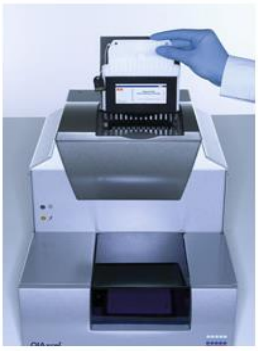
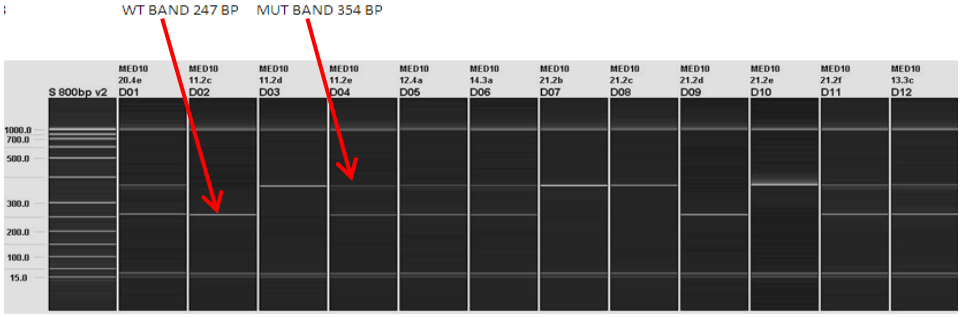
TTLL4 Genotyping Strategy

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.

TTLL4 gel based assay primers

WT Assay

TTLL4_WT_for1	ATGCTGCTGTTAGCGTCACC
TTLL4_WT_Rev4	TTGATCACAAGAACCATGACAA

Mutant Assay

TTLL4_WT_for1	ATGCTGCTGTTAGCGTCACC
TTLL4_MUT_Rev1	TTGGTGATATCGTGGTATCGTT

Internal control primers for use with WT and Mutant assay

1260_1	GAGACTCTGGCTACTCATCC
1260_2	CCTTCAGCAAGAGCTGGGGAC

PCR mix

WT and Mutant Assay

KAPA Taq PCR master mix	5µl
TTLL4_WT_for1 (20 µM)	0.5µl
TTLL4_WT_Rev4 (20 µM)	0.5µl
1260_1 (20 µM)	0.5µl
1260_2 (20 µM)	0.5µl
H_2O	2 µl
DNA	1µl

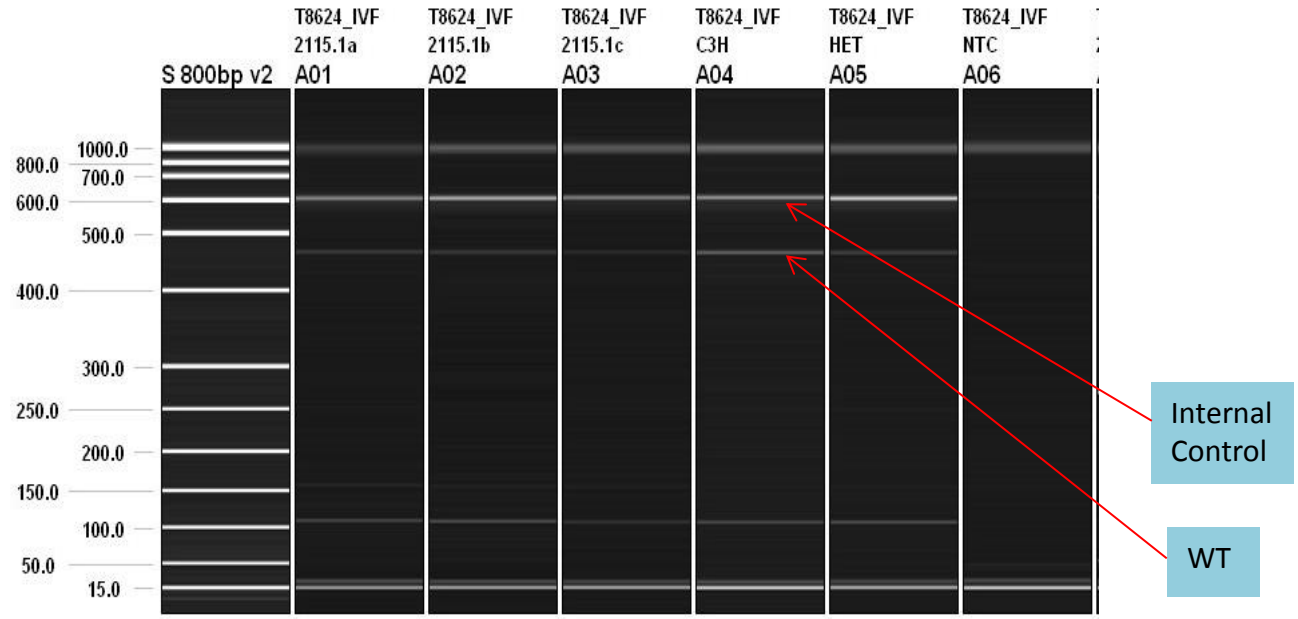
Mutant Assay

KAPA Taq PCR master mix	5µl
TTLL4_WT_for1 (20 µM)	0.5µl
TTLL4_MUT_Rev1 (20 µM)	0.5µl
1260_1 (20 µM)	0.5µl
1260_2 (20 µM)	0.5µl
H_2O	2 µl
DNA	1µl

Cycling conditions – (specifically for KAPA Fast Taq)
WT and Mutant Assay
56TM30FA

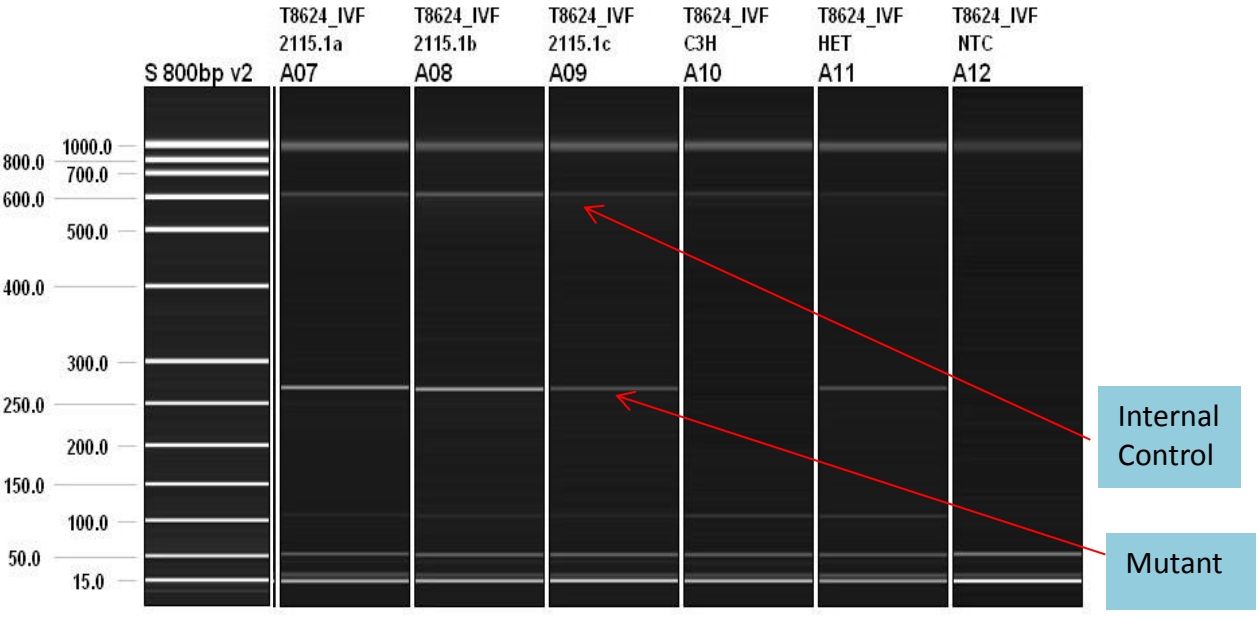
1. 95ºC 1min.
2. 95ºC 10sec.
3. **56**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC forever
8. end

Example of a TTLL4 WT gel based assay



WT band = ~439bp Internal Control Band = 585bp

Example of a Mutant gel based assay



Mutant band = 258bp Internal Control Band = 585bp