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Sample preparation for Illumina MiSeq Dual Index metabarcoding

In 2 collections

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ABSTRACT

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

- 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	TCGT CGGC AGCG TCAG ATGT GTAT AAGA GACA G
	TCGA TGAA GAAC GCAG CG
ITS4_Nextera	GTCT CGTG GCTC GGAG ATGT GTAT AAGA GACA G
	TCCT CCGC TTAT TGAT ATGC

Note

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	AATG ATAC GGCG ACCA CCGA GATC TACA CXXX XXXX X
	TCGT CGGC AGCG TC
Reverse indexing primer	CAAG CAGA AGAC GGCA TACG AGAT XXXX XXXX GTCT C
	GTGG GCTC GG

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

Note

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