Temperature Gradient Capillary Electrophoresis Using the Reveal Genetic Mutation Discovery System (Spectrumedix)

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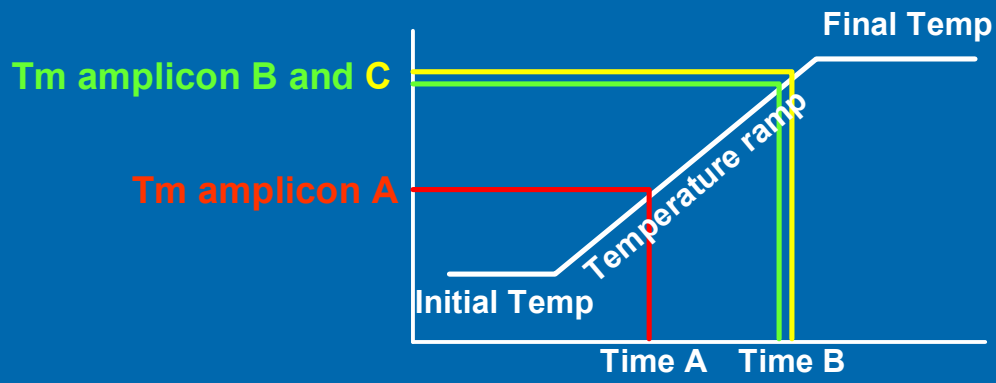
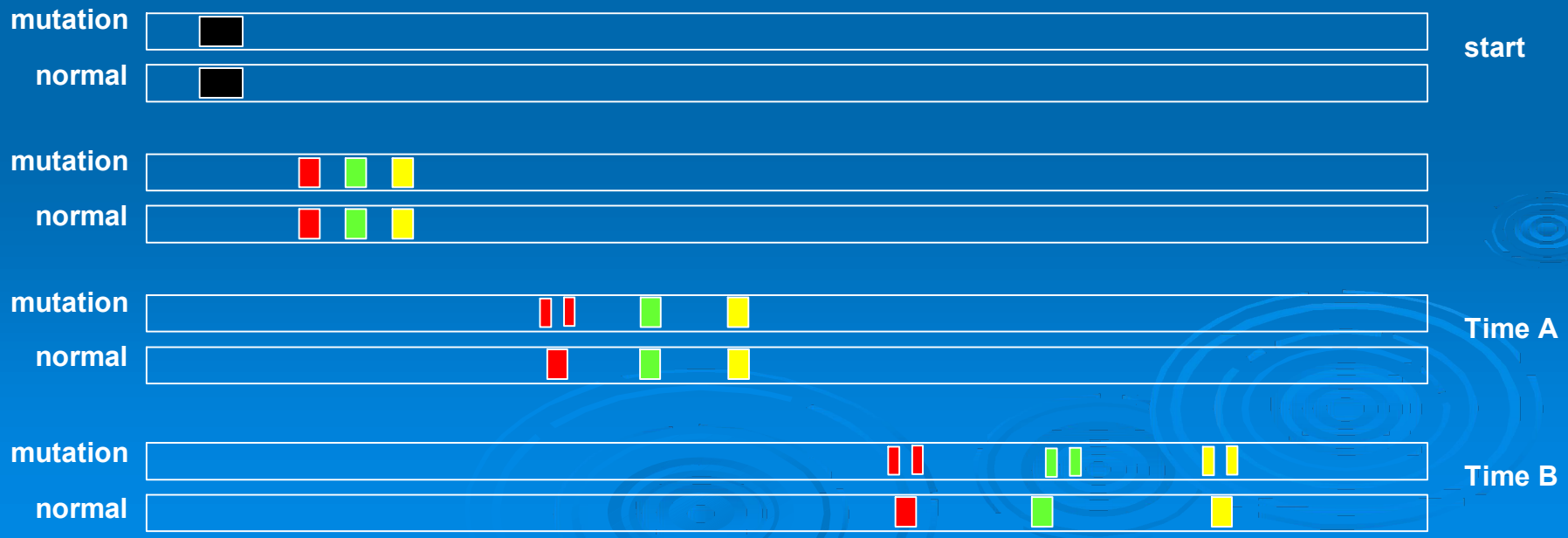
- Overview of Spectrumedix System
- Summary of our experience using it for mutation scanning at Guy's

Reveal Genetic Mutation Discovery System (Spectrumedix)

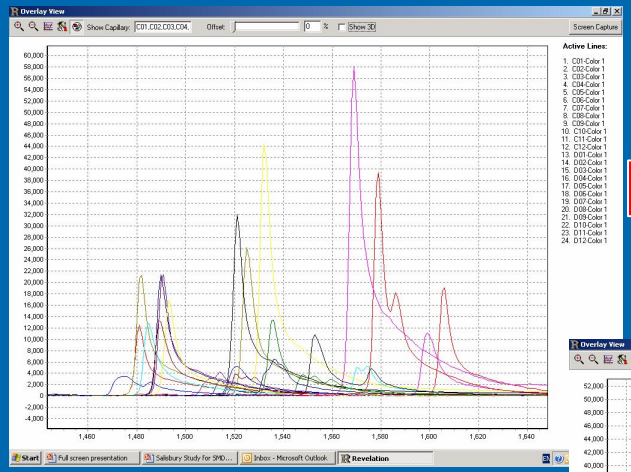
- Temperature gradient capillary electrophoresis (TGCE)
  - Fragments with different melting properties can be analysed together
  - Multiple DNA fragments can be analysed together
- Uses ethidium bromide to detect DNA
  - No need for fluorescent primers
- 96 Capillaries
- Specific software to compare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

Reveal Genetic Mutation Discovery System

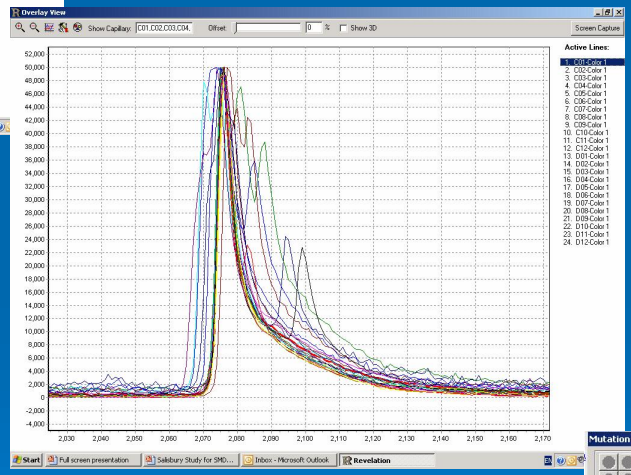




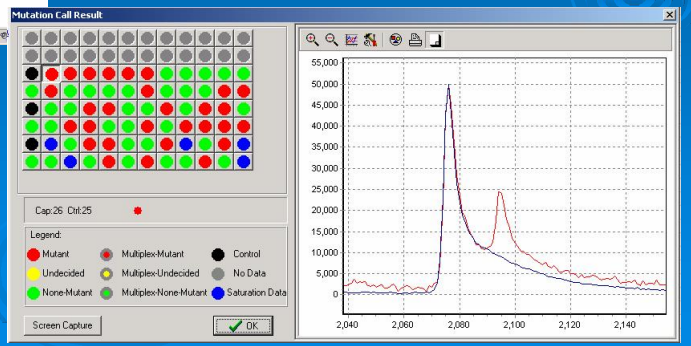
Revelation Mutation Detection Software



Raw data

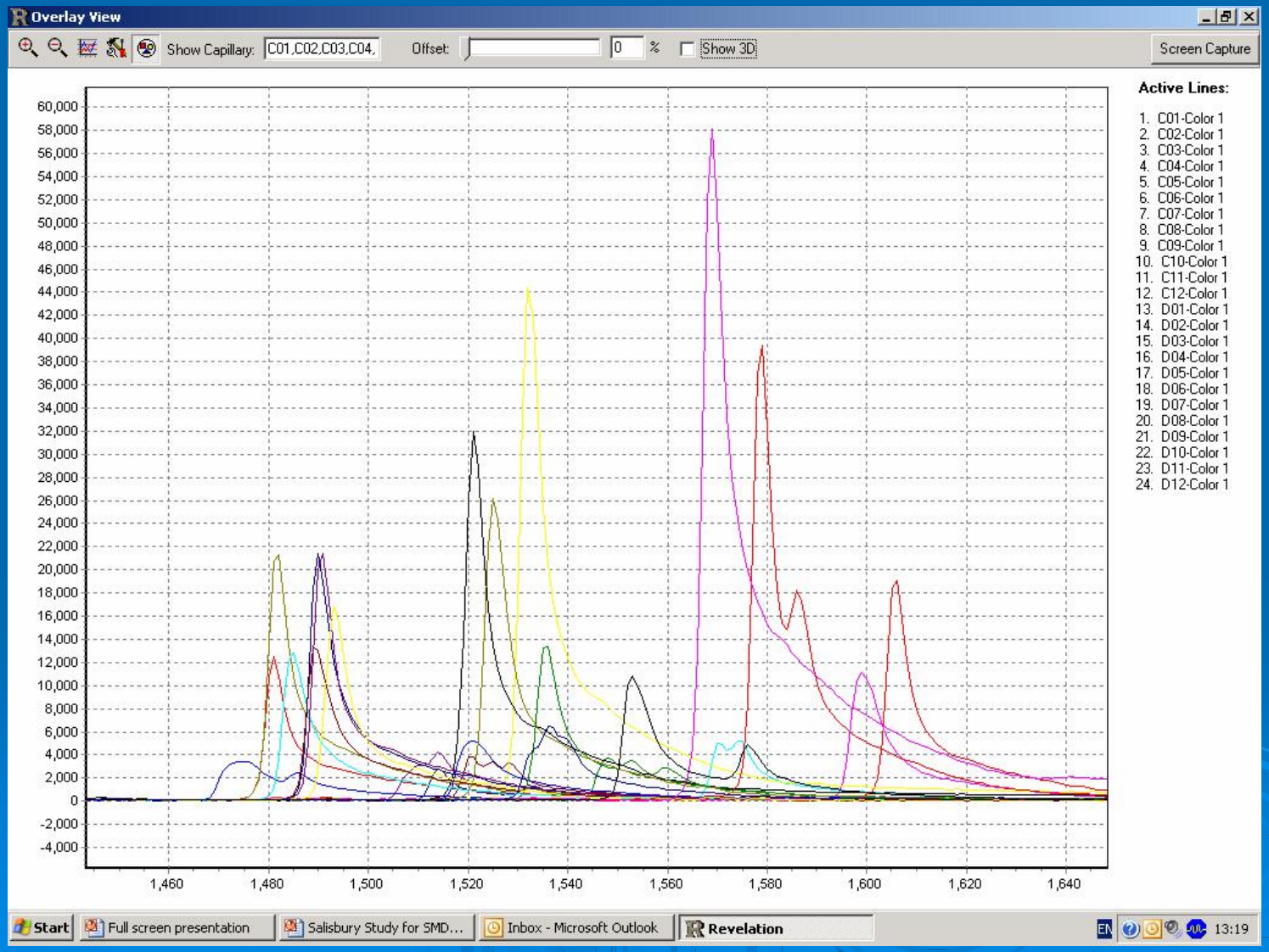


Normalised data

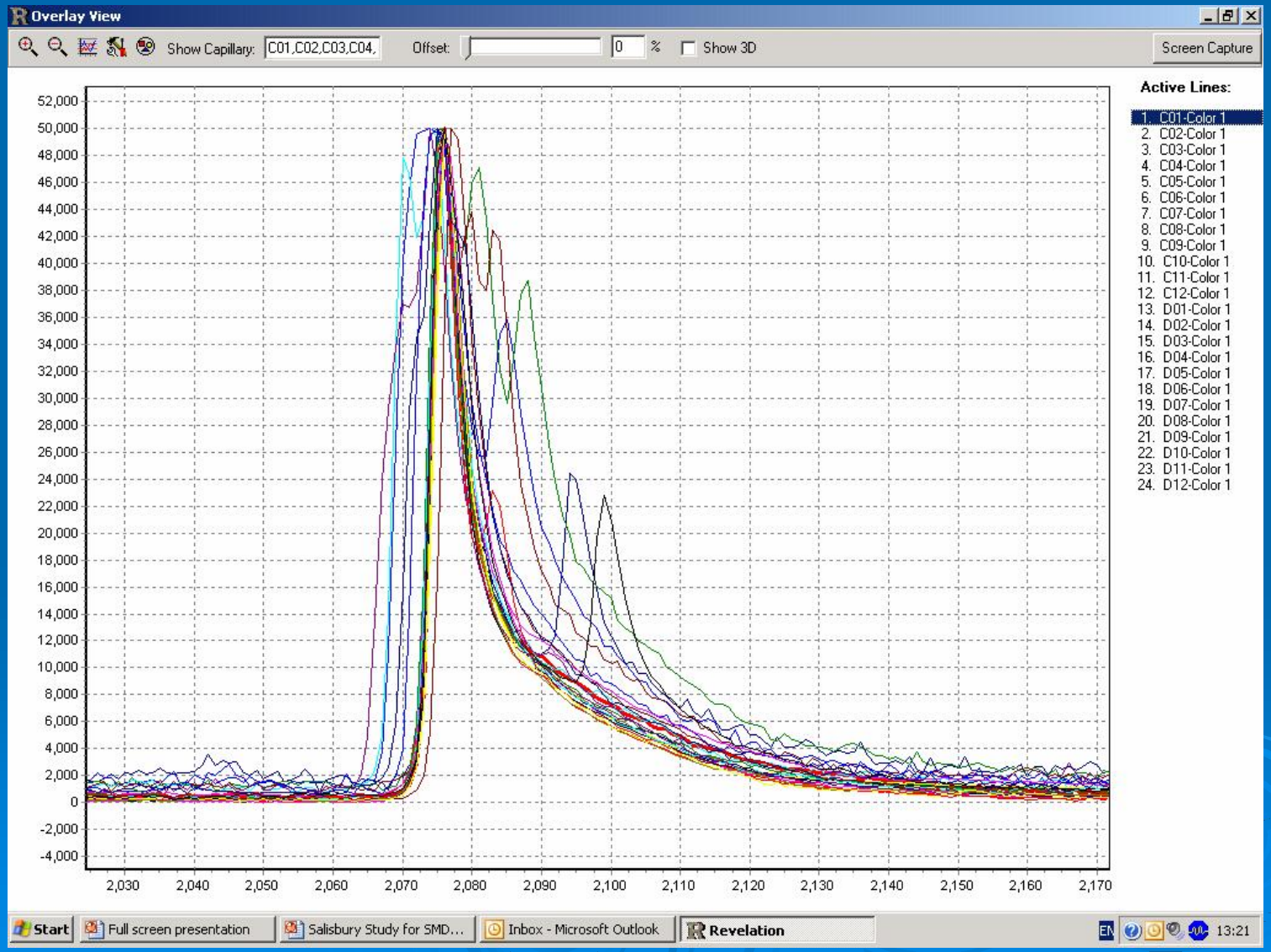


Mutation Report

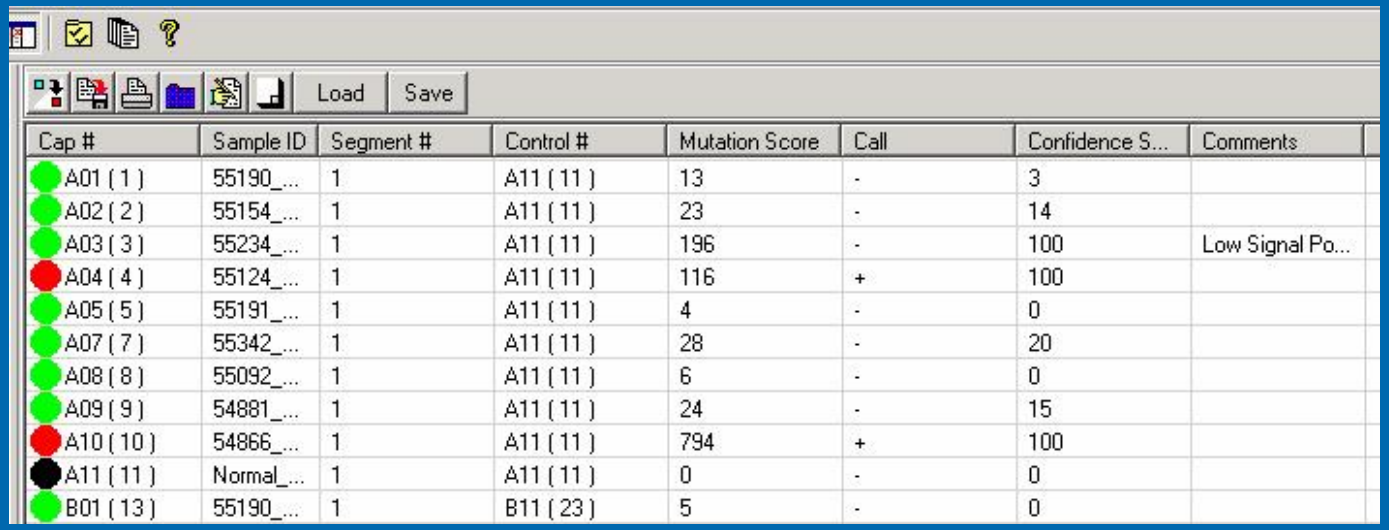
Revelation Mutation Detection Software



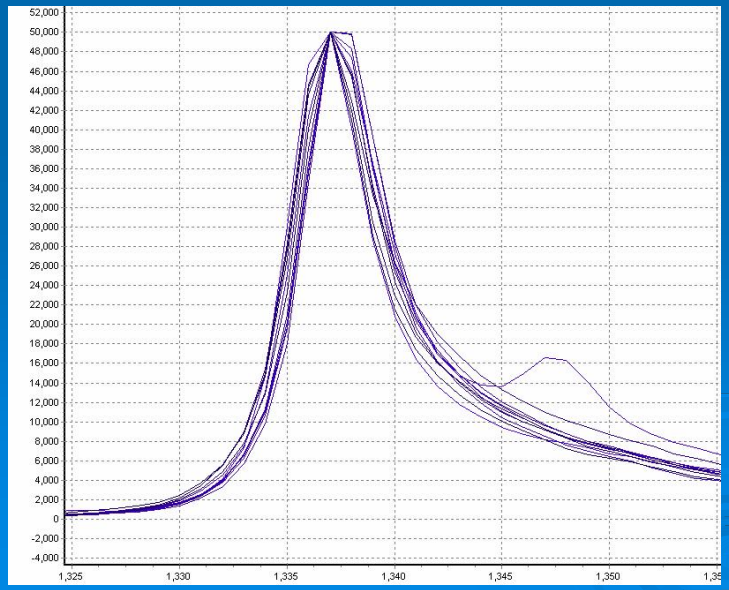
Revelation Mutation Detection Software

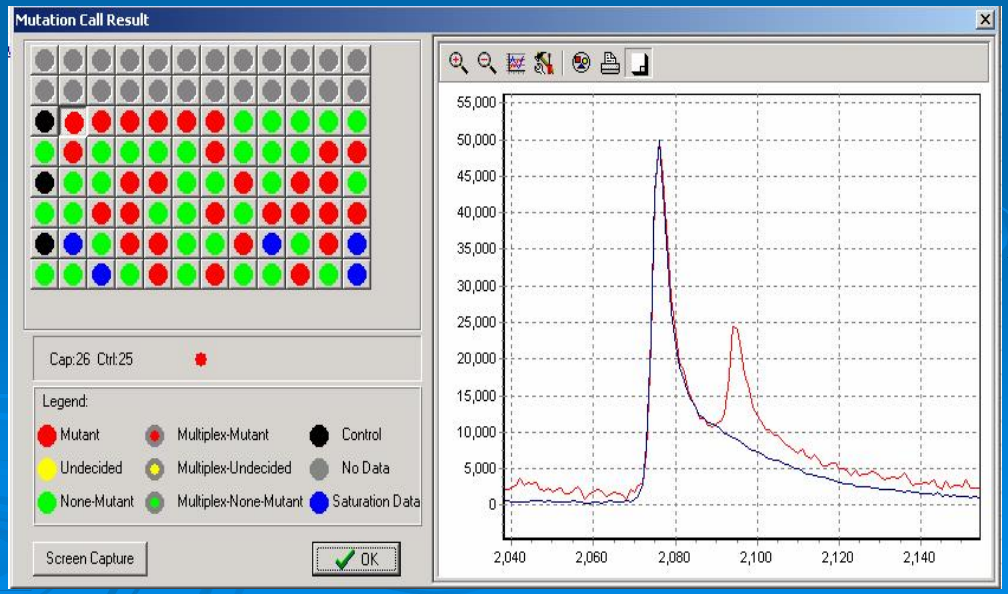


Revelation Mutation Detection Software

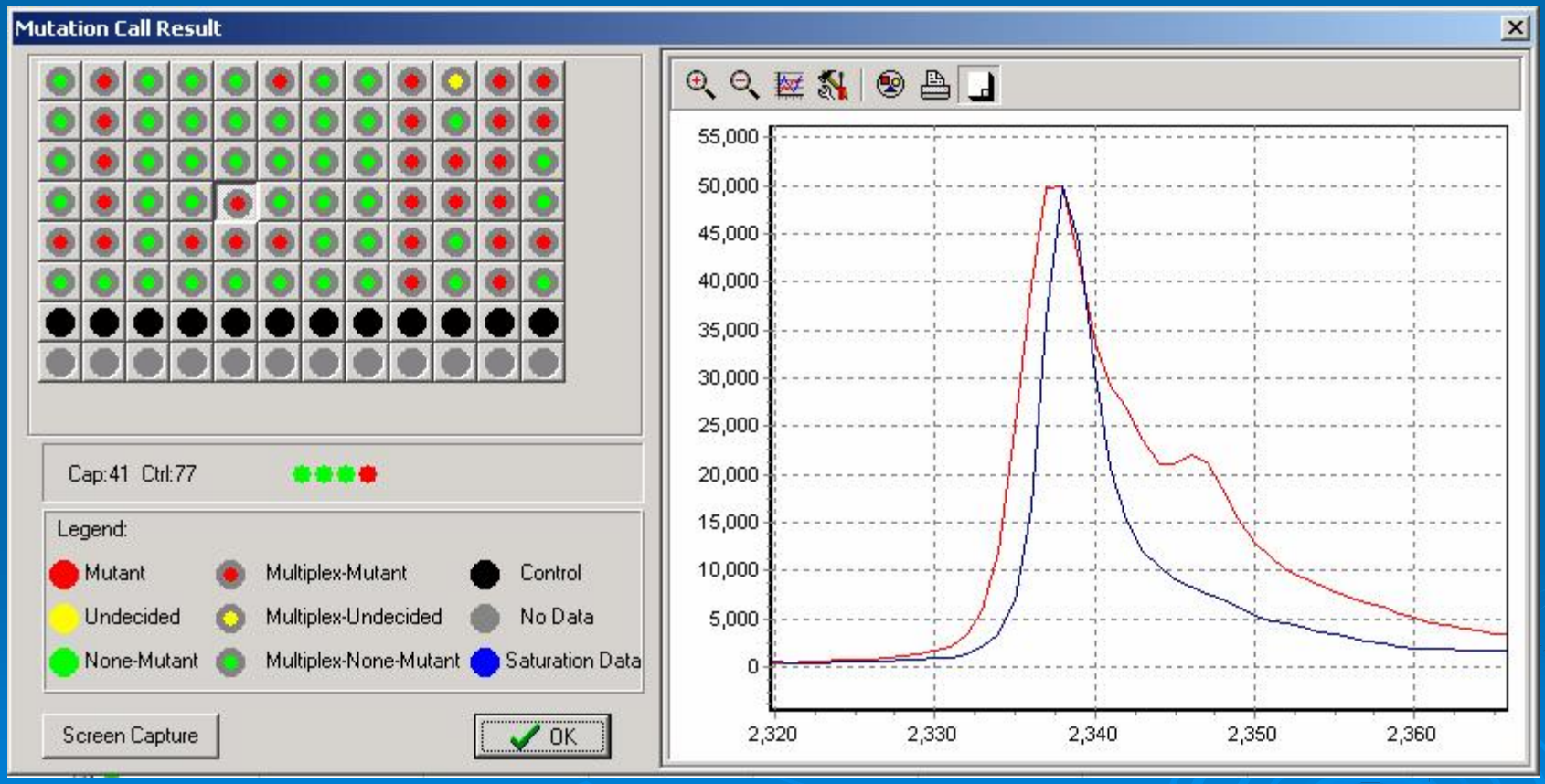


Cap #	Sample ID	Segment #	Control #	Mutation Score	Call	Confidence S...	Comments
A01 (1)	55190_...	1	A11 (11)	13	-	3	
A02 (2)	55154_...	1	A11 (11)	23	-	14	
A03 (3)	55234_...	1	A11 (11)	196	-	100	Low Signal Po...
A04 (4)	55124_...	1	A11 (11)	116	+	100	
A05 (5)	55191_...	1	A11 (11)	4	-	0	
A07 (7)	55342_...	1	A11 (11)	28	-	20	
A08 (8)	55092_...	1	A11 (11)	6	-	0	
A09 (9)	54881_...	1	A11 (11)	24	-	15	
A10 (10)	54866_...	1	A11 (11)	794	+	100	
A11 (11)	Normal_...	1	A11 (11)	0	-	0	
B01 (13)	55190_...	1	B11 (23)	5	-	0	





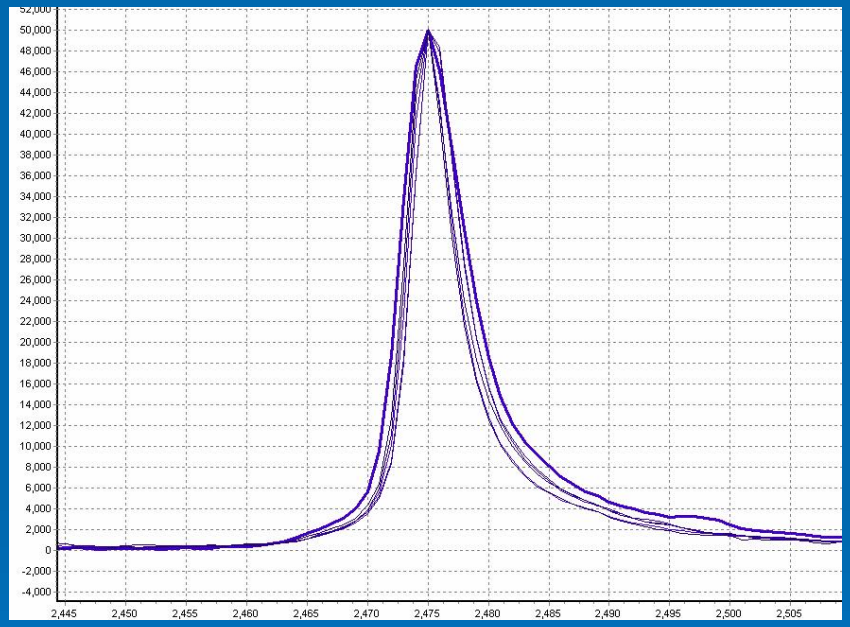
Revelation Mutation Detection Software



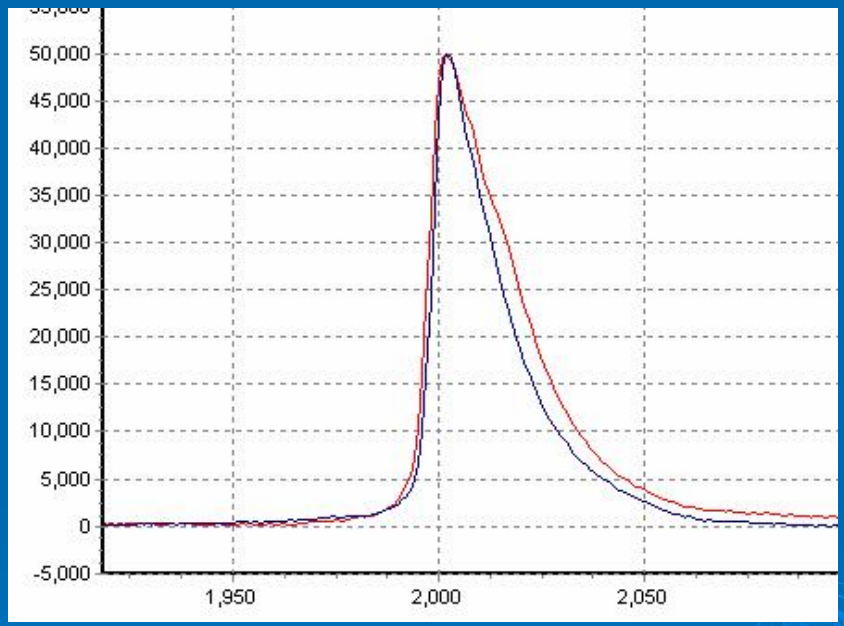
Sensitivity and Specificity

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

Some mutations give very subtle peak traces

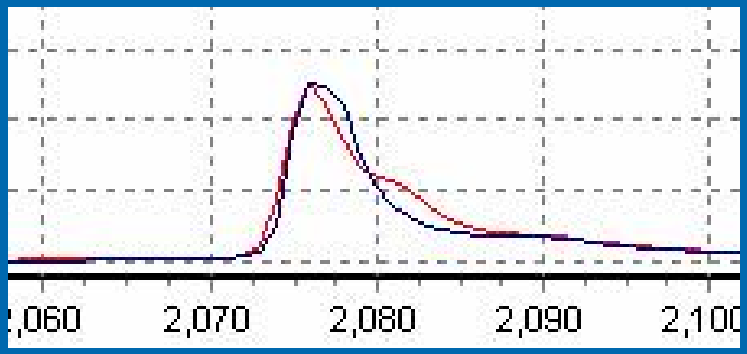


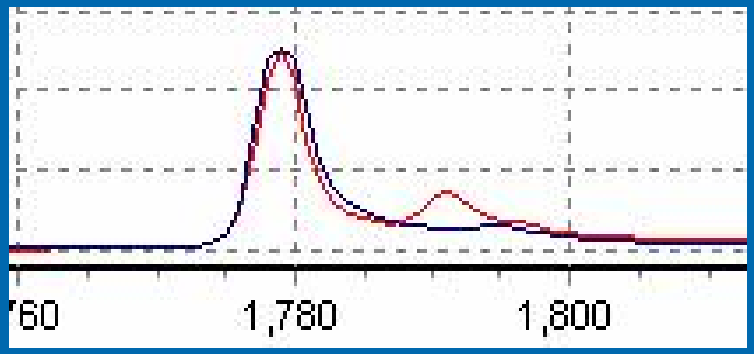
Dystrophin ex44 c.6438+2T>A

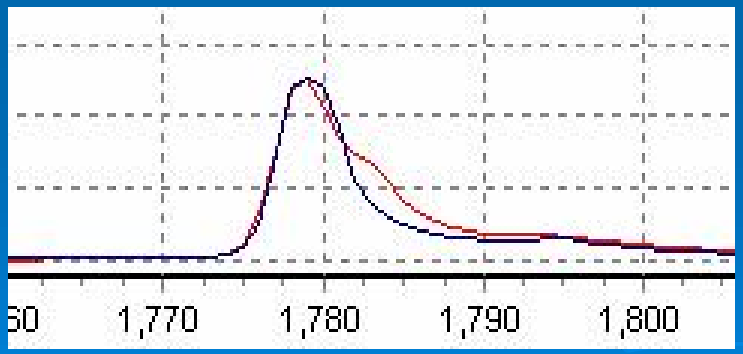


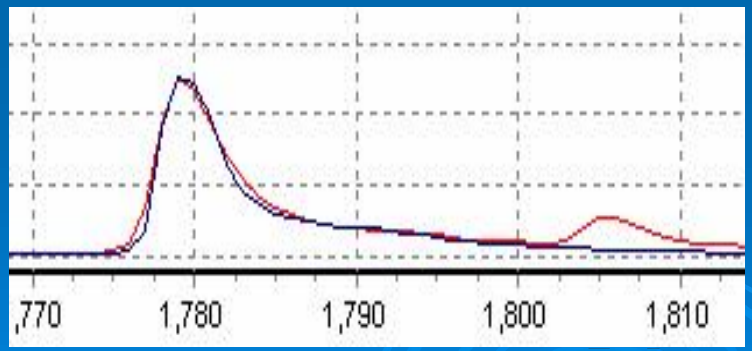
BRCA1 ex13 4327C>T

Revelation software doesn't detect all peak changes







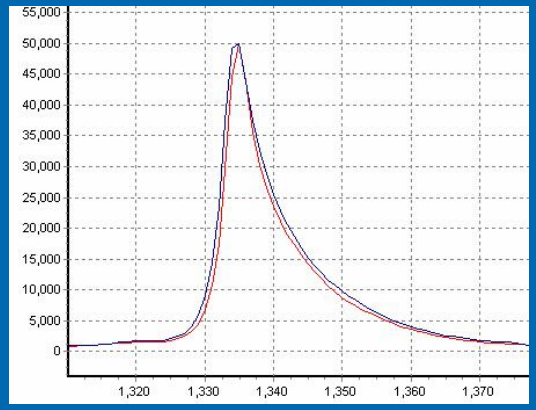


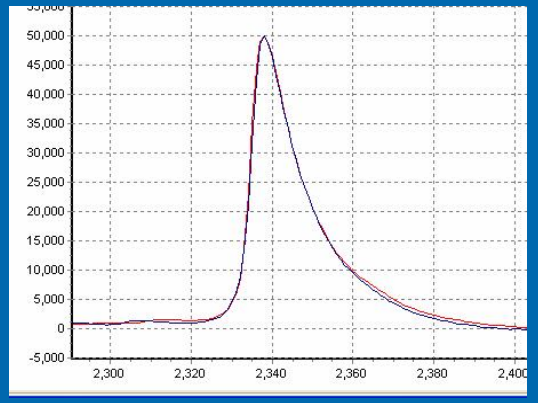
Some mutations are only detected at one temperature range

BRCA1 ex11I 3119G>A

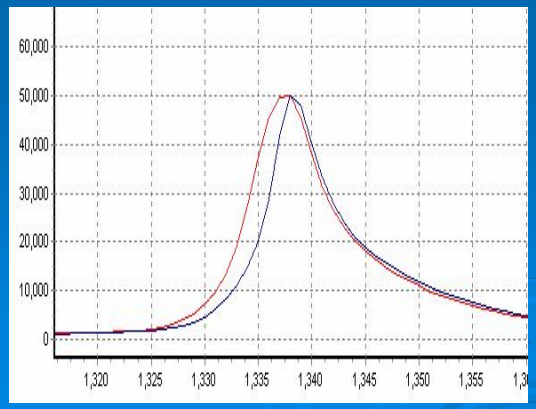
BRCA2 ex11PQ 4656T>C

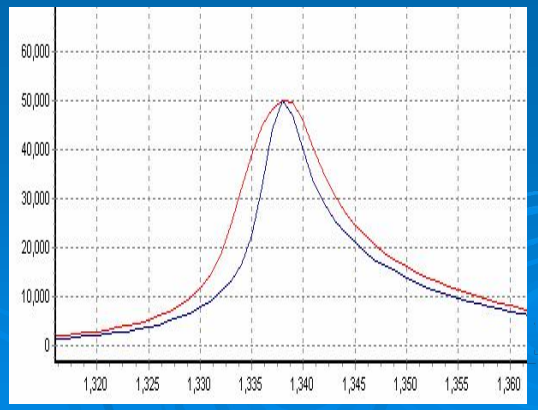
50-60 °C



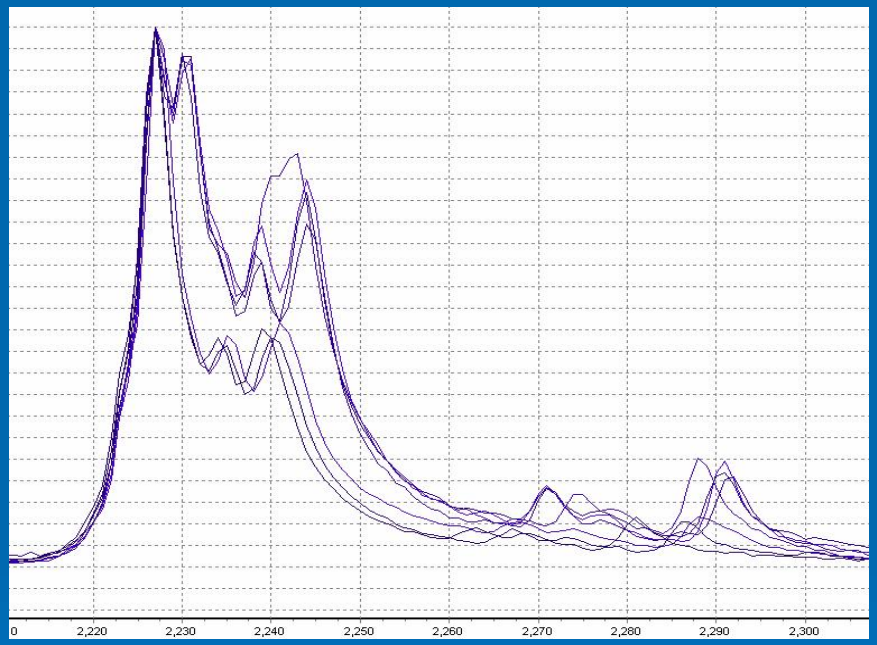


35-45 °C

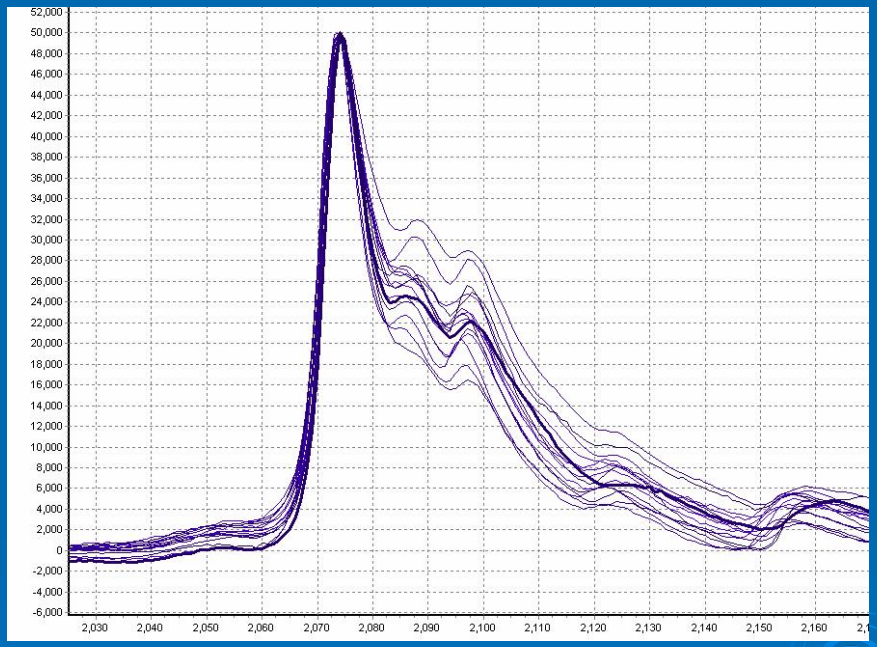




Some fragments are not suitable for TGCE



Dystrophin ex 26 Poly A tract



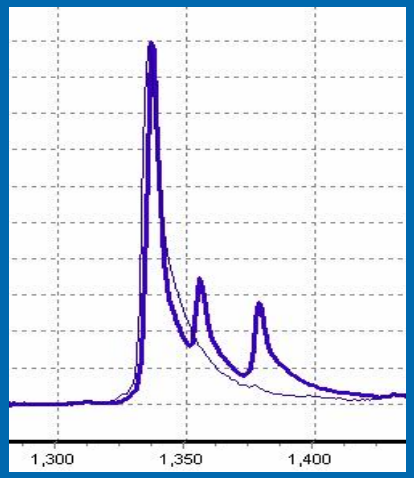
COL4A5 ex 26 Poly T tract
c.2041+1G>T

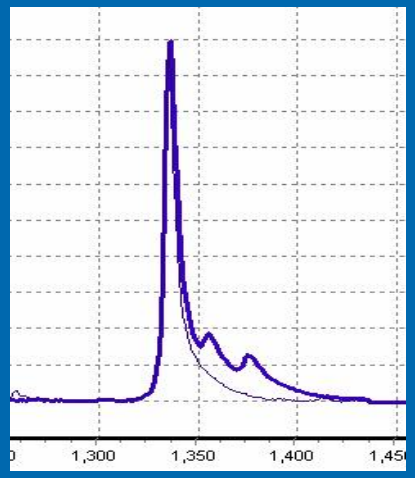
Using Tagged primers can alter peak traces

NO TAG

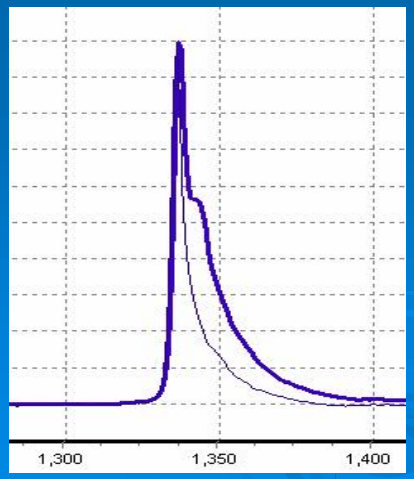
TAG

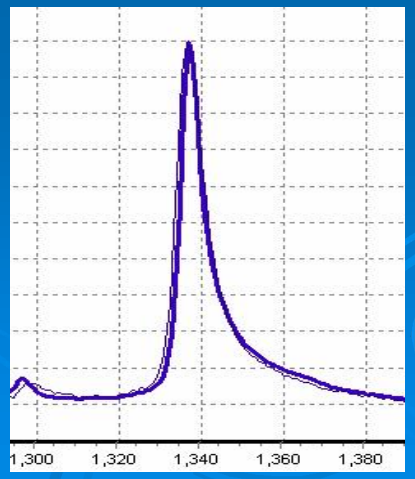
Dystrophin Ex59





Dystrophin Ex75





Other problems

- Peak strengths
- Reproducibility
- General Maintenance
- Technical support

Summary of mutation screening using the Spectrumedix at Guy's

- Congenital Muscular Dystrophy
  - POMT1, POMT2, POMGnT1, LARGE, Fukutin
  - LAMA2
- Hereditary Spastic Paraplegia
  - Spastin (SPG4)
- Hereditary Breast and Ovarian Cancer
  - BRCA1 and BRCA2
- Alports
  - COL4A5
- DMD/BMD
  - Dystrophin

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  - BRCA1 and BRCA2
- Alports
  - COL4A5
- DMD/BMD
  - Dystrophin

Summary

- Sensitivity is comparable to other techniques
- Specificity is low
- Software cannot be relied on for mutation calling
- Best for diseases with
  - Low level of polymorphism
  - A high mutation detection rate
  - Small numbers of samples

Acknowledgements

- Helen White
- Annabel Whibley
- Judith Pagan
- Rachael Mein
- Caroline Godfrey
- David Moore