Cell Maintenance

Introduction

The cell maintenance protocol is used to make bacterial glycerol stocks for long-term storage of plasmids. This way, the plasmid will already be in your desired bacterial strain and you will not need to obtain more competent cells and retransform. This technique allows addition of glycerol to stabilize 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.

Reference:
1. Addgene: Protocol - How to Create a Bacterial Glycerol Stock. (n.d.). Addgene. https://www.addgene.org/protocols/create-glycerol-stock/

Materials

- 50% glycerol
- Bacterial strain
- 2 mL screw top tube or cryovial

Procedure

Long-term stock

1. Inoculating an Overnight Liquid Culture.
2. add 1ml of the overnight culture to 0.5ml of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix.
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 37°C.

   Bacteria that are generally thawed will only be stored in a 4°C cold room for two weeks
   after two weeks, they need to be thawed again.

Short-term stock

6. Inoculating an Overnight Liquid Culture.
7. use sterile tip to streak the overnight culture on the agar medium with specfic antibiotics

8. store the medium in 4°C for less than two weeks.

9. redo the procedure before expiration to keep the cell available for immediate use.