Lot: 172

C3019H

# NEB 10-beta Competent *E. coli* (High Efficiency)

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# C3019H

**20 x 0.05 ml/tube** **Lot: 172**

**Store at –80°C**

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

**Features:**

- Transformation efficiency: 1–3 x $10^9$ cfu/µg pUC19 DNA
- DH10B™ derivative
- Transformation of large plasmids and BACs
- Efficient transformation of methylated DNA derived from eukaryotic sources or unmethylated DNA derived from PCR, cDNA and many other sources [*mcrA*Δ(*mrr-hsdRMS-mcrBC*)]
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (*fhuA2*)
- Suitable for blue/white screening without IPTG by α-complementation of the β-galactosidase gene
- Reduced recombination of cloned DNA (*recA1*)
- K12 Strain
- Free of animal products

**Reagents Supplied:**

20 x 0.05 ml/tube of chemically competent NEB 10-beta 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 NEB 10-beta Competent *E. coli* following the high efficiency protocol provided. 1–3 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. Cells are resistant to streptomycin, and sensitive to ampicillin, chloramphenicol, kanamycin, nitrofurantoin, spectinomycin and tetracycline. The cells were shown to be suitable for blue/white screening by α-complementation of the β-galactosidase gene using pUC19.

## High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.

1. Thaw a tube of NEB 10-beta 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 30 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 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

The following protocol results in only 10% efficiency compared to the High Efficiency Transformation Protocol. Perform steps 1–6 in the tube provided.

1. Remove cells from –80°C freezer and thaw in your hand.
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 2 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 2 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37–42°C. NOTE: Selection using antibiotics other than ampicillin may require some outgrowth before plating on selective media. Colonies develop faster at temperatures above 37°C, however some constructs may be unstable at elevated temperatures.

## Transformation Protocol Variables

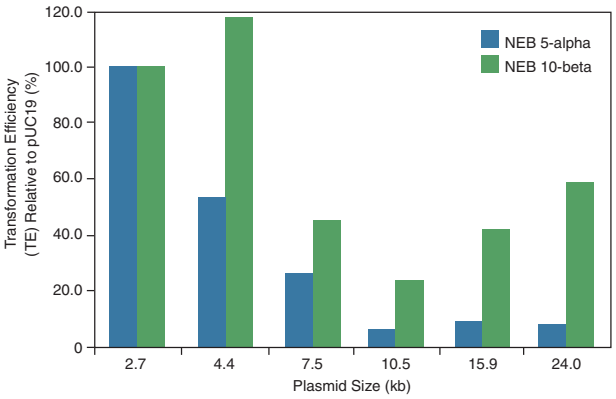
**Thawing:** Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

**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 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°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 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 Plasmid Size on Transformation Efficiency:*** *NEB 10-beta chemically competent cells are more efficiently transformed with large plasmids than NEB 5- alpha cells. The difference in TE between the two cell lines increases with the size of the plasmid being transformed.*

**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.

**Calculation of Transformation Efficiency**

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by transforming 1 µg of plasmid into a given volume of competent cells. The term is somewhat misleading in that 1 µg 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 = Colonies/µg/Dilution
Colonies = the number of colonies counted on the plate
µg = the amount of DNA transformed expressed in µg
Dilution = the total dilution of the DNA before plating

TE calculation example:

Transform 2 µl (100 pg) of control pUC19 DNA into 50 µl of cells, outgrow by adding 250 µl of SOC and dilute 10 µl up to 1 ml in SOC before plating 30 µl. If you count 150 colonies on the plate, the TE is:
Colonies = 150
µg DNA = 0.0001
Dilution = 10/300 x 30/1000 = 0.001
TE = 150/0.0001/0.001 = $1.5 \times 10^9$ cfu/µg

**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 |

Blue/White Screening:

| | |
|---|---|
| X-gal | 80 µg/ml |
| IPTG* | 0.3 mM |

*Omit IPTG for potentially toxic genes

**Antibiotics for Plasmid Selection**

| Antibiotic | Working Concentration |
|---|---|
| Ampicillin | 100 µg/ml |
| Carbenicillin | 100 µg/ml |
| Chloramphenicol | 33 µg/ml |
| Kanamycin | 30 µg/ml |
| Tetracycline | 15 µg/ml |

**Genotype:** *araD*139 Δ(*ara-leu*)7697 *fhuA lacX74 galK* (ϕ80 Δ*(lacZ)M15) mcrA galU recA1 endA1 nupG rpsL* ($Str^R$) Δ(*mrr-hsdRMS-mcrBC*)

**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 (ϕ80 Δ*(lacZ)*M15): makes ω-fragment of β-gal; *lacX74* deletes the β-gal gene on the chromosome. pUC19 and similar plasmids code for the α-peptide of β-galactosidase (*lacZ*). The α-peptide can combine with the ω-fragment of β-galactosidase which is carried on the F´ (α-complementation). When β-galactosidase is reconstituted in this manner it can cleave X-gal and results in blue colonies on an X-gal plate. Inserts cloned into the plasmid polylinker disrupt the α-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*$^+$ 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 [Δ(*mrr-hsdRMS-mcrBC*)]: 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 deletion described above eliminates both the methylase and the endonuclease.

Methyl Restriction Deficient [*mcrA* Δ(*mrr-hsdRMS-mcrBC*)]: *E.coli* has a system of enzymes, *mcr*A, *mcr*B and *mrr* which will cleave DNA with methylation patterns found in higher eukaryotes, as well as some plant and bacterial strains. DNA derived from PCR fragments, cDNA or DNA previously propagated in *E. coli* will not be methylated at these sites and will not be cleaved. All three Mcr enzymes have been inactivated in NEB 10-beta allowing the introduction of eukaryotic DNA of genomic origin (e.g. primary libraries) if desired.

T1 Phage Resistant (*fhuA*): 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.

**Companion Products Sold Separately:**

SOC Outgrowth Medium
#B9020S 4 x 25 ml medium