

Development and implementation of diagnostic services using NGS

Helen Lindsay

Leeds DNA lab

helen.lindsay@leedsth.nhs.uk

NGS in the Leeds DNA lab

- Development and Translational Facility
- Illumina GAIIx platform
 - single 80bp reads
- Instrument is run once a fortnight
- Each run is shared between University and NHS
- Target enrichment for diagnostic samples achieved by long PCR
- Multiple samples run per lane through use of tagged adapters (barcodes)

NGS in the Leeds DNA lab

- **Familial breast/ovarian cancer**
 - NGS replaced existing Sanger service for *BRCA1* and *BRCA2* for all diagnostic referrals in February 2010
 - First UK NGS diagnostic reports issued in March 2010
- **Hereditary non-polyposis colorectal cancer**
 - NGS replaced existing Sanger service for *MLH1*, *MSH2* and *MSH6* for all diagnostic referrals in October 2010

Validation

- **Parallel study**

- 53 patients tested in parallel with conventional BRCA panels
 - 437 *heterozygous variants*
 - 106 *homozygous variants*
 - 41 *distinct variants*

100% concordance

- **Morgan et al (2010); Human Mutation 31:1-8**

- analysis of *TP53*, *BRCA1* and *BRCA2* mutations in 55 patients previously screened by conventional sequencing
 - 604 *variants detected*

100% concordance

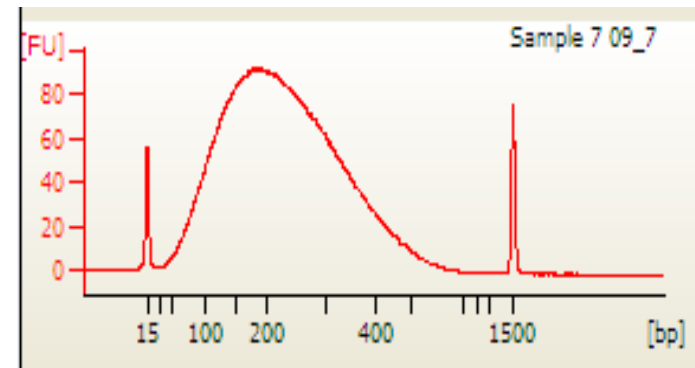
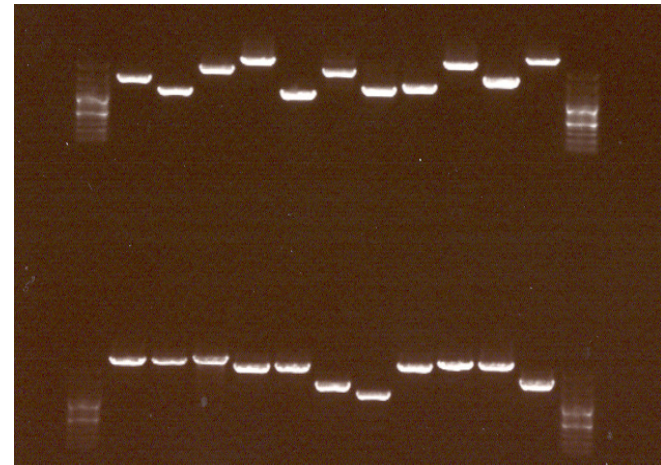
- **Test sensitivity**

- calculated by considering the number of unique variants detected in validation studies

95% confidence that false negative rate is <5%

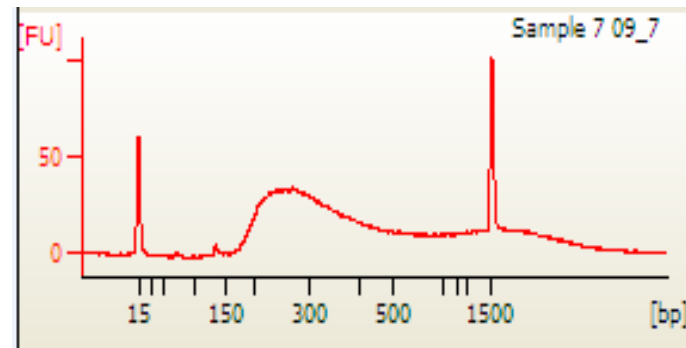
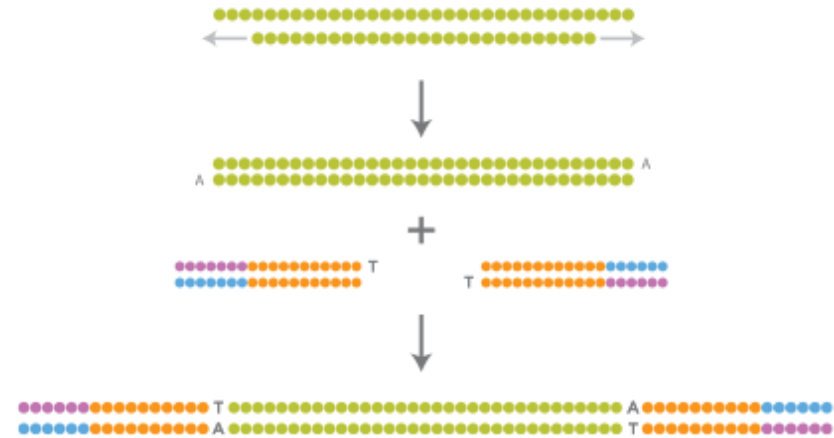
Library preparation I

- Target enrichment
 - long PCR of the complete CDS
- Clean-up and quantification of PCR products
- Shearing of pooled PCR products using the Covaris S2 instrument and verification using the Agilent Bioanalyzer

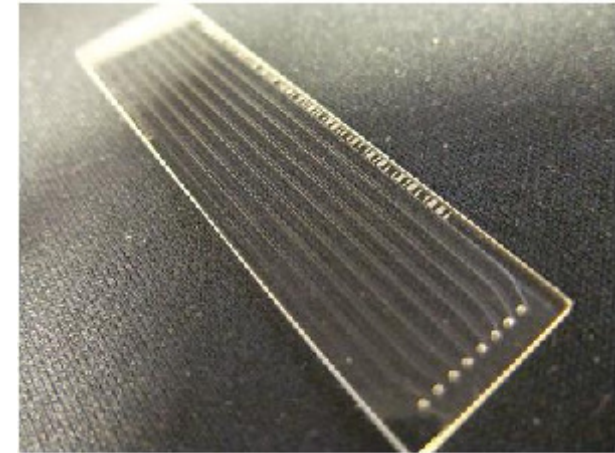
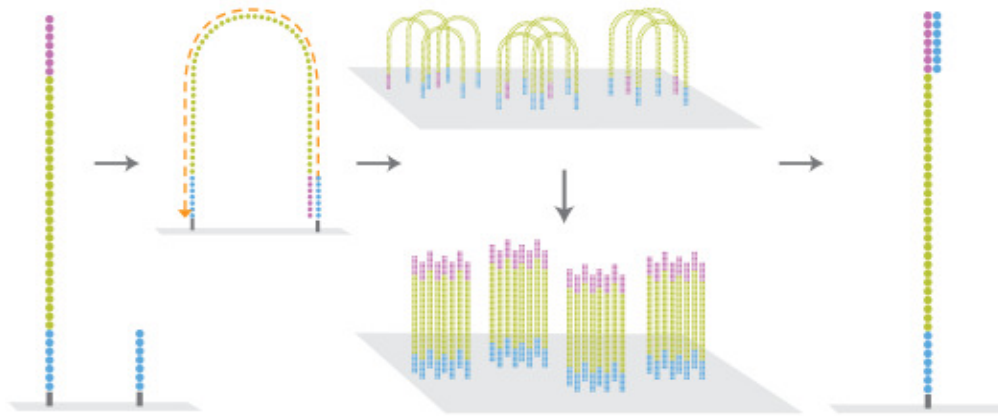


Library preparation II

- End repair, A addition and ligation of tagged (6bp barcode) adapters
- SPRI bead clean-up
- Enrichment PCR and verification on the Agilent Bioanalyzer
- Quantification and pooling of tagged samples



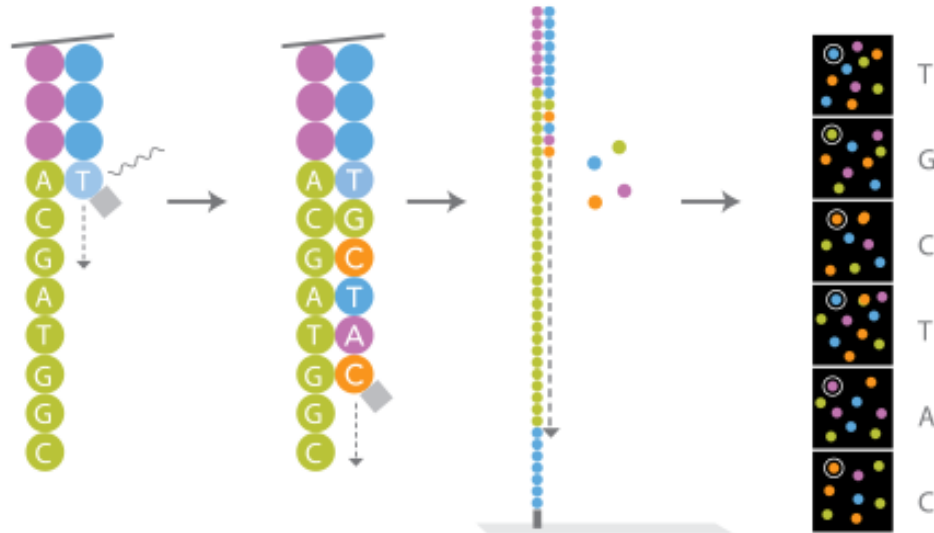
Cluster generation



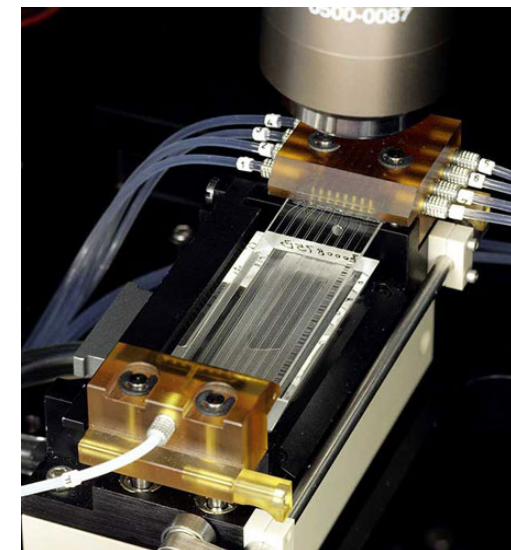
- Hybridise to flow cell
- Bridge amplification
- Cleave reverse strands
- Hybridise sequencing primer



Sequencing



- Copy template using fluorescently labelled, reversibly terminated nucleotides
- Capture fluorescence signal after each incorporation step
- Remove blocking group to allow addition of next base



Data analysis

- NextGENe (SoftGenetics) software
 - conversion to appropriate format
 - removal of low quality files
 - tag sorting
 - alignment to reference sequence(s)
 - detection of substitutions, small insertions and deletions

Data analysis

- Generation of mutation reports
 - manual inspection to confirm variants and exclude artefacts
- Generation of read depth information; custom spreadsheets automatically check depth at each base over the region of interest
 - a minimum threshold of 50-fold read depth is applied for diagnostic screening

Post analysis

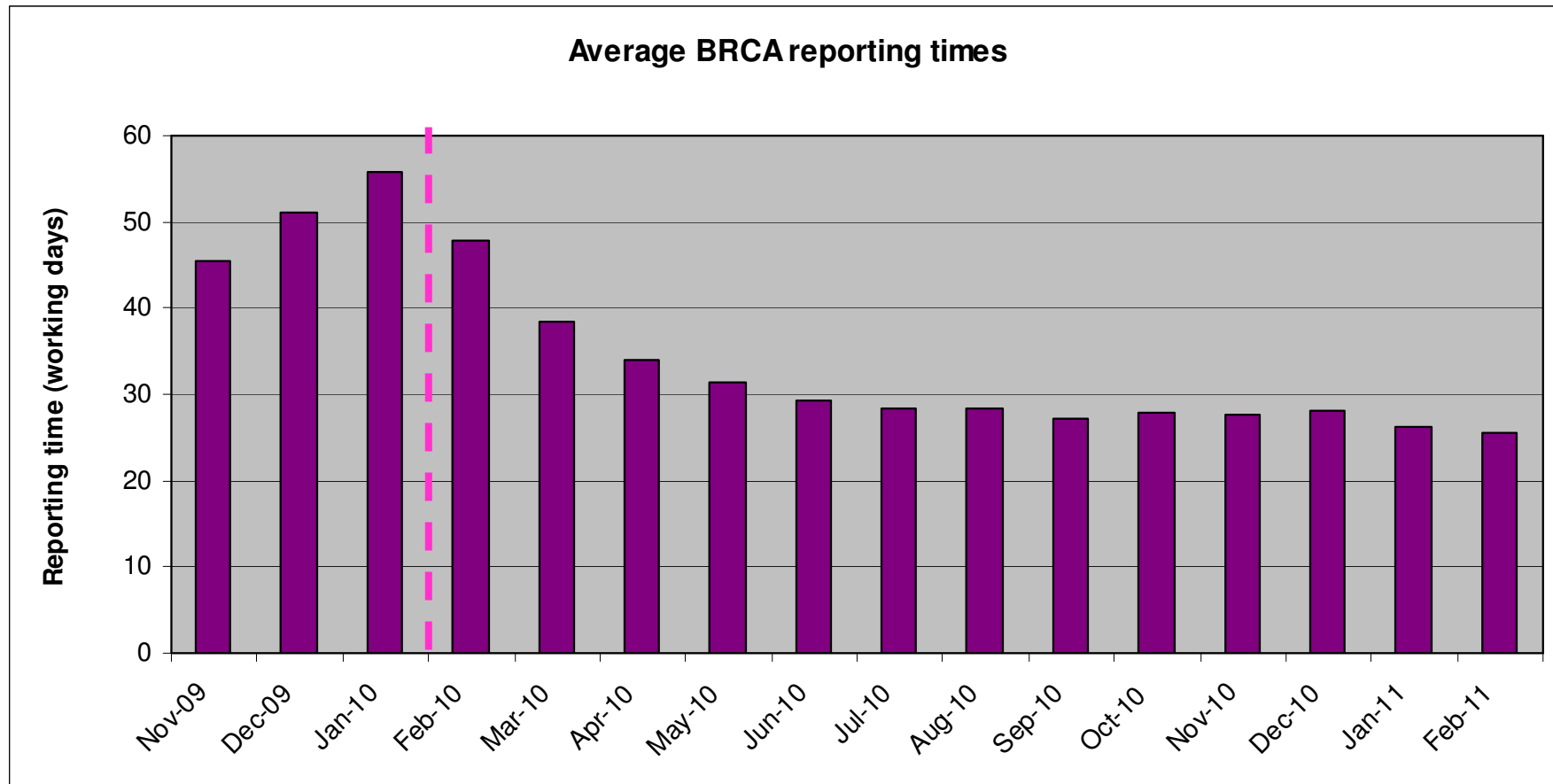
- Confirmation of all sequence variants other than known neutral polymorphisms by Sanger sequencing
- Sanger sequencing of any regions with less than 50-fold read depth
- Creation of a diagnostic report combining sequence data with MLPA results

Discussion

- To date we have sequenced and reported over 500 patients
- All mutations and UVs currently confirmed by Sanger sequencing
- 40% reduction in average test cost
 - *50% reduction in hand-on staff time*
 - *most significant reduction in sequence checking time*
 - *30-40% reduction in consumables costs*
- Process is CPA accredited
- Participated in 2010 NEQAS scheme for *BRCA2*

Discussion

- Improvement in turnaround times



Challenges

- Validation
- Long PCR design
- Limitations of sequencing strategy
 - large deletions
 - homopolymer regions
- Training
- Bioinformatics and data storage
- Maximising capacity

Future developments

- Alternative enrichment techniques
 - high throughput long PCR
 - hybridisation based target capture (SureSelect)
- Further automation of library preparation

Future developments

- More diseases
 - Cardiomyopathy
 - *MYBPC3, MYH7, TNNI3, TNNT2*
 - Pheochromocytoma & paraganglioma
 - *PRKAR1A, RET, SDH5, SDHB, SDHC, SDHD, TMEM127, VHL*
 - Marfan syndrome
 - *FBN1*
 - Long QT syndrome
 - *KCNQ1, KCNH2, KCNE1, KCNE2, SCN5A*
- We charge **£530** per sample for NGS testing; please contact the laboratory for further information.

Acknowledgements

- Leeds DNA lab
 - Nick Camm
 - Rachel Robinson
 - Ruth Charlton
- Leeds Institute of Molecular Medicine
 - Graham Taylor
 - Jo Morgan

