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Automated DNA/RNA Extractions from a stony coral (*Acropora palmata*) using ZymoBIOMICS DNA/RNA Magbead Kit and the Kingfisher Flex V.1

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ABSTRACT

This is the protocol used to extract DNA and RNA from the same piece of coral tissue and skeleton using the Zymo DNA/RNA MagBead and ZymoBIOMICS DNA/RNA MagBead kits with automation on the KingFisher Flex. Protocol goes from coral sampling and storage to sample prep for the DNA and RNA protocols on the KINGFISHER Flex.

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KEYWORDS

DNA/RNA Dual Extraction, Zymo MagBead, Kingfisher Flex, Coral DNA/RNA Extraction

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DISCLAIMER:

Coral Tissue Sampling and Storage

1 For each sample, bonecutters and tweezers were cleaned with 80% bleach and then flame sterilized with 70% ethanol. The bench space was also cleaned between each sample with 80% bleach and 70% ethanol.

2 All coral samples were stored in 2ml cryo vial tubes (Thermofisher Nalgene 2ml Cryo Vial Tubes) with 1.5ml of RNA later (Thermofisher RNAlater) at -80^{o}C.

Tissue Homogenization and Prep for Kingfisher Flex 1h 15m 30s

3 Thaw stored coral samples at room temperature for 45 minutes. 45m
During this time prep Zymo bead beating tubes (Zymo Bashingbead Lysis Tube) by filling with 750µl DNA/RNA shield.

4 Remove coral skeleton from cryo vial tubes and place in Zymo bead beating tubes filled with 750µl DNA/RNA shield. Bleach and flame with 70% ethanol tools between each coral sample.

5 Using a Genie 2 vortex with 24 tube holder attachment (Vortex 24 Tube Attachment), bead beat 18 tubes MAX (per 24 tube holder attachment) for 30 minutes. 18 samples per attachment is to maintain as high a vortex speed as possible. 30m

5.1 During bead beating step, prep the consumables and reagents needed for the Kingfisher DNA parallel protocol.
DeepWell plates -
Tip plate -
Tip comb -

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Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
Sample in DNA/RNA shield	200	-	Reagent
DNA/RNA Lysis buffer	500	-	Reagent
MagBinding Beads	30	-	Reagent

DNA/RNA Wash 1 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
MagBead DNA/RNA Wash 1	500	-	Reagent

DNA/RNA Wash 2 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
MagBead DNA/RNA Wash 2	500	-	Reagent

Ethanol Wash 1 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

Ethanol Wash 2 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

DNA Elution Plate - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
ZymoBIOMICS DNase/RNase free water	50	-	Reagent

Tip Plate - **96 standard plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
-	-	-	-

NO MANUAL DISPENSED AGENTS

Consumable and Reagent Prep for KingFisher Flex DNA Parallel Protocol.

Make sure all reagents are prepared accordingly following ZymoBIOMICS protocol
- DNA/RNA Wash 1 and 2 are concentrates and require addition of isopropanol.

5.2 Reconstitute lyophilized Proteinase K following ZymoBIOMICS protocol. Store aliquots at -20^{o}C
Reconstitute DNASE following ZymoBIOMICS protocol and storre aliquots at -20^{o}C
- 1 vial of DNASE = 2.3ml DNase/RNase free water
- 2 vials of DNASE (for 96 well plate) = 4.6ml DNase/RNase free water.

6 Centrifuge bead beating tubes for 30 seconds at 10,000g, and transfer 200µl of each sample to a KingFisher deep well plate (LINK). This is the sample plate. 30s

6.1 Bead beating tubes with remaining 550µl DNA/RNA shield, beads and coral tissue can be restored at -80^{o}C indefinitely for future use.

7 To each well with 200µl supernatant, add
- 10µl reconstituted Proteinase K

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- 500µl DNA/RNA lysis buffer
- 30µl ZymoBIOMICS MagBinding beads.

KingFisher Flex DNA Parallel Protocol

8 Initiate the KingFisher DNA Parallel Protocol on the Kingfisher Flex instrument and load all plates as instructed by the machine.

BindIT Script for KingFisher Flex

R2135_ZymoBIOMICS DNARNA Magbead_KingFisherFlex_DNA Parallel Purification_wPause_v2-2.bdz

PDF From BindIT Software

dna.pdf

8.1 After initial binding step, the KingFisher Flex will pause and allow removal of the sample plate. Sample plate with supernatant will be used for subsequent RNA Parallel extraction.

Seal plate and then either
- place on ice if extracting RNA the same day as DNA
- place at -20^{o}C (short term) or -80^{o}C (long term) if extracting RNA at a different point.

8.2 After removal of sample plate, and if running KingFisher RNA Parallel extraction same day, prep all consumables and reagents.

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KF RNA parallel **protocol Plate Information**

Sample plate - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
Supernatant from DNA purification	350	-	Reagent
95-100% Ethanol	350	-	Reagent
MagBinding Beads	30	-	Reagent

DNA/RNA Wash 1 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
MagBead DNA/RNA Wash 1	500	-	Reagent

DNA/RNA Wash 2 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
MagBead DNA/RNA Wash 2	500	-	Reagent

Ethanol Wash 1 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

Ethanol Wash 2 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

RNA Elution Plate - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
ZymoBIOMICS DNase/RNase free water	50	-	Reagent

Tip Plate - **96 standard plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
-	-	-	-

KingFisher RNA Parallel Protocol Reagents and Consumables P1/2

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DNase Treatment - 96 DW Plate

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
DNase I	5	-	Reagent
DNA Digestion Buffer	50	-	Reagent
DNase/RNase free water	40	-	Reagent

Ethanol Wash 3 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

Ethanol Wash 4 - **96 DW Plate**

Name	Well Volume (µl)	Tot Reagent Vol (µl)	Type
95-100% Ethanol	500	-	Reagent

Manual Dispensed Agents

AFTER DNASE TREATMENT

Name	Step	Well Volume (µl)	Tot Reagent Vol (µl)
DNA/RNA prep buffer	Pause	500	-

KingFisher RNA Parallel Protocol Reagents and Consumables P2/2

9 On completion of KingFisher DNA Parallel, remove plate with eluted DNA and place on a magnetic plate and allow to sit for 3 minutes (this step is to remove any residual beads in eluted DNA which can interfere with downstream protocols)

After 3 minutes transfer supernatant to a new 96-well PCR plate, seal and store at -80^{O}C until ready for downstream processing.

A good idea is to aliquot ~6µl to another 96 well PCR plate for quality control (nanodrop, qubit, tapestation) thus reducing freeze thaw cycles of DNA.

KingFisher Flex RNA Parallel Protocol 15m

15m

10 Remove sample plate from ice and allow to sit at room temperature for 15 minutes.
Vortex gently after 15 minutes incubation

11 Transfer 350µl of the supernatant to a new Kingfisher DeepWell plate.
Add 350µl 95-100% EtoH and 30µl MagBinding Beads

11.1 Original sample plate can be resealed and stored at -80^{O}C to be used for failed RNA extractions (remaining volume should be ~350µl).

12 Initiate the KingFisher RNA Parallel Protocol on the Kingfisher Flex instrument and load all plates as instructed by the machine.

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BindIT Script for KingFisher Flex

R2135_ZymoBIOMICS DNARNA Magbead_KingFisherFlex_RNA Parallel_350 uL input_wPause_v2-2.bdz

PDF From BindIT Software

rna_350.pdf

12.1 Prep DNase treatment following manufacturere protocol, and keep on ice until KingFisher Flew pauses to allow addition.
- 1 vial of DNASE = 2.3ml DNase/RNase free water
- 2 vials of DNASE (for 96 well plate) = 4.6ml DNase/RNase free water.

13 After Ethanol Wash 2, Kingfisher Flex will pause and allow addition of the prepped DNase plate. Reinitiate Kingfisher RNA Parallel Protocol on addition of DNase plate.

14 After DNase digestion, KingFisher will again pause and rotate the sample plate to the entry door to allow manual addition of 500µl of DNA/RNA Prep Buffer to each well.

After addition, reinitiate KingFisher Flex RNA Parallel Protocol.

15 Kingfisher will pause one more time to allow switching of consumable deep well plates with reagents.

Reinitiate the KingFisher RNA Parallel protocol after successful replacement of reagents.

16 On completion of KingFisher Flex RNA Parallel protocol, remove the plate with eluted RNA and place on a magnetic stand. Allow to sit for 3 minutes (this is to remove any residual beads which could interfere with down stream protocols).

On completion of 3 minutes, transfer supernatant to a new 96-well PCR plate.

Aliquot ~5µl to another 96-well plate for quality control (nanodrop, qubit, tapestation) and then seal both plates and place at -80^{o}C until ready for downstream processing.

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