

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS**

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 530-754-MMRRC

**NAME OF PCR:** STOCK Tg(Slc6a5-cre)KF91Gsat/Mmucd **MMRRC #** 034815-UCD

**Protocol:**

| Reagent/ Constituent  | Volume (µL) |
|---|-------------|
| Water   | 11.275      |
| 10x Buffer  | 2.5         |
| MgCl <sub>2</sub> (25mM)  | 1.7         |
| Betaine (5M)  | 6.5         |
| dNTPs (10mM)  | 0.5         |
| DMSO  | 0.325       |
| Primer 1 (20µM)   | 0.5         |
| Primer 2 (20µM)   | 0.5         |
| Taq Polymerase (5Units/µL)  | 0.2         |
| DNA extracted with <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input type="checkbox"/> Other: | 1.0         |
| <b>TOTAL VOLUME OF REACTION:</b>  | <b>25µL</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 <span style="float:right">HOT START? <input type="checkbox"/></span> | 94                    | 5:00        | 1           |
| 2. Denaturation  | 94                    | 0:15        | } 40x       |
| 3. Annealing   | 65 to 55 (↓1°C/cycle) | 0:30        |             |
| 4. Elongation  |                       | 0:40        |             |
| 5. Amplification   | 72                    | 5:00        | 1           |
| 6. Finish  | 4                     | ∞           | n/a         |

**Primers:**

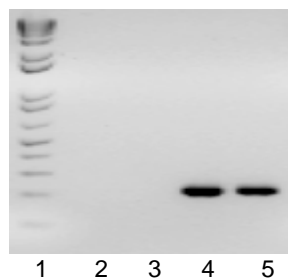
| Name                 | Nucleotide Sequence (5' - 3') |
|----------------------|-------------------------------|
| 1: Slc6a5 (34815) F1 | ggATTgCAgTgCTCCCAAgg          |
| 2: CreGS R1          | CGGCAAACGGACAGAAGCATT         |

**Electrophoresis Protocol:**

**Agarose:** 1.5% **V:** 90

**Estimated Running Time:** 90 min.

| Primer Combination | Band   | Genotype   |
|--------------------|--------|------------|
| 1 and 2            | 200 bp | transgenic |



Lanes

- 1: 1kb+ ladder (Invitrogen, Cat. #10787-026)
- 2: ntc
- 3: wild-type & Cre
- 4-5: Slc6a5+