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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 *Col*E1 or pMB1 origins of replication (e.g., pUC and pET type vectors). This constitutively expressed gene, *pcn*B (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 [*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)] for efficient cloning of methylated 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. 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$^-$ *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 *ton*A Δ*pcnB dhfr*.

Quality Control:

- The electrocompetent CopyCutter EPI400 *E. coli* have a transformation efficiency of >1 x 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 x 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 *ton*A gene, and phenotypically by resistance to bacteriophage T5 infection.

3. Kit Contents

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

4. General Considerations

1. **Copy number variability:** The copy number of vectors containing ColE1 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.

E. coli 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.

2. **Random plasmid distribution:** Plasmids containing *Col*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.
3. **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 220-230rpm 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)].

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. We recommend the Colony Fast-Screen™ Kit (PCR Screen) for preparing PCR-ready DNA in 10 minutes directly from colonies on a plate. Visit http://www.epicentre.com for more information on this product.

To rapidly screen induced CopyCutter EPI400 clones, we recommend the Colony Fast-Screen Kit (Size Screen). Briefly, colony picks are induced in microcentrifuge tubes, processed using the Colony Fast-Screen Kit (Size Screen), and analyzed by agarose gel electrophoresis. The size of the cloned insert is determined by comparing the migration of the supercoiled plasmid DNA with a size-standard of supercoiled DNAs. A modified Colony Fast-Screen Kit (Size Screen) protocol is given below and more information on this product can be found at http://www.epicentre.com.

1. For each colony to be screened, prepare 300 µl of LB medium + antibiotic in a 1.5 ml microcentrifuge tube.
2. Using a sterile tip transfer a portion of the colony into one of the 1.5-ml tubes.

   Note: *If the colony size is = 1 mm diameter, pick the whole colony; if the size is >1 mm diameter, only pick approximately half of the colony, because too much cell material may interfere with the screening process.*
3. Incubate at 37°C for 30 minutes with vigorous shaking.

   Important: *Vigorous shaking is crucial! For example, tape the tubes horizontally to the shaker. Placing the tubes in a rack and taping the rack to the bottom of the shaker is not sufficient.*
4. After 30 minute incubation, transfer 100 µl of the cell cultures into separate 1.5 ml microcentrifuge tubes. These 100 µl aliquots can be used as inocula for the clone induction process. Do not add the CopyCutter Induction Solution to these tubes.
5. To the remaining 200 µl, add 0.2 µl of thawed and thoroughly mixed 1000X CopyCutter Induction Solution (or 2 µl of a 1:10 dilution of the CopyCutter Induction Solution in sterile water) to a final concentration of 1X.
6. Shake both sets of tubes very vigorously for 4 to 5 hours at 37°C.

   Important: *Vigorous shaking is crucial! For example, tape the tubes horizontally to the shaker. Placing the tubes upright in a rack is not sufficient. Store the tubes containing the 100 µl aliquot at 4°C.*

   Note: *If these cells are not going to be used within 48 hours, streak them onto an LB + antibiotic plate for long term storage.*
7. Spin down the cells from the induced 200 µl of cell cultures, for 5 minutes at 12,500 rpm.
8. Discard the supernatant carefully, so as not to disturb the cell pellet. Resuspend the cell pellets in 15 µl of the EpiBlue™ Solution. Vortex to completely resuspend the cells. It is critical to completely resuspend the cell pellets to maximize the amount of DNA released from the cells.
9. Add 15 µl of the EpiLyse™ Solution, vortex vigorously and heat 10 minutes at 70°C.
10. Load 15 µl of the lysed cell solutions on the appropriate percentage agarose gel. If the lysed cell solutions are too viscous to load onto a gel, add more EpiLyse Solution until loading is manageable. It is important to make sure the lysed cell solution stay in the wells since the viscous solution may be easily pulled out of the wells when retracting the pipette tips. The remaining lysed cell solutions can be stored at room temperature for up to 1 week in the event additional gels are run.

11. Load the appropriate volume of a supercoiled DNA size marker (not supplied in the kit), to lanes of the gel adjacent to the lysed cell extracts. Run the gel for 45-60 minutes. Stain with a $1:10^4$ dilution of SYBR® Gold in gel running buffer or water, or prolonged staining with ethidium bromide.

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 OD_{600}. Based on this reading, dilute the overnight culture to a final OD_{600} of 0.2 in LB + antibiotic + 1X CopyCutter Induction Solution.

   For example, if the undiluted overnight culture has an OD_{600} 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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