ARIA

Glycerol stock for preservation Protocol

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Glycerol stock for plasmid preservation

Introduction

Within 2 weeks of receiving the plasmid, a glycerol stock should be created, which is needed for long-term storage of plasmids.

Materials

- Streaking and Isolating Bacteria on an LB Agar Plate
- Sterile toothpicks or wire loop
- Bunsen burner (or other small flame source)
- Incubator
- LB agar plate (with appropriate antibiotic)
- Bacterial stab

Procedure

Creating Bacterial Glycerol Stocks

1. 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.

   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.*

2. **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.

3. 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!

4. 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 the tube [1].

Bibliography

1. Addgene: Protocol - how to create a bacterial glycerol stock. (n.d.). Retrieved October 18, 2021, from Addgene.org website: https://www.addgene.org/protocols/create-glycerol-stock/