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Creating Bacterial Glycerol Stocks for Long-term Storage

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Behavioural Genomics

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We use this protocol and it's working

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Abstract

Bacterial glycerol stocks are important for long-term storage. Bacteria on 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.

Steps

1 Follow the protocol for *Inoculating a Liquid Bacterial Culture*

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 cryotube 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 thaw! Follow the protocol *Streaking and Isolating Bacteria on a LB Agar Plate*

Notes

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- Make the 50% glycerol solution by diluting 100% glycerol in dH20
- Snap top tubes are not recommended as they can open unexpectedly at -80°C
- 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
- 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!