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

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530-754-MMRRC

NAME OF PCR: **B6;129P2-*Srgap2*$^{Gt(XH102)Byg}$/Mmucd** **MMRRC: 030115-UCD**

Protocol: ***(PCR protocol provided by Research Investigator)***

Reagent/Constituent	Volume (µL)
Water	15.0
10x Buffer	2.5
$MgCl_2$	2.5
dNTPs	1.0
Primer 1. (#328 common forward)	1.0
Primer 2. (#329 WT reverse)	1.0
Primer 3. (#324 mutant reverse)	1.0
Taq Polymerase 5Units/µL	0.1
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.100 µL**

Comments on protocol:

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Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	96	3:00	**1**
2. Denaturation	96	0:15	**30x**
3. Annealing steps 2-3-4 cycle in sequence	55	0:30	
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 328 Foward	CTCTCAGGACTTTACTCTGC
2. 329 WT reverse	CAAAGATAGAGCTGCAACCAC
3. 324 mutant reverse	ACCGGCTAAAACTTGAGACC

Electrophoresis Protocol:

Argarose: **1.5** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	Band	Genotype
1 and 2	512 bp	WT
1 and 3	396 bp	Mutant (-/-)

Generic ß-Geo protocol developed by MMRRC at University of California, Davis attached below

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NAME OF PCR: B6;129P2-*Srgap2*$^{Gt(XH102)Byg}$/Mmucd **MMRRC #** 030155-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.

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:

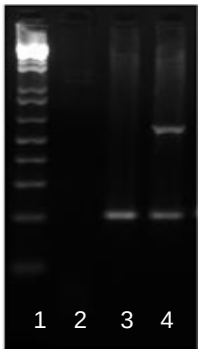
Name	Nucleotide Sequence (5' - 3')
1: B-Geo F	CAA ATG GCG ATT ACC GTT GA
2: B-Geo R	TGC CCA GTC ATA GCC GAA TA
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

Agarose: 2% **mV:** 80

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 provided by Research Investigator