GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6-*Tlr2*^{lngd}/Mmcd, (*languid*)** **MMRRC #** **012838-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	12.675
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) languid PCR F1	0.5
Primer 2 (stock concentration is 20µM) languid PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- The *languid* mutation introduces an *Mse* I restriction enzyme site in the *Tlr2* genomic DNA sequence. *Languid* genotyping is performed by amplifying the region containing the mutation using PCR, followed by *Mse* I restriction enzyme digestion.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: languid PCR F1	CTC AGA CGC TGG AGG TGT TGG ATG TTA G
2: languid PCR R1	GCA GCC TGG TGA CAT TCC AAG ACG

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
1 and 2	400 bp	WT
the novel *Mse* I site is highlighted in gray		
Restriction Digest w/ *Mse* I	94 bp, 306 bp	*languid*

Mutation site (red) and flanking sequence:

WT tccag aaAtaagctg
languid tccag aaTtaagctg