NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : **KAPA Stranded mRNA-Seq Kit (KK8420)**
Manufacturer : **KAPA BIOSYSTEMS**
Application : ***de novo* RNA-seq (stranded mRNA-Seq) from total RNA derived from invertebrates (stranded mRNA-Seq)**

The following application data were provided by the courtesy of Dr. Yohei Sasagawa, Bioinformatics Research Unit, Advanced Center for Computing and Communication, RIKEN, Japan.

Method

By using KAPA Stranded mRNA-Seq Kit (KK8420; KAPA BIOSYSTEMS), *de novo* RNA-seq was conducted from total RNA derived from invertebrates.

The main points to check were as follows:
(1) Whether expected library size distribution can be obtained through fragmentation
(2) Whether expected amount of library can be obtained with as few cycles as possible
(3) Whether sufficient data can be obtained as a result of sequencing

Starting material : Total RNA derived from invertebrates (species A and B) 4μg
RNA purification method : RNA was extracted using a phenol extraction reagent and then purified using RNeasy Mini kit (additional DNase treatment; Qiagen).
Library preparation kit : KAPA Stranded mRNA-Seq Kit (KK8420)
Next-generation sequencer : illumina HiSeq2500 (Rapid mode, Paired end 171bp)

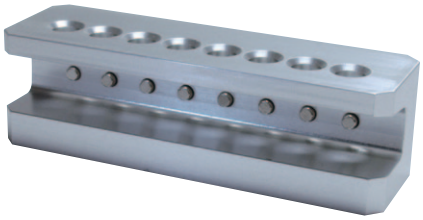
＜KAPA Stranded mRNA-Seq Kit workflow＞

- 4μg total RNA (50μL)
- ↓
- mRNA capture by KAPA mRNA Capture Bead
- ↓
- Elution and fragmentation of mRNA
- ↓
- 1st strand synthesis
- ↓
- 2nd strand synthesis (marking with dUTP)
- ↓
- AMPureXP cleanup
- ↓
- A-tailing
- ↓
- Adapter ligation*2
- ↓
- AMPureXP cleanup (first purification)
- ↓
- AMPureXP cleanup (second purification)

*1 (1st strand synthesis through AMPureXP cleanup (second purification))

- ↓
- Optimal number of cycles was determined*3
- ↓
- Library amplification*3
- ↓
- AMPureXP cleanup
- ↓
- Library quantification using KAPA Library Quantification kit

*1: By using the KAPA's proprietary with-beads protocol, these steps were conducted in one tube.



For the step of purification using magnetic beads, a magnet stand for trace samples (Magna Stand YS-Model; for 8 series×0.2mL PCR tube, Cat#FG-SSMAG2) was used.

*2: Concentration of the adapter added: 100 nM (final concentration)

*3: In order to determine the optimal number of cycles for library amplification, "KAPA library amplification kit (real-time PCR kit KK2701)" was used.
(1) Using the 1/10 amount of the library sample, real-time PCR was conducted with KAPA library amplification kit (real-time PCR kit).
(2) The optimal number of cycles was determined as per the kit protocol*
(3) By using the remaining 9/10 amount of the library sample, library amplification was conducted with KAPA HiFi HotStart ReadyMix contained in KAPA Stranded mRNA-Seq kit.

* When using the "KAPA library amplification kit (real-time PCR kit KK2701)", a Ct value on the amplification curve positioned between the fluorescent standard 1 and 3 can be determined as the optimal number of cycles.
Since the remaining 9/10 amount was to be used in the actual library amplification, the number of cycles near the standard 1 was determined as the optimal number of cycles in this case.

Results of library preparation

Data on library preparation derived from species A and B

[Condition 1] Fragmentation of the insert size 200-300bp

Fragmentation condition: 94°C, 7 min
(Library amplification after ligation was conducted at 4 cycles)

*In the protocol of this kit, recommended condition of fragmentation of 200-300bp is 94°C for 6min.
In the present application, approximately 200bp was targeted with the fragmentation condition of 94°C for 7min.

[Condition 2] Fragmentation of the insert size 300-400bp

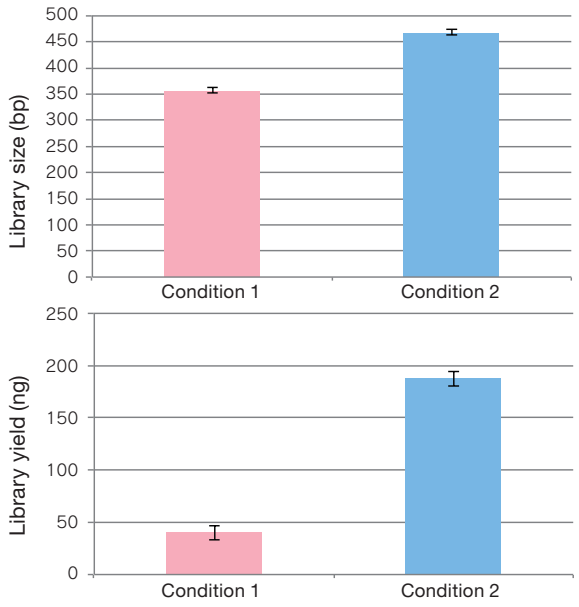
Fragmentation condition: 85°C, 6 min
(Library amplification after ligation was conducted at 5 cycles)

Mean library size (bp)

		[Condition 1] Insert size: 200-300bp	[Condition 2] Insert size: 300-400bp
Species A	A1	348	463
	A2	349	468
Species B	B1	359	468
	B2	365	464
Ave.		356	466
SD		8.18	2.68

Library yield (ng)

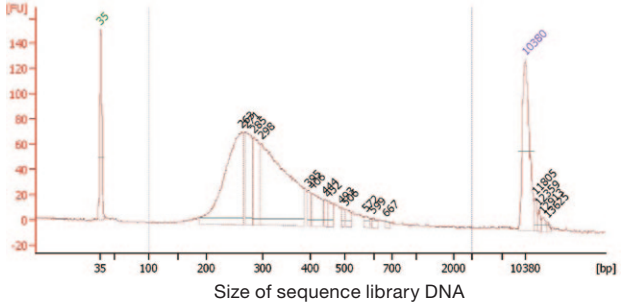
		[Condition 1] Insert size: 200-300bp	[Condition 2] Insert size: 300-400bp
Species A	A1	30.96	172.69
	A2	44.65	181.65
Species B	B1	46.35	191.13
	B2	38.80	204.31
Ave.		40.19	187.44
SD		6.95	13.53



Based on the data on library preparation, homogenous library was obtained in terms of both the size and yield. The mean library size was 356 bp under Condition 1 (insert size: 235 bp) and 466 bp under Condition 2 (insert size: 345 bp), indicating that libraries of targeted size were obtained.
In addition, stable amount of library was obtained with a small number of cycles.

[Condition 1]
Insert size: 200-300bp

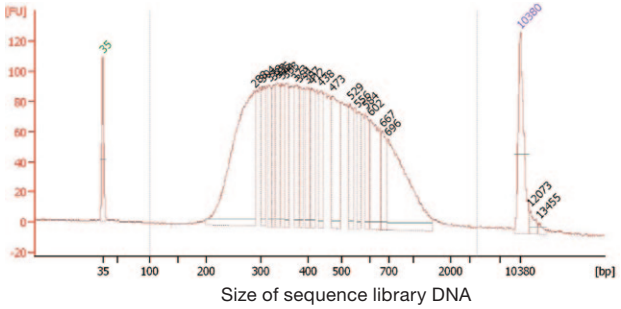
Library concentration (nM)	12.84



Size of sequence library DNA

[Condition 2]
Insert size: 300-400bp

Library concentration (nM)	11.92



Size of sequence library DNA

Results of sequencing (Species A, A1+A2)

Total sequence length (bp)	1.15508E+11
Total sequence reads	337,743,242
Total trinity 'genes'	155014
Total trinity transcript	177892
Percent GC (%)	39.47

Contig N10	5289
Contig N20	3708
Contig N30	2698
Contig N40	1951
Contig N50	1351

Median contig length (bp)	363
Average contig (bp)	737.71
total assembled bases	131232666

In the table above, for libraries prepared using insert size of 300-400 bp (Condition 2), data from analysis on libraries derived from species A (A1 + A2) are shown.
It was judged that enough transcripts were obtained.
In addition, based on these coding data, target genes were successfully found.

<Customer's comments>

Since this product adopts the with-beads protocol, reactions steps can be conducted in one tube. I tried this method for the first time, I could introduce this simple and convenient method comfortably. In addition, this product is designed to avoid the removal of A-rich sequences after fragmentation following poly-A RNA purification, and the benefits of such design could be seen in the data obtained. I could obtain library DNA with stable target size and sufficient yield. High performance of this product was confirmed trough *de novo* assembly taking the orientation of transcripts into consideration.

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