

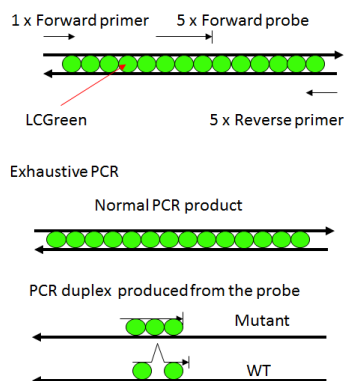
Hickory (EcsitN209I)

Introduction

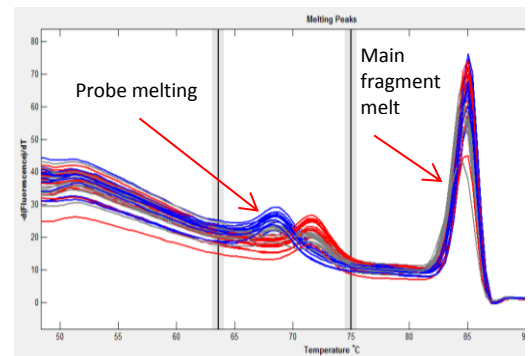
The Idaho Technology LightScanner is a system used to perform high throughput DNA melting analysis. PCR is performed in the presence of the double stranded DNA binding dye LCGreen. After PCR samples are then heated on the LightScanner and the fluorescence emitted by bound LCGreen is monitored. As the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



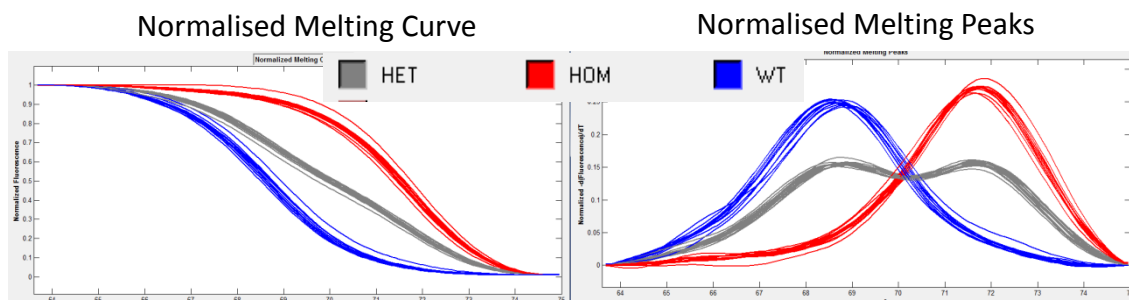
Unlabelled probe genotyping is used to distinguish between different homozygote samples at a given SNP where scanning analysis may not have enough sensitivity. Here a 3' blocked oligonucleotide (LunaProbe) is designed that sits directly over the SNP. Asymmetric exhaustive PCR is performed using 5 times 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.





Group: DMAT
Mutation type: SNP in EcsitN209I
Mutant allele: T (Highlighted in **Bold** in the sequence below)
WT allele: A
Assay Type: Unlabelled probe
Probe direction: Forward

Fragment sequence

GATTCAGATATTCGGGCGCAAAAGTTACCCCA**TGCTCAAGTTCCTGCGGAT**GAAGCTGTG**GTTCAACCC**
GATTCAAGATTATCAACCCCTACCCAGTGCCCCGAGATCTTCCCCAGGACCC**TTTGGACCTGGCCAAG**
CTAGGCCTGCGACACATGGAGCCTGACCTCAGTGCTAAGGTCACCTGTCTACCAGGTATGCATTCCTGT

Primers/Probe sets 5'>3'

EcsitN209I_LS_F1 TGCTCAAGTTCCTGCGGAT
EcsitN209I_LS_R1 CTAGCTTGGCCAGGTCCAAA
EcsitN209I_LS_Pr.F1 GTTCACCCGATTCAAGAT**T**TATCAACCC

PCR mix

HotShot master mix	5µl
LCGreen	1µl
EcsitN209I_LS_F1 (20ng/µl)	0.1µl
EcsitN209I_LS_R1 (20ng/µl)	0.5µl
EcsitN209I_LS_Pr.F1 (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 | |



Example

