KAPA Library Preparation Kits
with Real-Time PCR Library Amplification
Illumina series

Product Description

The KAPA Library Preparation Kit provides all of the enzymes and reaction buffers required for constructing libraries from fragmented dsDNA via the following steps:

1. **End repair:** Produce blunt-ended, 5′-phosphorylated fragments.
2. **A-tailing:** Add dAMP to the 3′-ends of the dsDNA library fragments.
3. **Adaptor ligation:** Ligate dsDNA adaptors with 3′-dTMP overhangs to library fragments.
4. **Library amplification:** Real-time amplification of library fragments carrying appropriate adaptor sequences on both ends.

Reaction buffers are supplied in convenient, concentrated "master mix" formats comprising all of the required reaction components except oligonucleotide adaptors and PCR primers. Similarly, a single enzyme mixture is provided for each step of the library construction process, reducing the number of pipetting steps.

High fidelity PCR is used to selectively enrich library fragments carrying appropriate adaptor sequences and to amplify the amount of DNA prior to sequencing. During PCR enrichment of libraries, minimizing amplification bias is critical to ensure more uniform sequence coverage. Amplification bias occurs when a DNA polymerase is unable to amplify all targets within a complex population of library DNA with equal efficiency. Bias is further exacerbated when libraries are over-amplified.

KAPA HiFi Real-Time PCR Library Amplification Kits are designed to address both sources of PCR-induced bias. The novel KAPA HiFi DNA Polymerase, engineered for high fidelity and processivity, is capable of balanced amplification of complex library DNA. Real-time monitoring of library amplification provides additional information required to optimize the number of amplification cycles and minimize over-amplification. The benefits of performing high fidelity, real-time PCR for next-generation sequencing library amplification include:

- Real-time monitoring of amplification allows precise control over the optimal number of PCR cycles.
- Real-time amplification workflows are amenable to automation.
- Real-time amplification plots provide quality metrics for individual enriched libraries, eliminating expensive and time-consuming post-enrichment gel electrophoresis and identifying inconsistencies in library preparation.
- Seamless integration with KAPA Library Quantification Kits.

Kit codes and components	
KK8220 10 reactions	KAPA Library Preparation Kit with Real-Time PCR Library Amplification - Illumina - End Repair Enzyme Mix (50 µL) - 10X End Repair Buffer with dNTPs (100 µL) - A-Tailing Enzyme (30 µL) - 10X A-Tailing Buffer (50 µL) - DNA Ligase (50 µL) - 5X Ligation Buffer (100 µL) - 2X KAPA HiFi HotStart Real-Time PCR Master Mix (250 µL) - 4 x Fluorescent Standards (1500 µL each)
KK8221 50 reactions	KAPA Library Preparation Kit with Real-Time PCR Library Amplification - Illumina - End Repair Enzyme Mix (250 µL) - 10X End Repair Buffer with dNTPs (500 µL) - A-Tailing Enzyme (150 µL) - 10X A-Tailing Buffer (250 µL) - DNA Ligase (250 µL) - 5X Ligation Buffer (500 µL) - 2X KAPA HiFi HotStart Real-Time PCR Master Mix (1250 µL) - 4 x Fluorescent Standards (1500 µL each)

Storage and handling

Store all components at -20 °C. KAPA HiFi HotStart Real-Time PCR Master Mix (2X) and fluorescent standards 1 – 4 are light sensitive and should be protected from light during storage, thawing, and reaction setup. Please refer to Section 6 for full details.

Quick Notes

- Reaction components should be **mixed fresh** and used on the same day.
- Reaction cleanup between successive enzymatic reactions may be accomplished via your method of choice. We recommend Agencourt Ampure XP Beads or Qiagen MinElute Reaction Cleanup Kits.
- Optimal real-time library amplification corresponds to the region between fluorescent standard 1 and 3. The termination cycle number should be adjusted accordingly without the requirement for performing gel electrophoresis (see Figure 1, page 9).
- It is critical that the correct data acquisition temperature is adhered to, to minimize background fluorescence due to inter- and intra-primer interaction (see Table 1, page 12).
- When using primers that differ in sequence from those listed in Table 1, we recommend performing gradient PCR to optimize the annealing temperature.

KAPA HiFi Real-Time PCR Library Amplification Kits contain KAPA HiFi HotStart Real-Time PCR Master Mix (2X), a ready-to-use cocktail containing all components for PCR, except primers and template. The 2X Master Mix contains KAPA HiFi HotStart DNA Polymerase in a proprietary reaction buffer, dNTPs, $MgCl_2$ (2.5 mM at 1X), SYBR® Green I dye and stabilizers. Four fluorescent standards are supplied, and are used to define a window for optimal amplification (Figures 1 and 2).

This kit is primarily intended for the construction of genomic, paired-end, and paired-end multiplex (indexed/barcoded) Illumina DNA libraries, but may be used for other applications requiring efficient end-repair, A-tailing, ligation, and/or library amplification steps.

1. End Repair

Materials required but not supplied in this kit:

- Reaction tubes. Reactions may be assembled and processed in PCR plates, PCR tubes, or microcentrifuge tubes.
- Pipette tips. We strongly recommend the use of high quality filter-plugged tips to prevent contamination of reagents and library samples.
- Reaction cleanup. We recommend Agencourt AMPure XP Beads (cat. # A63881) or Qiagen MinElute Reaction Cleanup Kits (cat. # 28204) for this purpose. AMPure XP Beads require the preparation of an elution buffer (10 mM TRIS-Acetate, pH 8.0, reagent grade water).

1.1 Assemble the End Repair reaction:

Water to 100 µL	x µL
10X End Repair Buffer	10 µL
End Repair Enzyme Mix	5 µL
1 - 5 µg sheared dsDNA	1 - 85 µL
Total	100 µL

1.2 Incubate for 30 min @ 20 °C
1.3 Proceed immediately to cleanup.

End Repair Cleanup

We recommend either Agencourt AMPure XP Beads, **or** Qiagen MinElute Reaction Cleanup Kits. Below are suggested protocols for the recommended methods:

AMPure XP Beads

1.4 Ensure that the AMPure XP Beads are equilibrated to room temperature, and that they are thoroughly resuspended.
1.5 Add AMPure Beads to the End Repair reaction:

End Repair reaction	100 µL
AMPure XP Beads	**160 µL**
Total	260 µL

1.6 Mix thoroughly on a vortex mixer or by pipetting up and down at least ten times.
1.7 Incubate at room temperature for 15 minutes to allow DNA to bind to the beads.
1.8 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
1.9 Carefully remove and discard 255 µL of the liquid. Take care not to disturb or discard any of the beads. Some liquid may remain visible in the tube/well.
1.10 Keeping the tube/plate on the magnetic stand and without disturbing the beads, wash the beads in 200 µL of 80% EtOH for at least 30 seconds.
1.11 Carefully remove and discard the ethanol without disturbing the beads, and repeat the process for a total of 2 washes in 80% EtOH.
1.12. Remove the tube/plate from the magnetic stand, and allow the beads to dry at room temperature for 15 minutes.
1.13 Resuspend the beads thoroughly in 32.5 µL elution buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.
1.14 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
1.15 Recover the DNA in 30 µL of supernatant and transfer to the tube/well in which you intend to perform the A-tailing reaction.

1. End Repair (cont.)

OR

Qiagen MinElute Reaction Cleanup Kit

The procedure for reaction cleanup using Qiagen MinElute columns described below is abbreviated and is intended for users who are already familiar with the method. Please consult the documentation provided by the supplier for full details.

1.4 If the End Repair reaction was performed in a PCR tube/plate, transfer the End Repair reaction to a microcentrifuge tube before proceeding.
1.5 Follow the MinElute protocol:

End Repair reaction	100 µL
Add Buffer ERC	**300 µL**
Total	400 µL

1.6 Apply the mixture to a column and centrifuge or apply vacuum as appropriate. Discard the flow-through.
1.7 Wash with 750 µL Buffer PE. Discard the flow-through.
1.8 Centrifuge for 2 minute at ≥ 10,000 *x g* to remove all traces of ethanol.
1.9 To elute, transfer the column to a clean, sterile microcentrifuge tube and add 31 µL buffer EB. Incubate for 1 minute at room temperature, and centrifuge to recover ~30 µL.

Safe Stopping Point

If you are not proceeding to A-Tailing immediately, the protocol can be safely stopped here. Store at -20 °C for up to seven days.

2. A-Tailing

2.1 Assemble the A-Tailing reaction:

Water	12 µL
10X A-Tailing Buffer	5 µL
A-Tailing Enzyme	3 µL
End repaired DNA	30 µL
Total	50 µL

2.2 Incubate for 30 min @ 30 °C
2.3 Proceed immediately to cleanup.

A-Tailing Cleanup

We recommend either Agencourt AMPure XP Beads, **or** Qiagen MinElute Reaction Cleanup Kits. Below are suggested protocols for the recommended methods:

AMPure XP Beads

2.4 Ensure that the AMPure XP Beads are equilibrated to room temperature, and that they are thoroughly resuspended.
2.5 Add AMPure XP Beads to the End Repair reaction:

A-Tailing reaction	50 µL
Add AMPure XP Beads	**90 µL**
Total	140 µL

2.6 Mix thoroughly on a vortex mixer or by pipetting up and down at least ten times.
2.7 Incubate at room temperature for 15 minutes to allow DNA to bind to the beads.
2.8 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
2.9 Carefully remove and discard 135 µL of the liquid. Take care not to disturb or discard any of the beads. Some liquid may remain visible in the tube/well.
2.10 Keeping the tube/plate on the magnetic stand and without disturbing the beads, wash the beads in 200 µ L of 80% EtOH for at least 30 seconds.
2.11 Carefully remove and discard the ethanol without disturbing the beads, and repeat the process for a total of 2 washes in 80% EtOH.
2.12 Remove the tube/plate from the magnetic stand, and allow the beads to dry at room temperature for 15 minutes.
2.13 Resuspend the beads thoroughly in 32.5 µL elution buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.
2.14 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
2.15 Recover the DNA in 30 µL of supernatant and transfer to the tube/well in which you intend to perform the adaptor ligation reaction.

2. A-Tailing (cont.)

OR

Qiagen MinElute Reaction Cleanup Kit

The procedure for reaction cleanup using Qiagen MinElute columns described below is abbreviated and is intended for users who are already familiar with the method. Please consult the documentation provided by the supplier for full details.

2.4 If the end repair reaction was performed in a PCR tube/plate, transfer the End Repair reaction to a microcentrifuge tube before proceeding.
2.5 Follow the MinElute protocol:

A-Tailing Reaction	50 µL
Add Buffer ERC	**300 µL**
Total	350 µL

2.6 Apply the mixture to a column and centrifuge or apply vacuum as appropriate. Discard the flow-through.
2.7 Wash with 750 µL Buffer PE. Discard the flow-through.
2.8 Centrifuge for 2 minute at ≥ 10,000 *x g* to remove all traces of ethanol.
2.9 To elute, transfer the column to a clean, sterile microcentrifuge tube and add 31 µL buffer EB. Incubate for 1 minute at room temperature, and centrifuge to recover ~30 µL.

Safe Stopping Point

If you are not proceeding to Adaptor Ligation immediately, the protocol can be safely stopped here. Store at -20 °C for up to seven days.

3. Adaptor Ligation

3.1 Assemble the Adaptor Ligation reaction:

5X Ligation Buffer	10 µL
DNA Ligase	5 µL
DNA Adaptor (30 µM)*	5 µL
A-Tailed DNA	30 µL
Total	50 µL

*Not supplied

3.2 Incubate for 15 min @ 20 °C
3.3 Proceed immediately to cleanup.

Adaptor Ligation Cleanup

Note:

- If using AMPure XP Beads for DNA recovery and purification, we recommend that you follow the protocol outlined below to perform a total of **TWO** successive cleanup procedures after adaptor ligation. If you are using the MinElute Reaction Cleanup Kit, then a **single** cleanup procedure is recommended.

AMPure XP Beads

First AMPure XP Bead Cleanup

3.4 Ensure that the AMPure XP Beads are equilibrated to room temperature, and that they are thoroughly resuspended.
3.5 Add AMPure Beads to the End Repair reaction:

Adaptor Ligation reaction	50 µL
Add AMPure XP Beads	**50 µL**
Total	100 µL

3.6 Mix thoroughly on a vortex mixer or by pipetting up and down at least ten times.
3.7 Incubate at room temperature for 15 minutes to allow DNA to bind to the beads.
3.8 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
3.9 Carefully remove and discard 95 µL of the liquid. Take care not to disturb or discard any of the beads. Some liquid may remain visible in the tube/well.
3.10 Keeping the tube/plate on the magnetic stand and without disturbing the beads, wash the beads in 200 µL of 80% EtOH for at least 30 seconds.
3.11 Carefully remove and discard the ethanol without disturbing the beads, and repeat the process for a total of 2 washes in 80% EtOH.
3.12 Remove the tube/plate from the magnetic stand, and allow the beads to dry at room temperature for 15 minutes.
3.13 Resuspend the beads thoroughly in 52.5 µL elution buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.
3.14 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
3.15 Recover the DNA in 50 µL of supernatant and transfer to the tube/well in which you intend to perform the second cleanup procedure (see below).

3. Adaptor Ligation (cont.)

Second AMPure XP Bead Cleanup

3.16 Ensure that the AMPure XP Beads are equilibrated to room temperature, and that they are thoroughly resuspended.
3.17 Add AMPure Beads to the End Repair reaction:

Library DNA from first cleanup	50 µL
Add AMPure XP Beads	**50 µL**
Total	100 µL

3.18 Mix thoroughly on a vortex mixer or by pipetting up and down at least ten times.
3.19 Incubate at room temperature for 15 minutes to allow DNA to bind to the beads.
3.20 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
3.21 Carefully remove and discard 95 µL of the liquid. Take care not to disturb or discard any of the beads. Some liquid may remain visible in the tube/well.
3.22 Keeping the tube/plate on the magnetic stand and without disturbing the beads, wash the beads in 200 µL of 80% EtOH for at least 30 seconds.
3.23 Carefully remove and discard the ethanol without disturbing the beads, and repeat the process for a total of 2 washes in 80% EtOH.
3.24 Remove the tube/plate from the magnetic stand, and allow the beads to dry at room temperature for 15 minutes.
3.25 Resuspend the beads thoroughly in 32.5 µL elution buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.
3.26 Capture the beads by placing the tube/PCR plate on an appropriate magnetic stand at room temperature for 15 minutes or until the liquid is completely clear.
3.27 Recover the DNA in 30 µL of supernatant and transfer to the tube/well in which you intend to store the library DNA until you carry out size selection.

OR

Qiagen MinElute Reaction Cleanup Kit

The procedure for reaction cleanup using Qiagen MinElute columns described below is abbreviated and is intended for users who are already familiar with the method. Please consult the documentation provided by the supplier for full details.

3.4 If the end repair reaction was performed in a PCR tube/plate, transfer the End Repair reaction to a microcentrifuge tube before proceeding.
3.5 Follow the MinElute protocol:

Adaptor Ligation reaction	50 µL
Add Buffer ERC	**300 µL**
Total	350 µL

3.6 Apply the mixture to a column and centrifuge or apply vacuum as appropriate. Discard the flow-through.
3.7 Wash with 750 µL Buffer PE. Discard the flow-through.
3.8 Centrifuge for 2 minute at ≥ 10,000 x *g* to remove all traces of ethanol.
3.9 To elute, transfer the column to a clean, sterile microcentrifuge tube and add 31 µL buffer EB. Incubate for 1 minute at room temperature, and centrifuge to recover ~30 µL.

Safe Stopping Point

If you are not proceeding to Size Selection immediately, the protocol can be safely stopped here. Store at -20 °C for up to seven days.

4. Size Selection

It is important to remove unligated adaptor molecules prior to library amplification to prevent the formation of "adaptor dimers" and other short adaptor-derived molecules, which may cause problems downstream during cluster amplification and sequencing. For many sequencing libraries/protocols, it is also helpful to select a relatively narrow and precisely defined size range of library fragments. Depending on your needs and options, you may choose to perform this size selection via a variety of common methods including:

- "SPRI-bead" size selection.
- "Double-SPRI-bead" size selection.
- Manual agarose gel electrophoresis, excision, and purification.
- Automated DNA size selection and collection (e.g. Sage Science Pippin Prep™).

Due to the wide variety of viable alternatives for this procedure we do not provide any specific protocols for size selection.

5. Real-Time Library Amplification

5.1 Overview of real-time high fidelity amplification of next-generation DNA sequencing libraries.

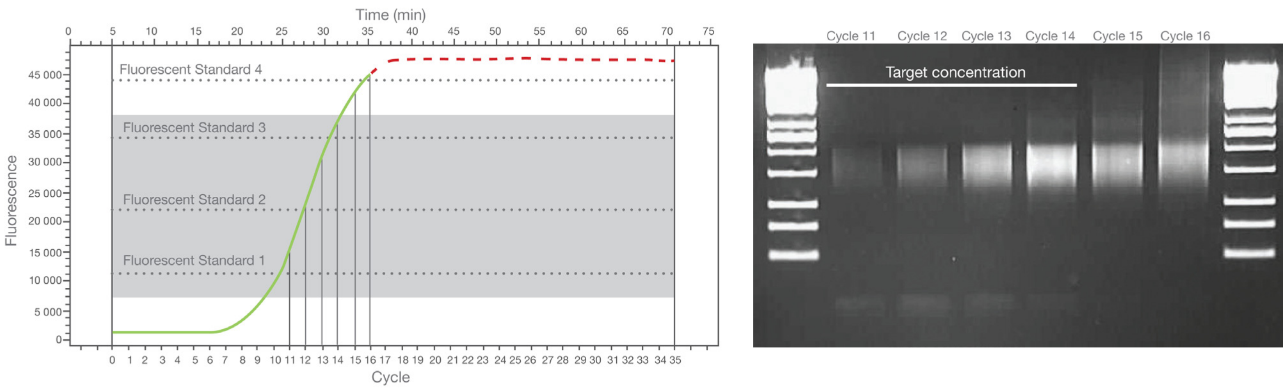

Figure 1. Libraries are amplified using a SYBR® Green-based real-time, high fidelity PCR master mix (left panel). Four triplicate wells of the PCR plate contain fluorescent reference standards representing a range of distinct DNA concentrations. Reactions terminated between standards 1 and 3 represent the optimal library amplification range (grey box), depicted here from cycles 10 - 14. A gel image of a typical library stopped at different amplification cycles is shown in the right panel. Low- and high-molecular weight artifacts increase progressively with additional cycles.

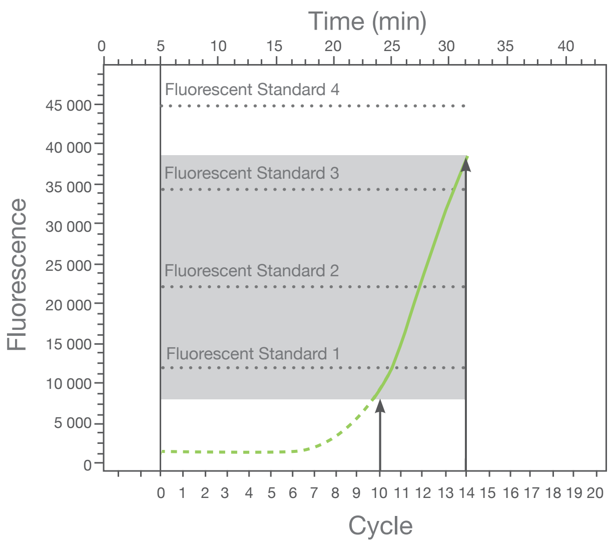

Figure 2a. Superimposed amplification plots for reactions terminated at the lower bound (hashed line, cycle 10) or upper bound (solid line, cycle 14) of the targeted concentration range (grey box). Library amplification reactions should ideally be terminated anywhere within the indicated target concentration range.

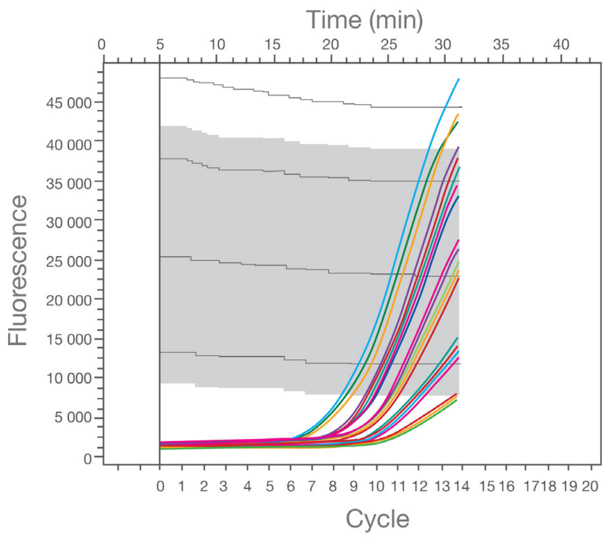

Figure 2b. Example of real-time high fidelity amplification of multiple libraries. 20 libraries, spanning a ~64-fold concentration range (6 cycles), were simultaneously amplified and terminated after 14 cycles. 14 of the 20 libraries fall within the targeted amplification range. The remaining 6 libraries could either be used as is, noting that they may be outside the optimal concentration range, or they could be re-amplified individually or in high- or low-concentration groups.

5. Real-Time Library Amplification (cont.)

5.2 Preparation

Note: KAPA HiFi HotStart Real-Time PCR Master Mix (2X) and fluorescent standards 1 - 4 are light sensitive and should be protected from light.

- Thaw the primers required for PCR enrichment (PCR primers are not supplied in the KAPA Library Preparation Kit), a tube of KAPA HiFi HotStart Real-Time PCR Master Mix (2X), and fluorescent standards 1 - 4 at room temperature.
- Mix and briefly centrifuge the thawed KAPA HiFi HotStart Real-Time PCR Master Mix (2X), primer and fluorescent standards 1 - 4.
- Thaw, gently mix, and briefly centrifuge the adaptor-ligated, size-separated purified library DNA for 5 seconds at 600 xg.
- Pre-program the real-time thermal cycler with the appropriate cycling protocol (see Table 1) for each specific set of library amplification PCR primers.

5.3 Reaction setup

Each plate must contain a set of fluorescent standards 1 - 4 (50 µL each loaded in triplicate) in addition to a single 50 µL real-time PCR reaction for each library requiring amplification.

In order to maintain optimal library diversity it is necessary to add sufficient adaptor-ligated library DNA to each enrichment PCR reaction. The optimal cycle number is dependant on the volume and concentration of library material added to each 50 uL PCR reaction. High background fluorescence may result if >100 ng dsDNA template is added per 50 µL real-time PCR reaction.

Refer to Table 1 (page 12) for the suggested reaction setup for specific library preparation protocols.

Fluorescent standard set up requires the addition of 50 µL of each fluorescent standard (1 - 4) in triplicate to wells of the real-time PCR plate.

Change tips after each pipetting step. Seal each reaction, mix gently and centrifuge briefly.

5.5 Cycling protocol

Refer to Table 1 for the thermal cycling protocol for specific library types.

- If conventional end-point PCR has previously been used successfully and the same amount and type of library is added to the KAPA HiFi HotStart Real-Time PCR reactions, then program the real-time thermocycler with the same number of cycles as previously used. If the optimal cycle number is not known refer to section 5.7 for more information.

5.6 Clean up PCR

- After enrichment PCR, clean up each reaction using either Agencourt AMPure XP beads (Beckman Coulter Genomics part # A63881) or Qiagen MinElute PCR Purification Kit (Qiagen, part # 28004).

5. Real-Time Library Amplification (cont.)

5.7 Validate library

Initially, the raw data (i.e., not background subtracted) linear real-time amplification plots can be used as a built-in quality metric to validate the level of amplification of each amplified library.

- If the linear amplification profile of the library is significantly below fluorescent standard 1 at the end of qPCR cycling, then it is unlikely that there will be sufficient library material to sequence after PCR purification.

- If the linear amplification profile of the library is significantly above fluorescent standard 3 at the end of qPCR cycling, then the library has been over-amplified. This may lead to 1) amplification bias, 2) higher error rates, and/or 3) the presence of chimeric PCR products.

This data is also useful as a quality control metric for identifying inconsistencies during library preparation between multiple libraries.

NOTE: The amplification plots can also be used in real-time to select the optimal cycle without a pre-programmed termination cycle. To do this:

1. Program 30 cycles into the real-time thermocycler.

2. After starting the real-time thermocycler, wait until the desired fluorescence of the library is achieved before terminating the real-time reaction.

NOTE: It is critical to terminate the reaction directly after data acquisition at 72ºC **and before the tube ramps to 95ºC** for the start of the next cycle. This will ensure that the enriched library DNA remains double-stranded for efficient downstream purification.

To verify the size of the PCR enriched fragments, check the size distribution by performing gel electrophoresis.

5.8 Library Quantification

Accurate quantification of amplifiable library molecules is critical for the efficient use of next-generation sequencing platforms. Overestimation of library concentration results in lower cluster density after bridge PCR. Underestimation of library concentration results in too many clusters on the flow cell, which can lead to poor cluster resolution. Both scenarios result in suboptimal sequencing capacity. Accurate library quantification is equally important when pooling indexed libraries for multiplexed sequencing to ensure equal representation of each library.

Integrate KAPA HiFi Real-Time PCR Library Amplification Kit with the appropriate KAPA Library Quantification Kit (KK4824, KK4835, KK4844, KK4854) to accurately quantify the number of PCR-competent molecules. If libraries have been terminated between fluorescent standards 1 – 3, a single 1:1,000 dilution of each library will be required for library quantification using the KAPA Library Quantification Kits.

5. Real-Time Library Amplification (cont.)

Table 1. Recommended reaction setup and cycling parameters for KAPA HiFi HotStart Real-Time PCR Master Mix (2X) reactions:

Library	Component	Final Conc.	Volume/50 µL rxn	Cycling Protocol	
Genomic DNA / ChIP	PCR grade water		As needed	Denaturation	45 sec at 98 ºC
	2X KAPA HiFi HS RT-PCR MM	1X	25 µL	Cycling*	15 sec at 98 ºC 30 sec at 65 ºC 30 sec at 72 ºC
	PCR Primer 1.1	500 nM	1 µL		
	PCR Primer 2.1	500 nM	1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 ºC
Paired-End	PCR grade water		As needed	Denaturation	45 sec at 98 ºC
	2X KAPA HiFi HS RT-PCR MM	1X	25 µL	Cycling*	15 sec at 98 ºC 30 sec at 65 ºC 30 sec at 72 ºC
	PE PCR Primer 1.0	500 nM	1 µL		
	PE PCR Primer 2.0	500 nM	1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 ºC
Paired-End Multiplex	PCR grade water		As needed	Denaturation	45 sec at 98 ºC
	2X KAPA HiFi HS RT-PCR MM	1X	25 µL	Cycling*	15 sec at 98 ºC 30 sec at 65 ºC 30 sec at 72 ºC
	PE PCR Primer InPE 1.0	500 nM	1 µL		
	PE PCR Primer InPE 2.0	10 nM	1 µL		
	PCR Primer Index 1 - 12	500 nM	1 µL		
	Library DNA		As needed	Final Extension	1 min at 72 ºC
TruSeq™ DNA				Denaturation	45 sec at 98 ºC
	2X KAPA HiFi HS RT-PCR MM	1X	25 µL	Cycling*	15 sec at 98 ºC 30 sec at 60 ºC 30 sec at 72 ºC
	PCR Primer Cocktail (PPC)	500 nM each	5 µL		
	Library DNA		20 µL	Final Extension	1 min at 72 ºC

* The optimal cycling number will depend upon the volume and concentration of adaptor-ligated, size separated, purified library DNA added to each enrichment PCR reaction. Typically this is in the 10-18 cycle range but may require optimization.

Appendix A: Composition of Materials

10X KAPA End Repair Buffer - contains dNTPs and ATP
500 mM Tris-HCl
100 mM $MgCl_2$
100 mM DTT
10 mM ATP
4 mM dATP
4 mM dCTP
4 mM dGTP
4 mM dTTP
pH 7.5 @ 25 °C

KAPA End Repair Enzyme Mix
3,000 U/mL T4 DNA Polymerase
10,000 U/mL T4 Polynucleotide Kinase
Supplied in: 100 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% Glycerol, pH7.4 @ 25 °C

10x KAPA A-Tailing Buffer - contains dATP
100 mM Tris-HCl
100 mM $MgCl_2$
500 mM NaCl
10 mM DTT
2 mM dATP
pH 7.9 @ 25 °C

KAPA A-Tailing Enzyme
5,000 U/mL (0.5 mg/mL) Klenow Fragment (3’→ 5’ exo-)
Supplied in: 20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 °C

5X KAPA Ligation Buffer
330 mM Tris-HCl
50 mM $MgCl_2$
5 mM DTT
5 mM ATP
30% PEG 6000
pH 7.6 @ 25 °C

KAPA DNA Ligase
600,000 U/mL (2 mg/mL) T4 DNA Ligase
Supplied in: 10 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 °C

KAPA HiFi HotStart Real-Time PCR Master Mix
KAPA HiFi HotStart DNA Polymerase is an antibody-based hot start formulation of KAPA HiFi DNA Polymerase, a novel B-family DNA polymerase engineered for increased processivity and high fidelity. 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 calculated at 1 error in 3.54×10^6 bases covered (2.82×10^{-7}). The SYBR® Green I intercalating dye contained within the master mix does not affect the fildelity of the polymerase. DNA fragments generated with KAPA HiFi HotStart ReadyMix may be used for routine downstream analyses or applications, including restriction enzyme digestion and sequencing. PCR products generated with KAPA HiFi HotStart ReadyMix are blunt-ended, but may be 3’-dA-tailed for cloning into TA cloning vectors.

6. Storage, handling and specifications

6.1 Shipping, storage and handling

KAPA Library Preparation 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.

Please note that certain components in KAPA Library Preparation Kits (e.g. End Repair Mix, DNA Ligase, buffers containing dNTPs and/or ATP, etc.) are particularly sensitive to temperature and freeze-thaw cycles, and should be handled with special care. Minimize exposure of the KAPA HiFi HotStart Real-Time PCR Master Mix (2X) and Flourescent Standards 1 - 4 to direct light. Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity.

The KAPA HiFi HotStart Real-Time PCR Master Mix (2X) contains isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the KAPA HiFi HotStart Real-Time PCR Master Mix is fully thawed and has been vortexed before use.

KAPA HiFi HotStart Real-Time PCR Master Mix (2X) 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. Please note that reagents stored above -20 °C are more prone to degradation when contaminated by the user; storage at such temperatures is therefore at the user's own risk.

6.2 Quality control

All kit components are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination. Detailed quality control information for individual kit components is available upon request, please contact support@kapabiosystems.com.

6.3 Product use limitations and licenses

KAPA Library Preparation 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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