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 We use this protocol and it's working

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Illumina GAII Library Construction and Sequencing for RNA Seq

In 1 collection

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ABSTRACT

Illumina GAll Library Construction and Sequencing for RNA Seq

MATERIALS

STEP MATERIALS

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

DNaseI NEB

Dynabeads mRNA purification kit Life
Technologies

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 GAI lane.
- 12 Read length on the GAIx 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.