Evaluation of a Targeted NGS Panel using Single-Vial Amplification of Candidate Genes in Solid Tumors

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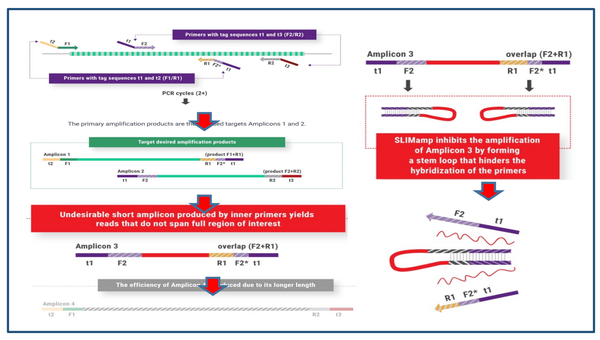
INTRODUCTION

The use of Targeted Next Generation Sequencing (NGS) assays for detection of variants with therapeutic, diagnostic and prognostic potential is well established. Recently, the association of variants in genes leading to DNA damage repair deficiency with response to immunotherapy has expanded the utility of NGS panels. We customized a 47 gene panel using single tube Stem-Loop Inhibition Mediated Amplification (SLIMamp™) technology (Pillar Biosciences) for detection of informative variants in tumors including , but not limited to NSCLC, colorectal and pancreatic cancer, GIST, melanomas, gliomas and thyroid tumors. The 24kb panel, covered hotspots in most genes and entire CDS in 3 genes with oncogenic potential. Robust performance with minimal DNA input and limited neoplastic material was central to the experimental design.

METHODS

Accuracy, precision, reproducibility and sensitivity were assessed using 110 patient samples and 15 controls. The assay was challenged with total DNA input of 2-3ng obtained from tumors with 10-20% of neoplastic cells. The variant allelic fractions of the samples interrogated ranged from 2.5% to >80%. Specificity was checked using GIAB NA12878. Quality Control was monitored using a engineered control with multiple targeted variants having VAF ranging from 4% - 15%. For each run, up to 24 samples were normalized, pooled and run using the MiSeq reagent kit V2 (Illumina). Data analysis including sequence alignment, variant calling and annotation was performed using FASTQ files, with the Pillar Variant Analysis Toolkit (PiVAT).



Schematic of SLIMamp technology: Increased specificity is obtained by inhibition of amplification of the stem-loop structure.

ACCURACY

VARIANT	V-20-03252019 NA12878 15ng VAF	V-20-03252019 NA12878 15ng Total Coverage	V-21-03252019 NA12878 10ng VAF	V-21-03252019 NA12878 10ng Total Coverage	V-22-03252019 NA12878 5ng VAF	V-22-03252019 NA12878 5ng Total Coverage	V-15-03282019 NA12878 5ng VAF	V-15-03282019 NA12878 5ng Total Coverage	V-16-03282019 NA12878 5ng VAF	V-16-03282019 NA12878 5ng Total Coverage	V-17-03282019 NA12878 5ng VAF	V-17-03282019 NA12878 5ng Total Coverage	AVERAGE VAF(%)	SD
EGFR NM_005228.4: c.1498+22A>T	99.62	7986	99.73	8288	99.76	7406	99.68	6844	99.55	7942	99.61	9334	99.66	0.08
EGFR NM_005228.4: c.2361G>A	49.92	17692	50.52	17776	50.35	19146	49.66	14266	51.88	18882	49.48	21198	50.30	0.87
FGFR3 NM_000142.4: c.1953G>A	99.08	5886	99.23	5740	99.44	5702	99.41	4756	99.64	6194	99.38	7102	99.36	0.19
PDGFRA NM_006206.5: c.1701A>G	99.92	7068	99.85	6654	99.81	5274	99.87	5978	99.97	6004	99.83	7252	99.88	0.06
PDGFRA NM_006206.5 c.2472C>T	50.24	15670	46.92	13870	47.86	13966	50.07	11736	48.85	13962	48.7	17106	48.77	1.27
POLE NM_006231.3: c.3156G>A	99.52	13846	99.62	13302	99.48	13124	99.65	11364	99.47	13878	99.6	15500	99.56	0.08
POLE NM_006231.3: c.1359+43G>A	99.78	2712	98.79	2806	99.31	2906	99.52	2490	99.28	3056	99.34	3322	99.34	0.33
POLE NM_006231.3: c.916G>T	48.7	13044	50.33	13348	50.12	13808	50.94	11108	49.45	14122	49.26	15656	49.80	0.81
PTEN NM_000314.4: c.1026+32T>G	47.5	2720	48.13	2722	50.77	2734	53.39	1210	49.19	1732	48.02	1920	49.50	2.23
RAC1 NM_006908.4: c.107+27C>T	50.95	8600	48.68	8048	51.63	7224	51.21	7490	54.09	7802	49.76	9152	51.05	1.84
RAC1 NM_006908.4: c.107+39C>T	50.87	8602	48.66	8052	51.59	7226	51.15	7492	54.04	7802	50.01	9162	51.05	1.79
RET NM_020975.4: c.2307G>T	99.85	19010	99.81	18016	99.82	19184	99.78	14502	99.79	18246	99.8	22426	99.81	0.02
TERT NM_198253.2: c.-3397>C	52.87	1706	56.31	1790	52.77	1838	50.97	1334	48.01	2008	54.05	2272	52.50	2.82

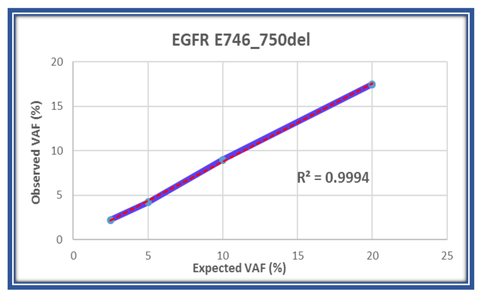
Accuracy of GIAB NA12878 for determination of well-characterized variants in triplicate at varying DNA input

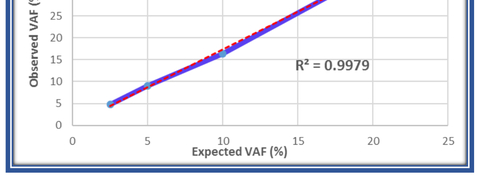
REPEATABILITY

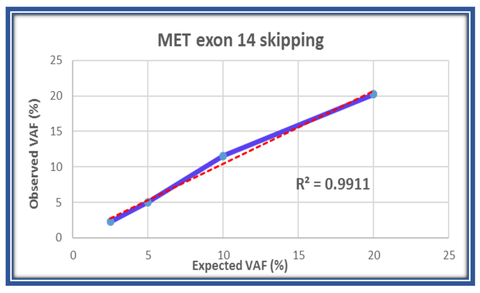
	DAY 1 REPLICATES 1	2	3	DAY 2 REPLICATES 1	2	3	DAY 3 REPLICATES 1	2	3	AVERAGE	ST. DEV
	VAF %			VAF %			VAF%			VAF(%)	
EGFR L858R	9.2	10.93	9.52	10.56	10.41	10.57	9.96	9.72	10.19	10.12	0.56
BRAF V600E	10.22	9.89	9.32	9.89	10.02	9.7	10.37	10.55	10.54	10.06	0.41
KRAS G12V	4.98	4.35	4.69	4.73	5.12	5.07	4.89	4.42	5.54	4.87	0.37
PIK3CA H1047L	6.05	6.63	6.23	5.85	6.76	6.29	6.44	6.75	6.32	6.37	0.31
KRAS G13D	12.73	12.36	12.23	11.92	11.9	12.06	12.55	11.96	11.56	12.14	0.36
EGFR E746_A750del	9.7	9.11	9.31	10.09	9.95	9.72	9.32	9.7	8.75	9.52	0.43
MET c.3082+1G>T	5.48	5.76	5.97	6.09	6.29	6.33	6.31	6.3	6.89	6.16	0.40
TERT C228T	9.2	9.11	8.29	8.13	7.8	7.3	8.6	8.42	8.95	8.42	0.63
KIT L576P	18.04	16.43	17.7	17.84	17.23	17.44	17.37	16.93	17.49	17.39	0.49
KIT Q554_L558del	17.11	16.73	16.31	16.9	17.51	17.03	16.01	16.43	16.07	16.68	0.51
IDH1 R132C	5.16	6.04	5.87	4.4	4.55	4.93	5.38	5.38	5.36	5.23	0.54
NRAS G12D	17.53	18.22	18.61	17.13	17.97	17.92	17.97	17.19	17.43	17.77	0.49

Repeatability (Precision and Reproducibility) for detection of low VAF(%) actionable variants

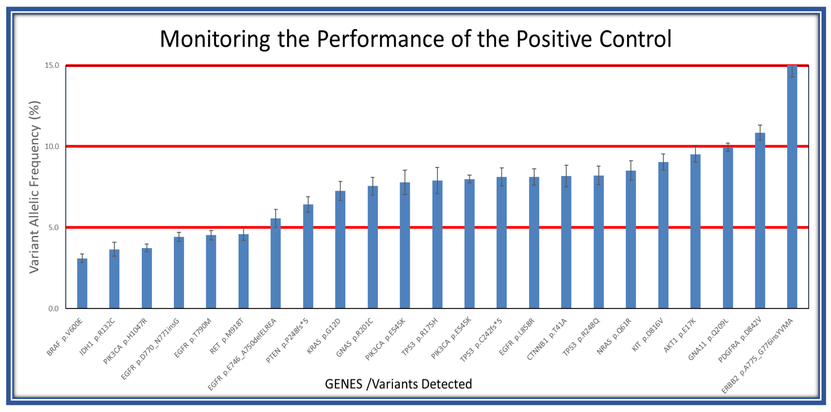
SENSITIVITY



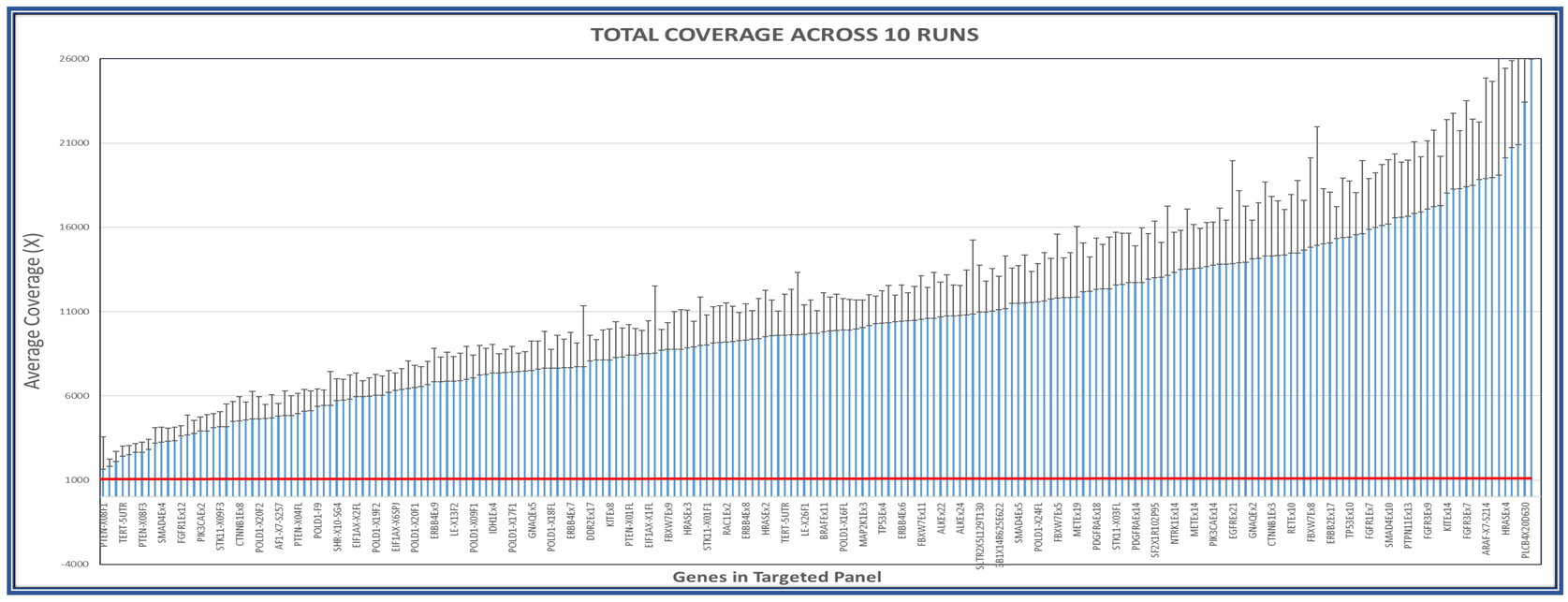




Sensitivity for accurate identification of complex variants; Indels in EGFR; promoter variants in TERT and exon 14 skipping in MET, present in patient samples diluted in a background of wildtype DNA



Quality Control for monitoring of 23 clinically relevant variants, including indels at VAF(%) in the Positive Control close to the lower limit of detection



Coverage Distribution across 221 amplicons present in the assay. The graph represents the average + standard deviation for 225 samples in 11 runs

CONCLUSIONS

- ❑ Interrogation of variants in solid tumors using the SLIMamp technology for identification actionable alterations, including missense variants and indels in tumors with minimal amount of neoplastic tissue showed robust analytical performance.
- ❑ Assays that use the technology can reliably detect variants with 2.5% VAF in samples with low input DNA.
- ❑ The easy workflow of a single-vial library preparation coupled with a rapid turn-around-time of 3-4 days from sample to answer, allows for viable implementation of SLIMamp technology in molecular laboratories.
- ❑ Single-vial technology reduces workflow errors.
- ❑ Variant analysis using PiVAT software provides rapid annotation and interpretation of genomic alterations.

RESULTS

- All samples that were previously reported in clinically validated assays showed concordant results in the NGS assay using SLIMamp™ technology
- Total DNA input for library preparation ranged from 1ng → 60 ng. The accuracy (PPV and NPV) for determination of variants was 100%
- The mutant allele fraction (MAF) percentage in the samples tested, ranged from 3% to 80%.
- The average coverage obtained across all specimens tested was >10,000X. (Range of coverage = 1639X →25,970X)
- The “on target” percentage of the assay was >99%
- The accuracy for determination of well-characterized NA12878 variants was excellent
- The standard deviation for Precision and reproducibility studies ranged from 0.3-0.6
- Sensitivity studies demonstrated that missense variants and indels with VAF of 2.5% or more were reliably detected at 2.5 ng input DNA.