

Making Glycerol Stocks

1. Inoculate cultures and grow for ~16h or until stationary.
2. After you have bacterial growth, add 500 μL of the overnight culture to 500 μL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.
3. Freeze the glycerol stock tube at -80°C .
 - a. 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.
 - a. Do not let the glycerol stock unthaw!
 - b. Streak the bacteria onto an LB agar plate.
5. Grow your bacteria overnight at the appropriate temperature.
 - a. Growth conditions, including copy number and growth temperature, can be found on your plasmid's information page.
 - b. The next day you will be able to start an overnight culture for plasmid DNA prep the following day.