KAPABIOSYSTEMS

Technical Data Sheet

KAPA Library Amplification Kit

Illumina® platforms

KR0408 – v5.14

Product Description

KAPA Library Amplification Kits for Illumina® platforms are designed for the amplification of next-generation sequencing libraries prepared for Illumina® sequencing.

To maximize sequence coverage uniformity and maintain relative transcript abundance, it is critical to limit library amplification bias. KAPA HiFi DNA Polymerase is designed for low-bias, high-fidelity PCR, and is the polymerase of choice for NGS library amplification.[1, 2, 3] KAPA Library Amplification Kits include KAPA HiFi HotStart ReadyMix (2X), and KAPA Library Amplification Primer Mix (10X) for library amplification.

KAPA HiFi HotStart ReadyMix (2X) is a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X ReadyMix contains KAPA HiFi HotStart DNA Polymerase in a proprietary reaction buffer, including dNTPs and $MgCl_2$ (2.5 mM at 1X).

KAPA Library Amplification Primer Mix comprises a forward and a reverse primer, and is suitable for the amplification of all Illumina® libraries flanked by the P5 and P7 flow cell sequences. The Primer Mix is formulated to achieve optimal amplification efficiency.

1. Oyola, S.O. et al. *BMC Genomics* **13**, 1 (2012).
2. Quail M.A. et al. *Nature Methods* **9**, 10 – 11 (2012).
3. Quail M.A. et al. *BMC Genomics* **13**: 341 (2012).

Product Applications

KAPA Library Amplification Kits for Illumina® platforms are suited for high-efficiency, high-fidelity, low-bias amplification of libraries prior to Illumina® sequencing. This includes libraries prepared for:

- Whole-genome shotgun sequencing
- Targeted sequencing (pre- and post-capture amplification)
- Amplicon sequencing
- ChIP-seq
- RNA-seq

Product Specifications

Shipping and storage

KAPA Library Amplification Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, store the entire kit at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, all kit components will retain full activity until the expiry date indicated on the kit label.

Kit Codes and Components	
KK2620 50 reactions	2X KAPA HiFi HotStart ReadyMix (1.25 ml) 10X KAPA Library Amplification Primer Mix, Illumina® (0.25 ml)
KK2621 250 reactions	2X KAPA HiFi HotStart ReadyMix (6.25 ml) 10X KAPA Library Amplification Primer Mix, Illumina® (1.25 ml)
KK2611 50 reactions	**PCR mix only:** 2X KAPA HiFi HotStart ReadyMix(1.25 ml)
KK2612 250 reactions	**PCR mix only:** 2X KAPA HiFi HotStart ReadyMix (6.25 ml)
KK2623 250 reactions	**Primers only:** 10X KAPA Library Amplification Primer Mix, Illumina® (1.25 ml)

Quick Notes

- KAPA HiFi HotStart ReadyMix is specifically designed to minimize amplification bias, while maintaining industry-leading fidelity.
- KAPA Library Amplification Primer Mix contain primers that target the P5 and P7 regions of Illumina® TruSeq™ and dual-indexed adapters.
- The Primer Mix is formulated to limit primer depletion and over-amplification.
- Kits without primers (KK2611 and KK2612) are available for library amplification with user-supplied primers.

Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. Keep all reaction components and master mixes on ice whenever possible during handling. KAPA HiFi HotStart ReadyMix (2X) may not freeze completely, even when stored at -20 °C. Nevertheless, always ensure that the KAPA HiFi HotStart ReadyMix is fully thawed and thoroughly mixed before use. Kit components may be stored at 4 °C for regular, short-term use (up to 1 month). Long-term storage at room temperature or 4 °C is not recommended.

Quality control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination. Please contact **support@kapabiosystems.com** for more information.

Library Amplification Protocol

Table 1 provides recommendations for amplification using the 10X KAPA Library Amplification Primer Mix supplied in KAPA Library Amplification Kits (KK2620, KK2621 and KK2623), while Table 2 provides reaction setup and cycling protocol recommendations for several Illumina® library types with user-supplied primers. When using primers not listed in the tables below, annealing temperature optimization may be required.

NOTE: Use the fewest number of cycles that ensures a sufficient yield of amplified library for the next step in the workflow, plus the amount of library needed for QC and/or archiving. Please refer to the **Important Parameters: Cycle number** for more information.

Table 1: Reaction setup and cycling protocols for library amplification with KAPA Library Amplification Primer Mix

Reaction setup			Cycling protocol		
Component	**Final conc.**	**50 µl rxn[1]**	**Step**	**Temp.**	**Time**
PCR grade water	N/A	As required	Initial denaturation	98 °C	45 sec
2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Cycling: Denaturation	98 °C	15 sec
10X KAPA Library Amplification Primer Mix	10X	5 µl	Annealing	**60 °C**	30 sec
Library DNA	N/A	Up to 20 µl	Extension	72 °C	30 sec
			Final extension	72 °C	1 min

Table 2: Reaction setup and cycling protocols for library amplification with user-supplied primers

Library (Illumina®)	Reaction setup			Cycling protocol		
	Component	**Final conc.**	**50 µl rxn[1]**	**Step**	**Temp.**	**Time**
Genomic DNA	PCR grade water	N/A	As required	Initial denaturation	98 °C	45 sec
	2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Cycling: Denaturation	98 °C	15 sec
	PCR Primer 1.1	0.5 µM	1 µl	Annealing	**65 °C**	30 sec
	PCR Primer 2.1	0.5 µM	1 µl	Extension	72 °C	30 sec
	Library DNA	N/A	As required	Final extension	72 °C	1 min
Paired-End (PE)	PCR grade water	N/A	As required	Initial denaturation	98 °C	45 sec
	2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Cycling: Denaturation	98 °C	15 sec
	PE PCR Primer 1.0	0.5 µM	1 µl	Annealing	**65 °C**	30 sec
	PE PCR Primer 2.0	0.5 µM	1 µl	Extension	72 °C	30 sec
	Library DNA	N/A	As required	Final extension	72 °C	1 min
Paired-End (PE) Multiplex	PCR grade water	N/A	As required	Initial denaturation	98 °C	45 sec
	2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Cycling: Denaturation	98 °C	15 sec
	PE PCR Primer InPE 1.0	0.5 µM	1 µl	Annealing	**65 °C**	30 sec
	PE PCR Primer InPE 2.0	10 nM	1 µl	Extension	72 °C	30 sec
	PCR Primer Index 1 – 12	0.5 µM	1 µl	Final extension	72 °C	1 min
	Library DNA	N/A	As required			
TruSeq™	PCR grade water	N/A	As required	Initial denaturation	98 °C	45 sec
	2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Cycling: Denaturation	98 °C	15 sec
	PCR Primer Cocktail	0.5 µM each	5 µl	Annealing	**60 °C**	30 sec
	Library DNA	N/A	As required	Extension	72 °C	30 sec
				Final extension	72 °C	1 min
Nextera®	PCR grade water	N/A	As required	Pre-PCR hold	72 °C	3 min
	2X KAPA HiFi HotStart ReadyMix	1X	25 µl	Initial denaturation	98 °C	30 sec
	Nextera™ Primer Cocktail	As supplied	5 µl	Cycling: Denaturation	98 °C	10 sec
	Index 1 Primer	As supplied	5 µl	Annealing	**63 °C**	30 sec
	Index 2 Primer	As supplied	5 µl	Extension	72 °C	3 min
	Library DNA[2]	N/A	As required	Final extension	72 °C	1 min

[1] Reaction volumes of 50 µl are recommended. Larger reaction volumes may result in lower reaction efficiency.

[2] For Nextera® library amplification, the maximum volume of library DNA that can be used per 50 µl reaction is 10 µl. A 1X SPRI cleanup can be used to concentrate DNA prior to library amplification.

Important Parameters

Cycle number

Excessive library amplification should be avoided to minimize the following adverse effects:

- Increased duplicate reads
- Uneven coverage depth and sequence dropout
- Chimeric library inserts
- Nucleotide substitutions
- Heteroduplex formation

To minimize over-amplification and associated unwanted artifacts, the number of amplification cycles should be optimized to ensure a sufficient amount of amplified library for the next step in the workflow (capture or sequencing), plus the amount needed for library QC and/or archiving. Depending on the sequencing application and degree of multiplexing, 100 ng – 1.5 µg of amplified library is typically required.

Table 3 indicates the number of cycles typically required to generate 100 ng or 1 µg of amplified library DNA, in workflows with or without size-selection. Size-selection of libraries at any part in the library construction process results in significant loss of material and as a result, 2 – 4 additional cycles are required for workflows which include a size-selection step prior to library amplification.

These guidelines are for libraries prepared with the KAPA LTP or HTP Library Preparation Kit; the actual optimal number of cycles may be higher, depending the reagents and protocol used for library construction, and the quality of the input DNA. For libraries prepared from FFPE DNA or other challenging samples, or libraries with a broad fragment size distribution, 1 – 3 additional cycles may be required.

Primer quality and concentration

To achieve optimal amplification efficiency and avoid primer depletion, it is critical that an optimal concentration of high quality primers are used. Primers should be used at a final concentration of 0.5 – 2 µM each. For libraries constructed from ≥100 ng input DNA, the highest final concentration (2 µM of each primer) is recommended.

To ensure the highest primer quality, primers should be HPLC-purified, and modified to include a phosphorothioate bond at the 3'-terminal of each primer (to prevent degradation by the strong proofreading activity of KAPA HiFi DNA Polymerase). Furthermore, primers should always be stored and diluted in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0 – 8.5), and the number of freeze-thaw cycles should be limited. To achieve the latter, primers should be stored at 4 °C for short-term use, or as single-use aliquots at -20 °C.

Table 3: Recommended library amplification cycles to generate 100 ng or 1 µg of amplified library, with or without size-selection (SS)

Input DNA*	Cycles for 100 ng amplified library DNA		Cycles for 1 µg amplified library DNA	
	No SS	With SS	No SS	With SS
1 µg	0	2 – 45	2 – 4	6 – 7
500 ng	0 – 1	3 – 5	3 – 5	7 – 8
250 ng	1 – 2	4 – 6	4 – 6	8 – 9
100 ng	2 – 4	6 – 8	6 – 8	9 – 11
50 ng	5 – 7	8 – 10	8 – 10	12 – 14
25 ng	6 – 8	9 – 11	9 – 11	13 – 15
10 ng	7 – 9	11 – 13	11 – 13	14 – 16
5 ng	8 – 10	12 – 14	12 – 14	16 – 18
1 ng	11 – 13	14 – 16	14 – 16	18 – 20

* Input into library construction.

Primer depletion and library over-amplification

In library amplification reactions, primers are typically depleted before dNTPs. When DNA synthesis can no longer take place due to primer depletion, subsequent rounds of DNA denaturation and annealing result in the separation of complementary DNA strands, followed by imperfect annealing to non-complementary partners. This presumably results in the formation of so-called "daisy-chains" or tangled knots, comprising large assemblies of improperly annealed, partially double-stranded, heteroduplex DNA. These species migrate slower and are observed as secondary, higher molecular weight peaks during the electrophoretic analysis of amplified libraries. However, they are typically comprised of library molecules of the desired length, which are separated during denaturation prior to target enrichment (capture) or cluster amplification. Since these heteroduplexes contain significant portions of single-stranded DNA, over-amplification leads to the under-quantification of library molecules with assays employing dsDNA-binding dyes. qPCR-based library quantification methods, such as the KAPA Library Quantification assay, quantify DNA by denaturation and amplification, thereby providing a more accurate measurement of the amount of adapter-ligated molecules, even in the case of over-amplified libraries.

Please refer to the **KAPA NGS Library Preparation Technical Guide** for a more detailed discussion of factors that can affect the efficiency of library amplification, and the impact of over-amplification on library quantification.

Important Parameters (continued)

Optimization of library amplification

Quantification of adapter-ligated libraries prior to library amplification can greatly facilitate the optimization of library amplification parameters, for new library construction workflows or sample types. The qPCR-based KAPA Library Quantification Kit is suitable for the quantification of adapter-ligated libraries prepared with full-length adapters. Once the actual amount of library available for amplification is known, the optimal number of amplification cycles can be determined theoretically. Please contact **support@kapabiosystems.com** for more information.

DNA polymerase

KAPA HiFi HotStart ReadyMix contains KAPA HiFi, a novel B-family DNA polymerase engineered for increased processivity and high fidelity. In the ReadyMix, an antibody-based hot start formulation of KAPA HiFi DNA Polymerase is used. KAPA HiFi HotStart DNA Polymerase has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activities, but no 5'→3' exonuclease activity. The strong 3'→5' exonuclease activity results in superior accuracy during DNA amplification. The error rate of KAPA HiFi HotStart DNA Polymerase is 2.8×10^{-7} errors/base, equivalent to 1 error per 3.5×10^{6} nucleotides incorporated.

Note to Purchaser: Limited License

KAPA Library Amplification Kits are developed, designed and sold exclusively for research purposes and in vitro use. Neither the product, nor any individual component, has been tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, which is available on request.

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