

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 Mir1943 LRPCR **MMRRC #** 036349-UCD

Protocol: SequelPrep Long PCR Kit, Invitrogen A10498

| Reagent/ Constituent | Volume (µL) |
|--|----------------|
| Sterile H ₂ 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 |
| TOTAL VOLUME OF REACTION: | 20.00µL |

Comments on protocol:

- 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 | |
| 6. Annealing | 60 | 0:30 | } 32x |
| 7. Elongation } steps 5-6-7 cycle in sequence | 68 | 9:00 (↑20sec/cycle) | |
| 8. Finish | 4 | ∞ | |

Primers:

| Name | Nucleotide Sequence (5' - 3') |
|---------------------------|--------------------------------|
| 1. 5' common rev | ATAGCATACATTATACGAAGTTATCACTGG |
| 2. 5' gene specific (LR1) | CTTTTGAGTCTTGAATCTGTCTTTTAGTT |
| 3. 3' common fwd | TCTAGAAAGTATAGGAACTTCCATGGTC |
| 4. 3' gene specific (LR4) | GGATTAAAGTAATTTCTACAACACTTTTCC |

Electrophoresis Protocol:

Agarose: 0.8% **V:** 90

Estimated Running Time (min): 90

| Primer Combination | Band (bp) | Genotype |
|--------------------|-----------|----------|
| 1 and 2 (5') | 5918 | Targeted |
| 3 and 4 (3') | 3880 | Targeted |