# Investigating mutations in Homologous Recombination Repair(HRR) Genes with genomic loss of heterozygosity (LOH) to characterize cause and consequence of HRD

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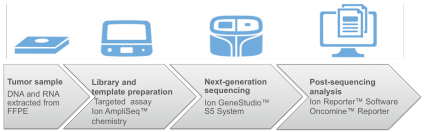
## INTRODUCTION

Alterations in genes in the Homologous Recombination Repair (HRR) pathway interfere with the ability to repair DNA double-strand breaks (DSBs), leading to Homologous Repair Deficiency (HRD) [1]. In certain cancers like ovarian and prostate, HRD leads to genomic instability and can be characterized by measuring loss of heterozygosity (LOH) across the genome (%LOH). HRD is an emerging biomarker with research supporting potential sensitivity to cisplatin and for inhibitors of poly(ADP-ribose) polymerase (PARP). Therefore, understanding the relationship between HRR gene alterations and genomic instability is critical to support clinical research for the future of precision medicine.

## MATERIALS AND METHODS

We used the Oncomine Comprehensive Assay Plus or OCA Plus (500+ gene targeted NGS assay using Ion Ampliseq™ library chemistry) on Ion GeneStudio ™ S5 system to generate sequencing data for three group of formaldehyde fixed paraffin embedded (FFPE) tumor samples (pan-cancer, ovarian, prostate). In each group, we used OCA Plus to characterize alterations in HRR genes and used an algorithm to estimate percent LOH (%LOH). In parallel, we determined %LOH using the Affymetrix Oncoscan™ CNV assay (microarray based whole genome assay) and compared those results to the %LOH results from OCA Plus. Further, we investigated mono-allelic vs. bi-allelic mutations in BRCA1/2 genes and their impact on %LOH.

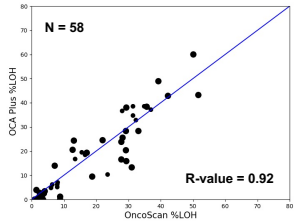
**Figure 1. Schematic flow-diagram of OCA Plus sequencing and informatics workflow**




The OCA Plus workflow uses a recommended 10 ng of input FFPE material per amplicon pool and uses two pools. The assay can leverage manual or automated library preparation and templating on the Ion Chef. Up to four samples can be multiplexed on the Ion 550 chip to achieve sufficient read depth. Ion Reporter software analyzes the sample using custom variant calling optimized for solid-tumor samples and CN analysis to generate tumor cellularity and LOH estimates.

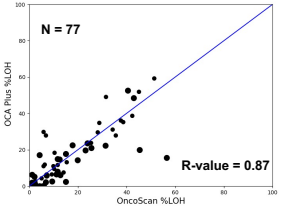
## RESULTS

**Figure 2. High Correlation between genome-wide LOH (%LOH) estimated by OCA Plus and Microarray data in FFPE samples in pan-cancer group**



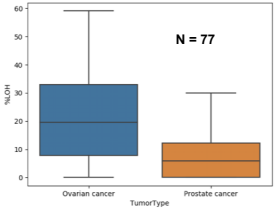

Affymetrix OncoScan™ CNV assay was used as an independent measure of genome-wide LOH for pan-cancer group of FFPE samples that consists of a variety of solid tumor tissue types. High correlation, R-value of 0.92 was observed between the genome-wide LOH (%LOH) estimated by OCA Plus and OncoScan.

**Figure 2. High Correlation between genome-wide LOH (%LOH) estimated by OCA Plus and Microarray data in FFPE samples in ovarian and prostate group**



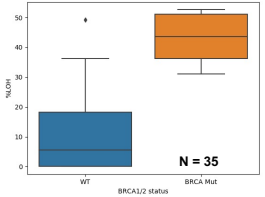

Affymetrix OncoScan™ CNV assay was used as an independent measure of genome-wide LOH for ovarian and prostate tumor group of FFPE samples. High correlation, R-value of 0.87 was observed between the genome-wide LOH (%LOH) estimated by OCA Plus and OncoScan.

**Figure 4. Comparison of %LOH in ovarian and prostate tumor group**



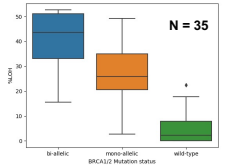

%LOH in the ovarian tumor group was found to be significantly higher compared to the prostate tumor group (p-value of 0.00037 using student's t-test) indicating higher average genomic instability in ovarian tumor samples.

**Figure 5. %LOH in ovarian tumor group stratified by mutations in BRCA1/2**




BRCA1/2 mutations have been investigated as a source of defect in homologous recombination and therefore increased genomic instability. This was supported by our investigations, namely in the ovarian tumor group where samples with BRCA1/2 mutations have significantly higher %LOH (p-value of 0.000082 using student's t-test) compared to BRCA1/2 wide-type samples.

**Figure 6. Comparison of %LOH in ovarian tumor samples with bi-allelic/mono-allelic mutations and wild-type**




Samples in ovarian tumor group with bi-allelic BRCA1/2 mutations (loss-of-function due to SNV/Indel and LOH) had higher %LOH compared to mono-allelic mutation (either SNV/Indel or LOH) and wild-type samples.

## CONCLUSIONS

We developed a comprehensive genomic profiling assay that supports genomic %LOH as one measurement of HRD. Our data supports that ovarian tumor samples with BRCA1/2 alterations exhibit significantly higher %LOH relative to samples without BRCA1/2 alterations. Further, samples with bi-allelic BRCA1/2 mutations in this group have demonstratively higher %LOH relative to mono-allelic mutations. Combining HRR pathway status with genomic instability in the same targeted NGS assay enables measurement of both cause and consequence of HRD and will advance HRD translational research for the future of precision medicine.

## REFERENCES

1. Wang, Z. *et al.*(2012) 'Profiles of Genomic Instability in High-Grade Serous Ovarian Cancer Predict Treatment Outcome', *Clin Cancer Res*, 18(20): 5806-5815
2. Shen Ronglai, Seshan Venkatraman E.: FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Research* 2016, Vol.44, No.16

## TRADEMARKS/LICENSING



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