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Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent *E. coli*

TransforMax™ EPI300™-T1^R Chemically Competent *E. coli*

Cat. Nos. EC02T15, EC02T110, and CT1C0210

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1. Introduction

Phage T1-Resistant TransforMax™ EPI300™-T1^R Electrocompetent and Chemically Competent *E. coli* have been specifically engineered for use with Epicentre's CopyControl™ Cloning Systems.* The cells contain the *trf*A gene, whose protein product is required for initiation of replication from the *ori*V origin of replication contained on the CopyControl vectors and on clones retrofitted with an EZ-Tn5™ <*ori*V/KAN-2> Transposon. The *trf*A gene is under tightly regulated control of an inducible promoter. When grown on standard LB plates or in LB or SOC culture medium, expression of the *trf*A gene is repressed. Addition of the CopyControl Induction Solution (provided) induces expression of the *trf*A gene and subsequent utilization of the *ori*V origin of replication and high copy amplification of the CopyControl clones. In addition, these cells are resistant to bacteriophage T1 and T5 infections (*ton*A genotype).

Benefits:

- *trf*A gene under tightly regulated control of an inducible promoter for copy number control of CopyControl clones.
- *ton*A for resistance to bacteriophage T1 and T5 infections.
- Supports blue/white screening of vectors.
- Readily accepts large DNAs for construction of large-insert libraries.
- Restriction minus [*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)] for efficient cloning of methylated (e.g. mammalian genomic) DNA.
- Endonuclease minus (*end*A1) to ensure high yields of plasmid clones.
- Recombination minus (*rec*A1) to ensure the stability of large cloned inserts.

2. Kit Contents

Cat. #	Quantity
Phage T1-Resistant TransforMax™ EPI-300™-T1R Electrocompetent *E. coli* are available in two sizes:	
EC02T15	5 x 100 µl
EC02T110	10 x 100 µl
Phage T1-Resistant TransforMax™ EPI-300™-T1R Chemically Competent *E. coli*:	
CT1C0210	10 x 50 µl

Each is supplied with CopyControl™ Induction Solution and pUC19 Control DNA (100 pg/µl).

3. Product Specifications

Storage: Store Phage T1-Resistant TransforMax EPI300-T1^R *E. coli* cells at -70°C. The CopyControl Induction Solution and the pUC19 Control DNA can be stored at either –20°C or –70°C. Warm the CopyControl Induction Solution to room temperature and mix thoroughly before use.

Genotype:

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*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 *trf*A *ton*A *dhf*r.

Quality Control:

- The electrocompetent Phage T1-Resistant TransforMax EPI300-T1^R *E. coli* have a transformation efficiency of >10^{10} cfu/µg DNA using 10 pg of pUC19 and an Eppendorf Multiporator with setting of 2.5 KV at 5 milliseconds, fast charge rate using 2 mm cuvettes.
- The chemically competent Phage T1-Resistant TransforMax EPI-300-T1^R *E. coli* have a transformation efficiency of >5 x 10^8 cfu/µg DNA using 10 pg of pUC19.
- Both Phage T1-Resistant TransforMax EPI300-T1^R *E. coli* cell types are tested:
  - for induced expression of the *trf*A gene using transformants harboring a plasmid with an *ori*V origin of replication.
  - to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin and chloramphenicol.
  - for bacteriophage T1 resistance: genotypically, by diagnostic PCR of the *ton*A gene, and phenotypically by resistance to bacteriophage T5 infection.

Electroporation of Phage T1-Resistant TransforMax EPI300-T1^R Electrocompetent *E coli*

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 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 EPI300-T1^R Electrocompetent *E. coli* cells on ice. Mix by gentle tapping or vortexing. Use the cells immediately. Unused cells can be refrozen at –70°C.

   Note: *Refrozen cells may have reduced transformation efficiency.*
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 1 hour to recover the cells and allow expression of the antibiotic resistance marker.
10. Dilute and plate the cells on appropriate medium (e.g. LB agar plates) and antibiotic. For cells transformed with the pUC19 Control 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 x 10^9 cfu/ µg or [(250 cfu/0.05 pg DNA) x (10^6 pg/µg)].

Transformation of Phage T1-Resistant TransforMax EPI300-T1^R Chemically Competent *E. coli*

This procedure was written for the transformation of 50 µl of TransforMax EPI300 Chemically Competent *E. coli*. The procedure can be scaled down as needed.

1. Prepare 250 µl of SOC medium (do not include antibiotic in the medium) for each transformation to be performed. Maintain the medium at room temperature.
2. Chill 1.5 ml microcentrifuge tubes or microplate at 4°C and heat a water bath or other temperature-controlled apparatus to 42°C.
3. Thaw TransforMax EPI300-T1^R Chemically Competent *E. coli* cells on ice. Mix by gentle tapping. Use the cells immediately.

   Note: *Refreezing chemically competent cells will result in a greatly reduced transformation efficiency.*
4. Transfer 1-5 µl of DNA and 50 µl of cells to a pre-chilled, microcentrifuge tube or into the wells of a microplate. Cover the cells and incubate on ice for 30 minutes.
5. Transfer the tubes or plate to 42°C and heat shock for 30 seconds.
6. Transfer the cells back to ice and cool for 2 minutes.
7. Add 250 µl of the room temperature SOC medium to each tube.
8. Recover the cells by incubating at 37°C for 60 minutes with shaking at 220-230 rpm.
9. Plate the cells on the appropriate media (e.g. LB agar plates) and antibiotic.

Induction of CopyControl Fosmid and CopyControl PCR Clones to High Copy Number

CopyControl Fosmid and PCR clones and fosmid clones retrofitted with the EZ-Tn5 <*ori*V/KAN-2> Transposon and grown in TransforMax EPI300-T1^R cells can be amplified to 10-50 copies per cell for fosmids and up to 200 copies per cell for PCR clones. The induction process can be done in any culture volume desired depending on the needs of the user. Generally, a 1 ml induced culture will provide a sufficient amount of DNA for most applications including sequencing and fingerprinting. Here we provide the procedure for amplifying the clones in 1 ml, 5 ml and 50 ml cultures.

Important: *The Growth Media for amplifying CopyControl Fosmid clones and CopyControl PCR clones and low copy fosmid clones retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon are different. Be sure to use the appropriate Growth Media for the clones that your are amplifying.*

Growth Media for CopyControl Fosmid Clones and CopyControl PCR Clones

LB + chloramphenicol (12.5 µg/ml)

Growth Media for Fosmid Clones Retrofitted with an EZ-Tn5 <oriV/KAN-2> Transposon

LB + chloramphenicol† (12.5 µg/ml) + kanamycin (50 µg/ml)

† or other selectable marker present on the cloning vector backbone

1. Add 5 ml of the appropriate Growth Media to 15 ml tubes for each fosmid or PCR clone that will be induced to high copy number.
2. Individually inoculate the media with a small portion of the desired fosmid or PCR clones grown on an overnight plate.
3. Grow the cultures overnight at 37°C with shaking. These cultures will be used as inocula for the copy number amplification procedure.
4. From the table below, combine the appropriate volumes of fresh Growth Media, the overnight culture and the CopyControl Induction Solution for the desired volume of induction culture. Aeration of the induction cultures is critical. Therefore, to maximize the surface area of the culture solution in the tube, perform the induction in the largest volume tubes that reasonably meets your needs and resources. For example, induce clones to high copy number in 1 ml of culture, using 1.5 ml tubes or larger, 5 ml cultures in 15 ml tubes and 50 ml cultures in 125 ml flasks.

Total volume of clone induction culture	Volume of fresh LB + chloramphenicol (12.5 µg/ml)	Volume of overnight 5 ml culture	Volume of 1000X CopyControl Induction Solution ‡
1 ml	800 µl	200 µl	1 µl
5 ml	4.5 ml	500 µl	5 µl
50 ml	45 ml	5 ml	500 µl

‡ Mix thoroughly after thawing.

5. Vigorously shake the tubes at 37°C for 5 hours. Aeration is critical! Shake the tubes in a manner that will maximize aeration of the cultures (for example 1.5 ml tubes can be taped horizontally to the shaking table).
6. Centrifuge the cells and purify the DNA by your standard lab methods.

Induction of CopyControl BAC Clones from Single Copy to High Copy Number

CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ-Tn5 <*ori*V/KAN-2> Transposon and grown in TransforMax EPI300-T1^R cells can be amplified to 10-20 copies per cell using the procedures described here. Generally, 1 ml of an induced culture will provide a sufficient amount of DNA for most applications including sequencing and fingerprinting. Procedures for amplifying CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ-Tn5 <*ori*V/ KAN-2> Transposon in 1.5 ml tubes and in deep-well, 96 well plates are provided. The induction volumes can be scaled up as required by the user.

Important: *The Growth Media for amplifying CopyControl BAC clones and low copy number BAC clones retrofitted with an EZ::TN <oriV/KAN-2> Transposon are different. Be sure to use the appropriate Growth Media for the type of BAC clones that you are amplifying.*

Growth Media for CopyControl BAC Clones
LB + chloramphenicol (12.5 µg/ml)

Growth Media for BAC Clones Retrofitted with an EZ-Tn5 <*ori*V/KAN-2> Transposon
LB + chloramphenicol§ (12.5 µg/ml) + kanamycin (50 µg/ml)

§ or other selectable marker present on the cloning vector backbone

Amplification of BAC clones in 1.5 ml tubes

1. Dispense 1 ml of the appropriate Growth Media into 1.5 ml tubes. Inoculate each tube with an isolated single BAC clone from an overnight plate.
2. Incubate the cultures at 37°C overnight without shaking.
3. Following overnight incubation, mix each tube and then aspirate off 800 µl of culture medium from each and discard.
4. Add 800 µl of fresh Growth Media into each tube containing the remaining 200 µl of the overnight culture. Mix by vortexing.
5. Incubate the tubes for 30 minutes at 37°C with shaking at 250 rpm. After 30 minutes, the $O.D_{600}$ will be at 0.4 - 0.6.
6. Thaw the CopyControl Induction Solution and mix thoroughly. Add 1 µl of 1000X CopyControl Induction Solution (to a 1X final concentration) to each tube. Incubate each for 2 hour at 37°C with vigorous shaking. Aeration is critical! Shake the tubes in a manner that will maximize aeration of the cultures (for example 1.5 ml tubes can be taped horizontally to the shaking table).
7. Isolate DNA from the induced culture by your method of choice.

Amplification of CopyControl BAC clones in deep-well (2 ml) 96 well plates

1. Dispense 1 ml of the appropriate Growth Media into each well of a deep-well plate. Inoculate each well with an isolated single BAC clone from an overnight plate.
2. Seal the plate with porous microtiter plate sealer, incubate at 37°C overnight without shaking.
3. Following overnight incubation, mix the cultures by shaking and then aspirate off 800 µl of culture medium from each well and discard.
4. Add 800 µl of fresh Growth Media into each well containing the remaining 200 µl of the overnight culture. Mix by shaking or vortexing.
5. Incubate the plate for 30 minutes at 37°C with shaking at 250 rpm. After 30 minutes, the $O.D_{600}$ will be at 0.4 - 0.6.
6. Thaw the CopyControl Induction Solution and mix thoroughly. Add 1 µl of 1000X CopyControl Induction Solution (to a 1X final concentration) to each well. Incubate each for 2 hour at 37°C with vigorous shaking. Aeration is critical!
7. Isolate DNA from the induced culture by your method of choice.

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