

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129P2-Wnk3-ps<sup>Gt(RR.J530)Byg</sup>/Mmucd MMRRC # 032830-UCD

### Protocol: (β-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) β-Geo F                                             | 0.5              |
| Primer 2 (stock concentration is 20μM) β-Geo R                                             | 0.5              |
| Primer 3 (stock concentration is 20μM) Tcrd F                                              | 0.5              |
| Primer 4 (stock concentration is 20μM) Tcrd R                                              | 0.5              |
| Taq Polymerase 5Units/μL                                                                   | 0.2              |
| DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.0              |
| <b>TOTAL VOLUME OF REACTION:</b>                                                           | <b>25.000 μL</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.
- The TCRD rxn is an internal control for testing for the presence of DNA.
- 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.
- 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"/> | 94                    | 5:00        | 1           |
| 2. Denaturation                                           | 94                    | 0:15        |             |
| 3. Annealing steps 2-3-4 cycle in sequence                | 65 to 55 (↓1°C/cycle) | 0:30        | 40x         |
| 4. Elongation                                             | 72                    | 0:40        |             |
| 5. Amplification                                          | 72                    | 5:00        | 1           |
| 6. Finish                                                 | 15                    | ∞           | n/a         |

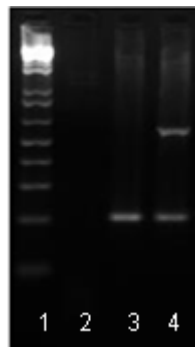
### Primers:

| Name       | Nucleotide Sequence (5' - 3') |
|------------|-------------------------------|
| 1. β-Geo F | CAA ATG GCG ATT ACC GTT GA    |
| 2. β-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: 90  
 Estimated Running 90 min.

| Primers | Band   | Genotype          |
|---------|--------|-------------------|
| 1 and 2 | 581 bp | β-Geo +           |
| 3 and 4 | 200 bp | DNA Reference rxn |



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