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NEB expressions

a scientific update

IN THIS ISSUE

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FEATURE ARTICLE ~

Advances in next generation sequencing: How researchers at NEB are working to improve our understanding of the genome, epigenome and transcriptome.

by Joanne Gibson, Ph.D. & Betsy Young, Ph.D.

Conducting innovative basic research has always been integral to New England Biolabs' philosophy; our scientists have contributed to the advancement of science and modern molecular biology technologies for almost 50 years and, as a result, have authored or co-authored over 1,500 publications.

Basic research at NEB has led to the development of new technologies, streamlined workflows, and has helped facilitate a deeper understanding of the scientific questions we seek to answer. This empowers not only our scientists but also the scientists we serve – scientists working for scientists.

Advances in NGS are a major area of focus for NEB researchers. As scientific questions evolve, our research team works to fill technological gaps to enable more insightful genomic, epigenomic and transcriptomic analysis. Most recently, our scientists have applied their expertise in enzymology and NGS to address unmet needs in the analysis of genome-wide methylation, transcriptional start sites, full-length transcriptomes, chromatin accessibility, and much more.

This article gives an overview of some of the exciting sequencing technologies developed at NEB and discusses the applications they enable.

Methylation analysis

The most abundant form of epigenetic modification in the genomes of both prokaryotes and eukaryotes is methylation, which plays a role in gene regulation and cell differentiation. Methylome analysis has traditionally been restricted to sodium bisulfite treatment, which causes extreme damage, followed by short-read sequencing using platforms like Illumina. With the recent introduction of NEBNext Enzymatic Methyl-seq (EM-seq) (NEB #E7120) [1], it has become possible to analyze methylation across a genome without the challenges of conversion-induced DNA damage with sub-nanogram amounts of DNA.

Long-Read EM-seq (LR-EM-seq)

Long-read sequencing has steadily grown in popularity. Companies like Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio) are facilitating ever-longer sequencing read lengths. LR-EM-seq, developed in the Ettwiller lab, preserves the integrity of DNA using a highly effective enzyme-based conversion that minimizes damage [2]. It allows long-range methylation profiling of 5mC and 5hmC within amplicons up to 5 kb using a long-read sequencing protocol. When applied to biologically relevant, differentially methylated genomic regions (DMR) with various methylation percentages and contexts, the result from LR-EM-seq is in accordance with previous studies.

Long-range phasing of methylated cytosines is essential for several applications, including studying DMRs of the genome, particularly where methylation status is a known disease biomarker. LR-EM-seqcan also support haplotyping of methylation patternsand targeted methylation analysis.

This new sequencing technology is a comprehensive solution for analyzing 5mC and 5hmC methyl marks in various contexts (e.g., CpG, CHG, CHH). LR-EM-seq only requires a small amount of starting material – as low as a few ng of DNA.
It uses the same analytical strategies developed for bisulfite sequencing.

Rapid Identification of Methylase Specificity (RIMS-seq)

Also developed in the Ettwiller Lab, RIMS-seq seamlessly combines shotgun sequencing of bacterial genomes with 5mC methylase detection in a single experiment using Illumina sequencing [3]. There are three types of methylation in bacteria: 5mC (5-methylcytosine), 4mC (N^4-methylcytosine), and m6A (N^6-methyladenine). While PacBio single molecule real-time (SMRT) sequencing can easily detect m6A and 4mC, it is more challenging to detect 5mC. This is because the polymerase stalls at the methylation site for a certain amount of time; however, the pause of the polymerase is much shorter for 5mC, which makes it more challenging to identify.

Inspired by the success of SMRT sequencing in revealing epigenetic landscapes, researchers in the Ettwiller lab modified the Illumina library preparation protocol and added an alkaline treatment incubation step. This treatment induces a level of deamination large enough to reveal 5mC methylation specificity commonly found in bacterial-RM systems while still producing sequence quality comparable to standard Illumina DNA sequencing. RIMS-seq can identify the 5mC methylome of mixed communities of unknown bacteria.

Because of the simplicity of this approach and its broad applicability, RIMS-seq has the potential to replace standard DNA-seq for bacterial genome sequencing (Figure 1).

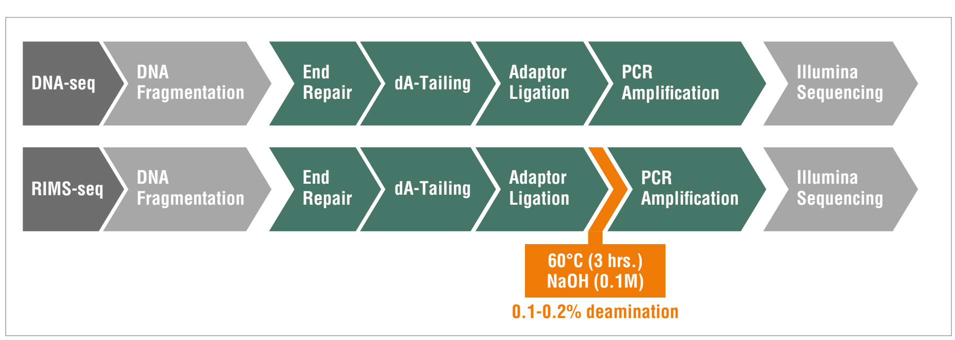
Identification of Transcription Start Sites (TSS)

Identifying transcription start sites (TSS) gives vital information relating to RNA transcripts, regulatory regions, promoters, and transcription factor binding sites in a sequence.

Cappable-seq

Developed in the Ettwiller and Schildkraut labs, Cappable-seq is a sensitive and robust method for directly enriching the 5´ end of primary transcripts from bacteria and microbiomes [4]. This method enables the determination of transcription start sites (TSS) at a single base resolution. Prokaryotes have a unique triphosphate at the beginning of the RNA transcript. One of the advantages of this technique is that it directly targets 5´ triphosphorylated RNA – the first nucleotide incorporated by the RNA polymerase

Figure 1: **Comparison of the RIMS-seq and DNA-seq workflow**



upon initiation of transcription – in total RNA preparations. Cappable-seq uses this feature to capture the 5´ end of the molecule. The overwhelming majority of a total RNA sample is made up of processed RNA, such as ribosomal RNA, but by targeting 5´ triphosphorylated RNA, the rRNA population is reduced to just 3%, and the need to perform rRNA depletion beforehand is eliminated; therefore, it offers the ability to investigate the triphosphorylated RNA molecules that would otherwise be overwhelmed by processed RNA. This reduces the complexity of the transcriptome to a single quantifiable tag per transcript resulting in the ability to sequence the enriched 5´ triphosphorylated RNA population at a much deeper level at a lower cost, enabling the profiling of gene expression in a microbiome.

ReCappable-seq

The Ettwiller and Schildkraut labs built upon Cappable-seq with ReCappable-seq [5] to capture the TSS of non-RNA Polymerase II transcripts in addition to the TSS of 7-methyl G-capped transcripts derived from RNA Polymerase II. Therefore, ReCappable-seq overcomes the limitation of other methods that only determine RNA Polymerase II transcripts, which entirely exclude the TSS derived from eukaryotic RNA Polymerase I, RNA Polymerase III and mitochondrial RNA Polymerase that all produce uncapped non-coding RNA. To achieve this, they took advantage of the property of the yeast scavenger decapping enzyme (yDcpS) to convert capped RNA into di-phosphorylated RNA that can be "re-capped" by the Vaccinia Capping Enzyme, hence the name ReCappable-seq. Recappable-seq enriches both RNA Polymerase II- and non-RNA Polymerase II- derived transcripts and provides the ability to comprehensively evaluate both mRNA and non-capped primary transcripts all in one library, genome-wide, at single nucleotide resolution. It allows a unique opportunity to simultaneously interrogate the regulatory landscape of coding and non-coding RNA in biological processes and diseases.

Like Cappable-seq, ReCappable-seq produces sequencing libraries from total RNA without the need to deplete rRNA beforehand. Because it is species agnostic, it can be used with complex communities composed of both prokaryotic and eukaryotic organisms.

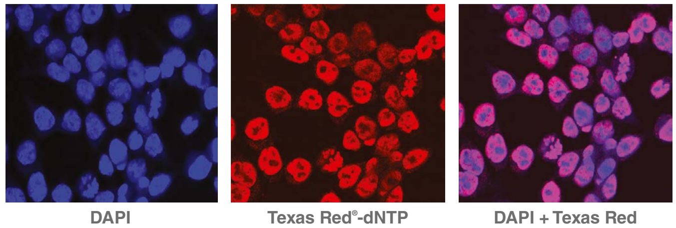
SMRT-Cappable-seq

This high-throughput technique was derived from Cappable-seq in the Ettwiller lab, but it differs in that it generates a snapshot of the full-length bacterial transcriptome at base resolution, whereas Cappable-seq identifies the TSS only [6]. To achieve this, the triphosphorylated 5´ ends of unfragmented transcripts are captured using an adapted Cappable-seq methodology, again removing the need to perform RNA depletion beforehand.

Because the transcripts do not need to be fragmented PacBio single-molecule long-read sequencing from TSS to the termination sites can be carried out. This is valuable when trying to gain information regarding bacterial operons, which are made up of a group of genes under the control of a common promoter – with short sequencing reads, much of the operon complexity is overlooked or hidden. Long-read sequencing keeps the information on the 5´ and 3´ ends of operons intact, facilitating their identification.

Additionally, SMRT-Cappable-seq can be used on complex microbiomes for which reference genomes are not readily available.

Figure 2: **Confocal microscopic images of colon carcinoma cell line (HCT116)**



Left: DAPI-stained nuclei, Middle: Texas Red-dNTP-stained accessible chromatin using NicE-viewSeq technology, Right: DAPI + Texas Red-dNTP

Analysis of chromatin accessibility

Within the nucleus, mammalian DNA is packaged as chromatin, along with essential proteins and RNA. The chromatin of the nuclear genome must be accessible to the transcriptional machinery to produce RNA for translation to cellular proteins. The dynamic nature of chromatin accessibility in cellular function is vital to gene expression and development.

Universal Nicking Enzyme-assisted Sequencing (UniNicE-seq) and Nicking Enzyme-assisted Viewing and Sequencing (NiCE-view-seq)

Developed in the Pradhan lab, UniNicE-seq captures and reveals open chromatin sites (OCS) and transcription factor occupancy at single nucleotide resolution; it reveals the transcriptionally active genome [7,8].

UniNicE-seq utilizes a labeling mix containing a sequence-specific nicking enzyme (Nt.CviPII), DNA polymerase I and dNTPs (containing biotin-conjugated dATP, dCTP and 5-methyl-dCTP). This mix labels the open chromatin region in the nucleus; then, the DNA is extracted and sonicated/digested to ~200 bp. This DNA undergoes library preparation, and Streptavidin magnetic beads capture the biotinylated library components for further PCR library preparation and sequencing.

UniNicE-seq may be used with cell lines (formalin-fixed and unfixed), mammalian- or plant-tissue nuclei, frozen tissue sections, and formalin-fixed paraffin-embedded (FFPE) tissue sections. It can be used with high resolution and a broad range of cell number inputs (25-250,000). This technology is capable of automation and cell-to-library preparation in one tube.

Lending an added dimension for analysis, NicE-viewSeq (Figure 2) includes a biotinylated dCTP mix and a Texas Red tagged dATP mix that enables visualization of regions of accessible chromatin and subsequent sequencing for genome analysis, enabling pharmacological studies of chromatin-modifying drug efficacy [9].

Protect-seq

At the periphery of the metazoan nucleus, the nuclear chromatin becomes less accessible to transcriptional machinery and, in some cases, in direct apposition to the nuclear lamina (in what are known as lamina-associated domains, or LADs). Understanding which sequences tend to become arrayed in these LADs is an essential step toward understanding their functions.

Using a familiar technique in a new way, the Pradhan lab developed Protect-seq [10]. It relies on a cocktail of nucleases targeted at degrading and removing the open and accessible chromatin, as in NicE-seq, but with the goal of leaving the less-accessible, sonication-resistant LADs for sequencing. It is an efficient way to identify constitutive heterochromatin around the nuclear periphery. Protect-seq is a simple, easy-to-use, cost-and-time-effective method that does not require actively dividing cells, specialized equipment, or reagents. The entire protocol can be performed in a day.

Techniques that strive to answer similar questions require particular constructs, genome modifications, the establishment of cell lines, actively dividing cells or highly specific antibodies. In contrast, Protect-seq requires only an enzyme cocktail and nuclei from fixed cells or tissues.

This sequencing technique is compatible with short-read Illumina sequencing and long-read ONT sequencing, broadening its accessibility for researchers.

Analysis of DNA damage and modification

All living cells are exposed to DNA-damaging agents that are found exogenously, such as UV radiation, and endogenously, such as reactive oxygen species. These DNA-damaging agents can cause the formation of a wide variety of DNA lesions that can be mutagenic and cytotoxic to the cell. Cells have evolved several DNA repair pathways that recognize, remove and repair these DNA lesions. In higher eukaryotes, the formation of DNA lesions and faulty repair has been shown to cause cancer, neurological disorders and premature aging.

RAre DAmage and Repair sequencing (RADAR-seq)

To understand the formation, persistence and repair of DNA lesions, NEB scientists have developed RADAR-seq [11,12]. This technique replaces a DNA lesion with a patch of modified bases that PacBio SMRT sequencing can detect. RADAR-seq can measure the frequency and map the locations of various rare DNA lesions genome-wide without requiring DNA amplification or enrichment. To understand *in vivo* DNA damage and repair pathways in a particular organism, RADAR-seq can be used to determine the DNA damaging effects of a specific DNA damaging agent, locate DNA damage hotspots across a genome, or locate the specific genomic site of any DNA nicking enzyme.

Several currently used DNA damage detection techniques employ short-read next generation sequencing methods that amplify damaged DNA; the drawback is that only the enriched regions with damaged, and not undamaged, DNA are sequenced. This gives a relative measure of DNA damage without information regarding absolute levels of damage in a genome. In contrast, RADAR-seq utilizes the PacBio long-read sequencing platform, which provides information on both damaged and undamaged DNA. In addition, most DNA damage detection techniques are tailored to locate a specific DNA lesion (i.e., ribonucleotides or cyclobutane pyrimidine dimers). RADAR-seq can locate any DNA lesion with an associated nicking enzyme or repair glycosylase. Furthermore, RADAR-seq can detect rare DNA damage, as low as one lesion per 1 Mb bases sequenced. Finally, RADAR-seq library construction is fast and can be completed in less than one day (Figure 3).

EcoWI-seq

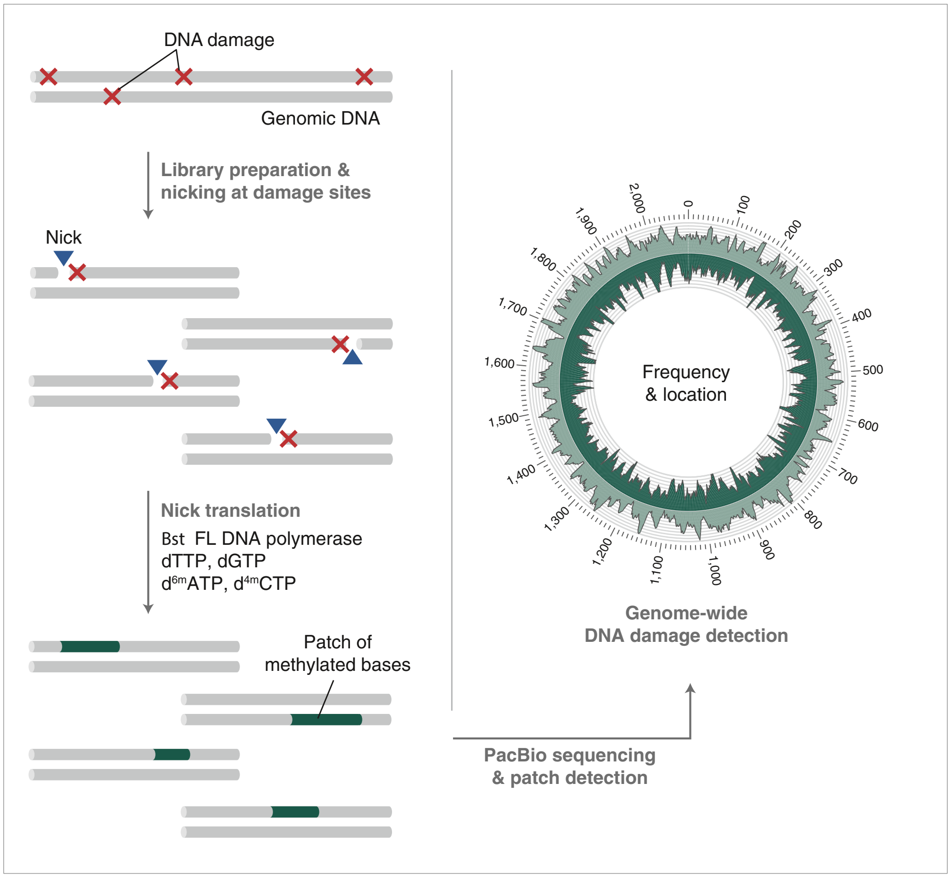
This sequencing method was also developed in the Ettwiller lab [13] and can determine the pattern of phosphorothioate (PT) modifications in bacteria. PT modifications occur on the DNA sugar-phosphate backbone rather than on the nucleotide (a non-bridging oxygen is replaced by sulfur) and are maintained at a particular density in a genome. The PT modification is widespread in prokaryotes and is a horizontally transferred epigenetic system that makes the phosphorothioate oligonucleotide more resistant to degradation. The modification occurs on a small proportion of the EcoWI recognition sequences in the genome, and whether they occur stochastically or deterministically is an area of investigation.

The restriction enzyme EcoWI is a PT-dependent endonuclease with recognition sequence GAAC/GTTC. EcoWI creates a double-stranded break only when a PT modification is present on both DNA strands. Subsequent sonication generates short sequences compatible with Illumina sequencing. The ability to detect PT modifications relies on the mapping pattern: fragments generated from sonication will map to the genome at random positions and provide sequencing information that can be used for genome assembly; fragments generated by EcoWI digestion map to fixed pattern ends, which provide the location of the modification at base resolution. This methodology is scalable and requires little starting material.

Enzyme discovery

The discovery and characterization of new enzymes aids the development of new technologies. Microbiomes are an untapped resource for discovering enzymes that can be harnessed for industrial purposes. Metagenomic, epigenomic, and transcriptomic pipelines are being used to rapidly discover novel enzymes.

Figure 3: **RADAR-seq overview. Details in Current Protocols of Molecular Biology [12]**



Metagenomics Genome-Phenome Association (Meta-GPA)

The field of microbiome research has evolved rapidly and is a topic of great scientific and public interest. Nonetheless, microbiome studies are often limited to shotgun sequencing providing detailed descriptions of species composition and gene content, but direct links to function are missing. In other words, while we now understand *who* (species) are out there, it remains very difficult to understand *what* they are doing.

MetaGPA bridges this fundamental gap between genetic information and functional phenotype using next generation shotgun metagenomic sequencing [14]. MetaGPA is conceptually close to Genome-Wide Association Studies (GWAS), where control and case cohorts are compared to identify associated variants in the case cohorts. Likewise, metaGPA associates genetic data with phenotypic traits at the level of an entire microbiome. The association analysis can be done at the pathway, protein, or even single amino acid resolution level, pinpointing the residues within a protein domain that underlie a respective phenotype within a microbiome, irrespective of whether the organisms within that microbiome are known or culturable.

Because sequencing is conducted on environmental matter, there is no reference genome. Therefore, once the DNA is isolated and sequenced at a deep level, a *de novo* reference meta-genome must be created using a *de novo* assembler. The case and control genomes are plotted to reveal those with a high frequency in the test group. An enrichment score calculation associates the contigs with the likelihood of being a feature of the case group. These contigs are then analyzed for functional units.

Conclusion

At NEB, our scientists are passionate about developing sequencing technologies that can keep pace with their imaginations. As they continue developing new and exciting methodologies for diverse applications to analyze the genome, epigenome and transcriptome, they also continue to foster NEB's ongoing commitment to sharing knowledge and collaborating with the broader scientific community.

Learn more about the exciting research performed in the Ettwiller, Pradhan, Gardner and Schildkraut labs at www.neb.com/research.

References

1. Technical note, NEBNext Enzymatic Methyl-seq (EM-seq)
2. Sun, Z. et al. (2021) Genome Res., 31(2):291-300
3. Baum, C. et al. (2021) Nucl. Acids Res., 8;49(19):e113
4. Ettwiller, L. et al. (2016) BMC Genomics, 17:199
5. Yan, B. et al. (2022) Genome Res., 32(1):162-174
6. Yan B. et al. (2018) Nat. Commun., 9(1):3676
7. Chin, H.G. et al. (2020) Clin. Epig., 12:143
8. Vishnu, U.S. et al. (2021) Epig. & Chrom., 14(53)
9. Estève, P-O. et al. (2020) J. Mol. Biol., 5304-5321
10. Spracklin, G. and Pradhan, S. (2020) Nucl. Acids Res., 48(3)e16
11. Zatopek, K.M. et al. (2019) DNA Repair, 36-44
12. Zatopek, K.M. et al. (2022) Curr. Prot., 2:11
13. Yang, W. et al. (2022) PLoS Genet., 18(9)
14. Yang, W. et al. (2021) Elife, 8;10:e70021

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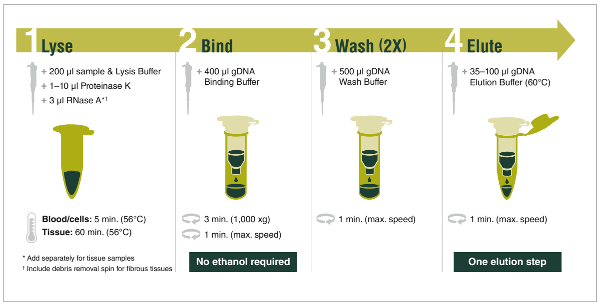
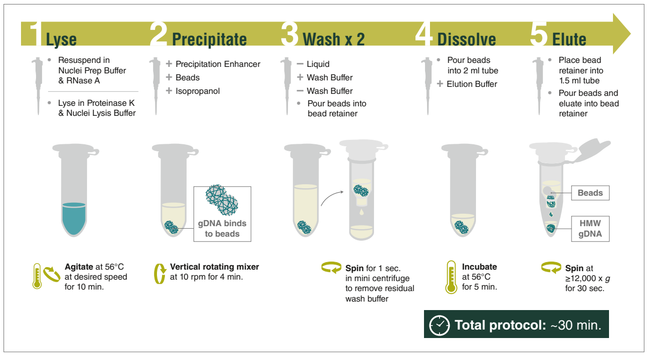
- Elute DNA easily and completely

Workflow of Monarch gDNA Purification Kit (#T3010)

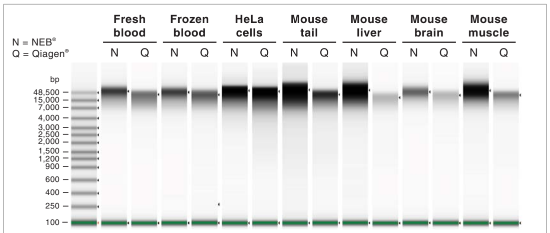


Workflow of Monarch HMW DNA Extraction Kit (#T3050)



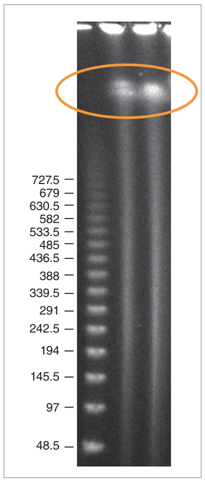
The Monarch Genomic DNA Purification Kit provides excellent yields of higher quality, higher molecular weight DNA than the Qiagen DNeasy Blood & Tissue Kit

N = NEB®
Q = Qiagen®



	Fresh blood N	Fresh blood Q	Frozen blood N	Frozen blood Q	HeLa cells N	HeLa cells Q	Mouse tail N	Mouse tail Q	Mouse liver N	Mouse liver Q	Mouse brain N	Mouse brain Q	Mouse muscle N	Mouse muscle Q
Peak size (kb)	54	28	50	27	57	54	>60	28	59	20	51	25	57	26
DIN	9.1	8.0	8.7	7.9	8.8	8.2	8.3	7.4	9.0	7.2	8.9	7.6	9.2	7.8
A_{260}/A_{280}	1.85	1.82	1.83	1.85	1.84	1.89	1.85	1.87	1.85	1.88	1.86	1.90	1.87	1.87
A_{260}/A_{230}	2.19	2.17	2.43	1.21	2.33	1.91	2.36	1.98	2.43	1.31	2.47	1.52	2.40	1.57

Agilent Technologies 4200 TapeStation Genomic DNA ScreenTape was used for analysis of gDNA purified from blood, cultured cells and tissue samples using the relevant protocols of the Monarch Genomic DNA Purification Kit and the Qiagen DNeasy Blood & Tissue Kit. For more technical data, please visit www.neb.com/t3010.



A unique workflow employing glass beads allow for highly pure HMW DNA Extraction and result in DNA size ranges from 50 kb into the Mb range (orange circle), depending on the agitation speed used during lysis. Right: Extracted HMW DNA from HEK293 cells analysed on an agarose gel. For more technical data, please visit www.neb.com/t3050 or www.neb.com/t3060.

View more technical data, please visit **NEBMonarch.com**

Ordering information:

PRODUCT	NEB #	SIZE
Monarch Genomic DNA Purification Kit	T3010S/L	50/150 preps
Monarch HMW DNA Extraction Kit for Cells & Blood	T3050S/L	5/50 preps
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Available with or without beads?	• With beads: NEB #E7103 • Without beads: NEB #E7645	• With beads: NEB #E6177 • Without beads: NEB #E7805
Modules available?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
ULTRA II PCR-FREE INPUT AMOUNTS	250 ng – 1,000 ng of sheared DNA	50 ng – 500 ng of intact DNA
Available with or without beads?	• With beads: NEB #E7415 • Without beads: NEB #E7410	• With beads: NEB #E7435 • Without beads: NEB #E7430
Modules available?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
COMPATIBLE WITH FFPE DNA?	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)
COMPATIBLE WITH METHYLOME ANALYSIS?	Yes – Methylome analysis is supported; however, we recommend NEBNext EM-seq (NEB #E7120)	No – not compatible due to potential for loss of methyl marks
COMPATIBLE WITH OXFORD NANOPORE TECHNOLOGIES?	Yes. We recommend the NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing (NEB #E7180).	

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NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 reactions
NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 reactions
NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 reactions

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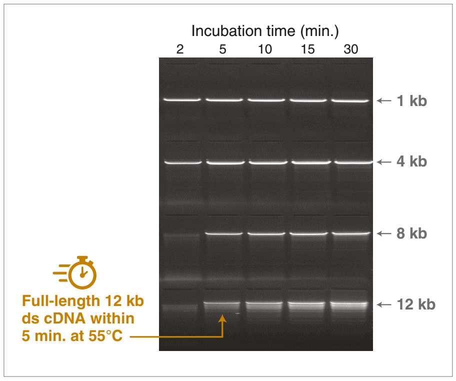
NEBNext Ultra II RNA Library Prep with Purification Beads	E7775S/L	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep Kit Illumina	E7760S/L	24/96 rxns
NEBNext Ultra II Directional RNA Library Prep with Purification Beads	E7765S/L	24/96 rxns
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with Purification Beads	E7405S/L/X	6/24/96 rxns
NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns

Induro Reverse Transcriptase

Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors for the synthesis of cDNA from RNA. It is an ideal enzyme for challenging cDNA synthesis from long transcripts (>8 kb), RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro Reverse Transcriptase can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows.

Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis



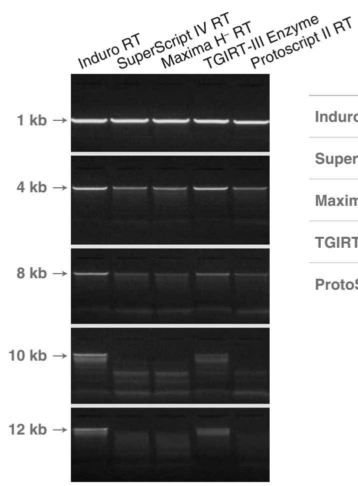
Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.

Benefits

- Rapidly generate high yields of long cDNA with our unique group II intron-encoded RT
- Strong inhibitor tolerance enables robust cDNA synthesis performance
- Support direct RNA sequencing and long read cDNA sequencing workflows
- Generate cDNA at higher temperatures, which is ideal for challenging sample types
- Experience comparable fidelity to retroviral RTs

View more technical data and recommended protocol at **www.neb.com/m0681**

Induro Reverse Transcriptase generates highest yields of long cDNA



	Temp.	Time	Yield of full length cDNA
Induro™ RT	55°C	10 min.	12 kb
SuperScript® IV RT	55°C	10 min.	4 kb / 8 kb
Maxima™ H⁻ RT	50°C	15 min.	4 kb / 8 kb
TGIRT®-III Enzyme	60°C	60 min.	8 kb / 12 kb
ProtoScript® II RT	42°C	60 min.	4 kb / 8 kb

Higher yield / Lower yield

Induro Reverse Transcriptase generates the highest product yields for cDNA ≥ 8 kb. RNA templates were in vitro transcribed poly(A) RNA (1 kb, 4 kb, 8 kb, 10 kb or 12 kb). After first strand cDNA synthesis, RNA was degraded and the second strand cDNA synthesis was performed in the presence of a 5´ specific primer.

Applications

- Challenging cDNA synthesis or when other RTs fail, including long cDNA synthesis (>8 kb), RNA with many isoforms or difficult structures, and RNA samples with impurities/inhibitors
- RNA-seq workflows, including direct RNA sequencing on the ONT platform, direct long read cDNA sequencing on the ONT platform, and RNA-seq studies of modifications and structures
- Applications where intron RTs are used (e.g., 3´ template switching)

View our NEBTV webinar

Induro Reverse Transcriptase: A Robust, Thermostable, Intron-Encoded RT for Full-Length cDNA Synthesis.

Ordering information:

PRODUCT	NEB #	SIZE
Induro Reverse Transcriptase	M0681S/L	4,000/ 10,000 units
Companion Products		
Oligo d(T)23 VN	S1327S	1 A_{260} units
Random Primer Mix	S1330S	100 µl
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol

Reverse Transcriptases (RTs)
from New England Biolabs®

NEB has an extensive portfolio of RTs, which are available as standalone products or have been incorporated into convenient master mixes or kits. For more details and access to the full product listing, visit ww.neb.com/rt.

RETROVIRAL RTs

For general cDNA synthesis

LunaScript® RT SuperMix
NEB #E3010/M3010

Product notes:
Single-tube supermix, fast 13-minute protocol. Contains random primers, ideal for RNA targets up to 3 kb.

LunaScript RT Master Mix Kit (Primer-free)
NEB #E3025

Product notes:
First strand cDNA synthesis with user-supplied primers

ProtoScript® II RT
NEB #M0368

Product notes:
RNase H⁻ mutant of M-MuLV RT with increased thermostability and reduced RNase H activity. Ideal for RNA targets up to 12 kb.

ProtoScript II First Strand cDNA Synthesis Kit
NEB #E6560

Product notes:
Kit contains ProtoScript II, Murine RNase Inhibitor, primers and dNTPs

M-MuLV RT
NEB #M0253

AMV RT
NEB #M0277

Product notes:
Robust RTs for a variety of templates

ProtoScript First Strand cDNA Synthesis Kit
NEB #E6300

Product notes:
Kit contains M-MuLV RT, Murine RNase Inhibitor, primers and dNTPs

For niche cDNA synthesis

LunaScript RT SuperMix
NEB #E3010/M3010

Recommended use:
Two-step RT-qPCR, amplicon sequencing

Luna® WarmStart RT
NEB #M3001*

Recommended use:
One-step RT-qPCR, One-step RT-PCR

Luna One-Step RT-qPCR reagents
- Luna Universal One-Step RT-qPCR Kit
  NEB #E3005
- Luna Universal Probe One-Step RT-qPCR Kit
  ROX: NEB #E3006
  No ROX: NEB #E3007
- Luna Probe One-Step RT-qPCR 4X Mix with UDG
  ROX: NEB #M3019
  No ROX: NEB #M3029
- LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG
  NEB #L4001

LunaScript Multiplex One-Step RT-PCR Kit
NEB #E1555

Product notes:
Features Luna WarmStart RT and Q5® Hot Start High-Fidelity DNA Polymerase

Template Switching RT Enzyme Mix
NEB #M0466

Recommended use:
cDNA amplification with a template switching oligo, 5´ RACE, second strand cDNA synthesis

WarmStart® RTx
NEB #M0380

Recommended use:
Optimized for RT-LAMP

LAMP Master Mixes
- WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)
  NEB #M1800
- WarmStart Colorimetric LAMP 2X Master Mix with UDG
  NEB #M1804
- WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
  NEB #M1708
- WarmStart LAMP Kit (DNA & RNA)
  NEB #E1700
- WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)
  NEB #E1708

* Available through OEM & Customized Solutions

GROUP II INTRON RTs

NEW

Induro™ RT
NEB #M0681

Recommended use:
- Long cDNA synthesis (up to 20 kb)
- Impure RNA samples
- RNA-seq workflows

Product notes:
Group II Intron-encoded RTs are distinct in sequence and domain organization from common retroviral RTs. They exhibit unique features such as high processivity, increased thermostability, and strong inhibitor tolerance.

Lighting the way.

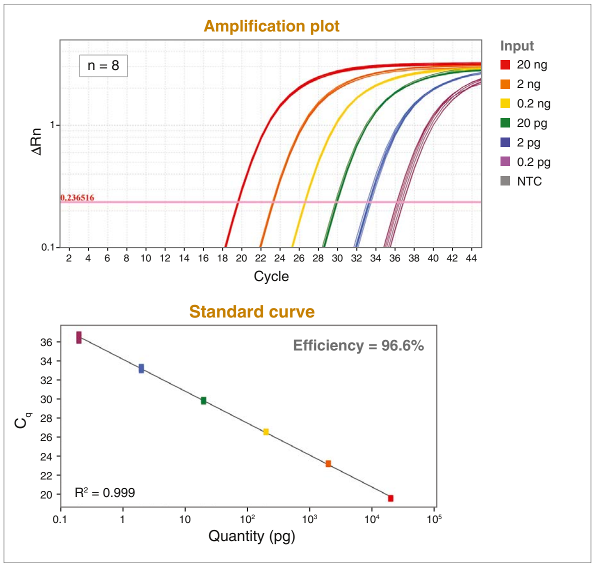
Luna Universal (RT)-qPCR products: unrivaled performance for all your (RT)-qPCR needs!

Luna (RT-)qPCR products offer best-in-class performance and robustness, and are available for intercalating dye or probe-based detection methods. They are compatible with all leading qPCR cyclers incl. ROX/dye-dependent machines. Luna Universal qPCR mixes contain dUTP, enabling carryover prevention when reactions are treated with Antarctic Thermolabile UDG (NEB #M0372).

LunaScript RTase is a unique designer enzyme and allows for a fast (13 min) and robust performance in RT-qPCR settings. It is available as LunaScript Super Mix or Super Mix Kit (NEB #M3010/#E3010) – your preferred choice in two-step workflows – or readily "built-in" as part of the Luna One-Step RT-qPCRs Kits.

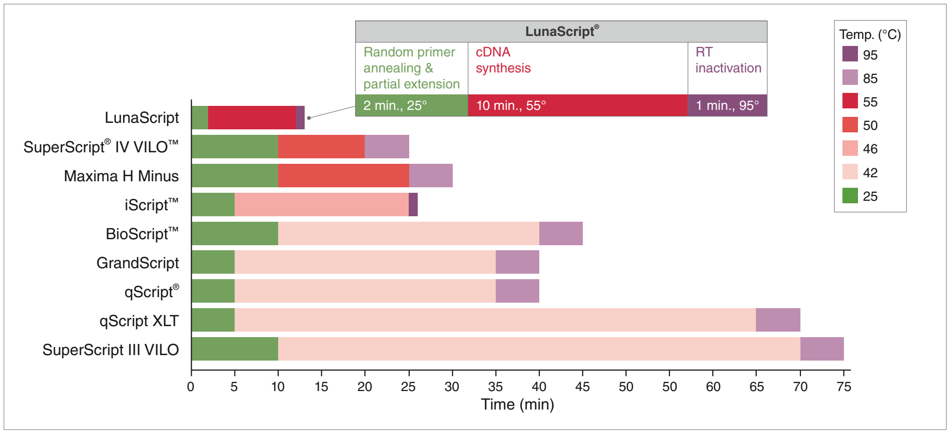
In addition, the "Luna Cell Ready" products and kits are designed for direct RNA quantification from cell lysate, bypassing traditional RNA extraction and purification steps.

Luna products offer exceptional sensitivity, reproducibility and qPCR performance



qPCR targeting human GAPDH was performed using the Luna Universal Probe qPCR Master Mix over a 6-log range of input template concentrations (20 ng – 0.2 pg Jurkat-derived cDNA) with 8 replicates at each concentration. cDNA was generated from Jurkat total RNA using the NEB Protoscript II First Strand cDNA Synthesis Kit (NEB #E6560). NTC = non-template control

At just 13 minutes, the LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol



Comparison of recommended protocols for cDNA synthesis. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures, reducing complications from RNA secondary structure.

Benefits

- Novel enzyme technology by pairing a unique thermostable reverse transcriptase (RT) with optimized Taq and unique aptamer technology increasing reaction specificity, sensitivity, accuracy, reproducibility and robustness
- Products perform consistently across a wide variety of sample sources
- Convenient master mix formats with user-friendly protocols allow for UDG-dependent carry-over-prevention and simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors
- Skip RNA purification and go direct from cells to RT-qPCR analysis with Luna Cell Ready Module and Kits
- Readily available as lyophilized kit – more lyophilization options available as part of our customized solutions offering
- Excellent value

What customers are saying:

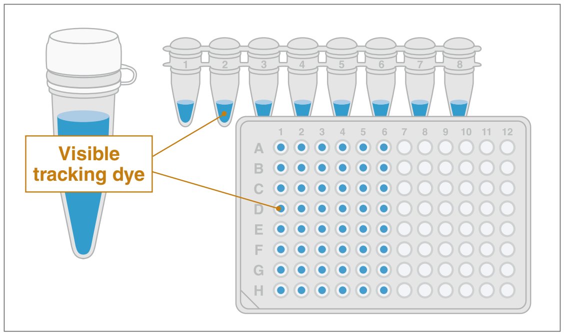
The choice of One-step kit is critical for sensitivity and at the same time a major cost factor. **We recommend the NEB Luna Universal Probe One-Step RT-qPCR Kit (E3007), which is attractively priced and performs among the most sensitive One-step kits we tested.**

VIENNA COVID-19 DETECTION INITIATIVE (VCDI)

It's really that easy to find the right Luna product for your application:

		2 Select your detection method	
		Dye-based	**Probe-based**
1 Select your target	**Genomic DNA or cDNA**	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
	Purified RNA **One-Step RT-qPCR**	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006) • Kit (no ROX) (NEB #E3007) • 4X Mix with UDG (NEB #M3019)
	Two-Step RT-qPCR	LunaScript® RT SuperMix Kit (NEB #E3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix Kit (NEB #E3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
	RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)

Luna products feature a blue tracking dye eliminating pipetting errors

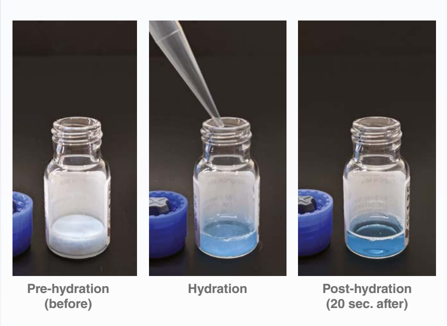


Ordering information:

Product	NEB #	Size
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1,000/2,500 rxns
Luna Probe One-Step RT-qPCR Kit (No ROX)	E3007E	2,500 rxns
Luna Probe One-Step RT-qPCR 4X Mix with UDG	M3019S/L/X/E	200/500/1,000/2,000 rxns
Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)	M3029S/L/E	200/500/2,000 rxns
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit	E3019S/L	96/480 rxns
LunaScript RT Master Mix Kit (Primer-free)	E3025S/L	25/100 rxns
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns
LunaScript RT SuperMix	M3010L/X/E	100/500/2,500 rxns
LunaScript Multiplex One-Step RT-PCR Kit	E1555S/L	50/250 rxns
Luna Cell Ready One-Step RT-qPCR Kit	E3030S	100 rxns
Luna Cell Ready Probe One-Step RT-qPCR Kit	E3031S	100 rxns
Luna Cell Ready Lysis Module	E3032S	100 rxns (50 µl)

Also available: LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG – lyophilized, storage at room temperature

Bringing together expertise in enzyme development, manufacturing and lyophilization, NEB Lyophilization Sciences™ – a new NEB subsidiary – has created shelf-stable, lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts.



PRODUCT	NEB #	SIZE
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG	L4001S	120 rxns

Learn more about lyophilization and access our on-demand webinar discussing considerations for lyophilizing reagents at **www.neb.com/LyoPrime** or contact your local distributor.

Free samples are available for many Luna products.
Request yours at **www.LUNAqPCR.com** or contact your local distributor.

From ORF to protein in less than 2 hours: Robust protein expressions using cell-free protein synthesis systems

NEB's cell-free protein synthesis systems offer robust and extremely rapid workflows to get from an open-reading-frame/cDNA to the actual protein/gene product in only a few hours.

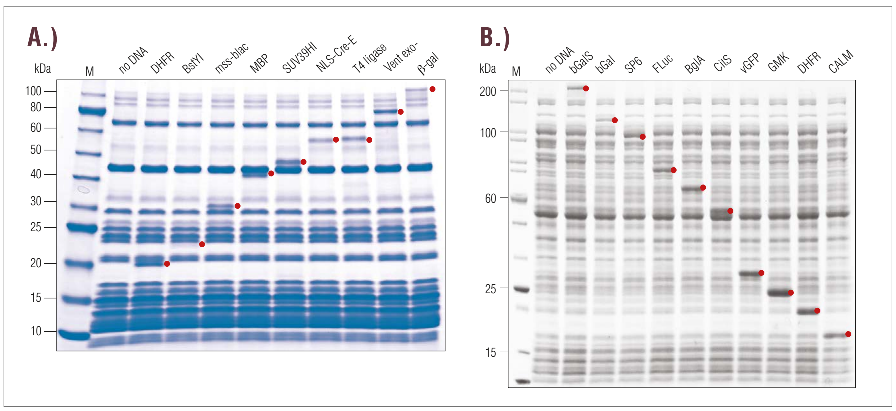
Choose your favorite:

A.) ***PURExpress in vitro Protein synthesis system:*** a reliable cell-free transcription/translation system reconstituted from highly purified *E. coli* components; allows for reverse affinity purification of protein of interest.

B.) ***NEBExpress Cell-free E. coli Protein Synthesis System:*** utilizes *E.coli* cell extracts; high yields at a value price; amendable for scale up.

Both systems offer robust expression of a wide range of proteins free of modification or degradation by simply mixing two tubes, followed by the addition of template DNA. With results available in a few hours, both systems save valuable laboratory time and are ideal for high throughput technologies.

NEB's protein synthesis systems can be used to express a wide range of proteins



(A) 25 µl reactions of PureExpress in vitro Protein Synthesis Kit containing 250 ng template DNA and 20 units RNase Inhibitor were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. M is a molecular weight marker.

(B) 50 µl reactions of NEBExpress Cell-free E. coli Protein Synthesis System containing 250 ng template DNA were incubated at 37°C for 3 hours. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad Range (NEB #P7717).

Ordering information:

PRODUCT	NEB #	SIZE
PURExpress In Vitro Protein Synthesis Kit	E6800S/L	10/100 rxns
NEBExpress Cell-free *E. coli* Protein Synthesis System	E5360S/L	10/100 rxns

For a complete list of protein expression tools, please visit *neb.com/proteinexpression*

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