GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

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Telephone **848-565-4854**	FAX **732-235-5562**	
Strain Name **Modvl4 Subcongenic 2**	MMRRC Stock Number **42091**	

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NAME OF PCR: **Modvl4 Sub2 genotyping using SSLP MIT markers** **MMRRC:** **0-UCD**

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

Reagent/Constituent	Volume (µL)
Water sddH2O	4
2x Buffer **GoTaq Green (Promega); contains Taq DNA polymerase**	10
$MgCl_2$ (stock concentration is 3mM) included in Buffer	
Betaine (stock concentration is 5M) *Optional*	
dNTPs (stock concentration is 10mM) 2X GoTaq Buffer contains 400uM dNTPs	
DMSO *Optional*	
Primer 1. (stock concentration is 10µM)	2
Primer 2. (stock concentration is 10µM)	2
Primer 3. (stock concentration is 10µM)	
Primer 4. (stock concentration is 10µM)	
Taq Polymerase 5Units/µL **included in Buffer**	
DNA (50-200ng/ µL) extracted w/ "Promega Wizard SV Genomic DNA Purification System"	2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**20.000 µL**

Comments on protocol:

- One primer pair is used for each reaction; to genotype the whole Modvl4 region, we used 14 SSLP MIT markers that are evenly distributed within the Modvl4 55 Mb interval. Expected band size below indicate MOLF/Ei background in Modvl4 region.
- Genotyping PCR was carried out using Promega GoTaq Green Master Mix; concentrations listed in the table above are approximations of Master Mix contents

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	1:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	51	0:15	**30x**
4. Elongation		72	0:45	
5. Amplification		72	10:00	**1**
6. Finish		4	∞	n/a

Primers

Name	Nucleotide Sequence (5' - 3')
1. D15MIT51	ACCTTGTTTCAGAACGAATAAATG
2.	GATGCTCAGAGAGTGCACCA
3. D15MIT81	ATGAAAACACAAACGTAAAGTTATACA
4.	ATCTTCTTTTATGCCTGTGTAGGC
5. D15MIT130	CATATTTTGCAATTTTTAGTAATAGGC
6.	CAACACAGAAATAAAAGTGAGAGAGG
7. D15MIT252	CTTCAAACATGTTATCATTGTCACA
8.	CTTCTGTATTCACAGGTGCTCG
9. D15MIT267	CTGAAAACTTCCAAGTCACTCTG
10	CAAGCAGAAAAGCAAACCAG
11 D15MIT94	CCACTTCTGACCTTCACATGT
12	TGCCTATGTATGTGGTATGTATGA
13 D15MIT204	GACTTTGTCTTATGTGATATGGTGTG
14	TTCTAGACTTGCCTAACATGAAACA
15 D15MIT49	TGTACTCATGTGTACAGACCCAC
16	GGTGACCCTGTGAAAACGAT
17 D15MIT24	TCAACAGCTTTATTAGGAACCTCC
18	CTTACCATCCCTTGCCAAAA
19 D15MIT86	TACACTTATCTGTTGGAACATCCC

Polyacrylamide: **8%** V: **150**
Estimated Running:Time: **120** min.

Primer Combination	**Band** (bp)	Genotype
D15MIT51	124	MOLF/MOLF
D15MIT81	132	MOLF/MOLF
D15MIT130	185	MOLF/MOLF
D15MIT252	147	MOLF/MOLF
D15MIT267	310	MOLF/MOLF
D15MIT94	160	MOLF/MOLF
D15MIT204	188	MOLF/MOLF
D15MIT49	158	C3H/C3H
D15MIT24	246	C3H/C3HF
D15MIT86	196	C3H/C3H
D15MIT59	116	C3H/C3H
D15MIT195	186	C3H/C3H
D15MIT27	208	C3H/C3H
D15MIT257	122	C3H/C3H

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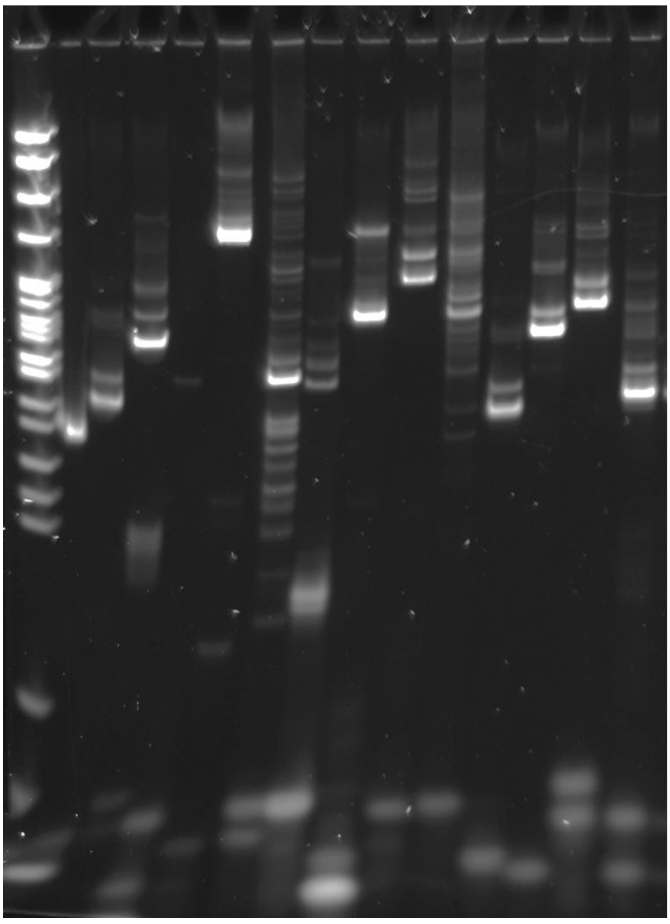
20	ATATAGATAAACACACGCACACCA			
21 D15MIT59	ATGGCTATGATTAAGAAGACAGCC			
22	ATACCATGAATGAGGGTTCACC			
23 D15MIT195	CAAGAGAGATTCAGTGACCACG			
24	GATGCAAGAGCCTCACCAG			
25 D15MIT27	GTTAGGCCCTGTGGTTTTGA			
26	ATCCTTTCTAAACATGGACTTTGG			
27 D15MIT257	AAGAAGGTCTATGAAGTCTGCAGG			
28	CTTCACCAGATGCCATGCTA			

Protocol / Gel Comments:

PCR products are resolved on a 8% polyacrylamide gel prepared as follows:
- H2O: 13.25 mL
- 10X Tris-Borate-EDTA (TBE) buffer: 2.0 mL
- 40% stock acrylamide solution (Acrylamide:Bis-acrylamide 29:1): 4.0 mL
- 10% stock Ammonium persulfate (APS): 0.25 mL
- Tetramethylethylenediamine (TEMED): 0.025 mL

Molecular Weight Marker used is pBR322 MspI from New England Biolabs
Gel run: 150V, 75 min

Gel pictures:



Lane
1 – pBR322 MW marker
2 – D15MIT51
3 – D15MIT81
4 – D15MIT130
5 – D15MIT252
6 – D15MIT267
7 – D15MIT94
8 – D15MIT204
9 – D15MIT49
10 – D15MIT24
11 – D15MIT86
12 – D15MIT59
13 – D15MIT195
14 – D15MIT27
15 – D15MIT257