

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: B6.129-Rnf212^{tm1Nhtr}/Mmucd MMRRC # 036647-UCD

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

Reagent/ Constituent	Volume (μL)
Water	18.2
10x Buffer (contains / without 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10 mM)	0.6
Primer 1 (stock concentration is 10 μM)	0.6
Primer 2 (stock concentration is 10 μM)	0.6
Primer 3 (stock concentration is 10 μM)	0.6
Primer 4 (stock concentration is 10 μM)	0.6
Taq Polymerase 5 U/ul	0.3
Additives / Other (if applicable):	0.0
DNA sample extracted ☐ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25.000μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	5:00	1
2. Denaturation	95	0:30	} 35x
3. Annealing	60	0:30	
4. Elongation	72	0:45	
5. Amplification	72	10:00	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Exon Forward	5'-CGCTGGAATGAACGCAGGCGC-3'
2. Exon Reverse	5'-CAGGGGAGTGAAGCCACGGTC-3'
3. pH530	5'-TCCATGGGCTTAAACCAGTGC-3'
4. VM3	5'-GCGCATGCTCCAGACTGCCTTG-3'

Electrophoresis Protocol:

Agarose: 2 % V: 100 ml

Estimated Running Time: 45 min.

Primer Combination	Expected Bands	Genotype
1 and 2	290 bp	WT
1, 2, 3 and 4	290 / 383 bp	Het
3 and 4	383 bp	Mut

