January 2014

Pick, Wash and Hatch Freshwater Sponge Gemmules Handbook

For

RNAi Treatment
Chemical inhibition
Other uses

NSF/EDEN Grant number IOS # 0955517

Required Equipment

Sample	
Maternal tissues containing gemmules	
Solutions	
Strekal's Medium	10 x 15mL
Strekal's Medium	14,5mL
Strekal's Medium	1mL/well
Hydrogen peroxide 30 wt % in water	500µL
Tools	
Recipient with ice	x2
Little Petri dish	x1
Microscope	x1
Teasing needle	x2
15mL Falcon conical tube	x1
Pipette (P1000)	x1
12-well Plate	
Circle cover glass (Ø18mm)	1/well

Sample storage

Sponges samples were collected near Prince William Forest National Park during late fall/early winter and stored in sampling water or cold Strekal's media for one year or more at 4°C and preserved from the light. Gemmules are aerated week and water replaced monthly.

Streakal's Medium

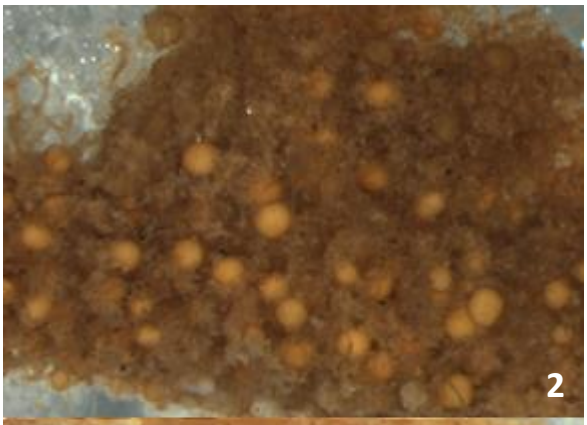
Strekal's Medium is an aqueous solution enabling gemmules attachment (Strekal and McDiffit, 1974) . 10X Strekal's Medium contains 0.9mM of $MgSO_4$-$7H_2O$, 0.5mM $CaCO_3$, 0.1mM Na_2SiO_3-$9H_20$ and 0.1mM KCl.

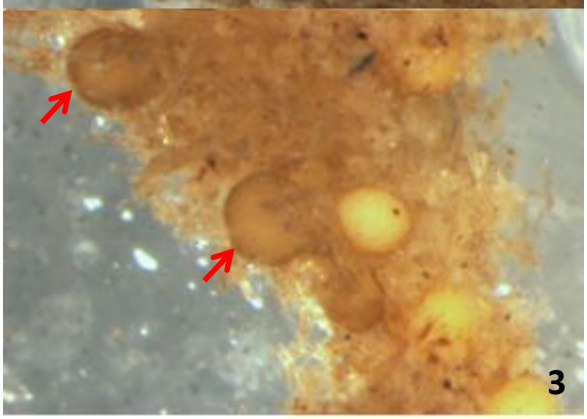
Pick gemmules

Important note before starting, all steps should be done on ice and keep Strekal's Medium cold during the procedure.

1. **Remove a small piece of maternal tissue from sampling water and pull it in a little Petri dish containing cold Strekal's Medium.**

2. **Using the microscope and teasing needles, carefully collect gemmules from maternal tissue (Pictures 1 and 2).**
   Sometimes, some gemmules coats are empty (picture 3) and do not contain cells. Squeezing them can confirm if the gemmule coat has cells on the inside, but will lead to popping the gemmule. A trained eye will lead to the ability to differentiate.





3. **Change Strekal's solution to remove all remaining maternal tissues and empty gemmules.**

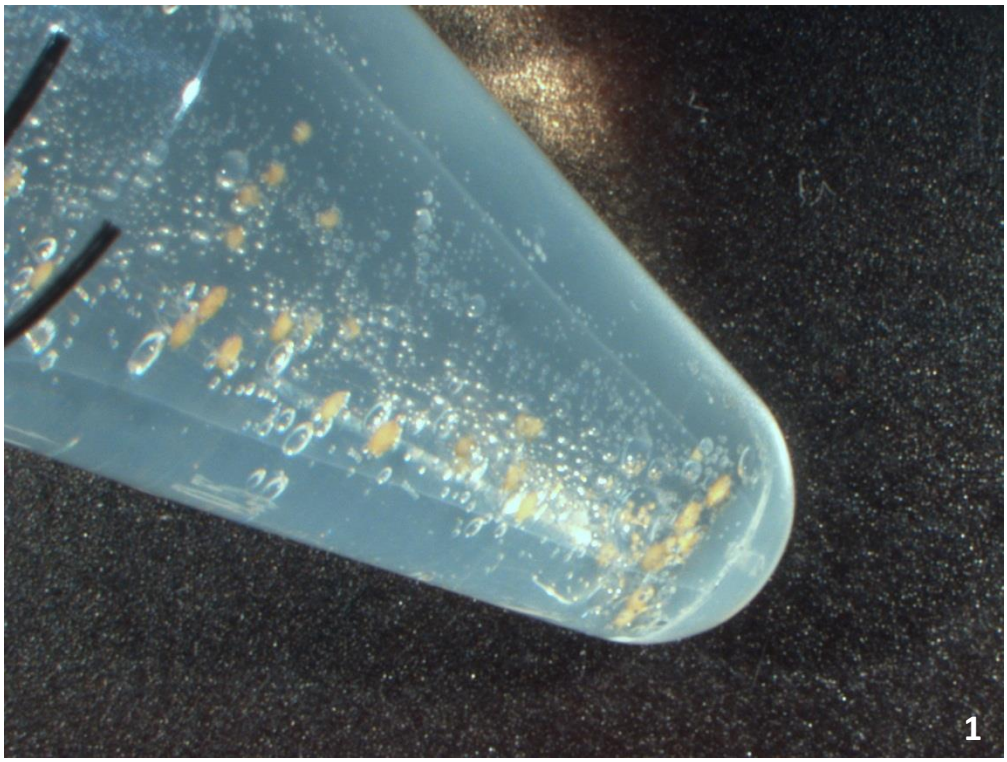
Wash gemmules

1. **Prepare hydrogen peroxide solution in a 15mL Falcon conical tube by diluting 500µL of 30% hydrogen peroxide solution in 13.5mL.**

2. **Gather gemmules by executing circles with the Petri dish.**
   Centripetal force will regroup gemmules in the center of the Petri dish.

3. **Pipette gemmules in 1mL of Strekal.**
   At least, adding 1mL of Strekal will permit to reach a hydrogen peroxide concentration of 1%.

4. **Add gemmules to the falcon containing the hydrogen peroxide solution and invert tube every minutes during 3 minutes.**
   This step permit removal of algae and to weaken gemmules membrane. Bubbles should appear (Picture 1).


1

5. **Help gemmules to go down by softly slapping the Falcon.**
   Some of them will not go down because of bubbles, don't worry and continue the procedure.

6. **Remove the solution by inverting the Falcon above the Petri dish, rapidly pick up the few gemmules from the Petri dish, pull them with others in the falcon, fill it with Strekal and invert it immediately several times.**
   Once at Falcon tip, gemmules stay there when you invert it (Picture 2).


2

7. **Change the solution using the same method and incubate gemmules 3 minutes. Invert falcon every minutes.**
   All gemmules which are still floating can be removed at this step.

8. **Proceed to step 7. 6 or 8 times more.**

9. **Gemmules are washed and ready to use. They can also be stored at 4°C at this time for later use.**

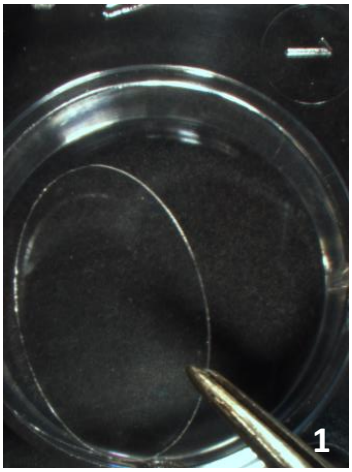
Hatch gemmules

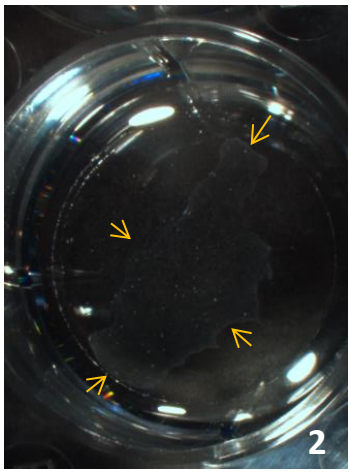
1. **Remove gemmules and Strekal's from ice. And let them reach room temperature.**
   During this time, proceed to next steps

2. **Open a 12-well plate and put a circular cover glass in each well (Picture 1).**

3. **Add 1mL of Strekal's Medium in each well.**

4. **Remove any air between well and cover-glass with a pipette tip (Picture 2 and 3).**
   Cover-glasses tend to float.



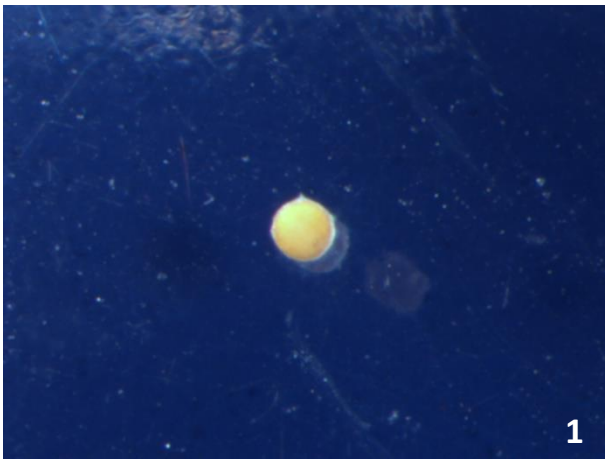


5. **Once gemmules and Strekal's reached room temperature, place 3-4 gemmules in each well.**
   Try to separate gemmules one from an other and from cover-glass edge.

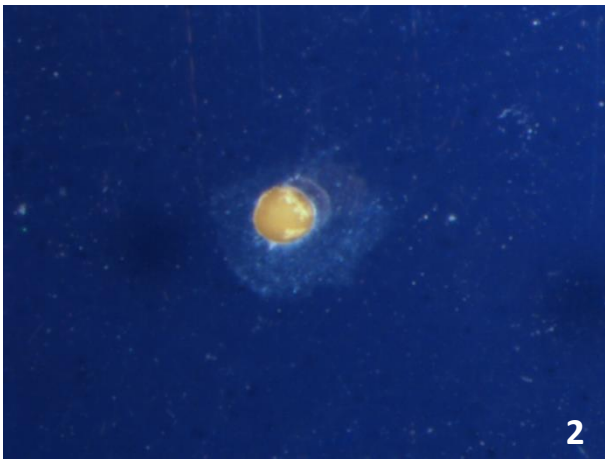
6. **Let gemmules to hatch for 2-3 days at room temperature in the dark.**

7. **Treat them at the desire stage (Pictures 1 to 6) (see Y27632 and RNAi treatment handbook).**
   Even without treatment, change the solution every 24h after attachment.

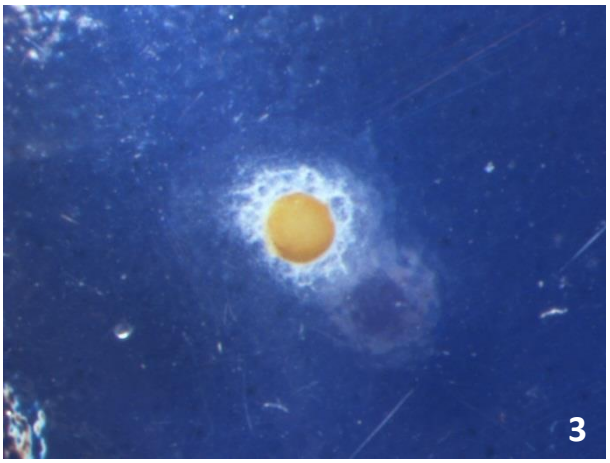
Stage 1



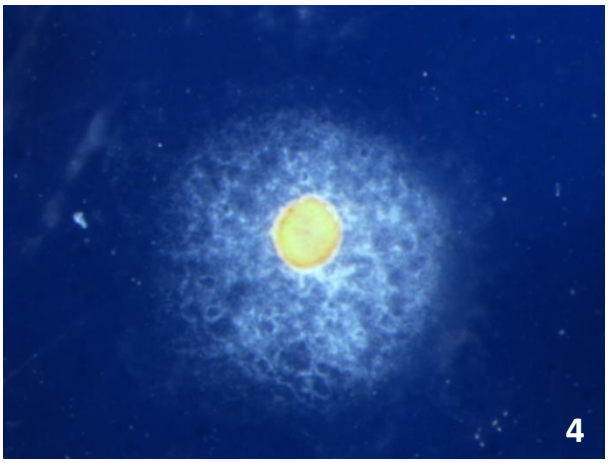
Stage 2



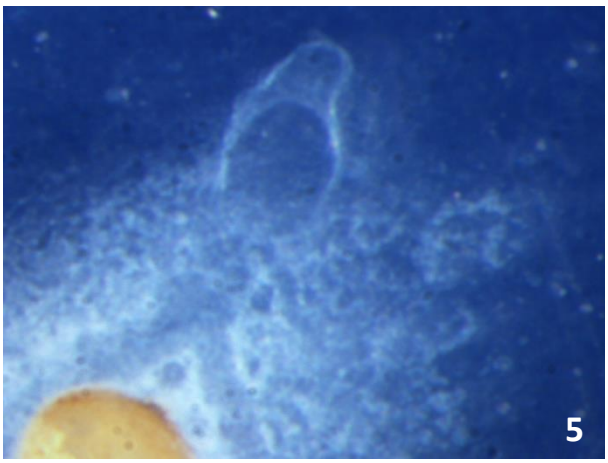
Stage 3

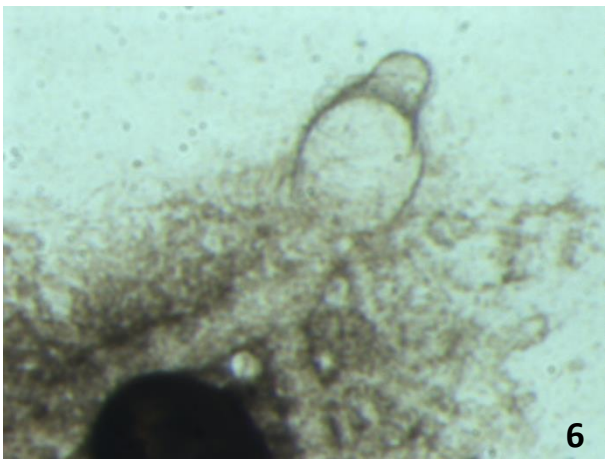


Stage 4



Stage 5





April Hill, *Department of Biology, University of Richmond, Richmond, VA, USA (ahill2@richmond.edu)*

Quentin Schenkelaars, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (quentin.schenkelaars@univ-amu.fr)*

Fernando Gomez, *Department of Biology, University of Richmond, Richmond, VA, USA (fgomez@richmond.edu)*

Chelsea Hall, *Department of Biology, University of Richmond, Richmond, VA, USA (chelsea.hall@richmond.edu)*

Laura Fierro, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (laura.fierro@univ-amu.fr)*

Emmanuelle Renard, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (emmanuelle.renard@univ-amu.fr)*

Carole Borchiellini, *Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille-Université, Marseille, France (carole.borchiellini@univ-amu.fr)*

NSF/EDEN Grant number IOS # 0955517