行政院所屬各機關因公出國人員出國報告書

（出國類別：開會考察）

參加「美國微生物學會第104屆年會」並順道考察「分析食因性病原菌之實驗室」

出國人　服務機關：衛生署藥物食品檢驗局

職稱姓名：薦任技正黃翠萍

薦任技士張育彰

出國地區：美國新澳爾良及洛杉磯

出國期間：九十三年五月二十一日至六月三日

報告日期：九十三年八月三十日

系統識別號:C09301971

公 務 出 國 報 告 提 要

頁數: 23　含附件: 否

報告名稱:

赴美國參加[美國微生物學會104屆年會]

主辦機關:

行政院衛生署藥物食品檢驗局

聯絡人／電話:

陳婉麗／02-26531300

出國人員:

黃翠萍　行政院衛生署藥物食品檢驗局　第五組　薦任技正
張育彰　行政院衛生署藥物食品檢驗局　第五組　薦任技士

出國類別: 其他

出國地區: 美國

出國期間: 民國 93 年 05 月 21 日 -民國 93 年 06 月 03 日

報告日期: 民國 93 年 09 月 02 日

分類號/目: J0／綜合（醫藥類）　J0／綜合（醫藥類）

關鍵詞: 美國微生物學會, ASM

內容摘要: 美國微生物學會（American Society for Microbiology，ASM）規模龐大歷史悠久，會員遍佈全球，從1899年59位科學家創立至今會員超過42000名，在美國分爲7個區域（Region）36分部（Branch），會員中有百分之三十爲國際會員，第104屆年會（General Meeting）自2004年5月23日至27日，於美國路易士安那州（Louisiana, LA）新澳爾良市（New Orleans）的Ernest N. Morial會議中心（Ernest N. Morial Convention Center）舉行，參加會議的人員主要是美國微生物學會成員和來自世界各國的微生物學者與專家，據大會統計參加人數超過15,000人。大會包括依專業領域或行政執掌之分組會議（meetings）與學術論文發表，儀器設備、書籍、試劑與相關基金會等之展示（exhibits），以及會前研習會（workshops）。學術論文發表分口頭及壁報兩種方式，討論主題分爲四組（Groups）：一、診斷微生物學暨流行病學（包括C、F、L、U、Y分組）；二、致病力與宿主反應機制（包括A、B、D、E、G、V、Z分組）；三、一般微生物學（包括I、N、O、P、Q、R、W分組）；四、分子生物學、生理學、病毒學（包括H、J、K、M、S、T、X、AA分組）。依專長領域及研究興趣再細分爲27組（Divisions）：A-抗生素化學治療（Antimicrobial Chemotherapy）156篇；B-微生物的致病性（Microbial Pathogenesis）503篇；C-臨床微生物（Clinical Microbiology）370篇；D-一般醫學微生物（General Medical Microbiology）287篇；E-免疫學（Immunology）103篇；F-醫用黴菌學（Medical Mycology）102篇；G-黴漿菌學（Mycoplasmology）28篇；H-遺傳與分子生物學（Genetics and Molecular Biology）215篇；I-一般微生物（General Microbiology）152篇；J-超微構造與功能（Ultrastructure and Function）40篇；K-微生物生理與代謝（Microbial Physiology and Metabolism）187篇；L-院內感染（Nosocomial infections）13篇；M-噬菌體（Bacteriophage）34篇；N-微生物生態學（Microbial Ecology）355篇；O-發酵與生物技術（Fermentation and Biotechnology）121篇；P-食品微生物（Food

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美國微生物學會歷史悠久、會員遍佈全球，例行年會除舉辦學術性的壁報論文及專題討論發表會，並有儀器設備、材料試劑、書籍及相關基金會之參展。與會專家學者來自世界各地，參展單位也涵蓋全球，藉此機會可汲取世界各地科學工作者的研究心得、蒐集食因性病原菌及其毒素之最新檢驗資訊及未來技術發展之趨勢，並建立國際資訊技術交流之管道。此行參加第104屆美國微生物學會(ASM)年會之任務尚包括發表本局所完成重要食因性病原菌之研究成果「A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from sea to sep」；研習「Modern Phenotypic Testing Approaches to Complement Genomics」，以分子生物學爲基礎之技術，應用於微生物檢測，提高檢驗速度及準確性；研習「Conducting Research with Category A Bacterial Select Agents: Requirements and Opportunities」，瞭解美國對於研究A類生物戰劑之實驗室相關的管理措施與法令規定。另安排順道參訪考察活動，包括位於新澳爾良市的圖拉內大學（Tulane University）醫學中心（Medical Center）、美國食品藥物管理局（Food and Drug Administration, FDA）太平洋區域西南實驗室（Pacific Regional Laboratory Southwest, PRL-SW）及加州Pomona科技大學（California State Polytechnic University, Pomona）生物科學系（Department of Biological Sciences），藉由參觀考察活動，擷取其他研究室之優點並建立人際關係。

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摘　要

美國微生物學會（American Society for Microbiology，ASM）規模龐大歷史悠久，會員遍佈全球，從 1899 年 59 位科學家創立至今會員超過 42000 名，在美國分為 7 個區域（Region）36 分部（Branch），會員中有百分之三十為國際會員，第 104 屆年會（General Meeting）自 2004 年 5 月 23 日至 27 日，於美國路易士安那州（Louisiana, LA）新澳爾良市（New Orleans）的 Ernest N. Morial 會議中心（Ernest N. Morial Convention Center）舉行，參加會議的人員主要是美國微生物學會成員和來自世界各國的微生物學者與專家，據大會統計參加人數超過 15,000 人。大會包括依專業領域或行政執掌之分組會議（meetings）與學術論文發表，儀器設備、書籍、試劑與相關基金會等之展示（exhibits），以及會前研習會（workshops）。學術論文發表分口頭及壁報兩種方式，討論主題分為四組（Groups）：一、診斷微生物學暨流行病學（包括 C、F、L、U、Y 分組）；二、致病力與宿主反應機制（包括 A、B、D、E、G、V、Z 分組）；三、一般微生物學（包括 I、N、O、P、Q、R、W 分組）；四、分子生物學、生理學、病毒學（包括 H、J、K、M、S、T、X、AA 分組）。依專長領域及研究興趣再細分為 27 組（Divisions）：A-抗生素化學治療（Antimicrobial Chemotherapy）156 篇；B-微生物的致病性（Microbial Pathogenesis）503 篇；C-臨床微生物（Clinical Microbiology）370 篇；D-一般醫學微生物（General Medical Microbiology）287 篇；E-免疫學（Immunology）103 篇；F-醫用黴菌學（Medical Mycology）102 篇；G-黴漿菌學（Mycoplasmology）28 篇；H-遺傳與分子生物學（Genetics and Molecular Biology）215 篇；I-一般微生物（General Microbiology）

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場，此行藉由參與各項相關活動，除自發表之成果中更加了解目前執行業務所涉及之重要食因性病原菌，並吸收相關研究之精華以拓展視野，同時蒐集參展廠商多方面資訊，對於未來業務推動及處理新興議題均有很大的幫助。訪美期間並順道參訪鄰近從事食因性病原菌或分子生物學相關研究之大學及研究機構實驗室，如新澳爾良市圖內拉大學（Tulane University）醫學中心（Medical Center）、美國食品藥物管理局（Food and Drug Administration, FDA）太平洋區域西南實驗室（Pacific Regional Laboratory Southwest, PRL-SW）、加州科技大學（California State Polytechnic University, Pomona）生物科學系（Department of Biological Sciences）。第 105 屆美國微生物學會年會將於 2005 年 6 月 5 日至 9 日，在喬治亞（Georgia）的亞特蘭大（Atlanta）會議中心（Convention Center）舉行，投稿期限自今年 10 月 15 日至 12 月 13 日至 16 日，截止期限依分組領域不同。

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美國微生物學會歷史悠久、會員遍佈全球，例行年會除舉辦學術性的壁報論文及專題討論發表會，並有儀器設備、材料試劑、書籍及相關基金會之參展。與會專家學者來自世界各地，參展單位也涵蓋全球，藉此機會可汲取世界各地科學工作者的研究心得、蒐集食因性病原菌及其毒素之最新檢驗資訊及未來技術發展之趨勢，並建立國際資訊技術交流之管道。此行參加第 104 屆美國微生物學會(ASM)年會之任務尚包括發表本局所完成重要食因性病原菌之研究成果「A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from *sea* to *sep*」；研習「Modern Phenotypic Testing Approaches to Complement Genomics」，以分子生物學為基礎之技術，應用於微生物檢測，提高檢驗速度及準確性；研習「Conducting Research with Category A Bacterial Select Agents: Requirements and Opportunities」，瞭解美國對於研究 A 類生物戰劑之實驗室相關的管理措施與法令規定。另安排順道參訪考察活動，包括位於新澳爾良市的圖拉內大學（Tulane University）醫學中心（Medical Center）、美國食品藥物管理局（Food and Drug Administration, FDA）太平洋區域西南實驗室（Pacific Regional Laboratory Southwest, PRL-SW）及加州Pomona科技大學（California State Polytechnic University, Pomona）生物科學系（Department of Biological Sciences），藉由參觀考察活動，擷取其他研究室之優點並建立人際關係。

貳、 行程及紀要

美國微生物學會（American Society for Microbiology，ASM）規模龐大歷史悠久，會員遍佈全球，從 1899 年 59 位科學家創立至今會員超過 42000 名，在美國分為 7 個區域 (Region) 36 分部 (Branch)，**組織架構**，會員中有百分之三十為國際會員，第 104 屆年會（General Meeting）自 2004 年 5 月 23 日至 27 日，於美國路易士安那州（Louisiana, LA）新澳爾良市（New Orleans）的 Ernest N. Morial 會議中心（Ernest N. Morial Convention Center）舉行，年會議程如表一。參加會議的人員主要是美國微生物學會成員和來自世界各國的微生物學者與專家，參加人數據大會統計超過 15,000 人。大會於 23 日下午六時舉辦開幕典禮，包括會員大會、專題演講及頒獎。專題演講由哈佛大學 Dr. R.J. Collier 主講近年微生物致病的新發現與展望，特別是細菌分泌毒素蛋白及毒素進入哺乳類細胞膜機制等研究，及利用遺傳、生化及生理等方法對毒素蛋白進行研究，其研究成果之完整性及深入性值得我們學習與讚佩。由於會場大、參加人數多，大會於會場前面特別準備了兩個大銀幕，現場將典禮進行情形直接播映，使所有參加者均能清楚的看到整個過程。參與此次年會者有來自世界各國的微生物學者與專家。雖名為美國微生物學會年會，實際上比一般國際會議有過之而無不及。會後還安排盛大的歡迎接待晚會"A Unique Cultural Experience in the Big Easy"， 會中有新澳爾良市(New Orleans, The Big Easy, The Crescent City)聞名的爵士音樂(Jazz)及美食，大會不但作了成功的觀光文化宣傳，與會者也藉此機會互相熟悉，共同迎接未來四天的豐富之旅。

此次美國微生物學會於會議前兩天舉辦26場研習會(Workshop)，半天的研習會有8場，一天的研習會共有18場，其中有7場除演講外另含實際操作，內容主要針對臨床微生物之篩選鑑別及其抗藥性之檢測，各研習會場次如表二。考量經費及業務需要僅報名「WS-11 Modern Phenotypic Testing Approaches to Complement Genomics」及「WS-26 Conducting Research with Category A Bacterial Select Agents: Requirements and Opportunities」兩場，均為半天之研習會。前者22日上午舉辦，此研討會共邀三位專家學者進行演講，分別是 Biolog 公司 Barry Bochner 博士、美國農業部(USDA) Jean Petter-Bouldin 博士及美國 Miami 大學 Kenneth Rudd 教授。Barry Bochner 博士由微生物的生化代謝反應與基因間的因果，介紹一個新技術評估微生物的基因型與表現型的關係，借由"OmniLog PM system"之助分析待測目標的基因型與表現型的關係。由此系統亦可進行基因調控的研究。Jean Petter-Bouldin 博士首先介紹微生物對農畜業的影響，並以沙門氏菌為例，說明以 phenotype microarray 分析沙門氏菌的特色。Kenneth Rudd 教授 1981 年於加州大學柏克萊分校取得博士學位，在大腸桿菌(*Escherichia coli*)研究的領域中是一位家喻戶曉的人物。著名的 *Escherichia coli* K-12 株的基因體已被全部解出，他即參與此任務。另外他亦對一些未知功能的基因進行研究。其相關資料如附件一。「Conducting Research with Category A Bacterial Select Agents: Requirements and Opportunities」研習會於23日下午舉辦，講授者為威斯康辛州立威斯康辛－麥迪森大學（University of Wisconsin–Madison Madison, WI）食品研究機構(Food Research Institute, FRI)之教授 Eric A. Johnson 及資深研究員 Ann E. Larson，演講內容含蓋管控特定生物戰劑相關規定（the Select Agent rules，ＳＡ）綜述、A

類生物戰劑（Category A Bacterial Select Agents）界定及危害、申請註冊認證之規定、人員訓練及安全防護、文件管理及標準作業程序、實驗室儀器及設備之安全措施、A類生物戰劑（Category A Bacterial Select Agents, SA）之移轉規定及可追溯之紀錄等，因兩位講師之研究實驗室已跨越學術範疇及商業經營多年，並取得A類管控生物戰劑中與筆者業務直接相關之「肉毒桿菌及其毒素」之認證許可，講師不但具備專業素養，行政管理之實務經驗也非常豐富，所準備之講義十分完整，包括演講的 slide、各式申請表單及相關法令條文，可以提供實務演練者之參考，其相關資料如附件二。特定生物戰劑管制措施涵蓋政府單位、大專院校、研究機構及商業團體，凡持有者均需註冊登錄列管，且其軟硬體均應符合相關管制措施，並接受美國疾病管制局（ＣＤＣ）及美國農業部之管理稽核方可繼續運作，本研討會參加學員約 30 位，多數來自美國生技公司或學校研究機關，大部分正進行或將從事相關業務，因此更引起熱烈的討論與心得交換。

壁報論文、專題演講於 5 月 24 日至 27 日同步進行，學術論文發表分兩大部份，為使能對微生物各領域之重要性有所瞭解，特將各分組研究範疇及此次年會發表之個別論文數目列於表三、表四，討論主題分為四組(Group)：一、診斷微生物學暨流行病學（Diagnostic Microbiology and Epidemiology）634 篇，包括 C-臨床微生物(Clinical Microbiology)370 篇；F-醫用黴菌學(Medical Mycology)102 篇；L-院內感染(Nosocomial infections)13 篇；U-分枝桿菌學(Mycobacteriology)99 篇；Y-公共衛生(Public Health)50 篇；二、致病力與宿主反應機制（Pathogenesis and Host Responses Mechanism）1164 篇，包括 A-抗生素化學治療(Antimicrobial Chemotherapy)156 篇；B-微生物的致病性(Microbial Pathogenesis)503 篇；

D-一般醫學微生物(General Medical Microbiology) 287 篇；E-免疫學(Immunology)103 篇；G-黴漿菌學(Mycoplasmology)28 篇；V-臨床診斷免疫學(Clinical and Diagnostic Immunology)37 篇；Z-動物健康微生物學(Animal Health Microbiology) 45 篇；三、一般微生物學（General Microbiology）1396 篇，包括 I-一般微生物(General Microbiology)152 篇；N-微生物生態學(Microbial Ecology)355 篇；O-發酵與生物技術(Fermentation and Biotechnology)121 篇；P-食品微生物(Food Microbiology)125 篇；Q-環境與一般應用微生物(Environmental and General Applied Microbiology)525 篇；R-演化及基因體微生物學(Evolutionary and Genomic Microbiology) 87 篇；W-微生物教育(Microbiology Education)31 篇；四、分子生物學、生理學、病毒學（Molecular Microbiology, Physiology, and Virology）564 篇，包括 H-遺傳與分子生物學(Genetics and Molecular Biology)215 篇；J-超微構造與功能(Ultrastructure and Function)40 篇；K-微生物生理與代謝(Microbial Physiology and Metabolism)187 篇；M-噬菌體(Bacteriophage)34 篇；S-去氧核糖核酸病毒(DNA Viruses)12 篇；T-核糖核酸病毒(RNA Viruses)35 篇；X-真核生物的分子、細胞及普通生物學(Molecular, Cellular and General Biology of the Eukaryotes)23 篇；AA-自營、共生、寄生之單細胞生物（Free-living, Symbiotic, and Parasitic Protists）18 篇。不管主題及對象之差異，切入觀點大多由遺傳及分子生物角度著手。大會另外以各領域之熱門題材，邀請專家做專題演講或座談會。本屆年會所發表的壁報論文約三千七百多篇，而邀請之專題演講約有三百餘場，同時間內常有多個演講進行，故只能選擇較有興趣之題目聽講。

相關廠商機關單位展示期間為 5 月 24 日至 26 日，展示場地約與臺北市信義路世貿中心相當，約有三百個展示攤位，幾乎在微生物範圍可能

用到的儀器設備、試劑等均可在展示場內看到，國內也有廠商派員參觀，以掌握最新產品趨勢。在此可找到最新的實驗器材等資料，有助於研究工作的進行。另外。為方便參觀者索取資料，大會為每位參加者準備了參觀卡 (Expocard) ，只要在廠商攤位的電腦刷卡，並告知個人需求，參觀者的基本資料，包括姓名、住址、工作單位及工作性質等資料就進入電腦中，日後廠商即可據以寄資料給參觀者。在會場展示攤位尚包括相關領域之圖書出版社，因此可收集到最新產品資料以及最新出版書籍資料，尤其很多在國內代理商無法解決的問題，原廠專家均能完滿回答。另外美國微生物學會也出版了十一種期刊，如表五，均為極優良之期刊，對於國內的研究極俱參考價值。除了廠商外，很多與微生物有關之機構亦設有攤位，如美國菌株保存中心 (American Type Culture Collection, ATCC)、美國農業部食品安全研究資訊機構(USDA，National Food Safety Research Information Office，FSRIO)、美國疾病管制局（Centers for Disease Control and Prevention，US-CDC）、美國食品藥物管制局（Food and Drug Administration，FDA）、美國國家衛生組織 (National Institute of Health, NIH)、美國國家科學院 (National Academy of Science, NAS)等，均在展示場設有攤位，使參觀者對其有所瞭解。

另外會程期間亦同時進行參訪事宜之聯繫，會程首日依事先之約定與鄭崇明博士、蘇意誠博士、林維真博士等旅美學者相聚，確認 ASM 會後參訪實驗室之相關事宜，並一起參加「美國微生物學會華人學會」（Chinese American Microbiology Society）之年度聚會，會見多位來自台灣及大陸的微生物學家。經路易士安那台灣新澳爾良市分會會長曾東松博士之安排，於 25 日下午參訪與我國學術交流密切的圖內拉大學，主要行程為參訪位於新澳爾良市北方的圖內拉大學基因治療中心

（Genetherapy Center）實驗室，如附件三。90 年 3 月立法院厚生基金會曾邀請立法委員、陳建仁署長及學者專家，參加圖內拉大學於美國新澳爾良市舉辦之「新世紀基因治療國際研討會」。我國立法院於 90 年 6 月舉辦之「21 世紀生醫科技國際研討會」，亦邀請圖內拉大學基因治療中心之專家作專題演講。包括 Dr. Darwin Prockop (現職於圖內拉大學擔內科教授及基因治療中心主任)及 Dr. Brian T. Butcher (現職是圖內拉大學基因治療中心之副主任)。圖內拉大學基因治療中心的走道上還可以發現貼有「21 世紀生醫科技國際研討會」的宣傳海報及旗幟。該中心實驗室設備新穎擁有流式細胞分析儀、PCR 系統、放射線標定、各式電泳系統等等設備。實驗室安全標示及管理做的相當不錯值得我們學習。基因治療中心主任 Dr. Darwin Prockop 的研究專長為膠原蛋白生物合成、構造、功能、基因及引起軟骨硬骨之疾病。目前，他主要的研究方向為細胞基因治療。

本局發表之研究報告「A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from *sea* to *sep*」，如附件四，引起許多研究金黃色葡萄球菌腸毒素專家學者的高度興趣，雖然被安排在最後一天展示（5 月 27 日），但一早便有來自法國研究室的學者等待我們將壁報論文貼上指定的版面，隨後即勤以筆記之，多位在會程中認識的朋友也來捧場，在熱烈的問題與回答之討論互動中雙方都獲得許多靈感。

回程至洛杉磯拜訪曾任職本局微生物組同事鄭崇明博士，因其任職美國 FDA 太平洋區域西南實驗室(Pacific Regional Laboratory Southwest, PRL-SW)，目前負責業務與本局關係密切，藉此機會請教美國國內食品安全管理機制（如附件五），及對於內銷及進口產品的管制措施及病原菌檢驗情形及分型技術，同時針對其發表之沙門氏桿菌檢測方法及檢出

率交換心得，並安排參觀去年七月才搬遷至新落成之辦公室，此位於爾灣（Irvine）實驗室（PRL-SW）與行政部門（Los Angeles District, LOS-DO）合併之聯合辦公室含兩層樓之褐色透明建築物，室內之辦公室與實驗室以透明玻璃完全區隔開，戶外為空曠之平面停車場，約有 180 個員工，編制及任務大致與本局相似（如附件六、七），行政上食品與藥品分組，局長（Elizabeth A. Keville, Director）以下有組長（Ted Dunn, Director, Microbiology & Dennis Farley, Director, Food Chemistry）及科長（如 Richard M. Ruby, Supervisory Microbiologist），實驗分化學與微生物分析，其中從事微生物實驗者約 30 位，工作性質則研究計劃與例行業務區分，鄭博士以研究為主（Food Pathogen Specialist），目前正評估 RT-PCR 在檢測病原性微生物之應用及利用 PCR 方法取代 BAM 上建議之沙門氏桿菌傳統檢測方法；而主要之例行業務為檢驗進口及國內食品之病原性微生物，採樣及驗畢之行政處理均屬於行政部門負責，主要依據 IOM（Investigation Operation Manual）操作手冊（如附件八）之規定。此辦公室雖距離 FDA 總部美國馬里蘭大學學院園分校（College Park）遙遠，但藉由定期舉行之視訊會議（會議室可同時容納 300 人），可以即時進行政策宣導及同步討論熱門議題。

目前從事肉毒桿菌及其毒素之研究的旅美校友林維真博士的博士論文乃 Dr. Eric Jonhson（履歷如附件九）所指導，林博士研究室去年通過 CDC 之認證，因肉毒桿菌列入 A 類生物戰劑最可能應用之材料，各研究單位均採保守方式秘密進行研究，官方也嚴加管制，此行很幸運的得到她的同意，參觀其任教之加州科技大學 POMONA 分校研究室（詳如附件八），並熱心提供多種培養厭氧菌的材料，及親自示範正確的操作方式，經此深感本局多年來因此菌發生率不高、送驗檢體不多、多未

檢出，對於此菌之重視程度不如其他病原菌，但依目前生物恐怖主義瀰漫，除了食品中毒案方面的考量外更需防範生物性的恐怖攻擊，對於此類厭氧菌之檢驗技術需迎頭趕上。

參、心得與建議

一、擬定出國開會考察計劃

出席國際會議前充分的準備工作是需要、必要而且十分重要。首先要掌握年會投稿報名期限及相關之規定，美國微生物學會年會舉辦的時間歷年約在五、六月間；地點則以美國本土東、西、南、北、中各大城市輪流，例如奧蘭多（May 20-May 24, 2001, Orlando, Florida）、鹽湖市（May 19-May 23, 2002, Salt Lake City, Utah）、華盛頓特區（May 18-May 22, 2003, Washington DC）、新澳爾良市（May 23-May 27, 2004, New Orleans, L A）（附件十一）、亞特蘭大市（June 5-June 9, 2005, Atlanta, Georgia）；投稿期限在前一年的十月至十二月間，目前一律採行網路線上投稿，且逾期不受理，投稿之作者中至少有一名需為現任會員或將會完成報名手續並出席大會；出席大會之線上報名在早期報名（early registration）出席費用上有很大的優惠，但因故無法參加則有退費的問題，現場報名（on-site registration）則比照 late registration 費用高；研習會一律採預約方式，均需事先報名且繳交費用；另外，大會還為攜眷參加者安排了許多活動及幼兒託寄中心，與會者可同時享有專業與親情兼顧的假期（詳見 http://www.asm.org）。

事前的準備工作需大力仰賴專業素養及語言能力，而兩者的養成均需日積月累，尤其國際共通語言-英語，精通外語不僅在專業知識的吸收方面有所助益，在規劃出國行程時更突顯其重要性：1.便於事前資訊蒐集，尤其網際網路資訊發達，舉凡交通航線選擇、當地旅館之地點、價位及設備、服務項目，參訪機關及其任務，或機票附帶之各種套裝旅遊...等琳瑯滿目，若用心搜索可以規劃出更圓滿的行程；2.便於個人於非母

語國家從事除食衣住行外之各項活動，如旅遊、購物、參觀博物館等；3.與國際組織或國外友人之通訊，主要仍依賴英語的溝通，尤其此行充分發揮 E-mail 便利迅速的優點，雖出國計畫擬定期間匆促且變數很多，尤其在短期內安排參訪考察更加困難，終於透過往返頻繁的 E-mail，進行有效率的互動而一一迎刃而解。

二、參加 ASM 之相關活動

參加定期舉辦的 ASM 年會，研究成果的發表有助於研究團隊士氣的鼓舞，而且不但可以提昇個人工作成究感，達到學術交流的目的，更可以展現本局在專業方面的實力，本局投稿之「A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from *sea* to *sep*」乃本局近三年之研究成果之一。除了參與壁報論文、專題討論之外，各專業領域的分組會議及聯誼會也值得鼓勵，因為可以發表意見的機會較多，影響力也較直接，又可趁機結交活躍於國際各專業領域的人士，當然要同時達到這些目的除在專業領域、語文能力需精通外，還需俱備樂觀、積極、進取的心態及精湛的談話技巧，另外諸如各組之社交晚會或短程之觀光活動，雖不屬於專業知識之增長，卻有助於促進相同領域學者專家間之人際關係。

美國微生物學會規模龐大歷史悠久，不愧大型國際會議的典範，歷年來國人參加此會議的目的主要為發表自己的研究報告、聽專題講座、見見難得碰面的朋友、逛逛壁報論文、看各類儀器及試劑與出版品的展覽。由於國際網路發達，雖 ASM 在美國國外並無分會之設置，但會員與總部或各專業領域分組組織之聯繫卻十分頻繁，事實上欲直接參與會務的機會也不困難。尤其在美國慘遭「九一一恐怖攻擊」事件之後，新

成立之 Y 組「公共衛生組」（Y Division，Public Health），其成立時間雖短，但成長速度卻非常快速，主要成員包括從事與大眾衛生安全相關之專家學者，尤其官方如美國 FDA、CDC、USDA、NHI 等單位之專業及行政部門人員，本局未來任務導向將逐漸朝行政管理方面著手，這一方面的訓練卻是注重檢驗工作之技術層面的我們所欠缺的，此行雖未達預期目標，但自分組報名至今由 Y 組的網路通訊負責人 Dr. Brian D. Sauders （Cornell University, Department of Food Science, Food Safety Laboratory; 405 Stocking Hall, Ithaca, NY 14853. Phone: (607) 255-1266 Fax: (607) 254-4868; Email: bds26@cornell.edu; http://www.foodsci.cornell.edu）善盡職守不斷的寄來 Y 組各項活動、資訊或徵詢意見可略知其運作之概況，可惜其中許多需俱會員資格方可參與，若能以「藥物食品檢驗局」的名義加入美國微生物學會，可以本局身份發表意見，藉此登上國際舞臺，同時出席所屬各項國際會議或研討會的費用也較省，同仁也有較多的機會參與大型國際性活動。且藉由積極參與 Y 組之各項活動，可以藉此民間機構達到官方交流之實質目的，事實上 Dr. Sauders 今年在與會期間曾多次表示會務遽增徵求自願者幫忙。

壁報論文方面有多篇與致病性息息相關，其中幾篇令人印象深刻，如介紹側鞭毛的新研究: 有兩種類型的鞭毛可使嗜中溫性氣單胞菌具有游移的能力。一種為無鞘的端鞭毛，它可讓菌體在液態的環境中游動(swim)但在固態的培養基上卻不能驅使菌體移動（swarm）。另一類為有鞘的側鞭毛，它可使菌體在固態的培養基上移動，側鞭毛增進氣單胞菌對細胞的附著、入侵及生物膜的形成。以 *A. caviae* 側鞭毛基因序列為基礎設計的探針，用於南方墨點法之分析，顯示與探針有陽性反應之

菌株與具有側鞭毛及移動能力的菌株有一致性。以更便捷的 PCR 方法偵測側鞭毛基因，其結果亦與側鞭毛的產生及移動能力具有一致性。由於並非所有嗜中溫性的氣單胞菌皆產生側鞭毛，因此將帶有側鞭毛基因（*laf*; lateral flagella）的質體送入不產側鞭毛的菌株，結果顯現以顯微鏡可觀察到側鞭毛的產生，且該菌株具增強對 HEp-2 細胞的吸附及入侵能力和生物膜的形成能力。另外一篇是與我們發表壁報論文同一時段的金黃色葡萄球菌毒素基因的調控論文:金黃色葡萄球菌產生各種毒性因子，包括菌體表面蛋白及胞外蛋白。這些毒性因子的分泌藉由一些調控基因如 *agr*、*sar*、*sig*β、*sae* 及 *arl* 等的基因產物和受一些與 SarA 具同源性之蛋白嚴謹的調控著。調控因子 *rot* 的發現是將金黃色葡萄球菌蛋白酵素和 α-毒蛋白缺陷的 *agr* 突變株藉由跳躍子造成回復突變而發現。Rot 是一個與 SarA 同源的蛋白，所有文獻顯示與 SarA 同源的蛋白皆具有綜合調控毒性基因的功能。因此作者藉由本篇文章探討 Rot 對金黃色葡萄球菌毒性基因在轉錄時的調控情形。作者利用生物晶片比對 *agr* 突變株與 *agr rot* 雙突變株在轉錄時的差異。結果顯示，Rot 不僅扮演抑制者的角色，它亦綜合性地調控著金黃色葡萄球菌的基因。另一方面 Rot 與 *agr* 對目標基因有著相反的調控結果。這結果進一步暗示 Rot 在金黃色葡萄球菌毒性因子基因的調控上扮演重要角色。藉由觀摩其他專家學者的研究不僅可啟發我們的研究方向更可體會研究者的用心值得我們效法。

另外，對於 ASM 所辦理之終身教育學分（Continuing Education Credit，CE Credit），需在大會結束前付費($30)，出席證明則可自行到會場 E-central 登錄後列印，或 6 月 11 日起上網（www.asm.org）登錄產生列印，此制度可以記錄所參加的講座、提高參與者的意願、並提昇參與

者在職教育機會，近年來國內許多研討會亦採此方式進行，消費者付費及榮譽感、自律性的觀念也漸為國人所接受。其中包含研習會（workshop），含實驗操作者所需費用雖然較高，但在技術面的收獲卻遠比僅有演講來得深刻易了解吸收，今年參加兩場研習會雖均為演講方式，但與講者面對面的溝通，將有助於未來專業需求的互動。除了年會外，在 ASM 的網站上尚有許多遠距教學進修的機會，有心者可上網瀏覽，其中有許多俱有參考價值的網站，可惜多數限定會員才可以登錄，另外有幾個屬於開放給一般非會員的廣大民眾如www.Microbe.org 專為小孩設計，www.MicrobeLibrary.org 乃針對推動微生物方面的教育者，www.MicrobeWorld.org 則範疇較大，較偏重於專業人員或科學家。

三、參訪考察之相關活動

美國食品藥物管理局執掌及編制任務與本局十分相似，其病原菌分析檢驗執全球之牛耳，而擬訂之「細菌分析手冊」（Bacterial Analysis Manual, BAM）亦為本局檢驗方法之重要參考依據。此行可與該單位負責專家研討食品檢驗之最新技術以及未來技術發展之趨勢。且因應本局未來業務轉型為食品衛生行政理導向，有必要了解該局及相關機構對食品中毒之預防預警、管理及發生時之處理機制，作為降低本國食品中毒案件及管理食品衛生之參考。且時代變遷快速，國際間交流頻繁，對於未來應更積極規劃因應的措施，針對本局職掌的食品安全議題，WHO 已經將食品安全列為重要的公共衛生問題，也擬定了『全球食品安全戰略』，證明此議題的重要性與急迫性，目前全球已有許多食品安全相關的監控系統，我們需要更積極的參與，蒐集相關資訊並建立本土的背景值與資料庫。因此 FDA 一向是本局出國研習或開會順道參訪的首要目

標，近年來由於反恐策略限制，無法如過去般容易申請進入各相關機構，進行較深入之研修或較長時間的參訪活動，此行參加 ASM 年會託曾任職於本局微生物組之同事鄭崇明博士鼎力相助，除安排導覽其目前工作所在 FDA 太平洋區域西南實驗室（PRL-SW）外，因美國西岸加州洛杉磯一帶幅員遼闊，都會地區距離遠，且大眾運輸系統不若東岸各大城市方便，只好接受鄭博士好意的安排，請休假親自帶領我們進行在加州的參訪活動，包括加州科技大學 POMONA 分校林維真博士研究室之行程，在交通住宿各方面細節也全部偏勞規劃，至於加州爾灣大學（California State University, Irvine）（附件十二）雖距離 FDA 新址最近，因時間匆促聯繫不及，僅在校園遊覽拍照紀念。對於大會所在地新澳爾良參訪目標的選定，則全賴曾東松博士之安排。身歷其境的參訪活動確實留下深刻的印象且獲益匪淺，但在規劃與進行的過程中更深深體會到人脈資源遠比物質資源更珍貴。

四、結語

此行所見所聞不論專業新知或異國風俗民情均深切體會『讀萬卷書、行萬里路』的重要。我們應多參與此類國際性會議，將同仁辛苦研究的成果發表在全球微生物專家學者聚集的重要會議上，不僅可宣揚我政府在微生物領域的努力及重視，亦可藉由參加會議達到交流及吸取新知的目的。因此建議鼓勵同仁將研究成果多發表於美國微生物年會，並且每年都派人員參加。

參、 謝誌

感謝局裡同仁在辦理出國計畫各方面行政需要的配合。感謝施組長養志及王科長貞懿的全力支持，本組同仁尤其食品中毒科同仁方紹威技正、郭荔平技正、楊怡真技士及王肇馨技士，在行前提供相關之資訊，以及出國期間辛勞代理職務。感謝鄭崇明博士、林維真博士、蘇意誠博士、曾東松與林慧宜博士賢伉儷、許素菁博士於訪美期間之帶領參訪實驗室及熱心款待與細心照顧。感謝 Y 組的網路通訊負責人 Brian D. Sauders 與會期間熱情接待，與會前後持續提供許多資訊。

表一、美國微生物學會第一0四屆年會議程

General meeting Program-at-a-Glance

	Saturday May 22	Sunday May 23	Monday May 24	Tuesday May 25	Wednesday May 26	Thursday May 27
Workshop - Registration	7:30 am - 2:00 pm	7:30 am - 2:00 pm				
Attendee Registration		12:00 noon -5:00 pm	7:00 am - 5:00 pm	7:00 am - 5:00 pm	7:00 am - 5:00 pm	7:00 am -12:00 noon
Workshops	8:30 am - 4:30 pm	8:30 am - 4:30 pm				
Colloqua and Symposia			8:00 am- 10:30 am 2:30 am - 5:00 pm	8:00 am- 10:30 am 2:30 pm- 5:00 pm	8:00 am- 10:30 am 2:30 pm - 5:00 pm	8:00 am- 10:30 am
Poster Sessions			9:00 am - 12:00 noon 1:00 am - 4:00 pm	9:00 am- 12:00 noon 1:00 am- 4:00 pm	9:00 am - 12:00 noon 1:00 am- 4:00 pm	9:00 am - 12:00 noon
Exhibits			9:00 am - 4:00 pm	9:00 am - 4:00 pm	9:00 am - 4:00 pm	
General Sessions		Opening Sessions 6:00 pm- 7:30 pm	President's Address 5:30 pm - 6:30 pm		President's Forum 5:30 pm- 7:00 pm	
Award Lectures or Student Presentations			10:45 am- 11:45 am 1:00 pm- 2:00 pm	10:45 am- 11:45 am 1:00 pm- 2:00 pm	10:45 am- 11:45 am 1:00 pm- 2:00 pm	
Social Events		Opening Reception 7:30 pm- 10:00 pm			President's Forum Reception New Orleans Marriott 7:30 pm- 10:00 pm	

表二、研習會(Workshop)場次及時刻表(5/22)

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01. May 22, 2004 8:30am - 4:30pm

Workshops WS-01. Introductory Clinical Mycology: Help for the Beginner

02. May 22, 2004 8:30am - 4:30pm

Workshops WS-02. Concepts for Establishing and Operating a Microbial Culture Collection

03. May 22, 2004 8:30am - 4:30pm

Workshops WS-03. Rapid Cycle, Real-Time PCR for the Clinical Microbiological Laboratory (2-day)

04. May 22, 2004 8:30am - 4:30pm

Workshops WS-04. Rapid Cycle, Real-Time PCR for the Clinical Microbiology Laboratory

05. May 22, 2004 8:30am - 4:30pm

Workshops WS-05. The Laboratory Information System (LIS): Maximizing Its Value in the Clinical Microbiology Laboratory

06. May 22, 2004 8:30am - 4:30pm

Workshops WS-06. Anaerobic Bacteriology for the Clinical Laboratory

07. May 22, 2004 8:30am - 4:30pm

Workshops WS-07. Susceptibility Testing Update for the Clinical Microbiology Laboratory

08. May 22, 2004 8:30am - 4:30pm

Workshops WS-08. GMP Series: Significance and Approach to Objectionable Organisms in a GMP Environment

09. May 22, 2004 8:30am - 4:30pm

Workshops WS-09. Regulatory Update on Changes in Coding and Reimbursement

10. May 22, 2004 8:30am - 4:30pm

Workshops WS-10. Algorithms and Streamlining for the Clinical Microbiology Laboratory

11. May 22, 2004 8:30am - 12:00pm

Workshops WS-11. Modern Phenotypic Testing Approaches to Complement Genomics

12. May 22, 2004 8:30am - 12:00pm

Workshops WS-12. The Gram-Positive Challenge: Clinical Importance of Aerobic Catalase-Negative Gram-Positive Cocci

13. May 22, 2004 1:00pm - 4:30pm

Workshops WS-13. Clinical Mycobacteriology: What is Old, Still Used, and New

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表二、研習會(Workshop)場次及時刻表(5/23)

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14. May 23, 2004 8:30am - 4:30pm

Workshops WS-14. Validation, Verification and Accreditation

15. May 23, 2004 8:30am - 4:30pm

Workshops WS-15. Verification of Training and Ongoing Competency in the Clinical Microbiology Laboratory

16. May 23, 2004 8:30am - 4:30pm

Workshops WS-16. In Vitro and In Vivo Test Methods Used to Assess the Efficacy of Topical Antimicrobial Products

17. May 23, 2004 8:30am - 4:30pm

Workshops WS-18. Gram Positive Rods in the 21st Century: An Updated Look at Bacillus, Listeria, Corynebacterium and Other Related Aerobic Gram-Positive Rods

18.May 23, 2004 8:30am - 4:30pm

Workshops WS-19. Biofilms V: Molecular Biology and Reporters

19. May 23, 2004 8:30am - 4:30pm

Workshops WS-20. Microbial Source Tracking Using Indicator Organisms

20. May 23, 2004 8:30am - 4:30pm

Workshops WS-21. Current Advancements in Predictive Microbiology Tools

21. May 23, 2004 8:30am - 4:30pm

Workshops WS-22. Rapid, Cost-Effective Identification of Gram-Negative Rods

22. May 23, 2004 8:30am - 12:00pm

Workshops WS-23. Select Agents: How to Prepare Your Laboratory for Inspections

23. May 23, 2004 8:30am - 12:00pm

Workshops WS-24. Modern Molecular Microbiology

24. May 23, 2004 8:30am - 12:00pm

Workshops WS-25. Microbiological Applications of RNAi Technologies

25. May 23, 2004 1:00pm - 4:30pm

Workshops WS-26. Conducting Research with Category A Bacterial Select Agents: Requirements and Opportunities

26. May 23, 2004 1:00pm - 4:30pm

Workshops WS-27. Staphylococcal Small Colony Variants (SCVs)

表三、美國微生物學會依專長領域或興趣分組（A-Z）

Division Descriptions

- Division A

  Antimicrobial Chemotherapy
  Division A is concerned with the discovery, mode of action, development and use of antimicrobial agents, and the mechanisms by which infective agents develop resistance to these compounds.

- Division B

  Microbial Pathogenesis
  Division B is concerned with understanding (i) the genetic, biochemical, and structural basis of the pathogenesis of bacterial and protozoan diseases (including toxins, colonization, invasion, immunity avoidance, and other virulence mechanisms) and (ii) host factors in the infectious process.

- Division C

  Clinical Microbiology Web Site
  Division C is involved with methods for detection, isolation, identification, characterization, and antimicrobial susceptibility testing of clinically significant microbial pathogens or their products of diagnostic significance, e.g., toxins, antigens, nucleic acids. Also involved with diagnosis-oriented investigations of these microorganisms.

- Division D

  General Medical Microbiology
  Division D is concerned with in vitro studies of medically-important bacteria including the genetics and physiology of pathogens (their surface structures and antigens), mechanisms of adherence, phagocytes and phagocytosis, and the etiology and classification of new agents.

- Division E

Immunology Web Site
Division E is interested in immunity to bacteria, fungi, parasites and viruses, cellular and molecular mechanisms of humoral and cellular immunity, phagocytic cells and constitutive host defenses, cytokines, immunomodulation by microbes, microbial products and other factors (e.g. stress, nutrition), adjuvants and vaccine development.

- Division F

Medical Mycology Web Site
Division F encompasses the biochemistry, molecular biology, genetics, morphogenesis, pathogenesis, immunology, epidemiology, laboratory identification, in situ detection, and taxonomy of fungi, especially those known to cause disease in man and other animals, and the therapy of those diseases.

- Division G

Mycoplasmology Web Site
Division G encompasses the genetic, pathogenic, immunogenic, taxonomic, biochemical, and clinical aspects of the animal, human, plant and insect mycoplasmas (Mollicutes).

- Division H

Genetics and Molecular Biology
Division H encompasses genetic and molecular biological studies of the regulation and detailed mechanisms of transcription, translation, and replication in microbial systems.

- Division I

General Microbiology
Division I encompasses a diverse range of interests including the growth, development, behavior and ecology of the entire spectrum of microorganisms.

- Division J

Ultrastructure and Function Web Site

Division J is concerned with ultrastructural analyses of microbial cells and of communities of microbial cells adherent to surfaces using biochemical, genetic, and microscopical techniques which yield information concerning organization on the molecular, cellular, and community levels.

- Division K

  Microbial Physiology and Metabolism Web Site
  Division K encompasses the integration of biophysical, biochemical, molecular biological, genetic and other approaches to understanding structure/function relationships of diverse microorganisms. Microbial physiology includes the study of microbial metabolism, enzymology, cell envelopes, transport, responses to environmental fluctuations, growth, differentiation, and other related processes.

- Division L

  Nosocomial Infections
  Division L encompasses the microbiology and epidemiology (including pathogenesis, diagnosis, control and treatment) of hospital and institutionally related infections and all levels of basic through applied research and clinical trials of interventions to reduce the occurrence or provided prompt diagnosis and treatment of such infections.

- Division M

  Bacteriophage Web Site
  Division M is composed of researchers dedicated to the study of bacterial viruses. Current topics of interest are: assembly and structure, genome structure, initiation of infection, regulation of transcription and translation, replication, recombination, repair, viral-host interactions, new phage systems and molecular cloning technology.

- Division N

  Microbial Ecology
  Division N encompasses the ecology of natural microbial assemblages

and laboratory approaches that help us understand microorganisms in natural environments, such as water, soils and in higher organisms.

- Division O

  Fermentation and Biotechnology Web Site
  Division O serves members with interests in the molecular biology, genetics, biosynthesis, and bioconversions of natural products including antibiotics, xenobiotics, and macromolecules produced by procaryote and eucaryote microorganisms and animal cell cultures. Programming is directed toward modern molecular aspects of biotechnology and industrial microbiology.

- Division P

  Food Microbiology Web Site
  Division P is concerned with fundamental and applied microbiology on food-associated organisms: their growth, identification, biosyntheses, control, interaction with hosts, genetics, toxin production, influence on food quality and safety, and application in food fermentations.

- Division Q

  Environmental and General Applied Microbiology
  Division Q serves microbiology from both applied and environmental fields, including the traditional fields (public health microbiology; disinfection; environmental virology; water and wastewater microbiology) and developing fields (biodegradation of xenobiotics; corrosion; microbial interactions with metals; biofouling; aerosolized microorganisms; environmental considerations for genetically engineered microorganisms; soil and subsurface microbiology).

- Division R

  Evolutionary and Genomic Microbiology Web Site
  Division R is a forum for the study of microbial diversity and systematics, and development of the laboratory, bioinformatic and

conceptual tools required to characterize and understand the evolution of genes, genomes and organisms.

- Division S

  DNA Viruses
  Division S is concerned with basic and applied microbiology of animal viruses with DNA genomes.

- Division T

  RNA Viruses
  Division T represents all ASM members interested in the structure replication, pathogenesis, and epidemiology of RNA-containing viruses of prokaryotic and eukaryotic cells.

- Division U

  Mycobacteriology Web Site
  Division U is composed of members involved with mycobacteria and its diseases, on a research, diagnostic, public health, or teaching basis.

- Division V

  Clinical and Diagnostic Immunology
  Division V (i) promotes research toward understanding the processes involved in the host immune system and its responses; encourages development and application of antibody, antigen, and molecular-based diagnostic procedures to assess the integrity and functioning of components of the host immune system, and supports clinical approaches to immune-mediated diseases; (ii) promulgates information on antibody, antigen and molecular-based diagnostic procedures, including the significance, interpretation and limitations of these assays; and (iii) encourages standardization and quality control of procedures and reagents used in clinical and diagnostic immunology laboratories.

- Division W

Microbiology Education Web Site
Division W provides a forum for members interested in microbiology education at all levels, including pre-college, college and university, and health professional curricula.

- Division X

Molecular, Cellular and General Biology of Eukaryotes
Division X encompasses researchers dedicated to the study of nucleated cells of both microbial and higher organisms. Current topics of interest include molecular mechanisms of basic cellular processes, structure and function of subcellular oganelles, and evolutionary biology and ecology of eukaryotic microbes.

- Division Y

Public Health Web Site
Division Y serves members with a primary interest in public health practice and infectious diseases. Involves the contributions of microbiology to surveillance, epidemic investigations and other public health activities.

- Division Z

Animal Health Microbiology
Division Z is the forum for investigators whose interests encompass the diseases of animals (e.g. companion, food and exotic) and the control or treatment of those diseases using antimicrobial agents, vaccines, probiotics, etc. Current topics of interest include animal pathogen diagnostics, veterinary or zoonotic pathogen antimicrobial susceptibility testing, surveillance/ epidemiological studies, new technologies to reduce on farm zoonotic pathogens, immunology and pathogenesis.

- Division AA

Free-Living, Symbiotic, and Parasitic Protists
Division AA's purpose is to bring together those with interests in all aspects (e.g., behavior, biochemistry, cell biology, chemotherapy,

cultivation, ecology, evolution, genetics, life cycle, molecular biology, morphogenetics, natural history, pathogenesis, parasitology, phylogenetics, physiology, systematics, taxonomy, and ultrastructure) of eukaryotic microbes that include those known as the "single-celled, unicellular or acellular organisms," protozoans, the lower algae, and the lower fungi.

表四、美國微生物學會第一０四屆年會各領域發表壁報論文篇數

領域 (發表壁報論文篇數)
Division A: Antimicrobial Chemistry（156）
Division AA : Free-living, Symbiotic, and Parasitic Protists（18）
Division B: Microbial Pathogenesis（503）
Division C: Clinical Microbiology（370）
Division D: General Medical Microbiology（287）
Division E: Immunology（103）
Division F: Medical Mycology（102）
Division G: Mycoplasmology（28）
Division H: Genetics and Molecular Biology（215）
Division I: General Microbiology（152）
Division J: Ultrastructure and Function（40）
Division K: Microbial Physiology and Metabolism（187）
Division L: Nosocomial Infections（13）
Division M: Bacteriophage（34）
Division N: Microbial Ecology（355）
Division O: Fermentation and Biotechnology（121）
Division P: Food Microbiology（125）
Division Q: Environmental and General Applied Microbiology（525）
Division R: Evolutionary and Genomic Microbiology（87）
Division S: DNA Viruses（12）
Division T: RNA Viruses（35）
Division U: Mycobacteriology（99）
Division V: Clinical and Diagnostic Immunology（37）
Division W: Microbiology Education（31）
Division X: Molecular, Cellular and General Biology of the Eukaryotes（23）
Division Y: Public Health（50）
Division Z: Animal Health Microbiology（45）

表五、美國微生物學會所出刊之期刊

Journal	Production Editor	Phone No. & E-Mail
Antimicrobial Agents and Chemotherapy	Arthur Gelmis	(202)-942-9231 agelmis@asmusa.org
Applied and Environmental Microbiology	Barbara Slinker	(202)-942-9219 bslinker@asmusa.org
Clinical and Diagnostic Laboratory Immunology	Anastacia Thomasian	(202)-942-9215 tthomasian@asmusa.org
Clinical Microbiology Reviews	Arthur Gelmis	(202)-942-9231 agelmis@asmusa.org
New Journal in 2002 -- Eukaryotic Cell	Arthur Gelmis	(202)-942-9231 agelmis@asmusa.org
Infection and Immunity	Diane Smith	(202)-942-9288 dsmith@asmusa.org
International Journal of Systematic Bacteriology (* No longer published by ASM)		
Journal of Bacteriology	Jack Kenney	(202)-942-9243 jkenney@asmusa.org
Journal of Clinical Microbiology	Anastacia Thomasian	(202)-942-9215 tthomasian@asmusa.org
Journal of Virology	Judith Nedrow	(202)-942-9234 jnedrow@asmusa.org
Microbiology and Molecular Biology Reviews (formerly Microbiological Reviews)	Arthur Gelmis	(202)-942-9231 agelmis@asmusa.org
Molecular and Cellular Biology	Becky Zwadyk	(202)-942-9214 bzwadyk@asmusa.org

*Now Published by the Society for General Microbiology

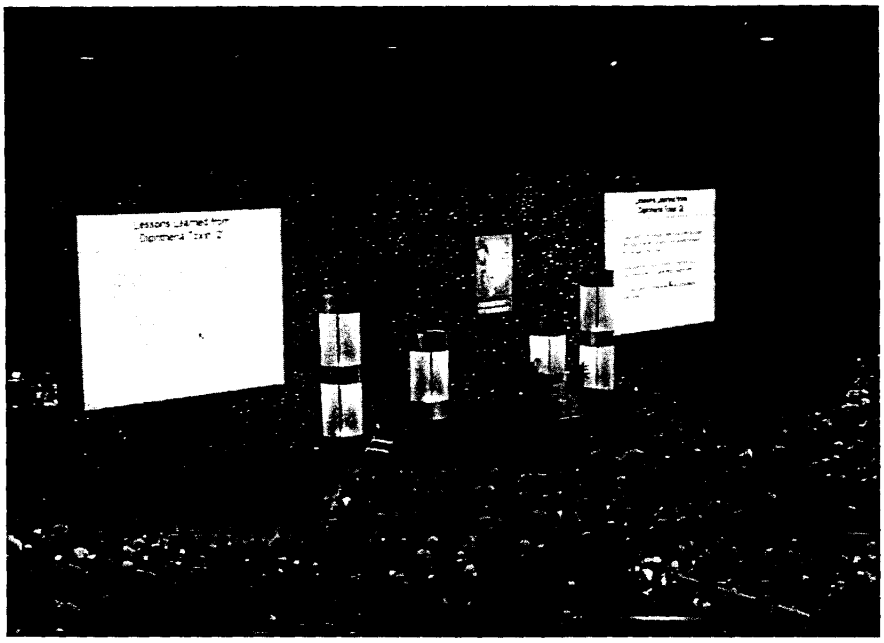


圖 1 (上)、美國微生物學會開幕典禮

圖 2 (下)、開幕典禮上哈弗大學教授 Dr. R.J. Collier 進行專題演講

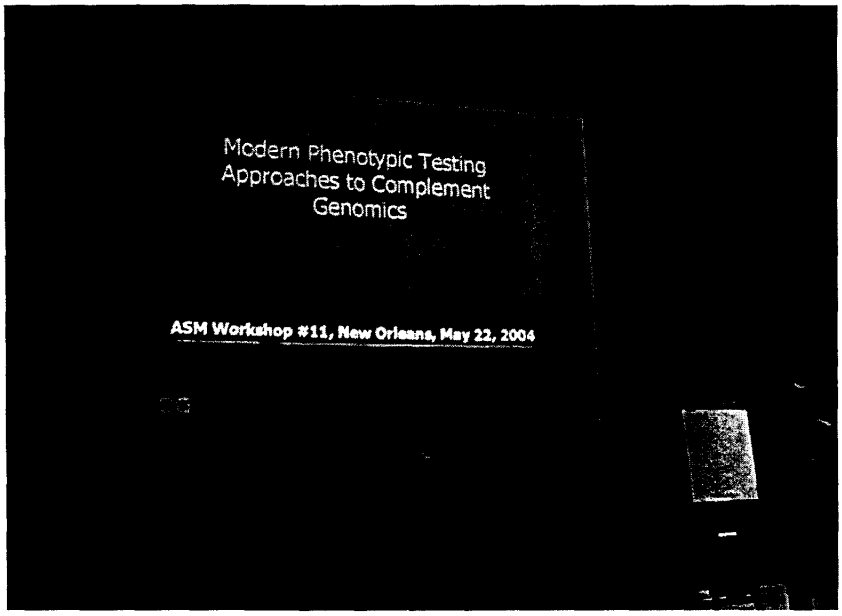
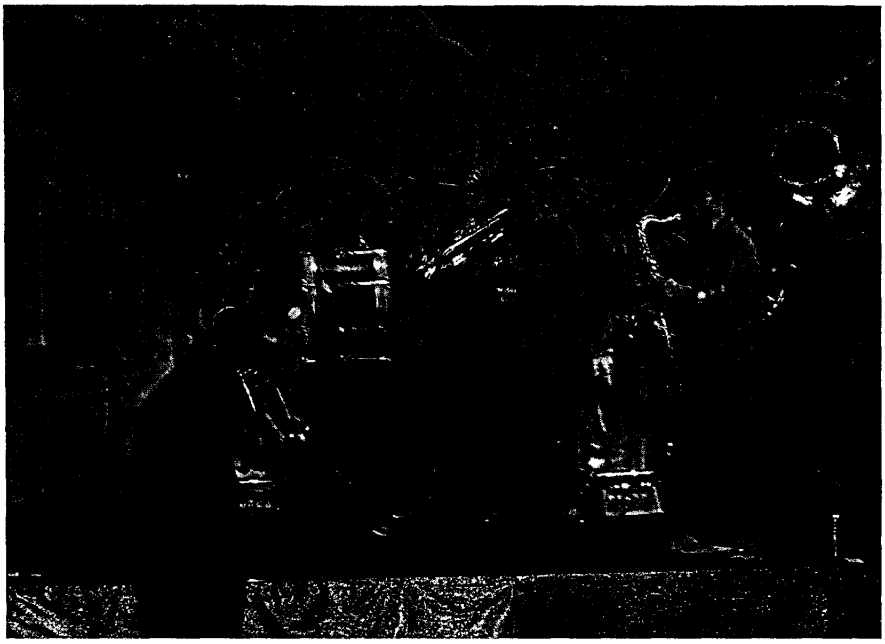


圖 3（上）、ASM 大會會場 A 入口

圖 4（下）、參加 WS-11 研習會



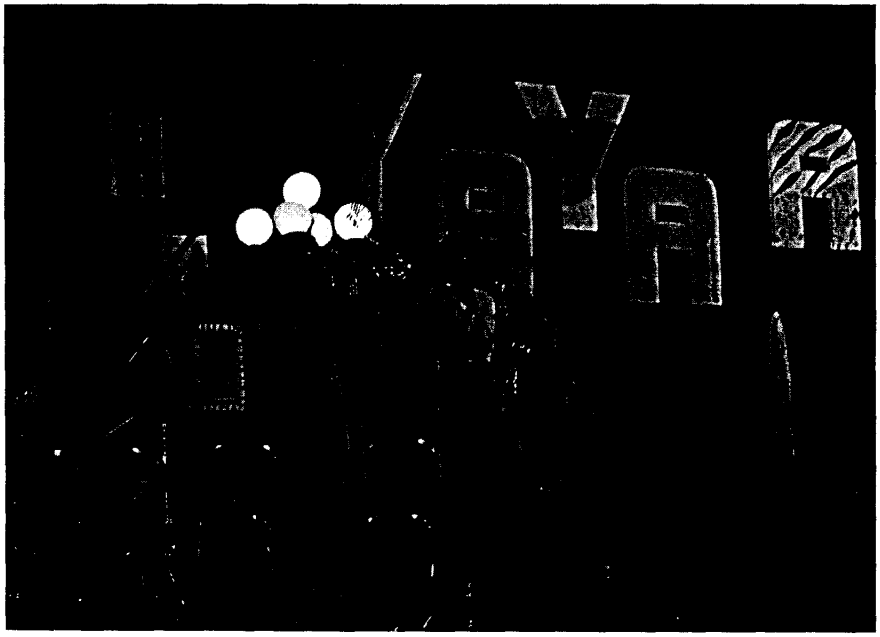
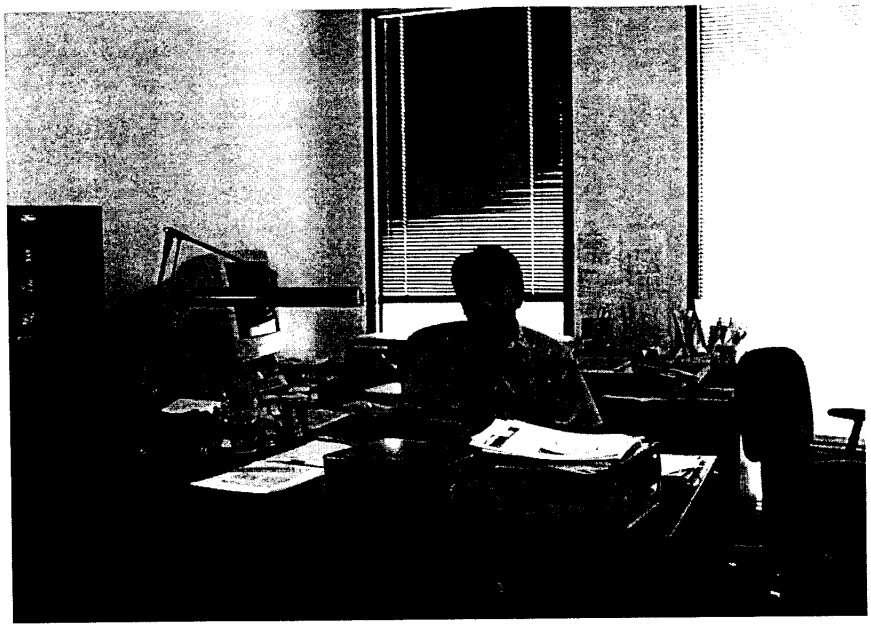


圖 5（上）、歡迎接待晚會精采的爵士表演

圖 6（下）、歡迎接待晚會精采的主秀（YAYAA's 年輕藝人)



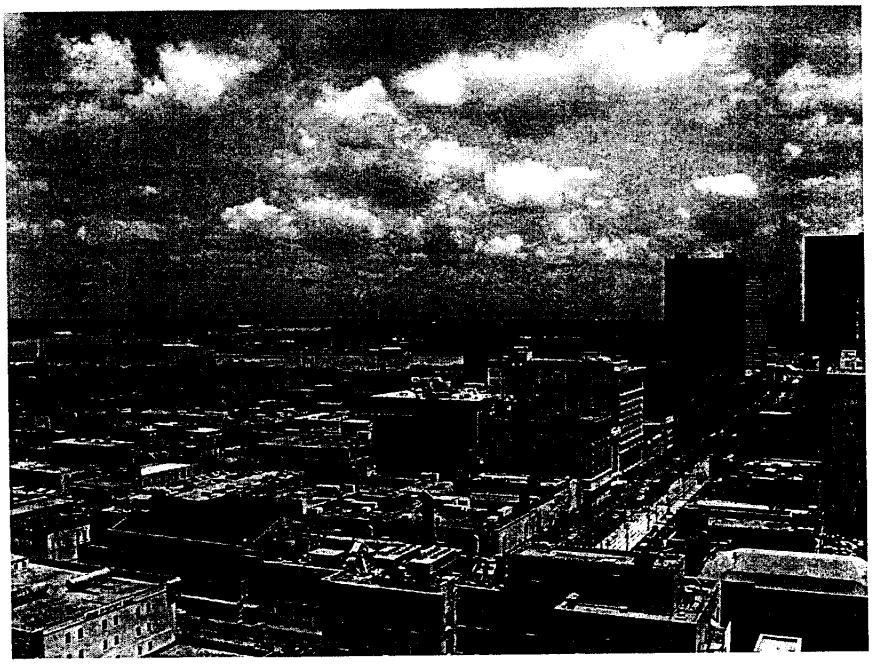
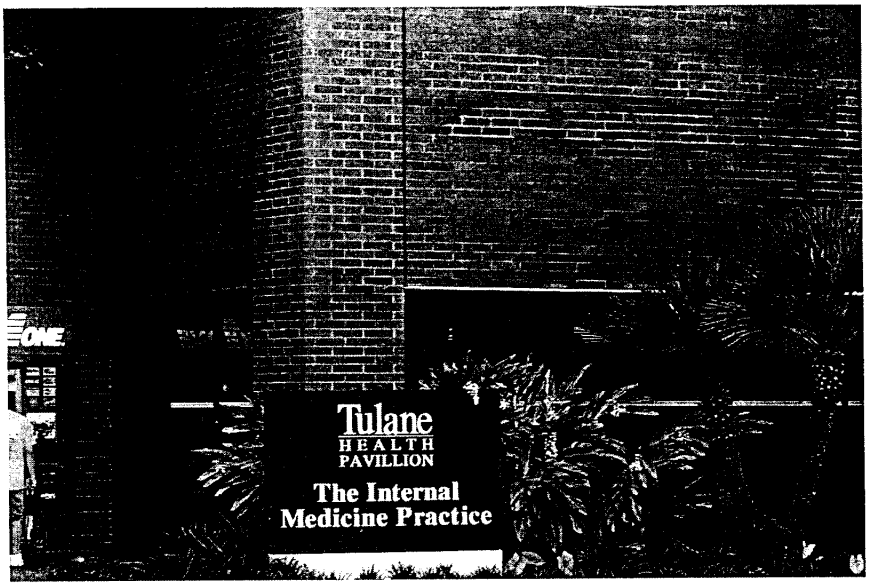


圖 7（上）、美國路易士安那台灣同學會新澳爾良分會會長（Dr 曾東松）

圖 8（下）、由會址辦公室俯瞰新澳爾良市及密西西比河



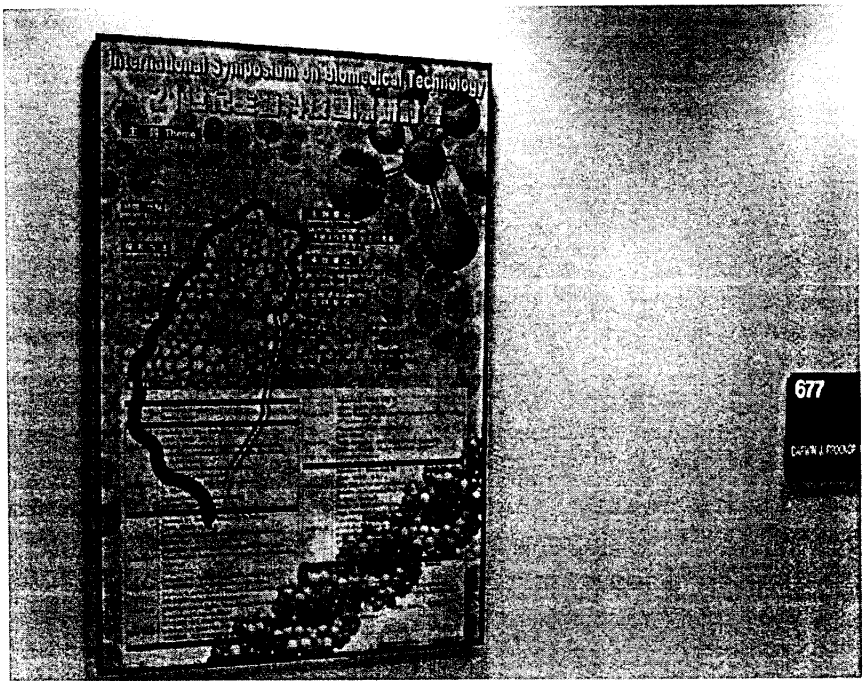
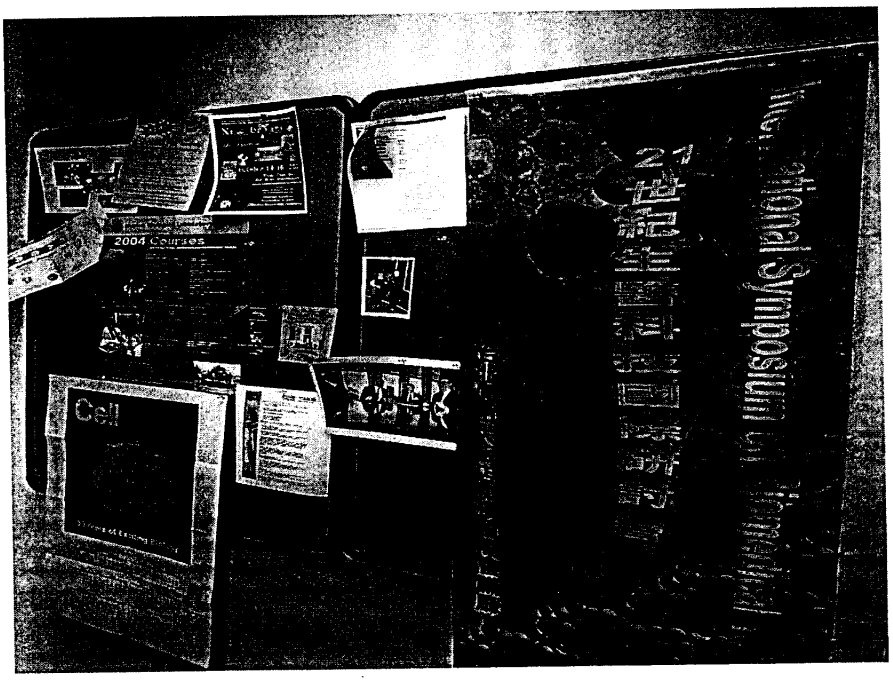


圖 9（上）、圖內拉大學基因治療中心位於 Tulane Health Pavillion 內

圖 10 (下)、我國舉辦之「21 世紀生醫科技國際研討會」宣傳海報



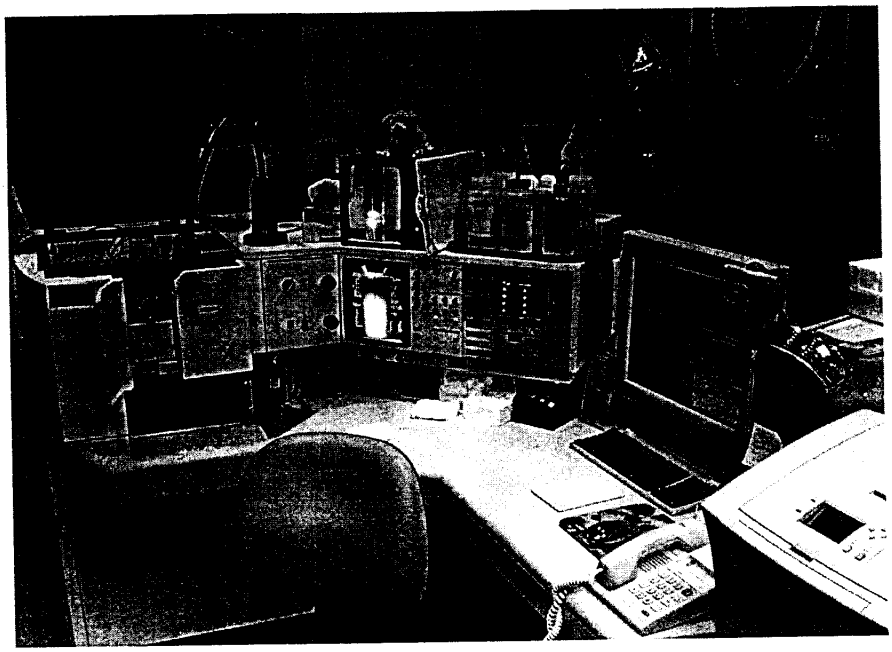


圖 11 (上)、我國舉辦之「21 世紀生醫科技國際研討會」宣傳旗幟

圖 12 (下)、圖內拉大學基因治療中心實驗室內之流式細胞分析儀

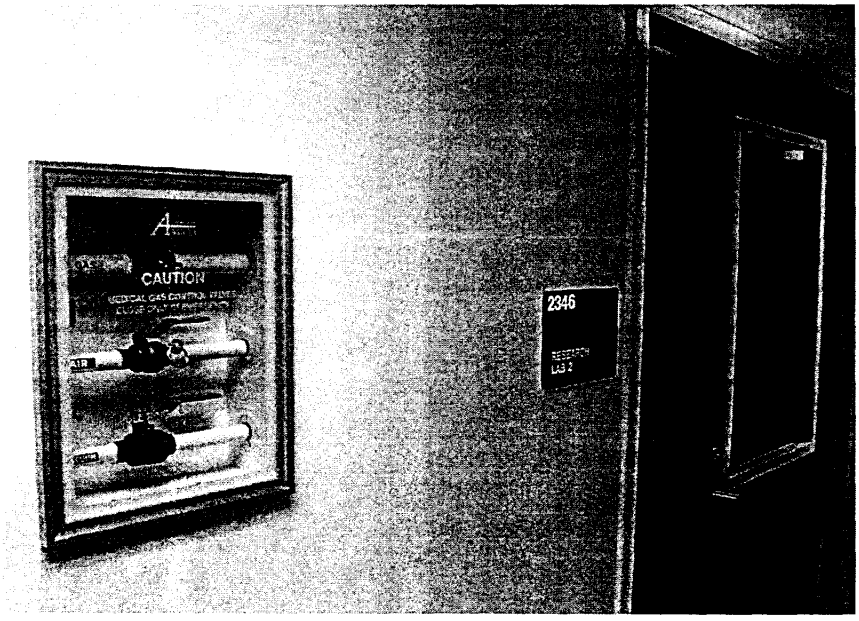
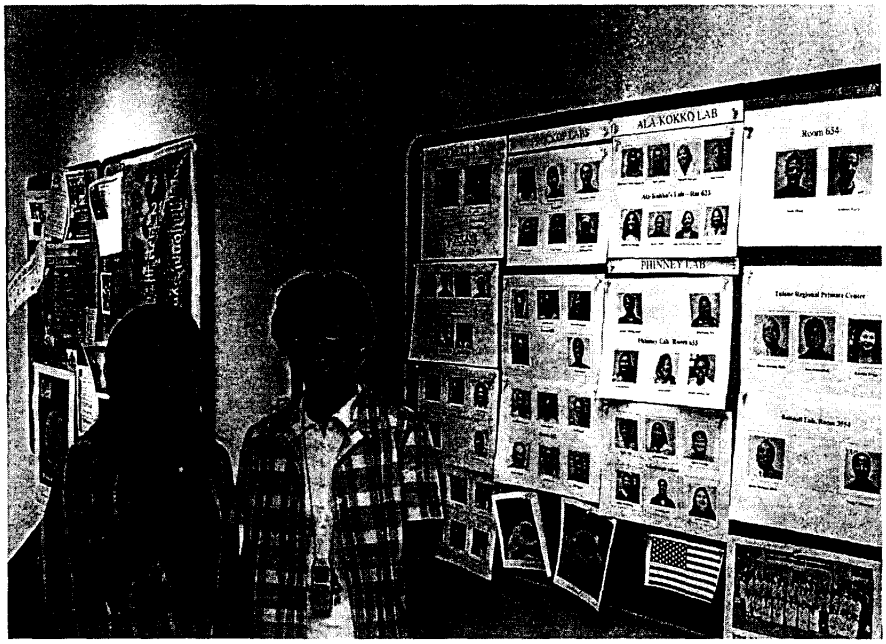


圖 13 (上)、圖內拉大學基因治療中心實驗室內一排 PCR 儀器

圖 14 (下)、圖內拉大學基因治療中心實驗室外之氣體控制閥



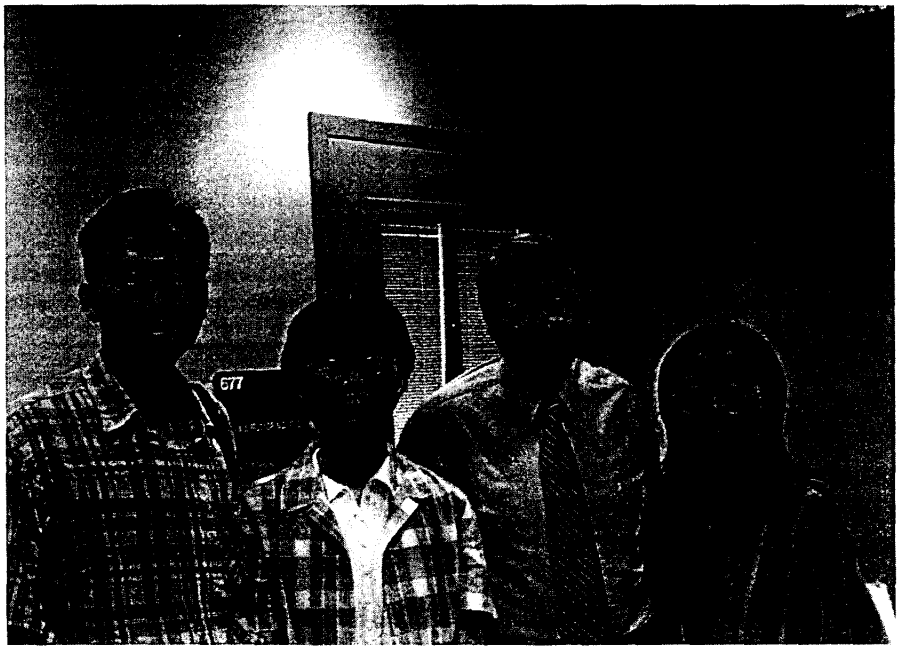
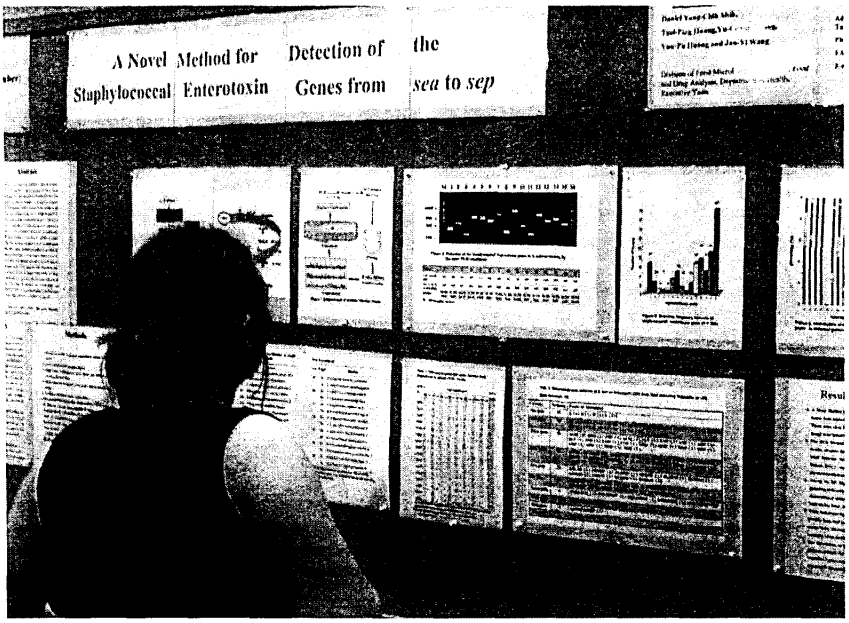


圖 15（上）、許素菁博士介紹圖內拉大學基因治療中心成員（佈告欄相片）

圖 16（下）、與基因治療中心主任 Dr. Darwin Prockop(右二)合影

圖 17（上）、有興趣的法國專家壁報未張貼好即以文字及圖畫問問題

圖 18（下）、去而復返討論其他問題並互留聯絡方式



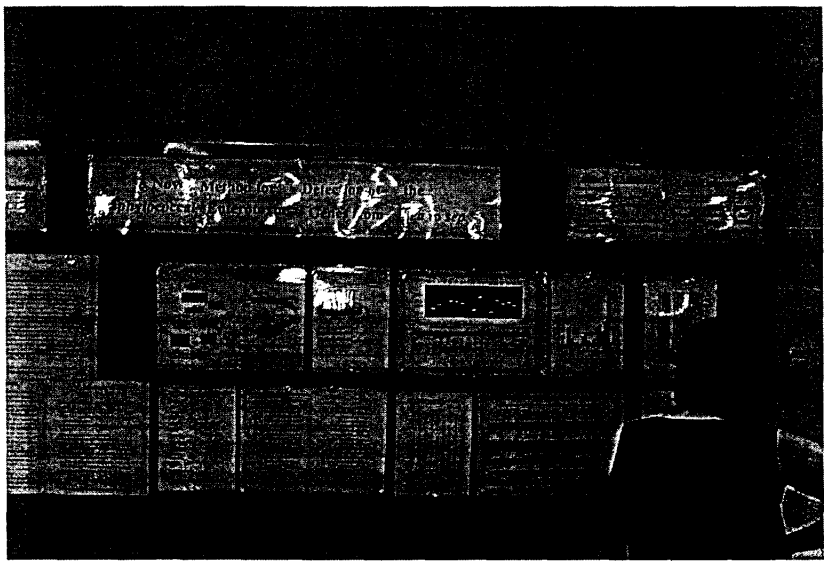
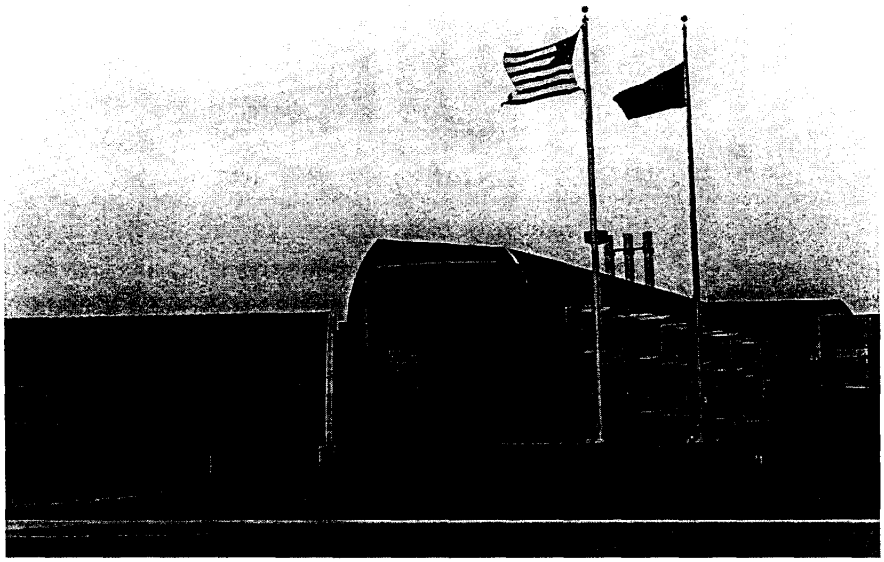


圖 19（上）、有興趣的專家詳讀內容

圖 20（下）、觀看完整篇報告後發問並留下聯絡方式

圖 21（上）、鄭崇明博士(左一)及蘇意誠博士(右二)觀看我們發表之壁報論文

圖 22（下）、各國專家學者觀看我們發表之壁報論文



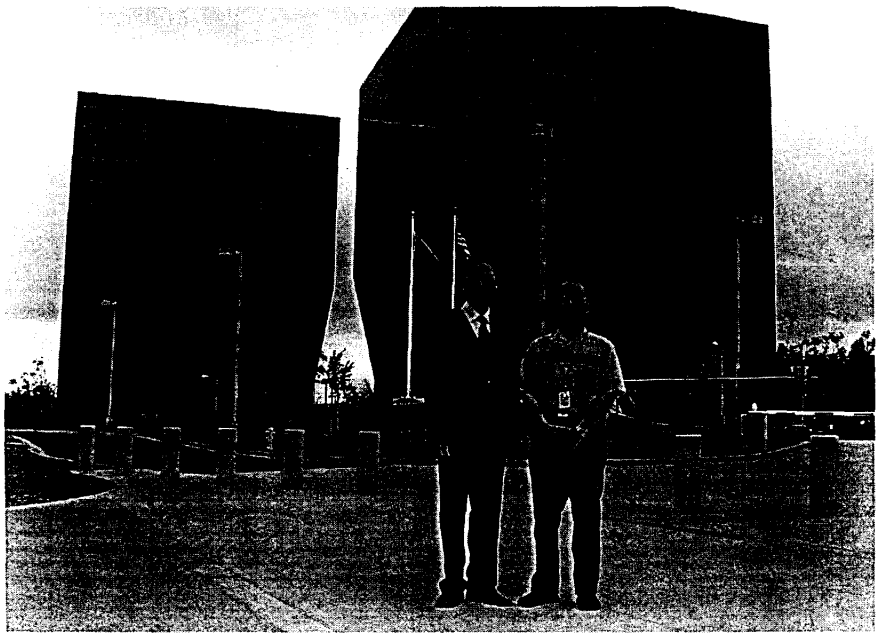
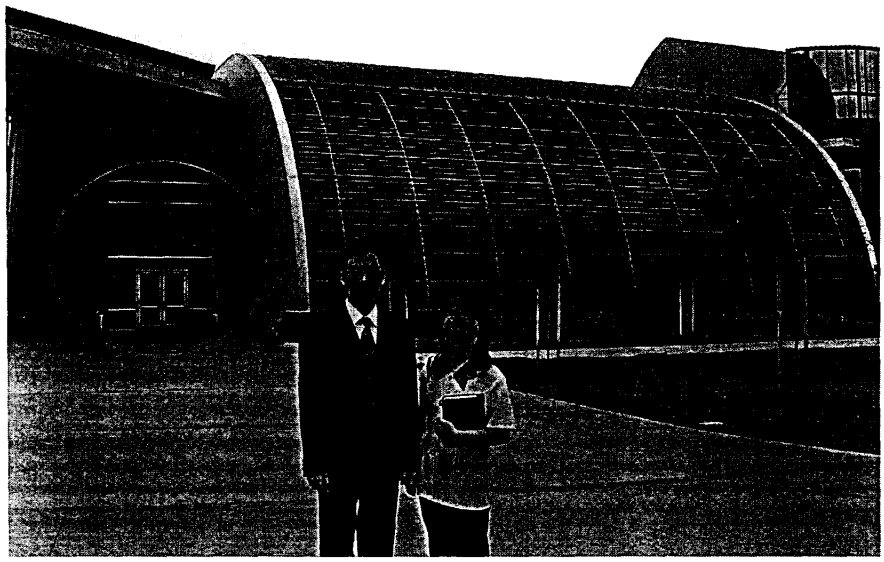


圖 23（上）、FDA 門牌地址、大門、鐵圍欄及國旗、局徽
圖 24（下）、由停車場向大門、鐵圍欄拍照



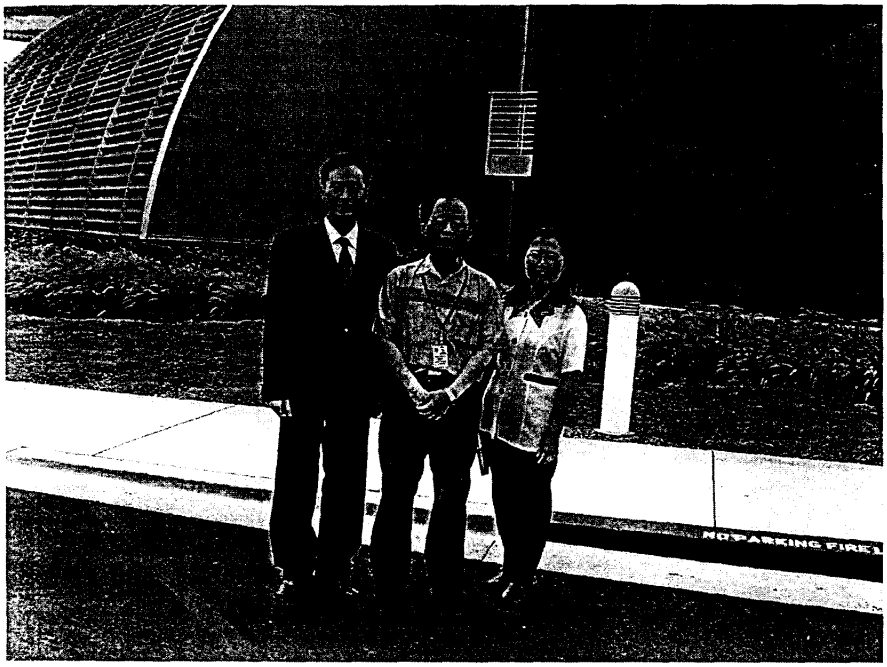
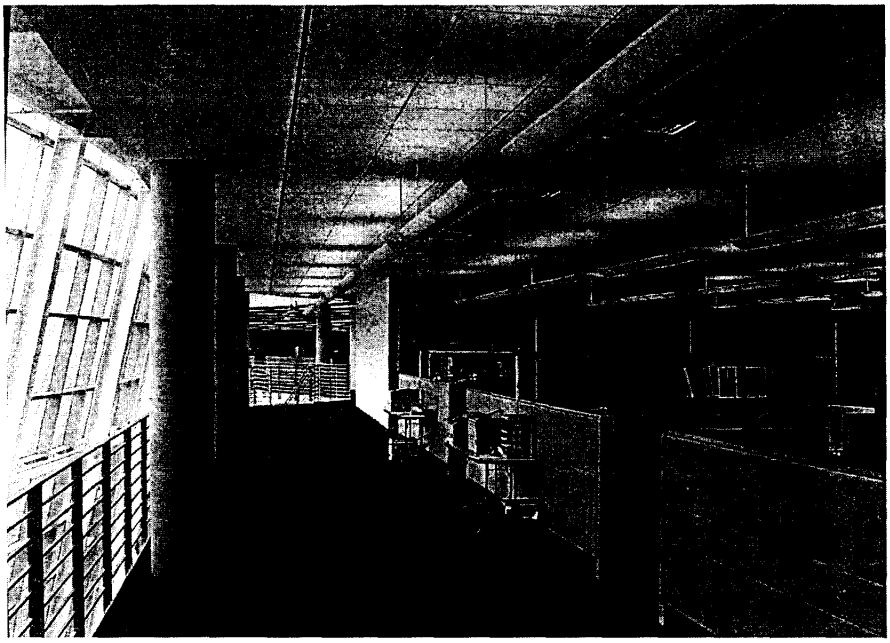


圖 25（上）、FDA 主建築物入口

圖 26（下）、FDA 戶外平面停車場



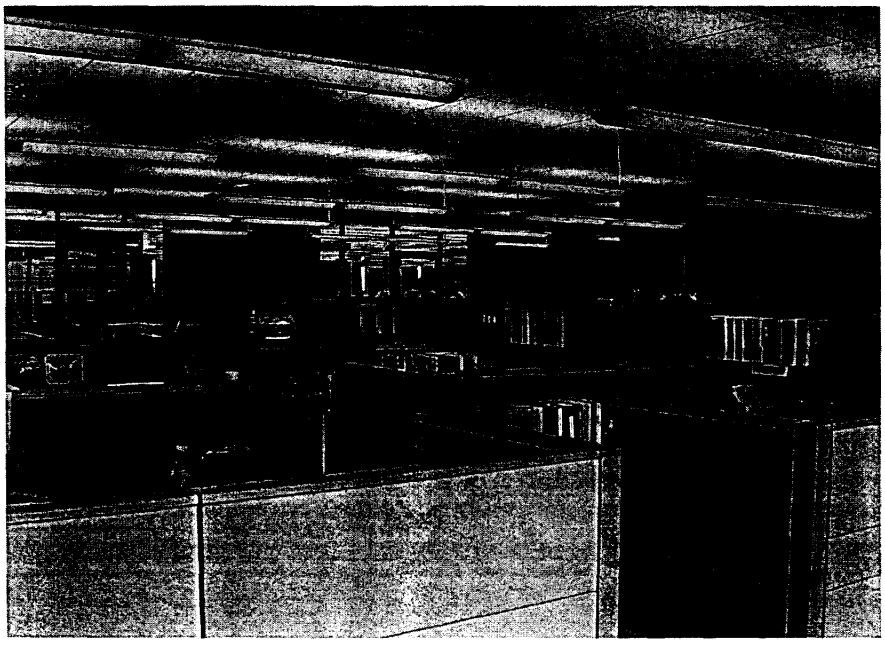


圖 27（上）、FDA 二樓辦公室

圖 28（下）、FDA 一樓辦公室

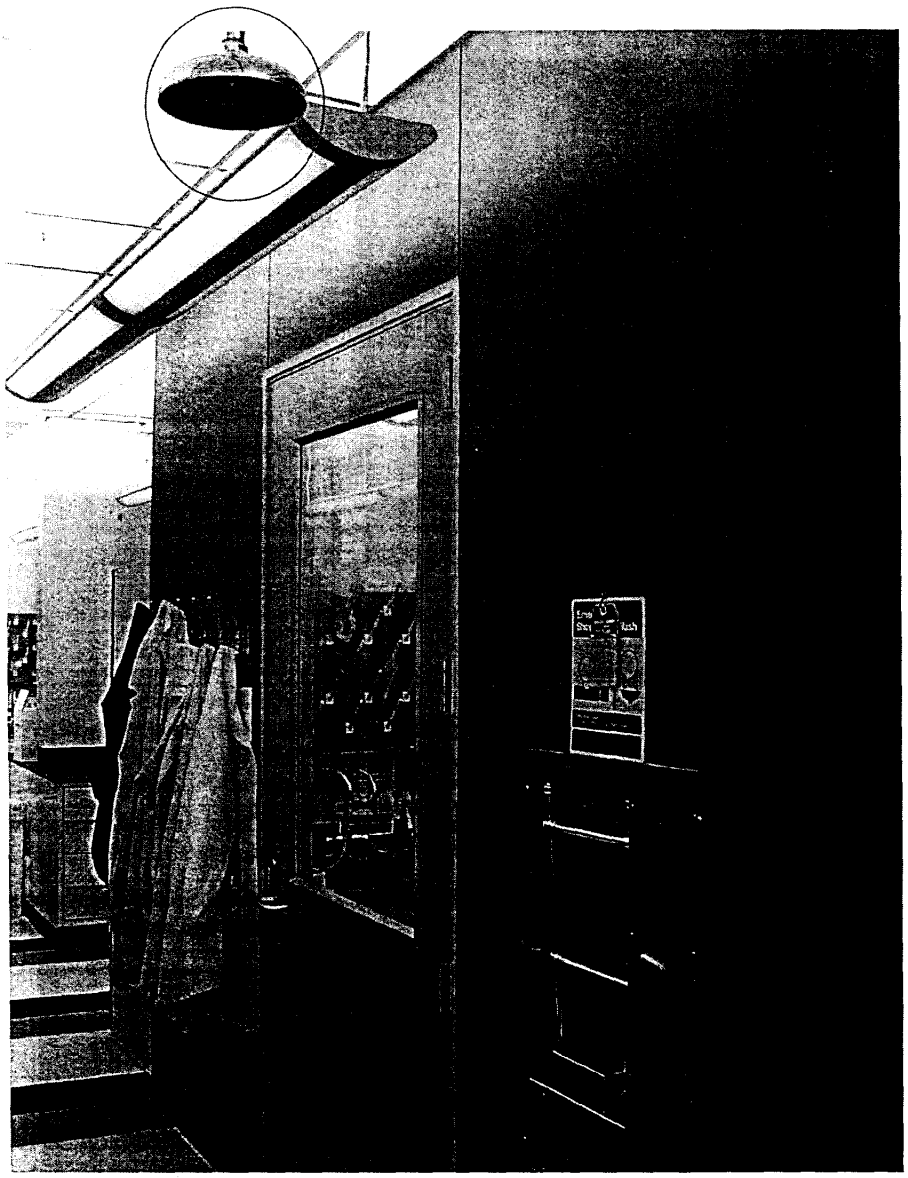


圖 29、FDA 實驗室入口更換實驗衣及緊急淋浴裝置

圖 30（上）、實驗室（左側）與辦公室（右側）以透明玻璃完全區隔

圖 31（下）、與 FDA 專家於實驗室內進行討論

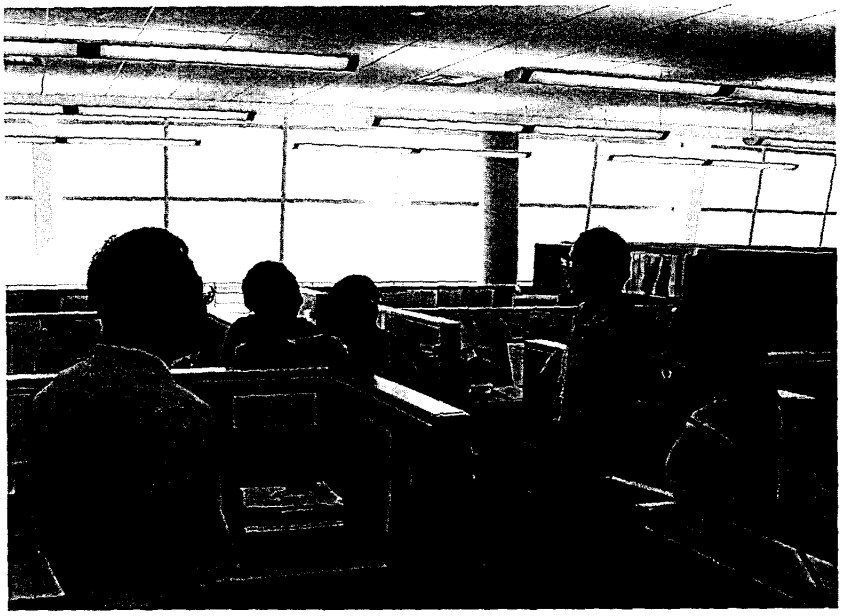


圖 32（上）、與最資深的科長 Richard M. Ruby 於實驗室合照

圖 33（下）、與微生物組專家於辦公室討論

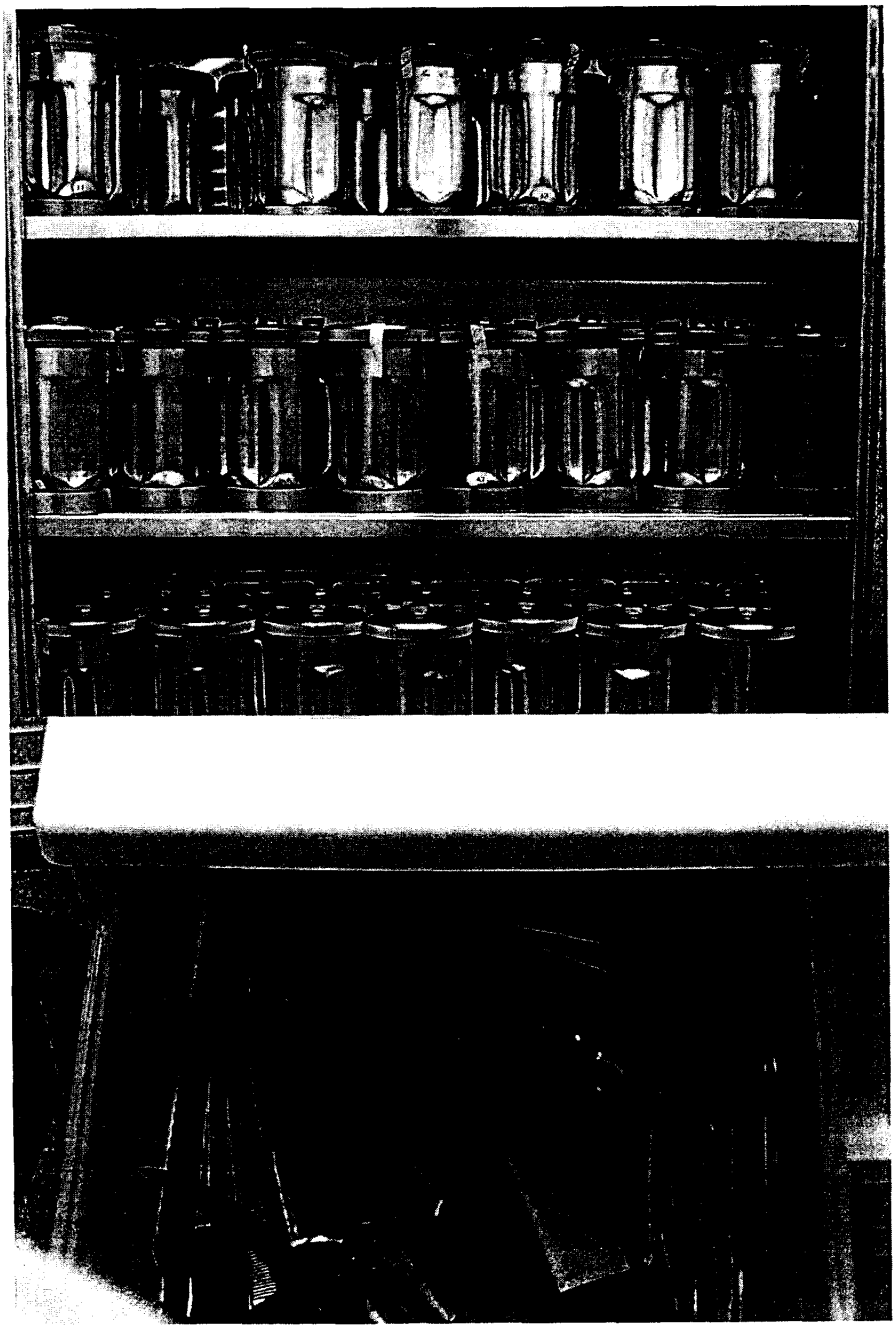
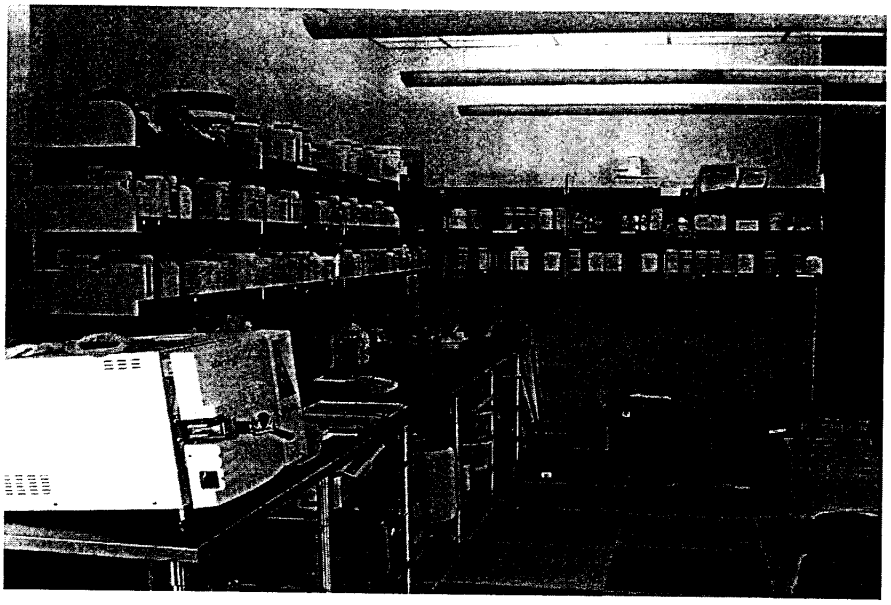


圖 34（上）、檢體均質前處理使用之鋼杯

圖 35（下）、檢體取樣用之各式刀具

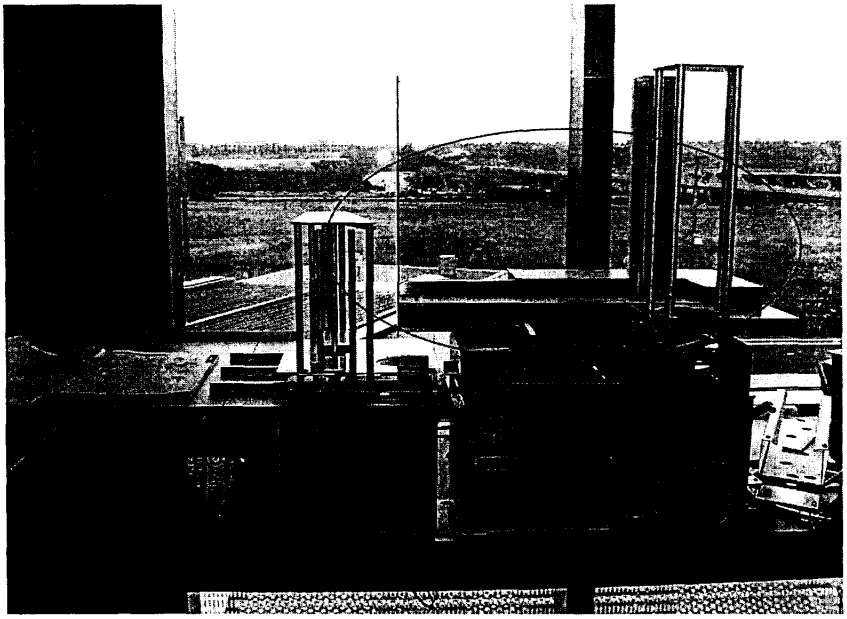


PRL-SW MEDIA INVENTORY SHEET DATE 5/11/07 PAGE 2

MEDIA	ACRONYM	EXPIRATION	MINIMUM STOCK AMT	TUBE	QUARANTINE	READY FOR USE	PREPARE
Brain Heart Infusion Broth	BHI	6 months	2 racks	13 mm		2	
EC-4 Broth	EC	4 months	6 racks as needed	16 mm		10	
Kosac's Citrate	KC	4 months	as needed	13 mm		4	
Luria Bertani Broth	LB	4 months	2 racks	16 mm		1F 5T	2F 15T
Lauryl Tryptose Broth	LST	4 months	15 racks	16 mm		1F 5T	
Long Term Preservation Media +	LTP+0.5% NaCl	4 months	1 rack	13 mm		2 1/2	
Long Term Preservation Media -	LTP+3.0% NaCl	4 months	as needed	13 mm		1	
Lysine Iron Agar	LIA (slant)	2 months	3 racks	13 mm		3F 9T	2 1/2 racks
M-ENDO M Broth	M Broth	4 months	6 racks	16 mm		8 racks	Frank
Motility Test Medium w/Screw Cap	MTMsc	6 months	1 rack	13 mm		1	
Motility Test Medium	MTM	6 months	1 rack	16 mm		1	
Methyl Red Voges Proskauer Broth	MRVP	2 months	as needed	13 mm		4	
Phosphate PO4	PO4	4 months	1 racks	20 mm		1	
Rappeport Vassiliadis Medium	RV	1 month	for research only	16 mm		5 racks	
Trypticase Soy/Tryptose Broth	TSTB	4 months	2 racks	16 mm		1	
Triple Sugar Iron Agar	TSI	2 months	2 racks	16 mm		4 1/2	
Tryptose Agar Slants	TryA	4 months	1 rack	16 mm		1	
Tryptose Broth	TryB	4 months	1 rack	16 mm		1/2	
Trypticase Soy Agar Slants	TSA	4 months	2 racks	13 mm		4 1/2	
Tryptic Soy Agar Slants w/Screw Cap	TSAsc	4 months	2 racks	13 mm			
Trypticase Soy Agar w/Yeast Slants	TSAYE	4 months	2 racks	13 mm		2	
TSAYE w/Screw Caps	TSAYEsc	4 months	1 rack	13 mm		1/2	
Trypticase Soy Broth w/Yeast	TSBYE	4 months	1 rack	13 mm		6 1/2	
1% Trytone Broth	T1N0	4 months	1 rack	13 mm		3	
1% Trypton Broth + 3% NaCl	T1N3	4 months	1 rack	13 mm		2 1/2	
Add Antibiotic							
0.5% Acriflavin			100 mls	BTL			
0.5% Nalidixic Acid			100 mls	BTL			
1 % Cycloheximide			100 mls	BTL			

圖 36（上）、一般固體培養基置於開放架上分類管理

圖 37（下）、培養基配製工作表單



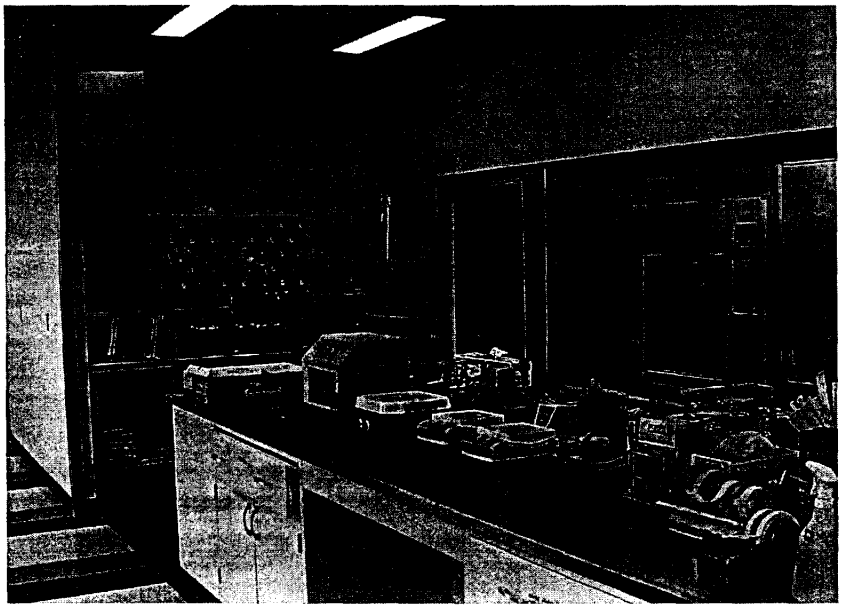
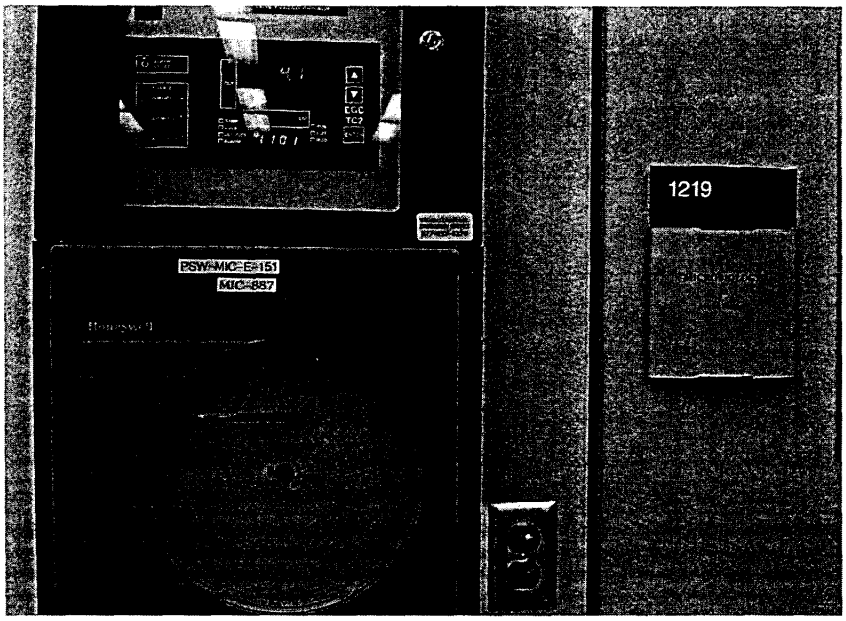


圖 38（上）、平板培養基自動製備裝置
圖 39（下）、液態培養基自動分注裝置



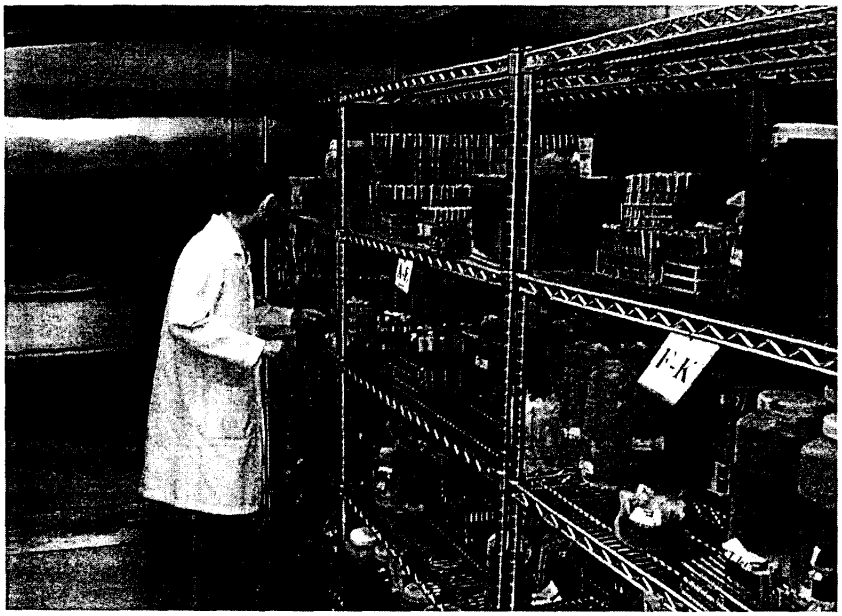


圖 40（上）、預先配製之培養基冷藏庫溫度監控

圖 41（下）、預先配製之培養基分類標示上架冷藏庫

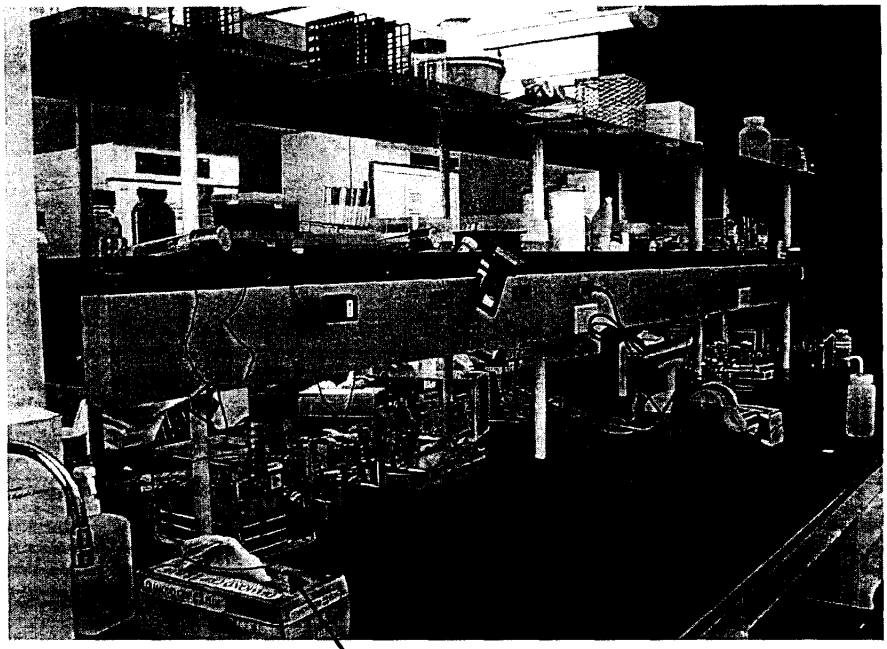
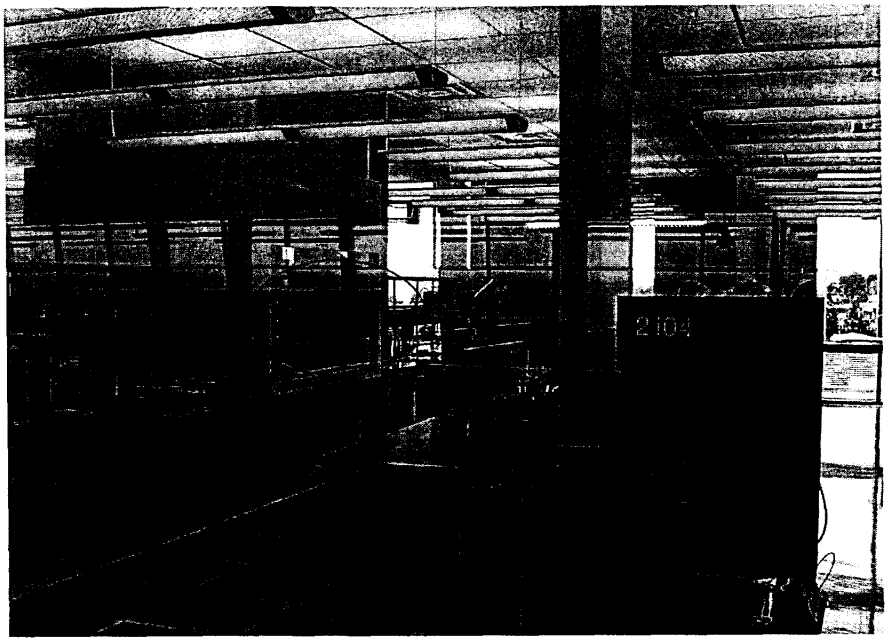


圖 42（上）、開放式微生物實驗操作檯

圖 43（右）、每六個月更新之內部對照菌株 1 保存在上鎖的透明壓克力箱內置於實驗檯上



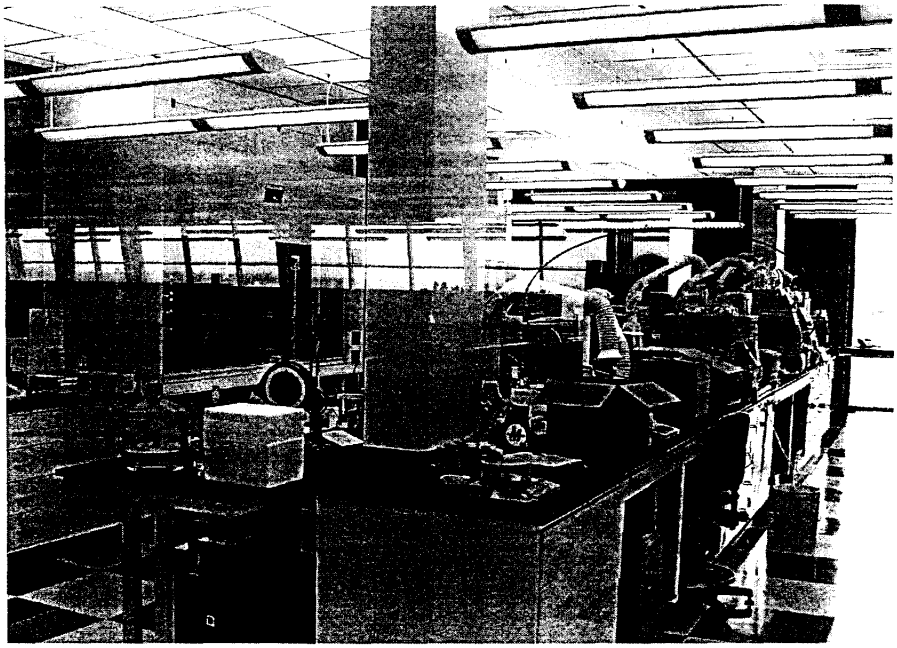


圖 44（上）、FDA 隔透明玻璃可見化學實驗室分散之抽氣櫃

圖 45（下）、FDA 隔透明玻璃可見化學實驗室檯面上許多抽氣裝置

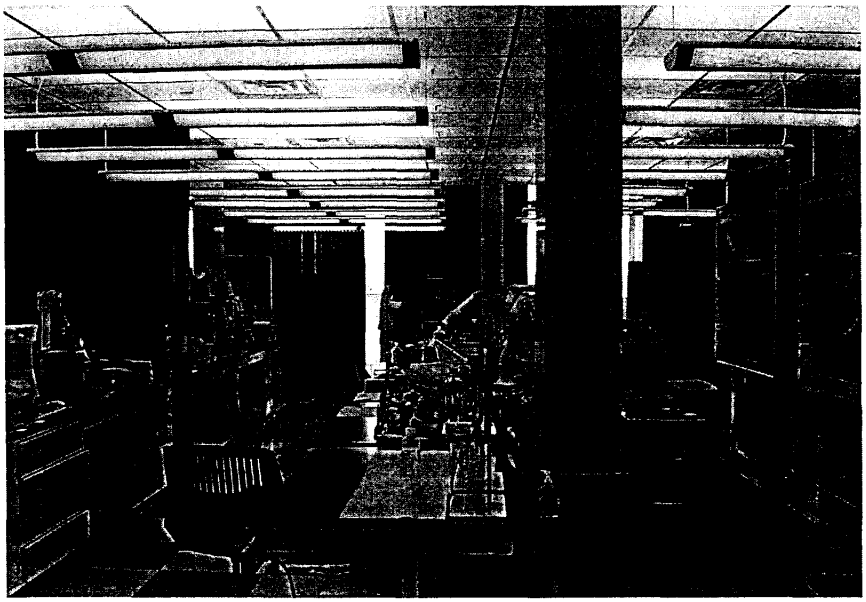
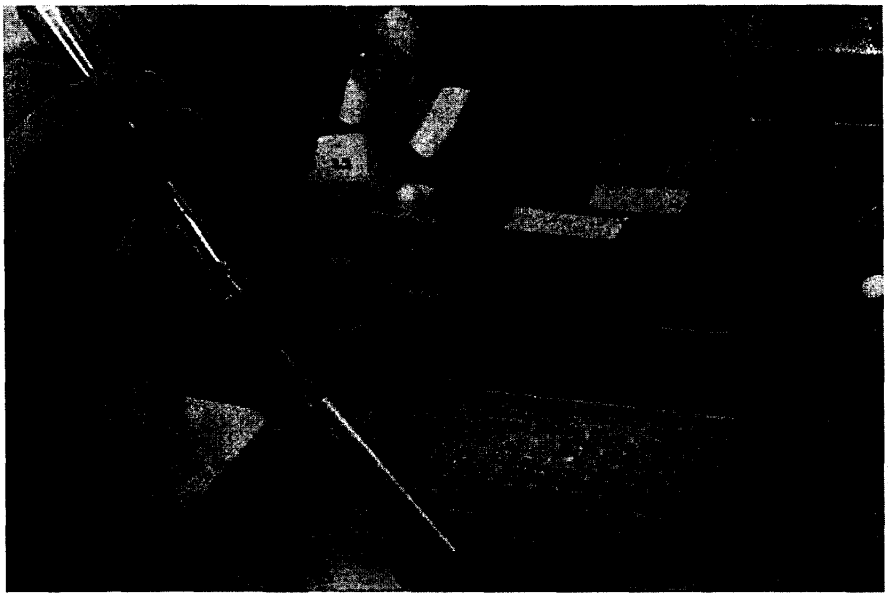


圖 46（上）、FDA 化學實驗室之水槽、檯面上有滴定架、抽氣裝置

圖 47（下）、FDA 化學實驗室抽氣櫃未單獨隔間

圖 48（上）、加州州立大學 POMONA 分校生物技術研究室留影

圖 49（下）、管制試劑置於保險箱加鎖並以鋼索固定於冷凍櫃



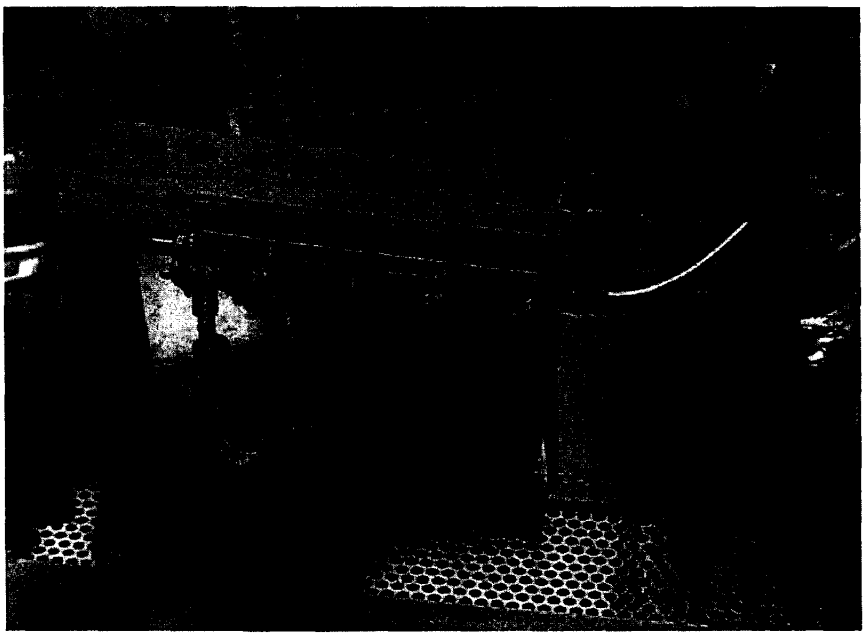
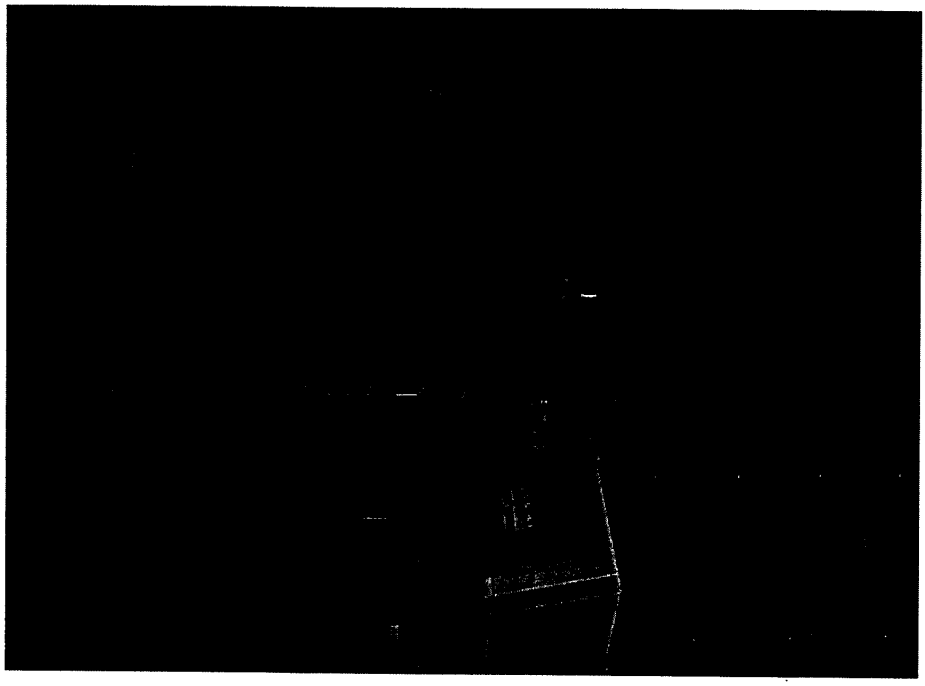


圖 50 (上)、林維真博士示範厭氧菌之接種方式

圖 51 (下)、林維真博士示範厭氧菌之保存方法



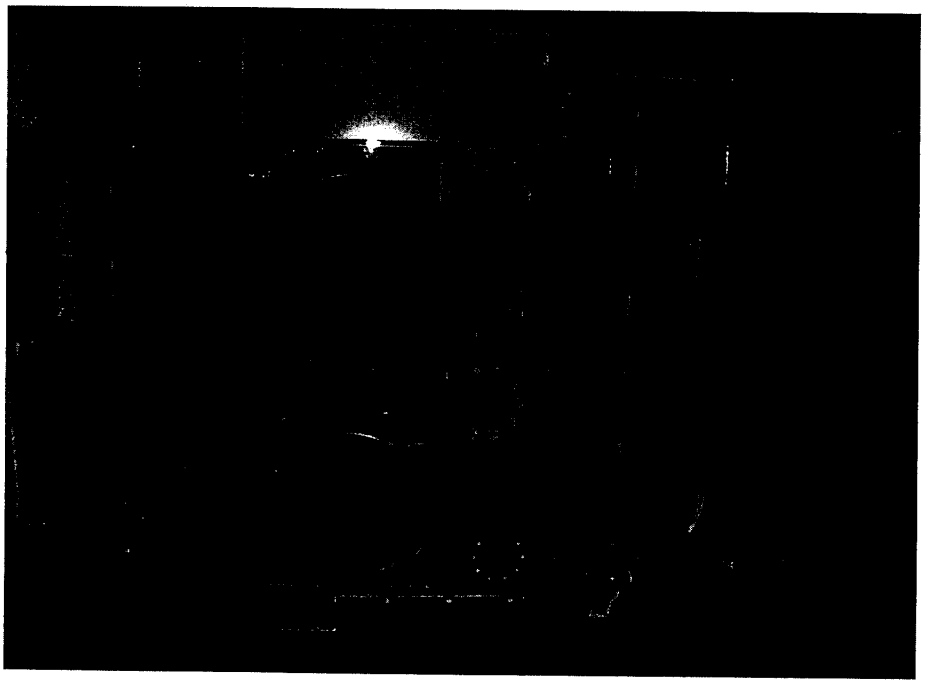
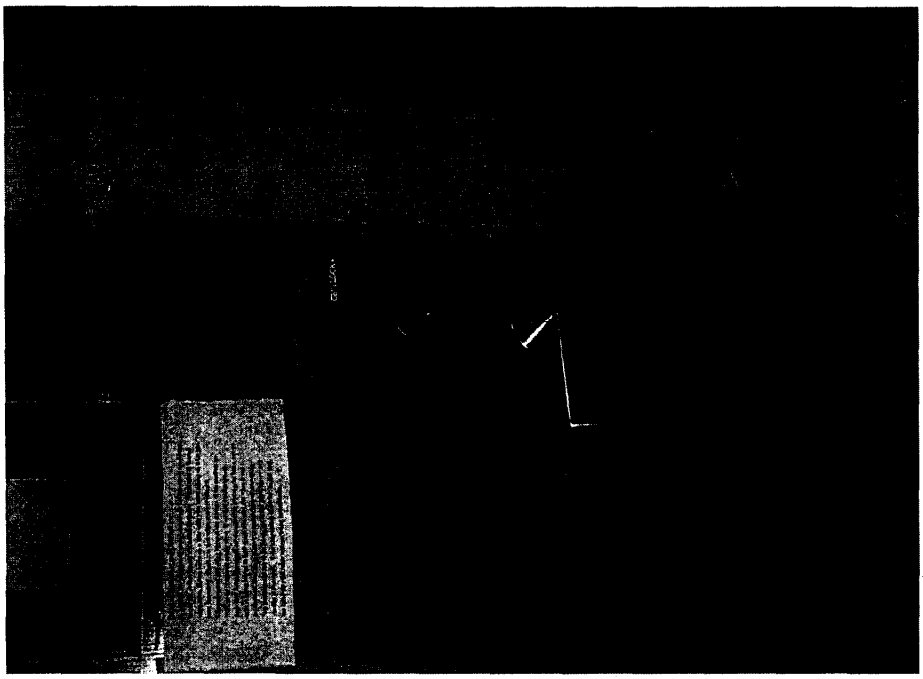


圖 52（上）、管制試劑實驗室內淋浴設施

圖 53（下）、小型厭氧操作檯



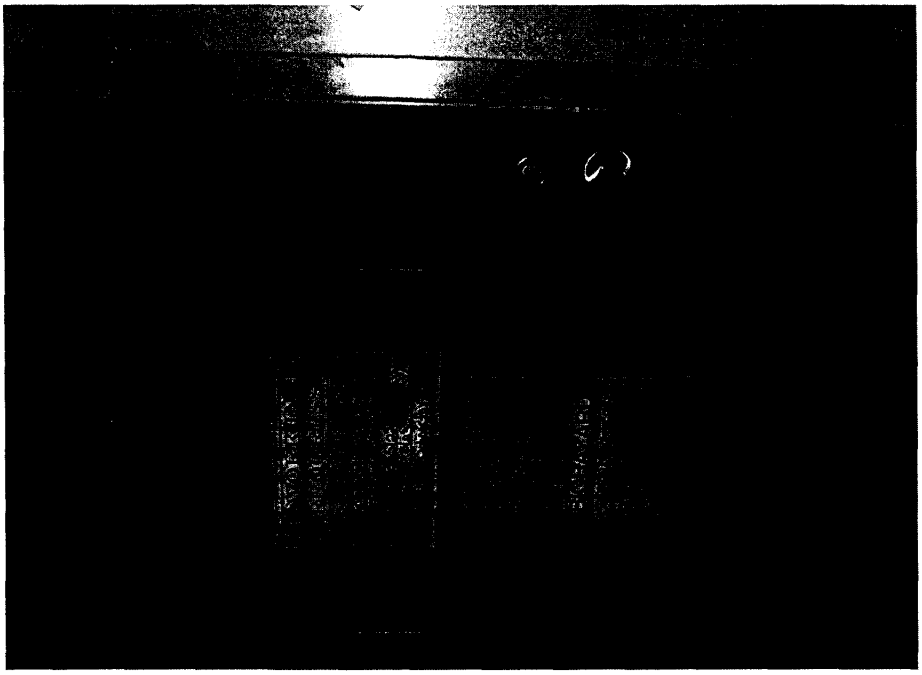
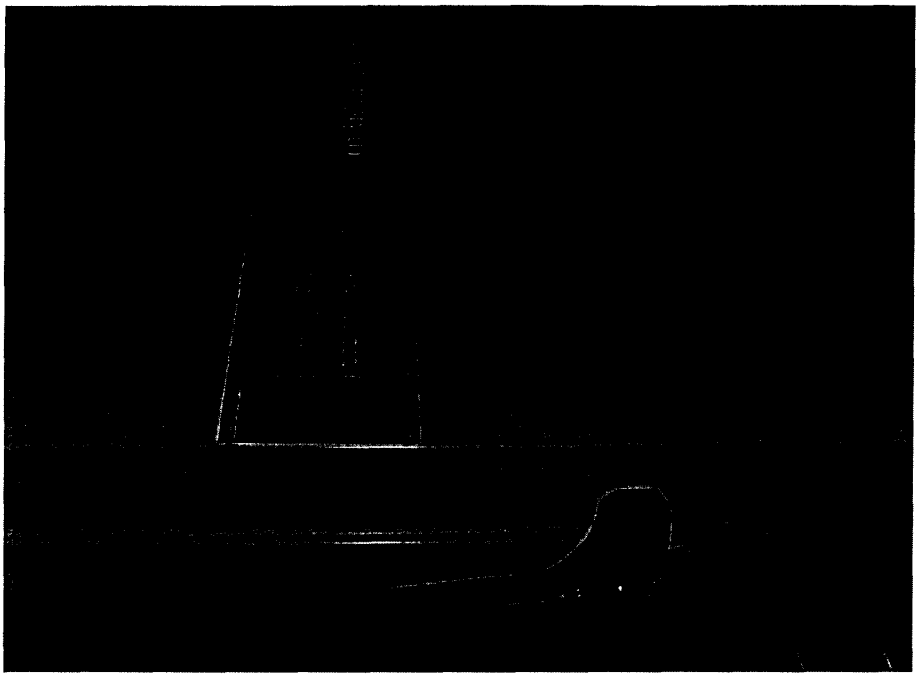


圖 54（上）、管制試劑研究實驗室門禁鎖

圖 55（下）、管制試劑研究實驗室入口貼生物危害警示



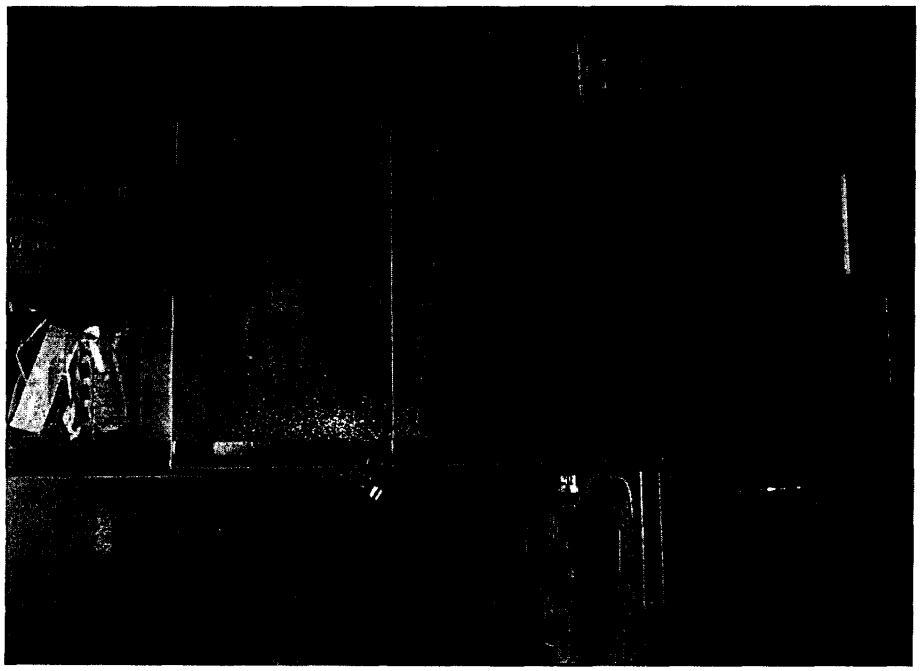


圖 56（上）、管制試劑研究實驗室冷凍櫃加鎖

圖 57（下）、管制試劑研究實驗室冰箱加鎖

附　　件　　一

Modern Phenotypic Testing Approaches to Complement Genomics

ASM Workshop #11, New Orleans, May 22, 2004

BIOLOG

Workshop #11 Faculty

Barry Bochner, Biolog, Inc.
Jean Petter-Bouldin, USDA
Ian Paulsen, TIGR
Kenn Rudd, Univ. of Miami

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Workshop #11 Agenda

8:30 – 9:45 History of phenotypic testing, development and application of modern methods. Bochner

9:45 – 10:00 Break

10:00 -10:30 Phenotypic analyis of Salmonella strains: a chicken and egg story. Bouldin

10:30 – 11:00 Phenotypic analysis meets genomics: transporters in P. aeruginosa. Paulsen

11:00 – 11:15 Break

11:15 – 12:00 Phenotypic analysis and genomics meet bioinformatics: tools and approaches. Rudd

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A Brief History of Phenotypic Testing in Microbiology

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An Old and Difficult Problem

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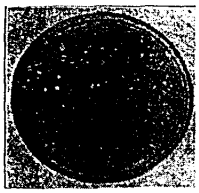
The Complexity of Microbial Life



Microoganisms Found in 1 Milligram of Soil

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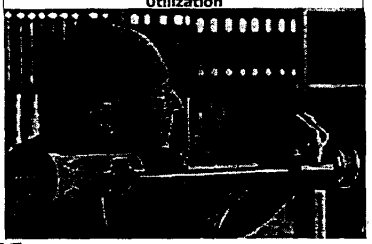
Microbial Identification by Carbon Source Utilization



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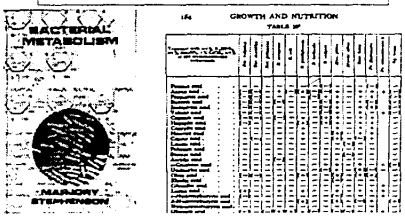
L. E. den Dooren de Jong

Microbial Identification by Carbon Source Utilization



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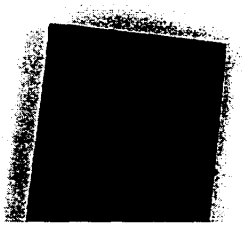
Bergey's Manual – first edition 1923



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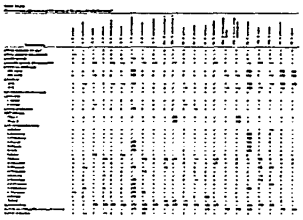
Bergey's Manual Identification Tables



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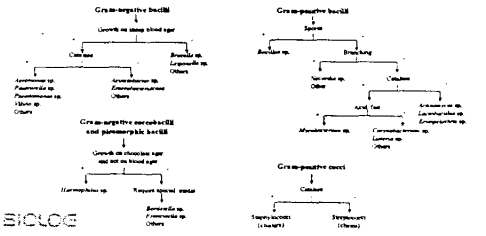
Identification Using Sequential Testing

Standard Procedure for Identifying Bacteria from Clinical Specimens



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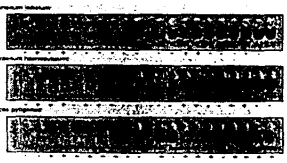
API panels circa 1960s



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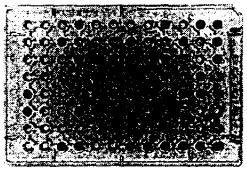
Biolog's Multi-Trait Redox Test Format



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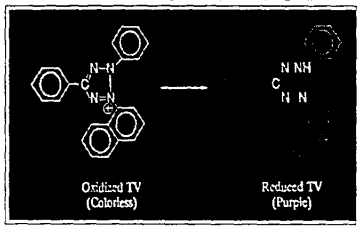
The Number of Possible Patterns in a MicroPlate is Vast

$$2^{95} = 4 \text{ X } 10^{28}$$

BIOLOG

Reduction of Tetrazolium Violet



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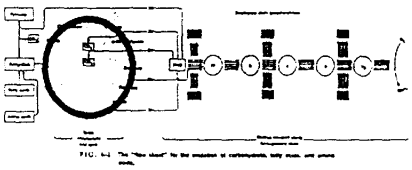
Reduction of Tetrazolium Violet



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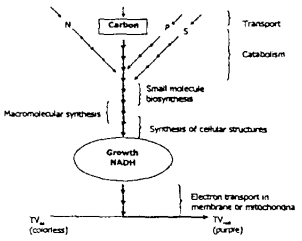
Cell Respiration: Universal Reporter



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Phenotypic vs Genotypic
Testing in Microbiology

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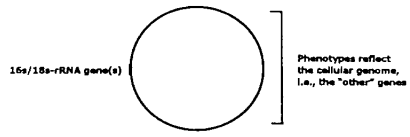
Phenotypes Reflect the Genome



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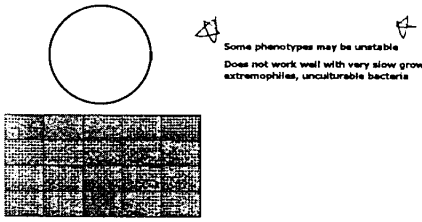
Disadvantages of Phenotypic Testing



- Some phenotypes may be unstable
- Does not work well with very slow growing microbes, extremophiles, uncultivable bacteria

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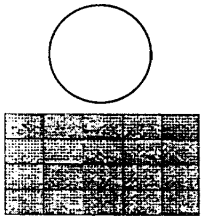
Advantages of Phenotypic Testing



- Tests thousands of genes instead of one
- Strong theoretical basis – phenotypes reflect the composition of the genome
- Tests have biological importance
- Easy and inexpensive to perform

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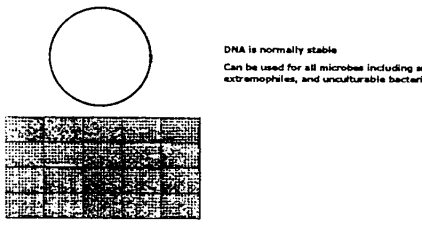
Advantages of 16s/18s Testing



- DNA is normally stable
- Can be used for all microbes including slow growers, extremophiles, and uncultivable bacteria

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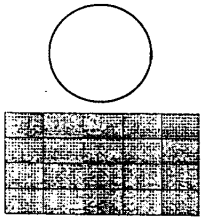
Disadvantages of 16s/18s Testing



- Tests only one gene
- Single gene phylogenies can be inaccurate
- Fails to make some important distinctions, e.g. E. coli vs E. coli O157 and Shigella or B. cereus vs B. thuringiensis and B. anthracis
- No *a priori* theoretical basis for species identification
- Results contain no biological information
- Expensive to perform

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Phenotype MicroArrays™

Assay 2000 Cellular Traits Simultaneously

Provide Metabolic/Physiologic Scans of Cellular Pathways in Living Cells

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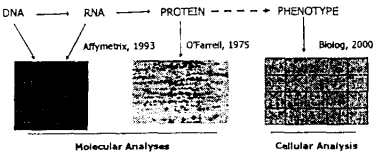
Global Cellular Analysis



Phenotypes result from an integration of genomics and metabolism

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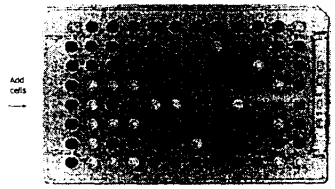
Assay of Cellular Pathways by Respiration



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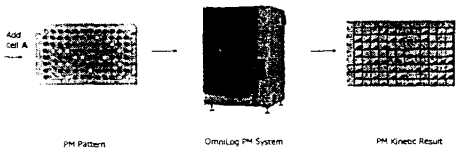
Generating Kinetic Data from Phenotype MicroArray Results



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PM Technology to
Integrate Metabolism and Genomics:
Carbon Metabolic Pathways of
Helicobacter pylori

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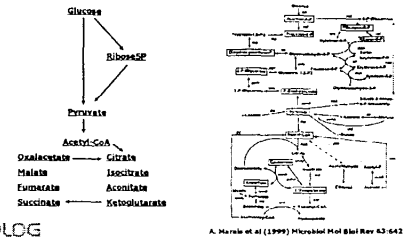
Predicted H. pylori Metabolism



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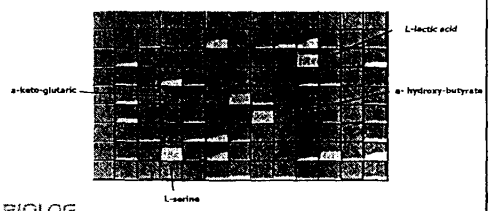
A. Marais et al (1999) Microbiol Mol Biol Rev 63:642
F. Doig et al (1999) Microbiol Mol Biol Rev 63:675

Carbon Metabolism of H. pylori in PM



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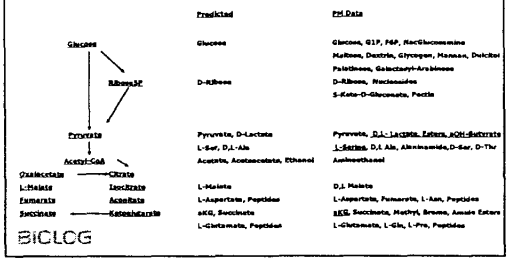
Carbon Metabolism in H. pylori



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Conclusions

1. Genomics gives a "sterile" view of metabolic pathways.
2. H. pylori, E. coli, S. cerevisiae, human cells all have the glycolytic pathway and TCA cycle but metabolize glucose very differently.
3. We cannot yet interpret regulation of metabolism from the genome sequences.
4. PM technology allows us to see what pathways are present and which are active. It provides needed biological information to improve genome annotation.

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Expansion of Phenotypic Testing
beyond Carbon Metabolism
and Comparison of 2 Strains
with PM Technology

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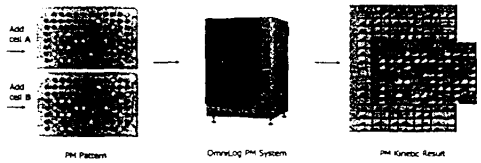
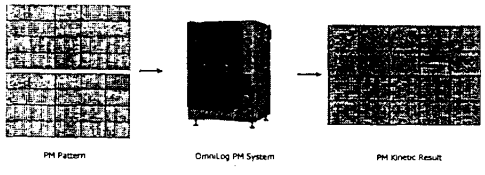
Using Phenotype MicroArrays™ to Compare Two Cell Lines



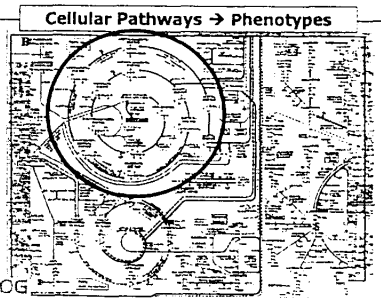
BIOLOG

2000 Test Phenotype MicroArray™



BIOLOG

Cellular Pathways → Phenotypes



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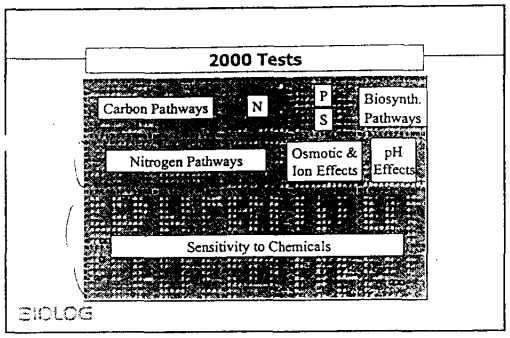
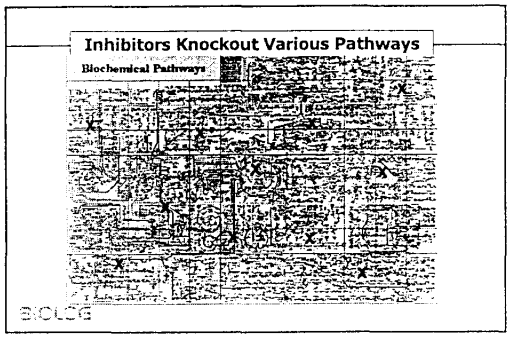
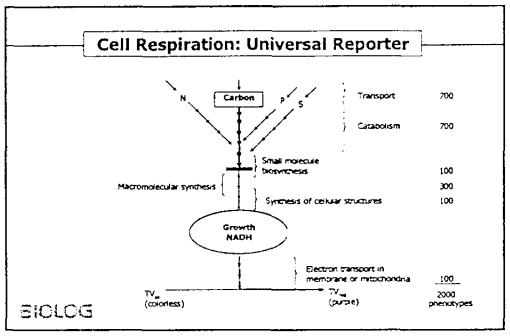
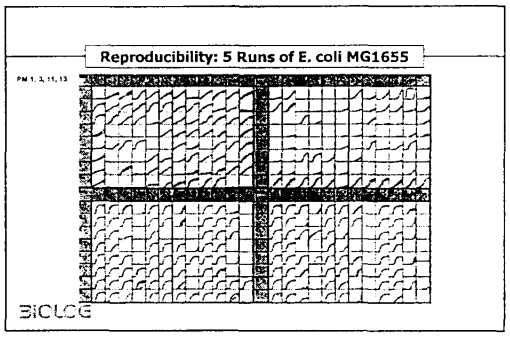
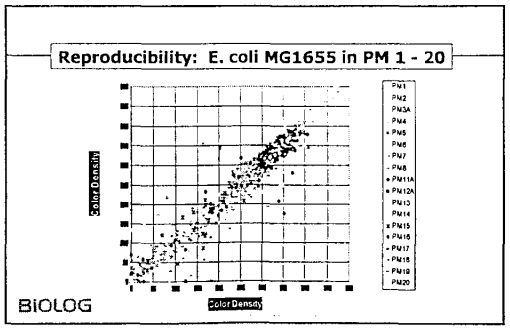










Determining Nutritional Preferences and Needs of Microbial Cells

BIOLOG

What Cells Need to Grow

C	carbon and energy
N	proteins, nucleic acids
P	phospholipids, nucleic acids
S	proteins, glutathione, CoA
K	
Mg	enzymes, ATP
Ca	
Fe	enzymes, cytochromes
Trace minerals	
Water	
Osmolarity (NaCl)	
pH (Buffer)	
Temperature	

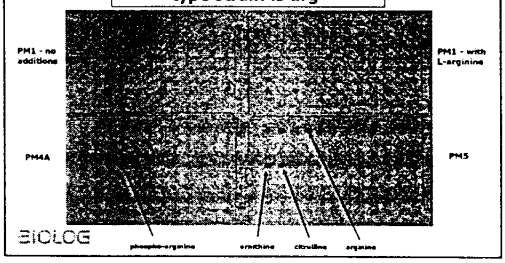
BIOLOG

What Cells Need to Grow

	PM1-2	PM3-8	PM4	PM5	PM9	PM10
C		+	+	+	+	+
N	+		+	+	+	+
P	+	+		+	+	+
S	+	+		+	+	+
Mg	+	+	+	+	+	+
K	+	+	+	+	+	+
Ca	+	+	+	+	+	+
Fe						
Trace minerals						
Water						
Osmolarity (NaCl)					X	
pH (Buffer)						X
Temperature						

BIOLOG

Yersinia pseudotuberculosis type strain is arg-



BIOLOG

PM9

Combination of ionic/non-ionic osmolytes
as well as biologically/environmentally important ions

NaCl, KCl, Na2SO4
NaCl with osmolytes
PO4
NH3
Urea, Ethylene glycol
Formic acid, Lactic acid, Benzoic Acid
Nitrate, Nitrite

BIOLOG

PM10

pH range of growth

pH control at acid range (4.5) with decarboxylases

pH control at alkaline range (9.5) with deaminases

BIOLOG

PM11+

Antimicrobial sensitivity is best tested in a lower nutrient, lower salt medium

BIOLOG

PM Comparison of Isogenic

Strain Pairs to Determine

Function of Genes

E. coli

BIOLOG

Assaying Genetic Changes with Phenotype MicroArrays

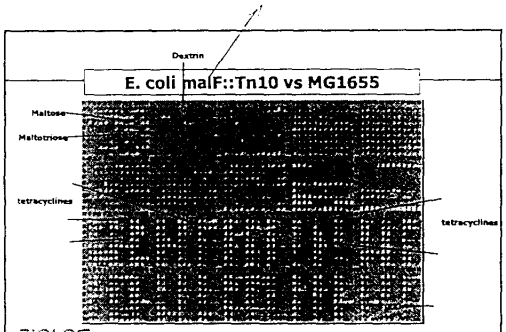
Genotype ⟶ **Phenotype**

Phenotype MicroArrays

Knock out a gene ⟶ Which phenotypes change?

BIOLOG

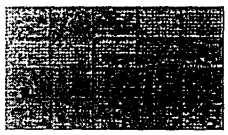
E. coli malF::Tn10 vs MG1655



BIOLOG Red = Phenotypes Lost Green = Phenotypes Gained

Cognate Pair: arcA and arcB

E. coli arcA vs MG1655



E. coli arcB vs MG1655

BIOLOG

Summary of E. coli PM Studies

1. **Most expected phenotypes can be detected.**
2. **Unexpected phenotypes are often detected as well.**
3. **For genes of unknown function we detect at least one phenotype in about 2/3.**

BIOLOG

Pathogenicity and Metabolism:

Comparison of

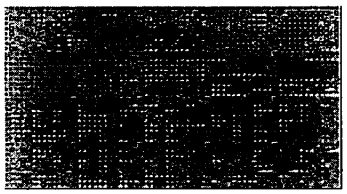
Pathogenic vs Non-Pathogenic

Microbial Strains

Vibrio, Listeria, Yersinia

BIOLOG

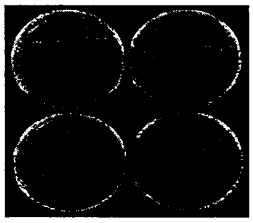
Comparison of Less (Classical O1) and More Dominant (El Tor O1) Vibrio cholerae



BIOLOG

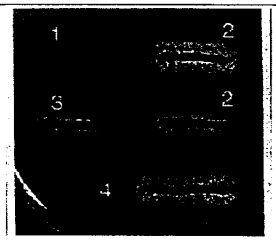
Mekalanos and coworkers have reported very little difference in the genomes of these strains

Stimulation of V. cholerae "Classical" by Tween 40



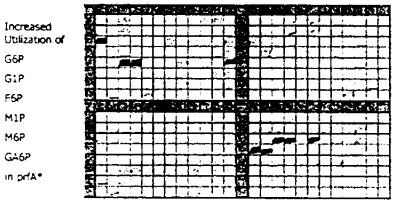
BIOLOG

Haemolysin Production by prfA* Listeria Strains



BIOLOG

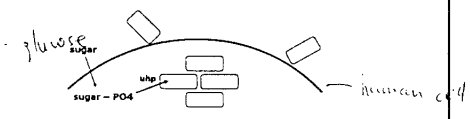
PM Analysis of L. monocytogenes prfA* Pathogenicity Allele



BIOLOG

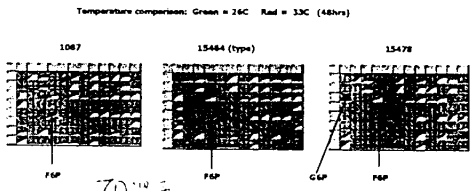
Listeria strains from Jose Vasquez-Boland

Sugar-PO4 Metabolism Aids Intracellular Growth



BIOLOG

Yersinia pseudotuberculosis



BIOLOG

Using PM Technology to Study

Human Diseases

S. cerevisiae

BIOLOG

Human Disease: Metabolic Basis and Correspondence of Microbial Genes

Best Sequence Similarity Matches to Date Between Positionally Cloned Human Genes and S. cerevisiae Proteins

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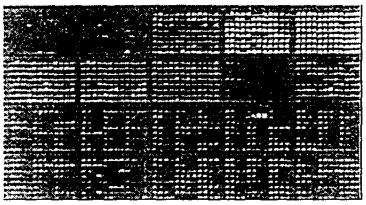
S. cerevisiae ira2 vs ho

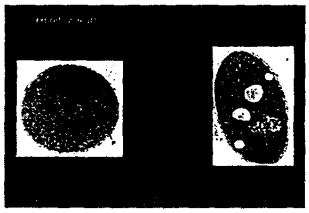


Homologous to human NF1 gene (Neurofibromatosis, type 1). Mutations in NF1 result in multiple discrete dermal neurofibromas, and Iris Lisch nodules.

BIOLOG

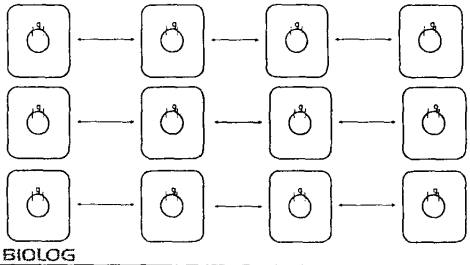
Increased metabolism of sugars: glucose, mannose, fructose, sucrose, raffinose
Sensitivity to myosin, trideromycin

Changes in S. cerevisiae Induced by Growth on Oleic Acid



BIOLOG

PMs: Cells in 2000 Growth Conditions



BIOLOG

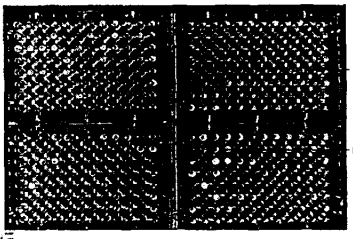
Screening for Metabolic Regulation

by Testing 2000 Growth Conditions

Streptomyces sp.

BIOLOG

4 Actinomycetes: Pigment Production



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Conclusions

PM Technology can be used to optimize culture conditions for production of:

- Antibiotics, Secondary metabolites
- Vitamins, Amino acids
- Ethanol, Lactic Acid, other organics
- Enzymes and Toxins

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Regulation of Nitrogen Metabolism &
Integration with Carbon Metabolism

S. aureus ≠ E. coli

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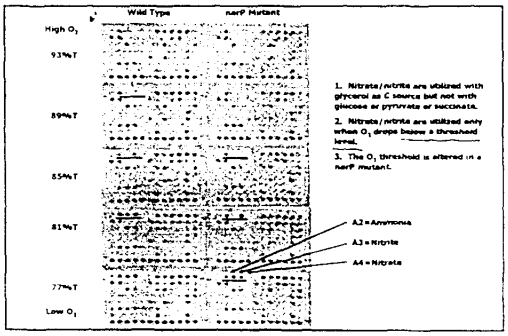
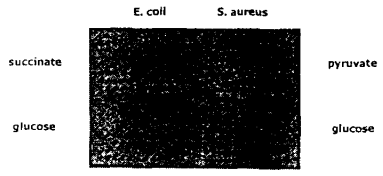


Effect of C-Source on N-Metabolism



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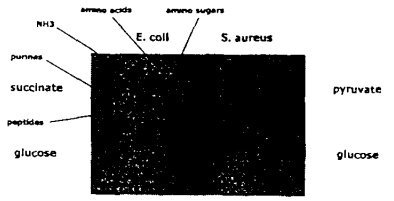
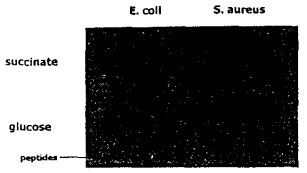
Effect of C-Source on N-Metabolism



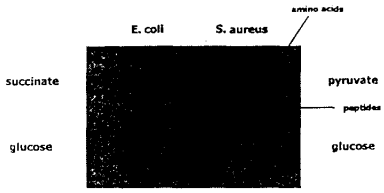
BIOLOG

Effect of C-Source on N-Metabolism



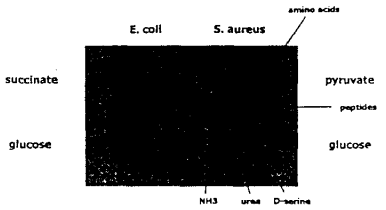
BIOLOG

Effect of C-Source on N-Metabolism



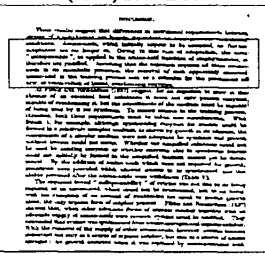
BIOLOG

Effect of C-Source on N-Metabolism



BIOLOG

G. P. Gladstone, British J. Expt. Pathol. (1937) 18:322-333



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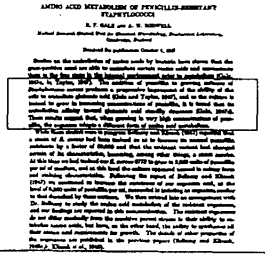
Antibiotic Resistance and

"Metabolic Adaptation"

S. aureus

BIOLOG

Gale & Rodwell, J. Bact (1948)55:161



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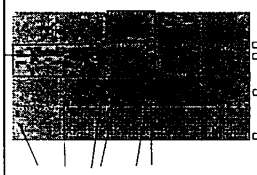
S. aureus VP32 vs. HIP5827



Phenotypic changes in van(R) Staph:

- Decreased catabolism of amino acids
- One specific exception is arginine
- Increased resistance to cell wall inhibitors penicillins, cephalosporins, bacitracin, glycine
- Increased sensitivity to piperacillin, amikacin

November 8, 2002
Staphylococcus strains from Gordon Archer
Test Green VP32 (from BHI Vancomycin 16ug/ml)
Reference Red HIP5827 (from BHI Vancomycin 4ug/ml)

BIOLOG

PM Analysis vs Genechip Analysis

BIOLOG

PM Technology Detects Only Biologically Significant Changes

	reproducible changes	biologically significant
Gene expression experiment	↑↑↑↑↑↑↑↑↑↑↓↓↓↓	↑↑↑↓↓
Phenotype MicroArray experiment	↑↑↑↓↓	↑↑↑↓↓

BIOLOG

PM and Gene Expression Technology are Complementary

	genes tested	cellular conditions
Gene expression experiment	thousands	one
Phenotype MicroArray experiment	one	thousands

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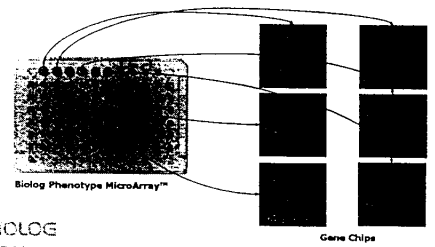
Biolog Phenotype MicroArrays™ Can Provide Cells With Diverse Protein Patterns



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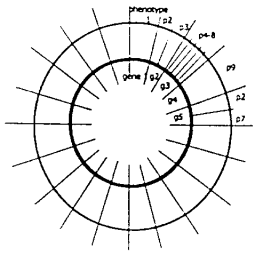
Genotypic and Phenotypic Maps



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Conclusions/Opinions

1. We cannot yet do a good job of "system integration" even after adding the new genomic data.
2. We need more fundamental knowledge of pathways that are present, their interaction and regulation.
3. Tools like gene chips, metabolomics, PM technology and other technologies are needed to supply basic information that is still missing.

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Advantages of PM Technology

1. Complements genomics and gene expression data by providing needed biological data (quantitative readout of *in vivo* pathway function).
2. Phenotypes represent the integration of metabolism and other biological pathways.
3. PM testing requires no modeling or assumptions.
4. With PM Technology you can do one experiment and get interesting and important results.

BIOLOG

Species Validated for PMs

Gram Negatives	Other Bacteria
Escherichia coli	Deinococcus radiodurans
Salmonella typhimurium	Helicobacter pylori
Pseudomonas aeruginosa	
Burkholderia cepacia	
Sinorhizobium meliloti	
Gram Positives	**Fungi**
Listeria monocytogenes	Saccharomyces cerevisiae
Staphylococcus aureus	Candida albicans
Streptococcus pneumoniae	Ustilago maydis
Enterococcus faecalis	Aspergillus nidulans

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Acknowledgements

Genetics:

Barry Wanner, Jose Vazquez-Boland, Gary Schoolnik, Gordon Archer, Sandy McCutcheon, and their coworkers

PMs:

Xiang-He Lei (E. coli), Amalia Franco-Buff (Listeria,Vibrio), John Argyle (Helicobacter) Michael Ziman (S. cerevisiae) at Biolog

Support:

NIH and NASA

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PM Publications

2-Component Genes of E. coli

Journal of Bacteriology (2003) 185:4956

Overview/Review

Nature Reviews Genetics (2003) 4:309

Initial Publication

Genome Research (2001) 11:1246

BIOLOG

INNOVATIONS

New technologies to assess genotype–phenotype relationships

Barry R. Bochner

The accelerating pace of the discovery of genes has far surpassed our capabilities to understand their biological function — in other words, the phenotypes they engender. We need efficient and comprehensive large-scale phenotyping technologies. This presents a difficult challenge because phenotypes are numerous and diverse, and they can be observed and annotated at the molecular, cellular and organismal level. New technologies and approaches will therefore be required. Here, I describe recent efforts to develop new and efficient technologies for assessing cellular phenotypes.

Ever since Gregor Mendel used the observable traits of pea plants to define and follow units of genetic inheritance, the definition and testing of phenotypes has had a key role in genetic analysis. Phenotypes are important for several reasons. They allow us to observe genetically inherited traits and events, and aid in genetic manipulations. Genetic changes that confer a growth or survival advantage, or a trait that can be scored physically, have been exploited to great advantage. Examples include the use of selectable drug-resistance genes (with drugs such as tetracycline, kanamycin and geneticin) and the selection and scoring of clones on the basis of β-galactosidase activity[1]. Phenotypes that confer a growth or survival disadvantage are also useful. They allow dissection of functional relationships by providing conditions for selecting suppressors that compensate for the disadvantage. Finding, identifying and understanding suppressors has been an important method for getting from a gene of interest to other genes (proteins) that interact with it. Phenotypes are also crucial because they are the expression of genotypes and reveal gene function. In this regard, phenotypes are an essential intermediate in the pathway from basic genetics to biological understanding.

Importance of phenotypes in genomics

In the past decade, we have witnessed an explosion in the availability of new genetic analysis tools and genomic information. Sequencing technology has provided us with complete genomic sequences for species ranging from microbes to plants and animals[2–8] — including that of the human[9,10]. These projects were accompanied by efforts to locate, enumerate and annotate genes and to assign known or putative biochemical functions to them. However, from the most thoroughly studied and 'simple' bacterial cells[2] to man[9,10], only about two-thirds of all genes have an assigned biochemical function and only a fraction of those are associated with a phenotype[11–13]. Even when phenotypes are assigned, they might represent only a partial understanding of the role of the gene. The function of a gene cannot be fully understood until it is possible to predict, describe and explain all the phenotypes that result from the wild-type and mutant forms of that gene.

Phenotypes often cannot be predicted on the basis of the biochemical function of a gene alone because it is not clear how a catalytic or regulatory activity will affect the biology of the cell or the whole organism. However, if a gene has a biological function then, for every identified gene, it should be possible to define at least one phenotype. A second layer of genomic annotation could then follow, in which every gene is described biologically by the phenotypes that it produces (shown conceptually in FIG. 1). A first step in producing a so-called 'phenomic map' has been made for *Escherichia coli* by LaRossa[14] who has tabulated ~1,000 phenotypes that correspond to various genes that have been studied. In diploid and higher organisms in particular, this will be complicated by the fact that several genes can affect gene expression[15], and the resulting phenotypes[16] of each other, leading to epistasis, complex traits and multifactorial diseases.

Along with phenomic maps, there is a need for phenotypic standardization that has already been recognized by breeding and stock centres[17]. Several projects[18,19] have begun to develop a standardized approach to developing annotation and databases. Just as comparative genomics has allowed powerful extrapolation of gene and protein function from one cell type to another[12,13], it will be important to develop a coordinated effort to standardize phenomic nomenclature to facilitate database searches, comparisons and extrapolations. Such a system of comparative phenomics would facilitate the progression of knowledge throughout model biological systems from bacteria to humans.

Many scientists are coming to the conclusion that advances in genetic and genomic analysis are being hindered by the slow pace at which our understanding of biology is progressing. Simply put, biological (that is, phenotypic) information is not keeping pace with genomic information. In 1989, I predicted that global phenotypic analysis would soon be needed to complement the massive amounts of genetic data being obtained[20], and, in 1996, Brown and Peters called attention to 'the phenotype gap' in mouse research[21]. The Nobel laureate Sydney Brenner, in a recent keynote address (at a joint Cold Spring Harbor Laboratory/Wellcome Trust Genome Informatics Conference held at Hinxton in the UK on 9 September 2002) emphasized

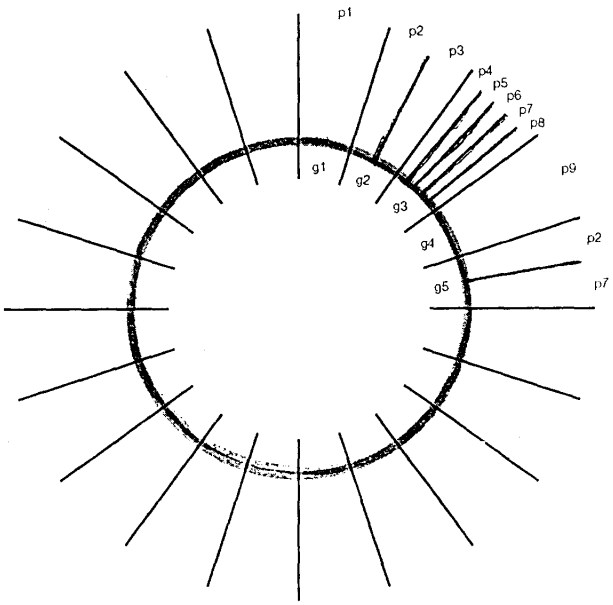


Figure 1 | **Genotypic and phenotypic maps.** A phenotypic map (yellow) can be generated to correspond to any genomic map (green). Some genes, such as gene1 (g1), have only one corresponding phenotype (p1), whereas most genes have many corresponding phenotypes. Phenotypes can be coded for by more than one gene, as shown by p2, which is affected by g2 and g5.

that approaches that relied heavily on genome sequences and bioinformatic extrapolation had too much noise and were becoming non-productive. Instead, he called for a renewed focus on cellular studies and the creation of function-based cell maps in a variety of cell types by the year 2020.

However, generating phenotypic maps will not be easy. Scientists generally test and measure phenotypes one at a time, which is too slow. Almost every model system in which the genome has been sequenced has functional genomics projects to associate the genome with the biology, and this typically includes some efforts that involve phenomics. Many large-scale projects are being carried out both in publicly funded research projects (for example, for animals[22–24] and for plants[25]) and in corporations (such as Lexicon Genetics, Inc., Deltagen, Inc., Phenomix Corporation, SurroMed, Inc. and Paradigm Genetics, Inc.). These projects generally use and adapt diverse existing phenotypic technologies that range from animal autopsies to MASS SPECTROMETER analysis of cellular metabolites.

Although cellular phenotyping does not replace plant or animal phenotyping, it can provide a more rapid, efficient and cost-effective method by which to begin to understand the phenotypes of the tens of thousands of non-annotated genes. The testing of cell suspensions is more amenable to large-scale high-throughput testing and can be implemented with modern robotics and instrumentation. However, so far, robotics has been used primarily to automate small numbers of phenotypic assays. There are few reports of efforts to test many phenotypes simultaneously. To maintain momentum and productivity in biological research, we need much more comprehensive and efficient tools for testing cellular phenotypes. The remainder of this article discusses recent efforts to develop better technologies for assessing genotype–phenotype relationships in cellular systems.

"...a system of comparative phenomics would facilitate the progression of knowledge throughout model biological systems from bacteria to humans."

Phenotyping in single-cell systems

The most complete gene annotation is available for simple microbial-cell model organisms such as *E. coli*[2] and *Saccharomyces cerevisiae*[3]. There are many advantages to large-scale phenotyping in single-cell systems, especially microbial cells, in which it is easier to standardize the biology and to alter genes and assess phenotypes. The phenotypes that are measured are typically biochemical and, therefore, can be easily related to specific enzymatic activities. Gene functions that are initially determined in these models can provide the basis for extrapolation to more complex life forms in which phenotypic testing presents further levels of complexity.

S. cerevisiae researchers have taken the lead in 'genomic-scale phenotyping'. Efforts began in 1996, when a consortium of yeast researchers undertook a project to construct ISOGENIC knockouts of most of the ~6,000 known genes[26]. Hampsey[27] published an overview of yeast phenotypes, and several groups took up the challenge of phenotyping knockout strains as a method of determining the function of various genes. The approaches that were taken are summarized in TABLE 1.

Although the efforts with yeast set a direction for large-scale phenotyping, their results have left many open issues and unanswered questions. A high percentage of the knockout strains that were assayed showed phenotypic changes. This was surprising, as the largest number of phenotypes assayed was 300 and most studies measured ≤20 phenotypes. For example, Hegemann and co-workers[28] tested just 20 phenotypes but found changes in one-third of the strains, and two-thirds of the conditional mutants had multiple phenotypes. Clearly, one problem with most of these approaches is that the phenotypes that were tested, such as growth in rich or minimal media, were not specific. When a change is detected, we can postulate little, if anything, about the gene function. Hegemann and co-workers concluded that the provision of the mutants to the scientific community was likely to be of more use than the phenotypes that were detected, but they expressed the hope that "...experts in specific areas of yeast cell biology will be able to analyze the relatively few phenotypes in which they are experts"[28].

Table 1 | **Large-scale phenotyping projects in *Saccharomyces cerevisiae***

Laboratory	Number of strains tested	Number of phenotypes tested	Test format	References
Brown	268	7	Growth assays	34
Davis	5,916	2–6	Growth competition assays with strain bar-coding	35–37
Slonimski	100	300	96-well microplates with agar media	38–40
Hegemann	Hundreds	20	96-well microplates with agar media	28,41
Snyder	8,000	20	Growth in 96-well microplates replica-plated to agar media	42
Lindquist	14	150	Growth on agar media	43
Harashima	465	11	Suspensions in 96-well microplates replica-plated to agar media	44
Blomberg	–	98	Growth in microwell plates in 350 µL liquid cultures	45

In general, the efforts to phenotype yeast mutants have not provided a basis for solving the general need for comprehensive and detailed cellular phenotyping. At most, 300 phenotypes were tested, the specific tests used are not readily adaptable for other types of cells, the technologies are still cumbersome for high-throughput applications and, in many cases, the phenotypes are still qualitative rather than quantitative.

Phenotype MicroArray technology

In 1998, our group began a programme to devise a phenotyping technology that had attributes that were missing from previous approaches: it could assay ~2,000 distinct culture traits; it could be used with a wide range of microbial species and cell types; it would be amenable to high-throughput studies and automation; it would allow phenotypes to be recorded quantitatively and stored electronically, to facilitate comparisons over time; it would give a comprehensive scan of the physiology of the cell; and, by providing global cellular analysis, it would provide a complement to genomic and proteomic studies (FIG. 2).

Instead of using growth-based assays, we have used a TETRAZOLIUM REDOX CHEMISTRY that produces a colour change in response to cell respiration[29] in each well of 96-well microplates. This gives an accurate reflection of the physiological state of the cell, and can be used in some important assays that do not depend on growth. The technology is feasible for high-throughput analyses because the microplates are manufactured with a stable dry chemistry ready for inoculation. Also, the monitoring and recording of data is automated, standardized and quantitative. The result of these efforts is a new technology that we have called Phenotype MicroArrays (PMs) (REF 30; BOX 1).

The initial objective of PM technology was to allow the testing of thousands of phenotypes. One simple reason for having thousands of tests is that microbial cells have thousands of genes, and we expect that each gene will be responsible for one or more phenotypes. Furthermore, we wanted our selection of phenotypes to provide a comprehensive analysis of the basic physiology of the cell, and to use specific phenotypes that could point towards specific cellular pathways and biological functions. Nearly 2,000 tests could be accomplished by using 20 96-well microplates, tested simultaneously, and with detailed kinetics recorded.

Expanded phenotypic analyses

An example of a phenotypic comparison of two isogenic strains of *E. coli* is shown in FIG. 3. In this example, MG1655 — the genomically sequenced strain[2] — is compared with an isogenic derivative that contains a knockout of the *malF* gene caused by the insertion of a Tn10 (tetracycline resistance) transposon. The *malF* gene encodes a protein that is involved in the uptake of maltosides, so we would expect to see phenotypic defects related to maltose metabolism as well as resistance to tetracyclines. The PM analysis detects both types of phenotypic changes: the loss of maltose, maltotriose and dextrin metabolism (red lines in FIG. 3) and the gain of resistance to a variety of tetracyline antibiotics (green lines in FIG. 3).

Whereas mutation of a specific transport or metabolic function might result in a small number of easily interpretable phenotypic changes, mutation of a global regulatory gene might alter many phenotypes, so interpretation might be complex. We have previously published an example of an adenylate cyclase (*cya*) mutant of *E. coli* (REF. 30). More recently, Xiang-He Lei in our laboratory has analysed knockouts of 32 two-component regulatory genes of *E. coli* in collaboration with Zhou and Wanner at Purdue University (L. Zhou and B. Wanner, unpublished observations); nineteen of these were found to have detectable phenotypic changes. The number of phenotypes ranged from as few as one change, to as many as 50 changes for *arcA* and *arcB* deletions. Some of the phenotypes were expected, but others were not and remain to be explained. We have also analysed mutant strains for a number of other laboratories working on *E. coli* and have completed a phenotypic comparison of several wild-type *E. coli* strains that are in common use (X.-H. Lei *et al.*, unpublished observations). Applications of this technology are not limited to *E. coli*.

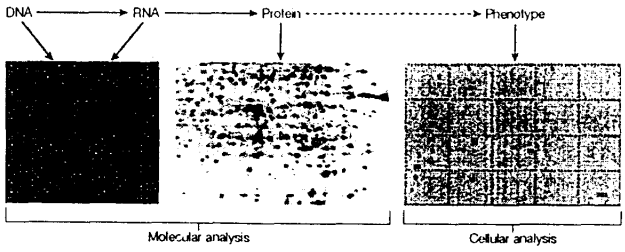


Figure 2 | **Global cellular analysis.** The information in cells flows from the level of genotype to the gene and protein expression levels, and results in cellular phenotypes. Modern tools for global analysis are beginning to provide a way to study and understand this process in greater detail.

Amalia Franco-Buff has analysed isogenic strains with alterations in the *prfA* gene of *Listeria monocytogenes* in collaboration with Jose Vazquez-Boland at the University of Bristol (A. Franco-Buff, unpublished observations). This is a particularly interesting regulatory gene because it regulates the biological functions that are essential for pathogenicity in this bacterium[31]. In another project, Richard Kostriken in our laboratory has analysed gene knockouts of human disease gene homologues in *S. cerevisiae* (R. Kostriken, unpublished observations). Over the past year, we have shown that we can use our current set of PMs to test other Gram-negative genera such as *Salmonella*, *Pseudomonas*, *Burkholderia*, *Vibrio* and *Sinorhizobium*, Gram-positive genera such as *Bacillus*, *Staphylococcus*, *Streptococcus* and *Enterococcus*; yeast such as *Candida* and *Cryptococcus*; and filamentous fungi such as *Aspergillus nidulans*. We have also had success in adapting this technique for bacteria that require incubation in special gas atmospheres (such as *Helicobacter pylori*).

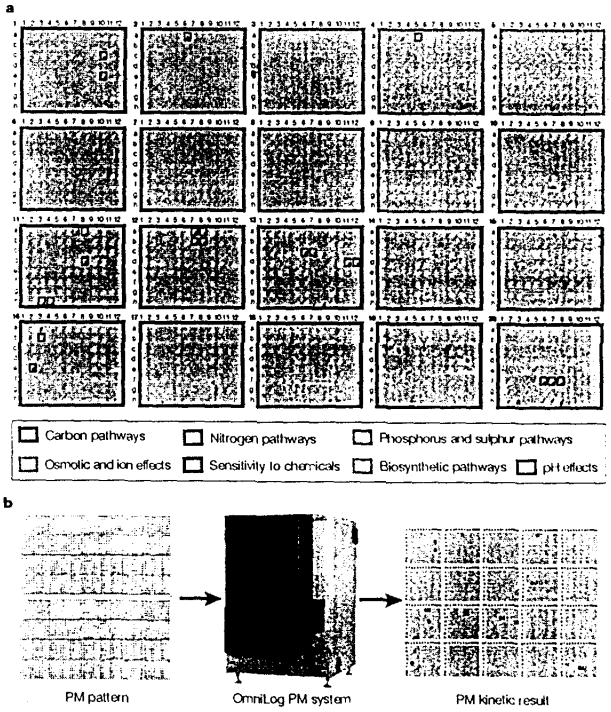
Comprehensive phenotyping with PM technology is useful for many other types of comparison. In addition to knockouts, it is possible to compare the phenotypic consequences of gene underexpression or overexpression, as well as interesting alleles of genes such as those that encode regulatory proteins that lock the circuitry in which they are involved in the 'on' or 'off' state. Modern controllable promoters, such as the arabinose system in bacteria[32], can be used to vary the level of gene expression, including that of essential genes. Alternatively, a controllable promoter can be used to produce varying amounts of antisense RNA *in vivo*[33]. Another possibility involves the introduction of one or more genes into a cell line to determine the phenotypic consequences of their expression, which can reveal their function. An example of detecting the function of introduced genes (in this case, the tetracycline

Box 1 | Phenotype MicroArray technology

Phenotype MicroArrays (PMs) are a simple tool for testing hundreds or thousands of cellular traits simultaneously. The PMs that are available at present contain ~2,000 tests that are selected to approximate a comprehensive scan of the known cellular pathways.

The layout of the 2,000 PM tests is summarized in panel a. PMs 1–8 test the main catabolic pathways in cells for carbon, nitrogen, phosphorus and sulphur, as well as biosynthetic pathways. PM9 tests osmotic and ion effects on the cell. PM10 primarily tests pH growth range and pH regulation. The remaining 10 PMs test the sensitivity of cells to a wide range of chemicals, including antibiotics, anti-metabolites, membrane-active agents, respiratory inhibitors and toxic metals. Antibiotics and anti-metabolites, with different modes of action, target the cell wall, membranes, ribosomes, RNA and DNA polymerases, and diverse metabolic pathways. Membrane-active agents and respiratory inhibitors probe the chemistry, structure and function of membrane-associated processes, such as respiration and protein localization. Toxic metals can be present in the environments of most cells, which are likely to have cellular systems for handling them.

To analyse a microbial strain, a cell suspension is prepared and inoculated into the set of microarrays. As shown in panel b, in which a pair of isogenic strains are compared, the PM panel sets are then placed inside the OmniLog — an incubator/reader instrument that cycles the arrays in front of an imaging head every 15 minutes, measuring and recording the colour formed from reduction of the tetrazolium dye in each well. Computer software plots kinetic graphs of colour formation against time for each well and each strain. When two strains are compared, the reference strain is plotted in red and the mutant strain in green. This is analogous to labelling the RNA from two strains with red and green dyes in gene-expression analysis. The software can compare the kinetic phenotypes by overlaying the kinetic graphs and colouring areas of overlap (no change) in yellow. The result is a red–green–yellow array in which phenotypes that are lost are coloured red, phenotypes that are gained are coloured green and unchanged phenotypes are coloured yellow. Thresholds can be set to disregard small and insignificant changes, and all of the wells with changes that exceed this threshold are marked with a black box.



resistance transposon Tn10) is shown in FIG. 3. Many laboratories have libraries of HETEROLOGOUS GENES that have been cloned in *E. coli*. If the cloned genes have promoters that are operative in *E. coli*, it might be possible to assay directly for the function of these genes using the bacterial cell as a surrogate. Other interesting blocks of DNA, such as plasmids, viruses and PATHOGENICITY ISLANDS, can be added to cells and tested for phenotypes that they have engendered. Isogenic cells can be compared for epigenetic effectors, such as changes in DNA methylation, histone acetylation, prion effects and so on. Useful information can also be gained by comparing non-isogenic cells such as multiple isolates from one species, pathogenic versus non-pathogenic strains and PASSAGED STRAINS versus recent natural isolates.

Limitations of large-scale phenotyping

An appealing aspect of phenotypic analysis is the simplicity and directness of its interpretation. For many applications, it relies on the validity of comparing the biology of isogenic strains, which has a substantial and proven record. A change in genotype leads to one or more changes in phenotype. To fully understand the function of a gene, we need to be able to enumerate and explain all the phenotypic changes that result from changes in that gene. But implicit in these types of isogenic analysis is that the cells, plants or animals that are being compared are truly isogenic (with the exception of the intended genetic change). This means that the genetic techniques that are used to create the strains must be precise, otherwise some of the phenotypes that are detected might be due to other unintended genetic differences between mother and daughter cell. As large-scale phenotyping technologies move towards the goal of measuring all the phenotypes of a cell, they also approach a level of sensitivity at which they can serve to assay the precision of genetic manipulation. In fact, in our work over the past two years using PM technology on *E. coli*, we have found several examples of strains that had been produced by proven genetic techniques that contained extraneous genetic changes. Even with accurate genetic manipulation, the accumulation of secondary suppressors in isogenic lines can be problematic. Two methods can be used to gain confidence that a phenotype is tied to a genotype: restoring the allele back to wild type and showing that the phenotypic change goes away, or assaying at least two independently constructed strains and showing that both isolates show the same phenotypic changes.

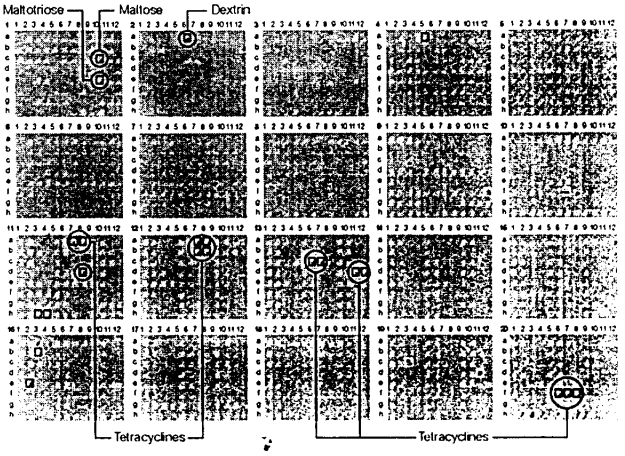


Figure 3 | **Phenotype MicroArray comparison of two isogenic strains of *E. coli*.** Phenotype MicroArray analysis of isogenic *E. coli* strains *E. coli* malF::tn10 versus MG1655. The mutant strain is shown in green and the parental MG1655 strain is shown in red. Knockout of the *malF* gene leads to the loss of catabolism of maltose, maltotriose and dextrin. Insertion of the TN10 CASSETTE leads to the gain of resistance to a number of tetracycline antibiotics.

An important impetus for the development of large-scale phenotypic analysis has been to determine the function of the remaining genes for which no function is known. We can expect these efforts to be partially successful — our limited experience, so far, using PM technology with genes of unknown function indicates a success rate of ~65% in *E. coli* (B.B. *et al.*, unpublished observations). When phenotypes are found, they can indicate anything from a precise enzymatic function to a vague allocation of the gene to an area of cellular physiology.

Surveying all of the phenotypes of a cell is a theoretical concept and goal. In reality, cells have too many phenotypes for us to be able to define, let alone test. It is important to acknowledge and be aware of the limitations of large-scale phenotyping techniques such as PM analysis. There are a number of reasons why, in its present form, PM analysis and other phenotyping technologies will not discover all of the phenotypes. First, the phenotyping sets available at present are not all-inclusive. For microbial cells, PM technology is likely to miss phenotypes that specifically involve intracellular structures (for example, the cytoskeleton and organelles) and surface structures and functions such as flagella, attachment, biofilm formation, motility and chemotaxis, as well as functions turned on only under anaerobic

Glossary

HETEROLOGOUS GENE
A gene that is transferred into a cell but originated in a cell from a different species.

ISOGENIC
Cells or organisms that are derived from the same parent and have almost identical genomes.

MASS SPECTROMETRY
An analytical tool for determining the molecular weight of a chemical.

MULTI-STATE AUTOMATON
A self-acting and self-responding machine that has the ability to change itself into multiple states.

PASSAGED STRAINS
Cells that have been repeatedly subcultured, typically under artificial *in vitro* laboratory-culture conditions and not in more natural *in vivo* conditions.

PATHOGENICITY ISLAND
A contiguous block of genes, found in pathogenic microorganisms, in which at least a subset of the genes code for virulence factors.

TETRAZOLIUM REDOX CHEMISTRY
A dye chemistry that absorbs the electrons produced by cellular respiration, causing a colour change as the tetrazolium dye is reduced.

TN10 CASSETTE
A contiguous block of genes that is derived from the bacterial transposon Tn10, which confers resistance to tetracycline antibiotics.

conditions. Also, it is not possible to test the cellular functions that still remain to be discovered — there are undoubtedly gaps in our knowledge of the whole spectrum of cellular functions. Second, the effects of some genes might be cryptic and only have a function under highly specific cellular conditions. Many microbial phenotypes might be expressed only when the microbe interacts with an animal or plant. And third, we might not be able to discern phenotypes for some genes because there are redundant cellular functions that compensate in their absence.

Phenotypic analyses are likely to provide an important complement to gene-expression and proteomic analyses of genetically altered cells. Molecular analyses enumerate a large number of biochemical changes, but cellular analyses show what these changes mean at the biological level. To illustrate the complementarity of these two approaches, consider the comparison of a mutant and wild-type cell tested by gene-expression analysis. Data from this analysis compares the level of thousands of genes under a single growth condition and state of the cell. The same comparison done by PM analysis looks at only a single gene, but under thousands of growth conditions and cell states. It is very important to appreciate that a cell line is not a single static entity. Every cell is a MULTI-STATE AUTOMATON with the capacity to change in minutes. A cell constantly senses its environment and adapts to changes by altering its gene-expression pattern, protein content, membrane constitution, surface receptors and so on. In each growth state, the cell becomes a different cell, sometimes markedly different. Understanding this fluid 'landscape' will challenge biologists for many decades to come. FIGURE 2 depicts PM analysis as a technology in the stream of, and complementary to, DNA microarrays and proteomic analysis. By using these genomic technologies, and others that are derived from and added to this set, we will continue to move our knowledge forward.

Phenotyping in higher eukaryotes

Here, I have emphasized studies of microbial cells as model systems. Other, more complex model systems, such as *Caenorhabditis elegans*, that can be cultured in microwells could be amenable to modified versions of these phenotypic technologies. Higher plants and animals could certainly be targeted next by adapting the technologies for cell cultures. We are working to extend PM technology to mouse and human cells, and prototype PMs for testing carbon and energy metabolism in human and mouse liver and blood cells have already been devised and successfully tested (A. Morgan, unpublished observations). In the near future, we will see the capability for simulating part of the metabolic and cell biology of a mouse or human by using the large-scale phenotypic analysis of cells derived from most of the main tissues. Another approach would involve the detailed phenotyping of embryonic stem cells in culture, to see how useful the cellular phenotypes are in predicting the phenotypes of animals. Relevant to this technology are the recent advances in gene inactivation using RNAi, which provide a method of specifically inactivating gene function. We forsee immediate applications of phenotypic analysis in genetics, physiology, toxicology and the study of ageing, differentiation and disease.

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Phenotype Microarray Analysis of *Salmonella enterica*

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Microbial Ecology in the 21st century

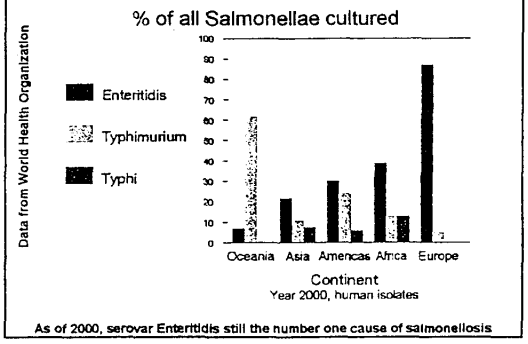
- A common theme of some of our most urgent research needs in agriculture that impacts:
  - Food Safety
  - Animal Health
  - Sustainable Agriculture
  - Biosecurity Issues
- What technological advances does microbial ecology need in order to make the next leap in knowledge?

Phenotype Microarray Analysis with Omnilog ©, Biolog Inc.

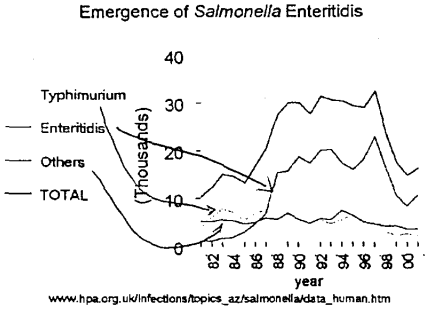
- Measures and collates growth under 1910 conditions that assay physiology and resistances.
  - Some conditions are arranged in dilution series
- Options:
  - Media used
  - Temperature of incubation
- Collects data as a growth curve – 36-48 hrs.
- Allows strain comparison
- Evaluates growth substrates and inhibitors.
- Could evaluate microbial communities
  - Differences in growth characteristics between mixed strains has to be considered.

The Egg Contamination Problem

- *Salmonella enterica* serovar Enteritidis.
- Involves a complex infection pathway.
- Current intervention is primarily biosecurity.
  - Vaccination is adjunctive control measure.
- The problem is endemic
  - Some decrease from peak, but has not declined to pre-1980 levels.



As of 2000, serovar Enteritidis still the number one cause of salmonellosis



Type of data obtained

- 3 images of plate (2 replicates and an average).
- Scatter plot of replicate 1 vs 2 data to assess reproducibility.
- 45 pages of readouts; 1900+ reactions; pos controls/background wells per strain
- 225 pages of data; 9,000+ datapoints.
- Synopsis of results from PMServices.

Approach to Data Analysis

- Analyze background/control data to establish thresholds of growth for each strain
  - Average, std dev, Ttest pvalue
- Sort and align data columns in spreadsheet.
- Frequency analysis to sort data points into bins
  - (lysis; no/slow growth; growth; stimulated growth)
- Evaluate classes of compounds
  - Fatty acid metabolism
  - Antibiotic resistances
  - Preferred C, N, P, S source

Frequency Analysis

- Determine Bin (category) parameters
  - 0, no or slow growth, growth, stimulated
- Follow excell instructions for data entry
- Choose chart presentation (pie)
- Set background limits
  - Strain specific
- Evaluate results in context of known biology

Frequency Analysis (cont.)

- Develop a standard approach for BINS
  - All 0 values are special class (lysis)
  - Round average UP for all other classes to closest 25 increment
    - 25 will be lowest increment that differentiates between no/slow growth and positive growth
    - Robust growers may have lower limit of 50
    - Evaluate data for upper limits by two methods
    - Consider the biology of the organism

	SE	SH	STM	SP1	SP2
NEGATIVE CONTROL ANALYSIS					
Average	43.2	48.1	8.9	9.9	7.0
Std Dev	27.9	29.8	5.2	6.9	5.8
Ttest pval	—	0.46	0.01	0.02	0.01
Ttest pval	0.01	0.01	0.15	0.09	—
thresholds	50	50	25	25	25
CATEGORIES (Bins)	SE	SH	STM	SP+	SP-
lysis	0	0	0	0	0
no growth	50	50	25	25	25
growth	225	225	200	150	50
stim growth	>225	>225	>200	>150	>50

	SE	SH	STM	SP1	SP2
POSITIVE CONTROL ANALYSIS					
	232.9	228.7	198.8	148.2	50.0
	42.4	39.6	39.1	78.7	56.1
	—	0.84	0.15	0.02	0.00
	0.00	0.00	0.00	0.12	—
	225	225	200	150	50
STIMULATED GROWTH VALUE					
	227	228	149	109	40

Fixed Costs

- Equipment. US version with computer plus video FG; battery operated pipettor; turbidimeter; colony magnifier lamp; 1 year on-site warranty. On-site installation and validation.
- Validation Package and strain set.

Yearly Costs

- $12,000 per year in standards
- $100,000 per year in panels
  - Total flexibility in choosing panels
    - C,N,P,S panels
    - Biosynthetic pathway panels
    - Nutrient stimulation
    - Osmotic/Ionic response
    - pH response
    - Chemical sensitivity assays (antibiotics, fungicides, detergents, chelators etc)
- Yearly service agreement
  - $9,000

$121,000 yearly costs

Cost considerations for phenotype microarray

- Coordination and management is required.
- Strain accession log maintained.
- Biosecurity guidelines apply (BSLIII ?).
- With a support scientist, yearly may be about $200,000 (100 strains per year).

Summary

- Powerful new technology for microbial ecology research.
- Has potential to redefine our knowledge of microbial ecology.
- Should consider a USDA center for conduct of phenotype microarrays.
- Putting control of parameters in the hands of the scientist increases research potential.

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Ph.D. (1981) University of California a Berkeley

Bacterial Genetics, Bioinformatics, Functional Genomics, Small Proteins in E. coli

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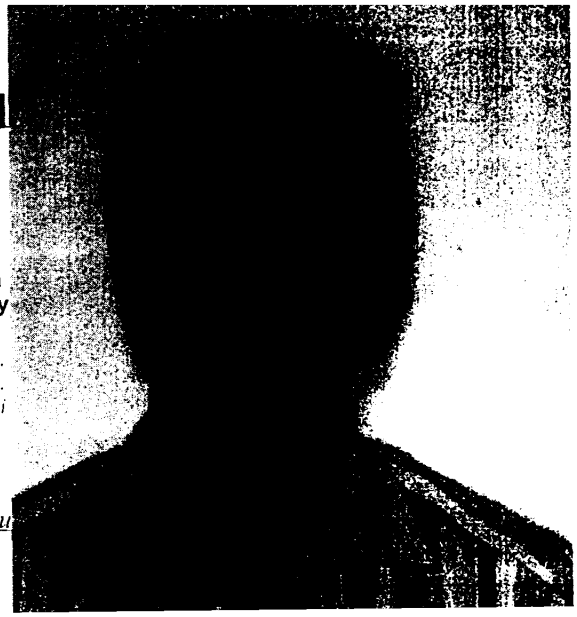


Our laboratory is engaged in the functional characterization of a selected set of genes and gene products of *Escherichia coli*. The 4.6 Mb genome of *E. coli* is now completely sequenced and contains over 4100 protein-encoding genes. Less than half of these genes have been functionally characterized. Most protein sequences can be organized into families based upon homologous relationships. Paralogous families have multiple members encoded in the *E. coli* genome; orthologous families have only one member in *E. coli*, but genes of similar function, orthologs, exist in other species. Some families are restricted to the bacterial domain of life whereas others contain Ancient Conserved Regions (ACRs) and are present in both bacteria and eucaryotes, including some human genes. Cloistered paralogous families do not have homologs in any other organisms, but do have homologs (paralogs) within *E. coli*. Loner proteins do not appear to have any homologs whatsoever, apparently having drifted far away from their ancestor proteins. All of the protein sequences derived from the *E. coli* genome are being organized into classes based upon types of homologous family relationships and functional predictions that can be associated with a family.

Our characterization of *E. coli* ORFs of unknown biological function is directed at selected proteins that fall into the different categories of homologous relationships. In some cases, a functional prediction can be made based on functions attributed to homologs in *E. coli* or other species. Sometimes, the functional prediction is limited to a general activity associated with a common protein motif. In other cases, no functions are attributed to any member of the homologous family, even though the family might be quite widespread in nature. Our approach includes determining the phenotype associated with mutations in the genes of interest as well as localizing, cloning, overproducing and purifying the proteins of interest. We are particularly interested in proteins of less than 150 amino acids in length as they are among the most difficult to analyze using bioinformatic approaches alone. Some of the proteins we are characterizing have predicted functions that include protein phosphorylation, nucleotide binding, protein-protein interactions, and protease activity. Other proteins have no function predicted, but appear to be soluble proteins with signal peptides that would localize them to the periplasm. We hope that this selective top-down approach to functional genomics will illuminate important new functions, not just in *E. coli*, but in organisms with related proteins as well.

The EcoGene database of sequences and annotations for all *E. coli* genes is available on a website:

http://bmb.med.miami.edu/EcoGene/EcoWeb

Representative Publications

- Rudd, K. E. and Menzel, R. 1987. The *his* operons of *E. coli* and *S. typhimurium* are regulated by DNA supercoiling, Proc. Natl. Acad. Sci. USA, **84**:517-521.

- Borodovsky, M., Koonin, E. V. and Rudd, K. E. 1994. New genes in old sequence: a strategy for finding genes in the bacterial genome, Trends in Biochem. Sci., **19**:309-313.

- Rudd, K. E., Rouviere, P. E., Lazar, S., Sofia, H., Plunkett, G., Koonin, E. V. 1995. A new family of peptidyl-prolyl isomerases. Trends in Biochem. Sci., **20**:12-14.

- Berlyn, M. B., Low, K. B. , and Rudd, K. E. 1996. Integrated linkage map of *Escherichia coli* K-12, edition 9. pp. 1715-1902. In F.C. Neidhardt, et .al (ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed., ASM Press, Washington, D.C.

- Koonin, E. V., Tatusov, R. L. and Rudd, K. E. 1996. Protein sequence comparison at genome scale. Meth. Enzymol., **266**:295-322.

- Rudd, K. E., Humphery-Smith, I., Wasinger, V. C. and Bairoch, A. 1998. Low molecular weight proteins: a challenge for post-genomic research, Electrophoresis, **19**:536-544.

- Rudd, K. E. 1998. Linkage map of *Escherichia coli* K-12, edition 10: the physical map. Microbiol. Mol. Biol. Rev., **62**:985-1019.

- Rudd, K. E. 1999. Novel intergenic repeats of *Escherichia coli* K-12, Res. Microbiol. **150**:653-664.

- Rudd, K. E. 2000. EcoGene: a genome sequence database for *Escherichia coli* K-12. Nucleic Acids Res., **28**:60-64.

Honors and Professional Activities

- Member, American Society for Microbiology

- Member, Genetics Society of America.

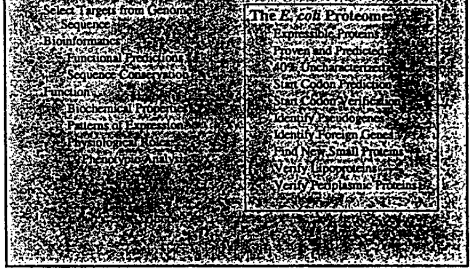
- Member, American Association for the Advancement of Science

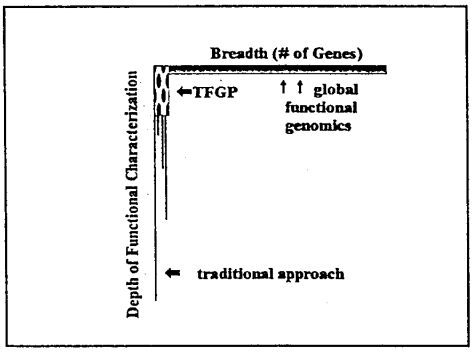
- Editorial Board, Journal of Bacteriology, 1992-2000

Phenotypic Analysis of *E. coli* ORFs of Unknown Function

Kenneth E. Rudd, Ph.D.
Department of Biochemistry and Molecular Biology
University of Miami School of Medicine
Miami, Florida

Targeted Functional Genomics and Proteomics of *E. coli*





Targeting Strategies

- Small proteins
- Periplasmic proteins
- Lipoproteins
- Novel enzyme mechanisms
- Genes with human homologs
- Paralogous genes: duplication and divergence
- Highly expressed genes
- Unusual amino acid composition proteins
- New structural or regulatory RNA genes
- Essential genes

Some Pitfalls of Post-Genomics

- Database versions of genes may not be accurate
- 167 *E. coli* genes appear to be pseudogenes
- 700 *E. coli* genes have incorrect or unlikely start codons annotated in Genbank; fixed in EcoGene
- Many functional predictions are inaccurate
- New structural RNA genes are hard to predict
- Not all overproduced purified proteins are soluble
- Mutant phenotypes may not be apparent
- Small genes and proteins are elusive
- Membrane proteins are largely excluded from Proteomics
- Uncertainty of high-throughput results

High-throughput essential gene identification is problematic

- Gerdes et al. performed global insertion mutagenesis and target sequencing to identify essential genes (*J. Bacteriol.* 185:5673-84, 2003)
- Many results at odds with published or known essentiality results
- *ompA* listed as non-essential, but essential nature of *ompA* is well established and published (M. Deutscher)
- Several genes we had no problem deleting are listed as essential from high-throughput screening

Functional Characterization of *E. coli* ORFs

Protein overexpression
- Purify, characterize and assay enzyme activity
- *Overexpression phenotype*
- Genetic complementation assay

In-Frame Deletion Mutations
- Phenotypic Analysis
  - PM screening (B. Bochner)
    - Detailed analysis to confirm and extend results
- Multiple mutations to counter possible redundancy
- Essential genes more difficult: depletion and death

Two Case Studies

- Singer-Gross Tn10 collection mutants
- PenP: SMR-family Penicillin Pump

Mutants in the Singer-Gross collection

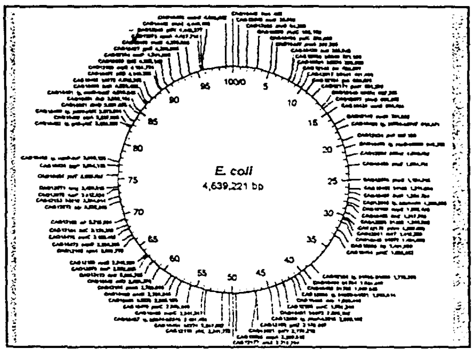
Singer et al. (Microbiol Rev. 53:1-24, 1989)
genetically isolated and mapped a set of 88 Tn10
insertions all around the chromosome that are used to
transduce linked mutations between strains

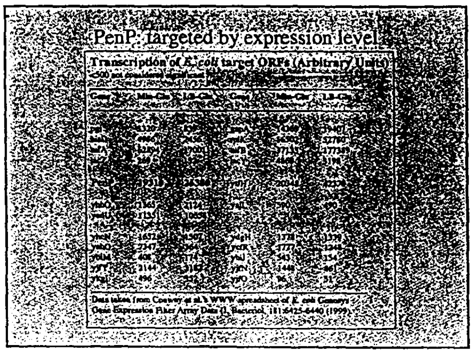
Nichols et al. (J Bacteriol. 180:6408-11, 1998) sequenced all
88 insertion points: 27 interrupted genes

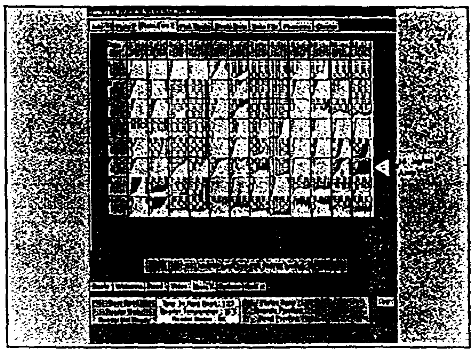
We chose 12 Tn10 insertion mutants for phenotypic analysis
using Phenotype Microarray technology

E.g., ytfN920::Tn10 is sensitive to the anti-fungal agent
patulin on PM plate-media (~2X LB) but not on LB

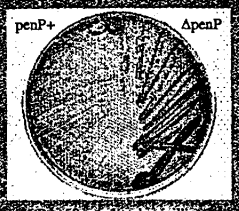
polA1 is also reported to confer patulin sensitivity, suggesting
that YtfN may be involved with DNA metabolism





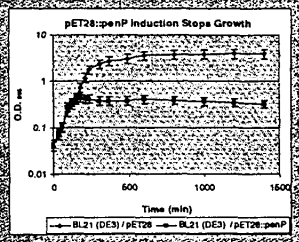


Deletion of penP Confers Resistance to β-Lactam Antibiotics

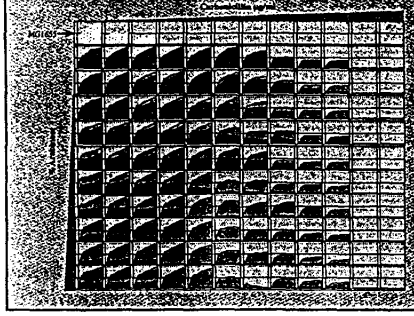


LB + 50μg/ml Carbenicillin

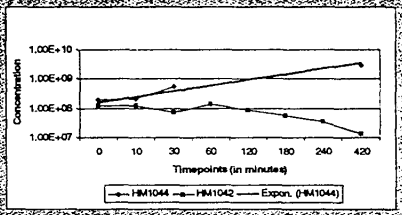
pET28::penP Induction Stops Growth



penP Complementation and Growth Inhibition



Overexpression of PenP Kills Cells



Targeted Functional Genomics and Proteomics of *E. coli*

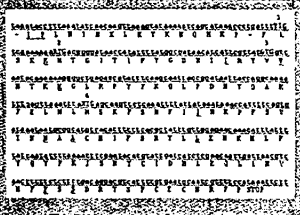
- Select Targets from Genome Sequence
- Bioinformatics
  - Functional Predictions
  - Sequence Conservation
  - Function
  - Biochemical Properties
  - Patterns of Expression
  - Physiological Roles
  - Phenotypic Analysis

The *E. coli* Proteome:

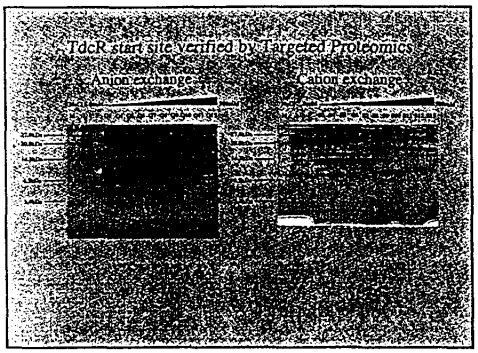
- Expressible Proteins
- Proven and Predicted
- 40% Uncharacterized
- Start Codon Prediction
- Start Codon Verification
- Identify Pseudogenes
- Identify Foreign Genes
- Find New Small Proteins
- Verify Lipoproteins
- Verify Periplasmic Proteins

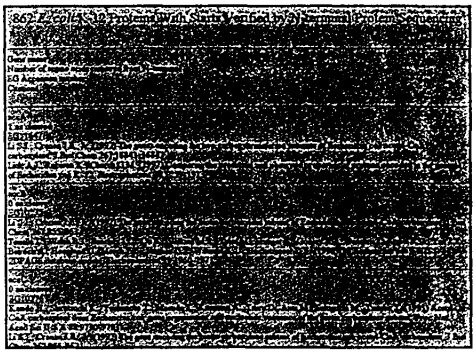
Translation Start Site Assignments can be erroneous or ambiguous

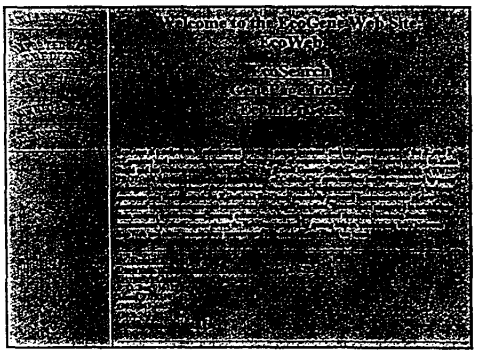
Four different versions of TdcR in Genbank

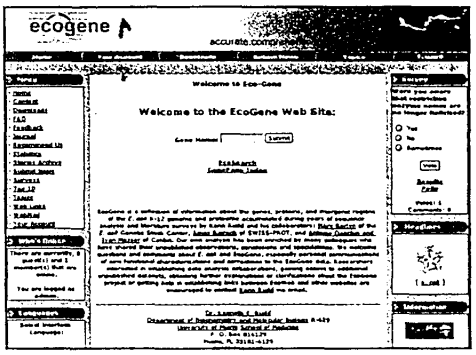


TdcR has no homologs to compare









Recent Collaborators

- Mary Berlyn - Coli Genetic Stock Center
- Pedro Gonnet - Swiss Federal Institute of Technology
- Federique Lisacek - Geneva Bioinformatics
- Tom Schneider, Ryan Shultzaberger - NCI
- Amos Bairoch - SWISS-PROT
- Barry Bochner - Biolog
- BMB Labs: Malhotra, Deutscher, Ofengand

The Rudd Lab

- Grad Students
  - Joshua Ballanco
  - Helen Yancey
  - Jr Huber
  - Rick Mitchell
- Undergrad Students
  - George Marzoulis
  - Julia Gonzalez
  - Adianez Garcia
  - Pedro Warfi
  - Linda Vick
  - Sylvia Oberrein
- Technicians
  - Ha-Ming Peng
  - Gypsy Hernandez
- Informatics
  - Matthew Hagen
  - Joshua Ballanco
  - Duncan Ross
- Rotation Grad Stud
  - Payan Vaidyanathan
  - Mark Del Campo
  - Luhong Zuo
  - Gokhan Tolun
  - Kenneth Ariz
  - Claudia Nelersa
  - Ilda Pedroso

MultiFun (E. coli, M. Riley) mapped to GO (Gene Ontology, initially eukaryotes only) by Ashburner and Lomax
There are not yet GO terms for all the MultiFun categories

http://www.geneontology.org/external2go/multifun2go
http://genprotec.mbl.edu

!From MultiFun site 2003-09-29
!typos etc in MultiFun corrected
!Michael Ashburner & Jane Lomax September 29 2003
!version 1.2
MultiFun:1 Metabolism > GO:metabolism ; GO:0008152
MultiFun:1.1 Carbon compound utilization > GO:.
MultiFun:1.1.1 Carbohydrates/Carbon compounds > GO:carbohydrate catabolism ; GO:0016052
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MultiFun:1.1.1.2 2,5-ketogluconate metabolism > GO:ketogluconate metabolism ; GO:0019522
MultiFun:1.1.1.3 D-arabinose catabolism > GO:D-arabinose catabolism ; GO:0019571
MultiFun:1.1.1.4 D-galactarate catabolism > GO:D-galactarate catabolism ; GO:0019582
MultiFun:1.1.1.5 D-galacturonate catabolism > GO:D-galacturonate catabolism ; GO:0019698
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MultiFun:1.1.1.9 L-idonate catabolism > GO:L-idonate catabolism ; GO:0046183
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MultiFun:1.1.1.15 Mannose catabolism > GO:mannose catabolism ; GO:0019309
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MultiFun:1.1.1.17 Sorbitol degradation > GO:sorbitol catabolism ; GO:0006062

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MultiFun:1.1.1.22 Ribose degradation > GO:ribose catabolism ; GO:0019303
MultiFun:1.1.1.23 Galactose degradation > GO:galactose catabolism ; GO:0019388
MultiFun:1.1.1.24 Sorbose degradation > GO:sorbose catabolism ; GO:0042848
MultiFun:1.1.1.25 L-ascorbate degradation > GO:L-ascorbic acid catabolism ; GO:0019854
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MultiFun:1.1.2.1 Degradation of short-chain fatty acids > GO:short-chain fatty acid catabolism ; GO:0019626
MultiFun:1.1.2.2 3-phenylpropionate and 3-(3-hydroxyphenyl)propionate degradation > GO:3-phenylpropionate catabolism ; GO:0019380 > GO:3-(3-hydroxy)phenylpropionate catabolism ; GO:0019622
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MultiFun:1.1.3.4 Glutamate degradation > GO:glutamate catabolism ; GO:0006538
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MultiFun:1.1.4.3 Ornithine degradation > GO:ornithine catabolism ; GO:0006593
MultiFun:1.1.5 Others > GO:.
MultiFun:1.1.5.1 Phenyl acetic acid degradation > GO:phenylacetate catabolism ; GO:0010124
MultiFun:1.1.5.2 Ethanol degradation > GO:ethanol catabolism ; GO:0006068
MultiFun:1.1.5.3 Eugenol catabolism > GO:eugenol catabolism ; GO:0042856
MultiFun:1.1.5.4 Beta-ketoadipate pathway > GO:beta-ketoadipate pathway ; GO:0042952
MultiFun:1.1.5.5 Mandelate catabolism > GO:mandelate catabolism ; GO:0019596
MultiFun:1.2 Macromolecule degradation > GO:macromolecule catabolism ; GO:0009057
MultiFun:1.2.1 RNA > GO:RNA catabolism ; GO:0006401
MultiFun:1.2.2 DNA > GO:DNA catabolism ; GO:0006308
MultiFun:1.2.3 Proteins/peptides/glycopeptides > GO:proteolysis and peptidolysis ; GO:0006508 > GO:glycopeptide catabolism ; GO:0009050
MultiFun:1.2.4 Polysaccharides > GO:polysaccharide catabolism ; GO:0000272
MultiFun:1.2.4.1 Glycogen catabolism > GO:glycogen catabolism ; GO:0005980
MultiFun:1.2.4.2 Chitin catabolism > GO:chitin catabolism ; GO:0006032
MultiFun:1.3 Energy metabolism (carbon) > GO:energy derivation by oxidation of organic compounds ; GO:0015980
MultiFun:1.3.1 Glycolysis > GO:glycolysis ; GO:0006096
MultiFun:1.3.2 Pentose phosphate shunt, oxidative branch > GO:pentose-phosphate shunt\, oxidative branch ; GO:0009051
MultiFun:1.3.3 Pyruvate dehydrogenase > GO:acetyl-CoA biosynthesis from pyruvate ; GO:0006086
MultiFun:1.3.4 Tricarboxylic acid cycle > GO:tricarboxylic acid cycle ; GO:0006099
MultiFun:1.3.5 Fermentation > GO:fermentation ; GO:0006113
MultiFun:1.3.6 Aerobic respiration > GO:aerobic respiration ; GO:0009060
MultiFun:1.3.7 Anaerobic respiration > GO:anaerobic respiration ; GO:0009061
MultiFun:1.3.8 ATP proton motive force interconversion > GO:ATP synthesis coupled proton transport proton transport ; GO:0015986
MultiFun:1.3.9 Entner-Doudoroff pathway > GO:Entner-Doudoroff pathway ; GO:0009255
MultiFun:1.4 Energy production/transport > GO:.
MultiFun:1.4.1 Electron donor > GO:electron donor activity ; GO:0009053
MultiFun:1.4.2 Electron acceptor > GO:electron acceptor activity ; GO:0009054

MultiFun:1.4.3 Electron carrier > GO:electron carrier activity ; GO:0009055
MultiFun:1.5 Building block biosynthesis > GO:.
MultiFun:1.5.1 Amino acids > GO:amino acid biosynthesis ; GO:0008652
MultiFun:1.5.1.1 Glutamate > GO:glutamate biosynthesis ; GO:0006537
MultiFun:1.5.1.2 Glutamine > GO:glutamine biosynthesis ; GO:0006542
MultiFun:1.5.1.3 Arginine > GO:arginine biosynthesis ; GO:0006526
MultiFun:1.5.1.4 Proline > GO:proline biosynthesis ; GO:0006561
MultiFun:1.5.1.5 Aspartate > GO:aspartate biosynthesis ; GO:0006532
MultiFun:1.5.1.6 Asparagine > GO:asparagine biosynthesis ; GO:0006529
MultiFun:1.5.1.7 Lysine, diaminopimelate > GO:lysine biosynthesis via diaminopimelate ; GO:0009089
MultiFun:1.5.1.8 Threonine > GO:threonine biosynthesis ; GO:0009088
MultiFun:1.5.1.9 Methionine > GO:methionine biosynthesis ; GO:0009086
MultiFun:1.5.1.10 Glycine > GO:glycine biosynthesis ; GO:0006545
MultiFun:1.5.1.11 Serine > GO:serine biosynthesis ; GO:0006564
MultiFun:1.5.1.12 Cysteine > GO:cysteine biosynthesis ; GO:0019344
MultiFun:1.5.1.13 Phenylalanine > GO:phenylalanine biosynthesis ; GO:0009094
MultiFun:1.5.1.14 Tyrosine > GO:tyrosine biosynthesis ; GO:0006571
MultiFun:1.5.1.15 Tryptophan > GO:tryptophan biosynthesis ; GO:0000162
MultiFun:1.5.1.16 Histidine > GO:histidine biosynthesis ; GO:0000105
MultiFun:1.5.1.17 Alanine > GO:alanine biosynthesis ; GO:0006523
MultiFun:1.5.1.18 Isoleucine/valine > GO:isoleucine biosynthesis ; GO:0009097 > GO:valine biosynthesis ; GO:0009099
MultiFun:1.5.1.19 Leucine > GO:leucine biosynthesis ; GO:0009098
MultiFun:1.5.1.20 Chorismate > GO:chorismate biosynthesis ; GO:0009423
MultiFun:1.5.1.21 Homoserine > GO:homoserine biosynthesis ; GO:0009090
MultiFun:1.5.2 Nucleotide > GO:nucleotide biosynthesis ; GO:0009165
MultiFun:1.5.2.1 Purine biosynthesis > GO:purine nucleotide biosynthesis ; GO:0006164
MultiFun:1.5.2.2 Pyrimidine biosynthesis > GO:pyrimidine nucleotide biosynthesis ; GO:0006221
MultiFun:1.5.2.3 Purine ribonucleotide biosynthesis > GO:purine ribonucleotide biosynthesis ; GO:0009152
MultiFun:1.5.2.4 Pyrimidine ribonucleotide/ribonucleoside biosynthesis > GO:pyrimidine ribonucleotide biosynthesis ; GO:0009220 > GO:ribonucleoside biosynthesis ; GO:0042455
MultiFun:1.5.3 Cofactor, small molecule carrier > GO:coenzymes and prosthetic group biosynthesis ; GO:0046138

MultiFun:1.5.3.1 Biotin > GO:biotin biosynthesis ; GO:0009102
MultiFun:1.5.3.2 Folic acid > GO:folic acid biosynthesis ; GO:0046656
MultiFun:1.5.3.3 Lipoate > GO:lipoate biosynthesis ; GO:0009107
MultiFun:1.5.3.4 Molybdenum (molybdopterin) > GO:Mo-molybdopterin cofactor biosynthesis ; GO:0006777
MultiFun:1.5.3.5 Coenzyme A > GO:coenzyme A biosynthesis ; GO:0015937
MultiFun:1.5.3.6 Pyridoxine (vitamin B6) > GO:pyridoxine biosynthesis ; GO:0008615
MultiFun:1.5.3.7 Nicotinamide adenine dinucleotide (NAD) > GO:nicotinamide adenine dinucleotide biosynthesis ; GO:0009435
MultiFun:1.5.3.8 Thiamine (Vitamin B1) > GO:thiamin biosynthesis ; GO:0009228
MultiFun:1.5.3.9 Riboflavin (Vitamin B2), FAD, FMN > GO:vitamin B2 biosynthesis ; GO:0009231 > GO:FAD biosynthesis ; GO:0006747 > GO:FMN biosynthesis ; GO:0009398
MultiFun:1.5.3.10 Glutathione > GO:glutathione biosynthesis ; GO:0006750
MultiFun:1.5.3.11 Menaquinone (MK), ubiquinone (Q) > GO:vitamin K2 biosynthesis ; GO:0009234 > GO:ubiquinone biosynthesis ; GO:0006744
MultiFun:1.5.3.12 Heme, porphyrine > GO:heme biosynthesis ; GO:0006783 > GO:porphyrin biosynthesis ; GO:0006779
MultiFun:1.5.3.13 Cobalamin (Vitamin B12) > GO:vitamin B12 biosynthesis ; GO:0009236
MultiFun:1.5.3.14 Enterochelin (enterobactin) > GO:enterobactin biosynthesis ; GO:0009239
MultiFun:1.5.3.15 Chrysobactin > GO:chrysobactin biosynthesis ; GO:0042858
MultiFun:1.5.3.16 Achromobactin > GO:achromobactin biosynthesis ; GO:0042861
MultiFun:1.5.3.19 Isoprenoid biosynthesis > GO:isoprenoid biosynthesis ; GO:0008299
MultiFun:1.5.3.20 Tetrahydrobiopterin biosynthesis > GO:tetrahydrobiopterin biosynthesis ; GO:0006729
MultiFun:1.5.3.21 Pyochelin biosynthesis > GO:pyochelin biosynthesis ; GO:0042864
MultiFun:1.5.4 Fatty acid and phosphatidic acid > GO:fatty acid biosynthesis ; GO:0006633 ; GO:phosphatidic acid biosynthesis ; GO:0006654
MultiFun:1.6 Macromolecules (cellular constituent) biosynthesis > GO:macromolecule biosynthesis ; GO:0009059
MultiFun:1.6.1 Phospholipid > GO:phospholipid biosynthesis ; GO:0008654
MultiFun:1.6.2 Colanic acid (M antigen) > GO:colanic acid biosynthesis ; GO:0009242
MultiFun:1.6.3 Lipopolysaccharide > GO:lipopolysaccharide biosynthesis ; GO:0009103
MultiFun:1.6.3.1 O antigen > GO:O antigen biosynthesis ; GO:0009243
MultiFun:1.6.3.2 Core region > GO:lipopolysaccharide core region biosynthesis ; GO:0009244
MultiFun:1.6.3.3 Lipid A > GO:lipid A biosynthesis ; GO:0009245

MultiFun:1.6.4 Enterobacterial common antigen (surface glycolipid) > GO:enterobacterial common antigen biosynthesis ; GO:0009246
MultiFun:1.6.5 K antigen > GO:K antigen biosynthesis ; GO:0009248
MultiFun:1.6.6 Osmoregulated periplasmic glucan > GO:glucan biosynthesis ; GO:0009250
MultiFun:1.6.7 Peptidoglycan (murein) > GO:peptidoglycan biosynthesis ; GO:0009252
MultiFun:1.6.9 Polysaccharides, cytoplasmic > GO:polysaccharide biosynthesis ; GO:0000271
MultiFun:1.6.10 Lipoprotein > GO:lipoprotein biosynthesis ; GO:0042158
MultiFun:1.6.11 Glycoprotein > GO:glycoprotein biosynthesis ; GO:0009101
MultiFun:1.6.12 Flagella > GO:flagella biogenesis ; GO:0009296
MultiFun:1.6.13 Fimbria, pili, curli > GO:fimbrial biogenesis ; GO:0009297
MultiFun:1.6.15 Large molecule carriers > GO:protein biosynthesis ; GO:0006412
MultiFun:1.6.15.1 Cytochromes > GO:cytochrome biogenesis ; GO:0017004
MultiFun:1.6.15.2 Thioredoxin, glutaredoxin > GO:thioredoxin biosynthesis ; GO:0042964 > glutaredoxin biosynthesis ; GO:0042965
MultiFun:1.6.15.3 Biotin carboxyl carrier protein > GO:biotin carboxyl carrier protein biosynthesis ; GO:0042966
MultiFun:1.6.15.4 Acyl carrier protein > GO:acyl carrier protein biosynthesis ; GO:0042967
MultiFun:1.6.16 Cellulose biosynthesis > GO:cellulose biosynthesis ; GO:0030244
MultiFun:1.7 Central intermediary metabolism > GO:.
MultiFun:1.7.1 Unassigned reversible reactions > GO:.
MultiFun:1.7.2 Glyoxylate bypass > GO:glyoxylate cycle ; GO:0006097
MultiFun:1.7.3 Pentose phosphate shunt, non-oxidative branch > GO:pentose-phosphate shunt\, non-oxidative branch ; GO:0009052
MultiFun:1.7.6 Glycerol metabolism > GO:glycerol metabolism ; GO:0006071
MultiFun:1.7.7 Galactose metabolism > GO:galactose metabolism ; GO:0006012
MultiFun:1.7.8 Gluconeogenesis > GO:gluconeogenesis ; GO:0006094
MultiFun:1.7.9 Misc. glucose metabolism > GO:glucose metabolism ; GO:0006006
MultiFun:1.7.10 Sugar nucleotide biosynthesis, conversions > GO:nucleotide-sugar biosynthesis ; GO:0009226
MultiFun:1.7.12 Amino sugar conversions > GO:amino sugar biosynthesis ; GO:0046349
MultiFun:1.7.13 Amino acid conversion > GO:amino acid metabolism ; GO:0006520
MultiFun:1.7.14 Polyamine biosynthesis > GO:polyamine biosynthesis ; GO:0006596
MultiFun:1.7.15 2'-deoxyribonucleotide/ribonucleoside metabolism > GO:2'-deoxyribonucleotide biosynthesis ; GO:0009265
MultiFun:1.7.17 Formyl-tetrahydrofolate biosynthesis > GO:10-formyltetrahydrofolate biosynthesis ; GO:0009257

MultiFun:1.7.18 Betaine biosynthesis > GO:betaine biosynthesis ; GO:0006578
MultiFun:1.7.19 Incorporation of metal ions > GO:.
MultiFun:1.7.20 S-adenosyl methionine biosynthesis > GO:S-adenosylmethionine biosynthesis ; GO:0006556
MultiFun:1.7.21 Glyoxylate degradation > GO:glyoxylate catabolism ; GO:0009436
MultiFun:1.7.22 Carnitine metabolism > GO:carnitine catabolism ; GO:0042413
MultiFun:1.7.23 Methylglyoxal metabolism > GO:methylglyoxal metabolism ; GO:0009438
MultiFun:1.7.24 Cyanate catabolism > GO:cyanate catabolism ; GO:0009440
MultiFun:1.7.25 Glycolate metabolism > GO:glycolate metabolism ; GO:0009441
MultiFun:1.7.26 Allantoin assimilation > GO:allantoin assimilation ; GO:0009442
MultiFun:1.7.27 Pyridoxal 5'-phosphate salvage > GO:pyridoxal 5'-phosphate salvage ; GO:0009443
MultiFun:1.7.28 Pyruvate catabolism > GO:pyruvate catabolism ; GO:0042867
MultiFun:1.7.29 Acetate catabolism > GO:acetate catabolism ; GO:0045733
MultiFun:1.7.31 Aminobutyrate catabolism > GO:aminobutyrate catabolism ; GO:0009450
MultiFun:1.7.32 Putrescine catabolism > GO:putrescine catabolism ; GO:0009447
MultiFun:1.7.33 Nucleotide and nucleoside conversions > GO:nucleobase\, nucleoside and nucleotide interconversion ; GO:0015949
MultiFun:1.7.34 Peptidoglycan (murein) turnover, recycling > GO:peptidoglycan metabolism ; GO:0000270
MultiFun:1.7.35 Lactate oxidation > GO:lactate oxidation ; GO:0019516
MultiFun:1.7.36 Trehalose biosynthesis > GO:trehalose biosynthesis ; GO:0005992
MultiFun:1.7.37 C1 assimilation, serine pathway > GO:serine-isocitrate lyase pathway ; GO:0019496
MultiFun:1.7.38 Methionine salvage pathway > GO:methionine salvage pathway ; GO:0019509
MultiFun:1.8 Metabolism of other compounds > GO:.
MultiFun:1.8.1 Phosphorous metabolism > GO:phosphorus metabolism ; GO:0006793
MultiFun:1.8.2 Sulfur metabolism > GO:sulfur metabolism ; GO:0006790
MultiFun:1.8.3 Nitrogen metabolism > GO:nitrogen metabolism ; GO:0006807
MultiFun:2 Information transfer > GO:.
MultiFun:2.1 DNA related > GO:DNA metabolism ; GO:0006259
MultiFun:2.1.1 DNA replication > GO:DNA dependent DNA replication ; GO:0006261
MultiFun:2.1.2 DNA restriction/modification > GO:DNA modification ; GO:0006304 > GO:DNA restriction ; GO:0009307
MultiFun:2.1.3 DNA recombination > GO:DNA recombination ; GO:0006310
MultiFun:2.1.4 DNA repair > GO:DNA repair ; GO:0006281
MultiFun:2.1.5 DNA degradation > GO:DNA catabolism ; GO:0006308

MultiFun:2.2 RNA related > GO:RNA metabolism ; GO:0016070
MultiFun:2.2.2 Transcription related > GO:transcription ; GO:0006350
MultiFun:2.2.3 RNA modification > GO:RNA modification ; GO:0009451
MultiFun:2.2.4 RNA degradation > GO:RNA catabolism ; GO:0006401
MultiFun:2.2.5 tRNA > GO:tRNA metabolism ; GO:0006399
MultiFun:2.2.6 rRNA, stable RNA > GO:rRNA metabolism ; GO:0016072
MultiFun:2.2.7 Antisense RNA > GO:antisense RNA metabolism ; GO:0042868
MultiFun:2.3 Protein related > GO:protein biosynthesis ; GO:0006412
MultiFun:2.3.1 Amino acid-activation > GO:amino acid activation ; GO:0006418
MultiFun:2.3.2 Translation > GO:protein biosynthesis ; GO:0006412
MultiFun:2.3.3 Posttranslational modification > GO:protein modification ; GO:0006464
MultiFun:2.3.4 Chaperoning, folding > GO:protein folding ; GO:0006457
MultiFun:2.3.5 Export, signal peptide cleavage > GO:protein targeting ; GO:0006605 > GO:intracellular protein transport ; GO:0006886 > GO:protein processing ; GO:0016485
MultiFun:2.3.6 Turnover, degradation > GO:proteolysis and peptidolysis ; GO:0006508
MultiFun:2.3.7 Nucleoproteins, basic proteins > GO:.
MultiFun:2.3.8 Ribosomal proteins > GO:structural constituent of ribosome ; GO:0003735
MultiFun:2.3.9 Non-ribosomal peptide synthetase > GO:nonribosomal peptide biosynthesis ; GO:0019184
MultiFun:3 Regulation > GO:.
MultiFun:3.1. Type of regulation > GO:.
MultiFun:3.1.1 DNA structure level > GO:.
MultiFun:3.1.1.1 DNA bending, supercoiling, inversion > GO:DNA bending activity ; GO:0008301 > GO:DNA supercoiling activity ; GO:0009387
MultiFun:3.1.1.2 Methylation > GO:DNA methylation ; GO:0006306
MultiFun:3.1.2 Transcriptional level > GO:regulation of transcription\, DNA-dependent ; GO:0006355
MultiFun:3.1.2.1 Sigma factors, anti-sigmafactors > GO:sigma factor activity ; GO:0016987 > GO:sigma factor antagonist activity ; GO:0016989
MultiFun:3.1.2.2 Activator > GO:transcriptional activator activity ; GO:0016563
MultiFun:3.1.2.3 Repressor > GO:transcriptional repressor activity ; GO:0016564
MultiFun:3.1.2.4 Complex regulation > GO:.
MultiFun:3.1.2.4.1 More than one signal needed > GO:.

MultiFun:3.1.2.4.2 Regulons or multilayer component regulatory systems > GO:.
MultiFun:3.1.2.4.3 Two-component regulatory systems (external signal) > GO:two-component sensor molecule activity ; GO:0000155 > GO:two-component response regulator activity ; GO:0000156
MultiFun:3.1.2.4.4 Quorum sensing > GO:quorum sensing response regulator activity ; GO:0009370 > GO:quorum sensing signal generator activity ; GO:0009369 > GO:quorum sensing ; GO:0009372
MultiFun:3.1.2.5 Action unknown > GO:.
MultiFun:3.1.3 Posttranscriptional > GO:.
MultiFun:3.1.3.1 Translation attenuation and efficiency > GO:translational attenuation ; GO:0009386
MultiFun:3.1.3.2 Covalent modification, demodification, maturation > GO:protein modification ; GO:0006464 > GO:protein processing ; GO:0016485
MultiFun:3.1.3.3 Inhibition / activation of enzymes > GO:enzyme inhibitor activity ; GO:0004857 > GO:enzyme activator activity ; GO:0008047
MultiFun:3.1.3.4 Proteases, cleavage of compounds > GO:peptidase activity ; GO:0008233
MultiFun:3.1.3.5 Multilayer regulatory systems > GO:.
MultiFun:3.1.3.6 Antisense RNA > GO:RNA interference ; GO:0016246
MultiFun:3.1.4 Regulation level unknown > GO:.
MultiFun:3.3 Genetic unit regulated > GO:.
MultiFun:3.3.1 Operon (regulation of one operon) > GO:.
MultiFun:3.3.2 Regulon (a network of operons encoding related functions) > GO:.
MultiFun:3.3.3 Stimulon (ie. environmental stimulus) > GO:.
MultiFun:3.3.4 Global > GO:.
MultiFun:3.4 Trigger (some information added) > GO:.
MultiFun:3.5 Trigger modulation (some information added) > GO:.
MultiFun:4 Transport > GO:transporter activity ; GO:0005215 > GO:transport ; GO:0006810
MultiFun:4.1 Channel-type Transporters > GO:channel/pore class transporter activity ; GO:0015267
MultiFun:4.1.A. alpha-type channels > GO:.
MultiFun:4.1.A.1. The Voltage-gated Ion Channel (VIC) Superfamily > GO:voltage-gated ion channel activity ; GO:0005244
MultiFun:4.2 Electrochemical potential driven transporters> electrochemical potential-driven transporter activity ; GO:0015290
MultiFun:4.2.A. Porters (Uni-, Sym- and Antiporters) > GO:symporter activity ; GO:0015293 > GO:antiporter activity ; GO:0015297 > GO:uniporter activity ; GO:0015292
MultiFun:4.2.C Ion-gradient driven energizers > GO:ion-gradient-driven energizer activity ; GO:0015404

MultiFun:4.3 Primary Active Transporters > GO:porter activity ; GO:0015291
MultiFun:4.3.A. Pyrophosphate Bond (ATP; GTP; P2) Hydrolysis-driven Active Transporters > GO:ATPase activity ; GO:0016887
MultiFun:4.3.A.1 The ATP-binding Cassette (ABC) Superfamily + ABC-type Uptake Permeases > GO:ATP-binding cassette (ABC) transporter activity ; GO:0004009
MultiFun:4.3.A.2 The H+/Na+-translocating F-, V- and A-type ATPase (F-ATPase) Superfamily > GO:hydrogen-translocating F-type ATPase complex (sensu Bacteria) ; GO:0045256 > GO:hydrogen-translocating V-type ATPase complex ; GO:0016471
MultiFun:4.3.A.3 The P-type ATPase (P-ATPase) Superfamily > GO:ATPase activity\, coupled to transmembrane movement of ions\, phosphorylative mechanism ; GO:0015662
MultiFun:4.3.A.4 The Arsenite-Antimonite (Ars) Efflux Family > GO:arsenite-transporting ATPase activity ; GO:0015446 > GO:antimonite-transporting ATPase activity ; GO:0042961 > GO:antimonite transport ; GO:0015699 > GO:arsenite transport ; GO:0015700
MultiFun:4.3.A.5 The Type II (General) Secretory Pathway (IISP) Family > GO:type II protein (Sec) secretion system ; GO:0015628 > GO:type II protein secretor activity ; GO:0015447
MultiFun:4.3.A.6 The Type III (Virulence-related) Secretory Pathway (IIISP) Family > GO:type III protein secretion system ; GO:0030254 > GO:type III protein (virulence-related) secretor activity ; GO:0015448
MultiFun:4.3.A.7 The Type IV (Conjugal DNA-Protein Transfer) Secretory Pathway (IVSP) Family > GO:type IV protein secretion system ; GO:0030255 > GO:type IV protein (DNA-protein) secretor activity ; GO:0015449
MultiFun:4.3.D. Oxidoreduction-driven Active Transporters > GO:oxidoreduction-driven active transporter activity ; GO:0015453
MultiFun:4.4 Group Translocators > GO:group translocator activity ; GO:0015455
MultiFun:4.4.A Phosphotransferase Systems (PEP-dependent PTS) > GO:phosphoenolpyruvate-dependent sugar phosphotransferase system ; GO:0009401
MultiFun:4.8.A Accessory Factors Involved in Transport > GO:.
MultiFun:4.9.A Transporters of Unknown Classification > GO:transporter activity ; GO:0005215 > GO:transport ; GO:0006810
MultiFun:4.9.A Recognized transporters of unknown biochemical mechanism > GO:transporter activity ; GO:0005215 > GO:transport ; GO:0006810
MultiFun:4.9.A.1 The Polysaccharide Transporter (PST) Family > GO:polysaccharide transporter activity ; GO:0015159 > GO:polysaccharide transport ; GO:0015774
MultiFun:4.9.A.4 The Nicotinamide Mononucleotide (NMN) Uptake Permease (PnuC) Family > GO:nicotinamide mononucleotide transporter activity ; GO:0015663 > GO:nicotinamide mononucleotide transport ; GO:0015890
MultiFun:4.9.A.8 The Ferrous Iron Uptake (FeoB) Family > GO:ferrous iron transporter activity ; GO:0015093 > GO:ferrous iron transport ; GO:0015684

MultiFun:4.9.A.13 The Short Chain Fatty Acid Transporter (scFAT) Family > GO:short-chain fatty acid transporter activity ; GO:0015635 > GO:short-chain fatty acid transport ; GO:0015912
MultiFun:4.9.A.16 The Septal DNA Translocator (SDT) Family > GO:.
MultiFun:4.9.A.17 The Metal Ion Transporter (MIT) Family > GO:metal ion transporter activity ; GO:0046873 > GO:metal ion transport ; GO:0030001
MultiFun:4.9.B Putative uncharacterized transport protein > GO:transporter activity ; GO:0005215 > GO:transport ; GO:0006810
MultiFun:4.S substrate > GO:transporter activity ; GO:0005215 > GO:transport ; GO:0006810
MultiFun:4.S.1 (D)-glucarate/galactarate > GO:D-glucarate transport ; GO:0042871 > GO:D-galactarate transporter activity ; GO:0042877 > GO:D-glucarate transporter activity ; GO:0042878
MultiFun:4.S.10 allose/ribose > GO:allose transport ; GO:0015754 > GO:ribose transport ; GO:0015752 > GO:allose transporter activity ; GO:0015593 > GO:ribose transporter activity ; GO:0015591
MultiFun:4.S.100 L-arabinose > GO:L-arabinose transport ; GO:0042882 > GO:L-arabinose transporter activity ; GO:0015147
MultiFun:4.S.101 L-arabinose /H+ > GO:L-arabinose/beta-D-thiogalactopyranoside\:hydrogen antiporter activity ; GO:0015524
MultiFun:4.S.102 L-arabinose/ isopropyl-beta-D-thiogalactopyranoside > GO:L-arabinose/beta-D-thiogalactopyranoside\:hydrogen antiporter activity ; GO:0015524
MultiFun:4.S.103 L-asparagine > GO:asparagine transport ; GO:0006867 > GO:asparagine transporter activity ; GO:0015182
MultiFun:4.S.104 lipooligosaccharides > GO:lipopolysaccharide transport ; GO:0015920 > GO:lipopolysaccharide transporter activity ; GO:0015221
MultiFun:4.S.105 lipopolysaccharide > GO:lipopolysaccharide transport ; GO:0015920 > GO:lipopolysaccharide transporter activity ; GO:0015221
MultiFun:4.S.106 lipoprotein > GO:lipoprotein transport ; GO:0042953 ; lipoprotein transporter activity ; GO:0042954
MultiFun:4.S.107 L-lactate > GO:lactate transport ; GO:0015727 > GO:lactate transporter activity ; GO:0015129
MultiFun:4.S.108 L-leucine/L-valine/L-iso-leucine > GO:isoleucine transport ; GO:0015818 > GO:leucine transport ; GO:0015820 > GO:valine transport ; GO:0015829 > GO:leucine/isoleucine/valine porter activity ; GO:0015602
MultiFun:4.S.109 L-rhamnose/H+ > GO:rhamnose transport ; GO:0015762 > GO:rhamnose transporter activity ; GO:0015153
MultiFun:4.S.11 alpha-ketoglutarate > GO:alpha-ketoglutarate transport ; GO:0015742 > GO:alpha-ketoglutarate transporter activity ; GO:0015139
MultiFun:4.S.110 L-threonine/L-serine > GO:threonine transport ; GO:0015826 > GO:serine transport ; GO:0015825 > GO:threonine transporter activity ; GO:0015195 > GO:serine transporter activity ; GO:0015194
MultiFun:4.S.111 lysine > GO:lysine transport ; GO:0015819 > GO:lysine transporter activity ; GO:0015189
MultiFun:4.S.112 lysine/arginine/ornithine > GO:histidine/arginine/lysine/ornithine porter activity ; GO:0015597

MultiFun:4.S.113 maltose > GO:maltose transporter activity ; GO:0005363 > GO:maltose transport ; GO:0015768
MultiFun:4.S.114 maltose/maltodextrin > maltodextrin transport ; GO:0042956 > GO:maltodextrin transporter activity ; GO:0042958 > GO:maltose transporter activity ; GO:0005363 > GO:maltose transport ; GO:0015768
MultiFun:4.S.115 mannitol > GO:mannitol transport ; GO:0015797 > GO:mannitol transporter activity ; GO:0015575
MultiFun:4.S.116 mannose > GO:mannose transport ; GO:0015761 > GO:mannose transporter activity ; GO:0015578
MultiFun:4.S.117 melibiose > GO:melibiose transporter activity ; GO:0015156 > GO:melibiose transport ; GO:0015769
MultiFun:4.S.118 methionine > GO:methionine transport ; GO:0015821 > GO:methionine transporter activity ; GO:0015191
MultiFun:4.S.119 methylgalactoside/galactose > GO:galactose transporter activity ; GO:0005354 > GO:galactose transporter activity ; GO:0005354 > GO:methylgalactoside transporter activity ; GO:0015592 > GO:methylgalactoside transport ; GO:0015765
MultiFun:4.S.12 amino acid > GO:amino acid transporter activity ; GO:0015171 > GO:amino acid transport ; GO:0006865
MultiFun:4.S.120 Mg++ > GO:magnesium ion transport ; GO:0015693 > GO:magnesium ion transporter activity ; GO:0015095
MultiFun:4.S.121 Mg2+/Ni2+/Co2+ > GO:nickel ion transport ; GO:0015675 > GO:cobalt ion transport ; GO:0006824 > GO:magnesium ion transport ; GO:0015693 > GO:nickel ion transporter activity ; GO:0015099 > GO:cobalt ion transporter activity ; GO:0015087 > GO:magnesium ion transporter activity ; GO:0015095
MultiFun:4.S.122 microcin B17 > GO:microcin uptake permease activity ; GO:0015638 > GO:microcin B17 transport ; GO:0042885
MultiFun:4.S.123 Mn+/H+ > GO:manganese ion transporter activity ; GO:0005384 > GO:manganese ion transport ; GO:0006828
MultiFun:4.S.124 molybdate > GO:molybdate ion transport ; GO:0015689 > GO:molybdate ion transporter activity ; GO:0015098
MultiFun:4.S.125 molybdenum > GO:molybdenum ion transporter activity ; GO:0042888
MultiFun:4.S.126 multidrug > GO:multidrug transporter activity ; GO:0015239 > GO:multidrug transport ; GO:0006855
MultiFun:4.S.127 multidrug/bicyclomycin > GO:bicyclomycin transport ; GO:0015905 > GO:bicyclomycin transporter activity ; GO:0015545
MultiFun:4.S.128 muropeptide > GO:peptidoglycan peptide transporter activity ; GO:0015640 > GO:muropeptide transport ; GO:0015834
MultiFun:4.S.129 myo-inositol > GO:myo-inositol transport ; GO:0015798 > GO:myo-inositol transporter activity ; GO:0005365
MultiFun:4.S.13 amino acid/amide > GO:amide transport ; GO:0042886
MultiFun:4.S.130 Na+ > GO:sodium ion transporter activity ; GO:0015081 > GO:sodium ion transport ; GO:0006814
MultiFun:4.S.131 Na+/ alanine/glycine > GO:alanine transport ; GO:0015808 > GO:glycine transport ; GO:0015816 > GO:alanine transporter activity ; GO:0015180 > GO:glycine transporter activity ; GO:0015187
MultiFun:4.S.132 Na+/ H+ > GO:sodium\:hydrogen antiporter activity ; GO:0015385
MultiFun:4.S.133 Na+/Ca+ > GO:calcium:sodium antiporter activity ; GO:0005432
MultiFun:4.S.134 Na+/dicarboxylate > GO:sodium\:dicarboxylate/tricarboxylate symporter activity ; GO:0005311

MultiFun:4.S.135 Na+/glutamate/aspartate > GO:glutamate/aspartate\:sodium symporter activity ; GO:0015372 > GO:L-glutamate transport ; GO:0015813 > GO:aspartate transport ; GO:0015810
MultiFun:4.S.136 Na+/H+
MultiFun:4.S.137 Na+/leucine/valine/iso-leucine > GO:isoleucine transport ; GO:0015818 > GO:leucine transport ; GO:0015820 > GO:valine transport ; GO:0015829 > GO:leucine/isoleucine/valine porter activity ; GO:0015602
MultiFun:4.S.138 Na+/pantothenate > GO:pantothenate transport ; GO:0015887 > GO:pantothenate\:sodium symporter activity ; GO:0015498
MultiFun:4.S.139 Na+/proline > GO:proline:sodium symporter activity ; GO:0005298
MultiFun:4.S.14 ammonium > GO:ammonium transport ; GO:0015696 > GO:ammonium transporter activity ; GO:0008519
MultiFun:4.S.140 Na+/serine/threonine > GO:threonine transport ; GO:0015826 > GO:serine transport ; GO:0015825 > GO:threonine/serine\:sodium symporter activity ; GO:0015500
MultiFun:4.S.141 N-acetylgalactosamine > GO:N-acetylgalactosamine transport ; GO:0015763 > GO:N-acetylgalactosamine transporter activity ; GO:0015571
MultiFun:4.S.142 N-acetylglucosamine > GO:N-acetylglucosamine transport ; GO:0015764 > GO:N-acetylglucosamine transporter activity ; GO:0015572
MultiFun:4.S.143 Ni++ > GO:nickel ion transport ; GO:0015675 > GO:nickel ion transporter activity ; GO:0015099
MultiFun:4.S.144 nicotinamide mononucleotide > GO:nicotinamide mononucleotide transport ; GO:0015890 > GO:nicotinamide mononucleotide transporter activity ; GO:0015663
MultiFun:4.S.145 nitrite > GO:nitrite transport ; GO:0015707 > GO:nitrite transporter activity ; GO:0015113
MultiFun:4.S.146 nucleoside > GO:nucleoside transport ; GO:0015858 > GO:nucleoside transporter activity ; GO:0005337 > GO:nucleoside transport ; GO:0015858
MultiFun:4.S.147 nucleoside/H+ > GO:nucleoside transport ; GO:0015858 > GO:nucleoside transporter activity ; GO:0005337 > GO:nucleoside transport ; GO:0015858
MultiFun:4.S.148 oligopeptide > GO:oligopeptide transport ; GO:0006857 > GO:oligopeptide transporter activity ; GO:0015198
MultiFun:4.S.15 antibiotic > GO:antibiotic transport ; GO:0042891 > GO:antibiotic transporter activity ; GO:0042895
MultiFun:4.S.150 p-aminobenzoyl-glutamate > GO:p-aminobenzoyl-glutamate transport ; GO:0015814 > GO:p-aminobenzoyl-glutamate transporter activity ; GO:0015569
MultiFun:4.S.151 Pb/Cd/Zn/Hg > GO:lead ion transport ; GO:0015692 > GO:lead ion transporter activity ; GO:0015094 > GO:zinc ion transport ; GO:0006829 > GO:zinc ion transporter activity ; GO:0005385 > GO:cadmium ion transport ; GO:0015691 > GO:cadmium ion transporter activity ; GO:0015086 > GO:zinc ion transport ; GO:0006829 > GO:zinc ion transporter activity ; GO:0005385 > GO:mercury ion transport ; GO:0015694 > GO:mercury ion transporter activity ; GO:0015097

MultiFun:4.S.152 peptide > GO:peptide transport ; GO:0015833 > GO:peptide transporter activity ; GO:0015197
MultiFun:4.S.153 phenylalanine > GO:phenylalanine transport ; GO:0015823 > GO:phenylalanine transporter activity ; GO:0015192
MultiFun:4.S.154 phenylalanine/ tyrosine > GO:aromatic amino acid transport ; GO:0015801 > GO:aromatic amino acid transporter activity ; GO:0015173
MultiFun:4.S.155 phosphate > GO:phosphate transport ; GO:0006817 > GO:phosphate transporter activity ; GO:0015114
MultiFun:4.S.156 polymyxin > GO:polymyxin transport ; GO:0042893 > GO:polymyxin transporter activity ; GO:0042897
MultiFun:4.S.158 proline > GO:proline transport ; GO:0015824 > GO:proline transporter activity ; GO:0015193
MultiFun:4.S.159 proline/betaine > GO:betaine transport ; GO:0015838 > GO:proline transport ; GO:0015824 > GO:glycine betaine/proline porter activity ; GO:0015596
MultiFun:4.S.16 arabinose polymer > GO:arabinose polymer transport ; GO:0042899 > GO:arabinose polymer transporter activity ; GO:0042900
MultiFun:4.S.160 protein > GO:protein transport ; GO:0015031 > GO:protein transporter activity ; GO:0008565
MultiFun:4.S.161 protein/DNA > GO:DNA-protein complex transport ; GO:0015869 > GO:DNA-protein complex transporter activity ; GO:0015219
MultiFun:4.S.162 purine/xanthine > GO:purine transport ; GO:0006863 > GO:purine transporter activity ; GO:0005345
MultiFun:4.S.163 putrescine > GO:putrescine transport ; GO:0015847
MultiFun:4.S.164 putrescine/ornithine > GO:putrescine\:ornithine antiporter activity ; GO:0015496
MultiFun:4.S.166 putrescine/spermidine > GO:putrescine transport ; GO:0015847 > GO:spermidine transport ; GO:0015848 > GO:spermidine transporter activity ; GO:0015606 > GO:putrescine transporter activity ; GO:0015489
MultiFun:4.S.167 serine > GO:serine transport ; GO:0015825 > GO:serine transporter activity ; GO:0015194
MultiFun:4.S.168 shikimate/dehydroshikimate > GO:shikimate transporter activity ; GO:0015530 > GO:shikimate transport ; GO:0015733
MultiFun:4.S.169 sialic acid > GO:sialic acid transport ; GO:0015739 > GO:sialic acid transporter activity ; GO:0015136
MultiFun:4.S.17 arginine > GO:arginine transport ; GO:0015809 > GO:arginine transporter activity ; GO:0015181
MultiFun:4.S.170 S-methylmethionine > GO:S-methylmethionine transport ; GO:0015806 > GO:S-methylmethionine transporter activity ; GO:0000100
MultiFun:4.S.172 sucrose > GO:sucrose transporter activity ; GO:0008515 > GO:sucrose transport ; GO:0015770
MultiFun:4.S.173 sugar > GO:carbohydrate transport ; GO:0008643 > GO:carbohydrate transporter activity ; GO:0015144
MultiFun:4.S.174 sulfate > GO:sulfate transport ; GO:0008272 > GO:sulfate transporter activity ; GO:0015116
MultiFun:4.S.175 taurine > GO:taurine transport ; GO:0015734 > GO:taurine transporter activity ; GO:0005368
MultiFun:4.S.176 tellurite > GO:tellurite transport ; GO:0015710 > GO:tellurite transporter activity ; GO:0015118

MultiFun:4.S.177 thiamine > GO:thiamin transport ; GO:0015888 > GO:thiamin transporter activity ; GO:0015234
MultiFun:4.S.178 thiosulfate > GO:thiosulfate transport ; GO:0015709 > GO:sulfate transport ; GO:0008272 > GO:thiosulfate transporter activity ; GO:0015117
MultiFun:4.S.179 thiosulfate/sulfate > GO:sulfate/thiosulfate porter activity ; GO:0015419
MultiFun:4.S.18 arginine/ornithine > GO:L-ornithine transport ; GO:0015822 > GO:arginine transport ; GO:0015809
MultiFun:4.S.180 threonine > GO:threonine transport ; GO:0015826 > GO:threonine transporter activity ; GO:0015195
MultiFun:4.S.181 trehalose > GO:trehalose transport ; GO:0015771 > GO:trehalose transporter activity ; GO:0015574
MultiFun:4.S.182 tripeptide > GO:tripeptide transport ; GO:0042939 > GO:tripeptide transporter activity ; GO:0042937
MultiFun:4.S.183 tryptophan > GO:tryptophan transport ; GO:0015827 > GO:tryptophan transporter activity ; GO:0015196
MultiFun:4.S.184 tyrosine > GO:tyrosine transport ; GO:0015828 > GO:tyrosine transporter activity ; GO:0005302
MultiFun:4.S.185 uracil > GO:uracil transport ; GO:0015857 > GO:uracil transporter activity ; GO:0015210
MultiFun:4.S.187 vitamin B12 > GO:vitamin B12 transport ; GO:0015889 > GO:vitamin B12 transporter activity ; GO:0015235
MultiFun:4.S.188 water > GO:water transport ; GO:0006833 > GO:water transporter activity ; GO:0005372
MultiFun:4.S.189 xanthosine > GO:xanthosine transport ; GO:0015863 > GO:xanthosine transporter activity ; GO:0015553
MultiFun:4.S.19 arsenite > GO:arsenite transport ; GO:0015700 > GO:arsenite transporter activity ; GO:0015105
MultiFun:4.S.190 xylose/H+ > GO:D-xylose transport ; GO:0015753
MultiFun:4.S.191 Zn > GO:zinc ion transport ; GO:0006829 > GO:zinc ion transporter activity ; GO:0005385
MultiFun:4.S.192 chrysobactin > GO:chrysobactin transport ; GO:0042932 > GO:chrysobactin transporter activity ; GO:0042933
MultiFun:4.S.193 achromobactin > GO:achromobactin transport ; GO:0042935 > GO:achromobactin transporter activity ; GO:0042934
MultiFun:4.S.2 2-keto-3-deoxy-D-gluconate > GO:2-keto-3-deoxygluconate transport ; GO:0046411 > GO:2-keto-3-deoxygluconate\:hydrogen symporter activity ; GO:0015649
MultiFun:4.S.20 benzoate > GO:benzoate transport ; GO:0042919 > GO:benzoate transporter activity ; GO:0042925
MultiFun:4.S.21 lactose/glucose > GO:lactose/glucose efflux transporter activity ; GO:0015543
MultiFun:4.S.22 beta-glucoside > GO:beta-glucoside transport ; GO:0015759 > GO:beta-glucoside transporter activity ; GO:0015573
MultiFun:4.S.25 Ca+/ H+ > GO:calcium ion transport ; GO:0006816 > GO:calcium\:hydrogen antiporter activity ; GO:0015369
MultiFun:4.S.26 cadaverine/lysine > GO:lysine transport ; GO:0015819 > GO:cadaverine transport ; GO:0015839 > GO:cadaverine\:lysine antiporter activity ; GO:0015497
MultiFun:4.S.27 carnitine > GO:carnitine transport ; GO:0015879 > GO:carnitine transporter activity ; GO:0015226
MultiFun:4.S.28 cation > GO:cation transport ; GO:0006812 > GO:cation transporter activity ; GO:0008324

MultiFun:4.S.29 cellobiose/arbutin/salicin > GO:salicin transport ; GO:0042948 > GO:salicin transporter activity ; GO:0042950 > GO:cellobiose transport ; GO:0019533 > GO:cellobiose transporter activity ; GO:0019191 > GO:salicin transporter activity ; GO:0042950 > GO:salicin transporter activity ; GO:0042950
MultiFun:4.S.3 3-hydroxyphenylpropionic acid > GO:3-hydroxyphenylpropionic acid transport ; GO:0042920 > GO:3-hydroxyphenylpropionic acid transporter activity ; GO:0042926
MultiFun:4.S.31 chloramphenicol > GO:chloramphenicol transport ; GO:0042892
MultiFun:4.S.32 chloride > GO:chloride transport ; GO:0006821 > GO:chloride transporter activity ; GO:0015108
MultiFun:4.S.33 choline > GO:choline transport ; GO:0015871 > GO:choline transporter activity ; GO:0015220
MultiFun:4.S.34 citrate/succinate > GO:succinate transport ; GO:0015744 > GO:citrate transport ; GO:0015746 > GO:citrate\:succinate antiporter activity ; GO:0015515
MultiFun:4.S.35 colicin > GO:colicin transport ; GO:0042914 ; GO:colicin transport activity ; GO:0042912
MultiFun:4.S.36 Cu+ > GO:copper ion transport ; GO:0006825 > GO:copper ion transporter activity ; GO:0005375
MultiFun:4.S.37 curli subunit > GO:.
MultiFun:4.S.38 cyanate > GO:cyanate transport ; GO:0015704 > GO:cyanate transporter activity ; GO:0015110
MultiFun:4.S.39 cysteine > GO:L-cysteine transport ; GO:0042883
MultiFun:4.S.4 3-phenylpropionic acid > GO:3-phenylpropionic acid transport ; GO:0042889
MultiFun:4.S.40 cysteine/O-acetyl-L-serine/cysteine metabolites
MultiFun:4.S.41 cytosine > GO:cytosine transport ; GO:0015856 > GO:cytosine transporter activity ; GO:0015209
MultiFun:4.S.42 D-alanine/D-serine/glycine > GO:D-serine transport ; GO:0042942 > GO:D-alanine transport ; GO:0042941 > GO:D-alanine transporter activity ; GO:0042944 > GO:D-serine transporter activity ; GO:0042945
MultiFun:4.S.43 D-galactonate > GO:D-galactonate transport ; GO:0042875 > GO:D-glucuronate transporter activity ; GO:0042880
MultiFun:4.S.44 D-glucarate > GO:D-glucarate transport ; GO:0042871 > GO:D-glucarate transporter activity ; GO:0042878
MultiFun:4.S.45 D-glucose/trehalose > GO:trehalose transport ; GO:0015771 > GO:glucose transport ; GO:0015758 > GO:trehalose transporter activity ; GO:0015574 > GO:glucose transporter activity ; GO:0005355
MultiFun:4.S.46 dicarboxylate > GO:dicarboxylic acid transport ; GO:0006835 > GO:dicarboxylic acid transporter activity ; GO:0005310
MultiFun:4.S.47 dipeptide > GO:dipeptide transport ; GO:0042938 > GO:dipeptide transporter activity ; GO:0042936
MultiFun:4.S.48 D-ribose > GO:ribose transport ; GO:0015752 > GO:D-ribose transporter activity ; GO:0015591
MultiFun:4.S.49 drug > GO:drug transport ; GO:0015893 > GO:drug transporter activity ; GO:0015238
MultiFun:4.S.5 alkanesulfonate > GO:alkanesulphonate transport ; GO:0042918 > GO:alkanesulphonate transporter activity ; GO:0042959

MultiFun:4.S.50 D-xylose > GO:xylose transport ; GO:0015753 > GO:D-xylose transporter activity ; GO:0015148
MultiFun:4.S.51 enterochelin > GO:enterobactin transport ; GO:0042930 > GO:enterobactin transporter activity ; GO:0042931
MultiFun:4.S.52 fatty acid > GO:fatty acid transport ; GO:0015908 > GO:fatty acid transporter activity ; GO:0015245
MultiFun:4.S.53 Fe > GO:iron ion transport ; GO:0006826 > GO:iron ion transporter activity ; GO:0005381
MultiFun:4.S.54 Fe++ > GO:ferric iron transport ; GO:0015682 > GO:ferric iron transporter activity ; GO:0015091
MultiFun:4.S.56 ferric enterobactin > GO:ferric-enterobactin transport ; GO:0015685 > GO:ferric-enterobactin transporter activity ; GO:0015620
MultiFun:4.S.57 ferric hydroxamate > GO:ferric-hydroxamate transport ; GO:0015687 > GO:ferric-hydroxamate transporter activity ; GO:0015622
MultiFun:4.S.58 ferrichrome > GO:ferrichrome transport ; GO:0042928 > GO:ferrichrome transporter activity ; GO:0042929
MultiFun:4.S.59 formate > GO:formate transport ; GO:0015724 > GO:formate transporter activity ; GO:0015499
MultiFun:4.S.60 formate/oxalate > GO:formate transport ; GO:0015724 > GO:oxalate transport ; GO:0019532 > GO:formate transporter activity ; GO:0015499 > GO:oxalate transporter activity ; GO:0019531
MultiFun:4.S.61 fosmidomycin/H+ > GO:fosmidomycin transport ; GO:0042894 > GO:fosmidomycin transporter activity ; GO:0042898
MultiFun:4.S.63 fructose > GO:fructose transport ; GO:0015755 > GO:fructose transporter activity ; GO:0005353
MultiFun:4.S.64 fucose > GO:fucose transport ; GO:0015756 > GO:fucose transporter activity ; GO:0015150
MultiFun:4.S.65 galactitol > GO:galactitol transport ; GO:0015796 > GO:galactitol transporter activity ; GO:0015577
MultiFun:4.S.66 galactose/H+ > GO:galactose transport ; GO:0015757 > GO:galactose\:hydrogen symporter activity ; GO:0015517
MultiFun:4.S.67 gamma-aminobutyrate > GO:gamma-aminobutyrate transport ; GO:0015812 > GO:gamma-aminobutyrate transporter activity ; GO:0015185
MultiFun:4.S.68 glucitol/sorbitol > GO:glucitol transport ; GO:0015795 > GO:glucitol transporter activity ; GO:0015576
MultiFun:4.S.69 gluconate> gluconate transport ; GO:0015725 > GO:gluconate transporter activity ; GO:0015128
MultiFun:4.S.70 gluconate/L-idonate > GO:gluconate transport ; GO:0015725 > GO:L-idonate transport ; GO:0015726 > GO:gluconate transporter activity ; GO:0015128 > GO:L-idonate transporter activity ; GO:0015568
MultiFun:4.S.71 glucose > GO:glucose transport ; GO:0015758 > GO:glucose transporter activity ; GO:0005355
MultiFun:4.S.72 glucose/maltose > GO:glucose transport ; GO:0015758 > GO:maltose transport ; GO:0015768 > GO:maltose transporter activity ; GO:0005363 > GO:glucose transporter activity ; GO:0005355
MultiFun:4.S.73 glucuronide > GO:glucuronoside transport ; GO:0015779 > GO:glucuronoside transporter activity ; GO:0015164
MultiFun:4.S.74 glutamate/aminobutyric acid > GO:gamma-aminobutyrate transport ; GO:0015812 > GO:glutamate transport ; GO:0015813 > GO:gamma-aminobutyrate transporter activity ; GO:0015185 > GO:L-glutamate transporter activity ; GO:0005313

MultiFun:4.S.75 glutamate/aspartate > GO:glutamate transport ; GO:0015813 > GO:aspartate transport ; GO:0015810 > GO:glutamate transporter activity ; GO:0005313 > GO:aspartate transporter activity ; GO:0015183
MultiFun:4.S.76 glutamine > GO:glutamine transport ; GO:0006868 > GO:glutamine transporter activity ; GO:0015186
MultiFun:4.S.77 glycerol > GO:glycerol transport ; GO:0015793 > GO:glycerol transporter activity ; GO:0015168
MultiFun:4.S.78 glycerol-3-P > GO:glycerol transport ; GO:0015793 > GO:glycerol-3-phosphate transporter activity ; GO:0015169
MultiFun:4.S.79 glycine betaine choline transport > GO:betaine transport ; GO:0015838 > choline transport ; GO:0015871
MultiFun:4.S.8 alkylphosphonate > GO:alkylphosphonate transport ; GO:0042916 > GO:alkylphosphonate transporter activity ; GO:0042917
MultiFun:4.S.80 glycine betaine/proline > GO:glycine betaine/proline porter activity ; GO:0015596
MultiFun:4.S.81 group A colicins > GO:group A colicin transport ; GO:0042915 > GO:group A colicin transporter activity ; GO:0042913
MultiFun:4.S.82 H+ > GO:proton transport ; GO:0015992 > GO:hydrogen ion transporter activity ; GO:0015078
MultiFun:4.S.83 H+/acridine > GO:acridine transport ; GO:0042909 > GO:acridine:proton antiporter activity ; GO:0042962
MultiFun:4.S.84 H+/lactose/glucose > GO:lactose transport ; GO:0015767 > GO:glucose transport ; GO:0015758 > GO:lactose/glucose efflux transporter activity ; GO:0015543
MultiFun:4.S.85 heme > GO:heme transport ; GO:0015886 > GO:heme transporter activity ; GO:0015232
MultiFun:4.S.86 hexose phosphate > GO:hexose phosphate transport ; GO:0015712 > GO:hexose phosphate transporter activity ; GO:0015119
MultiFun:4.S.87 hexuronate > GO:hexuronate transport ; GO:0015736 > GO:hexuronate transporter activity ; GO:0015134
MultiFun:4.S.88 histidine > GO:histidine transport ; GO:0015817 > GO:histidine transporter activity ; GO:0005290
MultiFun:4.S.89 histidine/lysine/arginine/ornithine > GO:histidine transport ; GO:0015817 > GO:lysine transport ; GO:0015819 > GO:arginine transport ; GO:0015809 > GO:ornithine transport ; GO:0015822 > GO:histidine/arginine/lysine/ornithine porter activity ; GO:0015597
MultiFun:4.S.9 allantoin > GO:allantoin transport ; GO:0015720 > GO:allantoin transporter activity ; GO:0015206
MultiFun:4.S.90 homoserine/lactone > GO:homoserine transport ; GO:0042968 > GO:homoserine transporter activity ; GO:0042970 > GO:lactone transport ; GO:0042969 > GO:lactone transporter activity ; GO:0042971
MultiFun:4.S.91 hydrophilic molecule > GO:.
MultiFun:4.S.92 hydrophilic molecules > GO:.
MultiFun:4.S.93 ion > GO:ion transport ; GO:0006811 > GO:ion transporter activity ; GO:0015075
MultiFun:4.S.95 iron dicitrate > GO:iron chelate transport ; GO:0015688 > GO:iron chelate-transporting ATPase activity ; GO:0015623

MultiFun:4.S.96 K+ > GO:potassium ion transport ; GO:0006813 > GO:potassium ion transporter activity ; GO:0015079
MultiFun:4.S.97 K+/H+ > GO:potassium\:hydrogen antiporter activity ; GO:0015386
MultiFun:4.S.98 lactate > GO:lactate transport ; GO:0015727 > GO:lactate transporter activity ; GO:0015129
MultiFun:4.S.99 lactose > GO:lactose transport ; GO:0015767 > GO:lactose transporter activity ; GO:0015155
MultiFun:5 Cell processes > GO:cellular process ; GO:0009987
MultiFun:5.1 Cell division > GO:cytokinesis ; GO:0000910
MultiFun:5.2 Cell cycle physiology > GO:cell cycle ; GO:0007049
MultiFun:5.3 Motility (incl. chemotaxis, energytaxis, aerotaxis, redoxtaxis) > GO:taxis ; GO:0042330
MultiFun:5.4 Genetic exchange, recombination > GO:DNA recombination ; GO:0006310
MultiFun:5.5 Adaptation to stress > GO:response to stress ; GO:0006950
MultiFun:5.5.1 Osmotic pressure > GO:response to osmotic stress ; GO:0006970
MultiFun:5.5.2 Temperature extremes > GO:response to temperature ; GO:0009266
MultiFun:5.5.3 Starvation response > GO:response to starvation ; GO:0042594
MultiFun:5.5.4 pH response > GO:response to pH ; GO:0009268
MultiFun:5.5.5 Dessication > GO:response to dessication ; GO:0009269
MultiFun:5.5.6 Other stresses (mechanical, nutritional, oxidative) > GO:response to mechanical stimulus ; GO:0009612 > GO:response to nutrients ; GO:0007584 > GO:response to oxidative stress ; GO:0006979
MultiFun:5.5.7 Fe aquisition > GO:iron ion transport ; GO:0006826
MultiFun:5.6 Protection > GO:response to stress ; GO:0006950
MultiFun:5.6.1 Radiation > GO:response to radiation ; GO:0009314
MultiFun:5.6.2 Detoxification (xenobiotic metabolism) > GO:xenobiotic metabolism ; GO:0006805
MultiFun:5.6.3 Cell killing > GO:.
MultiFun:5.6.4 Drug resistance/sensitivity > GO:response to drug ; GO:0042493
MultiFun:5.8 SOS response > GO:SOS response ; GO:0009432
MultiFun:5.10 Defense/survival > GO:defense response ; GO:0006952
MultiFun:5.11 DNA uptake > GO:cellular DNA uptake ; GO:0009290
MultiFun:5.12 Biofilm production > GO:biofilm formation ; GO:0042710
MultiFun:5.13 Virulence associated > GO:pathogenesis ; GO:0009405
MultiFun:6 Cell structure > GO:cellular_component ; GO:0005575
MultiFun:6.1 Membrane > GO:cell wall (sensu Bacteria) ; GO:0009274
MultiFun:6.2 Peptidoglycan (murein) > GO:cell wall (sensu Bacteria) ; GO:0009274

MultiFun:6.3 Surface antigens (ECA, O antigen of LPS) > GO:cell surface antigen activity\, host-interacting ; GO:0042280
MultiFun:6.4 Flagellum > GO:flagellum ; GO:0019861
MultiFun:6.5 Pilus > GO:fimbria ; GO:0009289
MultiFun:6.6 Ribosome > GO:cytosolic ribosome (sensu Bacteria) ; GO:0009281
MultiFun:6.7 Capsule (M and K antigens) > GO:capsule (sensu Bacteria) ; GO:0030113
MultiFun:7 Location of gene products > GO:cellular_component ; GO:0005575
MultiFun:7.1 Cytoplasm > cytoplasm ; GO:0005737
MultiFun:7.2 Periplasmic space > GO:periplasmic space ; GO:0042597
MultiFun:7.3 Inner membrane > GO:inner membrane ; GO:0019866
MultiFun:7.4 Outer membrane > GO:external outer membrane (sensu Gram-negative Bacteria) ; GO:0009279
MultiFun:7.5 Extracellular > GO:extracellular ; GO:0005576
MultiFun:8 extrachromosomal > GO:.
MultiFun:8.1 Prophage genes and phage related functions > GO:.
MultiFun:8.1.1 DNA packaging, phage assembly > GO:DNA packaging ; GO:0006323 > GO:phage assembly ; GO:0042963
MultiFun:8.1.2 Replication > GO:DNA dependent DNA replication ; GO:0006261
MultiFun:8.1.3 Regulation > GO:.
MultiFun:8.1.4 Integration, recombination > GO:DNA integration ; GO:0015074 > GO:DNA recombination ; GO:0006310 ; GO:provirus integration ; GO:0019047
MultiFun:8.1.5 Lysis > GO:lytic viral release ; GO:0019077
MultiFun:8.1.6 Structural component > GO:structural molecule activity ; GO:0005198
MultiFun:8.2 Plasmid related > GO:.
MultiFun:8.2.1 replication and maintenance > GO:DNA dependent DNA replication ; GO:0006261 > GO:plasmid maintenance ; GO:0006276
MultiFun:8.2.2 plasmid transfer > GO:unidirectional conjugation ; GO:0009291
MultiFun:8.3 Transposon related > GO:.
MultiFun:8.3.1 transposases > GO:transposase activity ; GO:0004803
MultiFun:8.3.2 regulation of mobility > GO:regulation of DNA transposition ; GO:0000337
MultiFun:8.4 Colicin related > GO:.
MultiFun:9. DNA sites > GO:.
MultiFun:10. cryptic genes > GO:.

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Fine-tuning the prediction of sequences cleaved by signal peptidase II: A curated set of proven and predicted lipoproteins of *Escherichia coli* K-12

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A curated set of 81 proven and 44 predicted lipoproteins of *Escherichia coli* K-12 was defined with the combined use of a literature survey, a variety of predictive tools and human expertise. The well-documented Gram-negative proteome of *E. coli* K-12 was chosen to assess how the different approaches complement each other and to ensure a stable definition of a consistent set of lipoproteins. The results of detailed analysis of such proteins at the level of a single proteome are presented, corroborated and rationalized.

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1 Introduction

Sequence annotation at the level of a single proteome is often automated. Fast processing is particularly needed in the case of prokaryotic proteomes given the current intensive effort for sequencing complete genomes. However, speed and quantity are often achieved to the detriment of quality. This issue is well introduced in [1] where high standards for producing reliable bacterial protein annotations are set. Among others, a relevant strategy involves gathering sequences into consistent families while carefully defining similarity criteria. Several independent initiatives dedicated to grouping bacterial protein into families were launched such as TIGRfam [2], HOBACGEN [3] and HAMAPfam [1] for maximum coverage of protein functions or AraC/XylS [4], among others, for specified functions. Grouping criteria do not necessarily reflect a global similarity of amino acid sequences. Some proteins can be functionally equivalent though structurally very diverse, including at the sequence level. Lipoproteins, with their Type II signal peptides, fall into such a category.

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Abbreviation: SPI, signal peptidase I

A variety of bacterial lipoprotein prediction schemes are currently available. An early PROSITE pattern (accession number: PS000013, http://www.expasy.org/prosite/) based on [5] and [6] and subsequent InterPro family signature (accession number: IPR000437, http://www.ebi.ac.uk/interpro/) were defined. Scanning tools associated with PROSITE [7] and InterPro [8] can be run to detect the patterns characterizing bacterial lipoproteins. More recently, dedicated computer programs were described [9, 10]. In both cases, the methodology relies on a learning phase during which the program is trained with examples to determine and recognize characteristics of lipoproteins, in other words, to extract and validate a pattern or a motif. A critical issue remains the careful selection of a representative set of examples to ensure the validity of the identified characteristics. In the particular case of bacterial sequences, new instances of motifs are often determined as a result of the presence of orthologues in genome-wide comparisons. Consequently, related and conserved bacterial sequences are commonly gathered into a training set, irrespective of further specificities of each of the organisms. However, distinctive features can also be of interest and such an issue is addressed in the present work.

We have deliberately chosen to focus on the most documented Gram-negative proteome of *Escherichia coli* K-12 and study how the selection of different training sets

bears on the quality of recognition. This strategy helped identify the weaknesses and strengths of published methods as well as the reliability of information found in various sources such as SWISS-PROT [11] and DOLOP [12]. A stable and consistent set of lipoproteins was finally defined which enhances annotation in EcoGene [13], an *E. coli* K-12 dedicated database.

2 Materials and methods

2.1 Training data

We focused on the *E. coli* K-12 proteome. According to publications available at the time of our study, less than 30 *E. coli* lipoproteins were experimentally verified. But recently, Dr. Shin-ichi Matsuyama of the University of Tokyo, experimentally proved the existence of additional *E. coli* lipoproteins. Although these results are not yet formally published, a list of 75 verified lipoproteins provided by Dr. Matsuyama was introduced in the recent LipoP publication [10]. Twenty-one proteins of this list belong to the set of 27 validated lipoproteins as annotated in EcoGene (Version 17). A nonredundant set of 81 proven lipoproteins could potentially be defined as a training set though *FtsI* [14] was omitted since only 15% of *FtsI* is a lipoprotein due to an anomalous cleavage site with a charged arginine residue at position −7 relative to the lipidated cysteine residue. Finally, 81 experimentally verified lipoproteins were used for the positive training set. They are listed along with their corresponding citations in Table 1. None of the signal sequences shared high percentage of similarity, therefore no homology reduction was necessary.

Table 1. Eighty-one experimentally verified *E. coli* K-12 lipoproteins

EG Acc	Gene	SP Acc	Len	Protein Description	SP Annotation	DOLOP	References
EG11703	acrA	P31223	397	AcrAB-TolC efflux pump, membrane-fusion lipoprotein	IPR000437, PS00013	Yes	[10, 26]
EG10266	acrE	P24180	385	AcrEF-TolC efflux pump, membrane-fusion lipoprotein	IPR000437, PS00013	Yes	[10, 53]
EG12073	apbE	P33944	351	Lipoprotein involved in alternative pyrimidine biosynthetic in Salmonella	IPR000437, PS00013	No	[10, 28]
EG12474	blc	P39281	177	Outer membrane lipoprotein, stationary phase inducible	IPR000437, PS00013	Yes	[10, 29]
EG13637	borD	P77330	97	Lipoprotein in DLP12 prophage, phage lambda bor gene homolog	IPR000437, PS00013	Yes	[10, 30]
EG13413	csgG	P52103	277	Possible assembly or transport protein for curli, novel lipoprotein	IPR000437, PS00013	Yes	[10, 31]
EG14233	cusC	P77211	457	Silver and copper efflux, outer membrane lipoprotein component	IPR000437, PS00013	Yes	[10]
EG10178	cyoA	P18400	315	Cytochrome *c* oxidase subunit II, lipoprotein	IPR000437, PS00013	Yes	[32]
EG14372	ecnA	P56548	41	Lipoprotein antidote to bacteriolytic lipoprotein entericidin B	IPR000437, PS00013	No	[33]
EG14345	ecnB	P56549	48	Bacteriolytic lipoprotein entericidin B	IPR000437, PS00013	No	[33]
EG13897	emtA	P76009	203	Membrane-bound transglycosylase, lipoprotein involved in murein hydrolysis	non-EcoGene start	No	[34]
EG10341	ftsI	P04286	423	Septal peptidoglycan synthesis, transpeptidase, 15% of FtsI is lipoprotein	No	No	[14]
EG20264	flgH	P75940	232	Flagellar synthesis, basal body L-ring lipoprotein	IPR000437, PS00013	No	[10, 35]
EG13181	hslJ	P52644	140	Heat-inducible lipoprotein involved in novobiocin resistance	IPR000437	No	[10]
EG11293	lolB	P24208	207	OM lipoprotein required for cell growth and localization of lipoproteins	IPR000437, PS00013	Yes	[10, 36]
EG10544	lpp	P02937	78	Murein lipoprotein	IPR000437, PS00013	Yes	[37, 42]
EG12240	mdtE	P37636	385	MdtEF-TolC multidrug resistance efflux transporter, MFP lipoprotein	IPR000437, PS00013	Yes	[10]
EG11504	metQ	P28635	271	L, D-methionine transporter, methionine-binding lipoprotein receptor	IPR000437, PS00013	Yes	[10]

Table 1. Continued

EG Acc	Gene	SP Acc	Len	Protein Description	SP Annotation	DOLOP	References
EG13085	mltA	P46885	365	mbrane-bound lytic transglycosylase MltA, periplasmic OM lipoprotein	IPR000437, PS00013	Yes	[10, 38]
EG12699	mltB	P41052	361	Membrane-bound lytic transglycosylase MltB, periplasmic OM lipoprotein	IPR000437, PS00013	Yes	[10, 39]
EG12986	mltC	P52066	359	Membrane-bound lytic transglycosylase MltC, periplasmic OM lipoprotein	IPR000437, PS00013	Yes	[10]
EG10246	mltD	P23931	452	Membrane-bound lytic transglycosylase MltD, periplasmic OM lipoprotein	IPR000437, PS00013	Yes	[10]
EG10657	nlpA	P04846	272	Lipoprotein in outer membrane vesicles	IPR000437, PS00013	Yes	[10, 40]
EG10658	nlpB	P21167	344	Lipoprotein in outer membrane vesicles	IPR000437, PS00013	No	[10, 41]
EG11133	nlpC	P23898	154	NlpC lipoprotein	IPR000437, PS00013	Yes	[10]
EG12111	nlpD	P33648	379	Lipoprotein possibly involved in cell wall formation, metalloprotease homolog	IPR000437, PS00013	No	[10, 27]
EG12137	nlpE	P40710	236	Outer membrane lipoprotein, activates Cpx response in response to adhesion	IPR000437, PS00013	No	[10, 54]
EG12371	nlpI	P39833	294	Minor lipoprotein, mutation causes osmotic sensitivity and filamentation	IPR000437, PS00013	Yes	[10, 44]
EG10679	osmB	P17873	72	OsmB lipoprotein	IPR000437, PS00013	Yes	[10, 45]
EG10044	osmE	P23933	112	Lipoprotein regulated by growth phase and osmotic pressure	IPR000437, PS00013	Yes	[10]
EG10684	pal	P07176	173	Lipoprotein associated with peptidoglycan	IPR000437, PS00013	Yes	[10, 46, 47]
EG11502	rcsF	P28633	134	Lipoprotein, overexpression increases capsule synthesis	IPR000437	Yes	[10]
EG10854	rlpA	P10100	362	Minor lipoprotein, suppressor of prc	IPR000437, PS00013	Yes	[10, 48]
EG10855	rlpB	P10101	193	Minor lipoprotein	IPR000437, PS00013	No	[10, 48]
EG11890	slp	P37194	188	Outer membrane lipoprotein, stationary phase inducible	IPR000437, PS00013	No	[10, 49]
EG13409	slyB	P55741	155	Novel lipoprotein, Mg(2+)-stimulated	IPR000437, PS00013	Yes	[10, 50]
EG14076	spr	P77685	188	Suppressor of prc mutants at low osmolality, lipoprotein	IPR000437, PS00013	No	[10]
EG14276	vacJ	P76506	251	Surface-exposed lipoprotein, required for intercellular spreading in Shigella	IPR000437, PS00013	Yes	[10]
EG13566	wza	P76388	379	Outer membrane auxillary lipoprotein, capsular poly-saccharide translocation	IPR000437, PS00013	Yes	[10, 51]
EG13332	yafT	P77339	261	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG13253	ybaY	P77717	190	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG13660	ybfN	P75734	108	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG14158	ybfP	P75737	164	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG12875	ybhC	P46130	427	Novel lipoprotein, pectinesterase homolog, function unknown	No	Yes	[10]
EG13685	ybjP	P75818	171	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG13687	ybjR	P75820	276	Novel lipoprotein, homologous to AmpD, function unknown	IPR000437	Yes	[10]
EG13133	ycaL	P43674	254	Novel lipoprotein, metalloprotease homolog, function unknown	IPR000437	No	[10]
EG13728	yccZ	P75881	379	Novel lipoprotein, Wza paralog, function unknown	IPR000437, PS00013	Yes	[10]
EG13864	ycdR	P75906	672	Polysaccharide deacetylase-like lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG11117	yceB	P09995	186	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG12689	yceK	P45806	75	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG13431	ycfM	P75947	213	Novel lipoprotein, function unknown	IPR000437	Yes	[10]

Table 1. Continued

EG Acc	Gene	SP Acc	Len	Protein Description	SP Annotation	DOLOP	References
EG13911	ycjN	P76042	430	Putative ABC transporter periplasmic binding lipoprotein, function unknown	IPR000437	Yes	[10]
EG13755	ydcL	P76101	222	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG13794	yddW	P76130	439	Novel lipoprotein, function unknown	IPR000437, PS00013	No	[10]
EG13511	yeaY	P76255	193	Novel lipoprotein, slp paralog, function unknown	IPR000437, PS00013	Yes	[10]
EG14036	yecR	P76308	107	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG11659	yedD	P31063	137	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG12004	yehR	P33354	153	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG14166	yfeY	P76537	191	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG14204	yfgH	P76572	172	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG14208	yfgL	P77774	392	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG11152	yfiB	P07021	160	Putative outer membrane lipoprotein, ompA homolog, function unknown	IPR000437, PS00013	Yes	[10]
EG12446	yfiL	P11289	121	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG14222	yfiO	P77146	245	Novel lipoprotein, homologous to N. gonorrhoeae ComL, function unknown	IPR000437, PS00013	Yes	[10]
EG13081	ygdI	Q46924	75	Novel lipoprotein, ygdR paralog, function unknown	IPR000437, PS00013	Yes	[10]
EG13076	ygdR	Q46932	72	Novel lipoprotein, ygdI paralog, function unknown	IPR000437, PS00013	No	[10]
EG13048	ygeR	Q46798	251	Novel lipoprotein, metalloprotease homolog, function unknown	IPR000437, PS00013	No	[10]
EG12991	yghG	Q46835	136	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG12833	yhdV	P45765	73	Novel lipoprotein, function unknown	IPR000437, PS00013	Yes	[10]
EG12907	yhfL	P45538	55	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG12271	yiaD	P37665	219	Novel lipoprotein, ompA homolog, function unknown	IPR000437, PS00013	Yes	[10]
EG11860	yiiG	P32151	351	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG11924	yjbF	P32687	212	Novel lipoprotein, ymcC paralog, function unknown	IPR000437, PS00013	No	[10]
EG12471	yjeI	P39278	117	Novel lipoprotein, function unknown	IPR000437	No	[10]
EG13731	ymcC	P75884	214	Novel lipoprotein, yjbF paralog, function unknown	IPR000437, PS00013	Yes	[10]
EG14298	ynbE	P76075	61	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG13841	ynfC	P76171	236	Novel lipoprotein, function unknown	IPR000437, PS00013	No	[10]
EG14304	yoaF	P76244	84	Novel lipoprotein, function unknown	IPR000437	Yes	[10]
EG13018	yqhH	Q46860	85	Novel lipoprotein, lpp paralog, function unknown	IPR000437, PS00013	Yes	[10]
EG12781	yraP	P45467	191	Novel lipoprotein, osmY paralog, function unknown	IPR000437, PS00013	No	[10]

EcoGene includes a compilation of all *E. coli* proteins whose *N*-terminal sequences were experimentally determined. This verified set currently contains 862 proteins: http://bmb.med.miami.edu/EcoGene/EcoWeb/CESS Pages/VerifiedProts.htm. Each protein is associated with the corresponding primary literature citations and the number of amino acids removed post-translationally, if any. Two negative training sets were derived from this verified set: (i) a set of 135 exported proteins cleaved by signal peptidase I (SPI), and (ii) a set of 722 proteins that are either not cleaved or cleaved by methionine aminopeptidase that liberates the *N*-terminal methionine residue. The second set was used for training the motif parameters whereas the first was used only for later verifications.

The EcoGene database includes revised predicted start sites for 730 *E. coli* proteins. Numerous proteins needed to be shortened as a result of an original annotation strategy that favored the longest ORF as opposed to the most likely [5]. Most of these corrections have been communicated to SWISS-PROT from EcoGene as part of a

collaborative annotation effort. Incorrect or unlikely annotation of translation start sites can cause many problems in postgenomics research, including the identification of potential lipoproteins.

2.2 Lipoprotein motif

We define a motif as a linear sequence of attributes or tokens. Each token describes one or more characteristics of a single amino acid or subsequence. Different token types were set. They are listed in Table 2. The different characteristics are shown in Table 3. The lipoprotein motif used for training is very similar to the one used in [9] for the detection of lipoproteins in *Bacillus subtilis*. In the PATOSEQ syntax it is written as:

M [p,3:3](0,25) ![R,K} [h~{R,K},15:15](6,20) {} {} {} C *

Table 2. Different token types and their interpretation. Each token can be weighted by prefixing with a numerical value. Tokens prefixed with a ! are locked for refinement

A	A fixed amino acid (anchor).
a	A variable amino acid (distance measure used: $Dayhoff_{250}$).
{S = 0.4, T = 0.6}	A frequency vector specifying the frequency of each amino acid. If no residue is specified, the natural frequencies of each amino acid are used. If residues are listed with no specified frequency, the relative natural frequency is assumed. If this token is preceded by a tilde (~), values are inverted (exclusive vector).
[x, 10:1]	A sequence of length 10 with variance 1 and the characteristics x. A range (min, max) can optional be appendend.
	Any single amino acid
	Any sequence of amino acids

Table 3. The variety of characteristics for sequence tokens. Each characteristic can be weighted by prefixing with a numerical value

p, n, u	positive, negative or no charge
o, y	hydrophobic or hydrophilic
l, s	large or small (volume)
a, b, i	amphipatic alpha helix, beta sheet or volume-helix
{A, G}	frequency vector
*	no characteristics

which should be read as an initial methionine residue (M), followed by a positively charged region of length 0 to 25 ([p,3:3](0,25)), followed by either an arginine or lysine residue (![R,K}[1]), followed by a hydrophobic region of length 6 to 20 residues characterized by a frequency vector initially not containing positively charged residues ([h~{R,K}, 15:15](6,20)[2]), followed by three residues characterized by frequency vectors ({} {} {}), followed by a fixed cysteine residue (C) which is the lipid binding site, followed by anything (*).

2.3 Motif scoring

Once a motif is aligned to an amino acid sequence, a corresponding score is calculated as the sum of the partial scores for each aligned token:

$$score(m) = \sum_i score_i(t_i) \quad (1)$$

where t_i is the i^{th} token in the motif m and $score_i$ its scoring function. For subsequences containing more than one characteristic, the partial score for each characteristic is summed. To ensure the consistency of the alignment, partial scores must all be in the same unit of measurement, independently of the characteristic being scored. Consequently, the partial score is defined as the relative log-probability of the aligned subsequence fitting the characteristics associated with the given token as opposed to a random match:

$$score(t_i) = \log(P^+(t_i)) \quad (2)$$

The total score can then be interpreted as the log-probability of the entire aminoacid sequence matching the entire motif:

$$e^{score(m)} = \prod_i P^+(t_i) \quad (3)$$

Since some features in a motif can be more important than others, they are optionally weighted. These weights are multiplied with the partial log-probabilities:

$$score(t) = \sum_i w_i \log(P^+(t_i)) \quad (4)$$

$$e^{score(m)} = \prod_i P^+(t_i)^{w_i} \quad (5)$$

where w_i is the assigned weight of the i^{th} token. Two tokens t_1 and t_2 with weights w_1 and w_1 are interpreted as the characteristics in t_1 occur $\frac{w_1}{w_2}$ times more often than those in t_2.

The functions for the different $P^+(t_i)$ are defined separately for each token type and each characteristic in a token. For the token types any (*) and space (_), this probability is

[1] The ! modifier locks this token so that it is not modified during training, effectively forcing the motif to match either R or K.
[2] ~ modifier inverts a given frequency vector.

always 1. For fixed amino acids (A), the probability is 1 in the case of a match and 0 otherwise.

For frequency vector tokens, $P^+(t_i)$ is given by the relative probability of an amino acid a matching the frequency vector f_v compared with natural occurrence f_n:

$$P_f^+(a) = \frac{f_v(a)}{f_v(a) + f_n(a)} \tag{6}$$

For frequency vectors within sequence tokens, the average relative probability is used:

$$P_f^+(s) = \frac{\prod_i f_v(s_i)}{\prod_i f_v(s_i) + \prod_i f_n(s_i)} \tag{7}$$

where s_i is the i^{th} amino acid in the subsequence s. To allow for flexibility (*i.e.*, during refinement), a noise factor ϵ can be added to the relative frequency:

$$P_f^+(a) = \frac{f_v(a) + \epsilon f_n(a)}{f_v(a) + f_n(a)} \tag{8}$$

which also avoids "trapping" the total score at 0 and hindering refinement, as seen later.

The length of a subsequence is similarly scored. The length of a given subsequence is assumed to be Poisson distributed (insertions and deletions being discrete cumulative events). If in the random case the subsequence length is evenly distributed over an interval (a, b), then P_l^+ is defined as:

$$P_l^+(s) = \frac{\pi_\mu(|s|)}{\pi_\mu(|s|) + (b - a + 1)^{-1}} \tag{9}$$

where $\pi_\mu(x)$ is the Poisson probability of x with $\lambda = \mu$. Values for a and b are set to 1 and 100, unless specified otherwise.

The scoring of the other tokens (*i.e.*, charge, hydrophobicity, volume, *etc.*) is somewhat more complicated, since they correspond to a more abstract concept of high or low as opposed to fixed values of these characteristics. Charge, hydrophobicity and volume are estimated from the normal distribution of their expected value, *e.g.*, for charge:

$$\mu_{ch} = \sum_a f_r(a) ch(a) \tag{10}$$

$$\sigma_{ch}^2 = \sum_a f_r(a)(\mu_{ch} - ch(a))^2 \tag{11}$$

where $ch(a)$ is the net charge of the amino acid a. The same scoring scheme is applied for amino acid volume and hydrophobicity indices, and in fact, any index that models a physicochemical characteristic.

Given the average charge of a subsequence s, P_p^+ (positive charge) is then calculated as the inverse probability of the charge being greater in the random case:

$$P_p^+(s) = CDF(\mathrm{N}(\mu_{ch}, \sigma_{ch}), ch(s)) \tag{12}$$

where $\mathrm{N}(\mu, \sigma)$ is the normal (Gaussian) distribution over μ and σ and $ch(s)$ is the average charge *per* residue of the subsequence s. This scoring method can be applied to any value for which the distribution can be derived analytically or experimentally (for more examples see [9]).

Finally, the optimal alignment of any motif with an amino acid sequence is achieved using dynamic programming (Fig. 1). This alignment scoring technique significantly differs from scoring with regular expressions and other grammars, or with weight matrices. In our method, a protein sequence is compared to a description of a protein sequence, so that the score is the maximized probability of the sequence fitting the description. In other words, we are not evaluating some abstract numerical score, but the ratio of the probability of the given sequence matching the motif over the probability of a random sequence matching the motif. The resulting score is not binary, as in the case of grammars and regular expressions. Moreover, it is statistically and biologically interpretable, unlike weight matrix scores.

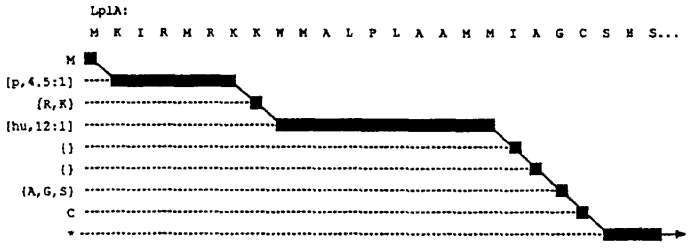

Figure 1. Motif Alignment: the motif is aligned to the sequence using dynamic programming much in the same way as pairwise sequence alignment.

2.4 Motif refinement

Given a set of known (or expected) positive and negative examples and a motif, a classification score is calculated to assess how well the given motif discriminates between the two sets. The motif can then be parameterized to maximize this score. Abundant literature is describing widely used scoring methods (Mathews correlation coefficient [16], Fisher linear discriminant [17], Linear-Classify [18], Precision/Recall [19] to quote the most popular). As pointed out in [20], these approaches are almost all directly based on the effective number of misclassified sequences. In the present work, the scoring scheme for classification does not depend on this quantity.

Scores of sequences in a positive or negative dataset can be plotted in a distribution. Such score distributions can be modelled as beta-distributions. The expected percentage of misclassified sequences can be calculated as the overlap between both distributions relative to a cut-off value c:

$$disc(c) = \frac{|S^-|}{|S^-|+|S^+|} CDF(\beta(\mu^-, \sigma^-), c) + \frac{|S^+|}{|S^-|+|S^+|}(1 - CDF(\beta(\mu^+, \sigma^+), c)) \quad (13)$$

where S^+ and S^- are the positive and negative sets and $\beta(\mu^+, \sigma^+)$ and $\beta(\mu^-, \sigma^-)$ their beta-distributions. The cut-off value c is chosen such as to maximize this score.

This approach is justified since sequences used for training are mere samples of an exhaustive set (*i.e.* an entire proteome). The classification score corresponds to the expected extent of the overlap. The method relies more on the average characteristics of our sequences and outliers are effectively treated as such (*i.e.* exceptions are tolerated). This will later avoid overfitting of the motif to the training set. The classification score is maximized over the motif parameters (frequency vectors, lengths and weights), which is nontrivial. Indeed, any change in the motif can affect the partial scores hence the alignment outcome, leading to discontinuities in the scoring surface.

Refinement is based on a heuristics presented in [9]. The frequency vector values and subsequence lengths are adjusted according to values observed in aligned positive examples. Weights are adjusted according to their relative discriminative power or through a least-square minimization of the overlapping distributions (approximate).

Optimization is iterative. Each parameter change is followed by sequence realignment and the change is kept only if the classification score improves. The motif is initially trained as detailed in [9] using the verified positive and negative training sets described above. The validity of this prediction is then tested using a *k*-fold validation test over the positive training sequences.

The refined motif is then applied to all sequences of the EcoProt (translated EcoGene) database (Version 17). This initial prediction is used for bootstrapping over the EcoProt set of sequences. Bootstrapping involves using the results of a prediction to re-refine the initial motif. It is followed by a new prediction over the same data set based on the re-refined motif. Practically, false positives and false negatives are considered respectively as positives and negatives until no further refinement is possible. The procedure is illustrated in Fig. 2. Bootstrapping is not used to optimize the motif itself, but to optimize the partition of the proteome given an initial motif. Intuitively speaking, such a partition can and should be assumed since the cell is likely to distinguish

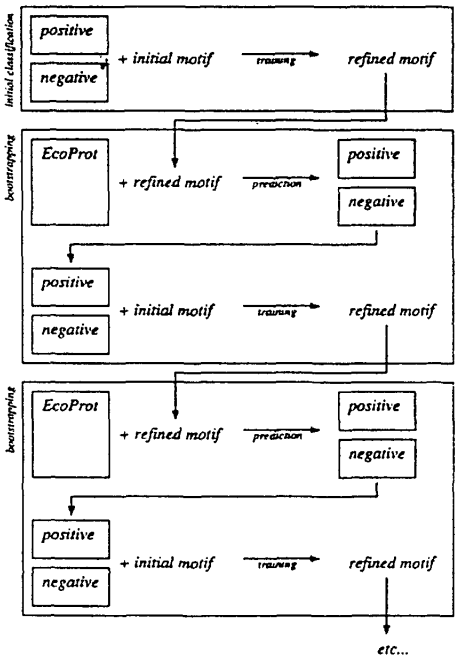

Figure 2. The different steps in motif refinement. Starting from an initial motif and an initial training set, the motif is refined. The refined motif is then used to partition a proteome into positive and negative predictions which are in turn used to re-refine the initial motif and so on, until the predictions remain stable.

between lipoproteins and nonlipoproteins. Moreover, the partition is not likely to degenerate and shift away from a lipoprotein/nonlipoprotein classification, given that only the parameterization of the initial motif is optimized during refinement. As the results show, this is effectively the case.

3 Results

3.1 Motif refinement

The motif was trained as described in Section 2.4. Both sets are perfectly distinguished with the refined motif. The lowest scoring positive is 30.139% and the highest scoring negative, 3.339%. The total classification score over the training data is 100%. In other words, all training sequences are discriminated correctly, and the distributions of scores do not discernibly overlap.

For the *k*-fold cross-validation, the set of known positive sequences was divided into 10 groups of eight sequences and the motif was retrained using all combinations of nine of these 10 groups and tested against the remaining one. The groups were created deterministically by sorting and splitting the data according to EcoGene IDs. This approach is equivalent to a random partition of the data set and was chosen to ensure unbiasedness and reproducibility.

Of the resulting predictions, six positive sequences (7.5%) are missed: *nlpE*, *rlpA*, *rlpB*, *mltC*, *apbE* and *yddW*. All of these sequences except for *rlpA* have singular residues in the lipobox and *RlpA* is the only sequence containing a tryptophan residue in the transmembrane region. These weak characteristics are difficult to predict if they are not represented in the training set. The refined motif was then applied to all sequences of the EcoProt (translated EcoGene) database (Version 17), yielding 120 predicted lipoproteins and 1457 nonlipoproteins. The remaining sequences could not be aligned due to the missing required methionine and cysteine residues and are therefore also considered nonlipoproteins, although they could no longer be used for this analysis.

After a first bootstrapping run, the initial motif was modified to be more specific in the lipobox:

{I,L,M,F,T,V} {} {A,G,S} C *

for faster convergence. Since the content of the frequency vectors is also subject to refinement, the exibility of the lipoprotein signal motif is not altered. Bootstrapping converged after two iterations towards 118 predicted lipoproteins with a classification score of 99.98%. *nanE* and *yjiK* were reclassified as nonlipoproteins. The lowest scoring positive sequence is *yddW* with 36.573% and the highest scoring negative is *nanE* with 31.886%. The distribution of the scores can be seen in Fig. 3. The complete set of predicted lipoproteins and their scores are shown in Table 4.

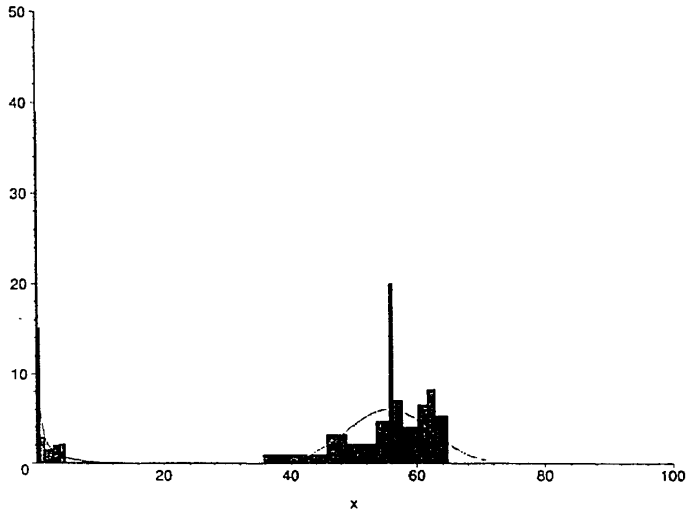

Figure 3. Distribution of the scores of the positive and negative predictions with the fitted β-distributions.

Table 4. The 118 predicted lipoprotein signals found in the EcoGene *E. coli* protein sequences using PATOSEQ

Name	Score	Aligned sequence
mltB	65.10412	SSK ...
mdtE	64.84520	DDK ...
yajG	64.76101	AKP ...
ybjR	64.49154	AGE ...
ecnA	64.17996	NTA ...
yafT	64.09877	ASE ...
yfbK	64.01340	GPQ ...
yhdV	63.77605	AHD ...
yfgL	63.63713	SLF ...
yfgH	63.58306	QSN ...
yfiO	63.44926	SGS ...
yggG	63.36798	QNM...
yfhM	63.35255	DNN ...
ybjP	62.65524	TTV ...
ycfM	62.44483	VGQ ...
slyB	62.34039	VNN ...
mltA	62.32953	SSK ...
ycfL	62.17904	RSH ...
ycaL	62.12220	QNT ...
lpp	62.09211	SSN ...
ynfC	62.03280	DRT ...
ymbA	61.93177	SSG ...
yifL	61.87079	GLK ...
yfeY	61.69214	STM ...
yccZ	61.39632	TLV ...
yedD	61.30897	AEV ...
rzoR	61.23163	TSK ...
rzoD	61.23163	TSK ...
ycdR	60.95369	ISQ ...
ybfN	60.77116	AQS ...
ybfN	60.68041	DDR ...
vacJ	60.55754	ASS ...
hslJ	60.49410	VSN ...
yhfL	60.47031	TGH ...
dcrB	60.20883	DDK ...
yegR	60.02550	AVH ...
nlpI	59.93968	SNT ...
borD	59.80778	AQQ ...
yehR	59.78834	GDK ...
wza	59.65025	TVL ...
yghJ	59.60455	DGG ...
emtA	59.38242	SSK ...
ycjN	58.98437	KEE ...
ygdI	58.50586	SGS ...
slp	58.37951	SSE ...
cyoA	58.23754	NSA ...
yfiL	58.10022	QID ...
cusC	57.70727	SLA ...
yafY	57.57113	DDR ...
yiaD	57.31151	TTN ...
yoaF	57.18330	STP ...
ydcL	57.11211	ASK ...
csgG	57.10907	LTA ...
mltD	56.92914	QST ...
yfiB	56.72722	QSP ...
nlpE	56.70599	NNR ...
nlpC	56.68813	SHH ...
yghG	56.66591	ASH ...
nlpA	56.60377	DQS ...
yjbF	56.56772	SAT ...
osmB	56.47267	SNW...
yceK	56.43614	GSI ...
ymcC	56.40653	THS ...
rcsF	56.36003	SML ...
metQ	56.33563	CQD ...
fecD	56.24603	ALL ...
yeaY	56.22610	VTV ...
yecR	56.15763	TVT ...
yajI	56.12183	VQQ ...
ygdR	56.08548	SSD ...
mltC	56.07822	STT ...
ygeR	55.96281	SGS ...
yceB	55.86755	NQL ...
yjeI	55.82614	SSS ...
ybhC	55.80707	SST ...
yidX	55.46699	ISY ...
pal	55.39019	SSN ...
ydbJ	55.32699	SSE ...
ecnB	55.22635	NTT ...
ybfP	55.15352	AQP ...
yfiM	55.05734	SHM...
mdtP	54.84027	ALV ...
smpA	54.76355	STL ...
yiaF	54.74585	FDK ...
ynBE	54.70140	TPR ...
rlpA	53.21251	TSD ...
acrE	52.72669	NDK ...
fepG	52.51321	VVA ...
yaeF	52.40728	TVD ...
ybaY	52.12813	ADK ...
yliB	51.92793	AFA ...
yaiW	51.48457	SSQ ...
yebF	51.48306	ASV ...
yiiG	50.81708	DDK ...
osmE	50.61636	TAY ...
ydjY	50.50179	DQQ ...
yqhH	49.55094	VNE ...
fliL	49.10648	ASA ...
nlpD	49.09852	SDT ...
yraP	48.60274	VAA ...
yjfO	48.43271	SAL ...
lolB	48.15941	SVT ...
blc	47.84833	SSP ...
ypdI	47.50745	NVF ...
nlpB	47.26330	SSD ...
apbE	47.21592	DQK ...
rlpB	46.96210	GWH...
acrA	45.26031	DDK ...
arnT	44.25810	YYL ...
spr	44.23807	SAN ...
flgH	44.14161	AWI ...
yqiG	43.01175	ASA ...
rtn	42.89918	LQF ...
ampC	42.79648	STF ...
yraM	41.24680	GTH ...
yecT	40.69571	QAY ...
ytcA	37.32636	SLS ...
yddW	36.63137	KST ...

3.2 Comparison of various sources of lipoprotein predictions

The results of a variety of lipoprotein prediction programs and existing lipoprotein database entries were combined and corroborated to obtain a final compilation predicting a total of 125 lipoproteins in *E. coli* K-12. These were derived from a variety of sources and assessed for reliability. The LipoP training sets included 63 verified lipoproteins, 328 SPI-cleaved proteins, and 388 cytoplasmic proteins [10] selected in a variety of Gram-negative bacteria, *i.e.*, not just *E. coli*. Fifteen of the 63 verified lipoproteins in the LipoP positive training set were *E. coli* sequences. LipoP could detect an additional seven *E. coli* lipoproteins when SWISS-PROT protein sequences were used as opposed to those in GenBank [21], given that start sites were corrected in the SWISS-PROT version of the sequences [10].

In total, 134 *E. coli* SWISS-PROT and TrEMBL entries (release 42) are cross-linked to the PS000013 PROSITE pattern or the InterPro IPR000437 (signature characterizing bacterial lipoproteins) and 86 sequences are stored in DOLOP as the predicted set corresponding to *E. coli* K-12. The complete list of predicted lipoproteins reaches 101 items in [10]. The 81 proven lipoproteins listed in EcoGene (Table 1) do not exactly match data found in other databases. In SWISS-PROT, 78 of these *E. coli* entries are cross-linked with InterPro IPR000437. In DOLOP, 51 sequences are common to the set of 81. Furthermore, the published list of predicted proteins in [10] coincides only for 76/81 sequences used for training. Table 1 contains the citations to the experimental verification publications.

Table 5 lists all lipoproteins that were predicted by the various sources, excluding the entries in Table 1, reaching a total of 88 predicted, but as yet unproven, lipoproteins. In fact, 11/88 are proven not to be lipoproteins. Such recognized false positives are referenced in Table 5. One putative lipoprotein gene, *yahH*, listed in DOLOP only, has been removed from EcoGene as it is an unlikely translation of a REP element. Thirty-two out of 88 are evaluated as probable false positives based on homology analysis and other considerations listed in the 'Comments and False Positive References' column in Table 5. These are not infallible exclusionary considerations and some of these may in fact turn out to be lipoproteins, although this seems quite unlikely. This leaves 44/88 predicted as lipoproteins as shown in Table 3. Sixteen out of 44 possible lipoproteins did not have enough homologues to support the lipoprotein predictions. A conservative estimate for the predicted set, *i.e.*, probable lipoproteins, amounts to 28. Added to the verified lipoproteins (Table 1) the final *bona fide* set of lipoproteins contains a total of 109 proteins.

Table 5. Predicted *E. coli* K-12 lipoproteins

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
LipoP and PATOSEQ hits								
EG12218	dcrB	P37620	185	Resistant to lytic phage C1	PATO/ LipoP-Yes	IPR000437	No	Probable lipoprotein, Cys conserved
EG11952	mdtP	P32714	488	Putative outer membrane factor for MdtNOP efflux pump	PATO/ LipoP-Yes	IPR000437, PS00013	Yes	Probable lipoprotein, Cys conserved
EG14380	rzoD	P58041	60	Homolog to lambda Rz1 lipoprotein in prophage DLP1 2	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved, homologous to Rz1
EG14381	rzoR	P58042	61	Homolog to lambda Rz1 lipoprotein in prophage Rac	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved, homologous to Rz1
EG10952	smpA	P23089	113	Putative lipoprotein, OmlA homolog, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	Yes	Probable lipoprotein, Cys conserved
EG12138	yaeF	P37056	274	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved
EG13608	yaiW	P77562	364	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437	Yes	Probable lipoprotein, Cys conserved
EG12182	yajG	P36671	192	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved

Table 5. Continued

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
EG12874	yajI	P46122	179	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved
EG13430	ycfL	P75946	125	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437	Yes	Probable lipoprotein, Cys conserved
EG13182	ydbJ	P52646	88	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437	No	Probable lipoprotein, Cys conserved
EG14095	yfbK	P76481	575	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437	Yes	Probable lipoprotein, Cys conserved
EG13394	yfhM	P76578	1653	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	Yes	Probable lipoprotein, Cys conserved
EG12857	yfiM	P46126	107	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	non-EcoGene start	No	Probable lipoprotein, Cys conserved
EG11291	yggG	P25894	252	Putative metalloprotease lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved
EG12994	yghJ	Q46837	1520	Putative lipoprotein, AcfD homolog, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	Yes	Probable lipoprotein, Cys conserved
EG12273	yiaF	P37667	236	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	non-EcoGene start	No	Probable lipoprotein, Cys conserved
EG11719	yidX	P31461	218	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437	No	Probable lipoprotein, Cys conserved
EG12353	yifL	P39166	67	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved
EG12489	yjfO	P39297	109	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Probable lipoprotein, Cys conserved
EG13719	ymbA	P75866	187	Putative lipoprotein, function unknown	PATO/ LipoP-Yes	non-EcoGene start	No	Probable lipoprotein, Cys conserved
EG12778	yraM	P45464	678	Putative lipoprotein, LppC homolog, function unknown	PATO/ LipoP-Yes	No	No	Probable lipoprotein, Cys conserved
EG13337	yafY	P77365	147	Function unknown	PATO/ LipoP-Yes	non-EcoGene start	No	Possible lipoprotein, yfjS is the only homolog
EG14001	ydjY	P76220	225	Function unknown	PATO/ LipoP-Yes	No	No	Possible lipoprotein, no homologs
EG14061	yegR	P76406	105	Function unknown	PATO/ LipoP-Yes	non-EcoGene start	No	Possible lipoprotein, no homologs
EG13205	yfjS	O52982	147	Function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Possible lipoprotein, yafY is the only homolog
EG14376	ypdI	O32528	91	Function unknown	PATO/ LipoP-Yes	IPR000437, PS00013	No	Possible lipoprotein, no homologs
EG10322	fliL	P06973	154	Affects rotational direction of flagella during chemotaxis	PATO/ LipoP-Yes	No	No	Probable false positive, Cys not conserved
PATOSEQ, not LipoP hits								
EG10178	cyoA	P18400	315	Cytochrome *c* oxidase subunit II, membrane-bound	LipoP-No	IPR000437, PS00013	No	Verified lipoprotein [32], 2 CM TMS predicted
EG14401	ytcA	None	91	Putative lipoprotein, function unknown	LipoP-No	Not in SWISS-PROT	No	Probable lipoprotein, lipobox conserved in Yersina
EG14316	yecT	P76296	162	Function unknown	LipoP-No	No	Yes	Possible lipoprotein, EC and distant homologs only

Table 5. Continued

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
EG14093	arnT	P76473	550	4-amino-4-deoxy-L-arabinose: Lipid A transferase	LipoP-No	IPR000437	No	Probable false positive, 12 CM TMs predicted, Cys not conserved
EG10289	fecD	P15029	318	Ferric citrate transport membrane permease	LipoP-No	IPR000437	Yes	Probable false positive, 9 CM TMs predicted, Cys not conserved
EG10298	fepG	P23877	330	Ferrienterobactin permease, membrane-bound	LipoP-No	IPR000437	Yes	Probable false positive, 9 CM TMs predicted, Cys conserved in enterics
EG14077	rtn	P76446	518	Overexpression confers resistance to lambda and N4	LipoP-No	IPR000437	No	Probable false positive, 2 CM TMs predicted, Cys not conserved
EG11807	yebF	P33219	122	Function unknown	LipoP-No	IPR000437, PS00013	Yes	Probable false positive, Cys not conserved, probable SPI substrate
EG13473	yliB	P75797	512	Putative periplasmic binding protein, function unknown	LipoP-No	IPR000437, PS00013	No	Probable false positive, Cys not conserved, possible SPI substrate
EG14228	yqiG	P76655	822	Function unknown, fimbrial usher homolog	LipoP-No	non-EcoGene start	No	Probable false positive, Cys not conserved, IS21 insertion
EG10040	ampC	P00811	377	Intrinsic weak bet a-lactamase activity	LipoP-No	IPR000437	No	False positive, verified SPI substrate [55]
LipoP, not PATOSEQ hits								
EG12020	mdtQ	P33369	478	Putative OM lipoprotein of tripartite efflux pump	PATO-No	IPR000437, PS00013	No	Probable lipoprotein signal, Cys conserved, paralogs MdtP and CusC
EG12139	yfhG	P37328	237	Putative lipoprotein, function unknown	PATO-No	IPR000437	No	Probable lipoprotein signal, Cys is conserved
EG11712	yidQ	P31454	110	Putative lipoprotein, function unknown	PATO-No	IPR000437	No	Probable lipoprotein, Cys is conserved and yceK paralog is lipoprotein
EG11917	yjaH	P32681	231	Function unknown	PATO-No	No	No	Possible lipoprotein, Cys conserved, 30 aa long signal predicted
EG11926	yjbH	P32689	698	Function unknown, ymcA paralog	PATO-No	IPR000437, PS00013	No	Possible lipoprotein, Cys somewhat conserved
EG14400	ysaB	None	99	Function unknown	PATO-No	Not in SWISS-PROT	No	Possible lipoprotein, Cys conserved, no charged residue
EG11164	ygiB	P24195	223	Function unknown	PATO-No	non-EcoGene start	No	Possible lipoprotein signal, Cys is conserved, long signal (35aa)
EG12258	bcsZ	P37651	368	Endo-1,4-D-glucanase, periplasmic cellulase	PATO-No	No	No	Probable false positive, Cys somewhat conserved, soluble periplasmic

Table 5. Continued

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
EG14240	kefA	P77338	1120	Mechanosensitive channel protein MscK(KefA)	PATO-No	No	No	Probable false positive, 12 CM TMs, Cys not conserved
EG11950	nrfG	P32712	198	Required for Nrf pathway, function unknown,	PATO-No	No	No	Probable false positive, Cys not conserved
EG11333	visC	P25535	400	Putative FAD-dependent oxidoreductase, function unknown	PATO-No	No	No	Probable false positive, Cys not conserved, probably cytoplasmic
EG11769	ybbC	P33668	122	Function unknown	PATO-No	IPR000437, PS00013	No	Probable false positive, Cys not conserved
EG13650	ybeT	P77296	184	Function unknown	PATO-No	No	No	Probable false positive, Cys not conserved
EG11780	ydeK	P32051	1325	Putative OM autotransporter adhesin, function unknown	PATO-No	IPR000437, PS00013	No	Probable false positive, Cys not conserved in other autotransporters
EG11840	yihN	P32135	421	Putative MFS family permease, function unknown	PATO-No	No	No	Probable false positive, 10 CM TMs, Cys not conserved
EG10530	lepB	P00803	324	Signal peptidase I (for nonlipoproteins)	PATO-No	No	No	False positive, shown to have no signal peptide [58]
EG13271	panE	P77728	303	Ketopantoate reductase, NADPH-dependent	PATO-No	No	No	False positive, unprocessed, verified by mass spectrometry [62]
EG10971	srlD	P05707	259	Sorbitol-6-phosphate dehydrogenase	PATO-No	No	No	False positive, unprocessed, verified amino terminus [43]
Swiss-Prot/InterPro only								
EG13149	yafL	Q47151	249	Putative lipoprotein, function unknown	PATO/ LipoP-No	IPR000437	No	Probable lipoprotein, NlpC paralog
EG11488	ydhA	P28224	109	Putative lipoprotein, function unknown	PATO/ LipoP-No	IPR000437	No	Probable lipoprotein, lipobox conserved, EcoGene had unlikely start codon
EG14383	mgrB	P76267	47	Mg(2+)-starvation-stimulated gene, function unknown	PATO/ LipoP-No	IPR000437	No	Possible lipoprotein, Cys conserved in Salmonella
EG13477	yliF	P75801	442	Function unknown	PATO/ LipoP-No	IPR000437	No	Possible lipoprotein, no N-domain homologs
EG13729	ymcA	P75882	698	Function unknown, yjbH paralog	PATO/ LipoP-No	IPR000437, PS00013	No	Possible lipoprotein, Cys somewhat conserved
EG14007	ynjE	P78067	435	Rhodanese-like protein, function unknown	PATO/ LipoP-No	IPR000437	No	Possible lipoprotein, Cys conserved
EG13178	rseC	P46187	159	Required for the reduction of SoxR	PATO/ LipoP-No	IPR000437	Yes	Probable false positive, lipobox not conserved
EG13643	ybdJ	P77506	82	Function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, 2 CM TMs predicted

Table 5. Continued

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
EG12395	ybgE	P37343	97	Fourth gene in cydAB operon, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved
EG13312	ybgP	P75749	242	Putative periplasmic pilus chaperone, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, likely SPI substrate
EG13710	ycbR	P75856	233	Putative periplasmic pilus chaperone, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, likely SPI substrate
EG11735	ycdB	P31545	423	Function unknown, peroxidase homolog	PATO/ LipoP-No	IPR000437, PS00013	No	Probable false positive, Cys not conserved, predicted Tat substrate
EG13970	ydiK	P77175	370	Putative membrane permease, function unknown	PATO/ LipoP-No	IPR000437	Yes	Probable false positive, Cys not conserved, 9 CM TMs predicted
EG14164	yfeW	P77619	434	Putative periplasmic esterase, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, likely SPI substrate
EG10018	yhdA	P13518	646	Function unknown	PATO/ LipoP-No	IPR000437, PS00013	No	Probable false positive, Cys not conserved
EG11267	yiaB	P11286	113	Inner membrane protein, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, 4 CM TMs predicted
EG12281	yiaM	P37674	157	Putative membrane permease, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, 4 CM TMs predicted
EG14229	yqiH	P77616	249	Putative periplasmic pilus chaperone, function unknown	PATO/ LipoP-No	IPR000437	No	Probable false positive, Cys not conserved, likely SPI substrate
EG10315	fimH	P08191	300	Minor type 1 fimbrial adhesion subunit	PATO/ LipoP-No	IPR000437	No	False positive, verified SPI substrate [56]
EG10374	ggt	P18956	580	gamma-Glutamyltranspeptidase	PATO/ LipoP-No	IPR000437	No	False positive, Verified SPI substrate [52]
DOLOP only								
EG12816	nanE	P45426	229	Putative ManNAc-6-Pto GlcNAc-6-P epimerase	PATO/ LipoP-No	No	Yes	Possible lipoprotein, Cys conserved
EG14386	yaaY	P75620	72	Function unknown	PATO/ LipoP-No	No	Yes	Possible lipoprotein, EC-ST only
EG11052	uhpB	P09835	500	Membrane protein controlling UhpA activity, sensor kinase	PATO/ LipoP-No	No	Yes	Probable false positive, Cys not conserved, 10 CM TMs predicted
EG12097	yfiH	P33644	243	Function unknown	PATO/ LipoP-No	No	Yes	Probable false positive, Cys not conserved
EG14163	yfeV	P77272	474	Putative PTS system IIBC component, function unknown	PATO/ LipoP-No	No	Yes	Probable false positive, Cys not conserved, 9 CM TMs predicted
EG13003	yghS	Q46843	237	Function unknown	PATO/ LipoP-No	No	Yes	Probable false positive, Cys not conserved
EG13839	ynfA	P76169	108	Inner membrane protein, function unknown	PATO/ LipoP-No	No	Yes	False positive (K.E.R., unpublished)

Table 5. Continued

EG Acc	Gene	SP Acc	Len	Description	PATOSEQ/ LipoP	SP Annotation	DOLOP	Comments and False Positive References
EG10120	bioD	P13000	225	Dethiobiotin synthase	PATO/ LipoP-No	No	Yes	False positive, verified amino terminus, Met is cleaved [59]
EG10202	dacB	P24228	477	D-alanine D-alanine carboxypeptidase PBP4	PATO/ LipoP-No	No	Yes	False positive, verified SPI substrate [60]
EG10306	fhuE	P16869	729	Outer membrane receptor for ferric-rhodotorulic acid	PATO/ LipoP-No	No	Yes	False positive, verified SPI substrate [61]
EG11481	tatD	P27859	260	Mg-dependent cytoplasmic DNase	PATO/ LipoP-No	No	Yes	False positive, unprocessed, verified amino terminus [57]
EG13592	yahH	P75690	106	YahH is no longer in EcoGene	PATO/ LipoP-No	No	Yes	Defunct gene, unlikely translation of REP sequences

4 Discussion

Automatic classification of protein sequences depends, in most cases, on the presence of patterns and motifs. Motifs are generally determined as regions of conserved positions in the optimized alignment of amino acid sequences. In other words, regularities identified in a conserved region are expressed as positional constraints. Such denoted consensus sequences often correspond to binding sites for substrates or regions involved in modification, transport, degradation, *etc.* In protein regions identified as cleavage sites in protein processing pathways, even though amino acid regularity is visually obvious, the variability of sequence length affects the quality of alignment through the introduction of a substantial number of gaps. Furthermore, many of the features of protein binding sites are given in terms of characteristics, such as net charge, hydrophobicity, size, *etc.*, which are not necessarily well represented by the presence or absence of a specific amino acid residue.

Such problems are exemplified in the case of the cleavable *N*-terminal regions of bacterial proteins. Searches for signal peptide patterns, irrespective of their type, were formalized along three main guidelines. Regular expressions were used to accommodate length variability but their implementation usually generates a binary answer (presence/absence of a motif) [19]. Alternatively, strategies using neural nets were defined to provide scoring functions but users are deprived from an explicit and rational biological explanation for an output [10, 22]. Rule-based systems [23] ideally circumvent the cited shortcomings associated with the use of regular expressions and neural nets but can only reproduce the limitations of human understanding.

PATOSEQ introduced in [9] was set as an attempt to identify further explicit rules and constraints that might not only be positional and would reflect a biological phenomenon. We first suggested to change the alphabet used for describing motifs in order to include partial information on positional constraints in the descriptors. Secondly, we set interdependent matching and scoring procedures that would guarantee stable and optimized scores. Given a motif description, scoring was set as the maximized probability for a sequence to match this description. But, as mentioned early in Section 2.1, in all cases, the most critical step remains the initial selection of a reference or a training set.

In the framework of bacterial lipoprotein study, attention has first been focussed on the consensus defining the so-called lipobox as initially identified in [5, 6]. The presence of this consensus has set the basis of all patterns used for lipoprotein recognition. At the time, much fewer sequences were available than nowadays. Incoming genome data spurred further direct investigations of sequence patterns with *ad hoc* methods in Gram-positive bacteria [24, 25] as well as all bacteria indiscriminately [12]. The latter updated resource provides a looser definition of the PROSITE pattern for searching potential lipoprotein signal sequence that allows a high number of false positives. The regular expression defined as the PROSITE pattern and complemented with *ad hoc* rules is more stringent.

We considered the most documented bacterial proteome, *i.e.* that of *E. coli* K-12. An initial set of 81 lipoproteins was carefully checked and crosschecked for maximum guarantee to comply with the standards of annotation in EcoGene [13]. It is justified in Section 3. This set

was used for training PATOSEQ to search lipoproteins. In parallel, 134 SWISS-PROT and TrEMBL entries cross-linked to the PS000013 PROSITE pattern or the InterPro IPR000437 were retrieved as well as the 86 predicted lipoproteins of *E. coli* K-12 in DOLOP. The variability of sources (EcoGene, SWISS-PROT, DOLOP, InterPro and PROSITE) and the differences of the prediction schemes provided by LipoP and PATOSEQ motivated the validation of each sequence.

Unsurprisingly, the quality of annotation is uneven and proportional to the level of human input. In particular, improper protein starts are a significant cause for inconsistencies that bear on the accuracy of database information as well as the performance of predictive methods. Indeed, feeding LipoP with EcoGene protein sequences that included recently revised predicted translation start sites, relative to GenBank or SWISS-PROT, enhanced the efficiency of the program: six additional lipoproteins were predicted (noted in the LipoP predictions of Table 5 as "non-EcoGene starts"). The EcoGene start site prediction revisions are presented in the EcoGene records for these genes in the "Gene Quality" field (http://bmb.med.miami.edu/EcoGene/EcoWeb).

Each item of the predicted lipoproteins in *E. coli* K-12 listed in this paper was analyzed in detail. Explanations correspond to the best of our knowledge *via* the use of heuristic rules (conservation in orthologues, prediction of transmembrane regions and other topological criteria). Using the corrected start sites from EcoProt, LipoP and PATOSEQ yield 109 matching predictions. Quite logically, PATOSEQ recognized the 81 proven lipoproteins. It also predicted another 37, 28 of which we consider to be correct predictions. The remaining nine predictions which we consider to be false are either proven or supposed Type I secreted proteins. It should be noted that these are not selected by the LipoP predictor since a filter for Type I signals is applied prior to processing sequences for Type II.

The explicit performance of PATOSEQ helped identify some specific features of the lipoprotein signal in *E. coli* K-12, proper. In particular the nonappearance of particular amino acids in the lipobox influences the prediction and may provide further constraints justifying protein secretion. Conversely, PATOSEQ is inflexibly sensitive to the absence of positively charged residues between the initial methionine and the helical part of the signal. The investigation of apparently minute discrepancies in signal peptide sequences can lead to a more precise recognition. In fact, unpublished tests with slight variations on the motif in Gram-positive bacteria led to fine-tune descriptions depending on the organism. Such small differences matched published observations in [25]. Given the physiological differences between organisms, variations in the properties of secretion are to be expected. We looked into characteristics that would distinguish pathogenic from nonpathogenic strains that could not be tested so far.

5 Concluding remarks

The careful sorting of *E. coli* K-12 lipoproteins led to the selection of a restricted set of candidates that could be tested. It also provided a benchmark set for evaluating predictive methods and their sensitivity. As a common trend in bioinformatics applications, the combined use of several methods is equivalent to merging several viewpoints; most of the time, it reinforces the reliability of prediction and shows that various methods complement each other.

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Linkage Map of *Escherichia coli* K-12, Edition 10: The Physical Map

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INTRODUCTION

EcoMap10, the physical map of edition 10 of the *Escherichia coli* K-12 linkage map, is a map of restriction sites and genomic positions of a set of bacteriophage lambda clones and includes a graphic representation of EcoGene10, a refined annotation of the *Escherichia coli* genome sequence. The previous version, EcoMap7, was published as part of edition 9 of the *Escherichia coli* K-12 linkage map (4). A brief description of EcoMap10 is provided here, and a more detailed description of EcoMap10 and the EcoGene10 data set will be published separately (16). The most significant change in EcoMap construction is that it is now based upon the complete genome sequence of *E. coli* K-12 strain MG1655 version M52 (4,639,221 bp) as determined by Blattner et al. (5). EcoMap10 features, including the predicted restriction sites, Kohara clone alignments, protein coding regions, gene and open reading frame (ORF) designations, insertion sequence (IS) elements, and repetitive extragenic palindrome (REP) clusters, have all been derived by using version M52 of the MG1655 DNA sequence (GenBank/EMBL/DDBJ accession no., U00096). The tables and references in the traditional map of edition 10 of the *Escherichia coli* K-12 linkage map (3) also apply to the genes displayed in the physical map.

EcoMap10

Restriction Enzyme Recognition Sites

The recognition sites for the eight restriction enzymes used to create the whole genome restriction map of Kohara et al. (10) are predicted from the genomic DNA sequence. These 6-bp recognition sites are mapped at the position of their first base pair. Although any set of restriction sites can now be used to create a restriction map of the entire chromosome, this set of sites was used in order to retain continuity with the original Kohara/Isono genomic restriction map and previous EcoMap versions. This set of commonly used enzymes provides a convenient pattern of restriction sites and includes a wide range in the number of predicted recognition sites in the MG1655 genome: *Bam*HI, 495; *Kpn*I, 516; *Hin*dIII, 556; *Eco*RI, 645; *Pst*I, 958; *Pvu*II, 1,778; *Bgl*I, 1,919; and *Eco*RV, 2,040. The expected number of 6-bp restriction enzyme recognition sites in a randomly generated DNA sequence of this length and composition would be 1,133. The mean number of predicted sites for this set of eight enzymes is 1,113.

Kohara/Isono Miniset Clones

The Kohara/Isono miniset is a widely used collection of ordered *E. coli* bacteriophage lambda clones derived from strain *E. coli* K-12 W3110 (10). Four hundred and seventy-three of the original 476 miniset clones have been aligned to EcoMap10. Seven of the clones were split into two portions labeled A and B because they crossed the 0-min point, the IN(*rrnD-rrnE*)1 inversion endpoints, or the sites of a duplication and translocation of the *tdc* region specific to the Kohara/Isono version of W3110, as previously described (4, 11, 14, 17, 19, 21). One hundred and eighty-six of the clones are present in GenBank/EMBL/DDBJ as individual sequence entries, and these clones are precisely aligned to the genomic DNA sequence since their chromosomal DNA inserts have been sequenced (1, 9, 13, 22). The remaining clones were positioned by using the gel electrophoresis-derived restriction enzyme map of Kohara et al. (10) as previously described (14, 17). These clones are referred to as "unsequenced" because there are no individual GenBank/EMBL/DDBJ records available for them, even though many of them may in fact have already been sequenced. When additional information about the remaining clones becomes available, this information will be incorporated into the EcoMap alignments. Most of the miniset clones are *Sau*3A partial restriction fragments cloned into the *Bam*HI site of lambda EMBL4, and no attempt was made to align the ends of the unsequenced clones to specific *Sau*3A sites in the genomic sequence. Twenty-four of the miniset clones depicted in EcoMap10 are *Eco*RI partial fragments cloned into the *Eco*RI site of lambda 2001, identified by clone names that begin with the designations E1 to E25. Fourteen of these have GenBank/EMBL/DDBJ entries and have terminal *Eco*RI restriction sites in the database entries that are all aligned to *Eco*RI sites in the genomic DNA sequence. The alignments of the 10 unsequenced *Eco*RI clones were manually adjusted so that their ends align to *Eco*RI restriction sites in the genomic sequence. The orientations depicted for the Kohara/Isono clone inserts indicate that the right arm of lambda is to the

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right of the insert's restriction map as depicted in EcoMap10 (positive orientation, rightward arrow) or to the left (negative orientation, leftward arrow).

Caution must be taken if EcoMap10 is used as the source of a restriction map for the Kohara/Isono miniset clone since the miniset was derived from W3110. In addition to the rare occurrence of DNA sequence errors and strain-specific DNA sequence polymorphisms that might lead to minor restriction map differences, there are major differences due to genome rearrangements (noted above) and the W3110-specific IS elements (see below) (reviewed in reference 6). Solutions to this problem include using the DNA sequence database entries for the sequenced clone subset or using the original Kohara/Isono W3110 restriction map (10) for the unsequenced subset of clones. In either case, the experimental verification of critical restriction sites is recommended.

IS Elements and REP Clusters

IS and REP (also called PU) elements are repeated DNA sequences and major extragenic features of the *E. coli* chromosome (2, 6). The positions of the IS elements present in MG1655 are determined by searching the complete genomic MG1655 DNA sequence with representative IS family member sequences. The positions of the W3110-specific IS element insertion points are determined from the sequenced W3110 clones whenever possible or estimated from the physical mapping data as previously described (14). The orientations of the IS elements indicate the direction of transcription of the transposase gene, as previously described (4, 6). The IS5 family element orientations were depicted incorrectly in EcoMap7 (4), and this error has been corrected in EcoMap10. Three putative IS-related sequences of unknown origin were identified and are temporarily designated ISX (2793.3 kb), ISY (2714.1 kb), and ISZ (1293.8 kb). The IS-encoded genes are not considered *E. coli* genes in EcoGene, and it is the full length of the IS element that is represented, not the coding regions contained within them.

REP elements have been postulated to have a variety of RNA- and DNA-related functions, but the stabilization of mRNA is the only firmly established function (2). The positions of individual REP elements were determined by a variety of pattern searches, as will be described elsewhere (16). This approach identified nearly all previously reported REP elements (2) and was used to locate new REP elements. The few REP elements identified earlier that were missed by this approach were annotated manually. Individual REP elements occur in intergenic REP clusters, also called bacterial interspersed mosaic elements (BIMEs) containing from 1 to 12 REP elements interspersed with other small conserved sequences (2, 8). Three hundred and fifty-five REP clusters (BIMEs) containing a total of 697 individual REP elements were identified. Particular attention was given to the detection of a class of REP-like putative bidirectional transcription terminators referred to as PU* or Y* (2, 7). A total of 108 individual Y* elements are included in the REP tabulation (16). Y* elements can also be found as subsequences of a number of other REP elements, but these overlapping Y* elements are not counted separately in the REP tabulation. The serially numbered REP clusters (BIMEs) identified in the MG1655 genome sequence are denoted R1 to R355 directly under the restriction map portion of EcoMap10 along with the minute position labels.

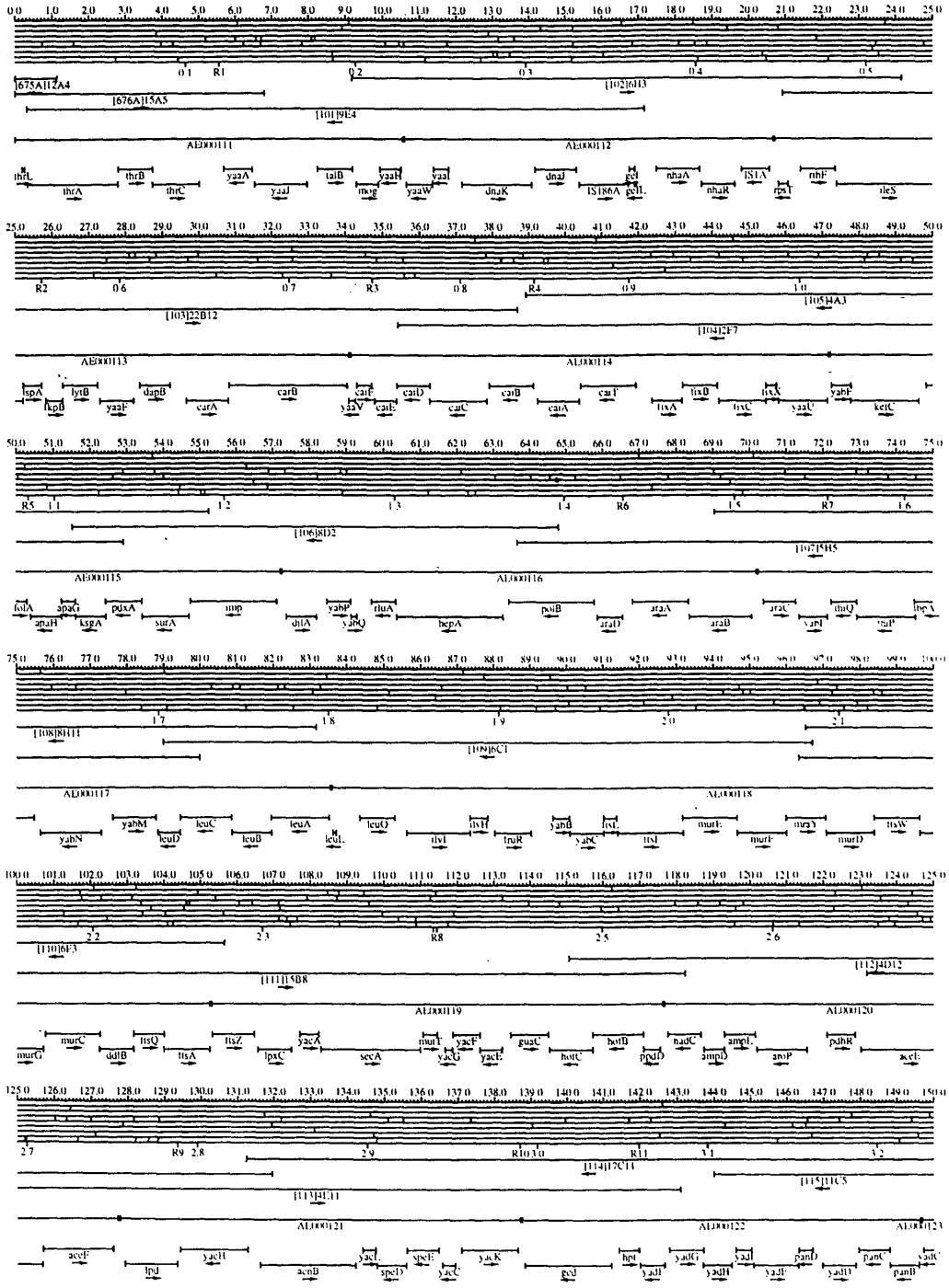
Genes and ORFs

A detailed description of the annotation of genes and functionally uncharacterized ORFs in EcoGene10 is presented in a separate publication (16). The entire genome sequence annotation of protein coding regions has been reviewed, and revisions have been made to approximately 15% of them. The most frequent revisions were the choice of an alternative translation start site, although sequences encoding small proteins were added and deleted from the set of coding regions as well. These two areas were acknowledged as difficult aspects of protein coding region annotation (5), and the EcoGene annotation should be thought of as one view of the *E. coli* K-12 genome. Producing a set of predicted protein sequences as accurately as possible was the goal of the reannotation effort, but experimental verification is the only way to establish the coding regions definitively. Published experimental data was used to establish gene intervals as much as possible. Anyone wishing to communicate additional prepublication information directly is encouraged to do so, especially if he or she has no objection to the information being made publicly available in the EcoGene and SWISS-PROT databases as a personal communication. The *E. coli* genome sequence annotation refinement has been a close collaboration with the curator of the SWISS-PROT database, Amos Bairoch.

Partial or frameshifted ORFs and genes are marked in Fig. 1 (see the figure legend). In most cases, but not all, the presence of a frameshift or deletion is based on sequence analysis alone and thus should be considered a prediction. It is not known if any particular putative frameshift or deletion is the result of a DNA sequencing error, a cloning artifact, an adaptation to the laboratory environment, natural evolutionary pressure, or pseudogene formation. Errors introduced during the reannotation process are also possible, and everyone is encouraged to contact this author or SWISS-PROT if he or she thinks an error has been made; we will take appropriate steps to update our databases. These sequence-based frameshift predictions should assist in the experimental determination of the source of the frameshifts.

The traditional and physical maps of edition 10 of the *Esch-*

FIG. 1. EcoMap10, a DNA sequence-derived map depicting restriction sites, Kohara/Isono clones, genes, ORFs, IS elements, and REP clusters of the *E. coli* K-12 chromosome. The derivation of this map from the complete genome sequence of *E. coli* K-12 strain MG1655 is briefly described in the text. The map depicts sites for eight restriction enzymes (top line to bottom line: *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II). Above the restriction map are position coordinates in kilobases; immediately below the map are minute coordinates (in 0.1-min increments). Also immediately below the map are the designations R1 to R355 referring to the 355 serially numbered REP clusters, placed at the genomic position of the base pair at their left ends. Some minute designations were omitted as they overlapped with the REP serial numbers, but the tick marks for these unlabeled 0.1-minute positions are present, and their values can be easily determined from the flanking minute values. The first set of spanning lines below the map represent the genomic positions and clone insert orientations of the Kohara miniset clones. Those Kohara miniset clone W3110 chromosomal DNA inserts that have been completely sequenced are additionally labeled with their GenBank/EMBL/DDBJ accession numbers, D90699 to D90892 (1, 9, 13, 22). The second set of spanning lines, labeled with database accession numbers AE000111 to AE000510, represent the locations of the GenBank/EMBL/DDBJ complete-genome MG1655 sequence entries of Blattner et al. (5). The third set of spanning lines depict the positions and orientations of the genes, ORFs, and IS elements that constitute EcoGene10. An asterisk following a gene or ORF name indicates that a frameshift or in-frame stop codon that prevents the EcoGene10 representation of the coding region from being translated is present in the genome sequence. A prime indicates a partial EcoGene entry, i.e., a deletion or IS element insertion is predicted to have disrupted the ancestral complete gene, ORF, or IS element. This figure was created by using the PrintMap Postscript drawing program, which implements the Plasmid Description Language developed by Craig Werner (18).



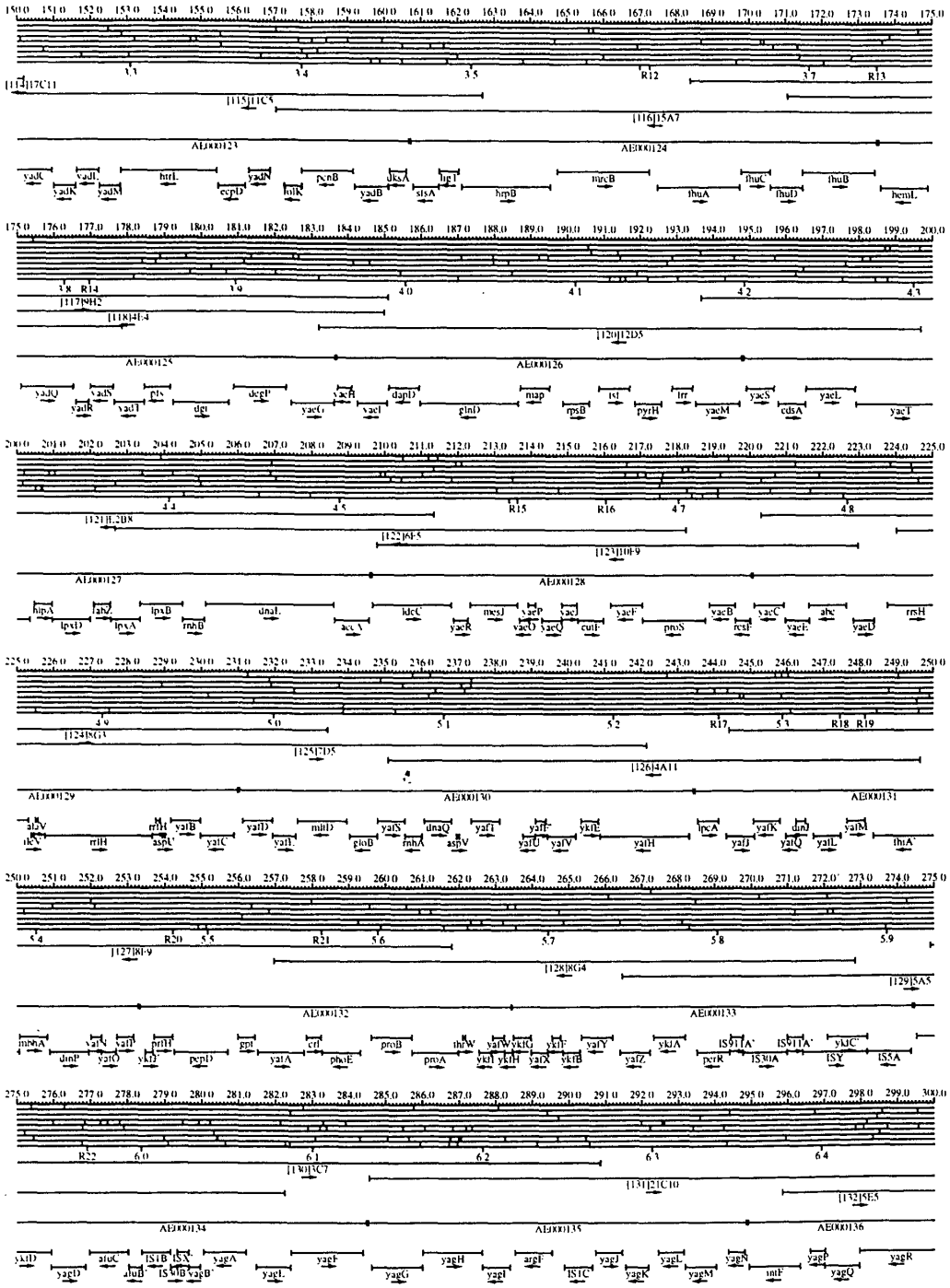


FIG. 1—*Continued.*

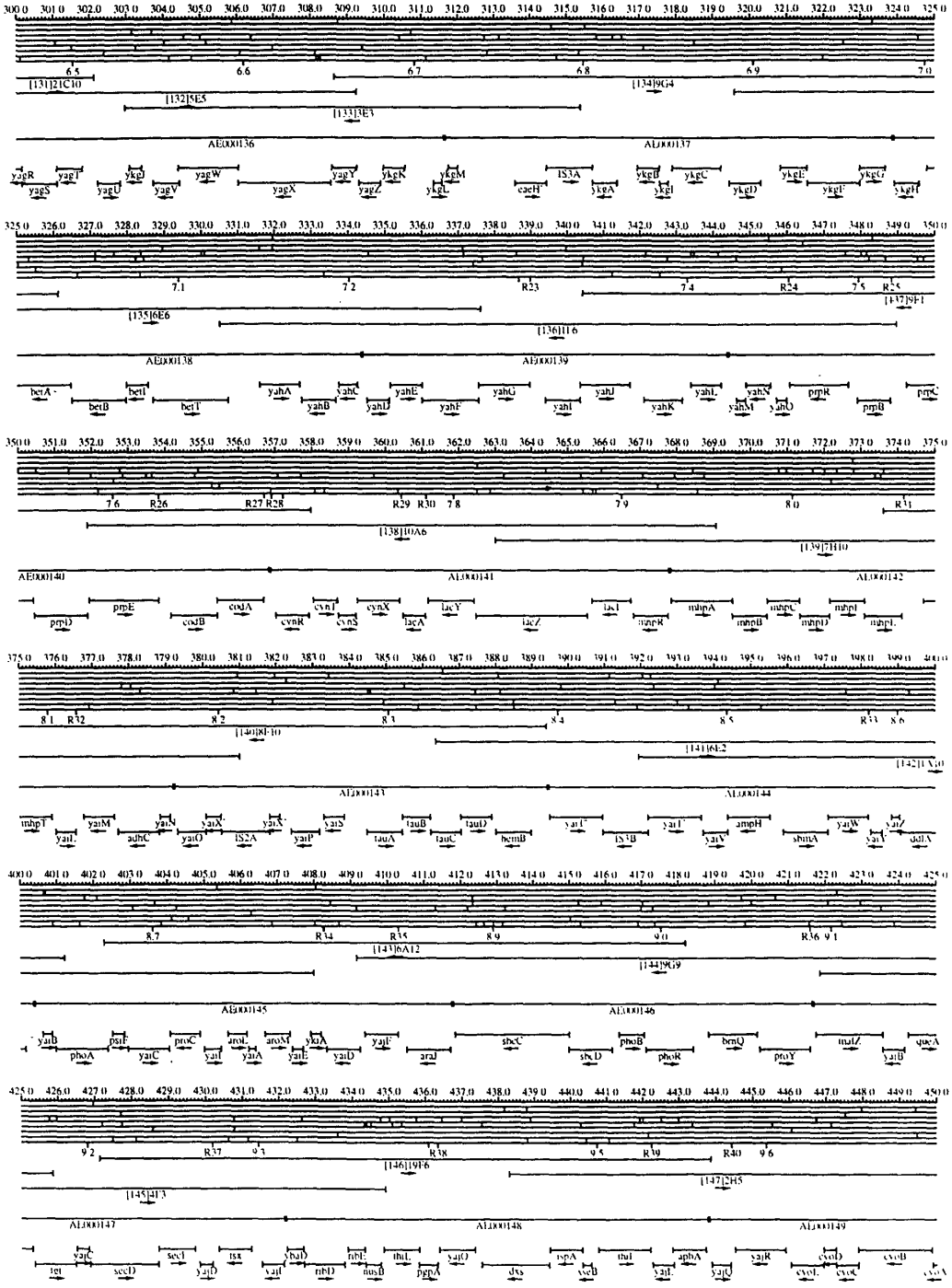


FIG. 1—*Continued.*

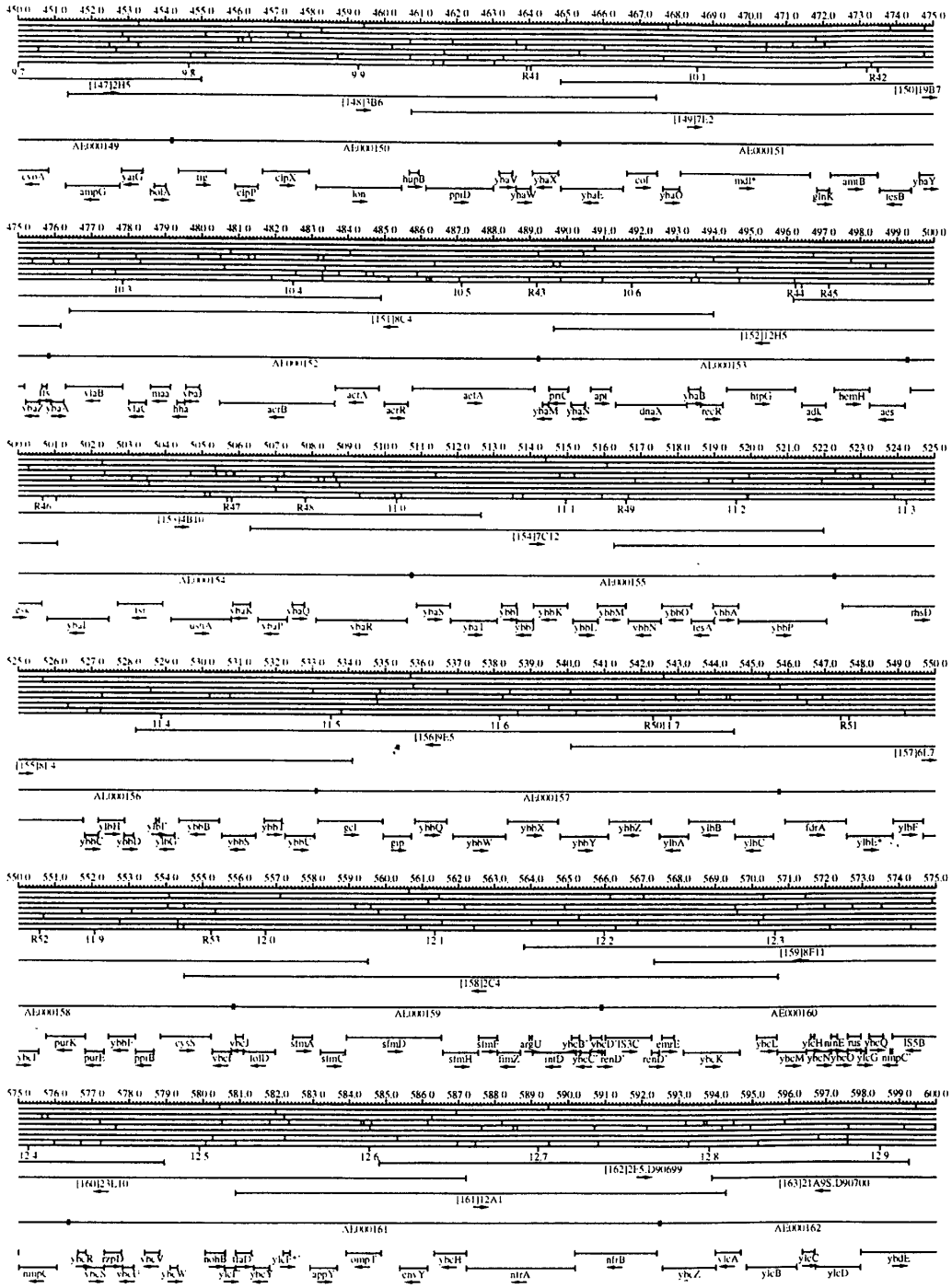


FIG. 1—*Continued.*

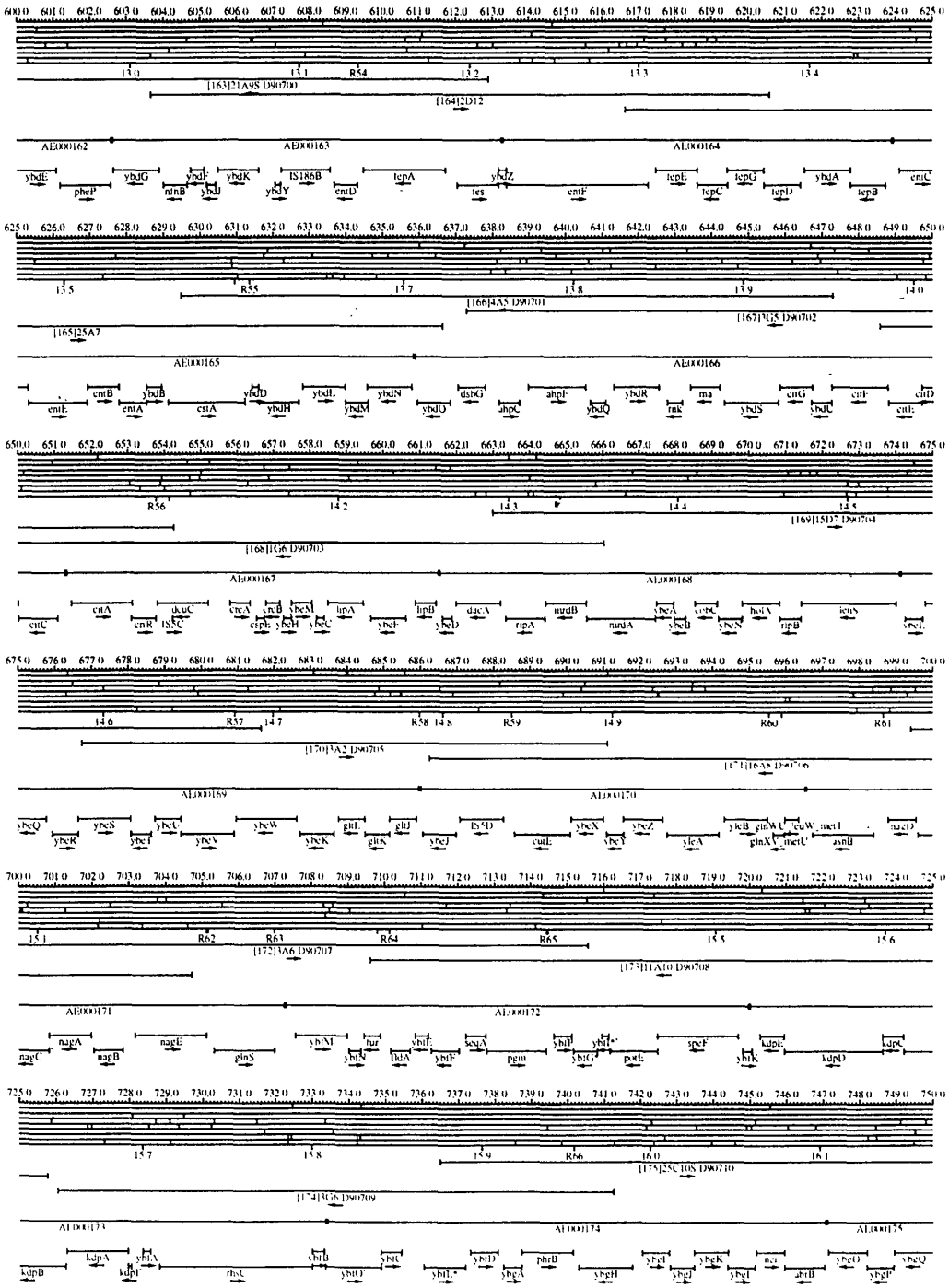


FIG. 1—*Continued.*

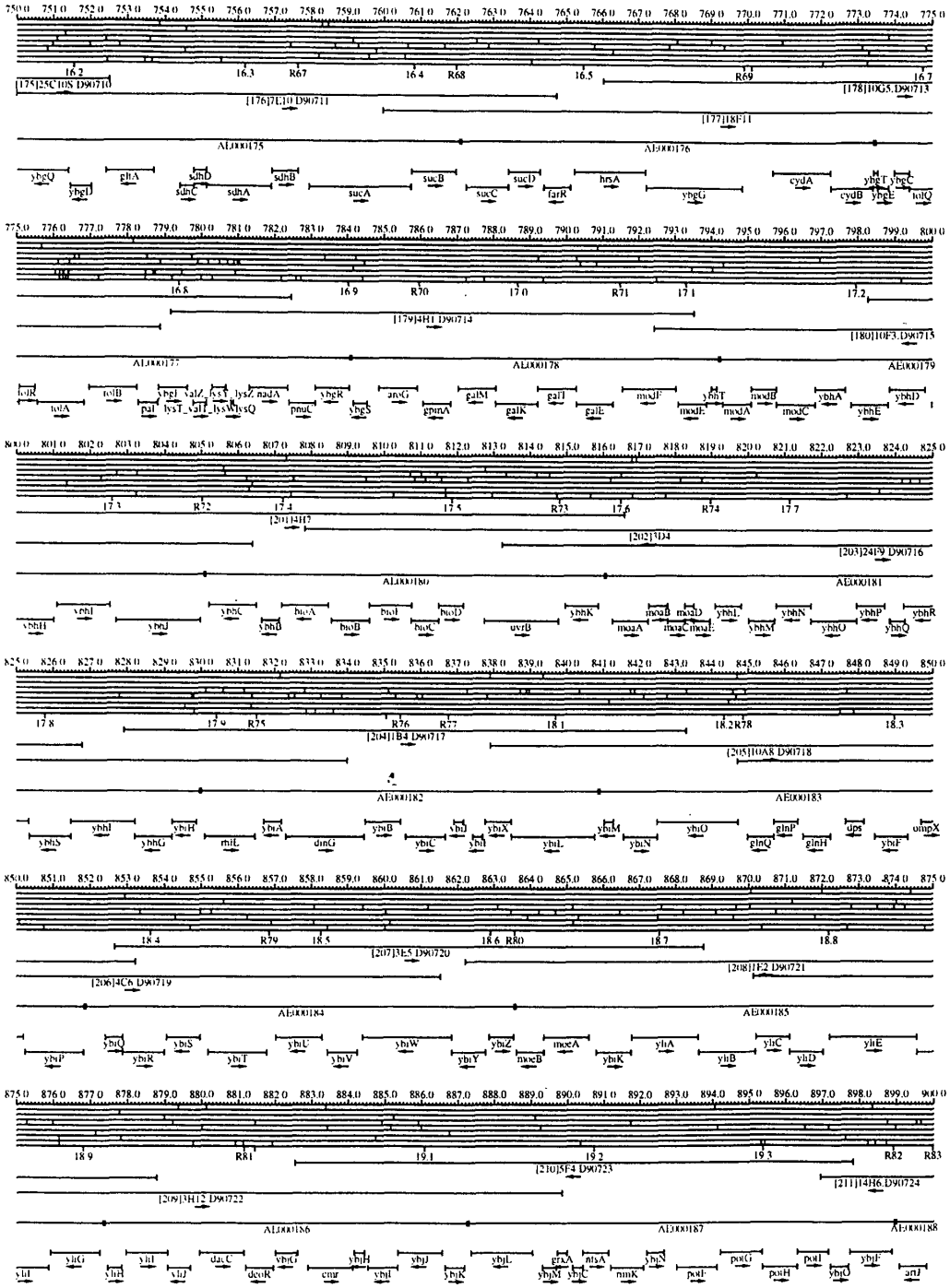


FIG. 1—*Continued.*

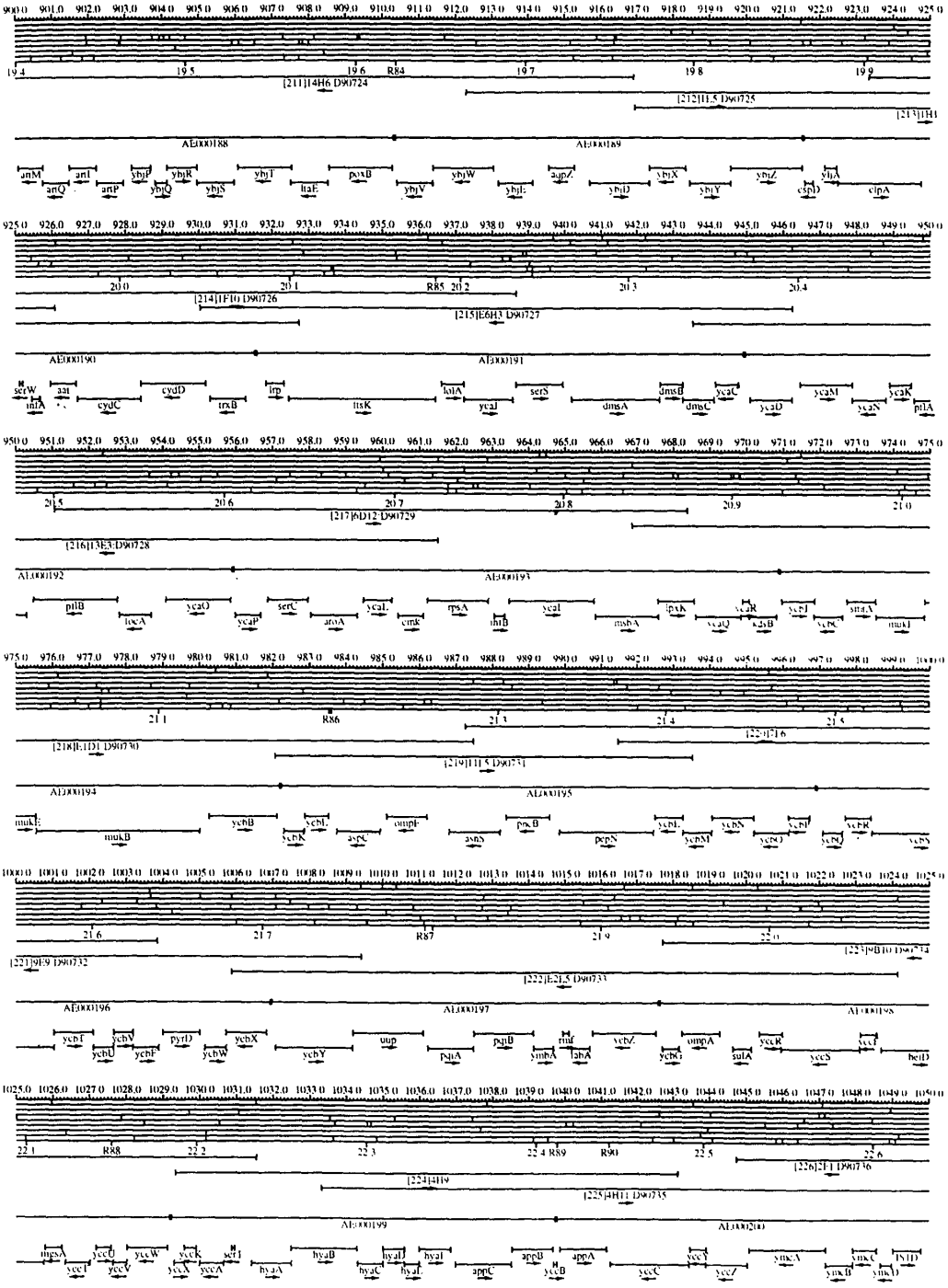


FIG. 1—*Continued.*

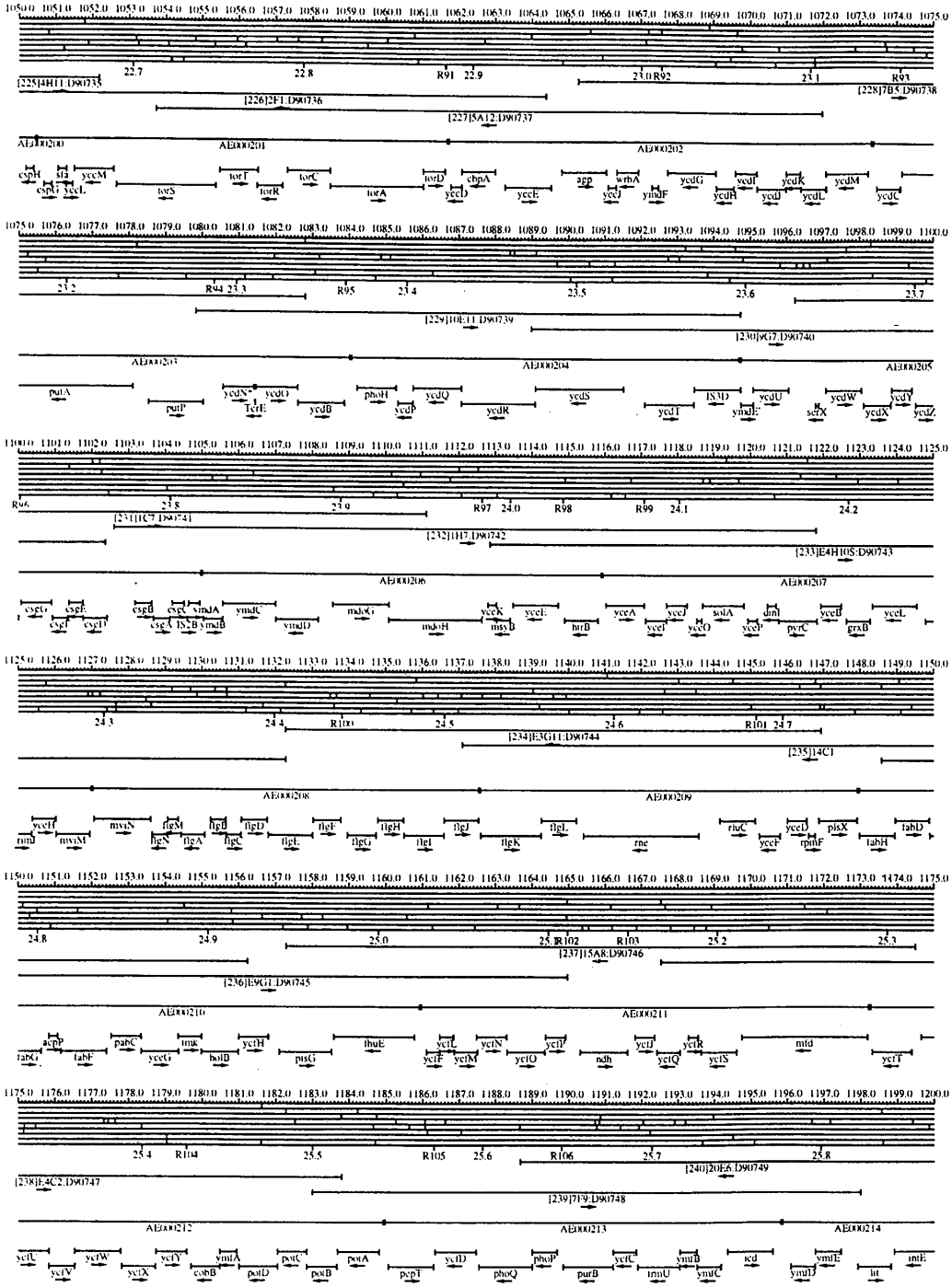
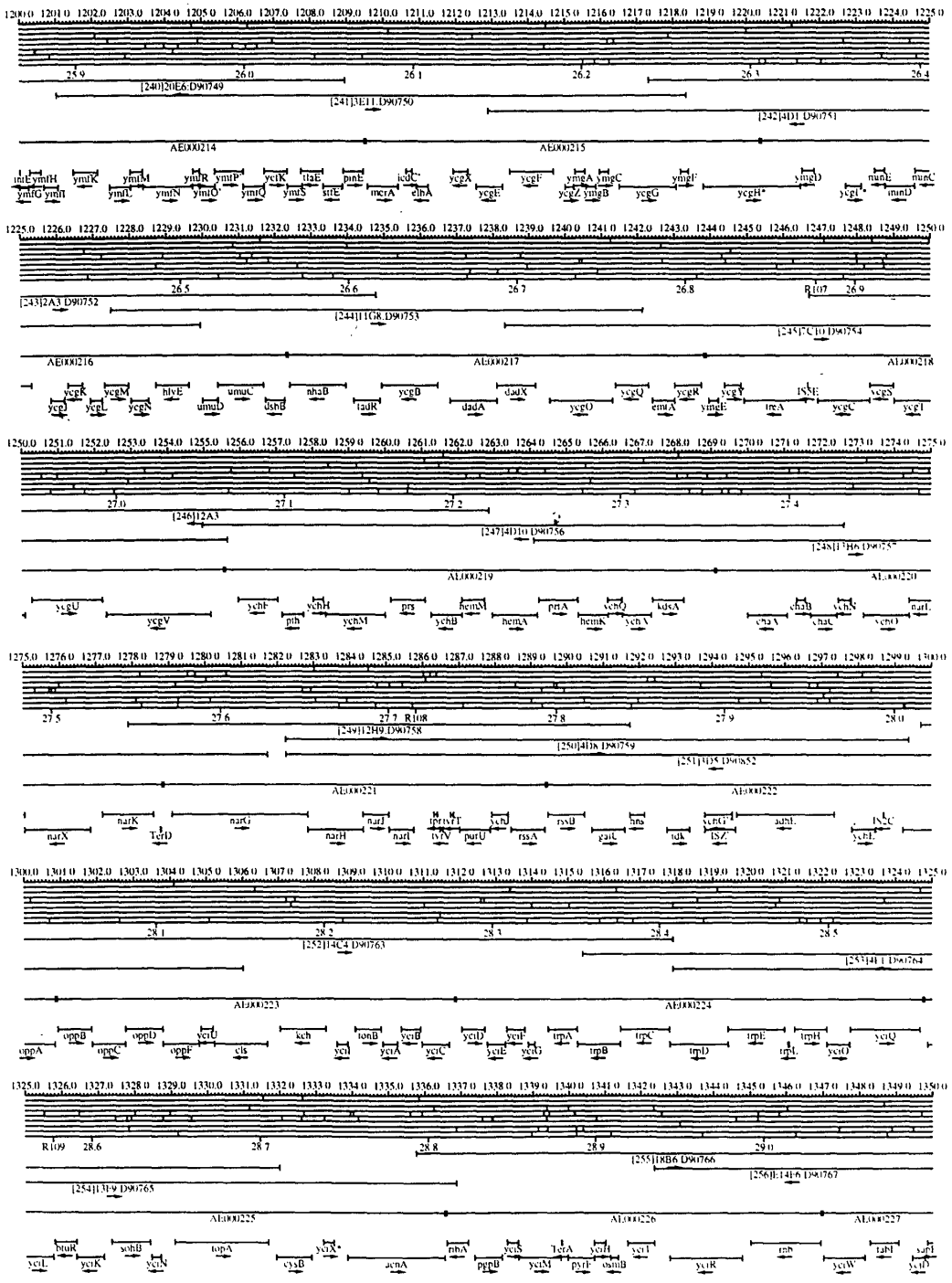


FIG. 1—*Continued.*



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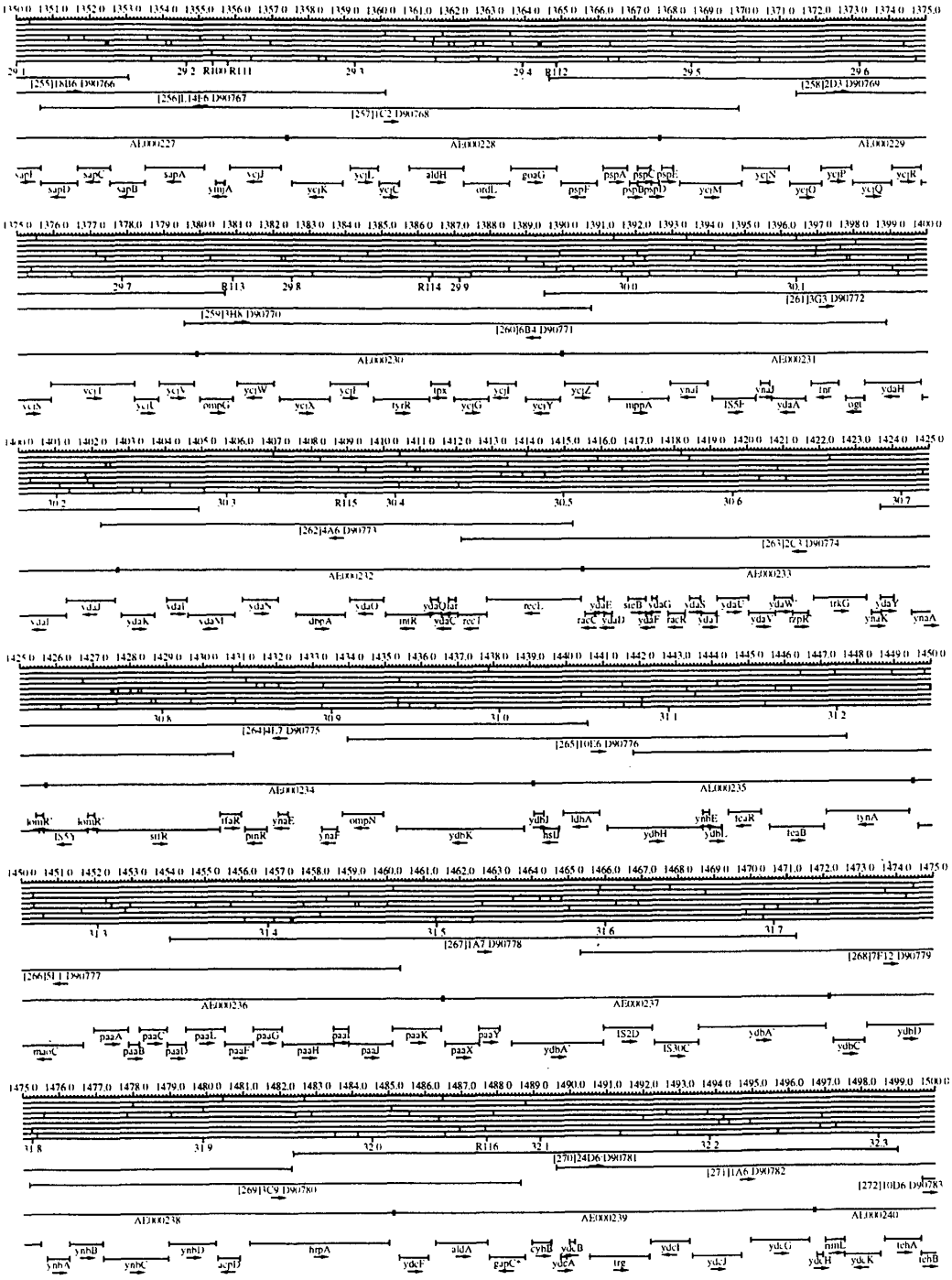

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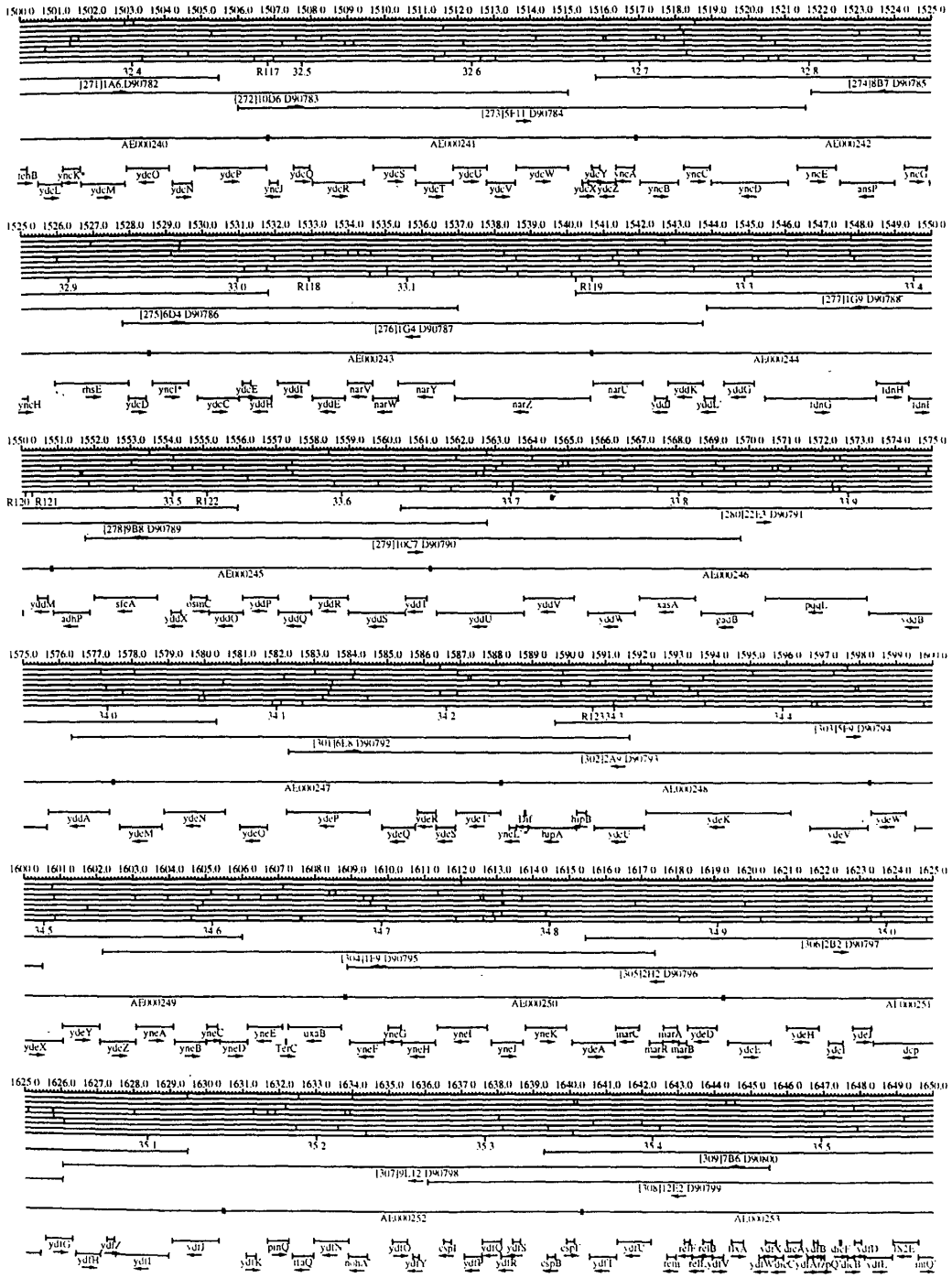

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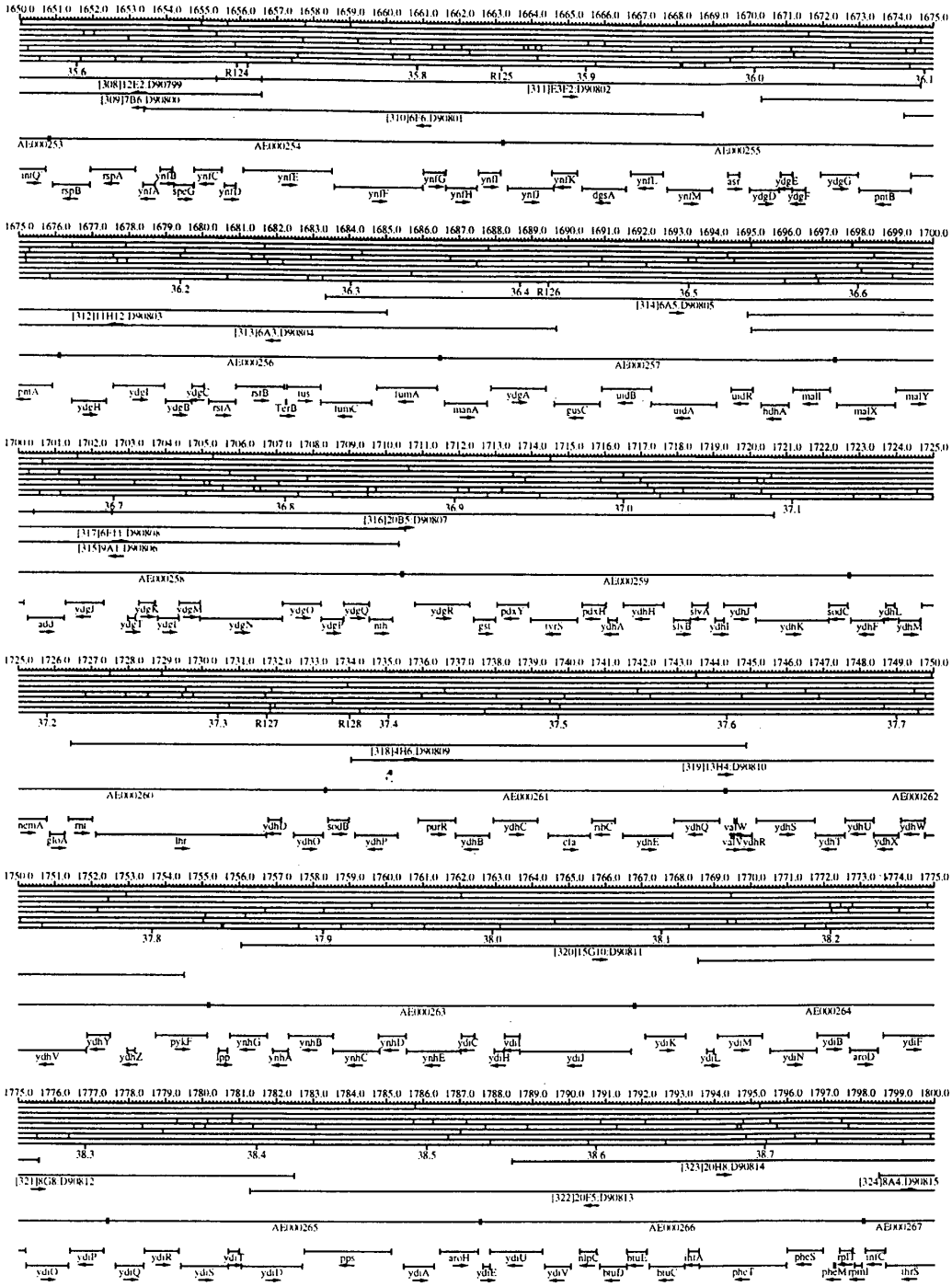

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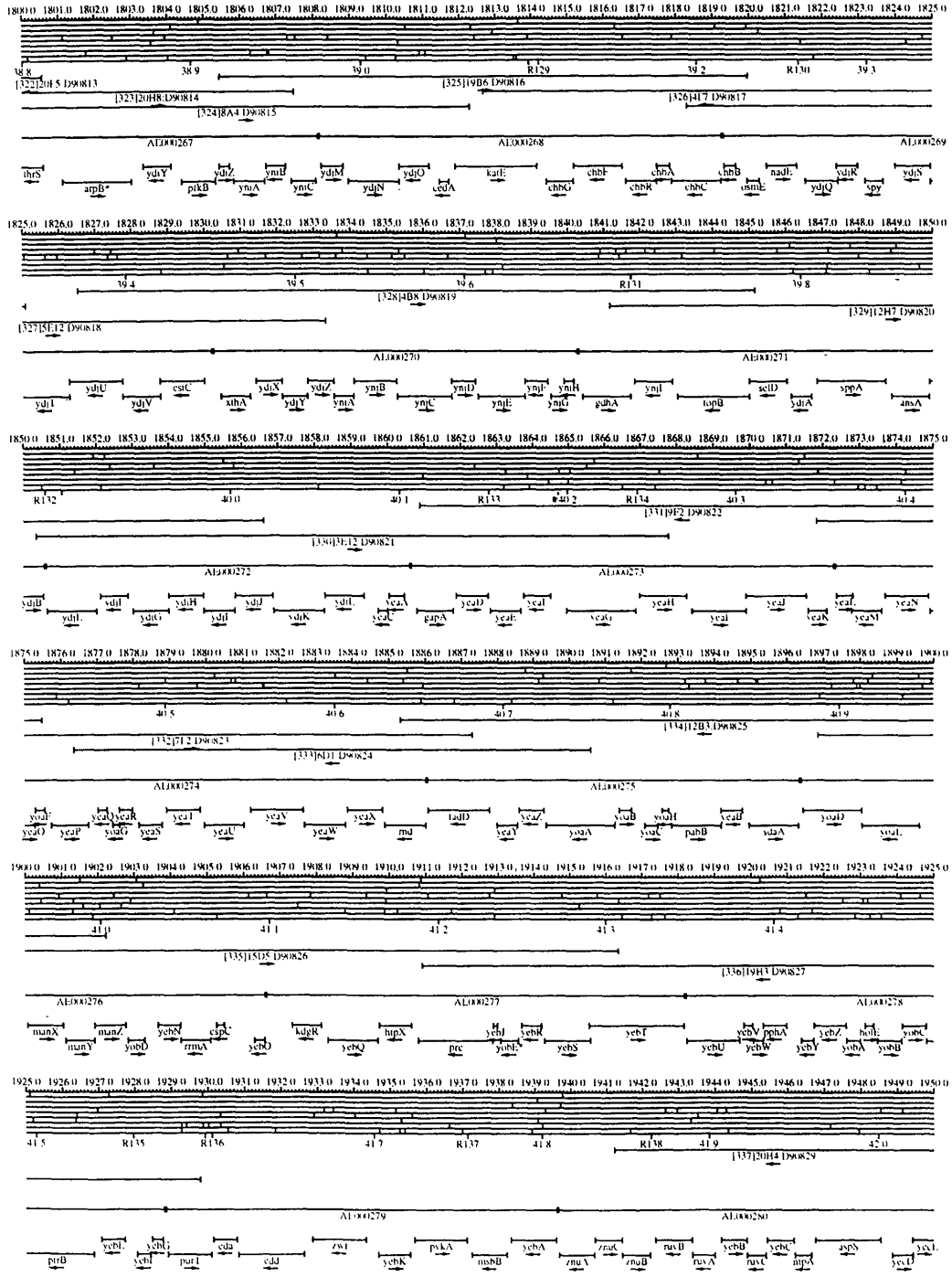

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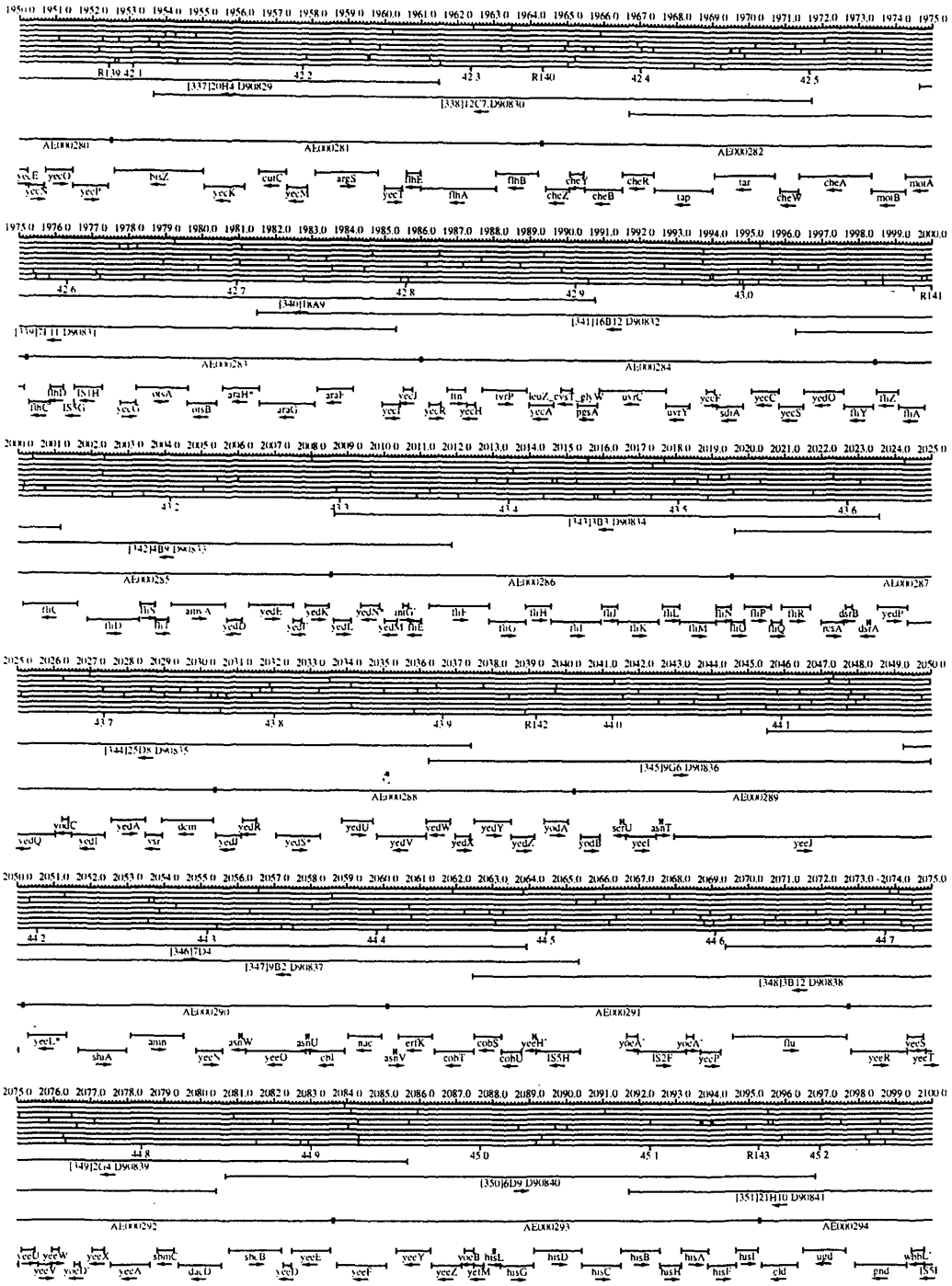

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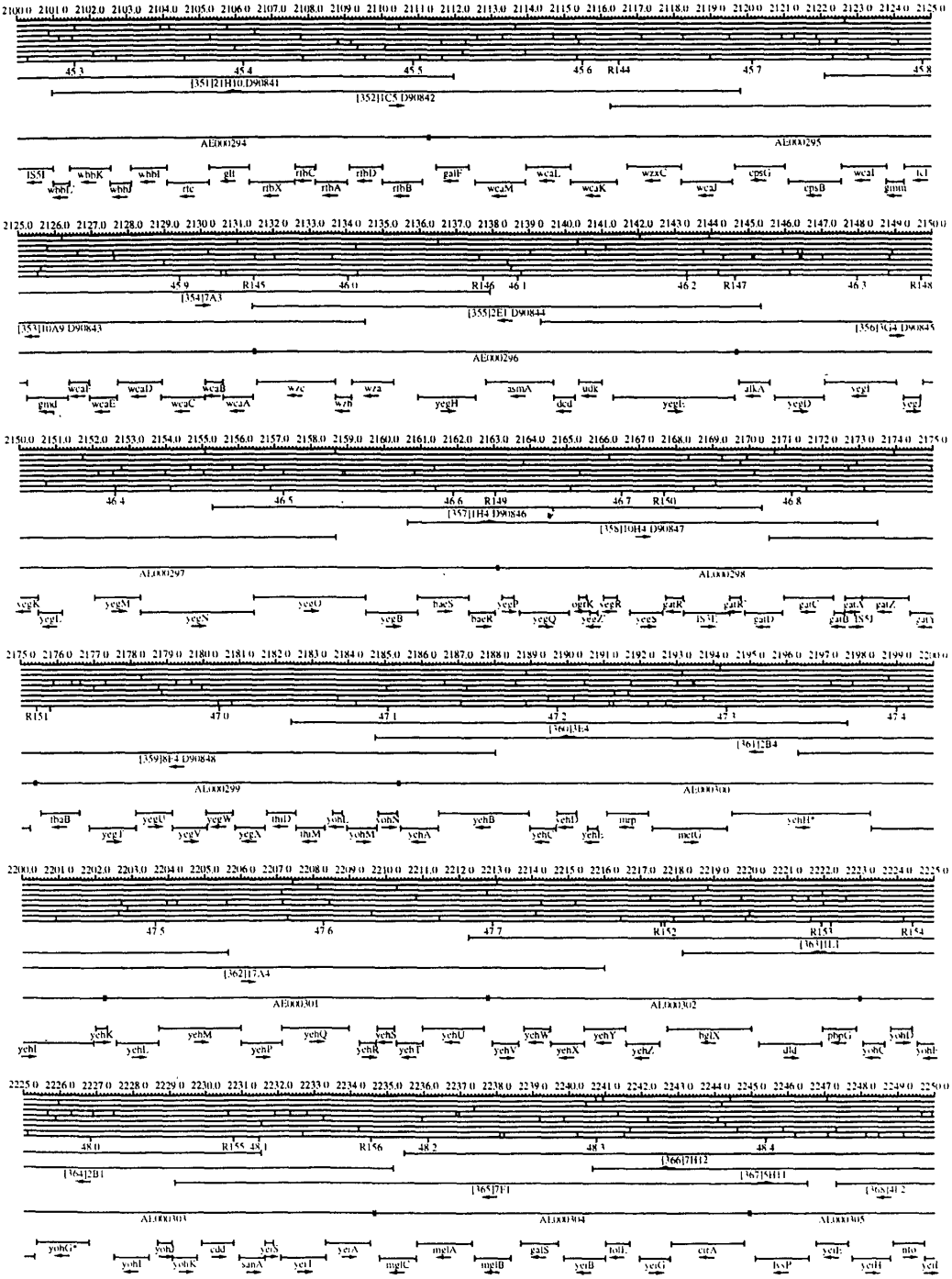

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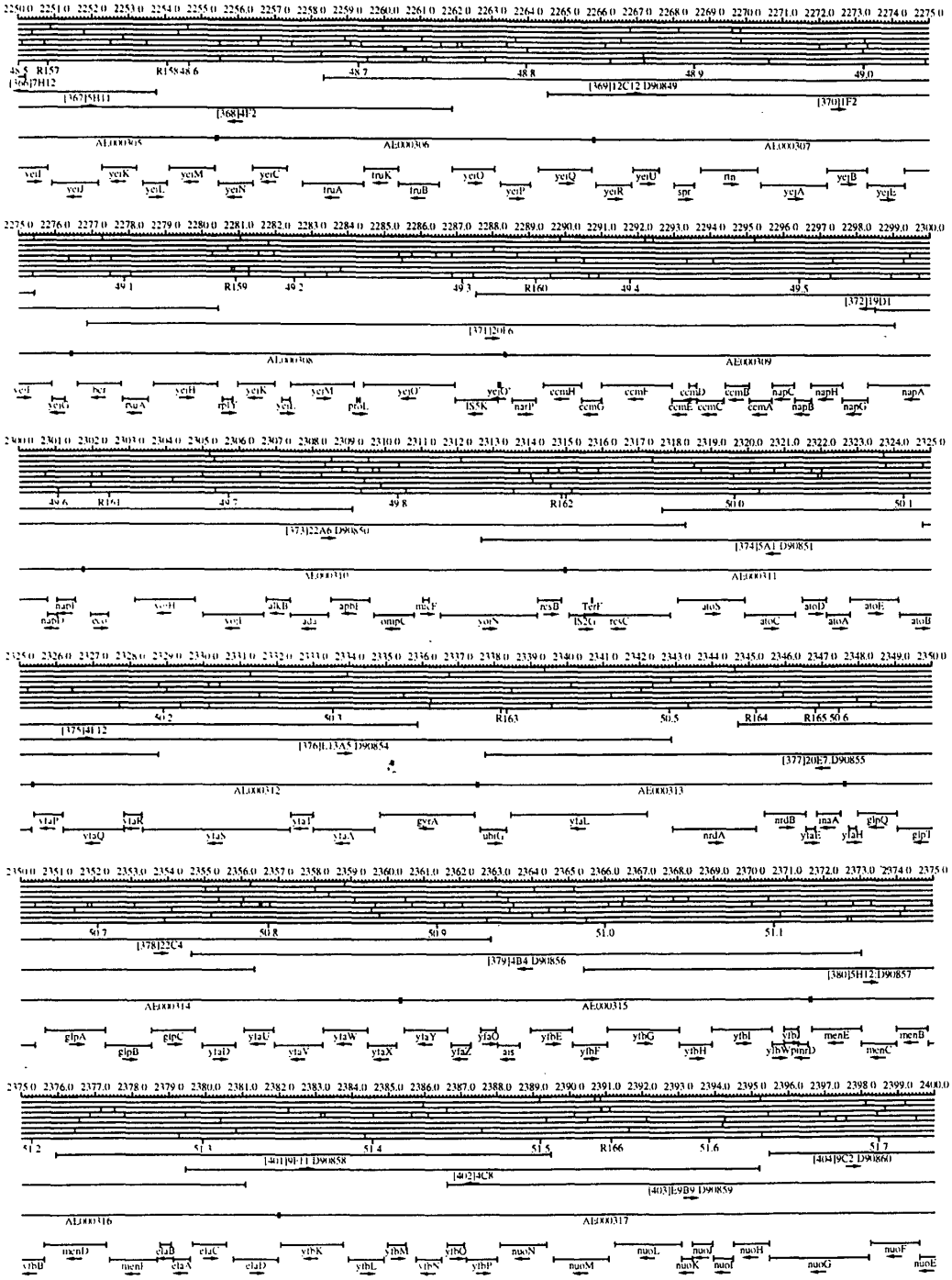

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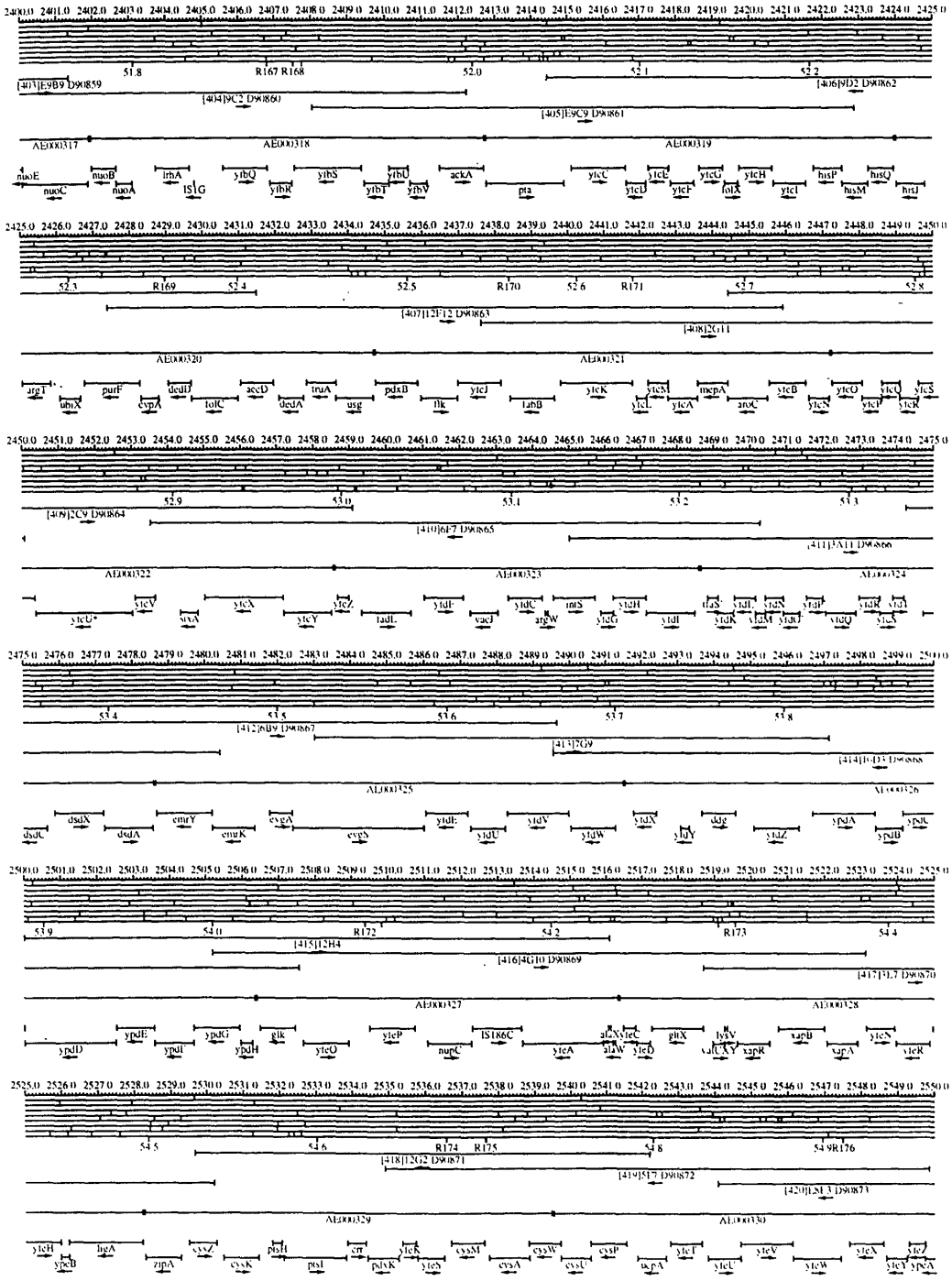

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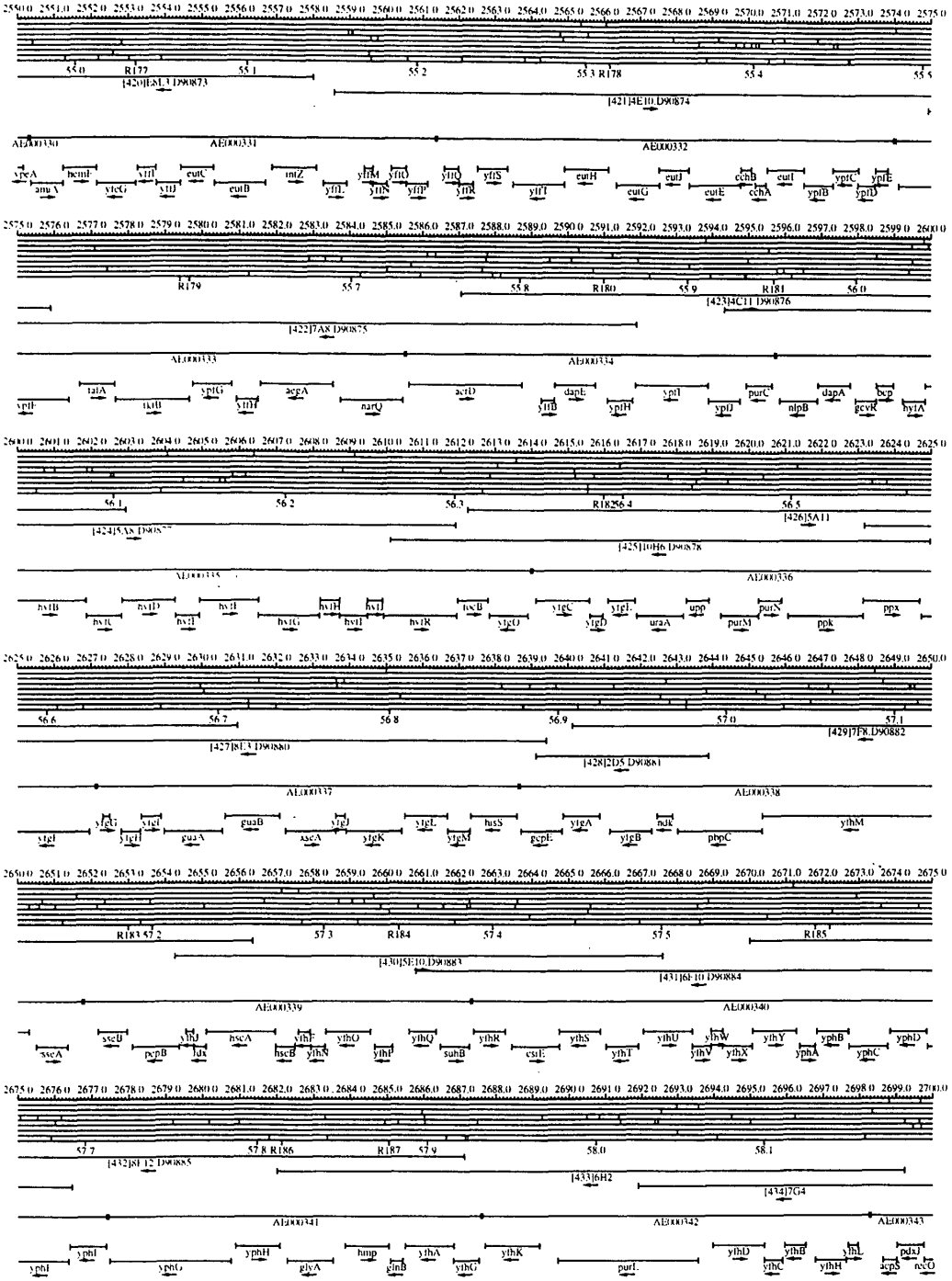

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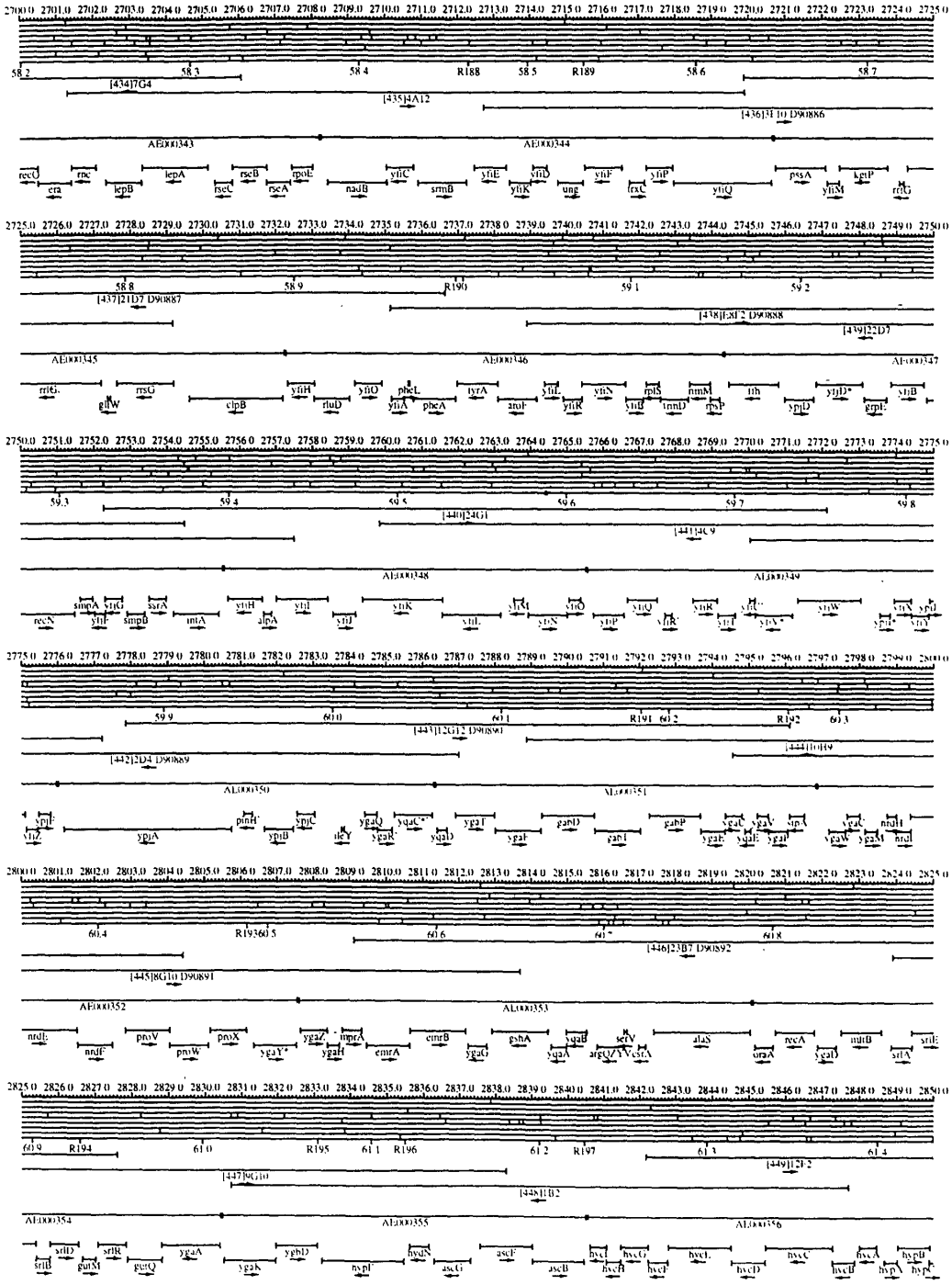

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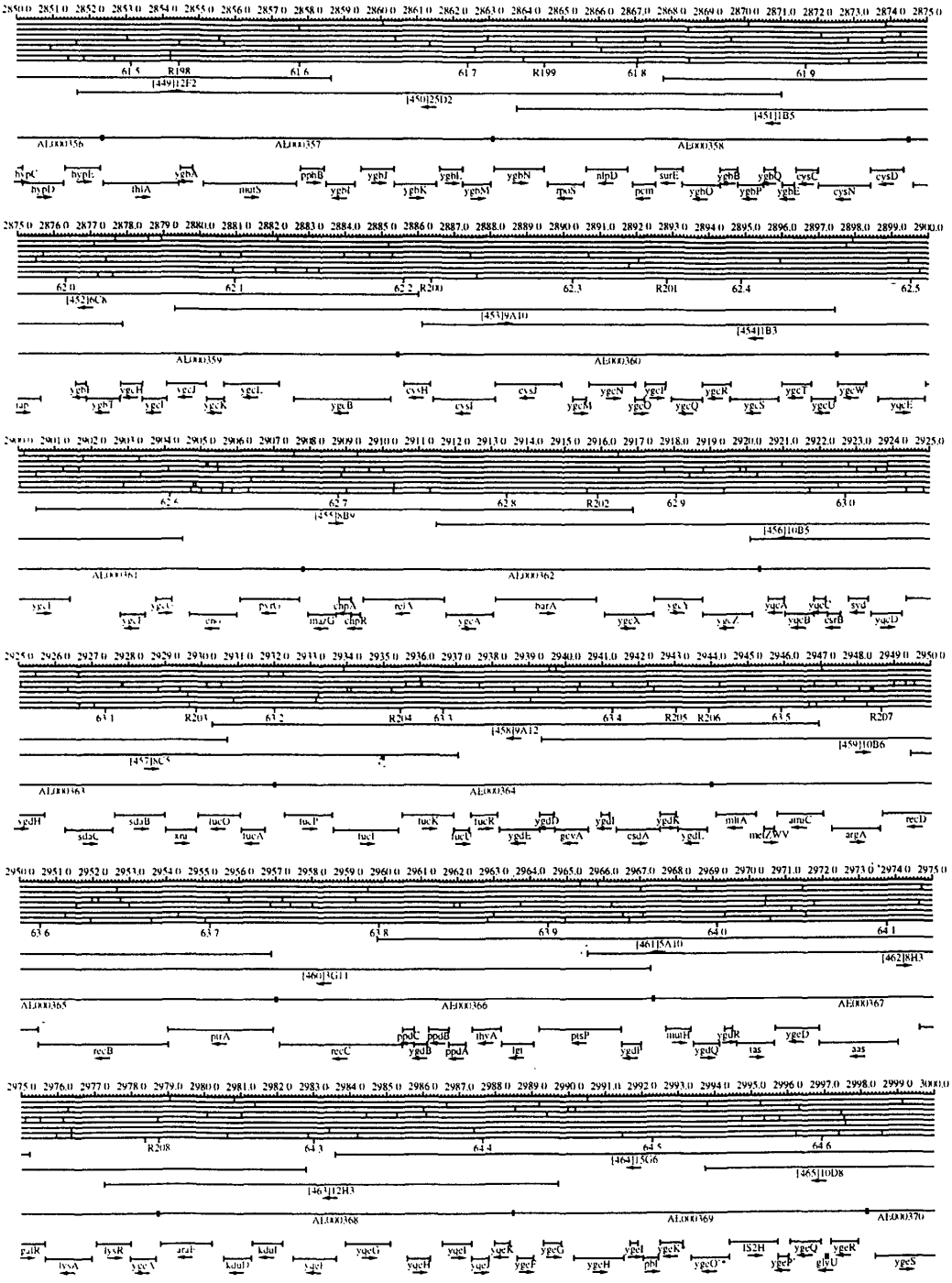

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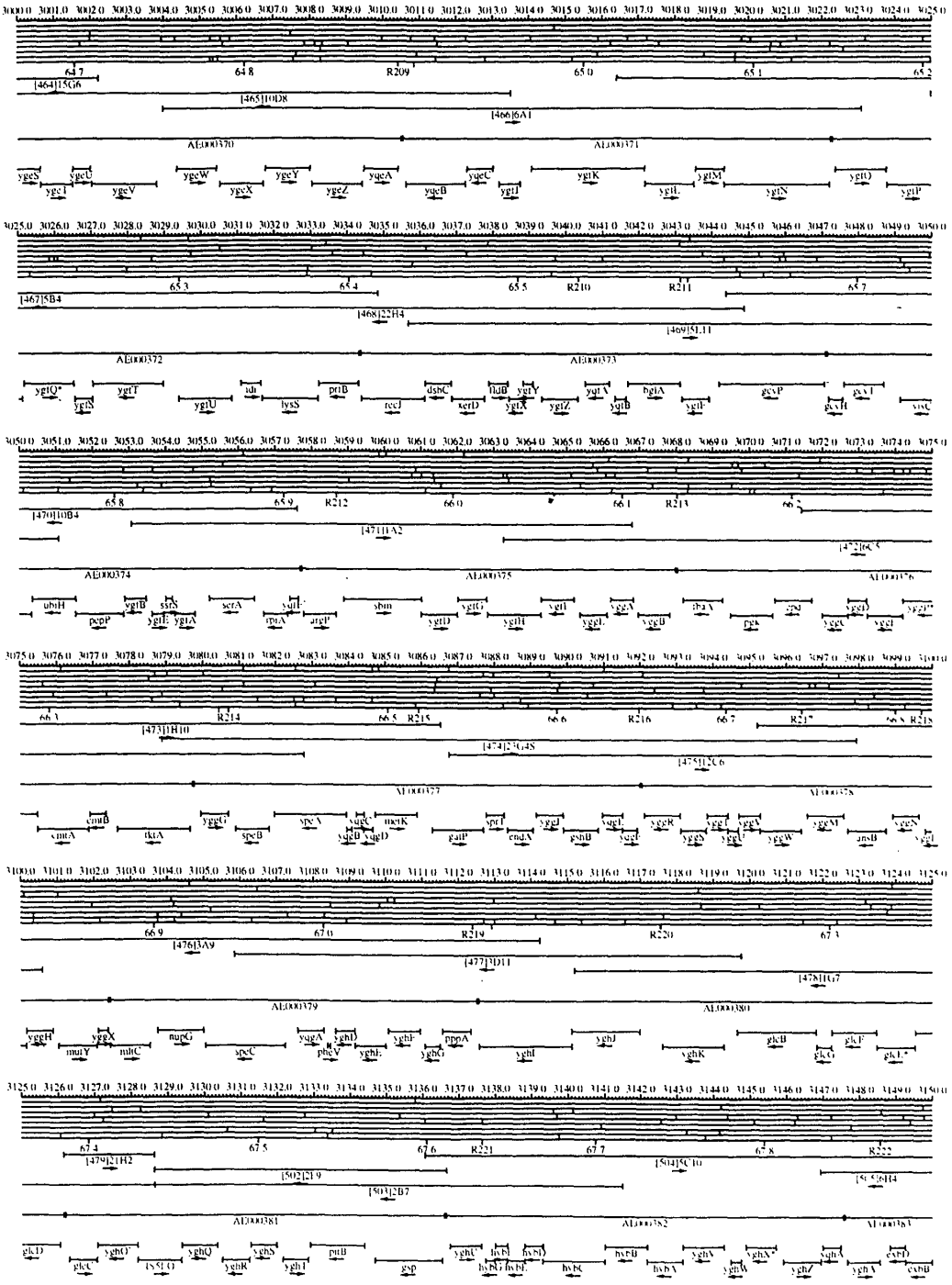

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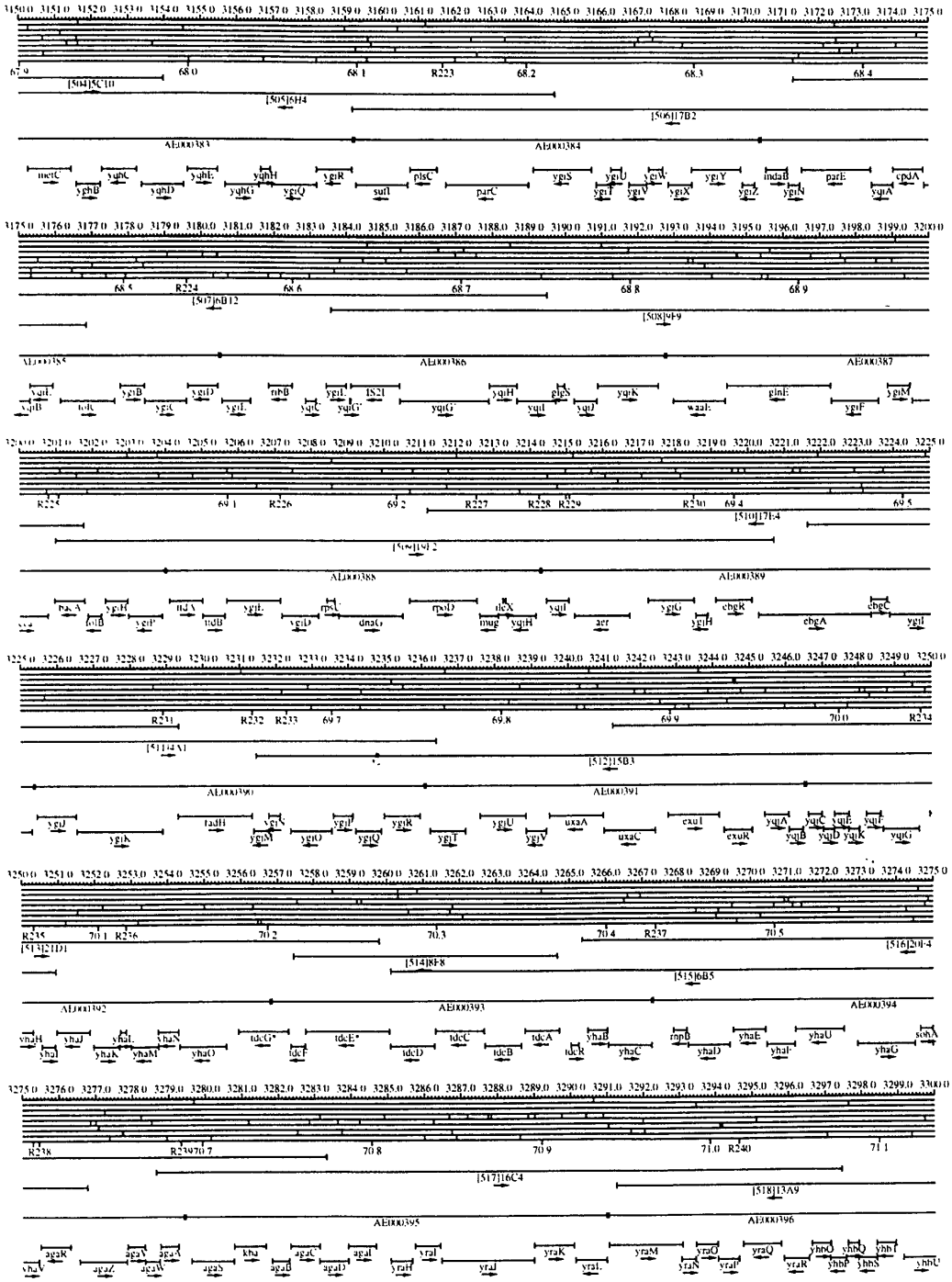

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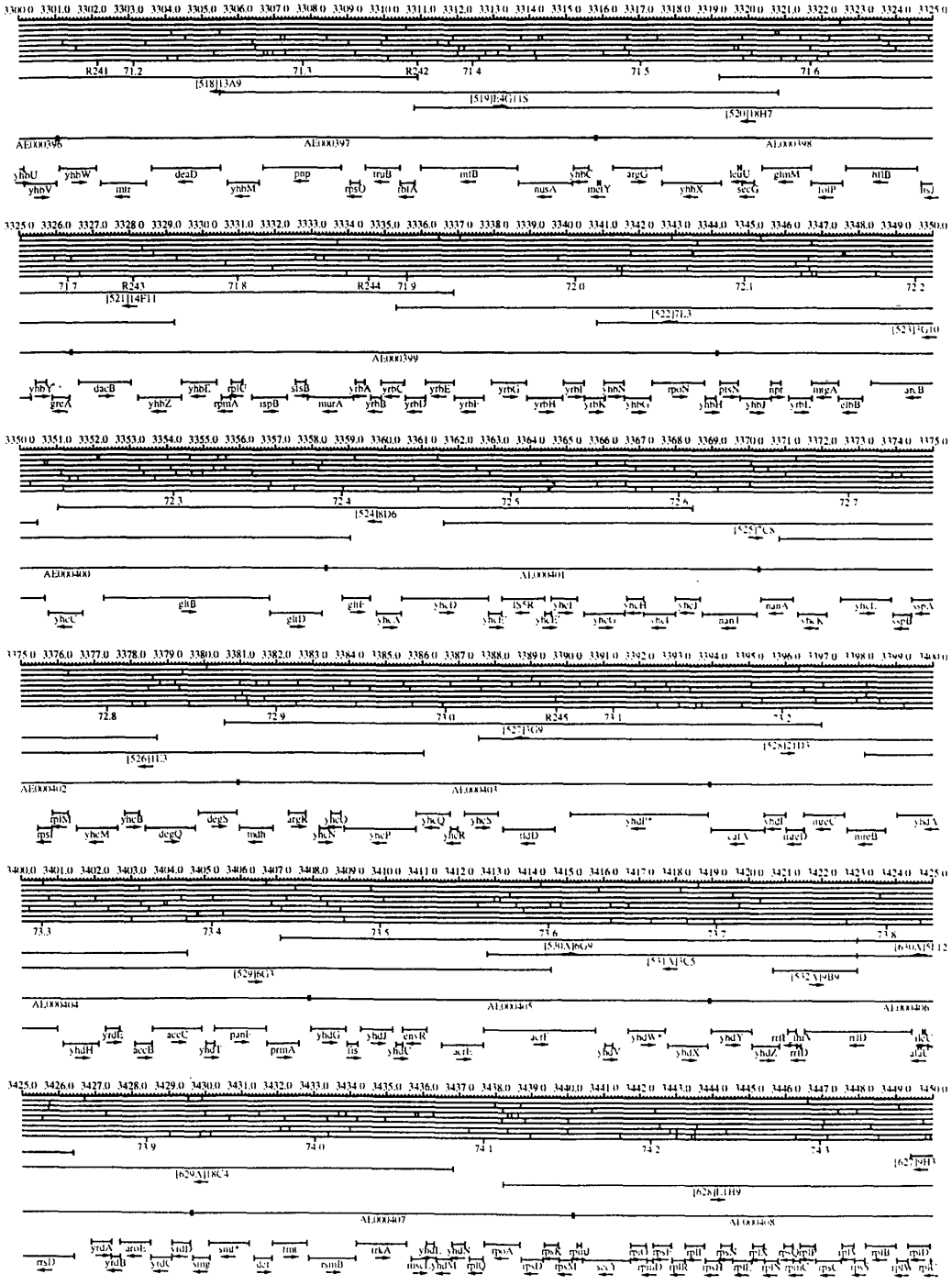

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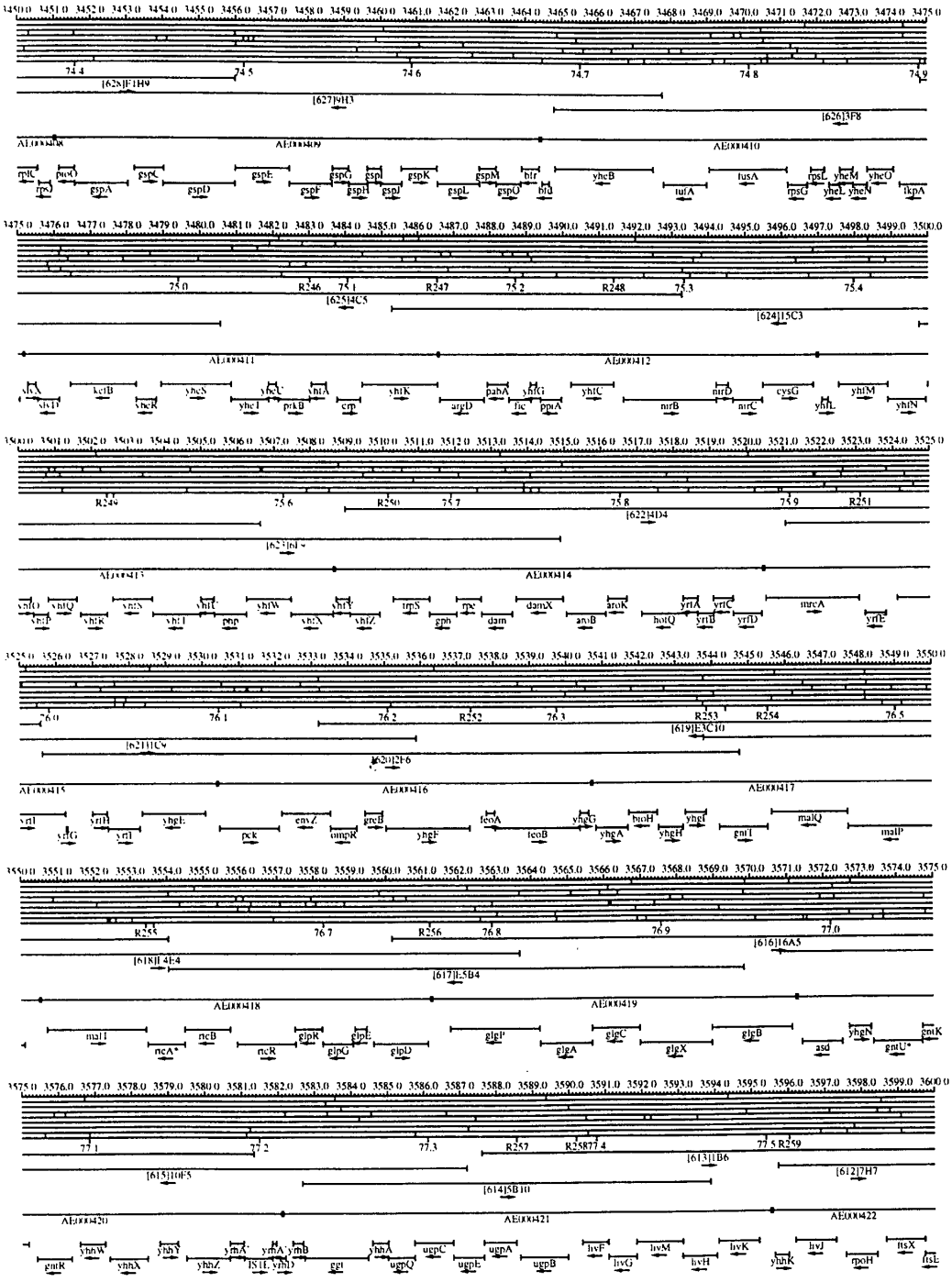

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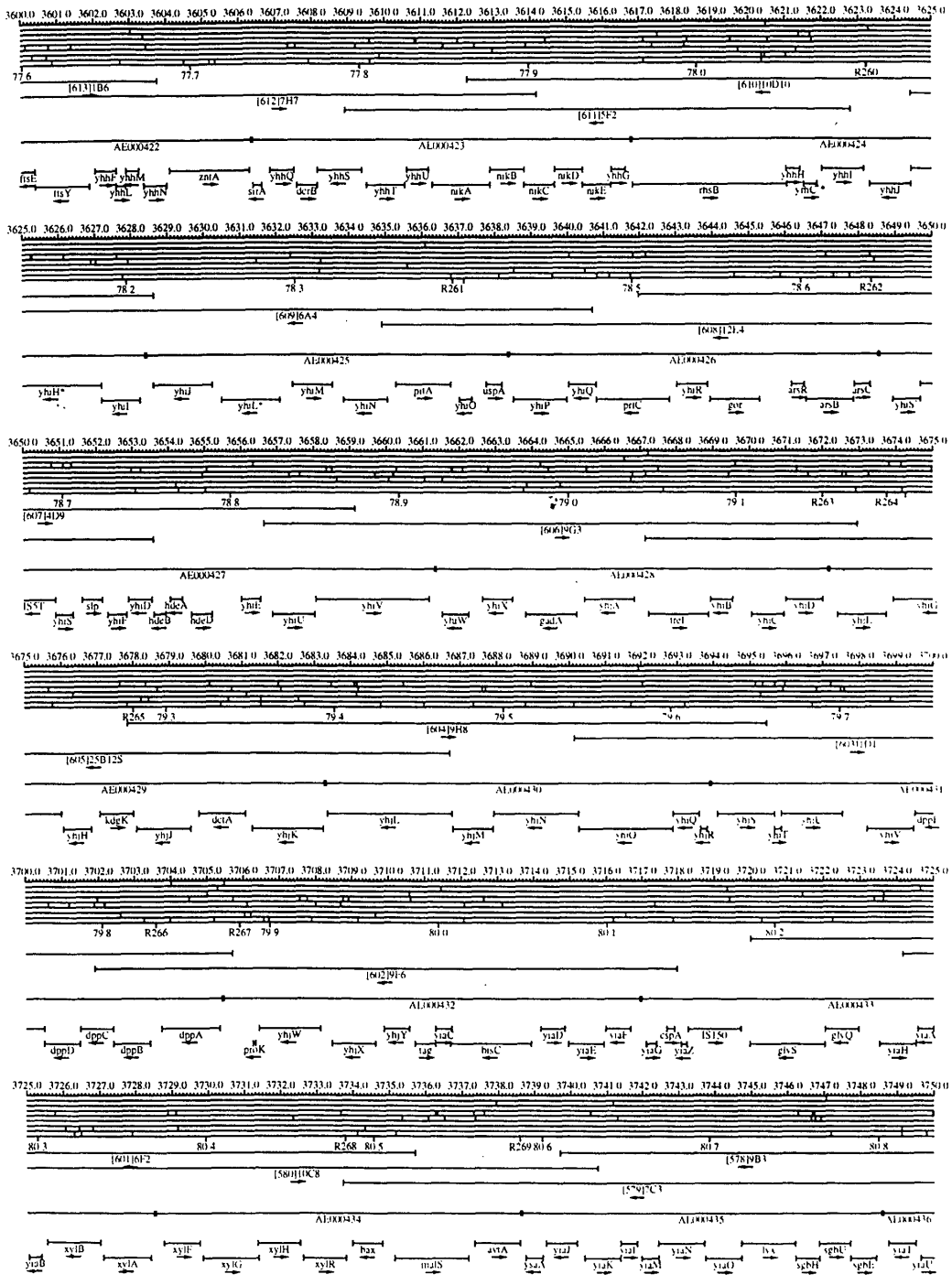

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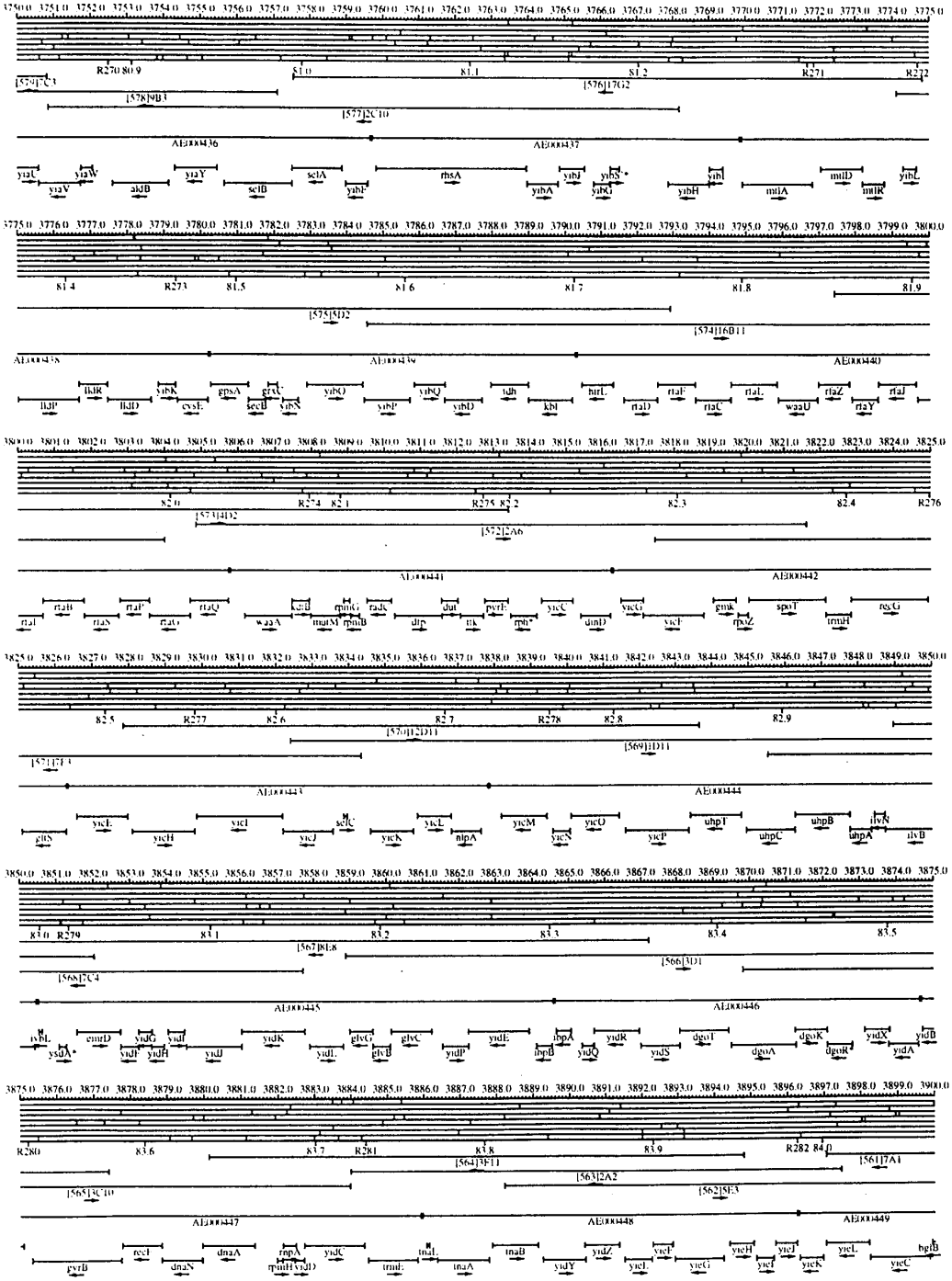

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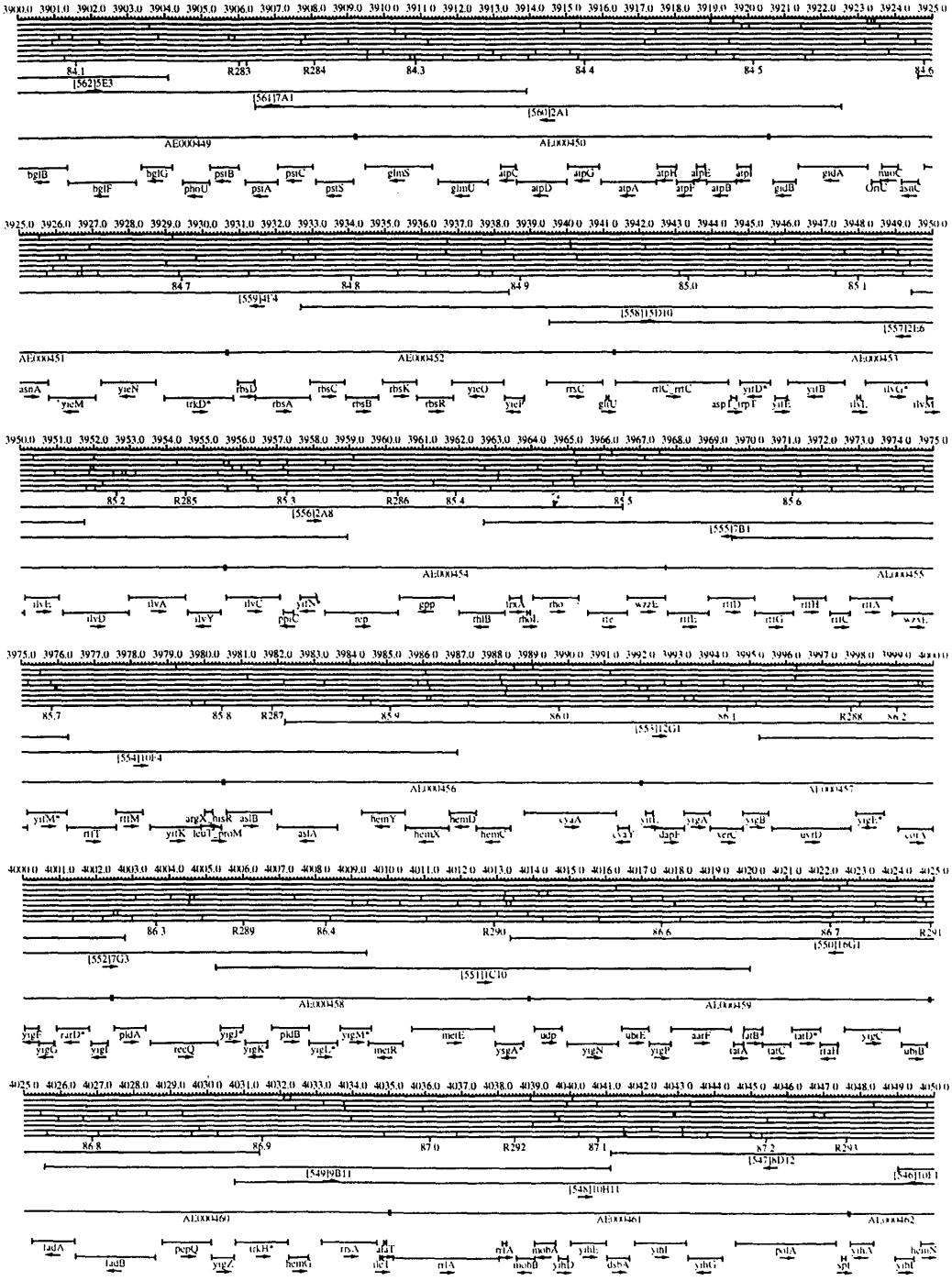
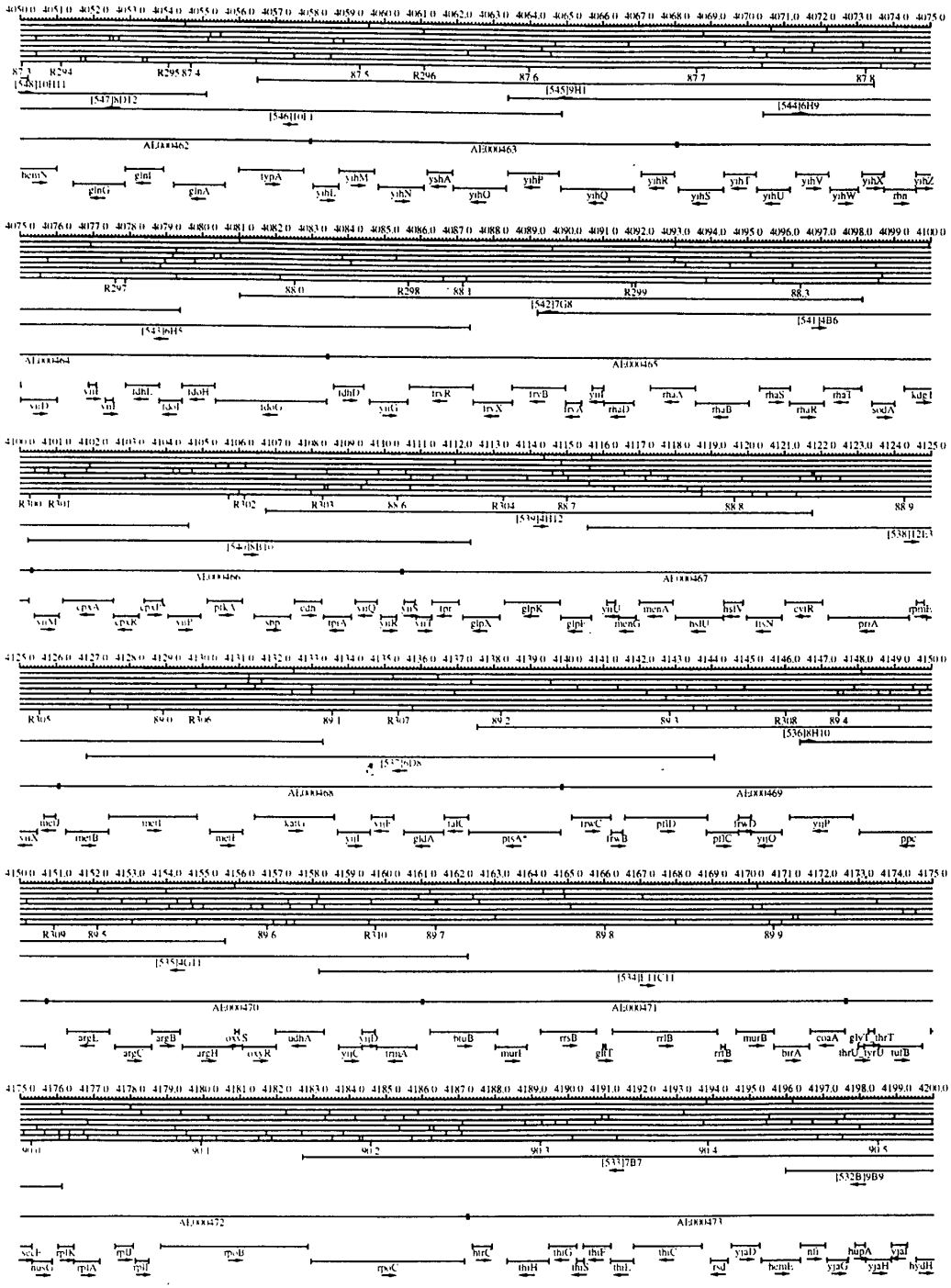


FIG. 1—*Continued.*



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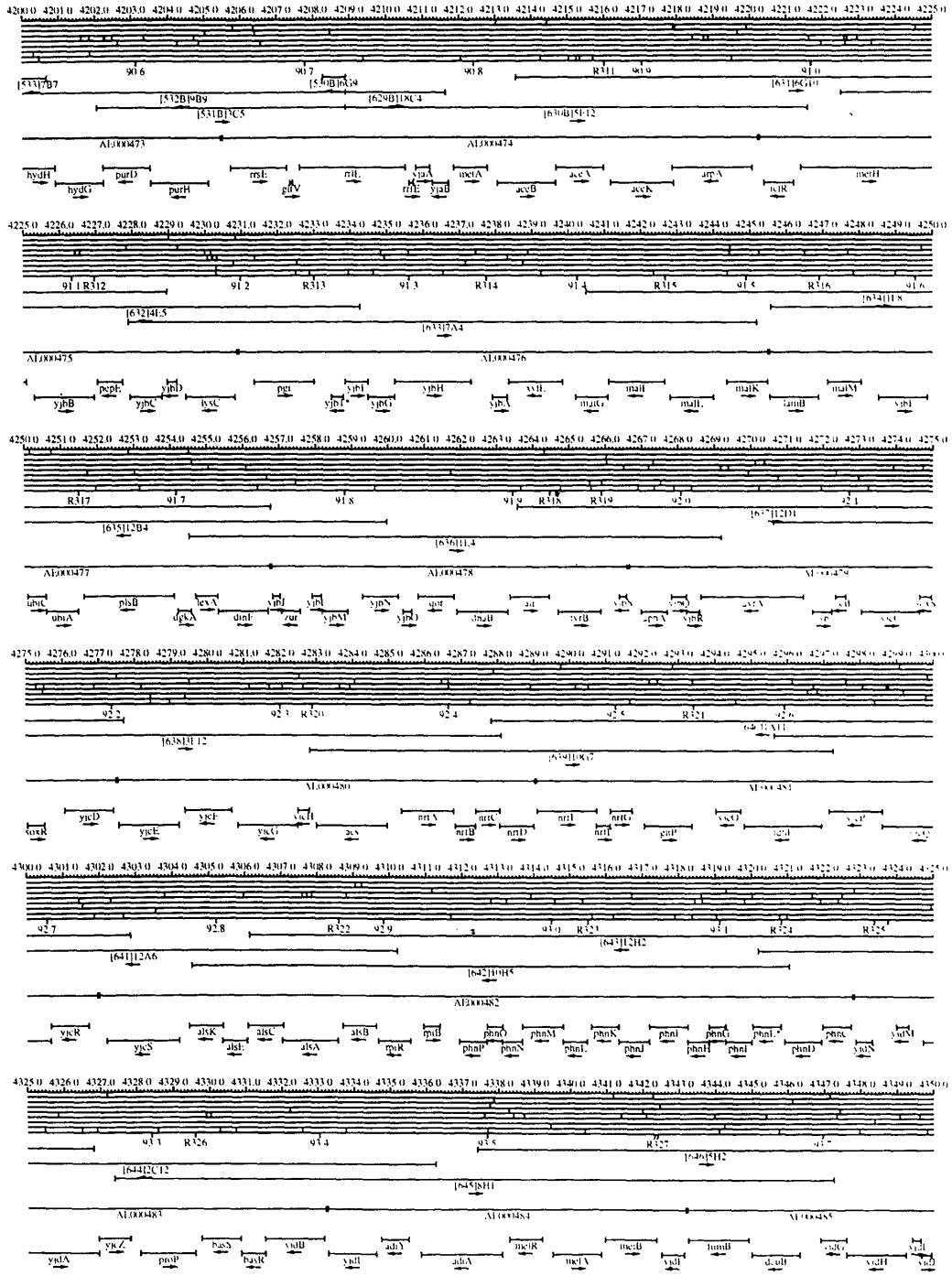

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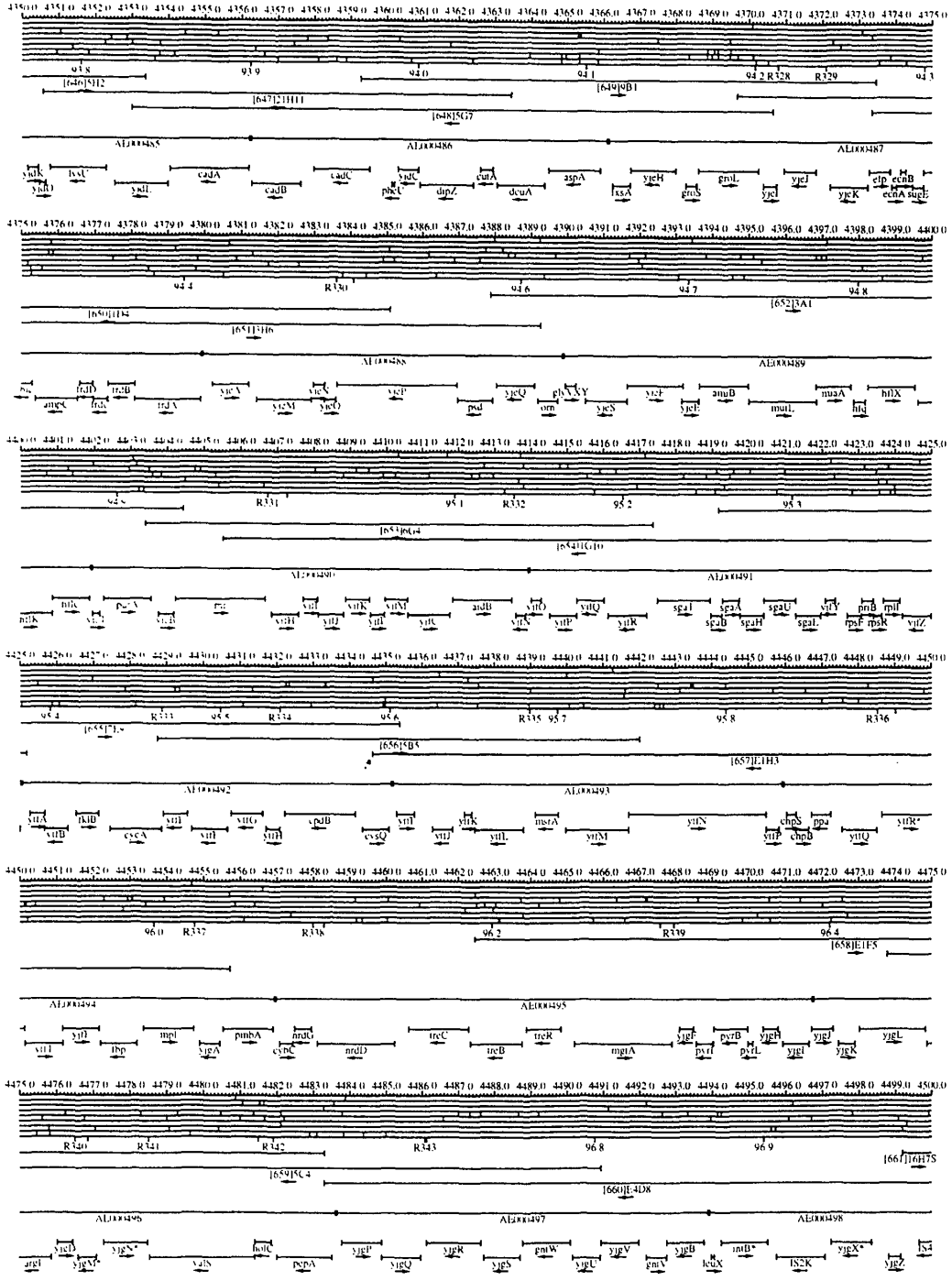

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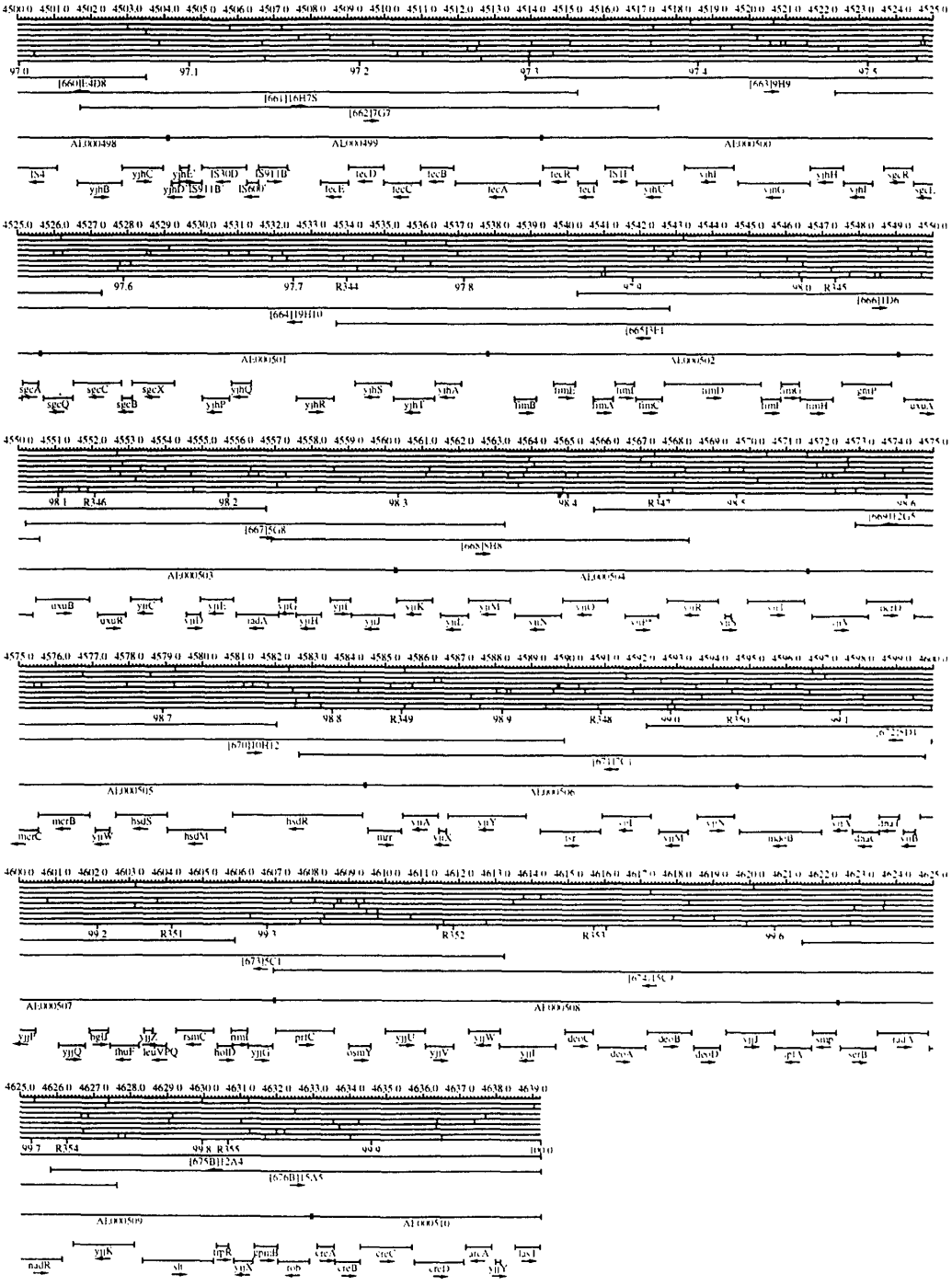

crichia coli K-12 linkage map are closely correlated. When there is a choice of several synonyms to use as the primary gene name, the physical map uses the same primary gene name as the traditional map. The primary names of genes not yet in the *E. coli* Genetic Stock Center (CGSC) database are considered provisional primary gene names. When choosing names for genes that are being functionally characterized for the first time, gene names already present in the database at the CGSC or the EcoGene database should be avoided. Guidance on naming and renaming genes is given in the paper containing the traditional map (3).

For the cases in which no standard-format gene name was assigned to a functionally uncharacterized gene or ORF, a systematic ORF nomenclature, the "y" naming system, was used to generate a provisional name (4, 15, 20). The first three letters of a "y" name are based on the map position of an ORF at the time the name was assigned. Similar to the "z" naming system for transposon insertions, ya[a to j]A to Z designates ORFs in the 0- to 10-min region of the chromosome, yb[a to j]A to Z designates ORFs in the 11- to 20-min region, and so on. The fourth letters (A to Z) can be assigned in any order within the 1-min interval. If all 26 names in any 1-min interval are exhausted, a new second letter is assigned to generate another 26 possibilities; additional ORFs after yaaZ would be ykaA, ykaB, and so on; additional ORFs after ybaZ would be ylaA, ylaB, and so on. The "y" names are not reused if a "y" ORF is given a new gene name or if an ORF becomes defunct, e.g., if a frameshift correction fuses two adjacent ORFs. Map locations provide a convenient and systematic method for naming ORFs, and the "y" names can guide one to an approximate map position. However, to avoid unnecessary renaming the "y" name of an ORF is not changed if a map revision moves it into an adjacent minute interval. The "y" names are now assigned to all the functionally uncharacterized, unnamed ORFs in EcoGene10. Once a new function is established for an *E. coli* gene, the provisional "y" name should be abandoned and a new gene name should be chosen.

Information concerning the availability of the EcoMap10 and EcoGene10 electronic datasets in various formats, including the Colibri database management program (12), can be obtained at http://cesspit.med.miami.edu. Additional information about the genes and ORFs in EcoGene10 is contained in SWISS-PROT records (http://www.expasy.ch/sprot) that can be accessed by using the names that are depicted on EcoMap10 and that are indexed in a master file (http://www.expasy.ch/cgi-bin/lists?ecoli.txt.

ACKNOWLEDGMENTS

This work was supported by funds made available to K.E.R. from a Lucille P. Markey Charitable Trust grant to the Department of Biochemistry and Molecular Biology at the University of Miami School of Medicine.

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附　　件　　二

Workshop 104-26

Conducting Research with Select Agents

May 23, 2004
1:00 p.m. – 4:30 p.m.

Morial Convention Center
New Orleans, LA

American Society for Microbiology
1752 N Street, N.W.
Washington, DC 20036

Conducting Research With Category A Bacterial Select Agents

Workshop #104-26

American Society for Microbiology Annual Meeting
May 23, 2004

General Outline

- Regulations
- Registration process
- Personnel
- Documentation
- Training
- Security
- Biological safety
- Funding Opportunities

Disclosure Statements

1. The following speakers indicated that there were no financial relationships to be disclosed:

Theresa M. Koehler, Ph.D.

Eric A. Johnson, Sc.D

Ann E. Larson, M.S.

2. The following speakers disclosed financial relationships:

Conducting Research With Category A Bacterial Select Agents:
Requirements and Opportunities

ASM Annual Meeting
May 23, 2004

Conveners

Dr. Eric Johnson
Ann Larson
Food Research Institute
University of Wisconsin-Madison

Dr. Theresa Koehler
University of Texas Health Center
Houston, TX

2

Brief Outline

- Regulations
- Registration process
- Personnel
- Documentation
- Training
- Security
- Biological safety
- Funding opportunities

3

4

Antiterrorism and Effective Death Penalty Act of 1996

- HHS required to:
  - Generate list of biological agents that have the potential to pose a severe threat to public health and safety
  - Establish procedures for the transfer of these agents
    - Included training and facility requirements
- Led to 42 CFR 72.6

5

42 CFR 72.6

- "The Select Agent Rule"
- Effective April 15, 1997
- Included
  - List of Select Agents
  - Facility registration
  - EA-101 for SA transfers
  - Inspection & agent disposal requirements
  - Research and clinical exemptions

6

2001 Patriot Act

- "No restricted person shall ship, possess, or receive a Select Agent"
- Includes definition of "restricted persons"
- Provides for penalties for unjustified possession of Select agents (SA)
- Led to:
  - The Public Health Security and Bioterrorism Preparedness and Response Act of 2002
  - The Agricultural Bioterrorism Protection Act of 2002

7

Public Health Security and Bioterrorism Preparedness and Response Act of 2002

- Changed the regulatory authority of HHS regarding select agents
- Agricultural Bioterrorism Protection Act of 2002
  - Granted regulatory authority to USDA for certain agents/toxins that threaten animals and plants, or animal and plant products
- Required cooperation between USDA and HHS on "overlap agents"

8

Public Health Security and Bioterrorism Preparedness and Response Act of 2002

- Signed June 12, 2002
- Major changes from previous legislation
  - Requires registration for possession and use of select agents, not just transfer
  - Security requirements, not just safety
  - Required background check (SRA) for entity and individual
  - Restricted persons cannot work with SA
  - required immediate one-time notification of possession

9

Public Health Security and Bioterrorism Preparedness and Response Act of 2002

- Other changes
  - Narrowed exemptions from regulations
  - Specified additional criminal penalties
  - Required notification of theft, loss, or release of select agent
  - Provided federal nondisclosure protection of sensitive site-specific information

10

Notification of Possession

- All facilities possessing select agents and toxin had to notify HHS by September 10, 2002, and/or USDA by October 11, 2002.
- Notification was different than registration
- If you don't already possess or use SA (and aren't registered), but you would like to, you need to complete the SA registration process first.
- If you possess SA now and aren't registered, contact CDC or USDA (after you call your lawyer)

11

Interim Final Rules, December 13, 2002

- 42 CFR 73
  - DHHS
  - "Possession, Use, and Transfer of Select agents and Toxins; Interim Final Rule"
- 7 CFR 331 + 9 CFR 121
  - USDA/APHIS
  - "Agricultural Bioterrorism Protection and Response Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins; Interim Final Rule"

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Interim Final Rules-2002

- Include detailed requirements for possession, use, and transfer of select agents and toxins
- Requirements in both documents are similar
- Set dates to phase in new requirements
  - Allowed labs already working with select agents to continue without seriously impeding progress of research
  - Full compliance required by November 12, 2003

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Interim Final Rules-2002

- In reality, the new requirements had a significant impact on many labs already working with select agents, especially:
  - Increased security measures
  - Increased reporting requirements
  - Security risk assessments

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Amendments to Interim Final Rules

- Published November 3, 2003
- Provided additional time to U.S. Attorney General to complete security risk assessments for those who had submitted all necessary info by November 12, 2003.
- Allowed issuance of provisional registration certificates for entities allowing access/use of select agents
- Assured that ongoing research was not disrupted
- Does not allow provisional authorization for entities or personnel starting the application process after November 12, 2003.

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Covered Agents and Toxin

- DHHS/CDC human select agents and toxins
  - May affect public health and safety
- APHIS High Consequence Livestock Pathogens and Toxins
  - Overlap--also are human pathogens
  - Non-overlap--not pathogenic to humans
- Certain APHIS plant pathogens

16

Select Agent Registration

- Required for any entity that possesses, uses, or will receive or transfer any select agent or toxin to or from entities within the U.S. or outside the U.S.

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Select Agent Registration

- DHHS/CDC
  - HHS non-overlap select agents and toxins
  - Overlap agents
  - ~80% of entities currently registered with CDC
- USDA/APHIS
  - Non-overlap high consequence livestock pathogens or toxins
  - Overlap agents
  - Listed plant pathogens
  - ~20% of entities currently registered with APHIS
- Registration forms are the same for both

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Select Agent Registration-Overlap Agents

- Registration for overlap agent can be with CDC or APHIS
  - Don't register with both for use of overlap agent
  - Your entity may already be registered
- ~85% of entities registered for overlap agents
- "Cooperation" between CDC and APHIS mandated by legislation

19

Select Agent Registration

- An entity with both CDC-only and APHIS-only agents must register with both agencies

20

Category A Select Agents

- Criteria
  - Easily disseminated or transmitted from person to person
  - High mortality
  - Potential for major public health impact
  - Potential to cause public panic/social disruption
  - Require special action for public health preparedness

21

Category A Bacterial Select Agents

- Overlap agents (CDC or APHIS)
  - Botulinum-neurotoxin-producing strains of Clostridia
    - *C. botulinum* + some strains of *C. baratii, C. butyricum*
  - Botulinum neurotoxins
  - *Bacillus anthracis*
  - *Francisella tularensis*
- DHHS human agent (CDC)
  - *Yersinia pestis*

22

Examples of Regulated Genomic Material

- Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed if the nucleic acids:
  - Are in vector or host chromosome, and/or
  - Can be expressed in vivo or in vitro
- Listed viruses, bacteria, fungi, and toxins that have been genetically modified

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Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms

- Clarification in 42 CFR 73
- Prior approval from HHS required:
  - Utilizing recombinant DNA to deliberately transfer drug resistance traits to SA that are not known to acquire the trait naturally, if this could subsequently compromise control of the agent
  - Deliberate formation of recombinant DNA containing genes for SA toxins highly lethal for vertebrates

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Exclusions & Exemptions

- Select agents in their naturally occurring environment, as long as they have not been intentionally introduced, cultivated, collected, or otherwise extracted from their natural source
  - Example of exempt item
    - Potato naturally contaminated with spores of *C. botulinum*, which are ubiquitous in soil
  - Example of regulated items
    - Potato samples packaged and incubated in a manner to encourage growth or any existing *C. botulinum* spores
    - Potato samples inoculated with *C. botulinum* spores as part of a research project
    - *C. botulinum* spores isolated from potato samples

25

Exclusions & Exemptions

- Non-viable ("non-replicating") select agents
- Nonfunctional toxins
- Certain attenuated strains of select agents or toxins
  - If explicit exemption granted
- Exempt amounts of biological toxins

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Examples of Exempt Amounts of Toxins

- 0.5 mg botulinum neurotoxins
- 5 mg staphylococcal enterotoxins
- 100 mg ricin or abrin
- 100 mg saxitoxin
- 100 mg shigatoxin
- 100 mg tetrodotoxin

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Exempt Amounts of Toxins

- Select Agent registration is not required if the total amount of a toxin under the control of a PI is below the specified amount
- Based on potency and quantity

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Exempt Amounts of Toxins

- Highly recommend treating exempt amounts of toxins with high level of security and (obviously) biological safety requirements
- Exemption can allow for easier collaborations
  - However, if providing exempt amounts of toxin to another lab, good idea to verify training & biological safety, protocols, etc. of the receiving lab

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Attenuated Strains Exempt from SA Regulations

- Current lists available
  - www.cdc.gov/od/sap/exclusion
  - www.aphis.usda.gov/vs/ncie/bta.htm
- Applications can be made in writing to CDC or APHIS to include other strains
- Strains subject to regulations if there is any reintroduction of factors associated with virulence, or manipulations that modify the attenuation such that virulence is restored or enhanced

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Examples of Currently Exempt Attenuated Strains

- *Bacillus anthracis*
  - Devoid of both plasmids pX01 and pX02
  - Devoid of plasmid pX02
- *Francisella tularensis*
  - *F. tularensis* subspecies *novicida*
    - strain, Utah 112 (ATCC 15482)
  - *F. tularensis* subspecies *holartica*
    - LVS (live vaccine strain)
    - Note: LVS requires VS-16-3 permit if used for off-label purpose
  - *F. tularensis* ATCC 6223 (also known as B38)

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Other exemptions

- Clinical or Diagnostic Laboratories
  - Exemption for diagnosis, verification, or proficiency testing
  - After being identified, agent must be transferred to registered facility or destroyed
  - Notification to feds required within 7 days of identification
    - CDC form 0.1318
    - APHIS form 2040
  - Positive controls and reference samples containing SA are no longer exempt.

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Other exemptions

- Exemption for products approved under specific laws
- Certain investigational products
  - Must apply for exemption
    - CDC form 0.1317
    - APHIS form 2042
- Domestic or foreign public health or agricultural emergency
  - Must apply for exemption

33

Select Agent Registration-General

- Difficulties and time involved in SA registration will vary widely between labs/facilities
- Most significant changes will probably involve increased security and recordkeeping
- SA registration process may be less painful for some labs:
  - Training documentation/SOP's up-to-date
  - Current full compliance with biological safety requirements
  - SA would be used by limited # personnel in limited areas
  - Existing modern secure BSL-3 high containment facility
  - In entities that already have registered labs

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New Select Agent Registrations

- Bad news
  - You can't work with select agent until the entire process is complete, including individual security assessments
- Good news
  - You won't have to suffer through <u>all</u> of the growing pains involved in implementation of the new regulations during 2003

35

Time Involved in SA Compliance

- Initial registration and training
  - Substantial amount of time
  - 20-50% of PI or lab manager for 2-6 months?
  - ~20% RO/ARO
- Ongoing training, recordkeeping
  - 10-30% FTE for each?
  - ~20% RO/ARO

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Time Involved in SA Compliance

- Will vary widely depending on:
  - Facility design, etc.
  - Number of areas
  - Number of registered personnel
  - Amount of agent

37

What is an "entity"

- Any government agency, university, corporation, company, partnership, association, firm, sole proprietorship, or other legal entity, including an individual acting on his or her own.

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Responsibilities of the Entity

- Register the entity (and personnel) with CDC or USDA/APHIS for each select agent or toxin it possesses, uses, or transfers
- Designate a Responsible Official (RO)
- Notify CDC or APHIS of any changes in registration

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Responsibilities of the Entity

- Make sure access to agents limited to approved individuals
  - Track individual security risk assessments
- Prohibit access by "restricted persons" as defined by Patriot Act
- Provide select agent training
- Regulate select agent transfers

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Responsibilities of the Entity

- Develop and implement emergency response plan.
- Conduct regular inspections
  - Document
  - Make sure deficiencies addressed
- Make sure accurate records are kept
  - Approved individuals
  - Training
  - Access to agents
  - Inspections
  - Inventories
  - Transfer documents

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Responsible Official (RO)

- Delegated responsibility and authority to make sure that all responsiblilities of the entity are met
  - Ensure compliance with regulations
- Must also designate alternate RO (ARO)
  - Same qualifications as RO
  - Acts on behalf of RO in his absence
- Optimally, RO:
  - should have good working knowledge of biological safety
  - Should not be individual working with SA
- Implementation of SA requirements will vary based on approach of RO

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Oversight of Compliance with Select Agent Regulations

- Ultimately the RO
- Large academic institutions with multiple SA labs
  - May have committee that includes RO & ARO
  - UW-Madison Biosecurity Task Force
    - RO, ARO
    - Members of UW Police, UW Communications, Office of Biological Safety, Division of Information Technology, Dean's office
- Smaller entities
  - RO may be owner, CEO, department head, etc.

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Select Agent Registration

- Information/forms
  - CDC Select Agent Program
    - www.cdc.gov/od/sap
    - 404-498-2255 (phone)
    - 404-498-2265 (fax)
    - lrsat@cdc.gov
  - USDA/APHIS
    - www.aphis.usda.gov.vs.ncie/bta/html
    - 301-734-3277 (phone)
    - 301-734-3652 (fax)

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Entity Registration Forms

- "Application for Laboratory Registration for Possession, Use, and Transfer of Select Agents or High Consequence Livestock Pathogens and Toxins"
  - CDC Form 0.1319
  - APHIS form 2044
  - Joint reporting system between CDC and APHIS
- Plans for secure web-based SA application in near future
- Information in SA application not subject to Freedom of Information Act

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Overall Process of Entity Registration For Previously Unregistered Entity

- Facility risk assessment
  - Ensure that facility meets requirements to handle agent
  - Biosafety and security
- Implement biosafety & security measures
- Submit SA Application form to CDC and/or APHIS
- Submit FD-961 for entity/RO/ARO to FBI
  - Submit fingerprint packets for RO/ARO to FBI
- FD-961 & fingerprinting for PI/individuals
  - After receiving unique identifying
- CDC or APHIS inspection

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Facility Risk Assessment

- By RO before submitting initial SA registration
  - Detailed internal audit of each area
- Ensure that facility meets requirements to handle agent
- Include security risk assessment
  - Institutional security
  - Biosecurity consultants
- Correct deficiencies before registration

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Entity Registration Form

- Section 1
  - Entity information
    - Addresses
    - Name of RO, ARO
- Section 2
  - Signatures of RO, ARO
- Section 3
  - Specific agents used, possessed, or transferred
- Section 4A
  - List of specific areas where each agent used
    - BSL, general type of use

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Entity Registration Form

- Section 4B
  - Information on personnel working with Select Agents
  - Name, address, PI, rooms, agents, job title
- Section 5A
  - Source information for agents
  - Include CV for PI
  - Specific biosafety checklist for each registered area
- Section 5B
  - Training & security information
- Section 5C
  - Quantity of infectious agent + decontamination

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Entity Registration Form

- Section 5D
  - Recombinant DNA info
- Sections 5E & 5F
  - Animal use
- Sections 5G
  - Toxin information

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What rooms in facility have to be registered

- Any room where Select Agent is used or stored
  - Even if very minor or infrequent use
- May include:
  - Labs
  - Rooms containing equipment used for select agent research or used to store SA
    - freezers, incubators, centrifuges, microscopes, PCR, etc.
  - Animal rooms
  - Areas shared with other, non-registered labs
    - Under very strict conditions
  - Autoclave rooms?

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What rooms have to be registered

- Not included, unless used for agent storage:
  - hallways
  - departmental offices
  - PI offices outside of labs

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Animal Facilities

- Animal rooms or facilities used for research with select agents must be registered
- If agent can be recovered from animals, then access to animals must be limited to approved (i.e. select agent registered) personnel
- If agent not recoverable after treatment, may not need to consider animals themselves as "select agent"
  - extremely difficult to recover purified botulinum toxin from mice after injection
  - May vary according to RO interpretation

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Animal Facilities

- Registering animal caretakers
  - Yes, if agent recoverable from animals
    - e.g. *B. anthracis, C. botulinum, F. tularensis*
  - May not be necessary--EAJ labs--caretakers treated as visitor until BoNT injections complete, then they can have access to animals

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FBI Form for Entity/RO/ARO

- FD-961
  - Bioterrorism Preparedness and Response Act FBI Information Form
- Fill out for entity, RO, ARO
  - Also any individual who owns or controls the entity
  - Same form will be used for individual & PI registration

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FD-961-Entity/RO/ARO

- Section I
  - All entities seeking SA registration
- Section II
  - Commercial, private, academic, and other entities
- Sections III and IV
  - RO/ARO
- RO/ARO fingerprint packets
  - Submit to FBI along with FD-961 after CDC or APHIS provides unique identifier number

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Entity Registration

- SA Registration valid for 3 years
- Can be denied or revoked
  - Training of RO not adequate
  - RO/entity does not have lawful purpose to possess, use, or transfer agents
  - Repeated violations of biosafety, containment, or security requirements
  - Facility does not comply with provisions of the regulations

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How long until I can work with Select Agents?

- Initial facility risk assessment by RO
  - Correction of presumed deficiencies
  - Months?
- Collecting initial information
  - Weeks/months
- Organizing documentation, writing SOP's, etc
  - Weeks/months

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How long until I can work with Select Agents?

- SA Application
  - "allow at least 8 weeks for processing"
- Individual security risk assessments
  - Hypothetically, usually complete in less than 45 days
  - Realistically, could be up to 6 months (?)
- Permits to receive SA
  - USDA or PPQ
- Overall
  - Start to finish--maybe 6 months to a year

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Amendment of Select Agent Registration

- Facility
  - Change in floor plans
    - i.e. moving equipment
  - Adding/deleting rooms
- Personnel changes
  - New hires/individuals leaving
  - Changes in name, address, job title
- Agent
  - Addition/deletion of agent/toxin used at entity or facility and/or by an individual
- Research
  - Significant changes in protocols or objectives

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Amending Select Agent Registration

- Most amendments require prior notification in writing from CDC or APHIS
- Includes:
  - Significant changes in floor plan of registered room, such as:
    - Moving or adding large equipment (incubators, freezers)
    - Installing new eyewash station

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Personnel in Select Agent facilities

- Two types:
  - Authorized/Approved
    - Provisional authorization or
    - Completed SA approval process
  - Unauthorized
    - Visitors
    - Custodians; maintenance staff; repairmen
    - Restricted persons

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Authorized/Approved Personnel

- May handle and work with agents/toxins
  - Supervision not required after training complete
- May have unescorted access to work and storage areas
- May be issued access control device(s)
- May serve as "escort" for unauthorized individuals
- Are responsible for maintaining agent security

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Provisional Authorization

- Original deadline for approval of personnel was November 12, 2003
  - Backlog in processing or individual security risk assessments by U.S. Attorney General (DOJ/FBI)
  - Approval for individuals not completed by deadline
- November 3, 2003--amendments to Select Agent Regulations
  - 42 CFR 73
  - 7 CFR 331
  - 9 CFR 121

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Provisional Authorization

- Allowed issuance of provisional registration certificates for entities allowing access/use of select agents
- Assured that ongoing research was not disrupted
- Applied to entities and personnel if registration process already ongoing
  - Only if SRA materials had already been successfully submitted by November 12, 2003

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Provisional Authorization

- Does not apply to entities or individuals starting the application process now.
- SA approval now requires approval from the DHHS/USDA upon completion of DOJ Individual Risk Assessment and FBI background checks
- Regardless of how long this process takes, affected individuals are "unauthorized"
- They must be handled as "visitors"; labs must satisfy all 'visitor' requirements
- Exceptions may NOT be granted

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Provisional Authorization

- Thus, entities and individuals starting the select agent registration process now must wait until the full approval process is complete before working with select agents
  - Time variation between CDC and APHIS(?)
  - Can have tremendous effect on new hires, rotating graduate students, visiting professors, etc.

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Record of Access to Select Agents

- Entities must maintain records of:
  - Each individual who has actually accessed a select agent or toxin
    - Can be recorded on inventory record
  - Each individual who has actually accessed any area where select agents are used or stored
    - Approved employee logs (manual or electronic)
    - visitor logs
- Save records for at least 3 years

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Authorized/Approved Personnel

- Need to record access to registered rooms
  - electronic card access or biometric devices
    - automatic
  - Manual records
    - Traditional key access
    - Sign-in and sign-out sheets
    - Need to ensure compliance
    - Sign-in sheets should be in easily accessible location

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Adding personnel to existing registration

- PIs are responsible for obtaining authorization for personnel in their labs BEFORE granting access
- Contact RO to see how they want to handle this
- Transfer of individual SA registration between registered facilities
  - streamlined

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Departure of Personnel

- PI's must immediately notify the RO when personnel depart lab, regardless of reason
- Amend entity registration
- Secure access devices
  - If access codes are used, they MUST be changed upon departure

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Unauthorized personnel

- May NOT have access to agent/toxins
  - access =
    - "physical ability to lay your hands on a select agent or toxin"
    - "the freedom or ability to obtain or make use of"
  - No physical control of agent
  - Cannot handle agent, even if supervised
- Must be escorted at all times by authorized personnel when select agent accessible
  - Even if a new hire that's been around for months
  - If you (escort) has to leave the area to use the restroom, they have to leave, too.

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Unauthorized Personnel

- May NOT be issued access control device(s)
- Must register arrival and departure in Visitor Log
  - Every time, not just in once and out once for the day
- Note: two different things
  - Visitor log
  - Manual log of access to registered areas by approved individuals

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Unauthorized personnel

- Includes custodial and maintenance personnel
  - Any custodial and maintenance service MAY ONLY be provided during times when authorized personnel are present.
    - May result in minimal routine cleaning performed by lab personnel
      - Changing trash, sweeping the floor
  - Use of higher quality materials in high containment facilities may decrease need for maintenance

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Visitors

- Authorized/approved escort must:
  - Minimally "train" visitors
    - Entry/exit requirements
    - Security precautions
    - Personal safety; PPE
  - Make sure accurate info in Visitor Log
  - Observe visitor directly at all times
  - Make sure visitors cannot access select agents
- Visitor must:
  - Accept and understand training
  - Follow instructions of escort

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Examples of Visitors

- New personnel before approval process complete
- Custodians
- Repairmen
- Office staff (unless registered)
- Delivery personnel
- RO/ARO
  - Unless specifically registered for those areas
- Inspectors
  - including CDC/APHIS select agent inspectors

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Visitors

- Should have legitimate need to be there
- Not:
  - Friends or roommates
  - Family members
- (Probably) can be in room when someone else is handling agent (assuming not biological safety issue), but cannot handle it themselves.
  - Some RO's may interpret this differently

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Visitor Protocol

- Written visitor protocol
  - May want to post on door or give to visitors to read before entering
- Escort must provide information
  - PPE, entry/exit requirements
  - Hazards
  - Specific risk groups
    - e.g. pregnant, immunocompromised
      - May need to have method to keep this information confidential
  - Where can they set materials (e.g. tools for repairmen)

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Visitor log

- Should be in easily accessible location near door in each registered area
- Need to record
  - Date, person, time in & out, escort (authorized personnel)
- May also want to record reason for visit

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"Restricted Persons"--Patriot Act

- May not be granted *any* access to covered agents
- Definition of restricted person:
  - Indicted of a crime punishable by imprisonment exceeding 1 year
  - Convicted of a crime punishable by imprisonment exceeding 1 year
  - A fugitive from justice
  - An unlawful user of any controlled substance
  - An illegal alien
  - Adjudicated a mental defective or committed to a mental institution
  - An alien from country determined to support terrorism (Iraq, etc.)
  - Dishonorable discharge from the US military

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Problems with Definition of Restricted Person

- Vague
- Persons indicted of crime punishable by imprisonment exceeding one year
  - Even if not convicted
- Person convicted of crime punishable by imprisonment exceeding one year
  - Even if they didn't serve a year in prison
- No exceptions for youthful indiscretions
  - A relatively minor crime committed 40 years ago may not allow you to ever work with select agents

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Problems with Definition of Restricted Person

- "Unlawful user of any controlled substance"
  - Vague, although according to FBI (verbal communication), this doesn't apply to marijuana use unless the person has used marijuana more than 15 times during their lifetime
  - Includes one-time use of any other controlled substance

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Problems with Definition of Restricted Person

- (Legal) alien from country designated to support terrorism.
  - If you are legally a citizen of Iran, you are ineligible to work with select agents, even if your family moved to the U.S. when you were a baby.
  - May need to ask citizenship of everyone interviewing for a position in your lab

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Problems with Definition of Restricted Person

- Dishonorable discharge from U.S. military
  - For any reason
    - At least one person has been turned down for select agent usage because they received a dishonorable discharge due to sexual preference
    - Deserters from Vietnam

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Problems with Definition of Restricted Person

- "Adjudicated mentally defective….. "
  - Definition of "mentally defective" ?
- "Committed to mental institution"
  - Doesn't have to be recent
  - Someone briefly hospitalized once for clinical depression?

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Individual Security Risk Assessment

- Required for:
  - Individuals requiring access to select agents and toxins
  - Also RO and ARO of each entity
  - "any individual who owns or controls the entity"
    - "partner, owner, director, holder, or owner of 50 percent or more of its voting stock and is in a managerial or executive capacity with regard to select agent possessed, used, or transferred by the entity"
    - For accredited academic institution:
      - Refers to RO, but not owner of private accredited academic institution

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Security Risk Assessment

- Performed by FBI for Department of Justice
  - CJIS--Criminal Justice Information Services Division
  - www.fbi.gov/hq/cjisd/cjis.htm
- Generally a database search
  - More extensive for some individuals?
- Non-U.S. citizens
  - Additional information may be obtained from State Department, Interpol, Department of Justice, and antiterrorism groups
  - State Department has final word on approval

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Process of Obtaining a Security Risk Assessment for Individual

- Described at FBI website
  - www.fbi.gov/terrorinfo/bioterrorfd961.htm
- RO will add name to Table 4B on CDC or APHIS Entity registration form
- CDC or APHIS will assign unique identifying number to each individual and report number to RO.

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Process of Obtaining a Security Risk Assessment for Individual

- RO/ARO should then provide individuals with instructions on how to fill out FD-961 (sections III and IV) and get fingerprinted
  - Need unique identifying number first
- RO/ARO submits completed FD-961 to FBI
- Law enforcement submits fingerprint packages to FBI
  - Address information in packet
  - All materials for each individual should be in one package

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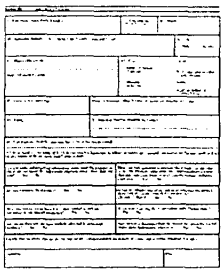

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Individual Security Risk Assessment

- Information needed for FD-961 (Section III)
  - Full legal name
  - Current resident address
  - Date of birth
  - SSN
    - If don't have, may be able to use alternate (check with CDC/USDA)
  - Sex
  - Place of birth
  - Race
  - Citizenship/Alien Registration Number
    - Useful to submit photocopy of INS documents
  - Registering entity
  - Unique identifying # (provided by RO)

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Individual Security Risk Assessment

- Any changes in personnel information at any time must provided to RO
  - Address, phone
  - Name change upon marriage
  - Job title
- Students must provide current local address, which may not be their permanent (hometown) address

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FD-961

- Certifications
  - Basically questions that cover criteria for designation as restricted person
  - Should be answered truthfully, even though some information may not be verifiable
    - Drug use
  - If you need to check "yes" on any of the questions, don't bother submitting
- Form must be signed (two places) by person seeking SA authorization
- Bioterrorism Help Line
  - 304-625-4900

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Fingerprinting

- RO should make arrangements for fingerprinting with local law enforcement agency
  - Agency will probably charge a fee for this service
- Fingerprint card packets can be obtained by requesting (by fax) from FBI at:
  - 304-625-3984
  - Request must contain name of entity & RO, mailing address & phone number, and number of fingerprint card packets requested

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Fingerprinting

- Two legible fingerprint cards must be prepared for each individual
  - If not legible, FBI will request that they be re-done, which will delay SRA
  - Have law enforcement agency double-check that the prints on both cards are legible before sending them out
- Law enforcement agencies forward directly to FBI
  - Entities may want to provide express mail labels to expedite process
- Instructions for submission of fingerprints
  - www.fbi.gov/hq/cjisd/cjis/htm

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Potential Problems with Security Risk Assessment

- Frequent causes of delayed SRA's
  - Incomplete or incorrect applications
  - Illegible fingerprint cards
  - Loss of information/forms by government agencies
    - Expect that this may happen and keep copies of everything
  - Sending FD-961 and fingerprint cards to FBI separately for each individual
- PI's may want to check with RO before starting SRA process if they or someone in their lab is concerned with passing SRA

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Results of SRA

- Sent from CJIS to CDC/APHIS
  - CDC or APHIS reports results to entity
- Designation as "restricted personnel" can be appealed if an error has potentially been made during individual security risk assessment
  - Designation may be overturned (rare)
  - If an error has not been made, you may be out of luck
- SRA for individual valid for 5 years
  - Can be revoked

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Hiring New Personnel for SA Lab

- Include in job description (?)
  - "Must be able to comply with select agent regulations and pass Department of Justice security risk assessment for use of select agents"
  - May be better to include in initial assessment/interview
- During waiting period before SRA completed
  - New hires must be treated as visitors
    - Sign in
    - Supervision at all times by authorized personnel in registered areas
  - Cannot handle any SA, but may be able to be in same room while someone else working with SA under certain conditions

98

Hiring New Personnel for SA Lab

- May need to write new grants assuming that new hires can't work directly with SA for first 3-6 months
- Undergraduate student workers can be registered for use with Select Agents
  - Recommend hiring students that can take regulations seriously
    - Contact references and discuss this specifically
- Potential new personnel must be informed of SA regulations and biological safety hazards from SA at some point before hiring

99

Personnel Management

- SA research can be stressful
  - Potential for lost or stolen agent
  - Unannounced inspections
  - Increased security requirements
  - Fingerprinting/background checks

100

Personnel management

- Morale in the lab is important
  - can indirectly affect biosafety & security compliance
  - Try to keep sense of humor about regulations
  - Listen to personnel
    - Complaints
    - Suggestions for improving safety/security
  - Positive reinforcement
    - If they are doing a good job, tell them!
  - Ensure frequent social interactions with others

101

Personnel Management

- Train them well and treat them well
- Replacing registered personnel costly
  - Re-training
  - Delay before replacement can work with select agents

102

Repeated noncompliance of SA regulations by personnel

- SA personnel must take regulations seriously
  - Even undergraduate students
  - Everyone must be made aware of the potentially serious consequences of noncompliance with reg's
- SA regulations + biological safety requirements
- Keep records
  - Document even minor infractions

103

Repeated noncompliance by personnel

- RO can revoke authorization if personnel are unable to comply with SA regulations
- Noncompliance with SA regulations can threaten everyone's select agent registration
  - Your lab
  - Entire entity
- If they are repeatedly cutting corners with safety/security compliance requirements, are they conducting sloppy research as well?

104

Potential Penalties

- Violation of Public Health Security and Bioterrorism Preparedness Response Act of 2002
- Criminal
  - Up to 5 years in prison, or a fine, or both for:
    - Transfer of SA to unregistered person
    - Possession of SA by unregistered person
    - Knowingly making a false statement
- Civil
  - $\leq$ $250,000 per individual per violation
  - $\leq$ $500,000 per organization per violation

105

Cooperation with other labs

- Your SA registration and SA requirements may have significant effect on other labs
  - Shared rooms/equipment
  - Give general info to other PI's & lab managers
- Be polite & appreciative
  - "Thank you for helping us out on this"
- Do not take hostile attitude
  - "you have to do this whether you like it or not"
- If you are appreciative and acknowledge their cooperation, they will be more likely to comply (i.e. locking doors to shared rooms, signing in as visitors).

106

Cooperation with other personnel

The select agent regulations may add tremendously to your workload.

However, recognize that the regulations also add to the workloads of the RO/ARO, your office staff, and potentially others within your institution.

107

Recordkeeping

- Keep organized and complete records of everything associated with SA
- Document EVERYTHING!!!!.
  - If it isn't documented, it hasn't happened.
- Takes a significant amount of time
  - Varies according to entity, PI, lab situation, # personnel, type of research, etc.

108

Documentation

- Training
  - Initial (interview questions + initial training checklist)
  - Ongoing
  - Security
  - SA-specific
  - biological safety
- Certifications
  - Varies with institution
  - Animal use, biological, SA-specific training by RO, other training courses (BSC usage, e.g.)
  - Shipping SA

109

Documentation (cont'd)

- Incidents
- Current personnel records
  - Addresses
  - E-mail
  - Title changes
- Visitor logs
- Access records for approved personnel
- Inventory
  - Current amounts & locations of SA
  - Usage
- SA access control

110

Documentation (cont'd)

- Health records
  - Immunizations
  - Ab titres
- SOP's/policies
  - Demonstrate that personnel are familiar with all that apply
- Autoclave records
  - Adequacy of autoclave function should be documented regularly (monthly, bimonthly)
    - *B. stearothermophilus* vials--commercially available
  - Overall log of autoclave usage?
- Efficacy testing of disinfection/decontamination methods
- Cybersecurity Plan

111

Documentation (cont'd)

- Equipment certifications
  - BSC certification
  - Emergency equipment testing
    - Eyewash testing
    - Emergency showers
- SA acquisition and transfers
- Animal protocols & records
- Inspection records

112

Documentation

- Must be available to RO and CDC/APHIS inspectors
- PI should be familiar with locations & contents of all relevant documents
- Recommend keeping training documentation & other documentation in central location
  - Protocols/SOPs should be readily accessible in all areas where agent or toxin used or stored

113

Keep all records organized!

114

Written Policies and SOP's

- Recommend extensive and thorough written policies for almost everything
- Modify as necessary
  - Must re-train & document re-training
- Consider worst-case scenarios

115

Policies/SOP's

- Inventory control & reconciliation procedure
- Incident policy
- Accident policy (handling & reporting injuries)
- Visitor policy
- Animal use policy
- Destruction of agent
- Sanitation/Disinfection of lab areas
- BSL-2 & BSL-3 procedures
- BSC usage
- Handling of Sharps

116

Incident policy

- "Catch-all" for lots of little things
- Reporting Select Agent incidents
  - Theft, loss, release of agent
- Suspicious personnel
  - At what point do police need to be called
  - What if you call the police on someone delivering pizza
- Loss of access devices
- Injuries
  - Even if SA not involved
- Handling inquiries from the press

117

Examples of Reportable Incidents

- Keys lost at party by undergraduate student
- Needle-stick when injecting a mouse with botulinum toxin
- Unknown person discovered alone in registered area
- Spill of agent in hallway leading to autoclave room

118

Training of SA Personnel

- SA regulations in general
  - RO/ARO may have specific requirements
- Biological safety
  - Spills
  - Specific risks and handling issues for your agent(s)
  - General BSL-2, BSL-3 practices
- All lab policies/SOP's
- Physical security
- Cybersecurity

119

Training of SA Personnel

- Maintaining records
- Inventory control
- Animal usage with Select agents
- Visitor policies

120

Information Readily Available to Personnel

- Examples
  - Lab protocols/SOP's
    - Spills, visitor policies, incident policies, etc.
  - Relevant sections of BMBL
  - MSDS for specific agents
    - Health Canada--www.hc-sc.gc.ca
- Good idea to keep in readily available location in each registered area
- Copies for each individual

121

If training hasn't been documented, it hasn't happened!

122

How to Document Personnel Training

- Keep detailed records of lab meetings
  - When
  - Who attended (signatures)
  - Agenda--what was discussed
  - Excellent method--recommend frequent lab meetings
  - Supervisor or PI may have to individually train persons not in attendance
    - Document this individual training
- Post memos & have people sign & date
  - Good for short memos only that people will actually read
  - Not good for long policies, etc.
    - People may sign without reading

123

How to document training

- Individual training-
  - Specifics of what was included
    - Outline form acceptable
    - Interview questions
    - Initial training of new hires
  - Who was trained (signature)
  - Date
  - Who did training?
- Certifications from all training courses

124

Training of Non-Lab and/or Non-SA Personnel

- Emergency Personnel
  - Fire, police, EMS
  - Layout of areas, risks from agent
  - Don't want EMS to hesitate accessing areas because of fear, yet they need to have enough info and training to safely enter and exit areas
  - Be pro-active--contact them before agent is present if at all possible
  - Conduct periodic drills?

125

Training of Non-Lab and/or Non-SA Personnel

- Personnel in other labs
  - Safety issues
  - Access
    - Why can't they come in your lab anymore?
    - Why do shared areas need to be locked, even if agent not present?
- Office staff
  - Handling inquiries from press
- Security staff
- On-site maintenance and custodial staff
- Reduce fears through education

126

Media Coverage of SA Research

- Trade-off between security and promotion of research
- Potential problems
  - Security issues
    - May identify agent and general location (building)
    - This info probably readily available through Internet search
  - Public perception regarding bioterrorist agents
    - Education can help

127

Handling Inquiries from Press

- Relatively easy to determine if SA research is conducted in academic institution
- What if a reporter calls the lab and asks about your research?
- Open records requests
  - One academic institution has been asked for their select agent registration information by the student newspaper
  - SA registration not subject to Freedom of Information Act
- Plan in advance how you will handle inquiries from the press or other interested parties
  - Lab personnel
  - Receptionist

128

Select Agent Inventory Records

- Accurate, up-to-date written inventory must be maintained for all select agents and toxins
- Format may vary widely due to number of items, frequency of usage, etc.
  - RO may have recommended or required format
  - Must allow for changes

129

Select Agent Inventory Records

- Handwritten or electronic format
  - If small inventory, may be able to easily keep handwritten inventory in a lab notebook
  - Electronic format may be more adaptable if large numbers of items
  - Entries should be made in indelible ink (blue or black)

130

Select Agent Inventory Records

- Should include:
  - Number of items
  - Strains or producing organisms (for toxins)
  - Date obtained or produced (& by whom)
  - CFU/g or MLD_{50}/g
  - Volumes
  - Where stored
  - Any other descriptive data
    - Type of container (cryovial, eppendorf tube, etc.)
  - Usage/destruction information

131

Select Agent Inventory Records

- Some information may not be available
  - e.g. exact CFU/g for each stock culture, original source data for old strains
  - May be acceptable to estimate
    - Records should indicate that it is an estimate
- Inventory records must be made available upon request to RO/ARO and CDC or APHIS inspectors
- Optimally, copy of inventory record should be kept in all select agent storage locations
  - May not always be possible
- Records should be maintained for at least 3 years

132

Records of Permanent vs. Transient Inventory

- Items in long-term storage
  - Must be listed on inventory record
  - Stock cultures, toxins
  - Always included in master select agent inventory records

133

Records of Permanent vs. Transient Inventory

- transient", "experimental", "working" cultures
  - Examples
    - Food samples inoculated with *C. botulinum* spores
    - Multiple cultures generated for short-term use
  - Highly recommend keeping accurate records
    - May be able to keep records of these items separate from long-term inventory items
    - Determined by RO
    - Detailed records in individual lab notebooks acceptable?

134

Tracking Inventory Changes

- Must keep detailed records of usage
  - Who (signatures of user and witness)
  - What (what items, how much)
  - When
  - Why (i.e. project--brief phrase may be adequate)
  - Amount remaining
  - Details of destruction if applicable
- Entries should be made in indelible ink
  - Preferably blue or black ink
- Optimal format for tracking changes will vary widely between labs

135

Tracking Inventory Changes

- Changes in Select Agent inventory must be documented when they happen!
  - Should be witnessed by second approved person
    - Witness should sign and date

136

Tracking Inventory Changes

- Destruction of agents must be documented
  - May require prior approval from CDC/APHIS
    - Vague wording on regulations
    - Long-term items
      - Strains or toxins
    - Not required to gain prior approval before destruction of every transient sample
  - Autoclaving/Inactivation
    - Detailed records when SA autoclaved
      - Time/temp, type of cycle, which autoclave
      - May be easier to keep log of all autoclave usage

137

Security of Select Agent Inventory Record

- Record of items in your select agent inventory record is a high-risk document
  - Describes what specfic agents you have and where they are located
  - Handle with same security as agent itself, except that inspectors can handle
- Electronic (computer) inventory record
  - Versatile, easy to change, but security risk if hard-drive accessed
  - One method--keep on dedicated laptop never connected to server
    - Easy to lock up laptop + storage devices
  - Must write and implement cybersecurity plan

138

Security of Select Agent Inventory Record

- Backup records
  - Written--make copies frequently (monthly?)
  - Electronic--disks, flash drives (must be secured)
  - Backup record should be stored in secondary secure location
    - If agent & all versions of inventory stolen, how will you know what is missing?
    - Fire can destroy records even if agent not destroyed
    - One method
      - securely fax copy of inventory record to RO monthly after reconciliation with physical inventory

139

Written Inventory of Select Agents

- Reconciliation with physical inventory
  - Checking that all items on select agent inventory are present.
  - Have written SOP or policy for reconciliation procedure
  - Frequency
    - RO will determine
    - Weekly, Monthly?
    - When personnel with SA access leave the lab for other employment

140

Written Inventory of Select Agents

- Reconciliation with physical inventory
  - Difficulties
    - Freeze/thaw of frozen stocks
    - Delays due to unclear labeling
      - Ice on frozen stock may make it difficult to check labels
    - Significant time involved
    - Sloppy recordkeeping by personnel

141

Written Inventory of Select Agents

- Reconciliation with physical inventory
  - By PI or designated individual(s)
  - If usually performed by one person, make sure alternate (and PI) knows where everything is
    - Recommend second person helps, at least on regular basis
  - Record signature/date of person(s) reconciling

142

Written Inventory of Select Agents

- Reconciliation with physical inventory
  - Easier if:
    - Consistent packaging & labeling of both individual items and containers
    - Clear descriptions of items on written inventory record
    - Tamper-evident packaging used whenever possible
      - Infrequently used items
      - May be as simple as racks/boxes heat-sealed in plastic bag with signature across seal
      - Other forms of tamper-evident packaging commercially available

143

Suspected Loss or Theft of Select Agent

- Must have written SOP or policy for handling missing or potentially missing items
  - Serious reportable incident if something really missing
  - Due to human nature, people may forget to write down that they used some or all of an item
    - Make sure personnel well aware of serious consequences if SA potentially lost or stolen
  - Depending on situation, before calling RO/police/FBI, may want to check first (immediately) with all lab personnel to make sure item is actually missing
    - Double check & triple-check that item hasn't fallen out of container

144

Loss or Theft of Select Agent

- Upon discovery of theft, loss (or unintentional release) of SA
  - Immediately notify RO and/or police
    - Need to have 24/7 method of contacting RO or ARO
  - RO should contact CDC or APHIS immediately
    - Phone, fax, or e-mail
  - RO may need to notify police, DOJ (through FBI), state veterinarian, state health department, etc.
  - Within 7 calendar days, written report filed by RO to CDC or APHIS
    - CDC Form 0.1316, APHIS form 2043

145

Shipment of Select Agents

- Within U.S., you can only ship Select Agents to another registered lab
- Keep accurate records in central location
- Complicated process using EA101 forms
- Requires prior approval from CDC or APHIS before shipment
  - Plan ahead
- Cannot leave packaged SA with unregistered office staff until FedEx arrives
- Packaging requirements detailed in 49CFR192.800

146

Process of Transferring Select Agents

- EA-101
  - Recipient's RO fills out sections 1 and 2, sends to sender's RO
  - Sender's RO fills out secion 3 and faxes to CDC or APHIS
  - CDC or APHIS will verify informaiton and fax form back to sender with approval confirmation number
  - Within 2 days of receipt of agent, recipient's RO fills out section 4 and sends to both sender's RO and CDC or APHIS

147

Shipment of Select Agents

- May be training courses available for some institutions
- Info on CDC/APHIS websites
- Exporting to other countries
  - Requirements will vary widely
  - Contact CDC/APHIS, U.S. Customs, and import agency in receiving country for guidance
- Import requirements

148

Methods of transferring SA

- Use commercial carrier that can maintain security
  - FedEx
  - Others?
- Closely track shipments and save shipping documentation
- Handing to personnel from another lab at your entity
  - EA101 not required for intrafacility transfer, assuming both labs registered for that agent
  - Appropriate secure packaging required
- Burden on shipper to make sure legal transfer

149

Acquiring/Transferring Agents

- Approvals involved
  - EA101
  - VS 16.3 for APHIS High Consequence Livestock Pathogens
  - PPQ Form 526 needed for relevant APHIS Plant Pathogens
  - Valid import permit needed to obtain agent from overseas
    - U.S. Customs
    - 42 CFR 71.54, Importation of etiologic agents, hosts, and vectors

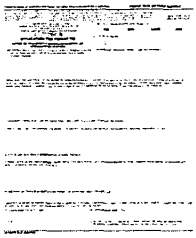
150

VS 16.3

- USDA/APHIS
  - Application to Import or Transport Controlled Material or Organisms or Vectors
- Applies to APHIS High Consequence Livestock Pathogens
  - Required to receive these strains, including:
    - *C. botulinum*
    - *B. anthracis*
    - *F. tularansis*
  - Not required to ship these strains
  - Does not apply to botulinum neurotoxin

151

VS 16.3



- Form
  - Phone (APHIS)
    - 301-734-3277
  - www.aphis.usda.gov/library/forms
- Fees
  - Application ($94)
  - Renewal ($61)
  - Amendment ($47)

152

VS 16.3

- Required for overlap agents, even if your SA registration is with CDC
- May take months to receive permit
- Will probably require APHIS inspection
  - Results of general SA inspection may suffice if you are registered with APHIS
- Permit valid for one year; can be renewed
- May be able to use non-specific wording on application that will allow receipt of different shipments from different sources during that year.

153

Biodefense and Emerging Infections Research Resources Repository

- "BEI Resources"
- Source of Select Agents & information
- Established by NIAID in September 2003
- Intended to be "Federal government's national resource and clearinghouse for specimens, reagents, and information on these organisms"
- Coordinated effort between NIAID, CDC, USDA, ATCC
- www.beiresources.org

154

Biosecurity Plan

- Required of each entity registering for SA use
  - Create & implement before submitting registration
  - Required to submit plan to CDC or APHIS
- Policies and procedures ensuring the security of areas containing select agents and toxins
- A single biosecurity plan may cover all facilities (labs) at that entity

155

Biosecurity Plan

- Includes
  - Inventory control procedures
  - Minimum education/experience for lab personnel
  - Provisions for cleaning, maintenance, repair
  - Provisions for personnel security training
  - Provisions for physical security
  - Provisions for loss of access devices
  - Package inspection
  - Protocol for intra-entity transfers

156

Select Agent Security--Why?

- Obvious
  - Theft of agent
    - Because many agents replicate, theft of very small amount could be problem
- Less obvious
  - Theft or intentional release of agent
    - Halt or significantly disrupt ongoing research
    - Public relations nightmare
  - Shutting down electrical, HVAC, etc.
    - Halting research

157

What needs to be protected?

- Agents
- Information
  - Written inventory
  - Security codes
  - Building blueprints
    - Blueprints and detailed floor plans for new buildings
    - Often available on-line
- Keys or other access devices
- Facility
  - Air handling, steam, electrical
- Personnel

158

Select Agent Security

- May be limited by financial resources, especially in academic setting
- Should be "risk-based"
- Aims:
  - Significantly delaying bad guy
  - Making theft of agent readily apparent
- Illicit use or theft of SA by authorized person
  - Almost impossible to prevent

159

Select Agent Security

- Should concentrate on agent and work outwards
  - Agent
  - Storage device
  - Anteroom door
  - Exterior lab door
  - Corridor
  - Exterior building door
  - Other
    - Fencing, guards, etc.

160

BMBL, Appendix F

- Laboratory Security and Emergency Response for Microbiological and Biomedical Laboratories
- Updated December 2002
- Useful for labs working with select agents

161

Select Agent Security

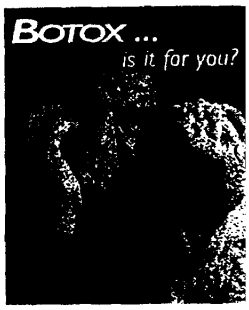
- Limit access to building or corridor containing registered labs
- In addition, need at least two other barriers to agent access
  - Methods will vary for each situation
  - Two secure doors?
  - Secure door + securely locked freezer/incubator?
- Physical security very important, but integrity, responsibility and training of personnel are vital to overall security

162

Select Agent Security

- Working with Select Agents will have significant effect on how you conduct research in your lab
  - Must find balance between securing agent and allowing relatively unimpeded research.
- "Security should not impede the operation"
  - Goal is minimal disruption of vital research
- May seem frustrating
  - Significant effort to protect against something that can often be found in the environment

163



164

Select Agent Security

- Must account for human behavior
  - If security measures too difficult, compliance will drop
    - e.g. "bootleg" stashes of working SA stocks in labs
  - Hard to break old habits
  - The best door locks are useless if the door is propped open
  - The long supervision required for new hires may be frustrating for everyone

165

Physical Security

- Building, rooms, agents
- Written inventory
- Security assessment
  - Institutional security personnel
  - Outside consultants
- May be expensive to implement
- Should be risk-based
  - Stocks, purified toxins highest risk

166

Laboratory Security

- Security awareness training recommended
  - Private consultants
  - Institutional security personnel
- Before personnel leave a registered area, they have to double-check that agent is secured if no one else is present
- End-of-the-day checks

167

Building/Lab Security

- May be necessary to question visitors in non-registered areas under certain circumstances
  - "Can I help you find someone?"
  - Use of ID tags
- Contingency plan if people locked out of lab after hours (if access devices locked in lab)

168

Security vs. Safety

- Trade-off's
  - Access 24/7 by unregistered police, fire, EMS
  - Biohazard signs on doors identifying agent
    - Biological safety vs. security
    - Two-door systems
      - Signage on inner door, not visible from outside?

169

Security vs. Safety

- Biohazard signs on units within lab
  - Incubators, freezers
  - Same issues--biological safety vs. security
  - Coded labels on stocks in storage
    - Hard for outsider to identify SA items

170

Security Risk Assessment

- Walk around your facility/labs
  - If you were a bioterrorist, how could you access agent?
- Recommendations from police, institutional security, consultants
- Very important to listen to feedback from all laboratory personnel
- Risk based
  - Likelihood of an event vs. the consequences from that event
- Where are you most vulnerable
  - "A chain is only as strong as its weakest link"

171

Security Tests

- Recommend periodically testing security
  - Agent locked?
  - Doors locked?
  - How easy is it for a visitor to talk their way into the lab?
- Should not be punitive, but should be used as a learning experience

172

Autoclave Security

- If autoclave room located outside of registered lab(s), materials containing select agent cannot be left unattended if autoclaves not immediately available
- Risk if unauthorized person aborts autoclave cycle and removes items?
  - Heat-tolerant spores may still be viable
  - Should you "babysit" autoclave during entire cycle?

173

Door Security

- Access control devices
- Hinge pins removable?
- Breakable/removable windows?

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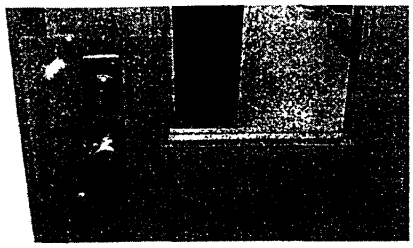

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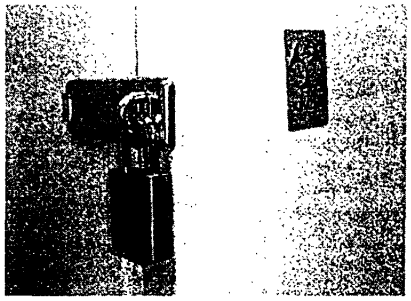

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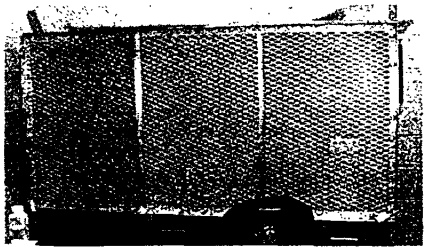

177

Inspection of Packages

- Regulations vague
  - "require the inspection of all packages upon entry and exit from area"
  - May be easy for some facilities
- Clarification (CDC website)
  - Minimum--method of inspecting unexpected or suspicious packages in or out of laboratory or facility

178

Other Security Measures

- ID tags
- Guards
- Remote cameras
- Alarms

179

Access control devices

- Keys
  - Loss of keys can be major headache
  - May be only option, especially if cost an issue
- Electronic card systems
  - Easy to track personnel access to registered areas
  - Easy to re-program
  - Expensive
- Biometric devices
  - Retinal scanning devices
  - Fingerprint recognition devices
    - Won't work in areas where glove use mandatory
  - Extremely expensive

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181

Access control devices

- Who gives out keys or programs access control devices?
  - That person may need to be registered
- Emergency personnel
  - Must make some provision to allow 24/7 access to select agent areas
    - Implementation will vary widely
    - "universal key" = fire ax
  - Optimally, should be in contact with police, EMS, fire before registering
    - Describe agent, layout of rooms, determine access

182

Access control devices

- Keep accurate, up-to-date records of registered personnel with access control devices
  - Who
  - Which areas
- Make sure turned in when employees/students leave for other employment
- Keys to secondary storage devices for SA must be secured
  - i.e. keys to locked incubators, freezers

183

Loss of Access Control Devices

- Due to human nature, it is likely that this will happen eventually
- Try to prevent
  - Make sure personnel know what will happen if they lose their keys or key cards
- Reportable incident--emergency!
- Have policy in place and verify that personnel know how to handle this

184

Loss of Access Control Devices

- Agent must be secured immediately
  - Notify police/security, PI, lab supervisors
  - Registered personnel & police/security may need to babysit agent until locks or key codes changed
- Depending on circumstances & length of time missing, it may be necessary to conduct inventory of agents to verify nothing missing
- Change access control devices
  - Easier with electronic access

185

Personal Safety

- Training,
- Incidents
  - Suspicious persons
    - Never compromise personal safety to confront someone
- Sharing SA information

186

Cybersecurity Plan

- E-mailing sensitive documents
  - What is a sensitive document?
- Inventory record --most sensitive document
- Firewalls, encryption, other security measures
- Assume hard drive can be accessed?
- Change passwords often
- Complex issue
  - Work with local IT experts

187

CDC/APHIS Inspections

- Announced and unannounced
  - Written notification of announced inspections
- Reason
  - Verify information in entity registration
  - Ensure compliance with biosafety & security requirements
  - Review records
- CDC or APHIS will:
  - document findings of inspections
  - Require that deficiencies be remedied

188

CDC/APHIS Inspections

- Should include:
  - Standard operating procedures
  - Access control
  - Visitor logs
  - Inventory records
  - Emergency response plans
    - PPE, spill kits, eyewash stations

189

CDC/APHIS Inspections

- Should include:
  - Physical laboratory
  - Accuracy of registration
    - Floor plans
    - Registered personnel
    - Agents used in each area
  - Building HVAC, electrical
  - Anything and everything

190

Inspection Recommendations

- Perform an internal audit before inspection
  - Review records
  - Organize documentation
  - Detailed visual check of all registered areas
    - Include all personnel
- Perform routine internal audits

191

Inspection Recommendations

- An inspection is usually not the time to argue policy or regulations
  - Let RO deal with these issues later
- Cooperate
- Be truthful, but don't volunteer information if not asked
- Make sure all documentation available
- APHIS inspectors
  - veterinarians

192

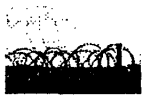
USDA Checklists

- Different biosafety levels for labs & animal facilities
- Personal security
- Physical security
- Cybersecurity
- Inventory security
- Incident response

193

USDA Checklists

- May be hard for academic institutions and some other facilities to comply with all items
  - Traditionally open community at public universities
  - Age of facilities
  - Difficulties with security issues
    - Public access to many areas
  - Significant costs



194

CDC/APHIS Inspections

- Treat inspectors like visitors
  - Check ID's
  - PPE & other entry requirements
  - Explain visitor policy
  - Sign them in
  - Supervise at all times
  - Inspectors cannot handle SA
    - They are not listed on your SA registration
    - Same may be true of RO and ARO
  - Inspectors can examine written SA inventory records

195

Unannounced APHIS Inspections

- 7 CFR 331.15
  - APHIS inspectors must "be allowed, without previous notification, to enter and inspect the entire premises, all materials and equipment, and all records required to be maintained by the regulations"

196

Unannounced CDC Inspections

- 42 CFR 73.16
  - "The HHS Secretary, without prior notification and with or without cause, shall be allowed to inspect any site at which activities regulated by this part are conducted and shall be allowed to inspect and copy any records relating to the activities covered by this part"

197

Unannounced CDC/APHIS Inspections

- RO may want to be present
  - Find out in advance how RO wants to handle these
- May need to temporarily deny access until RO/ARO present and identify of inspector verified
  - Be polite & explain what is going on
  - Check identification
- Make sure lab personnel know how to handle unannounced inspection
  - Assume it will happen
    - If fully compliant at all times, should not be problem

198

Inspections by RO/ARO

- Required at least annually
  - May be more frequent
  - Both announced and unannounced
  - Results must be documented
  - Deficiences must be corrected
    - You may not agree with RO's interpretation of requirements

199

Inspections

- Unannounced inspections by both your RO and CDC or APHIS will occur!
  - If you are fully compliant at all times, and your records are kept up-to-date, they shouldn't be a problem.

200

After CDC/APHIS Inspections

- Ask for exit interview (informal) so you can start correcting potential deficiencies.
- RO will receive report with timeline for correcting deficiencies
  - May take significant length of time to receive report
- Review results with all personnel
  - Retraining if changes in lab SOP's/policies

201

Potential Negative Indirect Effects of Select Agents Regulations

- Reducing or restricting collaborations
  - Between or within institutions
- Difficulties transferring personal SA registration
  - Effect on rotating sabbaticals, visiting scientists
- Visa problems or delays
  - new hires, students, or visiting scientists
- Loss of documents or processing errors by USDA or CDC can delay or prevent SRA's
- Effect on lab planning and design

202

Potential Negative Indirect Effects of Select Agents Regulations

- Potential graduate students
  - Short-term rotations
- Increased biosecurity may delay useful clinical information
- Talented scientists scared away from research on select agents
  - Fear inability to pass SRA
  - Unable or unwilling to try to meet other requirements
- Destruction of stock cultures by labs unwilling to register for SA use

203

Relevant Articles in Science

- R. T. Mulcahy
  - "An Uncertain Partnership"
  - 302:949, November 7, 2003
- J. Gaudioso and R.M. Salerno
  - "Biosecurity and Research: Minimizing Adverse Impact"
  - 304:687, April 30, 2004

204

Publication or Dissemination of Sensitive Data

- "dual use dilemma"
  - Same information used for good or bad purposes
  - Concept--"sensitive but unclassified"
  - Addressed in NAS draft document--2003
    - "Risk that the research results, knowledge, or techniques could facilitate the creation of "novel" pathogens with unique properties or create entirely new classes of threat agents"
    - Challenge to scientific community to develop system allowing unimpeded fundamental research, while identifying research with great potential for misuse

205

Dual Use Dilemma

- Limiting dissemination of research results to protect against use by bioterrorists could restrict communication between scientists
- What should be restricted?
  - Theoretically, results of almost any biological research could be used for both good and bad purposes.

206

Proposed Review Board

- NSAAB
  - National Science Advisory Board for Biosecurity
  - Proposed review board to improve biosecurity measures related to "dual use" research
  - Managed by NIH
  - Wide variety of members, including bioethics, law enforcement, vet medicine
- Voluntary (?) guidelines
- www.biosecurityboard.gov

207

Publication or Dissemination of Sensitive Data

- Future
  - "eyes-only" or classified publications?
    - Already being discussed
  - Closed seminars?
    - Or monitored attendance

208

Publication Issues for Select Agent Research

- Limits on publications may lead to:
  - Difficulty obtaining tenure
  - Difficulties obtaining future grants
- Extended review process could significantly delay publication
- Restrictions on publication may not always make sense
  - Plenty of information already available in published literature
    - Purification of botulinum toxin
    - Isolating *C. botulinum* from environmental samples
    - Virulence plasmid transfer between strains of *B. anthracis*

209

Costs of Select Agent Research

- Time for paperwork
  - PI/lab manager
  - RO/ARO
  - Initial and ongoing
- Time for training
  - Initial and ongoing
- Improving physical security
  - Initial costs may be high
  - Assistance available from entity?
- Installation of BSL-3 labs

210

Costs of Select Agent Research

- Purchase of new equipment formerly shared with other labs
  - Also may be space issue due to consolidation of equipment within registered labs
- Inability of new hires to immediately work with Select Agents
  - Try to hire well before actual start date and start SRA as soon as possible
  - May need to find something else for them to do in the interim
  - Try to write into new grants

211

Indirect Costs-Select Agent Research

- Research takes longer (assume ~20% average)
  - Locking doors
  - Signing in & out
  - documenting everything
- Full compliance with basic biological safety requirements

212

Is it worth it?

- Proposed FY2005 HHS biodefense budget is $4.1 billion
  - Significant amount of research & development funds
- Current NIAID Biodefense Research Funding Opportunities
  - www2.NIAID.nih.gov/Biodefense/Research/funding.htm#B

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NIAID Biodefense Research Funding Categories

- Basic research
  - Pathogen biology, host response, microbial genomic sequencing, proteomics
- Target identification
  - Potential vaccines, diagnostics, immunotherapy targets
- Preclinical development
  - Product refinement testing
- Clinical evaluation
  - Biodefense therapies, vaccines, diagnostics
- Research resources
  - Biodefense research capacity and infrastructure

214

Category A Agents

- NIAID Biodefense Research Agenda for CDC Category A Agents
  - February 2002

215

Select Agent Researchers

- SA registration certificate for U.S. institutions is required prior to spending NIH/NIAID funds on SA research
- Growing pressure for collaboration if SA registered
- Some major institutions not allowing SA researcher

216

Biological Safety

- BMBL recommendations are treated as requirements for SA research
  - Compliance in non-SA labs may vary
    - Academia, older facilities
- Check with institutional biological protocol requirements

217

Biological Safety Guidelines for Labs working with Listed Toxins

- 29 CFR 1910.1450
  - "Occupational Exposure to Hazardous Chemicals in Laboratories"
- Biosafety in Microbiological and Biomedical Laboratories (BMBL)
  - Appendix F
  - Appendix I--"Guidelines for Working with Toxins of Biological Origin"
- NIH or BMBL guidelines
  - Toxin-producing organisms or recombinant DNA coding for toxins
- Your institution's biological safety office

218

Biological Safety Guidelines for Labs working with (Infectious) Select Agents

- BMBL
- "NIH Guidelines for Research Involving Recombinant DNA Molecules"
- Again, check with your institution's Office of Biological Safety
  - Highly recommend an open, non-adversarial relationship with biological safety administrators

219

BMBL, 4th edition

- Biosafety in Microbiological and Biomedical Laboratories
- Available at
  - www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
- 5th edition anticipated September 2004

220

Other helpful resources

- Biological Safety: Principles and Practices
- American Biological Safety Association
  - www.absa.org
  - Many biological safety publications available
- CDC/NIH publication
  - Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets
- CDC, NIH, USDA websites

221

Other helpful resources

- RO/ARO
  - Labs should have close working relationship with RO
  - May have training available
- Collaborators that already work with SA
  - May have recommendations for specific problems
- Some institutions may have committees that handles Select Agent issues & reviews
  - UW-Madison Biosecurity Task Force

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UW-Madison Biosecurity Task Force

- Committee handling SA regulatory compliance and policy development at University of Wisconsin-Madison
- Interpret regugulations and try to fit in local environments (labs)

223

UW-Madison Biosecurity Task Force

- Members
  - RO, ARO
  - Select members of Dean's office, UW-Police, UW-Communications, Office of Biological Safety, Division of Information Technology
- Prepared useful, mandatory SA training course for all persons registering for SA use

224

Door Signage--BSL-2 or higher

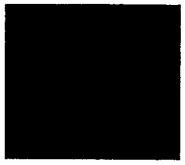
- Must include
  - Biosafety level
  - Biological material(s) in use
  - Special procedures or precautions for entry
  - Name of PI
    - Work & emergency phone numbers
- Recommend
  - Alternate contact information (name, phone)

225

226

Biohazard Symbol

- Use sparingly
  - Door to laboratory
  - Cages of infected animals
  - Equipment
  - Pathogen storage areas



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BSL-1 Practices

- No eating, drinking, applying makeup
- No mouth pipetting
- Safety glasses worn
- PPE
  - lab coat (stays in lab)
  - Gloves
  - Face/eye protection
- Handwashing
- Safe handling of Sharps
- Decontamination of cultures and waste
- Lab access limited when work in progress

228

BSL-2 Practices

- BSL-1 practices plus
  - Biohazard or restricted access sign on door
  - Door closed
  - Limit access to lab
  - Minimize aerosols
  - Use of biosafety cabinet
  - PPE--gloves, lab coats, respirators if needed
  - High degree of caution with Sharps

229

BSL-2 practices (continued)

- Decontaminate surfaces and equipment
- Biosafety manual specific to lab
- Policies and procedures for entry
- Leakproof transport containers
- Training with annual updates

230

BSL-2 Facility

- Work surfaces impervious and easily cleaned
- Lockable lab doors
  - Restricted access when work in progress
- Sink for handwashing
- Sturdy furniture
- Nonabsorbent surfaces on chairs
- Eyewash readily
- Negative airflow
- Adequate illumination

231

BSL-2 Facility

- Autoclave available
- Lab separate from public areas
- BSC (class II) may be needed
  - Aerosols
  - High concentrations
  - Large volumes

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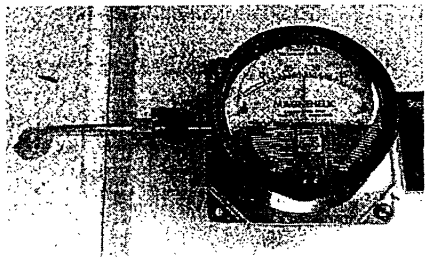

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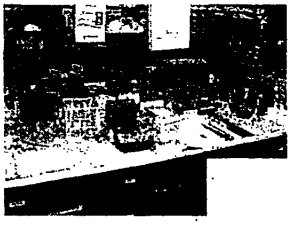


BSL-3

- Work with infectious agents or toxins that may cause serious or potentially lethal disease as a result of aerosol exposure

235

Generation of Aerosols

- Centrifugation
- Vortexing
- Pipetting
- Sonication
- Electroporation
- Popping tube caps
- Flame sterilization micro tools
- Flow cytometry
- Infected animals

236

BSL-3 Requirements

- BSL-1 and BSL-2 plus:
  - Separate building or isolated zone
  - Double door entry
  - Directional inward airflow
  - Single-pass air, 10-12 air exchanges per hour
  - Enclosures for aerosol-generating equipment
  - Room penetrations sealed

237

BSL-3 Requirements (continued)

- Walls, floors, ceilings water resistant for easy cleaning
- Vacuum lines protected by HEPA filters or liquid disinfectant traps
- Certified BSC class II or III for manipulations
- May need respiratory protection
- Prompt decontamination of spills
- Adequate supervision
- Personnel
  - Trained
  - Demonstrate proficiency
  - Medical surveillance
  - Report incidents

238

BSL-2 facility with BSL-3 practices

- Existing facilities that don't have all facility features for BSL-3.
- Strict adherence to BSL-3 practices in BSL-2 facility result in acceptable level of safety for routine procedures
  - BMBL

239

Biological Safety Cabinets

- Protect workers and materials from infectious or toxic substances
- The protection provided by a biosafety cabinet is only as good as the practices of the person using it
  - Training in proper use of BSL highly recommended
    - Institutional
    - ABSA, etc.

240

Types of BSC's

- Class I
  - Modified chemical hood with HEPA filtered exhaust
  - No product protection
- Class II
  - Type A1
    - recirculating cabinet with HEPA filtered exhaust
    - Provides product and personnel protection
  - Type A2: 30% exhause
  - Type B1: 70% exhaust
  - Type B2: 100% exhaust
- Class III
  - Fully contained glove box

241

Emergency Equipment

- Eyewash stations
- Emergency showers
- Spill kits

242

Clostridium botulinum

- "botulinum neurotoxin-producing strains of Clostridia"
- Risks
  - Spores
    - Generally not toxic to adults
    - Infant botulism, wound infection
    - Cultures may contain high levels of BoNT
  - Botulinum neurotoxin
    - Most potent toxin known to man
    - Lethal dose as low as 1 ng/kg
    - Aerosol, ingested, wounds, skin, eyes, mucous membrane
  - No person-to-person transmission

243

C. botulinum & BoNT

- BSL-2
  - Spores/vegetative cells
  - Materials potentially containing BoNT
  - Animals-mouse bioassays
- BSL-3
  - High potential for aerosol
  - BoNT production
  - Purified toxins

244

Clostridium botulinum

- Inactivation
  - Moist heat
    - spores-121°C, 15 minutes
    - Toxin-100°C 10 minutes
  - Disinfectants
    - Spores-1% sodium hypochlorite, 70% ethanol
    - Toxin--0.1% sodium hypochlorite, 0.1N NaOH

245

BoNT

- Medical
  - Immunization with pentavalent toxoid
    - Pros & cons
  - Treatment after possible exposure
    - Monitor for symptoms
    - Antitoxin--may be effective if treated early
    - Assist with mechanical ventilation
  - <5% fatality from botulism if treated
- Two reported laboratory-acquired cases of botulism

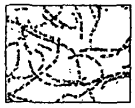
246

C. botulinum and BoNT

- Recommended precautions
  - Lab coat, gloves, gown
  - BSL-2
    - *C. botulinum* spores, small amount of cultures
  - BSL-3
    - Aerosol-generating manipulations
    - Toxin production
- Recommend lab workers shower & change clothing before handling infants

247

Bacillus anthracis



- Anthrax
  - Cutaneous
    - 20% fatality if untreated)
  - Inhalational
    - mortality close to 100%; early tratement improves prognosis
  - Gastrointestinal
    - 50-100% mortality, even if treated
- Transmission from person to person rare

248

Bacillus anthracis

- Spore inactivation
  - Resistant to many disinfectants
    - Susceptible to 2% glutaraldehyde, 5% formalin, 0.5% hypochlorite
  - Physical inactivation
    - Requires direct exposure to 121°C for at least 30 minutes
    - incineration

249

Bacillus anthracis

- Medical
  - Susceptible to many antibiotics (ciprofloxacin), requires prompt treatment if exposed
  - Infective dose
    - 8,000-15,000 spores
  - FDA-licensed vaccine available (CDC)
    - May help post-exposure
  - At least 45 cases of laboratory-acquired infections reported
    - 5 deaths
    - Large accidental release in 1979 from military facility in Soviet Union

250

Bacillus anthracis

- Special handling precautions
  - BSL-2
    - clinical materials and diagnostic quantities of infectious cultures
  - BSL-3 practices, equipment, facilities
    - Production quantities
    - Concentrated cultures
  - Adequate protective clothing
    - Gloves, gowns with tight wrists and ties in back
  - Facilities for washing and changing clothes
  - Routinely cleaning equipment, surfaces instruments with sporicidal solutions

251

Francisella tularensis

- Risks
  - Inhalation, skin, eyes, mucosal surfaces
  - 30-35% mortality if untreated (<10% if treated)
  - Not directly transmitted person to person
  - Highly virulent organisms (esp. type A)
    - Low infectious dose (1-10 org's, aerosol or through skin)
- Inactivation
  - Susceptible to many disinfectants
    - 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde
  - Readily inactivated by autoclaving, dry heat

252

Francisella tularensis

- Medical
  - Investigational vaccine available from CDC
    - ~80% efficacy
  - Exposure prophylaxis
    - High risk of infection (spill, needle-stick)
      - Tetracycline or other oral antibiotics for 14 days after exposure
    - Low risk of infection
      - Fever watch + treatment if symptoms develop
  - Laboratory-acquired infections commonly reported
    - Esp. from handling cultures

253

Francisella tularensis

- Handling precautions
  - BSL-2 practices and containment
    - Routine diagnostic procedures for clinical samples
  - BSL-3 practices and containment, and facilities
    - Culture manipulations
    - Animal experiments
    - Potentially aerosol production
  - Lab coat, gloves, gown
    - Fit-tested face masks--infectious materials in BSC
    - Impervious gloves--direct contact with infectious materials, infected animals

254

Yersinia pestis

- Plague
  - Bubonic (lymph nodes)
    - 50-60% mortality if untreated
    - Not usually transmitted person-to-person
  - Septicemic
  - Pneumonic
    - 100% mortality if untreated
    - Easily transmitted person-to-person under certain conditions
      - Aerosol droplets

255

Yersinia pestis

- Medical
  - Exposure
    - Aerosol, parenteral, ingestion
    - Monitor for symptoms
    - Antibiotic therapy
      - Doxycycline, gentamycin,, others
  - Vaccine no longer manufactured in U.S.
    - Not protective against pneumonic plague
  - 10 reported lab-acquired infections
    - 4 deaths

256

Yersinia pestis

- Risks
  - Aerosol
  - Careless manipulation of cultures
- Inactivation
  - Susceptible to many disinfectants
    - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodines, phenolics, formaldehyde
  - Readily inactivated by heat

257

Yersinia pestis

- Specific handling recommendations
  - BSL-2
    - Simple clinical materials
    - potentially infecious cultures
  - BSL-3 practices, containment equipment, and facilities
    - Potentially aerosol-generating manipulations
    - Concentrated infectious materials, production quantities
    - Antibiotic-resistant strains
  - Gloves, gown
    - Mask--aerosol risk

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Spills of Agent or Toxin in Lab

- Similar procedure
  - *C. botulinum*, BoNT, *B. anthracis*, *F. tularensis*, *Y. pestis*
- Allow aerosols to settle (≥ 30 min)
  - May not be necessary with small spills of *C. botulinum* spores
- Wear protective clothing
- Gently cover spill with paper towels
- Apply disinfectant, starting at perimeter and working in
- Allow sufficient contact time before clean-up

259

Spills of Agent or Toxin in Lab

- If spill of aerosol transmitted pathogen, also
  - Evacuate lab
  - Post "DO NOT ENTER" sign(s)
  - Re-enter with PPE after 30 minutes

260

Spills of Agent or Toxin

- Spills will happen
  - All lab personnel should be very familiar with spill protocols
  - Spill protocols specific to your agent(s) should be readily available
  - Practice--spill drills
- Know how to handle large and small spills within the lab and outside the lab

261

Reporting Spills of SA

- Required to report "Theft, Loss, or Release of Select Biological Agents or Toxins"
  - To RO, who will notify CDC or APHIS
  - CDC form 0.1316, APHIS form 2043
- Definition of "release"
  - Spills of agent/toxin exceeding containment
  - Accidental inoculation of someone with SA
- May also require notification of police, DOJ, state Veterinarian, state health departments

262

Spills

- Prepare readily accessible spill kits for every room where agents/toxins used or stored
  - Hypochlorite--need fresh solutions
- Prevention is key
  - Use unbreakable & leakproof materials when possible
    - Tubes, etc.
  - Transport in secondary leakproof containment devices if possible
  - Use carts for transport

263

Summary

- DOCUMENT EVERYTHING!!!
- Get compliant and stay compliant with all relevant regulations & requirements
  - Biosafety, security, recordkeeping
- Select Agent registration and full compliance require a tremendous amount of work and cooperation beween all parties involved.
  - It can be done

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Many thanks.....

- American Society for Microbiology
- UW-Madison Biosecurity Task Force
  - Dr. Tim Mulcahy, Assistant Dean, CALS
  - Jan Klein, Biological Safety Officer
- University of Texas, Environment Health and Safety

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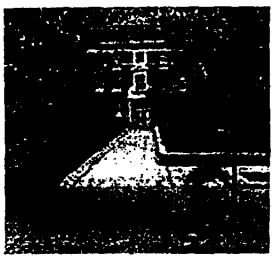
附　　件　　三

附件三、圖內拉大學（Tulane Uinversity）網路資訊

Tulane University Home Page



Tulane University

New Orleans · Saturday, August 28, 2004 · Cloudy · Temp 78°

Calendar of Events

SCHOOLS AND COLLEGES Architecture | A. B. Freeman School of Business | Engineering | Law | Liberal Arts and Sciences | Medicine | Public Health and Tropical Medicine | Social Work | Graduate School | Newcomb College | Tulane College | University College

Spotlight
170 Years of Health Sciences

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Experts Called on for HIV/AIDS Training

Online Reference Center Benefits Teachers

■ The Pace-Willson Glass Studio at Tulane University is a world-class facility

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Tulane

Text Only

Center for Gene Therapy

Overview | Research | Educational Activities | Services | Facilities | Faculty | Employment | News | CGT Home | Matrix DNA Diagnostics

TULANE Center for Gene Therapy
Tulane Center for Gene Therapy
Tulane University Health Sciences Center
J. Bennett Johnston Building
1324 Tulane Avenue, SL-99
New Orleans, LA 70112-2699
Phone: (504) 988-7711
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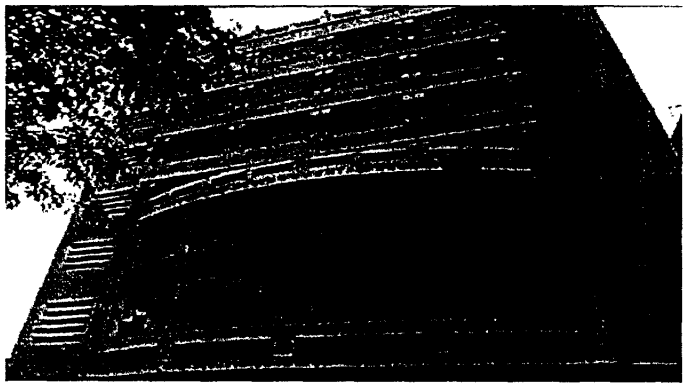
Email: cgt@tulane.edu



The Center for Gene Therapy is housed in the state-of-the-art J. Bennett Johnston Building on the campus of the Tulane University Health Sciences Center in downtown New Orleans

Overview

Overview | Research | Educational Activities | Services | Facilities | Faculty | Employment | News | CGT Home

The Tulane Center for Gene Therapy was formed in July of 2000.

The major aim of the Center is to develop new therapies for a series of common diseases that include osteoporosis, osteoarthritis, parkinsonism, spinal cord injury, stroke and Alzheimer's disease. The primary strategy of the Center is to use adult stem cells that can easily be obtained from a patient and then used for therapy of the same patient. The Center will also provide educational programs for career development, job training and life long learning of citizens; establish a forum to evaluate the social, legal and ethical implications of gene therapy; and develop commercial applications of gene therapy with an emphasis on commercial developments within the State of Louisiana. The Center is supported by research funds from the federal government via National Institutes of Health grants, from the state of Louisiana via the Louisiana Gene Therapy Research Consortium and the Louisiana Board of Regents, from the Tulane University Health Sciences Center, the HCA - Healthcare Company and several private foundations. The Center is a major participant in the Louisiana Gene Therapy Research Consortium that includes gene therapy centers at the LSU Health Sciences Centers in New Orleans and in Shreveport. The Center was launched with a staff of 15 who moved with Dr. Prockop from Philadelphia. It now has a staff of over 30 with plans to increase the staff to about 50 within the next year or two. The Center is housed in 14,000 sq. ft. of modern laboratory space in the Tulane University Health Sciences Center's J. Bennett Johnston Building, located at 1324 Tulane Avenue, New Orleans, Louisiana.

The Tulane Center for Gene Therapy is under the directorship of Darwin J. Prockop, MD, PhD. Dr. Prockop has a distinguished career and his pioneering research is recognized throughout the world. He has been honored by his peers in many ways, including election to the National Academy of Science, two honorary degrees, and the Lee C. Howley Prize of the Arthritis Foundation for research on arthritis.

The therapies being developed by the Center are based on the discoveries largely made by Dr. Prockop and his associates that adult stem cells from a patient's own bone marrow can be gene engineered and then potentially used in the same patient to target the genes of the central nervous system, the

bones, cartilage and many other tissues. The Center staff is doing research both on the basic biology of adult stem cells and developing procedures for use of the cells in patients with devastating diseases.

The interest in adult stem cells by the Center staff began after they and others had identified mutations in collagen genes that cause brittle bone disease in children (osteogenesis imperfecta) and inherited cartilage disorders (chondrodysplasias). We have also identified mutations in collagen genes in common connective tissue disorders such as lumbar disc disease, osteoporosis, osteoarthritis and aortic aneurysms.

Identifying the mutations that cause the diseases led to developing new therapies. Preliminary results from a collaborative clinical trial being carried out at St. Jude's Children's Hospital in Memphis suggests that the adult stem cells will be useful in treating osteogenesis imperfecta and perhaps osteoporosis.

Research

Studies in Progress:

Stem Cells

Characterization of the biological properties of adult stromal cells is essential for a better understanding of their normal function in adults as well as to revealing their potential in treating disease. Stem cells are so named because they are like the stems on a tree that can produce new leaves and flowers each year. Each stem cells has the remarkable property that is can divide so as to produce a perfect copy of itself together with a second cell that can become a "workhorse" cell of the body such as a bone cell or a nerve cell. Because the stem cell produced by the division is a perfect copy of the original stem cell, stem cells seem to be able to divide and live indefinitely, perhaps forever. Understanding stem cells is now one of the most important problems of biology. The Center staff is working at the forefront of research on stem cells using cutting edge technologies to define them in terms of the genes they express. Also, they have developed new procedures that make it possible to begin with a small sample of stem cells from a patient's bone marrow and grow extremely large numbers of the cells in the laboratory. The ability to grow the cells rapidly, in turn, makes it possible to gene engineer the cells with simple techniques that do not involve use of a virus.

To test the cell's potentials for therapy of patients, the Center is using the adult stromal stem cells to treat mice and rats that represent models of human diseases. Plans are being made to carry out similar experiments in non-human primates at the nearby Tulane University Primate Center, the largest N. I. H. sponsored primate center in the country. In one series of experiments, cells are transplanted into mice that undergo repeated spontaneous bone fractures because of a genetic defect. The aim of these experiments is to determine if the stem cells can travel to the site of a bone fracture and strengthen the bone and prevent further fractures. Results from these studies should define the most effective ways the cells can be used to treat human bone diseases, such as osteogenesis imperfecta and osteoporosis. In other experiments, the Center staff is pursuing their discovery that the adult stem cells can differentiate into cells that make up the

brain. Therefore, the cells are being transplanted directly into the brains of mice that exhibit progressive neurodegeneration due to lack of a critical protein. The aim of these experiments is to determine if the stem cells can replace the missing protein and reverse the degeneration of the brain. If the experiments succeed, they will suggest that the cells can be used to treat serious neurological diseases in children such as Tay-Sachs disease. Other studies are evaluating if the stem cells can replace brain cells lost in common diseases of adults. Promising preliminary results were recently obtained in a rat model for parkinsonism. Similar experiments are underway to test the effectiveness of the cells in animal models for Alzheimer's disease and brain tumors (gliomas). The procedures for the experiments in animal models are being developed so as to conform to reporting requirements of the Food and Drug Administration and other agencies in order that the therapies can be introduced as clinical trials in patients as soon as possible

Genetic Deficiencies

Another major interest of the Center is to identify the genetic causes of both common and diseases of connective tissues such as bone and cartilage. The Center staff was among the first to show that mutations in collagen genes can carry mutations causing diseases of bone and cartilage. Their interest in collagen genes was based on a large background of work they and others had done on the structure and function of the proteins and their biosynthesis. Members of the Center staff and their previous associates isolated the first gene for a series of human collagens. They then used the genes to find mutations that caused osteogenesis imperfecta and severe disorders of cartilage that cause dwarfism and associated problems (Stickler syndrome, spondyloepiphyseal dysplasia, and achondrogenesis type II, Kniest dysplasia). They went on to find mutations in collagen genes that caused the defects in a subset of patients with osteoporosis, a subset of patients with early onset osteoarthritis, and a subset of patients with aortic aneurisms that were prone to rupture. More recently, one faculty member of the Center found mutations in collagen genes that cause or predispose sciatica because of intervertebral disk herniation. The genes in which these mutations have been found are complex and the identification of the mutations has in part depended on technology developed by the Center staff for rapid scanning of genes for mutations, a technology known as conformation sensitive gel electrophoresis (CSGE). The DNA diagnostic tests for these and other diseases are being developed for several reasons. One is that a definitive diagnosis as to cause of a disease is frequently an important guide as to which existing therapies or changes in expectations or lifestyle may help the patient. The second reason is that knowing the exact cause of a disease is frequently the first step in developing new therapies, such as gene therapy, that may provide a cure for previously untreatable diseases.

Educational Activities

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Another major role of the Center is the training of new scientists. Annually, the Center mentors a dozen or more undergraduate and graduate students. Each student has a specific project that is a part of the over-all aim of characterizing adult stromal cells or evaluating their potential for treatment of diseases. Students have regular mentoring meetings, as well as regular seminars and journal club. In addition, the Center conducts an active program for postdoctoral fellows. Over the years, over 15 former students and postdoctoral fellows mentored by Dr. Prockop or his associates have become heads of their own departments. The Center is dedicated to continuing this tradition. In addition, the Center is planning open house forums and other programs to provide opportunities to inform local citizens about recent progress in gene therapy.

Services

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Core Laboratories and Services Provided (memo from Dr. Darwin J. Prockop):

Virtual Tour

The core laboratories have been established with funds provided by Tulane University Health Sciences Center, HCA, the Health Care Company and Louisiana Gene Therapy Research Consortium, Inc. We would like to make the services available to all scientists supported by the Consortium and to all academic scientists in this geographic region. For some of the services of the Core, we of necessity must make a charge. For others, there will be no charge for either uses or special situations in which scientists need preliminary data for grant applications.

Brief description of our core laboratories and the services they can provide:

A. Morphology Cores

1. DNA Sequencing. We have two ABI 3100 16-capillary sequencers that have been producing superb results. The sequencers have a large capacity, far larger than we in our Center can use. Justin Manges (504-988-7063) is ready to provide DNA sequencing for anyone at Tulane or in the general vicinity. He can also provide excellent advice in DNA template issues and designing primers to carry out sequencing.

2. DNA Diagnostics. The core carries out very specialized mutation detection for diseases of the skeleton. We have been offering diagnoses of mutations causing osteogenesis impefecta on a fee-for-service basis for many years. The technology was largely developed in our own laboratory and involves a protocol that make it possible to quickly scan multi-exon genes and find the appropriate PCR product to sequence. Please call (504-988-7706) for any questions about the DNA Diagnostics laboratory.

3. Microscopy Core. The Core supplies access to a series of microscopes that include visible light microscopy and ultraviolet microscopy. Dr. Phinney has just had installed a three-dimensional deconvolution microscope that

promises to be better and easier to use than a confocal microscope. It makes it possible to collect sharply focused images of sections and then reconstitute cells and other structures in three dimensions. One of the microscopes is also equipped with a computerized stage and environmental chamber that makes it possible to follow the growth and differentiation of cells in culture in real time.

4. Flow Cytometry Core. Our Flow Cytometry Core lab is equipped with the most advanced instruments available on the market today. We have a Becton-Dickinson FACSVantage SE cell sorter with 3 lasers plus most of the options offered for this model including TURBO-MACRO sort and Clone-cyte cell deposition hardware. We have also recently added a Beckman-Coulter benchtop phenotyper to help handle our increasing use of this technology. The core is operated and administered by Alan Tucker (504-988-7741), and is offered to outside investigators when time is available.

5. MicroPET Core. The MicroPET Core will become available on July 1. There will also be a small laboratory for temporary housing of animals and preparing ligands with radionucleotides with short-half lives. The MicroPET is an instrument that has only been available for about one year and has the amazing ability to do imaging of mice or rats at a resolution of 2 mm3. It promises to revolutionize research in a number of areas. One example of its use is to insert a gene such as the herpes simplex gene for thymidine kinase into a virus or cells that are about to be infused into a mouse or a rat. One can then anesthetize the animal and inject a fluoride -18 labeled analog of gancyclovir and visualize the location of the tagged virus or cells. The imaging is in real time and will be carried out repeatedly on the same animal. There are currently only about seven instruments available in the world and we hope to take delivery of ours in about a year. Any interested faculty members should consult a recent review by Michael Phelps (PNAS 97: 9226, 2000)

B. Stem Cell/Vector Cores

1. Microarray Core. Justin Manges (504-988-7063) and Joni Ylostolo (504-988-7071) have an operational Affymetrix microarray instrument for assays of mRNAs. They have already produced some very exciting results with it. They would like investigators to bring their samples of cells to him so that they would carry out the extraction of the RNA and the labeling. They would then provide the data. We think the Affymetrix instrument has an advantage over competing instruments because it uses specifically designed oligonucleotides instead of cDNAs. However, the chips cost about a thousand dollars each and the reagents are expensive. Also, it is frequently necessary to run duplicates. Therefore, the best strategy is to use the Affymetrix chips to identify mRNAs of interest and make a cheaper cDNA chip for genes of special interest.

2. Mass Spectrometry Core. Carl Gregory (504-988-7716) has set up an amazing mass spectrometer for protein analysis. The instrument can detect as little as one picogram of protein and indicate molecular weight within two

or three Daltons. By analysis of both the intact proteins and of tryptic peptide of the same proteins, it is possible to identify about half the proteins contained in most cells.

3. Circular Dichroism Spectro polarimeter Core. Dr. Leena Ala-Kokko (504-988-7709) and Joni Ylostalo (504-988-7071) have made this instrument operational. It provides a rather elegant assay of the conformation of macromolecules.

4. Biosensor Assay Core. Dr. Leena Ala-Kokko (504-988-7709) and her student Joni Ylostalo (504-988-7071) have set up an instrument for real-time bioassay of molecular binding. The Fison instrument is simpler to use than the Pharmacia BioCore. These instruments give real time assays of on rates and off rates as well as equilibrium constance. The data are elegant but some care must be taken as to the chemical steps involved in binding the first ligand to the solid support.

5. Cell Culture And Repository Core. We have set up a suite of three tissue culture rooms to handle a modestly large volume of cultured cells. We welcome occasional use by any faculty member, but we are not certain as yet how much of the capacity of this Core will be available for sharing. The cell repository in the Core contains almost 2,000 samples of cells from patients with a variety of diseases.

6. Laser-Assisted Microscopy Core. Joni Ylostalo (504-988-7071) and Justin Manges (504-988-7063) operate an elegant instrument for laser dissection of single cells from microscopic slides. The instrument is a P.A.L.M. that we think is much superior to competing instruments from other companies. It is fast and a delight to use.

7. Real-Time PCR Core. Dr. Donald Phinney (504-988-7725) has set up a real time PCR assay machine from ABI. Although I was originally skeptical about some of the claims made by the company, the instrument does provide quantitative assays of either messenger RNA levels and of rare DNA sequences. A key to proper use of the instrument is the design of the necessary probes. Don Phinney can provide expert assistance in the designing of these.

Photos

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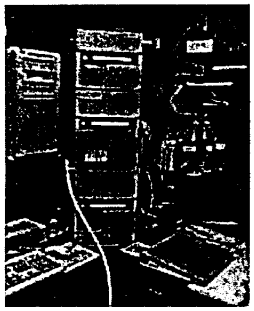
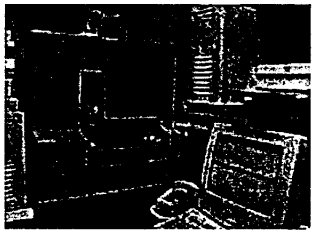
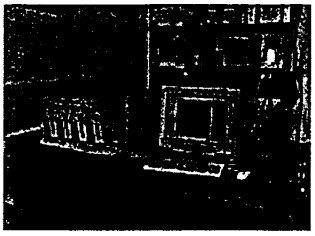
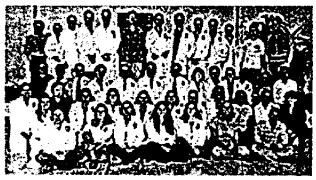
Click the photo for a larger version.









Employment

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Endowed Recruitment Programs for Fellows, Faculty and Visiting Professors in STEM CELL RESEARCH

The Tulane Center for Gene Therapy offers a series of endowed programs for research in stem cell biology and use of stem cells in gene therapy. The Center is supported by a five-year program funded by Tulane University, HCA-the Healthcare Company, and the Louisiana Gene Therapy Research Consortium, Inc. The Center provides a series of core facilities that include DNA sequencing, micro array assays, deconvolution microscopy, laser assisted microdissection, protein mass spectrometry, biosensor assays of ligand binding, CD spectropolarimetry, and FACS. In addition, a core laboratory is available at the affiliated Tulane University Primate Research Center and there is access to an NIH-funded General Clinical Research Center. A microPET core for imaging of small animals will be available within the year.

POSTDOCTORAL FELLOWSHIPS: Highly competitive stipends are offered for up to 3 years. Successful candidates should be graduates of well-recognized institutions and have an excellent command of English.

FACULTY POSITIONS: Tenure-track appointments are available at the level of assistant professor. The program is fully funded to provide salary, laboratory space, access to graduate students and up to four years of support for a postdoctoral fellow, a technician and research supplies for each faculty member.

SABBATICAL YEAR PROGRAM FOR VISITING PROFESSORS: Positions provide up to one year of full support for salary and research expenses for highly qualified scientists. Send (1) curriculum vitae, (2) brief summary of research interests, and (3) two or more letters of recommendation to Dr. Darwin J. Prockop, Director, Center for Gene Therapy, Tulane University Health Sciences Center, 1430 Tulane Avenue, SL-99, New Orleans, LA 70112; Fax (504) 988-7710; E-mail: dprocko@tulane.edu

Gene Therapy Center News

Current News: NIH Grant to Prepare Adult Stem Cells

- Tulane to Study Repair of Heart Damage Using Stem Cells
- Tulane Receives NIH Grant for Center to Prepare Adult Stem Cells
- Tulane at Epicenter of Stem Cell Research
- NIH Press Release: New Center for Preparation and Distribution of Adult Stem Cells
- Video News Release: Tulane Receives NIH Grant for Gene Therapy Center to Prepare Adult Stem Cells (You must have the RealPlayer software to view the movie)
- The Times-Picayune: Tulane Becomes Stem-Cell Supplier
- The Times-Picayune Editorial: Stem Cell Standard
- The Advocate: Tulane Wins Stem-Cell Work Grant

Press Releases

- Helping Children with Brittle Bone Disease - June 25, 2002
- Researcher Gets "Mini Nobel" - February 1, 2002 (see a photo from the award presentation)
- Tulane Receives One Million for Research into Genetic Therapies - November 15, 2001
- Tulane Doctor Receives Anders Jahre Award for Medical Research - October 12, 2001
- Tulane Receives Grant to Work on Cure for Brittle Bone Disease - October 10, 2001
- Tulane Center for Gene Therapy Receives $500,000 for Alzheimer's Research - May 21, 2001
- Medical Conference Focuses on Use of Stem Cells for Tissue Transplant - March 21, 2001
- Genetic Risk Factor Identified for Lumbar Disk Disease - April 11, 2001

Presentations/Publications

Press Kit

- PR Contacts
- Photos/logos

Links

- NIH Gene Therapy
- Louisiana Gene Therapy Research Consortium

Virtual Tour

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Dr. Prockop with students in laboratory

Best viewed with Quicktime

Hold your Mouse over the image above. Click and drag to investigate the panorama. Use the shift and control keys to zoom in and out.

- Fluorescence Activated Cell Sorter and Histology Cores
- Tissue Culture Laboratories
- Common Equipment Area

Virtual Tour

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Fluorescence Activated Cell Sorter and Histology Cores

Best Viewed with Quicktime

Hold your Mouse over the image above. Click and drag to investigate the panorama. Use the shift and control keys to zoom in and out.

- Dr. Prockop with students in laboratory
- Tissue Culture Laboratories
- Common Equipment Area

附　　件　　四

附件四、壁報論文內容

AMERICAN SOCIETY FOR MICROBIOLOGY
1752 N STREET, NW, WASHINGTON, DC 20036-2904

27 February 2004

Tsui-Ping Huang
Division of Food Microbiology, Bureau of Food and Drug Analysis, Department of Health,
Executive Yuan, R.O.C.
National Laboratories of Food and Drugs, Departmen
Taipei, 11513
Taiwan Republic of China

Re: Abstract number - 970

Dear Dr. Huang

I am pleased to inform you that your abstract has been accepted for a **poster** presentation at the 104th General Meeting, which will be held at the Ernest N. Morial Convention Center, from May 23 through May 27, 2004 in New Orleans, LA.

Following is information pertaining to the above abstract:

Abstract Title: **A Novel Method for Detecting the Staphylococcal Enterotoxin genes from sea to sep**
Session No.: 327
Room/Day/Time: Poster Hall/May 27, 2004 9:00 AM
Presentation Number (to be included in your poster title): D-252

Poster Placement: **The size of the posterboard is 4 feet tall by 8 feet wide (1.2 m x 2.4 m).**

Two poster sessions are scheduled each day (except Thursday). The morning session is from 9:00 a.m. to 12:00 p.m. and the presenter must stand at the poster from 10:30 a.m. until 12:00 p.m. The afternoon session is from 1:00 p.m. to 4:00 p.m. and the presenter must stand at the poster from 1:00 p.m. until 2:30 p.m. The period between 12:00 and 1:00 p.m. is reserved for removing the morning posters and placing the afternoon posters. The poster area of the Exhibit Hall is only open to the public from 9:00 a.m. to 4:00 p.m. **Therefore, you must bring this letter with you and show it to Security in order to place your poster between 7:30 and 9:00 a.m. for the morning session or to remove it between 4:00 and 5:30 p.m. for the afternoon session. On Thursday, admittance will be until 12:30 p.m. for poster removal/retrieval.**

Please recall that you agreed to present your poster as scheduled. If you fail to do so, you will be prohibited from submitting abstracts to ASM-sponsored meetings for 3 years. If unable to present your poster, notify ASM before March 5, 2004, so that your abstract will not be published.

Please check our website at http://www.asm.org/Meetings/index.asp?bid=697 for information regarding presentation hints. Select " Poster Guidelines" from the sidebar. While you are at the website, do not forget to register for the General Meeting. The link to the registration company can be found under "Registration and Housing" at the URL listed above.

Please note that for those who submitted a request for a student travel grant, confirmation notices will be sent under separate cover March 10.

We look forward to your participation and to seeing you in New Orleans.

Sincerely,

104th General Meeting

A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from *sea* to *sep*

Daniel Yang-Chih Shih, Tsui-Ping Huang,
Yu-Chang Chang, Yun-Pu Huang and Jan-Yi Wang,

Division of Food Microbiology, Bureau of Food and Drug Analysis,
Department of Health, Executive Yuan
161-2, Kuen-Yang Street, Nankang 115, Taipei, Taiwan, R.O.C.

Staphylococcus aureus ranked the second place among the foodborne pathogens that were responsible for the food poisoning outbreaks in Taiwan during the late twenty years. Since the staphylococcal enterotoxins (SEs) were the main causes, in this study we established a novel method by polymerase chain reaction (PCR) technique to detect the genes of 15 SEs, including *sea, seb, sec, sed, see, seg, seh, sei. sej, sek, sel, sem, sen, seo,* and *sep*. We designed the specific primers to obtain particular fragments from 156 bp (*sel*) to 880 bp (*sej*) , that were varied to each other, so we could adapt into many combination of multiplex PCR. We cloned each genotype of SE gene from the isolated strains into the plasmid and confirmed it by sequencing, for established an in-house standard strain. Then, the method was applied to screen 222 strains isolated from 85 samples of 34 outbreaks. The results showed that there were harboring several SE genes, and from one gene to 10 different genes could be found in the same strain. According to the different combination of the 15 SE genes, we divided these strains into 84 patterns. Among the tested strains, the top three groups were two-gene, three-gene and one-gene strains, and the percentages were 27.5%, 20.3% and 18.9%, respectively. There are one nine-gene strain and one ten-gene strain, their SE genotypes were AGHKLMNOP and BGHIKLMNOP respectively. The top five detection rates in each SE gene were *sep* (85%), *seo*(44%), *sem*(35%), *sel*(32%), and *sea* (28%) .

Keywords: *Staphylococcus aureus*, outbreak, staphylococcal enterotoxins, polymerase chain reaction (PCR), genotype.

Introduction

Staphylococcus aureus is one of the common causes of food poisoning worldwide, and moreover it also ranked in top three causes in Taiwan for the last twenty years. Staphyolcoccal food poisoning is characterized by vomiting and diarrhea resulting from the ingestion of foods or beverages contaminated with one or more preformed enterotoxins (SEs). Several types of Staphylococcal enterotoxins have been described and it is ordinary for *Staphylococcus aureus* to produce one or more of these toxins at the same time. In addition to the well-recognized SEA, SEB, SEC, SED, and SEE enterotoxins, new types of SEs (SEG, SHE, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, and SEP) have been identified, but their role in food poisoning is not clarified. Based on amino acid sequence comparisons, SEs have been divided into several groups; one includes SEA, SED, SEE, SEJ, and SHE, and another SEB and SEC, whereas SEG and SEI could not be clearly attributed to a specific group.

The *se* genes are carried either by plasmids (*sed* and *sej*), by phages (*sea*, and *see*), or by the chromosome (*seb, sec, seg, she, sei, sek, sel, sem, seo, sep, and seq*). Also, different *se* genes are implicated in pathogenicity islands such as the *sec*-bovine gene, or the *seb, sek* and *seq* genes. In addition, a cluster named *egc* encodes for toxins SEO, SEM, SEI, SEN and SEG, and two pseudogenes £ *ent1* and £ *ent2* localized between the *sei* and *sen* genes.

The aim of this study was to establish a rapid method to detect the *sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo,* and *sep*. We also investigated the presence of these 15 *se* genes among 222 *Staphylococcus aureus* isolates collected from 35 food poisoning outbreaks in Taiwan.

Methods

1. Stratagem for detection of genes and establishment of reference strains as figure 1.
2. Flowchart for genomic DNA isolation as figure 2.
3. Sample sources of *Staphylococcus aureus* isolated from food poisoning outbreaks in Taiwan as table 1.
4. Specific primers used for detection of SE genes in *S. aureus* by PCR methods as table 2.
5. Establishment of in-house reference strains as figure 3 .
6. The *Polymerase Chain Reaction (PCR)* : The extracted DNA (1 μL) as template was added with a reaction mixture containing 1X reaction buffer (10 mM Tris-HCl, pH 8.8 at 25℃, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X -100), 200 μM deoxynucleotide triphosphates, 500 nM each primer, and 1U/100 μL Recombinant *Taq* polymerase (Takara Shuzo Co., LTD, Shiga, Japan) to a final volume of 100 μL. Table 2 lists the Primer sequences. A PCR reactor （GeneAmp, PCR system 9700, Applied Biosystems, Foster City, CA, USA）was used. Reaction temperatures were programmed as follows: (1) at 95℃ for 10 min; (2) at 95℃for 1 min; (3) at 58℃ for 1.5 min; (4) at 72℃ for 1.5 min. The steps from (2) to (4) were repeated for 35 cycles and then finished with the step at 72℃ for 7 min. The resulting products were analyzed using a 2% agarose gel electrophoresis.

Table 1. Sample sources of *Staphylococcus aureus* isolated from food poisoning outbreaks in Taiwan

Sample group	No. of Outbreak	No. of sample	No. of strain
Fishes	11	17	42
Fishery products	2	2	6
Meats and their products	11	15	42
Eggs and their products	0	0	0
Milks and their products	0	0	0
Crops and their products	4	4	13
Fruits & vegetables and their products	2	3	7
Cookies & Candies and their products	0	0	0
Lunch boxes	4	12	29
Complex dishes	7	9	25
Swabs from environmental sample	11	18	44
Swab from hands of food handlers	2	5	14
Total	35*	85	222

* because there was more than one sample in the same outbreak.

Table 2. Specific primers used for detection of specific factors in *S. aureus* by PCR methods

Gene	Primer	Sequence
sea	A1*	5'-AAAGTGCCGATCAATTTAATGGCTA-3'
	A2*	5'-ATTAACGGAAGGTTCTGTAG-3'
seb	B*	5'-GATATTATTTCGCATCAAACTGAC-3'
	BC*	5'-GATTGGTCAAATTATCTCCTGG-3'
sec	C21	5'-AATAAGAGTCGATTTATTTCATGC-3'
	C22	5'-GTACCAGTAAACTCACTTGA-3'
sed	D	5'-GCAGATAAAAATCCAATAATAG-3'
	D1	5'-TTTCGGGAAAATCACCCTTAAC-3'
see	E1	5'-TTACAAAGAAATGCTTTAAGC-3'
	E2	5'-TACCGCCAAAGCTGTCTGAG-3'
seh	H1	5'- GTTAATGAAATATATTGAGGAGT- 3'
	H2	5'- TATGTCGAATGAGTAATCTC- 3'
sei	I1	5'- AAGATCTTACGTATGCTCAA- 3'
	I2	5'- ATTTACTTATTTCGTCCC- 3'

Table 2. (continued)

Gene	Primer	Sequence
sej	J1	5'-AAGGAGTTAACACAATGAA-3'
	J2	5'-GATAGATGTACTACGTATATG-3'
sek	K1	5'-CTGATATAACGTGGCAATT-3'
	K2	5'-TTAAATACATTAACGCCTA-3'
sel	L1	5'-ATTCAGCAGATATTCCAT-3'
	L2	5'-TCAAGTGTAGACCCTATTGC-3'
sem	M1	5'-TCGGAGTTTTGAATCTTAGG-3'
	M2	5'-TCAACTTTCGTCCTTATAAG-3'
sen	N1	5'-AATCTGATCTAGATAGTAGT- 3'
	N2	5'-TTAAATCGAACTTTAGTGTC- 3'
seo	O1	5'-CCCTATTGCTTTACATAATATT- 3'
	O2	5'-CCGAATGAGAATGAAATTTAATA- 3'
sep	P1	5'-ATCCTCAACTGTGTATCTGG'- 3'
	P2	5'-AGTGGATTTATATGGTGTTT- 3'

* Primer source reference are Shiau et al. (BFDA, 1996)

** All primers except * are designed by the specific gene from GenBank in this study.

Table 3. Enterotoxin genes and toxins detected from *Staphylococcus aureus* CCRC strains

CCRC* No.	Enterotoxin gene															RPLA result
	sea	*seb*	*sec*	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*	*sel*	*sem*	*sen*	*seo*	*sep*	*seq*	
14942#		✓	✓	✓		✓			✓			✓			✓	✓	B
12653#		✓	✓	✓					✓			✓			✓	✓	B
12654#			✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		C
12656#			✓	✓	✓	✓			✓	✓	✓				✓		E
12656#	✓		✓	✓		✓			✓		✓	✓	✓	✓	✓		AD
14943#	✓		✓	✓		✓		✓	✓		✓	✓		✓	✓		A
13824#	✓	✓	✓	✓		✓			✓		✓	✓					A
13825#	✓	✓	✓	✓		✓			✓	✓		✓					B
13828#			✓	✓		✓			✓			✓			✓	✓	C
13829#			✓	✓		✓			✓		✓	✓					D
13830#			✓	✓	✓				✓		✓	✓					E
11863#	✓		✓	✓		✓		✓	✓	✓	✓	✓	✓	✓			A
14944#				✓		✓		✓	✓	✓	✓	✓	✓	✓	✓		-
14980#			✓	✓		✓			✓	✓	✓	✓					-
13826#			✓			✓	✓	✓		✓		✓	✓				C
1382※			✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	✓		BCD

* *S. aureus* strains, obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC.

 # sec or *sed* was detected by PCR method, RPLA result did not match.

※ SEB detected by RPLA, *seb* did not detected by PCR method.

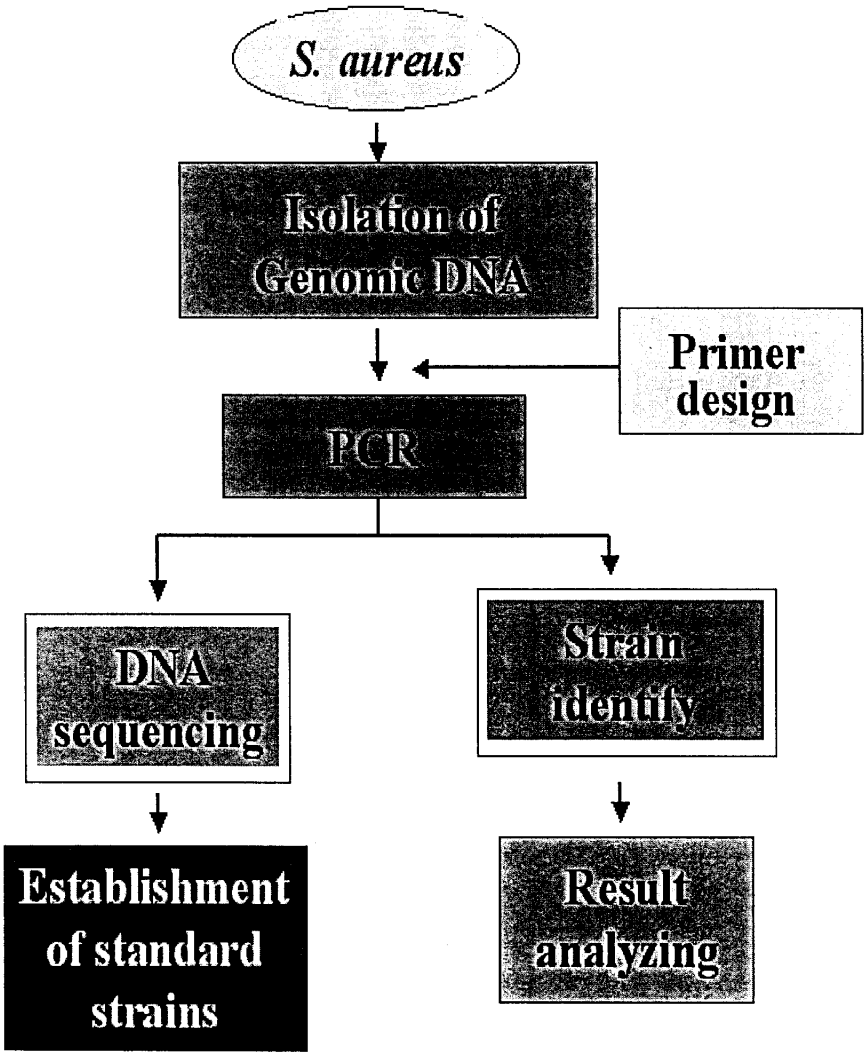

Figure 1. Stratagem for detection of genes and establishment of reference strains.

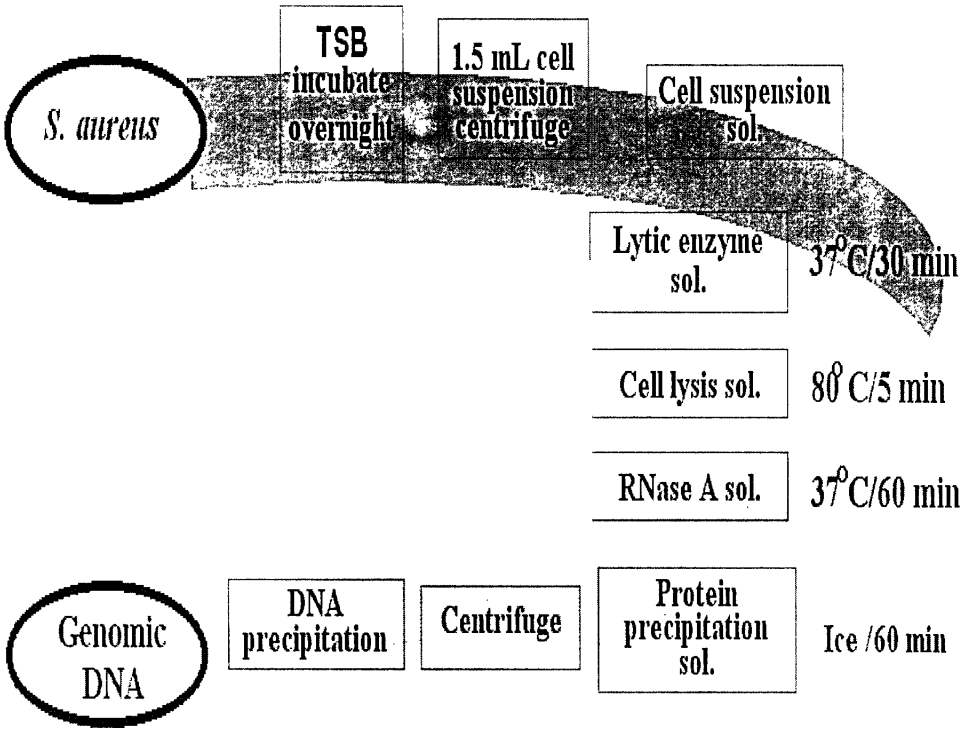

Figure 2. Flowchart for genomic DNA isolation.

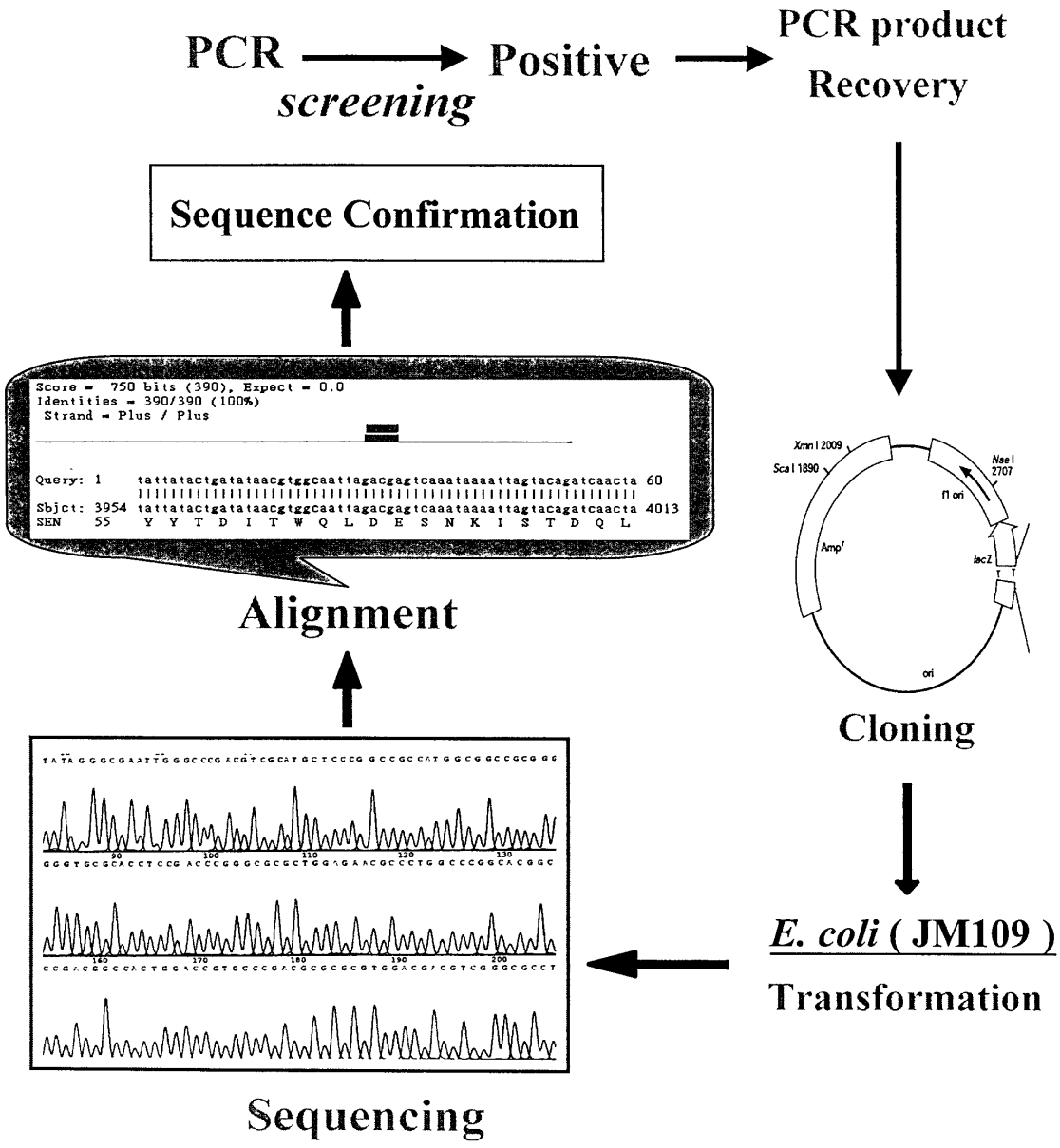

Figure 3. Establishment of in-house reference strains.

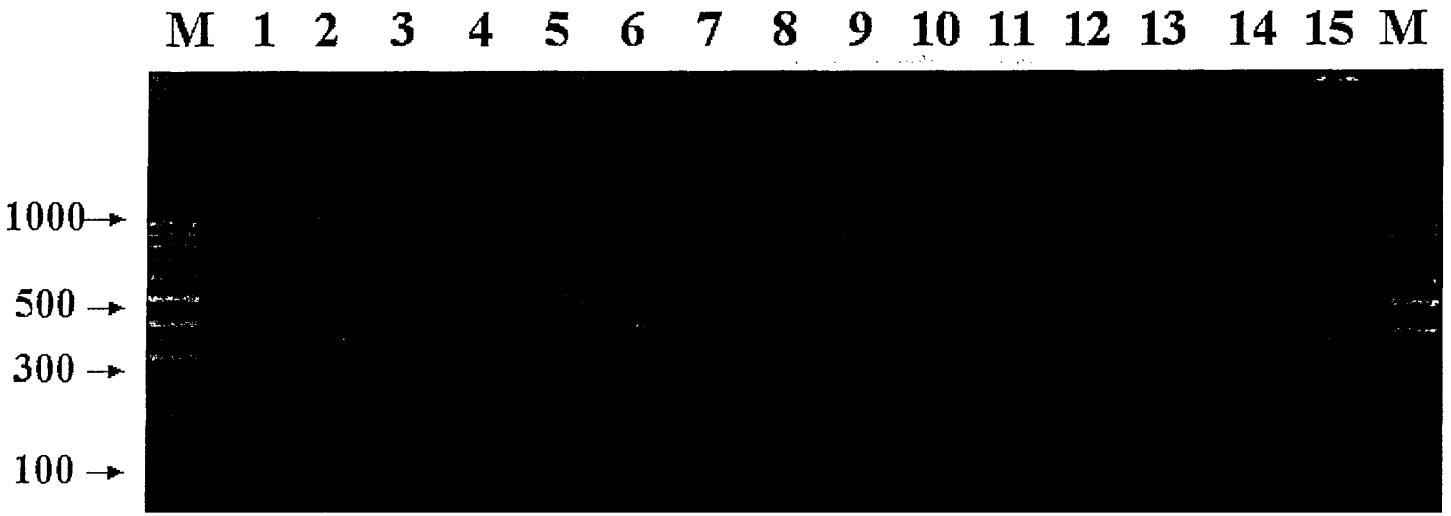

Figure 4. Detection of the Staphylococal Enterotoxin genes in *S. aureus* strains by the same PCR conditions.

Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Enterotoxin gene	*sea*	*seb*	*sec*	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*	*sel*	*sem*	*sen*	*seo*	*sep*
Size of PCR product(bp)	219	332	137	533	481	409	643	186	880	309	156	650	438	514	383
Strains	NLFD Sa16	NLFD Sa21	NLFD Sa144	NLFD Sa140	CCRC 13830	NLFD Sa21	NLFD Sa21	NLFD Sa21	NLFD Sa140	NLFD Sa21	NLFD Sa21	NLFD Sa22	NLFD Sa22	NLFD Sa22	NLFD Sa229

M : 100 bp ladder markers.3

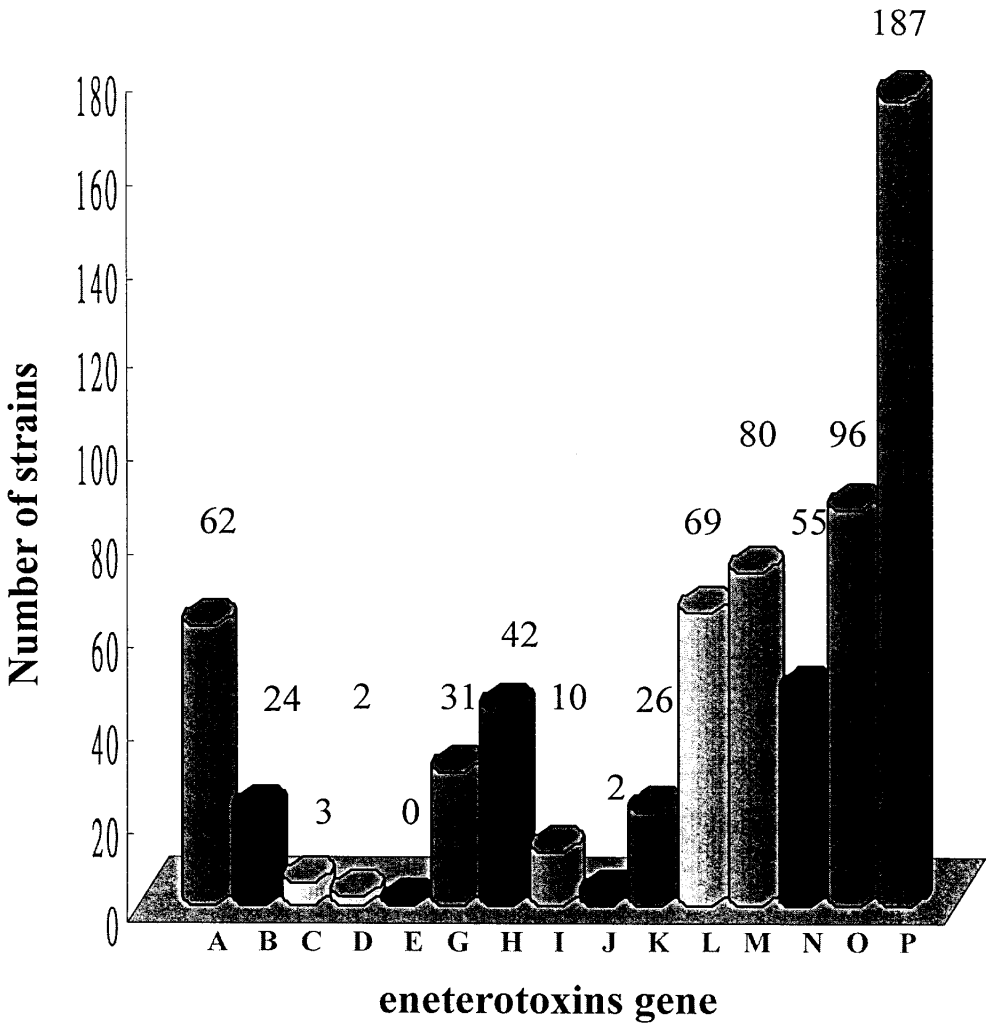

Figure 5. Detection frequency between 15 staphylococcal enterotoxin genes (N = 222).

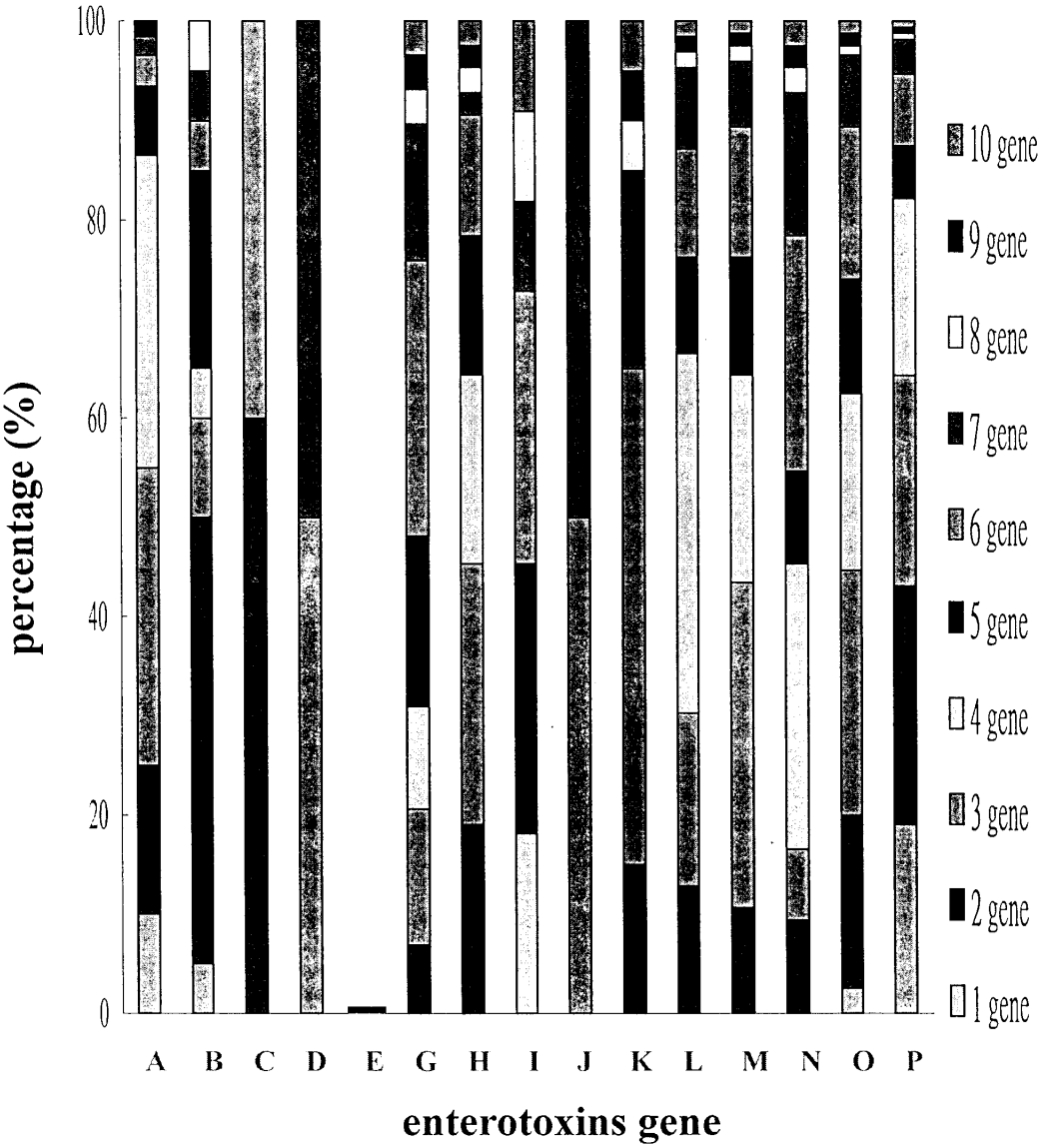

Figure 6. Distribution of staphylococcal enterotoxin genes in different patterns.

Results & Discussion

1. A Novel Method for Detection of the Staphylococcal Enterotoxin Genes from *sea* to *sep* was established by PCR method in this study. There were clear PCR products showed in the gel electrophoresis image as we tested the 15 enterotoxin genes individually (figure 4).
2. This PCR Method was run in 16 CCRC *Staphylococcus aureus* strains, obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC) , and the result revealed that there were more than one enterotoxin genes detected between these strains (table 3).
3. Among the 222 *Staphylococcus aureus* strains isolated from food poisoning outbreaks in Taiwan, the detection frequency between 15 staphylococcal enterotoxin genes (*sea~sep*) told that *sep*, *seo* and *sem* were the top three , then the *sel*, *sea* and *sen* (figure 5).
4. While looked into the Enterotoxin gene patterns of *S. aureus* isolates (N=222) from food poisoning outbreaks (N=35), the patterns in each group were diversely. Among these patterns, the *sep* was the majority, which distributed in 33 strains (table 4).
5. The distribution of staphylococcal enterotoxin genes in different patterns showed: the *sep* and *sea* were usually found in the strains which less 5 genes detected in the same time, on the contrary, the *sek* appeared in the strains contained more than 5 genes (figure 6).

附　　件　　五

附件五、美國食品安全機構聯合監管方法網路資訊

FDA/CFSAN: Food and Cosmetic International / Other Language Documents -- Chinese

FDA Food and Cosmetic International / Foreign Language Documents

Chinese

Overview | Foodborne Illness | Imports, Exports | Additional FDA Information

Overview

- 食品安全暨應用營養中心
- 美國食品暨藥物管理局(FDA)有關食品及化妝品規章的責任
- 食品安全：機構聯合監管方法

Foodborne Illness

- 靠食物傳播的十種最需避免的病原體
- 可能給你惹麻煩的菌體
- 人人都能*對付細菌!*™
- Fight BAC! Public Service Announcement (Cantonese) (Mandarin) (Video 30 sec)

Imports, Exports

- 食品暨藥物管理局進口程序

Additional FDA Food and Cosmetic Information

- FDA Food and Cosmetic Information on the World Wide Web (in English)

U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition

美國食品藥物管理局
食品安全暨應用營養中心

食品安全暨應用營養中心

(This document in English) | (Help with Asian Fonts)

概述

食品安全暨應用營養中心 (簡稱CFSAN) 是 FDA 六個分管不同產品的中心之一，與全美國範圍內的地區分支一起共同履行 FDA 的宗旨。FDA是一個科學管理機構，負責全美國內銷和進口食品、化妝品、藥物、生物製品、醫療設備和放射性產品的安全。這個機構是歷史最悠久的聯邦機構之一，主要任務在於保護消費者。FDA 的工作與全美國民眾息息相關，並且對每個人的生活產生直接的影響。該機構是世界公認的食品和藥物領導管理機構，許多國家在改善和監控食品安全時，都曾要求 FDA 提供協助。FDA是美國政府的衛生暨社會福利部 (DHHS) 和公共衛生服務處 (PHS) 的行政分支機構。 *若需要有關 FDA 和 HHS 的詳細資訊，請至： FDA網站，和 HHS網站。*

任務

CFSAN 和 FDA 的現場人員除了共同確保全國食品供應的安全性、衛生、健康性和誠實標示外，還負責確保化妝品的安全正確標示，以期促進和維護民眾的健康。

責任範圍

消費者每消費 1 元，就有 25 分錢是花在 FDA 管理的產品上，而其中用來購買食品的支出就佔了大約 75%。

本中心負責管理銷售於美國各州的 2 ,400 億美元內銷食品、150 億美元進口食品和 150 億美元的化妝品。這樣的管理從產品輸入美國或運至銷售地點時就已開始，範圍遍及大約 50,000 個食品營業所 (包括超過30,000家的美國食品製造商和加工廠，以及 20,000 座以上的食品倉庫) 和 3,500 家化妝品公司。這些數字不包括大約 600,000 家餐廳和公共團體的食品服務單位及 235,000 家超級市場、雜貨店，和其他由州和地方政府機構管理的食品通路商。這些機構都受到 FDA 的指

導、適用其標準規範，並且接受 FDA 的其他技術協助，而 FDA 也透過訓練和指導等方式來支援州和地方政府機構，期使 FDA 的計劃更爲落實，貫徹對食品營業所和零售商的各項管理工作。

美國食品工業的產值約佔國民生產毛額的 20%，雇用了 1,400 萬名員工，並且提供相關產業 400 萬個額外的工作機會，在經濟上具有舉足輕重的地位。

在 2000 會計年度裏 (1999 年 10 月 1 日到 2000 年 9 月 30 日)，FDA花在食品和化妝品安全業務上的經費超過2.8 億美元。本中心的主要職責包括：

- 確保食品中各類添加物的安全性，例如食品添加劑 (包括電離輻射) 和色素添加劑。
- 確保利用生物科技開發出來的食品和原料的安全 。
- 海鮮危險分析和重要控制點 (HACCP) 的管理。
- 擬定與食品內化學物質和生物污染物的防治有關的衛生法令及研究計劃。
- 擬定與食品和化妝品的正確標示 (例如成份、營養健康說明等) 有關的法令，並且落實管理工作 。
- 擬定補充食品、嬰兒配方和醫療食品安全等相關管理法令和政策
- 確保化妝品的成份和產品受到安全而且正確的標示。
- 對食品業進行售後監督，以確保符合相關規範。
- 消費者教育與產業升級。
- 擬定與州和地方政府的合作計劃。
- 國際食品標準和安全協調事宜。

雖然美國的食品供應是全世界最安全的，但隨著食品種類和速食產品的增加，關心公眾健康的人士仍然非常注意美國食品供應的安全性。食品業的複雜性更甚於以往，食品製造商也在食品的生產和包裝上運用了更多的科技。由於美國的食品進口比例日益增加，因此 CFSAN 不但與國際組織 (WHO、FAO、Codex) 合作，而且偶而還會直接和外國政府溝通，以便與出口國就國際標準事務進行協調，並且確保這些國家都能瞭解美國的要求。

食品污染源幾乎和污染物本身的數量和變化一樣多，其來源甚至可追溯至收割前的環境，以及在加工、包裝、運輸和備製過程中造成的污染。茲就 CFSAN 目前負責的部分食品安全業務說明如下：

- 生物病原體 (例如細菌、病毒、寄生蟲)
- 自然產生的毒素 (例如黴菌毒素、甲藻魚毒素、麻痺性甲殼類毒素)
- 飲食補充物 (例如麻黃素)
- 殺蟲劑殘留物 (例如戴奧辛)
- 有毒金屬 (例如鉛、水銀)
- 分解和污物 (例如昆蟲殘骸)
- 食品過敏原 (例如蛋、花生、小麥、牛奶)

- 營養品問題 (例如維他命D服用過量、幼兒鐵中毒)
- 飲食成份 (例如脂肪、膽固醇)
- 物理放射性核種
- TSE型疾病 (例如麋鹿的慢性消耗性疾病)
- 產品填充物

法律授權

FDA 的食品和化妝品管理權的法源如下：

- 1906 年聯邦食品藥物管制法
- 1927 年聯邦牛奶進口法
- 1938 年聯邦食品、藥物及化妝品修正法
- 1944 年公共衛生服務法
- 1966 年完整包裝標示法
- 1980 年幼兒配方修正法
- 1990 年營養標示與教育法
- 1994 年補充食品衛生與教育法
- 其他相關法令

有關上述法令的詳細資訊，請至：

- *美國食品安全制度*
- *FDA 執行的法律及其他相關法令*
- *美國食品藥物管制法的歷史哩程碑*
- *1906年立法的艱辛奮鬥史*
- *標籤背後的法律典故，第一部*
- *標籤背後的法律典故，第二部*

FDA 在食品方面的管理職責涵蓋所有內銷和進口食品，但肉類、禽肉及冷凍、乾燥和液態蛋由美國農業部 (也就是美國農業部食品安全檢驗處 [USDA]) 負責管理，酒精飲料 (酒精濃度超過 7%) 和菸草的標示由美國財政部煙酒槍砲管理局 (ATF) 管理，而食品中殺蟲劑殘留物的容忍值和飲用水的安全則由美國環保署 (EPA) 負責管理。

FDA 和這些管理機構及美國商業部的國家海洋漁業署、疾病管理防治中心 (CDC)、美國財政部的海關、聯邦貿易委員會 (FTC)、美國運輸部 (DoT)、消費者產品安全委員會 (CPSC) 和美國司法部 (DoJ) 等其他聯邦機構均保持密切的聯繫。FDA 曾多次與上述機構簽訂協議，明確規範各機構之間的職權。*如需有關上述聯邦機構和各州在食品安全方面的詳細職權資訊，請至：*

- *食品安全：團隊途徑*

- *www.FoodSafety.gov*
- *聯邦/州食品計劃*

FDA 負責管理州與州之間販賣的食品，而完全由某一州在其境內製造和販賣的食品則由該州自行管理。食品中心人員與各州的農業和衛生部門合作，共同解決食品安全問題和經濟詐欺案件，例如。*如需有關州管理機構的詳細資訊，請至：*

- *食品安全：團隊途徑 - 州和地方政府*
- *www.FoodSafety.gov：政府機構*

越來越多的國際組織希望與本中心合作，例如食品暨農業組織 (FAO) 和世界衛生組織 (WHO) 轄下的一個國際食品標準建立組織「食品準則委員會」(CAC) 和外國政府，都希望本中心能協助建立獲得國際認可的進口食品安全標準、準則和法令。本中心以往在建立標準時，通常都以美國產品作爲對象，但隨著近幾年來國際條約簽約次數的日益增加，這種情況已有所改變。現在有越來越多的食品在國際市場上交易流通。*如需有關食品準則的詳細資訊，請至：*

- *食品和化妝品的國際活動*
- *FDA的食品準則活動*
- *美國食品準則處*

如需有關 FAO 和 WHO 的詳細資訊，請至： FAO 網站, 和 WHO 食品安全計劃。

雖然 CFSAN 的任務在於保護和促進民眾的健康，但這仍然需要其他機構或相關人員的配合才能有所成效。雖然學術界、健康食品供應商、其他政府機構、受法令規範的業界，以及消費者本身一直在扮演著自己的角色，但今日社會的需求和複雜性更加突顯了這樣的角色配合關係。協作、聯盟、合作或夥伴關係對本中心來說已不陌生。本中心正積極進行具有前瞻性的合作計劃，例如與馬里蘭大學共同成立的食品安全暨應用營養聯合研究所 (JIFSAN)，以及與伊利諾科技研究所共同成立的國家食品安全暨科技中心 (NCFST)。這個中心是在產官學界共同努力下成立的機構，旨在提供重要資訊，使食品的管理更具成效，進而確保食品的安全。*如需有關 JIFSAN 和 NCFST 的詳細資訊，請至：JIFSAN 網站 和 NCFST 網站。*

- *給消費者的資訊*
- *給兒童、青少年和教育工作者的資訊*
- *給老年人的資訊*
- *更多關於婦女健康的資訊*

此外，與各州有關檢查措施的正式協定亦強化了本中心執行公共衛生任務的能力。

FDA 用來確保食品安全的工具

- 檢查營業所
- 樣品的收集與分析
- 進口監控
- 上市前的複檢 (例如食品和色素添加劑)
- 公告計劃 (例如食品接觸物質、幼兒配方)
- 法令/協定 (例如諒解備忘錄)
- 消費者研究、重點團體
- 實驗室研究
    - 研發/改良偵測食品中的病原體和化學污染物的方法
    - 確定食品污染物對健康的影響
    - 確定加工對食品成份的影響
    - 確定飲食因素對健康的影響
    - 研究調查造成生物污染物中毒的因素
- 指導工廠從事食品加工、包裝和生物科技的研究
- 合作行動/技術協助
- 資訊的收集與分析
- 透過教育和公開會議提升相關人員的瞭解程度
- 提供有關中心活動的資訊和發展資料

有關 CFSAN 關於化妝品的職責與活動的詳細資訊

組織

CFSAN 的理事長是 Joseph A. Levitt。Levitt 先生以優異的成績畢業於康乃爾大學，接著又以優異的成績取得波士頓大學法學博士學位。Levitt 先生是 FDA 的專業人員，曾多次獲頒獎項，包括 1992 和 1999 年的最佳經理人獎。Levitt 先生自 1998 年起擔任本中心理事長一職。

本中心員工人數超過 800 名，包括秘書和其他後勤人員，以及化學家、微生物學家、毒物學家、食品科技專家、病理學家、分子生物學家、藥理學家、營養學家、傳染病學家、數學家和公共衛生學家等具有高度專業素養的專家。

本中心的其他部門除了提供消費者、國內外業界和其他外部團體有關現場規劃、機關行政業務、科學分析與支援的服務外，還針對重大食品議題提供政策擬訂、規劃和處理等服務。本中心的大部份員工都在位於華盛頓特區的總部工作，但自 2001 年的秋季開始，大部份的總部員工將調至馬里蘭大學學院園分校 (College Park) 的新辦公室上班。本中心在馬里蘭州的勞瑞爾 (Laurel) 和阿拉巴馬州的多芬島(Dauphin Island) 設有研究機構，其他單位還包括位於馬里蘭大學學院園分校的 JIFSAN 和伊利諾州芝加哥附近的 NCFST。

(This document in English)

美國食品暨藥物管理局(FDA)有關食品及化妝品規章的責任

對在美國國內生產或從國外進口的食品及化妝產品之製造和分銷有影響的法律要求簡介

美國食品暨藥物管理局(FDA)管制所有食品和與食品有關的產品，但是商業化生產的蛋類、肉類和禽類產品除外，其中包括混合產品(例如炖煮類和披薩)，禽或禽製品含量百分之二或以上、肉或肉製品含量百分之三或以上的產品，這類產品由美國農業部下屬的食品安全檢驗局(FSIS)管制。水果、蔬菜和其他植物由該局下屬之動植物健康檢驗局(APHIS)管制，以避免將植物疾病和害蟲帶進美國。水果和蔬菜的自動分級工作由美國農業部下屬的農業市場局(AMS)進行。

所有非酒精飲料，以及酒精含量低於7%的葡萄酒飲料都屬於FDA的責任範圍。除酒精含量低於7%的葡萄酒飲料(即發酵過的果汁)之外的所有酒精飲料則歸財政部的煙酒火器局管制。

此外，環境保護局(EPA)管制農藥。環境保護局根據聯邦食品藥物和化妝品法案(FD&C Act)的有關規定，決定農藥產品的安全程度，規定食品中農藥殘留量的可容忍程度。它還出版指導安全使用農藥的書籍。執行EPA規定的可容忍程度是FDA的責任。

在美國，通過定期檢查設備和產品，分析樣品，舉行教育活動以及執行法律程序來保證FD&C Act得到遵守。FDA可以採取一些管制程序或行動，從而有助於保護民眾的健康、安全和幸福。

摻假或貼假標簽的食品也許會由運貨商自行銷毀或從市場召回，或者會由FDA根據聯邦地區法院的命令派美國警官加以沒收。對違法行爲負責的個人或公司可能會被聯邦法院起訴，如果發現有罪，可能被罰款和/或坐牢。聯邦法院可能會對連續違法者發出禁令。違反禁令可能會被定爲藐視法庭而受到懲罰。究竟採取一種或全部管制程序將視情況而定。

製造商或運貨商可以自願或根據FDA的要求採取行動，召回食品商品。FD&C Act對召回嬰兒代乳粉有特別規定。廠商或運貨商與FDA合作，召回產品，也許可以免於執行法院程序，但卻不能免去個人或公司因違法而要承擔的賠償責任。

在跨州商業中，食品的主人有責任保證食品符合 FD&C Act 和公平包裝及標簽法案(FPLA)的條例及其補充規定。一般說來，這些法案都要求食品產品是安全、清潔、健康的產品，而且其標簽要誠實，要提供有關信息。

根據 FD&C Act，FDA 有權設立並實施合理的食品生產衛生標準。內附一份聯邦規則法典第 21 篇第 110 節(21 CFR Part 110)，其中包括現行的制造、包裝和保存人類食品之行業“良好製造實踐”規定(GMP)，由於這些規定涉及人員、樓房和設施、設備，及產品工藝控制等，所以如果認真遵守，廠商可以在某種程度上保證食品的安全和衛生。FDA 在 21 CFR §110.110 中認識到不可能種植、收獲和加工完全沒有自然缺陷的農作物，因此該機構發行了“食品缺陷行動水準”。這些缺陷水準是在對健康無危害的基礎上規定的。如果某些案例不存在相應的缺陷行動水準，則逐個對案例的缺陷作出管制性決定。

另一個確定食品中自然缺陷水準的替換方法是堅持增加使用化學物品，以控制害蟲、鼠類和其他自然污染物。FDA 出版了關於有毒或有害物質的“行動水準”以控制在人類食品和動物飼料中污染物質的含量。(參閱內附的小冊子)。不過，美國法庭以程序方面的理由阻止 FDA 對有毒或有害物質制定的“行動水準”生效。在此期間，在人類食品和動物飼料中污染物質含量的行動水準只能作我們的指南，而不具法律“力量和效力”。該機構已闡明，行動水平是程序性指南，而不是實質性規章。

對於在跨州商業流通的國內產品，FDA 不執行批准、發執照、或發放許可證等工作。不過，所有商業加工商，凡用熱力加工低酸罐裝食品(LACF)然後盛於密封容器內，或是加工酸化食品(AF)，不論他們是外國的還是美國的加工商，都要按照規定登記他們的每一個加工廠。此外，LACF 或 AF 食品的每個加工工藝都要先呈報 FDA，並由 FDA 接受歸檔後才能進入跨州商業分銷。

低酸食品的定義是除酒精料外、最後酸鹼衡量 pH 大於 4.6、水活性大於 0.85 的任何食品。許多罐裝食品都是低酸罐裝食品(LACF)，因此包裝商要受此登記和工藝呈報要求的約束。僅有的例外是最後酸鹼衡量 pH 小於 4.7 的西紅柿和西紅柿產品。酸化食品(AF)是添加了酸或酸性食品後產生的最後酸鹼衡量 pH 等於或小於 4.6 的低酸食品。

FDA 關於 LACF 的規定要求，盛裝低酸加工食品的密封容器，每一個都要標有肉眼能看見的永久性識別編碼。要求的識別標簽應以編碼的形式表明產品的包裝地、里面盛裝的內容、包裝的年日、以及產品是在一天的什麼時間包裝的(21 CFR §113.60 (c))。至於一種產品是否必須在其生產日期之後一定時間內運出美國則沒有要求。如果一種低酸罐裝食品(LACF)或酸化食品(AF)的加工工藝適當，則不必要求任何特別的運輸及儲存條件。

我們的規章要求，加工低酸食品的預定工藝過程必須由合格人員來制定，這些人員除了應具有熱加工並保存在密封容器內的低酸食品的專門知識，還應擁有必需的相關設施(21 CFR §113.83)。有關此過程的所有關鍵因素都要由在預定的加工工藝方面有權威的人士指定。加工商應按要求將所有關鍵因素都控制在預定工藝規定的範圍以內。

FDA有責任爲一些食品商品規定美國識別標簽、品質和容器塡充物的標準。食品標準--從根本上來說是對食品內容和品質的定義--是根據FD&C Act的規定來確定的。現在許多種類的產品都已經制定了標準。這些標準爲消費者提供了這些產品所含主要成份的種類和份量方面的某些保障。一種食品若號稱爲一種有已公佈食品標準的產品，那麼此食品必須滿足這種標準，否則就會被認不符合標準，因而導致受到管制行動。

FD&C Act的修正案制定了嬰兒代乳粉營養要求，並授權予FDA規定良好製造實踐，規定營養成份份量、營養成份質量控制、保存記錄、以及報告制度等種種要求。根據這些修正案，FDA檢查工廠的權力已經擴大到製造商記錄、品質控制記錄、以及確定是否符合FD&C Act規定所必需的檢驗結果。

FDA規定海味行業必須執行危害分析關鍵控制點(HACCP)程序，以便保證做到安全加工、安全包裝、安全儲存和安全分銷國產的和進口的魚及魚產品。食品加工商可以根據HACCP這個系統來判定可能影響其產品的危害屬於什麼種類，從而建立必要的控制措施，避免危害的發生，監視這些控制措施的表現，並以常規的作業方式保存這些監視記錄。建議實行HACCP規定的目的，是確立命令性的預防性控制，以便保證在美國國內銷售和出口國外的海味產品的安全性。FDA在執行其傳統的檢查活動之外，還將審核是否充分執行了HACCP控制措施。

21 CFR 101和105內的食品標簽法規包含有一些要求，如切實遵守，即可達到食品標簽誠實且提供有關信息的要求。命令性食品標簽包括識別聲明（產品的通用或常用名稱 - 21 CFR §101.3）；所裝物品淨重的聲明（21 CFR § 101.105）；製造商、包裝商或分銷商的名稱和業務地點（21 CFR §101.5）；如果是用兩種或兩種以上成份製成，必須按顯著性順次列出每一種成份的通用或常用名稱（21 CFR §101.4和§101.6）。如果香料，調味素和某些染色素不是純以該種物質出售，可籠統標香料，調味素和染色素而不需逐一列名。不過，如食品中含有的染色添加劑需有FDA證書，則必在所含成份說明書中標明含有該種色素。

1993年1月6日，根據營養標簽和教育法規(NLEA)的規定，FDA頒發了有關食品標簽的最後規定，這些規章--附在食品標簽小冊子內--對現行食品標簽規定的許多方面，主要是營養標簽及其與食品的有關說法方面，作了顯著的修改。NLEA的規定適用於跨州商業中流通的國內食品和進口美國的食品。出口外國的食品，其標簽必須符合該國的要求。

如果一種食品產品的標簽不包括FD&C Act、FPLA、及其相關法規要求的所有聲明，或標簽對產品作出未經證實的描述，這種食品即被認爲貼了假標簽。對貼假標簽行爲FD&C Act允許進行民事和刑事訴訟，FPLA則允許進行沒收和禁令。當商品進入跨州商界流通時，製造商，包裝商或分銷商有法律責任徹底遵守這些法令的條款及其規章中有關標簽的規定。一種食品產品的標簽可以包括"通用產品碼(UPC)"和表示以下幾種情況的一些符號，即：該商標已經在美國專利辦公室登記過；標簽的文字和美術內容受美國版權法的保護不受侵犯；而且該食品按照某些宗教團體的飲食規定進行調制和/或遵循某些宗教團體的飲食規定。但很重要的一點要注意，UPC或以上提及的任何符號都不是美國食品暨藥物管理局的任何法規所要求的，也不在其管轄之下。

FD&C Act要求食品添加劑（指那些如果被使用後會導致或根據合理判斷會導致--直接地或間接地--其自身變成食品的一部分，或影響食品特征的物質）獲得面市前批准。批准過程包括對該添加劑在預期的使用中的安全性進行非常仔細的審核。食品添加劑獲得批准後，描述其使用情況的規定會發表在聯邦管制法典(CFR)中。正如CFR所定義的，安全的或安全這個詞的"……意思是，稱職的科學家有合理的理由肯定相信這種物質在預期的使用中是無害的。目前科學知識還不可能完全肯定使用某種物質是絕對無害的。但是，A FD&C Act要求在一種物質面市之前先取得批准，的確能使這種食品添加劑在使用時可能產生的負作用控制在可接受的最低水平。

關於飲食補充品的章程是FDA根據1994年"飲食補充品健康與教育法案"授權規定的。它保證產品都是安全的並適當地貼了標簽，而且保證任何與疾病和健康有聯系的說法都經過科學驗證。管轄飲食補充品安全性的法律條款取決於產品從法律角度來看是食品還是藥物。但不論哪一種情況，製造商都有義務生產安全的產品。FDA要求新藥物應進行面市前的安全審核。

飲食補充品的標簽，除了其所有資訊都應真實、不誤導外，還應說明產品含有什麼成份、含多少成份、應該怎麼使用、以及爲保證使用安全應該有些什麼必要的預防方法。如果此飲食補充品是一種食品，將根據NLEA健康提法規定對與疾病和健康有關的說法進行審核。

在美國推銷一種化妝品不需經過FDA的批准。除了色素添加劑和少數受禁止和限制的成份以外，化妝品製造商可以自己負責，使用任何原材料作化妝品的成份，不經批准即推銷該種產品。

在化妝品的標簽中提起治療效果，不論是實際的還是暗示的，都是不恰當的。有些產品既是化妝品，也可用來治療和預防疾病，或影響人體結構或人體功能。這種產品被同時當作藥物和化妝品，必須符合藥物和化妝品的法律條款規定。

若您需進一步了解如何遵循FDA的法律要求，或需了解具體某種受FDA管制的食品和化妝品產品，可聯系：

美國哥倫比亞特區華盛頓，20204 西南C 街200號

食品暨藥物管理局食品安全和應用營養中心行業行動工作人員 (HFS-565)
電話： (202) 205-5251 國際網路網址：http://www.fda.gov

Industry Activities Staff (HFS-565)
Center For Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

U.S. Food and Drug Administration
FDA Backgrounder
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(This document in English)

食品安全：機構聯合監管方法

一九九八年九月二十四日

美國的食品供應是世界上最安全的，這主要是由於美國實行機構聯合監管制度，在每一個層面（地方、州和全國）監督食品生產與流通。

各市縣衛生局、各州衛生機構以及聯邦政府的許多部門和機構，都雇用食品檢查員、微生物學家、流行病學家及其他食品科學家，執行持續監管。地方、州和聯邦法律、準則及其他法令對這些監管人員的權限有明確的規定。有些人員只監管一種食品，例如牛奶或海鮮。有些人員的權限只限於某個特定的地理區域。有些人員只負責監管某一類食品企業，例如飯店或肉品加工廠。這些工作人員攜手合作，則形成美國食品安全監管系統。

柯林頓政府於一九九七年發起「食品安全運動」，加強全國食品安全監管人員的工作，進一步防止由食品傳染的疾病。這類疾病每年影響到六百五十萬至三千三百萬美國人。一九九八年五月，食品安全運動開始實施一項重要計劃 -- 美國衛生部（包括其下屬機構FDA，即食品與藥物管理局）、農業部和環境保護總署聯合簽署一份備忘錄，決定建立「食品傳染疾病發生反應協調組」，英文簡稱爲FORC-G。這個新機構的職責是:

- 加強聯邦、州和地方食品安全機構之間的協調與聯絡。
- 在疾病發生時引導資源和技術力量的有效使用。
- 採取措施防止危害美國食品供應的新的和潛在的威脅。

除了聯邦政府官員之外，FORC-G還包括食品與藥物官員協會、全國市縣衛生官員協會、州與領地公共衛生實驗室主任協會、州與領地流行病學家委員會以及全國各州農業局協會。

下面的表格詳細地列出美國食品安全監管體制的組織結構。表格所列的各機構還與其他政府部門合作，例如，與消費產品安全委員會共同執行防毒包裝法，與聯邦調查局（FBI）共同執行聯邦防止私拆包裝法，與運輸部共同執行衛生食品運輸法，以及與美國郵政總局共同執行反郵件欺詐法。

美國衛生部*

食品與藥物管理局 (FDA) (Food and Drug Administration)

監管

- 各州際貿易中出售的國內生產及進口食品，包括帶殼的蛋類食品，但不包括肉類和家禽。
- 瓶裝水。
- 酒精含量低於7%的葡萄酒飲料。

食品安全權限

執行與國內生產及進口食品（肉類和家禽除外）有關的食品安全法律，具體方式如下:

- 檢查食品生產企業和食品倉庫，並且採集和分析樣品，以確定是否有物理、化學或細菌污染。
- 在市場行銷之前檢查食品添加劑和色素之安全性。
- 檢查動物藥物對接受藥物的動物之安全性，以及對食用此類動物食品的人類之安全性。
- 監管食用類動物所用飼料的安全性。
- 制定指導性規程、法規、準則及法律解釋，協助各州共同執行，以便監管牛奶、貝殼類海鮮和食品零售業，例如飯店和雜貨店。指導性「食品規程」即是一個具體例子。該規程向零售店、護理所和其他機構提供參考資訊，指導如何烹調食品以防止食品傳染疾病。
- 制定切實有效的食品生產方式和其他生產標準，例如工廠衛生、包裝要求、以及危險分析及關鍵控制點計劃等。
- 與外國政府合作，確保某些進口食品之安全性。
- 要求生產商回收某些不安全的食品，並監管回收計劃之執行。
- 採取適當的實施行動。
- 進行食品安全研究。
- 教育食品廠商和消費者瞭解食品安全使用方法。

詳情請洽

消費者:
FDA Headquarters (食品與藥物管理局)
Office of Consumer Affairs
HFE-88
5600 Fishers Lane
Rockville, MD 20857

各地的FDA辦事處，列在電話簿藍頁「美國政府」欄下

媒體採訪: 202-205-4144

消費者:
FDA食品資訊與海鮮熱線電話
1-800-FDA-4010 (1-800-332-4010)

http://www.cfsan.fda.gov/~mow/ctfoodte.html

疾病控制與防治中心 (Centers for Disease Control and Prevention)

監管

- 所有食品。

食品安全權限

- 與地方、州和其他聯邦官員一起調查由食品傳染的疾病之病源。
- 管理全國食品傳染疾病監視系統：設計和部署食品傳染疾病快速電子報告系統。與其他聯邦和州機構一起監視食品傳染疾病的發病率和趨勢。發展能使州和地方各級快速識別食品傳染病原體之先進技術。
- 制定和宣傳旨在防止食品傳染疾病的公共衛生政策。
- 進行研究以防止食品傳染疾病。
- 訓練地方和州的食品安全監管人員。

詳情請洽

Centers for Disease Control and Prevention（疾病控制與防治中心）
1600 Clifton Rd., N.E.
Atlanta, GA 30333

媒體採訪：404-639-3286

公眾：404-639-3311

www.cdc.gov

* 衛生部下屬的國立衛生研究院也進行食品安全研究。

美國農業部**

(U.S. Department of Agriculture)(USDA)

食品安全與檢查局 (Food Safety and Inspection Service)

監管

- 國內生產與進口的肉類、家禽及相關產品，例如含肉類或家禽肉的湯料、皮薩餅及冷凍食品。
- 蛋類加工產品（通常爲液態、冷凍和乾燥消毒的蛋類產品）

食品安全權限

執行與國內生產和進口的肉類及家禽產品有關的食品安全法律，具體方式如下：

- 在屠宰之前和之後，檢查食用類動物是否染有疾病。
- 檢查肉類加工廠和家禽屠宰廠。
- 與農業部農業市場行銷服務局共同監視和檢查蛋類加工產品。
- 採集和分析食品樣品，檢查是否有細菌、化學污染物、傳染病菌及毒性物質。
- 制定生產標準，用於監管肉類和家禽產品生產與包裝中食品添加劑和其他成分之使用、工廠衛生、熱處理工序以及其他工序。
- 檢查並確定向美國出口的所有外國肉類和家禽加工廠都達到美國標準。
- 要求肉類和家禽加工廠商自願回收不安全的產品。
- 資助肉類和家禽安全研究工作。
- 教育食品廠商和消費者瞭解食品安全使用方法。

詳情請洽

FSIS Food Safety Education and Communications Staff（食品安全與檢查局食品安全教育與通訊處）
Room 1175, South Building,
1400 Independence Ave., S.W.
Washington, DC 20250

媒體採訪：202-720-9113

消費者
肉類與家禽熱線電話1-800-535-4555
（首都華盛頓地區，請撥202-720-3333）
聽力殘障者專線：1-800-256-7072

www.fsis.usda.gov

各州研究、教育與擴展服務合作處 (Cooperative State Research, Education, and Extension Service)

監管

- 所有國內生產的食品以及某些進口食品。

食品安全權限

- 與美國各大學合作，制定以農民和消費者爲對象的食品安全研究與教育計劃。

詳情請洽

各地的擴展服務合作處，列在電話簿藍頁「縣政府」欄下

Cooperative State Research, Education and Extension Service（各州研究、教育與擴展服務合作處）
U.S. Department of Agriculture
Washington, DC 20250-0900
202-720-3029

www.reeusda.gov

國立農業圖書館 (National Agricultural Library) 美國農業部/食品與藥物管理局食品傳染疾病教育資訊中心 (USDA/FDA Foodborne Illness Education Information Center)

監管

- 所有的食品。

食品安全權限

- 管理一個關於防止食品傳染疾病的資料庫，包括電腦軟體、錄音和錄影材料、宣傳招貼、遊戲、教師指南及其他教育資料。
- 幫助教育工作者、食品服務訓練員和消費者尋找防止食品傳染疾病的教育資料。

詳情請洽

USDA/FDA Foodborne Illness Education Information Center (美國農業部/食品與藥物管理局食品傳染疾病教育資訊中心)
Food and Nutrition Information Center
National Agricultural Library/USDA
Beltsville, MD 20705-2351

301-504-5719

www.nal.usda.gov/fnic/

** 美國農業部下屬的其他許多機構也從事食品安全活動。

美國環境保護總署 (U.S. Environmental Protection Agency)

監管

- 飲用水。

食品安全權限

用植物、海鮮、肉類和家禽生產的食品。

- 制定飲用水安全標準。
- 監管毒性物質和廢物，防止它們進入環境和食品鏈。
- 幫助各州監視飲用水品質及尋找防止飲用水污染的方法。
- 測定新殺蟲劑的安全性，制定食品中可容許的殺蟲劑殘餘量標準，並公佈殺蟲劑安全使用指示。

詳情請洽

Environmental Protection Agency (環境保護總署)
401 M St., S.W.
Washington, DC 20460

202-260-2090

各地的EPA辦事處，列在電話簿藍頁「美國政府」欄下

www.epa.gov

美國商業部 (U.S. Department of Commerce)

全國海洋和大氣管理局 (National Oceanic and Atmospheric Administration)

監管

- 魚類和海產品。

食品安全權限

- 經由收費的「海鮮檢查計劃」，檢查漁船、海鮮加工廠和零售商店是否符合聯邦衛生標準，並頒發檢查證書。

詳情請洽

Seafood Inspection Program (海鮮檢查計劃)
1315 East-West Highway
Silver Spring, MD 20910

1-800-422-2750

seafood.nmfs.noaa.gov

美國財政部 (U.S. Department of the Treasury)

菸酒與火器管理局 (Bureau of Alcohol, Tobacco and Firearms)

監管

- 含酒精飲料，但不包括酒精含量低於7%的葡萄酒飲料。

食品安全權限

- 執行與含酒精飲料之生產和流通有關的食品安全法律。
- 調查含酒精產品摻假案件，有時和食品與藥物管理局一起辦案。

詳情請洽

Bureau of Alcohol, Tobacco and Firearms (菸酒與火器管理局)
Market Compliance Branch
650 Massachusetts Ave., N.W.
Room 5200
Washington, DC 20226

202-927-8130

http://www.cfsan.fda.gov/~mow/ctfoodte.html

www.atf.treas.gov/alcohol/index.htm

美國海關總署 (U.S. Customs Service)

監管

- 進口的食品。

食品安全權限

- 與聯邦管制機構合作，確保所有貨物在進入和離開美國時都符合美國法規條例的要求。

詳情請洽

U.S. Customs Service (美國海關總署)
P.O. Box 7407
Washington, DC 20044

媒體採訪: 202-927-1770

公眾: 請接洽當地進口港，列在電話簿藍頁「美國政府，海關」欄下

www.customs.ustreas.gov

美國司法部 (U.S. Department of Justice)

監管

- 所有的食品。

食品安全權限

- 起訴有違反食品安全法律嫌疑的公司及個人。
- 透過美國聯邦保安局，根據法院命令，扣押尚未進入市場的不安全食品。

詳情請洽

美國聯邦檢察官辦公室，列在電話簿藍頁「美國政府」欄下

www.usdoj.gov

聯邦貿易委員會 (Federal Trade Commission)

監管

- 所有的食品。

食品安全權限

- 執行各種法律，保護消費者，防止不公平的、虛假的或欺詐性的行爲，包括虛假和不實的廣告。

詳情請洽

FTC（聯邦貿易委員會）
Consumer Response Center, CRC-240
Washington, DC 20580

媒體採訪: 202-326-2180
聽力殘障者專線: 202-326-2502

消費者: 202-FTC-HELP
(202-382-4357)

www.ftc.gov

州與地方政府

監管

- 其司法管轄區域內的所有食品。

食品安全權限

- 和食品與藥物管理局及其他聯邦機構合作，對本州境內生產的魚類、海鮮、牛奶和其他食品實施食品安全標準。
- 檢查本地司法管轄區域裡的飯店、雜貨店和其他食品零售店，以及奶牛場和牛奶加工廠、穀物加工廠和食品生產廠。
- 禁售（停止銷售）本州境內生產或流通的不安全的食品。

詳情請洽

市、縣和州的衛生、農業及環保機構，列在電話簿藍頁「市、縣和州政府」欄下

(BG 98-7)

Home

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www.FoodSafety.gov
Gateway to Government Food Safety Information

News & Safety Alerts
Consumer Advice
Kids, Teens, & Educators
Industry Assistance
Report Illnesses & Product Complaints
Foodborne Pathogens
National Food Safety Programs
Federal & State Gov't Agencies
Other Topics
Search & Site Index

Foodborne Pathogens

Federal Government Web Sites

- The Bad Bug Book (FDA)
  Brings together information on 40 pathogen toxins from FDA, CDC, FSIS, and NIH.
- Morbidity and Mortality Weekly Report (CDC)
- Question & Answer Fact Sheets (CDC)
  Foodborne diseases, pathogens and toxins
- Foodborne Disease Fact Sheet (NIAID)
- Foodborne Infections: Frequently Asked Questions (CDC)
- Emerging Infectious Diseases Journal (CDC)
- Searchable database: Foodborne Disease Outbreaks from 1990-1995 (CDC)
- Bovine Spongiform Encephalopathy
- *Listeria monocytogenes*
- Outbreak Response and Surveillance Unit (CDC)
- Product Liability and Microbial Foodborne Illness (ERS)
- Resources for Health Professionals
- USDA Agricultural Research Service Food Safety Activities
- Achievements in Public Health, 1900-1999: Safer and Healthier Foods (CDC)
- Foodborne Pathogens (ERS)
- Children and Microbial Illness (available in PDF) (ERS)
- Healthy Pets Healthy People: Diseases People Can Get from Pets (CDC)
- *Vibrio vulnificus* Health Education Kit (FDA)

Federal Government/Private Sector Partnership Web Sites

- Ten Least Wanted Foodborne Pathogens (Partnership for Food Safety Education)
  (English) (Chinese (Big 5)) (Chinese (GB)) (French) (German) (Japanese) (Korean) (Russian (1251)) (Russian (KOI-8)) (Spanish)

- Organisms That Can Bug You (Partnership for Food Safety Education)
(English) (Chinese (Big 5)) (Chinese (GB)) (French) (German) (Japanese) (Korean) (Russian (1251)) (Russian (KOI-8)) (Spanish)

- Everyone can Fight BAC! (Partnership for Food Safety Education)
(English) (Chinese (Big 5)) (Chinese (GB)) (French) (German) (Korean) (Russian (1251)) (Russian (KOI-8)) (Spanish)

- Diagnosis and Management of Foodborne Illness: A Primer for Physicians (AMA, FDA, CDC, FSIS)

State & Local Government Web Sites

- About Food Poisoning (Department of Agriculture and Consumer Services, Virginia)
- Bad Bug Book for Kids (Department of Agriculture, North Carolina)
- Communicable Diseases Investigation (Department of Health, Pennsylvania)
- Disease Fact Sheets / Informacion Sobre Enfermedades (Department of Health, Texas)
- Epidemiology (Health and Human Services, Nebraska)
- Foodborne Illness in Rhode Island (Department of Health, Rhode Island)
- Massachusetts Foodborne Illness Investigation and Control Reference Manual (Department of Health, Massachusetts)
- Microbiology of Foodborne Illness Review (Rhode Island Cooperative Extension)
- Organisms of Concern (Cooperative Extension Service, North Carolina)
- Pfiesteria
    - About *Pfiesteria piscicida* (Sea Grant Extension Program, Maryland)
    - Quick Facts on Pfiesteria (Department of Health and Human Services, North Carolina)
- What You Should Know about Pfiesteria and Virginia's Water (Department of Health, Virginia)

More Web Sites

- Other Languages

- Video Library

Additional links to federal, state and local government agencies.
See Other Topics for other food safety issues.

www.FoodSafety.gov

Privacy | Accessibility | Webmaster | Last updated on 2004-APR-07 by dav/cms/cjm

Bad Bug Book

U.S. Food & Drug Administration
Center for Food Safety & Applied Nutrition

Foodborne Pathogenic Microorganisms and Natural Toxins Handbook

The *"Bad Bug Book"*

This handbook provides basic facts regarding foodborne pathogenic microorganisms and natural toxins. It brings together in one place information from the Food & Drug Administration, the Centers for Disease Control & Prevention, the USDA Food Safety Inspection Service, and the National Institutes of Health.

Some technical terms have been linked to the National Library of Medicine's Entrez glossary. Recent articles from Morbidity and Mortality Weekly Reports have been added to selected chapters to update the handbook with information on later outbreaks or incidents of foodborne disease. At the end of selected chapters on pathogenic microorganisms, hypertext links are included to relevant Entrez abstracts and GenBank genetic loci. A more complete description of the handbook may be found in the Preface.

PATHOGENIC BACTERIA

- *Salmonella* spp.
- *Clostridium botulinum*
- *Staphylococcus aureus*
- *Campylobacter jejuni*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*
- *Listeria monocytogenes*
- *Vibrio cholerae* O1
- *Vibrio cholerae* non-O1
- *Vibrio parahaemolyticus* and other *vibrios*
- *Vibrio vulnificus*
- *Clostridium perfringens*
- *Bacillus cereus*
- *Aeromonas hydrophila* and other spp.
- *Plesiomonas shigelloides*
- *Shigella* spp.
- Miscellaneous enterics
- *Streptococcus*

ENTEROVIRULENT ESCHERICHIA COLI

GROUP (EEC Group)

- *Escherichia coli* - enterotoxigenic (ETEC)
- *Escherichia coli* - enteropathogenic (EPEC)
- *Escherichia coli* O157:H7 enterohemorrhagic (EHEC)
- *Escherichia coli* - enteroinvasive (EIEC)

PARASITIC PROTOZOA and WORMS

- *Giardia lamblia*
- *Entamoeba histolytica*
- *Cryptosporidium parvum*
- *Cyclospora cayetanensis*
- *Anisakis* sp. and related worms
- *Diphyllobothrium* spp.
- *Nanophyetus* spp.
- *Eustrongylides* sp.
- *Acanthamoeba* and other free-living amoebae
- *Ascaris lumbricoides* and *Trichuris trichiura*

VIRUSES

- Hepatitis A virus
- Hepatitis E virus
- Rotavirus
- Norwalk virus group
- Other viral agents

NATURAL TOXINS

- Ciguatera poisoning
- Shellfish toxins (PSP, DSP, NSP, ASP)
- Scombroid poisoning
- Tetrodotoxin (Pufferfish)
- Mushroom toxins
- Aflatoxins
- Pyrrolizidine alkaloids
- Phytohaemagglutinin (Red kidney bean poisoning)
- Grayanotoxin (Honey intoxication)

OTHER PATHOGENIC AGENTS

- Prions

APPENDICES

- Infective dose
- Epidemiology summary table
- Factors affecting microbial growth in foods
- Foodborne Disease Outbreaks, United States 1988-1992
- Additional Foodborne Disease Outbreak Articles and Databases.

Foods Home | FDA Home | HHS Home | Search/Subject Index | Disclaimers & Privacy Policy | Accessibility/Help

Hypertext last updated by las/dav 2003-JAN-30

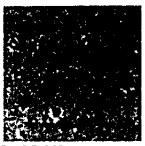
The Partnership for Food Safety Education

The Problem of Foodborne Illness

(This document in English)

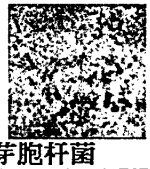
靠食物傳播的十種最需避免的病原體

美國衛生部根據其致病的嚴重程度或引發病例的數目，將下列微生物列爲引發食物中毒的罪魁。當心這些病原體：*與細菌做鬥爭！ (Fight BAC!)*

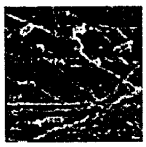


空腸彎形菌
是腹瀉最常見的病因；來源：*生的或未煮熟的肉*、禽，生牛奶，和未經處理的水。

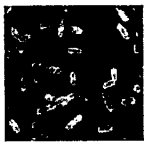
了解其所在及避免感染的方法



梭狀芽胞杆菌
該生物體可產生一種引發肉毒中毒的毒素。病症是肌肉麻痺；來源：家製飯菜及植物油。



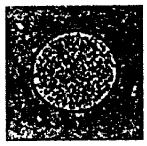
大腸杆菌 0157：H7
一種可以產生致命毒素的細菌；來源：肉，尤其是未煮熟的或生的漢堡包，水果、蔬菜、或生牛奶。



單核細胞增多性利斯特菌
可引發利斯特菌病，是一種孕婦、新生兒和免疫系統差之成人易得的嚴重疾病；病源：土壤和水。發現於包括軟奶酪在內的乳製品，生的或未煮熟的肉、禽類、海產品、及水果蔬菜中。

沙門菌
食物中毒的第二大病源。每年有數百萬個病例是沙門菌引起的。來源：生的或未煮熟的蛋、禽、肉，以及奶製品、海產品、水果和蔬菜。

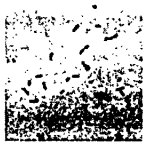
志賀杆菌
引發約300,000個腹瀉病例。不良衛生使志賀杆菌極易在人與人之間傳播。來源：沙拉、牛奶和奶製品，及不乾淨的水。



葡萄球菌耳
該細菌可產生一種毒素，食後很快就會引起嘔吐；來源：蛋白含量高的熟食(如熟火腿、沙拉、烤製的麵食、奶製品)。

兔弓形蟲
一種引發弓形體病的寄生蟲，弓形蟲病是一種非常嚴重的疾病，可導致中樞神經系統紊亂，尤其是可導致兒童痴呆、視力衰退。來源：肉，主要是豬肉。

弧菌
可引起腸胃炎或一種被稱爲初期敗血病的綜合症。有肝病的人尤其容易被感染。來源：生的或未煮熟的海產品。



耶爾辛血清菌
可引發小腸結腸炎。病症爲腹痛、嘔吐，或兩者並發。來源：豬肉、奶製品，水果和蔬菜。

關於這些及其他通過食物傳播的病原體，可查閱網際網路上的 "Bad Bug Book"，網址 "http://vm.cfsan.fda.gov/~mow/intro.html".

Partnership for Food Safety Education
FDA Center for Food Safety and Applied Nutrition

For More Information . . .

USDA's Meat and Poultry Hotline:
1-888-MPHotline (1-888-674-6854)

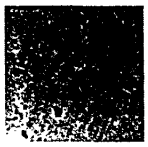
FDA's Food Safety Information Hotline:
1-888-SAFEFOOD (1-888-723-3366) 24 hr

Foodborne Illness: Ten Least Wanted Foodborne Pathogens

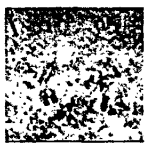
Updated on April 10, 2003

The U.S. Public Health Service has identified the following microorganisms as being the biggest culprits of foodborne illness, either because of the severity of the sickness or the number of cases of illness they cause. Beware of these pathogens: *Fight BAC!*

LEARN WHERE THEY ARE AND HOW TO AVOID THEM

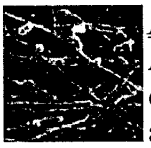


Campylobacter
Most common bacterial cause of diarrhea in the United States; Sources: *raw and undercooked meat and poultry*, raw milk and untreated water

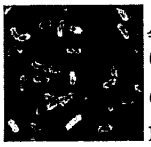


Clostridium botulinum
This organism produces a toxin which causes botulism, a life-threatening illness that can prevent the breathing muscles from moving

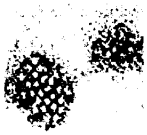
air in and out of the lungs. Sources: home-prepared foods and herbal oils; honey should not be fed to children less than 12 months old



***E. coli* O157:H7**
A bacterium that can produce a deadly toxin and causes approximately 73,000 cases of foodborne illness each year in the U.S.; Sources: meat, especially undercooked or raw hamburger, produce and raw milk

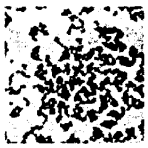


Listeria monocytogenes
Causes listeriosis, a serious disease for pregnant women, newborns and adults with a weakened immune system; Sources: soil and water. It has been found in dairy products including soft cheeses as well as in raw and undercooked meat, in poultry and seafood, and in produce



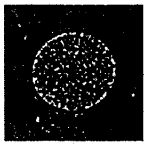
Norovirus
This virus is the leading cause of diarrhea in the United States. Any food can be contaminated with norovirus if handled by someone who is infected with this virus.

Salmonella
Most common cause of foodborne deaths. Responsible for millions of cases of foodborne illness a year; Sources: raw and undercooked eggs, undercooked poultry and meat, dairy products, seafood, fruits and vegetables

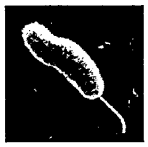


Staphylococcus aureus
This bacterium produces a toxin that causes vomiting shortly after ingesting; Sources: cooked foods high in protein (e.g. cooked ham, salads, bakery products, dairy products)

Shigella
Causes an estimated 300,000 cases of diarrhea illnesses. Poor hygiene causes *Shigella* to be easily passed from person to person. Sources: salads, milk and dairy products, and unclean water.



Toxoplasma gondii
A parasite that causes toxoplasmosis, a very severe disease that can produce central nervous system disorders particularly mental retardation and visual impairment in children. Pregnant women and people with weakened immune systems are at higher risk; Sources: meat, primarily pork



Vibrio vulnificus
Causes gastroenteritis or a syndrome known as primary septicemia. People with liver diseases are especially at high risk; Sources: raw or undercooked seafood

For more information on these and other foodborne pathogens, check out the "Bad Bug Book" on the World Wide Web at: **http://vm.cfsan.fda.gov/~mow/intro.html.**

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The Partnership for Food Safety Education

The Problem of Foodborne Illness

(This document in English)

可能給你惹麻煩的菌體

疾病及引發疾病的菌體	病源	症狀
細菌		
肉毒中毒 梭狀芽胞杆菌毒素(由*梭狀芽胞杆菌*產生)	此類細菌的孢子分布很廣。但它們只有在一種少酸性的無氧環境中才會產生毒素。見於相當多的罐裝食品，如玉米、豆莢、湯、甜菜、蘆筍、蘑菇、鮪魚和肝餅，也見於午餐肉、火腿、香腸、夾餡茄子、龍蝦、燻魚、及鹹魚。	**發病期**：通常在食後4—36 小時。 **症狀**：神經中毒的症狀，包括複視、不能吞咽、言語困難、呼吸系統逐漸癱瘓。 **應立即就醫。肉毒中毒可能致命。**
空腸畸形菌病 *空腸畸形菌*	家禽、牛羊上的細菌可以污染這些動物的肉和奶。主要的生食病源：生的禽、肉、及未經消毒的牛奶。	**發病期**：通常在食後 2—5 天。 **症狀**：腹瀉、腸痙攣、發燒、有時大便中帶血。持續7—10 天。
利斯特菌病 *單核細胞增多性利斯特菌病*	見於軟奶酪、未經消毒的牛奶、進口的海產品、冷凍熟蟹肉、熟蝦、及熟的人造海鮮肉。*利斯特菌*比許多其他微生物抗熱、鹽、亞硝酸鹽及酸性的能力都強，能在低溫下存活，生長。	**發病期**：食後 7—30 天，但大多數症狀在食用受污染的食物之後 48—72 小時即出現。 **症狀**：發燒、頭痛、惡心、嘔吐。主要影響到孕婦及其胎兒、新生兒、老年人、癌症患者、及免疫系統差的人，可導致胚胎和嬰兒死亡。

http://www.foodsafety.gov/~fsg/bac/ctbug.html

產氣莢膜杆菌食物中毒 *產氣荚膜梭狀芽胞杆菌*	多數情況是由於沒有將食物保溫引起的。一些生物體往往在烹飪後仍然存活，在食物冷卻和儲存過程中繁殖到有毒性的程度。它們最常產生於肉和肉製品。這些生物體在 120－130 華氏度之間比其他細菌生長得好。所以，肉汁和餡必須保持在華氏 140 度以上。	**發病期**：通常食後 8－12 小時。 **症狀**：腹痛、腹瀉、有時惡心、嘔吐。 症狀持續不過一天，通常不很嚴重。年老體弱的人症狀會重一些。
沙門菌病 *沙門菌*	最常見於生肉、禽、奶及其他奶製品、蝦、田雞腿、酵母、椰子、冷面沙拉、巧克力等。	**發病期**：通常食後 8－12 小時 **症狀**：腹痛、腹瀉、有時惡心、嘔吐。症狀持續不過一天，通常不很嚴重。年老體弱的人症狀會重一些。
志賀杆菌病(杆菌性痢疾) *志賀杆菌*	見於牛奶及奶製品、禽肉、和土豆沙拉中。當人不洗手就拿食物或湯水，而過後食物又沒有徹底煮透時，食物就會被污染，細菌就在室溫下在食物中繁殖。	**發病期**：食後 1－7 天。 **症狀**：胃痙攣、腹瀉。發燒，有時嘔吐，大便中有膿、血或粘液。
葡萄球菌食物中毒 葡萄球菌腸毒素(由*金色葡萄球菌* 產生)	將被細菌污染的食物置於室溫中太久，就會產生這種毒素。肉、禽、蛋製品、鮪魚、馬鈴薯和通心粉沙拉、加奶油的糕點，均是這種細菌製造毒素的好場所。	**發病期**：通常在食後 30 分鐘至 8 小時。 **症狀**：腹瀉、嘔吐、惡心、腹痛、痙攣和疲憊。持續 24－48 小時，一般無生命危險。
弧菌感染 *弧菌*	這種細菌生長於海濱水域，通過傷口或食用污染的海產傳染給人。這種細菌在溫暖天氣中最多。	**發病期**：突發。 **症狀**：寒顫、發燒、有時伴有疲憊的症狀。肝臟有問題、胃酸少、免疫力差的人最易感染此病。

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原生動物門		
阿米巴病 *溶組織內阿米巴(痢疾阿米巴)*	生存於人體腸道內，通過排泄物排出體外。污染的水及在污染的土壤中生長的蔬菜傳播這種病菌感染。	**發病期**：接觸後 3—10 天。 **症狀**：嚴重痙攣性疼痛、結腸或肝部有觸痛，早晨大便失禁、腹瀉不止、體重減輕、乏力，有時有貧血的症狀。
蘭氏賈第鞭毛虫 *賈第鞭毛虫病*	往往與飲用受污染的水有關。可以由在生長過程中受到污染而又未經燒煮的食物傳染，或煮熟後被已受感染的人接觸過的食物傳染。涼、潮的環境宜於此細菌的生存。	**發病期**：1—3 天。 **症狀**：突然發作，突發性水瀉、胃痙攣、厭食、惡心、嘔吐、長途跋涉者、兒童、旅行者、及需特殊護理的病人特別易受感染。
病毒		
甲型肝炎病毒	當水域被未經處理的污水污染時，軟體動物，(如牡蠣、蛤、海扇、鮮貝、螺)，即成爲此種病毒的載體，生的貝殼動物尤是此種病毒的極好載體，而烹調有時殺不死這種病毒。	**症狀和發病期**：開始是不適、欠爽，沒有食欲，惡心、嘔吐、發燒。 3—10 天後，患者會出現黃疸，深色尿。情況嚴重的可導致肝臟損壞和死亡。

Register to be a BACFighter!

For More Information . . .

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Organisms That Can Bug You

Disease and Organism That Causes It	Source of Illness	Symptoms
Bacteria		
Botulism Botulinum toxin (produced by *Clostridium botulinum* bacteria)	Spores of these bacteria are wide-spread. But these bacteria produce toxin only in an anerobic (oxygenless) environment of little acidity. Found in a considerable variety of canned goods, such as corn, green beans, soups, beets, asparagus, mushrooms, tuna, and liver paté. Also in luncheon meats, ham, sausage, stuffed eggplant, lobster, and	**Onset:** Generally 4-36 hours after eating. **Symptoms:** Neurotoxic symptoms, including double vision, inability to swallow, speech difficulty, and progressive paralysis of the respiratory system. **Get Medical Help Immediately. Botulism Can Be Fatal.**

	smoked and salted fish.	
Campylobacteriosis *Campylobacter jejuni*	Bacteria on poultry, cattle, and sheep can contaminate meat and milk of these animals. Chief raw food sources: raw poultry, meat, and unpasteurized milk.	**Onset:** Generally 2-5 days after eating. **Symptoms:** Diarrhea, abdominal cramping, fever, and sometimes bloody stools. Lasts 7-10 days.
Listeriosis *Listeria monocytogenes*	Found in soft cheese, unpasteurized milk, imported seafood products, frozen cooked crab meat, cooked shrimp, and cooked surimi (imitation shellfish). The *Listeria* bacteria resist heat, salt, nitrite, and acidity better than many other micro-organisms. They survive and grow at low temperatures.	**Onset:** From 7-30 days after eating, but most symptoms have been reported 48-72 hours after consumption of contaminated food. **Symptoms:** Fever, headache, nausea, and vomiting. Primarily affects pregnant women and their fetuses, newborns, the elderly, people with cancer, and those with impaired immune systems. Can cause fetal and infant death.
Perfringens food	In most	**Onset:** Generally

poisoning *Clostridium perfringens*	instances, caused by failure to keep food hot. A few organisms are often present after cooking and multiply to toxic levels during cool down and storage of prepared foods. Meats and meat products are the foods most frequently implicated. These organisms grow better than other bacteria between 120-130° F. So gravies and stuffing must be kept above 140° F.	8-12 hours after eating. **Symptoms:** Abdominal pain and diarrhea, and sometimes nausea and vomiting. Symptoms last a day or less and are usually mild. Can be more serious in older or debilitated people.
Salmonellosis *Salmonella* bacteria	Raw meats, poultry, milk and other dairy products, shrimp, frog legs, yeast, coconut, pasta and chocolate are most frequently involved.	**Onset:** Generally 8-12 hours after eating. **Symptoms:** Abdominal pain and diarrhea, and sometimes nausea and vomiting. Symptoms last a day or less and are usually mild. Can be more

		serious in older or debilitated people.
Shigellosis (bacillary dysentery) *Shigella* bacteria	Found in milk and dairy products, poultry, and potato salad. Food becomes contaminated when a human carrier does not wash hands and then handles liquid or food that is not thoroughly cooked afterwards. Organisms multiply in food left at room temperature.	**Onset:** 1-7 days after eating. **Symptoms:** Abdominal cramps, diarrhea, fever, sometimes vomiting, and blood, pus, or mucus in stool.
Staphylococcal food poisoning Staphylococcal enterotoxin (produced by *Staphylococcus aureus* bacteria)	Toxin produced when food contaminated with the bacteria is left too long at room temperature. Meats, poultry, egg products, tuna, potato and macaroni salads, and cream-filled pastries are good environments for these bacteria to produce toxin.	**Onset:** Generally 30 minutes-8 hours after eating. **Symptoms:** Diarrhea, vomiting, nausea, abdominal pain, cramps, and prostration. Lasts 24-48 hours. Rarely fatal.

Vibrio Infection *Vibrio vulnificus*	The bacteria live in coastal waters and can infect humans either through open wounds or through consumption of contaminated seafood. The bacteria are most numerous in warm weather.	**Onset:** Abrupt. **Symptoms:** Chills, fever, and/or prostration. At high risk are people with liver conditions, low gastric (stomach) acid, and weakened immune systems.
Protozoa		
Amebiasis *Entamoeba histolytica*	Exist in the intestinal tract of humans and are expelled in feces. Polluted water and vegetables grown in polluted soil spread the infection.	**Onset:** 3-10 days after exposure. **Symptoms:** Severe crampy pain, tenderness over the colon or liver, loose morning stools, recurrent diarrhea, loss of weight, fatigue, and sometimes anemia.
Giardiasis *Giardia lamblia*	Most frequently associated with consumption of contaminated water. May be transmitted by uncooked foods that become contaminated while growing or after cooking by infected food	**Onset:** 1-3 days. **Symptoms:** Sudden onset of explosive watery stools, abdominal cramps, anorexia, nausea, and vomiting. Especially infects hikers, children, travelers, and

	handlers. Cool, moist conditions favor organism's survival.	institutionalized patients.
Virus		
Hepatitis A virus	Mollusks (oysters, clams, mussels, scallops, and cockles) become carriers when their beds are polluted by untreated sewage. Raw shellfish are especially potent carriers, although cooking does not always kill the virus.	**Symptoms and Onset:** Begins with malaise, appetite loss, nausea, vomiting, and fever. After 3-10 days patient develops jaundice with darkened urine. Severe cases can cause liver damage and death.

The Partnership for Food Safety Education

Spread the Word

(This document in English)

人人都能*對付細菌!*™

(*Fight BAC!*)™

看不見的敵人：細菌

儘管美國是世界上食品供應最安全的國家之一，令人倒胃的事實是：我們所吃的食物仍會使我們生病。爲什麼呢？因爲在正常情況下，一種看不見的敵人“BAC”（細菌）可能在我們購買食物時附在食物上，或是在我們收拾、烹調、進食或儲存的過程中進入食物。實際上，儘管我們看不見，甚至聞不到、摸不到它，這傢伙和成千上萬個它的同類可能已經附在海綿塊上、砧板上、或食物上。

如果收拾、準備食物的每一個人都學會如何對付細菌™，多數與食物有關的病例是可以避免的。雖然細菌無處不在，一點知識和一些日常的“武器”，如肥皂、熱水、冰箱和食物溫度計，就可以對付它們。

關於細菌的事實

科學家對引發與食物有關的疾病的細菌及其他微生物進行了長期研究。他們發現了這些重要事實：

- 細菌是所有生物的一部份，存在於所有生的農產品上；

- 有害的細菌可以在人與食物之間交叉傳染，或食物之間傳染。
- 細菌可以在室溫下快速生長；
- 冷藏或冷凍可以減緩或阻止食物中有害菌的生長；
- 與食物有關的疾病可以引起輕微的症狀，也可以引起非常嚴重的症狀。發病可從食用帶有害細菌的食物後 30 分鐘到兩週之間開始。
- 最易因食物得病的是嬰幼兒、老年人和免疫力差的人。

對付細菌的四個簡單步驟

食品衛生安全專家建議，爲了*對付細菌!*™每個人在處理食物的過程中，每一步都要考慮到食品安全的問題 — 從購買到儲存剩餘食物。

這意味著隨時遵循以下四個簡單步驟：

清潔 — 在準備食物之前與之後，尤其在處理肉、禽、蛋和海產之後，都用熱肥皂水清洗手、器皿和其他接觸到的地方，以充分保證不受細菌感染。用消毒劑或漂白粉與水混合擦洗桌面、板面，用消毒肥皂洗手，可增強預防效果。

隔離 — 將生的肉、禽、蛋和海產及其湯汁與熟食分開；絕不要把做好的飯放在盛過生肉、禽、蛋或海產的盤子裡。

烹調 — 烹調食物一定要煮透(根據肉禽的種類和切塊的形狀、大小掌握火候)，用食物溫度計測量食物的生熟程度。煮雞蛋時一定要等蛋黃和蛋白都凝固變硬。

冷凍 — 在兩小時內冷藏或冷凍易腐壞的食物、做好了的食物和剩飯，一定要把冷藏室溫度設得不高於華氏 40 度，冷凍室設在華氏零度。

所以，不要疏忽大意，這幾個簡單步驟可以幫你減少與食物有關的疾病。

我們有
能力
對付細菌!
FIGHT BAC!

Partnership for Food Safety Education
FDA Center for Food Safety and Applied Nutrition

Register to be a BACFighter!

For More Information . . .

USDA's Meat and Poultry Hotline:
1-888-MPHotline (1-888-674-6854)

FDA's Food Safety Information Hotline:
1-888-SAFEFOOD (1-888-723-3366) 24 hr

Community Activity

Supermarket

Groups and Club

Everyone Can Fight BAC!TM

THE INVISIBLE ENEMY: BACTERIA

Despite the fact that America's food supply is among the safest in the world, the unappetizing fact is that sometimes, the food we eat can make us sick. Why? Because under the right conditions, an invisible enemy called "BAC" (bacteria) may be present on foods when purchased or get into food during preparation, cooking, serving or storage. In fact, even though we may not see BAC - or smell him or feel him - this creature and millions more like him may already be on a sponge, a cutting board, or the food itself.

Most cases of food-related illness can be prevented if everyone who handles and prepares food learns how to Fight BAC!TM Although BAC is everywhere, he can be stopped with a little know-how and such everyday weapons as soap and hot water, a refrigerator and a food thermometer.

BAC:The FACTS

Scientists have been studying bacteria and other tiny organisms that cause food-related illness for a long time. They have learned these important

facts:

- Bacteria are a part of all living things and are found on all raw agricultural products;
- Harmful bacteria can be transferred from food to people, people onto food, or from one food to another;
- Bacteria can grow rapidly at room temperature;
- Growth of harmful bacteria in food can be slowed or stopped by refrigerating or freezing;
- Food-related illness can produce symptoms from mild to very serious. Illness can occur from 30 minutes to two weeks after eating food containing harmful bacteria;
- People who are most likely to become sick from food-related illness are infants and young children, senior citizens and people with weakened immune systems.

FIGHTING BAC!: FOUR SIMPLE STEPS

To Fight BAC!TM, food safety experts recommend that everyone think about food safety at each step in the food handling process - from shopping to storing leftovers. What this really means is always following these four simple steps:

CLEAN - Wash hands, utensils and

surfaces in hot soapy water before and after food preparation, and especially after preparing meat, poultry, eggs or seafood to protect adequately against bacteria. Using a disinfectant cleaner or a mixture of bleach and water on surfaces and antibacterial soap on hands can provide some added protection.

SEPARATE - Keep raw meat, poultry, eggs and seafood and their juices away from ready-to-eat foods; never place cooked food on a plate that previously held raw meat, poultry, eggs or seafood.

COOK - Cook food to the proper internal temperature (this varies for different cuts and types of meat and poultry) and check for doneness with a food thermometer. Cook eggs until both the yolk and white are firm.

CHILL - Refrigerate or freeze perishables, prepared food and leftovers within two hours and make sure the refrigerator is set at no higher than 40°F and that the freezer unit is set at 0°F.

So, don't risk problems when these simple steps will help you reduce food-related illness.

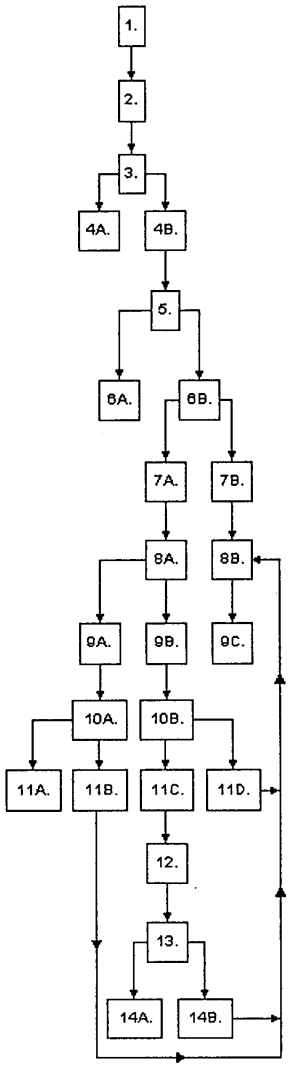
WE HAVE THE POWER TO FIGHT BAC!

U.S. Food and Drug Administration
Center for Food Safety & Applied Nutrition
Industry Activities Staff Flyer: 1996

美國食品暨藥物管理局
食品安全與營養中心
行業活動人員宣傳品：1996

(This document in English)

食品暨藥物管理局進口程序



1. 進口商或代理商在貨物到達五日之內向入境口岸海關遞交申報單；

2. 食品暨藥管局通過以下途徑獲知監管食品之入境：

   - 海關入境申報單複印件(CF 3461，CF 3461 ALT，CF 7501 或其替代件)，
   - 商業發票的副本，以及
   - 擔負責任、稅務和接收處罰之保證。

3. 食品暨藥管局審核進口商之入境申報單以確定是否要進行實物檢查、碼頭檢查或抽樣檢查。

4A. 決定不做抽樣檢查。食品暨藥管局向美國海關和提出申請之進口商發函“可以進行”。對於食品暨藥管局來說，該貨物即放行。

4B. 決定根據以下項目作抽樣檢查：

   - 貨物之性質；
   - 食品暨藥管局之優先次序，以及
   - 該商品的歷史。

   食品暨藥管局向美國海關和該進口商發出“抽樣通知”。該貨物必須保持原封不動，等待進一步通知。取樣之後，進口商可以將貨物移至其他碼頭或倉庫(詳情請與美國海關聯繫)。

5. 食品暨藥管局獲取實物樣本。樣本送食品暨藥管局區實驗室進行檢驗分析。

6A. 食品暨藥管局經分析確認樣本符合要求，食品暨藥管局向美國海關和進口商簽發“放行通知”。

6B. 食品暨藥管局分析認定樣本“似乎違反 FD & C 法以及其他相關法”。食品暨藥管局向美國海關和進口商簽發“扣留和聽證通知”，該通知

- 說明違法性質，
- 給進口商十個工作日陳述可以接收該貨物的理由。

這個聽證是進口商爲該批進口進行辯護和/或提供證據使其合法入關的唯一機會。

7A. 收貨人，實際貨主，進口商或其指定代表對“扣留和聽證 通知”作出反應。針對該貨物是否可以接收作出口頭或書面證詞。

7B. 收貨人，實際貨主，進口商或其指定代表對“扣留和聽證 通知”既不作出反應，又不要求延長聽證期限。

8A. 食品暨藥管局對該產品是否可以接收舉行聽證。這個聽證是陳述相關事務的機會，僅限於提供相關的證據。

8B. 食品暨藥管局向進口商簽發“拒絕入境”通知。這是曾向其簽發“抽樣通知“的同一個人或公司。所有收到“抽樣通知”以及“扣留和聽證通知”者皆發給一份“拒絕入境”通知。

9A. 進口商提供證據，證明該產品符合要求。提供經可靠實驗室檢驗、符合已公布的人類食物中污染物和殘缺標準的抽樣結果。

9B. 進口商提出“改善或採取其他措施授權(FDA FD 766 表)”申請。該表要求允許將摻假或誤貼商標的食品通過重新貼標簽或採取其他措施使其符合要求，或將其轉換成非實用物品。必須提出使該食品符合要求的具體辦法。

9C. 食品暨藥管局收到美國海關出口或銷毀該批貨物的核準。對“拒絕接收通知單”上所列商品的出口或銷毀在美國海關指導下進行。

10A. 食品暨藥管局採集經處理之食品樣本以決定其是否符合標準。

10B. 食品暨藥管局審核進口商提出的改善程序。對於清算損失的賠償須訂立契約。

11A. 食品暨藥管局認定樣品“合格”。向美國海關和進口商發出標有“原來扣留、現在予以放行”字樣的“放行通知”。

11B. 食品暨藥管局認定樣品“不合格”。進口商可以遞交“改善或採取其他措施授權”(參閱 9B)申請，否則，食品暨藥管局將簽發“拒絕接收通知”(參閱 8B)。

11C. 食品暨藥管局批准進口商之改善程序。經批准的申請含有“等待食品暨藥管局之放行通知，商品須保持原樣”的聲明。

11D. 如果過去的經驗顯示提出的辦法不會成功，食品暨藥管局會否決申請人之改進程序。第二次即最後一次請求中除非提出有意義的改進實施辦法以保證相當的成功可能性，食品暨藥管局將不予考慮。申請人從 FDA FD 766 表上得到通知。

12. 進口商完成所有改進程序，通知食品暨藥管局貨物可以檢查或抽樣了。

13. 食品暨藥管局進行後繼檢查、採樣以決定其是否符合改進授權條款。

14A. 食品暨藥管局分析認爲樣本合格。向進口商和美國海關發出“放行通知”。食品暨藥管局監管收費在 FDA FD790 表中估算。副本送美國海關。美國海關負責收取總費用，包括海關人員所需的費用。

14B. 食品暨藥管局認定樣本仍然不合格。食品暨藥管局監管收費在 FDA FD790 表中估算。美國海關負責收取總費用，包括海關人員所需的費用。

進口商可以加快商品入境!

- 在貨物起運之前確定待進口之產品是合法的。
- 請私人實驗室檢驗待進口食品樣品並核實對加工廠的分析。雖然這些分析不是最後結果，但是可能顯示該加工廠具備生產滿意和合法產品的能力。
- 簽訂貨運合同之前，熟悉食品暨藥管局之法律要求。
- 請求負責你處入境口岸的食品暨藥管局地區辦公室協助。
- 熟悉本文所述之食品進口程序。

附　　件　　六

附件六、FDA 簡介（A Tour of FDA）

A Tour of FDA

The Food and Drug Administration (FDA) touches the lives of virtually every American, every day. FDA regulates a host of products, from the most common food ingredients to complex medical and surgical devices, lifesaving drugs, and radiation-emitting consumer and medical products. These products are worth about a trillion dollars a year and make up 25% of all consumer spending.

Most of the Agency's nearly 10,000 employees are scientists, consumer safety officers, medical officers, and other professionals. FDA's budget is $1.6 billion a year, or about $4 a year per taxpayer.

After completing this course, you will be able to recognize FDA's public health mission and how the Agency is organized to carry out its mission. You will be able to recognize the history of FDA and the products that it regulates. You will also recognize the work of FDA's program Centers and offices, and how the Agency enforces its regulations.

This course addresses the following:

Food and Drug Administration Home Page
http://www.fda.gov/

21 CFR, Chapter 9
Federal Food, Drug, and Cosmetic Act
http://www.access.gpo.gov/uscode/uscmain.html

Food and Drug Administration Modernization Act of 1997
http://www.fda.gov/cdrh/modact97.pdf

What is FDA?
A federal science-based law enforcement agency mandated to protect public health and safety

The mission

The FDA Modernization Act (FDAMA) of 1997 affirmed FDA's public health protection role and defined the Agency's mission:

- to promote public health by promptly and efficiently reviewing clinical research and taking appropriate action on the marketing of regulated products in a timely manner.
- to protect public health by ensuring: foods are safe, wholesome, sanitary, and properly labeled; human and veterinary drugs are safe and effective; there is reasonable assurance of the safety and effectiveness of devices intended for human use; cosmetics are safe and properly labeled; and public health and safety are protected from electronic product radiation.
- to participate with representatives of other countries to reduce the burden of regulation, coordinate regulatory requirements, and achieve appropriate equivalent arrangements.
- as determined to be appropriate by the Secretary of Health and Human Services, to carry out the tasks above by consulting with experts in science, medicine, and public health, and by cooperating with consumers, users, manufacturers, importers, packers, distributors, and retailers of regulated products.

FDA accomplishes its mission by establishing and enforcing high product standards and other regulatory requirements authorized or mandated by the Federal Food, Drug and Cosmetic Act (FD&C Act), its amendments, and other public health laws.

A brief history

FDA is one of our nation's oldest public health agencies. Drag the red slider to learn more about the history of FDA.

With more than 80% favorable rating in public opinion polls, the Agency is cooperating with it stakeholders in the US and abroad to continue protecting consumers and the public health in the new area of technological and scientific advances.

In the wake of terrorist attack on September 11, 2001, FDA has also been entrusted with two critical functions in the nation's war on terrorism:

* To prevent the willful contamination of all regulated products, including foods.
* To improve the availability of medications to prevent or treat injuries caused by biological, chemical or nuclear agents.

Regulated products

Products regulated by FDA include: all foods except for meat and poultry [regulated by the US Department of Agriculture (USDA)]; prescription and non-prescription drugs; blood products, vaccines, and tissues for transplantation; medical devices and radiological products, including cellular telephones; animal drugs and feed; and cosmetics.

Areas that FDA does not handle include restaurant food and sanitation, air or water pollution, alcoholic beverages, and drug abuse. Although FDA doesn't directly regulate restaurant food and sanitation, it develops the policies (model code) that local authorities use. FDA also plays a strong role in the training of local investigators conducting restaurant inspections. Although FDA does not regulate pesticides, it does regulate products that are contaminated with pesticides. FDA doesn't regulate the practice of pharmacies or medical practitioners either, but there are certain sales and distribution practices of pharmacies and doctors that it does regulate.

Does FDA regulate product labeling?

In addition to setting product standards, FDA regulates the labeling of products under its jurisdiction. This information, which must be valid, well documented, and not misleading, plays a major role in protecting consumers and the public health. The FDA-regulated food label helps shoppers eat a healthy diet, and the labeling of drugs and medical devices gives prescribers and patients reliable guidance about the safety and effectiveness of healthcare products.

The next time you are in a grocery store, compare the nutritional information labels on three different foods or drinks. You will notice that the format and content of the labels are the same. FDA requires food producers to provide this specific information in the exact format that you see. So whether you are buying bottled water or a package of cupcakes, you — the consumer — are provided with nutritional information that can help you determine how that product fits into your dietary needs.

Reporting

FDA welcomes consumer reports and complaints about regulated products. Timely reporting by consumers, health professionals, and FDA-regulated companies allows the Agency to take prompt action. Information concerning ways for the public to report problems to FDA can be found on the FDA's Web site at:

http://www.fda.gov/opacom/backgrounders/problem.html

A collection of FDA-enforced laws and related statutes can be found at:

http://www.fda.gov/opacom/laws/lawtoc.htm

How is FDA organized?

Into 8 offices and program centers

Office of the Commissioner

The FDA falls within the executive branch of the US government, under the Department of Health and Human Services (DHHS). FDA is headed by the Commissioner of Food and Drugs, who is appointed by the President of the United States, confirmed by the US Senate, and serves at the President's discretion. The Office of the Commissioner (OC) oversees all the Agency's components and is responsible for the efficient and effective implementation of FDA's mission.

The Office of the Commissioner is made up of several components. Among these are:

Office	Responsibilities
Office of the Chief Council	Handles of the Agency's legal matters
Office of Policy, planning, and Legislation	Responsible for managing the Agency's policies
Office of International and Constituent Relations	Responsible for special health issues, consumer affairs, and international programs
Office of External Relations	The Agency's primary point of contact for the news media
Office of the Ombudsman	Services are available to any company or individual with a dispute with FDA
Office of Crisis Management	The Agency's focal point for coordinating emerhency and crisis response activities

	and counter-terrorism activities

Office of Regulatory Affairs

The Office of Regulatory Affairs (ORA) is the lead office for FDA's field activities. Headed by Associate Commissioner for Regulatory Affairs (ACRA), John M. Taylor III, the Office of Regulatory Affairs strives to achieve effective and efficient compliance of regulated products through high-quality, science-based work that maximizes consumer protection.

Specialized program Centers

FDA non-field activities are organized into several specialized program Centers that are responsible for protecting the public's health. Click on each dot in the image below to learn more about these Centers.

1. Center food safety and applied nutrition (CFSAN)
2. Center for drug evaluation and research (CDER)
3. Center for biologics evaluation and research (CBER)
4. Center for Devices and radiological health (CDRH)
5. National center for toxicological research (NCTR)
6. Center for veterinary medicine (CVM) : CVM's authority

以上為不分區域之特殊任務中心共有六個其中 CFSAN 與本組業務最相關

1.Center food safety and applied nutrition (CFSAN)

Foods

CFSAN develops the policy that regulates $240 billion worth of domestic food, $15 billion worth of imported foods, and $15 billion worth of cosmetics sold across state lines. This regulation takes place from the products' point of US entry or processing to their point of sale,

with approximately 50,000 food establishments and 3,500 cosmetic firms. These figures do not include the roughly 600,000 restaurants and institutional food service establishments and the 235,000 supermarkets, grocery stores, and other food outlets regulated by state and local authorities that receive guidance, model codes, and other technical assistance from FDA. FDA enhances its programs by supporting state and local authorities with training and guidance to ensure uniform coverage of food establishments and retailers.

CFSAN is organized into several main offices, including the Offices of Cosmetics and Colors, Food Additive Safety, Plant and Dairy Foods and Beverages, Seafood, and Nutritional Products, Labeling, and Dietary Supplements.

CFSAN has several responsibilities:

◎ Safety of food substance : Safety of food substance (e.g., food additives, including ionizing radiation and color additives) and the safety of foods and ingredients developed through biotechnology.

◎ Seafood HACCP: Seafood Hazard Analysis and Critical Control Point (HACCP) regulations, and activities dealing with the proper labeling of foods (e.g., ingredients, nutrition health claims) and comestics.

◎ Regulatory and research programs: Regulatory and research programs to address health risks associated with foodborne chemical and biological contaminants, and regulations and policies governing the safety of dietary supplements, infant formulas and medical foods.

◎ Education: consumer education and industry outreach, cooperative programs with state and local governments, and international food standard and safety harmonization efforts.

Ensuring food safety

A food is contaminated and considered adulterated if it contains a poisonous or harmful substance that could cause a health risk. Also, if a food has been prepared, packed, or stored in unsanitary conditions, it is considered a health risk.

FDA has several tools at its disposal to ensure that our food supply is safe:

◎ Monitoring and inspection: ORA's consumer safety officers (CSO's) inspect food production establishments and food warehouses. These inspections may be random or directed due to a complaint or CFSAN program goal. FDA also monitors imported foods. Formal agreements with the states for conducting inspections enhance the Center the ability to meet its public health mission.

◎ Sample collection: CSO's may collect samples to determine if any contaminants are presented in unacceptable amounts. These samples are then tested at a laboratory and analyzed for physical, chemical, and microbial contamination.

◎ Corrective action: If an unacceptable amount of contaminant is identified, FDA will take corrective action to enforce food safety regulations.

◎ Premarket review: Food and color additives must be reviewed by CFSAN before they can be marketed.

◎ Studies and research: CFSAN uses consumer studies and focus groups, laboratory research, and pilot plants for food processing and packaging and biotechnology studies in order to collect and analyze information regarding food safety.

◎ Outreach: CFSAN enhances stakeholder awareness through education and public meetings, cooperative activities and technical assistance, and information and outreach on Center activities.

Cosmetic safety

FDA is only able to regulate cosmetics after products are released to the marketplace. Neither cosmetic products nor cosmetic ingredients are reviewed or approved by FDA before they are sold to the public.

FDA cannot require companies to do safety testing of their cosmetic products before marketing. If, however, the safety of a cosmetic product has not been substantiated, the product's label must read "WARNING: The safety of this product has not been determined."

USDA

FDA does *not* monitor meat and poultry, which are regulated by the US Department of Agriculture (USDA) and monitored by its Food Safety and Inspection Service (FSIS). Thanks to the efforts of FDA, FSIS, and scores of state and local public health authorities, the US has one of the world's safest food supplies.

2.Center for drug evaluation and research (CDER)

Fulfilling the mission

CDER fulfills its mission by overseeing the research, development, manufacture, and marketing of drugs. It reviews the clinical trial evidence of the safety and effectiveness of new drugs before approving them for marketing and monitors their performance for unexpected health risks. CDER ensures that drug labeling, drug information for patients, and drug promotion are truthful, helpful, and not misleading.

CDER is organized into four main functional areas:

1. New drug development and review
2. Post- market drug surveillance

3. Generic drug review
4. Over-the-counter drug review

Good Manufacturing Practices

To make sure that drugs are manufactured to the same high standards that are required for their approval, FDA has developed a set of regulations called the current Good Manufacturing Practices (CGMPs). The law requires ORA's periodic inspections of all drug firms for compliance with CGMPs.

Adverse event reporting

FDA maintains several reporting systems that alert the Agency to side effects that were not detected during clinical trials, but emerged when the product became widely used. One of these programs is CDER's MedWatch, which encourages health professionals to report serious adverse events involving any medical product (including drugs, devices, and biologics). If necessary, FDA can take regulatory actions to protect consumers. Regulatory actions may include restrictions on the product's use or its withdrawal from the market. About 1%-3% of products approved each year have to be removed later because of rare, but serious side effects.

Prescription Drug User Fee Act (PDUFA)

In the Prescription Drug User Fee Act of 1992 (PDUFA) the US Congress, pharmaceutical industry, and FDA agreed on specific review goals for certain drugs and biologics, to be achieved with the help of user fees paid to FDA by the products' manufacturers. The program has been instrumental in reducing FDA's median drug review times by more than one-half. Today, typical drug applications are processed by FDA in one year or less; priority applications for breakthrough medications are usually approved in six months.

PDUFA user fees, however, do not cover FDA's expenses connected with generic and non-prescription drugs, plant inspections, post-market surveillance, and monitoring of drug advertisements.

Do all drugs have side effects?

Every drug that affects the body has some side effects. Since FDA approves only those drugs whose benefits outweigh their risks, the side effects of properly used drugs usually are not serious. To further mitigate the potential risks, FDA includes emphatic warnings about possible adverse events in product labeling and drug information that are routinely included with the packaged product

Accelerated approval

Many of the drugs currently used to treat life-threatening conditions, such as cancer, were approved through an accelerated FDA review process. In accelerated approval, FDA approves the drug on the condition that the applicant studies and reports findings of the clinical benefit of the drug. FDA continues to review new information and data about these drugs as the data becomes available. If the findings are negative, the appropriate actions are taken.

3.Center for biologics evaluation and research （CBER）

Regulating biologics

CBER's activities include:

- monitoring the pre-clinical and clinical testing of new biological products, and evaluating their safety and effectiveness before marketing.
- licensing biological products and manufacturing establishments, including blood banks.
- research on AIDS medications, diagnostic tests, and vaccines.
- compliance monitoring, lot releasing, and post-market surveillance.

CBER is organized into the Offices of Communication, Training, and Manufacturing Assistance; Blood Research and Review; Vaccines Research and Review; Cellular, Tissue, and Gene Therapies; and Compliance and Biologics Quality.

Approving a biologic

CBER staff reviews clinical research and laboratory testing data to determine if the biologic is safe and effective for its intended use. In order for a biological product to be approved for marketing in the US, an applicant must submit a Biologics License Application (BLA). The BLA must include information on the following:

- animal studies and human clinical trials performed.

- how the biologic is manufactured, processed, and packaged, including information on the quality control methods used during its manufacture.
- labeling that will be used with the product.

Once a biological product is approved, its identity (or make up) and manufacturing process cannot change without prior FDA approval.

Blood supply

Assuring the safety of, and the public confidence in, the nation's blood supply is one of CBER's main priorities. There are five overlapping safeguards in place to help protect the safety of blood.

1/. Quarantine of untested blood
Blood products may not be used until all test results are back.
2/. Donor screening:
A screener must ask donors about their health and risk factors.
3/. Donor deferral registries
Establishments must check donor names against a current list of deferred donors.
4/. Blood testing
Blood is tested for infectious disease.
5/. Investigation of problems
Establishments must investigate breeches of safeguards and correct any deficiencies.

4.Center for Devices and radiological health（CDRH）:

Medical devices

The FD&C Act defines a medical device as any healthcare product that does not achieve its principal intended purposes by chemical action or by being metabolized.

Under this definition, a "device" can be as simple as a tongue depressor or a thermometer, or as complex as a kidney dialysis machine. Medical devices are classified and regulated according to their degree of risk to the public.

Regulatory classes

Because each device is different, FDA establishes three different regulatory classes to ensure that each device is subject to regulations that are appropriate.

1/. General Controls

2/. Special Controls

3/. Premarket Approval

5.Center for veterinary medicine (CVM) : CVM's authority

CVM's authority is derived from the FD&C Act, which was amended in 1968 to include sections that specifically address animal drugs. These amendments were designed to ensure that animal drugs and medicated feed are safe and effective for their intended uses, and that they do not leave any unsafe drug residues in foods, such as eggs or milk.

In partnership with other federal and state agencies, CVM protects animal health and the safety of human food derived from animals. One of CVM's highest priorities is ensuring the safety of the food supply. This is accomplished primarily by preventing Bovine Spongiform Encephalopathy (i.e., BSE or mad cow disease), countering the risk of food-associated antibiotic resistance in humans, and ensuring the safety of food derived from genetically modified animals.

Requirements for animal drugs

CVM's staff is organized into major functional areas:

1/. Premarketing

2/. Investigational New Animal Drug exemption (INAD)

3/. New Animal Drug Application review (NADA)

4/. Post- marketing

5/. Research

6.National center for toxicological research (NCTR) : Strategic goals

NCTR's fundamental and applied research is designed to find the underlying biological issues that cause toxicity in products regulated by FDA. The Center's goals include:

- developing new strategies and methods to test/predict toxicity and assess/detect risk for FDA-regulated products, both new and those already

on the market.

- developing computer-based systems (knowledge bases) that predict human risk to enhance the efficiency and effectiveness of premarket product reviews.
- conducting research to understand mechanisms of toxicity, assess new product technology, and provide methods for use in FDA standards development and product risk surveillance.

How NCTR meets its goals

1/. Achieving the goals: by 8 research areas (biochemical toxicology, biometry and risk assessment, chemistry, neurotoxicology, molecular epidemiology, genetic and reproductive toxicology, microbiology, and veterinary services)

2/. Using new scientific technology: by 5 centers (Functional Genomics, Structural Genomics, Toxicoinfomatics, Phototoxicology, and Hepatoxicology)

What is ORA's mission? (The Office of Regulatory Affairs (ORA) is the lead office for FDA's field activities)

To ensure that FDA-regulated products comply with appropriate public health laws and regulations

Compliance

ORA's principal job is to survey and inspect regulated firms in order to assess their compliance with public health laws. ORA's compliance strategies include: providing information to industry; highlighting areas of significant violations and impact on public health; prioritizing and targeting high-risk areas; cooperating with state and local public health authorities and regulators; and focusing on covering products imported into the US through border coverage and foreign inspections.

ORA is responsible for the following:

- managing and operating the field offices.
- coordinating and managing all Agency field operations.
- providing advice and assistance on regulations and compliance policy matters that impact policy development, implementation, and long-range goals.
- working with additional federal agencies on issues of compliance and evaluating proposed legal actions.
- directing and conducting criminal investigative activities in coordination

with FDA headquarters units and other federal, state, and local law enforcement agencies.

Headquarters Offices

ORA Headquarters is composed of four offices. Each office has its own responsibilities, but they all work together to achieve ORA's mission. Click on each button below to learn more about the responsibilities of ORA's four individual offices.

1/. Office of Resource Management (ORM)

2/. Office of Regional Operation (ORO)

3/. Office of Enforcement (OE)

4/. Office of Criminal Investigation (OCI)

Field components 分區架構共分 5 區域

ORA's field staff is organized into five regions, each of which is headed by a Regional Food and Drug Director (RFDD).

1.The Pacific Region includes nine states: Montana, Idaho, Washington, Oregon, California, Nevada, Arizona, Alaska, Hawaii, there are three district offices and two regional labs in the Pacific Region 三個行政轄區兩個區域 lab

2.The Southwest Region: Lowa, Nebraska, Wyoming, Utah, Colorado, Kansas, Missouri, Arkansas, Oklahoma, Texas, and New Mexico are the states in the Southwest Region. This region includes three domestic district offices, the Southwest Import District (SWID)

3. The Southeast Region is comprised of eight states: North Carolina, South Carolina, Tennessee, Georgia, Alabama, Mississippi, Louisiana, and Florida. It also includes the islands of San Juan. There are four ditrict offices and a regional laboratory in the Southeast Region.

4. The Central Region: The states of New Jersey, Pennsylvania, Delaware, Maryland, Virginia, West Virginia, Kentucky, Ohio, Indiana, Illinois, Michigan, Wisconsin, Minnesota, North Dakota, and South Dakota are in the Central Region. The Central Region consists of seven district offices and the Forensic Chemistry Center.

5.The Northeast Region encompasses the states of Maine, New Hampshire, Vermont, New York, Massachusetts, Connecticut, and Rhode Island. It consists of

two district offices, a regional lab, and the Winchester Engineering and Analytical Center (WEAC).

Public cooperation

Each ORA district office has specific field personnel available to receive and handle calls from consumers and other stakeholders.

- Consumer Complaint Coordinators cover a specific geographic area. A list of these contact phone numbers can be found at:

  http://www.fda.gov/opacom/backgrounders/complain.html

- Public Affairs Specialists help answer a variety of questions and provide information about FDA. A listing of these people can be found at:

  http://www.fda.gov/ora/fed_state/dfsr_activities/fdapas.html

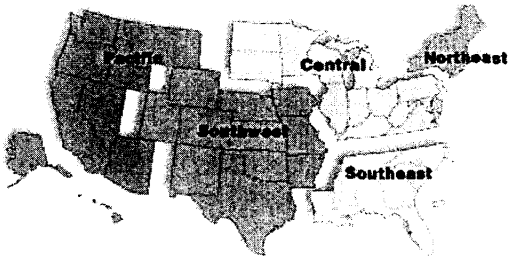
Small Business Representatives can be found in each ORA region. Small Business Representatives provide technical assistance to small companies, hold exchange meetings to hear the views and perspectives of small businesses, conduct educational workshops, develop informational materials, and provide an accessible, efficient channel through which small businesses can acquire information from the FDA. A contact list can be found at:

附　　件　　七

附件七、參訪 FDA 太平洋西北實驗室 (PRL-SW)) 相關資訊

Overview of the Office of Regulatory Affairs

Last Update: December 6, 2002



Organization Overview

The Office of Regulatory Affairs (ORA) is the lead office for all Field activities of the Food and Drug Administration. The map above shows the regions of the organization (note: the "Midwest" and "Midatlantic" regions were merged into a "Central Region".) The duties and functions of the Office and components of ORA can be viewed by clicking on the component name in the list below. The offices that report directly to ORA are shown here in UPPER CASE. Divisions within each office are in lower case. The letter-number combinations "HFC-xx" are mail routing symbols.

OFFICE OF REGULATORY AFFAIRS (HFC-1)

OFFICE OF RESOURCE MANAGEMENT (HFC-10)

- Division of Management Operations (HFC-20)
- Division of Information Systems (HFC-30)
- Division of Planning, Evaluation, & Management (HFC-40)
- Division of Human Resource Development (HFC-60)

OFFICE OF REGIONAL OPERATIONS (HFC-100)

- Division of Emergency & Investigational Operations (HFC-130)
- Division of Field Science (HFC-140)
- Division of Federal - State Relations (HFC-150)
- Division of Import Operations (HFC-170)

OFFICE OF ENFORCEMENT (HFC-200)

- Division of Compliance Management & Operations (HFC-210)
- Division of Compliance Policy HFC-230)
- Medical Product Quality Assurance Staff (HFC-240)

OFFICE OF CRIMINAL INVESTIGATIONS

REGIONAL & DISTRICT OFFICE CONTACTS

OFFICE OF REGULATORY AFFAIRS

a. Advises and assists the Commissioner and other key officials on regulations and compliance-oriented matters that have an impact on Policy development and execution, and long-range program goals.

b. Coordinates, interprets, and evaluates the Agency's overall compliance efforts; as necessary, establishes compliance policy or recommends policy to the Commissioner.

c. Stimulates an awareness within the Agency of the need for prompt and positive action to assure compliance by regulated industries; works to assure an effective and uniform balance between voluntary and regulatory compliance and Agency responsiveness to consumer needs.

d. Evaluates and coordinates all proposed legal actions to ascertain compliance with regulatory policy and enforcement objectives.

e. Executes direct line authority over all Agency field operations, develops, issues, approves, or clears proposals and instructions affecting field activities; serves as the central point within the Agency through which Headquarters offices obtain field support services.

f. Provides direction and counsel to Regional Food and Drug Directors in the implementation of policies and operational guidelines that form the framework for management of Agency field-activities.

g. Develops, and/or recommends to the Commissioner policy, programs, and plans for activities between the Agency and State and local agencies; administers the Agency's overall Federal-State program and policy, coordinates the program aspects of Agency contracts with State and local counterpart

agencies.

h. Evaluates the overall management and capabilities of the Agency's field organization; initiates action to improve the management of field activities and coordinates the formulation and management of career development plans.

i. Directs and coordinates the Agency's emergency preparedness and civil defense programs.

j. Operates the Federal Medical Products Quality Assurance Program for the Agency.

OFFICE OF RESOURCE MANAGEMENT (HFC-10)

a. Serves as the Agency lead office, in cooperation with the Office of Health Affairs,in initiating, coordinating, and offering specific regulatory bilateral agreements and Memoranda of Understanding (MOUS) to foreign countries.

b. Provides policy direction to other Agency components in the initiation, development, and recommendation of specific domestic regulatory bilateral agreements and MOUs with other governments.

c. Provides technical input for the Office of Regulatory Affairs quality assurance program as it pertains to assuring the consistency and adequacy of field investigational and inspectional operations.

d. Develops proposed overall field manpower allocations and long- and short-range operational program plans; identifies management data requirements for information systems; analyzes and evaluates field performance data and overall accomplishments.

e. Advises the Associate Commissioner and the Regional Food and

Drug Directors on all areas of management, including financial management, management analysis, and administrative operations.

f. Designs, develops, and manages the equal employment opportunity program and a comprehensive career development and training program for the Office of Regulatory Affairs Headquarters, field employees and State employees.

g. Develops and implements nationwide information storage and retrieval systems for data originating in the field offices.

Division of Management Operations (HFC-20)

Division of Information Systems (HFC-30)

Division of Planning, Evaluation, & Management (HFC-40)

Division of Human Resource Development (HFC-60)

OFFICE OF REGIONAL OPERATIONS (HFC-100)

a. Coordinates and manages all Agency field operations on behalf of the Associate Commissioner; develops, issues, approves, or clears proposals and instructions affecting field activities; serves as the central point within the Agency through which Headquarters offices obtain field support services.

b. Establishes field compliance and enforcement posture, based on Agency policy.

c. Develops and/or recommends to the Associate Commissioner policy, programs, and plans for activities between the Agency and State and local agencies; administers the

Agency's overall Federal-State program and policy; coordinates the program aspects of FDA contracts with State and local counterpart agencies.

d. Coordinates field consumer affairs and information programs; distributes timely information to the field; coordinates activities with Agency counterpart organizations.

e. Coordinates nationwide health fraud activities between the field, States, and Headquarters organizations.

f. Evaluates the overall management and capabilities of the Agency's field organization; initiates action to improve the management of field activities.

g. Serves as the Agency focal point in developing and maintaining international regulatory policy and activities to assure the safety, efficacy, and wholesomeness of various imported products. Coordinates Agency procedures with Headquarters and field offices and is the primary contact with U.S. Customs Service and other Federal agencies responsible for regulating imported products and assuring consistent programs among those offices.

h. Develops and/or recommends to the Associate Commissioner policy, program, and plans for applied research that relates to Agency enforcement problems and that will be conducted by field installations; coordinates such research efforts with appropriate agency components.

i. Directs and coordinates the Agency's emergency preparedness and civil defense programs.

j. Provides other Agency components with laboratory support in various highly specialized areas.

k. Recommends priorities for all field construction, repair, improvement, and renovation and recommends short- and

long-range field facility utilization plans.

Division of Emergency & Investigational Operations (HFC-130)

Division of Field Science (HFC-140)

Division of Federal - State Relations (HFC-150)

Division of Import Operations (HFC-170)

OFFICE OF ENFORCEMENT (HFC-200)

a. Advises and assists the Associate Commissioner and other key officials on regulations and compliance matters that have an impact on policy development, implementation, and long range program goals.

b. Coordinates, interprets, and evaluates the Agency's overall compliance efforts; as necessary, establishes compliance policy and recommends policy to the Associate Commissioner.

c. Stimulates an awareness within the Agency of the need for prompt and positive action to assure compliance by regulated industries; works to assure an effective and uniform balance between regulatory compliance and Agency responsiveness to consumer needs.

d. Acts as liaison with other Federal agencies on Agency compliance matters and encourages an effective and appropriate balance between voluntary and regulatory compliance.

e. Evaluates and coordinates proposed legal actions to ascertain compliance with regulatory policy and enforcement objectives.

f. Directs and coordinates with the Office of Regional

Operations (ORO), other Agency components, and Office of the Chief Counsel, new or novel cases which may be precedent setting.

g. Resolves appeals when proposed compliance actions are disapproved by the centers or the Office of the Chief Counsel.

h. Coordinates development of the Agency-wide bioresearch monitoring activities; monitors compliance activities to assure uniform application of compliance policy; serves as liaison with other Federal agencies and outside organizations relating to such Agency wide activates.

i. Serves as the Agency focal point for activities relating to the Federal Medical Products Quality Assurance Program and maintains liaison with other Government agencies procuring medical supplies; issues final administrative approval for quality assurance of specific products and firms.

Division of Compliance Management & Operations (HFC-210)

Division of Compliance Policy (HFC-230)

Medical Products Quality Assurance Staff (HFC-240)

Office of Criminal Investigations (HFC-300)

a. Advises and assists the Associate Commissioner and other key officials on regulations and criminal matters that affect the Agency.

b. Directs, plans, and develops criminal investigation activities in coordination with other Agency components and with other Federal, State, and local law enforcement agencies.

c. Develops, coordinates, and implements Agency policy related to criminal investigations.

d. Initiates and conducts criminal investigations under all statutes administered by the Food and Drug Administration, through area offices located throughout the United States; coordinates assignments involving undercover and surveillance personnel and activities.

e. Assures coordination of criminal investigation activities with FDA Regional Field Offices and District Offices and adherence to Agency's enforcement priorities; develops and maintains cooperative relationships with field and Headquarters components.

f. Provides recommendations to the Office of Chief Counsel on referrals of criminal cases to the Department of Justice for further investigation and/or prosecution, or directly to the U.S. Attorney when such direct reference is authorized.

g. Develops automated data processing systems to be used for criminal investigations and related enforcement matters.

h. Develops, reviews, and approves training programs for FDA's criminal investigators and related personnel.

i. Participates in Grand Jury investigations and serves as agents of the Grand Jury.

REGIONAL & DISTRICT OFFICE CONTACTS

Contents

HEADQUARTERS

ORA FIELD DIRECTORY

NORTHEAST REGION	**CENTRAL REGION**
New York Regional Office	Philadelphia Regional Office
New England Regional Office	Chicago Regional Office
Northeast Regional Lab	Forensic Chemistry Center
Winchester Engineering and Analytical Center	Philadelphia District
New England District	Baltimore District
New York District	Cincinnati District
New York City Office	New Jersey District
	Chicago District
	Detroit District
	Minneapolis District
SOUTHEAST REGION	**SOUTHWEST REGION**
Atlanta Regional Office	Dallas Regional Office
Southeast Regional Laboratory	Kansas City Regional Office
Atlanta District	Arkansas Regional Laboratory
New Orleans District	Dallas District
Florida District	Denver District
San Juan District	Kansas City District
	Southwest Import District
PACIFIC REGION	
San Francisco Regional Office	
Seattle Regional Office	
Pacific Regional Laboratory Northwest	
Pacific Regional Laboratory Southwest	
Los Angeles District	

San Francisco District Seattle District Office	

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SUBCHAPTER 800 - RECALLS

801 - DEFINITIONS

801.01 - Recall

A Recall is a firm's removal or correction of a marketed product that FDA considers to be in violation of the laws it administers, and against which the Agency would initiate legal action (e.g., seizure). Recall does not include a market withdrawal or a stock recovery. See the Agency recall policy outlined in 21 CFR 7.1/7.59 - Enforcement Policy - General Provisions, Recalls (Including Product Corrections) - Guidance on Policy, Procedures, and Industry Responsibilities.

Recall Classification - Means the numerical designation, i.e., I, II, or III, assigned by the FDA to a particular product recall to indicate the relative degree of health hazard presented by the product being recalled.

There are three possible classifications.

Class I - A situation in which there is a reasonable probability that the use of, or exposure to, a violative product will cause serious adverse health consequences or death.

Class II - A situation in which use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote.

Class III - A situation in which use of, or exposure to, a violative product is not likely to cause adverse health consequences.

Recall Type - A designation based on whether the recall is Voluntary, FDA Requested (at the request of the Commissioner or his designee), or ordered under section 518(e) of the FD & C Act [21 U.S.C 360h (e)].

Recall Strategy - A planned specific course of action to be taken in conducting a specific recall, which addresses the depth of recall, need for public warnings, and extent of effectiveness checks for the recall.

Depth of Recall - Depending on the product's degree of hazard and extent of distribution, the recall strategy will specify the level in the distribution chain to which the recall is to extend, i.e., wholesaler, retailer, user/consumer.

Recall Number - Number assigned by a responsible Center for each recalled product they initiate. This number consists first of a letter designating the responsible Center (see letter Codes below), a 3-digit sequential number indicating the number of recalls initiated by that Center during the fiscal year, and a 1-digit number (the Center for Devices and Radiological Health (CDRH) uses 2-digit numbers) indicating the fiscal year the recall was initiated. For example: F-100-2 identifies the 100th recall initiated by the Center for Food Safety and Applied Nutrition (CFSAN) in FY-2002. The following letters are used to identify the Centers.

Letter	Center/Office
F	Foods - CFSAN
D	Drugs - Center for Drug Evaluation and Research (CDER)
Z	Medical Devices & Radiological Health - CDRH
V	Veterinary Medicine - Center for Veterinary Medicine (CVM)
B	Biologics - Center for Biologics Evaluation and Research (CBER)
N	Medical Devices (Voluntary Safety Alerts & Notifications)
A	Audit Numbers issued by the District performing the recall, the Centers, Office of Enforcement (Division of Compliance Management and Operations [DCMO], or the Division of Field Investigations (DFI) to monitor recalls requiring audit checks.

CHAPTER 9 - INVESTIGATIONS

CONTENTS

CHAPTER 10 - REFERENCE MATERIALS

SUBCHAPTER 1000 - LAW, REGULATION AND GUIDANCE

This chapter will help you to locate regulatory references and FDA staff.

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Bioterrorism Act),the Medical Device User Fee and Modernization Act of 2002 (MDUFMA), the FDA Modernization Act of 1997, (FDAMA), the International Conference on Harmonization (ICH), the Mutual Recognition Agreement (MRA), national emergencies and initiatives, and other forces continue to impact FDA inspectional operations as changes in law, regulation, guidance and internal procedures issue. As ICH members (Japan, U.S. and European Union) reach consensus agreements, ICH guidelines are adopted by all three governments. In the United States, they may replace outstanding FDA guidance in the medical device, human and animal drug areas. Unless exempted, the Bioterrorism Act and implementing regulations require most domestic food facilities and foreign food facilities who export to the U.S. to register as of December 12, 2003; FDA began accepting registrations on October 16, 2003. The Bioterrorism Act requires that FDA receive prior notice of food imported into the United States, beginning on December 12, 2003. The 2002 MDUFMA authorizes FDA to charge user fees for medical device premarket review; it allows third party medical device inspections, sets out new regulatory requirements for single-use devices, and directs FDA to establish the Office of Combination Products. FDA drug GMP initiative and Process Analytical Technology (PAT) efforts are underway.

In conducting inspections and investigations according to changing policies, in order to be effective, FDA regulators must understand the difference between regulatory requirements and guidance.

Laws or statutes, enacted by Congress, and regulations or rules, promulgated by Federal agencies, contain regulatory requirements.

FDA's guidance documents, on the other hand, have a different legal status and serve purposes different from laws and regulations. The purposes of guidance documents are to:

1. Provide assistance to the regulated industry by clarifying requirements that have been imposed by Congress or issued in regulations by FDA, and by explaining how industry may comply with those statutory and regulatory requirements, and

2. Provide specific review and enforcement approaches to help ensure that FDA's employees implement the agency's mandate in an effective, fair, and consistent manner.

FOOD ADDITIVE STATUS LIST

FOREWORD

This Food Additives Status List organizes additives found in many parts of 21 CFR into one alphabetized list. Additives included are those specified in the regulations promulgated under the FD&C Act, under Sections 401 (Food Standards), and 409 (Food Additives). The list also includes selected pesticide chemicals from 40 CFR 180 for which EPA has set tolerances in food. FDA enforces those tolerances. Within the space available, the Food Additives Status List includes use limitations and permitted tolerances for each additive. For complete information on its use limitations, refer to the specific regulation for each substance. To access 21 CFR, see http://www.access.gpo.gov/nara/cfr/index.html. To access 40 CFR 180, "Tolerances and Exemptions from Tolerances for Pesticide Chemicals in Food", see http://www.access.gpo.gov/nara/cfr/waisidx_01/40cfr180_01.html. New regulations and revisions are published in current issues of the Federal Register as promulgated. To access the Federal Register, see http://www.access.gpo.gov/su_docs/aces/aces140.html. Also refer to the CFSAN website on Food Additives and Premarket Approval to review several FDA databases of additive categories. See http://www.cfsan.fda.gov/~lrd/foodadd.html. For example, EAFUS (Everything Added to Food in the United States) is a helpful reference within the limitations described at the beginning of the database.

The Food Additive Status List omits certain categories of additives. Here are the omissions:

1. Obviously safe substances not cited in a regulation as Generally Recognized as Safe (GRAS). You may find such substances on an FDA web site, see http://www.cfsan.fda.gov/~rdb/opa-gras.html. It contains GRAS notifications received from companies since 1998, and FDA' s response.
2. Synthetic flavoring substances in 21CFR 172.515. The CFR does not contain a complete list of permissible flavorings. Certain trade groups such as the Flavor Extract Manufacturers Association have established expert panels to evaluate and make determinations on the GRAS status of their products. If you need help in determining the acceptability of a flavoring after consulting 21 CFR 172.515, contact CFSAN Office of Food Additive Safety (HFS-200) at (202) 418-3100.
3. Those pending administrative determination.
4. Substances granted prior sanction for specific use prior to enactment of the Food Additives Amendment. For additional information on these substances, contact the CFSAN Office of Food Additive Safety (HFS-200) at (202) 418-3100.
5. Indirect food additives, 21 CFR Parts 175, 176, 177, & Part 178 (except that sanitizing agents for food processing equipment as listed in 178.1010 are included in the Food Additives list.) Be aware that as a result of the Food Quality Protection Act of 1996 and Antimicrobial Regulation Technical Corrections Act of 1998, EPA now has jurisdiction over sanitizing solutions applied to permanent or semi-permanent food contact surfaces, other than food packaging.

To look up indirect food additives in Parts 175, 176, 177 and 178 go to FDA's "List of Indirect Additives Used in Food Contact Substances" See http://www.cfsan.fda.gov/~dms/opa-indt.html. Use it to locate the regulation in which its use is fully described.

FDA has recently implemented a new way to market, called "Premarket Notification", for certain food contact substances. These notifications are effective only for the manufacturer or supplier identified in the notification. A list of effective notifications is available on the FDA website. See http://www.cfsan.fda.gov/~dms/opa-fcn.html.

6. Color additives, 21 CFR Parts 70, 71, 73, 74, 80 & 82. Go to the Color Additives Status List following the Food Additives Status list in Appendix A.

NOTE: The Food Additives Status List is provided only as a quick look-up on the use limitations for a food additive or pesticide chemical. It is possible that mistakes or omissions could have occurred. Additionally, there may be cases where the agency has offered interpretations concerning specific provisions of the regulations. For example, in the case of boiler water additives or other minor ingredients, processing aids, or indirect additives, FDA has not objected, in certain cases, to the substitution of ammonium, calcium, magnesium, potassium, or sodium salts for each other when only one is listed in a regulation. The Food Additive Status list is updated annually, so it may not reflect the latest information. For all these reasons, take care before advising a firm that a use of a particular food additive is prohibited or otherwise limited. Read the actual regulation. If there are any doubts or if a particular situation is unclear, you or your supervisor should consult with the CFSAN, Office of Food Additive Safety (HFS-200) at (202) 418-3100, or the Division of Petition Review (HFS-265) at (202) 418-3042, or the Division of Food Contact Substance Notification Review HFS-275 at (202) 418-3080, or the Division of Biotechnology and GRAS Notice Review HFS-255 at (202) 418-3090.

Please send corrections or additions to the list, to Alan Gion FDA/Division of Field Investigations (DFI) (HFC-130), 5600 Fishers Lane, Rockville, Maryland 20857 or e-mail them to IOM@ORA.FDA.GOV.

ABBREVIATIONS USED

Type	(kind, effect or use of additive)
AC	- Anticaking agent
AF	- Antifoaming (or defoaming) agent
AOX	- Antioxidant
BC	- Boiler compound
BL	- Bleaching agent or flour-maturing agent
B&N	- Buffer and neutralizing agent
CTG	- Component or coating for fruits & vegetables
DS	- Dietary supplement
EMUL	- Emulsifier
ENZ	- Enzyme
ESO	- Essential oil and/or oleoresin (solvent free)
FEED	- Substances under the Food Additives Amendment added directly to feed
FLAV	- Natural flavoring agent
FL/ADJ	- Substance used in conjunction with flavors
FUM	- Fumigant
FUNG	- Fungicide
HERB	- Herbicide
HOR	- Hormone
INH	- Inhibitor
MISC	- Miscellaneous
NAT	- Natural substances and extractives
NNS	- Non-nutritive sweetener
NUTR	- Nutrient
NUTRS	- Nutritive Sweetener
PEST	- Pesticide other than fumigant
PRES	- Chemical preservative
SANI	- Sanitizing agent for food processing equipment
SDA	- Solubilizing and dispersing agent
SEQ	- Sequestrant
SOLV	- Solvent
SP	- Spices, other natural seasonings & flavorings
SP/ADJ	- Spray adjuvant
STAB	- Stabilizer
SY/FL	- Synthetic flavor
VET	- Veterinary drug, which may leave residue in edible tissues of animals or in edible animal products

Status

BAN	- Substances banned prior to the Food Additives Amendment (FAA) because of toxicity. These substances are bolded and italicized.
FS	- Substance permitted as optional ingredient in a standardized food
GRAS	- Generally recognized as safe. Substances in this category are by definition, under SEC. 201(s) of the FD&C Act, not food additives. Most GRAS substances have no quantitative restrictions as to use, although their use must conform to good manufacturing practices. Some GRAS substances, such as sodium benzoate, do have a quantitative limit for use in foods.
GRAS/FS	- Substances generally recognized as safe in foods but limited in standardized foods where the standard provides for its use.
ILL	- Substances used or proposed for use as direct additives in foods without required clearance under the FAA. Their use is illegal. These substances are bolded and italicized.
PD	- Substance for which a petition has been filed but denied because of lack of proof of safety. Substances in this category are illegal and may not be used in foods.
PS	- Substance for which prior sanction has been granted by FDA for specific uses. There are a number of substances in this category not listed herein because they have not been published in the FEDERAL REGISTER.
REG	- Food additive for which a petition has been filed and a regulation issued.
REG/FS	- Food additive regulated under the FAA and included in a specific food standard.

Other

&	- and
amt	- amount
art	- artificially
avg	- average
ca	- about, approximately
calc	- calculated
CFR	- Code of Federal Regulations
cnd	- canned
cond	- conditions
comb.	- w/ in combination with; combined with
comp	- component
ctg	- coating for fruits, vegetables, tablets
do	- Same CFR reference as appears earlier in paragraph
dr	- dried
F.R.	- Federal Register
g	- gram(s)
GMP	- In accordance with good manufacturing practices; or sufficient for purpose; or quantity not greater than required
incl	- including
mfr	- manufacture
mg	- milligram(s)
min	- mineral
ml	- milliliter

21 CFR - SELECTED PARTS

PART 7, Subpart C - Recalls (Including Product Corrections) - Guidance on Policy, Procedures, and Industry Responsibilities

SOURCE: 43 FR 26218, June 16, 1978, unless otherwise noted.

7.40 Recall policy.

(a) Recall is an effective method of removing or correcting consumer products that are in violation of laws administered by the Food and Drug Administration. Recall is a voluntary action that takes place because manufacturers and distributors carry out their responsibility to protect the public health and well-being from products that present a risk of injury or gross deception or are otherwise defective. This section and 7.41 through 7.59 recognize the voluntary nature of recall by providing guidance so that responsible firms may effectively discharge their recall responsibilities. These sections also recognize that recall is an alternative to a Food and Drug Administration-initiated court action for removing or correcting violative, distributed products by setting forth specific recall procedures for the Food and Drug Administration to monitor recalls and assess the adequacy of a firm's efforts in recall.

(b) Recall may be undertaken voluntarily and at any time by manufacturers and distributors, or at the request of the Food and Drug Administration. A request by the Food and Drug Administration that a firm recall a product is reserved for urgent situations and is to be directed to the firm that has primary responsibility for the manufacture and marketing of the product that is to be recalled.

(c) Recall is generally more appropriate and affords better protection for consumers than seizure, when many lots of product have been widely distributed.

Seizure, multiple seizure, or other court action is indicated when a firm refuses to undertake a recall requested by the Food and Drug Administration, or where the agency has reason to believe that a recall would not be effective, determines that a recall is ineffective, or discovers that a violation is continuing.

[43CFR26218, June 16, 1978, as amended at 65 FR 56476, Sept. 19, 2000]

7.41 Health hazard evaluation and recall classification.

(a) An evaluation of the health hazard presented by a product being recalled or considered for recall will be conducted by an ad hoc committee of Food and Drug Administration scientists and will take into account, but need not be limited to, the following factors:

(1) Whether any disease or injuries have already occurred from the use of the product.

(2) Whether any existing conditions could contribute to a clinical situation that could expose humans or animals to a health hazard. Any conclusion shall be supported as completely as possible by scientific documentation and/or statements that the conclusion is the opinion of the individual(s) making the health hazard determination.

(3) Assessment of hazard to various segments of the population, e.g., children, surgical patients, pets, livestock, etc., who are expected to be exposed to the product being considered, with particular attention paid to the hazard to those individuals who may be at greatest risk.

(4) Assessment of the degree of seriousness of the health hazard to which the populations at risk would be exposed.

(5) Assessment of the likelihood of occurrence of the hazard.

(6) Assessment of the consequences (immediate or long-range) of occurrence of the hazard.

(b) On the basis of this determination, the Food and Drug Administration will assign the recall a classification, i.e., Class I, Class II, or Class III, to indicate the relative degree of health hazard of the product being recalled or considered for recall.

7.42 Recall strategy.

(a) General.

(1) A recall strategy that takes into account the following factors will be developed by the agency for a Food and Drug Administration-requested recall and by the recalling firm for a firm-initiated recall to suit the individual circumstances of the particular recall:

(i) Results of health hazard evaluation.

(ii) Ease in identifying the product.

(iii) Degree to which the product's deficiency is obvious to the consumer or user.

(iv) Degree to which the product remains unused in the market place.

(v) Continued availability of essential products.

(2) The Food and Drug Administration will review the adequacy of a proposed recall strategy developed by a recalling firm and recommend changes as appropriate. A recalling firm should conduct the recall in accordance with an approved recall strategy but need not delay initiation of a recall pending review of its recall strategy.

(b) Elements of a recall strategy. A recall strategy will address the following elements regarding the conduct of the recall:

(1) Depth of recall. Depending on the product's degree of hazard and extent of distribution, the recall strategy will specify the level in the distribution chain to which the recall is to extend, as follows:

(i) Consumer or user level, which may vary with product, including any intermediate wholesale or retail level; or

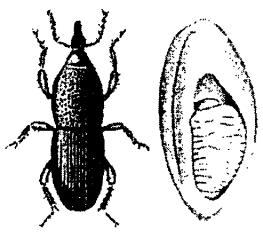
(ii) Retail level, including any intermediate wholesale level; or

(iii) Wholesale level.

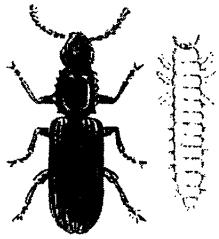
(2) Public warning. The purpose of a public warning is to alert the public that a product being recalled presents a serious hazard to health. It is reserved for urgent situations where other means for preventing use of the recalled product appear inadequate. The Food and Drug Administration in consultation with the recalling firm will ordinarily issue such publicity. The recalling firm that decides to issue its

PRINCIPAL STORED GRAIN INSECTS

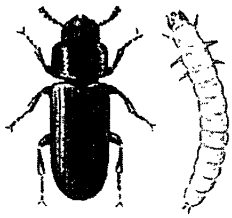
For safe and effective use of insecticides, always identify the problem correctly.



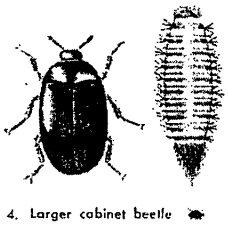
1. Granary weevil



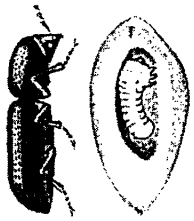
2. Saw-toothed grain beetle



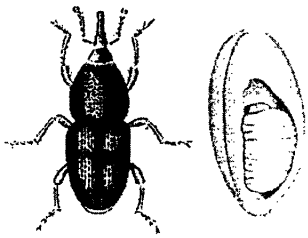
3. Red flour beetle



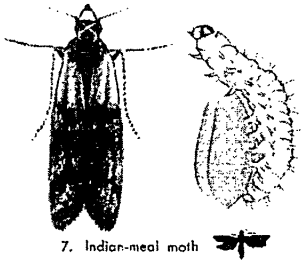
4. Larger cabinet beetle



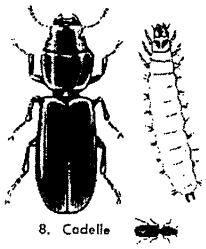
5. Lesser grain borer



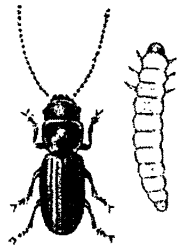
6. Rice weevil



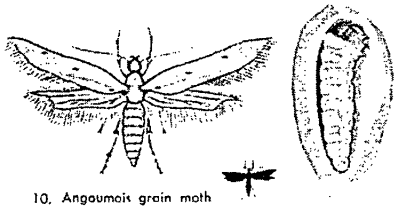
7. Indian-meal moth



8. Cadelle



9. Flat grain beetle



10. Angoumois grain moth

Some of these stored grain insects are also KITCHEN PESTS.

The saw-toothed grain beetle, red flour beetle, larger cabinet beetle, and Indian-meal moth develop in flour, cake mixes, corn meal, breakfast foods and similar products. The Angoumois grain moth infests popcorn.

Prepared by Extension Entomologists of the North Central States in cooperation with the Federal Extension Service, U. S. Department of Agriculture

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PRINCIPAL RESEARCH INTERESTS

- *Clostridium botulinum*: Control, Toxins, Genetics
- Use of Naturally Occurring Antimicrobials in Food

EDUCATION

- BS 1976, Fermentation Science, University of California, Davis
- MS 1978, Food Science, University of California, Davis
- ScD 1983, Food Microbiology, Massachusetts Institute of Technology
- Postdoc, 1985, Bacterial Physiology and Genetics, Harvard Medical School

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CURRENT RESEARCH PROJECTS

- Interspecies transfer of the gene coding for botulinum toxins.
- Regulation of expression of botulinum toxins.
- Purification, stabilization, and characterization of botulinum toxins.
- Development of botulinum toxin as a pharmaceutical.
- Behavior and control of *Clostridium botulinum* in foods.
- Assessment of the safety of new food processing procedures, e.g., modified atmosphere packaging, on safety from botulism.
- Characterization and application in foods of naturally occurring antimicrobials, including lactoperoxidase, lactoferrin, monoglycerides, polyacetylenes, lysozyme, and other lytic enzymes.
- Behavior and control of *Listeria monocytogenes* in foods.
- Control of pathogens in reduced fat cheeses.

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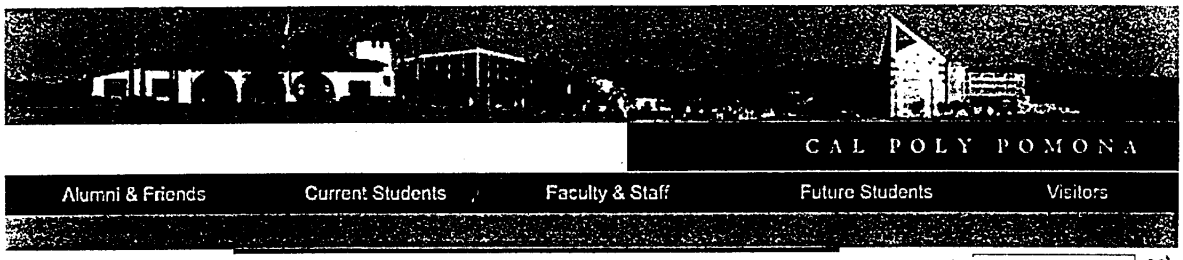
附　　件　　十

附件十、加州州立 POMONA 科技大學網路資訊

Cal Poly Pomona



Academics
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Why Cal Poly Pomona

BRONCODIRECT

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Campus Map
BroncoFusion
Supporting Cal Poly Pomona
Streaming Video
University Library

Budget 04/05

August 28, 2004

U.S. News & World Report Ranks Cal Poly Pomona Fifth in Public Universities in the West

Cal Poly Pomona ranked among the top universities in the West in the newly released 2005 edition of "America's Best Colleges" by *U.S. News & World Report*. The university placed fifth on the list of "Top Public Universities-Master's" in the Western United States, which includes all public schools whose highest degrees are bachelor's or master's. Read more...

Summer Bridge Program Celebrates 20th Year of Helping Students Prepare for College

Julio Molina moved from Mexico to the United States just five years ago, and when it came time to attend college he realized he still lacked some of the skills essential to having a successful college experience. To better prepare himself for his first year, Molina took part in the 2004 Summer Bridge Program at Cal Poly Pomona. Read more...

History of Cal Poly Pomona

Cal Poly Pomona opened in the fall of 1938 as the Voorhis Unit of the California Polytechnic School, with an all-male enrollment of 110 students. The campus was located on the 150-acre site of the former Voorhis School for Boys in San Dimas.

In 1949, breakfast cereal magnate W.K.Kellogg deeded 813 acres of land located three miles south of the Voorhis campus to the State of California. In 1956, 550 students and 30 faculty members moved to the Kellogg campus. The student body included women for the first time in 1961, when 322 women enrolled.

In 1966, Cal Poly Pomona separated from the San Luis Obispo campus to become California's 16th state college. University status was granted in 1972.

Today, the campus covers about 1,438 acres and is the second largest in area of the California State University's 20 campuses. More than 2,300 people are employed as members of the university's faculty and staff.

The university has been served by five Presidents in its 52-year history. Julian A. McPhee served as president of Cal Poly San Luis Obispo and Cal Poly Pomona from 1938 to 1966. Robert C. Kramer held the office from 1966 to 1977; and Hugh O. La Bounty served from 1978 to 1991. Bob H. Suzuki, held the office from 1991 to 2003. J. Michael Ortiz assumed the president's office on August 1, 2003.

Historical Milestones:

1925 Initial 377 acres purchased for $250,000 by W. K. Kellogg

1926 The W. K. Kellogg Arabian Horse ranch opened to the public.

1930 The W. K. Kellogg Foundation established.

1938 The Voorhis School for Boys becomes the Southern California branch of California Polytechnic College, San Luis Obispo, and is eventually donated to the state by Charles Brown Voorhis. Julian A. McPhee serves as president of Cal Poly San Luis Obispo and Cal Poly Pomona.

1943 Kellogg Ranch is temporarily transferred to the War Department during World War II, serving as a remount station where soldiers were trained in horsemanship.

1949 Breakfast cereal magnate W. K. Kellogg deeds his 813-acre ranch to the State of California for use as an expansion of the San Luis Obispo campus, California State Polytechnic College, Kellogg Unit.

1949 First student-built float entered in Tournament of Roses parade.

1956 550 students and 30 faculty members move to the Kellogg campus.

1957 California State Polytechnic College, Pomona's first graduating class, spring 1957

1961 In a first for the all-male campus, 329 women join the student body.

1966 The Pomona campus separates from the San Luis Obispo campus to become California State Polytechnic College, Kellogg-Voorhis, the 16th college in the California State College system.

1972 University status granted as California State Polytechnic University, Pomona.

Other links of Interest

Historical photographs from the Cal Poly Pomona History Exhibit

The Voorhis Connection

Cal Poly Pomona		About Cal Poly Pomona	
Welcome from President Ortiz		Campus Issues	
Academic Programs	History of Cal Poly Pomona	Contacting the University	Facts and Stats
Applying to the University	Location - How to get here	Tours and Visitor Services	University Catalog

University Library - Cal Poly Pomona
University Archives

Cal Poly Pomona History Exhibit

INTRODUCTION

This exhibit features many of the interesting events related to the founding and development of Cal Poly Pomona. The photographs have been printed from historic negatives kept in the University Archives and the W.K. Kellogg Arabian Horse Library.

The Cal Poly Pomona campus grounds and some of its buildings were once part of an Arabian horse ranch belonging to breakfast cereal pioneer Will Keith Kellogg. W.K. Kellogg was 64 when he took a leisurely trip from his home in Battle Creek, Michigan to Palm Springs, California in November 1924 for some resting, reading and sunbathing. A visit to Chauncey D. Clarkes Point Happy Ranch in Indio ultimately led Mr. Kellogg in 1925 to purchase a group of Arabian horses from the Clarke ranch. Mr. Kellogg then bought acreage in Pomona on which to build a ranch. While the stables were under construction, the horses were boarded at the Los Angeles County Fairgrounds.

For more information on the history of the Kellogg Arabians and the Kellogg Ranch, consult the following materials:

The Kellogg Arabian Ranch: The First Sixty Years: A Chronicle of Events, 1925-1985 by Mary Jane Parkinson

The Kellogg Arabians: Their Background and Influence by Herbert H. Reese in collaboration with Gladys Brown Edwards.

University Library - Cal Poly Pomona
University Archives

Cal Poly Pomona History Exhibit

Photo selection and narrative by Danette Cook Adamson
Web production by Ivano Aviandi

INTRODUCTION

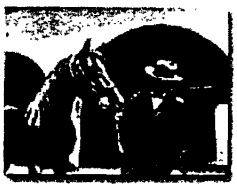
HISTORICAL PHOTOGRAPHS:

Part I - Part II - Part III

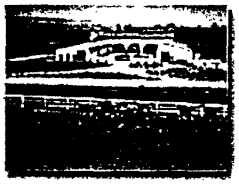
Historical Photographs: Part I

Each image below links to a larger version of the photograph and additional descriptive text.

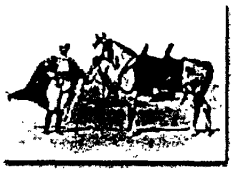
1926 - Killah, Sotamm, Sherlet, and Amham running in the hill pasture to the west of the original horse stables site.



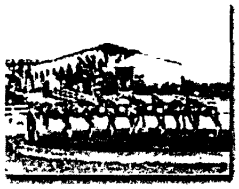
1929 - W.K. Kellogg with Antez.



1926 - Looking east at the original horse stables.



1926 - Valentino and Jadaan.



c. 1934 - The Liberty Drill
with trainer Mark Smith.

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Cal Poly Pomona History Exhibit

Historical Photographs: Part II

Each image below links to a larger version of the photographs and more text.

May 17, 1932 - Kellogg Ranch Presentation Ceremony.

June 7, 1946 - Decoration ceremony for Major Clayton

Voorhis Chapel in San Dimas.

1950 - Dedication of Voorhis Rock.

1955 - Voorhis College Men.

Introduction - Part I - Part III
Main Page

University Library - Cal Poly Pomona
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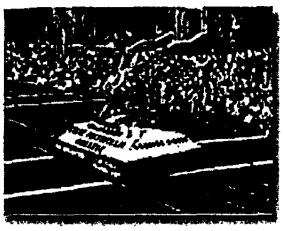
Cal Poly Pomona History Exhibit

Historical Photographs: Part III

Each image below links to a larger version of the photographs and more text.



January 1, 1949 - Cal Poly's First Rose Float

c1962- Graduation in the Rose Garden

1960 's - Women in Dormitory room

October 24, 1967 - President Robert Kramer Inaugurated

December 1968 - Library Moves to New Building

Introduction - Part I - Part II
Main Page

Welcome to the Home Page of Dr. Wei-Jen Lin

California State Polytechnic University, Pomona, CA

Classes	MIC 201 Basic Microbiology MIC301 Germs and You BIO 560 Bacterial Physiology BIO 499 Pharmaceutical Lab Practices BIO 499 Bioinformatics My Office Hour
Biotechnology Internship	General information and job posting Information for students Internship resources for students Career service resources Industry resources
About myself	Research interest Current research projects Curriculum Vitae Office Hour
Web resource	Literature Search On-line Journals Funding resource

Cal Poly Home	Biology Home	Graduate Program	Cal Poly Foundation	Cal Poly Library

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附件：林維真博士簡歷（共三頁）

Wei-Jen Lin, Ph.D

Cal Poly Pomona.

http://www.csupomona.edu/~weijenlin/

Research Interest

My research area is primarily focus on the molecular mechanisms of bacteria in the pathogenesis, spread, and the host response. Including protein-protein interaction of bacterial toxins, cross-species transfer of pathogenic genes, controls of bacterial infection and intoxication, food safety and host immune defense. Bacterial gene expression system with focus on promoter function and reporter gene activity.

Current Research Projects

1. *Clostridium botulinum* neurotoxins

   [for more background information, click botulinumweb]

   - Protein-protein interaction in the toxin complex
   - Application in clinical treatment of neuromuscular disorders

     [interesting link: Botox]
   - Gene regulation in *C. botulinum*

2. Avian botulism of California brown pelicans at Salton Sea [for more info, click Salton Sea]

   - Investigation of the correlation of intestinal microflora and avian botulism

3. Antimicrobial activity of probiotic bacteria [see probiotic articles]

   - Characterization of the antimicrobial substances
   - Heat and acid tolerance of formulated probiotic bacteria
   - DNA fingerprinting of probiotic bacteria

Curriculum Vitae

Education

Post-Doctoral and NIH-National Research Service Award fellow
Brigham and Women's Hospital and Harvard Medical School
Ph.D. University of Wisconsin - Madison
M.S. University of Minnesota, Twin City
B.S. National Taiwan University, Taipei, Taiwan

Professional Experience

2000-present
Assistant Professor of Microbiology
Department of Biological Sciences
California State Polytechnic University, Pomona
1998-2000
Scientist, Neurotoxin Research Program, Allergan, Inc. Irvine, CA.
Studied recombinant botulinum neurotoxin and its clinical use in treating neuromuscular disorders.
1995-1998
Scientist, Division of Immunology and Cancer Biology, Gilead Sciences (Formerly NeXstar Pharmaceuticals and Supragen Inc.), Lakewood, CO.
Studied bacterial superantigens and their role in autoimmune diseases.
1992-1994
NIH National Research Service Award Fellow, Channing Laboratory for Infectious Disease, Brigham and Women's Hospital, and Department of Medicine, Harvard Medical School.
Studied the regulation of late gene expression of *Staphylococcal* superantigens.
1988-1992
Research Assistant, University of Wisconsin-Madison.
Studied the regulation of botulinum neurotoxin in *Clostridium botulinum* with the development of various genetic tools.
1985-1988
Research Assistant, University of Minnesota, Twin City.
Studied the synergistic growth and the ß-galactosidase activity in a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Professional Activities

InterAgency Botulism Research Coordinating Committee (IBRCC)
American Society for Microbiology

Publications

1. Lin, W.-J., D. A. Savaiano, and S. K. Harlander. 1988. A method for determining ß-galactosidase activity of yogurt cultures in skim milk. J. Dairy Sci. 72:351-359.
2. Martini, M. C., E. C. Lerebours, W.-J. Lin, S. K. Harlander, N. M. Berrada, J. M. Antoine, and D. A. Savaiano. 1991. The effect of lactic acid bacterial strains and species in fermented milk (yogurt) on in vivo lactose digestion. Amer. J. Clinic Nutrition, 45:1041-1046.
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7. Johnson, E. A., W.-J. Lin, Y. T. Zhou, and M. Bradshaw. 1997. Characterization of neurotoxin mutants in *Clostridium botulinum*. type A. Clin. Infect. Dis. 25 (Suppl. 2):S168-S170.
8. Liu, C.-P., K. Jiang, C.-H. Wu, W.-H. Lee, and W.-J. Lin. 2000. Detection of glutamic acid decarboxylase (GAD) activated T cells with I-Ag7 tetramers. Proc. Natl. Acad. Sci. USA. PNAS, 97(26):14596-14601.
9. Lin, W.-J., D. A. Norris, M. Troetsch, B. L. Kotzin, and B. Tomkinson. 2001. Oligoclonal expansion of intraepidermal T cells in psoriasis skin lesions. Journal of Investigative Dermatology, 117:1546-1553.
10. Zhang, L., W.-J. Lin, S. Li, K. R. Aoki. 2003. Complete DNA sequences of the botulinum neurotoxin complex of *Clostridium botulinum* type A Hall strain. Gene 315:21-32.

Things that I do at spare time (if any!)

Most likely I will be driving my kids to various activities and volunteering for school and community activities

Things that I enjoy but have no time to do..... gardening, music, reading novel, etc.

Contact me at:
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California State Polytechnic University
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(909) 869-4078 (FAX)

附　　件　　十一

附件十一、新澳爾良市（New Orleans）簡介

Visitors Guide

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簡介新澳爾良市

http://www.geocities.co.jp/SilkRoad-Desert/2526/Cities/NewOrleans.html

美國路易士安那州新澳爾良市位於路易士安那州（Louisiana, LA）東南部的新澳爾良市（New Orleans）為美國第一大港，也是本州最大的城市。市區位在密西西比河三角洲，北臨龐恰特雷恩湖 (Lake Pontchartrain)，水道縱橫，地勢低窪，平均海拔僅 1.5 米，不少地方更低於海平面，沿河築有 209 公里長的防洪堤壩，由 112 個抽水站組成的排水系統，通過泄水道分水引入龐恰特雷恩湖。50 萬人口中，約一半為黑人。大都會區包括奧爾良、傑弗遜等 4 縣，面積 7661 平方公里，人口約占全州人口 30%。本地地處亞熱帶濕潤氣候，7 月平均氣溫 27.7□，1 月 11.6□，年降水量 1440 毫米，夏季多暴雨。城市鄰近地區石油、天然氣、硫磺、鹽礦豐富，盛產木材和棉花、甘蔗、稻米等。

此地原為印第安人居留地。1682 年法國探險家溯密西西比河航行至此，1718 年始建城，1722 年為法屬大路易士安那首府。1762 年後歸屬西班牙，1800 年複歸法國，1803 年隨同法屬大路易士安那賣給美國，1805 年正式設市。19 世紀上半葉成為重要棉花輸出港和黑奴貿易中心，曾兩度為路易士安那州首府。1840 年人口已逾 10 萬，居全國第四位。19 世紀中葉後，由於鐵路運輸競爭，黃熱病流行以及南北戰爭等因素，城市發展停滯，19 世紀末開始重建和復興。第二次世界大戰後，城區向西、向北擴展，市政建設加快，迅速發展成為現代化港市。

市中心區主要在密西西比河左岸。老城法國區(French Quarter)具有歐洲古城風貌。以傑克遜廣場為中心，保留著許多早期法國、西班牙式建築，如聖路易士大教堂、西班牙時期的市政廳和法院(現已闢為博物館)、烏蘇萊修道院、法國市場以及古老的公寓住宅等。西南部是著名的花園區，波本街(Bourbon St)的夜總會及皇家街(Royal St)的古董店和 Royal Cafe 頗為著名。"法國區"以西，隔運河大街(Canal St)是新城行政和商業區，州、市主要行政辦公機構在此組成市政中心建築群。運河大街和聖查爾斯大街(St. Charles Ave)是新城最繁華的商業街，前者南部聳立著 33 層的世貿大樓。普伊德拉斯大街兩側，高層建築林立，有許多銀行、辦公大樓和旅館。住宅區主要分佈在市中心區西、北、東部，並向郊區伸展。龐恰特雷恩湖區為遊覽勝地，建有占地 7 公頃的市立公園。

新澳爾良市是美國南方的主要工業城市，集中全州 1/4 的工廠企業，也是州內最大的零售、批發和金融中心。有紡織、食品、木材加工、煉油、石油化工、化學等工業部門；並是全國重要的造船和航太工業基地，阿馮爾達船廠和生產火箭、宇

航設備的米喬德廠是最大的企業。紐奧良旅遊業興盛，在城市經濟中的地位僅次於運輸業。這裡文化教育事業發達，亦富音樂傳統，為爵士音樂的誕生地，有許多音樂團體和劇場、音樂廳等；當然，市內也有許多博物館和新澳爾良市大學、圖拉內大學等高等學府以及可容納 7 萬多觀眾的路易士安那體育館。每年二月底左右，具有法國傳統的 Mardi Gras"油膩的星期二"嘉年華會更是盛況空前，吸引數以百萬計國內外遊客前來共襄盛舉。期間最熱鬧即是波本街，所有人都利用炫麗珠珠項鍊和面具，來做嘉年華打扮。更有人甘冒法網，以裸露胸部或屁股來換取別人的珠珠項鍊。

新澳爾良市是密西西比河流域的出海門戶，與中、南美洲貿易聯系密切。港區主要分佈於密西西比河和通往龐恰特雷思湖的運河沿岸，碼頭泊位總長 40 多公里，入港航道水深 9.12 米，60 年代建成密西西比河直通墨西哥灣水道，供遠洋海輪使用，使港口的入海距離縮短 60 多公里。1982 年貨物吞吐量 1.71 多億噸，居全國各港之首。這裡以轉口貿易為主，港區內設對外貿易帶，占地 7.6 公頃，進口貨物可免稅在此儲存、加工或展覽，也是 7 條鐵路幹線的交會點，通連洛杉磯、芝加哥、紐約等大城市。水陸聯運方便，是三角洲地區高速公路網的樞紐。多座大橋跨越密西西比河兩岸。著名的龐恰特雷思湖堤壩(Lake Pontchartrain Causeway)長達 39 公里，溝通市區與湖北岸的聯繫。有 1 個國際機場和 2 個國內機場。

航空方面

新澳爾良市國際機場 (MSY)位在市區西方 20 公里，為二層樓建築，共有 A~D 四個航站大廳，國際線集中在 C 大廳。從機場到市中心約三十分鐘，可以利用 Airport Shuttle，車資$10；您也可以選擇使用計程車到市中心，要價一人$24，超過三人每人$10。以上在航站大廈下層搭乘。如果使用$1.5 的 Jefferson Transit 或灰狗巴士，則在航站大廈上層 7 號入口處外面乘坐。

市內交通方面

新澳爾良市的市區運輸局(RTA)管理市內所有的公車，包括 St. Charlesc（green line）和 Riverfront（red line）兩條電車(Streetcar)路線。別小看這兩條電車線，可是從 1926 年起就開始了它的載客任務。雖然 1964 年 Riverfront 這條線被廢止，但是在有心人士的努力下，於 1988 年 8 月 14 日重新開始營業，連接法國區、水族館、世貿等密西西比河畔各個重要景點，可以稱的上是一條觀光路線。建議觀光客到指定地點購買$5 的一日票或$12 的三日票，可以無限搭乘 RTA 各路線公車。路線圖及時刻表

附　　件　　十二

UCI scientists to develop vaccine to combat bioterrorism threat from deadly bacteria

NIH awards $5.8M grant to support research

Irvine, Calif, August 25, 2004

Researchers at UC Irvine will develop a vaccine against the bacterium *Burkholderia pseudomallei*, an organism that can be dispersed by an aerosol spray and used as an agent for biological terrorism. The research will be supported by a $5.8 million grant from the National Institute of Allergy and Infectious Diseases, part of the National Institutes of Health.

B. pseudomallei, which is resistant to many antibiotics, causes melioidosis, an infectious and deadly disease that affects humans and animals. At present, no vaccine against melioidosis exists.

"The development of a vaccine against *B. pseudomallei* is a national and worldwide goal, and is the best way to blunt a bioterrorist threat," said Philip Felgner, principal investigator of the research project and director of the proteomics laboratory within the Center for Virus Research. "Even if we have antibiotics, it will be difficult to treat everyone affected. With the availability of a safe and effective vaccine, however, terrorists may not even proceed to develop weapons that use *B. pseudomallei*."

Related Links

The Center for Virus Research
School of Biological Sciences

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Melioidosis occurs primarily in tropical regions, such as Southeast Asia and northern Australia. It is currently the leading cause of sepsis in northeastern Thailand, where infection rates are high during the rainy season since the bacterium thrives in water. Transmission occurs when humans and animals inhale dust bearing the bacteria, when they drink contaminated water, or when their skin abrasions come into direct contact with contaminated soil. Contact with the blood or body fluids of an infected person also can spread the disease.

Symptoms of melioidosis include fever, anorexia, muscle aches and chest pain. The disease can result in pulmonary infections ranging from mild bronchitis to severe pneumonia. Some patients also suffer septic shock.

"We can learn from what is happening in Thailand, where melioidosis is causing many people to die from septic shock," said Felgner. "An outbreak of the disease in the United States would be devastating, made worse by how difficult it is to treat patients suffering from this disease."

B. pseudomallei, an intracellular bacterium, grows and replicates within mammalian cells. After it has entered a cell, it is hard for antibodies to kill the bacterium without also permanently damaging the cell.

The research at UCI will be conducted in Felgner's proteomics laboratory, which belongs to a group of on-campus biodefense laboratories developing vaccines and other countermeasures that target infectious microorganisms. Felgner's research group will generate the *B. pseudomallei* proteome, i.e., all the proteins encoded by the genes in *B. pseudomallei*, to identify antigens useful for developing a vaccine against the bacterium.

UCI's Center for Virus Research is in the School of Biological Sciences. Felgner will be joined in the research at UCI by Luis Villarreal, director of the center, and D. Huw Davies, an

immunologist and an associate project scientist in the Department of Molecular Biology and Biochemistry. The three scientists will also collaborate with the laboratories of Dr. Richard Titball at the Defence Science and Technology Laboratories at Porton Down, United Kingdom; Dr. Gregory Bancroft at the London School of Hygiene and Tropical Medicine; and Dr. Ganjana Lertmemongkolchai at Khon Kaen University, Thailand.

About the University of California, Irvine: The University of California, Irvine is a top-ranked public university dedicated to research, scholarship and community service. Founded in 1965, UCI is among the fastest-growing University of California campuses, with approximately 24,000 undergraduate and graduate students and about 1,300 faculty members. The third-largest employer in dynamic Orange County, UCI contributes an annual economic impact of $3 billion.

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