NEB EXPRESSIONS

A scientific update

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SCIENCE

INNOVATION

EDUCATION

NEW ENGLAND Biolabs®

REDEFINING NGS LIBRARY PREP FOR THE REAL WORLD:

Streamlining for Speed and Savings

Photo credit: The Koues lab

How NEBNext UltraExpress® helps address key challenges at a University Core Genomics Center

By Betsy Young, Ph.D., Senior Product Marketing Manager for Next Generation Sequencing, New England Biolabs

In today's rapidly evolving genomics landscape, core facilities and research groups often need to adapt to evolving demands – such as managing high sample volumes or accelerating experiments to generate sequencing data more quickly. Genomics is a field where technological advancements continuously and rapidly reshape the landscape, necessitating faster and better reagents for faster and better libraries. This is the origin story of the NEBNext UltraExpress library prep kits for DNA, FS DNA, and RNA – innovative solutions designed to meet sequencing demands with unparalleled speed, accuracy and efficiency. These kits offer a fast workflow, single-condition setup for adaptor concentration and PCR cycle number, minimal hands-on time, and reduced consumables usage and costs. NEBNext UltraExpress is truly streamlined for speed.

CHALLENGING TO THE CORE

At the University of Michigan's Advanced Genomics Core, the sample variety is astounding – from reptile skin from the Museum of Zoology, to surgical samples from the University Medical Center. All demand the same attention to detail and high-quality treatment to transform them into genomic insights. Dr. Olivia Koues, Director of the Advanced Genomics Core, is well acquainted with the challenges of building processes to support the diverse needs of genomic research, where adaptability is crucial. "It's never the same from day to day. It feels like there's always something new coming out, so if you don't like change, it's a problem in my facility because we are constantly evolving. We're trying to stay not just cutting edge but a leader in those fields."

Given the variety and volume of samples processed at Dr. Koues' facility, the streamlined efficiency of the NEBNext UltraExpress kits becomes even more important. Whether in a core laboratory like the Advanced Genomics Core, which serves as a shared technology resource providing guidance, expertise and services across the organization, or in individual research labs seeking to optimize their sequencing workflows, the adaptability of the NEBNext UltraExpress kits is invaluable. Dr. Koues explains, "We're a large facility, and the samples we receive run the gamut of all the research done at the University of Michigan, which is a large institution. Everything from bulk RNA and DNA to single-cell and spatial tissue blocks and cell suspensions."

THE NEED FOR SPEED

Time is critical in any laboratory experiment, and in the Koues lab the number and variety of samples must be quickly and meticulously prepared before sequencing. The NEBNext UltraExpress library prep kits address this challenge head-on with their short workflow times. For example, the NEBNext UltraExpress RNA library prep workflow has transformed what was once a multi-day process into a single-day protocol. This significantly enhances lab productivity, allowing more samples to be processed and sequenced in a shorter timeframe.

The workflow times for each of the NEBNext UltraExpress kits are as follows:

- **NEBNext UltraExpress DNA Library Prep Kit: 1.8 hours**
- **NEBNext UltraExpress FS DNA Library Prep Kit: 1.75 hours**
- **NEBNext UltraExpress RNA Library Prep Kit: 3 hours**

For Dr. Koues, the flexibility and speed of NEBNext UltraExpress initially caught her attention. "When we consider new kits and methods that we're going to adopt, we try to pick and choose based on what we think can accommodate the most samples. We're constantly trying to streamline and make things efficient because we try to get people the best data possible as quickly as possible." The projects submitted to the sequencing core may be large or small, but resources are always in demand, and every project is important. Setting expectations for data delivery across the range of projects can be challenging, but the scientists relying on the data have no time to waste.

"The time and robustness of the kit are key because we only have a couple of technicians. Those technicians are prepping all our libraries, not just the bulk RNA or the bulk DNA projects, but they're doing the single-cell library preps, the spatial library preps - anything that feeds onto a sequencer goes through the hands of these two individuals". Continues Dr. Koues, "Larger projects that come in can be a challenge too. Shorter workflows mean we can prep more samples and turn them around quickly. As the sequencing platforms increase in throughput, we can sequence more samples at once, and we're able to keep that four-week turnaround time for almost all projects." By combining rapid turnaround and reliable performance, NEBNext UltraExpress helps the University of Michigan Advanced Genomics Core to consistently meet tight deadlines while delivering excellent data quality.

A SINGLE-CONDITION WORKFLOW

Speed alone speaks to both research and core labs engaged in high-throughput NGS, but simplicity is another feature most users can appreciate. One of the stand-out features of the NEBNext UltraExpress kits is the single-condition workflow with providing a universal protocol that works across a wide range of input masses, sample types, and downstream applications. By removing the requirement for individually adjusted adaptor concentrations and fine-tuned PCR cycle numbers, NEBNext UltraExpress works for most samples within the kit's stated input range:

- **NEBNext UltraExpress DNA Library Prep Kit: 10 – 200 ng pre-sheared DNA**
- **NEBNext UltraExpress FS DNA Library Prep Kit: 10 – 200 ng intact DNA**
- **NEBNext UltraExpress RNA Library Prep Kit: 25 – 250 ng total RNA**

PERFORMANCE WITHOUT COMPROMISE

While the speed and flexibility were initial draws for Dr. Koues to test the NEBNext UltraExpress Kits, the consistent data quality ultimately made the difference, especially for challenging sample types that can sometimes require extensive work due to suboptimal performance. This is a significant challenge at the University of Michigan Advanced Genomics Core, tasked with the acceptance of samples derived from a broad spectrum of RNA extraction methods, which can result in inconsistent quality of RNA. "We don't control how extractions are performed externally, and not all samples come to us DNase-treated or of high quality... I can't control those metrics. Kits that handle contaminants are very important to us, as well as those that can accommodate a range of inputs," explains Dr. Koues. The Core often deals with what are termed 'fringe' or 'marginal' samples - those samples that others might reject based on not meeting acceptable input amounts and sample quality. It is here that the NEBNext UltraExpress kits have truly made a difference, enhancing outcomes for challenging samples. Dr. Koues said, "It's really helped what we would consider lower input, low-quality samples that have been challenging. We've found that the kits are performing better than anything we've tried in the past, minimizing cleanups, re-preps, or random failures."

Figure 1: **NEBNext UltraExpress workflows are designed for speed**

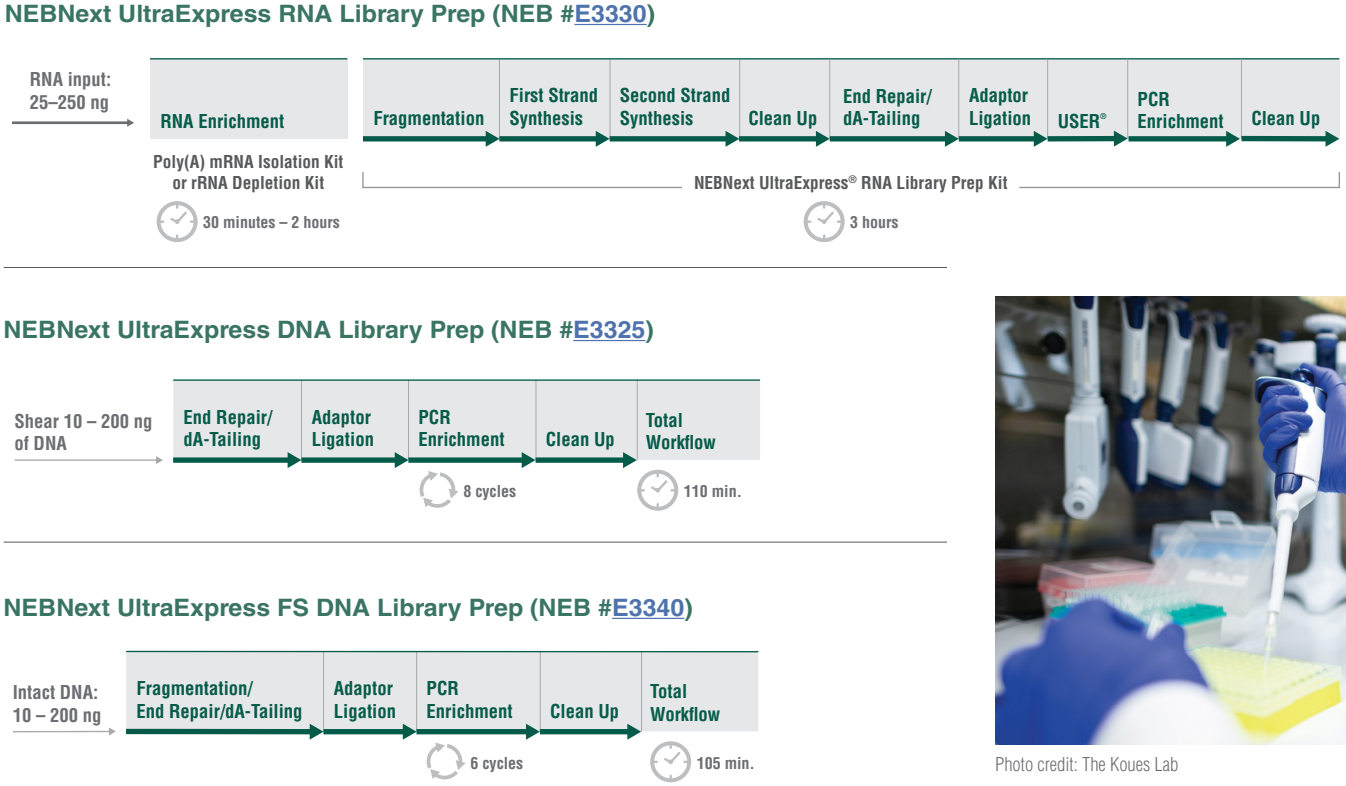


Photo credit: The Koues Lab

USING FEWER CONSUMABLES

In addition to the technical advantages of the NEBNext UltraExpress kits, there is a significant reduction in plastic usage, benefiting a lab's bottom line and its ecological impact (Figure 2). These kits reduce the overall number of tips and tubes by streamlining the workflow and minimizing the number of components required. Whether you're motivated by the cost savings of needing to purchase fewer tips and tubes, or you're inspired by a more ecologically friendly library prep kit, NEBNext UltraExpress saves.

HAPPY CUSTOMERS, HAPPY LAB

Implementing the NEBNext UltraExpress Kits marked a significant improvement at the University of Michigan Advanced Genomics Core. Dr. Koues reflects on the transition: "I think there was a lot of buy-in once they ran it and we didn't have some of the issues that we've been having in the past with fall-out samples or excess adaptor dimer - I can't stress that enough, that's the bane of my lab…when you do 96 samples or more, and then QC results come back and a third of them look like junk. Then we have to clean them up, or maybe we have to fail them or re-prep them. That makes work challenging because you're a little disheartened at the end of that process, especially if it happens over and over again."

Keeping both the scientific community of the University of Michigan and the 28 core lab staff members satisfied and motivated is a priority for Dr. Koues. The UltraExpress Kits are playing an important role. "The staff are really happy with it right now and I'm not the one doing the hands-on work. I'm glad that I'm not getting a lot of complaints. People don't like change, but we've been running this since it launched. We made the switch pretty quickly because it fixed a lot of our issues."

NEBNext UltraExpress library prep kits for DNA, FS DNA, and RNA represent a leap forward in speed and efficiency. As genomics continues to evolve towards faster sequencing and data interpretation, and researchers ask more questions about the genomic underpinnings of health and disease in a range of organisms, NEB and the NEBNext UltraExpress kits ensure they have the support they need to answer them faster and with lower resource expenditure than ever before.

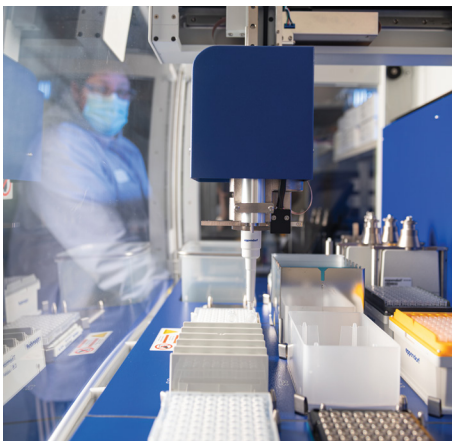
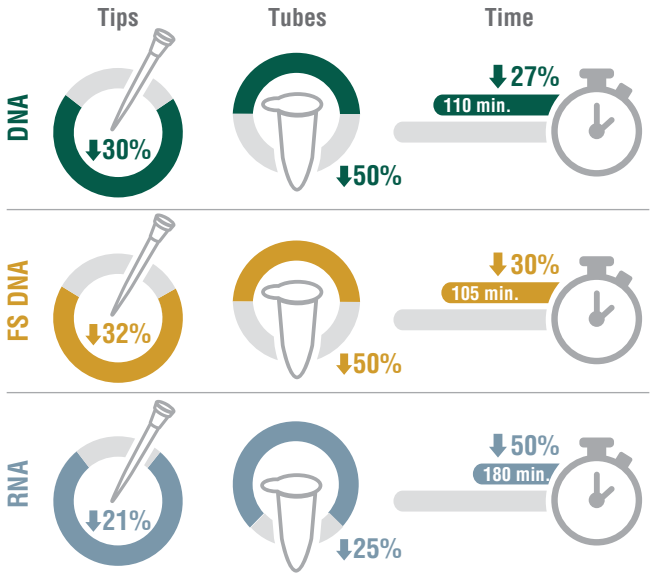


Photo credit: The Koues Lab

Figure 2: **Savings with NEBNext UltraExpress***



* As compared to NEBNext® Ultra™ II

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext UltraExpress DNA Library Prep Kit	E3325S/L	24/96 rxns
NEBNext UltraExpress FS DNA Library Prep Kit	E3340S/L	24/96 rxns
NEBNext UltraExpress RNA Library Prep Kit	E3330S/L	24/96 rxns

5 Reasons to choose NEBNext UltraExpress

1. Fast workflow
2. High-quality libraries from a wide input range
3. Single protocol for all input amounts
4. Fewer steps, consumables, and clean ups
5. Automation friendly

To explore the full NEBNext portfolio, visit

www.NEBNext.com

Featured product:

NEBNext® Enzymatic Methyl-seq v2 Kit

NEBNext Enzymatic Methyl-seq (EM-seq™) is a high-performance enzyme-based alternative to bisulfite conversion for the identification of 5mC and 5hmC. Unlike bisulfite conversion, this highly efficient method minimizes DNA damage, resulting in superior detection of methylated cytosines, with fewer sequencing reads.

The new NEBNext Enzymatic Methyl-seq v2 Kit expands upon the capabilities of the original EM-seq kit (NEB #E7170), offering a wider in input range (0.1 ng - 200 ng), with improved efficiency, sensitivity and accuracy.

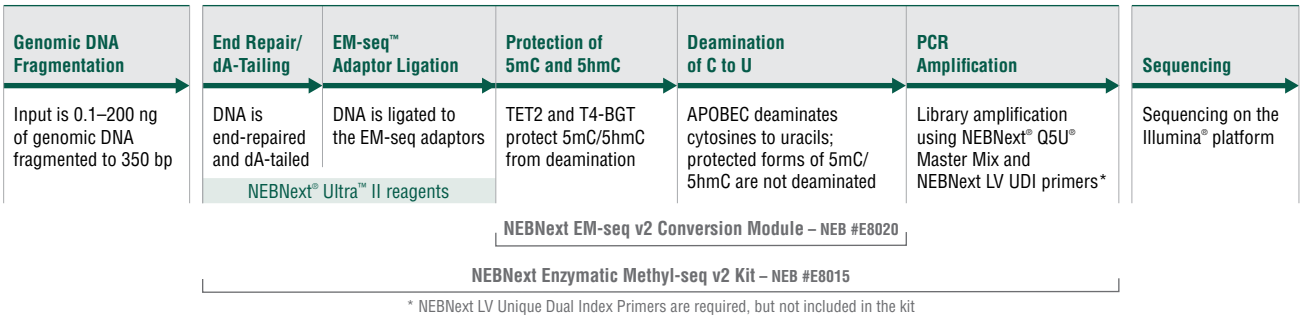
Learn more at: **www.neb.com/E8015**

Superior Performance when compared to bisulfite sequencing

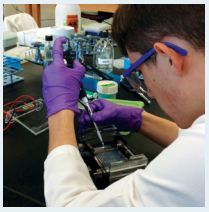
- Utilize a gentle enzymatic approach to identify methylated cytosines at the single-base level
- Experience greater mapping efficiency
- Generate more uniform GC coverage
- Detect more CpGs with fewer sequence reads
- High-efficiency library preparation, with larger library insert sizes

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext Enzymatic Methyl-seq v2 Kit	E8015S/L	24/96 rxns



* NEBNext LV Unique Dual Index Primers are required, but not included in the kit



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This intensive two-week lecture and laboratory course requires no previous experience in molecular biology. Forty participants will be selected from a variety of disciplines and academic backgrounds, including principal investigators, medical doctors, research scientists, postdoctoral fellows, graduate students, research assistants, sales associates, equipment engineers, patent attorneys, etc. Applications will be accepted on a first-come, first-served basis.

Learn more and apply today!

TOPICS/TECHNIQUES

- Gene cloning (genomic and cDNA)
- DNA, RNA and protein isolation and purification
- DNA/cDNA library construction
- Restriction enzyme digestion and DNA ligation
- PCR and real-time quantitative RT-PCR
- Gene expression analysis
- Protein expression systems
- Introduction to bioinformatics (no coding)
- Next Generation Sequencing (DNA and RNA)
- CRISPR/Cas9 and genome editing

COURSE INFORMATION

Date: July 13 – July 26, 2025.
Application deadline: June 1, 2025 (applicants are accepted on a first-come, first-served basis).

Tuition: The fee for tuition, room and board for two weeks is $4800 if paid by June 1, 2025. After June 1, a late fee of $300 will be added.

Location: Clark Science Center, Smith College, Northampton, MA

Facilitating Purification and Detection of Viral Nucleic Acids from Milk

Burcu B. Minsky, Ph.D., Anagha Kadam, Ph.D., Nathan A. Tanner, Ph.D., Eric Cantor, Ph.D., Gregory C. Patton, Ph.D., New England Biolabs

INTRODUCTION

Outbreaks of Highly Pathogenic Avian Influenza (HPAI) A (H5N1) have been reported worldwide in wild birds and poultry since 2022, with incidences of sporadic spillover into mammalian species (1-5). On March 25th, 2024, a multi-state outbreak of HPAI A (H5N1) bird flu in dairy cows was first reported in the United States with 46 herds in nine states reported infected as of May 14, 2024 (6,7). The spread of H5N1 in cattle poses a health risk to the animals as well as our agricultural workforce that has occupational exposure to livestock. The CDC, FDA and USDA have provided situation updates and measures to limit the impact of H5N1 spread including implementation of surveillance systems, guidance for H5N1 testing in cattle, and safety assessment of milk and dairy products available for public consumption (8).

In the Testing Guidance for Influenza A in Livestock (Version.2024.05.01), the USDA recommends using milk and nasal swabs for H5N1 testing in cows (9). Additionally, initial reports by the FDA on April 25th, 2024, indicated that 1 in 5 retail milk samples tested positive for HPAI viral fragments (10). In this evolving scenario, broader testing of milk samples and convenient research tools are needed to better understand this public health challenge.

Here we demonstrate use of milk as a sample type. Viral nucleic acid was extracted from milk containing inactivated Influenza A and SARS-CoV-2 viruses using NEB's Monarch® Mag Viral DNA/RNA Extraction Kit (NEB #T4010) and subsequently amplified using RT-qPCR or RT-LAMP. Additionally, we demonstrate the tolerance of milk with NEB's Luna® RT-qPCR and RT-LAMP reagents to support direct detection assays.

Considering the current supply challenges of H5N1 test material, this app note focuses on detecting Influenza A and SARS-CoV-2 as a representative proxy input.

RESULTS

Milk is a compatible sample type for Monarch Mag Viral DNA/RNA Extraction Kit (NEB #T4010)

The Monarch Mag Viral DNA/RNA Extraction Kit provides a simple, magnetic bead-based workflow for extracting viral nucleic acids. The procedure employs an optimized buffer chemistry in a lyse-bind-wash-elute workflow which has been previously demonstrated to be compatible with swabs, saliva and wastewater samples. Here we report that the Monarch Mag Viral DNA/RNA Extraction Kit protocol can be used to extract viral nucleic acids from milk using manual and automated workflows.

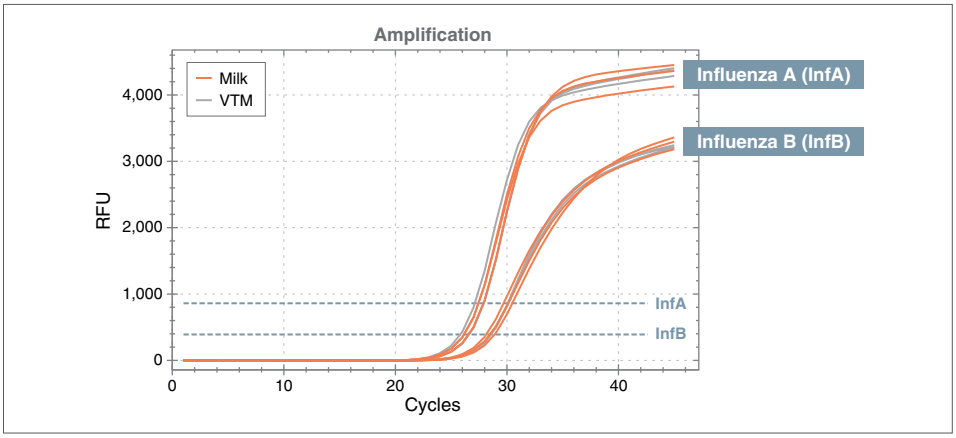
To simulate infected milk, mock samples were created by spiking store-bought pasteurized whole milk with known inactivated viruses and used as the starting point for extraction. Given the complex composition of milk in terms of high protein and fat content, different points of optimization were explored, including phase separation, additional Proteinase K treatment and other workflow modifications. Here we present an optimized manual and automated protocol for extracting viral nucleic acids from milk.

Manual protocol for extraction of viral nucleic acids from milk

Our recommendation for manual extraction for viral nucleic acids from milk follows the current kit protocol provided on www.neb.com: Protocol for Manual Isolation of Viral DNA/RNA in Microfuge Tubes (1.5 or 2.0 ml).

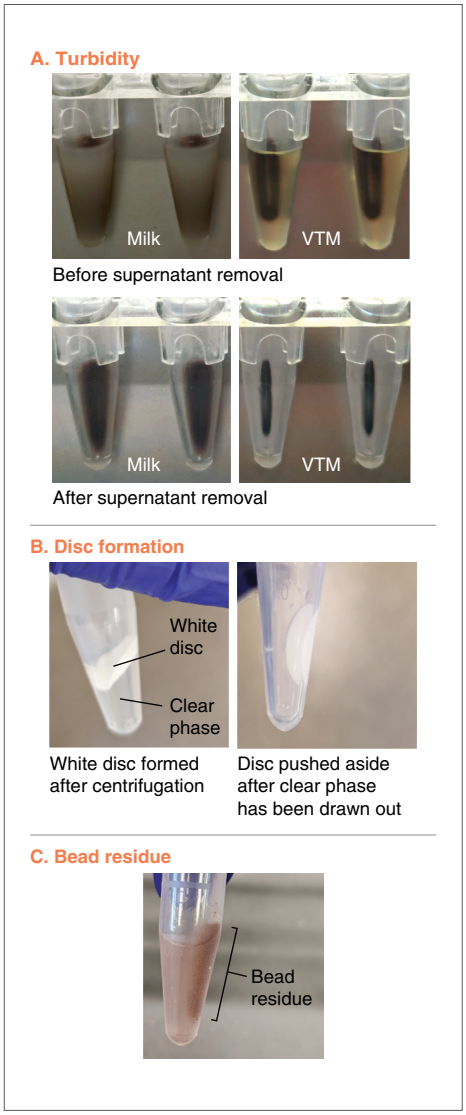
We evaluated this protocol using virus-spiked milk and Viral Transport Medium (Hardy Diagnostics®) samples spiked with swabs containing inactivated Influenza A, Influenza B, Respiratory Syncytial Virus and SARS-CoV-2 (Microbiologics®, Cat 8246). After extraction, the eluates were subjected to RT-qPCR using NEB's Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) with primers/probes targeting Influenza A and Influenza B, as described in CDC's influenza SARS-CoV-2 (Flu SC2) multiplex assay granted an Emergency Use Authorization (EUA) in 2020 (11). Results from the RT-qPCR indicate that the Monarch Mag Viral DNA/RNA Extraction Kit enables successful extraction of viral nucleic acids from milk, with no Cq delay in the mock samples compared to the VTM controls (Figure 1).

FIGURE 1: **Milk matrix is not inhibitory to extraction of viral nucleic acids**



RT-qPCR amplification curves showing detection of InfA and InfB from viral RNA extracted using Monarch Mag Viral DNA/RNA Extraction Kit from virus-spiked milk (orange) and virus-spiked VTM (gray) samples. Milk and VTM were spiked with swabs containing inactivated Influenza A, Influenza B, Respiratory Syncytial Virus and SARS-CoV-2 and RT-qPCR was performed using Luna Probe One-Step RT-qPCR 4X Mix with UDG in a 20 µL reaction with CDC InfA (FAM) and InfB (HEX) primers/probes on a Bio-Rad® CFX-96 qPCR instrument. Dotted light blue lines represent the instrument-defined threshold for InfB (HEX) and InfA (FAM).

FIGURE 2: **Practical observations when processing milk samples with the Monarch Mag Viral DNA/ RNA Extraction Kit**



Useful guidance for milk extraction

Below we summarize some observations specific to milk as a sample type and helpful tips to achieve optimal results.

1. The sample will appear in two phases when the lysis buffer bead mix is added. At this point, vortex the sample thoroughly to ensure the even dispersion of reagents within the sample.

2. During the steps on the magnet, the milk samples may retain a level of turbidity. This does not impact the magnetic separation and bead-free supernatant can still be successfully removed after the magnetic separation is complete (Figure 2A).

3. In our testing, centrifugation of milk did not improve end results and is therefore not recommended. Milk samples are directly compatible with our extraction kit. If, however, centrifugation of milk is strongly desired, we suggest 16000 x g for two minutes at room temperature, and proceeding with the clear phase in the next steps, after carefully pushing the top layer (disc) aside (Figure 2B).

4. Milk sample tubes may appear to have a bead residue along the tube wall, even at stages away from the magnet (Figure 2C). Vortex vigorously to resuspend the beads as much as possible. Vortexing at the elution step removes the bead residue and does not have an impact on performance.

5. Based on our testing, additional Proteinase K or higher incubation temperatures for protein digestion were not helpful. Therefore, we recommend performing the Proteinase K incubation step at room temperature with 5 µl of Proteinase K per sample as outlined in the protocol.

FEATURED WEBINAR

In our recent webinar, we discussed the full workflow for detecting viral particles from milk, demonstrating the efficiency of our Monarch Mag Viral DNA/ RNA Extraction Kit and Luna RT-qPCR, RT-LAMP reagents. These tools are designed to integrate seamlessly into existing testing protocols, offering rapid and accurate results crucial for managing such outbreaks.

Access the webinar on-demand at: **www.neb.com/webinar/neb-tv-webinar-series**

Ordering Information

PRODUCT	NEB #	SIZE
Monarch Mag Viral DNA/RNA Extraction Kit	T4010S/L/X	100/600/1800 preps

Scan code to access the complete application note and read about the automated workflows and viral detection in RT-qPCR & RT-LAMP assays

References

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4. Baechlein, C., et al. (2023) *Emerg. Infect. Dis.* 29(12), 2509.
5. Burrough, E., et al. (2024) *Emerg. Infect. Dis.* 30(7).
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9. *Testing Guidance for Influenza A in Livestock* (2024) U.S. Department of Agriculture, Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/sites/default/files/hpai-livestock-testing-recommendations.pdf
10. *Updates on Highly Pathogenic Avian Influenza* (2024) U.S. Food & Drug Administration. https://www.fda.gov/food/alerts-advisories-safety-information/updates-highly-pathogenic-avian-influenza-hpai

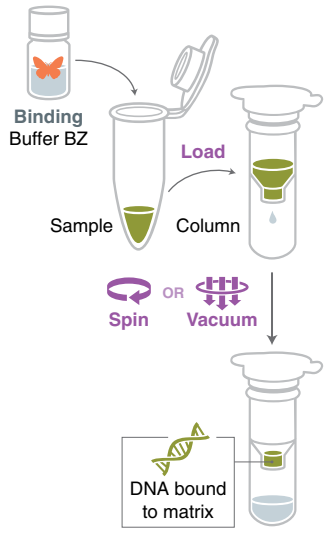
Essential Tips for PCR & DNA Cleanup: Maximizing DNA Purity and Yield

Purifying DNA after PCR amplification or enzymatic reactions is a critical step in molecular biology workflows. Removing enzymes, primers, nucleotides, and buffer components ensures your DNA is ready for downstream applications like cloning, sequencing or labeling. While PCR cleanup protocols are generally straightforward, following best practices can significantly enhance the yield and purity of your DNA. This technique is simple and speedy with our Monarch® Spin PCR & DNA Cleanup Kit (NEB #T1130). The kit delivers best-in-class DNA cleanup with less plastic waste. Here are some expert tips and tricks for each step of the DNA cleanup process: Bind, Wash, and Elute.

STEP	DO ✓	DON'T ✗

BIND your DNA effectively

Efficient binding of DNA to the purification matrix is crucial for high recovery rates. This step ensures that your DNA is securely attached to the column matrix, allowing impurities to be washed away in subsequent steps.



1. **Monitor sample volume and DNA quantity:**
   Sample volume: A starting volume of 20–100 µl is recommended. If your sample volume is less than 20 µl, adjust it by adding nuclease-free water or TE buffer.
   DNA quantity: Do not exceed the column's binding capacity (e.g., 5 µg for the Monarch Spin Column S1A provided with Monarch Spin PCR & DNA Cleanup Kit). Overloading can lead to poor binding and lower yields.
2. **Use the correct binding buffer:**
   Use the binding buffer supplied with your kit, such as the Monarch Buffer BZ (supplied with the Monarch Spin PCR & DNA Cleanup Kit), which is optimized for efficient DNA binding without the need to monitor pH.
3. **Ensure proper mixing:**
   Thoroughly mix your sample with the binding buffer to facilitate optimal interaction between the DNA and the matrix.
4. **Adjust for small DNA fragments:**
   If purifying small DNA fragments (50 bp) or oligonucleotides, modify the protocol by adding additional alcohol to the sample before binding, increasing the binding efficiency for small DNA. With Monarch Spin PCR & DNA Cleanup Kit, a separate protocol is provided for small DNA or oligonucleotide cleanup.

1. **Overload the column:**
   Exceeding the maximum DNA capacity can result in inefficient binding and reduced yield. If you have more DNA, split the sample across multiple columns.
2. **Skip incubation times:**
   Allow sufficient time for the DNA to bind to the matrix per the protocol. Rushing this step can decrease binding efficiency.
3. **Use incompatible buffers**:
   Avoid using binding buffers not specified for your kit, as they may not support optimal DNA binding.

ADDITIONAL TIPS FOR OPTIMAL DNA PURIFICATION

Monitor DNA Quality:

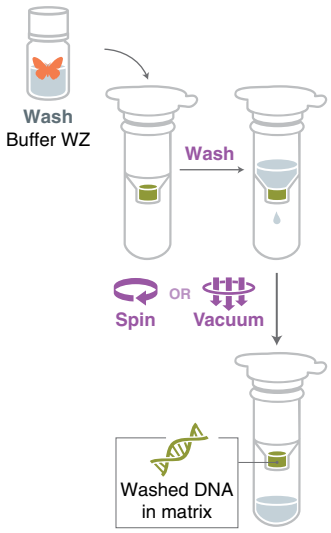
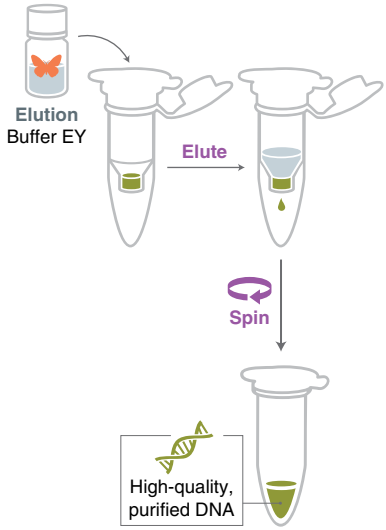
Check Purity Ratios:
A Use a spectrophotometer to measure A_{260}/A_{280} and A_{260}/A_{230} ratios. A pure DNA sample typically has an A_{260}/A_{280} ratio around 1.8 and an A_{260}/A_{230} ratio between 2.0 – 2.3.

Assess DNA Integrity:
Run an aliquot on an agarose gel to verify the size and integrity of your DNA.

Handle DNA Gently:

Avoid Vortexing:
During binding and elution steps, mix by gentle inversion to prevent shearing of DNA, especially if working with large fragments.

Prevent DNA Denaturation:
Overexposure to chaotropic salts can denature DNA. Follow incubation times carefully.

STEP	DO ✓	DON'T ⊗
WASH your DNA thoroughly Proper washing is essential to remove impurities such as salts, enzymes, and unincorporated nucleotides that can interfere with downstream applications.  	1. **Use the provided wash buffers:** **First wash:** Use the initial wash buffer, such as Monarch Buffer WZ, to remove most contaminants. **Second wash:** Perform a second wash to eliminate all residual impurities. 2. **Ensure complete removal of wash buffer:** Use the binding buffer provided with your kit, such as the Monarch Buffer BZ (supplied with the Monarch Spin PCR & DNA Cleanup Kit), which is optimized for efficient DNA binding without the need to monitor pH. **Spin for the full time:** After the final wash, centrifuge the column for the recommended time (typically 1 minute) to remove all traces of ethanol. **Avoid column tip contamination:** Ensure the column tip does not touch the flow-through in the collection tube during transfers. Any residual ethanol from the wash buffer can affect downstream applications. If in doubt, perform an additional spin.	1. **Skip wash steps:** Omitting any wash steps can leave residual contaminants that may inhibit downstream enzymatic reactions. 2. **Rush the process:** Insufficient washing or centrifugation can result in the carryover of impurities. 3. **Let column touch flow-through:** Contaminating the column tip with wash buffer flow-through can eintroduce impurities.
ELUTE your DNA carefully Elution is the final step where purified DNA is released from the column matrix. Proper elution ensures you recover DNA in a suitable concentration and volume for downstream applications.  	1. **Use the recommended elution buffer:** **Monarch Buffer EY:** This buffer (10 mM Tris, 0.1 mM EDTA, pH 8.5) is ideal for eluting DNA and provides long-term stability. 2. **Optimize elution volume:** The optimal range is 5–20 µl. To obtain highly concentrated DNA, elute in less volume, as little as 5 µl with Monarch Spin kits. 3. **Pre-warm elution buffer (if necessary):** For larger DNA fragments (>10 kb), pre-warm the elution buffer to 50°C and extend the incubation time on the column to at least 5 minutes to enhance recovery. 4. **Apply elution buffer correctly:** **Center the buffer:** Apply the elution buffer directly to the center of the column matrix to ensure even distribution. **Incubate properly:** Allow the buffer to sit on the column for at least 1 minute before centrifugation to maximize elution efficiency.	1. **Use incompatible elution buffers:** If using water instead of the provided buffer, ensure it is nuclease-free and pH-adjusted to 7–8.5. Milli-Q® water can be slightly acidic and may require pH adjustment. 2. **Shorten incubation times:** Skipping or shortening the recommended incubation can lead to incomplete elution and lower DNA yield. 3. **Store DNA in unstable conditions:** Contaminating the column tip with wash buffer flow-through can reintroduce impurities. 4. **Avoid magnesium-containing solutions:** Do not store DNA in solutions with magnesium, as it can promote degradation. even distribution. **Improper storage temperatures:** For long-term storage, keep your DNA at –20°C to maintain its integrity.

For more information and troubleshooting tips, you can access a full list of FAQs for the Monarch Spin PCR & DNA Cleanup Kit here:
www.neb.com/T1130

Advancing Research with our Enzymes for Innovation Initiative

In a recent episode of our podcast Lessons from Lab & Life, host Lydia Morrison spoke with Nathan Tanner, Associate Director of Research, about the groundbreaking work in our Enzymes for Innovation program. Below is an excerpt from their discussion.

Lydia Morrison: *Could you start by giving us an overview of the Enzymes for Innovation program at New England Biolabs?*

Nathan Tanner: Enzymes for Innovation, or EFIs, as we call them, really stem from us being scientists at NEB. We work in the lab with new enzymes every day, and our CSO, Rich Roberts, really wanted to formalize the collaboration between our research labs and the outside world. The goal is to get the innovative tools we discover into the hands of people who can develop new technologies with them. So, as we study them in the lab, we find ones we think are really interesting and commercialize them, offering them as NEB products. Our normal products are geared around a specific application, like PCR, or new library prep methods. But the EFIs are really unique. They perform activities that no other enzymes on the market do. We're excited to see what researchers and developers will do with them. We want to enable researchers and developers to innovate with these enzymes and explore their potential applications.

Lydia Morrison: *Why is it important for the scientific community to be aware of the Enzymes for Innovation program?*

Nathan Tanner: No one has commercialized an enzyme that does what the EFIs do. Sometimes we can imagine their use; other times, it's not as clear. They're unique. We like to think we're talented scientists at NEB, but we're only a small piece of the biology and biotech world. There are people out there with incredible ideas that can't do the things they want because the enzyme that would help them doesn't exist. But if they check the EFI page, they can see all these cool new activities and potentially develop new technologies.

Lydia Morrison: *How does NEB identify and develop these unique enzymes with functionality that we don't fully understand?*

Nathan Tanner: So, they all come from a research lab that specializes in that type of activity or enzyme. The lab identifies an enzyme that does something interesting, and then, importantly, we ensure these are NEB-quality products. They need to be expressible, purifiable, and stable – just like all our products.

Lydia Morrison: *Can you explain what TelN is and its significance in the Enzymes for Innovation context?*

Nathan Tanner: TelN started as an EFI and now has been promoted up the chain to a full NEB product, and is offered as GMP-grade* product. It's a protelomerase; it cuts double-stranded DNA at a really long recognition site. Instead of producing blunt double-stranded ends, the ends are covalently attached to each other. It turns out it's really useful for making protected DNA molecules for therapeutic applications – because they're protected, nothing can act on the ends. TelN has found a lot of utility for producing therapeutic DNAs, and that's why we have promoted to our GMP-grade manufacturing, because that's the quality of product that people need for those applications. It was our first graduate from the program due to its success.

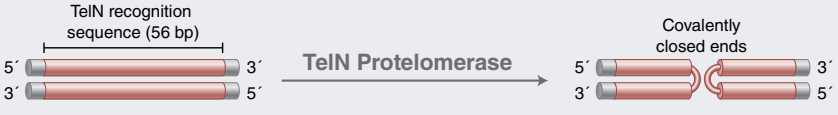
To view full list of enzymes available, visit:
www.neb.com/enzymesforinnovation

Scan code to access the full podcast for more insights into how these cutting-edge enzymes are transforming research

RECENT EFI GRADUATES

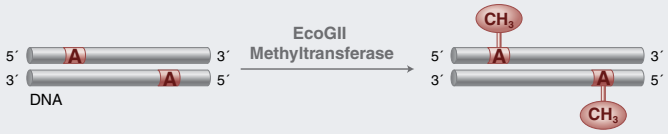
TelN Protelomerase (NEB #M0651)

Creates closed-ended DNA, facilitates hairpin DNA cleavage, and supports in vitro DNA template generation. Now available in GMP-grade formulation.*



EcoGII Methyltransferase (NEB #M0603)

*Enables m6A methylation to mark open chromatin (e.g. RASAM**), advancing epigenetics research.*



Thermostable FEN1 (NEB #M0645)

Removes single-stranded 5´ DNA flaps, creating ligatable ends for DNA repair workflows.

* "GMP-grade" is a branding term NEB uses to describe products manufactured or finished at NEB's Rowley facility. The Rowley facility was designed to manufacture products under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Products 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.

** Ostrowski, M.S., 2023 *BioRxiv*, doi.org/10.1101/2023.10.09.56158

Brewing a better understanding of the MICROBIAL WORLD

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

Dr. Anne A. Madden, a pioneering microbiologist and the founder of The Microbe Institute (microbeinstitute.org) is transforming our understanding of the microbial world. Sparked by a simple yet profound question during her Ph.D. research — "What good is a wasp?" — Madden's explorations have led to groundbreaking discoveries, including the innovative Wasp Beer Project. By exploring unconventional sources for beneficial microbes, she challenges scientific assumptions and passionately advocates for democratizing microbial research. Her work questions the notion that valuable scientific discoveries can only come from expected sources and underscores the potential of microbes as allies in innovation and sustainability. Her vision for The Microbe Institute is akin to creating a "NASA for microbes," aiming to involve the public in microbial discovery to find the transformative technology of tomorrow while helping anyone — regardless of their background — find inspiration in the microbial cosmos.

The First Wasp Yeast Beer

Brewing beer relies on yeast — an organism responsible for fermenting sugars into alcohol and producing flavors. Although thousands of yeast species exist, commercial beer production has long relied on just a few: predominantly ale yeasts (*Saccharomyces cerevisiae*) and lager yeasts (*Saccharomyces pastorianus*). These domesticated strains have been honed for centuries to create flavorful, consistent beer. However, the craft brewing industry craves new flavors.

The story of Wasp Beer, in some ways, began when Dr. Madden was studying the microbiome of paper wasps and became involved in an outreach project collaborating with brewing scientist John Sheppard and applied ecologist Rob Dunn at North Carolina State University. Contrary to the common view of wasps as pests, they uncovered that these insects harbor wild yeast strains capable of unique fermentative properties for the brewing world. Typically, wild yeasts are poor performers in brewing due to their inability to ferment maltose or tolerate high alcohol concentrations, and because they produce undesirable flavors. However, when Dr. Madden isolated *Lachancea thermotolerans*, she found it efficiently metabolized maltose and tolerated higher alcohol levels, ultimately producing a beer with delightful tart notes, with hints of honey and tropical fruit. It became a game-changing yeast for the brewing industry.

2024 Passion in Science Awards® recipient Anne Madden.
The Microbe Institute, Yarmouth, ME, USA

The debut of this wasp yeast beer at the World Beer Festival in Raleigh, NC in 2014 marked a significant innovation in brewing. The yeast's ability to impart a clean sourness to the beer without the need for additional souring agents simplified production processes and reduced risks of contamination. It cut down on sour beer brewing time and costs — advantages quickly recognized and appreciated by amateur and professional brewers. This breakthrough demonstrated how rethinking assumptions about microbial habitats can lead to significant technological advancements.

What began as a short-term outreach project soon evolved into a full-fledged scientific breakthrough. Within months, Dr. Madden and her team worked with commercial brewers to bring lactic acid yeast beers to market and were on the path to what would eventually lead to a patent on the yeast. Media outlets took notice, and the project received widespread coverage on PBS NewsHour, National Geographic, and Dr. Madden was invited to give TED and TEDx Talks.

Lachancea thermotolerans is now globally available to the brewing market through one of the world's largest yeast providers, under AB Biotek's Pinnacle Crisp Sour label. Brewers worldwide have adopted this yeast to create award-winning sour beers, as well as new styles of cider and sake. While the original wasp yeast was the first primary souring yeast discovered, this method is now an industry standard for making sour beer.

The Broader Mission to Democratize Microbial Discovery

While Wasp Beer is one of Dr. Madden's most well-known projects, it's just a small part of her larger mission. In 2020, she founded The Microbe Institute, a nonprofit organization dedicated to engaging the public in microbial science through participatory art, education, and science projects. The initiatives are designed to bridge the gap between science and community and integrate microbial research into everyday life.

One notable project involved partnering with colleagues at North Carolina State University to enable students to grow and analyze their own sourdough starters, learning about microbial ecology in the process, and submitting data to scientists at the same time (microbeinstitute.org/wild-sourdough-project). Another initiative included working with a broader network of nonprofits, artisans, storytellers, and scientists to focus on identifying naturally occurring pigment that can be used as sustainable textile dyes — a project that collaborated with Moroccan weavers to replace synthetic dyes with microbial and plant alternatives, thereby reducing environmental impact while supporting local economies. This project not only applies microbial science to traditional crafts but also demonstrates the practical benefits of microbial research in global sustainability efforts (microbeinstitute.org/morocco-natural-dyes).

Despite their critical role in everything from brewing to medicine, microbes are often misunderstood by the public. Dr. Madden is determined to change that through her talks, art collaborations, and citizen science programs. She encourages people to see microbes not as threats but as vital partners in improving our world.

By exploring the microbial world — whether it's in a wasp nest or a sourdough starter — we can unlock new possibilities for food, medicine and sustainable living. Through The Microbe Institute, Dr. Madden continues to inspire a new generation to explore and appreciate the vast potential of the microbial world. Her work reveals that the greatest innovations often come from unexpected places and that, by embracing the unknown and questioning the accepted, science can advance in truly remarkable ways, uncovering new solutions and revealing vast opportunities for innovation and sustainability.

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