**Eliminating detergent**

Detergent is a major inhibitor of competent cell growth and transformation. Glass and plastic must be detergent free for these protocols. The easiest way to do this is to avoid washing glassware, and simply rinse it out. Autoclaving glassware filled 3/4 with DI water is an effective way to remove most detergent residue. Media and buffers should be prepared in detergent free glassware and cultures grown up in detergent free glassware.

## *BASIC PROTOCOL 1:* TRANSFORMATION USING CALCIUM CHLORIDE

*Escherichia* ***coli*** **cells** are grown to log phase. **Cells** are concentrated by centrifugation and resuspended in a solution containing calcium chloride. Exposure to calcium ions renders the **cells** able to take up DNA, or ***competent***. Plasmid DNA is mixed with the **cells** and presumably adheres to them. The mixture of DNA and **cells** is then heat shocked, which allows the DNA to efficiently enter the **cells**. The **cells** are grown in nonselective medium to allow synthesis of plasmid-encoded antibiotic resistance proteins, then plated on antibiotic-containing medium to allow identification of plasmid-containing colonies.

### Materials

Single colony of ***E. coli*** **cells**
LB medium (*UNIT 1.1*)
$CaCl_2$ solution (see recipe), ice cold
LB plates (*UNIT 1.1*) containing ampicillin (Table 1.4.1)
Plasmid DNA (*UNITS 1.6 & 1.7*)

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 (*UNIT 1.2*)

*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; see *UNIT 1.2*).

*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 $OD_{590}$ 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 baffle flask can be used instead of the 2-liter flask. Overgrowth of culture (beyond* $OD_{590}$ *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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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-ul aliquots are convenient). Freeze immediately at -70°C.

***Assess competency of cells***

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

10. Calculate the number of transformant colonies per aliquot volume (ul) × $10^5$: this is equal to the number of transformants per microgram of DNA.

*Transformation efficiencies of $10^7$ to $10^8$ and $10^6$ to $10^7$ 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 ul 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 ug 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 (see UNIT 3.16).*

12. Rapidly thaw **competent cells** by warming between hands and dispense 100 ul immediately into test tubes containing DNA.

12a. Gently swirl tubes to mix, then place on ice for 20 min.

13. Heat shock **cells** by placing tubes into a 42°C water bath for 45 seconds. Accurate timing affects efficiency.

14. Add 0.9 ml LB medium to each tube. Place each tube on a roller drum at 250 rpm for 1 hr at 37°C.

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.