Adapted from a UCLA protocol.
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∅∅∅∅∅ Making LB Plates

This protocol will generate plates containing LB nutrient broth to grow bacteria, agar, and your choice of antibiotic. While generating the initial LB is does not require strong sterile technique as it will be sterilized in the autoclave, a strongly bacteria-free environment will be necessary for pouring the plates.

After agar LB is autoclaved, the liquid should be handled very carefully-any introduced bacterial will contaminate the stock.

This protocol will produce 25 plates. The external protocol on *autoclaving* will be used in addition to this one. A 1 hour gap separates procedures *I* and *II*, where *I* prepares the agar solution and *II* fills the plates.

Compounds
dH2O
LBagar powder
Your favorite antibiotic

Materials
1000mL graduated cylinder
500mL Erlenmeyer flask
10mL Autopipettes
Pipettes capable of 250μL, tips
Weighing boats
Petri dishes
Permanent markers
Masking tape

*You will also need access to a*n
Autoclave
Refrigerator
Scale

External protocols
Autoclaving

Protocol I

1. Mass **10g LB agar powder** in a **weighing boat**.
2. Pinching the sides of the boat, pour the entire contents of the boat into an empty **500mL Erlenmeyer flask**.
3. Fill a **1000mL graduated cylinder** with **250mL dH2O**.
4. Empty the contents of the graduated cylinder into the Erlenmeyer flask.

⇒Autoclave in accordance with the **autoclaving protocol.**

Protocol II

1. Let the autoclaved **LB agar solution** sit on a counter until cool enough to hold without a glove.
2. **Ensuring the work area is sterile**, remove the sterile **Petri dishes** from the bag and stack in 5-high piles.
3. Carefully remove the cover on the **LB agar solution.**

4. Add **250µL** of **your favorite antibiotic** to the LB solution.

5. Transfer **10mL LB agar solution** to one of the plates with a **10mL autopipette.** Carefully lift the lid of the plate just high enough to fit the pipette under. Swirl each plate to distribute the agar. Once the pipette has been emptied, set the plate off to the side, spreading them out over the counter.

6. Repeat **step 5** with the other **24 plates**. Wait **20 minutes** or until the agar is solid.

7. Flip each plate over so the agar side of the plate is oriented upwards to prevent condensation. Stack and store in a sterile plastic bag, making sure to label the bag.

Despite being treated with antibiotic, each plate can still be contaminated and should be handled carefully and in a sterile manner.