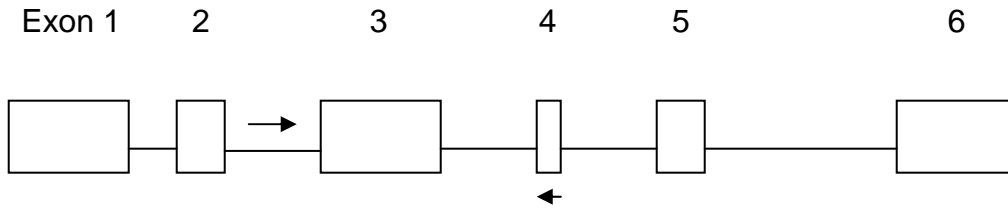


# PCR Screening for MyD88 Targeted Mice

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

Principle: The murine Myeloid Differentiation Factor 88 (MyD88) gene contains 6 exons (transcript length is 1911 bps, and translation length is 296 bps) 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)



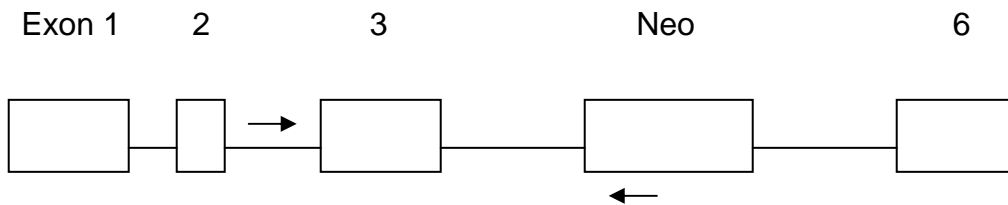
Intron 2-3 `gtaagggcccagtagtactgtgcccctaggtagaataggtgggcccacagcctcaaacatgtga  
cctgcagagggcatggataccggaagcagatggatggacctgggaccttgggcaagctgc  
tcattctgagccttggtttccccatctaagaaatgggaataatggcagtcctctcccaga  
gtggttctgagactttaatgggcatgcctccatcatagttaaccgggatttcatctgggag  
gaagtatctgttcactggtagagagggcatgtatgatgacattgctttgatatggataca  
ggcccagggtcccttgatggaagactccagggtgggctccttccagccttctgcagaggg  
tgattgattcccttgtcccctgtcctcag`

Exon 3 `GACAAACGCCGGAAC TTTTCGATGCCTTTATCTGCTACTGCCCAACGATATCGAGTTTG  
TGCAGGAGATGATCCGGCAACTAGAACAGACAGACTATCGGC TTAAGTTGTGTGTGTC  
CCG ACCGTGACGTCTGCCGGGCACCTGTGTCTGGTCCATTGCCAGCGAGCTAATTGAGAAA  
G`

Exon 3-4 `gttggttaaacatctaagagggtaggtgggtgaatgcatgaaaccagaggtccagatgc  
aaggactgtcctgctagctgggctctgtcccgcctgggtaatgtagtccttccctgacccc  
atcctctgaaggaagtcccgagtgccactctccctcag`

Exon 4 `GTGTCGCCGCATGGTGGTGGTTGTTTCTGACGATTATCTACAGAGCAAGGAATGTGACTT  
CCAGACCAAGTTTGCACTCAGCCTGTCTCCAG`

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

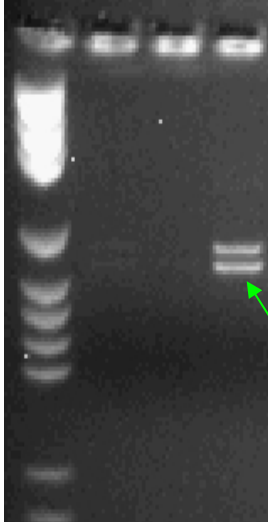


## Primers:

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

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

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



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

## Reaction Components:

DNA template	2.0 $\mu$ l
MyD88 Sense	0.1 $\mu$ l
MyD88 Anti-Sense	0.1 $\mu$ l
MyD88 Neo	0.2 $\mu$ l
*Promega Master Mix	10.0 $\mu$ l
*Taq polymerase	0.1 $\mu$ l
H <sub>2</sub> O	7.5 $\mu$ l
	20.0 $\mu$ l total

## PCR Program:

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

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

## Electrophoresis Conditions:

Run on a 2-3% agarose gel loading 15ul at 100-110 Volts for ~1 hour in order to achieve a clear separation of the bands due to only 50bp difference between the WT and KO band.

Modified: 09-15-07 APO

Updated: 04-07-08 BMK