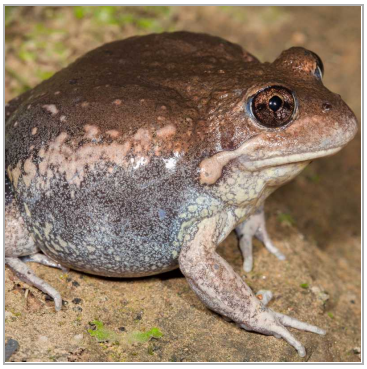

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Construction and sequencing of DNA libraries on Hiseq 2000 platform for the eastern banjo frog

In 2 collections

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ABSTRACT

This protocol is used for construction and sequencing of DNA libraries which include short-insert libraries and mate-paired libraries on the Hiseq 2000 platform for the eastern banjo frog.

Keywords: DNA seq, paired-end sequencing, Hiseq 2000, the eastern banjo frog, Library construction

1 **The following step is the protocol for construction and sequencing of short-insert libraries (170 bp, 250 bp, 500 bp, and 800 bp).**

Genomic DNA interruption

1.1 The extracted 1 μg genomic DNA was randomly fragmented by Covaris E220 ultrasonicator (Covaris, Brighton, UK) to obtain ~170 bp, ~250 bp, ~500 bp, and ~800 bp fragments (for 170 bp, 250 bp, 500 bp, and 800 bp libraries respectively).

End-repair

1.2 Repair by 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

1.3 T-tailed adapters were ligated to both ends of these DNA fragments and amplified.

PCR amplification

1.4 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.

00:03:00
95 °C
00:00:20

98 °C
00:00:15
60 °C
00:00:00
72 °C
00:10:00 more
72 °C

Library purification

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

Sequencing

1.6 After purification, the library was qualified by the Agilent Technologies 2100 bioanalyzer and ABI StepOnePlus Realtime PCR System.
Finally, the qualified libraries were sequenced paired-end using Hiseq System (Illumina).

2 **The following step is the protocol for construction and sequencing of mate-paired libraries (2 kb, 5 kb, 10 kb, and 20 kb).**

Genomic DNA interruption

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

End-repair

2.2 Repair by using T4 DNA polymerase (ENZYMATIC, Beverly, the U.S.) 30 min at 20 °C.

00:30:00
20 °C

Biotin Label

2.3 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

2.4 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

2.5 The T3 DNA ligase was used to connect the ring. And then, Covaris LE220 was used to cyclize DNA fragments.

T3 DNA ligase **Enzymatics**

End-repair

2.6 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

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

01:00:00
20 °C
00:00:15

PCR amplification

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

95 °C
00:03:00
98 °C
00:00:20
60 °C
00:00:15
72 °C
00:00:45
72 °C
00:10:00
4 °C

Library purification

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

Sequencing

2.10 After purification, the library was qualified by the Agilent Technologies 2100 bioanalyzer and ABI StepOnePlus Realtime PCR System.
Finally, the qualified libraries were sequenced paired-end using Hiseq System (Illumina).