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The PacBio libraries preparation for the *Scapharca broughtonii*.

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We use this protocol and it's working

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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 fragments, and verified with Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA).

Note

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 procedures were kept 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 concentration 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 Binding Kit (Pacific Biosciences, p/n 100-372-700), and ready for sequencing.