TruSight™ RNA Fusion Panel

Comprehensive gene fusion detection in formalin-fixed, paraffin-embedded (FFPE) tissues and other cancer research samples.

Highlights

- **One Simple Assay with Discovery Power**
  Industry-guided content for a comprehensive view of cancer-related genes, detecting common and novel fusions
- **Streamlined RNA to Data Workflow**
  Transition from RNA to results in 4 days, including on-instrument software that displays fusion calls
- **Economical, Scalable Fusion Detection**
  Gene fusion analysis on the MiniSeq™ and MiSeq™ Systems, also scalable to higher throughput systems
- **Robust, Reproducible, and Low-Input Fusion Assay**
  Optimized RNA sequencing for all sample types, including FFPE tissues

Introduction

Gene fusions, first discovered in the early 1980s, are frequently key events in tumorigenesis. Fusions can result from chromosomal rearrangements, such as translocations, interstitial deletions, and chromosomal inversions, or from posttranscriptional events. The Mitelman database alone currently holds over 10,180 total gene fusions.[1] Identifying fusions is challenging, especially since multiple distinct fusions can be associated with a particular cancer type.[2] The TruSight RNA Fusion Panel is a next-generation sequencing (NGS) solution that simplifies this task by offering the ability to capture hundreds of potential fusions in a single assay.

To help clinical researchers gain a deeper understanding of fusions in cancer classification and progression, the TruSight RNA Fusion Panel offers in-depth assessment of selected genes cited in public databases and implicated in cancer, including solid tumors, sarcomas, and hematological malignancies.[1] The TruSight RNA Fusion Panel is designed to enable cost-effective analysis of limited quantity and degraded samples, such as FFPE tumor tissues, on a benchtop sequencer.

Efficient, Comprehensive Coverage of Relevant Genes

The content of the TruSight RNA Fusion Panel represents 507 genes implicated in cancer fusions (Table 1). Covering hundreds of genes in a single assay increases the possibility of fusion detection in the first round of testing. Yet the data output is simple and easy to understand, with only the detected fusions displayed. By focusing only on genes commonly associated with fusion transcripts, gene fusions can be analyzed with deep coverage on a benchtop sequencer, providing a robust, cost-effective NGS solution that small labs can access.

Advantages of Targeted RNA Sequencing

RNA sequencing (RNA-Seq) using a targeted NGS approach enables researchers to focus on key genes of interest while maintaining the ability to detect novel fusion partners. Compared to traditional array-based approaches, targeted RNA-Seq provides higher analytical sensitivity and a broader dynamic range, enabling robust detection of low-abundance transcripts.[3-5] NGS complements arrays and fluorescent in situ hybridization (FISH). Not only can NGS detect balanced translocations, but it can also detect single nucleotide variants, as well as known and novel gene fusions. Another key advantage of RNA analysis is the detection of expressed fusion genes resulting from both chromosomal abnormalities and posttranscriptional events.[6,7] By focusing on RNA, the panel detects gene fusions that most DNA panels would not recognize.

Table 1: Coverage Details

Parameter	Value
No. of target genes	507
Targeted exonic regions	7690
No. of probes	21,283

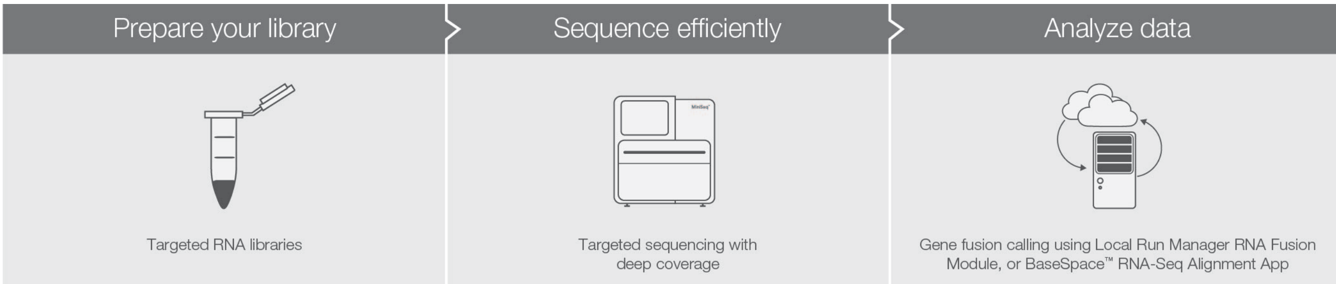


Figure 1: Simple TruSight RNA Fusion Workflow—The TruSight RNA Fusion Panel follows a simple workflow that is fully integrated and scalable.

Simple, Scalable Workflow

The TruSight RNA Fusion Panel provides a simple workflow that can be scaled according to the number of samples. RNA samples can be multiplexed and sequenced on a benchtop system to maximize lab budgets. The integrated workflow includes library preparation, sequencing, and data analysis (Figure 1).

The capture chemistry used by the TruSight RNA Fusion Panel enables simple isolation of target regions of interest from total RNA. Unique oligonucleotide indexes are added to each library (Figure 2A), providing the option for downstream multiplexed sequencing. Libraries are hybridized to biotin-labeled probes specific for targeted RNA regions (Figure 2B). These targets are captured by adding streptavidin beads that bind to the biotinylated probes (Figure 2C). Magnets are used to remove the bound fragments efficiently from solution (Figure 2D). After amplification, a targeted library is ready for cluster generation and sequencing.

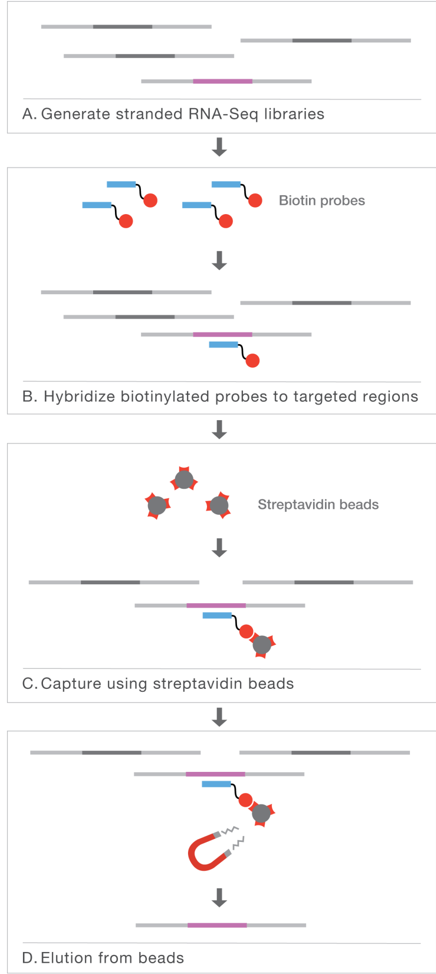
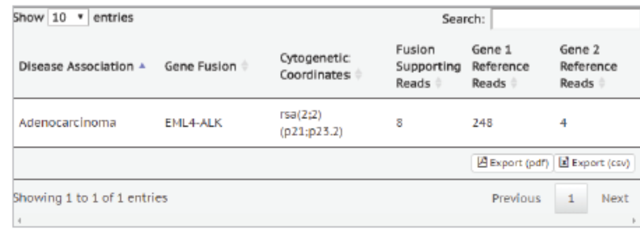


Figure 2: TruSight RNA Fusion Capture Chemistry—The TruSight RNA Fusion Panel provides a simple, streamlined method for isolating targeted regions of interest from total RNA.

With instruments that perform both sequencing and analysis with fusion calling, TruSight RNA Fusion provides simplified solutions that inexperienced users can quickly interpret. The fusion calling software aligns reads, indicates fusion partners (both known and novel) automatically, and determines fusion junctions. Analysis also provides a list of detected fusions, relevant disease associations (as identified by the Mitelman database[1]), and evidence of fusion-supporting reads (Figure 3). Results can be exported in .csv or .pdf format. Researchers also have the option to explore variants and expression information further in the BaseSpace Sequence Hub with the RNA-Seq Alignment App.

A. High Confidence Fusion Calls



Show 10 entries Search:

Disease Association	Gene Fusion	Cytogenetic Coordinates	Fusion Supporting Reads	Gene 1 Reference Reads	Gene 2 Reference Reads
Adenocarcinoma	EML4-ALK	rsa(2;2) (p21;p23.2)	8	248	4

Export (pdf) Export (csv)

Showing 1 to 1 of 1 entries Previous 1 Next

B. Low Confidence Fusion Calls

Show 10 entries Search:

Disease Association	Gene Fusion	Cytogenetic Coordinates	Fusion Supporting Reads	Gene 1 Reference Reads	Gene 2 Reference Reads
Chronic myeloid leukemia, t(9;22)	NUP214-XKR3	rsa(9;22) (q34.13;q11.1)	5	99	0

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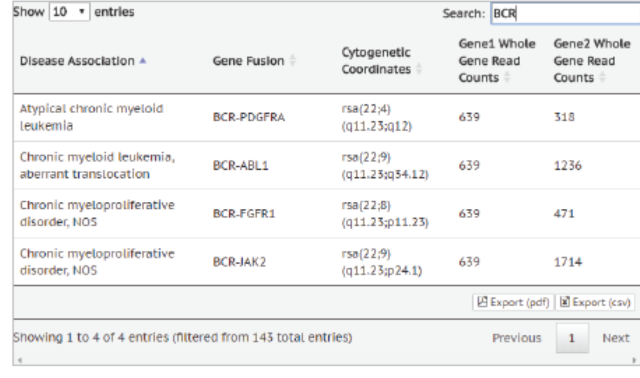
C. Recurrent Fusion Not Called



Show 10 entries Search: BCR

Disease Association	Gene Fusion	Cytogenetic Coordinates	Gene1 Whole Gene Read Counts	Gene2 Whole Gene Read Counts
Atypical chronic myeloid leukemia	BCR-PDGFRA	rsa(22;4) (q11.23;q12)	639	318
Chronic myeloid leukemia, aberrant translocation	BCR-ABL1	rsa(22;9) (q11.23;q34.12)	639	1236
Chronic myeloproliferative disorder, NOS	BCR-FGFR1	rsa(22;8) (q11.23;p11.23)	639	471
Chronic myeloproliferative disorder, NOS	BCR-JAK2	rsa(22;9) (q11.23;p24.1)	639	1714

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Showing 1 to 4 of 4 entries (filtered from 143 total entries) Previous 1 Next

Figure 3: Data Output from Local Run Manager—The fusion-calling software provides the following information: (A) High confidence fusion calls are detected fusions that meet a high-confidence score. (B) Low-confidence fusion calls, if detected, are reads where there is some evidence suggesting there may be a fusion, but where the supporting reads do not meet a high-confidence score. (C) Recurrent fusions not called represent common fusions of interest where no fusion-supporting reads were detected. For example, *BCR-ABL1* fusions are frequent in chronic myeloid leukemia (CML). The searchable, sortable table allows customers to assess whether any reads for the native transcripts were detected. This feature serves as a control to determine whether regions of interest were successfully detected by the panel when fusions are not called.

Table 2: Concordance of TruSight RNA Fusion Detection with FISH and RT-PCR

	TruSight RNA Fusion					RT-PCR	FISH	
Sample	TruSight RNA Fusion Results	Fusion-supporting Reads	Reference Reads (Gene 1)	Reference Reads (Gene 2)	Cytogenetic Coordinates[a]	RT-PCR Results	FISH Results	FISH Images
1	*BCR-ABL1* fusion *ABL1-BCR* fusion	205 7	462 23	67 392	rsa(22;9)(q11.23;q34.12) rsa(9;22)(q34.12;q11.23)	*BCR-ABL* fusion	*BCR-ABL1* fusion (77/100 interphase nuclei)	
2	*AFF1-KMT2A* fusion *KMT2A-AFF1* fusion	57 16	443 22	59 272	rsa(4;11)(q21.3;q23.3) rsa(11;4)(q23.3;q21.3)	*KMT2A-AFF1* fusion (or *MLL-AF4*)[b]	*KMT2A* (*MLL*)* rearrangement (87/100 interphase nuclei)	
3	*WWTR1-CAMTA1* fusion	21	178	5	rsa(3;1)(q25.1;q36.23)	*WWTR1-CAMTA1* fusion	FISH not performed	

Samples were assessed with combinations of FISH, RT-PCR, and NGS to demonstrate concordance between these assays. FISH and RT-PCR detected a *BCR-ABL1* fusion in sample 1. TruSight RNA Fusion results support this finding. With FISH, sample 2 shows an *MLL* rearrangement using *MLL* break-apart probes. The rearrangement was identified as being an *KMT2A-AFF1* fusion by RT-PCR. TruSight RNA Fusion also detects this fusion. FISH was not performed on sample 3, but RT-PCR demonstrated a *WWTR1-CAMTA1* fusion, which was supported by the TruSight RNA Fusion results. Data courtesy of the MLL Munich Leukemia Laboratory and Mount Sinai Hospital, Toronto.

a. Data are in the same format as output from the software.
b. *MLL* is another common name for the *KMT2A* gene, and *AF4* is another name for the *AFF1* gene.

Deep Coverage and Economical Targeted Sequencing

Compared to whole-genome approaches, targeted sequencing increases target coverage while reducing the data analysis burden and sequencing cost per sample. Focusing on content enriched with cancer-associated transcripts provides more sequencing reads for the regions of interest, allowing detection of rare fusion transcripts. RNA-Seq also enables discovery of both somatic variants and gene fusions. TruSight RNA Fusion data shows concordance with other assays such as RT-PCR and FISH (Table 2). Yet gene fusion detection by NGS also provides more insight than standard approaches because the precise breakpoint can be identified. The fusion sequence is reported under the Contig column in the fusions CSV file.

Efficient Analysis of Difficult Samples

Although archival FFPE tissues provide a valuable repository of information for cancer research, the nucleic acids preserved within these samples are often highly degraded. This degradation poses a challenge during molecular analysis. To overcome these challenges, the TruSight RNA Fusion Panel is optimized for high performance from both high- and low-quality RNA sample types, such as bone marrow or FFPE tumor tissue. Libraries can be prepared from as little as 10 ng total RNA, or 20 ng FFPE RNA. This low input requirement makes the TruSight RNA Fusion Panel ideal for reliable targeted analysis of limited quantity samples (Table 3).

Table 3: Fusion Detection in FFPE Samples Using the TruSight RNA Fusion Panel

Sample Name	DV_{200}	Total RNA Input	PF Reads Per Sample	Expected Fusion	Result
FFPE-1	32	100 ng	2.99 M	*EWSR1-FLI1*	Fusion detected
FFPE-2	64	50 ng	3.02 M	*EWSR1-FLI-1*	Fusion detected
FFPE-3	72	20 ng	3.39 M	*SS18-SSX2*	Fusion detected
UHR	Not needed	10 ng	3.95 M	*BCR-ABL1* *BCAS4-BCAS3* *NUP214-XKR3*	Fusion detected
FFPE-5	74	50 ng	3.99 M	*EML4-ALK*	Fusion detected
FFPE-6	57	50 ng	3.12 M	*SS18-SSX1*	Fusion detected
FFPE-7	Not determined	50 ng	3.25 M	*RP2-BRAF*	Fusion detected
FFPE-8	Not determined	20 ng	2.68 M	*LMNA-NTRK1*	Fusion detected[a]

Eight samples were sequenced on the MiSeq System and fusion calls were made with the Local Run Manager RNA Fusion Module. DV_{200}, a value used to assess RNA quality from FFPE samples, is the percentage of RNA fragments > 200 nucleotides.[8] PF = passing filter. UHR = Universal Human Reference.

a. Fusion detected by lowering the Local Run Manager confidence score threshold from 0.6 to 0.5.

Summary

The TruSight RNA Fusion Panel provides a reproducible, and economical solution for studies of gene fusions in many cancer types. With comprehensive detection of expressed gene fusions, researchers can avoid hypothesis-driven approaches, while gaining a focused view of the functionally relevant changes occurring in cancer. The panel is compatible with several Illumina benchtop sequencers and provides on-instrument fusion calling, making NGS-based fusion detection accessible to labs without the need for additional bioinformatics support. The assay is also compatible with FFPE tissue, accommodating as little as 10 ng fresh-frozen total RNA, or 20 ng FFPE RNA input.

Learn More

For more information about the TruSight RNA Fusion Panel, visit www.illumina.com/RNAFusion.

References

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Ordering Information

Product	Catalog No.
TruSight RNA Fusion Panel Set A Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-304-1002
TruSight RNA Fusion Panel Set B Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-304-1003

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