

Background

- With the development of a new family of saturating double stranded DNA binding dyes, high resolution melt curve analysis (HRM) has been identified as a new and potentially useful method of high throughput mutation scanning.
- Recent publications suggest that HRM has a mutation detection sensitivity which is comparable or superior to currently available pre-screening techniques
- We have evaluated 3 machines that are specialised for HRM analysis: Rotor-Gene™ 6000 (Corbett Life Science), HR-1™ and 384 well LightScanner™ (Idaho Technology)
- Eleven different amplicons were analysed. Seven amplicons were generated from the NGRL (Wessex) panel of generic mutation detection control plasmids and 4 were generated from genomic DNA: hMLH1 Exons 1, 7 & 13 and hMSH2 Exon 10.
- The amplicons varied in size from 139 to 449bp and had GC contents ranging from 22 – 79% and the types of mutations analysed included all possible point mutation base substitutions and 1 and 2bp insertions and deletions.
- A total of 624 blinded samples (including controls) were amplified in the presence of the saturating ds DNA binding dye LCGreen® Plus (Idaho Technology) using the Rotor-Gene™ 6000 (Corbett Life Science).
- Identical PCR products were analysed using HRM on the Rotor-Gene™ 6000, HR-1™ and 384 well LightScanner™ platforms. Analysis of the Rotor-Gene™ 6000 & HR-1™ melt curves was undertaken manually by two operators and the LightScanner™ data was analysed with the software supplied using both high & normal sensitivity settings.
- Data were unblinded and the sensitivity and specificity of mutation detection were determined for each amplicon and platform.

HRM Platforms Evaluated

HR-1™ Instrument (Idaho Technology)

- Single sample
- Temp Control +/- 0.05 °C
- 5-20 µl Capacity
- 35 samples per hour
- 0.3°C ramp rate
- HRM only



384 well LightScanner (Idaho Technology)

- 96 or 384 microtiter plate
- Temp Control +/- 0.1 °C
- 5-20 µl Capacity
- 15 minutes per run
- HRM only



Rotor Gene™ 6000 (Corbett Life Science)

- 36 / 72 / 100 well rotor format
- Thermal uniformity ±0.01°C
- Resolution ±0.02°C
- 5-20 µl Capacity
- 15 minutes per run
- HRM, real time PCR and allelic discrimination (5 colours)



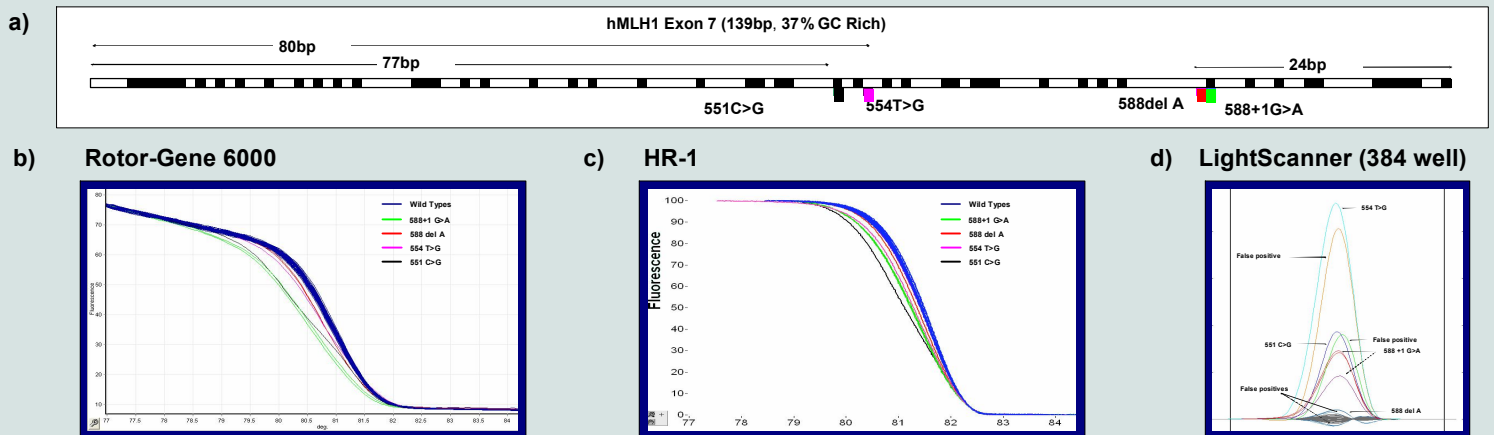
Result and Conclusions

Amplicon	No. of wild type samples	No. of mutated samples	No. of unique mutations	Rotor-Gene™ 6000 (Corbett Life Science)		HR-1™ (Idaho Technology)		LightScanner™ 384 well (Idaho Technology)			
				Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	High sensitivity		Normal sensitivity	
								Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
20L	23	24	12	100	82.6	89.6	97.8	87.5	100	75	100
20S	23	24	12	100	100	100	91.3	100	100	100	100
40L	23	24	12	100	100	95.8	97.8	100	100	75	100
40S	23	24	12	100	100	95.8	97.8	100	100	71	100
60L	23	24	12	100	90.9	95.8	84.4	Not analysed	Not analysed	Not analysed	Not analysed
60S	23	24	12	100	100	100	97.7	100	100	69.6	100
80S	23	24	12	100	87	100	93.5	100	87	98.5	87
hMLH1 x1	60	9	5	100	96.7	100	94.2	100	80	80	90
hMLH1 x7	62	7	5	100	100	100	95.2	100	90.5	71.4	96.8
hMLH1 x13	55	14	5	100	98.2	100	85.5	100	96.4	50	100
hMSH2 Combined	55	14	6	100	96.4	100	96.3	100	80	100	90.9

Table 1: % Sensitivity (true positive / (true positive + false negative)) and specificity (true negative / (true negative + false positive)) of mutation detection for eleven amplicons analysed using the three HRM platforms. LightScanner™ data are given for the 2 software settings: high & normal sensitivity.

- The overall sensitivity and specificities for each machine were comparable at 100% & 95% (Rotor-Gene 6000, Corbett Life Science), 98.4% & 95% (HR-1, Idaho Technology) and 99% & 88% (LightScanner, Idaho Technology)
- We conclude that HRM is an extremely sensitive and specific technique for mutation scanning which could be easily integrated into clinical diagnostic pre-screening strategies.
- The technique has the potential to allow large genes to be screened and reported within the UK Genetics White Paper guidelines of 6-8 weeks
- Further work is required to determine the feasibility of analysing many different exons for small batches of patients within the same HRM run

Examples of high resolution melt curves for hMLH1 Exon 7



a) Schematic diagram of hMLH1 Exon 7 Amplicon. G/C and A/T bases within the amplicons are represented by black and white bars respectively. The coloured bars indicate the position of the different types of mutations analysed. High resolution melt curves are shown for 69 samples analysed using b) Rotor-Gene 6000, c) HR-1 and d) LightScanner. All mutations were successfully detected including those located 24bp from the end of the amplicon. The most subtle mutation was 588delA which appears to change the temperature at which the amplicon melts rather than altering the overall shape of the melt curve. Data from the HR-1™ and LightScanner™ are first normalised and then temperature shifted. When the data are temperature shifted the curve for 588del A becomes nearly superimposed on the wild type samples making detection difficult. Data from the Rotor-Gene™ 6000 is normalised, but not temperature shifted as the machine has excellent well-to-well thermal uniformity and temperature can be more accurately plotted. Therefore mutations such as these (and homozygous mutations) can be more easily detected using the Rotor-Gene 6000 as the melt curves do not become overlaid on the wild type curve.