



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
CreI _x	TTTGGTGCACGGTCAGCAGATTGG

PCR mix

KAPA Taq PCR master mix	5µl
Mel135F	0.5µl
Mel684R	0.5µl
CreI _x	0.5µl
H ₂ O	2.5µl
DNA	1µl

Example of a GE Cre gel based assay



WT band = 615bp Mutant band = 185bp



Cycling conditions

65TM35FA

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