GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: C57BL/6J-*Irf8*^{m1Btlr}/Mmcd, (*Gemini*) **MMRRC #** 034294-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	20.0
10x Buffer (contains 15mM $MgCl_2$)	2.5
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) Gemini(F)	0.5
Primer 2 (stock concentration is 20µM) Gemini(R)	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	0.5
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

PCR products are verified to contain the correct amplicon size by running ~10µl of the reaction on a gel and the remaining 15µl purified via column based PCR purification method for sequencing.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	**1**
2. Denaturation	94	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	60	0.20	} **35x**
4. Elongation	72	1:00	
5. Amplification	72	5:00	**1**
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Gemini(F)	AGA GGA CTT TGC CAA AGC TGC ATC
2: Gemini(R)	TGC ATC ACA GAC TTC AGC AGA GCC
3: Gemini_seq(F)	CAA ATT CCA GCT TTG CTG AGT G

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	824 bp	*gemini*
SNP found at position ~ 320		

Mutation site (red) and flanking sequence:

wt ggaagaa**C**aaaaatgtaact

gemini ggaagaa**T**aa aaatgtaact