Oct 08, 2020

Homogenate of A. cervicornis

Stephanie Rosales[1]
[1](University of Miami)

In Development This protocol is published without a DOI.

Stephanie Rosales

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.

PROTOCOL CITATION

Stephanie Rosales 2020. Homogenate of A. cervicornis. **protocols.io** https://protocols.io/view/homogenate-of-a-cervicornis-biupkevn

CREATED

Jul 21, 2020

LAST MODIFIED

Oct 08, 2020

PROTOCOL INTEGER ID

39535

MATERIALS

NAME	CATALOG #	VENDOR
Filtered and autoclaved seawater (FSW; artificial seawater also works)		
Razor blade		
RNaseZap™ RNase Decontamination Solution	AM9780	Thermo Fisher Scientific
Serological pipette		
DNA/RNA Shield	R1100-50	Zymo Research
10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)		
Airbrush		
1 gallon ziploc bag		
serological pipette controller		
ZR BashingBeadTM Lysis Tubes (0.1 & 0.5 mm)		Zymo Research

SAFETY WARNINGS

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

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.

BEFORE STARTING

- Prepare sterilized and 0.2 micron filtered seawater.
- Collect healthy coral fragments

- Collect diseased coral fragments
- Randomly assign tanks to treatments

1 Sterile razor with bleach and RNA removal reagent then collect coral tissue near the base of the fragment.

1.1 Place tissue in ZR BashingBeadTM Lysis Tubes (0.1 & 0.5 mm) with DNA/RNA shield (lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures)

2 Collect fragments of *A. cervicornis* showing signs of active white-band disease. These fragments are preferably collected from the same area and with similar disease progression.

3 Hold diseased fragment over Ziploc bag (** this will require two people**) and remove tissue by air brushing. Per fragment, remove ~10 cm^2 of live tissue from the diseased fragment by airbrushing off the tissue within 5 cm of the advancing band with 0.2 microns of filter-sterilized seawater.

4 Mix disease homogenate in the bag.

5 With a serological pipette apply 100 mL of homogenate above each coral fragment in the randomly selected disease experimental tanks.

5.1 Save at least 500 ul of homogenate for sequencing.

6 Repeat steps 1-5 with healthy coral samples and add healthy coral homogenate to the control tanks.