

Preparation and Revival of Glycerol Stocks

Introduction

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.

[https://www.addgene.org/protocols/create-glycerol-stock/?](https://www.addgene.org/protocols/create-glycerol-stock/?gclid=Cj0KCQjwoKzsBRC5ARIsAITcwXHQRf7D4Rt9DFX15OFrWV7ZouBvLgPwniPJjCEue65R-Qzego2vpFsaApL_EALw_wcB)

[gclid=Cj0KCQjwoKzsBRC5ARIsAITcwXHQRf7D4Rt9DFX15OFrWV7ZouBvLgPwniPJjCEue65R-Qzego2vpFsaApL_EALw_wcB](https://www.addgene.org/protocols/create-glycerol-stock/?gclid=Cj0KCQjwoKzsBRC5ARIsAITcwXHQRf7D4Rt9DFX15OFrWV7ZouBvLgPwniPJjCEue65R-Qzego2vpFsaApL_EALw_wcB)

Materials

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Procedure

Create a glycerol stock

1. Add 500 µL of an overnight culture (or OD > 0.6) to 500 µL of 50% glycerol in a 2 mL screw top tube or cryovial and gently mix by pipetting up and down.
2. Freeze the glycerol stock tube at -80°C.

Revive a glycerol stock

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! Streak the bacteria onto an agar plate, or do liquid inoculation.