FEB 05, 2021

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

In 2 collections

Xiaohuan Sun¹, Yuehua Hu², Zewei Song¹

¹BGI-Shenzhen; ²CAS Key Laboratory of Tropical Forest Ecology

Xiaohuan Sun

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bn95mh86

Protocol Citation: Xiaohuan Sun, Yuehua Hu, Zewei Song 2021. Library construction of metabarcoding at DNBSEQ-G400 with MGIEasy universal DNA library prep Kit.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.bn95mh86>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Oct 31, 2020

Last Modified: Feb 05, 2021

PROTOCOL integer ID:
 44061

ABSTRACT

Library construction steps following MGIEasy universal DNA library prep set user manual started from end repair and A-tailing to single strand circularization in chapter 3. 500 ng of gDNA is used in this Library Construction Protocol. The steps were described below.

<https://en.mgitech.cn/Uploads/Temp/picture/20191023/5db00efef2bd7.pdf>

- 1 End-repair and A-tailing steps 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 19 μ L 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 . 9m 5s

- 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-iT™ PicoGreen® dsDNA Assay Kit. The required yield for PCR products is ≥ 1 pmol. For pooled sequencing, please follow instructions provided by MGIEasy DNA Adaptors User Manual. The total yield after pooling should be 1 pmol, with a total volume $\leq$ 48 μ L .

- 7 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 . 5m

- 8 Single strand circularization is prepared with single strand circularization reaction mixture consisting of 11.6 μ L splint buffer and 0.5 μ L 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. 30m

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