

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol: **NAME OF PCR** B-Geo Tcrd Duplex

Reagent/ Constituent	Vol. 1(ul)
Water	12.4
10x Buffer w/ 15 mM MgCl ₂	2
10 mM dNTPs	0.4
Primer 1: B-Geo F -20 uM	1
Primer 2: B-Geo R -20 uM	1
Primer 3: Tcrd F - 20 uM	0.4
Primer 4: Tcrd R - 20 uM	0.4
Taq Polymerase	0.4
DNA Sample	2
TOTAL VOLUME OF REACTION:	20 ul

Comments on protocol: Use for BayGenomics ES Cell lines and other genetrapp constructs. Primers amplify fragment between Neomycin and B-Galactosidase fusion vector element..TCRD is an internal control for presence of DNA

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	2:30	1
2. Denaturation	94	1:00	\
3. Annealing	60	0:45	> 30
4. Elongation			
5. Amplification	72	5:00	1
6. Finish	4	hold	--

Primers:

Primer Name	Nucleotide sequence (5' - 3')																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1: B-Geo F	C	A	A	A	T	g	g	C	g	A	T	T	A	C	C	g	T	T	g	A															
2: B-Geo R	T	g	C	C	C	A	g	T	C	A	T	A	g	C	C	g	A	A	T	A															
3: Tcrd F	C	A	A	A	T	g	T	T	g	C	T	T	g	T	C	T	g	g	T	g															
4: Tcrd R	g	T	C	A	g	T	C	g	A	g	T	g	C	A	C	A	g	T	T	T															

Electrophoresis Protocol:

% Agarose: 2 mV : 80

Estimated Running Time (min): 90

Number	Band (bp)	genotype
1	No band	No dna
2	200	Wild type dna only
3	200/581	B-Geo positive

