



# Clone Unstable DNA by Lowering the Copy Number of Common Vectors Using CopyCutter™ EPI400™ *E. coli* Cells\*

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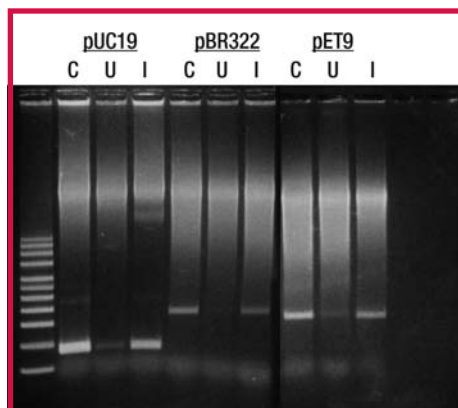
## Introduction

If you clone DNA on a regular basis, chances are good that you've been caught in the lab, gel image in hand, mumbling to yourself "Where's the insert?" Your controls look great and you've optimized everything you can think of, but your clones are "empty" or they contain an insert that's too small or that doesn't map correctly. Often this problem arises because the insert can not be stably maintained in a high-copy number vector.

The insert may code for a protein that interferes with normal cellular functions and inhibits cell growth. As a result, the clone either dies or is overtaken by "empty" or mutated recombinants that can grow faster. Similarly, although for reasons less well understood, AT- and GC-rich sequences or sequences with strong secondary structure, can also be unstable at high-copy number and are often selected against.

One of the easiest solutions to such cloning impasses is to use a lower-copy number vector. But you probably chose the vector you did for a reason. Besides the high-copy number that makes plasmid purification easier, the vector's multiple cloning site works well with your cloning strategy and/or the vector contains other sequences that you need for downstream applications. Keeping this in mind, EPICENTRE engineered our new CopyCutter™ EPI400™ *E. coli* cells.

Here we demonstrate how the CopyCutter EPI400 cells can significantly lower the copy number of a wide variety of popular, high-copy number vectors so that you



**Figure 1. The copy number of ColE1-type plasmids is lowered 4- to 25-fold in CopyCutter™ EPI400™ *E. coli* cells.** Lane C, TransformaMAX™ EC100™ cells; Lanes U and I, uninduced and induced CopyCutter EPI400 cells, respectively. Crude extracts of plasmid DNA were prepared from cells grown in selective media and analyzed by agarose gel electrophoresis. Approximately the same number of lysed cells (based on OD<sub>600</sub>) were loaded per lane.

can clone "toxic" genes or unstable DNA sequences into your favorite vectors. Moreover, following a short incubation in the presence of the CopyCutter™ Induction Solution, you can increase the copy number of the vector to improve plasmid yields.

## Methods and Results

The CopyCutter EPI400 cell line was derived from our high-transformation efficiency phage T1-resistant TransformaMAX™ EC100™ *E. coli* strain by manipulating a gene that controls the copy number of ColE1-type plasmids. This constitutively

expressed gene, *pcnB* (plasmid copy number), was deleted from the TransformaMAX EC100 strain and replaced with a modified *pcnB* gene linked to an inducible promoter, creating the CopyCutter EPI400 strain.

### Lower copy number

ColE1-type plasmids include the naturally occurring ColE1, pMB1, and p15A, as well as pBR322, the pUC plasmids, the pET series, the pBluescript series, and many others. In standard *E. coli* strains the copy number of pUC19 can reach well over 100 copies per cell.<sup>1</sup> As shown in Table 1, the copy number of this vector is reduced by approximately 25-fold in the CopyCutter EPI400 strain compared to the parental TransformaMAX EC100 strain, grown under the same conditions. Since lower-copy number plasmids, like pBR322 and many pET-derivatives contain an additional control element, their copy number is kept at 30 to 70 copies per cell in standard strains.<sup>2</sup> In the CopyCutter EPI400 strain the copy number of these vectors is reduced approximately 4-fold compared to the parental TransformaMAX EC100 strain (Table 1).

### Raise copy number

To induce plasmids in CopyCutter cells to higher copy number, a single clone was grown overnight in selective medium. Following overnight growth, cells were diluted into fresh medium to an OD<sub>600</sub> of 0.2 and CopyCutter Induction Solution was added. Cultures were incubated for 4 hours at 37°C with shaking. As shown in Table 1 and Figure 1, raising the copy number of pUC19, pBR322 or pET9

**Table 1. Comparing Plasmid Load in CopyCutter™ EPI400™ and TransformaMAX™ 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)
TransformaMAX™ 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 10<sup>10</sup> 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 as described in the text.

increased the plasmid load per ampicillin or kanamycin-resistant cell by approximately 22-, 4-, or 2-fold, respectively.

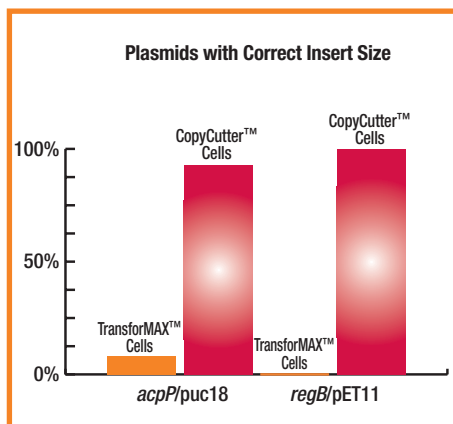
### Random plasmid distribution

Because ColE1-type plasmids are randomly distributed to daughter cells during cell division, some daughter cells will receive plasmids and others will not.<sup>1</sup> After overnight growth in the presence of ampicillin, CopyCutter EPI400 cells containing pBR322 or pUC18 were plated on LB plates and on LB-ampicillin plates. The number of colonies obtained on LB plates was 40 to 60% higher than the number of colonies on LB-ampicillin plates, indicating the percentage of daughter cells that did not receive a plasmid. After a 4-hour induction in the presence of ampicillin, similar percentages of plasmid-free cells were observed with these vectors in CopyCutter EPI400 cells.

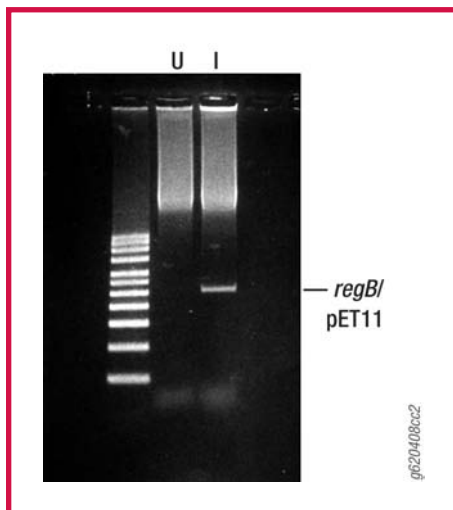
Kanamycin selection is more stringent in this application and fewer daughter cells were found to be plasmid-free. Comparisons were made between uninduced and induced cultures of the CopyCutter EPI400 strain containing a pET-derivative with a kanamycin marker. The number of colonies on LB plates was no more than 10% higher than the number of colonies on LB-kanamycin plates.

### Clone toxic genes

To demonstrate the utility of the CopyCutter EPI400 strain we used two "potent" gene sequences that others have been unable to maintain in ColE1-type vectors. The first gene, *acpP*, encodes *E. coli* acyl carrier protein (ACP). When ACP is overproduced from multicopy plasmids, an unmodified form of the protein accumulates and inhibits cell growth.<sup>3</sup> A 423-bp PCR product containing *acpP* and its native promoter was ligated into pUC18 and aliquots of the reaction were electroporated into TransforMAX EC100 and CopyCutter EPI400 cells. Although 3 of the 36 TransforMAX EC100 clones screened contained a full-length insert, sequencing showed that each contained multiple point mutations, resulting in non-conserved amino acid substitutions. Quite remarkably, 27 of the 29 *acpP* CopyCutter EPI400 clones screened contained inserts of the expected size (Figure 2). Plasmid DNA purified from 3 clones induced to a higher copy number was also of the expected size and the sequenced inserts contained no mutations.



**Figure 2. DNA inserts encoding toxic gene products were successfully cloned into high-copy number vectors using CopyCutter™ EPI400™ *E. coli* cells.** After sequencing, the full-length *acpP* clones in TransforMAX™ EC100™ cells were found to contain multiple point mutations.



**Figure 3. Uninduced CopyCutter™ EPI400™ *E. coli* cells containing a *regB* clone (Lane U) are induced to higher-copy number (Lane I) using the CopyCutter™ Induction Solution.** Crude extracts of plasmid DNA were prepared from cells grown in selective media and analyzed by agarose gel electrophoresis. Approximately the same number of lysed cells (based on OD<sub>600</sub>) were loaded per lane.

Cloning the *regB* gene from phage T4 provided a second set of dramatic results. This gene encodes a restriction endonuclease that cleaves vital bacterial messages and is therefore highly toxic to *E. coli* even in very small quantities.<sup>4</sup> A 461-bp PCR fragment containing a promoterless *regB* gene was ligated into the T7 expression vector, pET11a, and aliquots of the reaction were electroporated into TransforMAX EC100

and CopyCutter EPI400 cells. Although neither strain contains a T7 RNA polymerase gene, basal expression of *regB* in pET-derivatives has been observed in similar hosts, presumably from cryptic *E. coli* promoters on the plasmid.<sup>4</sup> Of the 19 TransforMAX EC100 clones screened, 17 contained no insert, and 2 had large deletions. In contrast, all 29 CopyCutter EPI400 clones screened contained a full-length insert (Figure 2). Inducing a *regB* clone to higher copy number severely retarded cell growth but plasmid DNA purified from the induced culture was the correct size and the sequenced insert was free of mutations (Figure 3).

### Summary

EPICENTRE's new CopyCutter EPI400 *E. coli* cells significantly lower the copy number of many common vectors so that you can more readily clone unstable DNA sequences. Since you often can't predict whether the gene or chromosomal region you are cloning will be unstable, we supply these cells in a convenient, single-use format so you can easily incorporate them into your cloning regimen.

### References

1. Summer, D. (1998) *Mol. Microbiol.* **29**, 1137.
2. Atlung, T. et al. (1999) *Plasmid* **41**, 110.
3. Keating, D. et al. (1995) *J. Biol. Chem.* **270**, 22229.
4. Saida, F. et al. (2003) *Biotechnol. Progr.* **19**, 727.

\* Patent pending.

[www.epicentre.com/copycutter.asp](http://www.epicentre.com/copycutter.asp)

**CopyCutter™ EPI400™ Electrocompetent *E. coli*\***

C400EL10	10 X 50 µl
Includes CopyCutter™ Induction Solution and pUC19 Control DNA.	

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**CopyCutter™ EPI400™ Chemically Competent *E. coli*\***

C400CH10	10 X 50 µl
Includes CopyCutter™ Induction Solution and pUC19 Control DNA.	

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**CopyCutter™ Induction Solution**

CIS40025	25 ml
1000X concentrated solution. Filter sterilized.	