GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: FVB.Cg-Mbtm1Jsc/Mmucd **MMRRC: 41495-UCD**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	
4. Elongation		68	2:00	
				Go to 2 (9x)
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	
7. Elongation		68	2:00 (+20s/cycle)	
				Go to 5 (24x)

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 41495-mutF	CTTGCAAAACCACACTGCTC
2. 41495-mutR	GAGGGAGCTGGTGTCAACAG
3. 41495-WtF	GAGGACCTGAAGAAGCATGG
4. 41495-WtR	TGAGTGGCTAAGCTGGGATT

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

Estimated Running **90** min.

Primers	**Band** (bp)	Genotype
1 & 2	~1100	mutant
3 & 4	198	wildtype

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Electrophoresis Protocol: