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

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NAME OF PCR: **B6;129P2-*6530418L21Rik*$^{Gt(XL577)Byg}$/Mmucd** **MMRRC #** **036557-UCD**

Protocol: (B-Geo Tcrd Duplex)

Reagent/ Constituent	Volume (µL)
Water	10.275
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	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 ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25µL**

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? ☐	95	2:30	**1**
2. Denaturation	steps 2-3-4 will cycle in sequence	94	1:00	**34x**
3. Annealing		60	0:45	
4. Elongation		72	1:00	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

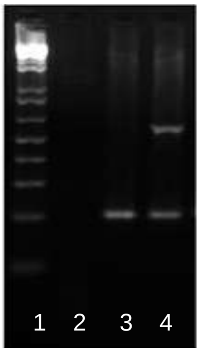
Name	Nucleotide Sequence (5' - 3')
1: B-Geo F	CAA ATG GCG ATT ACC GTT GA
2: B-Geo R	TGC CCA GTC ATA GCC GAA TA
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time (min): 90

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



Lanes:
1. 1Kb+ ladder
2. H_2O control
3. Wild-type control
4. B-Geo control

PCR protocol developed by MMRRC at University of California, Davis