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18S and 16S rRNA genes amplicon generation for eukaryotic and prokaryotic metabarcoding

In 1 collection

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1 *Works for me* dx.doi.org/10.17504/protocols.io.qwhdxb6

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ABSTRACT

This protocol describes the 18S and 16S rRNA genes amplicon generation for eukaryotic and prokaryotic metabarcoding for the *Tara* Oceans expedition and is part of Viral to metazoan marine plankton nucleotide sequences from the *Tara* Oceans expedition.

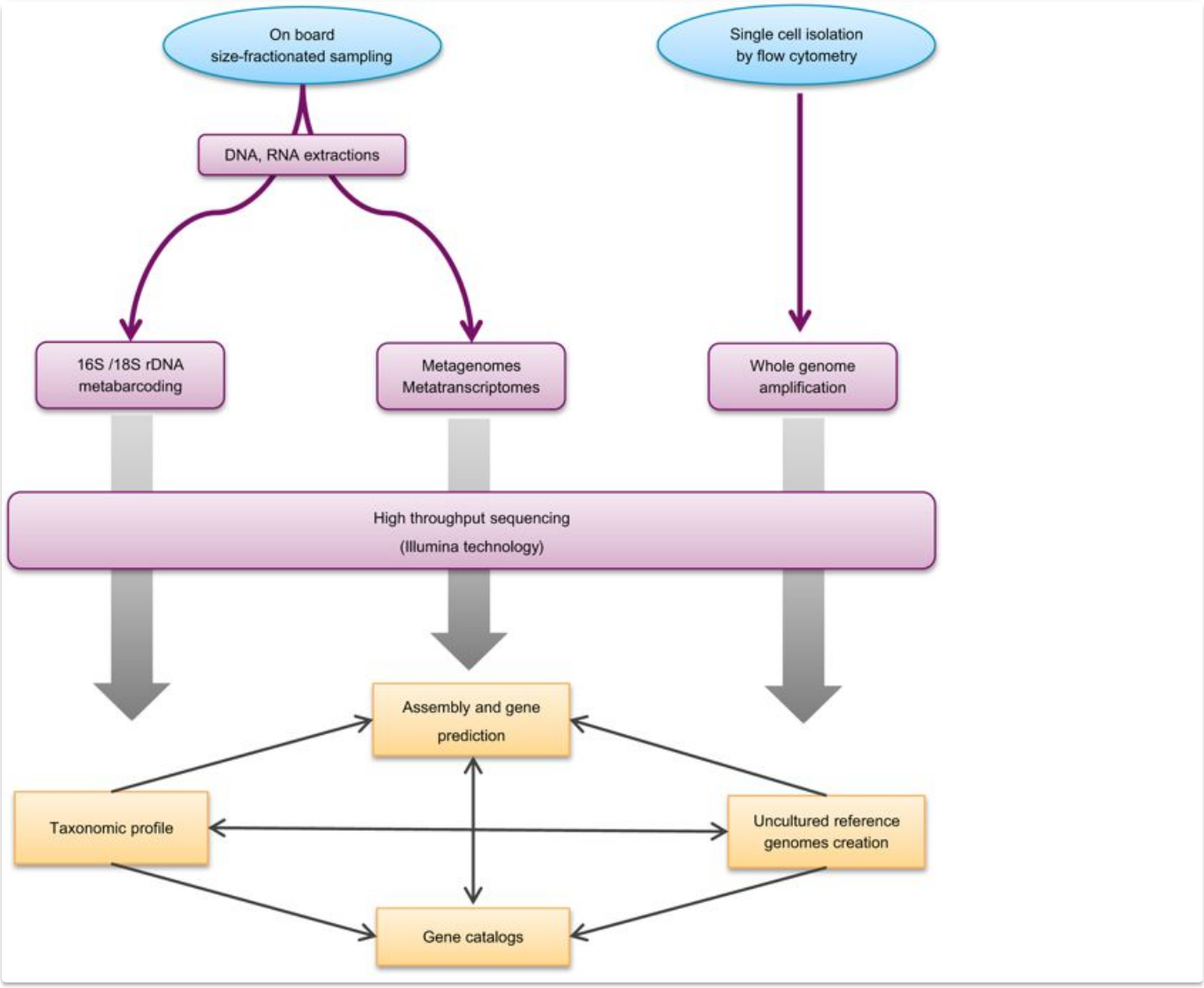

Figure 1: Overview of -omics analysis strategy applied on Tara Oceans samples.

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

GUIDELINES

To address general questions of eukaryotic biodiversity over extensive taxonomic and ecological scales, the hypervariable loop V9 of the 18S rRNA gene was targeted for amplicon generation using DNA extracted from eukaryote-enriched fractions (0.8–5 µm or 0.8–3 µm, 5–20 µm or 3–20 µm, 20–180 µm and 180–2,000 µm) as template. For unravelling prokaryotic biodiversity, V4 and V5 hypervariable loops of 16S rRNA genes were co-amplified from the same DNA templates used for 18S barcoding and from DNA obtained from prokaryote-enriched fractions (0.2–1.6 µm and 0.2–3 µm).

Both these barcodes present a combination of advantages: (i) they are universally conserved in length and simple in secondary structure, thus allowing relatively unbiased PCR amplification across eukaryotic and prokaryotic lineages followed by Illumina sequencing, (ii) they

include both stable and highly variable nucleotide positions over evolutionary time frames, allowing discrimination of taxa over a significant phylogenetic depth, (iii) they are extensively represented in public reference databases across the eukaryotic and prokaryotic tree of life, allowing taxonomic assignment amongst all known lineages.

At the time of publication of this paper, generation of 16S rRNA genes amplicon is still under progress. In Metadata Record, an example of datasets produced by this strategy and available at ENA can be found.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
AMPure XP beads		Beckman Coulter Genomics
NucleoSpin® Gel and PCR Clean-up	740609.10	Macherey and Nagel
Agencourt AMPure XP beads		

SAFETY WARNINGS

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

1 Please select from the following two cases:

1. Eukaryotic 18S rRNA gene amplicon generation (Method ID: 18S_PCR)

2. Prokaryotic 16S rRNA gene amplicon generation (Method ID: 16S_PCR)

step case

18S_PCR

Eukaryotic 18S rRNA gene amplicon generation

18S_PCR

2 For generation of 18S barcodes, perform PCR amplifications with the Phusion High Fidelity PCR Master Mix with GC buffer (ThermoFisher Scientific) and the forward/reverse primer pair 1389F 5'- TTGTACACACCGCCC-3' and 1510R 5'-CCTTCYGCAGGTTCACCTAC-3' as described by Amaral-Zettler et al, 2009.

This primer pair yields a product of around 160 bp.

3 Prepare a PCR mixture containing 5 to 10 ng of total DNA template with 0.35 µM final concentration of each primer, 3% of DMSO and 1X Phusion Master Mix in 25 µl final volume.

PCR is performed in triplicate in order to smooth the intra-sample variance and to obtain sufficient amount for Illumina sequencing.

4 Carry out PCR amplifications with the following program:

initial denaturation at 98 °C for 30 s
25 cycles of 10 s at 98 °C, 30 s at 57 °C, 30 s at 72 °C
final extension at 72 °C for 10 min

PCR is performed with a reduced number of cycles to avoid the formation of chimeras during the plateau phase of the reaction.

5 Purify PCR products by a modified 0.6x AMPure XP beads cleanup in which after binding of DNA to the beads, the supernatant containing larger DNA fragments is kept and subsequently purified with the NucleoSpin Gel and PCR Clean-up kit.

AMPure XP beads
by Beckman Coulter Genomics

NucleoSpin® Gel and PCR Clean-up
by Macherey and Nagel
Catalog #: 740609.10

6 Check amplicons length and amount.

Run aliquots of purified amplicons on an Agilent Bioanalyzer using the DNA High Sensitivity LabChip kit to check their lengths and quantify with a Qubit Fluorometer.

7 Use 100 ng of amplicons to prepare a standard Illumina library avoiding the first DNA fragmentation step.

16S_PCR

step case

16S_PCR

Prokaryotic 16S rRNA gene amplicon generation

2 For generation of 16S barcodes, perform PCR amplifications with the Phusion High Fidelity PCR Master Mix with GC buffer (ThermoFisher Scientific) and the forward/reverse primer pair 515F-Y (5'- GTGYCAGCMGCCGCGGTAA-3') and 926R (5'- CCGYCAATTYMTTTRAGTTT-3') 16S described by Parada *et al*.

This primer pair encompasses the V4 and V5 hypervariable regions, yielding a product of 400 bp.

3 Prepare PCR mixture containing 5 to 10 ng of total DNA template with 0.35 µM final concentration of each primer, 3% of DMSO and 1X Phusion Master Mix in 25 µl final volume.

Perform PCR from each sample in triplicate in order to smooth the intra-sample variance and to obtain sufficient amount for Illumina sequencing.