BIOTECH 101 Protocols

Written by Avery Louie

Table of Contents

Procedure for Autoclaving:

materials/equipment:

autoclave

autoclave bags (for waste)

things to be autoclaved

1. Prepare your materials to be autoclaved
   Gather up the things you want to autoclave. If anything is sealed air-tight, loosen the cap or top so that it is not air tight. air-tight containers in the autoclave tend to explode, which can damage the autoclave, destroy whatever you are working on, and possibly injure people. If anything is leaking or dripping, or will melt in the autoclave (like agar plates), put them in an autoclave bag. Cleaning the autoclave is hard, and agar clogs up the autoclave.
2. Prepare the autoclave
   make sure the autoclave has water in it, and that the waste water container is only filled to the low mark. Turn the autoclave power on, and set the temperature and time to 121 C for 15 min. This is the same as setting it to 15 psi for 15 min, because pressure and temperature are linearly related. 15 min at 15 psi is the "standard" cycle used for sterilization. Sometimes, you need to run it for a little longer for large (500 ml +) aliquots of liquid media.
3. Put your stuff in
   Get the basket out of the autoclave, load it with the items to be autoclaved, and then carefully lower it into the chamber.
   NB: To ensure sterility of instruments and parts, make sure they are fully exposed to steam and NOT touching anything else. Double check that your lids are loose.
   Caution: The autoclave is shaped kind of like a top loading washer. Do not drop glass or small, loose objects into the autoclave. The glass can shatter and the items could clog the plumbing.
4. Close the door
   Swing the door shut, and turn the handle until it is very tight (not just until the door seal light

comes on). Attach the magnetic safety to the magnetic safety button on the backslash of the machine.

5. Start the Cycle

   Press the start button. During the cycle, there will be various beeps indicating coming up to temperature and pressure, and when the machine is venting. You want to wait until there are five beeps, because that means the cycle is done. Once the cycle is done, don't do **anything** until the temperature is below 80C and the pressure has dropped to 0. Opening the machine before then is dangerous and can result in steam burns.

6. Remove your stuff

   At this point, your stuff should be sterile. Carefully remove it in a way that keeps it sterile. Don't tear off any wrappers or open any boxes until the item is ready for use. It is generally OK to tighten the lids of bottles now. You probably want to use the orange autoclave gloves to pick your stuff up, because it could still be quite hot.

   NB: if the autoclave gloves get wet, the water forms a heat-conductive path to your hands. they are USELESS if wet. be careful about that, and just wait to pick up your items if they are wet.

7. Shut down the autoclave

   Turn the autoclave off, drain the waste water to the low mark, and swing the door shut and tighten it slightly- this helps to keep stuff from getting into the autoclave and growing there, which smells awful.

Procedure for pouring plates:

materials/equipment:

sterile petri dishes

sterilized solid media

permanent marker

alcohol burner

1. melt your media
   If your media has frozen (become solid) melt it in the microwave or in water bath.
   NB: if you are microwaving your media, be sure to loosen the cap! Or it will explode in the microwave, leaving you with an unpleasant mess to clean up. Microwaving will also leave a "plug" of agar in the center. There is no need to get it all melted! Just melt what you need.
2. Prepare your bench
   Wipe your bench down if it looks dirty. light your burner, and make sure you have easy access to your plates. Don't worry, your media will stay hot for a long time (10-20 min).
3. stack your plates
   This is helpful if you are pouring a lot of plates. Stack 4-5 (depending on how many you can palm) plates with the lids (the big side) facing down, and with the tray (the small part) facing up. This is so you can pick up the top of the bottom plate and all the plates on top of it. This means you can very quickly pour stacks of plates, as you will see.
4. pour your plates
   open your media bottle, and hold it in one hand. with the other hand, pick up the top of the bottom-most unreported plate, and all the plates on top of it. You only need to take the top off far enough so you can pour the media into the tray of the plate. Pour slowly to avoid bubbles. As soon as you are done with the pour, put the lid back on and move to the next plate up in the stack.

NB: you only want a thin layer of media on the bottom of the plate. Too much media is a waste, and an excess of media can cause the top and bottom plates to be bridged by liquid, which is an invitation for contamination.

NB: When you take the lid off your media bottle and place it down on the table, so that the rim of the cap is in contact with the table. This prevents things from falling into the cap.

5. let plates cool

   let the plates cool for a few minutes. They cool quicker if you spread them out or put them on something cold.

6. flame the bottle

   flame the mouth of the bottle before putting the cap back on. At this point, you should put out the burner.

7. Label

   label your plates with the marker. use the format: media name, date, initials. So "LB AGAR 4/3/13 AL" means the lb agar plate was poured on April 3rd, 2013 by 'AL'.Later, when the plate is used, the user will write the bacteria or organism they are growing on the plate.

Procedure for Plating e. coli (or any Bacteria)

materials/equipment:

source of e. coli (plate or liquid culture)

plate to put the e coli onto (target plate)

alcohol burner

inoculating loop

1. set up the bench
   wipe off the bench, light your burner. Put the two plates down next to each other, bottom down.
2. sterilize the loop
   put the end of the loop into the flame until it is red hot. This sterilizes it.
3. pick up the bacteria from the source plate
   Stab the end of the loop into a non-populated part of the source plate, or into the target plate. This cools the loop so that you don't kill the bacteria that you are trying to pick up.
   once the loop is cool, scoop up a tiny bit of bacteria. it doesn't take much!
   NB: Depending on the species and temperature it is grown at, you will have to adjust you plating technique to get single colonies.
4. Streak
   Take your inoculated loop and swipe it back and forth across the target plate. This smears the bacteria across the surface, allowing them to grow!
5. flame your loop and clean up
   Flame your loop again and store your plate somewhere safe. don't forget to put out the burner!

Procedure for transforming e. coli

Procedure for Overnight Culture:

materials/equipment:

sterile 50 ml beaker

shaker

source culture (liquid or solid)

liquid media

alcohol burner

loop or micropipettor and tips

1. prep the bench
   wipe down the bench and light the burner.
2. add media to the beaker
   add media to the beaker. Use a pipette-aid and a serological pipette, or just pour it from the bottle. don't forget to flame the mouth of the bottle before putting the lid back on!
3. Inoculate
   Inoculate the media with either a few ul of your liquid culture, or by scraping some bacteria off the plate with a sterile loop.
4. Incubate
   clean up your workspace and tape the beaker to the shaker. Be sure to cover the beaker with whatever it was sealed with when sterilized (often times, foil is used)

Procedure for Extracting pDNA

Bioline PDF

Procedure for Running a Gel

materials/equipment:

50 ml 1% Agarose in 1X TBE (Or 1X TAE)

50 ml 1X TBE (Or 1X TAE)

Gel box with casting tray, comb

Gel power supply

! WARNING !

The gel electrophoresis power supply can supply voltages up to many hundreds of volts. This much voltage can kill you before the circuit breaker shuts off the power. **ALWAYS** turn the power on to the supply **AFTER** you have everything connected, and shut off the power before you open the lid of the gel box.

1. add DNA dye (Gelgreen)
   Melt the agarose in the microwave. Pour 50ml into a Erlenmeyer flask and add 5 ul of gelgreen. Gently swirl flask to mix.
2. cast the gel
   Put the casting tray into the gel box, with the comb in. Pour the gel into the tray until the comb is a few mm deep in gel. more gel means more reagents, and more time to cool. the pre-stained gel can be saved for later use.
3. let the gel set
   Let the gel set. After it has solidified, you can gently remove the comb and turn the gel so that it faces the correct orientation in the box. Remember, the DNA "runs to red", so you want the red wire to be the one furthest from the wells left by the comb.
4. add samples
   Pre mix dna samples with loading dye. Depending on the dye and the DNA, you may need more or less. pipette up the mix and put it in a well, recording which well gets what sample.

5. add buffer

   carefully add 50 ml of your buffer (TAE or TBE) to the gel box. You do not want to wash away your samples, but it is ok if the buffer runs over the top of the gel.

6. add lid

   The lid is attached to the power cables. push the lid onto the box

7. attach power supply

   attach the red cable to the red jack on the power supply, and the black one to the black jack on the power supply. Turn the supply on (big switch in the back of the supply), and then adjust the voltage and current. To adjust the voltage or current, press set and the voltage or current button. the dial should now change the number, which is the setting.

8. verify power settings

   I like to run mini-gels at 100v and 250mA. once those settings are in the box, flip the power switch from off to on. in a minute or so, you should notice the loading dye migrating into the gel.

9. stop the gel

   at any time you can stop the gel and look at it. after a reasonable amount of time, (an 30 min-hr) you should probably do so. To do this, first turn off the power supply. Then you can disconnect the lid from the box to wipe the condensation off of it. Finally, you should look for bands with a blue LED (look through the top filter).

10. Clean up

    Once you are satisfied with your test (or not), you should clean up. again, turn off the power supply off and disconnect the lid before opening the box. The buffer and gel can go in the sink and trash can respectively, and the box should be rinsed to remove any leftover salt. Let the pieces air dry near the power supply, which can be left plugged in but turned off.