

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129-Rapsn^{tm1Jrs}/Mmucd

MMRRC: 015108-UCD

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (μL)
Water	18.5
10x Buffer	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer 1. (stock concentration is 20μM) FWD	1.0
Primer 2. (stock concentration is 20μM) REV	1.0
Taq Polymerase 5Units/μL Clontech Titanium Taq	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.0 μL

Comments on protocol:

- Run mutant and wild-type as two simplex reactions.
- Mutant allele: both primer sequences are in the neo cassette.
- Wild-type allele: both sequences are in exon 3 of the *rapsn* gene, 32250 sequence is deleted in the mutant allele

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	1:00	1
2. Denaturation	94	0:30	
3. Annealing steps 2-3-4 cycle in sequence	68	1:30	35x
4. Elongation	68	1:30	
5. Amplification	68	5:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.2 V:120		
1. 32901 F	CATTGCATCAGCCATGATGGATAC	Estimated Running:Time: 90 min.		
2. 32902 R	CTATTCGGCTATGACTGGGCACAAC	Primer Combination	Band	Genotype
3. 32251 F	GACTATGAGAAAGCCCTGTTCTTTCC	1 and 2	305 bp	MUTANT
4. 32250 R	CTCACAACACTCCATGGCACTGCC	3 and 4	160 bp	WT

Image not Available