GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: C57BL/6J-*Ap3b1*^{m1Btlr}/Mmcd, (*bullet gray*) **MMRRC #** 030291-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	37.0
10x Buffer	5.0
dNTPs (stock concentration is 25mM)	2.5
Primer 1 (stock concentration is 20µM) bullet gray PCR F	1.0
Primer 2 (stock concentration is 20µM) bullet gray PCR R	1.0
Taq Polymerase	2.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**50µL**

Comments on protocol:

- *Bullet gray* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transversion.
- Confirm the size and presence of the amplicon on a 1X TAE gel. Purify using spin dialysis or the QIAGEN QIAquick PCR purification kit. Sequence the purified DNA fragments utilizing the Sequencing Primers listed.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	2:00	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	0:30	} **29x**
3. Annealing }	56	0:30	
4. Elongation }	72	1:00	
5. Amplification	72	7:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: bullet gray PCR F	ACC CTG GCT TGA AAA TGT CCC TTT G
2: bullet gray PCR R	CGA CTT CCA CGC ATG ACT AGG AAA C
3: bullet gray_Seq (F)	ACG TTA AGC AAT CAT TTG GGG C
4: bullet gray_Seq (R)	TAT GAA GCA GTG CTT AGT GAA TG

Electrophoresis Protocol:

Agarose: 2% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	621 bp	*bullet gray*
SNP found at position ~ 266 of sequencing		

Mutation site (red) and flanking sequence:

WT ggatg**G**tgag
bullet gray ggatg**T**tgag