

TransforMax™ EC100™ Electrocompetent *E. coli*

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1. Introduction

TransforMax™ EC100™ Electrocompetent *E. coli* are ideal for all cloning applications. Their higher efficiency, lack of size bias and other features make them ideal for transformation of EZ-Tn5™ Transposon insertion clones or for generating deletion libraries using the pWEB-TNC™ cosmid. Since TransforMax EC100 cells are restriction minus and lack transformation size bias against large inserts, they can be used to generate complete and unbiased primary bacterial artificial chromosome (BAC) libraries.

Important Phenotypes and Applications

- Ultra-high transformation efficiency ($>1 \times 10^{10}$ cfu/ μ g of supercoiled DNA).
- Compatible with vectors expressing the *LacZ'* α -complementing peptide for “blue/white” screening of recombinants.
- Restriction minus for efficient cloning of methylated genomic DNA.
- Accepts large clones for unbiased, primary cosmid and BAC clone library production.
- Endonuclease minus (*endA1*) to ensure high yields of plasmid clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

2. Kit Contents

Cat. #	Concentration	Quantity
TransforMax™ EC100™ Electrocompetent <i>E. coli</i> are available in two sizes:		
EC10005	(10 Electroporations)	5 x 100 μ l
EC10010	(20 Electroporations)	10 x 100 μ l

Each is supplied with 10 μ l (100 pg/ μ l) of pUC19 Control DNA in TE Buffer.

3. Related Products

The following products are also available:

- EZ-Tn5™ Transposons
- EZ-Tn5™ Transposome™ Kits
- TransforMax™ EC100D™ *pir*⁺ and *pir*-116 Electrocompetent *E. coli*
- TransforMax™ EC100-T1^R Electrocompetent *E. coli*
- Fast-Link™ DNA Ligation Kits

4. Product Specifications

Storage: Store TransforMax EC100 *E. coli* cells at -70°C and the pUC19 Control DNA at either -20°C or -70°C .

5. Genotype

F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80d/*lacZ*ΔM15 Δ*lacX74 recA1 endA1 araD139* Δ(*ara, leu*)7697 *galU galK* λ⁻ *rpsL nupG*.

Quality Control: TransforMax EC100 Electrocompetent *E. coli* yield >1 x 10¹⁰ transformants per microgram of supercoiled DNA. Transformation efficiency is determined using 10 pg of pUC19 control DNA, an Eppendorf Multiporator (2.5 kV, fast charge rate), and a 2-mm cuvette. TransforMax EC100 Electrocompetent *E. coli* are tested to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin, and chloramphenicol.

6. Electroporation of TransforMax EC100 Electrocompetent *E. coli*

Note: The electroporation procedure described here uses 50 µl of electrocompetent cells. A different volume of cells can also be used based on the experiences and needs of the user.

1. DNA should be in water or very low salt buffer (e.g., TE Buffer: 10 mM Tris-HCl [pH 7.5], 1 mM EDTA) to prevent arcing during electroporation. The pUC19 Control DNA is provided in TE at 100 pg/µl. If running a transformation control, dilute the pUC19 Control DNA 1:10 (to a final concentration of 10 pg/µl) with sterile, deionized water and use 1 µl for electroporation.
2. Prepare 1 ml of SOC or LB medium (**do not** include antibiotic in the medium) for each electroporation to be performed. This medium will be used for post-electroporation outgrowth of transformed cells. Maintain the medium at room temperature.
3. Pre-chill electroporation cuvettes and 1.5-ml tubes on ice.
4. Set-up the electroporation device according to the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
5. Thaw TransforMax EC100 Electrocompetent *E. coli* cells on ice. Mix gently. Use the cells immediately. Unused cells can be refrozen at -70°C.

Note: Refrozen cells may have reduced transformation efficiency, but for most cases, the reduction is not significant enough to interfere with the desired results.

6. Transfer the desired amount of DNA and 50 µl of cells to a pre-chilled microcentrifuge tube.

Note: A smaller volume of cells can be used based on the needs and experiences of the user. Mix the cells and DNA by pipetting up and down 2-3 times.
7. Transfer the cell/DNA mix to the electroporation cuvette. Be sure that there are no air bubbles in the cuvette. Wipe the cuvette of any condensation. Place into the electroporator and apply the electric pulse at the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
8. Immediately after electroporation, add 950 µl of room temperature SOC medium [Hanahan, D., (1983) *J. Mol. Biol.*, **166**, 557] to the cuvette. Mix gently by pipetting up and down 2-3 times.
9. Transfer the cells to a 15-ml tube and incubate at 37°C with shaking at 220-230 rpm for one hour to recover the cells and allow expression of the antibiotic resistance marker.

10. Dilute and plate the cells on appropriate medium and antibiotic. For cells transformed with the control pUC19 DNA, plate on LB agar containing 100 µg/ml of ampicillin. The remaining cell outgrowth can be stored at 4°C in the event additional cell dilutions are plated.

Control (Optional): Dilute the control reaction 1:20 and plate 100 µl (equivalent to 0.05 pg DNA) to LB-ampicillin (100 µg/ml) plates. If 250 colonies are observed on the plate, the transformation efficiency is 5×10^9 cfu/µg or [(250 cfu/0.05 pg DNA) x (10^6 pg/µg)].

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