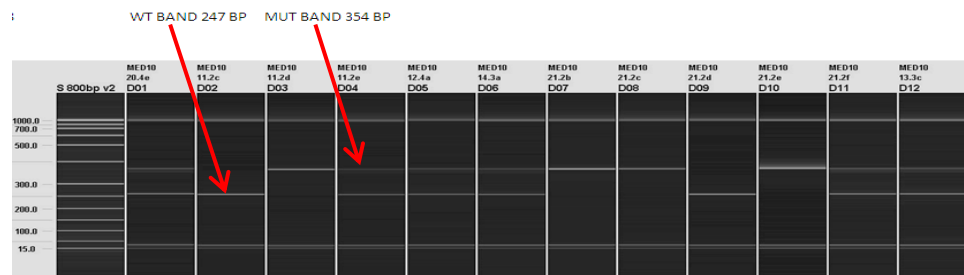




Lac Z Genotyping Strategy

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 KAPA2G Fast Taq polymerase, although alternatives may be used.

Lac Z gel based primers

LacZ_2_small_F ATCACGACGCGCTGTATC
LacZ_2_small_R ACATCGGGCAAATAATATCG

PCR mix

KAPA Fast Taq PCR master mix 5µl
LacZ_2_small_F 1µl
LacZ_2_small_R 1µl
H₂O 2µl
DNA 1µl

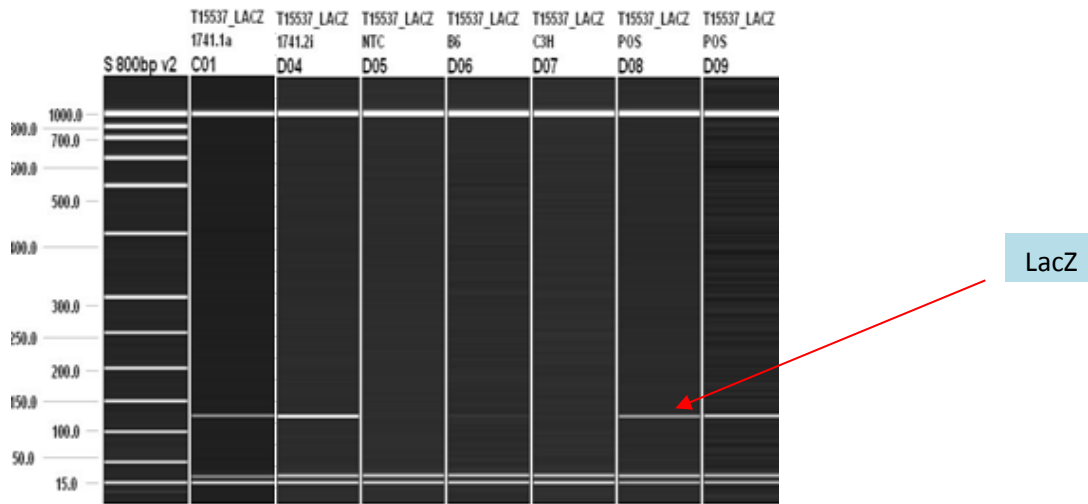
Cycling conditions

60TM35FA-(PCR programme specific for KAPA2G Fast mix)

1. 95°C 1 min.
2. 95°C 10 sec.
3. 60°C 10 sec.
4. 72°C 1 sec.
5. Go to 2 for 34 cycles
6. 72°C 30 sec.
7. 16 °C forever
8. end



Example of LacZ mutant gel based assay



LacZ band =108bp