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Hiseq 2000 Library Construction and Sequencing for RNA Seq

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



ABSTRACT

Hiseq 2000 Library Construction and Sequencing for RNA Seq

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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

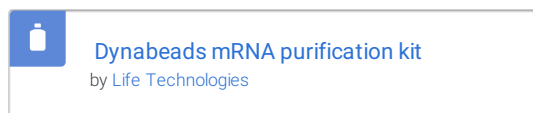
STEPS MATERIALS

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

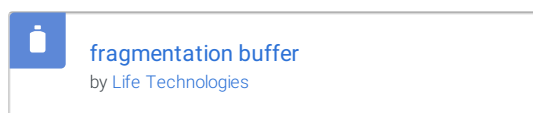


using



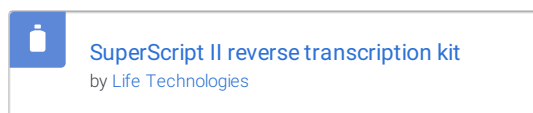
.It is best to use up to 50 µg as the use of a lower mass (typically 20 µg) has been insufficient for successful library construction. This can be assessed by running final PCR products on an agarose gel; the library construction is considered to have failed when there was no visible band. It is possible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

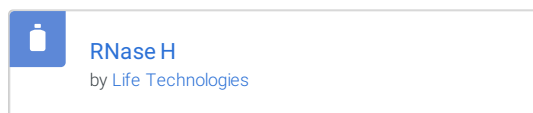


at  70 °C for  00:01:30 to 200–300 nt fragment sizes.

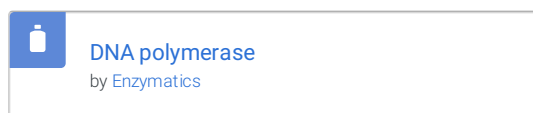
3 The first cDNA strand is then synthesized with random hexamer primers using the



4 The second-strand synthesis is performed by incubation with

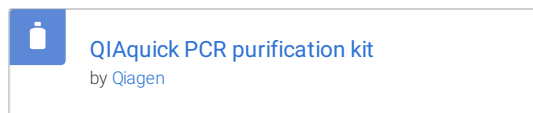


and

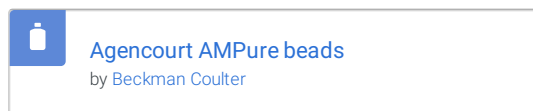


5 Short double-stranded cDNA fragments are then purified using one of two methods.

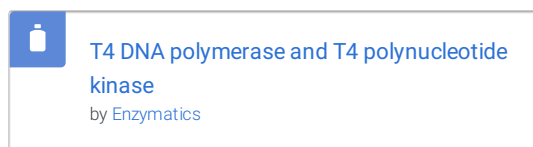
6 Our standard procedure was to use the



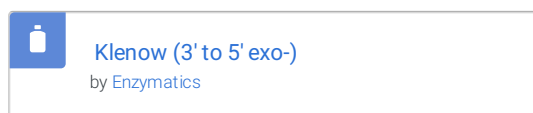
, whereas for samples with low RNA mass it is better to use



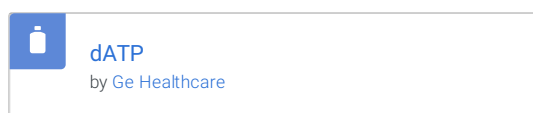
7 Both methods are then followed by end-repair with Klenow polymerase,



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

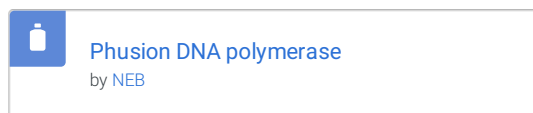


and



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 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.



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