

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129P2-*Pan3*<sup>Gt(BGB533)Byg</sup>/Mmcd

MMRRC # 030154 - 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> (25mM)                                                      | 1.7         |
| Betaine (5M) <i>Optional</i>                                                  | 6.5         |
| dNTPs (10mM)                                                                  | 0.5         |
| DMSO <i>Optional</i>                                                          | 0.325       |
| Primer 1 (20μM)                                                               | 0.5         |
| Primer 2 (20μM)                                                               | 0.5         |
| Primer 3 (20μM)                                                               | 0.5         |
| Primer 4 (20μM)                                                               | 0.5         |
| Taq Polymerase (5Units/μL)                                                    | 0.2         |
| DNA extracted with "Qiagen DNeasy columns or other similar silica based kits" | 1.0         |
| <b>Total Volume of Reaction:</b>                                              | <b>25.0</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 | 94                     | 5:00        | 1            |
| 2. Denaturation       | 94                     | 0:15        | } <b>40x</b> |
| 3. Annealing          | 65 to 55 (↓ 1°C/cycle) | 0:30        |              |
| 4. Elongation         |                        | 0:40        |              |
| 5. Amplification      | 72                     | 5:00        |              |
| 6. Finish             | 15                     | ∞           | 1            |

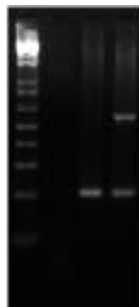
### Primers:

| Primer Name | Nucleotide Sequence (5' - 3') |
|-------------|-------------------------------|
| 1. B-Geo F  | CAAATGGCGATTACCGTTGA          |
| 2. B-Geo R  | TGCCAGTCATAGCCGAATA           |
| 3. Tcrd F   | CAAATGTTGCTTGTCTGGTG          |
| 4. Tcrd R   | GTCAGTCGAGTGCACAGTTT          |

### Electrophoresis Protocol:

Agarose: 1.5%      V: 90  
 Estimated Running Time: 90 min.

| Expected Band | 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