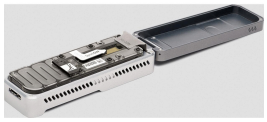
Apr 21, 2019

The Nanopore libraries preparation for the Scapharca broughtonii



GigaScience

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.z8bf9sn

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DOI: dx.doi.org/10.17504/protocols.io.z8bf9sn

External link: https://doi.org/10.1093/gigascience/giz067

Protocol Citation: Chang-Ming CM Bai 2019. The Nanopore libraries preparation for the Scapharca broughtonii. **protocols.io https://dx.doi.org/10.17504/protocols.io.z8bf9sn**

Manuscript citation:
Chang-Ming Bai, Lu-Sheng Xin, Umberto Rosani, Biao Wu, Qing-Chen Wang, Xiao-Ke Duan, Zhi-Hong Liu, Chong-Ming Wang, Chromosomal-level assembly of the blood clam, *Scapharca* (*Anadara*) *broughtonii*, using long sequence reads and Hi-C, *GigaScience*, Volume 8, Issue 7, July 2019, giz067, https://doi.org/10.1093/gigascience/giz067

Protocol status: Working
We use this protocol and it's working

Created: April 21, 2019

Last Modified: April 21, 2019

Protocol Integer ID: 22499

Abstract

This protocol is used to outline the process of Nanopore library preparation for *Scapharca broughtonii* genome.

1 About 6 µg genomic DNA was fragmented using a Megaruptor (Diagenode, Seraing, Belgium) to obtain ~20kb frequments, and verified with Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA).

Note

The sizes of the main fragments should >17 Kb.

2 Fragments with size > 15 Kb were selected using BluePippin Size-Selection system (Sage Science, Beverly, the U.S.), and further purified using AMPure XP beads (Agencourt, Beverly, the U.S.).

3 DNA fragments were end-repaired using NEBNext FFPE Repair Mix (NEB, M6630). The precedures was keeped at 20℃, 15 min.

4 The repaired fragments were purified using AMPure XP beads (Agencourt, Beverly, the U.S.).

5 DNA fragments were end-repaired and dA-tailed by using the NEBNext End repair / dA-tailing Module (NEB, E7546). The precedures was keeped at 20℃, 30 min, and then at 60℃, 30 min.

6 The repaired fragments were purified using AMPure XP beads (Agencourt, Beverly, the U.S.).

7 Nanopore adaptors were ligated to the dA-tailed DNA fragment by using Ligation Sequencing Kit 1D R9 Version (Nanopore, SQK-LSK108) and NEB Blunt/TA Ligase Master Mix (NEB, M0367). The precedures was keeped at 20℃, 60 min.

8 The concerntration of the library was detected with Qubit 3.0 (Thermo Fisher Scientific Inc., Carlsbad, CA, USA), and proved to be qualified for sequencing.

9 This was carried out with Ligation Loading Bead Kit R9 Version (EXP-LLB001) and MinION Flow Cell (FLO-MIN106) according to the user manual.

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