University of Oregon
Zebrafish International Resource Center

TITLE: Embryo Surface Sanitation

Purpose: To minimize the risk of pathogen transmission within a fish population.

Supplies:

- Spray bottle of 70% alcohol (EtOH) or disinfectant solution
- Mesh baskets or strainers (see Fig. 1)
- 5 containers to hold solutions (see Fig. 2)
- Pipettes (for moving eggs/embryos)
- 3-display timer
- Petri dishes
- Squirt bottle containing 0.5X E2 embryo media

Stock Solutions:

0.5X Embryo Media (E2) w/ Methylene Blue:
http://zebrafish.org/zirc/documents/protocols/pdf/Fish_Nursery/E2_solution.pdf

Sodium Hypochlorite (5.65-6%): Fisher Scientific, Catalog # SS290-1

Sodium Thiosulfate Solution: 1 g sodium thiosulfate into 2 L 0.5X E2 embryo medium

Pronase *Stock* (Concentrate) Solution: 30 mg protease (from *Streptomyces griseus*, Sigma P-6911) into 1 ml 0.5X E2 embryo medium.

- To prepare a 1-2 month supply, add 0.60 grams of protease powder into 20ml nanopure water. Stir VERY gently by hand until completely dissolved. Keep solution on ice throughout preparation and aliquot process.
- For storage, aliquot 0.68 ml of pronase stock solution into 1.5 ml eppendorf tubes, avoiding the creation of bubbles during pipetting, and place into freezer storage at -62˚C (-80˚F) for long term or -28˚C (-20˚F) for short term.

Working Solutions:

Load and Rinse Solution (Containers 1 and 5): 0.5X Embryo Media (E2) w/ Methylene Blue

Bleach Solution (Containers 2): 0.59 ml sodium hypochlorite (chemical grade bleach) into 1 L RO water (make fresh daily and do not use expired bleach)

Sodium Thiosulfate Solution (Container 3): Use stock solution listed above

Pronase Solution in Embryo Media (Container 4): 0.68 ml pronase stock solution into 500 ml embryo media (make fresh daily or each time embryos need to be bleached and use within 2 hours of preparation)

Note: All bottles should be changed at least once per week. The pronase bottle should be changed daily.

Procedure:

1. If needed, acclimate your embryos and solutions to approximately 28.5°C prior to bleaching. Ideally, embryos should be bleached between 24 and 30hpf, and should not be bleached if they are beginning to hatch.

2. Sanitize the counter with 70% alcohol (EtOH) or disinfectant.

3. Set up containers with the following solutions (recipes above and see Fig. 2):
    a. **Container 1 (Load)**: Embryo media with methylene blue
    b. **Containers 2 (Bleach)**: Bleach solution
    c. **Container 3 (Neutralize)**: Sodium thiosulfate solution
    d. **Container 4 (Pronase Chorions)**: Pronase solution
    e. **Conatiner 5 (Rinse)**: Embryo media with methylene blue

    Starting with container 1, use the smallest volume of solution necessary to cover the eggs; each subsequent container should have a larger volume than the previous container.

    Note: The pronase working solution is of low concentration and therefore does not remove the chorions immediately. Allow at least 24 hours for the embryos to hatch following pronase exposure.

4. **Containers 1 and 2** (Load and Bleach Embryos/Eggs):
    a. First, remove the unfertilized or dead embryos before bleaching (VERY IMPORTANT).
    b. Place the required size and number of mesh baskets into container 1 which contains 0.5X embryo media with methylene blue (see Figures). When bleaching more than one stock, label each mesh basket so that you can keep track of the embryos/eggs or stock in each basket.
    c. Pipette up to 100 un-hatched embryos into the small mesh baskets, 200 into the medium baskets and 300 embryos into the large mesh baskets. **Be sure to change the pipette between stocks.**
    d. Lift the mesh baskets out of container 1 and place into container 2 (bleach solution).
    e. Start the timer and **bleach for 10 minutes.** Move the eggs as little as possible or death could occur.
    f. Transfer baskets to container 3**.**

5. **Container 3** (Neutralize Bleach in Sodium Thiosulfate Solution)**:**
    a. Swirl eggs **for 5 seconds**.
    b. Transfer baskets to container 4.

6. **Container 4** (Pronase Chorions):
    a. Swirl eggs **for 1 minute**.
    b. Transfer baskets to container 5.

7. **Container 5** (Rinse Embryos):
    c. Swirl eggs **for 2 minutes**.

8. Transfer embryos to Petri dishes:
    a. Rinse the eggs out of the mesh basket into Petri dishes or finger bowls using a squirt bottle of 0.5X E2 embryo media with methylene blue.

    b. Label the Petri dishes or finger bowls appropriately.

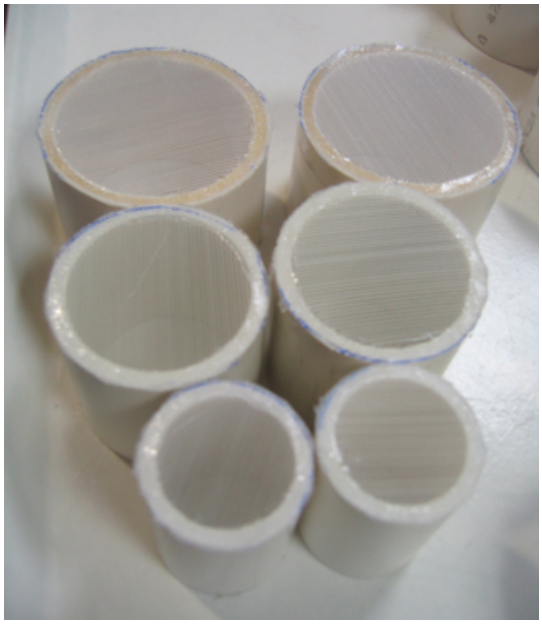

Fig 1. Mesh baskets. Made using hot glue to adhere nylon mesh (~400 microns) to the bottom of PVC pipe. Various sizes can be made to hold larger numbers of eggs.

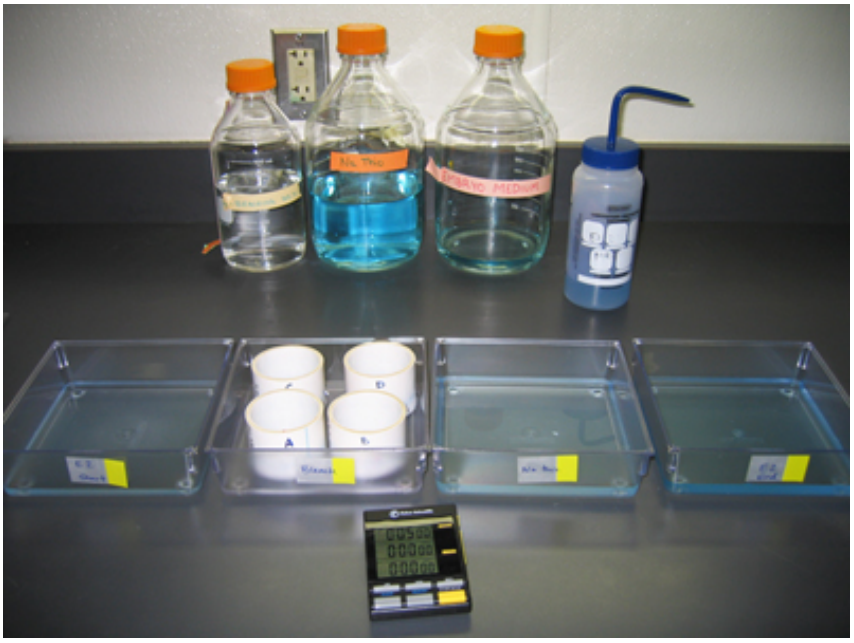

Fig 2. Embryo sanitation station. 5 containers are set-up for 0.5X embryo media, bleach, sodium thiosulfate, pronase, and rinse solutions. Pre-made large volume solutions are shown in the background.