NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : **KAPA HyperPlus Kit (for illumina) (KK8510, KK8512, KK8514)**
Manufacturer : **KAPA BIOSYSTEMS**
Application : **Evaluation of library preparation method using KAPA HyperPlus Kit including fragmentation method with enzyme**

- **Examination of conditions for fragmentation using DNAs of tardigrade (*Hypsibius dujardini*) and Joro spiders (*Nephila clavata*)**
- **Library preparation using cDNA from *Hypsibius dujardini* (1 individual) and a minute amount of DNA (1 ng)**

The following data were provided by the courtesy of Dr. Kazuharu Arakawa of the Institute for Advanced Biosciences, Keio University, Japan.

Background

As the data from any manufactures are usually insufficient, DNA sequences for non-model invertebrate animals need individual verification. KAPA Hyper Plus Kit states that (1) due to its enzymatic method, the high randomness of the fragmentation position is obtained, allowing the change of the fragmentation length according to the reaction time, and (2) its ligation efficiency is higher than the products from other manufacturers. These advantages are considered effective especially in the parallelism needed when many samples are treated at the same time (e.g., RNA-Seq), as well as in the experiments that are started with a minute amount of samples. In this application note, these 2 features are verified using non-model invertebrates (a Tardigrade and spider).

Experiment 1 Fragmentation of genomic DNA

Method

Species :
Hypsibius dujardini (Tardigrade : obtained from the U.K.) GC=45%
Nephila clavata (Joro spider) GC=30%
Input DNA extraction method : *Hypsibius dujardini* → MagAttract HMW DNA Kit (Qiagen)
Nephila clavata → Quick-gDNA MicroPrep (Zymo)
Composition of input DNA buffer : 10mM Tris-HCl (pH7.5)
Fragmentation method :

- Covaris M220/XTU microTUBE-15
- Fragmentation enzyme (from another manufacturer)
- KAPA HyperPlus Kit Thermal cycler : LifeEco (Bioer)

Fragmentation condition : 37°C
2.5 min
5 min
7.5 min
10 min
15 min
20 min
25 min
30 min

Input DNA condition

Fragmentation	Amount of input DNA [ng]
Covaris	150
Fragmentation enzyme from another manufacture	50
KAPA HyperPlus Kit	150

Fragmentation conditions of KAPA HyperPlus Kit

Fragmentation time [min]	Target size [bp]] (KAPA protocol)
2.5	
5	600
7.5	
10	350
15	
20	200
25	
30	150

<KAPA HyperPlus Kit fragmentation workflow>

1. Preparation of the conditioning solution
(Prepare the solution according to the EDTA concentration in the sample)

Composition of the input DNA buffer used under these conditions:
10 mM Tris-HCl (pH 7.5) (no EDTA)
Therefore, no conditioning solution was used.

Example) When the sample was dissolved in TE (5 μM Tris-HCl, 1 mM EDTA, pH 8.0)

final EDTA conc. = 0.1mM
EDTA 0.1mM Conditioning solution :
Conditioning solution (per 100μL) 6.5μL
PCR-grade water (per 100μL) 93.5μL

Composition of conditioning solution

Final EDTA concentration in 50μL rxn	Dilution Factor	Volume of Conditioning Solution (per 100μL)	Volume of PCR-grade water (per 100μL)
0.02-0.05 mM	32.0	3.1 μL	96.9 μL
0.1 mM	15.4	6.5 μL	93.5 μL
0.2 mM	7.4	13.5 μL	86.5 μL
0.3 mM	4.8	21.0 μL	79.0 μL
0.4 mM	3.3	30.0 μL	70.0 μL
0.5 mM	2.6	38.8 μL	61.2 μL
0.6 mM	2.2	46.5 μL	53.5 μL
0.7 mM	1.8	56.0 μL	44.0 μL
0.8 mM	1.6	64.0 μL	36.0 μL
0.9 mM	1.4	72.0 μL	28.0 μL
1.0 mM	1.3	80.0 μL	20.0 μL

2. Fragmentation reaction was conducted for each sample with the following conditions.
(The solution used in this condition did not contain EDTA; therefore, no conditioning solution was used.)

Component	Volume	Volume recommended by KAPA
Double-stranded DNA	1 μL	5 μL
PCR grade water	6 μL	30 μL
KAPA Frag Buffer (x10)	1 μL	5 μL
KAPA Frag Enzyme	2 μL	10 μL
Total volume	10 μL	50 μL

Note
When the solution containing EDTA is used, use the conditioning solution consisting of:
5 μL the conditioning solution prepared in 1 and
25 μL PCR grade water,
instead of 30 μL PCR grade water.

3. Incubate with a thermal cycler.

Step	Temp	Time
Pre-cool block	4 ℃	N/A
Fragmentation	37 ℃	
HOLD	4 ℃	∞

Note: Turn OFF the heat lid of the thermal cycler

4. Prepare the solution of the fragmented samples for reaction of End Repair and A-tailing

Component	Volume	KAPA protocol volume
Fragmented, double-stranded DNA	10 μL	50 μL
End Repair & A-Tailing Buffer*	1.4 μL	7 μL
End Repair & A-Tailing Enzyme Mix*	0.6 μL	3 μL
Total volume	12 μL	60 μL

5. Incubate using a thermal cycler.

Step	Temp	Time
End repair and A-tailing	65 ℃	30 min
HOLD	4 ℃	∞

Note: Set thermal cycler heat lid at 85° C.

Results

Results of fragmentation with TapeStation are shown below.

Hypsibius dujardini genomic DNA (GC=45%)

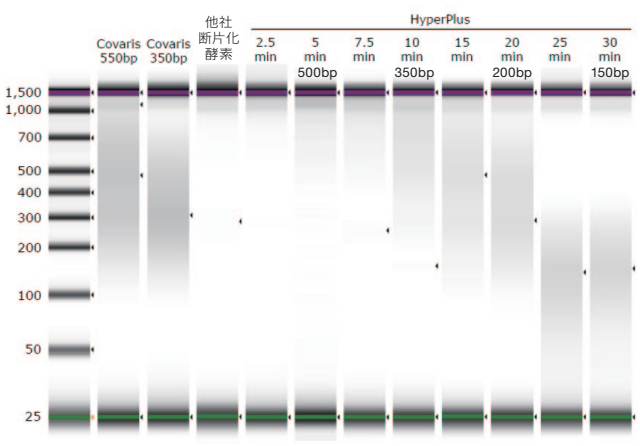

Fig.1. Results of fragmentation,by TapeStation (D1000)

Possibly because GC is higher than human, etc., the results were little diverged from those in the catalogue both in Covaris and HyperPlus, but the fragment size was approximately in proportion to time for HyperPlus.

Nephila clavata genomic DNA (GC=30%)

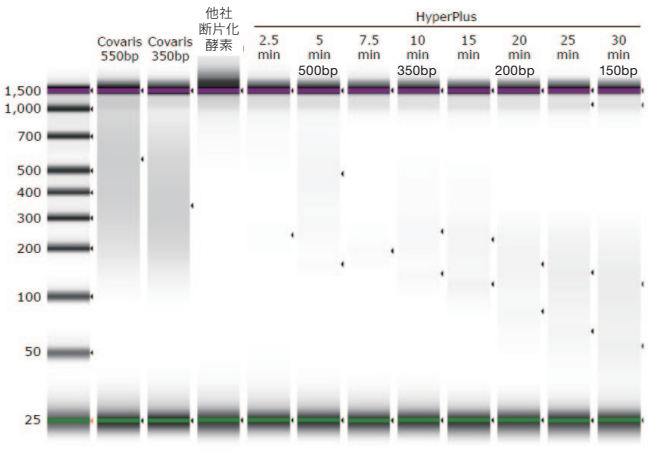

Fig.2. Results of fragmentation,by TapeStation (D1000)

Possibly because of the many AT-rich repeats, the fragmentation efficiencies are remarkably lower for fragmentation enzymes obtained from other manufactures. Covaris resulted in the size shown in the catalogue, and HyperPlus showed the size approximately in proportion to time.

Experiment 2 Preparation of single individual RNA-seq library for *Acutuncus antarcticus* (Tardigrade: obtained from the South Pole)

Method

Amount of initial sample	: Several hundred pg
Species	: *Acutuncus antarcticus* (Tardigrade: obtained from the South Pole)
RNA extraction method	: Direct-zol, tissue (1 individual) (L: ≈300 μm, W: ≈100 μm)
cDNA preparation	: SMART-Seq v4 Ultra Low Input RNA Kit
Composition of input cDNA buffer	: 10mM Tris-HCl (pH7.5)
Fragmentation method of cDNA	: KAPA HyperPlus Kit
Fragmentation condition	: 37℃, 20 min
Library preparation	: KAPA HyperPlus Kit illumina® platforms (KAPA BIOSYSTEMS)
Adapter	: TruSeq RNA Sample Prep Kit v.2 Adapter
Sequencer	: MiSeq (illumina)

Results

＜Workflow＞

1. Totral RNA extraction ..... After extracting with Direct-zol

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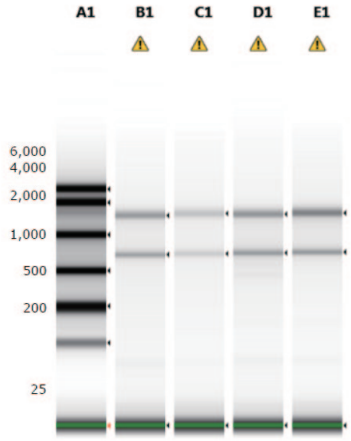
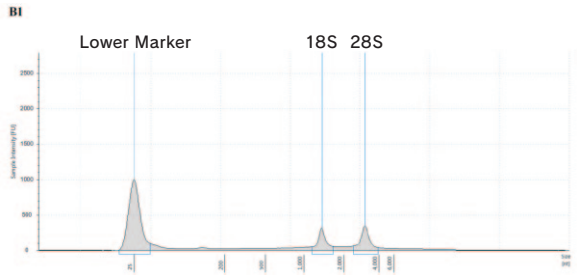
2. quantification ..... Qubit RNA HS Assay Kit (Thermo Fisher Scientific) was used.
   QC ..... Nanodrop 2000 (Thermo Fisher Scientific) was used.
   QC ..... TapeStation High Sensitivity RNA Screen Tape (Agilent) was used.

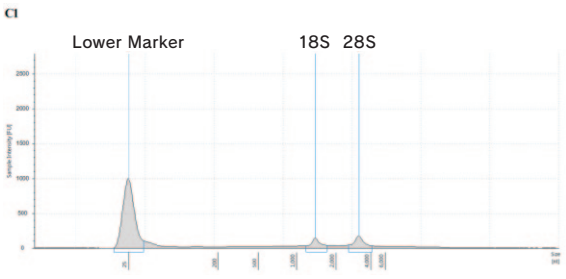


Well	RINe	28S/18S (Area)	Conc. [pg/μl]	Sample Description	Alert	Observations
A1	-	-	3750			Ladder
B1	8.4	1.3	335		⚠	RNA concentration outside recommended range for RINe
C1	7.3	1.4	235		⚠	RNA concentration outside recommended range for RINe
D1	7.9	1.2	370		⚠	RNA concentration outside recommended range for RINe
E1	8.7	1.6	360		⚠	RNA concentration outside recommended range for RINe

RIN 8.4



RIN 7.3



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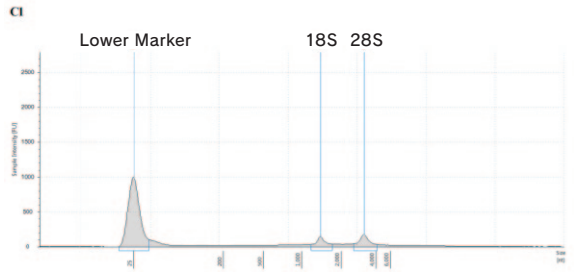

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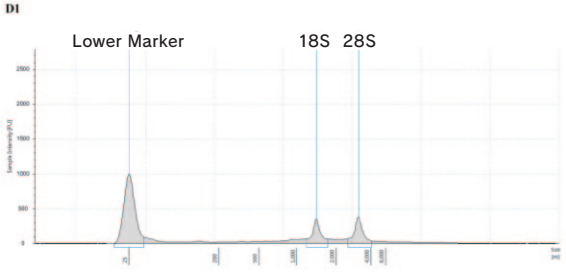

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3. cDNA synthesis (input RNA: several hundred pg)..... SMART-Seq v4 Ultra Low Input RNA Kit was used
   Amplification cycle: 20

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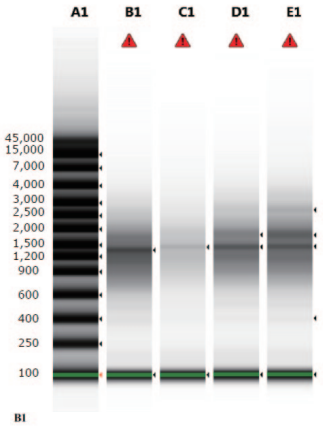
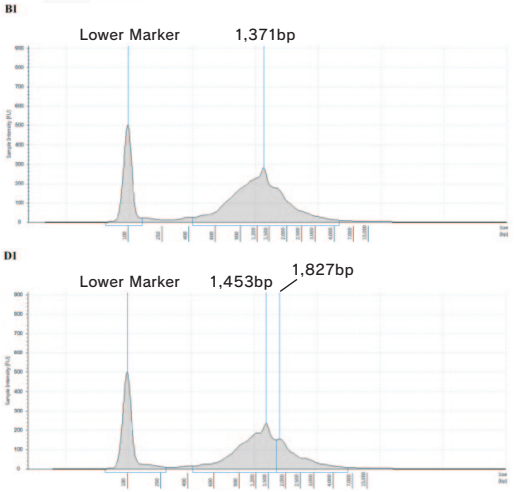
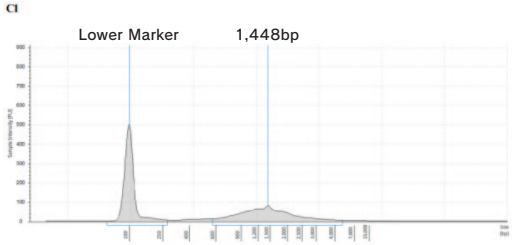
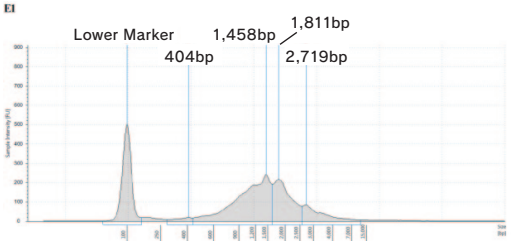
4. quantification ..... Qubit dsDNA HS Assay Kit （Thermo Fisher Scientific）
QC ..... TapeStation Genomic DNA ScreenTape （Agilent） was used.



Well	DIN	Conc. [ng/μl]	Sample Description	Alert	Observations
A1	-	112	Ladder		Ladder
B1	-	27.2		⚠	The original ladder for this lane had too few peaks
C1	-	7.88		⚠	The original ladder for this lane had too few peaks; Sample concentration outside recommended range
D1	-	19.2		⚠	The original ladder for this lane had too few peaks
E1	-	25.5		⚠	The original ladder for this lane had too few peaks







5. Enzymatic fragmentation (fragmentation conditions: 37°C, 20 min)

Component	Volume	KAPA protocol volume
Double-stranded DNA (with KAPA Frag Conditioning Solution, if needed)	7 μL	35 μL
KAPA Frag Buffer (x10)	1 μL	5 μL
KAPA Frag Enzyme	2 μL	10 μL
Total volume	10 μL	50 μL

6. End Repair and A-tailing

Component	Volume	KAPA protocol volume
Fragmented, double-stranded DNA	10 μL	50 μL
End Repair & A-Tailing Buffer	1.4 μL	7 μL
End Repair & A-Tailing Enzyme Mix	0.6 μL	3 μL
Total volume	12 μL	60 μL

7. Adapter Ligation

Component	Volume	KAPA protocol volume
End repair and A-tailing reaction product	12 μL	60 μL
Adapter stock (concentration as required)	1 μL	5 μL
PCR-grade water	1 μL	5 μL
Ligation Buffer	6 μL	30 μL
DNA Ligase	2 μL	10 μL
Total volume	22 μL	110 μL

8. Post-ligation Cleanup

Component	Volume	KAPA protocol volume
Adapter ligation reaction product	22 μL	110 μL
Agencourt® AMPure® XP reagent	17.6 μL	88 μL
Total volume	39.6 μL	198 μL

9. Library Amplification

Component	Volume	KAPA protocol volume
2X KAPA HiFi HotStart ReadyMix	12.5 μL	25 μL
10X KAPA Library Amplification Primer Mix	2.5 μL	5 μL
Adapter-ligated library	10 μL	20 μL
Total volume	25 μL	50 μL

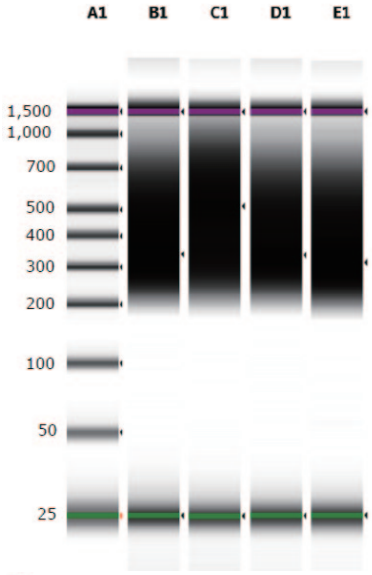
10. Library Amplification（continued）

Step	Temp	Duration	Cycles
Initial denaturation	98℃	45 sec	1
Denaturation	98℃	15 sec	10
Annealing	60℃	30 sec	
Extension	72℃	30 sec	
Final extension	72℃	1 min	1
HOLD	4℃	∞	1

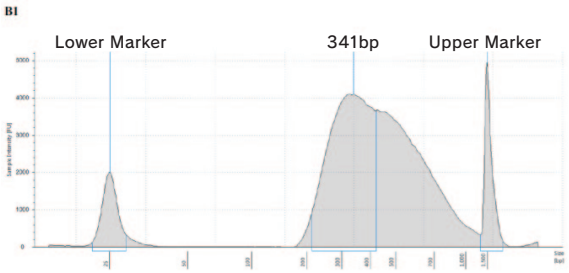
} Minimum number required for optimal amplification

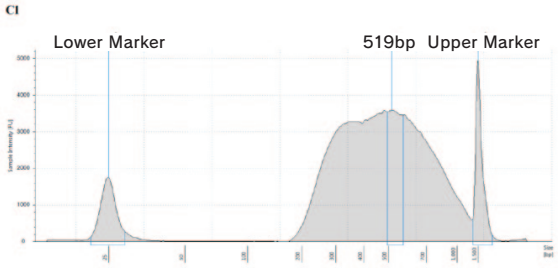
11. Post-amplification Cleanup

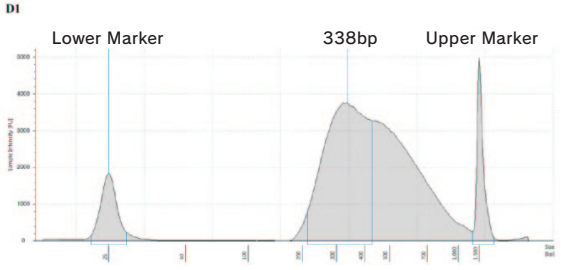
12. quantification ..... Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) was used.
QC ..... TapeStation D1000 ScreenTape (Agilent) was used.

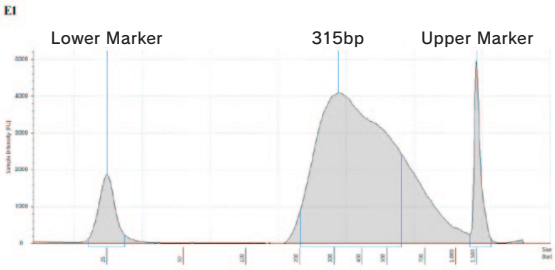


Fragmentation time: 20 min
Target fragment size: 200 bp
Target library size: 320 bp









Well	input ds cDNA(ng)	Final Adapter conc(nM)	PCR cycle amplification Library conc(ng/ul)	total Library(ng)
B1	50 ng	25 μM	86.7 ng/μL	1300.5 ng
C1	50 ng	25 μM	91 ng/μL	1365 ng
D1	50 ng	25 μM	102 ng/μL	1530 ng
E1	50 ng	25 μM	98.2 ng/μL	1473 ng

Experiment 3 Preparation of genomic DNA library for a tardigrade species

Method

Amount of initial sample : Genomic DNA: 1 ng
Species : A Tardigrade species
Input DNA extraction method : MagAttract HMW DNA Kit (QIAgen)
Composition of input cDNA buffer : 10 mM Tris-HCl (pH7.5)
DNA fragmentation method : Covaris M220/XTU microTUBE-15
KAPA HyperPlus Kit
Fragmentation condition : 37°C, 5 min
Library preparation method:

- Low input library preparation kit from Covaris and other manufactures
- KAPA HyperPlus Kit

Results

＜Workflow＞

Sample fragmented with Covaris

Library was prepared following the protocol specified for the low input library preparation kits from other manufacturers.
1 ng input DNA (Covaris: before fragmentation)

Sample fragmented with KAPA HyperPlus Kit

1. As in the case of Experiment 1 and Experiment 2, 1/5 volume of the reaction mixtures in the processes from the fragmentation to adaptor ligation was prepared.

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2.

Component	Volume	KAPA protocol volume
2X KAPA HiFi HotStart ReadyMix	25 μL	25 μL
10X KAPA Library Amplification Primer Mix	5 μL	5 μL
Adapter-ligated library	20 μL	20 μL
Total volume	50 μL	50 μL

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3.

Step	Temp	Duration	Cycles
Initial denaturation	98℃	45 sec	1
Denaturation	98℃	15 sec	14
Annealing	60℃	30 sec	
Extension	72℃	30 sec	
Final extension	72℃	1 min	1
HOLD	4℃	∞	1

} Minimum number required for optimal amplification

⬇

4. QC ..... Tape Station Genomic DNA Screen Tape (Agilent) was used.

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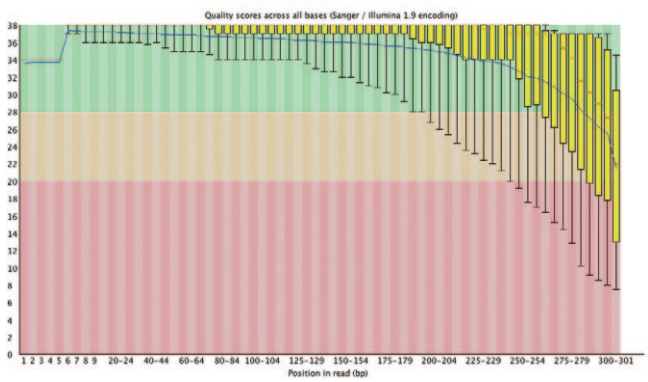
5. size selection
E-gel 2% + NEB QuickLoad 2-Log DNA Ladder were used. Slicing was conducted with a cutter.
Nucleospin Gel and PCR clean-up kit were used for purification. Final extraction was 10 mM Tris-HCl ph 7.5.

⬇

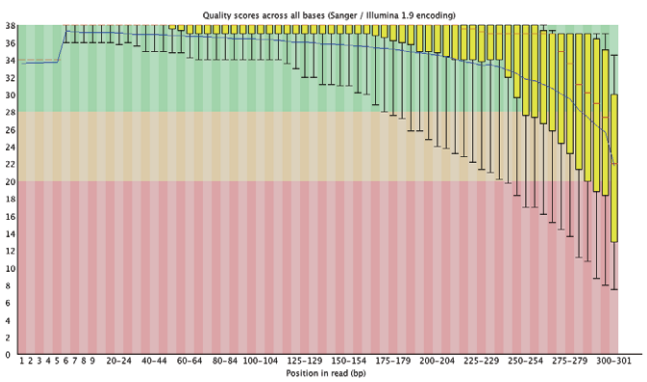
6. Next-generation sequence
FASTQC data

Covaris and other low input library preparation kit

Per base sequence quality

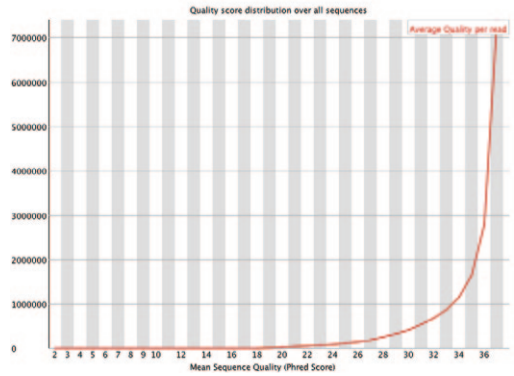


KAPA HyperPlus Kit

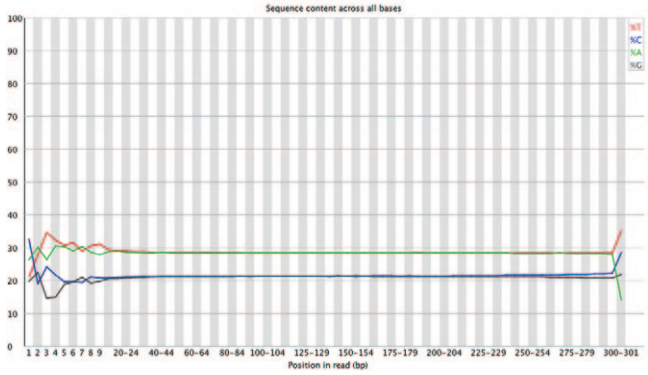


Covaris and other low input library preparation kit

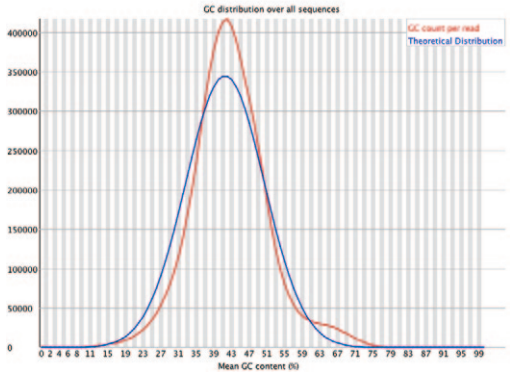
Per sequence quality scores



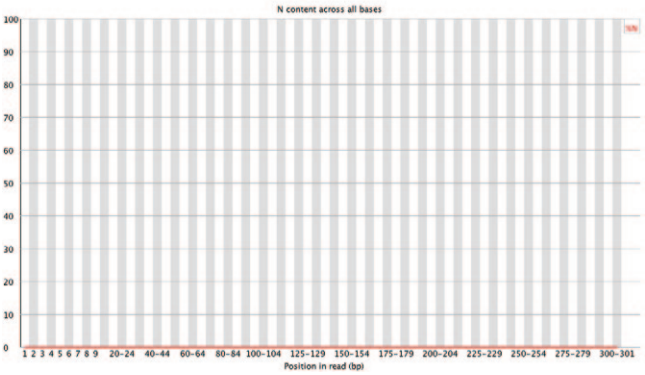
Per base sequence content



Per sequence GC content

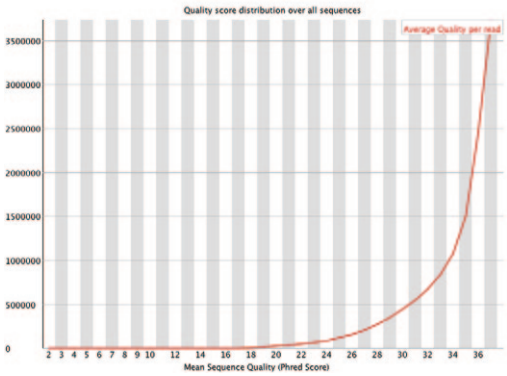


Per base N content



KAPA HyperPlus Kit

Per sequence quality scores



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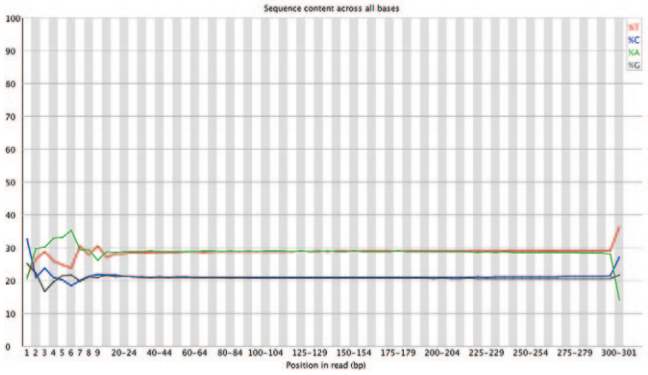

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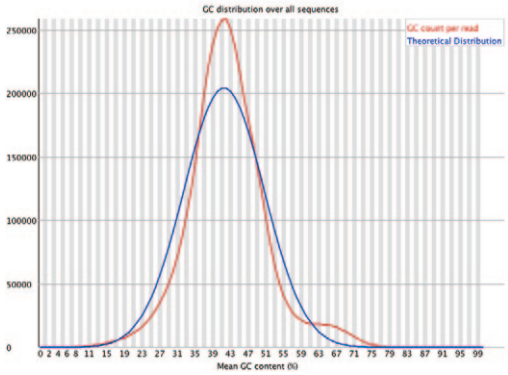
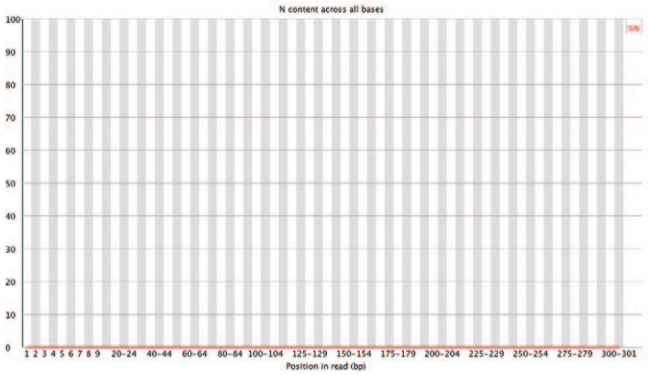


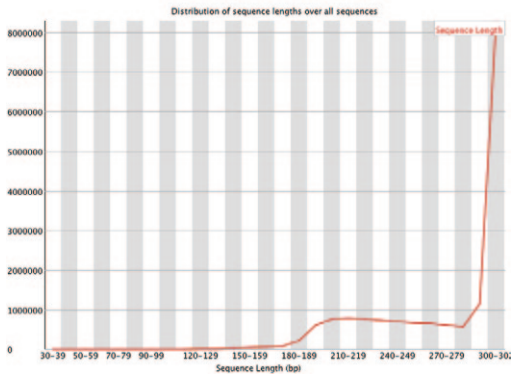
Per base N content

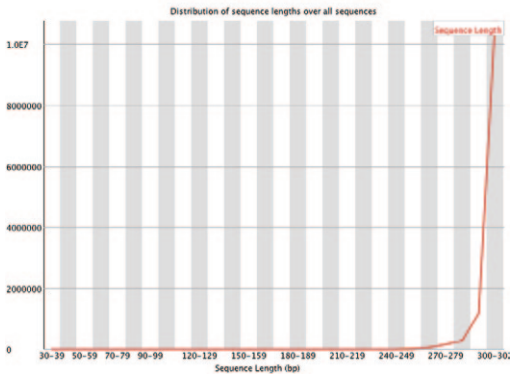


Covaris and other low input library preparation kit

KAPA HyperPlus Kit

Sequence Length Distribution





● Summary

Based on the all data, no difference was observed between KAPA HyperPlus Kit and the low input library preparation kit from Covaris and other manufactures.
Although KAPA HyperPlus showed wider deviation than Covaris in the per-base sequence content, no remarkable differences were observed in the results.
(In the case of fragmentation enzymes from other manufacturers, a little more strong bias is seen at the first ≈10 bp (no data).)

As for the sequence length distribution, it should be noted that the libraries are selected before the sequence by size under the following conditions:
Covaris + other low input library preparation kits: cut with 300–1,000 bp
KAPA HyperPlus Kit: cut with 400–1000 bp
These should be the reason for the slightly shorter fragments in Covaris and other low input library kits.

	Covaris and other low input library preparation kits	KAPA HyperPlus Kit
Number of reads	34,648,529	26,180,631
Mapped reads	27,566,045 / 79.56%	20,253,892 / 77.36%
Duplication rate	43.77%	23.25%
GC Percentage	40.84%	40.1 %
Coverage mean	39.99	32.62

<Customer's comments>

1. These experiments were conducted using large volumes of genomic DNA because visualization of TapeStation for small volume was difficult. However, as seen in the SMART-Seq v4 library adjustment and sample adjustment from 1 ng, we were able to stably prepare a library with a minute amount of the initial sample.
2. Although a certain level of fluctuation can occur in the fragmentation time due to the GC contents and/or sequence characteristics of the DNA, a specific length of fragments were obtained in proportion to time when the same DNA was used. I have an impression that a little larger amount of target fragments were obtained with Covaris. For RNA-Seq (quantity-targeted), where the length is not a matter as long as a certain length is secured and an extremely many samples are used (for high-throughput), HyperPlus will be the first choice.
3. Because KAPA HyperPlus Kit uses the enzymatic method, samples can be treated simultaneously, and thus the treatment can be done extremely easily and efficiently compared with the case when Covaris is used.
4. While most of the kits from other manufacturers require Adapter ligation reaction with a small amount (e.g., 10 µL), KAPA uses 50 µL at the fragmentation step. That is, downsizing of the reaction system is possible with the KAPA system. This is a big advantage when using a minute sample (with a 1/5 reaction volume, concentration can be practically quintuplicated); it is very economical.
5. Since a certain level of yield is needed, the last PCR amplification is not enough to decrease the reaction volume to 1/5. To solve this problem, we have verified that self-prepared oligo nucleotides that support PCR Illumina adapter and KAPA HiFi HS Ready Mix can make amplification inexpensively (it did not work well with the enzyme obtained from other manufactures).

   Primer-1 AATGATACGGCGACCACCGAGA
   Primer-2 CAAGCAGAAGACGGCATACGAG OPC purification (Eurofins, Japan.)

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