

Cold-shock protocol to bleach Aiptasia

Pringle lab

Abstract

This protocol describes how to use a short-term cold shock to render Aiptasia aposymbiotic. After the cold shock it includes two possibilities to proceed one faster one with usage of DCMU and a slower and more gentle one with only keeping the animals in the dark.

Citation: Pringle lab Cold-shock protocol to bleach Aiptasia. **protocols.io**

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Guidelines

Preparing DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) stock solution:

0.0313 M DCMU in 95-100% ethanol.

For 50 ml, dissolve 7.3 mg in 50 ml ethanol.

For an end concentration of 50 μ M add 32 μ l of the stock to 200 ml of artificial seawater.

Before start

Fluorescence filters:

Every wavelength between 400 and 550 nm efficiently excites the algal chlorophyll.

The chlorophyll fluorescence emission peaks between 650 and 720 nm.

This means that most RFP filter sets with a wide bandpass or longpass emission filter at above 600 nm will work well.

GFP longpass filter sets will work as well but only in combination with an RGB camera to distinguish red algal fluorescence and green autofluorescence of the anemones themselves.

These numbers are only rough estimations based on a few algal strains. For purchasing new filter sets it will be still best to test them before buying.

Materials



DCMU D2425 by Sigma - Aldrich



Artificial seawater by Contributed by users



Fluorescence stereomicroscope by
Contributed by users

Protocol

Preparation

Step 1.

Prepare a tank of anemones.

It is best (but not critical) if you let them sit in the tank until they stick to the sides/bottom of the tank.

Preparation

Step 2.

Chill a large volume of seawater to 4°C.

Initial treatment

Step 3.

Pour out the seawater in the Aiptasia tank and replace it with the 4°C seawater. Place the entire tank into a 4°C refrigerator (or cold room).

Initial treatment

Step 4.

Incubate at 4°C for 4 hours.

 DURATION

04:00:00 Additional info: Cold-shock

Initial treatment

Step 5.

Remove the tank from 4°C, pour out the cold water, and replace with room-temperature seawater.

The animals will be spitting out boluses of algae, which you will see as dark brown balls next to the anemones

Initial treatment

Step 6.

Repeat the water change 2-3 times by pouring out the tank water and replacing it with room-temperature seawater.

Initial treatment

Step 7.

Place the tank into darkness (we use dark cabinets) at room temperature for 1 - 2 days. Continue to replace seawater with room-temperature seawater once a day.

Complete bleaching

Step 8.

You now have two options: a faster method or a slower method. The faster method works quickly but is harsher on the anemones; the slower method is more gentle on the anemones but takes longer.

Fast bleaching

Step 9 - Fast and harsh bleaching (Using DCMU to remove algae faster but with more stress to the animals).

Prepare seawater containing DCMU (or 'diuron': 3-[3,4-dichlorophenyl]-1,1-dimethylurea).

We generally use a final DCMU concentration in seawater between 10 μ M and 50 μ M.

Higher concentrations work faster, but are harsher on the anemones so you may need more frequent water changes.

Fast bleaching

Step 10 - Fast and harsh bleaching (Using DCMU to remove algae faster but with more stress to the animals).

Remove the tank from the dark cabinet. Replace tank seawater with the DCMU-seawater. Incubate at room temperature in constant light for several weeks.

Fast bleaching

Step 11 - Fast and harsh bleaching (Using DCMU to remove algae faster but with more stress to the animals).

Replace the water with DCMU-seawater once or twice a week (depending on how the anemones look: when they appear very unhappy, replace the water). Feed anemones with brine shrimp at least once a week; more feeding is better, so feed 2 - 3 times a week if possible.

Importantly, after each feeding the water must be changed the next day.

Fast bleaching

Step 12 - Fast and harsh bleaching (Using DCMU to remove algae faster but with more stress to the animals).

You can start screening the anemones under a fluorescence stereoscope after 2 - 3 weeks to check for the presence of algae. Continue incubating with DCMU until anemones appear aposymbiotic.

See guidelines section for information about fluorescence filters.

Fast bleaching

Step 13 - Fast and harsh bleaching (Using DCMU to remove algae faster but with more stress to the animals).

When anemones appear aposymbiotic, incubate first a few of them in room-temperature seawater in the light (without DCMU) for 1 week and then check for algal presence under a fluorescence stereoscope.

This step ensures there are no algae still in the anemones: if any are present, they will populate

quickly over the week and become detectable.

If they still contain algae, continue the DCMU treatment. If they remain free of algae, they are fully aposymbiotic.

Bleaching in darkness

Step 9 - Slower and more gentle bleaching (Keeping the anemones in darkness until they are aposymbiotic).

Leave tank in complete darkness with regular room-temperature seawater. Continue to change the water every 1 - 2 days. Feed animals at least once a week, but 2 - 3 times a week if possible: whenever you feed, change the water the next day.

Bleaching in darkness

Step 10 - Slower and more gentle bleaching (Keeping the anemones in darkness until they are aposymbiotic).

You can start screening the anemones under a fluorescence stereoscope after 1.5 months to check for the presence of algae. Continue incubating in the dark until anemones appear aposymbiotic.

Bleaching in darkness

Step 11 - Slower and more gentle bleaching (Keeping the anemones in darkness until they are aposymbiotic).

When anemones appear aposymbiotic, incubate first a few of them in room-temperature seawater in the light for 1 week and then check for algal presence under a fluorescence stereoscope.

This step ensures there are no algae still in the anemones: if any are present, they will populate quickly over the week and become detectable.

If they still contain algae, continue the dark treatment. If they remain free of algae, they are fully aposymbiotic.

Warnings

DCMU is toxic and suspected to be carcinogenic.

[https://chemicalsafety.com/sds1/sdsviewer.php?id=30234487&name=3-\(3%2C4-Dichlorophenyl\)-1%2C1-dimethylurea](https://chemicalsafety.com/sds1/sdsviewer.php?id=30234487&name=3-(3%2C4-Dichlorophenyl)-1%2C1-dimethylurea)