Sensitive One-Tube Solution for Cell-Free DNA-RNA Detection Based on Targeted SLIMamp™ NGS Technology

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ABSTRACT

Introduction Sensitive profiling of cfDNA-cfRNA (cfTNA) has shown promise in precision oncology. By making serial sampling for monitoring tumor progression possible, cfTNA variant detection can provide insights on actionable genomic alterations during clinical treatment., These technologies allow for MRD detection, which requires a lower limit of detection (LoD). We present an ultra-sensitive one-tube solution for (cfTNA) detection based on targeted NGS, where cfDNA (SNV/Indel) and cfRNA (fusion) are detected without separate preparations. Our SLIMamp™-based oncoReveal cfTNA Panel makes automated streamlined operation possible for rapid and robust cfTNA profiling.

Methods The 131-amplicon based cfDNA section covers 9378 hotspots across 31 genes. Unique molecule barcodes (UIDs) are attached on the reversed primers. The cfRNA section covers common fusions between 27 genes.
To evaluate the sensitivity of the cfTNA Panel, we studied 7 confirmed positive samples, including 4 SNV/Indel samples and 3 fusion samples. Three out of the 4 positive SNV/Indel samples were known to have 2 variants. 24 normal samples were pooled to one baseline sample, which was mixed with each positive sample with different ratios to create lower variant frequencies. All SNV/Indel and negative samples were extracted from fresh plasma. The SNV/Indel frequency ranges from 0.07% to 1% and fusion frequency ranges from 0.2% to 20%. All diluted SNV/Indel samples were tested at 5, 10 and 30ngs, and each input had 3 independent replicates. Input of fusion samples was 10ng. The final library was sequenced to an average depth of 200,000x and lower depths were achieved by downsampling. Fastq files were directly processed by our proprietary software, PiVAT, where both SNV/Indel and Fusion calls are reported.

Results For cfDNA, the UID molecular rescue rate is ~100% for 5ng input and ~60% for 30ng with ~50,000x average sequencing depth. When running 3 replicates, the sensitivity at 0.07%-0.09% for SNVs is 100% with 5ng input. Without running replicates, the sensitivity at 0.07%-0.09% is 77.8% with 10ng input and 100% with 30ng input. For mutations at higher VAFs, the sensitivity is 100% across all inputs. The sensitivity and the LoD are consistent for average sequencing depth from 50,000x to 200,000x. For cfRNA, we detected fusion at 1% reliably with similar sequencing depths.

Conclusions The oncoReveal cfTNA Panel demonstrates 0.07% LoD for SNV/Indel detection with 30ng input and 1% LoD for fusion detection with 10ng input. The complete profiling of cfTNA enables one-step tumor progression monitoring. This one-tube solution for cfTNA detection simplifies lab processing and reduces required input and testing cost. With increased input, our panel has the potential to achieve 0.01% LoD for MRD detection.

ASSAY DESIGN AND ADDITIONAL CLAIMS

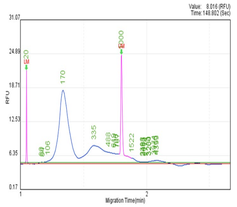
PillarHS cfTNA Panel Specification	
Enrichment chemistry	SLIMamp™
Number of pools	1 pool
Sample types	cfTNA/cfDNA/ctDNA/ccfDNA
SNV/Indel: Number of genes/amplicons	**31/131**
Fusion: # of Driver genes/amplicons	**8/28**
Variant types	SNVs, indels
Average amplicon size	71bp
DNA input range	5 ng to 50 ng
Mapping rate	98.4% ± 0.7%
% on-target aligned reads	96.3% ± 0.3%
Coverage uniformity (% targets with >0.2x mean coverage)	95.7% ± 0.3%
Total assay time (from DNA to sequencer)	<9-10 hours
Sequencing platforms	Illumina®

PillarHS cfTNA Gene List (SNV/Indel)					
AKT1	EGFR	FGFR2	KIT	NTRK1	PTEN
ALK	ERBB2	GNAS	KRAS	NTRK3	ROS1
APC	ERBB3	HRAS	MAP2K1	PDGFRA	SF3B1
ARAF	ESR1	IDH1	MET	PIK3CA	SMAD4
BRAF	FBXW7	IDH2	NRAS	PPP2R1A	TP53
CTNNB1					

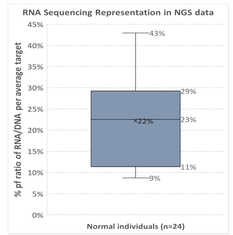
Driver	Fusion Partners			
ALK	EML4	KIF5B	TPM3	STRN
RET	CCDC6	KIF5B	NCOA4	PRKAR1A
ROS1	CD74	SLC34A2	EZR	
FGFR3	TACC3	BAIAP2L1		
NTRK1	TPM3	TPR	LMNA	
NTRK2	STRN			
NTRK3	ETV6			
MET	Ex14 skipping			

Panel Performance

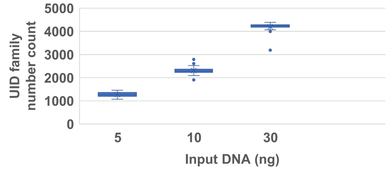
cfDNA QC (Representative)



RNA Sequencing Representation in NGS data



UID family numbers at mean coverages of ~ 50K x



Fusion Detection (Sample Input: 10ng)			
Expected VAF	0.50%	1.0%	4.0%
Fusion Sample [KIF5B (ex15)-RET(ex12)]#	0.58	17.15	29.12

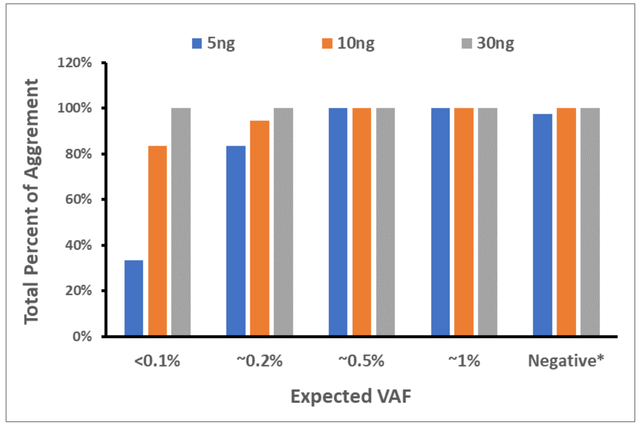
#:The numerical value in the cell is Fusion reads per 100,000 mapped DNA+RNA reads

Input	Mean of Average Accepted UID	Min. Average Accepted UID	Max Average Accepted UID	Total Lib#	GE	Conversion Rate
5 ng	1270	1068	1458	27	1500	84.7%
10 ng	2304	1902	2787	35	3000	76.8%
30 ng	4195	3186	4389	27	9000	46.6%*

*: one sample analyzed with deeper sequencing results in 76%
- When sequencing depth is sufficient our cfTNA assay has a ~80% efficiency for converting unique molecules into filtered sequencing reads

RNA yield evaluation:
cfRNA is difficult to quantitate with traditional methods (representative cfDNA Bioanalyzer electropherogram shown above). Therefore, RNA yield is measured by the ratio of the mean coverage of the RNA expression controls to the mean coverage of the DNA amplicons in the NGS assay.

RESULTS AND CONCLUSIONS



EGFR Exon 19 deletion					
Expected VAF	5ng	10ng	30ng	Expected# in each input	Agreement with all inputs
~0.2%	3	3	3	3	100% (9/9)
~0.5%	6	6	6	6	100% (18/18)
~1%	3	3	3	3	100% (9/9)
Negative	15	15	15	15	100% (45/45)

EGFR L858R					
Expected VAF	5ng	10ng	30ng	Expected# in each input	Agreement with all inputs
~0.2%	6	6	6	6	100% (18/18)
~0.5%	6	6	6	6	100% (18/18)
Negative*	14	15	15	15	98% (44/45)

** the one false positive was detected below 0.04% and all other positives.*

EGFR T790M					
Expected VAF	5ng	10ng	30ng	Expected# in each input	Agreement with all inputs
<0.1%	2	5	6	6	72% (13/18)
~0.2%	6	8	9	9	85% (23/27)
~0.5%	3	3	3	3	100% (9/9)
Negative	9	9	9	9	100% (27/27)

Conclusions:
Pillar's oncoReveal™ cfTNA Panel demonstrates:
- ~ 0.1% LoD for SNV/Indel detection with 30ng input.
- Detects fusion RNA down to 0.50% in DNA VAF with 10ng input.
- Simplified workflow with **robust SLIMamp™ chemistry** that tests cfDNA and cfTNA in **one tube.**