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Working

The PacBio libraries preparation for the Scapharca broughtonii.

In 1 collection

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ABSTRACT

This protocol is used to outline the process of the PacBio libraries preparation for the *Scapharca broughtonii*.

1 The genomic DNA was fragmented using a g-TUBE (Covaris, Brighton, UK) to obtain ~20kb frequments, and verified with Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA).

The sizes of the main fragments should >17 Kb.

2 End-repair and adaptor-ligation were carried out using SMRTbell Template Prep Kit (Pacific Biosciences, p/n 100-259-100). The precedures was keeped at 37°C, 60 min for damage repair; 25°C, 10 min for end repair, and 25°C, over night for adapter ligation.

3 These fragments were firstly selected into size ranges of 12-50 kb using BluePippin Size-Selection system (Sage Science, Beverly, the U.S.), and further purified using AMPure PB beads (Agencourt, Beverly, the U.S.).

4 The purified fragments were re-repaired with SMRTbell Damage Repair Kit (Pacific Biosciences, p/n 100-465-900), and purified using AMPure PB beads.

5 The concerntration and size were detected with Qubit 3.0 (Thermo Fisher Scientific Inc., Carlsbad, CA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA).

6 The qualified fragments were combined with primers, polymerase and Magbeads (Pacific Biosciences, p/n 100-676-500) using PacBio Bingding Kit (Pacific Biosciences, p/n 100-372-700), and ready for sequencing.