Evaluation of High Resolution Melt Analysis for Mutation Scanning

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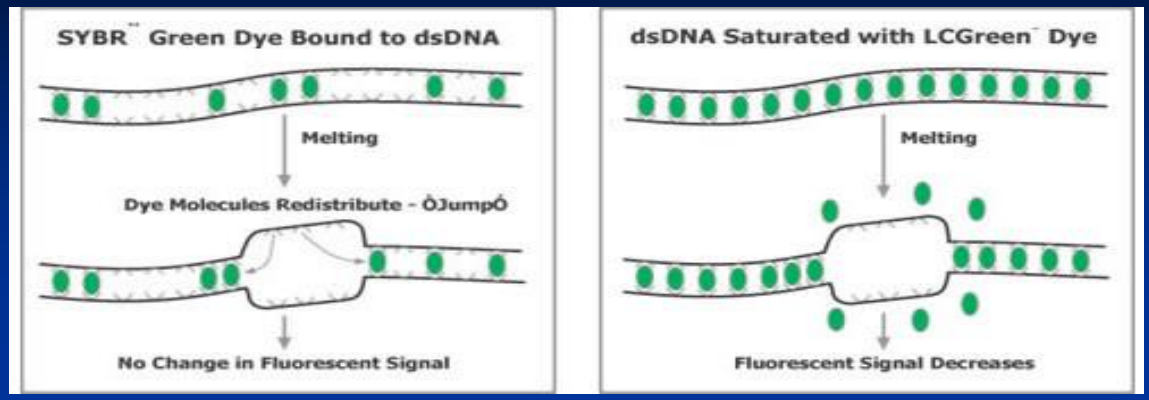
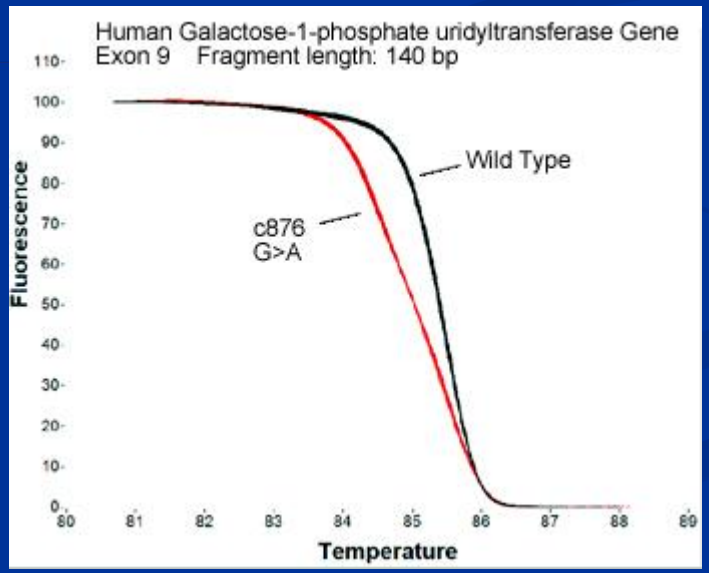
High Resolution Melt Analysis

- Use Saturating dye in PCR



- Analysis of fluorescence as amplicon is melted
- Heteroduplexes and homoduplexes will melt with different profiles



Evaluation protocol

- PCR optimised with LC Green Plus
- 11 different amplicons analysed; 7 plasmid based, 4 genomic DNA
  - Size range: 139bp – 449bp
  - GC content: 22% - 79%
  - Types of mutation: All possible heteroduplex types
    ins C, ins AA
    del A, del C, del CA
- Amplicons amplified using RotorGene 6000 in 20ul reactions
- Amplification efficiency monitored using real time PCR
- Same amplicons analysed using HRM
- Machines evaluated:
  - HR-1 (Idaho Technology)
  - 384 well LightScanner (Idaho Techonology)
  - Rotor Gene 6000 (Corbett Research)

HR-1™ Instrument (Idaho Technology)

Single sample (glass capillary tubes)

Temp Control +/- 0.05 °C

5-20 µl Capacity

35 samples per hour with a 0.3°C ramp rate (after amplification)

HRM only

LightScanner™ Instrument (Idaho Technology)

Standard 96 or 384 microtiter plate

Temp Control +/- 0.1 °C

5-20 µl Capacity

15 minutes per run (after amplification)

HRM only

Rotor Gene™ 6000 (Corbett Research)

36 / 72/ 100 well rotor format

Thermal uniformity ±0.01°C, Resolution ±0.02°C,

HRM data acquisition (read) rate: 20 reads for each 0.02°C increment

5-20 µl Capacity

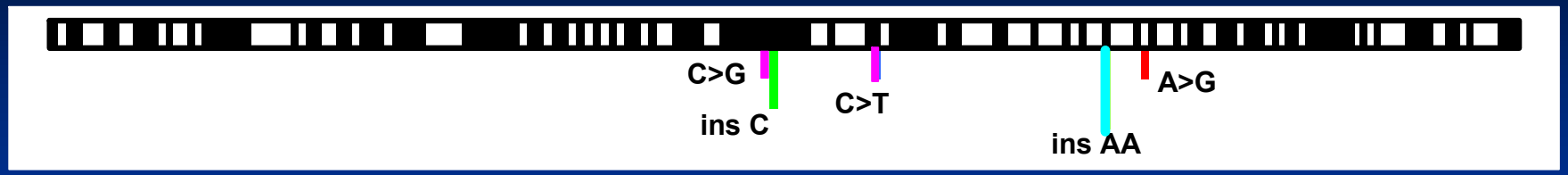
15 minutes per run (after amplification)

HRM, real time PCR and allelic discrimination (5 colours)

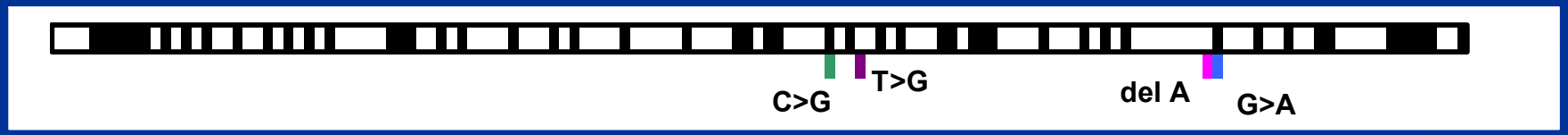
Amplicons and Mutations Analysed I

Amplicons and Mutations Analysed II

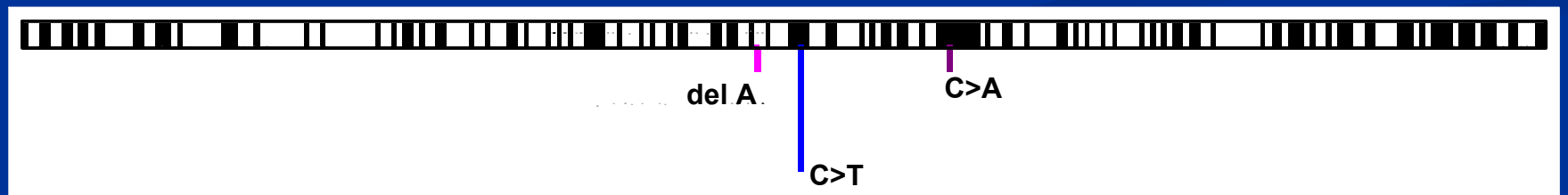
hMLH1 Exon 1 (193bp, 57% GC Rich)



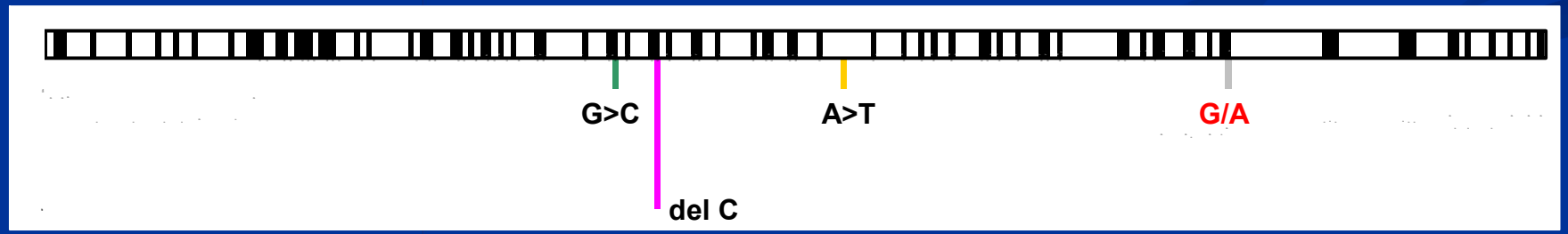
hMLH1 Exon 7 (139bp, 37% GC Rich)



hMLH1 Exon 13 (277bp, 44% GC Rich)



hMSH2 Exon 10 (249bp, 34% GC Rich)



Analysis

Plasmid based samples (47 samples total)

- 24 mutated samples (duplicates of each mutation at each position)
- 23 wild type samples
- 1 NTC
- Randomised differently for each GC content

Genomic DNA samples (70 samples total)

- 35 known HNPCC mutation carriers for exons tested
- 32 anonymised normal controls
- 1 wild type plasmid contol
- 1 hetrozygous plasmid control
- 1 homozygous plasmid control
- 2 NTC
- Randomised

ALL AMPLICONS WERE VERIFIED BY SEQUENCING

HR-1 and RotorGene 6000

- Samples analysed manually by 2 operators

LightScanner

- Samples analysed using software provided with High and Normal Sensitivity

Data unblinded and true and false positive and negatives recorded

Sensitivity and Specificity

Sensitivity = true positive / (true positive + false negative)

Specificity = true negative / (true negative + false positive)

	RotorGene 6000		HR-1		LightScanner 384 well			
					High Sensitivity		Normal Sensitivity	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
20L	100	82.6	89.6	97.8	87.5	100	75	100
20S	100	100	100	91.3	100	100	100	100
40L	100	100	95.8	97.8	100	100	75	100
40S	100	100	95.8	97.8	100	100	71	100
60L	100	90.9	95.8	84.4	-	-	-	-
60S	100	100	100	97.7	100	95.7	69.6	100
80S	100	87	100	93.5	100	87	98.5	87
hMLH1 x1	100	96.7	100	94.2	100	80	80	90
hMLH1 x7	100	100	100	95.2	100	90.5	71.4	96.8
hMLH1 x13	100	98.2	100	85.5	100	96.4	50	100
hMSH2 x10 Combined	100	96.4	100	96.3	100	80	100	90.9

False Negative Results

HR-1

20L	Position 1	A to T	G to C	G to A
40L	Position 3	A to T		
40S	Position 3	G to A	G to C	

LightScanner (Analysed with High Sensitivity)

20L	Position 1	A to T	G to C	G to A

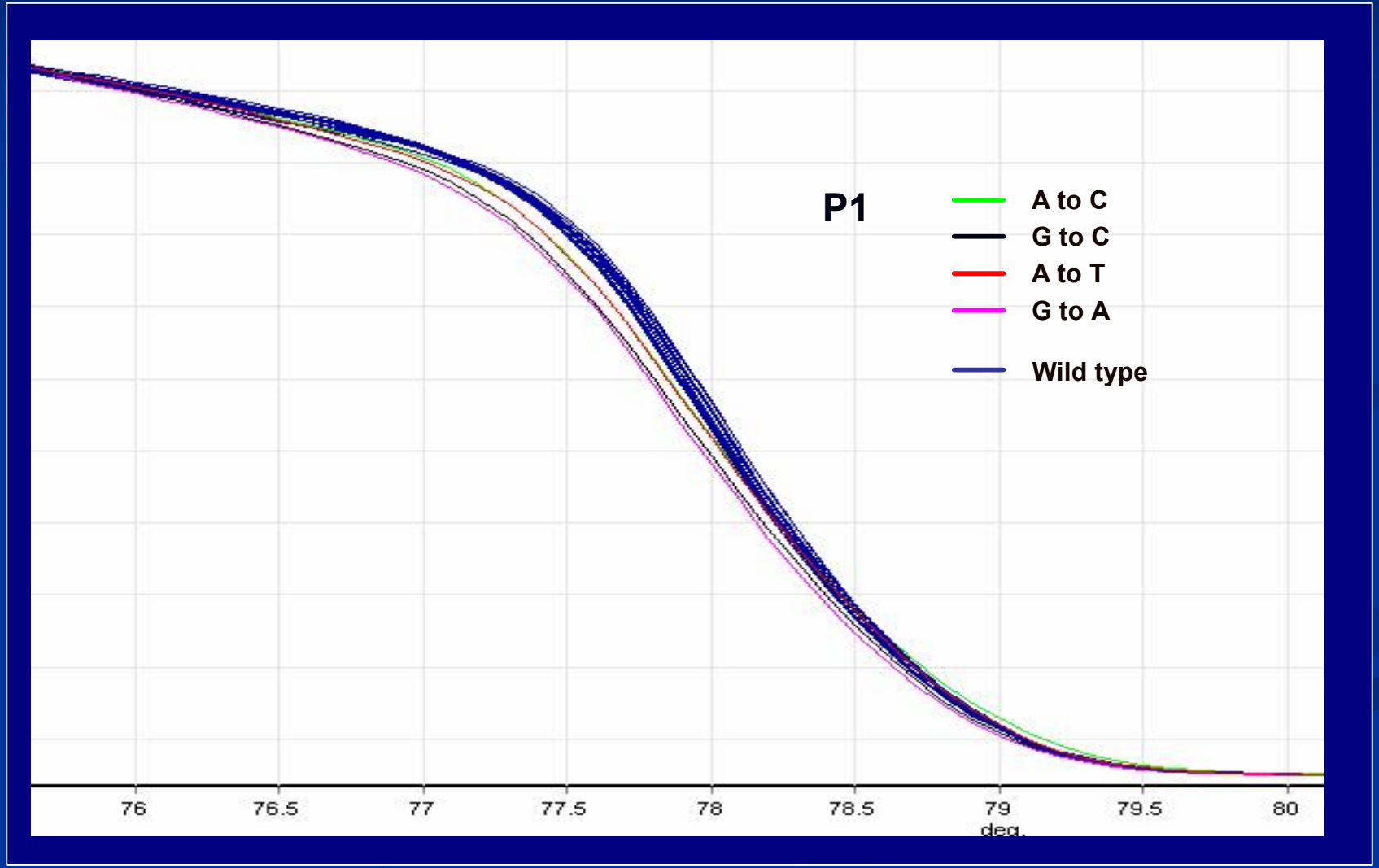
Rotor Gene 6000

No false negatives

20% GC long P1

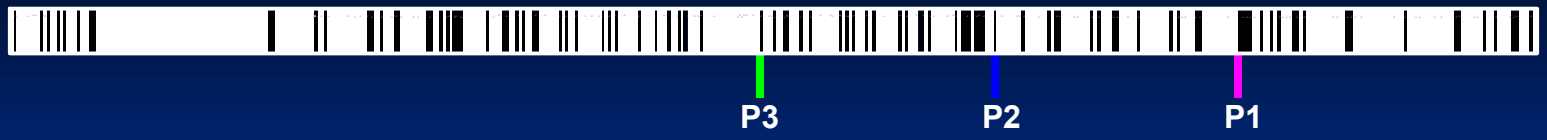
(449 bp, 22% GC)

84bp

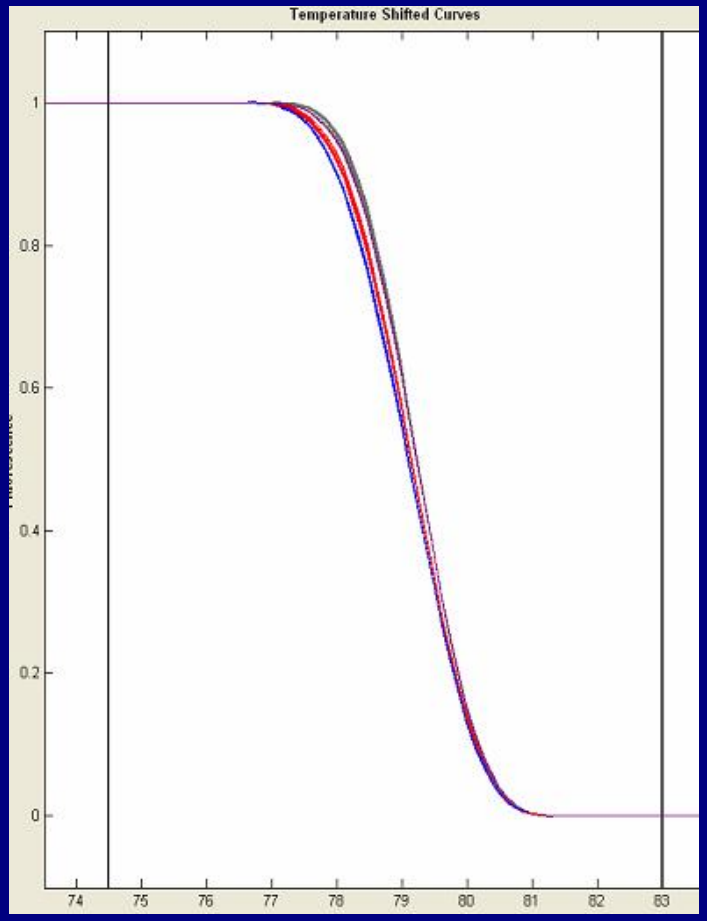


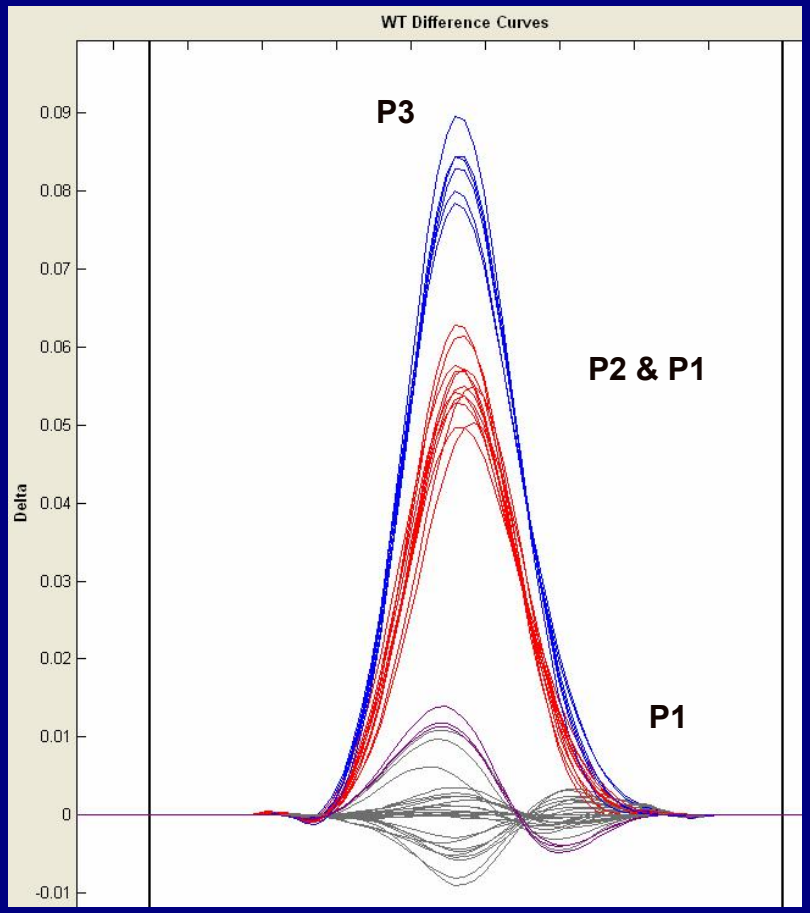
20% GC long

(449 bp, 22% GC)



LightScanner 384 well

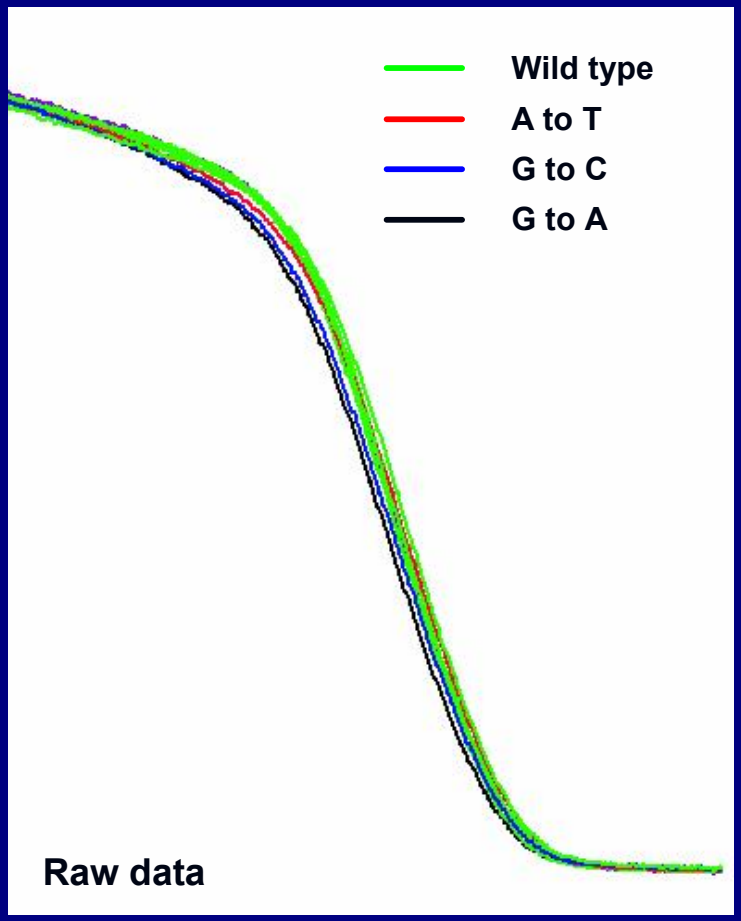


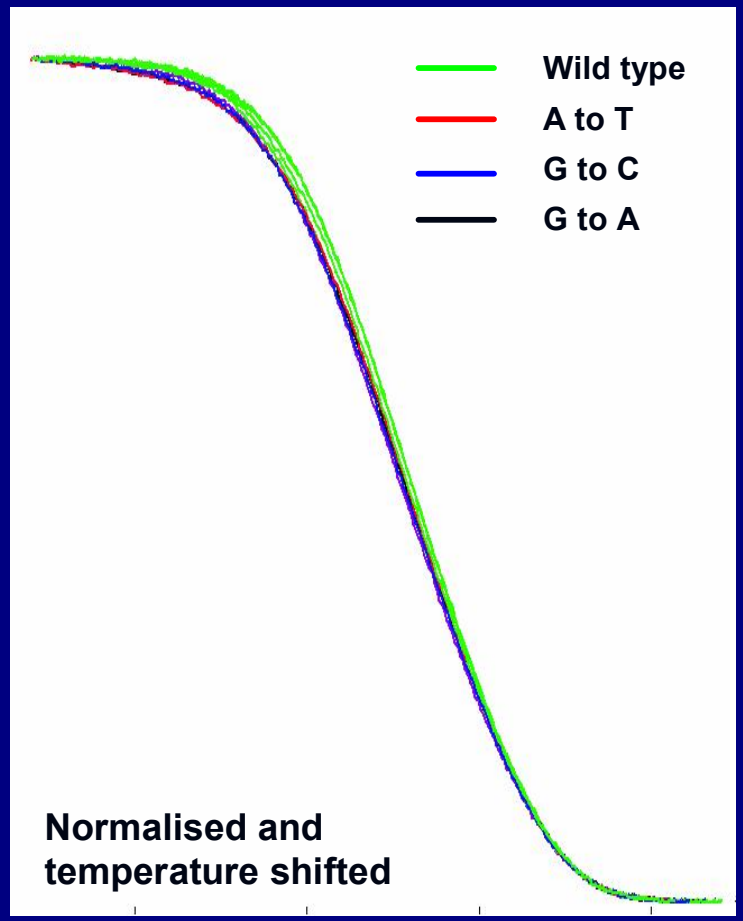


20% GC long

(449 bp, 22% GC)

HR-1





Summary

- 624 samples analysed in total
- 11 amplicons tested: 139bp – 449bp with GC contents of 22-79%
- Mutations included all possible point mutation base substitutions and 1 and 2bp insertions and deletions
- The same PCR reaction was analysed using the HR-1 and 384 well LightScanner (Idaho Technology) and RotorGene 6000 (Corbett Research)

Sensitivity and Specificity of mutation detection for each platform were:

	Sensitivity	Specificity
RotorGene 6000	**100.0**	**95.3**
HR-1	**98.4**	**95.0**
LightScanner 384 well (High)	**99.0**	**88.0**
LightScanner 384 well (Normal)	**83.9**	**95.3**

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