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COI library preparation for Illumina MiSeq eDNA metabarcoding - rocky intertidal seawater samples

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We use this protocol and it's working

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Abstract

This protocol describes library preparation for COI amplicon metabarcoding with the Illumina MiSeq system. Seawater samples were collected from California rocky intertidal habitats, filtered using Sterivex capsules, and extracted with Qiagen DNeasy Blood & Tissue kits (see related protocols). This protocol is modified from:

Illumina 16S Sample Preparation Guide
CALeDNA Metabarcoding Library Preparation
Wangensteen, O.S., Palacín, C., Guardiola, M. and Turon, X., 2018. **DNA metabarcoding of littoral hard-bottom communities: high diversity and database gaps revealed by two molecular markers.** *PeerJ*,*6*, p.e4705.

Materials

This protocol requires a laboratory set up for standard PCR, gel electrophoresis and imaging, DNA quantitation, and cold storage.

See CALeDNA **Library Preparation** protocols and Illumina **16S Sample Prep Guide** for detailed lists of instruments and consumables.

Before start

Order Leray XT primer set (Wangensteen et al. 2018) with Illumina overhangs:

Forward overhang: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
Reverse overhang: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG

Forward primer (mlCOIintF-XT): 5′-GGWACWRGWTGRACWITITAYCCYCC
Reverse primer (jgHCO2198): 5′-TAIACYTCIGGRTGICCRAARAAYCA

https://www.idtdna.com/pages/products/custom-dna-rna/dna-oligos/custom-dna-oligos

Amplicon PCR

1 Dilute primers to 5 uM in PCR water. Dilute BSA to 4 mg/ml in PCR water. Set up triplicate PCRs for each sample.

2 Perform PCRs in 20 ul volumes with the following reaction chemistry:

10 ul 2X AmpliTaq Gold 360 Master Mix (Applied Biosystems)
0.75 ul 4 mg/ml bovine serum albumin (BSA)
1 ul 5 uM fwd primer
1 ul 5 uM rvs primer
1.25 ul undiluted DNA template
6 ul PCR grade water

Thermocycling conditions:

Initial denaturation, 95C for 10 min

35 cycles of denaturation, 94C for 1 min; annealing, 45C for 1 min; extension, 72C for 1 min

Final extension, 72C for 5 min, then hold at 4C

3 Pool triplicate reactions for a total 60 ul per sample.

See CALeDNA **Replicate Pooling** protocol

Check PCR products

4 Run gels for all samples to check for successful amplification.

See CALeDNA **Gel Electrophoresis** protocol

Amplicon PCR clean-up

5 Clean PCR products using 0.8X ratio and AMPure XP beads (Beckman Coulter). Use fresh 70% ethanol and elute DNA in 30 ul ultra pure PCR water.

Bead prep: x samples * (0.8)(45 ul sample) * 1.1(10% error) = x ul beads

See CALeDNA **PCR Purification** protocol

Product QC and quantification

6 Check PCR product sizes for a random mix of samples and controls.

Quantify all pooled sample yields.

Normalize

7 Using product yields, dilute samples in a new plate to 10 ng in up to 11.25 ul PCR water per sample. If yield is less than 10 ng, then use 11.25 ul product for indexing reactions.

Index PCR

8 Use IDT for Illumina DNA/RNA Unique Dual Indexes (Sets A-D, 20027213-6).

For each sample, perform PCR in 25 ul reactions with the following reaction chemistry:

12.5 ul KAPA HiFi Hot Start Ready mix (Roche Diagnostics 07958927001)
1.25 ul Illumina UD indexing primer mix
11.25 ul normalized product

Thermocycling conditions:
Initial denaturation, 95C for 5 min

8 cycles of denaturation at 98C for 20 sec; annealing at 56C for 30 sec; extension at 72C for 3 min.

Final extension, 72C for 5 min, then hold at 4C.

See CALeDNA **Indexing PCR** protocol

Index PCR clean-up

9 Check product sizes again (see Step 6).

Determine bead ratio using observed fragment sizes after index PCR. Use bead prep calculation from Step 5, adjusted for 25 ul product and appropriate bead ratio from product size check. We used 0.8X.

Clean PCR products with AMPure XP beads (Beckman Coulter). Use fresh 70% ethanol and elute DNA in 30 ul ultra pure PCR water.

See CALeDNA **PCR Purification** protocol

10 Quantify all indexed sample yields.

Pool libraries

11 Pool cleaned, indexed samples by equal mass, targeting a final library concentration of 10-15 nM in at least 200 ul. Use fragment size to calculate nM from ng/ul.

See CALeDNA **Library Pooling** protocol

12 Quantify pooled library and check fragment size (same methods as in previous steps).

Sequencing

13 Follow Illumina **16S Sample Preparation Guide** starting at page 17.

Prep PhiX Control Kit v3 for 15% spike-in.

Sequence with MiSeq v3 600-cycle kit, 2 × 300 PE reads.

Protocol references

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