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Poster #565

Performance of TruSight® Tumor 170 for Fusions and Splice Variants in FFPE RNA Tumor Samples

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INTRODUCTION

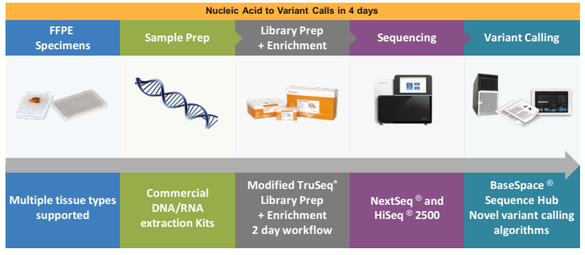
Recent studies have highlighted the importance of gene fusions and splice variants in solid tumor profiling. Next-generation sequencing (NGS) can be an effective means of detecting these alterations in formalin-fixed, paraffin-embedded (FFPE) samples when using RNA rather than DNA, as a single RNA transcript could result from numerous alterations in DNA. To that end, Illumina developed TruSight Tumor 170, a comprehensive, hybrid capture-based NGS assay targeting 170 key cancer genes. Along with a DNA workflow, the assay includes a RNA workflow for the identification of splice variants and gene fusions.

There is not yet a standard definition for the limit of detection (LoD) in detecting gene fusions and splice variants from NGS data. We propose to define the LoD of a fusion calling and splice variant NGS panel as the lowest molecule count of a chimeric transcript that could be reliably detected with a sufficient number of supporting sequencing reads. To determine the LoD of TruSight Tumor 170 using this definition, we mixed multiple cell lines expressing a panel of known fusions and splice variants to measure the copy number of each chimeric transcript.

MATERIALS AND METHODS

Next-generation sequencing (NGS) offers the ability to assess variants in multiple genes using one sample. To that end, Illumina is developing a comprehensive, hybrid capture-based NGS assay targeting 170 key cancer genes for sequencing on the NextSeq® platform or HiSeq® 2500 at equivalent sequencing depth. The TruSight Tumor 170 panel consists of a DNA workflow for the identification of single-nucleotide variants (SNVs), small insertions and deletions (indels), and CNVs, as well as a panel of 55 genes for an RNA workflow for the identification of splice variants and gene fusions. As this is an enrichment assay, fusions and splice variants do not need to be known *a priori*. As long as a fusion event or splicing event happens near an exon from a gene in the panel, the assay can pull down reads supporting the event.

Figure 1: TruSight Tumor Workflow



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MATERIALS AND METHODS

Table 1. Variant calling results for samples in mixes

Sample ID 1 - Sample ID 2	Variant	Min CN/ng detected (ddPCR)	Minimum CN/ng tested (ddPCR)	Called in all 5 CN/ng mixes
LC-2/ad – 22RV1	CCDC6-RET	5	5	Yes
	ARv7	4.2	1.8	Yes
Hs746T - RT-4	MET exon 14 skipping	3.5	3.5	Yes
	FGFR3-TACC3	9.4	1.7	Yes
VCaP - RL	ARv7	3.1	2.1	Yes
	BCL2-IGHJ6	257.1	2.4	No[1]
	GFOD2-ERG[2]	1.4	0.7	Yes
MCF7 - NCI-H228	ALK-PTPN3	4	1.4	Yes
	RPS6KB1-VMP1	5.1	2.4	No[3]
	EML4-ALK	0.5	0.5	Yes
343-621	TMPRSS2-GNPTN	4.6	2.5	Yes
	RPS6KB1-VMP1	1.5	0.3	Yes
	TMPRSS2-ERG	0.02	0	Yes
	RPS6KB1-DIAPH3	1.9	0.6	Yes
	CCDC170-ESR1	18.8	0.3	Yes
664-623	EML4-ALK	6.1	2.1	Yes
	TMPRSS2-ERG	0.4	0.4	Yes
	ALK-PTPN3	2.1	2.1	Yes
	GFOD2-ERG	6.9	0.04	No[4]
434-497	TMPRSS2-ERG	2.7	2.7	Yes
449-344	EML4-ALK	2.9	2.9	Yes
	TMPRSS2-ERG	0.3	0.3	Yes

1. BCL2-IGHJ6 has higher limit of detection than other fusion types due to unexpected insertion between two halves of fusion. See text for more detail.
2. GFOD2-ERG is a lowly expressed unexpected fusion in the VCaP cell line at 4.1 copies per ng RNA input. Due to this, detection within the mixes was highly variable. Although the fusion could be detected even at 1.4 copies per ng of input, it was only able to be reliably called within the unmixed cell line.
3. RPS6KB1-VMP1 missed due to informatics error caused by the middle of the contig not being correctly reported by the fusion caller.
4. GFOD2-ERG at 6.9 missed due to informatics error caused by error in assembly overlapping repeat region

Table 2. Variant calling results for unmixed samples

Sample ID	Variant	CN/ng RNA	Variant called
504	EGFR vIII	0.5	Yes
355	EGFR vIII	0.3	No
461	EWSR1-FLI1	2.9	Yes
455	EWSR1-FLI1	2.9	Yes
462	EWSR1-FLI1	0.5	Yes
466	FOXO1-PAX3	1.3	Yes

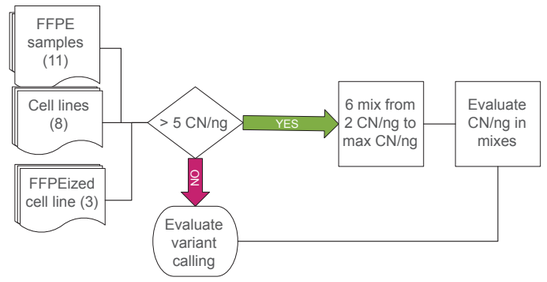
Table 3: Overall sensitivity and specificity

Criteria	Requirement	Result
Sensitivity for Fusions	≥ 95%	98.10%
Sensitivity for Splice variants	≥ 95%	100%
Specificity for Fusions	≥ 95%	96%
Specificity for Splice Variants	≥ 95%	100%

Fusion and splice variant calling were first optimized against simulated read data from 349 fusions from COSMIC cancer database matched to a gene in the TruSight RNA panel and a set of 13 known splicing variants which are cancer relevant. A hybrid approach of read alignment and assembly was used to enhance the fusion calling sensitivity. After initial prototyping, further enhancements were made based on data from FFPE samples to handle error modes such as false positive (FP) calling from sequence homologs, polymerase read-through, or FFPE artifacts.

To demonstrate the analytical sensitivity and specificity of this NGS based assay, we compiled a panel of 49 mixed samples and validated the molecule count of variant transcripts to be near the LoD of 5 copies per ng RNA input (CN/ng). by Droplet Digital PCR (ddPCR). For understanding the limit of blank (LoB) of the assay, another panel of 40 samples not harboring these fusions and splice variants was also assessed by TruSight Tumor 170. Any passing fusions were considered FPs and any known oncogenic splice variants were considered FPs.

Figure 2: LoD mixing scheme



RESULTS

As shown in Table 1, every fusion and splice variant was able to be called at 5 copies per ng of RNA with the exception of two which were due to informatics limitations and BCL2-IGHJ6, which was found to be a unique fusion with a 23bp insert in between the two fusion genes. Sensitivity and specificity for both splice variant calling and fusion calling is above 95%. These results indicate that the TruSight Tumor 170 panel analysis can identify lowly expressed fusions and splice variants from a small amount of compromised RNA from solid tumor samples at high sensitivity and specificity.

CONCLUSION

Through examining limit of detection in the context of RNA expression, this study shows that TruSight Tumor 170 provides high sensitivity and specificity in RNA variant calling down to 5 copies of transcript per ng of input.

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