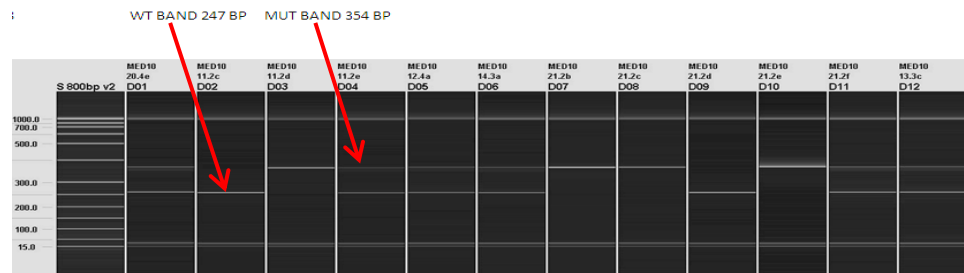




## SMAD8 Genotyping Strategy

### 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      GCAGTGCCTTGGGGTTTTCA  
SMAD8R3      AAGCAGTGGGGTCCGATTCAA

### PCR mix

KAPA Taq PCR master mix    5µl  
SMAD8\_F1                        1µl  
SMAD8\_R1                        1µl  
H<sub>2</sub>O                                2.0µl  
DNA                                 1µl

### 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