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Electro-transformation of *E. coli* with M13 DNA

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Introduction

M13 vectors have been used extensively for subcloning mainly because the abundant, easily isolated single-stranded form of the mp series M13 phage DNA is a superior starting material for sequencing and *in vitro* mutagenesis. In addition, the blue/white selection process afforded by the LacZ fusion cloning scheme allows easy detection of recombinant phage over background. During the process of evaluating and optimizing different techniques of transforming *E. coli* with M13 DNA, including conventional chemical methods and electroporation, we found significant inhibition of *E. coli* XL1-Blue/M13mp19 transformation efficiency by the addition of routine amounts of X-Gal* and IPTG** to electroporated cells. This inhibition is not observed with plasmid or conventional transformations. The problem was circumvented by adding the X-Gal and IPTG to the bottom agar, or by allowing the cells to recover for 20 minutes before the addition of X-Gal and IPTG. Electroporation proved to be the most efficient technique for transforming these cells with M13 DNA.

Materials and Methods

Electroporation Competent Cells

Fresh streaks of XL1-Blue[1] from a frozen glycerol stock were grown overnight at 37 °C on L-broth (1% tryptone, 0.5% yeast extract, 170 mM NaCl[2]; pH 7.5) agar plates containing 10 µg/ml tetracycline. Five or six single colonies were dispersed in 1 ml SOB medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM each $MgCl_2$ and $MgSO_4$[2]; pH 7.5) and then added to 1 liter SOB medium with 10 µg/ml tetracycline. Bacteria were grown at 37 °C with moderate agitation until they reached an $O.D._{600}$ of approximately 0.3. Flasks were chilled on ice for 30 minutes, and cells were spun at 3,000 rpm in 250 ml bottles. The supernatants were decanted, and cells were dispersed by gentle inversion in an equal volume of cold 1 mM HEPES, pH 7.0. Cells were washed 3 more times as above with the following: one volume cold water, one-half volume cold water, and 30 ml cold 20% glycerol. The final cell pellet was resuspended in 2 ml cold 10% glycerol. Cells were distributed in 50 µl aliquots into chilled Eppendorf tubes and then immediately flash-frozen in a dry ice/ethanol bath. Frozen electroporation competent cells were stored at -70 °C.

Electro-transformation

Electroporation competent cell aliquots (above) were thawed on ice and appropriate quantities of DNA in water (see Discussion section) were added in 1 µl total volume. Mixtures were pulsed with the Gene Pulser® apparatus and Pulse Controller at various voltages with 25 µF and 200 Ω in chilled 0.1 cm electroporation cuvettes (Bio-Rad); immediately after pulsing, 200 µl of an overnight or late log-phase culture of XL1-Blue cells were added to each cuvette and mixed with the pulsed cells. Mixtures were transferred to Falcon 2059 tubes and mixed with 3 ml melted 0.83% L-top agar at 50 °C with or without X-Gal (in N, N-dimethylformamide; DMF) and IPTG (in water), then plated immediately on warmed L-agar plates or L-agar plates with 6 mg/ml X-Gal and 9 mg/ml IPTG. Plaques were visible after 6 hours at 37 °C.

Competent Cells Prepared by Conventional Methods

XL-1 Blue cells were prepared according to the RF1/RF2 method of Hanahan[2] and frozen in 500 µl aliquots. Competent INV1αF´ *E. coli* cells (DH1 derivatives) were purchased from Invitrogen (San Diego).

Transformantion without Electroporation

Competent cells were thawed on ice and 100 µl aliquots were distributed into chilled Falcon 2059 tubes. DNA in TE (10 mM Tris-HCl, 1 mM EDTA; pH 7.5) was added and allowed to incubate on ice for 30 minutes. Mixtures were heat shocked for 90 seconds at 42 °C and replaced on ice immediately. For transformation with M13 DNA, 200 µl of growing cells were added and the mixture was plated as above. For plasmid DNA transformation, SOC medium (SOB medium with 2% glucose) was added to 1 ml and the mixture was shaken at 37 °C for 1 hour before plating on L-agar plates with 100 µg/ml ampicillin.

* X-Gal: 5-Bromo-4-chloro-3-indoxyl β-D galactoside
** IPTG: Isopropyl β-D-thiogalactopyranoside

Table 1. Electroporated Transformations§

Sample	mp19 DNA (pg)	Electroporation Volts	Electroporation Ω in parallel	X-Gal and IPTG	# of Plaques	Extrapolated Transformation Efficiency (transformants/µg)
1	10	1,880	200	0	3,558	3.6×10^8
2	1	1,880	200	0	541	5.4×10^8
3	10	1,880	200	bottom agar	3,220	3.2×10^8
4	1	1,880	200	bottom agar	489	4.9×10^8
5	10	1,880	200	4 µl/4 µl*	3,140	3.1×10^8
6	10	1,880	200	50 µl/10 µl**	328	3.2×10^7
7	10	1,880	200	50 µl DMF***	1,620	1.6×10^8
8	0	1,880	200	0	0	0
9	10	0	0	0	0	0
10#	10	1,880	200	50 µl/10 µl	1,046	1.1×10^8
11##	1 pg pKS^+	1,880	200	bottom agar	880	8.8×10^8

§ Data from one batch of XL1-Blue electroporation competent cells
* 4 µl 200 mg/ml X-Gal in DMF + 4 µl 200 mg/ml IPTG in water
** 50 µl 20 mg/ml X-Gal in DMF + 10 µl 20 mg/ml IPTG in water
*** DMF only
\# Sample 10 was allowed to recover at 37 °C for 20 minutes before plating with X-Gal and IPTG.
Sample 11 was transformed with the Bluescript plasmid pKS^+ (Stratagene, San Diego).

Table 2. Non-electroporated Transformations
(All transformants without X-Gal and IPTG)

Sample	*E. coli* Cells	DNA	Extrapolated Transformation Efficiency (transformants/µg)
1	XL1-Blue	M13mp18	1.0×10^6
2	XL1-Blue	pKS^+	3.7×10^7
3	INV1αF'	M13mp18	3.3×10^7
4	INV1αF'	pKS^+	9.0×10^7

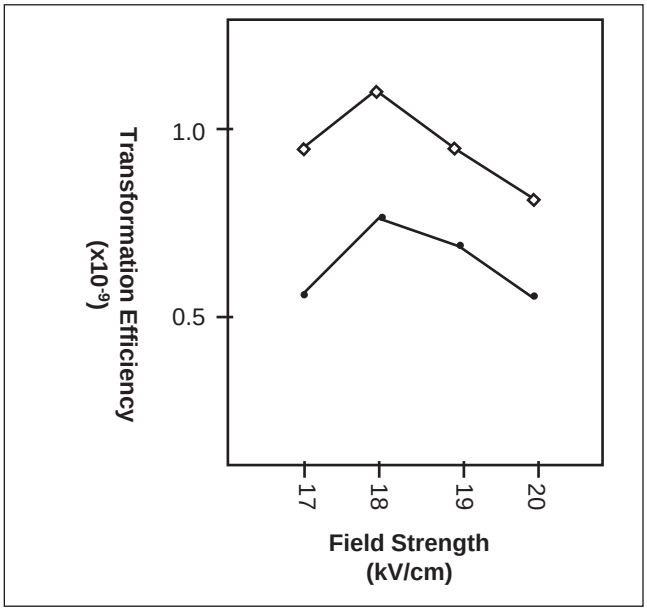

Fig. 1. Transformation efficiency as a function of field strength. Data from one batch of electroporation competent cells: 0.5 picograms of M13mp19 DNA were electrotransformed with 1,700-2,000 volts in 0.1 cm cuvettes at 200 Ω and 25 µF, and then plated on L-agar (◊) or L-agar containing X-Gal and IPTG (•).

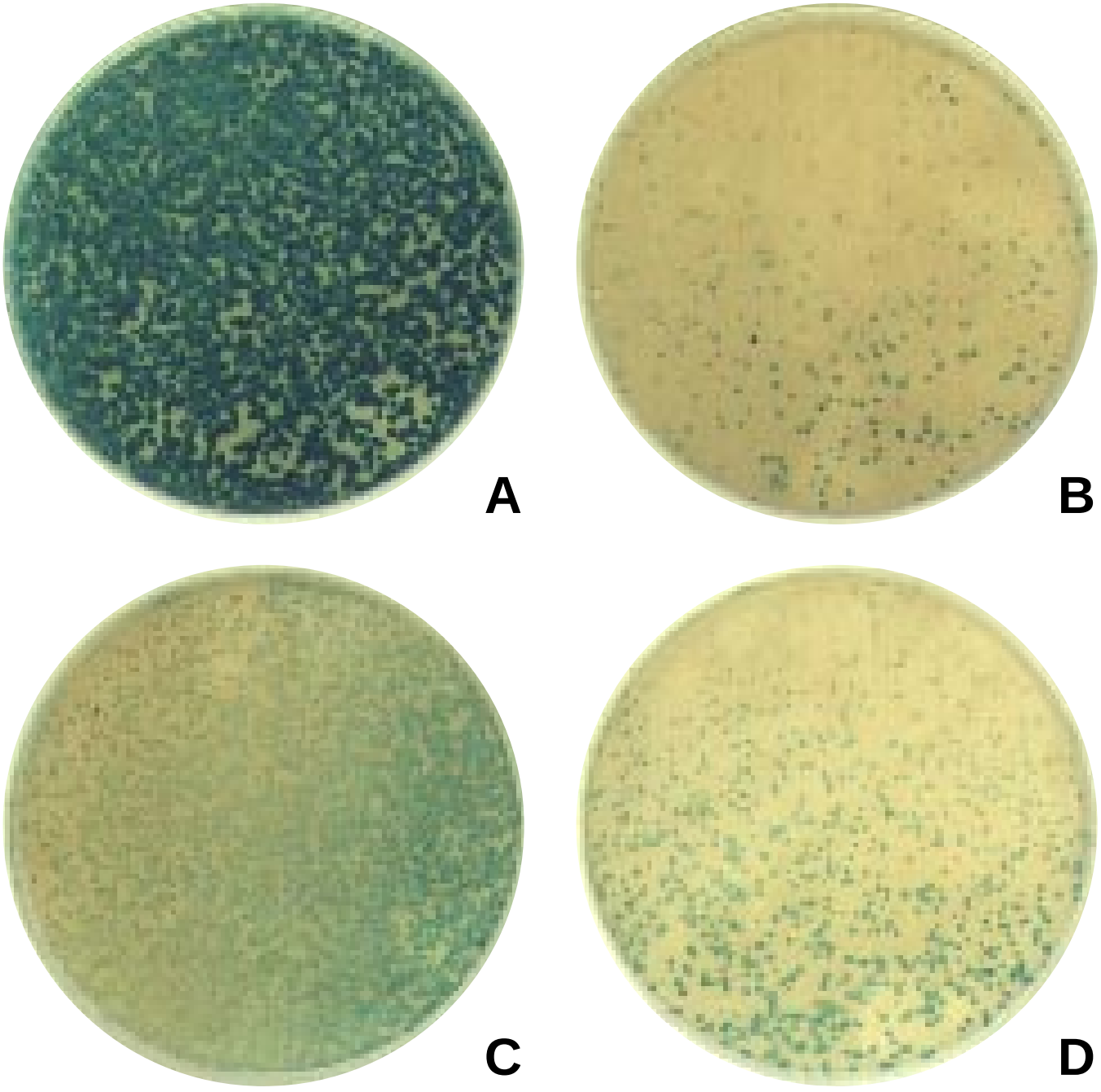

Fig. 2. Blue plaques resulting from different X-Gal and IPTG treatments of electro-transformed *E. coli* cells. All pictured samples received 10 pg M13mp19 DNA. A) Sample 3, Table 1: electro-transformation plated on an X-Gal and IPTG plate. B) Sample 6, Table 1: 50 µl 20 mg/ml X-Gal and 10 µl 20 mg/ml IPTG added to cells after electroporation. C) Sample 5, Table 1: 4 µl 200 mg/ml X-Gal and 4 µl 200 mg/ml IPTG added to cells after electroporation. D) Sample 10, Table 1: 50 µl 20 mg/ml X-Gal and 10 µl 20 mg/ml IPTG added to electroporated cells after a 20 minute recovery at 37 °C.

Results

When *E. coli* cells transformed with M13mp19 DNA by electro-transformation are plated with a routine amount of X-Gal and IPTG (Table 1, sample 6), a reduction of about ten-fold is seen in the transformation efficiency of our electroporation competent XL-1 Blue cells. To determine if the X-Gal solvent (DMF) was the source of this reduction, cells were plated with DMF only (sample 7). These cells fare better than those with the routine amount of X-Gal and IPTG, although the efficiency is lowered by about 50% from that of cells with no additives (sample 1). This indicates that although the DMF contributes to the problem, it is not the major factor. Reducing the volume of the additives by increasing their concentration helps significantly (sample 5), but the transformation efficiency still does not equal that of untreated cells. In fact, the reduced volume causes some problems due to the increased difficulty of dispersing the small volume evenly throughout the plating agar (resulting in uneven distribution of blue plaques, or no blue plaques at all). Adding the X-Gal and IPTG to the bottom agar has proven to be a reasonable solution: the efficiency is reduced by only about 10-30%, and the blue color is distributed evenly across the plates. Note that allowing the transformed electroporated cells to recover for 20 minutes before adding the X-Gal and IPTG (sample 10) increases the transformation efficiency somewhat. However, longer incubations should be avoided as the transformed cells will begin to produce infectious virus after about 30 minutes.

We have not been routinely successful in generating conventional competent cells with transformation efficiencies greater than 5×10^8/µg. However, our electroporation competent cells in the efficiency range of 10^9-10^{10}/µg are easily and reproducibly generated with the protocol described above. In addition, conventional competent XL1-Blue cells take up M13mp18 DNA at a significantly reduced efficiency from that of plasmid DNA (Table 2, samples 1 and 2) which can not be explained with the decreased molar concentration of M13 due to its larger size. This effect is not seen with electroporated XL1-Blue cells (Table 1, samples 4 and 11), or with conventional competent INV1αF' cells (Table 2, samples 3 and 4).

Discussion

While attempting to increase electro-transformation efficiencies with M13 DNA, we found several critical factors that influence the process: 1) cells should be started from a fresh streak from a frozen stock; 2) cells must be kept scrupulously near 0 °C during preparation as well as during electroporation; 3) growing cells must be added to the electroporated cells immediately after electroporation; 4) DNA should be added in a small volume of water to keep ionic contaminants at a minimum (ions interfere with the electroporation process and also increase the chance of high voltage arcing in the sample); and 5) growing cells must be in good condition, preferably before stationary phase. Each batch of electroporation competent cells seems to vary somewhat in efficiency relative to field strength; we routinely titer each batch accordingly (Figure 1).

We found a decrease in the number of M13 vector transformant plaques obtained from electroporated XL1-Blue cells treated with X-Gal and IPTG which does not appear to be due solely to the presence of the X-Gal solvent, DMF. The suppressive effect varies with the amount of X-Gal and IPTG added to the electroporated cells, but is minimized when the reagents are added in a small volume or to the bottom agar only. The highest electro-transformation efficiencies of XL1-Blue with M13-derived vectors, however, are always obtained when X-Gal and IPTG are avoided entirely. We found no significant X-Gal/IPTG sensitivity of XL1-Blue electro-transformations with plasmids (data not shown). We have not fully investigated the effect with other strains of *E. coli*, but preliminary work with INV1αF' shows only limited sensitivity to X-Gal and IPTG. We have not been as successful obtaining high electro-transformation efficiencies ($>10^8$/µg) with this strain.

There is good motivation for using X-Gal and IPTG when subcloning into the M13 mp series vectors because the blue/white color selection allows easy detection of recombinants over background. There is also good motivation for electroporating XL1-Blue to transform M13 DNA, as electroporation seems to maximize the transformation efficiency of this strain. With conventional transformation techniques, blue/white selection does not present a problem; however, electroporated XL1-Blue cells show a significant sensitivity to even small amounts of these reagents present in top agar. If blue/white selection is necessary when electro-transforming XL1-Blue with M13 vectors, we suggest addition of the X-Gal and IPTG to the bottom agar rather than (traditionally) to the top agar in order to minimize the decrease in efficiency.

References

1. Bullock, W., *et al., BioTechniques,* 5, 376 (1987).
2. Hanahan, D., *DNA Cloning,* Volume I, page 109, D.M. Glover, ed. IRL Press, 1985.

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