Feb 07, 2023

Growing freshwater sponges from gemmules in the laboratory

DOI
dx.doi.org/10.17504/protocols.io.x54v9dkj4g3e/v1

Scott Nichols[1]

[1]University of Denver

Scott Nichols
University of Denver

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9dkj4g3e/v1

Protocol Citation: Scott Nichols 2023. Growing freshwater sponges from gemmules in the laboratory. **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9dkj4g3e/v1**

Protocol status: Working

Created: January 31, 2023

Last Modified: February 07, 2023

Protocol Integer ID: 76122

Keywords: Freshwater sponge, porifera, Ephydatia, Spongilla, freshwater sponges from gemmule, growing freshwater sponge, freshwater sponge, cleaning gemmule, contaminating protist, ephydatia muelleri, gemmule, bacteria, fungi, laboratory

Funders Acknowledgements:
National Science Foundation
Grant ID: 2310071

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.

Attachments

631-1308.docx
9KB

Guidelines

Guidelines

Gemmules are temperature sensitive. Keep gemmule stocks at 4 °C 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.

A note about gemmule storage:

Field-collected gemmules should be stored at 4 °C 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.

Materials

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

Troubleshooting

Before start

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.

Gemmule sterilization

1 Place a 40-70 µm cell strainer into a clean 10 cm Petri dish filled with cold, lake/spring water.

2 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.

Gemmule sterilization: sterilize with hydrogen peroxide, then rinse

3 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).

4 Transfer the cell strainer containing gemmules into the hydrogen peroxide solution and incubate for 00:05:00 . 5m

> Note
>
> Lift the cell strainer up and then submerge it again a few times to agitate the gemmules periodically through incubation.

5 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 00:01:00 . 1m

> Note
>
> 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.

Gemmule sterilization: select gemmules for use

6 Place the cell strainer into a clean 10 cm Petri dish containing lake/spring water.

7 Using a new p1000 tip, transfer the gemmules from the cell strainer to the surrounding dish.

Note

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.

Gemmule plating: sterilize in Anti-Anti for 24-48 hours

8

Note

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).

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.

9 Place the desired amount of spring/lake water containing Anti-Anti 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 .

Note

We have successfully grown *Ephydatia* at temperatures ranging from 15-30 °C .

10 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).

Note

Anti-Anti seems to slow gemmule hatching and may affect tissue development.

Gemmule plating: position gemmules in the center of the culture dish

11 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.

12 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.

Gemmule plating: Mature sponges are best used between days 7-10

13 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.

14 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.

15 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.