Pouring LB Agar Plates

Introduction

The media must be autoclaved before you pour plates. This is not something for iGEMers to do; ask an advisor.

Materials

- One sleeve of empty plates per 500 ml of media
  - Make sure you are using **the Weiss lab's bacteriological plates, *not tissue culture plates***.
- LB-Agar media, either solid or already melted
- 1000X stock solution of the antibiotic
  - (Amp)icillin, (Kan)amycin, (Chlor)amphenicol
- X-gal in DMF (optional)
  - stored in the -20, in a brown bottle

Procedure

Making media

1. Make sure you don't already have a bunch of the plates already in the fridge
2. Fill a 1-liter bottle with 500 ml of water.(You can make up to 900mL for a 1-L bottle)
3. Weigh out **XXXX** of powdered LB-Agar (check the bottle for the weight.)

   IF YOU MAKE A MESS OF THE WEIGH STATION, CLEAN UP AFTER YOURSELF.
4. Ask an instructor to autoclave your media.

Melting media

5. Solid media must be melted in the microwave. **Be careful -- it gets very hot.**
6. **Loosen the cap to prevent the bottle from exploding.** 1 full turn of the cap is sufficient.
7. Microwave 2-3 minutes, **watching carefully**, until the media begins to melt and bubble.
8. Continue microwaving in short bursts until the media is completely melted.

   IF YOU MAKE A MESS OF THE MICROWAVE, CLEAN UP AFTER YOURSELF.
9. Cool the media to <= 60°C before adding the antibiotics.

   You can either swirl it under a cold tap until you can handle it without burning yourself, or you can leave it for a few hours in the 55°C water bath.

To check the media temperature, swirl the bottle, wait ~30 seconds and measure with an IR thermometer. (Pew pew.)

Pouring plates

10. Add antibiotic.

    Unless otherwise noted, it is 1000X -- so for 500 ml of media, add 500 μl of an tibiotic.

11. (Optional) add X-gal. X-gal's concentration is 500x, so for 500 ml of media add 1 ml of X-gal.

12. Pour all of the media you add these too. Refreezing and thawing has unpredictable results on the amount of antibiotic destroyed by the heat, so to avoid over/under dosing don't refreeze. 500 ml makes a sleeve of plates.

13. Tighten the lid to avoid spills and swirl the bottle to mix

14. Use a seriological pipette to add 20ml to each plate.

    Use sterile practices
    If you get bubbles, use the pipette to try and suck the air out of the bubbles, this removes them
    20ml is a rough estimate, what is important is that you get a solid layer with no holes.

15.

16. Dry on the bench, with the lids cracked, for 30 minutes or so.

17. Re-stack the plates upside-down. Mark the plates with a stripe of marker up the side, based on which antibiotic(s) you added:

    Blue: Amp
    Red: Kan
    Green: Cm
    Black: Non-antibiotic additive (like X-gal)

18. Slip the plastic sleeve onto the plates. Label the sleeve with the antibiotics, your initials and the date.