Plate Pouring

C. elegans are generally maintained on plates containing a soft agar media called nematode growth media (NGM). NGM contains agar, along with several salts, peptone, and cholesterol. We use a simplified NGM formula called NGM Lite in most of our laboratory applications. This comes pre-mixed with everything that is needed except for cholesterol. Other additives, such as drugs (i.e. dopamine, levamisole, etc.) or compounds (i.e. IPTG for RNAi), can be added to the agar during the pouring process. The media is mixed, autoclaved, and poured in liquid form into 3, 6, or 10 cm plates depending on the specific needs of the experiment. After drying for 1-2 days, the NGM plates are seeded with a bacterial food source - typically the *E. coli* strain OP50. After another 1-2 days of drying, worms can be maintained on the plates.

Standard Maintenance Plates

1. Ensure that the autoclave machine in SCA 210 is not currently being used. If it is not in use, turn on the machine by pushing the green button. Fill in your name on the clipboard, along with the date and time. *NOTE: It will take about 10-15 minutes for the autoclave to start up, so make sure to turn it on before preparing your media.*
2. Measure out **14.75 grams of NGM Lite powder** using a weigh boat and scale.
3. Add the powder to a **1L-size Erlenmeyer flask**.
4. Add **500 mL of distilled water** from the white tap next to the sink at the south of the lab. Swirl to mix the agar.
5. Place aluminum foil over the mouth of the flask and add a piece of autoclave tape.
6. Put the flask in a glass baking sheet, and slide it into the autoclave.
7. Perform a **30 minute liquid cycle** by selecting the appropriate protocol and pushing 'Enter'.
8. Push the green rectangular 'Start' button in the lower right hand corner to begin the cycle. You should hear the door lock and see the cycle begin on the screen. The process should take between 1.5 - 2 hours to complete.
9. Prepare the lab bench by cleaning it with 70% ethanol. Light a small ethanol lamp or burner to maintain sterile conditions. Acquire approximately 50 sterile petri dishes and assemble stacks of 5 plates each.
10. After the autocycle cycle is finished, collect the flask containing 500 mL of molten NGM.

11. Swirl the molten NGM and ensure that it is cooled to approximately 65° by running it under cold water.
12. Add **500 uL of 5 mg/mL cholesterol** stock solution with a micropipette directly to the NGM using sterile technique. Replace the foil on the mouth of the flask.
13. Add any other additives that may be necessary for your experiment, i.e. antibiotics, IPTG, dopamine, etc.
14. Swirl the flask for about 30 seconds to ensure the components are well-mixed.
15. Remove the foil from the flask and flame the mouth.
16. Starting from the lowest dish in a stack of 5, remove the lid and all other dishes to reveal the petri dish. Pour in the liquid NGM mixture until it reaches about halfway up the dish.
17. Replace the lid (and the other dishes in the stack), then move up to the next lid in the stack and remove it (along with the other dishes above it. Pour in the NGM and repeat the process until all plates in the stack have been filled with NGM.
18. Push the stack aside and avoid moving or touching the plate until the NGM has fully solidified.
19. Sterilize the mouth of the flask after pouring each stack, and repeat steps 16-18 for the remaining plates.
20. Once you pour out all of the NGM media from the flask, quickly wash it with hot water and a drop of liquid soap before the agar solidifies.

Dopamine Plates

Plates containing dopamine are used to determine locomotion and adaptation phenotypes in *C. elegans* (Chase et al. 2004). Dopamine is a monoamine neurotransmitter that is water-soluble and comes in powder form. Unfortunately, some of the components in NGM cause a spontaneous breakdown of dopamine into melanin, a dark pigment that no longer exhibits neurotransmitter effects. In order to stabilize the dopamine, we must pour plates containing standard agar supplemented with acetic acid, which helps stop the breakdown of dopamine. The overall procedure for pouring dopamine plates follows that described above, with a few modifications:

1. Pour **smaller batches** of plates (100 mL volume, 10-12 plates) that are used within 5-7 days.
2. Mix the agar at 1.7% by adding **1.7 grams to 100 mL of distilled water** in a 500 mL Erlenmeyer flask.

3. Microwave the mixture instead of autoclaving. Ensure that the agar crystals are fully solubilized by heating in short 20-30 sec bursts.
4. After cooling the agar to ~65 deg C, add **200 uL of 1 M glacial acetic acid**.
5. Add the **dopamine powder** to the desired concentration.
6. Swirl the flask to ensure the powder is mixed evenly.
7. Pour plates as described above.

There are two major types of dopamine plates that are used in the lab: test plates and adaptation plates. The **test plates** contain 30 mM dopamine, which is a concentration that produces almost 100% paralysis in wild type worms. These are used to test locomotion and are NOT seeded with OP50 bacteria. They are typically poured in the small 3 cm dishes.

Adaptation plates contain a sub-paralyzing dose of 10 mM dopamine, and are used to culture worms for 1-2 days to induce the adaptation response. These should be used in parallel with control plates that lack dopamine. All adaptation plates (dopamine and control) are seeded with OP50 bacteria, and are typically poured in medium (6 cm) dishes.

Commonly Used Amounts and Supplements

Plate Type	Volume	Agar	Cholesterol	Acetic Acid	Dopamine
Maintenance	500 mL	14.75 g NGM-Lite	500 uL	none	none
Dopamine Test Plates	100 mL	1.7 g agar	none	200 uL	30 mM (0.6 g)
Dopamine Adaptation Plates	100 mL	1.7 g agar	none	200 uL	10 mM (0.2 g)
Control Adaptation Plates	100 mL	1.7 g agar	none	200 uL	none