

APPENDIX: Instructions for Use

Store the contents in a dry place (at -20 or -80°C if possible) until reconstituted in purified H₂O. Thereafter, store at -20 or -80°C. Subdividing the reconstituted primers into aliquots is recommended to prevent degradation and contamination.

Reconstituting the primers:

Add 1000 µL of purified H₂O to each tube. Vortex the tube for 1 minute to dissolve the primers. When dissolved in this volume, the primers will be at a 25× concentration relative to the final reaction concentration. Users needing a different concentration can vary the amount of water added.

The UN primer is always used in combination with at least one other primer in equal volumes. Primers (other than UN) may be omitted if certain members of the complex are not expected or of interest.

Typical reaction components for a 25 µL reaction:

9.05 µL	Deionized pure water
5 µL	GoTaq® 5× PCR Buffer (w/ 15 mM MgCl ₂)
2.5 µL	10 mM dNTP solution*
0.3 µL	25 mM MgCl ₂
1 µL	IMP-UN, F
1 µL	AR-3T, R
1 µL	GA-3T, R
1 µL	ME-3T, R
1 µL	QD-3T, R
1 µL	IMP-S1, R
1 µL	IMP-M1, R
0.15 µL	5 U/µL GoTaq® DNA Polymerase
24 µL	Total (to each 24 µL reaction, add 1 µL template DNA)

*2.5 mM each dATP, dCTP, dGTP, dTTP

Template DNAs can be prepared by various methods: purified DNA, by inclusion of a tarsus in the reaction, or by rapid heating in buffer.

Thermal cycling conditions:

Conditions may vary, but typical conditions are shown:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	5 minutes	1
Denaturation	95°C	30 seconds	30 cycles of these three steps together
Annealing	58°C	30 seconds	
Extension	72°C	30 seconds	
Final Extension	72°C	5 minutes	1
Hold	12°C	Indefinite	1