Mate-pair large libraries preparation for assembly of the Lateolabrax maculatus genome

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Abstract

This protocol is used to clarity the process of the mate-pair large libraries preparation for the L. maculatus.

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Protocol

Genomic DNA interruption

Step 1.

The genomic DNA was fragmented using a Covaris E220 ultrasonicator (Covaris, Brighton, UK) to obtain 2kb (for 2 kb library) and a Hydroshear (GeneMachines, CA, USA) to obtain 5 kb, 10 kb and 20kb fragments (for 5 kb, 10 kb and 20 kb libraries respectively).

EQUIPMENT
Equipment brand:
Covaris
SKU:
1
Specifications:
E220 ultrasonicator

EQUIPMENT
Equipment brand:
GeneMachines
SKU:
1
Specifications:
Hydroshear

End-repair

Step 2.

Repaired using T4 DNA polymerase, (ENZYMATICS, Beverly, the U.S.) 30 min at 20 ℃.

TEMPERATURE
20 °C :

REAGENTS

T4 DNA polymerase by Enzymatics

DURATION

00:30:00 :

Biotin Label

Step 3.

Add Biotin Label by Biotin dNTP Mix (5mM) 30 min at 20 ℃.

TEMPERATURE

20 °C :

REAGENTS

Biotin dNTP Mix by Invitrogen - Thermo Fisher

DURATION

00:30:00 :

Fragment selection

Step 4.

These fragments were further selected into size ranges of 2–2.4 kb, 5–5.5 kb, 10–11 kb or 20-23 kb by agarose gel electrophoresis.

Fragment cyclizing

Step 5.

The T4 and T3 DNA ligase were used to connect the ring. And then, Covaris LE220 was used to cyclizing DNA fragments.

REAGENTS

T3 DNA ligase by Enzymatics

End-repair

Step 6.

Fragmented DNA labeled with biotin was captured on M280 streptavidin beads (Invitrogen, CA, USA), followed by end repair (30 min. at 20°C, 1000 rotation per minute, rpm, vibrate for 15 sec. per 2 min.), A-tailing (30 min. at 37°C, 1000 rpm vibrate for 15 sec. per 2 min.).

TEMPERATURE

20 °C :

TEMPERATURE

37 °C :

REAGENTS

M280 streptavidin beads by Invitrogen - Thermo Fisher

DURATION

00:30:00 :

DURATION

00:00:15 :

DURATION

00:30:00 :

DURATION
00:00:15 :

Add adapter

Step 7.

Adaptor ligation (1h at 20°C, 1000rmp vibrate for 15 sec per 2 min.).

TEMPERATURE
20 °C :
DURATION
01:00:00 :
DURATION
00:00:15 :

PCR amplification

Step 8.

PCR amplifications on beads 95°C 3 min., (98 °C 20 sec., 60 °C 15 sec., 72 °C 45 sec.) for N cycles, 72 °C 10 min., 4°C hold (For 2 kb library, N=16; For 5 kb library, 10 kb library and 20 kb library, N=18)] using Enzymatics (MA, USA) and NEB (MA, USA) reagent.

TEMPERATURE
95 °C :
DURATION
00:03:00 :