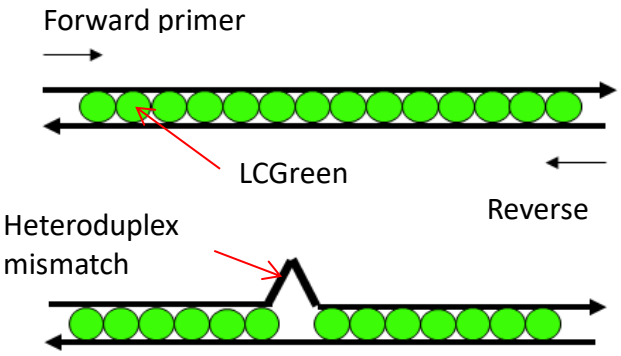
RKS2real Genotyping Strategy

Introduction

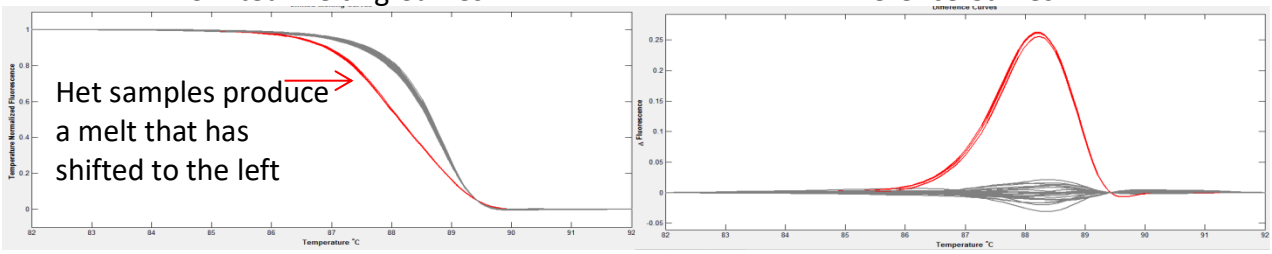
The Idaho Technology LightScanner is a system used to perform high throughput DNA melting analysis. PCR is performed in the presence of the double stranded DNA binging dye LCGreen. After PCR, samples are then heated on the LightScanner and the fluorescence emitted by bound LCGreen is monitored. As the 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.

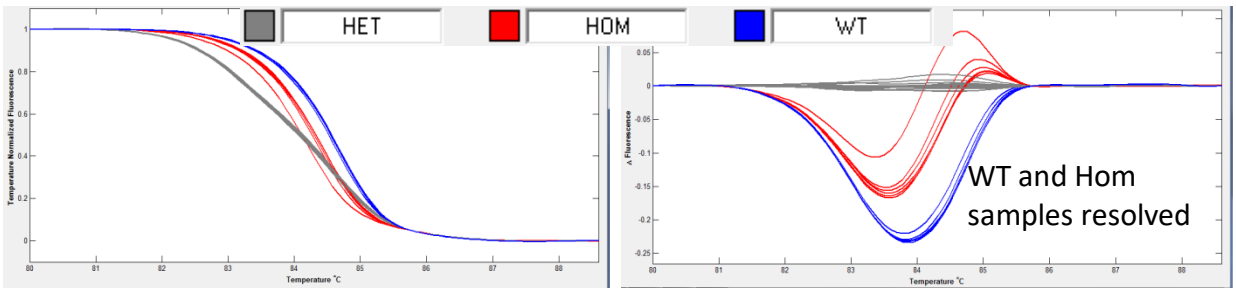


Scanning analysis can be used to detect samples that are heterozygous at a particular SNP. These samples will produce a melt curve that is shifted to the left as the instability created by the mismatch causes DNA to melt faster releasing the bound LCGreen. Homozygous WT and mutant samples will occasionally 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.

Shifted Melting Curves

Difference Curves





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

Fragment sequence

GGCCACCAGCAGGATCGGCAGTGTGGTCTCTGAACCCCACCTCATCAGGGTGCAGAAGAGAATCATGGCCAA
TCGACTTGTGTCCACTGCCTCAGCCCTGGTAAATGCCAATGTGTCCTTTGAGTGCAGGCTCAACTTTGGCACTG
ATGTAGCCTACCTTTGGAACTTTGGAGGTGACACCATTGAGTTGGGCAGCAGCTCCTCCAGCCATGTCTACAG
CAGGTGAGCAGTAAGGGACAGGTGACAGCTGATTGCAGGTGTACACGTGGCTGCCTTCTGAAGGATCGATAC
TTAAAACCCCACAGTCAATCTTGCAGATGACAAATGTTGACTGGAAAGAAGGCAGGCTGAGCTAGGGC

Primers/Probe sets 5'>3'

RKS2realF	AGGCTCAACTTTGGCAC
RKS2realR	CTGTCACCTGTCCCTTACT

PCR mix

HotShot master mix	5µl
LCGreen	1µl
RKS2realF (20ng/µl)	0.1µl
RKS2realR (20ng/µl)	0.1µl
DNA (1/10 dil ABI)	2µl
ddH2O	1.8µl

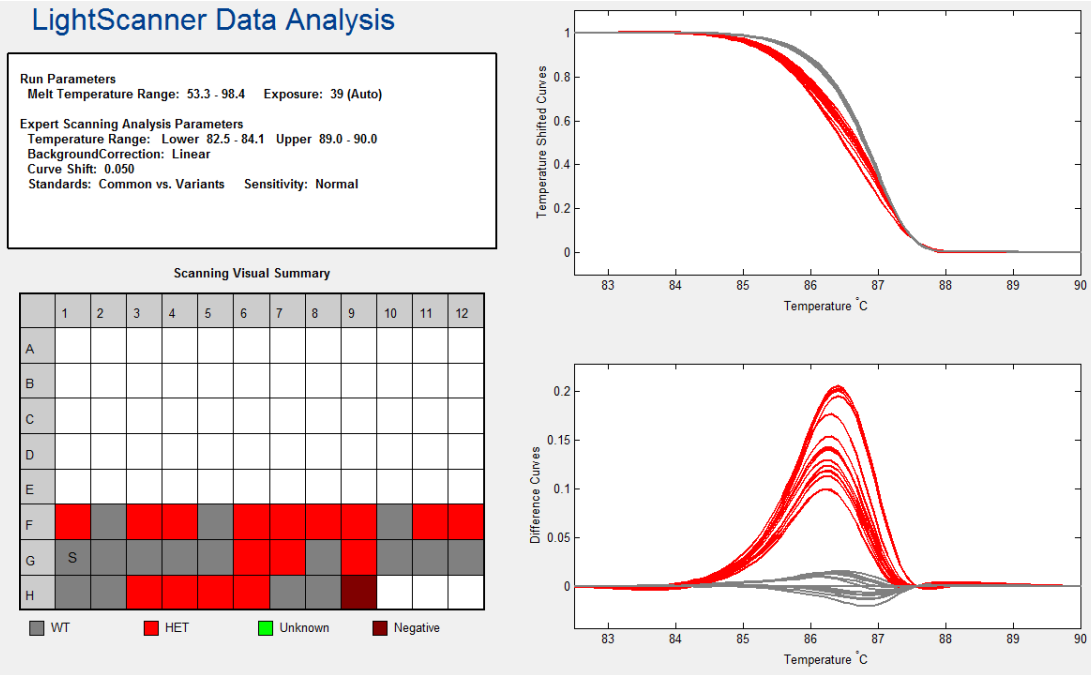
PCR program

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


LightScanner Data Analysis
Run Parameters
Melt Temperature Range: 53.3 - 98.4 Exposure: 39 (Auto)
Expert Scanning Analysis Parameters
Temperature Range: Lower 82.5 - 84.1 Upper 89.0 - 90.0
BackgroundCorrection: Linear
Curve Shift: 0.050
Standards: Common vs. Variants Sensitivity: Normal
Scanning Visual Summary
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G S
H
WT
HET
Unknown
Negative
Temperature Shifted Curves
Temperature °C
Difference Curves
Temperature °C