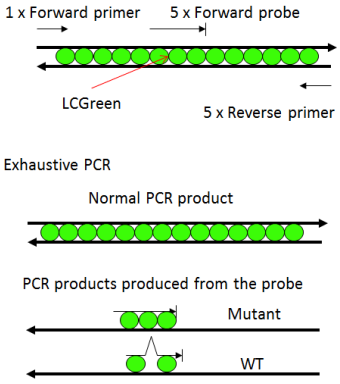
MPC151-Wars2 V117L

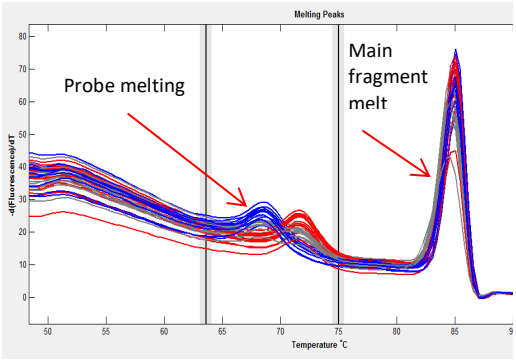
Introduction

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.

Unlabelled probe genotyping is used to distinguish between different homozygote samples at a given SNP where scanning analysis may not have enough sensitivity. Here a 3' blocked oligonucleotide (lunaProbe) is designed that sits directly over the SNP. Asymmetric exhaustive PCR is performed using 5 times the amount of probe and opposite primer. This creates two products, one is the full PCR product between the normal primers and the other is the probe that is bound to the opposite strand.

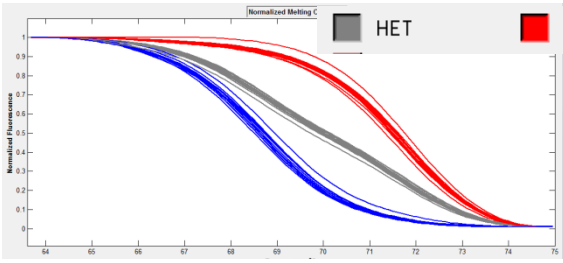


- 3' blocked oligo spanning the SNP (LunaProbe) added to PCR reaction.
- Exhaustive, asymmetric PCR performed with unequal primer concentrations.
- Probe designed to mutant allele. Mutant allele produces the greatest fluorescence.

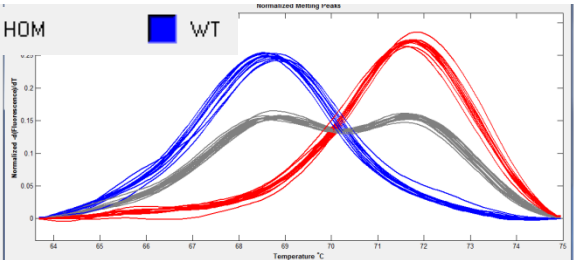


When the products are melted the probe melts at a lower temperature and by focussing analysis on this section hom, het and wt samples can be resolved.

Normalised Melting Curve



Normalised Melting Peaks



Group: Diabetes
Mutation type: ENU mutation in the first codon of exon 3 in Wars2. Leads to a Valine (GTG) to Leucine (TTG) change
Mutant allele: T
WT allele: G
Assay Type: Light Scanner unlabelled probe
Probe direction: Forward

Fragment sequence

TAAATCACTGCTTAGTTATCCTTATCTGTATATCAGGTATCATGGCCCAGCCCCTCACCTGTTTCCTTTTAATAA
CCTTGACTTAGGTTATTTTCAACTGTTTCAGCCTATCCCTGTTGTCTAGTTAGTACTGCTTAGATGCAAAAAGAC
ACTGGTCACCTTTCTTTCTCTCCTTCCTTTTAGTTGTCTGAACACACTCAGTTAAGTTGGATCCTCACCTGCATG
GTGAGACTGCCTCGATTGCAGCATTTACACCAGTGGAAGGTAAATGCCAGAACCTGAGTGTTAATGGGAAGGAAG
GTTTCATTTGTTCTGTGCCAGGTGCTAGGCATCCTTTAATCAATAAGCTGTCTTGCCCACCCTAGCAGCAGCTTT

Primers/Probe sets 5'>3'

Wars2V117L-LSF1	TCAGCCTATCCCTGTTGTCTA
Wars2V117L-LSR1	TGGTGTAAATGCTGCAATCG
Wars2V117L-PrF1	CCTTCCTTTTAGTTGTCTGAACACACTCAG

PCR mix

HotShot master mix	5µl
LCGreen	1µl
Wars2V117L-LSF1 (20ng/µl)	0.1µl
Wars2V117L-LSR1 (20ng/µl)	0.5µl
Wars2V117L-PrF1 (20ng/µl)	0.5µl
DNA (1/10 dil ABI)	2µl
ddH2O	0.9µl

PCR program

LSGENO60H (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 55 times
6) 95 °C for 30 sec Hybridisation
7) 25 °C for 30 sec
8) 15 °C for 30 sec

WARS2 V117L unlabelled probe