

Introduction to small molecule screens in the zebrafish

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Lab overview

For this lab, you will conduct parts or all of three separate small molecule screens:

1. Developmental screen. In this experiment, you will screen through a small chemical library to identify small molecules that disrupt specific aspects of development. Wild-type zebrafish embryos and standard stereomicroscopy will be used. Each student will work independently and conduct all steps of the screen, including embryo handling, small molecule pin transfer, and phenotype assessment.

2. Behavioral screen – known compounds. In this experiment, you will screen through a small collection of previously-characterized, behavior-modifying small molecules to familiarize yourself with a range of zebrafish behaviors and the acute effects of small molecules on them.

3. Behavioral screen – novel compounds (optional). In this experiment, you will have the opportunity to participate in a behavioral screen using novel small molecules. Those who participate will seek to identify novel sedatives and stimulants and are likely to experience the excitement (and frustrations) of real small molecule screens. This experiment is optional.

Procedures

Developmental Screen

After the pit talk, you will begin setting up your developmental screen as follows:

Fill a 96-well plate with embryo buffer using a multi-channel pipettor, adding 200uL to each well. For screens that will involve use of a dissecting microscope (like this one), it is important to use round bottom 96-well plates because the round bottoms keep the embryos near the center of the well, where they can be visualized easily from above. We are using unbuffered E3, but buffered media and media with PTU can also be used.

Question: What impact might the composition of the medium (buffered or not, salt composition, etc.) have on the screen outcome?

Array embryos into the wells of the plate, 3 embryos per well, using eggs that were laid that morning. Leave columns 1 and 12 empty. To achieve this, draw 50-100 healthy embryos into a pasteur pipette using a manual pipette pump and let the embryos settle to the tip of the pipette. Then, gently touch the pipette tip

on the surface of the water in a well. There should be no need to expel any water from the pipette—surface tension should simply pull 1-2 embryos out of the pipette and into the well. It is important to keep the volume of solution in the wells constant at 200 μ L, so don't force water out of the pipette. If embryos become stuck in the pipette, gentle tapping or drawing in should remedy the situation. Repeat until all wells in columns 2-11 have 3 embryos in each.

Pin transfer the library into the 96-well plate containing embryos. The library is preformatted in 96-well plates. The small molecules are dissolved in DMSO at stock concentrations of 10 mM, sealed with foil seals, and frozen at -80 C. The library plate should be thawed completely in a desiccator and spun down in a plate spinner before removing the foil. A clean, 96-pin transfer device is inserted into the plate until the pins touch the bottoms of the wells. The device is then raised slowly out of the wells without allowing the pins to touch the sides of the wells. The device is then inserted into the plate containing zebrafish, taking some care to ensure that embryos are not crushed in the process. Hold the pins in the zebrafish medium for about 30 seconds to give the DMSO time to release from the pins. Then, remove the pin transfer device from the plate and clean. To clean the pins, rinse first in a DMSO bath, then in an ethanol bath, and finally, flame the pins BRIEFLY to remove excess ethanol. **Once you have flamed the pins, be certain that you do not pass the pin transfer device over the ethanol bath—it can easily catch fire!** Set the pin transfer device down on its side to cool before its next use, being careful not to bend any of the pins.

Questions: Why use DMSO as solvent? What happens to the DMSO and the small molecules once they are transferred to the water? What factors might influence the dose(s) you choose for performing a screen?

Cover your plate of embryos with a lid or another plate and place it in an incubator at 28 degrees C.

After you finish setting up your developmental screen, you should spend the rest of the afternoon conducting the behavioral screens. On the following day, you will screen through your developmental screen plate to identify the phenotypes that have emerged. Using a dissecting microscope, screen through your plate looking for any consistent developmental defects. Pay attention to as many organs and structures as you are comfortable with, including but not limited to the brain, skin, heart, blood, eye, notochord, ear, kidney, and general body plan. Make note of any observations on your scoring sheet, and if you find any phenotypes of note, identify the responsible chemical structures from the database. Remember to pace yourself, and try to discriminate between phenotypes that are small molecule induced (typically consistent among all embryos in a well) and those that may have other causes.

Questions: What phenotypes were easiest/most difficult to detect? How can you discriminate between specific and non-specific effects? Between primary and

secondary effects? How do small molecule-induced perturbations in development differ from those induced by genetic mutation? Did you notice any similarity between phenotypes induced by different small molecules? How did the structures of the small molecules compare? What value might a small molecule that disrupts development have? What could you do to identify the target of one of these small molecules?

Behavioral Screens

For the behavioral screens, you will fill a 96-well plate with embryos just as you did for the developmental screen except that you will use older embryos (~30 hpf). If you only plan to screen known compounds, you will only need to array embryos into column 1 of your plate. If you plan to screen both known and novel compounds, you will need to fill columns 1-11.

Treating the embryos for the behavioral screens will be done with a multichannel pipette, rather than the pin transfer device. You should use the small volume pipette to transfer 1 uL of the known compounds into column 1 of your plate, making sure to preserve the orientation of the master plate.

If you are performing a screen of novel compounds, select one of the library plates and make a note of which plate you chose. This will be essential for determining the identity of any hits you might find. While wearing gloves, carefully unseal the plate by holding the plate firmly on the benchtop and carefully peeling off the foil label. **Pulling the label off too quickly or failing to hold the plate down firmly will cause the compounds to “bounce” out of the wells.** Then, use the small volume pipette to transfer 1 uL of the library compounds into columns 2-11 of your plate, making sure to preserve the orientation of the library plate.

Mix the treated wells by gently pipetting up and down in the wells using the larger volume multichannel pipette.

After allowing your plate to settle for a few minutes, begin your behavioral observations by watching the embryos under your dissecting microscope. First, watch for changes in the spontaneous motion of the animals, noting wells with increased, decreased, or qualitatively different spontaneous motion. After observing spontaneous motion, test the animals' touch response by gently probing them through the chorion using a plastic pipette tip. Again, watch for increased, decreased, or qualitatively different responses to the touch stimulus, making note of any well that exhibits altered behaviors.

Questions: What does the rapid onset of the behavioral phenotypes tell you about their mechanisms of action? What seems to be more common – sedating or stimulating compounds? How can you distinguish between a compound that is sedating and one that makes the embryos sick? What human benefit might you imagine could come from behavioral screens like this one?