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Mate-pair large 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 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**
- T4 DNA polymerase **Enzymatics**
- Biotin dNTP Mix **Invitrogen - Thermo Fisher**
- T3 DNA ligase **Enzymatics**
- M280 streptavidin beads **Invitrogen - Thermo Fisher**

Protocol materials

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

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).

Equipment	
new equipment	NAME
Covaris	BRAND
1	SKU
E220 ultrasonicator	SPECIFICATIONS

Equipment	
new equipment	NAME
GeneMachines	BRAND
1	SKU
Hydroshear	SPECIFICATIONS

End-repair

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

T4 DNA polymerase **Enzymatics**

00:30:00

20 °C

Biotin Label

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

Biotin dNTP Mix **Invitrogen - Thermo Fisher**

00:30:00

20 °C

Fragment selection

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

5 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

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.).

M280 streptavidin beads **Invitrogen - Thermo Fisher**

00:30:00

20 °C

00:00:15

00:30:00

37 °C

00:00:15

Add adapter

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

01:00:00

20 °C

00:00:15

PCR amplification

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.

00:03:00

95 °C