illumina®

TruSight® RNA Pan-Cancer Panel

Comprehensive assessment of cancer-related RNA transcripts and fusion detection in FFPE tissues and other oncology samples.

Highlights

- **Comprehensive View of Cancer Pathways**
  Illumina RNA enrichment chemistry enables interrogation of gene expression levels, variants, and gene fusions
- **Low-input Protocol for All Sample Types**
  Optimized for sequencing RNA from all sample types, including formalin-fixed, paraffin-embedded tissues
- **Focus on Relevant Transcripts and Fusion Genes**
  Industry validated content for a comprehensive view of 1,385 cancer-related RNA transcripts
- **Economical, Targeted RNA-Seq on a Desktop Sequencer**
  Enables RNA sequencing on the MiniSeq™ or MiSeq® Systems for affordable, sensitive analysis

Introduction

To date, at least 138 known driver genes for cancer have been discovered.[1] However, large numbers of variants are often detected in DNA of unexpressed genes, highlighting that RNA expression can provide key insights into the functionally relevant genes in cancer. Cancer can arise from epigenetic changes, expression level changes, and gene fusions that are undetectable by standard sequencing.[2,3] RNA sequencing (RNA-Seq) using next-generation sequencing (NGS) offers the ability to capture all relevant transcripts in a single assay. Compared to traditional array-based approaches, targeted RNA-Seq is a highly sensitive approach, with a broader dynamic range, that robustly detects RNAs of low abundance. In addition, RNA-Seq can identify gene fusions from both known and novel fusion gene partners.

To help clinical researchers gain a deeper understanding of the gene expression patterns in cancer classification and progression, the TruSight RNA Pan-Cancer Panel offers in-depth assessment of cancer-related RNA transcripts, including measurement of expression levels and detection of fusion genes. The panel includes 1,385 genes that have been cited in public databases and implicated in cancer, including solid tumors, soft tissue cancers, and hematological malignancies. TruSight RNA Pan-Cancer Panel enables cost-effective analysis of limited and degraded samples, such as formalin-fixed, paraffin-embedded (FFPE) tumor tissue, on a desktop sequencer.

Comprehensive Coverage of Relevant Genes

The content of the TruSight RNA Pan-Cancer Panel represents 1385 genes implicated in cancer pathways (Table 1). In a single assay, researchers can assess all relevant RNA transcripts for multiple cancer types, regardless of origin. The focus on a subset of relevant genes enables RNA-Seq with high sensitivity on a desktop sequencer, allowing cost effective access to NGS for any lab. TruSight RNA Pan-Cancer Panel can be used for gene expression profiling and variant calling, highlighting functionally relevant mutations. The panel also detects fusion genes that most DNA panels would not detect. The TruSight RNA Pan-Cancer Panel provides a comprehensive assessment of cancer-related RNA transcripts for a more detailed view of cancer biology.

Table 1: Coverage Details

Parameter	Specification
Number of target genes	1,385
Targeted exonic regions	21,043
Number of probes	57,010

Efficient Analysis of Difficult Samples

Archival FFPE tissues provide a valuable repository of information for cancer research, but the RNA preserved within these samples is often highly degraded. This degradation poses a challenge when creating libraries for NGS. To overcome these challenges, the TruSight RNA Pan-Cancer 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 Pan-Cancer Panel ideal for reliable targeted analysis of limited samples (Figure 1).

Simple, Scalable Workflow

The TruSight RNA Pan-Cancer workflow first creates RNA-Seq libraries. This method adds unique oligonucleotide indexes to each library, tagging them for downstream multiplexed sequencing (Figure 2A). Libraries are hybridized to biotin-labeled probes specific for targeted RNA regions (Figure 2B). These targets are then captured by adding streptavidin beads that bind to the biotinylated probes (Figure 2C). Magnets pull the bound fragments from solution (Figure 2D). Captured fragments are eluted from the beads and a second hybridization and elution are performed. After amplification, a targeted library is ready for cluster generation and sequencing.

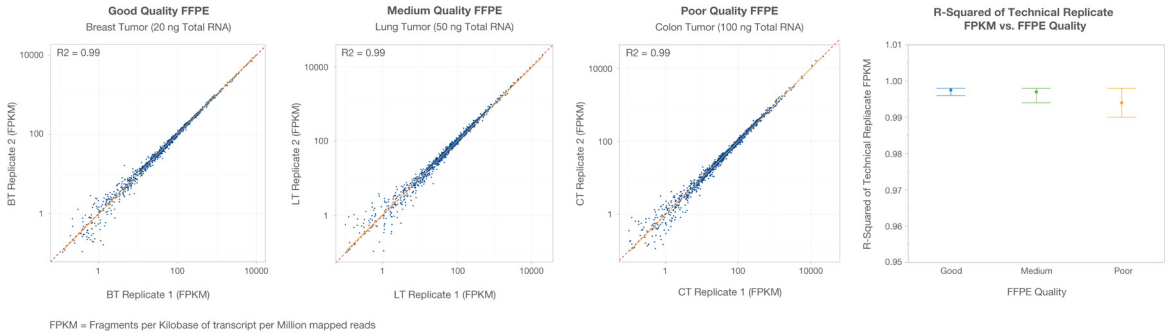


Figure 1: Highly reproducible expression data obtained from poor quality FFPE samples—Degraded FFPE RNA samples of poor, medium, and high quality (as determined by DV_{200} values) were sequenced and data reproducibility measured by R-squared of technical replicate FPKM. DV_{200} = the percentage of RNA fragments > 200 nucleotides. FPKM = fragments per kilobase of transcript per million mapped reads. Each error bar is constructed using the minimum and maximum data values.

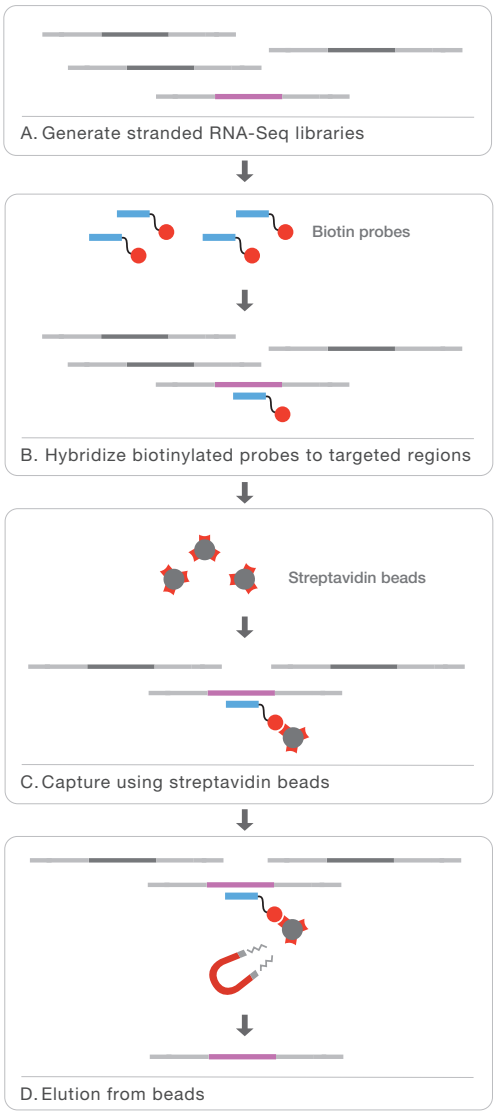


Figure 2: TruSight Pan-Cancer Capture Chemistry—The TruSight RNA Pan-Cancer Panel provides a simple, streamlined method for isolating targeted regions of interest from total RNA.

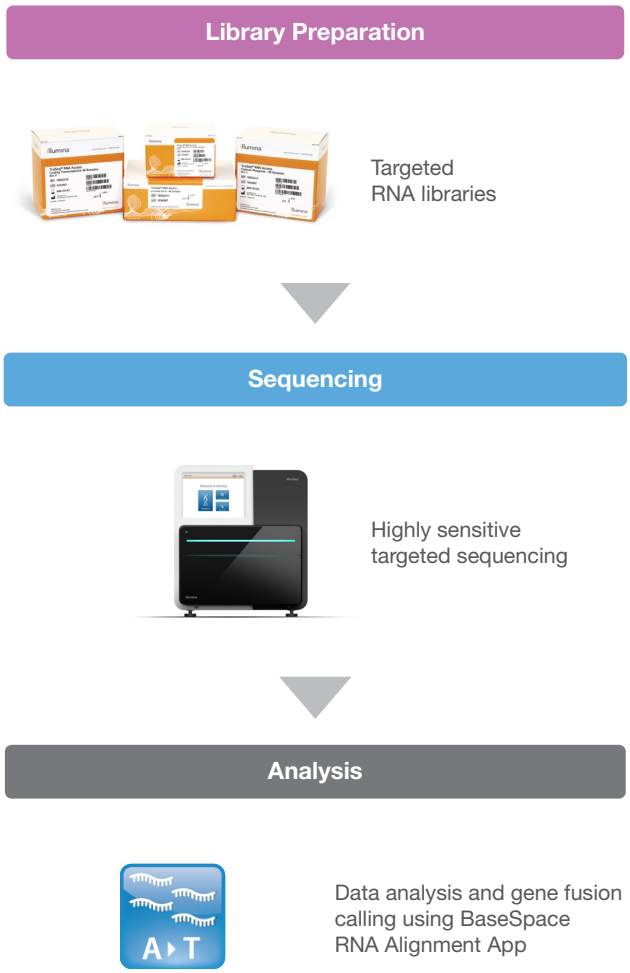


Figure 3: Simple TruSight RNA Pan-Cancer Workflow—The TruSight RNA Pan-Cancer Panel follows a simple workflow that is fully integrated and scalable to large sample numbers.

The TruSight RNA Pan-Cancer Panel is optimized to provide a simple workflow that can be scaled according to the number of samples. RNA samples can be multiplexed and sequenced on a desktop system to maximize lab budgets. The integrated workflow includes library preparation, sequencing, and easy data analysis using BaseSpace® Apps for RNA (Figure 3).

Highly Sensitive and Economical Targeted Sequencing

By focusing on key genes of interest, targeted sequencing enables researchers to study high-value content enriched with cancer-associated transcripts, while retaining the ability to discover novel somatic variants and gene fusions. This approach yields more sequencing reads for the regions of interest, delivering expression level quantification with the sensitivity needed to detect rare transcripts and fusions. Gene fusion detection is highly quantitative, as shown with spike-in experiments using synthetic fusion constructs (Figure 4). Novel fusions can be detected even when one of the fusion partners is not targeted in the panel (Figure 5). With the focused content of the TruSight RNA Pan-Cancer Panel, RNA-Seq is now possible on the Illumina family of desktop sequencers.

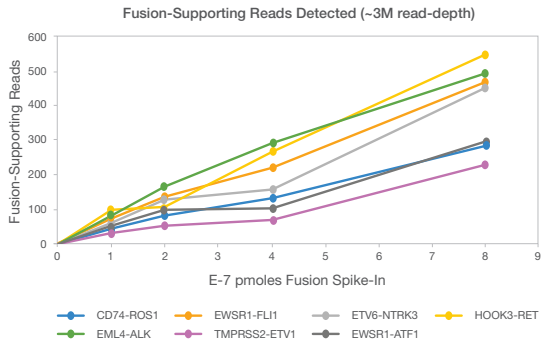
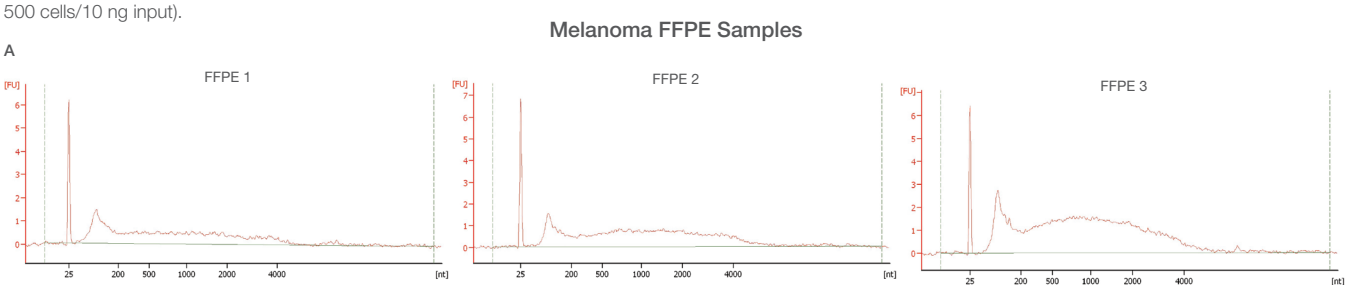


Figure 4: Quantitative detection of synthetic fusions—In-vitro transcribed fusions were spiked into 10 ng MCF7 control RNA, and the number of fusion-supporting reads is shown. Fusion supporting reads decrease linearly with decreasing spike-in amounts and can be detected as low as concentrations equivalent to $1x10^{-7}$ pmoles, or ~13 fusion copies per cell (based on 500 cells/10 ng input).

Sequencing focused content generates a smaller data set with easier data analysis and data management. TruSight RNA Pan-Cancer data sets can be analyzed using the new RNA-Seq Alignment app in the BaseSpace environment (Table 2). Providing expert-preferred data analysis tools packaged in an intuitive interface designed for biologists, the RNA-Seq Alignment app provides gene expression profiles, identifies gene fusions, and reports single nucleotide variants and small indels. Simple-to-follow prompts guide users through the entire process, starting from selecting files generated by the sequencer, to filtering analyzed data, and visualizing results (Figure 6).

Table 2: Features of RNA-Seq Data Analysis App

BaseSpace App	Description
RNA-Seq Alignment	• RNA-Seq alignment using STAR-Manta, and cSNP calling using Isaac Variant Caller • Works with all samples, including fresh, frozen, and FFPE • Results seamlessly integrated into the Cufflinks Assembly and Differential Expression App • Read mapping, variant calling (SNVs and small indels), and gene fusion calling with STAR-Manta



B

Sample Name	Total RNA Input	N-Term Gene	C-Term Gene	Chromosomal Location	Fusion-Supporting Reads
FFPE 1	20 ng	LMNA	NTRK1	chr1-chr1	51
FFPE 2	50 ng	LMNA	NTRK1	chr1-chr1	189
FFPE 3	50 ng	RP2	BRAF	chrX-chr7	89

Figure 5: Identification of novel gene fusion partners—(A) The size distribution of total RNA from 3 different FFPE samples derived from melanoma are shown on a Bioanalyzer. (B) Table shows the detection of the gene fusion partner in FFPE samples when only one gene (highlighted) of the fusion pair is targeted.

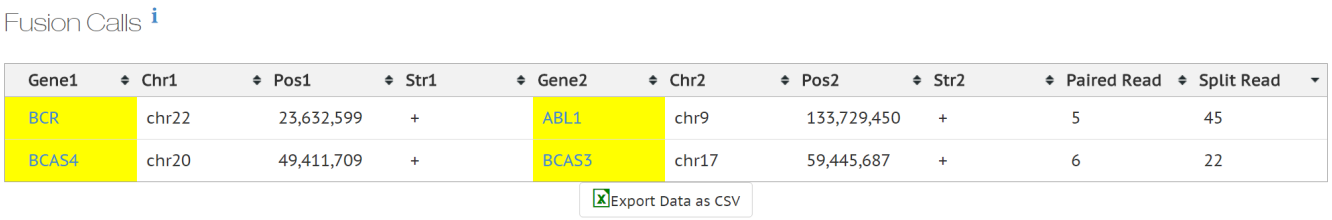


Figure 6: Screenshot of BaseSpace software—The RNA-Seq Alignment App provides easy access to sample results including a report of detected fusion genes. Highlighted genes represent those that the panel targets.

Summary

The TruSight RNA Pan-Cancer Panel enables comprehensive detection of gene fusions and gene expression changes, providing researchers with a focused view of the functionally relevant changes occurring in cancer. The panel is compatible with FFPE tissue and accommodates as little as 10 ng fresh-frozen total RNA, or 20 ng FFPE RNA input. The TruSight RNA Pan-Cancer Panel provides a sensitive, reproducible, and economical solution for studies of expression dynamics and functional mechanisms in cancer.

Ordering Information

Product	Catalog No.
TruSight RNA Pan-Cancer Panel Set A Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-303-1002
TruSight RNA Pan-Cancer Panel Set B Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-303-1003
TruSight RNA Pan-Cancer Set A MiniSeq Kit Includes library preparation consumables and oligos for 48 samples with 12 indexes, plus 6 MiniSeq High Output Kits (150 Cycles)	20005611

Learn More

For more information about the TruSight RNA Pan-Cancer Panel, visit www.illumina.com/RNAPanCancer.

Reference

1. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science.* 2013;339:1546-1558.
2. Green MR, Vicente-Duenas C, Romero-Camarero I, et al. Transient expression of BCL6 is sufficient for oncogenic function and induction of mature B-cell lymphoma. *Nat Commun*. 2014;5:3904 doi:10.1038/ncomms4904.
3. Piskol R, Ramaswami G, Li JB. Reliable identification of genomic variants from RNA-seq data. *Am J Hum Genet.* 2013;93(4):641-651.

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

illumina®