

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: B6;129S5-Rtn4^{tm1Lex}/Mmcd **MMRRC #** 032547-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	10.275
10x Buffer (15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20μM) UNQ330-17	0.5
Primer 2 (stock concentration is 20μM) UNQ330-18	0.5
Primer 3 (stock concentration is 20μM) UNQ330-R2	0.5
Primer 4 (stock concentration is 20μM) Neo3a	0.5
Taq Polymerase 5Units/μL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	94	5:00	1
2. Denaturation	94	0:15	} 40x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: UNQ330-17	CTG CAG TAC CTC TAC CTA CAA G
2: UNQ330-18	CTG ATG AGG AAC CTCG GAA CTT C
3: UNQ330-R2	GGA ATG TCA CCT CAC TTC CATATG TG
4: Neo3a	GCA GCG CAT CGCCTT CTA TC

Electrophoresis Protocol:

% Agarose: 1.5 **V:** 90

Estimated Running Time (min): 90

Primers	Expected Bands	Genotype
1 and 2	385 bp	Wild-type
3 and 4	288 bp	Mutant