C3020K Lot: 40

NEB 10-beta Electrocompetent *E. coli*

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C3020K

6 x 0.1 ml/tube Lot: 40

Store at –80°C

Description: NEB 10-beta electrocompetent *E. coli* cells are optimized for high efficiency transformation by electroporation. These cells are ideal for DNA library constructions and all cloning purposes.

Features:

- Transformation efficiency: 2–4 x 10^{10} cfu/µg pUC19
- Accomodation of large plasmids including BAC and cosmid constructs
- Efficient transformation of methylated DNA derived from eukaryotic sources and unmethylated DNA derived from PCR, cDNA and other sources
- Activity of nonspecific endonuclease I eliminated for highest quality plasmid preparations (*endA1*)
- Suitable for blue/white screening by α-complementation of the β-galactosidase gene (ϕ80Δ*lacZM15*)
- Resistance to T1 lytic phage (*fhuA2*)
- Reduced recombination of cloned DNA (*recA1*)
- K-12 Strain

Reagents Supplied:

6 x 0.1 ml/tube of NEB 10-beta Electrocompetent *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: 10 pg of pUC19 plasmid DNA was electroporated into 25 µl NEB 10-beta Electrocompetent *E. coli* following the protocol described below. 2–4 x 10^{10} colonies formed/µg pUC19 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. They were shown to be suitable for blue/white screening by α-complementation of the β-galactosidase gene using pUC19.

Electroporation Protocol

1. Prepare 17 mm x 100 mm round-bottom culture tubes (e.g. VWR #60818–667) at room temperature. Place SOC recovery medium in a 37°C water bath. Pre-warm selective plates at 37°C for 1 hour.
2. Place electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. As a positive control for transformation, dilute the control pUC19 by 1:5 to a final concentration of 10 pg/µl using sterile water. Heat-denatured ligation reactions can be used for electroporation directly; however, column purification is recommended.
4. Thaw NEB 10-beta Electrocompetent cells on ice (about 10 min) and mix cells by flicking gently. Transfer 25 µl of the cells (or the amount specified for the cuvettes) to a chilled microcentrifuge tube. Add 1 µl of the DNA solution.

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.

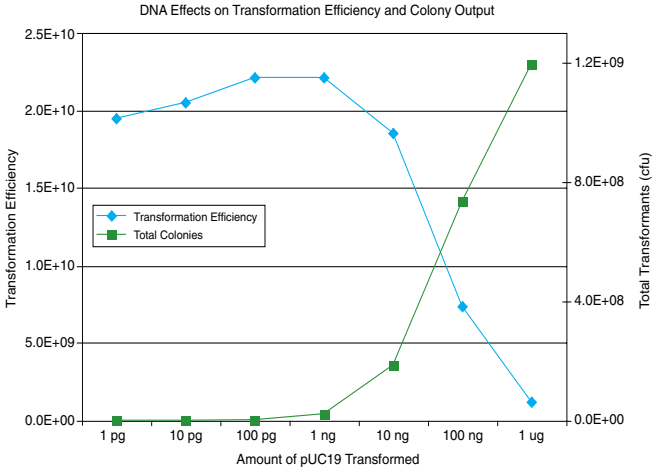
5. Carefully transfer the cell/DNA mix into a chilled cuvette without introducing bubbles and make sure that the cells deposit across the bottom of the cuvette. Electroporate using the following conditions for BTX ECM 630 and Bio-Rad GenePulser electroporators: 2.0 kV, 200 Ω, and 25 µF. The typical time constant is 4.8 to 5.1 milliseconds.
6. Immediately add 975 µl of 37°C SOC to the cuvette, gently mix up and down twice, then transfer to the 17 mm x 100 mm round-bottom culture tube.
7. Shake vigorously (250 rpm) or rotate at 37°C for 1 hour.
8. Dilute the cells as appropriate then spread 100–200 µl cells onto a pre-warmed selective plate.
9. Incubate plates overnight at 37°C.

Calculation of Electrotransformation 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 electrocompetent cells. For example, if a 10 pg transformation with pUC19 yields 100 colonies when 100 µl of a 1:100 dilution is plated, then the cfu/µg is 1.0 x 10^{10} (100 cfu/10 pg x 10^{6} pg/µg x 1 ml/0.1 ml plated x 100 dilution).

Electroporation Tips

- Electroporation cuvettes and microcentrifuge tubes should be pre-chilled on ice.
- Electrocompetent cells should be thawed on ice and suspended well by carefully flicking the tubes.
- Once DNA is added to the cells, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.
- Contaminants such as salts and proteins can lower electroporation efficiency. Ideally, DNA for transformation should be purified and suspended in water or TE. Transformation efficiency is more than10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporator. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1 mm gap are recommended (e.g. BTX Model 610/613 and Bio-Rad #165–2089). Higher voltage is required for cuvettes with 2 mm gap.
- Arcing may occur due to high concentration of salts or air bubbles.
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.



DNA Effects on Transformation Efficiency and Colony Output: *Electrotransformation efficiency remains extremely high up to about 10 ng of input DNA, then decreases precipitously at higher DNA concentrations. Total colony output continues to increase with increasing DNA input up to at least 1 µg.*

Electroporation Tips (continued)

- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at –80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

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: *araD139* Δ(*ara,leu)7697 fhuA lacX74 galK16 galE15 mcrA* ϕ*80d(lacZ*Δ*M15)recA1 relA1 endA1 nupG rpsL rph spoT1*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 that 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 which 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 that are *recA*$^-$ 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