# NEB EXPRESSIONS

*A scientific update*

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### INNOVATION



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

*By Rebecca Kucera, M.S.,*
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*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 GGA. Statistically,

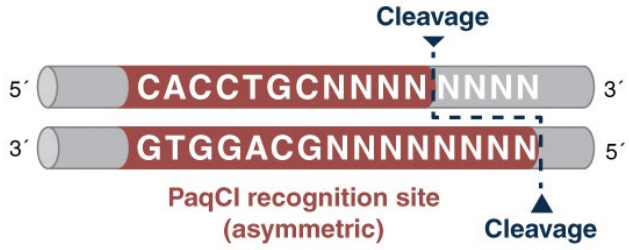


***Figure 1:*** *Type IIS enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence.*

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 highlighing 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 PaqCI binding site, which functions in *trans* as an activator for PaqCI 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 PaqCI 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 PaqCI 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 PaqCI and T4 DNA Ligase. In addition, PaqCI 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 PaqCI, 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 PaqCI, 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 PaqCI 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 20 µM stock of the activator is provided with the PaqCI enzyme.

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

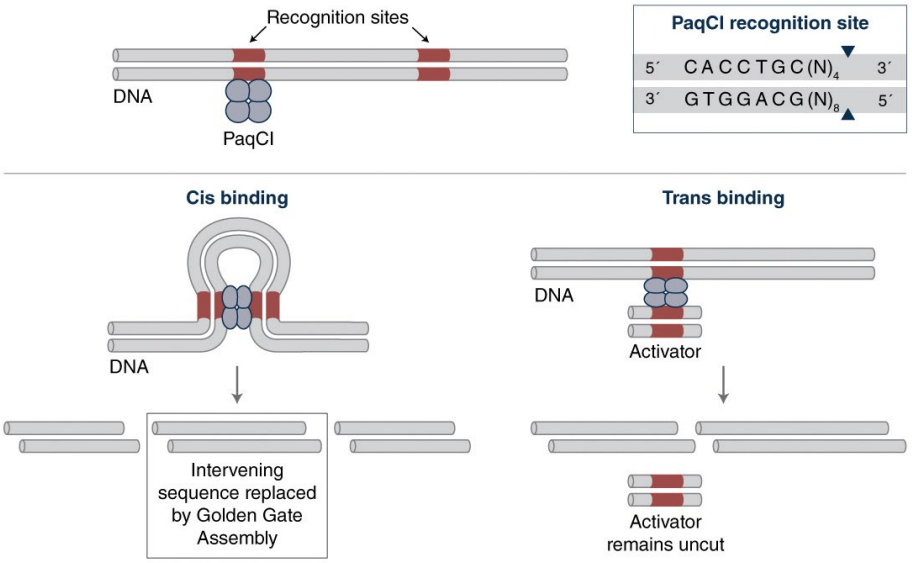


***Figure 2:*** *Presumed mechanism for how the PaqCI activator assures complete cutting via trans binding if needed*

**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 PaqCI by visiting **www.neb.com/R0745**.

For additional information about Golden Gate Assembly, visit **neb.com/goldengate**.

**Table 1:** Recommendations for PaqCI Golden Gate Assembly

| Assembly Complexity | PaqCI | T4 DNA Ligase | PaqCI 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 ul 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.)

# You heard the message.

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

We've told you before that we offer 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:**

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RNA-seq

RNA Detection

RNA Synthesis

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- Find extensive technical resources, including brochures, technical notes and usage guides
- Request your copy of our RNA Metro Map Poster

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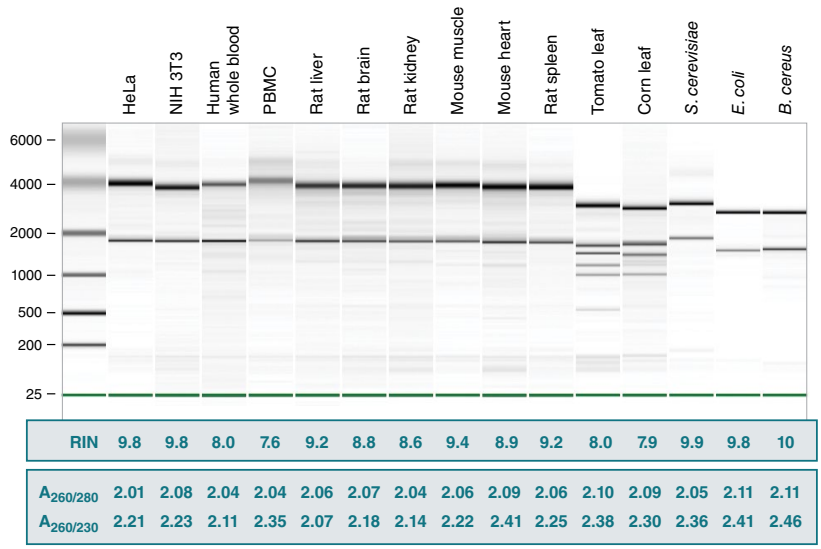
## Monarch® Total RNA Miniprep Kit

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**




| | HeLa | NIH 3T3 | Human whole blood | PBMC | Rat liver | Rat brain | Rat kidney | Mouse muscle | Mouse heart | Rat spleen | Tomato leaf | Corn leaf | *S. cerevisiae* | *E. coli* | *B. cereus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RIN | 9.8 | 9.8 | 8.0 | 7.6 | 9.2 | 8.8 | 8.6 | 9.4 | 8.9 | 9.2 | 8.0 | 7.9 | 9.9 | 9.8 | 10 |
| $A_{260/280}$ | 2.01 | 2.08 | 2.04 | 2.04 | 2.06 | 2.07 | 2.04 | 2.06 | 2.09 | 2.06 | 2.10 | 2.09 | 2.05 | 2.11 | 2.11 |
| $A_{260/230}$ | 2.21 | 2.23 | 2.11 | 2.35 | 2.07 | 2.18 | 2.14 | 2.22 | 2.41 | 2.25 | 2.38 | 2.30 | 2.36 | 2.41 | 2.46 |

*Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit (NEB #T2010). Aliquots were run on an Agilent® Bioanalyzer® 2100 using the Nano 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:

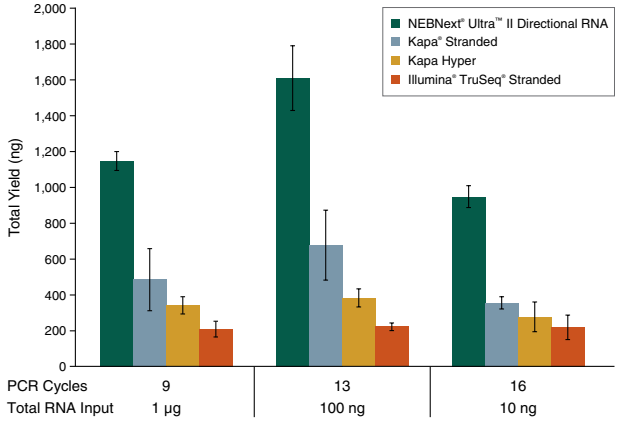
| Product | NEB # | Size |
|---|---|---|
| Monarch Total RNA Miniprep Kit | T2010S | 50 preps |



# 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 and primers (12- and 96-index) sold separately

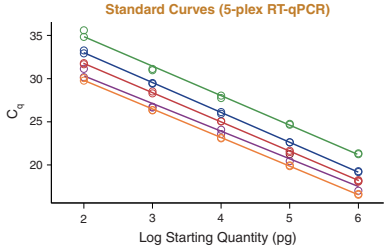
Ordering information:

| 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 | Cy®5 | 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 RT-qPCR 4X Mix with UDG | M3019 S/L/X/E | 200/500/ 1,000/2,000 reactions |


## 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.

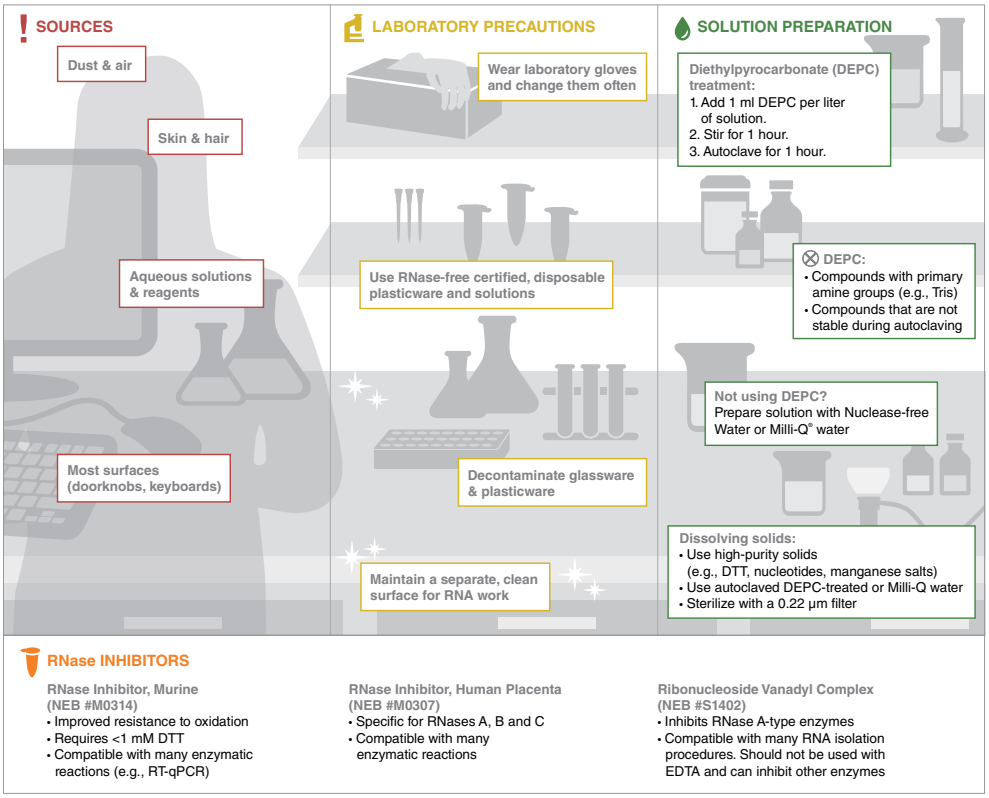
### ENABLING GRAM-SCALE RNA SYNTHESIS

*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 *in vitro* 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 (*E. coli*) | 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 | Separate components available in GMP-grade format |

*"GMP-grade" is a branding term NEB uses to describe reagents manufactured at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.*

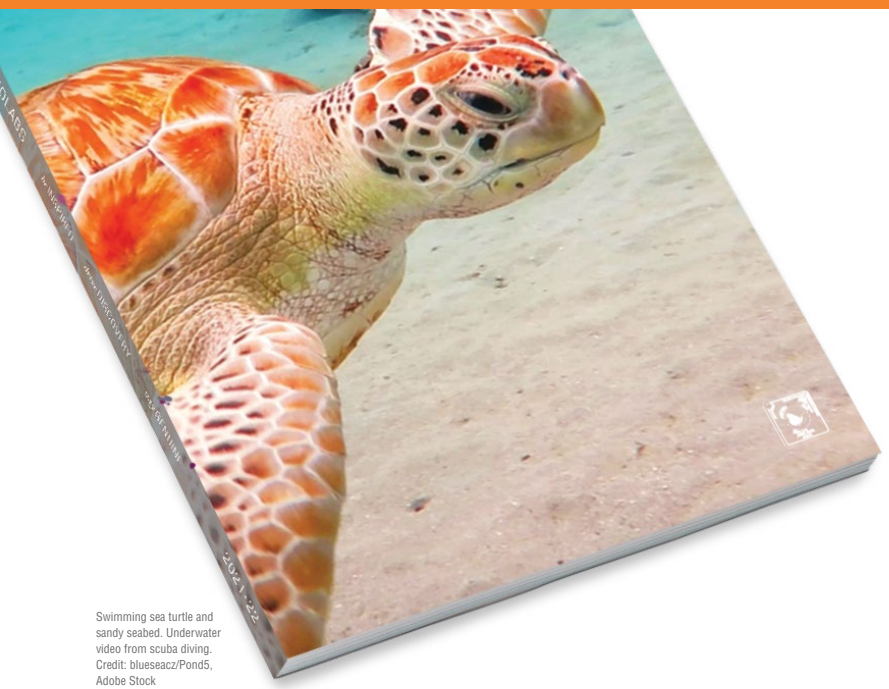
## Avoiding RNase Contamination




To view full text for Avoiding RNase Contamination, please visit

**www.neb.com/AvoidingRNaseContamination**

# The 2021-22 NEB Catalog & Technical Reference is now available!



Swimming sea turtle and sandy seabed. Underwater video from scuba diving. Credit: blueseacz/Pond5, Adobe Stock

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- Troubleshooting guides
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Each edition of the catalog contains a collection of mini-reviews that addresses various scientific, environmental and/ or humanitarian topics. This year, we are dedicating the catalog to our Founder, **Donald G. Comb**, and are sharing some of the values that he was passionate about.

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# Restriction enzymes from NEB — same high performance, now with BSA-free reaction buffer

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For more details visit
**www.neb.com/BSA-free**

# New products supporting ARTIC workflows

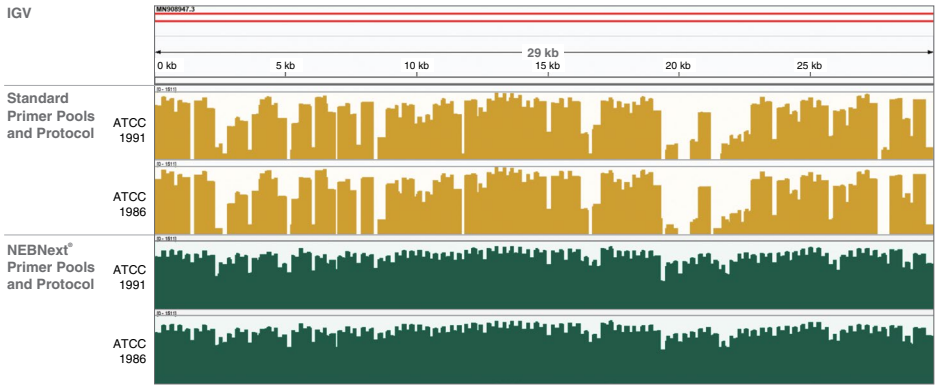
The NEBNext® ARTIC kits were developed in response to the critical need for reliable and accurate methods for SARS-CoV-2 sequencing, especially with the ongoing emergence of SARS-CoV-2 variants that affect virus transmission. These kits, for long and short read sequencing, were based on the original 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.

## Visit www.neb.com/ARTIC to:

- Learn more about ARTIC products for SARS-CoV-2 sequencing
- Request a sample
- View workflows for Oxford Nanopore Technologies sequencing
- Check out NEB's COVID-19 Researcher Spotlight podcast, featuring Joshua Quick, Ph.D., of the University of Birmingham and the ARTIC network

### 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 QS0639) 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.*

## 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

Ordering information:

| Product | NEB # | Size | Features |
|---|---|---|---|
| NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina) | E7658S/L | 24/96 rxns | • Incorporates enzymatic cDNA fragmentation<br>• Produces Illumina-compatible libraries with library inserts in the 150 bp range<br>• Compatible with 2 x 75 sequencing on Illumina instruments |
| NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) | E7650S/L | 24/96 rxns | • Produces Illumina compatible libraries with library inserts in the 400 bp range<br>• Compatible with 2 x 250 sequencing on Illumina instruments |
| NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) | E7660S/L | 24/96 rxns | • Compatible with sequencing on the Oxford Nanopore Technologies (ONT) platform<br>• 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 | • Contains the reagents required for cDNA synthesis and targeted cDNA amplification from SARS-CoV-2 genomic RNA |

1. Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io **https://dx.doi.org/10.17504/protocols.io.bdp7i5rn**.

# Primer Monitor: an online tool to track SARS-CoV-2 variants that may impact primers used in diagnostic assays

*Matthew A. Campbell, Ph.D., Yanxia Bei, Ph.D., Nicole M. Nichols, Ph.D. and Bradley W. Langhorst, Ph.D., New England Biolabs, Inc.*

## Introduction

While the SARS-CoV-2 genome seems to be less variable (1) than some other retroviral genomes, variants with potential effects on amplification efficiency have arisen and become prevalent in local areas. Some regions (e.g., Brazil; Madera County, CA, USA (2)) report greater than 15% of observed sequences with variants in genomic loci commonly used by diagnostic tests. The challenge for diagnostic developers is to demonstrate that SARS-CoV-2 assays continue to detect the virus variant circulating in the population being tested. Nucleic acid diagnostic tests for SARS-CoV-2, whether based on RT-qPCR, RT-LAMP or other amplification technologies, all depend on primers. We developed an online Primer Monitor Tool to track SARS-CoV-2 variants as a function of geography and map those variants against user-defined and commonly used primer sets, such as those provided by the Centers for Disease Control and Prevention (CDC).

## Description and Use of the Primer Monitor Tool

As shown in Figure 1, the main page of the tool (Primer Variant Summary tab) shows an overview of the primer set of interest within the context of the SARS-CoV-2 genome (Fig 1A), and a broad view of variants that may impact primer binding, as observed across geographic region (Fig 1B). Data is regularly uploaded (multiple times per week) to the tool directly from GISAID, an initiative that promotes the rapid sharing of data from all influenza viruses and the coronavirus causing COVID-19. To highlight emerging variants of interest, a variant fraction of at least 10% is depicted in dark blue. Note that only primer loci with variant fractions that meet user defined minimum thresholds are shown in this panel. Logged in users can subscribe to be notified when a variant overlapping a primer set reaches a threshold fraction of observed sequences in any geographic region.

To further investigate variant position as a function of primer location, a second visual is presented at the bottom of the main page (Figure 2). In this visual, variants are shown in the context of the full primer/probe sequences, enabling a more complete assessment of potential impacts to primer/probe annealing dynamics. With qPCR primers, variants that occur closer to the 3´ end of primers/probes are typically more disruptive to assay performance than variants that occur closer to the 5´ end.

By evaluating both geographic and genomic regional variation, specific hotspots can be detected where primer assessment might be warranted. In the data below, a significant variant in South Korea with a mutation occurring near the 3´ end of the CDC N2 forward primer was detected. To further investigate the potential impact of this variant, RNA representing the N2 region from the wild-type SARS-CoV-2 sequence (Wuhan-Hu-1) and from the mutant S. Korean variant were assessed experimentally. Using the Luna SARS-CoV-2 Multiplex Assay Kit (NEB #E3019) based on the CDC N1 and N2 primer/probe sets described previously (https://www.neb.com/faqs/2020/12/22/what-targets-do-the-sars-cov-2-primer-probe-mix-detect-how-were-these-primers-chosen), we observed a minor impact of the variant on assay sensitivity (from an LOD of 5 copies per reaction to 25 copies per reaction), and a consistent $C_q$ delay across different RNA input amounts.

For LAMP assays, most single point mutations are not disruptive enough to result in significant assay performance perturbations (3, 4), suggesting that this technique may offer additional benefits in the face of emerging SARS-CoV-2 variants. Please find additional information on NEB's LAMP based SARS-CoV-2 assay here: **www.neb.com/e2019**.


***Figure 1:*** *Primer Variant Summary. Users can choose from a variety of pre-loaded primer sets using a drop-down menu (A). The location of the chosen primer set is noted in the context of the SARS-CoV-2 genome by orange arrows. (B) shows an overview of variants that may impact primer binding across various geographic regions. Users can specify minimum thresholds using the right-hand panel. Hovering over shaded squares will reveal additional information, including total sequences deposited, to enable further evaluation of potential impact.*

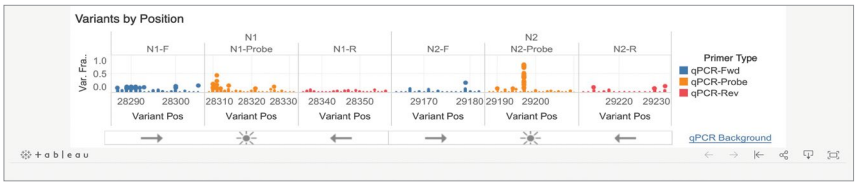



***Figure 2:*** *Variants by Position. At the bottom of the main page, positions of the variants are shown in the context of the full primer/probe sequences (A). Dots indicate data points from the figure above and each dot reveals additional information upon hovering. A simple pictogram helps to orient the user to the forward and reverse primers and probe sequences (B). Data sharing, downloading, and further manipulation are all enabled using tools at the bottom right corner of the page.*

## Primer Sets

The tool is pre-loaded with commonly used primer sequences from SARS-CoV-2 qPCR and LAMP assays and ARTIC sequencing workflows (currently v3). Users who create a free account can also upload additional primer sets, which will become publicly available for any/all to monitor after a simple review and mapping process. Users who subscribe will also be able to receive notifications should variations within a specific primer set region cross a specified threshold in a geographic region of interest.

## Conclusion

This tool provides diagnostic assay developers with additional resources to evaluate SARS-CoV-2 assay effectiveness by providing up-to-date data highlighting potential issues around primer/probe binding. It has been released quickly in response to ongoing concerns and needs in the scientific and diagnostic community in the hopes that this task will be made easier for all. It is under active development at GitHub and additional features including time-course assessment, and primer-centric scoring are in progress or planned. Visualization is enabled by Tableau. Contributions, problem reports, and feature requests are welcome and requested.

*The full white paper can be downloaded by visiting* ***neb.com/primer-monitor-SARS-CoV-2****.*

*You can access our Prime Monitor Tool here:* ***primer-monitor.neb.com****.*

***Acknowledgements***

*We are thankful for the team at GISAID and all who are working tirelessly to provide data, serve patients and otherwise lessen and shorten the impact of this global pandemic.*

References

1. Kupferschmidt, Kai. "The Pandemic Virus Is Slowly Mutating. But Does It Matter?" Science 369, no. 6501 (July 17, 2020): 238–39. https://doi.org/10.1126/science.369.6501.238.
2. V Vanaerschot, Manu, Sabrina A. Mann, James T. Webber, Jack Kamm, Sidney M. Bell, John Bell, Si Noon Hong, et al. "Identification of a Polymorphism in the N Gene of SARS-CoV-2 That Adversely Impacts Detection by a Widely-Used RT-PCR Assay." Journal of Clinical Microbiology Dec 2020, 59 (1) e02369-20. https://jcm.asm.org/content/59/1/e02369-20.
3. Deguo Wang (2016) Effect of internal primer–template mismatches on loop- mediated isothermal amplification, Biotechnology & Biotechnological Equipment, 30:2, 314-318, DOI: 10.1080/13102818.2015.1125765
4. Tanner, N., personal communication

# The Unseen Ecosystem Benefits of Salt Marshes

*By Jennifer Bowen, Associate Professor, Marine and Environmental Sciences, Northeastern University*

Salt marshes, coastal wetlands that line the shores throughout the temperate zone, play critically important, though often undervalued, roles in healthy coastal communities. These coastal habitats provide many important ecosystem services — benefits to humans that are provided by natural ecosystems. Some of these services are evident in our daily lives, while others often go unheeded. Their calm waters, hidden away from the crashing surf, offer nursery grounds for a diverse number of commercially important fish and shellfish species that populate our fish markets. Their location between land and sea allows them to intercept storm surges and decrease the force of coastal storms, protecting coastal communities. People enjoy kayaking through their sinuous channels, watching for passing birds and other wildlife, and connecting with the nature that exists in their communities.

The ecosystem benefits provided by marshes that often go unnoticed are perhaps the most important ones. Amazingly, it is the tiniest residents of marshes — the microbes that live in the waterlogged soils of marshes — that are the unsung heroes that provide these services. Microbes are amazing in the diversity of ways that they can gain energy for growth. Like humans, there are microbes who can decompose organic materials (which is basically what we are doing when we eat food) using oxygen to facilitate the process. However, unlike humans, there are microbes that can continue to decompose organic materials in the absence of oxygen, although the process is much less efficient when there is no oxygen available. The result of these different metabolisms and their efficiencies have important consequences for marsh ecosystems.

The first consequence that results from slowed microbial metabolisms is that marshes are able to store an amazing amount of carbon. In fact, an order of magnitude more carbon is stored in a square meter of salt marsh than in a square meter of tropical rain forest. Marshes are highly productive grasslands — through photosynthesis they draw a lot of carbon dioxide out of the atmosphere and store it in the soils. However the microbes that live in the saturated soils don't have access to much oxygen so they only very slowly decompose that organic matter, allowing it to build up over millennia. Thus, marshes are considered a carbon sink. Healthy robust marshes help offset global warming by sucking up carbon dioxide, converting it to organic matter and burying it in the sediments where it can no longer contribute to global warming. Thus, conservation of existing salt marshes and restoration of degraded marshes can be one part of the multifaceted approaches we need to combat anthropogenic climate change.

The second function that microbial metabolisms play in the ecosystem services provided by marshes is that they help remove nutrients that can have harmful effects in coastal waters. Reactive nitrogen that we use in fertilizers on our lawns and gardens and that is an important component of wastewater, can lead to degraded water quality — a condition known as eutrophication — when it is provided in large amounts to coastal waters. But microbes in the marsh can convert that reactive nitrogen, in the form of nitrate, to inert nitrogen gas, preventing it from causing harm to coastal waters. They do this because they can use that nitrate, instead of oxygen, to facilitate decomposition. In marshes that receive a lot of nutrients from land, the microbes are able to remove a portion of those nutrients before they enter coastal waters, helping prevent the negative economic consequences that result from degraded water quality.

The host of ecosystem services provided by marshes, some obvious, others more difficult to observe, render marshes economically and ecologically essential for coastal communities. Yet, these critical habitats are under siege by numerous forces.

*Globally, 25% of marshes have been destroyed since the 1800s and in New England the loss of marshes exceeds 35%.*

In regions where salt water flow paths have changed because of the development of roads and rails, salt marshes are vulnerable to invasive species that are able to outcompete marsh grasses in lower salinity areas. As warming of the Earth continues, sea levels are rising due to a combination of melting ice sheet and glaciers and the expansion of sea water as it warms. Marshes historically were able to keep pace with sea level rise by accumulating biomass and trapping sediments, however it is unknown whether they will be able to keep up with the accelerated rate of sea level rise that is occurring as a result of recent warming. Urban development on upland boundaries prevents marshes from migrating landward as sea level rises, which could mean that accelerated loss of marsh area and the many ecosystem services associated with that marsh. All these threats underscore how important it is to conserve the marshes that remain, and a push to restore degraded marshes. Our coastal communities depend on it.

*Microbes in action – a decomposing leaf in a pool of standing water on the marsh surface. The white, pink and dark green shades are different kinds of microbes that are able to grow in that environment. Photograph by Chris Lynum.*

*The Great Marsh is the largest continuous stretch of salt marsh in New England. Photograph by David Johnson.*

# Why are so few life sciences companies Certified B Corps?

*Brian Tinger, Corporate Controller, New England Biolabs, Inc.*

Earlier this year, NEB announced that it had achieved Certified B Corporation™ status – one of 4,000 pioneering companies worldwide that meet the highest verified standards for social and environmental responsibility, legal accountability and public transparency. Together the B Corp community works to reduce inequality and poverty, build stronger communities, create high-quality jobs, and promote a healthier environment.

B Corporation certification was a natural progression for NEB, which, as a company, has embraced these core values since the company's founding in 1974. In fact, in 1975, we printed our first catalog on 100% recycled paper.

Only a handful of life science companies are B Corps, and recently, NEB's Corporate Controller, Brian Tinger, was interviewed to answer some questions about why we pursued B Corp certification.

The following is an excerpt from an interview conducted by Christopher Marquis for Forbes.com on May 7th, 2021. For the full article, see *https://bthechange.com/why-are-so-few-life-sciences-companies-b-corps-7d6affa665cc.*

***Christopher Marquis:*** *Why did NEB pursue B Corp certification?*

**Brian Tinger:** NEB was founded with the advancement of science and stewardship of the environment as its highest priorities. Since the mid-1970s, NEB has worked on a number of initiatives through the company and the community that speak to this ideal, including establishing the first shipping box recycling program in the U.S., creating the New England Biolabs Foundation to foster community-based conservation, commissioning the design of a LEED® (Leadership in Energy and Environmental Design)-certified laboratory, hosting science events for the community and engaging with art-based programs worldwide.

*"When we took a hard look at the B Corp mission, we recognized that our core values align closely, so it was a logical step in terms of formalizing the certification."*

***Marquis:*** *What benefits did NEB see from the certification and what did you learn by going through the process?*

**Tinger:** The B Corp assessment helped to provide a detailed roadmap for improvements that we could incorporate into our business. We examined several aspects of our business — from the way we work with our customers to the impact on our community to the happiness and satisfaction of our employees — to really understand what we were doing right and what area we could improve upon. We found that we lacked documentation of certain metrics referenced in the B Corp assessment and by measuring these aspects of the business we'll be in a better position to monitor our performance going forward.

*"We also discovered opportunities to improve our supply chain management by encouraging improved social and environmental performance of our suppliers."*

However, attaining certification is only the first step. We see the B Corp assessment as a way to challenge NEB to evaluate its actions and enhance its ability to use business as a force for good™, not just today but 20 or 30 years from now. We foresee many more businesses embarking on this journey because awareness of our social and environmental responsibility will continue to grow.

***Marquis:*** *Why do you think there are not more life science and healthcare companies that have become B Corps?*

**Tinger:** There are a number of reasons why more life science companies are not B Corps. Through our own research, we noticed that B Corp status is more often associated with and pursued by consumer brands, such as Patagonia and Ben & Jerry's, so there may be a general lack of awareness in our industry. Secondly, since the life science industry is capital intensive and typically requires significant outside investment, I suspect that it's challenging to find the right investor or corporate structure to support the policies and procedures that B Corp certification requires. In addition, every organization is different but there are always competing priorities that a business has to consider and becoming B Corp may simply not be a priority.

Ultimately, however, B Corp certification is recognition that a company is meeting very high standards of social and environmental accountability, which is something that all life science and healthcare companies should strive for.

***Marquis:*** *Why should (or should not) more science based companies become B Corps?*

**Tinger:** As B Corporations continue to gain prominence and visibility, I think the value of B Corp will resonate strongly within the scientific community.

*"Life science companies, intrinsically, are on a mission to use science to improve the world and make it a better place."*

However, just like any other industry, life sciences can also leave behind a carbon footprint, which is why it's critical to have a more holistic approach towards corporate responsibility. As a result, aiming for B Corp certification can further expand that mission by placing more emphasis on social responsibility, corporate culture and environmental sustainability.

Long term, this has great benefits for the company as well. From attracting new talent who are equally invested in sustainability to partnering with customers who are seeking companies committed to the values expressed by B Corp.

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# Using business as a force for good™

NEB is excited to announce that it has become a Certified B Corporation™, a recognition awarded to organizations with the highest standards for social and environmental performance, transparency and accountability.
B Corp™ certification goes beyond evaluating a product or service; it looks at the overall impact of a company by evaluating how a company treats:

Certified B Corporation®

- its employees
- its customers
- its community
- the environment

Learn more at **bcorporation.net**

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