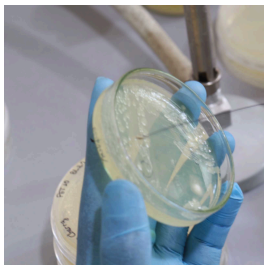
Jun 14, 2022 Version 1

Preparation of Bacteria Glycerol Stocks V.1



This protocol is a draft, published without a DOI.

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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.

Freezing is an efficient way of storing bacteria. Glycerol allows to reduce the harmful effect of ice crystals on bacteria which can damage cells by dehydration through the a localized increase in salt concentration leading to denaturation of proteins. Additionally, ice crystals can also puncture cellular membranes.

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 1.5ml Eppendorf tubes or cryo-tubes
- P-1000 micropipette
- Sterile 1000ul pipette tips
- 50ml Erlenmeyer flask
- Inoculation wire loop
- Sterile 0.2µm micro filter
- Sterile 20ml syringe
- Sterile3ml plastic dropper
- Sterile 50ml falcon tube

Protocol materials

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

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Safety warnings

Endeavor to segregate the waste generated and discard appropriately.

Preparing liquid culture of the bacteria to be stored

1 3h

- Prepare LB following this **protocol** depending on the desired amount of LB and subsequent number of glycerol stock tubes needed.
- Using an inoculating wire loop, pick up some bacteria colonies from a culture plate and inoculate in an Erlenmeyer flask containing the LB (with the right antibiotic if applicable).
- Grow the cells by incubating in an incubator at 37 °C for 03:00:00 to obtain maximum cell growth.

Diluting Pure Glycerol to 50% with Distilled water 5m

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

Filtering the 50% glycerol 2m

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

Aliquoting the bacterial culture into 50% glycerol and storing

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- To make 1ml of Bacteria glycerol stocks, aliquot 500µl of the filtered 50% glycerol into separate 1.5ml 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 (The final proportions of your glycerol stock should be 50% bacteria and 50% diluted 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.

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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.