

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

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 530-754-MMRRC

NAME OF PCR: B6;129P2-*Elovl5*<sup>Gt(CC0725)Wtsi</sup>/Mmucd

MMRRC # 031820-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 <span style="float:right">HOT START? <input type="checkbox"/></span>	95	2:30	1
2. Denaturation	94	1:00	} 34x
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:45	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

**Primers:**

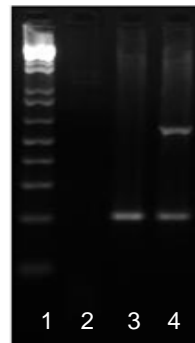
Name	Nucleotide Sequence (5' - 3')
1: B-Geo F	CAA ATG GCG ATT ACC GTT GA
2: B-Geo R	TGC CCA GTC ATA GCC GAA TA
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

**Electrophoresis Protocol:**

Agarose: 1.5% V: 90

Estimated Running Time (min): 90

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



Lanes:

- 1Kb+ ladder
- H<sub>2</sub>O control
- Wild-type control
- B-Geo control