

NIH-1841 Genotyping Strategies

Reaction Components	Vol (ul)
5X GoTaq Buffer	10.0
25mM MgCl ₂	3.5
10mM dNTPs	1.0
Primer 20 uM	1.0
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5 U/ul Taq polymerase	0.5
Water	28.0
Tail lysate (1:20 dilution)	5.0
Total reaction volume	50.0

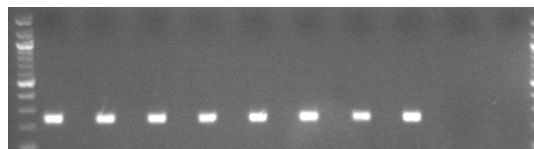
Step	Temp	Time	Note
1	94C	4'	
2	94C	15"	
3	65C	30"	Decrease 1C/cycle
4	72C	40"	Go to 2, 10 cycles
5	94C	15"	
6	55C	30"	
7	72C	40"	Go to 5, 30 cycles

Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 1841-11, 247 bp	
Recommended Wt PCR: Primer 1841-10 and Primer 1841-11, 399 bp	
Neo3a	GCAGCGCATCGCCTTCTATC
1841-11	TCCAACATTAAAGCGATAACC
1841-10	CCGAATCTCTCCGATCACTAC

Well	Sample	Genotype
1	166	het
2	175	het
3	176	het
4	178	het
5	179	het
6	184	het
7	187	het
8	es DNA	het
9	wt lysate	wt
10	water	no amp

Mutant PCR

M 1 2 3 4 5 6 7 8 9 10 M



*2% Agarose, NEB 100 bp ladder