GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6J-*Tlr6*^{m2Btlr}/Mmucd, *(m2sd1)*** **MMRRC #** **030959-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:

- *M2sd1* 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.
- 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	*m2sd1*
SNP found at position ~ 1501 **of sequencing**		

Mutation site (red) and flanking sequence:

WT agagat **A**gcagac
M2sd1 agagat **T**gcagac