Lot: 89

C2984I

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

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6 x 0.2 ml/tube **Lot: 89**

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 rapid colony growth.

Features:

- Transformation efficiency 1–3 x10^9 cfu/µg pUC19 DNA
- Tight control of expression by *lacI*^{q} allows potentially toxic genes to be cloned
- Highest growth rate on agar plates - visible colonies 6.5 hours after transformation
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (*fhuA2*)
- Suitable for blue/white screening by α-complementation of the β-galactosidase gene
- Suitable for 5 minute transformation protocol with *Amp*^{R} plasmids
- EcoKr$^-$m$^-$, McrBC$^-$
- K12 Strain
- Isolate DNA after 4 hours of growth from a single overnight colony
- Free of animal products

Reagents Supplied:

6 x 0.2 ml/tube of chemically competent NEB Turbo Competent *E.coli* cells **(Store at –80°C)**

25 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 Turbo Competent *E. coli* following the high efficiency protocol provided. 1–3 x 10^9 colonies formed/µg after a 12 hour 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 nitrofurantoin, and sensitivity to ampicillin, chloramphenicol, kanamycin, spectinomycin, streptomycin and tetracycline. They were shown to be suitable for blue/white screening by α-complementation of the β-galactosidase gene using pUC19.

High Efficiency Transformation Protocol

1. Thaw a tube of NEB Turbo Competent *E. coli* cells until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
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 8–12 hours to overnight at 37°C. Alternatively, incubate at 30°C for 16 hours or 25°C for 24 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.

1. Thaw a tube of NEB Turbo Competent *E. coli* cells until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
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 8–12 hours 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

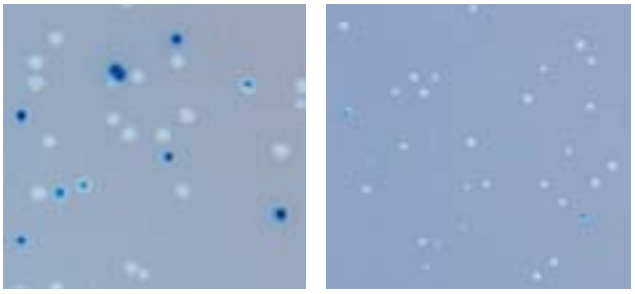
Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

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.



12 hour colony growth of NEB Turbo (left) vs DH5α™ (right). *Competent cells from each strain were transformed with a ligation mix containing a 1 kb blunt-end fragment cloned into pUC19. Turbo cells formed visible colonies within 8 hours of transformation. After 12 hours of growth at 37°C, blue and white colonies could easily be discriminated.*

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 = 1.5×10^9 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
Streptomycin	25 µg/ml
Tetracycline	15 µg/ml

Genotype: F´ *proA*$^+$*B*$^+$ *lacI*^{q} Δ *lacZ* M15/ *fhuA2* Δ*(lac-proAB) glnV gal R(zgb-210::Tn10)*Tet^S *endA1 thi-1* Δ*(hsdS-mcrB)5*

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 (F´ Δ(*lacZ*)M15): makes ω-fragment of β-gal; Δ(*lac-proAB*) 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 plus (*recA*$^+$): *E. coli* has a repair system that will recombine homologous sequences. Although genomic clones often have duplicated regions, they are generally less than 200 bp. The RecA repair system will not cause rearrangements or deletions under these circumstances. Strains which have the *recA* function intact tend to be more healthy and to grow faster 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*(hsdS-mcrB)5*): Wild type *E. coli* K12 strains carry the EcoK Type I 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 of *hsdS* eliminates both the endonuclease and methyl-ltransferase activities of EcoK.

Partially Methyl Restriction Deficient (Δ*(hsdS-mcrB)5*): *E. coli* has system of enzymes encoded by *mcrA*, *mcrB* and *mrr* which will cleave DNA with methylation patterns typical in eukaryotic cells. 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. This strain has functional McrA and Mrr endonucleases and may not be suitable for direct cloning of eukaryotic DNA.

M13 phage sensitive (F´): Infection by M13 and other similar phage requires *E. coli* surface features conferred by the F plasmid carried by some *E. coli* strains. Infection by these phage allows production of single-stranded DNA and the generation of phage display libraries. The F plasmid is frequently modified to carry other useful DNA in the cell [e.g. Δ*(lacZ)*M15 in this cell line] and when modified is called F´.

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.

Lac Promoter Control (*lacI*^{q}): The Lac repressor blocks expression from *lac tac* and *trc* promoters frequently carried by expression plasmids. If the level of Lac repressor in *E. coli* cells is not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression can reduce transformation efficiency and select against desired transformants. The extra molecules of Lac repressor in *lacI*^{q} strains help to minimize promoter activity until IPTG is added.

Companion Products Sold Separately:

SOC Outgrowth Medium
#B9020S 4 x 25 ml medium