

1. Mix the following in a 25 microL reaction

PCR mix

DNA 1 microL

10X Taq Buffer 2.5 microL

2.5 mM dNTP mix 2 microL

Taq polymerase 0.5 microL

DH20 16.9 microL

MyD88 extra new (20 microM) 1 microL

MyD88 wild new (20 microM) 1 microL

Neo1500 (20 microL) 1 microL

2. PCR conditions:

94° C 2 min X1

94° C 30 sec, 60° C 30 sec, 72° C 60 sec X35

72° C 10 min X1

4° C forever

Primers

MyD88 extra new: 5' – ATCGGCTTAAGTTGTGTGTGTCGACC–3'

MyD88 wild new: 5' – AAGGCCAAAGGGTGTGGTATTAGGACC –3'

Neo1500: 5' – ATCGCCTTCTATCGCCTTCTTGACGAG –3'

Wild-type allele is amplified as about 500 bp product, and mutant allele is amplified as about 300 bp product.

Wild-type mice: 500 bp product alone

Heterozygote: both 300 bp and 500 bp products

MyD88 KO mice: 300 bp product alone.