GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR: C57BL/6-*Tlr4*$^{lps\text{-}2Btlr}$/Mmcd (*Lps4*), MMRRC #031073-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): *Lps4* 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 lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs).

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [**x**]	94	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0.50	30
3. Annealing	56	0.50	30
4. Elongation	72	1	30
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

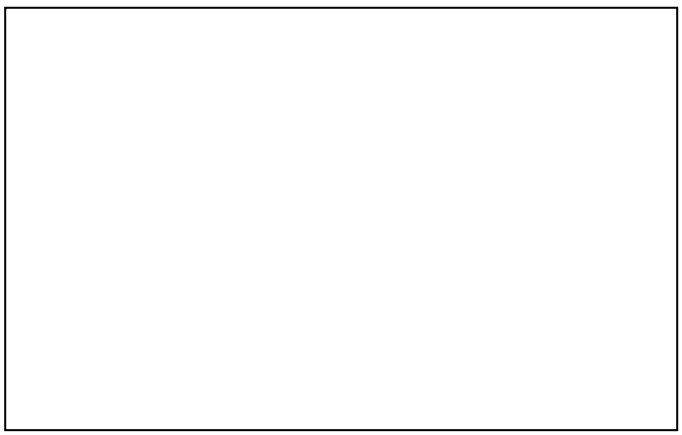
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Lps4 (F)	TCTTGGCTTGACCAGTCTCAACAC
2: Lps4 (R)	TTGTCCTCCCATTCCAGGTAGGTG
3: Lps4 -seq (F1)	GGGGTATTTGACACCCTCCATAG
4: Lps4 _seq (F2)	ACAATCATCAGTGTGTCAGTGG
5: Lps4 _seq (R)	GTTTCTGCTAAGAAGGCGATAC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.999	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 999 nucleotides (from Genbank genomic region **NC_000070** for linear DNA sequence of *Tlr4*) is amplified:

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12556                tcttg gcttgaccag tctcaacaca ttaaaaatgg ctggcaattc
12601 tttcaaagac aacacccttt caaatgtctt tgcaaacaca acaaacttga cattcctgga
12661 tctttctaaa tgtcaattgg aacaaatatc ttgggggggta tttgacaccc tccatagact
12721 tcaattatta aatatgagtc acaacaatct attgtttttg gattcatccc attataacca
12781 gctgtattcc ctcagcactc ttgattgcag tttcaatcgc atagagacat ctaaaggaat
12841 actgcaacat tttccaaaga gtctagcctt cttcaatctt actaacaatt ctgttgcttg
12901 tatatgtgaa catcagaaat tcctgcagtg ggtcaaggaa cagaagcagt tcttggtgaa
12961 tgttgaacaa atgacatgtg caacacctgt agagatgaat acctccttag tgttggattt
13021 taataattct acctgttata tgtacaagac aatcatcagt gtgtcagtgg tcagtgtgat
13081 tgtggtatcc actgtagcat ttctgatata ccacttctat tttcacctga tacttattgc
13141 tggctgtaaa aagtacagca gaggagaaag catctatgat gcatttgtga tctactcgag
13201 tcagaatgag gactgggtga gaaatgagct ggtaaagaat ttagaagaag gagtgccccg
13261 ctttcacctc tgccttcact acagagactt tattcctggt gtagccattg ctgccaacat
13321 catccaggaa ggcttccaca agagccggaa ggttattgtg gtagtgtcta gacactttat
13381 tcagagccgt tggtgtatct ttgaatatga gattgctcaa acatggcagt ttctgagcag
13441 ccgctctggc atcatcttca ttgtccttga gaaggttgag aagtccctgc tgaggcagca
13501 ggtggaattg tatcgccttc ttagcagaaa cacctacctg gaatgggagg acaa
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated G is shown in red text.