# NEB EXPRESSIONS

*A scientific update*

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## 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.*

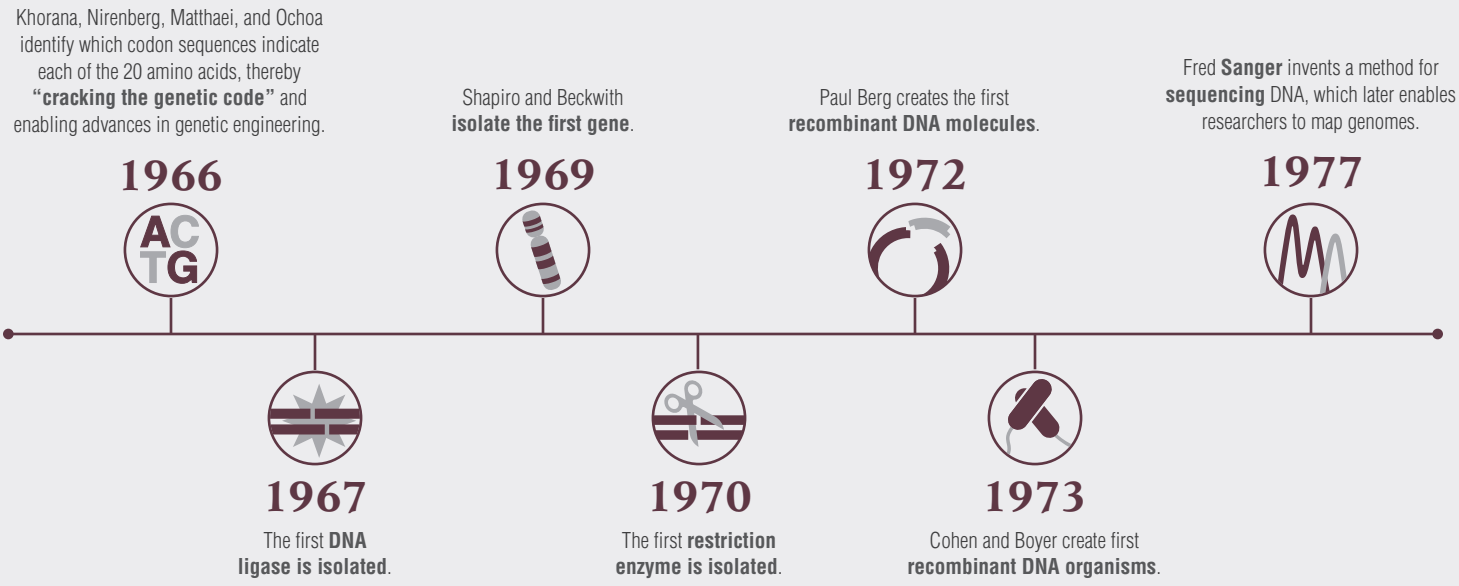
New England Biolabs® (NEB®) has been integrally involved in expressing and purifying proteins since the dawn of the recombinant DNA era in the 1970s – whether it be for our own research interests for our manufacturing processes. In 1978, NEB began screening microorganisms for restriction enzymes. Our 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.

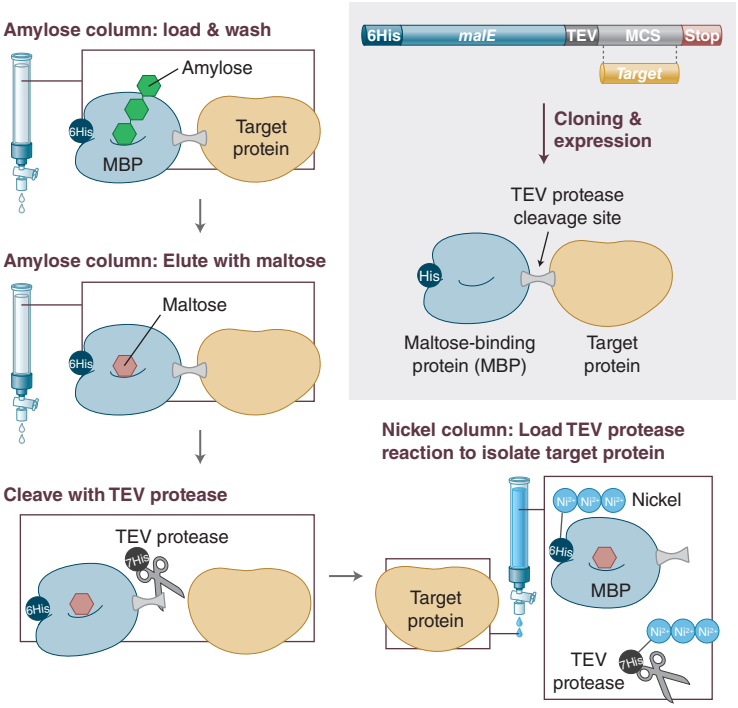
Figure 1:
**Advances in DNA Understanding were Foundational for Protein Overexpression***




**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*.

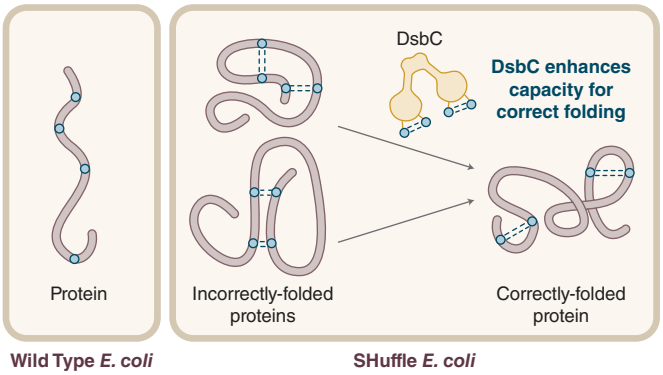
In the following years, the interest around affinity tags exploded. Additional fusion proteins (e.g., glutathione S-transferase [GST], chitin binding domain [CBD]) and many small peptide tags (poly-His-, FLAG-, S-tag-, Strep II- and poly-Arg-) were developed and used. Of these, the most influential was 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 called "inteins". An intein was first

*continued on page 4...*

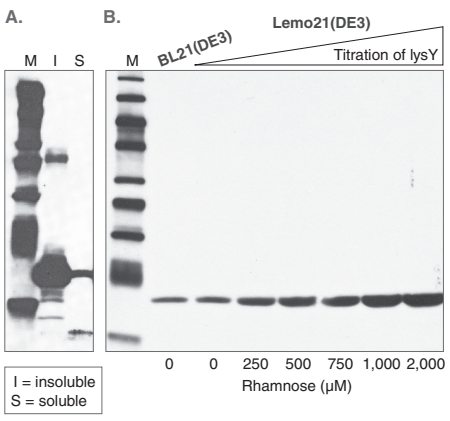
Figure 3:
**Expression of protein with multiple disulfide bonds using SHuffle® Competent *E. coli***




*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.*

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.

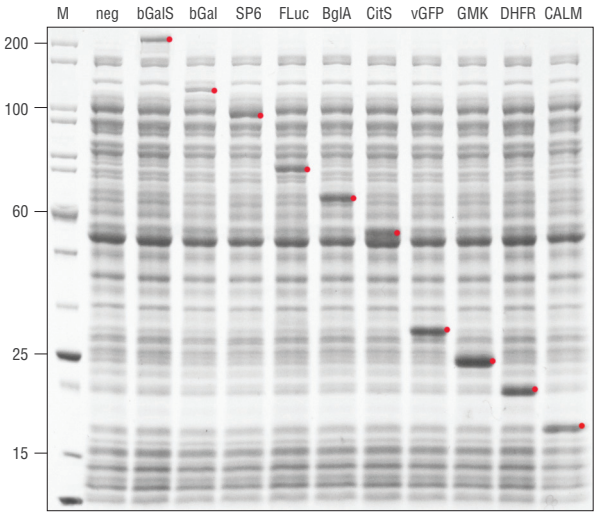
For over 40 years, New England Biolabs has been developing and using recombinant protein technologies in *E.coli* for our own manufacturing processes. Our NEBExpress™ portfolio of products includes solutions for expression and purification and is supported by access to scientists with over 40 years of 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.*

## 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:

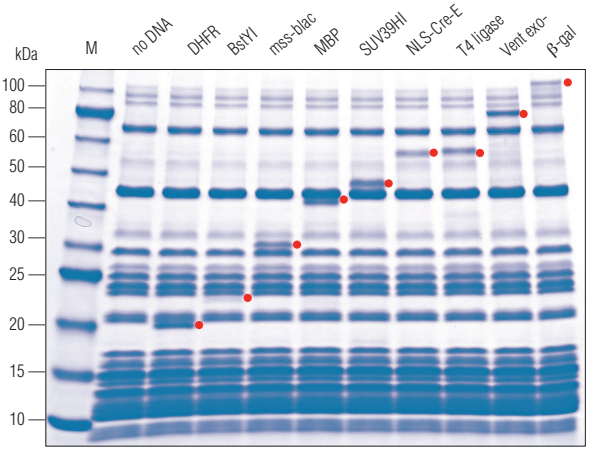
| Product | NEB # | Size |
|---|---|---|
| NEBExpress Cell-free *E. coli* Protein Synthesis System | E5360S/L | 10/100 rxns |
| NEBExpress GamS Nuclease Inhibitor | P0774S | 75 µg |

*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 novel 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. Choose from several kits depending on your desired application.

**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:

- Suitable for circular or linear DNA templates
- Visualize directly on a Coomassie-stained gel
- Protein expression in approximately 2 hours
- Transcription/translation components can be removed by affinity chromatography

## Applications:

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

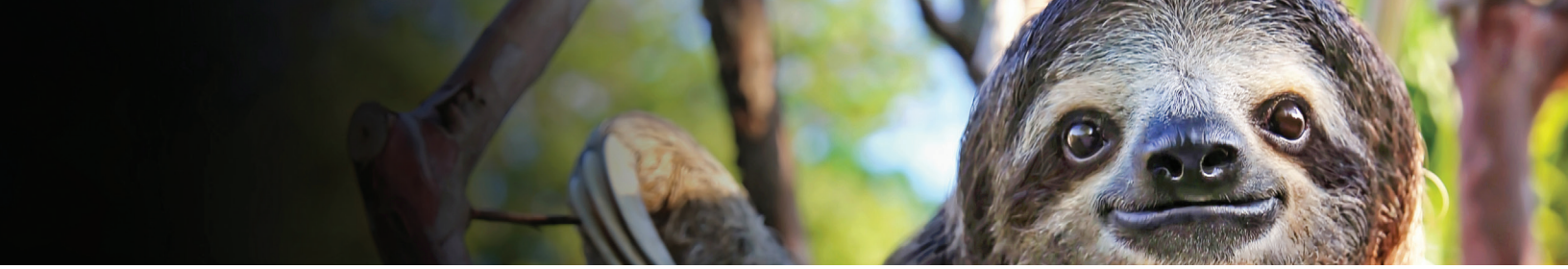
Ordering information:

| 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.

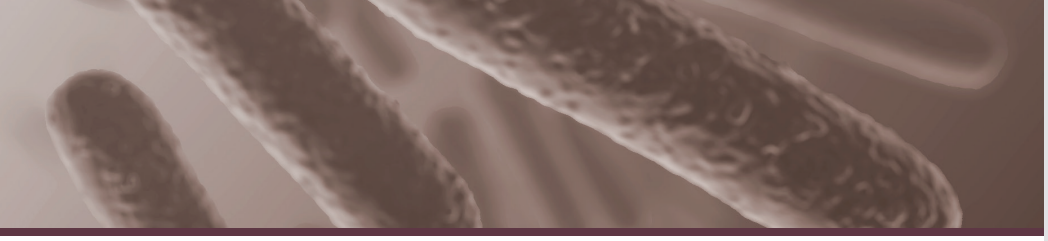
- The **NEBExpress Ni-NTA Magnetic Beads** (NEB #S1423S/L) enable small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats.
- The **NEBExpress Ni Spin Columns** (NEB #S1427S/L) are ready-to-use, and can purify ≥ 1 mg of His-tagged protein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



# 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.

| | 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<br>• Protease deficient | C2523H/I | 20 x 0.05 ml/<br>6 x 0.2 ml |
| **NEB Express *I*$^{q}$ Competent *E. coli*** | • Control of IPTG induced expression from $P_{tac}$, $P_{lac}$ and $P_{trc}$<br>• Protease deficient | C3037I | 6 x 0.2 ml |
| **T7 Express Competent *E. coli*** | • Most popular T7 expression strain<br>• Protease deficient | C2566H/I | 20 x 0.05 ml/<br>6 x 0.2 ml |
| **T7 Express *lysY* Competent *E. coli*** | • T7 expression<br>• Protease deficient<br>• Better reduction of basal expression | C3010I | 6 x 0.2 ml |
| **T7 Express *lysY/I*$^{q}$ Competent *E. coli*** | • T7 expression<br>• Protease deficient<br>• 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<br>• 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<br>• T7 expression<br>• Protease deficient/B strain | C3029J | 12 x 0.05 ml |
| **SHuffle T7 Express *lysY* Competent *E. coli*** | • T7 expression<br>• Protease deficient/B strain<br>• Tightly controlled expression of toxic proteins<br>• 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<br>• T7 expression/K12 strain | C3026J | 12 x 0.05 ml |
| **BL21 Competent *E. coli*** | • Routine expression for non-T7 Vectors<br>• Protease deficient | C2530H | 20 x 0.05 ml |
| **BL21(DE3) Competent *E. coli*** | • Routine T7 Expression<br>• Protease deficient | C2527H/I | 20 x 0.05 ml/<br>6 x 0.2 ml |
| **Lemo21(DE3) Competent *E. coli*** | • Tunable T7 Expression for difficult targets<br>• Protease deficient | C2528J | 12 x 0.05 ml |
| **NiCo21(DE3) Competent *E. coli*** | • Expression and purification of His-tagged proteins<br>• 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).

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

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.

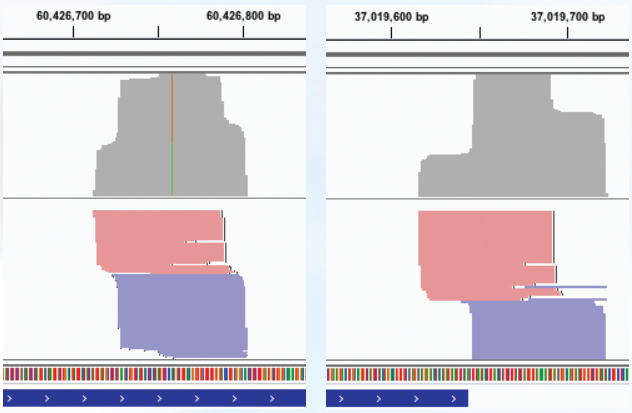
## *Sample Indexing and Multiplexing*

With 96 pre-capture sample indexes and 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. 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.

## *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.

**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.

## Features:

- 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

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# The role of voice-activated assistants in the laboratory

*- Joanne Gibson, Ph.D., New England Biolabs*

Voice-activated assistants, such as Google Home and Amazon Alexa, are widely used in homes and provide users with valuable information, such as the weather forecast, news of the day, or even compiling shopping lists. The home chef can ask for measurement conversions or set a timer, and the trivia buff can brush up on random facts. Voice-activated assistants can even be programmed to turn lights on and off with a voice command. In fact, we consult our smart devices so often, and so casually, that most of us don't consider the complexity of the underlying technology.

The foundation of voice-activated home systems is Natural Language Processing (NLP). For example, Alexa, the NLP system by Amazon, converts speech into words, sounds and ideas, which are then analyzed and deconstructed into individual sounds. These sounds are used to consult a database to find corresponding words and sounds, and then a relevant application is accessed. For example, after going through these steps, the command: "Alexa, play 80s music" would ultimately open a music app. Information is then sent back to the user through the same process.

Upgrading NLP with specific, customized "skill sets" for various occupational fields is being tackled not by Amazon or Google, but by software development companies, such as HelixAI. This start-up company develops apps that can help NLP systems recognize scientific jargon, reagents and calculations, and NEB is leading the way as one of Helix's first customers. The voice-activated laboratory assistant myNEB® contains extensive information about NEB's library of restriction enzymes and their associated properties: compatible buffer, incubation and inactivation temperatures, cut site and methylation sensitivity – to name a few.

A pilot test of myNEB highlighted its convenience and accuracy; with ease, life science researchers can access not only NEB restriction enzyme information, but also scientific calculations and cloning tips. Additionally, researchers can find NEB's podcasts – Lessons from Lab & Life™ – for some entertaining and thought-provoking insights, while their hands are busy at the bench.

A voice-activated lab assistant is just another avenue for scientists to access information. It can help to avoid the frustrating scenario when working in a lab under aseptic conditions and realizing that you need to access essential information that is not within reach. Consequently, you need to take off your gloves to turn the page of a lab notebook or access a computer keyboard, find the information you need, put gloves back on (often needing to discard the previous gloves and get fresh ones) and then resume your work. It is evident from this chain of events that a voice-activated lab assistant can both save time AND improve accuracy. With a voice-activated laboratory assistant, the scenario can play out more like this: you realize you need to access essential information that is not at your bench. You can simply ask a question aloud, such as, "Alexa, what is the recommended buffer for PacI and BglII?" or another restriction enzyme protocol-related question or scientific calculation. Alexa provides a prompt, accurate answer and you can quickly continue with your work.

The scope of myNEB's capabilities will increase in the future, expanding to include other NEB product areas and hence provide even greater convenience for the bench scientist. In a world of ever-evolving smart technology, voice-activated assistants are now being used for so much more than they were originally conceived, and are becoming an extremely valuable tool in the workplace by providing access to information more easily.

## With myNEB, you can:

- Hear the **Tech Tip of the Day** to help with your cloning
- Get **restriction enzyme information**, including:
  - The recommended buffer for double digestions
  - Our "tell me about" prompt that highlights enzyme characteristics
  - Cutsite, concentration, catalog number, recommended NEBuffer, heat inactivation, etc.
- Make **simple lab calculations** (e.g., molarity, ligation, or dilution)
- Access a **lab timer**
- Listen to NEB's podcast, **Lessons from Lab & Life™**

To learn more about myNEB, visit:
**www.neb.com/myNEB**

To download the free myNEB skill, visit "Skills and Games" in your Alexa app.

# Climate change under the microscope

*Written by Joanne Gibson, Ph.D., New England Biolabs and background photograph by Rick Cavicchioli.*

When we think about climate change, we might think about polar bears on melting ice, the effect of ocean acidification on reef ecosystems, or threats to the global food supply, but how often do we ponder the impact of climate change on microorganisms? And conversely, the effect that microorganisms have on climate change? How much could tiny, invisible-to-the-naked-eye microorganisms matter in the enormous global issue of climate change? The answer is – they matter A LOT! Microbiologists know this, and they know that the world is not paying enough attention to microorganisms, so they have issued a warning to humanity:

*"The impact of climate change will rely heavily on the responses of microorganisms"*

We know that human activity is responsible in large part for climate change. The downstream effects of increased $CO_2$ (and other greenhouse gases) and warmer environments is an unprecedented increase in the rate of plant and animal extinction. Much research goes into studying extinction, and the threat of extinction of many larger plants and animals, as well as the ripple effects that this can cause throughout the ecosystems that they inhabit. What is not well studied is the community of microorganisms that underpin all of these ecosystems. Thus, changes to the diversity of microbial populations and the impact this will have on the resilience of other organisms are unknown.

Life on Earth evolved from microorganisms, and they are essential for sustaining a healthy global ecosystem; they exist in every ecosystem on Earth that is occupied by macroorganisms, as well as in niches where no larger organisms are found. Microorganisms occupy both terrestrial and marine environments, and they both contribute to climate change and mitigate it.

Microbes represent 90% of the biomass in Earth's oceans. Marine phytoplankton sequester as much $CO_2$ as all terrestrial plants even though they make up only 1% of the global plant biomass. Marine microorganisms are also responsible for the cycling of other elements, such as nitrogen, and they produce and consume $CH_4$ on the seafloor. Additionally, marine microorganisms recycle nutrients, which subsequently enter the food web and release $CO_2$ to the atmosphere.

Terrestrial microorganisms have the potential to contribute to a climate change solution: plants absorb $CO_2$ from the atmosphere, and microorganisms convert the atmospheric carbon into soil carbon through the carbon cycle. Here, they decompose organic matter and release nutrients for plant growth as well as $CO_2$ and $CH_4$ into the atmosphere. Soil has the potential to act as a carbon sink; however, the topsoil that can help solve the climate crisis is rapidly disappearing due to modern, intensified farming practices. There is hope among soil scientists that proper land management practices can rebuild topsoil and return much of this carbon to the soil.

Microorganisms release a massive amount of $CO_2$ to the atmosphere through natural processes. However, there has always been a balance in $CO_2$ cycling between the biosphere and atmosphere – or there was before humans started burning fossil fuels at a rate that is severely disproportionate to the rate at which they are formed. Our knowledge of carbon fluctuation is incomplete because current climate change models do not include changes to microbial communities and the processes they perform in response to increased atmospheric $CO_2$ and warmer temperatures. Microorganisms are not considered in climate change policy development, and so the rate of carbon flow through the atmosphere, biosphere and oceans is no longer predictable. Questions we need to start asking are:

- What will be the response of microorganisms?
- How adaptable are microorganisms?
- Will microbial communities undermine a sustainable future because of their responses to the drastic changes occurring to global ecosystems?

Is this lack of attention a case of "out of sight, out of mind"? Scientists from Australia, Europe, USA and Canada have rallied together to form a consensus statement that essentially puts humanity on notice: pay attention because the response of microorganisms to climate change will largely impact the extent to which we will be affected (1).

These scientists emphasize that we need to recognize the importance of microorganisms as the support system of the biosphere; we need to form models that incorporate microorganisms which will enable us to better understand their current role and make predictions about how they will be affected in the future and how this will consequently affect us.

Specifically, they are calling for:

– Greater recognition that all multicellular organisms, including humans, rely on microorganisms for their health and functioning.

– The inclusion of microorganisms in mainstream climate change research, particularly research addressing carbon and nitrogen fluxes.

– Experimental design that accounts for environmental variables and stresses (biotic and abiotic) that are relevant to the microbial ecosystem and climate change responses.

– Investigation into the physiological, community and evolutionary microbial responses and feedbacks to climate change.

– A focus on microbial feedback mechanisms in the monitoring of greenhouse gas fluxes from marine and terrestrial biomes, as well as agricultural, industrial, waste and health sectors and investment in long-term monitoring.

– Incorporation of microbial processes into ecosystem and Earth System models to improve predictions under climate change scenarios.

– The development of innovative microbial technologies to minimize and mitigate climate change impacts, reduce pollution, and eliminate reliance on fossil fuels.

– The introduction of teaching microbiology in school curricula, including the personal, societal, environmental and sustainability relevance, to achieve a more educated public and appropriately trained scientists and workforce.

*– Explicit consideration of microorganisms for the development of policy and management decisions.*

– A recognition that all key biosphere processes rely on microorganisms and are greatly affected by human behavior, necessitating an integration of microbiology in the management and advancement of United Nations Sustainable Development Goals.

Concerned scientists worldwide urge humanity to improve literacy regarding microorganisms with a view to understanding, inspiring curiosity and ultimately formulating some general principles and theories about the role of microorganisms and their response to environmental variables that contribute to climate change. Most microorganisms are incredibly adaptable because of their large population sizes and rapid asexual generation times; however, until we include them in our climate change predictions, the accuracy of these models is called into question.

To learn more, visit the Microbiologists' Warning (www.babs.unsw.edu.au/research/microbiologists-warning-humanity) and read the Consensus Statement (www.nature.com/articles/s41579-019-0222-5)

**Reference**

1. Cavicchioli, R., et al. (2019) *Nat. Rev. Micro.,* 17, 569-586.

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