



CopyCutter™ EPI400™
Electrocompetent *E. coli*
CopyCutter™ EPI400™
Chemically Competent *E. coli*
CopyCutter™ Induction Solution

Cat. Nos. C400EL10, C400CH10, and CIS40025

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1. Introduction

CopyCutter™ EPI400™ Electrocompetent and Chemically Competent *E. coli** cells were developed to significantly lower the copy number of a wide variety of common vectors so that you can more readily clone unstable DNA sequences. Moreover, following a short incubation in the presence of the CopyCutter Induction Solution, you can subsequently raise copy number to improve plasmid yields without compromising stability.

The CopyCutter EPI400 cell line was derived from our high-transformation efficiency phage T1-resistant TransforMax™ EC100™ *E. coli* strain by manipulating a gene that controls the copy number of vectors containing *ColE1* or *pMB1* origins of replication (e.g., pUC and pET type vectors). This constitutively expressed gene, *pcnB* (plasmid copy number), was deleted from the TransforMax EC100 strain and replaced with a modified *pcnB* gene linked to an inducible promoter, creating the CopyCutter EPI400 strain.

Additional Benefits:

- High transformation efficiency with clones of all sizes.
- Supports blue/white screening of vectors.
- Restriction minus [*mcrA* Δ (*mrr-hsdRMS-mcrBC*)] for efficient cloning of methylated DNA.
- Endonuclease minus (*endA1*) to ensure high yields of plasmid clones.
- Recombination minus (*recA1*) to ensure the stability of large cloned inserts.

2. Product Specifications

Storage: Store CopyCutter EPI400 *E. coli* cells at -70°C . The CopyCutter Induction Solution and pUC19 Control DNA can be stored at either -20°C or -70°C . Warm the CopyCutter Induction Solution to room temperature and mix thoroughly before use.

Genotype:

F⁻ *mcrA* Δ (*mrr-hsdRMS-mcrBC*) ϕ 80*dlacZ* Δ M15 Δ *lacX74* *recA1* *endA1* *araD139* Δ (*ara*, *leu*)7697 *galU* *galK* λ ⁻ *rpsL* *nupG* *tonA* Δ *pcnB* *dhfr*.

Quality Control:

- The electrocompetent CopyCutter EPI400 *E. coli* have a transformation efficiency of $>1 \times 10^{10}$ 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 CopyCutter EPI400 *E. coli* have a transformation efficiency of $>1 \times 10^7$ cfu/ μg DNA using 10 pg of pUC19.
- Both CopyCutter EPI400 *E. coli* cell types are tested for copy number repression and induction using transformants harboring pUC19.
- Both CopyCutter EPI400 *E. coli* cell types are tested to be free of contaminating DNA rendering resistance to ampicillin, tetracycline, kanamycin and chloramphenicol.
- Both CopyCutter EPI400 *E. coli* cell types are tested for bacteriophage T1 resistance: genotypically, by diagnostic PCR of the *tonA* gene, and phenotypically by resistance to bacteriophage T5 infection.

3. Kit Contents

Desc.	Quantity
CopyCutter™ EPI400™ Electrocompetent <i>E. coli</i> C400EL10 (10 Electroporations)	10 × 50 µl
CopyCutter™ EPI400™ Chemically Competent <i>E. coli</i>: C400CH10 (10 Transformations) Each is supplied with 1 ml (1000X) CopyCutter™ Induction Solution and 10 µl (100 pg/µl) pUC19 Control DNA	10 × 50 µl
CopyCutter™ Induction Solution CIS40025 1000X concentrated solution. Filter sterilized.	25 ml

4. General Considerations

- Copy number variability:** The copy number of vectors containing Co/E1 or pMB1-based origins in the CopyCutter EPI400 strain is dependent on several different factors, including the type of vector used as well as the size and orientation of the cloned insert. As an example, the table below compares the copy number of three different cloning vectors in the CopyCutter EPI400 strain compared to the parental TransforMax EC100 strain.

Table 1. Comparing Plasmid Load in CopyCutter EPI400 and TransforMax EC100 *E. coli* Cells.

<i>E. coli</i> Host Cells	Growth Condition	Approximate Number of Vector Copies Per Cell *		
		pUC19 (Amp)	pBR322 (Amp)	pET9 (Kan)
TransforMax EC100 cells	Normal	~216	~71	~33
CopyCutter EPI400 cells	Uninduced	~9	~17	~9
CopyCutter EPI400 cells	Induced	~200	~66	~19

*Based on the molar amount of plasmid DNA obtained from at least 1010 ampicillin or kanamycin resistant cells. Cultures were grown overnight in selective media (EC100 and EPI400-uninduced) or induced for 4 hours with the CopyCutter Induction Solution.

- Random plasmid distribution:** Plasmids containing Co/E1 or pMB1-based origins are randomly distributed to daughter cells during cell division. Consequently, some daughter cells will receive plasmids and others will not. The number of plasmid-free cells in a CopyCutter culture can vary from 10-60%, dependent on the copy number of the vector and the type of antibiotic used for selection. Kanamycin selection, for example, results in many fewer plasmid-free cells than ampicillin selection.
- Tetracycline selection:** Using CopyCutter cells to lower the copy number of vectors that require tetracycline selection is not recommended

A. Electroporation of CopyCutter EPI400 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 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.
4. Set-up the electroporation device according to the manufacturer's recommendations for bacterial (*E. coli*) electroporation.
5. Thaw CopyCutter EPI400 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 to 50 μl of chilled cells.
Note: *A smaller volume of cells can be used based on the needs and experiences of the user. Mix the cells and DNA thoroughly but gently so as not to introduce air bubbles.*
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 LB 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 5×10^9 cfu/μg or $[(250 \text{ cfu}/0.05 \text{ pg DNA}) \times (10^6 \text{ pg}/\mu\text{g})]$.

B. Transformation of CopyCutter EPI400 Chemically Competent *E. coli*

Two procedures for transforming the CopyCutter EPI400 Chemically Competent *E. coli* are presented. The Standard Transformation Procedure will provide the highest transformation efficiency. The 5 Minute Transformation Procedure is more rapid but may yield 10-fold or more lower transformation efficiency. The 5 Minute Transformation Procedure should only be used with ampicillin selection of the clones. Both procedures were written for transformation of 50 μl of CopyCutter EPI400 Chemically Competent *E. coli*.

Note: Once thawed, do not refreeze the cells.

A different volume of cells can also be used based on the experiences and needs of the user.

Standard Transformation Procedure

1. Prepare 250 µl of SOC medium [Hanahan, D., (1983) *J. Mol. Biol.*, **166**, 557] for each transformation to be performed. Maintain the media at room temperature.
2. Heat a water bath or other temperature-controlled apparatus to 42°C.
3. Thaw the appropriate number of tubes of CopyCutter EPI400 Chemically Competent *E. coli* cells on ice. Mix by gentle tapping. Use the cells immediately.
4. Transfer 1-5 µl of DNA or ligation reaction into each tube. Cap the tubes and incubate on ice for 5-30 minutes.
5. Transfer the tubes to 42°C and heat shock for 30 seconds.
6. Transfer the cells back to ice and cool for 2 minutes.
7. Remove the cover of the tubes and add 250 µl of SOC Media.
8. Recover the cells by incubating at 37°C for 60 minutes with horizontal shaking (e.g. 225 rpm).
9. Plate the cells on the appropriate media and antibiotic, and grow overnight at 37°C.

5 Minute Transformation Procedure

The rapid 5 minute transformation procedure may yield 10-fold or more lower transformation efficiency than the Standard Transformation Procedure described above. Importantly, only selection with ampicillin can be used with the 5 Minute Transformation Procedure.

1. Thaw the appropriate number of tubes of CopyCutter EPI400 Chemically Competent *E. coli* cells on ice. Mix by gentle tapping. Use the cells immediately.
2. Transfer 3-5 µl of DNA or ligation reaction into each tube. Cap the tubes and incubate on ice for 5 minutes.
3. Spread the entire cell/DNA mixture onto a pre-warmed LB+ampicillin (100 µg/ml) plate and grow overnight at 37°C.

C. Rapid Screening of CopyCutter EPI400 Clones

Plasmids in CopyCutter EPI400 cells can be screened for the correct insert size using small amounts of either uninduced or induced cells. A PCR-based screen is generally used to analyze uninduced clones since there are so few plasmid copies per cell.

D. Inducing CopyCutter EPI400 Clones to High Copy Number

1. To prepare inocula for the induction process, add 5 ml of LB + antibiotic to a 15 ml tube. Inoculate with an isolated colony or use 5-10 µl from Part C, Step 6. Shake overnight at 37°C.
2. The induction process can be done in any culture volume desired depending on user need. Dilute the overnight culture 1:10 and measure the OD₆₀₀. Based on this reading, dilute the overnight culture to a final OD₆₀₀ of 0.2 in LB + antibiotic + 1X CopyCutter Induction Solution.

For example, if the undiluted overnight culture has an OD₆₀₀ of 2.70 then add 3.7 ml of the overnight to 50 ml of LB + antibiotic + 1X CopyCutter Induction Solution.
3. Incubate at 37°C for 4 hours with vigorous shaking.
Important: *Vigorous shaking is crucial for the best possible induction of copy number. 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. Induce clones to high copy number in a 5 ml cultures in at least 15 ml tubes, and 50 ml cultures in at least 125 ml flasks.*
4. Isolate plasmid DNA from the induced culture by your method of choice.

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