

SEQuoia Express Stranded RNA Library Prep Kit

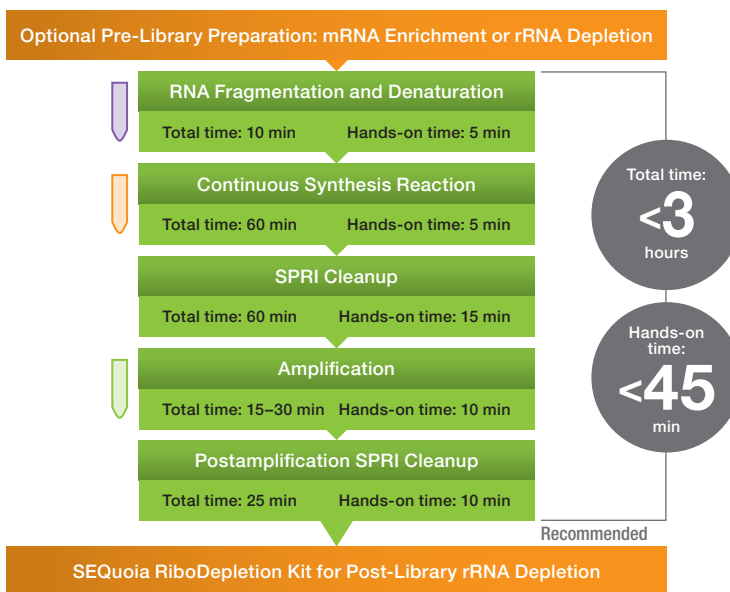
3-Tube, 3-Hour Workflow

- Simplified workflow can be completed in less than 3 hours
- Convenient 3-tube configuration minimizes pipetting steps and hands-on time
- Efficient and unbiased capture of mRNA and lncRNA

The SEQuoia Express Stranded RNA Library Prep Kit constructs high-quality, stranded libraries composed of mRNA and long noncoding RNA (lncRNA) fragments >200 bp, using just three reagents and a protocol that can be completed in less than 3 hours. Featuring SEQzyme, a proprietary engineered enzyme that couples cDNA synthesis with ligation-free adapter addition in a one-tube continuous synthesis reaction, this kit affords high capture efficiency and low sequencing bias with a range of input types and quantities.

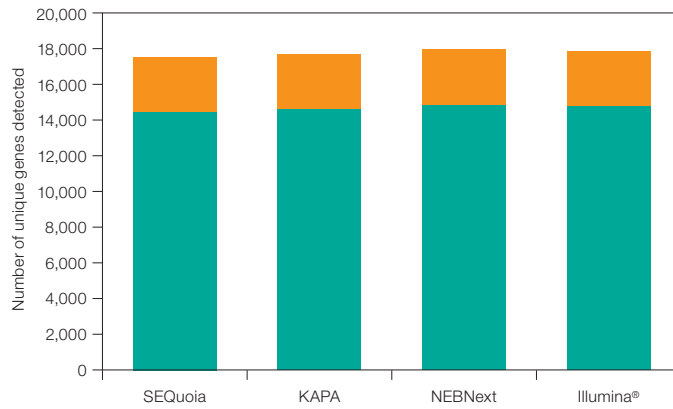
The kit includes the reagents required to assemble complex libraries using either a manual or automated workflow and is compatible with upstream and downstream depletion methods. When used in conjunction with SEQuoia Dual Indexed Primers and the post-library preparation SEQuoia RiboDepletion Kit, the SEQuoia Express workflow can greatly reduce the total cost of library preparation without compromising data integrity or quality.

SEQuoia Express Stranded RNA Library Prep Kit Workflow



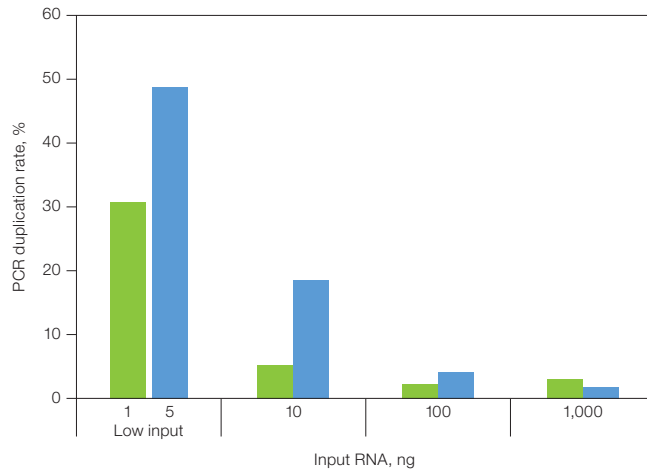
| Key Features | Benefits |
|---|---|
| 3-tube configuration | Produce more consistent results by reducing the number of pipetting steps |
| 3-hour protocol | Increase throughput and reduce overall experiment cost |
| Compatible with paired-end reads | Generate more accurate alignments of reads to the genome |
| Compatible with a range of input types and quantities | Generate high-quality, biologically significant data |

Efficient Capture of mRNA and lncRNA Biotypes



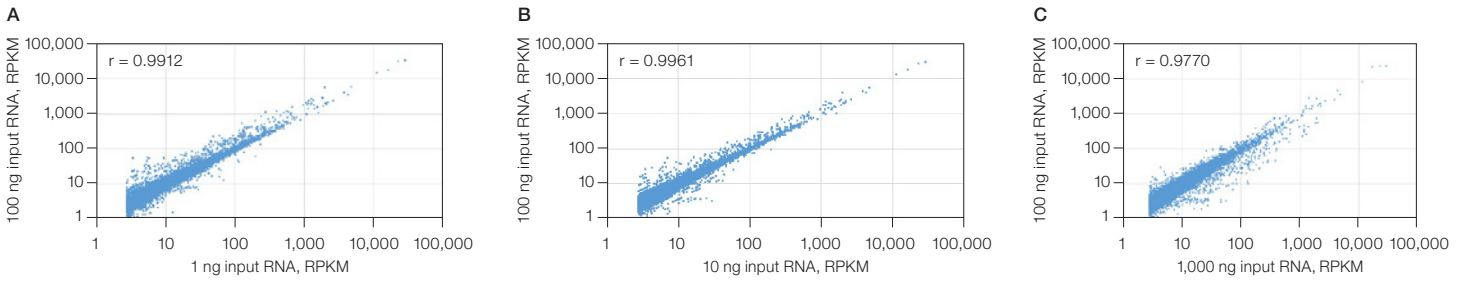
The SEQuoia Express Stranded RNA Library Prep Kit effectively captures mRNA and lncRNA transcripts. Libraries were constructed with the SEQuoia Express Stranded RNA Library Prep Kit (Bio-Rad™ Laboratories, Inc., catalog #12017297 or 12017265), NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, Inc. [NEB], E7760S), KAPA RNA HyperPrep Kit (Roche Molecular Systems, Inc., KK8540), and Illumina Stranded Total RNA Prep with Ribo-Zero Plus Kit (Illumina, Inc., 20040525). One hundred nanograms of Universal Human Reference RNA (Agilent Technologies, Inc., #740000) was used and the libraries were sequenced on an Illumina® NextSeq® 500 System using a NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles) (Illumina, #20024904) with paired-end mode to a read depth of 10 million per library. Reads were aligned to the human reference genome hg38 using the SEQuoia Express Analysis Toolkit. The quantity of mRNA and lncRNA detected at ≥5 reads in libraries prepared using the SEQuoia Express Stranded RNA Library Prep Kit was equivalent to that of libraries prepared using other kits. mRNA (■); lncRNA (■).

Low PCR Duplication Rate



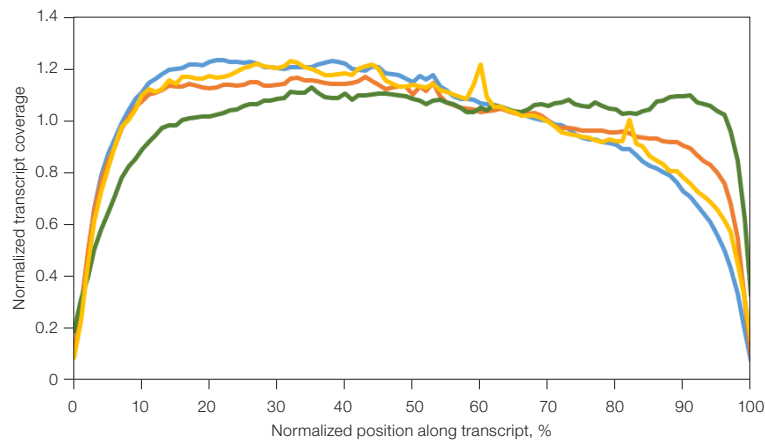
The SEQuoia Express Stranded RNA Library Prep Kit produces high-quality libraries with low duplication rates. A varying amount of Universal Human Reference RNA (Agilent) was used to construct libraries with the SEQuoia Express Stranded RNA Library Prep Kit (Bio-Rad) and the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB), following the manufacturer’s recommendations for the PCR amplification step. Libraries were then sequenced on an Illumina NextSeq 500 System using a NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles) (Illumina) with paired-end mode to a read depth of 10 million per library. The low duplication rates achieved with the SEQuoia Express Kit are indicative of diverse libraries, even with libraries constructed from very low input amounts. SEQuoia (●); NEBNext (●).

Consistent Results across a Wide Range of Input RNA



The SEQuoia Express Stranded RNA Library Prep Kit produces libraries with consistent gene detection across a broad range of input RNA quantities. Libraries were constructed using varying amounts of Human Placenta Total RNA (Thermo Fisher Scientific Inc., #AM7950) ranging from 1 to 1,000 ng, depleted of rRNA-derived constructs using the SEQuoia RiboDepletion Kit (Bio-Rad, #17006487), then sequenced on an Illumina NextSeq 500 System using a NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles) (Illumina) with paired-end mode. The number of reads per kilobase of transcript per million mapped reads (RPKM) for the 10,000 highest-expressing genes derived from a 100 ng library (y-axis) is plotted against RPKM derived from 1 ng (A), 10 ng (B), and 1,000 ng (C) libraries (x-axes). Correlation between the libraries, calculated as the Pearson correlation coefficient (r), indicates exceptional consistent gene detection across the different input amounts, even with low input.

Efficient Coverage across Transcripts with Minimal Bias



| Library Prep Kit | Coverage Uniformity, median CV |
|--|--------------------------------|
| SEQuoia Express Stranded RNA Library Prep Kit | 0.49 |
| NEBNext Ultra II Directional RNA Library Prep Kit for Illumina | 0.46 |
| KAPA RNA HyperPrep Kit | 0.45 |
| Illumina Stranded Total RNA Prep with Ribo-Zero Plus Kit | 0.56 |

The SEQuoia Express Stranded RNA Library Prep Kit provides exceptional coverage uniformity. Libraries were constructed with the SEQuoia Express Stranded RNA Library Prep Kit (Bio-Rad), NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB), KAPA RNA HyperPrep Kit (Roche), and Illumina Stranded Total RNA Prep with Ribo-Zero Plus Kit (Illumina). One hundred nanograms of Universal Human Reference RNA (Agilent) was used and the libraries were sequenced on an Illumina NextSeq 500 System using a NextSeq 500/550 Mid Output Kit v2.5 (150 Cycles) (Illumina) with paired-end mode to a read depth of 10 million per library. 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 constructed using other commercial kits, the SEQuoia Express libraries had a uniform distribution of reads per transcript and better coverage at the 3' end of transcripts. SEQuoia (—); NEBNext (—); KAPA (—); Illumina (—).

Ordering Information

| Catalog # | Description |
|-----------|---|
| 12017297 | SEQuoia Express Stranded RNA Library Prep Kit, 24 reactions |
| 12017265 | SEQuoia Express Stranded RNA Library Prep Kit, 96 reactions |

Related Products

| | |
|----------|--|
| 17006487 | SEQuoia RiboDepletion Kit, 24 reactions |
| 12011928 | SEQuoia Dual Indexed Primers Set, 12 vials of unique dual indexed primers, 96 reactions |
| 12011930 | SEQuoia Dual Indexed Primers Plate, 96-well plate of unique dual indexed primers, 96 reactions |
| 12016658 | CFX Opus Deepwell Real-Time PCR System |
| TBC0802 | 0.2 ml 8-Tube PCR Strips and Domed Cap Strips, high profile, clear |
| TBC1202 | 0.2 ml 12-Tube PCR Strips and Domed Cap Strips, high profile, clear |

Visit bio-rad.com/SEQuoiaExpress for more information.

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