

## 转化质粒 DNA 到 *E.coli*\* (CaCl<sub>2</sub> 法制备感受态)

### *Materials*

Single colony of *E. coli* cells  
LB medium  
CaCl<sub>2</sub> solution (see recipe), ice cold  
LB plates containing ampicillin  
Plasmid DNA  
Chilled 50-ml polypropylene tubes  
Beckman JS-5.2 rotor or equivalent  
42°C water bath  
Additional reagents and equipment for growth of bacteria in liquid media  
*NOTE:* All materials and reagents coming into contact with bacteria must be sterile.

### *Prepare competent cells*

**1. Inoculate a single colony of *E. coli* cells into 50 ml LB medium. Grow overnight at 37°C with moderate shaking (250 rpm).**

*Alternatively, grow a 5-ml culture overnight in a test tube on a roller drum.*

**2. Inoculate 4 ml of the culture into 400 ml LB medium in a sterile 2-liter flask. Grow at 37°C, shaking at 250 rpm, to an OD<sub>590</sub> of 0.375.**

*This procedure requires that cells be growing rapidly (early- or mid-log phase). Accordingly, it is very important that the growing cells have sufficient air. A 1-liter baffled flask can be used instead of the 2-liter flask. Overgrowth of culture (beyond OD<sub>590</sub> of 0.4) decreases the efficiency of transformation*

**3. Aliquot culture into eight 50-ml prechilled, sterile polypropylene tubes and leave the tubes on ice 5 to 10 min.**

*Cells should be kept cold for all subsequent steps.*

*Larger tubes or bottles can be used to centrifuge cells if volumes of all subsequent solutions are increased in direct proportion.*

**4. Centrifuge cells 7 min at 1600 × g (3000 rpm in JS-5.2), 4°C. Allow centrifuge to decelerate without brake.**

*We have not attempted to determine whether deceleration without braking is critical to this procedure. However, we do not use the brake for this step or for the subsequent centrifugation steps.*

**5. Pour off supernatant and resuspend each pellet in 10 ml ice-cold CaCl<sub>2</sub> solution.**

*Resuspension should be performed very gently and all cells kept on ice.*

**6. Centrifuge cells 5 min at 1100 × g (2500 rpm), 4°C. Discard supernatant and resuspend each pellet in 10 ml ice-cold CaCl<sub>2</sub> solution. Keep resuspended cells on ice for 30 min.**

**7. Centrifuge cells 5 min at 1100 × g, 4°C. Discard supernatant and resuspend each pellet in 2 ml ice-cold CaCl<sub>2</sub> solution.**

*It is important to resuspend this final pellet well. The suspension may be left on ice for several days. For many strains (e.g., DH1) competency increases with increasing time on ice, and reaches a maximum at 12 to 24 hr. This is not true for MC1061 cells, which should be frozen immediately.*

**8. Dispense cells into prechilled, sterile polypropylene tubes (250- $\mu$ l aliquots are convenient). Freeze immediately at -70°C.**

*Assess competency of cells*

**9. Use 10 ng of pBR322 to transform 100  $\mu$ l of competent cells according to the steps provided below. Plate appropriate aliquots (1, 10, and 25  $\mu$ l) of the transformation culture on LB/ampicillin plates and incubate at 37°C overnight.**

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**10. Calculate the number of transformant colonies per aliquot volume ( $\mu$ l)  $\times$  10<sup>5</sup>: this is equal to the number of transformants per microgram of DNA.**

*Transformation efficiencies of 10<sup>7</sup> to 10<sup>8</sup> and 10<sup>6</sup> to 10<sup>7</sup> are obtained for E. coli MC1061 and DH1, respectively. Competency of strains decreases very slowly over months of storage time.*

***Transform competent cells***

**11. Aliquot 10 ng of DNA in a volume of 10 to 25  $\mu$ l into a sterile 15-ml round-bottom test tube and place on ice.**

*Plasmid DNA can be used directly from ligation reactions. When this is done, more DNA is usually used. However, if there is >1 g of DNA in the ligation reaction, or if the ligation reaction is from low gelling/melting temperature agarose, it is wise to dilute the ligation mix.*

**12. Rapidly thaw competent cells by warming between hands and dispense 100  $\mu$ l immediately into test tubes containing DNA. Gently swirl tubes to mix, then place on ice for 10 min.**

*Competent cells should be used immediately after thawing. Remaining cells should be discarded rather than refrozen.*

**13. Heat shock cells by placing tubes into a 42°C water bath for 2 min.**

*Alternatively, incubate at 37°C for 5 min.*

**14. Add 1 ml LB medium to each tube. Place each tube on a roller drum at 250 rpm for 1 hr at 37°C.**

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**15. Plate aliquots of transformation culture on LB/ampicillin or other appropriate antibiotic-containing plates.**

*It is convenient to plate several different dilutions of each transformation culture. The remainder of the mixture can be stored at 4°C for subsequent platings.*

**16. When plates are dry, incubate 12 to 16 hr at 37°C**

### ***CaCl<sub>2</sub> solution***

60 mM CaCl<sub>2</sub>

15% glycerol

10 mM PIPES [piperazine-*N,N*-bis(2-hydroxypropanesulfonic acid)], pH 7.0 Filter sterilize using a disposable filter unit, or autoclave

Store at room temperature (stable for years)

\*:出自 *Current Protocols in Molecular Biology* [B], Wiley Online Library,2007