

# A fast-throughput service for Familial Hypertrophic and Dilated Cardiomyopathy using High resolution melt curve analysis on the Lightscanner.

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## Introduction

- We have provided a molecular genetic service for Familial Hypertrophic Cardiomyopathy (HCM) and Familial Dilated Cardiomyopathy (DCM) since April 2004.
- HCM has a prevalence of approximately 1 in 500 and has been associated with mutations in 13 genes, all components of the cardiac sarcomere.<sup>[1]</sup>
- The screen currently comprises analysis of *MYH7*, *MYBPC3*, *TNNI2* and *TNNI3*, which are thought to account for up to 60% of familial HCM<sup>[1]</sup> and 10% of familial DCM<sup>[2]</sup>.
- In January 2007 all analysis was transferred from dHPLC WAVE to high resolution melt curve analysis on the Lightscanner.
- High-resolution melt curve analysis is a new technology that allows mutation scanning using the unique dsDNA binding dye 'LCGreen', which is able to saturate the DNA molecule. See figure 1.
- The binding of the dye is disrupted in the presence of a heteroduplex. The variation in fluorescence is detected by the camera in the Lightscanner as the DNA is melted.
- The Lightscanner is able to generate results for a 96 well plate within 6 minutes and can be run immediately after PCR. The potential throughput of the technique is expected to increase the efficiency of the service and assist in achieving the Department of Health Genetics Target Reporting times.

## Methods

- Primers were designed for 86 exons of the 4 genes using Idaho primer design software and optimised for Lightscanner use. Four exons are yet to be optimised.
- A bank of 16 samples with a known genotype were then screened on the Lightscanner for all 78 fragments. Where possible a known heterozygous or homozygous variant was run for all fragments; all common polymorphisms were tested.
- 98% (60/61) of variants were detected, including 4 homozygous variants.
- The missed variant was found to be within the primer binding site of the Lightscanner primer.
- Two exons were found to be too polymorphic. *MYH7* exon 12 and *MYBPC3* exon 13 (See figure 2-F) both contain 4 or more common polymorphisms. These were taken off the Lightscanner screen and are now analysed by direct sequencing.
- Over the validation period quality control measures were determined to establish when contamination was present and the strength of samples required to provide a result.
- Robotic methods were designed and validated for all PCR set-up on the Beckman Coulter Biomek NX 8 Robot.

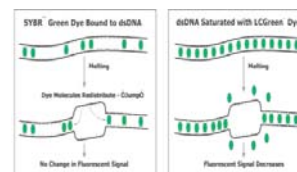


Figure 1 - LCGreen dye saturates DNA thus allowing detection of heteroduplexes.

<http://www.idahotech.com/LifeScience/HR-App-WhyLCG.htm>

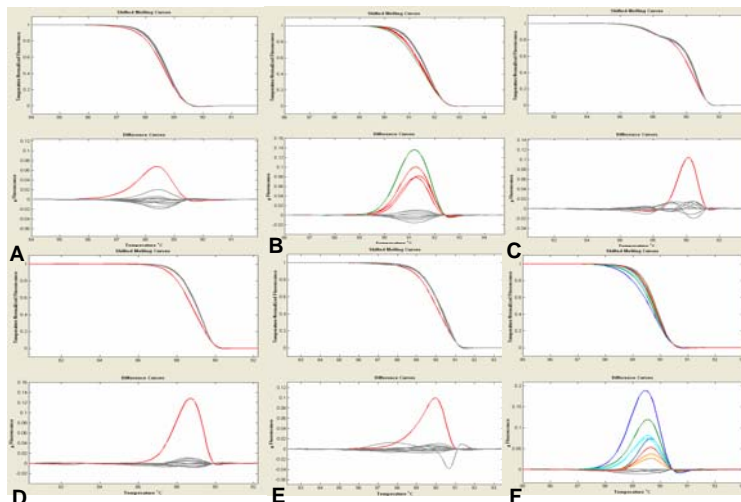


Figure 2 – Examples of Lightscanner traces. Shifted Melting Curves (above) and Difference Curves (below); A – *MYBPC3* c.1504C>T (p.R502W) het. B – *MYBPC3* c.655G>C (p.V219L) het. C – *TNNI3* c.586G>A (p.D196N) het. D – *TNNI2* c.237G>A (p.S79S) het. E – *TNNI2* c.200-4C>G het. F – *MYBPC3* Exon 13 showing multiple polymorphisms. In all cases red line represents the variant except in B where red lines represent the p.S236G polymorphism and green line represents p.V219L variant.

## Results

- In the last 8 months a total of 270 gene screens have been carried out using the Lightscanner.
- Throughput** - The Lightscanner has significantly reduced technical analysis times leading to increased throughput, this is shown in Table 1. Lightscanner results can be generated for a complete gene (up to 35 fragments) in 1 day whereas similar analysis by dHPLC would take 5 days. Using the Lightscanner only 1 set of data is generated for each fragment thus reducing data analysis time. In comparison dHPLC generally requires multiple sets of data to be generated per fragment.
- Cost** – Analysis by high-resolution melt curve analysis on the Lightscanner is considerably cheaper than dHPLC WAVE. The day to day running costs of dHPLC are high, approximately £108 per person for the complete gene screen (92 fragments). In comparison the Lightscanner has negligible day to day running costs. The cost of the PCR reactions which includes the cost of the LCGreen dye are identical, both approximately £39. Using the Lightscanner has enabled us to refine our process and reduce the charge for the service from £2027 in 2006 to £1332 in 2007.
- Ease of Analysis** - Analysis of data on the Lightscanner is quick and easy. The data in Figure 2 shows that the majority of variant traces can be easily picked out. However, discerning variants in the presence of polymorphisms can be difficult. Figure 2- B shows that traces with the same variant can be widely spaced thus reducing confidence in distinguishing variants from common polymorphisms. In some situations we have found it necessary to sequence all patients with the polymorphism trace.
- Sensitivity** - The data in Table 1 shows that no reduction in mutation pick-up has occurred due to transfer to High-resolution melt curve analysis. Variation is likely to be due to variability with in the patient cohort.
- Turn-around times** – The 4 genes are currently screened sequentially. The average reporting time per gene screen from January to August 2007 was 36 days. To further increase efficiency we aim to analyse all 4 genes concurrently and issue a single report for each patient.

	dHPLC WAVE (Samples analysed Apr 04- Dec 06)			Lightscanner (Samples analysed Jan 07-Aug 07)		
	Samples Analysed	Mutations Detected	% Mutation Pick-up	Samples Analysed	Mutations Detected	% Mutation Pick-up
<i>MYH7</i>	224	23	10	71	14	20
<i>MYBPC3</i>	178	54	30	85	22	26
<i>TNNI2</i>	162	8	5	57	3	5
<i>TNNI3</i>	-	-	-	57	2	3.5
<b>TOTAL</b>	<b>564</b>	<b>85</b>	-	<b>270</b>	<b>41</b>	-

Table 1 Comparison of samples analysed and mutations detected by dHPLC WAVE and by high-resolution melt-curve analysis on the Lightscanner. NB. Samples analysed on WAVE carried out between April 2004 and December 2006 (33 months) and on Lightscanner between January 2007 and August 2007 (8 months).

## Summary

- In-house validation has shown High-resolution melt curve analysis on the Lightscanner to be a highly sensitive technique suitable for screening all but the most polymorphic of fragments.
- Since January 2007 all gene screens for HCM and DCM have been carried out on the Lightscanner.
- Using the Lightscanner has enabled us to manage higher numbers of referrals and make significant progress in meeting the White Paper target reporting times.