Streaking and Isolating Bacterial on an LB Agar Plate

KEY POINTS:

1. Use dry plates
2. Be gentle with the agar
3. Streak multiple paths
4. Use a new toothpick for each path
5. Work near a flame

INTRODUCTION:

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

PROCEDURE:

1. Obtain an LB agar plate with appropriate antibiotic.
    a. Make sure the plate is **dry**; if the plate has condensation, place the plate next to the flame with lid open for ~ 10 min to dry it out.
2. Labe the bottom of the plate with the plasmid name and the date.
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. Obtain the appropriate glycerol stock.
5. Using a **sterile** toothpick, immerse the toothpick into semi-frozen glycerol stock solution.
6. **Gently** spread the bacteria over a section of the plate, as shown in the diagram above, to create streak #1.
    a. Hold your toothpick at an angle, the way you would hold a pencil, so that you can make a broad stroke. Only touch the surface of the plate, do NOT dig into the agar.
7. Using a **fresh**, sterile toothpick drag through streak #1 and spread the bacterial over a second section of the plate, to create streak #2.
8. Using a third sterile toothpick drag through streak #2 and spread the bacteria over the last section of the plate, to create streak #3.

9. Incubate plate overnight (12-18 hours) at **37 °C.**
10. In the morning, single colonies should be visible.
    a. 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.
11. The plate can be stored at **4 °C.**

Further reading at https://www.addgene.org/protocols/streak-plate/

Inoculating a Liquid Bacterial Culture

INTRODUCTION:

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. A liquid culture is capable of supporting a higher density of bacteria and is used to grow up sufficient numbers of bacteria necessary to isolate enough plasmid DNA for experimental use.

PROCEDURE (work near a flame):

1. Add liquid LB to a tube and add the appropriate antibiotic to the correct concentration
    a. Add **2 ml LB** into each tube
    b. Dilute the antibiotic stock 1000 times (e.g. add **2 µl** Ampicillin stock into 2 ml liquid LB)
    c. For each plasmid, prepare **3 tubes** (2 for bacterial culture; 1 for negative control)
2. Using a sterile toothpick, select a single colony from your LB agar plate
    a. Pick two single colonies, labeled as A and B, respectively
3. Drop the toothpick into the tube
    a. A good negative control is LB media + antibiotic with a clean toothpick
4. Loosely cover the culture with a cap that is not air tight
5. Incubate bacterial culture at **37 °C** for **8 hr** in a shaking incubator (**300 rpm**)

6. After incubation, check for growth, which is characterized by a cloudy haze in the medium; this is the **starter culture**
7. Add **25-50 µl** starter culture (from either A or B; select the better one) into **25 ml** liquid LB; culture at **37 °C** for **12-16 hr** in a shaking incubator (**300 rpm**)
8. The bacterial culture can be used for plasmid DNA isolation, following the Plasmid Extraction (**QIAGEN Plasmid Midi Kit**) protocol

Further reading at https://www.addgene.org/protocols/inoculate-bacterial-culture/

TIMELINE

1st day: Streak bacterial on a LB agar plate at 5 PM

2nd day: Pick up the single colony at 9 AM; add starter culture to 25 ml liquid LB at 5 PM

3rd day: Collect bacterial culture at 9 AM; isolate plasmid DNA