

Electrotransfer: direct transfer of bacterial plasmid DNA by electroporation

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Electroporation is an increasingly popular method for introducing plasmid DNA into bacterial cells. A report that electroporation can also be used to cure bacterial cells of plasmids (1) caused us to wonder whether plasmid DNA might transfer directly from a plasmid-bearing to a plasmid-free strain under the influence of an electric pulse. We describe here the 'electrotransfer' of plasmid pBR322 (which confers ampicillin and tetracycline resistance) from *Escherichia coli* JC8679 (AB1157 *recBC sbcA*) to a kanamycin-resistant derivative of DS941 (AB1157 *recF argR*::Tn*5*).

Stationary phase cultures of donor and recipient were prepared for electroporation by the method of Heery and Dunican (2). 20 μl aliquots of the prepared cells were mixed in a microfuge tube before transfer to an ice-cold 0.2 cm electroporation cuvette. The mixture was subjected to a single pulse of 2.4 kV, 25 μF (time constant 4.6 msec) in a Gene Pulser unit (Bio-Rad Laboratories, Richmond, CA). 1 ml SOC medium (2) was added and the pulsed cells were incubated at 37°C for one hour. Ten-fold and one hundred-fold dilutions were spread onto L-agar plates containing kanamycin (25 μg ml^{-1}) and tetracycline (5 μg ml^{-1}). Only kanamycin resistant recipient cells which had acquired the plasmid should grow on these plates. Typically, 3×10^3 colonies were obtained per electroporation. In control experiments where the 2.4 kV pulse was omitted, no colonies were obtained. We have also used electrotransfer with the ampicillin resistance plasmid pUC8 and obtained 2×10^5 colonies on selection plates containing kanamycin and carbenicillin. We find it desirable to use carbenicillin (10 μg ml^{-1}) in place of ampicillin in these experiments to reduce background growth of donor cells on the selection plates.

To exclude the possibility that the colonies resistant to both kanamycin and tetracycline were kanamycin resistant mutants of the donor strain induced by the pulse treatment, we examined their plasmid DNA. The donor strain carries the *sbcA* mutation which stimulates plasmid recombination and the formation of plasmid oligomers (Fig. 1; lane 1). This plasmid ladder would also appear in kanamycin-resistant donor mutants. The recipient, however, carries the *recF* mutation which abolishes plasmid recombination. The doubly-resistant colonies lacked the plasmid ladder, and were thus the result of true plasmid transfer. Approximately equal numbers of colonies contained plasmid monomers and dimers, with occasional higher oligomers (Fig. 1; lanes 2, 3 and 4, respectively), reflecting the plasmid composition of the donor strain.

Electroporation has proved effective at introducing DNA into a wide range of prokaryotic and eukaryotic cells. Electrotransfer, because it depends upon a physical rather than biological mechanism has the potential to transfer non-conjugative plasmid DNA across species barriers and may be particularly useful for the movement of shuttle vectors between *E. coli* and other species.

REFERENCES

1. Heery,D.M. *et al.* (1989) *Nucl. Acids Res.* **17**, 10131.
2. Heery,D.M. and Dunican,L.K. (1989) *Nucl. Acids Res.* **17**, 8006.

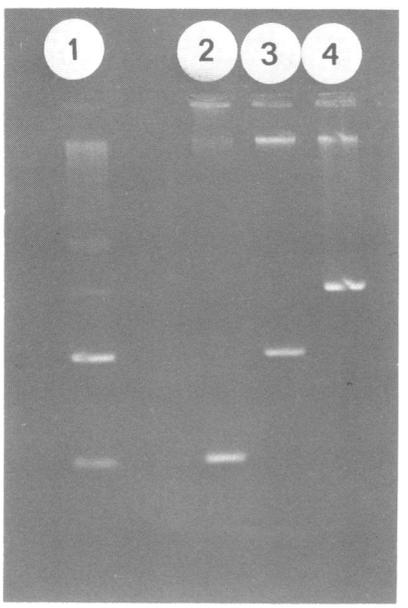

Figure 1. Plasmid DNA from donor (lane 1) and recipient (lanes 2, 3 and 4) cells.