Developing a high-quality, sample-to-result Hereditary Breast and Ovarian Cancer panel assay pipeline for a novel sequencing platform

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INTRODUCTION

Hereditary Breast and Ovarian Cancer (HBOC) testing has remained inadequate, plagued by medical history–based testing, high test cost, and challenges in NGS data quality. This has led to a model of centralized testing on high-throughput sequencers that always do not fit well with independent clinical testing. We explore herein alternative solutions and improvement approaches.

METHODS

- Compared 5 Coriell cell line and 46 patient samples across two short read sequencing platforms (Element and Illumina NextSeq550)
- Used TruSight Hereditary Cancer Panel (113 genes) that contain HBOC Genes to compare performance on Illumina platform for SNV and CNV calls to identify improvement areas

RESULTS

- Target read depth highly correlated across the two sequencer platforms
- High concordance across clinically significant variants: 277/279 sites
- Benchmark Study: Performance Metrics on Illumina Sequencer: High Sensitivity [TP/(TP+FP)] and PPV [TP/(TP+FP)]/Specificity [TN/(TN+FP)]
- Improved FG Panel design for CNV detection

20,406 total variants in 51 samples with high concordance between platforms

Clinically Classified Variants by FG	Concordant Genotypes (Element/Illmn)	Discordant Genotypes (Element/Illmn)
279	277	2

Only two discordant variants. Both located at sites next to a long homopolymer, where read mapping can be difficult and classified as benign variants

Samples used in the study

5 Coriell
46 patients
51 samples

	Sample ID	Description	Size	Gene
1	NA14091	5382insC (exon20)	1 bp	BRCA1
2	NA11630	317ins5	5 bp	MEN1
3	NA11410	3149delC (resulting in truncation of gene product)	1 bp	APC
4	NA10080	781C>T (Gln261Ter)	1 bp	PTEN
5	NA16533	19-bp deletion in exon 2	19 bp	CDKN2A
6	Patient 1			
7	Patient 2			
...	...			
51	Patient 46			

FG PAN CANCER panel (113 genes)

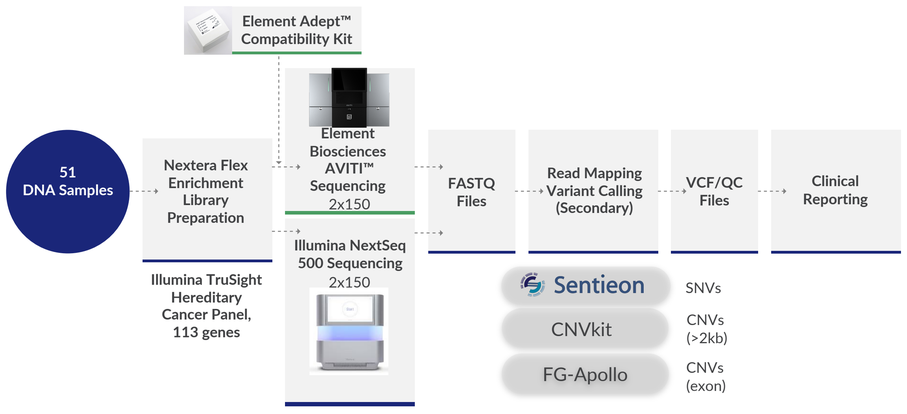
HBOC PANEL

Breast Cancer	Ovarian Cancer	Breast & Ovarian Cancer (HBOC)
ATM	ATM	ATM
BARD1		BARD1
BRCA1	BRCA1	BRCA1
BRCA2	BRCA2	BRCA2
	BRIP1	BRIP1
CDH1		CDH1
CHEK2		CHEK2
	EPCAM*	EPCAM*
	MLH1	MLH1
	MSH2	MSH2
	MSH6	MSH6
		NBN
NF1		NF1
PALB2	PALB2	PALB2
	PMS2*	PMS2*
PTEN		PTEN
RAD51C	RAD51C	RAD51C
RAD51D	RAD51D	RAD51D
STK11		STK11
TP53		TP53

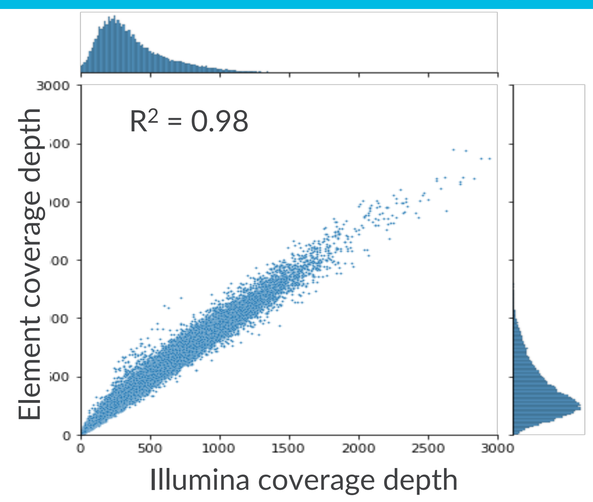
NCCN v1.2022 Risk and Management, Evidence
* NCCN v1.2022 Risk and Management, Limited Evidence

- Colorectal
- Pancreatic
- Melanoma
- Pediatric
- Brain

Sequencing Platform Workflows



Correlation: Read depth per target



BRCA1 mapped reads (IGV)

DISCUSSION

- Improvements in CNV calling (secondary) and panel design is expected to boost performance (high sensitivity) in short read sequencing platforms

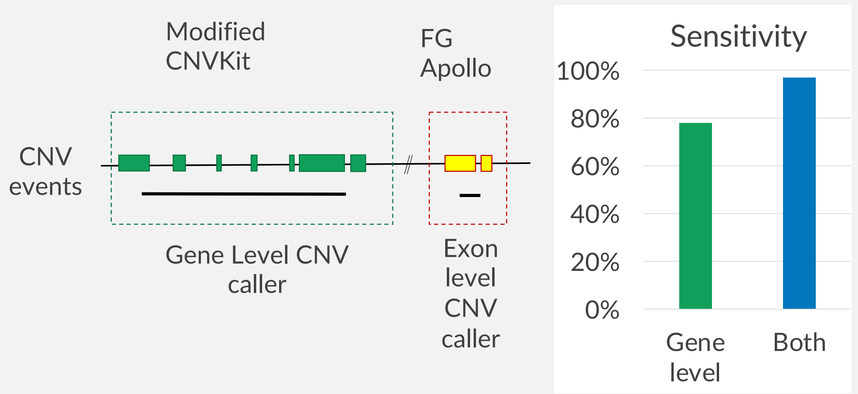
CONCLUSION

- Combination of joint CNV calling and panel design is expected to provide a high-quality short-read sequencing solution for HBOC and pan cancer testing

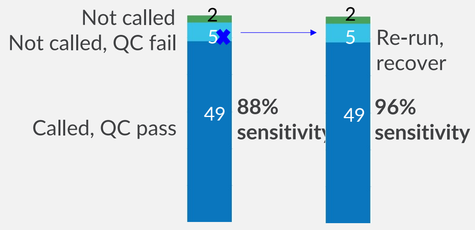
Performance on Illumina Sequencer

Variant Type	Reference Material	Performance
SNVs and small Indels in Genome-in-a-Bottle consortium +/- 10-bp	NIST ID HG001, HG002,HG003, HG004 (Coriell ID NA12878, NA24385, NA24149, NA24143), across 20 HBOC genes (see above)	▪ Sensitivity: 99.38% for HBOC and 98.49% for 113 gene panel ▪ PPV HBOC or panel: ~99%
SNVs and small Indels, Challenging variants	Seracare Seraseq™ Inherited Cancer DNA Mix v1 of 23 variants across (*BRCA1*, *BRCA2*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *PMS2*)	Sensitivity: 100% with the exception of 2 challenging indel variants-126-bp insertion and a 300-bp AluY insertion in BRCA2 that require visual review
Large and Small CNVs	Orthogonally validated collection of cases from (a) 33 Coriell samples spanning large and small CNVs. (b) ICR 96 dataset for 56 CNVs across 14 genes (*ATM*, *BRCA1*, *BRCA2*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *NF1*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *TP53*)	▪ Sensitivity: ~97% for HBOC; ~95% for 113 gene panel ▪ Specificity: >99.98 %

CNV Calling on Illumina TruSight/Illumina Sequencer



FG Apollo (CNV exon) Performance

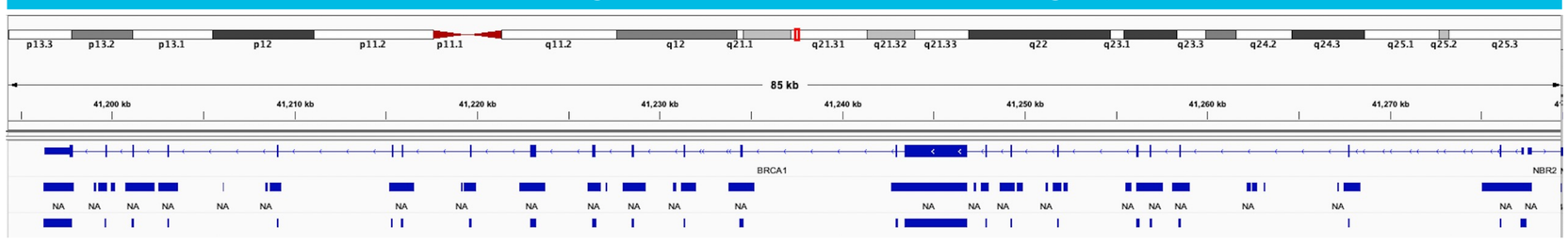


- ICR96 dataset[1]
- 56 small CNVs
- 20 HBOC genes
- CNV calling with FG-Apollo
- Verified by MLPA

Total # of exons in CNV neutral regions in 20 HBOC genes across 56 subjects

FPs	TNs	Specificity (%)
4	19009	99.9

Panel Probe Design for FG Panel vs Illumina TruSight (BRCA1)



REFERENCES

1. Mahamdallie S, Ruark E, Yost S et al. The ICR96 exon CNV validation series: a resource for orthogonal assessment of exon CNV calling in NGS data. Wellcome Open Res 2017, 2:35 (doi.org/10.12688/wellcomeopenres.11689.1)