NIPPON Genetics Co.,Ltd.

Product feedback from users

Method of Mutiplex Library Preparation from Trace Samples (0.1-10 ng) - LIMprep (Ligation based Illumina Multiplex library PREParation method)- [A successful case of library preparation by next-generation sequencing] (MiSeq, Illumina Inc.)

Product name : KAPA Library Preparation Kit
: KAPA Real-Time Library Amplification Kit
: KAPA Library Quantification Kit

The data in this document were provided by the courtesy of Dr. Youhei Sasagawa of Functional Genomics Unit, RIKEN Center for Developmental Biology, Kobe, Japan.

Introduction (Customer's comments)

I have successfully established a highly sensitive, robust method for multiplex-library preparation from trace samples (0.1-10 ng) by combining a series of KAPA products available from Nippon Genetics with a self-made indexed adaptor DNA. For the validation of the application note, I prepared several library DNAs using the same starting material and examined the degree of variation in yield, distribution and content. The validation results demonstrated the high sensitivity and robustness of the library preparation method. Although the validation was conducted using genomic DNAs with an average length of 100 bp, library preparation efficiency can be increased by using an average DNA length of 200 bp or larger. Using this method, library DNAs were obtained from various starting materials and detected by HiSeq 1000/2000 to achieve satisfactory results. (MiSeq is used in this experiment)

KAPA products have two advantages over other products, one of which is the KAPA real-time library amplification kit. The kit is capable of preventing over-amplification during PCR-enrichment. Over-amplification causes library DNAs to stick together, making it difficult to identify their precise sizes, and creates excessive PCR bias. The engineered enzyme withstands the inhibition by SYBR Green, so the number of PCR cycle serving as the index substantially reflects the expected yield. Another advantage is the superiority of the enzymes included in the kit, such as the highly active engineered PCR enzyme KAPA HiFi DNA polymerase (see the corresponding application notes for these kits). All lots of the KAPA products I used were stable and of high quality.

The only bottleneck of the method was the self preparation of the adaptor. If you are interested in this method, please contact me via Nippon Genetics. Successful sequencing comes from successful library preparation. Good luck with you sequencing.

July 27, 2012 Youhei Sasagawa

For details and questions about the protocol, contact NIPPON Genetics Co., Ltd. info@genetics-n.co.jp

Method

A library (n=9) was prepared from 10 ng fragmented mouse genome DNA using the following kit and evaluated using MiSeq (Illumina Inc.).

Starting material	: 10 ng fragmented mouse genomic DNA
Genome fragmentation method	: Covaris S220 (M&S Instruments Inc.) used with standard settings for 100 bp
Library preparation kit	: KAPA Library Preparation Kit (includes KAPA Library Amplification kit), KK8201
Validation of library amplification cycle	: KAPA Real-Time Library Amplification kit, KK2701
Library amplification reagent	: KAPA Library Amplification kit (includes KAPA HiFi HotStart enzyme), includes KK8201
Library quantification kit	: KAPA Library Quantification kit, KK4835
Next-generation sequencer	: MiSeq (Illumina Inc.)

[LIMprep Workflow]

<Starting material>	10ng fragmented mouse genomic DNA (n=9)
↓	
<KAPA library preparation kit>	End-repair, dA-tail, adaptor ligation
↓	
<KAPA real-time library amplification kit>	Determination of PCR cycle
↓	
<KAPA library amplification kit>	PCR enrichment
↓	
<Resulted Library DNA>	Quantified by pico-green Average: 73.8±4.8 ng, CV: 0.066
↓	
<Quantified by qPCR>	1ng Library DNA / gene locus (n=9) Detected 8 gene locus (A-H)
↓	
<KAPA library quantification kit>	Final QC
↓	
<MiSeq Run>	SE50bp

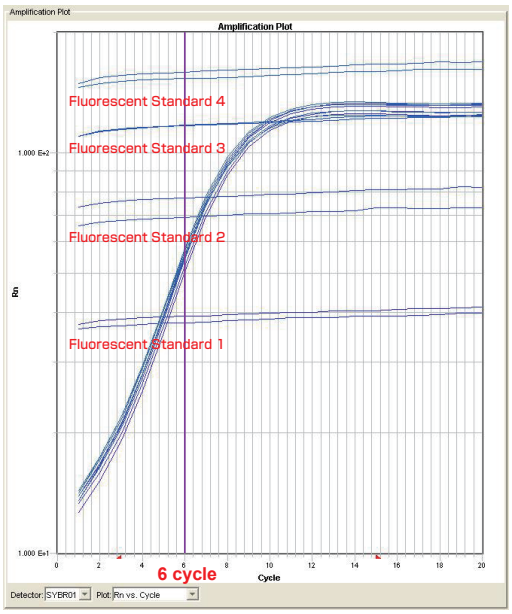
＊**LIMprep**: Ligation-based Illumina Multiplex library **PREP**aration method for low amount DNA using TruSeq adaptor and KAPA library preparation kit

Results

[LIMprep Workflow]

<Starting material>	10ng fragmented mouse genomic DNA (n=9)
↓	
<KAPA library preparation kit>	End-repair, dA-tail, adaptor ligation
↓	
<KAPA real-time library amplification kit>	Determination of PCR cycle ❶
↓	
<KAPA library amplification kit>	PCR enrichment
↓	
<Resulted Library DNA>	Quantified by pico-green Average: 73.8±4.8 ng, CV: 0.066 ❷,❸
↓	
<Quantified by qPCR>	1ng Library DNA / gene locus (n=9) Detected 8 gene locus (A-H)
↓	
<KAPA library quantification kit>	Final QC
↓	
<MiSeq Run>	SE50bp

❶ Determination of PCR cycle



<KAPA real-time library amplification kit>

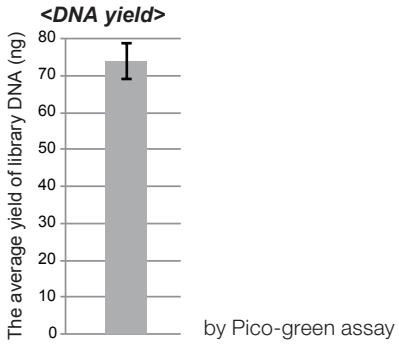
Determination of PCR cycle

For library amplification, it is important to determine the optimal cycle number for achieving the library volume required for next-generation sequencing. The cycle number should be as small as possible to minimize amplification bias.
From the results of the present experiment, the optimal minimum cycle number was determined to be 4-6 cycles.
(The optimal minimum cycle number was 6 cycles for this experiment.)

[Note (Nippon Genetics)]

KAPA Real-Time Library Amplification Kit has been developed for validating the optimal minimum cycle number through highly sensitive real-time PCR based on the "KAPA HiFi HotStart enzyme" engineered for library amplification.
The master mix (2×) contains four fluorescent standards of different concentrations and is designed to yield an optimal library concentration between standards 1 and 3. This enables the user to constantly obtain libraries with stable concentrations at minimum cycle numbers, which should enhance the stability of library quality.

❷ PCR enrichment (after 6 cycles)



by Pico-green assay

<KAPA library amplification kit>

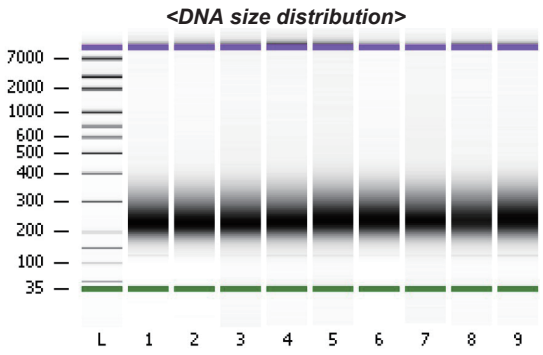
PCR enrichment
Quantified by pico-green Average: 73.8±4.8 ng, CV: 0.066

In this experiment, a stable amount (73.8±4.8 ng, CV: 0.066) of library DNAs could be obtained with a stable size distribution through library preparation (n=9) using a single DNA sample.

[Note (Nippon Genetics)]

KAPA Library Preparation Kit includes an End-repair enzyme, a dA-tail enzyme and an Adaptor ligation enzyme as well as the Kapa Library Amplification Kit (KAPA HiFi HotStart enzyme).
KAPA HiFi HotStart enzyme offers high fidelity, minimizing the bias when amplifying the sequences with high GC/AC contents and yielding stable amplification results.
The Wellcome Trust Sanger Institute has demonstrated that this engineered enzyme effectively reduces bias in NGS libraries.
Michael A Quail et al., Optimal enzymes for amplifying sequencing libraries, Nature Methods 9, 10-11 (2012)

❸ PCR enrichment (after 6 cycles)



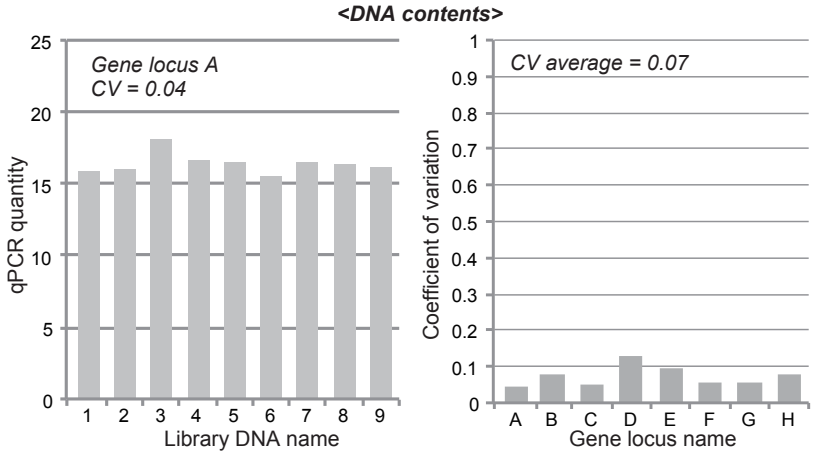
by Bioanalyzer

Results

[LIMprep Workflow]

<Starting material> 10ng fragmented mouse genomic DNA (n=9)
↓
<KAPA library preparation kit> End-repair, dA-tail, adaptor ligation
↓
<KAPA real-time library amplification kit> Determination of PCR cycle
↓
<KAPA library amplification kit> PCR enrichment
↓
<Resulted Library DNA> Quantified by pico-green Average: 73.8±4.8 ng, CV: 0.066
↓
<Quantified by qPCR> 1ng Library DNA / gene locus (n=9) Detected 8 gene locus (A-H) ····· 4
↓
<KAPA library quantification kit> Final QC
↓
<MiSeq Run> SE50bp ····· 5

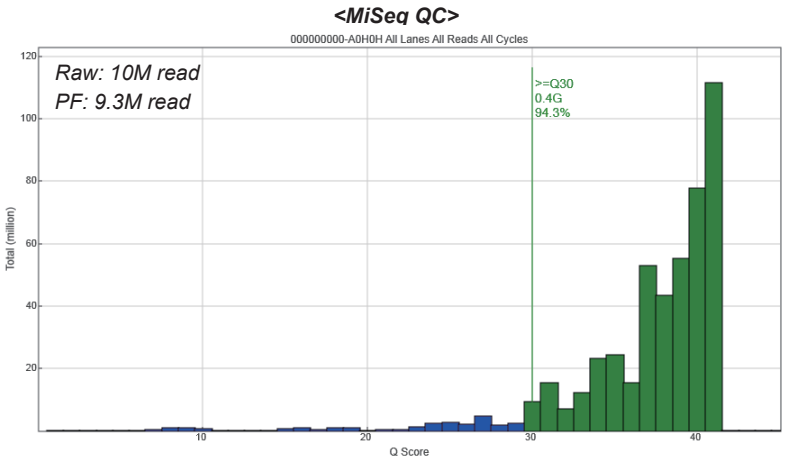
4 Quantified by qPCR



<Quantified by qPCR>
1 ng Library DNA / gene locus (n=9) Detected 8 gene locus (A-H)

In order to confirm the absence of amplification bias among the library DNAs amplified by the KAPA HiFi HotStart enzyme from the same DNA sample, real-time PCR was performed to quantify eight loci (A through H).
In this experiment, the CV for nine library DNAs for locus A was 0.04, and the average CV for loci A through H was 0.07, which demonstrated that the library DNAs has a low amplification bias.

5 Sequencing by MiSeq



<KAPA library quantification kit>
<MiSeq Run>
SE50bp

For final validation, the library concentration was determined using KAPA Library Quantification Kit, and sequencing was performed by MiSeq with 50-bp single-end run.
As a result, a high-quality sequence data could be obtained, with 10M RAW reads (9.3M reads passing filter) and 0.4 G bases (94.3%) equal to or higher than Q30.

[Note (Nippon Genetics)]
KAPA Library Quantification Kit is intended for library quantification by real-time PCR based on an engineered enzyme withstanding the inhibition by SYBR Green, a substance known to inhibit PCR.
The kit includes the "Master mix" enabling stable amplification of heterogeneous sequences with biased GC/AT contents, "six diluted standard DNAs" of strictly controlled quality and the "primer", yielding consistent, stable library quantification results.

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