GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: **FVB/N-*AU040320*^{em1Janc}/Mmucd** **MMRRC: 043535-UCD**

Protocol:

GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1.43535-snpF	CTGAAAATTTACTGGCAGGGATGAA
2.43535-snpR	TGACAACCACAGAACGCTGAAGC
3.43535-snpFS	ACATTTTTCTAGCATTCCACATTTCC

Electrophoresis Protocol:

Agarose: **1.5%** V: **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	315	*

***The mutation of MMRRC Strain 43535 is the loss of a single nucleotide. The T is missing in the mutant. PCR is 315bp, sequence with snpFS and SNP is at ~123rd nt.**

KO: tgggagtcaagccaagtcccgcttcc**X**gggttttgccaggatattg

WT: tgggagtcaagccaagtcccgcttcc**T**gggttttgccaggatattg