

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K X Other _____

Protocol: NAME OF PCR: MMRRC Strain 30238-UCD, C57BL/6J-*Tlr4*^{m1Btr}, (Lps3)

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl ₂)	5
dNTPs (stock concentration is 25mM)	0.4
Primer 1 (stock concentration is 20 uM)	1
Primer 2 (stock concentration is 20 uM)	1
Primer 3 (stock concentration is 20 uM)	
Primer 4 (stock concentration is 20 uM)	
Taq Polymerase	2.5
water	39.1
TOTAL VOLUME OF REACTION:	
	50 ul

Comments on protocol (e.g., different concentration of MgCl₂, etc): using primers 1 & 2 for amplification and 3 & 4 for sequencing.

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []	94	2	1
2. Denaturation	94	15 seconds	30
3. Annealing } steps 2-3-4 will cycle in sequence	60	30 seconds	
4. Elongation	72	1 min	
5. Amplification (i.e., 72°C, 10 min)	72	7	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Lps3_typeF	AGCACCTGGATTTTCAGCACTCTAC
2: Lps3_typeR	AAGGCCCACTGAAGACTGGTTCTC
3: Lps3_seqF	GGGGTATTTGACACCCTCCATAG
4: Lps3_seqR	TAGGTAATTCATACCCCTGGAAAGG

Electrophoresis Protocol:

% Agarose: _____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	1528	
(i.e. 3&4)		
(i.e. 1&2&3)		

The flanking sequences of mutation sites:

Wt ACAGAG**A**CTTTAT
 Lps3 ACAGAG**T**CTTTAT