Spring/Summer **2018**

NEB expressions

a scientific update

in this issue

SPECIAL PRICES

Did you get the message?

NEW ENGLAND BioLabs®

be **INSPIRED**
drive **DISCOVERY**
stay **GENUINE**

Improving Enzymatic DNA Fragmentation for Next Generation Sequencing Library Construction

by Lynne Apone, Ph.D., Eileen Dimalanta, Ph.D., and Fiona Stewart, Ph.D., New England Biolabs, Inc.

INTRODUCTION

The Human Genome Project (HGP), which was officially completed in 2003, was considered to be one of the world's largest collaborative projects of its time (1). This involved many research groups worldwide and had the lofty goal of deciphering all 3 billion bases of the human genome. The project cost almost $4 billion dollars and took 13 years to complete with the available technology. Over a decade later, advancements in next generation sequencing (NGS) technologies have enabled sequencing of a human genome to become routine, taking less than two days, and at a tiny fraction of the cost of the original HGP.

The ability to quickly and inexpensively sequence whole genomes has truly revolutionized genomics research. Where once single genes or families of genes were studied, now whole genomes, exomes, transcriptomes and epigenomes are interrogated. With recent advances, such as the ability to multiplex and sequence many samples at once, NGS has transitioned from a basic research tool into the clinic, where it impacts discovery, diagnostics and treatment of disease.

Advances in genomics driven by NGS, as well as advances in the technology itself, continue at an amazing pace and move us closer to the realization of personalized medicine, where clinical decisions are tailored to an individual's genome. However, if this pace is to continue, advances in all aspects of the technology must also continue. This includes early steps of the sequencing workflow, specifically in the preparation of samples, before they are sequenced.

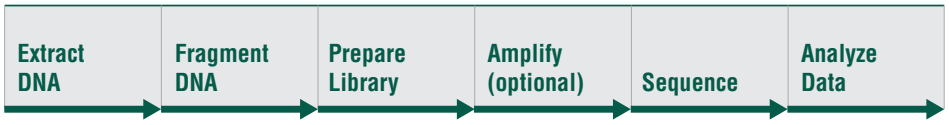
To date, there are no sequencing platforms that can sequence intact DNA. Therefore, prior to sequencing, DNA molecules must be fragmented, or broken, into smaller pieces. These DNA fragments are then converted into libraries, by different methods depending on the sequencing platform to be used (Figure 1). In all cases, the libraries generated consist of the fragments of the unknown DNA to be sequenced, flanked by pieces of known DNA (adaptors), which are specific to each sequencing platform.

DNA FRAGMENTATION APPROACHES

One of the major bottlenecks to sample prep is the first step: DNA fragmentation.

The size of the DNA fragments generated depend on the sequencing platform being used, and can range from several hundred base pairs for short read sequencing technologies (e.g., Illumina®, Ion Torrent™) to >10 kb pieces for long read sequencing technologies (e.g., Pacific Biosciences® and Oxford Nanopore Technologies®). Methods for fragmenting DNA are broadly split into two basic categories: mechanical and enzyme-based. Mechanical shearing methods include acoustic shearing, hydrodynamic shearing and nebulization, while enzyme-based methods include transposons, restriction enzymes and nicking enzymes. Although many different options exist to fragment DNA, final fragment size, amount of starting material, upfront capital investment, and scalability must be considered when choosing a fragmentation method. Critically, in order to be useful for NGS, the method used must shear the DNA sufficiently randomly, so that the library being sequenced is fully representative of the original sample.

FIGURE 1:
Traditional library preparation workflow



Mechanical Shearing

Options for mechanical fragmentation of DNA range from small plastic nebulizer devices to sophisticated electronic instruments. The most commonly used technique utilizes **focused acoustic shearing** devices, such as the instruments made by Covaris®. This involves focused transmission of high-frequency, short wavelength acoustic energy on the DNA sample. The size of the DNA fragments generated (150 – 5,000 bp) is controlled by changing both the intensity and the duration of the acoustic waves, and the protocols used are the same regardless of the amount or GC content of the DNA. Cost, challenges of scalability and sample loss (often caused by sample transfer after shearing), are some of the reasons that users of this method seek alternatives, especially as throughput increases.

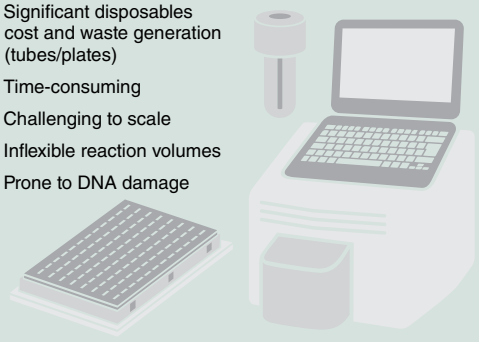
If larger DNA fragments are required, **hydrodynamic shearing** can be used. In this method, hydrodynamic shear forces are applied by pushing DNA through the small orifice of a syringe. Size is controlled by altering the speed at which the DNA is pushed through the syringe. Centrifugation can also be used to create hydrodynamic force, by pulling the DNA sample through a hole with a defined size. Here, the rate of centrifugation determines the degree of DNA fragmentation. DNA fragments generated with hydrodynamic shear forces are typically in the

DNA fragmentation landscape prior to NEBNext Ultra II FS

Focused acoustic shearing

\+
- Historical "gold standard"
- Simple control of fragment size
- Same protocol regardless of GC content or input amount

–
- Significant upfront instrument cost
- Significant disposables cost and waste generation (tubes/plates)
- Time-consuming
- Challenging to scale
- Inflexible reaction volumes
- Prone to DNA damage



Enzymatic fragmentation

\+
- Scalable
- Flexible reaction volumes
- Ease of use
- Minimized DNA damage

–
- Prone to sequence bias
- May be sensitive to contaminants in the DNA sample
- Significant optimization may be required
- Transposases can have very specific input requirements

range of 1-75 kb, but require large DNA input amounts (> 1 µg) and throughput is low.

Nebulization is another method used to mechanically fragment DNA. Nebulization uses compressed air to force DNA through a small hole in a nebulizer unit and DNA fragment size is determined by the pressure used. Although this method is inexpensive and fragment size is somewhat tunable (typically 700 – 5000 bp in size), microgram quantities of DNA are required for starting material, and the method is most suitable for small numbers of samples.

Enzymatic Fragmentation

Enzyme-based fragmentation of DNA is an attractive alternative to mechanical shearing methods, as it does not require upfront capital expense, is amenable to quickly processing many samples at the same time, and reduces sample loss. Historically, the main concern with this method has been sequence bias, as many enzymes that cleave DNA have recognition sequences or sequence preferences.

Transposases fragment DNA by cleaving and inserting a short double-stranded oligonucleotide to the ends of the newly cleaved molecule. The inserted oligonucleotide must contain a sequence that is specific to the particular transposase being used. While this method is fast and has low input requirements, the known sequence bias associated with transposases make them incompatible with some applications.

The great majority of **restriction enzymes** have very specific recognition and cleavage sites, and therefore are not suitable for the random cleavage required for most NGS applications. However, restriction enzymes do have utility in a workflow called RAD-Seq (Restriction-site Associated DNA Sequencing). Here, the sequence bias from the restriction enzyme cut site is exploited to target certain regions for sequencing.

Lastly, **non-specific nicking enzymes** can be used to fragment DNA. These enzymes have less sequence bias than transposases or restriction enzyme-based methods, and generate fragments of different sizes (generally 50 bp-1 kb) in a time-dependent manner: the longer the reaction time, the smaller the fragments. Historically, these enzymes have required significant reaction optimization. They have been sensitive to the buffering conditions of the DNA sample, and required different reaction conditions for different DNA input amounts and for varying GC content. While appealing in theory, this has made enzymatic fragmentation methods more challenging to implement, especially in laboratories where a variety of sample types and amounts are used.

Emerging drawbacks to the gold standard

Of all the techniques described, acoustic shearing has traditionally been the method of choice for short read sequencing technologies, such as Illumina; its popularity a result of robust shearing with low bias. However, this method requires a significant financial investment in equipment, which can be prohibitive for many researchers. In addition, throughput is low and sample loss high, creating bottlenecks and limitations for users of the technology. Interestingly, recent studies have also shown that oxidative damage can occur during mechanical shearing processes (2,3).

FIGURE 2:
NEBNext Ultra II FS Kit workflow

IMPROVING DNA FRAGMENTATION FOR NGS LIBRARY CONSTRUCTION

To address the challenges associated with existing fragmentation approaches, NEB has developed a fragmentation system, the NEBNext® Ultra™ II FS DNA Library Prep Kit (NEB #E7805, E6177), in which a unique enzymatic fragmentation reagent is fully integrated into library preparation to generate low bias, high quality NGS libraries, with a simple, scalable workflow (for more information see page 6).

In order to reduce the NGS sample prep bottleneck, improvements in both performance and ease of use were necessary. In this work, we have focused on the DNA fragmentation step. Our new DNA fragmentation reagent is combined with end repair and dA-tailing reagents, and subsequent adaptor ligation is also carried out in the same vial (Figure 2). For low input samples, PCR amplification is performed prior to sequencing.

Importantly, enzymatic shearing of DNA with this method does not introduce bias into the library, and this method is suitable for input DNA with a full range of GC content. Reduced sample loss and increased efficiencies of the workflow enable use of lower input amounts, with a range of 100 pg – 0.5 µg, and insert sizes of 100 bp to 1 kb can easily be generated.

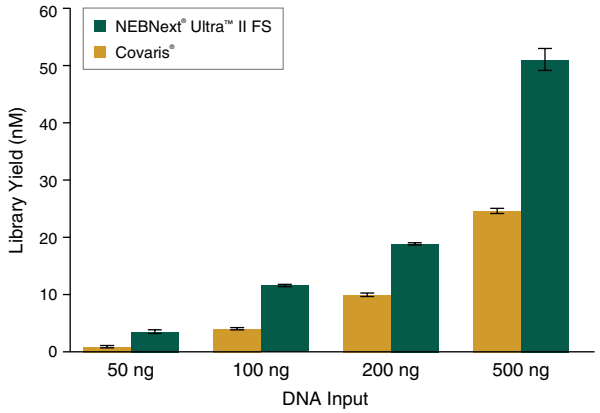
PERFORMANCE

Increased Library Yields

The use of enzymatic fragmentation can result in higher library yields as compared to mechanical shearing workflows, as the latter results in sample loss and DNA damage. Achieving sufficient library yields for high quality sequencing from very low input amounts can be especially challenging with mechanical shearing of DNA, a situation compounded by the preference to amplify libraries using as few PCR cycles as possible. Integration of our unique fragmentation reagent with end repair and dA-tailing, removing sample cleanup prior to ligation and eliminating multiple transfer steps all help to minimize sample loss. When combined with the high reaction efficiences of each step in the workflow and lack of DNA damage cuased by mechanical shearing, NEBNext Ultra II FS generates higher yields than library preparation using mechanical shearing methods. High library yields can be achieved with input amounts as low as 100 pg of human genomic DNA with amplification, or as low as 50 ng for PCR-free workflows (Figure 3, page 3).

FIGURE 3:
NEBNext Ultra II FS DNA produces higher yields of PCR-free libraries

Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown, without amplification. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing each input amount in 1X TE Buffer to ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Library yields were determined by qPCR using the NEBNext Library Quant Kit for Illumina (NEB #E7630). Error bars indicate standard deviation for an average of 2 libraries.



Improvements in Library Quality

As mentioned above, a historical concern regarding the use of enzymatic fragmentation methods was the potential for introduction of bias into a sequencing library, and ultimately into the sequencing data. As shown, this new fragmentation reagent provides consistent uniformity of GC coverage at the full range of input amounts (Figure 4) and over a broad range of GC composition (Figure 5).

In addition, oxidative damage markers typically associated with mechanical-shearing methods (2,3) are observed at significantly lower frequency in libraries made using this new fragmentation system (Figure 6, page 5). Importantly, a greater difference between the observance of these markers is seen with lower input amounts. This highlights the higher quality of libraries constructed with this new method compared to Covaris-sheared DNA libraries, especially at low input amounts.

EASE OF USE

Robustness of DNA Fragmentation:

Consistent and reliable fragmentation is critical for a new method to be adopted. We optimized this new fragmentation system to be insensitive to variables such as input amounts, GC content, and DNA buffer conditions. In practice, these details are often unknown for a sample, requiring clean up and quantification prior to traditional enzymatic DNA fragmentation methods. Even when all of the variables are known, traditional enzymatic methods require different fragmentation parameters for each type of sample and DNA input amount. This new fragmentation system addresses all of these issues by requiring just a single-fragmentation protocol for the full range of input amounts (100 pg – 0.5 µg) (Figure 7, page 5) and for the full range of GC content (Figure 5). Also, input DNA can be in water, Tris, 0.1X TE or 1X TE (Figure 8, page 5). Fragmentation using the new system is time dependent, and final library sizes ranging from 100 bp – 1 kb can be generated by simply changing the fragmentation time.

CONCLUSION:

The continued expansion of the use of next generation sequencing depends in significant part on overcoming the limitations and bottlenecks associated with high-quality library preparation, including the initial DNA fragmentation step. While acoustic shearing has for some time been the method of choice for NGS, limitations in terms of instrumentation, throughput and sample damage necessitate sourcing an alternative solution for many users. This new method for enzymatic DNA fragmentation and library

FIGURE 4:

NEBNext Ultra II FS DNA provides uniform GC coverage with human DNA over a broad range of input amounts

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and number of PCR cycles shown, and a 20-minute fragmentation time was used. Libraries were sequenced (2 x 76 bp) on an Illumina® MiSeq®. Reads were mapped to the hg19 reference genome using Bowtie 2.2.4 and 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.

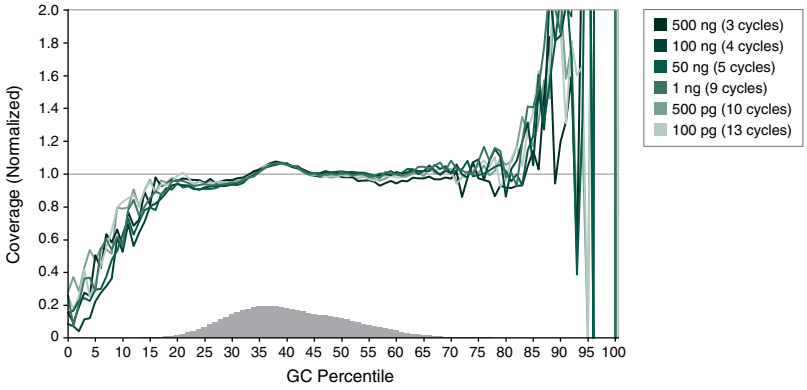


FIGURE 5:

NEBNext Ultra II FS DNA provides uniform GC coverage for microbial DNA over a broad range of GC composition

Libraries were prepared using 1 ng of a mix of genomic DNA samples from *Haemophilus influenzae, Escherichia coli* (K-12 MG1655) and *Rhodopseudomonas palustris*, with 9 PCR cycles, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing 1 ng of DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Reads were mapped using Bowtie 2.2.4 and 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.

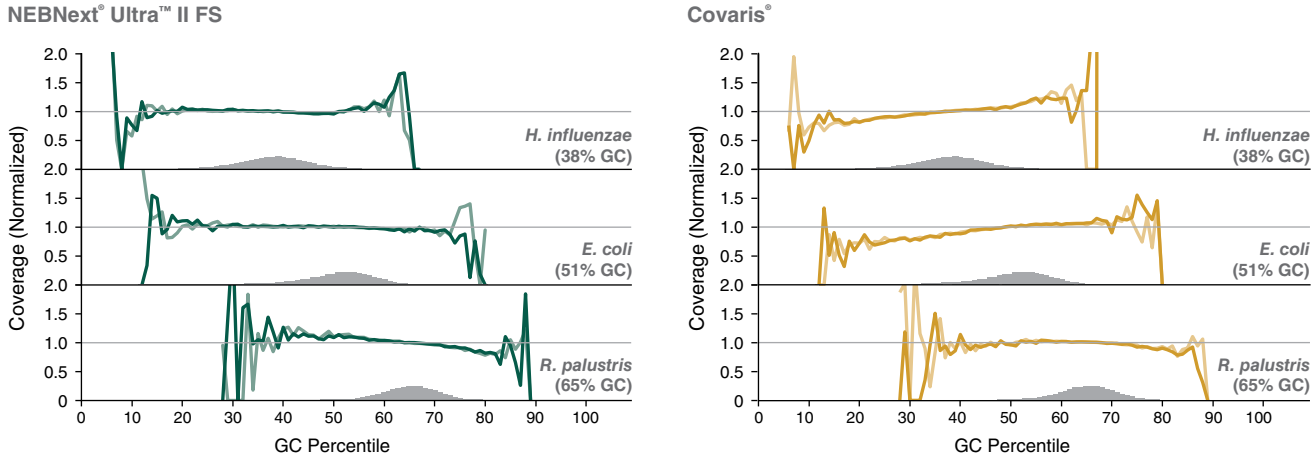
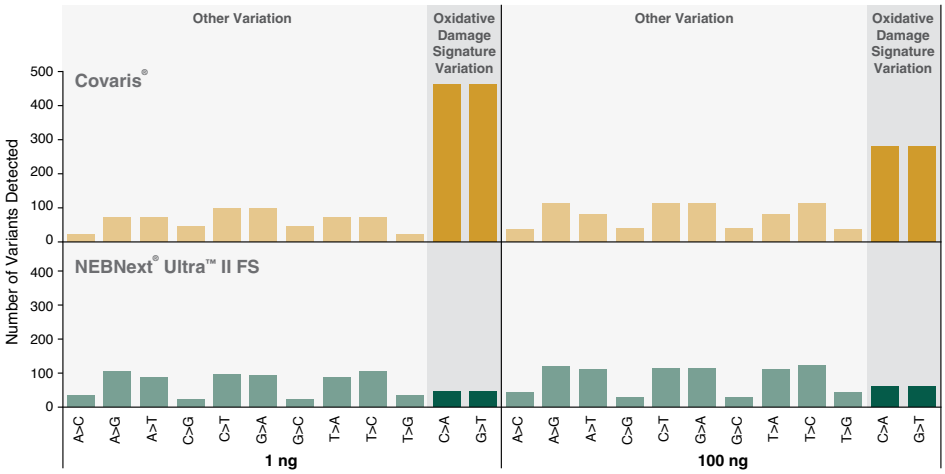


FIGURE 6:

NEBNext Ultra II FS DNA libraries show reduced markers of oxidative damage compared to libraries produced by mechanical shearing

Libraries were prepared from 1 ng and 100 ng Human NA19240 genomic DNA, using 9 and 4 PCR cycles, respectively. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing each input amount in 1X TE Buffer to ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Libraries were sequenced on an Illumina® HiSeq® 2500 (2 x 75 bp). 723M reads were randomly sampled (seqtk) and aligned to the GRCh38 full reference genome using bwa (0.7.15). Adaptors were trimmed prior to alignment using trimadap (r9). Duplicates were marked using samblaster (0.1.24). Variants were called on chromosome 1 using freebayes (1.0.2.29) with frequency based options requiring at least 10 reads per site. More variants are observed for C>A and G>T transversions compared with all other variants for PCR-amplified Covaris libraries. These variants indicative of oxidative damage are not pronounced in NEBNext Ultra II FS libraries.



preparation addresses these issues, further streamlines the process and improves the quality of NGS libraries. The broadening of the input amount range to as low as 100 picograms enables access to high-quality sequencing of samples not achievable by previous methods, and the reliability and ease of use of the method not only allows automation, but also successful adoption by users with a wide range of laboratory skills.

References:

1. https://www.battelle.org/docs/default-source/misc/battelle-2011-misc-economic-impact-human-genome-project.pdf
2. M. Costello et al. (2013) *Nucleic Acids Research*, 41, e67.
3. L. Chen, et al. (2017) *Science*, 17, 355: 752-756.

FIGURE 7:

NEBNext Ultra II FS DNA provides consistent fragmentation regardless of input amount

Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.

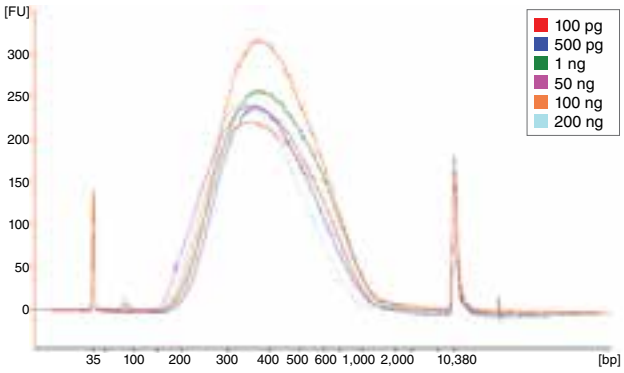
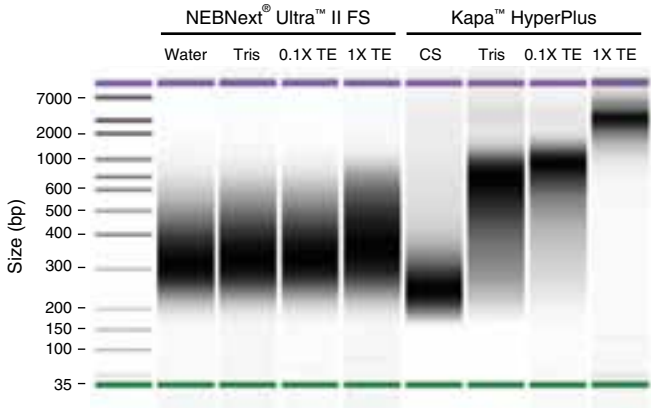


FIGURE 8:

NEBNext Ultra II FS DNA provides consistent fragmentation of DNA in water, Tris or TE

Libraries were made using 100 ng Human NA19240 genomic DNA using the NEBNext Ultra II FS kit or the Kapa HyperPlus Kit. Fragmentation conditions targeting ~200 bp inserts were used, which would generate ~320 bp libraries. For the NEBNext Ultra II FS kit, input DNA was in H2O, Tris, 0.1X TE or 1X TE . For the Kapa HyperPlus kit, libraries were made using the recommended dilution of the supplied Conditioning Solution (CS), or using DNA in Tris, 0.1X TE or 1X TE, in the absence of either Conditioning Solution or 3X bead clean up. Library size distribution was assessed using the Agilent Bioanalyzer. Fragmentation is consistent for the NEBNext Ultra II FS kit for DNA in H_2O, Tris, 0.1X TE or 1X TE.



Novel Enzymatic Fragmentation System

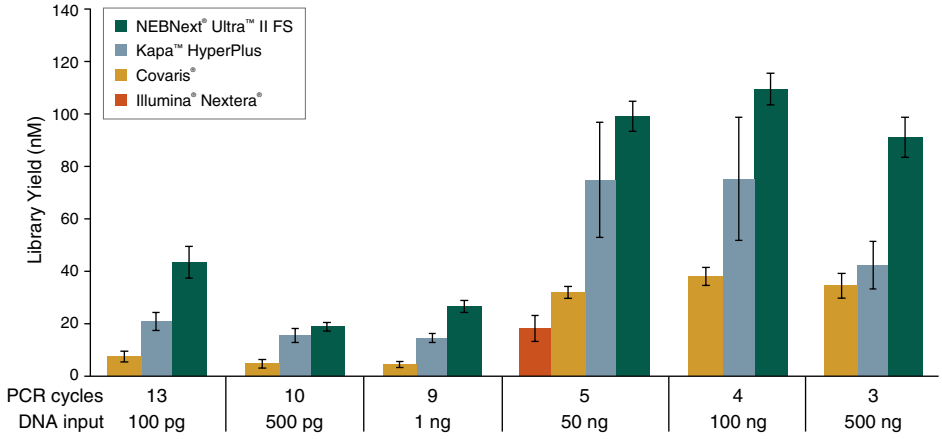
Do you need a faster or more reliable solution for DNA fragmentation and library construction? Our new NEBNext Ultra II FS DNA Library Prep Kit meets the dual challenges of constructing high quality next gen sequencing libraries from ever-decreasing input amounts, and simple scalability. Further, this kit provides a fragmentation and library prep solution that can handle different input amounts and sample types, quickly and reliably.

The Ultra II FS kit includes a new fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-up steps or sample loss. The same fragmentation protocol is used for any input amount (100 pg–500 ng), or GC content.

You'll be thrilled to pieces with the result – a reliable, flexible, high-quality library prep that is fast and scalable.

FIGURE 1:
NEBNext Ultra II FS DNA produces the highest yields, from a range of input amounts

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa™ HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. Illumina® recommends 50 ng input for Nextera®, and not an input range; therefore, only 50 ng was used in this experiment. "Covaris®" libraries were prepared by shearing each input amount in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.



View more performance data by downloading our technical notes at
NEBNextUltraII.com

High-yield, Scalable Library Preparation with the NEBNext Ultra II FS DNA Library Prep Kit

Next generation DNA library construction (LC) for high-throughput genomics – *Data presented by Peter Ellis, Senior Staff Scientist at the Wellcome Trust Sanger Institute*

Advantages

- Perform fragmentation, end repair and dA-tailing with a **single enzyme mix**
- Experience **reliable fragmentation with a single protocol**, regardless of DNA input amount or GC content
- Prepare high quality libraries from a **wide range of input amounts**: 100 pg–500 ng
- Use with DNA in standard buffers (e.g., TE, Tris-HCl) and water
- Save time with a **streamlined workflow**: ~ 2.5 hours, with < 15 minutes hands-on time
- Experience **reliable fragmentation**, even with very low input amounts
- Generate **high yields** with increased reaction efficiencies and minimized sample loss
- Vary incubation time to generate a **wide range of insert sizes**

Free Sample

For more information and to request a sample, visit
NEBNextUltraII.com

NEBNext® Ultra™ II FS DNA Library Prep Kit

FIGURE 2:

NEBNext Ultra II FS DNA provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition

Libraries were prepared using 1 ng of a mix of genomic DNA samples from *Haemophilus influenzae, Escherichia coli* (K-12 MG1655), *Rhodopseudomonas palustris* and the library prep kits shown with 9 PCR cycles for consistency across samples, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, followed by a 25-minute fragmentation time. "Covaris" libraries were prepared by shearing 1 ng of DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Reads were mapped using Bowtie 2.2.4 and 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.

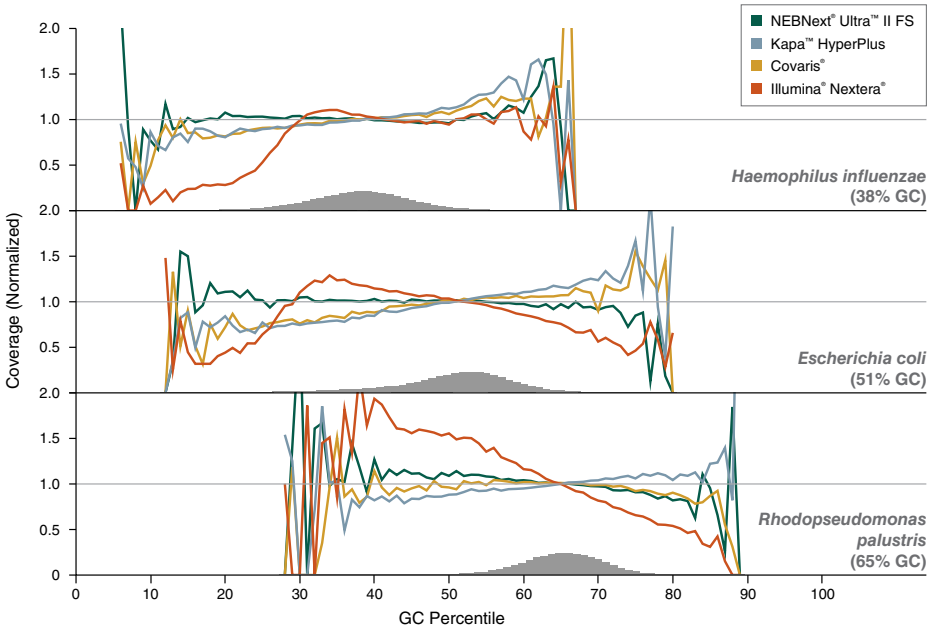


TABLE 1:

NEBNext Ultra II FS DNA Library Prep Workflow

	Fragmentation/ End Repair/ dA-Tailing	Adaptor Ligation	Clean Up/ Size Selection	Amplification	Clean Up	Total Workflow
Hands-On	2 min.	1 min.	5 min.	0–1 min.	0–5 min.	**8–14 min.**
Total	37–62 min.	16–31 min.	27–37 min.	0–34 min.	0–27 min.	**1.3–3.2 hr.**

ORDERING INFORMATION

PRODUCTS	NEB #	SIZE	PRICE
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 rxns	695 € / 2.640 €
NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S/L	24/96 rxns	765 € / 2.890 €
NEBNext Ultra II FS DNA Module	E7810S/L	24/96 rxns	305 € / 1.090 €

ALSO AVAILABLE	NEB #	SIZE	PRICE
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3, 4)	E7335, E7500, E7710, E7730S/L	24/96 rxns	106 € / 384 €
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns	680 € / 2.720 €
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns	461 €

Available products:

- **NEBNext Ultra II FS DNA Library Prep Kit for Illumina**
  Includes optimized mixes for DNA library preparation (enzymatic fragmentation/end repair/dA-tailing, adaptor ligation and PCR enrichment steps) for sequencing on the Illumina platform. This kit includes a new DNA fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-ups or sample loss.
  NEB # E7805S/L **24/96 rxns**

- **NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads**
  Includes optimized mixes for DNA library preparation (enzymatic fragmentation/end repair/dA-tailing, adaptor ligation and PCR enrichment steps) plus SPRIselect® beads for size selection and cleanup. This kit includes a new DNA fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-ups or sample loss.
  NEB # E6177S/L **24/96 rxns**

- **NEBNext Ultra II FS DNA Module**
  This module is part of the NEBNext Ultra II FS workflow, and includes optimized mixes for DNA library preparation (enzymatic fragmentation/end repair/dA-tailing, adaptor ligation and PCR enrichment steps).
  NEB # E7810S/L **24/96 rxns**

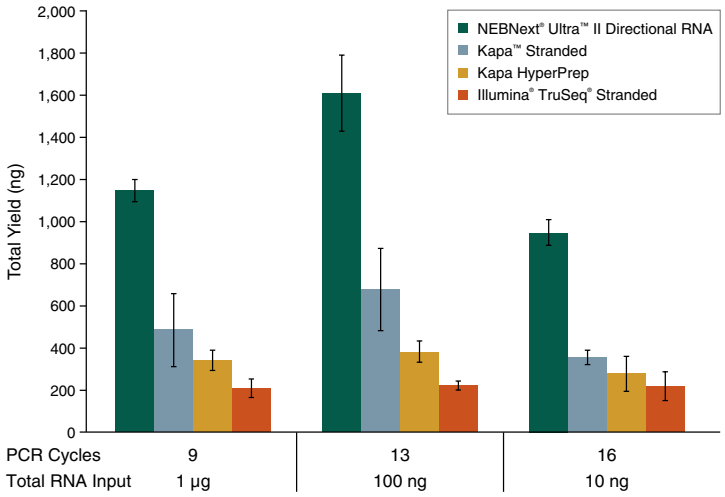
Easy, Fast & Extremely Efficient Library Prep Kits for RNA-Seq

NEBNext® Ultra™ II RNA Library Prep

Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, our next generation of RNA library prep kits have been reformulated at each step, resulting in several fold higher yields of high quality libraries, enabling use of lower input amounts and fewer PCR cycles. NEBNext Ultra II RNA Kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method"(1,2)) and non-directional library prep, with the option of SPRISelect® beads for size selection and clean-up steps.

NEBNext Ultra II Directional RNA produces the highest yields, from a 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 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.



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

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	PRICE
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns	1.028 € / 3.495 €
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns	1.140 € / 3.880 €
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns	980 € / 3.325 €
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns	1.080€ / 3.685 €

OTHER PRODUCTS YOU MIGHT BE INTERESTED IN:

NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns	330 € / 1.200 € / 4.320 €
NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns	374 € / 1.375 € / 4.950 €
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns	67 € / 242 €
NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns	165 € / 528 €
NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns	390 € / 1.245 €
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns	295 € / 1.180 €
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3, 4)	E7335, E7500, E7710, E7730S/L	24/96 rxns	106 € / 384 €
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns	680 € / 2.720 €
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns	461 €

Advantages

- **Generate high yield**, high-quality libraries even with limited amounts of RNA:
  - 10 ng – 1 µg total RNA (poly(A) mRNA workflow)
  - 5 ng – 1 µg total RNA (rRNA depletion 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 (12-, 96-, and dual index) 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

Free Sample

For more information and to request a sample, visit **UltraIIRNA.com**

Gene Editing

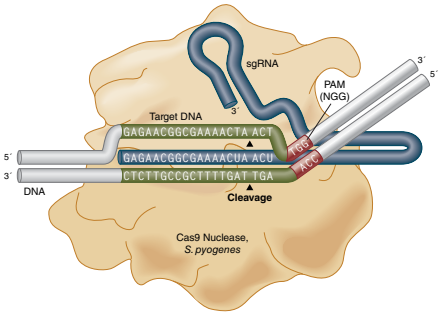
NEW PRODUCTS

Programable CRISPR/Cas9 Nucleases

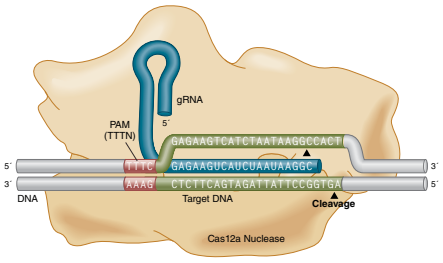
NEB now offers **EnGen® Spy Cas9 Nickase** and **EnGen Spy dCas9 (SNAP-tag®)** with nuclear localization sequences for genome editing studies. Cas nucleases have been adapted for use in genome engineering, because they can be easily programmed for target specificity by supplying gRNAs of any sequence. 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 latest addition to our EnGen toolbox is **EnGen Lba Cas12a (Cpf1),** which has a different PAM sequence compared to Cas9. It leaves 5' overhangs after digestion presenting an opportunity for novel CRISPR-based cloning methodologies. It's optimal activity starts already at 16°C making it an extremely efficient choice for researchers working with ectothermic organisms such as zebrafish and xenopus.

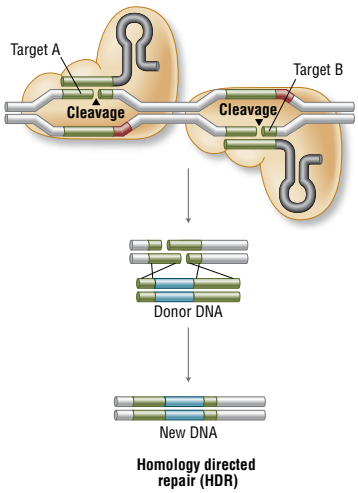
EnGen Cas9 Nuclease, *S. pyogenes* (Spy)
Standard genome editing



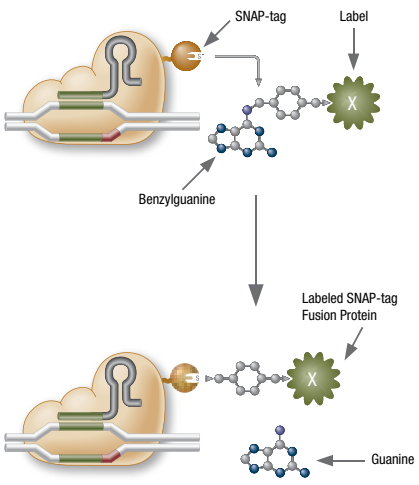
EnGen Lba Cas12a ("Cpf1")
AT-rich PAM, expanded temperature range



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



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



Available products:

- **EnGen Cas9 NLS, *S. pyogenes***, is an RNA-guided endonuclease that catalyzes site-specific cleavage of double-stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif).
- **EnGen Spy Cas9 Nickase** is a variant of Cas9 nuclease differing by a point mutation (D10A) in the RuvC nuclease domain, which enables it to nick, but not cleave, DNA. Double-stranded breaks can be generated with reduced off-target cleavage by targeting two sites with EnGen Cas9 Nickase, NLS in close proximity.
- **EnGen Spy dCas9 (SNAP-tag®)** is an inactive mutant of Cas9 nuclease that retains programmable DNA binding activity. The N-terminal SNAP-tag allows for covalent attachment of fluorophores, biotin, and a number of other conjugates useful for visualization and target enrichment.
- **EnGen Lba Cas12a (Cpf1)** is a programmable DNA endonuclease guided by a single guide RNA (gRNA).Targeting requires a gRNA complementary to the target site as well as a 5' TTTN protospacer adjacent motif (PAM) on the DNA strand opposite the target sequence. Cleavage by EnGen Lba Cas12a (Cpf1) occurs ~18 bases 3' of the PAM and leaves 5' overhanging ends.

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	PRICE
EnGen Spy Cas9 Nickase	M0650S/T	70/400 pmol	63 € / 158 €
EnGen Spy dCas9 (SNAP-tag)	M0652S/T	70/400 pmol	63 € / 158 €
EnGen Cas9 NLS, *S. pyogenes*	M0646T/M	400/2,000 pmol	158 € / 632 €
EnGen Lba Cas12a (Cpf1)	M0653S/T	70/2,000 pmol	74 € / 264 €

Did you get the message?

Everything You Need for Your RNA-Related Workflows

In recent years, the discovery of new classes and modifications of RNA has ushered in a renaissance of RNA-focused research. Did you know that 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.

Utilize NEB's RNA Toolbox for your RNA related experiments and research and benefit from excellent quality and performance.

Moreover, benefit from our Special Prices for many RNA related products featured in this newsletter.

25% DISCOUNT ON SELECTED RNA PRODUCTS*

PROMO CODE: **RNA18**
Valid until 30.06.2018

DID YOU GET THE MESSAGE?
Get special prices on selected NEB RNA Products!
All eligible discounted products are marked with the "Special Price" icon within this newsletter (pages 10-15). Campaign ends June 30th, 2018.
See conditions on www.neb-online.fr

FEATURE ARTICLE – PDF Download

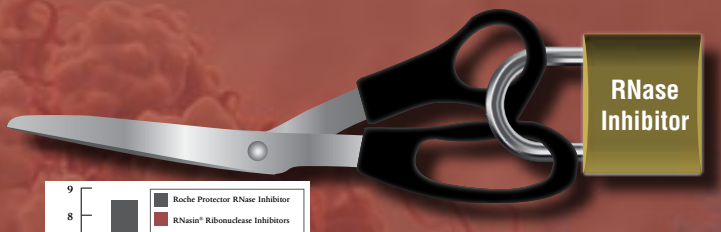
Minding your caps and tails – considerations for functional mRNA synthesis

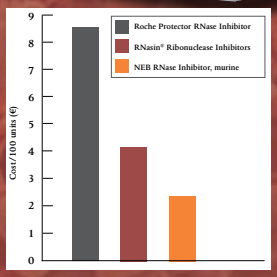
Applications of synthetic mRNA have grown and become considerably diversified in recent years. Examples include the generation of pluripotent stem cells (1-3), vaccines and therapeutics (4), and CRISPR/Cas9 genome editing applications (5-7). The basic requirements for a functional mRNA – a 7-methylguanylate cap at the 5´ end and a poly(A) tail at the 3´ end – must be added in order to obtain efficient translation by eukaryotic cells. Additional considerations can include the incorporation of modified bases, modified cap structures and polyadenylation strategies. Strategies for *in vitro* synthesis of mRNA may also vary according to the desired scale of synthesis. This article discusses options for selection of reagents and the extent to which they influence synthesized mRNA functionality...

Read the full feature article as a PDF on:
www.neb-online.fr/mRNA-Synthesis

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

- 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 polymerase activity used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3)





25% discount* PROMO CODE: RNA18

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE*
RNase Inhibitor, murine	M0314S	3.000 units	53,25 €
RNase Inhibitor, murine	M0314L	15.000 units	213,00 €

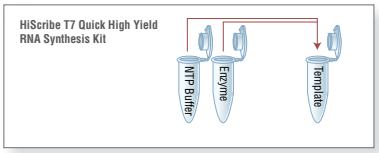
RNA Special Price Campaign

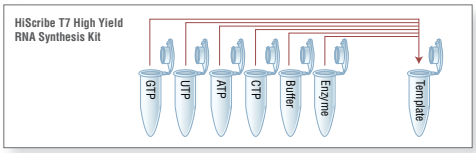
High Yield RNA Synthesis in all Flavors

In vitro RNA synthesis requires a DNA template, RNA polymerase, NTPs and other factors. 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.

HiScribe™ T7 High Yield RNA Synthesis Kit & HiScribe™ T7 *Quick* High Yield RNA Synthesis Kit

The **HiScribe T7 High Yield RNA Synthesis Kit (NEB #E2040)** delivers robust RNA synthesis for a wide range of template sizes. Flexible protocols ensure that performance is maintained even under demanding conditions, such as extended reaction time using very low amounts of template. Protocols are included for partial or complete incorporation of modified or labeled nucleotides in the transcript body, and cap analogs at the RNA 5' end. The **HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050)** utilizes a master mix format, allowing for faster reaction setup. DNase I and lithium chloride are included for DNA template removal and quick RNA purification.





HiScribe™ T7 ARCA mRNA Synthesis Kit

Most eukaryotic mRNAs require a 7-methyl guanosine (m7G) cap structure at the 5' end and a poly(A) tail at the 3' end for efficient translation to occur. The **HiScribe™ T7 ARCA mRNA Synthesis Kit (NEB #E2060S**) is designed to synthesize capped and tailed mRNAs for a variety of applications. Capped mRNAs are synthesized by co-transcriptional incorporation of Anti-Reverse Cap Analog, ARCA, using T7 RNA Polymerase. A poly(A) tail is then added by *E. coli* Poly(A) Polymerase. **This kit is also available without *E. coli* Poly(A) Polymerase (NEB #E2065S)** for use with DNA templates encoding a poly(A) stretch or not requiring a poly(A) tail. Both kits include DNase I and LiCl for DNA template removal and quick mRNA purification.

Overview of mRNA synthesis work flow with the HiScribe T7 ARCA mRNA Kit (with tailing)

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE*
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 rxns	166,50 €
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 rxns	202,50 €
HiScribe T7 ARCA mRNA Kit (with Tailing)	E2060S	20 rxns	279,00 €
HiScribe T7 ARCA mRNA Kit	E2065S	20 rxns	239,25 €
HiScribe SP6 RNA Synthesis Kit	E2070S	50 rxns	202,50 €

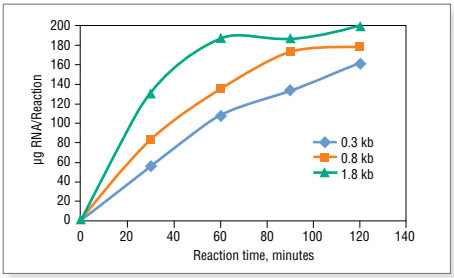
25% discount* PROMO CODE: RNA18

** Offer ends on June 30th, 2018. See conditions on www.neb-online.fr.*

Advantages

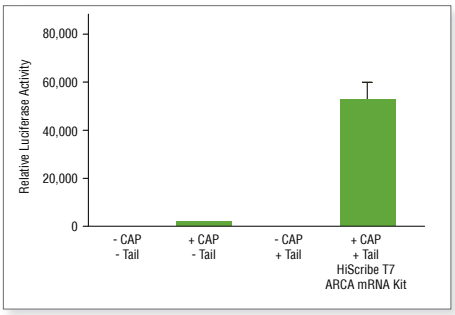
- Streamlined format & Quick Workflows
- Flexibility – enables incorporation of cap analogs, radiolabeled and modified nucleotides
- High Yield – up to 180 µg of RNA from a standard 20 µl reaction (e.g. #E2050)
- High Quality Transcripts – optimized formulation for increased transcript integrity
- Get the best translation efficiencies with correctly oriented ARCA caps (#E2060 & #E2065)

Robust RNA Synthesis from a Variety of Template Sizes using the HiScribe T7 High Yield RNA Synthesis Kit



Time course of standard RNA synthesis from three DNA templates of different sizes using HiScribe T7 High Yield RNA Synthesis Kit assembled according to the protocol and incubated at 37°C for the indicated time.

Both cap and tail are required for mRNA function in cell culture.



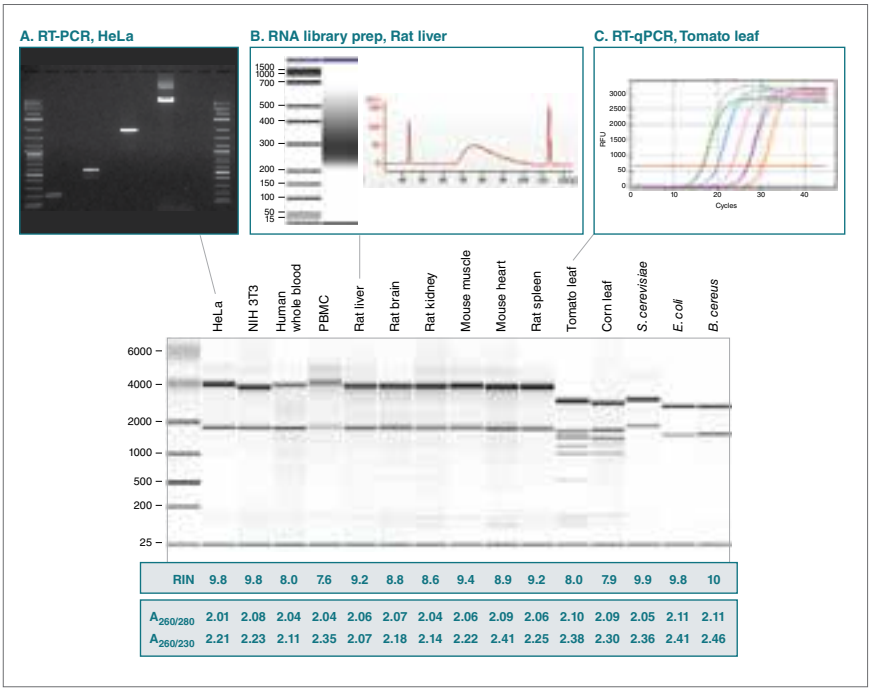
Purified Cypridina luciferase RNA produced as indicated was co-transfected into U2OS cells with purified Gaussia luciferase mRNA. mRNAs produced using the HiScribe T7 ARCA mRNA Kit (with Tailing) are 5´-capped and have 3´ poly(A) tails. After 16 hours incubation at 37°C, cell culture supernatants from each well were assayed for CLuc and GLuc activity.

Did you get the message?

Monarch Total RNA Miniprep Kit

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 plant, 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 scores, 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, Northern blot analysis, etc.

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



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).

Advantages

- Use with a wide variety of sample types
- Purify RNA of all sizes, including miRNA & small RNAs >20 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent
- Protocols available for RNA fractionation and RNA cleanup
- Save money with value pricing for an all-in-one kit

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	✓	×[1]	×[1]	✓
DNase I	✓	×	×	×
Proteinase K	✓	×	×	×
RNA Protection Reagent	✓	×	✓	×

✓ = Included [1] Not included and not sold separately

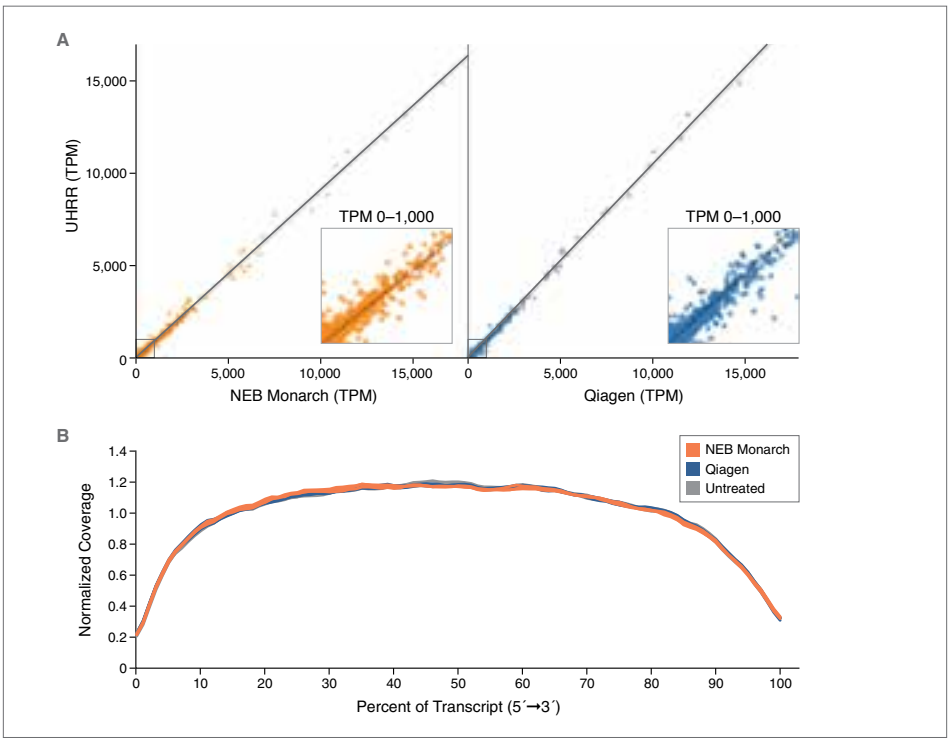
Time for change – try Monarch for free.

Free Sample

For a limited time, try one of our Monarch Nucleic Acid Purification Kits by visiting **NEBMonarch.fr**

RNA Special Price Campaign

Monarch-purified RNA can be used to prepare high quality RNA-seq libraries for gene expression analysis



Transcript levels in Universal Human Reference RNA (UHRR, Agilent) are compared before and after re-purification using either Qiagen RNeasy® or the Monarch Total RNA Miniprep Kit. Strong correlation with untreated UHRR is observed for both methods (Pearson R > 0.99 for both samples). All samples display consistent end-to-end coverage of transcripts indicating an absence of detectable degradation during purification. Poly-A selected RNA was selected from 100 ng of untreated, Qiagen and Monarch samples using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). RNA-seq libraries were then prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina® (NEB #E7760) before sequencing on a Miseq® instrument (2 x150). 1.6M reads were randomly sampled from each library and adapter trimmed (Seqprep v1.1). Levels of all Gencode v26 transcripts were assessed using Salmon (0.4) and plotted above (panel A). Average 5'-3' Coverage of Gencode v26 transcripts (assessed by Picard's CollectRnaSeqMetrics 1.56 after mapping to the GRCh38 reference genome with Hisat v2.0.3 and marking duplicates with Picard's MarkDuplicates 1.56) is shown below (panel B).

TIPS FOR SUCCESSFUL RNA EXTRACTIONS

- **Inactivate RNases after harvesting your sample:** Nucleases in your sample will lead to degradation, so inactivating them is essential. Process samples quickly, or use preservation reagents, and always ensure nuclease-free working environments.
- **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.
- **Ensure samples are properly homogenized/ disrupted:** Samples should be disrupted and homogenized completely to release all RNA.
- **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.

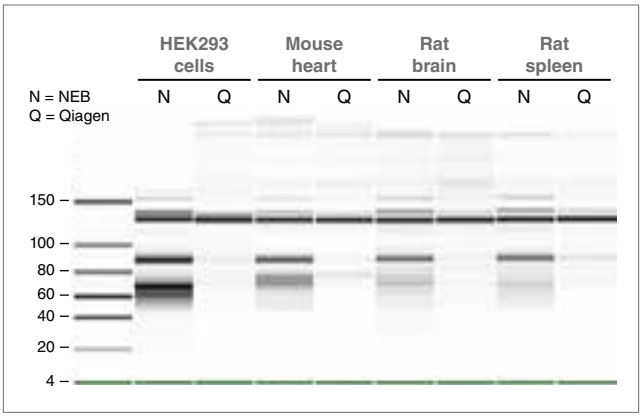
NEB also offers an extensive range of Monarch DNA Purification Kits and products. Visit ***NEBMonarch.fr*** to learn more!

Free Sample

Request a free sample !**
Visit **NEBMonarch.fr**

25% discount*
PROMO CODE: RNA18

The Monarch Total RNA Miniprep Kit successfully purifies small RNAs below 200 nucleotides, enabling a more accurate representation of the total RNA pool



RNA preps were performed on HEK293 cells, mouse heart, rat brain, or rat spleen using the Monarch Total RNA Miniprep Kit (N) (NEB #T2010) and the RNeasy® Mini Kit from Qiagen (Q). Equivalent amounts were resolved on a Bioanalyzer 2100 using the Small RNA chip. Monarch-purified RNA contains significantly more RNA in the sub-200 nucleotide pool.

ORDERING INFORMATION

RNA PURIFICATION KIT	NEB #	SIZE	SPECIAL PRICE*
Monarch Total RNA Miniprep Kit	T2010S	50 preps	186,00 €
COLUMNS AVAILABLE SEPARATELY			
Monarch RNA Purification Columns	T2007L	100 columns and tubes	153 €
Monarch gDNA Removal Columns	T2017L	100 columns and tubes	128 €
Monarch Collection Tubes II	T2018L	100 tubes	26 €
BUFFERS & REAGENTS AVAILABLE SEPARATELY			
Monarch DNA/RNA Protection Reagent	T2011L	56 ml	128 €
Monarch RNA Lysis Buffer	T2012L	100 ml	61 €
Monarch Total RNA Miniprep Enzyme Pack (contains DNase I, Prot K, and associated buffers)	T2019L	1 pack	61 €
Monarch RNA Priming Buffer	T2013L	56 ml	61 €
Monarch RNA Wash Buffer	T2014L	50 ml	61 €
Nuclease-free Water	B1500S/L	25 ml/100 ml	25 € / 61 €

** Offer ends on June 30th, 2018. See conditions on www.neb-online.fr.*

**Limit one per customer, while supplies last.

Did you get the message?

RNA Quantitation by RT-qPCR: Novel enzyme technology for superior RT-qPCR Performance

New England Biolabs offers a bright new choice for your qPCR and RT-qPCR. Luna products have been optimized for robust performance on diverse sample sources and target types. Available for dye-based or probe-based detection, Luna products can be used across a wide variety of instrument platforms.

For superior RT-qPCR Performance, experience a novel, more thermostable "Designer" Reverse Transcriptase ("WarmStart RT") as an integral part of the Luna Universal One-Step RT-qPCR Kits. This new technology highly improves the performance and is paired with Hot Start *Taq* which increases reaction specificity and robustness.

A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates.

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.

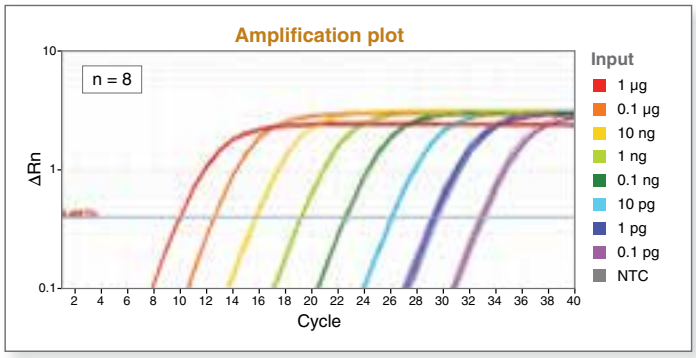
OPTIMIZE YOUR RT-qPCR WITH LUNA WARMSTART® REVERSE TRANSCRIPTASE

- Novel, thermostable reverse transcriptase (RT) improves performance.
- WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness.
- Convenient and fast One-Step RT-qPCR set-up reducing the risk of pipetting errors

MAKE A SIMPLER CHOICE

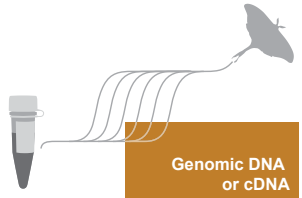
- One product per application simplifies selection.
- Convenient master mix formats and user-friendly protocols simplify reaction setup.
- Non-interfering, visible tracking dye helps to eliminate pipetting errors.

Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility & RT-qPCR performance



RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit (Input: 1 µg – 0.1 pg Jurkat total RNA); NTC = non-template control

Find the right Luna product for your application:



1 Select your target

2 Select your detection method

	Dye-based	Probe-based
Genomic DNA or cDNA	Luna Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)

Request a free sample!**
Visit **LUNAqPCR.fr**

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE*
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/ 1,000/2,500 rxns	84,75 € / 189,00 € / 337,50 € / 748,50 €
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/ 1,000/2,500 rxns	71,25 € / 159,00 € / 290,25 € / 645,00 €
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/ 1,000/2,500 rxns	175,50 € / 395,25 € / 686,25 € / 1524,75 €
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/ 1,000/2,500 rxns	158,25 € / 356,25 € / 624,75 € / 1374,75 €

** Offer ends on June 30th, 2018. See conditions on www.neb-online.fr.*

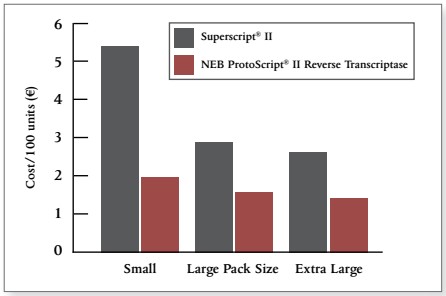
**Limit one per customer, while supplies last.

RNA Special Price Campaign

Affordable Performance cDNA Synthesis: ProtoScript® II RTase & cDNA Kits

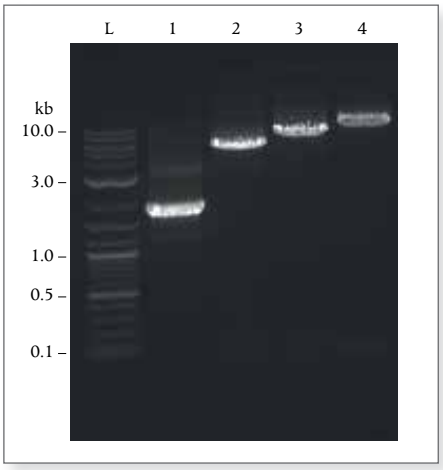
ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild-type M-MuLV. The enzyme is active up to 48°C, providing higher specificity, higher yield of cDNA and more full-length cDNA product, up to 12 kb in length.

Take advantage of the low cost per unit with ProtoScript II

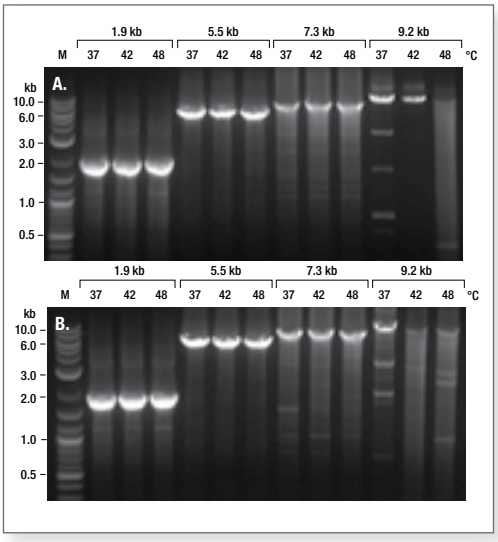


Based on published list prices as of 01/2018; prices may differ in various European countries!

cDNA synthesis for RT-PCR up to 10 kb using the ProtoScript II First Strand cDNA Synthesis Kit



Jurkat total RNA (250 ng) was converted to first strand cDNA using the ProtoScript II First Strand cDNA Synthesis Kit. Using 10% of the first strand cDNA reactions, amplicons representing four different mRNAs were amplified using LongAmp® Taq 2X Master Mix (NEB #M0287). Lane 1, (mRNA) SDHA, 1.9 kb; lane 2, (mRNA) HERC1, 5.5 kb; lane 3, (mRNA) XRN1, 7.3 kb; lane 4, (mRNA) FBN1, 9.2 kb; Ladder (L) is a 2-log DNA ladder (NEB #N3200).



Jurkat total RNA (1 µg) was used in a 20 µl first strand cDNA synthesis. Mixtures of all reaction components, except for reverse transcriptase, were held at different temperatures for 3 min. 200 units NEB's ProtoScript® II Reverse Transcriptase (A) or SuperScript® II (B) was added and incubated at the indicated temperature for 50 minutes, followed by heat inactivation for 5 min at 80°C. 1 µl of cDNA was used in a 25 µl PCR using LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) for 35–40 cycles. Ladder M is the Quick-Load® 2-Log DNA Ladder (NEB #N0469).

Advantages

- High cDNA yield
- Superior performance for longer templates (up to 12 kb)
- Increased thermostability (up to 50°C)
- Value pricing
- Also available as affordable first strand cDNA synthesis kit

TIPS FOR SUCCESSFUL cDNA Synthesis

- You can increase the yield of long cDNA products by doubling the amount of enzyme and dNTP.
- Intact RNA of high purity is essential for generating cDNA for cloning applications.
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for cDNA synthesis reactions. However, if desired, mRNA can be easily obtained using a polyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for cDNA cloning depends on the abundance of the transcript-of-interest. In general 1 ng to 1 µg total RNA or 0.1 ng to 100 ng mRNA are recommended.

25% discount* PROMO CODE: RNA18

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE*
ProtoScript II First Strand cDNA Synthesis Kit	E6560S/L	30/150 rxns	121,50 € / 486,00 €
ProtoScript II Reverse Transcriptase	M0368S/L/X	4.000/10.000/40.000 units	59,25 € / 118,50 € / 426,00 €
AVAILABLE SEPARATELY:			
Random Primer 6 (5'd(N_6)3') ~14.6 nmol	S1230S	1.0 A_{260} unit	72,00 €
Random Primer 9 (5'd(N_9)3') ~11.6 nmol	S1254S	1.0 A_{260} unit	72,00 €
Oligo d(T)23 VN	S1327S	1.0 A_{260} unit	72,00 €
Random Primer Mix	S1330S	100 µl	72,00 €
Oligo d(T)18 mRNA Primer	S1316S	5.0 A_{260} units	72,00 €

** Offer ends on June 30th, 2018. See conditions on www.neb-online.fr.*

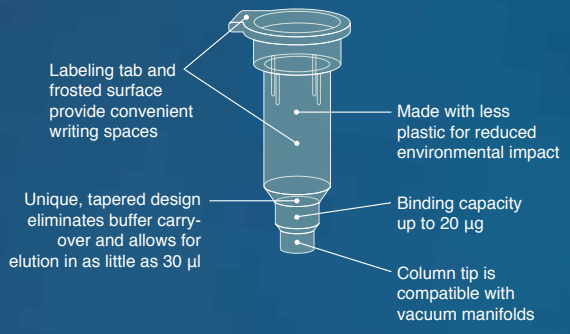
NEW ENGLAND BioLabs® Inc.

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723

It's time to transform your DNA purification experience.

NEB's Monarch Nucleic Acid Purification Kits are optimized for maximum performance and minimal environmental impact. Our unique thin-walled column design uses less plastic, prevents buffer retention, eliminates the risk of carryover contamination, and enables elution in smaller volumes.

Unique design of Monarch Miniprep Columns



"These kits might be the best I have used for the price. The best part is that it uses less plastic for production!! Thank you for caring about our environmental impacts, NEB!!!"

– NEB customer

ORDERING INFORMATION

PRODUCT	NEB #	SIZE	SPECIAL PRICE*
Monarch™ Plasmid Miniprep Kit	T1010L	250 preps	243,75 €
Monarch™ DNA Gel Extraction Kit	T1020L	250 preps	298,50 €
Monarch™ PCR & DNA Cleanup Kit (5µg)	T1030L	250 preps	298,50 €

**Offer ends on June 30th, 2018. See conditions on www.neb-online.fr*

25% DISCOUNT ON L-SIZE KITS*

France
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 EVRY cedex
Toll Free Telephone: 0800 100 632
Toll Free FAX : 0800 100 610
info.fr@neb.com
www.neb-online.fr

USA (Headquarter)
New England Biolabs, Inc.
Telephone: (978) 927-5054
Toll Free: (U.S. Orders) 1-800-632-5227
Toll Free: (U.S. Tech) 1-800-632-7799
Fax: (978) 921-1350
info@neb.com

United Kingdom
New England Biolabs (UK), Ltd.
Call Free 0800 318486
info.uk@neb.com

Germany & Austria
New England Biolabs GmbH
Free Call 0800/246 5227 (Germany)
Free Call 00800/246 52277 (Austria)
info.de@neb.com

Canada
New England Biolabs, Ltd.
Toll Free: 1-800-387-1095
info.ca@neb.com

China, People's Republic
New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
info@neb-china.com

Japan
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info@neb-japan.com

Singapore
New England Biolabs Pte. Ltd.
Telephone +65 6776 0903
sales.sg@neb.com

www.neb-online.fr