

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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530-754-MMRRC

NAME OF PCR: Sanger MirKO ES cell line Mir709 LRPCR MMRRC # 036596-UCD

Protocol: *PCR protocol provided by Donating Investigator*

| Reagent/ Constituent                                                                                                                                       | Volume (μL)    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Sterile H <sub>2</sub> O                                                                                                                                   | 13.44          |
| 10X Buffer (green tube)                                                                                                                                    | 2.0            |
| Enhancer A (red tube)                                                                                                                                      | 1.0            |
| Enhancer B (yellow tube)                                                                                                                                   | 1.0            |
| DMSO (brown tube)                                                                                                                                          | 0.2            |
| Gene Specific Primer (10μM)                                                                                                                                | 0.5            |
| Universal Primer (10μM)                                                                                                                                    | 0.5            |
| Enzyme (black tube)                                                                                                                                        | 0.36           |
| DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Qiagen DNEasy | 1.0            |
| <b>TOTAL VOLUME OF REACTION:</b>                                                                                                                           |                |
|                                                                                                                                                            | <b>20.00μL</b> |

**Comments on protocol:**

- SequelPrep Long PCR Kit, Invitrogen A10498
- May decrease Elongation time for shorter PCR fragments per manufacturer's suggestion, i.e. 1 minute/kb of sequence.

**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. 5' common rev          | ATAGCATACATTATACGAAGTTATCACTGG |
| 2. 5' gene specific (LR1) | GACTGCTCTAGTCTCTCAGCTCATATTT   |
| 3. 3' common fwd          | TCTAGAAAGTATAGGAAGTCCATGGTC    |
| 4. 3' gene specific (LR4) | TAATAAAGATGGAAAGCATTATAGATTGAA |

**Electrophoresis Protocol:**

Agarose: 0.8% V: 90

Estimated Running Time (min): 90

| Primer Combination | Band (bp) | Genotype |
|--------------------|-----------|----------|
| 1 and 2 (5')       | 5637      | Targeted |
| 3 and 4 (3')       | 3674      | Targeted |

Note: the gene specific primers in this protocol have been provided by Wellcome Trust Sanger Institute. Expected band size not provided.