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Sample preparation for Illumina MiSeq Dual Index metabarcoding

In 2 collections

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1 Works for me dx.doi.org/10.17504/protocols.io.bn97mh9n

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ABSTRACT

The protocol shows amplicon sequencing samples preoared by Illumina Dual Index strategy, and sequened with Illumina MiSeq (RRID:SCR_016379) for bacteria and microeukaryotes.

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Protocols for " Efficient and stable metabarcoding sequencing data using DNBSEQ-G400 sequencer validated by comprehensive community analyses"

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1 Perform the first PCR (duplicates of each sample) using Illumina adaptor attached primers that target the gene of your choice. Here we present the protocol using the eukaryotic primers 5.8S and ITS4.

The primer sequences	
5.8SR_Nextera	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
	TCGATGAAGAACGCAGCG
ITS4_Nextera	GTCTCGTGGCTCGGAGATGTGTATAAGAGACAG
	TCCTCCGCTTATTGATATGC

The PCR recipe was as follows: **3 mL** of diluted template DNA, **0.3 mL** of forward primer (10 M), **0.3 mL** of reverse primer (10 M), **0.48 mL** of nuclease-free water, **1.2 mL** of 5X KAPA HiFi Buffer, **0.18 mL** of KAPA dNTPs mix (10mM), and **0.12 mL** of KAPA HiFi Hot Start Polymerase, **0.3 mL** DMSO, **0.12 mL** ROX dye (25mM), and **0.003 mL** 1000x SYBR Green dye. We amplified samples using the following cycling conditions: **98 °C** for **00:05:00** ; 20 cycles of **98 °C** for **00:00:20** , **55 °C** for **00:00:15** , and **72 °C** for **00:01:00** .

2 Check PCR products with Agarose gel electrophoresis (optional).

3 Pool PCR duplicate samples together.

4 Second PCR is conducted for attaching standard illumina handles and index primers.

The primer sequences	
Forward indexing primer	AATGATACGGCGACCACCGAGATCTACACXXXXXXXX
	TCGTCGGCAGCGTC
Reverse indexing primer	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTCTC
	GTGGGCTCGG

Where X indicates the positions of the 8-bp indices.

The PCR recipe was as follows: **5 mL** of diluted template DNA, **0.5 mL** of forward primer (10 M),

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0.5 mL of reverse primer (10 M), **1 mL** of nuclease-free water, **2 mL** of 5X KAPA HiFi Buffer, **0.3 mL** of KAPA dNTPs mix (10mM), **0.5 mL** DMSO, and **0.2 mL** of KAPA HiFi Hot Start Polymerase. We amplified samples using the following cycling conditions: **98 °C** for **00:05:00** ; 10 cycles of **98 °C** for **00:00:20** , **55 °C** for **00:00:15** , and **72 °C** for **00:01:00** ; and then a final extension at **72 °C** for **00:10:00** .

5 All mixtures were purified using Agencourt AMPure XP (Beckman Coulter).

6 Pool the PCR samples in equal DNA amount (ng) or for unequal length amplicons, in equal molecule amount (mol).

7 The pooled samples were size selected at 427 bp ± 20%, denatured with NaOH, diluted to 8 pM, and then spiked with 15% PhiX.

8 Load the denatured, diluted pooled library into the loading position of the Illumina reagent cartridge (V3, 600 cycle kit). Load reagent cartridge, flow cell, and PR2 buffer into Miseq instrument, confirm the metrics and start the run.

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