

Lucigen's electrocompetent cells are very efficiently transformed with BAC clones carrying large DNA inserts

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Introduction

Introduction of DNA into living cells is one of the most important and widely used procedures in molecular biology. Several methods have been developed to make bacterial cells competent to take up DNA. Chemical preparation of competent cells involves treatment with CaCl_2 or other chemicals (Hanahan, 1983). Recently, electroporation has become a method of choice to transform *E. coli* and other bacteria (Dower et al., 1988), because electrocompetent cells are very convenient to use and are commercially available at high efficiencies of transformation.

Efficiency of transformation depends largely on the competent cells; however, the size and amount of the transforming DNA also have an effect. Competent cells that efficiently take up small plasmids like pUC19 or pBR322 may not be efficiently transformed with large plasmids. In this study, we compare transformation efficiencies of commercially available electrocompetent cells using Bacterial Artificial Chromosome (BAC) clones of different sizes.

Results and Discussion

Single-copy BAC plasmids are the vectors of choice for construction of stable genomic DNA libraries with large inserts (Shizuya et al., 1992). However, clones obtained using single-copy vectors yield little DNA, which is often insufficient for end sequencing, restriction fingerprinting, or optical mapping. We had re-engineered the original pBAC vector by adding an inducible replication element (*oriV*) allowing "on demand" amplification of DNA (Szybalski et al., 2000, 2003; Wild et al., 2002). We and others have shown that clones obtained in such amplifiable pBAC/*oriV* vectors are maintained at single copy, but upon induction they produce multiple copies of the cloned DNA (Wild et al., 2002; Wild and Szybalski, 2004a). The pBAC/*oriV* vector is well suited for genomic library production and gene expression studies (Szybalski et al., 2002, Wild et al., 2004b). Derivatives of pBAC/*oriV* include the CopyRight™ vectors (Lucigen), which have been further optimized for construction of low-bias BAC libraries. They likewise allow induction of copy number (Figure 1).

A major consideration in constructing a BAC library is the quality of the competent cells, because plasmids with large DNA inserts are inefficient at transforming cells. To test the efficiency of electrocompetent cells for BAC transformation, we transformed pBAC/*oriV* clones carrying inserts of 40, 77, 108, or 140 kb into *E. coli*® Electrocompetent Cells (Lucigen) and competent cells from two other suppliers.

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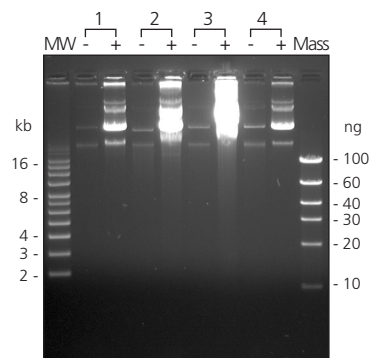


Figure 1. Copy number amplification of CopyRight™ clones. Genomic DNA from *Anopheles gambiae* was sheared to 40 kb, end-repaired, and cloned into the CopyRight pSMART VC vector. Fosmid clones (#s 1 - 4) were grown either with (+) or without (-) induction of copy number by L-arabinose. Uncut plasmid DNA from random clones was analyzed by gel electrophoresis.

MW = size marker (kb)
- = no induction
+ = copy number induction
Mass = mass marker (ng)

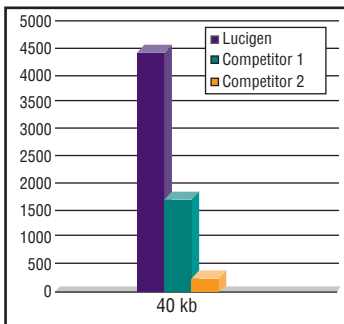


Figure 2. Relative transformation efficiency of a pBAC/oriV clone carrying a 40 kb insert of DNA from soil microorganisms.

DNA was electroporated into *E. coli* cells (Lucigen) and into competent cells from two other companies (Competitor 1 and 2). Electrocompetent cells (25 μ l) were mixed with 2 μ l of plasmid DNA and transferred into a chilled Gene Pulser cuvette (0.1 cm). Electroporations were performed using a BTX Transporator Plus Electroporation System at 1.8 V. After the pulse, 1 ml of Recovery Medium was added, and cells were incubated for 1 hour at 37°C. Cells were diluted 100-fold, and 0.1 ml was plated.

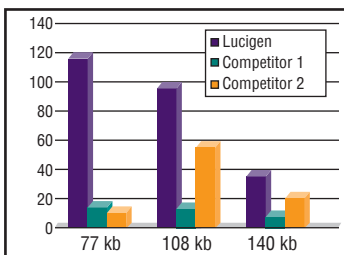


Figure 3. Relative transformation efficiencies for different sizes of BAC clones. The DNA of pBAC/oriV clones carrying 77 kb of rice DNA, 108 kb of wheat DNA, or 140 kb of wheat DNA were electroporated into *E. coli* cells (Lucigen) and into competent cells from two other companies. Conditions for electroporations were as described in the legend to Figure 2, but different dilutions were used.

Upon transformation with a pBAC/oriV clone carrying a 40-kb insert of DNA from soil microorganisms, the Lucigen cells yielded three times more transformants than those of competitor 1 and over 10 times more than those of competitor 2 (Figure 2).

Similar results were obtained in a separate experiment using larger clones that contained DNA from other sources. Transformation of a BAC containing 77 kb of rice DNA produced about 10 times more transformants using Lucigen's competent cells as compared to competent cells from competitor 1 or 2 (Figure 3). Efficiency of transformation using a 140 kb wheat DNA insert was five times higher than that of competitor 1 and 30% higher than that of competitor 2.

Conclusions

We have shown here that the source of electrocompetent cells has a profound influence on the efficiency of transformation. Using *E. coli* Electrocompetent Cells from Lucigen resulted in an increase of 3-10 fold in the number of colonies produced from BAC clones. In addition, the Replicator™ Cells from Lucigen were proficient at amplifying the copy number of plasmids containing the *oriV* inducible origin, such as pBAC/oriV and the CopyRight™ vectors from Lucigen.

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