

Protocol for Changing Media in Gametophyte Cultures

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
 - ☐ Media Vessel
 - ☐ F/2
 - ☐ Pasteurized seawater
 - ☐ Waste vessel
 - ☐ Parafilm
3. Ensure all supplies are sterile.
4. Prepare some fresh culture media with F/2 and pasteurized seawater.
 - Keep the media partially covered parafilm to prevent splashes from contaminating it.
 - You will likely need to make new media many times during the culture refreshment process, so make sure you have enough sterile water and nutrients.
5. Remove the culture from red light.
6. Visually inspect the culture and record what you see on a culture maintenance data sheet. (If there is an issue with the culture, it should be set aside until clean cultures are refreshed to reduce risk of contamination spreading.)
7. Remove the top and slowly pour out the water into the dump vessel; dump as much as possible, ideally more than 75%. (Avoid dumping out any of the gametophytes and be mindful of the splash.)
8. Refill the culture with fresh media with F/2.
9. Replace the top back on the culture.
10. Make sure the label on the culture is clear and legible; replace if it isn't.
11. Record the water refreshment in a culture maintenance data sheet.
 - See **Culture Log and Maintenance Data Sheet** template.
12. Return the culture to red light.
13. Dispose of waste in accordance with biosecurity protocols