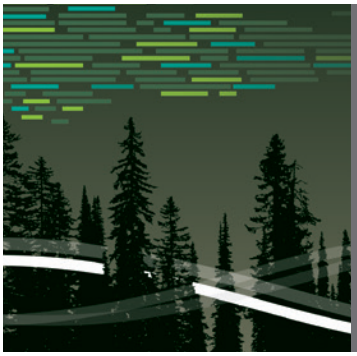


SEQuoia RiboDepletion Kit

A Stand-Alone Post–Library Depletion Solution

- Effectively eliminate rRNA-derived fragments from an RNA-Seq library
- Retain rare transcripts commonly lost in pre–library RNA depletion strategy
- Pool barcoded libraries into a single depletion reaction to save time and money

The SEQuoia RiboDepletion Kit eliminates fragments derived from cytoplasmic ribosomal RNA (rRNA) and mitochondrial ribosomal RNA (mt rRNA) sequences from an RNA-Seq library. Ribosomal RNA is abundant, constituting 80–90% of total RNA. Efficient removal of rRNA is critical to enable cost-effective sequencing of RNA samples. Depletion of total rRNA before library construction can result in the loss of rare or low-abundance transcripts. The post–library preparation depletion strategy employed in the SEQuoia RiboDepletion Kit is ideal for researchers working with a limited sample, targeting rare transcripts, or needing to generate sequencing data that are more representative of the complete transcriptome.

The SEQuoia RiboDepletion Kit exhibits superior performance in profiling the whole transcriptome, retaining small RNAs while minimizing the loss of rare transcripts and RNA from limited samples. This stand-alone depletion kit is optimized to work with a broad input (0.1–20 ng of RNA-Seq library) and is compatible with most available library prep kits. With the innovative post–library depletion technology, multiple pre-indexed libraries can be pooled and depleted in a single reaction, maximizing the depletion efficiency and saving significant time and cost.

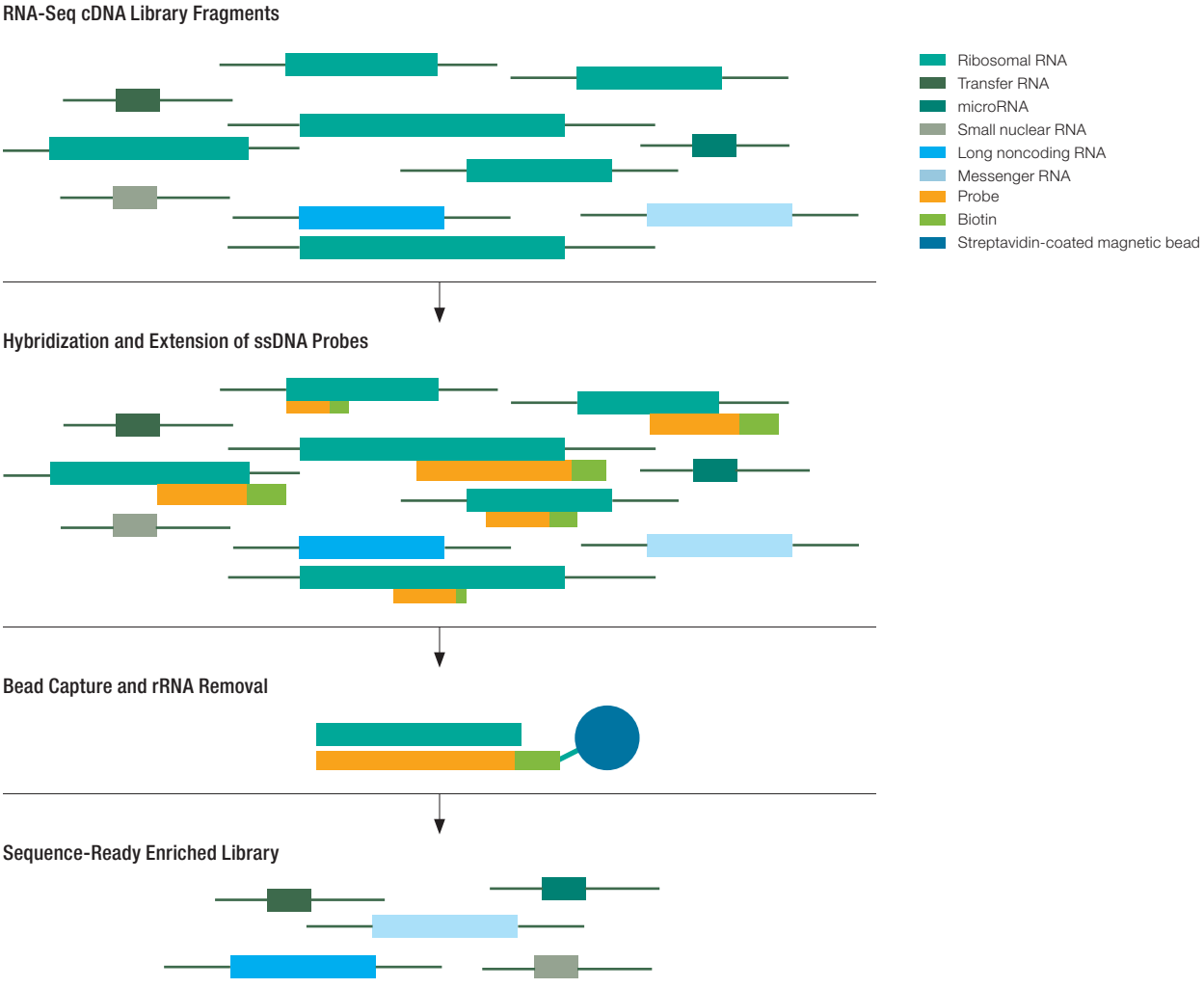
Key Features	Benefits
Post–library depletion strategy	Obtain sequencing data from more of the transcriptome by retaining rare and small transcripts commonly lost in pre–library depletion strategies
Deplete multiple libraries in one reaction	Save time and cost by depleting up to 96 pooled libraries in a single reaction Maximize sequencing efficiency and flexibility by depleting pooled libraries constructed using different available library preparation kits
Broad dynamic input range	Obtain biologically relevant sequencing data from libraries with an input of 0.1–20 ng cDNA
Streamlined workflow	Produce more consistent results with a simple four-tube kit Increase throughput and generate more data faster with a 2 hour protocol
Stand-alone kit that is compatible with most available RNA-Seq library prep kits	Tailor library preparation and depletion strategies to accommodate specific experimental needs

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

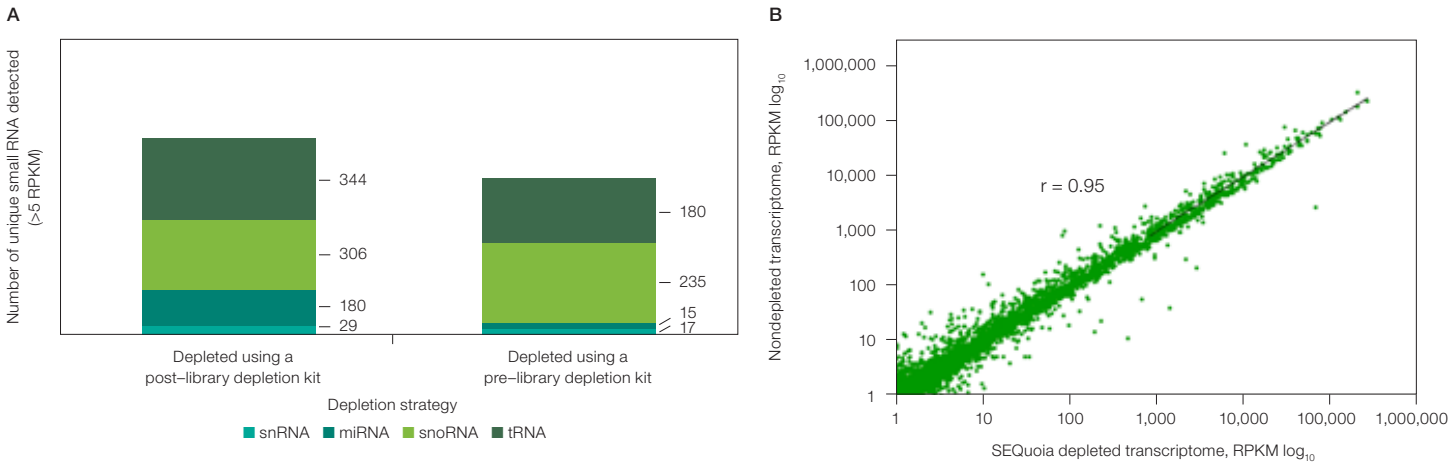
BIO-RAD

Streamlined Workflow

The SEQuoia RiboDepletion Kit uses a proprietary blend of synthetic biotinylated oligonucleotides with homology to cDNA produced from human, mouse, and rat cytoplasmic rRNA (5S, 5.8S, 18S, and 28S rRNA) and mt rRNA (12S and 16S mt rRNA). Target cDNA sequences hybridize to complementary biotinylated oligonucleotides and are extended through a DNA synthesis reaction. The resulting double-stranded DNA is captured using streptavidin-coated paramagnetic beads and removed from the library. This streamlined workflow can be completed in 2 hours.

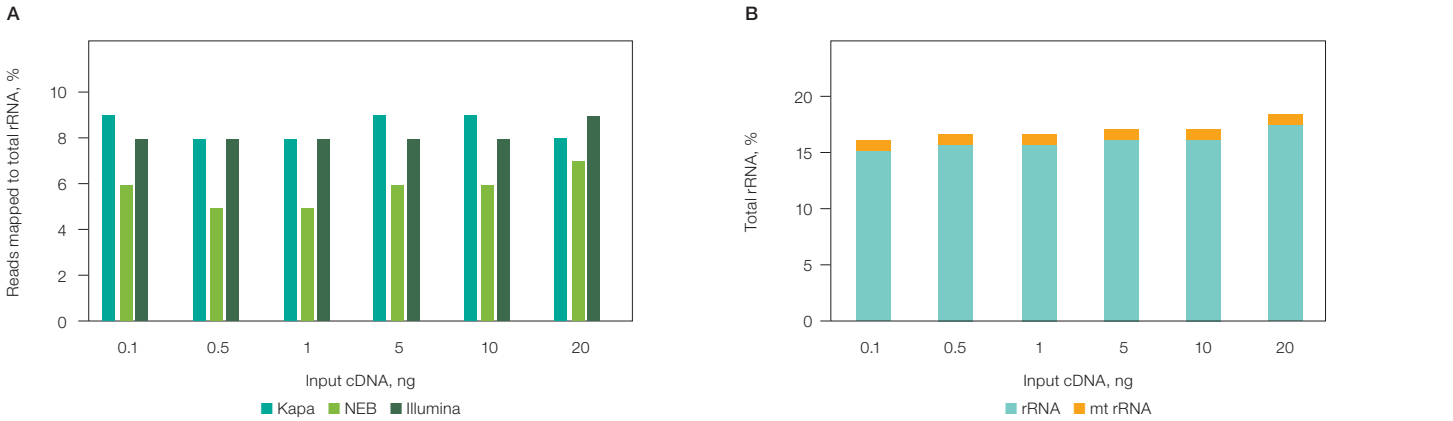


Superior Preservation of the Whole Transcriptome



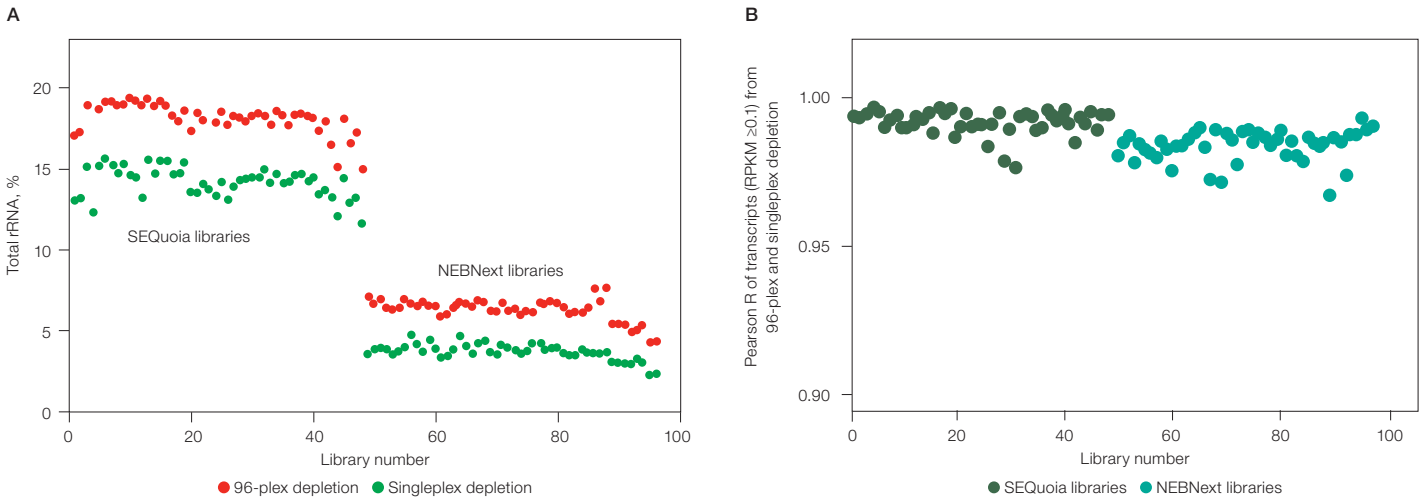
Depletion of rRNA after RNA-Seq library preparation enriches for RNAs of interest and preserves the diversity of the transcriptome. A, a significantly greater number of small RNAs (<200 bases) is retained using a post–library depletion strategy compared to a pre–library depletion strategy. Libraries were constructed using the SEQuoia Complete Stranded RNA Library Prep Kit (catalog #17005726) and either rRNA depleted (NEBNext rRNA Depletion Kit, New England Biolabs [NEB], #E6350L) Human Placenta Total RNA (Thermo Fisher Scientific Inc., #AM7950) or nondepleted Human Placenta Total RNA. The library constructed using nondepleted total RNA was subsequently depleted using the SEQuoia RiboDepletion Kit. Libraries were then sequenced on a NextSeq® 500 Sequencing System (Illumina, Inc.) to a read depth of 10 million. The number of unique small RNAs detected (RPKM ≥5) was significantly more in the post–library depletion sample, resulting in a more complex library and richer dataset. **B**, treatment with the SEQuoia RiboDepletion Kit does not affect the abundance of nontargeted transcripts. RNA-Seq libraries constructed with the SEQuoia Complete Stranded RNA Library Prep Kit, using 100 ng of Human Placenta Total RNA, were either depleted of rRNA using the SEQuoia RiboDepletion Kit or left nondepleted. Libraries were sequenced to a read depth of 10 million (depleted library) or 29 million (nondepleted library). The RPKM of the transcriptome for the nondepleted library was plotted against the SEQuoia depleted library. The concordance calculated using the Pearson correlation coefficient, R >0.95, indicates rRNA depletion after library preparation preserves the whole transcriptome without the bias of the quantification of non-rRNA transcript. miRNA, microRNA; RPKM, reads per kilobase per million mapped reads; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; tRNA, transfer RNA.

Consistent Depletion across a Broad Input Range of RNA-Seq Libraries Constructed Using Different Kits



Effective depletion of rRNA from libraries constructed using a variety of RNA library prep kits and a broad input range. A, the SEQuoia RiboDepletion Kit effectively removes rRNA fragments from libraries constructed using the NEBNext Ultra II Directional RNA Library Prep Kit (#E7760S), Illumina® TruSeq® Stranded Total RNA Library Prep Kit (#20020596), or KAPA RNA HyperPrep Kit (Roche Sequencing, #KK8540). Each library was constructed using 100 ng of Human Placenta Total RNA according to the manufacturer's instructions. A broad range of input cDNA (0.1–20 ng) from each library was depleted using the SEQuoia RiboDepletion Kit and sequenced to a read depth of 10 million. On average, <10% of sequencing reads map to rRNA and mt rRNA, indicating efficient and consistent depletion across a broad input range. **B**, libraries constructed using the SEQuoia Complete Stranded RNA Library Prep Kit (#17005726), which captures more of the transcriptome and includes smaller RNA fragments that are commonly lost with other library preparation kits, consistently had <20% of sequencing reads mapping to rRNA and mt rRNA. This result is consistent across a broad range of input cDNA (0.1–20 ng). mt rRNA, mitochondrial ribosomal RNA; rRNA, ribosomal RNA.

Efficient Multiplex Depletion



RNA-Seq libraries can be depleted in a 96-plex pooled reaction without affecting library quality. A, similar depletion efficiency is obtained when libraries are depleted individually compared to when they are pooled and depleted in one multiplexed reaction, even if the libraries are constructed using different library preparation kits. Ninety-six individual pre-indexed libraries were constructed using 100 ng Human Placenta Total RNA: 48 libraries were constructed using the SEQuoia Complete Stranded RNA Library Prep Kit and 48 libraries with the NEBNext Ultra II Directional RNA Library Prep Kit. An aliquot of each library was either individually depleted (singleplex) using the SEQuoia RiboDepletion Kit or pooled (multiplex) by mixing an equal molar ratio of the libraries and then depleted. Libraries were sequenced on the NextSeq 500 Sequencing System to a read depth of 3 million reads. Consistently, the percentage of reads mapping to rRNA in a library that was depleted in the multiplex reaction was comparable to those libraries that were depleted individually. **B**, transcriptome profiling is preserved when libraries are depleted in a pooled reaction. The number of total unique transcripts (RPKM >0.1) in the libraries depleted in the pooled reaction was compared to the number in the individually depleted libraries. For each library, the correlation R was >0.97. These data suggest RNA-Seq libraries can be depleted in one multiplexed reaction without compromising depletion efficiency or transcript expression. RPKM, reads per kilobase per million mapped reads; rRNA, ribosomal RNA.

Ordering Information

Catalog #	Description
17006487	**SEQuoia RiboDepletion Kit**, 24 reactions
Related Products	
17005726	**SEQuoia Complete Stranded RNA Library Prep Kit**, 24 reactions
17005710	**SEQuoia Complete Stranded RNA Library Prep Kit**, 96 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
1851197	**C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module**
1854095	**CFX96 Touch Deep Well Real-Time PCR System**
TBC0802	**0.2 ml 8-Tube PCR Strips and Domed Cap Strips**, high-profile, clear

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

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.

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 7460 Ver A US/EG *21-0212 0321 Sig 0121*