Drug Assay on *C. elegans*

Reagents Needed:

M9
0.5M alkaline hypochlorite solution
S-basal medium complete
2.4X drug solution(s) to be tested
cholesterol (5 mg/mL in EtOH)
100X Penicillin/Streptomycin
100X Nystatin (10,000 units/mL)

Procedure:

I. Synchronizing Worms

Bleaching worms allows synchronizing worms because only the eggs are resistant to the bleach. Once the eggs hatch development will be stalled at the L1 stage because there is no food.

1. Wash a plate full of eggs and adults with eggs with 5 ml of M9.
2. Transfer the worms and eggs into a 15 mL Falcon tube and spin for 1 minute at max speed.
3. Remove supernatant and either:
    a. Resuspend pellet in 1 mL of M9 and transfer it in an Eppendorf tube, spin again, remove supernatant and add 1 mL of bleach solution.
    b. Add 20 ml of bleach solution directly to the falcon tube.
4. Mix the tube gently, vortexing occasionally, for a maximum of 5 minutes.
5. Spin tube at maximum speed for 1 minute.
6. Remove supernatant, being careful not to disturb the pellet.
7. Wash either 10 times with 1 ml of M9 or 3 times with 40 mL of M9 depending on the bleaching procedure used.
8. Let the L1s hatch overnight on a nutator.

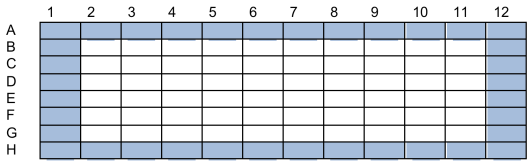
II. Set-Up of 96-Well Plate

To test the effects of a drug on synchronized L1 worms living in liquid on a 96-well plate and eating dead bacteria.

1. Pellet a 50 mL tube of fresh OP50 bacteria, remove the supernatant, and freeze the pellet at -80°C.
2. Take 10 mL of freshly prepared S-medium complete and add:
    - 8 µL Cholesterol
    - 50 µL 100X Penicillin/Streptomycin
    - 100 µL 100X Nystatin
3. Resuspend the frozen bacterial pellet with about 4 mL of S-media complete with antibiotics and cholesterol.
4. Dilute the bacteria 1:1000 with S-medium complete and measure the absorbance using a spectrophometer. Dilute the sample until you obtain an OD600 between 0.5 and 0.6.

* NOTE: Make sure the resuspended bacteria reach the room temperature because lower temperature affects worm development.

5. Put a tape on the top and left rows of a clear, flat-bottomed 96-well plate. You will not use these rows and you can indicate on it the strains and the compounds at various concentrations you are testing.
6. Add ddH_2O to the wells on the edges of the plate to avoid the plate drying over time:



7. Put the bacteria and S-medium complete preparation in a trough.
8. Using an automatic pipette, transfer 25 µL of this food preparation into all the wells where you are testing drugs.
9. Add 25 µL of 2.4X Drug and control solutions to the appropriate wells.
10. Remove the tube with L1s from the nutator and calculate the density of L1 worms. Pipet 10 µL of the worms and M9 onto a plate and count the worms using a dissecting microscope. You want between 15-20 worms per 10 µL. If you do not have the correct density, dilute or spin down the worms and add less volume of solution.
11. Add 10 µL of the L1 worm solution (of the correct density) to the corresponding wells of the 96-well plate.
12. Put the 96-well plate in a plastic box, secure the plate to the bottom of the box with tape, and transfer to a shaker (170-180 rpm) of the appropriate temperature for the suitable length of time.
    * NOTE: The temperature of the room affects the growth of the worms. The temperature should be around 20°C. If lower, worms will grow slower and vice-versa. Obviously the circumstances are different for temperature sensitive worm strains.

III. <u>Counting Aggregates</u>

Example: For Q35 worms, counting aggregates begins on day 2 and ends on day 6.

1. On the appropriate day, transfer the 60 µL of worms per well onto a plate and let the liquid soak into the agar.
2. Count aggregates using a fluorescence microscope.
    * NOTE: *Do not* count worms that are 'bagging' (i.e. contain larvae) or are dead/noticeably sick.
3. Count at least 25 worms per condition tested to be able to do statistical analysis.

Recipes:

M9 (1L)
* Common lab stock in worm room.
5.8g $Na_2HPO_4•7H_2O$
3.0g KH_2PO_4
5.0g NaCl
0.25g $MgSO_4•7H_2O$
ddH_2O to 1L
• Filter (0.22μm) and bottle.

0.5M Bleach Solution (50mL)
10mL Bleach (non-germicidal bleach)
15mL ddH_2O
25mL 1M NaOH

S-Basal Medium (1L)
5.85g NaCl
1.0g K_2HPO_4
6.0g KH_2PO_4
1mL cholesterol (5 mg/mL in ethanol)
ddH_2O to 1L
• Bottle and autoclave.

S-Basal Medium Complete (~100mL)
To 100mL of S-Basal Medium add:
300μL 1M $MgSO_4$
300μL 1M $CaCl_2$
1mL 100X trace metal solution
1mL 1M potassium citrate (pH 6)
• Use sterile technique. Do not autoclave.

Trace Metals Solution (500mL)
0.346g $FeSO_4•7H_2O$
0.93g Na_2EDTA
0.098g $MnCl_2•4H_2O$
0.012g $CuSO_4•5H_2O$
ddH_2O to 500mL
• Bottle and autoclave.
• Store in the dark.

References:

Voisine C, Varma H, Walker N, Bates EA, Stockwell BR, Hart AC. Identification of potential therapeutic drugs for huntington's disease using Caenorhabditis elegans. PLoS ONE. 2007 Jun 6; 2(6):e504.

Garcia SM, Casanueva MO, Silva MC, Amaral MD, Morimoto RI. Neuronal signaling modulates protein homeostasis in Caenorhabditis elegans post-synaptic muscle cells. Genes Dev. 2007 Nov 15; 21(22):3006-16.

Modified by Barbara Calamini, 2008.