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Hiseq 2000 Library Construction and Sequencing for RNA Seq

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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**

Protocol materials

- Dynabeads mRNA purification kit **Life Technologies**
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- 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 (±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
[M] 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.