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Plate Pouring V.2

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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. Smaller plates (35 mm diameter) are useful for mating or when using expensive drugs. Medium size plates (60 mm diameter) are useful for general strain maintenance, and larger plates (100 mm diameter) are useful for growing larger quantities of worms, such as for certain mutant screens. 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.

Reference: http://www.wormbook.org/

MATERIALS TEXT

Nematode Growth Medium (NGM) agar- Molten with temperature between 55C-70C

Equipments:
1. Hood- Hera Guard Heraeus, Serial no. 1932
2. Peristaltic pump- Jencons Permatic GP
3. Tubings- 8mm for 60mm and 90mm diameter plates, 3mm for 35mm imaging plates

Preparation of the Hood

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- 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.

Actual Pouring

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

Post Pouring

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- 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