



Now includes  
NEBNext UltraExpress® RNA Library Prep

# NEBNext® for RNA Library Prep

FOR THE ILLUMINA® PLATFORM





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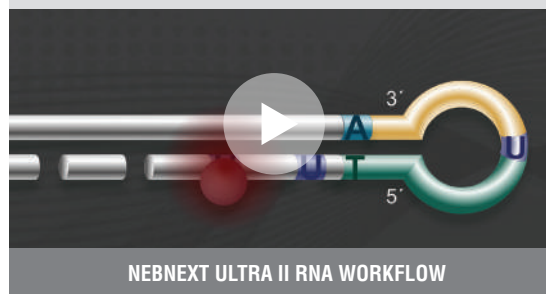
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#### Visit [NEBNext.com](https://nebnext.com) to find:

- 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
- Over 30,000 NEBNext citations
- Protocols & FAQs
- Refer to NEBNext Automation-Compatibility Table for details about successful kit automation.



Streamlined  
for speed.

NEBNext UltraExpress



# Why Choose NEBNext for RNA?

Next generation sequencing (NGS) has been adopted by a wide range of laboratories and scientists, each with their own experimental question, unique samples, and level of NGS expertise. NEBNext's suite of library prep kits, modules, and reagents offer a range of solutions for every user. Below are just a few of the ways that NEBNext can support your RNA library prep needs, now and into the future:

## 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).

Our expanding selection of reagents for mRNA enrichment and depletion of abundant RNAs bring flexibility to your library prep workflow.

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 2009, the NEBNext brand has stood for quality you can count on, with extensive QC's 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 30,000 peer-reviewed publications.

## Flexible Formats

NEBNext library prep reagents are available in multiple kit and workflow formats, for maximum convenience and flexibility.

### Kits and modules

Kits include reagents for an entire library prep workflow, whereas modules provide reagents for individual steps in a workflow.

Kits\* do not include adaptors and primers to enable maximal customization; see [www.neb.com/oligos](http://www.neb.com/oligos) for the NEBNext multiplex oligo selection chart.

\*with the exception of Small RNA

### Large volume & 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. We encourage consultation with the Customized Solutions team at NEB.

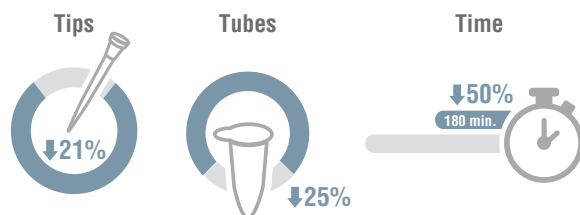
### WHAT'S NEW IN NEBNext FOR RNA?

- **NEBNext UltraExpress RNA Library Prep Kit** combined with our Express Poly(A) enrichment workflow for libraries in <3.5 hours
- **NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)** offers improved depletion of abundant ribosomal RNAs
- **NEBNext RSV Primer Module** enables sequencing of respiratory syncytial virus
- Even more UDIs and UMI for optimal multiplexing



Visit [NEBNextSelector.neb.com](http://NEBNextSelector.neb.com) to access the **NEBNext Selector Tool**, our online tool for help with selecting the NEBNext product that fits your needs

### Savings\* with NEBNext UltraExpress® RNA



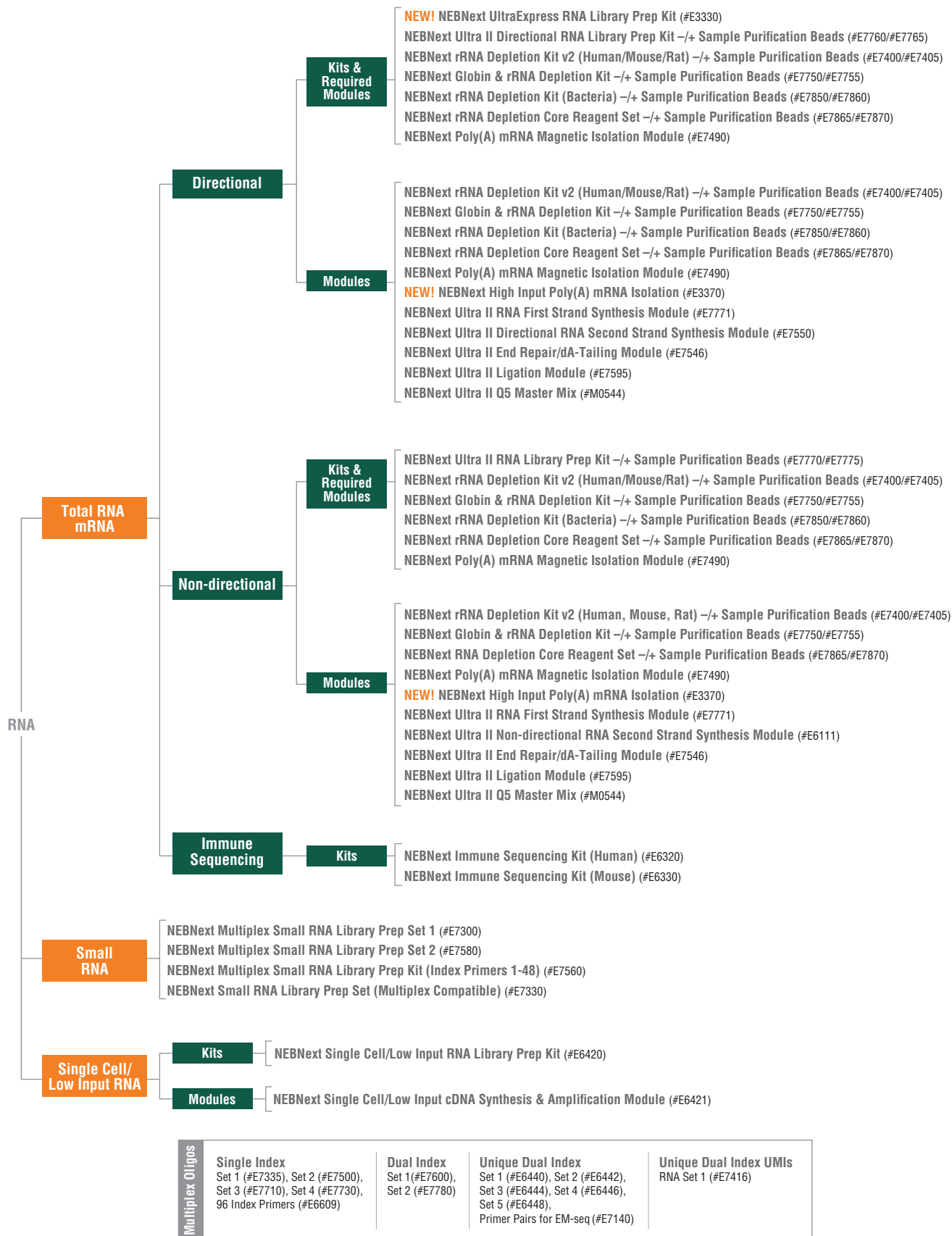
\* As compared to NEBNext® Ultra™ II RNA



Please complete the NEB Custom Contact Form at [www.neb.com/CustomContactForm](http://www.neb.com/CustomContactForm) to learn more.

# Illumina® RNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your Illumina RNA prep needs. For the most up-to-date product and pricing information, visit [NEBNext.com](https://www.nebnext.com).





# Tips for Working with RNA

## RNA Sample Input Guidelines

### Integrity of RNA

- Assess the quality of the input RNA using an Agilent® TapeStation® Instrument or a similar device.
- RNA should be completely free of DNA; isolation of purified RNA via the Monarch® Total RNA Miniprep Kit is recommended.
- Total RNA with an RNA Integrity Number (RIN) of 7 or higher is required for workflows reliant on the poly(A) tail (e.g., poly(A) mRNA Enrichment, Single Cell/Low Input, and Immune Sequencing library prep kits).
- Partially degraded RNA or degraded RNA (RIN < 7) is compatible with all rRNA depletion products/workflows.
- The use of the DV200 metric (percentage of fragments >200 nucleotides) is recommended for degraded RNA.

### Quantitation of RNA

- Accurate quantification of RNA samples is essential prior to library construction. RNA concentration can be determined using fluorescence-based quantitation with a Qubit® Fluorometer or similar devices, or by measuring absorbance at 260 nm ( $A_{260}$ ) with a spectrophotometer. Note that free nucleotides or organic compounds used in some RNA extraction methods may lead to an overestimation of RNA concentration when using spectrophotometry.

### Indices/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.
- For help choosing the right indices/barcodes, visit [www.neb.com/oligos](http://www.neb.com/oligos).

## NEBNext Magnetic Separation Rack

NGS library prep 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

# *Choose the right kit for your needs*

## NEBNext UltraExpress RNA or NEBNext Ultra II RNA

Choosing the right NEBNext kit for your RNA library prep doesn't need to be complicated!  
By answering just a few questions, you'll be able to confidently select the right kit for your needs.

### 1 What's your sample type/quality?

Whether you're working with an established model organism with a well-annotated reference genome or you're breaking new ground with a non-model organism, either NEBNext RNA library prep kit is an excellent place to start. Both have been rigorously analyzed with a range of samples from different organisms, but we encourage the user to confirm whether a kit works on any sample types that aren't explicitly identified in the supporting data.

Sample quality can range from a RIN of 1 to 10 and low-quality RNA can make it difficult to produce high quality libraries.

If you're pushing the lower limits of library prep input amounts, the NEBNext Single Cell/Low Input RNA Library Prep Kit can be used with as little as 2 pg of RNA. Where analysis of small RNAs is the goal, the NEBNext Small RNA library prep kits are available.

### 2 How much sample can you spare?

The answer to this question is critical to your choice between NEBNext UltraExpress and NEBNext Ultra II. If your samples are within the 25–250 ng total RNA input range, we'd strongly recommend choosing NEBNext UltraExpress due to its speed and more streamlined workflow. Spending less time on library prep means you can spend more time on answering your underlying questions. If, instead, your samples are rare, precious, or require significant time and/or effort to procure, available only in low amounts, or of low quality (see above), NEBNext Ultra II offers a workflow that works with inputs as low as 10 ng, stretching your sample further.

### 3 How important is speed?

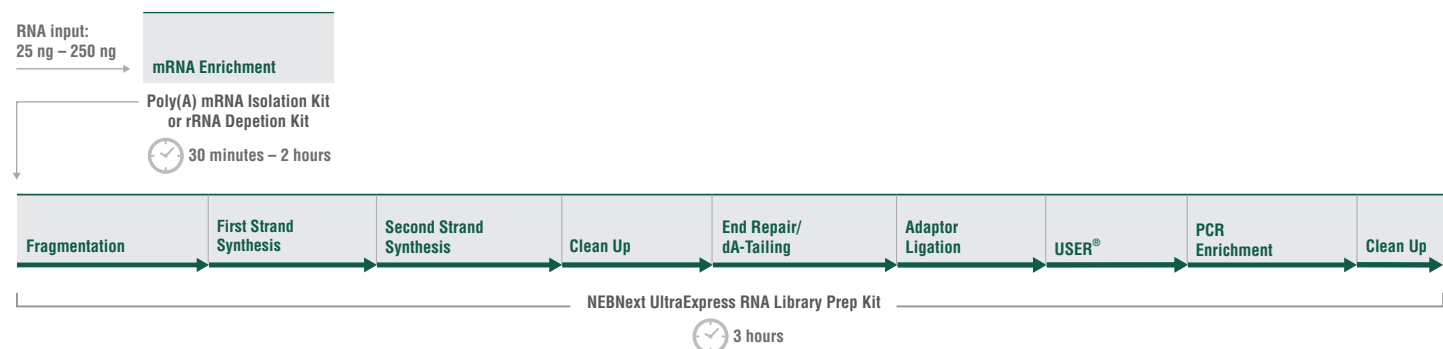
NEBNext UltraExpress was intentionally developed to further streamline the library prep workflow.

NEBNext UltraExpress RNA Library Prep, when combined with NEBNext Poly(A) mRNA Enrichment, enables RNA library prep in 3.5 hours.

NEBNext Ultra II Directional RNA Library Prep can also be combined with the 30-minute Express Poly(A) mRNA Enrichment step, but the total standalone workflow is a 6–6.5 hour protocol. The speed of the NEBNext UltraExpress RNA Library Prep Kit is a differentiator. The choice is yours!

# NEBNext RNA Reagents for Illumina Sequencing

NEBNext UltraExpress RNA offers a faster, more streamlined RNA library prep workflow. NEBNext Ultra II RNA Kits are available for directional (strand-specific) and non-directional library preparation, and for bulk RNA and single cell samples. These kits utilize streamlined workflows and have been designed for performance with input amounts as low as 5 ng. Note that reagents for rRNA depletion and poly(A) mRNA enrichment are supplied separately, as are adaptors and primers. In addition to stringent QCs on individual components, the NEBNext RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing.

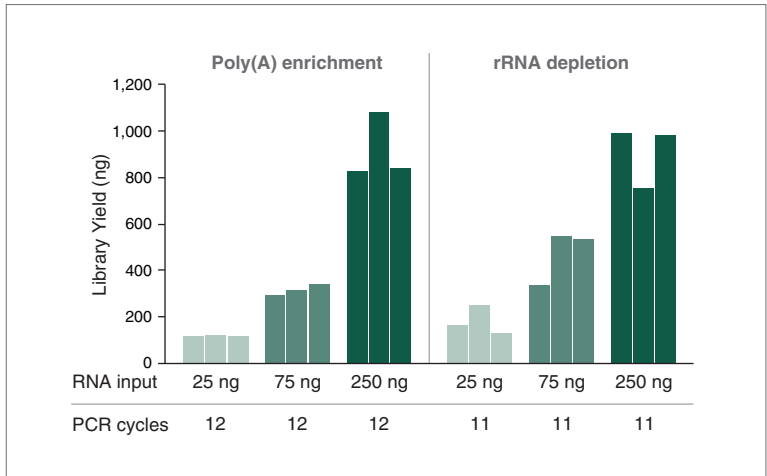
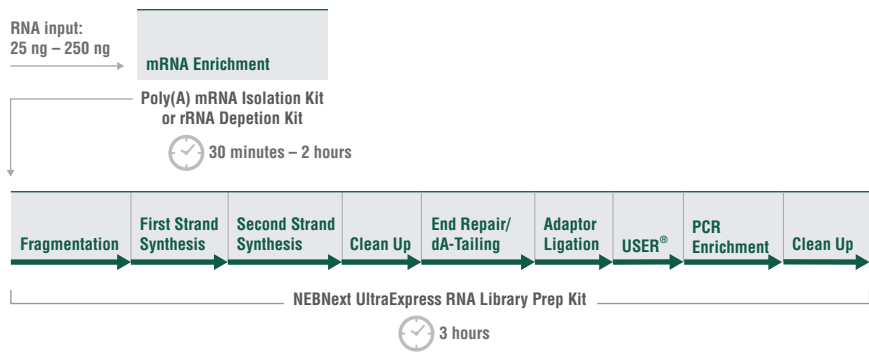


High quality RNA-Seq libraries in a day.

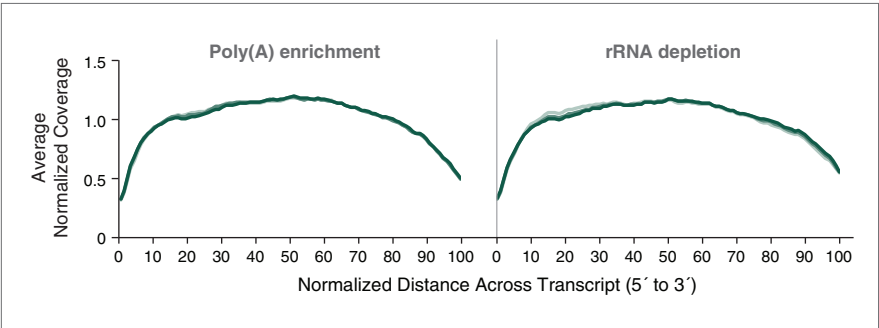
Input		Poly(A) mRNA Workflow: 10 ng – 1 µg		rRNA Depletion Workflow: 10 ng – 1 µg							
mRNA Isolation/ rRNA Depletion		mRNA Fragmentation	First Strand cDNA Synthesis	Second Strand cDNA Synthesis	End Repair/ dA Tailing	Adaptor Ligation	Size Selection	PCR Enrichment	Clean Up	Total Workflow	
NEBNext Ultra II Directional Kits	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) – with Sample Purification Beads (NEB #7765)										 <b>Hands-On</b> 27 min <b>Total</b> 5.5 – 5.7 hrs* 6.6 – 6.8 hrs**
	NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770) – with Sample Purification Beads (NEB #E7775)										 <b>Hands-On</b> 27 min <b>Total</b> 5.5 – 5.7 hrs* 6.6 – 6.8 hrs**
NEBNext Ultra II Non-directional Kits	rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405)	Magnesium RNA Fragmentation Module (NEB #E6150)	Ultra II RNA First Strand Synthesis Module (NEB #E7771)	Ultra II Directional RNA Second Strand Synthesis Module (NEB #E7550)	NEBNext Ultra II End Repair/ dA-Tailing Module (NEB #E7546)	NEBNext Ultra II Ligation Module (NEB #E7595)		NEBNext Ultra II Q5 Master Mix (NEB #M0544)		* Including poly(A) mRNA isolation ** Including rRNA depletion	
				Ultra II Non- directional RNA Second Strand Synthesis Module (NEB #E6111)							
mRNA Isolation/rRNA Depletion (continued)											
Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750, #E7755)		NEBNext RNA Depletion Core Reagent Set (NEB #E7865, #E7870)		Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)		High Input Poly(A) mRNA Isolation (NEB #E3370)					

# NEBNext UltraExpress RNA Library Prep Kit

The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library prep, with a fast, streamlined workflow. The kit is compatible with mRNA isolation and rRNA depletion workflows and a wide range of sample types. With a 3-hour library prep protocol, the kit enables creation of high-quality RNA libraries in a single day, in conjunction with mRNA or rRNA depletion kits.



**The NEBNext UltraExpress RNA Library Prep Kit produces high library yields for a range of inputs, in poly(A) enrichment and rRNA depletion workflows.** Universal Human Reference RNA with the indicated input amounts was (A) enriched for poly(A) mRNA (NEB #E7490) or (B) depleted of ribosomal RNA (NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400), followed by creation of strand-specific libraries using the NEBNext UltraExpress RNA Library Preparation Kit. Library yields were assessed using TapeStation® 4200 and values shown are for three replicates for each input amount.



**NEBNext UltraExpress RNA provides even transcript coverage with a range of input amounts.** Universal Human Reference RNA with the indicated input amounts was (A) enriched for poly(A) mRNA (NEB #E7490) or (B) depleted of ribosomal RNA (NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400), followed by creation of strand-specific libraries using the NEBNext UltraExpress RNA Library Preparation Kit. Libraries were sequenced on an Illumina NextSeq® 500 (2x75 bp). 9M reads were sample per library. Reads were mapped to the hg38 reference genome using RNA STAR v2.7.8a and 5' to 3' transcript coverage was calculated from the first 1000 highest abundance transcripts using the CollectRnaSeqMetrics (Picard) tool v2.18.2.2.



Even more  
from less, for  
RNA.

- Fast workflows (3 hours)
- Fewer steps and consumables
- Fewer cleanups
- Single protocol for all inputs
- Compatible with a range of sample types including human genomic RNA, bacterial RNA and human blood RNA
- Automation friendly

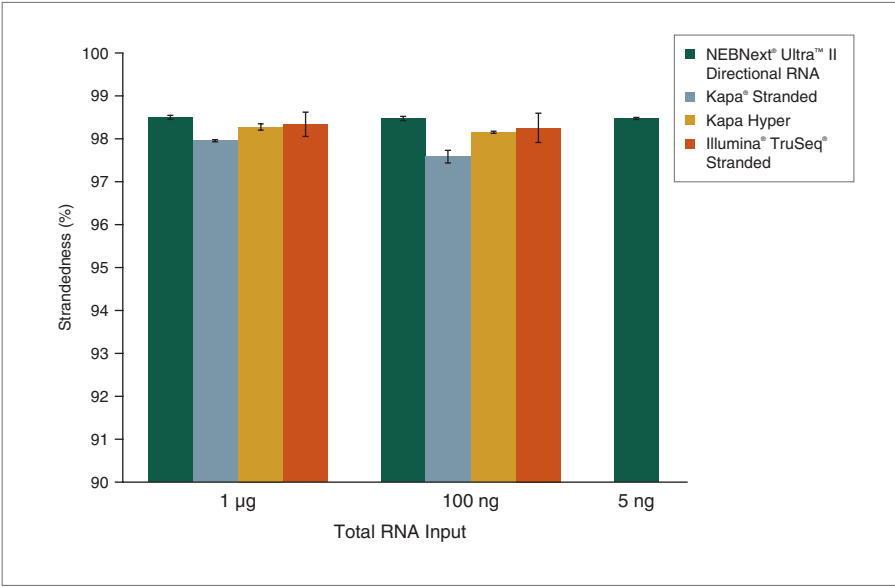
PRODUCT	SIZE
NEBNext UltraExpress RNA Library Prep Kit (NEB #E3330S/L)	24/96 rxns




# NEBNext Ultra II Library Prep Kits for RNA

Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, NEBNext Ultra II RNA library prep kits generate several fold higher yields of high quality libraries and enable use of lower input amounts and fewer PCR cycles. The kits have streamlined, automatable workflows and are available for directional (strand-specific, using the “dUTP method”) and non-directional library prep, with the option of SPRISelect™ beads for size-selection and clean-up steps.

## NEBNext Ultra II Directional RNA libraries have high directionality/strandedness



Ribosomal RNA (rRNA) was depleted from Human Universal Reference RNA (Agilent #740000) and libraries were made using 1 µg, 100 ng and 5 ng input with NEBNext Ultra II Directional RNA Kit (plus the NEBNext rRNA Depletion Kit (Human/Mouse/Rat)), and using 1 µg and 100 ng input with 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 (2x76 bp). Reads were mapped to the hg19 reference genome using Hisat 2.0.3 and directionality was calculated using the infer experiment tool from the RSeQC Quality Control Package. Ultra II Directional RNA retains high directionality at low ng input amounts.



### Even more from less, for RNA.

- Get more of what you need, with the broadest input range
- Generate high quality libraries with limited amounts of RNA: 10 ng–1 µg Total RNA (polyA mRNA workflow); 10 ng–1 µg (rRNA depletion workflow)
- Minimize bias, with fewer PCR cycles required
- Maximize the flexibility to order reagents for your specific workflow needs
- Directional (strand-specific, using the “dUTP method”) and non-directional workflow options available
- rRNA Depletion and poly(A) mRNA isolation reagents are available separately
- Automation compatibility
- Enjoy the reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need
- Rely on robust performance, even with low quality RNA

PRODUCT	SIZE
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760S/L)	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (Illumina) (NEB #E7765S/L)	24/96 rxns
NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770S/L)	24/96 rxns
NEBNext Ultra II RNA Library Prep with Sample Purification Beads (NEB #E7775S/L)	24/96 rxns

# NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)

Highly expressed transcripts with minimal biological interest, such as ribosomal RNA (rRNA) can dominate readouts, masking detection of more informative low-abundance transcripts. This second-generation NEBNext rRNA Depletion Kit has been further optimized to incorporate reagent, probe and protocol improvements to the RNaseH-based workflow, resulting in superior depletion performance. The efficient RNase H-based workflow, and close spacing of probes, enables effective depletion from both low- and high-quality RNA, with a broad range of input amounts.

The NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) employs the NEBNext RNase H-based RNA depletion workflow to deplete the following:

- Superior depletion of rRNA from human, mouse and rat RNA
- Depletion of cytoplasmic (5S, 5.8S, 18S, 28S, human ITS, ETS) and mitochondrial (12S and 16S) rRNA
- Compatible with a broad range of input amounts: 10 ng - 1 µg
- Suitable for low-quality or high-quality RNA
- Fast workflow: 2 hours, with less than 10 minutes hands-on time

The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded. The resulting depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis.


This kit can also be used following poly(A) mRNA enrichment, with or without the 30-minute Express protocol (e.g., using the NEBNext poly(A) mRNA Magnetic Isolation Module, NEB #E7490), so that the final depleted RNA contains only mRNA of interest and no non-coding RNA.

This kit is available with or without RNAClean® beads.

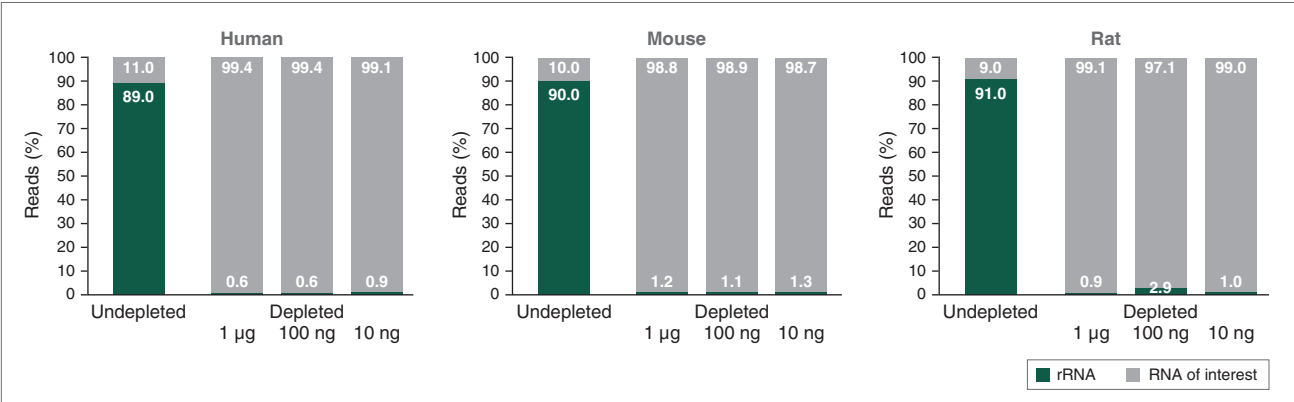
## ADVANTAGES

- Efficient, specific depletion of ribosomal RNA from human, mouse, and rat samples
- Suitable for low- and high-quality RNA
- Broad range of input amounts: 10 ng–1 µg
- Superior deletion of abundant RNAs
- Fast workflow: 2 hours, with less than 10 minutes hands-on time

## TOOLS & RESOURCES



Use the **NEBNext Custom RNA Depletion Design Tool** to design probes for use with NEBNext Depletion Kits.



**Universal human, mouse and rat reference total RNA (1 µg, 100 ng and 10 ng) was depleted of rRNA using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat).** 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 were sampled (seqtk) from each library and reads were identified as ribosomal using mirabait (6 or more shared 25-mers) with rRNA sequences for human baits (5S, 5.8S, 12S, 16S, 18S, 28S, ETS/ITS) or mouse/rat baits (5S, 5.8S, 12S, 16S, 18S, 28S). 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 NEBNext rRNA Depletion Kit v2 is efficient at depleting rRNA across species and input amounts.

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 & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750S/L/X)	6/24/96 rxns
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) 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

# NEBNext Poly(A) mRNA Magnetic Isolation Module

The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from 1–5 µg of previously isolated total RNA. The technology is based on the coupling of Oligo d(T)25 to 1 µm paramagnetic beads which is then used as the solid support for the direct binding of poly(A)+ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)+ transcripts in the eluent. Using the Express protocol, intact poly(A)+ RNA which is fully representative of the mRNA population of the original sample can be obtained in 30 minutes.

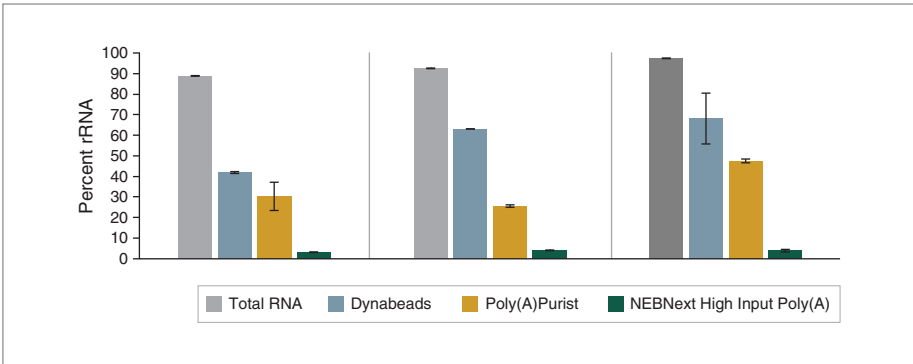
Even more  
*from less, for*  
RNA.

- Inputs: 1 – 5 µg total RNA per reaction
- Low elution volume
- New Express protocol
- Automation compatible

PRODUCT	SIZE
NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490S/L)	24/96 rxns

# NEBNext High Input Poly(A) mRNA Isolation Module

The NEBNext High Input Poly(A) mRNA Isolation Module is designed to isolate intact poly(A)+ RNA from high inputs (5-50 µg per reaction) of previously isolated total RNA, using oligo d(T)25-coupled paramagnetic beads. Intact mRNA can be obtained in approximately one hour and eluted in small volumes. For inputs less than 5 µg, the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490) is recommended.



**The NEBNext High Input Poly(A) mRNA Isolation Module produces low ribosomal RNA retention, across sample types.** Poly(A) RNA was enriched using Dynabeads® mRNA Purification Kit (Invitrogen®), Poly(A)Purist™ MAG (Invitrogen) or the NEBNext High Input Poly(A) mRNA Magnetic Isolation Module from 50 µg Universal Human Reference RNA (UHR, Agilent) or RNA extracted from mouse kidney tissue or *S. cerevisiae* (yeast) using the Monarch Total RNA Miniprep Kit. Percent ribosomal RNA (rRNA) of total or poly(A)-enriched RNA samples was determined from sequencing of triplicate (UHR and mouse poly(A) samples) or duplicate (total RNA and yeast poly(A) RNA samples) experiments, with standard deviation. Libraries were prepared from 40 ng poly(A)-enriched RNA using the NEBNext Ultra II Directional RNA Library Prep Kit and sequenced on an Illumina NextSeq 550 instrument. Six million reads were sampled from each library.

PRODUCT	SIZE
NEBNext High Input Poly(A) mRNA Isolation Module (NEB #E3370S)	24 rxns

COMPANION PRODUCTS	SIZE
NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490S/L)	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760S/L)	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (NEB #E7765S/L)	24/96 rxns

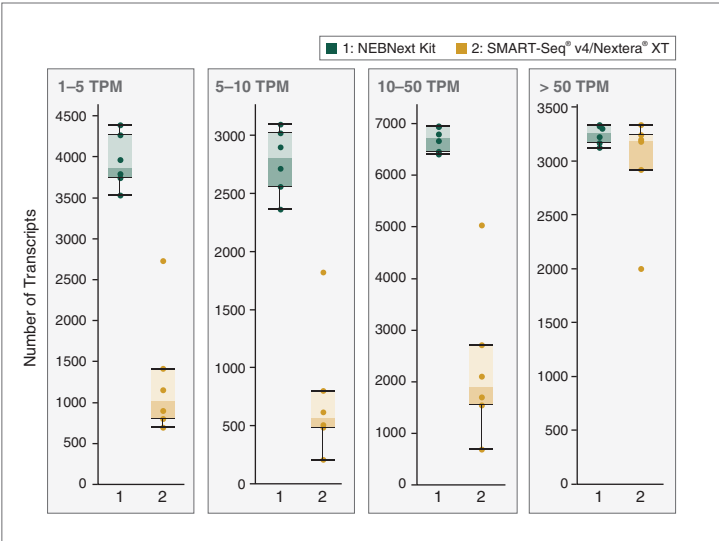


# NEBNext Single Cell/Low Input RNA Library Prep

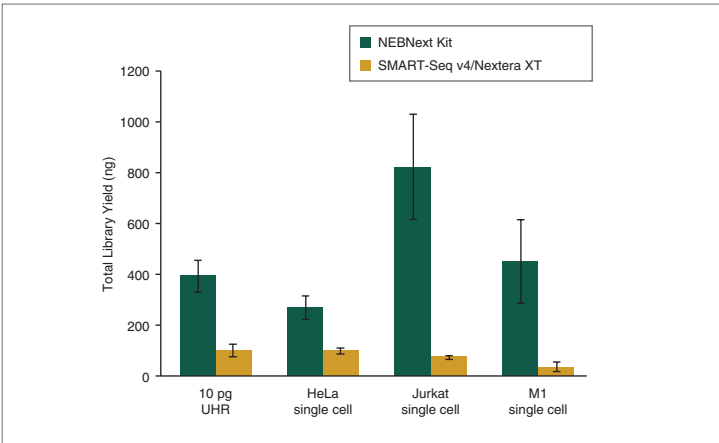
The unique workflow of the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, and utilize a unique protocol and suite of reagents.


cDNAs are generated directly from single cells or 2 pg–200 ng RNA, and even low-abundance transcripts are represented in the high yields of cDNA obtained. This is followed by library construction that incorporates the Ultra II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.



**Increased transcript detection with the NEBNext Single Cell/Low Input RNA Library Prep Kit.** Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq<sup>®</sup> v4 Ultra Low Input RNA Kit for Sequencing (Clontech<sup>®</sup> #634891) plus the Nextera<sup>®</sup> XT DNA Library Prep Kit (Illumina #FC-131-1096). Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate and method. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1–5, 5–10, 10–50 and > 50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.



**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 1 (Thermo Fisher Scientific<sup>®</sup> #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. Error bars indicate standard deviation for 6–11 replicates. 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.



## How low can you go?

- 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
- Utilize a single-tube protocol from cell lysis to cDNA
- Enzymatic DNA fragmentation, end repair and dA-tailing reagents are in a single enzyme mix, utilizing a single protocol, regardless of GC content
- Available with or without library construction reagents

PRODUCT	SIZE
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420S/L)	24/96 rxns
NEBNext Single Cell Lysis Module (NEB #E5530S)	96 rxns
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module (NEB #E6421S/L)	24/96 rxns

# NEBNext Products for Infectious Disease Sequencing

## NEBNext ARTIC Products for SARS-CoV-2 Sequencing

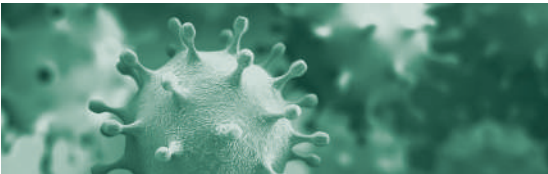
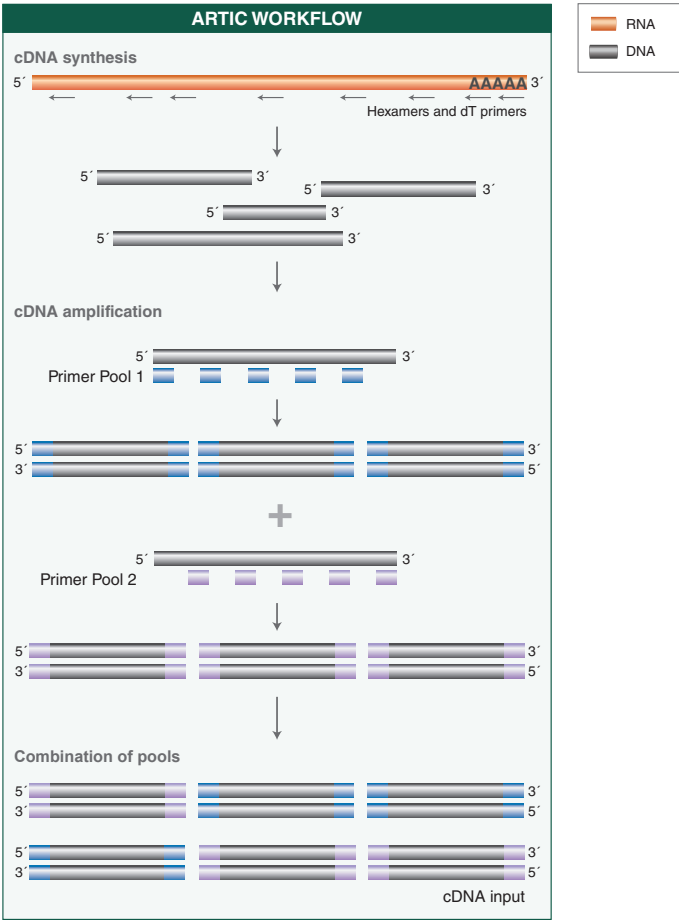
The NEBNext ARTIC kits were developed in response to the critical need for reliable and accurate methods for sequencing viral pathogens, specifically SARS-CoV-2. These kits, for long and short read sequencing, were based on the original work of the ARTIC Network (1). The ARTIC SARS-CoV-2 sequencing workflow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

### 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 FS Library Prep Kit (Illumina) (NEB #E7658L)	96 rxns
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) (NEB #E7660L)	96 rxns
NEBNext ARTIC SARS-CoV-2 RT-PCR Module (NEB #E7626L)	96 rxns
NEBNext RSV Primer Module (NEB #E9642S/L)	24/96 rxns



This system continually monitors registered primer sets for overlapping variants in sequences of SARS-CoV-2 and RSV from NCBI GenBank®.

<https://primer-monitor.neb.com/>





**Now available! NEBNext RSV Primer Module for Respiratory Syncytial Virus sequencing.**

References  
1. Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io <https://dx.doi.org/10.17504/protocols.io.bdp7i5m>

## 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

				
	<b>SINGLE INDEX</b>	<b>DUAL INDEX</b>	<b>UNIQUE DUAL INDEX</b>	<b>UNIQUE DUAL INDEX UMIs</b>
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# E7416
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 combinatorial 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



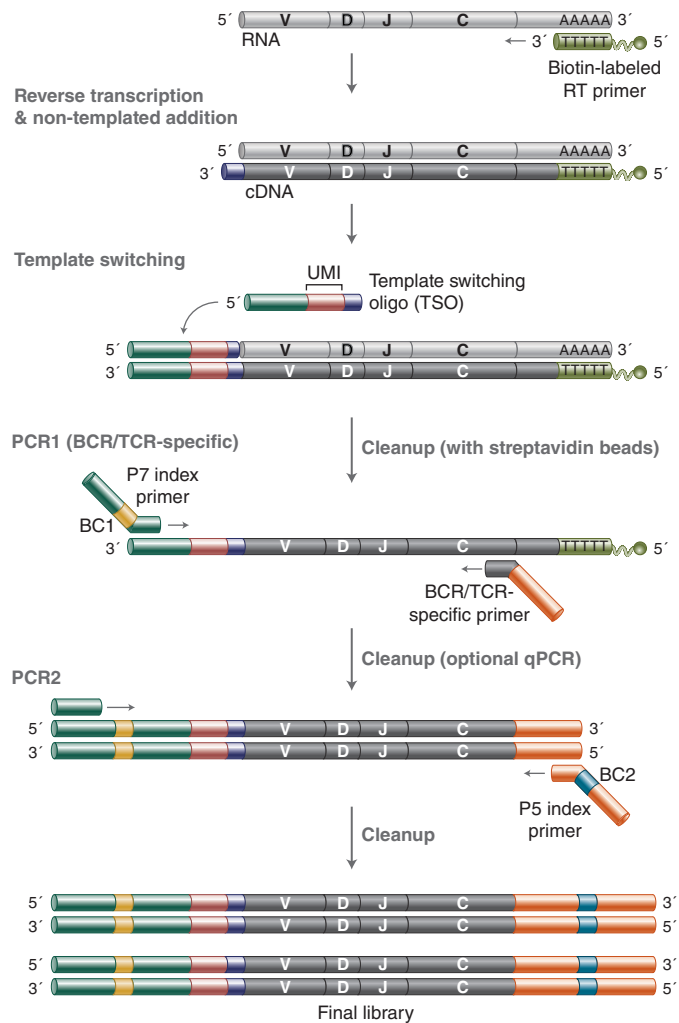
# NEBNext Immune Sequencing Kits (Human & Mouse)

The NEBNext Immune Sequencing Kits (Human & Mouse) enable exhaustive profiling of somatic mutations in the full-length immune gene repertoires of B cells and T cells, via the expression of complete antibody chains. A unique, UMI-based mRNA barcoding process allows PCR copies derived from an individual molecule to be converted to a consensus sequence. This improves sequence accuracy and eliminates PCR bias.

Immune repertoire sequencing is frequently used to analyze immune responses, both current and distant. Areas of particular interest include characterization of autoimmune diseases, oncology, discovery of neutralizing antibodies against infectious disease, tumor-infiltrating lymphocytes and use as a tool to study residual disease. Recent improvements in read lengths and throughputs of next-generation sequencing (NGS) platforms have resulted in a rise in the popularity of immune repertoire sequencing.

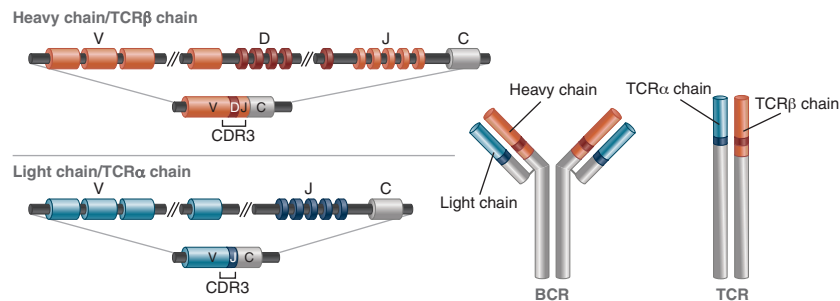
## ADVANTAGES

- Generation of full-length variable sequences (including isotype information), allowing downstream antibody synthesis and functional characterization not possible with approaches sequencing only the CDR3 region
- Eliminated use of variable region primers, reducing primer pool complexity and realizing unbiased and simultaneous recovery of B-cell and T-cell receptor transcripts
- Minimized PCR bias and improved sequencing accuracy by allowing a consensus to be generated from duplicate sequencing reads originating from the same transcript; UMIs enable accurate quantitation of each clone present in the sample
- Optimized high target-capture efficiency for immune repertoire sequencing and analysis from sub-microgram quantities of total RNA



NEBNext Immune Sequencing Kit Workflow.

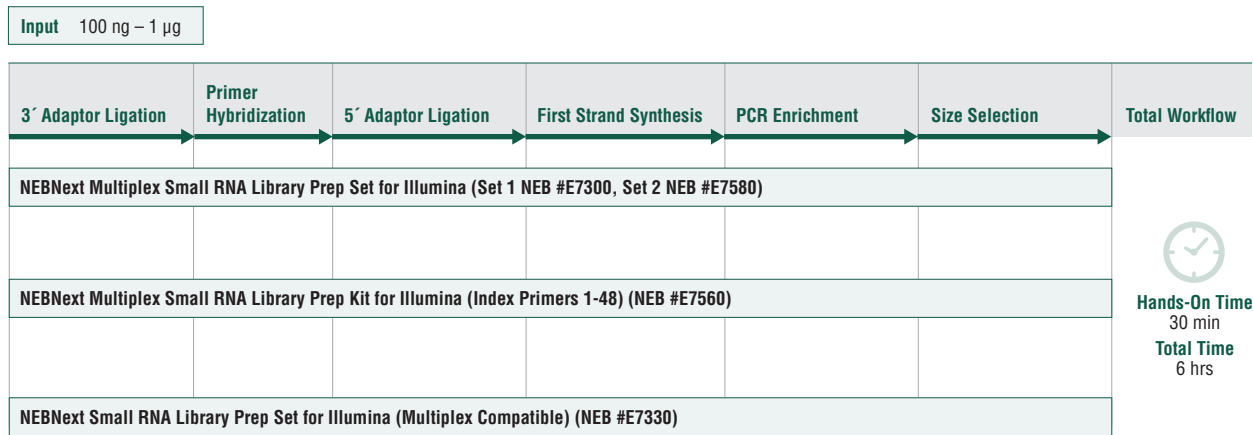
PRODUCT	SIZE
NEBNext Immune Sequencing Kit (Human) (NEB #E6320S/L)	24/96 rxns
NEBNext Immune Sequencing Kit (Mouse) (NEB #E6330S/L)	24/96 rxns



Simplified representation of the structure of an antibody or TCR. Simplified representation of the structure of an antibody or TCR showing the outcome of V(D)J recombination in mature lymphocytes.

# NEBNext Small RNA Library Prep Kits

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



ADVANTAGES

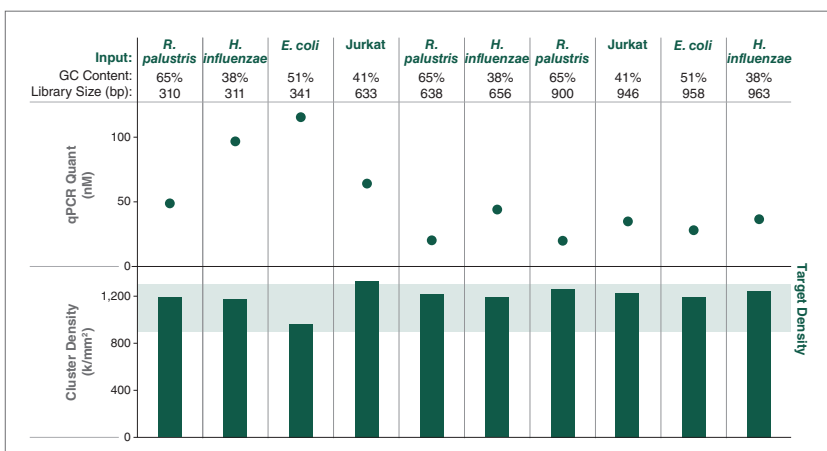
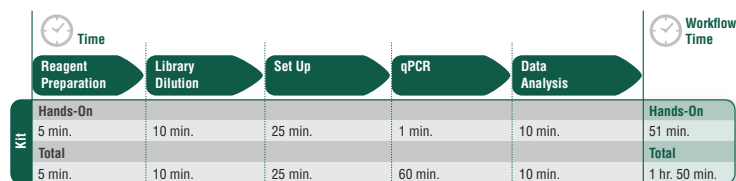
- Minimal adaptor-dimer formation
- High yields
- Input RNA can be total RNA
- Suitable for methylated small RNAs (e.g, piRNAs) as well as unmethylated small RNAs
- Flexibility for use with NEBNext library preparation kits and other standard, Illumina-compatible library

PRODUCT	SIZE
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) (NEB #E7300S/L)	24/96 rxns
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2) (NEB #E7580S/L)	24/96 rxns
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560S)	96 rxns
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330S/L)	24/96 rxns

# NEBNext Library Quant Kit for Illumina

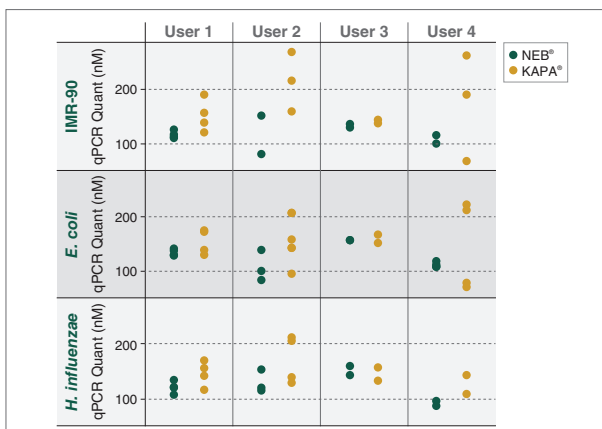
Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence output. 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



With NEBNext, optimal cluster density is achieved from quantitated libraries with a broad range of library size and GC content. Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq® (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.

## 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 (green) versus those from the Kapa kit (yellow).

## Count on it.

- 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](http://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](http://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



## Ordering Information

Kits for Illumina RNA Library Preparation		NEB #	Size
Directional RNA	NEBNext UltraExpress RNA Library Prep Kit	E3330S/L	24/96 rxns
	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
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
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
	NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)	E7658L	96 rxns
Modules & Enzymes		NEB #	Size
RNA	NEBNext ARTIC SARS-CoV-2 RT-PCR Module	E7626L	96 rxns
	NEBNext RSV Primer Module	E9642S/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 Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 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 Single Cell/Low Input cDNA Synthesis & 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 Ligation Module	E7445L	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/X	50/250/500 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, Sets 1–5)	E6440S/L	96/384 rxns
		E6442S/L	
		E6444S/L	
		E6446S/L	
		E6448S/L	
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Sets 1, 2)	E7600S	96 rxns
		E7780S	
	NEBNext Multiplex Oligos for Illumina (Index Primers, Sets 1–4)	E7335S/L	24/96 rxns
		E7500S/L	
		E7710S/L	
		E7730S/L	
	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 for Illumina		NEB #	Size
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	15 ml

Products for Oxford Nanopore DNA Library Preparation		NEB #	Size
RNA	NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660L	96rxns

Magnetic Separation		NEB #	Size
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

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