

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: Keck MirKO ES cell line Mir138-1 (miR-138-1) MMRRC # 036839-UCD

Protocol: **FRT**

*PCR protocol provided by Donating Investigator*

| Reagent/ Constituent                                                                                                                                     | Volume (μL)    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Sterile H <sub>2</sub> O                                                                                                                                 | 14.6           |
| 10X Buffer                                                                                                                                               | 2.5            |
| dNTPs (stock concentration is 10mM)                                                                                                                      | 0.5            |
| DMSO                                                                                                                                                     | 1.2            |
| Primer 1 (stock concentration is 10μM) F-pr                                                                                                              | 1.3            |
| Primer 2 (stock concentration is 10μM) R-pr                                                                                                              | 1.3            |
| Primer 3 (stock concentration is 10μM) Universal primer                                                                                                  | 1.3            |
| Taq Polymerase                                                                                                                                           | 0.3            |
| DNA extracted w/ <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Qiagen DNEasy | 2.0            |
| <b>TOTAL VOLUME OF REACTION:</b>                                                                                                                         | <b>25.00μL</b> |

*Comments on protocol:*

**Strategy:**

| Steps                                                                | Temp (°C)             | Time (m:ss)         | # of Cycles |
|----------------------------------------------------------------------|-----------------------|---------------------|-------------|
| 1. Initiation/Melting HOT START? <input checked="" type="checkbox"/> | 93                    | 3:00                | 1           |
| 2. Denaturation                                                      | 93                    | 0:15                | } 8x        |
| 3. Annealing } steps 2-3-4 cycle in sequence                         | 68 to 60 (↓1°C/cycle) | 0:30                |             |
| 4. Elongation                                                        | 68                    | 9:00                |             |
| 5. Denaturation                                                      | 93                    | 0:15                | } 32x       |
| 6. Annealing } steps 5-6-7 cycle in sequence                         | 60                    | 0:30                |             |
| 7. Elongation                                                        | 68                    | 9:00 (↑20sec/cycle) |             |
| 8. Finish                                                            | 4                     | ∞                   | n/a         |

**Primers:**

| Name                 | Nucleotide Sequence (5' - 3') |
|----------------------|-------------------------------|
| 1. F                 | GTACATTGGTTGGTGGATACTGAA      |
| 2. R                 | GGTGCTTACAGATCTTCCTTTTCAT     |
| 3. Reverse Universal | GTGGTATCGTTATGCGCCTT          |

**Electrophoresis Protocol:**

Agarose: 1% V: 90

Estimated Running Time (min): 90

| Genotype  | Expected Band |
|-----------|---------------|
| Wild-type | 1768 bp       |
| Mutant    | 1526 bp       |

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**NAME OF PCR:** Keck MirKO ES cell line Mir138-1 (miR-138-1) **MMRRC #** 036839-UCD

**Protocol:** loxP *PCR protocol provided by Donating Investigator*

| Reagent/Constituent                                                                        | Volume (μL)     |
|--------------------------------------------------------------------------------------------|-----------------|
| Water                                                                                      | 16.0            |
| 10x Buffer                                                                                 | 2.5             |
| dNTPs (stock concentration is 10mM)                                                        | 0.5             |
| DMSO                                                                                       | 1.2             |
| Primer 1 (stock concentration is 10μM) F-pr                                                | 1.3             |
| Primer 2 (stock concentration is 10μM) R-pr                                                | 1.3             |
| Taq Polymerase                                                                             | 0.2             |
| DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 2.0             |
| <b>TOTAL VOLUME OF REACTION:</b>                                                           | <b>25.00 μL</b> |

**Comments on protocol:**

**Strategy:**

| Steps                                                     | Temp (°C)             | Time (m:ss) | # of Cycles |
|-----------------------------------------------------------|-----------------------|-------------|-------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 94                    | 5:00        | <b>1</b>    |
| 2. Denaturation                                           | 94                    | 0:15        |             |
| 3. Annealing steps 2-3-4 cycle in sequence                | 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') |
|------|-------------------------------|
| 1. F | TGAGGCTGCTGTTTATTGTAACAC      |
| 2. R | AGCTCTAGTTTCAGCAGATTGGAC      |

**Electrophoresis Protocol:**

**Agarose:** 1% **V:** 90

**Estimated Running Time:** 90 min.

| Genotype  | Expected Band |
|-----------|---------------|
| Wild-type | 439 bp        |
| Mutant    | 531 bp        |