NEB EXPRESSIONS

A scientific update

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IN THIS ISSUE

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Accelerating DNA Construction to Protein Expression: A Rapid 1-Day Workflow Using NEBridge® Golden Gate Assembly

Sean Lund, Ph.D., Colby Stoddard, Ph.D., Matthew Norton, Ph.D., New England Biolabs®, Inc.

Introduction

Molecular biology tools enable the custom generation of proteins with complete control of sequence, purification tags, secretion signals, and other performance characteristics. While the breadth of tools allows researchers to create their desired protein, this process often involves a low-throughput and time-consuming, multi-day workflow using live cells. To overcome these limitations, we have demonstrated a completely *in vitro* workflow that combines Golden Gate DNA Assembly, rolling circle amplification (RCA), and cell-free protein expression (CFPE) to rapidly screen the impact of multiple protein designs simultaneously (Figure1, page 2). This workflow enables researchers to generate an array of protein variants in a single day using a basic set of custom DNA vectors or insertion fragments. It also provides a means to assess the engineering constraints that are unique to each protein of interest and allows for the rapid identification of soluble protein.

Golden Gate Assembly (GGA) utilizes Type IIS restriction enzymes to cleave DNA in a manner that permits scarless assembly in a modular fashion, thereby facilitating the rapid creation of protein variant libraries. Efficient assemblies from low complexity (2 fragments) to high complexity assemblies (7–50+ fragments) are supported by NEB's optimized NEBridge® reagents and accompanying protocols (1, 2). While GGA has been widely used for over a decade, recent computational advances (3) by NEB have made this DNA assembly approach more robust and user-friendly, thereby enabling the creation of simplified workflows with a high probability of generating the desired DNA construct after a single round of assembly. As such, the workflow demonstrated here can be leveraged to generate many variants of any desired protein with minimal customization of DNA sequences.

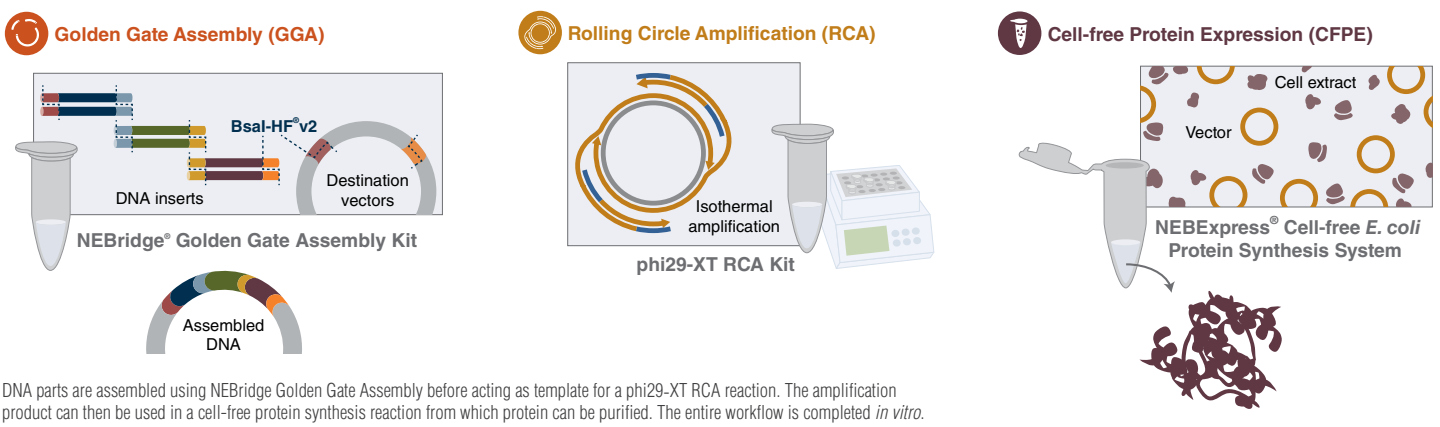
While GGA provides a standardized and efficient approach for gene assembly, RCA provides an ideal method to rapidly amplify assembled genes. RCA is a robust and highly sensitive isothermal amplification approach that continuously amplifies circular DNA, resulting in a high yield without specific PCR primers. Two-hour amplification times are achieved by employing a new phi29 polymerase engineered by NEB called phi29-XT. This polymerase generates more DNA product in a shorter amount of time versus wild-type phi29 polymerase, and maintains key qualities that are ideal for RCA applications, including high processivity, strong strand displacement activity, and high fidelity. phi29-XT Polymerase is also more thermostable, with an optimal reaction temperature of 42°C, and has improved sensitivity, supporting amplification down to 1 fg of DNA input (4).

Materials

- NEBridge Ligase Master Mix (NEB #M1100)
- BsaI-HF®v2 Type IIS restriction enzyme (NEB #R3733)
- Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494)
- phi29-XT RCA Kit (NEB #E1603)
- NEBExpress Cell-free *E. coli* Protein Synthesis System (NEB #E5360)
- NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)
- SNAP-Capture Magnetic Beads (NEB #S9145)
- Monarch® DNA Gel Extraction Kit (5 µg) (NEB #T1020)

The use of RCA over PCR shortens workflow time while generating ample amounts of template that are amenable to cell-free protein expression with the NEBExpress® Cell-free *E. coli* Protein Synthesis System. This system is an extract-based, transcription/translation (TXTL) system derived from *E. coli* cells engineered for high *in vitro* synthesis performance. It is designed to synthesize proteins ranging in size from 17–230 kDa in just 2–4 hours under the control of T7 RNA

FIGURE 1: **Overview of workflow**



DNA parts are assembled using NEBridge Golden Gate Assembly before acting as template for a phi29-XT RCA reaction. The amplification product can then be used in a cell-free protein synthesis reaction from which protein can be purified. The entire workflow is completed *in vitro*.

Polymerase (5). It removes the need for the costly and time-consuming steps of live-cell transformation and screening (6). Together, GGA, RCA, and cell-free protein synthesis provide a valuable tool set for maximizing laboratory productivity, especially when combined with automation, as high-throughput screening has become increasingly prevalent in research labs.

Methods

Please visit www.neb.com/appnote-DNAtoProtein for full methods used in workflow.

Results

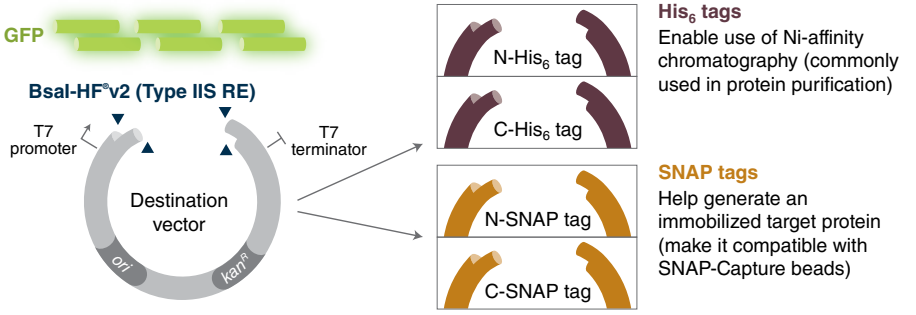
DNA constructs were rapidly generated and expressed in a 24-hour, *in vitro* workflow. Assemblies were constructed using 3-insert fragments to further demonstrate the utility and efficiency of GGA with modular systems. The three-fragment assemblies were constructed into expression vectors that were subjected immediately to RCA to generate templates for cell-free expression. After assembly construction, the entire workflow was conducted on a Thermomixer® providing support that such workflows could be scaled and automated. By using GGA with standardized vectors, multiple assemblies could be performed in parallel using the same DNA inputs with different accepting vectors. This plug-and-play methodology enabled rapid screening of various expression constructs without the need to order custom DNA for each expression.

By cloning multiple constructs at once, researchers investigating a single target or multiple targets in a molecular foundry can rapidly construct, express, and assay their target proteins. Here we show a rapid workflow expressing tagged constructs with minimal perturbance to native sequences from amplification through protein purification.

NEBridge Ligase Master Mix performs high-efficiency and high-fidelity GGA with a broad assortment of NEB Type IIS restriction enzymes. NEB has optimized the conditions that allow for convenient, efficient, and accurate GGA; users need only choose their preferred NEB Type IIS restriction enzyme and add DNA substrates to be assembled. By using NEBridge Ligase Master Mix, minimal ligation sequences of 4 bp can be added to 5´ and 3´ ends of inserts, thereby facilitating the use of common overhangs between destination vectors adding different functionality. If homologous sequence cloning methods were used, adding approximately 6 amino acids to each N- and C-terminus would be required, which could greatly disrupt the protein sequence to be investigated.

Four destination vectors were designed: N-terminal His_6, C-terminal His_6, N-terminal SNAP, and C-terminal SNAP (Figure 2). The His_6 tags enable the use of Ni-affinity chromatography commonly used in protein purification. The SNAP tags functionalize the target protein with a fusion that is compatible with NEB SNAP-Capture beads to generate an immobilized protein through a covalent linkage in the presence of a reducing agent. Using these two systems, we aimed to show that this construction-to-expression workflow is compatible with both soluble protein purification as well as immobilized products.

FIGURE 2: **Vector and construct design using Golden Gate Assembly**



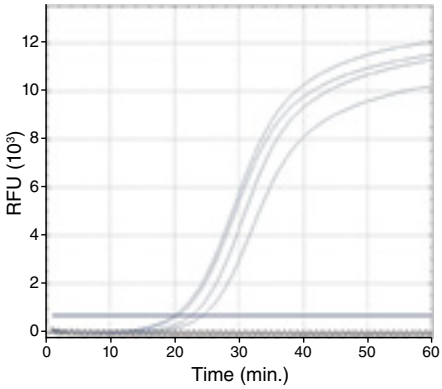
NEBridge Golden Gate Assembly allows for plug and play assembly of multiple vector constructs. The GFP protein is seamlessly cloned and assembled from 3 fragments into the vector containing either N- or C-terminal His tags, or N- or C-terminal SNAP tags, including the necessary T7 promoter, RBS, T7 RNAP terminator sequences, origin of replication and antibiotic resistance marker.

Destination vectors containing an origin and an antibiotic marker were designed to utilize the same overhangs when treated with the Type IIS restriction enzyme, BsaI-HFv2 (Figure 2). The multiple cloning sites were all flanked with a T7 promoter sequence upstream and a T7 terminator sequence downstream. The 5´ overhang was designed as "ACCA" where "A" represents the start codon of the ORF being investigated. This tag adds an additional threonine between any N-terminal tag and the investigated ORF. When using destination vectors not encoding an N-terminal tag, the "ACC" in the overhang is part of the spacer sequence between the ribosomal binding site and the coding sequence (CDS), generating a seamless CDS without an N-terminal addition. The 3´ overhang is "ATTC" wherein the stop codon is not included. The C-terminus has an isoleucine followed by a leucine ("CTG"). For vectors generating a C-terminal tag, the tag sequence immediately follows this isoleucine-leucine (IL) linker. If the vector does not encode a C-terminal tag, a stop codon (*) follows the IL linker sequence adding a total C-terminal linker of: IL*.

Inserts were designed using NEBridge SplitSet® Tool to divide the desired sequence into three fragments. The overhangs used were then verified with NEBridge Ligase Fidelity Viewer® with the added overhangs: "ACCA and ATTC" to ensure the robustness of the assembly. Fragments were next amplified using Q5 Hot Start High-Fidelity 2X Master Mix from dsDNA fragments. The amplified fragments were purified using the Monarch DNA Gel Extraction kit and quantified by absorbance. Stoichiometric amounts of each piece and backbone were then used in a GGA reaction with BsaI-HFv2 and NEBridge Ligase Master Mix.

Following the assembly of 3 parts into the destination vectors, the assembly mixes were diluted into a phi29-XT amplification reaction performed on a temperature block without shaking, and a high yield of DNA was quickly obtained (Figure 3, page 4). After amplification for 2 hours and heat inactivation at 65°C for 10 minutes, the reaction was cooled to room temperature, and a master mix of NEBExpress Cell-free *E. coli* Protein Synthesis was directly added to the tube. The mixture was incubated overnight with vigorous shaking. The NEBExpress Cell-free *E. coli* Protein Synthesis System was developed using several strategies to enhance performance, ease of use, and ensure robustness. These include using an *E. coli* strain genetically engineered to maximize the stability of template DNA and RNA and the protein products, a highly optimized reaction buffer, and a stringent biomanufacturing process.

FIGURE 3: **Rolling Circle Amplification quickly generates high yields of DNA from NEBridge Golden Gate Assembly reactions**



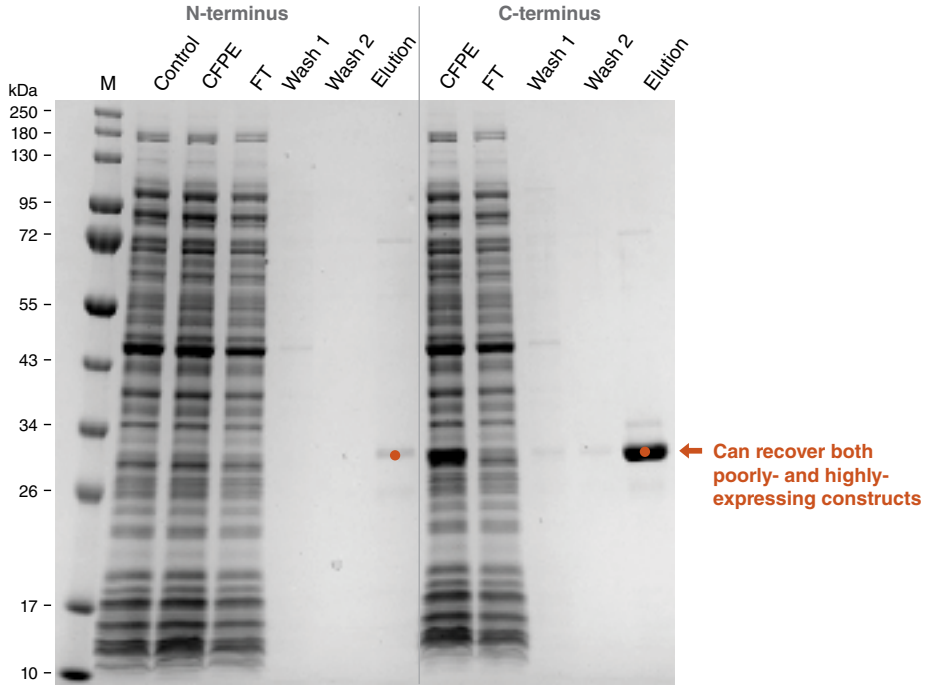
Real-time PCR using phi29-XT DNA Polymerase demonstrates fast and efficient amplification of DNA template within 2 hours. Image of real-time fluorescence results shows saturation at 60 minutes or less. Black triangles are non-templated amplification.

The following morning, after 14 hours of incubation, protein synthesis was verified by SDS-PAGE. In one workflow, 100 µl of the His_6-tagged reactions were purified using 50 µl NEBExpress Ni-NTA Magnetic Beads (Figure 4, page 4). A stark contrast in yield was observed between the N- and C-terminally His_6-tagged proteins. Despite the low yield of the N-terminally tagged construct, the purified protein was isolated and observed. The difference in yield demonstrates the need for screening multiple constructs in parallel. In a separate workflow, the SNAP-tagged expressed proteins were affixed to 50 µl SNAP-Capture Magnetic beads in the presence of DTT (Figure 5, page 4). These two workflows demonstrated the compatibility of the material from NEBExpress Cell-free *E. coli* Protein Synthesis with our protein purification products.

Conclusion

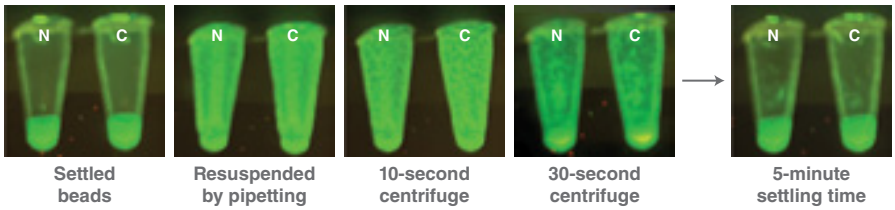
The workflow described herein represents a seismic shift in the time it takes to go from a cloning reaction to purified protein relative to typical cloning and subsequent protein expression protocols. NEBridge Golden Gate Assembly reactions were successfully used as templates for amplification by phi29-XT. The amplified material was suitable as an input for cell-free protein synthesis as shown by the demonstration of purifying His_6-tagged constructs as well as SNAP-Capture. Due to the precision of assemblies using NEBridge Golden Gate Assembly, the rapid amplification of the phi29-XT RCA kit, and the high yield of the NEBExpress Cell-free *E. coli* Protein Synthesis system, genes can be cloned into expression constructs and expressed within a day.

FIGURE 4: **GFP is highly pure following cell-free protein expression and purification**



SDS-PAGE showing the different His_6-tagged GFP proteins that were expressed by NEBExpress Cell-free *E. coli* protein synthesis system and purified using NEBExpress Ni-NTA Magnetic Beads. While the N-terminally tagged construct expressed poorly, protein was still obtained after purification. The C-terminally tagged construct expressed well resulting in high yield of purified protein.

FIGURE 5: **GFP is immobilized and functionalized on magnetic beads**



SNAP-functionalized proteins were captured using SNAP-Capture Magnetic Beads. As shown, SNAP-tagged GFP is detected on the beads when they are settled. After resuspension and pelleting, GFP fluorescence is only detected on the beads showing a strong association between the protein of interest and bead. N = N-terminus, C = C-terminus.

Learn more about NEBridge Golden Gate Assembly at **www.neb.com/goldengate**. Try our NEBridge Ligase Fidelity Tools for Golden Gate Assembly at **ligasefidelity.neb.com**

For more information about the phi29-XT RCA Kit, please visit **www.neb.com/E1603**

Learn more about protein expression at NEB at **www.neb.com/proteinexpression**

View the entire article
www.neb.com/appnote-DNAtoProtein

References

1. Pryor, J.M., et al. (2022) *ACS Synth. Biol.* 11: 2036-2042.
2. Pryor, J. M., et al. (2020) *PLoS One*, 15(9): e0238592.
3. Potapov, V. et al. (2018) *ACS Synth. Biol.*, 7: 2665–2674.
4. *phi29-XT RCA Kit Instruction Manual*: New England Biolabs, Inc.
5. Magnelli, P., et al. (2020) Technical Note: *NEBExpress cell-free E. coli protein synthesis system – a high performance E. coli cell lysate-based system for in-vitro protein synthesis.* New England Biolabs, Inc.
6. Buss, J., et al. (2023) Application Note: *Scaling down to scale up – Miniaturizing cell-free protein synthesis reactions with the Echo 525 Acoustic Liquid Handler.* New England Biolabs, Inc.

NEW PRODUCT HIGHLIGHT:

Authenticase™

Authenticase (NEB #M0689) is a new, unique blend of structure-specific nucleases that can recognize and cleave outside mismatch and indel (insertion and/or deletion) regions, ranging from 1-10 base pairs on double-stranded DNA. The unique combination of enzymes in Authenticase gives it superior recognition capabilities. Authenticase identifies seven of the eight possible single-base mismatch combinations: C/C, T/C, A/C, T/G, G/G, T/T and A/A. The only single-base mismatch it does not recognize is A/G.

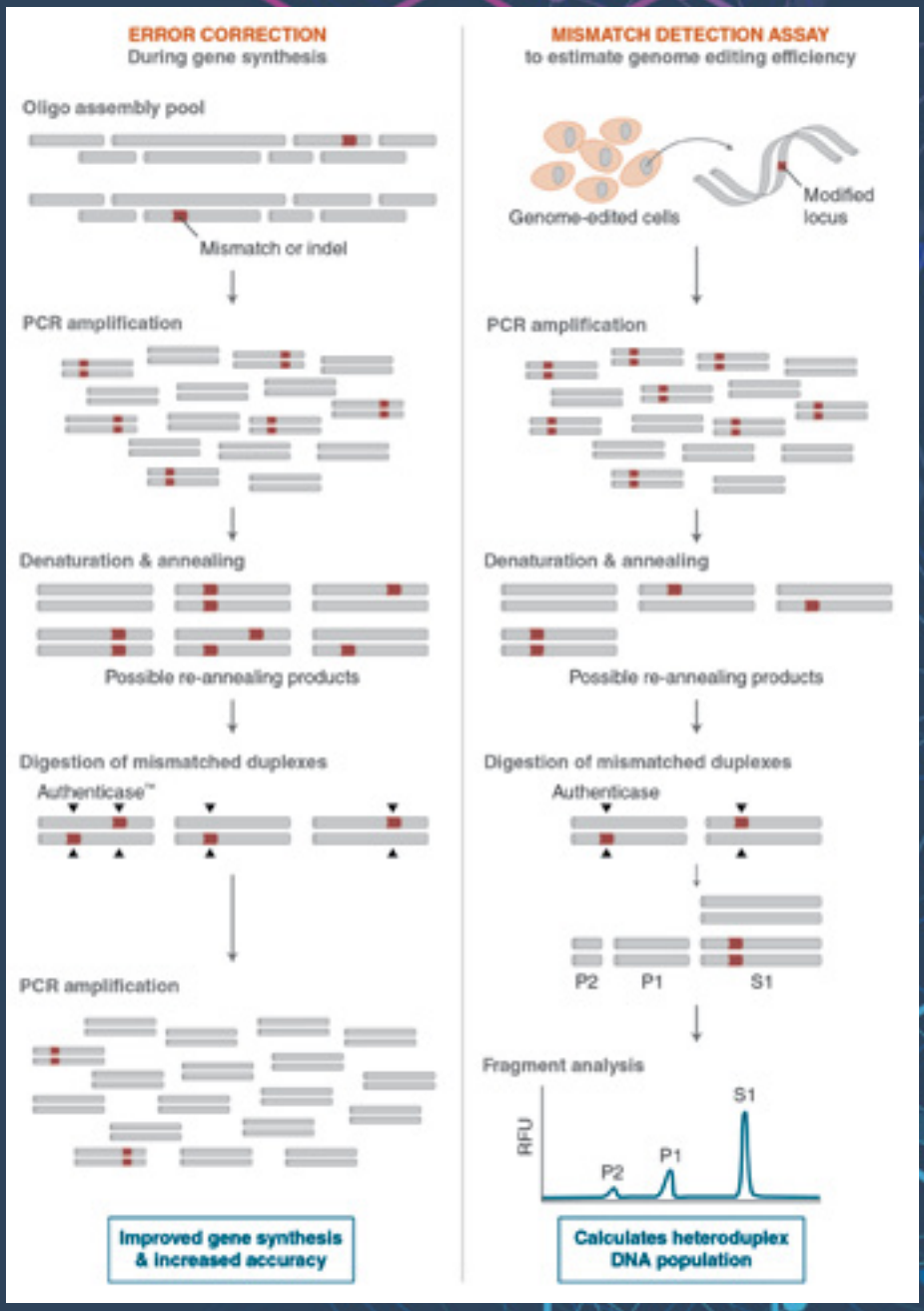
Authenticase has two primary applications:

1. Error correction during gene synthesis – oligo-based PCR gene assembly workflows encounter a significant rate of residual errors. Authenticase can enzymatically remove these errors prior to the final renaturation and amplification step.

2. Mismatch detection assay – quickly screen heterogenous cell populations to estimate genome editing efficiency by identifying regions containing mismatches and indels. Authenticase recognizes a more comprehensive set of structures than the commonly used T7 Endonuclease I.

Ordering Information

PRODUCT	NEB #	SIZE
Authenticase	M0689S/L	25/125 rxns



Authenticase is a mixture of structure-specific nucleases capable of recognizing and cleaving outside mismatch and indel (insertion and/or deletion) regions, ranging from 1–10 basepairs (bp) on double-stranded DNA. The formulation has limited non-specific activity on homoduplex regions of DNA. Authenticase can be used as an error-correction reagent in oligo-based PCR gene assembly by enzymatically removing "mistakes" prior to the final renaturation and amplification step. Alternatively, Authenticase can replace T7 Endonuclease I in the mismatch detection assay used to assess the efficiency of genome editing (S1 is the starting material. P1 and P2 are products of Authenticase digestion.).

NEBinspired

Learn more about how Authenticase can enhance error correction during gene synthesis in our NEB Inspired blog post
www.neb.com/superior-error-detection

NEBNext® for FFPE DNA Library Prep

Archiving of clinical materials as Formalin-Fixed, Paraffin-Embedded (FFPE) samples is a common practice. However, DNA extracted from FFPE samples poses many challenges for library preparation, including low input amounts and highly variable damage from fixation, storage, and extraction methods. As a result, it can be challenging to construct high quality libraries in sufficient quantity to achieve good sequence data at the desired depth of coverage. NEB scientists have been working on repair and fragmentation solutions that result in high-quality and high-yield DNA libraries.

The NEBNext FFPE DNA Library Prep Kits address these challenges with a unique combination of optimized reagents:

- NEBNext FFPE DNA Repair v2 Module offers improved ability to repair FFPE-induced DNA damage
- Library prep reagents and protocols have been optimized for FFPE DNA
- The new NEBNext MSTC™ FFPE Master Mix has been optimized for robust library amplification
- NEBNext UltraShear™ FFPE DNA Library Prep Kit (#E6655 only) provides enzymatic fragmentation tailored for FFPE DNA

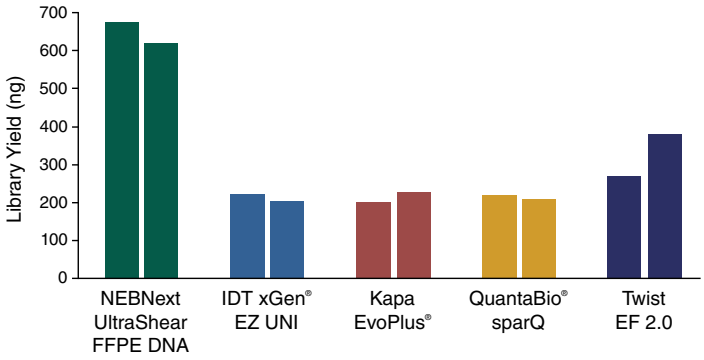
Benefits

- Increased library yields
- Improved sequencing metrics
- Greater sensitivity of somatic variant calling
- 5-250 ng input range
- Automation-friendly workflows

NEBNext UltraShear FFPE DNA Library Prep Kit workflow

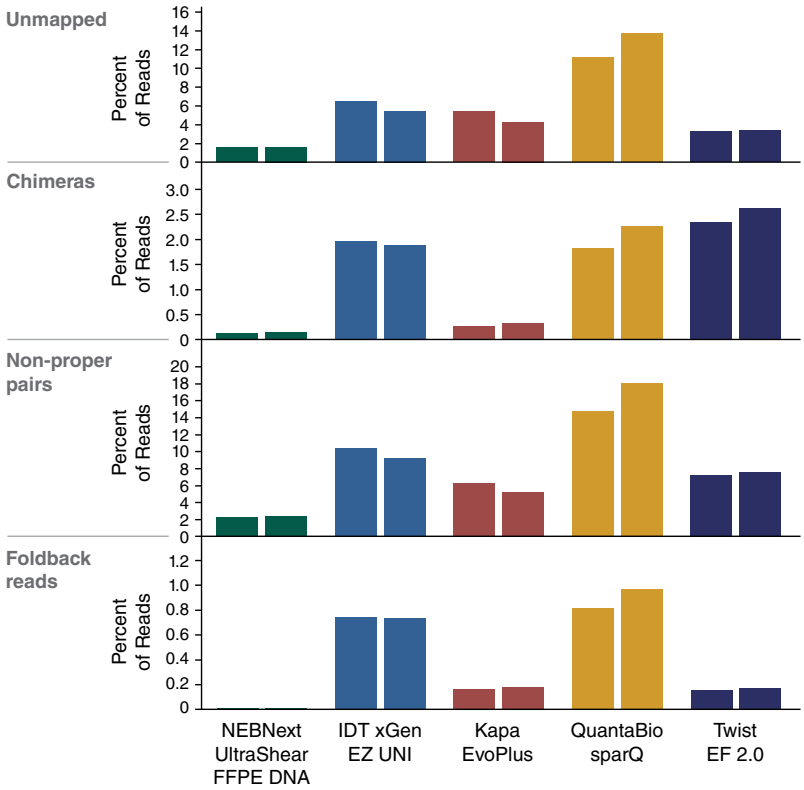
NEBNext FFPE DNA Library Prep Kit workflow

The NEBNext UltraShear™ FFPE DNA Library Prep Kit and the NEBNext® FFPE DNA Library Prep Kit have streamlined workflows with minimal hands-on time across a range of inputs from 5-250 ng. The protocols have been optimized for the user to safely store the reaction after any step in the workflows overnight at -20°C without affecting library yield or quality.



NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields

Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear™ FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors (IDT® xGen® EZ UNI, Kapa EvoPlus® Library Prep Kit, QuantaBio® sparQ DNA Library Prep Kit, and Twist Library Preparation EF 2.0 kit). Library yields (total ng) were quantified using the Qubit® High-Sensitivity dsDNA assay (Thermo Fisher Scientific®). The NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields, sufficient for target enrichment library input.



NEBNext UltraShear FFPE DNA Library Prep Kit improves library equity

Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors (IDT xGen EZ UNI, Kapa EvoPlus Library Prep Kit, QuantaBio sparQ DNA Library Prep Kit, and Twist Library Preparation EF 2.0 kit). Libraries were sequenced on the Illumina NovaSeq 6000 (2 x 100 base reads) and downsampled to 5 million paired-end reads. Reads were mapped using Bowtie2 (version 2.3.2.2) to the GRCh38 reference and duplicates marked using Picard MarkDuplicates (version 1.56.0). Library quality metrics were assessed using Picard Alignment Summary Metrics (version 1.56.0). The level of foldback reads was calculated using Seq_frag_remap (version 0.2). The NEBNext UltraShear FFPE DNA Library Prep Kit improves library quality by reducing the percentage of unmapped, chimeric, non-properly paired, and foldback reads.

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext Ultrashear FFPE DNA Library Prep Kit	E6655S/L	24/96 rxns
NEBNext FFPE DNA Library Prep Kit	E6650S/L	24/96 rxns
NEBNext FFPE DNA Repair v2 Module	E7360S/L	24/96 rxns

Learn how to overcome the challenges in FFPE DNA library prep in our recent NEB inspired blog post

For more information, visit **www.neb.com/ffpe-dna**

NEB's commitment to the scientific community through enhanced web tools

At New England Biolabs (NEB), we are steadfast in our commitment to support scientists in their research endeavors. Part of this commitment is offering an array of software tools tailored to streamline scientific processes. At their core, our tools aggregate important product information and offload tedious or error-prone calculations. Notably, these tools aren't commercial products for sale. Instead, they are collaborative innovations stemming from the collective expertise across various departments at NEB - from research scientists to marketing professionals.

In this article, Senior Software Engineer Sanjay Kumar answers questions about the collaborative nature of tool development at NEB, the testing and validation processes, and their impact on advancing science.

How collaborative is the process of developing a new web tool?

Almost every tool is a collaborative project with multiple contributions from a broad range of expertise at NEB. Core algorithmic design and functional specifications usually come from research scientists, product advocacy groups, or technical support specialists. Our Marketing Communications group provides invaluable advice on user interface design. Software design architecture and implementation are handled by software developers, who usually are or were research scientists themselves. The process is very iterative, with multiple interactions between all the aforementioned groups.

Who tests the tools to ensure they are ready for use?

The tools are tested at several levels. Primary testing is done by the software developers who almost always have strong knowledge in the tool's application domain. At the next level, the tools are tested in-house by domain experts in NEB's Research and Application & Product Development divisions. Finally, in some cases, we enlist potential users from outside of NEB to evaluate the tools prior to general release.

What is the most popular NEB web tool?

The occupant of the top spot in popularity varies over time. Recently, it's been NEBioCalculator®, with Tm Calculator and NEBcutter® close behind. The top four tools consistently are NEBioCalculator, NEBcutter, TmCalculator, and NEBcloner®.

Are the tools subscription-based? Do I need to download them?

Neither. The tools are almost all web-based applications and are freely accessible from any modern browser. Many will work comfortably on even small platforms like phones, though most will do better on desktop or tablet browsers. The only tools that require download are the NEB tools mobile app and the NEB AR mobile app that runs on iPhone® iPad® and Android™ devices.

Are the tools complicated to use? Are there any resources to explain how to use them?

Every effort is made to ensure our tools are intuitive and easy to use, but sometimes, additional resources are helpful to fully understand all the features available for use. Many tools have a "Help" link, which is often overlooked but loads a page that is surprisingly readable (and useful) as software documentation goes. In addition, several tools have video tutorials, which can be found on their "home" page, "help" page, or in our video library (www.neb.com/tool-tutorials).

Are the tools regularly updated with new features?

Yes. New features are added as new products or applications for the products a tool supports are developed. Recurring problems, questions, or suggestions reported through NEB Technical Support are another source for new features. In addition, tools requiring on-board data sets are updated when the data changes. Most recently, we have updated the capabilities of NEBaseChanger, our web tool that helps researchers design site-directed mutagenesis experiments, with options for batch inputs, the ability to use ambiguous bases, and the ability to make substitutions based on either nucleotide or amino acid sequences.

We also make available beta versions of tools that our scientists use in their research (neb.com/research/nebeta-tools). These tools are in the process of optimization for design and usability, and so feedback from users in the scientific community on their functionality and utility helps us improve them for future use.

Do you have any examples of how NEB tools have enabled science?

One notable example of how NEB web tools have advanced science is during the COVID-19 pandemic. NEB's computational biologists recognized that mutations in the SARS-CoV-2 virus might render detection primers ineffective. In response, they developed the Primer Monitor Tool, which collates SARS-CoV-2 genome sequences and offers visualization methods to ensure diagnostic primers can detect virus variants. While initially designed for COVID-19, the underlying technology has broader applications, and the NEB Bioinformatics team envisions expanding its use to other viruses, enhancing research and diagnostics globally.

To view all web tools, visit **www.neb.com/tools-and-resources/web-tools**

Choose NEBuilder® HiFi for DNA assembly

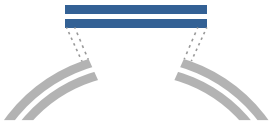
NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5´- and 3´-end mismatches. Available with and without competent *E. coli*, this flexible kit allows simple and fast seamless cloning utilizing a new proprietary high-fidelity polymerase. Find out why NEBuilder HiFi is the next generation of DNA assembly and cloning.

NEBuilder HiFi DNA assembly offers unique features over other DNA assembly methods and can be used for:

- 3´- and 5´-end mismatch removal before fragment assembly. It is excellent for removing restriction sites or other sequences from plasmids or other DNA constructs
- Bridging two ds-fragments with a ssDNA oligo, which is useful for sgRNA arrays
- Performing multi-site mutagenesis or combinatorial mutagenesis for diverse multi-site mutant library creation and screening
- Experiments involving miniaturization and nanoliter scale volumes

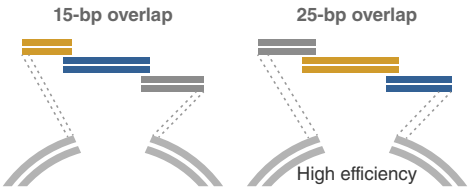
Not your average DNA assembly reagent

2-fragment assembly

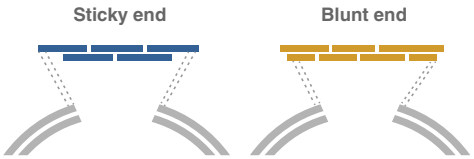


ssOligo & dsDNA assembly

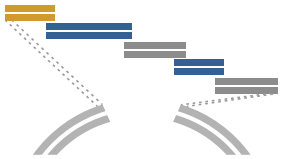
4-fragment assembly



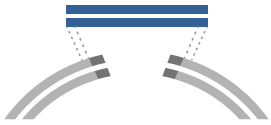
Annealed-oligo assembly



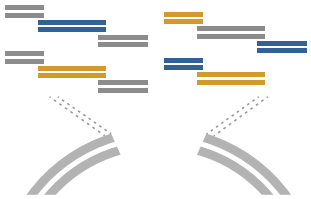
6-fragment assembly



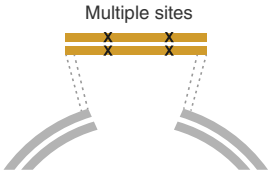
3´- and 5´-end mismatch assembly



12-fragment assembly



Site-directed mutagenesis



6 Reasons to choose NEBuilder HiFi

1 Save time

Enjoy simple and fast seamless cloning in as little as 15 minutes.

2 Flexibility

Use one system for both "standard-size" cloning and larger gene assembly products, up to 12 fragments.

3 Compatible with downstream applications

DNA can be used immediately for transformation or as template for PCR or RCA.

4 Adaptable

Adapts easily for multiple DNA manipulations, including mismatch and ssOligo assembly.

5 Site-directed mutagenesis

Use to perform multi-site mutagenesis.

6 Increased stability

Store at -20°C, with improved stability over competition.

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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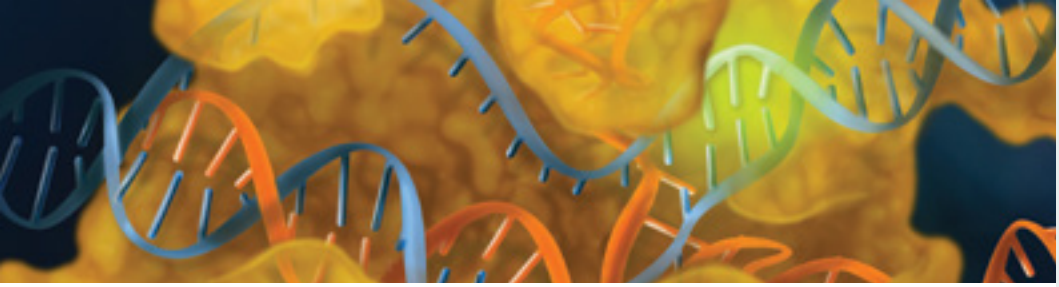
***Sample available in US only.*



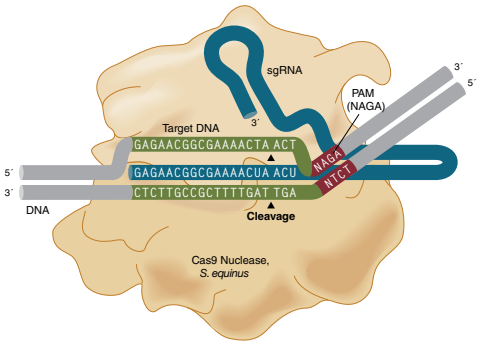
New genome editing product highlights

NEB is focused on discovering unique properties of Cas nucleases. Our recent offerings include EnGen® Spy Cas9 HF1 and EnGen Seq1 Cas9, RNA-guided DNA endonucleases that catalyze site-specific cleavage of double-stranded DNA (dsDNA). Both products feature the Simian virus 40 (SV40) T antigen nuclear localization signals (NLS) at both the N- and C-termini of the protein and are ideal for the direct introduction of Cas9/sgRNA complexes, underscoring their utility in precision genetic editing.

EnGen Seq1 Cas9

- From *Streptococcus equinus*
- 5´- NAGA -3´ PAM sequence allows targeting of additional genomic regions
- Ideal for direct introduction of Cas9/sgRNA complexes
- Compatible with the EnGen Mutation Detection Kit (NEB #E3321S)
- Active in *in vitro* reactions from 20°C to 45°C

EnGen Seq1 Cas9
Schematic representation of *S. equinus* Cas9 nuclease with a single guide RNA and target DNA.



EnGen Spy Cas9 HF1

- From *Streptococcus pyogenes*
- High-fidelity variant of Spy Cas9 nuclease differing by four point mutations (N497A/R661A/Q695A/Q926A) reduces off-target cleavage
- Targets Cas9 to the region immediately upstream of a 5′-NGG-3′PAM
- Ideal for direct introduction of Cas9/sgRNA complexes
- Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322S) and the EnGen Mutation Detection Kit (NEB #E3321S)

Ordering Information

PRODUCT	NEB #	SIZE
EnGen® Spy Cas9 HF1	M0667T/M	500/2,500 pmol
EnGen® Seq1 Cas9	M0668T	500 pmol

FEATURED BLOG

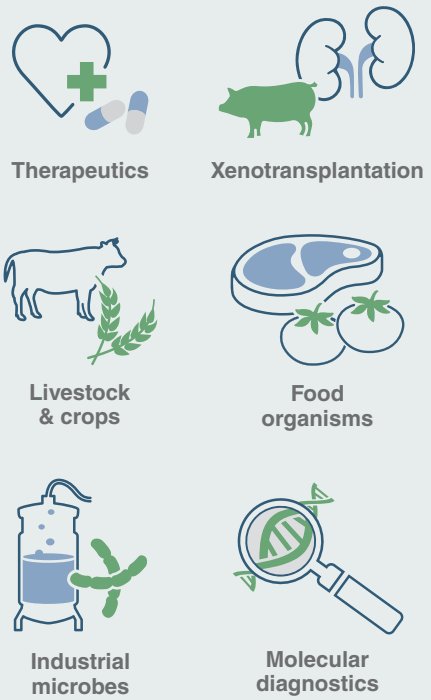
Adding capabilities to CRISPR-Cas-based biotechnology

by Nicole Kelesoglu, New England Biolabs

NEB collaborates with both academic and industry researchers in their mission of discovery, characterization, and application development efforts with CRISPR-Cas systems. It's always galvanizing to look to the origin of CRISPR tools and consider how this scientific field has expanded into multiple substrate types and applications over time. While NEB produces many useful Cas nucleases to support research and development, our researchers are always pursuing novel, distinctive properties – inspired by the abundance and diversity of Cas nucleases found in nature.

visit **neb.com/crispr-capabilities** to read or listen to this blog

CRISPR APPLICATIONS



by Aiden Beauglehole, Australian National University

The environmental impact of plastics: a global concern

Globally, we possess 8.3 billion tons of plastic. The magnitude of this plastic's impact on the world cannot be overstated. By 2050, plastics alone will contribute to 15% of the global carbon budget, hastening irreversible damage caused by climate change. For comparison, this is equivalent to the current CO_2 emissions from deforestation. Furthermore, plastics exert a tangible impact on the environment. According to the United Nations, an estimated 23 million tons of plastic contaminate our aquatic ecosystems annually. This leads to significant consequences for both marine and terrestrial wildlife, contributing to ecosystem degradation and loss of life.

Despite the negative impacts of plastics, they remain an integral and valuable aspect of global society. They have revolutionized our products and culture, and their influence will continue to be deeply embedded in our society. It is imperative that we develop strategies to mitigate their global impact.

Understanding the chemistry of plastics: the persistence of plastic and the challenge of recycling

To comprehend the issues associated with plastics, it is essential to understand their synthesis. Plastics are composed of monomers that are polymerized through chemical reactions to form polymers. Monomers are typically derived through the refining process of crude oil, serving as the primary source of plastics' contribution to the carbon budget. Specific combinations of monomers give different plastics their unique properties. For example, single use drink bottle plastic, PET, is made from the monomers ethylene glycol and terephthalic acid. These monomer combinations confer unique properties upon plastics, including their remarkable durability, as it is incredibly hard to reverse the polymerization reaction. However, their durability is also problematic as they are resistant to environmental degradation. This hampers recycling efforts, necessitating the production of virgin-grade monomers for further plastic creation. This establishes a detrimental unidirectional cycle, resulting in an accumulation of plastics while production of virgin plastics continues.

Addressing plastic accumulation through enhanced recycling efforts

One viable solution to tackle the accumulation of plastics involves a concerted effort to increase current recycling endeavours. With its considerable abundance, plastic offers a substantial feedstock capable of sustaining recycling initiatives across generations. Recycling the plastic already created promotes circularity, effectively halting its re-entry into the environment. This turns plastic into a valuable commodity, serving as the foundation for generating new plastic products. Embracing this approach holds significant promise for mitigating the impact of plastics on our planet. However, the current global plastic recycling rate remains disheartening low, standing at a mere 9%. Adding to the challenge, existing recycling techniques only allow plastic to be recycled between one to ten times, contingent upon the plastic type.

Advancing recycling techniques: an enzymatic approach to enhancing recycling efforts

The key to advancing current recycling techniques lies in embracing enzymatic recycling. Samsara Eco (www.samsaraeco.com) has developed enzymes that depolymerize plastics into their core monomers. The broader scientific community is familiar with enzymes that degrade plastic, having been discovered in various microbial species. However, Samsara has elevated enzymatic plastic recycling to a higher level through synthetic biology. They have created a proprietary algorithm to design new-to-nature enzymes specific for different types of plastics and specific for industrial processes. These enzymes are subsequently produced using microbial fermenters, yielding ample quantities for the chemical engineering recycling process.

The benefits of the enzymatic recycling process

Samsara's enzymatic recycling process offers notable advantages. The primary advantage is the ability to infinitely recycle polymers into monomers. The plastic is recycled into virgin-grade monomers, ensuring no loss of quality between batches. Unlike previous methods with a recycling number cap, Samsara's process for recycling plastic can undergo this process an unlimited number of times. Moreover, it possesses an equivalent economic viability to traditional plastic synthesis methods. Through meticulous chemical engineering, this enzyme recycling process enables the sale of monomers at a competitive cost, comparable to oil-based methods. This is a pivotal factor enabling the adoption of recycled monomers in the market, while still preserving the cost advantage of plastic production.

In the face of an escalating plastic crisis, enzymatic recycling emerges as a groundbreaking solution. The method gives hope for a sustainable future, blending environmental responsibility with economic viability. The future of recycling, it seems, lies in the incredible power of enzymes, heralding an era of genuine circularity in plastic usage.

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