Volume 16 Number 24 1988 Nucleic Acids Research

High voltage *E.coli* electro-transformation with DNA following ligation

Tracy A.Willson and Nicholas M.Gough

The Walter and Eliza Hall Insitute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia
Submitted November 17, 1988

High efficiency transformation of E.coli with plasmid DNA has recently been achieved by electroporation [1], which has been reported to give transformation frequencies approaching 10^{10} transformants/µg plasmid DNA [1], some 2 orders of magnitude greater than most conventional techniques. Thus electroporation makes feasible the construction of large recombinant libraries in plasmid vectors. However, the electroporation technique requires that the DNA be in a low ionic strength buffer such as TE [10mM Tris-HCl, pH8, 1mM EDTA]. If the salt concentration of a DNA solution is too high, arcing occurs across the cuvette and no electroporation ensues. Currently available literature [1] does not address the problem of how to deal with DNA in high salt, such as that encountered under ligation conditions. We have therefore investigated ways to circumvent this problem.

Bacteria were prepared for electroporation as described [1], except that after harvest, the first two washes were in 10% glycerol, 1mM HEPES pH7, in order to reduce cell lysis. Thus we have routinely prepared batches of E.coli MC1061 [2] which can be transformed at a frequency of $5x10^8$ to 10^{10} transformants per µg of pUC12, although some batches display a lower frequency [10^7/µg]. Batches of NM522 [3] were obtained at frequencies between $4x10^7$ and $3x10^8$/µg pUC12.

Although the obvious means of removing salt is ethanol precipitation, in our hands such DNA caused arcing, despite repeated washings with 70% ethanol. Consequently, we explored the option of dilution. The Table details the transformation efficiency after dilution of a ligation mix containing 100ng of religated pUC12. Using 1-5µl of undiluted ligation mix, no electroporation was obtained due to arcing. Our general experience is that it is possible to electroporate up to 5µl of a 1/10 dilution: for example in the experiment in the Table, a frequency of 2×10^6/µg was obtained at this dilution, irrespective of whether the electroporated cells were finally diluted into 1500µl [a] or 300µl [b] of growth medium. Although we routinely dilute ligation mixes only 10-fold, we note that in general a 1/100 dilution yields a higher transformation efficiency [$3x10^7$ compared with $2x10^6$ in this experiment] and thus where the maximum recovery from a ligation mix is required, such a dilution would be recommended.

SAMPLE	TRANSFORMATION FREQUENCY/µg
Uncut pUC12	3×10^8
1 to 5µl undiluted ligation	arcing - no electroporation
5 µl 1/10 diluted ligation [a]	2×10^6
5 µl 1/10 diluted ligation [b]	2×10^6
5 µl 1/100 diluted ligation	3×10^7

Ligation mix: 100 ng EcoR1-cut pUC12 religated at 15°C for 16 hrs in 50 mM Tris-HCl pH8.0, 10 mM $MgCl_2$, 1 mM ATP, 10 mM DTT, 3 Units T4 DNA ligase

ACKNOWLEDGEMENT This work was supported by AMRAD Corporation.

References:

1. W.J. Dower et al., Nucl. Acids Res. 16, 6127-6145 [1988].
2. M.J. Casadaban et al., J. Mol. Biol. 138, 179-207 [1980].
3. J.A. Gough et al., J. Mol. Biol. 166, 1-19 [1983].