

Standard PCR amplification protocol used for data base primer sets.

All primer sets listed on this web site amplify unique bands under the standard conditions listed below. The same PCR protocol is used for both the initial primer testing (on agarose gels) and the polymorphic testing (on acrylamide gels),

Basic PCR reaction mix (10 µl)

Reagents	Amount added (1 X)	Final concentration
Temple DNA(20ng/µl)	1.0 µl	1.33 ng/µl
PCR compatible Dye ¹	2.0 µl	
Forward primer (10 µM)	0.5 µl	0.5 µM
Reverse Primer (10 µM)	0.5 µl	0.5 µM
dNTP (2 mM)	1.0 µl	0.2 mM
Buffer (10 X)	1.0 µl	1 X
MgCl ₂ (25 mM)	0.6 µl	1.5 mM
Taq (5 U/µl)	0.15 µl	0.067 Units/µl
dH ₂ O	4.25 µl	
Total	10.0 µl	

PCR amplification protocol

Step	Temperature	Time	Cycles
Hot start	94° C	4 min.	1
Denaturing	94° C	1 min.] 30
Annealing	58° C	1 min	
Elongation	72° C	1 min.	
Final elongation	72° C	5 min.	1

¹. PCR compatible Dye contains 12% sucrose, 0.8 mg/ml tartrazine, 0.1mg/ml cresol red. Filter and aliquot in 1.5 ml tubes and store at -20°C. Adding PCR compatible dye saves time by allowing the mix to be loaded directly onto a gel