

Next generation sequencing for BRCA1 mutations

Natalie Chandler

Abid Sharif and Gareth Cross

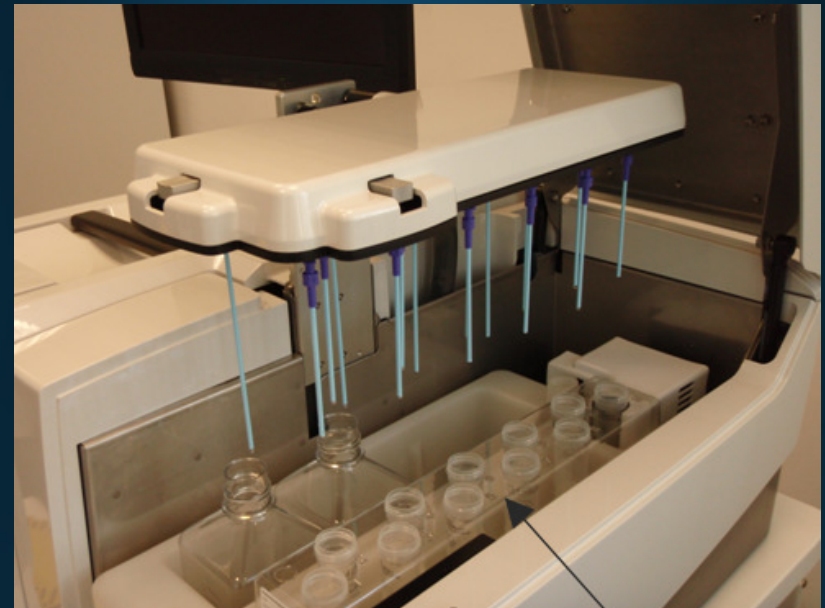
East Midlands Regional Genetics Service

Next generation sequencing- Roche GS-FLX 454

- Based on pyrosequencing



Camera



Reagents

Nottingham DeepSeq centre



Workflow

Library preparation



Emulsion PCR



Sequencing run



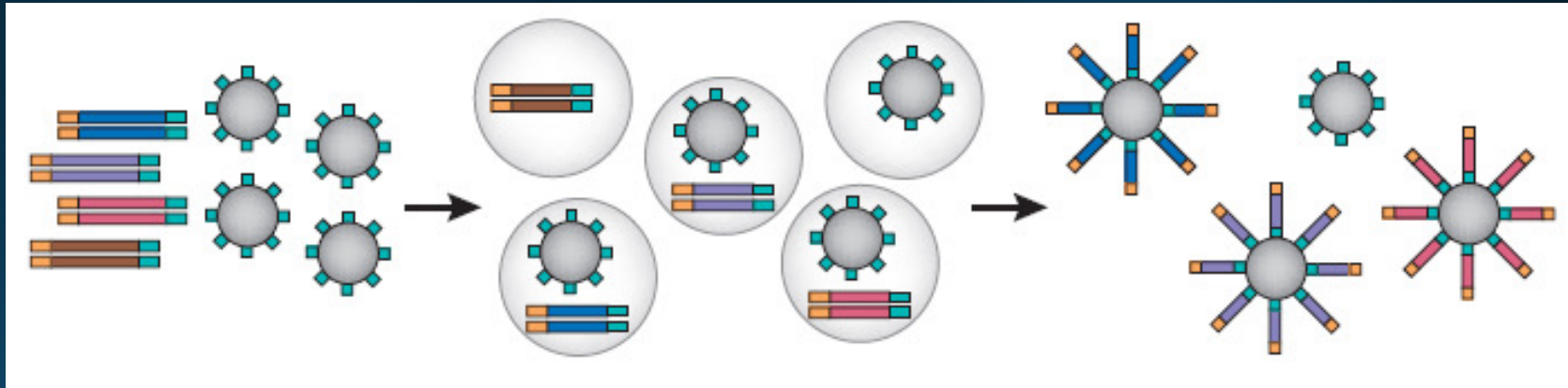
Sequence analysis



Library preparation

- PCR amplification
- Purification-Ampure
- Quantification- PicoGreen
- Creation of library
- Multiplex samples

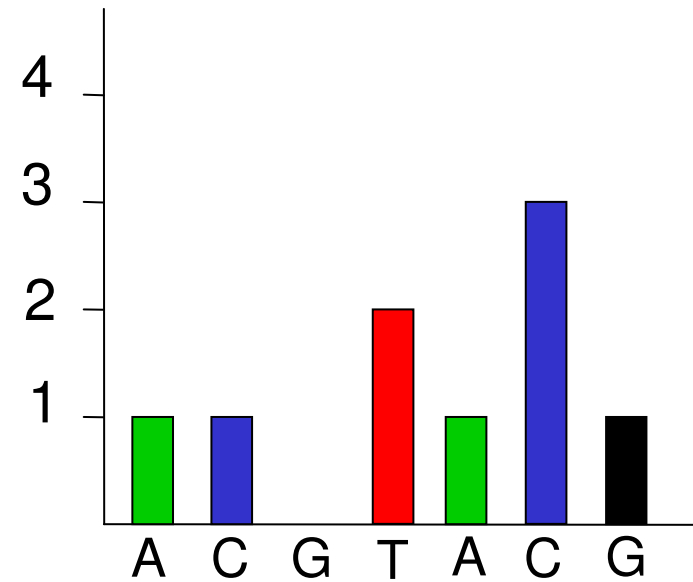
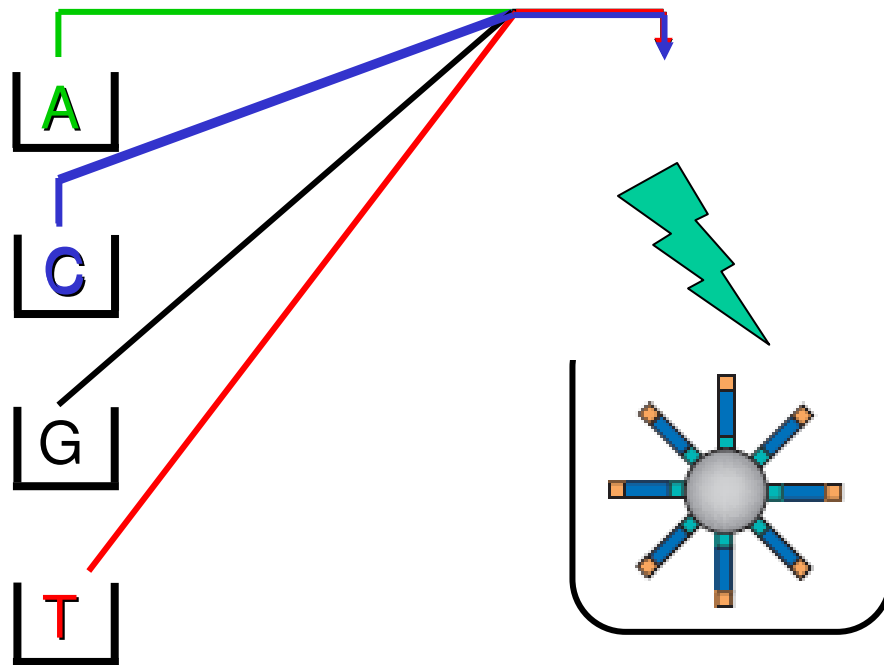
Emulsion PCR



Each beads binds a single fragment, PCR performed, emulsion is broken then bead enrichment

Sequencing

- Each bead= one well on PicoTitre plate



ACTTACC CG



Data analysis

- Sequencing reads align to reference sequence
- Any variants can be viewed in both tabular or graphical forms
- Forward and reverse reads



Aim

- To compare library preparation methods
- Fluidigm access array versus manual library creation

Primer design

Barcode primer

Sequence of interest

Target specific primer

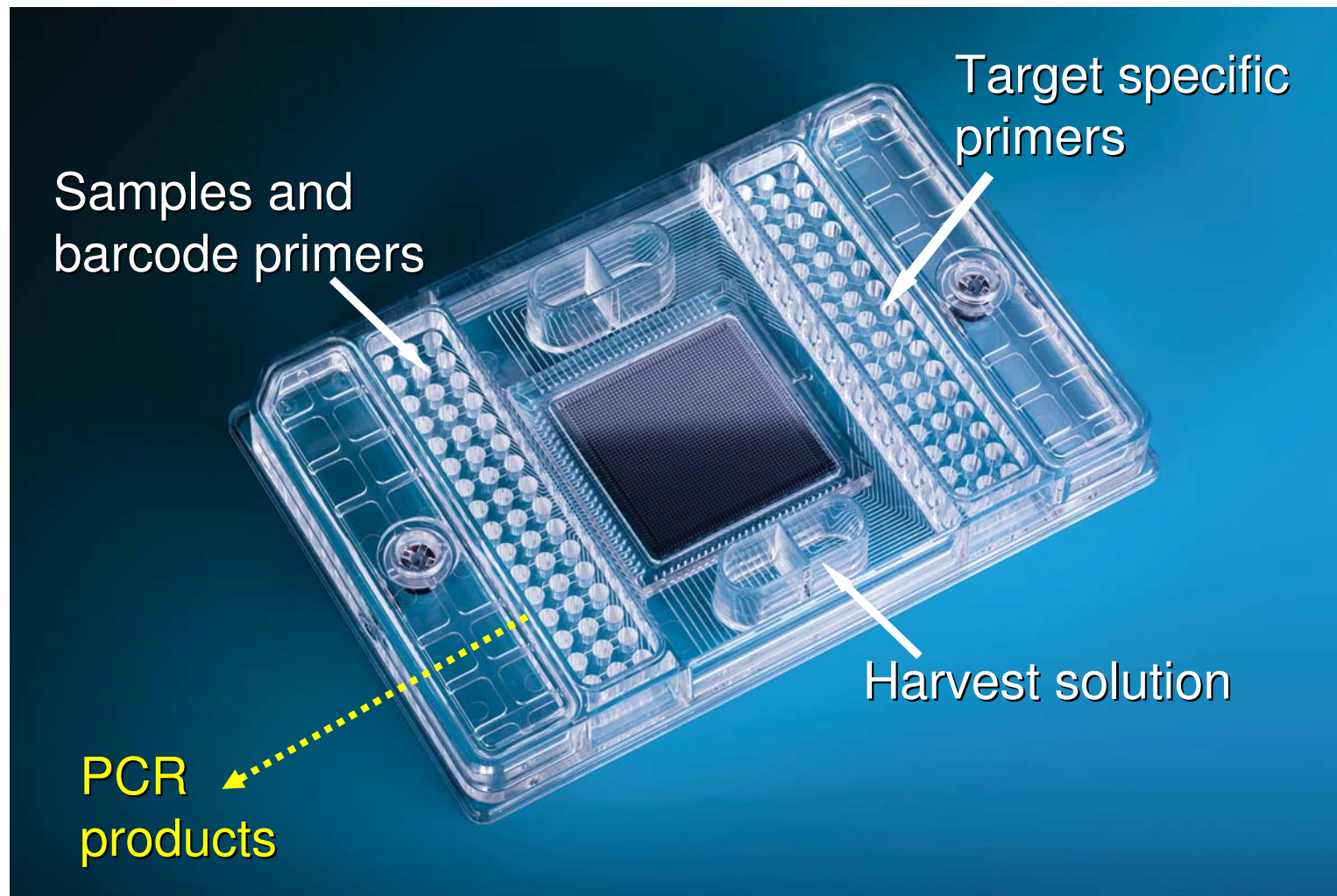


Amplification (e.g. PCR)



Resultant amplicons

Fluidigm access array



48 primers x 48 samples with unique barcodes
2304 unique products

Mutation detection

Exon/intron	Mutation	Detected by software?
Exon 2	c.187_188delAG	Yes
Exon 7	c.427G>T	Yes
Exon 11	c.1961dupA	No
Exon 11	c.3954delT	No
Exon 11	c.4065_4068delTCAA	Yes
Intron 18	c.5153-26A>G	Yes
Exon 24	c.5503C>T	Yes

Other mutations detected by software

c.427G>T

```
CTA-CAGAGT-GAA-CCC-GAAA-T
CTA-CAGAGT-GAA-CCC-GAAAAT
CTA-CAGAGT-GAA-CCC-GAAAAT
CTA-CAGAGT-GAA-CCC-TAAAAT
CTA-CAGAGT-GAA-CCC-GAAAAT
CTA-CAGAGT-GAA-CCC-GAAAAT
CTA-CAGAGT-GAA-CCC-TAAAAT
CTA-CAGAGT-GAA-CCC-TAAAAT
```

c.4065_4068delTCAA

```
GGCTTGG AAGAAAAT AA - - - - GAAGA G
GGCTTGG AAGAAAAT AA - - - - GAAGA G
GGCTTGG AAGAAAAT AA - - - - GAAGA G
GGCTTGG AAGAAAAT AA - - - - GAAGA G
GGCTTGG AAGAAAAT AAT - CAAGAAGA G
GGCTTGG AAGAAAAT AAT - CAAGAAGA G
GGCTTGG AAGAAAAT AAT - CAAGAAGA G
GGCTTGG AAGAAAAT AAT - CAAGAAGA G
```

c.5153-26A>G

```
T--GT-A-TGT-AGCC-T--GTC-TTT
T--GT-A-TGT-AGCC-T--GTC-TTT
T--GT-A-TGT-AGCC-T--GTC-TTT
T--GT-A-TGT-AGCC-T--GTC-TTT
T--GT-A-TGT-AA CC-T--GTC-TTT
T--GT-A-TGT-AA CC-T--GTC-TTT
T--GT-A-TGT-AA CC-T--GTC-TTT
T--GT-A-TGT-AA CC-T--GTC-TTT
```

c. 5503C>T

```
CT-GTGGT-GACC C -G-AGA-G-TGGG-T
CT-GTGGT-GACC C -G-AGA-G-TGGG-T
CT-GTGGT-GACC C -G-AGA-G-TGGG-T
CT-GTGGT-GACC C -G-AGA-G-TGGG-T
CT-GTGGT-GACC T -G-AGA-G-TGGG-T
CT-GTGGT-GACC T -G-AGA-G-TGGG-T
CT-GTGGT-GACC T -G-AGA-G-TGGG-T
CT-GTGGT-GACC T -G-AGA-G-TGGG-T
```

c.3954delT

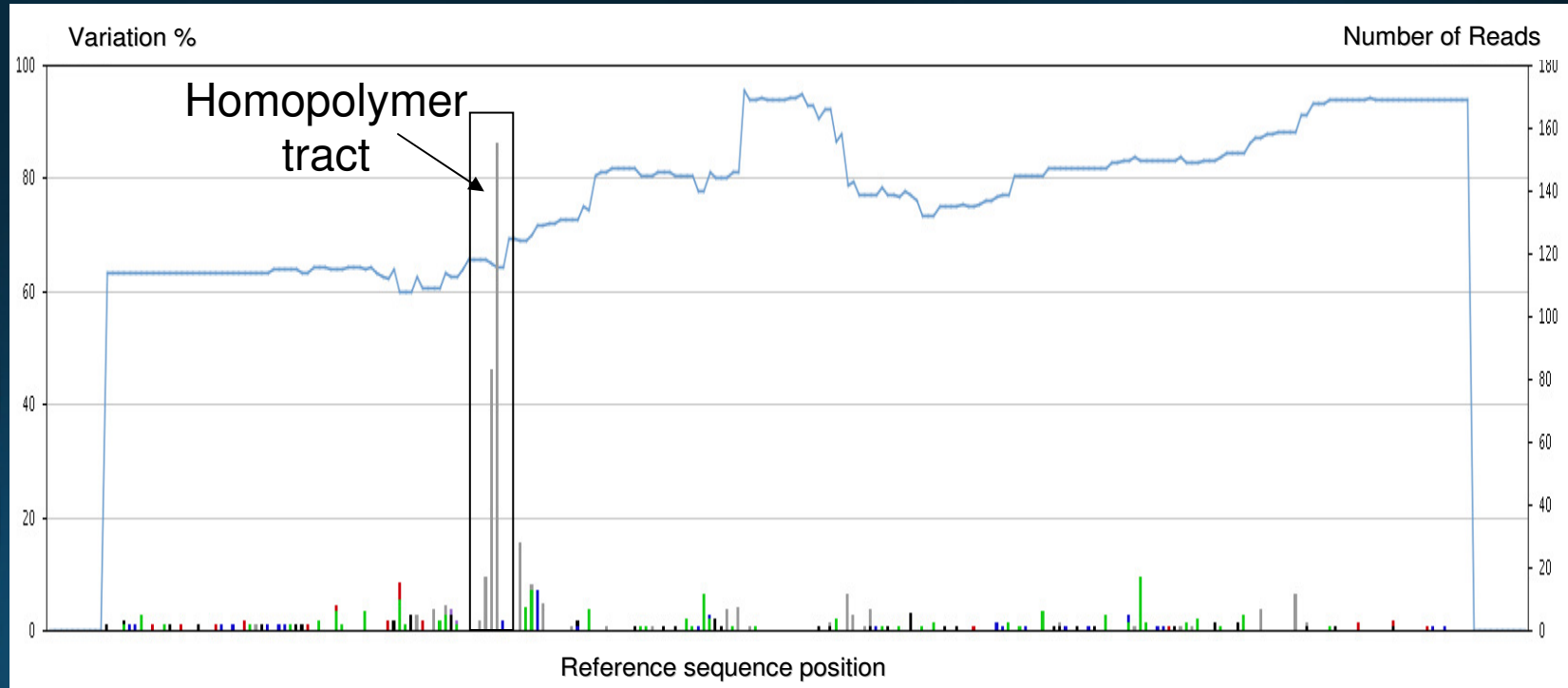
Not detected by software



T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A
T	-	C	C	T	-	T	T	-	C	-	T	T	-	-	G	-	A	-	T	-	T	-	G	G	T	T	C	-	T	T	-	C	C	-	-	A	A	A	-	C	-	A	A	A

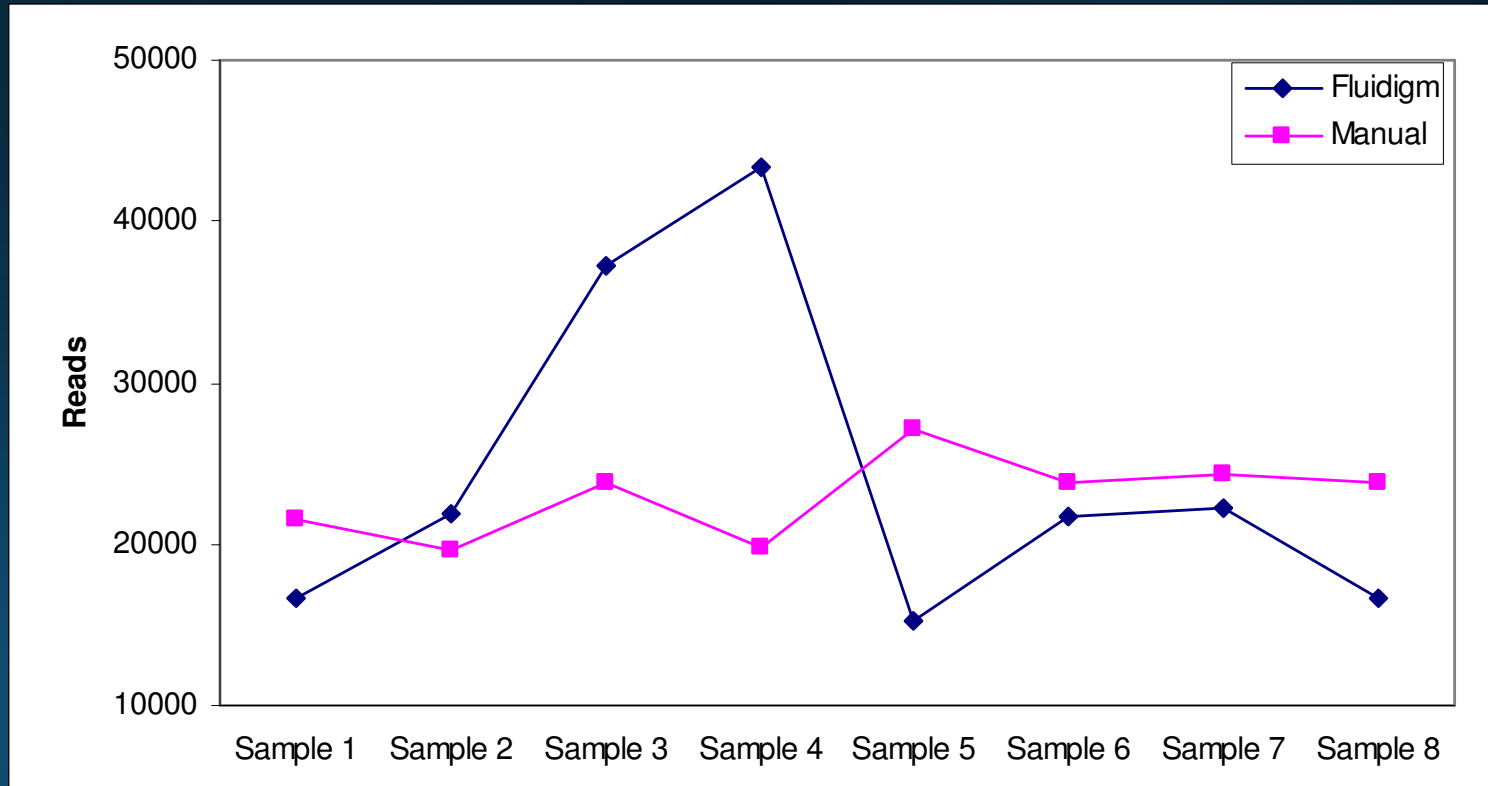
Select @ 37155: T (50%)
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Homopolymer tract- c.1961dupA



AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
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AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C
AGAT	-	AAAG	-	AAAAAAA	-	GT	-	A	-	C	-	AAC	C	AAA	-	T	-	G	-	CC	AG	T	C

Fluidigm Vs Manual



No drop-out of any amplicon or sample for either method



Costs

- Costs for 21 samples for BRCA 1 & 2 full screen (without MLPA), not including analysis time
- Sanger sequencing ~ £5785
- Manual library sequencing ~£5224
- Fluidigm access array sequencing ~£2673



Conclusions

- Fluidigm Access Array system cheaper & more high throughput than manual library preparation
- Both approaches worked well
- Roche software –sequencing errors
- Pyrosequencing homopolymer tract, try another sequencing platform?



Acknowledgements

- All at the East Midlands Regional Genetics laboratory
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 - Gareth Cross
- Nottingham University DeepSeq Centre
 - Sunir Malla
 - Martin Blythe

Thank you, any questions??