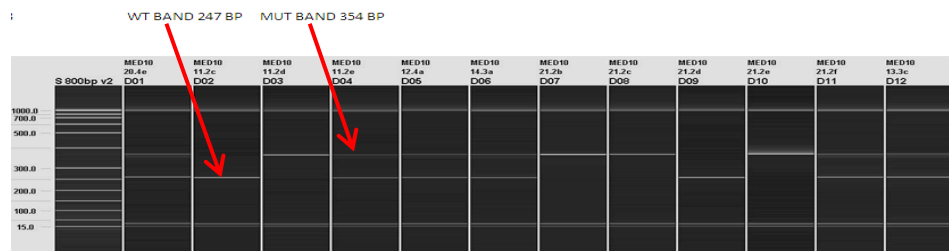




IL19 TM2E Genotyping Strategy

Introduction

The gel based assays are normally run on the Qiagen QIAxcel. This is a capillary based system that provides clearer resolution and is quicker than running standard agarose gels. Different size ladders maybe loaded onto runs depending on the fragment sizes being analysed. Typically samples are run with a 50-800bp size ladder.



PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

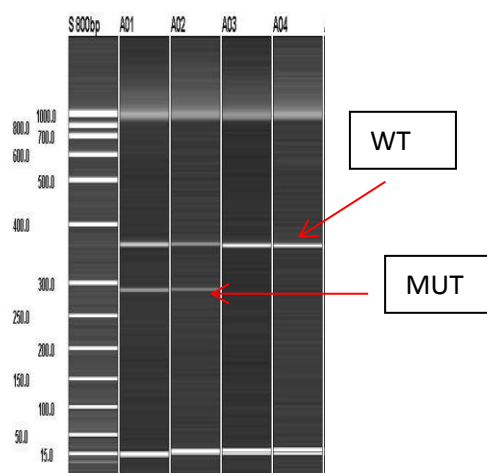
IL19 TM2E gel based primers

IL19-5arm-WTF CTTCTCTGGGCTGTGCTTTG
 IL19-3arm-WTR ATCCCTCCAAGAACCAGGTG
 5mut-R1- GAACTTCGGAATAGGAACTTCG

PCR mix

KAPA2G Fast Hotstart Readymix (2X) 5µl
 5arm-WTF (20µM) 0.5µl
 3arm-WTR (20µM) 0.5µl
 5mut-R1 (20µM) 0.5µl
 H₂O 2.5µl
 DNA (~30ng) 1µl

Example of a gel based assay



WT band = 332bp MUT band = 265bp

Cycling conditions

62TM30FA

1. 95°C 1min.
2. 95°C 10sec.
3. 62°C 10sec.
4. 72°C 1sec.
5. Go to 2 for 29 cycles
6. 72°C 30sec.
7. 16 °C forever
8. end



Sequences of products

IL19 'Knockout'-First and WT sequences

Genomic sequence

Black sequence is missing in the construct due to the insertion of loxP sites .

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GCTTGAAGAAAGCTTCCC GGCTTCTCTGGGCTGTGCTTTGAGCATCTGGAGTCTTGTCTCAGAGGCATCTGCTTT
CTGTTGCAGACCTTTGACCTTCCCAGATGTGCCTGCCACCTCCTAGTCTTGCAAACCCCATCCCGCCCCGCCCC
AATTTAAGGGGACAAACTGCAAGGGAAGTCAAGTGTGCGGTCCAGGGCTCAGTGGCTAAGATAAAATATATTGGTC
ATGCCTGGTAGGATACCTGATGTCCAGGAGCCAAGGACATTGTGGGCTGGGAGACTACAGAGCTGACAACCTTGG
CTTGTGGGATACAAACCATCTCCTAAAGGAGG CACCTGGTTCTTGGAGGGATTTTAGAGTTTCCTTATATCATGC
TGGGGATGCATGCTGTGGAACAAACCTTGTCTGTGATAGGATCCAAGCTTTACTCTTTGTA
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5' homology arm and cassette sequence

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GCTTGAAGAAAGCTTCCC GGCTTCTCTGGGCTGTGCTTTGAGCATCTGGAGTCTTGTCTCAGAGGCATCTGCTTT
CTGTTGCAGACCTTTGACCTTCCCAGATGTGCCTGCCACCTCCTAGTCTTGCAAACCCCATCCCGCCCCGCCCC
AATTTAAGGGGACAAACTGCAAGGGAAGTCAAGTGTGCGGTCCAGGGCTCAAGGCGCATAACGATACCACGATAT
CAACAAGTTTGTACAAAAAAGCAGGCTGGCGCCGGAAC CGAAGTTCCTATTCCGAAGTTCCTATTCTAGAAAAG
TATAGGAACTTC
```