

GENOTYPING BY PCR PROTOCOL FORM #30110

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: DNA was extracted from mouse tail by using DNeasy Blood & Tissue Kit (Cat. No. 69506) from QIAGEN Inc.

Protocol: NAME OF PCR: **mutCRE-bcl2 PCR condition**

Reagent/ Constituent	Volume (uL)
DNA Sample	2
5x AccuPrimer™ GC-Rich Buffer A (contains dNTPs and MgSO4)	5
Primer 1 (stock concentration is 5 uM)	1
Primer 2 (stock concentration is 5 uM)	1
AccuPrimer™ GC-Rich DNA Polymerase	0.5
H ₂ O	15.5
Additives if applicable:	n/a
TOTAL VOLUME OF REACTION:	25 ul

Comments on protocol: AccuPrimer™ GC-Rich DNA Polymerase Kit (including 5x AccuPrimer™ GC-Rich Buffer A and AccuPrimer™ GC-Rich DNA Polymerase) was purchased from Invitrogen (Cat. No. 12337-016).

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?...CHECK HERE []	95	3	1
2. Denaturation	95	30 sec	
3. Annealing	60	35 sec	
4. Elongation	72	1	32 cycles
5. Amplification (i.e., 72°C, 10 min)	72	10	1
6. Finish (i.e., 4°C, indefinite)		n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1:	5'-CTCCACGGTGCTGTCCAATGAC-3'
2:	5'-GGATCTGATGCCCTCTTCTG-3'

Electrophoresis Protocol:

% Agarose: 1% V : 110 v

Estimated Running Time (min): **90 min**

Primer combination	Band (kB)	genotype
1 & 2	458 bp	Knock in
1 & 2	303 bp	Wild-type

1kb plus DNA ladder from Fisher Scientific Inc.
(Cat. No. BP2579100).

**PASTE GEL PICTURE HERE, CLEARLY
INDICATING LADDER, WATER CONTROL, DNA
CONTROL, & DIAGNOSTIC SAMPLES**