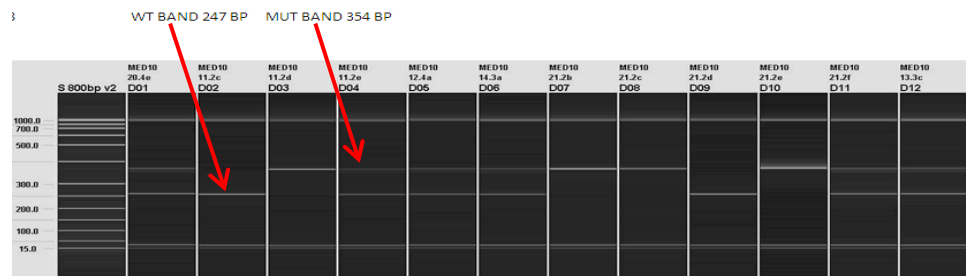




FLPe Genotyping Strategy

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.



CRE primers run alongside an internal control Slc40a1. PCR is performed using KAPA2G Fast Taq polymerase, although alternatives may be used.

CRE gel based primers

FLPe-LS_F	GGTTTGAAAGACCTTCAGGG
FLPe-LS_R	CTCTTGATTGCTGTTCCG
Slc40a1_sh_F (control)	CCTTTGTAACCTCCTCTGTGTC
Slc40a1_sh_R (control)	CTGAAGTCTTTCATGATAACTGCATT

PCR mix

KAPA2G Fast Taq PCR mix	5µl
FLPe-LS_F (20 µM)	0.5µl
FLPe-LS_R (20 µM)	0.5µl
Slc40a1_sh_F (control) (20 µM)	0.5µl
Slc40a1_sh_R (control) (20 µM)	0.5µl
H ₂ O	2µl
DNA	1µl

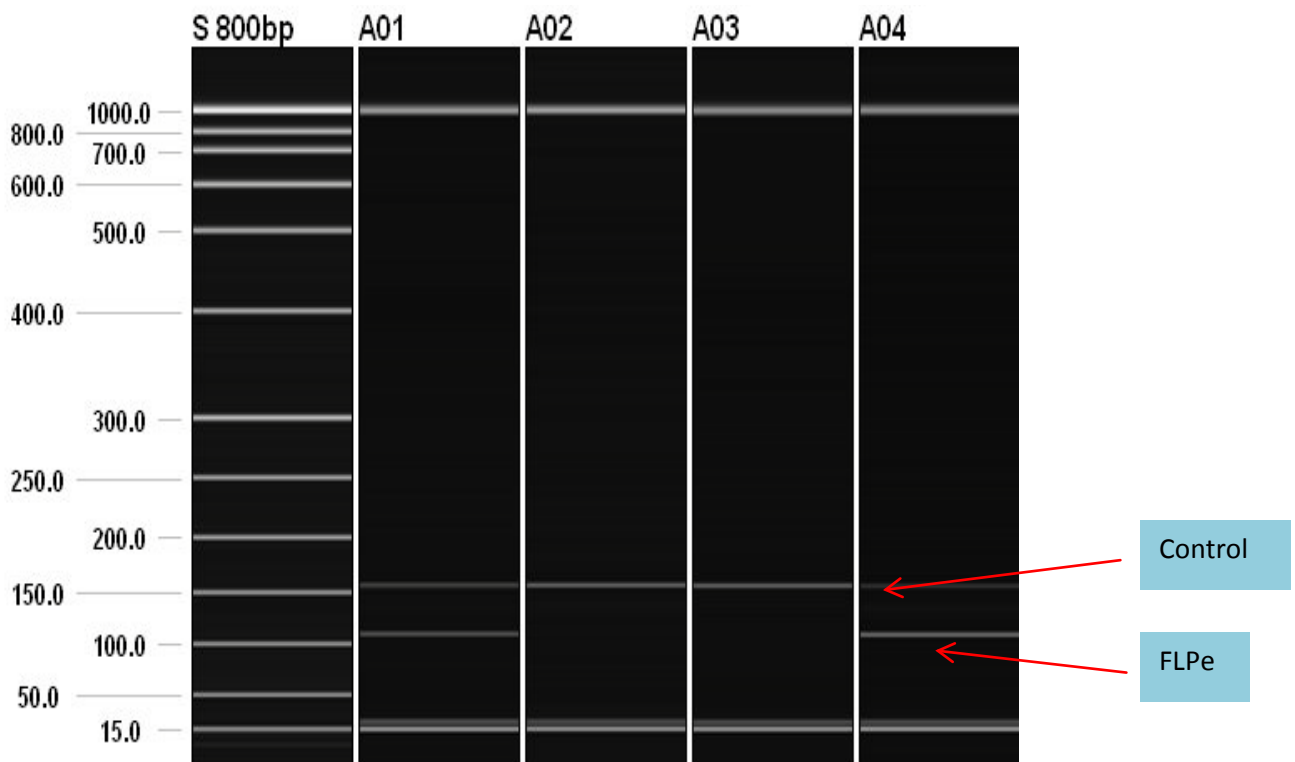


Cycling conditions

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

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 CRE gel based assay



FLPe band = ~97bp

Internal control band size = 147bp