NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : **KAPA RNA HyperPrep Kit with RiboErase (KK8560, KK8561)**
Manufacturer : **KAPA BIOSYSTEMS**
Application : **Evaluation test for selecting a library preparation kit for effective standard RNA-Seq**
～ rRNA depletion RNA-Seq using embryonic stem cells (ES cells) and primitive endoderm cells (PrE cells) ～

The following application data are through the courtesy of Ms. Mana Umeda, Mr. Tetsutaro Hayashi, Mr. Yohei Sasagawa, and Mr. Itoshi Nikaido, Bioinformatics Research Unit, RIKEN Advanced Center for Computing and Communication, Japan (Laboratory URL: http://bit.riken.jp/).

Introduction (NIPPON Genetics Co., Ltd.)

KAPA RNA HyperPrep Kit* is the new kit enabling the streamlined workflow by integrating several steps of enzymatic reactions, which are performed using the conventional standard RNA-Seq library preparation kit.

* The kits attached with the module of mRNA Capture or rRNA Depletion (target: human, rat, and mouse) are also available.)

The streamlined workflow reduces complication of operation and risk of sample loss by purification, and thus enables a highly reproducible, strand-specific expression analysis in shorter operation time than ever before. Here we introduce the result of the evaluation of RNA-Seq with rRNA depletion (rRNA removal) in order to select a library preparation kit for effective standard RNA-Seq. Using KAPA RNA HyperPrep Kit with RiboErase, RNA-Seq with rRNA depletion (rRNA removal) was performed separately on mouse embryonic stem cells (ES cells) and differentiated cells thereof, primitive endoderm cells (PrE cells).

Evaluation design

RNA extraction → QC (Result ①)
↓
rRNA depleted RNA → QC (Result ②)
↓
Library preparation → QC (Result ③)
↓
Sequencing
↓
Data analysis → Efficiency of rRNA depletion (Result ④)
Coverage (Result ⑤)
Reproducibility (Result ⑥)
Marker gene analysis (Result ⑦)

Characteristics of KAPA RNA HyperPrep Kit

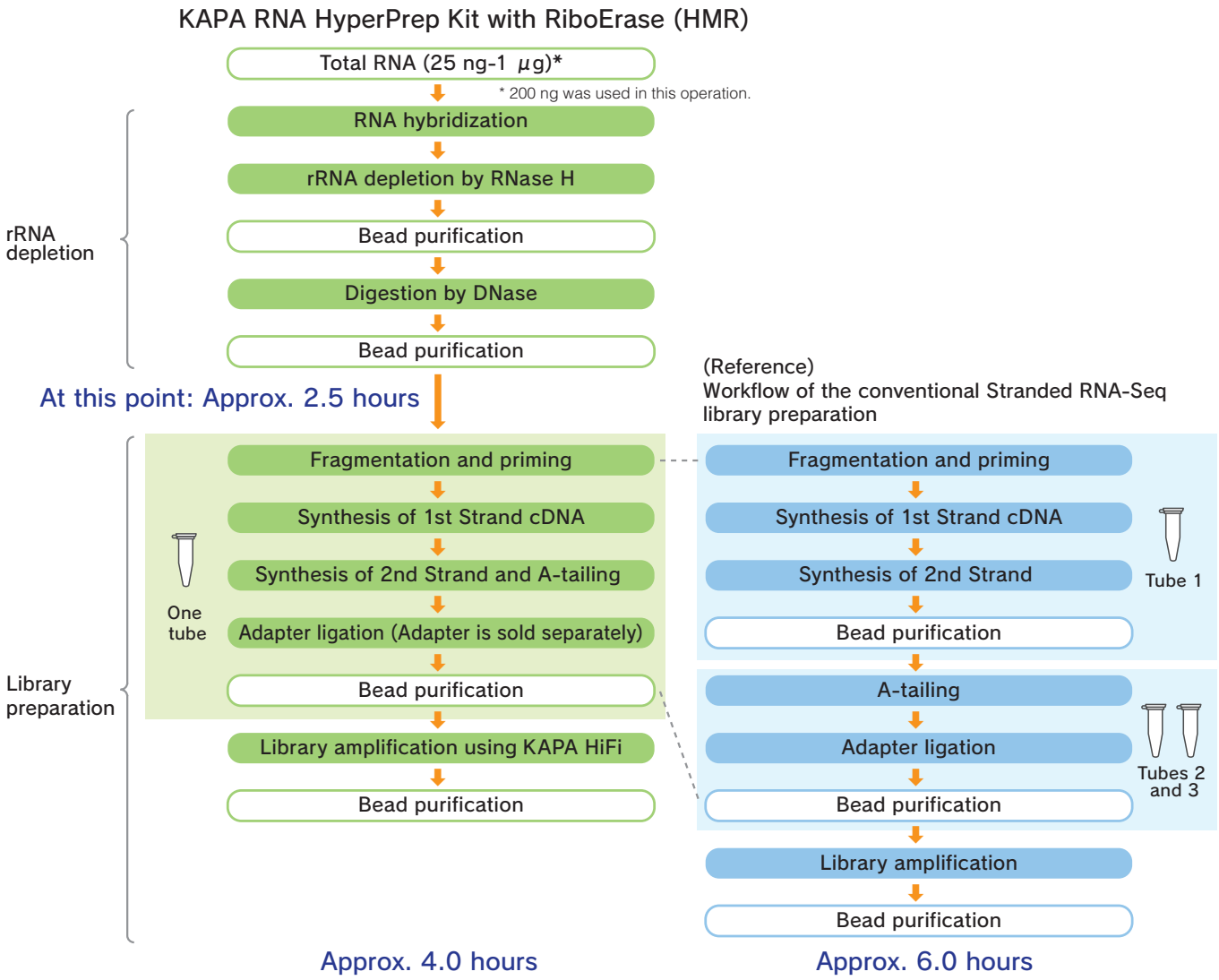
- Library preparation can be labor-saving by reducing steps of enzymatic reactions and purification compared with the conventional operation.
- A library can be prepared in a short time (approx. 4 hours).
- The kits with following modules are available:
  mRNA Capturer
  rRNA Depletion (Human, rat, and mouse)

Notice: The kit includes no adaptor.

〈Experimental conditions〉

- Initial samples (cells): Mouse embryonic stem cells (ES cells)
  Mouse primitive endoderm cells (PrE cells)
  * PrE cells were induced and differentiated from ES cells treated with dexamethasone for 72 hours.
- RNA extraction method: Total RNA was extracted from 1.0 × 10^6 cells, which were dissolved in QIAzol Lysis Reagent (Qiagen) and DNase I-treated with Direct-zol™ RNA MiniPrep Kit (Zymo Research).
- Check the extracted RNA: Quality: Bioanalyzer RIN > 9.5; Concentration measurement: Nano-drop
- Library preparation kit: KAPA RNA HyperPrep Kit with RiboErase
  * All were performed in a half of the reaction volume recommended by the protocol.
- Preparation of input RNA:
  i. ES cell total RNA
  ii. ES cell poly (A) selected RNA(*)
  iii. PrE cell total RNA
  iv. PrE cell poly (A) selected RNA(*)
  * For future verification, the samples, whose poly (A) RNA was purified using the kit by New England Biolabs Japan Inc., were also prepared.The operation was started from the step of rRNA deletion to ensure equal conditions.
- Amounts of input RNA: 200 ng each for i- iv; n = 3
  * For poly (A) selected RNA samples, the reduced amount of 200 ng total RNA was used.
- Spike RNA for external evaluation: ERCC RNA Spike-In Mix (ThermoFisher)
  * Was added under the recommended conditions (2 μL of a 0.00001-fold diluted solution was added per 1ng
- RNA fragmentation conditions: 85℃, 6 min (Target size: 300-400 bp)
- Adapter used: SeqCap Adapter Kit (Roche)
- Concentration added to the adapter: 1.5 μM
- Number of library amplification cycles: 7～8cycle
- Sequencer: HiSeq 2500

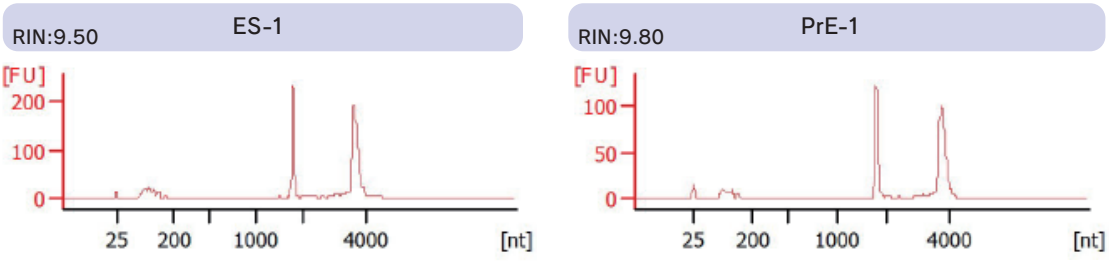
Workflow of KAPA RNA HyperPrep Kit with RiboErase



Several enzymatic reaction steps, which are performed in the conventional Stranded RNA-Seq library preparation kit, are integrated to streamline the workflow.

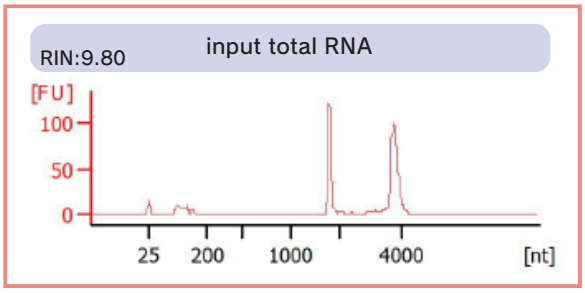
Results

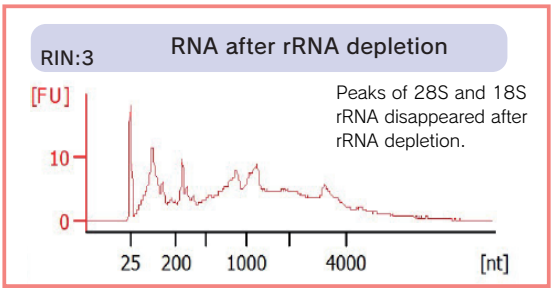
① Quality of the extracted RNA (part) (Agilent Bioanalyzer RNA 6000 Nano Kit)



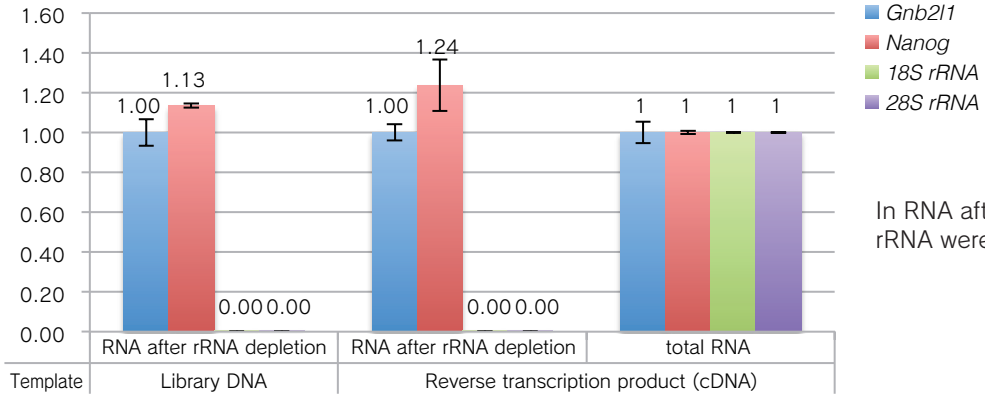
RIN > 9.5 was confirmed for all samples.

②-1 Quality of the RNA after rRNA depletion (Agilent Bioanalyzer RNA 6000 Pico Kit)





②-2 Quality of the RNA after rRNA depletion (qPCR assay for specific genes)



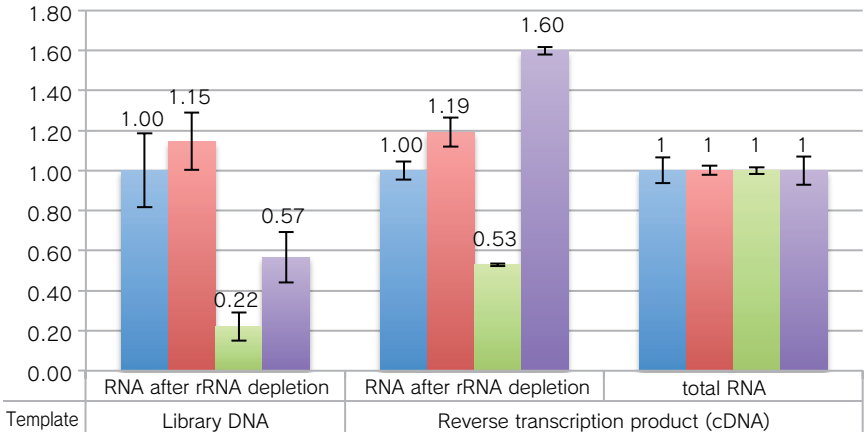
In RNA after rRNA depletion, 28S or 18S rRNA were not detected by qPCR.

* Relative value, when the reverse transcription product of total RNA is 1 using the value corrected by the detected amount of Gnb2l1

(Reference) Case in which no appropriate results were obtained by the quality check of RNA after rRNA depletion

Total RNA 200 ng extracted from mouse ES cells was used as input RNA. The operation from rRNA depletion to 2nd strand synthesis was performed using a competitor's kit. Then, the library was prepared using KAPA HyperPrep Kit (for DNA-Seq).
In several operations the expected quality was not obtained by the QC after rRNA depletion, as shown by the following data.
The subsequent library construction resulted in no intended size distribution.
Finally, the library was stably obtained in an evaluation of KAPA RNA HyperPrep Kit with RiboErase using the same samples.

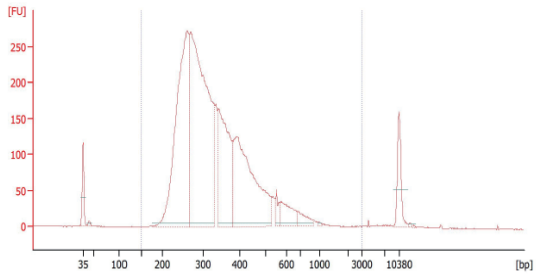
Quality of RNA after rRNA depletion (qPCR assay for specific genes)



In RNA after rRNA depletion, 28S and 18S rRNA were detected by qPCR.
This result suggested that rRNA was not sufficiently removed.

* Relative value, when the reverse transcription product of total RNA is 1 using the value corrected by the detected amount of Gnb2l1

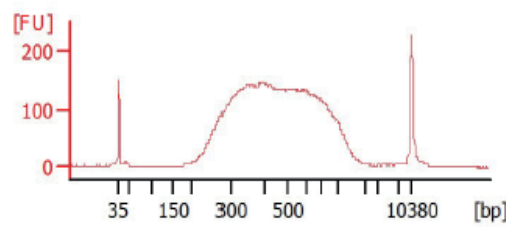
Quality of the constructed sequence library DNA



The check of the size distribution of the library DNA showed the size distribution differed from the intended one.

③ Quality of the constructed sequence library DNA -- Intended size distribution and stable yields

Size distribution of library



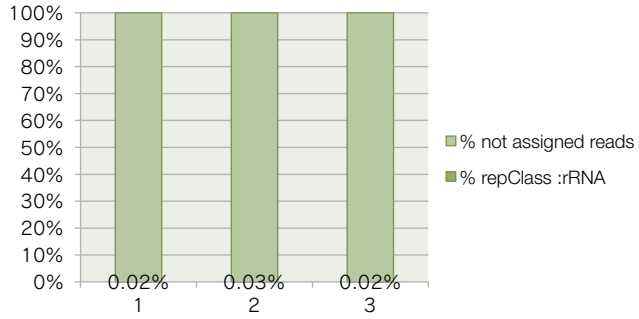
Yield of library DNA	Mean (ng)	Standard deviation (ng)	PCR cycle
i. ES cell total RNA	59.92	5.70	7
ii. ES cell poly (A) selected RNA	48.13	3.79	8
iii. PrE cell total RNA	61.33	4.44	7
iv. PrE cell poly (A) selected RNA	47.27	4.89	8
(Reference) Performed separately; ES cell total RNA	41.64	1.73	6

* n = 3 each

④ Efficiency of rRNA depletion

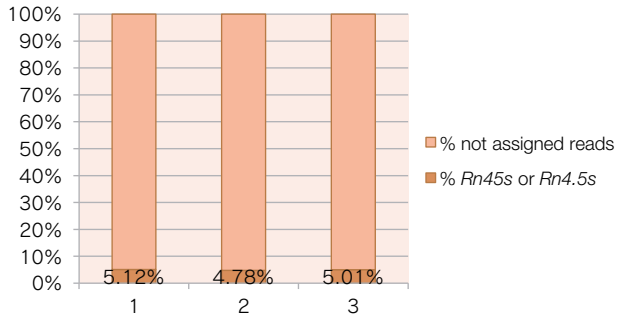
Verification of the rate of rRNA-derived reads included in the obtained sequence reads, compared with the reference data of rRNA, showed that rRNA was effectively removed and the rate was sufficiently low.

RepeatMasker repClass: rRNA



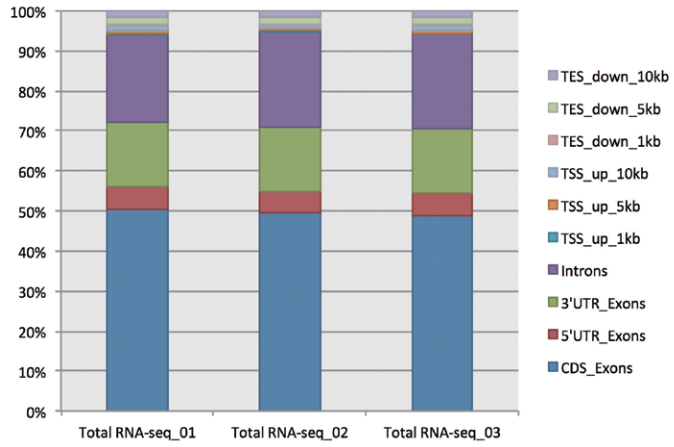
repClass of RepeatMasker: Calculated referring to rRNA (total 1564 sites).
Using this calculation method, almost rRNA was removed.

RefSeqGene: *Rn45s* or *Rn4.5s*



Calculated referring to Rn45s (45S pre-ribosomal RNA) and Rn4.5s (4.5S RNA) of RefSeqGene.
Using this calculation method, approx. 5% of rRNA was included.

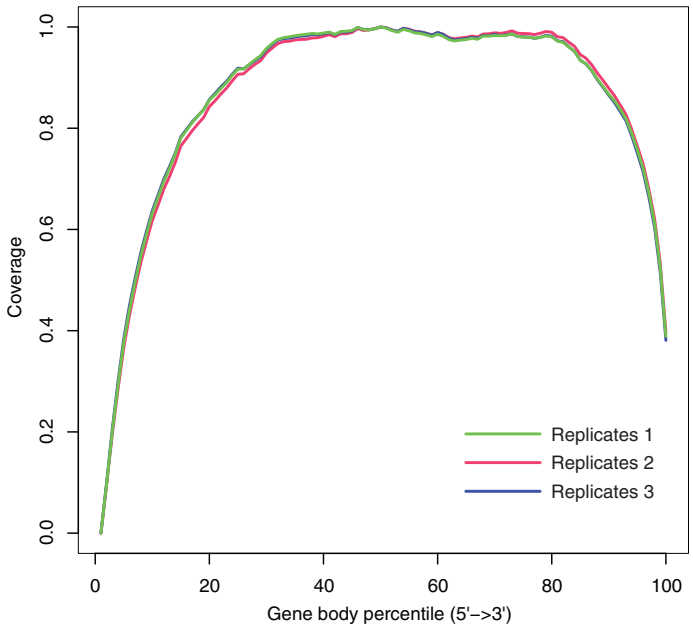
Constitutive reads of RNA-Seq after RNA depletion



The examination of the constitutive sequence reads showed that 20% or more of the reads were Introns (■ purple) and not only poly (A) RNA but non-poly (A) RNA were efficiently sequenced.

⑤ Coverage

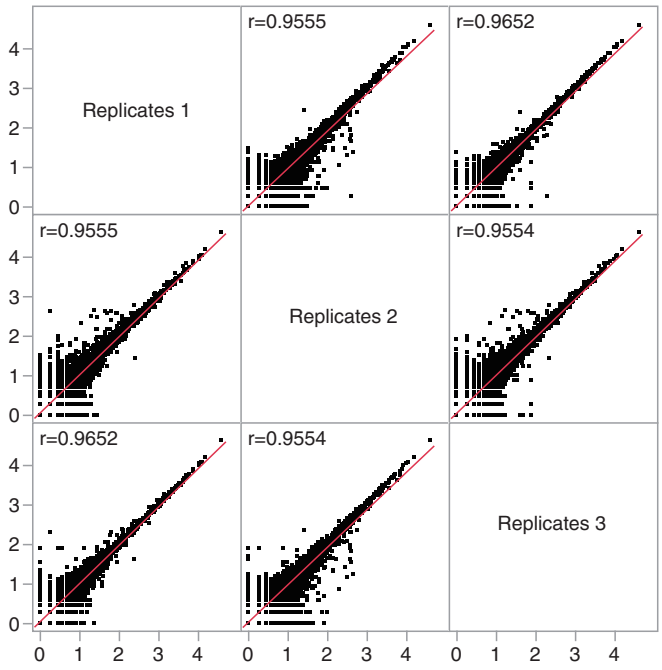
Coverage of Transcripts, which is important for identification of gene structures, was very uniform.



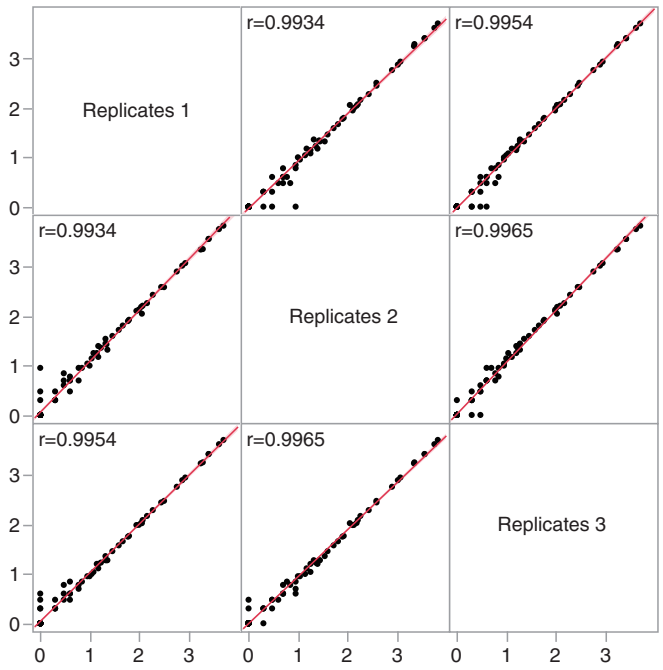
⑥ Reproducibility

The scatter plots of level of expression showed that gene expression was sufficiently reproducible.
The external evaluated RNA, including ERCC spike, showed sufficient correlation.

(Ensembl transcripts)



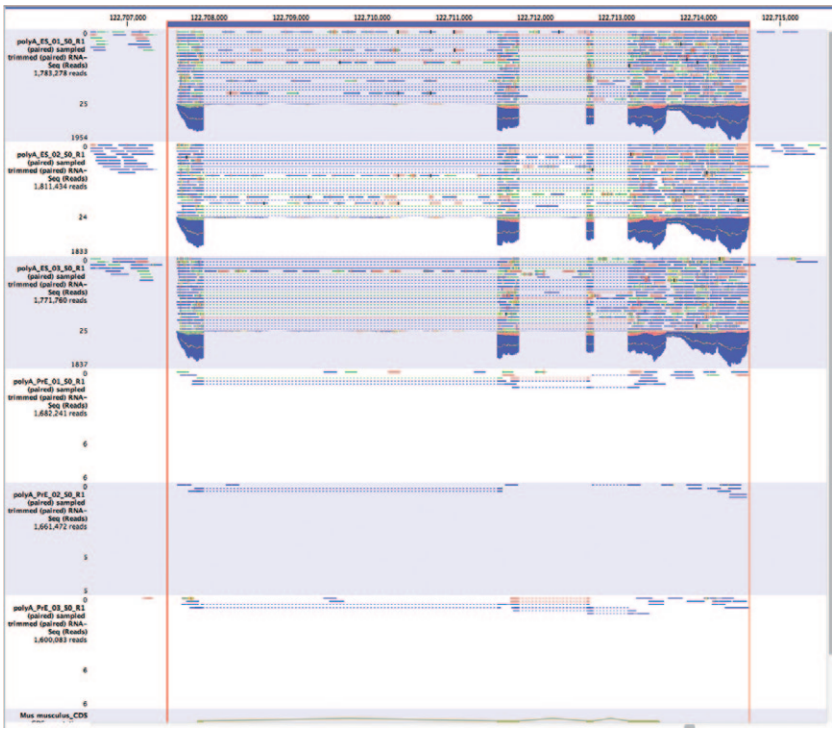
(ERCC RNA Spike)



⑦ Analysis of marker genes

The analysis was performed practically using the genome viewer. The RNA-Seq identified the marker genes specific to each of ES and PrE cells.

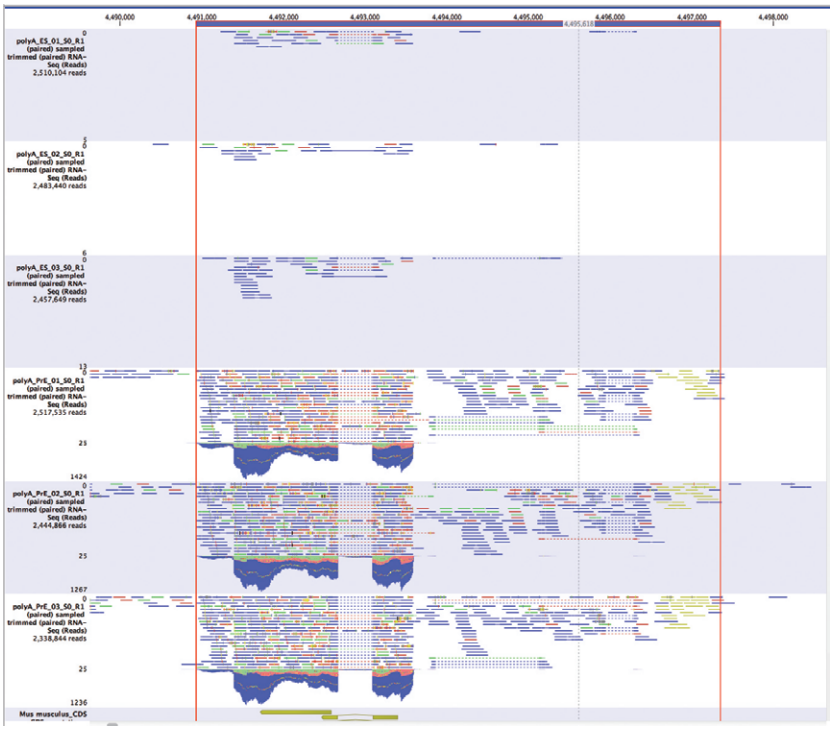
ES cell



ES cell-specific marker genes

PrE cell-specific marker genes

PrE cell



ES cell-specific marker genes

PrE cell-specific marker genes

<Customer's comments>

As the step of library preparation after the rRNA-depletion was more simplified and speeded up, it seemed that it was easier to perform the practical operation.
This product is expected to make the use of RNA-seq more stable compared with the conventional product.
It seemed for rRNA-depletion by the RNaseH method that the step in which RNA solution maintained at 45°C was mixed with the next reagent was relatively complicated However, I was very satisfied that the rRNA-depletion had reproducibility.
Poly-A RNA-seq is easier because the complicated process mentioned above is not needed. It seemed that there was no problem for the practical operation as long as the operation was performed according to the protocol specifying the precautions. This product, stably providing the sufficient accuracy, will be the first choice for a RNA-seq kit with rRNA-depletion.

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