Lucigen®
Simplifying Genomics

Biozym®
SCIENCE IS OUR BUSINESS

TransforMax™ EC100D™ *pir+* Electrocompetent *E. coli*

TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*

Cat. Nos. ECP09500 and EC6P095H

Available exclusively thru Lucigen.
lucigen.com/epibio

epicentre®
an Illumina® company

1. Introduction

The TransforMax™ EC100D™ *pir*$^+$ and the TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli* each express the Π protein (pir gene product) for replication of vectors containing the R6Kγ conditional origin of replication (R6Kγori). The cells are derived from Lucigen's TransforMax EC100™ *E. coli* by P1 phage transduction with a strain containing either the *pir* or *pir*-116 gene linked to a dihydrofolate reductase (DHFR) marker. Both types of cells can be used for "rescue cloning" of genomic DNA fragments transposed with an EZ-Tn5™ <R6Kγ*ori* /KAN-2>Transposon using either the EZ-Tn5 <R6Kgori /KAN-2> Insertion Kit or the EZ-Tn5 <R6Kgori/KAN-2>Tnp Transposome Kit. The TransforMax EC100D *pir*$^+$ Electrocompetent *E. coli* will maintain R6Kγ*ori*-containing vectors at approximately 15 copies per cell[1] for cloning of potentially toxic or unstable DNA sequences. The TransforMax EC100D *pir*-116 Electrocompetent *E. coli* are for high copy number propagation of up to 250 rescue plasmid copies per cell.[1]

2. Genotypes

TransforMax EC100D *pir*$^+$

Electrocompetent *E. coli*

F$^-$ *mcr*A Δ(*mrr*-hsdRMS-*mcr*BC) φ80d*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara*, *leu*)7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G *pir*$^+$(DHFR).

Maintains plasmids at ~15 copies per cell.

TransforMax EC100D *pir*-116 Electrocompetent *E. coli*

F$^-$ *mcr*A Δ(*mrr*-hsdRMS-*mcr*BC) φ80d*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara*, *leu*)7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G *pir*-116(DHFR).

Maintains plasmids at ~250 copies per cell.

Transformation Efficiency:

Greater than 5 × 10^9 cfu/µg of supercoiled DNA.

Important Phenotypes and Applications

- Expresses the Π protein (*pir* gene product) for rescue cloning and propagation of vectors containing an R6Kγ origin of replication.
- 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 propagation and stability of large rescue clones.
- Endonuclease minus (*end*A1) to ensure high yields of plasmid clones.

Storage: Store TransforMax EC100D *pir*$^+$ and *pir*-116 *E. coli* cells at –70°C and the pR6Kan Control DNA at either –20°C or –70°C.

Quality Control: TransforMax EC100D *pir*$^+$ and *pir*-116 Electrocompetent *E. coli* yield >5 × 10^9 transformants/µg of supercoiled DNA. Transformation efficiency is determined using

10 pg of pR6Kan control DNA, an Eppendorf Multiporator (2.5 kV, fast charge rate), and a 2-mm cuvette. TransforMax EC100D *pir*$^{+}$ and *pir*-116 Electrocompetent *E. coli* are tested to free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin, and chloramphenicol.

3. Kit Contents

Cat. #	Quantity
TransforMax™ EC100D™ *pir*$^{+}$ Electrocompetent *E. coli*	
ECP09500	5 x 100 µl
TransforMax™ EC100D™ *pir*-116 Electrocompetent *E. coli*	
EC6P095H	5 x 100 µl

Each is supplied with 10 µl (100 pg/µl) of pR6Kan Control DNA in TE Buffer.

4. Electroporation Procedure

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. It is critical that the DNA, cuvettes, electroporation chamber (if applicable), and microcentrifuge tubes are thoroughly chilled on ice.*

Caution: *DNA should be prepared in water or very low ionic strength buffer (e.g., TE Buffer: 10 mM Tris-HCl [pH 7.5], 1 mM EDTA) to prevent arcing during electroporation. DNA from an Epicentre Fast-Link DNA ligation reaction can be diluted and used directly, without desalting or ethanol precipitation.*

1. Thaw TransforMax EC100D *pir*$^{+}$ or *pir*-116 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.*

2. 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.*

   Control (Optional): *Dilute the pR6Kan control DNA 1:10 with sterile, distilled water. Add 1 µl (10 pg) to 50 µl of cells as described.*

3. 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.
4. Immediately after electroporation, add 950 µl of the room temperature SOC medium[2] to the cuvette. Mix gently by pipetting up and down 2-3 times.
5. Transfer the cells to a sterile culture tube and incubate at 37°C with shaking at 220-230 rpm for 1 hour to recover the cells and allow expression of the antibiotic resistance marker.
6. We recommend diluting the cells 1:10 and 1:100 and plating 100 µl of each dilution to the appropriate medium and antibiotic plate. Incubate the plates at 37°C overnight.

Control (Optional): Dilute the control reaction 1:20 and plate 100 µl (equivalent to 0.05 pg DNA) to LB-kanamycin (50 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) × (10^6 pg/µg)].

6. References

1. Metcalf, W.W. *et al.*, (1994) *Gene* **138**, 1.
2. Hanahan, D. (1983) *J. Mol. Biol.*, **166**, 557.