

# Manual

## TransforMax EC100 Electrocompetent *E. coli*

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For Research Use Only. Not for use in diagnostic procedures.

TransforMax™ EC100™ Electrocompetent *E. coli* is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

# Manual

## TransforMax EC100 Electrocompetent *E. coli*

### 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. 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/ $\mu$ g of supercoiled DNA).
- Compatible with vectors expressing the *LacZ'*  $\alpha$ -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. Product specifications

<b>Transformation efficiency:</b>	Electrocompetent cells: $> 1 \times 10^{10}$ cfu/ $\mu$ g of supercoiled DNA*
<b>Genotype</b>	F <sup>-</sup> <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\phi$ 80d <i>lacZ</i> $\Delta$ M15 $\Delta$ <i>lacX74</i> <i>recA1</i> <i>endA1</i> <i>araD139</i> $\Delta$ ( <i>ara, leu</i> )7697 <i>galU galK</i> $\lambda$ <i>rpsL nupG</i>
<b>Quality control:</b>	TransforMax EC100 Electrocompetent <i>E. coli</i> yield $>1 \times 10^{10}$ transformants per microgram of supercoiled DNA. Transformation efficiency is determined using 10 pg of pUC19 control DNA. TransforMax EC100 Electrocompetent <i>E. coli</i> are tested to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin and chloramphenicol.

### 3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
TransforMax EC100 Electrocompetent <i>E. coli</i>	10 x 100 $\mu$ L*	EC10010	TransforMax EC100 Electrocompetent <i>E. coli</i>	SS001008-D	10 x 100 $\mu$ L
			pUC19 DNA **	SS000200-D	10 $\mu$ L

\* 20 Electroporations.

\*\* Each is supplied with 10  $\mu$ L (100 pg/ $\mu$ L) of pUC19 Control DNA in TE Buffer.

### 4. Storage conditions

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

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## 5. Electroporation of TransforMax EC100 Electrocompetent *E. coli*

**NOTE:** The electroporation procedure described here uses 50  $\mu\text{L}$  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/ $\mu\text{L}$ . If running a transformation control, dilute the pUC19 Control DNA 1:10 (to a final concentration of 10 pg/ $\mu\text{L}$ ) with sterile, deionised water and use 1  $\mu\text{L}$  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 1.5ml 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  $\mu\text{L}$  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  $\mu\text{L}$  of room temperature SOC medium<sup>1</sup> 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  $\mu\text{g}/\text{mL}$  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  $\mu\text{L}$  (equivalent to 0.05 pg DNA) to LB-ampicillin (100  $\mu\text{g}/\text{mL}$ ) plates. If 250 colonies are observed on the plate, the transformation efficiency is  $5 \times 10^9$  cfu/ $\mu\text{g}$  or  $[(250 \text{ cfu}/0.05 \text{ pg DNA}) \times (10^6 \text{ pg}/\mu\text{g})]$ .

## 6. Reference

1. Hanahan D, 1983, *J. Mol. Biol.*, 166, 557.

## 7. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: [techsupport@lgcgroup.com](mailto:techsupport@lgcgroup.com).



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