

The optimization of preparations of competent cells for transformation of *E. coli*

Xiaoren Tang, Yasuhiko Nakata, Hai-Ou Li, Mingdi Zhang, Hui Gao, Akiko Fujita, Osamu Sakatsume, Tomohiko Ohta and Kazushige Yokoyama*

Gene Bank, Tsukuba Life Science Center, RIKEN (The Institute of Physical and Chemical Research), 3-1-1 Koyadai, Tsukuba, Ibaraki 305, Japan

Received May 4, 1994; Accepted June 9, 1994

Most methods for the transformation of bacteria are based on the observation of Mandel and Higa (1) and Cohen *et al.* (2), who showed that bacteria treated with ice-cold solution of CaCl_2 and then brief-heat treatment, could be transfected with bacteriophage and plasmid DNAs. These treatments induce a transient state of 'competence' in recipient bacteria during which they are able to take up DNAs. The methods have been refined to optimize the efficiency of transformation of different bacterial strains by DNA (2,3). Many variations of this basic technique have been reported so far and all directed toward optimizing the efficiency of transformation of different strains by plasmids. Furthermore, it is widely accepted that the density of cultures of *E. coli* cells for transformation at high frequencies should not exceed 1×10^8 viable cells per milliliter ($\text{OD}_{600} = 0.3-0.4$). However, it is unknown why this density of *E. coli* cells (1×10^8 viable cells/ml) is so suitable for inducing a transient state of 'competence'. Therefore, we prepared competent cells from various cultures with densities from 5×10^7 to 4×10^8 cells ($\text{OD}_{600} = 0.3-0.98$) and transformed them with supercoiled pBR322 plasmid DNA by a 'calcium chloride' method with slight modifications in order to investigate the competence of the cells (2). Five strains of *E. coli*, namely, HB101, C600, XL1-blue, JM105 and JM109, were precultured and inoculated separately into 100-ml aliquots of LB liquid medium and shaken at 37°C for 2 hrs. The optical density at 600 nm was monitored every 20 min to examine growth of each culture. Samples at different times during the growth cycle, between optical densities at 600 nm of 0.1 and 0.98, were collected. After brief centrifugation, cell pellets were resuspended in 10 ml of an ice-cold solution of 80 mM CaCl_2 and 50 mM MgCl_2 and stored on ice for 10 min. This treatment was repeated twice. Finally, each cell pellet was resuspended in ice-cold 0.1 M CaCl_2 at a concentration of about 5×10^9 cells per milliliter and then the suspension was mixed with an equal volume of 50% glycerol to yield competent cells. For transformation, 80 μl of each suspension of competent cells and 50 pg of pBR322 plasmid DNA were mixed in a tube and kept on ice for 30 min. Then heat-shock at 42°C for 40 seconds was carried out and the mixture was rapidly transferred to an ice bath for 1 min. Then 400 μl of SOC liquid medium (4) were added and the mixture was incubated for 30 min at 37°C . A 100- μl aliquot of each sample, that is about one fifth of the total, was transferred to agar-SOB medium (4) that contained

20 mM MgSO_4 and the appropriate antibiotic, spread over the surface of the agar plate, and incubated at 37°C overnight. The number of transformants was calculated and the relative transformation frequencies for different preparations of competent cells were compared. To our surprise, we found that the efficiency of transformation was greatest for cells from cultures at two optical densities (OD_{600}), namely, 0.3–0.4

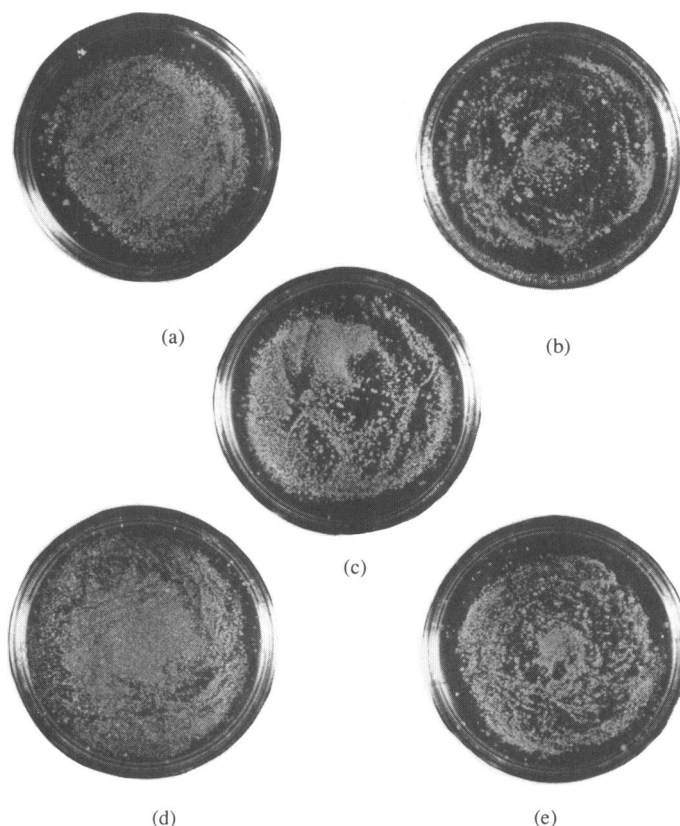


Figure 1. Transformation by 50 pg of pBR322. Transformation was carried out with competent cells of HB101 harvested at different times during the growth cycle, namely, at OD_{600} 0.4 (a), 0.5 (b), 0.6 (c), 0.8 (d) and 0.94 (e).

*To whom correspondence should be addressed

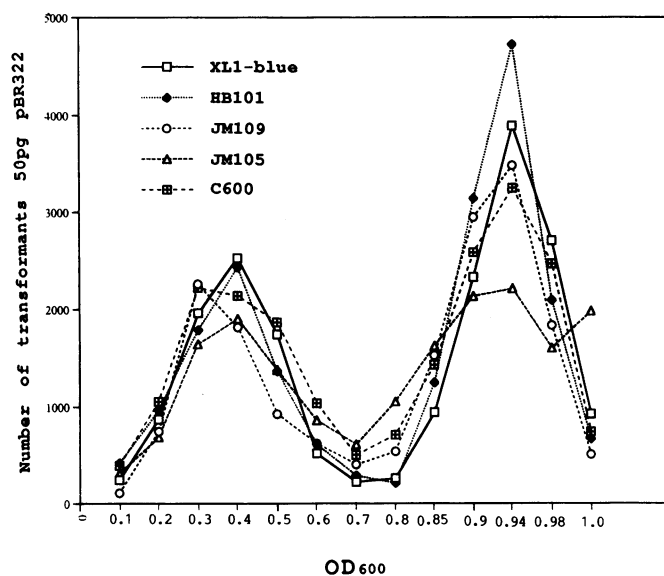


Figure 2. A graph showing the numbers of transformants per 50 pg of supercoiled pBR322 DNA for competent cells of five different *E. coli* strains during the course of culture. The cell density of cultures was monitored in terms of OD₆₀₀ or by counting cells. The graph shows the results for XL1-blue (□), HB101 (◆), JM109 (○), JM105 (△) and C600 (⊞). An OD₆₀₀ of 0.94–0.95 during the growth cycle of *E. coli* cells seems to represent the best time for harvesting, potentially competent cells of most strains of *E. coli*.

(5×10^7 – 1×10^8 cells/ml) and 0.94–0.95 (4×10^8 cells/ml). Moreover, the cells harvested at an optical density of 0.94–0.95 gave twice the transformation efficiency of cells harvested at an optical density of 0.3–0.4 (7×10^7 versus 4×10^7) (Figure 1).

This new unexpected observation allows us to prepare competent cells that are able to take up DNA at higher efficiency than when the usual protocol is followed (5×10^7 – 1×10^9 versus 2×10^7 – 5×10^8 per microgram DNA) (4), and to harvest competent cells at 4–8 times higher levels. In addition, this method can be used irrespective of the strain of bacteria, such as Rec A⁺ or rec A⁻, as host. Therefore, we strongly recommend this improved method for the preparation of active competent cells for the transformation of *E. coli*.

ACKNOWLEDGEMENTS

This work has been supported by the Life Science Research Project of RIKEN and Special Coordination Funds of the Science and Technology Agency of Japan.

REFERENCES

1. Mandel, M. and Higa, A. (1970) *J. Mol. Biol.* **53**, 159–163.
2. Cohen, S.N., Chang, A.C.Y. and Hsu, L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110–2118.
3. Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557–563.
4. Sambrook, D., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd. edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.