

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6N;129S6-Raf1^{tm1.1Bgn}/Mmucd MMRRC # 036521-UCD

Protocol: *(Protocol provided by donating investigator)*

Reagent/Constituent	Volume (μL)
Water	18.25
10x Buffer donor's lab use the 10X buffer attached with Taq polymerase (Gene Choice)	2.5
dNTPs (stock concentration is 10mM)	1.0
Primer 1 (stock concentration is 10μM) L613V-F2	0.5
Primer 2 (stock concentration is 10μM) L613V-R2	0.5
Taq Polymerase 5Units/μL	0.25
DNA	2.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1
2. Denaturation	94	0:30	
3. Annealing steps 2-3-4 cycle in sequence	61	0:30	40x
4. Elongation	72	0:45	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. L613V-F2	TTG TAT ACC TCT GGA GCT GAG CAG G
2. L613V-R2	ACG TGT ACT CGT ACG GCA TCG TGC T

Restriction Digest: Incubate at 37 °C for 1-1.5 hours

Reagent/Constituent	Volume (μL)
PCR Product	10.0
Water	11.75
NEB Buffer 3	2.5
Dra III	0.5
BSA	0.25
TOTAL VOLUME OF REACTION:	25.00 μL

Electrophoresis Protocol:

Agarose: 2% 1X TAE V: _____

Estimated Running Time: _____ min.

Expected Band	Genotype
~400 bp + 550 bp	Mutant
950 bp	WT