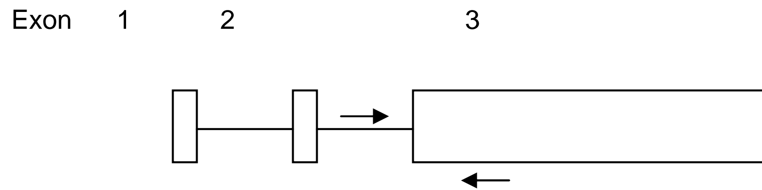
PCR Screening for TLR4 Targeted Mice

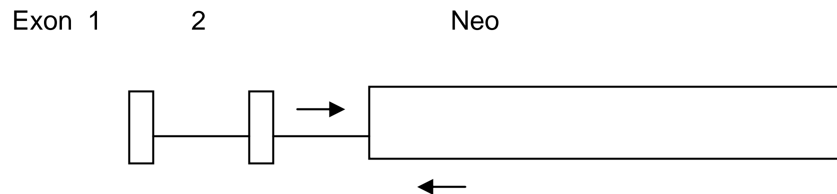
Purpose: To identify Toll receptor 4 (TLR4) deficient mice.

Principle: The murine Tlr4 gene contains 3 exons (transcript length is 3843 bps, and translation length is 835 bps) and is located in mouse chromosome 4 (ensemble: ENSMUSG00000039005, NCBI: NM_021297.2, location: 66,313,972-66,328,954). The gene has been disrupted by insertion of a Neomycin (***Neo***) cassette (unknown origin) sequence in replacement of exon 3. The screen amplifies a section of intron 2-3 (Forward primer HC-26 attaches in intron 2-3 from position 4372-4399) and either the ***Neo*** cassette to yield a band specific to the KO (1300bp) or a section of exon 3 (Reverse primer HC-27 attaches in exon 3 from position 503-477) for the wild-type band (1300bp).

Wild-Type Band (~1300bp)



Targeted Band (~1300bp)

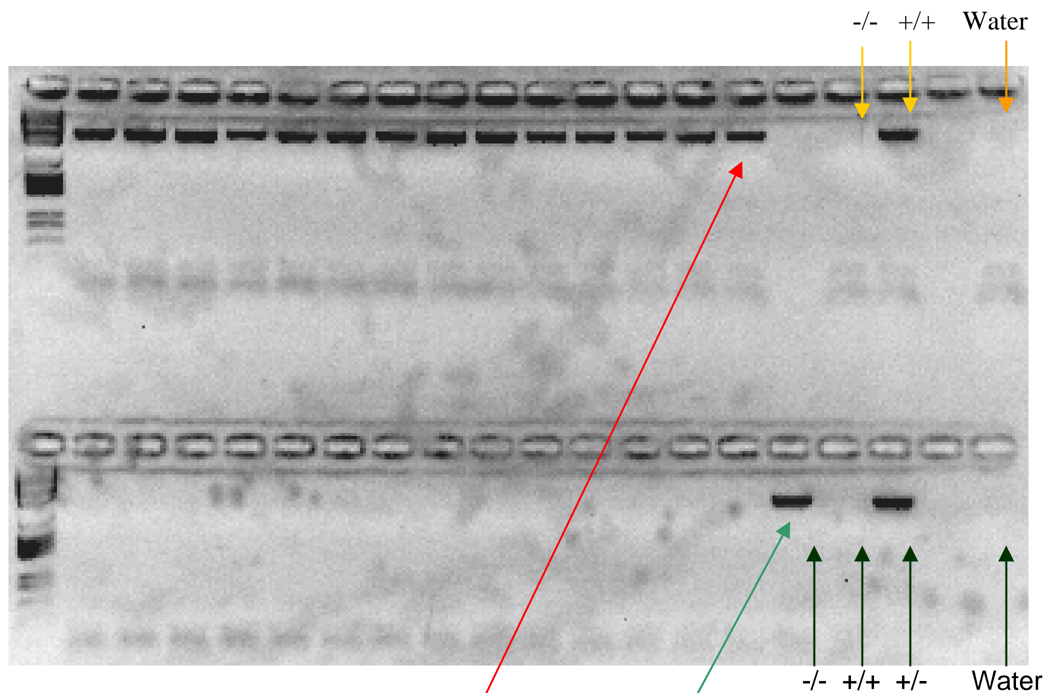


Primers:

TLR4 HC-26 F: `5'-TGTTGCCCTTCAGTCACAGAGACTCTG-3'` 27-mer

TLR4 HC-27 R: `5'-CGTGTAAACCAGCCAGGTTTTGAAGGC-3'` 27-mer

TLR4 HC-28 NEO: `5'-ATCGCCTTCTATCGCCTTCTTGACGAG-3'` 27-mer



Wild type gel is run on the top,
deficient gel is run on the bottom.
All mice in this screen were TLR4+/+.

Wild-type locus will produce a 1300 bp product
Targeted locus will produce a 1300 bp product

Reaction Components:

Wild-type Reaction

		PCR Program:
DNA template	2.0 µl	94° for 4 minutes
TLR4 HC-26 Forward	0.2 µl	
TLR4 HC-27 Reverse	0.2 µl	94˚ for 1 minute (30 cycles)
*Promega Master Mix	10.0 µl	62˚ for 1 minute (30 cycles)
*Taq	0.1 µl	72˚ for 2 minutes (30 cycles)
H_2O	7.5 µl	
	20.0 µl total	72˚ for 10 Minutes

Targeted Reaction

DNA template	2.0 µl	94° for 4 minutes
TLR4 HC-26 Forward	0.2 µl	
TLR4 HC-28 NEO	0.2 µl	94˚ for 1 minute (30 cycles)
*Promega Master Mix	10.0 µl	62˚ for 1 minute (30 cycles)
*Taq	0.1 µl	72˚ for 2 minutes (30 cycles)
H_2O	7.5 µl	
	20.0 µl total	72˚ for 10 Minutes

*Note: If you are using Promega GoTaq Green Master Mix, you do not have to add Taq polymerase

Electrophoresis Conditions:

Since the two products come out at exactly the same (1300bp), you must make sure to run on a two row gel, or make two gels. Run with 2% agarose loading 15-20ul at 100-110 Volts for ~30 minutes in order to achieve separation.

This protocol was adapted from Dr. Peter Tobias at the Scripps Institute.

Modified: 09-16-07 APO
Updated: 07-10-08 APO