GENProtocols

Protocol

# Filter Capture, Extraction, and Sequencing of Coastal Metagenomes

OMICS

Elizabeth Murguia, California State University San Marcos
Ciara I. Sanders, California State University San Marcos
Kevin H. García, California State University San Marcos
Jared M. Helms, California State University San Marcos
Matthew L. Cope-Arguello, California State University San Marcos, Current: University of California Davis
Christian Lopezguerra, California State University San Marcos, Current: University of North Carolina Greensboro
Lakmé Caceres, California State University San Marcos, Current: University of California San Diego
Haven Johansen, California State University San Marcos, Current: Western Washington University
Anahita Rahimi, California State University San Marcos, Current: California State University East Bay
Leanna Apodaca, California State University San Marcos
Nicole DeNamur, California State University San Marcos, Current: Tune Therapeutics
Mackenzie V. Pylant, California State University San Marcos
Dennis Zanesco, California State University San Marcos
Uriel U. Rivera, California State University San Marcos
Brad T. Hunter, California State University San Marcos
Sarah N. Eltiste, California State University San Marcos
Elinne Becket, California State University San Marcos

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## Abstract

In order to analyze the metagenomes of coastal microbial communities, we have standardized a protocol to collect, extract, and sequence near-shore microbiome samples. This procedure involves on-site filter capture of microbiomes from coastal water, DNA extraction via ceramic bead processing and silica column purification, and shotgun DNA library preparation to generate high-quality metagenome sequencing libraries for Illumina-based sequencing. Modifications can be made to pursue other sample and sequencing types as needed.

## Introduction

Coastal microbiomes are dynamic and complex, with limited clonality (Pachiadaki et al. 2019). Microbes within these communities readily interact with one another across domains (Wietz et al., 2019). Therefore, cross-domain investigations are essential to interpreting the potential functionality of these communities. Shotgun ("metagenomic") sequencing permits species-level multi-domain analyses with genomic information of abundant organisms (or less abundant organisms as well, depending on sequencing depth) (Ma et al., 2014). Additionally, metagenomic sequencing enables screening for genes of interest within a community, such as antibiotic resistance genes, mobile genetic elements, prophages, and virulence factors, which are important in community function and evolution. While amplicon sequencing (i.e. 16S, 18S, ITS) is a scalable and

affordable approach that provides high-depth taxonomic information, this approach is limited in investigating mid- to high-multiplex loci, across domains, and discovery-based analyses (Ranjan et al., 2016). Here, we developed a collection, isolation, and shotgun metagenome sequencing approach for near-shore microbiomes. However, isolated metagenomic DNA from this protocol can be used for amplicon sequencing or other analyses, if desired.

In this protocol, we describe on-site filter capture of near-shore coastal microbiomes, in order to obtain the most accurate depiction of the microbiome at a selected site and avoid changes to the metagenome composition if filter-captured off-site. Because we are most interested in cellular composition of these communities, we utilized a 0.22 µm pore size for filter capture, but 0.022 µm pore sizes can be used for studies including virus particles as well. While coastal microbiomes are dilute and require filter capturing to obtain needed biomass, listed captured volumes (50 mL) yield enough DNA for metagenomic analyses while minimizing materials needed and multiple filter passes, thereby minimizing potential contamination and field work steps required. Lastly, we use a mechanical lysis approach with bead-beater instrument-specific protocols in order to maximize lysis efficiency while minimizing genomic shearing. Collectively, the protocol described generated high-quality metagenome sequencing libraries for studying coastal metagenomes.

## Procedure

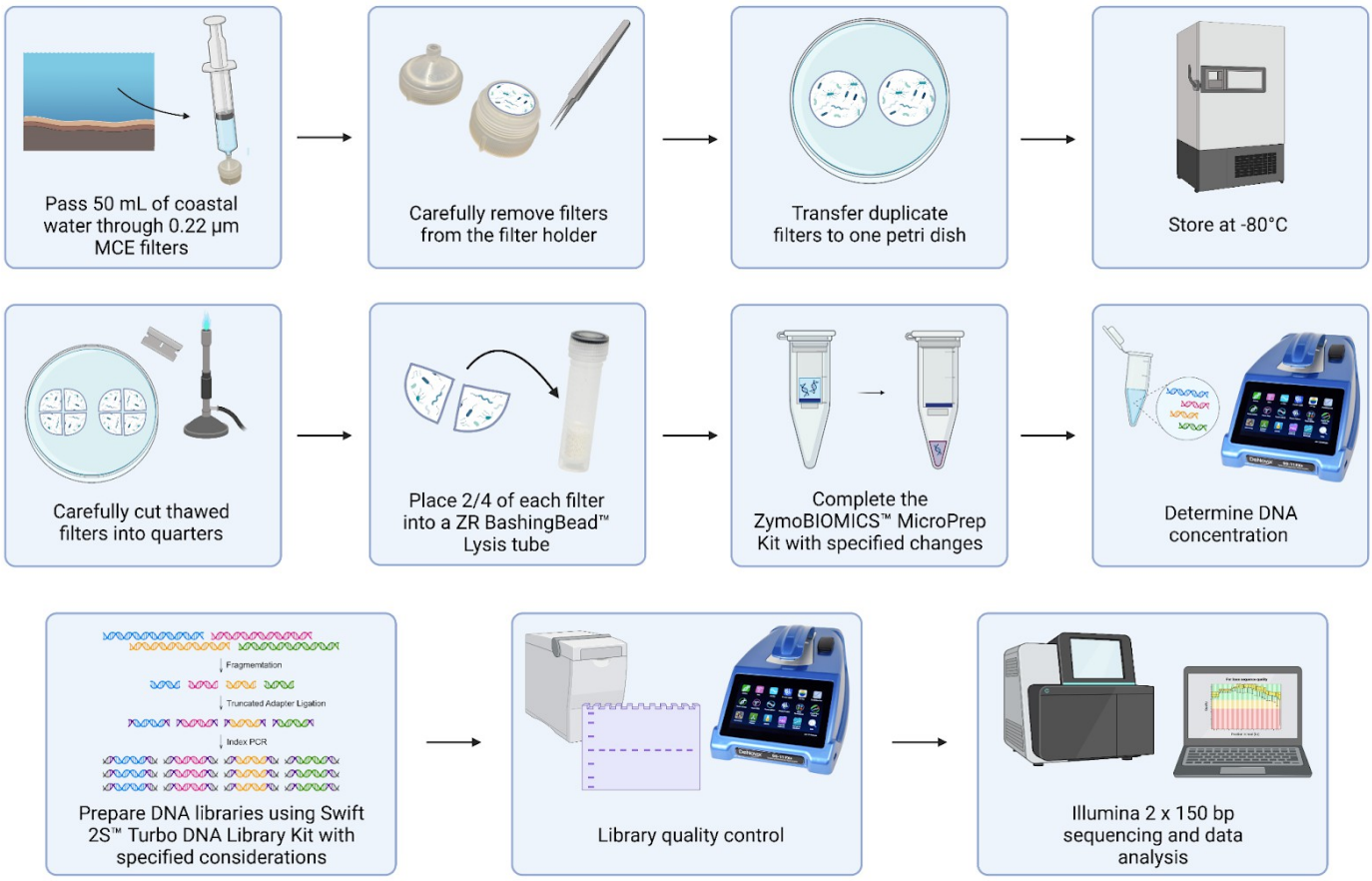


**Fig. 1.** Illustrated workflow described herein.

**On-Site Sample Collection**

1. Beforehand, clean, wrap in foil, and autoclave 25 mm filter holders (EMD Millipore cat # SX0002500) containing 0.22 µm mixed cellulose ester (MCE) membrane filters (Allpure Biotechnology, Amazon cat # B07TNZ52LP). MCE filters were chosen for their high flow rates, hydrophilicity, and high recovery rates.

2. Collect two 50 mL tubes of coastal water (as replicates) approximately knee-deep in the water, submerging the tubes to collect. Careful collection of water is necessary to limit human contamination and collection of sand/sediment.
3. Pass each 50 mL aliquot of coastal water through using sterile 60 mL syringes (FisherSci cat # 14-955-461) and autoclaved filter holders containing MCE membrane filters.
4. After passing the coastal water through the filter, remove filter holders from the syringe and unscrew to open. Carefully transfer the filter membrane to a sterile 60 mm petri dish using sterile tweezers.
5. Collect a duplicate sample on another filter by repeating steps 2 and 3, thus each set of replicates will have one petri dish containing two filters.
6. Tape the petri dish closed, label with the date and time, and keep on ice during transport until stored at -80°C. After all samples throughout the sampling period have been collected, the filters are ready for DNA extraction.

**DNA Extraction**

1. Prepare materials required for the extraction, including:
    a. Sterile tweezers, a sterile razor blade, and 70-100% ethanol to sterilize the tweezers/razor after each use.
    b. ZymoBIOMICS™ DNA MicroPrep Kit (Zymo Research cat # D4301). Be sure that there are enough columns and reagents necessary for the amount of samples you are extracting.
    c. Prepare needed ZR BashingBead Lysis tubes by adding 800 µL of ZymoBIOMICS™ Lysis Solution (cat # D4300-1-40) into each lysis tube.
2. Thaw the filters at room temperature in the closed petri dishes. An ongoing flame is kept in close proximity to the filters to create an updraft.
3. Using the sterile tweezers and razor blade, cut each thawed captured filter into quarters, and place two quarters (one half) of each filter into a ZR BashingBead Lysis tube prepared in step 1c. The remaining half filters are stored in -80°C for backup or subsequent analyses.
4. Follow the protocol for the ZymoBIOMICS™ DNA MicroPrep kit as instructed with the following changes/parameters used below:
    a. The processing time, speed, and number of cycles set using the Fisherbrand Bead Mill 24 Homogenizer (Fisher Scientific cat # 15-340-163) is as follows. Follow the Zymo kit Appendix D instructions if using other bead-beating devices.
        i. Speed = 4.20 m/s. Cycle run time = 1:00 minute. Cycles = 4. Rest between cycles = 3:00 minutes
    b. During each wash buffer step, be sure to invert samples prior to centrifugation to rinse walls of spin column with wash buffer.
    c. After the second Wash Buffer 2 centrifugation, discard the flow through and run samples one more time in the microcentrifuge at 16,000 x *g* speed for 1 minute. We include this "dry spin" to further remove any remaining wash buffer from the column before proceeding to the elution step.
    d. Use an elution volume of 20 µL per sample. Low EDTA buffer (pre-warmed to 60°C) is preferred for elution instead of DNase/RNase Free Water.
    e. Samples were stored in -20°C before sequencing library preparation.

**Library Preparation**

1. To prepare DNA libraries for Illumina sequencing, we used Swift 2S™ Turbo DNA Library Kit (cat # 44024) with the following considerations.
2. Perform DNA quantification of the isolated DNA using the Qubit™ HS dsDNA Assay Kit (Invitrogen™ cat # Q32851) to determine DNA input concentration and the fragmentation time required:
    a. <u>DNA INPUT CONCENTRATION:</u> Choose a standard/target concentration of DNA that all samples will be diluted to. This amount depends on the lowest sample concentration you have, as you will be taking more volume from this sample and will need to make sure that you will have enough.
    b. <u>FRAGMENTATION TIME:</u> Select a fragmentation time depending on the DNA input chosen. The following fragmentation times reflect the most optimal conditions we have found within our past library preparations of these samples:

i. 1 ng - 12 minutes
ii. 5 ng - 12 minutes
iii. 10 ng - 11 minutes

3. The Swift 2S™ Turbo DNA Library Kit protocol was used with no major changes but using the following parameters:
    a. All steps that utilized varying reagent volumes or time points, use "Direct Sequencing" parameters
    b. Parameters chosen for this protocol:
        i. 5 ng initial concentration was used for each sample
        ii. 12 minute fragmentation time
    c. Index PCR:
        i. 8 PCR cycles were used
        ii. Swift Combinatorial Dual Indexing Primers (cat # X8096) were used. Be sure to only use a unique combination of primers by keeping track of which combinations of primers have already been used, else reagents will be used unevenly or can cause limitations in library pooling.
4. For cleanup steps, use Omega BioTek Mag-Bind® TotalPure NGS (cat # M1378), as they behave equivalently to leading magbeads at a lower cost.
5. Final prepared libraries were quality-controlled using the Qubit™ HS dsDNA Assay Kit, and average library fragment size was determined either using 1) end-point PCR + agarose gel using P5/P7 Illumina adapter primers (P5: 5′-AATGATACGGCGACCACCGA-3′, P7: 5′-CAAGCAGAAGACGGCATACGA-3′) or 2) the Agilent Bioanalyzer high sensitivity DNA Analysis kit.

**Results**

Filtering 50 mL of coastal water (Fig 2, left) resulted in visible captured debris (Fig 2, right) on the 0.22 µm MCE filters. DNA extraction resulted in 15-100 ng of DNA per sample, ranging in size from 6-10 kb. Variation in yield was greatest across weather conditions, and yields were similar across replicates. Library preparation using the described parameters resulted in uniform library smears with a 450-700 bp size range (Fig 3). Bioanalyzer analysis revealed no detectable adapter contamination using the described workflow. Low-pass QC sequencing on the Illumina iSeq 100 revealed heterogeneous and consistent signal intensity across all sequencing cycles, and >90% of bases had a Q Score of >=30 (Fig 4).

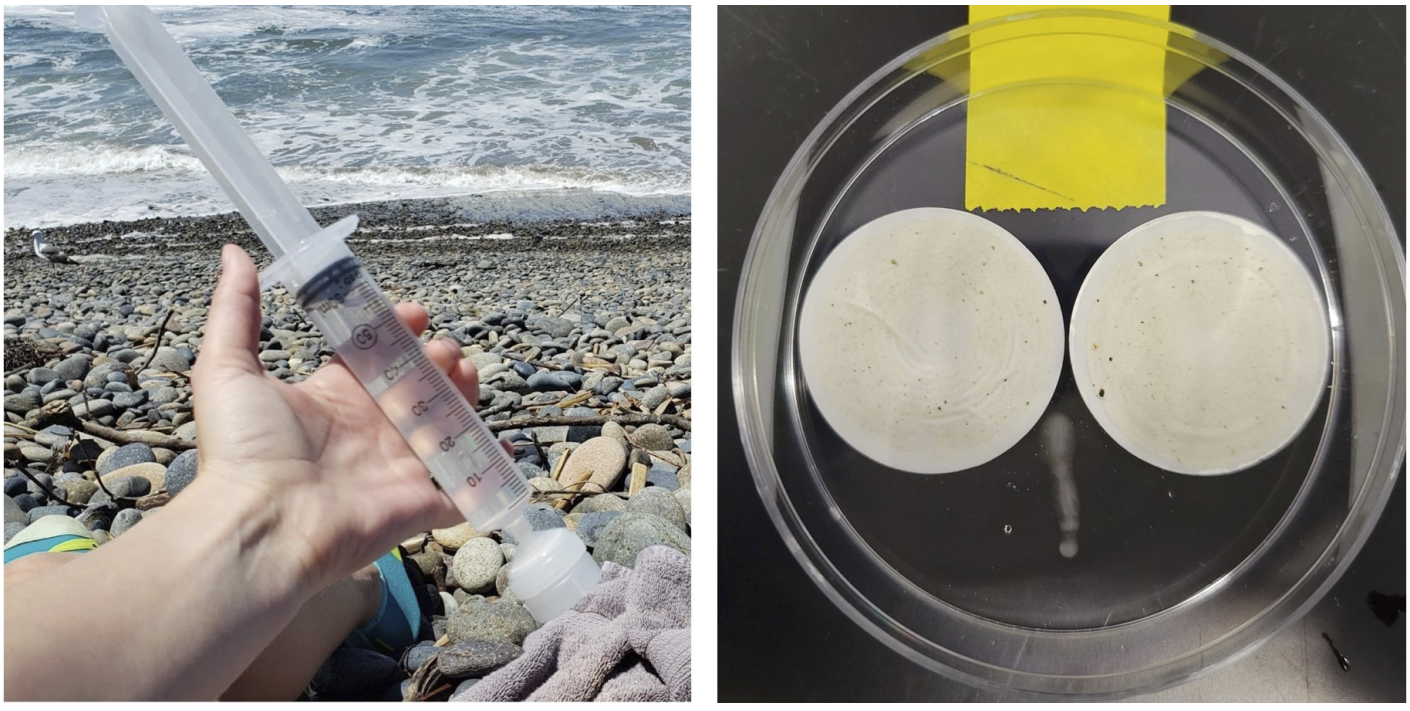

**Fig. 2.** Images of coastal water sampling methods. Left - Photo of the 60 mL syringe filter with coastal water with attached filter

holder. Right - Replicate MCE filters with captured coastal microbiome samples.

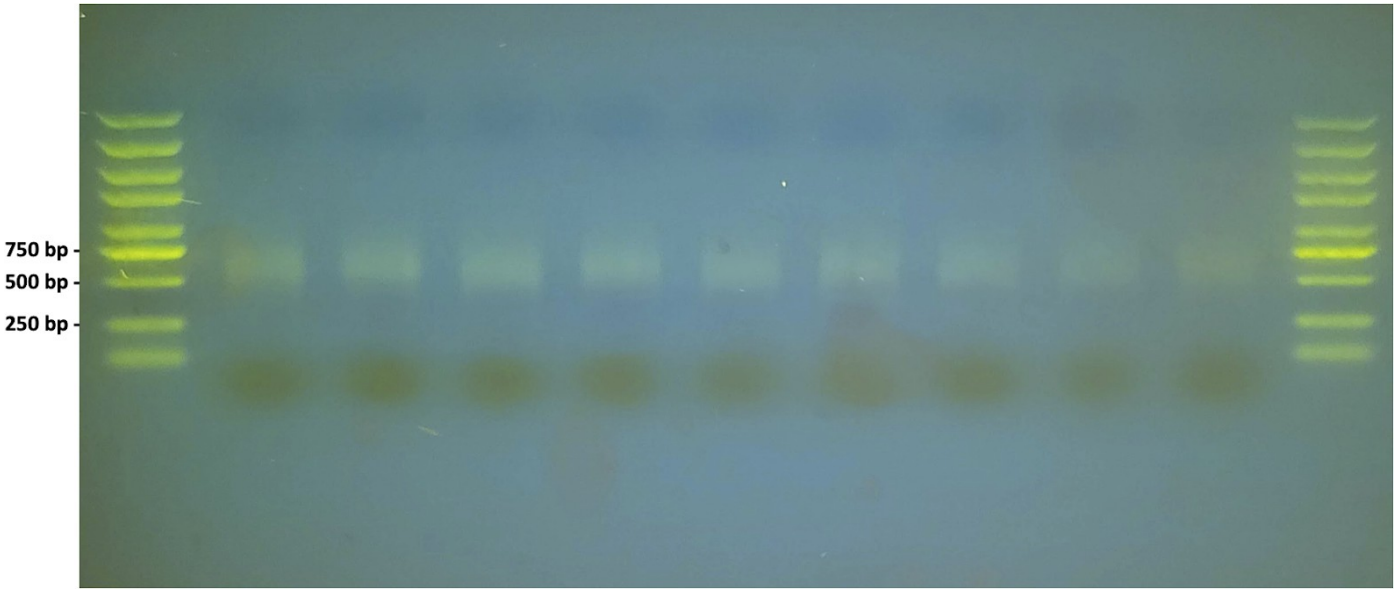


**Fig. 3.** Image of sequencing library fragment size distribution on an agarose gel. 1 µL of each sequencing library was amplified using primers targeting the Illumina P5/P7 adapters for 15 cycles and visualized on an agarose gel on the miniPCR bio™ GELATO™ to determine library fragment length distribution. Initial library preparations underwent analysis via the Agilent Bioanalyzer to ensure sequencing adapter contamination was minimal/not detected.

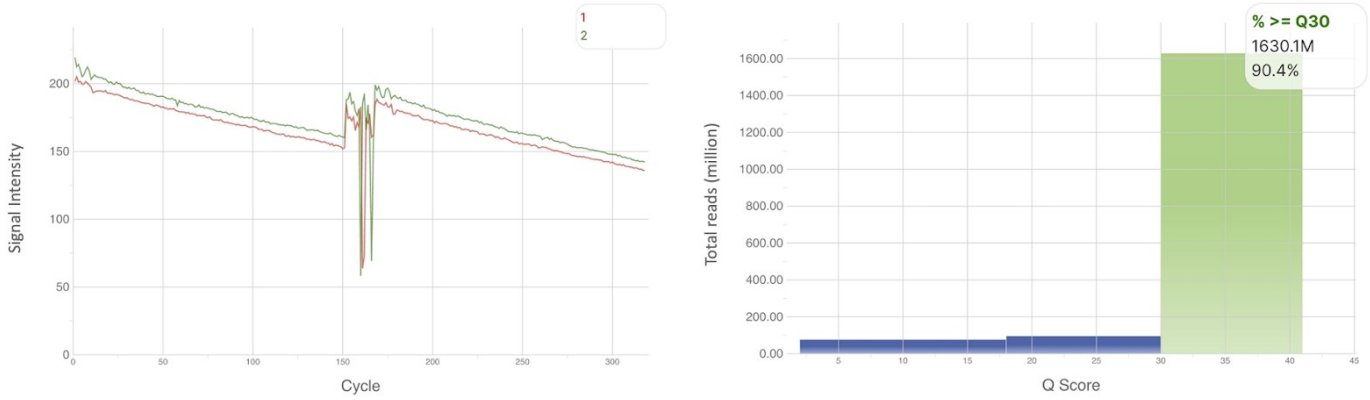


**Fig. 4.** Example quality metrics of an iSeq 100 sequencing run using prepared coastal metagenome libraries. Left - Intensity by color and cycle of the 90% percentile of the data for each tile. Right - The percentage of bases with a quality score of >30.

## Time Taken

1.5 days total, from sampling (1-2 hours with time added for distance), DNA extraction and QC (3-4 hours), to prepared sequencing libraries and QC (7-8 hours).

## Notes and Comments

The o-ring in the filter holders need to be properly cleaned between each use. Small sediment particles will break the seal when filtering. Take extra autoclaved filter holder/filter membranes on site in case of failure. Failure is determined by a faster flow

rate/easier syringe filtration, resulting in low and biased yields.

## References

Jutkina, J., Rutgersson, C., Flach, C. F., & Joakim Larsson, D. G. (2016). An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. The Science of the total environment, 548-549, 131–138. doi: 10.1016/j.scitotenv.2016.01.044.

Ma, J., Prince, A., & Aagaard, K. M. (2014). Use of whole genome shotgun metagenomics: a practical guide for the microbiome-minded physician scientist. Seminars in reproductive medicine, 32(1), 5–13. doi: 10.1055/s-0033-1361817.

Pachiadaki, M. G., Brown, J. M., Brown, J., Bezuidt, O., Berube, P. M., Biller, S. J., Poulton, N. J., Burkart, M. D., La Clair, J. J., Chisholm, S. W., & Stepanauskas, R. (2019). Charting the Complexity of the Marine Microbiome through Single-Cell Genomics. Cell, 179(7), 1623–1635.e11. doi:10.1016/j.cell.2019.11.017

Ranjan, R., Rani, A., Metwally, A., McGee, H. S., & Perkins, D. L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochemical and biophysical research communications, 469(4), 967–977. doi: 10.1016/j.bbrc.2015.12.083.

Wietz, M., Lau, S.C., & Harder, T. (2019). Socio-Ecology of Microbes in a Changing Ocean. Frontiers in Marine Science, 6, 190. doi:10.3389/fmars.2019.00190.

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