NEB EXPRESSIONS

A scientific update

Issue I • 2021

IN THIS ISSUE

SCIENCE

INNOVATION

INSPIRATION

NEW ENGLAND BioLabs® Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

Think differently about your molecular diagnostics supply chain


By Tom Evans, Ph.D. and Salvatore V. Russello, Ph.D., New England Biolabs, Inc.

New England Biolabs® (NEB®) partners with customers globally to address the challenges faced by innovators developing the molecular diagnostics (MDx) technologies required to address public health and pandemic preparedness.

The current COVID-19 pandemic has impacted nearly every aspect of daily life. It has elevated many of the challenges faced by clinical labs, and new and innovative solutions are required to address them. One strategy to help public health professionals understand and control the spread of SARS-CoV-2 is the widespread testing of millions of people around the world. Conventional RT-qPCR based tests performed in large, centralized testing facilities have been the backbone of testing to date. Despite the rapid development of these SARS-CoV-2 assays, dozens of new modalities are being introduced to help close the gap between the number of cumulative tests that can be performed daily and the desired testing capacity required to control and track the spread of the virus.

Numerous companies, diagnostic testing facilities, and academic institutes have introduced SARS-CoV-2 assays under the FDA's Emergency Use Authorization (EUAs). Based upon recent FDA guidance, the priority of new EUAs reviewed by FDA will be on tests that increase testing accessibility or significantly increase capacity. Additionally, new SARS-CoV-2 assays can be introduced by Clinical Laboratory Improvement Amendments (CLIA) laboratories without going through the EUA process. Still, such rapid progress has not been without challenges—it has exposed weaknesses in diagnostics supply chains and has belied the need for innovation and thinking differently about how diagnostics should be developed, manufactured, and deployed.

Many scientists know NEB as a trusted reagent provider to the life science community. What many do not know is that we also offer a portfolio of products that serve as critical components for a wide array of diagnostics products and services. Extensive molecular biology and enzymology experience provide NEB with the unique ability to help customers solve the challenges inherent in technology development and ultimately in scale-up and commercialization.

Leveraging NEB's research program to influence product development for SARS-CoV-2 testing

NEB's founder, Dr. Donald Comb, prioritized basic research ever since NEB was founded in the early 1970s, and this has influenced the product development direction of the company. Our research interests include finding new enzyme activities, engineering enzymes specifically for biotechnology applications, and understanding how enzymes behave. This level of expertise and knowledge is then harnessed by our development and production teams to create robust enzymes and optimized workflows for commercialization. For example, NEB's expertise in amplification has resulted in an extensive portfolio of reagents for RT-qPCR and isothermal amplification, two technologies essential in today's molecular testing landscape. In fact, many of NEB's products have been already cited in numerous publications and EUA protocols.

Currently, the gold standard for diagnostics testing is RT-qPCR, and most of today's testing infrastructure is based on this technology. It's highly sensitive and robust, and NEB offers a number of products in this area, as do many other suppliers. However, like any technology, it has strengths and weaknesses. For example, it requires use of expensive equipment (a thermal cycler with fluorescence detection) and, in some cases, has longer turnaround times.

At NEB, our emphasis on long-term research resulted in us evaluating and working with loop-mediated isothermal amplification (LAMP), an alternate approach originally developed at the Eiken Chemical Co., Ltd. Over the last decade we combined a number of breakthroughs to make the technology even more suitable for the molecular diagnostics community. This included novel engineered DNA polymerases, a new reverse transcriptase, the ability to set up reactions at room temperature using "WarmStart®" enzymes, multiplexing, and the ability to perform carryover prevention. We also introduced a version of this technology that enables the visual detection of products amplified by LAMP and RT-LAMP. We have also published extensively in this area (1).

Partnering with you from R&D to production

NEB is a company that scientists know and trust. We pride ourselves on being a resource—not just through our product offerings and production capabilities, but also through the support we provide to diagnostics assay development scientists—from R&D through to scale-up and commercialization. For this reason, NEB is an ideal partner as new testing technologies are moved from the bench into production.

Our support starts early in the R&D process, as customers obtain information about our products and technologies through our catalog, extensive web resources and support staff, and ultimately obtain material to evaluate. As questions arise about how a product might be used in a given technology, customers can speak directly with scientists

who have played a role in developing or producing these products. And in those instances where a customer wishes to optimize performance for a particular detection modality, modify a product, or request a customized packaging format, the OEM & Customized Solutions Team is brought in. We quickly assemble a cross-functional team of researchers, product developers, project managers, and logistics staff to assess and make recommendations as to how best to address our customers' needs.

Identifying the pain points involved in developing diagnostics

Our customers range from some of the largest molecular diagnostics organizations to early stage technology companies. Their challenges differ. In some cases they are looking to build out redundancy in their reagent supply chain, while in others they may need NEB to work collaboratively to further develop and help bring their technology to market.

That said, there are several common challenges that many customers have cited repeatedly over the past several months. These are scale, supply chain resilience, and product quality, performance, and consistency.

Regardless of what technology is ultimately incorporated into a molecular diagnostics product, a consistent and reliable supply chain is essential, especially in today's landscape. Product demand is higher than ever before, and dozens of organizations are pursuing similar approaches with the need for the same reagents and associated consumables. Further, the quality and consistency of reagents can vastly impact the performance of an assay.

Enabling your solution

Unlike other reagent providers of similar size and capacity, NEB has made the decision to enable science and not compete with our diagnostics customers in the markets that they serve. As one of the few privately held molecular biology tools providers, our goal is to establish partnerships that advance our customers' science and business objectives—100% of our production capacity is earmarked for the customers we serve, and not for the manufacture of our own diagnostics products.

Over the past decade, NEB has made significant investment in its manufacturing scale-up and operations, as well as in its quality systems. Our ISO 13485 facilities in Ipswich, Rowley, and Beverly, Massachusetts, have the capacity to provide reagents to enable many millions of molecular assays, whether they be for RT-qPCR or isothermal amplification.

NEB was founded to serve the scientific community with humility, integrity and transparency—principles which we believe are more important

now than ever. We look to our diagnostics customers as the real innovators. These customers engage with NEB scientists to understand what our products do and don't do, to make good decisions quickly about whether or not we are a fit for their technology platform. We also provide clarity about capacity, turn-around-times and, when possible, pass along cost savings to our customers in the event that scale can be achieved to reduce the price of our products.

In summary, we would like the molecular diagnostics community to think differently about NEB and consider us as their partner for future assay development and scale-up needs.

1. Anahtar, M.N. et al (2020) Open Forum Infectious Diseases, doi.org/10.1093/ofid/ofaa631.

If you want to learn more about how NEB can support your molecular diagnostics needs, contact us at **custom@neb.com** or visit **www.neb.com/MDx**.

Amplification-based Molecular Diagnostics

NEB has a long history in the development of reliable and convenient tools for amplification, and offers a large selection of products for PCR, qPCR, RT-qPCR and isothermal amplification. Our extensive expertise in this area has allowed us to develop optimized enzymes for a variety of applications, including incorporation into diagnostics. The table below summarizes some of the products available from NEB for molecular diagnostics applications. Bulk and/or custom formats are available for all products, with more specific customization details included below:

APPLICATION		PRODUCTS	PRODUCT NOTES	CUSTOM FORMULATIONS AVAILABLE
PCR APPLICATIONS	qPCR/ RT-qPCR	**DNA, Probe** Luna® Universal Probe qPCR Master Mix (NEB #M3004) **DNA, Dye** Luna Universal qPCR Master Mix (NEB #M3003)	• Sensitive, reproducible and reliable performance • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours	• ROX-free • Blue-dye-free • Lyo-compatible
		RNA (1-step), Probe Luna Universal One-Step RT-qPCR Kit (NEB #E3005) **RNA (1-step), Dye** Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) 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)	• Luna WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours • High conc. ideal for viral targets (NEB #M3019) • Includes carryover prevention (NEB #M3019)	• ROX-free • Blue-dye-free
		RNA (2-step) LunaScript® RT SuperMix Kit (NEB #E3010)	• Novel thermostable RT • Single-tube format • 13-minute protocol	• Blue-dye-free
	PCR/ RT-PCR	**Master Mixes** Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494) Q5 High-Fidelity 2X Master Mix (NEB #M0492) **Standalone Enzyme & Buffer** Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) Q5 High-Fidelity DNA Polymerase (NEB #M0491)	• ~280X fidelity of *Taq* • Consistent, fast, reliable performance • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours	• High conc. • Glycerol free
		Hemo KlenTaq® (NEB #M0332)	• Amplification direct from blood	• +/- Hot Start • High conc.
		Hot Start *Taq* DNA Polymerase (NEB #M0495) Hot Start *Taq* 2X Master Mix (NEB #M0496)	• Unique aptamer-based enzyme control supports fast protocols • Compatible with automation and reaction miniaturization	• High conc. • Glycerol free
ISOTHERMAL APPLICATIONS	LAMP	WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB #M1800) WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804)	• Fast, clear pink-to-yellow visible detection of amplification • Results in approximately 30 minutes	
		SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB #E2019)	• Simple, colorimetric detection of amplification of SARS-CoV-2 nucleic acid • Automation-compatible when coupled with absorbance plate reader	
		WarmStart LAMP Kit (DNA & RNA) (NEB #E1700)	• Master mix for LAMP and RT-LAMP workflows • Supports multiple detection methods, including fluorescence and turbidity • Automation compatible	• Lyo-compatible • High conc.
		Bst 2.0 WarmStart DNA Polymerase (NEB #M0538) *Bst* 2.0 DNA Polymerase (NEB #M0537)	• Improved reaction properties compared to wild-type *Bst* DNA Polymerase • Increased dUTP tolerance enables carryover prevention	• Glycerol-free • High conc.
		Bst 3.0 DNA Polymerase (NEB #M0374)	• DNA binding domain fusion supports robust performance • Significantly increased RT activity up to 72°C enables single enzyme RT-LAMP	• Glycerol-free • High conc.
		WarmStart RTx Reverse Transcriptase (NEB #M0380)	• *In-silico* designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C	• Glycerol-free • High conc.
	Strand Displacement	Nt.BstNBI (NEB #R0607)	• High purity, high quality nicking endonuclease	• Glycerol-free • High conc.
	Helicase-dependent Amplification	*Tte* UvrD Helicase (NEB #M1202)	• Thermostable • Improves specifically of problematic fluorescent LAMP reactions	• High conc.
		IsoAmp II Universal tHDA Kit (NEB #H0110)	• Requires only two primer • Produces short, discrete DNA products	
	Other	*Bsu* DNA Polymerase Large Fragment (NEB #M0330)	• Enables low temperature isothermal applications	• High conc.
		T4 Gene 32 Protein (NEB #M0300)	• Can increase yield and efficiency of amplification reactions	• Glycerol-free
		Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)	• Highly pure • Individual mixes available	• Custom conc.
		Antarctic Thermolabile UDG (NEB #M0372)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• High conc.
		Proteinase K, Molecular Biology Grade (NEB #P8107)		• Custom conc.
		Thermolabile Proteinase K (NEB #P8111)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• Custom conc.

NGS-based
Molecular Diagnostics

Sequencing is enabling scientists to make rapid advances in epidemiology and surveillance, basic and clinical research, and diagnostics. A fast-growing number of methods are being developed to address whole genome, as well as targeted approaches. In all cases, streamlined workflows that result in high-quality, high yield libraries are critical towards optimizing your next generation sequencing (NGS) results. The table below summarizes some of the products available from NEB for molecular diagnostics applications. Bulk and/or custom formats are available for all products, with more specific customization details included below:

APPLICATION			PRODUCTS	NOTES
NGS-BASED MOLECULAR DIAGNOSTICS	DNA Sequencing	DNA library preparation with enzymatic DNA fragmentation	NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina® (NEB #E7805) NEBNext Ultra II FS DNA Module (NEB #E7810) NEBNext Ultra II Q5® Master Mix (NEB #M0544) NEBNext Adaptors and Primers	• Range of packaging options including 96-well plates • Component and kit customization • Kitting • Automation-compatible • Streamlined workflows
		DNA library preparation for cfDNA or pre-sheared DNA	NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB #E7645) NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546) NEBNext Ultra II Ligation Module (NEB # E7595) NEBNext Ultra II Q5 Master Mix (NEB #M0544) NEBNext Adaptors and Primers	
	RNA Sequencing	Depletion of abundant RNAs	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400) NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750) NEBNext rRNA Depletion Kit (Bacteria) (NEB #E7850) NEBNext RNA Depletion Core Reagent Set for customized depletion (NEB #E7865)	
		RNA library preparation	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) NEBNext Ultra II RNA First Strand Synthesis Module (NEB #E7771) NEBNext Ultra II Directional RNA Second Strand Synthesis Module (NEB #E7550) NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546) NEBNext Ultra II Ligation Module (NEB #E7595) NEBNext Ultra II Q5 Master Mix (NEB #M0544) NEBNext Adaptors and Primers	
		Virus sequencing and detection	LunaScript RT SuperMix Kit (NEB #E3010) Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494) NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546) Blunt/TA Ligase Master Mix (NEB #M0367) NEBNext Quick Ligation Module (NEB #E6056) NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770) WarmStart LAMP Kit (DNA & RNA) (NEB #E1700)	

Find out how NEB's Customized Solutions Team can help streamline your molecular diagnostics product development. Contact us at custom@neb.com or visit www.neb.com/customized-solutions.

Supporting COVID-19 Research

Visit www.neb.com/COVID19 to learn more

As the SARS-CoV-2 virus continues to impact our communities, our manufacturing and distribution teams continue to be fully operational, and we are working closely with our suppliers and distribution partners to ensure uninterrupted access to our products and technical support.

NEB's products are available for research purposes only. However, we are supplying and supporting customers who are working diligently to develop better diagnostic tools and vaccines for the SARS-CoV-2 virus, and are ready to supply additional customers with the reagents they need to validate and develop them as diagnostic tools for lab-based or point-of-care settings.

Learn more about our growing selection of products available for these applications:

RNA Extraction | Virus Detection | Sequencing & Epidemiology | Vaccine Development | Viral Biology

Visit www.neb.com/COVID19 to:

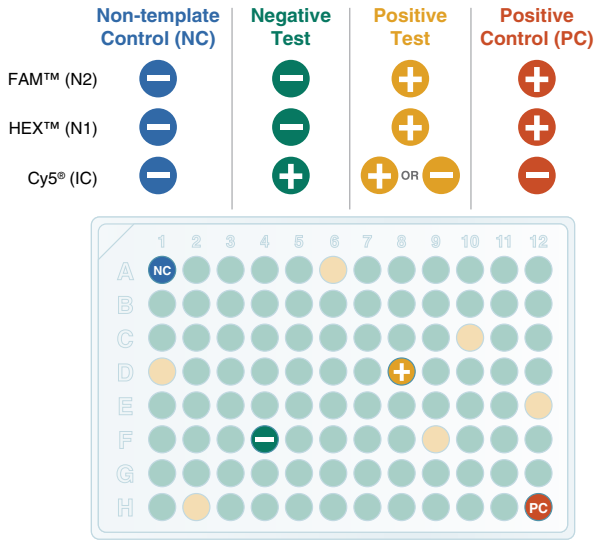
- View NEB products being used in COVID-19 related research
- View citations and EUA protocols utilizing NEB products
- Check out our COVID-19 Researcher Spotlight podcast/video series
- Tell us about your own COVID-19 related research

Featured Products

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit

The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit is a research use only (RUO) kit optimized for real-time qualitative detection of SARS-CoV-2 nucleic acid using hydrolysis probes.

The kit features a primer/probe mix specific to two regions of the SARS-CoV-2 virus N gene [based on sequences provided by the Centers for Disease Control and Prevention (CDC)]. The probes have been modified to contain different fluorophores (N1: HEX; N2: FAM) to enable multiplexing. An internal control primer and probe set, designed to amplify the human RNase P gene, is also included in the primer mix. The reverse primer of this target has been modified from the CDC design to target an exon/exon boundary to reduce background amplification from possible contaminating genomic DNA.



Using the Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit, up to 94 different samples can be assessed in a single 96-well plate. Anticipated results for each sample type are shown (in each fluorophore channel).

Advantages:

- Multiplex detection of 2019-nCoV_N1 and 2019-nCoV_N2 targets and human RNase P gene enables high throughput workflows
- Reduce background amplification from genomic DNA by use of a modified RNase P Internal Control reverse primer to target an exon-exon boundary
- Increase sensitivity with Luna Probe One-Step RT-qPCR 4X Mix with UDG allowing for more sample input
- Compatible with low reaction volumes including 384-well plate formats

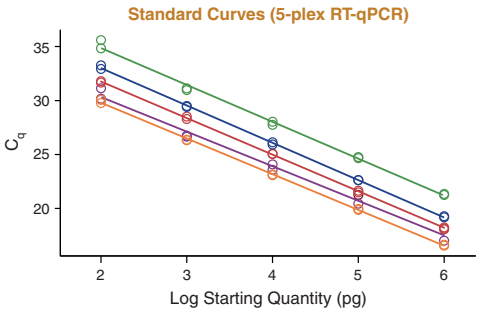
Ordering information:

Product	NEB #	Size
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit	E3019S/L	96/480 rxns

Luna Probe One-Step RT-qPCR 4X Mix with UDG

The Luna Probe One-Step RT-qPCR 4X Mix with UDG supports robust, sensitive detection and quantitation of up to 5 targets in a multiplexed reaction. It is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where RNA present in low abundance is of interest, such as pathogen detection. The Dual WarmStart/Hot Start enzyme formulation enables room temperature setup and stability for up to 24 hours. The single tube master mix format includes thermolabile UDG and dUTP for reduced risk of carryover contamination.

Multiplex detection (5 targets) with the Luna Probe One-Step RT-qPCR 4X Mix with UDG



Target	Fluorophore (channel)	Efficiency	R^2
SDHA	HEX™	96.1%	0.996
SMG1	FAM™	94.7%	0.999
RPL32	Cy®5	97.2%	1.000
GAPDH	Tye™ (Quasar® 705)	105.1%	0.990
ACTIN	Texas Red®	100.5%	0.999

Multiplex RT-qPCR was performed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG over a 5-log range of Jurkat total RNA (100 ng to 10 pg) on a Bio-Rad® CFX96 real-time instrument. Amplification standard curves and efficiencies for each of the 5 human targets are shown. Reactions (20 µl) included primers and probes at 200 nM each, and followed the product recommended cycling conditions. All five targets were detected linearly in the multiplex reactions with strong efficiency and R2 values.

Learn about how the Innovative Genomics Institute (https://innovativegenomics.org/) at University of California, Berkeley has designed a novel SARS-CoV-2 assay using this product. View PDF at https://www.medrxiv.org/content/10.1101/2020.12.10.20247338v1.full.pdf.

Ordering information:

Product	NEB #	Size
Luna Probe One-Step RT-qPCR 4X Mix with UDG	M3019 S/L/X/E	200/500/ 1,000/2,000 reactions

From research to therapeutic production, NEB's *in vitro* transcription portfolio will meet your needs

NEB's portfolio of research-grade and GMP-grade* reagents enables bench-scale to commercial-scale mRNA manufacturing. Our optimized HiScribe™ kits enable convenient *in vitro* transcription (IVT) workflows. When it is time to scale up and optimize reaction components, our standalone reagents are readily available in formats matching our GMP-grade offering, enabling a seamless transition to large-scale therapeutic mRNA manufacturing.

ENABLING GRAM-SCALE RNA SYNTHESIS

NEB manufactures and inventories the following enzyme specificities at GMP-grade, meeting customer needs with short lead times:

Product	NEB #	Feature
Vaccinia Capping Enzyme	M2080S	A full system for enzymatic capping based on the Vaccinia virus Capping Enzyme (VCE)
T7 RNA Polymerase	M0251S/L	RNA Polymerase used for *in vitro* mRNA synthesis, and is highly specific for the T7 phage promoter
mRNA Cap 2′-O-Methyltransferase	M0366S	mRNA Cap 2´-O-Methyltransferase adds a methyl group at the 2′-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA
RNase Inhibitor, Murine	M0314S/L	RNase Inhibitor, Murine, specifically inhibits RNases A, B and C
Pyrophosphatase, Inorganic (*E. coli*)	M0361S/L	Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate
DNase I (RNase-free)	M0303S/L	DNA-specific endonuclease used for removal of contaminating genomic DNA from RNA samples and degradation of DNA templates in transcription reactions
HiScribe T7 High Yield RNA Synthesis Components	E2040S	Separate components available in GMP-grade format

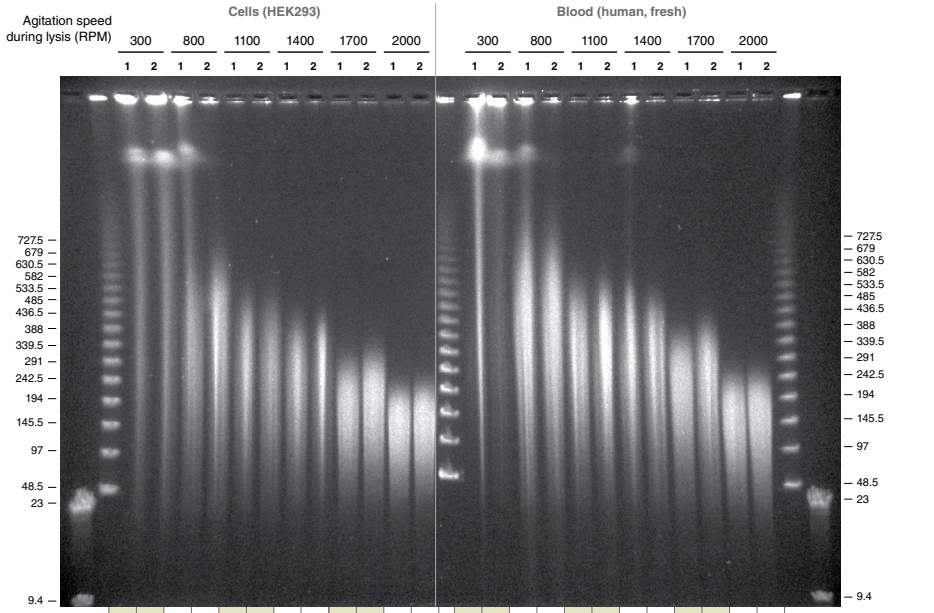
"GMP-grade" is a branding term NEB uses to describe reagents manufactured at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured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

NEW PRODUCTS

Monarch® HMW DNA Extraction Kits

NEB is pleased to announce the release of a powerful new solution for high molecular weight (HMW) DNA extraction. The Monarch HMW DNA Extraction Kits employ a novel glass bead-based approach, allowing users to extract DNA into the megabase (Mb) range quickly and easily, with best-in-class yields and purity from cells, blood, tissue, bacteria and other sample types. Extracted HMW DNA is easy to dissolve and is often ready to use on the same day, significantly shortening the extraction process, which has long been a bottleneck for long read sequencing technologies.

DNA fragment size is tunable based on agitation speed during lysis



	Cells (HEK293)												Blood (human, fresh)											
Yield (µg)	11.3	11.6	11.1	12.1	11.7	12.4	13.2	12.0	12.7	12.9	12.0	13.5	22.5	22.5	22.5	22.2	24.4	26.0	24.7	23.5	24.4	24.9	24.0	25.6
260/280	1.86	1.85	1.87	1.87	1.87	1.87	1.88	1.87	1.87	1.86	1.87	1.87	1.87	1.87	1.87	1.86	1.87	1.86	1.87	1.86	1.86	1.86	1.86	1.86
260/230	2.31	2.28	2.36	2.44	2.39	2.47	2.49	2.50	2.52	2.53	2.48	2.44	2.50	2.57	2.54	2.42	2.40	2.44	2.35	2.48	2.37	2.37	2.46	2.38
DIN	9.7	9.8	9.8	9.8	9.9	9.8	9.8	9.7	9.8	9.8	9.8	9.7	9.8	9.9	9.8	9.9	9.7	9.7	9.8	9.7	9.8	9.8	9.8	9.8

Best-in-class yields and purity

Preps were performed on duplicate aliquots of 1 x 10^6 HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad CHEF-DR III System). Yield anpurity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #T3041 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.

> *"We've had great success with obtaining HMW DNA for long read sequencing from a variety of cell types, using less input and obtaining a comparable yield… It is straightforward and easy to use."*
>
> – Inswasti Cahyani & Matt Loose, DeepSeq, University of Nottingham

> *"Excellent fragment length as per TapeStation, excellent sequencing on Oxford Nanopore Minion. This solved our bottleneck."*
>
> – Dr. Scott Lindner, Pennsylvania State University

Highlights:

- Fast workflows (cells: 30 minutes, blood: 60 minutes, tissue & bacteria: 90 minutes)
- Extract DNA into the megabase (Mb) range from cells, blood, soft organ tissues, and bacteria
- Tune DNA size based on agitation speed during lysis
- Reproducibly achieve excellent yields and purity
- Excellent performance in long read sequencing

Validated sample types:

Monarch HMW DNA Extraction Kit for Tissue:

- Mouse brain
- Mouse liver
- Mouse muscle
- Mouse kidney
- Mouse tail
- Mouse ear punch
- Rat kidney
- *E. coli*
- *B. cereus*
- *M. luteus*
- *X. laevis*
- *S. cerevisiae*
- *C. elegans*
- *A. aegypti*

Monarch HMW DNA Extraction Kit for Cells & Blood:

CELLS

- K293
- HeLa
- NIH3T3
- Jurkat
- K562 (suspension cells)
- HCT116
- A549
- U5Os
- HepG2
- NCI-460
- SK-N-SH
- Aa23

MAMMALIAN BLOOD

- Human
- Mouse
- Rat (fresh only)
- Rabbit
- Pig
- Horse
- Cow
- Rhesus money
- Goat (fresh only)
- Sheep (fresh only)

NUCLEATED BLOOD

- Chicken
- Turkey

For more information, visit
www.neb.com/monarchhmwdnainputs

Ordering information:

Product	NEB #	Size
Monarch HMW DNA Extraction Kit for Tissue	T3060S/L	5/50 preps
Monarch HMW DNA Extraction Kit for Cells & Blood	T3050S/L	5/50 preps

A faster workflow for the assessment of genomic loci in mice using a novel HMW DNA extraction technology upstream of Cas9 targeted sequencing

Simon Lesbirel, Ph.D.[1], Jeremy R. Charette, B.S.[1], Chia-Lin Wei, Ph.D.[1], Eric Cantor, Ph.D.[2], Danielle Freedman, M.S.[2], Giron Koetsier, Ph.D.[2]
1: The Jackson Laboratory, Bar Harbor, ME/Farmington, CT and 2: New England Biolabs, Inc., Ipswich, MA

Introduction

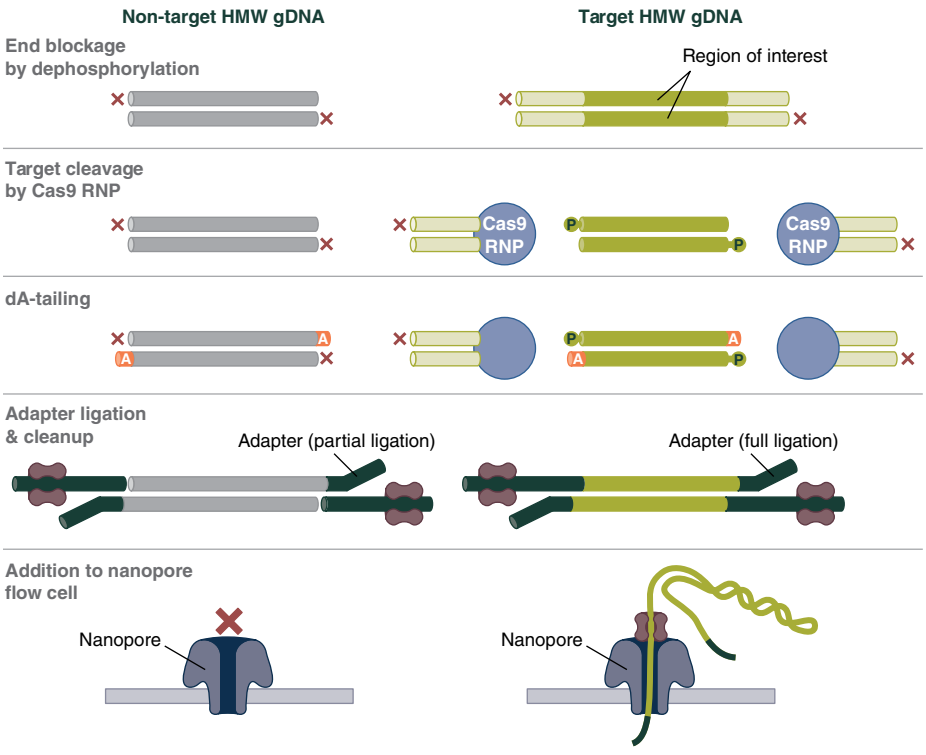
Generation of transgenic mice through random or targeted integration of DNA fragments can lead to structural variation and integration mutagenesis (1), both of which are undesirable outcomes. Due to the significant labor required for their characterization, it is estimated that only around 5% of transgenic mouse models published in the Mouse Genome Database have an annotated chromosomal location (1). Therefore, a technique capable of quickly and cost-effectively identifying chromosomal location and confirming the transgene sequence integrity is essential. Further to this, the interrogation of large loci at the base level between strains remains difficult without using whole genome sequencing. A recently described technique, Cas9 no-amplification enrichment (2), has the potential to fulfill that need.

Traditionally, the genomic modifications required to generate mouse models leverage PCR-based assays and Sanger sequencing for validation. However, in many cases, the structure and the sequence of the gene or its chromosomal integration site hinder analysis by these methods. Loops and sequence repeats prevent effective assessment of DNA sequence. The CRISPR/Cas9-mediated amplification-free enrichment approach for Oxford Nanopore Technologies® sequencing is an alternative method for interrogation of loci of interest or transgene sites. The method is relatively low-cost and can enrich regions of interest over native sequences without the need for PCR amplification (Figure 1).

In standard ligation-based whole genome sequencing approaches, desired loci/transgenes will be sequenced only once or a few times per nanopore sequencing run, but not with enough coverage to collect reliable sequence information. The Cas9 no-amplification enrichment workflow allows for specific enrichment of targeted regions by reducing undesired fragments from the sequencing process via dephosphorylation of their phosphate ends. Lacking terminal 5´ phosphate groups, they do not participate in adapter ligation. The target region, however, is subsequently cleaved using a Cas9-sgRNA (single guide RNA) ribonucleoprotein complex (RNP) making it accessible for sequencing adapter ligation. The resulting libraries allow for enriched sequence generation from the region of interest against a minimal background of genomic DNA sequences typically resulting from off-target Cas9 cleavage and non-specific adapter ligation. Furthermore, multiple sgRNAs can be used to enrich a variety of targets in a single library, thereby increasing efficiency and decreasing cost (3).

At The Jackson Laboratory, one of our priorities has been to establish assays to standardize analysis for routine assessment of genomic alterations such as targeted mutagenesis and transgene integrations. Cas9 enrichment has proven to be an effective approach. Simultaneous Cas9 enrichment analysis of 2 to 4 targeted sequences has now been established as a standard workflow, with targeted regions typically being around 5 kb in size and sometimes up to 30 kb.

Figure 1: **Overview of the Cas9 no-amplification enrichment library prep workflow**



Successful use of the Cas9 enrichment protocol relies on using high-quality, high molecular weight (HMW) gDNA as an input material. Working with the longest possible DNA fragments increases the chance that the entire region of interest remains intact after DNA extraction. The initial Cas9 enrichment sequencing workflow implemented within the Genome Technologies group at The Jackson Laboratory was dependent on phenol/chloroform DNA extraction, which initially fulfilled the requirements. However, while the phenol/chloroform-based workflow is effective for ultralong sequencing, it proved to be laborious and time consuming when applied to the Cas9 enrichment protocol. The sample lysis, phenol extraction and DNA precipitation take approximately one full day. Subsequently, this method requires up to 3 days of "rest time" to allow the isolated HMW gDNA to return to solution, resulting in the whole extraction process taking several days. Accordingly, the Cas9 enrichment can be started around day 5 (Figure 3). In addition, the increased frequency of extractions produced excessive amounts of hazardous waste. Therefore, we sought a faster and more environmentally friendly DNA extraction alternative.

In this work, we leveraged the novel glass bead-based approach employed by the New England Biolabs Monarch® HMW DNA Extraction Kits to significantly reduce the time required for generating HMW gDNA from mouse tissue samples. With this new approach, the HMW DNA extraction process from tissues is complete in about 90 minutes, and DNA is ready to use shortly after, thereby significantly reducing the overall time required to perform the Cas9 enrichment workflow. Yield, purity, and integrity of the isolated HMW DNA is compared to phenol-extracted DNA and its efficient use in the optimized Cas9 sequencing workflow is demonstrated.

Results & Discussion

Comparison of HMW DNA Extraction

The HMW DNA that was used for initial Cas9 enrichment studies was isolated using phenol/chloroform extraction. For later studies, the Monarch HMW DNA Extraction Kit for Tissue was introduced. Yield and purity data are shown for DNA extracted from liver and kidney samples with both methods (Table 1, page 10)

For phenol-extracted samples, spectrophotometric readings revealed that around 1.6 µg DNA per mg tissue was isolated for liver and 4-6 µg per mg tissue for kidney. However, Qubit values (data not shown) were significantly lower, particularly for the liver

Continued on page 10...

TABLE 1: **DNA Extraction with phenol/chloroform and the Monarch HMW DNA Extraction Kit**

HMW DNA samples purified with either phenol/chloroform or the Monarch kit were analyzed on a Nanodrop 2000 to determine the concentration and purity ratios, and to determine yield per mg. DNA samples from kidney purified with the Monarch kit using 500 rpm agitation speed during were bound for 8 minutes on the rotator.

Sample	Sample and Input Amount (Lysis Agitation)	Yield Per mg (µg)	A_{260}/A_{280} and A_{260}/A_{230}
Phenol/ Chloroform	A–Liver, 15 mg	1.9	1.85, 1.33
	B–Liver, 15 mg	1.4	1.84, 1.12
	C–Kidney, 20 mg	5.9	1.83, 2.08
	D–Kidney, 20 mg	4.2	1.86, 1.77
	E–Kidney, 20 mg	3.8	1.85, 2.07
Monarch	4–Liver, 15 mg (1700 rpm)	1.4	1.86, 2.27
	5–Liver, 10 mg (500 rpm)	1.3	1.84, 2.14
	6–Kidney, 17 mg (2000 rpm)	2	1.85, 2.27
	7–Kidney, 20 mg (2000 rpm)	2.7	1.83, 2.25
	10–Kidney, 12 mg (500 rpm)	1.4	1.85, 2.35
	11–Kidney, 10 mg (500 rpm)	1.9	1.84, 2.36
	12–Kidney, 10 mg (500 rpm)	2.1	1.85, 2.34

samples. Purity grade was intermediate; though A_{260}/A_{280} purity ratios were in the normal range (1.83-1.85), the A_{260}/A_{230} ratios were lower than optimal, with 1.8-2.1 for kidney and only 1.1-1.3 for liver.

For Monarch-extracted samples, spectrophotometric measurements revealed that good yields were obtained for both liver and kidney, with around 1.4 µg DNA per mg tissue for liver and 2-3 µg per mg tissue for kidney. Consistent high purity was observed among all extracted samples as A_{260}/A_{280} purity ratios were greater than 1.83 and A_{260}/A_{230} ratios were >2.1.

Tapestation analysis was carried out using the Genomic DNA Screen Tapes. Densitometric region analysis revealed that the portion of DNA >50 kb for the Monarch-extracted DNA was 81% while that of phenol-chlrorofom-extracted DNA was 69% (data not shown).

Cas9-enrichment for assessment of transgene insertion sites

The efficiency of the Cas9 enrichment approach is demonstrated in Figure 2, showing analysis of Sample 5 (homozygous 30 kb insert) and Sample 4 (heterozygous 5 kb insert). Sample 5 contains a large insert and delivered 44X coverage over the region of interest. In Samples 4 and 9 (another mouse line with a 5 kb transgenic insert, data not shown), the Cas9 enrichment approach was used to check the integrity of the insertion site as well as the transgene sequence. The sgRNAs were designed 1 kb up and downstream of the transgene, and the enrichment approach worked well with a 92X and 74X coverage, respectively. A slight overrepresentation of the shorter allele not containing the transgene was observed.

Sample 6 and 7 (both heterozygous 5 kb inserts) proved to be difficult targets; even with the Cas9 enrichment approach, it was challenging to generate reads (data not shown). Monarch HMW DNA extraction was repeated with larger input amounts and lysis was carried out at maximal agitation speed (2000 rpm) to reduce the size of the HMW DNA fragments and the accompanying viscosity. This modification enabled 20X/23X coverage of the region of interest, respectively, sufficient for our purposes. This example provides a compelling case of how having access to a rapid extraction method enables troubleshooting experiments without significant time loss.

FIGURE 2: **Analysis of 30 kb homozygous transgene insertion and 5 kb heterozygous transgene insertions**

(A) Sample 5: Homozygous 30 kb insert; coverage across the region of interest was 44X. (B) Sample 4: a mouse line with a 5 kb heterozygous insertion, coverage depth of 92x. Note: mean coverage depth does not include endogenous chromosomal reads located at the 5´ and 3´ ends of the transgenic insertion.

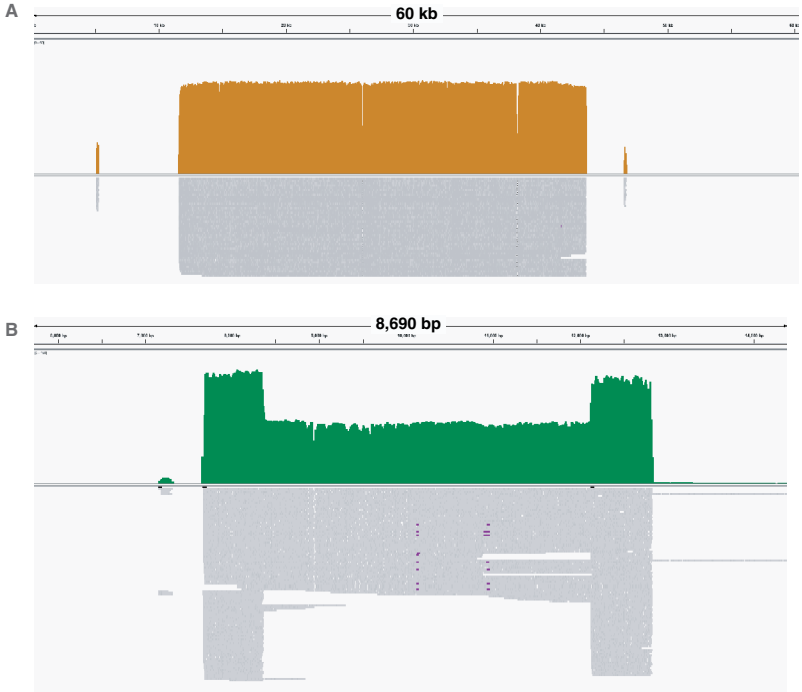
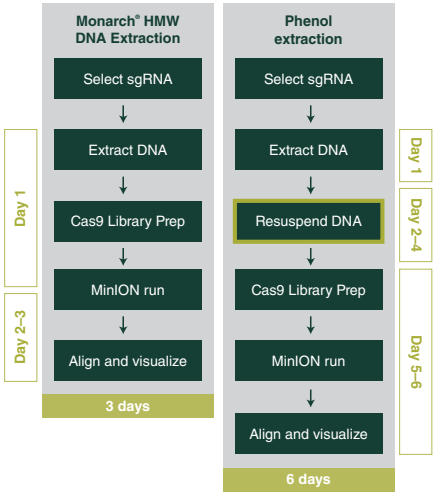


FIGURE 3: **Comparing the Cas9 sequencing workflow duration for phenol/chloroform and Monarch HMW DNA extraction**



Conclusion

Until recently, Cas9 enrichment workflows have been described using phenol/chloroform extracted HMW DNA when working with animal tissues. The drawbacks of this extraction approach are numerous, including significant handling, hazardous chemicals, and excessive time required for dissolving. Because this method requires several days to obtain usable DNA, it is not conducive to rapid transgene/strain analysis and limits the flexibility needed for troubleshooting or tweaking of parameters.

The new Monarch HMW DNA extraction workflow provided DNA with high yields, high purity, and high DNA integrity which was ready to use in only a few hours. The Monarch-extracted DNA performed well in Cas9 sequencing and resulted in a significant time savings in the Cas9 enrichment sequencing workflow of up to 3 days (Figure 3). The Monarch workflow also enables tunable fragment length generation by having the user change the agitation speed of the thermal mixer during lysis, empowering the user to adjust the size of the DNA to optimize conditions for the relevant downstream application. Overall, the Cas9 enrichment approach is a powerful tool for interrogation of genomic loci. Having a rapid high-quality method like Monarch for HMW DNA extraction enables a significant reduction in the workflow time and facilitates troubleshooting efforts without adding several days of work.

The full technical note including methods and data can be downloaded by visiting the "Protocols, Manuals & Usage" tab at www.neb.com/T3050 and www.neb.com/T3060.

References:
1. Goodwin, L.O. et al. (2019) Genome Research. https://doi.org/10.1101/gr.233866.117.
2. Gilpatrick, T. et al. (2020) Nature Biotechnology 38, 433–438.
3. Oxford Nanopore Technologies (2020). "Cas9 Targeted Sequencing Before Start Checklist Cas9 Targeted Sequencing," 1–6.

Additional COVID-19 Resources

COVID-19 Researcher Spotlight: mRNA Vaccine Development

Bijoyita Roy, Ph.D.
NEB Senior Scientist

In our recent COVID-19 Researcher Spotlight, Lydia Morrison chatted with NEB Senior Scientist Bijoyita Roy about the landscape and timeline of COVID-19 vaccine development, focusing on the mRNA vaccine platform and how NEB is helping improve mRNA vaccine development and production.

You can read an excerpt from our interview with Bijoyita below. You can access the COVID-19 Researcher Spotlight Series at www.neb.com/COVID19 to watch the entire interview and to learn about two of the vaccine candidates that were recently approved by the FDA.

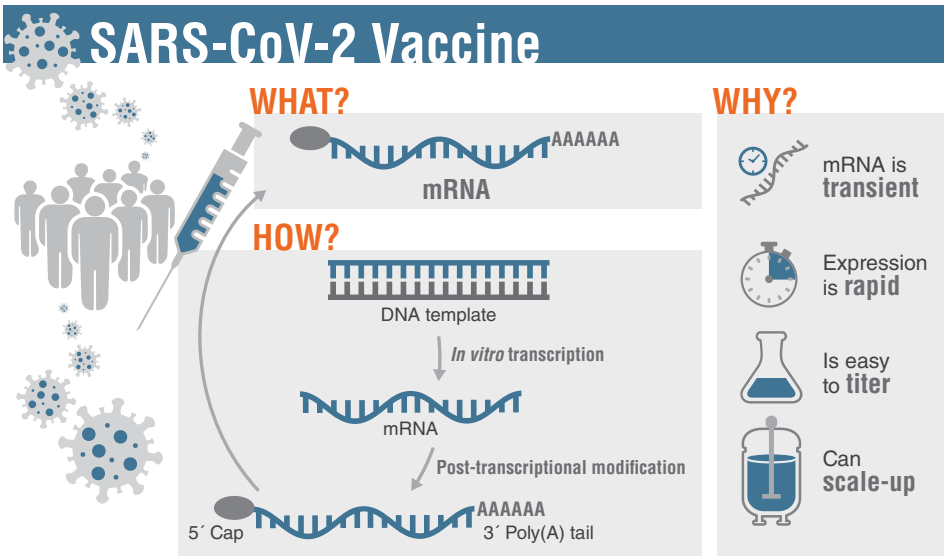
Lydia Morrison: mRNA vaccines are relatively new. What are the advantages of that platform?

Bijoyita Roy: Messenger RNA is transient in expression, and its expression is rapid. It's easy to titer and it can also be synthesized, standardized and easily scaled. So, these are some of the most prominent advantages of the mRNA based platform. Now, mRNA based vaccines are emerging as an alternative to conventional vaccine approaches. So, the idea here is really simple. You make an RNA or a messenger RNA in a tube, and you introduce it into a cell to hijack the cells machinery, to make any protein you are interested in. And once it is delivered in the cell, it gets translated by the cellular machinery resulting in the synthesis of the protein antigens. Now these antigens are then recognized by the immune system and you see immune responses. So, what's happening here is, the cell itself acts as a bioreactor to make any protein of interest.

So all you really need is the DNA sequence to make the RNA that you are interested in. So, you use the same process for any protein, and that makes this entire platform really lucrative, and it streamlines a lot of early development and discovery work. As an example, once the sequence of SARS-CoV-2 genome was released, the DNA sequence of interest that could potentially be used as a vaccine target was generated in a matter of few days, and the mRNA molecule that could actually encode for the spike protein was actually synthesized in a matter of weeks.

Lydia Morrison: So what are the similarities and differences between the two FDA approved vaccines?

Bijoyita Roy: The main similarity is that both of these vaccines contain the genetic instructions for building a specific Coronavirus protein, the spike protein. So, when injected into the cell the vaccine causes them to make spike proteins, which then gets released into the body, and it involves an immune response from the immune system. What is really interesting is that for the two independent vaccines, the mRNA sequence is very different, but the mechanism of action for both the Pfizer – BioNTech, as well as the Moderna vaccines, is exactly the same and they are showing similar efficacy.



Doing COVID-19 related research?

Let us know what you are working on, so we can keep you informed on new COVID-19 related products and publications available from NEB.

Visit www.neb.com/COVID19

Upcoming episode:

Bas Oude Munnink

Lydia Morrison and Senior Application Scientist, Bjoern Textor of NEB Germany, will talk to **Bas Oude Munnink** of Erasmus MC in the Netherlands about the impact of multiple circulating strains of SARS-CoV-2 and what he's learned from his work on COVID-19 that could aid in identifying and stopping outbreaks of COVID-19 or other pathogens in the future.

Getting back to science

We hope that you and your families are in good health. Thank you to those who have switched focus to COVID-19 related projects – we applaud your efforts and will do our very best to support you. For those returning to the lab, we want to help you quickly get back to the important science that you do, and have prepared some tips and tools to help you Reboot Your Bench.

www.neb.com/RebootYourBench

NEW ENGLAND BioLabs® Inc.

240 County Road, Ipswich, MA 01938-2723

LOCATIONS

USA
New England Biolabs, Inc.
Telephone: (978) 927-5054
Toll Free: (U.S. Orders) 1-800-632-5227
Toll Free: (U.S. 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

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

United Kingdom
New England Biolabs (UK), Ltd.
Call Free 0800 318486
info.uk@neb.com

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