KAPABIOSYSTEMS

Poster Note
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KAPA HYPER PREP:

A next-generation kit for fast and efficient library construction from challenging DNA samples.

Authors

Victoria van Kets[1] , Maryke Appel[1], Jacob Kitzman[2], Matthew Snyder[2], Jay Shendure[2], Phillip Gray[3], Pei-Fang Tsai[3], Jia Yan Hoo[3], Eric van der Walt[1], John Foskett[1], Paul McEwan[1]

1 Kapa Biosystems, 200 Ballardvale St, Suite 250, Wilmington, MA
2 Genome Sciences, University of Washington, Seattle, WA
3 Ambry Genetics, Aliso Viejo, CA

UNIVERSITY *of* WASHINGTON

Ambry Genetics

ATCGGCA
TTTGGCC
CGCATCG
GACTACG
NGS

PCR

qPCR

INTRODUCTION

Kapa Biosystems has previously developed a robust and versatile library preparation kit, that is currently regarded as the best sample preparation solution for Illumina® sequencing. A combination of ultra-pure, optimally formulated enzymes and a highly-optimized "with-bead" protocol enables the construction of high-quality libraries from challenging samples such as FFPE, ChIP and cell-free DNA.

Rapid growth in sequencing capacity, falling costs and the implementation of next-generation sequencing (NGS) in clinical and diagnostic settings are driving a relentless demand for NGS library construction solutions that are faster, simpler, more cost-effective, and easier to scale This is evidenced by the growing interest in streamlined "single-tube" adapter ligation-mediated library construction workflows, and ransposase-mediated "tagmentation" technology. Typically, single-tube ligation- or tagmentation-based library construction methods represent considerable compromises in performance: they convert less input DNA into library fragments, resulting in libraries with lower complexity and reduced coverage uniformity; and are more sensitive to the amount and/or quality of input DNA. This presents a particular challenge for projects involving challenging samples of variable input and quality.

To address these requirements, Kapa Biosystems has developed an optimized, single-tube library construction method (Fig. 1) that enables fast and highly efficient library construction from a range of sample types and inputs.

Preliminary results demonstrated similar or better performance with the existing KAPA Library Preparation Kit, and improved results for FFPE samples.

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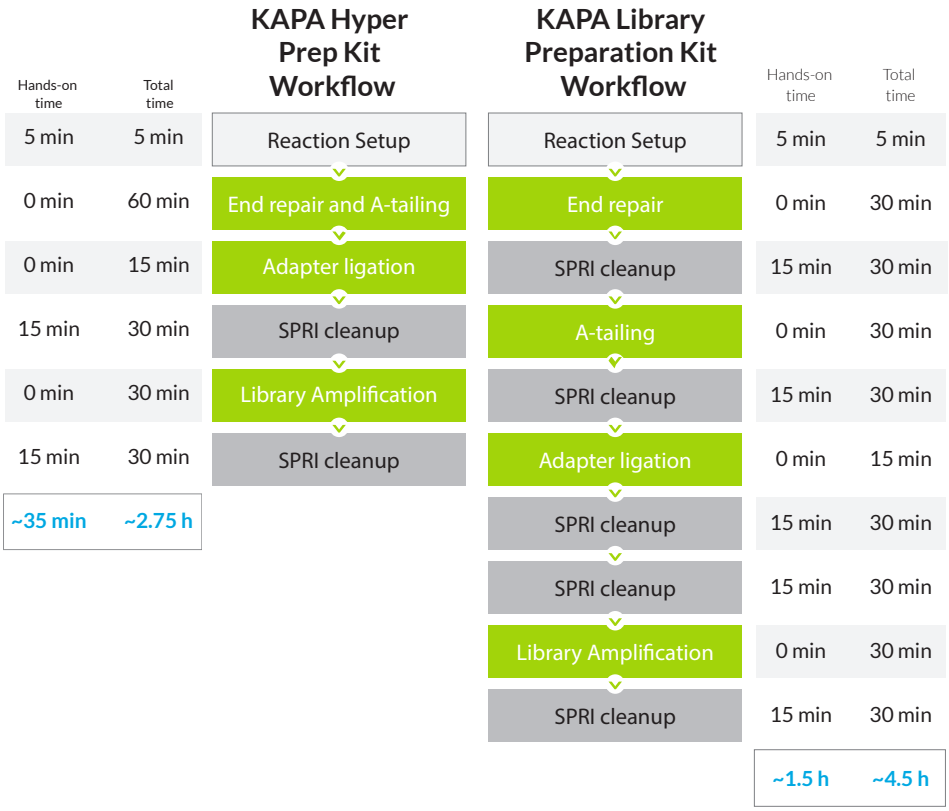
FIGURE 1

Comparative workflow diagrams for the KAPA Library Preparation and KAPA Hyper Prep Kits



In the KAPA Hyper Prep protocol, end repair and A-tailing are performed with a novel enzyme mix and buffer, in a single tube, with a 2-step temperature profile (end repair for 30 min at 20°C, followed by A-tailing for 30 min at 65°C). Adapters, ligation buffer and enzyme are added directly to the ER & AT reaction product, for a total ligation reaction volume of 110 µL. The novel ER & AT reagent formulation inhibits adapter-dimer formation. As a result, adapter:insert molar ratios can be increased to improve ligation efficiency for low-input and challenging samples (see Fig. 2), without the need for rigorous post-ligation cleanup.

Like the KAPA Library Preparation Kit, KAPA Hyper Prep Kits contain KAPA HiFi HotStart ReadyMix, which has become the gold standard for NGS library amplification. Library amplification is optional, and depends on the sample type and input, adapter design and sequencing application. KAPA Hyper Prep Kits also contain a new, highly optimized Library Amplification Primer Mix (for Illumina® platforms), which is formulated to eliminate or delay primer depletion, thereby ensuring optimal amplification efficiency with KAPA HiFi.

KAPA Hyper Prep Kits are available in 24-reaction and automation-friendly 96-reaction pack sizes.

ACHIEVE HIGHER LIBRARY YIELDS THAN WITH COMPETITOR STREAMLINED KITS

To assess the performance of the KAPA Hyper Prep Kit, libraries were prepared from Covaris-sheared *E. coli* genomic DNA, over a range of inputs (10 ng – 1000 ng). Duplicate libraries were prepared with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs). All libraries were made with indexed Y-adapters, at three different adapter: insert molar ratios for each input.

The KAPA Library Quantification Kit was used to quantify library yields after ligation. This data was used to calculate the efficiency of library construction, expressed as the % of input DNA converted to adapter-ligated library. Results are given in Fig. 2.

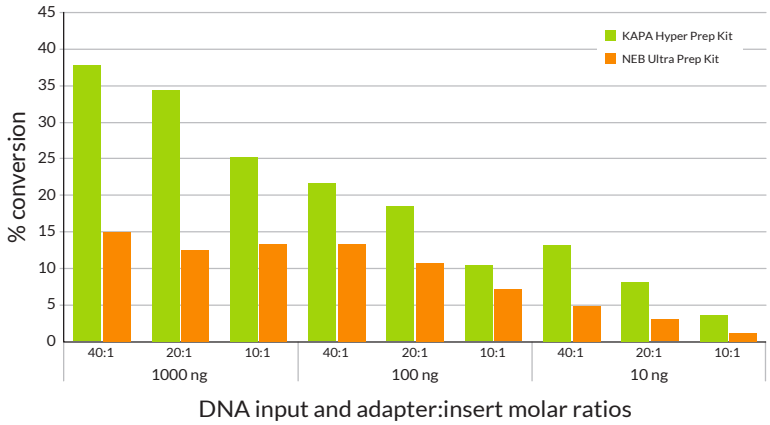
Yields of adapter-ligated library achieved with the KAPA Hyper Prep Kit were 1.5 – 2.8 times higher than those obtained with the NEBNext kit. Kapa outperformed NEB across the entire range of inputs and adapter:insert ratios. Since the two kits employ similar protocols, the data indicated that Kapa's single-tube chemistry is superior to NEB's.

Since the efficiency of library construction up to adapter ligation determines the number of unique molecules in a library, as well as the number of PCR cycles needed to generate a sufficient amount of DNA for subsequent processes, the KAPA Hyper Prep Kit is expected to yield higher-quality libraries and improved sequence coverage as compared to competitor kits.

FIGURE 2

The KAPA Hyper Prep Kit achieves higher yields of adapter-ligated library than streamlined competitor kits

2A



E. coli genomic DNA was Covaris-sheared to an average size of ~150 bp. Libraries were prepared with the KAPA Hyper Prep Kit and the NEBNext Ultra DNA Library Prep Kit (New England Biolabs), according to the standard protocol for each kit. DNA inputs and adapter:insert molar ratios are indicated on the x-axis.

A Conversion rates (% input DNA converted to adapter-ligated library; calculated without compensation for the mass added during adapter ligation) are represented by the green (Kapa) and orange (NEB) bars. Actual conversion rates are given above the bars.

B Because of the higher yields of adapter-ligated molecules, libraries generated with the KAPA Hyper Prep Kit needed 1–2 fewer cycles of library amplification to achieve ~500 ng of amplified library.

2B

DNA INPUT	ADAPTER: INSERT MOLAR RATIO	CYCLE NUMBER TO GENERATE 500 NG AMPLIFIED LIBRARY	
		KAPA	NEB
1000 ng	40:1	3	4
	20:1	3	4
	10:1	3	4
100 ng	40:1	7	8
	20:1	8	9
	10:1	9	10
10 ng	40:1	12	14
	20:1	13	15
	10:1	14	16

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CONSTRUCT HIGH-QUALITY LIBRARIES FROM CHALLENGING, LOW-INPUT SAMPLES

To assess the performance of the KAPA Hyper Prep Kit with challenging, low-input samples, libraries were prepared from 2 or 10 ng cell-free DNA, isolated from plasma. Duplicate libraries were made with the KAPA Hyper Prep Kit or KAPA Library Preparation Kit, using different adapter:insert molar ratios. Libraries where amplified with KAPA HiFi in the presence of SYBR® Green I to ensure optimal amplification of each library. Multiplexed sequencing was performed on an Illumina® MiSeq. Key sequencing metrics are given in Fig. 3.

The library prepared from 2 ng input DNA with the KAPA Library Preparation Kit, using the lowest adapter: insert molar ratio (10:1), produced the worst overall results. As expected, increasing adapter concentrations yielded better quality libraries, but also resulted in significantly more adapter-dimer carry-over in libraries prepared with the traditional method. This underlines the benefits of the novel, single-tube chemistry for low-input library construction.

Libraries prepared with the KAPA Hyper Prep Kit displayed higher diversity than those prepared with the existing KAPA Library Preparation Kit.

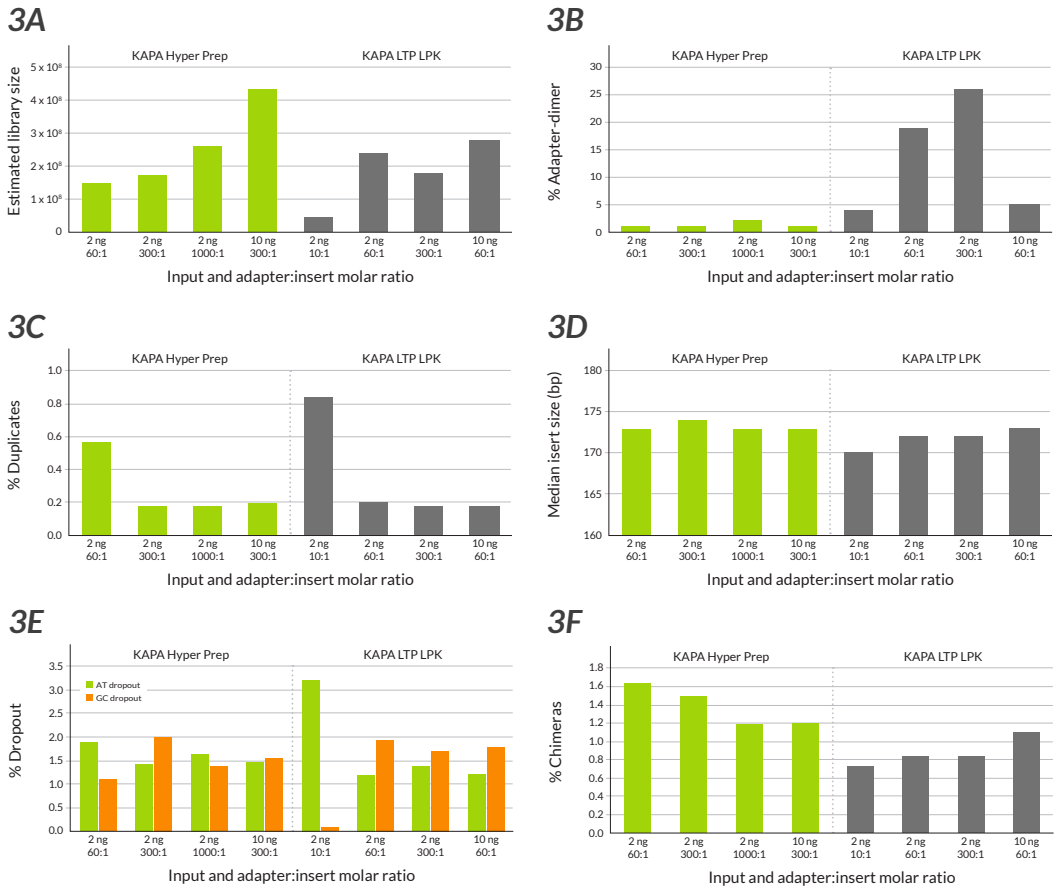
Libraries prepared with the two kits had similar median insert sizes, and were of comparable quality with respect to:

- Duplication rates, particularly for libraries prepared with higher adapter:insert molar ratios, and 10 ng input DNA.
- % AT- and GC-dropout (with the exception of the library prepared with the traditional chemistry from 2 ng input and an adapter:insert molar ratio of 10:1).

Interestingly, the Hyper Prep libraries contained almost double the % of chimeric molecules; a phenomenon that appears to be associated with streamlined, single-tube protocols.

FIGURE 3

Key sequencing metrics for libraries prepared from low-input cell-free DNA



Libraries prepared with the KAPA Hyper Prep Kit (green) and existing KAPA Library Preparation Kit (grey) were compared. The amount of input DNA and adapter:insert molar ratio for each library is given on the x-axis. Standard protocols were followed, with the following exceptions: KAPA Hyper Prep Kit—amplified libraries were incubated on SPRI beads for 40 min during the post-amplification cleanup; KAPA Library Preparation Kit—the second post-ligation cleanup was omitted. Picard was used for sequence data analysis.

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IMPROVE SEQUENCING RESULTS FOR FFPE SAMPLES

Formalin-fixed paraffin embedded (FFPE) tissue is an important source of DNA for cancer genomics studies and clinical diagnostics. DNA extracted from FFPE samples is, however, typically limited to nanogram quantities and riddled with molecular damage that further lowers usable input. This impacts the ability to construct high-quality libraries for NGS.

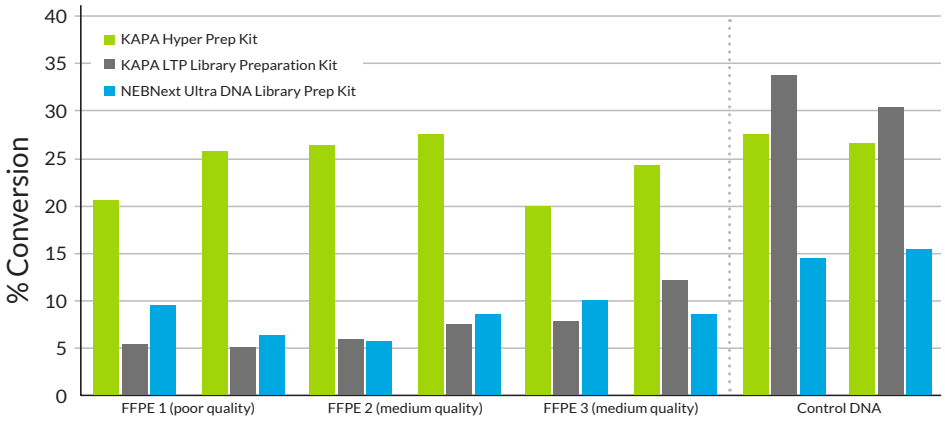
To confirm the benefits of the newly developed single-tube chemistry and streamlined protocol for FFPE library construction, libraries were prepared from three FFPE DNAs of variable quality using the KAPA Hyper Prep Kit, KAPA Library Preparation Kit, and NEBNext® Ultra™ DNA Library Prep Kit. Adapter-ligated libraries were quantified with the KAPA Library Quantification Kit, and conversion rates calculated as outlined previously. Results are given in Fig. 4A.

The KAPA Hyper Prep Kit achieved significantly higher yields of adapter-ligated library than the other two kits. To confirm that these benefits translate to improved sequence quality, two additional FFPE libraries were prepared for targeted sequencing on the Illumina® platform. Post-capture sequencing metrics for these libraries are given in Fig. 4B.

FIGURE 4

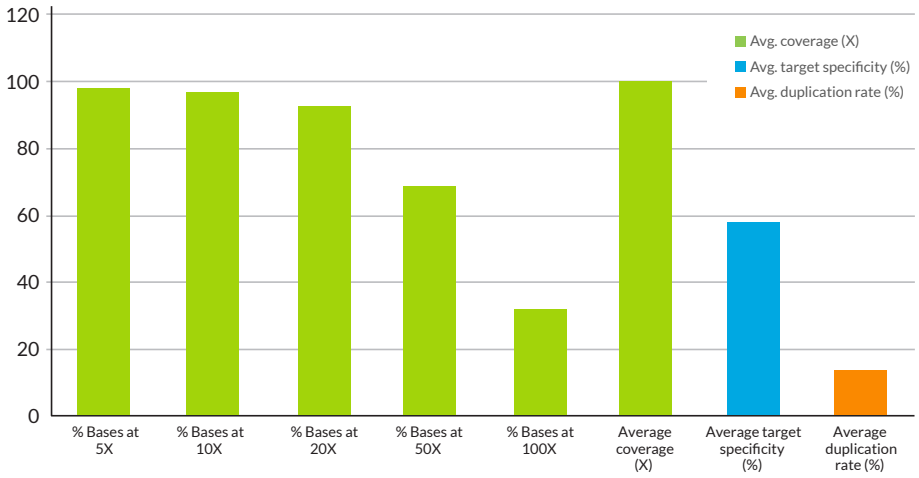
The KAPA Hyper Prep Kit enables improved sequencing quality for FFPE libraries

4A



A Conversion rates (% input DNA converted to adapter-ligated library; calculated without compensation for the mass added during adapter ligation) for libraries prepared from three FFPE samples of variable quality with the existing KAPA Library Preparation Kit (grey), KAPA Hyper Prep Kit (green), and NEBNext Ultra DNA Library Prep Kit (blue). Q(129/41)-ratios, generated with the KAPA Human Genomic Quantification and QC Kit were 0.26, 0.56 and 0.96 for FFPE 1, FFPE 2 and FFPE 3, respectively. DNA was Covaris-sheared to an average size of ~180 bp. Libraries were prepared from 80 – 100 ng DNA using SureSelectXT2 adapters (Agilent Technologies) at an adapter:insert molar ratio of 50:1. The control DNA was a commercial human genomic DNA preparation.

4B



B Post-capture sequencing metrics and duplication rates for libraries prepared from two FFPE samples with the KAPA Hyper Prep Kit for whole exome sequencing. The input into library construction was 100 ng. Pre- and post-capture amplification (8 and 13 cycles, respectively) was performed with KAPA HiFi HotStart ReadyMix. Exome capture was performed with the SeqCap EZ HGSC VCRome Design (Roche NimbleGen).

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CONCLUSIONS

We have developed a highly efficient, streamlined, single-tube library preparation method for the rapid construction of DNA libraries for Illumina® sequencing. The KAPA Hyper Prep Kit:

- Outperforms streamlined library preparation kits from other vendors across a range of DNA inputs and sample types.
- Can be used with higher adapter:insert molar ratios, which contributes to improved library yields and sequence quality for low-input samples.
- Achieves significantly higher conversion rates for FFPE samples, which translates to lower duplication rates and higher coverage.

Contact Us at:

Headquarters, United States
Wilmington, Massachusetts
Tel: 781.497.2933
Fax: 781.497.2934
sales@kapabiosystems.com

International Office
Cape Town, South Africa
Tel: +27.21.448.8200
Fax: +27.21.448.6503
sales@kapabiosystems.com

Kapa Technical Support
kapabiosystems.com/support

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