



NEXT-GENERATION SEQUENCING

SEQuoia Complete Stranded RNA Library Prep Kit

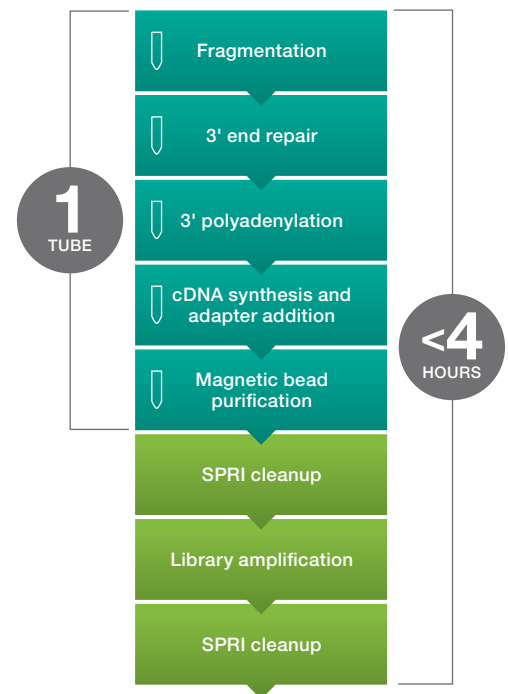
- Full protocol can be completed in less than 4 hours
- Captures long and short RNAs in a single preparation
- Low input sample requirements
- Validated with FFPE and other low-quality samples

The Holistic Approach to Transcriptome Research

The SEQuoia Complete Stranded RNA Library Prep Kit is a high-performance RNA-Seq kit that captures both long and short RNAs in a single library, even from limited and low-quality samples. Featuring SEQzyme, a proprietary engineered enzyme that couples cDNA synthesis with adapter addition in a one-tube continuous synthesis reaction, this kit allows construction of libraries that are >99% stranded and suitable for next-generation sequencing on Illumina® platforms.

The unique enzymatic properties of SEQzyme effectively capture fragments from highly degraded RNA (for example, formalin-fixed paraffin-embedded [FFPE] samples) and short RNA fragments (for example, small nucleolar RNA [snoRNA], microRNA [miRNA], and transfer RNA [tRNA]), which are commonly missed by other commercial RNA library prep kits. The efficient simultaneous capture of diverse RNA fragments results in a library that more accurately represents the complete transcriptome. The streamlined workflow minimizes the number of pipetting steps and reduces the overall protocol time to less than 4 hours.

SEQuoia Complete Stranded RNA Library Prep Kit Workflow



Key Features	Benefits
Efficiently captures short and long RNAs in a single prep, even from degraded or limited samples	Generate robust and reproducible results, even from FFPE and other challenging samples
	Construct comprehensive, diverse libraries from a wide range of starting materials
	Discover biologically relevant markers beyond mRNA
	Reduce cost and time requirements for studies targeting multiple RNA biotypes
Enables library construction in 4 hours or less	Produce more consistent results by reducing the number of pipetting steps
	Increase throughput and reduce overall experiment cost



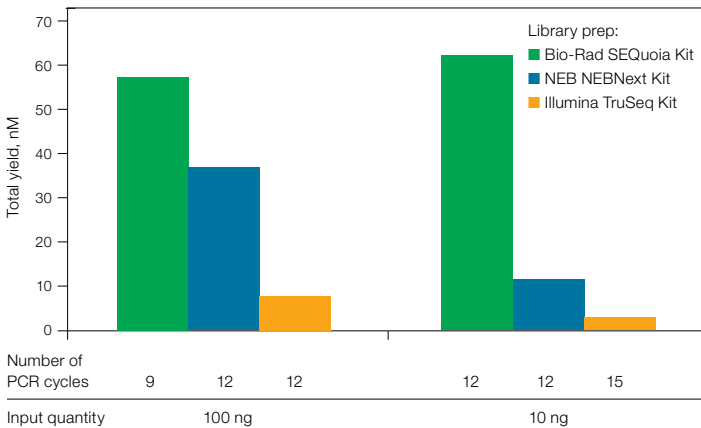
Streamlined Workflow Produces More Data in Less Time, Even with Challenging Samples

Key Attributes	SEQuoia Complete Stranded RNA Library Prep Kit	New England Biolabs (NEB) NEBNext Ultra II Directional RNA Library Prep Kit	Illumina® TruSeq® Stranded Total RNA Library Prep Kit
Library preparation protocol time	<4 hours	~7 hours	~6 hours
Minimum input (rRNA-depleted RNA)	100 pg	5 ng	100 ng
RNA subtypes captured	mRNA, long noncoding RNA (lncRNA), miRNA, snoRNA, tRNA, and more	mRNA, lncRNA	mRNA, lncRNA
Compatible with FFPE and unprocessed RNA samples	*****	***	*
Tubes per kit	7	15	23
Automation friendly	Yes	Yes	Yes
Dedicated analysis solution and toolkit	Yes	No	No

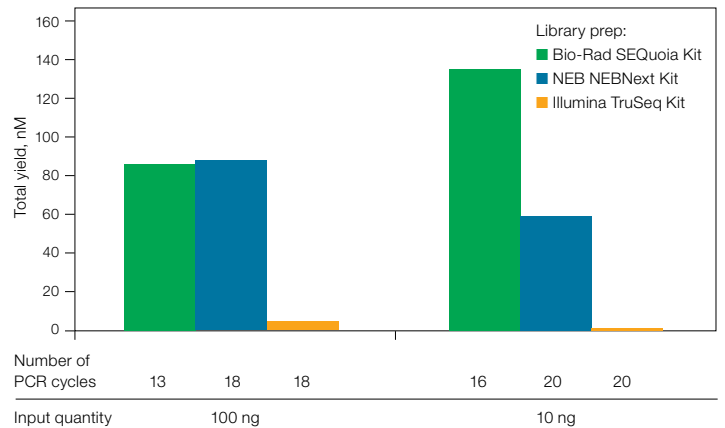
* Poor compatibility. *** Good compatibility. ***** Excellent compatibility.

Better Library Yield with Fewer PCR Cycles

A. RNA from frozen tissue

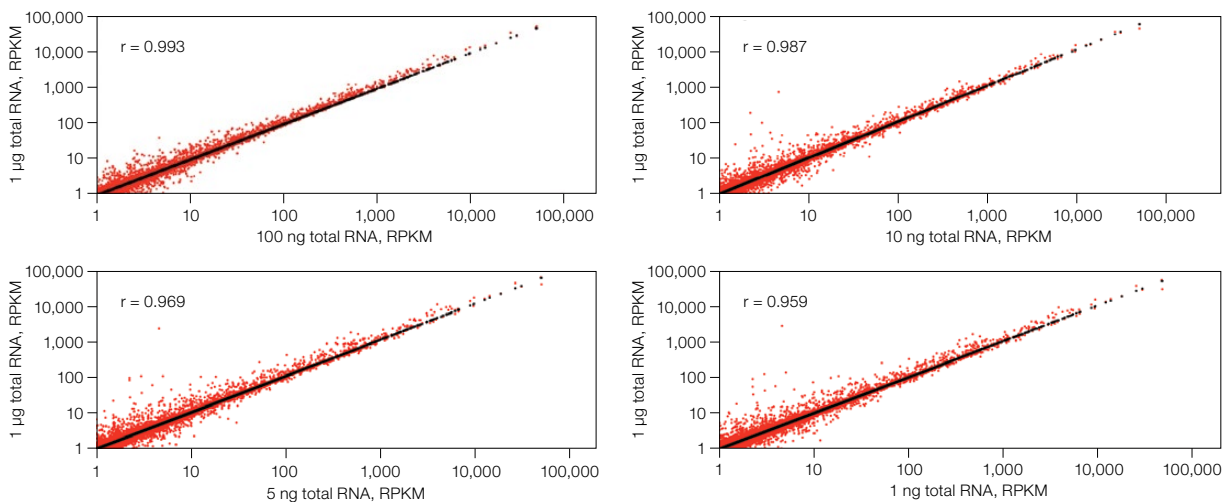


B. RNA from FFPE tissue



The SEQuoia Complete Stranded RNA Library Prep Kit produces substantially higher yields with fewer PCR cycles than other commercial kits when low quantities of degraded RNA are used as input. Ribosomal RNA was depleted using an NEBNext rRNA Depletion Kit (NEB catalog #E6310) from Frozen (A) and FFPE (B) Matched Pair Total RNA: Human Adult Normal Tissue: Liver (BioChain Institute Inc. #R8234149-FP). Libraries were constructed using 100 ng or 10 ng of input with the SEQuoia Complete Stranded RNA Library Prep Kit, NEB NEBNext Ultra II Directional RNA Library Prep Kit, or Illumina TruSeq Stranded Total RNA Library Prep Kit. The total yields relative to the input RNA amount and number of PCR cycles are indicated.

Consistent Results across a Wide Range of Input RNA

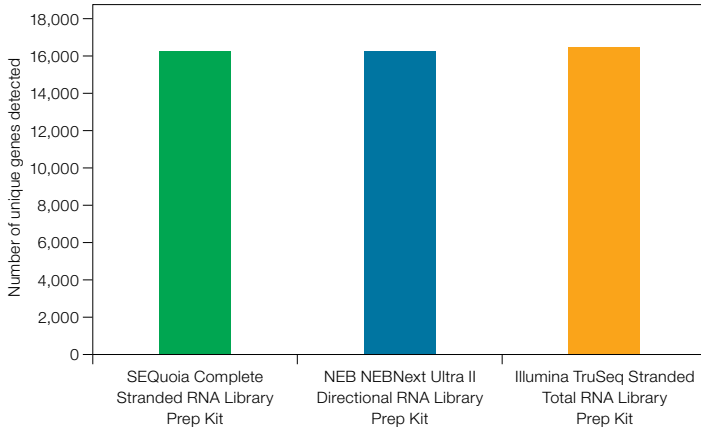


The SEQuoia Complete Stranded RNA Library Prep Kit produces libraries with consistent gene detection across a broad range of input RNA quantities.

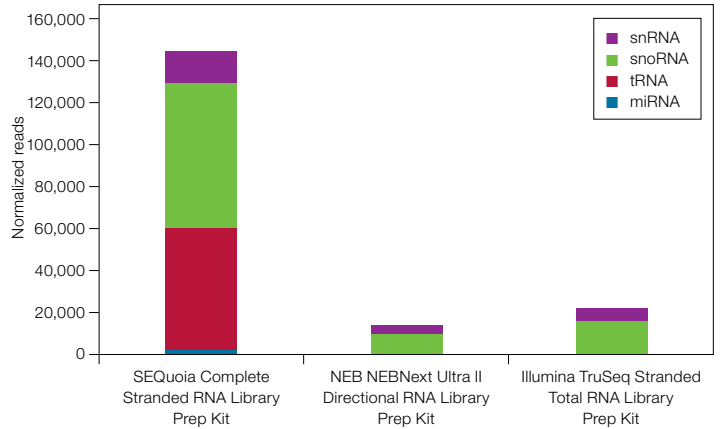
Varying amounts of Human Placenta Total RNA (Thermo Fisher Scientific Inc. #AM7950) ranging from 1 µg to 1 ng were rRNA depleted (NEBNext rRNA Depletion Kit, NEB catalog #E6310), used to construct libraries, and then sequenced. The numbers of reads per kilobase of transcript per million mapped reads (RPKM) derived from each library are plotted against RPKM derived from the 1 µg library (y-axis). Correlation between the libraries, calculated as the Pearson correlation coefficient (r), indicates exceptional detection concordance between the different input amounts, even with limited input.

Unbiased Capture of Long and Short RNA Biotypes

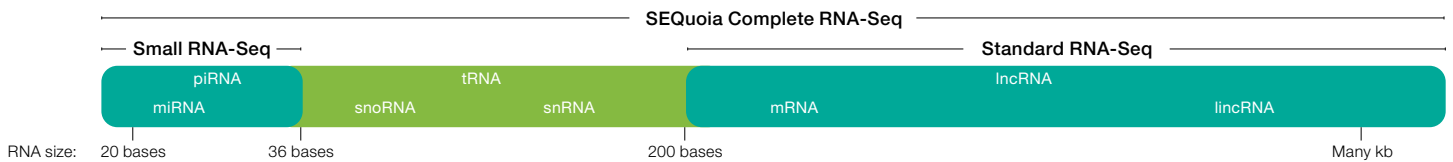
A. Capture of mRNA



B. Capture of small RNA (<200 bases)

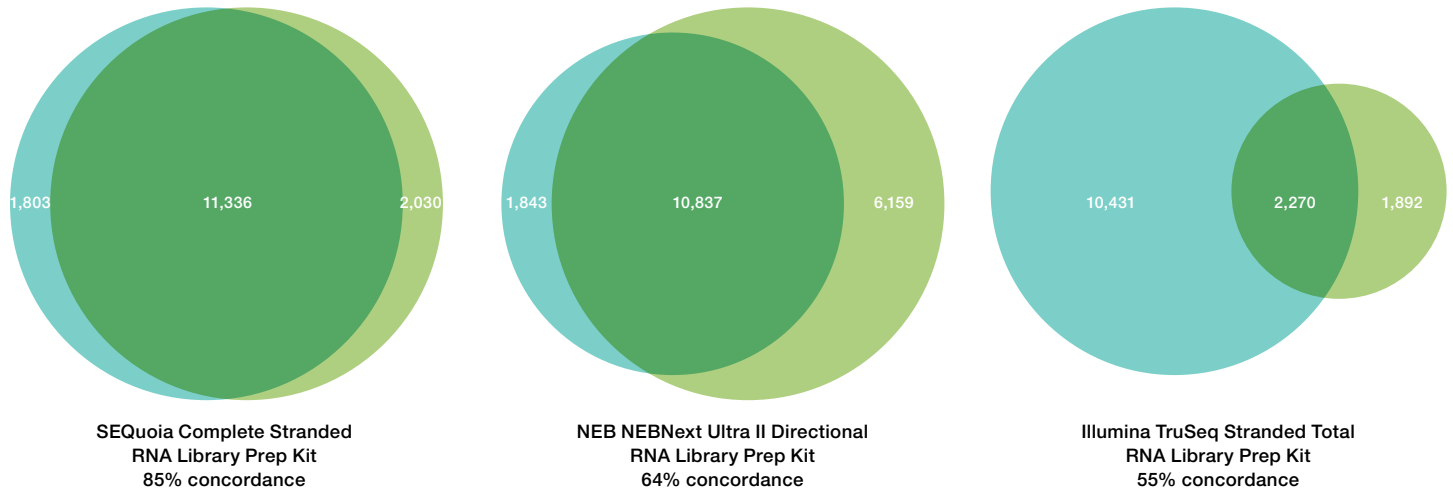


C.



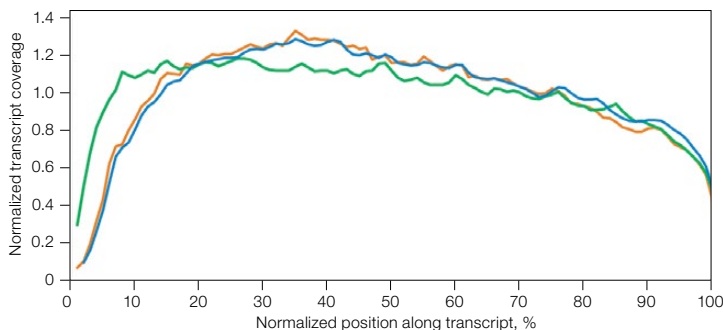
Libraries prepared using the SEQuoia Complete Stranded RNA Library Prep Kit produce richer datasets. Libraries were prepared with either the SEQuoia Complete Stranded RNA Library Prep Kit or other commercial kits using 10 ng of rRNA-depleted RNA and sequenced to a read depth of 10 million reads. **A**, the number of genes detected at >5 RPKM in libraries prepared using the SEQuoia Complete Stranded RNA Library Prep Kit was equivalent to that of libraries prepared using other kits; **B**, in addition to effectively capturing mRNA transcripts, libraries prepared with the SEQuoia Complete Stranded RNA Library Prep Kit included a far greater number of unique reads mapping to RNAs shorter than 200 nucleotides in length, indicating the SEQuoia Kit creates a richer, more complex library than other commercial kits; **C**, the SEQuoia Complete Stranded RNA Library Prep Kit captures a greater diversity of RNA species than standard or small RNA-Seq kits combined. lincRNA, long intervening noncoding RNA; lncRNA, long noncoding RNA; miRNA, microRNA; mRNA, messenger RNA; piRNA, piwi-interacting RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; tRNA, transfer RNA.

Highly Accurate Sequencing Results from FFPE Samples



Gene discovery from limited FFPE samples exhibits high concordance with matched frozen samples using the SEQuoia Complete Stranded RNA Library Prep Kit. Overlap (■) in genes detected at a read depth of 1 RPKM in libraries constructed from 10 ng of rRNA-depleted Frozen (■) or FFPE (■) Matched Pair Total RNA: Human Adult Normal Tissue: Liver (BioChain catalog #R8234149-FP) using the SEQuoia Complete Stranded RNA Library Prep Kit or other commercial kits. Eighty-five percent of genes identified in SEQuoia libraries from the FFPE sample were also found in libraries from the frozen sample, highlighting the biological relevance of the FFPE sequencing results obtained using the SEQuoia Complete Stranded RNA Library Prep Kit.

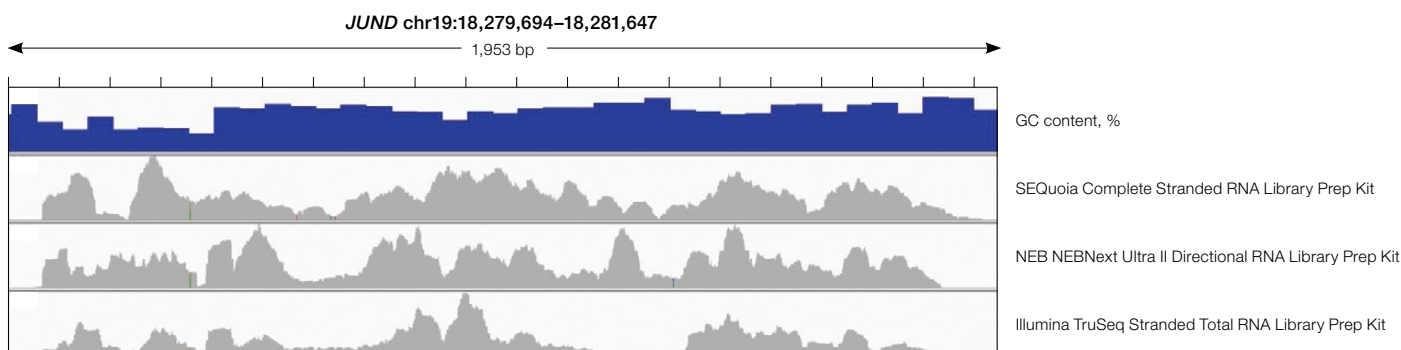
Even Coverage across Transcripts with Minimal Positional Bias



Library Prep Kit	Coverage Uniformity, median CV
SEQuoia Complete Stranded RNA (—)	0.46
NEB NEBNext Ultra II Directional RNA (—)	0.64
Illumina TruSeq Stranded Total RNA (—)	0.66

The SEQuoia Complete Stranded RNA Library Prep Kit provides exceptional coverage, especially at the 5' end. Human Placenta Total RNA (100 ng) was depleted of rRNA, used to prepare libraries, and then sequenced. The median coefficient of variation (CV) for coverage of the 1,000 most highly expressed transcripts is shown as a measure of transcript coverage. Relative to libraries produced using other commercial kits, the library produced using the SEQuoia Complete Stranded RNA Library Prep Kit produced a more uniform distribution of reads per transcript and had better coverage on the 5' end of transcripts.

Improved Coverage of GC-Rich Transcripts



The SEQuoia Complete Stranded RNA Library Prep Kit facilitates detection of GC-rich transcripts. Coverage tracks for the *JUND* transcript, which has a GC content of 70%, are shown. Libraries prepared with the SEQuoia Complete Stranded RNA Library Prep Kit attain more even coverage (97%) than libraries prepared with the equivalent Illumina and NEB Kits (91% and 94%, respectively).

Ordering Information

Catalog #	Description
17005726	SEQuoia Complete Stranded RNA Library Prep Kit, 24 reactions
17005710	SEQuoia Complete Stranded RNA Library Prep Kit, 96 reactions

Related Products

12011928	SEQuoia Dual Indexed Primers Set, 12 vials of unique dual indexed primers, 96 reactions
12011930	SEQuoia Dual Indexed Primers Plate, one 96-well plate of unique dual indexed primers, 96 reactions
1863040	ddPCR Library Quantification Kit for Illumina TruSeq, 200 x 20 µl reactions, includes ddPCR Library Quantification Assay and ddPCR Supermix for Probes (No dUTP), for quantification of Illumina TruSeq libraries using the QX100 or QX200 Droplet Digital PCR System
1851197	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module
TBC0802	0.2 ml 8-Tube PCR Strips and Domed Cap Strips, high-profile, clear

Visit bio-rad.com/SEQuoiaComplete to learn more.



Bio-Rad PCR reagents and analytical instruments are manufactured under an ISO 13485:2016 certified Quality Management System and are quality control tested to ensure consistent product performance you can trust.

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