CZAR - Embryo Bleaching Protocol

TITLE: Embryo Bleaching Protocol

PURPOSE: To describe the procedures for bleaching embryos prior to transporting between various fish facilities on campus in order to reduce the risk of spreading pathogens between systems. All embryos going into the CZAR nursery incubator should either be bleached using this protocol, or Ovadine treated using the CZAR Ovadine protocol. This Bleaching protocol followed by the Ovadine protocol must be used for all embryos coming out of the CZAR QX room.

All users must read the "Sanitizing Embryos for Nursery Incubator" policy prior to putting embryos into the nursery incubator.

1. Supplies and Solutions:
   a. Fresh petri dishes
   b. Fresh pipettes
   c. 6 plastic dishes for bleaching (clean mating tanks work well)
   d. Sterile tea strainer
   e. Egg water (E3 w/o methylene blue) from the sterilized containers in the injection rooms or the QX, or ddH_2O water for rinsing.
      i. System water may **NOT** be used.
      ii. The use of methylene blue egg water is not recommended during the first 24 hrs after bleaching.
      iii. When doing multiple batches of embryos, prepare replacement rinse water containers as the rinse water will accumulate the bleach rinsed from the previous batch of embryos.
   f. Pronase : Pronase stock (30 mg/mL) or forceps for dechorionating embryos. (Pronase stocks are in the -20 freezers in both injection rooms.)
      i. Add 1-2 µL pronase to 40 ml of E3 to soften chorions. Place in incubator overnight at 28 °C, embryos can then hatch on their own.
      ii. Alternately, pronase dechorionate (3 ul in 20 ml of E3 for 12-15 min. at 28.5°C.) or manually dechorionate your embryos.
   g. Bleach solution: Mix 380 ul of concentrated bleach provided by CZAR in 1L of ddH_2O. Add tea strainer to sterilize for 10 minutes, rinse in ddH2O. Dump out the bleach. Make a fresh batch of bleach and split it into two containers for embryo bleaching.

	Sodium Hypochlorite (% Concentration)	**Chlorine (% Available)**	**Microliters of bleach per 500 mL of egg water**
Sigma-Aldrich bleach solution	N/A	*10-15	*125
Concentrated household bleach	8.25	7.85	**190

* When using Sigma-Aldrich Sodium Hypochlorite solution (product # 425044), use the "Certificate of Analysis" feature under the product page to determine that lot number's % available chlorine. The above calculation was based on 11.7% available chlorine.

**Concentrated bleach provided by CZAR.

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Protocol:

1- Dilute 190 µL bleach into 500 ml of ddH_2O in each bleach container. (or 380 ul/L of ddH_2O).
2- Put 1 L ddH_2O into rinsing containers 1 and 2. Put 500 ml of E3 (No m-blue) in rinsing containers 3 and 4.
3- Pour embryos into tea strainers, place directly into bleach-water for 5 minutes.
    a. Do not agitate or stir the embryos while in bleach solution.
4- Transfer embryos to rinsing ddH_2O container for 5 minutes.
5- Transfer into 2nd bleach-water for 5 minutes.
    a. Do not agitate or stir the embryos while in bleach solution.
6- Transfer embryos to rinsing ddH_2O container 2 for 5 minutes.
7- Transfer embryos to rinsing E3 container 3 for 5 minutes.
8- Transfer embryos to rinsing E3 container 4 for 5 minutes.
9- Return embryos to petri dishes in E3 w/o methylene blue.
(Move to the Ovadine protocol here if doing both protocols. Then proceed with steps 10 or 11 below.)

10- Add 1 µL pronase to 40 ml of E3 to soften chorions, embryos can then hatch on their own.
    a. Utilize hand dechorionation using watchmaker's forceps (or equivalent) for any fish still inside their chorion.
    b. Wash embryos and place in new Petri dish with water (egg or blue) and store in an incubator.
11- Alternately, pronase dechorionate (3 ul in 20 ml of E3 for 12-15 min. at 28.5°C.) or manually dechorionate your embryos.
    1. Get the embryos out as soon as the chorions come off or the Pronase will start to break down the embryos.
    2. Rinse the embryos 3 times in E3 (w/o methylene blue) before placing them in a new petri dish.
    3. Utilize hand dechorionation using watchmaker's forceps (or equivalent) for any fish still inside their chorion. Wash embryos and place in new Petri dish with E3 (+/- blue) and store in an incubator.

Bleach 1 → ddH_2O Rinse-1 → Bleach-2 → ddH_2O Rinse-2 → E3 Rinse-3 → E3 Rinse-4

Figure 1. The order and steps in the bleaching process.