Competent Cells

CLONING & PROTEIN EXPRESSION

FREE
Next Day
Delivery
(UK mainland)

NEW ENGLAND
Biolabs®

Cloning Strains

NEB's growing line of competent cells includes several popular strains for cloning. Our cloning strains include derivatives of the industry standards, DH5α™ and DH10B™. NEB Turbo is unique to NEB and allows colony growth after 6.5 hours. NEB's *dam*⁻/*dcm*⁻ strain enables isolation of plasmids free of Dam and Dcm methylation. NEB Stable is recommended in all difficult cloning experiments. Our cells are all extensively tested for phage resistance, antibiotic resistance and sensitivity, blue/white screening and transformation efficiency. High efficiency, 5 minute transformation and electroporation protocols are provided, when applicable.

ADVANTAGES

- Compatible with NEBuilder® HiFi DNA Assembly and Gibson Assembly® reactions, as well as ligation reactions
- Strains for cloning toxic genes
- Free of animal products
- T1 phage resistance (*fhuA2*)
- Media and control plasmid included
- A variety of convenient formats
- Custom packaging available for bulk orders

Routine Cloning

FEATURED PRODUCT: NEB 5-ALPHA COMPETENT *E. COLI*
High Efficiency and Sub cloning Efficiency available
DH5α™ derivative
Transformation efficiency: 1 - 3 x 109 cfu/µg pUC19 DNA
Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsd*R)
Activity of nonspecific endonuclease I (*endA*) eliminated for highest quality plasmid
Suitable for blue/white screening by α-complementation of the β-galactosidase gene
Reduced recombination of cloned DNA (*recA*)
K12 Strain

Large Construct Cloning

FEATURED PRODUCT: NEB 10-BETA COMPETENT *E. COLI*
Clone large plasmids and BACs
DH10β™ derivative
Transformation efficiency: 1 - 3 x 109 cfu/µg
Efficient transformation of methylated DNA derived from eukaryotic sources or unmethylated DNA derived from PCR, cDNA and many other sources [*mcrAΔ*(*mrr-hsdRMS-mcrBC*)]
Activity of nonspecific endonuclease I (*endA*) eliminated for highest quality plasmid preparations
Suitable for blue/white screening by α-complementation of the β-galactosidase gene (No IPTG required if plasmid does not encode the lacI gene, only X-gal required)
Reduced recombination of cloned DNA (*recA1*)
K12 Strain

Unstable Construct Cloning

FEATURED PRODUCT: NEB STABLE COMPETENT *E. COLI*
High-quality plasmid preparations due to endA mutation
Rapid growth recA strain
T1 phage resistance (fhuA)

Rapid Cloning

FEATURED PRODUCT: NEB TURBO COMPETENT *E. COLI*
High-quality plasmid preparations due to endA mutation
Rapid growth (colonies visible after 6.5 hours); Isolate DNA after 4 hours of growth from a single overnight colony
Tight control of expression by *lacI*^{q} allows potentially toxic genes to be cloned

DOWNLOAD THE NEB AR APP*

Find tips for successful transformation.

Download the NEB AR App for iPhone® or iPad®. Scan the augmented reality butterfly icon located on the corner of the page to find videos, tutorials and immersive experiences.

FEATURED PRODUCT

NEB 5-alpha Competent *E. coli* (High Efficiency) – choice of convenient formats available

A versatile *E. coli* strain, NEB 5-alpha is a derivative of DH5α™ and has the same genetic features as this popular cloning strain. NEB 5-alpha offers high transformation efficiencies, convenient formats and value pricing. Whether you are doing routine cloning, subcloning or looking for a high efficiency (electrocompetent) format, NEB 5-alpha is the ideal strain for you.

ADVANTAGES

- A derivative of the popular DH5α strain
- Free of animal products
- No dry ice charges with any NEB competent cell shipments
- High efficiency and sub cloning formats

NEB 5-alpha tube strips and plate formats

96-well plate format	384-well plate format	12x8-tube strip format

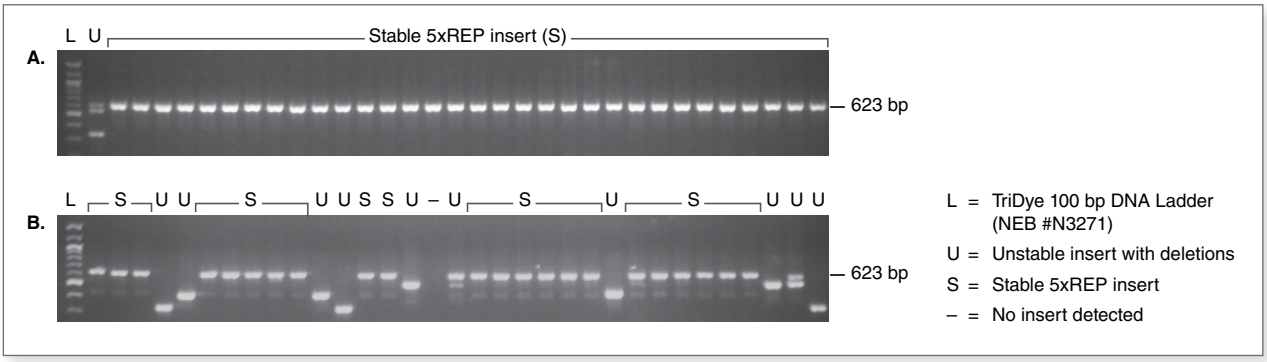
96-well plate format is also available for NEB 10-beta Competent *E. coli* (High Efficiency)
NEB #C3019P

FEATURED PRODUCT

NEB Stable Competent *E. coli*

NEB Stable Competent *E. coli* are chemically competent *E. coli* cells suitable for high-efficiency transformation and isolation of plasmid clones containing repeat elements. NEB Stable Competent *E. coli* are recommended for use when cloning genes into retroviral/lentiviral vectors.

NEB Stable Enables the Isolation of Plasmid Clones Containing Repetitive DNA Elements



Plasmid pUC-5xREP contains five 32-bp repeats, making it unstable in a recombination-proficient strain.
A) NEB Stable competent cells or B) Stbl3™ competent cells were transformed with 2 µl of a pUC-5xREP Gibson Assembly® reaction containing 2.2 ng (0.00125 pmol) pUC19 vector and approximately 1 ng (0.0028 pmol) 5xREP insert. Transformed cultures were plated on LB plates containing 100 µg/ml ampicillin and incubated overnight at 30°C. The next day, colony PCR was performed using M13/pUC polylinker primers to analyze 5xREP insert stability.

Cloning Strain Selection Chart

STRAIN	TRANSFORMATION EFFICIENCY	PRODUCT NO.	PACK SIZE
NEB 5-alpha Competent *E. coli* (High Efficiency)	1-3 x 10^9 cfu/µg	C2987H	20 x 0.05 ml
		C2987I	6 x 0.2 ml
		C2987P	1 x 96 well plate (20 µl/well)
		C2987R	1 x 384 well plate (10 µl/well)
		C2987U	96 x 50 µl/tube (12 x 8-tube strips)
NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)	>1 x 10^6 cfu/µg	C2988J	6 x 0.4 ml
NEB 10-beta Competent *E. coli* (High Efficiency)	1-3 x 10^9 cfu/µg	C3019H	20 x 0.05 ml
		C3019I	6 x 0.2 ml
		C3019P	1 x 96-well plate (20 µl/well)
NEB 10-beta Electrocompetent *E. coli*	2-4 x 10^{10} cfu/µg	C3020K	6 x 0.1 ml
NEB Turbo Competent *E. coli* (High Efficiency)	1-3 x 10^9 cfu/µg	C2984H	20 x 0.05 ml
		C2984I	6 x 0.2 ml
NEB 5-alpha F´I^q Competent *E. coli* (High Efficiency)	1-3 x 10^9 cfu/µg	C2992H	20 x 0.05 ml
		C2992I	6 x 0.2 ml
dam–/dcm– Competent *E. coli*	1-3 x 10^6 cfu/µg	C2925H	20 x 0.05 ml
		C2925I	6 x 0.2 ml
NEB Stable Competent *E. coli* (High Efficiency)	1-3 x 10^9 cfu/µg pUC19 DNA	C3040H	20 x 0.05 ml/tube
		C3040I	6 x 0.2 ml/tube

For additional information, companion products and kit components sold separately, please visit www.neb.com. Licensing information for these products can be found on our website.

Not sure which strain to choose? Try the NEB Cloning Competent *E.coli* Sampler

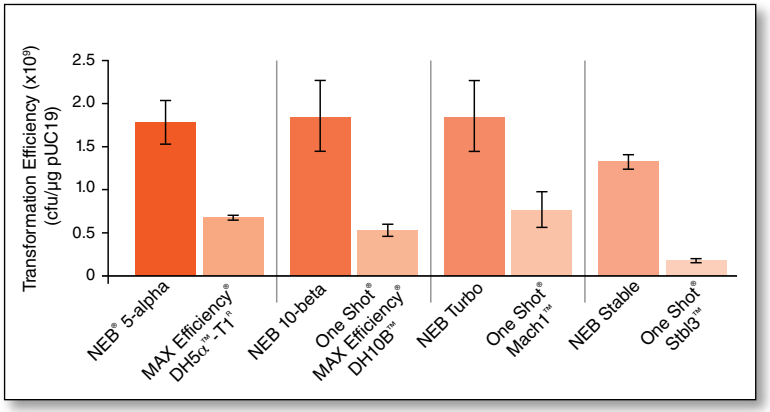
The NEB Cloning Competent *E.coli* Sampler allows you to sample 4 of our popular chemically competent strains: NEB 5-alpha (High Efficiency), NEB 10-beta, NEB Stable and NEB Turbo. The Sampler includes two 0.05 ml tubes of each of the following strains (as well as outgrowth medium and control):

NEB 5-alpha (High Efficiency): Our best-selling strain for basic cloning; Derivative of the popular DH5α. It is T1 phage resistant and *endA1* deficient for high-quality plasmid preparations.

NEB 10-beta: derivative of the popular DH10B. It is T1 phage resistant and endonuclease I (*endA1*) deficient for high-quality plasmid preparations.

NEB Stable: Ideal for isolation of plasmid clones containing repeat elements and unstable inserts. Useful for isolating and propagating retroviral/lentiviral clones.

NEB Turbo: Features fast colony growth (6.5 hours) and tight expression control (*lacIq*). Isolate DNA after only 4 hours of growth.



The transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.

Ordering Information

PRODUCT	PRODUCT NO.	PACK SIZE
Cloning Competent *E. coli* Sampler	C1010S	8 Tubes

Proteins Expression Strains

Routine Expression

NEB offers a wide variety of competent cell strains ideal for many protein expression applications. These strains address the needs of protein expression control, toxic protein expression, cytoplasmic disulfide bond formation, difficult targets and crystallography. NEB T7 Express and SHuffle® strains are available with varying levels of control. I^q strains feature the added control of IPTG-induced expression of non-T7 plasmids by *lacI*^{q}. Only NEB offers the exceptional control of expression from the *lysY* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Our Lemo21(DE3) strain features tunable T7 expression for difficult targets such as membrane proteins and proteins prone to insoluble expression (see feature opposite for more details). Each strain is provided with a detailed protocol for optimal expression.

ADVANTAGES

- Deficient in proteases Lon and OmpT
- Do not restrict methylated DNA
- Free of animal products
- T1 phage resistance (*fhuA2*)
- Media and control plasmid included
- A variety of convenient formats
- Bulk sales available for custom packaging

Routine Expression

FEATURED PRODUCT: T7 EXPRESS COMPETENT *E. COLI* (HIGH EFFICIENCY)
Transformation efficiency: 0.6-1 x 10^9 cfu/µg pUC19
Enhanced BL21 derivative, B Strain
T7 RNA Polymerase in the lac operon - no λ prophage
Deficient in proteases Lon and OmpT

Clone and Express in the Same Strain

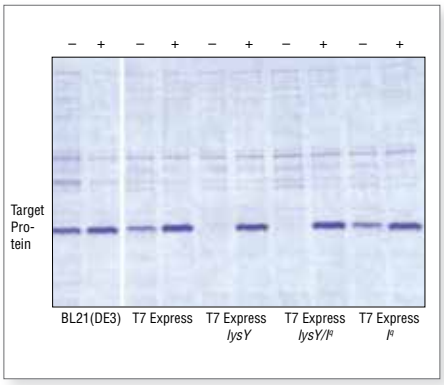
COMPARISON OF BL21 AND NEB T7 EXPRESS	
BL21	**T7 Express**
Express endonuclease 1 - direct cloning not advised	*endA1* - direct cloning an option (isolated plasmid clean enough for sequencing)
Basal level expression not tightly repressed	Basal level expression more tightly repressed

Controlled Expression

T7 expression of recombinant protein is often improved by the co-expression of T7 lysozyme which binds and inhibits T7 RNA polymerase function until the point of induction. NEB has constructed unique T7 Express derivatives with a single copy of a T7 lysozyme gene (*lysY*) or a single copy of *lysY* and *lacIq* genes on a mini-F plasmid, which is maintained without antibiotic selection. This enhancement is unique to NEB strains and makes them less susceptible to lysis during protein overexpression. *lysY* ensures complete repression of T7 expression in the absence of inducer molecule. Yet, T7 expression is activated within 30 minutes after induction. Our *lysY* strains offer maximal control of T7-mediated toxic protein expression.

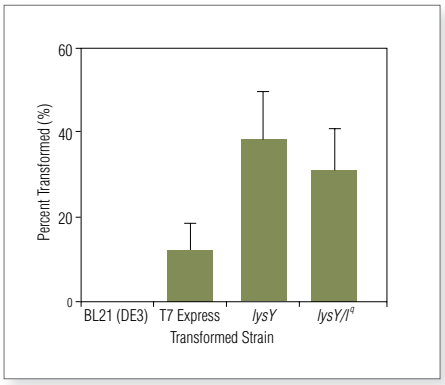
FEATURED PRODUCT: T7 EXPRESS *LYSY* COMPETENT *E. COLI* (HIGH EFFICIENCY)
Transformation efficiency: 0.6-1 x 10^9 cfu/µg pUC19 DNA
No Cam requirement
Fast growth from colonies
Lysozyme on miniF enhances stability
Control of T7 RNA Polymerase by mutant lysozyme allows potentially toxic genes to be expressed
LysY is a variant of T7 lysozyme lacking amidase activity – cells are not susceptible to lysis during induction
Maintenance of lysozyme plasmid does not require antibiotic selection
Enhanced BL21 derivative, B Strain
Deficient in proteases Lon and OmpT
T7 RNA Polymerase in the lac operon - no λ prophage

Superior control of basal expression in T7 Express strains.



T7-controlled expression of a non-toxic protein in E. coli hosts. A T7 expression plasmid containing a gene encoding an E. coli protein was transformed into each host, grown to an OD of 0.6 and induced for 3 hours. Comparison of soluble extracts from uninduced (-) and induced (+) cells shows superior control of basal expression in the T7 Express hosts while maintaining high levels of induced expression.

Improved transformation of toxic clones with T7 expression strains from NEB



A T7 expression plasmid and the same plasmid containing a gene encoding a toxic mammalian protein were transformed into each host. Comparison of the relative transformation efficiencies demonstrates that the T7 Express hosts provide the levels of control necessary for transformation of potentially toxic clones. BL21(DE3) could not be transformed with the toxic clone.

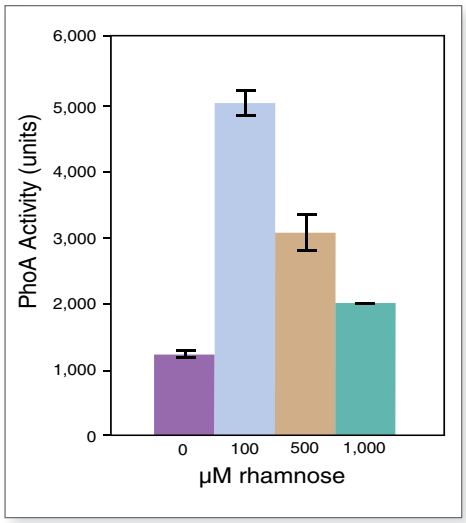
Expression of Difficult Proteins

Lemo21(DE3) Competent *E. coli* for Tunable Protein Expression

Lemo21(DE3) are chemically competent *E. coli* cells suitable for high efficiency transformation and protein expression. Fine tuning of T7 expression can alleviate inclusion body formation or growth inhibitory effects from toxic proteins. In many cases, less expression equals more protein of interest produced in the desired form. This is particularly true for membrane protein expression. Membrane protein expression and protein export in *E. coli* are both limited by the throughput capacity of the Sec translocase and in some cases the Tat translocase. T7 expression of proteins targeted to the Sec translocase often leads to accumulation of inclusion bodies or inhibition of cell division, if expression is not regulated.

Lemo21(DE3) offers the host features of BL21(DE3) while also allowing for tunable expression of difficult clones. Tunable expression is achieved by varying the level of lysozyme, *lysY*, the natural inhibitor of T7 RNA polymerase. The level of lysozyme is modulated by adding L-rhamnose to the expression culture at levels from zero to 2000 µM. When Lemo21(DE3) is grown without rhamnose, the strain performs the same as a pLysS containing strain. However, optional addition of rhamnose tunes the expression of the protein of interest. For difficult soluble proteins, tuning the expression level may also result in more soluble, properly folded protein.

Overnight expression of a membrane protein



PhoA fusion: *Lemo System™ enables simple, rapid optimization of membrane protein expression.*

The Lemo System™ is owned by Xbrane Biosciences AB (Stockholm, Sweden) and has been exclusively licensed to New England Biolabs, Inc. for the manufacture and distribution of Lemo21(DE3) Competent E. coli. The buyer and user have a non-exclusive sub-license to use this system or any component thereof for RESEARCH PURPOSES ONLY, based upon agreement to certain assurances. For full details please visit www.neb.com.

LEMO21(DE3) FEATURES

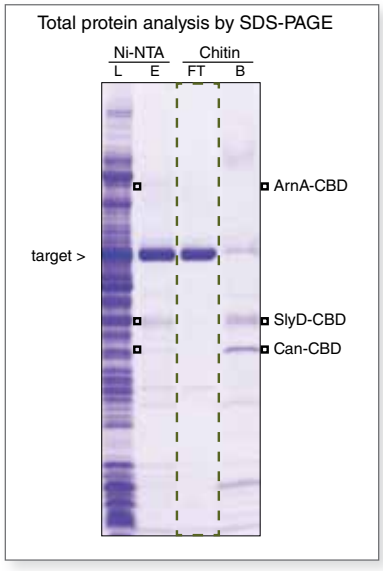
- Enhanced BL21(DE3) strain
- Fine control of expression
- Greatest range of expression of any T7 strain (0–2,000 mM rhamnose)
- Potential elimination of inclusion body formation
- Resistant to phage T1 (fhuA2)
- Rhamnose is provided
- Simple induction protocol
- Reagents Supplied: 6 x 0.05 ml/tube of chemically competent Lemo21(DE3) Competent *E. coli* cells, 12.5 ml of 0.5 M L-rhamnose solution, 0.025 ml of 50 pg/µl pUC19 Control DNA

NiCo21(DE3) Competent *E. coli*

For the expression of higher purity His-tagged proteins

His-tagged recombinant proteins that are isolated by immobilized metal affinity chromatography (IMAC) are often contaminated with significant amounts of endogenous *E. coli* metal binding proteins. NiCo21(DE3), a T7 expression strain and derivative of BL21(DE3) has been engineered to minimize this contamination resulting in a target with higher purity; GlmS is mutated to eliminate binding to IMAC resins and three other proteins (SlyD, ArnA and Can) are tagged with chitin binding domain (CBD) to enable rapid removal by chitin affinity chromatography.

Improved purity of His-tagged proteins with NiCo21(DE3)



Expression of Glutamyl tRNA Synthetase (6-His) in NiCo21(DE3) Competent E. coli followed by Ni-NTA purification. Eluent (E) from a Ni-NTA column was passed over a chitin column. The protein of interest elutes in the flow through (FT), while the CBD-tagged metal binding proteins remain bound (B) to the chitin resin (NEB #S6651S). Purifications were performed according to manufacturers' recommended conditions.

ADVANTAGES

- Superior alternative to BL21(DE3) for routine protein expression
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (fhuA2)
- No dry ice surcharge on competent cell shipments

SHuffle Competent *E. coli*

E. coli capable of oxidizing proteins in the cytoplasm to promote correct disulfide bond formation

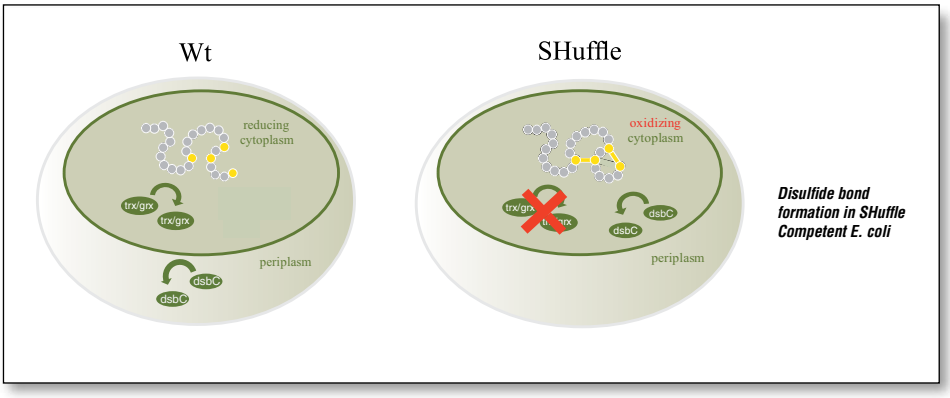
Normally reductases in the *E. coli* cytoplasm keep cysteines in their reduced form, thereby reducing any disulfide bond that may form in this compartment. SHuffle competent cells have deletions of the genes for glutaredoxin reductase and thioredoxin reductase (Δ*gor* Δ*trxB*), which allows disulfide bond formation in the cytoplasm. This combination of mutations is normally lethal, but the lethality is suppressed by a mutation in the peroxiredoxin enzyme (*ahpC**). In addition, SHuffle cells constitutively express a version of the periplasmic disulfide bond isomerase DsbC which lacks its signal sequence, retaining it in the cytoplasm. This enzyme has been shown to act on proteins with multiple disulfide bonds, to correct mis-oxidized bonds which significantly increases the capacity to correctly fold multi-disulfide bonded proteins. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam.

Shuffle is constructed in two *E. coli* strain backgrounds: *E. coli* K12 (Shuffle) and *E. coli* B (Shuffle Express) for enhanced protein expression. The T7 RNA polymerase engineered into the chromosome enables the expression of proteins from the T7 promoter (Shuffle T7 and Shuffle T7 Express). Shuffle T7 Express *lysY* contains an episome harboring a mutant T7 RNA polymerase inhibitor (*lysY*) lacking its lysozyme activity, thus allowing for the expression of toxic proteins.

SHUFFLE STRAIN FEATURES:

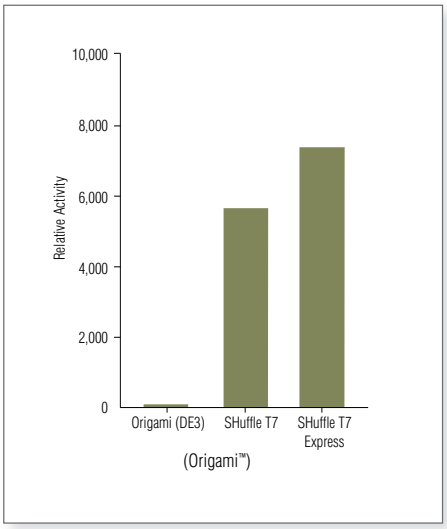
- Transformation efficiency: 1 x 106 cfu/µg pUC19 DNA
- Cytoplasmic expression of proteins
- Enhanced capacity to correctly fold proteins with multiple disulphide bonds
- Constitutively expresses a chromosomal copy of the disufide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form
- The cytoplasmic DsbC is also a chaperone that can assist in the folding of proteins that do not require disulfide bonds

Shuffle Competent *E. coli* promote correct disulfide bond formation



Disulfide bond formation in SHuffle Competent E. coli

PfCHT1 Chitinase Expression in Shuffle T7 Express: activity assayed from crude lysates



Plasmodium falciparum chitinase (PfCHT1) with three cysteines was expressed from a plasmid under the regulation of a T7 promoter. After induction, cells were harvested and crude cell lysates were prepared. PfCHT1 was assayed using a chromogenic substrate (CalBioChem #474550) and standardized to protein concentration using Bradford reagent.

Protein Expression Strain Selection Chart

STRAIN	CHARACTERISTICS	TRANSFORMATION EFFICIENCY	PRODUCT NO.	PACK SIZE
PROTEASE DEFICIENT B STRAINS				
BL21 Competent *E. coli*	• Routine non-T7 expression	1-5 x 10^7 cfu/µg	C2530H	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	• T7 expression	1-5 x 10^6 cfu/µg	C2527H/C2527I	20 x 0.05 ml/6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	• Tunable T7 expression • Expression of difficult targets including membrane proteins, toxic proteins and proteins prone to insoluble expression	1-3 x 10^7 cfu/µg	C2528J	12 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	• Expression and purification of His-tagged proteins • Improved purity of target proteins isolated by IMAC	1-5x10^7 cfu/µg	C2529H	20 x 0.05 ml
NEBExpress Competent *E. coli*	• Versatile non-T7 expression strain • Protease deficient	0.6-1 x 10^9 cfu/µg	C2523H/C2523I	6 x 0.2 ml/ 20 x 0.05 ml
NEBExpress *I^q* Competent *E. coli*	• Control of IPTG induced expression from Plac , Ptac, Ptrc and PT5-lac • Protease deficient	0.6-1 x 10^9 cfu/µg	C3037I	6 x 0.2 ml
T7 Express Competent *E. coli*	• Most popular T7 expression strain • Protease deficient	0.6-1 x 10^9 cfu/µg	C2566H/C2566I	20 x 0.05 ml/6 x 0.2 ml
T7 Express *lysY* Competent *E. coli*	• T7 expression • Protease deficient • Reduction of basal expression'	0.6-1 x 10^9 cfu/µg	C3010I	6 x 0.2 ml
T7 Express *lysY/I^q* Competent *E. coli*	• T7 expression • Protease deficient • Lowest basal expression • Highest level of expression control	0.6-1 x 10^9 cfu/µg	C3013I	6 x 0.2 ml
SHuffle Express Competent *E. coli*	• Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	1 x 10^8 cfu/µg	C3028J	12 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	• T7 expression • Protease deficient/B strain • Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^8 cfu/µg	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 capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^8 cfu/µg	C3030J	12 x 0.05 ml
K12 STRAINS				
SHuffle T7 Competent *E. coli*	• T7 expression • Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^6 cfu/µg	C3026J	12 x 0.05 ml

Featured Online Tools

The Tools & Resources tab, accessible on our homepage, contains a selection of interactive technical tools. These tools can also be accessed directly in the footer of every web page.

Competitor Cross-Reference Tool

Use this tool to select another company's competent cell product and find out which NEB strain is compatible. Choose either the product name or catalog number from the available selection, and this tool will identify the recommended NEB product, highlight its advantages, and provide a link for ordering the product.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation and transformation) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBioCalculator®

NEBioCalculator is a collection of calculators and converters that are useful in planning bench experiments in molecular biology laboratories.

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Transformation Tips

Thawing

- Cells are best thawed on ice.
- DNA should be added as soon as the last trace of ice in the tube disappears.
- Cells can be thawed by hand, but warming above 0°C decreases efficiency.

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium.
- Incubation with shaking or rotation results in 2-fold higher TE.

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE.
- Warm, dry plates are easier to spread and allow for the most rapid colony formation.

DNA

- DNA should be purified and resuspended in water or TE Buffer.
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency.
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal.
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA Contaminants to Avoid

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

RECOMMENDED PROTOCOLS

HIGH EFFICIENCY TRANSFORMATION PROTOCOL

1. Thaw cells on ice for 10 minutes
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 30 minutes
4. Heat shock at 42°C for 10–30 seconds or according to recommendations
5. Place on ice for 5 minutes
6. Add 950 µl of room temperature SOC
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle® strains) or according to recommendations

5 MINUTE TRANSFORMATION PROTOCOL

(10% efficiency compared to above protocol)

1. Thaw cells in your hand
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 2 minutes
4. Heat shock at 42°C for 30 seconds or according to recommendations
5. Place on ice for 2 minutes
6. Add 950 µl of room temperature SOC. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37°C. (30°C for SHuffle strains)

NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

For tips on protein expression with T7 Express Strains, visit **www.neb.com**

Cloning Strain Properties

STRAIN PROPERTIES	CHEMICAL TRANSFORMATION EFFICIENCY (cfu/µg)	ELECTRO-COMPETENT TRANSFORMATION EFFICIENCY (cfu/µg)	AVAILABLE FORMATS [3]	OUTGROWTH MEDIUM & CONTROL PLASMID INCLUDED?	STRAIN BACKGROUND	LIBRARY CONSTRUCTION	BLUE/WHITE SCREENING	*lacI*^{q}	*endA*$^-$ [1]	F´	*recA*$^-$	DRUG RESISTANCE [2]	METHYLATION PHENOTYPE
***dam*$^-$/*dcm*$^-$**	1-3 x 10^6	n/a	50, 200	●	K12		–	–	●	–	–	cam, str, nit	*Dam*$^-$, *Dcm*$^-$, M. EcoKI$^+$
NEB Turbo (High Efficiency)	1-3 x 10^9	n/a	50, 200	●	K12	●	●	●	●	●	–	nit	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
NEB 5-alpha (High Efficiency)	1-3 x 10^9 [4]	> 1 x 10^{10}	50, 200, 96, 384, Strips	●	K12	●	●	–	●	–	●	none	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
NEB 5-alpha F´ I^q (High Efficiency)	1-3 x 10^9	n/a	50, 200	●	K12	●	●	●	●	●	●	tet	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
NEB 10-beta (High Efficiency)	1-3 x 10^9 [5]	> 2 x 10^{10}	50, 200, 96	●	K12	●	●	–	●	–	●	str	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
NEB Stable (High Efficiency)	1-3 x 10^9	n/a	50, 200	●	K12	●	●	●	●	●	●	tet, str	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
NEB 5-alpha (Subcloning Efficiency)	> 1 x 10^6	n/a	400	–	K12		●	–	●	–	●	none	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$

(1) Important for high-quality plasmid preparation.
(2) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(3) Legend 50 = 50 µl tubes; 200 = 200 µl tubes; 96 = 96 well plate; 384 = 384 well plate; strips = 96 tube strips (50 µl/tube); 400 = 400 µl tubes
(4) 1-5 x 10^8 for R-format.
(5) 1-3 x 10^8 for P-format.

Protein Expression Strain Properties

STRAIN PROPERTIES	CHEMICAL TRANSFORMATION EFFICIENCY (cfu/µg)	AVAILABLE FORMATS [7]	OUTGROWTH MEDIUM & CONTROL PLASMID INCLUDED?	STRAIN BACKGROUND	LIBRARY CONSTRUCTION	*lacI*^{q}	*lysY*	*endA*$^-$ [2]	PROTEASE DEFICIENT [3]	F´	T7 RNA POLYMERASE	CYTOPLASMIC DISULFIDE BOND FORMATION [4]	DRUG RESISTANCE [5]	METHYLATION PHENOTYPE
NEBExpress	0.6-1 x 10^9	50, 200	●	B	●	–	–	●	●	–	–	–	nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
BL21(DE3)	1-5 x 10^7	50, 200	●	B		–	–	–	●	–	●	–	none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
Lemo21(DE3)	1-3 x 10^7	50	●[1]	B		–	●	–	●	–	●	–	cam	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
NiCo21(DE3)	1-5 x 10^7	50	●	B		–	–	–	●	–	●	–	none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
BL21	1-5 x 10^7	50	●	B		–	–	–	●	–	–	–	none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
T7 Express	0.6-1 x 10^9	50, 200	●	B	●	–	–	●	●	–	●	–	nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
T7 Express *lysY*	0.6-1 x 10^9	200	–	B	●	–	●	●	●	–	●	–	cam, nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
T7 Express *lysY*/I^q	0.6-1 x 10^9	200	–	B	●	●	●	●	●	–	●	–	cam, nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
SHuffle T7	1 x 10^6	50	–	K12		●	–	–	–	●	●	●	str, spec, nit	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
SHuffle Express	1 x 10^7	50	–	B		●	–	●	●	–	–	●	spec[6], nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
SHuffle T7 Express	1 x 10^7	50	–	B		●	–	●	●	–	●	●	spec[6], nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
SHuffle T7 Express *lysY*	1 x 10^7	50	–	B		●	●	●	●	–	●	●	cam, spec[6], nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
NEBExpress I^q	0.6-1 x 10^9	200	–	B	●	●	–	●	●	–	–	–	cam, nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$

(1) Rhamnose solution is provided instead of SOC; control plasmid is included.
(2) Important for high-quality plasmid preparation.
(3) Lacks Lon and OmpT protease activity.
(4) Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC.
(5) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(6) Resistance to low levels of streptomycin may be observed.
(7) Legend 50 = 50 µl tubes; 200 = 200 µl tubes; 96 = 96 well plate; 384 = 384 well plate; strips = 96 tube strips (50 µl/tube); 400 = 400 µl tubes

Competent Cells Product Comparison Chart

Find NEB's equivalent to competent cells from other suppliers: see the Competitor Cross-reference tool at neb.com for a more comprehensive list.

STRAIN NAME	COMPANY	CAT. #	VIALING	NEB NAME	NEB #	NEB VIALING
MAX Efficiency™ DH10B™ Competent Cells*	ThermoFisher	18297010	5 x 200 µl	NEB 10-beta Competent *E. coli* (High Efficiency)	C3019I	6 x 0.2 ml
One Shot™ MAX Efficiency™ DH5α™-T1^R Competent Cells	ThermoFisher	12297016	20 x 50 µl	NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987H	20 x 0.05 ml
MAX Efficiency™ DH5α™ T1^R Competent Cells	ThermoFisher	12034013	5 x 200 µl	NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987I	6 x 0.2 ml
Subcloning Efficiency™ DH5α™ Competent Cells	ThermoFisher	18265017	4 x 500 µl	NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)	C2988J	6 x 0.4 ml
One Shot™ TOP10 Chemically Competent *E. coli*	ThermoFisher	C404003	21 x 50 µl	NEB 10-beta Competent *E. coli* (High Efficiency)	C3019H	20 x 0.05 ml
BL21-AI™ One Shot™ Chemically Competent *E. coli*	ThermoFisher	C607003	21 x 50 µl	Lemo21(DE3) Competent *E. coli*	C2528H	6 x 0.05 ml
One Shot™ BL21(DE3) Chemically Competent *E. coli*	ThermoFisher	C600003	21 x 50 µl	BL21(DE3) Competent *E. coli*	C2527H	20 x 0.05 ml
One Shot™ BL21(DE3)pLysS Chemically Competent *E. coli*	ThermoFisher	C606003	21 x 50 µl	T7 Express *lysY* Competent *E. coli* (High Efficiency)	C3010I	6 x 0.2 ml
One Shot™ StbI3™ Chemically Competent *E. coli*	ThermoFisher	C737303	21 x 50 µl	NEB Stable Competent *E. coli* (High Efficiency)	C3040H	20 x 0.0 ml
XL1-Blue MR Supercompetent Cells	Agilent	200229	5 x 200 µl	NEB 10-beta Competent *E. coli*	C3019I	6 x 0.2 ml
XL1-Blue Supercompetent Cells	Agilent	200236	5 x 200 µl	NEB 5-alpha F´I^q Competent *E. coli*	C2992I	6 x 0.2 ml
BL21(DE3) Competent Cells	Agilent	200131	5 x 200 µl	BL21(DE3) Competent *E. coli*	C2527I	6 x 0.2 ml
BL21(DE3) pLysS Competent Cells	Agilent	200132	5 x 200 µl	T7 Express *lysY* Competent *E. coli*	C3010I	6 x 0.2 ml

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USA
New England Biolabs, Inc.
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