

PCR Protocol: Identification of *P. infestans*

Primers (5' – 3'):

Pinf2: CTCGCTACAATAGCAGCGTC

ITS5: GGAAGTAAAAGTCGTAACAAGG

Master Mix:

Reagent	Volume per Reaction (µl) (1X)	____X
ddH ₂ O	35.25	
10X PCR buffer	5	
dNTP mix (2mM each)	2.5	
Pinf2 (10µM)	2	
ITS5 (10µM)	2	
MgCl ₂ (50mM)	1.8	
BSA (20mg/ml)	0.25	
Taq (5U/µl)	0.2	

Master mix recipe is for 50µl but can be reduced to 25µl if needed. Combine 49 or 24 µl of master mix with 1-2µl DNA.

Thermal cycling protocol:

Initial denaturation:	94C	2 min
35 cycles:	94C	15 sec
	56C	15 sec
	72C	15 sec
Final extension	72C	5 min
Hold	4C	Forever

The reaction will produce an amplicon approximately 600bp long in the presence of *P. infestans* DNA. The Pinf2 primer is specific to *P. infestans* on potato and tomato hosts.