

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S1-Syngap1^{tm1.1Mabk}/Mmucd

MMRRC: 037374-UCD

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

Reagent/Constituent	Volume (μL)
Water	18.275
10x Buffer (Qiagen)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1. (stock concentration is 20μM)	0.325
Primer 2. (stock concentration is 20μM)	0.325
Primer 3. (stock concentration is 20μM)	0.325
Taq Polymerase 5Units/μL	0.25
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	2.5
TOTAL VOLUME OF REACTION:	25 μL

Comments on protocol:

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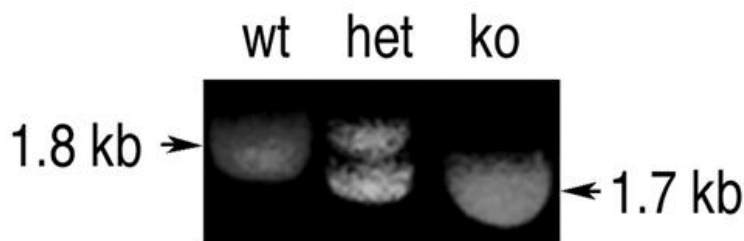
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	1:30	1
2. Denaturation	95	0:30	
3. Annealing steps 2-3-4 cycle in sequence	53	0:30	32x
4. Elongation	72	2:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1% V:150
1. Lox-DS	GAAGAGGAGTTTACGTCCAGCCAAGCT	Estimated Running:Time: 50 min.
2. MGIN-11	GAGAGAGATGGAGGGTCACTTGAG	Primer Combination
3. MGEX9-1	CGGATGCTATGTGCAGTGCTGGA	Band
		Genotype
		1 and 2 1700 bp KO
		2 and 3 1800 bp WT



Run gel until dye front runs off bottom of gel.

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