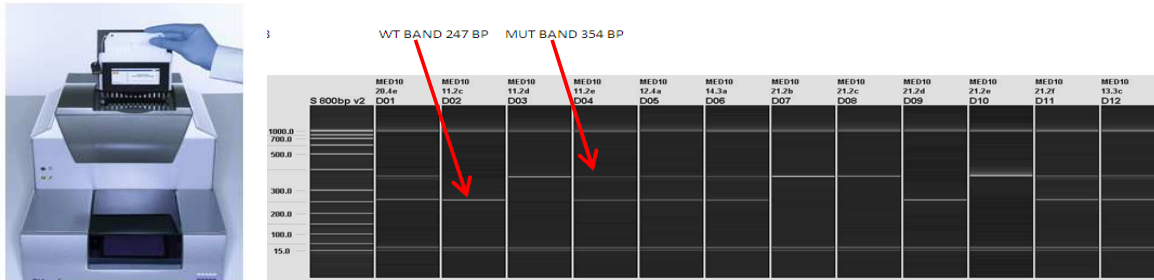




Pde4b Genotyping Strategy

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 Taq PCR master mix, although alternatives may be used.

Pde4b gel based primers

PDE4B (E) TCAGCAGTGCCTCTCCCACTAGAG
 PDE4B (T) GGGTGGGATTAGATAAATGCCTGCTCT
 PDE4B (E,T) ATGAGAACTGATTGGAGACTCCAGG

PCR mix

KAPA PCR master mix	5µl
PDE4B (E) (20 µM)	0.5µl
PDE4B (T) (20 µM)	0.5µl
PDE4B (E,T)	0.5µl
H ₂ O	2.5µl
DNA	1µl

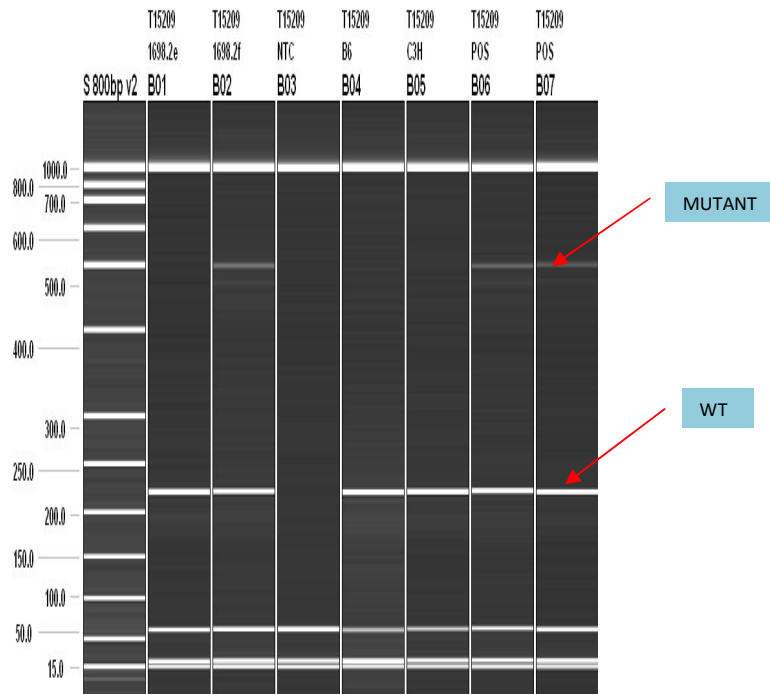
Cycling conditions

60TM35FA

1. 95°C 1min.
2. 95°C 10sec.
3. **60°C** 10sec.
4. 72°C 1sec.
5. Go to 2 for 29 cycles
6. 72°C 30sec.
7. 16 °C forever
8. end



Example of a Pde4B gel based assay



WT ~210bp, MUTANT ~500bp