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DNA/RNA extraction and cleanup protocol for PCR and tag seq applications with coral samples

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Carsten Grupstra[1], Ally Swank[2], Sarah W. Davies[2]

[1]Florida Atlantic University; [2]Boston University


Carsten Grupstra

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We use this protocol and it's working

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Abstract

The extraction of high-quality genomic material from corals can be difficult due to high concentrations of lipids and carbohydrates in coral tissues. This is especially the case for some corals in the genus *Porites.* This protocol uses a modified PCA protocol to extract combined DNA and RNA from coral tissues stored in 100% ethanol and frozen. The DNA can be diluted and used in varied PCR applications. If the end goal is RNA sequencing, the RNA cleanup protocol can be followed to acquire clean high-quality RNA.

Materials

Materials needed:

DNA/RNA extraction protocol:
a. 100% molecular grade EtOH
b. Water (molecular grade)
c. Phenol:Chloroform:Isoamyl alcohol (PCA; we used Invitrogen 15-593-031)
d. DNA digest buffer (see Table 1)
e. Proteinase K (20 mg/ml)
f. Microcentrifuge tubes (1.5 ml, DNAse and RNAse free)
g. 3M NaOAC (see Table 1)
h. Kimwipes

RNA cleanup protocol:
a. Zymobiomics RNA clean [redacted] concentrator kit (R1013)
b. 100% molecular grade EtoH
c. Molecular grade water
d. Microcentrifuge tubes (1.5 ml, DNAse and RNAse free)
e. Kimwipes

Equipment needed:
i. Vortex
j. Refrigerated centrifuge
k. Heat block or hot water bath
l. Fume hood
m. PCA waste container
n. Ice bucket with crushed ice
o. Sterile forceps and razor blades
p. Pipettes and filter tips

Troubleshooting

Safety warnings

CAUTION: PCA will burn skin, be careful.

Before start

Notes: The PCA protocol results in a combined eluate of DNA and RNA. The DNA is suitable for downstream applications after dilution to eliminate contaminants (try 10-100x dilution). To acquire concentrated high-quality RNA, additional clean up and DNase steps are required (see RNA cleanup protocol below).

Combined DNA and RNA extraction protocol

1 Turn on required equipment: 4°C centrifuge and 42°C heat block/water bath. Sterilize working surfaces and equipment using a 10% bleach solution (remake weekly) for 20 minutes. Then, remove the bleach with a dry paper towel and clean benches and equipment with a 70% ethanol solution.

2 For each sample, add 600 µl of DNA digest buffer and 6 µl proteinase K to a labeled 1.5 ml tube and put on ice.

3 Using sterilized forceps and razor blades, scrape off coral tissue the size of a large rice grain, and grind it to a fine dust in a sterile petri dish. Transfer to the tube from step 2.

4 Vortex all samples for 1-2 minutes.

5 Incubate for 30 mins in 42°C heat block, vortex, and incubate for at least another 30 mins.

6 While incubating, label sterile 1.5 ml tubes and add 30 µl 3M NaOAC and 750 µl 100% EtOH.

7 When the incubation is done, vortex all samples briefly.

8 In the fume hood: Add 600 µl of PCA to each tube and ensure cap is on tight. Make sure to use the lower layer of PCA.

CAUTION: PCA will burn skin, be careful.

9 Vortex tubes for several seconds inside the fume hood. Make sure the sample is properly mixed (looks milky white). Return every sample to ice immediately after mixing.

10 Thoroughly vortex all samples again and return to ice for one minute.

11 Centrifuge all samples for 5 minutes at max speed (we used 32,000 x g) at 4°C and return samples to ice.

12 In the fume hood: pipette 300 µl of the upper aqueous phase from each sample into the tubes from step 6.

Note: It helps to do this 150 µl at a time with a 200 µl pipette and by holding the pipette tip at the top of the top layer of liquid. If you accidentally suck up mucus, eject and restart from step 11. Discard PCA waste in dedicated PCA waste container.

13 Briefly vortex tubes with sample and return to ice.

14 Spin all tubes with sample at max speed for 20 minutes at 4°C. This will pellet the genomic material.

15 Pour off supernatant into liquid waste.

Note: You should be able to see the genetic material in the bottom of the tube as white or red specks. If not, it is advisable to add more tissue in step 3 during future extractions.

16 Add 100 µl 80% EtOH and ensure lid is on tight.

17 Manually invert tubes several times to mix, but don't vortex as this might break the RNA and DNA.

18 Centrifuge at max speed for 5 minutes at 4°C.

19 Pour off ethanol supernatant. Make sure you get as much waste out as possible by tapping or flicking gently. Place tube upside down on kimwipe.

20 Dry tubes for 15-30 minutes until no drops of liquid can be seen in the tube.

21 Leave tubes upright, covered by a Kimwipe, in your tube rack for 10 additional minutes to ensure all EtOH is evaporated.

22 Re-suspend pellet in 30µl molecular grade water and vortex for up to ten seconds. Be aware that vortexing can break the genetic material.

23 If using DNA for PCR, dilute samples 100-fold (e.g., 1 µl of eluate in 99 µl of molecular grade water) and proceed to PCR protocol.

24 If using RNA for RNA sequencing or tag-seq, continue to RNA cleanup and DNAse step.

RNA cleanup protocol

25 **Notes for RNA cleanup**:
- Because the resultant genomic material from Step 24 has high levels of contaminants from Porites mucus, the protocol has been modified to improve contaminant removal. Notably, the RNA is diluted before the initial filter step. Additionally, 80% EtOH is used instead of wash buffer to eliminate the risk of salts in the wash buffer reducing the efficacy of the kit.

- All centrifugation steps are at 14,000 x g for 30 seconds at 4C unless otherwise indicated.

26 Take 25 µl of the final eluate of the previous protocol (step 24) and dilute it with 175 µl molecular grade water (final volume 200 µl). Vortex briefly.

27 Add two volumes of ZymoBiomics RNA binding buffer (400 µl) and pipette up and down to mix.

28 Add an equal volume of 100% EtOH (600 µl) and pipette up and down to mix.

29 Transfer half of the sample to a Zymo-Spin IC column in a collection tube and centrifuge. Pour out the flow-through.

30 Repeat step 5 with the rest of the sample so that all genomic material is bound to the filter.

31 Perform the in-column DNase I step as described in the manual.

32 Add 400 µl RNA Prep buffer to the column and centrifuge. Discard the flow-through.

33 Add 700 µl 80% EtOH to the column, centrifuge and discard the flow-through.

34 Add 400 µl 80% EtOH to the column, centrifuge for 1 minute and discard the flow-through.

35 Centrifuge for two minutes to ensure the filter is dry (dry-spin step). Note: When removing the spin columns from the collection tube, ensure that the eluate does not splash onto the spin column.

36 Transfer the spin column to a sterile 1.5 ml tube.

37 Add 20 µl molecular grade water to the spin column, wait 5 minutes for the RNA to dissolve, and centrifuge.

38 **Table 1. Buffer recipes.**

Recipe for DNA digest buffer
compound Final concentration in DI water
NaCl 100 mM
Tris-Cl 10 mM
EDTA(aq) 25 mM
SDS 0.50%

Recipe for 3M NaOAc pH 5.2 (50 mL)
20.412 g NaOAc in a 50 ml conical
add DI water to fill to 50 ml
6ml Glacial Acetic Acid