NEB expressions

a scientific update

IN THIS ISSUE

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Over 40 years in protein expression and purification – a historical perspective

by Christopher H. Taron, Ph.D., James C. Samuelson, Ph.D., and Lydia Morrison, M.S., New England Biolabs, Inc.

New England Biolabs® (NEB®) has been integrally involved in expressing and purifying proteins since the dawn of the recombinant DNA era in the 1970s – whether it be for our own research interests for our manufacturing processes. In 1978, NEB began screening microorganisms for restriction enzymes. Our scientists remember the challenges involved in purifying limited amounts of restriction enzymes and other proteins from these native organisms isolated from the environment. The efforts of NEB scientists to clone, overexpress and purify restriction enzymes from recombinant systems greatly advanced the field of molecular biology. Many of the original methods used by NEB scientists have endured and have been applied by countless scientists to study the structure and function of individual proteins. Now NEB scientists are striving to develop faster, simplified methods for recombinant protein expression and purification which rely on engineered protein expression hosts or optimized cell-free systems.

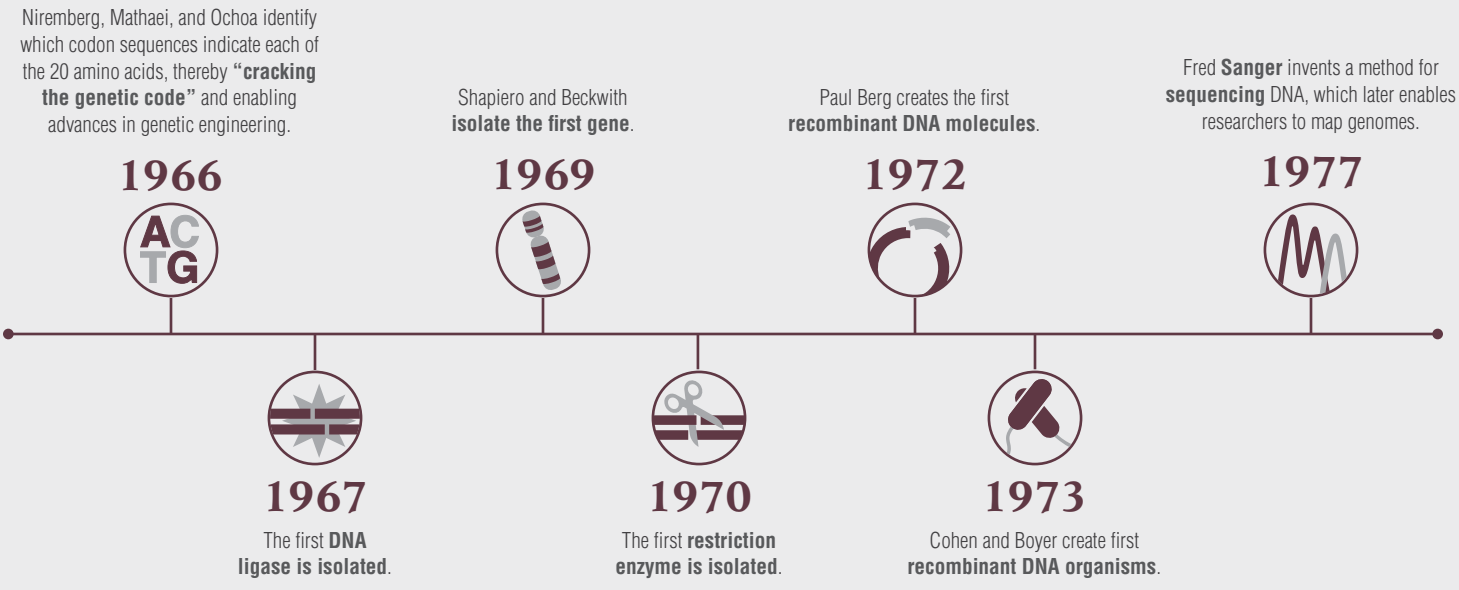
The period from 1966-77 saw a series of remarkable scientific breakthroughs. During this time, the genetic code was correctly interpreted, the first gene was isolated, and enzymes that both cut DNA at specific sequences (restriction enzymes) and that paste DNA pieces together (DNA ligases) were discovered. These discoveries ultimately enabled the cloning of the first genes and the creation of the first genetically modified microorganisms. Finally, in 1977, DNA sequencing technologies advanced beyond the laborious extension of just a few bases at a time and gave scientists the ability to unlock the genetic information encoded in any piece of DNA. The remarkable scientific advances of this decade, which made possible protein overexpression and purification, forever changed the course of biological and medical research, and enabled the emergence of the biotech industry (Figure 1).

NEB was founded in the midst of this era (1974) with the goal of providing researchers purified restriction enzymes, DNA ligases and other tools needed to clone and express genes. Restriction enzymes were the cornerstone of our early product offering. At that time, restriction enzymes were purified from bacteria isolated from the environment. This presented many challenges for commercial-scale production. For example, native restriction enzymes are generally not abundantly expressed and must be purified free from many other nucleases produced by an organism. Additionally, there were difficulties associated with large-scale culturing of various obscure microorganisms. Thus, to meet a steadily growing demand for these molecular tools, and to lower costs for our customers, NEB turned to recombinant DNA technology to clone and express enzymes in the laboratory bacterium, *Escherichia coli* (*E. coli*). This effort resulted in NEB producing some of the first recombinant enzymes available for commercial sale, and was the beginning of NEB's long-tenured experience with the process of recombinant protein expression.

Since these early days, recombinant protein expression has been integral to the success of NEB. Over the past forty years, we have continuously worked to invent and adopt new expression methodologies to improve the production of recombinant proteins. Our expertise has enabled the commercialization of over 550 recombinant enzymes to date. In this article, we highlight some of the major innovations in protein expression that have impacted our company's journey, with both a historical view and an eye to the future.

Figure 1:

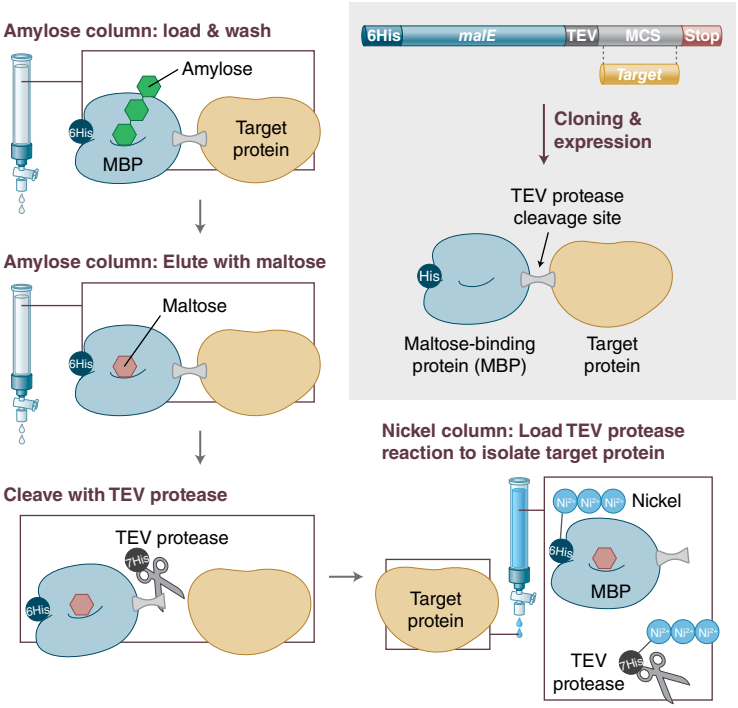
Advances in DNA Understanding were Foundational for Protein Overexpression*



**Created referencing the National Science Teaching Association's "Cloning Timeline".*

Figure 2:

Overview of the NEBExpress™ MBP Fusion and Purification System (previously known as the pMAL Protein Fusion and Purification System)



The target protein is fused to MBP, enhancing solubility and expression, which is followed by an easy and effective purification strategy.

Early recombinant protein expression in *E. coli*

NEB's interest in recombinant proteins was clearly evident by 1980. That year, the first recombinant enzymes were offered for sale. These enzymes were *E. coli* DNA Polymerase (Pol I), which was cloned by Bill Kelly in Noreen Murray's lab at Edinburgh University, and T4 DNA Ligase, cloned by Geoff Wilson in the same lab several years earlier. Dedicated research on protein expression at NEB also commenced that year – including efforts to create a vaccine against malaria using recombinant parasite surface antigens. The cloning and expression methodology being used was quickly adopted for use with restriction enzymes to increase yields, enable higher purity, and permit better characterization of restriction enzyme structure and function. NEB's early work involved establishing methods and tools to enable restriction enzyme cloning in *E. coli*, which had already become the standard for cloning and expression, and remains so today (1). In order to clone foreign restriction-modification systems in *E. coli* and over-produce individual restriction enzymes, it was necessary to characterize and eliminate the native methyl-dependent restriction systems of *E. coli*. Many of the key relevant discoveries were made by NEB scientists, who then genetically-tailored *E. coli* strains to be tolerant of restriction enzymes (2).

Cloning Vectors and Promoters

NEB's first efforts in cloning used the *E. coli* plasmid pBR322, an early plasmid vector made by Francisco Bolivar and Ray Rodriguez, who were post-docs in Herb Boyer's lab at the University of California, San Francisco. Incidentally, it was Herb Boyer who discovered EcoRI and demonstrated that the "sticky" ends it created could join DNA fragments from different sources, making it the first restriction enzyme useful for DNA cloning. NEB used derivatives of pBR322 that carried λPL, a powerful leftward promoter from bacteriophage Lambda, which is controlled by temperature ("off" at 32°C and "on" at 42°C). As pBR322 had only a moderate copy number (~30-40 copies per cell), NEB quickly adopted use of the higher copy number plasmid, pUC19, after its development by Jo Messing at the University of California Davis. The pUC19 vector offered multiple cloning sites, a much higher copy number (~250 copies per cell) and employed a promoter from the lac operon. In 1984, William Studier of Brookhaven National Labs developed an inducible T7 promoter system. With this method, a target gene is cloned downstream of the T7 promoter that is recognized by T7 RNA Polymerase (whose gene is integrated into the *E. coli* genome in expression strains). This strong promoter system is often capable of producing heterologous proteins, comprising up to 50% of total cellular protein. This approach became popular both at NEB and throughout the field.

NEB's internal efforts on recombinant restriction enzymes soon paid off. In 1982, PstI became the first product cloned and expressed by NEB scientists. The recombinant strain overexpressed PstI ~100-fold relative to the native organism. This allowed NEB to reduce the unit price of PstI 20-fold (i.e., supplying 20 times more enzyme for the same price). Following PstI, NEB cloned, overexpressed and sold an increasing number of restriction enzymes each year, beginning with EcoRI, HaeII, HindIII, followed by many more. Today, nearly all of the over 250 restriction enzymes we sell are purified from overexpression clones made at NEB.

Purification using Affinity Chromatography

Soon after NEB began producing recombinant restriction enzymes, there was a desire to couple more facile purification to the expression process. In the mid-1980's NEB began research on one of the first affinity-tagging systems. This approach employed fusing the gene encoding the *E. coli* maltose binding protein (MBP) in-frame with the target gene of interest. The resulting "fusion" protein can then be purified on amylose chromatography resin and the fusion tag can be removed using a site-specific protease. This system (the pMAL™ Protein Fusion and Purification System) was released in 1988 and was NEB's first kit that enabled customers to perform protein expression and purification with the same system. As an added benefit, it was later discovered that MBP has the natural ability to significantly increase the solubility of fused target proteins in *E. coli*.

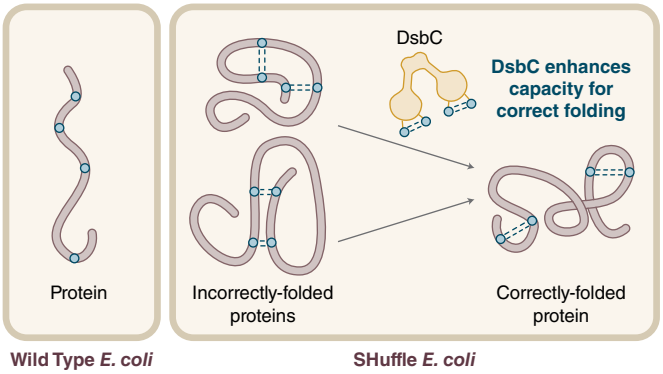
In the following years, the interest around affinity tags exploded. Additional fusion proteins (e.g., glutathione S-transferase [GST], chitin binding domain [CBD]) and many small peptide tags (poly-His-, FLAG-, S-tag-, Strep II- and poly-Arg-) were developed and used. Of these, the most influential was poly-His-tagging, which was developed by Roche in the late 1980's. His-tagged fusion proteins can be recovered using immobilized metal affinity chromatography (IMAC), which typically employs Ni^{2+} beads or resin. To the present day, poly-His-tagged protein expression and IMAC is the most common approach to affinity-based protein purification, as it tolerates a wide range of conditions, including the presence of protein denaturants, high salt and detergents. It can also be used with many common cell lysis reagents and a variety of buffer additives.

The removal of an affinity tag/fusion partner from a purified recombinant protein is commonly performed using digestion with site-specific proteases. A drawback to this approach is that the released target protein needs to be purified from the liberated tag and the protease through additional chromatography steps. If the fusion partner contains the same affinity tag as the protease, this simplifies purification of the target protein. An increasingly popular approach is to remove both the fusion partner (e.g., 6His-MBP) and the protease (His-tagged TEV) by a single IMAC capture step. This technique is employed in the NEBExpress MBP Fusion and Purification System (Figure 2).

Another NEB approach to affinity protein purification involved the use of auto-splicing protein domains

continued on page 4...

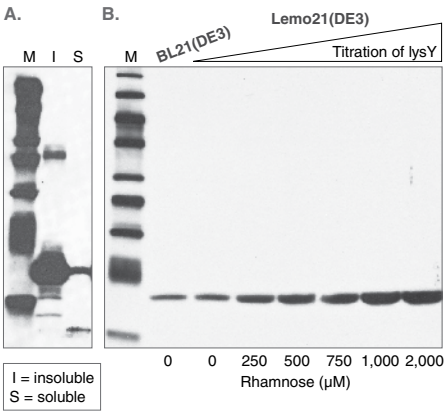
Figure 3:
Expression of protein with multiple disulfide bonds using SHuffle® Competent *E. coli*



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.

called "inteins". An intein was first described in 1988 in the context of protein splicing. In 1990, the first proof was provided that defined an intein as a protein domain that can catalyze its own excision from a protein. NEB researchers were studying inteins due to their presence in certain hyperthermophilic DNA polymerases, and described the intein reaction mechanism. Soon after, this research converged with protein expression and resulted in a new intein-mediated strategy for fusion protein removal without the need for protease cleavage. In this approach, *E. coli* expression of a target protein carrying an intein-chitin binding domain (intein-CBD) tag permits one-step purification using chitin resin. Upon passage of a cell lysate over chitin resin, the fusion protein becomes immobilized, after which the target protein can be released from CBD by inducing intein auto-cleavage with addition of a thiol-containing buffer or by pH shift. This work was commercialized as NEB's IMPACT™ (Intein Mediated Purification with an Affinity Chitin-binding Tag) Kit in the late-1990s (3).

Figure 4:
Western analysis of 6-His tagged Brugia malayi protein



A) B. malayi protein expressed at 20°C in BL21(DE3). B) Soluble fractions of B. malayi protein expressed at 30°C in BL21(DE3) or Lemo21(DE3).

Solving Protein Expression Problems

As NEB has grown, so has our need to express classes of proteins outside of restriction enzymes. This has presented new challenges – as not all proteins express well, or at all, in *E. coli*. In addition to offering the popular BL21 and BL21(DE3) expression strains, NEB has focused on solving expression of "difficult" proteins. We have sought to improve the ability of *E. coli* to express various challenging proteins, including those with multiple disulfide bonds, with transmembrane domains, or that are toxic to the host.

Expressing Proteins Containing Disulfide Bonds

Disulfide bonds are post-translational covalent linkages formed by the oxidation of a pair of cysteines. Native disulfide bonds increase the stability of a protein and are often found in proteins that reside outside the chaperone rich environment of the cytoplasm, such as secreted peptides, hormones, antibodies, interferons and extracellular enzymes. When proteins are expressed in *E. coli*, it can be difficult for them to fold correctly. In 2009, NEB commercialized SHuffle® expression strains, which are engineered to support correct folding of proteins with multiple disulfide bonds in the cytoplasm (Figure 3). These strains constitutively express DsbC disulfide isomerase within the cytoplasm to promote the correction of mis-oxidized proteins (4).

Membrane or Toxic Protein Expression

Expression of membrane proteins is challenging for most heterologous systems, and often results in protein aggregation and misfolding due to the hydrophobic nature of transmembrane segments. When working with *E. coli* as a host, it is advantageous to express membrane proteins in moderation to avoid saturation of the membrane protein biogenesis pathway. NEB's Lemo21(DE3) Competent *E. coli* strain was designed for tunable protein expression to achieve optimal assembly of transmembrane proteins or the optimal folding of soluble proteins (Figure 4) (5).

In cases where the heterologous protein is toxic to cells, tightly controlling gene expression can improve host viability by maintaining expression levels of a toxic target protein just below a host strain's tolerance. In strong T7 promoter-based systems, an effective means to control expression is to employ a host strain that expresses a T7 RNA Polymerase inhibitor protein (LysY) as in NEB's Lemo21(DE3) or T7 Express *lysY/I*^{q} strains (see page 8 for details).

To express a highly toxic protein, it may be necessary to employ a cell-free expression system: NEB's PURExpress® *In Vitro* Protein Synthesis Kit is reconstituted from purified components necessary for *E. coli* translation (see page 6 for details). This kit can also be used with the PURExpress Disulfide Bond Enhancer to improve protein folding. Alternatively, the NEBExpress™ Cell-free *E. coli* Protein Synthesis System utilizes a cell lysate which provides high-level expression of target proteins from linear or plasmid DNA templates (see page 5 for details).

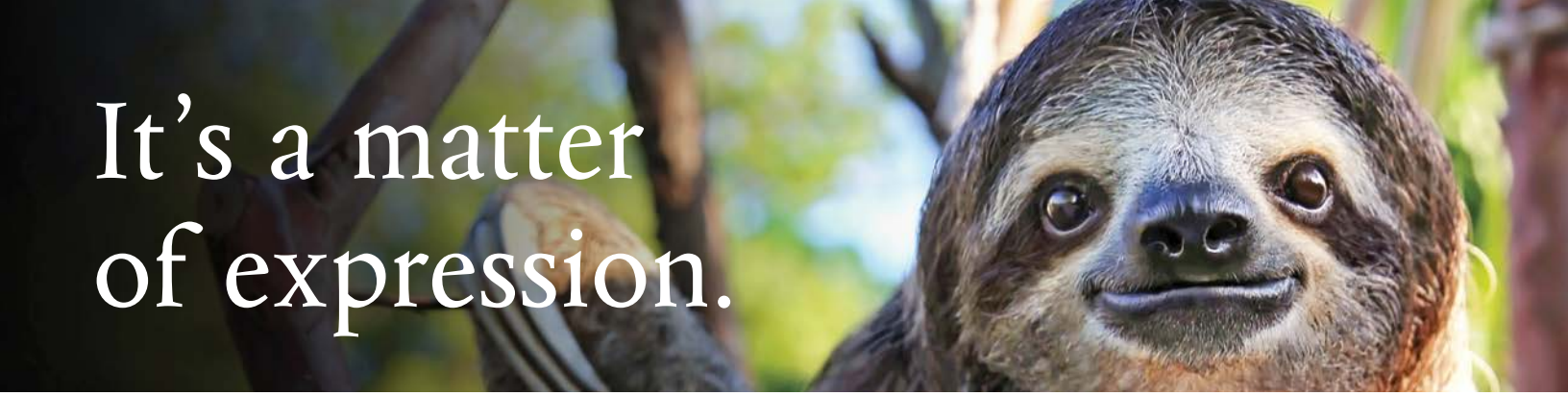
The Future of Protein Expression

The protein expression field is constantly evolving. Applications such as protein engineering and synthetic biology are driving the field toward high throughput protein expression. Scientists now desire to test hundreds, if not thousands, of expressed proteins in a single day to quickly narrow their focus to the most interesting variants. As the standard method of cloning, vector introduction into a host strain, and cell propagation takes multiple days, it is becoming clear that cell-free protein expression, which can be accomplished in as little as one hour, will become increasingly important in the coming years. Just as *in vivo* protein expression started from humble beginnings and has progressed to highly engineered host strains and regimented bioprocessing, we anticipate a similar revolution in cell-free protein expression systems. A new generation of NEB scientists are dedicated to advancing cell-free expression by engineering novel cell lines, developing improved cell-free system manufacturing processes (such as those employed for PURExpress or NEBExpress), optimizing cell-free system formulations and exploring the potential for system scale up for production of milligram to gram quantities of protein.

References

1. Rosano, G.L. and Ceccarelli, E.A. (2014) *Front. Microbio.* 5, 172.
2. Raleigh, E.A. and Wilson, G. (1986) *PNAS,* 83, 9070–9074.
3. Chong, S. et al. (1997) *Gene,* 192, 271-281.
4. Lobstein, J. et al. (2012) *Microb. Cell. Fact.* 11, 56.
5. Wagner, S. et al. (2008) *PNAS,* 105, 14371-14376.

For more information on products mentioned in this article, please visit
www.neb.com/ProteinExpression



It's a matter of expression.

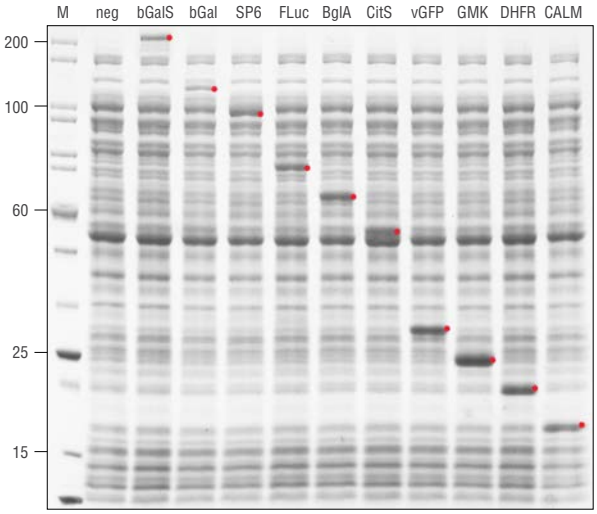
For over 40 years, New England Biolabs has been developing and using recombinant protein technologies in *E. coli* for our own manufacturing processes. Our NEBExpress™ portfolio of products includes solutions for expression and purification and is supported by access to scientists with over 40 years of experience in developing and using recombinant protein technologies in *E. coli*. We use these solutions in our own research and manufacturing processes and know that quality and performance are critical – all of our products are stringently tested so that you can be sure they will work optimally for your solution, just as we rely on them to work in ours.

Synthesize high yields of protein with the NEBExpress™ Cell-free *E. coli* Protein Synthesis System

The NEBExpress Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA template under the control of a T7 RNA Polymerase promoter. The system offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost-effective for high-throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening.

The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain of *E. coli*, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

The NEBExpress Cell-free *E. coli* Protein Synthesis System can be used to express a wide range of proteins



50 µl reactions 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); "neg" = negative control, no DNA.

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Free Sample

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Advantages:

- Synthesize high yields of protein (typically 0.5 mg/ml) in approximately 2–4 hours
- Express a wide variety of target proteins ranging from 17 to 230 kDa
- Templates can be plasmid DNA, linear DNA, or mRNA
- RNase contamination can be inhibited by the supplied RNase inhibitor, eliminating clean-up steps
- Flexible reaction conditions achieve maximum yield; protein synthesis can be sustained for 10 hours at 37°C or up to 24 hours at lower temperatures
- Reactions can be miniaturized or scaled up to yield milligram quantities of protein

Applications:

- Quickly generate analytical amounts of protein for further characterization
- Use for high throughput screening and liquid handling
- Study epitope mapping and protein folding
- Express toxic proteins

Ordering information:

Product	NEB #	Size
NEBExpress Cell-free *E. coli* Protein Synthesis System	E5360S/L	10/100 rxns
NEBExpress GamS Nuclease Inhibitor	P0774S	75 µg

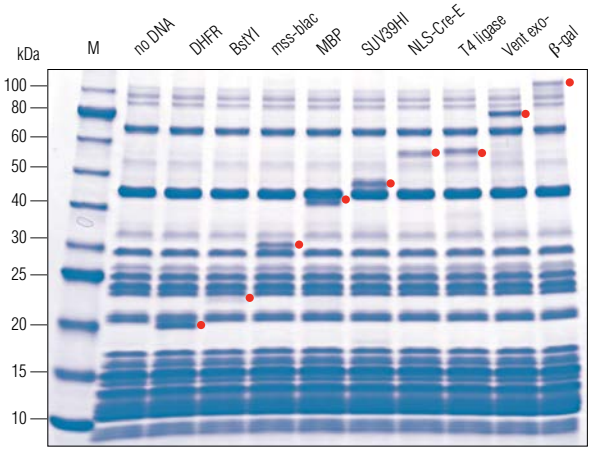
Explore our expression and purification portfolio at

www.neb.com/ProteinExpression

Express high yields of protein with the PURExpress® *In Vitro* Protein Synthesis Kit

A rapid method for gene expression analysis, PURExpress is a reliable cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Express a wide range of proteins free of modification or degradation by mixing two tubes, followed by the addition of template DNA. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies. Due to reconstituted nature, synthesized protein can be reverse-purified or subject to direct functional analysis. Also, several kits are offered where translation factors or macromolecules have been omitted to facilitate specific studies.

The PURExpress *In Vitro* Protein Synthesis Kit can be used to express a wide range of proteins



25 µl reactions 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. Marker M is the Protein Ladder.

PURExpress is based on the PURE system technology originally developed by Dr. Takuya Ueda at the University of Tokyo and commercialized as the PURESYSTEM™ by Biocomber (Tokyo, Japan).

Advantages:

- Reconstituted from purified & His-tagged *E. coli* translation components with only minimal nuclease and protease activity
- Protein can be synthesized in a few hours and directly visualized on a Coomassie-stained gel
- Synthesized protein can be reverse-purified or subject to direct functional analysis

Applications:

- Quickly generate analytical amounts of proteins for further characterization
- Generate truncated proteins to identify active domains and functional residues, or confirm open reading frames
- Introduce modified, unnatural or labeled amino acids
- Study ribosome structure and function release factor function, or epitope mapping

Ordering information:

Product	NEB #	Size
PURExpress *In Vitro* Protein Synthesis Kit	E6800S/L	10/100 rxns
PURExpress Δ Ribosome Kit	E3313S	10 rxns
PURExpress Δ (aa, tRNA) Kit	E6840S	10 rxns
PURExpress Δ RF123 Kit	E6850S	10 rxns
PURExpress Disulfide Bond Enhancer	E6820S	50 rxns
E. coli Ribosome	P0763S	1 mg

Simplify your Ni purification with NEBExpress

NEB offers a selection of products for affinity chromatography using polyhistidine-tagged (His-tagged) fusion proteins. Generate highly pure protein (>95%) with high specific binding under native or denaturing conditions.

- The **NEBExpress Ni-NTA Magnetic Beads** (NEB #S1423S/L) enable small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats.
- The **NEBExpress Ni Spin Columns** (NEB #S1427S/L) are ready-to-use, and can purify ≥ 1 mg of His-tagged protein in as little as 15 minutes.
- The **NEBExpress Ni Resin** (NEB #S1428S) can be used in gravity or pressure flow columns, and batch purifications.

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See page 7.

Let us help you find the purification beads, columns and resins that will work best for you

Isolation of pure substrates or proteins for downstream experiments is a common, yet time consuming, task. New England Biolabs offers a variety of resins and magnetic beads that are easy-to-use, highly specific, and available in several different formats for rapid isolation and purification of proteins, nucleic acids and immunoglobulins.

SPECIAL PRICE* until 30.04.2020

Please look at www.neb-online.fr/nebexpress20 for details!

	Protein Purification	Large-Scale Purifications	Use In Automated Chromatography	High-Throughput	Biotinylated Substrate Binding	Protein Pull-Down	Nucleic Acid Pull-Down	mRNA Purification/Pull-Down	Immunoprecipitation	Cell Separation/ Cell Sorting
NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)	● (His-tag)			●		●				
NEBExpress Ni Spin Columns (NEB #S1427)	● (His-tag)			●		●				
NEBExpress Ni Resin (NEB #S1428)	● (His-tag)	●	●			●				
Amylose Resin (NEB #E8021)	● (MBP)	●				●				
Amylose Resin High Flow (NEB #E8022)	● (MBP)	●	●			●				
Amylose Magnetic Beads (NEB #E8035)	● (MBP)			●		●				
Anti-MBP Magnetic Beads (NEB #E8037)	● (MBP)			●		●				
Chitin Resin (NEB #S6651)	● (intein-CBD tag)	●				●				
Chitin Magnetic Beads (NEB #E8036)	● (intein-CBD tag)			●		●				
Oligo d(T)$_{25}$ Magnetic Beads (NEB #S1419)				●			●	●		
Streptavidin Magnetic Beads (NEB #S1420)				●	●	● (biotinylated bait)	● (biotinylated bait)			
Hydrophilic Streptavidin Magnetic Beads (NEB #S1421)				●	●	● (biotinylated bait)	● (biotinylated bait)			
Protein A Magnetic Beads (NEB #S1425)				●					●	
Protein G Magnetic Beads (NEB #S1430)				●					●	
Goat Anti-Mouse IgG Magnetic Beads (NEB #S1431)				●					● (Mouse IgGs)	●
Goat Anti-Rabbit IgG Magnetic Beads (NEB #S1432)				●					● (Rabbit IgGs)	●
Goat Anti-Rat IgG Magnetic Beads (NEB #S1433)				●					● (Rat IgGs)	●
Magnetic mRNA Isolation Kit (NEB #S1550)				●				●		

Try NEB's competent cells for expression of a wide range of proteins

NEB offers a wide selection of competent cell strains ideal for expression of a variety of proteins. Proteins with multiple disulfide bonds are correctly oxidized to significantly higher yields with SHuffle strains. Tunable T7 expression is achieved with Lemo21(DE3), an ideal strain for difficult targets, including membrane proteins. NiCo21(DE3) is designed for the expression and purification of His-tagged proteins. NEB Express and T7 Express Competent *E. coli* are offered with varying levels of control. Only NEB offers exceptional control of T7 expression by the *lysY* gene, which is ideal for proteins that are difficult to express or toxic to the cell. Each strain is provided with a protocol for optimal expression.

Strain	Characteristics	NEB #	Size
NEB Express Competent *E. coli*	• Versatile non-T7 expression strain • Protease deficient	C2523H/I	20 x 0.05 ml/ 6 x 0.2 ml
NEB Express *I^q* Competent *E. coli*	• Control of IPTG induced expression from P_{tac}, P_{lac} and P_{trc} • Protease deficient	C3037I	6 x 0.2 ml
T7 Express Competent *E. coli*	• Most popular T7 expression strain • Protease deficient	C2566H/I	20 x 0.05 ml/ 6 x 0.2 ml
T7 Express *lysY* Competent *E. coli*	• T7 expression • Protease deficient • Better reduction of basal expression	C3010I	6 x 0.2 ml
T7 Express *lysY/I^q* Competent *E. coli*	• T7 expression • Protease deficient • Highest level of expression control	C3013I	6 x 0.2 ml
SHuffle Express Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	C3028J	12 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	C3029J	12 x 0.05 ml
SHuffle T7 Express *lysY* Competent *E. coli*	• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	C3030J	12 x 0.05 ml
SHuffle T7 Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	C3026J	12 x 0.05 ml
BL21 Competent *E. coli*	• Routine expression for non-T7 Vectors • Protease deficient	C2530H	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	• Routine T7 Expression • Protease deficient	C2527H/I	20 x 0.05 ml/ 6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	• Tunable T7 Expression for difficult targets • Protease deficient	C2528J	12 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	• Expression and purification of His-tagged proteins • Protease deficient	C2529H	20 x 0.05 ml

Note: *Store Competent Cells at –80°C. Once thawed, do not refreeze. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.*

Troubleshooting tips for protein expression with T7 express strains

No Colonies or No Growth in Liquid Culture

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In *I^q* strains over-expression of the *LacI^q* repressor reduces basal expression of the T7 RNA Polymerase.
  - In *lysY* strains, mutant T7 lysozyme is produced, which binds to T7 RNA Polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA Polymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues.
- Check antibiotic concentration (test with control plasmid).

No Protein Visible on Gel or No Activity

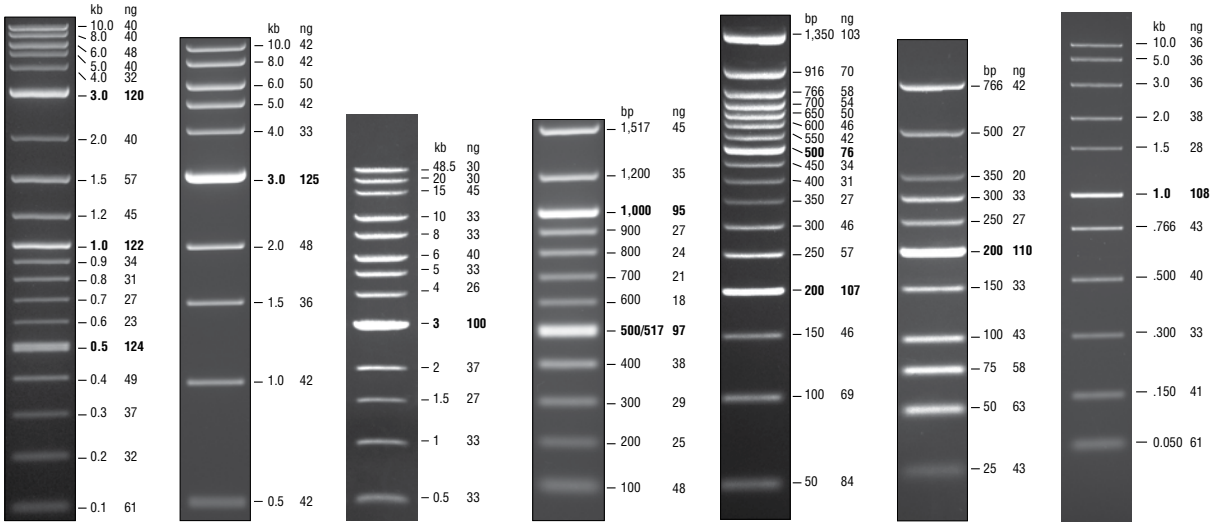
- Check for toxicity – the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test *I^q* and/or *lysY* strains to reduce basal expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced Protein is Insoluble

T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:

- Induce at lower temperatures (12–15°C overnight).
- Reduce IPTG concentration to 0.01 mM – 0.1 mM.
- Induce for less time (as little as 15 minutes).
- Induce earlier in growth (OD_{600} = 0.3 or 0.4).

NEB's Markers and Ladders – built for perfect migration!



1 kb Plus
1.0% TBE agarose gel. Mass values are for 1 µg/lane.

1 kb
0.8% TAE agarose gel. Mass values are for 0.5 µg/lane.

Quick-Load 1 kb Extend DNA Ladder
0.5% TBE agarose gel. Mass values are for 0.5 µg/lane.

100 bp
1.3% TAE agarose gel. Mass values are for 0.5 µg/lane.

50 bp
3% TBE agarose gel. Mass values are for 1 µg/lane.

Low Molecular Weight
3% TBE agarose gel. Mass values are for 0.5 µg/lane.

Fast DNA Ladder***
1.2% TBE agarose gel. Mass values are for 0.5 µg/lane.

AVAILABLE FORMATS:

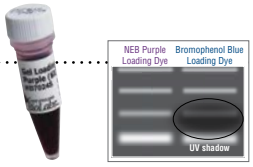
Standard*	#N3200	#N3232		#N3231	#N3236	#N3233	#N3238
Quick-Load® Purple*	#N0550	#N0552		#N0551	#N0556	#N0557	
Quick-Load®	#N0469	#N0468	#N3239	#N0467			
TriDye™	#N3270	#N3272		#N3271			
For Safe Stains**:	#N0559						

* All Quick-Load Purple Ladders include a free vial of Gel Loading Dye, Purple (6X), no SDS.
** Safe stain format is compatible with GelRed®, GelGreen® and SYBR® precast gels.
*** Fast DNA Ladder can be used for fast electrophoresis systems (e.g., E-Gels®) as well as standard electrophoresis.

Ready-to-Load – pre-diluted for added convenience:

- Quick-Load Purple: containing purple loading dye
- Quick-Load: containing Bromophenol Blue
- TriDye: containing three dyes for better visualization
- For Safe Stains: compatible with GelRed®, GelGreen® and SYBR® precast gels

***No UV shadow and sharper bands!:**
All "Standard" Ladders and all Quick-Load Purple Ladders include a free vial of Gel Loading Dye, Purple (6X), no SDS (#B7025).



Protein Markers from NEB

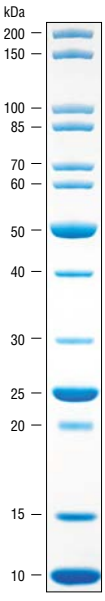
Built for perfect migration & "ready-to-load"

Advantages

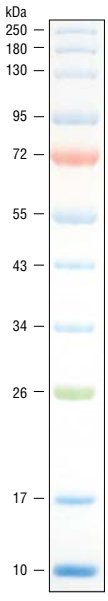
- Suitable for a wide range of proteins (from 10 to 250 kDa)
- "ready-to-load" – no heating prior to gel loading required
- Uniform band intensities
- Easy-to-identify reference bands
- Shelf life: 24 months at –20°C; 12 months at 4°C; 2 weeks at RT (25 °C)

Ordering information:

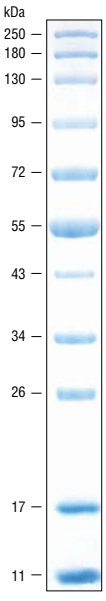
Product	NEB #	Size
Unstained Protein Standard, Broad Range (10-200 kDa)	P7717S/L	150 / 750 lanes
Blue Prestained Protein Standard, Broad Range (11-250 kDa)	P7718S/L	150 / 750 lanes
Color Prestained Protein Standard, Broad Range (10–250 kDa)	P7719S/L	150 / 750 lanes



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE Gel
#P7717



Color Prestained Protein Standard, Broad Range (10–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel
#P7719

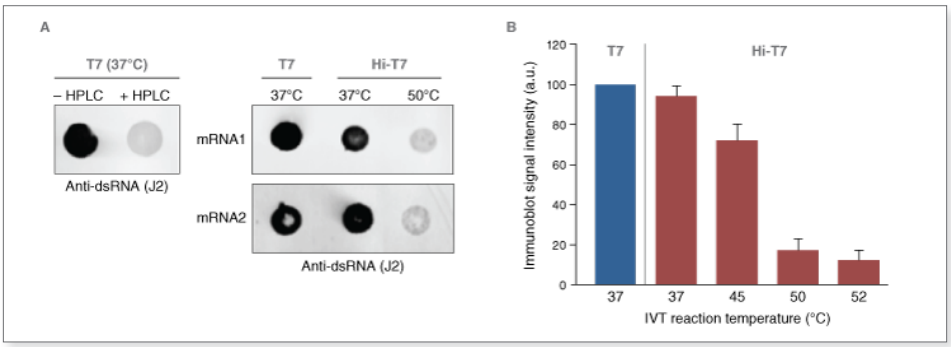


Blue Prestained Protein Standard, Broad Range (11–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel
#P7718

Limited Offer: Contact us via info.fr@neb.com for a free sample!

New: Hi-T7® RNA Polymerase (High Concentration) for reduced dsRNA by-product formation

Hi-T7 RNA Polymerase is an engineered DNA-dependent RNA polymerase that is highly specific for T7 phage promoters, designed for *in vitro* transcription of RNA at higher temperatures and recommended for experienced users interested in building and optimizing their own *in vitro* transcription reactions.



Immunoblot using an anti-dsRNA antibody (J2) shows presence of dsRNA by-products in the IVT reactions for both T7 and Hi-T7 RNA Polymerases when IVT is performed at 37°c. HPLC purification of the IVT RNA eliminates dsRNA by-products. dsRNA by-products are reduced when IVT is performed at 50°c (or higher temperatures) with Hi-T7.

Advantages

- Active from 37-56°C, optimal incubation temperature is 50-52°C.
- Increased co-transcriptional capping efficiency with cap analogs
- Decreased unwanted immunogenicity from RNA synthesized at higher temperature due to reduced dsRNA by-product formation

Ordering information:

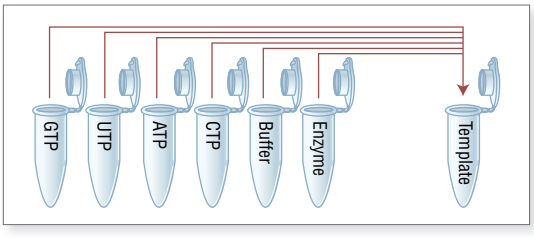
Product	NEB #	Size
Hi-T7 RNA Polymerase (High Concentration)	M0470T	50.000 units
Hi-T7 RNA Polymerase	M0658S	5.000 units

Fast and reliable RNA synthesis up to 180 µg per reaction: HiScribe™ T7 (Quick) High Yield RNA Synthesis Kit

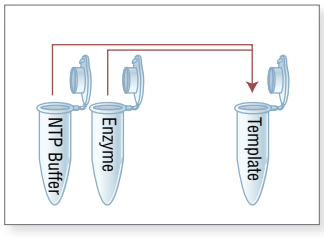
The HiScribe T7 High Yield RNA Synthesis Kit (NEB #E2040) delivers robust RNA synthesis for a wide range of template sizes. Flexible protocols ensure that performance is maintained even under demanding conditions, such as extended reaction time using very low amounts of template. Protocols are included for partial or complete incorporation of modified or labeled nucleotides in the transcript body, and cap analogs at the RNA 5' end.

The HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050) utilizes a master mix format, allowing for faster reaction setup. DNase I and lithium chloride are included for DNA template removal and quick RNA purification.

HiScribe T7 High Yield RNA Synthesis Kit



HiScribe T7 Quick High Yield RNA Synthesis Kit



Advantages

- Streamlined format & Quick Workflows
- High Yield – up to 180 µg of RNA from a standard 20 µl reaction
- Flexibility – enables incorporation of cap analogs, radiolabeled and modified nucleotides
- Also available as kits to include ARCA-capping and poly(A) tailing (#E2060 & #E2065)

Ordering information:

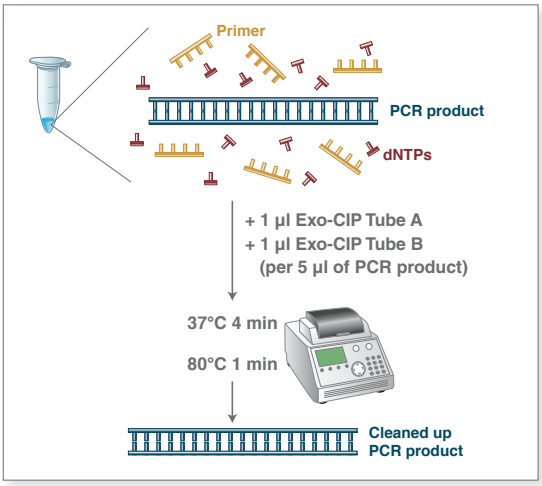
Product	NEB #	Size
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 rxns
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 rxns
HiScribe T7 ARCA mRNA Kit (with Tailing)	E2060S	20 rxns
HiScribe T7 ARCA mRNA Kit	E2065S	20 rxns
HiScribe SP6 RNA Synthesis Kit	E2070S	50 rxns

Doing Sanger Sequencing? Experience the benefits of the Exo-CIP™ Rapid PCR Cleanup Kit & get your free sample now!

The Exo-CIP Rapid PCR Cleanup Kit contains thermolabile versions of Exonuclease I (Exo I) and Calf Intestinal Phosphatase (CIP) that will degrade residual PCR primers and dephosphorylate excess dNTPs after PCR amplification. Degradation occurs in 4 minutes at 37°C, and is immediately followed by rapid inactivation of the enzymes by heating for 1 minute at 80°C.

In just 5 minutes, the PCR product is ready for downstream analysis, such as Sanger Sequencing, SNP detection or library prep for next generation sequencing.

Exo-CIP Rapid PCR Cleanup Kit workflow



Limited Offer: Contact us via info.fr@neb.com for a free sample or visit www.neb.com/E1050!

Advantages:

- Save time with our 5-minute protocol
- Eliminate buffer exchange step, as kit is compatible with commonly-used reaction buffers
- Use with PCR products of varying length
- Enables high quality sequencing results

Ordering information:

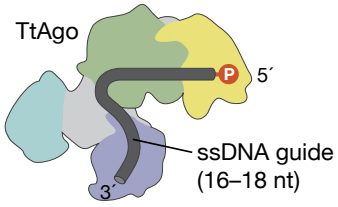
Product	NEB #	Size
Exo-CIP Rapid PCR Cleanup Kit	E1050S	100 reactions
Exo-CIP Rapid PCR Cleanup Kit	E1050L	400 reactions

Enzyme for Innovation

Tth Argonaute (TtAgo) – the first programmable, ssDNA-dependent Endonuclease!

Are you looking for a cool new enzyme with hot exciting activities that spurs your innovation? Here it is: the first commercially available prokaryotic argonaute, *Tth* Argonaute (TtAgo), which functions as a programmable DNA-guided endonuclease when provided with a 16–18 nucleotide long 5'-phosphorylated single-stranded DNA oligonucleotide guide. Unlike Cas9 and Cas12a used for CRISPR/Cas gene editing experiments, TtAgo's guide/target sequence selection is not limited by the requirement of an adjacent sequence motif. It is highly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates) between 65–85°C. *Tth* Argonaute is recommended for *in vitro* application.

TtAgo binds a short, 5´-phosphorylated ssDNA guide



Visit www.neb.com and look for more cool "Enzymes for Innovation"!

Advantages:

- Short 16–18 nucleotide 5'-phosphorylated ssDNA guides
- Guide/target sequence selection is not limited by the requirement of an adjacent sequence motif
- Highly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates)

Ordering information:

Product	NEB #	Size
Tth Argonaute (TtAgo)	M0665S	50 pmol

The *core* of your NGS Library Prep

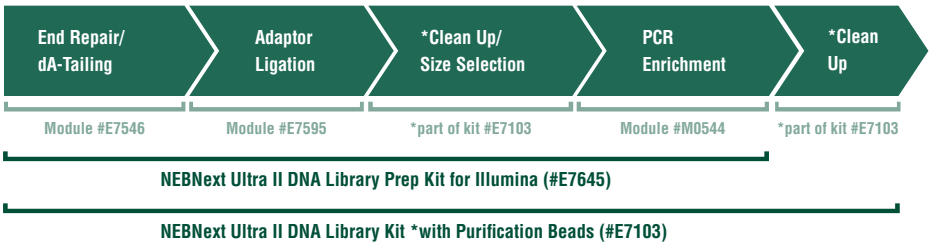
One central workflow for a wide range of applications: NEBNext® Ultra™ II reagents & kits

The NEBNext Ultra II reagents and kits for Illumina platforms compose the core of your NGS library preparation: a perfect combination of reagents, optimized formulations and simplified workflows. With incredibly short "hands-on" time you create libraries of highest quality and yields even when starting from extremely low input quantities.

Easily scalable and already automated on various robotic platforms, the **Ultra II DNA workflow** is *the* centerpiece of the modular NEBNext system: You will find this central workflow in many other NEBNext solutions, such as the Fragmentation System, RNA-seq, Single Cell/Low Input RNA-seq and more. The NEBNext Ultra II workflow is available in a convenient kit format or as separate NEBNext modules, adding additional flexibility to your experimental design.

Library Prep couldn't be better and easier!

The ULTRA II DNA WORKFLOW is availble in convenient kit formates or modules:



Your benefits:

- Highest library yields and quality
- Fewer PCR-cycles
- Low input amounts

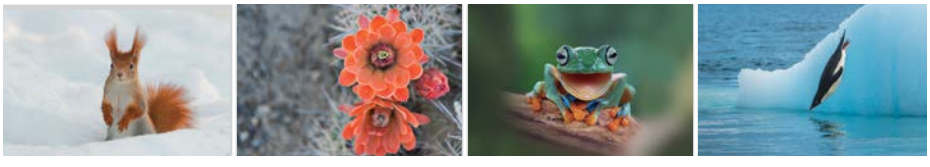
- „Hands on" time : <15 minutes
- Total time: Just ~2:30 – 3:00 hrs

Choose the convenient NEBNext Ultra II DNA Library Prep Kit for:

- Whole Genome Seq
- Standard & Low Input Seq
- ChIP-seq, NICE-seq, Cut&Run-Seq
- Exome Capture
- Targeted Sequencing
- FFPE-Material
- cfDNA ...

The ULTRA II DNA WORKFLOW IS ALSO THE CORE OF:

Enzymatic Methyl-Seq (bisulfite-free)	Directional & non-directional RNA-seq	Enzymatic DNA Fragmentation System	Single Cell/ Low Input RNA Library Prep



For more information incl. an overview of all NEBNext Ultra II Kits and modules etc., please visit www.NEBNext.com or contact us via info.fr@neb.com

Advantages:

- Learn one central workflow and apply it to a whole suite of different applications
- Save time with streamlined, modular workflows, reduced hands-on time, and automation compatibility
- Benefit from low input amount requirements, fewer PCR cycles and extremely uniform GC-coverage in all applications

TOOLS & RESOURCES

- View performance data in our Technical Notes, which can be downloaded at **NEBNextUltraII.com**
- View the NEBNext Ultra II DNA protocol video for protocol steps, and tips for optimization
- Find hundreds of peer reviewed publications citing use of NEBNext Ultra II DNA on the product pages at **NEB.com**

Ordering information:

Product	NEB #	Size
NEBNext Ultra II DNA Library Prep Kit for Illumina *	E7645S/L	24 /96 rxns
NEBNext Ultra II FS DNA Library Prep Kit for Illumina *	E7805S/L	24 /96 rxns
NEBNext Ultra II RNA Library Prep Kit for Illumina *	E7770S/L	24 /96 rxns
NEBNext Ultra II Directional RNA Library Prep Kit Illumina *	E7760S/L	24 /96 rxns
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24 /96 rxns
NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24 /96 rxns

*also available as Kit with Sample Purification Beads!

Selected popular applications utilizing the NEBNext Ultra II Workflow (or modules):

NEBNext Ultra II FS Library Prep Workflow:

Enzymatic Fragmentation & End Repair/ dA-Tailing → Adaptor Ligation → *Clean Up/ Size Selection → PCR Enrichment → *Clean Up

Module #E7810

NEBNext Ultra II FS Library Prep Kit (#E7805)

NEBNext Ultra II FS DNA Library Prep *with Sample Purification Beads (#E6177)

Limited Offer: Contact us via info.fr@neb.com for a free sample!

Ultra II DNA FS input amounts:
100 pg – 0.5 µg unsheared DNA

- "Hands on" time: <15 minutes
- Total time: ~ 2:30 hrs

NEBNext Ultra II directional or non-directional RNA Workflow:

Poly(A) enrichment OR rRNA depletion → RNA fragmentation and priming → First strand cDNA synthesis → Second strand cDNA synthesis → End Repair/ dA-Tailing → Adaptor Ligation → *Clean Up/ Size Selection → PCR Enrichment → *Clean Up

Module #E7490 or various depletion kits

Module #E6150 | Module #E7771 | Module #E7550 or #E6111

NEBNext Ultra II directional or non-directional RNA Library Prep Kits (#E7760 and #E7770)

NEBNext Ultra II directional or non-directional RNA Library Prep Kits *with Sample Purification Beads (#E7765 or #E7775)

Ultra II RNA input amounts:
5 ng – 1 µg total RNA (rRNA depletion)
10 ng – 1µg total RNA (poly(A) mRNA)

- "Hands on" time: <30 minutes
- Total time: ~ 5:30 – 6:30 hrs

NEBNext Single Cell/Low Input RNA Workflow:

Single Cell Lysis → Reverse Transcription → Template Switching → cDNA Amplification → End Repair/ dA-Tailing → Adaptor Ligation → Clean Up/ Size Selection → PCR Enrichment → Clean Up

Module #E5530

Sample Purification Beads not included

Sample Purification Beads not included

Module #E6421

NEBNext Single Cell/Low Input RNA Kit (#E6420)

Single Cell/Low Input, RNA input amounts:
2 pg – 200 ng total RNA

- "Hands on" time: <30 minutes
- Total time: 6 hrs – 7 hrs

NEBNext Enzymatic Methyl-seq Workflow:

Genomic DNA Shearing by Sonication → End Repair/ dA-Tailing → Adaptor Ligation → Oxidation of 5mC and 5hmC → Deamination of C to U → PCR Amplification → Clean Up

not included

Module #E7125 | Module #M0597

NEBNext Enzymatic Methyl-seq Kit (#E7120) incl. Sample Purification Beads

EM-seq input amounts:
10 ng – 200 ng

- Total time: 6 hrs – 7 hrs

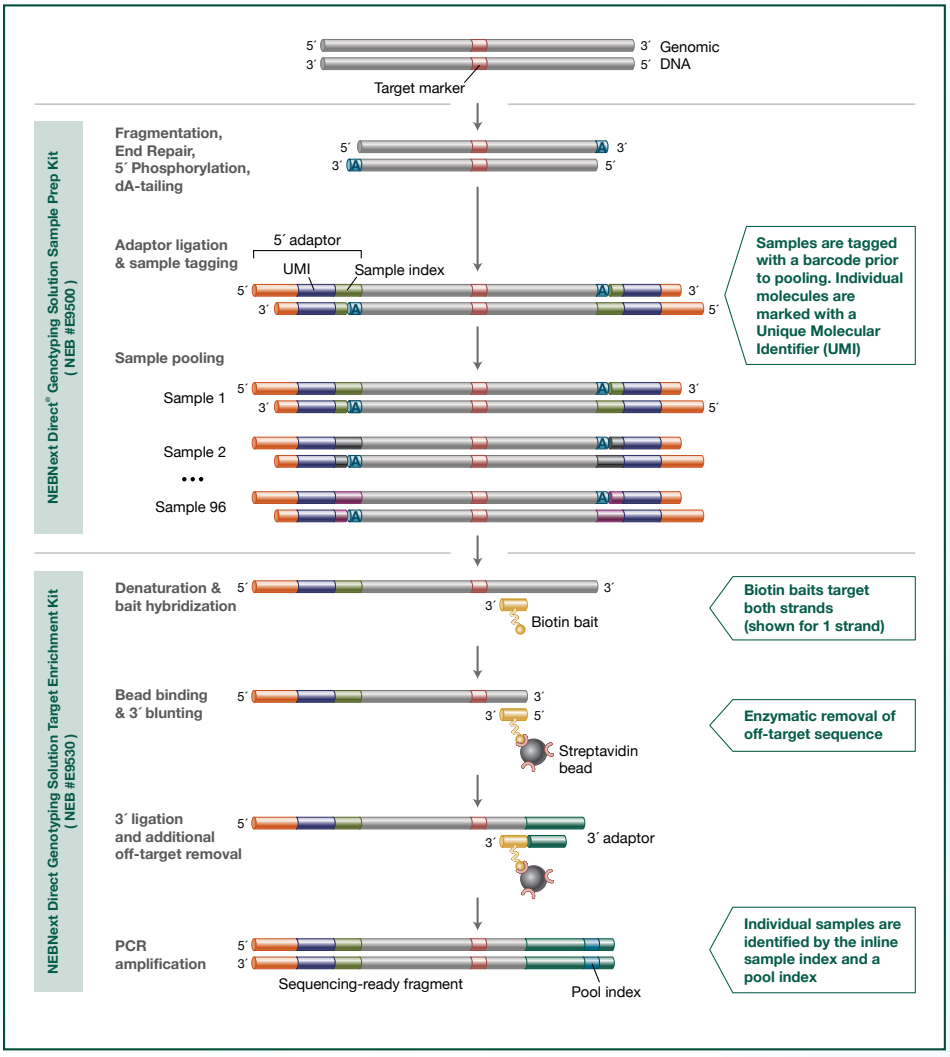
NEBNext Direct® Genotyping Solution – High throughput targeted genotyping for Illumina®

The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next generation sequencing to deliver cost-effective, high throughput genotyping for a wide variety of applications. Applicable for ranges spanning 100-5,000 markers, pre-capture multiplexing of up to 96 samples combined with dual indexed sequencing allows over 3.8 million genotypes in a single Illumina sequencing run.

Advantages:

- Single-day workflow
- 96-plex pre-capture sample multiplexing of hundreds to thousands of markers
- Bait design and sample multiplexing to maximize sequencer efficiency
- High specificity and coverage uniformity
- Already automated on the Biomek® i7 Workstation (Beckman Coulter Life Sciences)

NEBNext Direct Genotyping Solution Workflow:

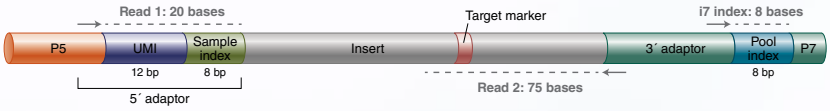


The NEBNext Direct Genotyping Solution begins with 25-100 ng of purified genomic DNA. The DNA molecules are enzymatically fragmented and 5′ tagged with an Illumina-compatible P5 adaptors, incorporating both an inline sample index to tag each sample prior to pooling and an inline Unique Molecular Identifier (UMI) to mark each unique DNA fragment within the samples. Up to 96 samples are subsequently pooled together prior to hybridization-based enrichment using biotinylated baits and captured on streptavidin beads. For the remainder of the protocol, up to 96 samples are processed as a single pool through ligation of a 3′ adaptor, removal of off-target sequence and final PCR, which amplifies the material and adds a second pool index to produce the final sequencing-ready fragment.
An expanded post-capture pool index strategy is available upon request to process a maximum of 9216 samples in a single Illumina sequencing run.

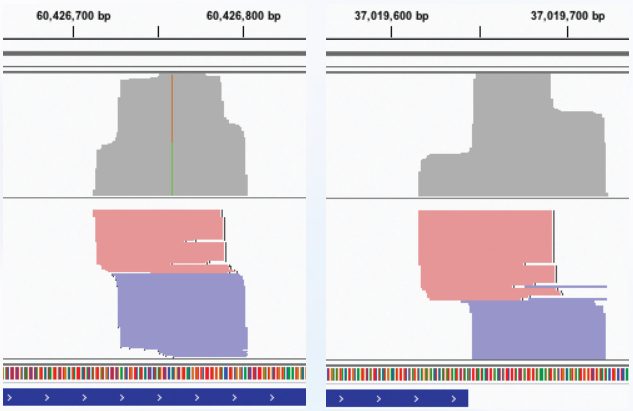
Optimized Bait Design

The NEBNext Direct Genotyping Solution employs a purpose-built bait designer that has been optimized to provide both highly specific capture of target loci and maximized sequencer efficiency. By designing baits independently to each target DNA strand with proximity to the target loci, shorter sequence reads can be utilized for genotyping calls. Further, by removing upstream off-target sequence, individual baits can be unambiguously linked to their corresponding sequencing read, presenting opportunities for bait optimization on a per target level and resulting in extremely uniform coverage levels across markers.

NEBNext Direct Genotyping Solution Sequencing Read Structure



Marker coverage across DNA strands



Two examples of the coverage of targeted markers within a single sample from the 96-plex enrichment as visualized in the Integrative Genome Browser (IGV)[1,2]. Reads shown are de-duplicated using UMIs. Baits target both strands of the input DNA, as indicated by the red and blue aligning reads.

1. Robinson, J.T., et al (2011) *Nat Biotech.*, 29, 24-26.
2. Thorvaldsdottir, H., et al (2013) *Briefings in Bioinformatics.* 14, 178-192.

Sample Indexing and Multiplexing

With 96 pre-capture sample indexes and standard 8 post-capture pool indexes available, up to 96 samples can be combined for a single capture, and 768 samples can be pooled into a single Illumina sequencing run. An expanded post-capture pool index strategy is available upon request to process a maximum of 9216 samples all at a time. Additionally, a 12 bp Unique Molecular Identifier (UMI) is added prior to sample pooling and enrichment, allowing for accurate assessment of input coverage and improving the accuracy of genotyping calls. Finally, sequencing cycle numbers are optimized to sequence only the necessary target region, indexes and UMI required for marker genotyping. The NEBNext Direct Genotyping Solution is compatible with the full range of Illumina sequencers.

NGS-based targeted genotyping for a wide range of applications

PLANT

Marker Assisted Selection / Breeding

Quantitative Trait Locus (QTL) Screening

ANIMAL

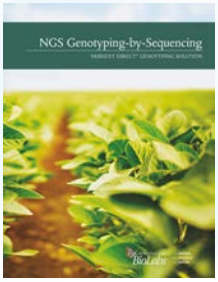
Mouse Genotyping

Livestock Breeding

HUMAN

Biobanking

NGS Sample Tracking



For more information and to download the full brochure, visit **www.neb.com/E9500** or contact us via **bulks.fr@neb.com**

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Go Directly to RNA Quantitation Without Purification:

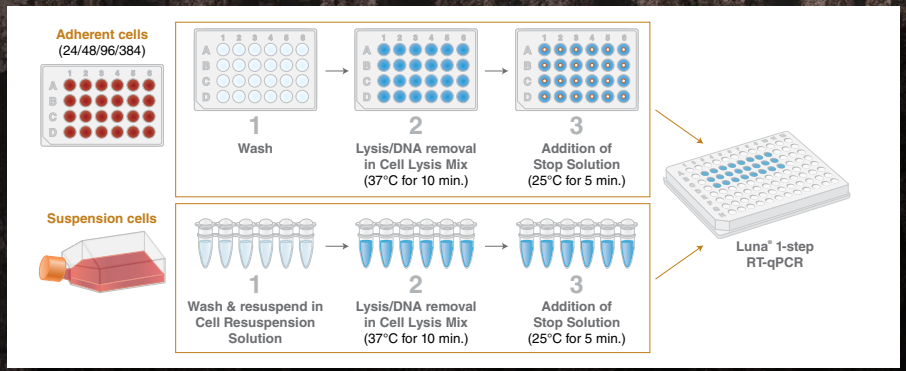
New: Luna® Cell Ready One-Step RT-qPCR Kits

The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct RNA detection and quantitation from cultured mammalian and insect cell lines.

Advantages:

- Increased convenience and minimal sample loss
- Obtain reliable and precise results comparable to purified RNA
- Linear RNA detection across a 5-log range from 10 to 100,000 cells
- Coordinated cell lysis, RNA release, and genomic DNA removal in a fast 15-minute protocol
- Non-interfering, visible tracking dye eliminates pipetting errors

Luna Cell Ready Workflow



Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple workflow resulting in effective cell lysis, RNA release, and genomic DNA removal simultaneously in a 15-minute protocol. Up to 2 µl lysate can be transferred into 20 µl downstream RT-qPCR reactions.

Ordering information:

PRODUCT	NEB #	SIZE
Luna Cell Ready One-Step RT-qPCR Kit (Dye)	E3030S	100 lysis & 500 PCR rxns
Luna Cell Ready Probe One-Step RT-qPCR Kit	E3031S	100 lysis & 500 PCR rxns
Luna Cell Ready Lysis Module	E3032S	100 lysis rxns

Use NEB's leading qPCR reagents for all your (RT)-qPCR Experiments:

Luna Universal qPCR Master Mix (Dye)	M3003S/L/X/E	200/500/1000/2500 rxns (20 µl)
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1000/2500 rxns (20 µl)
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns
Luna Universal One-Step RT-qPCR Kit (Dye)	E3005S/L/X/E	200/500/1000/2500 rxns (20 µl)
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1000/2500 rxns (20 µl)

You haven't tested our superior Luna qPCR reagents yet? Request a free sample from info.fr@neb.com, or visit: LunaqPCR.fr!

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Toll Free FAX : 0800 100 610
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www.neb-online.fr

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Toll Free: (U.S. Tech) 1-800-632-7799
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Free Call 00800/246 52277 (Austria)
info.de@neb.com

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