

MMRRC # 10473 – Lackdaisical

*Lackadaisical* is a missense mutation in the *MyD88* gene. A single nucleotide A to G transition at residue 116 results in the replacement of a Tyrosine with a Cysteine (Y116C)

For genotyping, the DNA fragment in which the point mutation resides will be amplified by PCR from genomic DNA, subsequent sequencing will be applied to detect the particular point mutation.

Amplification primers: Amplicon size 426bp  
Up: GCAGTCAGTGCTCTTACCGGCTGAG  
Dn: AATGAGCAGCTTGCCCAAGGTCCC

Sequencing primers:  
Forward: TCTTACCGGCTGAGCCATCTC  
Reverse: AAGGTCCCAGGTCCATCCATC

**PCR Protocol:**

For a 50µl reaction, use the following:

**1X:**

5µl 10X PCR Buffer  
2.5µl 10mM dNTPs (D-7295- Sigma)  
1µl 10µM Up primer  
1µl 10µM Dn primer  
1µl 50-100ng/µl gDNA template  
2.5µl JumpStart REDAccuTaq LA DNA Polymerase (D-1313- Sigma)  
37µl DNase-free water  
50µl total volume

**Thermocycler Conditions:**

94°C 2min

94°C 15sec }  
60°C 20sec } 35X  
68°C 1min }

68°C 5min

4°C ∞

Confirm the size and presence of the amplicon on a 1X TAE gel. Purify using spin dialysis or the QIAGEN QIAquick PCR purification kit. Sequence the purified DNA fragments utilizing the Sequencing Primers listed above for genotyping.

The mutation and flanking sequences are: to

Wild type: AAA TAC TTA

*Lackadaisical*: AAA TGC TTA