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

In 1 collection

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ABSTRACT

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

MATERIALS

STEP MATERIALS

- T4 DNA polymerase Enzymatics
- Biotin dNTP Mix Invitrogen - Thermo Fisher
- T3 DNA ligase Enzymatics
- M280 streptavidin beads Invitrogen - Thermo Fisher
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PROTOCOL MATERIALS

- M280 streptavidin beads Invitrogen - Thermo Fisher
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Protocol status: Working

We use this protocol and it’s working

Created: Aug 22, 2018
Genomic DNA interruption

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).

End-repair

2. Repaired using T4 DNA polymerase, (ENZYMATICs, Beverly, the U.S.) 30 min at 20 °C.

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

Biotin dNTP Mix Invitrogen - Thermo Fisher
00:30:00
20 °C

Fragment selection

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

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

T3 DNA ligase Enzymatics

End-repair

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.).

M280 streptavidin beads Invitrogen - Thermo Fisher
00:30:00
20 °C
00:00:15
00:30:00
37 °C
00:00:15

Add adapter

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

01:00:00
20 °C
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.