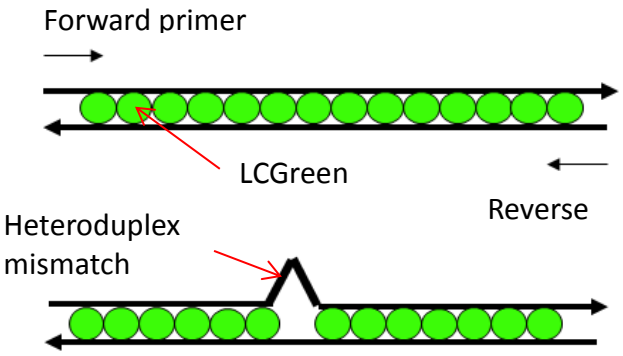
NESSIE Genotyping Strategy

Introduction

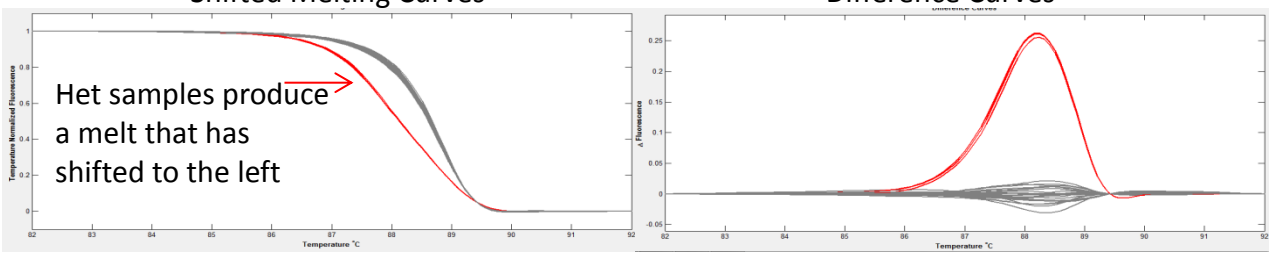
The Idaho Technology LightScanner is a system used to perform high throughput DNA melting analysis. PCR is performed in the presence of the double stranded DNA binding dye LCGreen. After PCR, samples are then heated on the LightScanner and the fluorescence emitted by bound LCGreen is monitored. As the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

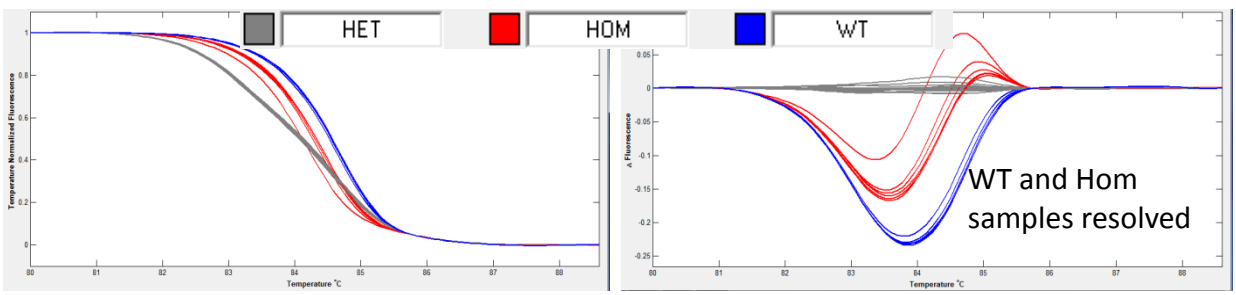


Scanning analysis can be used to detect samples that are heterozygous at a particular SNP. These samples will produce a melt curve that is shifted to the left as the instability created by the mismatch causes DNA to melt faster releasing the bound LCGreen. Homozygous WT and mutant samples will occasionally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

Shifted Melting Curves

Difference Curves





Group: FESA
Mutation type: SNP
Mutant allele: A
WT allele: T
Assay Type: Scanning

Fragment sequence

CGTGTGCGAGTACGCGGCGGCGCCGGTGGCTCGGCTGGCGTCTCACTGGGACCTGCCGATGCTGTCCGCAGG
AGCGCTGGCCGCCGGTTTCCAGCACAAGGACACGGAATACTCGCACCTCACGCGCGTGGCGCCTGCCTACGC
CAAGATGGGAGAGATGATGCTCGCTCTGTTTCGCCACCACCACTGGAGCCGTGCAGCCCTGGTCTACAGCGAC
GACAAACTCGAGAGGAACTGT**(T/A)**ATTTCACCCTCGAGGGGGTCCACGAGGTTTTTCAGGAGGAGGGGTTG
CACACGTCTGCCTACAATTTCGACGAGACCAAAGACTTGGACCTGGACGACATAGTGCGCTACATCCAAGGCA
GCGAGCGAGGTGAGCGGGAGCGGGACCAGGGGGGTCCTTGGCTCTGACTCAGCGGTTCTCAGTGGCTCTCC
CCCAAACCCCACCAAGTCAATCTTCTGCAGACCCCACCCTTCCCCGTAAC

Primers/Probe sets 5'>3'

Nessie_LS_For	CGCCAAGATGGGAGAGATGA
Nessie_LS_Rev	AGGCAGACGTGTGCAAC

PCR mix

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

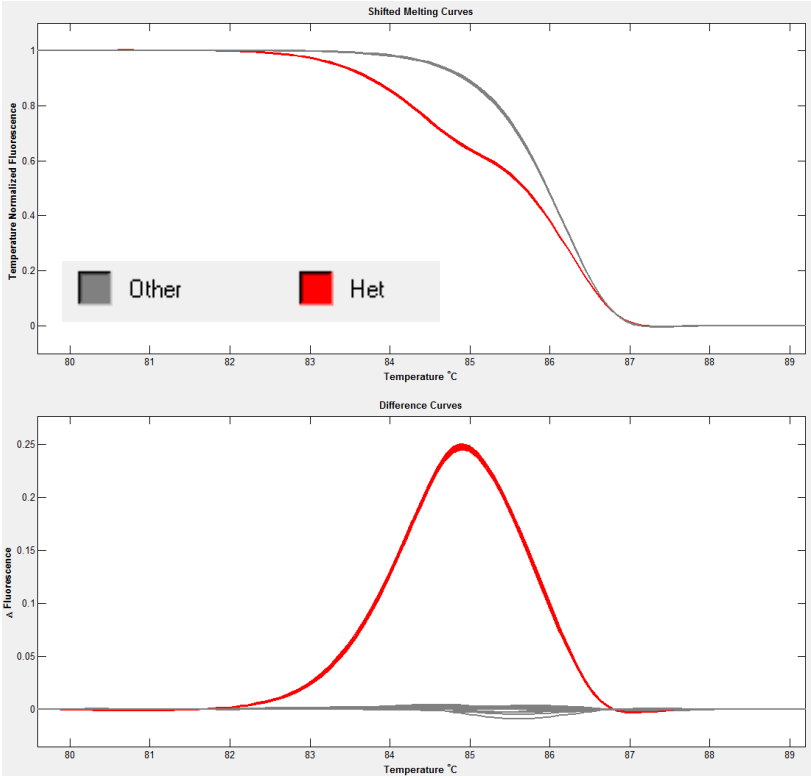
PCR program

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



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