# Conformation Sensitive Capillary Electrophoresis

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Confirmation Sensitive Capillary Electrophoresis
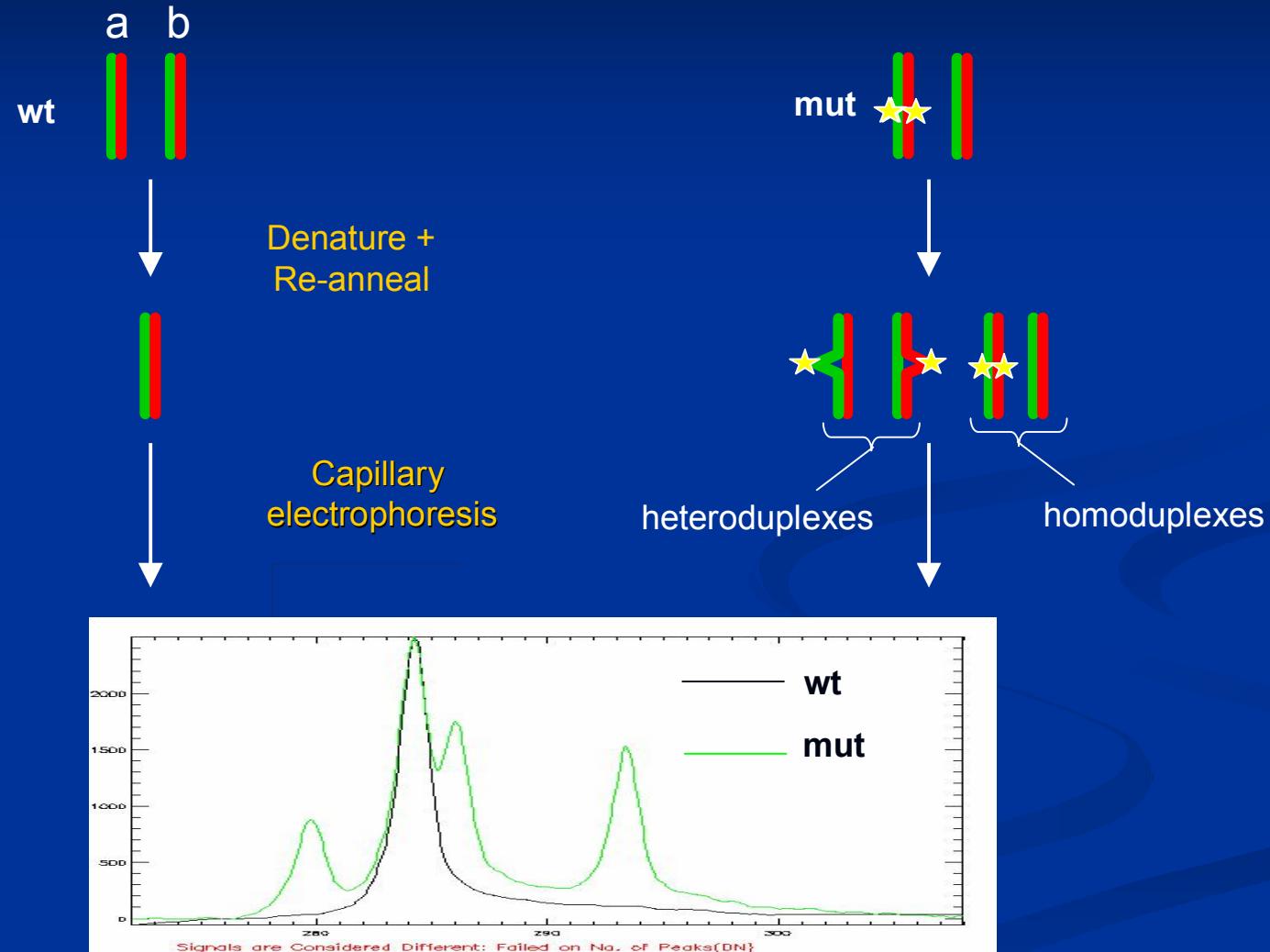
CSCE examples (BRCA1)

CSCE workflow

Sample → PCR → Pool products and dilute → Electrophoresis
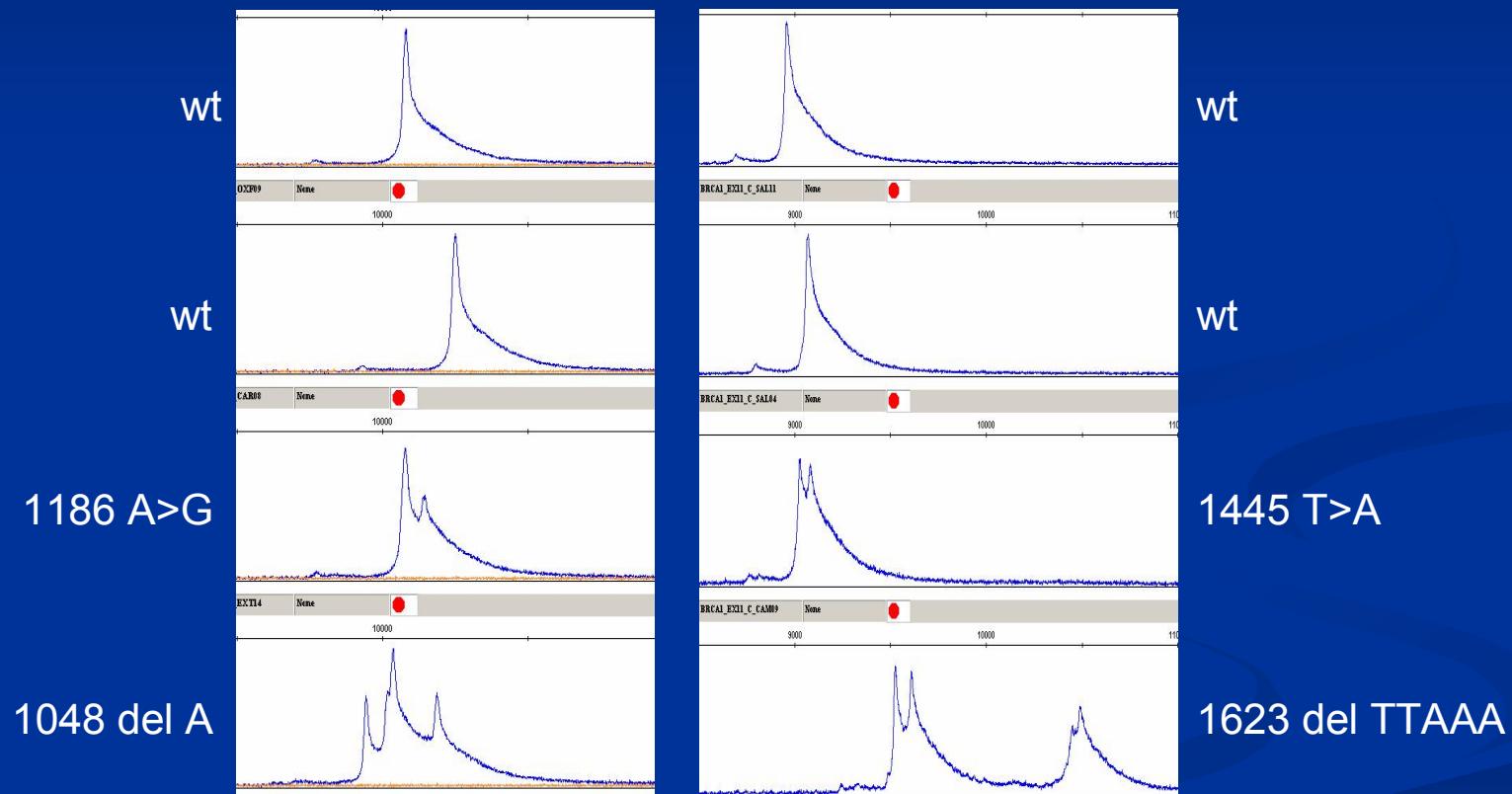
- Variable no of samples
- Variable no of PCRs (fragments) per sample
- 3 fluorescent labels (pooling x3)
- 96 well plate format

Batch example

- All PCRs to work at one annealing temperature (61°C)
- Any fragment can be labeled in any colour

Universally tagged PCR

```
M13F = tgt aaa acg acg gcc agt
M13R = cag gaa aca gct atg acc
```
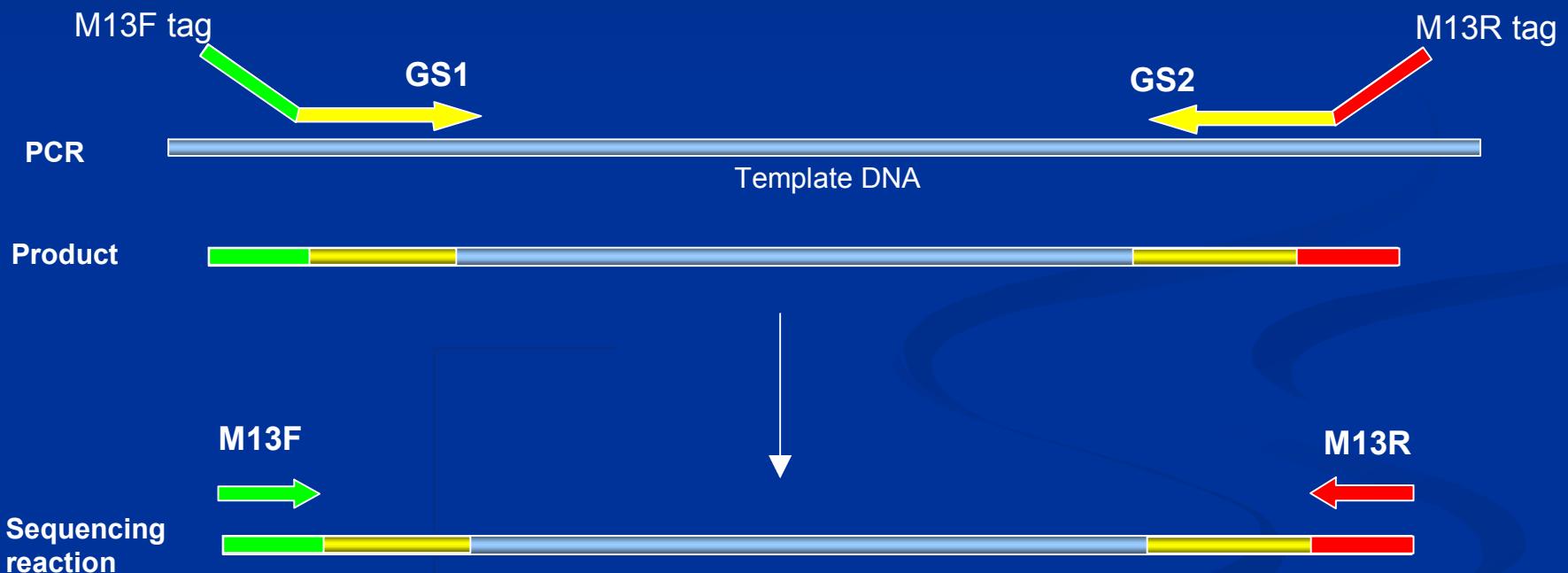
Assay Design: Standardised primer optimisation and design specification
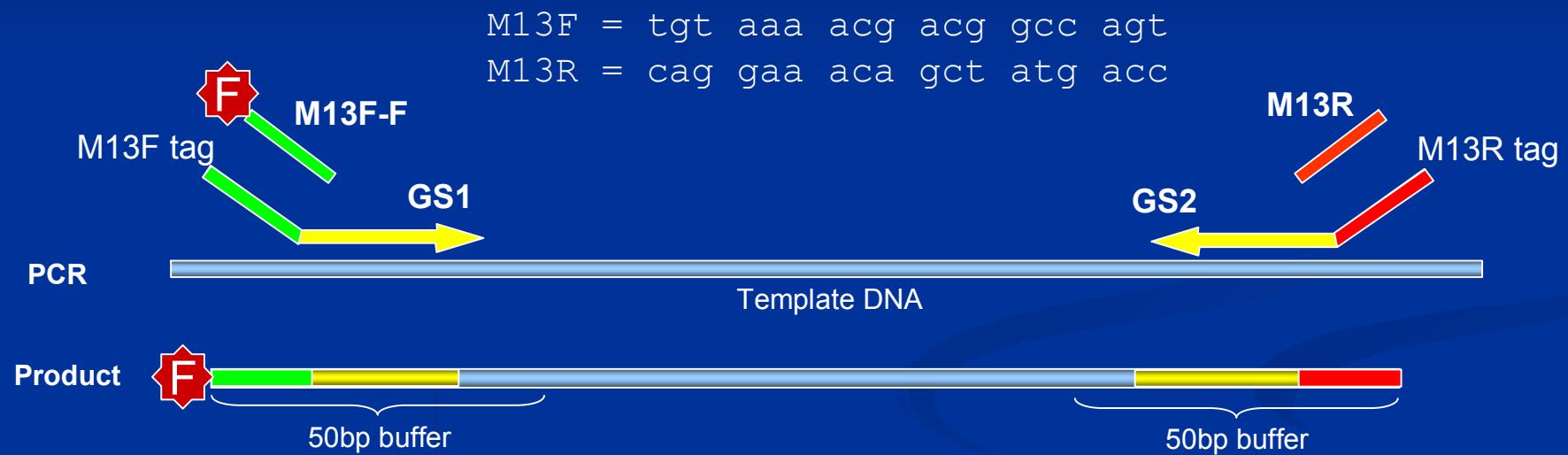
Assay Design: Standardised primer optimisation and design specification
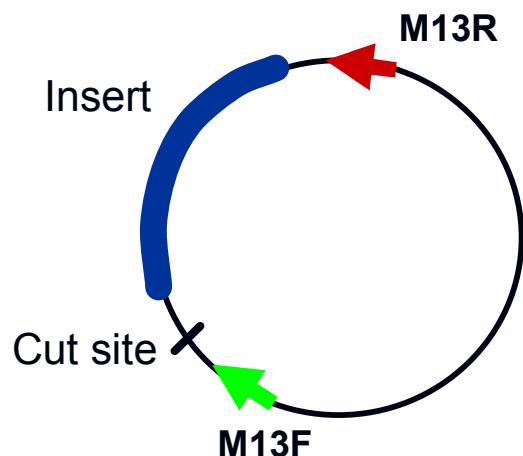
Approximate quantities in PCR

Plasmid 50,000 copies
Primers 24,000 copies each

Assay Design: Standardised primer optimisation and design specification
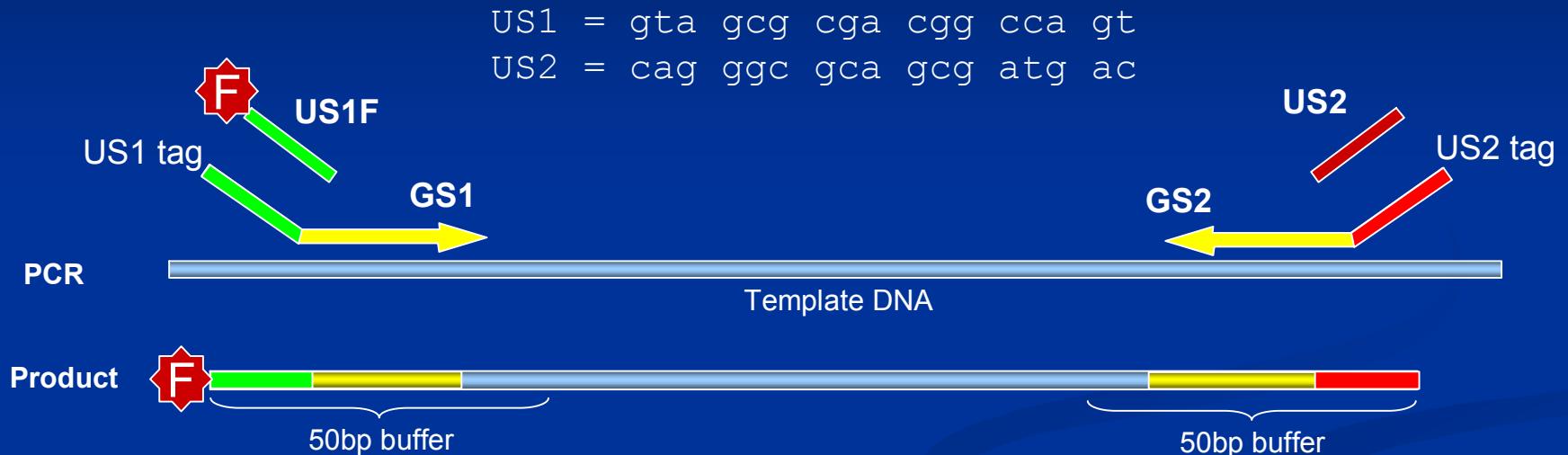
```
US1 = gta gcg cga cgg cca gt
US2 = cag ggc gca gcg atg ac
```


Step	°C	Time		Cycle
Taq activation	95	10	min	
Denature	95	0	sec	
GS annealing	61	30	sec	x40 cycles
Primer extension	72	30	sec	
Final extension	72	5	min	
Hold	15	∞		

Assay Design: Standardised primer optimisation and design specification
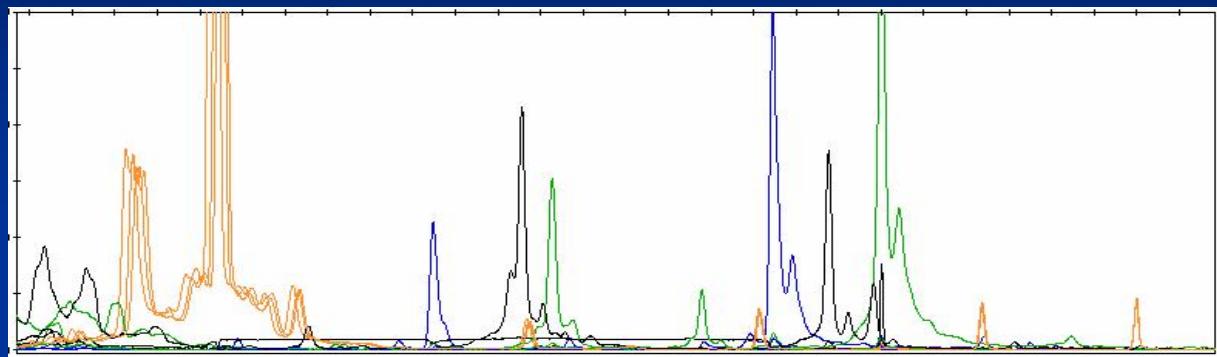
35bp   50bp   75bp   100bp
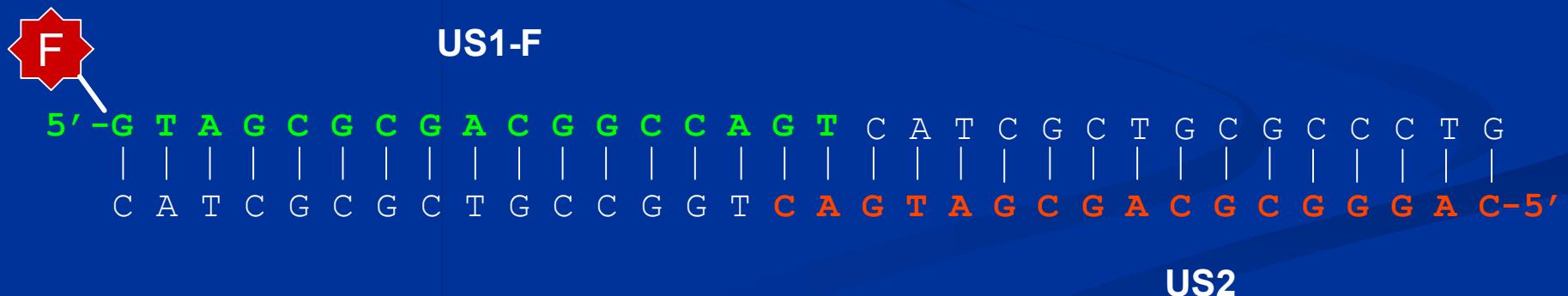
←——————————————————— 32bp ———————————————————→

US1-F

F

```
5'-G T A G C G C G A C G G C C A G T C A T C G C T G C G C C C T G
     | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
    C A T C G C G C T G C C G G T C A G T A G C G A C G C G G G A C-5'
```

US2

Assay Design: Standardised primer optimisation and design specification

US1 = gta gcg cga cgg cca gt
US2 = cag ggc gca gcg atg ac
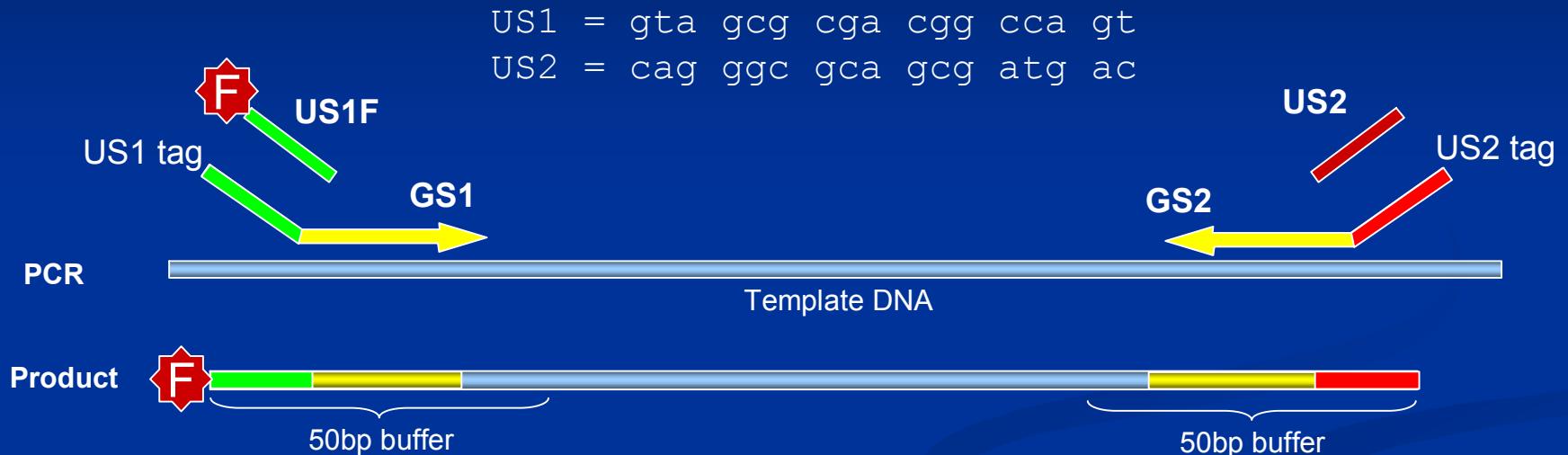
| Step	°C	Time		Cycle
Taq activation	95	10	min	
Denature	95	0	sec	
GS annealing	61	30	sec	x40 cycles
Primer extension	72	30	sec	
Final extension	72	5	min	
Hold	15	∞		

Primer optimisation

Aim: <u>clean trace with single peak within analysis window (1000 to 25000 RFU for 3730)</u>

- Primary optimisation [US1F]:[GS1]:[GS2]
  - Determine fixed [US1F]   (15 fmol/µl reaction)
  - Determine titration ranges for individual optimisations
    [GS1] 3, 9, 27, 81 fmol/µl reaction
    [GS2] 40, 80, 160, 320 fmol/µl reaction
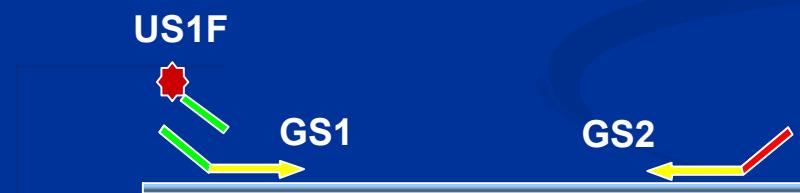
- Individual fragment optimisation [GS1]:[GS2]

BRCA2 Ex03

US1F

GS1

GS2

[GS2] fmol/µl rxn

	40	80	160	320

[GS1] fmol/µl rxn

3

9

27

81

BRCA2 Ex23

BRCA2 Ex11E

US1F
GS1
GS2

[GS2] fmol/µl rxn

| 40 | 80 | 160 | 320 |

[GS1] fmol/µl rxn

3

9

27

81
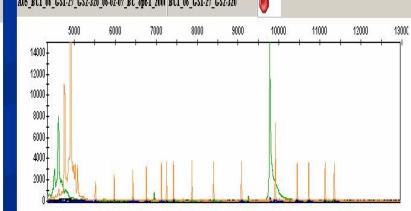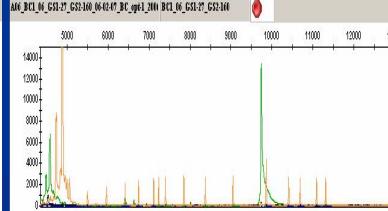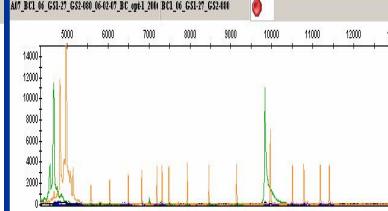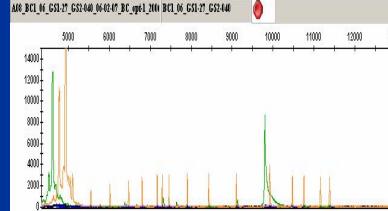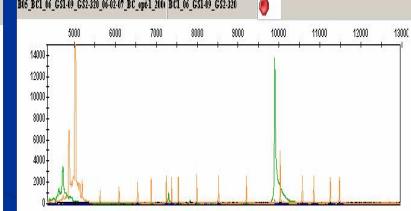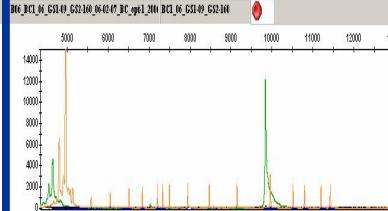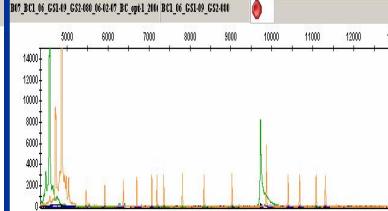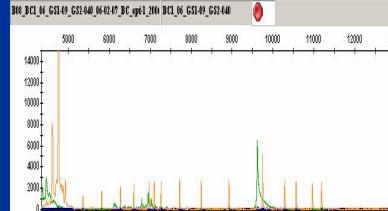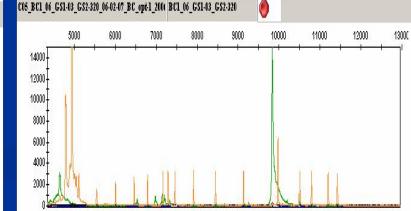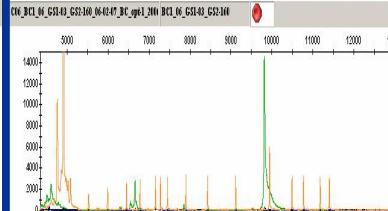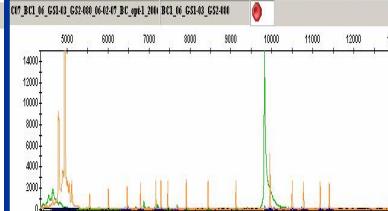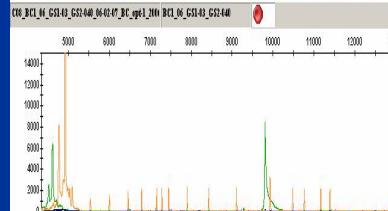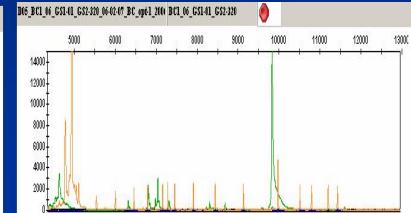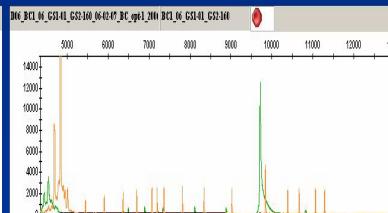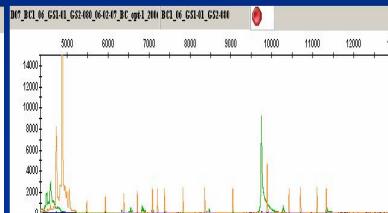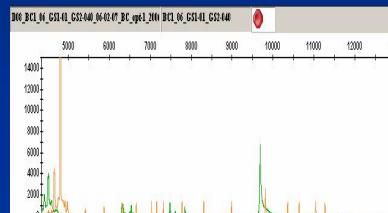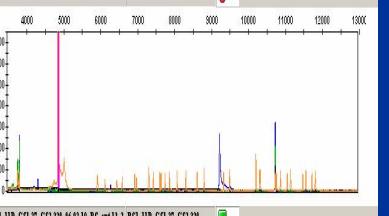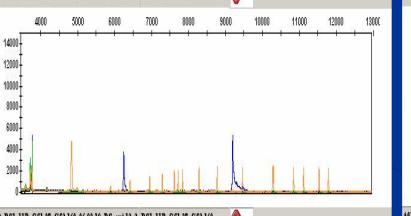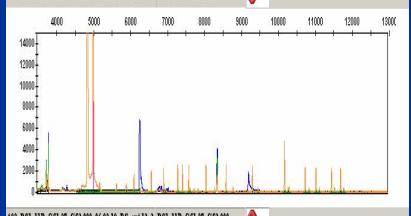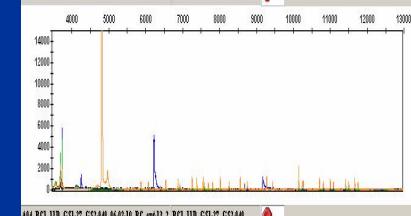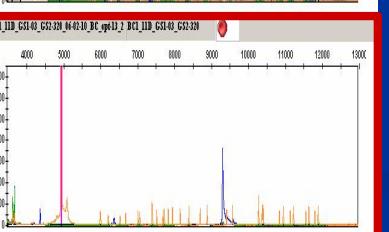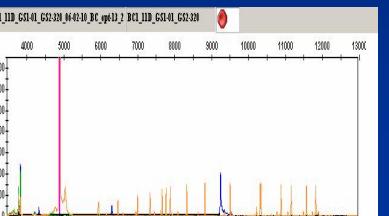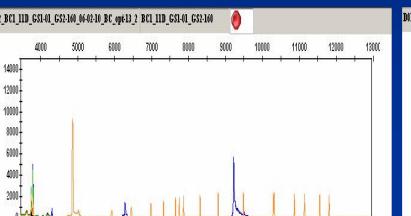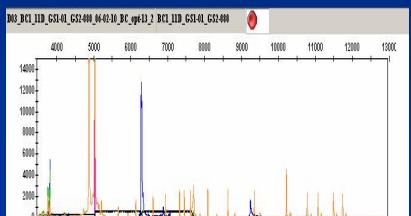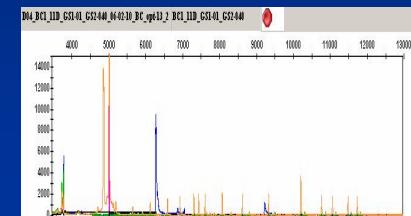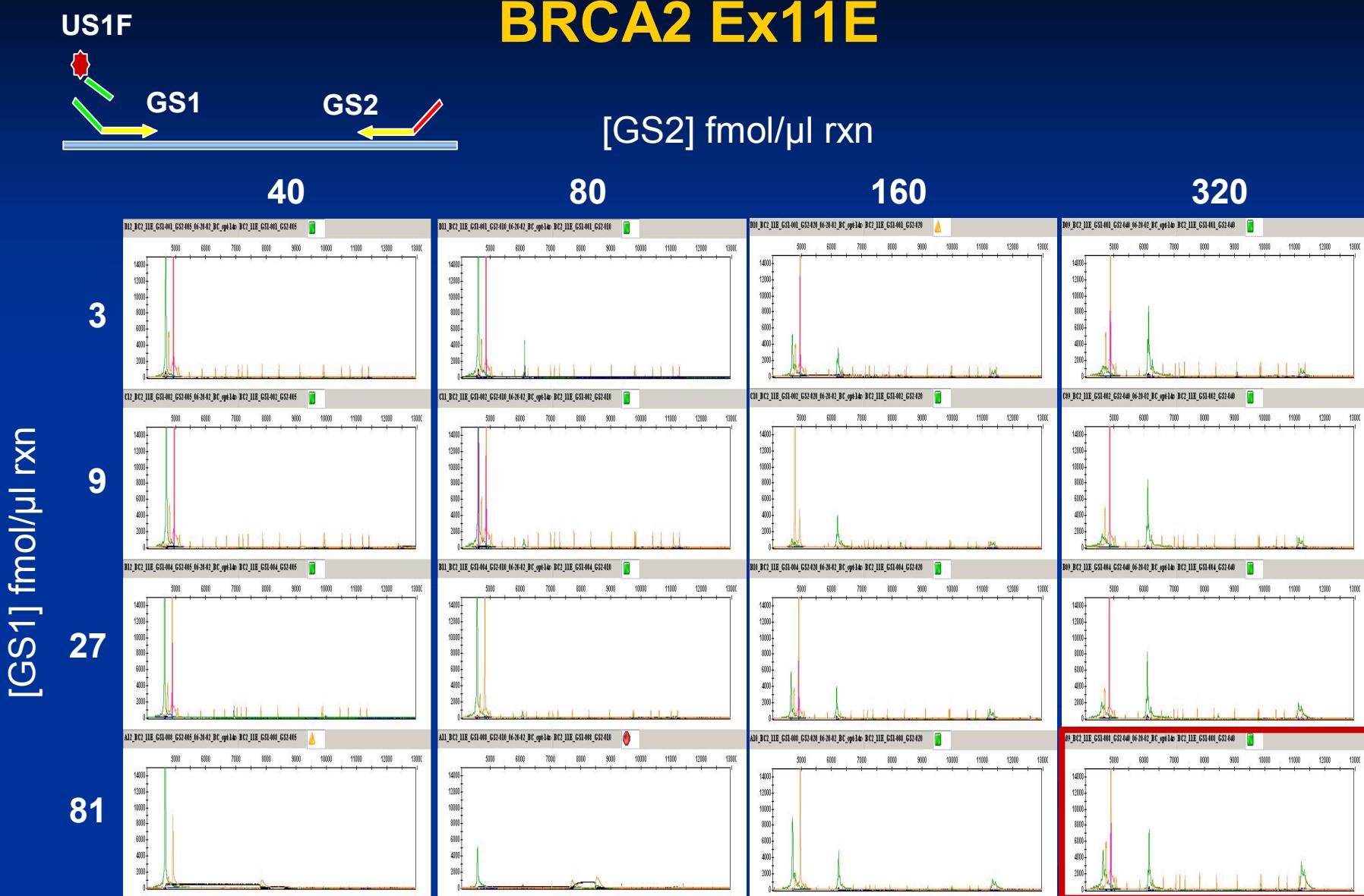
Optimisation

- Experience gained over 2yrs used to refine process
- Automated protocol

| Disease	Gene	Fragments	Designed	Optimised (%)	1° (%)	2° (%)	3° (%)
Breast cancer	BRCA1	33	33	100	64	29	7
Breast cancer	BRCA2	46	46	100	84	15	1
Marfans	FBN1	61	61	92	92	-	-

- FBN1 (61 fragments)
  - Design – 1 week
  - Wait for primers!
  - Optimisation - 2 days
  - 1° Re-design requirement – 5 fragments (8%)

Controls

- Per fragment
  - Mutation positive plasmid control
  - Mutation negative plasmid control
  - Polymorphism control
  - Water control

- Per run
  - 60.1 G>A reference control (x1)

- Per week
  - 60.1 G>A reference control (every capillary)

60.1 G>A
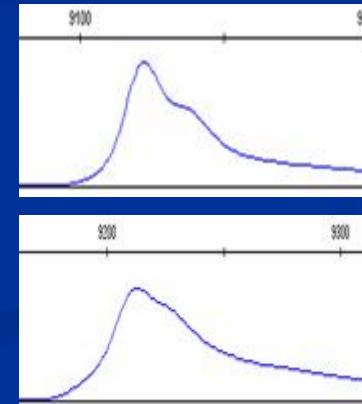
Post PCR processing

- Dilution ~1:50 in 0.05mM EDTA

- Mix products by colour (and/or size)
  - Currently 3-plex by colour (FAM, VIC, NED)
  - Potential up to 20 analyses per capillary (4x colour, 5x size)

- Add size standard (0.02µl LIZ 500 per load)

- Loading volume 10 µl
- Wax overlay

Mixed products
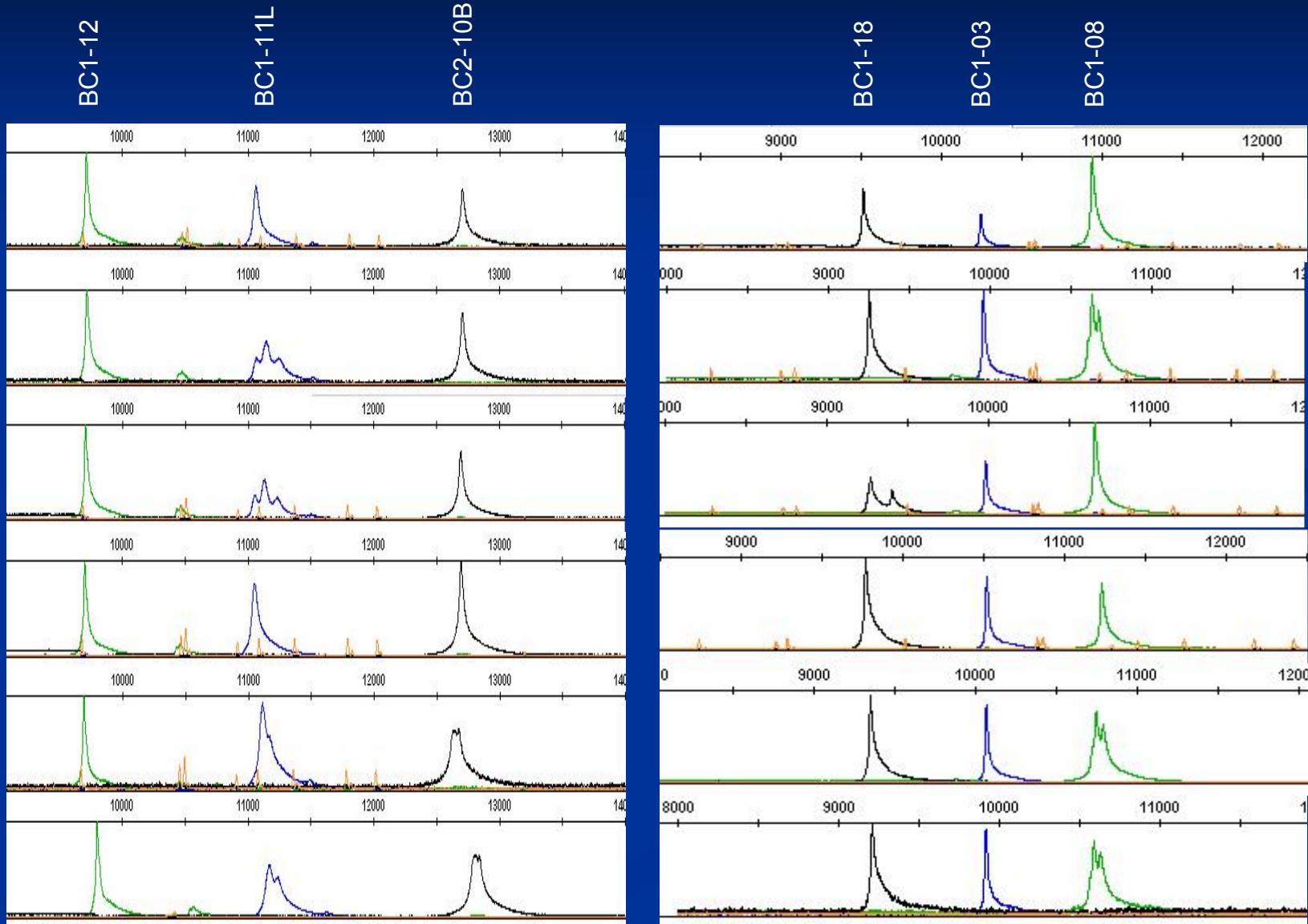
# Special cases - Compound heterozygosity
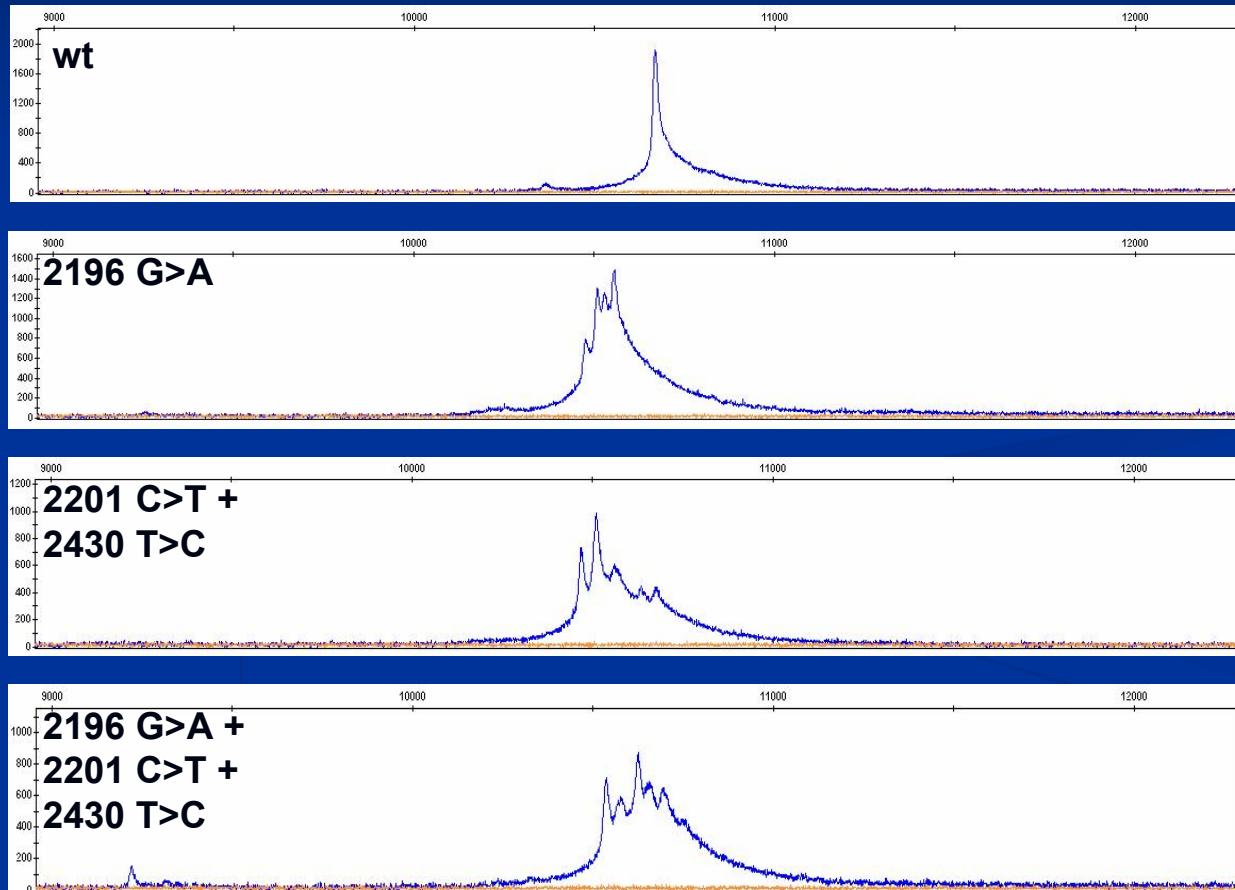
Special cases - Homozygotes

- May give mobility shift (esp. insdels)
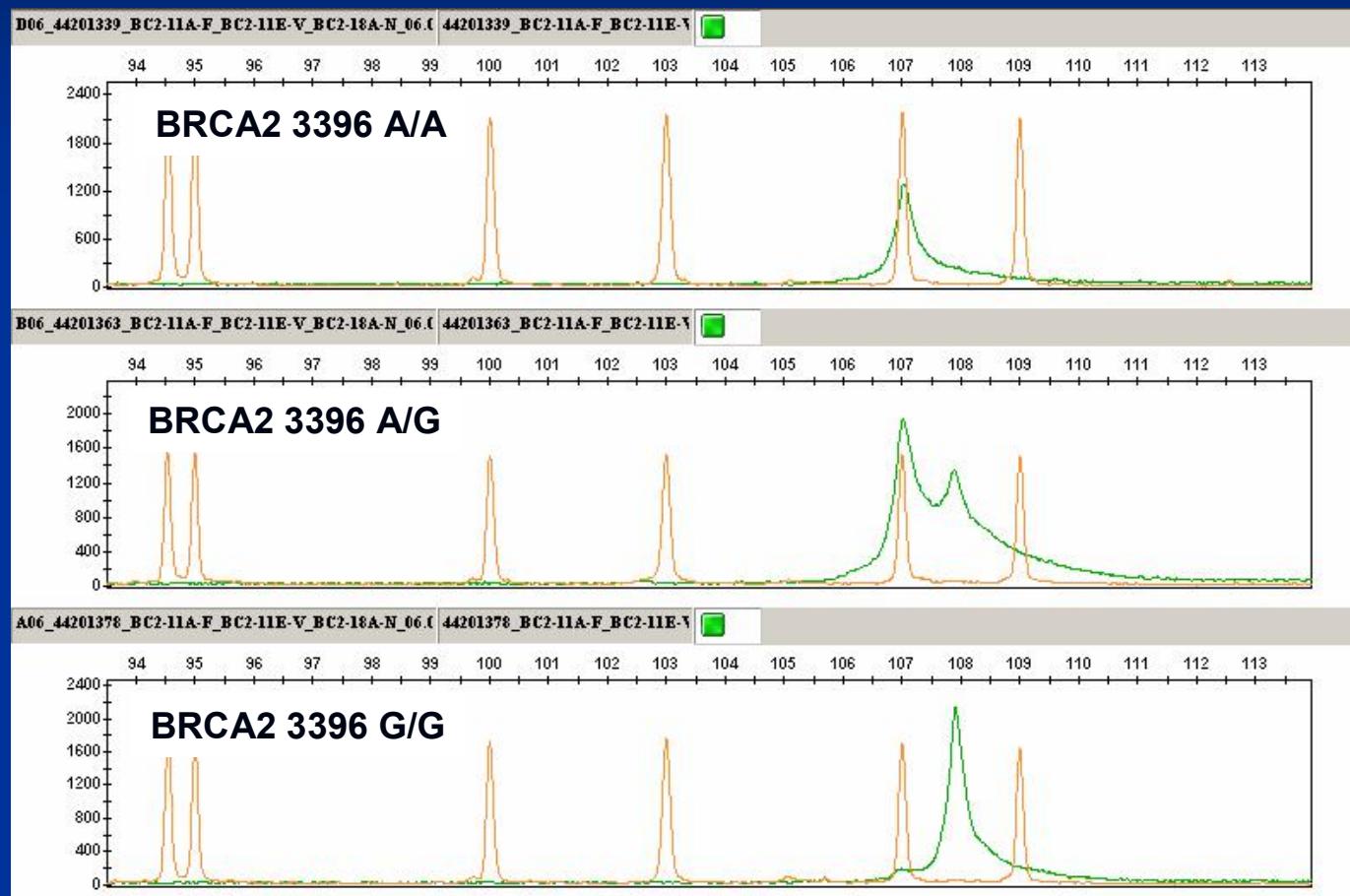
Sequential loading
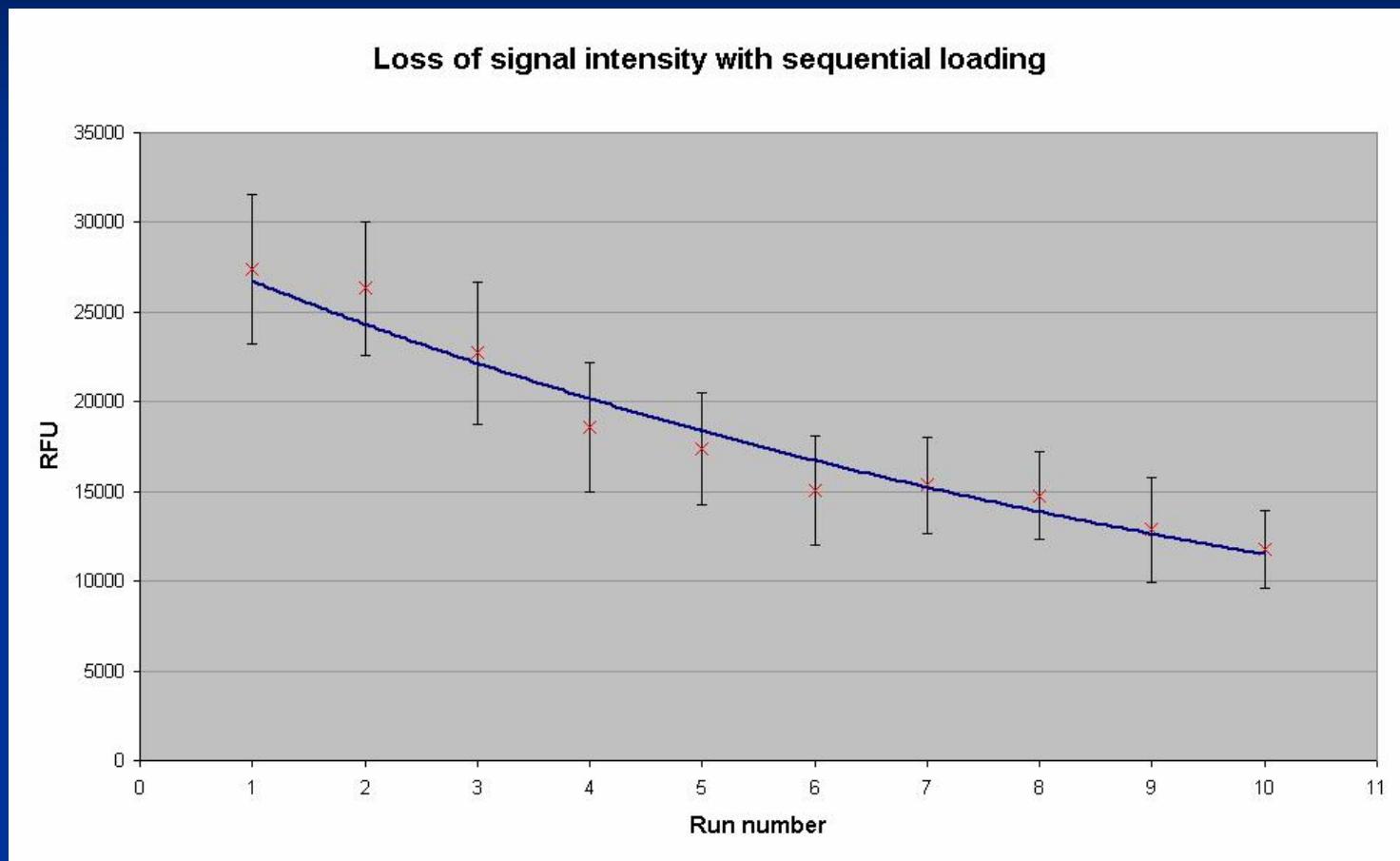
Dilution of products

Dilution in water
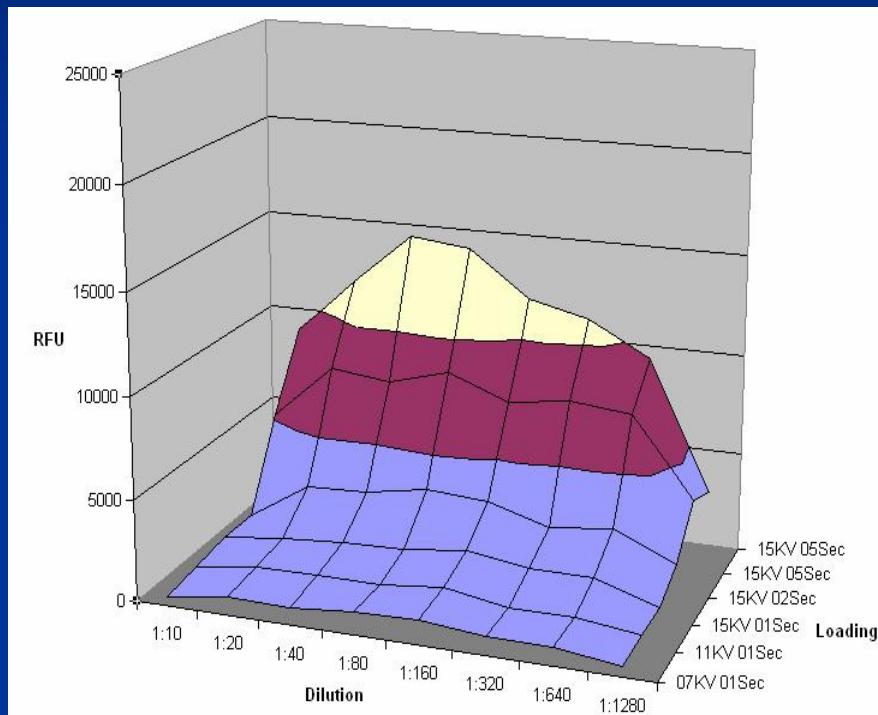
Dilution in 0.05 mM EDTA
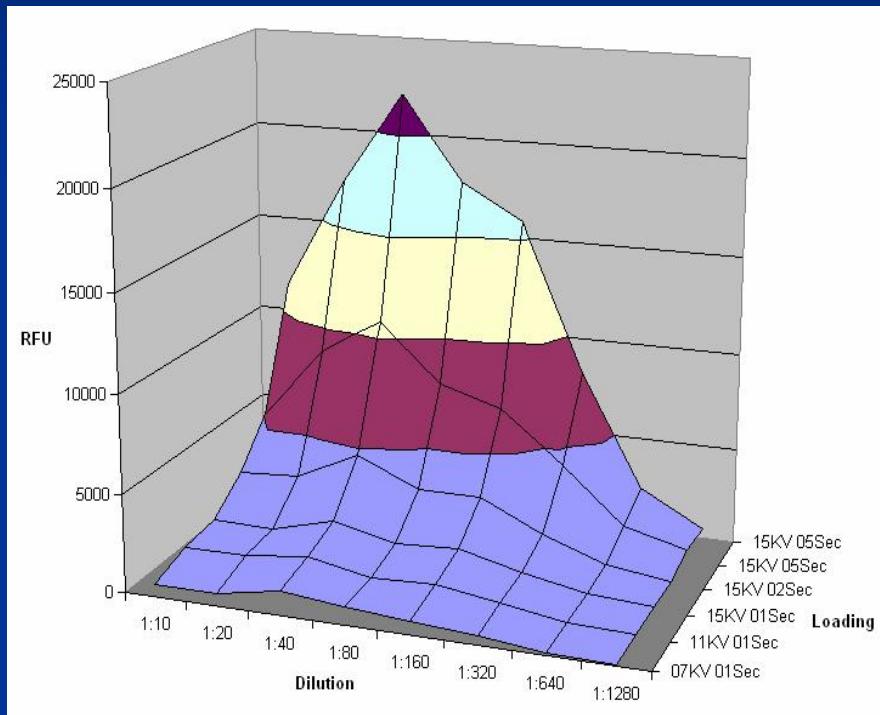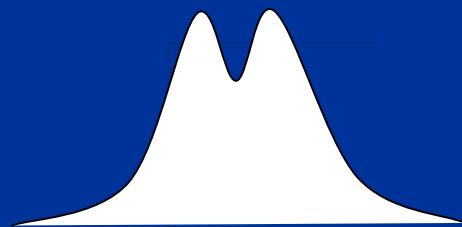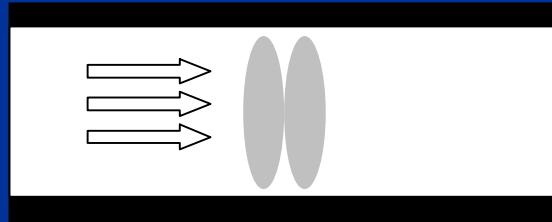
Loss of resolution
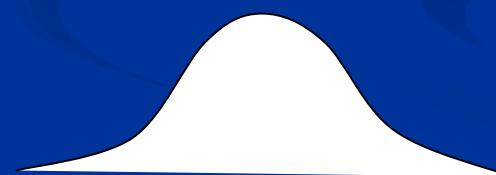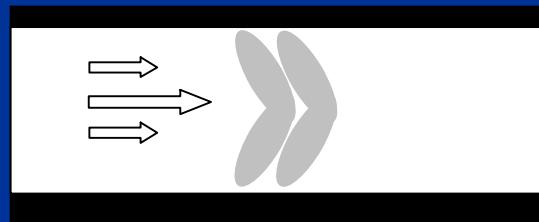
- Break down of the dynamic coating of the internal walls of the capillary
- Resolution can be recovered by leaving the instrument in an idle state (currently four hours)

Validation

- CSCE setup using Sanger protocol (Davies *et al* 2006) which gives general conditions for CSCE

- Generic Mutation Detection controls (GMD controls)
    - four different amplicons (20%, 40%, 60% and 80% GC rich)
    - At 3 different positions in each ampilcon four different mutations have been introduced
    - 48 mutant controls and 4 wild type (WT) controls

- GMD set passed through the system and scored manually

# Validation

- Scoring system for CSCE traces
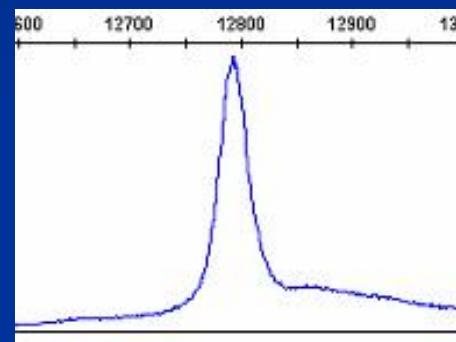
WT and 0
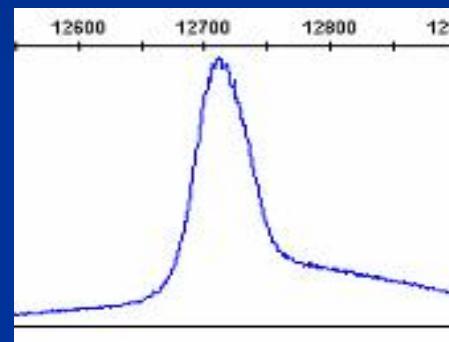
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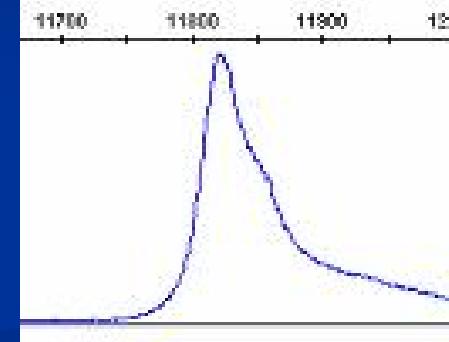
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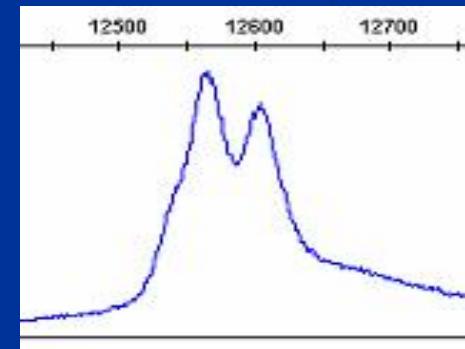
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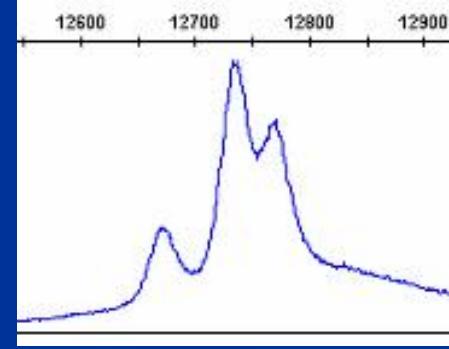
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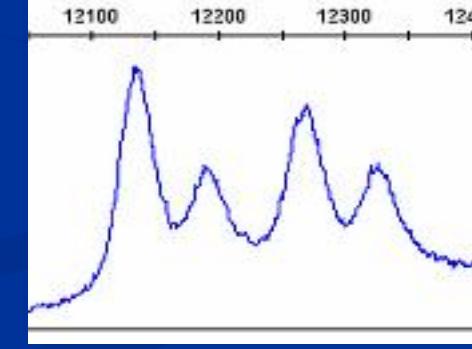
5

Validation

■ Testing the Sanger protocol using the GMD controls gives a sensitivity of 98% (47/48); the only mutation not detected was 80.1G>A

■ Two main factors affecting detection capability are the GC content of the fragment and the position of the mutation in the fragment
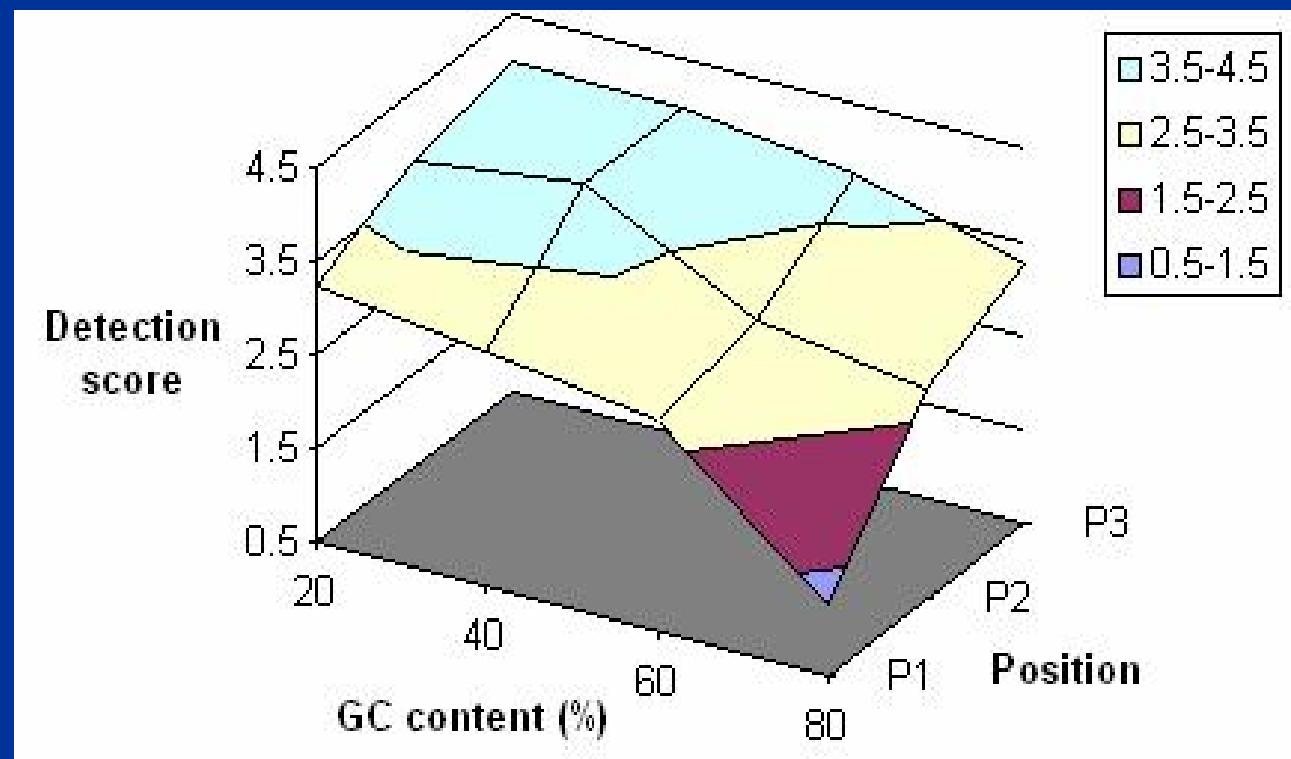
Conclusions

- We have developed an ampilfication system that allows:
  - Single PCR annealing temperature
  - Flexible fragment labelling
  - Reduced primer cost
  - Simple and informative optimisation

- BRCA1 & 2 screen set up and operational (79 fragments)
- Marfans screen (FBN1) in optimisation (~60 fragments)
- Next targets HNPCC and NOTCH1

Acknowledgements

CSCE
- Chris Mattocks - NGRL (Wessex)
- Helen Davies - Cancer Genome group, Sanger institute
- Nick Owen - NGRL (Wessex)

Generic and disease specific mutation controls
- Helen White - NGRL (Wessex)
- Vicky Hall - NGRL (Wessex)
- Gemma Potts - NGRL (Wessex)

HTSF
- Julie Sillibourne
- Tracey Merrifield
- Anne-Marie Coupe
- Alison Skinner
- Stacey Sandell