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Working

The Illumina libraries preparation for the Scapharca broughtonii

In 1 collection

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ABSTRACT

This protocol is used to detail the process of Illumina libraries preparation for the *Scapharca broughtonii* genome.

1 The extracted DNA was sheared into fragments about 350 bp in size using a Bioruptor Pico Sonication System (Diagenode, Seraing, Belgium), and verified by agarose gel electrophoresis.

The sizes of the main fragments should between 200 bp and 500 bp.

2 Fragments with size > 300 bp were purified using VAHTSTM DNA Clean Beads (Vazyme Biotech Co., Ltd, Nanjing, China).

3 Repaired using NEBNext® End Prep Enzyme Mix (NEB, E6091) and NEBNext® End Repair Reaction Buffer (NEB, B6052) keeped at 20°C, 30min; 65°C, 30min with the end-repaired fragments to obtain blunt ends which were then 3'-adenlyated to create sticky ends.

4 These DNA fragments were ligated at both ends to T-tailed adapters and amplified.

5 PCR was performed using NEBNext® Ultra™ II Q5® Master Mix (NEB, M0544), and the Index i7 and Universal i5 preimers. The temperature profile was 30 sec. at 98 °C followed by 10 cycles of 10 sec. at 98 °C, 75 sec. at 65 °C, and more 5 min. at 65 °C for further elongation.

INDEX i7 PRIMER: 5´-CAA GCA GAA GAC GGC ATA CGA GAT ATGACGTC GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC*T-3´
NEBNext Universal i5 PCR Primer 5´-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC*T-3´

6 VAHTSTM DNA Clean Beads (Vazyme Biotech Co., Ltd, Nanjing, China) was used to purify the PCR production.

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