

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

**Protocol Name:** Modvl4 (Modifier of vacuolated lens 4) Subcongenic2/Mmucd **MMRRC:** 042091-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             |
| <b>TOTAL VOLUME OF REACTION:</b>                                                      | <b>15.00 µL</b> |

### Comments on protocol:

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

### Strategy:

| Steps                                                                                        | Temp (°C )            | Time (m:ss) | # of Cycles |
|----------------------------------------------------------------------------------------------|-----------------------|-------------|-------------|
| 1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span> | 94                    | 5:00        | <b>1</b>    |
| 2. Denaturation                                                                              | 94                    | 0:15        |             |
| 3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>                | 65 to 55 (↓1°C/cycle) | 0:30        | <b>40x</b>  |
| 4. Elongation                                                                                | 72                    | 0:40        |             |
| 5. Amplification                                                                             | 72                    | 5:00        | <b>1</b>    |
| 6. Finish                                                                                    | 15                    | ∞           | n/a         |

### Primers

| Name         | Nucleotide Sequence (5' - 3') | Agarose: 1.5% V: 90             |                  |                 |
|--------------|-------------------------------|---------------------------------|------------------|-----------------|
| 1. D15MIT51  | ACCTTGTTTCAGAACGAATAAATG      | Estimated Running Time: 90 min. |                  |                 |
| 2.           | GATGCTCAGAGAGTGCACCA          | <b>Primer Combination</b>       | <b>Band (bp)</b> | <b>Genotype</b> |
| 3. D15MIT81  | ATGAAAACACAAACGTAAAGTTATACA   | D15MIT51                        | 124              | MOLF/MOLF       |
| 4.           | ATCTTCTTTTATGCCTGTGTAGGC      | D15MIT81                        | 132              | MOLF/MOLF       |
| 5. D15MIT130 | CATATTTTGCAATTTTGTAGTAATAGGC  | D15MIT130                       | 185              | MOLF/MOLF       |
| 6.           | CAACACAGAAATAAAAGTGAGAGAGG    | D15MIT252                       | 147              | MOLF/MOLF       |
| 7. D15MIT252 | CTTCAAACATGTTATCATTGTCACA     | D15MIT267                       | 310              | MOLF/MOLF       |
| 8.           | CTTCTGTATTCACAGGTGCTCG        | D15MIT94                        | 160              | MOLF/MOLF       |
| 9. D15MIT267 | CTGAAACTTCCAAGTCACTCTG        | D15MIT204                       | 145              | MOLF/MOLF       |
| 10           | CAAGCAGAAAAGCAAACCAG          | D15MIT49                        | 158              | C3H/C3H         |
| 11 D15MIT94  | CCACTTCTGACCTTCACATGT         | D15MIT24                        | 246              | C3H/C3H         |
| 12           | TGCCTATGTATGTGGTATGTATGA      | D15MIT86                        | 196              | C3H/C3H         |
| 13 D15MIT204 | GACTTTGTCTTATGTGATATGGTGTG    | D15MIT59                        | 116              | C3H/C3H         |
| 14           | TTCTAGACTTGCCTAACATGAAACA     | D15MIT195                       | 186              | C3H/C3H         |
| 15 D15MIT49  | TGTACTCATGTGTACAGACCCAC       | D15MIT27                        | 208              | C3H/C3H         |
| 16           | GGTGACCCTGTGAAAACGAT          | D15MIT257                       | 122              | C3H/C3H         |
| 17 D15MIT24  | TCAACAGCTTTATTAGGAACCTCC      |                                 |                  |                 |
| 18           | CTTACCATCCCTTGCCAAAA          |                                 |                  |                 |

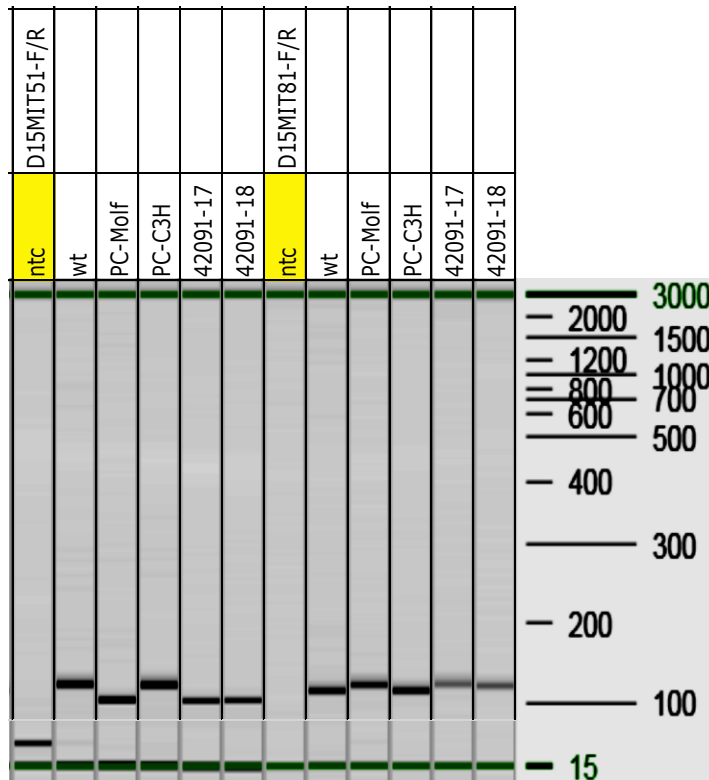
# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

|              |                          |  |  |  |
|--------------|--------------------------|--|--|--|
| 19 D15MIT86  | TACACTTATCTGTTGGAACATCCC |  |  |  |
| 20           | ATATAGATAAACACACGCACACCA |  |  |  |
| 21 D15MIT59  | ATGGCTATGATTAAGAAGACAGCC |  |  |  |
| 22           | ATACCATGAATGAGGGTTCACC   |  |  |  |
| 23 D15MIT195 | CAAGAGAGATTCAGTGACCACG   |  |  |  |
| 24           | GATGCAAGAGCCTCACCAG      |  |  |  |
| 25 D15MIT27  | GTTAGGCCCTGTGGTTTTGA     |  |  |  |
| 26           | ATCCTTTCTAAACATGGACTTTGG |  |  |  |
| 27 D15MIT257 | AAGAAGGTCTATGAAGTCTGCAGG |  |  |  |
| 28           | CTTCACCAGATGCCATGCTA     |  |  |  |



# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

