Protocol for Creating Mixed Gametophyte Cultures

Note: If you are making clonal cultures at the same time, be sure to read the "Creating Clonal Cultures" protocol beforehand.

1. Ensure your work area is clean and sterilize any surfaces that will be used with a generous amount of ethanol.
2. Gather supplies:
    - ☐ Gloves
    - ☐ Ethanol spray bottle
    - ☐ Paper towels or kimwipes
    - ☐ Permanent marker
    - ☐ Pasteur pipettes
    - ☐ Pasteurized seawater
    - ☐ Media vessel
    - ☐ Small beakers (100-200 mL)
    - ☐ F/2
    - ☐ Kanamycin
    - ☐ Germanium dioxide (GeO2)
    - ☐ Culture containers
    - ☐ Microscope
    - ☐ Hemocytometer
    - ☐ Cell counter
    - ☐ Tweezers
    - ☐ 20-40 um sieves
    - ☐ Release Calculator Sheet - Mixed Cultures tab
3. Ensure all supplies are sterile.
4. Prepare release media with F/2, kanamycin, and GeO2 according to the media preparation protocols.
5. Set up the Culture Release Sheet to meet your requirements.
    - Adjust the culture container volume to match the amount of liquid that will be in one of your culture containers.
    - Fill in all other fields that are highlighted yellow.
6. Place the prepared pieces of sorus into small beakers with labels.
7. Pour the release media into the beaker until it covers the sorus.
8. Let the beaker sit, swirling occasionally, and monitor for color change in the water.
    - Most of the time, there will be a slight color change when the sorus releases. However, this does not always happen and a blade may release without a clear change in color of the media.
    - Keep the temperature of the water as close to 12C as possible.
9. After a piece of sorus has released, pour the release water through a 20-40 um sieve into a new culture container.
10. Note the volume of release water in the Release Calculation Sheet.
11. Using a pipette, sample the release water and load the hemocytometer.
    - Place a coverslip over the base.
    - Hold the pipette close to the valley in the glass.
    - Squeeze gently so a drop on the release water is at the end of the pipette.
    - Move the drop up to the valley until the capillary action fills the hemocytometer.

12. Under the microscope, begin counting the number of swimming spores seen in the large corner squares.
    - A hemocytometer has 9 large squares.
    - Each of those 9 large squares is composed of 16 small squares.
    - Typically, you count 2-4 of 9 large squares - the corners are easiest.
13. Count 2-4 of the squares and average the numbers.
14. Input the average into the Release Calculation Sheet in the “Spore Count” column.
15. Look at the “Number of Containers” column in the sheet and pull out that many containers - minus one since you have your release water in one already.
16. Label the containers with the collection location, date of collection, and blade number of the sorus.
17. Split the release water evenly among the containers according to the “Volume of Release Water per Culture” column.
18. Fill the containers to the volume that you used in the “Volume of Container” column.
19. Seal the container and move it to a no light area.
20. Repeat above until all releases are done.
21. Leave the new cultures in the dark for 14 days.
22. On day 14, scrape the cultures and move them to red light.
23. On day 28, change the water in the cultures.
24. Change the water monthly and move the culture into circulation.
    - Be sure to put the right label on the culture and add it to your culture collection log sheet once it is in circulation.