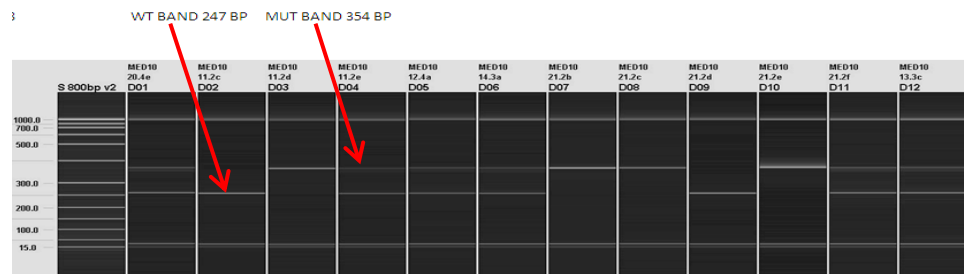




EX1A-T-CON Genotyping Strategy

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 Qiagen Taq PCR master mix, although alternatives may be used.

EX1A-T CON gel based primers

WT Assay

| | |
|------------------|------------------------|
| EX1AM_WT_Exon1aF | CAGTCGCGTCGGCACCGCGGAG |
| EX1AM_WT_Exon1aR | GACGCACTCACACGCAAAGCAG |

Mut Assay

| | |
|----------------------|-----------------------------------|
| EX1AM_MUT_PRAYR | TAAGGGTTATTGAATATGATCGG |
| EX1AM_MUT_EXON 1AKO3 | CCGCTCGAGCCTTGGTTACTTTCAATCGCTTTC |

PCR mix

| | |
|-----------------------|-------|
| Qiagen PCR master mix | 5µl |
| Exon1aF (20 µM) | 0.5µl |
| Exon1aR (20 µM) | 0.5µl |
| H ₂ O | 3µl |
| DNA | 1µl |

Cycling conditions

55TM35CY

1. 95°C 5min.
2. 94°C 30sec.
3. **55°C** 30sec.
4. 72°C 45sec.
5. Go to 2 for 34 cycles
6. 72°C 5min.
7. 16 °C forever
8. end



Example of a gel based assay

WT band = ~150bp

Mutant band = ~400bp