

EXPERIENCES

A scientific update

Issue I • 2023

IN THIS ISSUE

SCIENCE

- 2** **Feature article**
Enhancing molecular diagnostics in regulated markets with lyophilized assays
- 5** **Automate with confidence**
Learn about NEB's solutions for automated DNA assembly, mutagenesis, sequencing, protein synthesis and more.
- 10** **New product alert – phi29-XT RCA Kit**
Learn about our new engineered phi29-XT DNA Polymerase

INNOVATION

- 8** **Featured web tools**
Read about how NEBcutter® and NEBaseChanger® are simplifying DNA analysis
- 9** **From primer design to analysis**
Discover how bioinformatics drives sequencing success at NEB

INSPIRATION

- 11** **Young scientists launch their ideas into space**
Learn how one student's assay for bio-monitoring DNA changes in astronauts is set to launch

Enhancing Molecular Diagnostics in Regulated Markets with Lyophilized Assays

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

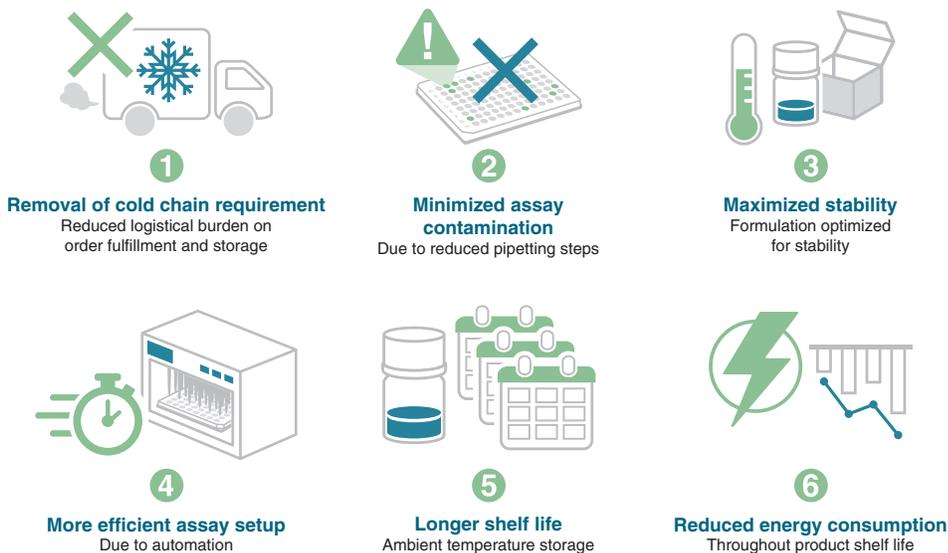
Molecular diagnostic assays are developed to identify and quantitate specific DNA, RNA, or protein biomarkers found within samples of interest. In many regions, molecular diagnostic testing may only be performed by procuring assays subject to regulatory approval, or with validated tests and workflows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

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

Obtaining regulatory approval for human *in vitro* diagnostic tests requires significantly greater time and consideration than developing Research Use Only (RUO) assays typically used in general research laboratories. Nonetheless, it is important to highlight that the developmental steps, performance and safety information, and the data needed for review by regulatory authorities remain consistent regardless of whether wet or lyophilized reagents are utilized.

Transitioning an assay that utilizes wet reagents to an assay in a dry form is not as simple as just lyophilizing the wet reagents. Commonly-used liquid reagent formulations contain components such as glycerol or co-solvents that either do not allow for lyophilization or impact the stability and performance of a lyophilized version of the formulation. Many of these liquid-form components help with enzyme stabilization and assay sensitivity, which makes removing components problematic. However, many alternatives to these materials can restore assay performance, and these components can be identified through substitution testing. An additional consideration for lyophilization is the need for excipients and cryoprotectants that protect the macromolecules during freezing and provide for the preservation of the dried material. While it is critical to ensure optimal assay performance when excipients are included in the formulation, it should be noted that they may also act as alternative assay enhancers (Figure 1).

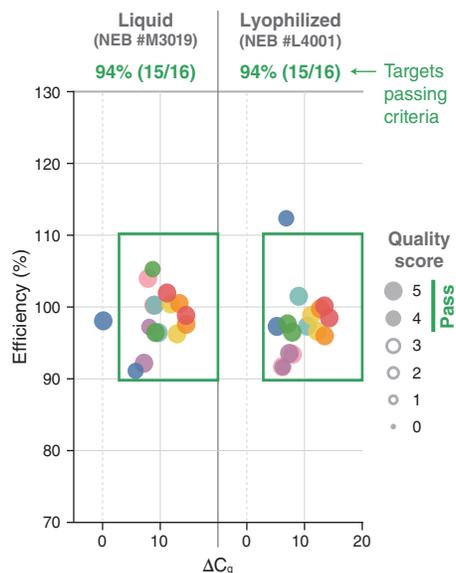
BENEFITS OF LYOPHILIZED PRODUCTS



Just like when building a diagnostic assay with liquid reagents, the developer of a lyophilized assay must consider who their end user will be; how the assay will be incorporated into the laboratory's workflow; which instruments are critical for compatibility; and what quality and regulatory requirements will govern its use. When working with multiple vendors and components to build the final assay, it is important to understand any reconfiguration the developers may undertake when the lyophilized assay is supplied as a

sub-component of a workflow or device. Understanding the requirements for handling, transfer and repacking will help the development team make the appropriate product. Vendors specializing in lyophilization can guide development teams on design-for-manufacturing (DFM) options. Ensuring efficient communication of this information at the start of the development process will help the team stay on track as the assay developer completes the product realization process.

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



LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) was compared to Luna® Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) and tested on eight human RNA targets varying in abundance, length, and %GC. Data were collected by two users, and results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔCq = average Cq of non-template control – average Cq of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). The LyoPrime Luna Probe RT-qPCR Mix with UDG (NEB #L4001) yielded results comparable to those of the liquid format Luna Probe RT-qPCR Mix with UDG (NEB #M3019).

Learn more about our comprehensive qPCR/RT-qPCR testing and "dots in boxes" data visualization at www.neb.com/tools-and-resources/video-library/dots-in-boxes-visualization-of-qpcr-data

Packaging

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

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

Optimizing lyophilization conditions and process

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

Process Validation

The ISO 13485 standard requires that the processes used to make a product are robust. In some instances, where measurements may not be easily applied, process validation shall be required. For example,

simple temperature and pressure measurements in a freeze dryer will not provide sufficient information that the drying process is robust.

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

Figure 2: Overview of the lyophilization process



Optimized assay
Including assay components and excipients



Drying

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



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

DESIGN • DEVELOP • MANUFACTURE



Product Validation and Verification

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

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

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

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

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

Amplification-based Molecular Diagnostics

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

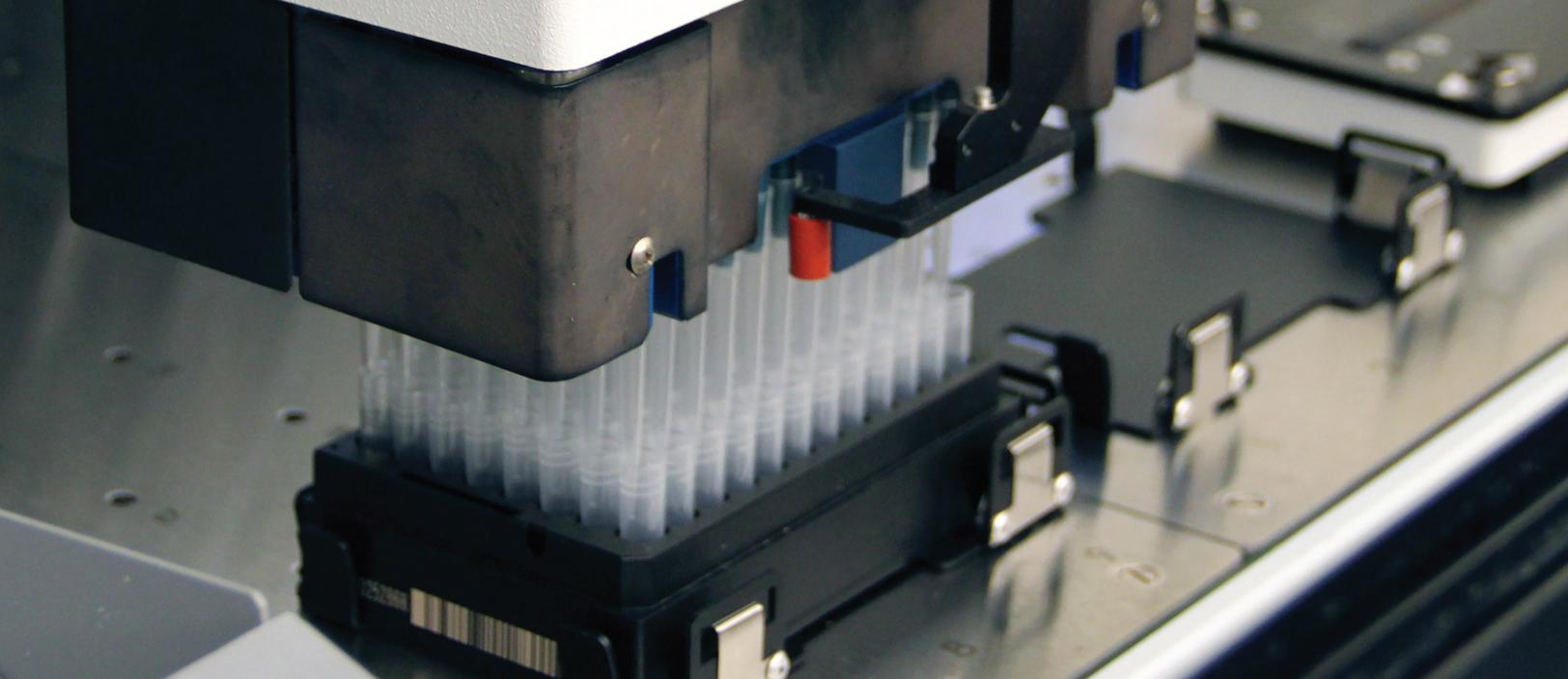
	APPLICATION	PRODUCTS	PRODUCT NOTES	CUSTOM FORMULATIONS
PCR APPLICATIONS	qPCR/ RT-qPCR	DNA, Dye <ul style="list-style-type: none"> Luna Universal qPCR Master Mix (NEB #M3003) DNA, Probe <ul style="list-style-type: none"> Luna Universal Probe qPCR Master Mix (NEB #M3004) 	<ul style="list-style-type: none"> Compatible with automated liquid handling and reaction miniaturization Room temperature stable for ≥ 24 hours 	<ul style="list-style-type: none"> Blue-dye-free Lyo-compatible
		RNA (1-step), Dye <ul style="list-style-type: none"> Luna Universal One-Step RT-qPCR Kit (NEB #E3005) RNA (1-step), Probe <ul style="list-style-type: none"> Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX) (NEB #M3029) Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019) LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) 	<ul style="list-style-type: none"> Luna WarmStart RT paired with Hot Start <i>Taq</i> increases reaction specificity and robustness Compatible with automation and reaction miniaturization Room temperature stable for ≥ 24 hours Lyophilized format (NEB #L4001) 	<ul style="list-style-type: none"> Blue-dye-free Lyo-compatible Add primers and probes (NEB #L4001)
		RNA (2-step) <ul style="list-style-type: none"> LunaScript® RT SuperMix (NEB #E3010/#M3010) 	<ul style="list-style-type: none"> Novel thermostable RT Single-tube format 13-minute cDNA synthesis protocol 	<ul style="list-style-type: none"> Blue-dye-free
PCR/ RT-PCR		Master Mixes <ul style="list-style-type: none"> Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494) Q5 High-Fidelity 2X Master Mix (NEB #M0492) Standalone Enzyme & Buffer <ul style="list-style-type: none"> Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) Q5 High-Fidelity DNA Polymerase (NEB #M0491) 	<ul style="list-style-type: none"> ~280X fidelity of <i>Taq</i> Consistent, fast, reliable performance Compatible with automation and reaction miniaturization Room temperature stable for ≥ 24 hours 	<ul style="list-style-type: none"> High conc. Glycerol-free Custom mixes
		<ul style="list-style-type: none"> Q5 Blood Direct 2X Master Mix (NEB #M0500) 	<ul style="list-style-type: none"> Amplification direct from blood 	
		<ul style="list-style-type: none"> Hot Start <i>Taq</i> DNA Polymerase (NEB #M0495) Hot Start <i>Taq</i> 2X Master Mix (NEB #M0496) 	<ul style="list-style-type: none"> Unique aptamer-based enzyme control supports fast protocols 	<ul style="list-style-type: none"> High conc. Glycerol-free
ISOTHERMAL APPLICATIONS	LAMP	<ul style="list-style-type: none"> WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB #M1800) WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804) 	<ul style="list-style-type: none"> Fast, clear pink-to-yellow visible detection of amplification Results in approximately 30 minutes Automation-compatible when coupled with absorbance plate reader 	
		<ul style="list-style-type: none"> SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB #E2019) 	<ul style="list-style-type: none"> Simple, colorimetric detection of amplification of SARS-CoV-2 nucleic acid 	
		<ul style="list-style-type: none"> WarmStart LAMP Kit (DNA & RNA) (NEB #E1700) WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708) WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) (NEB #E1708) 	<ul style="list-style-type: none"> Master mix for LAMP and RT-LAMP workflows Supports multiple detection methods, including fluorescence and turbidity 	<ul style="list-style-type: none"> Lyo-compatible High conc.
		<ul style="list-style-type: none"> <i>Tte</i> UvrD Helicase (NEB #M1202) 	<ul style="list-style-type: none"> Improves specificity of fluorescent LAMP reactions 	<ul style="list-style-type: none"> High conc.
		<ul style="list-style-type: none"> <i>Bst</i> 2.0 WarmStart DNA Polymerase (NEB #M0538) <i>Bst</i> 2.0 DNA Polymerase (NEB #M0537) 	<ul style="list-style-type: none"> Improved reaction properties compared to wild-type <i>Bst</i> DNA Polymerase Increased dUTP tolerance enables carryover prevention 	<ul style="list-style-type: none"> Glycerol-free High conc.
		<ul style="list-style-type: none"> <i>Bst</i> 3.0 DNA Polymerase (NEB #M0374) 	<ul style="list-style-type: none"> DNA binding domain fusion supports robust performance Significantly increased RT activity up to 72°C enables single enzyme RT-LAMP 	<ul style="list-style-type: none"> Glycerol-free High conc.
		<ul style="list-style-type: none"> WarmStart RTx Reverse Transcriptase (NEB #M0380) 	<ul style="list-style-type: none"> <i>In-silico</i> designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C 	<ul style="list-style-type: none"> Glycerol-free High conc.
		Strand Displacement <ul style="list-style-type: none"> Nt.BstNBI (NEB #R0607) WarmStart Nt.BstNBI (NEB #R0725) 	<ul style="list-style-type: none"> High purity, high quality nicking endonuclease 	<ul style="list-style-type: none"> Glycerol-free High conc.
		Helicase-dependent Amplification <ul style="list-style-type: none"> IsoAmp® II Universal tHDA Kit (NEB #H0110) 	<ul style="list-style-type: none"> Requires only two primers Produces short, discrete DNA products 	
		Other <ul style="list-style-type: none"> <i>Bsu</i> DNA Polymerase, Large Fragment (NEB #M0330) T4 Gene 32 Protein (NEB #M0300) Deoxynucleotide (dNTP) Solution Mix (NEB #N0447) Nuclease-free Water (NEB #B1500) Antarctic Thermolabile UDG (NEB #M0372) Proteinase K, Molecular Biology Grade (NEB #P8107) Thermolabile Proteinase K (NEB #P8111) 	<ul style="list-style-type: none"> Enables low temperature isothermal applications Can increase yield and efficiency of amplification reactions Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows 	<ul style="list-style-type: none"> High conc. Glycerol-free Custom conc. High conc. Custom conc. Custom conc.

Clone with Confidence.®

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



Explore the wise choice at
CloneWithNEB.com



Automate with Confidence – High-throughput Solutions for Cloning and Automation

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

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

NEB offers a wide variety of products that enable highly efficient and accurate DNA assembly and mutagenesis, sequencing, cell-free protein synthesis and purification that are all amenable to high-throughput workflows and automation devices. To learn more, visit www.neb.com/AutomateWithConfidence.

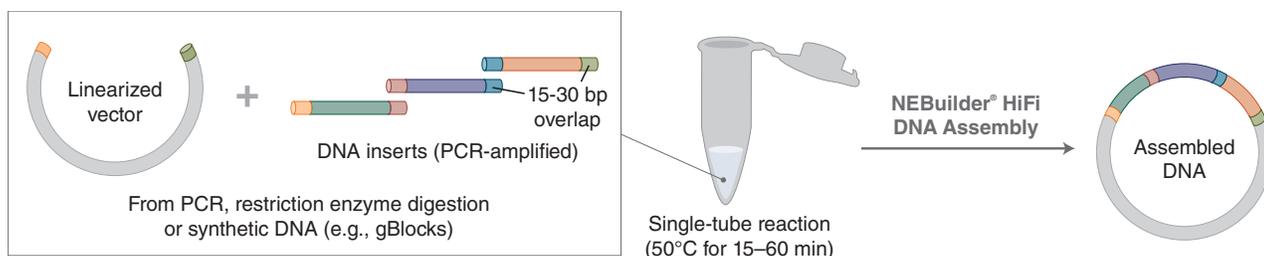
FEATURED PRODUCTS FOR HIGH-THROUGHPUT DNA ASSEMBLY

NEBuilder HiFi DNA Assembly

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

Ordering Information

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

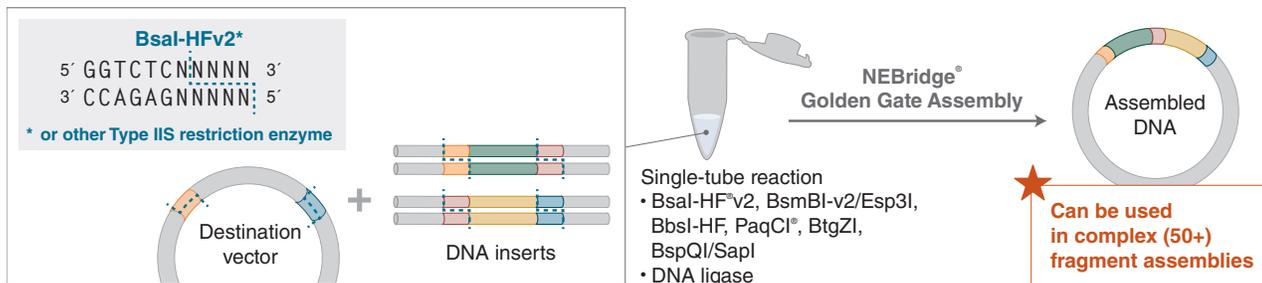


NEBridge Golden Gate Assembly

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

Ordering Information

PRODUCT	NEB #	SIZE
NEBridge Golden Gate Assembly Kit (BsmBI-v2)	E1602S/L	20/100 reactions
NEBridge Golden Gate Assembly Kit (Bsal-HF [®] v2)	E1601S/L	20/100 reactions
NEBridge Ligase Master Mix	M1100S	50 reactions



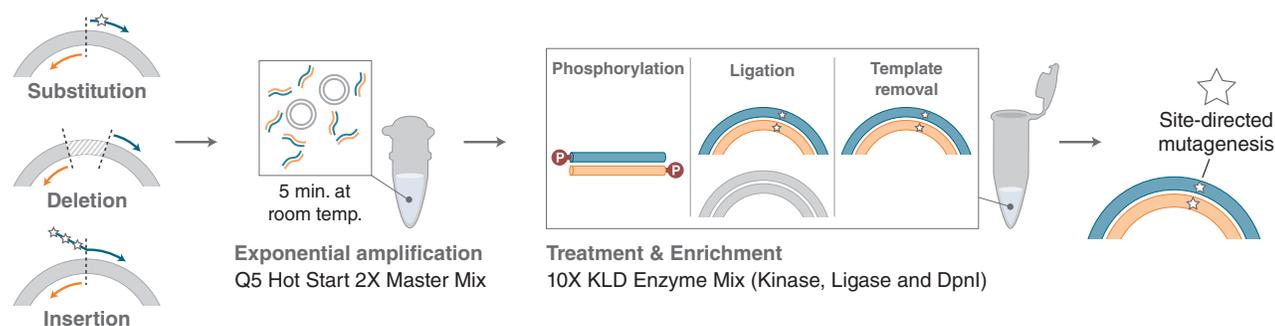
FEATURED PRODUCTS FOR HIGH-THROUGHPUT MUTAGENESIS

Single site-directed mutagenesis

- Quickly create mutant libraries using primers designed with our free online tool, NEBaseChanger[®]
- Scale-up, or miniaturize with individual master mix formats compatible with automation
- Use Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494) for PCR amplification
- Use KLD Enzyme Mix (NEB #M0554) for kinase, ligase and DpnI enzymatic activities in single mix
- Reduce screening of correct mutants with extremely accurate and robust Q5 High-Fidelity DNA Polymerase
- Take advantage of automation compatible room temperature reaction set up using a hot start polymerase

Ordering Information

PRODUCT	NEB #	SIZE
Q5 [®] Hot Start High-Fidelity 2X Master Mix	M0494S/L/X	100/500/500 reactions
KLD Enzyme Mix	M0554S	25 reactions

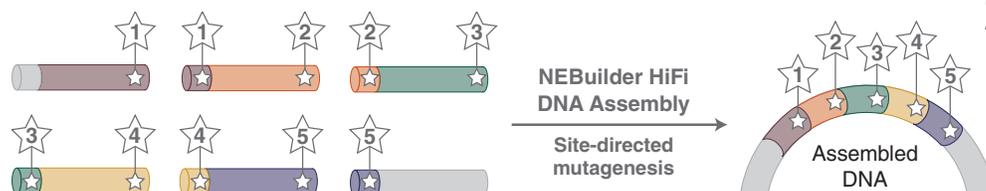


Multi site-directed mutagenesis

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

Ordering Information

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



Updated NEB Web Tools

Achieve effortless DNA restriction digests and site-directed mutagenesis reactions with our updated web tools, designed to ensure trouble-free experimentation.

NEBaseChanger®

NEBaseChanger helps researchers design site-directed mutagenesis experiments using NEB's Q5 Site-Directed Mutagenesis Kit.

- Design primers for use in site-directed mutagenesis reactions
- Calculate a custom annealing temperature taking into account mismatches
- Download a summary of primer information, sequence of final construct and recommendation protocol

We have recently enhanced the capabilities of NEBaseChanger. New features include the following:



Generate multiple primers using ambiguous bases



Opt for mutation sequences in primer tails



Substitutions based on nucleotide or amino acid position



Batch input to simultaneously generate multiple primer sets

Visit [NEBaseChanger.neb.com](https://nebasechanger.neb.com) to get started, and check out these online tutorials for help with some common NEBaseChanger applications:

-  **NEBaseChanger®: Designing primers for use with the Q5® Site-Directed Mutagenesis Kit**
-  **Overview of the Q5® Site-directed Mutagenesis Kit**
-  **Troubleshooting tips for Q5® Site-Directed Mutagenesis kit**

NEBcutter®

NEBcutter was originally designed to assist users with restriction digests and traditional molecular cloning workflows, and quickly became one of our most popular online tools.

- Find restriction enzyme sites within a DNA sequence
- Visualize a virtual restriction digest on a gel to compare against results at the bench
- Determine whether DNA methylation will impact your restriction digest

Additionally, NEBcutter V3.0 offers an improved user interface and includes the following features:



Download results as a PDF



Filter by types of REs (i.e., blunt, 8-base cutter, etc)



Add multiple enzymes at one time



Log in to save your projects and view from various browsers



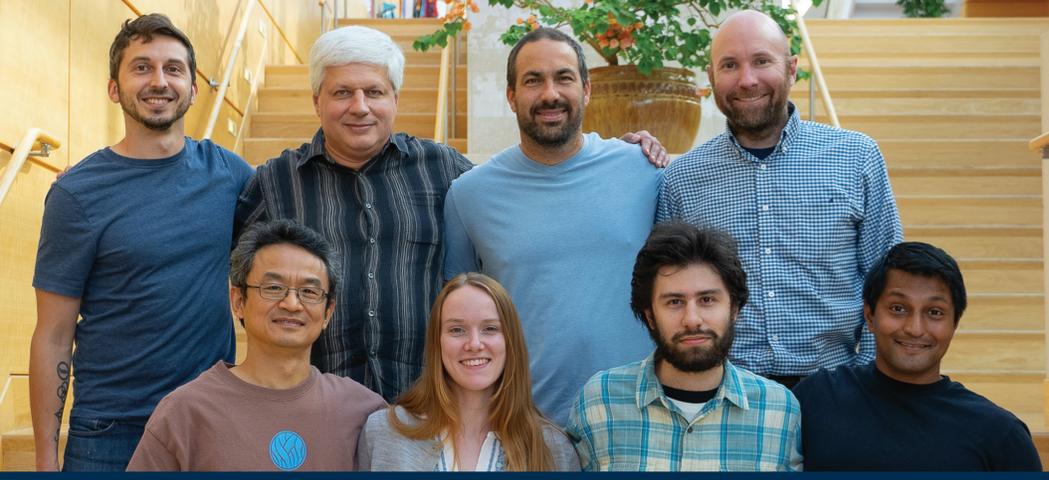
Visualize digestion on a virtual gel



Quickly find open reading frames with our enhanced ORF Finder

Visit [NEBcutter.neb.com](https://nebcutter.neb.com) to get started, and check out these online tutorials for help with some common NEBcutter applications.

-  **How to determine methylation effects on restriction digests**
-  **Determine the restriction enzymes that cut your DNA**
-  **Visualize a restriction digest on a virtual gel**
-  **Find restriction enzymes to remove a specific DNA sequence**



TOP ROW: Jake Miller, Timur Shtatland, Ariel Erijman, Brad Langhorst BOTTOM ROW: Jian Sun, Laura Blum, Caiden Kumar, Gautam Naishadham
NOT PICTURED: Matt Angel, Matt Campbell

How Bioinformatics Informs Sequencing at NEB

In a recent Lessons from Lab & Life™ podcast, Lydia Morrison chatted with NEB Development Group Leader Brad Langhorst about how bioinformatics informs sequencing, from primer design to analysis. An excerpt from Lydia's interview with Brad is included below. Access the Lessons from Lab & Life podcast at www.neb.com/podcasts to hear the entire interview and to learn more about how the bioinformatics team at NEB support R&D.

How does the bioinformatics team at NEB support research and development for the NEBNext portfolio?

People are moving pretty quickly in different areas of sequencing. So we have a bunch of computational folks working closely with the lab folks. They sit together with the lab people, so we don't have all the computational people in one place. I think it's important that everybody be in physical proximity and work closely together to design experiments, analyze results, and figure out what the next iteration of the experiments is as we're trying to figure out how to make better RNA-Seq products or how we are going to evaluate the next pathogenic virus that's coming along. How do we design and test it? It's really important to be close to it.

Your team developed the primer monitor tool during the COVID pandemic. Can you share a bit about that and how you envision it growing or changing?

There are qPCR assays, LAMP assays, and sequencing methods that depend on primers sitting in specific places on the SARS-CoV-2 genome. But the virus was moving fast and modifying itself quickly over time, and we were worried that all these primers wouldn't bind.

So, we needed to stay up on that, and I was surprised to find that there was not a resource in the world where I could say, okay, here are the primers we're using, tell us if something breaks. So I decided to build it. Matt, one of my teammates, built the backend pieces, and we had to fetch all the new viral sequences. Others were sequencing the virus in Southeast Asia, Japan, Africa, Europe,

and California and submitting all those viral sequences to central databases. So, every day, we pull down the latest viral sequences, put them in our database, and evaluate where they're different and how they are changing so we can understand where the primers are sitting.

We collected that information and then built some visualizations and a notification scheme.

Fairly recently, we saw a new variant in Singapore, and there was already one variant on the probe that most people use to detect SARS-CoV-2. We were worried that this might be a problem. So we were watching it in Singapore, and we started seeing it in other places and said, okay, we need to do some experimental testing here. Colleagues Guoping and Greg in the qPCR group used that information from the primer monitor to evaluate if this was going to be a big problem. Fortunately, no. We found it, saw it early, and had enough time to experiment to evaluate whether we needed to modify the assay. And it turns out there was still good detection even though there are two variants underneath that probe in that qPCR set.

Are there any plans to grow the primer monitor tool or plans for future changes to it?

Absolutely. We've had lots of requests from different groups at the CDC and other places in the world interested in viral sequences. So, Zika or Ebola or whatever it is, people want to know, could we monitor this? Mpox was a hot one for a while there too. And so, we didn't design the tool specifically for SARS-CoV-2. We just designed something to compare if sequences are changing and what differences we see.

I wanted to switch gears a bit and talk about indices, UMIs, and de-multiplexing, which seem to cause a lot of confusion for people new to next-generation sequencing. Can you share a bit about how you envision it growing or changing?

Sure. As the sequences get big and they can produce tons and tons of data, we want to be able to study more than one individual at a time, more than one virus, more than one bacteria, and more than one person. So, to do that, we need to combine samples to run them together.

To do that, we need to label every sample with a sequence we know. We can look up that sequence and say that sample belongs to Person A, or this one belongs to Person B. This is important for clinical situations. There are some complex schemes to match up – labeling things twice, and both have to match to, say, Person B. If there is a conflict, we throw it away, say, wait a minute, we can't tell what this is. So we put that in its own bucket. Hopefully, that bucket stays small. That's what we want to measure, and that's part of the performance we're trying to look at and design into these sequences.

The UMIs are another layer to that. We can look at some of the technical effects if we add amplification. If we have a small sample, let's say it's a blood sample, and we want to look at just circulating DNA in blood, there's a very tiny amount of it. We want to evaluate how much there is or what its properties might be. We want to amplify that signal first. So we have to make a bunch of copies, but the copies aren't perfect. Sometimes if one gets started quickly, you might get a lot of copies of that one, and if another one starts slowly just by chance, you get fewer. But we really want to know the original abundance of the two things we're trying to evaluate. A UMI is a nice little trick where we can stick a random piece of sequence onto each of those two things we want to study. If we see many copies of that same random sequence, there's a good chance that they're actually from the same original molecule. So we can collapse it down and correct our counts to get an accurate picture of how much is A and how much is B in a sample.

We've been building a lot of tools to help both our customers understand what we have and how they can be applied. Customers want to do these more complicated barcoding and UMI schemes, and they need more examples of how this could be done. And it's great to be able to share that kind of information. So, we figure it out and then build a starting point analysis method. Then we can hand that out to the world and let people make it better, give us feedback, and hopefully give people a good starting point to use these products.

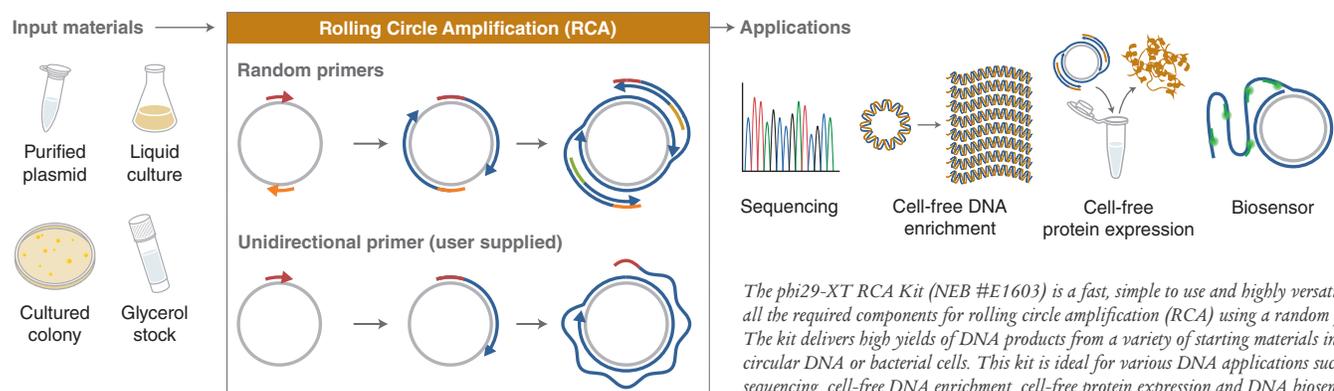
phi29-XT RCA Kit

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

Advantages

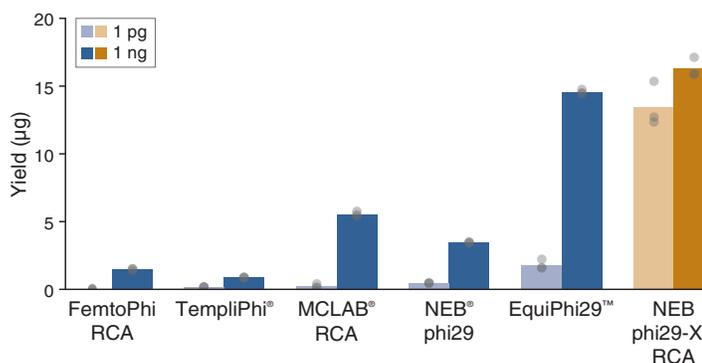
- Amplify from as little as 1 fg of circular DNA input
- Flexible protocol offers compatibility with different types of input material
- Enables multiple downstream applications without further processing steps
- Kit includes dNTPs and exo-resistant random primers

Overview of the phi29-XT RCA Kit



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

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



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

Ordering Information

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



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

Genes in Space™ is a unique opportunity for young scientists to launch their ideas into orbit

by Joanne Gibson, Ph.D., New England Biolabs

Pristine Onuoha is a high school student who has always been passionate about biology and is a curious person by nature. So, when she learned about the Genes in Space program (www.genesinspace.org) through her school's Women in STEM club, she was immediately intrigued. The idea of developing a research proposal related to space biology fascinated her. The fact that it was an application of biology she hadn't heard much about didn't deter her; she eagerly wanted to pursue and explore it.

Genes in Space is a free competition, founded and run by miniPCR bio™ (www.minipcr.com) and Boeing®, for 7th- to 12th-grade students, challenging them to come up with experiments evaluating how living things react to space conditions. Every year, about 200 schools participate in the contest, but students can enter independently with sponsorship from an adult (a teacher or parent).

Pristine began exploring ideas and came across The NASA Twins Study (www.nasa.gov/twins-study), a comprehensive physiological study of identical twin brothers, both astronauts, one who spent a year on the ISS and one who remained on Earth. One surprising genetic level result from this study was that telomeres, which shorten with age, were observed to lengthen in space. Drawing upon her prior knowledge of biology, Pristine thought the proliferation of stem cells might underlie this process since they tend to have longer telomeres than other cell types. The panel of judges thought this was an exceptional proposal for its ability to build upon prior space biology research while taking it a step further.

During the initial round of proposals, students are afforded a significant degree of freedom in developing their experimental concepts. They do, however, need to demonstrate an understanding of the relevant scientific principles. Students design proposals that use the Genes in Space toolkit (www.genesinspace.org/toolkit), a suite of biotechnology tools available on the ISS that includes PCR, BioBits® cell-free protein synthesis, and fluorescence visualization.

Pristine was one of five finalists selected to present her idea to contest judges at the ISS Research & Development Conference. Prior to presenting, each finalist is paired with a mentor who helps students deepen their knowledge of the scientific areas they've chosen for their proposals. At this juncture, more stringent constraints are imposed. For example, students must adjust their experimental plans to conform to feasibility constraints imposed by the space environment, such as microgravity limiting basic operations such as micropipetting.

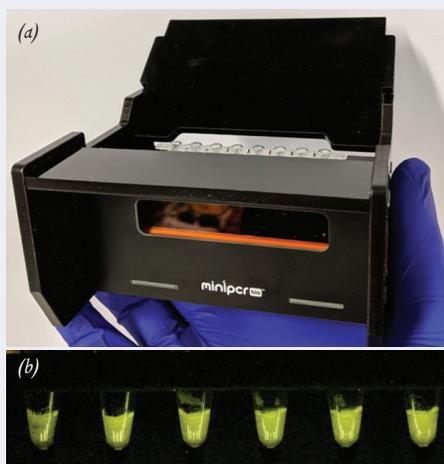
With guidance from her mentor, Harvard Graduate Student Ana Karla Cepeda Diaz, Pristine refined her proposal, which she subsequently presented to a panel of judges at the conference. Her hard work paid off when she emerged as the 2022 Genes in Space winner.

“My favorite part is working with the students and seeing all their excitement and unique ideas. One of the things that I loved about Pristine’s experiment from the beginning was how creative she was. Her ability to tie different aspects of different fields of biology all together in this experiment was very impressive.”

Ana Karla Cepeda Diaz, Harvard Grad Student & Pristine Onuoha’s Genes in Space mentor

Implementing Pristine’s experiment would require an assay to measure telomere length in space. Existing methods of assessing the length of a nucleic acid segment are not well suited to spaceflight. Gel electrophoresis, for example, requires large volumes of liquid, so cannot be used in the space environment. The task at hand became designing a proof-of-concept assay to measure DNA segment length suitable for the space context.

As with so many things in science, one thing leads to another, and suddenly, Pristine’s experiment took on a more impactful direction of expanding our toolkit of DNA technologies in space.



(a) The Genes in Space Fluorescence Viewer, which will be used in tandem with the miniPCR® to carry out a qPCR-like protocol for Pristine’s experiment.

(b) The fluorescent output through the viewer window when samples are illuminated inside.



Pristine Onuoha joined the Genes in Space team in Boston at a Space Biology Camp event.

Pristine and Ana Karla collaborated with miniPCR bio scientist Dr. Ally Huang to establish a space-ready assay that could detect length differences in DNA. They combined miniPCR amplification and a fluorescence viewer from the Genes in Space toolkit to look at the length of a DNA segment with an easy-to-interpret visual readout. The fluorescence reader uses blue light to illuminate fluorescent biomolecules in a tube; more molecules make for brighter fluorescence. This is a simplified method that is relatively robust even for novice users. In Pristine’s experiment, the fluorescence viewer will be used to visualize synthetic DNA segments of different lengths as they are amplified via PCR. Earlier detectable fluorescence should indicate a greater abundance of DNA, so Pristine expects longer segments to fluoresce after fewer PCR cycles. If successful, this assay will be useful as an indicator of changes in the length of a DNA sequence.

This assay is relevant for telomere measurement, but there are also other very relevant space applications that require measuring different DNA segments. One salient application is that space travel-associated cosmic radiation can cause DNA mutations, such as insertions and deletions, which may manifest as fluorescence that is visible earlier or later than expected, using the method Pristine established.

So, the initial scope of Pristine’s project has expanded in a direction that lays the groundwork to create an assay that is more broadly applicable than originally thought, with far-reaching implications for bio-monitoring DNA changes in astronauts as they travel in space.

The first launch window for Pristine’s experiment is June 2023, when she will be present at Kennedy Space Center to see her research project lift off.

The Genes in Space competition offers a unique opportunity for students to unleash their creativity and contribute to the field of space biology. As the field is still in its infancy, these experiments can potentially make a real impact unlocking new discoveries and pushing the boundaries of science in space.

About Genes in Space

Genes in Space is a national STEM contest that challenges students in grades 7 through 12 to design biotechnology experiments for space. Winning experiments are performed in the International Space Station National Lab, a platform for cutting-edge research and technology development that enables future space exploration. Genes in Space accepts applications between January and April each year. The contest is a collaboration between miniPCR bio and ISS prime contractor Boeing, with generous support from the ISS U.S. National Laboratory and New England Biolabs. To learn more visit genesinspace.org.

For U.S. customers only:



- ✓ Correct this address
- ✓ Unsubscribe from future mailings
- ✓ Remove name from organization

Simply go to www.wastefreemail.com and enter Code below:



or fax this cover to 571-970-6072



The estimated CO₂ emissions from the paper used for this publication have been offset with Verified Emissions Reductions from the Hudson Farms Forestry project in New Jersey.

Products and content are covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc (NEB). The use of trademark symbols does not necessarily indicate that the name is trademarked in the country where it is being read; it indicates where the content was originally developed. See www.neb.com/trademarks. The use of these products may require you to obtain additional third-party intellectual property rights for certain applications. For more information, please email busdev@neb.com.

Your purchase, acceptance, and/or payment of and for NEB's products is pursuant to NEB's Terms of Sale at neb.com/support/terms-of-sale. NEB does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of NEB.

New England Biolabs is an ISO 9001, ISO 14001 and ISO 13485 certified facility.

gBlocks[™] is a trademark of Integrated DNA Technologies, Inc

miniPCR[™] bio is a trademark of Amplyus, LLC

Boeing[®] is a registered trademark of The Boeing Company

Genes in Space[™] is a trademark of

MCLAB[®] is a registered trademark of MCLAB

EQUIPHI29[™] is a trademark of Thermo Fisher Scientific

TEMPLIPH1[®] is a registered trademark of Global Life Sciences Solutions Operations UK LTD

© Copyright 2023, New England Biolabs, Inc.; all rights reserved.



COMING SOON!

**The 2023-2024
NEB catalog**

Request yours today!



www.neb.com/support/catalog-and-literature-request

USA

New England Biolabs, Inc.
Telephone (978) 927-5054
Toll Free (USA Orders) 1-800-632-5227
Toll Free (USA Tech) 1-800-632-7799
email: info@neb.com

Australia & New Zealand

New England Biolabs (Australia) PTY
Telephone: 1800 934 218
email: info.au@neb.com
NEB New Zealand Ltd
Telephone: 0800 437 209
email: info.nz@neb.com

Canada

Telephone (905) 665-4632
Toll Free 1-800-387-1095
email: info.ca@neb.com
www.neb.ca

China

New England Biolabs (Beijing), Ltd.
Telephone 010-82378265/82378266
Free Call 400-811-2220
Free Call 400-690-3366
Fax 010-82378262
email: info@neb-china.com
www.neb-china.com

France

New England Biolabs France SAS
Telephone +33 (0) 1 69 90 87 15
Free Call 0800 100 632
email: info.fr@neb.com
www.neb-online.fr

Germany & Austria

New England Biolabs GmbH
Telephone +49 (0) 69/305 23140
Free Call 0800/246 5227 (Germany)
Free Call 00800/246 52277 (Austria)
Fax +49 (0) 69/305 23149
Free Fax 0800/246 5229 (Germany)
email: info.de@neb.com
www.neb-online.de

Japan

New England Biolabs Japan, Inc.
Telephone +81 (0) 3 4545 1422
Fax +81 (0) 3 5669 6194
email: cs.jp@neb.com
www.nebj.jp

Singapore

New England Biolabs Pte. Ltd.
Telephone +65 638 59623
Fax +65 638 59617
email: sales.sg@neb.com
www.neb.sg

United Kingdom

New England Biolabs (U.K.), Limited
Telephone +44 (0) 1462 420616
Free Call 0800 318486
Fax +44 (0) 1462 421057
Free Fax 0800 435682
email: info.uk@neb.com
www.neb.uk.com

For contacts in other countries, please visit
www.neb.com/international-support

