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A novel method for increasing the transformation efficiency of *Escherichia coli*—application for bacterial artificial chromosome library construction

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ABSTRACT

Bacterial artificial chromosome (BAC) libraries play a pivotal role in genomics studies. A crucial step in BAC library construction is the transformation of *Escherichia coli* by electroporation. Absolute efficiency (cfu/μg DNA) is affected by a number of factors including the topological form and treatment of DNA samples. Here we report a simple new protocol using tRNA assisted precipitation that increased transformation efficiency by 70-fold for BAC ligations and up to 400-fold for plasmid ligations. The mechanism may involve altering or stabilizing the topographical form of the DNA molecules.

Large-insert libraries, particularly bacterial artificial chromosome (BAC) libraries, are increasingly being used as the foundation for physical mapping, map based cloning, and sequencing projects (1). BAC libraries are relatively straight forward to construct and have few chimeric clones (1–4). A crucial step in BAC library construction is transferring ligated vector-insert DNA into *Escherichia coli* cells. Electroporation has been proven to be the most efficient transformation method thus far (5). However, obtaining large insert size clones is offset by a significant and often unacceptable reduction in the number of clones obtained. In addition to insert size, efficiency (cfu/μg of DNA) is affected by factors such as buffer components, temperature (6) and electroporation conditions determined by users, including voltage gradient, resistance and capacitance (7–10). Furthermore, the genetic background of host cells (5), post-pulse treatment (11), the topological form (12) and treatment of DNA samples (13,14) also contribute to the efficiency observed. Here we describe a straightforward new protocol that increases the number of recombinant clones obtained (transformation efficiency) up to 70-fold for BAC ligation mixtures and 300–400-fold for plasmid ligation mixtures.

Electrocompetent cells (efficiency > 1.5×10^{10}/μg DNA), DH10B, were prepared as described by Hanahan (5). For BAC DNA ligations, 25 ng of size-selected high-molecular-weight DNA electroeluted from TAE agarose gel matrix (25–50 μl) and 20 ng vector DNA (pBACwich) in 1 μl TE were mixed and incubated at 56°C for 10 min (15). The mixture was cooled to room temperature and 9 μl of 10× ligation buffer (300 mM Tris–HCl pH 7.5, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP) and 12 U of T4 ligase (Promega, USA) were added to a final volume of 100 μl. After overnight ligation at 16°C, 5 μl of ligation product were mixed with 1 μl of 1 μg/μl yeast tRNA (dissolved in ultrapure water, Gibco BRL, USA), and 14 μl ultrapure water. Fifty microliters of 100% cold ethanol were added to precipitate the DNA. After 15 min at –20°C, the sample was centrifuged at 14 000 *g* for 10 min at 4°C. The pellet was washed once with 100 μl of 70% ethanol, air-dried, and resuspended in 1 μl of ultrapure water. Twenty microliters of competent cells were mixed with resuspended DNA and transformed using an Electroporator I (Invitrogen, USA) with the following conditions: 13 kv/cm for field strength and 2.4 ms for time constant. After recovery in 1 ml of SOC medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose pH 7.0) at 37°C for 1 h with shaking, the cells were plated on LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar) containing 12.5 μg/ml chloramphenicol, 7 μl of 200 μg/ml IPTG and 70 μl of 20 μg/ml X-gal per 100 mm diameter plate. The numbers of recombinant clones (white) were counted after incubation at 37°C for 24 h. As a control experiment, 1 μl of the ligation mix was transformed directly, and the number of recombinants determined. Transformation efficiencies (cfu/μg DNA) were calculated for each treatment shown in Table 1 based on the amount of insert DNA added to each ligation reaction.

In comparison with direct transformation of the BAC ligation mixture, the tRNA/precipitation method increased overall transformation efficiency by 70-fold (Table 1). Using this new method, we routinely obtained >1200 recombinant clones/5 μl of a 100 μl BAC ligation mix, resulting in an efficiency of 1.6×10^6 cfu/μg DNA. The proportion of recombinant BAC clones as estimated by the blue–white ratio was not affected by the addition of tRNA. Nor did the treatment damage large DNA ligation products. Average insert-sizes of BAC clones obtained using both tRNA/precipitation method and direct transformation were very similar with similar insert-size distribution profiles. A BAC library with an average insert-size of 130 kb was constructed for the rice blast fungus *Magnaporthe grisea* using the tRNA/precipitation method (15).

A variety of other treatments were included to elucidate the possible mechanisms responsible for this finding. Transformation of 1 μg of tRNA alone produced no colonies. Heat inactivation of T4 DNA ligase prior to direct transformation resulted in a 7-fold increase in efficiency, perhaps as a result of denaturing the DNA ligase. Adding tRNA after the heat treatment increased the efficiency a further 2-fold (Table 1). Neumann *et al.* (6) suggested that the topological form of circular DNA affects the transformation

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Table 1. Transformation efficiency of BAC and plasmid ligation products following different post ligation treatments

	BAC ligation mixtures			Plasmid ligation mixtures				
				Sticky-end cloning				TA cloning
[A]*	+	+	-	-	-	-	-	-
[B]	-	+	+	+	-	-	-	+
[C]	-	-	-	-	+	-	-	-
[D]	-	-	-	-	-	+	-	-
[E]	-	-	-	-	-	-	+	-
[F]	-	-	+	+	+	+	+	+
Efficiency (cfu/µg)	(1.6±0.2)# X10^5	(3.3±0.3) X10^5	(1.6±0.1) X10^6	(9.2±1.2) X10^9	(8.2±1.8) X10^8	(4.8±2.4) X10^8	(1.0±0.8) X10^8	(1.3±0.4) X10^9
Control (cfu/µg)	(2.3±1.0) X10^4	(2.3±1.0) X10^4	(2.3±1.0) X10^4	(2.3±0.4) X10^7	(1.9±1.3) X10^7	(1.9±1.3) X10^7	(1.9±1.3) X10^7	(4.2±1.0) X10^6
Fold increase	7±3	14±5	70±3	410±60	44±12	26±13	5±2	297±37

*Treatment (+ means that the treatment was performed): [A] ligation mixture was heated at 70°C for 20 min; [B] 1 µg of tRNA added; [C] 1 µg of double-stranded Herring sperm DNA added; [D] 1 µg of single-stranded Herring sperm DNA added; [E] 0.1 V, 3 M NaOAc added; [F] 2.5 × 100% ethanol added and pellet collected by centrifugation. Pellet was washed with 70% ethanol and then resuspended in ultrapure water.

#Mean ± standard deviation of at least three separate experiments using the same preparation of electrocompetent cells. Transformation efficiency was calculated based on the amount of DNA in each ligation reaction.

efficiency; supercoiled being more efficient than relaxed DNA. When purified DNA from BAC clones (in ultrapure water) was heated at 65°C for 20 min, transformation efficiency decreased 5-fold. However, when tRNA was added before the heat treatment, transformation efficiency remained the same as the unheated control (data not shown). This suggests that tRNA prevented the BAC DNA from relaxing and thus the transformation efficiency remained unaffected.

Similar results were obtained using plasmid ligations; however, the tRNA/precipitation method increased transformation efficiency by ~300-fold for TA cloning (pGem T-easy, Promega, USA) and ~400-fold for sticky-end cloning (Table 1). For sticky-end ligations, *Eco*RI linearized pUC18 was used as vector. Plasmid ligations were set up by mixing 20 ng of appropriately digested vector DNA, 20 ng of insert DNA, 2 µl 10× ligation buffer and 3 U of T4 DNA ligase in 20 µl reaction. Ligation and electroporation conditions for plasmids were the same as described for BAC ligations, except transformed cells were spread on LB plates containing 50 µg/ml ampicillin. When XL-1 Blue was used as host, the fold increase dropped to ~140-fold (data not shown).

The effects of other carriers or additives to facilitate precipitation by ethanol were also investigated. Plasmid ligated DNA was ethanol precipitated as described above except other carriers or additives were substituted for tRNA, including NaOAc (0.1 vol), double- or single-stranded Herring sperm DNA (1 µg; Sigma, USA). Double-stranded DNA was prepared by dissolving Herring sperm DNA in ultrapure water then autoclaved for 15 min followed by gradual cooling to room temperature. To prepare single-stranded DNA, double-stranded Herring sperm DNA was boiled for 5 min then snap cooled on ice. NaOAc assisted precipitation had only a modest effect and resulted in a 5-fold increase in transformation efficiency compared with direct transformation. This treatment and subsequent 70% ethanol wash may have removed salts from the ligation mixture that are known to negatively affect electroporation efficiency. High salt causes excessive heat during electroporation and *E.coli* cell death. Addition of either single- or double-stranded DNA, although resulting in increased electroporation efficiency, was 6–10 fold less efficient than adding tRNA (Table 1).

Overall, these results demonstrate that tRNA assisted ethanol precipitation substantially increases the number of recombinant clones that can be obtained by transformation. The mechanism may involve altering or stabilizing the topological form of the ligated DNA molecules. Moreover, the size of inserts derived from large insert ligation experiments appeared to be unaffected. This method should prove particularly valuable in BAC library construction for organisms with large genomes.

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