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Library construction of metabarcoding at DNBSEQ-G400 with MGIEasy universal DNA library prep Kit

In 2 collections

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ABSTRACT

Library construcion steps following MGIEasy universal DNA library prep set user manual started from end repair and A-tailing to single strand circularizaiton in chaptor 3. 500 ng of gDNA is used in this Library Construction Protocol. The steps were described bellow.
https://en.mgitech.cn/Uploads/Temp/picture/20191023/5db00efef2bd7.pdf

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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 End-repair and A-tailingsteps are performed with ERAT Enzyme Mix in MGIEasy Universal DNA Library Prep Set (Cat. No: 1000006985).

2 Adaptor ligation step following the instruction bellow by using **5 mL** MGIEasy DNA Adaptors.

3 Cleanup of Adapter-ligated DNA by using DNA clean beads and freshly prepared 80% ethanol, final DNA was elute by **40 mL** of TE buffer.

4 9m 5s

19 µl of purified Adapter-ligated DNA sample with **25 mL** PCR enzyme mix and **6 mL** PCR primer mix were used for PCR amplification. We amplified samples using the following cycling conditions: **98 °C** for **00:03:00** ; 7 cycles of **98 °C** for **00:00:20** , **60 °C** for **00:00:15** , and **72 °C** for **00:00:30** ; and then a final extension at **72 °C** for **00:05:00** .

5 PCR products are cleaned by DNA clean beads. **32 mL** of TE buffer is used to elute the DNA.

6 Quantify the purified PCR products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iTTM PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adapters User Manual. The total yield after pooling should be 1 pmol, with a total volume ≤ **48 µl** .

7 5m

PCR products are stored at **95 °C** for **00:03:00** for denaturation. After the reaction is complete, immediately place the PCR tube on ice for **00:02:00** .

8 30m

Single strand circularization is prepared with single strand circularization reaction mixture consisting of **11.6 µl** splint buffer and **0.5 µl** DNA rapid ligase. The mixture is incubated at **37 °C** for **00:30:00** . The mixture should be immediately placed on ice after the completeness of the reaction.

9 The circulized products are therefore used for sequencing at DNBSEQ-G400.

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