GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6J-*Tlr6*^{m3Btlr}/Mmucd, *(m2sd2)*** **MMRRC #** **030960-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	20.0
10x Buffer (contains 15mM $MgCl_2$)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer 1 (stock concentration is 20µM) PCR F	0.5
Primer 2 (stock concentration is 20µM) PCR R	0.5
Taq Polymerase	0.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	0.5
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- *M2sd2* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change. The genotyping protocol is the same as for *m2sd1*.
- lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma), 12.5 µl in a 25 µl reaction (includes Taq, buffer, dNTPs).
- 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? ☒	94	2:00	**1**
2. Denaturation	94	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	56	0:30	} **30x**
4. Elongation	72	1:00	
5. Amplification	72	7:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. M2sd1 PCR F	GGAGACAGCACTGAAGTCACTGATG
2. M2sd1 PCR R	TGGCACCACTCACTCTGGATGAAG
3. M2sd1 _Seq (F)	TTTCAAAGGAGGCGCTATACTCG

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	1.24 kB	*m2sd2*
SNP found at position ~ 1301 **of sequencing**		

Mutation site (red) and flanking sequence:

WT gtcttc **T**gatgct
M2sd2 gtcttc **A**gatgct