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Short insert size WGS libraries preparation for assembly of the Lateolabrax maculatus genome

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

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Abstract

This protocol is used to clarity the process of the short insert size WGS libraries preparation for the L. maculatus.

Materials

STEP MATERIALS

- AMPure XP beads **Beckman Coulter**
- T4 DNA polymerase **Enzymatics**
- AMPure XP beads **Beckman Coulter**
- T4 DNA polymerase **Enzymatics**

Protocol materials

- AMPure XP beads **Beckman Coulter**
- T4 DNA polymerase **Enzymatics**
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- T4 DNA polymerase **Enzymatics**

Genomic DNA interruption

1 The extracted DNA was sheared into fragments between 50 bp and ~800 bp in size using a Covaris E220 ultrasonicator (Covaris, Brighton, UK). Treat time 20s, Acoustic Duty Factor 25%, Peak Incident Power 500W, Cycles Per Burst 500, 24 cycles.

00:00:20

Fragment selection

2 Fragments between 150 bp and 250 bp or 200 to 500 bp were selected using AMPure XP beads (Agencourt, Beverly, the U.S.).

AMPure XP beads **Beckman Coulter**

End-repair

3 Repaired using T4 DNA polymerase, (ENZYMATICS, Beverly, the U.S.) 30 min. at 20 ℃ to obtain blunt ends which were then 3′-adenlyated to create sticky ends.

T4 DNA polymerase **Enzymatics**

00:30:00

20 °C

Add adapter

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

PCR amplification

5 The temperature profile was 3 min. at 95 ℃ followed by 8 cycles of 20 sec. at 98 ℃, 15 sec. at 60 ℃, 30 sec. at 72 ℃, and more 10 min. at 72 ℃ for further elongation.

Library purification

6 AMPure XP beads (Agencourt, Beverly, the U.S.) was used to purify the PCR production.