Pouring LB-Agar+Antibiotic Plates

This recipe is for **500 mL** of LB agar. This makes about 20, 10-cm plates (1 bag).

5 g bacto tryptone
2.5 g yeast extract
5 g NaCl
7.5 g bacto agar
500 mL Milli-Q water

-OR-

Materials you need:

- LB Agar Miller (Fisher, catalog #: BP1425-500)
  - 40 grams of LB Agar per 1L of MilliQ water
- 2000mL Erlenmeyer flask
  - Do not use plastic flasks
  - Jennifer's suggestion: Use 2L Erlenmeyer flask to hold 750mL of water and 30g of LB/Agar.
- Big stir bar placed in the flask

1. Combine the above solids and water in a large bottle or flask (typically >2x the volume of what you are autoclaving). Add a large stir bar and stir for ~5 minutes (Note: the bacto agar will not dissolve until melted in the autoclave, but all other ingredients will dissolve). Once well mixed, autoclave for 20 minutes on liquid cycle.
2. Allow flask to cool in a 55°C water bath or on the bench. Obviously, you don't want the agar to cool too much or it will start to solidify. You also don't want to add antibiotics when it is too hot since the heat will degrade the antibiotics. A good temperature to aim for is ~50-55°C (you can change the temperature of the water bath for this use – just email the lab to let them know you are doing so). At this temperature, the flask will still be pretty warm to the touch, but cool enough that you can place your hands on it and hold them there for several seconds.
3. Prepare the area where you will be pouring the plates – wipe bench surface thoroughly with 70% ethanol and be sure there is a flame and sparker available. Remove the petri dishes from their sleeve, taking care to leave the lids in place at all times to ensure sterility. Stack plates 2-3 tall. Keep the sleeves for storage.
4. When agar is ~55°C, add the antibiotics. Most of the stock solutions are prepared as a 1000X concentration, so you will add 0.5 mL of stock antibiotic per 500 mL of solution. (Aliquots of Carbenicilin and Kanamycin are located in the -20C fridge by Bay D)
   Typical antibiotic FINAL concentrations;
   Carbenicllin 100 μg/mL
   Ampicillin 100 μg/mL
   Kanamycin 50 μg/mL
5. Using a stir-plate, mix the agar and antibiotic slowly, but thoroughly. You DO NOT want to introduce air bubbles!
6. Pour plates. It is best to do this next to a flame for reasons of sterility. Wear gloves and flame the lip of the bottle or flask prior to pouring. Fill each plate at least ½ full and gently swirl the stack of plates to ensure the agar is evenly distributed in the dish.
7. If air bubbles formed while pouring the plates, flame the air bubbles BRIEFLY to pop them. Solidified air bubbles are difficult to spread bacterial culture over.
8. Allow plates to solidify at room temperature. Then, invert the plates and allow them to dry at room temperature overnight. You may need to cover the plates with tinfoil, as light inactivates many antibiotics.