

Protocol for Grinding Gametophyte Biomass

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
- ☐ 1.5 mL microcentrifuge tubes or tissue grinder
- ☐ Pasteur pipettes
- ☐ Pasteurized seawater
- ☐ Media vessel
- ☐ F/2



3. Ensure all supplies are sterile.

4. Prepare some media with F/2 and pasteurized seawater.

5. Remove the culture from red light.

6. Visually inspect the culture for signs of contamination. (Contaminated cultures should not be ground until contamination is under control. In the case of ectocarpus, grinding the culture will cause the ectocarpus to fragment and grow back in many new colonies.)

7. Prepare and label a new container for the ground biomass to be put in.

8. Remove the lid of the culture.

9. Using a pasteur pipette, move biomass from the culture into the grinding instrument with as little excess water as possible. (For single tufts, use a microcentrifuge tube and a pestle, for larger amounts of biomass, use a tissue grinder.)

10. If you are grinding with a microcentrifuge tube and pestle, fill the tube to ~300uL. Gently press the pestle down and spin it to grind the biomass until there are no large chunks left. (This usually takes less than 10 presses.)

11. If you are grinding with a tissue grinder, fill the grinder about half way. Plunge the pestle down and back up until the solution is homogeneous. If the chunks are very large, you may have to dilute the mixture and grind more slowly. (Be careful not to be too aggressive because this will cause biomass to overspill.)

12. After the biomass is ground, dump it into the new container.

13. Rinse the grinder or microcentrifuge tube with sterile seawater to make sure you get all of the remaining biomass and dump it into the new container.

14. Fill the new container with new media.

15. Replenish the used water in the old container.

16. Replace the lids for both containers and make sure they are labeled properly.

17. Put the cultures back into red light.

18. Rinse and sterilize pestles and grinders between uses. Microcentrifuge tubes can be thrown out.

