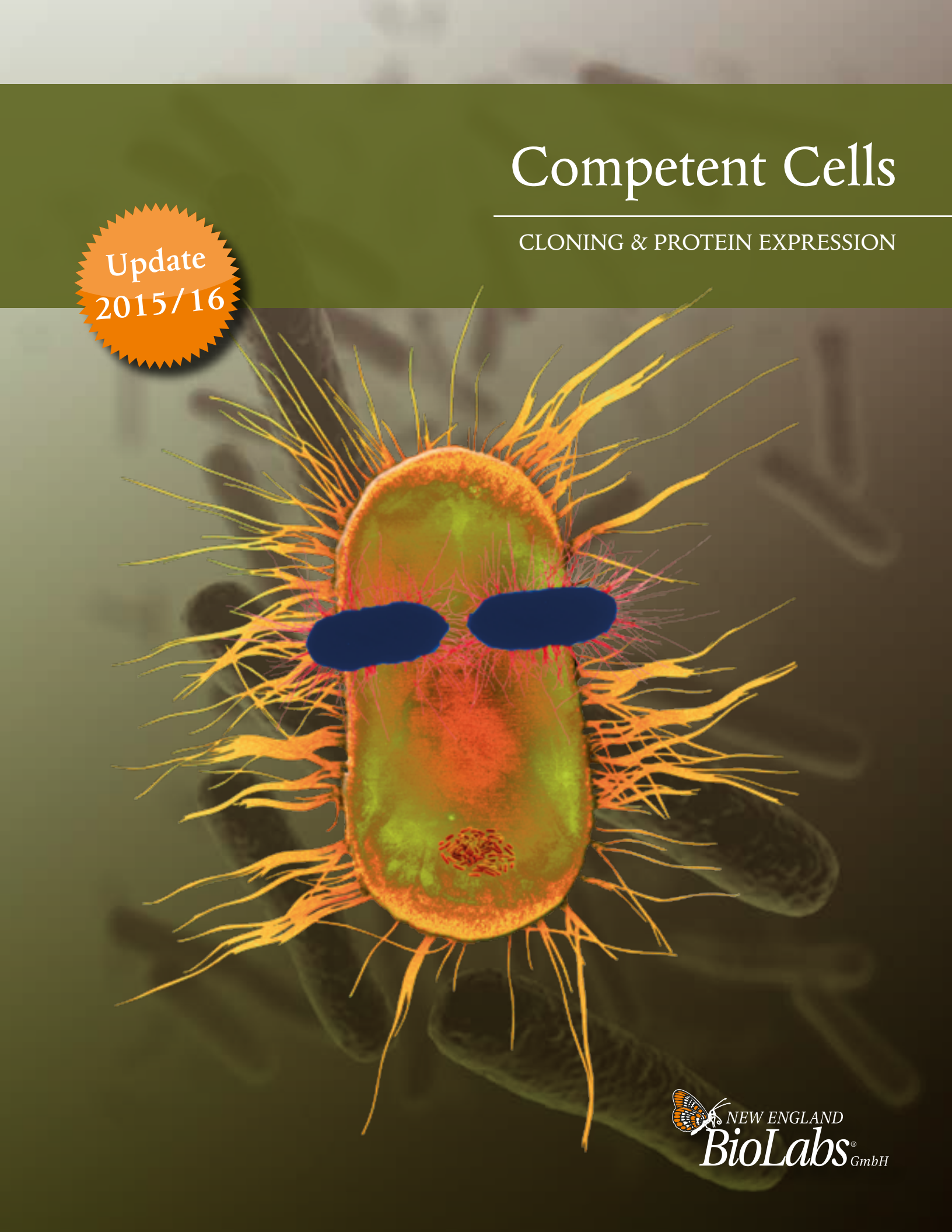
Competent Cells

CLONING & PROTEIN EXPRESSION

Update 2015/16



NEW ENGLAND BioLabs® GmbH

Strain Properties

There are many properties to consider when choosing a strain for your experiments. Requirements such as high-quality plasmid preparations, blue/white screening, cytoplasmic disulfide bond formation, regulated protein expression and fast colony growth necessitate specific strain choices. The following selection chart highlights the characteristics of NEB's strains to help select the optimal strain for a particular experiment.

Cloning Strain Properties

STRAIN PROPERTIES	TRANSFORMATION EFFICIENCY (cfu/µg)[1]	STRAIN	T1 PHAGE RESISTANT	BLUE/WHITE SCREENING	*lacI*^{q}	*lysY*	COLONIES VISIBLE AFTER 6.5 HRS.	ENDO I DEFICIENT[2]	PROTEASE DEFICIENT[3]	F′	RecA$^-$	T7 RNA POLYMERASE	CYTOPLASMIC DISULFIDE BOND FORMATION[4]	DRUG RESISTANCE[5]
NEB Turbo	1–3 x 10^9	K12	●	●	●	–	●	●	–	●	–	–	–	nit
NEB 5-alpha	1–3 x 10^9	K12	●	●	–	–	–	●	–	–	●	–	–	none
NEB 5-alpha F′ I^q	1–3 x 10^9	K12	●	●	●	–	–	●	–	●	●	–	–	tet
NEB 10-beta	1–3 x 10^9	K12	●	●	–	–	–	●	–	–	●	–	–	str
***dam*$^-$/*dcm*$^-$**	1–3 x 10^6	K12	●	–	–	–	–	●	–	–	–	–	–	cam, str, nit
NEB Stable	1–3 x 10^9	K12	●	●	●	–	–	●	–	●	●	–	–	tet, str

Protein Expression Strain Properties

NEB Express	0.6–1 x 10^9	B	●	–	–	–	–	●	●	–	–	–	–	nit
NEB Express I^q	0.6–1 x 10^9	B	●	–	●	–	–	●	●	–	–	–	–	cam, nit
T7 Express	0.6–1 x 10^9	B	●	–	–	–	–	●	●	–	–	●	–	nit
T7 Express *lysY*	0.6–1 x 10^9	B	●	–	–	●	–	●	●	–	–	●	–	cam, nit
T7 Express *lysY*/I^q	0.6–1 x 10^9	B	●	–	●	●	–	●	●	–	–	●	–	cam, nit
T7 Express Crystal	0.6–1 x 10^9	B	●	–	–	–	–	●	●	–	–	●	–	nit
SHuffle® Express	1 x 10^7	B	●	–	●	–	–	●	●	–	–	–	●	spec
SHuffle T7 Express	1 x 10^7	B	●	–	●	–	–	●	●	–	–	●	●	spec[6]
SHuffle T7 Express *lysY*	1 x 10^7	B	●	–	●	●	–	●	●	–	–	●	●	cam, spec[6]
SHuffle T7	1 x 10^6	K12	●	–	●	–	–	–	–	●	–	●	●	str, spec
BL21	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	–	–	–
BL21(DE3)	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	●	–	–
Lemo21(DE3)	1–3 x 10^7	B	●	–	–	●	–	–	●	–	–	●	–	cam
NiCo21(DE3)	1–5 x 10^7	B	●	–	–	–	–	–	●	–	–	●	–	–

(1) TE are given high-quality for high efficiency chemically competent strains. TE for electrocompetent strains are 1-4 x 10^{10} cfu/µg. TE for subcloning strains are >1 x 10^6 cfu/µg.
(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.

Convenient Formats

NEB® competent cell strains are available in various formats for your convenience. Most are available as 50 µl single-use transformation tubes and many are offered in larger, 200 µl tubes for multiple simultaneous reactions. Our most popular cloning strains are available as electrocompetent cells. SOC outgrowth media and control plasmids are provided with many of these formats. For even greater value, NEB 5-alpha is also available in a lower efficiency, subcloning format, as well as a 96-well plate format. 96-well plate formats are also available for other NEB strains upon request. For inquiries regarding bulk sales or custom packaging options including 96-well plate format, please contact NEBsolutions@neb.com.

TOOLS & RESOURCES

Visit **www.neb.com** to find:

- Tips for enhancing transformation efficiencies
- Troubleshooting transformation reactions
- Product comparisons

Cloning Formats

FORMATS	50 µl SINGLE-USE (H-FORMAT)	200 µl TUBES (I-FORMAT)	ELECTROCOMPETENT (K-FORMAT)	SUBCLONING (F-FORMAT)	96-WELL PLATE (P-FORMAT)	SOC & CONTROL PLASMID INCLUDED
NEB Turbo	●	●	●	–	–	●
NEB 5-alpha	●	●	●	●	●	●
NEB 5-alpha F′ I^q	●	●	–	–	–	●
NEB 10-beta	●	●	●	–	–	●
dam⁻/*dcm*⁻	●	●	–	–		●
NEB Stable	●	●	–	–	–	●

Protein Expression Formats

NEB Express	●	●	–	–	–	●
NEB Express I^q	–	●	–	–	–	–
T7 Express	●	●	–	–	–	●
T7 Express *lysY*	–	●	–	–	–	–
T7 Express *lysY*/I^q	–	●	–	–	–	–
T7 Express Crystal	–	●	–	–	–	–
SHuffle® Express	●	–	–	–	–	–
SHuffle T7 Express	●	–	–	–	–	–
SHuffle T7 Express *lysY*	●	–	–	–	–	–
SHuffle T7	●	–	–	–	–	–
BL21	●	–	–	–	–	●
BL21(DE3)	●	●	–	–	–	●
Lemo21(DE3)	●	–	–	–	–	●(1)
NiCo21(DE3)	●	–	–	–	–	●

(1) Rhamnose solution is provided instead of SOC, control plasmid is included.

Cloning Strains

NEB's growing line of competent cells includes several popular strains for cloning and protein expression, in addition to strains with unique properties including fast colony growth, tight control of expression and disulfide bond formation. 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 Dam and Dcm methylation-free plasmid growth. 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
- Bulk sales available for custom packaging

	NEB 5-alpha Competent *E. coli* (NEB #C2987)	NEB Turbo Competent *E. coli* (NEB #C2984)	NEB 5-alpha F′ *I*^{q} Competent *E. coli* (NEB #C2992)	NEB 10-beta Competent *E. coli* (NEB #C3019)	*dam*⁻/*dcm*⁻ Competent *E. coli* (NEB #C2925)	NEB Stable Competent *E. coli* (NEB #C3040)
FEATURES						
Versatile	•					•
Fast growth (< 8 hours)		•				
Toxic gene cloning		•	•			•
Large plasmid/BAC cloning				•		•
Dam/Dcm-free plasmid growth					•	
Retroviral/lentiviral vector cloning						•
FORMATS						
Chemically competent	•	•	•	•	•	•
Electrocompetent	•	•		•		
Subcloning	•					
96-well plate format	•					

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.

Protein Expression Strains

NEB also 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 Express, T7 Express and SHuffle strains are available with varying levels of expression control. Only NEB offers the exceptional control of expression from the *lysY* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Lemo21(DE3) features tunable T7 expression for difficult targets such as membrane proteins and proteins prone to insoluble expression. Our NiCo21(DE3) strain is designed for the expression and purification of His-tagged proteins. Each strain is provided with a detailed protocol for optimal expression.

ADVANTAGES

- Deficient in proteases Lon and OmpT
- Free of animal products
- T1 phage resistance (*fhuA2*)
- Media and control plasmid included
- A variety of convenient formats
- Bulk sales available for custom packaging

STRAIN	CHARACTERISTICS
PROTEASE DEFICIENT B STRAINS	
BL21 Competent *E. coli*	• Routine non-T7 expression
BL21(DE3) Competent *E. coli*	• T7 expression
Lemo21(DE3) Competent *E. coli*	• Tunable T7 expression • Expression of difficult targets including membrane proteins, toxic proteins and proteins prone to insoluble expression
NiCo21(DE3) Competent *E. coli*	• Expression and purification of His-tagged proteins
NEB Express Competent *E. coli*	• Versatile non-T7 expression strain
NEB Express I^q Competent *E. coli*	• Control of IPTG induced expression from Plac , Ptac, Ptrc and T5/lac
T7 Express Competent *E. coli*	• Most popular T7 expression strain
T7 Express *lysY* Competent *E. coli*	• T7 expression • Better reduction of basal expression
T7 Express *lysY*/I^q Competent *E. coli*	• T7 expression • Lowest basal expression
T7 Express Crystal Competent *E. coli*	• T7 expression • SeMet labeling of protein for crystallography
SHuffle Express Competent *E. coli*	• Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm
SHuffle T7 Express Competent *E. coli*	• T7 expression • Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm
SHuffle T7 Express *lysY* Competent *E. coli*	• T7 expression • Tightly controlled expression of toxic proteins • Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm
K12 STRAINS	
SHuffle T7 Competent *E. coli*	• T7 expression • Enhanced capability to correctly fold proteins with multiple disulfide bonds in the cytoplasm

Protein Expression Strains for Difficult Targets

Expression of Toxic Proteins

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 *lacI*^{q} 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.

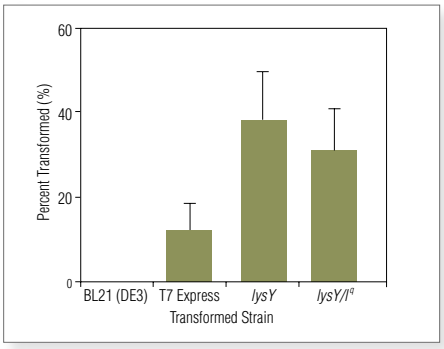
Proteins with Multiple Disulfide Bonds

SHuffle strains from NEB are engineered *E. coli* strains capable of expressing proteins with increased disulfide bond complexity in the cytoplasm. SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. DsbC isomerizes mis-oxidized substrates into their correctly folded state greatly enhancing the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.

Difficult to Express Proteins

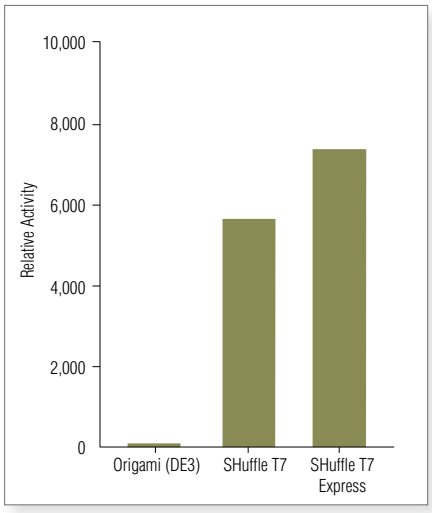
Lemo21(DE3) is a tunable T7 expression strain designed for the expression of challenging proteins. A derivative of BL21(DE3), Lemo21(DE3) offers the host features of this popular expression strain, with the added benefit of being able to control expression levels by varying the level of T7 lysozyme (*lysY*), the natural inhibitor of T7 RNA Polymerase. The fine control of expression makes Lemo21(DE3) ideal for membrane proteins, toxic proteins, secreted proteins and proteins prone to insoluble expression.

Improved transformation of toxic clones with T7 expression strains from NEB



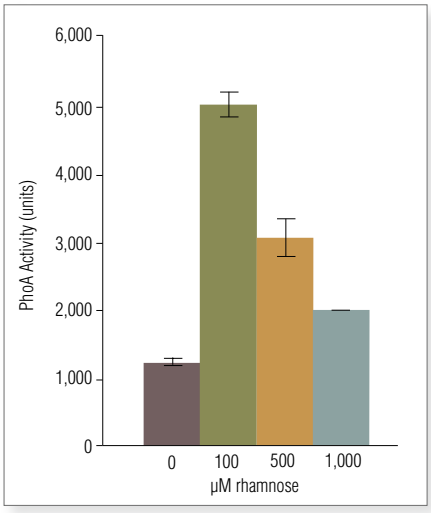
A T7 expression plasmid and the same plasmid containing a gene encoding a toxic mammalian protein were transformed into each host. Comparison of the relative transformation efficiencies demonstrates that the T7 Express hosts provide the levels of control necessary for transformation of potentially toxic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

PfCHT1 chitinase 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.

Overnight expression of a membrane protein – PhoA fusion



Lemo21(DE3) System™ enables simple, rapid optimization of membrane protein expression.

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, please visit:
http://international.neb.com

Ordering Information

Cloning Strains

PRODUCT	NEB #	SIZE
NEB Turbo Competent *E. coli*	C2984H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB Turbo Electrocompetent *E. coli*	C2986K	6 x 0.1 ml
NEB 10-beta Competent *E. coli* (High Efficiency)	C3019H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB 10-beta Electrocompetent *E. coli*	C3020K	6 x 0.1 ml
NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987H/I/P	20 x 0.05 ml / 6 x 0.2 ml/ 1 x 96-well plate
NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)	C2988J	6 x 0.4 ml
NEB 5-alpha Electrocompetent *E. coli*	C2989K	6 x 0.1 ml
NEB 5-alpha F´ I^q Competent *E. coli* (High Efficiency)	C2992H/I	20 x 0.05 ml / 6 x 0.2 ml
dam$^-$/*dcm*$^-$ Competent *E. coli*	C2925H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB Stable Competent *E. coli* (High Efficiency)	C3040H/I	20 x 0.05 ml / 6 x 0.2 ml
Component Sold Separately: SOC Outgrowth Medium	B9020S	4 x 25 ml

Protein Expression Strains

PRODUCT	NEB #	SIZE
NEB Express Competent *E. coli* (High Efficiency)	C2523H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB Express I^q Competent *E. coli* (High Efficiency)	C3037I	6 x 0.2 ml
T7 Express Competent *E. coli* (High Efficiency)	C2566H/I	20 x 0.05 ml / 6 x 0.2 ml
T7 Express *lysY* Competent *E. coli* (High Efficiency)	C3010I	6 x 0.2 ml
T7 Express *lysY*/I^q Competent *E. coli* (High Efficiency)	C3013I	6 x 0.2 ml
T7 Express Crystal Competent *E. coli* (High Efficiency)	C3022I	6 x 0.2 ml
SHuffle T7 Competent *E. coli*	C3026H	6 x 0.05 ml
SHuffle Express Competent *E. coli*	C3028H	6 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	C3029H	6 x 0.05 ml
SHuffle T7 Express *lysY* Competent *E. coli*	C3030H	6 x 0.05 ml
BL21 Competent *E. coli*	C2530H	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	C2527H/I	20 x 0.05 ml / 6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	C2528H	6 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	C2529H	20 x 0.05 ml
Component Sold Separately: SOC Outgrowth Medium	B9020S	4 x 25 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.

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enabling technologies in the life sciences

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