

Preparing Media and Growing Cultures

Many biology experiments involve microorganisms such as bacteria and yeast. Because these organisms are so small, we need specialize techniques to handle them. **Growth media** for bacteria and yeast are solutions containing all the nutrients needed for the cells to grow; they may be prepared as liquid media (**broth**) for growing cells in tubes or flasks or, by adding **agar** as a solidifying agent, as solid media (**plates**) for growing individual colonies on a surface.

The type of media to use depends on the specific microorganism you want to grow. For growing bacteria such as *Escherichia coli* (*E. coli*) or *Salmonella*, we most commonly use **LB medium** (Luria-Bertani medium), which is a rich medium suitable for many different bacterial species. For yeast, **YPD medium** (yeast extract, peptode and dextrose) is a common growth medium. However, a particular experiment or an unusual organism may require specific media.

Preparing Liquid Media

A particular growth medium may be available pre-mixed, or you may have to mix the ingredients yourself. If you are using a pre-mixed medium, the bottle (which you will find on the media shelf in SC 224) will tell you the amount to use, in grams per liter. Obviously, you may not need an entire liter, so calculate smaller volumes accordingly. For other media, you will need a recipe, either from the box in SC 222 or (for more unusual media) from a journal article or a book of media ingredients such as the *Difco Manual*.

Media should always be made in the media flasks on the shelf in SC 224. Other flasks could contain chemical residue which might be harmful to your cells. If you are planning to grow your microorganisms in a flask, find out from your instructor if you should grow them directly in a media flask or if a culture flask is needed. If you will be growing the bacteria in tubes, you will want to either transfer the media to the tubes before autoclaving or to an appropriate-sized screw-cap bottle.

1. Obtain a media flask of an appropriate size from the shelf in SC 224.
2. Weigh out the desired amount of media mix or media ingredients.

◇ *Some media mixes include agar, some do not—and sometimes we have both versions of a particular medium on hand. Since you are making liquid medium, be sure you're not using a mix that contains agar!*

3. Pour the powdered media into the flask, then add the desired volume of dH₂O (measured in a media cylinder) from the outlet in SC 224. Don't worry about accounting for the volume of the powder.
4. Swirl the flask well to get most of the powder off the bottom.

◇ *In most cases, you don't need a stir bar and you don't need to worry about dissolving the powder completely: that will happen in the autoclave. However, if you will need to transfer your medium to a bottle or tubes before autoclaving, you will need to dissolve it completely first.*

5. If you will be growing organisms directly in the flask, add a foam plug and then cover the top with a square of foil. If not, the foil alone will be sufficient.
6. Use two small pieces of autoclave tape to tape the bottom of the foil to the glass.
7. Autoclave your medium (see directions below).
8. After autoclaving, swirl the flask thoroughly before use to ensure complete mixing.
9. If you need to add antibiotics, amino acids, vitamins or other heat-sensitive components to your medium, do this after autoclaving and after the medium has cooled to near room temperature.

10. Rinse your flask thoroughly with tap water and then three times with dH₂O and then return it to its shelf. Media flasks never go through the dishwasher and normally shouldn't be cleaned with soap.

Pouring Plates

A particular growth medium may be available pre-mixed, or you may have to mix the ingredients yourself. If you are using a pre-mixed medium, the bottle (which you will find on the media shelf in SC 224) will tell you the amount to use, in grams per liter. For other media, you will need a recipe, either from the box in SC 222 or (for more unusual media) from a journal article or a book of media ingredients such as the *Difco Manual*. Obviously, you may not need an entire liter, so calculate smaller volumes accordingly. One plate requires about 25 ml of medium, and Petri plates come in sleeves of 20.

Media should always be made in the media flasks on the shelf in SC 224. Other flasks could contain chemical residue which might be harmful to your cells.

1. Obtain a media flask of an appropriate size from the shelf in SC 224.
2. Weigh out the desired amount of media mix or media ingredients.

◇ *Some media mixes include agar, some do not—and sometimes we have both versions of a particular medium on hand. Since you are making solid medium, be sure you're using a mix that contains agar, or that you add agar to your mix. You need 15 g/ml of agar for normal plates.*

3. Pour the powdered media into the flask, then add the desired volume of dH₂O (measured in a media cylinder) from the outlet in SC 224. Don't worry about the volume of the powder.
4. Swirl the flask well to get most of the powder off the bottom.

◇ *You don't need a stir bar and you don't need to worry about dissolving the powder completely: that will happen in the autoclave.*

5. Cover the top with a square of foil. Use two small pieces of autoclave tape to tape the bottom of the foil to the glass.
6. Turn on the 50°C water bath next to the autoclave.
7. Autoclave your medium (see directions below).
8. While the medium is autoclaving, obtain sleeves of sterile Petri dishes from the boxes in the back hall behind SC 222. Lay out as many plates as you need on a benchtop and label them on the bottom (smaller half) with a Sharpie.

◇ *Don't label plates on the top, because the tops could get mixed up. The agar is in the bottom half, so you can't make a mistake.*

◇ *Don't return partially used sleeves of plates to the boxes. No one will want to use them later because they won't know if they've been kept sterile. If you're using less than a full sleeve, tape the bag shut, label it for your own use and put it somewhere appropriate.*

9. After autoclaving, place the flask in the 50°C water bath and allow it to cool down to this temperature. At this point, the agar will still be liquid but it won't be too hot to handle.

◇ *When you put your flask(s) in the water bath, they will raise the temperature above 50 °. You can tell that they've cooled to 50 °C when the bath temperature comes back down to this point.*

10. Swirl the flask thoroughly before use to ensure complete mixing, but try not to create a lot of bubbles.

◇ *Melted agar is heavier than water and will tend to stay on the bottom of the flask. If you don't swirl it well at this point, you'll wind up pouring some plates that are too thick and some that are too runny.*

11. If you need to add antibiotics, amino acids, vitamins or other heat-sensitive components to your medium, do this after autoclaving and after the medium has cooled to 50°C. Don't forget to mix thoroughly after adding any additional components.
12. Open one plate at a time and pour in enough agar that the liquid will just cover the bottom on its own without swirling. This should be about 25 ml. If you look at the plate from the side, there is a line about halfway up the bottom half of the plate; you should have about this much medium in the plate.
13. Let the plates stand until they have hardened completely, usually about 15 min. if your agar was cooled to 50°C when you started.
14. Wash the media flask **immediately** so the agar won't harden in it. Rinse thoroughly three times with tap water and then three times with dH₂O. Use a brush if there is any agar residue remaining in the flask. Return the flask to its shelf in SC 224.
15. **Turn off the 50°C water bath.** If left on, the water evaporates quickly and the heating element can then burn out.
16. Dry your plates by placing them in a 37°C incubator overnight.
17. Put your plates in the plastic sleeve they came in, tape the top shut, label with the type of medium and the date and store the plates in the refrigerator.

Using the Autoclave

The autoclave is like a giant pressure cooker: it uses steam under pressure to raise the temperature to 121°C. After 15 minutes at this temperature, media, glassware or instruments can be considered **sterile**: free of any living organisms, including highly resistant bacterial spores. We usually autoclave for 20 min. to ensure that everything in the autoclave has actually reached 121°C and stayed there for 15 min.; you can increase the time if you have large volumes of media.

1. Check the jacket pressure on the gauge on the lower part of the autoclave. This must read at least 15 psi or the autoclave will not run correctly. If it is lower, either someone recently turned the machine on and you need to wait for it to pressurize, or there is a problem. Consult a faculty member if this happens.
2. Place the materials in the autoclave. Dry items (pipet tips, empty glassware, surgical instruments, etc.) can be placed directly on the wire autoclave racks; liquids must always be placed on a tray.

◇ *If you are autoclaving any liquids, ensure that they are in containers filled no more than half full. When the pressure comes down at the end of the autoclave cycle, over-full containers can boil over and spill or crack the container. If you are using a screw-cap bottle, the cap must be loose: tighten it normally and then back it off ¼ turn.*

3. Be sure there is a piece of autoclave tape somewhere on the materials being autoclaved. The markings on the tape will turn dark when the autoclave reaches the correct temperature. If the tape fails to turn dark, something went wrong and your materials are not sterile.
4. Close the door and turn the wheel to extend the metal flanges to hold it shut under pressure. Keep turning the wheel until it is tight.

5. Choose the desired cycle by pressing the appropriate colored button.

◇ *The **fluid** cycle must be used any time you are autoclaving liquids, even if you're also autoclaving dry materials. In this cycle, the pressure is released slowly after sterilizing, reducing boilovers. If all of your materials are dry, you may choose the **fast** cycle, which releases the pressure rapidly at the end, or the **dry** cycle, which releases the pressure fast and then puts the contents under vacuum for the amount of time you specify to help dry the items.*

6. Set the desired sterilizing time (usually 20 min.) on the dial on the upper right. Set the desired drying time if you are using the dry cycle.
7. Press the **start** button. You should see an immediate increase in pressure on the pressure gauge and a slower increase in temperature.

◇ *If you hear whistling or see steam escaping around the door, the door is not tight enough; tighten it quickly. A "pop" sound as the autoclave starts is normal.*

8. When the cycle is complete, a buzzer will sound. Check that the pressure gauge is at zero, then slowly open the door. A small amount of steam will escape.

◇ *Always double-check the pressure gauge even though you hear the buzzer. It would be extremely hazardous to open the door if there is still pressure in the chamber.*

9. Use autoclave gloves (be sure they're not wet!) to remove your items. Use caution, as they are extremely hot.
10. Clean up any spills from the autoclave trays or the bottom of the autoclave.

Making a Streak Plate

When bacteria are grown on a solid surface, they form **colonies**: groups of literally billions of bacteria (Figure 24). All the cells in a colony are produced by the division of a single original cell that landed in that spot. If two bacteria land close together, their colonies will merge, but if they can be spread out far enough, then you can be confident that one colony represents the descendants of one single cell.

How do we spread the cells out this well? One common way is to use a **streak plate**. When you "**streak**" a plate, you start with a small amount of a concentrated sample and spread it out so that individual cells will land far enough apart to form distinguishable colonies.

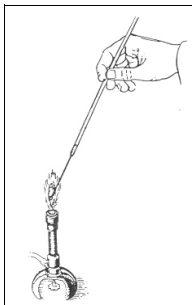


Figure 2. Flaming a loop.

To make a streak plate, you will use one of the microbiologist's favorite tools: a wire **loop**. This is just what it sounds like: a thin wire bent into a loop at one end and inserted into a handle. The wire can be sterilized ("flamed") in a Bunsen burner flame by heating until it is red hot (Figure 25). Because it is a thin wire, it doesn't take long to cool. The loop is convenient for scooping up a bacterial colony from a plate or picking up a loopful of liquid from a broth culture. When flaming a loop, hold it vertically in the flame so that the entire length of



Figure 1. A streak plate. Notice that there are isolated colonies in the last streaked region.

the wire gets red hot. Then, don't touch it to any surface before using it. The steps in making a streak plate are described below and illustrated in Figure 26.

1. Flame the loop red-hot, let it cool in the air for several seconds, then pick up a small amount of the bacterial sample with the loop. Only a very tiny amount is needed: a single loopful of broth or a small amount of a single colony from a plate.

⚠ *If you hear a "hiss" when your loop touches the sample, it's still too hot! This can not only kill the bacteria, but can also create aerosols, sending bacteria into the air to be breathed in by an unsuspecting human or to fall into someone's experiment (maybe your own!). Cool your loop enough that this does not happen!*

2. Gently streak the loop back and forth over a small area of the plate, as shown in part (a) of Figure 26. Use only $\frac{1}{4}$ of the plate or less. Be careful not to gouge into the agar; just run the loop along the surface. Make your streaks close together to spread out the bacteria as far as possible.

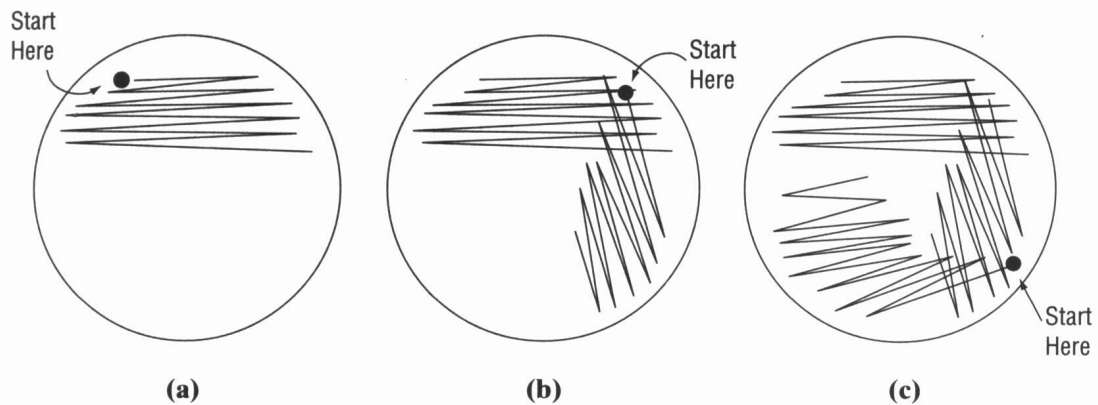


Figure 3. Technique for making a streak plate to obtain isolated colonies.

3. Flame the loop again. From here on, the bacteria that you have already transferred to the plate will be diluted out so that single colonies can form.
4. Rotate the plate a little so you can make a second streak at right angles to the first. For the first two or three sweeps of the loop, cross back into the area of the first streak as shown in part (b) of Figure 26. Then, continue streaking without touching the already streaked area. (You're spreading some of the bacteria from the first area over a larger area.)
5. Flame the loop again. When it cools, make a third streak, as shown in Figure 26 (c). Again, sweep back into area #2 a couple of times, but then continue streaking without touching the previously streaked areas. For this streak, use as much of the remaining untouched part of the plate as possible. This is probably the area in which your isolated colonies will be found.
6. Flame the loop again before putting it away.
7. Label your plate on the bottom (the side with the agar) with a Sharpie. Every plate should have the date, your initials and some kind of a descriptive label written on it. Unlabeled plates found in the incubator will be discarded without warning.

⚠ *Always label plates on the bottom, not the top. Lids can get switched!*

8. Incubate your plate upside-down (agar side up) in an incubator set to the desired growth temperature.

⚠ *Because the agar plate is moist, water will tend to evaporate from it and condense on the lid. Droplets falling from the lid back onto the agar can smear*

bacteria around and spoil your beautiful isolated colonies! By incubating the plate upside down, we avoid this problem. Always incubate your plates upside-down unless specifically instructed otherwise.

Starting a Culture in Broth

It is often convenient to grow bacteria in broth culture, instead of on a plate. The steps for starting a broth culture are described below and illustrated in Fig. 27.

1. Flame your loop, let it cool, and touch it to a colony on a plate that you want to use. Be careful not to touch any other colonies or create aerosols. Alternatively, you can pick up a loopful of an existing broth culture for transfer to a new tube.

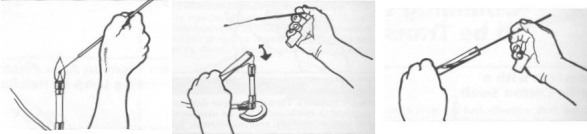


Figure 4. Starting a broth culture.

2. Remove the cap from a broth culture tube and pass the top of the tube quickly through the burner flame to reduce the chance of contamination from the air. (Just a quick pass; don't let it get hot!) Try to hold the cap in your other hand as shown so that you don't have to set it down. (If you are transferring bacteria from one broth tube to another, the best technique is to hold both tubes in your non-dominant hand while manipulating the caps with your other hand. This takes some practice.)
3. Dip the loop carrying the bacteria into the broth. Tip the tube so that you can touch the loop to the liquid without letting the non-sterile handle contact the inside of the tube. Swish the loop around for a few seconds to dislodge the bacteria.
4. Pass the top of the tube quickly through the flame again and replace the cap. The whole procedure should happen fast to minimize the chance of any contamination.
5. Flame the loop again before setting it down.
6. You could also use a micropipettor and a sterile tip to transfer bacteria from one broth tube to another. Remember that only the bottom portion of the tip itself is sterile!