

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

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PCR Protocol: STOCK Tg(Hpcal4-EGFP)IZ36Gsat MMRRRC:031120-UCD

| Reagent/ Constituent | Volume (µL) |
|----------------------------------|--------------|
| Water | 11.275 |
| 10x Buffer | 2.5 |
| 25 mM MgCl ₂ | 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 |
| TOTAL VOLUME OF REACTION: | 25 µl |

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 MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

| Steps | Temp (°C) | Time (m:ss) | # of Cycles |
|---|-----------------------|-------------|---------------|
| 1. Initiation/Melting | 94 | 5:00 | 1 |
| 2. Denaturation | 94 | 0:15 | \ |
| 3. Annealing steps 2-4 will cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30 | > x 40 |
| 4. Extension | 72 | 0:40 | / |
| 5. Final Extension | 72 | 5:00 | 1 |
| 6. Finish | 4 | Hold | -- |

Primers:

| Primer Name | Nucleotide sequence (5'- 3') |
|---------------------|------------------------------------|
| 1: Hpcal4 (31120) F | GGACAGATGCTGACAGATGTTAGGAAG |
| 2: GS eGFP R3 | GGTCGGGGTAGCGGCTGAA |

Electrophoresis Protocol:

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

| Primer Combinations | Band size (bp) | genotype |
|---|----------------|------------|
| 1 & 2 | 410 | transgenic |
| Note: Tg copy number is ~ 8/genome | | |

