Creating Bacterial Glycerol Stocks for Long-term Storage of Plasmids

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Protocol

1. Follow the steps for Inoculating an Overnight Liquid Culture.
2. After you have bacterial growth, add 750 µL of the overnight culture to 250µL of 80% glycerol in a 2 mL screw top tube or cryovial and gently mix.

   Note: *Make the 80% glycerol solution by diluting 100% glycerol in dH20 and sterilize by autoclaving.*

   Note: *Snap top tubes are not recommended as they can open unexpectedly at -80°C. Saying that, I use them as they are easier to label.*
3. Immediately put on ice and transfer to the -80 as quickly as possible.

   Note: *Cells don't like living in this solution so you want to freeze them as quickly as you can.*
4. Note in Airtable which box you have placed your stock in.
5. To recover bacteria from your glycerol stock, open the tube and use a sterile loop to scrape some of the frozen bacteria off the top and streak on a plate. **Don't let your glycerol stock thaw!**

   Note: *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.*

   Note: *You can streak plates out in the -80 room using a portable Bunsen burner but I don't recommend doing this if not using antibiotics.*

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.
- 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!