NGMA Plate Pouring Protocol

General Notes:

- 6 cm plates take 12 mL of solution
- 10 cm plates take ~25 mL of solution

1. Prepare media using following recipe.
   NOTE: The dry ingredients for the media can be pre-measured into the plastic bottles labeled "For NGMA Prep" (Bench#1, Shelf D3). Label the bottle with a piece of tape stating: Type of media (e.g. NGMA or HGMA), date of preparation, and your initials.

NGMA Recipe ***(NOTE: If making HGMA2 media, refer to the recipe at the end of this protocol)***

	1 L	2 L
Peptone	2.5 g	5 g
NaCl	3 g	6 g
Agarose	7 g	14 g
Agar	10 g	20 g
Sterile water	975 mL	1950 mL

2. Combine all reagents in a flask that can hold 2X the volume of your media.
   NOTE: If using a pre-measured aliquot of NGMA from a plastic bottle, follow these steps:
   1. Put a plastic funnel in the mouth of the flask
   2. Dump the contents of the bottle into the flask
   3. Pour sterile water into the bottle and swirl to get any powder reagents off the sides
   4. Pour the water from the bottle into the flask, making sure to rinse any powder stuck on the funnel into the flask
   5. Repeat Steps 3 and 4 until you've poured in all the sterile water

3. Tape the foil cap to the flask using three strips of autoclave tape, and label flask with a piece of tape stating: Type of media (e.g. NGMA or HGMA), date of pour, and your initials
   NOTE: you can simply move the tape label from the plastic bottle to the flask, making sure to also write the date of pour on the label

Two out of the three autoclave tape strips.

4. Gently swirl to mix.

5. Autoclave on liquid cycle for 45 minutes (Pancoe Third floor autoclave: cycle #10; Fourth floor autoclave: cycle #9)
   NOTE: If you are autoclaving after hours (before 9 AM and after 5 PM), you can use the Cook 3rd Floor Autoclave Room - make sure to use the big autoclave, Cycle # 2 (Liquid, 45 min).

6. Turn on the water baths in both the lab and the plate pouring room. Make sure the lab water bath is set to 65°C, and the plate pouring room water bath is set to 59°C (the thermometers should read ~63-65°C).

7. Bring the autoclaved media back to the lab. Place flask in 65°C oven or waterbath for a minimum of one hour and a maximum of four hours. Media in the 65°C oven must first be cooled at room temp or in a waterbath to 55°C to use.
   NOTE: As of March 2016, we use 60-65°C water baths rather than a 55°C water bath. We noticed the media was solidifying while in the 55°C water bath, even when the thermometer read 55 - 60°C. The media should now be kept in water baths that read between ~60-65°C on the thermometer, and does not have to be cooled to 55°C to use.

8. After the media has reached 55°C, add the following **IN THE ORDER LISTED** using a plastic, disposable pipette:
   a. Make sure to add the reagents in the correct order! Otherwise they will precipitate.
   b. Gently swirl the flask to mix after adding each reagent

		1 L	**2 L**
1)	1 M KH_2PO_4 (K Phosphate Buffer)	25 mL	50 mL
2)	Cholesterol (5 mg/ml in Ethanol)	1 mL	2 mL
3)	1 M $CaCl_2$	1 mL	2 mL
4)	1 M $MgSO_4$	1 mL	2 mL

9. Bring one flask of media, plates and a nozzle to the plate pourer in Cook 3120 (across the hall from the lab). Leave any remaining flasks of media in the lab water bath until you are ready to pour the plates to avoid solidification of the media.
   a. For 2 L of 6 cm plates, you need about 180-190 plates
   b. For 2 L of 10 cm plates, you need about 80 plates

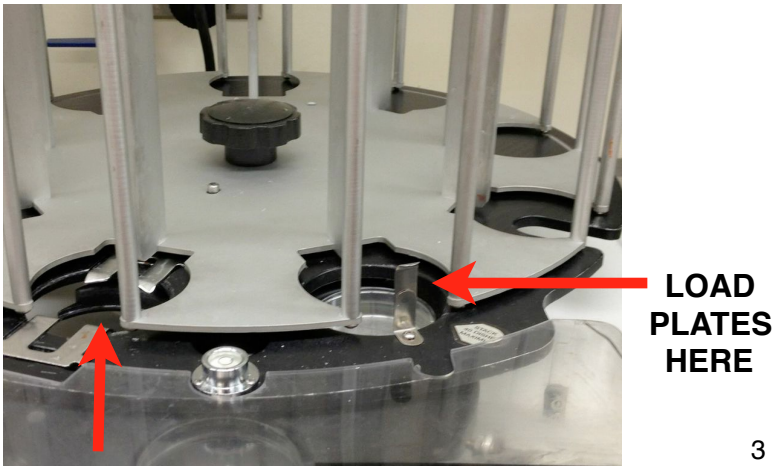
10. Place your flask of media in the water bath in the plate pouring room.

11. If there are plates on the carousel, move them to the foil on the right side of the table and also move the pieces of tape indicating the type of plates and who poured them.
    a. DO NOT move plates if they are not solidified. If the person who poured those plates left an indication of time, make sure it has been 15 minutes since the plates were poured (30 minutes if they are 10 cm plates)
    b. If the plates are still liquid, leave your media in the water bath until you can safely move the plates.

12. Turn on the plate pourer with the white switch on the right side. The press the “carousel” button to rotate the carousel and align it correctly. (note: do not try to turn the carousel manually. Instead, press the carousel button to rotate the carousel).

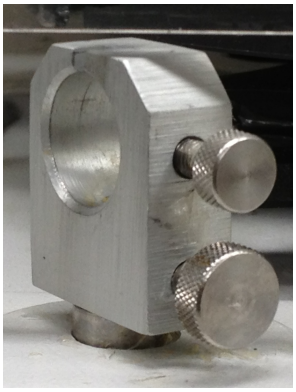
13. Put empty plates on the carousel starting with the column to the right of the column with the “elevator”.
    a. Plates can be loaded by spreading apart the column bars at the *top* of the column.
    b. **Put no more than 40 6cm plates or 25 10cm plates in each column.**
    c. **Make sure to note if there are any empty plates in your first column. If there are, be sure to include this plate in the count of 40.**
    d. Try to check for inverted or cracked plates.
    e. Load as many plates as you can to begin. This will be about three columns worth or about 120 plates. Fill the remaining columns as necessary while the plate pourer is operating. **DO NOT open plate bags until you actually need more plates.**

Elevator from the top down

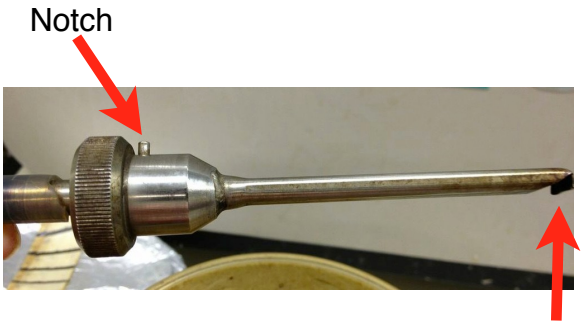


Elevator

14. Put the nozzle into the holder - because of a notch on the nozzle, the nozzle can only go in one direction. Tighten the screws to hold the nozzle in place.
    a. **MAKE SURE TO NOT TOUCH THE TIP OF THE NOZZLE OR ANY TUBING THAT TOUCHES THE MEDIA!**

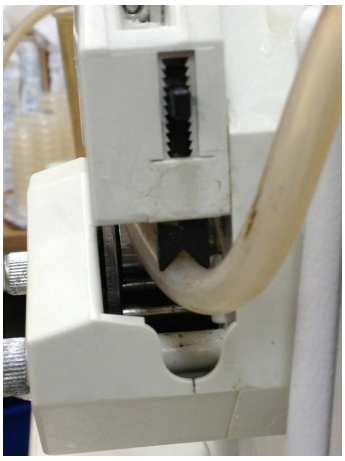


Nozzle holder



15. Feed the tubing through the peristaltic pump and close the clamp for the pump.
    a. Make sure to leave some slack in the tubing between the pump and the nozzle. If you don't have enough slack, the media might not be drawn into the tubing, and you'll see that your plates aren't getting filled with the proper volume. Try increasing the slack to see if this solves the problem.

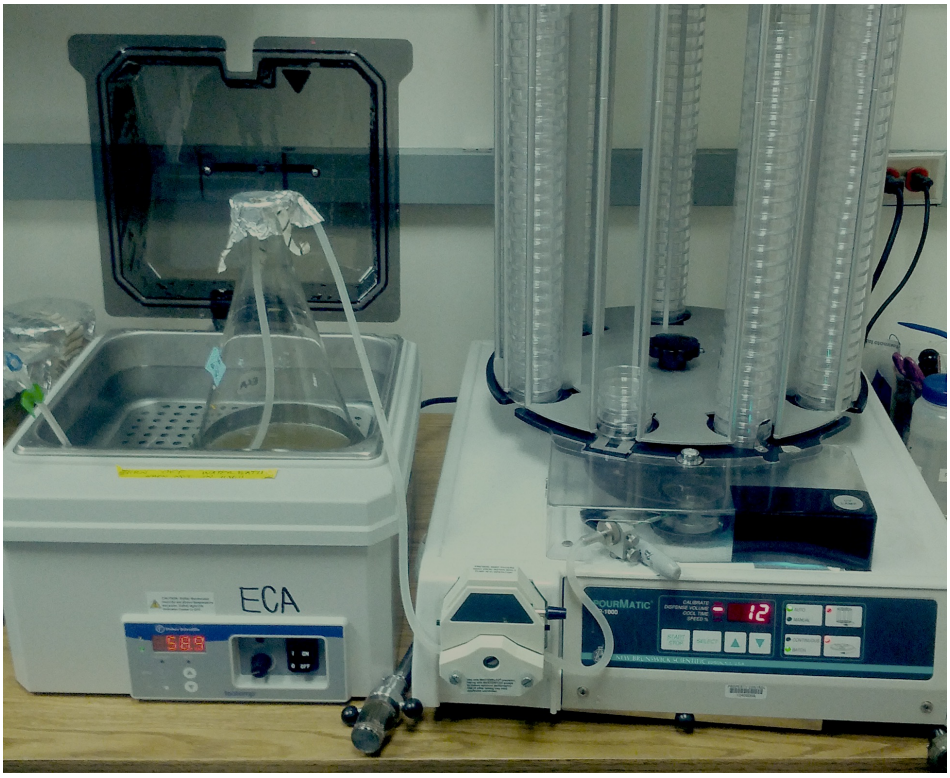
Open peristaltic pump



Tubing threaded through the teeth of the pump

Tubing closed in the pump

16. Place the other end of the tubing in the the flask so that the end is submerged in the media. Keep the foil on the flask.
    a. You will have to pull the water bath forward to the end of the table for the tubing to reach the flask.
    b. Be careful when handling the tubing - remember that it is sterile!



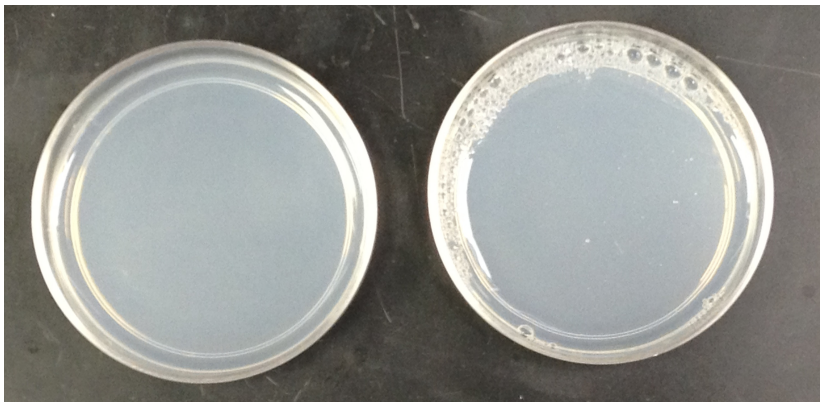
17. Set the dispense volume. Use the select button to move the red line to “Dispense Volume”, and then use the up and down buttons to set the volume.

    • If pouring 6 cm plates, the dispense volume should be set to “12”.
    • If pouring 10 cm plates, the dispense volume should be set to “25”.
    • To change the dispense volume, use the arrow keys.

18. Press “Start/Stop” button to begin pouring.

19. Stay with the pourer as plates begin to fill. Watch out for any inverted plates or plates with cracks.
    a. If a filled plate cracks for any reason, stop the pourer and immediately clean up all media before it solidifies. Clean up using dampened paper towels (above the sink)

20. When there is not much media left, you might have to tip the flask to make sure that media is getting into the tubing. You can take it out of the water bath to make it easier to do this.
    a. If the opening to the tubing is not completely covered by media, air will get into the tubing, which results in bubbles in the plates. **Plates with bubbles are not useable**



GOOD **BAD**

21. You can add more plates if there is enough media. Stop the pourer when there is not enough media.

22. If you have another flask of media, retrieve it from the water bath in the lab and bring it to the plate pouring room. Take the empty flask out of the plate pouring room water bath. Carefully move the tubing from the empty flask into the new flask, so that the end of the tubing is submerged in the media. Move the new flask into the water bath and start pouring.
    a. You can pour up to 4L of media using the same nozzle. This will be a little over one carousel of plates.
    b. Make sure to work quickly so that media doesn't solidify in the tubing while you are preparing the new flask.
    c. Rinse the empty flask out with **HOT** water

23. When there is not enough media to cover the tubing, stop the pourer and disconnect the nozzle. Squeeze the tubing, starting from the nozzle side, to push out any media left in the tube. Bring **both the flask and nozzle/tubing** back to Cook 3117. Rinse using **HOT** water.
    a. Remember to rinse these items immediately as you don't want media to solidify in either.
    b. To rinse the nozzle, hold the the metal tip in the water stream with the opening facing up. You should see water coming out the other end of the tubing.

Rinsing the nozzle

Finished plates!

24. Rinse the nozzle and tubing with distilled water. Also rinse out the beaker that holds the nozzle. Cover the beaker with foil and bring it to the autoclave (dry cycle, 10 min).
    a. To rinse the tubing with distilled water, you can insert the nozzle tubing into the tube coming off the distilled water tap. Gently hold the nozzle tubing in place while you turn on the water (otherwise, the nozzle tubing will just come off when you turn on the water). You should see water come out of the nozzle.
    b. If water doesn't come out of the nozzle, media probably solidified in it. To get the media out, place the nozzle in a 250ml bottle filled with distilled water. Bring the water to a boil, and let the nozzle sit for at least 20 minutes. Periodically try to rinse out the nozzle as described in a. until the solidified media is out and water comes out of the nozzle.

Insert nozzle tubing into distilled H_2O tubing

Hold the nozzle tubing in place while running the water

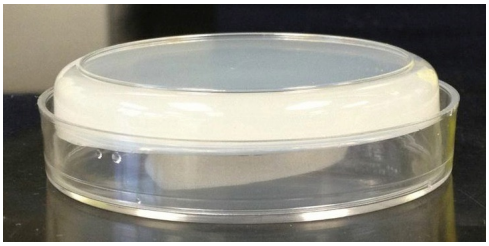
25. Leave a piece of tape in front of the carousel with the following information:
    a. Type of medium in the plates
    b. Date
    c. Your initials
    d. Andersen Lab
    e. Time that the pour finished

26. 15 minutes after your pour finished (or 30 minutes if you poured 10 cm plates), retrieve plates from the pourer using a box or autoclave bin. Put the plates in the box **lid-side up**.

27. Bring the plates back to the lab and stack all plates, **lid-side up**, in the Drying Hood. Allow plates to dry with air flow on: **for 10 cm plates, dry overnight (12-18 hours) in clean hood; if 6 cm plates, dry 48 hours (2 overnights) in hood** *(Refer to the posted sign in the hood for Drying Details)***.**
    a. Be sure to label the stacks of plates with the label you made for the flasks

28. After the plates have dried for the appropriate amount of time, clean a plastic storage box by rinsing with a 10% bleach solution. Wipe down all sides of the box until it is dry. Then repeat with distilled water.
    a. Wear a lab coat to protect your clothes from bleach!

29. Stack all plates into the cleaned plastic storage box, lid-side down. Move the labeled tape (with the type of plates, date and your initials) onto the box. If you cannot fit all the plates in one box, put them in a box labeled “mixed plates”.



Lid-side down plates

~314 6 cm plates fit in a large box

30. Store the box of plates in the 4°C room.

Troubleshooting

Elevator Problems

1. If the elevator is stuck, attempt to push it back down using the long metal rod with the black knob that is next to the plate pourer. By pressing down with slow, steady pressure, you should be able to make the elevator go back down.

2. If the elevator is stuck and cannot be manually pushed down, remove all plates and take the carousel off. Remove the large and small rotating platforms(reference protocol for changing the carousel). Then find and turn a small wheel in the bottom left of the plate pouring machine.

Wheel inside plate pouring machine

Plate Quality Issues

2. If the plates look precipitated after drying, the problem was most likely that one of the water baths was not set to a high enough temperature. Set the lab water bath to 65 degrees and set the plate pouring room water bath to the highest temperature that it can go. It usually does not exceed 50 degrees.

Recipes

HGMA2 Recipe

If making HGMA2, u*se this recipe for STEP 1, then proceed through the next steps exactly as above*.

	1 L	2 L
Peptone	20 g	40 g
NaCl	3 g	6 g
Agarose	20 g	40 g
Sterile water	975 mL	1950 mL