Creating Bacterial Glycerol Stocks for Long-term Storage of Plasmids

Background Information

Bacterial glycerol stocks are important for long-term storage of plasmids. Although you can store your plasmid DNA at -20°C, many labs also create bacterial glycerol stocks of their plasmids. This way, when you want to make more plasmid DNA, the plasmid will already be in your desired bacterial strain and you will not need to obtain more competent cells and retransform.

Bacteria on an LB agar plate can be stored at 4°C for a few weeks. However, if you want to store bacteria for a longer time, you will need to establish glycerol stocks. The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at -80°C for many years.

Protocol

1. Follow the steps for Inoculating an Overnight Liquid Culture.
2. After you have bacterial growth, add 500 µL of the overnight culture to 500µL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.

   Note: *Make the 50% glycerol solution by diluting 100% glycerol in dH20.*

   Note: *Snap top tubes are not recommended as they can open unexpectedly at -80°C.*

3. Freeze the glycerol stock tube at -80°C. The stock is now stable for years, as long as it is kept at -80°C. Subsequent freeze and thaw cycles reduce shelf life.
4. To recover bacteria from your glycerol stock, open the tube and use a sterile loop, toothpick or pipette tip to scrape some of the frozen bacteria off of the top. Do not let the glycerol stock unthaw! Streak the bacteria onto an LB agar plate.
5. Grow your bacteria overnight at the appropriate temperature. Growth conditions, including copy number and growth temperature, can be found on your plasmid's information page. The next day you will be able to start an overnight culture for plasmid DNA prep the following day.

Tips and FAQ

- The optimal concentration of long-term glycerol storage is unknown. Most labs store bacteria in 15-25% glycerol.
- You can prepare the glycerol stock the same time you prepare your plasmid DNA. In the morning, when you retrieve your liquid bacterial culture, take 500µL of culture to make your glycerol stock before you begin your plasmid mini-prep.
- Try not to freeze/thaw your glycerol stock too many times. Placing the glycerol stock on dry ice while streaking onto LB agar will prevent it from thawing completely and will improve the shelf life.
- It is very important that you shake the glycerol before freezing (5-6 times). Make sure that you see one uniform solution, and there are no layers present.
- Be sure to label both the lid and the tube of a glycerol stock before you place the sample at -80°C. Frozen tubes are hard to write on and samples stored for long periods at -80°C can lose labels stuck to tube!