出國報告（出國類別：國際會議）

參加 2021 年第 73 屆美國鑑識科學學會年度線上會議報告書

服務機關：法務部法醫研究所
姓名職稱：賴詠淳助理研究員
派赴國家：美國（線上會議）
期間：110 年 2 月 15 日至 110 年 2 月 19 日
報告日期：110 年 4 月 21 日

摘要

本所派員參加第 73 屆美國鑑識科學學會（American Academy of Forensic Sciences, AAFS）年度會議，本屆年會會議期間為美國時間 2021 年 2 月 15 日至 19 日，為期 5 日，因新冠肺炎疫情在全球嚴重擴散，本次會議本應在美國休士頓舉辦，為因應疫情，更改為線上會議，於美國科羅拉多州的科羅拉多泉市 2021 年 2 月 15 日早上 7 時舉行，AAFS 學會克服了艱難的挑戰，以不同以往之方式，利用虛擬模式舉辦會議，是一項與眾不同的非凡體驗。

美國鑑識科學學會成立於 1948 年，至今已 73 年，本次會議主題內容包括: 人類學（Anthropology）、犯罪學（Criminalistics）、數位及多媒體科學（Digital & Multimedia Sciences）、工程學（Engineering Sciences）、一般刑事鑑識（General）、法律裁判學（Jurisprudence）、法醫齒科學（Odontology）、病理/生物學（Pathology/Biology）、精神及行為科學（Psychiatry & Behavioral Science）、問題文書（Questioned Documents）及毒物學（Toxicology）等。參加者分別來自世界各地鑑識人員，並邀請各專家學者發表專題演講（seminars），開設 560 餘場演講及 19 場不同領域之專題討論（workshops）。許多學術、產業及實務單位亦透過年會進行最新科技交流，使整個年會更加熱絡，是相互學習的好機會。

本所亦利用此次機會，於年會中發表研究論文 1 篇：「NGS 技術應用於法醫 DNA 混合型別研判之探討」（The Study of Using Next-Generation Sequencing Technologies to Analyse Mixed DNA Pattern），此研究利用 NGS 技術分析實務案例，並將案件分析結果與毛細管電泳數據比較，評估 NGS 技術的正確性與可靠性，透過 DNA 定序結果，有效輔助鑑別二人混合檢體，改善法醫 DNA 混合型別的鑑別能力。

感謝法務部每年支持本所參與此項國際會議，參與國際會議除了可拓展視野及對多元鑑識深入認識之外，更可提升我國於國際之能見度，雖然從去年開始全世界疫情嚴峻，無法像往年出國參加研討會，但仍可藉此方式促進交流，擴展

國際視野，未來可借鏡美國鑑識科學學會線上會議舉辦方式，舉辦國內及國際相關會議。

目次

摘要.....2

目次.....4

目的.....5

過程.....7

研討會專題內容.....8

一、專題研討會議題：「DNA 證據的詮釋」專題研討會.....8

二、專題研討會議題：「DNA 的證據力」專題研討.....10

三、專題研討會議題：「DNA 定序的崛起」專題研討會.....12

四、研討會專題演講：「Criminalistics Oral I」-血跡斑代謝物分析.....15

五、研討會專題演講：「Criminalistics Oral III」-VeriFiler 試劑之確效.....16

六、研討會專題演講：「Criminalistics Oral I」-Touch 微生物與接觸 DNA (Touch DNA)之鑑別力及局限性.....18

心得與建議.....21

附錄.....24

一、研究論文摘要.....24

二、本所發表研究成果之海報展示.....30

三、線上會議相關內容.....32

目的

美國鑑識科學學會（American Academy of Forensic Sciences）成立於 1948 年，總部位於科羅拉多州的科羅拉多泉市(Colorado Springs, Colorado State)。每年 2 月都會在美國不同城市舉辦年度會議，會議聚集了全世界的專家學者 ，本次會議參加人數超過 3700 人，相較去年增加 10%，共有 560 餘場演講；超過 380 件海報；19 個專題討論；特別會議和主題演講，除了發表各自專業領域的最新發現，也傳遞各領域對鑑識結果一致性的看法。

雖然全球因新冠肺炎疫情，世界各國皆在進行相關防疫措施，但美國鑑識科學學會仍然克服重重困難，用不同於以往之方式如期舉行，實屬不易。本次會議於美國標準中部時間 110 年 2 月 15 日早上 7 時以虛擬方式舉辦線上會議，在會議前及會議中，參加學員皆可透過 email 接收學會最新通知，以確保學員可順利參與課程，並設立虛擬 Lounge Chatrooms 及 Information center 等討論室，可線上互相討論，解決學員疑問，讓課程及展場順利進行，海報展示區可線上觀看、下載及提出問題，廠商展示區可了解最新儀器設備，另外，最重要的是在線上課程進行時，參加課程學員及老師可利用即時的 Live Chat 直接進行討論與交流，課程並不會因為是線上方式無法互動，仍可活潑生動的進行對談，這些都值得我們學習。

年會中每一篇研究報告及每一場演講皆為各自領域的專家經年累月研究成果。藉由參加年度會議可汲取這些寶貴的知識及經驗，了解國際上鑑識研究最新發展；此外，也可作為與會人員在職訓練，溫故知新。因此，本所每年編列預算派員參與年會，旨在於提升本所鑑識能力及鑑驗技術。而為了與各國交流互動並提升國際能見度，本所於 2020 年將科技計畫-先進 NGS 技術應用於混合型別分析之研究部份研究成果投稿該學會，經該學會審核後，將該研究成果發表於 2021 年第 73 屆年會中。

本所於年會發表之研究係以 NGS 技術分析 15 件實務案例，並將案件分析

結果與毛細管電泳數據比較，評估 NGS 技術的正確性與可靠性，透過定序結果，有效輔助鑑別二人混合檢體。其中 15 件法醫混合檢體，以上述二種方法分析 STR 及 Y-STR DNA 型別；另分析其中 4 件法醫混合檢體之人類粒線體 HV1 及 HV2 序列。人類 STR 部份:以 NGS 及毛細管電泳法分析法 15 件實務案件，其中 2 件毛細管電泳檢出率高於 NGS 該 2 件因 DNA 含量太少以致 NGS 之 STR DNA 型別檢出率低於傳統毛細管電泳，其餘 13 件 NGS 之 STR DNA 型別檢出率皆大於傳統毛細管電泳。人類粒線體 DNA 部份:以 NGS 分析法醫混合檢體粒線體 DNA，可突破毛細管電泳對於混合檢體只能研判其存在 2 種鹼基現象，而不易量化所含比例之困境。因此，本研究增加 NGS 定序深度，輔以 SNP 或粒線體位點鹼基所含比例情形，俾利有效解決混合型別問題。

此次參加線上國際會議，除希望能於國際會議中得到相關寶貴意見及回饋，更期待與國際經驗豐富之專家學者進行交流，使本所研究能更精進與深入有效解決實務案件中混合 DNA 型別的問題。

過程

110 年 02 月 15 日 美國中部時間上午 7 時舉行

110 年 02 月 15 日 參加「Interpreting and Communicating DNA Evidence in a Probabilistic Genotyping Universe」專題研討會（workshop）及 Live Chat

110 年 02 月 15 日 參加「When “Who” Doesn’t Matter as Much as “How”—DNA Testimony Given Activity Level Propositions」專題研討會（workshop）及 Live Chat

110 年 02 月 16 日 參加「STR Wars: The Rise of Sequencing」專題研討會（workshop）及 Live Chat

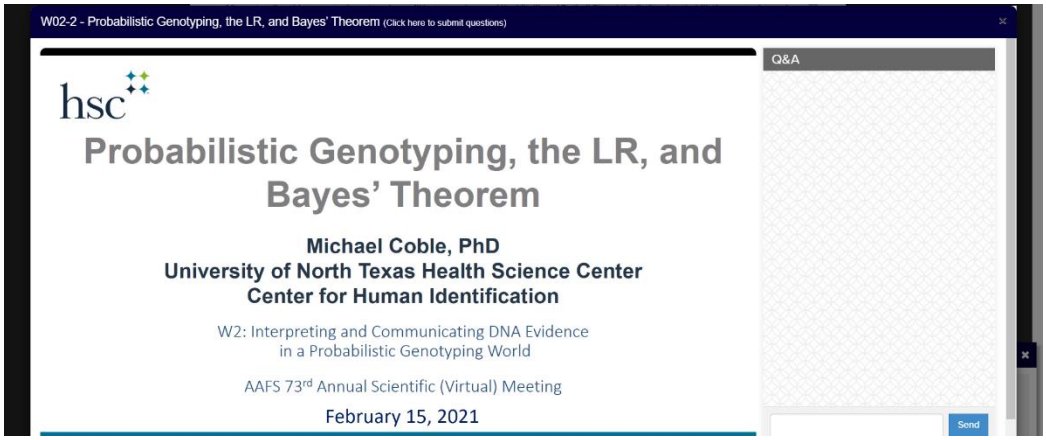
110 年 02 月 17 日 參加「Criminalistics Oral I 」及「Poster Sessions」

110 年 02 月 18 日 參加「Criminalistics Oral III」及「Poster Sessions」

110 年 02 月 19 日 參加「Criminalistics Oral I 」及「Poster Sessions」

研討會專題內容

一、 專題研討會議題：「DNA 證據的詮釋 ；Interpreting and Communicating DNA Evidence in a Probabilistic Genotyping Universe」專題研討會（workshop）



「DNA 證據的詮釋」線上課程實際參與情況。圖片來源：AAFS

隨著 STR 試劑及毛細管電泳儀器的敏感度提升，微量證物的來源研判相對的更加困難，特別是混合型別。概率性計算(Probabilistic Genotyping)是利用生物學模式、統計理論、計算概論等計算似然比(likelihood ratios；LRs)，計算各種組合的可能性，並考慮基因型別的遺失(drop out)及插入(drop in)等因素（依據 SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems）。

根據 Bayes 理論公式所示需考慮事前機率(prior probability)、事後機率(posterior probability) 及似然比（likelihood ratio），如圖 1。犯罪現場所收集到的證物可以利用似然比（likelihood ratio）公式（如圖 2）訂定假設理論(Hypotheses)及計算。

$$\frac{\Pr(H_1 \mid E,I)}{\Pr(H_2 \mid E,I)} = \frac{\Pr(H_1 \mid I)}{\Pr(H_2 \mid I)} \times \frac{\Pr(E \mid H_1,I)}{\Pr(E \mid H_2,I)}$$

Posterior Probability　　Prior Probability　　Likelihood Ratio

圖 1: Bayes 理論

圖片來源：AAFS

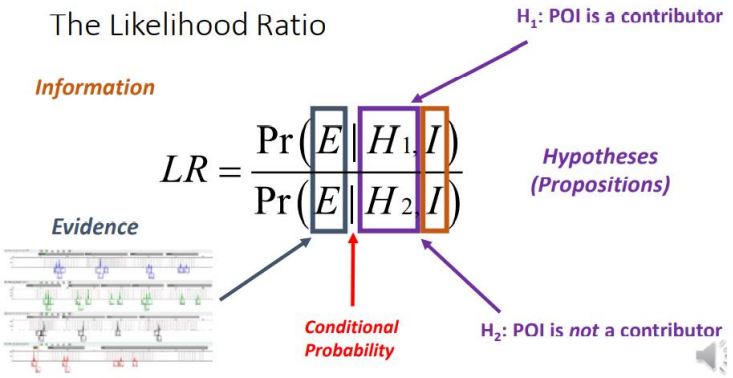


圖 2:似然比（likelihood ratio）

（POI: the DNA came from the person of interest） 圖片來源：AAFS

例如：在現場發現一基因型別為 14 及 15，則 LR=1/2*p_{14}*p_{15}。

關於概率性計算（Probabilistic Genotyping），在美國有 59 個實驗室使用 STRmix 軟體，10 家實驗室使用 TrueAllele 軟體，2 家實驗室使用 Lab Retriever 軟體，而概率性計算包括半連續性(semi-continuous)及連續性(continuous)計算模式。連續性計算模式則以圖譜上基因型別的峰高（Peak heights），考慮基因型別各種組合方式可能的機率。半連續性計算模式不考慮圖譜基因型峰高(peak height)，只考慮圖譜上出現那些基因型別。

似然比在法庭上的應用，可計算數值後依據 SWGDAM Verbal Scale 研判證據力。課程上透過各式的案例分析探討，解釋不同案件中證物之證據力及假設理論，收獲甚多。而概率性計算（Probabilistic Genotyping）軟體是有助於協助解釋 DNA 結果，並依 SWGDAM Guidelines 協助研判，然而計算軟體因算法及編輯，仍然是由“人”所設計，所以並非完全客觀，但可減少各實驗室間的差異及個人主觀性，而混合物型別計算仍需透過鑑識人員分析判別。

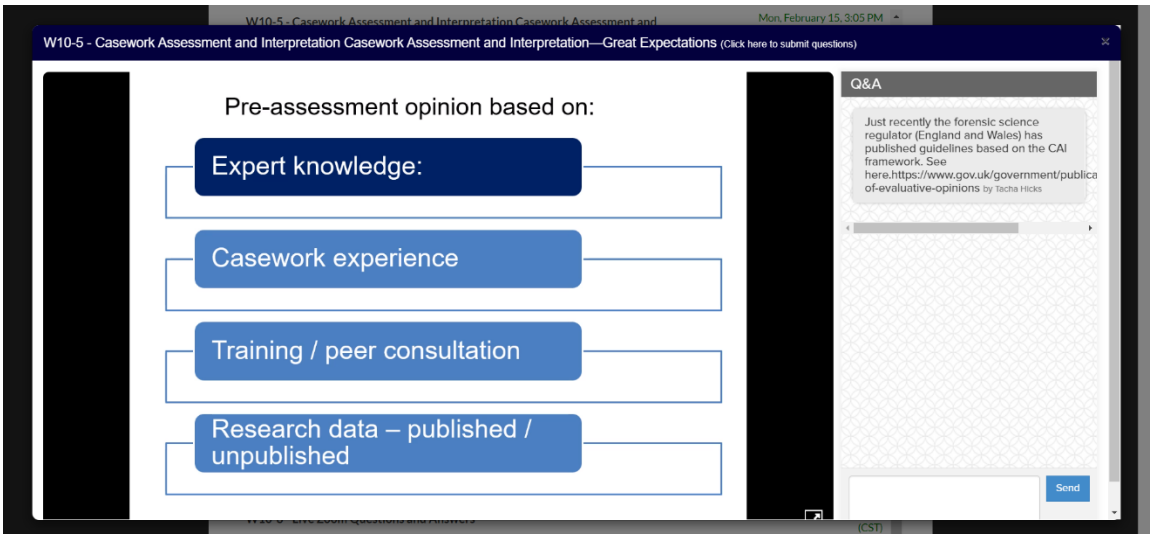
SWGDAM Verbal Scale

LR	Verbal Qualifier
1-2	Uninformative
2-99	Limited Support
100-9,999	Moderate Support
10,000-999,999	Strong Support
> 1,000,000	Very Strong Support

圖 3: SWGDAM 量表（WGDAM Verbal Scale）

圖片來源：AAFS

二、 專題研討會議題：「DNA 的證據力 ；When "Who" Doesn't Matter as Much as How"—DNA Testimony Given Activity-Level Propositions」專題研討（workshop）



「DNA 的證據力」線上課程實際參與情況。圖片來源：AAFS

從犯罪現場收集到的證物，在經過一連串的科學檢驗之後，最重要的是對案件的解讀，至於誰（Who）具有評估證據的能力？必需是具有法醫鑑識專業知識，以及在法醫 DNA 領域工作的實務經驗人員或是研究人員。案件的概率（Probabilities）取決於被告的相關資訊、案件相關資訊、科學信息、非科學信息等會影響，因此，如何建立一個邏輯性的假設思考，對於案件的偵查是非常重要的一件事，相對於在法庭上的陳述也是相當重要。

舉例來說: 犯罪現場收集到的性侵案 DNA 證物（陰道棉棒），必需要考慮到有多少量的 DNA 轉移到上面、上面的 DNA 來源是否來自於涉嫌人或

另有他人，而 DNA 圖譜是具有完整的型別、部分型別亦或是混合型別（如圖 4 及圖 5），如果為混合型別則需考慮誰是主要(major)及次要(minor)型別，以及 DNA 是否有降解的情形，然後進一步計算似然比，分析案件可能的來龍去脈，上述這些因素皆會影響到最後的結論及後續的偵查方向。

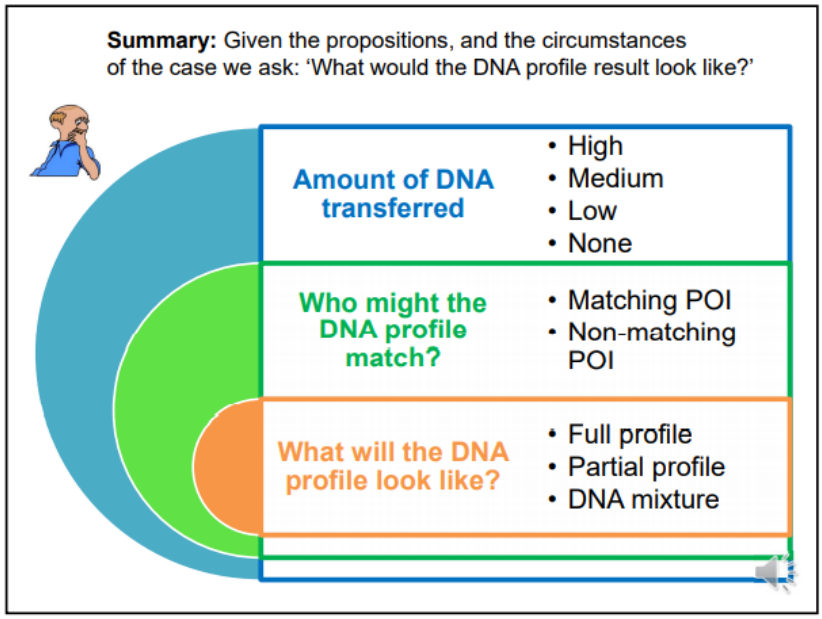


圖 4: DNA 圖譜結果的呈現（What would the DNA profile result look like?）圖片來源：AAFS

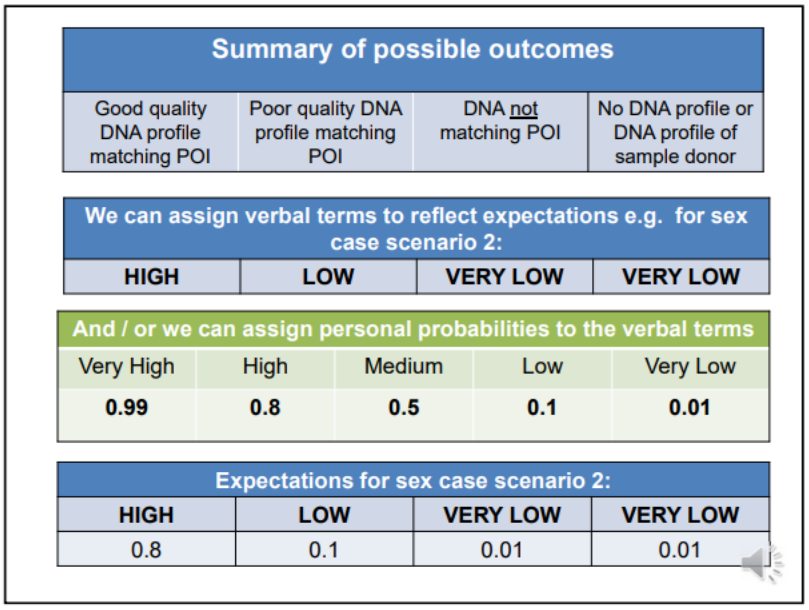
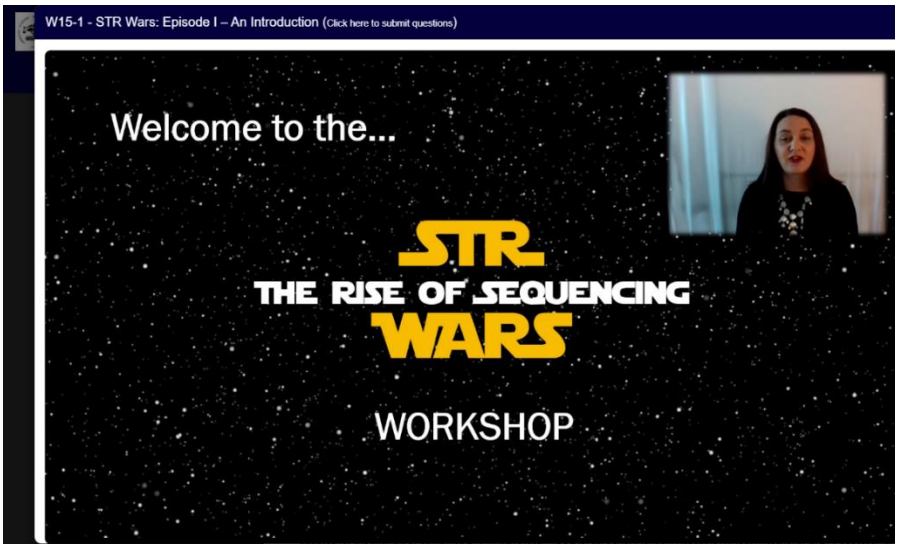


圖 5:總結（Summary of possible outcomes）圖片來源：AAFS

因此，通過考量所有案例中的關鍵問題及相關信息，了解是否有進一步需要去探討的問題，制定各個案件獨自的定位(propositions)，考慮潛在因素，了解證據的概率(Probabilities)及級別(Activity-Level)，有助於鑑識人員，更有效率的完成工作。

三、 專題研討會議題：「DNA 定序的崛起 ；STR Wars: The Rise of Sequencing 」專題研討會（workshop）



「DNA 定序的崛起」線上課程實際參與情況。圖片來源：AAFS

STR Wars: The Rise of Sequencing 之專題演講，具有各式各樣之議題，從現今法醫實驗室常使用之 Forenseq™ DNA Signature Preparation Kit 之確效線體基因之 NGS 分析、Y-染色體（Y-chromosome）DNA 分析、人類眼睛顏色之辨識及生物資訊學角度來看遺傳等，有許多豐富的課程都非常值得學習。

透過其中課程內容學習到有關法醫 Y-chromosome DNA 之分析應用，演講者表示 Y-chromosome DNA 可分成二個部份去探討應用方式:（1）Y-STRs: 父系血統鑑定（Paternal Lineage identification） (例如:性侵案件)、親子關係鑑定及親緣關係鑑定。（2）Y-SNPs : 父系血統鑑定及父系祖先起源鑑定。但 Y-SNPs 分析仍具有些限制性。因此，本研究利用大量平行定序 MPS(Massively Parallel Sequencing)分析 859 Y-SNPs 及 640 Y-haplogroups，並

經由確效去測試實驗的感度，結果顯示最適合分析之 DNA 濃度為 250 pg（圖 6），並再進一步使用軟體分析 Y-haplogroups （圖 7）及祖緣關係。

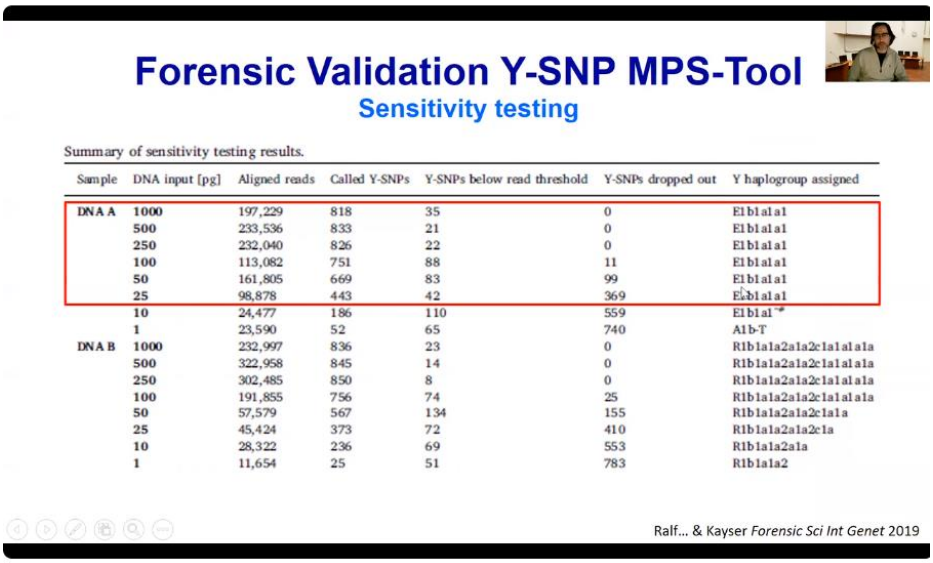


圖 6: Y-SNP MPS 確效（Forensic Validation Y-SNP MPS-Tool ）圖片來源：AAFS

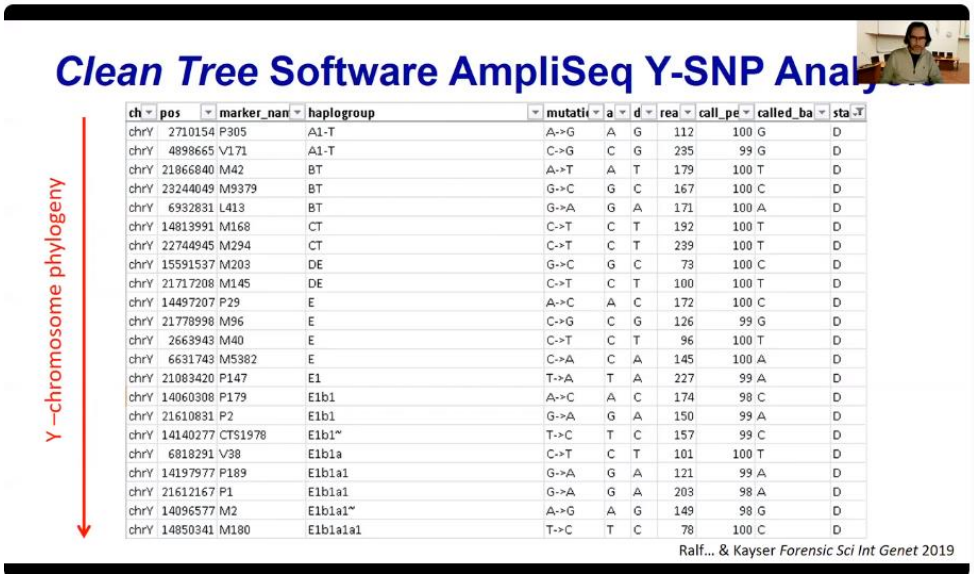


圖 7: Y-SNPs 分析（Y-SNPs Analysis）圖片來源：AAFS

另外，該研究收集全球世界各地 65 個組群，7800 個不具有血緣關係的隨機男性，並分析 17 Yfiler Y-STRs 及 13 RM Y-STRs （Rapidly Mutating Y-STRs）（圖 8），透過分析發現 New RM Y-STR 各基因位之突變率高於 10^{-2}（圖 9），未來可進一步應用於法醫及刑事鑑識實際案例，以協助判斷父系來源，提供更高的鑑別力。

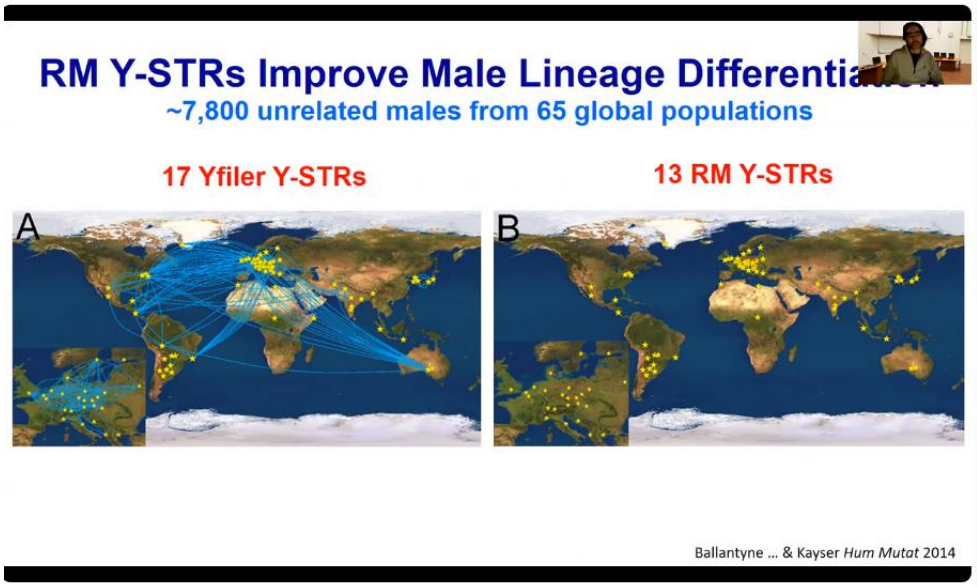


圖 8: Y-STRs 男性血統之差異（Y-STRs Male Lineage Different）圖片來源：AAFS

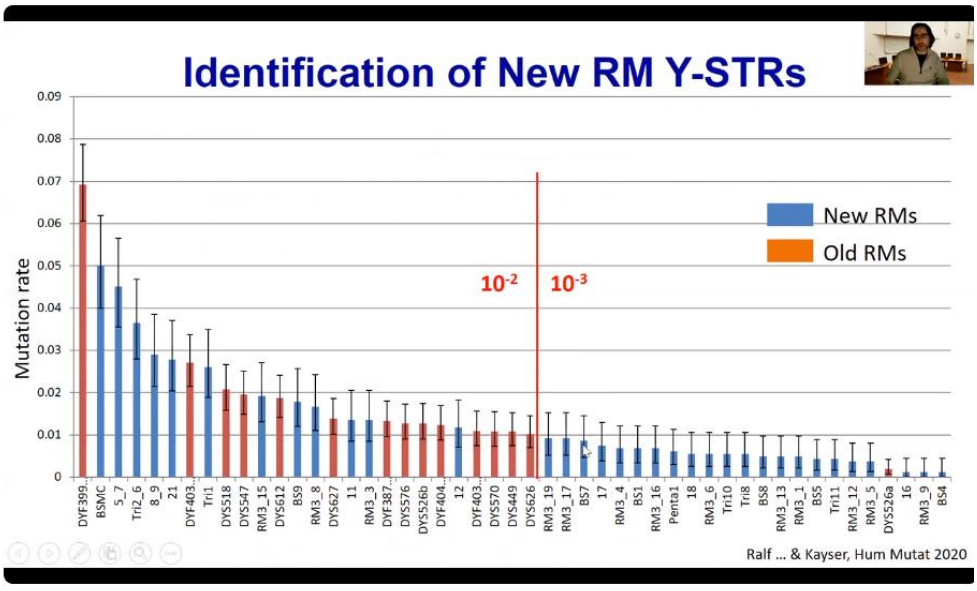
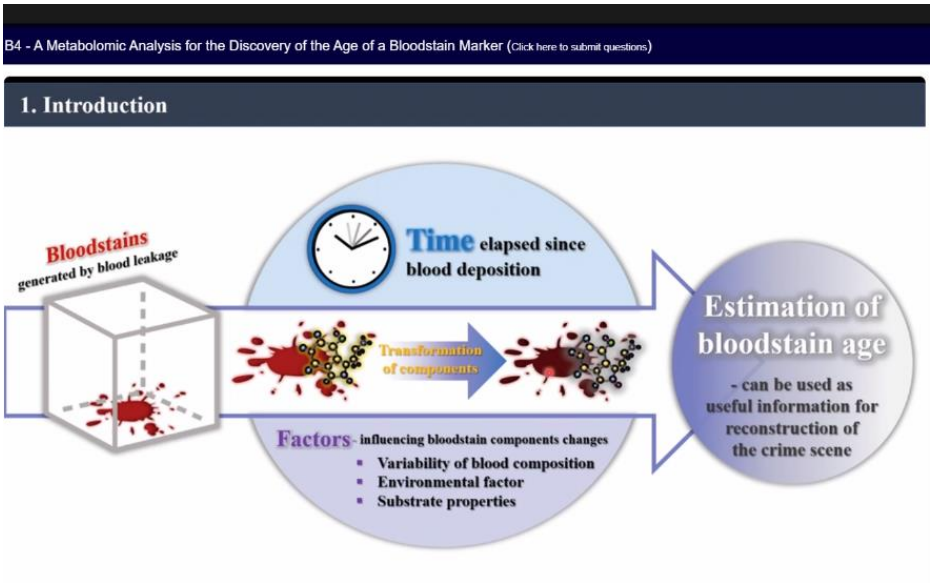


圖 9: 新 RM Y-STRs 鑑定（Identification of New RM Y-STRs）圖片來源：AAFS

四、 研討會專題演講：「Criminalistics Oral I」- 血跡斑代謝物分析 ；A Metabolomic Analysis for the Discovery of the Age of a Bloodstain Marker



「血跡斑代謝物分析」線上課程實際參與情況。圖片來源：AAFS

該研究共收集 20 名男女受試者之血液檢體，其中包括年輕人及老年人，將採集血液滴在濾紙上形成血跡斑，保存於室溫且避光，放置 28 天，並將溫度及濕度維持在同一固定條件。藉由液相層析質譜儀分析血色素代謝產物變化之情形，並進行天數之比較，分別為第 0 天、第 7 天、第 14 天、第 21 天及第 28 天。分析結果顯示，總共有 57 種代謝物具有顯著差異。第 0 天及第 7 天結果相比，並未發現明顯差異，但第 0 天和第 14 天、第 21 天及第 28 天相比具有顯著差異，而第 7 天和第 14 天、第 21 天及第 28 天相比同樣也具有顯著差異，血液中的代謝物隨著時間的增加而改變，且具有明顯的變化模式，因此，該研究可藉由分析血液代謝物，推測血跡斑可能出現的時間點（圖 10 及圖 11）。

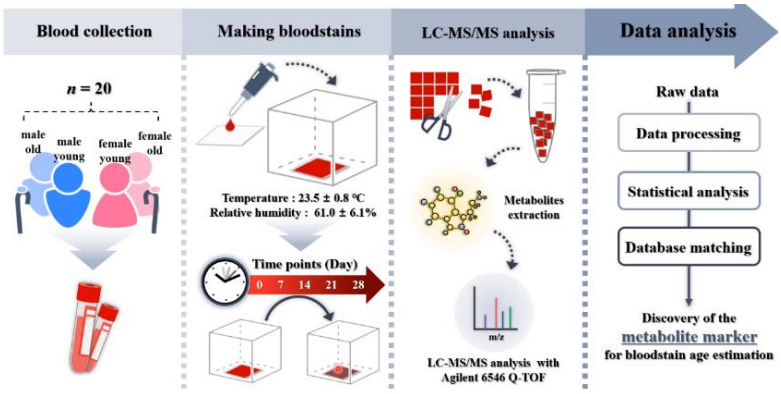


圖 10: 實驗方法流程圖。 圖片來源：AAFS

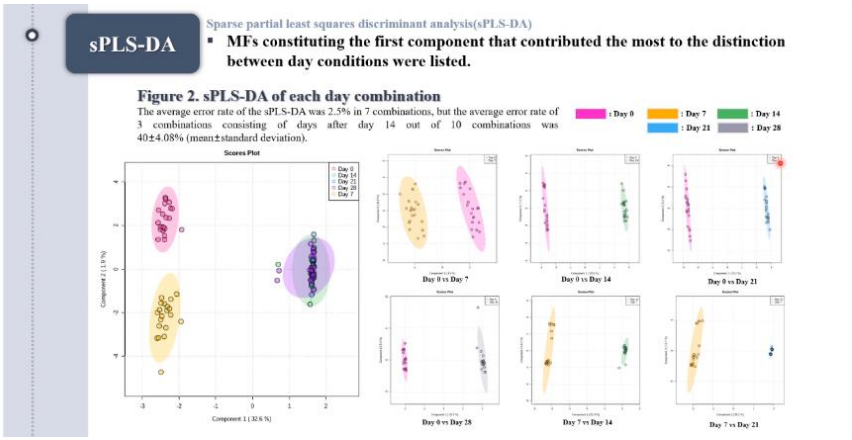


圖 11: 實驗結果。 圖片來源：AAFS

五、 研討會專題演講：「Criminalistics Oral III」- VeriFiler 試劑之確效 ；A Validation Study of Applied Biosystems™ VeriFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit

「VeriFiler 試劑之確效」線上課程實際參與情況。圖片來源：AAFS

該研究探討 PCR 擴增試劑盒(Amplification kit) 之內容物，包括內部品質控制標記(internal quality control markers)，可有效監控 PCR 效率、檢體品質以及是否有抑制物存在。市面上售有各式 PCR 擴增試劑盒，具統計資料顯示，截至 2017 年 1 月 1 日為止，只有 6 個 PCR 試劑盒具有內部品質控制標記，但只有 1 個試劑盒完成確效。Applied Biosystems（ABI）公司生產的 VeriFiler Plus PCR 擴增試劑盒是一種 6 色 dye，可擴增 23 個體染色體 STR 位點，包含 Y 染色體上的一個插入/缺失標記（Y indel），以及性別基因（amelogenin），並含有兩個控制標記（IQCS 和 IQCL，圖 12）。IQCS 和 IQCL 是兩個合成的 DNA 片段，一個是小分子量和一個是大分子量，可與樣品同時放大，並評估 PCR 成功率及樣品品質。有關 VeriFiler 確效方法流程如圖 13 所示。

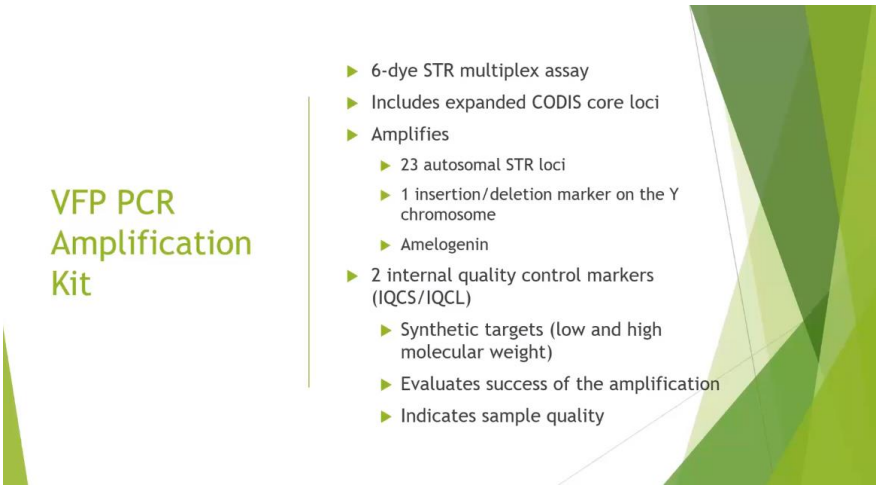


圖 12: VeriFiler 簡介。 圖片來源：AAFS

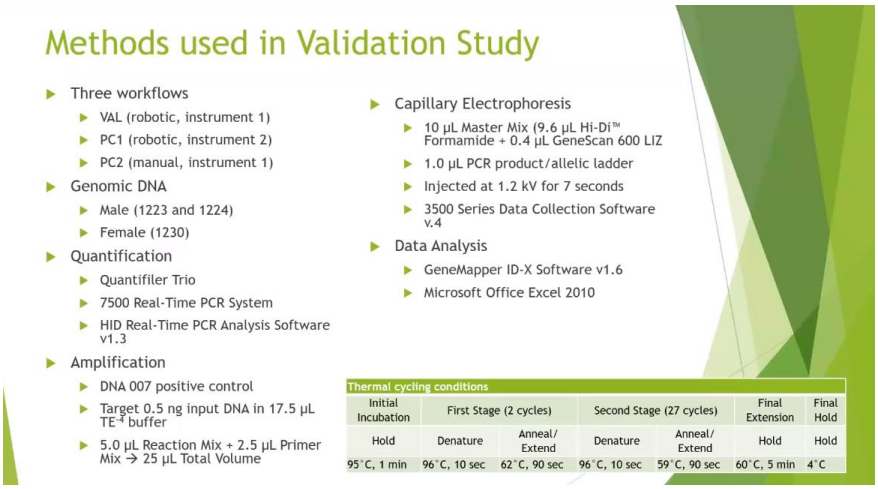
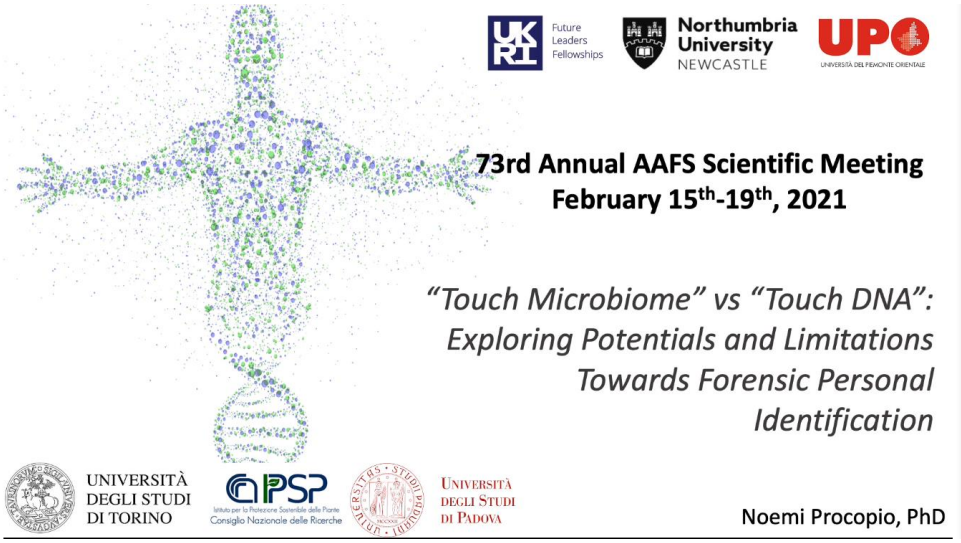


圖 13: VeriFiler 確效方法流程。 圖片來源：AAFS

實驗結果顯示，經評估 VeriFiler 試劑盒的敏感度、可靠性及正確性，並與現今常用之 PCR 試劑盒相互比較，結果發現 VeriFiler 試劑盒可得到一致的實驗結果，且具有相同的準確性，並無任何試劑汙染問題產生，雖然在實驗過程中發現一些 PCR 偽產物(artifacts)，但並非是 VeriFiler 試劑盒所導致，經查應該是 3500 series data collection v4 軟體分析 bug 所導致，對實驗結果並無影響。

六、 研討會專題演講：「Criminalistics Oral I」- Touch 微生物與接觸 DNA (Touch DNA)之鑑別力及局限性 ；“Touch Microbiome” vs. “Touch DNA”: Exploring Potentials and Limitations Toward Forensic Personal Identification



「Touch 微生物與 Touch DNA 之鑑別力及局限性」線上課程實際參與情況。

圖片來源：AAFS

該研究探討鑑識案件中所採得之微量證物，希望藉由皮膚上的微生物輔助接觸 DNA (Touch DNA)分析，以進行個化鑑識。我們從犯罪現場所採集的微量證物可分析其微生物足跡，以探討微生物及 DNA 的來源及轉移情形。微生物分析可作為法庭案件的證據，但目前缺乏準確的法醫參考數據，因此，

該方法在應用上有其限制。該研究收集 11 位受試者，包含不同性別及年齡，受試者填寫各自生活方式及習慣等資料，其相關採樣方式如圖 14 所示。

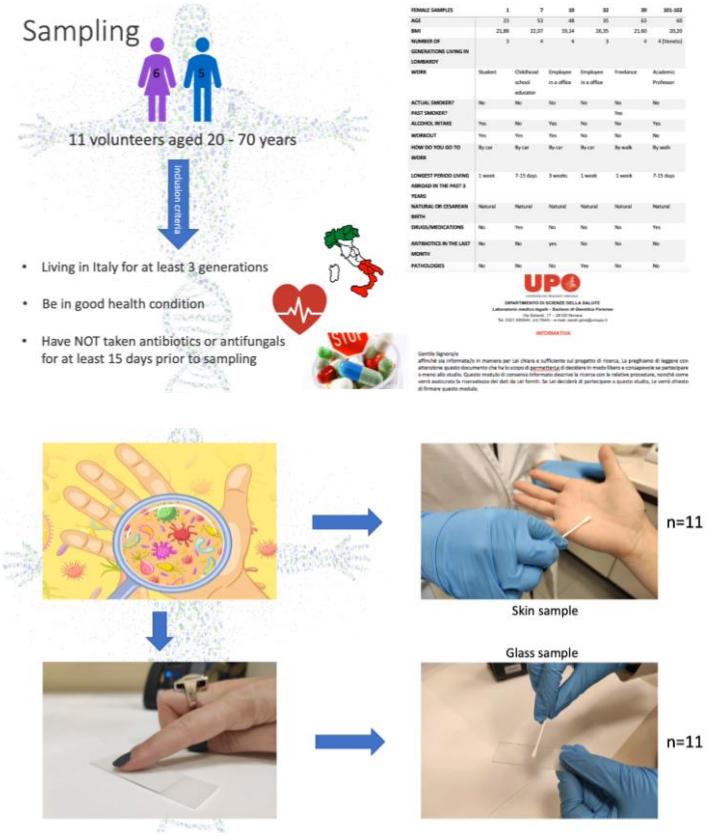


圖 14: 收集方式。 圖片來源：AAFS

分別分析上述受試者檢體 STR DNA 型別及 16S rRNA 定序，實驗結果顯示，22 個檢體中有 20 個檢體成功檢出微生物種類(例如：Actinobacteria、Proteobacteria、Firmicutes 等)，因此，如果當接觸 DNA (Touch DNA)無法成功的檢出 STR DNA 型別，則可利用微生物的種類輔助個化鑑定（圖 15 至圖 17），對於刑事鑑識領域之觀念及技術發展是一項重要突破。

STR profiles – "Touch DNA"

- 5/22 samples allowed for the extraction of a complete (or partially complete) STR profile
- Allelic dropouts observed for 2 loci in sample "M glass" – profile considered "complete" could show some homozygous loci that in the reality are heterozygous but that lost an allele

Samples	Quantification	Number of loci
D (glass)	21.2 ng/µL	12/16
H (skin)	8 ng/µL	16/16
L (glass)	4.5 ng/µL	16/16
M (skin)	7.9 ng/µL	16/16
M (glass)	6.7 ng/µL	16/16

same individual

圖 15: Touch DNA 定量結果及 STR 檢出情形。 圖片來源：AAFS

"Touch microbiome"

- 2/22 samples did not give a sufficient number of NGS reads –> excluded from the subsequent analysis together with their counterparts –> 18 samples (9 donors) were kept
- Excluded taxa present only on glass and not on skin, for each sample (environmental contamination)

Samples	Quantification skin	Quantification glass	Number of taxa from glass
A	6.4 ng/µL	5.9 ng/µL	92
B	2.9 ng/µL	6.4 ng/µL	85
D	3.8 ng/µL	5.5 ng/µL	42
F	3.4 ng/µL	5 ng/µL	53
G	6.1 ng/µL	5.3 ng/µL	40
H	3.9 ng/µL	5.4 ng/µL	99
I	2.3 ng/µL	5.4 ng/µL	111
L	6.2 ng/µL	4.6 ng/µL	63
M	6.9 ng/µL	4.6 ng/µL	89

圖 16: Touch 微生物檢出情形。 圖片來源：AAFS

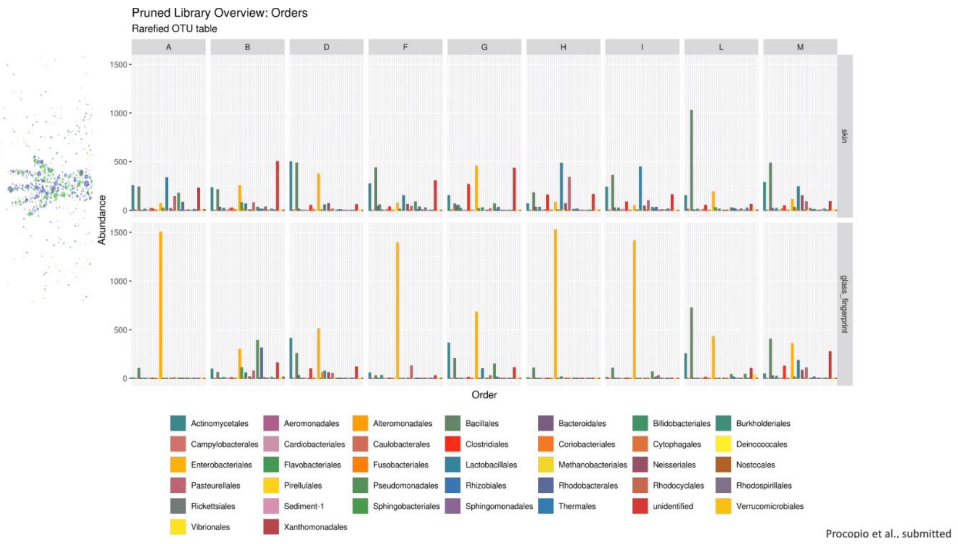


圖 17: Touch 微生物種類檢出情形。 圖片來源：AAFS

心得與建議

一、心得

這次有機會參加 2021 年第 73 屆美國鑑識科學學會（American Academy of Forensic Sciences, AAFS）年度會議，本屆年會原訂於美國科羅拉多州的科羅拉多泉市舉行，時間為 2021 年 2 月 15 日至 19 日，為期 5 日，雖然全球因新冠肺炎疫情，世界各國皆在進行相關防疫措施，但美國鑑識科學學會仍然克服重重困難，用不同於以往之方式如期舉行，實屬不易，該學會克服許多困難與挑戰，更改為線上會議，利用虛擬模式舉辦會議，是一項與眾不同的非凡體驗。

美國鑑識科學學會成立於 1948 年，至今已 73 年，本次會議主題內容包括: 人類學（Anthropology）、犯罪學（Criminalistics）、數位及多媒體科學（Digital & Multimedia Sciences）、工程學(Engineering Sciences)、一般刑事鑑識(General)、法律裁判學（ Jurisprudence ）、法醫齒科學（ Odontology ）、病理/生物學（Pathology/Biology）、精神及行為科學（Psychiatry & Behavioral Science）、問題文書（Questioned Documents）及毒物學（Toxicology）等。參加者分別來自世界各地鑑識人員，並邀請各專家學者發表專題演講（seminars），開設 560 餘場演講、19 場不同領域之專題討論（workshops）及特別會議和主題演講，除了發表各自專業領域的最新發現，也傳遞各領域對鑑識結果一致性的看法，參加演講及專題討論的學員可獲得美國刑事犯罪協會核可之繼續教育學分，以供累積在職進修教育學分之用。此外，許多學術、產業及實務單位也透過年會進行最新科技技術交流，除了於年會中展示研究成果或進行口頭演說，也讓與會者更能了解目前最新科技的發展應用，使整個年會更加熱絡，是相互學習的好機會。

本所以中華民國（臺灣）的名義投稿 2021 年美國鑑識科學學會年會，該學會有約 6600 位會員，分別來自全世界 60 幾個國家，本次年會約有 4000 位各國專家學者投稿，本所於前一年（2020 年）投稿該學會並脫穎而出，榮獲刊登，有幸於該年會發表研究論文 1 篇，題目為：「NGS 技術應用於法醫 DNA 混合型別

研判之探討」（The Study of Using Next-Generation Sequencing Technologies to Analyse Mixed DNA Pattern），本研究係利用 NGS 技術分析法醫混合檢體，並將檢體分析結果與傳統毛細管電泳結果比較，評估技術的正確性與可靠性，透過 NGS 技術可分析精液斑混合檢體，明確鑑別被害人及加害人 DNA 型別，對於二者 DNA 所占之比例加以量化，使 DNA 鑑定結果更為清楚與周延。該技術分析混合檢體，可改善傳統毛細管電泳之瓶頸，因實際案件中法醫檢體多屬微量檢體，若以傳統毛細管電泳進行個別基因位分析，需消耗較多 DNA 量。假使能以 NGS 技術分析檢體，在使用相同 DNA 量時，可同時獲得許多基因位訊息，遠比傳統毛細管電泳單次分析所得之訊息更多，不僅節省法醫 DNA 檢體用量，更可提高人別鑑定確定率，對於法醫鑑識技術是一項重要創新與突破。

本所每年均會藉由參加美國鑑識科學學會機會，發表本所研發最新技術之研究論文，以提昇我國法醫鑑識之能見度與國際地位。此次參加國際線上會議，除希望能於國際會議中得到相關寶貴意見及回饋，更期待與國際經驗豐富之專家學者進行交流，目的是為使研究成果能更精進與實用，有效解決實務案件中許多棘手的問題。目前本所所使用之 NGS 基因鑑定系統係為國際上法醫鑑識最先進鑑定技術，利用該技術可解決長久以來混合檢體 DNA 分型問題。因此，本次年會許多與會學者專家均給予本所高度肯定。

目前本所人力及經費相當困窘，於鑑驗案件業務壓力之下，每年均須爭取科技發展計劃經費，以提昇本所法醫鑑識技術。感謝法務部每年均支持本所發展最新鑑驗技術及參與國際會議，與世界各國專家進行交流，參與國際會議除了可拓展視野及對多元鑑識深入認識之外，更可提升我國於國際之能見度，雖然從去(2020)年開始全世界疫情嚴峻，無法像往年出國參加研討會，但仍可藉此方式促進與國際專業人士交流，著實獲益良多，創造多贏局面。

二、建議

1. 相關專業技術及研究成果作為推動法醫政策參考：

本所為加強法醫人才培訓工作，每年舉辦法醫科學學術研討會，除汲取各法醫鑑識單位經驗，提昇國內法醫鑑識專業人員鑑識水平外，亦可參考國外研討會相關專業技術及研究成果，作為推動法醫政策參考。

2. 辦理線上會議與專家學者溝通互動：

建議未來辦理線上會議可以汲取此次美國鑑識科學學會線上會議的模式，以促進與會者及專家學者的溝通和互動。

3. 賡續提供人員赴國外短期進修：

由於法醫鑑識科技日新月異，一日千里，隨社會的變遷，犯罪案件與日俱增，千變萬化，因此專業人員的在職訓練相當重要，除了參加國際會議外，宜安排人員赴國外作短期進修，培養人力，藉此強化本所鑑識技術能力。

4. 採購高精密度之儀器及最新試劑：

國際上大部份的實驗室及研究單位對於法醫 DNA 鑑驗品質要求越來越來高，以求最精準之實驗結果，因此採購高精密度之儀器及最新試劑，不僅有助於提升國內法醫 DNA 鑑驗品質，更可應用於司法偵審案件，彰顯司法正義，以達勿枉勿縱之目的。

5. 儘速補充本組預算員額:

本組目前人力及經費相當困窘，於鑑驗案件業務繁忙壓力之下，實在無法有多餘時間、精力及物力投入法醫 DNA 研究工作。建議法務部應補充 10-15 位預算員額，以投入法醫鑑驗及法醫研究工作，將人力分成研究組及鑑驗組，一方面可提高鑑驗品質，另一方面可更致力於研究工作，並將所研發之最新技術投入鑑驗案件，以期與國際鑑識領域一起脈動。

附錄

一、 研究論文摘要

The Study of Using Next-Generation Sequencing Technologies to Analyse Mixed DNA Pattern

Yung-Chun Lai[1], Chu-Chun Hsu, Chia-Hung Huang, Man-Chen Chang, Chun-Yen Lin,

[1]Institute of Forensic Medicine, Ministry of Justice, No.123, Min'an St., Zhonghe Dist., New Taipei City 235, Taiwan (R.O.C.)

In forensic science laboratories, capillary electrophoresis is currently employed to perform routine analysis of STR fragments and DNA sequences. However, the technique has limited discrimination for mixed forensic evidences. The DNA quantity of most forensic evidences is extremely small and the mixing ratio is unknown. Therefore, it is difficult to identify trace component of DNA in mixed samples by capillary electrophoresis. Next-Generation Sequencing can overcome the problem of excessively large proportions of DNA in mixed samples by increasing the sequencing depth, and analyze SNP or mitochondrial DNA sequence to assist in the study of DNA composition in mixed forensic evidences.

In this study, a total of 28 forensic cases were collected and analyzed through both capillary electrophoresis and NGS including human STR, human mitochondrial HV1 and HV2 sequence, and animal mitochondrial 12S rRNA, 16S rRNA, and Cyt b sequences. A comparison of the results obtained through two methods was performed to validate the accuracy and reliability of NGS technologies. Moreover, NGS technologies can further aid to identify the sources of two-person mixed samples. Among the 15 DNA mixed samples were analyzed STRs and Y-STRs DNA patterns using above two methods and four mixed samples were examined human mtDNA HV1 and HV2 sequence. In addition, nine animal cases were analyzed, which included mtDNA 12S rRNA, 16S rRNA, and Cyt b sequences.

The results of this research are respectively stated as follows. Human STR part: the 15 cases were analyzed by above two methods. The 15 cases can be correctly detected by capillary electrophoresis, however, the 13 cases can be correctly detected by NGS. The detection rate of NGS was lower than traditional capillary electrophoresis due to the low DNA quantity of two cases. The detection rate of STR DNA of the remaining 13 cases of NGS was higher than traditional capillary electrophoresis. The study found that increasing the sequencing depth or supplementing with SNP sites can assist in the judgment of mixed samples. Human mtDNA part: NGS analysis of forensic mixed evidences of mitochondrial DNA can break through the dilemma that capillary electrophoresis can only study the existence of two kinds of bases in mixed samples, and it is not easy to quantity the ratio. Animal species identification part: Capillary electrophoresis only detected five cases, and the remaining four cases were not detected. The NGS technologies are more sensitive than capillary electrophoresis. Except for one case that was not detected due to severe decomposition, the other eight cases were all detected mitochondrial DNA sequences.

In conclusion, the NGS method still needs to invest a lot of manpower, material resources and time, combined with molecular biology, forensic sciences and statistical analysis, to effectively use this huge amount of information to help identify forensic evidences. It is hoped that in the future, we can continue to refine NGS technologies and achieve a forensic energy that could not be achieved in the past.

Keywords: Next-Generation Sequencing (NGS), Mixed DNA, Human identification, Mitochondrial DNA

Introduction

The Illumina Miseq instrument is the most popular sequencing platform nowadays. Its feature is to provide multiple strand DNA sequences information, which can be used to identify the DNA source [1]. Therefore, NGS technology is suitable for DNA mixtures analysis. Traditional capillary electrophoresis method can not analyze the differences of STR DNA with the same length. If the length of sequence is the same, it will be identified as same pattern. In addition, capillary electrophoresis method cannot distinguish mixed mitochondrial DNA sequences. This problem of DNA mixture is the current challenges for forensic DNA identification.

Because the results of traditional capillary electrophoresis (CE) analysis cannot be quantified, samples with lower DNA concentrations are difficult to detect. However, NGS technology can be use for analyzing multiple DNA to identify the source of mixtures with lower DNA concentration. The results can aid to distinguish the mixture DNA samples with 2 or 3 sources [2]. This study used the Illumina Miseq sequencing platform to analyze the STR and mtDNA of 15 two-persons mixture samples. The results of STR and mtDNA will be compared with the traditional capillary electrophoresis and NGS technology.

Materials and Methods

Forensic samples

A total of 15 samples were collected: ten Vaginal swab samples (VS1-VS10), two Nail exogenous samples (NA1 and NA2), one anal swab sample (AS1), one bone samples (B1) and one teeth sample (T1).

STR analysis with capillary electrophoresis

For each sample,1ng DNA were amplified with AmpFℓSTR Identifiler Kit & AmpFℓSTR Yfiler Kit. The PCR products were analyzed with Applied Biosystems 3500xL Genetic Analyzer and GeneMapper ID-X software for STR pattern.

STR analysis with NGS

For each sample,1ng DNA were inputted for NGS analysis. The steps of NGS experiment for library preparation are including amplify, tag targets, enrich Targets, purify libraries, and normalize libraries. Then the libraries are utilized by ForenSeq™ DNA Signature Prep kit and sequenced by Illumina Miseq Platform .

mtDNA sequencing with capillary electrophoresis

For each sample, HV-1 and HV-2 region were amplified with primers L15997, H16401, L29, and H408. 1 ng template DNA was inputted for each region. The PCR products were purified with Genemark PCR Clean Up Kit and subjected to cycle sequencing with the above-mentioned primers. The PCR products were sequenced in both directions with BigDye Terminator v3.1 Cycle Sequencing Kit. The results were

analyzed with SeqScape v2.6 and compared with the Revised Cambridge Reference Sequence (rCRS).

mtDNA sequencing with NGS

The whole genome of mtDNA was amplified with Long range PCR method. The PCR product were dealed with Nextera DNA Flex Library Prep Kit for library preparation (for each sample,100 ng template DNA). The steps of libary preparation are including tagment DNA, post-tagmentation, PCR, and library cleanup. Then, the DNA quality was checked and sequenced by Illumina Miseq Platform.

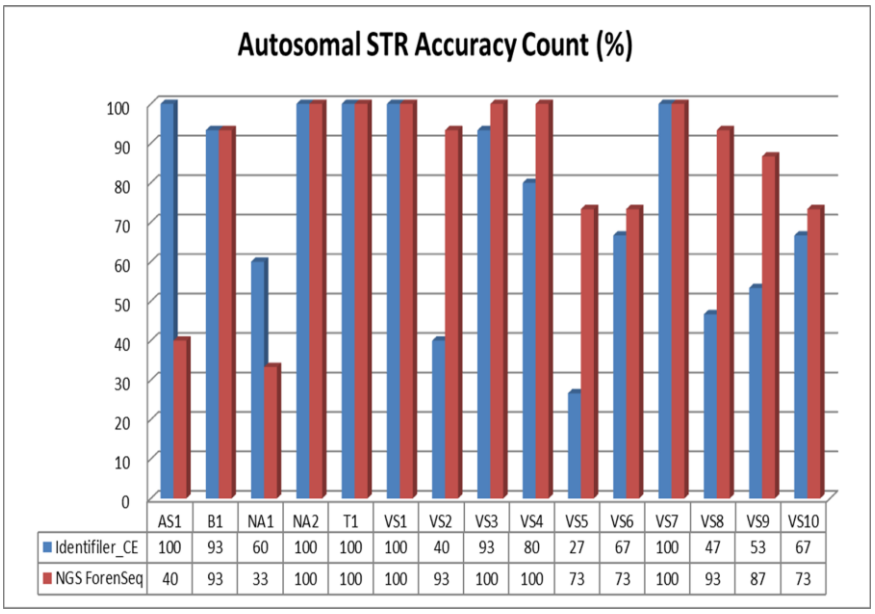
Results

The results of STR analysis

The results of 15 samples show that the NGS provides more excellent data than CE, except two samples (AS1 and NA1) with low amount of DNA (<1ng) for NGS analysis (Figure1).The results of sexual sample VS1 indicates that both CE and NGS could detect the complete 15 genotypes (Table1). In addition, we calculate the DNA percentage of victim and suspect of VS1 sample, and indicated the proportions of Contributor 1 and Contributor 2 were 45.1% and 54.9%, respectively.

The results of mtDNA

Capillary electrophoresis and NGS were used to analyze the Nail exogenous sample (NA1). The result of capillary electrophoresis show only two types of base was found that 16183 position. However, there were 11 positions with 2 types of base from the results of NGS, such as positions 146, 150 and 152 (Figure 2). In addition, the vaginal swab (VS1) analyzed by capillary electrophoresis was found only 2 kinds of base in 6 positions, while11 positions was shown by NGS analysis(Table 2).



	AS1	B1	NA1	NA2	T1	VS1	VS2	VS3	VS4	VS5	VS6	VS7	VS8	VS9	VS10
Identifiler_CE	100	93	60	100	100	100	40	93	80	27	67	100	47	53	67
NGS ForenSeq	40	93	33	100	100	100	93	100	100	73	73	100	93	87	73

Figure 1: The comparison the STR result of CE and NGS

Table 1: The STR results of sample (VS1) using Next-Generation Sequencing Technologies.

Sample / STR loci	Contributor 1 (Victim)	Contributor 2 (Suspect)	VS1 Vaginal swab (DNAinput; reads)		NA1 Nail exogenous DNA (DNAinput; reads)	
			Identifiler CE (1ng)	NGS ForenSeq (0.5 ng;1,103,826)	Identifiler CE (0.30 ng)	NGS ForenSeq (<0.05 ng;99,384)
D8S1179	14,16	10,11	10,11,14,16	10,11,14,16	10,11,14	10,11,14
D21S11	30,32.2	30,31	30,31,32.2	30,31,32.2	30,31,32.2	30,31,32.2
D7S820	10,12	8,11	8,10,11,12	8,10,11,12	8,10,11	8,11
CSF1PO	10,13	11	10,11,13	10,11,13	10,11	11
D3S1358	15,17	15,16	15,16,17	15,16,17	15,16,17	15,16
TH01	7,9	9	7,9	7,9	9	7,9
D13S317	8,11	8,10	8,10,11	8,10,11	8,10,11	8,10
D16S539	9,11	12	9,11,12	9,11,12	9,11,12	12
D2S1338	19,20	18,19	18,19,20	18,19,20	18,19,20	18,19,20
D19S433	13,15	15,15.2	13,15,15.2	13,15,15.2	13,15,15.2	13,15,15.2
vWA	18,19	14,16	14,16,18,19	14,16,18,19	14,16,19	16
TPOX	8,11	8	8,11	8,11	8	8
D18S51	12,15	13,16	12,13,15,16	12,13,15,16	12,13,15,16	13,15,16
D5S818	10,11	9,10	9,10,11	9,10,11	9,10,11	9
FGA	22,23	21.2,24	21.2,22,23,24	21.2,22,23,24	21.2,23,24	21.2,22,23,24
Amelogenin	X, X	X, Y	X, Y	X, Y	X, Y	X, Y

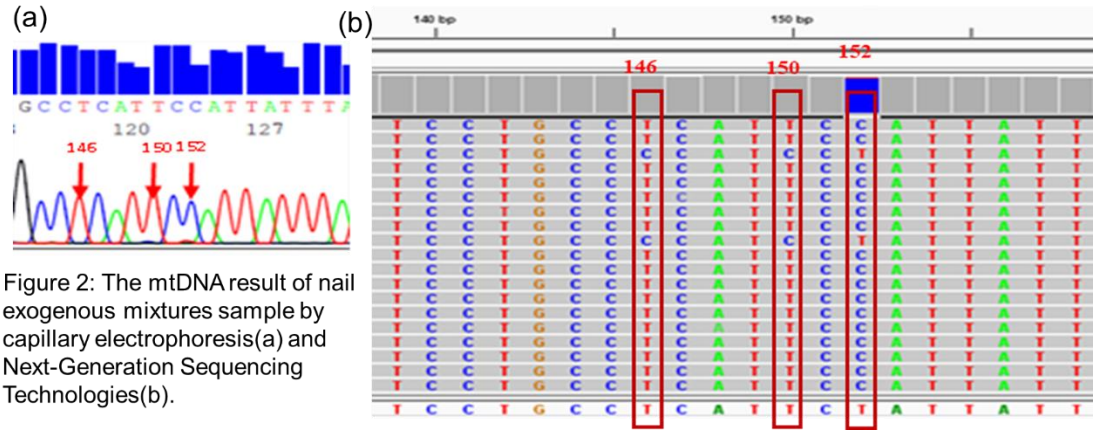


Figure 2: The mtDNA result of nail exogenous mixtures sample by capillary electrophoresis(a) and Next-Generation Sequencing Technologies(b).

Table 2: The comparison of mtDNA result of nail exogenous and suspect of mixtures sample by CE and NGS.

Position / Analysis method	146	150	152	249	16148	16183	16189	16256	16298	16327	16362
Contributor2 (Suspect)	T	T	C	A	T	C	C	T	T	C	C
NA1-CE	T	T	C	A	T	A/C	C	T	T	C	C
NA1-NGS (ratio)	C:T (5:95)	C:T (5:95)	C:T (95:5)	Del:A (4:96)	C:T (23:77)	A:C (29:71)	C:T (65:35)	C:T (22:78)	C:T (16:84)	C:T (88:12)	C:T (89:11)
VS1-CE	C/T	C/T	C/T	Del	C/T	A/C	C/T	C	C	T	T
VS1-NGS (ratio)	C:T (74:26)	C:T (75:25)	C:T (22:76)	Del:A (74:26)	C:T (92:8)	A:C (94:6)	C:T (7:93)	C;T (92:8)	C:T (86:14)	C:T (14:84)	C:T (16:83)

Discussions

STR analysis

The establishment of NGS technology for analyzing mixture specimens can improve the defects of capillary electrophoresis. In real cases, most of forensic evidences are in trace amounts. If we can use NGS technology for sequencing, we can obtain different genomic information at the same time , such as: 27 Autosomal STRs, 24 Y-STRs, 7 X-STRs and 94 SNPs.

In sexual case, suspect was the minor contributor of mixed sample and cannot be distinguished with the major contributor. Traditional capillary electrophoresis may cause pull-up due to the high amount of input DNA. However, if the NGS technology is used for analysis, it will be able to overcome the problems as mentioned above. NGS can input higher DNA amounts and increase the sequencing depth and reads of the minor contributor. Therefore, it can improve the accuracy of STR pattern of the minor contributor to solve the problems of mixture samples.

mtDNA

Heteroplasmy is often presence in mitochondrial DNA. It is difficult to distinguish heteroplasmy from mixture with two persons. There are three steps for identification of mixture of mtDNA. Firstly, we will check the result of STR analysis by NGS to confirm it is single source or not. If STR result is mixture samples, we will further analyze the mtDNA using NGS technology. So that, we can found the different base position and its mixing ratio. Finally, we can compare mtDNA sequences between suspect and victim to assist to identify the mixture mtDNA of forensic evidences.

Conclusions

NGS techniques can analysis mixture and overcome the problems of traditional capillary electrophoresis. Most evidences which were collected from forensic cases are trace samples. NGS can obtain more information such as 27 Autosomal STRs, 24 Y-STRs, 7 X-STRs, and 94 SNPs in one experiment than capillary electrophoresis. The NGS technology also can sequence of HV1 and HV2 regions of the mixture specimens. Compared with traditional capillary electrophoresis and NGS sequencing, the NGS technology can further confirm the composition ratio of mixture source . In summary,

this study establishes a method for analyzing mixed DNA specimens of forensic samples, and hopes that it can effectively solve problems of forensic mixture samples.

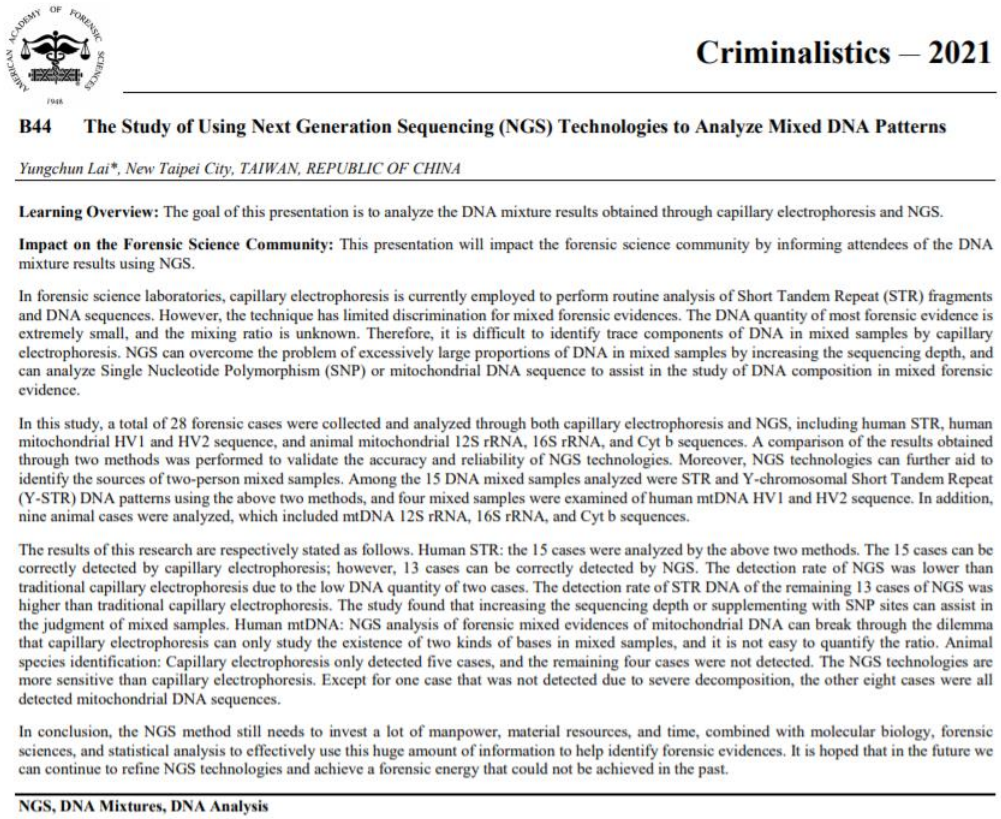
Acknowledgements

This study was supported by the grants No.107-1301-05-04-02 from Ministry of Justice,Taiwan, ROC.

References

1. 1.H. Shiono, [Personal identification using DNA polymorphism--the identification of forensic biological materials]. Nihon Hoigaku Zasshi 50, 320-330 (1996).
2. J. Purps et al., A global analysis of Y-chromosomal haplotype diversity for 23 STR loci. Forensic Science International. Genetics 12, 12-23 (2014).

二、 本所發表研究成果之海報展示



Criminalistics – 2021

B44 The Study of Using Next Generation Sequencing (NGS) Technologies to Analyze Mixed DNA Patterns

Yungchun Lai, New Taipei City, TAIWAN, REPUBLIC OF CHINA*

Learning Overview: The goal of this presentation is to analyze the DNA mixture results obtained through capillary electrophoresis and NGS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees of the DNA mixture results using NGS.

In forensic science laboratories, capillary electrophoresis is currently employed to perform routine analysis of Short Tandem Repeat (STR) fragments and DNA sequences. However, the technique has limited discrimination for mixed forensic evidences. The DNA quantity of most forensic evidence is extremely small, and the mixing ratio is unknown. Therefore, it is difficult to identify trace components of DNA in mixed samples by capillary electrophoresis. NGS can overcome the problem of excessively large proportions of DNA in mixed samples by increasing the sequencing depth, and can analyze Single Nucleotide Polymorphism (SNP) or mitochondrial DNA sequence to assist in the study of DNA composition in mixed forensic evidence.

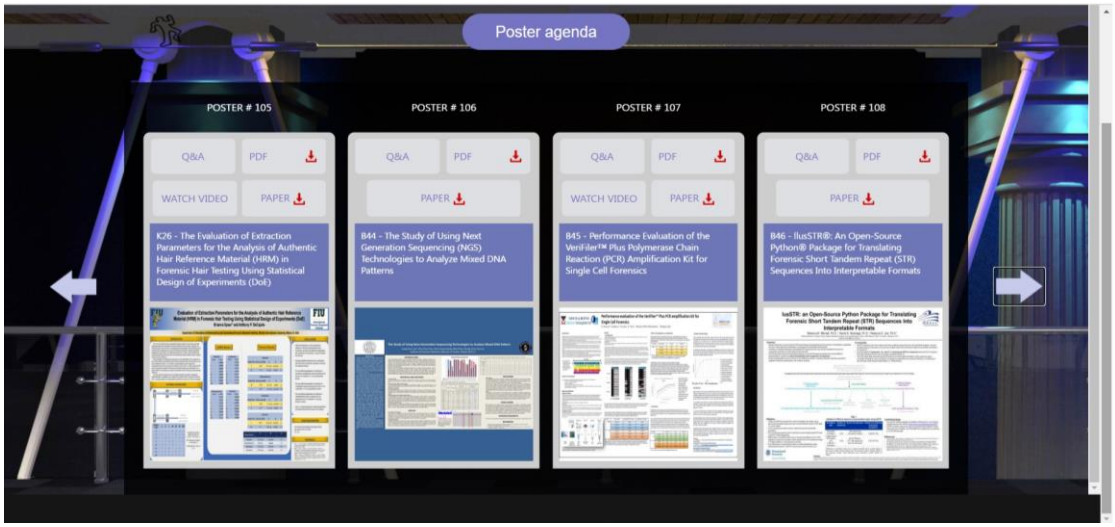
In this study, a total of 28 forensic cases were collected and analyzed through both capillary electrophoresis and NGS, including human STR, human mitochondrial HV1 and HV2 sequence, and animal mitochondrial 12S rRNA, 16S rRNA, and Cyt b sequences. A comparison of the results obtained through two methods was performed to validate the accuracy and reliability of NGS technologies. Moreover, NGS technologies can further aid to identify the sources of two-person mixed samples. Among the 15 DNA mixed samples analyzed were STR and Y-chromosomal Short Tandem Repeat (Y-STR) DNA patterns using the above two methods, and four mixed samples were examined of human mtDNA HV1 and HV2 sequence. In addition, nine animal cases were analyzed, which included mtDNA 12S rRNA, 16S rRNA, and Cyt b sequences.

The results of this research are respectively stated as follows. Human STR: the 15 cases were analyzed by the above two methods. The 15 cases can be correctly detected by capillary electrophoresis; however, 13 cases can be correctly detected by NGS. The detection rate of NGS was lower than traditional capillary electrophoresis due to the low DNA quantity of two cases. The detection rate of STR DNA of the remaining 13 cases of NGS was higher than traditional capillary electrophoresis. The study found that increasing the sequencing depth or supplementing with SNP sites can assist in the judgment of mixed samples. Human mtDNA: NGS analysis of forensic mixed evidences of mitochondrial DNA can break through the dilemma that capillary electrophoresis can only study the existence of two kinds of bases in mixed samples, and it is not easy to quantify the ratio. Animal species identification: Capillary electrophoresis only detected five cases, and the remaining four cases were not detected. The NGS technologies are more sensitive than capillary electrophoresis. Except for one case that was not detected due to severe decomposition, the other eight cases were all detected mitochondrial DNA sequences.

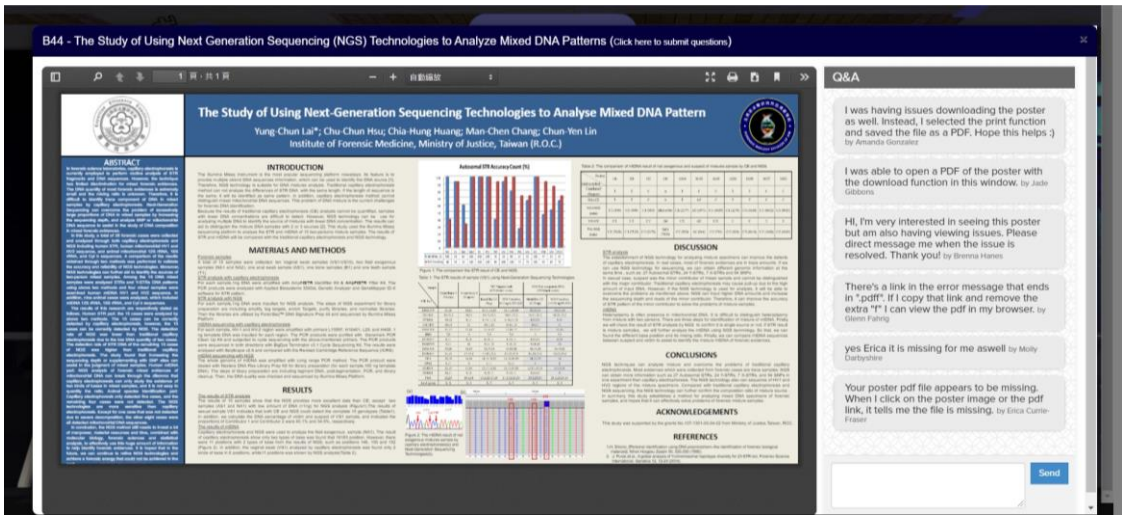
In conclusion, the NGS method still needs to invest a lot of manpower, material resources, and time, combined with molecular biology, forensic sciences, and statistical analysis to effectively use this huge amount of information to help identify forensic evidences. It is hoped that in the future we can continue to refine NGS technologies and achieve a forensic energy that could not be achieved in the past.

NGS, DNA Mixtures, DNA Analysis

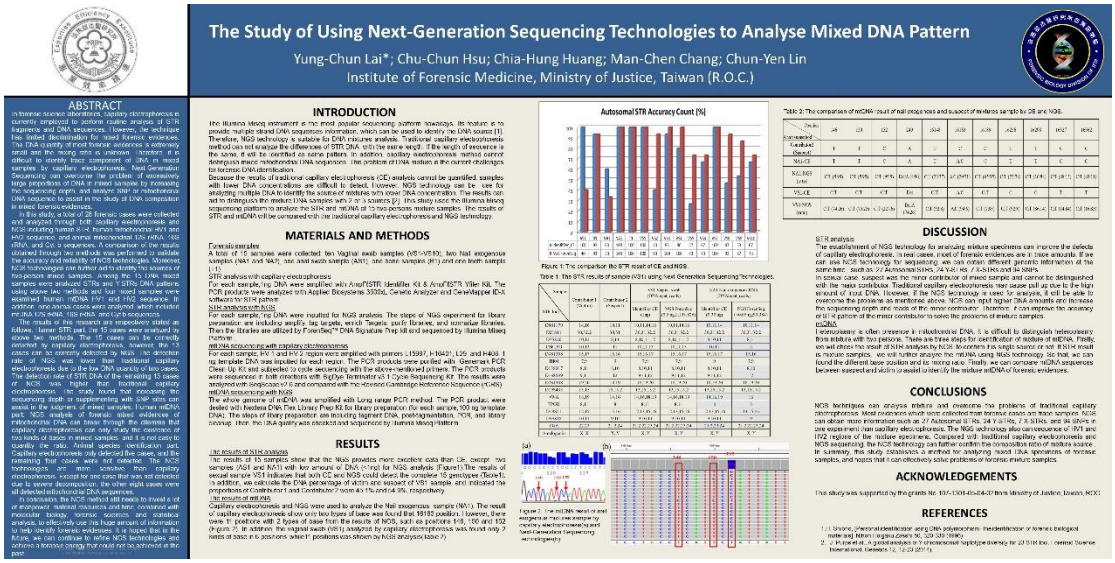
本所投稿論文摘要線上呈現。圖片來源：AAFS



本所海報展示線上呈現 圖片來源：AAFS



本所海報線上點選放大圖 圖片來源：AAFS



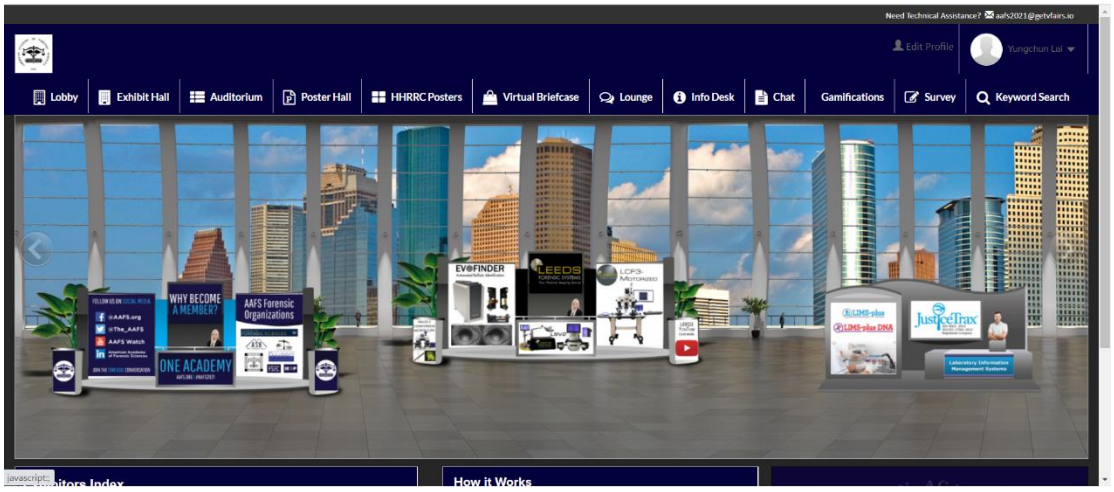
本所研究成果海報 圖片來源：AAFS

三、 線上會議相關內容

2021AAFS 線上會議開始倒數 1 天又 4 小時 33 分。圖片來源：AAFS

2021AAFS 線上會議會場入口。圖片來源：AAFS

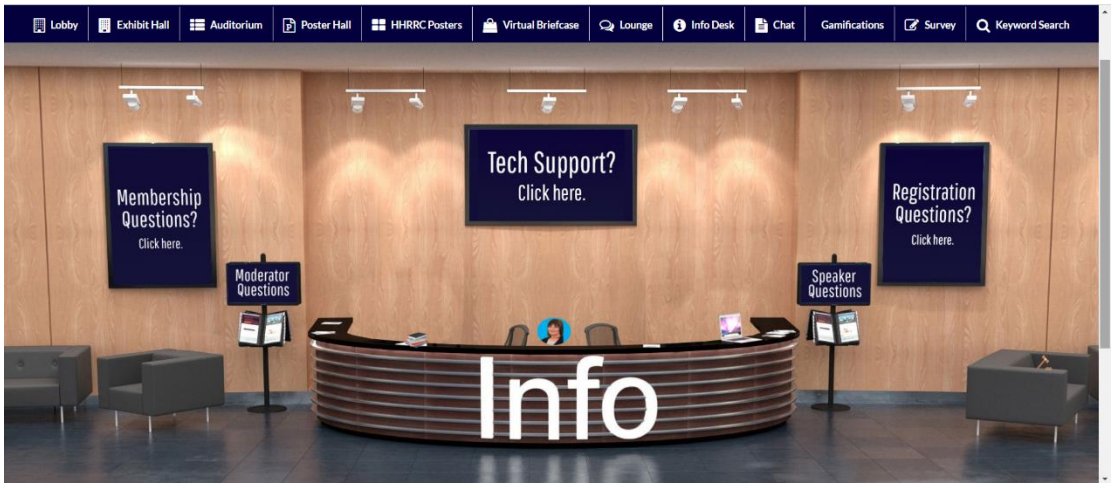
會議大廳（Lobby）。圖片來源：AAFS



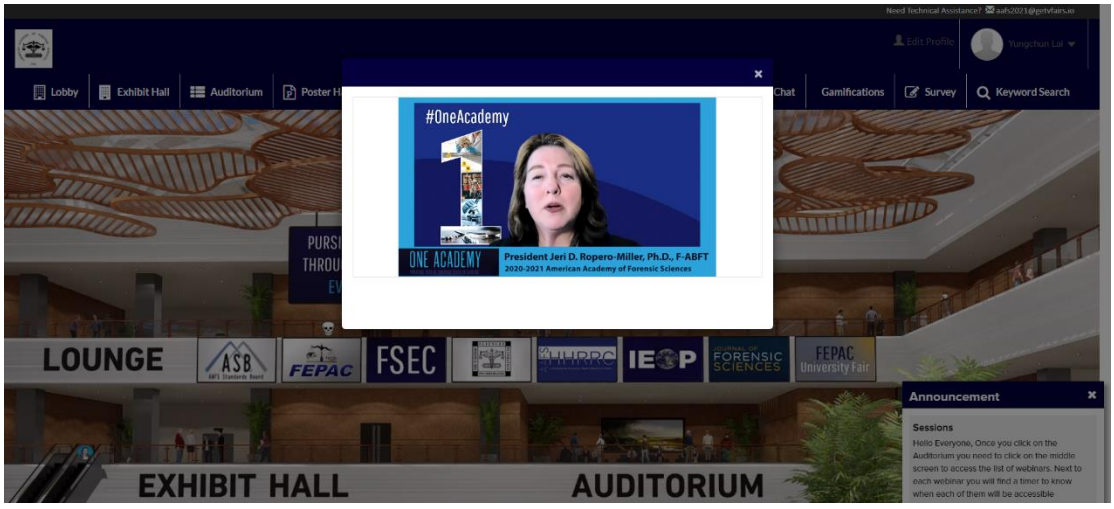
展覽廳(Exhibit Hall)。圖片來源：AAFS

會議廳(Auditorium)。圖片來源：AAFS

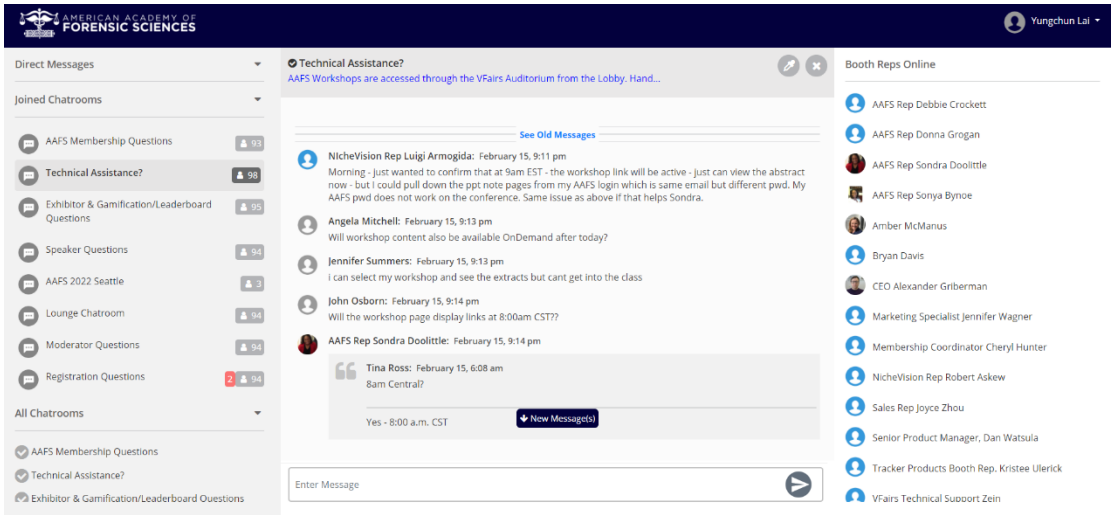
休息室(Lounge)。圖片來源：AAFS



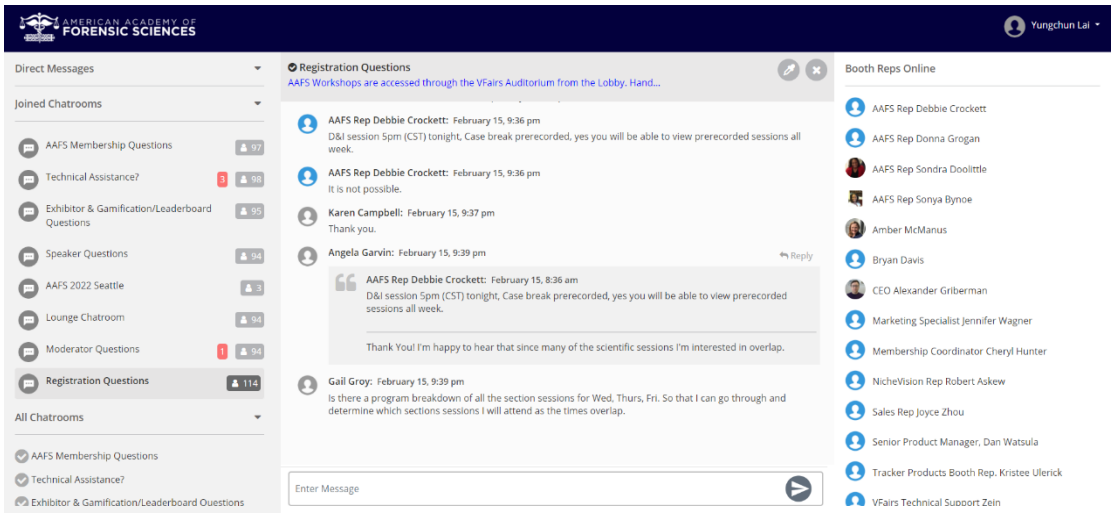
諮詢台（Info Desk）。圖片來源：AAFS



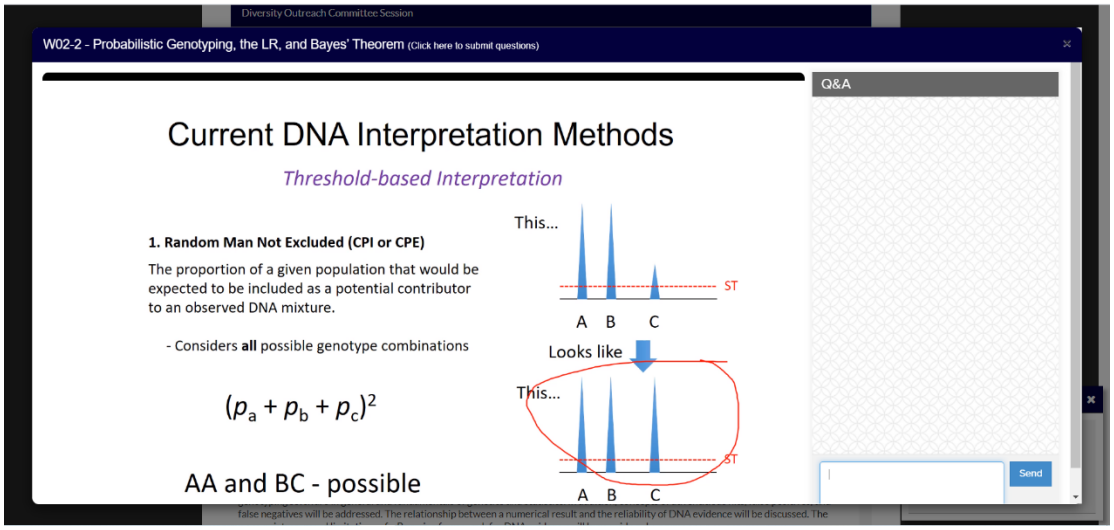
AAFS 主席開場演講。圖片來源：AAFS



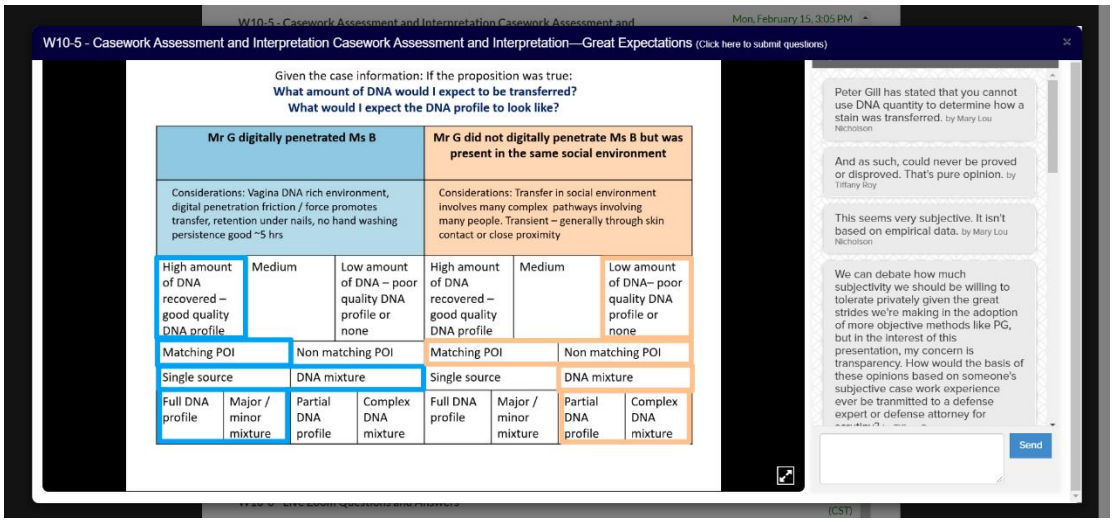
線上對談（Live Chat）。圖片來源：AAFS



線上對談（Live Chat）。圖片來源：AAFS

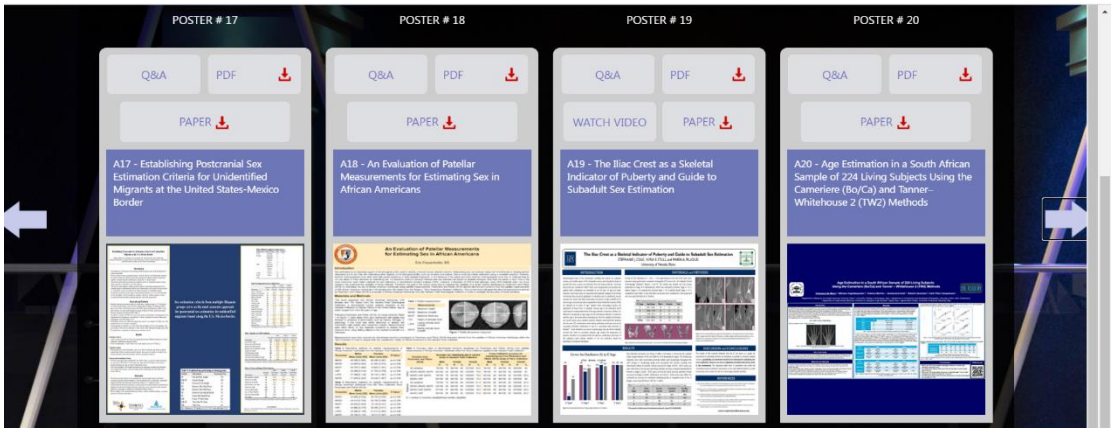


線上專題研討會（workshop）。圖片來源：AAFS

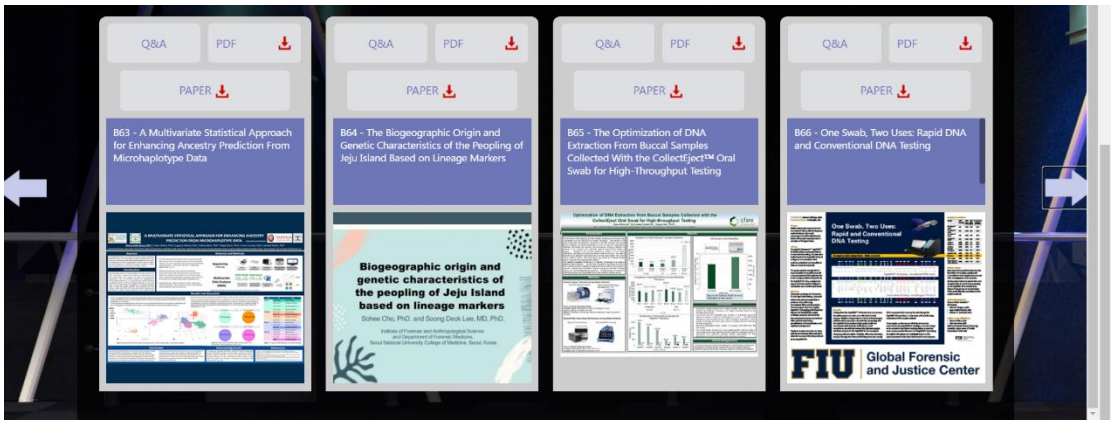


線上專題研討會（workshop）。圖片來源：AAFS

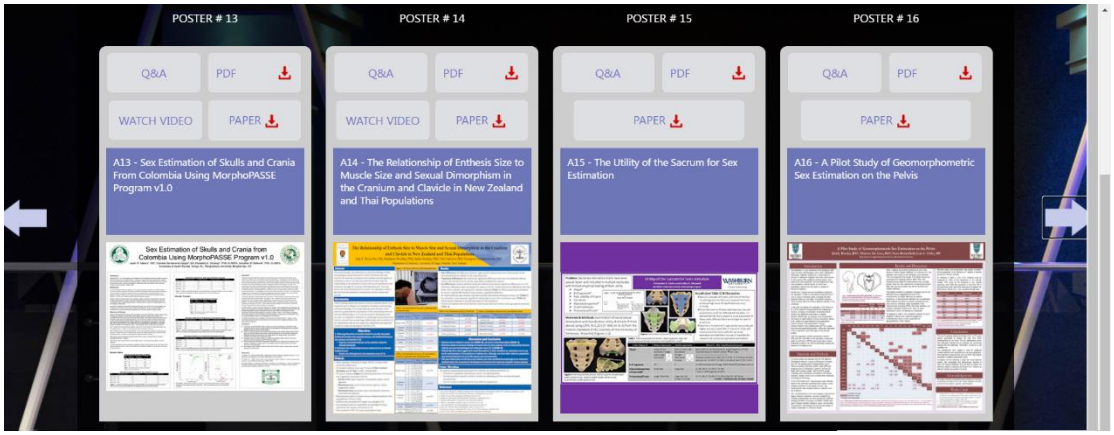
線上海報展示區(Poster Hall) 。圖片來源：AAFS



線上海報展示區(Poster Hall) 。圖片來源：AAFS



線上海報展示區(Poster Hall) 。圖片來源：AAFS



線上海報展示區(Poster Hall) 。圖片來源：AAFS

全文完