

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129P2-*Prkd2*^{Gt(RR380)Byg}/Mmcd

MMRRC # 030152 - 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) <i>Optional</i>	6.5
dNTPs (10mM)	0.5
DMSO <i>Optional</i>	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	94	0:15	} 40x
3. Annealing	65 to 55 (↓ 1°C/cycle)	0:30	
4. Elongation		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	TGCCAGTCATAGCCGAATA
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. H₂O control
 3. Wild-type control
 4. B-Geo control