**All PPCDL/Beltsville Workshop Description****s**

**Bioinformatics of Sanger Sequencing Workshop-Module SS**

The Bioinformatics Module SS workshop is taught over 3 days and has both lecture and hands-on activities. The lectures will cover a range of sanger sequencing topics from primer design to introductory sequence analysis, as well as the molecular classification and diagnostics of a wide range of pathogens including bacteria, fungi, nematodes, Phytoplasmas, viruses and *Phytophthora* species. The hands-on portion of the workshop allows participants to analyze provided sequence data and use various bioinformatic tools for detection and diagnosis of plant pathogens. Participants also may analyze their own Sanger sequence data.

In recent years, this workshop has been presented using a virtual delivery format. Participants must have access to a computer. Presentation slides are text heavy, therefore, phones and tablets are not recommended. Participants must have access to Geneious prime software for the activities. Trial versions and monthly rental may be available if your institution does not own a license.

**Bioinformatics of High Throughput Sequencing Workshop-Module HTS**

The Bioinformatics of High Throughput Sequencing (HTS) is a 3-day lecture and hands-on based training that familiarizes participants with the latest in HTS technologies and analysis tools. The lectures will cover introduction to HTS technologies, its applications in plant pathogen diagnostics and data analysis pipelines.

The hands-on module will allow participants to analyze example datasets using various open-source sequence analysis software publicly available on the Galaxy server for detection of plant pathogens.

**Experiential Bioinformatics of High Throughput Sequencing Workshop-Module EHTS 102**

The Experiential Bioinformatics Workshop Module EHTS 102 Workshop is a 3-day, primarily hands-on based training that familiarizes participants with the latest in HTS technologies and analysis tools. A small group of up to 5 participants will generate their own MinION sequence data and analyze it using various sequence analysis software for detection and diagnosis of plant pathogens.

Participants **must** have completed the core NPDN Bioinformatics of HTS training and should be proficient in plant DNA/RNA extraction, PCR, and other molecular biology techniques.

**Citrus Canker Molecular Detection Workshop**

The citrus canker molecular detection workshop is being offered in person for the first time in 2023. The workshop will have lecture and hands-on components. The lectures will cover the disease characteristics and an overview of citrus canker diagnostic testing at the USDA-APHIS-PPQ S&T Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL).

The hands-on portion of the training will focus on sample preparation, screening diagnostics and interpretation of results.

**Isothermal Amplification Workshop**

The isothermal amplification workshop for 2023 will be offered in-person and will have lecture and hands-on components. The lecture will cover isothermal amplification technologies and assay design. The hands-on portion of the workshop will include nucleic acid extraction and loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA) targeting select bacterial and fungal pathogens.

Pathogens covered include:

* Citrus Black Spot (Guignardia citricarpa)
* Citrus greening (*‘Candidatus* Liberibacter asiaticus’)
* Ash Dieback (*Hymenoscyphus fraxineus*)

**Phytophthora 101 Workshop**

The *Phytophthora* 101 Workshop is a 4.5-day workshop that has lecture and hands-on components. Lecture materials include a review of *Phytophthora ramorum, P. austrocedri,* and the *P. kernoviae* complex, DNA extraction from leaf and woody host tissue, the real-time PCR protocols used for screening detection of *P. ramorum* (ITS and Elicitin), *P. austrocedri* (ITS and COX assays), and the *P. kernoviae* complex (ITS1 and ITS2), and interpretation of results. Each day will consist of a combination of lecture and lab work, as well as discussion of participant results, troubleshooting, and good lab practices to obtain optimal results.

The Phytophthora 101 Workshop is one of the most requested workshops we offer. The workshops often fill the available spaces very quickly and we often have a waitlist.

***Phytophthora* *austrocedri* Workshop**

The *Phytophthora austrocedri* Workshop is a 1.5-day workshop that has lecture and hands-on components. Lecture materials include a review of *Phytophthora austrocedri*, DNA extraction from woody host tissue, and the real-time PCR protocol used for screening detection of *P. austrocedri*. The workshop will also include discussion of participant results, troubleshooting, and good lab practices to obtain optimal results. Participants must have taken Phytophthora 101 within the past three years to sign up for this workshop.

**Phytoplasmas Molecular Detection Workshop**

The Phytoplasma Molecular Detection Workshop will be returning in 2025. This 2-day workshop provides an introduction on Phytoplasmas, their taxonomy and disease symptoms associated with some of these bacterial pathogens of interest to APHIS PPQ. The hands-on portion of the training will focus on available phytoplasma-infected samples and cover sample preparation, DNA extraction/purification, molecular assays, and interpretation of results.

**Seed Pathogen Testing Workshop on CGMMV, ToBRFV, Popsiviroids**

Seed Pathogen Testing Workshop is a 2-day workshop that has lectures and a hands-on training component. Lectures include a background of seedborne viral diseases of cucurbits and solanaceous plants and review of methods for the detection and identification of seedborne viral pathogens, focusing on nucleic acid extraction principles and methods of manual and high throughput extraction from seeds. The hands-on portion of the training will focus on manual and high throughput nucleic acid extraction from cucurbit and solanaceous seeds. Robotic nucleic acid extraction training will be provided on ThermoFisher Scientific KingFisher Apex nucleic acid purification systems.

**General Best Laboratory Practices**

The good laboratory practices and quality management systems workshop is being offered for the first time in 2023. The workshop will be held virtually and will be a prerequisite for all other NPDN trainings this year. The quality management portion of the lecture will discuss quality management systems vs ISO 17025 accreditation and the importance of record management in all aspects of quality management. The good laboratory practices portion of the lecture will discuss aseptic technique, preventing contamination and general recommendations for working in a laboratory.

**Real-time PCR basics**

The TaqMan probe real-time PCR basics workshop is being offered for the first time in 2023. The workshop will be a virtual lecture. The lecture will introduce the basic principle of TaqMan Probe Real-time PCR, knowledge about the instruments, assay design / development, and method validation. Regarding applying the knowledge in problem solving, examples from our practices will be discussed in, e.g., methods for detection of HLB (aka. Citrus greening), Citrus Variegated Chlorosis (CVC), Bacterial Blight of rice.

Pathogens exemplified in the lecture include:

* Huanglongbing, aka. Citrus Greening (“*Candidatus*” Liberibacter asiaticus)
* Citrus Variegated Chlorosis (*Xylella fastidiosa* CVC strains)
* Bacterial Blight of rice (*Xanthomonas oryzae* *pv.* *oryzoe* *vs. X. oryzae USA strains*)

**Production and Validation of Diagnostic Assay Controls**

This training will discuss the need and benefit of using validated assay controls, type of the controls and their production, followed by validation schemes to evaluate homogeneity and stability, and how to maintain and document traceability. It will be approximately 2 hrs. including Q and A. Thanks!

**Sample Submission for Confirmatory Diagnostic Testing**

This virtual workshop will describe how to submit suspect samples, including Select Agents, for regulatory confirmatory diagnostic testing at the PPCDL. The presentation will focus on answering questions about the steps of the diagnostic process, sample quality, shipping procedures, and who needs to be contacted at various steps. Questions covered include: What determines if a sample needs confirmatory diagnostic testing? How to start the process of submission? Who needs to be informed or contacted? How are results reported and to whom? What materials need to be included in the shipment? Where is the suspect sample sent? How does one learn if steps are changed? Participants will also have ample time to ask their own questions.

**ELISA Fundamentals Workshop**

This 2-day in-person workshop will cover all aspects of ELISA testing using commercially available kits. Lectures will cover the fundamentals of ELISA, different testing approaches and advantages and disadvantages of ELISA types with an emphasis on Good Lab Practice (GLP). The hands-on portion will give participants exposure to processing samples, the ELISA workflow and results interpretation.

Note: This workshop is not required in order to take the ELISA proficiency testing panel.