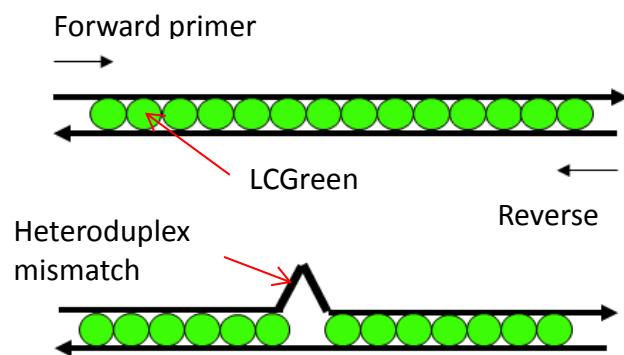


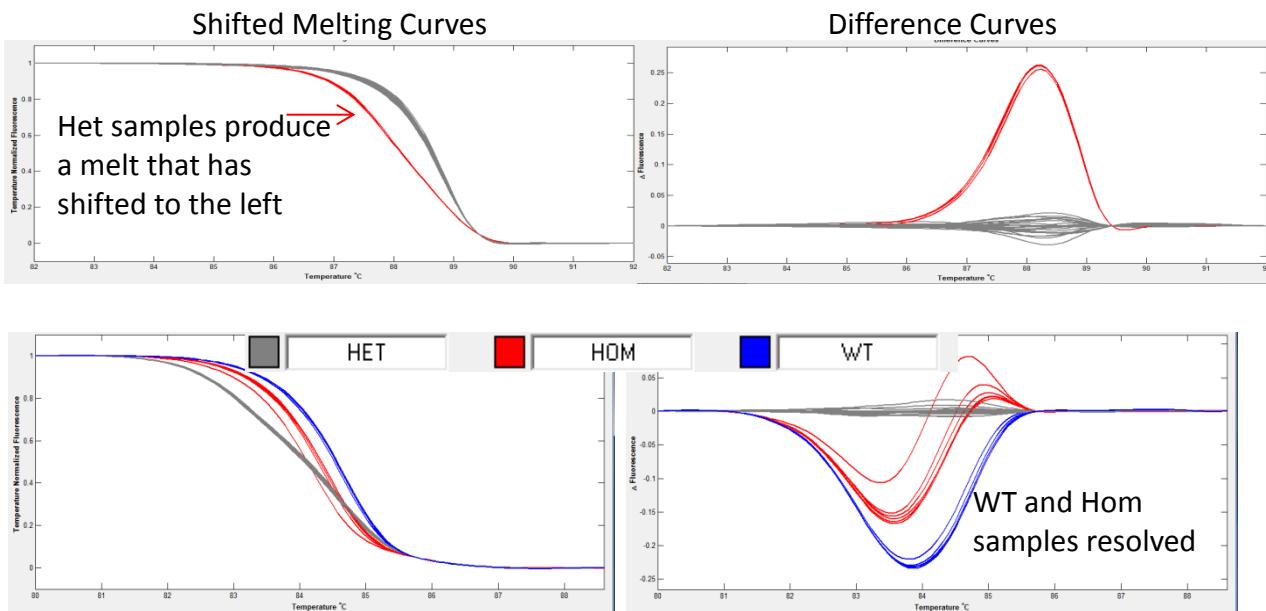
Lrm4 Genotyping Strategy

Introduction

The Idaho Technology LightScanner is a system used to perform high throughput DNA melting analysis. PCR is performed in the presence of the double stranded DNA binding dye LCGreen. After PCR, samples are then heated on the LightScanner and the fluorescence emitted by bound LCGreen is monitored. As the DNA melts the LCGreen is released and so the fluorescence decreases until all the DNA has melted and all LCGreen is unbound. There are several different genotyping methods that can be used on the LightScanner.



Scanning analysis can be used to detect samples that are heterozygous at a particular SNP. These samples will produce a melt curve that is shifted to the left as the instability created by the mismatch causes DNA to melt faster releasing the bound LCGreen. Homozygous WT and mutant samples will occasionally produce different melt traces to each other, but often this is not the case and using a lunaprobe and the unlabelled probe genotyping method is required to resolve all samples.





Group: Molecular Embryology
Mutation type: SNP
Mutant allele: G
WT allele: A
Assay Type: Scanning

Fragment sequence

AGAATCCTGACTCCTAAGCACACACTTCTGTAAACACGGAAACAAGGGTCAGGATTCCCCAGCTTG
TAACCCGAGATACTGGTTCTCACCTCATAGTGCAGGGACTGACCAGACAGACTTCCTTCTTGAGTCT
GTCTTGCATGTTAACCTAATTGTTGTCTTGCATTGTAGGTTATTGGCGGGGTTCCCAGCAACGGGTG
GCGTGGTACCCCTTGGCAGTTCAAGCCTGTAAAAGTATGATCCGCTATGTCACAGCCTTGATTTCTC
CTGGCAGCCTGTGAGATCATCTTTGTT

Primers/Probe sets 5'>3'

LRM4_F CTTGAGTCTGTCTTGCATGT
LRM4_R TGAAACTGCCAAGAGGGTACCA

PCR mix

HotShot master mix	5µl
LCGreen	1µl
LRM4_F (20ng/µl)	0.1µl
LRM4_R (20ng/µl)	0.1µl
DNA (1/10 dil ABI)	2µl
ddH2O	1.8µl

This assay only detects hets but line is hom lethal. Use for colony genotyping only

PCR program

LS60H (annealing temperature 60 °C with hybridisation step)

Control method Calculated
Lid control mode Off (no need for heated lid as sample is overlaid with oil)
Lid pressure Microplate

- 1) 95°C for 2 min
- 2) 95 °C for 30 sec PCR cycle
- 3) 60 °C for 30 sec
- 4) 72 °C for 30 sec
- 5) Cycle, step 2 44 times
- 6) 95 °C for 30 sec Hybridisation
- 7) 25 °C for 30 sec
- 8) 15 °C for 30 sec



Example

