Development and Characterization an NGS Multi-Cancer Panel plus CNV Detection; A Single-Tube, Multiplex-PCR Based NGS Assay with 309 Tiled Amplicons

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Abstract

Introduction: SLIMamp allows multiplex-PCR of tiled amplicons in a single tube, which enables targeting of large exons for NGS analysis with a streamlined process. CNVs have a high prevalence in the pathogenesis of cancer and their characterization is important to acquire a more comprehensive picture of the mutations present in a patient sample. Pillar developed a proprietary CNV algorithm and combined it with SLIMamp to develop an integrated multi-cancer plus CNV detection NGS panel which identifies CNVs in ERBB2, EGFR, MET, and MYC.

Methods: To assess the ability of the ONCO/Reveal Multi-Cancer with CNV Panel to detect ERBB2 CNV compared to DISH, libraries were created from 44 well-characterized FFPE breast cancer samples plus 3 GIAB samples. 23 tumor samples had known ERBB2 amplification, 21 were known non-amplified tumors, and 3 GIAB samples were used as negative controls. Libraries were sequenced on an Illumina MiSeq and data was analyzed by PiVAT.

Results: Overall the assay performed well with a mapping rate of 99.4%, on-target rate of 99.1%, and coverage uniformity >0.2x mean coverage of 93.5%. With respect to CNV calling, the assay and software detected 100% of ERBB2 amplification negative samples as confirmed by DISH. For amplification positive samples, the NGS assay detected 100% of positive samples. The software correctly identified 91% of amplification positive samples. PiVAT correctly called all samples with a normalized gene count >1.5.

Conclusions: The ONCO/Reveal Multi-Cancer with CNV Panel is a robust assay for the detection of CNVs, SNVs, and Indels across multiple cancer types. The workflow is streamlined, with same day loading of finished libraries when starting from as little as 5ng of isolated input DNA. The assay and software demonstrate detection of low CNV with the recommended cutoff set at 1.5. The detection and identification of CNV gives a more comprehensive overview of the mutations present in a sample, supporting better clinical management of patients.

Methods and Assay Design

ONCO/Reveal Multi-Cancer with CNV Panel (59 genes / 309 amplicons)						
ABL1	CTNNB1	FGFR3	IDH2	MPL	PIK3CA	SMO
AKT1	DDR2	FLT3	JAK2	MYC	PTEN	SRC
ALK	EGFR	FOXL2	JAK3	NOTCH1	PTPN11	STK11
APC	ERBB2	GNA11	KDR	NPM1	RAC1	TP53
ATM	ERBB4	GNAQ	KIT	NRAS	RB1	VHL
BRAF	EZH2	GNAS	KRAS	NTRK1	RET	
CDH1	FBXW7	HNF1A	MAP2K1	NTRK2	ROS1	
CDKN2A	FGFR1	HRAS	MET	NTRK3	SMAD4	
CSF1R	FGFR2	IDH1	MLH1	PDGFRA	SMARCB1	

Note: CNV detected for genes in red.

Figure 1: The ONCO/Reveal Multi-Cancer with CNV Panel is a 309-amplicon panel detecting SNVs and indels in 59 gene and CNV in four key genes: EGFR, ERBB2, MET, and MYC.

Panel Attributes	
Enrichment chemistry	Multiplex PCR using tiled amplicons
Number of pools	1 pool
Number of genes/amplicons	59 / 309
Total panel size	25 kb
Variant types	SNVs, indels, CNV
Average amplicon size	143 bp
Input DNA range	5 ng to 80 ng (10ng recommended)
Sample types	Tissue or blood; FFPE
Mapping rate	99.4% ± 2.1%
% on-target aligned reads	99.1% ± 0.4%
Coverage uniformity (% targets with >0.2X mean coverage)	93.5% ± 4.1%

Figure 2: The ONCO/Reveal Multi-Cancer with CNV panel demonstrated high mapping, on target, and coverage uniformity.

Results and Conclusions

NGS vs DISH ERBB2 amplification calls

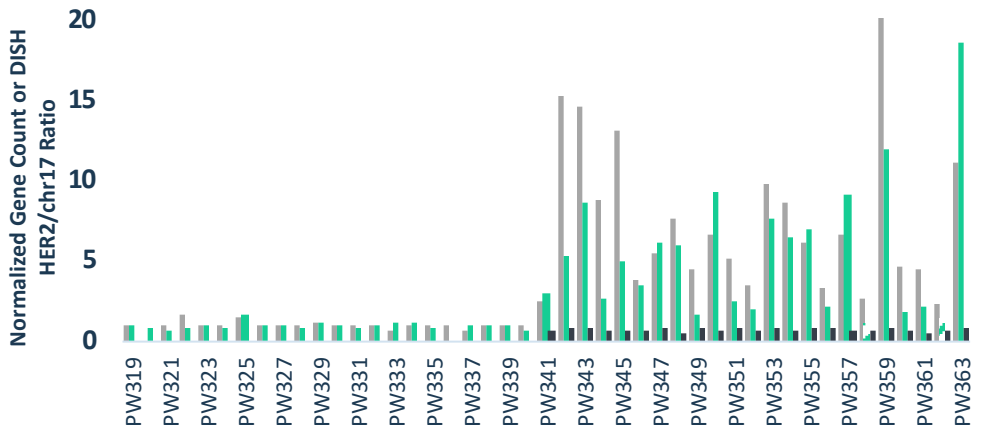

Figure 3: Concordance to DISH was demonstrated for amplification negative samples, amplification positive samples, and matched normals from the positive samples.

ONCO/Reveal Multi-Cancer with CNV Panel

Figure 4: The assay and software demonstrated concordance to DISH for all calls above 1.5x copies. The software missed two low positive results. However, with further algorithm refinement it is expected that the limit of detection can be further lowered.

DISH amplification call	NGS assay concordant call	NGS assay and software concordant call
Positive	100% (23/23)	91% (21/23)
Negative	100% (22/22)	100% (22/22)

ONCO/Reveal Multi-Cancer w CNV Panel Summary

- The assay and software are able to detect CNV down to a recommended cutoff of 1.5 and demonstrated high concordance to DISH
- The panel is robust and sensitive for the detection of CNVs, SNVs and indels across multiple cancer types
- The assay features a streamlined workflow to quickly provide actionable results to care team
- CNV detection is beneficial to support better clinical management of cancer patients