GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

NAME OF PCR: B6;129P2-*Gnpat*$^{Gt(RRM060)Byg}$/Mmucd **MMRRC #** 032831-UCD

Protocol: (β-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) 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
TOTAL VOLUME OF REACTION:	**25.000 µL**

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? ☐	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:

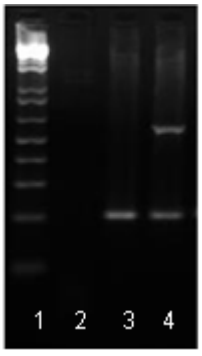
Name	Nucleotide Sequence (5' - 3')
1. β-Geo F	CAA ATG GCG ATT ACC GTT GA
2. β-Geo R	TGC CCA GTC ATA GCC GAA TA
3. Tcrd F	CAA ATG TTG CTT GTC TGG TG
4. Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

Agarose: 1.5% **V:** 90

Estimated Running Time: 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_2O control
3. DNA reference rxn
4. B-Geo control