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Drug treatment in Zebrabox

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ABSTRACT

How to do a standard Rihel lab behaviour tracking experiment with drug treatment.

Design your experiment

1 Figure out if the drug you will use has good solubility in water.

Ideal case is to find a publication which has used the drug in zebrafish larvae. Alternatively, you will often be able to find the solubility on PubChem.

The present protocol will assume the drug is soluble in water.

2 Decide which concentration(s) of the drug you will use.

Again, ideal case is to find publications which have used the drug in zebrafish larvae.

If it's any help, all of Rihel et al., 2010 drug screen was done at 10–30 µM.

The number of concentrations you choose may also be limited by the number of larvae you have available. Try to have at least N = 12 larvae for each concentration.

You typically want to pick concentrations which are separated by half-log steps (i.e. each concentration is 5× the previous one) or log steps (i.e. each concentration is 10× the previous one). The logic is that a typical dose-response curve (where X is Log(dose) and Y is the response) looks like a sigmoid. In an ideal case, you probably want (at least): one concentration at the end of the initial plateau / one concentration in the middle of the sharp increase / one concentration on the top plateau.

Say you have enough larvae to do 5 concentrations (including the 0 µM control);
Some examples of half-log steps:

- 0, 1, 5, 25, 125 µM
- 0, 0.3, 1.5, 7.5, 37.5 µM
- 0, 0.1, 0.5, 2.5, 12.5 µM

Some examples of log steps:

- 0, 1, 10, 100, 1000 µM
- 0, 0.3, 3, 30, 300 µM
- 0, 0.1, 1, 10, 100 µM

Prepare the drug stocks

3 We will now prepare stocks which are 500× the concentrations you chose.

For example, if we chose 0.1, 1, 10, 100 µM, we will prepare stocks which are 50 µM, 500 µM, 5000 µM (= 5 mM), 50000 µM (= 50 mM).

If you have the drug in solid form, find the molecular weight (in g/mol) online and calculate the mass you need to dilute in e.g. 2 mL (can use a 2 mL Eppendorf) to obtain the highest stock concentration (50 mM in the example). If this mass is so low it is difficult to weigh precisely, you can also dissolve the drug in e.g. 10 mL (in a small Falcon).

Vortex well and make sure all the drug is fully dissolved.

For example, pyrazinamide has a molecular weight of 123.11 g/mol (source: PubChem).

Therefore, to obtain a 50 mM stock, we need to dissolve 0.0123 g in 2 mL dH2O.

4 Once you have the most concentrated stock, serially dilute it to to obtain ~ 1 mL of the lower ones.

For example, we have a 50 mM stock of pyrazinamide, so we can obtain a 5 mM stock as:

- 50 mM stock 100 µL
- dH2O 900 µL

etc.

Plate the larvae

5 Use a standard Whatman square well plate.
Cut the very tip of a P1000 tip to make it wider.
Set the P1000 pipet to 650 µL.

6 Clean the Petri dish of larvae to remove any debris and replace the water.

7 Use the P1000 pipet with the cut-off tip to catch larvae and transfer each in a set volume of 650 µL.

8 Set up the plate in the Zebrabox.
Bring the drug stocks, a P10 (P2.5) pipet and tips.
Set up the experiment on Zebralab.

9 Add 1.3 µL on top of each well.
This will dilute the stock 500×, e.g. 50 mM will be diluted to 100 µM in the well.

Replace tip between concentrations, but no need to replace tip for every well (the little fish water you will bring into the drug stock is negligible).

10 Start the tracking and close the Zebrabox.

Top-up the water each morning with fish water, *without* drug.

Note, this is making the following assumptions:

- the drug is stable in fish water at 25–28˚C for a few days.
- only water evaporates, the drug remains in the well.

Even if these assumptions are correct, keep in mind that the drug concentration will fluctuate over the course of the experiment. The overall trend will be a decrease in drug concentration as it is being metabolised by the larva. During a single day/night, the concentration will *increase* as water evaporates until top-up.