**Growing freshwater sponges from gemmules in the laboratory**

**Author List**

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**Abstract**

This is a basic protocol for growing freshwater sponges from gemmules in the laboratory. We specifically developed this protocol for working with *Ephydatia muelleri*, but have used it for other species as well. This protocol is good for cleaning gemmules, and removing contaminating protists, fungi, and bacteria.

**Keywords**

Freshwater sponge, porifera, Ephydatia, Spongilla

**Guidelines**

Gemmules are temperature sensitive. Keep gemmule stocks at 4C to maintain their longevity. There are published reports of defined media for growing sponges, but we typically use store bought spring water, or sterile-filtered lakewater; both work well.

**Before Start**

Gemmules can be collected seasonally throughout the world. There are published records of freshwater sponge distributions, but it is also possible to use citizen science apps such as iNaturalist to identify locations where they have been reported. A map of freshwater sponge reports in the United States can be found at the following link: <https://www.inaturalist.org/projects/freshwater-sponges-of-the-united-states>.

**Safety Warning**

None

**Materials**

Freshwater sponge gemmules

Autoclaved lake water or spring water

10 cm petri dish

40 or 70 µm cell strainer

50 mL conical tubes

Any format cell culture plates / dishes (6-well, 12-well, 24-well)

Hydrogen peroxide

100x Antibiotic-Antimycotic (Sigma-Aldrich A5955-100ML)

p1000 pipette and tips

**Optional**

Incubator set at 25-28 C

Stereomicroscope

50 mg/mL Kanamycin or 100 mg/mL Ampicillin

**Equipment**

None

**A note about gemmule storage:** Field-collected gemmules should be stored at 4C in sterile-filtered or autoclaved lake water. Keep gemmules collected from different adults in separate containers to ensure that you don't accidentally mix different species, and to ensure that gemmules used in an experiment have the same genetic background. If stored in a deli-style fridge, place gemmules in an opaque container to limit the growth of algae.

Anecdotally, we have observed that gemmules remain viable longer if stored in a minimal volume of water, just covering their surface.

**Step 1: Gemmule sterilization**: Place a 40-70 µm cell strainer into a clean 10 cm Petri dish filled with cold, lake/spring water. Cut off the end of a p1000 pipette tip with scissors to increase the size of the opening. Using the trimmed pipette tip, transfer the isolated gemmules into the cell strainer.

1.1 sterilize with hydrogen peroxide, then rinse. Prepare a 60 ml solution of 1% hydrogen peroxide in lake water and place in a 50 ml conical tube (fill to the very top of the tube). Transfer the cell strainer containing gemmules into the hydrogen peroxide solution and incubate for 5 minutes. Remove the cell strainer from the hydrogen peroxide solution and rinse very thoroughly by placing under a flowing tap of RO water for at least 1 minute. This step is essential to remove all traces of hydrogen peroxide and bubbles attached to the surface of gemmules that will cause them to float.

1.2 select gemmules for use. Place the cell strainer into a clean 10 cm Petri dish containing lake/spring water. Using a new p1000 tip, transfer the gemmules from the cell strainer to the surrounding dish. This is an opportunity to spread the gemmules out and separate them from remaining debris leftover from the parent tissue. Also, at this point you can discard floating gemmules; even if they are viable they will not attach when plated.

**Step 2: Gemmule plating**: You can plate gemmules in essentially any dish. The limiting factor is the water volume; a single gemmule grows best in at least 500 µl of water. If you need to grow gemmules in a smaller format, such as in a 96-well culture dish, it is possible but you have to perform frequent water changes (maybe even twice daily).

2.1 sterilize in Anti-Anti for 24-48 hours. Even though you have sterilized the gemmules with hydrogen peroxide, it is still possible for cultures to contain contaminating fungi. To prevent fungal growth, dilute the antibiotic-antimycotic (Anti-Anti) to 1x final concentration in lake/spring water. Place the desired amount in the culture dish where you wish to grow the sponges, and then add the number of gemmules you wish to grow, and place at room temperature (we have successfully grown Ephydatia at temperatures ranging from 15 C - 30 C). Incubate in Anti-Anti for 24 - 48 hours, then replace with pure lake/spring water, or with lake/spring water containing another antibiotic such as 50 µg/mL kanamycin or 100 µg/ml ampicillin (depending upon your experimental goals). Anti-Anti seems to slow gemmule hatching and may affect tissue development.

2.2 position gemmules in the center of the culture dish. Sponges typically start to hatch between 72 and 96 hours after plating, at which point they attach to the culture well/dish and cannot be moved. It is important to make sure the gemmules are positioned according to your experimental needs (usually towards the center of the well to enable imaging). If placed near the edge of the well/dish, the sponges may grow vertically and be difficult/impossible to image. A trick for moving gemmules to the center of the well is to swirl the dish for several seconds to create a vortex within the well.

2.3 Mature sponges are best used between days 7-10. If the sponges are grown in at least 500 µl of lake/spring water per gemmule it is usually not critical to change the solution over the course of a 7-10 day experiment. If you wish to limit autofluorescence for subsequent imaging studies, it is essential to grow the sponges in a dark incubator or drawer to limit the growth of intracellular *Chlorella*-like algae. Without feeding, sponges cannot be maintained long beyond 10 days usually. As they age, their tissues often retract and/or the entire sponge will migrate away from the gemmule capsule, leaving behind sponging fibers and spicules. We avoid working with sponges at this stage.