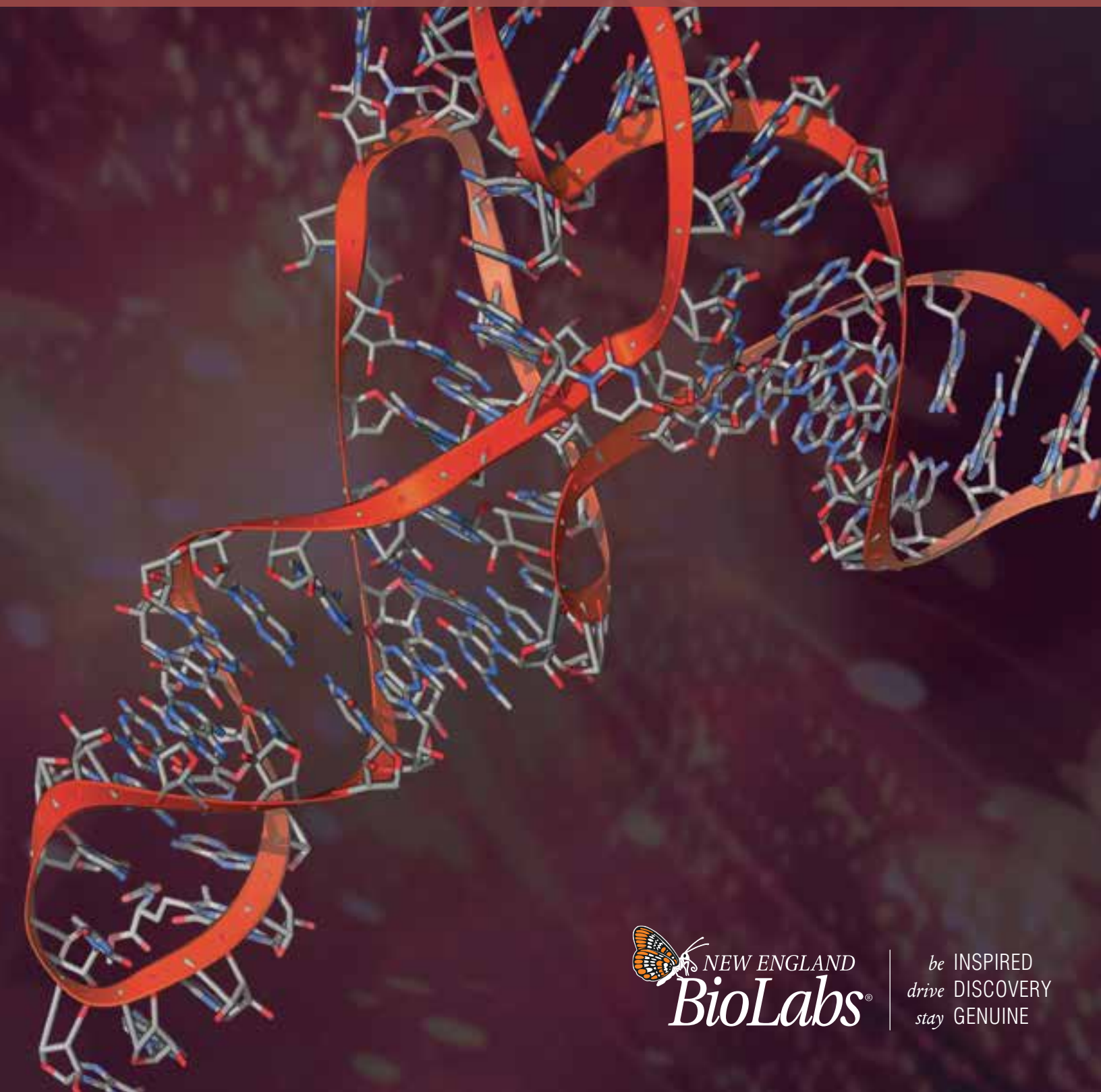
RNA Technical Guide

TOOLS TO STREAMLINE RNA – RELATED WORKFLOWS



NEW ENGLAND BioLabs®

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drive DISCOVERY
stay GENUINE

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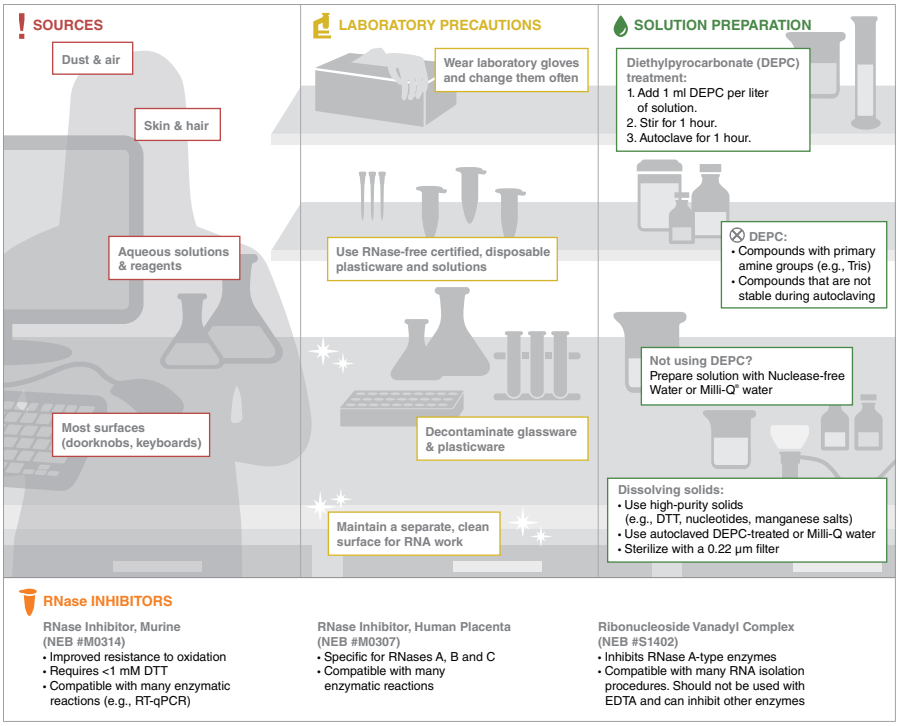
Let NEB Help Streamline Your RNA-related Workflows

Historically, our understanding of the function of RNA in the cell was limited to its role in translation of genetic information from DNA into protein. The major species of RNA described were; (A) messenger RNA (mRNA), which converts DNA into RNA, (B) transfer RNA (tRNA) that is charged with specific amino acids and, (C) ribosomal RNA (rRNA), a major component of the ribosome. More recently, RNA has been implicated in a diverse number of biological processes, including catalysis and transcriptional regulation. Technological advances and improvements in RNA analysis and detection have led to the discovery of many new classes of small and large non-coding RNAs with novel regulatory functions. Examples include, microRNA (miRNA), circular RNA, long non-coding RNA (lncRNA), small nucleolar RNA (snoRNA) and extracullular RNA (exRNA). In addition, RNA modifications have revealed added complexity to RNA. These biologically relevant modifications are an active area of exploration. These findings have helped usher in a renaissance of RNA-focused research in biology.

NEB offers a broad portfolio of reagents for the purification, quantitation, detection, synthesis and manipulation of RNA. Experience improved performance and increased yields, enabled by our expertise in enzymology. Get started today at **NEBrna.com**

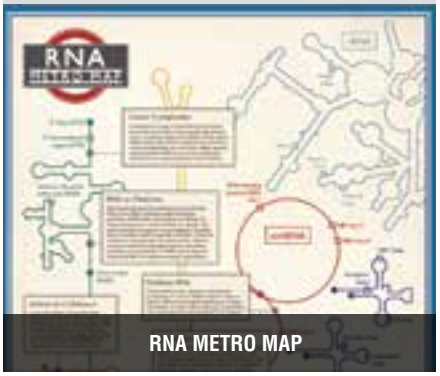
Getting Started: Avoiding RNase Contamination



FEATURED RESOURCES

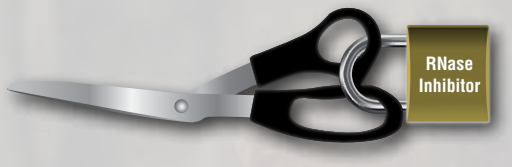
Visit NEBrna.com to find additional resources, including:

- **RNA Synthesis Brochure**
  Learn more about NEB's products for synthesis of RNA, which range from template generation to poly(A) tailing
- **Monarch Nucleic Acid Purification Brochure**
  It's time to transform your DNA and RNA purification experience! Learn about the advantages of choosing Monarch Nucleic Acid Purification Kits from NEB
- **Luna Universal qPCR And RT-qPCR Brochure**
  Make a simpler choice with Luna! Learn about the advantages of choosing Luna products for your qPCR & RT-qPCR, and see how Luna products compare to other commercially available reagents.
- **NEBNext for Illumina Brochure**
  Review NEB's extensive range of NGS sample prep products for RNA, Small RNA and DNA
- **RNA Metro Map**
  Download our RNA poster to learn more about the various RNA structures and recent applications



PRODUCT TIP

Do you want to be on the safe side?
Use NEB's murine RNase Inhibitor!



Schematic visualisation of RNA degradation: (A) RNA Ladder, (B) sample with RNase Inhibitor, (C) sample without RNase Inhibitor, sample has been degraded.

Murine RNase Inhibitor (NEB #M0314)

- Specifically inhibits RNases A, B and C.
- Improved resistance to oxidation compared to human/porcine RNase inhibitors
- Ideal for reactions where low DTT concentrations are required (e.g., Real-time PCR)
- Isolated from a recombinant source
- Tested for the absence of DNases and RNases
- No inhibition of polymerases like e.g. *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3)

RNA Extraction and Purification

Isolating high-quality RNA is crucial to many downstream experiments, such as cloning, reverse transcription for cDNA synthesis, RT-PCR, RT-qPCR and RNA-seq. There are various approaches to RNA purification including phenol-chloroform extraction, spin column purification, and the use of magnetic beads.

Transform your RNA purification experience with Monarch

The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plants, can be processed with additional steps that enhance lysis. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios > 1.8, high RIN values, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq and Northern blot analysis.

Quickly and easily purify up to 100 µg of high quality total RNA from multiple sample types – all with one kit!

- For use with blood, cells and tissues
- Also works with tough to lyse samples (bacteria, yeast, plants)
- Effectively purifies total RNA including small RNA < 200 nt
- Efficient genomic DNA removal (column and DNase I-based)
- Contains Proteinase K for processing of tissues and blood samples
- Includes RNA Protection Reagent for sample preservation
- Excellent value
- Kit components available separately

Request your sample at **NEBMonarch.com**

FEATURED PRODUCT

Monarch Total RNA Miniprep Kit
(NEB #T2010)

SPECIFICATIONS

- **Binding Capacity:** 100 µg RNA
- **RNA Size:** > 20 nt
- **Purity:** $A_{260/280}$ and $A_{260/230}$ usually ≥ 1.8
- **Input Amount:** up to 10^7 or 50 mg tissue*
- **Elution Volume:** 50 – 100 µl
- **Yield:** varies depending on sample type*
- **Compatible downsteam applications:** RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

**View manual for other sample types*

Kit Component	Monarch RNA Purification Kit	Qiagen RNeasy Kits		
		Mini	Protect Mini	Plus Mini
gDNA Removal Columns	✓	×*	×*	✓
DNase I	✓	×	×	×
Proteinase K	✓	×	×	×
RNA Protection Reagent	✓	×	✓	×

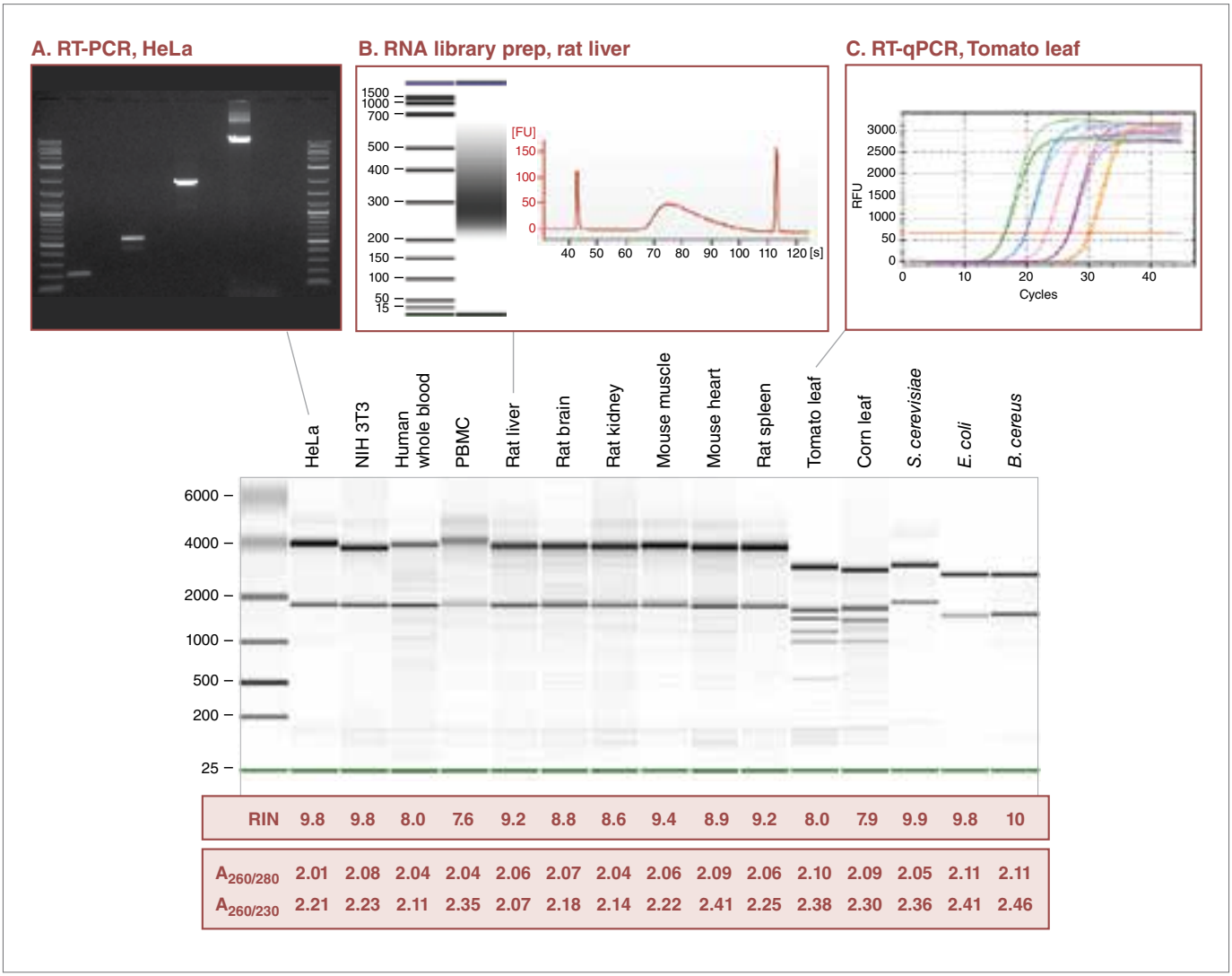
✓ = *Included* **Not included and not sold separately*

Monarch has been validated for the following sample types:

- HeLa Cells
- HEK 293 Cells
- NIH 3T3 Cells
- Human Blood
- Rat Blood
- PBMC's
- Rat Liver
- Rat Spleen
- Rat Kidney
- Rat Brain
- Rat Muscle
- Mouse Muscle
- Mouse Heart
- Mouse Kidney
- *S. cerevisiae*
- *E. coli*
- *B. cereus*
- Corn Leaf
- Tomato Leaf

For information on input amounts, yield, and RIN values, please refer to the product manual at **neb.com/T2010**.

Monarch-purified RNA is high-quality and compatible with a wide variety of downstream applications



RIN	9.8	9.8	8.0	7.6	9.2	8.8	8.6	9.4	8.9	9.2	8.0	7.9	9.9	9.8	10
$A_{260/280}$	2.01	2.08	2.04	2.04	2.06	2.07	2.04	2.06	2.09	2.06	2.10	2.09	2.05	2.11	2.11
$A_{260/230}$	2.21	2.23	2.11	2.35	2.07	2.18	2.14	2.22	2.41	2.25	2.38	2.30	2.36	2.41	2.46

Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit (NEB #T2010). Aliquots were run on an Agilent® Bioanalyzer® 2100 using the Nano 6000 RNA chip (S. cerevisiae RNA was run using a plant Nano assay). RIN values and O.D. ratios confirm the overall integrity and purity of the RNA. To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/– RT) (A) for detection of 4 different RNA species using Protoscript® II Reverse Transcriptase (NEB #M0368)/LongAmp® Taq DNA Polymerase (NEB #M0323), NGS library prep (B) using NEBNext Ultra II RNA Library Prep Kit (NEB #E7760) and RT-qPCR (C) using Luna One-Step RT-qPCR Reagents (NEB #E3005).

TIPS FOR SUCCESSFUL RNA EXTRACTIONS

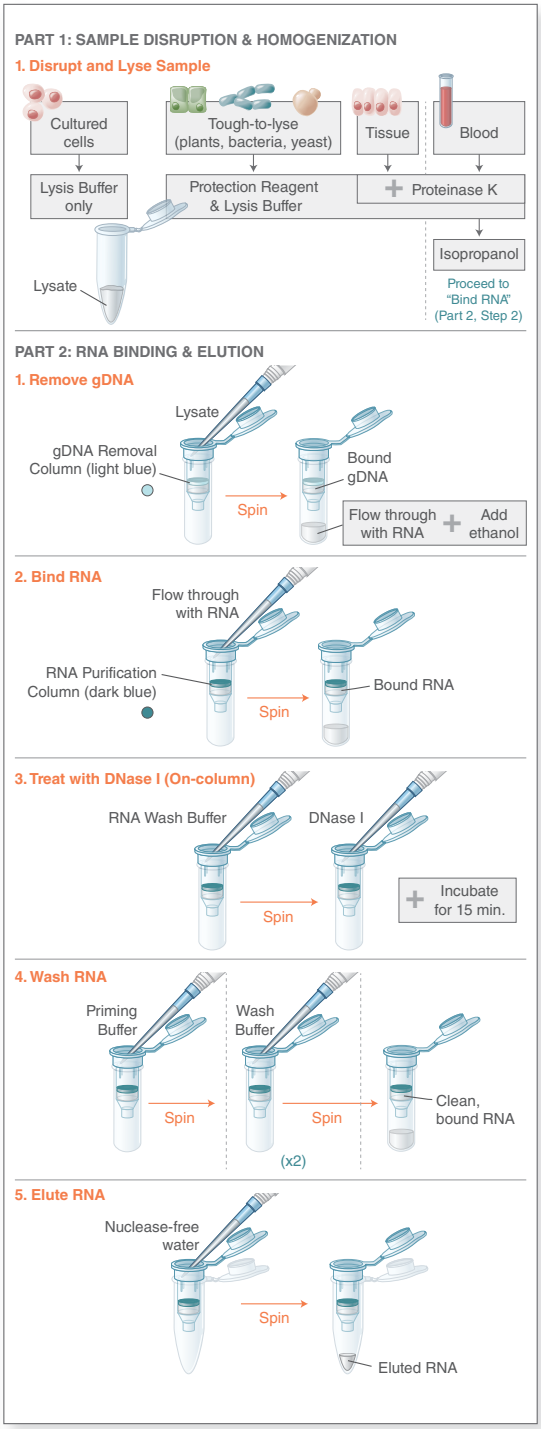
1. **Prevent RNase activity:** Nucleases in your sample will degrade your RNA, so inhibiting their activity is essential. Process samples quickly after harvest, use preservation reagents, and always ensure nuclease-free working environments.
2. **Do not exceed recommended input amounts:** Buffer volumes are optimized for the recommended input amounts. Exceeding these can result in inefficient lysis and can also clog the column. For guidance on sample input amounts, visit **neb.com/MonarchRNAinputs**
3. **Ensure samples are properly homogenized/disrupted:** Samples should be disrupted and homogenized completely to release all RNA.
4. **For sensitive applications, ensure proper gDNA removal:** gDNA removal is removed by the gDNA removal column and subsequent on-column DNase I treatment. Off-column DNase I treatment can also be employed.

For additional tips, including guidelines for RNA purification from various sample types, click the other tools and resources tab at **www.neb.com/T2010**

Guidance on Choosing Sample Input Amounts when Using the Monarch Total RNA Miniprep Kit

RNA yield, purity, and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples. It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

Sample type		Input	Avg. yield (µg)	Observed RIN	Maximum starting material
Cultured cells					
HeLa		1 × 10^6 Cells	12-15	9-10	1 × 10^7 Cells
HEK 293		1 × 10^6 Cells	12-14	9-10	1 × 10^7 Cells
NIH 3T3		1 × 10^6 Cells	8-12	9-10	1 × 10^7 Cells
Mammalian Blood					
Human	Fresh	200 µl	0.5-1.0	7-8	3 ml
	Frozen	200 µl	0.5-1.0	7-8	3 ml
	Stabilized	200 µl	0.5-1.0	7-8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml
Blood cells					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 × 10^7 Cells
Tissue					
Rat liver	Frozen, pulverized	10 mg	25	8-9	20 mg
	Stabilized solid	10 mg	50+60	8-9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40+50	9	20 mg
Rat kidney (Frozen pulverized)		10 mg	7-10	9	50 mg
Rat brain	Frozen, pulverized	10 mg	2-3	8-9	50 mg
	Stabilized solid	10 mg	0.5-1.5	8-9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5-8	8-9	50 mg
Rat muscle (Frozen pulverized)		10 mg	2.3	8-9	50 mg
Mouse muscle	Frozen, pulverized	10 mg	3	8-9	50 mg
	Frozen, pulverized with bead homogenizer	10 mg	5	7-8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8-10	9	50 mg
Mouse heart (stabilized w/bead homogenizer)		10 mg	5-6	8-9	50 mg
Yeast					
S. cervisiae	Frozen with bead homogenizer	1 × 10^7 Cells	50	9-10	5 × 10^7 Cells
	Fresh with Zymolyase®	1 × 10^7 Cells	60	9	5 × 10^7 Cells
Bacteria					
E. coli	Frozen	1 × 10^9 Cells	5	10	1 × 10^9 Cells
	Frozen with bead homogenizer	1 × 10^9 Cells	10	10	1 × 10^9 Cells
	Frozen with lysozyme	1 × 10^9 Cells	70	10	1 × 10^9 Cells
B. cereus	Frozen with lysozyme	1 × 10^8 Cells	20-30	9	1 × 10^9 Cells
	Frozen with bead homogenizer	1 × 10^8 Cells	8	9-10	1 × 10^9 Cells
Plant					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg



S. cerevisiae total RNA was run on an Agilent® Nano 6000 Chip using plant assay. Zymolyase® is a registered trademark of Kirin Brewery Co Ltd. Agilent® is a registered trademark of Agilent Technologies.

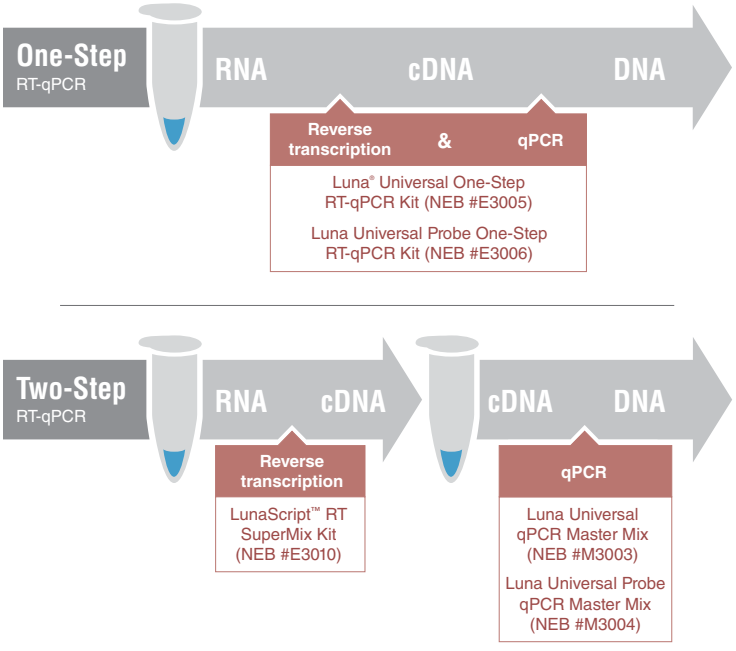
qPCR and RT-qPCR

Quantitative PCR (qPCR) uses real-time fluorescence to measure the quantity of DNA present at each cycle during a PCR. A wide variety of approaches have been developed for generating a fluorescent signal, the most common of which use either hydrolysis probes (e.g., TaqMan®), or a double-stranded DNA binding dye, (e.g., SYBR® Green). qPCR can be modified to detect and quantitate RNA by adding a reverse transcriptase (RT) step upstream of the qPCR assay to generate cDNA (i.e., RT-qPCR). Reverse transcription can be performed separately from qPCR or directly in the qPCR mix (i.e., one-step RT-qPCR). One-step workflows are commonly favored in molecular diagnostic assays and where sample inputs may be limiting. Separate cDNA synthesis followed by qPCR (i.e., two-step RT-qPCR) is preferred when multiple interrogations will be made of the same starting material or where archiving of cDNA may be required.

Lighting the way with Luna

Luna products from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. All Luna products provide robust performance on diverse sample sources and target types.

Find the right Luna product for your application



Doing one-step RT-qPCR?

The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

FEATURED PRODUCT

Luna Universal qPCR Master Mix (NEB #M3003)

Luna Universal Probe qPCR Master Mix (NEB #M3004)

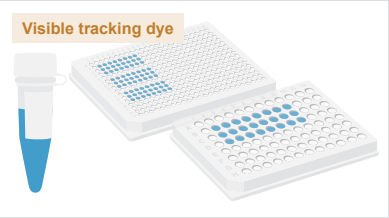
Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)

Luna Universal One-Step RT-qPCR Kit (NEB #E3005)

LunaScript™ RT SuperMix Kit (NEB #E3010)

Make a Simpler Choice

- One product per application simplifies selection
- Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors

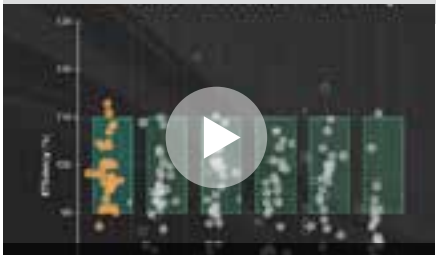


Experience Best-in-class Performance

- All Luna products have undergone rigorous testing to optimize specificity, sensitivity, accuracy and reproducibility
- Products perform consistently across a wide variety of sample sources
- A comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents demonstrates superior performance of Luna products

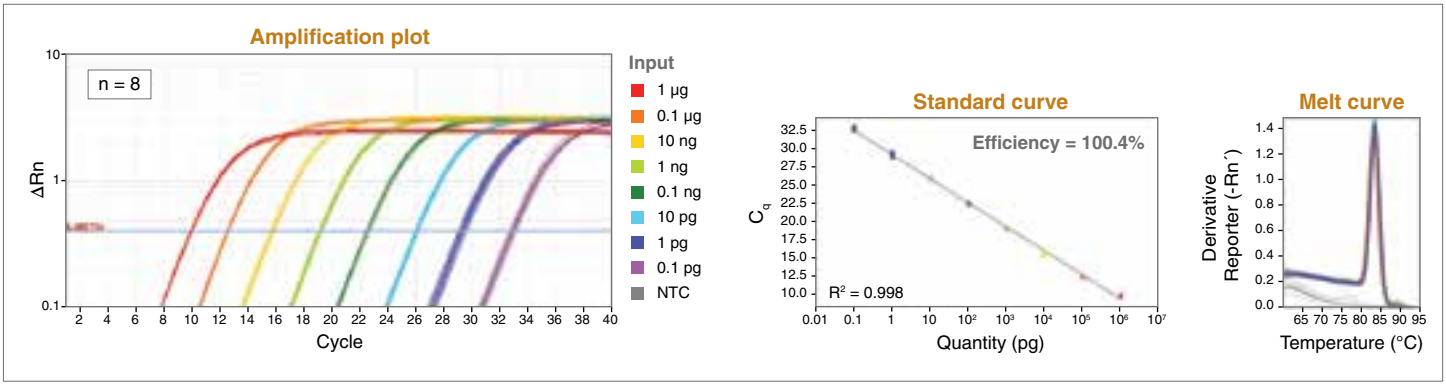
Optimize your RT-qPCR: Luna WarmStart® Reverse Transcriptase or LunaScript™ RT SuperMix Kit

- Employs novel, thermostable Reverse Transcriptases (RT) for fast protocols at elevated temperatures, exceptional robustness and sensitivity
- One-Step Kits: the unique WarmStart RT paired with Hot Start Taq increases reaction specificity and robustness
- Two-Step: the convenient LunaScript RT SuperMix Kit optimized for best-in-class two-step protocols includes dNTPs, primers and RNase Inhibitor



"DOTS IN BOXES" VISUALIZATION OF DATA

Luna WarmStart Reverse Transcriptase exhibits exceptional sensitivity, reproducibility and RT-qPCR performance



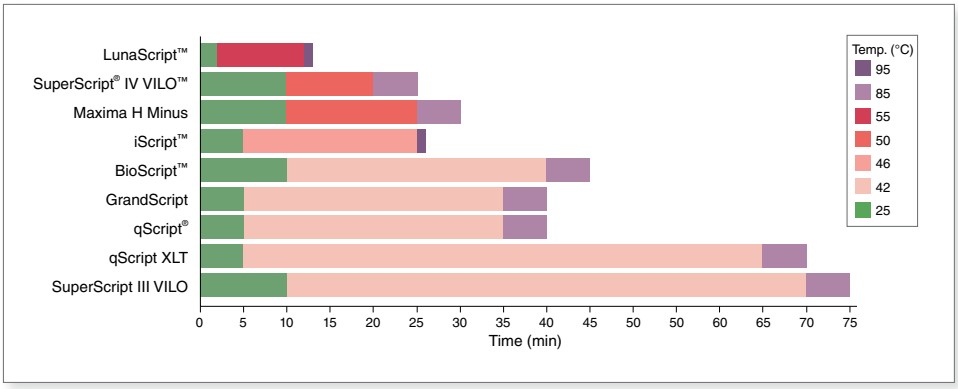
Doing two-step RT-qPCR?

The LunaScript RT SuperMix Kit is ideal for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. The latest edition to the Luna portfolio, LunaScript, delivers best-in-class performance, user-friendly protocols, and includes a convenient blue dye to track your sample throughout the RT-qPCR workflow. cDNA products generated by LunaScript have been extensively evaluated in qPCR using the Luna Universal qPCR Master Mix (NEB #M3003) and Luna Universal Probe qPCR Master Mix (NEB #M3004). In combination, these products provide a two-step RT-qPCR workflow with excellent sensitivity and accurate, linear quantitations.

ADVANTAGES

- Simplify reaction setup with convenient Single-tube SuperMix format
- Contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and unique in silico designed Luna® Reverse Transcriptase
- Synthesize cDNA in less than 15 minutes at elevated temperatures (up to 65°C)
- Eliminate pipetting errors with non-interfering, visible tracking dye
- Experience best-in-class performance, as all Luna products have undergone rigorous testing to optimize specificity, sensitivity, accuracy and reproducibility
- Enjoy consistent linearity, sensitivity, and capacity for reliable RNA quantification

The LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol at elevated temperatures



Comparison of recommended protocols for cDNA synthesis. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures (default 55° C, up to 65° C), reducing complications from virtually any RNA secondary structure.

LOOKING FOR REVERSE TRANSCRIPTASES (RTs) FOR OTHER APPLICATIONS?

NEB offers several RTs, including ProtoScript II Reverse Transcriptase (NEB #M0368) and WarmStart RTx Reverse Transcriptase (NEB #M0380), which can be used in isothermal amplification (e.g., LAMP). See page 18 for the full list of RTs available.

Visit **LUNAqPCR.com** to request a sample.

Optimization Tips for RT-qPCR with Luna

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**.
The following tips can be used to help optimize your One-Step RT-qPCR.

TARGET SELECTION

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA TEMPLATE

- Use high quality, purified RNA templates whenever possible. Luna qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

PRIMERS

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's Tm calculator. (**Tmcalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

HYDROLYSIS PROBES

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

MULTIPLEXING

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

REVERSE TRANSCRIPTION

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

CYCLING CONDITIONS

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

REACTION SETUP

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

ASSAY PERFORMANCE

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template.
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

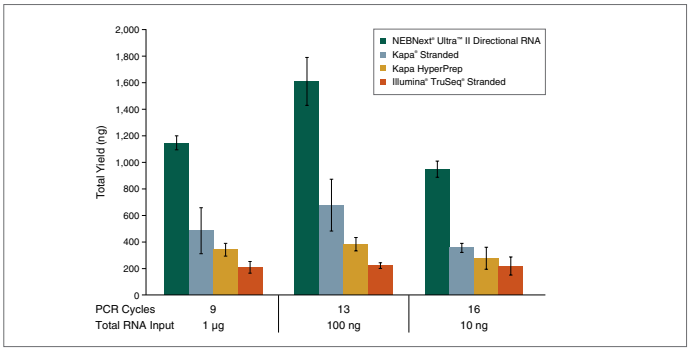
RNA-Seq

Next generation sequencing (NGS) can be used to determine the presence and quantity of RNA species in a sample, enabling sensitive and accurate gene expression analysis. For the Illumina sequencing platform, mRNA libraries are prepared by removal of ribosomal RNA, then cDNA synthesis followed by DNA Library preparation steps: end repair, addition of a non-templated dA overhang, adaptor ligation, and PCR amplification.

Get even more from less with NEBNext Ultra II

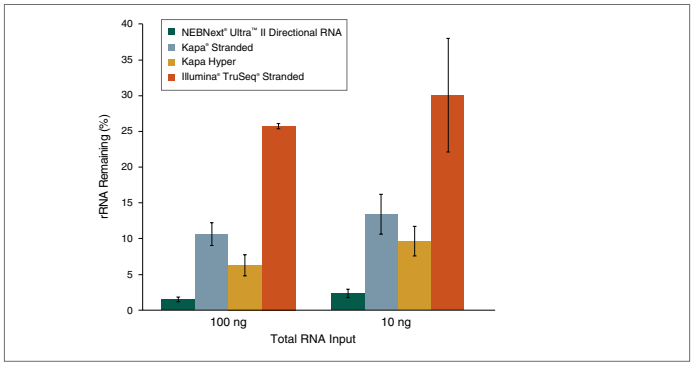
NEBNext Ultra II RNA Library Prep Kits for Illumina are available for both directional and non-directional (non-strand-specific) RNA library construction, and deliver significantly increased sensitivity and specificity from your RNA-seq experiments, from ever-decreasing amounts of input RNA. In conjunction with ribosomal RNA (rRNA) depletion or poly(A) mRNA enrichment, the kits enable the production of high quality libraries from 5 ng or 10 ng of Total RNA, respectively, up to 1 µg.

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



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

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



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

FEATURED PRODUCTS

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760)

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (NEB #E7765)

NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770)

NEBNext Ultra II RNA Library Prep with Sample Purification Beads (NEB #E7775)

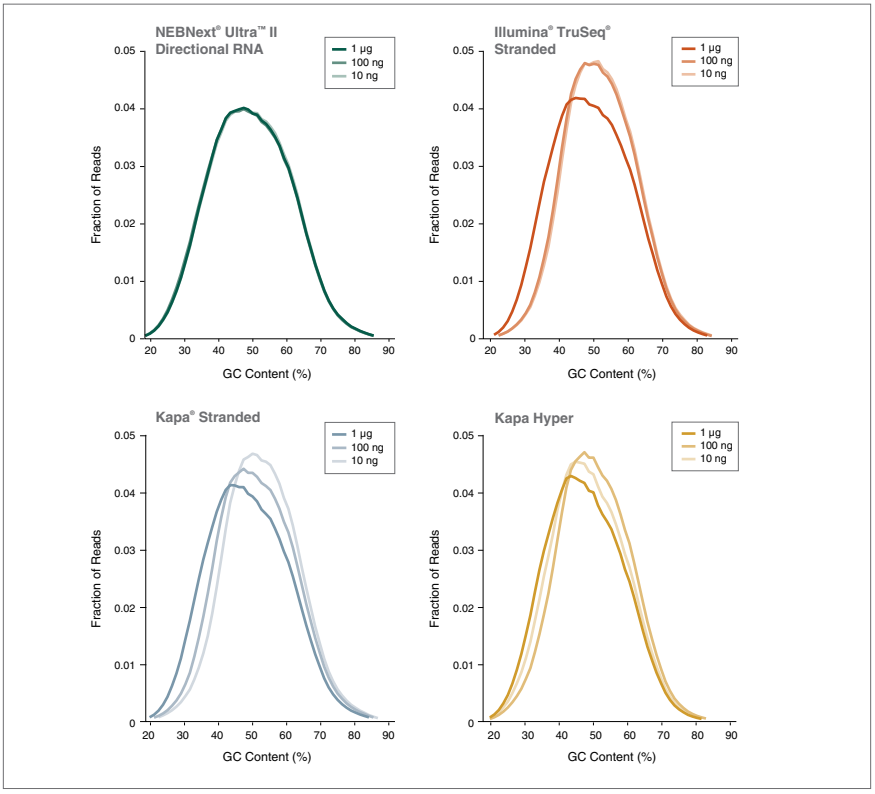
NEBNext rRNA Depletion Kit (Human/Mouse/Rat (NEB #E6310) with RNA Sample Purification Beads (NEB #E6350)

NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)

- Get more of what you need, with the **highest library yields**
- Generate **high quality libraries** even when you have only limited amounts of input RNA
  - 10 ng – 1 µg Total RNA (polyA mRNA workflow)
  - 5 ng – 1 µg Total RNA (rRNA depletion workflow)
- **Minimize bias**, with fewer PCR cycles required
- Increase the **complexity and transcript coverage** of your libraries
- **Save time** with streamlined workflows, reduced hands-on time, and automation compatibility
- Rely on **robust performance**, even with low quality RNA, including FFPE
- Compatible with NEBNext poly(A) mRNA isolation, rRNA depletion reagents and multiplexing adaptors and primers
- Use our NEBNext selector tool at **NEBNextSelector.neb.com** for help with selecting the right NEBNext product for your needs

Visit **NEBNext.com** to see if you qualify for a free sample.

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



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

SEQUENCING SMALL RNAs?

The unique workflow of the NEBNext Small RNA library prep kits addresses the challenge of minimization of adaptor-dimers while achieving production of high-yield diverse multiplex libraries in a simple protocol.

- Minimized adaptor-dimer formation
- High yields
- Input can be total RNA
- Suitable for methylated small RNA's (e.g. RNAs as well as unmethylated small RNAs)
- 48 Indices available
  - **NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) (NEB #E7770)**
  - **NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2) (NEB #E7580)**
  - **NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1–48) (NEB #E7560)**
  - **NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)**
- Visit **NEBNext.com** for more information

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® or similar instrumentation. The RNA sample should have a RIN value higher than 7.
- Integrity and size distribution of total RNA can be checked by electrophoresis on a denaturing agarose gel and staining with ethidium bromide. The ribosomal RNA bands should appear as sharp bands on the stained gel. For eukaryotic samples, intact total RNA will have sharp, clear bands corresponding to 28S and 18S. The 28S rRNA band should be approximately twice as intense as the 18S rRNA band. This 2:1 ratio (28S:18S) is a good indication that the RNA is completely intact. Partially degraded RNA will have a smeared appearance, will lack the sharp rRNA bands, or will not exhibit the 2:1 ratio of high quality RNA. Completely degraded RNA will appear as a very low molecular weight smear.
- 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 on a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. 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.

RNA Synthesis

In vitro synthesis of single-stranded RNA molecules is a widely used laboratory procedure that is critical to RNA research, as well as to RNA biopharmaceuticals. This technique is versatile in that it allows the researcher to tailor synthesis and introduce modifications to produce a transcript. Downstream applications include biochemical and molecular characterization of RNA for RNA-protein interactions and structural analyses, generation of RNA aptamers, synthesis of functional mRNAs for expression, and generation of small RNAs for alteration of gene expression (e.g., guide RNAs, RNAi). Furthermore, the use of *in vitro* synthesized RNA has been instrumental in the development of RNA vaccines and CRISPR/Cas9 genome editing tools, generation of pluripotent stem cells, screening of RNA inhibitors, as well as development of RNA amplification-based diagnostics.

High-yield robust reactions require optimization of each reaction component. NEB offers five *in vitro* RNA synthesis kits, all of which have been optimized to generate reproducible yields of quality RNA. Additionally, individual components can be purchased for *in vitro* transcription (IVT) and mRNA capping.

For more information on products available for RNA synthesis, visit **NEBrna.com** and download our RNA Synthesis Brochure

mRNA synthesis workflow example & available NEB products

Template Generation	*In vitro* Transcription	RNA Capping	Poly(A) Tailing
Q5® High-Fidelity DNA Polymerase	HiScribe T7 ARCA mRNA Synthesis Kit (with tailing)		
dNTP solution mixes	HiScribe T7 ARCA mRNA Synthesis Kit		*E. coli* Poly(A) Polymerase
Type IIs restriction enzymes & cloning reagents	HiScribe T7 High Yield RNA Synthesis Kit	Vaccinia Capping System	
	HiScribe T7 Quick High Yield RNA Synthesis Kit	mRNA Cap 2´-O-Methyltransferase	
	HiScribe SP6 RNA Synthesis Kit	ARCA and other mRNA cap analogs	
	T3, T7 and SP6 RNA Polymerases		

Companion Products

- RNase inhibitors
- Pyrophosphatases
- DNase I
- NTPs

Generate Microgram Quantities of RNA with HiScribe

NEB offers a selection of HiScribe RNA Synthesis Kits for the generation of high yields of high quality RNA, that can be used in a wide variety of applications. Use the chart below to determine which HiScribe Kit will work best for you.

		T7 KITS				SP6 KITS
	APPLICATION	**HISCRIBE T7 HIGH YIELD RNA SYNTHESIS KIT** (#E2040)	**HISCRIBE T7 QUICK HIGH YIELD RNA SYNTHESIS KIT** (#E2050)	**HISCRIBE T7 ARCA mRNA KIT** (#E2065)	**HISCRIBE T7 ARCA mRNA (WITH TAILING)** (#E2060)	**HISCRIBE SP6 RNA SYNTHESIS KIT** (#E2070)
Probe labeling	Fluorescent labeling: FAM, Cyanine (Cy) dyes, etc. • Fluorescent *in situ* hybridization (FISH)		✓			✓
	Non-fluorescent labeling: Biotin, Digoxigenin • *In situ* hybridization • Blot hybridization with secondary detection • Microarray		✓			✓
	High specific activity radiolabeling • Blot hybridization • RNase protection	✓				✓
mRNA & RNA for transfection	Streamlined mRNA synthesis with ARCA co-transcriptional capping and enzymatic poly(A) tailing • Transfection • Microinjection • *In vitro* translation				✓	✓
	Streamlined ARCA capped RNA synthesis • Template encoded poly(A) tails • Non polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation			✓		✓
	Co-transcriptional capping with alternate cap analogs • Transfection • Microinjection • *In vitro* translation		✓			✓
	Post-transcriptional capping with Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	✓	✓			✓
	Complete substitution of NTPs: 5-mC, pseudouridine, etc. • Induction of stem cell pluripotency • Modulation of cell fate or phenotype • Post translational capping with Vaccinia mRNA Capping System	✓				✓
	Partial substitution of NTPs: 5-mC, pseudouridine, etc.		✓	✓	✓	✓
	Unmodified RNA		✓			✓
	Hairpins, short RNA, dsRNA • Gene knockdown		✓			✓
Structure, function, & binding studies	Complete substitution of NTPs • Aptamer selection • Isotopic labeling	✓				✓
	Partial substitution of one or more NTPs • Aptamer selection • Structure determination		✓			✓
	Unmodified RNA • SELEX • Structure determination		✓			✓

Generating Guide RNA for CRISPR/Cas9 Experiments

Cas nucleases are central components of CRISPR-based immunity, a mechanism used to protect a bacterial or archaeal cell from invading viral and foreign DNA. CRISPRs (clustered regularly interspaced short palindromic repeats) are DNA loci that contain multiple, short, repeated sequences, separated by unique "spacer DNA". The CRISPR locus is transcribed and processed into short guide RNAs (gRNAs) that are incorporated into Cas nuclease. The RNA corresponding to the spacer DNA guides the Cas nuclease to its target by complementary base pairing; double-stranded DNA cleavage results.

Cas nucleases have been adapted for use in genome engineering because they can easily be programmed for target specificity by supplying gRNAs of any sequence. In cells and animals, genome targeting is performed by expressing nucleases and gRNA from DNA constructs (plasmid or virus), supplying RNA encoding Cas nuclease and gRNA, or by introducing RNA-programmed Cas nuclease directly.

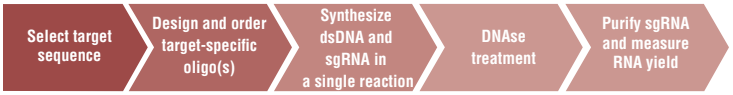
The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

FEATURED PRODUCT

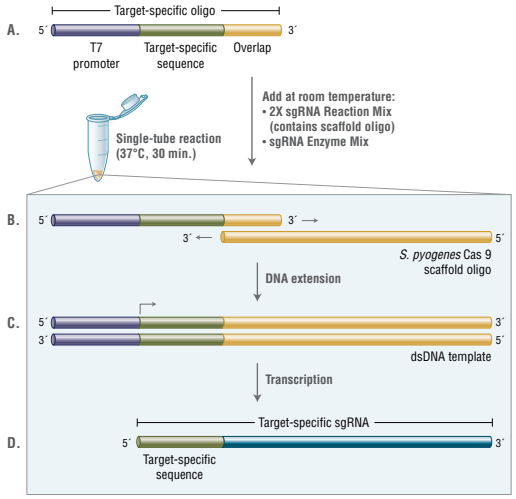
EnGen sgRNA Synthesis Kit (NEB #E3322)

- Reduce protocol time with single-reaction format
- Generate up to 25 µg of sgRNA
- Facilitate troubleshooting with included control oligo provided
- Save money with reduced cost per reaction

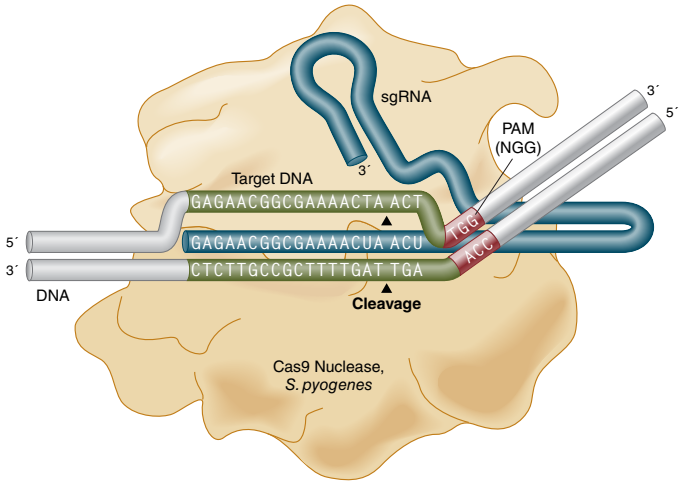
General workflow for the EnGen sgRNA Synthesis Kit, *S. pyogenes*.



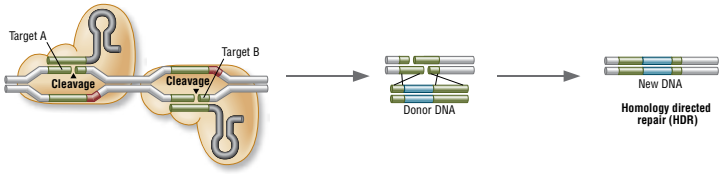
sgRNA Synthesis with EnGen is complete in less than one hour



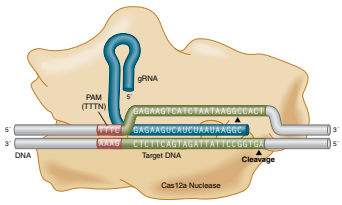
Schematic representation of Cas9 Nuclease, *S. pyogenes* recognition and DNA cleavage



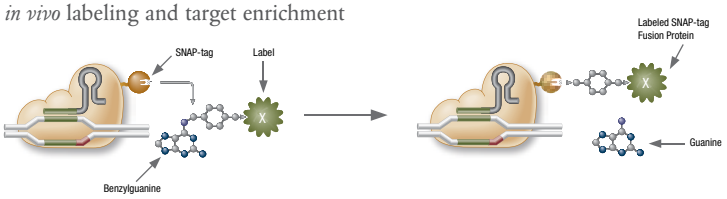
EnGen Spy Cas9 Nickase
increased specificity homology directed repair, dual guide sequence



EnGen Lba Cas12a ("Cpf1")
AT-rich PAM, expanded temperature range



EnGen Spy dCas9 (SNAP-tag)
in vivo labeling and target enrichment



Interested in Designing Your Own Workflows?

NEBs broad portfolio of enzyme specificities and reagents enables development of creative workflows for your RNA research. For example, RNA can be modified with enzymes and reagents that act selectively depending on the existence of particular structures, and hence are useful tools for characterizing RNA species. The selective properties of RNA modifying enzymes enable researchers to distinguish different RNA species. Some unique end modifications can be used to selectively degrade or isolate particular RNA species when treated sequentially with the appropriate series of enzymes. For instance, 5´-capped RNAs are not substrates for polynucleotide kinases, or RNA ligases, but are substrates for decapping enzymes. Similarly, ligation and poly-adenylation of RNA 3´ end requires a free 3´ -OH.

Featured product: RNA ligases

NEB offers a wide selection of ligases with varying activities to support a variety of applications. Use the selection chart to choose the best RNA ligase for your needs.

	RNA LIGASES							
	T4 RNA LIGASE 1 (#M0204)	**T4 RNA LIGASE 2** (#M0239)	**T4 RNA LIGASE 2 TRUNCATED** (#M0242)	**T4 RNA LIGASE 2, TRUNCATED K227Q** (#M0351)	**T4 RNA LIGASE 2 TRUNCATED KQ** (#M0373)	**THERMO-STABLE 5´ APP DNA/RNA LIGASE** (#M0319)	**5´ ADENYLATION KIT** (#E2610)	**RtcB LIGASE** (#M0458)
RNA APPLICATIONS								
Nicks in dsRNA		✓✓✓						
Labeling of 3´ Termini of RNA	✓✓✓		✓	✓	✓	✓		
Ligation of ssRNA to ssRNA	✓✓✓							
Ligation of Preadenylated Adaptors to RNA	✓✓		✓✓	✓✓	✓✓✓	✓✓		
5´ Adenylation							✓✓✓	
Ligation of 3´ P and 5´ OH of ssRNA								✓✓✓
DNA APPLICATIONS								
Ligation of Preadenylated Adaptors to ssDNA						✓✓✓		
DNA/RNA APPLICATIONS								
Joining of RNA and DNA in a ds-structure		✓✓						
Ligation of RNA and DNA with 3´ P and 5´ OH								✓✓
NGS APPLICATIONS								
NGS Library Prep dsDNA-dsDNA (Ligation)								
NGS Library Prep ssRNA-ssDNA (Ligation)	▲		▲	▲	▲	▲		
NGS Library Prep ssRNA-ds-Adaptor Splinted Ligation		▲						
FEATURES								
Thermostable						●	●	
Recombinant	●	●	●	●	●	●	●	●

KEY			
✓✓✓ Optimal, recommended ligase for selected application	✓✓ Works well for selected application	✓ Will perform selected application, but is not recommended	▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

Featured protocol: Cappable-seq

Cappable-seq is a method for directly enriching the 5´ end of primary transcripts developed at NEB. This is achieved by capping the 5´ triphosphorylated end of RNA with the Vaccinia Capping System (NEB #M2080) and 3´-Desthiobiotin-GTP (NEB #N0761). The primary transcripts are enriched by binding to Hydrophilic Streptavidin Magnetic Beads (NEB #S1421), followed by washing and eluting with biotin. This method enables determination of transcription start sites at single base resolution (1).

1. Ettwiller, L. et al. (2016). *BMC Genomics.* 17, 199.

FEATURED PRODUCT

Vaccinia Capping System (NEB #M2080)

3´ -Desthiobiotin-GTP (NEB #N0761)

Hydrophilic Streptavidin Magnetic Beads (NEB #S1421)

PROTOCOL: CAPPABLE-SEQ FOR PROKARYOTIC TRANSCRIPTION START SITE (TSS) DETERMINATION

DESTHIOBIOTIN-GTP CAPPING OF PROKARYOTIC RNA:

1. Prepare total RNA from prokaryotic source at a concentration of 300 ng/µl in water or 1.0 mM Tris pH 7.5, 0.1 mM EDTA.

2. Prepare the capping reaction as follows:

COMPONENT	REACTION
RNA	10 µl
10X VCE Buffer	5 µl
ddH_2O	25 µl
Total	**40 µl**

3. Incubate reaction for 2 minutes at 70°C.

4. Place the reaction on ice,

5. Add the following to above reaction:

COMPONENT	REACTION
5 mM 3'DTB-GTP	5 µl
Vaccinia Capping Enzyme (10 units/µl)	5 µl
Total	**50 µl**

6. Incubate the reaction at 37°C for 30 minutes. Immediately proceed to cleanup.

Note: We have recently found that capping with DTB-GTP is more efficient in the absence of S-Adenosylmethionine (SAM).

RNA CLEANUP:

1. Purify RNA on a Zymo Research's Clean and Concentrator™-5 column using manufacturer instructions for > 200 nucleotide RNA; with a total of 4 washes with RNA wash buffer. *(Note: Wash the sides of the column that may have come into contact with the capping reaction, reducing the carryover of DTB-GTP.)*

2. Elute the RNA in 100 µl of 1 mM Tris pH 7.5, 0.1 mM EDTA (low TE).

Note: it is essential to completely remove unincorporated DTB-GTP (less than 0.01% DTB-GTP remaining). Alternative methods of RNA cleanup such as AMPure beads can also be employed. If the RNA is going to be directly bound to streptavidin before the following fragmentation step, an additional cleanup step should first be employed.

ENRICHMENT OF RNA:

1. Fragment desthiobiotin-GTP-capped RNA by setting up the following reaction:

COMPONENT	REACTION
10X Polynucleotide Kinase Buffer	2.5 µl
Capped RNA	100 µl

2. Incubate for 5 minutes at 94°C. Put on ice.

3. Clean up RNA as follows:

A) Bind RNA to AMPure XP Beads:
Add 1.8 volumes of AMPure beads to the eluted RNA volume and add 1.5 volumes of 100% ethanol to the resulting volume of the AMPure/RNA mix (i.e., if volume of RNA is 100 µl, add 180 µl of AMPure beads and 420 µl of ethanol). Incubate the beads on the bench for 5 minutes, then expose to a magnet and wash beads 2 times with 80% ethanol while confined with the magnet. Remove the tube from the magnet and elute the RNA in 75 µl of low TE.

B) Remove 3´ phosphates from fragmented RNA:

To 75 µl of the eluted RNA, add the following:

COMPONENT	REACTION
10X T4 Polynucleotide Buffer	8.8 µl
ATP-free T4 Polynucleotide Kinase	4 µl
Total	**87.8 µl**

Incubate the reaction at 37°C for 15 minutes. Directly proceed to streptavidin enrichment.

C) 1st round of streptavidin enrichment:
Hydrophilic Streptavidin Magnetic Beads (NEB #S1421) are prepared by washing 2 times with 400 µl of 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA and 2 times with 400 µl of 10 mM Tris-HCl pH 7.5, 500 mM NaCl, 1 mM EDTA and suspended in their original suspension concentration of 4 mg/ml in the 500 mM NaCl wash buffer. Add the DTB-GTP capped RNA from Step B to 30 µl of the prewashed streptavidin beads and incubate at room temperature with occasional resuspension for 20 minutes. Wash the beads 2 times with 200 µl of 10 mM Tris-HCl pH 7.5, 500 mM NaCl, 1 mM EDTA, and 2 times with 200 µl of 10 mM Tris-HCl pH 7.5, 50 mM NaCl and 1 mM EDTA to remove unbound material.

D) Elute RNA from the streptavidin beads:
Resuspend the beads in 30 µl of 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA and 1 mM biotin. Incubate the beads for 20 minutes at room temperature with occasional resuspension. Collect the biotin-eluted RNA by placing the tube on the magnetic rack.

E) Clean-up eluted RNA:
Bind the eluted RNA to AMPure XP Beads by adding 1.8 volumes of AMPure beads to one eluted RNA volume. Add 1.5 volumes of 100% ethanol to the resulting volume of the AMPure/RNA mix (ie., if 30 µl of RNA was recovered from the beads, add 54 µl of AMPure beads and 126 µl of ethanol). Wash the beads 2 times with 80% ethanol, air dry for 5 minutes on bench, and elute the RNA with 30 µl low TE.

F) 2nd Round of streptavidin enrichment:
Add 30 µl of the RNA eluate to 30 µl of prewashed streptavidin beads for a second round of enrichment. Wash and elute the streptavidin beads as above. Collect and bind the biotin-eluted RNA to AMPure beads as above, and elute with 30 µl low TE.

G) Decapping (prior to 5´ end ligation):
Remove the desthiobiotin cap to leave a 5´ monophosphate terminus by adding 3.3 µl of 10X ThermoPol Buffer (NEB #B9004) and 3 µl (15 units) of RppH (NEB #M0356) and incubate for 60 minutes at 37°C. Terminate the reaction by adding 0.5 µl of 0.5 M EDTA and heat to 94°C for 2 minutes. Bind the RNA to AMPure beads as described above. Wash and elute in 20 µl low TE. The eluted RNA is the starting RNA for library preparation using the NEBNext Small RNA Library Prep Set for Illumina (NEB #E7330).

RNA SEQUENCING LIBRARY PREP

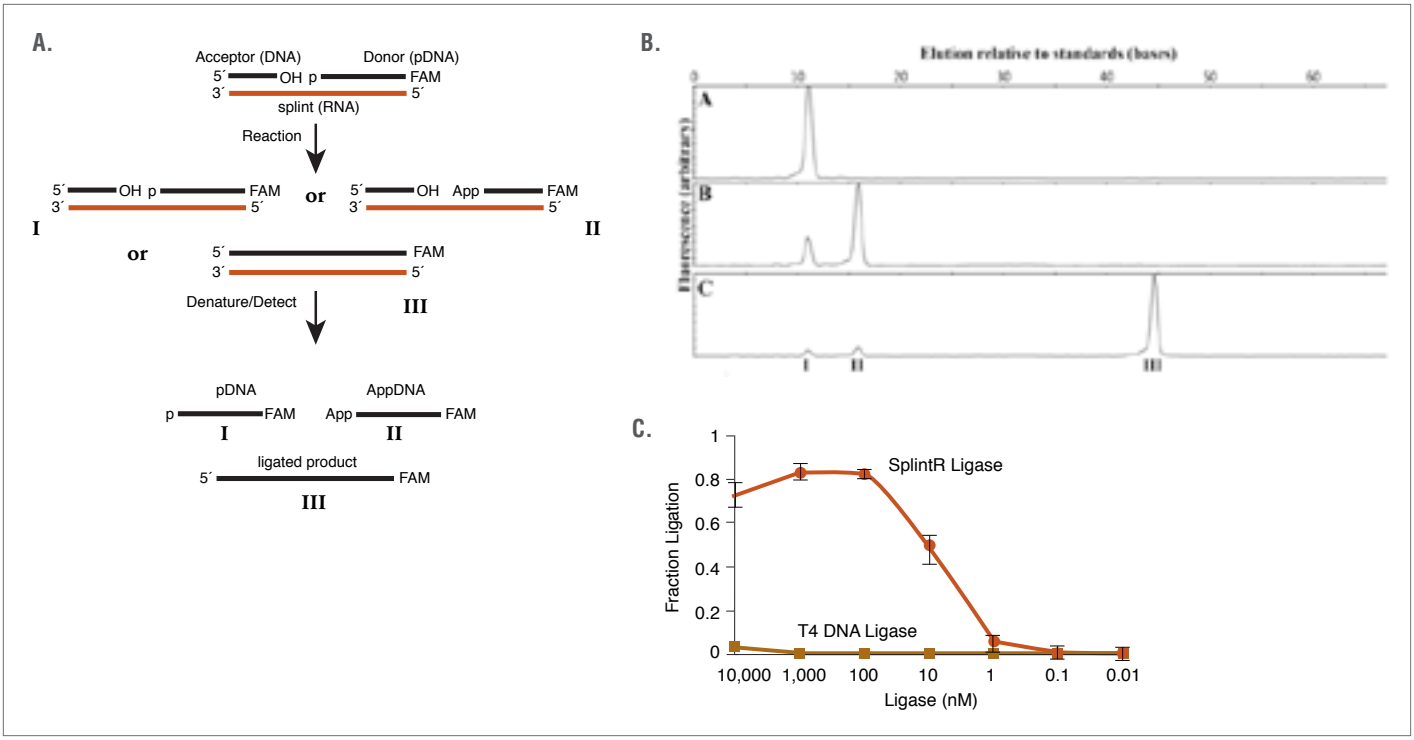
The NEBNext Small RNA Library Prep Set for Illumina® (NEB# E7330) can be used to generate an Illumina sequencing library. The library is amplified through 15 cycles of PCR. RNA sequencing can be performed on an Illumina MiSEQ® with single reads of 100 bases using V3 illumina platform. Visit **www.neb.com/E7330** to access library preparation protocols.

Featured product: SplintR Ligase

Our broad portfolio of RNA ligases includes unique specificities such as SplintR Ligase, which efficiently catalyzes the ligation of adjacent, single-stranded DNA splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Learn more about SplintR Ligase in our webinar at **NEBTV.com/NEBTVwebinars**

Ligation of DNA splinted by RNA



(A) Outline of the ligation assay: a 5´-phosphorylated, 3´-FAM labeled DNA "donor" oligonucleotide and an unmodified DNA "acceptor" oligonucleotide are annealed to a complementary RNA splint. This substrate is reacted with a ligase to form a mixture of unreacted starting material (I), adenylylated DNA (II), and ligated product (III). These products are denatured, separated by capillary electrophoresis and detected by fluorescence. (B) Ligation of the RNA-splinted substrate in SplintR Ligase Reaction Buffer for 15 minutes at 25°C with (a) no enzyme, (b) 1 µM T4 DNA Ligase and (c) 100 nM SplintR Ligase. Indicated peaks correspond to starting pDNA (I), AppDNA (II) and ligated product (III) as determined by co-elution with synthetically prepared standards. (C) The fraction of ligated product catalyzed by either SplintR Ligase or T4 DNA Ligase was analyzed by performing sets of ligations with both ligases at concentrations between 10 pM and 10 µM for 15 minutes at 25°C. SplintR Ligase is clearly much more efficient at ligating RNA-splinted DNA than T4 DNA Ligase.

Ordering Information

RNA Synthesis

PRODUCT	NEB #	SIZE
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 reactions
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 reactions
HiScribe SP6 RNA Synthesis Kit	E2070S	50 reactions
HiScribe T7 ARCA mRNA Kit	E2065S	20 reactions
HiScribe T7 ARCA mRNA Kit (with tailing)	E2060S	20 reactions
T3 RNA Polymerase	M0378S	5,000 units
T7 RNA Polymerase	M0251S/L	5,000/25,000 units
SP6 RNA Polymerase	M0207S/L	2,000/10,000 units
E. coli Poly(A) Polymerase	M0276S/L	100/500 units
Poly(U) Polymerase	M0337S	60 units
E. coli RNA Polymerase, Core Enzyme	M0550S	100 units
E. coli RNA Polymerase, Holoenzyme	M0551S	50 units
Ribonucleotide Solution Set	N0450S/L	10/50 µmol of each
Ribonucleotide Solution Mix	N0466S/L	10/50 µmol of each
Pyrophosphatase, Inorganic (*E. coli*)	M0361S/L	10/50 units
Pyrophosphatase, Inorganic (yeast)	M2403S/L	10/50 units
Thermostable Inorganic Pyrophosphatase	M0296S/L	250/1,250 units
Vaccinia Capping System	M2080S	400 units
Anti-Reverse Cap Analog 3´-O-Me-m^7G(5´)ppp(5´)G	S1411S/L	1/5 µmol
Standard Cap Analog m^7G(5´) ppp(5´)G	S1404S/L	1/5 µmol
Unmethylated Cap Analog G (5´) ppp(5´)G	S1407S/L	1/5 µmol
Methylated Cap Analog for A + 1 sites m^7G(5´)ppp(5´)A	S1405S/L	1/5 µmol
Unmethylated Cap Analog for A + 1 sites G(5´)ppp(5´)A	S1406S/L	1/5 µmol
mRNA Cap 2´-*O*-Methyltransferase	M0366S	2,000 units
3´-Desthiobiotin-GTP	N0761S	0.5 µmol

cDNA Synthesis

PRODUCT	NEB #	SIZE
ProtoScript II Reverse Transcriptase	M0368S/L/X	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase	M0253S/L	10,000/50,000 units
AMV Reverse Transcriptase	M0277S/L	200/1,000 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
ProtoScript II First Strand cDNA Synthesis Kit	E6560S/L	30/150 reactions
ProtoScript First Strand cDNA Synthesis Kit	E6300S/L	30/150 reactions

RNA Quantitation

PRODUCT	NEB #	SIZE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1,000/2,500 reactions
LunaScript RT SuperMix Kit	E3010S/L	25/100 reactions

RNA Ligases & Modifying Enzymes

PRODUCT	NEB #	SIZE
T4 RNA Ligase 1 (ssRNA Ligase)	M0204S/L	1,000/5,000 units
T4 RNA Ligase 1 (ssRNA Ligase), high concentration	M0437M	5,000 units
T4 RNA Ligase 2 (dsRNA Ligase)	M0239S/L	150/750 units
T4 RNA Ligase 2, truncated	M0242S/L	2,000/10,000 units
T4 RNA Ligase 2, truncated K227Q	M0351S/L	2,000/10,000 units
T4 RNA Ligase 2, truncated KQ	M0373S/L	2,000/10,000 units
RtcB Ligase	M0458S/L	25 reactions
Thermostable 5´ AppDNA/RNA Ligase	M0319S/L	10/50 reactions
5´ DNA Adenylation Kit	E2610S/L	10/50 reactions
SplintR Ligase	M0375S/L	1,250/6,250 units
RNA 5´ Pyrophosphohydrolase (RppH)	M0356S	200 units
5´ Deadenylase	M0331S	1,000 units
RNase I_f	M0243S/L	5,000/25,000 units
RNase H	M0297S/L	250/1,250 units
RNase HII	M0288S/L	250/1,250 units
Quick Dephosphorylation Kit	M0508S/L	100/500 units
Antarctic Phosphatase	M0289S/L	1,000/5,000 units
Alkaline Phosphatase Calf Intestinal (CIP)	M0290S/L	1,000/5,000 units
Shrimp Alkaline Phosphatase (rSAP)	M0371S/L	500/2,500 units
T4 Polynucleotide Kinase	M0201S/L	500/2,500 units
ShortCut RNase III	M0245S/L	200/1,000 units
XRN-1	M0338S/L	20/100 units
Exonuclease T	M0265S/L	250/1,250 units

gRNA Synthesis

PRODUCT	NEB #	SIZE
EnGen sgRNA Synthesis Kit, *S. pyogenes*	E3322S	20 reactions

RNase Control

PRODUCT	NEB #	SIZE
RNase Inhibitor, Murine	M0314S/L	3,000/15,000 units
RNase Inhibitor, Human Placenta	M0307S/L	2,000/10,000 units
Ribonucleoside Vanadyl Complex	S1402S	10 ml (200 mM)

RNA Library Preparation for Next Generation Sequencing

PRODUCT	NEB #	SIZE
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 reactions
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 reactions
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 reactions
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 reactions
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 reactions
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 reactions
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 reactions
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 reactions
NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers 2)	E7780S	96 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 reactions
NEBNext Singleplex Oligos for Illumina	E7350S/L	12/60 reactions
DNase I	S1402S	10 ml (200 mM)

RNA Purification & Detection

PRODUCT	NEB #	SIZE
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch RNA Purification Columns	T2007L	100 columns
Monarch DNA/RNA Protection Reagent	T2011L	56 ml
Magnetic mRNA Isolation Kit	S1550S	25 isolations
Epimark N6-Methyladenosine Enrichment Kit	E1610S	20 reactions
Oligo $d(T)_{25}$ Magnetic Beads	S1419S	25 mg
Oligo $d(T)_{25}$ Cellulose Beads	S1408S	250 mg
Streptavidin Magnetic Beads	S1420S	5 ml (20 mg)
Hydrophilic Streptavidin Magnetic Beads	S1421S	5 ml (20 mg)
polyA Spin mRNA Isolation Kit	S1560S	8 isolations
p19 siRNA Binding Protein	M0310S	1,000 units

RNA Markers & Ladders

PRODUCT	NEB #	SIZE
dsRNA Ladder	N0363S	25 gel lanes
microRNA Marker	N2102S	100 gel lanes
ssRNA Ladder	N0362S	25 gel lanes
Low Range ssRNA Ladder	N0364S	25 gel lanes
RNA Loading Dye (2X)	B0363S	4 ml
Universal miRNA Cloning Linker	S1315S	0.83 nmol

CRISPR/Cas, Genome Editing

PRODUCT	NEB #	SIZE
EnGen Cas9 NLS *S. pyogenes*	M0646T/M	400 / 2.000 pmol
EnGen Cas9 *S. pyogenes*	M0386S/T/M	70 / 400 / 2.000 pmol
EnGen Lba Cas12a (Cpf1)	M0653S/T	70 / 2.000 pmol
EnGen Spy Cas9 Nickase	M0650S/T	70 / 400 pmol
EnGen Spy dCas9 (SNAP-tag®)	M0652S/T	70 / 400 pmol

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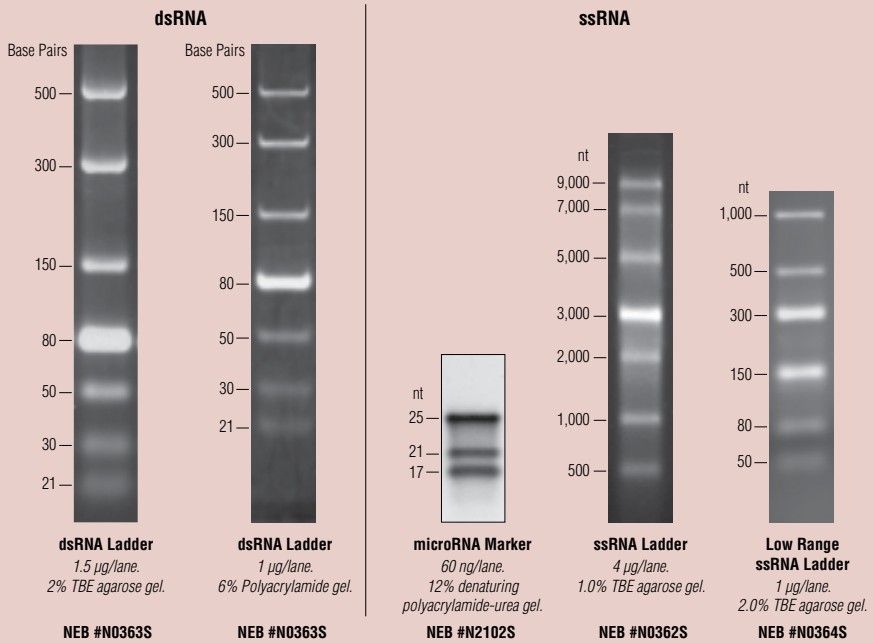
RNA Markers & Ladders



dsRNA Ladder
1.5 µg/lane.
2% TBE agarose gel.
NEB #N0363S

dsRNA Ladder
1 µg/lane.
6% Polyacrylamide gel.
NEB #N0363S

microRNA Marker
60 ng/lane.
12% denaturing polyacrylamide-urea gel.
NEB #N2102S

ssRNA Ladder
4 µg/lane.
1.0% TBE agarose gel.
NEB #N0362S

Low Range ssRNA Ladder
1 µg/lane.
2.0% TBE agarose gel.
NEB #N0364S

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