Comparison of standard bacterial transformation protocols

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Abstract
Bacterial transformation plays an important role in molecular genetics, but tends to be a source of great frustration when carried out by students in a teaching laboratory situation. This study compared the transformation efficiencies of four common transformation protocols and the effects of storage on the cells’ competency. The TSS single step protocol proved the most efficient (1.58 x 10^6 CFU/µg plasmid DNA) when used on the same day while the Manganese Chloride method produced cells that retained their full competency even after storage (1.45 x 10^5 CFU/µg plasmid DNA).

Introduction
Bacterial transformation, the ability to take up and express exogenous DNA, is a critical process in molecular cloning (Sharma et al. 2007). Some cells have a natural ability to take up and express linear DNA. This process is termed “natural competency”. This is primarily carried out to help meet nutritional needs, but can be used for the acquisition of new genes that promote genetic diversity (Dubnau 1999). Griffith (1928) and Avery (1944) first observed natural competence when the non-lethal strain (R) of Streptococcus pneumoniae was transformed into the lethal one (S) by uptaking DNA from heat-killed S strain. By this mechanism new functionality, and competitive advantage, may be achieved, as the cell incorporates the extraneous DNA into its
chromosome followed by the expression of the newly acquired genes. It has been estimated that only about 1% of all identified bacterial strains are able to become naturally competent (Lorenz and Wackernagel 1994, Solomon and Grossman 1996). Competency can be artificially induced in many bacterial strains; this state has been elucidated for many bacteria, especially those commonly used in a molecular biology laboratory. There are two general methods used in a molecular biology laboratory to introduce foreign DNA into bacterial hosts. Competency can be induced by exposing bacterial cells to a variety of inducing agents, including divalent cations, polyethylene glycol (PEG) and Dimethyl Sulfoxide (DMSO) (Hanahan et al 1991); these solutes help to render the membrane more permeable to negatively charged molecules such as DNA. Alternatively, an electric current can be used to introduce transient pores in the membrane through which DNA can enter; this process has been termed “electroporation.”

One of the first observations of induced competency was made by Mandel and Higa (1970) who serendipitously noted that Escherichia coli was capable of uptaking phage λ DNA when placed in the presence of calcium chloride. Cohen et al (1972) further explored these observations and developed the calcium chloride transformation protocol that serves as the standard for induced competency in E. coli today. Tu et al (2005) later attempted to describe the mechanism of induced competency and suggested that as Cl⁻ ions enter the cell they draw water into the cytosol causing the bacterium to swell. As the cell swells it creates a favorable environment for the uptake of the plasmid. In addition in the presence of divalent cations the negative charge of DNA is neutralized. The actual transport of the DNA through the polar membrane is carried out by a DNA translocation machinery. This complex has three major components; the first one being the DNA receptor protein which located on the outside of the cell membrane whose function is to recognize and bind to DNA. Then there is an aqueous membrane channel
through which the bound DNA will be transported upon hydrolysis of APT by the third component, the ATP binding protein (Chen and Dubnau 2002). This whole process requires some time and in generally carried out during an incubation step of the competent cells with the plasmid DNA.

In 1989 Chung et al outlined a single step transformation protocol that uses Mg$^{2+}$ and required the use of PEG and DMSO to simplify the transformation process and to generate competent cells that could easily be prepared and stored in the same solution. Further modifications have made use of other divalent cations such as Ca$^{2+}$, Mn$^{2+}$ or Zn$^{2+}$ ions (Lacks et al 1974 and Chung et al 1989).

Sharma et al (2007) added ethanol to the original Cohen et al (1972) CaCl$_2$ procedure to increase transformation rates. According to their hypothesis, ethanol disrupts the bacterial cell wall and facilitates the movement of plasmid DNA into the cell.

In 1982, Zimmerman and Vienken investigated the use of an electric field to induce cell to cell fusion; they noted that when cell membranes are polarized at very high voltage the membrane tended to break down with a reversible increase in permeability. Subsequent studies demonstrated that this electrically-induced permeability could be used to introduce DNA into a cell. In 1988 Dower developed a standard protocol for electroporation using the Bio-Rad Gene Pulser®. Electroporation must employ cells suspended in a nonionic buffer to prevent arcing and to generate pores of adequate size to promote successful transformation. Electroporation is initiated in a specialized cuvette that possesses two aluminum electrodes on either side of a gap into which washed cells and DNA are mixed immediately prior to the discharge of a 2.5kV electric current through the cuvette. Electroporation has been extensively used to introduce recombinant DNA into bacteria, yeasts, plant and mammalian cells (Miller et al 1988).
The success rate of a bacterial transformation is determined by several factors. For example the growth phase at which the bacterial culture is harvested (Ryu and Hartin 1990), quality of chemical additives, the temperature at which cells are maintained during preparation, the speed at which cells are centrifuged, and the degree of shear forces generated upon resuspension may all affect transformation efficiency. Dargert and Ehrlich (1979) also demonstrated that temperature and duration of competent cell storage may affect efficiency.

While bacterial transformations are central to a successful molecular biology project, they can frequently be a major source of frustration, especially to those who are inexperienced in the preparation and use of competent cells. These problems are amplified when used in a teaching lab environment. Considering all of the variables that can be modified and adjusted to obtain quick and reliable results, the question of which protocol to use for routine and inexpensive transformations in a student molecular biology laboratory arises. This work compares four commonly used transformation protocols to assess reliability and ease in a student laboratory scenario.

Materials and Methods

Bacterial strains and plasmids. *Escherichia coli* JM109 was used as the recipient strain for all transformations. All culturing was performed in Luria Bertani media (Bertani 1951, LB) containing 35µg/mL of chloroamphenical, where appropriate, at 37°C with vigorous shaking for routine manipulations. The bacterial plasmid, pACYC184, which confers resistance to chloramphenical (Cm), was obtained from New England Biolabs (Beverly, MA) and used for all transformations.

Plasmid isolation. Plasmid was isolated using the Qiaprep® Miniprep kit (Qiagen, Inc., Valencia, CA) and stored in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.8) at -20°C.
**Bacterial Transformation.** Four commonly used *E. coli* transformation protocols were compared for transformation efficiency, reproducibility, ease of use, and survivability upon storage. These protocols including the single step TSS method (Chung *et al* 1989), the ethanol-modified TSS method (Sharma *et al* 2007), the manganese chloride method (Lacks *et al* 1974), and electroporation (Dower 1988). To ensure a direct comparison between transformation efficiencies, the experiments were carried out in parallel using four replicates. Half the volume of competent cells prepared by each method were used in transformation reactions immediately following their preparation; the other half was preserved in 10% glycerol, frozen rapidly by submersion in liquid nitrogen, and then stored for 24 h at -70°C prior to using for transformation. Following transformation, successful transformants were selected by plating 100 µL of the cell suspension on LB plates containing 35 µg/mL Cm; plates were incubated overnight at 37°C. The number of colony forming units (CFU) were recorded for each attempt.

Statistical analysis was performed using WINKS statistics software.

**TSS transformation.** *E. coli* JM109 was initially grown overnight at 37°C. A 1% inoculum of this culture was used to initiate growth in 50 mL LB; incubation continued at 37°C until the culture reached an optical density (O.D$_{600nm}$) of 0.6 ± 0.1. Cells were pelleted by centrifugation (10,000xg, 15 min, 4°C) and resuspended in 5mL of Transformation Storage solution (TSS; 5% DMSO, 1% MgSO$_4$, 0.5% MgCl$_2$, 10% PEG, 10% Tryptone, 5% yeast extract, 5%NaCl). The mixture was incubated on ice for 1h. For transformation 100 µL of TSS competent cells were mixed with 1µg of pACYC184 was added and incubated on ice for 1 h. SOC media media (2% Bacto-tryptone, 0.5% Bacto-yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl$_2$, 10 mM MgSO$_4$, 20 mM Glucose, pH 7.0) was then added to a final volume of 1 mL and the culture was incubated
for an hour at 37°C. The cells were pelleted (13,000 rpm for 1 minute) and resuspended in 100 µL of supernatant.

**Ethanol mediated transformation.** The TSS single step protocol, as described above, was modified by adding 10% ethanol to the competent cells immediately before use. All other steps were conducted as previously outlined.

**Manganese Chloride transformation.** *E. coli* JM109 from an overnight culture was used to inoculate 100 mL of 2XL media (2% Tryptone, 1% Yeast extract and 0.1% NaCl) at a 1% inoculation level; the culture was incubated at 30°C to an OD$_{595}$ of 0.2. Sterile MgCl$_2$ was then added to a final concentration of 20 mM. Incubation of this culture continued at 30°C to an OD$_{595}$ of 0.5 upon which the culture was placed in an ice-water bath for 2 h. The cells were pelleted by centrifugation (900x g, 5 minutes) and resuspended in 1/20$^{th}$ of the original volume in CaMn media (100 mM CaCl$_2$, 70 mM MnCl$_2$ and 40 mM Sodium acetate, pH 5.5). For transformation, 50 µL cell suspension was mixed with 1µg of plasmid and incubated on ice for 1 h. The cells were heat shocked at 42°C for 2 min and incubated on ice for 5 min; 1 mL of 2XL media was added followed by incubation at 37°C for 1h.

**Electroporation.** *E. coli* JM109 from an overnight culture was used to inoculate 30 mL LB media at a 1% inoculation level and the culture was incubated at 37°C to an O.D$_{600nm}$ of 0.6. The culture was then chilled on ice for 10 min and transferred to pre-chilled centrifuge tubes. Cells were pelleted by centrifugation (6,000x g, 20 min, 4°C), the supernatant was discarded, and the pellet was resuspended in 30 mL of cold sterile deionized H$_2$O. The washing step was repeated three times. Cells were resuspended in 300 µL of cold sterile 10% glycerol. Cold, washed cells (50 µL) and 1µg of pACYC184 were mixed in a pre-chilled 0.2 cm gap electroporation cuvette. The cuvette was then incubated on ice for 5 min. A 2.5kV pulse (200Ω) was applied using a Bio-Rad Gene.
Pulser® and 900 µL of SOC was immediately added to the cell suspension. The culture was then incubated at 37°C for 1 h while shaking (225 rpm).

**Results and discussion**

Successful bacterial transformation often proves challenging in a student laboratory. Inexperience, poor technique, errors in measurements, and sub-optimal laboratory conditions all combine to make transformations difficult in the hands of students. With the increased use of comprehensive teaching laboratory projects, wherein one laboratory protocol must be mastered before the next can be attempted, simple and reliable bacterial transformations are critical to a complete molecular biology learning experience. Bacterial transformations play a key role in numerous molecular applications including cloning, mutagenesis, gene linkage studies and genetic mapping, and a number of transformation methods have been described in the scientific literature, each offering their own benefits and limitations. When considering a transformation protocol for use in a student laboratory, ease of use, reliability, transformation efficiency, and expense, are all important considerations.

The TSS single step method has been widely used as it is inexpensive, reliable, and reproducible under a wide variety of conditions. This method does not require specialized equipment and uses reagents easily found in any molecular biology laboratory; this is also a very rapid technique that requires very little preparation time. The limitations of this technique include a limited transformation efficiency of about $10^7$ CFU per microgram of plasmid DNA under optimal conditions and is generally applicable to only a very few bacterial species. Under the conditions employed in this study, this protocol was the most effective averaging $3.2 \times 10^5$ transformants/µg of DNA when used for transformation immediately after preparation (Figure 1).
Adding Ethanol to enhance the TSS single step transformation method is just one of the many modifications that reportedly increase transformation rates. However, in the current comparison, the addition of ethanol failed to significantly increase transformation efficiency, yielding an average of $1.61 \times 10^5$ CFU/µg DNA ($\sigma 5.27 \times 10^4$).

The Manganese Chloride method is a relatively simple and reproducible protocol that seeks to increase the transformation efficiency of *E. coli* through the combined use of Mn$^{2+}$, Mg$^{2+}$ and Ca$^{2+}$. This method was found to require considerably more preparation time than any of the other protocols, and yielded an average of $1.35 \times 10^5$ CFU/µg DNA.

Electroporation proves to be quite efficient as the same general principle of generating transient pores in the cell membranes can be used successfully with a wide variety of different cell types and DNA forms and sizes. The parameters of the cuvette, gap, voltage and duration of the pulse can be altered to optimize transformation efficiency for a variety of bacterial, plant, and mammalian cell types. The major
drawback is the cost of the electroporator and of non-reusable cuvettes. Such apparati are not found in all laboratories. However, it has been a reliable and efficient technique reportedly yielding $10^8$-$10^{10}$ CFU/µg DNA (Ryu and Hartin 2005). In the current comparison, however, electroporation yielded the lowest number of transformants at an average of $5.6 \times 10^4$ CFU/µg DNA ($\sigma 1.1 \times 10^4$).

In order to verify that the transformed cells carried pACYC184, the cells were grown overnight at 37°C in LB/Cm broth (35 µg/mL) from which the plasmids were isolated using the Qiaprep® Miniprep kit. The visualization on a 0.8% agarose gel of the isolated plasmids (Figure 2) in comparison with the commercial pACYC184 confirmed the success of the transformations.

![Figure 2 Gel electrophoreses of isolated plasmids from transformants](image)
Another important factor to consider is the effect of storage on the ability of the cells to remain competent. As the preparation of the cells can be time consuming it is often advantageous to prepare them in large quantities and to store them for future use. Based on the Newman-Keul multiple comparison test, the TSS method was the most reliable transformation technique if used within 24 h, while the MnCl₂ competent cells were the most effective in maintaining their previous competency after storage (p= 0.38).

Bacterial transformation will continue to be a critical tool in molecular processes and using the most convenient one will remain a priority. In the hands of students, the TSS single step method is an appropriate method to induce competency in *Escherichia coli*. The Manganese Chloride method on the other hand provides competent cells practically unaffected by storage.

**Works cited**

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