illumina®

METHOD GUIDE

# Methods for RNA sequencing

## Illumina solutions for profiling RNA, from targeted panels to the whole transcriptome

RNA SEQUENCING

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# Introduction

As researchers gain deeper insights into the genome, they learn how factors beyond the genetic sequence influence development, disease states, and more. To truly understand what is occurring, scientists are turning towards a multiomics approach, looking at the transcriptome, epigenome, and proteome, in addition to the genome, to find comprehensive answers. In this guide, we will focus on next-generation sequencing (NGS) workflows for studying the transcriptome.

NGS-based RNA sequencing (RNA-Seq) offers a highly sensitive method for analyzing gene expression across the transcriptome, illuminating changes and characterizing various RNA forms with no prior knowledge of the transcriptome required. This technique uncovers transcript isoforms, gene fusions, and single nucleotide variants (SNVs) in a single experiment.[1-3] Bulk RNA-Seq is a well-established method that measures average RNA expression in cell populations. Ideal for newcomers to NGS, bulk RNA-Seq is increasingly used in translational and clinical cancer research, benefiting from its simplicity and the depth of information RNA-Seq provides.[4]

## Overview of the RNA-Seq workflow

Illumina RNA-Seq workflows integrate RNA extraction, library preparation, sequencing, and data analysis to support transcriptome studies (Figure 1).

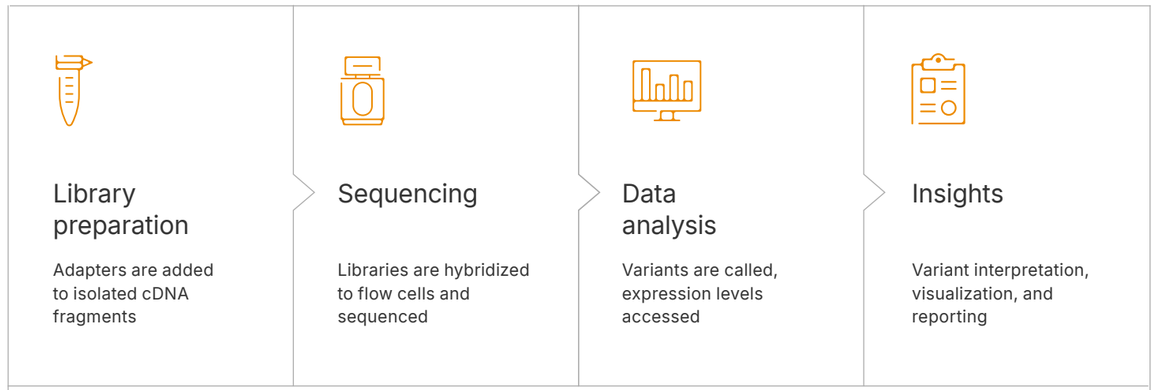



Figure 1: RNA sequencing workflow

## Key benefits:

**Comprehensive analysis:** RNA-Seq offers several advantages over other RNA analysis methods, such as qPCR and gene expression arrays, including:

- Covering a wider dynamic range than gene expression arrays, resulting in greater sensitivity and accuracy[5]
- Capturing both known and novel features, enabling analysis of the transcriptome without a reference[2]
- Offering a rich view of the transcriptome beyond expression profiling, detecting alternative splice sites, gene fusions, and allele-specific expression





STEP 1

## Nucleic acid extraction

Many commercial DNA and RNA extraction and purification kits are available. Choose a kit or method that produces the highest quality nucleic acids possible for your specific targets and from your specific sample types. Illumina has tested a variety of commercially available kits, but other kits or methods work better for your targets and samples.

STEP 2

## Library preparation

Following nucleic acid isolation, scientists can prepare nucleic acid libraries—a collection of similarly sized fragments that have known oligonucleotide adapter sequences attached to the 5′ and 3′ ends of the strands that are loaded onto an instrument for sequencing. The Illumina RNA library prep portfolio offers a range of solutions to support various sequencing methods and sample types, including formalin-fixed paraffin-embedded (FFPE) tissue (Table 1). Illumina RNA library prep solutions offer flexibility, scalability, and performance with a rapid, automation-friendly workflow option to prepare sequencing-ready libraries in a single day. The RNA library preparation products mentioned in this guide are representative of the portfolio and are not meant to be a comprehensive list of all available options. View the full RNA library prep portfolio.

Table 1: A comparison of select Illumina RNA preparation kits[a]

| Parameter | Illumina Stranded mRNA Prep | Illumina RNA Prep with Enrichment | Illumina Stranded Total RNA Prep with Ribo-Zero Plus |
|---|---|---|---|
| Area of interest | Coding transcriptome with high-quality samples | Coding transcriptome with FFPE samples | Noncoding and coding RNA |
| Description | Quantify gene expression, identify known and novel isoforms, detect gene fusions, and measure allele-specific expression | Analyze expression in a focused set of genes of interest, obtain quantitative expression information, detect small variants and gene fusions | Measure genes and transcripts while detecting known and novel features; targeted hybridization removes abundant rRNA to focus on high value portions of the transcriptome |
| Mechanism of action | PolyA selection upfront of ligation-based RNA library prep | Tagmentation-based RNA library prep followed by hybrid–capture enrichment | rRNA depletion ahead of ligation-based RNA library prep |
| Input | 25–1000 ng high-quality RNA | 10 ng high-quality RNA, 20 ng FFPE RNA | 1 ng high-quality RNA, 10 ng FFPE RNA |
| Hands-on time | < 3 hr | < 2 hr | < 3 hr |
| Total assay time | 6.5 hr | < 9 hr | ~ 7 hr |
| Automation capability | Yes | Yes | Yes |

a. More RNA library preparation kits are available at illumina.com/techniques/sequencing/ngs-library-prep/rna.html.

STEP 3

## Sequencing

After libraries are prepared, they are ready for sequencing. Regardless of your research question, flexible Illumina sequencing systems can help you find answers using simple push-button workflows (Table 2). For studies focused on a small amount of information, ie, prokaryotic species or small targeted RNA oncology panels, researchers can use a benchtop sequencing system, such as the MiSeq™ i100 Series or NextSeq™ 1000 and NextSeq 2000 Systems. For large-scale studies, researchers can use high-throughput instruments like the NovaSeq™ 6000 System and NovaSeq X Series, and multiplex up to 384 samples with unique dual indexes.

Table 2: Select examples of sequencing systems


| System | MiSeq i100 Series | NextSeq 1000 and NextSeq 2000 Systems | NovaSeq 6000 System | NovaSeq X Series |
|---|---|---|---|---|
| System overview | Simplified operations and fast, flexible sequencing | Expansive application breadth and proven performance | Immense discovery power for deeper insights | Extraordinary throughput and transformative economics |
| Output range | 1.5–30 Gb | 10–540 Gb[a] | 65 Gb–6 Tb | 165 Gb–16 Tb |
| Single reads per flow cell | 5–100M | 100M–1.8B | 800M–10B | 1.6–26B |
| Maximum read length | 2 × 500 bp | 2 × 300 bp | 2 × 250 bp | 2 × 150 bp |

a. Maximum specifications based on a P4 flow cell run; P4 flow cells are available for the NextSeq 2000 System only.





STEP 4

## Data analysis

RNA-Seq data can be easily and securely transferred, stored, and analyzed in the Illumina cloud-computing platforms: BaseSpace™ Sequence Hub and Illumina Connected Analytics. For users without bioinformatics expertise, BaseSpace Sequence Hub is recommended for its intuitive interface, easy run setup and monitoring, and simplified push-button secondary analysis. For more advanced users, Illumina Connected Analytics supports customization with highly configurable, scalable analysis. Both platforms offer in-cloud access to DRAGEN™ secondary analysis pipelines for accurate and efficient analysis of RNA-Seq data.

STEP 5

## Insights

Outputs from secondary analysis pipelines can be ingested into reporting and exploration software, such as:

- Correlation Engine—analyze private omics data with highly curated public data to help put data into biological context
- Illumina Connected Insights—streamline variant interpretation and reporting for somatic oncology research applications
- Partek™ Flow™ software—perform statistical analysis and explore data with interactive, customizable, and publication-ready visualizations

# Method 1: mRNA-Seq

Messenger RNA sequencing (mRNA-Seq) sensitively and accurately quantifies gene expression, identifies known and novel isoforms in the coding transcriptome, and measures allele-specific expression. Protein-coding genes with polyA tails are selected ahead of library preparation in this method. Using mRNA-Seq to study the coding transcriptome, researchers can focus on a smaller, more manageable portion of the transcriptome that will provide information relevant to their area of interest.

## Relevant applications

### Gene expression profiling for disease research

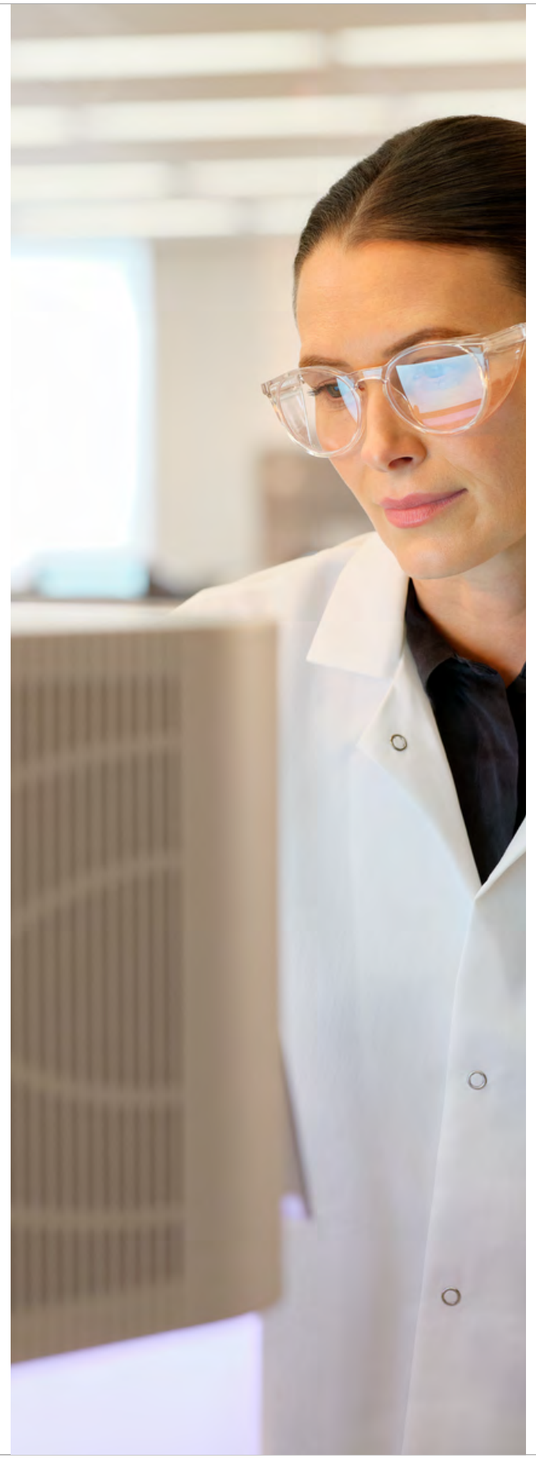
To understand normal cell development and disease mechanisms, researchers frequently investigate differential expression during development, in specific tissues, or in response to varying conditions. mRNA-Seq shows exceptional performance in profiling genes with low expression levels. It is being used to assess gene expression profiles for the study of complex diseases and laying the groundwork for advances in precision medicine by identifying potentially therapeutic biomarkers.[6]

Data from mRNA-Seq experiments can offer insight into the gene networks and pathways involved in complex disease and cell biology mechanisms.[7,8] For example, transcriptomic analysis is helping researchers compare brain regions with different pathology to identify meaningful gene expression changes in Alzheimer's disease (AD).[7,9] Differential expression profiling is also revealing the pathogenesis of heart failure and identifying gene signatures to detect heart disease.[10-14]

### Biomarker profiling for drug development

mRNA-Seq is increasingly being utilized to discover and profile RNA-based drug response biomarkers with the aim of improving the efficiency and success rate of the drug development process. While several technologies have been used for this application, the capabilities of mRNA-Seq promise to be of particular benefit.[15-17]

Differential expression profiles and gene fusions detectible through RNA analysis have been shown to associate with a range of response characteristics, including efficacy, the incidence of adverse effects, pharmacodynamics, and other attributes.[18-21] Such biomarkers have therefore become an invaluable tool in multiple components of the development process, such as informing the interpretation of clinical trial data, allowing more efficient stratification of trial cohorts, and identifying neoantigen candidates for immunotherapy.[22,23] RNA-based biomarkers may also provide a foundation for the development of companion diagnostics, including for compounds that have previously failed clinical trials.



## Step-by-step overview

mRNA-Seq has five basic steps: RNA extraction, library preparation, sequencing, analysis, and insights (Figure 2).

01
### RNA extraction
QIAGEN RNeasy Kit

02
### Library preparation
Illumina Stranded mRNA Prep

03
### Sequencing
Illumina sequencing system

04
### Data analysis
DRAGEN RNA app

05
### Insights
Partek Flow software
Correlation Engine
Illumina Connected Insights

Figure 2: mRNA-Seq workflow.

**STEP 1**
## RNA extraction

For mRNA-Seq, it is crucial to use high-quality mRNA as input. There are several commercial options for mRNA isolation, depending on the sample type; the QIAGEN RNeasy for RNA kit is recommended for use with Illumina mRNA-Seq workflows. Effectively evaluating mRNA quality is a critical step in successful RNA sequencing and can be achieved by measuring mean mRNA fragment size.

**STEP 2**
## Library preparation

Illumina Stranded mRNA Prep delivers accurate, unbiased detection of the coding transcriptome with precise measurement of strand information (Table 3).

Table 3: Illumina targeted amplicon sequencing analysis software

| Feature | Specification |
|---|---|
| RNA input amount | 25–1000 ng high-quality RNA |
| Total assay time | 6.5 hr |
| Hands-on time | < 3 hr |

STEP 3

## Sequencing

Several sequencing systems can be used for mRNA-Seq. The one chosen depends on several factors, including the application, study size, throughput requirements, and more (Table 4).

Table 4: Experimental parameters for performing mRNA-Seq on different sequencing systems

| System | Flow cell | Single reads per flow cell | No. of samples per flow cell[a] | Recommended read length |
|---|---|---|---|---|
| MiSeq i100 Series | 50M | 50M | 2 | 2 × 75 bp |
| | 100M | 100M | 4 | |
| NextSeq 1000 and NextSeq 2000 Systems | P1 | 100M | 4 | 2 × 75 bp |
| | P2 | 400M | 16 | |
| | P3[b] | 1.2B | 48 | |
| | P4[b] | 1.8B | 72 | |
| NovaSeq 6000 System | SP | 1.6B | 64 | 2 × 75 bp |
| | S1 | 3.2B | 128 | |
| | S2 | 8.2B | 328 | |
| | S4 | 8–10B | 384 | |
| NovaSeq X Series | 10B | 10B | 384 | 2 × 75 bp |
| | 25B | 26B | 384 | |

a. Based on 25M reads per sample. Sufficient gene expression and most use cases of fusion calling.
b. Flow cells are only available on the NextSeq 2000 System.

STEP 4

## Data analysis

For secondary analysis of mRNA-Seq data, Illumina recommends using the DRAGEN RNA pipeline, which performs RNA quantification, gene fusion detection, and small variant calling in one integrated workflow (Table 5). This pipeline is available on-premises with a DRAGEN Server, on the cloud-based BaseSpace Sequence Hub and Illumina Connected Analytics platforms, and onboard select sequencing systems, including the NextSeq 1000 and NextSeq 2000 Systems and NovaSeq X Series.

STEP 5

## Insights

After secondary data analysis, results can be transferred to Correlation Engine to understand the biological effects of gene expression changes (Table 5). Correlation Engine contains knowledge-based gene sets and results from thousands of public studies that inform biological interpretation. Connect differential gene expression data from RNA-Seq experiments with disease associations or visualize correlated genes. Gain further insights with Partek Flow software using robust statistical algorithms and information-rich visualizations. Additionally, Illumina Connected Insights can be used to streamline interpretation and reporting for research applications.

Table 5: Illumina mRNA-Seq analysis software

| Pipeline | Application | Input | Access point |
|---|---|---|---|
| DRAGEN RNA | Offers multiple operating modes, including reference-only alignment and annotation-assisted alignment with gene fusion detection. The gene fusion module uses the DRAGEN RNA spliced aligner to perform split-read analysis on supplementary (chimeric) alignments to detect potential breakpoints. | FASTQ<br><br>Optional: Custom reference Gene Annotation File (GTF, GFF, or GFF3) | • DRAGEN Server<br>• BaseSpace Sequence Hub<br>• Illumina Connected Analytics<br>• Onboard MiSeq i100 Series, NextSeq 1000/2000 Systems, NovaSeq X Series |
| RNA-Seq interpretation | Processes sequencing data from mRNA to estimate transcript abundance and identify differentially expressed transcripts across samples | csv, txt, xlsx files | • Correlation Engine |
| RNA-Seq visualization and statistical analysis | Provides differential analysis, clustering, and data exploration plots | tsv, csv, txt, gz, FASTQ, BAM | • Partek Flow software |
| Illumina Connected Insights | Supports streamlined interpretation and reporting from DRAGEN software for oncology research applications | VCF | • Illumina Connected Insights with automated ingestion of VCF files |

# Method 2: Targeted RNA-Seq

Targeted RNA-Seq is a highly accurate method for selecting and sequencing specific transcripts of interest that offers both quantitative and qualitative information. Targeted RNA-Seq can be achieved via either enrichment or amplicon-based approaches, both of which enable gene expression analysis in a focused set of genes of interest. Here, we focus on enrichment assays, which provide the ability to detect both known and novel gene fusion partners in many sample types, including FFPE tissue.

## Relevant applications

### Variant detection for cancer research

Targeted RNA-Seq is a critical tool for direct measurement of the functional consequences of mutations. Despite the average cancer containing about 46 mutations, only five to eight are necessary for initiation.[24] Genomic profiling alone is insufficient to differentiate these driver mutations from passenger mutations, or those that do not influence cancer initiation or progression. Measurement of gene expression patterns and mutation consequences using targeted RNA-Seq enables large-scale, unbiased differentiation of factors crucial for cancer progression, resulting in more thorough and accurate cancer modeling.

### Detection and characterization of respiratory pathogens

Targeted RNA-Seq provides an effective method for rapid and accurate identification of respiratory pathogens. Combining Illumina RNA Prep with Enrichment with target-specific probe panels enables targeted sequencing of different subsets of respiratory pathogens. For example, the Respiratory Virus Oligo Panel v2 targets SARS-CoV-2 and other common respiratory viruses in a single assay.[25] Alternatively, Illumina offers the Respiratory Pathogen ID/AMR Panel, which targets ~280 respiratory pathogens, including viruses, bacteria, and fungi, and associated antimicrobial resistance (AMR) markers.[26]






## Step-by-step overview

Targeted RNA-Seq has five basic steps: RNA extraction, library preparation and enrichment, sequencing, data analysis, and insights (Figure 3).

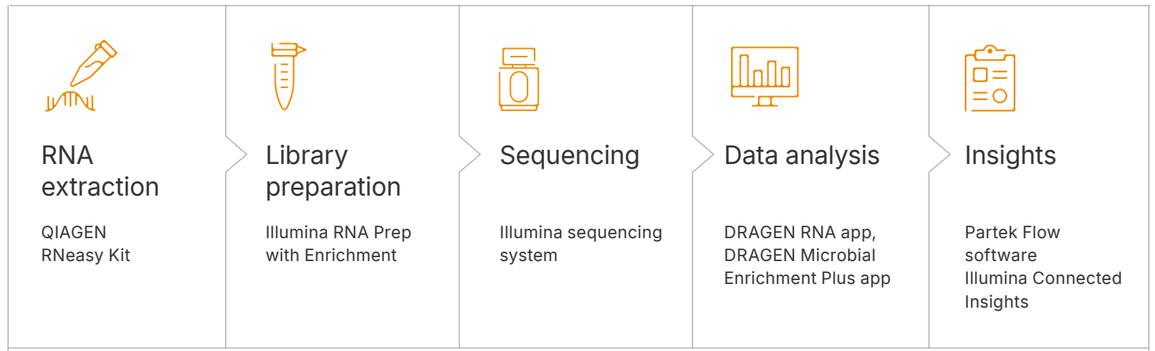



Figure 3: Targeted RNA-Seq workflow.

**STEP 1**

### RNA extraction

For targeted RNA-Seq, high-quality intact RNA or RNA isolated from FFPE samples can be used as input. There are several commercial options for RNA isolation, depending on the sample type; the QIAGEN RNeasy for RNA kit is recommended for use with intact samples and QIAGEN RNeasy FFPE for use with FFPE tissue. Effectively evaluating RNA quality is a critical step in successful RNA-Seq and can be achieved by measuring mean RNA fragment size before library prep.

**STEP 2**

### Library preparation

Illumina RNA Prep with Enrichment provides a fast, integrated workflow for producing enriched and indexed sequencing libraries (Table 6). The kit enables rapid, targeted interrogation of an expansive number of target genes with exceptional capture efficiency and coverage uniformity using various prebuilt probes or custom probes for maximum flexibility.[27]

Table 6: Illumina RNA Prep with Enrichment specifications

| Feature | Specification |
|---|---|
| RNA input amount | 10 ng high-quality RNA, 20 ng FFPE RNA |
| Total assay time | < 9 hr |
| Hands-on time | < 2 hr |

STEP 3

## Sequencing

There are many panels that can be used to select the targets of interest. In this example workflow, we focus on using the Illumina Exome Panel to analyze the coding transcriptome. The sequencing system used depends on several factors, including the application, study size, throughput requirements, and more (Table 7).

Table 7: Experimental parameters for performing targeted RNA-Seq on different sequencing systems

| System | Flow cell | Single reads per flow cell | No. of samples per flow cell[a] | Recommended read length |
|---|---|---|---|---|
| MiSeq i100 Plus System | 25M | 25M | 8 | 2 × 100 bp |
| | 50M | 50M | 16 | |
| | 100M | 100M | 33 | |
| NextSeq 1000 and NextSeq 2000 Systems | P1 | 100M | 33 | 2 × 100 bp |
| | P2 | 400M | 133 | |

a. Based on 3M clusters per sample using the TruSight RNA Pan-Cancer Panel.

STEP 4

## Data analysis

Illumina recommends using the DRAGEN RNA pipeline or the Enrichment panel-specific analysis workflow, ie, DRAGEN Microbial Enrichment Plus (Table 8). These pipelines are available on-premises with a DRAGEN Server, on cloud-based platforms, including BaseSpace Sequence Hub and Illumina Connected Analytics, and onboard select sequencing systems.





STEP 5

## Insights

After secondary analysis, further explore data with Partek Flow software using statistical analysis and interactive visualizations. For somatic oncology applications using targeted RNA-Seq, Illumina Connected Insights enables QC, annotation, interpretation, curation of SNVs, splice variants, and fusion variants from RNA (expression analysis coming in future) with subsequent report generation. RNA may be assessed together with DNA or sequentially, and with either option, results may be merged into a single research report if desired.

Table 8: Illumina mRNA-Seq analysis software

| Pipeline | Application | Input | Access point |
|---|---|---|---|
| DRAGEN RNA | Offers multiple operating modes, including reference-only alignment and annotation-assisted alignment with gene fusion detection. The gene fusion module uses the DRAGEN RNA spliced aligner to perform split-read analysis on supplementary (chimeric) alignments to detect potential breakpoints. | FASTQ<br><br>Optional: Custom reference Gene Annotation File (GTF, GFF, or GFF3) | • DRAGEN server<br>• BaseSpace Sequence Hub<br>• Illumina Connected Analytics<br>• Onboard MiSeq i100 Series, NextSeq 1000/2000 Systems, NovaSeq X Series |
| DRAGEN Microbial Enrichment Plus | Provides secondary analysis of common Illumina infectious disease and microbiology enrichment panels (ie, VSP, RVEK, RPIP) | FASTQ, BCL | • BaseSpace Sequence Hub<br>• Illumina Connected Analytics<br>• Onboard MiSeq i100 Series |
| RNA-Seq visualization and statistical analysis | Supports differential analysis, clustering, and data exploration plots | tsv, csv, txt, gz, FASTQ, BAM | Partek Flow |
| Illumina Connected Insights | Supports streamlined interpretation and reporting from DRAGEN software for oncology research applications | VCF | Illumina Connected Insights with automated ingestion of VCF files |

# Method 3: Total RNA-Seq

In addition to proteins, genes encode a vast array of nonprotein-coding elements that play an instrumental role in orchestrating how a cell is organized from a transcriptional regulation perspective. A major strength of total RNA-Seq lies in its ability to identify novel features of the transcriptome. NGS-based total RNA-Seq can sequence the whole transcriptome, including both coding and noncoding transcripts, without the limitation of probe design, delivering a high resolution, base-by-base view of coding and multiple forms of noncoding RNA activity. This provides a comprehensive picture of gene expression across the full transcriptome at a specific moment in time. With total RNA-Seq, the whole transcriptome—including both known and unknown regions—is captured.[1-4]

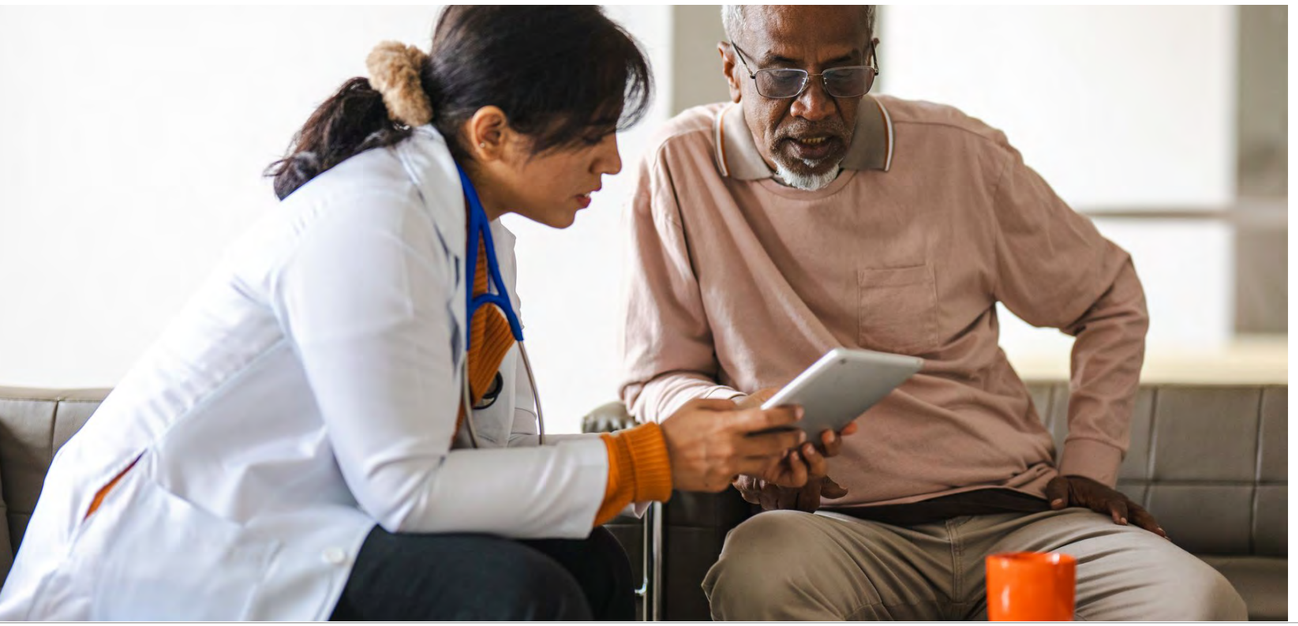
## Relevant applications

### Discovery of pathways associated with disease

Investigations into the transcriptomic differences between cancer samples and non-cancerous tissue have been shown to be useful in differentiating cancer subtypes, assessing the impact of mutations and identifying biomarkers and other variables. Combining total RNA-Seq with DNA sequencing and methylation analyses successfully classified meningioma tumors into distinctive molecular groups that correlated with predicted clinical outcomes, suggesting they are capable of informing medical therapies.[28]

### Assessment of therapy response

Total RNA-Seq has the potential to identify genes and pathways associated with biological response or lack of response to novel drug therapies in model systems or retrospective studies of tissue samples. As part of a multiomic profiling study on tumor samples from melanoma patients undergoing anti-PD-1 immunotherapy, total RNA-Seq identified an interferon gamma (INFγ) expression pattern that, along with other biomarkers, robustly predicted successful response to immunotherapy.[26]







## Step-by-step overview

Total RNA-Seq has five basic steps: RNA extraction, ribosomal RNA (rRNA) depletion (optional) and library preparation, sequencing, data analysis, and insights (Figure 4).

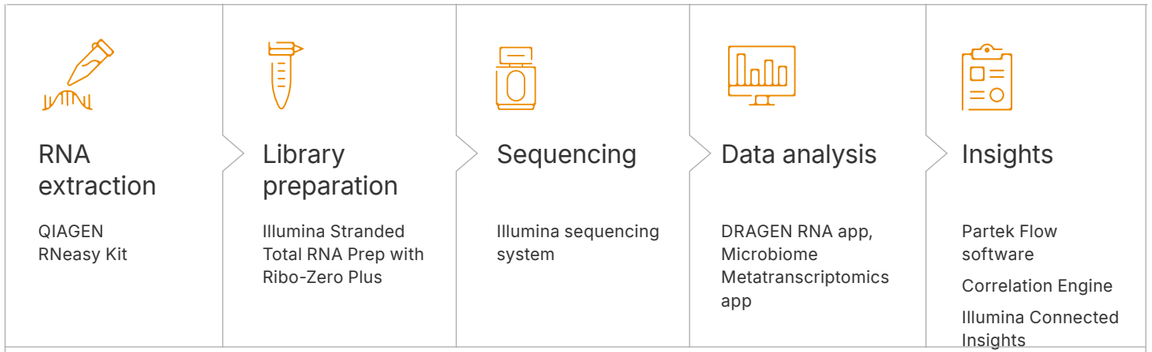



Figure 4: Total RNA-Seq workflow.

**STEP 1**

### RNA extraction

For whole-transcriptome methods, you can use high-quality RNA or degraded samples as input. There are several options for RNA isolation kits depending on your sample type. Illumina recommends the QIAGEN RNeasy for extraction of RNA from cells and QIAGEN RNeasy FFPE for extracting RNA from FFPE tissue. Effectively evaluating RNA quality is a critical step in successful total RNA-Seq and can be achieved by measuring mean RNA fragment size before library prep.

**STEP 2**

### Library preparation

Illumina Stranded Total RNA Prep with Ribo-Zero Plus enables preparation of whole-transcriptome sequencing-ready libraries from low RNA inputs and low-quality samples to support a wide range of RNA-Seq applications. The kit includes Ribo-Zero Plus for single-tube depletion of rRNA and globin RNA from multiple species to facilitate rich transcriptome analyses (Table 9).[30]

Table 9: Illumina Stranded Total RNA Prep with Ribo-Zero Plus specifications

| Feature | Specification |
|---|---|
| RNA input amount | 1 ng high-quality RNA, 10 ng FFPE RNA |
| Total assay time | ~7 hr |
| Hands-on time | < 3 hr |

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STEP 3

## Sequencing

The sequencing system used depends on several factors, including the application, study size, throughput requirements, and more (Table 10).

Table 10: Experimental parameters for performing total RNA-Seq on different sequencing systems

| System | Flow cell | Single reads per flow cell | No. of samples per flow cell[a] | Recommended read length |
|---|---|---|---|---|
| NextSeq 1000 and NextSeq 2000 Systems | P1 | 100M | 2 | 2 × 100 bp |
| | P2 | 400M | 8 | |
| | P3[b] | 1.2B | 24 | |
| | P4[b] | 1.8B | 36 | |
| NovaSeq 6000 System | SP | 1.6B | 32 | 2 × 100 bp |
| | S1 | 3.2B | 64 | |
| | S2 | 8.2B | 164 | |
| | S4 | 10B | 200 | |
| NovaSeq X Series | 10B | 10B | 200 | 2 × 100 bp |
| | 25B | 26B | 384[c] | |

a. Based on 50M reads per sample.
b. P3 and P4 flow cells are only available on the NextSeq 2000 System.
c. Based on Illumina indexes. Additional indexes can be added.

STEP 4

## Data analysis

Illumina recommends using the DRAGEN RNA pipeline for secondary analysis of whole-transcriptome libraries. The DRAGEN RNA pipeline is available on-premises with a DRAGEN Server, on the cloud-based BaseSpace Sequence Hub and Illumina Connected Analytics platforms, and onboard select sequencing systems (Table 11).

For studies that include analysis of metatranscriptomes from complex microbiome samples, such as human gastrointestinal (GI) tract, the Microbiome Metatranscriptome application on BaseSpace Sequence Hub provides taxonomic and functional profiling.

STEP 5

## Insights

For downstream tertiary analysis, output from the DRAGEN RNA pipeline can be automatically ingested into Illumina Connected Insights for variant interpretation and reporting. Results can also be transferred to Correlation Engine for gaining insights into the biological effects of gene expression changes, and to Partek Flow software for further statistical analysis and information-rich visualizations.

Table 11: Illumina total RNA-Seq analysis software

| Pipeline | Application | Input | Access point |
|---|---|---|---|
| DRAGEN RNA | Offers multiple operating modes, including reference-only alignment and annotation-assisted alignment with gene fusion detection<br><br>The gene fusion module uses the DRAGEN RNA spliced aligner to perform split-read analysis on supplementary (chimeric) alignments to detect potential breakpoints | FASTQ<br><br>Optional: Custom reference Gene Annotation File (GTF, GFF, or GFF3) | • DRAGEN Server<br>• BaseSpace Sequence Hub<br>• Illumina Connected Analytics<br>• Onboard NextSeq 1000/2000 Systems, NovaSeq X Series |
| Microbiome Metatranscriptomics | Performs taxonomic and pathway enrichment analysis on microbiome derived RNA libraries | FASTQ | • BaseSpace Sequence Hub |
| RNA-Seq interpretation | Processes sequencing data from mRNA to estimate transcript abundance and identify differentially expressed transcripts across samples | csv, txt, xlsx files | • Correlation Engine |
| RNA-Seq visualization and statistical analysis | Supports differential analysis, clustering, and data exploration plots | tsv, csv, txt, gz, FASTQ, BAM | • Partek Flow software |
| Illumina Connected Insights | Supports streamlined interpretation and reporting from DRAGEN software for oncology research applications | VCF | • Illumina Connected Insights with automated ingestion of VCF files |

## Support that never stops

Illumina strives to be the best partner possible. With a global presence, you can rely on our support to facilitate your success. Technical support is available via phone five days a week or access online support 24/7, worldwide and in multiple languages, with rapid response time near most major metropolitan areas. Illumina provides excellent product consistency, supply, and quality enabled by a mature global manufacturing infrastructure.

**Trusted technology, trusted partner**

As a preferred NGS platform provider, Illumina has shipped over 20,000 sequencing systems globally. Illumina NGS technology is cited in over 421,000 peer-reviewed publications—5× more than all other NGS technologies combined.[31] Building on decades of expertise, Illumina has a relentless commitment to innovation and building future NGS capabilities and applications.

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## Ordering information

### Library preparation

| Product | Catalog no. |
|---|---|
| Illumina Stranded mRNA Prep, Ligation (16 samples) | 20040532 |
| Illumina Stranded mRNA Prep, Ligation (96 samples) | 20040534 |
| Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples) | 20040536 |
| Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples) | 20040537 |
| Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (16 samples) | 20040525 |
| Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (96 samples) | 20040529 |
| Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus Microbiome (96 samples) | 20072063 |
| Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)[a] | 20091654 |
| Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)[a] | 20091656 |
| Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)[a] | 20091658 |
| Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)[a] | 20091660 |
| PhiX Control Kit, v3 | FC-110-3001 |

a. 384 indexes total available when combining sets A, B, C, and D.

## Sequencing systems

| System | Catalog no. |
|---|---|
| MiSeq i100 Plus System | 20115695 |
| NextSeq 1000 System | 20038898 |
| NextSeq 2000 System | 20038897 |
| NovaSeq 6000 System | 20012850 |
| NovaSeq X System | 20084803 |
| NovaSeq X Plus System | 20084804 |

## Data analysis

| Product | Catalog no. |
|---|---|
| BaseSpace Sequence Hub Professional Annual Subscription[a] | 20042109 |
| BaseSpace Sequence Hub Enterprise Annual Subscription[a] | 15066411 |
| Illumina Connected Analytics Professional Annual Subscription | 20044876 |
| Illumina Connected Analytics Enterprise Annual Subscription | 20038994 |
| Illumina Connected Insights–Oncology Genome Equivalent Sample – VCF | 20090138 |
| Illumina Connected Insights Starter Implementation Package | 20071787 |
| Illumina Connected Insights Expanded Implementation Package | 20071787 (as scoped) |
| Partek Flow software | Contact an Illumina sales representative |
| Correlation Engine | Contact an Illumina sales representative |
| DRAGEN Server | 20051343 |

a. BaseSpace Sequence Hub subscriptions include complimentary iCredits for running analysis apps and data storage. Additional iCredits are available for purchase.





# Appendix

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