

Paramecium Preparation SOP
Mayo Clinic Zebrafish Facility
Version: December 2015

1. PURPOSE

The purpose of this SOP is to describe the preparation routine for paramecium in the Mayo Clinic Zebrafish Facility.

2. POLICY

Using properly cultured paramecium, it is the policy of the Mayo Clinic Zebrafish Facility to provide the highest standard of young zebrafish feeding.

3. DEFINITIONS

- 3.1. **Paramecium:** any of a genus (*Paramecium*) of ciliate chiefly freshwater protozoans that have an elongate body rounded at the anterior end and an oblique funnel-shaped buccal groove bearing the mouth at the extremity. (<http://www.m-w.com/dictionary/paramecium>)
- 3.1.1. In the Mayo Clinic Zebrafish Facility, paramecium is fed only to young zebrafish from their arrival on the baby system up until post-fertilization day 14.

4. RESPONSIBILITY

- 4.1. It is the responsibility of the Animal Care Assistants and Research Technologists employed by the Zebrafish Facility to prepare paramecium each day following the Mayo Clinic Zebrafish Facility SOP.
- 4.2. It is the responsibility of the facility director and facility manager to oversee the adherence and training regarding this SOP.

5. MATERIAL

- 5.1. Protozoan pellets (Carolina Biological Supply Company)
- 5.2. Wheat seed (Carolina Biological Supply Company)
- 5.3. 4 to 8 deli containers (and lids), able to hold at least 1,500 mL water
- 5.4. Marking tape (purple, pink, yellow)
- 5.5. 1,000 mL of boiling water per deli container
- 5.6. 10 μ L pipette
- 5.7. Disposable stir sticks (do NOT reuse! Use one stick per culture)
- 5.8. Petri dishes
- 5.9. Medium-sized brine net
- 5.10. 1000 mL clean glass beaker
- 5.11. Microwave
- 5.12. Heat-protective gloves

6. Counting Paramecium

- 6.1. The facility paramecium is located to the left of the fish room entrance on the shelves across from the microscopes.
- 6.2. Each day pull down the week-old paramecium from the shelf above prep area, these will be counted for culture. There will be 4-8 containers.
- 6.3. Stir the week-old paramecium well, and put a line of ten 10 μ L samples (8

from the perimeter and 2 from the center) using the 10 μ L pipette onto a petri dish to be counted.

- 6.4. Under the microscope, located right behind prep area, count the total paramecium seen in the samples. Then go through each sample again counting coleps (hopefully there won't be any).
- 6.5. With yellow tape, label each container with the amount of paramecium (and coleps) per 10 μ L, your initials, and date. Record this same info on the paramecium QC log.
- 6.6. Do this to each container that is 1-week old (usually 4 or 8 containers). Select the best possible count of paramecium. Always select a lower paramecium with ZERO coleps over a high paramecium with any coleps.

7. Culturing Paramecium

- 7.1. Set aside your best count from the week old paramecium, do NOT stir it again. Let the culture "rest" for at least 3 hours. Settling sinks any coleps to the bottom of the container. Even if you didn't see any coleps while counting, use this precaution!
- 7.2. Carefully pour ONLY the TOP 400 to 800ml (depending on the number of media houses) of culture through the net and into the clean 1000ml beaker.
- 7.3. Distribute 100ml of culture into each of the media houses made the day before.
- 7.4. With pink tape, label each container as cultured "C" with the date and your initials.
- 7.5. Place all the cultured containers on the shelf above the prep area.

8. Paramecium Media Preparation

- 8.1. To prepare the next batch of paramecium media "houses", place four to eight clean deli containers in the prep area depending on the day (Mon-Thur 8, Fri-Sun 4).
- 8.2. With purple tape, label them each with "P&W" (meaning, protozoa and wheat), followed by the date and your initials
- 8.3. Place two protozoa pellets in each clean container
- 8.4. Place 20 wheat seeds in each clean container
- 8.5. Fill a separate deli container with 1000ml of fish system water from the hose, NOT the sump! Cover.
- 8.6. Microwave 9 minutes, or until at a rolling boil.
- 8.7. After the water has boiled, and while wearing the protective gloves, immediately dump it into one of the pellet & seed prepped deli containers.
- 8.8. Repeat this step for all prepped containers (4-8 total depending on the day).
- 8.9. Leave the containers to cool overnight. Culture will be added to them the following day.

9. TANK LABELS

- 9.1. Note that labeling paramecium with tape follows certain colors:
 - 9.1.1. **Yellow**= this tag implies that the container has been counted. On this tag, please place the amount of paramecium/coleps counted per 10

- μL , as well as the date and your initials
- 9.1.2. **Pink**= this tag implies that the container has been cultured. On this tag, please write "C" followed by the date and your initials
 - 9.1.3. **Purple**= this tag implies that the container is ready for culturing and contains protozoa and wheat. Please write "P&W" followed by the date and your initials. These containers will be cultured one day after preparation.

10. FEED TIME

- 10.1. Paramecium is fed twice a day during normal feeding hours to fish on the baby rack. **Feed approximately 1ml/ fish in a tank**
- 10.2. Use the paramecium from the beakers above the second sink. It has been harvested into a clean glass beakers by being run through a clean brine net
- 10.3. To fill these beakers use the paramecium containers from the very right side of the shelf above the fridge. Use the oldest, but not if it's over two weeks old!
- 10.4. These need to be rechecked under the microscope for any abnormal cilia, or coleps before being strained into the beakers with brine net.
- 10.5. Always dump any paramecium over two weeks of (cultured) age, and/or containing more than 4 coleps/10 μL .

11. CLEAN-UP

- 11.1. Scrub, then place all containers & beakers in the dishwasher
- 11.2. Rinse and autoclave all nets