Next Generation Sequencing of BRCA1 and BRCA2: One Year On

The Leeds Teaching Hospitals NHS Trust

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INTRODUCTION

Clonal sequencing of BRCA1 and BRCA2 using the Illumina GAII platform was introduced as a new diagnostic service in the Yorkshire Regional Genetics Service in February 2010, replacing conventional Sanger sequencing of BRCA1/2 and PTT analysis of BRCA2 exon 11 for all new referrals. This is the first CPA accredited UK service routinely using next generation sequencing. Our validation studies* demonstrate test sensitivity and specificity comparable to conventional sequencing and Mutation Surveyor analysis.

METHODS: THE BRCA WORKFLOW

Library preparation (figure 1)

- ❑ Target enrichment of BRCA1 and BRCA2: long PCR of the complete CDS of BRCA1 and BRCA2 in 22 amplicons ranging from 1.2-5.8kb
- ❑ PicoGreen quantification of PCR products and pooling in equimolar amounts
- ❑ Shearing of pooled PCR products using the Covaris S2 instrument and verification using the Agilent Bioanalyzer
- ❑ End repair, A addition and ligation of tagged (6bp barcode) adaptors
- ❑ Size selection using SPRI magnetic beads
- ❑ Enrichment PCR and verification on the Agilent Bioanalyzer
- ❑ Quantification and pooling of tagged samples
- ❑ Cluster generation and single read 80bp sequencing on the Illumina GAIIx

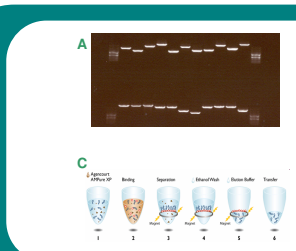


Figure 1. BRCA library preparation
A. Long PCR of BRCA1 & BRCA2 in 22 amplicons
B. Agilent Bioanalyzer trace of randomly sheared PCR products
C. Size selection of tagged fragments using magnetic beads
D. Agilent Bioanalyzer trace of post-enrichment PCR library

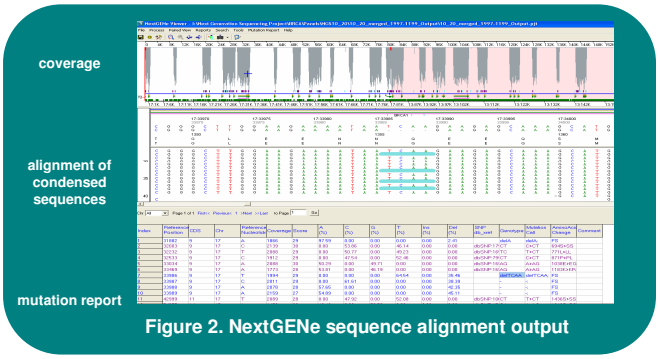


Figure 2. NextGENe sequence alignment output

Data analysis (figure 2)

- ❑ Data analysis using NextGENe (SoftGenetics) software
- ❑ Generation of mutation reports
  - manual inspection to confirm variants and exclude artefacts
- ❑ Generation of coverage information; custom spreadsheets automatically check coverage at each base over the region of interest
  - a minimum threshold of 50-fold coverage is required for diagnostic screening

Post analysis

- ❑ Confirmation of all sequence variants other than known neutral polymorphisms by Sanger sequencing
- ❑ Creation of a diagnostic report combining sequence data with MLPA results

RESULTS AND DISCUSSION

Patient data

Following a year of service we have sequenced and reported 461 patients on a diagnostic basis. At least one sequence variant (excluding neutral polymorphisms) was detected in 126 patients; these were confirmed by Sanger sequencing with 100% concordance. Excluding dosage abnormalities, we have observed a 13% pick-up rate of pathogenic mutations, including a heterozygous 40 base pair deletion in BRCA1 (Figure 3).

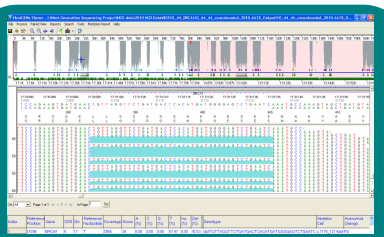


Figure 3. BRCA1 heterozygous 40bp deletion – c.1175_1214del het (p.Leu392fs)

Costing

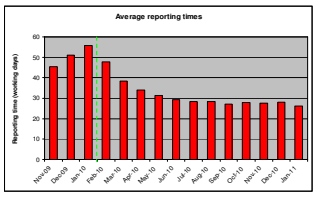
The Illumina workflow has made substantial savings to the BRCA screening pipeline. Average testing costs have been reduced by ~40%, reflecting a 50% saving in staff hands-on time and an overall saving of 30-40% on consumables. We currently charge £530 per BRCA screen. This includes BRCA1/2 clonal sequencing, any confirmations required, and MLPA. As the depth of sequence coverage currently achieved far exceeds the minimum required for diagnostic purposes, more samples will be pooled per sequence run in the future, lowering run costs.

Turnaround times

From sample receipt to reporting, the clonal sequencing method has resulted in a 50% reduction in hands-on staff time. This is due partly to the new lab workflows, but most significantly to the reduction in sequence checking time now required. The data analysis enables the generation of a single mutation report for both BRCA1 and BRCA2, compared with 59 Mutation Surveyor reports. This has had an immediate and sustainable effect on the lab turnaround times (figure 4), and therefore on patient care pathways.

Figure 3. The reporting time for BRCA diagnostic reports in working days over the last 15 months is shown. Each bar represents the mean reporting time for reports issued in the preceding three months.

The dashed line indicates the implementation of the BRCA clonal sequencing service.



Workflow developments

Over the last year the service has been modified to incorporate liquid handling robots to further reduce the amount of staff hands-on time. The introduction of SPRI beads for size selection has reduced library preparation times and removed the need for laborious manual gel excision and purification. We are currently investigating alternative target enrichment techniques, including high-throughput approaches to long PCR as well as target capture hybridisation methodology.

Services in development

We are in the process of implementing the following services and hope to be able to offer them at a similar price to the BRCA testing in the near future:

- ❑Hypertrophic Cardiomyopathy – *MYBPC3, MYH7, TNNT2 & TNNI3*
- ❑Pheochromocytoma – *PRKAR1A, RET, SDH5, SDHB, SDHC, SDHD, TMEM127 & VHL*
- ❑Marfan syndrome – *FBN1*
- ❑Long QT syndrome – *KCNQ1, KCNH2, KCNE1, KCNE2 & SCN5A*

SUMMARY

The implementation of BRCA clonal sequencing in Leeds has led to significantly reduced laboratory costs and turnaround times, with no compromise on test sensitivity as compared to conventional sequencing.

We are happy to accept samples for testing - please contact the laboratory for further information.

*Morgan *et al* (2010) Human Mutation 31(4): 484-491