

NIH-0824 Genotyping Strategies

Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	3.5
10mM dNTPs	1
Primer 20 uM	1
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5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer 0824-5' and Primer LTR-rev, 162 bp	
Recommended Wt PCR: Primer 0824-5' and Primer 0824-3', 147 bp	
Primer LTR-rev	ATAAACCCCTCTTGCAGTTGCATC
Primer 0824-5'	GCTCAGAGTTTCCTCAGAGAAACG
Primer 0824-3'	TTCTCTGTCAGGCTCGGTTCTTCC

Well	Sample	Genotype
1	211	het
2	ES DNA	het
3	wt lysate	wt
4	water	no amp

