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Making normal NGM for imaging plates (Cabreiro Lab)

Forked from Making low peptone NGM for imaging plates

Ida Barlow[1], Priota Islam[1], Saul Moore[1]

[1]Imperial College London

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Behavioural Genomics

Saul Moore

SUBMIT TO PLOS ONE

ABSTRACT

C. elegans is maintained in the laboratory on Nematode Growth Medium (NGM) agar which has been aseptically poured into petri plates. The NGM agar medium can be poured into petri plates easily and aseptically using a peristaltic pump. This pump can be adjusted so that a constant amount of NGM agar is dispensed into each petri plate. A constant amount of agar in the plates reduces the need for refocusing the microscope when you switch from one plate to another.

The imaging plates can be 35mm, 60mm or 90mm in diameter depending on the assay design.

PROTOCOL CITATION

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FORK NOTE

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Forked from Making low peptone NGM for imaging plates, Ida Barlow

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GUIDELINES

The quality of the imaging plates are crucial in producing good images that can later be analysed. It is very important to makesure there aren't any air bubbles trapped on the plates.

Also, since most imaging is done using 35mm diameter plates that uses a very thin 3mm tubing for pouring, extra care should be taken to not let the NGM cool too much and block the tubing.

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MATERIALS TEXT

Reagents:

For 500mL:

(A) Pre-autoclave:

- **1.5 g Sodium Chloride (Sigma-Aldrich-71376-1KG)**
- **8.5 g Bio Agar, Biogene-400-050**
- **1.25 g Difco Bactopeptone, BD-211820**
- **0.5 mL Cholesterol (5mg/ml in EtOH), Sigma-C1145-250MG [store at 4°C away from light]**
- **482.5 mL Sterile Water**

(B) Post-autoclave:

- **0.5 mL 1M CaCl2, Sigma-C3881-1KG**
- **0.5 mL 1M MgSO4, Fisher-M/1050/53**
- **12.5 mL 1M KPO4 (pH 6.0) [see solution protocol for how to make]**

1M KPO_4 pH6.0
by Ida Barlow

PREVIEW RUN

BEFORE STARTING

If the volume intended is more than 1000ml, it is better to prepare in more than one bottle.

Pre-Autoclave:

1
- Book the autoclave (notebook on top of the machine).
- Take clean flasks from the glass kitchen.
- Measure all the pre-autoclave reagents and add to the flask (Use a new weighing boat and spatula for each reagent. Also, the cholesterol is kept in the fridge.)
- Once water is added mix thoroughly and label with autoclave tape ('NGM Rm 5020'). Make sure the bottle is not screwed completely when placing it inside the autoclave machine.

Using the autoclave:

2
- Turn ON the autoclave
- Make sure that the autoclave's probe bottle is the same size as the largest bottle you use and fill it with water.
- Place the temperature probe in it.
- Fill up the autoclave with water until it reaches the grill.
- Place the bottles in the autoclave and make sure that the cap is not screwed completely.
- Check the waste flask is not too full
- Use 'media' program.
- Press START.
- It will take about 2 hours for a 500ml bottle to autoclave and about 2.5 hours for 1L or larger bottles.

Post autoclave:

3
- When autoclave is complete, remove the probe flask
- Make sure to wear gloves as the flask will be hot
- Let the agar to cool to around 55°C, ie the bottle is cool enough to hold for a second with a gloved hand.
- Add the post autoclave reagents.
- Mix it well and start pouring onto imaging plates following the Protocol for *Plate Pouring:*

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

3.1

- Sterilize the hood with Ethanol
- Count the number of plates to be poured and stack them inside the hood (A stack of 10 or 5 depending on convenience)
- Volumes to be poured for different sized plates are:
  Large plates 90mm: 45 mL – possible with 8mm tubing
  Medium plates 60mm: 15 mL – possible with 8mm tubing
  Small plates 35mm: 3.5 mL – possible with 3mm tubing
- 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 parameters:
  a) Setup -> Options ->Next -> Volume (15ml for medium plates) -> Accept
  b) Setup -> Options -> Next -> Tube -> 8mm (Usually 8mm for big and medium plates and 3mm for imaging plates)
  c) Setup -> Options -> Next -> Direction (Clockwise is preferred)
  d) Setup -> Options -> Next -> Profile (This is the mode of the flow and preferrably set to Slow-Slow-Slow)
- 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. Priming is very important to make the tubes warm and to avoid the agar being solidified.
- Be careful not to touch the ends of the tube.

3.2

- Place the nozzle perpendicluarly on top of the centre of the plate and press the foot pedal letting the agar to flow
- If some agar spills 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 into the next bottle to prevent wastage (Wash the empty bottle and get some hot water in it)
- Put both openings into the new bottle and prime again
- When you reach the end of the second/last bottle stop pouring and discard the residual agar in the waste bucket

3.3

- After pouring, wash the tubing (Prime) by passing 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 any agar in the waste bucket, wash the bottles and put them in the grey box in the lab
- Close all switches in the hood
- Return the pump
- Clean the hood for the next user
- Wrap and label the tube 2x in aluminium foil, label it with tube size and lab room number (eg 8mm Tubing R5020) 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, label them with the date poured and then store them in the cold room upside down
- On the same day of the pouring, measure the weights of 3 random poured plates with lids on from the stack and add to the plate weight data file in the shared folder

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