NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA Hyper Prep Kit (KK8500, KK8502, KK8504)
Manufacturer : KAPA BIOSYSTEMS
Application : Optimization of the library preparation protocol for KAPA Hyper Prep Kit using trace amounts of dsDNA (10-1,000 pg) (LIMprep2*)

Introduction

KAPA Hyper Prep Kit has been developed for preparing libraries from 1 ng to 1µg dsDNA. This application note introduces an example study (LIMprep2) in which the protocol has been optimized for even smaller amounts (10-1,000 pg) of input DNA.

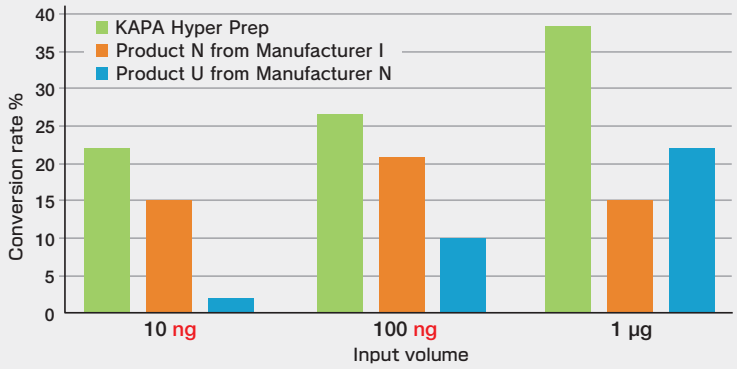
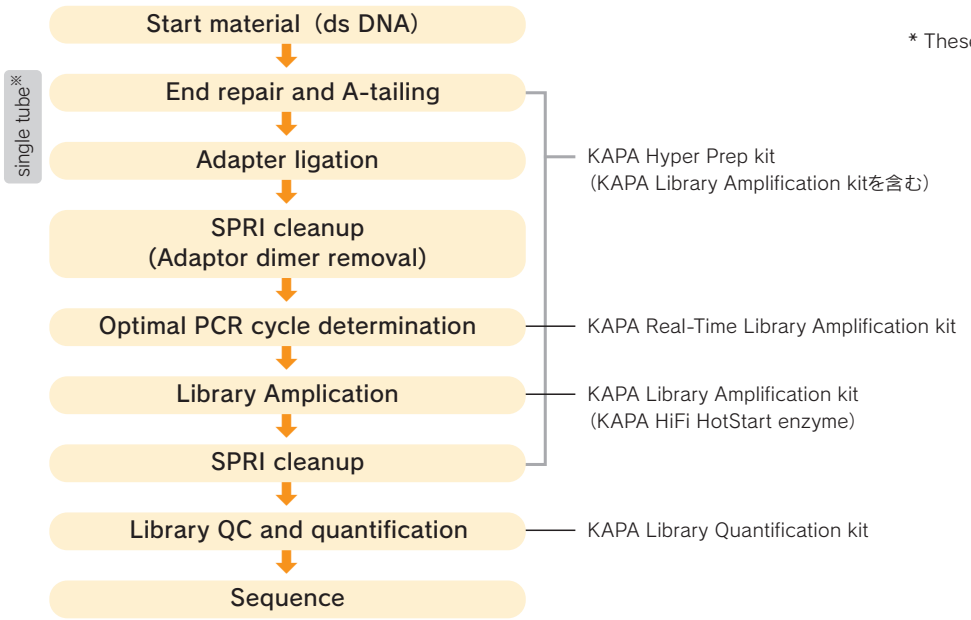


Fig. 1 Library conversion rate of input DNA

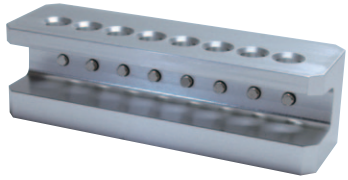
KAPA Hyper Prep Kit is capable of converting the input DNA into adaptor-ligated library at a high rate. The library prepared from Covaris-fragmented DNA was subjected to adaptor ligation and subsequently quantified by KAPA Library Quantification Kit (see figure on the left). Regardless of the amount of input DNA (10 ng, 100 ng or 1 µg), KAPA Hyper Prep Kit offered the highest rate of conversion into adapter-ligated library and required fewer amplification cycles for preparing 1µg of library. (Data provided by KAPA Biosystems)

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

LIMprep2 Workflow



* These steps were performed within a single tube



The magnetic bead-based cleanup steps were performed using MagnaStand YS-Model (holding eight 0.2 ml PCR tubes, Cat#FG-SSMAG2), a magnetic stand for trace samples.

* Detailed protocols (including the LIMprep2 protocol) for Quartz-Seq (RNA-Seq from single-cell RNA/trace RNA) in general can be downloaded from the protocol page (http://bit.accc.riken.jp/ptorocols/) provided by the Bioinformatics Research Unit, Advanced Center for Computing and Communication, RIKEN.

Results

Example data ①

The optimization of adaptor concentration was performed using 1 ng of fragmented 200bp genomic DNA.

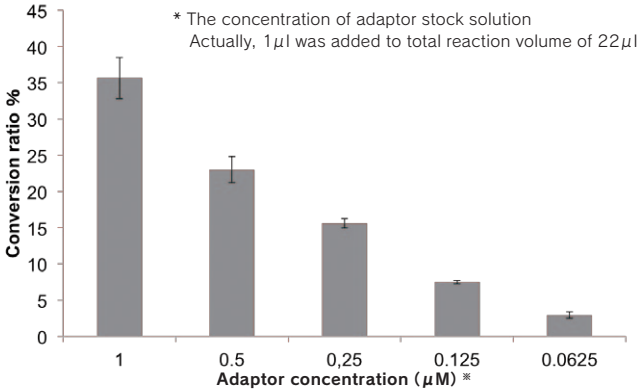


Fig. 2 Relationship between adaptor concentration and library conversion rate

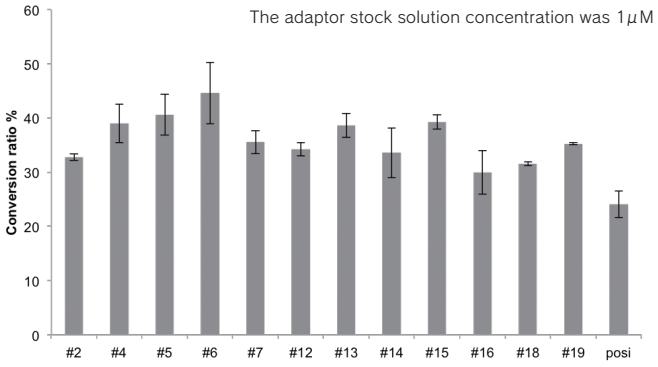
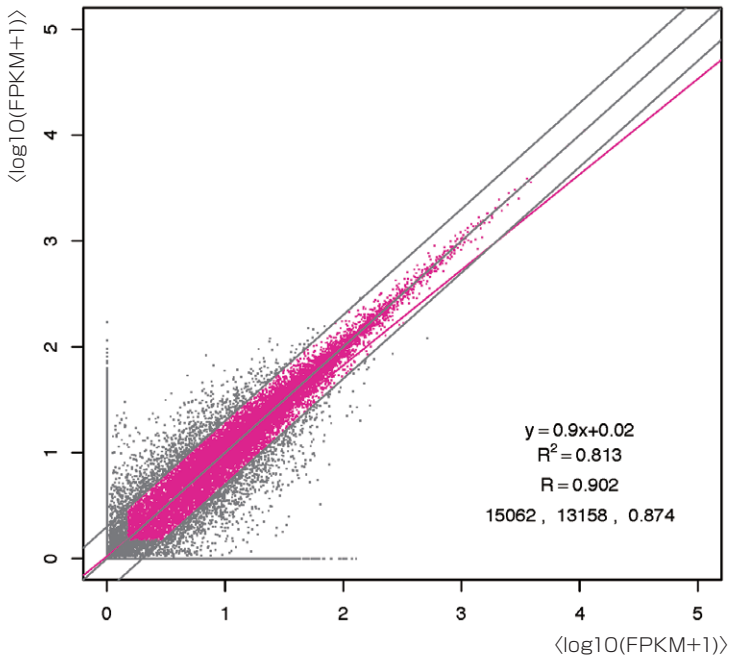


Fig.3 Comparison among adaptors with different indexes

Example data ②

As technical replicates, libraries were prepared several times from Quartz-seq* cDNA using the LIMprep2 protocol and sequenced by MiSeq. As a result, data of about 1-2M reads could be obtained, and a high correlation could be achieved even with few reads. It has been confirmed that high-accuracy sequencing is feasible with Quartz cDNA.

The expression was quantified and plotted on a scatter diagram.

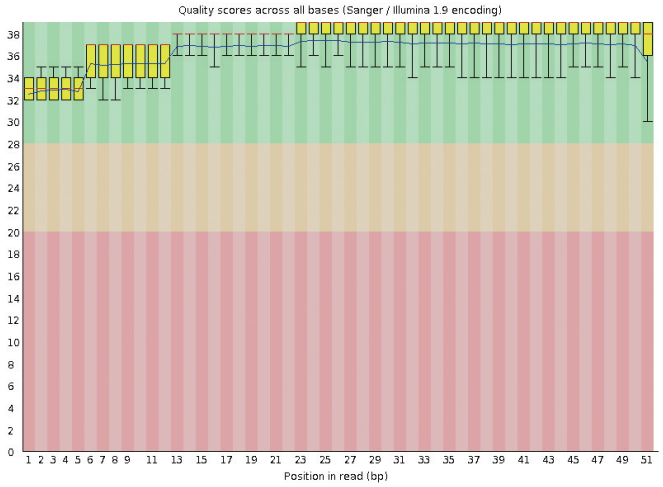


Sufficient correlation was achieved even with few reads

* Detailed protocols (including the LIMprepr2 protocol) for Quartz-Seq (RNA-Seq from single-cell RNA/trace RNA) in general can be downloaded from the protocol page (http://bit.accc.riken.jp/ptorocols/) provided by the Bioinformatics Research Unit, Advanced Center for Computing and Communication, RIKEN.

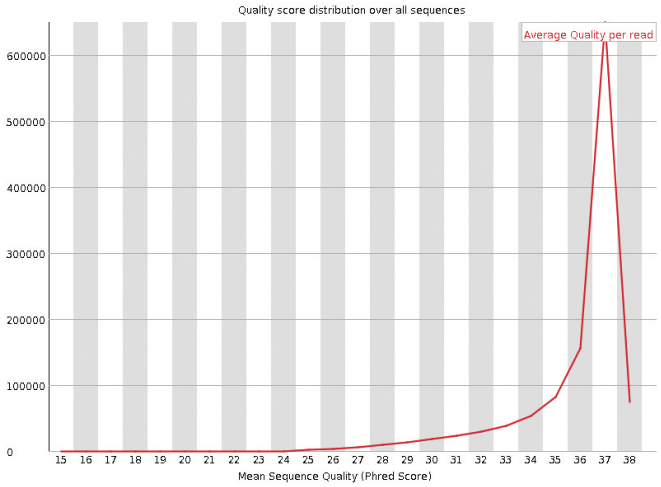
Data obtained by performing FASTQC after removing the adaptor

Per base sequence quality



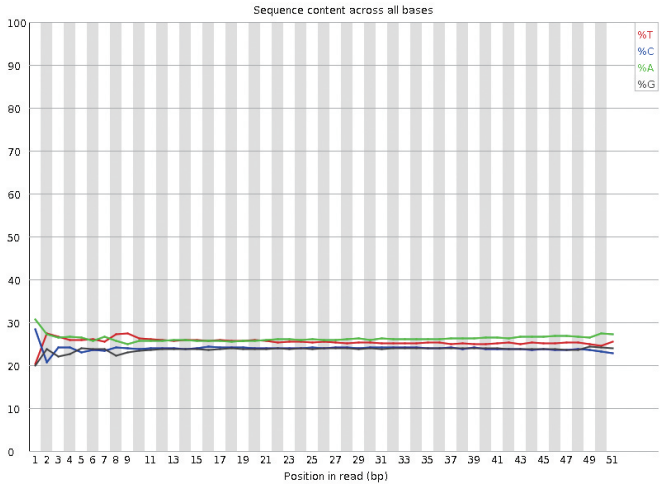
A high quality score was retained even at 50bp read

Per sequence quality scores



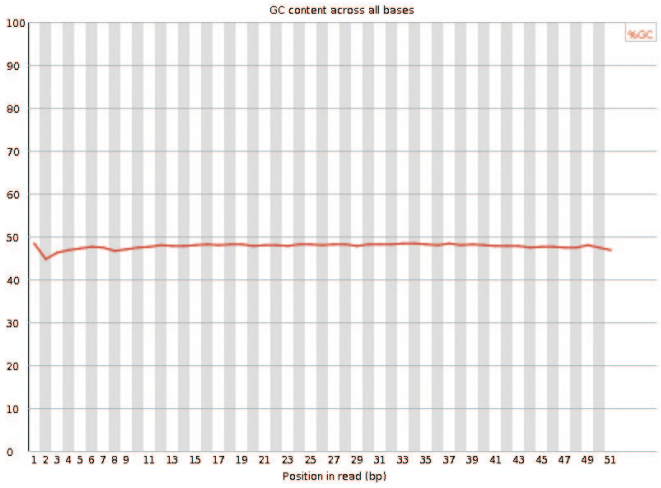
Most frequent quality score was 37

Per base sequence content



No gradient from start of read

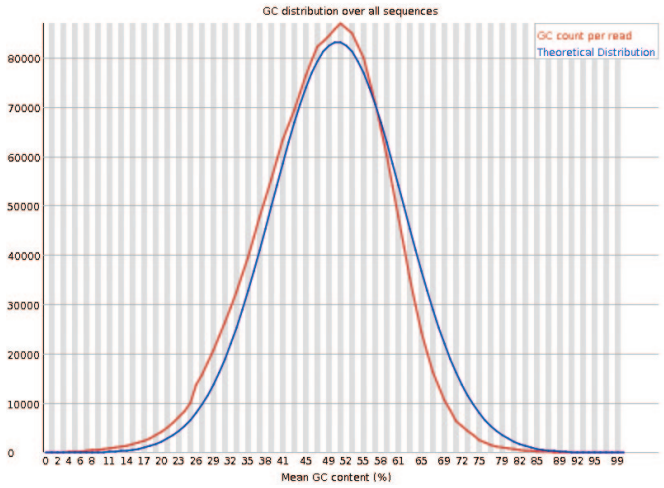
Per base GC content



No gradient of GC content up to 50bp

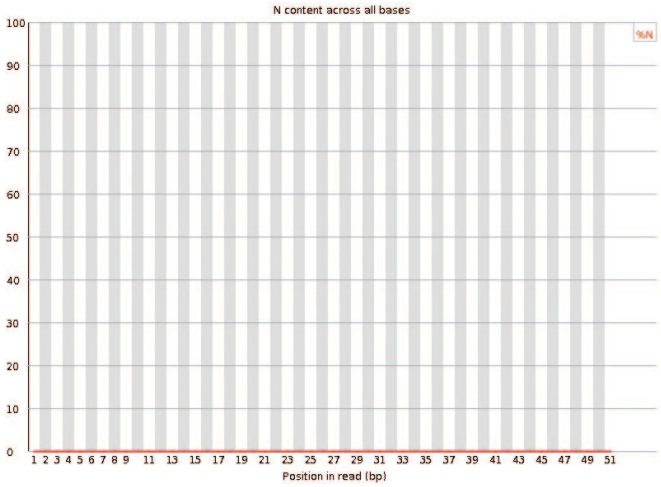
Data obtained by performing FASTQC after removing the adaptor

Per sequence GC content



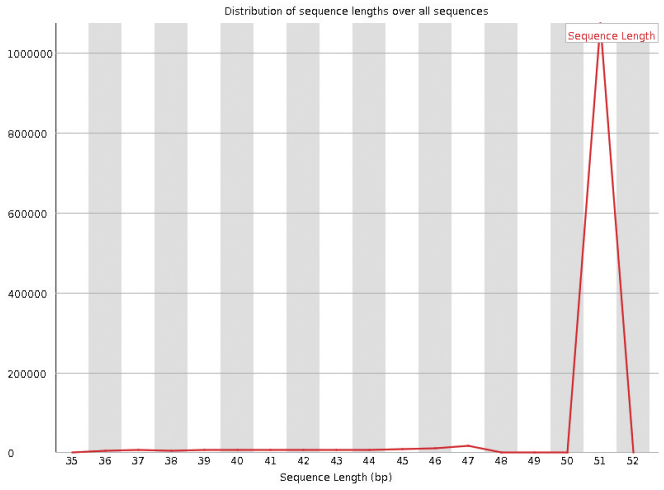
GC content per read showed a theoretical normal distribution

Per base N content



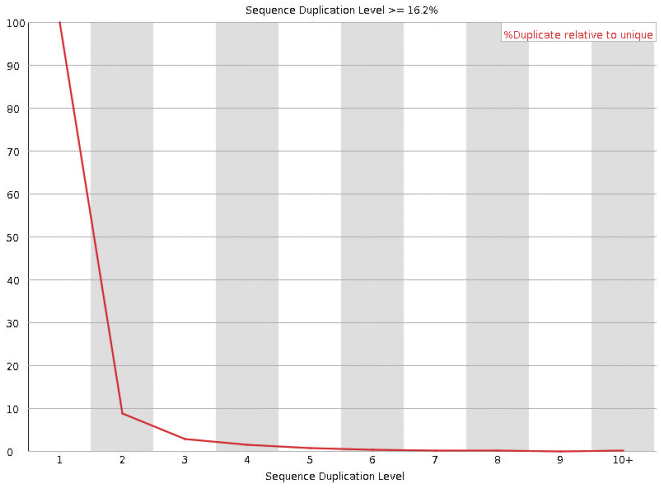
N was not detected

Sequence Length Distribution



The read length was 51bp

Sequence Duplication



Duplication was hardly observed

<Customer's comments>

The previous kit supplied by KAPA had many advantages such as high enzyme stability but required many purification steps. We expected the kit to be more simplified, as some manufacturers have released kits enabling the entire reaction to be completed within a single tube. KAPA Hyper Prep Kit is a good kit, achieving simplification of steps while improving and ensuring accuracy. Protocols supplied by KAPA tend to adopt large safety margins, so there was no data for trace input volumes of 1 ng or less. In this study, we confirmed that the high library conversion rate is retained even with trace input volumes. The optimized protocol can be downloaded from our laboratory's website. The protocol involves only few steps and can be easily completed just by adding the solutions one after another, so it should serve as the first choice protocol for many users.

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