Making normal NGM

Bonnie Evans[1]

[1]Imperial College London

Jun 14, 2021

1 Works for me Share dx.doi.org/10.17504/protocols.io.bvh7n39n

Behavioural Genomics

Bonnie Evans

ABSTRACT

C. elegans is maintained in the laboratory on Nematode Growth Medium (NGM) agar which has been aseptically poured into petri plates using a peristaltic pump. NGM (rather than no peptone NGM) may also be used for imaging plates when screening bacterial strains.

DOI

dx.doi.org/10.17504/protocols.io.bvh7n39n

PROTOCOL CITATION

Bonnie Evans 2021. Making normal NGM. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvh7n39n

CREATED

Jun 03, 2021

LAST MODIFIED

Jun 14, 2021

PROTOCOL INTEGER ID

50463

MATERIALS TEXT

Pre-autoclave	For 1L:	For 500mL:
Sodium chloride	3g	1.5g
BioAgar	17g	8.5g
Bacto Peptone	2.5g	1.25g
Sterile water	975mL	487.5mL

A	B	C
Post-autoclave	Fo 1L:	For: 500mL
5mg/mL cholesterol (store at 4°C and away from light)	1mL	500uL
1M CaCl2	1mL	500uL
1M MgSO4	1mL	500uL
1M KPO4 (see protocol)	25mL	12.5mL

Pre-autoclave

1 Book the autoclave via the notebook on top of the machine. It will take around 2 hours for a 500ml bottle and 2.5 hours for 1L or larger bottles.

2 Take clean bottles and measuring cylinders from the glass kitchen - you will also need one bottle for the autoclave probe, which should be the size of the largest bottle you are autoclaving. Get sterile water from the media room.

3 Measure out all the pre-autoclave reagents and add to the bottle. Use a new weighing boat and spatula for each reagent. Add water last and mix thoroughly.

4 Add equivalent amount of water to the empty probe bottle.

5 Make sure the lid of your media bottle is not screwed completely when placing it inside the autoclave machine. Label with autoclave tape (‘NGM agar Rm 5020’), and place over the lid to prevent it coming off in the autoclave.

Using the autoclave

6 Turn ON the autoclave and touch the screen

7 If 'Fill with water' is on the screen, fill up the autoclave with sterile water until it reaches the grill/'Water Level OK' appears on the screen.

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n

8 Place the probe bottle inside the autoclave with the temperature probe inside.

9 Place the bottles in the autoclave and make sure that the cap is not screwed completely.

10 Check the waste flask is not too full

11 Close the door

12 (On the screen) LOCK --> START --> MEDIA 121X15 BALLAST --> Start

Post-autoclave

13 When autoclave is finished, UNLOCK the door

14 Remove the probe and media bottles using gloves (the bottles will be hot)

15 Leave the agar on a lab bench to cool to around 55°C, ie the bottle is cool enough to hold for a second with a gloved hand

16 Add the post autoclave reagents

17 Try not to shake the bottle too much while mixing to avoid air bubbles.

18 The agar needs to be warm to be poured without blocking the tubings, so try to pour as quickly as possible and if not poured immediately put the bottle on a waterbath set to 60C until being used.

Pouring Petri dishes

19 For pouring maintenance/imaging Petri dishes:

Plate Pouring
by Priota Islam,
Imperial College London

PREVIEW RUN

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n

19.1
- Set the flow rate to 2 in the hood
- Turn on the lights and Plug switches
- Take the Pump and plug it in
- Set up the pump for the desired volume:
  a) Setup -> Options -> Volume (15ml for medium plates) -> Accept
  b) Setup -> Next -> Tube -> 8mm (Usually 8mm for big and medium plates and 3mm for imaging plates)
- Place the tubing around the pump making sure both ends are inserted into the agar bottle (Make sure the tubing is placed clockwise as the pump moves that way)
- Prime the tubes by: Setup-> Prime -> Accept (Leave it on for few mins till the tubes get warm), Press Accept to stop Prime

19.2
- Take plates in stack of 5/10 (as per convenience)
- Place the nozzle on top of the centre of the plate and press the foot pedal letting the agar to flow
- If some agar drops on the surface wipe it off with a paper towel after it solidifies
- When taking a pause of more than 1min put the pump back to prime
- When one bottle finishes, place the nozzle in the new bottle and prime to suck out the residual agar to prevent wastage (Wash the empty bottle and get some hot water in it)
- Put both openings into the new bottle and prime again

19.3
- After pouring, wash the tubing (Prime) by passing the hot water from the clean bottle to the other one (to remove any agar in the tube)
- Followed by priming air into the tubing making sure no liquid is remaining
- Discard the agar in the agar container and wash the bottles and put them in the grey box in the lab
- Close all switches in the hood
- Return the pump
- Wrap and label the tube 2x in aluminium foil and take it to the media kitchen to put in the autoclave basket (Collect the tube the following day)
- Order agar in the media kitchen for the next pouring day (i.e. Every other Monday)
- Let the plates solidify under the hood about an hour and then store it in the cold room

Pouring multi-well plates

20 For pouring multi-well imaging plates:

Dispensing agar into multiwell plates
by Saul Moore
PREVIEW RUN

20.1 Prepare a 250ml bottle of hot milliQ water in the microwave and keep in the waterbath along with the agar. The water is important to have on hand in case of tubing blockages.

20.2 Insert large cassette into the machine

20.3 Configure X, Y, and Z settings for the multiwell plate by clicking on tool symbol -> stage alignment.

For UNIPLATE96SQWLF 650U:

X = 95.6

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n

Y = 4.2
Z = -22.5

For 48WP:

20.3.1 Put the plate into the stage and then press 'Move' so that the plate moves so that it is under the dispensing cassette.

20.3.2 Use the up and down arrows to move the pipette tip so that they hover just over the plate and make note of the height (this will be entered into the dispensing program at a later step). Press 'Fast/Slow' button to switch between fast and slow movements.

20.3.3 Use the X, Y arrows to move the plate so that the pipette tips are centered in the middle of column 5.

20.3.4 Save all settings.

20.4 Exit settings by pressing the back button

20.5 Press on the program you wish to use (see later for configuring your own program)

20.6 Make sure that the correct cassette is listed and change if necessary

20.7 Select the volume you wish to dispense

For 96WP:
200 µL

20.8 Select 'set height' and set the appropriate height for tip height (usually all the same)

20.9 Place the end of the tubing from the casettee into the agar that is being kept warm in the water bath

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n

20.10 Press 'Prime' to prime the tubing and allow to finish so that agar flows from the pipette tips.

IMPORTANT:
Once the agar is in the tubing it is important to act quickly to avoid agar solidifying and causing blockages. If you are particularly concerned about agar cooling in the tubing, wrap the tubing in aluminium foil to keep hot.

Step 20.10 includes a Step case.
Unblocking the tubing

step case

Unblocking the tubing

If the tubing does block, clear the blockage by 'reverse priming' as much of the agar as possible.

Then place tube ends in the hot water and prime continuously with hot water until the water runs all the way through.

If you are having trouble getting the water through, squeeze and massage parts of the tubing where you can see blockages to force the agar along and allow the water to pass.

Once all cleared, 'reverse prime', and reprime with the agar

20.11 Place a clean plate in the stage

20.12 Press run and then plate should fill with agar

20.13 Repeat steps 11-12 until all the plates have been filled.

Little drops of agar can solidify on the tip ends. It is often good to remove these drops using a pipette tip every few runs so that blockages do not occur.

20.14 'Reverse prime' all the agar

20.15 Place the tubing ends into the hot water.

20.16 Prime so that the water runs through and clears all the agar

20.17 Reverse prime to remove the water

20.18 Release tension from the tubing and remove cassette

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n

10.19 Double wrap the cassette in aluminium foil for autoclaving

Citation: Bonnie Evans (06/14/2021). Making normal NGM. https://dx.doi.org/10.17504/protocols.io.bvh7n39n