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Benchtop and Microcentrifuge Preparation of *Pseudomonas aeruginosa* Competent Cells

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Although indispensable for the introduction of DNA fragments manipulated in vitro into bacteria, transformation using chemically prepared competent or electrocompetent cells is still a rather slow and labor-intensive technique, and apart from waiting for transformants to appear on selective plates, the preparation of competent cells is the slowest part of the whole process. Most published transformation protocols for use with *Pseudomonas aeruginosa* are modifications of protocols developed for *E. coli* and either involve electrocompetent cells (1–4,9,12) or cells made competent by chemical treatment (8,10). Conventional chemical transformation of *P. aeruginosa* has many drawbacks, most notably low transformation efficiencies and/or time-consuming procedures. Therefore, electroporation has become the method of choice for this bacterium.

However, the preparation of low-ionic strength DNA and competent cells for electroporation is still a critical and relatively time-consuming process. Here we describe the adaptation of a rapid benchtop- and microcentrifuge-based method previously reported (14) for obtaining low-efficiency competent *E. coli* cells to prepare highly competent *P. aeruginosa* cells for chemical transformation. For *P. aeruginosa*, this method yields transformation efficiencies that are comparable to those observed when using electroporation.

For preparation of competent *P. aeruginosa* cells, strain PAO1 cells were grown in 4 mL LB broth (Difco Laboratories, Detroit, MI, USA) at 37°C until they reached saturation (usually overnight). All of the following steps, except the centrifugations, were performed with the tubes sitting on ice. A 1.5-mL microcentrifuge tube was pre-chilled on ice for 3–5 min. Aliquots (1 mL) of a stationary phase or overnight *P. aeruginosa* culture were transferred to the chilled microcentrifuge tubes, and the cells were harvested at room temperature by a short centrifugation for 30 s at approximately 13 000× *g*. Centrifugations longer than 30 s are not recommended because they cause significant reduction of transformation efficiencies. The cell pellets were resuspended with a pipet tip in 1 mL cold (approximately 4°C) 0.1 M MgCl₂. The cell suspension was again centrifuged at approximately 13 000× *g* for 30 s at room temperature. The supernatant was removed, and the cell pellets were resuspended with a pipet tip in 1 mL cold transformation salts with glycerol (TG salts) solution (75 mM CaCl₂, 6 mM MgCl₂, 15% glycerol). The cell suspensions were kept on ice for 10 min and then centrifuged at approximately 13 000× *g* for 30 s at room temperature. After decanting the supernatant, the pellets were then resuspended with a pipet tip in 200 µL cold TG salt solution and kept on ice until use. At this stage, the cells were ready for transformation.

The transformation efficiency of *P. aeruginosa* cells prepared using this procedure was determined using the 4.2-kb broad-host-range plasmid pUC-P20T (11). Aliquots (100 µL) of the competent cells were transferred to

thin-walled 13 × 100 mm borosilicate glass tubes (VWR Scientific, South Plainfield, NJ, USA) that were pre-chilled on ice. Next, 2–5 µL aliquots containing 100–1000 ng pUCP20T DNA that has been purified using the Qiagen® Midiprep kit (Qiagen, Valencia, CA, USA) and suspended in either 10 mM Tris-HCl (pH 8.5) or sterile water were added to the cells (DNA in this buffer or water yielded the same transformation efficiencies). A negative control received sterile water instead of plasmid DNA. This DNA-cell mixture was incubated on ice for 15 min. The mixture was then heat-shocked at 37°C for 2 min, and 500 µL LB broth were immediately added. The tubes were incubated at 37°C for 1 h in a shaking incubator. After incubation, a 200-µL aliquot of each cell suspension was plated on LB agar containing 200 µg/mL carbenicillin (Gemini Bio-Products, Woodland, CA, USA). The remaining contents of each tube were transferred to microcentrifuge tubes, and cells were harvested by centrifugation at approximately 13 000× *g* for 1 min at room temperature, resuspended in 200 µL LB medium, and then spread on a LB-carbenicillin plate. The plates were incubated at 37°C for 20–24 h before colonies were counted.

Using the procedures described above for the preparation and transformation of competent *P. aeruginosa* PAO1 cells (Figure 1), we obtained 1 × 10⁵ transformants/µg pUCP20T DNA (Table 1), using an optimal DNA concentration of 200–400 ng. This transformation efficiency is comparable to the 1.1–1.5 × 10⁵ transformants/µg DNA when using most electroporation protocols (1–4,9,12). Only two other reports indicated higher transformation efficiencies [i.e., 1.2 × 10⁶ transformants/µg DNA by using chemically prepared competent cells and a heat shock at 50°C (8) and approximately 2 × 10⁶ transformants/µg DNA by employing an electroporation procedure (3)]. Although the transformation efficiency of competent cells prepared using our procedure is about 10-fold lower when compared to the most efficient procedures, it is comparable to those achieved with most published methods and therefore more than adequate for most laboratory applications. However,

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its simplicity and speed make it superior to most, if not all, previously described methods. The time spent from the start of preparation of competent cells to the plating of the transformation mixtures is about 2 h. The procedure is entirely performed at the bench, requires no large-volume refrigerated floor model centrifuge, no spectrophotometer to monitor cell growth, no other specialized equipment and laboratory supplies, and is therefore well-suited for most research and teaching laboratory applications.

In addition to transformation with the replicative plasmid pUCP20T, we also attempted to select for the integration of a non-replicative plasmid into the PAO1 chromosome via homologous recombination after preparation of competent cells and transformation using the rapid procedure. Non-replicative plasmids are generally used to transfer plasmid-borne mutant alleles into the *P. aeruginosa* chromosome after conjugal transfer from *E. coli* (13). In this study, we transformed strain PAO1 with a plasmid containing a $\Delta(mexAB-oprM)$ mutation, affecting one of the multidrug efflux pumps of *P. aeruginosa*. On this plasmid, a significant portion of the *mexAB-oprM* operon is replaced with *aacC1* and GFP, genes encoding gentamycin resistance (Gm^r) and the green fluorescent protein, respectively (13). One hundred and fifty nanograms of pPS951 DNA purified using a Qiagen Midiprep kit were added to 200 μ L competent PAO1 cells. The entire approximately 700 μ L transformation mixture was spread in two approximately 350- μ L aliquots onto Vogel-Bonner minimal medium agar plates (6) containing 30 μ g/mL gentamycin. A total of 29 Gm^r colonies was obtained, which corresponds to a transformation efficiency of approximately 2×10^2 (Table 1). To verify the presence of the Gm^r marker, 10 of the 29 colonies were randomly selected and subjected to colony PCR using a previously described procedure and *aacC1*-specific primers (6). All Gm^r colonies yielded an approximately 550-bp PCR fragment, indicating that they contained the *aacC1* gene integrated into their genome. Therefore, this newly described method is useful to transfer a suicide plasmid into *P. aeruginosa*

Table 1. Transformation Efficiency of *P. aeruginosa* and *E. coli* Competent Cells Using Replicative and Non-Replicative Plasmids

Competent Cells	Plasmid	Transformation Efficiency (Transformants/ μ g DNA)
<i>P. aeruginosa</i> PAO1	pUCP20T (r) ^a	1×10^5
<i>P. aeruginosa</i> PAO1	pPS951 (nr)	2×10^{2b}
<i>E. coli</i> DH5 α	pUCP20T (r)	1×10^6

^ar and nr denote replicative and non-replicative plasmids, respectively, in the indicated host.
^bSince pPS951 does not replicate in *P. aeruginosa*, antibiotic-resistant colonies obtained after transformation are the result of plasmid integration into the chromosome via homologous recombination of sequences present on the plasmid and the chromosome.

and to achieve homologous recombination between plasmid-borne and chromosomal DNA sequences.

Although we had previously reported the use of a similar method to obtain low-efficiency competent *E. coli* cells (14), we never optimized the method for *E. coli* or determined the transformation efficiencies. To do this and to compare the transformation efficiencies of *E. coli* and *P. aeruginosa*, pUCP20T was transformed into competent *E. coli* DH5 α cells and the transformants were selected on LB agar containing 100 μ g/mL ampicillin (Sigma, St. Louis, MO, USA). We obtained 1×10^6 transformants/ μ g pUCP20T DNA, using an optimal DNA concentration of 100–400 ng. This transformation efficiency is higher than the transformation effi-

ciency of $1.7 \pm 0.1 \times 10^4$ transformants/ μ g DNA obtained by electroporation of freshly plated *E. coli* cells, another rapid method (3). However, it is significantly lower than the 10^8 – 10^9 or 10^9 – 10^{10} transformants/ μ g DNA obtained by using cells that were chemically induced by conventional methods or using electrocompetent *E. coli* cells, respectively (5). Therefore, this method is mostly practical for preparation of *E. coli* competent cells for applications where high transformation efficiencies are not required (e.g., for the transformation of purified plasmid DNA into various mutant strains for complementation analyses or for the transfer of plasmids from one strain background to another). It is interesting to note that although plasmid DNA from purified

Figure 1. Flow scheme highlighting the steps involved in (A) the preparation of bacterial competent cells and (B) their transformation.

from *E. coli* must encounter significant restriction barriers when entering *P. aeruginosa*, the transformation efficiencies observed in *E. coli* were only 10 times higher than those observed with *P. aeruginosa*.

We have successfully used this procedure to transform many different *P. aeruginosa* mutant strains with vectors based on different replicons and also to simultaneously introduce two compatible plasmids into the same strain. However, we have not yet been able to obtain integrase-mediated insertion of integration-proficient vectors at the chromosomal *attB* site (7), either by transfer of the suicide integration vector using this new method or by electroporation, but only by its conjugal transfer from *E. coli*. For routine transformations, two-day-old *P. aeruginosa* cultures have been used to prepare competent cells with lower but acceptable transformation efficiencies. Furthermore, competent cells in TG salts can be frozen at -70°C for future use, although the transformation efficiency may decline slightly over time. In summary, because of its speed and efficiency, the procedure described here is an attractive alternative to the many other, more time-consuming methods available for the transformation of *P. aeruginosa*. With proper modification, it may be adaptable to other bacteria, as has already been demonstrated for *E. coli*.

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