Oct 18, 2019

Streaking and Isolating Bacteria on a LB Agar Plate

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1 Works for me dx.doi.org/10.17504/protocols.io.8euhtew

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ABSTRACT

Follow this protocol if you have a glycerol stock of the bacteria of interest and want to isolate an individual clonal population (single colony) of bacteria from this stock. Using a single colony from a freshly streaked agar plate to inoculate a bacterial culture for DNA purification will minimize the chance of having a mixture of plasmids in your purified DNA. This protocol explains how to isolate a single bacterial colony by streaking it onto a LB agar plate.

This is a general protocol for streaking bacteria on LB plate and the following parameters need to be mentioned of a specific type of bacterial streaking:
1. Name of the bacterial strain
2. Growth temperature
3. Incubation time
4. Antibiotic resistance (if any)
5. Rpm of the shaking incubator

MATERIALS TEXT

Equipment

Sterile pipette tip
Bunsen burner (or other small flame source)
Shaking Incubator
Marker

Reagents

LB agar plate (with appropriate antibiotic if required)
Frozen glycerol stock of the bacteria of interest

1 Obtain an LB agar plate with appropriate antibiotic if any.

2 Label the bottom of the plate with the strain name and the date. It is also a good idea to add the antibiotic resistance (if any) and your initials.

3 Sterilize your lab bench by spraying it down with 70% ethanol and wiping it down with a paper towel. Maintain sterility by working near a flame or bunsen burner.

4 Find the bacteria of interest from the bacterial stock database and obtain the appropriate glycerol stock from the -80C freezer (Freezer 6- Bacterial Stock box)

5 Using a sterile pipette tip touch the top of the glycerol stock to obtain the bacteria on the tip

6 Gently spread the bacteria over a section of the plate, as shown in the diagram below, to create streak #1
Using a fresh, sterile pipette tip, drag through streak #1 and spread the bacteria over a second section of the plate, to create streak #2
Using a third sterile pipette tip, drag through streak #2 and spread the bacteria over the last section of the plate, to create streak #3

The repeated streaking is done to dilute the bacteria and obtain single colonies.

7 Incubate the plate (lid side down) with the newly plated bacteria overnight (generally 12-18 hours) at the appropriate growth temperature for the particular strain. In case of antibiotic resistant plasmids, incubation should not exceed the recommended duration as it poses risk of the bacteria metabolising the antibiotic.

8 Following adequate incubation, single colonies should be visible. A single colony should look like a white dot growing on the solid medium. This dot is composed of millions of genetically identical bacteria that arose from a single bacterium. If the bacterial growth is too dense and you do not see single colonies, re-streak onto a new agar plate to obtain single colonies.

9 Store the streaked plates at 4C (lid side down to avoid condensation falling on the single colonies). The streaked plates can be used for up to a month to make liquid cultures.