

Egg Bleaching and Pronase Procedure

If needed, acclimate your embryos and working solutions (see below) to approximately 28.5°C prior to bleaching. Embryos should be bleached prior to 30 hpf and should not be bleached if they are beginning to hatch.

1. Sanitize the counter with 95% alcohol (EtOH) or disinfectant.
2. Set up solutions from pre-made, large-volume working solutions (recipes below):
 - a. **Container 1:** Load solution
 - b. **Container 2:** Bleach solution
 - c. **Container 3:** Sodium thiosulfate and pronase solution
 - d. **Container 4:** Final rinse solution

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.

3. Load and bleach eggs: First, remove dead eggs (VERY IMPORTANT). Place the required number and sizes of mesh baskets into container 1 (see Figures). When bleaching more than one stock, label each mesh basket with a unique letter so that you can keep track of which eggs are in which basket. Make a note of the stocks and corresponding mesh basket letters (Fig. 2 and 3). Then pipette up to 100 eggs into the small mesh baskets or up to 300 eggs into the large mesh baskets in container 1. **Be sure to change or rinse out the pipette between stocks.** Once all eggs are loaded, lift mesh baskets out of container 1 and place into container 2 (bleach solution). Start the timer and **bleach for 5 minutes.**
4. Lift the mesh baskets or swirl the container periodically to separate the eggs and allow the entire chorion to contact the bleach solution. Remove the mesh baskets of eggs after 5 minutes.
5. Neutralize bleach and pronase embryos: Transfer baskets to container 3 (**sodium thiosulfate and pronase solution**) for **1 minute.** Lift baskets or swirl eggs.
6. Final rinse: Transfer baskets to container 4 (**final rinse solution**) for **2 minutes.** Lift baskets or swirl eggs.
7. After final rinse in container 4, rinse the eggs out of the mesh holder using a squirt bottle of 0.5X E2 embryo medium with methylene blue. Follow your notes regarding which stocks are in which lettered baskets, rinse the eggs into a clean finger bowl or petri-dish, and label appropriately.
8. Wash all utensils (beakers, meshes, etc.) after use.

Large Volume Working Solutions

- 1. Methylene Blue Stock (Concentrate) Solution:** 1 g methylene blue powder into 1 L RO
- 2. Load and Final Rinse Solution:** 10 ml methylene blue stock solution into 20 L 0.5X E2 embryo medium
- 3. Bleach Solution:** 0.59 ml 5.25% sodium hypochlorite (chemical grade bleach) into 1 L RO water (make fresh daily)
- 4. Sodium Thiosulfate Stock Solution:** 1 g sodium thiosulfate into 2 L 0.5X E2 embryo medium
- 5. Pronase Stock (Concentrate) Solution:** 30 mg protease (from *Streptomyces griseus*, Sigma P-6911) into 1 ml 0.5X E2 embryo medium (freeze for long term storage or refrigerate for short term use)
- 6. Sodium Thiosulfate and Pronase Solution:** 0.68 ml pronase stock solution into 500 ml sodium thiosulfate stock solution (make fresh daily or every 2 hours, as needed)
- 7. Shipping Water (for Tissue Culture Flasks):** 10 ml methylene blue stock solution into 20 L 0.5X E2 embryo medium



Fig 1. Mesh baskets. Made by using hot glue to adhere nylon mesh (~500 microns) to the bottom of PVC pipe. Various sizes can be made to hold larger amounts of eggs.



Fig 2. Egg bleaching station. 4 containers are set up for bleach, sodium thiousulfate-pronase, and rinse solutions. Pre-made large volume solutions are shown in the background.

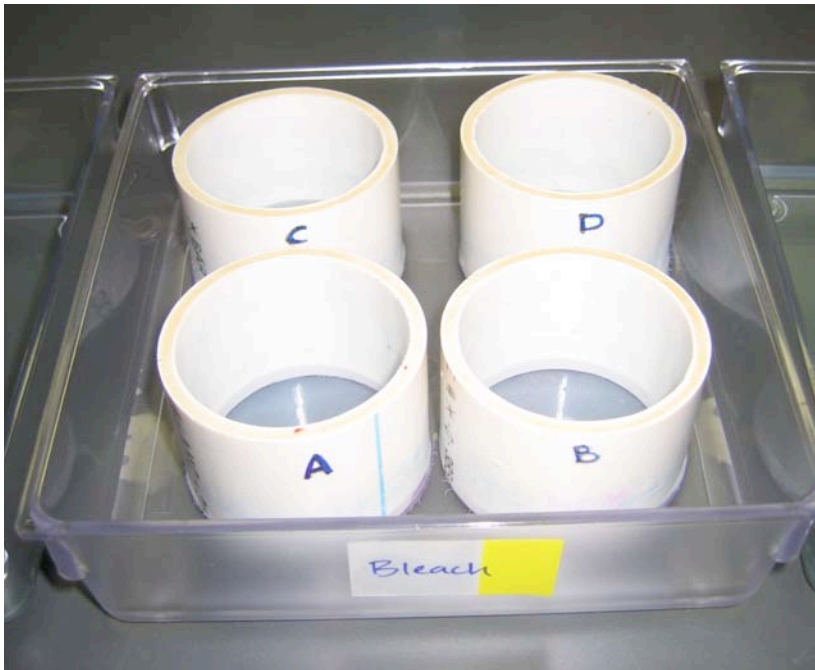


Fig 3. Mesh baskets are labeled with a letter for tracking of multiple stocks. Make a list of which stock is in each basket before loading the eggs.