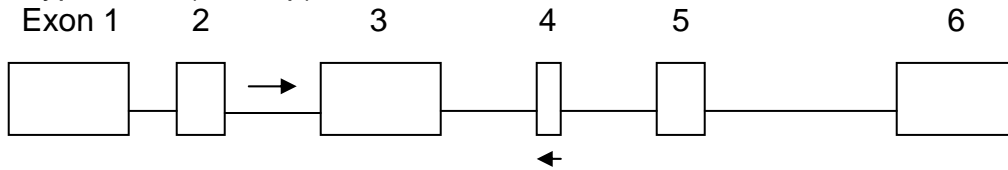


## Genotyping of MyD88 Deficient Mice by PCR

**Purpose:** To identify MyD88 deficient mice from wild-type mice.

**Gene Information:** The murine Myeloid Differentiation Factor 88 (MyD88) gene contains 6 exons (transcript length is 1911 bps, and translation length is 296 residues) and is located in mouse chromosome 9 (ensemble: ENSMUST00000035092, NCBI: NM\_010851, location: 119,184,642-119,188,700 ). The gene has been disrupted by insertion of a Neomycin (**Neo**) cassette (unknown origin) sequence in replacement of exons 4 and 5 (exon 6 is non-coding). The screen amplifies a section of the intron between exon 2 and 3 and either the **Neo** cassette to yield a unique band specific to the KO (600bp) or a section of exon 4 (550bp).

Wild-Type Band (~550bp)



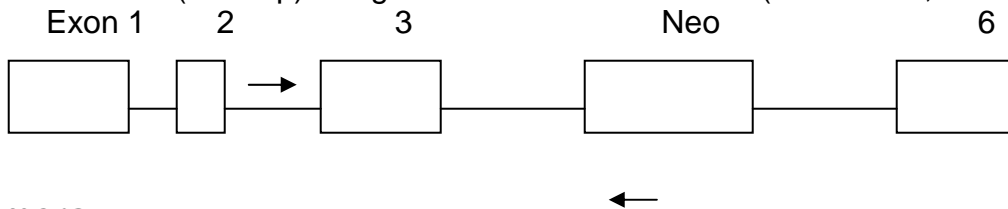
Intron 2-3 gtaagggcccagctactgtgcccctaggtagaataggtgggccacagcctcaaacatgtgac  
cctgcagagggcatggataccggaagcagatggatggacctgggacctgggcaagctgc  
tcattctgagccttggttccccatctaagaaatgggaataatggcagtcctctcccaga  
gtggttctgagactttaatggcatgcctccatcatagttaaccgggatttcatctgggag  
gaagtatctgttcaactggtagagagggcatgtatatgacattgctttgatatggataca  
ggcccagggtcccttgatggaagactccaggttgggctccttccagccttctgcagaggc  
tgattgatcccttgtcccctgtcctcag

Exon 3 GACAAACGCCGGAACCTTTTCGATGCCTTTATCTGCTACTGCCCAACGATATCGAGTTTG  
TGCAGGAGATGATCCGGCAACTAGAACAGACAGACTATCGGCTTAAGTTGTGTGTGCCG  
ACCGTGACGTCTGCCGGGCACCTGTGTCTGGTCCATTGCCAGCGAGCTAATTGAGAAAA  
G

Exon 3-4 gttggttaaacatctaagagggtaggtgggtgaatgcatgaaaccagaggtccagatgc  
aaggactgtcctgctagctgggctctgtcccgcctgggtaatgtagtcttccctgacccc  
atcctctgaaggaagtcaccgcagtgccactctccctcag

Exon 4 GTGTCGCCGCATGGTGGTGGTTGTTTCTGACGATTATCTACAGAGCAAGGAATGTGACTT  
CCAGACCAAGTTTGCACTCAGCCTGTCTCCAG

Knockout Band (~600bp) Targeted KO of exons 4 and 5 (Akira 1998, Immunity)



### Primers:

MyD88 F: 5' - TGGCATGCCTCCATCATAGTTAACC - 3' 25-mer

MyD88 R: 5' - GTCAGAAACAACCACCACCATGC - 3' 23-mer

MyD88 neo: 5' - ATCGCCTTCTATCGCCTTCTTGACG - 3' 25-mer

## PCR

### Reaction Components:

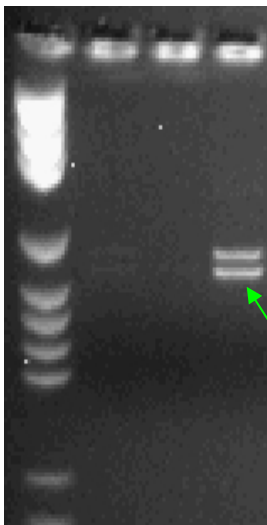
DNA template	1.0 $\mu$ l
MyD88 Sense primer	0.5 $\mu$ l
MyD88 Anti-Sense primer	0.5 $\mu$ l
MyD88 Neo primer	0.5 $\mu$ l
Promega Master Mix	10.0 $\mu$ l
Taq polymerase	0.1 $\mu$ l
H <sub>2</sub> O	<u>7.4 <math>\mu</math>l</u>
	20.0 $\mu$ l total

### Program:

95°C for 2 minutes	
95°C for 30 Sec.	} 30 cycles
60°C for 30 Sec.	
72°C for 30 Sec.	
72°C for 5 Minutes	

### Electrophoresis Conditions:

Run on a 3% agarose gel at 100-110 Volts for ~1½ hours in order to achieve a clear separation of the bands due to only 50bp difference between the WT and KO band.



Targeted locus will produce a 600 bp product  
Wild Type locus will produce a 550 bp product

Expected bands: 600bp for MyD88<sup>-/-</sup> animals and 550 for Wild-type animals.

Screening Compound: MyD88

Reference: Nature. 2002 Apr 11;416(6881):603-7

Filename: adlab\protocols and forms\mouse\PCR protocols\MyD88 PCR protocol