

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: MMRRC 71884 C57BL/6N-Nrp1em1(NRP1)Mbp/Mmucd

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

| Reagent/Constituent   | Volume (µL)    |
|---|----------------|
| Water   | 4.0            |
| GoTaq® G2 Colorless Master Mix,2X   | 7.5            |
| Primer 1. (stock concentration is 20µM) IVF   | 0.5            |
| Primer 2. (stock concentration is 20µM) IVR   | 0.5            |
| Primer 3. (stock concentration is 20µM) kiR   | 0.5            |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.5            |
| <b>TOTAL VOLUME OF REACTION:</b>  | <b>15.0 µL</b> |

**Comments on protocol:**

- Protocol may work with other DNA extraction methods.

**Strategy:**

| Steps  | Temp (°C)       | Time (m:ss)         | # of Cycles |
|--|-----------------|---------------------|-------------|
| 1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span> | 94              | 2:00                | <b>1x</b>   |
| 2. Denaturation  | 94              | 0:10                |             |
| 3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>                | 65 (↓1°C/cycle) | 0:30                | <b>10x</b>  |
| 4. Elongation  | 68              | 2:00                |             |
| 5. Denaturation  | 94              | 0:15                |             |
| 6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>                | 55              | 0:30                | <b>25x</b>  |
| 7. Elongation  | 68              | 2:00 (↑20sec/cycle) |             |
| 8. Finish  | 4               | ∞                   | n/a         |

**Primers:**

**Electrophoresis Protocol:**

| Name            | Nucleotide Sequence (5' - 3') | Agarose: 1.5%             | V: 90            |
|-----------------|-------------------------------|---------------------------|------------------|
| 1. C19-Nrp1-IVF | TTCTTAGGCGGACTTAAGAGCG        | Estimated Running         | 90 min.          |
| 2. C19-Nrp1-IVR | CTGACTTCTCCGTTCTTGCG          | <b>Primer Combination</b> | <b>Band (bp)</b> |
| 3. C19-Nrp1-kiR | GGATAACCAGGAGATGTAAGGTACC     | 1 & 2                     | 352              |
|                 |                               | 1 & 3                     | 247              |
|                 |                               |                           | <b>Genotype</b>  |
|                 |                               |                           | wildtype         |
|                 |                               |                           | mutant           |

WT: BL11234-324  
Het: BL11234-325

