

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK Tg(Vip-EGFP)JN37Gsat/Mmucd MMRRC # 031009-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)                                                                                               | 6.5             |
| dNTPs (stock concentration is 10mM)                                                                                               | 0.5             |
| DMSO                                                                                                                              | 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 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>25.000μL</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. 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        | <b>1</b>    |
| 2. Denaturation                                           | 94                    | 0:15        | <b>40x</b>  |
| 3. Annealing } steps 2-3-4 will cycle in sequence         | 65 to 55 (↓1°C/cycle) | 0:30        |             |
| 4. Elongation                                             | 72                    | 0:40        |             |
| 5. Amplification                                          | 72                    | 5:00        | <b>1</b>    |
| 6. Finish                                                 | 4                     | Hold        | n/a         |

### Primers:

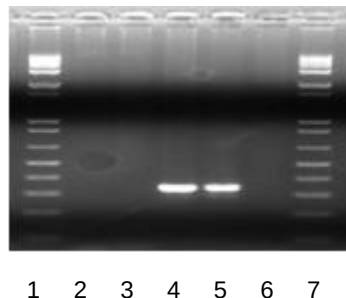
| Name              | Nucleotide Sequence (5' - 3') |
|-------------------|-------------------------------|
| 1. Vip (31009) F1 | GCTAGACCCTCTGAAATGTTGCCAA     |
| 2. GS eGFP R3     | GGTCGGGGTAGCGGCTGAA           |

### Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

| Primer Combination | Band   | Genotype   |
|--------------------|--------|------------|
| 1 and 2            | 350 bp | transgenic |
|                    |        |            |



Lanes  
 1 & 7: 1 kb+ ladder  
 (Invitrogen, Cat. #10787-026)  
 2: H<sub>2</sub>O  
 3: Wild-type Control  
 4 & 5: Vip tg/+  
 6: Other GENSAT line