MAKING AND POURING LB AGAR PLATES (STERILE)

(1 batch makes about 40 plates; adjust as needed)
Est. Total Time: 1-2 Hours.

Summary: Bacteria need nutrient plates to grow on. This protocol will show you how to make selective (with antibiotics) and nonselective (without antibiotics) plates.

SAFETY

- Wear appropriate PPE at all times; not only to protect yourself, but to avoid contamination.
- As an autoclave will be used for sterilization, have autoclave gloves ready.

Materials

- 1L Graduated Cylinder
- 1L Glass Autoclave Bottle, w/ Cap
- Autoclave
- Autoclave Gloves
- Autoclave Tape
- Analytical Mass Balance
- Weighing Boat(s)
- Distilled Water (DI H_2O)
- LB Agar, Miller (Fisher#:)
  - (40g/1L DI H_2O)
- LB Agar, Lennox (Fisher#:)
  - (32g/1L DI H_2O)
- Stir Bar and Stir Bar Remover
- Stir Plate
- Plastic Petri Dishes
- 70% Ethanol or Isopropanol (EtOH/IPrOH)

OPTIONAL:

- Water Bath at 55^{o}C
- Ampicillin, (Cell Center)
- Kanamycin,
- Tetracycline,
- X-Gal
- S-Gal
- IPTG
- Ammonium Iron (III) Citrate

Procedure:

Dissolve/Suspend the LB Agar

1. Add 250mL of DI H_2O to a graduated cylinder.
2. Mass out 20g of LB Agar, Miller or 16g of LB Agar, Lennox. Careful, the powder is displaced very easily.
3. Add the powder to the 1L glass autoclave bottle, then the 250mL of water.
4. Use a stir bar and a stir plate. Add any optional ingredients that are not heat-sensitive.
5. Remove the stir bar when suspension is achieved.
6. Add a further 250mL of DI H_2O to make a total volume of 500mL.
7. Label the glass bottle and place autoclave tape on the jar, a small piece on the cap, and a piece on the bottle itself.

Sterilization (IF YOU HAVE NEVER USED THE AUTOCLAVE, READ THE MANUAL AND CONSULT LAB STAFF!)

1. Before placing the bottle(s) in the autoclave, open the caps a little on the bottles so that the bottles will be vented. Otherwise, the bottles will explode in the autoclave.
2. Autoclave on the Liquid Setting.
3. With autoclave gloves, remove the agar from the autoclave, and let it cool (in a water bath, or without) to 55^{o}C.
4. After cooling, add any antibiotics one wishes to add.

Pouring & Storing Plates

1. Wipe down the bench top with 70% EtOH or 70% IPrOH.
2. Remove Petri dishes from plastic bag (you can save the bag for storage).

Pouring & Storing Plates (cont'd)

3. Pour a thin layer of LB Agar (~15-20mL) into each plate being careful to not lift the cover off excessively (you should be able to just open up enough to pour).
4. Swirl plate in a circular motion to distribute agar on bottom completely.
5. Let each plate cool until its solid (~20 minutes) then flip so as to avoid dripping condensation on the agar.
6. Store plates in plastic bags in fridge with: name, date and contents (note any additives).

Cleanup:

1. Put all materials back where they came from (Locations on Lab Equipment List).
2. Wash all glassware and containers used.
3. Replace all reagents in their proper locations.
4. Wipe down all surfaces used with 70% EtOH or IPrOH.
5. Consult Lab Staff if unsure about any of the above.