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 1 µg 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.

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 1 µg of library. (Data provided by KAPA Biosystems)

Fig. 1 Library conversion rate of input DNA

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

- Start material (dsDNA)
  - End repair and A-tailing
  - Adapter ligation
  - SPRI cleanup (Adaptor dimer removal)
  - Optimal PCR cycle determination
  - Library Amplification
  - SPRI cleanup
  - Library QC and quantification
  - Sequence

* These steps were performed within a single tube

* 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.acc.c.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.

![Graph showing the relationship between adaptor concentration and library conversion rate]

"Fig. 2 Relationship between adaptor concentration and library conversion rate"

![Graph showing comparison among adaptors with different indexes]

"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.

![Scatter diagram showing sufficient correlation achieved even with few reads]

"Sufficient correlation was achieved even with few reads"

* 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/protocols/] 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

<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.