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Version 2

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Acropora DNA extraction with Qiagen DNeasy tissue kit V.2

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1 *Works for me* This protocol may be deleted by the owner

Astrangia | Coral Genotyping Protocols

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ABSTRACT

DNA extraction protocol for Acropora or other coral tissue based on Qiagen DNAeasy kit. This extraction protocol works well for the Acropora SNPchip and other coral genotyping applications (such as microsatellite genotyping). It preferrentially extracts coral host DNA but some *Symbiodiniacea* DNA will be present.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Baums IB, Hughes CR, Hellberg MH (2005) Mendelian microsatellite loci for the Caribbean coral Acropora palmata. Mar Ecol Prog Ser 288:115-127. doi:10.3354/meps288115.

ATTACHMENTS

Acroporid tissue DNA extraction.docx

GUIDELINES

This protocol works well on coral tissue perserved in 95% non-denatured Ethanol. Do not overload the columns. A few polyps is enough. Make sure your centrifuge can spin high enough to complete this protocol before you start. Both the the 96-well plate version and the single 1.5 ml tube version of the QIAGEN kit work. For the 1.5ml tube version, we like the electronic mulit-channel MATRIX pipetor with flexible spacing to go from 1.5 ml tubes to PCR tubes/plates. Avoid defrost cycles of samples and extracted DNA.

MATERIALS

NAME	CATALOG #	VENDOR
QIAgen DNeasy Blood and Tissue Kit, 50 rxn	69504	Qiagen
Proteinase K, 2mL	19131	Qiagen
Incubator		
Razor blade		
Centrifuge	5415 D	Eppendorf Centrifuge

Sample preparation

1 Use sterile technique to cut 3-4 polyps (skeleton with tissue) off the coral fragment with a razor blade.

2 Transfer the pieces to a 1.5 ml tube/96-well plate.

3 Add 180 ul of Buffer ATL and 20 ul of proteinase K (from Qiagen kit) to each tube/well.

4 If using the tubes, invert the tubes several times to gently mix the reagents. *Do not vortex*

5 Incubate the mixture overnight at 56 °C.

6 a.The mixture will have a slight yellow hue after lysis from the algal symbionts.

7 b.The tubes or plate can be placed on a rocking platform for gentle agitation.

Qiagen DNeasy Blood and Tissue Kit protocol

8 Follow Qiagen's protocol with the following changes:
Following step 6 of the spin-column protocol, we perform the optional centrifugation for 1 min at 20,000 xg to dry the column membrane.

Elution 2x

9 Elution 1- Add 100 ul of low TE buffer directly to the column membrane, incubate for 1 min and centrifuge as recommended.
a.This elution typically contains smaller fragmented DNA.

10 Elution 2 – Move the column to a new 1.5 ml tube. Repeat elution as above, but increase incubation of 100 ul of low TE to 5 min.
a.This elution contains high molecular weight DNA and is used for downstream analyses.
b.Store at -20 °C.

Check extraction quality

11 Run DNA on a 1.5% agarose gel to check extraction quality of elution 1 and 2. Look for one high molecular weight band at the top of the gel with little smearing.