Transformation of *Pseudomonas aeruginosa* by electroporation

Anthony W.Smith and Barbara H.Iglewski[1]*

Microbiology Research Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, UK and [1]Department of Microbiology and Immunology, University of Rochester Medical Center, Box 672, 601 Elmwood Avenue, Rochester, NY 14642, USA
Submitted November 13, 1989

The use of an electric field to reversibly permeabilize cells (electroporation) has become an important technique to introduce nucleic acids into eukaryotic and prokaryotic cells (1). Here we report a method to reproducibly electroporate plasmid DNA into *P. aeruginosa* based on a modification of the protocol developed by Dower et al. (2) for *E. coli*, which has application where transformation by chemical methods is inefficient or when plasmids cannot be mobilized by conjugation. *P. aeruginosa* PA103 was grown in L-broth with vigorous shaking at 37°C until early log phase (ABS540 ~0.3-0.5). The cells were harvested by centrifugation at 7000 g for 10 min at 4°C, washed in the same volume of 300mM sucrose, re-centrifuged, washed in 0.5 volume of wash medium and finally resuspended in 0.01 volumes of 300mM sucrose (~1 x 10^{11} cfu/ml). The efficiency of electroporation was approximately 10^3-fold lower when either water or 1mM HEPES buffer was used as the wash medium, due to cell lysis. The cell suspension was chilled on ice for 30 min prior to electroporation. Plasmid pUC18 DNA containing an additional 1.8 kb stabilizing fragment from pRO1614 (3) was preprared by the alkaline lysis method and resuspended in water at 1 µg/ul. The exponential decay pulses were generated by a Gene Pulser and Pulse Controller (Bio-Rad Laboratories, Richmond, CA). Forty µl aliquots of cell suspension were mixed with 5 µl of DNA solution and transferred to chilled 0.2 cm gap cuvettes (Bio-Rad). Following delivery of the pulse, the cells were mixed with 3ml L-broth and shaken for 2h at 37°C. Preliminary experiments indicated that, in contrast to *E. coli* (2), electroporated *P. aeruginosa* cells could be held on ice for up to 10 min before addition of the outgrowth medium with only a 10% decrease in transformation efficiency. The cells were diluted in L-broth and plated on L-agar supplemented with carbenicillin at 200 µg/ml. Transformation efficiency was calculated at CFU/µg of plasmid DNA. Figure 1 shows the effect of field strength on transformation efficiency at an exponential decay constant of 5 msec. Maximum efficiency was reached at 8 kV/cm. The data in Fig. 2 shows the number of transformants obtained as a function of the DNA concentration, indicating that up to 10^4 transformants can be obtained with picogram quantities of plasmid DNA. Unlike *E. coli*, *P. aeruginosa* cells could not be electroporated with high efficiency after being frozen.

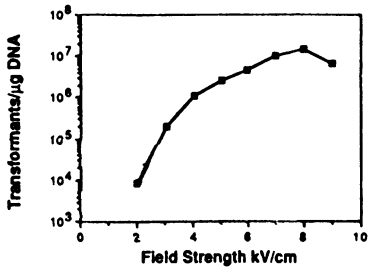

Figure 1. Electroporation of pUC181.8 into *P. aeruginosa* PA103.

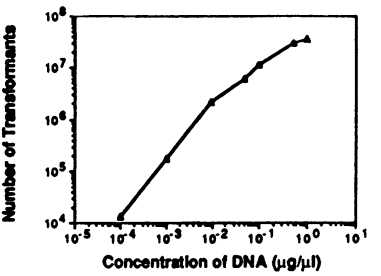

Figure 2. Effect of DNA concentration electroporated at 8 kV/cm, 5 msec decay constant.

Acknowledgements. AWS was supported by travel grants from the Royal Society and the Wellcome Trust and the research was supported by Public Health Service grant AI 25669 from the National Institutes of Health.

*To whom correspondence should be addressed

References. (1) Shikegawa, K. and W. J. Dower (1988) BioTechniques 6:742. (2) Dower, W. J., J. F. Miller and C. W. Ragsdale (1988) Nuc. Acids Res. 16:6127. (3) Olsen, R. H., G. DeBusscher and W. R. McCombie (1982) J. Bacteriol. 150:60.