Maintaining Worms

Escherichia coli (*E. coli)* is a bacteria most commonly found in the lower digestive tract of warm-blooded animals. In the lab, one of the ways we use *E. coli* is to feed *Caenorhabditis elegans* (*C. elegans*). In order to keep a stable stock of *C. elegans* for our experiments, we need to prepare the growth medium for the two species to grow on.

The medium we use to grow both organisms is called lysogeny broth (LB). We'll be using 2 main types of LB - liquid and solid. The liquid form (LB broth) is made by combining tryptone, yeast extract, sodium chloride, and water. Agar can be included in the recipe to create a solid medium (LB agar), which is a gel-like at room temperature, but melts at higher temperatures (50℃, for example). *E. coli* can grow in the LB broth or on the surface of LB agar. Liquid medium is used when we need a lot of bacteria due to the larger growth area for the bacteria. Solid medium is used when we need single colonies of bacteria or a stable surface.

LB medium:

Materials

- disposable spatula
- weigh boats
- 250 ml bottle
- 100 ml graduated cylinder
- 150 ml Erlenmeyer flask

LB Agar for Plates

1. Label a 250 ml bottle with "LB Agar," your initials, and today's date.
2. Put a weigh boat on the balance, then press TARE/ZERO - this will prevent the scale from adding the mass of the weigh boat to your measurement.
3. Add 3.5 g LB agar powder to the weigh boat with a disposable spatula.
   Note: Never put chemicals back to their storage container! Assume that chemicals are contaminated by the boat. Start with a small amount to avoid taking out too much.
4. Carefully transfer the weighed powder to your labelled bottle with the help of the spatula.
5. Measure 100 ml DI water with a 100 ml graduated cylinder.
6. Add the 100 ml water to the labelled bottle. Swirl slightly to mix the powder with water.

Note: Not all powder will dissolve.

LB Broth for Liquid Medium

1. Label a 150 ml Erlenmeyer flask with "LB broth" and today's date.
2. Using a 50 mL graduated cylinder, measure ~30 ml of DI water and add it to your labeled flask.
3. Place a weigh boat on the balance, then press TARE/ZERO.
4. Add 0.5 g tryptone to the weigh boat with a disposable spatula.
5. Carefully transfer the weighed powder to your labelled bottle with the help of the spatula. Discard the spatula into the red biohazard bin on the floor.
6. Swirl the labeled flask until all powder is dissolved.
7. Repeat steps 3-6 to measure out 0.25 g yeast extract and 0.25 g sodium chloride and add them to your flask, making sure to change spatulas for each new chemical.
   Note: Never put chemicals back to their storage container! Assume that chemicals are contaminated by the boat. Start with a small amount to avoid taking out too much.

8. Once all solid is dissolved, pour the contents of your labeled flask into a 50 ml graduated cylinder.
9. Fill the cylinder with DI water until the meniscus reaches the 50 mL mark. Then add the 50 ml of solution back into your labelled flask.
10. Pool the liquid in the flasks into the bottle provided.
11.

The bottles will be autoclaved and then stored at 4℃. (Why?)