

# GENOTYPING BY PCR PROTOCOL FORM

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: MMRRC

Address: 2795 2nd street suite 400 Davis, CA 95618

Contact: Reneé Araiza

Telephone: 530-757-3284

FAX: 530-757-3284

email: mmrrc@ucdavis.edu

### PCR Protocol: STOCK Tg(Ppp1r2-EGFP)IX45Gsat MMRRC:031121-UCD

| Reagent/ Constituent             | Volume (μL)  |
|----------------------------------|--------------|
| Water                            | 11.275       |
| 10x Buffer                       | 2.5          |
| 25 mM MgCl <sub>2</sub>          | 1.7          |
| 5 M Betaine                      | 6.5          |
| 10 mM dNTPs                      | 0.5          |
| DMSO                             | 0.325        |
| Primer 1: (20uM)                 | 0.5          |
| Primer 2: (20uM)                 | 0.5          |
| Taq Polymerase-5 Units/μl        | 0.2          |
| DNA Sample                       | 1.0          |
| <b>TOTAL VOLUME OF REACTION:</b> | <b>25 μl</b> |

#### Comments on protocol:

- 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/DMSO is standardized due to high GC content in promoter regions and 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                         | 94                    | 5:00        | <b>1</b>      |
| 2. Denaturation                               | 94                    | 0:15        | \             |
| 3. Annealing steps 2-4 will cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30        | > <b>x 40</b> |
| 4. Extension                                  | 72                    | 0:40        | /             |
| 5. Final Extension                            | 72                    | 5:00        | <b>1</b>      |
| 6. Finish                                     | 4                     | Hold        | --            |

#### Primers:

| Primer Name                | Nucleotide sequence (5'– 3') |
|----------------------------|------------------------------|
| <b>1: Ppp1r2 (31121) F</b> | <b>GTGGCGTTGCGGTCATTGTG</b>  |
| <b>2: GS eGFP R3</b>       | <b>GGTCGGGGTAGCGGCTGAA</b>   |

#### Electrophoresis Protocol:

**% Agarose: 1.5    Volts :    90**

**Estimated Running Time (min):    90**

| Primer Combinations                       | Band size (bp) | genotype   |
|-------------------------------------------|----------------|------------|
| 1 & 2                                     | 440            | transgenic |
| <b>Note: Tg copy number is ~ 3/genome</b> |                |            |

