

Overnight liquid culture

Introduction

This protocol describes how to grow an overnight culture of bacteria in LB (Luria-Bertani) liquid media broth.

Material

- Agar plate/Glycerol stock (bacterial source)
- Antibiotic (Kanamycin, Ampicillin or Chloramphenicol)
- LB media

Procedure

Note: ALWAYS have a bunsen burner at your station. Sterilize material by placing it under fire before use!

1. Pour 5-30mL of LB broth into your selected autoclaved conical container (not more than 30-40% of the total container volume)
2. Add antibiotic to the correct final concentration and mix.
 - Kanamycin: 50ug/mL (*E. coli*)
 - Ampicillin: 100ug/mL (*E. coli*)
 - Chloramphenicol: 25ug/mL (*E. coli*)
 - Chloramphenicol: 5ug/mL (*B. subtilis*)
 - MLS selection (*B. subtilis*): Erythromycin (1ug/mL) + Lincomycin (25ug/mL)
3. Place the inoculation loop under fire until it glows red and cool by air (close to the burner so it stays sterile) or by tapping the inner roof of the plates.
4. Scrape the top of the colony you want to use to inoculate or the top of the glycerol stock.
5. Shake the loop inside the liquid LB prepared in steps 1 and 2.
6. When selecting colonies from a plate, highlight which colony belongs to which culture. (Look at the image below)
7. Close the container loosely or cover with its sterilized tinfoil if it's a flask.
8. Place the tube/flask in an orbital shaker at ~140rpm at the optimal growing temperature of your bacterial species (37° C for *E. coli*).
9. Leave the liquid culture to grow overnight for approximately 12-14 hrs.