Protocol for Checking the Health of 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
    - ☐ Waste vessel
    - ☐ Pasteur pipettes
    - ☐ Microscope
    - ☐ Microscope slides
    - ☐ Coverslips
3. Ensure all supplies are sterile.
4. Remove culture from red light.
5. Visually inspect culture. (If there is an issue with the culture, it should be set aside until clean cultures are checked to reduce risk of contamination spreading.)
6. If you see any contamination, note it down in a culture maintenance data sheet. (Reference the Familiarizing Yourself with Types of Contamination in Cultures document to identify the contamination.)
7. Label a microscope slide with the culture ID.
8. Remove the lid of the culture.
9. Using a pasteur pipette, suck up a small amount of biomass.
    - If there are areas that look odd, get a sample of that too.
10. Drop the biomass onto a microscope slide and cover it with a coverslip.
11. Put the top back on the culture.
12. Gently press down on the coverslip to “smush” the gametophytes. (This creates a single layer of cells and makes it easier to check the health of the gametophytes.)
13. Move the slide to the microscope and look at it.
    - Use the Familiarizing Yourself with Types of Contamination in Cultures document to identify contamination.
    - Refer to “Identifying and Treating Health Issues” to identify possible health issues.
14. Mark down notes about the health of the cultures in a culture maintenance data sheet.
    - If the culture is clean and healthy, replace the top and move it back to red light.
    - If the culture is contaminated, determine if the contamination is treatable by using the Easy Reference Contamination Treatment Chart.
    - If the culture looks bleach or otherwise unhealthy, refer to the “Identifying and Treating Health Issues” section to determine the next steps.
15. Change the media in the culture according to the media change protocol and refill it with media containing treatment chemicals.
16. Replace the top of the culture and move it back to red light.
17. If necessary, change the water of the culture again after the specified time on the Easy Reference Contamination Treatment Chart.

*This process can be done at the same time as media changes to save water.