GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

2795 2nd Street, Suite 400, Davis, CA 95618
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530-754-MMRRC

NAME OF PCR: **C57BL/6J-*Tlr9*^{M8Btlr}/Mmcd, *(Meager)*** **MMRRC #** **034618-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:

- *Meager* 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).

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: Meager (F)	TGGCTGTTCCTGAAGTCTGTACCC
2: Meager (R)	AGATGCCGTTCATGTTCAGCTCC
3: Meager_Seq (F)	GTTTCTCTGCGGCAGCATC
4: Meager_Seq (R)	CTTGACAATGAGGTTATAGGACACC

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	985 bp	*Meager*

Mutation site (red) and flanking sequence:

WT tgaagtataac**A**acctcaca
Meager tgaagtataac**T**acctcaca

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NAME OF PCR: C57BL/6J-*Tlr9*^{M8Btlr}/Mmcd, *(Meager)* **MMRRC # 034618-UCD**

The following sequence of 985 nucleotides (NCBI Mouse Genome Build 37.1, Chromosome 9, bases 106,125,981 to 106,126,965) is amplified:

```
                                        tggctgtt cctgaagtct gtaccccgtt
tctctgcggc agcatcctgc tccaacatca cccgcctctc cttgatctcc aaccgtatcc
accacctgca caactctgac ttcgtccacc tgtccaacct gcggcagctg aacctcaagt
ggaactgtcc acccactggc cttagccccc tgcacttctc ttgccacatg accattgagc
ccagaacctt cctggctatg cgtacactgg aggagctgaa cttgagctat aatggtatca
ccactgtgcc ccgactgccc agctccctgg tgaatctgag cctgagccac accaacatcc
tggttctaga tgctaacagc ctcgccggcc tatacagcct gcgcgttctc ttcatggacg
ggaactgcta ctacaagaac ccctgcacag gagcggtgaa ggtgacccca ggcgccctcc
tgggcctgag caatctcacc catctgtctc tgaagtataa caacctcaca aaggtgcccc
gccaactgcc ccccagcctg gagtacctcc tggtgtccta taacctcatt gtcaagctgg
ggcctgaaga cctggccaat ctgacctccc ttcgagtact tgatgtgggt gggaattgcc
gtcgctgtga ccatgccccc aatccctgta tagaatgtgg ccaaaagtcc ctccacctgc
accctgagac cttccatcac ctgagccatc tggaaggcct ggtgctgaag gacagctctc
tccatacact gaactcttcc tggttccaag gtctggtcaa cctctcggtg ctggacctaa
gcgagaactt tctctatgaa agcatcaccc acaccaatgc ctttcagaac ctaacccgcc
tgcgcaagct caacctgtcc ttcaattacc gcaagaaggt atcctttgcc cgcctccacc
tggcaagttc ctttaagaac ctggtgtcac tgcaggagct gaacatgaac ggcatct
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Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is indicated in red.