Protocol: Nematode Growth Medium (NGM)

Adapted from Wormbook: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html

C. elegans are maintained in the laboratory on Nematode Growth Medium (NGM) agar which has been aseptically poured into petri plates (Brenner, 1974). These plates contain essential nutrients that the nematodes require to survive (including cholesterol, they are one of the few animals that cannot produce this on their own!) The NMG plates are "seeded" with bacteria, which is the preferred food source.

Several sizes of petri dishes are available. We use 60 mm dishes for our aging experiments and 100 mm dishes for maintaining stocks or growing up large numbers of worms for other experiments. **60mm plates hold 10ml of NGM agar, while 100mm dishes hold 25ml of NGM agar.**

Note: When autoclaving liquid, the rule of thumb is to make sure the final volume of your liquid is no more than ½ the max volume of the container (e.g., no more than 500 mL of liquid in a 1 L Pyrex bottle.)

1. To an autoclavable bottle, add:

Reagent	Amount per 500 mL final volume	Amount per 250 mL final volume
NaCl	1.5 g	0.75 g
Bacto Agar	8.5 g	4.25 g
Bacto Peptone	1.25 g	0.625 g
diH_2O	486 ml	243 ml

   a. Weigh out and add dry reagents to autoclavable Pyrex bottle.
   b. Using a graduated cylinder, measure out the appropriate volume of diH_2O.
   c. Add diH_2O to the dry reagents and swirl.
2. Autoclave the mixture **(You MUST get trained on how to use the autoclave before doing this!)**
   a. Make sure the screw-caps are **LOOSLEY** screwed on, allowing gas to escape the bottle.
   b. Place a piece of autoclave tape across the cap, making sure one end is taped to the bottle.
   c. Autoclave for 25 minutes (use "SLOW EXHAUST" setting for liquids).
3. Set the water bath to 55°C.
4. When the autoclave is done place the bottle in the 55°C water bath to cool. When the NGM has cooled to 55°C (bottle should be hot but not too hot to touch) add the following reagents:

Reagent	Amount per 500 mL final volume	Amount per 250 mL final volume
1M $CaCl_2$	500 µl	250 µl
5 mg/ml Cholesterol	500 µl	250 µl
1M $MgSO_4$	500 µl	250 µl
1M KPO_4 buffer	12.5 ml	6.25 ml
100 mg/ml Carbenicillin	500 µl	250 µl

The recipe for the 1M KPO_4 buffer is as follows:

Reagent	Amount per 250 mL final volume	Amount per 100 mL final volume
KH_2PO_4	27.08 g	10.83 g
K_2HPO_4	8.9 g	3.56 g
diH_2O	175 ml	75 ml
5M NaOH	dropwise to pH 6.0	dropwise to pH 6.0
diH_2O	to 250 ml	to 100 ml

NOTE: Stocks of calcium chloride, magnesium sulfate, and 1M KPO_4 buffer should all be filter sterilized. Use sterile technique when adding the buffers to the cooled NGM agar.

NOTE: FOR LIFE SPAN PLATES, include the following two additions:

a) FUDR prevents egg development. (**CAUTION: FUDR is toxic. Please handle carefully!**)
b) *****If RNAi bacteria is used***** IPTG to induce bacterial RNAi expression

Reagent	Amount per 500ml final volume	Amount per 250ml final volume
150 mM FUDR	165 µl	82.5 µl
1M IPTG	500 µl	250 µl

5. Swirl the media cooled to 55C well to mix the additives, all the while trying to avoid creating too many bubbles.
6. Add Sharpie stripes to the sides of each dish, following the color codes above for various additives (**BLUE** = Carbenicillin; **RED** = FUDR; **GREEN** = IPTG).
7. Using sterile technique, pipette either 10 mL of NGM into each 60 mm plate, or 25 mL of NGM into each 100 mm plate. Allow the plates to cool on the bench top.

Seeding NGM Plates with Bacteria

After plates are poured, start cultures of bacteria for food. Two kids of bacterial food serve different purposes: OP50 for maintenance and stock plates and RNAi clones for genetic screening. The OP50 bacteria is concentrated 10-fold, and OP50 plates are UV-treated (Stratalinker). RNAi bacteria is neither concentrated nor UV-treated.

FOR OP50:

1. For every 100 mL of NGM, we need 10 mL of OP50 culture. For example, if you are making 500 mL of NGM, we need 50 mL of OP50 bacteria. Using sterile technique, add the appropriate volume of LB media to an appropriately sized Erlenmeyer flask (**NOTE: the flask should be 5X larger than the volume of LB it is to hold in order to ensure proper aeration.**)

2. Place aluminum foil over the Erlenmeyer flask and affix a small piece of autoclave tape over the top. Autoclave for 25 minutes using "SLOW EXHAUST" settings.
3. Once the autoclave has finished the exhaust cycle, allow the flask and media to cool completely to room temperature.
4. Using a pipette tip, acquire a small amount of OP50 bacteria from the frozen stock kept in the -80°C freezer. Dab the tip of the pipette into the media in the flask, being careful not to touch the shaft of the pipettor to the flask.
5. Grow the culture overnight at 37°C in the orbital shaker/incubator at 200 RPM.
6. The following day (or 24 hours later), aliquot 50 ml of your cells grown to saturation into a suitable number of 50 ml conical tubes.
7. Spin the cells down in the swinging bucket centrifuge at max speed (or 3,000 x g) for 10 minutes.
8. Using a serological pipette, remove and dispose of 45 ml of the culture medium, being careful not to disturb your cell pellet.
9. Resuspend your cell pellets well, and then combine all of your 10X concentrated cells into a single conical tube.

To each NGM plate, add the following volume of bacteria to the CENTER of the NGM medium.

	Amount per 100 mm plate	**Amount per 60 mm plate**
10X Concentrated OP50	250 μl	100 μl

10. Carefully, but vigorously, swirl the dish in order to spread out the bacteria in the center of the dish. It is important that the bacteria do not reach the edge of the plastic petri dish, as this seems to encourage worms to climb up the plastic dish and dehydrate.

11. Allow the bacteria to dry on the plate at room temperature overnight. The following day expose the plates to UV light to arrest the growth of the bacteria. Using the Strategene Stratalinker:
    - Place plates in the Stratalinker. Remove the lid and place the lid upside-down in the Stratalinker next to the NGM dish.
    - Close the door and turn on the Stratalinker
    - Press 'Energy'
    - Enter '9999' using the keypad
    - Press 'Start'
    - The plates will be exposed to UV for approximately 5 minutes

Plates with UV-killed bacteria can be stored upside-down at 4°C for up to 1 month.

FOR RNAi CLONES:

- Grow bacteria cultures in the appropriate volume of LB with carbenicillin for 18 hours to select for the RNAi clone. Depending on your needs, 5ml per clone is enough in a disposable 15ml culture tube.
- Inoculate each tube with the appropriate bacterial clone. Set the shaker incubator to 37°C and 200 RPM and incubate 18 hours (or overnight).
- RNAi bacteria is ***NOT CONCENTRATED***. Instead, pipette 100μl of the unconcentrated bacteria straight from the overnight culture onto 60mm plates (or 250μl onto a 100mm plate).
- RNAi life span plates are ***NOT UV'd!!*** Store them at 4°C until use.

****Cheat sheet for NGM additives****

- Stock maintenance plates contain carbenicillin only. 
- Timed egg-laying (TEL) plates contain carbenicillin and IPTG. 
- Life span plates contain carbenicillin, IPTG, and FUDR.