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Making low peptone NGM for imaging plates

Forked from Making low peptone NGM for imaging plates

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

Behavioural Genomics

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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.

For imaging purposes, low peptone NGM is prepared which contains less concentration of bactopeptone as compared to the normal NGM. This is because we don't want the seeded bacteria to grow too much and obscure the images, rather just sufficient enough to keep the worms from crawling out of the plate due to lack of food. The imaging plates can be 35mm, 60mm or 90mm in diameter depending on the assay design.

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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.

MATERIALS TEXT

Reagents:

For 500ml

A) Pre-Autoclave

- Sodium Chloride- 1.5g, Sigma- Aldrich-71376-1KG
- Bio Agar- 10g, Biogene- 400-050
- Difco Bactopeptone- 0.065g, BD- 211820
- Cholesterol (5mg/ml in EtOH)- 0.5ml, Sigma- C1145-250MG [Cholesterol should be stored at 4C away from light]
- Sterile water- 482.5ml

B) Post-Autoclave

- 1M $CaCl_2$- 0.5ml, Sigma- C3881-1KG
- 1M $MgSO_4$- 0.5ml, Fisher- M/1050/53
- 1M KPO_4 (pH 6.0)- 12.5ml (see solution protocol for how to make)

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For imaging purposes, low peptone NGM is prepared which contains less concentration of bactopeptone as compared to the normal NGM. This is because we don't want the seeded bacteria to grow too much and obscure the images, rather just sufficient enough to keep the worms from crawling out of the plate due to lack of food. The imaging plates can be 35mm, 60mm or 90mm in diameter depending on the assay design.

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 ('Low peptone NGM Rm 5020'). Makesure 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.

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- 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 (See Protocol for plate pouring)
- 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.

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