

# NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)



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## C2988J

6 x 0.4 ml/tube

Lot: 0361502

Store at  $-80^{\circ}\text{C}$

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

**Description:** Chemically competent *E. coli* cells suitable for subcloning efficiency transformation in a wide variety of applications.

### Features:

- DH5 $\alpha^{\text{TM}}$  derivative
- Transformation efficiency  $> 1 \times 10^6$  cfu/ $\mu\text{g}$  pUC19 DNA
- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (*thiA2*)
- Suitable for blue/white screening by  $\alpha$ -complementation of the  $\beta$ -galactosidase gene
- Reduced recombination of cloned DNA (*recA1*)
- K12 Strain
- Free of animal products

### Reagents Supplied:

6 x 0.4 ml/tube of chemically competent NEB 5-alpha Competent *E. coli* cells (Store at  $-80^{\circ}\text{C}$ )

### Quality Control Assays

**Transformation Efficiency:** 100  $\mu\text{g}$  of pUC19 plasmid DNA was used to transform NEB 5-alpha Competent *E. coli* following the transformation protocol provided. Greater than  $1 \times 10^6$  colonies formed/ $\mu\text{g}$  after an overnight incubation on LB-ampicillin plates at  $37^{\circ}\text{C}$ .

Untransformed cells were also tested for resistance to phage  $\phi 80$ , a standard test for resistance to phage T1 and sensitivity to ampicillin, chloramphenicol, kanamycin, nitrofurantoin, spectinomycin, streptomycin and tetracycline. The cells were shown to be suitable for blue/white screening by  $\alpha$ -complementation of the  $\beta$ -galactosidase gene using pUC19.

### Transformation Protocol

1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50  $\mu\text{l}$  of cells into a transformation tube on ice.
2. Add 1–5  $\mu\text{l}$  containing 100  $\text{pg}$ –1  $\mu\text{g}$  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  $42^{\circ}\text{C}$  for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.

**STORAGE AND HANDLING:** Competent cells should be stored at  $-80^{\circ}\text{C}$ . Storage at  $-20^{\circ}\text{C}$  will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above  $-80^{\circ}\text{C}$ , even if they do not thaw.

6. Pipette 950  $\mu\text{l}$  of room temperature SOC into the mixture.
7. Place at  $37^{\circ}\text{C}$  for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to  $37^{\circ}\text{C}$ .
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100  $\mu\text{l}$  of each dilution onto a selection plate and incubate overnight at  $37^{\circ}\text{C}$ . Alternatively, incubate at  $30^{\circ}\text{C}$  for 24–36 hours or  $25^{\circ}\text{C}$  for 48 hours.

### 5 Minute Transformation Protocol

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

Follow the 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.

### Transformation Protocol Variables

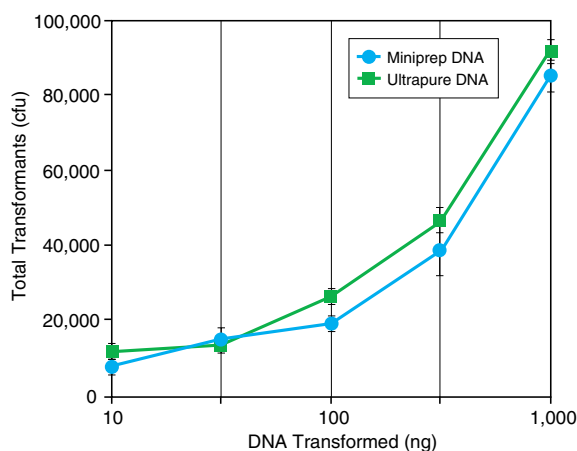
**Thawing:** Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above  $0^{\circ}\text{C}$  will decrease the transformation efficiency.

**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 this step is shortened.

**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, 30 seconds at  $42^{\circ}\text{C}$  is optimal.

**Outgrowth:** Outgrowth at  $37^{\circ}\text{C}$  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 this step is shortened. 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.



**Effect of DNA Purity on Transformation Efficiency and Colony Output:** The total colonies which can be obtained from a single transformation reaction with NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) are not significantly reduced when using miniprep DNA. Using 10 ng–1000 ng of clean, supercoiled pUC19 or pUC19 isolated with a QIAprep Spin Miniprep Kit, total colonies increase with increasing DNA concentration.

## 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  $\mu$ l 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.

## Calculation of Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by transforming 1  $\mu$ g of plasmid into a given volume of competent cells. The term is somewhat misleading in that 1  $\mu$ g of plasmid is rarely actually transformed. Instead efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. If you plan to calculate efficiency to compare cells or ligations, keep in mind the many variables which affect this metric.

Transformation efficiency (TE) equation:

$$TE = \text{Colonies}/\mu\text{g}/\text{Dilution}$$

Colonies = the number of colonies counted on the plate

$\mu$ g = the amount of DNA transformed expressed in  $\mu$ g

Dilution = the total dilution of the DNA before plating

TE calculation example:

Transform 1  $\mu$ l (1  $\mu$ g) of 1 mg/ml pUC19 DNA into 50  $\mu$ l of cells, outgrow by adding 950  $\mu$ l of SOC and dilute 10  $\mu$ l up to 1 ml in SOC before plating 100  $\mu$ l. If you count 250 colonies on the plate, the TE is:

$$\text{Colonies} = 250$$

$$\mu\text{g DNA} = 1.0$$

$$\text{Dilution} = 10/1000 \times 100/1000 = 0.001$$

$$TE = 250/1/0.001 = 2.5 \times 10^5 \text{ cfu}/\mu\text{g}$$

## Solutions/Recipes

SOB:

2% Vegetable peptone (or Tryptone)

0.5% Yeast Extract

10 mM NaCl

2.5 mM KCl

10 mM  $\text{MgCl}_2$

10 mM  $\text{MgSO}_4$

SOC:

SOB + 20 mM Glucose

LB agar:

1% Tryptone

0.5% Yeast Extract

0.17 M NaCl

1.5% Agar

Blue/White Screening:

X-gal 80  $\mu$ g/ml

IPTG\* 0.3 mM

\*Omit IPTG for potentially toxic genes

## Antibiotics for Plasmid Selection

Antibiotic	Working Concentration
Ampicillin	100 $\mu$ g/ml
Carbenicillin	100 $\mu$ g/ml
Chloramphenicol	33 $\mu$ g/ml
Kanamycin	30 $\mu$ g/ml
Streptomycin	25 $\mu$ g/ml
Tetracycline	15 $\mu$ g/ml

**Genotype:** *fhuA2*  $\Delta$ (*argF-lacZ*)U169 *phoA glnV44*  $\phi$ 80 $\Delta$  (*lacZ*)M15 *gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

## Strain Properties

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

Blue/White Screening ( $\phi$ 80 $\Delta$ (*lacZ*)M15): makes the  $\omega$ -fragment of  $\beta$ -galactosidase ( $\beta$ -gal); (*argF-lacZ*) deletes the  $\beta$ -gal gene on the chromosome. pUC19 and similar plasmids code for the  $\alpha$ -peptide of  $\beta$ -gal (*lacZ*). The  $\alpha$ -peptide can combine with the  $\omega$ -fragment of  $\beta$ -gal that is carried on  $\phi$ 80 ( $\alpha$ -complementation). When  $\beta$ -gal is reconstituted in this manner it can cleave 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactosidase (X-gal) and results in blue colonies on an X-gal plate. Inserts cloned into the plasmid polylinker disrupt the  $\alpha$ -peptide gene and the colonies are white.

Recombination Deficient (*recA1*): *E. coli* has a repair system that will recombine homologous sequences. Genomic clones often have duplicated regions, and RecA mediated rearrangements can be problematic, particularly when regions of homology are longer than 50 bp. Strains which have the RecA function deleted tend to grow more slowly than *recA*<sup>+</sup> strains.

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 (*hsdR17*): Wild type *E. coli* K12 strains carry a restriction endonuclease which cleaves DNA with sites (AAC(N6)GTGC and GCAC(N6)GTT. While *E. coli* DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The *hsdR* mutation eliminates this endonuclease activity. However, this strain has functional methyl restriction systems and may not be suitable for direct cloning of eukaryotic DNA.

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.

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