# On site Rapid Isothermal Detection of *Phytophthora* species

Frank Martin

USDA-ARS

Salinas, CA

Design of Marker System

- Comparative mitochondrial genomics for systematic development of diagnostic markers (see poster 60)
- Targets conserved mitochondrial gene order differences in *Phytophthora*
  - *atp9-nad9*
  - *trnM-trnP-trnM*
- Initially set up for TaqMan real time PCR
  - Bilodeau et al. Phytopathology 104:733-748
  - See poster 62 (Miles et al.)

TaqMan Real Time PCR Detection
Phytophthora

atp9-nad9
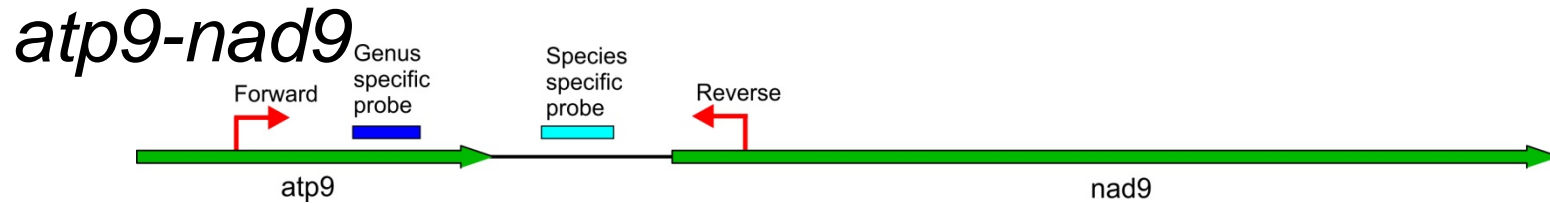
- Have 50 validated species specific TaqMan probes
- Believe can design specific probes for approximately 90% of genus
- Does not work for *P. bisheria* and *P. frigida*

trnM-trnP-trnM
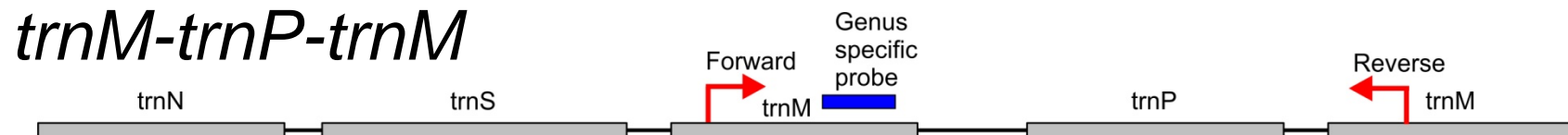
Internal plant control
- Plant *cox1* gene

Isothermal Detection

- Recombinant Polymerase Amplification (RPA)
 - TwistDx in Cambridge, UK
  - Also sold through Agdia (AMPLIFYRP)

Advantages of technology
- Primer and probe design similar to TaqMan, so not difficult to transfer TaqMan assays to RPA
  - May need to work with multiple primers
- Don't need to do DNA extractions, tolerant of inhibitors
- Can multiplex amplifications
- Can use portable fluorometer for data collection directly in the field.

Miles et al. Phytopathology105:265-278   **See poster 61**

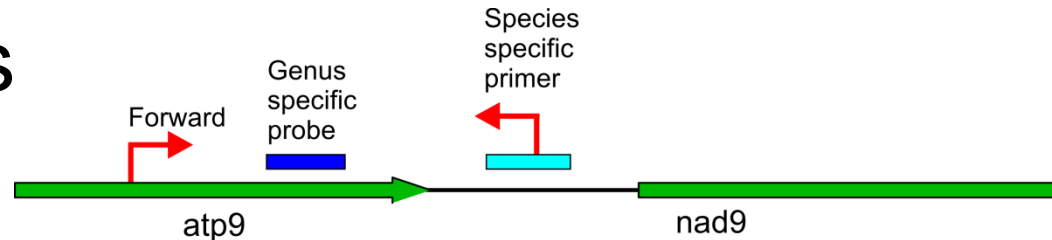# RPA *Phytophthora* Detection Genus Specific

trnM-trnP-trnM locus
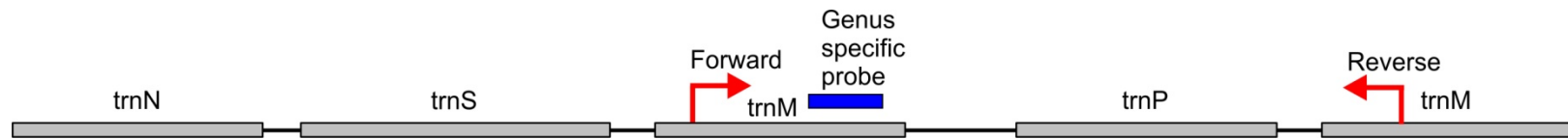
- Amplifies all *Phytophthora* spp.
  - 134 taxa tested
  - No false positives with plant or *Pythium*

Multiplex with plant control amplification to ensure template is amplifiable

RPA *Phytophthora* Detection Species Specific

atp9-nad9 locus

- Genus specific RPA probe designed from same location as genus specific TaqMan probe
  - So only one probe has to be ordered
  - Should be able to have systematic approach for designing species specific reverse primer based on TaqMan probes

Phytophthora Species Specific RPA Assays

Species specific RPA assays have been validated against 134 *Phytophthora* taxa for:

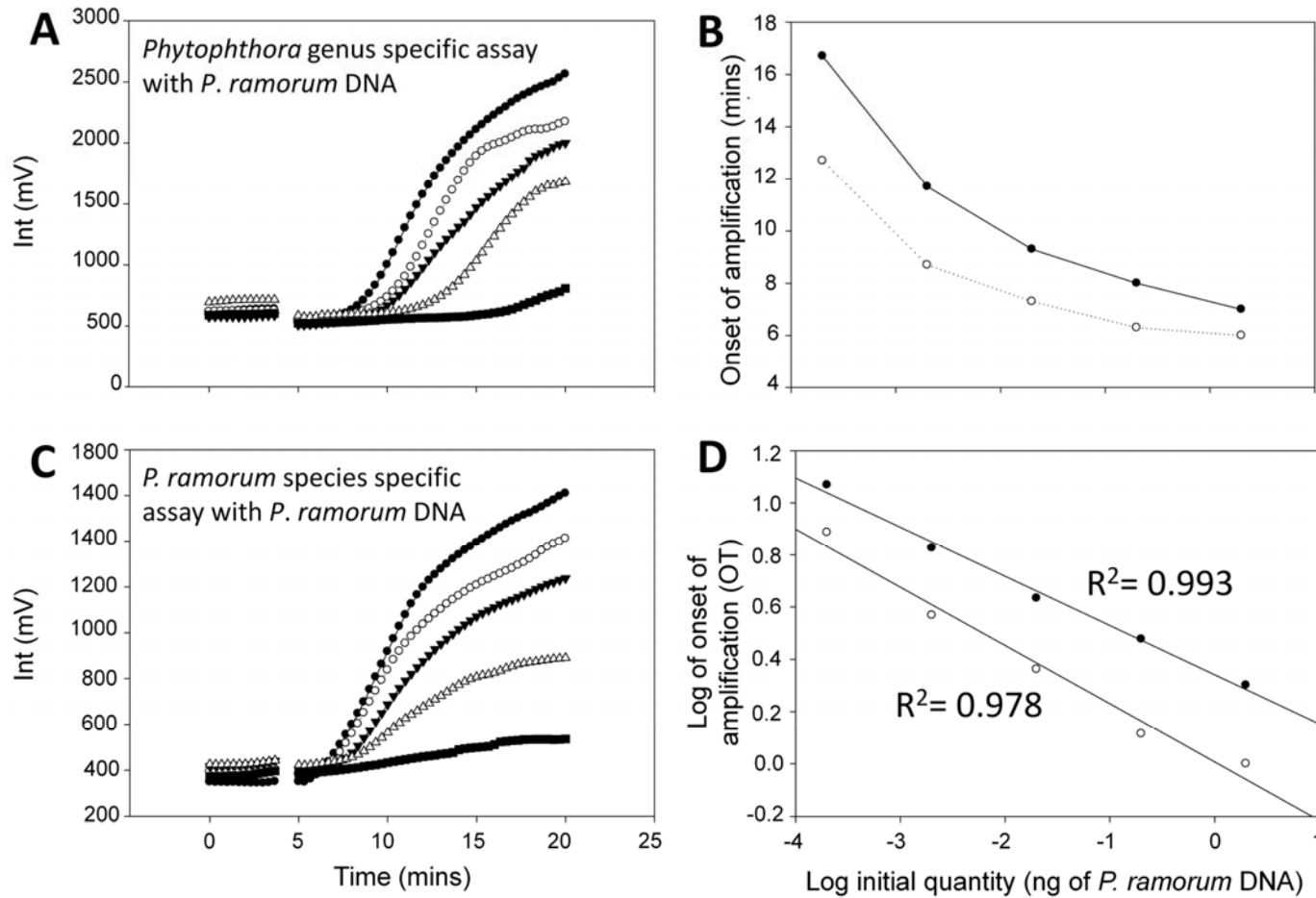|	
P. cactorum	*P. cinnamomi*
P. fragariae	*P. kernoviae*
P. ramorum	*P. rubi*

P. tentaculata

Martin Chilver's lab at Michigan State University

P. sansomeana	*P. sojae*

Amplification Results

Confirm Species Identification by Sequence Analysis

- Use RPA primers that amplify the *atp9-nad9* spacer

- Generate a sequencing template using nested PCR primers

- Have *atp9-nad9* database of over 800 isolates representing 134 *Phytophthora* taxa to support identification

Current Status of *Phytophthora* RPA Assay

- TwistDx formulated kits containing primers and probe in lyophilized pellet
  - Kits worked the same as when we added primers and probe in the lab
- Portable fluorometers
  - BioRanger/SmartDart

Rapid Identification of Strawberry Root Pathogens

- *Phytophthora* spp.
  - *P. cactorum, P. citricola, P. fragariae*
- *Verticillium dahliae*
- *Macrophomina phaseolina*
- *Fusarium oxysporum* f. sp. *fragariae*
- *Pythium* spp.
- *Rhizoctonia* spp.

Conclusion

- *Phytophthora* genus and species specific multiplexed detection with plant control
- Sensitivity of detection (200-300 fg)
- Can be done directly in the field with portable equipment in as little as 15 minutes
- Can PCR amplify sequencing template from RPA amplification for confirmation of species identification
- Designing diagnostic assays for soilborne strawberry pathogens

Systematic Approach for Marker Design

- Comparative Oomycete mitochondrial genomics (275 mitochondrial genomes assembled)
  - *Phytophthora*
  - *Pythium*
  - Downy mildews - TaqMan
    - *Bremia lactucae, Peronospora effusa, Ps. cubensis*
    - *Peronosclerospora philippinensis*

Frank.martin@ars.usda.gov

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