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United States Department of Agriculture

Animal and Plant Health Inspection Service (APHIS)

Plant Protection and Quarantine (PPQ)

Science &Technology (S&T)

Center for Plant Health Science and Technology (CPHST)

Beltsville Laboratory

Safeguarding U.S. Agriculture Against Plant Pathogens

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Director

USDA
United States Department of Agriculture   **CPHST**

Center for Plant Health Science and Technology
- *Provides scientific solutions for the PPQ regulatory decisions & operations*

- *Methods Development*
- *Scientific Investigation*
- *Pest Risk Analyses*
- *Technology*

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Timely, reliable diagnostics of quarantine plant pathogens to safeguard US agriculture from the introduction, spread and establishment of harmful pests

- Federal Confirmatory Diagnostics
- Development and Validation of Diagnostic Methods for Regulatory Pathogens
- Proficiency testing
- Provide Training
- Staff: thirty five (35)

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BSL-3 Enhanced Facility

- *Ralstonia solanacearum* R3B2
  - Brown rot of potato-Bacterial wilt
- *Rathayibacter toxicus*
  - Ryegrass gumming
- *Xanthomonas oryzae*
  - Bacterial leaf blight of rice
- *Synchytrium endobioticum*
  - Potato Wart
- *Peronosclerospora philippinensis (P. sacchari)*
  - Philippine Downy mildew of maize
- *Sclerophthora rayssiae*
  - Brown stripe downy mildew of maize
- *Phoma glycinicola*
  - Red Leaf Blotch of Soybean

- 12500 total square feet
- 10 greenhouse modules (3155 square feet)
- 1 Main diagnostic lab with Class II BSC and HEPA filtered fume hood
- 1 Fungal isolation room with Class II BSC
- 1 Pass-through autoclave
- 1 Ethylene oxide sterilizer
- 2 Refrigerated rooms
- 3 Walk-in growth chambers

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- *ISO/IEC 17025 accredited diagnostic lab and is registered through the ANSI-ASQ National Accreditation Board (ANAB).*
- *Unbiased, independent, third party assessments*
- *International Recognition of Test Results*
- *Demonstrated technical competence for accurate and precise test results*

USDA · United States Department of Agriculture — Operational Confirmatory Diagnostics

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Effective Date: 10/20/2011	Detection of *Citrus Leprosis Virus*-Cytoplasmic Type (CiLV-C) using a Multiplex One-Step Reverse Transcription (RT) Conventional PCR (K565 and K568)	Page 1 of 11

- *Phytophthora ramorum* (SOD)
- *Phytophthora kernoviae*
- *Phytophthora tentaculata*
- *Peronosclerospora philippinensis*
- *Sclerophthora rayssiae*
- Sweet Orange Scab (SOS)
- Citrus Black Spot (CBS)
- *Phoma glycinicola*

- Citrus Greening (HLB)
- Citrus Canker (CC)
- *Citrus Variegated Chlorosis* (CVC)
- *Ralstonia solanacearum* R3B2
- *Rathayibacter toxicus*
- *Xanthomonas oryzae*
- *Synchytrium endobioticum*
- *Citrus leprosis virus* (CiLV)
- *Plum pox virus* (PPV)
- Potato Cyst Nematode (PCN)

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Cooperative Agriculture Pest Survey (CAPS)

Pathogen	Disease
Candidatus Phytoplasma	Several diseases
Harpopora maydis	Late wilt of corn
Hymenoscyphus pseudoalbidus (Chalara fraxinea)	Ash dieback
Monilina fructigena	Brown rot
Phytophthora alni	Alder root & collar rot
Phytophthora austrocedrae	Cypress mortality

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Plant Germplasm Quarantine Program

Pathogen	Method
Nepoviruses subgroup A	RT-PCR
Nepoviruses subgroup B	RT-PCR
Nepoviruses subgroup C	RT-PCR
Black currant reversion virus (BRV)	RT-PCR & qRT-PCR
Tomato black ring virus (TBRV)	RT-PCR & qRT-PCR
Potato black ringspot virus (PBRSV)	RT-PCR & qRT-PCR
Tomato ringspot virus (ToRSV)	RT-PCR & qRT-PCR
Arabis mosaic virus (ArMV)	RT-PCR & qRT-PCR

Method Development

Next Generation Sequencing for Molecular Diagnostics

- What if the standard gene targets are not informative enough?
- What if plant symptoms are not associated with a known pathogen or strain?
- What if instead of targeting one gene we could target the entire genome of the pathogen?
- What if in addition to the presence/absence of a pathogen we could easily trace its origins?

All these can be supported by incorporating Next Generation Sequencing into the methods development and diagnostics work flow.
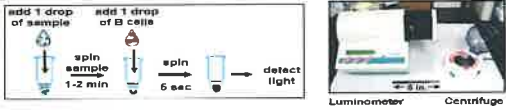
The MiSeq instrument is a benchtop sequencer for small scale labs that will allow us to start using NGS data for identifying better diagnostic targets.

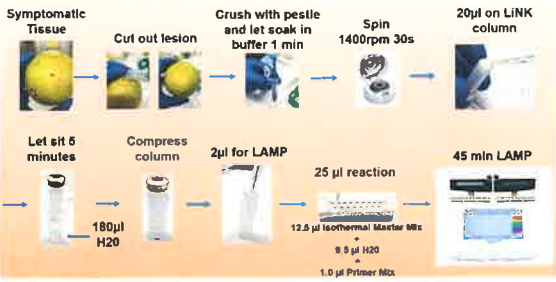## Evaluation and Adaptaion of New Technologies

CANARY Assay Operation

- Simple procedure
- Simple devices
- Fast: ~ 3 minutes
- Highly sensitive

- Diagnostic Laboratory
- Plant Inspection Station
- Field Survey

Loop-mediated Isothermal Amplification

LAMP Detection for Citrus Black Spot

Symptomatic Tissue → Cut out lesion → Crush with pestle and let soak in buffer 1 min → Spin 1400rpm 30s → 20µl on LiNK column

Let sit 5 minutes → Compress column → 2µl for LAMP → 25 µl reaction → 45 min LAMP

180µl H20

12.5 µl Isothermal Master Mix
8.5 µl H20
1.0 µl Primer Mix

12 minutes for sample preparation/ amplifications in 8-20 min of LAMP.

Proficiency Testing (PT)

- A method to verify that the performance of each network laboratory diagnostician is in line with other lab/s diagnosticians performing the same analysis.
- Provides external and independent assessment of accuracy of the results generated by each participant.

NPPLAP
National Plant Protection Laboratory Accreditation Program

Enhance PPQ ability to respond to and manage intentional and unintentional introductions of plant diseases and pests by increasing the reliability and speed of diagnostic tests.

- Accredit laboratories in the National Plant Diagnostic Network (NPDN), State Depts. of Ag., federal and private or commercial sectors
- Carry out diagnostic tests on plant pathogens or pests *of regulatory concern*
- Defined standards for facilities, equipment, personnel training, sample tracking, and methods.

Technology Transfer - Hands-on Training

March 2015

Beltsville Laboratory

Protecting U.S. Agriculture at the Molecular Level

Thank You