A partner agnostic approach for gene fusion detection with targeted next-generation sequencing

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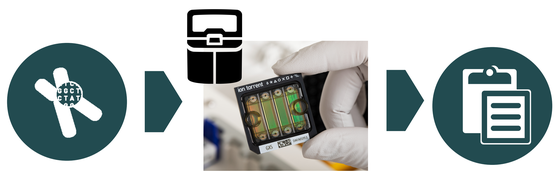
Introduction

Oncogenic fusions are present across multiple cancer types and are important biomarkers for cancer diagnosis and selection of targeted therapies. Ion AmpliSeq™ targeted next-generation sequencing technology successfully detects pre-defined fusion isoforms with high sensitivity and specificity in clinical research samples. Targeted approaches are advantageous in increasing the depth on the most informative regions to detect known clinically relevant fusion transcripts, relying on prior knowledge of partner genes and precise fusion breakpoint positions.

Here we present a complementary transcript-based expression imbalance assay designed to identify gene fusions in a partner agnostic manner. The method utilizes exon-tiling designs to leverage the enhanced expression observed downstream of the fusion breakpoint in the 3' end of driver genes in oncogenic fusion products. RNA sequencing is used to measure expression imbalance between the 3' and 5' ends of the driver gene, while correcting for expression variation and systematic biases.

Materials and Methods

- We designed ~1,000 RNA sequencing amplicons to detect known fusions (Fig. 1) as well as intragenic rearrangement events in *MET*, *EGFR* and *AR*, which are included in the Oncomine™ Precision Assay.
- We supplemented the panel with 60 amplicons tiling the exon junctions of *ALK*, *RET*, *NTRK1*, *NTRK2* and *NTRK3* to measure 3'/5' expression imbalance (Fig. 2).
- We constructed a baseline for imbalance analysis for each gene using normal samples from different tissues.
- Positive and negative fusion samples were assayed on the Ion Torrent™ Genexus™ Integrated Sequencer, followed by fully automated analysis software that performs sample QC, read-filtering, mapping, fusion calling and reporting.



Targeted Fusion Isoform Detection

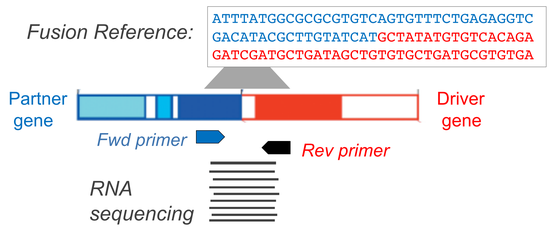


Figure 1. Targeted fusion junction assay design

Results

Confirmation of targeted fusion detection, FFPE control

Fusion RNA	Primary Cancer Tissue	Detected fusion isoform	10% dilution Read counts, r1	10% dilution Read counts, r2
EML4-ALK	Lung	EML4-ALK.E13A20.COSF408.2	578	591
KIF5B-RET	Lung	KIF5B-RET.K24R11.COSF1262.1	571	675
NCOA4-RET	Thyroid	NCOA4-RET.N7R12.COSF1491.1	881	978
CD74-ROS1	Lung	CD74-ROS1.C6R34.COSF1200.1	95	400
SLC34A2-ROS1	Lung, Stomach	SLC34A2-ROS1.S4R34.COSF1198	234	58
TPM3-NTRK1	Lung, Intestine	TPM3-NTRK1.T7N10.COSF1329	1043	1125
FGFR3-BAIAP2L1	Urinary tract	FGFR3-BAIAP2L1.F17B2.COSF1346	657	921
FGFR3-TACC3	Urinary tract, CNS	FGFR3-TACC3.F17T11.COSF1348.1	1116	1311
ETV6-NTRK3	Multiple	ETV6-NTRK3.E5N15.COSF571.2	547	960
LMNA-NTRK1	Skin	LMNA-NTRK1.L2N11	1102	908
SLC45A3-BRAF	Prostate	SLC45A3-BRAF.S1B8.COSF871	437	452
MET exon14 skip	Lung	MET-MET.M13M15.1	513	342
EGFRvIII	Brain	EGFRvIII.E1E8.Del1	677	731
ETV6-NTRK2		ETV6-NTRK2.E5N16	0	0
KLK2-FGFR2		KLK2-FGFR2.K1F5	0	0
TFG-ALK		TFG-ALK.T5A20.COSF426	0	0

Yellow rows indicate negative controls: fusion isoforms not present in the control sample

Table 1. Read counts, Seraseq® FFPE Tumor Fusion RNA Reference Material v2, 10% dilution into FFPE sample

Concordance between targeted isoform detection and imbalance assays, *ALK* fusion FFPE control

Sample	Detected Fusion isoform	ALK fusion imbalance assay result			Imbalance detected in other driver genes
		Predicted fusion breakpoint range	3'/5' Imbalance score	Imbalance p-value	
BON1056	KIF5B-ALK.K17A20.COSF1257	exon15-exon20	5.3	7.00E-04	NO
BON1057	EML4-ALK.E13A20.COSF1062.1	exon15-exon20	5.3	7.00E-04	NO
BON1068	EML4-ALK.E20A20.COSF409.2	exon15-exon20	5.23	2.70E-03	NO
AD1456	**EML4-ALK.E13A20.COSF1062.1**	**exon15-exon20**	**5.3**	**2.00E-03**	**NO**
BON1519	HIP1-ALK.H21A20	exon15-exon20	5.17	7.00E-04	NO
BON1184	EML4-ALK.E6aA20.AB374361.1	exon15-exon20	5.3	7.00E-04	NO
BON1029	EML4-ALK.E6bA20.AB374362.1	exon15-exon20	3.08	6.60E-03	NO
BON1031	EML4-ALK.E13A20.COSF1062.1	exon15-exon20	5.25	7.00E-04	NO
BON1040	EML4-ALK.E13A20.COSF1062.1	exon15-exon20	5.29	7.00E-04	NO
BON1041	EML4-ALK.E13A20.COSF1062.1	exon15-exon20	5.3	7.00E-04	NO
BON1053	KIF5B-ALK.K17A20.COSF1257	exon15-exon20	5.3	7.00E-04	NO
BON1056	KIF5B-ALK.K17A20.COSF1257	exon15-exon20	5.3	7.00E-04	NO
BON1057	EML4-ALK.E13A20.COSF1062.1	exon15-exon20	5.3	7.00E-04	NO
Neg. control	NA	exon4-exon5	0.63	NA	NA

Yellow rows indicate negative controls: fusion isoforms not present in the control sample

Table 3. ALK exon-tiling imbalance results in 11 fusion-positive FFPE controls.

Partner Agnostic Fusion Detection with Exon-Tiling Imbalance Assay

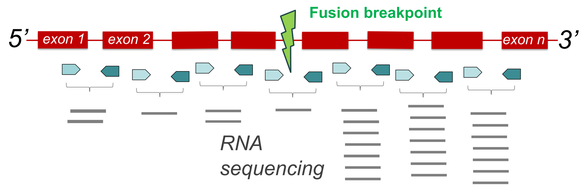


Figure 2. Exon-tiling method for 3'/5' imbalance measurement

Confirmation of NTRK targeted fusion detection, FFPE Control

5' partner	3' partner	Detected fusion isoform	10% dilution Read counts, r1	10% dilution Read counts, r2
TPM3	NTRK1	TPM3-NTRK1.T7N10.COSF1329	421	464
LMNA	NTRK1	LMNA-NTRK1.L11N11.1	366	308
IRF2BP2	NTRK1	IRF2BP2-NTRK1.I1N10	151	218
SQSTM1	NTRK1	SQSTM1-NTRK1.S5N10.1	493	316
TFG	NTRK1	TFG-NTRK1.T5N10.COSF1328	276	280
AFAP1	NTRK2	AFAP1-NTRK2.A14N12	381	456
NACC2	NTRK2	NACC2-NTRK2.N4N13.COSF1448	198	468
QKI	NTRK2	QKI-NTRK2.Q6N16.COSF1446	418	689
TRIM24	NTRK2	TRIM24-NTRK2.T12N15.1	184	362
PAN3	NTRK2	PAN3-NTRK2.P1N17	82	99
ETV6	NTRK3	ETV6-NTRK3.E4N14.1	400	663
ETV6	NTRK3	ETV6-NTRK3.E4N15.COSF823.2	400	330
ETV6	NTRK3	ETV6-NTRK3.E5N14.1	305	432
ETV6	NTRK3	ETV6-NTRK3.E5N15.COSF571.2	328	357
BTBD1	NTRK3	BTBD1-NTRK3.B4N14	217	433
		EML4-ALK.E6aA20.AB374361.1	0	0
		CD74-ROS1.C6R34.COSF1200.1	0	0
		KIF5B-RET.K24R11.COSF1262.1	0	0

Yellow rows indicate negative controls: fusion isoforms not present in the control sample

Table 2. Read counts, Seraseq® FFPE NTRK Fusion RNA Reference Material, 10% dilution into FFPE sample

Example: **Sample AD1456**
Fusion RNA: **EML4-ALK.E13A20.COSF1062.1**

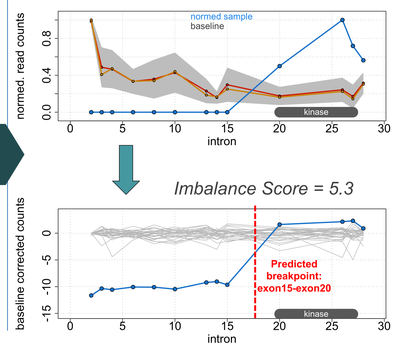


Figure 3. Example for imbalance assay in *ALK* (FFPE sample AD1456).
Top: exon-tiling normalized coverage profile of the sample (blue) relative to a baseline computed from 34 normal samples (grey).
bottom: exon-tiling coverage profile of the sample after baseline correction (blue), relative to negative samples (grey).

Concordance between targeted isoform detection and imbalance assays, *RET* and *NTRK1* diluted cell lines

Sample (cell line)	% Dilution	Rep.	Detected fusion isoform	fusion imbalance assay result			Example
				Gene	Predicted fusion breakpoint range	3'/5' Imbalance score	
LC2/ad	2%	1	CCDC6-RET.C1R12.COSF1271.1	*RET*	exon8-ex12	2.04	Fig. 4a
		2	CCDC6-RET.C1R12.COSF1271.1	*RET*	exon8-ex12	2.07	
KM12	5%	1	TPM3-NTRK1.T7N10.COSF1329	*NTRK1*	exon6-ex13	2.58	Fig. 4b
		2	TPM3-NTRK1.T7N10.COSF1329	*NTRK1*	exon6-ex13	2.57	
Negative FFPE Control			NA	*RET*	exon8-ex9	1.16	
			NA	*NTRK1*	exon4-ex5	0.91	

Yellow rows indicate negative controls: fusion isoforms not present in the control sample

Table 3. Imbalance assay results in RET and NTRK1 diluted cell lines

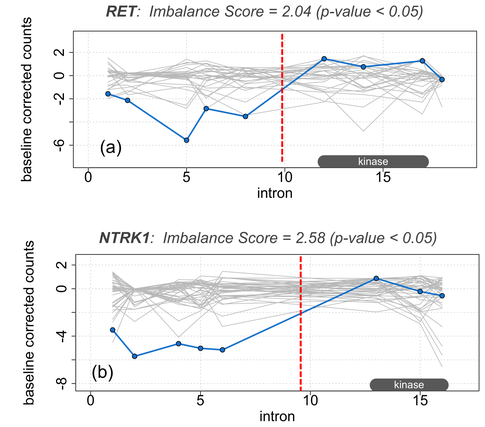


Figure 4. exon-tiling coverage profiles for diluted fusion cell lines (background: normal lung). blue: sample, grey: negative samples (a) *RET* cell line, 2% (b) *NTRK1* cell line, 5%

Conclusions

We demonstrated strong fusion detection capabilities with the Oncomine™ Precision Assay on the Ion Torrent™ Genexus™ Integrated Sequencer which included novel partner-agnostic fusion method utilizing exon-tiling amplicons in key driver genes.

The imbalance approach shows concordance with targeted fusion assays and is incorporated to complement multiplexed RNA sequencing panel to enhance research in oncology for detecting relevant RNA alterations from solid tumor FFPE samples.

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