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Methods for culturing the freshwater sponge *Ephydatia muelleri*: Growing sponges on agarose and priming gemmules

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1

dx.doi.org/10.17504/protocols.io.yxmvmn29bg3p/v1[Symbiosis Model Systems](#) [Transfection project](#)

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The freshwater sponge *Ephydatia muelleri* is an emerging sponge model system. This species has clonal asexual reproduction stages called gemmules that are long-lasting and easily maintained in the lab, and a chromosome-level assembly of the genome (Kenny *et al.*, 2020) with molecular resources (ephybase.ca), making this an easily used species for study by researchers around the world.

Sponges grow attached to substrates, and one technical difficulty when working with species are attached to a substrate, is how to transfer samples to and from different solutions and vessels for experimental manipulations. In the case of *E. muelleri*, transfer is usually done with gemmules before they hatch and attach, or with sponges that are attached to small coverslips.

Here, we outline a simple and effective method to grow *E. muelleri* on 2% agarose, a substrate that allows them to be peeled off with little damage, and transfer to other containers at any stage. Once transferred and left alone, they re-attach readily.

E. muelleri development from gemmules are referred to as stages: stage 1 is just prehatching; stage 2 is hatched with no aquiferous system; stage 3 has 'pockets' of water spaces that are growing and merging with each other; stage 4 has an incipient but not fully organized canal system; stage 5 has a fully developed canal system with osculum vent. Although all stages can be easily peeled off the agarose substrate, sponges at earlier stages (2-3) develop at the same rate as undisturbed sponges after transfer, allowing direct comparison with unmanipulated control samples.

This method allows different stages of *E. muelleri* to be transferred into cuvettes for electroporation, or into a solution for pharmacological treatment, or onto a new substrate for imaging.

We also describe the best mechanism of timing hatching of gemmules. Gemmules are

'dormant' when kept at 3°C. Once placed at room temperature they undergo development within the collagen capsule, that involves differentiation of different cell types prior to their emergence from the micropyle (opening), as described by Höhr in 1977. This development can be thought of as "priming", or readying them for hatching. 'Priming' gemmules to the same level of hatching readiness, allows more control over timing of experiments. Primed gemmules also appear to be more resilient to both physical and chemical treatments.

The gemmules slowly get primed once cleaned, or thawed from -80°C. A general guideline to prime gemmules is:

1 week in a 3°C incubator = 1 less day it takes for the gemmules to hatch after plating
1 day at room temperature = 1 less day it takes for the gemmules to hatch after plating
Once they are over-primed (usually after 4-5 weeks in the 3°C incubator), they will start hatching even when kept at 3°C.

We suggest that for reproducibility of experiments that concern the stage of development, experiments using *E. muelleri* gemmules would benefit from using primed gemmules or indicating how primed the gemmules were.

Reference

Kenny, N.J., Francis, W.R., Rivera-Vicéns, R.E. *et al.* Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge *Ephydatia muelleri*. Nature Communications. 2020. 11, 3676. <https://doi.org/10.1038/s41467-020-17397-w>.

Höhr, D. 1977. Differenzierungsvorgänge in der keimenden Gemmula von *Ephydatia fluviatilis*. Roux Arch. Dev. Biol. 182: 329-346.

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Freshwater sponge, *Ephydatia muelleri*, Porifera, agarose, Development, gemmules, transfection

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Development rates and best timing for transfer

Once hatched, every stage (stages 1-5) can be peeled off completely intact, quite easily using fine forceps.

Up until stage 5 (before full oscula formation) no recovery time is required, and sponges develop at the same rate as undisturbed controls.

A stage 5 sponge (osculum is fully formed) may take a couple days to recover (i.e. it might ball up -> spread out again), before looking like stage 5 again.

At any stage, if the sponges are left for 1-2hr or longer after peeling, they will start to ball up, which could delay their development afterwards.

Modified pipette tips

The wide bore Pasteur pipettes are easiest for transferring sponges, but this is not a useful option when the sponges need to be transferred in a specific volume of solution during experiments.

This can be easily achieved by modifying the plastic pipette tips.

The diameter of the tip of P1000 tips and other pipettes can be modified by cutting the tip off with scissors, and smoothening the tip using forceps to remove jagged edges that could damage sponges during transfer.



Modified P1000 tip with a wider diameter to reduce potential damage to sponges during transfer

Agarose (molecular grade)
Wide bore Pasteur pipettes and bulbs
Modified P1000 tip
Petri dishes (e.g. 5cm diameter)
Fine forceps (e.g. Dumont No.5)

Strekal's medium:

Strekal TA, McDiffett W. 1974. Factors affecting germination, growth, and distribution of the freshwater sponge, *Spongilla fragilis* Leidy (Porifera). Biological Bulletin 146:267-278.

Preparing agarose plates (2% in 1x Strekal's medium)

1 Make 2% agarose plates and allow to cool.

1.1 Weigh 2g agarose into a flask, add 100mL of 1x Strekal's medium, microwave for 30-40 seconds to dissolve, and pour enough to only coat the bottom of 5 cm diameter Petri dishes. Leave to cool for 2 hours.

*Note that a thin layer of agarose allows room for a greater volume of growth medium for growing the sponges.

1.2 Once the agarose is solidified, store the dishes inverted in an airtight Tupperware container or plastic bags at 4°C.

*Agarose plates should last for several months when stored in these conditions.

Cleaning and plating gemmules

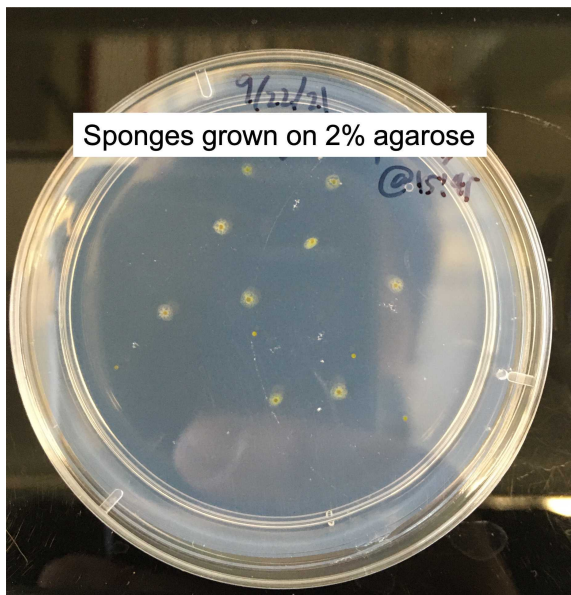
2 Clean gemmules for plating, or rinse gemmules stored at -80°C following the protocol - "Hatching and freezing gemmules from the freshwater sponge *Ephydatia muelleri*" by Sally P Leys, Lauren Grombacher, and April Hill (dx.doi.org/10.17504/protocols.io.863hzgn).

2.1 Pour 1x Strekal's medium onto the agarose Petri dish, and transfer gemmules onto the agarose using a wide-bore Pasteur pipette.

2.2 a) Let the gemmules hatch and develop in the dark (e.g. covered with tin foil).
b) Change the medium every 2 days after hatching.

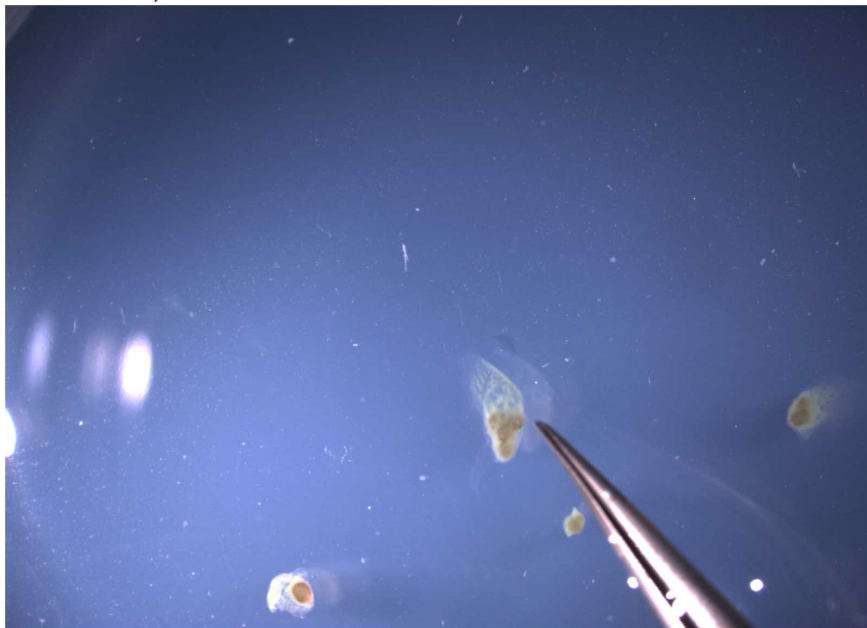
Peeling sponges and transferring them from the agarose dish

3 Once the sponges have reached the desired stage they can be transferred for experimental use or to new substrates.

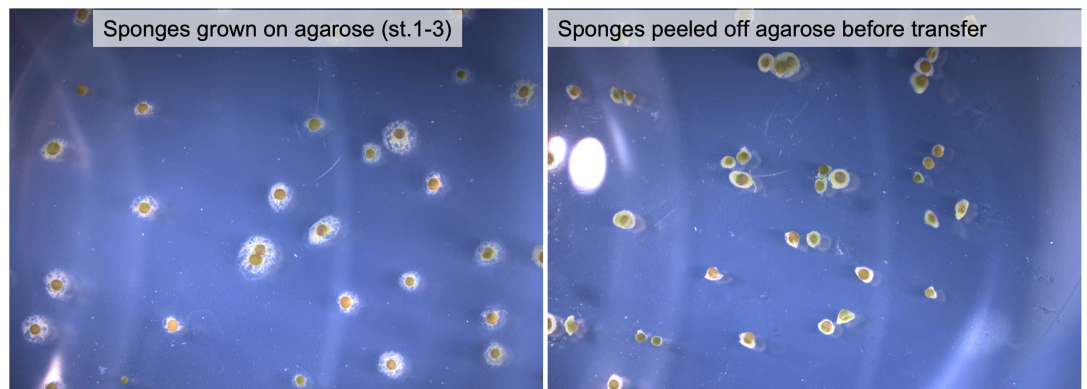


Sponges grown on 2% agarose

- 3.1 Gently peel the sponges off of the agarose by using a pair of fine forceps (e.g. Dumont No.5).

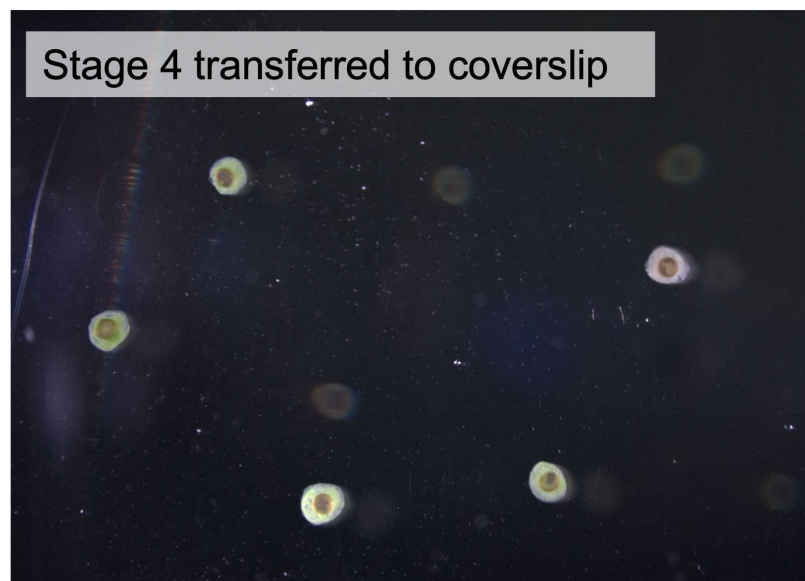


Peeling the sponges off of the agarose with a pair of fine forceps



Sponges of various stages before and after peeling off of agarose

- 3.2 Once the sponges have been peeled off the agarose, transfer them using a wide bore Pasteur pipette or a modified P1000 tip, to prevent damage to the sponge tissue.
- 3.3 Transfer to a new substrate or cuvette for treatment. On the new substrate adjust the the sponges to an upright position to speed attachment to the substrate.



Stage 4 sponges transferred on to a new coverslip

- 3.4 Grow until desired stage and carry on with imaging and/or fixing.



Sponges from previous image spread out and developed to stage 5

4 Priming gemmules for faster hatching and increased resilience to manipulation

Cleaning gemmules prompts the process of cellular differentiation inside the gemmule capsule. This process takes a few days. Cleaned gemmules will undergo cellular differentiation at 3°C, such that gemmules plated directly after cleaning hatch slower than gemmules plated a week after cleaning. Note, how vigorously gemmules are cleaned also affects how rapidly they undergo differentiation.

Whereas freshly cleaned gemmules do not always survive manipulations, primed gemmules hatch and develop well despite physical or chemical manipulation. Below are two treatments that are possible with primed gemmules.

4.1 General timeline of gemmule hatching when cleaned and kept at 3°C

Freshly cleaned gemmules - hatch after 2 days to one week at room temperature.

- 2 weeks after cleaning (kept at 3°C), gemmules usually start hatching by the next day.
- 4 weeks after cleaning (kept at 3°C), gemmules may hatch inside the incubator.

Gemmules stored at -80°C, thawed and rinsed, hatch after about 5 days at room temperature.

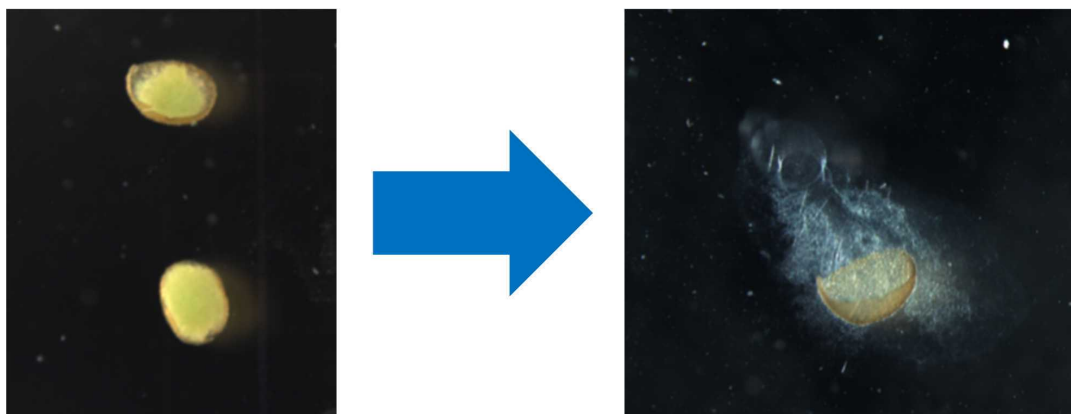
- One week after rinsing (kept at 3°C), gemmules start hatching within 3 days.
- 4 weeks after rinsing (kept at 3°C), gemmules may hatch inside the incubator.

*NOTE: Gemmules thawed from -80°C seem to prime faster than the gemmules from fresh sponges kept at 3°C, but those thawed from -80°C last longer in the 3°C incubator as primed gemmules.

5 Physical manipulations that are practical for use with primed gemmules *E. muelleri*.

5.1 Example 1: Cutting gemmules

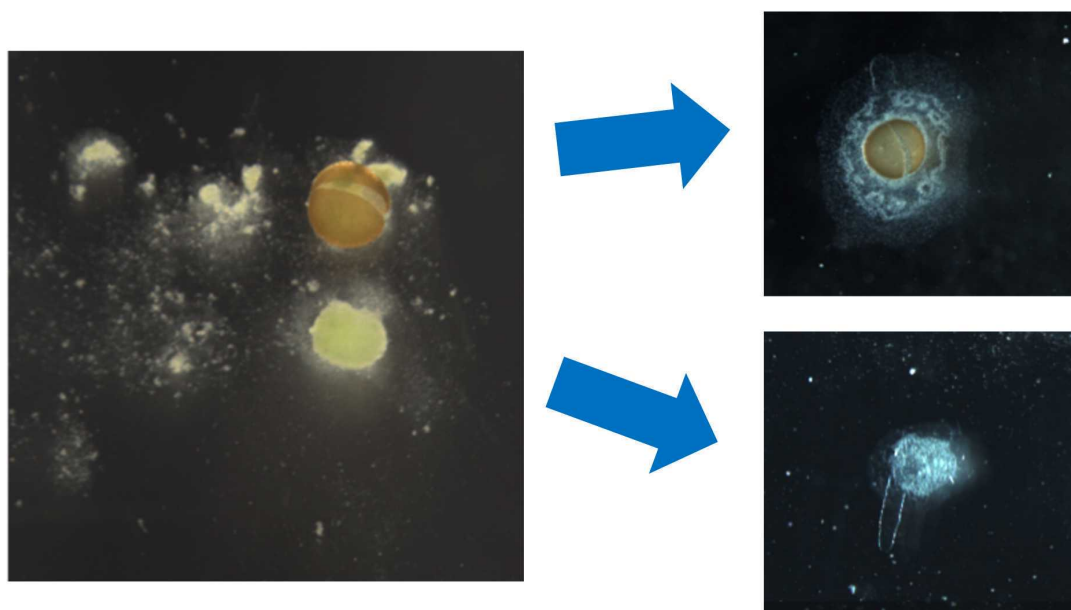
When the gemmules are primed, they can be cut in half with a scalpel blade and both halves will develop into a functional stage 5 sponge with an osculum.



A primed gemmule cut in half, developing into a functional stage 5 sponge with an osculum

5.2 Example 2: Squeezing cells from gemmules

When the gemmules are primed, they can be cut/squeezed with a pair of forceps to push out the cells and both halves (with or without the gemmule casing) will develop into a functional stage 5 sponge with an osculum.



A primed gemmule cut and squeezed, can develop into a functional stage 5 sponge with an osculum, with or without the gemmule casing

