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a scientific update

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 **NEW ENGLAND**
BioLabs®

Using PaqCI™ for Golden Gate Assembly – What Makes it a Special Addition to NEB's DNA Assembly Portfolio?



By Rebecca Kucera, M.S.,
NEB Principal Development Scientist,
New England Biolabs, Inc.

At NEB, we have placed focus on advancements in both the development of new enzymes and maximizing enzyme functionality for Golden Gate Assembly reactions. In that spirit, our Golden Gate Assembly choices now feature a new player: the exciting Type IIS restriction enzyme, PaqCI. In this article, read about how PaqCI (an AarI isoschizomer) can be used for simple to complex 24-fragment assembly, achieving our highest level of efficiency and fidelity yet, and with less concerns regarding the domestication of internal sites due to its 7 base-pair recognition sequence.

Golden Gate Assembly (GGA) is dependent on Type IIS restriction enzymes that have asymmetric DNA recognition sites and cleave outside of these sequences. NEB currently offers 50 Type IIS restriction enzymes, of which a subset have the necessary favorable characteristics for GGA. Enzymes such as BsaI-HF®v2, BsmBI-v2, and BbsI-HF have been Golden Gate workhorses, as they have historically been featured in published assembly protocols and NEB has extensive experience working with them. During this time, and with input from our customers, we recognized that it would be useful to offer an enzyme with a 7-base recognition site for assembly, along with fully optimized protocols and enzyme recommendations, for assemblies ranging from simple to complex, and at a reasonable price.

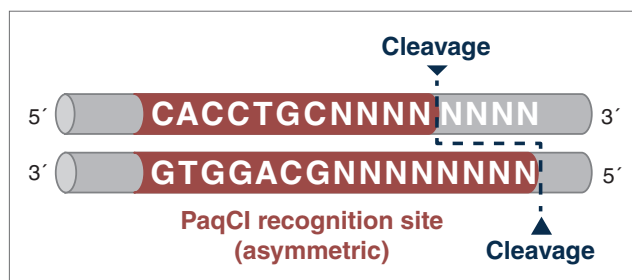
The advantage of a Type IIS restriction enzyme with a 7-base recognition site (see Figure 1) is that these sites are less likely to be present in the DNA sequences being assembled, yet they are capable of the full range of assembly complexity that scientists require for their experiments.

Through a collaboration between laboratories in NEB's Research, Applications Development, and Production Departments, PaqCI was identified and cloned, and its expression was optimized. A DNA activator for the enzyme was also optimized and protocols were developed for single inserts, as well as simple-to-complex assemblies.

The significance of PaqCI with regards to domestication

Domestication refers to converting any DNA fragment that will be part of an assembly into "Golden Gate-ready" form - flanking the DNA at both ends with the Type IIS restriction sites that will direct the assembly and removing any internal sites for that enzyme that might be present in the DNA and are not tolerated well in

Figure 1: Type IIS enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence



GGA. Statistically, a 7-base sequence will appear in any given DNA sequence less often than the 6-base sequence of the more commonly used Type IIS restriction enzymes. Internal sites significantly decrease GGA efficiency because they allow the finished construct to be susceptible to digestion by the restriction enzyme present in the assembly reaction, and could also lead to incorrect and unwanted assemblies.

This is less of an issue when using Golden Gate for single insert cloning because the overall efficiency for single inserts is high; the desired construct will be assembled even if many of the successfully cloned inserts became linearized and did not efficiently transform. But typically, researchers are using Golden Gate for multiple inserts – and the greater the assembly complexity, the more important the assembly efficiency becomes. For this reason, the presence of an internal recognition site of the chosen restriction enzyme, hinders the assembly.

There are proven methodologies for eliminating internal sites while domesticating DNA sequences: (1) site-directed mutagenesis to eliminate an internal site in advance of the assembly reaction, or (2) designing an assembly junction point right at the internal restriction site with a base change to eliminate the site upon assembly.

However, domestication of a DNA sequence is time consuming, further highlighting the benefit of a 7-base recognition site enzyme, which significantly decreases the probability of internal sites. PaqCI is a 7-base recognition restriction enzyme that has been optimized for Golden Gate Assembly, and is supplied at a concentration that enables use for complex assemblies up to 20+ fragments.

The mechanism of multi-site enzymes and why they benefit from the addition of an activator

Some enzymes have more intricate ways of interacting with their recognition sites in DNA than others. Most homodimeric enzymes, like the standard Type IIP restriction enzymes EcoRI and HindIII, have two identical subunits that bind cooperatively at the symmetric site with each subunit cutting one strand to result in a double-stranded cut. In contrast, multi-site enzymes like PaqCI have a more complex structure and mechanism. It is presumed that PaqCI utilizes multiple subunits to interact with two recognition sites in order to cleave a single target site. To be sure that PaqCI cuts all the sites during Golden Gate Assembly, NEB supplies an inert short

oligonucleotide activator containing an extra PqCI binding site, which functions in *trans* as an activator for PqCI cleavage (see Figure 2).

By definition, during Golden Gate Assembly, every insert and every destination plasmid has an assembly active DNA fragment flanked by two sites, implying that there is no need for any added sites. But Golden Gate is a very dynamic process, with concurrent cutting and ligating – situations arise where PqCI binds and cuts sites on different DNA molecules, leaving a remaining site on each molecule to be cut. So having an optimized number of extra sites available in the form of the PqCI activator ensures that complete cutting in the assembly reaction occurs. It should be noted that the activator does not get cut or interact in any way with the assembly – it only provides a second binding site that can activate cutting.

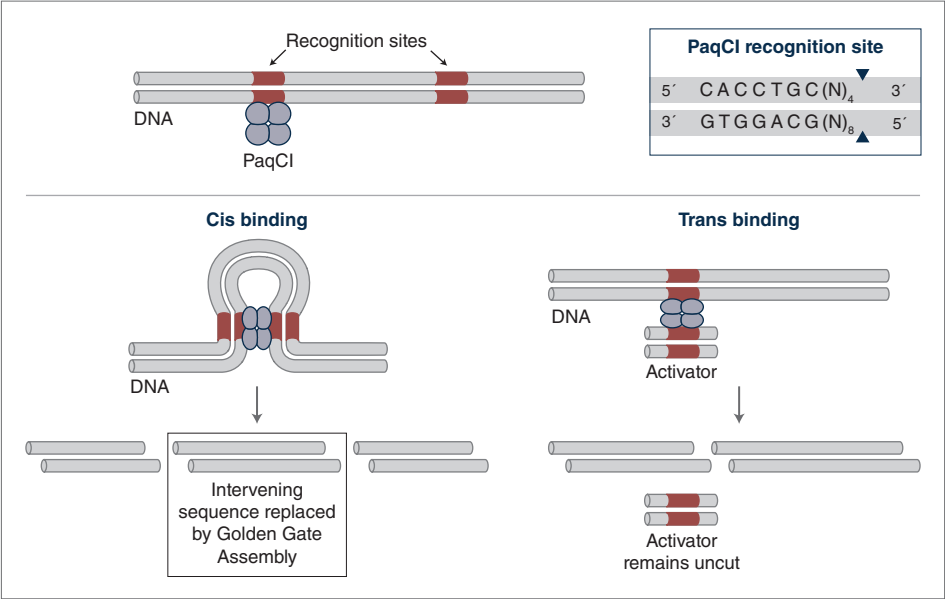
Different levels of complexity call for different levels of PqCI and T4 DNA Ligase. In addition, PqCI and activator amounts have been carefully optimized for different assembly complexities. The optimal amount of the activator can be different from what is recommended for a standard restriction digest with PqCI, where using 1 µl of the enzyme (10 U) requires 1 µl of the activator (20 pmoles). The reason for this is that cutting of DNA in a typical restriction digest, where cut DNA remains cut, is different than what occurs in Golden Gate assembly reactions, where overhangs can sometimes be reannealed and ligated, reconstructing the original recognition site. In the latter case, any one DNA cut site can require being cut more than once throughout the assembly reaction. Because of the dynamic nature of GGA, these regenerated sites translate to less supplementary sites in the form of the activator being needed.

From over a thousand test assembly reactions, NEB researchers have established the optimal amount of PqCI, activator, and T4 DNA Ligase for everything from simple single insert cloning to a complex 24-fragment assembly (see Table 1).

As assembly reactions increase in complexity, more units of enzyme are required for maximal performance; the range is from 5 to 20 U of PqCI paired with 200-800 U of T4 DNA Ligase. Recommendations for how much activator to add to each assembly reaction are within a range of 5-10 pmoles. A 20µM stock of the activator is provided with the PqCI enzyme.

One note regarding the buffer requirements: while rCutSmart Buffer is the recommended buffer for use in a simple DNA digest with PqCI, for Golden Gate Assembly, there are better efficiencies achieved by maximizing the PqCI and T4 DNA Ligase enzyme activities using T4 DNA Ligase Reaction Buffer.

Figure 2: Presumed mechanism for how the PqCI activator assures complete cutting via *trans* binding if needed



Golden Gate Assembly tools from New England Biolabs

At NEB, we have designed several online tools to help facilitate your Golden Gate workflows. After designating the DNA fragments for any given assembly, the NEB Golden Gate Assembly Tool can design optimal unique four base overhangs between the inserts that have been independently verified through T4 DNA Ligase fidelity studies to work at high fidelity. It will also automatically check your inserts for the presence of any internal sites that might affect the choice of Type IIS restriction enzyme to direct an assembly, or alert the user to remove such internal sites via domestication. The program will also automatically generate a set of primers for your inserts to add the flanking bases and recognition sites required either for amplicon generation of inserts to be directly used or for pre-cloning purposes. Finally, a report can be generated describing your full assembly with a

color-coded graphical read out, your final assembly sequence, and descriptions of each junction between inserts.

In addition, there are also useful programs available under the “Utility” tab within the tool. Those programs can take an uploaded sequence and make suggestions for different desired insert or module design and can also provide you with vetted lists of what overhangs have been found to support high efficiencies and fidelities during Golden Gate Assembly. Together the NEB Golden Gate Assembly Tool makes assembly design easy, even for the first time user! The NEB Golden Gate Assembly Tool is available at goldengate.neb.com.

Learn more about the Golden Gate Assembly workflow and usage guidelines for working with PqCI by visiting www.neb.com/R0745. For additional information about Golden Gate Assembly, visit neb.com/goldengate.

Table 1: Recommendations for PqCI Golden Gate Assembly

Assembly Complexity	PqCI	T4 DNA Ligase	PqCI Activator ³
Single Insert Cloning (10 min 37°C) or Library Prep (60 min 37°C)	5 U	200 U	+ 5 pmoles (1/4 µl 20 µM stock)
Simple to Moderate assembly ¹ (2-10 fragments)	5-10 U	200-400 U	+5 pmoles (1/4 µl 20 µM stock)
Complex assembly ² (11-20+ fragments)	10-20 U	400-800 U	+5-10 pmoles (1/4-1/2 µl 20 µM stock)

1. Based on 5 fragment assembly test system.
2. Based on 24 fragment assembly test system.
3. The activator solution is in a Mg-free buffer for best long-term storage. For short-term working stocks, if desired, dilute an appropriate amount in 1X T4 DNA ligase buffer to achieve more easily pipettable volumes (e.g., a four-fold dilution = 5 µM, 5 pmoles/µl activator).

Interested in fast and reliable DNA Cloning? Get started with Golden Gate Assembly.

Golden Gate Assembly is a molecular DNA assembly technique that utilizes simultaneous digestion with Type IIS restriction enzymes and ligation by a DNA ligase to enable the seamless, ordered assembly of multiple fragments (see page 2-3). Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence. The absence of internal sites in a sequence determines the choice of which Type IIS restriction enzyme to drive the assembly.

With over 45 Type IIS restriction enzymes available and constant advances in both the development of new enzymes and research on maximizing enzyme functionality (e.g., ligase fidelity), NEB is the industry leader in pushing the limits of Golden Gate Assembly and related methods.

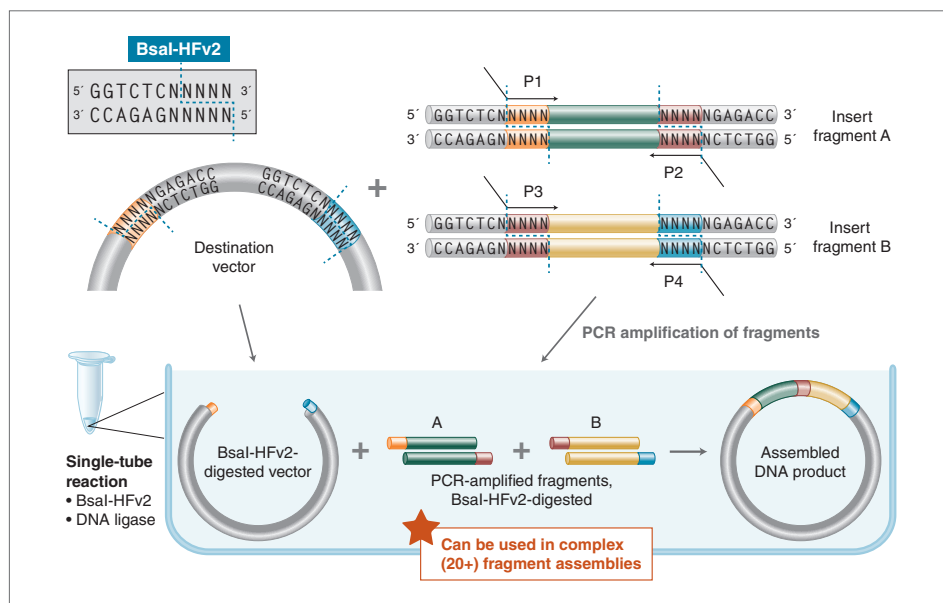
Get started today. To simplify the workflow, NEB offers two kits for Golden Gate Assembly featuring BsaI-HFv2 or BsmBI-v2. Both kits can be used to assemble 2 or more than 50 fragments in a single step.

BsaI-HFv2

5' GGTCTC NNNNN 3'
3' CCAGAG NNNNN 5'

Type IIS restriction enzymes are the basis of Golden Gate Assembly.

Golden Gate Assembly workflow



Advantages:

- Seamless & ordered cloning
- Assemble multiple fragments (2 to 50+) in a single reaction
- Perform single insert cloning in just 5 minutes using fast protocols
- Experience high efficiency, even with regions of high GC content and areas of repeats
- Generate libraries with high efficiencies
- Use with a broad range of fragment sizes (from less than 100 bp to over 15 kb)

Find the best assembly strategy/Type IIS REase

Introduce desired cut sites into fragments by PCR (design PCR primers using NEB Golden Gate Assembly tool)

Perform single-tube Golden Gate Assembly reaction

Cycle rxn mix at optimal cutting and ligation temperatures

Heat kill reaction and transform/ plate

Pick positive colonies



For more detailed information on Golden Gate Assembly incl. video tutorials, free primer design tool, ligase fidelity etc., please visit www.neb.com/GoldenGate

Get started!

Benefit from our special price offer:

Product	NEB #	Size
NEB Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 rxns
NEB Golden Gate Assembly Kit (BsaI-HFv2)	E1601S/L	20/100 rxns

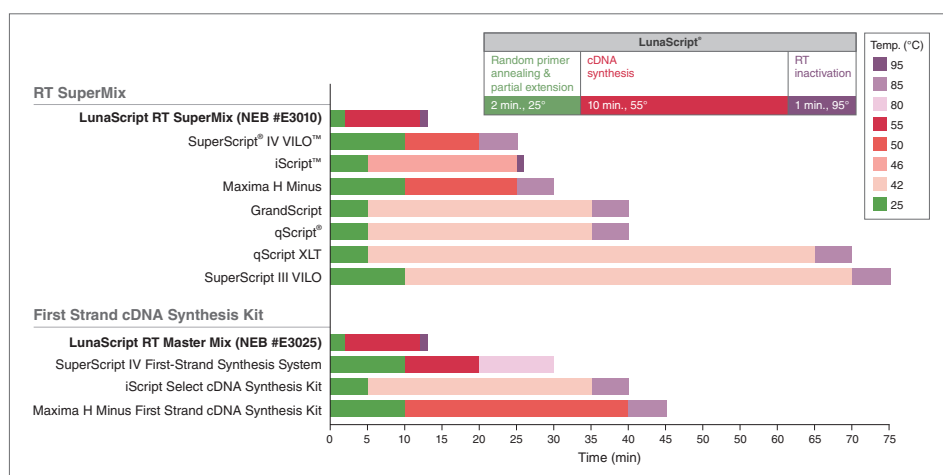
*Offer valid through 31/12/21 and not cumulative with other discount or special price.



LunaScript Reverse Transcriptase – The fastest and hottest cDNA Synthesis solution available!

Luna Reverse Transcriptase (RT) is a unique *in silico* designed thermostable RT with improved performance: It supports elevated temperatures, and extremely fast protocols making it the best RT available outperforming any competition. LunaScript RT is part of our convenient Luna Universal One-Step RT-qPCR Kits (see page 7) and is now also available in two separate Master Mix formats:

1. The LunaScript RT SuperMix Kit (#E3010) contains Luna Reverse Transcriptase plus random hexamer and oligo-d(T) primers, dNTPs, Murine RNase Inhibitor. The SuperMix is optimized for cDNA synthesis in a two-step RT-qPCR workflow. It is also featured in the ARTIC SARS-CoV-2 sequencing workflow and is a component of the NEBNext ARTIC SARS-CoV-2 kits (see page 10).
2. The LunaScript RT Master Mix Kit (Primer-free) (NEB #E3025) features an optimized 5X master mix containing all the necessary components for first strand cDNA synthesis, except primers. The mix is compatible with random primers, oligo d(T) primers, and gene-specific primers, thus enabling maximum cDNA synthesis flexibility. The cDNA product generated can be used in a variety of downstream applications incl. full-length cDNA analysis with cDNA products up to 9 kb.



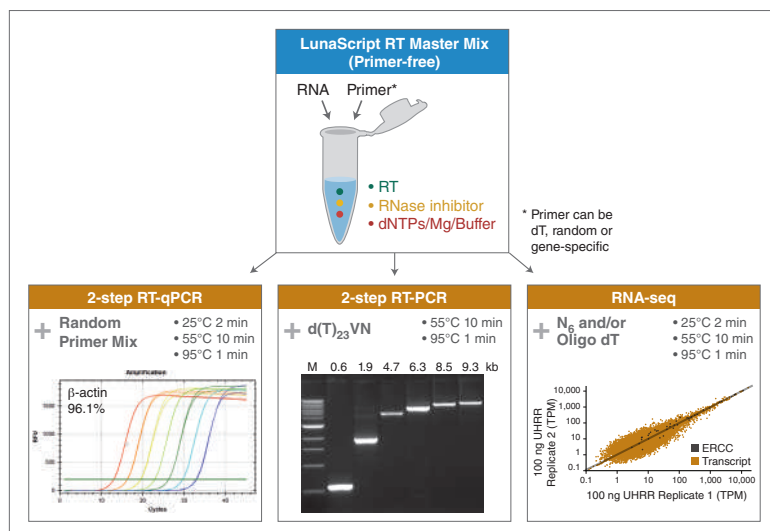
At just 13 minutes, LunaScript offers the shortest available first strand cDNA synthesis protocol!

Comparison of recommended protocols for cDNA synthesis. Both LunaScript RT SuperMix Kit and LunaScript RT Master Mix Kit require the shortest reaction time and tolerate elevated temperatures, reducing complications from RNA secondary structure.

Advantages:

- Single-tube 5X master mix contains Luna Reverse Transcriptase, dNTPs, and Murine RNase Inhibitor
- Primer-free format enables user flexibility for choice of primers for optimal cDNA synthesis
- Complete first strand cDNA synthesis protocol in less than 15 minutes
- Eliminate pipetting errors with non-interfering, visible tracking dye
- Includes No-RT Control Mix for increased confidence

LunaScript RT Master Mix (Primer-free) supports numerous applications



By adding different primers including a Random Primer Mix, d(T)₂₃VN oligos, or random hexamers, the LunaScript RT Master Mix can produce cDNA that is ideally suited for various downstream applications such as RT-qPCR, RT-PCR, and RNA-seq studies.

Ordering information:

Product	NEB #	Size
LunaScript RT Master Mix Kit (Primer-free)	E3025S/L	25/100 rxns
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns

You heard the message.

NEB has everything you need for your RNA-related workflows.

NEB offers you a broad portfolio of reagents for purification, quantitation, detection, synthesis and manipulation of RNA. But did you know that these products are available from bench-scale to commercial-scale to enable both academic and industrial needs? Further, we provide these products at quality levels that support vaccine and diagnostic manufacturing. Experience improved performance and increased yields, enabled by our expertise in enzymology.

Learn more about our growing selection of products for the following applications:



RNA
Purification



RNA-seq



RNA
Detection



RNA
Synthesis

Visit

NEBna.com to:

- View NEB products being used in RNA-related workflows
- Find extensive technical resources, including brochures, technical notes and usage guides
- Request your free copy of our RNA Metro Map Poster and the "RNase-free Zone" Sticker Pack

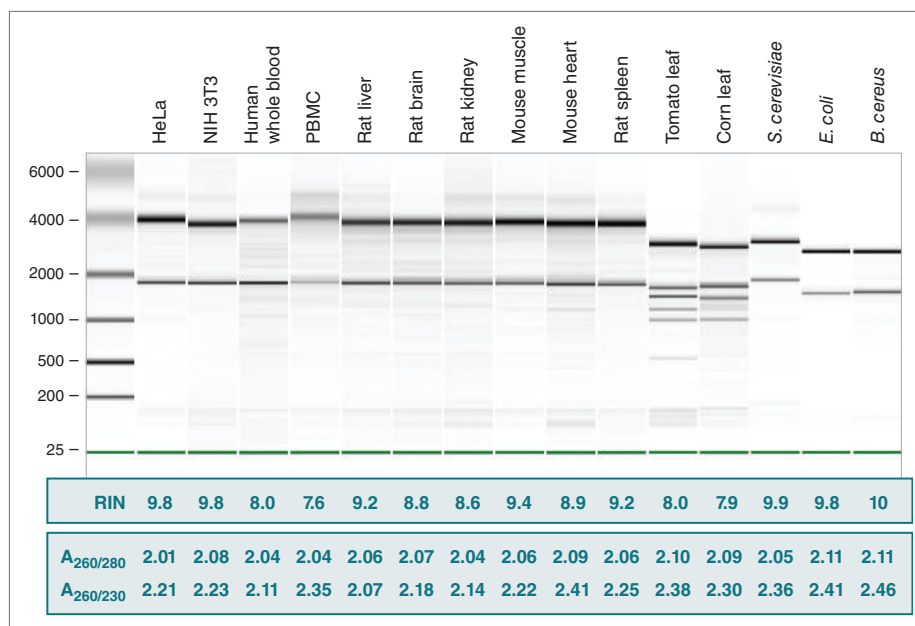
FEATURED PRODUCTS



Monarch[®] Total RNA Miniprep Kit

Purify high-quality total RNA from a wide variety of sample types with the Monarch Total RNA Miniprep Kit. This comprehensive kit includes genomic DNA removal columns, DNase I, Proteinase K and a stabilization/preservation reagent, all at a competitive price. Purified RNA ranges in size from full length RNAs down to intact miRNAs and is ready for use in downstream applications, including cDNA synthesis, RT-PCR, RT-qPCR and RNA-seq.

Purify high-quality RNA from a wide variety of sample types



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 or Pico 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.

Advantages:

- Compatible with blood, cells, tissues, plants, tough-to-lyse samples, saliva, swabs and many other samples
- Validated for viral RNA extraction from clinically-relevant samples (automatable on the QIAcube[®] and KingFisher[®] Flex)
- Effectively purify total RNA of all sizes, including small RNA (<200 nt)

Ordering information:

Product	NEB #	Size
Monarch Total RNA Miniprep Kit	T2010S	50 preps

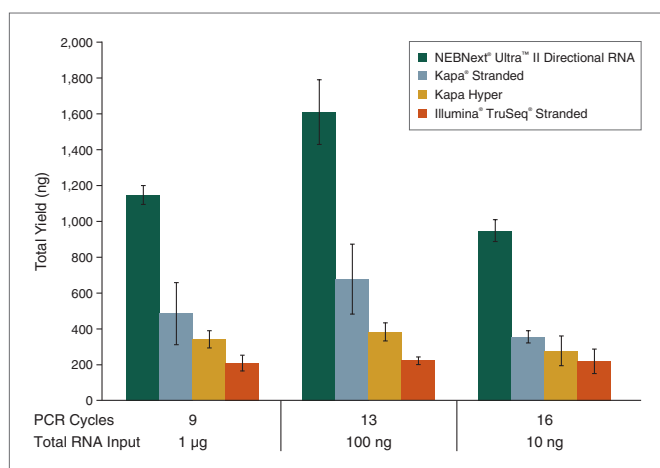


NEW PRODUCTS



NEBNext® Ultra™ II RNA Library Prep

Our NEBNext Ultra II RNA kits have streamlined, automatable workflows and also are available for directional (strand-specific, using the “dUTP method”) and non-directional library prep, and are compatible with poly(A) mRNA enrichment or rRNA depletion. The kits are available 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 Human Universal Reference RNA (Agilent #740000), and libraries were made using the NEBNext Ultra II Directional RNA Kit (plus the NEBNext Poly(A) mRNA Magnetic Isolation Module), Kapa Stranded mRNA-Seq Kit, Kapa mRNA HyperPrep Kit and Illumina TruSeq Stranded mRNA Kit. Library yields from an average of 3 replicates are shown.

Advantages:

- Save time with streamlined workflows, reduced hands-on time and automation compatibility
- Generate high quality libraries even with limited amounts of RNA
- Minimize bias, with fewer PCR cycles required
- Reagents, and adaptors & primers (12- and 96-index) sold separately

Ordering information:

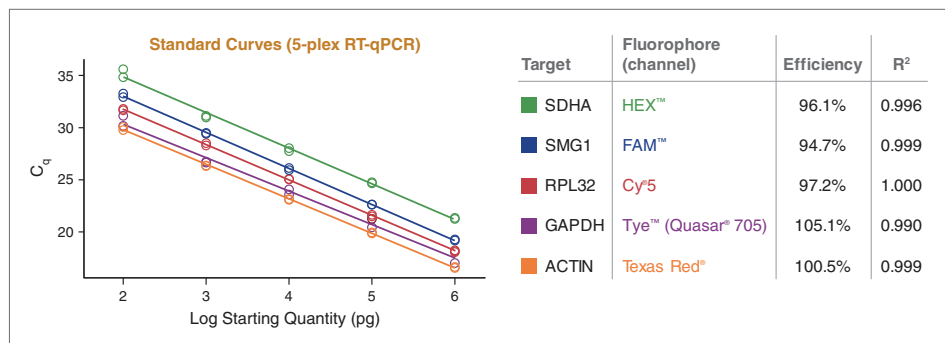
Product	NEB #	Size
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns



Luna® Probe One-Step RT-qPCR 4X Mix with UDG

Experience robust, sensitive detection and quantitation of up to 5 targets in a multiplexed reaction. Supplied at a 4X concentration, this mix enables higher amounts of sample input, which is relevant for applications where RNA is present in low abundance, such as pathogen detection. The Dual WarmStart®/Hot Start enzyme formulation enables room temp. setup and stability for up to 24 hours. This master mix also includes thermolabile UDG and dUTP for reduced risk of carryover contamination.

Multiplex detection (5 targets) with the Luna Probe One-Step RT-qPCR 4X Mix with UDG



Target	Fluorophore (channel)	Efficiency	R ²
SDHA	HEX™	96.1%	0.996
SMG1	FAM™	94.7%	0.999
RPL32	Cy5	97.2%	1.000
GAPDH	Tye™ (Quasar® 705)	105.1%	0.990
ACTIN	Texas Red®	100.5%	0.999

Multiplex RT-qPCR was performed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG over a 5-log range of Jurkat total RNA (100 ng to 10 pg) on a Bio-Rad® CFX96 real-time instrument. Amplification standard curves and efficiencies for each of the 5 human targets are shown. Reactions (20 µl) included primers and probes at 200 nM each, and followed the product recommended cycling conditions. All five targets were detected linearly in the multiplex reactions with strong efficiency and R² values.

Ordering information:

Product	NEB #	Size
Luna Probe One-Step T-qPCR 4X Mix with UDG	M3019 S/L/X/E	200/500/1,000/2,000 reactions

You heard the message.

NEB has everything you need for your RNA-related workflows.



From research to therapeutic production, NEB's *in vitro* transcription portfolio will meet your needs.

NEB's portfolio of research-grade and GMP-grade* reagents support bench-scale to commercial-scale mRNA manufacturing. Our optimized HiScribe™ kits enable convenient *in vitro* transcription (IVT) workflows. When it is time to scale up and optimize reaction components, our standalone reagents are readily available in formats matching our GMP-grade offering for a seamless transition to large-scale therapeutic mRNA manufacturing.

Enabling gram-scale RNA synthesis

NEB manufactures and inventories the following enzyme specificities at GMP-grade, meeting customer needs with short lead times:

Product	NEB #	Feature
Vaccinia Capping Enzyme	M2080S	A full system for enzymatic capping based on the Vaccinia virus Capping Enzyme (VCE)
T7 RNA Polymerase	M0251S/L	RNA Polymerase used for <i>in vitro</i> mRNA synthesis, and is highly specific for the T7 phage promoter
mRNA Cap 2'-O-Methyltransferase	M0366S	mRNA Cap 2'-O-Methyltransferase adds a methyl group at the 2'-O position of the first nucleotide adjacent to the cap structure at the 5' end of the RNA
RNase Inhibitor, Murine	M0314S/L	RNase Inhibitor, Murine, specifically inhibits RNases A, B and C
Pyrophosphatase, Inorganic (<i>E. coli</i>)	M0361S/L	Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate
DNase I (RNase-free)	M0303S/L	DNA-specific endonuclease used for removal of contaminating genomic DNA from RNA samples and degradation of DNA templates in transcription reactions
HiScribe T7 High Yield RNA Synthesis Components	E2040S	Flexible system for high yield synthesis of (m)RNA of up to 180 µg of RNA per reaction from 1 µg of control template. Separate components available in GMP-grade format

* "GMP-grade" is a branding term NEB uses to describe reagents manufactured at our Rowley, MA facility, where we utilize procedures and process controls to manufacture reagents under more rigorous conditions to achieve more stringent product specifications, and in compliance with ISO 9001 and ISO 13485 quality management system standards. NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor do we manufacture products in compliance with all of the Current Good Manufacturing Practice regulations.

Avoiding RNase Contamination

SOURCES

- Dust & air
- Skin & hair
- Aqueous solutions & reagents
- Most surfaces (doorknobs, keyboards)

LABORATORY PRECAUTIONS

- Wear laboratory gloves and change them often
- Use RNase-free certified, disposable plasticware and solutions
- Decontaminate glassware & plasticware
- Maintain a separate, clean surface for RNA work

SOLUTION PREPARATION

Diethylpyrocarbonate (DEPC) treatment:

1. Add 1 ml DEPC per liter of solution.
2. Stir for 1 hour.
3. Autoclave for 1 hour.

DEPC:

- Compounds with primary amine groups (e.g., Tris)
- Compounds that are not stable during autoclaving

Not using DEPC?

Prepare solution with Nuclease-free Water or Milli-Q® water

Dissolving solids:

- Use high-purity solids (e.g., DTT, nucleotides, manganese salts)
- Use autoclaved DEPC-treated or Milli-Q water
- Sterilize with a 0.22 µm filter

RNase INHIBITORS

RNase Inhibitor, Murine (NEB #M0314)

- Improved resistance to oxidation
- Requires <1 mM DTT
- Compatible with many enzymatic reactions (e.g., RT-qPCR)

RNase Inhibitor, Human Placenta (NEB #M0307)

- Specific for RNases A, B and C
- Compatible with many enzymatic reactions

Ribonucleoside Vanadyl Complex (NEB #S1402)

- Inhibits RNase A-type enzymes
- Compatible with many RNA isolation procedures. Should not be used with EDTA and can inhibit other enzymes

Get your free RNA Specials!



Request RNase-free Sticker Pack & RNA Metro Map Poster from your local distributor or at:
www.NEBrna.com



To view all tips for Avoiding RNase Contamination, please visit
www.neb.com/AvoidingRNaseContamination



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

Murine RNase Inhibitor (NEB #M0314)

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



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



Benefit from our
special price offer:

Product	NEB #	Size
Murine RNase Inhibitor	M0314S	3,000 units
Murine RNase Inhibitor	M0314L	15,000 units

*Offer valid through 31/12/21 and not cumulative with other discount or special price.

Restriction enzymes from NEB – same high performance, now with BSA-free reaction buffer.

To address the growing need for comparable performance using BSA-free reagents, we have begun switching our current BSA-containing reaction buffers (NEBuffer 1.1, 2.1, 3.1 and CutSmart® Buffer) to Recombinant Albumin (rAlbumin)-containing buffers (NEBuffer r1.1, r2.1, r3.1 and rCutSmart Buffer). These buffers have been rigorously tested, and there is no difference in performance when using either system. This switch started in April 2021, but may take months to complete. During this period, you may receive product with BSA- or rAlbumin-containing buffer – either will work for your reactions.



For more details visit

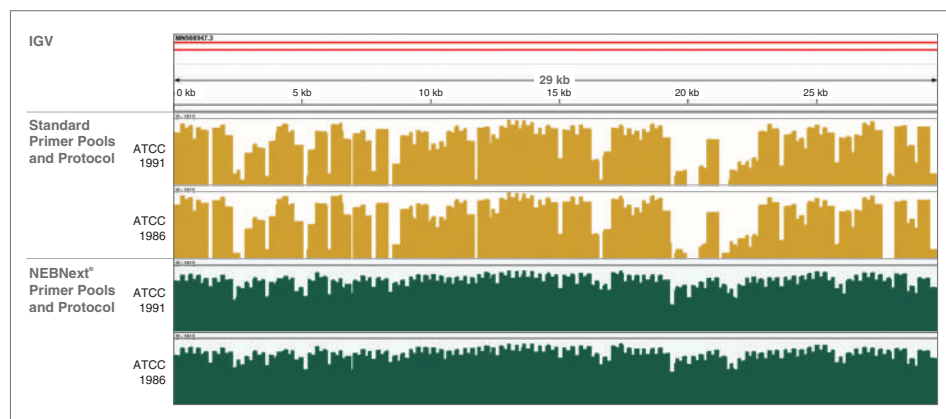
www.neb.com/BSA-free

Reliable and fast kits for SARS-CoV-2-Sequencing (ARTIC workflows)

The NEBNext® ARTIC kits were developed in response to the critical need for reliable and accurate methods for SARS-CoV-2 sequencing, especially with the ongoing emergence of SARS-CoV-2 variants that affect virus transmission. These kits, for long and short read sequencing, were based on the original work of the ARTIC Network (1). The ARTIC SARS-CoV-2 sequencing workflow is a multiplexed amplicon-based whole-viral-genome sequencing approach.

NEBNext ARTIC kits include primers and reagents for RT-PCR from SARS-CoV-2 gRNA and downstream library preparation for Illumina® and Oxford Nanopore Technologies® sequencing. The V3 ARTIC primers have been balanced, using methodology developed at NEB based on empirical data from sequencing, to provide greater uniformity of genome coverage from 10-10,000 SARS-CoV-2 genome copies. In combination with optimized reagents for RT-PCR, the kits deliver improved uniformity of amplicon yields from gRNA across a wide copy number range.

Fewer reads are required to completely cover the genome with the NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)



Integrative Genome Viewer visualization of read coverage across the SARS-CoV-2 genome. Amplicons were generated from 1,000 copies of SARS-CoV-2 viral gRNA inputs (ATCC VR-1986 and VR-1991) in 100 ng of Universal Human Reference RNA (ThermoFisher Q50639) using IDT ARTIC nCoV-2019 V3 Panel ("Standard") or the NEBNext balanced ARTIC SARS-CoV-2 primer pools. Libraries were constructed using the NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) and the Oxford Nanopore Technologies Native Barcoding Expansion kits 1-12 (EXP-NBD104) and 13-24 (EXP-NBD114), Ligation Sequencing Kit (SQK-LSK109) and SFB Expansion Kit (EXP-SFB001). Sequencing was on a GridION instrument using R9.4.1 flow cells. Minimap2 was used with 24500 reads or 250x data for the mapping against SARS-CoV-2 Wuhan-Hu-1.

Ordering information:

Product	NEB #	Size	Features
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)*	E7658S/L	24/96 rxns	<ul style="list-style-type: none">• Incorporates enzymatic cDNA fragmentation• Produces Illumina-compatible libraries with library inserts in the 150 bp range• Compatible with 2 x 75 sequencing on Illumina instruments
NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)	E7650S/L	24/96 rxns	<ul style="list-style-type: none">• Produces Illumina compatible libraries with library inserts in the 400 bp range• Compatible with 2 x 250 sequencing on Illumina instruments
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)*	E7660S/L	24/96 rxns	<ul style="list-style-type: none">• Compatible with sequencing on the Oxford Nanopore Technologies (ONT) platform• Used alongside kits from ONT to generate libraries with inserts in the 400 bp range, that can be sequenced on any ONT instrument, in 2-24 hour run times
NEBNext ARTIC SARS-CoV-2 RT-PCR Module*	E7626S/L	24/96 rxns	<ul style="list-style-type: none">• Contains the reagents required for cDNA synthesis and targeted cDNA amplification from SARS-CoV-2 genomic RNA

Visit www.neb.com/ARTIC to:

- Learn more about ARTIC products for SARS-CoV-2 sequencing
- Request a sample
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Advantages

- Streamlined, high-efficiency protocol
- Effective with a wide range of viral genome inputs (10-10,000 copies)
- Improved uniformity of SARS-CoV-2 genome coverage



Starting September:

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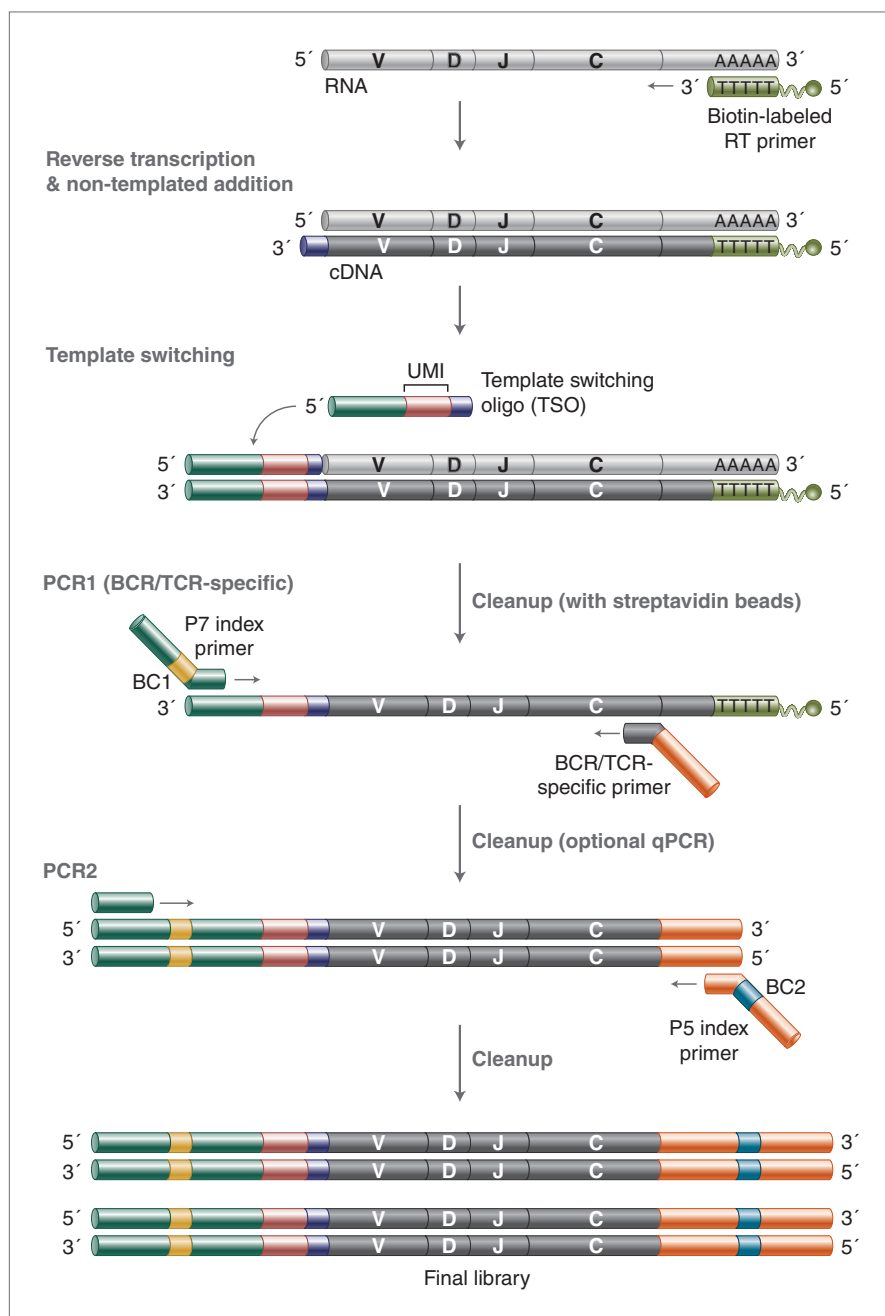
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1. Josh Quick 2020. nCoV-2019 sequencing protocol v2 (Guntt). protocols.io <https://dx.doi.org/10.17504/protocols.io.bdp7i5n>.

NEW: NEBNext Immune Sequencing Kits (Human & Mouse)

The NEBNext Immune Sequencing Kit (Human) (NEB #E6320) and NEBNext Immune Sequencing Kit (Mouse) (NEB #E6330) enable detailed profiling of somatic mutations in the full-length immune gene repertoires of B cells and T cells via the expression of complete antibody chains. This includes modular primer sets, information for complete V, D, and J segments and full isotype information analysis (IgM, IgD, IgG, IgA and IgE), and TCR chain characterization. A unique, UMI-based mRNA barcoding process allows PCR copies derived from an individual molecule to be converted to a consensus sequence; this improves sequence accuracy and eliminates PCR bias. Based on the open-source pRESTO toolkit, a workflow is available via the Galaxy platform to enable robust bioinformatic analysis locally or in a cloud environment. A workflow tutorial ensures that users who are not familiar with Galaxy can successfully use the analysis workflow.

NEBNext immune sequencing workflow



Advantages

- Generate full-length variable sequences, allowing downstream antibody synthesis and functional characterization
- Eliminate use of variable region primers, reducing primer pool complexity and enabling simultaneous recovery of BCR & TCR transcripts
- Minimize PCR bias and improve sequencing accuracy by allowing a UMI consensus to be generated from duplicate sequencing reads
- Optimize target-capture efficiency for immune repertoire sequencing and analysis from sub-microgram quantities of total RNA
- Analyze with an easy-to-use bioinformatic workflow based on the open-source pRESTO toolkit



For more details, visit
www.neb.com/ImmuneSequencing

Ordering information:

Product	NEB #	Size
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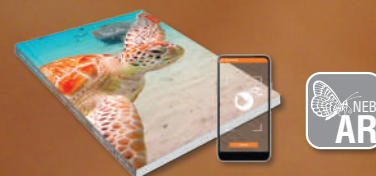
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