

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

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)  
530-754-MMRRC

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

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Strain Name <b>Modvl4 Subcongenic 1</b>		MMRRC Stock Number <b>42090</b>

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NAME OF PCR: Modv14 Sub1 genotyping using SSLP MIT markers MMRRC: 0-UCD

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

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

### Comments on protocol:

- One primer pair is used for each reaction; to genotype the whole Modv14 region, we used 14 SSLP MIT markers that are evenly distributed within the Modv14 55 Mb interval. Expected band size below indicate MOLF/Ei background in Modv14 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 (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	1:00	1
2. Denaturation	94	0:15	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	51	0:15	<b>30x</b>
4. Elongation	72	0:45	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

### Primers

Name	Nucleotide Sequence (5' - 3')	Polyacrylamide: 8%	V: 150
1. D15MIT51	ACCTTGTTTCAGAACGAATAAATG	Estimated Running Time: 120 min.	
2.	GATGCTCAGAGAGTGCACCA	<b>Primer Combination</b>	<b>Band (bp)</b>
3. D15MIT81	ATGAAAACACAAACGTAAGTTATACA	D15MIT51	134
4.	ATCTTCTTTTATGCCTGTGTAGGC	D15MIT81	132
5. D15MIT130	CATATTTTGCAATTTTGTAGTAATAGGC	D15MIT130	185
6.	CAACACAGAAATAAAAGTGAGAGAGG	D15MIT252	147
7. D15MIT252	CTTCAAACATGTTATCATTGTCACA	D15MIT267	310
8.	CTTCTGTATTCACAGGTGCTCG	D15MIT94	160
9. D15MIT267	CTGAAAACCTCCAAGTCACTCTG	D15MIT204	188
10	CAAGCAGAAAAGCAAACCAG	D15MIT49	158
11 D15MIT94	CCACTTCTGACCTTCACATGT	D15MIT24	246
12	TGCCTATGTATGTGGTATGTATGA	D15MIT86	196
13 D15MIT204	GACTTTGTCTTATGTGATATGGTGTG	D15MIT59	116
14	TTCTAGACTTGCTAACATGAAACA	D15MIT195	186
15 D15MIT49	TGTAATCATGTGTACAGACCCAC	D15MIT27	208
16	GGTGACCCTGTGAAAACGAT	D15MIT257	122
17 D15MIT24	TCAACAGCTTTATTAGGAACCTCC		
18	CTTACCATCCCTTGCCAAAA		
19 D15MIT86	TACACTTATCTGTTGGAACATCCC		

*PCR protocol provided by Donating Investigator*

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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:**

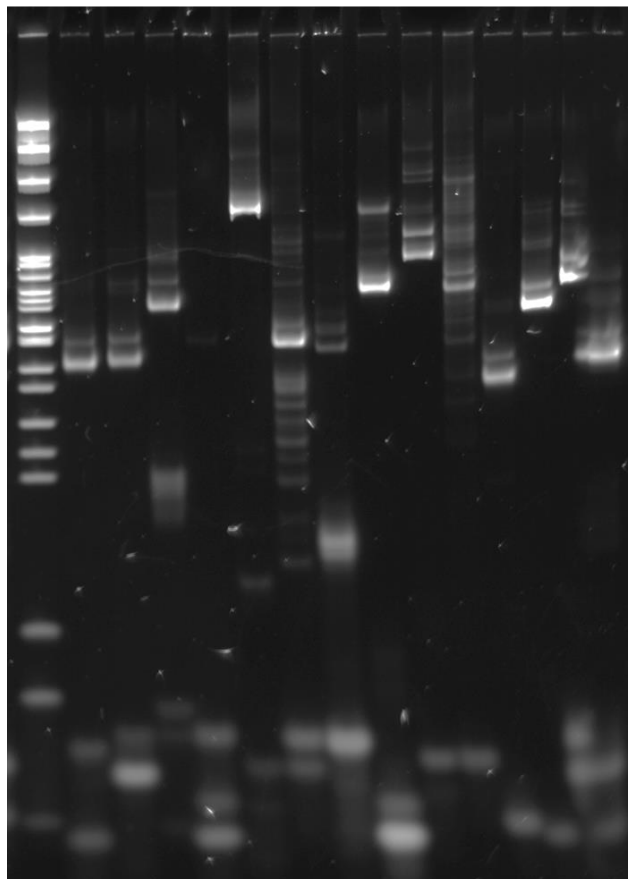
PCR products are resolved on a 8% polyacrylamide gel prepared as follows:

- H<sub>2</sub>O: 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