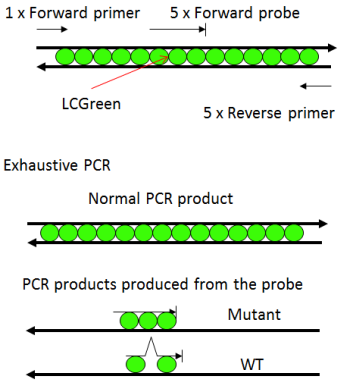
# CACNA1C-Q1466STOP

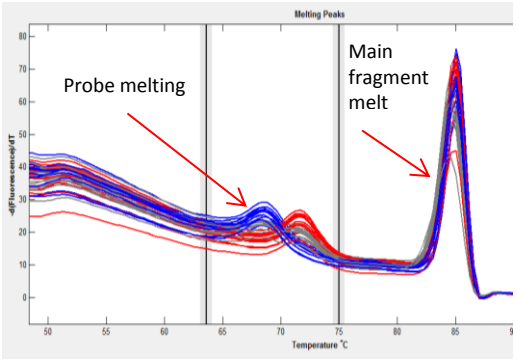
## Introduction

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.

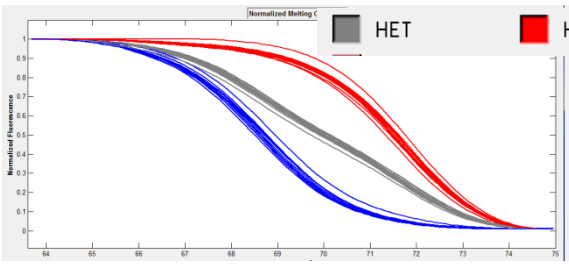



- 3' blocked oligo spanning the SNP (LunaProbe) added to PCR reaction.
- Exhaustive, asymmetric PCR performed with unequal primer concentrations.
- Probe designed to mutant allele. Mutant allele produces the greatest fluorescence.

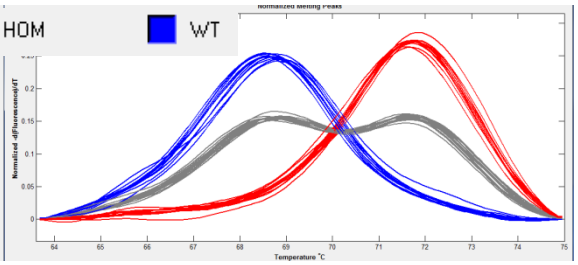



When the products are melted the probe melts at a lower temperature and by focussing analysis on this section hom, het and wt samples can be resolved.

Normalised Melting Curve




Normalised Melting Peaks



# LIGHTSCANNER GENOTYPING

| | |
|---|---|
| Group: | Neurobehavioural |
| Mutation type: | SNP |
| Mutant allele: | T |
| WT allele: | C |
| Assay Type: | LS LunaProbe |
| Probe direction: | Reverse |

Fragment sequence

GAAAAATGAAGGGAAAAAAAAAAACAGCCATGTACAGACAGGAACAAGCAGTTTAATAAATAAAAGAAGTTTTAGG
TGCTGAAAAATATCAAGGTAACCTGGTGGGATCACTCATCTTTTTCTCTTCTCCGTATGCCTTAGATGACGAGGT
CACAGTGGGCAAGTTCTATGCCACCTTCCTGATCCAAGAGTACTTCAGGAAATTCAAGAAGCGAAAAGAGCAGGG
GCTGGTGGGCAAGCCCTCACAAAGGAATGCACTGTCCCTCCAGGTGAGGGGCTGGAAGGGGGTGCCCACACTCAA
AGGTCCTGGTCACCTCCCACTGACCCTATTGAGGGTCCAAGCCCTGCTAGCTACCTAGAGGCTAAGTCCCATCAC
AAGAACCCCTGTGAGCACTGTAACCCGAAGGCGGCTATGGGAAGGCAGTGTGCTCTTCTTCCCCCTCCCCCTCAC

Primers/Probe sets 5'>3'

| | |
|---|---|
| CACNA1C-Q1466STOP-F | CCGTATGCCTTAGATGACGA |
| CACNA1C-Q1466STOP-R | GGGACAGTGCATTCCTTTG |
| CACNA1C-Q1466STOP-PrR | GAAGTACTCTTAGATCAGGAAGGTGGC |

PCR mix

| | |
|---|---|
| HotShot master mix | 5µl |
| LCGreen | 1µl |
| CACNA1C-Q1466STOP-F (20ng/µl) | 0.5µl |
| CACNA1C-Q1466STOP-R (20ng/µl) | 0.1µl |
| CACNA1C-Q1466STOP-PrR (20ng/µl) | 0.5µl |
| ddH2O | 0.9µl |
| DNA (1/10 dil ABI) | 2µl |

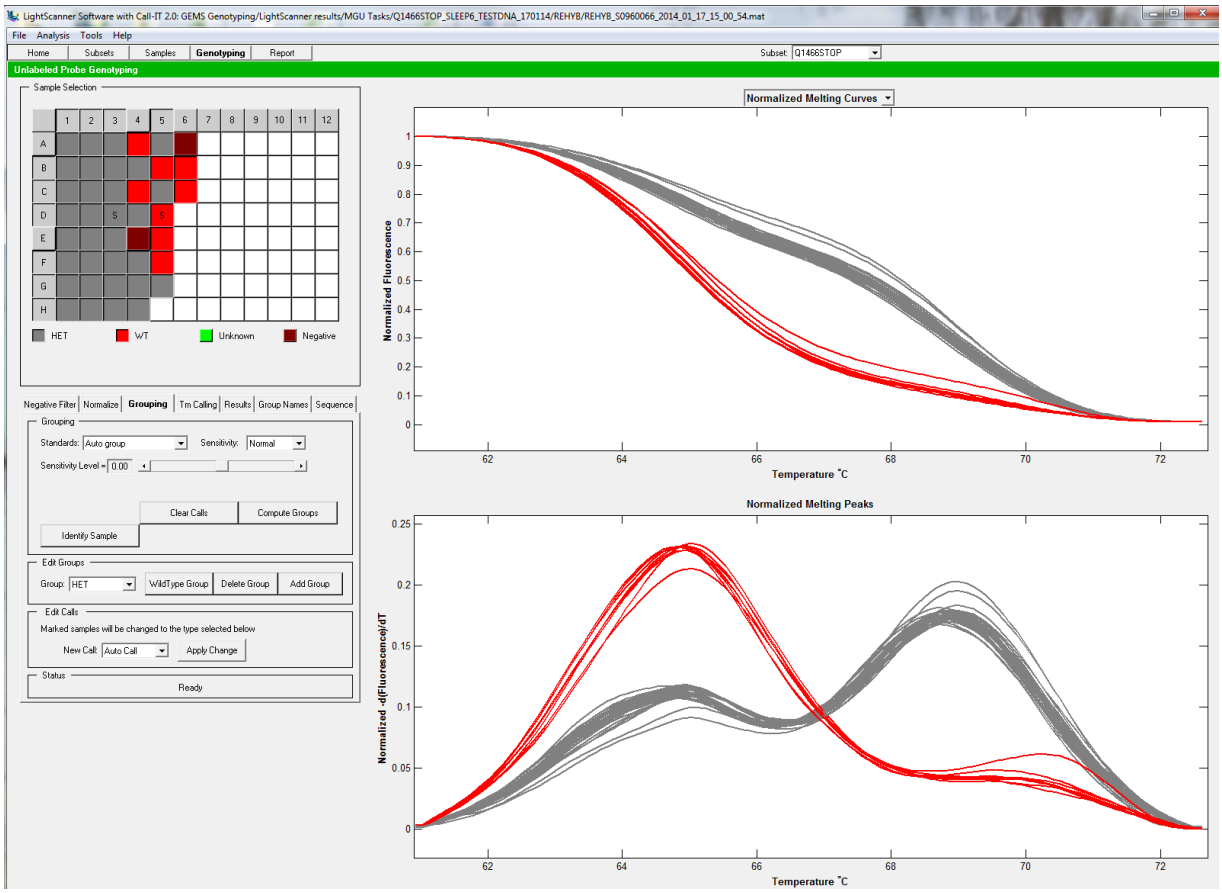
PCR program

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 | |

## Example


LightScanner Software with Call-IT 2.0: GEMS Genotyping/LightScanner results/MGU Tasks/Q1466STOP_SLEEP6_TESTDNA_170114/REHYB/REHYB_S0960066_2014_01_17_15_00_54.mat
File Analysis Tools Help
Home Subsets Samples Genotyping Report
Subset: Q1466STOP
Unlabeled Probe Genotyping
Sample Selection
HET WT Unknown Negative
Negative Filter Normalize Grouping Tm Calling Results Group Names Sequence
Grouping
Standards: Auto group
Sensitivity: Normal
Sensitivity Level = 0.00
Clear Calls
Compute Groups
Identify Sample
Edit Groups
Group: HET
WildType Group
Delete Group
Add Group
Edit Calls
Marked samples will be changed to the type selected below
New Call: Auto Call
Apply Change
Status
Ready
Normalized Melting Curves
Normalized Fluorescence
Temperature °C
Normalized Melting Peaks
Normalized -d(Fluorescence)/dT
Temperature °C