NEB Express Competent *E. coli* (High Efficiency)

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C2523H

20 x 0.05 ml/tube **Lot: 0131406**

Store at –80°C

CAUTION: This product contains DMSO, a hazardous material. Review the MSDS before handling.

Description: Chemically competent *E. coli* cells suitable for high efficiency transformation and protein expression.

Features:

- Transformation efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA
- Enhanced BL21 derivative
- Deficient in proteases Lon and OmpT
- Resistant to phage T1 *(fhuA2)*
- Does not restrict methylated DNA (McrA⁻, McrBC⁻, EcoBr⁻m⁻, Mrr⁻)

Reagents Supplied:

20 x 0.05 ml/tube of chemically competent NEB Express Competent *E. coli* cells **(Store at –80°C)**

20 ml of SOC Outgrowth Medium **(Store at room temperature)**

0.025 ml of 50 pg/µl pUC19 Control DNA **(Store at –20°C)**

Quality Control Assays

Transformation Efficiency: 100 pg of pUC19 plasmid DNA was used to transform one tube of NEB Express Competent *E. coli* following the high efficiency protocol provided. 0.6–1 x 10^9 colonies formed/µg after an overnight incubation on LB-ampicillin plates at 37°C.

Untransformed cells were also tested for resistance to phage ϕ80, a standard test for resistance to phage T1 and sensitivity to ampicillin, chloramphenicol, kanamycin, spectinomycin, streptomycin and tetracycline.

High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of NEB Express Competent *E. coli* cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 20 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24–36 hours or at 25°C for 48 hours.

STORAGE AND HANDLING: Competent cells should be stored at –80°C. 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.

5 Minute Transformation Protocol

A shortened transformation protocol resulting in approximately 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable.

Follow the High Efficiency Transformation Protocol above with the following changes:

1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Protocol for Expression Using NEB Express

1. Transform expression plasmid into NEB Express. Plate on antibiotic selection plates and incubate overnight at 37°C.
2. Resuspend a single colony in 10 ml liquid culture with antibiotic.
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6.
4. Induce with 40 µl of a 100 mM stock of IPTG (final concentration of 0.4 mM) and induce for 2 hours at 37°C.
5. Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight.

Transformation Protocol Variables

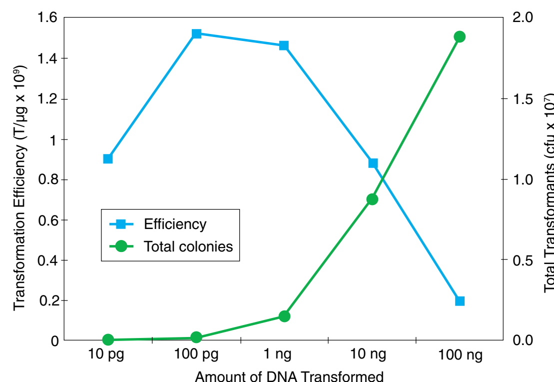
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes you shorten this step.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 20 seconds at 42°C is optimal.

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 transformation efficiency for every 15 minutes you shorten this step. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.



DNA Effects on Transformation Efficiency and Colony Output: *The optimal amount of DNA to use in a transformation reaction 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.*

CERTIFICATE OF ANALYSIS

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

*Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Troubleshooting Protein Expression

No colonies or no growth in liquid culture: There may be basal expression in the NEB Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in:

- NEB Express *I^q^* : over-expression of the LacI repressor reduces basal expression.

Incubation at 30°C or room temperature may also alleviate toxicity issues. In addition, check antibiotic concentration (test with control plasmid).

No protein visible on gel or no activity: Check for toxicity - no protein may mean the cells have eliminated or deleted elements in the expression plasmid.

- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, there will be a significant difference between the number of colonies on the plates. Fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.
- If toxicity is the problem test the above *I^q^* host to reduce basal level expression.

Induced protein is insoluble: Check for insolubility - this is important because high expression and high production of protein can result in the target protein becoming insoluble. Potential solutions for this are:

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

Solutions/Recipes

SOB:

2%	Vegetable peptone (or Tryptone)
0.5%	Yeast Extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgCl_2$
10 mM	$MgSO_4$

SOC:

SOB + 20 mM Glucose

LB agar:

1%	Tryptone
0.5%	Yeast Extract
0.17 M	NaCl
1.5%	Agar

Antibiotics for Plasmid Selection

Antibiotic	Working Concentration
Ampicillin	100 µg/ml
Carbenicillin	100 µg/ml
Chloramphenicol	33 µg/ml
Kanamycin	30 µg/ml
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Genotype: *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10*--Tet^S*)2 [dcm] R(zgb-210::Tn10*--Tet^S*) endA1 (mcrC-mrr)114::IS10*

Strain Properties

The properties of this strain that contribute to its usefulness as a protein expression strain are described below. The genotypes underlying these properties appear in parentheses.

Protease Deficient (*[lon] ompT*): *E. coli* B strains are "naturally" deficient in the *lon* protease which in K-12 strains serves to degrade misfolded proteins and to prevent some cell cycle-specific proteins from accumulating. The OmpT protease resides at the surface of wild type *E. coli* in both K-12 and B strains, presumably helping the cells to derive amino acids from their external environment. Cells deficient in both these proteases are much more amenable to the production of proteins from cloned genes.

Recovery from DNA Damage *(sulA11)*: *E. coli* cells can tolerate a substantial amount of chronic DNA damage as long as repair is allowed to proceed. This capacity is compromised if the cells are unable to divide following repair. In *lon*$^-$ cells, SulA, a cell division inhibitor, accumulates and causes cells to become hypersensitive to DNA damage. The *sulA* mutation introduced into the T7 Express strain allows cells to divide more normally in the absence of Lon protease.

Endonuclease I Deficient (*endA1*): The periplasmic space of wild type *E. coli* cells contains a nonspecific endonuclease. Extreme care must be taken to avoid degradation of plasmids prepared from these cells. The *endA* mutation deletes this endonuclease and can significantly improve the quality of plasmid preparations.

Restriction Deficient (Δ*(mcrC-mrr)114::IS10*): Wild type *E. coli* B strains carry a Type I restriction endonuclease which cleaves DNA with the site TGA(N8)TGCT. While *E. coli* DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The deletion described above eliminates both the methylase and the endonuclease.

Methyl Restriction Deficient (Δ*(mcrC-mrr)114::IS10 and R(mcr-73::miniTn10*--Tet^S*)2*): *E. coli* has a system of enzymes encoded by *mcrA*, *mcrBC* and *mrr* which will cleave DNA with methylation patterns found in higher eukaryotes, as well as some plant and bacterial strains. All three Mcr enzymes and Mrr have been inactivated in T7 Express allowing the introduction of eukaryotic DNA of genomic origin (e.g. primary libraries) if desired.

T1 Phage Resistant (*fhuA2*): T1, an extremely virulent phage requires the *E. coli* ferric hydroxamate uptake receptor for infectivity. Deletion of this gene confers resistance to this type of phage, but does not significantly affect the transformation or growth characteristics of the cell.