

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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NAME OF PCR: C57BL/6-Rc3h1^{san}/Mmcd, (San Roque) **MMRRC #** 011119-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	29.8
10x Buffer (contains 25mM MgCl ₂)	4.0
dNTPs (stock concentration is 10mM)	0.2
Primer 1 (stock concentration is 10μM) SRMsFa	5.0
Primer 2 (stock concentration is 10μM) SRRWT	7.0
Primer 3 (stock concentration is 10μM) SRRMut	2.0
Taq Polymerase 5 Units/μL	0.5
Triton x100 10%	0.5
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	50μL

Comments on protocol:

Do a primer titration to find the optimal combination of primer amounts to amplify the two products at equal intensity. The above volumes are what currently works in our lab. A non specific product is sometimes observed beneath the lower band.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1
2. Denaturation	94	0:20	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	62	0:20	
4. Elongation	72	0:30	
5. Amplification	72	5:00	1
6. Finish	10	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: SRMsFa	CATCAGGCTGGCCTCTAGTT
2: SRRWT	AAGACCAGCTTCAGAGCTTCCTCCAGCA
3: SRRMut	GAACCATCTTCTAAGGCCAGCGTGACCAGCTTCAGAGCTTCCTCCTACC

Electrophoresis Protocol:

% Agarose: 3 mV: 140

Estimated Running Time (min): 60

Expected Bands	Genotype
194 bp	Wild-type +/+
215 bp	Mutant s/s

