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Poster #3732

Analytical Performance of TruSight® Tumor 170 on Small Nucleotide Variations and Gene Amplifications Using DNA from Formalin-Fixed, Paraffin-Embedded (FFPE) Solid Tumor Samples

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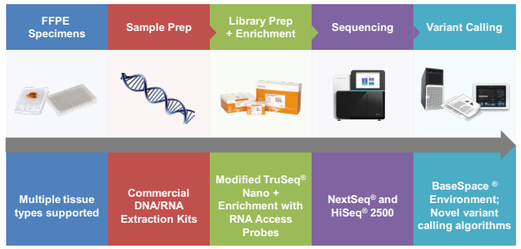
INTRODUCTION

Expanding the paradigm of solid tumor profiling from single-gene testing to comprehensive panels presents many challenges. One of these challenges is the ability of these panels to detect genetic alterations from FFPE samples, where the DNA is of low abundance and often heavily compromised. Despite these challenges, next-generation sequencing (NGS) offers the ability to assess variants in an ever-expanding list of relevant genes from one sample at one time. To that end, Illumina developed a comprehensive, hybrid capture-based NGS assay targeting 170 key cancer genes. The assay consists of a DNA workflow for the identification of single-nucleotide variants (SNVs), small insertions and deletions (indels), multiple nucleotide variants (MNVs), gene amplifications (CNVs), as well as a RNA workflow for the identification of splice variants and gene fusions.

Following sequencing on the NextSeq® or HiSeq® instruments, TruSight Tumor 170 offers push-button variant calling. The analytical sensitivity and specificity of TruSight Tumor 170 was assessed on 95 unique samples (each in duplicates), including FFPE samples of varying quality from multiple tissue types, reference standards, and cell line and FFPE mixes. We demonstrate the TruSight Tumor 170 is able to detect multiple variant types within a single sample at low nucleic acid input, while exhibiting high sensitivity and specificity for low allele fraction detection.

WORKFLOW AND BIOINFORMATICS



- 2-day flexible workflow
- Low input requirements
- GMP (Good Manufacturing Practice) manufactured probes for 170 genes
- Variant calling optimized for FFPE-extracted nucleic acid
  - Target ≥95% sensitivity and specificity with small variants at 5% allele frequency at positions with sufficient sequencing coverage (≥250x).
  - Target ≥95% specificity and sensitivity for copy number variants at a copy number change relative to diploid of ≥2.2, for example in samples with 30% tumor cells with 10 copies.

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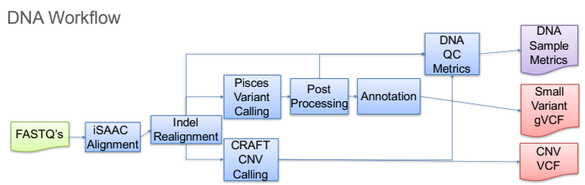
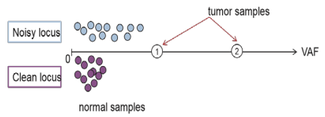
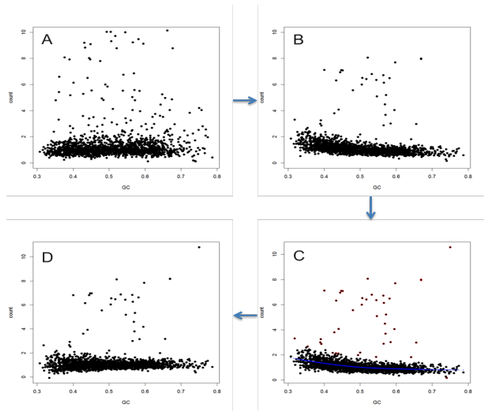
WORKFLOW AND BIOINFORMATICS

DNA Workflow





Variant post processing against a collection of normal samples to filter out false positive variant calls at noisy sites.



CRAFT (CNV Robust Analysis For Tumors) CNV calling normalization steps. (A) due to the large baseline effect, there is no visible relationship between exon bin count and GC. (B) after baseline correction, there is a visible negative trend between count and GC. (C) Outliers are identified and loess regression is fitted on outlier removed data. (D) Final normalization results after removing GC bias.

ANALYTICAL PERFORMANCE

Small variant calling sensitivity and specificity

A total of 614 small DNA variants, composed of 530 SNVs, 80 indels (including deletions up to 30 bp and insertions up to 31 bp), and 4 MNVs were tested with two reagent lots (B and D). The variant allele frequencies tested ranged from 3% to 35% for the 613 variants, with a small fraction (3%) of the variants below 5%, more than 2/3 (70%) between 5% and 15%, and almost 1/3 (27%) > 15%.

		Sensitivity (x/n) Lot B	Sensitivity (x/n) Lot D
small variants (SNVs, indels, MNVs)	Overall*	99.5% (596/599)	99.3% (606/610)
	VAF ≥5%**	99.7% (581/583)	99.5% (591/594)
SNVs	Overall*	99.8% (514/515)	99.6% (525/527)
	VAF ≥5%**	99.8% (505/506)	99.6% (516/518)
Indels	Overall*	98.8% (79/80)	98.7% (78/79)
	VAF ≥5%**	98.6% (73/74)	98.6% (72/73)
MNVs	Overall*	100% (3/3)	100% (3/3)
	VAF ≥5%**	100% (3/3)	100% (3/3)

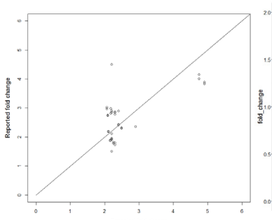
*Overall numbers include variants with VAF <5%, and are included for information use only.
**VAF (Variant Allele Frequency) measured by orthogonal methods.

Normal Sample	Sample Type	Lot B Passing Variants*	Lot B Passing Sites	Lot B Specificity	Lot D Passing Variants*	Lot D Passing Sites	Lot D Specificity
NA20431	Cell line	12	490419	100.00%	13	498453	100.00%
NA21070	Cell line	4	504193	100.00%	7	505087	100.00%
NA21660	Cell line	14	487971	100.00%	17	492410	100.00%
NA21677	Cell line	5	451154	100.00%	10	502014	100.00%
NA21687	Cell line	18	400194	100.00%	12	507277	100.00%
NA21730	Cell line	10	492429	100.00%	11	496694	100.00%
NA21731	Cell line	16	425851	100.00%	5	498931	100.00%
NA21781	Cell line	9	508985	100.00%	9	509714	100.00%
NA21833	Cell line	24	360927	99.99%	7	494516	100.00%
NA21846	Cell line	33	473596	99.99%	33	496078	99.99%
ASHSON	Cell line	105	457831	99.98%	106	496597	99.98%
ASNSON	Cell line	23	510555	100.00%	14	522038	100.00%
1914	FFPE	396	443340	99.91%	400	453184	99.91%
1944	FFPE	196	488259	99.96%	188	492577	99.96%
1945	FFPE	300	480429	99.94%	320	477363	99.93%
1954	FFPE	450	471933	99.91%	358	476728	99.93%
1955	FFPE	356	436322	99.92%	685	446763	99.85%
2111	FFPE	227	456570	99.95%	523	443216	99.88%
2112	FFPE	258	458854	99.94%	443	452766	99.90%
2121	FFPE	330	509289	99.94%	285	516577	99.95%
2122	FFPE	185	484966	99.96%	185	472330	99.96%
2127	FFPE	272	477934	99.94%	344	465750	99.93%
2135	FFPE	214	449637	99.95%	564	393814	99.86%
Overall Specificity			**99.97%**			**99.96%**	
				99.96%			

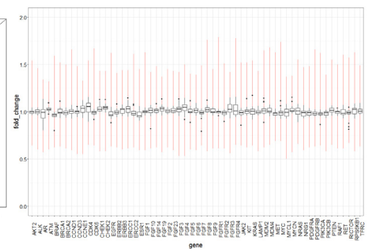
*excluding known polymorphism sites

Copy Number Variant calling sensitivity and specificity

		Lot B			Lot D		
	Total	Called	Missed	Sensitivity	Called	Missed	Sensitivity
CNV	43	21	1	95.50%	20	1	95.20%
Combined Sensitivity	95.30%						



*expected fold changes measured by orthogonal methods



No CNV called in 24 normal samples (in duplicates)
CNV specificity = 100%

CONCLUSION

TruSight Tumor 170 can achieve high sensitivity and specificity for the detection of somatic variants (small variants and CNVs) from DNA extracted from FFPE tissues.

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