Drug Behaviour Imaging on Phenix

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1 Works for me

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ABSTRACT

For imaging drug-treated young adult C. elegans in liquids using the Multiworm tracker. Worms are synchronised by picking L4s, and then the young adults are exposed to drugs for 4 hours prior to imaging for 15 mins in a liquid droplet on a coverslip mounted on a 3.5cm plate.

ATTACHMENTS

liquid imaging protocol_Ida.docx

MATERIALS

NAME	CATALOG #	VENDOR
25mm round coverslip	MIC3380	Scientific Laboratory Supplies Ltd

Preparing wroms (-5 days)

1

Bleach synchronisation of C. elegans
by Ida Barlow

PREVIEW RUN

1.1 Wash hermaphrodites off plate with several ml of M9 solution and transfer to 15ml falcon tube (Fisher Scientific-Falcon 352096)

1.2 Fill falcon tube up to 15ml with M9 solution

1.3 Centrifuge for 2 minutes at 1500 rpm (RCF:210, ascending 9; descending 7) – program 1

Program 1 retains the worms as pellets and the bacteria is suspended as the supernatant

The descending is slow as the worm pellet is lose at this stage which we don't want to break

1.4 Remove supernatant using a plastic Pasteur pipette taking care not to disturb pellet
Leave atleast 0.5ml M9 to avoid disturbing the pellet

1.5 Fill the tube with M9 upto 15ml

1.6 Spin program 1

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1.7 *Repeat steps 4-6*

1.8 On final wash remove as much supernatant as possible and add M9 upto 4ml

1.9 Add 4ml 2X Bleach solution (From here onwards try to work as quickly as possible to avoid over-exposure of the worms to the bleach)

USE FRESHLY PREPARED BLEACH EVERYTIME

2X Bleach solution:

5% Sodium hypochlorite solution - 4ml
Sterile water - 3.5 ml
1M NaOH solution - 2.5 ml
TOTAL - 10 ml

1.10 Vortex on maximum setting for 4 min (no more as this will damage the eggs)

Makesure the vortex forms

After vortexing, top up the tube with M9 till 15ml

1.11 Centrifuge for 2 mins at 2500rpm (RCF:590, ascending 9; descending 7) – program 2

(Always check the program on the centrifuge before using it)

1.12 Remove supernatant by pouring into waste bottle – pellet should be compact and yellow in colour at bottom of falcon, but be careful not to lose

1.13 Add 15ml M9

1.14 Centrifuge at program 2

1.15 Repeat steps 12-14 four more times

The number of washes is crucial here as we need to get rid of all the bleach

1.16 After final wash add 15ml M9 and store eggs/larvae in the falcon on the rotator that is constantly spinning at 20^{O}C, until feeding

L1 arrested larvae can be starved for up to 5 days before refeeding

1.17 Centrifuge larvae on program 2 to pellet

1.18 Remove supernatant with plastic Pasteur pipette

The pellet is lose here so makesure not to disturb it

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1.19 Add 15ml M9, spin to wash

1.20 On final wash leave 0.5ml M9 in falcon

1.21 Resuspend the pellet by gently tapping the tube/flicking it

1.22 Place droplet containing larvae onto seeded plate and allow to grow to desired developmental state (ie. 2 days for L4s, 2.5 days for young adults)

Use glass pipette to place the droplet onto seeded plate, avoid using plastic pipette as larvae will stick to it

Development times at 20^{o}C:

- 2 days for L4s
- 2.5 days for young adults

Note:

- If you feed larvae within 12hrs of bleaching then they develop faster than the longer arrested ones
- It is a good practice to bleach in two tubes in parallel
- If you drop the tube at any point of the process, makesure to transfer the contents into a new tube as the dropped tube may get cracked resulting in loss of worms during centrifugation/vortexing
- Any unused larvae can be topped up with M9 and stored spinning in the rotator to be re-used
- Use clean autoclaved rubber bulbs for the refeeding everytime to avoid contamination
- Put the used bulb in the box labelled 'Used Teets'

Stages	Grown at 20 C from L1	Grown at 25 C from L1
L1 division	11.7hrs	9hrs
Mid L1	16.9hrs	13hrs
First L2 division	22.1hrs	17hrs
Between L2 divisions	23.4hrs	18hrs
Second L2 divisions	24.3hrs	19hrs
Mid L2	29.9hrs	23hrs
L3 division	32.5hrs	25hrs
Mid L3	37.7hrs	29hrs
L4 division	42.9hrs	33hrs
Mid L4	49.4hrs	38hrs
Early adult	55.9hrs	43hrs
Adult	62.4hrs	48hrs

Table of Development times for different temperatures

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Preparing Behaviour plates (-3 day)

2

Making low peptone NGM for imaging plates
by Priota Islam,
Imperial College London

PREVIEW RUN

2.1
- 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.

2.2
- 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.

2.3
- 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.

Refeed worms (-2.5 day)

3 Refeed L1s to get young adults on the day of tracking

Making up the drugs (-1 Days)

4 First identify the drugs to be used in the study and ensure that they are correctly labelled and handled.

5 Calculate the desired weight and volume of solvent required for the desired concentration if using powdered compounds. Alternatively calculate the desired dilution for liquid compounds.

Important point – Ensure that your calculations are 1000 fold higher at this stage as your compound will be diluted when it is added to the plate. E.g. mM for uM active concentration

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6 Inside a fume hood with an analytical balance weigh out the desired amount of compound into an Eppendorf tube labelled with the compound and the final concentration.

7 While still in the fume hood add the desired amount of solvent (eg DMSO) to get the right compound concentration, and close the lid of the Eppendorf tube

8 Finally, to ensure the compound is dissolved fully, vortex the Eppendorf tubes (ensuring the lid is firmly in place) at setting 10 for 30 seconds.

NOTE: Some compounds require longer vortexing times (especially at higher concentrations) to ensure they completely dissolve. In these instances, repeat until fully dissolved.

Making drug plates (-1 Days)

9 Select the appropriate number of fresh unseeded 35mm NGM plates (no older than 3 days) and inspect to ensure an even level of agar.

NOTES: If plates are uneven the images can appear blurry as the different height can result in out of focus patched. Different level of agar indicates that there is a different volume and so the resultant concentration of drug will be affected

10 Label the side of each plate with the compound name, compound concentration and the date.

See the attached spreadsheet as a template for planning the imaging throughout the day **Phenix_template.xlsx**

11 Inside a flow hood apply 50ul of sterile water to the centre of the agar (this is to help the drug spread over the agar surface where the OP50 will be applied)

12 Before this dries apply 3.5ul of your compound solution (ensuring the correct compound and concentration is used).

13 Allow to dry in the flow hood with the lids off (this should take around 30 minutes).

14 Once dry seed the plates with 50ul OP50 into the centre of each drug plate from the fridge

NOTES:
Take a falcon tube of OP50 from the fridge and vortex on setting 10 until all the pellet has dissolved and not visible clumps remain in the solution.
Dilute the OP50 1:10 in M9 solution

Normally when doing this stage, you might have many plates to do, in these instances the ones that were drugged first will probably dry before you have finished. Therefore, check for plates that have dried and replace the lids to ensure that there is no overdrying.

15 Leave the plates overnight at room temperature in the dark.

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Drug exposure (Day 0, AM)

16 First identify and label plates in accordance with the experimental Excel template.

1. The plates will now be labelled with the set number and the rig position as well as half having a black dot annotation.

17 For each set pick 10 worms from your synchronised plates onto each of the drug plates. This should be completed within 10 minutes for each set to ensure equivalence.

18 After 3h 30m has elapsed transfer 10 worms to the drugged imaging plates

Leave to accimate o the new plate for 30 mins

Imaging (Day 0, PM)

19 Image for 15 minutes at 25fps with the .hdf5 file format.

20 Transfer the files to the server.

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