



EXPRESSIONS

A SCIENTIFIC UPDATE

Issue II | 2026

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CELL-FREE DNA SYNTHESIS OF dbDNA™ FOR A FASTER PATH TO RNA AND DNA THERAPEUTICS

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

Speed is a critical differentiator in therapeutic development. For teams developing DNA and RNA therapeutics, non-viral gene therapies, viral vectors, cell-free protein synthesis workflows, or CRISPR gene editing, shortening the design-to-test cycle can be the defining factor in program success.

Many therapeutic DNA development pipelines still rely on plasmid DNA (pDNA) produced through *E. coli* fermentation. While familiar and well-established, this process introduces delays and operational complexity that are increasingly at odds with rapid design-build-test cycles.

A need exists for innovation in DNA manufacturing as advanced therapies for common diseases move into clinical pipelines. The primary constraint is not just capacity, but also the need for scalable processes that ensure robust quality, safety, and regulatory compliance, while minimizing cost.

Cell-free dbDNA (doggybone™) technology is a fundamentally different path forward with significant benefits.

dbDNA technology replaces bacterial fermentation in plasmid-based systems with enzymatic synthesis, thereby cutting timelines and costs. Manufacturing is simplified by removing many constraints associated with traditional plasmid workflows, while delivering a cleaner DNA starting material for downstream RNA and DNA therapeutic applications.

dbDNA (doggybone DNA) is linear, double-stranded DNA with covalently closed ends



THE LIMITATIONS OF PLASMID DNA MANUFACTURING

Conventional plasmid production, even at a small scale, requires transforming bacteria, picking colonies, growing overnight cultures, purifying plasmids, and performing restriction digests before the DNA is ready for downstream use. This can be a rate-limiting step, especially at the early stages of project development, when multiple constructs are screened in parallel.

At a larger scale, this also includes complex cell-banking steps, a reliance on large-scale fermentation systems, and management of host-cell impurities (host-cell DNA/protein and endotoxin control).

Production-scale DNA manufacturing can take weeks to months, adding time, cost and risk.

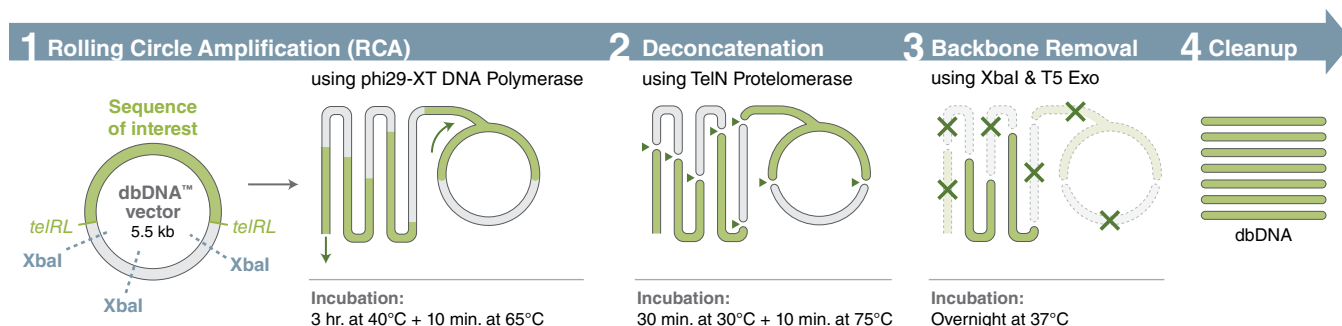
WHAT IS dbDNA?

dbDNA is linear double-stranded DNA with covalently closed hairpin ends, produced entirely through enzymatic synthesis.

Because dbDNA is generated without a bacterial host, it eliminates the drawbacks of working with plasmid systems, such as the presence of bacterial backbone sequences and antibiotic resistance markers, as well as endotoxins associated with Gram-negative bacterial production. dbDNA synthesis generates cleaner DNA material for therapeutic workflows.

The EnClose® Cell-free dbDNA™ Synthesis workflow combines the robust phi29-XT DNA Polymerase for high-yield rolling circle amplification (RCA) and EnClose TelN Protelomerase for deconcatenation and covalent closure of linear dsDNA ends, in a streamlined, one-day process. This enzymatic approach eliminates challenges associated with plasmid systems, enabling teams to move quickly from bench to clinic.

Overview of the EnClose Cell-free dbDNA Synthesis Kit workflow



dbDNA IVT template



Linearized pDNA IVT template



dbDNA enables template generation in 1 day, with just 1.5 hrs hands-on time compared to pDNA template generation in 3–4 days with 6 hrs hands-on time.

dbDNA production



Traditional plasmid production



dbDNA enables multi-gram DNA manufacture in weeks, rather than months.

RNA THERAPEUTICS AND IVT TEMPLATES

Using dbDNA technology, an IVT template can be generated in ~24 hours with only 1.5 hours of hands-on time. By comparison, linearized plasmid production can take 3 to 4 days with significantly more hands-on time for bacterial culture, plasmid purification and restriction digestion.

Use of dbDNA eliminates the presence of plasmid backbone sequences, reduces endotoxin and bioburden risk, and enables maintenance of long or repetitive elements such as poly(A) tails.

The enzymatic workflow enables seamless transition from DNA assembly to dbDNA synthesis without bacterial transformation or overnight culture. The faster design-build-test cycle allows more time to optimize untranslated regions, coding sequences, and delivery formats, and enables multi-gram manufacturing in weeks versus months.

DNA THERAPEUTICS AND VECTOR WORKFLOWS

For DNA therapeutics and viral vector manufacturing, dbDNA offers a simplified cell-free enzymatic workflow, elimination of bacterial sequences, lower endotoxin and contamination risks, a smaller laboratory footprint, and reduced regulatory burden.

Removing microbial dependencies makes dbDNA appealing for early-stage DNA therapeutic programs, including adeno-associated virus and lentiviral payload production, and other applications where clean DNA and rapid iteration are critical.

By reducing DNA production timelines from months to days, dbDNA helps accelerate early development with faster iterations and high-throughput screening, reduce operational complexity and footprint, simplify regulatory pathways, and ultimately move promising candidates to the clinic sooner.

TOOLS & RESOURCES



Learn more in our latest eBook:

Cell-free enzymatic solutions for DNA manufacturing: enhancing next-generation therapeutics with dbDNA synthesis



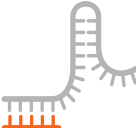
View this animation of the One-day Workflow for Synthesizing dbDNA using the EnClose Cell-free dbDNA Synthesis Kit:

To learn more, visit:

www.neb.com/e9301

Explore the Applications of dbDNA™

From viral vector production and RNA therapeutics to gene editing and protein expression, dbDNA provides a cell-free alternative to traditional plasmid DNA across a range of research applications. Discover how its purity and scalability could support your workflow.

APPLICATION		BENEFITS OF dbDNA
Adeno-associated viral (AAV) vector production		Increased Purity: Maintains inverted terminal repeats (ITRs) Improved Safety Profile: Reduced bacterial impurities (no KanR assays) Cost: Use 40% less DNA per transfection, rapid scale out and scale up
Lentiviral vector (LV) production		Increased Purity: Maintains long terminal repeats (LTRs) Improved Safety Profile: Reduced bacterial impurities (no KanR assays) Improved Packaging: Less empty or unloaded capsid for improved infectivity Cost: Improved infectivity by 4-fold, rapid scale out and scale up
DNA vaccines		Increased Purity: Increased copy number, demonstrated dose sparing Improved Safety Profile: Reduced bacterial impurities, improved immunogenicity vs. pDNA Cost: Reduced DNA requirements, rapid scale out and scale up
Non-viral gene therapy		Increased Purity: Compatible with multiple delivery methods Improved Safety Profile: Reduced toxicity in LNPs, improved tolerability via innate sensing, reduced bacterial impurities Cost: Reduced DNA requirements, rapid scale out and scale up
RNA therapeutics		Increased Purity: Maintains coded polyA tails, improved RNA yields Improved Safety Profile: Reduced bacterial impurities Cost: Reduced DNA requirements, rapid scale out and scale up
Gene editing		Increased Purity: Improved editing efficiency up to 2.5-fold, supports large DNA constructs (up to 20 kb), maintains high viability between donors Improved Safety Profile: Reduced bacterial impurities Cost: Reduced DNA requirements, rapid scale out and scale up
Protein expression		Increased Purity: Improved expression Improved Safety Profile: Reduced bacterial impurities Cost: Reduced DNA requirements, rapid scale out and scale up

Discover how the EnClose® Cell-free dbDNA™ Synthesis Kit can help you generate dbDNA for your research: www.neb.com/e9301

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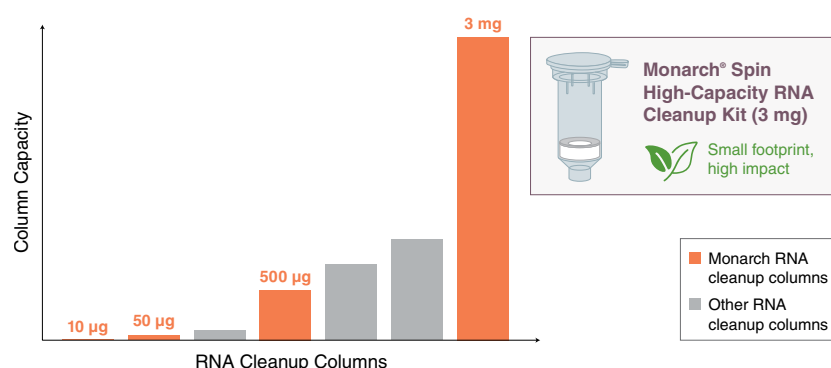


5 THINGS TO CONSIDER WHEN CLEANING UP LARGE AMOUNTS OF RNA

Milligram-scale RNA purification in a spin column format

The Monarch® Spin High-Capacity RNA Cleanup Kit (3 mg) provides an efficient, microcentrifuge-scale solution for purifying large amounts of RNA in a single spin column. Optimized for cleanup of RNA synthesized from high-yield *in vitro* transcription (IVT) reactions, this unique kit delivers highly pure RNA in low elution volumes for downstream applications like RT-PCR, transfection, RNA labeling, and RNA library prep for NGS.

High-capacity RNA purification in an easy spin column format



The Monarch Spin High-Capacity RNA Cleanup Kit (3 mg) efficiently purifies large amounts of RNA following in vitro transcription (IVT) and other enzymatic reactions using a silica spin column at the microcentrifuge scale.

BENEFITS:

- Purify up to 3 mg RNA in a single high-capacity silica spin column
- Recover highly pure, concentrated RNA in elution volumes as low as 250 µl
- Perform high-capacity RNA cleanup workflows using standard microcentrifuges – no additional equipment such as HPLC/FPLC required
- Confidently purify RNA synthesized with modified nucleotides and enzymatically capped RNA
- Simplify workflow with a user-friendly bind–wash–elute protocol using a single wash buffer

Ordering Information

PRODUCT	NEB #	SIZE
Monarch® Spin High-Capacity RNA Cleanup Kit (3 mg)	T2060S/L	10/50 preps

For more information, visit: www.neb.com/T2060

When working with large quantities of RNA, several factors can influence recovery, purity and suitability for downstream applications.

1 Match the cleanup capacity to your RNA input

Ensure the cleanup format can handle your total RNA mass. Standard columns suit smaller inputs, while Monarch high-capacity formats accommodate larger amounts, including milligram-scale RNA.

2 Remove contaminants effectively

Consider which impurities must be removed, such as enzymes, unincorporated nucleotides, salts, detergents, and other low-molecular-weight reaction components.

3 Balance RNA recovery and final concentration

Choose a format that provides both adequate recovery and a practical final volume. Lower elution volumes increase concentration but may affect recovery.

4 Confirm compatibility with your RNA size and sample type

Check whether the method suits your RNA size range and source, such as IVT RNA, RNA synthesized with modified nucleotides, extracted RNA, small RNAs, or long transcripts.

5 Meet downstream application requirements

Select a cleanup method that delivers the purity and concentration needed for applications such as RT-PCR, RNA-seq, transfection, etc.

Featured application notes for RNA analysis and purification

High-quality RNA begins with effective characterization and impurity control. Explore two recent application notes featuring workflows for mRNA 5' cap analysis and enzymatic removal of dsRNA contaminants.

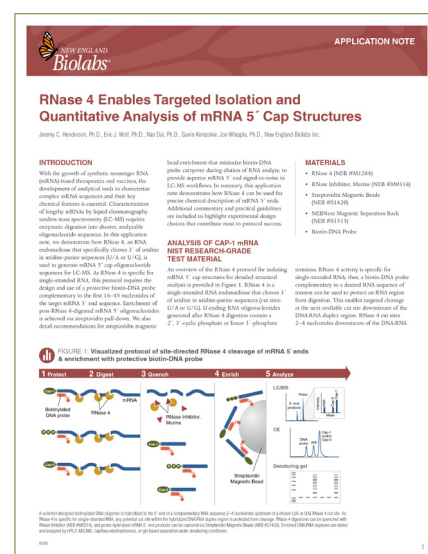


RNase 4 Enables Targeted Isolation and Quantitative Analysis of mRNA 5' Cap Structures

The 5' cap is a critical quality attribute of mRNA therapeutics and vaccines. This application note describes a targeted LC-MS workflow that uses RNase 4 and a custom biotinylated DNA probe to isolate and enrich cap-containing mRNA fragments for analysis. The workflow includes practical guidance for probe design, digestion, and enrichment, helping researchers obtain high-quality data for confident characterization of mRNA 5' cap structures.



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

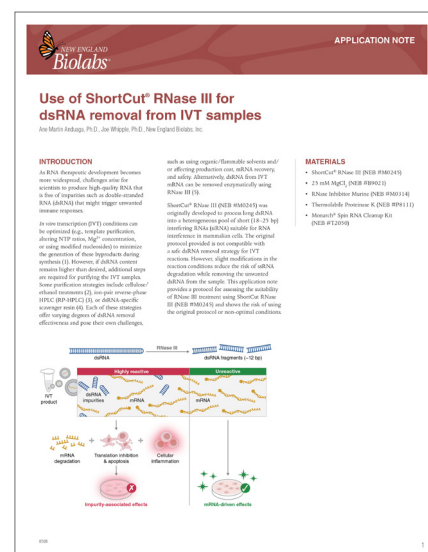


Use of ShortCut® RNase III for dsRNA removal from IVT samples

Double-stranded RNA (dsRNA) impurities generated during *in vitro* transcription can affect RNA quality and trigger unwanted immune responses. This application note demonstrates how optimized ShortCut RNase III treatment can significantly reduce dsRNA contaminants while preserving the integrity of the desired single-stranded RNA. The workflow offers a simple enzymatic alternative to more complex purification methods and provides guidance for balancing impurity removal with RNA recovery.



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



NEB products supporting RNA-related workflows



NEB offers a wide selection of reagents for purification, quantitation, detection, synthesis and manipulation of RNA. We can support all of your RNA-related workflows, from start to finish. We also offer samples for many of these reagents so that you can test directly in your workflow and see how they perform for you. View the table of products below, which also indicates whether samples are available, and access the sample request form at www.neb.com/products-for-rna-workflows to get started.



RNA Synthesis

RNA synthesis requires solutions for template generation, *in vitro* transcription, RNA capping, poly(A) tailing, RNA purification, and nucleotide solutions for synthesizing modified mRNAs. Our RNA synthesis products are available from bench-to-commercial-scale to serve both academic and industrial needs. Further, we provide these products at quality levels that support vaccine and therapeutic manufacturing.

Template Generation and Cleanup	In vitro Transcription	RNA Capping	Poly(A) Tailing	RNA Purification
EnClose® Cell-free dbDNA™ Synthesis Kit	HiScribe® T7 mRNA Kit with CleanCap® Reagent AG		E. coli Poly(A) Polymerase	Monarch Spin RNA Cleanup Kit (10 µg)*
Q5® Hot Start High-Fidelity DNA Polymerase	HiScribe T7 ARCA mRNA Synthesis Kit (with tailing)			Monarch Spin RNA Cleanup Kit (50 µg)*
phi29 DNA Polymerase	HiScribe T7 ARCA mRNA Synthesis Kit			Monarch Spin RNA Cleanup Kit (500 µg)*
TeIN Protelomerase	HiScribe T7 High Yield RNA Synthesis Components	Faustovirus Capping Enzyme		Lithium Chloride
EnClose® TeIN Protelomerase	HiScribe T7 Quick High Yield RNA Synthesis Kit	Vaccinia Capping System		Companion Products:
EnClose® TeIA Protelomerase	HiScribe SP6 High Yield RNA Synthesis Kit	mRNA Cap 2'-O-Methyltransferase		Monarch Buffer BX
		mRNA Cap N6 Methyltransferase (m6A)		
dNTP solution mixes	T3 & SP6 RNA Polymerases	ARCA, mRNA Cap N6 Methyltransferase (m6A), and other mRNA cap analogs		Monarch Buffer WX
BspQI	T7 RNA Polymerase	S-Adenosylmethionine (SAM)		Nuclease-free Water
BspQI-HF	Hi-T7 RNA Polymerase			
XbaI	Companion Products:			
NEBuffer™ 4	RNase Inhibitor (Murine)			
NEBuilder HiFi DNA Assembly*	RNase Inhibitor (Human Placental)			
NEBridge® Golden Gate*				
Monarch Spin PCR & DNA Cleanup Kit (5 µg)*	Pyrophosphatase, Inorganic (<i>E. coli</i>)			
Monarch Mag PCR & DNA Cleanup Kit (5 µg)				
Monarch Spin High-Capacity DNA Cleanup Kit (100 µg)	Pyrophosphatase, Inorganic (Yeast)			
	DNase I (RNase-free)			
	DNase I-XT			
	NTPs, Modified NTPs			

*Samples available

2026 PASSION IN SCIENCE AWARDS® RECIPIENTS

Meet the 2026 Passion in Science Award Recipients

From citizen-science biodiversity monitoring and STEM education to science communication through art, this year's Passion in Science Awardees once again inspire us with their creativity and their desire to make a meaningful difference.

The New England Biolabs Passion in Science Awards began in 2014, when we recognized that we wanted to celebrate scientists whose impact extends beyond the bench and creates positive change in their communities and the environment. This year's recipients remind us that discovery often begins with thinking outside the box, and that creativity and curiosity are often at the heart of scientific progress.

For the 2026 Passion in Science Awards, we recognized eleven scientists for their commitment to advancing science across four categories: Science Mentorship & Advocacy, Environmental Stewardship, Humanitarian Duty, and Arts & Creativity.

The full list of Passion in Science Awardees is as follows:

CATEGORY: SCIENCE MENTORSHIP AND ADVOCACY AWARD™

Richard O'Rorke

(University of Auckland, Auckland, New Zealand): co-led The DNA Drive (DNADRV) project, which explores the distribution and biodiversity of insects found in Aotearoa, New Zealand by swabbing for DNA traces that remain after insects collide with car number plates.

Alexandra-Teodora Szabo

(University of Tübingen, Tübingen, Germany): founder of the podcast PhD: The Experience, which provides real experiences, expert interviews & practical advice to help scientists navigate doctoral life and thrive in academia.

Hemanshi Galaiya

(The University of Queensland, Brisbane, Australia): founded Young Stripes (YS), an organization that delivers practical STEM education, mentorship and science communication programs that empower underrepresented students to see STEM education and careers as accessible, fun, creative and transformative.

CATEGORY: ENVIRONMENTAL STEWARDSHIP AWARD™

Homere Alves Monteiro

(University of California San Diego, San Diego, CA): conducted a population-genomic survey of ~1,000 *Hypoconera* ants across Madagascar, using low-cost whole-genome sequencing to delimit cryptic species and record an island fauna before deforestation erases it.

Chukwudi Ikegwu

(Howard University, Washington, D.C.): advanced amphibian conservation in Nigeria using eDNA and bioacoustics to non-invasively monitor biodiversity, detect threatened species, and support sustainable ecosystem protection.

CATEGORY: HUMANITARIAN DUTY AWARD™

Kehinde Abereojie

(University of Oxford, Oxford, UK): founded Purple Pact Initiative, a non-profit that uses evidence-based and peer-led sexual and reproductive health education programs to close knowledge gaps and promote autonomy among Nigerian women and girls in underserved communities.

Rojelio Mejia

(Baylor College of Medicine, Houston, Texas): founded My Poverty and Parasite Nexus project, which mentors trainees and collaborators across countries to connect parasites, poverty, climate change, sanitation, and child health through science and advocacy.

CATEGORY: ARTS AND CREATIVITY AWARD™

Saurja DasGupta

(University of Notre Dame, Notre Dame, IN): worked with collaborator Argha Manna to develop the graphic novel and science-comics exhibition "A Pilgrimage to Life's Beginning", which combines scientific rigor with compelling narrative and accessible visual art to tell Life's origin story.

Julianna "Jules" Ho

(Brown University and Rhode Island School of Design, Providence, RI): created Charismatic Critter Club, which uses character design, storytelling, and worldbuilding to translate biodiversity and ecological relationships into imaginative, scientifically grounded experiences.

Anne-Frances Miller

(University of Kentucky, Lexington, KY): developed courses that embed understanding of chemical concepts in practices of textile art, to engage students with limited access to laboratory learning opportunities with hands-on projects that use dyes from native plants, and investigate artisanal traditions.

Haolun (Allen) Tian

(HZNU, China and Queen's University, Ontario, Canada): created Algal Blooms: Aerial Brushstrokes, which captures the abstract beauty of algal blooms through drone photography. The project blends molecular ecology research with visual art to foster public environmental empathy and stewardship.

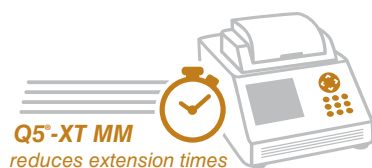
More information on the winners can be found at www.neb.com/passioninscience following the event



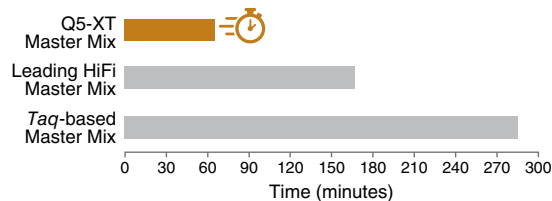
Fidelity at its Finest

The newest Q5[®] solution for faster long-range PCR

When DNA targets are long or complex, or speed is a priority, Q5-XT Hot Start High-Fidelity 2X Master Mix delivers faster, more robust amplification while maintaining the performance you expect from Q5.

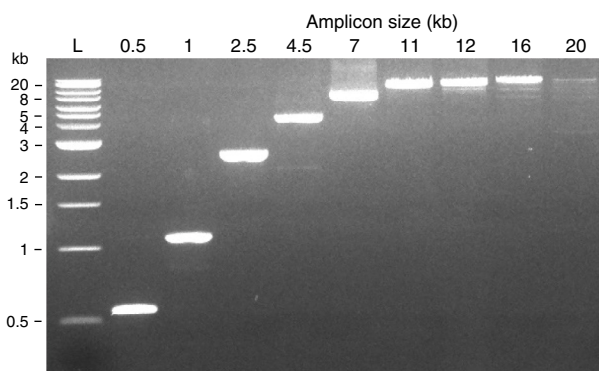


Comparison of protocol times for a 10 kb amplicon

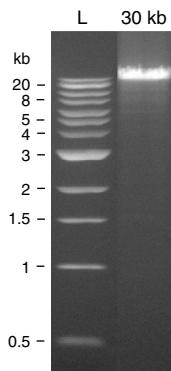


Q5-XT enables amplification on long DNA targets

A. Human genomic DNA



B. Lambda phage DNA



Amplification of specific sequences from human genomic DNA (A) or Lambda phage DNA (B) using Q5-XT High-Fidelity 2X Master Mix. Amplicon sizes are indicated above the gel. Ladder (L) is Quick-Load[®] 1 kb Extend DNA Ladder (NEB #N3239). All PCR products were visualized by ethidium bromide staining on 1.2% TAE agarose gel.

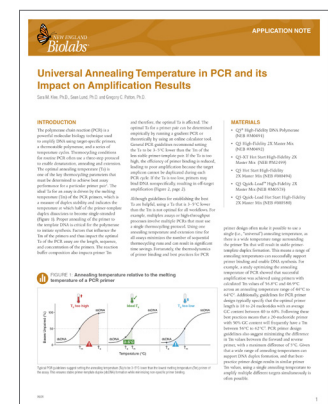
Ordering Information

PRODUCT	NEB #	SIZE
Q5 [®] -XT Hot Start High-Fidelity 2X Master Mix	M2499S/L	100/500 rxns

WITH Q5-XT, YOU CAN:

- Save time with fast cycling conditions and reduced extension times of 5–15 seconds/kb
- Amplify challenging targets, including high AT or high GC templates, up to 20 kb.
- Work with confidence using high-fidelity amplification, approximately 280X more accurate than *Taq*.
- Streamline setup with a hot-start aptamer for improved reaction specificity and room-temperature setup
- Compatible with a 62°C universal annealing temperature protocol

Application Note:



Scan here to read the application note:
Universal Annealing Temperature in PCR and its Impact on Amplification Results

To learn more about Q5-XT and request a sample, please visit www.neb.com/m2499



Exploring the Hidden Diversity of Unorthodox Genomes

The molecular biology of nucleic acids is typically discussed using conventional categories such as chromosomes, plasmids, viruses, and mobile genetic elements. But biology doesn't always conform to our classifications. Some genetic entities are smaller than expected; others are larger and more complex; and some depend on other viruses or host systems in ways that challenge simple classification.

These diverse entities were the focus of New England Biolabs' recent research symposium, Unorthodox Genomes. The free, one-day event was held in Boston, MA and was open to the broader scientific community, bringing together invited speakers from across the globe.

Obelisks: small genetic entities hiding in plain sight

At the small end of the genetic spectrum, Professor Andy Fire of Stanford University described computational strategies his group has used to uncover previously overlooked biological features in sequencing databases. His group identified small, apparently circular RNAs, about 1000 bases long, with predicted rod-like secondary structures that are prevalent in gut and oral metatranscriptomes, Zheludev et al. 2024. The team named these molecules "obelisks," inspired by their resemblance to ancient obelisk monuments.

Obelisks differ from viruses because they lack a protein shell and are more akin to viroids- small, naked circular RNAs that cause disease in plants. Unlike viroids, however, obelisks have been detected within bacterial cells and have distinct sequences and secondary structures.

These entities have been hidden in plain sight for decades within sequencing databases, but overlooked because they didn't contain any obvious signatures that researchers were initially investigating.. Their discovery shows what can be uncovered by returning to existing data with a new set of questions.

There is still much to learn about obelisks, including how they spread, whether they impact human health, and if they are descendants of the precellular world. After reading the seminal paper from the Fire Lab, Dr. Andrew Varble of the University of Rochester began developing experimental systems to investigate how small extrachromosomal elements propagate. This work aims to illuminate how these enigmatic RNAs maintain themselves and complements the computational approach used to discover them.

Phage satellites: genetic hitchhikers & parasites of parasites

Other talks shifted the focus from size to dependence. Phage satellites are mobile genetic elements that depend on helper bacteriophages for replication and spread. Typically 5 to 20 kb in size, they are far smaller than the bacteriophages they exploit and can be thought of as parasites of parasites.

Dr. José Penadés of Imperial College London explored how interconnected phage satellites are with their viral hosts. His group found that some phage satellites encode their own capsids but use the tails of their host's bacteriophages, thereby expanding the range of cellular hosts to which the satellites can migrate.

Dr. Kim Seed of the University of California, Berkeley, discussed phage satellites in the context of infectious disease. Her work explores the interactions among *Vibrio cholerae*, the bacteriophage ICP1 and phage-inducible chromosomal island-like elements, or PLEs, that reside within the bacterium. PLEs can interfere with ICP1 and redirect viral packaging to promote their own transfer into new host cells. This interaction between a bacterium, a resident mobile genetic element and a bacteriophage illustrates the complexity of microbial conflict and co-evolution.

When mobile genetic elements get unexpectedly large

While the symposium began with some of the smallest genetic entities, it concluded with the largest and most complex phage genomes. Scientists have known about the existence of jumbo phages and megaphages for decades, but advances in technology are helping researchers uncover more of their diversity and biological sophistication.



Left to right: Speakers Jill Banfield, Andy Fire, José Penadés, Kim Seed, Andrew Varble, and Joe Pogliano

Dr. Joe Pogliano of the University of California, San Diego, described how certain bacteriophages build a dynamic, selectively permeable pseudonucleus that shields their replicating DNA from host endonucleases. Using cryo-electron microscopy and image reconstruction, his team showed that this pseudonucleus is spatially dynamic and selective about which proteins pass through its surrounding shell. Viral capsid precursors were visualized accumulating like a bouquet of flowers around the phage pseudonucleus as they filled with DNA.

In contrast to using microscopy to visualize large viruses, Dr. Jill Banfield of the University of California, Berkeley, has developed sophisticated methodologies to reconstruct microbial genomes from complex natural communities. Her recent work has identified giant extrachromosomal elements up to one million base pairs, which her team

named Borgs. Based on their gene composition, Borgs appear to provide methane-oxidizing archaea access to genes involved in redox reactions and energy conservation. Their genes can be traced to diverse microbes, suggesting that these elements accumulate metabolic pathways as they move through microbial communities.

Together, the symposium talks highlighted how much remains to be discovered about the ways genetic information persists, moves and evolves. These systems are not united by a single structure, mechanism or evolutionary origin. Rather, each challenges familiar expectations about genome size, replication, mobility or biological independence.

The day concluded with a poster session showcasing work from students, postdoctoral researchers and scientists from NEB and the broader research community. Organized by

scientists in the NEB Research Department, the symposium reflects NEB's longstanding commitment to investing in basic science and creating opportunities for researchers to connect, exchange ideas and collaborate. More than fifty years after NEB's founding, the principle of being "created by scientists for scientists" continues to bring important areas of fundamental research to the forefront of scientific conversation.

Reference:

Zheludev IN et al. 2024. Cell. doi: 10.1016/j.cell.2024.09.033



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Toll Free (USA Orders) 1-800-632-5227
Toll Free (USA Tech) 1-800-632-7799
info@neb.com

Australia & New Zealand

New England Biolabs (Australia) PTY
Telephone: 1800 934 218 (AU)
info.au@neb.com
Telephone: 0800 437 209 (NZ)
info.nz@neb.com

Canada

New England Biolabs, Ltd.
Toll Free: 1-800-387-1095
info.ca@neb.com

China

New England Biolabs (Beijing), Ltd.
Telephone: 010-82378265/82378266
info@neb-china.com

France

New England Biolabs France
Telephone: 0800 100 632
info.fr@neb.com

Germany & Austria

New England Biolabs GmbH
Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

India

New England Biolabs India Private Ltd.
Telephone: +91 6827 421 085
info.in@neb.com

Japan

New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info.jp@neb.com

Singapore

New England Biolabs, Pte. Ltd.
Telephone: +65 638 59623
sales.sg@neb.com

Republic of Korea

New England Biolabs Korea Ltd.
Toll Free: +82 (70) 47318478
info.kr@neb.com

United Kingdom

New England Biolabs (UK), Ltd.
Call Free: 0800 318486
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