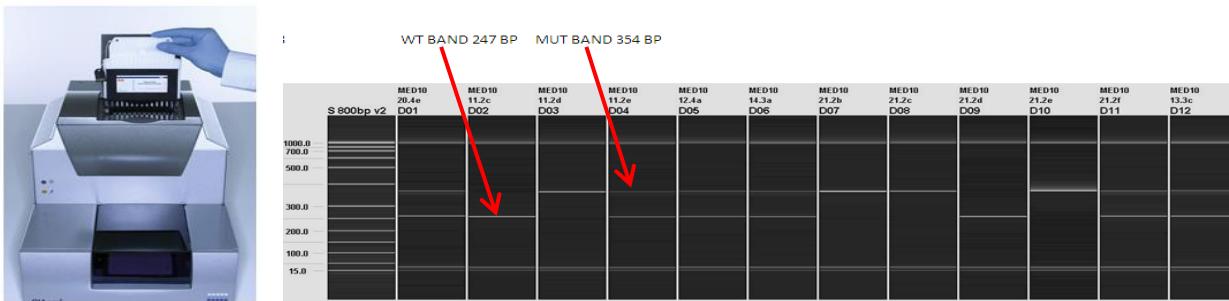


SMAD8 Genotyping Strategy

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.



Information for the assay was taken from:

<http://www.sciencedirect.com/science/article/pii/S0012160606007226>

PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

SMAD8 gel based primers:

SMAD8F3 GCAGTGCCTGGGGTTTCA
SMAD8R3 AAGCAGTGGGTCCGATTCAA

PCR mix

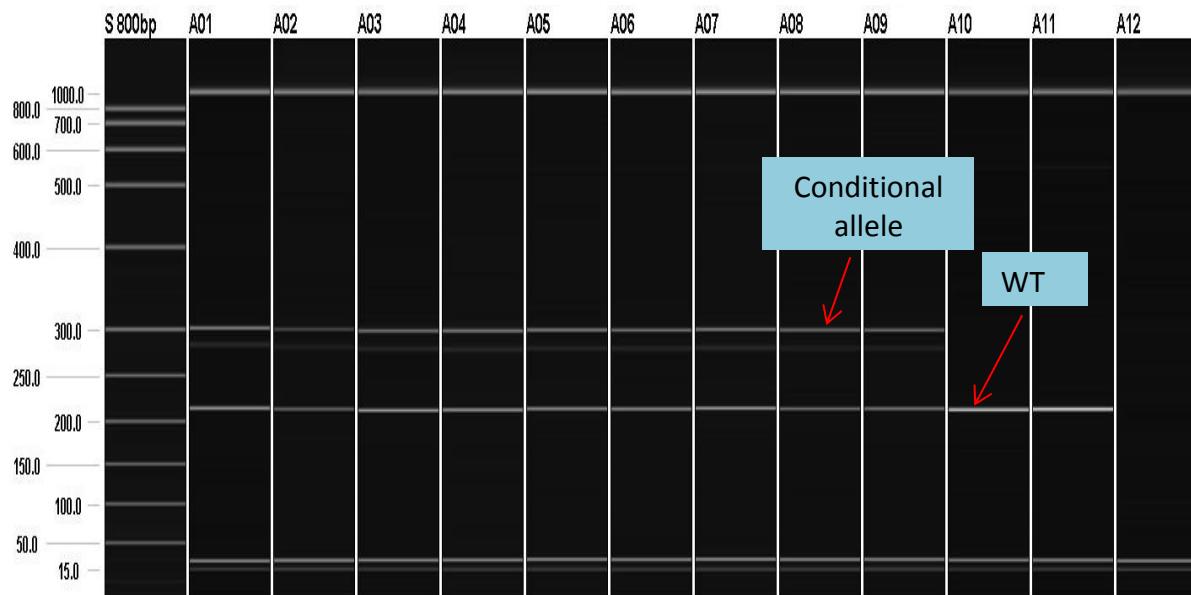
KAPA Taq PCR master mix 5 μ l
SMAD8_F1 1 μ l
SMAD8_R1 1 μ l
H₂O 2.0 μ l
DNA 1 μ l

Cycling conditions

56TM35FA

1. 95°C 1min.
2. 95°C 10sec.
3. **56°C** 10sec.
4. 72°C 1sec.
5. Go to 2 for 34 cycles
6. 72°C 30sec.
7. 16 °C forever
8. end

Example of a SMAD8 gel based assay result



Wild-type allele = 192bp

Conditional Allele = 244bp