Jan 14, 2020

Making LB NGM Plates

DOI
dx.doi.org/10.17504/protocols.io.bbbgiijw

Priota Islam[1]

[1]Imperial College London

Behavioural Genomics

Priota Islam
Imperial College London

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bbbgiijw

Protocol Citation: Priota Islam 2020. Making LB NGM Plates. **protocols.io https://dx.doi.org/10.17504/protocols.io.bbbgiijw**

Protocol status: Working
We use this protocol and it's working

Created: January 14, 2020

Last Modified: October 21, 2020

Protocol Integer ID: 31816

Abstract

C. elegans is maintained in the laboratory on Nematode Growth Medium (NGM) agar which has been aseptically poured into petri plates. Sometimes they can also be grown on LB-NGM, where the LB in the media will promote better growth of the bacteria the worms feed on.
The LB-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 LB-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.

Materials

Reagents:
For 1000ml
A) Pre-Autoclave
Sodium Chloride- 3g, Sigma- Aldrich-71376-1KG
Bio Agar- 17g, Biogene- 400-050
LB Broth Powder- 2.5g, Fisher BioReagents- BP- 1426-2
Cholesterol (5mg/ml in EtOH)- 1ml, Sigma- C1145-250MG [Cholesterol should be stored at 4C away from light]
Sterile water- 975ml

B) Post-Autoclave
1M Cacl2- 1ml, Sigma- C3881-1KG
1M MgSO4- 1ml, Fisher- M/1050/53
1M K2HPO4 (pH 6.0)- 25ml, Sigma-Aldrich- P0662-500G-M

Pre-Autoclave:

1 Book the autoclave (notebook on top of the machine).

2 Take clean flasks from the glass kitchen (Only the ones with autoclave tape on are sterile)

3 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.)

4 Once water is added mix thoroughly and label with autoclave tape ('LB NGM Rm 5020'). Makesure the bottle is not screwed completely when placing it inside the autoclave machine.

Using the Autoclave:

5 Turn ON the autoclave

6 Make sure that the autoclave's probe bottle is the same size as the largest bottle you use and fill it with water.

7 Place the temperature probe in it.

8 Fill up the autoclave with water until it reaches the grill.

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 Use 'media' program.

12 Press START.

13 It will take about 2.5 hours for 1L or larger bottles.

Post-Autoclave:

14 When autoclave is complete, remove the probe flask

15 Make sure to wear gloves as the flask will be hot

16 Let the agar to cool to around 55°C, ie the bottle is cool enough to hold for a second with a gloved hand.

17 Add the post autoclave reagents.

18 Mix it well and start pouring onto desired sized plates (See Protocol for plate pouring)

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

20 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.