GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: Modvl5 (Modifier of vl)/Mmucd **MMRRC:** 042070-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.
- One primer pair is used for each reaction; to genotype the whole Modvl5 region, we used 6 SSLP MIT markers that are evenly distributed within the Modvl5 15 Mb interval. Expected band size below indicate MOLF/Ei background in the Modvl5 region.

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. D18MIT181	CTTGAACTTCCTTCACAAGAACTG
2.	AATCATGCTACTTGGAATGATAATG
3. D18MIT140	AGATTCTGCCTCTTCCTCTGG
4.	AGGTTGCCTGAAAGGGAACT
5. D18MIT40	GGTAGGAGTCACTTTCCGTCC
6.	TTTTGTGAGCATTTTTATACCATT
7. D18MIT50	TCCCTAAATCACTCTTTCCATTG
8.	AACTCGGGGACTTTGACATG
9. D18MIT184	CACACATGTGTAGGTAGGTAGGTAGG
10	CGCACAAGGACTACTGAAACA
11 D18MIT141	CTAAAATGTTGGCAGATTTTAAATG
12	ATACAGTAGATTTAGTTCTCTGGCACC

Agarose: 1.5% **V:** 90

Estimated Running Time: 90 min.

Primer Combination	**Band** (bp)	Genotype
D18MIT181	148	MOLF/MOLF
D18MIT140	220	MOLF/MOLF
D18MIT40	145	MOLF/MOLF
D18MIT50	175	MOLF/MOLF
D18MIT184	185	MOLF/MOLF
D18MIT141	105	MOLF/MOLF

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