

RMRC Genotyping Protocol-PCR

No.	RMRC13250	Strain Name	C57BL/6- <i>Tlr4</i> ^{tm1c(GEMMS)<i>Narl</i>} / <i>Narl</i>
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A. Primers

No.	Primer name	Sequence
1	Tlr4LF	AGTCACAGAGACTCTGTTGTGGCTC
2	Tlr4LR	GGTGTCAAGAGTCAAGAATGGCAT

B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	10.8 μl	1	94	180	1
PCR buffer	10 x	1.5 μl	2	94	30	35
Formamide	–	0.5 μl	3	60	30	35
dNTP	10 mM	0.3 μl	4	72	30	35
Primer 1	20 μM (μmol/μl)	0.3 μl	5	72	180	1
Primer 2	20 μM (μmol/μl)	0.3 μl				
gDNA	50-250 ng/μl	1 μl				
Taq	5 U/μl	0.3 μl				
Total		15 μl				

C. PCR Product

No.	Primer set		Product size	Target region
1	Tlr4LF	Tlr4LR	340 bp	Wildtype
2	Tlr4LF	Tlr4LR	440 bp	Mutant

D. Gel Photo

