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

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530-754-MMRRC

NAME OF PCR:B6;129P2-*Trim32*$^{Gt(BGA355)Byg}$/Mmcd **MMRRC # 011810 - UCD**

Protocol: B-Geo Tcrd Duplex

Reagent/ Constituent	Volume (µL)
Water	10.275
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (25mM)	1.7
Betaine (5M) *Optional*	6.5
dNTPs (10mM)	0.5
DMSO *Optional*	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
Total Volume of Reaction:	25.0

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	} steps 2-3-4 will cycle in sequence	94	0:15	} **40x**
3. Annealing		65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		72	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	TGCCCAGTCATAGCCGAATA
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_2O control
3. Wild-type control
4. B-Geo control