Aiptasia spawning and embryo/larvae handling - Pringle Lab

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Abstract

This protocol seeks to provide all information needed to allow regular spawning of Aiptasia and safe handling of the spawn.

It is based on the protocol described in Grawunder et al., 2015.

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Guidelines

This protocol refers to three different sizes of polycarbonate plastic food containers to maintain Aiptasia in:

-**small (250 ml)**: Cambro 92CW
-**medium (750 ml)**: Cambro 62CW
-**large (2000 ml)**: Cambro 44CW

The artificial seawater (ASW) used in the Pringle lab is Coral Pro Salt (manufactured by Red Sea) dissolved in de-ionized water to a salinity of between 32 and 34 (usually ~37.4 g/l).

The Pringle lab uses the male, clonal Aiptasia strain CC7 to obtain sperm and the clonal female strains H2, PLF3/F003, PLF5 and used also PLF8 in the past to produce eggs. All strain combinations yield viable planula larvae.

Materials

- KimWipes by Fischer Scientific
- Artificial seawater by Contributed by users
- CORAL PRO SALT by Red Sea
- blue LEDs (450 nm) by Contributed by users
- Culture Dishes, Carolina, 4 1/2 in, 250 mL

741004 by Contributed by users

Thermo Scientific™ Samco™ Transfer Pipettes 13-711-5B by Fisher Scientific

PYREX™ Reusable Petri Dishes: Complete 08-747A by Fisher Scientific

Protocol

Preparing animals for spawning

Step 1.

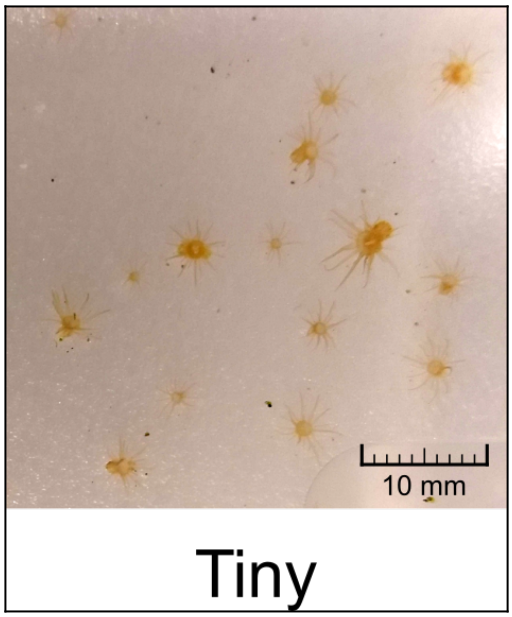
Collect pedal lacerates from the respective Aiptasia strain and transfer them into a medium tank with 500 ml ASW. Use 30-40 pedal lacerates per tank.

Preparing animals for spawning

Step 2.

Transfer fully developed small anemones into a large tank with 1.5 l ASW.

This is the 'tiny polyp' tank.



Preparing animals for spawning

Step 3.

To grow animals to a good spawning size numbers of animals will be reduced step-by-step as the anemones grow. All preparation steps can be done at room temperature (22-27°C) under cool-white light with 25 µmol photons/m^2/s in clear tanks of the respective sizes.

The list with sizes and models of containers used in this protocol can be found in the guidelines.

Feed anemones in all tanks twice a week and do water changes several hours later. You can swipe the sides of the tanks with clean, lint-free tissues (Kimwipes®) and remove pedal lacerates with disposable/clean spatulas.

Depending on temperature and feeding regimen, growth from a pedal lacerate to a spawning-sized animal takes 3-5 months. This protocol is optimized for obtaining a constant number of spawning-sized animals for several (in our case 4) spawning incubators while keeping the workload for maintanance at managable levels. If the spawning animals are needed faster, a higher feeding (and therefore cleaning) frequency and low numbers of anemones at all sizes will accelerate growth.

Preparing animals for spawning

Step 4.

Transfer polyps with a pedal disc diameter of 1-3 mm into a new large tank with 1.5 l ASW.

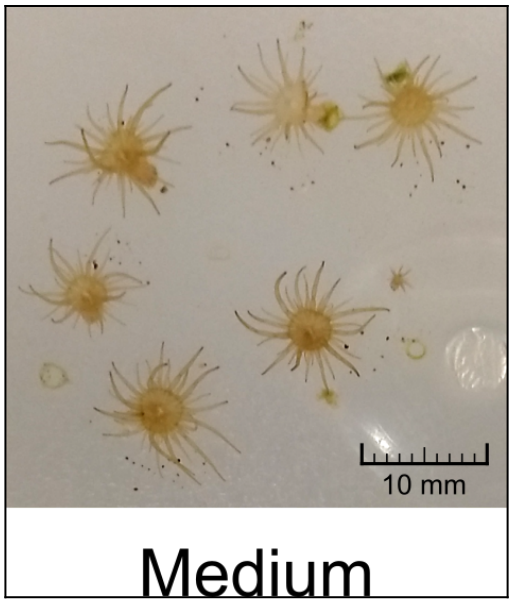
This is the 'small polyps' tank.

Preparing animals for spawning

Step 5.

Transfer polyps with a pedal disc diameter of 3-5 mm into a new large tank with 1.5 l ASW.

This is the 'medium polyps' tank.

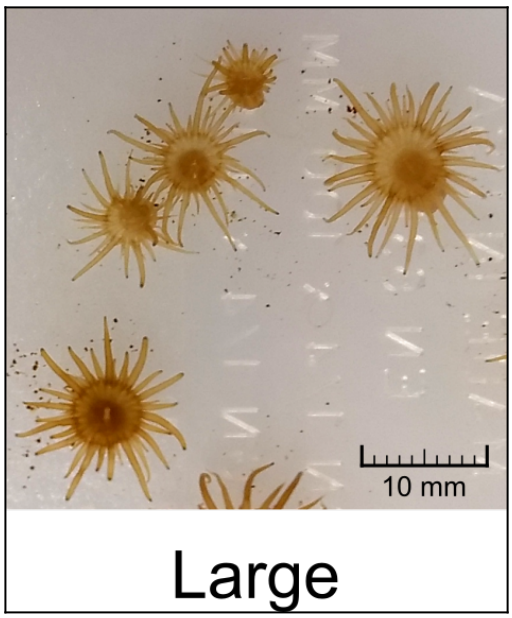


Preparing animals for spawning

Step 6.

Transfer anemones with a pedal disc diameter of 5-6 mm into a medium tank with 500 ml ASW and maximal 10 anemones per tank.

This is the 'large polyps' tank.

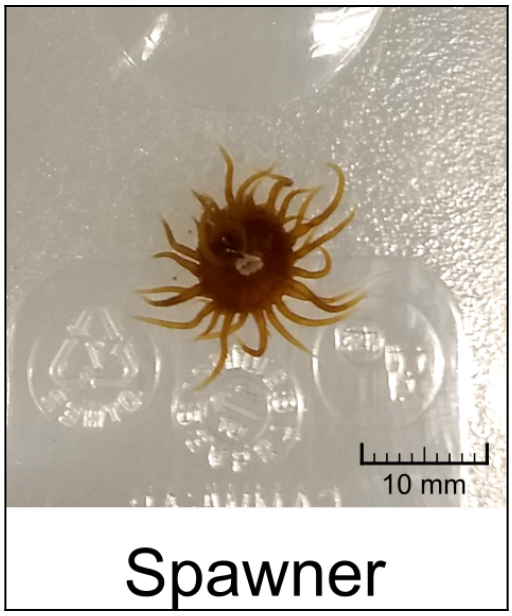


Preparing animals for spawning

Step 7.

When polyps from the 'large polyp' tank reach a pedal disc diameter >8 mm transfer 3 animals of the same strain and sex into a small tank with 200 ml ASW.

This tank is ready to be transferred into the spawning incubator.



Spawning incubator setup and maintenance

Step 8.

The spawning incubators are set to a temperature of 29°C and an inverted 12 h light: 12 h dark rhythm (light from 4 PM to 4 AM, dark from 4 AM to 4 PM). Light flux is 25 µmol photons/m^2/s for day light (cool white fluorescent tubes). A 28-day moon cycle is simulated by applying blue light (LEDs with 450 nm wavelength; we use 'Current USA TrueLumen LED Lunar Lights - 453nm Actinic Blue') with a photon flux of 10 µmol/m^2/s for the first five nights of the cycle followed by 23 dark nights.
The inverted day-night cycle allows to obtain spawing between 9 AM and 10 AM. Spawning usually occurs between 5 and 6 hours after onset of darkness.

To obtain robust spawning, each spawning incubator houses ~12 tanks, each with 3 anemones. The strains and sexes are separated for better control of fertilization. Usually, eggs are the critical factor, therefore 5 tanks with male polyps are in one incubator with 8 tanks of females.
If controlled fertilization is not needed and only developed planula larvae are desired, sexes might be mixed in one tank.

Spawning incubator setup and maintenance

Step 9.

Place new spawning tanks from step 7 into a spawning incubator seven days before full moon starts to acclimate. Each spawning tank is subjected to three moon cycles and then replaced.

Spawning incubator setup and maintenance

Step 10.

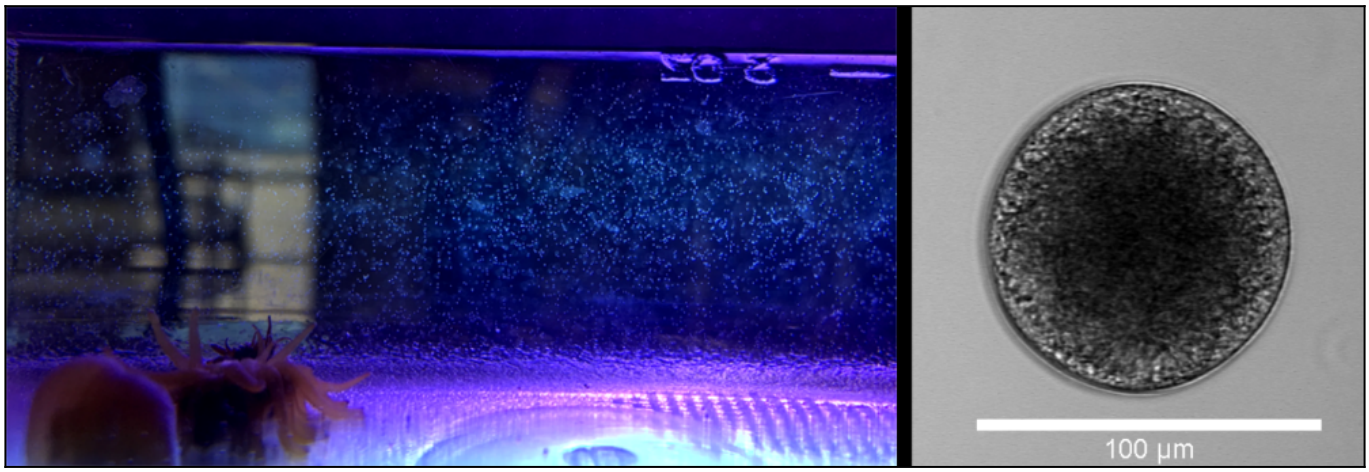
Feed all tanks in the spawning incubators at least four times per week with living brine shrimps in the morning. Change water and clean tanks in the afternoon after onset of 'daylight' in the incubators. Replace tanks if they get too dirty.

Spawning of Aiptasia

Step 11.

The spawning animals are very sensitive to 'out-of-cycle' light cues. Therefore check for spawning in the dark with a red light 5-6 h after onset of the dark phase. Usually, the animals spawn 10-13 days after end of the full-moon phase. Although, they sometimes spawn later during the cycle. Therefore, checking all incubators during on working days helps to not miss any spawning event.

Our full-moon phase is set to Tuesday to Saturday (with different spawning incubators at different weeks) and happens mostly from Tuesdays to Thursdays sometimes on Fridays but extremely rarely on Mondays. Aiptasia eggs are white, round and about 80 µm in diameter. They are visible by bare eyes if released to the water and often sink and form patches on the ground.



Spermatozoa are much smaller (the head is 3-3.5 µm long and around 1.5 µm thick, the tails is about 50 µm long) more difficult to see. If released in moderate to high amounts, the water in the tanks looks slightly opaque.

To confirm sperm in the water, a sample might be viewed on a light microscope with a total magnification of 600x or higher (the microscopic example image actually shows concentrated sperm; see below). A way to ensure that the sample doesn't dry out to fast and the spermatozoa don't get squashed is to use teflon-coated glass slides with multiple wells as often used for immunofluorescence. Another advantage of these slides is that the initial focus plane can be adjusted at the borders of the wells. Once a the focal plane is adjusted it might take some time to find spermatozoa, it might help to change the focus slightly during the search to find a tail and then follow it to the head. The density of the sperm in the water is usually very low (even if it looks cloudy by bare eye). To make observation of sperm more likely centrifugation of 50 ml of the sperm-containing water for 15 min at 3700 ×g can help. Pour out most of the liquid afterwards and resuspend the sperm in the remaining 0.5-2 ml of ASW. To get an even higher concentration a second

centrifugation can be done in microcentrifuge tube for 5 min at 4000 ×g.

Collecting gametes - eggs

Step 12.

If eggs are found and settled to patches on the ground of the tank, they can be transferred into a glass dish (4.5 inches diameter, 250 ml volume) with a disposable polypropylene transfer pipette and diluted with some ASW.

If eggs are floating in the water column, decant the egg-containing water into two of the 250 ml glass dishes.

Don't use polystyrene for handling the unfertilized eggs as this causes damage to them.

Collecting gametes - sperm

Step 13.

Filter the sperm-containig water by two stacked sieves (120 µm and 20 µm mesh width) to remove larger debris particles. Collect the sperm in a glass beaker (at least 250 ml capacity).

In vitro fertilization

Step 14.

To fertilize the eggs with sperm, add 10-15 ml of the filtered sperm-containing water to 100 ml of eggs.

Fertilized eggs can be distributed into 60×15 mm glass petri dishes. Again, don't use polystyrene as it will reduce fertilization and viability.

In vitro fertilization

Step 15.

If the fertilization is successful, embryos in the 4-cell stage should be observable within 1-3 h after fertilization at room temperature.

The lower the temperature, the slower are the divisions.

Ciliated blastulae are usually observable 6-8 h after fertilization and should be developed into planula larvae the next day.