

## PCR Protocol: Identification of *Phytophthora* species - ITS

### Primers (5' – 3') (From White et al. 1990):

ITS5: GGAAGTAAAAGTCGTAACAAGG

ITS4: TCCTCCGCTTATTGATATGC

### Master Mix:

Reagent	Volume per Reaction (µl) (1X)	____X
ddH <sub>2</sub> O	35.25	
10X PCR buffer	5	
dNTP mix (2mM each)	2.5	
ITS4 (10µM)	2	
ITS5 (10µM)	2	
MgCl <sub>2</sub> (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. If planning to run sequencing, a 50µl reaction is recommended. Combine 49 or 24µl 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 size of this amplicon may vary depending on the DNA amplified. Note that these primers are universal fungal primers, and so may amplify other species in a mixed sample (e.g. from an infected leaf).