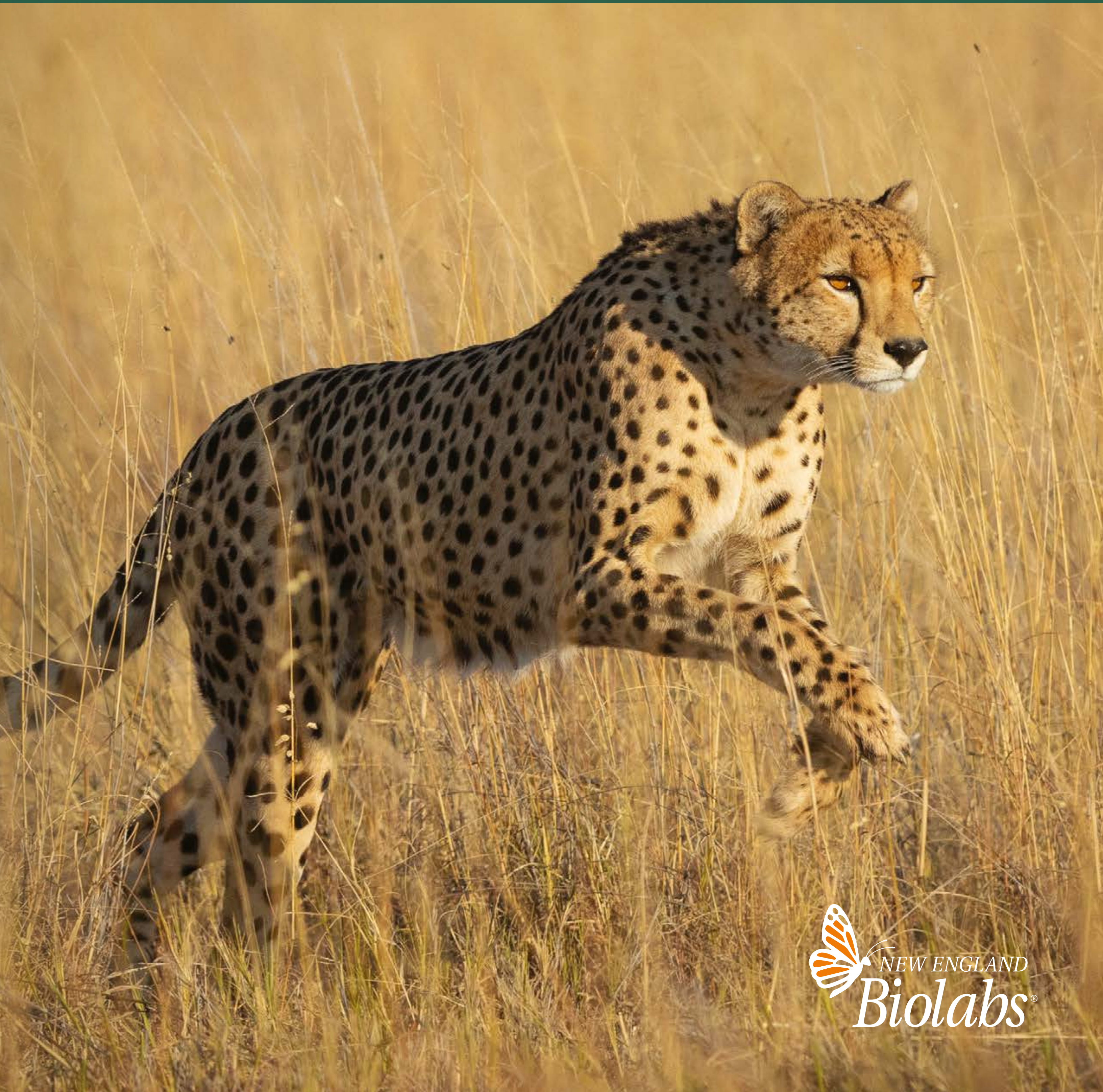
NEBNext UltraExpress® Library Prep Kits

STREAMLINED FOR SPEED



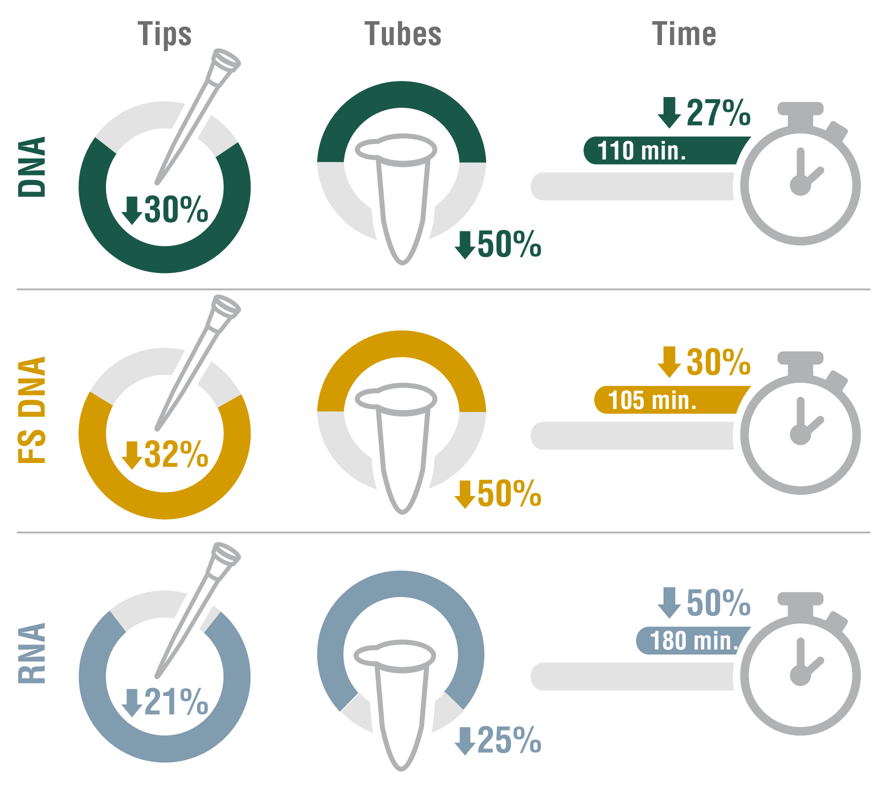
NEW ENGLAND Biolabs®

NEBNext UltraExpress
Streamlined for speed

Sometimes speed is required to set you apart from the pack. The NEBNext UltraExpress DNA and RNA Library Prep Kits have been carefully optimized for speed, while providing the high yields and high quality that you've come to rely on. Each kit has a single-condition protocol, with fixed universal adaptor concentration and number of PCR cycles, for the ultimate in streamlining.

With fewer workflow and cleanup steps and automation friendly transfer volumes, the kits were built for ease of use and automation compatibility. And, they do this all while reducing consumables waste, making your discoveries at the bench greener.

Savings* with NEBNext UltraExpress



** As compared to NEBNext Ultra II*

5 Reasons to choose NEBNext UltraExpress

1. Fast workflow
2. High quality libraries from a wide input range
3. Single protocol for all input amounts
4. Fewer steps, consumables, and cleanups
5. Automation friendly

> *"Protocols designed to save time usually only save 20 - 30 minutes. The NEBNext UltraExpress RNA Library Prep protocol saved us just over an hour in processing time. This is quite significant. We were especially impressed with the new cleanup approach that resulted in squeaky clean libraries."*
>
> ~ Director, Large Sequencing Core

TOOLS & RESOURCES

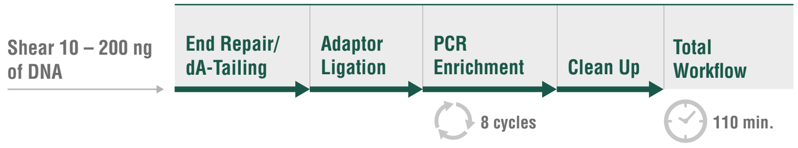
Visit NEBNext.com to find:

- The full list of products available
- Video protocols & Workflow animations
- Online tutorials to help with product selection, general handling tips and more
- Access to **NEBNext Selector Tool,** our online tool for help with selecting the right NEBNext product
- Over 30,000 NEBNext citations
- Protocols & FAQs
- Refer to NEBNext Automation Compatibility Selection Chart for details about successful kit automation

NEBNext UltraExpress DNA Library Prep Kit

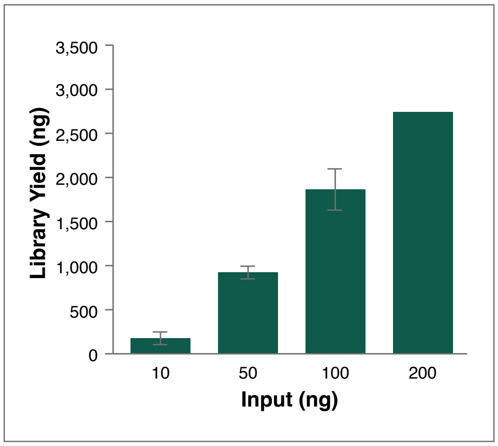
The NEBNext UltraExpress DNA Library Prep Kit features a fast, streamlined workflow that enables the creation of high yield, high quality libraries from a broad input amount range in under 2 hours. The workflow incorporates master mixed reagents, reduced incubation times, and fewer cleanup steps, all conducted in a single tube, which minimizes plastic consumable waste. With simplicity at the forefront, the kit includes a single protocol for all input amounts, making it well-suited for all throughputs.

DNA Library Prep Workflow



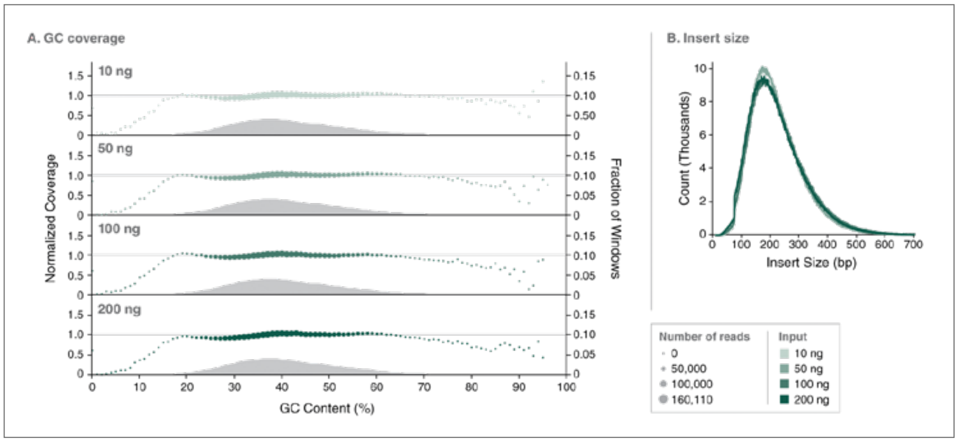
ADVANTAGES

- Fast workflow (< 2 hours)
- Fewer steps and consumables
- Fewer cleanups
- Single protocol for all input amounts
- High quality libraries from a wide input range (10 - 200 ng pre-sheared DNA)
- Single protocol for all input amounts
- Automation friendly



The NEBNext UltraExpress DNA Library Prep Kit provides robust library yields over a wide input range

Libraries were prepared from 10, 50, 100 or 200 ng of Human NA19240 genomic DNA (Coriell Institute for Medical Research) using the same adaptor amount and 8 PCR cycles. Yields exceeded the minimum requirement (40 ng) for a single Ilumina® NovaSeq® 6000 run to achieve whole genome sequencing with at least 30X coverage.



The NEBNext UltraExpress DNA Library Prep Kit produces libraries with uniform GC coverage and insert size from a range of input amounts

Libraries were prepared from 10, 50, 100 or 200 ng of Human NA19240 genomic DNA (Coriell Institute for Medical Research) using the same adaptor amount and 8 PCR cycles. Libraries were pooled and sequenced on an Illumina® MiSeq® (2 x 75 bases). Data showed consistent GC coverage (A) and insert size (B). 2 million paired-end reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1) and mapped to the GRCh38 reference (bowtie2 v2.4.5), and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v 1.56.0). In (A), the horizontal grey line indicates the expected normalized coverage of 1.0, and the dots in shades of green represent read numbers at each GC%. The grey area plot is a histogram representing the distribution of GC content in 100 bp windows of the reference genome.

Need help with product selection?

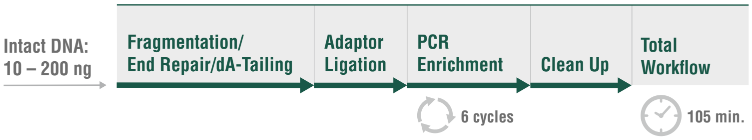
If you need assistance navigating the ever-growing NEBNext product line, NEBNext Selector Tool can help guide your product selection.

Visit **nebnextselector.neb.com**

NEBNext UltraExpress FS DNA Library Prep Kit

The NEBNext UltraExpress FS DNA Library Prep Kit offers a fast, streamlined workflow, including enzymatic fragmentation, end prep, and dA-tailing with a single enzyme mix. In under 2 hours, the protocol enables the creation of high yield, high quality libraries, from a broad input amount range, while generating less plastic waste. Designed for simplicity and efficiency, the kit features a single protocol for all input amounts, making NEBNext UltraExpress FS DNA Library Prep well suited for all throughputs.

FS DNA Library Prep Workflow



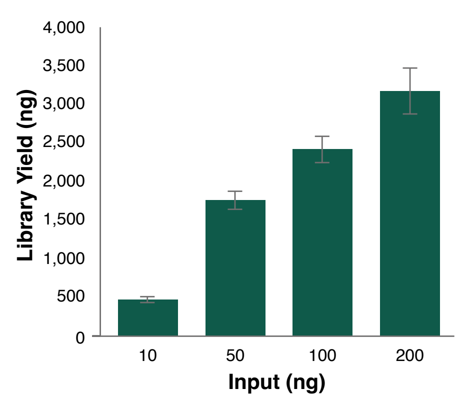
Intact DNA: 10 – 200 ng → **Fragmentation/ End Repair/dA-Tailing** → **Adaptor Ligation** → **PCR Enrichment** → **Clean Up** → **Total Workflow**

6 cycles

105 min.

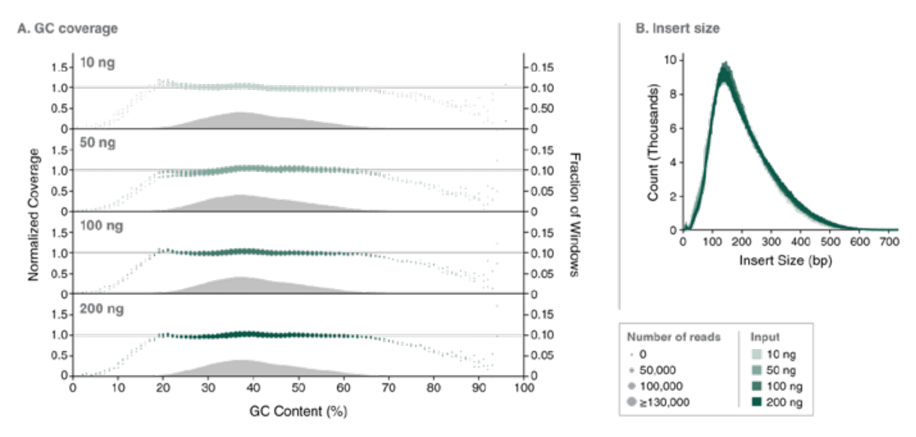
ADVANTAGES

- Fast workflow (< 2 hours), including FS "Fragmentation System" enzymatic fragmentation
- Fewer steps, requiring only a single reaction tube
- Fewer cleanup steps required
- High quality libraries from a wide input range (10 - 200 ng intact DNA)
- Single protocol for all input amounts
- Automation friendly



The NEBNext UltraExpress FS DNA Library Prep Kit provides robust library yields over a wide input range

Libraries were prepared in triplicate from 10, 50, 100 and 200 ng of a 9:1 Human NA19240 genomic DNA (Coriell Institute for Medical Research) and Escherichia coli gDNA (Lofstrand Labs Limited) mixed sample, using the NEBNext UltraExpress FS DNA single-protocol workflow (e.g., same adaptor amount and 6 PCR cycles for all input amounts). Yields exceeded the minimum requirement (40 ng) for a single Illumina® NovaSeq® 6000 run to achieve whole genome sequencing with at least 30X coverage.



The NEBNext UltraExpress FS DNA Library Prep Kit produces libraries with uniform GC coverage and insert size from a range of input amounts

Libraries were prepared from 10, 50, 100 and 200 ng of a 9:1 Human NA19240 genomic DNA (Coriell Institute for Medical Research) and Escherichia coli gDNA (Lofstrand Labs Limited) mixed sample, using the NEBNext UltraExpress FS DNA single-protocol workflow (e.g., same adaptor amount and 6 PCR cycles for all input amounts). Libraries were pooled and sequenced on an Illumina NextSeq® 500/550 (2 x 75 bases). Data showed consistent (A) GC coverage and (B) insert size. 2 million paired-end reads from each library were sampled (seqtk v1.0), adaptor-trimmed (seqprep v0.1) and mapped to a composite reference containing GRCh38 and E. coli MG1655 contigs (bowtie2 v2.5.0). GC coverage and insert size distributions were calculated using Picard's CollectGCBiasMetrics and Picard CollectInsertSizeMetrics (v1.56.0). Picard CollectGCBiasMetrics (v1.56.0) was run on human autosomes only due to the even copy number assumption of the tool. In (A), the horizontal grey line indicates the expected normalized coverage of 1.0, and the dots in shades of green represent read numbers at each GC%. The grey area plot is a histogram representing the distribution of GC content in 100 bp windows of the reference genome.

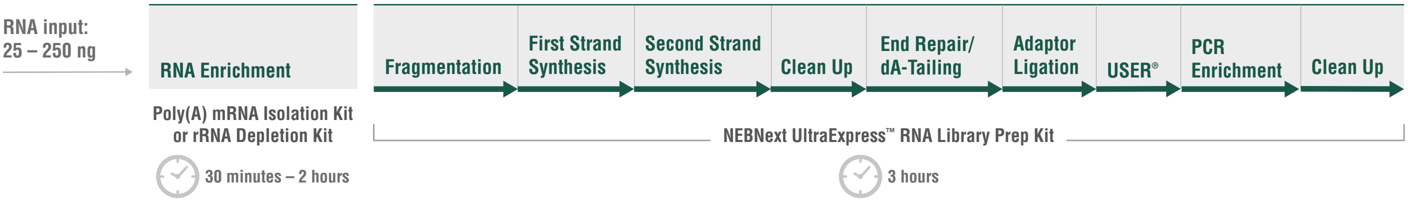
Did you know that many NEB products can be purchased in large volumes and custom formats?

Learn more at **www.neb.com/customizedsolutions**

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.

RNA Library Prep Workflow

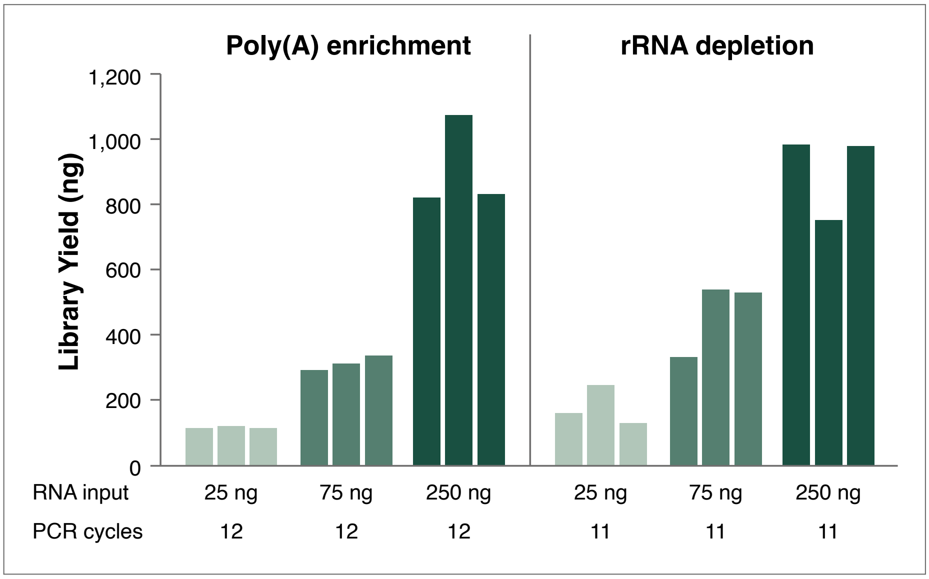


RNA input: 25 – 250 ng → **RNA Enrichment** → **Fragmentation** → **First Strand Synthesis** → **Second Strand Synthesis** → **Clean Up** → **End Repair/ dA-Tailing** → **Adaptor Ligation** → **USER®** → **PCR Enrichment** → **Clean Up**

RNA Enrichment: Poly(A) mRNA Isolation Kit or rRNA Depletion Kit – 30 minutes – 2 hours

Fragmentation to Clean Up: NEBNext UltraExpress™ RNA Library Prep Kit – 3 hours

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.

ADVANTAGES

- Fast workflow (3 hours)
- Fewer steps and consumables
- Fewer cleanups
- Single protocol for all input amounts
- High quality directional libraries from a broad input range: 25 - 250 ng total RNA
- Automation friendly

NEBNext Poly(A) mRNA Magnetic Isolation Module

- Isolation of intact poly(A)+ RNA from 1 - 5 µg of previously isolated total RNA
- Protocol can be adapted for automated high-throughput applications
- Permits elution of intact mRNA in small volumes

! **Fast workflow: streamlined express protocol of only 30 minutes**

Note that for input amounts >5 µg we recommend the NEBNext High Input Poly(A) mRNA Isolation Module.

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

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

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Ordering Information

DNA	NEB #	SIZE
NEBNext UltraExpress DNA Library Prep Kit	E3325S/L	24/96 rxns
NEBNext UltraExpress FS DNA Library Prep Kit	E3340S/L	24/96 rxns
NEBNext Magnetic Separation Rack	S1515S	24 tubes

RNA	NEB #	SIZE
NEBNext UltraExpress RNA Library Prep Kit	E3330S/L	24/96 rxns
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns
NEBNext High Input Poly(A) mRNA Magnetic Isolation Module	E3370S	24 rxns
NEBNext Magnetic Separation Rack	S1515S	24 tubes

NEBNext Multiplex Oligos Selection Chart

The chart simplifies the process of selecting the appropriate indexing primers and adaptors for your sequencing applications. It provides a comprehensive overview of available options, including single, dual, and unique dual indices and addresses specific needs like reducing index hopping and incorporating unique molecular identifiers (UMIs) for enhanced accuracy.

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