Abstract

This paper describes an efficient bacterial transformation system that was established for the preparation of competent cells, plasmid preparation, and for the storage in bacterial stocks in our laboratory. Using this method, a number of different plasmids have been amplified for further experiments. Competent cells for bacterial transformation were prepared by the calcium chloride method with an optimum concentration of 75 mM. Three different strains of Escherichia coli that were tested are DH5α, TG1 and XL1 blue, and the most efficient strain being XL1 blue. The optimal optical density (OD600) range for competent cell preparation varied for each of the strains investigated, and for XL1 blue it was 0.15-0.45; for TG1 it was 0.2-0.5; and for DH5α it was 0.145-0.45. The storage time of competent cells and its correlation to transformation efficiency has been studied, and the result showed that competent cells can be stored at -20°C for 7 days and at -70°C for 15 days. Three critical alterations to previous methods have been made, which are the changing of the normal CaCl2 solution to TB solution, the changing of the medium from LB to S.O.C., and addition of DMSO or PEG8000 during transformation of competent cells with plasmids. Changing the medium from LB to S.O.C., resulted in much faster growth of transformants, and the transformation efficiency was increased. Addition of DMSO or PEG8000 raised transformation efficiencies by 100-300 fold. Our improved bacterial transformation system can raise the transformation efficiency about 10³ times, making it becoming a highly efficient bacterial transformation system.

Keywords

competent cells, E. coli, plasmid, storage, transformation