Culture from Agar Stab (Inoculation)

Introduction

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Materials

- Liquid LB (2xYT)
  - $NaCl_{(S)}$
  - $Tryptone_{(S)}$
  - Yeast Extract
  - dH_2O
- Equitment:
  - Autoclave
  - Shaking Incubator set to 37 °C
- Total Wait Times:
  - Incubation: 12-18 hours

Procedure

Inoculating a Liquid Bacterial Culture

1. Prepare liquid LB. For example, to make 400 mL of LB, weigh out the following into a 500 mL glass bottle:

   4 g NaCl
   4 g Tryptone
   2 g Yeast Extract
   and dH_2O to 400 mL
   Note: *If your lab has pre-mixed LB agar powder, use the suggested amount, instead of the other dry ingredients above.*
   Media without growth (top) and with growth (bottom)
   Loosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) and then loosely cover the entire top of the bottle with aluminum foil. Autoclave and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at room temperature.

2. When ready to grow your culture, add liquid LB to a tube or flask and add the appropriate antibiotic to the correct concentration (see table below).

Antibiotic Concentrations

	A	B
1	Ampicillin	100 µg/mL
2	Bleocin	5 µg/mL
3	Carbenicillin	100 µg/mL
4	Chloramphenicol	25 µg/mL
5	Coumermycin	25 µg/mL
6	Gentamycin	10 µg/mL
7	Kanamycin	50 µg/mL
8	Spectinomycin	50 µg/mL
9	Tetracycline	10 µg/mL

Note: *If you intend to do a mini-prep you will usually want to start 2 mL in a falcon tube, but for larger preps you might want to use as much as a liter of LB in a 2 L Erlenmeyer flask.*

3. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.

4. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.

5. Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.

6. Incubate bacterial culture at 37°C for 12-18 (12–16 recommended by Qiagen) hr in a shaking incubator.

   Note: *Some plasmids or strains require growth at 30°C. If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.*

7. After incubation, check for growth, which is characterized by a cloudy haze in the media (see linked protocol for info).

   Note: *Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.*
   Note: *A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.*

8. *(Optional)* For long term storage of the bacteria, you can proceed with Creating a Glycerol Stock.

9. You can now isolate your plasmid DNA from the bacterial culture by following Isolating Your Plasmid DNA.

Procedure (Version 2 from Veronica's old lab notebook)

10. Remove the agar plates from the 37° C degree incubator

11. Obtain a 14-mL sterile plastic round-bottom cell culture tubes. Label the tubes. Using sterile technique, pipette 5 mL of the 2xYT media into each tube.

12. Add the appropriate amount of antibiotic (see table above)

Example: Add 5 µL of 100 mg/mL ampicillin stock solution into each tube so that the final concentration of ampicillin in the tube is 100 µg/mL.

13. Light the Bunsen burner

14. Using a sterile loop, pick a colony of cells from the plasmid transformation plate; then, swirl the loop in of the 5 mL media tube.

    Take a metal loop and flame it in the Bunsen burner until it becomes red hot.
    Cool the loop on a side portion of the agar in the petri dish that is free of bacteria so the heat does not kill it
    Scrape up a single large colony of cells.
    Swirl the loop in the media until the cells come off the loop and go into the media.
    Flame the loop again to sterilize the loop.

15. Place the 5 mL culture in a 37° C shaker to be incubated overnight with shaking at about 225-300 rpm.