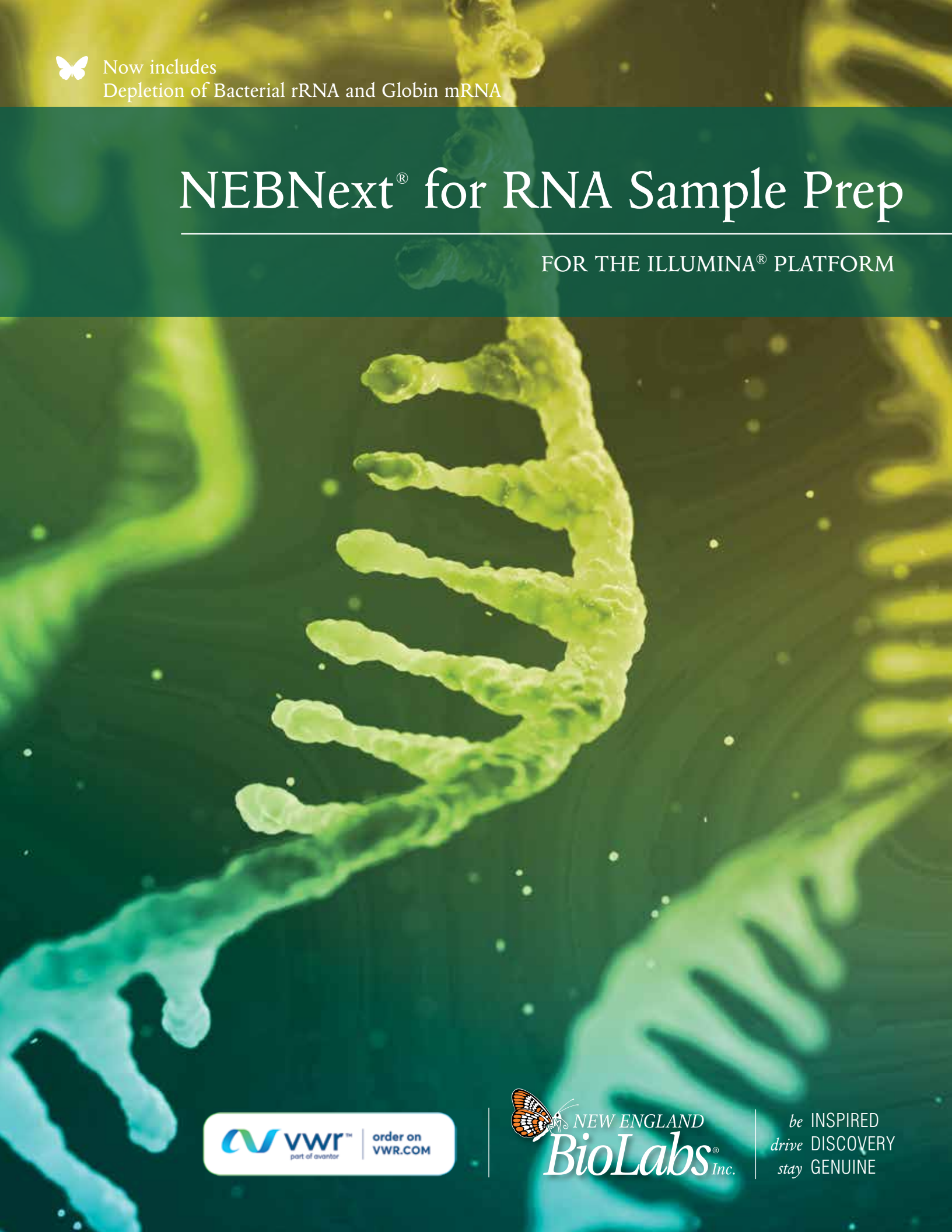
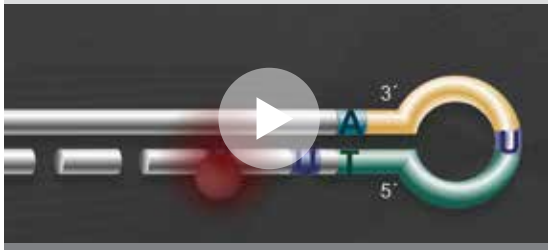

Now includes
Depletion of Bacterial rRNA and Globin mRNA
NEBNext® for RNA Sample Prep
FOR THE ILLUMINA® PLATFORM
VWR™ part of avantor
order on VWR.COM
NEW ENGLAND BioLabs® Inc.
be INSPIRED
drive DISCOVERY
stay GENUINE

Table of Contents

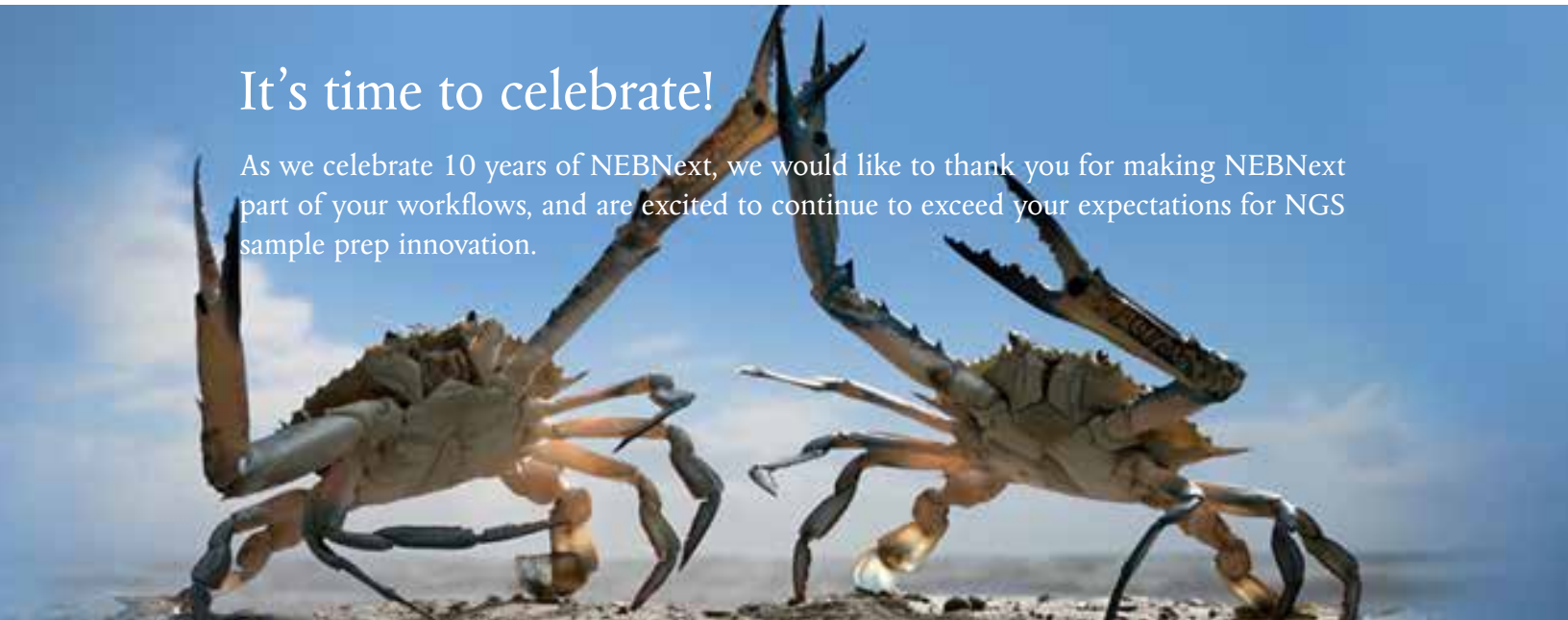
TOOLS & RESOURCES

Visit NEBNext online 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



It's time to celebrate!

As we celebrate 10 years of NEBNext, we would like to thank you for making NEBNext part of your workflows, and are excited to continue to exceed your expectations for NGS sample prep innovation.

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 samples). 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. To serve 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. In addition to the extensive QCs performed on individual kit components, all NEBNext kits for Illumina are functionally validated by preparation of a library, followed by Illumina sequencing. Additionally, NEBNext products have been cited in over 6,000 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 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.

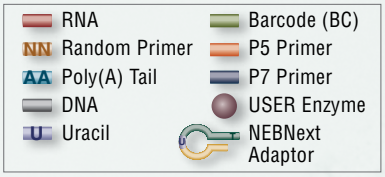
WHATS NEW IN NEBNEXT?

- Depletion of globin mRNA and rRNA for human, mouse and rat
- Bacterial rRNA depletion
- Unique dual index primer pairs
- The **NEBNext Magnetic Separation Rack:** custom designed for NGS magnetic bead separations

NEBNEXT MAGNETIC SEPARATION RACK

Workflow for RNA Library Preparation

PRODUCT	INPUT AMOUNTS
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	5 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	
NEBNext Ultra II RNA Library Prep Kit for Illumina	
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	
NEBNext Poly(A) mRNA Magnetic Isolation Module	
NEBNext Oligos (including 12-plex, 96-plex and dual index primers)	



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

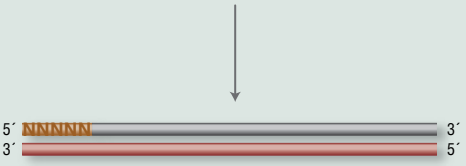
- Removal of rRNA (> 80% of total RNA) or enrichment for mRNA
- NEBNext Library Prep kits are compatible with either method

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

DIRECTIONAL

NON-DIRECTIONAL

5 End Repair, dA-Tailing & Adaptor Ligation

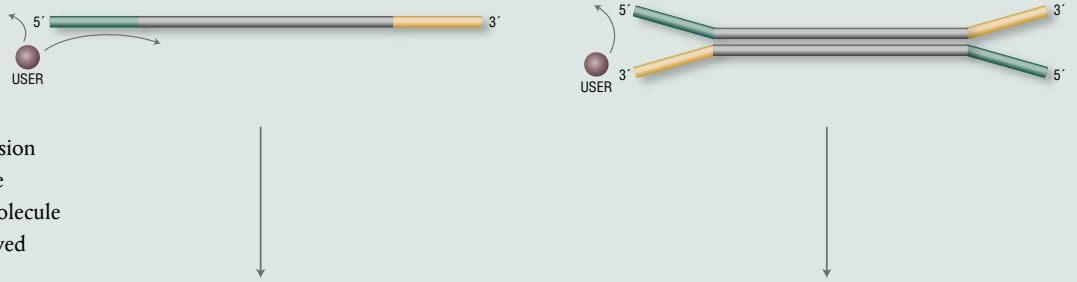
- Generation of blunt, phosphorylated ends
- Addition of single A 3´ overhang (enables ligation to adaptors with single T overhangs)
- Ligation of short adaptors (contain sequences required downstream)
- NEBNext adaptors increase ligation efficiency & minimize adaptor-dimer formation

6 U Excision

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

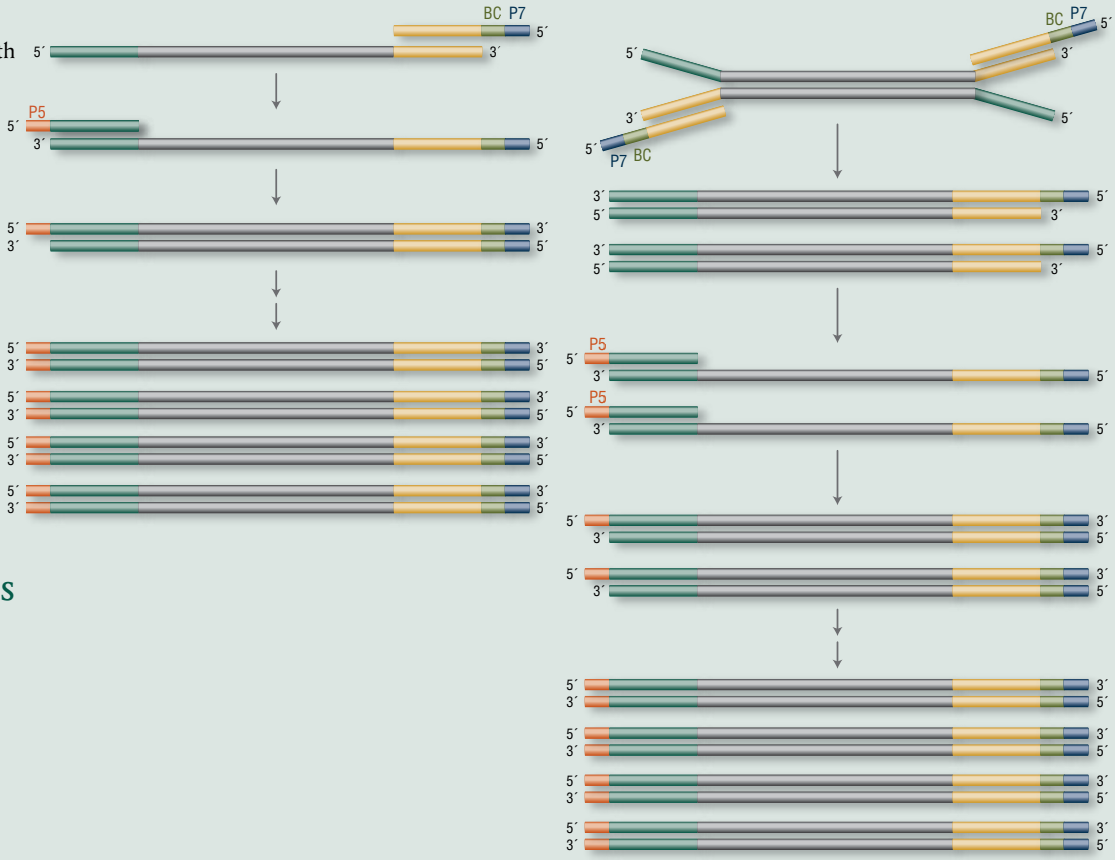
Directional Only

- 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



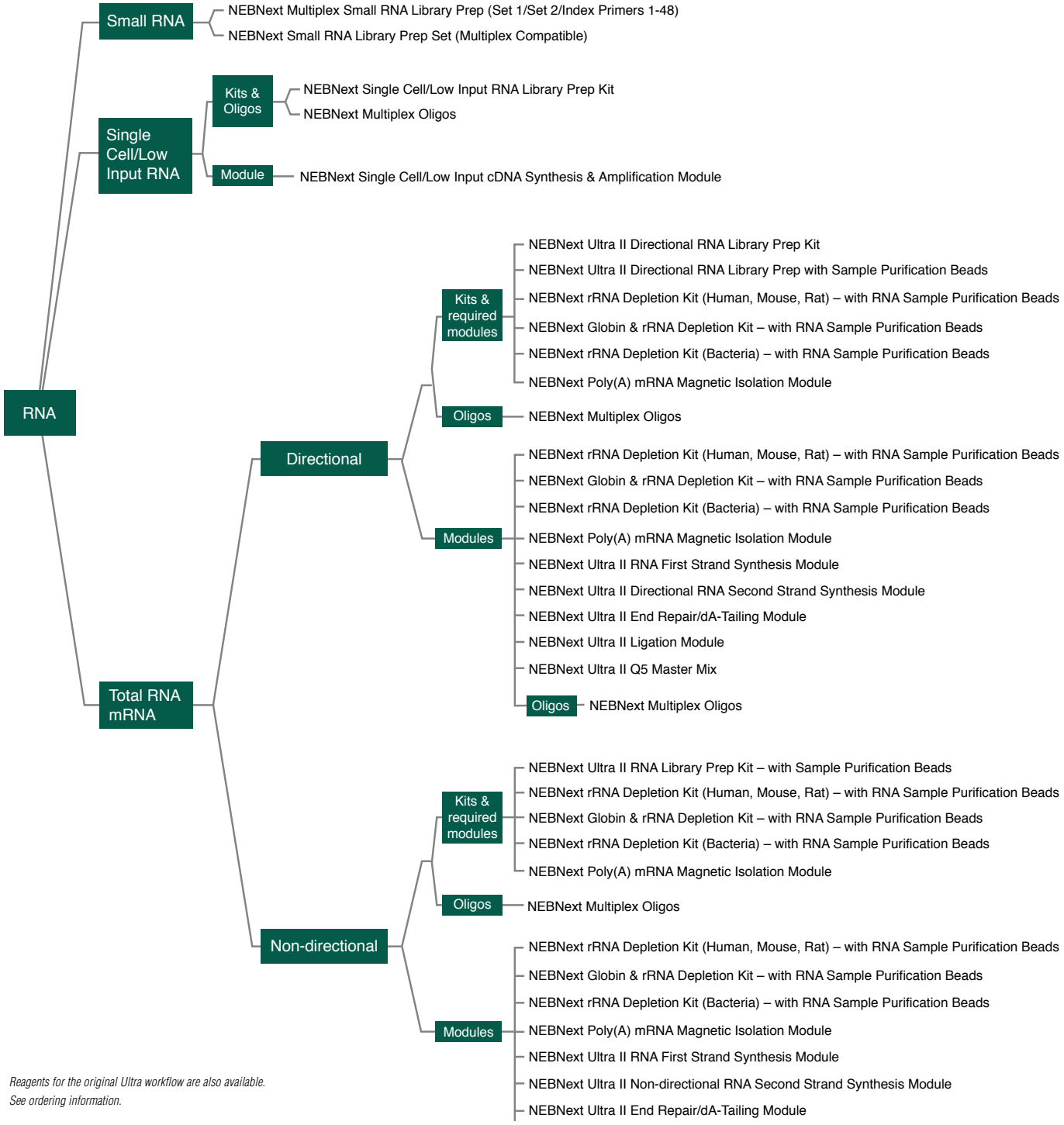
NEBNext Oligos

- 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**, to choose the best products for your needs.



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

- It is important to start with high quality RNA. The use of degraded RNA can result in low yield or failure to generate libraries. We recommend determining RNA quality using the RNA Integrity Number (RIN) estimated by the Agilent Bioanalyzer. The RNA sample should have a RIN value higher than 7.
- RNA should be completely free of DNA. DNase digestion of the purified RNA with RNase-free DNase 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®, TapeStation® or similar. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. However, free nucleotides or other organic compounds routinely used to extract RNA will also absorb UV light near 260 nm and will result in an over-estimation of the RNA concentration.

Bead-based clean-ups and size selection

- Be careful when transferring material not to disturb the bead pellet
- Be sure to vortex the beads just before use – they should be a uniform suspension
- Do not over-dry the beads. This can make resuspension difficult and reduce yield.
- Bead-based clean-ups and size-selection are explained in the Ultra II video

Barcodes

- When you are using a subset of the barcodes supplied in a kit, or using barcodes from more than one kit, it is important to optimize the combination of barcodes used, to ensure balanced sequencing reads. We provide recommendations for NEBNext barcode combinations at NEBNext.com.
- Open only one index primer vial at a time, to minimize the risk of contamination

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

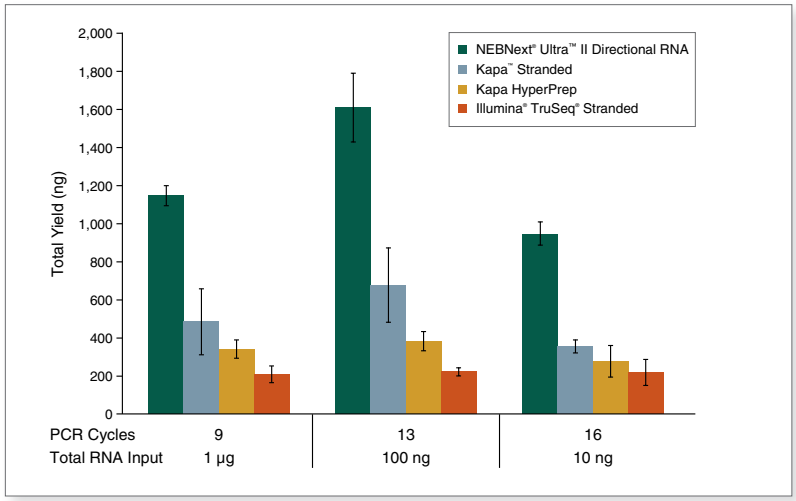
The latest generation of NEBNext kits for RNA enable directional (strand-specific) or non-directional library construction from low ng to µg input amounts, and are compatible with poly(A) mRNA enrichment or rRNA depletion. Workflows are streamlined with minimal hands-on time, and are automatable. Information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

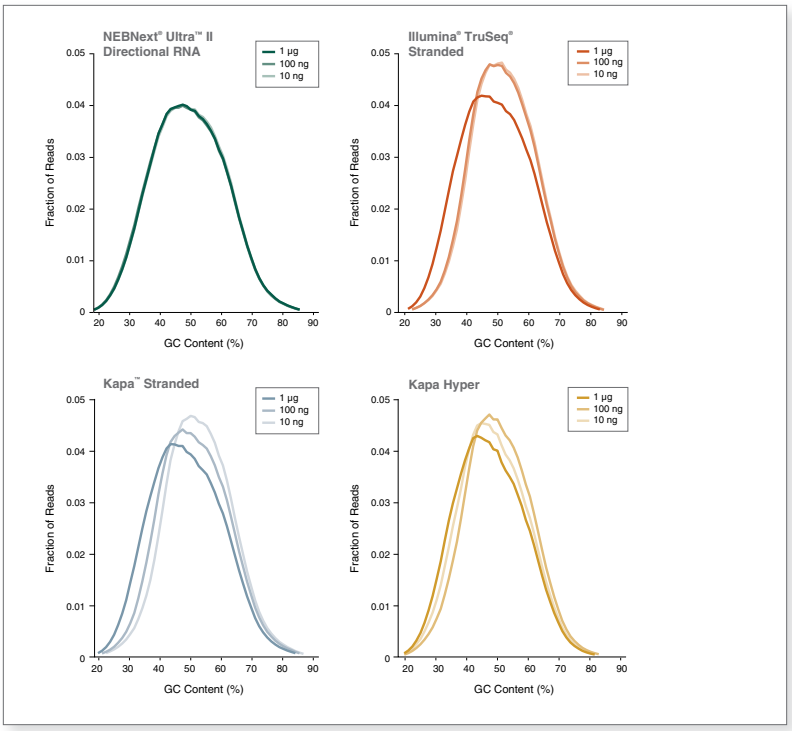
- **Generate high yield**, high-quality libraries, even with limited amounts of RNA:
  - 5 ng – 1 µg total RNA (rRNA depletion workflow)
  - 10 ng – 1 µg total RNA (poly(A) mRNA workflow)
- **Minimize bias**, with fewer PCR cycles required
- **Increase library complexity** and transcript coverage
- **Increase 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

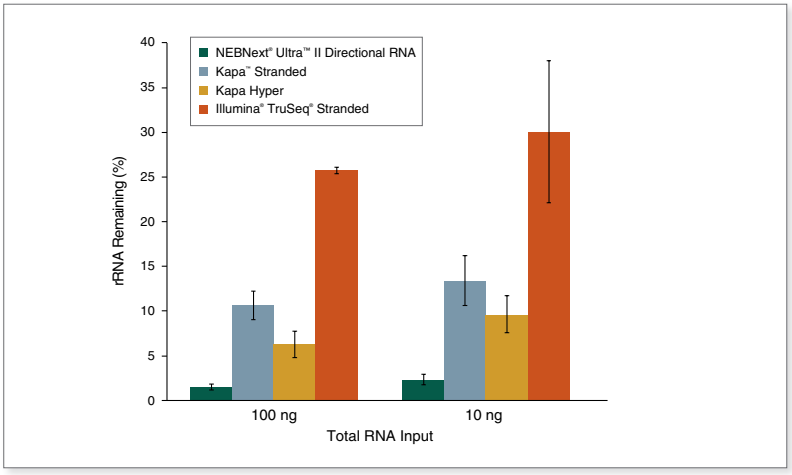
References
1. Parkhomchuk, D., et al. (2009) Nucleic Acids Res. 37, e123.
2. Levin, J.Z., et al. (2010) Nature Methods 7, 709–715.

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, 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 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: 5 ng – 1 µg

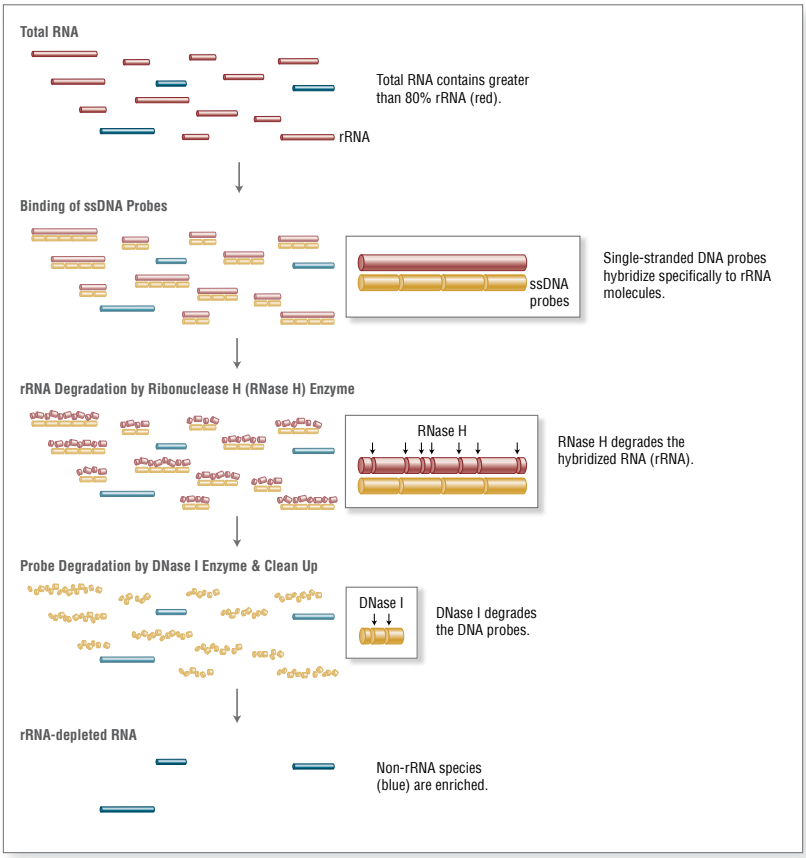
	mRNA Isolation/ rRNA Depletion	mRNA Fragmentation	First Strand cDNA Synthesis	Second Strand cDNA Synthesis	End Repair/ dA Tailing	Adaptor Ligation	Size Selection	PCR Enrichment	Clean Up	Total Workflow
Ultra II Directional Kits		**NEBNext Ultra II Directional RNA Library Prep Kit for Illumina**								
		NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads								
		• 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	• NEBNext Sample Purification Beads (SPRIselect)	• NEBNext Ultra II Q5 Master Mix	• NEBNext Sample Purification Beads (SPRIselect)	**Hands-On** 27 min **Total** 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
Ultra II Non-directional Kits		**NEBNext Ultra II RNA Library Prep Kit for Illumina**								
		NEBNext Ultra II RNA Library Prep with Sample Purification Beads								
		• 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	• NEBNext Sample Purification Beads (SPRIselect)	• NEBNext Ultra II Q5 Master Mix	• NEBNext Sample Purification Beads (SPRIselect)	**Hands-On** 27 min **Total** 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
Ultra II Modules	**rRNA Depletion Kit (Human/Mouse/Rat)** **Globin & rRNA Depletion Kit (Human/Mouse/Rat)** **rRNA Depletion Kit (Bacteria)** • RNase H/RNase H Reaction Buffer • rRNA Depletion Solution (unique to kit) • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water	**Magnesium RNA Fragmentation Module** • RNA Fragmentation Buffer • RNA Fragmentation Stop Solution	**Ultra II RNA First Strand Synthesis Module** • First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent	**Ultra II Directional RNA Second Strand Synthesis Module** • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer with dUTP **Ultra II Non-directional RNA Second Strand Synthesis Module** • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer	**NEBNext Ultra II End Repair/ dA-Tailing Module** • Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer	**NEBNext Ultra II Ligation Module** • Ultra II Ligation Master Mix • Ligation Enhancer		**NEBNext Ultra II Q5 Master Mix** • NEBNext Ultra II Q5 Master Mix		* Including poly(A) mRNA isolation ** Including rRNA depletion
	rRNA Depletion Kits with Sample Purification Beads • RNase H/RNase H Reaction Buffer • rRNA Depletion Solution (unique to kit) • Probe Hybridization Buffer • DNase I/DNase I Reaction Buffer • Nuclease-free Water • NEBNext RNA Sample Purification Beads									
	Poly(A) mRNA Magnetic Isolation Module • 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.

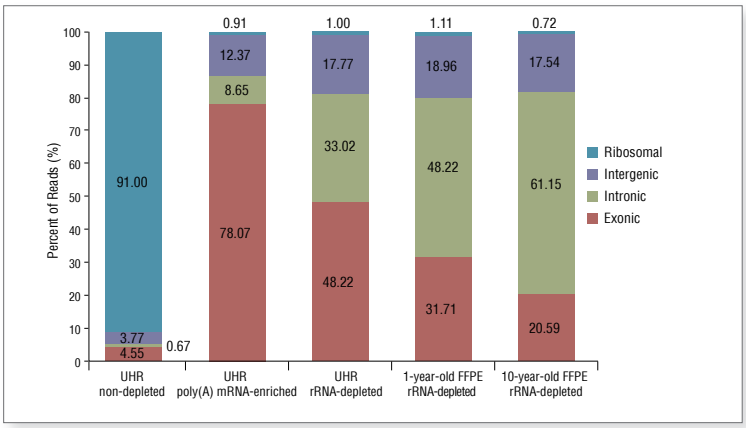
- Suitable for low-quality (e.g., FFPE) and high-quality RNA
- Compatible with a broad range of input amounts: 10 ng–1 µg
- Superior depletion of abundant RNAs, with retention of RNAs of interest
- Fast workflow: 2 hours, with less than 10 minutes hands-on time
- Depleted RNA is suitable for RNA-seq,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

PRODUCT	SIZE
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	6/24/96 rxns
NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	6/24/96 rxns
NEBNext Globin & RNA Depletion Kit	6/24/96 rxns
NEBNext Globin & RNA Depletion Kit with RNA Sample Purification Beads	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria)	6/24/96 rxns
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	6/24/96 rxns
ALSO AVAILABLE	SIZE
NEBNext Poly(A) mRNA Magnetic Isolation Module	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.

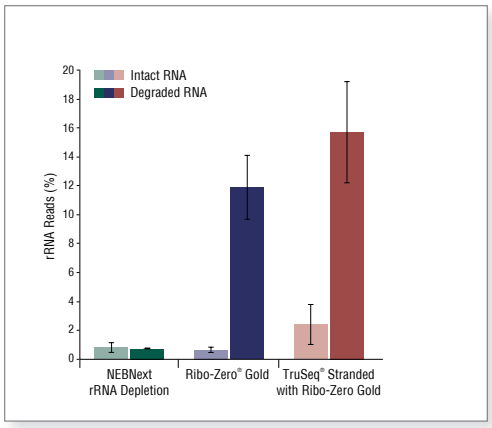
rRNA Depletion (Human/Mouse/Rat)

Efficient removal of rRNA from intact and degraded RNA (FFPE), while retaining coding and non-coding transcripts



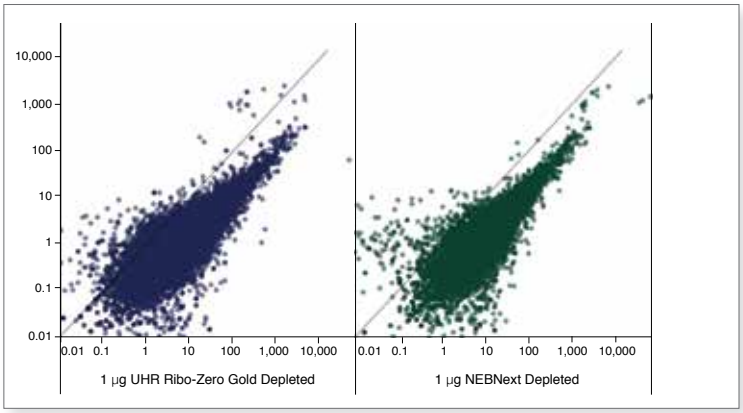
RNA-seq libraries were generated from Universal Human Reference Total RNA (UHR, Agilent) or Breast Cancer FFPE RNA (with an archive age of 1 year and 10 years). RNA was either untreated or treated with the NEBNext poly(A) mRNA Magnetic Isolation Module or the NEBNext rRNA Depletion Kit. RNA-seq libraries were made using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina. Reads were mapped to the hg19 genome and read distributions were determined using Picard RNA-seq Metrics. Libraries generated from rRNA-depleted RNA result in comparable low rRNA reads to poly(A) mRNA-enriched RNA, while also retaining more noncoding reads. rRNA depletion efficiency is achieved even with FFPE RNA.

rRNA Depletion efficiency with intact or degraded RNA



Ribosomal RNA was depleted from intact Universal Human Reference Total RNA (UHR, Agilent) (RIN > 9) and degraded UHR Total RNA (RIN < 3) using either the NEBNext rRNA Depletion Kit, Ribo-Zero® Magnetic Gold Kit (Human/Mouse/Rat) (Epicentre) or Ribo-Zero Gold provided within the TruSeq Stranded Total RNA Kit with Ribo-Zero Gold (Illumina). rRNA-depleted RNA libraries were made using either the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (Green and blue bars) or the TruSeq Stranded Total RNA Kit with Ribo-Zero Gold (Illumina, red bars). Total rRNA-aligned reads were determined using Bowtie 2.0 (local, sensitive).

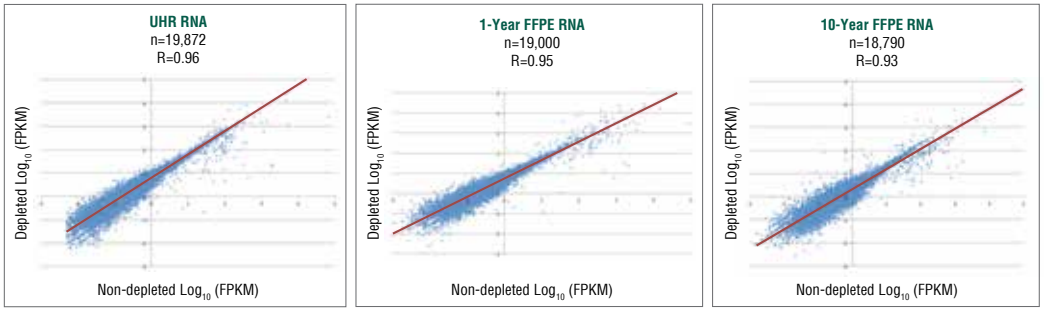
Transcription expression correlation with undepleted libraries



1 µg of Universal Human Reference Total RNA (Agilent) was depleted using either the NEBNext rRNA Depletion Kit or the Ribo-Zero Magnetic Gold Kit (Epicentre). Libraries were prepared using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina and sequenced on an Illumina NextSeq® 500 instrument. Reads were mapped to all CDS entries from the GRCh38 Enzembl release 81 reference using Salmon (1). Transcripts per million values were correlated between an undepleted sample and depleted libraries. Transcript levels from the NEBNext-depleted library were better correlated than those from the Ribo-Zero depleted library.

Reference
1. https://github.com/COMBINE-lab/salmon.

Transcript expression correlation with non-depleted libraries

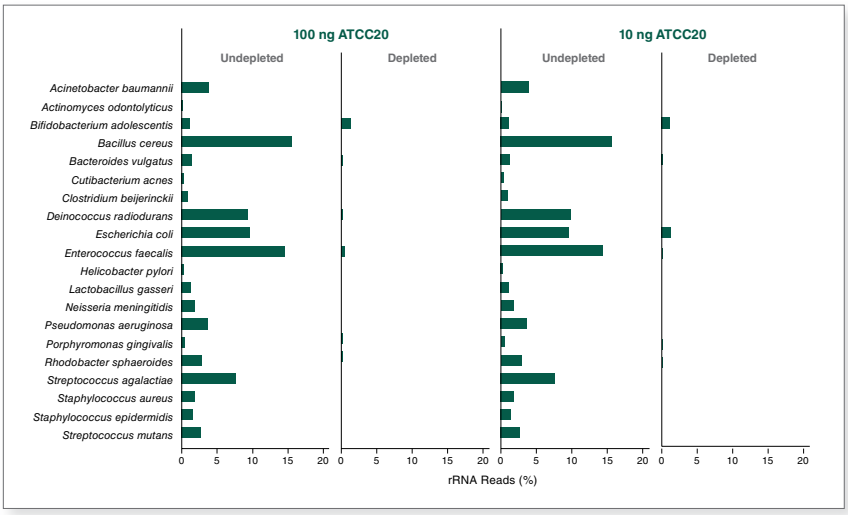


Libraries were made from UHR RNA (Agilent) and Breast Cancer FFPE RNA (with archive age of one year and 10 years), both non-depleted and depleted rRNA using the NEBNext rRNA Depletion Kit. All libraries were made using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina. TopHat2 and Cufflinks were used for read mapping and transcript assembly and quantification. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) correlation analysis indicates very good transcript expression correlation (R > 0.93) between Depleted and Non-Depleted libraries. NEBNext rRNA depletion does not affect transcript expression levels.

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



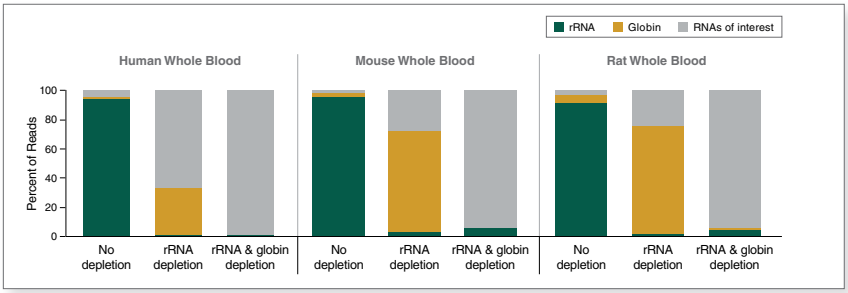
Total RNA was extracted from a lyophilized pool of 20 different bacterial organisms. Ribosomal RNA was depleted using the NEBNext rRNA Depletion Kit (Bacteria). RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed 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).

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.

What users are saying:

"The NEB Bacterial Depletion has depleted rRNA equally or better than our previous ribodepletion gold standard across a wide RIN quality range. We have been pleased with the flexibility of Total RNA input ranges and have routinely gotten effective ribodepletion at 100 ng Total RNA Input in both single isolates and metagenomic samples. The protocol is also more ergonomically friendly than bead based ribodepletion protocols. Of all the new bacterial ribodepletion methods we have tested, NEB was by far the best."

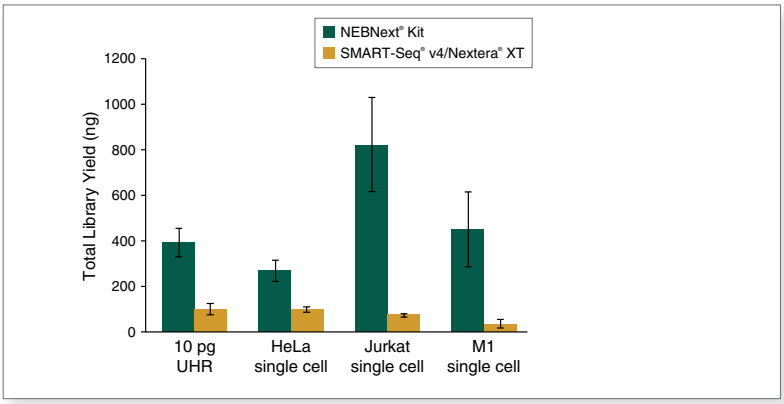
– Research Assistant,
Biomedical research institution

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 with recommended amounts of ERCC RNA Spike-In Mix I. The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq® v4 Ultra Low Input RNA Kit for Sequencing plus the Nextera® XT DNA Library Prep Kit 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

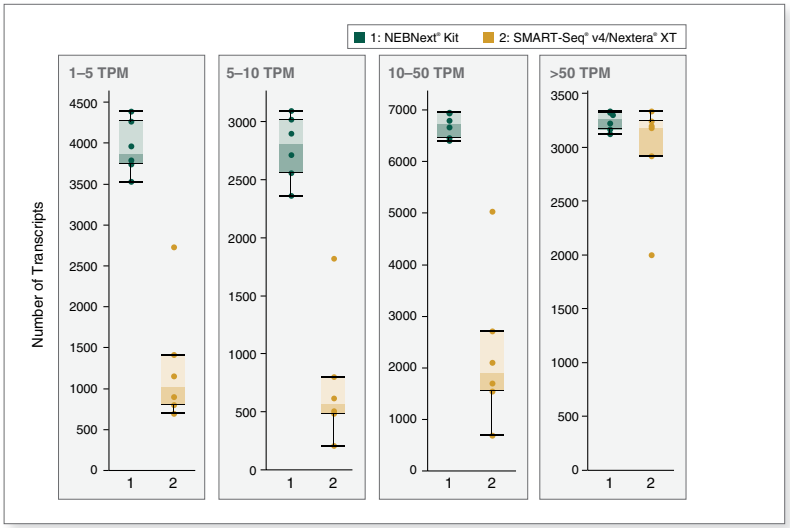
PRODUCT	SIZE
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	24/96 rxns

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."

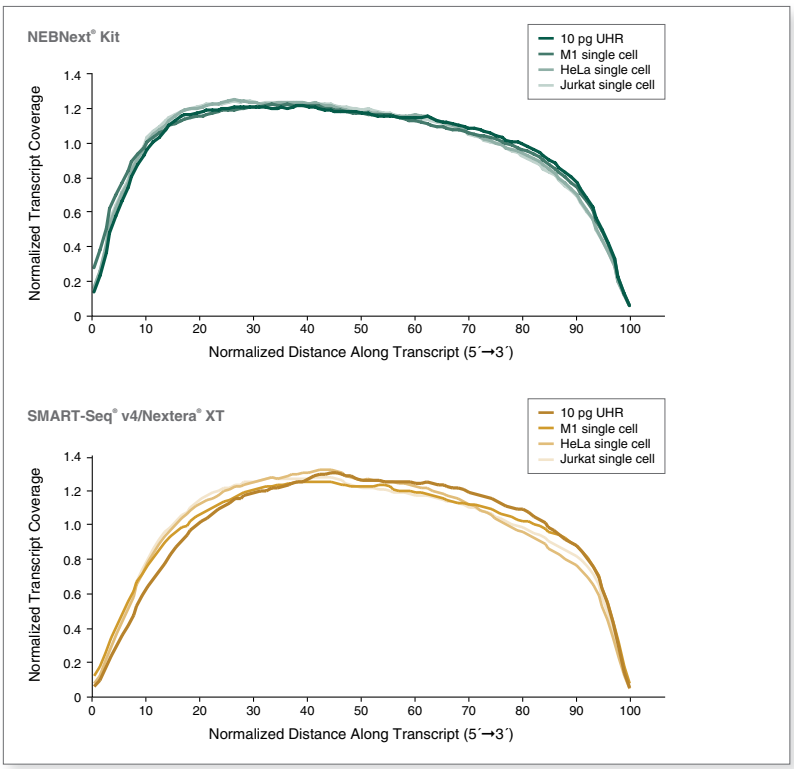
– Jon Preall, Ph.D.,
Research Assistant Professor at Cold Spring Harbor Laboratory and director of the CSHL Single Cell Sequencing Core.

The NEBNext Single/Cell Low Input RNA Library Prep Kit increases transcript detection



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 v4 Ultra Low Input RNA Kit for Sequencing plus the Nextera XT DNA Library Prep Kit. 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. 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.

The NEBNext Single Cell/Low Input RNA Library Prep Kit provides uniform coverage across the length of transcripts



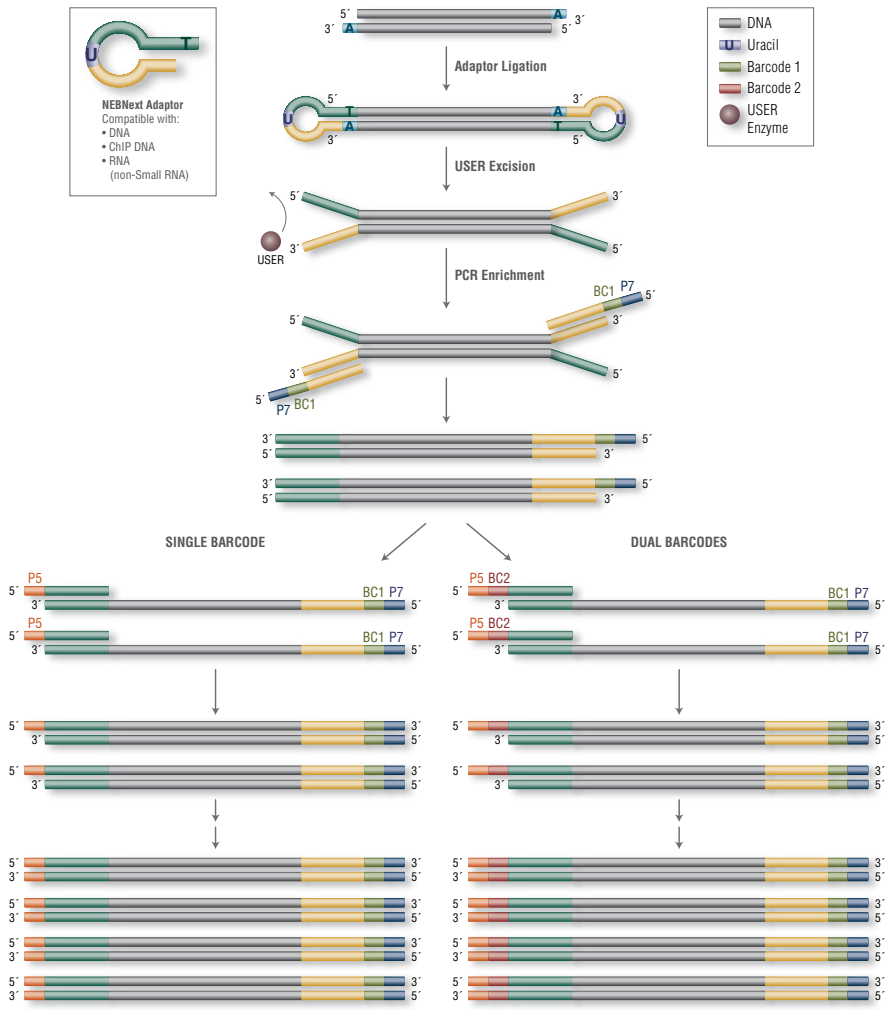
Sequencing libraries were generated from HeLa, Jurkat and M1 single cells, or 10 pg of Universal Human Reference (UHR) RNA with recommended amounts of ERCC RNA Spike-In Mix I. The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing plus the Nextera XT DNA Library Prep Kit were used. Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). Gene body coverage shown is an average of four replicates and was calculated using Picard tools. The global view of the 5´ to 3´ coverage of the RefSeq transcripts reveals both consistency across different sample types and uniformity across the transcript length in the NEBNext libraries.

NEBNext Adaptors and Primers

Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Adaptors enable high-efficiency adaptor ligation and high library yields, with minimized adaptor-dimer formation. Incorporating a novel hairpin loop structure, the NEBNext Adaptor ligates with increased efficiency to end-repaired, dA-tailed DNA. The loop contains a U, which is removed by treatment with USER® Enzyme (a mix of UDG and Endo VIII), to open up the loop and make it available as a substrate for PCR. During PCR, barcodes can be incorporated by use of the NEBNext index primers, thereby enabling multiplexing. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols. Single- or dual-barcode primer options are available.

Also the Unique Dual Index Primer Pairs address the "index hopping" seen with certain Illumina sequencing instruments. The unique combination of dual indices allows identification and complete filtering of index-swapped reads.

Workflow demonstrating the use of NEBNext adaptors and index primers



ADVANTAGES

- Increased ligation efficiency
- Minimized adaptor-dimer formation
- Increased library yields
- Multiple options
  - Unique dual indices (2 sets of 96 pairs)
  - Dual indices (2 sets)
  - Single indices (4 sets of 12 indices, one set of 96 indices)
- Convenient formats
  - Vials
  - Single-use 96-well plates with a pierceable foil seal
- Index pooling guidelines and sample sheets are provided

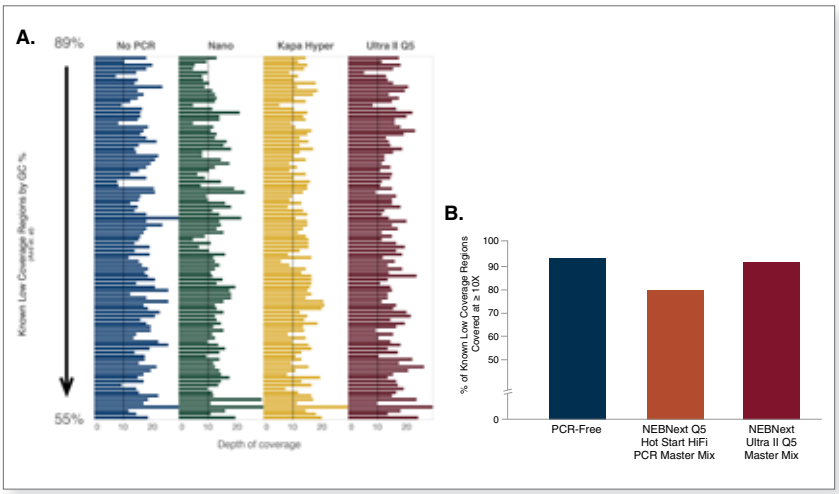
PRODUCT	# INDICES	SIZE
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs or 96 Unique Dual Index Primer Pairs Set 2) (NEB #E6440S/L, #E6442S/L)	96 unique pairs	96/384 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1 or 2) (NEB #E7600S, #E7780S)	8 x 12	96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers) (NEB #E6609S/L)	96	96/384 reactions
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 reactions
NEBNext Adaptor Dilution Buffer (NEB #B1430)		1 x 9.6 ml

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.

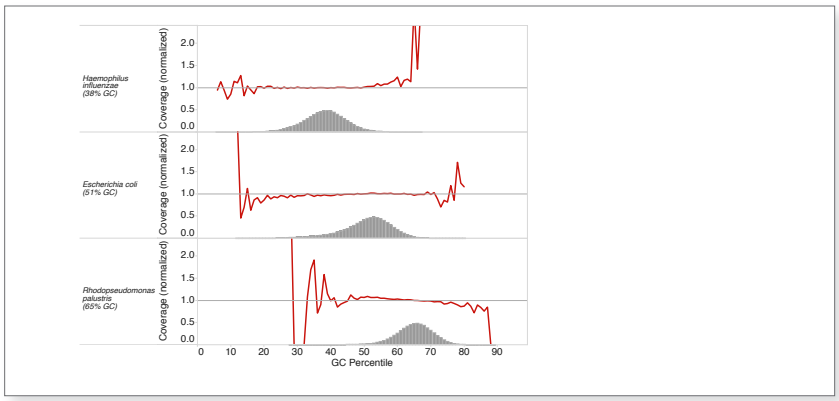
The NEBNext Ultra II Q5 Master Mix (NEB #M0544) is the latest formulation of Q5 DNA polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior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

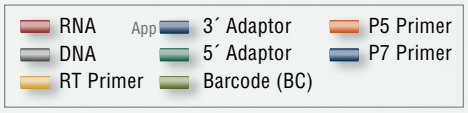
PRODUCT	SIZE
NEBNext Ultra II Q5 Master Mix	50/250 rxns

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.*

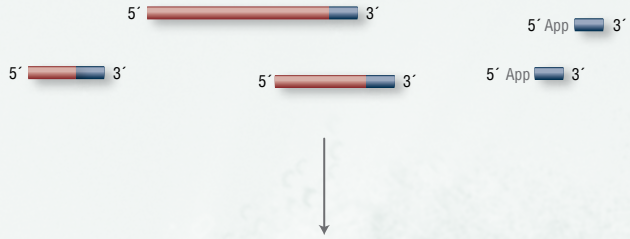
Workflow for Small RNA Library Preparation

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



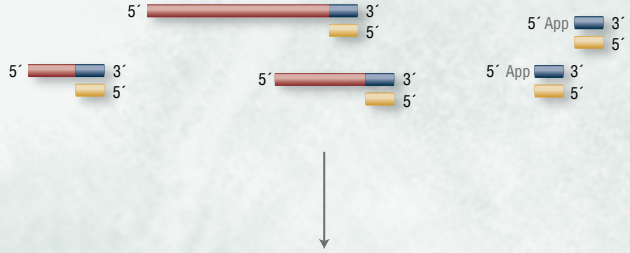
1 3´ Adaptor Ligation

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



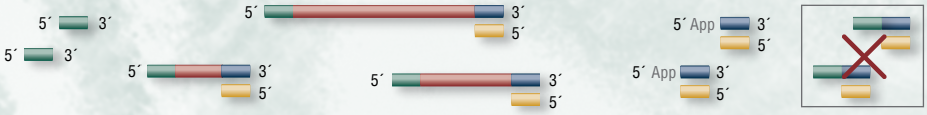
2 Primer Hybridization

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



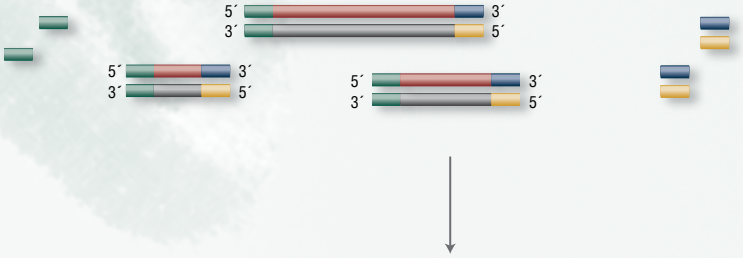
3 5´ Adaptor Ligation

- 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



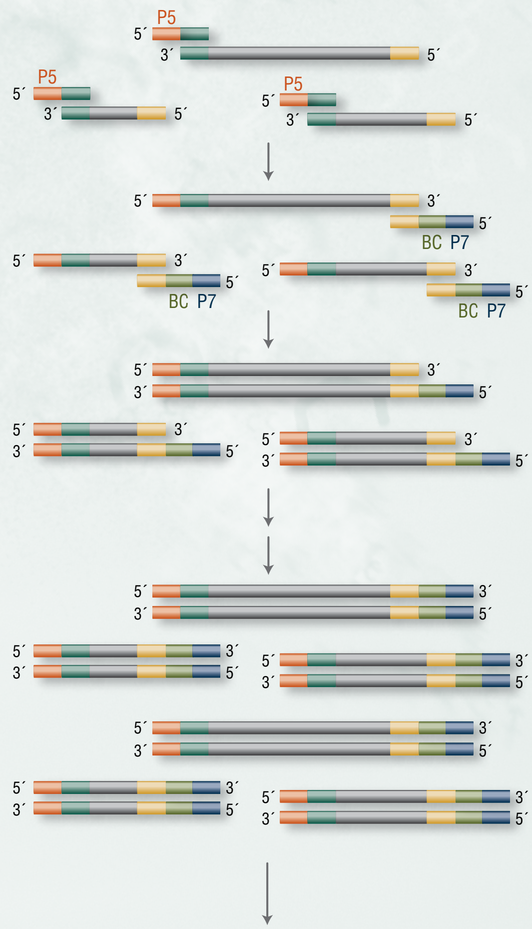
4 First Strand cDNA Synthesis

- 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

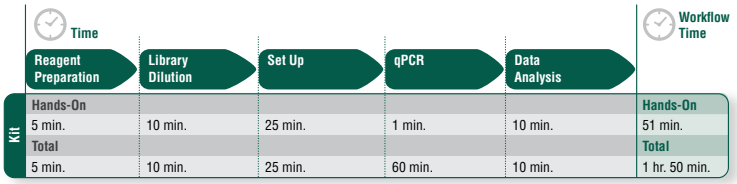
Input 100 ng – 1 µg

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, Set 2)						
• 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)						
• 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)						
• 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

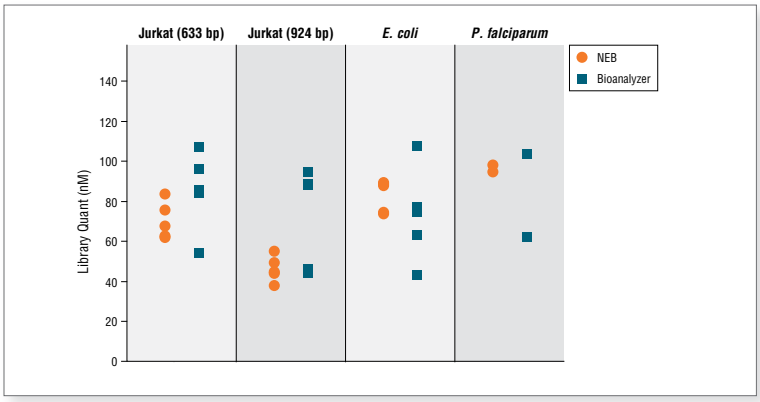
NEBNext Library Quant Kit for Illumina

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 workflow

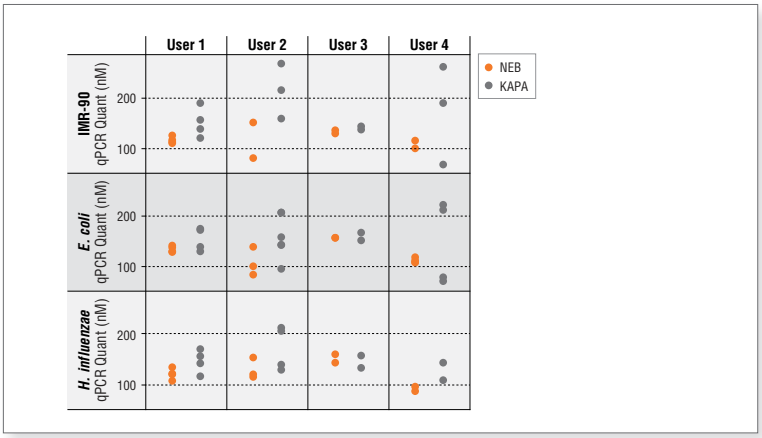


Comparison of quantitation by qPCR and electrophoretic methods

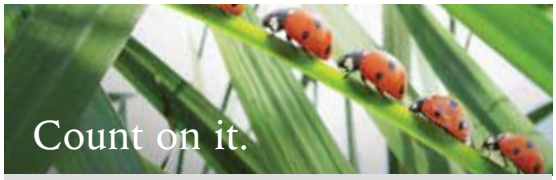


Concentrations of 4 libraries were determined by the NEBNext Library Quant Kit (orange) and compared to values measured using the Aglient Bioanalyzer (blue). Compared to NEBNext's qPCR-based method, the Bioanalyzer concentrations displayed a greater level of variation.

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, 4 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"

PRODUCT	SIZE
NEBNext Library Quant Kit for Illumina	100/500 rxns
NEBNext Library Dilution Buffer	7.5 ml

NEBNext Reagents for RNA Sample Preparation:

KITS FOR ILLUMINA RNA LIBRARY PREPARATION		VWR CAT. NO.	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	103501-464	24 reactions
		103501-462	96 reactions
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	103501-468	24 reactions
		103501-466	96 reactions
	NEBNext Ultra Directional RNA Library Prep Kit for Illumina	102715-920	24 reactions
		102715-918	96 reactions
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	103501-446	24 reactions
		103501-444	96 reactions
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	103501-456	24 reactions
		103501-454	96 reactions
	NEBNext Ultra RNA Library Prep Kit for Illumina	102715-924	24 reactions
		102715-922	96 reactions
Small RNA	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	102500-104	24 reactions
		102500-102	96 reactions
	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	102877-578	24 reactions
		102877-576	96 reactions
	NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	103258-562	96 reactions
	NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	102500-114	24 reactions
		102500-112	96 reactions
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	76293-216	24 reactions
		76293-214	96 reactions
MODULES & ENZYMES		**VWR CAT. NO.**	**SIZE**
RNA	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	102877-568	6 reactions
		102877-566	24 reactions
		102877-570	96 reactions
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	103501-482	6 reactions
		103501-480	24 reactions
		103501-484	96 reactions
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	76377-490	6 reactions
		76377-488	24 reactions
		76377-492	96 reactions
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	76377-496	6 reactions
		76377-494	24 reactions
		76377-498	96 reactions
	NEBNext rRNA Depletion Kit (Bacteria)	76407-334	6 reactions
		76407-332	24 reactions
		76407-336	96 reactions
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	76407-340	6 reactions
		76407-338	24 reactions
		76407-342	96 reactions
	NEBNext Poly(A) mRNA Magnetic Isolation Module	102715-962	24 reactions
		102715-960	96 reactions
	NEBNext Magnesium RNA Fragmentation Module	101710-296	200 reactions
	NEBNext Ultra II RNA First Strand Synthesis Module	102855-138	24 reactions
		102855-140	96 reactions
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	102855-116	24 reactions
		102855-132	96 reactions
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	101640-874	20 reactions
		101640-872	100 reactions
	NEBNext RNA First Strand Synthesis Module	102855-138	24 reactions
		102855-140	96 reactions
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	76293-220	24 reactions
		76293-218	96 reactions
	NEBNext Single Cell Lysis Module	76326-030	96 reactions

NEBNext Reagents for RNA Sample Preparation (cont.):

MODULES & ENZYMES		VWR CAT. NO.	SIZE
DNA	NEBNext Ultra II End Repair/dA-Tailing Module	102969-102	24 reactions
		102969-100	96 reactions
	NEBNext Ultra II Ligation Module	102969-106	24 reactions
		102969-104	96 reactions
	NEBNext Ultra End Repair/dA-Tailing Module	102855-134	24 reactions
		102855-136	96 reactions
	NEBNext Ultra Ligation Module	102855-128	24 reactions
		102855-130	96 reactions
	NEBNext End Repair Module	101640-834	20 reactions
		101640-832	100 reactions
	NEBNext dA-Tailing Module	101640-838	20 reactions
		101640-836	100 reactions
	NEBNext Quick Ligation Module	101640-842	20 reactions
		101640-840	100 reactions
	NEBNext Ultra II Q5 Master Mix	102969-090	50 reactions
		102969-088	250 reactions
	NEBNext Q5 Hot Start HiFi PCR Master Mix	102902-486	50 reactions
		102902-484	250 reactions
	NEBNext High-Fidelity 2X PCR Master Mix	102500-096	50 reactions
		102500-094	250 reactions
ADAPTORS & PRIMERS		**VWR CAT. NO.**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	102500-100	24 reactions
		102500-098	96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	102715-934	24 reactions
		102715-932	96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	103258-544	24 reactions
		103258-564	96 reactions
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	103258-548	24 reactions
		103258-546	96 reactions
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	103218-910	96 reactions
		103218-908	384 reactions
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	102877-580	96 reactions
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	76284-672	96 reactions
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	76284-670	96 reactions
		76284-192	384 reactions
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	76407-330	96 reactions
		76407-328	384 reactions
	NEBNext Adaptor Dilution Buffer	76326-062	96 ml
LIBRARY QUANTITATION		**VWR CAT. NO.**	**SIZE**
	NEBNext Library Quant Kit for Illumina	102942-110	100 reactions
		102942-108	500 reactions
	NEBNext Library Dilution Buffer	102969-094	7.5 ml

ISO 9001 Registered Quality Management

ISO 14001 Registered Environmental Management

ISO 13485 Registered Medical Devices

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0420 Lit. No. 140024W