Protocol: *E. coli* Glycerol Stock Preparing

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Reviewed by: Patrik

The protocol was originally provided by Protocols.io Website. Additional details, clarifications, and tips for practical use in the lab were provided.

Note:

- Work in a sterile area.
- Wear gloves.
- Use sterile tubes and pipette tips.

Materials and reactants:

- LB Medium (with or without ampicillin)
- Distilled Water
- Bacteria strain of interest
- Sterile 1.5 mL Eppendorf tubes
- Inoculation loop
- Sterile 0.2 µM filter and a syringe
- Sterile Falcon tube

Day 1: Inoculation of overnight culture

1. Prepare the desired amount of tubes containing ~5 mL LB Medium (with or without ampicillin)
2. Using an inoculation loop, pick up bacterial colonies from a culture plate and inoculate the tubes containing LB medium.
3. Grow the cells overnight in a shaking incubator at 37 C at 150 rpm.

Day 2: Preparation of Glycerol Stock

Preparing the 50% glycerol mixture

1. Use a measuring cylinder or serological pipette to measure 10 mL of distilled water and 10 mL of glycerol into a 50 mL falcon tube.
2. Close the falcon tube and shake thoroughly until evenly mixed
3. Use a sterile syringe to aspirate the 50% glycerol from the falcon tube
4. Plug in the sterile 0.2 µM filter and filter out the glycerol into a new sterile falcon tube.

Finishing the glycerol stocks

To prepare 1 mL of bacteria glycerol stock:

- Aliquot 500 µL (with a sterile pipette tip) of the filtered 50% glycerol stock into a separate 1.5 mL Eppendorf tubes
- Aliquot 500 µL (with a sterile pipette tip) of the overnight bacterial culture into the same Eppendorf tube
- Close the tube and mix until evenly mixed
- Store the vials in the -80C freezer

To prepare different volumes of bacterial glycerol stocks:

- Mix 50% of diluted glycerol and 50% of overnight bacterial culture.
- The final glycerol concentration should be around 25%

Note: When taking the cells out of the freezer, **don't thaw the entire tube**, but use an inoculation loop or a sterile pipette tip to scrape off a tiny bit of the frozen culture into an LB Agar plate.

Source:

Stephane Fadanka, Shalo Minette, Nadine Mowoh 2022. Preparation of Bacteria Glycerol Stocks. https://dx.doi.org/10.17504/protocols.io.x54v9ykd4g3e/v2 Version created by Nadine Mowoh.