

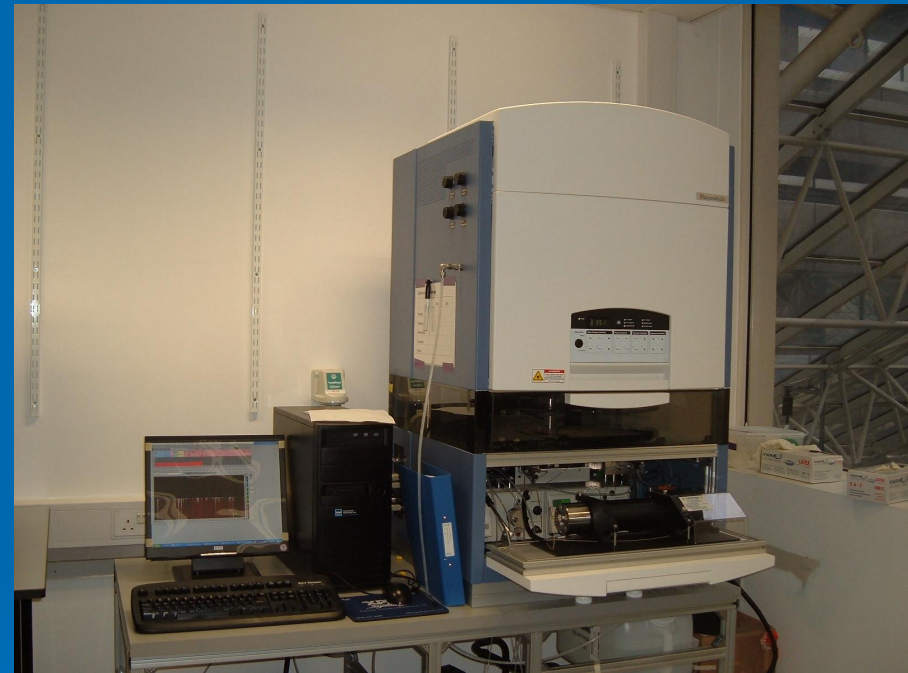
# Using the Reveal Genetic Mutation Discovery System (Spectrumedix) for Mutation Screening in a Diagnostic Laboratory

JK Campbell

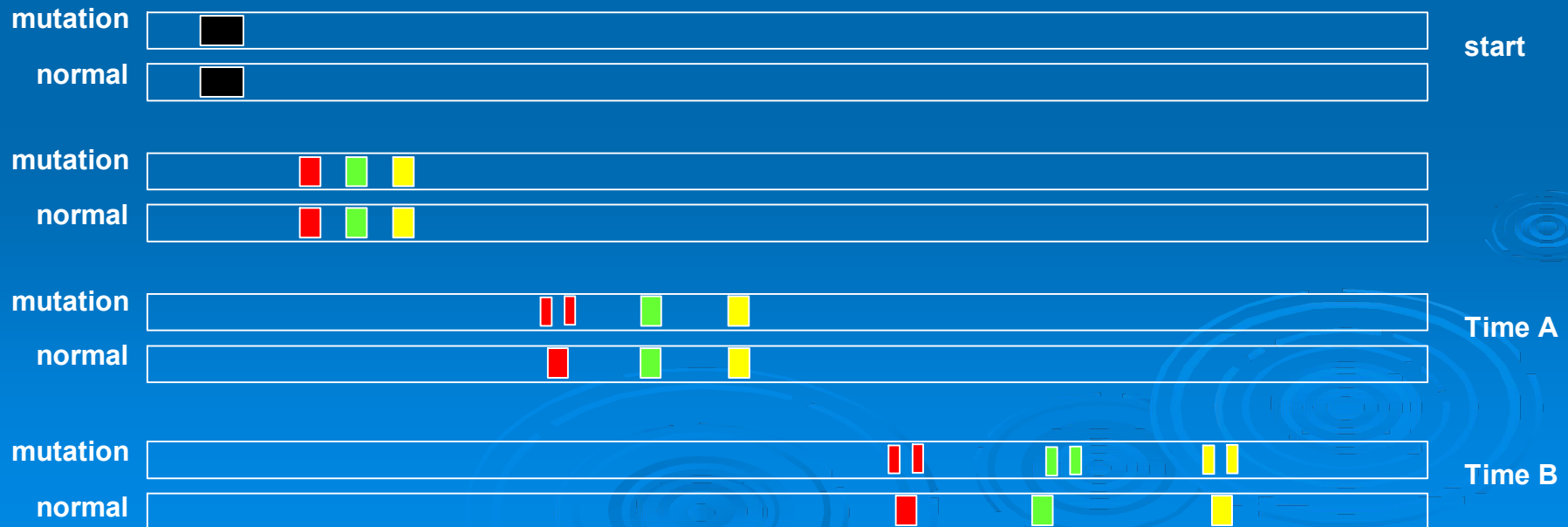
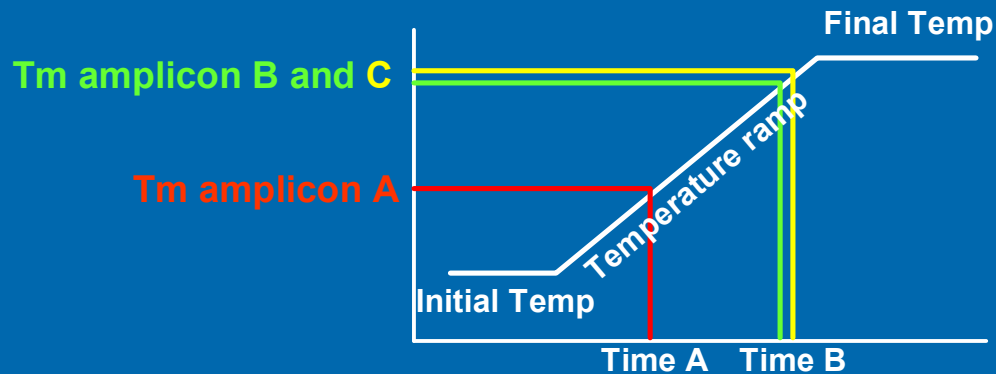
- Overview of Spectrumedix System
- Evaluation using mutation detection reference reagents
- Summary of current use at Guy's

# Reveal Genetic Mutation Discovery System (Spectrumedix)

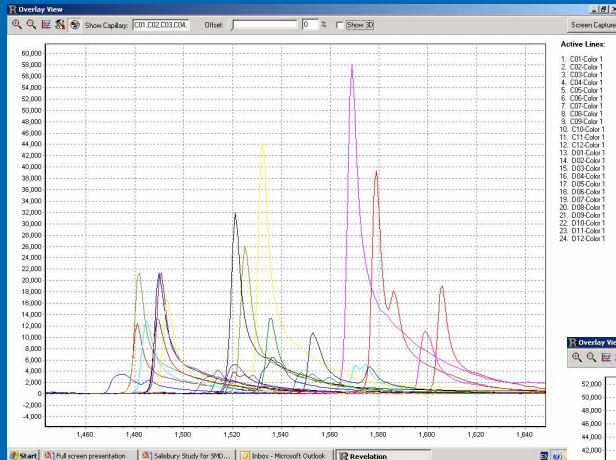
- Temperature gradient capillary electrophoresis (TGCE)
  - Fragments with different melting properties can be analysed together
  - Multiple DNA fragments can be analysed together
- Uses ethidium bromide to detect DNA
  - No need for fluorescent primers
- 96 Capillaries
- Specific software to compare peak traces and identify mutations
- Can also be used for genotyping and sequencing



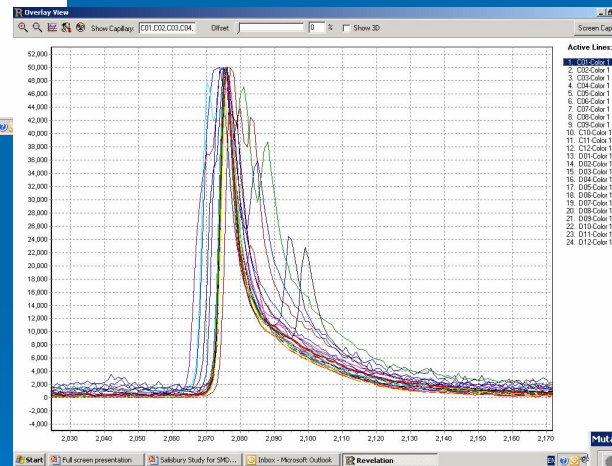
# Reveal Genetic Mutation Discovery System



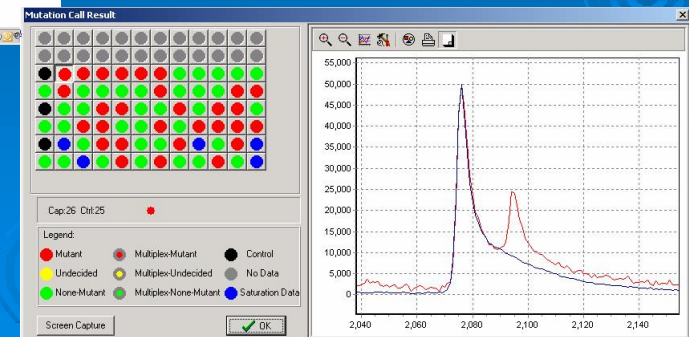
# Revelation Mutation Detection Software



Raw data



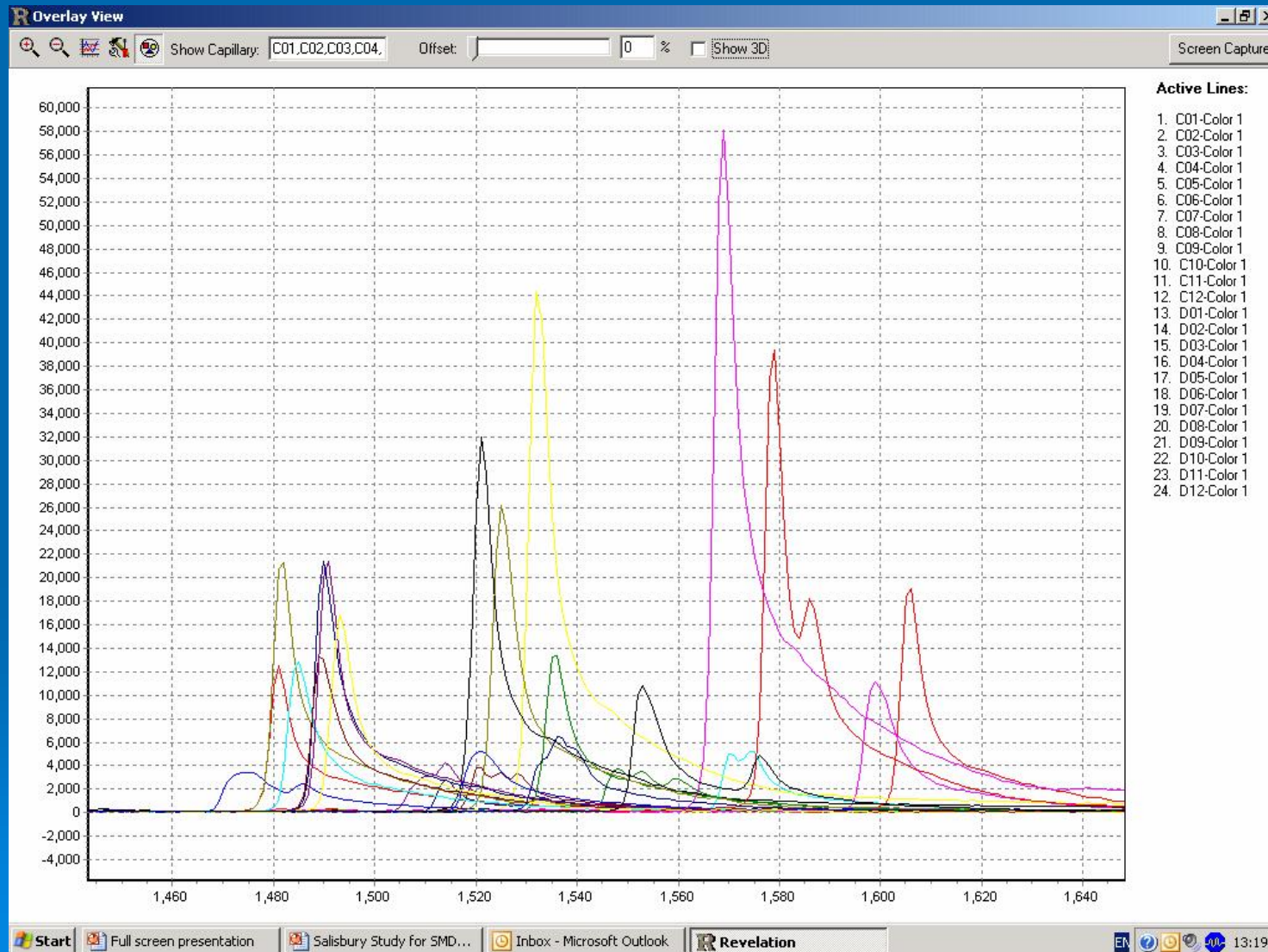
Normalised data



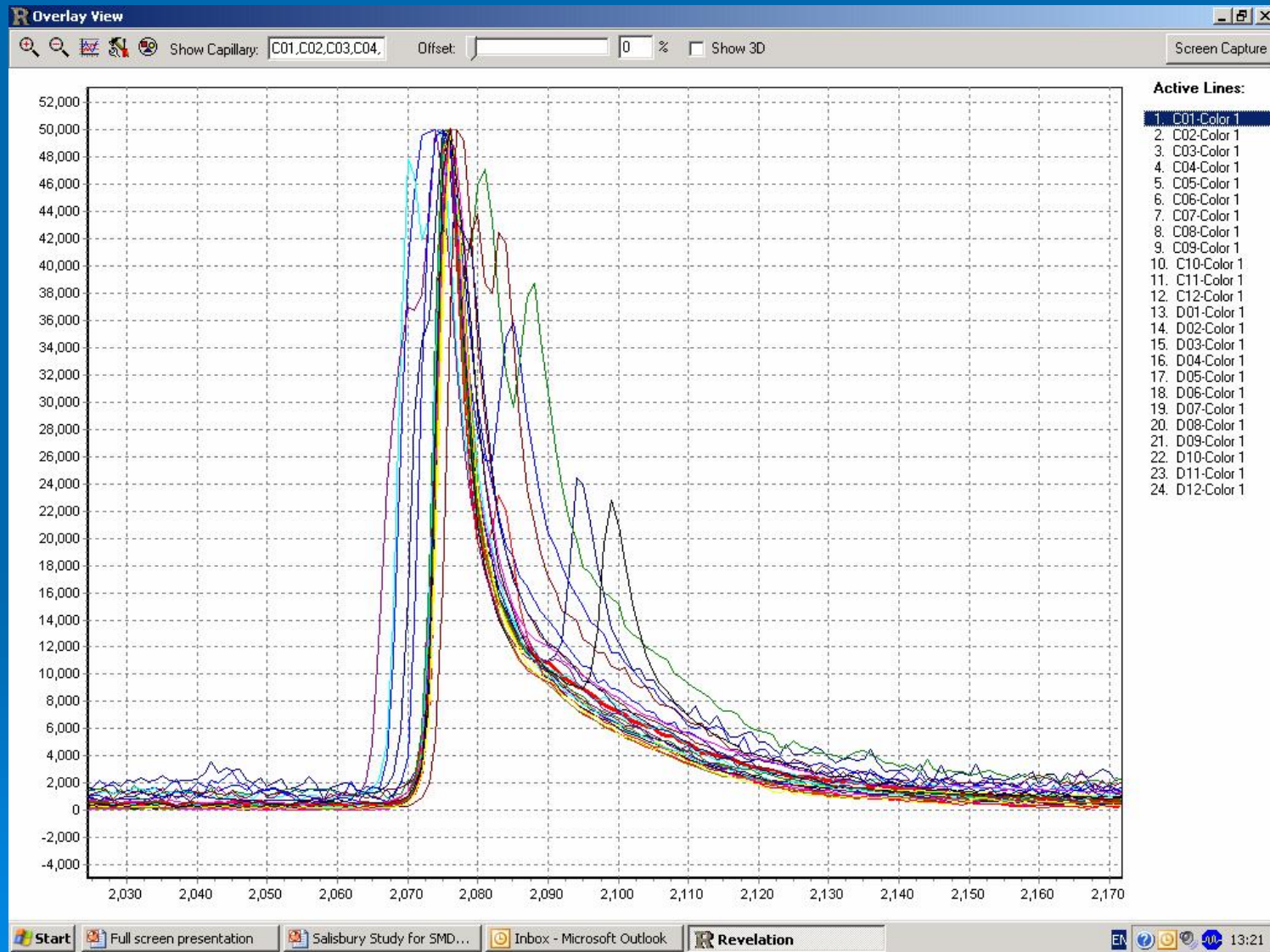
Mutation Report



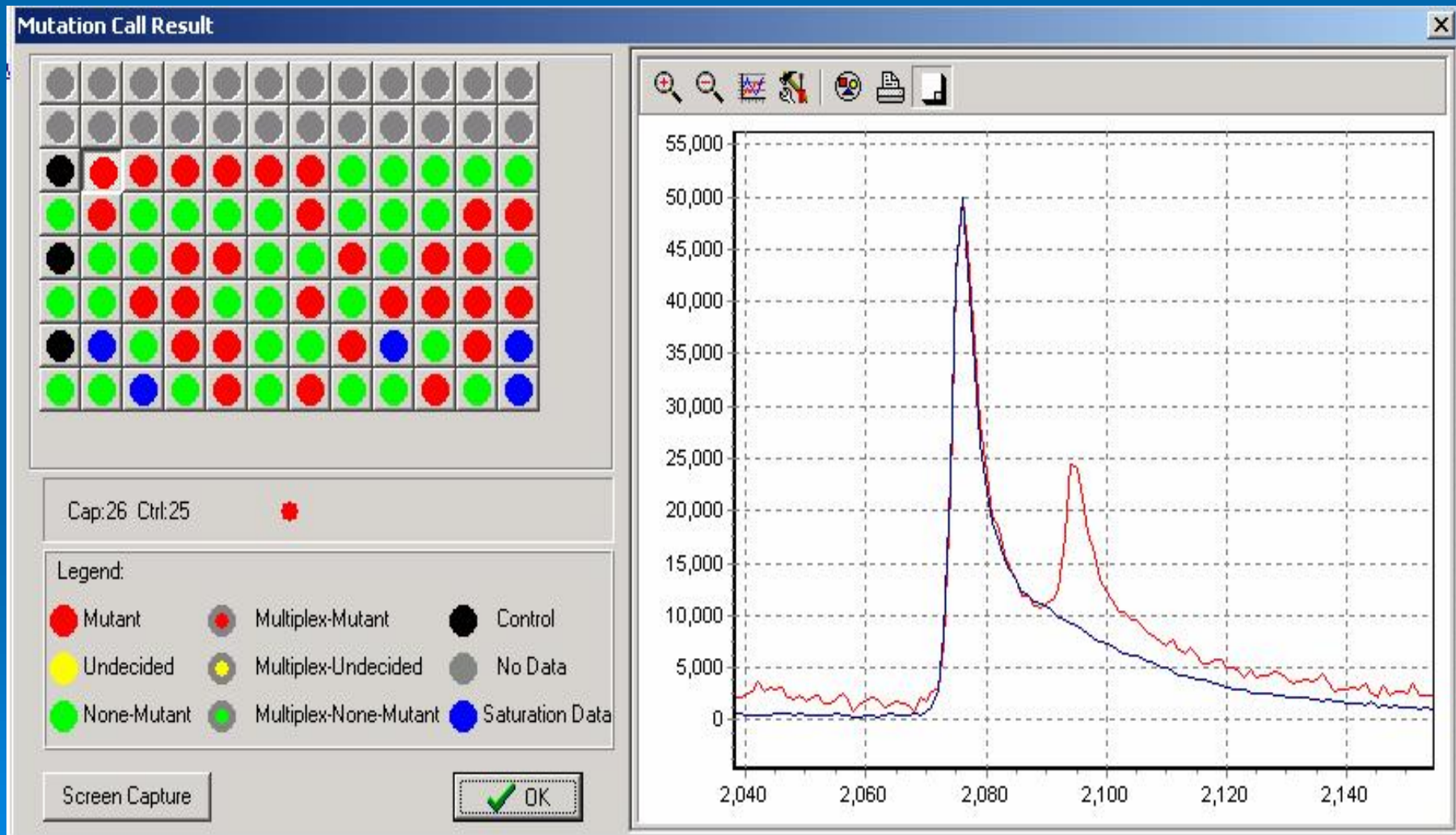
# Revelation Mutation Detection Software



# Revelation Mutation Detection Software



# Revelation Mutation Detection Software





# Evaluation using mutation detection reference reagents

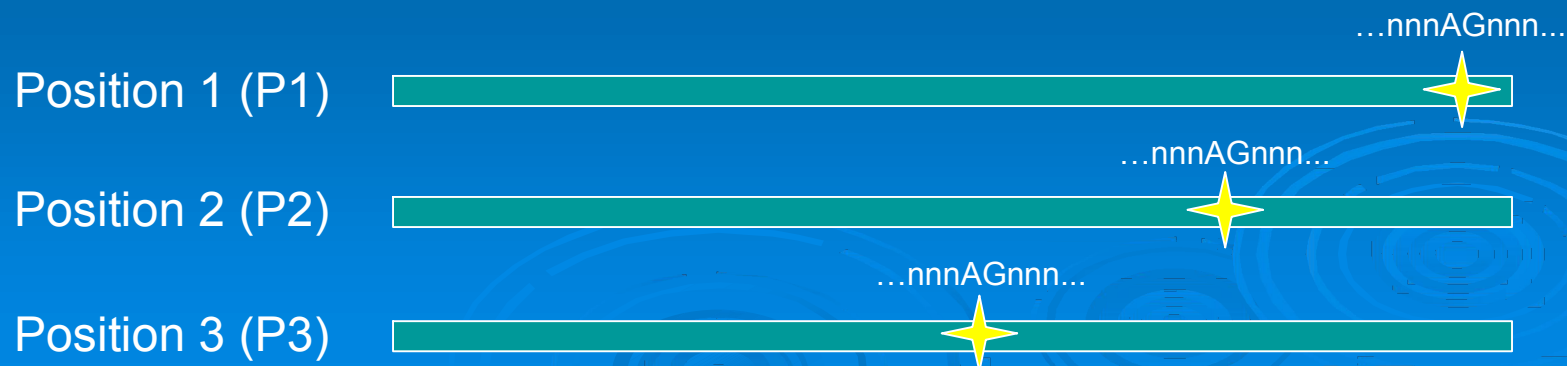
## Aims

- To determine sensitivity and specificity
- To determine the best temperature range for analysis
- To determine any correlation between type of mutation and position with ease of detection

# Study Design

- Generic mutation detection reference reagents supplied by The National Genetics Reference Laboratory (Wessex).
- 4 plasmid based fragments produced with an average GC content of 20, 40, 60 or 80%.
- Each of these sequences has been mutated to produce all possible base substitutions at three positions within the amplicon.

Mutation created	Sequence generated	Heteroduplex produced
A > C	nnn <b>C</b> Gnnn	C:T & G:A
A > T	nnn <b>T</b> Gnnn	T:T & A:A
G > A	nnn <b>A</b> Gnnn	A:C & G:T
G > C	nnn <b>C</b> Gnnn	C:C & G:G



# Results – Sensitivity and Specificity

	TGCE	CSCE
<b>True positive</b>	<b>84</b>	<b>89</b>
<b>True negative</b>	<b>54</b>	<b>73</b>
<b>False positive</b>	<b>26</b>	<b>6</b>
<b>False negative</b>	<b>4</b>	<b>7</b>
<b>Fails</b>	<b>8</b>	<b>1</b>
<b>Sensitivity</b>	<b>95%</b>	<b>93%</b>
<b>Specificity</b>	<b>68%</b>	<b>92%</b>

# Results – Best Analysis Temperature

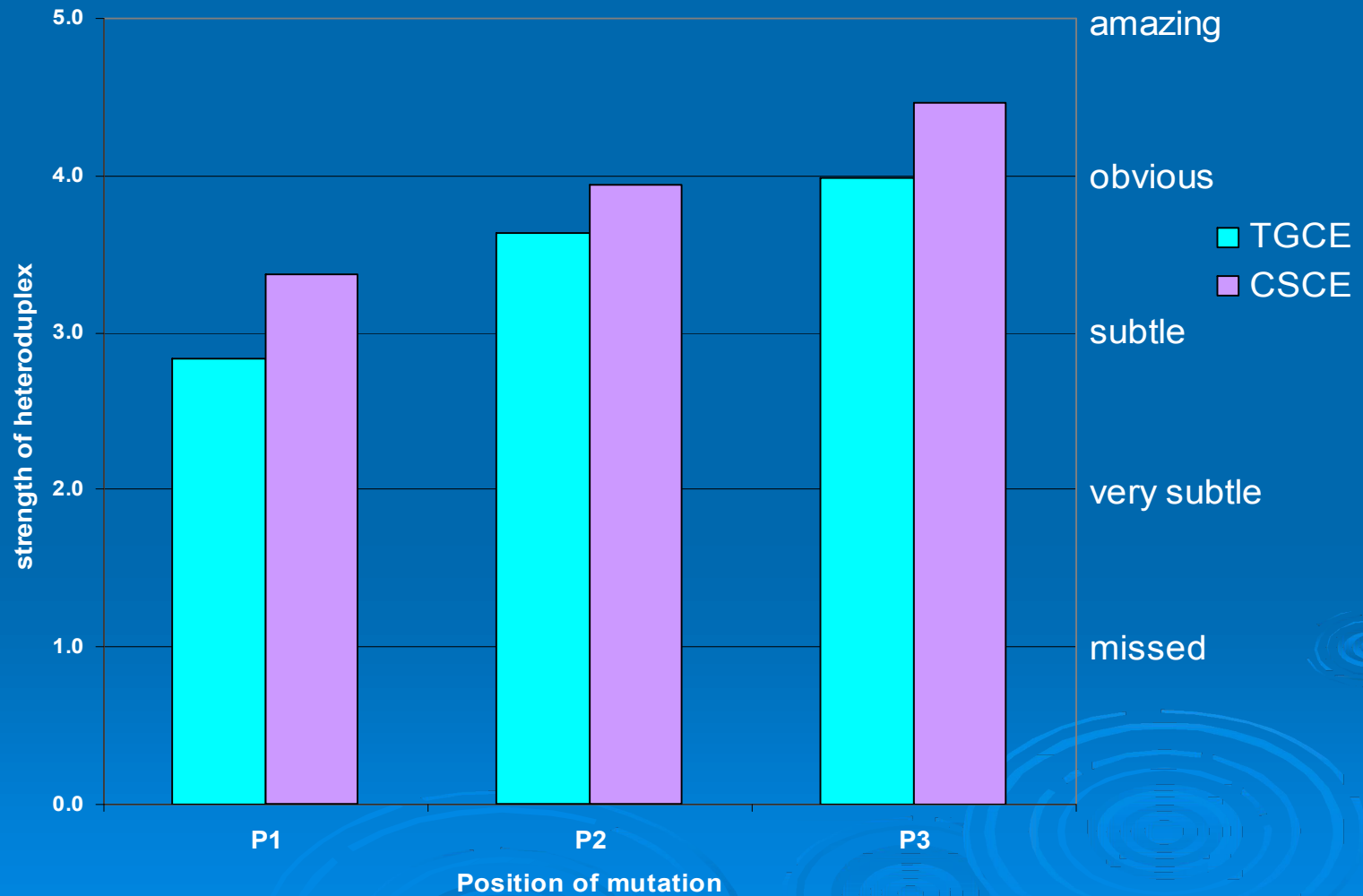
- Temperature range can be set by user
  - 35-45 °C
  - 50-60 °C (default)
  - 55-65 °C
  - 64-67 °C – recommended for high GC fragments
- No major differences in mutation detection at different temperature ranges, however
  - 20% GC fragments degraded at all temps except 35-45 °C
  - 40% GC fragments degraded at 64-67 °C
  - Several mutations produced only subtle traces at 35-45 °C
- Overall conclusion was that a single temperature range (50-60°C) was sufficient to detect all mutations



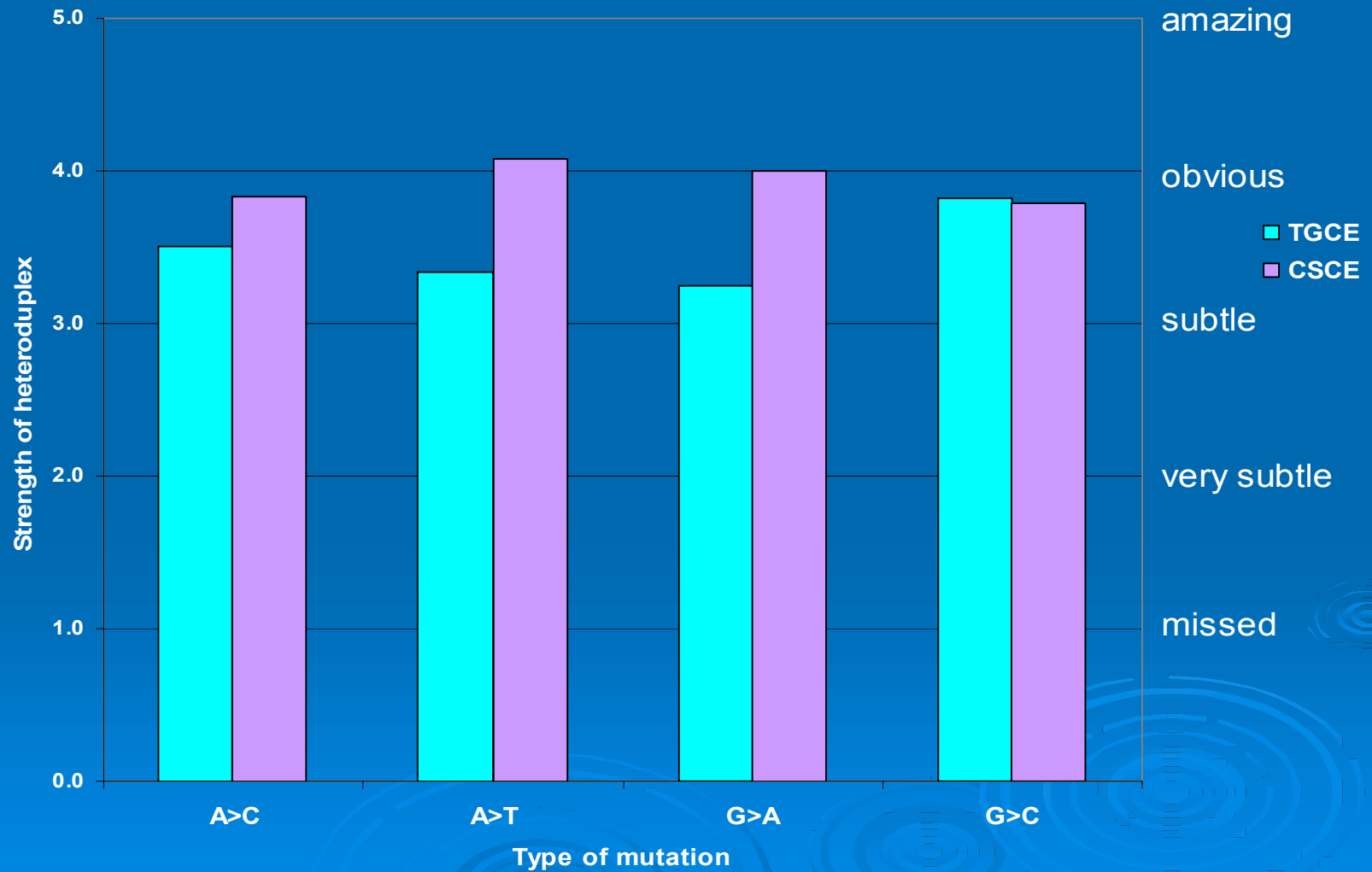
# Results - Correlations

- Heteroduplex traces were classified according to how clear they were:
  - 1 – mutation missed
  - 2 – very subtle (eg wider)
  - 3 – subtle (eg small shoulder)
  - 4 – Obvious
  - 5 – Amazing (eg 3 or 4 separate peaks)
- Data was stratified according to position and type of mutation to look for correlations.

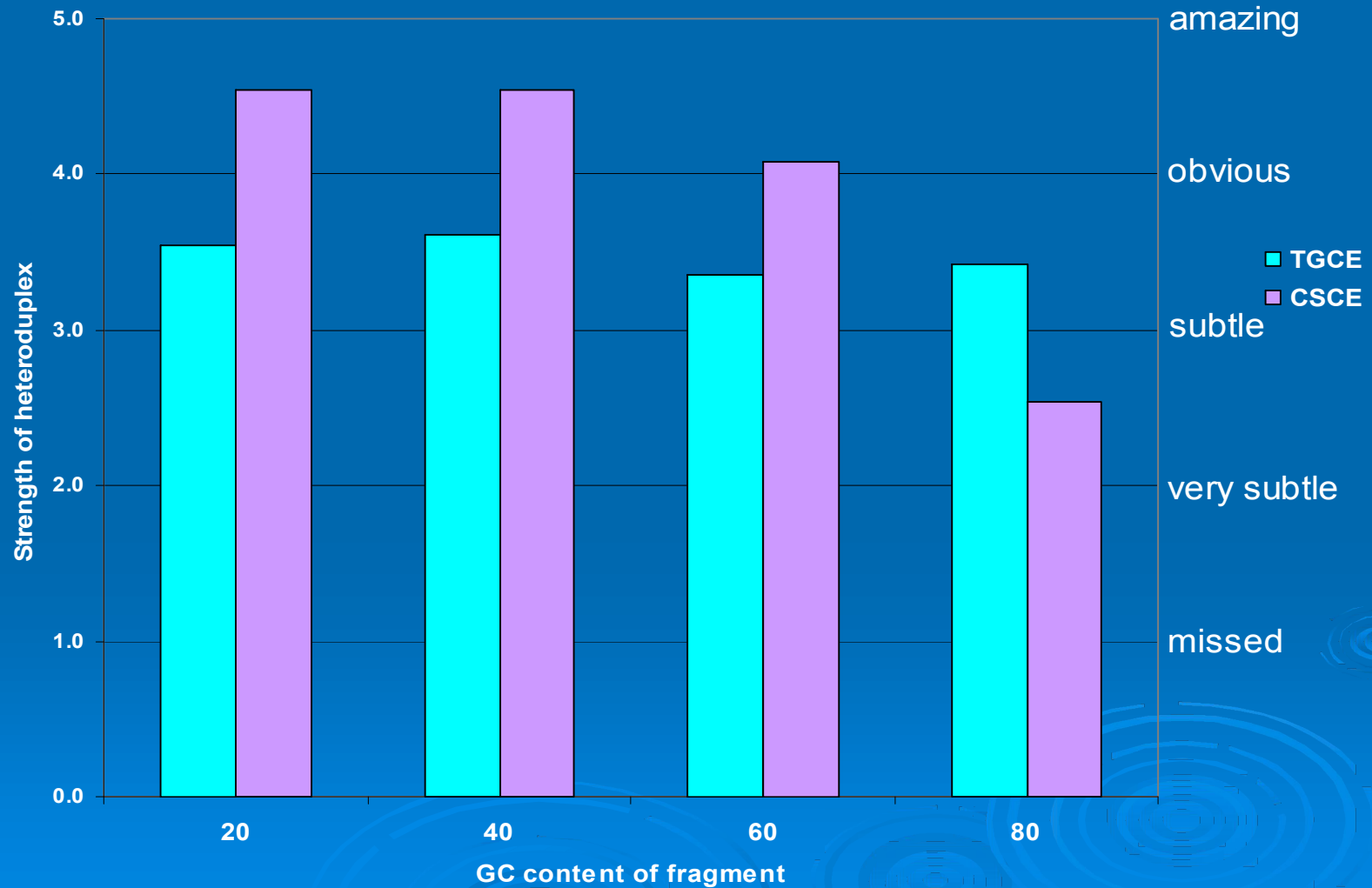
# Position of Mutation



# Type of Mutation



# GC Content of Fragment





# Summary of mutation screening using the Spectrumedix at Guy's

Disease	Congenital Muscular Dystrophy		HSP	Alports	HBOC	DMD/BMD
Gene(s)	POMT1, POMT2, POMGnT1, LARGE, Fukutin	LAMA2	Spastin (SPG4)	COL4A5	BRCA1 + BRCA2	Dystrophin
# fragments	80	65	16	51 (+1 control)	82	80
single/ multiplex	M	S	S	M	M	M
Analysis temperature	50-60	50-60	50-60	55-60	50-60 and 35-45	
sensitivity	100% (4/4)	100% (9/9)	95.8% (23/24)	90% (27/30)	97.7 (43/44)	
sequencing		14.4% (8.6% HD, 5.8% fails)				
Exons sequenced			1	1	2	

# Summary

- TGCE is being used successfully at Guy's for mutation scanning
- Sensitivity is comparable to other techniques
- TGCE has several advantages:
  - Analysis of fragments with different melt properties simultaneously
  - Analysis of multiplex data
  - No need for fluorescent primers
  - Analysis software with mutation calling

# Acknowledgements

- Helen White
- Judith Pagan
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- Annabel Whibley