

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK *Mfn2*<sup>tm3Dcc</sup>/Mmucd MMRRC # 029902-UCD

### Protocol:

| Reagent/Constituent  | Volume (μL)      |
|--|------------------|
| Water  | 11.275           |
| 10x Buffer   | 2.5              |
| MgCl <sub>2</sub> (stock concentration is 25mM)  | 1.7              |
| Betaine (stock concentration is 5M) <i>Optional</i>  | 6.5              |
| dNTPs (stock concentration is 10mM)  | 0.5              |
| DMSO <i>Optional</i>   | 0.325            |
| Primer 1 (stock concentration is 20μM)   | 0.5              |
| Primer 2 (stock concentration is 20μM)   | 0.5              |
| Taq Polymerase 5Units/μL   | 0.2              |
| DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0              |
| <b>TOTAL VOLUME OF REACTION:</b>   | <b>25.000 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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non-specific amplifications.

### Strategy:

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

### Primers:

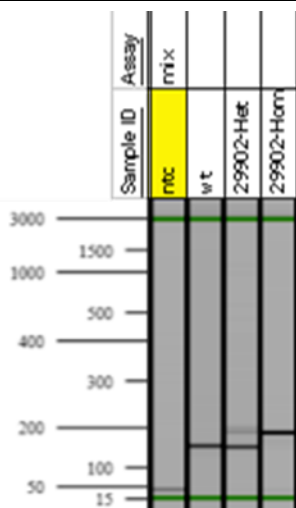
| Name       | Nucleotide Sequence (5' - 3') |
|------------|-------------------------------|
| 1. 29902-F | GAA GTA GGC AGT CTC CAT CG    |
| 2. 29902-R | AAC ATC GCT CAG CCT GAA CC    |

### Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination | Band   | Genotype |
|--------------------|--------|----------|
| 1 and 2            | 145 bp | WT       |
| 1 and 2            | 180 bp | floxed   |



Wild-type band out competes floxed band in heterozygous DNA and should expect weak floxed band.