Phytophthora qPCR worksheet – Plant Samples

Master mix

Reagents	Initial Concentration	Volume per reaction (µL)		
		1X	18 X	Check
Primers				
PhyG_ATP9_2FTail	10 µM	1.0	18	
PhyG-R6_Tail	10 µM	1.0	18	
Probes				
Phytophthora genus-specific probe	10 µM	0.05	0.9	
P. sojae species-specific probe	10 µM	0.2	3.6	
P. sansomeana species-specific probe	10 µM	0.1	1.8	
Plant Internal Control				
FMPI2b	1 µM	0.4	7.2	
FMPI3b	1 µM	0.4	7.2	
Plant-IC probe	1 µM	0.4	7.2	
Real Master Mix without Rox (5 Prime)	2.5X	8.0	144	
Mg^{++}	25 mM	2.0	36	
PCR-grade water		4.45	80.1	
Sample DNA		**2 µL**	**36 µL**	
Total volume		**20 µL**	**360 µL**	

Sample set up

16 wells (2 qPCR 8-well strips):

Well	1	Check
A	*100pg*	
B	*100pg*	
C	*10pg*	
D	*10pg*	
E	*1pg*	
F	*1pg*	
G	*100fg*	
H	*100fg*	

Well	2	Check
A	Unknown_1	
B	Unknown_1	
C	Unknown_2	
D	Unknown_2	
E	Unknown_3	
F	Unknown_3	
G	NTC	
H	NTC	

SDS – *Fusarium virguliforme* qPCR worksheet

Master mix

Reagent	Working concentration	Final concentration	Volume per reaction (µL)		Check
			1X	18X	
2X Taqman universal master mix	2X	1X	10	180	
F6-3 Primer	20 µM	500nM	0.5	9	
R6 Primer	20 µM	500nM	0.5	9	
Prb3 Probe	10 µM	250nM	0.5	9	
IC F primer(Internal Control)	20 µM	600nM	0.6	10.8	
IC R primer	20 µM	200nM	0.2	3.6	
IC Probe	10 µM	200nM	0.4	7.2	
IC DNA	3k copies/ul	600 copies	0.2	3.6	
BSA	20 mg/ul	200ng/µl	0.2	3.6	
H_2O	N/A		4.9	88.2	
Sample DNA	N/A		2	36	
Total Volume			**20 µL**	**360 µL**	

Sample set up

16 wells (2 qPCR 8-well strips):

Well	1	Check
A	*100pg*	
B	*100pg*	
C	*10pg*	
D	*10pg*	
E	*1pg*	
F	*1pg*	
G	*100fg*	
H	*100fg*	

Well	2	Check
A	Unknown_1	
B	Unknown_1	
C	Unknown_2	
D	Unknown_2	
E	Unknown_3	
F	Unknown_3	
G	NTC	
H	NTC	

Phytophthora genus-specific RPA worksheet

Master mix

Reagents	Initial concentration	RPA *Phytophthora* genus volume per reaction (µL)		
		1X	4.5X	Check
Genus-specific				
TrnM-F	1 µM	0.50	2.3	
TrnM-R	10 µM	1.45	6.5	
TrnM-Probe	10 µM	0.35	1.6	
Plant Internal Control				
Cox1-IPC-F	10 µM	0.63	2.8	
Cox1-IPC-R	10 µM	0.63	2.8	
Cox1-IPC-Probe	10 µM	0.30	1.4	
RPA Buffer		14.75	66.4	
Water		4.15	18.7	
Crude Plant Extract/DNA		1.00	4.5	
Total Volume*		**25 µL**	**112.5 µL**	

* This volume is after adding the magnesium acetate (1.25 µL), but this is added just before the start of the reaction on the cap of each tube.

Sample set up

Add 22.75 µL of master mix on each well:

Well	1	Check
A	*Sample 1 – Phyt genus*	
B		

Phytophthora sojae species-specific RPA worksheet

Master mix

Reagents	Initial concentration	RPA *Phytophthora sojae* volume per reaction (µL)		
		1X	4.5X	Check
*S*pecies-specific				
Atp9-F	1 µM	0.3	1.1	
Psojae-nad9-R	10 µM	2.1	9.3	
Atp9-Probe	10 µM	0.5	2.0	
RPA Buffer		14.8	66.4	
Water		5.30	23.9	
Crude Plant Extract/DNA		1.0	4.5	
Total Volume*		**25 µL**	**112.5 µL**	

* This volume is after adding the magnesium acetate (1.25 µL), but this is added just before the start of the reaction on the cap of each tube.

Sample set up

Add 22.75 µL of master mix on each well:

Well	1	Check
A		
B	*Sample 1 – P. sojae*	

RPA amplification of *Fusarium virguliforme* with lateral flow detection worksheet

Master mix

Reagents	Initial concentration	1X	8.5 X	__.5 X	Check
Rehydration buffer	-	14.75µL	125.375 µL		
Water	-	5.6µL	47.6 µL		
FvF30	10 µM	1.05 µL	8.925 µL		
FvR30	10 µM	1.05 µL	8.925 µL		
FvNfo	10 µM	0.3 µL	2.55 µL		
Crude Plant Extract/DNA		1.00 µL	-		
Total Volume*		**25 µL***			

** Total volume is 25 µL after adding the Magnesium acetate, but this is added just before the start of the reaction.*

Sample set up

2 wells:

Well	1	Check
A	*Sample 1*	
B	*Sample 2*	
C		
D		

Sample set up Bio-Rad (CFX96) platform – *Phytophthora* assay

	Group 4		Group 6				
	1	**2**	**3**	**4**	**5**	**6**	…
A	*100pg*	Unk_1	*100pg*	Unk_1			
B	*100pg*	Unk_1	*100pg*	Unk_1			
C	*10pg*	Unk_2	*10pg*	Unk_2			
D	*10pg*	Unk_2	*10pg*	Unk_2			
E	*1pg*	Unk_3	*1pg*	Unk_3			
F	*1pg*	Unk_3	*1pg*	Unk_3			
G	*100fg*	NTC	*100fg*	NTC			
H	*100fg*	NTC	*100fg*	NTC			

Sample set up ABI (StepOne Plus) platform – SDS assay

	Group 3		Group 5				
	1	**2**	**3**	**4**	**5**	**6**	…
A	*100pg*	Unk_1	*100pg*	Unk_1			
B	*100pg*	Unk_1	*100pg*	Unk_1			
C	*10pg*	Unk_2	*10pg*	Unk_2			
D	*10pg*	Unk_2	*10pg*	Unk_2			
E	*1pg*	Unk_3	*1pg*	Unk_3			
F	*1pg*	Unk_3	*1pg*	Unk_3			
G	*100fg*	NTC	*100fg*	NTC			
H	*100fg*	NTC	*100fg*	NTC			

Sample set up Smart-Cycler (Cepheid) platform – SDS and *Phytophthora* assay

Group 1 - Block A - SDS

	1		2
1	*100pg*	9	Unk_1
2	*100pg*	10	Unk_1
3	*10pg*	11	Unk_2
4	*10pg*	12	Unk_2
5	*1pg*	13	Unk_3
6	*1pg*	14	Unk_3
7	*100fg*	15	NTC
8	*100fg*	16	NTC

Group 2 - Block B - Phytophthora

	1		2
1	*100pg*	9	Unk_1
2	*100pg*	10	Unk_1
3	*10pg*	11	Unk_2
4	*10pg*	12	Unk_2
5	*1pg*	13	Unk_3
6	*1pg*	14	Unk_3
7	*100fg*	15	NTC
8	*100fg*	16	NTC

Group 7 - Block C - SDS

	1		2
1	*100pg*	9	Unk_1
2	*100pg*	10	Unk_1
3	*10pg*	11	Unk_2
4	*10pg*	12	Unk_2
5	*1pg*	13	Unk_3
6	*1pg*	14	Unk_3
7	*100fg*	15	NTC
8	*100fg*	16	NTC