Highly Sensitive and Cost-Effective Detection of BRCA1 and BRCA2 Cancer Variants in FFPE Samples Using Multiplicom's MASTR Technology & Single Molecule, Real-Time (SMRT®) Sequencing

Steve Kujawa[1], Anand Sethuraman[1], Primo Baybayan[1], Jurgen Del Favero[2] and Lien Heyrman[2]
[1]Pacific Biosciences, 1380 Willow Road, Menlo Park, CA 94025
[2]Multiplicom N.V., Galileilaan 18, 2845 Niel, Belgium

Introduction

Specific mutations in BRCA1 and BRCA2 have been shown to be associated with several types of cancers. Molecular profiling of cancer samples requires assays capable of accurately detecting the entire spectrum of variants, including those at relatively low frequency. Next-Generation Sequencing (NGS) has been a powerful tool for researchers to better understand cancer genetics.

Here we describe a targeted re-sequencing workflow that combines barcoded amplification of BRCA1 and BRCA2 exons from 12 FFPE tumor samples using Multiplicom's MASTR technology with PacBio SMRT Sequencing. This combination allows for the accurate detection of variants in a cost-effective and timely manner.

Workflow

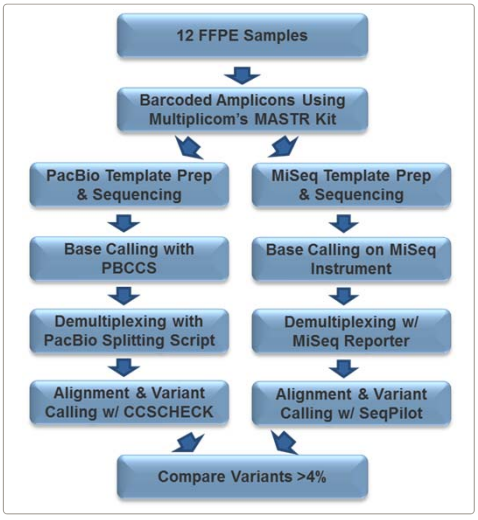

Figure 1. Twelve FFPE tumor samples were amplified, barcoded and sequenced on either the PacBio RS II or MiSeq instrument. Variants >4% were compared.

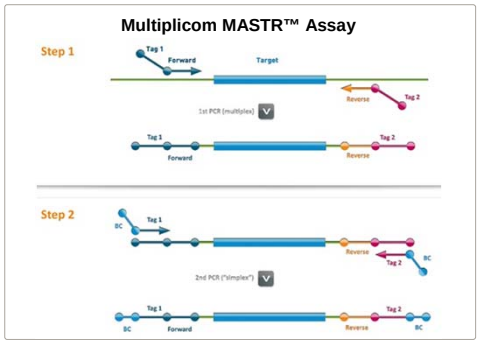

Figure 2. The MASTR™ assay employs a 2-step PCR protocol enabling specific amplification of the regions of interest (step 1) followed by the incorporation of molecular barcodes (step 2) to unambiguously link each read to the originating sample.

PacBio Coverage

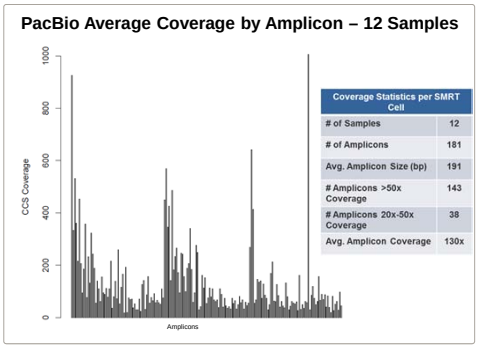

Figure 3. The Multiplicom BRCA Tumor MASTR™ plus assay generates 181 amplicons per sample.

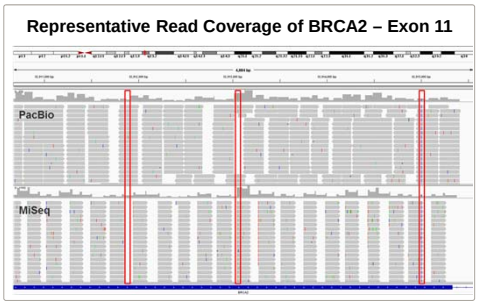

Figure 4. Representative read coverage of a ~4.5 kb region of exon 11 in BRCA2. Red boxes highlight concordant variants between PacBio (top) and MiSeq (bottom) reads. PacBio reads are slightly longer as the primers have not been trimmed in this view.

Comparison of Variants

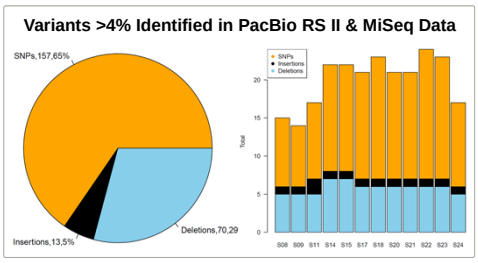

Figure 5. PacBio data identified all 240 distinct variants >4% that were identified by MiSeq data in the 12 samples analyzed. About 2/3 of the variants are SNPs and 1/3 are indels.

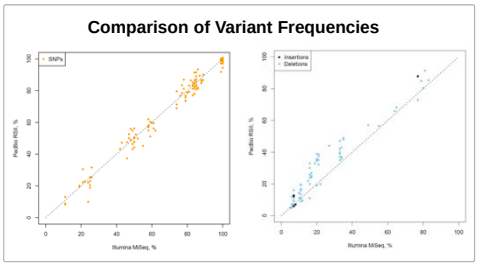

Figure 6. The 157 SNPs (left panel) show good concordance between the platforms. Of the 83 indels (right panel) identified by both platforms, there were 13 insertions and 70 deletions. The PacBio workflow calls indels at a higher frequency compared to the MiSeq workflow.

Description of Indels >4%

Homopolymer Regions	
Insertions ≥1 bp	0
Deletions ≥1 bp	44
Dinucleotide Repeats	
Insertions ≥1 unit	12
Deletions ≥1 unit	12
Non-Repetitive Regions	
Insertions	1
Deletions	14
TOTAL	**83**

Figure 7. Of the 83 total indels identified by both platforms, approximately 50% were deletions in homopolymer regions. In dinucleotide repeat regions, an equal number of insertions and deletions were observed.

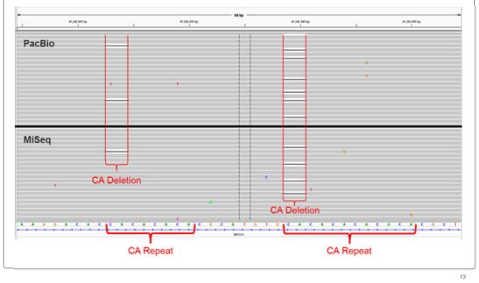

Figure 8. A comparison of reads from an intronic region in BRCA1 from Sample 14. In the two distinct CA repeat regions, similar dinucleotide deletions are observed in both the PacBio reads (top) and MiSeq reads (bottom).

Summary and Resources

Summary:

- This work describes a complete PacBio workflow for amplifying, sequencing and calling variants from multiplexed FFPE tumor samples
- SMRT Sequencing identifies all variants >4% that were detected with MiSeq sequencing
- With the recent launch of the PacBio Sequel instrument, approximately 8 barcoded samples can be sequenced in a 90-minute sequencing run, providing a more cost-effective workflow

Multiplicom Info Available Here:

http://www.multiplicom.com/products/brca-tumor-mastr-plus

PacBio Info Available Here:

http://www.pacb.com/applications/targeted-sequencing/

Data & Analysis Info Available Here:

https://github.com/PacificBiosciences/pbccs
https://github.com/PacificBiosciences/ccscheck
https://github.com/PacificBiosciences/PacBioFileFormats/wiki/BAM-recipes#splitting

Acknowledgements

The authors would like to thank Nigel Delaney and John Harting for their assistance with the data analysis and scripts.