

## **STORAGE MEDIA**

Bacteria can be stored for up to 2 years in stab cultures or indefinitely in cultures containing glycerol.

### **Stab Cultures**

Use glass vials (2–3 ml) with screw-on caps fitted with rubber gaskets. Add molten LB agar until the vials are two thirds full. Autoclave the partially filled vials (with their caps loosely screwed on) for 20 minutes at 15 lb/sq. in. on liquid cycle. Remove the vials from the autoclave, let them cool to room temperature, and then tighten the caps. Store the vials at room temperature until needed.

To store bacteria, pick a single, well-isolated colony with a sterile inoculating needle and stab the needle several times through the agar to the bottom of the vial. Replace and tighten the cap, and label both the vial and the cap. Store the vial in the dark at room temperature.

### **Cultures Containing Glycerol**

#### **BACTERIAL CULTURES GROWING IN LIQUID MEDIA**

To 0.85 ml of bacterial culture, add 0.15 ml of sterile glycerol (sterilized by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle). Vortex the culture to ensure that the glycerol is evenly dispersed. Transfer the culture to a labeled storage tube equipped with a screw cap and an air-tight gasket. Freeze the culture in ethanol–dry ice or in liquid nitrogen, and then transfer the tube to  $-70^{\circ}\text{C}$  for long-term storage.

To recover the bacteria, scrape the frozen surface of the culture with a sterile inoculating needle, and then immediately streak the bacteria that adhere to the needle onto the surface of an LB agar plate containing the appropriate antibiotics. Return the frozen culture to storage at  $-70^{\circ}\text{C}$ . Incubate the plate overnight at  $37^{\circ}\text{C}$ .

#### **BACTERIAL CULTURES GROWING ON AGAR PLATES**

Scrape the bacteria growing on the surface of an agar plate into 2 ml of LB medium in a sterile tube. Add an equal volume of LB medium containing 30% sterile glycerol. Vortex the mixture to ensure that the glycerol is completely dispersed. Dispense aliquots of the glycerinated culture into sterile tubes equipped with screw caps and air-tight gaskets. Freeze the cultures as described above.

This method is useful for storing copies of cDNA libraries established in plasmid vectors (for discussion, see Hanahan 1985).