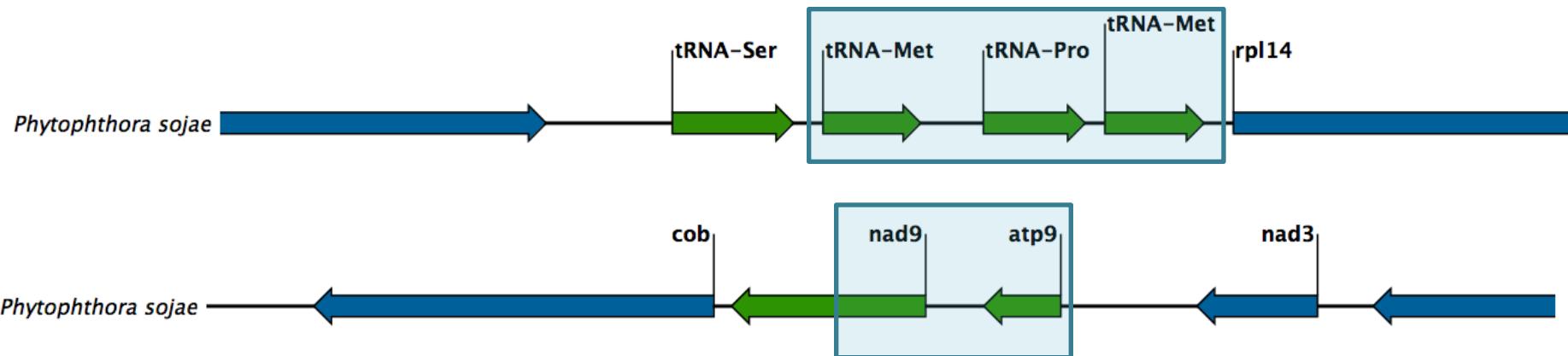
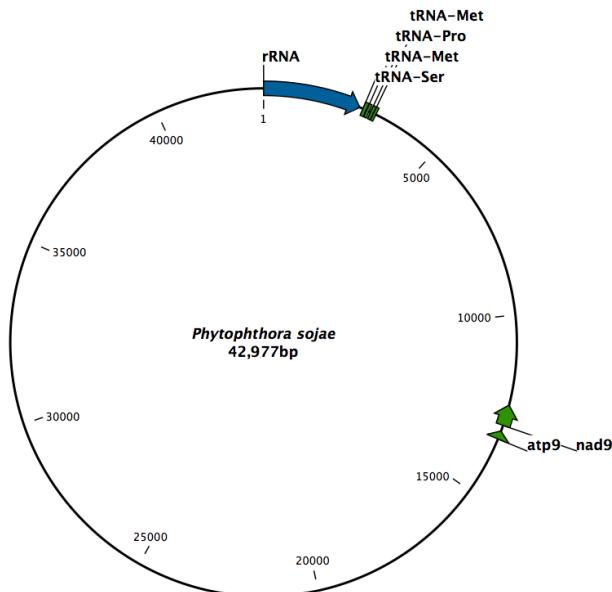


Multiplex quantitative PCR *P. sojae* and *P. sansomeana*

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

- New marker system developed by Bilodeau et al. (2014)
 - Gene order
 - Intra- and inter-specific variability
- Two systems:
 - trnM-trnP-trnM
 - nad9-atp9



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

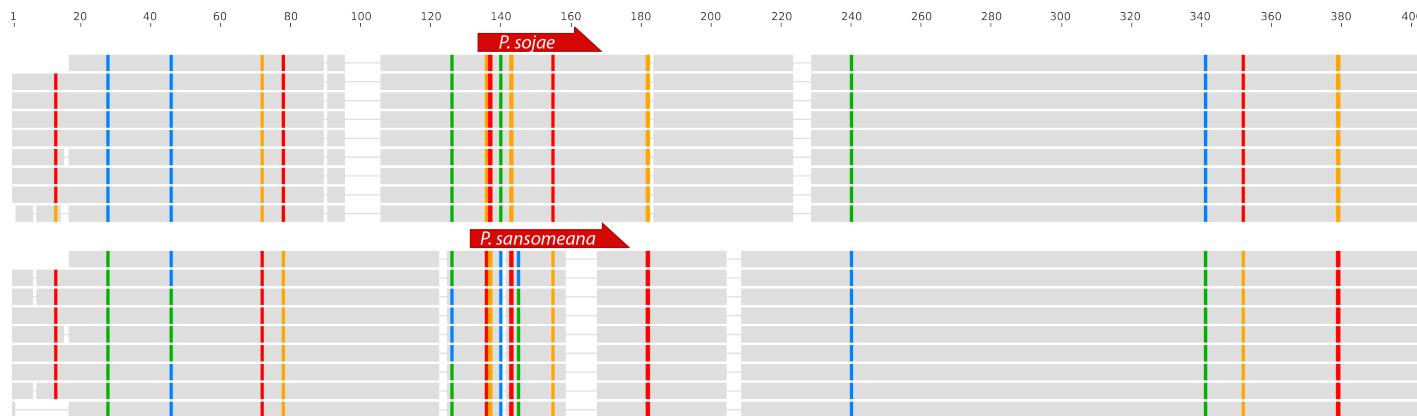


Species-specific probe: *P. sojae* – *P. sansomeana*

- Genus specific

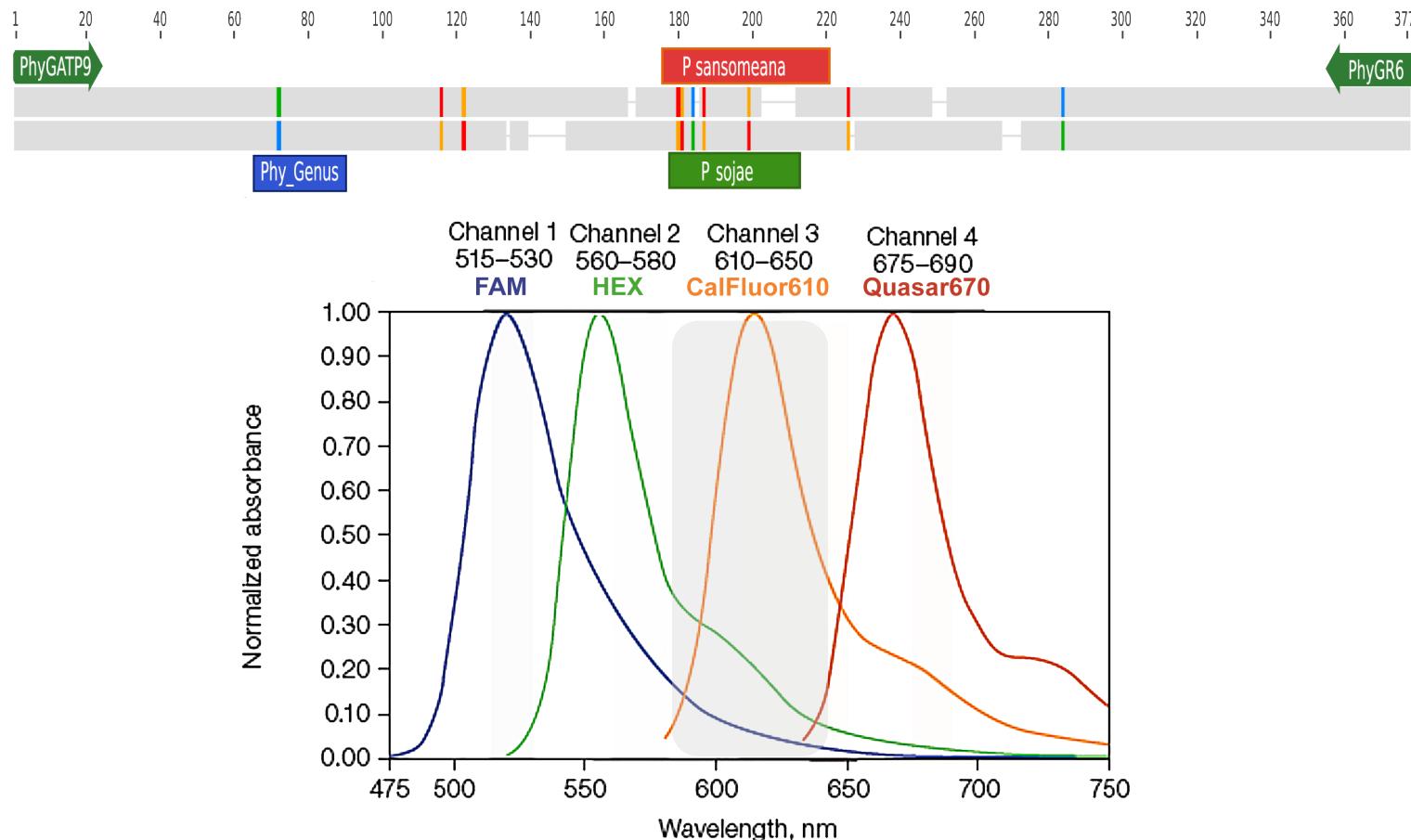


- Intraspecific variability
 - 67 different strains of *P. sojae*
 - 30 different strains of *P. sansomeana*



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

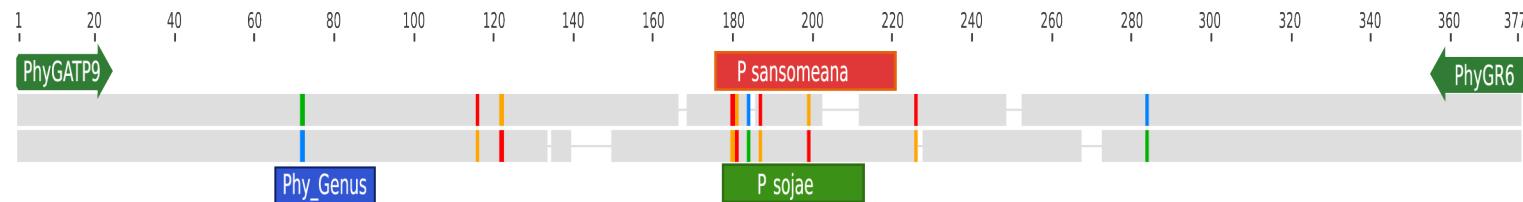
- Multiplex qPCR for the detection of *Phytophthora* on soybean



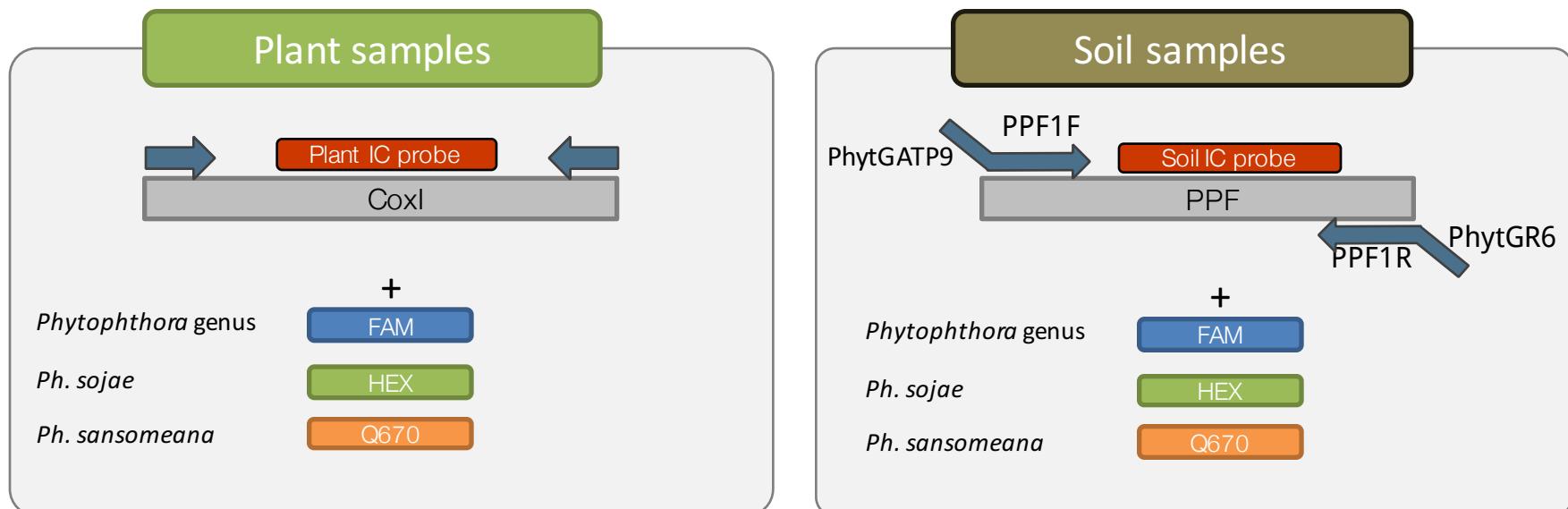
- Two internal controls

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

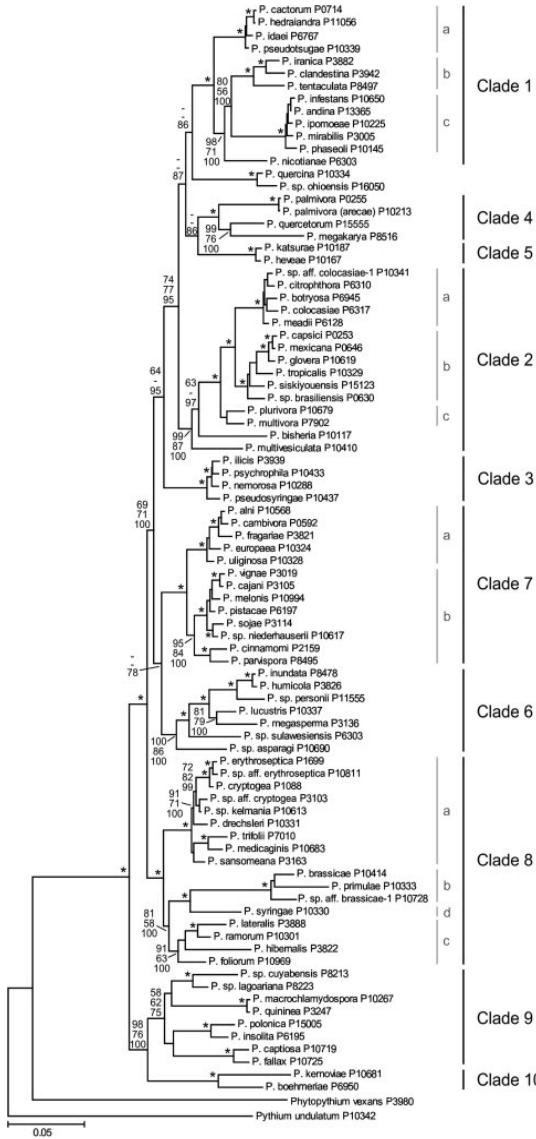
- Multiplex qPCR for the detection of *Phytophthora* on soybean



- Two internal controls

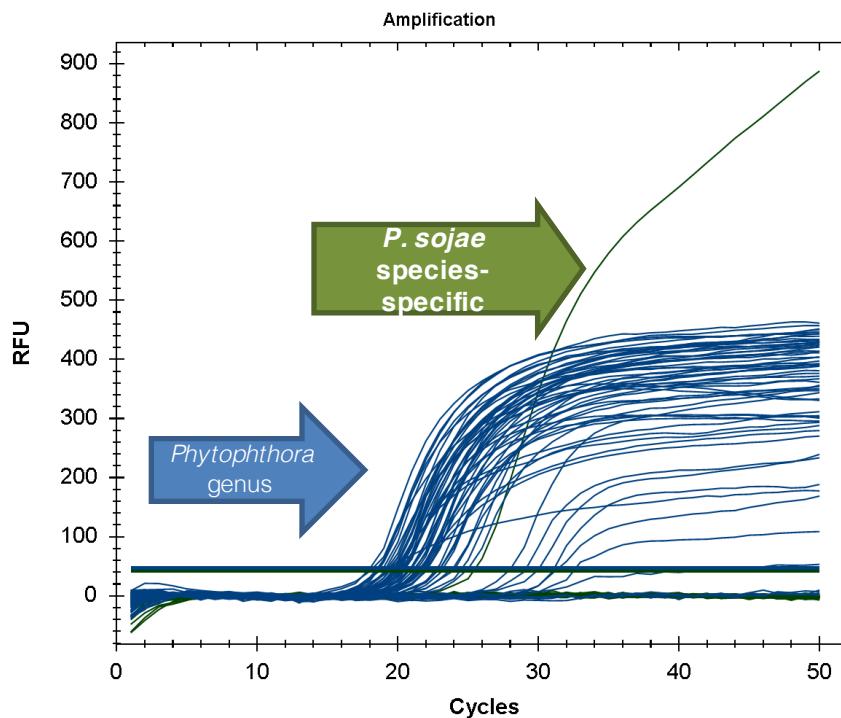


qPCR *Phytophthora* - validation qPCR

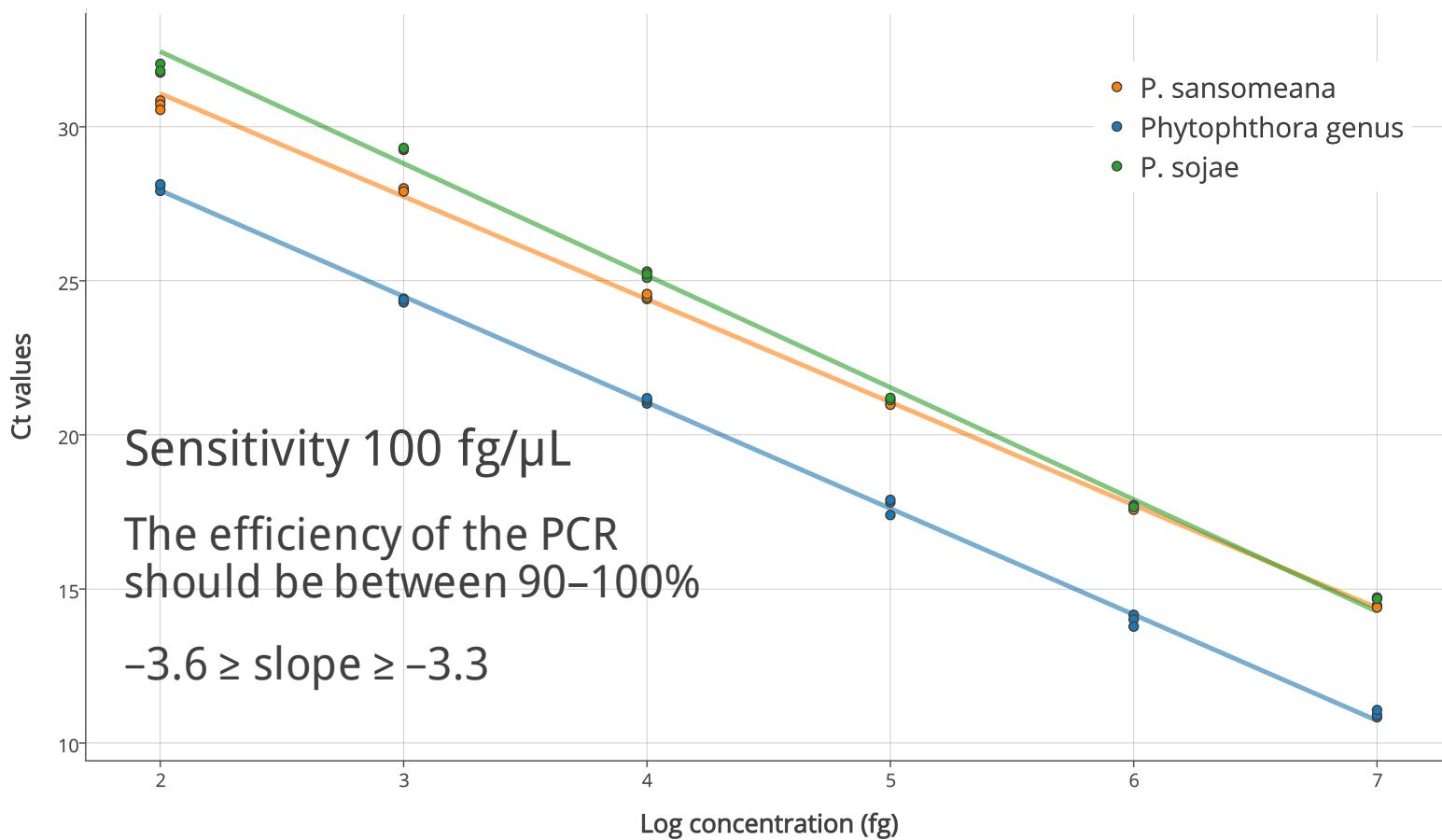


Specificity

- 96 different *Phytophthora* spp.
- 14 provisional *Phytophthora* species
- 10 *Pythium* spp.



qPCR *Phytophthora* - validation qPCR



Sensitivity

- Ten fold diluted standard curve
- Ranging from 10 ng to 1 fg

Cross-platform validation qPCR

- Transferability and consistency
- Validation on multiple platforms

Phytophthora sojae (HEX)

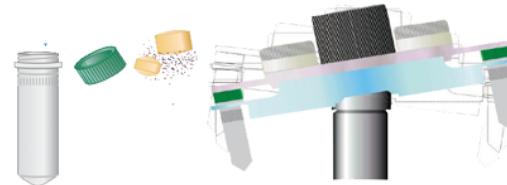
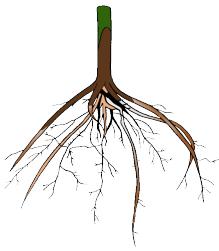
	Platforms		
	Step One Plus (ABI)	LightCycler 96 (Roche)	CFX96 (Bio-Rad)
Efficiency %	95.325	95.5	95.99
Slope	-3.439	-3.563	-3.422
Y-Intercept	41.175	39.35	38.746
R ²	0.998	1	0.997

Phytophthora sansomeana (Quasar670)

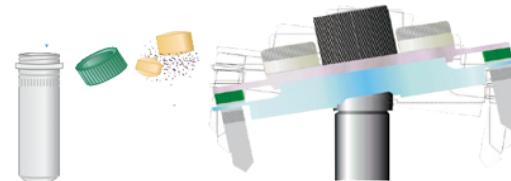
	Platforms		
	Step One Plus (ABI)*	LightCycler 96 (Roche)	CFX96 (Bio-Rad)
Efficiency %	NC	100.5	111.16
Slope	NC	-3.3094	-3.081
Y-Intercept	NC	37.59	36.656
R ²	NC	1	0.988

Samples processing

Plant samples



Soil samples



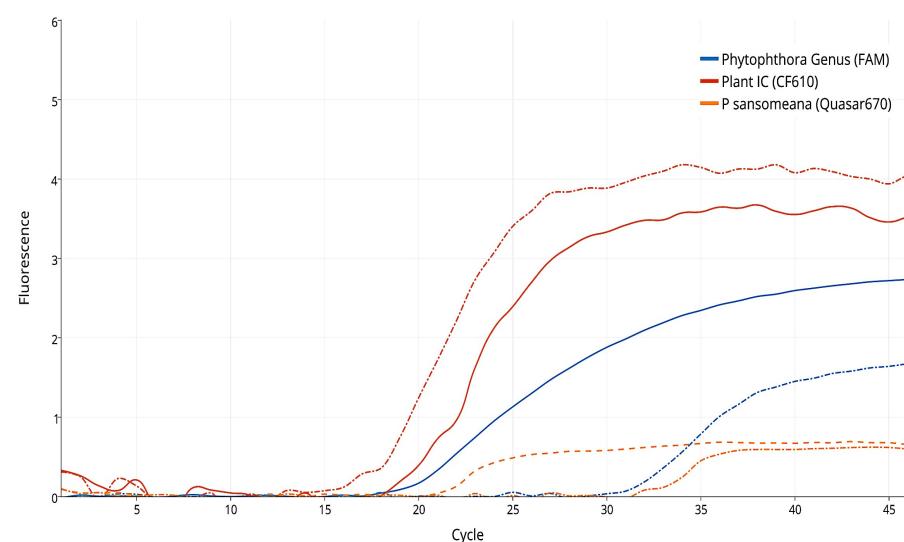
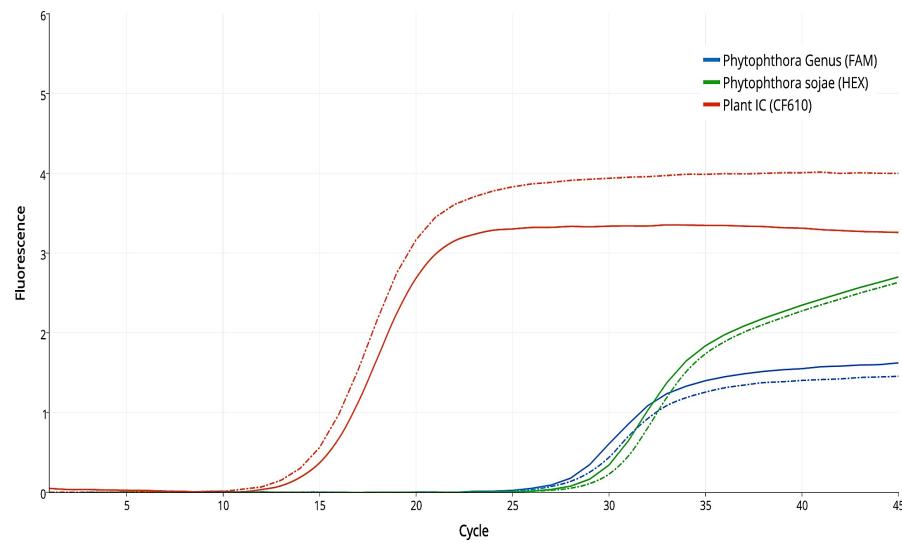
Diagnostic Samples: *Psojae* – *Psansomeana*



Soybean plant infected by *P. sojae* detected with multiplex qPCR assay.



Soybean plant infected by *P. sansomeana* detected with the multiplex qPCR assay.



Field Samples: *Psojae* – *Psansomeana*

Fields	Isolation	Phytophthora ELISA	Positive Samples qPCR*	Plant IC Ct Range	Phytophthora Genus		<i>P. sojae</i>		<i>P. sansomeana</i>	
					Ct range	DNA (fg)	Ct Range	DNA (fg)	Ct Range	DNA (fg)
MIPS2	+	+	(3/3)	9.7 - 12.6	23.9 - 28.7	2.28E+02	25.1 - 30.2	3.68E+02	ND**	-
MIPS3	+	+	(3/3)	11.8 - 12.3	20.6 - 26.2	1.44E+03	21.1 - 27.3	3.97E+03	ND	-
MIPS4	-	+	(3/3)	11.2 - 12	26.1 - 28.1	9.11E+01	27.3 - 29.8	1.42E+02	ND	-
MIPS5	+	+	(3/3)	13 - 14	20 - 21.3	4.05E+03	20.2 - 21.6	1.24E+04	ND	-
MIPS6	+	+	(3/3)	11.6 - 12.3	26.6 - 28.3	7.09E+01	27.5 - 29.5	1.29E+02	ND	-
MIPS7	+	NC	(3/3)	11.8 - 14.8	17.5 - 18	2.48E+04	18 - 18.4	7.49E+04	ND	-
MIPS8	+	NC	(2/3)	12.1 - 13.1	29.7 - 31.2	1.28E+01	31 - 31.7	2.01E+01	ND	-
MIPS9	+	+	(3/3)	13.2 - 13.5	27.3 - 31.5	3.18E+01	28.9 - 32.1	4.61E+01	ND	-
MIPS11	-	+	(3/3)	11.4 - 12.4	23.4 - 27.8	3.34E+02	23.5 - 28.3	1.00E+03	ND	-
MIPS12	-	+	(1/1)	12.9 - 13	24.1 - 24.3	4.87E+02	24.7 - 24.9	1.21E+03	ND	-
ONPS1	+	+	(2/3)	13.7 - 14.9	21.4 - 29.6	9.99E+02	22.3 - 30.2	2.80E+03	ND	-
ONPS2	+	+	(1/2)	15 - 15.3	18.1 - 18.2	2.27E+05	16.8 - 17.7	1.48E+06	ND	-
ONPS3	+	+	(3/3)	13.2 - 14.3	22 - 29	4.61E+02	23.1 - 29.7	1.21E+03	ND	-
ONPS4	+	+	(2/2)	12.2 - 12.4	25.9 - 29.3	6.13E+02	28 - 30.5	1.49E+03	ND	-
ONPS5	-	+	(0/2)	12.9 - 13.1	ND	-	ND	-	ND	-
ONPS6	-	+	(2/2)	13.8 - 14.7	27.5 - 28.5	3.45E+02	28.8 - 29.6	1.31E+03	ND	-