GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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530-754-MMRRC

NAME OF PCR: C57BL/6-*Rxra*^{pke}/Mmcd, (*pinkie*)   **MMRRC #** 012828-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	12.675
10x Buffer (contains 15mM MgCl$_2$)	2.5
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) Pink PCR F1	0.5
Primer 2 (stock concentration is 20µM) Pink PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- PCR products are verified to contain the correct amplicon size by running ~10µl of the reaction on a gel and the remaining 15µl purified via column based PCR purification method for sequencing.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0^o C; next 30 cycles anneal at 55° C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl$_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (^{o}C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐		94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing } steps 2-3-4 will cycle in sequence		65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing } steps 5-6-7 will cycle in sequence		55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Pink PCR F1	GCT TCT TCT GAT GAC CAC TGC CTT G
2: Pink PCR R1	ACA CGG CTG CAG AGG TCA CAT AGA C
3: Pinkie Sequencing	Use PCR primers for sequencing

Electrophoresis Protocol:

Agarose: 2%   **mV:** 80   **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	512 bp	*pinkie*

Mutation site (red) and flanking sequence:

WT   cctgttaccaaca**T**ctgtca

pinkie   cctgttaccaaca**A**ctgtca