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A rapid and convenient method for the preparation and storage of competent bacterial cells

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Several effective methods for bacterial cell transformation have previously been described. These methods involve treatment of cells with $CaCl_2$ (1-5) or cobalt hexamine chloride (6). Agents such as polyethylene glycol (PEG) have also been shown to induce bacterial cell transformation (7). We now describe a new method for the preparation of competent bacterial cells that yields high transformation efficiencies but is simpler and more convenient than other techniques.

Bacterial cells are grown to the early log phase (OD_{600} = 0.3-0.6) in LB broth, then pelleted by centrifugation (1,000 X g for 10 mins at 4°C). The cells are then resuspended in 1/10th volume of transformation and storage buffer (TSB): LB broth (pH 6.1) containing 10% PEG (MW=3,350), 5% DMSO and 20 mM Mg^{++} (10mM $MgCl_2$ + 10 mM $MgSO_4$) at 4°C, and incubated on ice for approximately 10 mins. For transformation, 0.1 ml aliquots of the cells are pipetted into cold polypropylene tubes and mixed with 100 pg of plasmid DNA. The cells are returned to ice for 5-30 mins. Heat shock is not necessary. Next, cells are grown to permit expression of the antibiotic resistance gene [0.9 ml of TSB with 20 mM glucose is added, and the cells incubated at 37°C with shaking (225 rpm) for 60 mins] and plated on antibiotic-containing agar plates for selection of transformants.

Using this protocol, we obtain transformation efficiencies of up to 2 X 10^8 transformants per microgram of DNA. Moreover, cells in TSB can be frozen (in a dry ice/ethanol bath) and stored at -70°C for use at a later date without a significant loss of cell viability or transformation efficiency. This technique provides a convenient and reproducible method for the preparation and storage of competent bacterial cells.

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