AUG 16, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.38kggruw

Protocol Citation: Eric J. Carpenter, Naim Matasci, Shuangxiu Wu, Jing Sun, Jun Yu, Fabio Rocha Jimenez Vieira, Chris Bowler, Richard G. Dorrell, Matt Gitzendanner, Ling Li, Wensi Du, Kristian Ullrich, Michael S. Barker, James H. Leebens-Mack, Gane Ka-Shu Wong 2019. Hiseq 2000 Library Construction and Sequencing for RNA Seq. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.38kggruw>

MANUSCRIPT CITATION:
<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0050226>

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Protocol status: Working
 We use this protocol and it's working

Hiseq 2000 Library Construction and Sequencing for RNA Seq

In 1 collection

Eric J. Shuangxiu
 Carpenter¹, Naim Matasci^{2,3}, Wu⁴, Jing Sun⁴,
 Chris
 Jun Yu⁴, Fabio Rocha Jimenez Vieira⁵, Bowler⁵,
 Richard G.
 Dorrell⁵, Matt Gitzendanner⁶, Ling Li⁷, Wensi Du⁷,
 Michael S. James H. Leebens-
 Kristian Ullrich⁸, Barker⁹, Mack¹⁰,
 Gane Ka-Shu
 Wong¹¹

¹Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada.;

²CyVerse, University of Arizona, Arizona, U.S.A.;

³Current address: Lawrence J. Ellison Institute for Transformative Medicine, University of Southern California, Los Angeles, CA 90033, U.S.A.;

⁴CAS Key Laboratory of Genome Sciences and Information, Beijing, Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China.;

⁵École Normale Supérieure, Paris.;

⁶Department of Biology, University of Florida, Gainesville, Florida 32611, USA.;

⁷BGI-Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen 518083, People's Republic of China.;

⁸Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Biology, Plön, Germany.;

⁹Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ 85721 USA.;

¹⁰Department of Plant Biology, University of Georgia, Athens, GA 30602, USA.;

¹¹Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada.BGI-Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen 518083, People's Republic of China.Department of Medicine, University of Alberta, Edmonton, Alberta, T6G 2E1, Canada.

GigaScience Press

BGI

Created: Jun 15, 2019

Last Modified: Aug 16, 2019

PROTOCOL integer ID: 24556

Keywords: Hiseq 2000, Library Construction, Sequencing, RNA Seq

Haorong Lu

ABSTRACT

Hiseq 2000 Library Construction and Sequencing for RNA Seq

MATERIALS

STEP MATERIALS

DNaseI NEB

Dynabeads mRNA purification kit Life Technologies

fragmentation buffer Life Technologies

SuperScript II reverse transcription kit Life Technologies

RNase H Life Technologies

DNA polymerase Enzymatics

QIAquick PCR purification kit Qiagen

Agencourt AMPure beads Beckman Coulter

T4 DNA polymerase and T4 polynucleotide kinase Enzymatics

Klenow (3' to 5' exo-) Enzymatics

dATP GE Healthcare

Phusion DNA polymerase NEB

1 Isolate polyA RNA from 20 µg of total RNA treated by

DNaseI NEB

using

Dynabeads mRNA purification kit Life Technologies

.It is best to use up to 50 µg as the use of a lower mass (typically 20 µg) has been insufficient for successful library construction. This can be assessed by running final PCR products on an agarose gel; the library construction is considered to have failed when there was no visible band. It is possible to use less than 20 µg of total RNA when isolation of an important sample yielded

low RNA mass but library construction was successful.

2 Purified polyA RNA is fragmented in a

fragmentation buffer Life
Technologies

at 70 °C for 00:01:30 to 200–300 nt fragment sizes.

3 The first cDNA strand is then synthesized with random hexamer primers using the

SuperScript II reverse transcription kit Life
Technologies

.

4 The second-strand synthesis is performed by incubation with

RNase H Life Technologies

and

DNA
polymerase Enzymatics

.

5 Short double-stranded cDNA fragments are then purified using one of two methods.

6 Our standard procedure was to use the

QIAquick PCR purification
kit Qiagen

, whereas for samples with low RNA mass it is better to use

Agencourt AMPure beads Beckman
Coulter

.

7 Both methods are then followed by end-repair with Klenow polymerase,

T4 DNA polymerase and T4 polynucleotide
kinase Enzymatics

.

8 A single 3' adenosine (A base) was added to the double-stranded cDNA using

Klenow (3' to 5' exo-
) Enzymatics

and

dATP GE Healthcare

.

- 9 The Illumina PE Adapter oligo mix is ligated onto the A base on repaired double-stranded cDNA ends and DNA fragments of a selected size are then gel-purified to make sure the insert size is 200 bp ($\pm 10\%$ deviation).
- 10 Thereafter, libraries were amplified by 15 cycles of PCR with
Phusion DNA
polymerase NEB
and "indexed" paired-end PCR primers; the prepared libraries were 322 bp long.
- 11 The amplified libraries were denatured with sodium hydroxide and diluted to
2.5 picomolar (pM) in hybridization buffer for loading into a HiSeq flowcell.
- 12 Read lengths viewed on the HiSeq platform were predominately 90 bp with a small number of sequences in the 84–87 bp range.
- 13 Samples are sequenced with paired-end reads, and up to eleven samples can be multiplexed into a single lane of the Illumina HiSeq flow cell. With average run time of three to twelve days depending on read length.