



Aug 16,
2019

Illumina GAI Library Construction and Sequencing for RNA Seq

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

1 Works for me dx.doi.org/10.17504/protocols.io.38mgru6



ABSTRACT

Illumina GAI Library Construction and Sequencing for RNA Seq

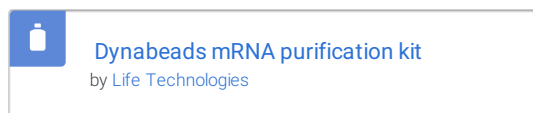
STEPS MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
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

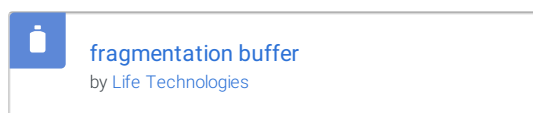


using



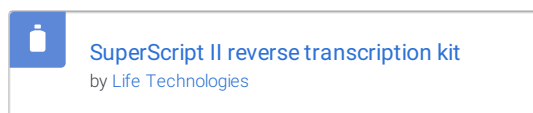
.It is best to use up to 50 µg as the use of a lower mass (typically 20 µg) has been insufficient for successful library construction. This can be assessed by running final PCR products on an agarose gel; the library construction is considered to have failed when there was no visible band. It is possible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

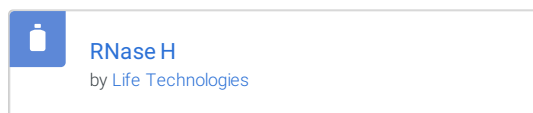


at  70 °C for  00:01:30 to 200–300 nt fragment sizes.

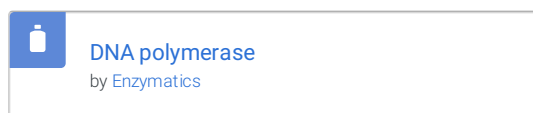
3 The first cDNA strand is then synthesized with random hexamer primers using the



4 The second-strand synthesis is performed by incubation with

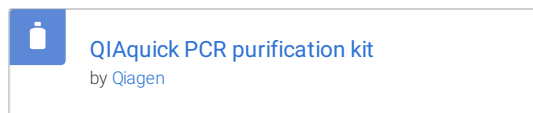


and

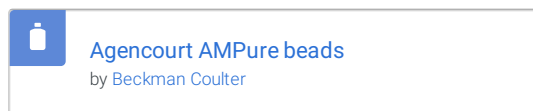


5 Short double-stranded cDNA fragments are then purified using one of two methods.

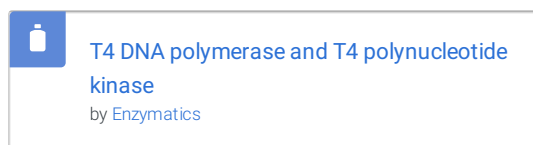
6 Our standard procedure was to use the



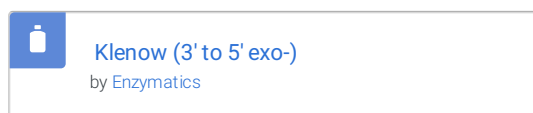
, whereas for samples with low RNA mass it is better to use



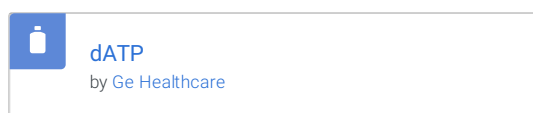
7 Both methods are then followed by end-repair with Klenow polymerase,



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

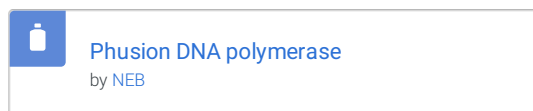


and



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



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 GAIi lane.

12 Read length on the GAIi platform are typically adjusted to 73–75 bp (mean=74 bp), but can be read at 100 bp..

13 Samples are sequenced with paired-end reads, and average run times are about 5 days.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited