

**GENOTYPING BY PCR PROTOCOL FORM**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

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**PCR Protocol: STOCK Tg(Rab3b-EGFP)LK217Gsat/Mmcd MMRRC:031188-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: Rab3b (31188) F</b> | <b>GTTCCACCCCAATCCTGAAACAAC</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                             | 400            | transgenic |
| <b>Tg copy # ~ 24 copy/genome</b> |                |            |

