

PCR Protocol: Identification of *P. infestans* - COXI

Primers (5' – 3'):

COX1F: GTATTTCTTCTTTATTAGGTGC

COX1R: CGTGAACATAATGTTACATATAC

Master Mix:

Reagent	Volume per Reaction (µl) (1X)	____X
ddH ₂ O	35.25	
10X PCR buffer	5	
dNTP mix (2mM each)	2.5	
COX1F (10µM)	2	
COX1R (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. 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	30 sec
	52C	30 sec
	72C	1 min
Final extension	72C	10 min
Hold	4C	Forever

The size of this amplicon may vary depending on the DNA amplified.