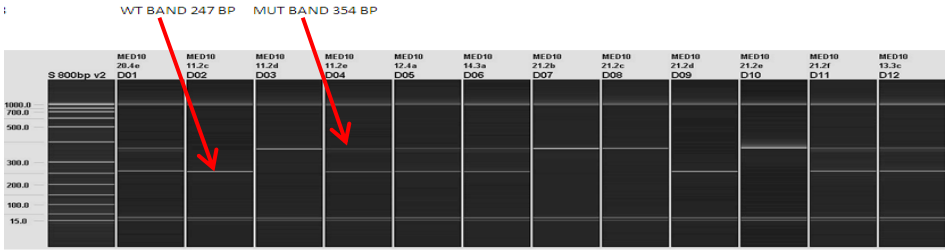
LMNB1 Genotyping Strategy

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.

LMNB1 gel based primers

Lmnb1_39066_F	AACGGATCAACTCTGTCCAGC
Lmnb1_39066_R	ACATTCCCCACATCACATGC
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

PCR mix – Multiplex Assay

KAPA Taq PCR master mix	5µl
Lmnb1_39066_F	0.5µl
Lmnb1_39066_R	0.5µl
CAS_R1_Term	0.5µl
H_2O	2.5µl
DNA	1µl

PCR mix –Lac Z Assay

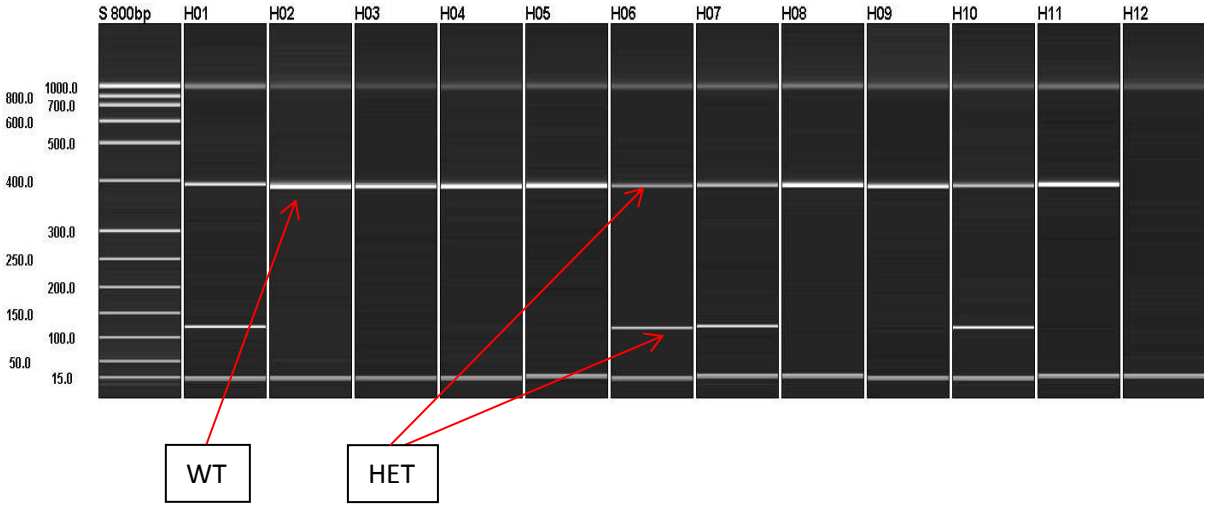
KAPA Taq PCR master mix	5µl
LacZ_2_small_F	0.5µl
LacZ_2_small_R	0.5µl
H_2O	3 µl
DNA	1µl

Cycling conditions

60TM30FA

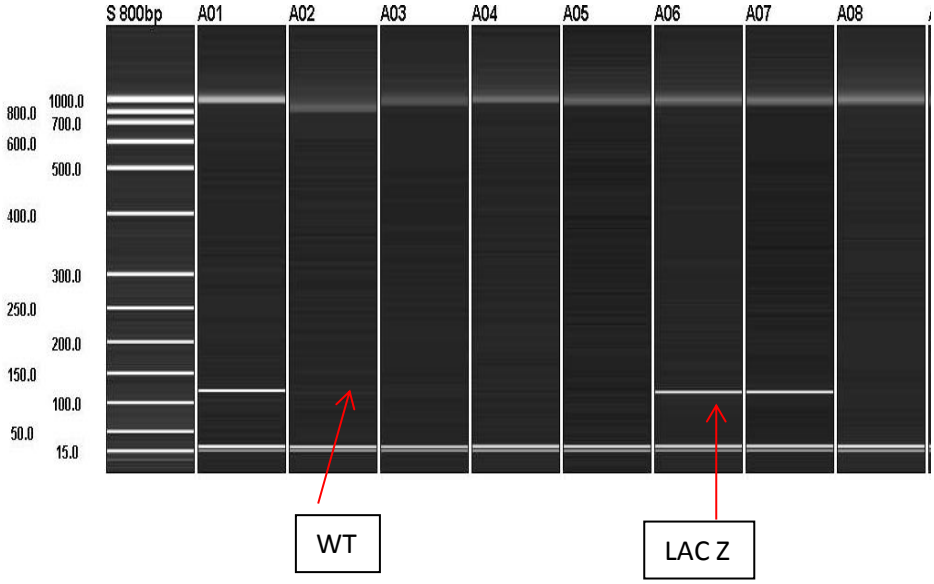
1. 95ºC 1min.
2. 95ºC 10sec.
3. **60**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC forever
8. end

Example of Multiplex assay



WT band = 380bp Mutant band = 120bp

Example of LAC Z assay



Lac Z band = 108bp