

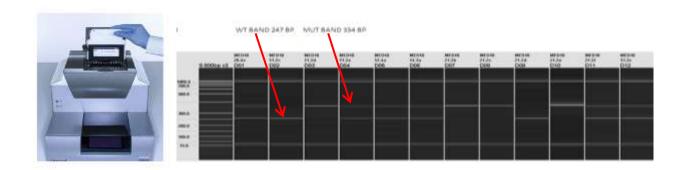
GEL BASED GENOTYPING



GE Cre 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.

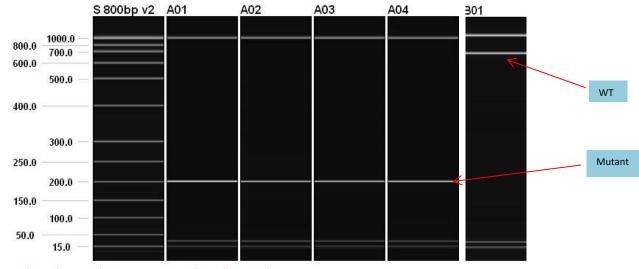
GE Cre gel based primers

Mel135F CATGACACCCCCACTGTCGTGTCC Mel684R TGGCACCACAGAAATGACCTCCAC Crelx TTTGGTGCACGGTCAGCAGATTGG

PCR mix

KAPA Taq PCR master mix	5µl
Mel135F	0.5μ
Mel684R	0.5μ
Crelx	0.5μ
H_2O	2.5μ
DNA	1μl

Example of a GE Cre gel based assay



WT band = 615bp Mutant band = 185bp



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Cycling conditions

65TM35FA

- 1. 95°C 1min.
- 2. 95ºC 10sec.
- 3. **65**ºC 10sec.
- 4. 72ºC 1sec.
- 5. Go to 2 for 34 cycles
- 6. 72ºC 30sec.
- 7. 16 °C forever
- 8. end