

Protocol for Spore Seeding Spools

Ensure your work area is clean and sterilize any surfaces that will be used with a generous amount of ethanol.

1. Gather supplies:

- ☐ Gloves
- ☐ Ethanol spray bottle
- ☐ Paper towels or kimwipes
- ☐ Spool log sheet
- ☐ Pasteur pipettes
- ☐ Pasteurized seawater
- ☐ Media vessel
- ☐ Release beaker/bucket x2
- ☐ F/2
- ☐ Kanamycin
- ☐ Germanium dioxide (GeO₂)
- ☐ Spools and release tank
- ☐ Microscope
- ☐ Hemocytometer
- ☐ Cell counter
- ☐ Tweezers
- ☐ 20–40 um sieve
- ☐ Spore Density Calculator

2. Ensure all supplies and the working surface are clean.

3. Set up the Spore Density Calculator sheet to meet your requirements.

- Choose your stocking density. GreenWave uses 2500 spores/mL for sugar kelp.
- Adjust the Volume of Inoculation Vessel cell to match the amount of liquid (in mL) that will be in one of your inoculation tanks.

4. Place the prepared sorus into a beaker with a label denoting blade number and location.

5. Pour the release media into the beaker until it covers the sorus.

6. Let the beaker sit, swirling occasionally, and monitor for color change in the water.

- Typically, there will be a color change when the sorus releases. The darker the release water, the more spores.
- Keep the temperature of the water as close to 12°C as possible.

7. After a piece of sorus has released, pour the release water through a 20–40 um sieve into a clean release container.

8. Note the volume of release water (in mL) in the Volume of Spore Solution cell.

9. 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 of the release water is at the end of the pipette.
- Move the drop up to the valley until the capillary action fills the hemocytometer.

10. Under the microscope, begin counting the number of swimming spores seen in the large corner squares.
 - Use the Instructions tab in the Spool Density Calculator sheet for directions on loading and using the hemocytometer.
11. Count 2–4 of the squares and average the numbers.
12. Input the average into the # of Spores Counted on the Hemocytometer cell in the Spore Density Calculator.
13. Look at the two large green cells in the Spore Density Calculator to see how much spore solution you need per inoculation vessel and how many inoculation vessels you can seed with this release.
14. Use a graduated cylinder to measure out the amount of spore solution indicated in the Volume of Spore Solution Needed to Inoculate row.
15. Pour the spore solution into the inoculation vessel.
 - For seeding tubes, place the seed spool in the tube. Fill the tube halfway with chilled, sterile seawater. Then, pour the solution around the outside seed spool, avoiding the inside of the spool. Top off the seeding tube with chilled, sterile water to mix the spore solution around in the water column. Cap the tube and store it in a cool place.
 - For seeding tanks, arrange your seed spools in the tank efficiently. Fill the tank halfway with chilled, sterile seawater. Pour the spore solution into the tank around the spools in an evenly dispersed pattern. Use a clean pipe or pump to stir the tank and begin filling the tank the rest of the way.
 - _ If you don't have pre chilled water and have to fill your tank ahead of time to chill, pour the solution through a pvc pipe into the tank so that the spore solution gets dispersed throughout the water column or have the aquarium pump running while you pour the spore solution in.
 - _ If you use chillers and pumps to cool your water, chill your tank room as low as possible and turn the chillers/pumps off for this process.
16. Leave the newly seeded spools in the dark at 10°C for 24 hours.
17. After the 24 hour settling period, move the spools into their assigned tanks.
18. Add nutrient media to the tanks.
19. Turn on the lights to low-light conditions (20 μ mol).
20. Set up/turn on any other tank equipment that you use, like water flow, aeration, or UV.