Combined Low-pass Whole Genome and Targeted Sequencing Identifies Causative Mutations and Associated Genomic Scarring Indicative of Homologous Recombination Deficiency

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INTRODUCTION

- Most targeted next generation sequencing (tNGS) approaches used in Comprehensive Genomic Profiling (CGP) are not designed to assess genome-wide copy number variation (CNV) or the genomic scars associated with homologous recombination deficiency (HRD), such as loss of heterozygosity (LOH).
- In this study, a low-pass whole-genome sequencing (LP-WGS) based assay was developed to run in parallel with tNGS to support simultaneous evaluation of HRD causative mutations and genome-wide scarring.

METHODS

- LP-WGS, tNGS and SNP Arrays Workflow (Figure 1)

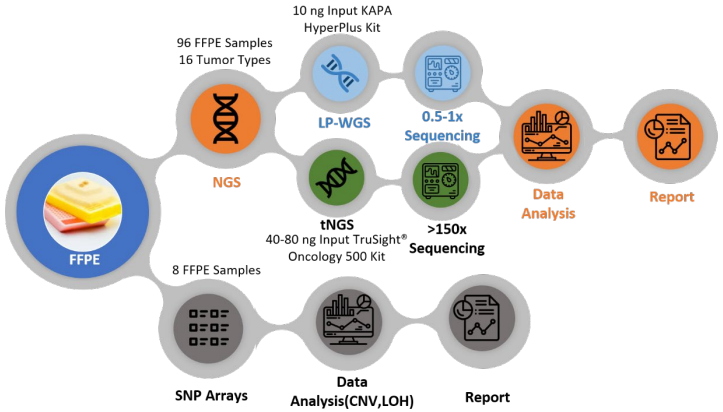

Figure 1: 96 FFPE tumor samples were processed for LP-WGS and tNGS using TruSight® Oncology 500 Assay. A subset of 8 samples were also evaluated using OncoScan™ CNV Plus Assay (SNP Arrays).

- Regions of CNV were determined using CNVKit v0.9.6 and regions of LOH were estimated using a proprietary ancestry-aware method.
- Small variant detection was performed using the TruSight® Oncology 500 v2.2.0.12 analysis pipeline.
- CNV and LOH estimates derived from LP-WGS, TSO500 (TruSight® Oncology 500 Assay) and SNP arrays (OncoScan™ CNV Plus Assay) were calculated using Jaccard similarity index (Figure 2)

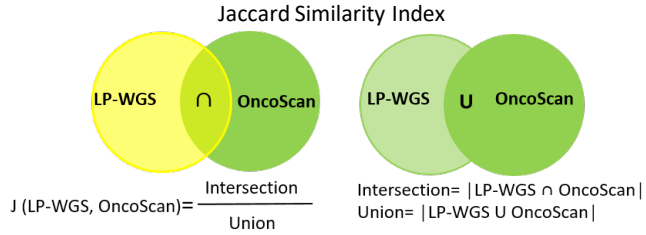

Figure 2: Jaccard similarity index for calculating CNV and LOH call concordance between LP-WGS and SNP arrays (OncoScan™ CNV Plus Assay) . Jaccard similarity index equal to or near 1 is considered near perfect similarity.

RESULTS

- Near perfect levels of regional concordance for CN gains and losses between LP-WGS assay and SNP arrays (OncoScan™ CNV Plus Assay) (Figure 3, Tables 1a and 1b).

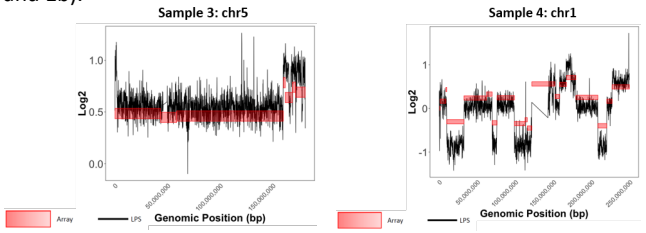

Figure 3: Examples of CNV calls detectable via both SNP arrays (OncoScan™ CNV Plus Assay) and LP-WGS assay demonstrating regional CNV call concordance.

CN Gains				
Sample	Intersection	Union	# of Segments	Jaccard Similarity Index
1*	0	50170	0	0
2	3032220	3032220	3	1.000
3	3520341	3520341	2	1.000
4	33839981	33839981	6	1.000
5	143861459	143861459	23	1.000
6	1899462	1899462	3	1.000
7	365595	365595	1	1.000
8	20034277	20034277	5	1.000
87.5% (7/8) samples have 100% regional concordance in CN gains.				

Note: Sample 1*- copy neutral, some small CNVs in sequence

CN Losses				
Sample	Intersection	Union	# of Segments	Jaccard Similarity Index
1	1344714	1344714	3	1.000
2	240128000	240128000	10	1.000
3*	0	0	0	N/A
4	363380567	363380567	32	1.000
5	361334918	361598944	21	0.999
6	26422711	26422711	7	1.000
7	818344520	818344520	37	1.000
8	953458143	953458143	39	1.000
87.5% (7/8) samples have near 100% regional concordance in CN losses.				

Note: Sample 3*- euploid on many chromosomes, other clean full-chromosome gains, some segments gains, one full chromosome loss

Table 1: Jaccard Similarity Index scores for a) CN gains, and b) CN losses demonstrating high concordance between LP-WGS and SNP arrays (OncoScan™ CNV Plus Assay) .

- High concordance between regions of the genome called LOH between LP-WGS assay and SNP arrays (Median Jaccard index=0.70, IQR=0.254), but noted an attenuation of sensitivity in samples where estimated tumor heterogeneity was high (Table 2).

LOH					
Sample	Intersection	Union	# of segments	Jaccard Similarity Index	Tumor Purity
1	33145382	48861155	11	0.678	35%
2	526916730	967829122	8	0.544	90%
3	1585749525	2049379785	18	0.774	80%
4	1621366824	1621366824	40	1.000	100%
5	510871862	872888460	17	0.585	95%
6	191569485	856853984	7	0.224	80%
7	767653201	1060690481	31	0.724	70%
8	977156343	980058838	19	0.997	60%
Median Jaccard similarity index= 0.70, IQR=0.254					

Table 2: Jaccard Similarity Index scores for genomic regions of LOH demonstrating high concordance between LP-WGS and SNP arrays (OncoScan™ CNV Plus Assay).

- High sensitivity (95.52%; 89.33%) and specificity (94.01%; 90.36%) for both CN gains and losses, respectively were observed by LP-WGS assay against TruSight® Oncology 500 Assay (TSO500) (Table 3).

LP-WGS Assay vs TSO500 Assay in CNV Calls for 96 FFPE Tumor Samples		
	CN Gains	CN Losses
Sensitivity	95.52%	89.33%
Specificity	94.01%	90.36%
Accuracy	94.04%	90.32%

Table 3: Sensitivity and specificity for both CNV calls by LP-WGS assay vs TruSight® Oncology 500 Assay (TSO500).

CONCLUSIONS

- CGP workflows incorporating LP-WGS with tNGS can support simultaneous evaluation of BRCA1/2 mutations, other HRD causative mutations and genome-wide scarring.
- This approach can provide a more complete assessment of HRD which is essential for identifying patients who may obtain clinical benefit from treatment with PARP inhibitors.

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Notice: the abstract has minor revisions since submission.