Transferring plasmid DNA between different bacterial species with electroporation

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After the discovery that treatment of carrot spheroplasts with high-voltage pulses of electricity permits the efficient introduction of extracellular DNA (1), the successful 'electroporation' of DNA into a wide variety of species has been reported. These recipients include the bacteria *Escherichia coli* (2, 3) and *Salmonella typhimurium* (3), model genetic organisms. Although Summers and Withers reported the electroporetic transfer of plasmids between stationary *E. coli* cultures (4), transfer between exponential cells occurs with higher efficiencies. As shown in Fig. 1, when a tetracycline-sensitive (Tc^S) donor strain carrying the chloramphenicol-resistant (Cm^R) plasmid pUC19cat (5) is mixed with a tetracycline-resistant (Tc^R) strain of *E. coli* (6), and the mixture is shocked, novel $Cm^R Tc^R$ segregants called 'electroductants' are recovered. These segregants have the auxotrophic genetic marker from the recipient and the plasmid from the donor. Efficient formation of these segregants depends on shocking a mixture of the two strains (e). Mixing without shock (a), or shock of the donor alone and subsequent mixing (b), does not yield electroductants. A few segregants are found after shocking only the recipient, then mixing (c). Shocking both strains, then mixing, yields a modest number of electroductants (d).

Electroductants can occur both intraspecifically and interspecifically. Efficiencies of transfer under optimal conditions (0.1 cm cuvette, 20 kV/cm, 200 Ω , 25 μ F) are: from *E. coli* to *E. coli*, 4×10^{-5} per recipient; from *S. typhimurium* to *E. coli*, 7×10^{-4} ; and from *S. typhimurium* to *S. typhimurium*, 4×10^{-3} . Optimal conditions for electroductants from *E. coli* to *S. typhimurium* (0.1 cm cuvette, 25 kV/cm, 200 Ω , 25 μ F) give efficiencies of 2×10^{-6} . This lower number is likely due to the presence of restriction systems in *S. typhimurium* LT2 absent from *E. coli* K12 (7). If cells are frozen prior to shock, typical efficiencies are two- to ten-fold less.

Electroductants is a fourth, new way in which genetic material may be transferred between bacteria, in addition to transformation, transduction, and conjugation. We speculate that sudden, natural electric discharges, such as lightning, may facilitate this novel kind of lateral transfer of genetic material.

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Figure 1. Electroductants of plasmid pUC19cat from *E. coli* to *E. coli*. Plasmid pUC19cat (5) confers chloramphenicol resistance. The tetracycline-resistant recipient *E. coli* MG1655 *thr::Tn10* and chloramphenicol-resistant donor *E. coli* SCS-1 (pUC19cat) were grown in L broth to a density of 10^8 /ml, washed twice in an equal volume of distilled water, washed once in 1/10 volume 10% glycerol, and concentrated 200-fold by centrifugation. Cells were shocked at 2.5 kV, 25 μ F, and 200 Ω in 40 μ l volume, using a Bio-Rad 0.2 cm cuvette and Gene Pulser system. Higher efficiencies are obtained with a 0.1 cm cuvette (see text); lower voltages or different time constants give lower efficiencies. In each case, 20 μ l of donor cells were mixed with 20 μ l of recipient cells, treated, grown in 1 ml SOC medium (2) for 1 h at 37°C, then spread on plates with chloramphenicol (20 μ g/ml) and tetracycline (20 μ g/ml). Numbers of $Cm^R Tc^R$ electroductants were scored after incubation for 12 h at 37°C. (a) Control with no shock; (b) donor shocked, then mixed with recipient; (c) recipient shocked, then mixed with donor; (d) donor and recipient shocked separately, then mixed; (e) donor and recipient shocked together. The efficiency of transfer (per recipient cell) was 4×10^{-5} for this experiment. Interspecific transfers were done in a similar way. In all cases, the highest efficiencies of transfer were obtained with equal titers of donor and recipient cells.

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- pUC19cat is an ampicillin-sensitive chloramphenicol-resistant derivative of pUC19 (Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* **33**, 103). From the collection of A. Greener.
- E. coli* K12 strains are SCS-1 (F^- *recA1*, *endA1*, *gyrA86*, *thi*⁻, *hsdR17* (r_k^- , m_k^-) *supE44*, *relA1*) (stratagene) and MG1655 *thr::Tn10* (Singer, M., Baker, T.A., Schnitzler, G., Deischel, S.M., Goel, M., Dove, W., Jaacks, K.J., Grossman, A.D., Erickson, J.W. and Gross, C. (1989) *Micro. Rev.* **53**, 1.) *S. typhimurium* LT2 strains, *hisD::Tn10* (recipient) and *leuA-am414* (donor) are from J. Roth and M.M. Susskind, respectively.
- For a recent review, see Bickle, T.A. (1987) In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* Neidhardt, F.C. (ed.) ASM, Washington, DC, pp. 692-696.