Video Article

Large Scale Zebrafish-Based *In vivo* Small Molecule Screen

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URL: http://www.jove.com/details.php?id=2243
DOI: 10.3791/2243
Citation: Hao J., Williams C.H., Webb M.E., Hong C.C. (2010). Large Scale Zebrafish-Based In vivo Small Molecule Screen. JoVE. 46. http://www.jove.com/details.php?id=2243, doi: 10.3791/2243

Abstract

Given their small embryo size, rapid development, transparency, fecundity, and numerous molecular, morphological and physiological similarities to mammals, zebrafish has emerged as a powerful *in vivo* platform for phenotype-based drug screens and chemical genetic analysis. Here, we demonstrate a simple, practical method for large-scale screening of small molecules using zebrafish embryos.

Protocol

1) Zebrafish Egg Collection

1. On the afternoon prior to the day of the chemical screen, set up 10 to 20 zebrafish breeding tanks. Fill each tank with water from the aquaculture system. Using a fish net, transfer one adult male and one to two adult females to inner container in each breeding tank. Separate the male and female fish from each other with a divider. Label the cages and put a lid over them.
2. On the morning of the screen, remove the dividers from breeding tanks and allow zebrafish to mate. Over the course of next 1 hour, allow fertilized eggs to fall through grid at the bottom of each inner container.
3. After 1 hour, return adult zebrafish back to permanent storage tanks, remove the inner container and collect the eggs by straining the water in each breeding tank through a plastic tea strainer.
4. Invert the strainer over a Petri dish and rinse the strainer gently to flush the eggs into the Petri dish by using a wash bottle containing the E3 medium.
5. All unfertilized eggs, which appear opaque, should be removed using a disposable plastic pipette. Each mating cross should yield approximately 200 embryos.

2) Arraying Embryos in 96-well Plates

1. Transfer about 5 embryos in E3 medium into each well of a 96-well plate by using a glass Pasteur pipette.
2. Once embryos are arrayed onto the 96-well plate, remove as much of the E3 medium as possible out of the wells using a 12-channel (30 - 300 μL) pipette, taking care not to puncture the embryo. Using the 12-channel pipette, deliver 250 μL of E3 medium containing 0.5μg/ml kanamycin to each well as quickly as possible so as not to allow embryos to dry up.
3. Put the 96-well plates into 28.5°C incubator until they reach the desired stage when the compounds to be added.

3) Transfer of Small Molecule Library

While compound transfer can be automated with robotic transfer methods, we will describe the manual transfer method.

1. Small molecule libraries are typically supplied in a 96-well format, with each compound stored in DMSO as a 10 mM stock. About 60 minutes before the embryos reach the stage when the compounds are to be added, thaw a desired number of 96-well plates containing aliquots of small molecules (source plate). Take note of the serial or other identification number of the source plates. To minimize condensation on the plates, thawing can occur in a desiccation chamber containing Drierite (W.A. HAMMOND DRIERITE CO, Xenia, OH).
2. Briefly spin down the plates in a tabletop centrifuge equipped with multi-well plate adaptor.
3. Remove the aluminum sealing tape from source plate. Using a 12-channel pipette, dilute the compounds in the source plate to the concentration of 0.5 mM (for example, if starting with 250 nL aliquots of 10mM stock, add 4.75 μL of DMSO to each well).
4. When the embryos in the 96-well plate (recipient plate) reach the desired stage, use a 12-channel (2-20 μL) pipette to transfer 2.5μL of compounds (0.5mM) from the source plates into the recipient plates containing the embryos.
5. Record the identification number of the source plates on the recipient embryo plates. Cover the recipient plates now containing the embryos and compounds with lids, gently mix the plates by gently swirling, and place them in a 28.5°C incubator.
6. Cover each source plate containing unused small molecules (0.5 mM) with an aluminum sealing tape and place in a -80°C freezer for long-term storage.

4) Screening for Effects of Small Molecules by Visual Inspection of Phenotypes

1. Prior to performing the screen, formulate a specific criterion for what would constitute a "hit".
2. At desired times in development, remove the 96-well plates containing compound-treated embryos from incubator and examine each well under a stereomicroscope. For better visualization of subtle changes, such as changes in circulatory pattern, a phase-contrast inverted

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microscope can be used. Fluorescent microscopy can be used to examine perturbation of expression of GFP or DsRed proteins under a tissue-specific promoter.
3. Quickly scan the 96-well plates for any well in which at least 3 out of 5 embryos exhibit the prescribed "hit" phenotype. Record the identity of the plate and the well location of each potential hit.
4. Reconfirm a potential hit by retesting the effects of the compound at several doses (1 uM, 5 uM, 10 μM and 50 μM). For each dose, 10 embryos are tested in 0.5 mL of E3 media in a 48-well plate format. The timing of compound addition for retesting should be identical to that of the original screening. A hit is confirmed when the elicited phenotype is reproduced on retesting of the compound
5. Identify the hit compound from the database of small molecules in the chemical library.

Discussion

When planning a zebrafish-based chemical screen, particular attention must be paid to the robustness of the phenotype under consideration and the background rate of such phenotype. This is particularly important for screens for chemical suppressors of an induced phenotype. For example, for a phenotype caused by heat-shock induction of a transgene, the condition that induces the phenotype reproducibly must be precisely mapped out prior to initiating the screen to avoid unacceptably high false positive rates. With careful planning, zebrafish chemical screens, which require modest capital investment, can lead to the discovery of novel modulator of cell signaling and physiology, as well as lead compound to reverse models of human diseases. Lastly, by analogy to the classic forward genetic screens, zebrafish-based chemical screens can be valuable for chemical genetic dissection of fundamental biological process.

Disclosures

No conflicts of interest declared.

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