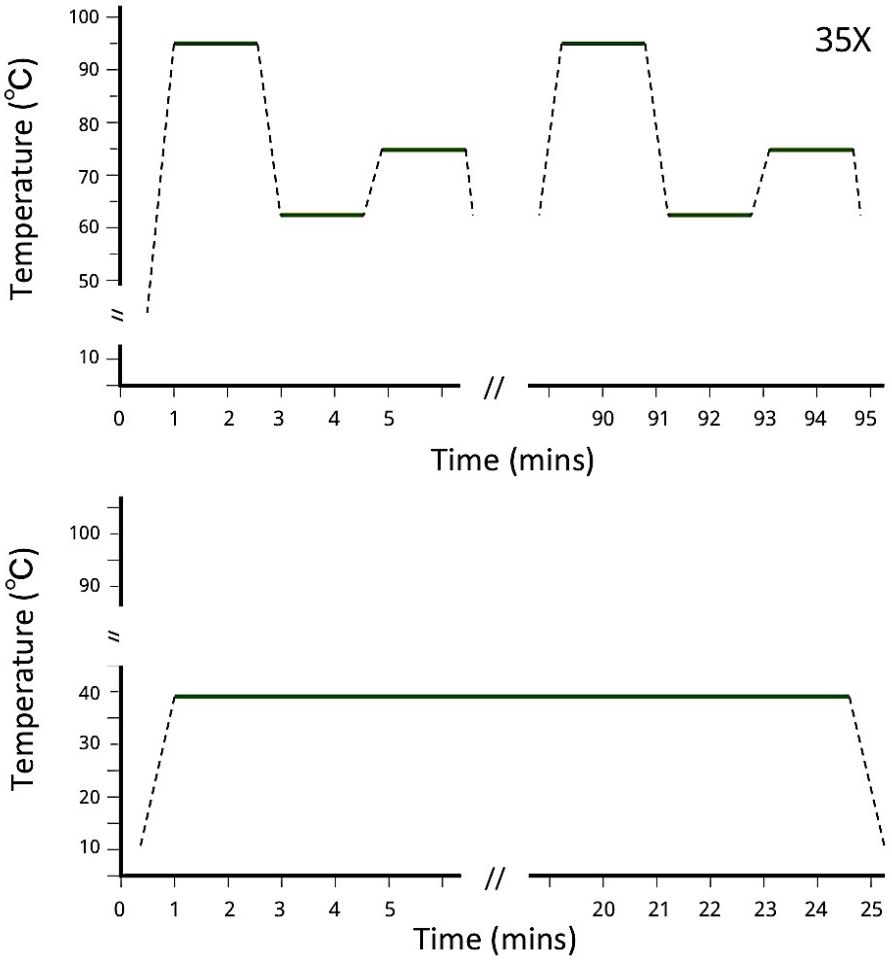
Isothermal amplification *P. sojae* and *P. sansomeana*

Isothermal Amplification

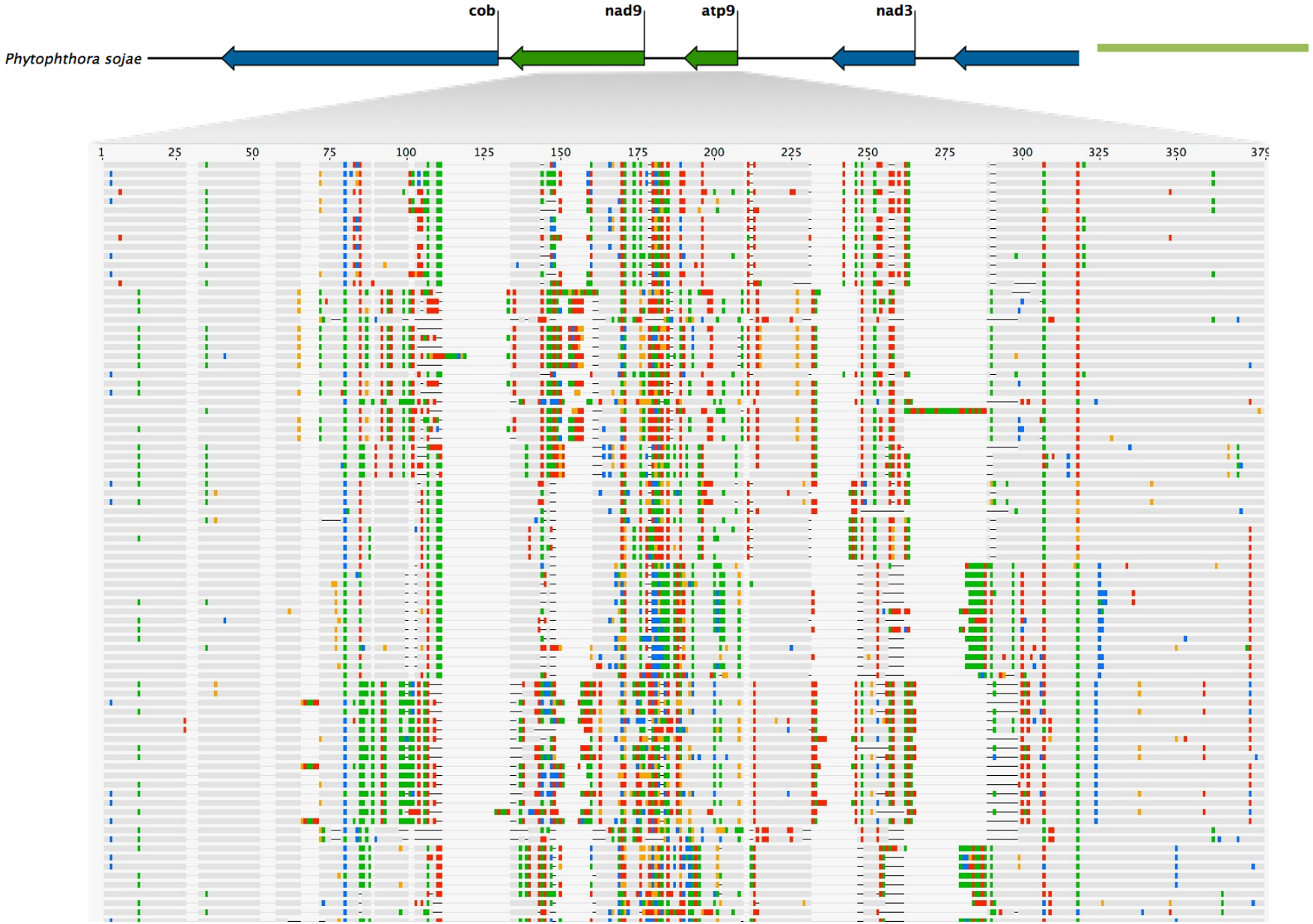


- Multiple isothermal methods developed
  - ~ 11 different techniques
- Loop-mediated isothermal amplification (LAMP)
- Recombinase Polymerase Amplification
- End-Point and Real-time detection

Isothermal Amplification

	SDA Strand Displacement	RCA Rolling Circle	LAMP Loop Mediated Isothermal	HDA Helicase dependent	RPA Recombinase Polymerase
Template	ssDNA	Circular ssDNA	ssDNA	dsDNA	dsDNA
Performance	~90 min >10 copies	~40 min >10 copies	~60 min 1-10 copies	~100 min 100 copies	~20 min 1-10 copies
Temperature	37°C	30 – 65°C	60 – 65°C	64°C	37 – 42°C
Primers	2-4	1	4 - 6	2	2
Tolerance to biological components	Yes	No	Yes	Yes	Yes
Disadvantage	Less efficient in long targets	Ligation is complicated	Complicated primer design	Complex buffer optimization	Stringent conditions**

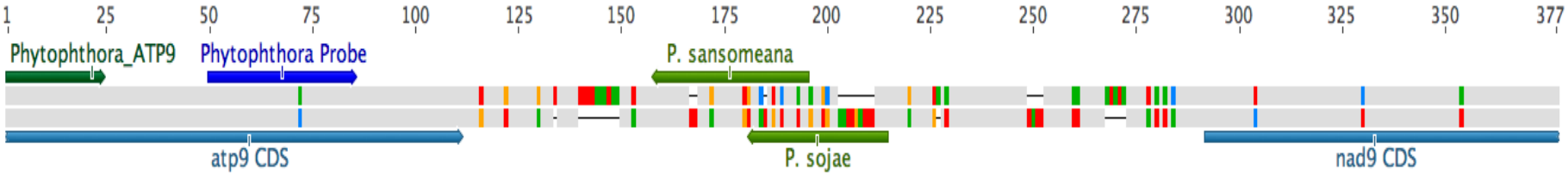
Diagnostic qPCR for *P. sojae* and *P. sansomeana*



Assay Background: RPA isothermal

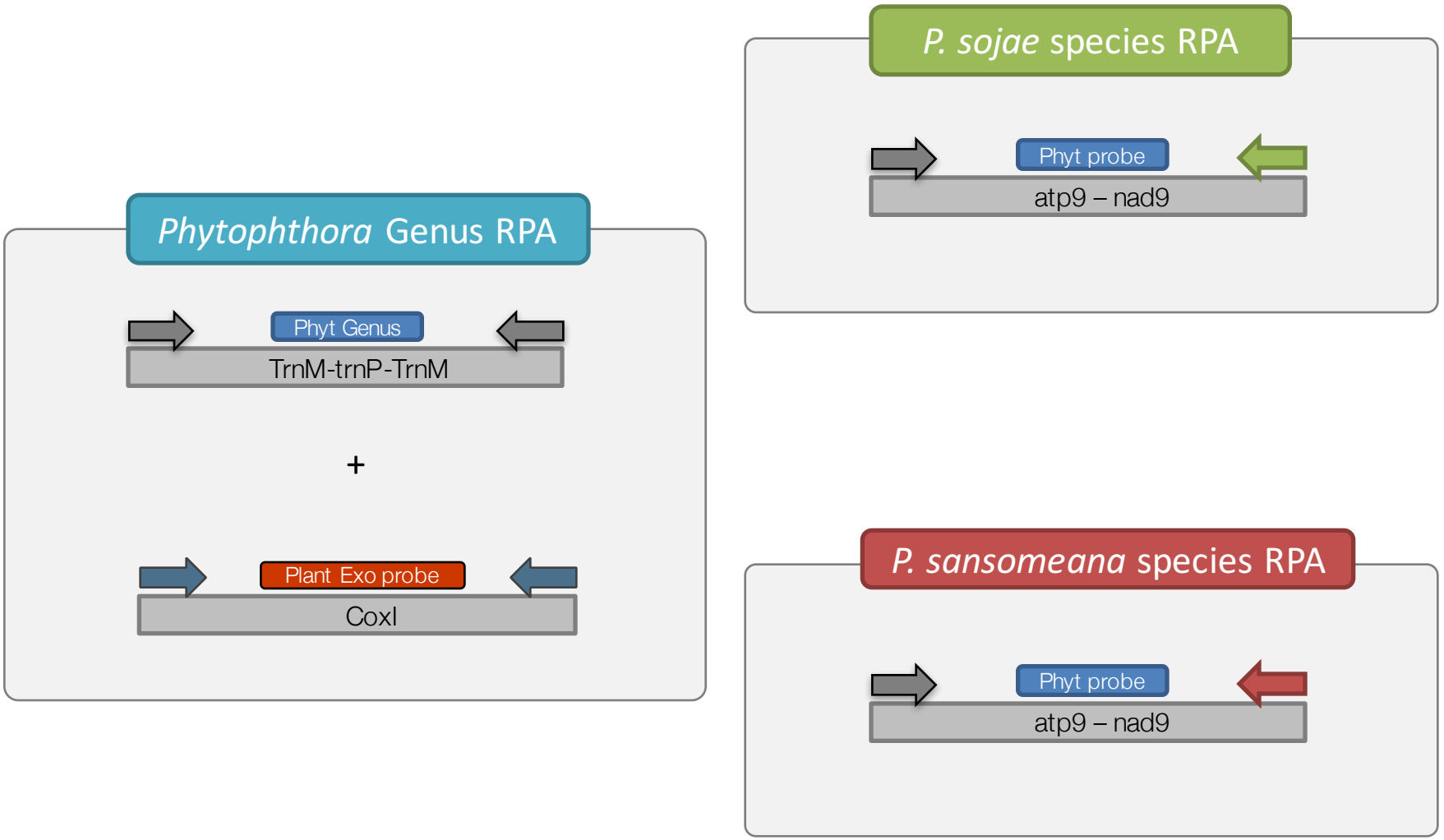
Phytophthora Genus-Specific

Phytophthora Species-Specific

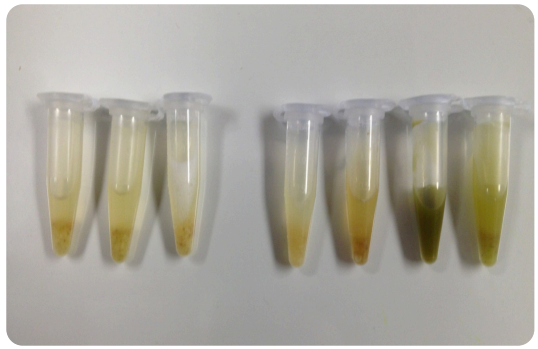


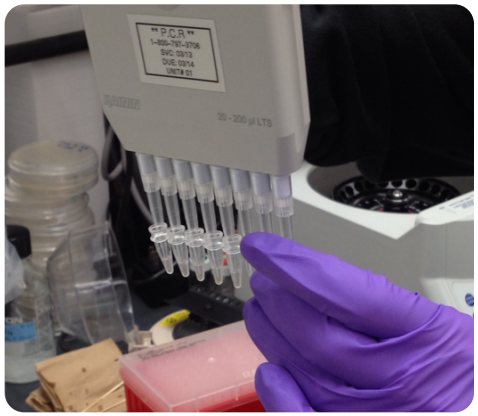
- Optimal primer size ~ 30 bp

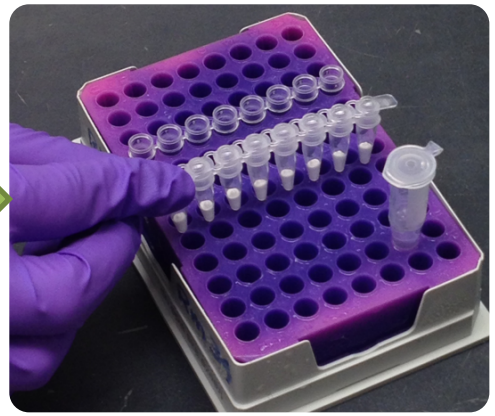
Diagnostic RPA for Genus and Species-specific



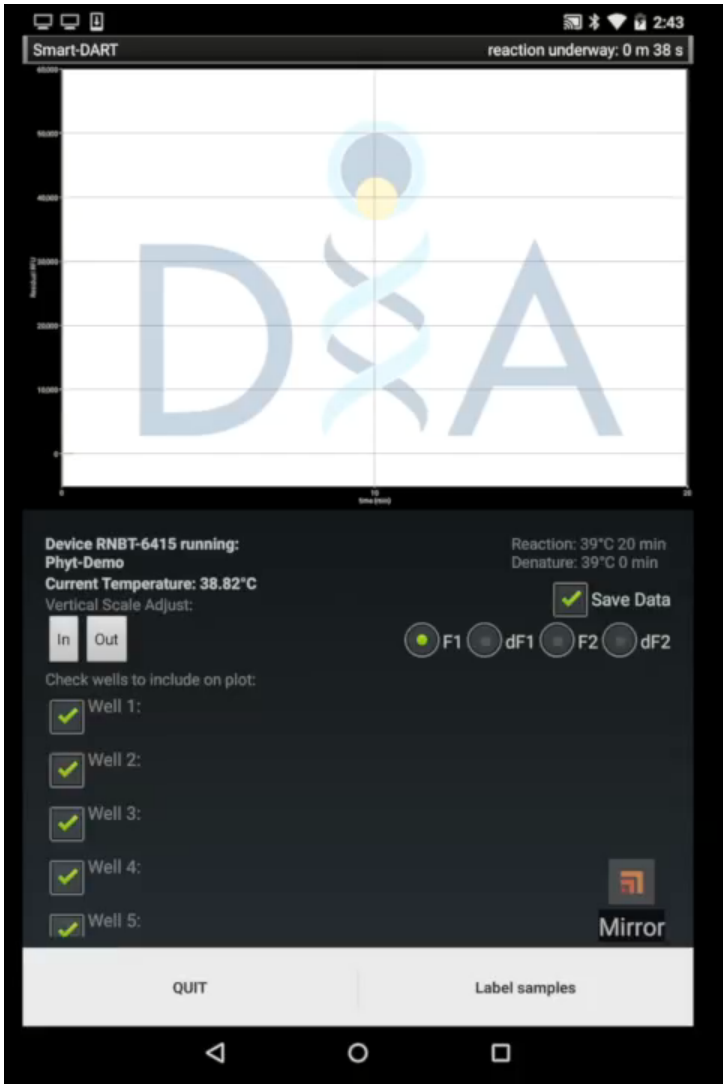
RPA process: *P. sojae* and *P. sansomeana*



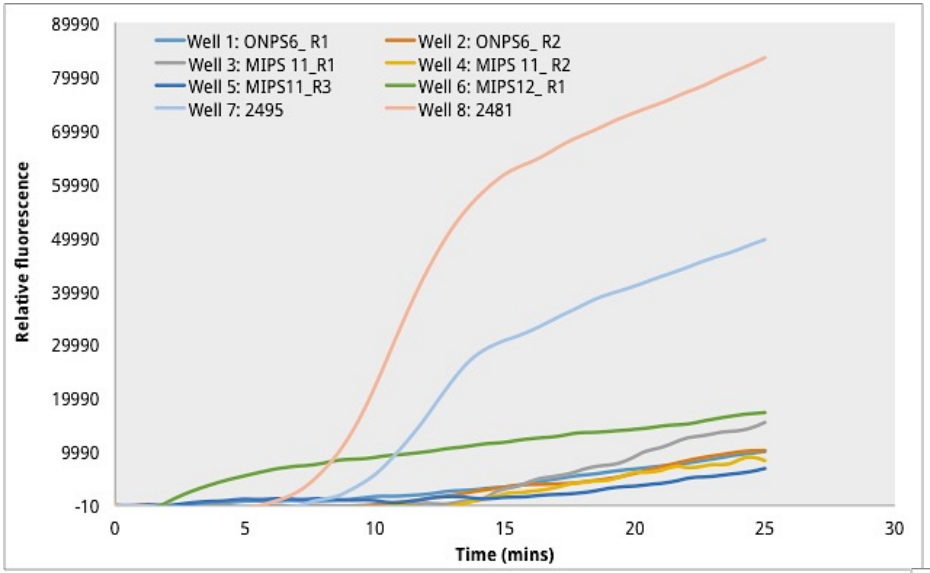




RPA process: *P. sojae* and *P. sansomeana*

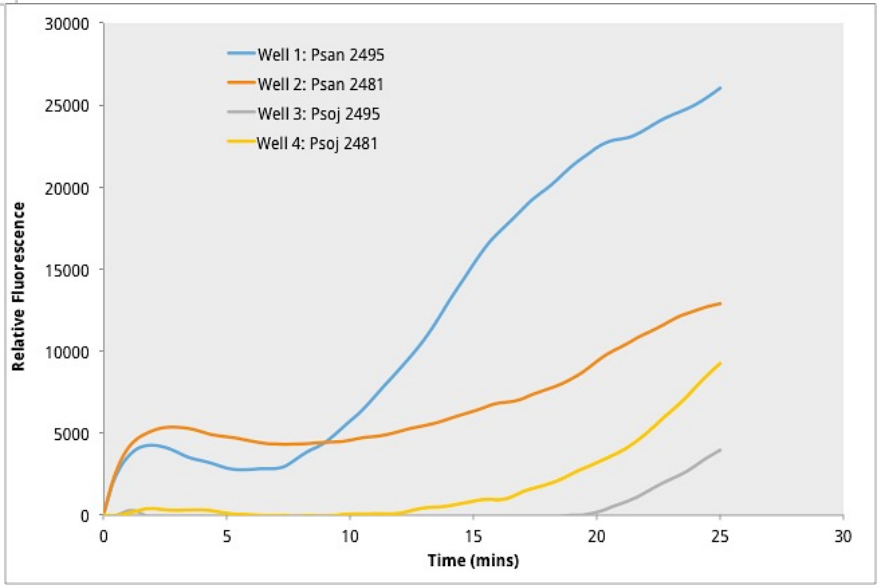


Field Samples: *P. sojae* – *P. sansomeana*



***Phytophthora* Genus-Specific**

***Phytophthora* Species-Specific**



Field Samples: *P. sojae* – *P. sansomeana*

Fields	Isolation	Phytophthora ELISA	RPA *Phytophthora* Genus	
MIPS2	+	+	+	(2/3)
MIPS3	+	+	+	(3/3)
MIPS4	-	+	+	(3/3)
MIPS5	+	+	+	(3/3)
MIPS6	+	+	+	(3/3)
MIPS7	+	NC	+	(2/3)
MIPS8	+	NC	+	(1/3)
MIPS9	+	+	+	(3/3)
MIPS11	-	+	+	(2/3)
MIPS12	-	+	+	(1/1)
ONPS1	+	+	+	(3/3)
ONPS2	+	+	+	(1/2)
ONPS3	+	+	+	(1/3)
ONPS4	+	+	+	(2/2)
ONPS5	-	+	-	(0/2)
ONPS6	-	+	+	(2/2)