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Transformation in *Escherichia coli*: Cryogenic Preservation of Competent Cells

D. A. MORRISON

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Illinois 60680

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Escherichia coli cells prepared for transformation by treatment with cold 0.1 M $CaCl_2$ remained viable and competent after storage at −82°C in 15% glycerol; thawed-cell samples yielded up to 10^6 transformants per μg of plasmid deoxyribonucleic acid.

A method for rendering *Escherichia coli* susceptible to transfection by purified phage deoxyribonucleic acid (DNA) was described in 1970 by Mandel and Higa (9), and, with minor modifications, shown to be successful for transformation with plasmid DNA by Cohen et al. (2). Similar modifications, and introduction of a *recB recC sbcB* genetic background, allowed Cosloy and Oishi (4) to demonstrate transformation of chromosomal genes. Lederberg and Cohen (7) showed that another modification also allowed transformation at similar or better efficiencies by plasmid DNA in both *Salmonella typhimurium* and *E. coli*. These methods have been of wide use in introducing purified plasmid DNA or recombinant plasmid DNA constructed in vitro into enterobacterial hosts.

All of these methods of rendering cells competent for uptake of DNA involve, briefly: (i) several successive washes in cold NaCl, $MgCl_2$, and/or $CaCl_2$ solutions, (ii) incubation in $CaCl_2$ at 0°C with DNA, and (iii) a short incubation at some higher temperature before (iv) dilution into growth medium and/or selection of transformants.

In the naturally occurring transformation systems, the routine use of frozen preparations of competent cells has provided the benefits of economy of effort in preparation, and of reproducibility of results (5, 6, 10, 11). The well-known utility of glycerol solutions in preserving viability of *E. coli* at low temperatures led us to explore the possibility of preserving competence in *E. coli* in the same way. The product of treatment (i), above, would seem to be a particularly valuable stage at which to be able to preserve competence. It is shown here that calcium-treated cells frozen and stored at −82°C display good competence after thawing.

Cells of the tetracycline-sensitive *E. coli* derivative RR1 (obtained from H. Boyer, as was the plasmid pMB9) were prepared for transformation essentially as described by Lederberg and Cohen (7). A log-phase culture in 1 liter of L broth (8) was chilled to 0°C; the cells were collected by centrifugation, washed with 250 ml of 0.1 M $MgCl_2$, suspended in 250 ml of 0.1 M $CaCl_2$ for 20 min, sedimented again, and finally suspended in a mixture of 42.5 ml of 0.1 M $CaCl_2$ plus 7.5 ml of glycerol (all steps at 0 to 4°C). This suspension was distributed in screw-cap culture tubes (13 by 100 mm), frozen in a bath of acetone and dry ice, and stored in a freezer at −82°C.

For transformation, a tube was thawed in ice water, and 1 volume of the cell suspension was added to a DNA sample in 1/3 volume of 0.1 M $CaCl_2$ at 0°C. The donor DNA was supercoiled plasmid pMB9, purified as described by Clewell (1). The mixture was held at 0°C for 30 min and heated to 42°C for 2 min before dilution into L broth for expression, as described (7). Expression of tetracycline resistance by transformed cells was assayed by embedding a sample directly in L agar (2%) containing 10 μg of tetracycline per ml. The number of colony-forming units (CFU) containing potential transformants was determined by an immediate double-overlay technique in which each petri dish (60 by 15 mm) is filled with four 3-ml L agar layers: L agar, L agar plus cell sample, L agar, and finally L agar plus 40 μg of tetracycline per ml. This procedure was designed so as to allow full expression of drug resistance by each potential transformant before diffusion carries the drug to the cell-containing layer of the plate.

Table 1 presents the results for one preparation of competent cells. Although the overall viability of the culture decreases during this procedure, as has also been reported for other such preparations (2, 3), it may be seen that part of this loss occurs during the calcium treatment, and the remainder occurs during the heat pulse. The freezing and thawing step itself has no effect on viability.

TABLE 1. *Transformation of frozen competent cells*

Prepn stage	CFU/ml[a]	Relative[a] viability	Potential[b] transformants	Drug-resistant transformants[c]
Exponentially growing culture	5.6×10^8	1.0		
Calcium treated and concentrated 20-fold	3.4×10^9	0.29		
Frozen to −82°C, thawed at 0°C	3.7×10^9	0.31		
Transformed,[d] and incubated in 100 volumes of L broth for				
2 min	1.8×10^7	0.16	46	1
20 min	2.5×10^7		83	13
60 min	5.7×10^7		153	176
Heat pulsed and diluted 100-fold without DNA			<1	<1

[a] Ratio of CFU in culture to CFU before initial harvest.
[b] After 100-times dilution, so that 100 CFU corresponds to 3.3×10^5 transformants per μg of DNA. Measured by immediate double-overlay method with 40 μg of tetracycline per ml.
[c] After 100-times dilution. Measured by embedding directly in agar with 10 μg of tetracycline per ml.
[d] Incubation was with about 0.03 μg of pMB9 DNA per ml for 30 min at 0°C and 2 min at 42°C.

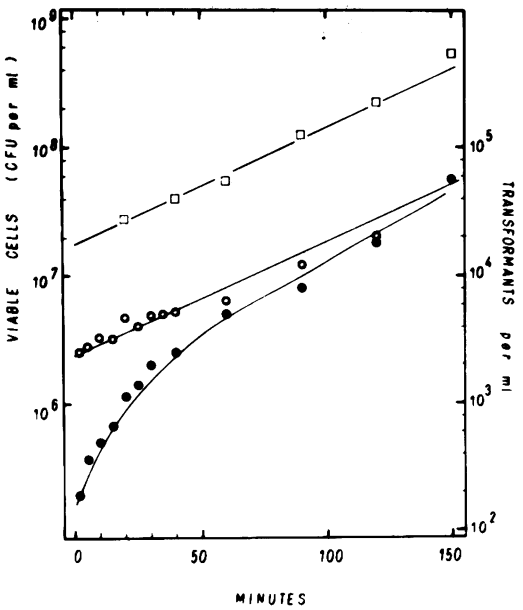

FIG. 1. *Segregation and expression of tetracycline resistance in frozen competent cells transformed with plasmid DNA. Competent cells (0.6 ml) were incubated with 0.2 ml of 0.1 M $CaCl_2$, containing 0.3 μg of supercoiled pMB9 DNA at 0°C, pulsed to 42°C, diluted to 30 ml with L broth, and incubated with aeration at 37°C. Samples were taken at the times indicated for determination of viable cells (□), potential transformants (○), and drug-resistant transformants (●), as described in the text.*

Figure 1 shows more details of the progress of expression and segregation of tetracycline-resistant transformants after the heat pulse in a transformation experiment with a frozen preparation of competent cells. Expression of the new tetracycline resistance phenotype appears to begin immediately, and to be essentially complete after 60 min. The number of CFU capable of becoming drug resistant, i.e., the potential transformants, also begins to increase immediately in L broth, and parallels the growth of the entire viable population. Thus, there appears to be no segregation lag in the division of these transformants. These competent cells, transformed after storage (for 30 days) at −82°C, could be described as yielding 2.5×10^5 or 1.0×10^6 transformants per μg of plasmid DNA, for assay of the culture after 2 or 90 min of incubation, respectively.

As these data show, $CaCl_2$-treated *E. coli* cells can be preserved without loss of viability, and with good competence, by freezing and storage at −82°C. Results are reproducible from tube to tube within such preparations for at least 3 months. This procedure should facilitate routine use of the transformation assay, under controlled conditions, of the quantity or quality of plasmid or other DNA species. For small plasmids, we note that a yield of 10^6 colonies per μg of DNA, with a background of less than one, even compares favorably with most conventional radioactive labels in sensitivity.

LITERATURE CITED

1. Clewell, D. B. 1972. The nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. 110:667-676.
2. Cohen, S. N., A. C. Y. Chang, and L. Hsu. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. 69:2110-2114.
3. Collins, C. J., D. A. Jackson, and F. A. J. DeVries. 1976. Biochemical construction of specific chimeric plasmids from ColE1 DNA and unfractionated *Escherichia coli* DNA. Proc. Natl. Acad. Sci. U.S.A. 73:3838-3842.
4. Cosloy, S. D., and M. Oishi. 1973. Genetic transformation in *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. 70:84-87.

5. **Dubnau, D., and R. Davidoff-Abelson.** 1971. Fate of transforming DNA following uptake by competent *Bacillus subtilis*. I. Formation and properties of the donor-recipient complex. J. Mol. Biol. 56:209-221.
6. **Fox, M. S., and M. K. Allen.** 1964. On the mechanism of deoxyribonucleate integration in pneumococcal transformation. Proc. Natl. Acad. Sci. U.S.A. 52:412-419.
7. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. 119:1072-1074.
8. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-206.
9. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. 53:159-162.
10. **Nickel, L., and S. H. Goodgal.** 1964. Effect of interspecific transformation on linkage relationships of markers in *Haemophilus influenzae* and *Haemophilus parainfluenzae*. J. Bacteriol. 88:1538-1544.
11. **Porter, R. D., and W. R. Guild.** 1969. Number of transformable units per cell in *Diplococcus pneumoniae*. J. Bacteriol. 97:1033-1035.