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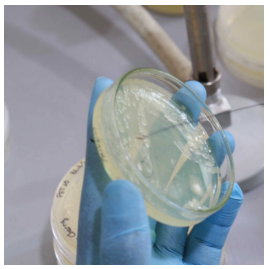
Preparation of Glycerol Stocks from storing microbes from Petri Dishes



Forked from Preparation of Bacteria Glycerol Stocks

This protocol is a draft, published without a DOI.

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Beneficial Bio | Low-cost, high-quality ...

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is meant to provide researchers with a step by step procedure on how to prepare glycerol stocks in order to preserve and store bacteria for long term.
Bacterial glycerol stocks are important for long-term storage of plasmids. 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 and -20°C for several months.

Glycerol reduces the harmful effects of ice crystals on bacteria which cause dehydration and damage cells through a localised increase in salt concentration leading to the denaturation of proteins.

Guidelines

This protocol describes the steps in preparing bacterial glycerol stocks. This protocol can be performed by anyone with basic molecular biology skills.

Materials

Reagents

- LB Broth (with or without antibiotic)
- Glycerol **Sigma – Aldrich**
- Sterile Distilled water
- Bacteria(E. coli) strain of interest (from a LB Agar plate)
- Antibiotic stock of choice (if required)

Equipment and glassware

- Refrigerator
- Incubator
- Timer
- Sterile 1.5 mL Eppendorf tubes or cryo-tubes
- P-1000 micropipette
- Sterile 1000 uL pipette tips
- 50 mL Erlenmeyer flask
- Inoculation wire loop
- Sterile 0.2 µM micro filter
- Sterile 20 mL syringe
- Sterile 3 mL plastic dropper
- Sterile 50 mL falcon tube

Protocol materials

Glycerol **Merck MilliporeSigma (Sigma-Aldrich)** Step 1

Safety warnings

Endeavor to segregate the waste generated and discard appropriately.

Before start

Make sure all materials and reagents needed for this protocol are available

Diluting Pure Glycerol to 50% with Distilled water

5m

1
- Use a clean measuring cylinder to measure 10 mL of distilled water and equal amount 10 mL of Glycerol **Sigma Aldrich** into a 50 mL falcon tube.
- Cork the tube and shake thoroughly until the liquids are evenly mixed

Filtering the 50% glycerol

2m

2
- Use a 20 mL sterile syringe to aspirate the 50% glycerol from the falcon tube
- Plug in a 0.2 µM micro filter and filter out the glycerol into a sterile falcon tube

From the plate to a PBS eppendorf

3m

3
- If you have colonies already growing in a Petri dish you can simply collect them using an inoculating wire loop and transfer to a eppendorf tube with 500 µL of 1x PBS
- Once you have collect enough pure colonies you can go to the next step.

3m

Aliquoting the bacterial culture into 50% glycerol and storing

4
- To make 1mL of Bacteria glycerol stocks, aliquot 500 µL of the filtered 50% glycerol into separate 1.5 mL Eppendorf tubes (triplicates or more depending on the quantity of glycerol stocks needed.
- Use sterile micropipette and tips to measure out equal volumes (500 µL) of the bacterial culture from the Erlenmeyer flask into the tubes containing the 50% glycerol (We now have equal proportion of bacteria and 50% glycerol).
- Keep your thumb pressed firmly against the lid of the Eppendorf tube and shake vigorously to make sure the 2 liquids mix completely.
- Use a marker pen to label the tubes with the name of the bacterial strain and date of preparation of the glycerol stock
- Store the vials in the freezer at -20°C until they are used.

Note

After pipetting the bacteria culture and 50% Glycerol, shake several times to ensure it mixes completely and uniformly.
Bacteria glycerol stocks prepared and stored in this manner are stable for up to year.
Avoid frequent freeze-thaw of the glycerol stocks.