Autumn/Winter 2023

NEB expressions

a scientific update

IN THIS ISSUE

SPECIAL PRICE* until 31.12.2023

NEW ENGLAND BioLabs®

Enhancing Molecular Diagnostics in Regulated Markets with Lyophilized Assays

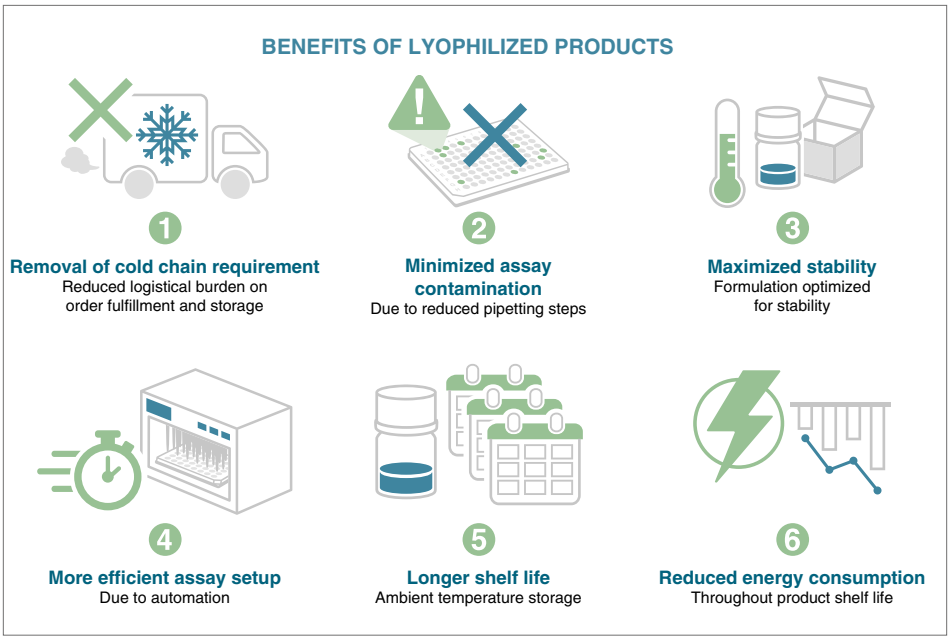
by Jenny Loeb, M.Sc., and Nathan Tanner, Ph.D., New England Biolabs®, & Martin Lee, Ph.D., New England Biolabs® Lyophilization Sciences™

Molecular diagnostic assays are developed to identify and quantitate specific DNA, RNA, or protein biomarkers found within samples of interest. In many regions, molecular diagnostic testing may only be performed by procuring assays subject to regulatory approval, or with validated tests and workflows in approved laboratories.

Historically, molecular diagnostic assays have utilized cold storage reagents, which require controlled temperature cabinets and dry or wet ice shipment. However, recently, there has been a dramatic increase in the need for molecular diagnostic testing in near-patient and point-of-care settings, and ambient-stored reagents are a key factor in enabling this testing. This requirement has driven the markets towards lyophilized reagents that can be stored and shipped at ambient temperatures.

Obtaining regulatory approval for human *in vitro* diagnostic tests requires significantly greater time and consideration than developing Research Use Only (RUO) assays typically used in general research laboratories. Nonetheless, it is important to highlight that the developmental steps, performance and safety information, and the data needed for review by regulatory authorities remain consistent regardless of whether wet or lyophilized reagents are utilized. Transitioning an assay that utilizes wet reagents to an assay in a dry form is not as simple as just lyophilizing the wet reagents. Commonly-used liquid reagent formulations contain components such as glycerol or co-solvents that either do not allow for lyophilization or impact the stability and performance of a lyophilized version of the formulation. Many of these liquid-form components help with enzyme stabilization and assay sensitivity, which makes removing components problematic. However, many alternatives to these materials can restore assay performance, and these components can be identified through substitution testing. An additional consideration for lyophilization is the need for excipients and cryoprotectants that protect the macromolecules during freezing and provide for the preservation of the dried material. While it is critical to ensure optimal assay performance when excipients are included in the formulation, it should be noted that they may also act as alternative assay enhancers (Figure 1).

Just like when building a diagnostic assay with liquid reagents, the developer of a lyophilized assay must consider who their end user will be; how the assay will be incorporated into the laboratory's workflow; which instruments are critical for compatibility; and what quality and regulatory requirements will govern its use. When working with multiple vendors and components to build the final assay, it is important to understand any reconfiguration the developers may undertake when the lyophilized assay is supplied as a subcomponent of a workflow or device. Understanding the requirements for handling, transfer and repacking will help the development team make the appropriate product. Vendors specializing in lyophilization can guide development teams on design-for-manufacturing (DFM) options. Ensuring efficient communication of this information at the start of the development process will help the team stay on track as the assay developer completes the product realization process.



Packaging

Product aging for a lyophilized material begins when it is exposed to the atmosphere. Part of the product manufacturing process should be to verify the stability

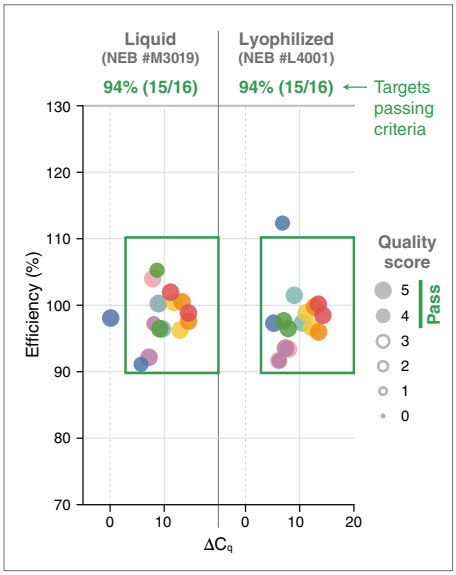


Figure 1: LyoPrime Luna® matches the performance of the liquid Luna reagent

LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) was compared to Luna® Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) and tested on eight human RNA targets varying in abundance, length, and %GC. Data were collected by two users, and results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔCq = average Cq of non-template control – average Cq of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) yielded results comparable to those of the liquid format Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019). Learn more about our comprehensive qPCR/RT-qPCR testing and "dots in boxes" data visualization at www.neb.com/tools-and-resources/video-library/dots-in-boxes-visualization-of-qpcr-data.

of the assay if it is exposed to the atmosphere. While not an obvious consideration, selecting the appropriate primary packaging format can also impact stability and performance of a lyophilized product over time. The optimal format and composition of the packaging is dependent on the workflow compatibility within the required integrated instruments. Most workflows and integrated instruments will have an assay housed in a molded plastic vessel designed to work with the associated instrumentation.

Many plastics are not resistant to moisture and oxygen ingress, and as a result, the lyophilized assay within the vessel will start to absorb moisture and oxygen even when sealed in this type of container. Therefore, secondary packaging that is resistant to both moisture and oxygen ingress is often used. Secondary packaging includes metalized polymer bags that may be zip-lock and/or heat-sealed. The secondary packaging is often sealed under an inert atmosphere, such as nitrogen gas, and may include a desiccant material to adsorb residual moisture. While this packaging should be designed to minimize the reagents' exposure to both moisture and air, in many cases it should also be designed to minimize exposure to light. Many fluorogenic assay components used in real-time analysis are light sensitive and, if not protected, can degrade over time, reducing assay performance.

Optimizing lyophilization conditions and process

As with optimizing the formulation of the assay components for performance, there is also a requirement to optimize the lyophilization process conditions (Figure 2). Lyophilization is conducted through a controlled process of temperature and pressure changes to facilitate drying. The overall time of each step and the total process will depend upon the formulation of the wet reagents, reagent volume, and container geometry. Poorly optimized processes can lead to incomplete drying and reagent collapse (i.e., the resulting reagent cake losing its intended structure). Products of incomplete drying and collapse can exhibit poor stability and other issues, such as poor dissolution characteristics and inconsistent assay performance. Just as it is possible to lyophilize an assay incompletely, it is also possible to lyophilize "too much" and make an assay that is too dry, resulting in poor enzyme(s) performance.

Process Validation

The ISO 13485 standard requires that the processes used to make a product are robust. In some instances,where measurements may not be easily applied, process validation shall be required. For example, simple temperature and pressure measurements in a freeze dryer will not provide sufficient information that the drying process is robust.

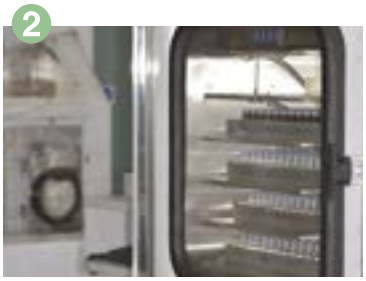
Process validation involves testing using pre-determined (outcome) values to demonstrate the process meets its requirements. Such processes include mixing reagents, dispensing methods, lyophilization, and packing processes such as the application of heat seals to plates and bag closures. For lyophilization, the drying process requires sampling plans for specific dryers to ensure that the process performs well at all locations on all shelves within the freeze dryer cabinet. These processes complement product validation to ensure that the test is robust in manufacturing, storage and intended use, and reduces any risks of the test affecting patient safety.

Figure 2: Overview of the lyophilization process

1

Optimized assay
Including assay components and excipients

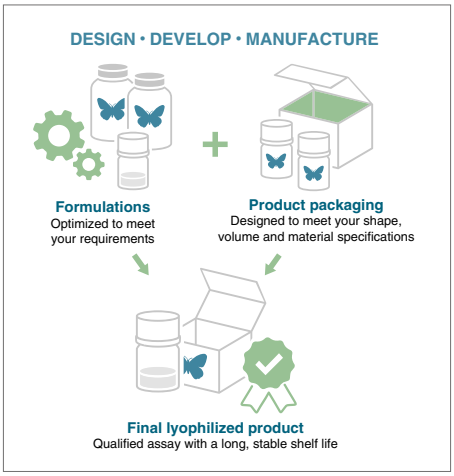
2



Drying
1. *Freeze assay by decreasing the temperature inside the dryer*
2. *Decrease the pressure inside the dryer to remove water vapor*
3. *Slowly raise the temperature inside the dryer while keeping the atmospheric pressure low*

3

Reconstitution
Ideal „cake" is easily reconsituted when assay diluent is added to vial



Product Validation and Verification

Multiple parameters must be demonstrated to show that an assay meets its intended use. Some parameters are assay reproducibility, analytical sensitivity and specificity, repeatability, false positive and false negative rates. These are carried out as part of pre-analytical studies, usually prior to clinical testing. For infectious disease testing, this will also include inclusivity and exclusivity testing. Inclusivity testing determines the percentage of target samples that correctly determines that a sample is positive. In contrast, exclusivity testing determines the percentage of non-target sample that correctly determines that a sample is negative. The testing of interfering substances on the test will also be carried out.

Clinical assay reproducibility looks at how consistent assay results are when performed at multiple trial sites using pre-defined population sample sets. Clinical trials typically determine and validate the products' clinical sensitivity, specificity, and the test's positive and negative predictive value.

Technological advances over the past few years have made it possible to shift some molecular diagnostic tests away from only being performed in specialized laboratories to point of care testing locations, including in-home testing. One of the advancements that has permitted this shift is the ability to create lyophilized assays that are stable at ambient temperatures and perform equivalently to traditional assays making point-of-care testing more accessible across the globe.

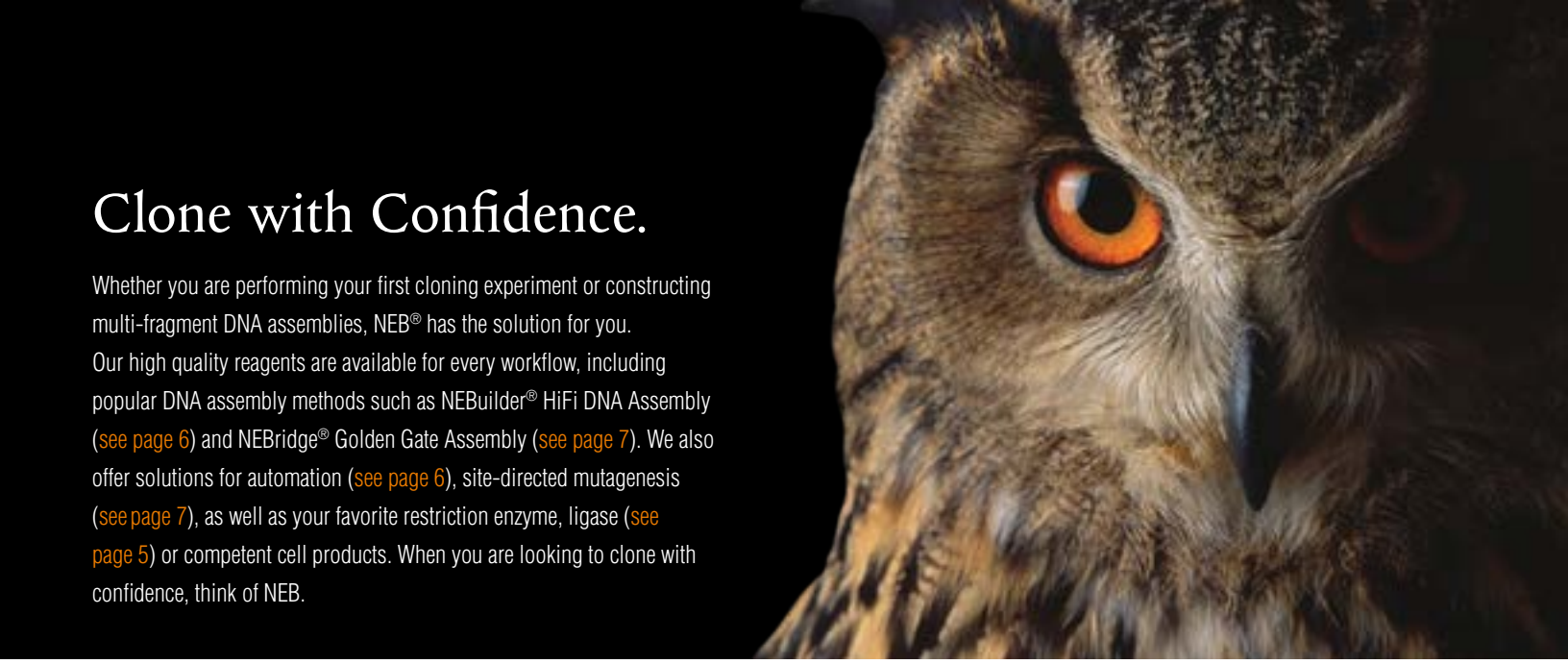
To learn more about lyophilization services available through NEB Lyophilization Sciences, visit
www.neb.com/lyosciences

To learn more about how we can work with you to develop your molecular diagnostic assay, contact us at
custom@neb.com

Amplification-based solutions for Molecular Diagnostics

Our extensive expertise in amplification, including PCR, qPCR, RT-qPCR and isothermal amplification has allowed us to develop optimized enzymes for a variety of applications, including incorporation into diagnostics. *Learn more at www.neb.com/MDx*.

	APPLICATION	PRODUCTS	PRODUCT NOTES	CUSTOM FORMULATIONS
PCR APPLICATIONS	qPCR/ RT-qPCR	**DNA, Dye** • Luna Universal qPCR Master Mix (NEB #M3003) **DNA, Probe** • Luna Universal Probe qPCR Master Mix (NEB #M3004)	• Compatible with automated liquid handling and reaction miniaturization • Room temperature stable for ≥ 24 hours	• Blue-dye-free • Lyo-compatible
		RNA (1-step), Dye • Luna Universal One-Step RT-qPCR Kit (NEB #E3005) **RNA (1-step), Probe** • Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) • Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX) (NEB #M3029) • Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) • Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) • Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019) • LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)	• Luna WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours • Lyophilized format (NEB #L4001)	• Blue-dye-free • Lyo-compatible • Add primers and probes
		RNA (2-step) • LunaScript® RT SuperMix (NEB #E3010/#M3010)	• Novel thermostable RT • Single-tube format • 13-minute cDNA synthesis protocol	• Blue-dye-free
	PCR/ RT-PCR	**Master Mixes** • Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494) • Q5 High-Fidelity 2X Master Mix (NEB #M0492) **Standalone Enzyme & Buffer** • Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) • Q5 High-Fidelity DNA Polymerase (NEB #M0491)	• ~280X fidelity of *Taq* • Consistent, fast, reliable performance • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours	• High conc. • Glycerol-free • Custom mixes
		• Q5 Blood Direct 2X Master Mix (NEB #M0500)	• Amplification direct from blood	
		• Hot Start *Taq* DNA Polymerase (NEB #M0495) • Hot Start *Taq* 2X Master Mix (NEB #M0496)	• Unique aptamer-based enzyme control supports fast protocols	• High conc. • Glycerol-free
ISOTHERMAL APPLICATIONS	RT-LAMP	• WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB #M1800) • WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804)	• Fast, clear pink-to-yellow visible detection of amplification • Results in approximately 30 minutes • Automation-compatible when coupled with absorbance plate reader	
		• SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB #E2019)	• Simple, colorimetric detection of amplification of SARS-CoV-2 nucleic acid	
		• WarmStart LAMP Kit (DNA & RNA) (NEB #E1700) • WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708) • WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) (NEB #E1708)	• Master mix for LAMP and RT-LAMP workflows • Supports multiple detection methods, including fluorescence and turbidity	• Lyo-compatible • High conc.
		• Tte UvrD Helicase (NEB #M1202)	• Improves specificity of fluorescent LAMP rxns	• High conc.
		• Bst 2.0 WarmStart DNA Polymerase (NEB #M0538) • Bst 2.0 DNA Polymerase (NEB #M0537)	• Improved reaction properties compared to wild-type Bst DNA Polymerase • Increased dUTP tolerance enables carryover prevention	• Glycerol-free • High conc.
		• Bst 3.0 DNA Polymerase (NEB #M0374)	• DNA binding domain fusion supports robust performance • Significantly increased RT activity up to 72°C enables single enzyme RT-LAMP	• Glycerol-free • High conc.
		• WarmStart RTx Reverse Transcriptase (NEB #M0380)	• *In silico* designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C	• Glycerol-free • High conc.
	Strand Displacement	• Nt.BstNBI (NEB #R0607) • WarmStart Nt.BstNBI (NEB #R0725)	• High purity, high quality nicking endonuclease	• Glycerol-free • High conc.
	Helicase-dependent Amplification	• IsoAmp II Universal tHDA Kit (NEB #H0110)	• Requires only two primers • Produces short, discrete DNA products	
	Other	• Bsu DNA Polymerase, Large Fragment (NEB #M0330)	• Enables low temperature isothermal applications	• High conc.
		• T4 Gene 32 Protein (NEB #M0300)	• Can increase yield and efficiency of amplification rxns	• Glycerol-free
		• Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)		• Custom conc.
		• Nuclease-free Water (NEB #B1500)		
		• Antarctic Thermolabile UDG (NEB #M0372)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• High conc.
		• Proteinase K, Molecular Biology Grade (NEB #P8107)		• Custom conc.
		• Thermolabile Proteinase K (NEB #P8111)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• Custom conc.



Clone with Confidence.

Whether you are performing your first cloning experiment or constructing multi-fragment DNA assemblies, NEB® has the solution for you. Our high quality reagents are available for every workflow, including popular DNA assembly methods such as NEBuilder® HiFi DNA Assembly (see page 6) and NEBridge® Golden Gate Assembly (see page 7). We also offer solutions for automation (see page 6), site-directed mutagenesis (see page 7), as well as your favorite restriction enzyme, ligase (see page 5) or competent cell products. When you are looking to clone with confidence, think of NEB.

Spot on Ligation: The last step in cloning workflows – Never compromise on quality and performance!

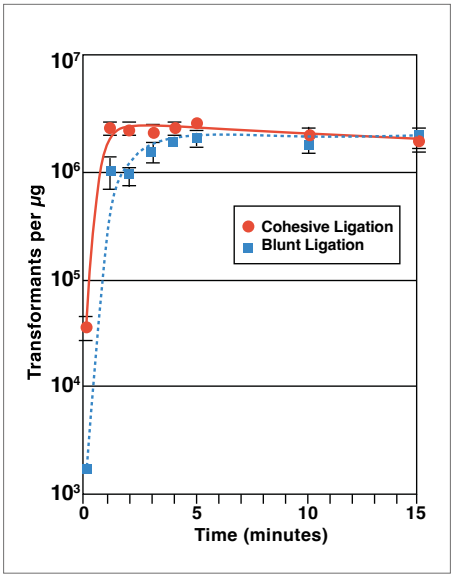
Any way you look at it, ligation of DNA fragments is the last and crucial step in DNA cloning regardless if you performed traditional restriction enzyme-based approaches or modern DNA assembly techniques. Never risk the outcome of your entire cloning or assembly approach just by compromising on quality and performance of your Ligase! Use NEB's Ligase products instead – trusted by your peers and cited in tens of thousands of publications.

Here, we present two quick solutions that accelerate your research.

Explore the wise choice at
CloneWithNEB.com

Quick Ligation Kit

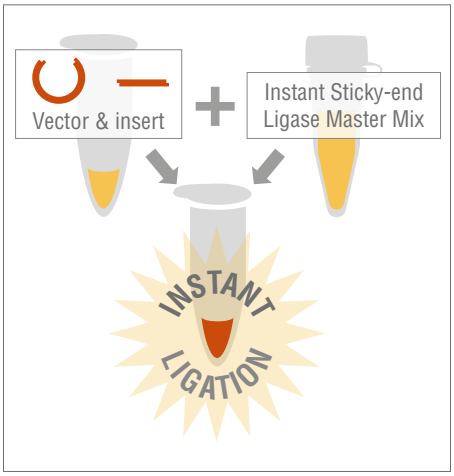
- Fast – 5 minutes for cohesive or blunt ends
- Convenient – ligation performed at RT
- Flexible – suitable for all common ligation rxns



Optimal ligation and transformation efficiency in just 5 min at room temperature (RT). For more experimental details visit www.neb.com/M2200

Instant Sticky-end Ligase Master Mix

- Fastest ligation possible – No incubation time necessary to achieve instant ligation
- No thawing of the master mix is required
- Simplified reaction setup with optimized ratio of enzyme and buffer components



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Ordering Information

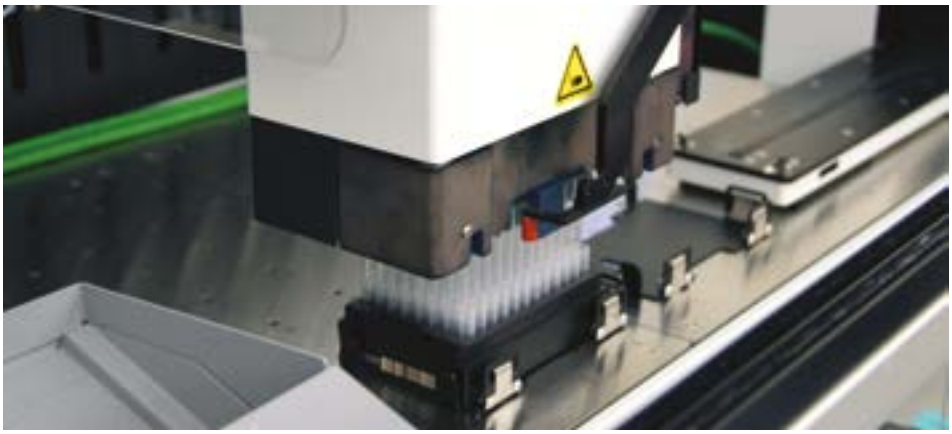
PRODUCT	NEB #	SIZE
Quick Ligation Kit	M2200S/L	30/150 rxns
Instant Sticky-end Ligase Master Mix	M0370S/L	50/250 rxns
Blunt/TA Ligase Master Mix	M0367S/L	50/250 rxns

Clone with confidence at any scale – from single experiments to high-throughput

High-throughput cloning is a molecular biology method for assembling large numbers of DNA sequences, such as genes, open reading frames (ORFs) or highly repetitive gRNAs, to create libraries and enable screening of constructs, protein expression or protein function.

By integrating automation, researchers can scale up and increase throughput to hundreds or thousands of rxns, save time and money with rapid workflows and miniaturized volumes, and improve reproducibility with automated complex mixing that reduces manual errors.

NEB offers a wide variety of products that enable highly efficient and accurate DNA assembly and mutagenesis, sequencing, cell-free protein synthesis and purification that are all amenable to high-throughput workflows and automation devices.



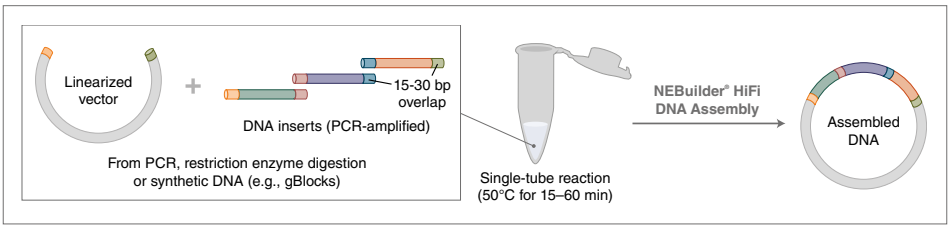
To learn more, visit
www.neb.com/AutomateWithConfidence

Recommended Products for Quick & Reliable DNA Assembly:

A) NEBuilder HiFi DNA Assembly

- Perform less sequencing and screening of constructs with high-fidelity, virtually error-free assembly
- Enjoy compatibility with synthetic dsDNA fragments, such as gBlocks™, and ssDNA oligos
- Save time by avoiding PCR clean-up, simplifying your workflow
- Supports miniaturization with nanoliter scale volumes
- Easily adaptable to multiple site-directed mutagenesis
- Design primers and assemblies quickly and easily with our free online tool, NEBuilder Assembly Tool

Explore the wise choice at
nebuilder.neb.com



Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L/X	10/50/250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 rxns
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 rxns

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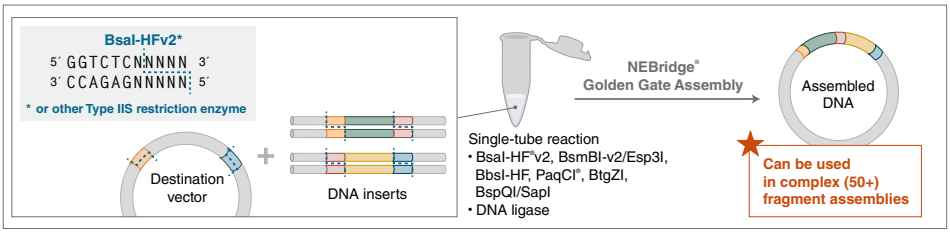
B) NEBridge Golden Gate Assembly

- Experience high efficiency within regions of high GC content and areas of repeats
- Supports miniaturization
- Enjoy compatibility with synthetic dsDNA fragments, such as gBlocks
- Find flexibility with NEBridge Ligase Master Mix (NEB #M1100) and your choice of Type IIS restriction enzymes
- Use our free online tools to design complex, high-fidelity Golden Gate Assemblies
  - NEBridge Golden Gate Assembly Tool – design primers, predict overhang fidelity and find optimal junctions
  - NEBridge Ligase Fidelity Tools – visualize overhang ligation preferences, predict high fidelity junction sets and split DNA sequences for scarless high-fidelity assembly



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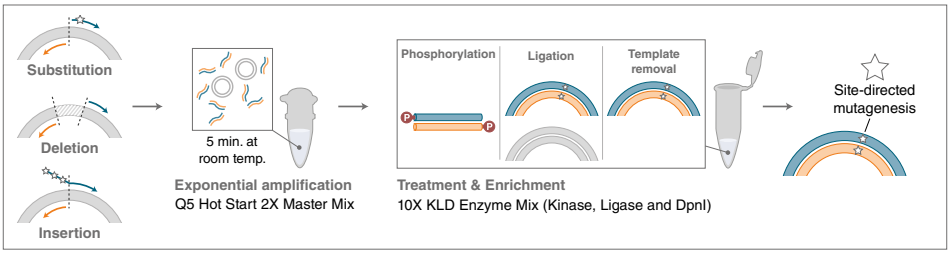
Ordering Information

PRODUCT	NEB #	SIZE
NEBridge Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 rxns
NEBridge Golden Gate Assembly Kit (BsaI-HF v2)	E1601S/L	20/100 rxns
NEBridge Ligase Master Mix	M1100S/L	50/250 rxns

Recommended Products for Site-directed Mutagenesis:

A) Single site-directed mutagenesis

- Quickly create mutant libraries using primers designed with the free online tool NEBaseChanger®
- For manual pipetting workflows, use the optimized Q5® Site-Directed Mutagenesis Kit that enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours
- Reduce screening of correct mutants and take advantage of room temperature reaction set up
- For scale-up, miniaturization or automation, use the separately available Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494) for PCR amplification followed by KLD Enzyme Mix (NEB #M0554) for kinase, ligase and DpnI enzymatic activities in a single mix



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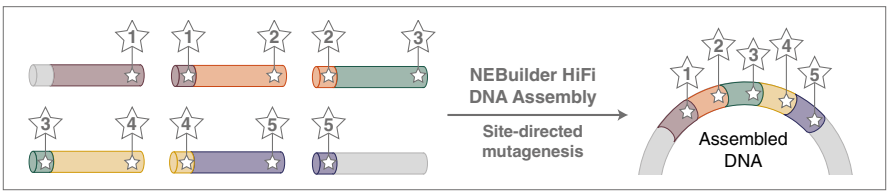
Ordering Information

PRODUCT	NEB #	SIZE
Q5 Site-Directed Mutagenesis Kit	E0554S	10 rxns
Q5 Site-Directed Mutagenesis Kit (Without Competent Cells)	E0552S	10 rxns
Q5 Hot Start High-Fidelity 2X Master Mix	M0494S/L/X	100/500/500 rxns

B) Multi site-directed mutagenesis

- Using NEBuilder HiFi DNA Assembly, perform multi-site mutagenesis or combinatorial mutagenesis for diverse multi-site mutant library creation and screening



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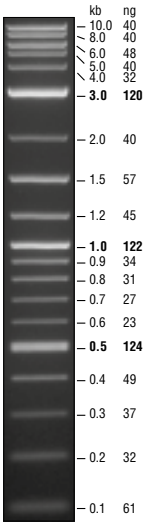
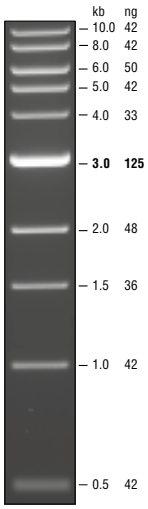
Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L/X	10/50/250 rxns

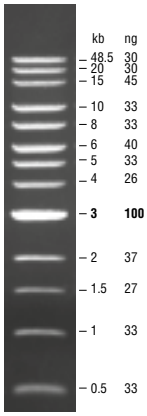
NEB's Markers and Ladders – built for perfect migration!



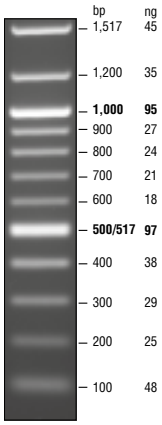
1 kb Plus
1.0% TBE agarose gel. Mass values are for 1 µg/lane.



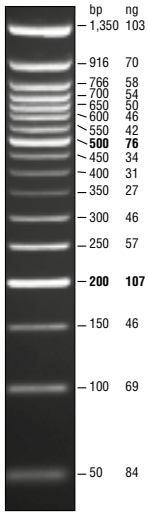
1 kb
0.8% TAE agarose gel. Mass values are for 0.5 µg/lane.



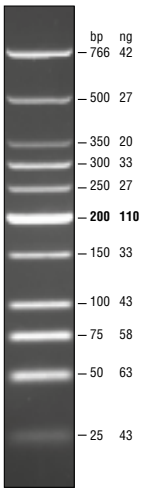
Quick-Load 1 kb Extend DNA Ladder
0.5% TBE agarose gel. Mass values are for 0.5 µg/lane.



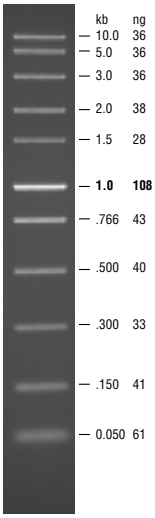
100 bp
1.3% TAE agarose gel. Mass values are for 0.5 µg/lane.



50 bp
3% TBE agarose gel. Mass values are for 1 µg/lane.



Low Molecular Weight
3% TBE agarose gel. Mass values are for 0.5 µg/lane.



Fast DNA Ladder*
1.2% TBE agarose gel. Mass values are for 0.5 µg/lane.

*Fast DNA Ladder can be used for fast electrophoresis systems (e.g., E-Gels®) as well as standard electrophoresis.

AVAILABLE FORMATS:

	1 kb Plus	1 kb	Quick-Load 1 kb Extend DNA Ladder	100 bp	50 bp	Low Molecular Weight	Fast DNA Ladder*
Standard	#N3200	#N3232		#N3231	#N3236	#N3233	#N3238
Quick-Load Purple	#N0550	#N0552		#N0551	#N0556	#N0557	
Quick-Load	#N0469	#N0468	#N3239	#N0467			
TriDye	#N3270	#N3272		#N3271			
For Safe Stains:	#N0559						

Ready-to-use, pre-diluted formats for added convenience:

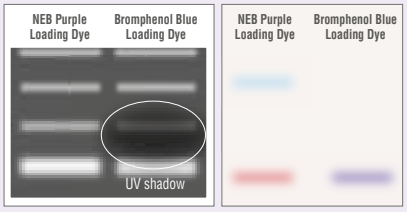
- Quick-Load® Purple: containing purple loading dye
- Quick-Load®: containing Bromophenol Blue
- TriDye™: containing three dyes for better visualization
- For Safe Stains: compatible with GelRed®, GelGreen® and SYBR® precast gels

SPECIAL PRICE* until 31.12.2023

Please ask your local distributor for details!

Included with NEB DNA Markers & Ladders: Gel Loading Dye, Purple (6X)

- Brighter, sharper bands vs. conventional loading buffers
- No UV shadow allows for publication-grade images
- Stops enzyme reactions



Gel Loading Dye, Purple (6X), no SDS, is included in most DNA ladders and markers. This unique loading buffer does not cast a UV shadow over the underlying bands, unlike conventional Bromophenol Blue containing loading buffers.

Ordering Information

PRODUCT	NEB #	SIZE
1 kb Plus:		
1 kb Plus DNA Ladder	N3200S/L	200/1,000 gel lanes
Quick-Load Purple 1 kb Plus DNA Ladder	N0550S/L	250/750 gel lanes
Quick-Load 1 kb Plus DNA Ladder	N0469S	250 gel lanes
TriDye 1 kb Plus DNA Ladder	N3270S	250 gel lanes
1 kb Plus DNA Ladder for Safe Stains	N0559S	250 gel lanes
1kb:		
1 kb DNA Ladder	N3232S/L	200/1,000 gel lanes
Quick-Load Purple 1 kb DNA Ladder	N0552S/L	125/375 gel lanes
Quick-Load 1 kb DNA Ladder	N0468S/L	125/375 gel lanes
TriDye 1 kb DNA Ladder	N3272S	125 gel lanes
Quick-Load 1 kb Extend DNA Ladder		
Quick-Load 1 kb Extend DNA Ladder	N3239S	125 gel lanes
100 bp:		
100 bp DNA Ladder	N3231S/L	100/500 gel lanes
Quick-Load Purple 100 bp DNA Ladder	N0551S/L	125/375 gel lanes
Quick-Load 100 bp DNA Ladder	N0467S/L	125/375 gel lanes
TriDye 100 bp DNA Ladder	N3271S	125 gel lanes
50 bp:		
50 bp DNA Ladder	N3236S/L	200/1,000 gel lanes
Quick-Load Purple 50 bp DNA Ladder	N0556S	250 gel lanes
Low Molecular Weight:		
Low Molecular Weight DNA Ladder	N3233S/L	100/500 gel lanes
Quick-Load Purple Low Molecular Weight DNA Ladder	N0557S	125 gel lanes
Fast DNA Ladder:		
Fast DNA Ladder	N3238S	50 gel lanes

NEW: phi29-XT RCA Kit – speeding up Sanger Sequencing or Protein Expression Workflows

Featuring phi29-XT DNA Polymerase, an engineered polymerase with improved thermostability, sensitivity, and capable of generating high product yield in a short reaction time, the new phi29-XT RCA Kit includes everything needed for sensitive and reliable rolling circle amplification (RCA).

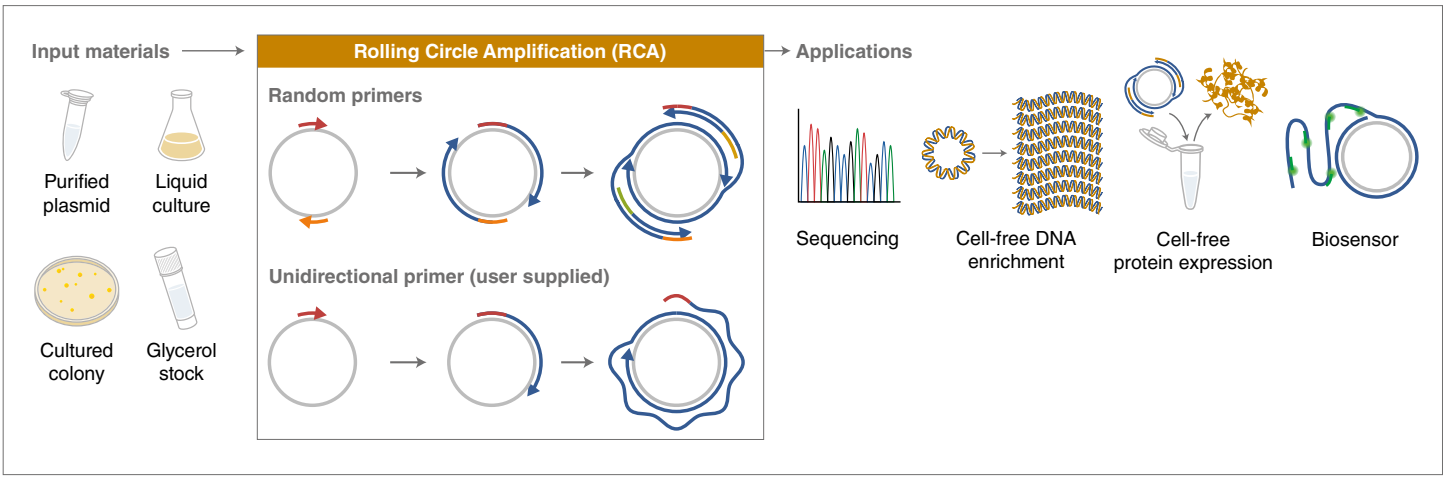
Input material can be purified circular DNA (single or double-stranded) or direct from liquid media culture, agar plate colonies, and glycerol stocks without the need for DNA extraction.

Moreover, RCA products can be used directly in downstream applications such as DNA Sanger sequencing, cell-free protein expression, cell-free DNA enrichment, and DNA biosensors, making the new kit the optimal DNA amplification tool.

Advantages

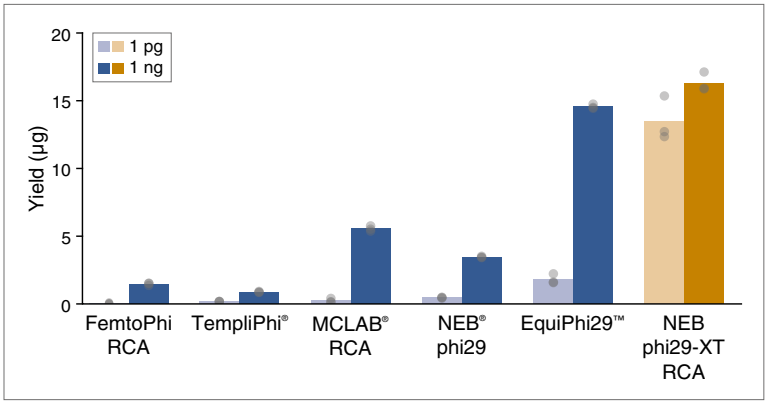
- Amplify from as little as 1 fg of circular DNA input in less than 2 hours at 42°C
- Flexible protocol offers compatibility with different types of input material incl. unpurified material
- Enables multiple downstream applications without further processing/purification steps
- Kit includes dNTPs and exo-resistant random primers

Overview of the phi29-XT RCA Kit



The phi29-XT RCA Kit (NEB #E1603) is a fast, simple to use and highly versatile kit containing all the required components for rolling circle amplification (RCA) using a random primer mix. The kit delivers high yields of DNA products from a variety of starting materials including purified circular DNA or bacterial cells. This kit is ideal for various DNA applications such as DNA sequencing, cell-free DNA enrichment, cell-free protein expression and DNA biosensors.

The phi29-XT RCA Kit offers exceptional sensitivity and product yield



Triplicate RCA rxns were carried out using commercially available phi29 DNA polymerases, according to manufacturers' protocols, for 2 hours with 1 pg or 1 ng pUC19 plasmid as the starting material. Reaction yields (dots) were quantified using Quant-iT® PicoGreen® dsDNA Reagent and averaged (bar). The phi29-XT RCA Kit (NEB #E1603) generates more product in less time than other commercially available products.

View more technical data and recommended protocol at
www.neb.com/e1603

Ordering Information

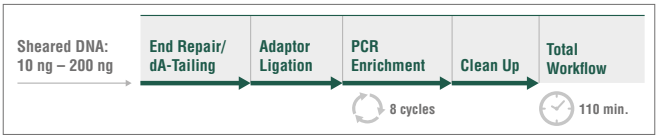
PRODUCT	NEB #	SIZE
phi29-XT RCA Kit	E1603S/L	100/500 rxns
COMPANION PRODUCTS		
NEBExpress Cell-free *E. coli* Protein Synthesis System	E5360S/L	10/100 rxns
T7 Endonuclease I	M0302S/L	250/ 1,250 rxns
Monarch DNA & PCR Cleanup Kit (5 µg)	T1030S/L	50/ 250 preps

New NEBNext Library Prep Solutions Coming Soon

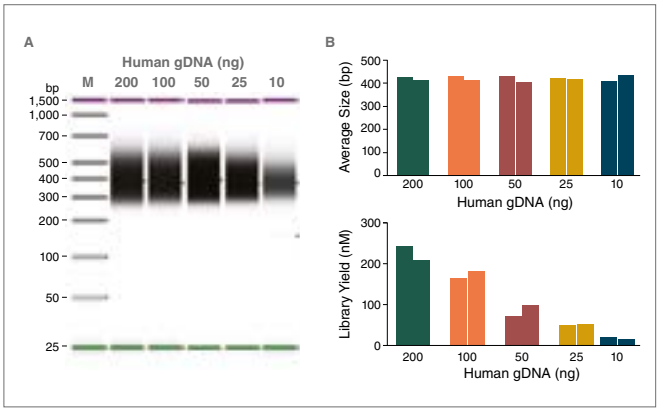
(please check with your distributor for availability)

NEBNext UltraExpress™ DNA Library Prep Kit

The NEBNext UltraExpress DNA Library Prep Kit is the latest generation of NEBNext DNA library prep, with a fast, streamlined workflow to generate high yields of high-quality libraries. The workflow allows processing of samples with a wide range of input amounts of pre-sheared DNA using a single protocol, without adjustment of reaction conditions.



NEBNext UltraExpress DNA Workflow



NEBNext UltraExpress DNA generates high yields of high quality libraries, across a broad input range.

A-B. Libraries were made using 10-200 ng Covaris®-sheared Human NA19240 genomic DNA and the NEBNext UltraExpress™ DNA Library Prep Kit, with the same amount of adaptor and the same PCR conditions (8 cycles) for each. Libraries were pooled and sequenced on the Illumina® MiSeq®.

Advantages

- Fast workflow (< 2 hours)
- Fewer steps and consumables
- Fewer cleanups
- Wide input range (10-200 ng pre-sheared DNA)
- Single protocol for all inputs
- Automation friendly

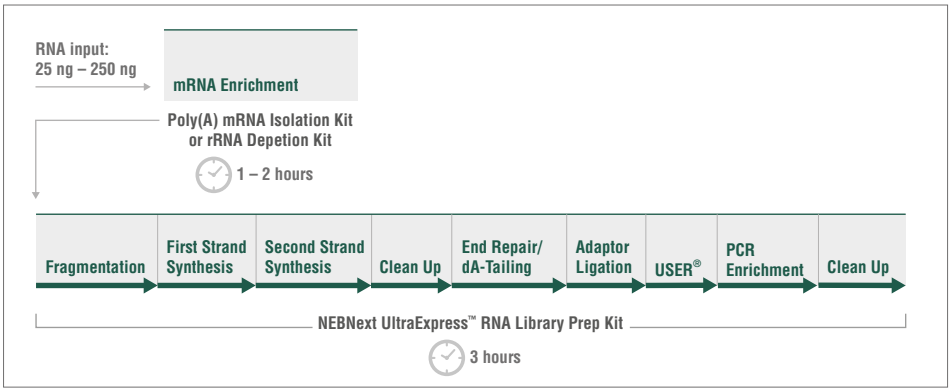
Ordering Information

PRODUCT	NEB #	SIZE
NEBNext UltraExpress™ DNA Library Prep Kit	E3325S/L	24/96 rxns

NEBNext UltraExpress™ RNA Library Prep Kit

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

High quality RNA-Seq libraries in a day.



Advantages

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

Ordering Information

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

For updates on the latest release dates, please visit NEBNext.com

NEBNext® FFPE DNA Library Prep & NEBNext UltraShear™ FFPE DNA Library Prep Kits

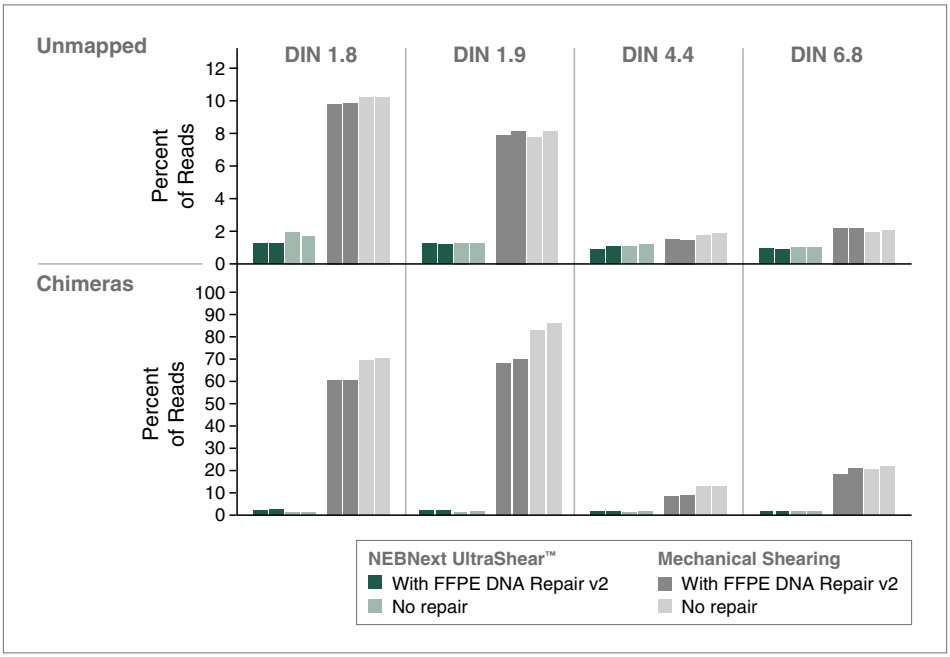
FFPE DNA poses many challenges for library preparation, including characteristically low input amounts and highly variable damage from fixation, storage, and extraction methods. Regions of interest are often enriched using hybrid capture-based approaches – these workflows require a high input of diverse, uniform DNA library.

The NEBNext FFPE DNA Library Prep Kit includes the NEBNext FFPE DNA Repair v2 Mix, an optimized cocktail of enzymes designed to repair FFPE DNA, library prep reagents featuring a new polymerase master mix, and a protocol optimized for FFPE DNA. The NEBNext UltraShear FFPE DNA Library Prep Kit also includes NEBNext UltraShear, a new solution designed for enzymatic fragmentation of challenging samples (e.g., FFPE DNA). This enzymatic shearing solution further increases library yields and quality, while improving scalability and ease of use.

Advantages

- Includes FFPE DNA repair reagents plus optimized library prep reagents and protocol
- Optional NEBNext UltraShear enzymatic fragmentation
- Increased library yields
- Improved sequencing metrics
- Greater sensitivity of somatic variant calling
- Automation-friendly workflows

The NEBNext UltraShear FFPE DNA Library Prep Kit improves the quality of data from low and high quality FFPE DNA samples



50 ng of FFPE DNAs with the DNA Integrity Numbers (DIN) shown were prepared using either the NEBNext UltraShear FFPE DNA Library Prep Kit (NEB #E6655), the NEBNext UltraShear Module (NEB #M7634), the NEBNext FFPE DNA Library Prep Kit (NEB #E6650) with Covaris-sheared DNA, or the NEBNext Ultra II DNA Library Prep Kit with Covaris-sheared DNA. Libraries were prepared using the NEBNext Multiplex Oligos Unique Dual Index Primer Pairs (NEB #E6440) with 10 PCR cycles, and sequenced on the Illumina NextSeq 500. Data was analyzed using 2 million paired-end reads, mapped using Bowtie 2 v2.3.2.2 end-to-end mapping, and analyzed using Picard Collect Alignment Summary Metrics v2.18.2.1. The NEBNext UltraShear FFPE DNA Library Prep Kit and the NEBNext UltraShear module increase the mapping rate and decrease the rate of chimeras.

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext FFPE DNA Library Prep Kit	E6650S/L	24/96 rxns
NEBNext UltraShear FFPE DNA Library Prep Kit	E6655S/L	24/96 rxns

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