BGISeq-500 library construction Protocol

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Abstract

This single-tube library construction protocol is for degraded DNA using BGISeq-500 sequencing platform.

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Before start

Calculate the DNA input and prepare the library adapters.

Protocol

End-repair

Step 1.

End-repair step following Carøe et al. 2017 with the addition of 1 µl "Reaction enhancer" consisting of 25% Poly-Ethylene Glycol (PEG4000); 2 µg/µL Bovine Serum Albumin (BSA) and 400 mM NaCl.

NOTES

GigaScience Database 02 Jun 2017

Carøe C, Gopalakrishnan S, Vinner L, Mak SST, Sindin MHS, Samaniego JA, et al. Single-tube library preparation for degraded DNA. Methods in ecology and evolution; 2017; in press.

Ligation

Step 2.

Ligation step following Carøe et al. 2017 by using 1 µl 10 µM BGISeq-500 adapters (Supplemental Table S4).

NOTES

GigaScience Database 02 Jun 2017

Carøe C, Gopalakrishnan S, Vinner L, Mak SST, Sindin MHS, Samaniego JA, et al. Single-tube library preparation for degraded DNA. Methods in ecology and evolution; 2017; in press. Supplemental Table S4 (This study)

Adpater Fill-in & library purification

Step 3.

Fill-in step of the adapter to complete library building and purification using 1:5 volume of PB binding buffer (Qiagen) and using Monarch DNA Cleanup Columns (New England Biolabs, Massachusetts, USA).

NOTES

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Adpater Fill-in & library purification

Step 4.

Wash with 750 µl buffer PE (Qiagen).

Adpater Fill-in & library purification

Step 5.

Incubate for 5 minutes at 37 °C.

DURATION

00:05:00

Adpater Fill-in & library purification

Step 6.

Elute in 40 µl buffer EB (Qiagen).

Quantitative real-time PCR (qPCR)

Step 7.

To determine the number of cycles used in index PCR, performe a qPCR in a 20 µl reaction volume using 1:20 dilution of:

- purified library template
- 0.2 mM dNTPs (Invitrogen)
- 0.04 U/µl AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California, USA)
- 2.5 mM MgCl2 (Applied Biosystems)
- 1X GeneAmp® 10X PCR Buffer II (Applied Biosystems)
- 1 µl SYBR Green (Invitrogen, Carlsbad, California, USA)

- 0.2 µM forward and reverse primers mixture(CommonprimerBGI forward primer and one of the indexed reverse primers) (Supplemental Table S4)
- 13.48 µl AccuGene molecular biology water (Lonza)

Quantitative real-time PCR (qPCR)

Step 8.

qPCR cycling conditions:

cycles	temprature	time
1	95 °C	10 min
40	95 °C	30 sec
	60 °C	60 sec
	72 °C	60 sec

(using the Agilent MX3005 qPCR machine)

PCR amplification

Step 9.

100 µl PCR reactions containing:

- 20 µl of purified library
- 0.2 mM dNTPs (Invitrogen)
- 0.1 U/µl AmpliTaq Gold DNA polymerase (Applied Biosystems)
- 2.5 mM MgCl2 (Applied Biosystems)
- 1X GeneAmp® 10X PCR Buffer II (Applied Biosystems)
- 0.4 mg/ml BSA (New England Biolabs Inc)
- 0.2 µM of each forward (CommonprimerBGI forward primer)
- indexed reverse primers (Supplemental Table S4)
- 51.2 µl AccuGene molecular biology water (Lonza, Basel, CH)

PCR amplification

Step 10.

PCR cycling conditions:

cycles	**temprature**	**time**
initial denaturation	95 °C	12 min
13-21	95 °C	30 sec
	60 °C	30 sec
	72 °C	40 sec

elongation step	72 °C	5 min

PCR amplification

Step 11.

Post-PCR, purify libraries with QiaQuick columns (Qiagen).

PCR amplification

Step 12.

Incubate libraries for 10 min at 37 °C.

DURATION
00:01:00

PCR amplification

Step 13.

Elute libraries with 30 µl buffer EB (Qiagen).

Bead purification (optional)

Step 14.

Use small amounts of aliquots after purification for concentration quantification and fragment size estimation with the High-Sensitivity DNA Assay kit 2100 expert High Sensitivity DNA Assay in Bioanalyzer 2100 (Agilent).

Bead purification (optional)

Step 15.

Split each purified library to 2 aliquots (12.5 µl each), and subject one of each aliquot to an extra purification to remove any residual primer dimers.

Bead purification (optional)

Step 16.

Extra purification using the AMpure XP system (Agentcourt, Beckman Counter, Indianapolis, USA) with 1.8X beads:library ratio, in order to remove any persisting primer dimers or other molecules with a fragment size of <100 bp.

Quantification

Step 17.

Use small amounts of aliquots after purification for concentration quantification and fragment size estimation with the High-Sensitivity DNA Assay kit 2100 expert High Sensitivity DNA Assay in Bioanalyzer 2100 (Agilent).

Quantification

Step 18.

Circularisation and sequencing in BGI.