NEB EXPRESSIONS

A scientific update

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IN THIS ISSUE

SCIENCE

INNOVATION

EDUCATION

NEW ENGLAND Biolabs®

A LETTER FROM OUR CEO

Dear Researcher,

We at NEB recognize the uncertainties many of us in the life sciences are facing today. These uncertainties stem from concerns about the impact of tariffs, the real potential for reductions in US government funding for science, geopolitical conflict, and a variety of other factors.

For many of you, this has already disrupted the important science you are doing. Unfortunately, unless corrected or minimized quickly, these issues are likely to have long-lasting impacts on life science innovation, the full extent of which may not be evident in the short term.

During times of uncertainty, we at NEB remain committed to the constants that guide us – our core values. For more than 50 years, we have prioritized the advancement of science in everything we do and have remained dedicated to supporting the scientific community in any way we can.

We recognize the essential role science plays in improving human health, in understanding the world around us, and in preserving that world for future generations. We are community of scientists, collaborators and supporters, all working together to advance science for the greater good.

Through the remainder of this year, NEB will be celebrating scientists and the important work they do across the globe. We will highlight their research in social media posts, videos, blogs, podcasts, and featured publications, including this issue of NEB Expressions in which Jenny Brown interviews our friends Tim Mercer and Seth Cheetham from the University of Queensland. This issue also highlights NEB Passion in Science Award® Winner Adewunmi Akingbola and his dedication to combatting viral hepatitis in underserved communities across Nigeria.

If you have a project or scientific accomplishment that you would like us to highlight, please feel free to reach out at customerstories@neb.com. By working together and showcasing the real value of the work we do, we can continue to shape the science of tomorrow.

Thank you and wishing you success in your research,

Sal Russello
Chief Executive Officer
New England Biolabs, Inc.

CLONING BEYOND CLASSIC RESTRICTION ENZYME METHODS

Gregory J. S. Lohman, Ph.D., Sean Lund, Ph.D., Stephanie M. Khairallah, Ph.D., Andrew P. Sikkema, Ph.D., S. Kasra Tabatabaei, Ph.D., New England Biolabs®

For over 50 years, NEB has been a leading supplier of restriction enzymes and other reagents for DNA cloning. While restriction enzyme-based methods for the generation of recombinant DNA have dominated for most of molecular biology's modern history, in the last 15 years new methods have emerged that enable seamless cloning and the joining of multiple inserts in a single reaction, some without the use of restriction enzymes at all. This article is an excerpt from "*Molecular Cloning Technology - Past, Present and Future*" on neb.com (see QR code to right), highlighting three widely adopted methods: USER® cloning, homology-directed assembly (NEBuilder®), and Golden Gate Assembly. Other approaches, including recombinational cloning, SLiCE, TOPO, and automation-enabled workflows, are also discussed in the full version available online.

View full article

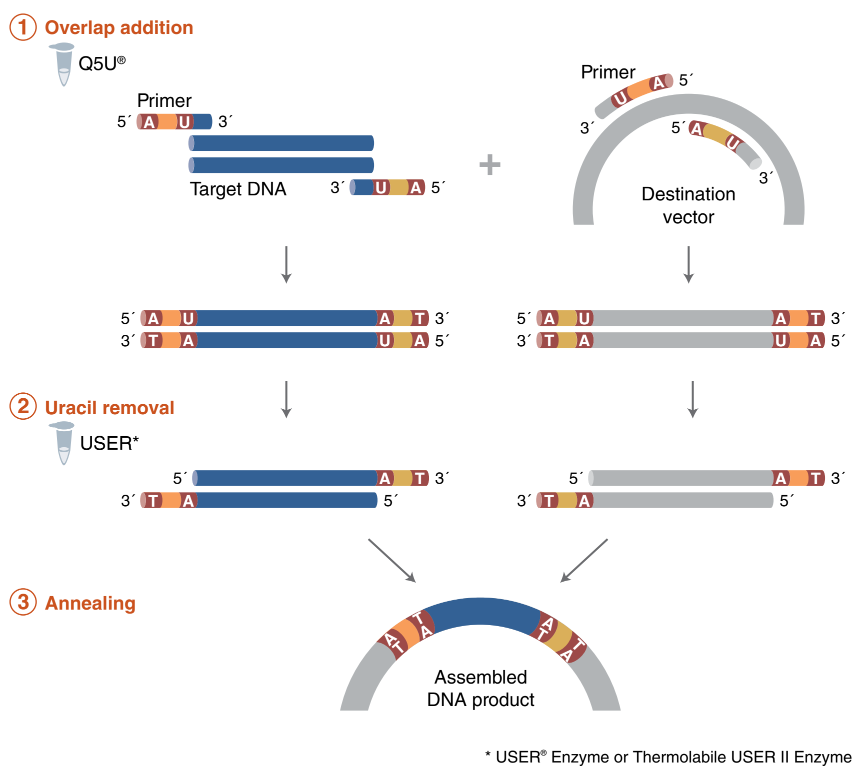
USER CLONING

USER (Uracil-Specific Excision Reagent) cloning (Figure 1) was developed at NEB in the mid-2000s (1). This method relies on PCR primers that contain deoxyuridine bases at defined positions. The PCR product is treated with uracil DNA glycosylase (UDG), which excises the uracil bases to generate abasic sites, and Endonuclease VIII (NEB #M0299), which cleaves the abasic nucleosides leaving a single-stranded 3´ overhang that can be annealed to a similarly treated vector. USER treatment can generate ssDNA overhangs of arbitrary length from almost any sequence, with the exception that the last base of the overhang before the dsDNA region must be an adenosine. Fragments are annealed and transformed, with the resultant nicks ligated *in vivo*. Thus, if overhang sequences are chosen carefully, this method permits the assembly of multiple fragments at once, allowing multiple PCR products to be inserted into a vector in a single step.

ISOTHERMAL HOMOLOGY-DIRECTED ASSEMBLY (GIBSON/NEBUILDER)

While pursuing the goal of producing a fully synthetic *Mycoplasma genitalium* genome (>500 kB in size), a one-step, isothermal assembly protocol was developed, combining a 5´→3´ exonuclease, a DNA polymerase, and a ligase in one reaction.

Figure 1: **Uracil-Specific Excision Reagent (USER) Cloning**



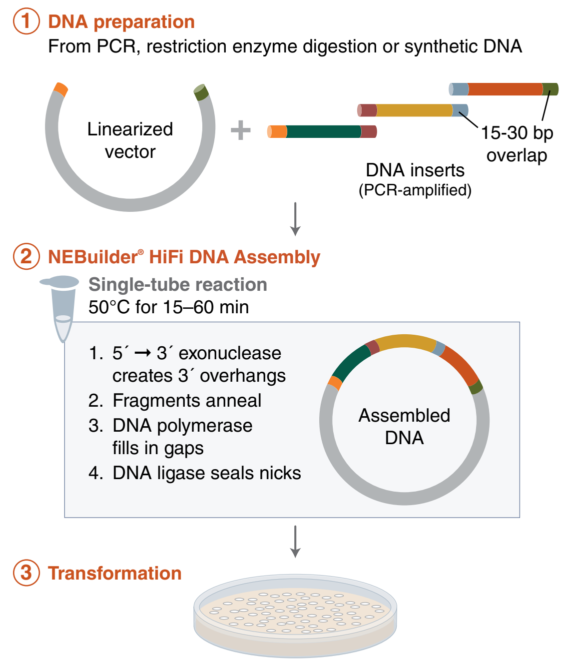
Primers containing a terminal A and an internal dU are used to append the homology regions to the insert; similar primers to generate compatible ends are used to linearize the vector (1). The resulting amplified vector and insert contain one dU base near each end of the amplified DNA. Treatment with USER enzyme removes the uracil base and excises the abasic site, generating long defined 3´ overhangs. Direct transformation of annealed products (3) allows for the joining of these ends in.

In this version, the exonuclease activity is used to generate complementary single-stranded regions that can anneal with complements in adjoining fragments. A DNA polymerase lacking strand displacement activity then extends the DNA to fill in gaps, halting upon encountering a 5´ end. The resultant nick is then sealed by *Taq* DNA Ligase (NEB #M0208). This method, commonly known as Gibson Assembly®, would go on to see application far beyond complex genome assembly and become one of the dominant cloning protocols in use today (2).

Homology-directed cloning offers significant advantages over traditional methods. It allows any insert to be cloned into any vector using an enzyme master mix in a single isothermal incubation step, taking as little as 15 minutes. This method can also join multiple linear DNA fragments in a user-defined order, provided 15–20 bp of homology are present at the joining sites. Vectors are linearized using restriction enzymes or PCR amplification. Homology can be introduced via PCR using primers that encode homologous sequences. Furthermore, primers can be designed to introduce mutations at the joining sites, enabling multi-site mutagenesis in a single reaction. Typically, five fragments can be routinely assembled using homology-directed isothermal assembly methods. Assembly design tools like Geneious® or the online NEBuilder Assembly Tool support error-free design of homology regions and PCR primers for multi-fragment assembly.

While a powerful technique, homology-directed assembly can be limited by incorporation errors within the overlap regions, derived from the repeated digestion/extension reactions in the overlap regions. This issue can be mitigated by using homology-directed assembly methods that employ high-fidelity polymerases, such as NEBuilder HiFi DNA Assembly (Figure 2). NEBuilder provides enhanced proof-reading activity and tolerance of both 3´ and 5´ terminal mismatches between homologous fragments. Additionally, the special formulation allows for the assembly of homologous parts using single-stranded bridges and requires shorter overlapping homology regions than the original method. Up to twelve fragments can be assembled in one step using NEBuilder.

Figure 2: **NEBuilder® HiFi DNA Assembly**



A high-fidelity homology-directed cloning method, NEBuilder permits the simultaneous, ordered joining of multiple fragments through 15-30 nt of sequence homology at their ends. After *in silico* design, fragments are generated via PCR to add the homology ends that will be used for assembly. Vectors are typically also prepared through PCR, but can be prepared by restriction digestion or obtained as synthetic DNA given the product contain the required homology regions to the inserts. The fragments are combined (2) with the enzyme master mix; homologous ssDNA overhangs are generated in the homology regions by an exonuclease, a DNA polymerase fills any gaps, and a DNA ligase seals the junctions. The assembled insert-containing vector is then transformed into competent cells (3), allowing the desired recombinant construct to be propagated.

GOLDEN GATE ASSEMBLY

Golden Gate Assembly (GGA), also known as Golden Gate Cloning, is another cloning method that permits multiple fragments to be joined in a single reaction, efficiently and in a defined order (3–5). The name was inspired by inserts "bridging" the two ends of a vector, along with a reference to the recombination-based Gateway® cloning for its use of standardized vectors. While it is often considered an alternative to long homology-dependent multi-fragment assembly, the method shares more in common with traditional restriction enzyme cloning than with exonuclease-dependent methods like NEBuilder.

GGA relies on the unique properties of Type IIS restriction enzymes, which had already been explored in precursor methodologies (6,7). Traditional cloning methods most often use Type IIP enzymes, which recognize and cut within a palindromic binding site to generate a self-complementary overhang. Ligation of two ends generated by the same Type IIP enzyme regenerates the restriction recognition/cut site. Type IIS enzymes, by contrast, bind a non-palindromic recognition site, and cut distal to the binding site (Figure 3, page 5). This generates a short overhang that can be any possible nucleotide sequence, determined by the DNA sequence of the cut site, not the recognition site. This property allows generation of overhangs that are not self-complementary, preventing dimerization and permitting the joining of multiple fragments in a desired order (6,7). Instead of a classic digestion, purification and ligation protocol, GGA combines the uncut DNA fragments and destination vector in a one-pot reaction mixture containing both the restriction and ligation enzymes. When the sites are cut, the Type IIS recognition sites are separated from the inserts and lost from the vector. If a fragment or vector re-ligates to the originally cut away piece of DNA, the Type IIS site is regenerated and can be cut again. However, if it ligates to its desired partner, the Type IIS recognition site is eliminated. By cycling between optimal temperatures for cleavage and ligation, high yields of assembled product can be achieved.

A drawback of the method is that GGA requires all naturally occurring instances of the recognition site for the enzyme to be removed from the native sequence, or these will be cut during assembly, greatly reducing the yield of the final assembly. The removal of these sites can be accomplished through a process known as "domestication," altering the sequence to remove these sites, e.g., by making silent mutations. When using synthetic DNA, these changes can be made *in silico*. When generating fragments by PCR, internal sites must be removed by first cloning the fragments then performing site-directed mutagensis (SDM), or by making the changes in the primers themselves by placing the GGA break points near the sites to be mutated. The latter process can be used to introduce any desired point mutations, permitting GGA to be used as a method of multisite mutagenesis as well as assembly (8).

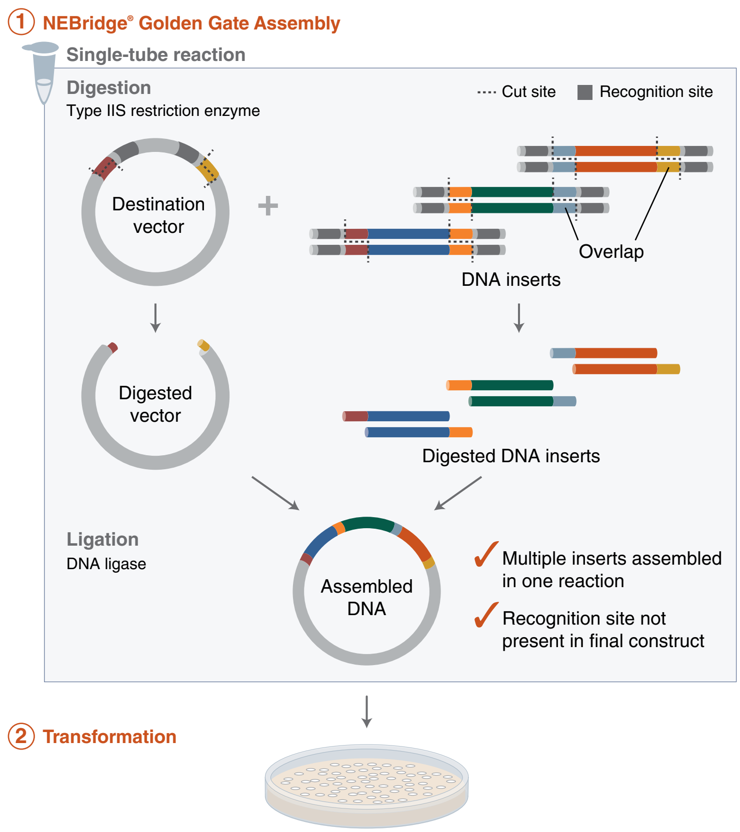
Commonly used restriction enzymes for GGA (BsaI-HF®-v2, BsmBI-v2, BbsI-HF®, PaqCI® and their isoschizomers) generate four base overhangs. In theory there are 120 possible four-base Watson-Crick pairs (excluding palindromes) that could be used to order fragments in a Golden Gate Assembly; in practice, most existing standards join only five to eight fragments per assembly reaction. Recent work at NEB has comprehensively explored the ligation of all possible three and four base overhangs, identifying all pairs prone to mismatch ligation (9,10).

In GGA, any mismatched overhangs that ligate will result in erroneous assembly, leading to reduce yield and products containing large insertions or deletions. Using the above studies, Data-optimized Assembly Design rules that allow for high complexity Golden Gate Assemblies of dozens of fragments to be joined in a single reaction step were developed. These design approaches permit the direct assembly of up to 50 kB from typical PCR fragments (1–5 kB) in a single step, and have been combined with PCR amplification of synthetic oligo pools to permit assembly of many genes in a cost-effective manner utilizing equipment available to most academic labs (11).

Online tools have been designed to enable the application of these design rules to any sequence (12,13).

While USER, NEBuilder, and Golden Gate Assembly represent some of the most popular and versatile modern cloning workflows, they are part of a growing toolbox of innovative techniques. For a deeper dive into additional methods like recombinational and ligation-in-dependent cloning, or to learn how synthetic DNA and automation are shaping the future of DNA assembly, visit the full article at **www.neb.com/tools-and-resources/feature-articles/foundations-of-molecular-cloning-past-present-and-future**.

Figure 3: **Golden Gate Assembly (GGA)**



GGA uses Type IIS restriction enzymes to generate compatible sticky ends, which are then joined by a DNA ligase. Type IIS enzymes have the unique property of cutting distal to their recognition site which allows for overhangs of any sequence to be generated. Therefore, the ligation of two overhangs generated by a Type IIS enzyme can result in loss of the recognition sequence. This property can be exploited to enable cutting and ligation to occur in the same reaction mixture with the desired ligation products protected from digestion. If the excised regions are ligated back to their original sequence, the recognition and cut sites are regenerated and can be cut again. Inserts for GGA are designed with flanking Type IIS sites that create overhangs of the desired sequence upon digestion (1). GGA compatible vectors typically have a sacrificial insert which contains the recognition sites and is cut away during the assembly reaction to expose the overhangs for insert ligation. The GGA protocol alternates between temperatures favoring digestion and ligation and over multiple cycles the desired assembly product accumulates in high yield. Transformation (2) permits the assembled construct to be propagated and isolated. Multiple overhang sequences can be chosen such that many fragments can be joined in a specified order by the DNA ligase.

PRODUCTS MENTIONED IN THIS ARTICLE

PRODUCT NAME	CATALOG	FUNCTION	APPLICATIONS
USER® Enzyme	NEB #M5505	• Generates a single nucleotide gap at the location of a uracil residue	• Directional RNA Seq • NEBNext adaptor cleavage • USER cloning
Thermolabile USER® II Enzyme	NEB #M5508	• Generates a single nucleotide gap at the location of a uracil residue • It can be 100% inactivated at temperatures >65°C	
Q5U® DNA Polymerase	NEB #M0515	• Uracil-tolerant version of Q5 Hot Start High-Fidelity DNA • Polymerase, for use with USER Cloning	• Amplifies uracil- or inosine containing DNA • USER cloning
NEBuilder® HiFi DNA Assembly Master Mix	NEB #E2621	• Seamlessly combines overlapping DNA fragments into a single construct via exonuclease, polymerase, and ligase activities	• Construction of plasmids for gene expression • Site-directed mutagenesis • Assembly of synthetic genes and operons • CRISPR/Cas9 vector construction • Fusion protein design
NEBridge® Ligase Master Mix	NEB #M1100	• Enables scarless DNA assembly using Golden Gate Assembly cloning	• Use with Type IIS restriction enzyme for seamless cloning • Cloning of single inserts and library construction
BsaI-HF-v2	NEB #R3733	• Cuts outside recognition site to create custom DNA overhangs • Recognizes the sequence 5´-GGTCTC	• Golden Gate Assembly
NEBridge Golden Gate Assembly Kit (BsaI-HFv2)	NEB #E1601	• For use in Golden Gate Assembly reactions using BsaI-HFv2	• Golden Gate Assembly • Cloning of single inserts and library preparations
BsmBI-v2	NEB #R0739	• Cuts outside recognition site to create custom DNA overhangs • Recognizes the sequence 5´-CGTCTC • Isoschizomer of Esp3I	• Golden Gate Assembly
NEBridge Golden Gate Assembly Kit (BsmBI-v2)	NEB #E1602	• For use in Golden Gate Assembly reactions using BsmBI-v2	• Golden Gate Assembly • Cloning of single inserts and library preparations
BspQI-HF®	NEB #R3712	• Cuts outside recognition site to create custom DNA overhangs • Recognizes the sequence 5´-GCTCTTC • Isoschizomer of SapI	• Golden Gate Assembly
PaqCI	NEB #R0745	• Recognizes the sequence 5´-CACCTGC • Isoschizomer of AarI	• Golden Gate Assembly

Visit **www.neb.com/tools-and-resources/interactive-tools** to find NEB's suite of online tools to support DNA Assembly

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A Streamlined Cell-Free Workflow for On-Demand Protein Expression Using NEBuilder HiFi DNA Assembly

Sean Lund, Ph.D., Jackson Buss, Ph.D., and Matthew Norton, Ph.D., New England Biolabs, Inc.

INTRODUCTION

NEBuilder HiFi DNA Assembly is a versatile tool used for seamless cloning and high-fidelity assembly of multiple DNA fragments into a desired construct. It supports applications such as construction of plasmids for recombinant protein expression, sgRNAs, cassettes as linear donors for yeast integration, and site-directed mutagenesis. Due to its high efficiency and accuracy, NEBuilder HiFi can be scaled to assemble many constructs in parallel over a wide range of volumes (1). Often, these assemblies are used to prototype variants for protein engineering or screening applications.

Traditionally, these assembled expression vectors are introduced into hosts through transformation, verified by sequencing, and subsequently expressed *in vivo*. While reliable, this method can be cumbersome and time-consuming, and many sequences pose difficulties for *in vivo* expression due to toxicity.

NEBExpress® Cell-free *E. coli* Protein Synthesis System expresses proteins from DNA sequences completely *in vitro* by providing both transcription and translational components in a single reaction. This system is compatible with various forms of DNA, including plasmid and linear templates. It can be miniaturized to submicroliter volumes for screening (2), or scaled up for automated purification using magnetic particle processors (3).

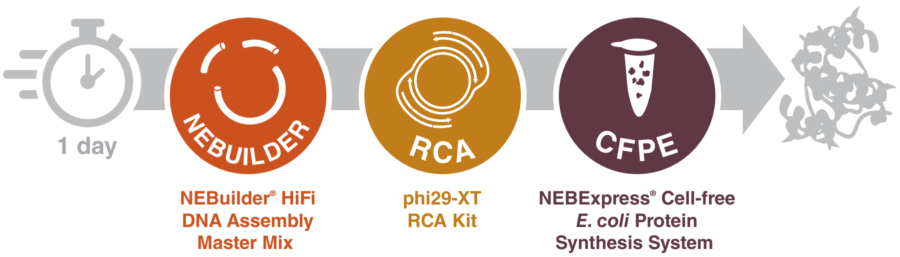
While convenient, most cell-free protein synthesis (CFPS) systems require significant amounts of DNA, which are not typically available directly from DNA assembly reactions. To overcome this challenge, rolling circle amplification (RCA) can be employed using universal primers to yield high concentrations of DNA (> 1 µg/µl) in just a few hours, providing ample template for CFPS reactions. RCA preferentially amplifies circular DNA, thus enriching only successful ligation products circumventing the need for additional cleanup steps. In this study, we show that NEBuilder HiFi assemblies can be used as templates for phi29-XT RCA, just as they can be used as templates for subsequent PCR amplification.

In this application note, which is available using the QR code below, we demonstrate that RCA-amplified NEBuilder HiFi assemblies from unpurified PCR products can be used with the NEBExpress Cell-free *E. coli* Protein Synthesis System to provide a rapid and streamlined workflow for assembling and expressing different target proteins.

MATERIALS

- Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494)
- NEBuilder® HiFi DNA Assembly Master Mix (NEB #E2621)
- phi29-XT RCA Kit (NEB #E1603)
- NEBExpress® Cell-free *E. coli* Protein Synthesis System (NEB #E5360)
- NEBExpress® Ni-NTA Magnetic Beads (NEB #S1423)
- 2X IMAC Buffer (NEB #B1076SVIAL, part of NEB #S1423)
- 2M Imidazole (NEB #B1077SVIAL, part of NEB #S1423)
- Blue Protein Loading Dye (NEB #B7703)
- Unstained Protein Standard, Broad Range (10-200 kDa) (NEB #P7717)
- Quick-Load® 1 kb DNA Ladder (NEB #N0468)
- NEBNext® Magnetic Separation Rack (NEB #S1515)
- Thermocycler
- Eppendorf® Thermomixer C

Streamlined workflow for rapid protein expression using NEBuilder HiFi DNA Assembly and NEBExpress Cell-free *E. coli* Protein Synthesis System



Scan here to read the full application note including methodology and results

References

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2. Buss, J.A. 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.

3. Buss, J.A. et al. (2024) Application Note: Automated Cell-free Protein Expression and Purification for High-Throughput Screening using NEBExpress® Cell-free E. coli Protein Synthesis System and NEBExpress Ni-NTA Magnetic Beads. New England Biolabs, Inc.

mRNA Synthesis Democratized with Open-source Approach by BASE Facility Researchers

New England Biolabs Sales Manager of Australia & New Zealand, Jenny Brown, interviewed Professor Tim Mercer and Associate Professor Seth Cheetham from the BASE mRNA Facility at the University of Queensland, Australia to find out more about their recently published novel protocol for mRNA synthesis in Nature Protocols, utilizing standard laboratory techniques and equipment to produce high-purity mRNA suitable for *in vitro* and *in vivo* preclinical studies.

Jenny Brown

Timothy Mercer

Seth Cheetham

Jenny Brown: Please tell us about the BASE facility.

Seth Cheetham: The BASE facility is a national facility that provides researchers around Australia with high-quality mRNA. We provide an end-to-end service, from mRNA design through to final formulation. This enables people to start using mRNA in their research to develop new vaccines and therapies.

In addition to supporting scientists with mRNA, we also have our own internal research program, where we explore new mRNA manufacturing methods, quality control of mRNA, new approaches to deliver mRNA to tissues, and develop our own pipeline of mRNA candidates.

Timothy Mercer: Everyone thinks about mRNA in terms of medicines, vaccines and therapies, but scientists are increasingly realizing that mRNA makes a fantastic experimental reagent. We see the scientific community increasingly adopting mRNA for use in their experiments, especially in fields such as immunology and neuroscience, that have been otherwise difficult to explore.

Jenny Brown: What specific issue in mRNA manufacturing were you aiming to solve with this protocol?

Seth Cheetham: So far, mRNA manufacturing has been regarded as being a very specialized approach, which has only been accessible through specialized facilities such as the BASE facility. What we wanted to do was to develop an open-source approach to democratize mRNA access. This allows researchers around the world to produce high-quality mRNA in their own lab, and develop new vaccines and therapies.

Timothy Mercer: This protocol has really been developed with the researcher in mind. It's designed to be able to produce mRNA in a standard laboratory, using standard equipment, in an affordable manner. First, we design the mRNA using the mRNArchitect software (app.basefacility.org.au/). After that, we use a PCR approach to prepare that DNA template, which is then used for *in vitro* transcription to synthesize the mRNA. Finally the mRNA can be formulated within the lipid nanoparticle at the end for *in vivo* delivery, or within your *in vitro* experiments, depending on what that final application is.

Jenny Brown: What are some of the technical hurdles labs commonly face when making mRNA?

Seth Cheetham: One of the issues in mRNA manufacturing was that it relied on plasma DNA, which is extracted from bacterial cells.

Timothy Mercer: A big challenge for plasmids is the bioburden, the contamination that they can sometimes introduce within the process. When you're making mRNA, you want to remove any of those contaminants, and so excluding the plasmid removes one of those primary sources of contaminants.

Seth Cheetham: We wanted to move away from this approach by using synthetic templates. So, we used PCR amplification to produce our templates either from a synthetic piece of DNA or from plasmid, which removes the need to grow large amounts of bacteria. This makes the process both cleaner and faster, and results in a higher quality mRNA.

Jenny Brown: Who specifically do you think it will benefit the most? What type of researcher?

Timothy Mercer: I think all researchers have a lot to benefit from mRNA. I envision mRNA assuming a position alongside plasmids and proteins in terms of experimental tool. We support a lot of scientists who work with non-dividing or fickle or difficult cells, and they are getting really good results using mRNA. It's a really powerful tool to express genes in those difficult cell lines experimental models.

Jenny Brown: What global impacts do you see this protocol having?

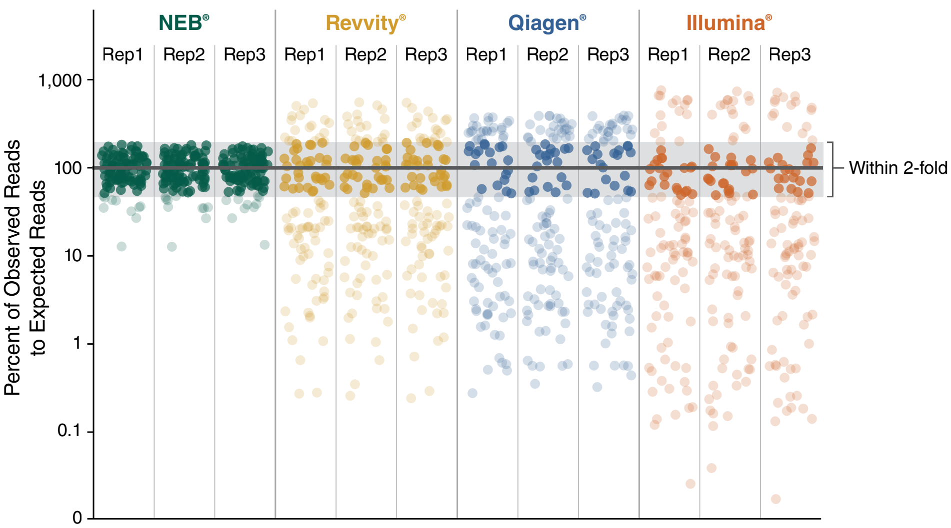
Timothy Mercer: We hope that this protocol allows people to start adopting and producing mRNA within their lab. In some ways, it demystifies the process of making mRNA. This protocol is pretty easy-to-follow and provides guidelines to make sure you produce good, high-quality and non-contaminated mRNA. We just hope it gets adopted by different scientists all over the world, so they can start generating their own mRNA and start using it in their experiments.

Kind of a big deal.

Successfully see more small RNAs with the NEBNext Low-bias Small RNA Library Prep Kit

The NEBNext Low-bias Small RNA Library Prep Kit reduces bias introduced during adaptor ligation, improving the accurate representation of RNA species present. What used to be a multi-day workflow can be accomplished in ~3.5 hours, without the need for gel purification. Starting with as little as 0.5 ng of total RNA, see more of what your small RNA is telling you.

NEBNext Low-bias Small RNA Library Prep Kit produces libraries with the lowest bias.



NEBNext Low-bias Small RNA libraries were made using a mix of 100 synthetic control miRNAs, including five that had 3´ 2´-O-methyl ends. Libraries were prepared from 0.3 ng of the synthetic miRNA mix to compare library-generated bias between the NEBNext Low-bias Small RNA Library Prep Kit and small RNA kits from Revvity® (NEXTFLEX® Small RNA Sequencing Kit V4), Qiagen® (QIAseq® miRNA Library Kit) and Illumina (TruSeq® Small RNA Library Prep). Libraries were sequenced on an Illumina NextSeq 500 (1 x 56 bases), and expected reads were calculated from total reads mapped to the synthetic controls, divided by the total number of control sequences (black line at 100%). Percent of observed reads to expected reads was calculated for each control sequence and plotted across replicates.

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- Derive insights from biologically relevant input amounts
- Plan ahead with 18-month shelf life
- Learn a single protocol for standard and 2´-O-methylated samples
- Expand your insights with up to 480 compatible UDI primer pairs, available separately

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext Low-bias Small RNA Library Prep Kit	E3420S/L	24/96 rxns

NEBNext Low-bias Small RNA Library Prep workflow

RNA input:
- **Total RNA: 1,000–0.5 ng**
- **Enriched Small RNA: 5–0.05 ng**

3´ Ligation	Excess Adaptor Removal	5´ Ligation	Reverse Transcription	Bead Size Selection	PCR Amplification	Bead Purification	Total Workflow
30 minutes	35 minutes	30 minutes	25 minutes	30 minutes	30–50 minutes	20 minutes	~3.5 hours

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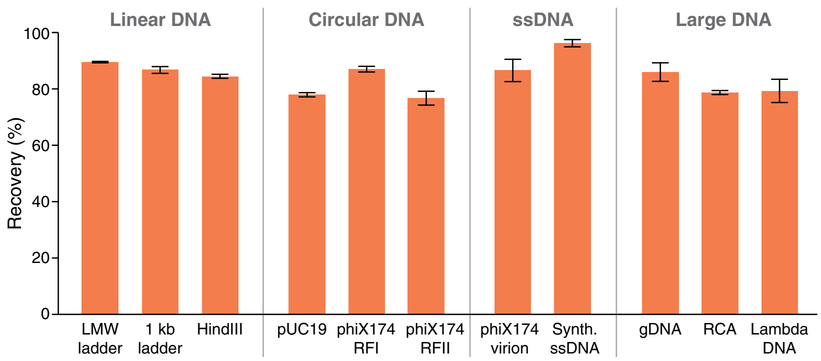
Ordering Information

PRODUCT	NEB #	SIZE
Monarch Spin High-Capacity DNA Cleanup Kit (100 µg)	T1135V/S/L	10/50/200 rxns

Monarch Spin High-Capacity DNA Cleanup Kit (100 µg) effectively recovers various DNA sample types, maximizing utility of the kit



DNA samples were purified in triplicate using the Monarch Spin High-Capacity DNA Cleanup Kit (100 µg), following either the standard protocol (for linear, circular, and ssDNA) or the supplemental protocol (for large DNA). Each sample, except for the RCA product (50 µl, 1 mg/ml), contained 10 µg of DNA and was eluted in 100 µl of Monarch Buffer EY. DNA concentrations for both input and eluted samples were measured using a Trinean DropSense 16, except for the RCA product, which was measured using the Qubit dsDNA Broad Range (BR) Assay Kit. Percent recovery was calculated based on the measured DNA concentrations and elution volumes, with results displayed in a bar graph. For linear DNA, the following ladders were utilized: LMW DNA ladder (25-766 bp, NEB #N3233), 1 kb DNA Ladder (0.5–10 kb, NEB #N3232), and HindIII digest (2–23 kb, NEB #N3012). For circular DNA, pUC19 (NEB #N3041), PhiX174 RF I (NEB #N3021) and PhiX174 RF II (NEB #N3022) were employed. For ssDNA, phiX174 Virion (NEB #N3023) and synthetic ssDNA (150 nt) were used.

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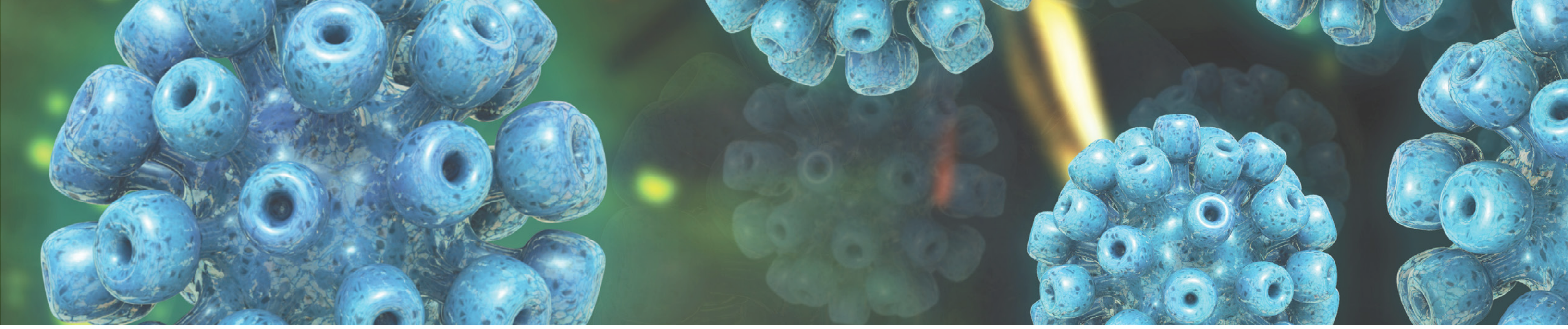

PROTECTING COMMUNITIES in the FIGHT AGAINST HEPATITIS B

Dr. Adewunmi Akingbola (King's College, Cambridge, UK) was the recipient of one of two New England Biolabs' 2024 Passion in Science Humanitarian Awards for his visionary and compassionate Community Hepatitis B Shield Project initiative. You can read about his efforts in the article below or scan the QR code to hear more in our recent Lessons from Lab & Life™ podcast episode.

Adewunmi Akingbola, MD, MPhil
Founder of HealthDrive Nigeria

The "Community Hepatitis B Shield Project," led by HealthDrive Nigeria, is an extraordinary initiative dedicated to combating viral hepatitis in underserved communities across Nigeria. Founded by Dr. Adewunmi Akingbola, the project addresses the pressing need for education, testing and vaccination against hepatitis B and C, particularly in low-income areas where access to healthcare is limited.

Dr. Akingbola's journey began with personal experiences of loss and intensified while attending medical school, where the prevalence of hepatitis among patients in gastroenterology clinics was alarmingly high. Out of every 10 patients, six tested positive for hepatitis B, and at least one for hepatitis C. This realization, coupled with the general lack of public knowledge about the disease, sparked a profound motivation to drive awareness and change.

Recognizing that education alone was not enough to prevent infections, Dr. Akingbola initiated a comprehensive campaign. This effort began with public speaking engagements and social media campaigns, which later evolved into offering rapid diagnostic screenings for hepatitis B and C. The screenings were particularly crucial, as hepatitis B, while manageable, is incurable, and hepatitis C, though treatable, remains a significant health threat.

HealthDrive Nigeria's innovative "B-Safe Model" further expanded to establish screening points in various communities, ensuring that community members were regularly tested and educated about hepatitis. Dr. Akingbola quickly realized that the risk of new infections persisted. This led to the formation of partnerships with local health clinics, hospitals, and pharmaceutical companies to make hepatitis vaccines more affordable and accessible. Through these collaborations, HealthDrive Nigeria was able to launch vaccination campaigns, offering subsidized hepatitis vaccines that significantly increased community uptake.

The project has since grown to include over 100 volunteers, including medical students from various states, all committed to eradicating hepatitis through community engagement and awareness. Professional nurses administer the vaccines, while trained volunteers conduct the tests, creating a comprehensive network of care and support.

The project's impact has been profound, with over 15,000 people screened and more than 10,000 vaccinated in southwestern Nigeria alone. Beyond the immediate health benefits, the project has also increased public awareness about hepatitis in the region, encouraging preventive measures in communities that were previously unaware of the disease's dangers.

Dr. Akingbola acknowledges the ongoing challenges, particularly in securing sustainable funding. However, the project continues to innovate, integrating other community services such as food and clothing distribution to enhance engagement and ensure the longevity of their health messages.

The initiative also focuses on addressing unsafe local practices, which significantly increase the risk of hepatitis transmission, such as unsterilized equipment in barbershops.

Operating from the UK, Dr. Akingbola provides guidance and support to the local team in Nigeria, ensuring the project's continued growth and impact. As the campaign expands its social media presence and seeks further partnerships with news agencies, the goal remains clear: to raise awareness about hepatitis B and C, reduce infections and ultimately save lives in the communities that need it most.

Scan here to listen to the full podcast now

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