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DNA and RNA backups

In 1 collection

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1 *Works for me* dx.doi.org/10.17504/protocols.io.qwpdxdn

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ABSTRACT

This protocol is part of Nucleic acids preparations for Viral to metazoan marine plankton nucleotide sequences from the *Tara* Oceans expedition.

EXTERNAL LINK

https://www.nature.com/articles/sdata201793#methods

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Alberti, A. (2017). Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. *Scientific Data***4**, 170093 (2017)
doi: 10.1038/sdata.2017.93

ATTACHMENTS

Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition.pdf

STEPS MATERIALS

NAME	CATALOG #	VENDOR
illustra™ GenomiPhi DNA Amplification Kit	25-6600	Ge Healthcare
RepliPhi phi29 DNA polymerase		Epicentre
S1 nuclease		Thermo Fisher Scientific
Agencourt GenFind V2 System	A41497	Beckman Coulter Genomics
Qubit		Invitrogen - Thermo Fisher
Agencourt GenFind V2 System	A41497	Beckman Coulter Genomics
DNA Polymerase I (E.coli) - 2,500 units	M0209L	New England Biolabs
DNA Polymerase I (E.coli) - 2,500 units	M0209L	New England Biolabs
Deoxynucleotide Solution Mix - 8 umol of each	N0447S	New England Biolabs

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for warnings and hazard information.

1 After nucleic acids extractions, prepare two RNA aliquots and three DNA aliquots for each sample.

- RNA: use one aliquot for the library preparation and sequencing process, and store the second one as a backup. If RNA quantity is <100 ng, omit backup copy.

- DNA: use the first aliquot for the librayr preparation and the second one for backup copy. The third aliquot is used to produce an amplified DNA backup by whole genome amplification (WGA) by using Illustra GenomiPhi DNA Amplification Kit (GE Healthcare, Little Chalfont, UK) with the procedure described herein

2 Briefly, dilute 10 ng of DNA in 25 µl sample buffer and denature for 3 min at 95 °C.

10 ng DNA

25 µl Sample buffer

95 °C Denaturation

00:03:00 Denaturation

illustra™ GenomiPhi DNA Amplification Kit
by Ge Healthcare
Catalog #: 25-6600

3 Cool samples on ice.

4 Mix samples to 22.5 µl reaction buffer containing random hexamers and 2.5 µl Phi29 enzyme mix and incubate at 30 °C for 3 hours.

22.5 µl reaction buffer containing random hexamers and 2.5 µl Phi29 enzyme mix

30 °C Incubation

00:03:00 Incubation

5 After amplification, heat inactivate Phi29 DNA polymerase during 10 min at 65 °C.

65 °C Heat inactivation

00:10:00 Heat inactivation

6 In order to reduce hyperbranched DNA regions generated by WGA process, incubate amplified DNA with RepliPhi phi29 DNA polymerase without any primer at 30 °C for 2 hours.

RepliPhi phi29 DNA polymerase
by Epicentre

30 °C Incubation

02:00:00 Incubation

7 Inactivate the enzyme at 65 °C for 3 min.

65 °C Incubation

00:03:00 Incubation

8 Digest by S1 nuclease at 37 °C for 30 min.

S1 nuclease
by Thermo Fisher Scientific

37 °C Incubation

00:30:00 Incubation

Clean up the reaction with Agencourt GenFind V2 System following the manufacturer protocol

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Agencourt GenFind V2 System
by Beckman Coulter Genomics
Catalog #: A41497

Omit the lysis step

Repair internal nicks by adding 100 U E. coli DNA polymerase I in100 µl 1X NEB buffer 2 with 4 mM dNTP. Incubate 30 min at 25 °C

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DNA Polymerase I (E.coli) - 2,500 units
by New England Biolabs
Catalog #: M0209L

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DNA Polymerase I (E.coli) - 2,500 units
by New England Biolabs
Catalog #: M0209L

Deoxynucleotide Solution Mix - 8 umol of each
by New England Biolabs
Catalog #: N0447S

25 °C

00:30:00 incubation

12 Purify DNA again with Agencourt GenFind V2 System and resuspend in 200 µl elution buffer.

200 µl Elution buffer

Agencourt GenFind V2 System
by Beckman Coulter Genomics
Catalog #: A41497

13 Quantify DNA with Qubit dsDNA BR and HS Assays and subject to quality check by running 1 µl on 0.7% agarose gel for 60 min at 100 V.

1 µl DNA

01:00:00 Agarose gel

Qubit
by Invitrogen - Thermo Fisher

Store DNA at -20 °C

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