

NIH-1304 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 1304-5' and Primer LTR-rev, 216 bp	
Recommended Wt PCR: Primer 1304-5' and Primer 1304-3', 152 bp	
Primer 1304-5'	CAGCCTTACAGTTCTGCTGATGC
Primer LTR-rev	ATAAACCCCTCTTGACAGTTGCATC
Primer 1304-3'	ACTGAGCAATGGACCAGACCAAGG

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

Mutant PCR
MK 1 2 3 MK

