NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA Hyper Prep Kit (KK8500, KK8502, KK8504)
Manufacturer : KAPA BIOSYSTEMS
Application : Effective preparation of RNA-Seq library from trace amounts of RNA (10 pg total RNA derived from invertebrate tissues)

The following data were provided by the courtesy of Mr. Takashi Watanabe of the Human DNA Analysis Group, Department of Research & Development, Kazusa DNA Research Institute, Japan.

Method

RNA-Seq has been a challenge for us in terms of input requirement.
Most kits require about 10-100ng of total RNA, which is difficult to prepare when only trace amounts of tissue samples are available.
To solve this problem, we validated the use of SMARTer® Ultra™ Low Input RNA Kit, which enables cDNA preparation from 10 pg of total RNA, in combination with KAPA Hyper Prep Kit for library preparation.

Initial sample volume : 10pg of total RNA
Species : Invertebrate
RNA extraction method : 1ml TRIzol (Life Technologies) used per 1mm^2 of invertebrate tissue, yielded about 5 ng of RNA
cDNA preparation : SMARTer® Ultra™ Low Input RNA Kit (634848, Clontech Labratories)
cDNA fragmentation method : Covaris S2
Library preparation : KAPA Hyper Prep Kit illumina® platforms (KK8502 KAPA BIOSYSTEMS)
Adapter* : SeqCap Adapter Kit A (07141530001,Roche)
Sequencer : HiSeq2000 (illumina)

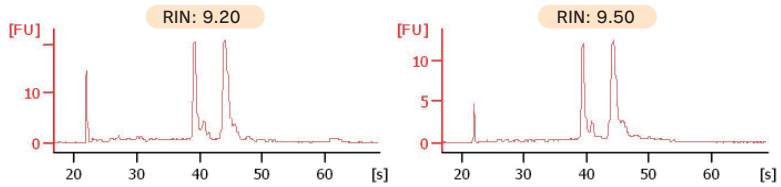
* Adapter volume added

Input DNA	Stock concentration	Adapter volume per reaction
0.5ng	150nM	5μl
1.0ng	300nM	5μl
2.5ng	750nM	5μl

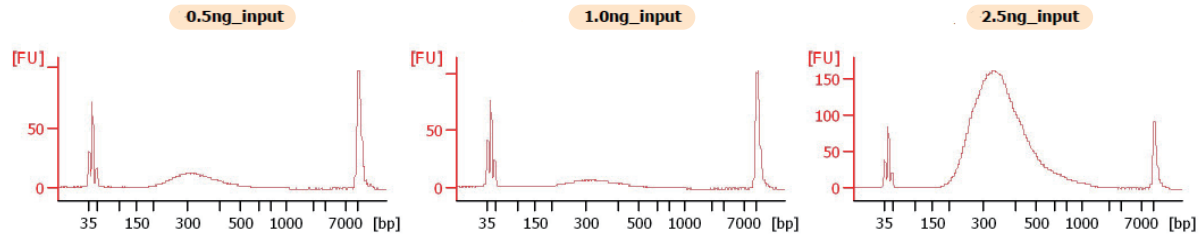
Method

＜**Workflow**＞

Total RNA extraction..... Extraction by TRIzol, followed by purification using RNAClean XP kit
⬇
QC by Agilent Bioanalyzer ..... Using RNA6000 Pico kit
⬇



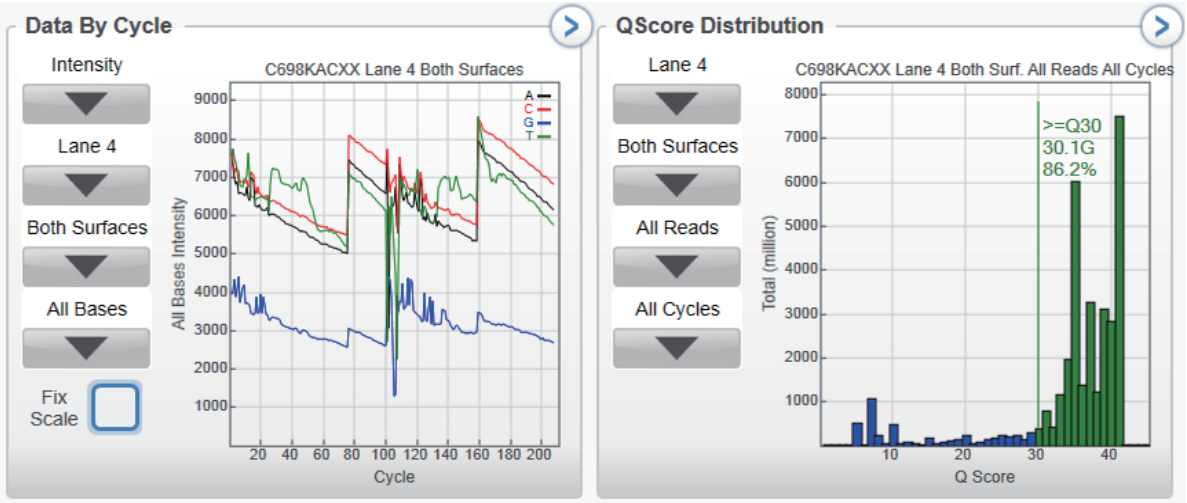
cDNA synthesis (input total RNA 10pg) ..... Using SMARTer® Ultra™ Low Input RNA Kit (634848)
Number of amplification cycles: 18 (recommended)
⬇
ds cDNA (about 5ng)
⬇
shearing (input 0.5 or 1 or 2.5ng) ..... Covaris S2 (200bp:175W 3min)
⬇
Library prep..... KAPA Hyper Prep Kit Illumina® platforms (KK8502)
⬇
QC ..... Qubit dsDNA HS Assay kit
Agilent Bioanalyzer High Sensitivity
⬇

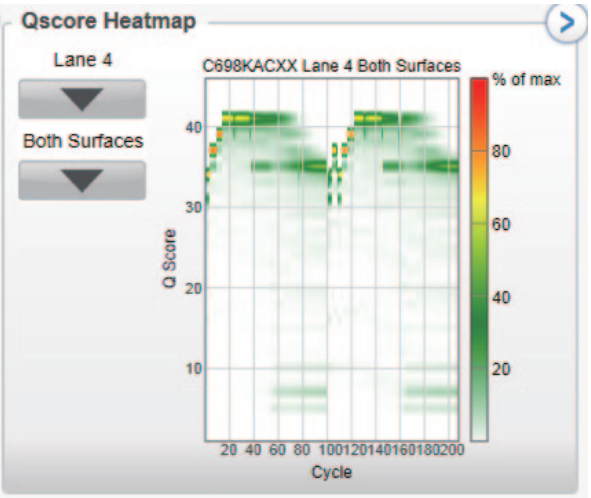


input ds cDNA(ng)	Final Adapter conc(nM)	PCR cycle	amplification Library conc(ng/ul)	total Library(ng)
0.5	7	16	0.427	21.35
1	14	14	0.211	10.55
2.5	34	12	4.34	217

Library pool (2nM) ..... The library prepared using 1ng of input cDNA was pooled, considering the feasibility of reanalysis using limited amount of ds cDNA samples and the fewer number of PCR cycles
⬇
Sequence..... HiSeq2000 High Out Put PE100

Results





	Lane	Tiles	Density (K/mm²)	Clusters PF (%)	Phas/Prephas (%)	Reads PF (M)	Intensity Cycle1	% Intensity Cycle20
Read 1	4	96	659 +/- 111	92.4 +/- 1.9	0.179 / 0.070	167.79	7650 +/- 823	80.6 +/- 1.5
Read 2(index)	4	96	659 +/- 111	92.4 +/- 1.9	0.000 / 0.000	167.79	7453 +/- 889	0.0 +/- 0.0
Read 3	4	96	659 +/- 111	92.4 +/- 1.9	0.143 / 0.058	167.79	7365 +/- 791	78.8 +/- 1.3

Sequence data

Total length (bp)	153,426,328
GC content(%)	41.6031543165134

Using the initial 10pg of RNA, an extremely high-quality data (Q30=86.2% 30.1G for PE100) could be obtained.
In the present study, we performed fragmentation to avoid quality deterioration of dT30 sequence and Read 2 resulting from the use of SMART adapter. As a consequence, paired-end read data could be used for *de novo* assembly.

<Customer's comments>
The advantages of this product are the low template requirement for library preparation (1ng of template) and the simplified reaction procedure due to its simple reagent composition. This is a great advantage when preparing libraries from multiple samples.
In the present study, a sufficient amount of library for performing sequencing could be prepared from a trace amount of template, and a sufficient amount of high-quality data for *de novo* assembly could be achieved.

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