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High efficiency 5 min transformation of *Escherichia coli*

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We report a simple, rapid, 5 min transformation protocol that produces ~100% more transformants than the original calcium competent protocol of Mandel and Higa (1). There have been improvements on the transformation efficiency of 10^5–10^6 colonies/μg plasmid DNA (2); the best probably that of Hanahan (3) yielding 10^7–10^9. The highest rates are achieved using electroporation (10^9–10^{10}). However, we experienced difficulties in electroporation of the repair deficient strain of *Escherichia coli* (BMH71-18 mut S) required in the Unique Site Mutagenesis (USE) technique (4) and examined factors effecting the efficiency of the calcium competent protocol. It is possible to transform cells in a fraction of the time recommended and vastly improve efficiency.

Mutagenesis protocols generally rely on a high transformation efficiency, but this is not of prime concern for the majority of cloning techniques. Standard calcium chloride transformations are simple, cheap and the competent cells may be stored at 4°C or frozen at –70°C, whereas Hanahan cells must be used immediately and electroporating equipment is expensive.

We have concentrated on three *E.coli* cell types, JM101 for general cloning steps, BL21(DE3) for subsequent protein expression and the repair deficient BMH71-18 mut S. Cells were grown at 37°C in 100 ml of 2xTY medium (16 g bacto tryptone, 10 g yeast extract, 5 g NaCl/l pH 7.4) containing 50 μg/ml tetracycline or ampicillin where appropriate. At an A_{600} ~0.7–0.8 they were chilled on ice, pelleted, resuspended in half volume of 0.1 M $CaCl_2$, cooled on ice for 30 min, repelleted and resuspended in 5 ml 0.1 M $CaCl_2$ (5). Competent cells were stored at 4°C or as 100 μl aliquots in 10% glycerol at –70°C. The plasmid used was a 4.8 kb construct of human gelsolin cDNA in pKN172 (a 2.6 kb ampicillin resistant pET based vector) (6) . In the 'standard protocol', ~1–10 ng of DNA was mixed with 100 μl of cells, left for 30 min on ice, heat shocked to 42°C for 2–3 min, cooled again on ice before 500 μl antibiotic free medium was added for a 30–60 min outgrowth at 37°C and final plating out on selective agar plates (5). Using these conditions we achieved ~5×10^6 colonies/μg plasmid.

The effect of salt concentration on electroporation has been well documented. Calcium competent *E.coli* were originally transformed using DNA solutions containing 20 mM NaCl (7), Figure 1 shows the effect NaCl has on these transformations. Optimum volumes for transformation were 8 ± 4 μl DNA for 100 μl cells. Larger volumes reduced transformation efficiency: 30 μl gave 70% of the colonies obtained using 5 μl. The length of time that cells were pre-incubated on ice with DNA was not important. JM101 cells incubated with plasmid on ice for 1–180 min before heat shock showed little variation in transformant numbers; at 180 min transformation was ~75% of that at 5 min.

Transformations with BMH71-18mutS cells were variable and often as low as 20% of those obtained from the other cell lines. Incubation of cells with the plasmid on ice followed by directly spreading onto plates at room temperature (i.e. no 42°C heat shock) was sufficient to restore the higher transformation efficiency. When this was repeated on plates pre-incubated to 37°C the efficiency was even higher. Figure 2A shows results for JM101 cells incubated with the GS construct where the plasmid/cell mixtures have been plated directly from the incubation on ice onto agar plates pre-incubated to different temperatures. By spreading directly onto plates pre-incubated to 37°C we routinely achieve double the transformation efficiencies of the 'standard protocol'.

Once heat shocked to 42°C as in the standard transformation protocol, there is an indifference to the pre-incubation temperature of the plates (Fig. 2B). This figure also demonstrates that cells give higher transformation rates following 3–4 days storage at 4°C and can be used for periods of up to 2 weeks for simple transformation of plasmid, although in agreement with Dagert and Ehrlich's original observations they are best used after 24 h (8).

Following 42°C heat shock, transformations were generally better with a 30–60 min 37°C non-selective medium recovery

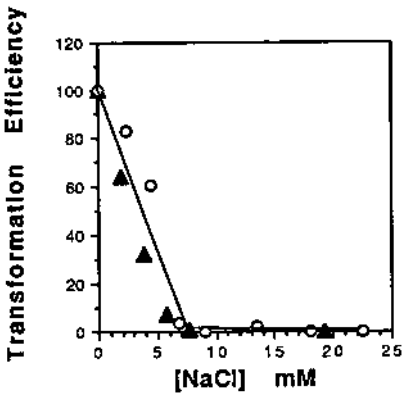

Figure 1. Results from two experiments on the transformation rates from JM101/DNA mixtures containing salt, expressed as percent of those obtained with zero salt. (400 and 1400 colonies at zero salt).

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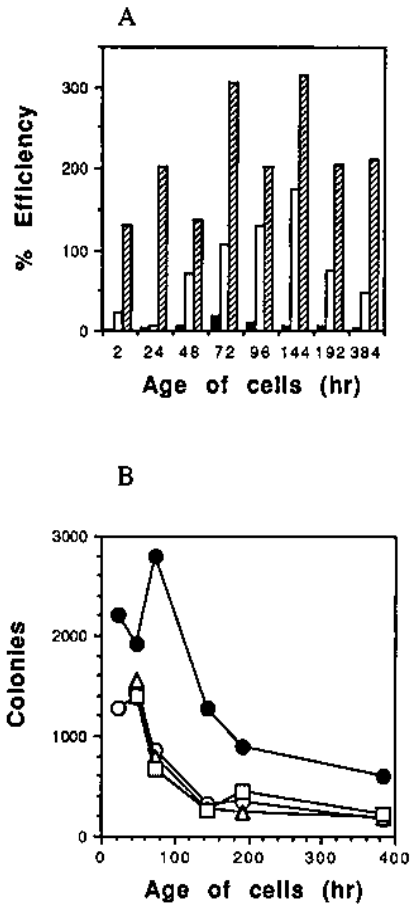

Figure 2. (**A**) JM101 cells incubated with DNA on ice and then spread onto plates pre-incubated at 4°C (solid bars), 20°C (open bars) and 37°C (hatched bars). Results expressed as percent of those for the standard heat shock and outgrowth protocol spread onto 20°C plates at each cell age point. Cells prepared and stored at 4°C for testing to 16 days old. Data averaged from three experiments with 100–1400 colonies for control plates. **(B)** Colony counts from the 'direct' v 'standard protocol' methods for cells stored for different periods at 4°C. 'Direct protocol' (solid circles), 'standard protocol' to 37°C plates (open circles), 20°C plates (squares) and 4°C plates (triangles).

period (7) . Using the direct spreading technique, there was no advantage from this outgrowth for cells carrying ampicillin, tetracycline or chloramphenicol resistance. However, transformations for kanamycin resistance by the direct method averaged only 30% of those by the standard protocol thus demonstrating a requirement for a recovery period with kan^r.

With the exception of kan^r, improved transformation rates using the direct method have been noted with all cell lines and plasmids thus far tested. Similar results have been obtained in pBluescript +/– inserts up to 3 kb and with a 6.2 kb transfection vector (CB6) +/– inserts up to 3 kb (M. Way, personal communication).

In summary it is possible to efficiently transform bacteria in <5 min using neither specialist equipment nor noxious solutions. The standard 1.5 h protocol can be condensed into a simple 1–5 min incubation of cells with DNA on ice and spreading onto selective agar plates pre-warmed to 37°C, resulting in speed and 2-fold increased efficiency.

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