Next Generation Sequencing Applications for Plant Protection

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How PPQ uses DNA information

- Pest identification
- Pest detection
- Germplasm screening
- Pathway analysis

- What is it?
- Where is it?
- Is this plant infected?
- Where did it come from?
History of DNA sequencing

Maxam-Gilbert chemical degradation sequencing 1977
Sanger chain termination sequencing 1977 First genome, Bacteriophage phiX174

PCR 1983

1977 First genome, Bacteriophage phiX174
1978 First plant pathogen, Potato spindle tuber viroid

ABI markets first automated DNA sequencer 1987
1990 Human Genome Project starts

Whole genome-shotgun sequencing 1995
1995 First bacteria genome, *Haemophilus influenzae*
1998 Google founded

454 LifeSciences markets NGS parallelized pyrosequencing 2004
2003 Human Genome Project complete

Solexa/Illumina NGS on the market 2006
2007 First iPhone

Life Technologies markets new NGS technologies 2010
2014 Illumina announces the $1000 human genome
2015 Real-time genome sequencing for Ebola surveillance
Comparison of Sequencing Technologies

Next generation sequencing

• Includes several high throughput DNA sequencing technologies capable of sequencing millions of molecules simultaneously
• Sequence numerous genes at a time or all DNA in a sample
• IT/data intensive

Sanger Sequencing

• Sequence one region at a time
• Better for sequencing a small number of regions
• Fast, low cost
DNA Sequencing Equipment

ACGT
There are known knowns. There are things we know we know.

We also know there are known unknowns. That is to say, we know there are some things we do not know.

But there are also unknown unknowns— the ones we don't know we don't know.

Donald Rumsfeld
Grapevine red blotch-associated virus

- Disease first recognized in 2008; NGS used to determine pathogen in 2012
- Found in grapevines across North America
- National Clean Plant Network supporting testing of germplasm

Sudarshana et al. 2015. Phytopathology 105: 1026-1032
Nectarine stem pitting-associated virus

- Detection of new Luteovirus in nectarine germplasm from France after clearing post entry quarantine.
- No obvious symptoms except stunting, but stem pitting observed after bark removed.
- NGS of extracted dsRNA detected a novel virus
- Current molecular assays only test for known pathogens

Bag et al. 2015. Phytopathology 105: 840-846
Boxwood blight diagnostics development

- NGS used to develop draft genomes of *Calonectria henricotiae* and *C. pseudonaviculata*, fungal pathogens causing boxwood blight
- Designed organism-specific markers/ LAMP diagnostic assays to quickly detect the two boxwood blight pathogens and exclude other common boxwood pathogens
- NGS/ whole genome sequencing used to show first report of *C. pseudonaviculata* causing blight on Sarcococca (sweet box)
- First application to identify causal agent of fungal disease; Less than a week; $700 in reagents

Detection of sugarcane viruses in quarantine programs

• PPQ Plant Germplasm Quarantine Program and ARS tested sugarcane samples by NGS and standard diagnostic tests.

• NGS detected all viruses found using standard tests and found an additional pathogen (Sugarcane bacilliform virus)

• NGS testing completed in 2.5 weeks (sent out) vs. 2-3 weeks of staff time to complete all of the in-house virus panel tests
How NGS can improve plant protection

• Improved detection of pests
  o Detection of unknown and cryptic pathogens
  o Faster method to detect pests and certify that post-therapy material is free from pests

• Certified plant material for export

• Pest identification
  o Identify novel or unknown pests without pre-existing sequence knowledge
  o Quickly identify DNA sequences that distinguish species, strains to design new diagnostic tests

• Pathway analysis
  o Rapidly sequence thousands of DNA regions to identify pest origin
How NGS can complicate plant protection

• Discovery of pathogen-like sequences in germplasm without biological evidence of disease
• Discovery of new pathogens without information on distribution and economic impact could have trade impacts
• Application limited by logistics and resources
• Lack of international standards on testing
• Could be required by other countries as a condition of entry for our exports
PPQ plans for NGS

- Developing roadmap for NGS implementation

Considerations
- Personnel expertise and training
- In-house equipment or commercial sequencing
- IT infrastructure
- Collaboration with stakeholders