Now includes
ARTIC Kits for Viral Sequencing and Customizable RNA Depletion

NEBNext® for RNA Sample Prep

FOR THE ILLUMINA® PLATFORM

NEW ENGLAND BioLabs® Inc.

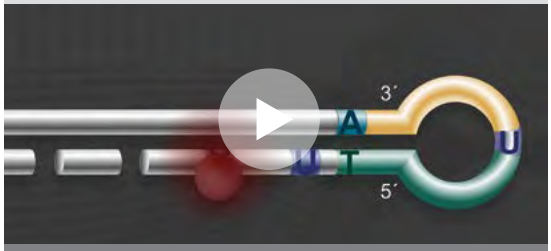
be INSPIRED
drive DISCOVERY
stay GENUINE

Table of Contents

TOOLS & RESOURCES

Visit NEBNext.com to find:

- The full list of products available
- Video protocols
- Workflow animations
- Online tutorials to help with product selection, general handling tips and more
- Access to **NEBNext Selector Tool**, our online tool for help with selecting the right NEBNext product
- NEBNext citations
- Protocols & FAQs



NEBNEXT ULTRA II RNA WORKFLOW

Why Choose NEBNext for RNA?

RNA-seq's increasing requirements for sensitivity and specificity, along with a desire to push boundaries on input amounts and quality, mean that sample prep needs not just to keep up, but to drive your next discovery. With so many options, we know you have choices, so these are just a few of the reasons to choose NEBNext.

High Performance and Streamlined Workflows

The NEBNext suite of products supports sequencing of multiple types of RNAs on the Illumina platform, with sample prep tools that streamline workflows, minimize inputs, improve library yields and quality, and allow you to sequence relevant RNAs. NEBNext RNA library prep kits are compatible with a wide range of inputs (single cell to a microgram of total RNA) and sample qualities (high- and low-quality). Options are also available for small RNA library prep.

Our expanding selection of reagents for upstream depletion of abundant RNA enables removal of ribosomal RNA from human, mouse, rat and bacterial samples, as well as depletion of globin mRNA from blood and customizable depletion of any RNA.

To meet your multiplexing needs, our list of indices (barcodes) continues to grow, and our qPCR-based library quantitation method provides accurate yield determination.

Reliable and Time Tested

Since our first product release in 2009, the NEBNext brand has stood for quality you can count on, with extensive QCs performed on individual kit components, plus functional validation by preparation of a library, followed by Illumina sequencing. Additionally, NEBNext products have been cited in over 17,000 peer-reviewed publications.

Flexible Formats

Kits and modules

Kits are the most convenient option, as they include reagents for the entire library prep workflow. Many kits are available with RNAClean® and SPRISelect® beads for clean-up and size-selection steps.

When flexibility is a priority, NEBNext modules contain reagents for individual steps in library preparation. These modules can be combined to cover the entire library prep workflow, or a subset of NEBNext modules can be combined with other reagents to enable a customized workflow for your specific needs.

Adaptors and primers are supplied separately from the NEBNext kits (as NEBNext Oligos),* allowing for increased customization in multiplexing options.

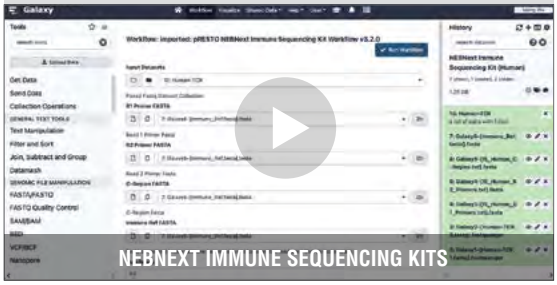
*except in the case of the small RNA kits, which include adaptors and primers.

Bulk & custom formats:

When your reagent needs exceed standard volumes, or you require a specialized formulation or kit, consider NEBNext's Customized Solutions options. As reagent manufacturers, we are able to provide customized components, kits and modules to meet your specific needs. For more information, please contact Custom@neb.com.

WHATS NEW IN NEBNEXT?

- **ARTIC Kits for SARS-CoV-2** sequencing and variant detection
- Customizable RNA depletion for any species
- 480 unique dual index primer pairs
- The **NEBNext Immune Sequencing Kits** for human or mouse samples



Visit NEBNextSelector.neb.com to access the NEBNext Selector Tool, our online tool for help with selecting the right NEBNext product

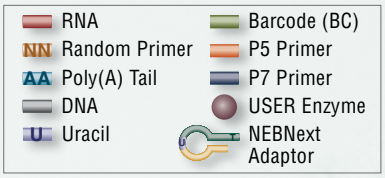
DOWNLOAD THE NEB AR APP*

Find an overview of NGS library preparation.

*see back cover for details

Workflow for RNA Library Preparation

PRODUCT	INPUT AMOUNTS
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760)	10 ng - 1 µg Total RNA (rRNA Depletion Workflow) 10 ng - 1 µg Total RNA (poly(A) mRNA workflow)
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (NEB #E7765)	
NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770)	
NEBNext Ultra II RNA Library Prep with Sample Purification Beads (NEB #E7775)	
NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)	
NEBNext Oligos (including 12-plex, 96-plex, dual and unique-dual index primers and UMI adaptors) (NEB #E6440, #E6442, #E6444, #E6446, #E6448, #E6609, #E7335, #E7416, #E7500, #E7600, #E7710, #E7730, #E7780)	



1 RNA Enrichment (rRNA Depletion or Poly(A) mRNA Isolation)

- Removal of rRNA (> 80% of total RNA) or enrichment for mRNA
- NEBNext Library Prep kits are compatible with either method

2 RNA Fragmentation & Random Priming

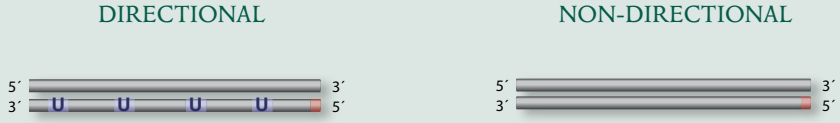
- Fragmentation by incubation with divalent cations (e.g., Mg^{++}) or enzymes (e.g., RNase III)
- Hybridization of random primers

3 First Strand cDNA Synthesis

- Reverse transcriptase lacking RNase H activity is optimal (does not degrade RNA in RNA:DNA complex)
- For directional RNA library preparation, Actinomycin D is added:
  – To inhibit DNA-dependent DNA Polymerase activity of RT & inhibit second strand synthesis/increase strand specificity

4 Second Strand cDNA Synthesis

- Generation of nicks & gaps in RNA by RNase H, enabling second strand synthesis by nick translation
- Sealing of breaks in second strand by *E. coli* DNA ligase
- For Directional RNA library preparation, second strand labeled with uracils by dUTP incorporation



5 End Repair, dA-Tailing & Adaptor Ligation

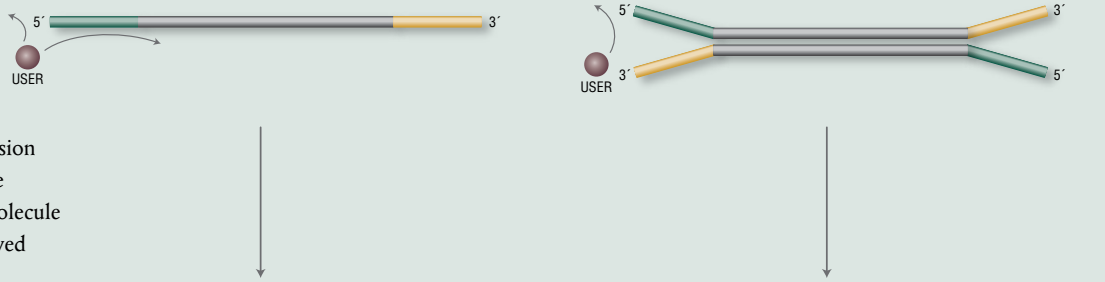
- Generation of blunt, phosphorylated ends
- Addition of single A 3´ overhang (enables ligation to adaptors with single T overhangs)
- Ligation of short adaptors (contain sequences required downstream)
- NEBNext adaptors increase ligation efficiency & minimize adaptor-dimer formation

6 U Excision

- Removal of uracils in NEBNext Adaptor loop by USER Enzyme (to make accessible for PCR)

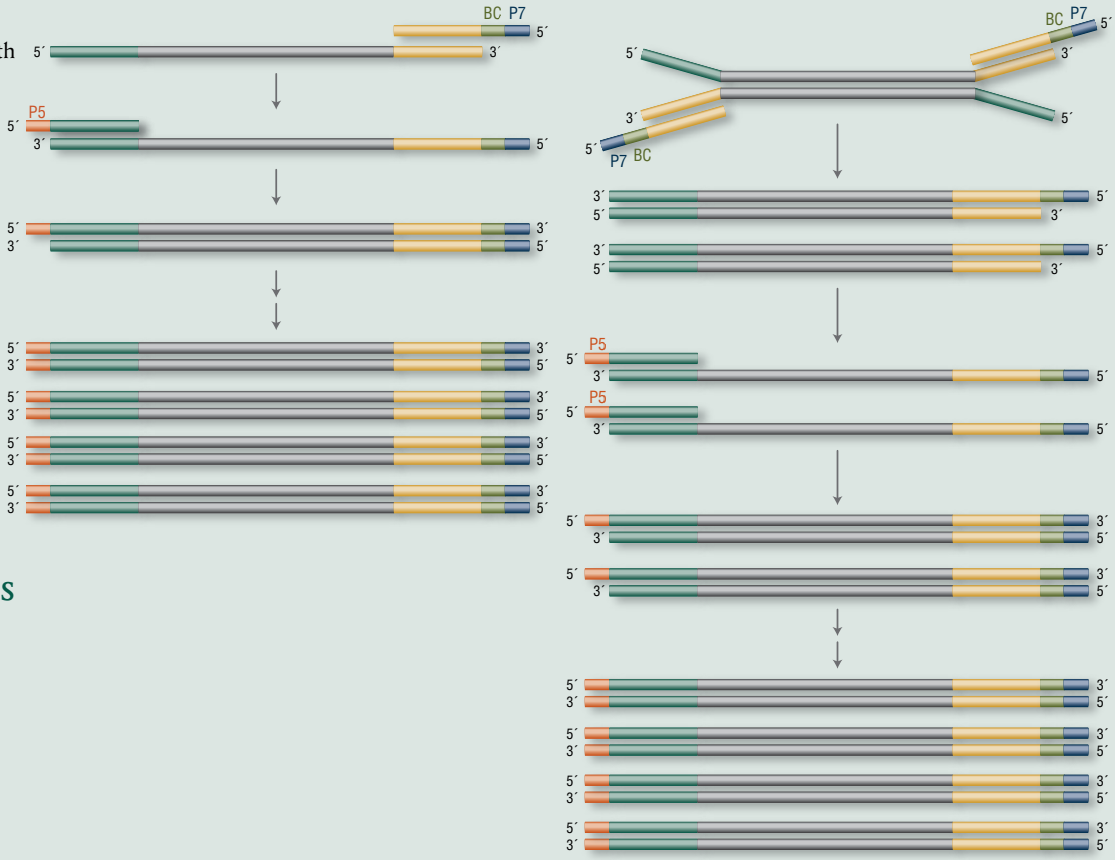
Directional Only

- Selective removal of second strand through excision of uracils by USER Enzyme
- Result is single-stranded molecule with different adaptor-derived sequences on each end



7 PCR Enrichment

- Amplification using a high-fidelity polymerase:
  - Selects for molecules with an adaptor at each end
  - Increases library yield
  - Incorporates barcodes/ indices to enable multiplexing, and P5 & P7 sequences required downstream



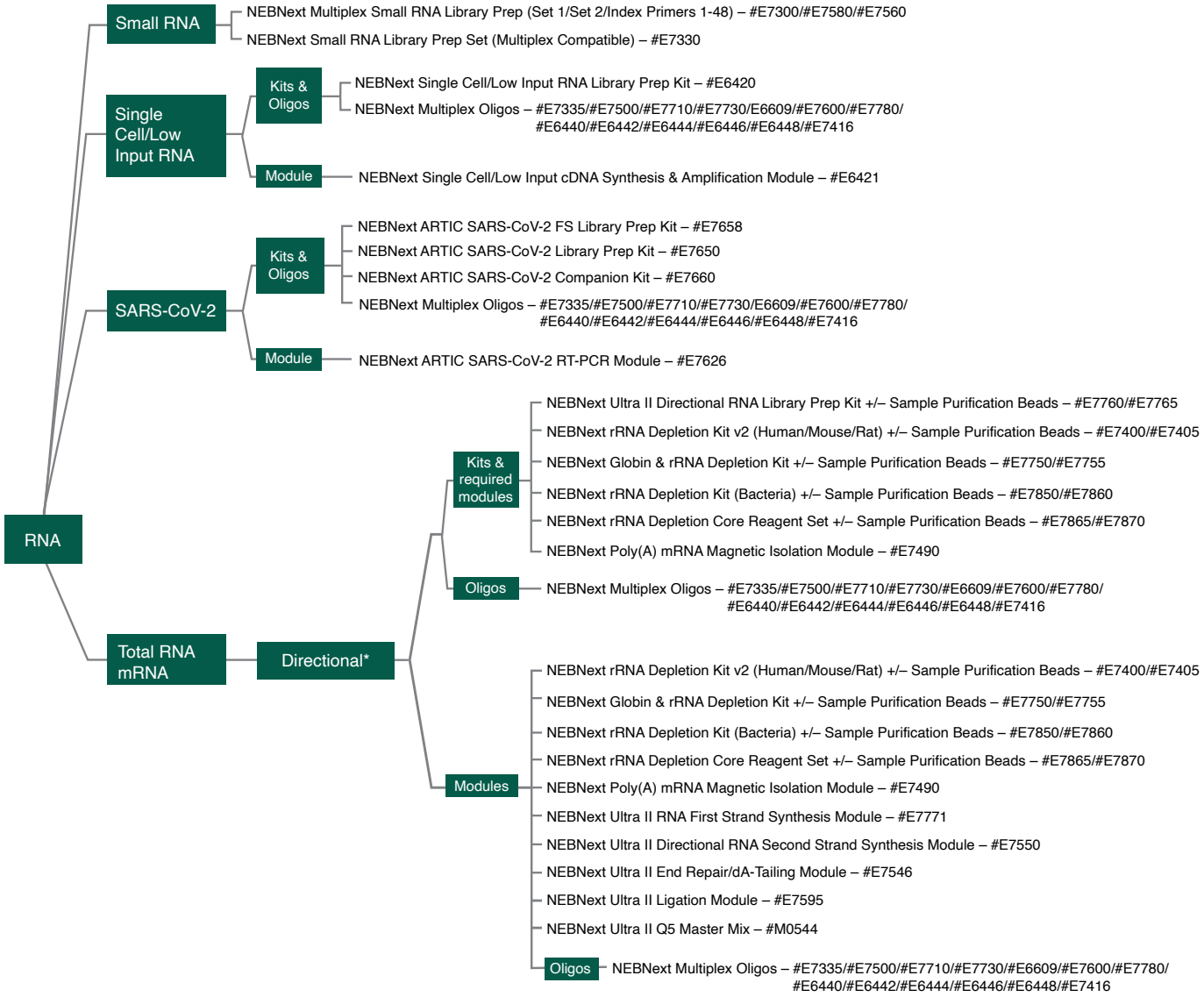
NEBNext Oligos

- Barcodes incorporated using NEBNext primers
- Unique dual-, dual-, and single- barcode primer options available

RNA Product Selection

NEBNext Ultra II RNA kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method") and non-directional library construction. The kits are compatible with poly(A) mRNA enrichment or ribosomal RNA depletion, for low nanogram to microgram total RNA inputs. The kits are also available with the option of SPRISelect beads for size-selection and clean-up steps. Our novel Small RNA workflow has been optimized to minimize adaptor-dimers, while producing high-yield, high-diversity libraries. See page 16 for more information. Modules offer the ability to customize sample preparation, and are available for directional and non-directional RNA library prep workflows. Adaptors and primers (NEBNext Oligos) are supplied separately.

Use this chart to determine the best NEBNext product for your Illumina RNA library preparation. You can also use our online tool, **NEBNext Selector at NEBNext Selector.com**, to choose the best products for your needs.



*Notes: *Products and workflows are also available for non-directional RNA library prep.*
Reagents for the original Ultra workflow are also available.
See ordering information.

NEBNext for RNA Library Prep & Tips for Working with RNA

RNA Sample Input Guidelines

Integrity of RNA

- We recommend determining the RNA Integrity Number (RIN) as estimated by the Agilent TapeStation or similar. Ideally, samples will have a RIN value of 7 or higher, but NEBNext RNA products are compatible for use even with low-RIN samples.
- RNA should be completely free of DNA, and DNA digestion of the purified RNA using RNase-free DNase I (such as that provided with the Monarch Total RNA Miniprep Kit) is recommended.

Quantitation of RNA

- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer or similar, using a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop. Note that free nucleotides or organic compounds used in some RNA extraction methods may cause an over-estimation of RNA concentration.

Bead-based clean-ups and size selection

- Be sure to vortex the beads well just before use. They should form a uniform suspension. When beads have not been used for several weeks, plan for extra time for bead vortexing and agitation.
- Do not over-dry the beads. Beads should still be dark brown and glossy when eluting. Over-drying can make resuspension difficult and reduce yield.
- Take care not to remove beads after separation.
- Remove all of the supernatant after the bind step. Incomplete supernatant removal can cause leftover adaptor dimer or PCR primers to remain in the libraries.
- When adding beads to the sample, aspirate slowly, remove any droplets from the outside of the tip and make sure to dispense the full volume into the sample.

Barcodes

- It is important to optimize the combination of indices used in order to ensure balanced sequencing reads. Refer to the product manual for recommendations.
- For index primers provided in vials, open only one vial at a time to minimize the risk of contamination.
- Be sure to change pipette tips for each index primer.
- For 96-well plate formats, NEBNext index primers are provided in single-use plates with pierceable foil lids; do not pipette from a well more than once.

NEBNext Magnetic Separation Rack

Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps and it is important for library yield and quality that bead separation be highly efficient and fast.

The NEBNext Magnetic Separation Rack was designed for this application and contains rare earth Neodymium Iron Boron (NdFeB) magnets, the most powerful commercially available magnets, in an anodized aluminium rack. The rack holds 24 0.2 ml tubes, and is compatible with single tubes or strip tubes.

ADVANTAGES

- Fast separations in purification and size-selection steps in next generation sequencing workflows
- 24 tube capacity

NEBNEXT MAGNETIC SEPARATION RACK

Ultra II for RNA

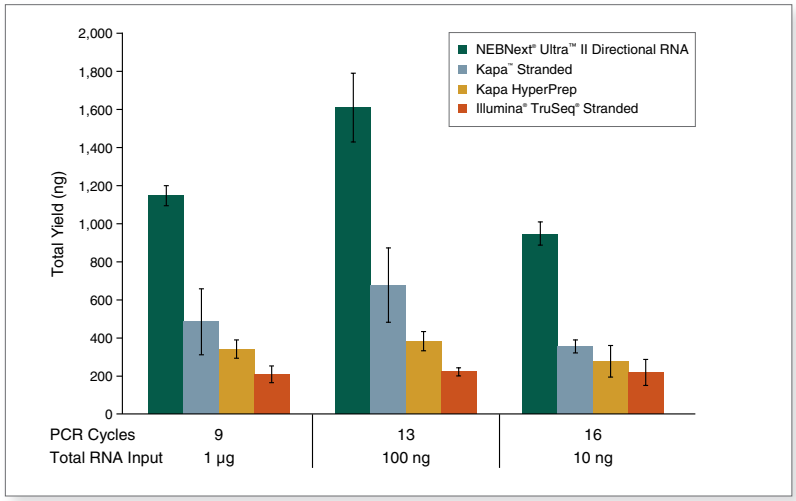
The latest generation of NEBNext kits for RNA enable directional (strand-specific) or non-directional library construction from low ng to µg input amounts, and are compatible with poly(A) mRNA enrichment or rRNA depletion. Workflows are streamlined with minimal hands-on time, and are automatable. Information on NEBNext rRNA Depletion and NEBNext Poly(A) mRNA enrichment is available on page 11.

Directional (Strand-specific) RNA Library Preparation

Non-directional methods for RNA library preparation do not retain information on the DNA strand from which the RNA strand was transcribed. However, the ability to obtain information on the originating strand is useful for many reasons including the identification of antisense transcripts, determination of the transcribed strand of noncoding RNAs, and determination of expression levels of coding or non-coding overlapping transcripts. Overall, the ability to determine the originating strand can substantially enhance the value of a RNA-seq experiment.

The NEBNext Ultra Directional RNA Library Prep Kit uses the high-performing "dUTP method" (1,2) for strand-specificity.

NEBNext Ultra II Directional RNA produces the highest yields, from a range of input amounts



Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent® #740000) and libraries were made using the NEBNext Ultra II Directional RNA Kit (plus the NEBNext poly(A) mRNA Magnetic Isolation Kit), Kapa™ Stranded mRNA-Seq Kit, Kapa mRNA HyperPrep Kit and Illumina TruSeq® Stranded mRNA Kit. The input RNA amount and number of PCR cycles are indicated. Library yields from an average of three replicates are shown.

- **Generate high yield**, high-quality libraries, even with limited amounts of RNA:
  - 10 ng – 1 µg total RNA (rRNA depletion workflow v2)
  - 10 ng – 1 µg total RNA (poly(A) mRNA workflow)
- **Minimize bias**, with fewer PCR cycles required
- **Increase library complexity** and transcript coverage
- **Increase flexibility** by ordering reagents specific to your workflow needs:
  - Directional and Non-directional kits available
  - rRNA depletion and poly(A) mRNA isolation reagents supplied separately
  - Adaptors and primers supplied separately
- Enjoy the **reliability of the gold standard SPRIselect size selection and clean-up beads**, supplied in just the amounts you need
- Save time with **streamlined workflows**, reduced hands-on time, and automation compatibility
- Rely on **robust performance**, even with low quality RNA, including FFPE

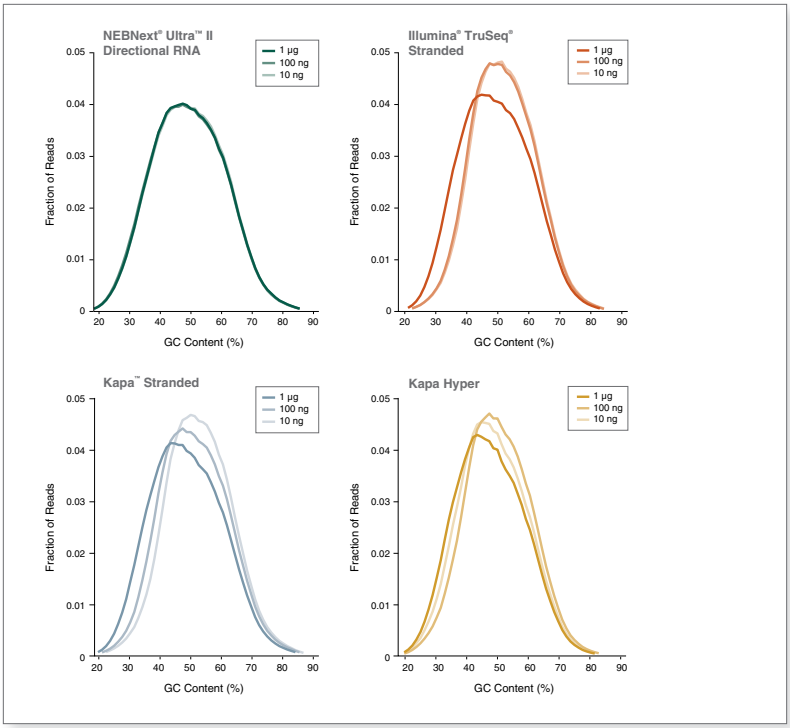
TOOLS & RESOURCES

- View and download performance data in our Technical Notes at **UltraIIRNA.com**

View the NEBNext Ultra II Directional RNA Workflow.

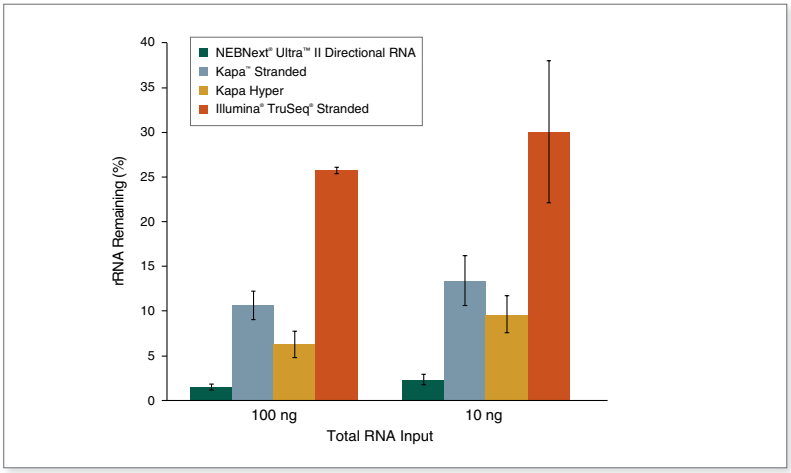
References
1. Parkhomchuk, D., et al. (2009) Nucleic Acids Res. 37, e123.
2. Levin, J.Z., et al. (2010) Nature Methods 7, 709–715.

NEBNext Ultra II Directional RNA libraries provide uniform GC content distribution, at a broad range of input amounts



Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (Agilent #740000), and libraries were made using the NEBNext Ultra II Directional RNA Kit (plus the NEBNext Poly(A) mRNA Magnetic Isolation Module), Illumina TruSeq Stranded mRNA Kit, Kapa Stranded mRNA-Seq Kit and Kapa mRNA HyperPrep Kit. Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2 x 76 bp). Reads were mapped to the hg19 reference genome. GC content distribution for each library was calculated using mapped reads. Ultra II Directional RNA libraries had uniform GC content distribution across a range of input amounts, whereas for other kits the GC content distribution changed with different input amounts, indicating the introduction of input-dependent sequence bias.

NEBNext Ultra II Directional RNA with NEBNext rRNA Depletion results in the lowest remaining ribosomal RNA levels with FFPE samples



Ribosomal RNA was depleted from human adult normal liver tissue FFPE Total RNA (Biochain # R2234149. RIN 2.5) and libraries were made using NEBNext Ultra II Directional RNA Kit (plus the NEBNext rRNA Depletion Kit (Human/Mouse/Rat)), Kapa Stranded RNA-Seq Kit with RiboErase®, Kapa HyperPrep Kit with RiboErase, and Illumina TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold. Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). Read pairs were assessed to be rRNA if they contain 6 or more 32 base matches to 18S, 28S, 5S, 5.8S, 16S or 12S human rRNA sequences (mirabait 4.9). Percent rRNA remaining was calculated by dividing rRNA reads by the total number of reads passing instrument quality filtering. Average percent rRNA remaining is shown for three replicates. Error bars indicate standard deviation. The NEBNext rRNA Depletion Ultra II Directional RNA workflow is the most efficient in removing rRNA from total FFPE RNA.

What users are saying:

"At The Earlham Institute we process many sample types; these include plant, microbial and animal. We trialed NEB's Ultra II Directional RNA Library Prep using three plant species that have previously been problematic with other RNA-seq kits. We were thrilled with how well and how consistently this kit performed. The NEBNext Poly(A) mRNA Magnetic Isolation Module was also effective and SortMeRNA showed that we had less than 2% contamination for each plant species. The strand specificity was also good at 98%. We were so pleased with the results that we are automating this protocol ready for production with in the EI Genomics Lab."

– Leah Clissold,
Platforms & Pipelines Team Leader,
The Earlham Institute,
Norwich, UK

"The Ultra II RNA kit has allowed us to reduce the input for directional polyA⁺ RNAseq libraries by a factor of 10 or more. We can now make a library with only 10 ng high quality total RNA and get the same gene expression profile as for 1 µg input. We've even pushed the input as low as 1 ng for very high quality total RNA. The new Ultra II RNA kit makes RNAseq achievable for low yield samples, we actually need more RNA for quality control than for library prep! Furthermore, the library prep protocol is streamlined compared to the previous Ultra RNA kits, including a reduction in AMpure bead cleanups and PCR cycles, resulting in better libraries for less time and resources."

– Jen Grenier, Ph.D.,
Director of RNA Sequencing Core (RSC),
Center for Reproductive Genomics,
Department of Biomedical Sciences,
College of Veterinary Medicine,
Cornell University

"I used the NEBNext Ultra II Directional RNA Library Prep Kit to process very low input (7–8 ng total RNA) samples from difficult to obtain tissue for one of our customers, and I am very pleased with the results."

– Brian James, Ph.D.,
Genomics Facility Director,
Sanford Burnham Prebys Medical Discovery Institute

Ultra II RNA Workflows and Product Details

In addition to stringent QCs on individual components, the NEBNext RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits. Adaptors and primers are supplied separately (NEBNext Oligos). For more information, see page 16.

NEBNext kit components

Input Poly(A) mRNA Workflow: 10 ng – 1 µg rRNA Depletion Workflow v2: 10 ng – 1 µg

	mRNA Isolation/ rRNA Depletion	mRNA Fragmentation	First Strand cDNA Synthesis	Second Strand cDNA Synthesis	End Repair/ dA Tailing	Adaptor Ligation	Size Selection	PCR Enrichment	Clean Up	Total Workflow
Ultra II Directional Kits		NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) – with Sample Purification Beads (NEB #7765)								Hands-On 27 min Total 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
		• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer with dUTP Mix • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• Sample Purification Beads (SPRIselect) – NEB #E7765 only	• Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) – NEB #E7765 only	
Ultra II Non-directional Kits		NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770) – with Sample Purification Beads (NEB #E7775)								Hands-On 27 min Total 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
		• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• Sample Purification Beads (SPRIselect) – NEB #E7775 only	• Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) – NEB #E7775 only	
Ultra II Modules	**rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405)** • RNase H/RNase H Reaction Buffer • rRNA Depletion Solution • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • RNA Sample Purification Beads (NEB #E7405 only)	**Magnesium RNA Fragmentation Module (NEB #E6150)** • RNA Fragmentation Buffer • RNA Fragmentation Stop Solution	**Ultra II RNA First Strand Synthesis Module (NEB #E7771)** • First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent	**Ultra II Directional RNA Second Strand Synthesis Module (NEB #E7550)** • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer with dUTP	**NEBNext Ultra II End Repair/ dA-Tailing Module (NEB #E7546)** • Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer	**NEBNext Ultra II Ligation Module (NEB #E7595)** • Ultra II Ligation Master Mix • Ligation Enhancer		**NEBNext Ultra II Q5 Master Mix (NEB #M0544)** • Ultra II Q5 Master Mix		* Including poly(A) mRNA isolation ** Including rRNA depletion
				Ultra II Non-directional RNA Second Strand Synthesis Module (NEB #E6111) • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer						

mRNA Isolation/rRNA Depletion (continued)

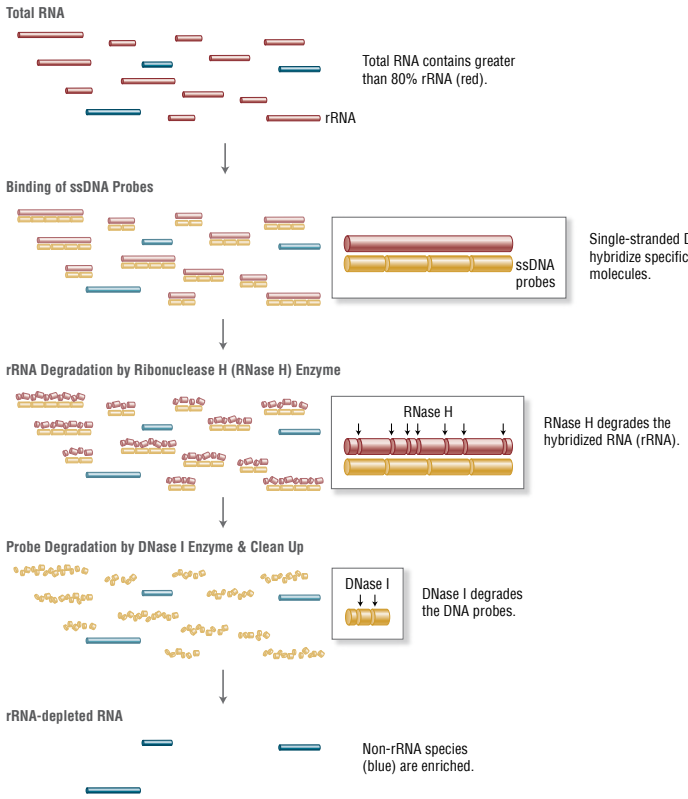
Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750, #E7755) • RNase H/RNase H Reaction Buffer • Globin & rRNA Depletion Solution • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • RNA Sample Purification Beads (NEB #E7775 only)	**NEBNext rRNA Depletion Kit (Bacteria) (NEB #E7850, #E7860)** • RNase H/RNase H Reaction Buffer • Bacterial rRNA Depletion Solution • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • RNA Sample Purification Beads (NEB #E7860 only)	**NEBNext RNA Depletion Core Reagent Set (NEB #E7865, #E7870)** • Probe Hybridization Buffer • Thermostable RNase H • RNase H & DNase I Reaction Buffers • DNase I • Nuclease-free Water • RNA Sample Purification Beads (NEB #E7870 only)	**Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)** • Oligo d(T)25 beads • RNA Binding Buffer • Wash Buffer • Nuclease-free Water

Note that reagents for the original Ultra workflow and standard workflow are also available. See ordering information.

NEBNext RNA Depletion

Abundant RNAs can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable. NEBNext RNA Depletion kits facilitate this removal, while ensuring retention of RNAs of interest. These kits employ the efficient RNase H method (1,2), as well as close probe tiling of abundant RNAs, thereby ensuring that even degraded RNA is hybridized and subsequently removed.

NEBNext rRNA Depletion Kit workflow



Total RNA (0.1-1 µg) is hybridized with single stranded DNA probes targeting cytoplasmic (5S, 18S, 28S, 5.8S rRNAs) and mitochondrial (12S and 16S rRNAs) ribosomal RNA, followed by RNase H digestion to degrade targeted RNA. Finally, DNA probes are digested with DNase I. The ribosomal-depleted RNA is purified using Agencourt RNAClean XP beads. Ribosomal RNA depletion can be immediately followed by RNA-seq library preparation.

NEBNext rRNA Depletion Kit workflow times

	Input Amount	Time				Workflow Time
		RNA/Probe Hybridization	RNase H Digestion	DNase I Digestion	Clean Up	
Kit	10 ng – 1 µg	Hands-On				Hands-On
		2 min.	2 min.	2 min.	2 min.	8 min.
		Total				Total
		22 min.	32 min.	32 min.	27 min.	1 hr., 53 min.

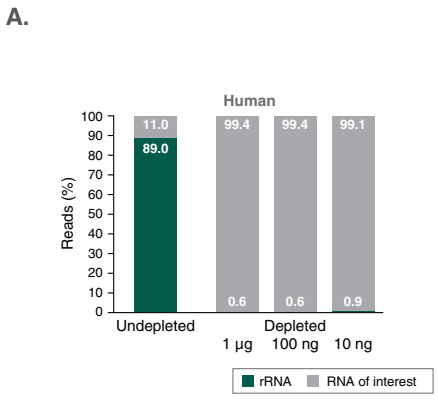
- Suitable for low-quality (e.g., FFPE) and high-quality RNA
- Compatible with a broad range of input amounts: 10 ng–1 µg
- Superior depletion of abundant RNAs, with retention of RNAs of interest
- Fast workflow: 2 hours, with less than 10 minutes hands-on time
- Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications
- Available with optional Agencourt® RNAClean® XP Beads for RNA Purification
- Customizable option to deplete unwanted RNA from any organism, using probe sequences designed with a user-friendly web tool

PRODUCT	SIZE
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400S/L/X)	6/24/96 rxns
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads (NEB #E7405S/L/X)	6/24/96 rxns
NEBNext Globin & RNA Depletion Kit (NEB #E7750S/L/X)	6/24/96 rxns
NEBNext Globin & RNA Depletion Kit with RNA Sample Purification Beads (NEB #E7755S/L/X)	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria) (NEB #E7850S/L/X)	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads (NEB #E7860S/L/X)	6/24/96 rxns
NEBNext RNA Depletion Core Reagent Set (NEB #E7865S/L/X)	6/24/96 rxns
NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads (NEB #E7870S/L/X)	6/24/96 rxns
ALSO AVAILABLE	SIZE
NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490S/L)	24/96 rxns

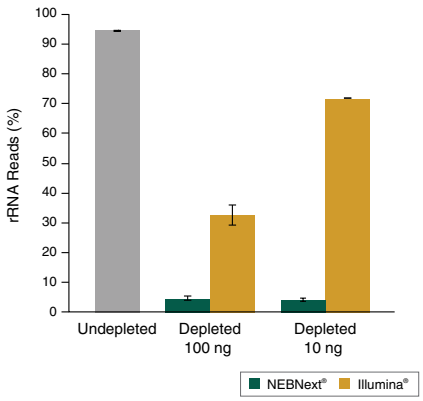
References
1. Adiconis, X. et al. (2013). Nature Methods 10; 623-629.
2. Morlan, J.D. et al. (2012). PLoS One 7, e42882.

rRNA Depletion v2 Kit (Human/Mouse/Rat)

The NEBNext rRNA Depletion Kit v2 enriches for RNAs of interest across a wide range of total RNA inputs in human



The NEBNext rRNA Depletion Kit v2 efficiently depletes rRNA from degraded FFPE total RNA while preserving transcript abundances



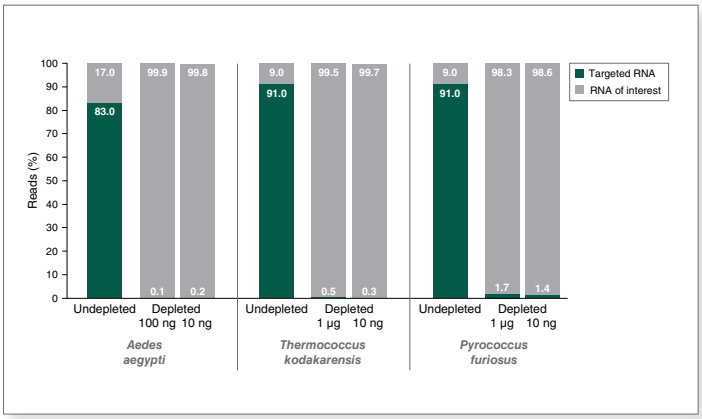
Universal human reference total RNA (A) or human adult normal liver tissue FFPE Total RNA, RIN 2.3 (B) was depleted of rRNA using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (A and B), or the TruSeq® Stranded Total RNA Gold kit (B). RNA-seq libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 10 Million reads (A) or 20 Million reads from depleted libraries and 200 million reads from undepleted libraries (B) reads were sampled (seqtk) and were identified as ribosomal using mirabait.

Customized Depletion of Unwanted RNA

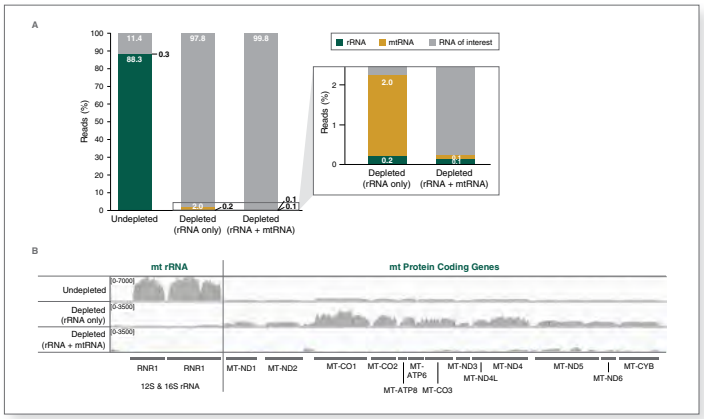
In RNA-seq, highly expressed transcripts with minimal biological interest, such as ribosomal RNA (rRNA) can dominate readouts and mask detection of more informative low-abundance transcripts. This challenge is amplified when working with sample types for which pre-designed RNA depletion kits are not available. The NEBNext RNA Depletion Core Reagent set provides a customized approach to deplete unwanted RNA from any organism, using probe sequences designed with the user-friendly NEBNext Custom RNA Depletion Design Tool.

NEBNext® Custom RNA Depletion Design Tool

Design oligos for depletion of unwanted RNA from any organism, when used in the NEBNext RNA depletion workflow. https://depletion-design.neb.com/



NEBNext Custom RNA Depletion enriches for RNAs of interest by efficiently removing targeted RNA from total RNA across species and a wide range of inputs. The NEBNext Custom RNA Depletion Design Tool was used to design probes against rRNA of the mosquito Aedes aegypti, and the archaeal species Thermococcus kodakarensis and Pyrococcus furiosus. Total RNA (1 µg, 100 ng or 10 ng) was used as input for rRNA depletion using the Core RNA Depletion Reagent Set with the designed probes. RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library. Read pairs were identified as ribosomal using mirabait (6 or more shared 25-mers), and levels of rRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. The data represents an average of 3 replicates. The method efficiently depletes targeted rRNA across species and a wide range of total RNA input amount (1 µg–10 ng).



Combined probe pools efficiently deplete human rRNA and mitochondrial mRNA using NEBNext Custom RNA Depletion. The NEBNext Custom RNA Depletion Design Tool was used to design probes against human mitochondrial mRNA. The probes were used in combination with the probe pool from the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat). 1 µg of total Universal Human Reference RNA (Agilent®) was depleted of mitochondrial RNA and rRNA using the Core RNA Depletion Reagent Set. RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library.

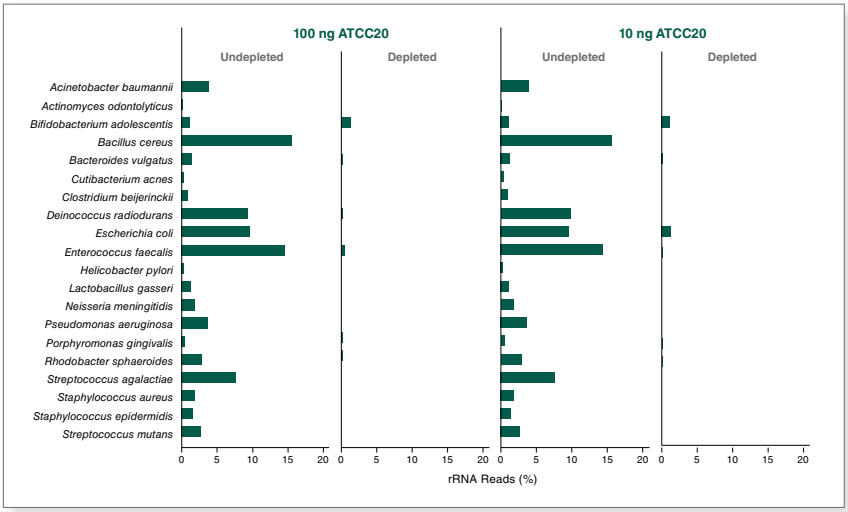
A. Read pairs were identified as ribosomal and mitochondrial using mirabait (6 or more, 25-mers), and levels of rRNA and mtRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. Both rRNA and mitochondrial RNA are efficiently depleted.

B. Integrative Genome Viewer (IGV) visualization of read coverage across the human mitochondrial genes.

rRNA Depletion Kit (Bacteria)

The NEBNext rRNA Depletion Kit (Bacteria) targets removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms. The kit is effective with both intact and degraded RNA preparations, from monocultures or samples with mixed bacterial species.

Depletion of ribosomal RNA enriches for RNAs of interest across a mock community of bacterial species and a range of input amounts



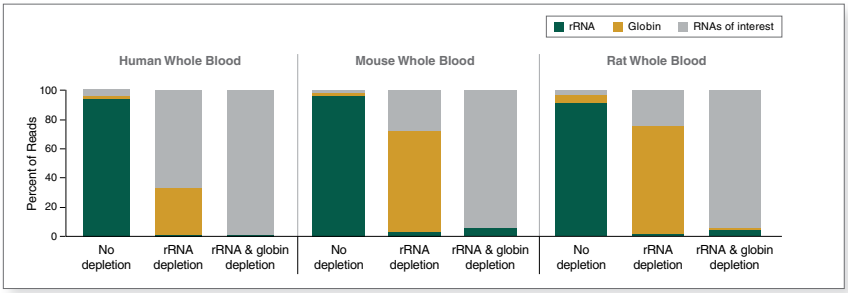
Total RNA was extracted from a lyophilized pool of 20 different bacterial organisms (ATCC® #MSA-2002). Ribosomal RNA was depleted using the NEBNext rRNA Depletion Kit (Bacteria). RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). Reads were aligned (Hisat 2) to a composite reference genome containing the best matching strains in the NCBI genome database. Alignments were duplicate marked (Picard) and assessed for transcript levels (ht-seq count). Effective depletion of sequences overlapping with annotated rRNA regions was observed at 100 ng and 10 ng of input RNA for most of the organisms.

Globin & rRNA Depletion Kits (Human/Mouse/Rat)

The NEBNext RNaseH-based depletion method can be applied to abundant RNAs beyond rRNA. In blood samples, the great majority of RNA is comprised of rRNA and globin mRNA, and the removal of both is desirable. The NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) depletes globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ), cytoplasmic rRNA (5S, 5.8S, 18S, 28S, ITS and ETS) and mitochondrial rRNA (12S, 16S). The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded.

When only mRNA (and not non-coding RNA) is of interest, the Globin & rRNA Depletion Kits can be used following poly(A) mRNA enrichment (e.g. using the NEBNext poly(A) mRNA Magnetic Isolation Module NEB #E7490).

Depletion of globin mRNA & ribosomal RNA enriches for RNAs of interest across species



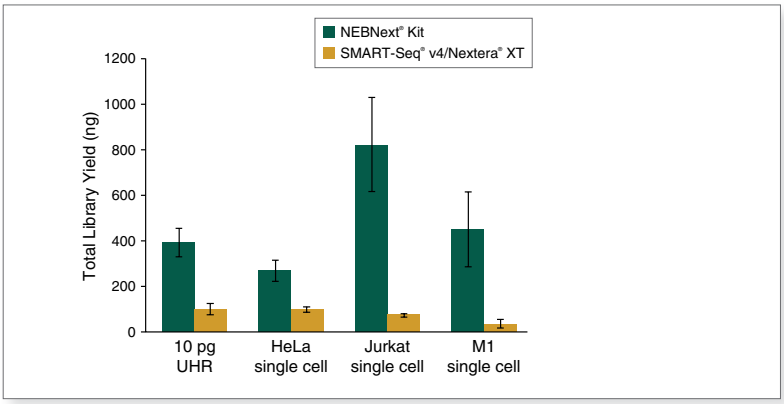
Human, mouse and rat whole blood total RNA (1 µg) was depleted of rRNA alone, or rRNA and globin mRNA transcripts, using the NEBNext Globin & rRNA Depletion Kit. RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). Reads were identified as rRNA or globin mRNA using mirabait (6 or more, 25-mers), and levels of rRNA and globin mRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering.

NEBNext Single Cell/Low Input RNA Library Prep

This unique workflow meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, as well as utilize a unique protocol and suite of reagents. Even low-abundance transcripts are represented in the high yields of cDNA obtained. Subsequent library construction incorporates the Ultra™ II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.

Generate higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit



Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq® v4 Ultra Low Input RNA Kit for Sequencing (Clontech® #634891) plus the Nextera® XT DNA Library Prep Kit (Illumina #FC-131-1096) were used. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.

- Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg–200 ng total RNA
- Experience unmatched detection of low abundance transcripts
- Rely on consistent transcript detection for a wide range of input amounts and sample types
- Obtain full length, uniform transcript coverage, regardless of input amount or sample type
- Use with cultured or primary cells, or total RNA
- Save time with a fast, streamlined workflow, minimal handling steps and hands-on time
  - Single-tube protocol from cell lysis to cDNA
  - Enzymatic DNA fragmentation, end repair and dA-tailing reagents in a single enzyme mix, with a single protocol, regardless of GC content
- Available with or without library construction reagents

TOOLS & RESOURCES

- View and download performance data in our Technical Note at **www.neb.com/E6420**

PRODUCT	SIZE
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420S/L)	24/96 rxns

What users are saying:

"We invested a lot of time and effort in my laboratory trying to "home-brew" our own single-cell and low-input RNAseq protocols based on published methods. We found ourselves having to re-optimize and troubleshoot multiple steps, and still struggled with reproducibility and robustness. When we tested the NEBNext Single Cell/Low Input RNA Library Prep Kit against our in-house protocol, we saw a substantial improvement in library quality and reproducibility. The product manual and documentation were very easy to follow but thorough and well annotated with clear quality control checkpoint examples. We were very pleased with the performance and ease of use."

– Jon Preall, Ph.D.,
Research Assistant Professor at Cold Spring Harbor Laboratory and director of the CSHL Single Cell Sequencing Core.

NEBNext ARTIC Products for SARS-CoV-2 Sequencing

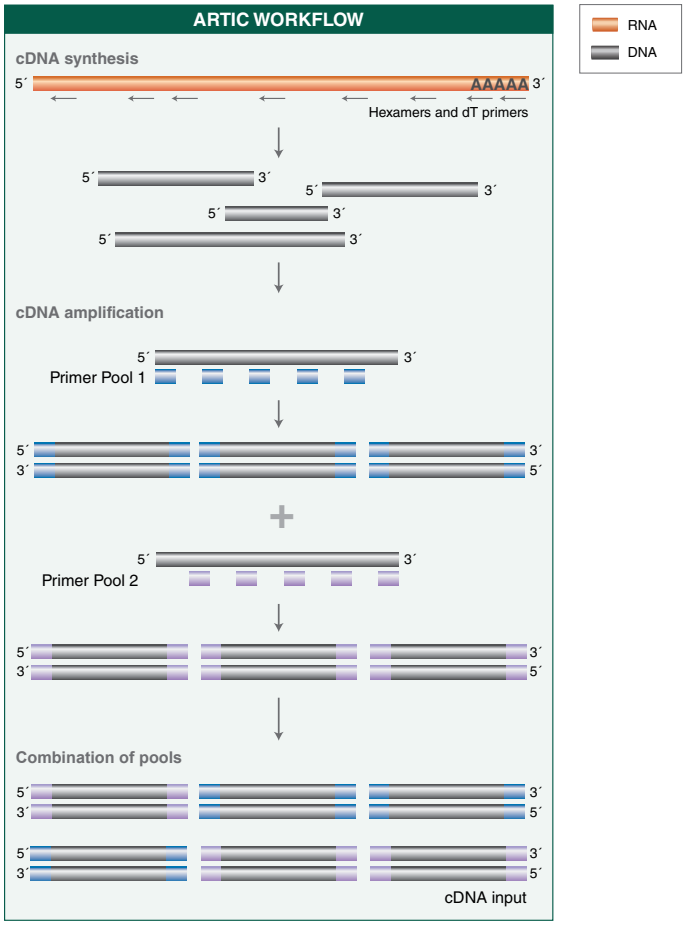
The NEBNext ARTIC kits were developed in response to the critical need for reliable and accurate methods for sequencing viral pathogens, specifically SARS-CoV-2. These kits, for long and short read sequencing, were based on the original work of the ARTIC Network (1). The ARTIC SARS-CoV-2 sequencing workflow is a multiplexed amplicon-based whole-viral-genome sequencing approach.

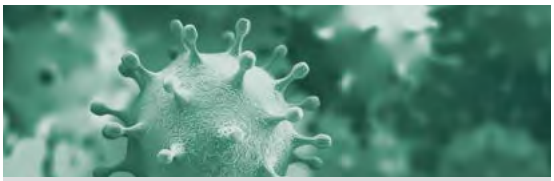
NEBNext ARTIC Companion kits include primers and reagents for RT-PCR from SARS-CoV-2 gRNA and downstream library preparation for Illumina and Oxford Nanopore Technologies sequencing. Primers optimized for performance with variants are included.

The optimized primers and reagents for RT-PCR deliver uniform, ample amplicon yields from gRNA across a wide copy number range, and library prep and sequencing can be performed downstream of a single RT-PCR procedure.

For Illumina applications, a novel DNA polymerase formulation for the enrichment of next-generation sequencing libraries eliminates the need to normalize amplicon concentrations prior to library preparation. Two library prep options are available: The NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina) incorporates enzymatic cDNA fragmentation, and generates libraries with inserts in the 150 bp range. The NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) does not include DNA fragmentation and library inserts are in the 400 bp range.

NEBNext ARTIC workflow





- Streamlined, high-efficiency protocol
- Ample amplicon yields from a wide range of viral genome inputs
- Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool
- Available for Illumina and Oxford Nanopore Technologies sequencing platforms
- No requirement for amplicon normalization prior to Illumina library preparation
- Optimized performance with SARS-CoV-2 variants
- Express protocol options available

PRODUCT	SIZE
NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) (NEB #E7650S/L/X)	24/96 rxns
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina) (NEB #E7658S/L)	24/96 rxns
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) (NEB #E7660S/L/X)	24/96 rxns
NEBNext ARTIC SARS-CoV-2 RT-PCR Module (NEB #E7626S/L)	24/96 rxns

References

1. *Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io https://dx.doi.org/10.17504/protocols.io.bdp7i5rn*

NEBNext Adaptors and Primers

Adaptors and Primers are an essential component of your NGS sample prep workflow, and NEBNext Multiplex Oligos offer flexibility in multiplexing; indexing options include unique dual indices (UDIs) with unique molecular identifiers (UMIs), unique dual indices (UDIs), combinatorial dual (CD) indices, and single indices in a range of formats and indexing strategies. For an overview of our Multiplex Oligos products, refer to the NEBNext Multiplex Oligos Selection Chart below.

NEBNext Multiplex Oligos Selection Chart

	 SINGLE INDEX	 DUAL INDEX	 UNIQUE DUAL INDEX	 UNIQUE DUAL INDEX UMIs
NEB PRODUCTS	NEB #E7335 NEB #E7500 NEB #E7710 NEB #E7730 NEB #E6609	NEB #E7600 NEB #E7780	NEB #E6440 NEB #E6442 NEB #E6444 NEB #E6446 NEB #E6448 NEB #E7140	NEB #E7395
Contains UMI	No	No	No	Yes
Addresses Index Hopping	No	No	Yes	Yes
Indexing Strategy	Index Primer	Index Primer	Index Primer	Index Adaptor
Applications	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	PCR-free DNA-seq, RNA-seq (except small RNA)
Number of Indices for Multiplexing	up to 144	up to 384	up to 480	up to 96
Compatible with ARTIC sequencing for Illumina®	Yes	Yes	Yes	No
Compatible with EM-seq™	Yes*	Yes*	Yes*	No
Compatible with EpiMark® Bisulfite Sequencing	Yes**	Yes**	Yes**	No
Number of Sets Available; Formats and Indices Available	Five; Sets 1-4 (12 indices/set): Individual vials 96 Index: premixed plate	Two; Individual vials containing 8 i5 primers and 12 i7 primers for combinitorial mixing	Five; 96 indices in premixed, foil-sealed 96-well plates, including a version for EM-seq (up to 120 indices, either 96-well plate or 24 vial format)	One; 96 indices in premixed, foil-sealed 96-well plate (DNA-seq OR RNA-seq) and primers

** Requires the use of the EM-seq Adaptor; Single, dual and unique dual index are all compatible; NEB recommends using the Unique Dual Index Primers found in the NEBNext Enzymatic Methyl-seq Kit (NEB #E7120) or the NEBNext Multiplex Oligos for EM-seq (NEB #E7140), both supplied with the NEBNext EM-seq Adaptor; For higher levels of multiplexing, Unique Dual Index Primers Sets 3 and 4 (NEB #E6444 and #E6446) are also validated for EM-seq.*

*** Requires use of NEBNext EM-seq adaptor from NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs, #E7140S/L or NEBNext methylated adaptor from NEBNext Multiplex Oligos for Illumina® (Methylated Adaptor, Index Primers Set 1, #E7535S/L).*

PRODUCT	# INDICES	SIZE
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1) (NEB #E7416S/L)	96 unique pairs	96/384 rxns
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs or 96 Unique Dual Index Primer Pairs Set 1, 2, 3, 4, 5) (NEB #E6440S/L, #E6442S/L, #E6444S/L, #E6446S/L, #E6448S/L)	96 unique pairs	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1 or 2) (NEB #E7600S, #E7780S)	8 x 12	96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers) (NEB #E6609S/L)	96	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3 or 4) (NEB #E7335S/L, #E7500S/L, #E7710S/L, #E7730S/L)	12	24/96 rxns
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs) (#E7140S/L)	96	24/96 rxns
NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1) (NEB #E7535S/L)	12	24/96 rxns
NEBNext Adaptor Dilution Buffer (NEB #B1430)		1 x 9.6 ml

ADVANTAGES

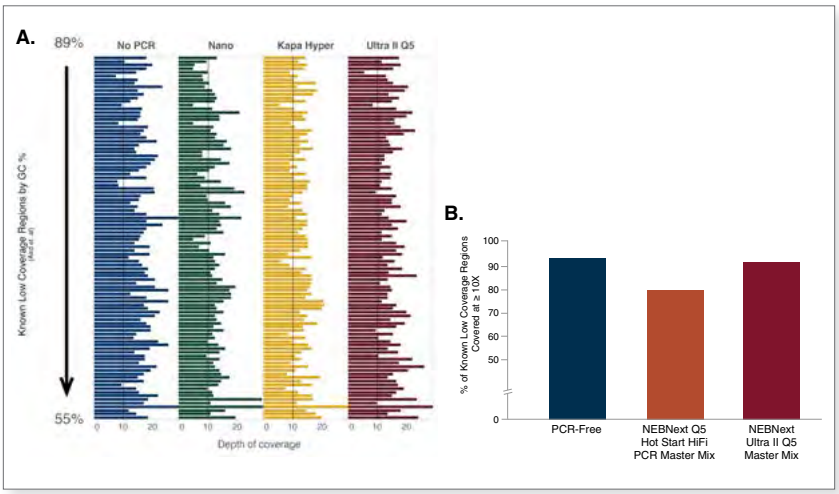
- Indexing strategies optimized by application
- Index Primers – For NGS Library Prep workflows that include an amplification step
- Index Adaptors – Enablement of PCR-free workflows and incorporation of UMIs for error correction/deduplication
- Extensively QC'd for purity and increased library yields
- Flexibility for use with NEBNext library preparation kits and other standard, Illumina-compatible library preparation methods
- Convenient formats (*e.g.*, vials and single-use 96-well plates with pierceable foil seal)
- Provided with index-pooling guidelines and sample sheets

High Yields and Minimized GC Bias with the NEBNext Ultra II Formulation of Q5® High-Fidelity DNA Polymerase

To ensure that sequence data reflects exactly the sequence of the original sample, it is essential that amplification of libraries be performed uniformly and with high fidelity. Historically, high-fidelity polymerases have been more susceptible to difficulties in PCR amplification of GC- rich and other challenging regions. If such bias occurs in library amplification, this can lead to uneven sequence coverage, challenges in sequence assembly and even "missing" sequence.

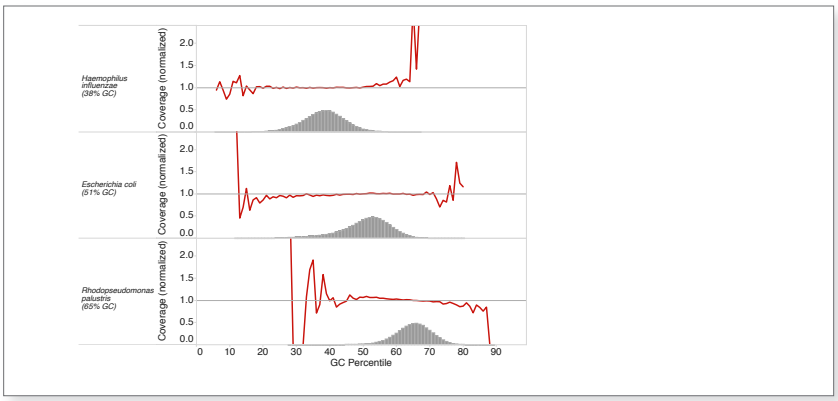
The NEBNext Ultra II Q5 Master Mix (NEB #M0544) is the latest formulation of Q5 DNA polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

NEBNext Ultra II Q5 Master Mix provides improved coverage of known low coverage regions of the human genome



Libraries were prepared from Human NA19240 genomic DNA. One library was not amplified. The other two libraries were amplified using 5 cycles of PCR with NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543) or with NEBNext Ultra II Q5 Master Mix (NEB #M0544). Libraries were sequenced on an Illumina NextSeq 500. 420 million 75 bp reads were randomly extracted from each dataset, representing an average coverage of 10X. Reads were mapped to the hg19 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1. ***A:*** *The number of reads overlapping distinct low coverage regions of the human genome (1) are shown for each library.* ***B:*** *From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The % of difficult regions covered at ≥ 10X are shown for each library. The NEBNext Ultra II Q5 Master Mix provides improved coverage of these known low coverage regions, without drop-outs, and shows similar coverage to the unamplified sample.*

NEBNext Ultra II Q5 Master Mix provides uniform GC coverage with a broad range of GC composition



Libraries were made using 100 ng of the genomic DNAs shown and the NEBNext Ultra II DNA Library Prep Kit. Libraries were amplified using the NEBNext Ultra II Q5 Master Mix, and sequenced on an Illumina MiSeq®. GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. NEBNext Ultra II Q5 Master Mix provides uniform GC coverage regardless of the GC content of the DNA.

ADVANTAGES

- Optimized for high yields in NGS library amplification
- Minimizes GC bias, with superior performance across the GC spectrum
- Ultra-high-fidelity amplification with Q5, the highest-fidelity polymerase (2)
- Aptamer-based hot start without a separate activation step, for room-temperature reaction set-up

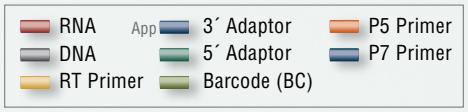
PRODUCT	SIZE
NEBNext Ultra II Q5 Master Mix (NEB #M0544S/L)	50/250 rxns

Reference:

1. *Aird, D. et al. (2011). Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. Genome Biology 12(2), R18.*
2. *Popatov, V. and Ong, J.L. (2017). Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS ONE. 12(1):e0169774.*

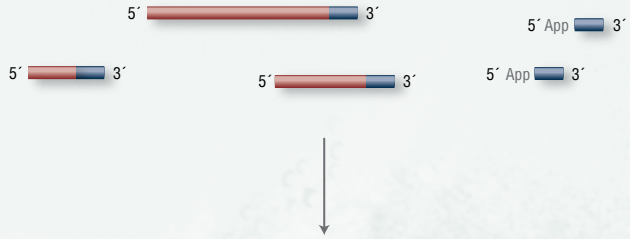
Workflow for Small RNA Library Preparation

PRODUCT	TOTAL RNA
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) (NEB #E7300)	100 ng–1 µg
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2) (NEB #E7580)	100 ng–1 µg
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1–48) (NEB #E7560)	100 ng–1 µg
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)	100 ng–1 µg



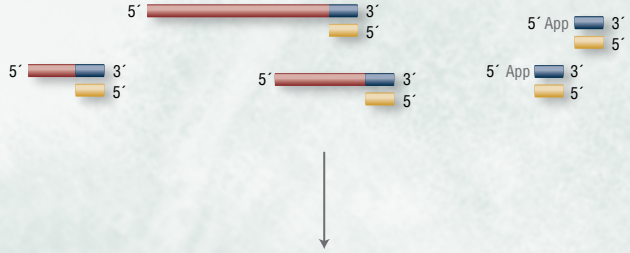
1 3´ Adaptor Ligation

- Input is purified total RNA
- Ligation of 5´-adenylated, 3´-blocked, single-stranded DNA adaptor to 3´ end of RNA



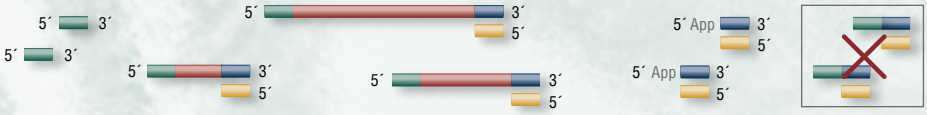
2 Primer Hybridization

- Hybridization of RT primer to 3´ adaptor-ligated molecules & any remaining 3´ adaptors



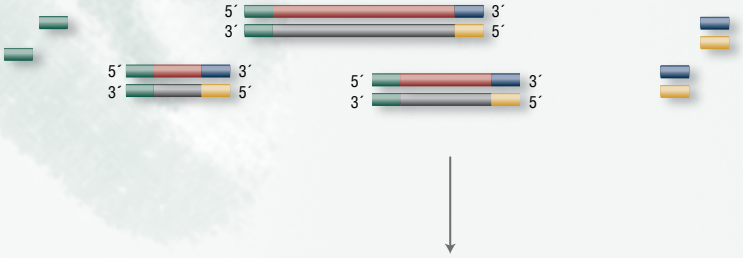
3 5´ Adaptor Ligation

- Preferential ligation of 5´ adaptor to single-stranded molecules (and therefore not to double-stranded 3´ adaptor:RT primer hybrid molecule)
- Result is minimized formation of adaptor-dimers



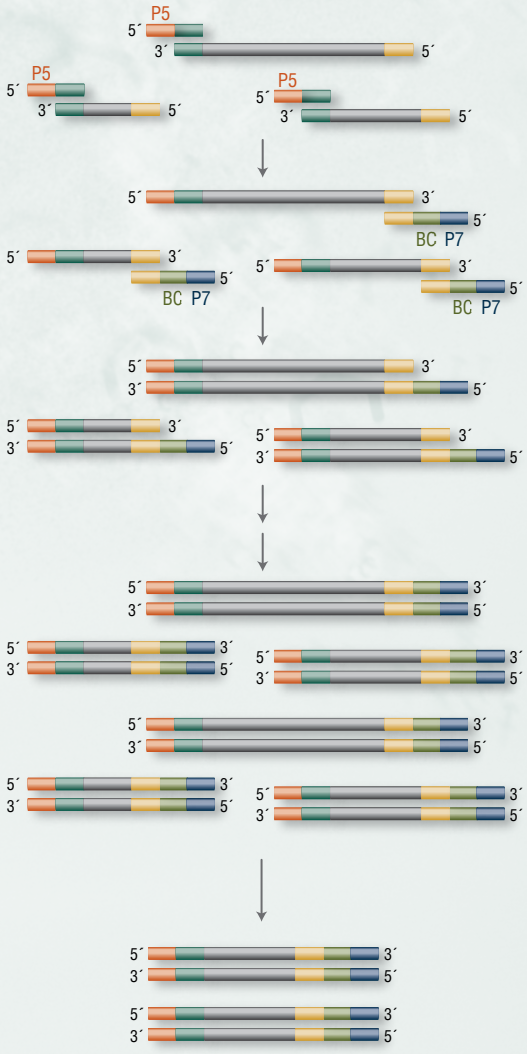
4 First Strand cDNA Synthesis

- Extension from RT primer synthesizes first strand cDNA
- Reverse transcriptase lacking RNase H activity is optimal (does not degrade RNA in RNA:DNA complex)



5 PCR Enrichment

- Amplification with a high-fidelity polymerase:
  - Selects for molecules with an adaptor at each end
  - Increases library yield
  - Incorporates barcodes/indices to enable multiplexing, and P5 & P7 sequences required downstream



6 Size Selection

- Ensures that only Small RNAs of interest are included in final library

Small RNA Workflow and Product Details

Our novel Small RNA workflow has been optimized to minimize adaptor-dimers while producing high-yield, high-quality libraries. Adaptors and primers are included in the Small RNA kits, and multiplexing options are available. The Multiplex kits contain index primers, and the Multiplex-Compatible kit enables use with your own barcode system.

In addition to stringent QCs on individual components, the NEBNext Small RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits.

NEBNext kit components

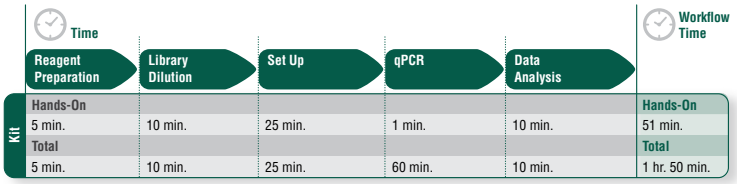
Input 100 ng – 1 µg

3´ Adaptor Ligation →	Primer Hybridization →	5´ Adaptor Ligation →	First Strand Synthesis →	PCR Enrichment →	Size Selection →	Total Workflow
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1 NEB #E7300, Set 2 NEB #E7580)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp® *Taq* 2X Master Mix • SR Primer • Index Primers 1–12 (Set 1) • Index Primers 13–24 (Set 2)	• Gel Loading Dye, Blue (6X) • Quick-Load® pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • NEBNext Index 1-48 Primers for Illumina	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • Index Primer 1	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs

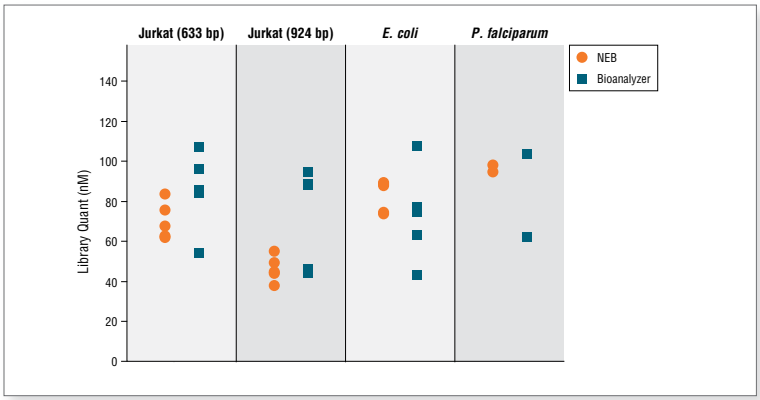
NEBNext Library Quant Kit for Illumina

Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence output. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility of quantitation. qPCR-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of the library molecules that can be sequenced. The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next-generation sequencing.

NEBNext Library Quant Kit for Illumina (NEB #E7630) workflow

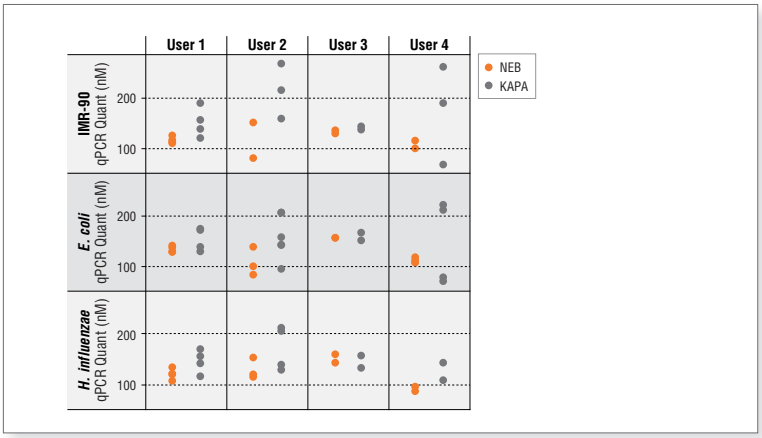


Comparison of quantitation by qPCR and electrophoretic methods

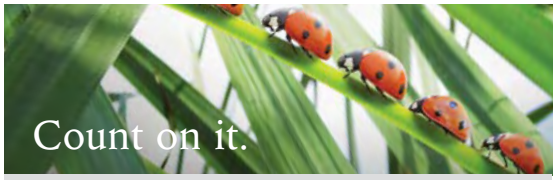


Concentrations of 4 libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured using the Aglient Bioanalyzer (blue). Compared to NEBNext's qPCR-based method, the Bioanalyzer concentrations displayed a greater level of variation.

Greater reproducibility of library quantitation with the NEBNext Library Quant Kit



Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or Kapa™ Library Quantification Kit (Universal). A notable improvement in quantitation consistency was observed for concentrations determined by the NEBNext Kit (orange) versus those from the Kapa kit (gray).



- Be confident in your quant values, as our kit provides **more accurate and reproducible results** than other methods and kits
- Get up and running quickly with our **easy-to-use kit**, containing Library Dilution Buffer, optimized master mix, 6 standards and ROX dye
- Simplify your reaction setup with **fewer pipetting steps** and a **single extension time** for all libraries
- Quantitate more libraries per kit, **as only 4 standards are required**
- **Use with all your libraries**, regardless of insert size, GC content and preparation method
- Save money with our value pricing

TOOLS & RESOURCES

Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate your qPCR-based library quant values

Download our application note, "Improved library quantitation for a broad range of library types using the NEBNext Quant Kit for Illumina" at **www.neb.com/E7630**

PRODUCT	SIZE
NEBNext Library Quant Kit for Illumina (NEB #E7630S/L)	100/500 rxns
NEBNext Library Dilution Buffer (NEB #B6118S)	7.5 ml

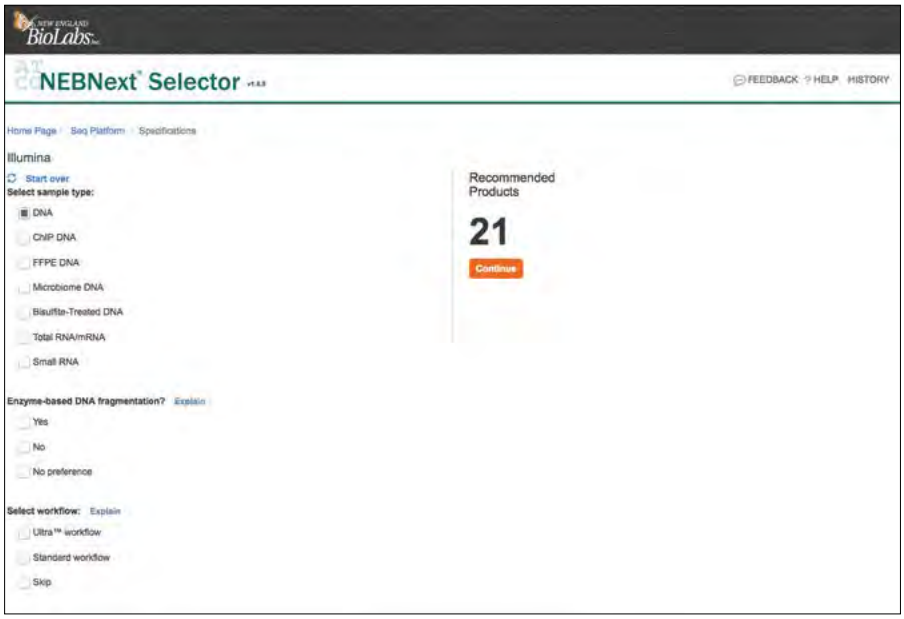
Illumina Platform:

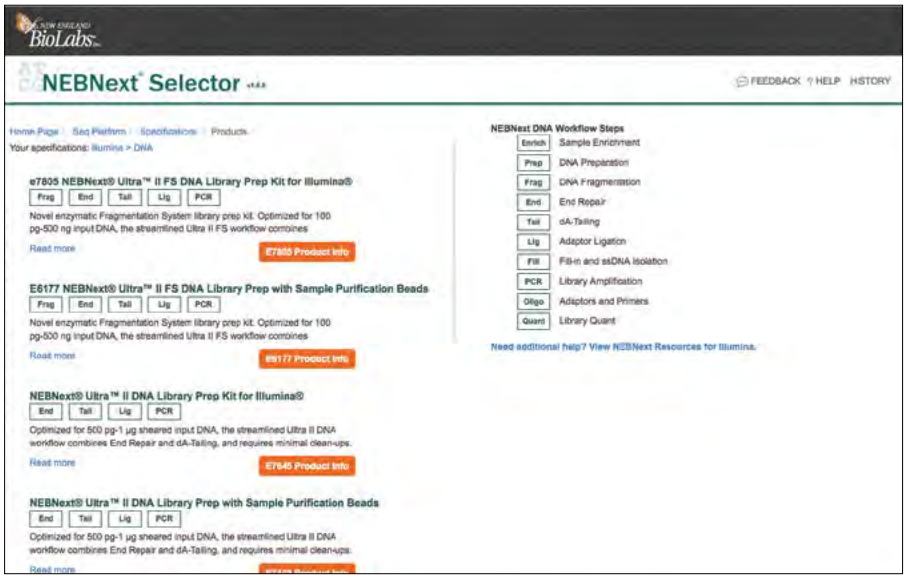
KITS FOR ILLUMINA RNA LIBRARY PREPARATION		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
	NEBNext Ultra Directional RNA Library Prep Kit for Illumina	E7420L	96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
	NEBNext Ultra RNA Library Prep Kit for Illumina	E7530L	96 rxns
Small RNA	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 rxns
	NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
SARS-CoV-2	NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)	E7650S/L	24/96 rxns
	NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)	E7658S/L	24/96 rxns
	NEBNext NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
RNA	NEBNext ARTIC SARS-CoV-2 RT-PCR Module	E7626S/L	24/96 rxns
	NEBNext RNA Depletion Core Reagent Set	E7865S/L/X	6/24/96 rxns
	NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	E7870S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext RNA First Strand Synthesis Module	E7525S/L	24/96 rxns
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	E6421S/L	24/96 rxns
	NEBNext Single Cell Lysis Module	E5530S	96 rxns
DNA	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra End Repair/dA-Tailing Module	E7442S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445S/L	24/96 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
ADAPTORS & PRIMERS		**NEB #**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)	E7416S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)	E6448S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml

LIBRARY QUANTITATION		NEB #	SIZE
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	15 ml
MAGNETIC SEPARATION		**NEB #**	**SIZE**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

NEBNext Selector

Use this tool to guide you through selection of NEBNext reagents for next generation sequencing sample preparation. Try it out at **NEBNextSelector.neb.com**





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NEBNext® Selector V1.0

For help with choosing the best reagents for your next generation sequencing sample preparation, try our NEBNext selector at **NEBNextselector.neb.com.**