Making LB Agar Plates

Background Information

Plasmids can carry one or more antibiotic resistance genes, which confer resistance to a specific antibiotic to the bacteria carrying them. The presence of an antibiotic resistance gene on a plasmid allows researchers to easily isolate bacteria containing that plasmid from bacteria that do not contain it by artificial selection (i.e. growing the bacteria in the presence of the antibiotic).

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. The addition of agar to LB results in the formation of a gel that bacteria can grow on, as they are unable to digest the agar but can gather nutrition from the LB within. The addition of an antibiotic to this gel allows for the selection of only those bacteria with the specific antibiotic resistance - usually conferred by a plasmid carrying the antibiotic resistance gene. The following protocol is for making LB agar plates for the purpose of bacterial selection.

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[Streaking and Isolating Bacteria on an LB Agar Plate](#)

[Bacterial Transformation](#)

[Creating Bacterial Glycerol Stocks for Long-term Storage of Plasmids](#)

Protocol To make 500mL of LB agar (makes about 25 LB agar plates):

1. Weigh out the following into a 1L Erlenmeyer flask:
   - 5g NaCl
   - 5g Tryptone
   - 2.5g Yeast Extract
   - 7.5g Agar
   - add dH_2O to 500mL

   Note: If your lab has pre-mixed LB agar powder, use the suggested amount instead of the other dry ingredients above.

2. Swirl to mix - the contents do not have to be completely in solution, but any powder left on the sides of the flask will caramelize on the glass during autoclaving.

3. Cover the top of the flask with aluminum foil and label with autoclave tape.

4. Autoclave on the liquid setting for 20 minutes or according to your autoclave's specifications.

5. After removing the solution from the autoclave, allow the agar solution to cool to 55°C.

   Note: This can be done by placing the flask in a 55°C oven or water bath, as this will hold the temperature and it can be left unattended for some time.

6. When pouring plates, keep your bench area sterile by working near a flame or bunsen burner.

7. Add the appropriate amount of desired antibiotic to the solution (500µL if you are using a 1,000x antibiotic stock) and swirl to mix.

8. Pour ~20mL of LB agar per 10cm polystyrene Petri dish.

   Note: Pour slowly from the flask into the center of the petri dish. When the agar has spread to cover about 2/3 of the dish stop pouring and the agar should spread to cover the entire plate. You may need to tilt the plate slightly to get the agar to spread out completely. If you pour in too much, the plate will be fine, but it will reduce the number of plates you can make per batch.

   Note: If bubbles are introduced during the pouring, these can be removed by quickly passing the flame of an inverted bunsen burner over the surface of the plate. Be careful, if you leave the flame too long it will melt the petri dish. Also be careful not to burn yourself.

9. Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified), then invert the plates. Let sit for several more hours or overnight.

10. Label the bottom of plates with antibiotic and date and store in plastic bags or sealed with parafilm at 4°C.

Antibiotic Concentrations

Commonly Used Antibiotics	Recommended Concentration

Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

Tips and FAQ

- **Why is it important to carefully monitor the temperature when making plates?**
  After removing the agar solution from the autoclave, it is recommended to leave the solution at room temperature. Make sure the agar solution is cool enough to proceed, but not too cold where it is fully solidified. If the solution is too warm when you add the antibiotic, it may degrade the antibiotic or damage the petri plates. If the solution is too cool, it may solidify before you have time to pour the plates.

- **How long are the plates viable?**
  Storage time will vary depending on antibiotic added, but plates are generally good for 1-2 months.