Splice site and exon deletion variants detection with Oncomine RNA sequencing research panel

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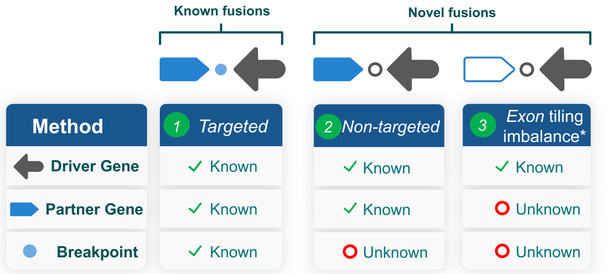
INTRODUCTION

Exon skipping events caused by aberrant RNA splicing, and exon deletion events may play an important role in oncogenesis. Herein, we describe a pan-cancer NGS RNA sequencing Oncomine™ panel, which features *FusionSync* (Fig. 1) technology for broad detection of known and novel gene fusions important in cancer research, and detection of >40 intragenic rearrangement variants (e.g., exon skip and deletion), including reporting of *MET* exon14 skip and *EGFR*vIII variants. The combined RNA assay strategy is aimed at providing a wide research scope and detection capacities of fusions, exon skip and exon deletion variants.



** Available for ALK, FGFR2, NTRK1, NTRK2, NTRK3, and RET fusion drivers*

Figure 1. *FusionSync* combined approach for detection of known and novel gene fusions.

MATERIALS AND METHODS

As part of Oncomine Comprehensive Assay Plus development, we designed an expanded Ion AmpliSeq™ RNA fusion panel (Fig. 2) that:

- Targets *MET* exon 14 skipping and *EGFRvIII* (exon deletion) and utilizes an end-to-end bioinformatic solution for reporting these intragenic variants in sample types where they may be detected (such as in NSCLC and Glioblastoma, respectively)
- Targets and reports > 1,300 fusion breakpoints in 49 driver genes
- Leverages the primers in the panel to support detection of non-targeted fusions (i.e., novel combinations of drivers and partners); thus, expanding the potential breadth of fusion isoform detection approximately 10 fold.
- Supports partner agnostic fusion detection in *ALK*, *RET*, *NTRK*1,2,3 and *FGFR*2
- In addition, the panel is supplemented with additional 39 RNA assays for detection of intragenic rearrangements across 7 driver genes: *AR*, *BRAF*, *BRCA1*, *EGFR*, *MET*, *NTRK1*, *RELA*.
- Additional 10 Wild Type (WT) assays in the 7 driver genes with intragenic assays are included in the panel for read normalization.

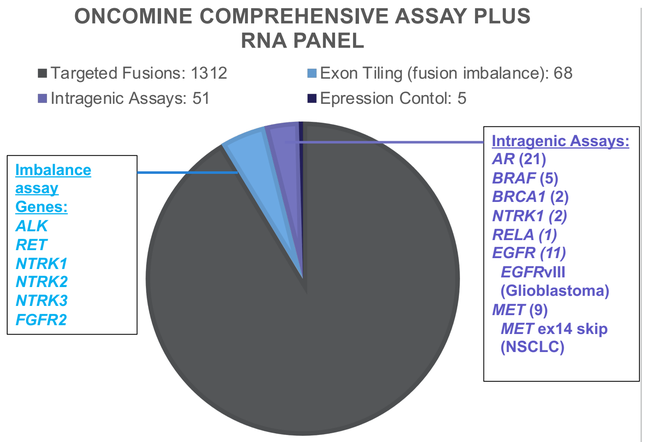


Figure 2. Oncomine Comprehensive Assay (OCA) Plus RNA panel content. Intragenic RNA variants are highlighted on the right. Driver genes with exon-tiling imbalance assays on the left.

RESULTS

Confirmation of *MET* ex14 skip and *EGFR*vIII detection in fusion RNA reference controls (SeraCare V4)

SeraCare V4 (% (Dilution)	Detected fusion isoform	Read Counts	% Normalized reads
100	*MET* ex14 skipping (MET-MET.M13M15.1)	21113	0.517
20		3586	0.061
5		861	0.027
100	*EGFR*vIII (EGFR-EGFR.E1E8.DelPositive)	7998	0.196
20		8424	0.143
5		2029	0.063

Table 1. *MET* ex14 skip and *EGFR*vIII coverage in SeraCare V4 control with and without dilution

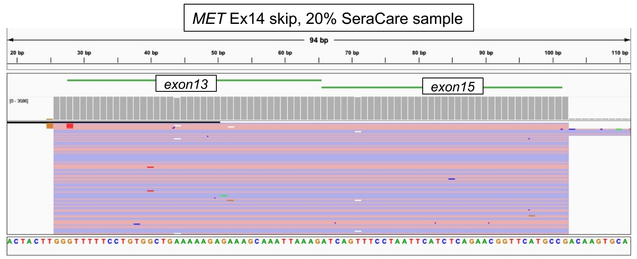


Figure 3. SeraCare V4 sample at 20% dilution, aligned to *MET* ex14 reference (MET-MET.M13M15)

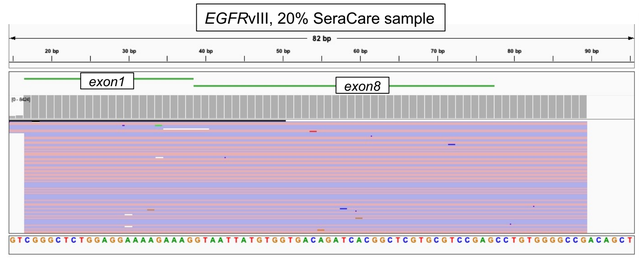


Figure 4. SeraCare V4 sample at 20% dilution, aligned to *EGFR*vIII (EGFR-EGFR.E1E8.DelPositive)

Segregation between positive and negative controls

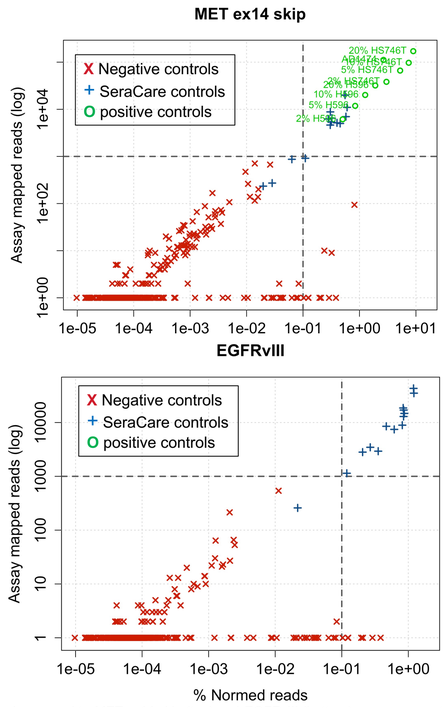


Figure 5. Reads mapped to *MET* ex14 skip (top) and *EGFR*vIII (bottom) versus percentage of normalized reads demonstrating separation between positive and negative controls. For MET ex14 skip, shown in green are 2 cell lines (HS746T, H596) at sample dilutions of 2-20% and an additional FFPE AD1474. Dashed lines mark recommended thresholds for the assay (reads >1000 & normalized reads > %0.1)

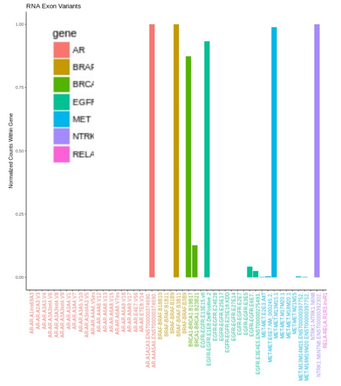
Positive *MET* ex14 skip and *EGFR*vIII detection in lung and brain FFPE research samples

Sample	Tissue	Detected isoform	Mapped reads	% Normalized reads
1	Lung	MET-MET.M13M15.1	208919	11.12
2	Lung	MET-MET.M13M15.1	405413	15.65
3	Lung	MET-MET.M13M15.1	4115	0.158
4	Lung	MET-MET.M13M15.1	2942	0.23
5	Brain	EGFR-EGFR.E1E8.DelPositive.2	376705	13.03
6	Brain	EGFR-EGFR.E1E8.DelPositive.2	8113	0.36
7	Brain	EGFR-EGFR.E1E8.DelPositive.2	66859	2.63
8	Brain	EGFR-EGFR.E1E8.DelPositive.2	232793	9.45
9	Brain	EGFR-EGFR.E1E8.DelPositive.2	32657	0.98
10	Brain	EGFR-EGFR.E1E8.DelPositive.2	161740	4.32
11	Brain	EGFR-EGFR.E1E8.DelPositive.2	169550	5.32
12	Brain	EGFR-EGFR.E1E8.DelPositive.2	21570	1.73

Table 2. Example assay results in FFPE research samples with read coverage and normalized reads at the thresholds depicted in Fig 5, for *MET* ex14 skip and *EGFR*vIII in Lung and Brain samples, respectively.

Visualization of other intragenic RNA assays in Ion Reporter

Figure 6. The assay makes calls only for *MET* ex14 skip and *EGFR*vIII on the basis of empirically derived thresholds. For the remaining 39 intragenic assays, Ion Reporter software users can view the assay results and normalization to WT assays in a barplot (example on the right for SeraCare control) that is generated as a part of the fusion workflow in OCA plus.



CONCLUSIONS

We demonstrate exon deletion and splice site detection technology with an expanded RNA sequencing Oncomine panel that includes over a thousand of gene fusion targets and assays for novel fusion detection.

The approach is compatible with Ion systems, requires low input material, and retains simple workflows and fast turn-around time.

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