

High efficiency electroporation of ligated DNA into bacteria

Eugene R.Zabarovsky[1,2] and Gösta Winberg[1]
[1]Department of Tumor Biology, Karolinska Institutet, Box 60400, S 10401 Stockholm, Sweden and
[2]Engelhardt Institute of Molecular Biology, Academy of Sciences of the USSR, 32 Vavilov Street, Moscow, 117984, USSR

Submitted July 31, 1990

The use of high voltage electroporation has proven a highly effective method for introducing genetic material into eucaryotic (1) as well as procaryotic cells (2). Using optimal procedure, the efficiency of bacterial transformation approaches 10^{10} colonies/μg of plasmid DNA. Although the true power of the method resides in its use for applications such as library construction where a quantitative yield of clones from ligated material is essential, it would also be a convenient replacement for CaCl2 mediated transformation procedures traditionally used, provided that ligated DNA could be used. Previous studies (3, 4) indicate that the use of ligated DNA for electroporation results in low transformation efficiencies. We have reinvestigated this, and find that ligation reactions can indeed be used for electroporation after precipitation with either ethanol or isopropanol yielding efficiencies of $0.5-2.5\times10^8$/μg of ligated DNA. In these tests, purified plasmid DNA yielded efficiencies of $1-9\times10^9$ colonies/μg. Bacterial cells were grown in 1 L of NZCY or L-broth to A600 0.5−0.7. Cells were washed three times in successively smaller volumes (300, 100, 50 mL) of ice cold 10% glycerol in water and the final pellet was resuspended by adding 1 mL of 10% glycerol. The glycerol solution was sterile-filtered rather than autoclaved to avoid the formation of aldehydes. It was found essential to rinse all glassware and filters meticulously in double-distilled water to remove traces of detergent. The cell suspension was frozen as 50 μL aliquots in liquid nitrogen and stored at −70°C. Aligquots of 5 ng HindIII cleaved pBS+ (Stratagene) was ligated in 50 μL of 50 mM Tris-HCl pH 8, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP and 8 U T4 DNA-ligase. In other experiments, 500 ng each of EcoRI cleaved pBS+ and a 2.6 kb cDNA fragment with EcoRI ends were ligated under the above conditions. After 16h at 15°C, potassium acetate pH 8 was added to 0.25M and 2.5 vol ethanol or 0.8 vol isopropanol was added. Ethanol precipitates were allowed to form at −20°C for 2 h. Isopropanol precipitates were left on ice for 2 h. The DNA was sedimented at 14000 rpm for 2 min. at 4°C in a refrigerated Eppendorf centrifuge. The pellets were rinsed twice with cold 70% ethanol and air dried before they were redissolved in 5 μL water. Electroporation was performed as described (2). To each aliquot of bacterial cells, 1 μL of precipitated ligation mix was added. At 2.5 kV, 25 μFD and 200 ohms parallel resistance, time constants around 4.7 msec were obtained with the Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, CA). Arcing (3) was observed infrequently, but correlated either with air bubles in the cell suspension, or with the cuvette used, rather than with the DNA solution. It was found to be essential to add SOC medium (2) immediately after the completion of the pulse. Therefore, the cuvettes were used without the lid, and SOC was added directly into the cuvette with a Pasteur pipette. Screening of 12 colonies from the ligation of the cDNA into pBS+ revealed that 3 clones were self-ligated pBS+, while 9 clones contained the insert. Thus, the conditions for a bimolecular ligation reaction had been attained. XL-1 Blue cells were from Stratagene.

REFERENCES

1. Potter,H. *et al.* (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7161−7165.
2. Dower,W.J. *et al.* (1988) *Nucl. Acids Res.* **16**, 6127−6145.
3. Willson,T.A. and Gough,N.M. (1988) *Nucl. Acids Res.* **16**, 11820.
4. Jacobs,M. *et al.* (1990) *Nucl. Acids Res.* **18**, 1653.

Table 1.

Host Cells	DNA	Efficiency CFU/μg
LE392	pBS+	$2-3\times10^9$
MC1061	pBS+	$6-9\times10^9$
TG-1	pBS+	$3-6\times10^9$
XL-1 Blue	pBS+	$9-10\times10^9$
XL-1 Blue	Self-ligated pBS+, Ethanol ppt.	$0.5-1\times10^8$
TG-1	Self-ligated pBS+, Isoprop. ppt.	$0.5-1\times10^8$
XL-1 Blue	Self-ligated pBS+, Isoprop. ppt.	$1-2.5\times10^8$
XL-1 Blue	cDNA + pBS+, Isoprop.ppt	$0.5-1.5\times10^8$