

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129P2-Sphk2<sup>Gt(NPX357)Byg</sup>/Mmucd MMRRC # 000402-UCD

### Protocol: (B-Geo Tcrd Duplex)

| Reagent/ Constituent                                                                                                              | Volume (μL) |
|-----------------------------------------------------------------------------------------------------------------------------------|-------------|
| Water                                                                                                                             | 10.275      |
| 10x Buffer (contains 15mM MgCl <sub>2</sub> )                                                                                     | 2.5         |
| MgCl <sub>2</sub> (stock concentration is 25mM)                                                                                   | 1.7         |
| Betaine (stock concentration is 5M) <i>Optional</i>                                                                               | 6.5         |
| dNTPs (stock concentration is 10mM)                                                                                               | 0.5         |
| DMSO <i>Optional</i>                                                                                                              | 0.325       |
| Primer 1 (stock concentration is 20μM) B-Geo F                                                                                    | 0.8         |
| Primer 2 (stock concentration is 20μM) B-Geo R                                                                                    | 0.8         |
| Primer 3 (stock concentration is 20μM) Tcrd F                                                                                     | 0.2         |
| Primer 4 (stock concentration is 20μM) Tcrd R                                                                                     | 0.2         |
| 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 this generic protocol for BayGenomics ES Cell lines and other gene trap constructs. Primers amplify fragment between neomycin and β-galactosidase fusion vector element. TCRD is an internal control for presence of DNA.
- Additional [BayGenomics Protocols](#) can be found at the International Gene Trap Consortium (IGTC) website.

### Strategy:

| Steps                                                     | Temp (°C) | Time (m:ss) | # of Cycles  |
|-----------------------------------------------------------|-----------|-------------|--------------|
| 1. Initiation/Melting HOT START? <input type="checkbox"/> | 95        | 2:30        | <b>1</b>     |
| 2. Denaturation                                           | 94        | 1:00        | } <b>34x</b> |
| 3. Annealing                                              | 60        | 0:45        |              |
| 4. Elongation                                             | 72        | 1:00        |              |
| 5. Amplification                                          | 72        | 5:00        | <b>1</b>     |
| 6. Finish                                                 | 4         | ∞           | n/a          |

### Primers:

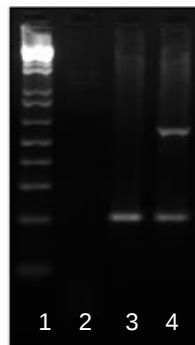
| Name       | Nucleotide Sequence (5' - 3') |
|------------|-------------------------------|
| 1: B-Geo F | CAA ATG GCG ATT ACC GTT GA    |
| 2: B-Geo R | TGC CCA GTC ATA GCC GAA TA    |
| 3: Tcrd F  | CAA ATG TTG CTT GTC TGG TG    |
| 4: Tcrd R  | GTC AGT CGA GTG CAC AGT TT    |

### Electrophoresis Protocol:

Agarose: 1.5% V: 80

Estimated Running Time (min): 90

| Expected Bands | Genotype |
|----------------|----------|
| 200 bp         | WT +/+   |
| 200 / 581 bp   | B-Geo +  |



Lanes:  
1. 1Kb+ ladder  
2. H<sub>2</sub>O control  
3. Wild-type control  
4. B-Geo control