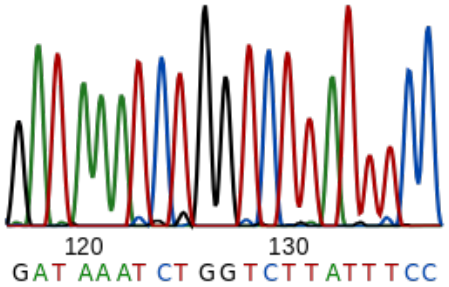
USDA
United States Department of Agriculture

Next Generation Sequencing Applications for Plant Protection

PHILIP BERGER, MARTHA MALAPI-WIGHT,
YAZMIN RIVERA, JENNIFER NICHOLSON
PLANT PROTECTION AND QUARANTINE
NATIONAL PLANT BOARD ANNUAL MEETING
AUGUST 3, 2016

USDA
United States Department of Agriculture

How PPQ uses DNA information



- Pest identification
- What is it?

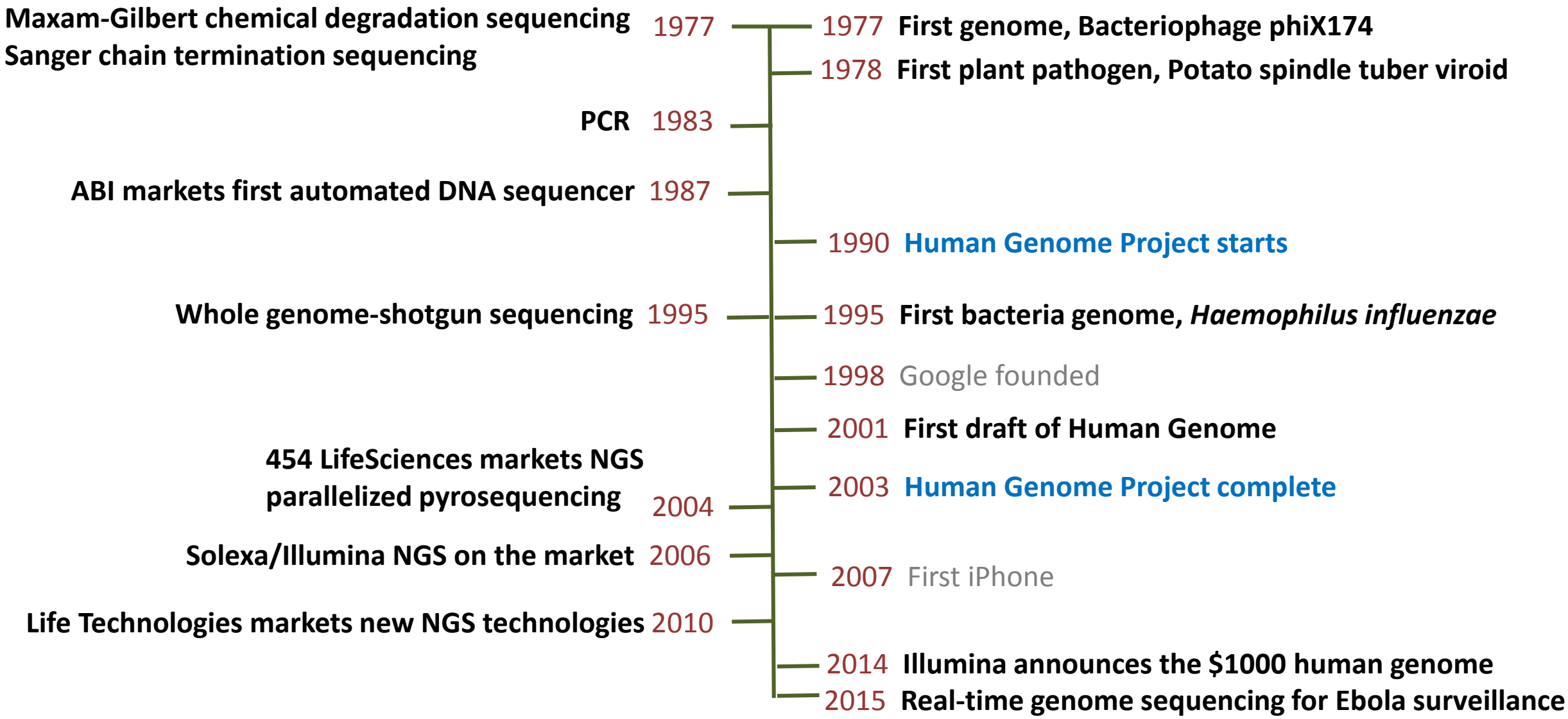
- Pest detection
- Where is it?

- Germplasm screening
- Is this plant infected?

- Pathway analysis
- Where did it come from?

USDA
United States Department of Agriculture

History of DNA sequencing



USDA
United States Department of Agriculture

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

USDA
United States Department of Agriculture

DNA Sequencing Equipment

ACGT

USDA
United States Department of Agriculture

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

Visible symptoms, with known pathogen

Standard diagnostics ✓

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

Visible symptoms, but unknown pathogen

NGS ✓

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

Donald Rumsfeld

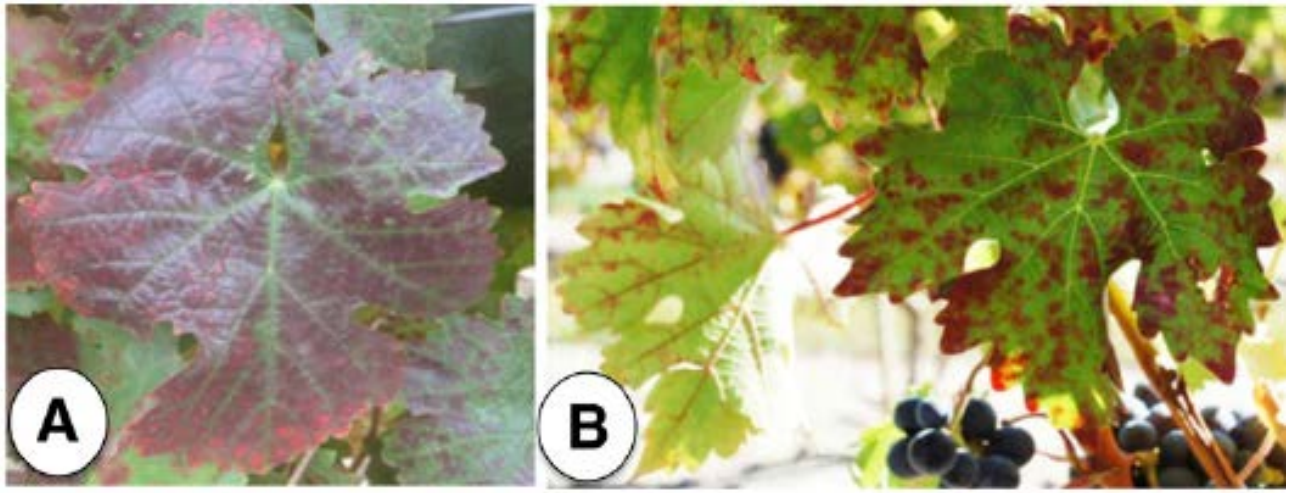
Cryptic symptoms, unknown pathogen

NGS ✓

USDA
United States Department of Agriculture

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



Grapevine red blotch-associated virus

Grapevine leafroll-associated virus 3

Sudarshana et al. 2015. Phytopathology 105: 1026-1032

USDA
United States Department of Agriculture

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

USDA
United States Department of Agriculture

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

Malapi-Wight et al. 2016. Nature Scientific Reports 6:26140
Malapi-Wight et al. 2016. Plant Disease. 100: 1093-1100

USDA
United States Department of Agriculture

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

USDA
United States Department of Agriculture

How NGS can improve plant protection

- Improved detection of pests
  - Detection of unknown and cryptic pathogens
  - Faster method to detect pests and certify that post-therapy material is free from pests
- Certified plant material for export
- Pest identification
  - Identify novel or unknown pests without pre-existing sequence knowledge
  - Quickly identify DNA sequences that distinguish species, strains to design new diagnostic tests
- Pathway analysis
  - Rapidly sequence thousands of DNA regions to identify pest origin

USDA
United States Department of Agriculture

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

USDA United States Department of Agriculture

PPQ plans for NGS

- Developing roadmap for NGS implementation

<u>Considerations</u>

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