

MMRRC # 10474 – Insouciant

Insouciant is a missense mutation of *Tlr6 gene*. A single nucleotide T to C transition at residue 327 results in a replacement of Valine with a Alanine (V327A) in the ectodomain.

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 436bp
Up: CTTTCAGATTCCCAATACCACCGTTCTC
Dn: GCATCTTGATGTTTCATCTCAGCAAACAC

Sequencing primers:
Forward: CCAATACCACCGTTCTCCATTTG
Reverse: ATGTTTCATCTCAGCAAACACCGAG

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:

Wild type: CAA GTG TTC
Insouciant s: CAA GCG TTC