How to Pour Bacterial Plates — Version 1.3

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1 Materials

1. 1 or 2 1 L Bottles w/ Caps
2. 1 or 2 Magnetic Stir Bars
3. Autoclave Tape
4. 25 g LB Broth Powder
5. 15 g Agar Powder (omit if making liquid media)
6. Antibiotic (Carbenicillin or Ampicillin), calculated for $100\,\mathrm{mg\,mL^{-1}}$ in 50% ethanol
7. Autoclave Safe Tray (if using 1 bottle)

2 Procedure

1. Weigh 25 g of LB Broth in weigh boat.
   (a) Folded weigh boat can be used to funnel broth powder into bottle.
2. Slowly pour broth powder into bottle.
3. Fill bottle to 1000 mL mark with DI water & shake to combine.
4. If using two bottles, pour half of the broth solution into each and add half of the agar and antibiotic to each in steps 5 and 10.
   (a) If only one bottle is used, the mixture will boil over in the autoclave.
5. Add stir bar & 15 g agar powder.
6. Stir until no dry clumps are present.
7. *Loosely* screw on cap & secure lid with autoclave tape.
   (a) Autoclave tape will show black stripes after heating.
8. Autoclave on cycle 3 (liquid) until complete (≈1 hour).
9. While the solution is being autoclaved, prepare the petri dishes.

(a) Arrange dishes on lab bench with the tops (larger half) covering about ⅓ of the plate with the rest resting on the bench.

(b) Be careful when opening the petri dish package, you will put your completed plates in there.

10. When mixture reaches 55–60 °C, pipette 100 µL (green box) of antibiotic into bottle.

    (a) Liquid media is complete at this point, and can be stored at ambient temperature (covered) or in walk-in fridge.

11. Return antibiotic to −80 °C freezer.

12. Stir to combine on magnetic hotplate.

13. Pour solution into petri dishes, each plate should be about ⅓ full of media.

    (a) A good rule of thumb is to pour enough solution into the plate so that the bottom is ¾ covered, then swirl the plate.

    (b) Rotating the bottle while pouring minimizes dripping.

    (c) Clean floor spills as soon as possible, any solution spilled on the lab bench will be easier to clean after it has hardened.

14. Once the plates have cooled (they will change color to a light yellow), put the lids on and stack the completed plates, *upside-down* in their original packaging.

15. Label the package with the name(s) of whoever poured them, iGEM, the date, as well as the concentration and identity of antibiotic ("100 µg mL^{-1} carb").

3 Condensed notation:

The condensed notation is an overview of how the procedure is done. It has the same information as section 2, but all the information is available at a glance. The columns are approximate points in time and the rows represent the components. For example, step 1 in column i applies to the 1000 mL bottle and the LB broth powder. Step 2 should happen at the same time, but it only applies to the antibiotic.

1 Liter Growth Media for 50-75 plates								
1000 ml Bottle I-1	i Weigh & pour into bottle 1	ii Fill to 1000 ml, shake to combine 3	iii Add & stir until no dry clumps remain 4	iv *Loosely* cap and seal with autoclave tape 5	v Autoclave on Cycle 3 (liquid) 6	vi Cool to between 55–60 °C 8	vii Pipette calculated amount of stock solution 9	viii Pour into plates 10
25 g LB Broth Powder I-2								
1000 ml DI Water I-3								
1 Magnetic Stir Bar I-4								
15 g Agar I-5								
Antibiotic (to taste) I-6	Remove from -80 2							
75 Petri Dishes, bags reserved I-7					Arrange on bench 7			