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Homogenate of A. cervicornis

Forked from Homogenate of A. cervicornis

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Stephanie Rosales[1], Ana M Palacio-Castro[1]

[1]University of Miami

Ana M Palacio-Castro
University of Miami

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is to study disease in Acropora cervicornis by using a disease homogenate method. These methods are adapted from methods presented in Muller 2018 https://elifesciences.org/articles/35066.

Materials

MATERIALS

- Filtered and autoclaved seawater (FSW; artificial seawater also works)
- Razor blade
- Falcon tube (50 mL)
- RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #**AM9780
- DNA/RNA Shield **Zymo Research Catalog #**R1100-50
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- Airbrush
- ZR BashingBeadTM Lysis Tubes (0.1 & 0.5 mm) **Zymo Research**
- Clippers
- Air compressor
- Tweezers
- Ruler
- Slate or sterile work surface
- Sterile containers for corals
- Ziplock bags
- Pipette (P1000) and tips

Safety warnings

Wear gloves and masks when dealing with pathogens especially those of unknown origin.

Before start

- Prepare sterilized and 0.2 micron filtered seawater (FSW).
- Collect healthy coral fragments
- Collect diseased coral fragments
- Randomly assign tanks to treatments
- Label and UV Ziploc bags and Falcon tubes and other material taht cannot be autoclaved

1 Obtain healthy and diseased *Acropora cervicornis* fragments. These fragments are preferably collected from the same area and with similar disease progression.

Note

The tissue areas should be similar between healthy and disease corals to prepare diseased and placebo blastates with similar concentrations.

2 Prepare placebo and disease **homogenates**

2.1 Grab healthy fragments from the holding tank.

Note

Sigle branch fragments are easier to blast and to estimate their tissue area.
If cutting different branches, use clean clippers.

2.2 Take a picture of every fragment with a size scale to estimate the amount of tissue blasted.

2.3 Rinse fragments with FSW, and place them in a sterile container with enough FSW to cover them.

2.4 Grab a fragment and hold it over a pre-labeled Ziploc bag (** this will require two people**). 5m

Airbrush the tissue with an airgun and FSW.

Approximately 00:05:00 per fragment

Number of fragments to blast

Note

Work in a fume hood if possible to avoid blasting aerosols.

When working with disease fragments, remove the tissue up to 5 cm above the lesion.

2.5 Pour homogenate in a pre-labeled 50mL falcon tube(s). Measure volume and store in the fridge.

2.6 Grab diseased fragments from the holding tank. And repeat steps 2.2 - 2.5.

2.7 Change gloves

2.8 Bring placebo and diseased homogenates to the same volume by adding FSW

2.9 Add 20 beads to each falcon tube containing ~ 40mL of homogenate and vortex for 10 minutes 10m

00:10:00 each tube

2.10 Save and preserve ~ 500 ul of both homogenates for sequencing.

3 **Dose** experimental corals with the placebo and disease homogenates

3.1 Sterile razor with bleach and scratch a small area of each experimental coral

Note

Create a ring around the coral located ~ 1cm from the bottom

3.2 Apply 500 uL of placebo and disease homogenate above each coral fragments in their respective treatments.

3.3 Add 16 L of seawater to each tank to completely cover the fragments

3.4 Maintained closed system for 3hours

4 **Clean -up**

4.1 Bleach tweezers, clippers, slates, airgun, surfaces

4.2 Discard bags and falcon tubes

Protocol references

Erinn M Muller, Erich Bartels, Iliana B Baums (2018) Bleaching causes loss of disease resistance within the threatened coral species Acropora cervicornis eLife 7:e35066. doi: 10.7554/eLife.35066