KAPA Library Amplification Kits

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

October 2025, Version 1.0

KAPA Library Amplification Kits

Kit Material Number	Component Material Number	Description	Volume
10613556001	10577711001	KAPA EvoAmp ReadyMix pwd by KAPA HiFi (24 rxn)	600 µL
10613572001	10577720001	KAPA EvoAmp ReadyMix pwd by KAPA HiFi (96 rxn)	2.4 mL
10613599001	10577738001	KAPA EvoAmp ReadyMix pwd by KAPA HiFi (384 rxn)	9.6 mL
07958960001	08202923001	KAPA HiFi HotStart ReadyMix (2X) (250 rxn)	6.25 mL
09420398001	09420711001	KAPA HiFi HotStart ReadyMix (2X) (384 rxn)	9.6 mL
09420444001	09420720001	KAPA HiFi HotStart ReadyMix (2X) (96-well plate)*	96 x 25 µL
07958978001	08202940001	KAPA HiFi HotStart ReadyMix (2X) (50 rxn)	1.25 mL
		KAPA Library Amplification Primer Mix (10X)	0.25 mL
07958986001	08202923001	KAPA HiFi HotStart ReadyMix (2X) (250 rxn)	6.25 mL
		KAPA Library Amplification Primer Mix (10X)	1.25 mL
07958994001	08204489001	KAPA Library Amplification Primer Mix (10X) (250 rxn)	1.25 mL
09420410001	09420738001	KAPA Library Amplification Primer Mix (10X) (384 rxn)	1.92 mL
09420479001	09420754001	KAPA Library Amplification Primer Mix (10X) (96-well plate)	96 x 5 µL

*10% overage + 5 µL is provided for KAPA HiFi HotStart (2X) (96- well plate)

Note: Accessory kits (KAPA Cleanup Beads, KAPA Universal Adapter & KAPA UDI Primer Mixes, KAPA Unique Dual-Indexed Adapter and KAPA Library Amplification Primer Mix) are sold separately.

Storage and Stability

- The components provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage.
- The KAPA Library Amplification Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, store the kit at -15°C to -25°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.

Application

KAPA EvoAmp ReadyMix contains KAPA HiFi HotStart DNA Polymerase that is specifically designed to minimize amplification bias, while maintaining extremely high fidelity.

The KAPA EvoAmp ReadyMix is designed for efficient library amplification of adapter-ligated libraries for next-generation sequencing (NGS) on Illumina® platforms.

KAPA EvoAmp ReadyMix is compatible with libraries constructed from input DNA amounts of 0.1 ng to 500 ng (high quality DNA). It is compatible with high-quality gDNA, cfDNA and low-quality DNA such as that extracted from formalin-fixed, paraffin-embedded tissue (FFPET) samples.

The KAPA EvoAmp ReadyMix Kits are ideally suited for high-efficiency, high fidelity, low-bias amplification of libraries prior to Illumina sequencing. This includes libraries prepared for Whole-genome sequencing (WGS) and Whole exome sequencing (WES) or targeted sequencing. KAPA EvoAmp ReadyMix Kits which are powered by KAPA HiFi build on the existing performance of KAPA HiFi which is ideally suited for high-efficiency, high fidelity, low-bias amplification of libraries prior to Illumina sequencing.

This includes libraries prepared for:

- whole-genome shotgun sequencing
- exome or targeted sequencing (pre- and post-capture amplification)
- RNA-seq
- ChIP-seq
- other sequencing applications

In order to maximize sequence coverage uniformity, it is critical to minimize library amplification bias. Amplification bias occurs when a DNA polymerase is unable to amplify all targets within a complex population of library DNA with equal efficiency. KAPA HiFi DNA Polymerase is a B-family DNA polymerase engineered for increased processivity, extremely high fidelity and low-bias, and is the reagent of choice for NGS library amplification.[1,2,3,4] KAPA HiFi HotStart DNA Polymerase has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activities. The error rate of KAPA HiFi HotStart DNA Polymerase is 2.8 x 10^{-7} errors/base, equivalent to 1 error per 3.5 x 10^{6} nucleotides incorporated. The enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This prevents nonspecific amplification during reaction setup, increases sensitivity, and improves reaction efficiency. KAPA EvoAmp ReadyMix, a ready-to-use Library Amplification mix comprising all the components for library amplification; except primers and template.

1. Oyola, S.O., et al., BMC Genomics 13, 1 (2012). 2. Quail, M.A., et al., Nature Methods 9, 10 (2012). 3. Quail, M.A., et al., BMC Genomics 13, 341 (2012). 4. Ross, M.G., et al., Genome Biology 14, R51 (2013).

Warnings and Precautions

- Wear the appropriate personal protective equipment, such as gloves and safety glasses, to avoid direct contact while handling the reagents.
- Use good laboratory practices to avoid contamination when working with the reagents.
- In the event of a spill, clean up the solution with absorbent pads, allow pads to dry, and dispose of pads. Observe all national, regional, and local regulations for waste disposal and management.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Safety Data Sheets (SDS) are available online or upon request from the local Roche office.

Ordering Information

For a complete overview of Roche Sequencing products, including KAPA EvoAmp ReadyMix Kits and KAPA HiFi HotStart ReadyMix Kits, go to ***sequencing.roche.com/products***.

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Contact and Support

If you have questions, contact your local Roche Technical Support. Go to ***sequencing.roche.com/support*** for contact information.

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Editions

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Preface

Regulatory Disclaimer

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Not for use in diagnostic procedures.

Contact Information

Technical Support

If you have questions, contact your local Roche Technical Support.

Go to *sequencing.roche.com/support* for contact information.

Manufacturing & Distribution

Manufacturer	Roche Diagnostics Cape Town, South Africa Roche Diagnostics GmbH
Distribution	Roche Diagnostics GmbH Mannheim, Germany
Distribution in USA	Roche Diagnostics Corporation Indianapolis, IN USA

Conventions used in this manual

Symbols

Symbols	Description
	**Important Note:** Information critical to the success of the procedure or use of the product. Failure to follow these instructions could result in compromised data.
	**Information Note:** Designates a note that provides additional information concerning the current topic or procedure.

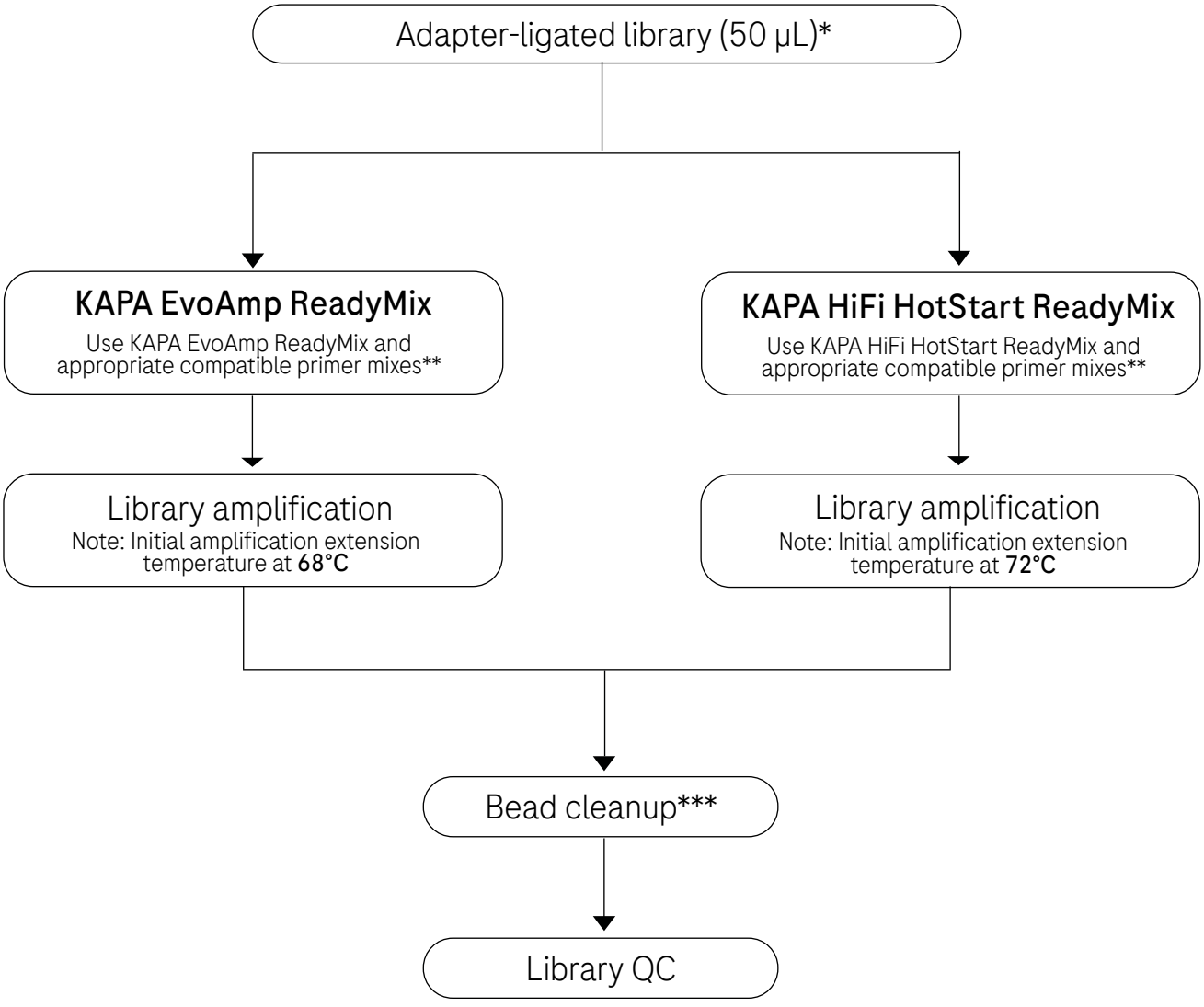
Text

Conventions	Description
Numbered listing	Indicates steps in a procedure that must be performed in the order listed.
Italic type, blue	Highlights a resource in a different area of this manual or on a web site.
Italic type	Identifies the external general resources or names.
Bold type	Identifies names of paragraphs, sections or emphasized words.

Chapter 1. Before You Begin

The KAPA EvoAmp ReadyMix Kit is powered by KAPA HiFi and provides:

- Improve accuracy and coverage uniformity over a broad GC range
- Ready-to-use and automation-friendly ReadyMix in tubes and bottle format
- KAPA EvoAmp has been validated as part of our DNA library prep kits; KAPA EvoPrep Boost kit and KAPA EvoPlus Boost kit and as part of KAPA RNA EvoPrep Kit
- KAPA EvoAmp has also been validated as part of KAPA HyperCap Evolved workflows
- If using the KAPA RNA EvoPrep workflow, please follow the entire workflow as recommended in the respective Instructions for Use. Failure to do so may impact the libraries and downstream sequencing data generated.
- Single vendor service when using the following accessory reagents:
  - KAPA HyperPure Beads
  - KAPA Unique Dual-Indexed (UDI) Adapters & KAPA Library Amplification Primer Mix or
  - KAPA Universal Adapter & KAPA UDI Primer Mixes 1 – 384



* This workflow is a representation of a DNA library preparation workflow
** Using KAPA EvoAmp ReadyMix or KAPA HiFi HS ReadyMix and either KAPA Library Amplification Primer Mix if KAPA UDI Adapters were used or KAPA UDI Primers Mixes if KAPA Universal Adapter was used
*** Follow respective library preparation instructions for use for bead cleanup recommendations

Protocol Information & Safety

- Wear gloves and take precautions to avoid sample contamination.
- Perform all centrifugations at room temperature (+15°C to +25°C).
- Unless otherwise specified, all mixing steps are listed as 'mix thoroughly' and indicate that mixing should be performed by vortexing for at least 10 seconds. Mixing should be optimized for automation as applicable.
- If liquid has collected in a tube's cap after mixing, gently tap or briefly centrifuge the sample to collect the liquid into the tube's bottom, ensuring that the mixture remains homogeneous before progressing to the next step.

References

- Thermocycler Manual
- Qubit Fluorometer Manual
- Qubit dsDNA HS Assay Kit Guide

Terminology

Sample Library: The initial shotgun library generated from DNA by mechanical or enzymatic fragmentation and ligation.

Amplified Sample Library: The sample library after amplification by PCR.

KAPA UDI Adapter: KAPA Unique Dual-Indexed Adapter.

KAPA UDI Primer Mixes: KAPA Unique Dual-Indexed Primer Mixes.

KAPA Universal Adapter: Truncated adapter containing a subset of sequencing motifs. Used in conjunction with KAPA UDI Primer Mixes.

Prepare Equipment and Reagents

To ensure streamlined workflow execution, thermocyclers can be programmed with the following prior to relevant reaction setup:

It is recommended to use a thermocycler with a programmable heated lid. If not possible, please use the default settings.

The following steps should be taken before beginning the workflow:

Verify you are using the most up-to-date version of these Instructions for Use, go to *sequencing.roche.com/support*.

Required Equipment, Labware and Consumables

You assume full responsibility when using the equipment, labware, and consumables described below. This protocol is designed for use with the specified equipment, labware, and consumables.

Laboratory Equipment

Equipment	Supplier
Tube Magnetic Rack	Multiple Vendors
Microcentrifuge (16,000 x *g* capability)	Multiple Vendors
Qubit Fluorometer	ThermoFisher
Electrophoretic device & associated assays and reagents	Multiple Vendors
Thermocycler	Multiple Vendors
Vortex mixer	Multiple Vendors
Plate Centrifuge (minimum 280 x *g* capability)	Multiple Vendors

Consumables Purchased from Other Vendors

Equipment	Supplier
10 mM Tris-HCl, pH 8.0 – 8.5	Multiple Vendors
Ethanol, 200 proof (absolute), for molecular biology	Multiple Vendors
Qubit dsDNA HS Assay Kit	ThermoFisher
Qubit Assay Tubes	ThermoFisher
Low binding Tubes: • 0.2 mL PCR tubes • 1.5 mL microcentrifuge tubes (optional)	Multiple Vendors
Nuclease-free, PCR-grade water	Multiple Vendors

Consumables Available from Roche

Description	Package Size	Material Number
KAPA EvoPrep Kit (PCR-free)	24 rxn	10153806001
	96 rxn	10153814001
	384 rxn	10153857001
	96 rxn, plated	10613688001
KAPA EvoPlus V2 Kit (PCR-free)	24 rxn	09420045001
	96 rxn	09420304001
	384 rxn	09420371001
KAPA HyperCapture Reagent kit	96 rxn	09075828001
KAPA HyperCapture Bead kit	96 rxn	09075798001
KAPA Universal Enhancing Oligos	96 rxn	09075852001
KAPA Library Quantification Kit for Illumina platforms	Various options	Various material numbers
KAPA HyperPure Beads	5 mL	08963835001
	30 mL	08963843001
	60 mL	08963851001
	4 x 60 mL	08963878001
	450 mL	08963860001
KAPA Unique Dual-Indexed Adapter Kit	96 x 20 µL	08861919702
KAPA Library Amplification Primer Mix	250 reactions (1.25 mL)	07958994001
KAPA Library Amplification Primer Mix	384 reactions (1.92 mL)	09420410001
KAPA Library Amplification Primer Mix, 96-well plate	96 x 5 µL	09420479001
KAPA Universal Adapter	96 reactions	09063781001
	384 reactions*	09063790001
KAPA UDI Primer Mixes 1 – 96	96 reactions	09134336001
KAPA UDI Primer Mixes 97 – 192	96 reactions	09329838001
KAPA UDI Primer Mixes 193 – 288	96 reactions	09329846001
KAPA UDI Primer Mixes 289 – 384	96 reactions	09329854001

* Virtual kit - consist of 4 x 96 reaction kits

Chapter 2. Store and Prepare the Reagents

Step 1. Store the Reagent Kits

Reagent Kit	Storage Temperature
KAPA EvoAmp ReadyMix	-15°C to -25°C
KAPA HiFi HotStart ReadyMix	-15°C to -25°C
KAPA HyperPure Beads*	+2°C to +8°C
KAPA UDI Adapter Kit	-15°C to -25°C
KAPA Universal Adapter	-15°C to -25°C
KAPA UDI Primer Mixes or KAPA UDI Primer Mixes (resuspended)	+2°C to +8°C or -15°C to -25°C
KAPA Library Amplification Primer Kit	-15°C to -25°C

*The KAPA HyperPure Beads kit must not be frozen.

Step 2. Prepare the KAPA UDI Primer Mixes

For multiplexing guidelines, please refer to either the KAPA UDI Adapter or KAPA UDI Primer Mixes Instructions for Use (available ***online*** on eLabDoc) as applicable.

Before use of the KAPA UDI Primer Mixes, undertake the following steps to resuspend the primers:

1. Retrieve the KAPA UDI Primer Mixes plate from storage (+2°C to +8°C).
2. Centrifuge the KAPA UDI Primer Mixes plate at 280 x *g* for 1 minute to ensure the contents are at the bottom of the wells.
3. Before removing the foil cover, please ensure the plate is in the correct orientation before proceeding. In order to have well position A1 on the top left corner, the notched corner must be facing the user on the bottom left as shown in ***Figure 1***.
4. Carefully remove the foil cover on the plate ensuring to avoid cross contamination. Discard the original foil cover.
5. Using a multichannel pipette, add 10 µL of Nuclease-free, PCR-grade water directly to the bottom of each well and discard tips after dispensing.

A new pipette tip must be used for each well to avoid cross contamination. Be sure to dispense water slowly to the bottom of each well to avoid liquid splash over to adjacent wells.

6. Ensure every well contains 10 µL of Nuclease-free, PCR-grade water and cover the plate with one of the adhesive foil seals provided in the kit.

Make sure the foil seal is properly aligned and fully covers all 96 wells. Failure to do so can lead to cross contamination of the KAPA UDI Primer Mixes.

7. Use a roller or appropriate tool to ensure the foil seal is evenly applied.
8. Centrifuge the plate at room temperature (280 x *g* for at least 30 seconds) to ensure the dispensed 10 µL are at the bottom of the well.
9. Thoroughly vortex the plate ensuring all wells are mixed well.

Ensure wells at the corners of the plate are mixed well by vortexing the corners of the plate. Keep the plate upright.

10. Centrifuge the plate at (280 x *g* for at least 1 minute) to ensure the contents are collected at the bottom of the wells.
11. The KAPA UDI Primer Mixes plate is now ready for use in the Library Amplification step.
12. Store any unused but already resuspended KAPA UDI Primer Mixes at -15°C to -25°C. To avoid repeated freeze/thaw cycles you may transfer the resuspended primers to separate tubes or tube strips for storage.

⚠ Ensure aliquoted KAPA UDI Primer Mixes are correctly labeled.

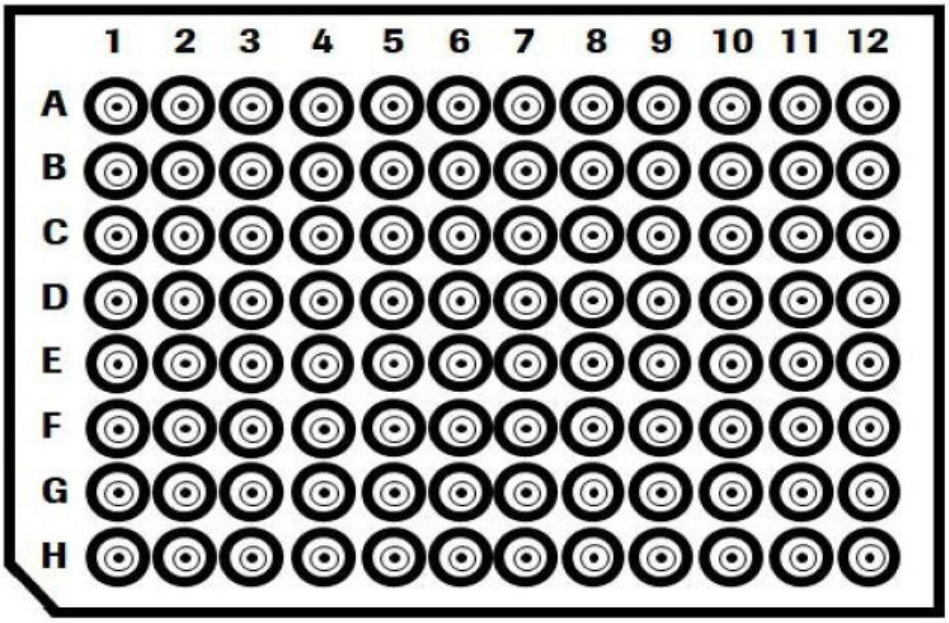


Figure 1: KAPA UDI Primer Mixes Plate layout

Chapter 3. Amplify the Sample Library

This chapter describes how to amplify the adapter-ligated library, if necessary, using either KAPA EvoAmp ReadyMix or KAPA HiFi HotStart ReadyMix, and appropriate compatible indexes or primer sets.

⚠ If KAPA UDI Adapters were used for adapter ligation, ensure KAPA Library Amplification Primer Mix is used for library amplification.

⚠ If KAPA Universal Adapters were used for adapter ligation, amplification is mandatory. Ensure a unique KAPA UDI Primer is added to each sample library.

Ensure that the following is available:

- Freshly-prepared 80% ethanol
- 10 mM Tris-HCl, pH 8.0 – 8.5
- Nuclease-free, PCR-grade water
- KAPA Adapter Dilution Buffer

Disclaimer: This KAPA EvoAmp Library Amplification Readymix has been validated using the KAPA EvoPrep Boost kit and KAPA EvoPlus Boost kit DNA Library Preparation workflows with KAPA UDI Adapters and KAPA Library Amplification Primer Mix or KAPA Universal Adapter with KAPA UDI Primer Mixes. Libraries prepared using third-party library preparation kits may require optimisation. KAPA EvoAmp ReadyMix has also been validated as part of the KAPA RNA EvoPrep Kit. Please follow the entire KAPA RNA EvoPrep workflow as recommended in the respective Instructions for Use. Failure to do so may impact the libraries and downstream sequencing data generated.

Important Parameters

Automation: The KAPA EvoAmp ReadyMix and KAPA HiFi HotStart ReadyMix are designed to be automation-friendly. Mixing steps should be optimized for automation as applicable.

KAPA EvoAmp ReadyMix and KAPA HiFi HotStart ReadyMix are available in ready-to-use formats including tubes, bottles, and plated configuration (note that the plated format is applicable to KAPA HiFi HotStart ReadyMix only) to facilitate automation.

General Handling & Equipment

Manual Mixing: For manual operation, vortex for 10-20 seconds or pipette mix 10 times.
Equipment: Plate centrifuges with appropriate g-force capabilities are suitable for automated processes.
Bead Handling: Equilibrate beads to room temperature and store protected from light.
Plated Component Preparation: For plated components like KAPA UDI Primer Mixes, prepare by sealing, vortexing, and centrifuging.

Optimization of Library Amplification

The quantification of adapter-ligated libraries (prior to library amplification) can greatly facilitate the optimization of library amplification parameters, particularly when a library construction workflow is first established. With the KAPA Library Quantification Kit, the amount of template DNA (adapter-ligated molecules) available for library amplification can be determined accurately. From there, the number of amplification cycles needed to achieve a specific yield of amplified library can be predicted theoretically. Please contact Technical Support at *sequencing.roche.com/support* for a calculator designed to assist with these calculations.

Cycle Number

Table 1 indicates the number of cycles typically required to generate 4nM of amplified library DNA for libraries prepared with tthe KAPA EvoPrep/Plus Boost or KAPA EvoPrep/Plus V2 Kits; the actual optimal number of cycles may be higher, depending on the reagents and protocol used for library construction, and the quality of the input DNA.

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.

Please consult the relevant library preparation kit instructions for use for specific recommendations.

Step 1. Prepare the Library Amplification Reaction

Make sure the KAPA HyperPure Beads and 10 mM Tris-HCl, pH 8.0 – 8.5 are removed from storage at least 30 minutes prior to starting the workflow to ensure they are equilibrated to room temperature. For best performance, store the beads protected from light when not in use.

KAPA EvoAmp ReadyMix and KAPA HiFi HotStart ReadyMix (2X) contains isostabilizers and may not freeze completely, even when stored at -15°C to -25°C. Nevertheless, always ensure that the ReadyMix is fully thawed and thoroughly mixed before use.

If applicable, retrieve and thaw the KAPA UDI Primer Mixes plate prepared in ***Chapter 2, Step 2***. Centrifuge the plate at 280 x g for 30 seconds to collect the contents to the bottom of thewells and peel off or pierce the foil seal for the appropriate number of wells needed. If only using a subset of the KAPA UDI Primer Mixes from the original plate, remove and discard residual primers from the well and apply a new adhesive foil seal provided in the kit.

If piercing the foil seal, avoid cross contamination by using a new pipette tip for every well.

Proper re-sealing and storage of the KAPA UDI Primer Mixes plate is necessary for unused primer mixes utilization at a later date.

The KAPA EvoAmp ReadyMix contains surfactant, and may foam. Ensure this solution is sufficiently mixed and collect the clear liquid. Mix by either vortexing for 10-20 seconds or pipette mix 10 times prior to use. Avoid “pipetting” the foam to avoid potential yield loss.

Keep the KAPA EvoAmp ReadyMix or KAPA HiFi HotStart ReadyMix (2X) on ice as long as possible during handling.

1. Assemble each library amplification reaction as follows:

Component	Volume per Individual Library
Adapter-ligated library	20 µL
KAPA EvoAmp ReadyMix OR KAPA HiFi HotStart ReadyMix (2X)	25 µL
KAPA Library Amplification Mix (10X)* OR KAPA UDI Primer Mix**	5 µL
Total volume	**50 µL**

* If KAPA UDI Adapters were used for adapter ligation, ensure KAPA Library Amplification Primer Mix is used for library amplification.
The KAPA EvoAmp ReadyMix or KAPA HiFi HostStart ReadyMix and KAPA Library Amplification Primer Mix should preferably be premixed and added in a single pipetting step.
**If KAPA Universal Adapters were used for adapter ligation ensure a unique KAPA UDI Primer Mix is added to each sample library

2. Mix thoroughly and centrifuge briefly. Proceed immediately to the next step.

Step 2. Perform the Library Amplification

The following section has been divided into two subsections:

If using KAPA EvoAmp ReadyMix- proceed to ***Step 2a*** for library amplification parameters

If using KAPA HiFi HotStart ReadyMix- proceed to ***Step 2b*** for library amplification parameters

Step 2a. Perform the Library Amplification using KAPA EvoAmp ReadyMix

1. Place the sample in the thermocycler and amplify the adapter-ligated sample library using the following Library Amplification program with the lid temperature set to +105°C:

Step	Temperature	Time	Cycles
Hold	+4°C	∞	1
Initial denaturation	+98°C	45 sec	1
Denaturation	+98°C	15 sec	Variable, see the *Table 1* depending on application/sample type**
Annealing	+60°C	30 sec	
Extension	+68°C*	30 sec	
Final extension	+72°C	1 min	1
Hold	+4°C	∞	1

* Different extension temperature compared to KAPA HiFi HotStart ReadyMix
** If amplifying libraries with total GC content <20%, see KAPA EvoAmp Library Amplification parameters in *Appendix E*

Step 2b. Perform the Library Amplification using KAPA HiFi HotStart ReadyMix

1. Place the sample in the thermocycler and amplify the adapter-ligated sample library using the following Library Amplification program with the lid temperature set to +105°C:

Step	Temperature	Time	Cycles
Hold	+4°C	∞	1
Initial denaturation	+98°C	45 sec	1
Denaturation	+98°C	15 sec	Variable, see the *Table 1* depending on application/sample type
Annealing	+60°C	30 sec	
Extension	+72°C*	30 sec	
Final extension	+72°C	1 min	1
Hold	+4°C	∞	1

*Different extension temperature compared to KAPA EvoAmp ReadyMix

Table 1: Recommended number of amplification cycles per DNA input for Whole-Genome Sequencing (WGS) applications if using either KAPA EvoAmp ReadyMix or KAPA HiFi HotStart ReadyMix:

Input DNA into library preparation	Number of amplification cycles* for WGS to achieve 4 nM** if using KAPA UDI Adapters	Number of amplification cycles* for WGS to achieve 4 nM** if using KAPA Universal Adapter & KAPA UDI Primer Mixes
50 - 500 ng	0 (PCR-free workflow)	3-4
10 ng	3-5	4-7
1 ng	7-9	8-10
0.1 ng	10-13	11-13

*The number of cycles needed depends on the specific adapter and amplification primer design, as well as input type, quality and whether double-sided size selection is performed. When using incomplete, or truncated, adapters in conjunction with indexed amplification primers (such as KAPA Universal Adapter & KAPA UDI Primer Mixes), a minimum number of amplification cycles (3) are required to complete adapter sequences for the next step in the process (target capture or sequencing), irrespective if a sufficient amount of library is available after ligation. The number of cycles needed depends on the specific adapter, downstream application and amplification primer design. Certain sample types, such as FFPET-derived DNA may require additional cycles of amplification to reach 4 nM threshold. This may also depend on the quality (low vs high) of the FFPET-derived DNA.

**Based on sequencing recommendations, 4 nM is the minimum starting concentration to proceed with sequencing. For input amounts ≥ 50 ng, PCR amplification should not be required to achieve the ~4 nM requirement for sequencing (unless libraries were constructed using KAPA Universal Adapter). Users requiring concentrations >4 nM can adjust the number of amplification cycles in 2 cycle increments until the target concentration is achieved. This may require optimization. Note: increasing cycle numbers ultimately decreases the library complexity by increasing the duplication rate.

Number of cycles required to achieve desired concentration may require optimization

For Targeted Sequencing PCR parameters conditions please refer to the corresponding KAPA HyperCap Evolved workflow Instructions for Use (IfU)

This is not a safe stopping point. Please proceed directly to the bead-based purification of the libraries

Step 3. Purify the Amplified Sample Library using KAPA HyperPure Beads

Follow respective Library Preparation Instructions for Use (IfU) for post-amplification cleanup ratios taking into account specific adapter types utilised.

For examples of a traditional 1X bead cleanup and modified 1X bead cleanup, please refer to ***Appendix A*** and ***Appendix B***, respectively.

For detailed instruction on performing double-sided size selection, see ***Appendix C.***

Appendix A. 1X Bead cleanup

Purify the Amplified Sample Library constructed using KAPA UDI Adapter & KAPA Library Amplification Primer Mix

1. Add 50 µL (1X) of room temperature, thoroughly resuspended, KAPA HyperPure Beads to each amplified sample library.
2. Mix the amplified sample library and KAPA HyperPure Beads thoroughly and centrifuge briefly to collect all droplets.

   ⚠ Do NOT allow beads to pellet.
3. Incubate the sample at room temperature for 5 minutes to allow the sample library to bind to the beads.
4. Place the sample on a magnet to capture the beads. Incubate until liquid is clear.
5. Carefully remove and discard the supernatant.
6. Keeping the sample on the magnet, add 200 µL freshly prepared 80% ethanol.
7. Incubate the sample at room temperature for ≥ 30 seconds.
8. Carefully remove and discard ethanol, without disturbing beads.
9. Keeping the sample on the magnet, add 200 µL freshly prepared 80% ethanol.
10. Incubate the sample at room temperature for ≥ 30 seconds.
11. Carefully remove and discard ethanol, without disturbing bead pellets.
12. Allow the beads to dry at room temperature, sufficiently for all remaining ethanol to evaporate.

    ⚠ Over-drying the beads may result in dramatic yield loss. Over-drying is indicated by a dry, cracked appearance on the surface of the bead pellet. The surface of the bead pellet should have a matte appearance when sufficiently dried.
13. Remove the sample from the magnet.
14. Thoroughly resuspend the beads in 25 µL (or appropriate volume) of 10 mM Tris-HCl, pH 8.0 – 8.5. Centrifuge briefly to collect all droplets.

    ⚠ If proceeding with double-sided size selection, resuspend the beads in 55 µL of elution buffer.
15. Incubate the sample at room temperature for 2 minutes to elute the library off the beads.
16. Place the sample on a magnet to capture the beads. Incubate until the liquid is clear.
17. Transfer the appropriate volume of the clear supernatant to a fresh tube(s)/well and proceed with size selection, library QC, target capture or sequencing, as appropriate.

    ⚠ The remaining 5 µL can be used for quality control purposes e.g., quantification using the KAPA Library Quantification Kit.
18. Purified, amplified sample libraries can be stored at +2°C to +8°C for 1 – 2 weeks or at -15°C to -25°C for up to 3 months.

Appendix B. Modified 1X Bead cleanup

Purify the Amplified Sample Library constructed using KAPA Universal Adapter & KAPA UDI Primer Mixes

A modified post-amplification cleanup is highly recommended when using KAPA Universal Adapter & KAPA UDI Primer Mixes with KAPA library preparation kits. Especially if WGS libraries will be sequenced on an Illumina NovaSeq or HiSeq X system (with patterned flow cells). This additional or modified cleanup will serve to remove any indexed primer carryover which may exacerbate index hopping.

1. Add 50 µL (1X) of room temperature, thoroughly resuspended, KAPA HyperPure Beads to each amplified sample library.
2. Mix the amplified sample library and KAPA HyperPure Beads thoroughly and centrifuge briefly to collect all droplets.

   Do NOT allow beads to pellet.
3. Incubate at room temperature for 5 minutes to allow the sample library to bind to the beads.
4. Place the sample on a magnet to capture the beads. Incubate until liquid is clear.
5. Carefully remove and discard the supernatant.
6. Remove the tubes from the magnet, and resuspend the beads in 50 µL of nuclease-free, PCR-grade water or 10 mM Tris-HCl pH 8.0 – 8.5. Incubate for 2 minutes to allow the sample to elute off from the beads.
7. Add 50 µL (1X) of KAPA HyperPure Beads to each sample.
8. Mix thoroughly by pipetting or vortexing, and centrifuge briefly.
9. Incubate the sample at room temperature for 5 minutes to allow the sample library to bind to the beads.
10. Place the sample on a magnet to capture the beads. Incubate until the liquid is clear.
11. Carefully remove and discard the supernatant.
12. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
13. Incubate the sample at room temperature for ≥30 seconds.
14. Carefully remove and discard the ethanol.
15. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
16. Incubate the sample at room temperature for ≥30 seconds.
17. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
18. Allow the beads to dry at room temperature, sufficiently for all of the ethanol to evaporate.

    Over-drying the beads may result in dramatic yield loss. Over-drying is indicated by a dry, cracked appearance on the surface of the bead pellet. The surface of the bead pellet should have a matte appearance when sufficiently dried.

19. Remove the sample from the magnet.
20. Thoroughly resuspend the beads in 25 µL (or appropriate volume) of 10 mM Tris-HCl, pH 8.0 – 8.5.

If proceeding with double-sided size selection, resuspend the beads in 55 µL of elution buffer.

21. Incubate the sample at room temperature for 2 minutes to elute the library off the beads.
22. Place the sample on a magnet to capture the beads. Incubate until the liquid is clear.
23. Transfer an appropriate volume of the clear supernatant to a fresh tube(s)/well and proceed with double-sided size selection, library QC, target capture or sequencing, as appropriate.
24. Purified, amplified sample libraries can be stored at +2°C to +8°C for 1 – 2 weeks or at -15°C to -25°C for up to 3 months.

Appendix C. Double-sided size selection

Size selection requirements vary widely for different sequencing applications. For sequencing on Illumina HiSeq X and NovaSeq instruments, narrow insert size distributions (in the range of 300 – 650 bp), and sequencing-ready libraries free of short fragments, such as unligated adapter and adapter-dimer are required. This is essential to ensure optimal cluster generation, mitigate the potential impact of index misassignment, and facilitate data analysis.

If required, any commonly used bead- or gel-based size selection techniques may be integrated in the library preparation workflow.

Size selection may be carried out at different points in the overall workflow, for example after the post-ligation cleanup, or after the library amplification cleanup.

Size selection inevitably leads to a loss of sample material. These losses can be dramatic (60 – 95%), and may significantly increase the number of amplification cycles required to generate sufficient material for the next step in the process (capture or sequencing).

The potential advantages of one or more size selection steps in a library construction workflow should be weighed against the potential loss of library complexity, especially when input DNA is limited. A well-optimized fragmentation protocol, especially for shorter insert libraries and/or read lengths, may eliminate the need for size selection, thereby simplifying the library construction process and limiting sample losses.

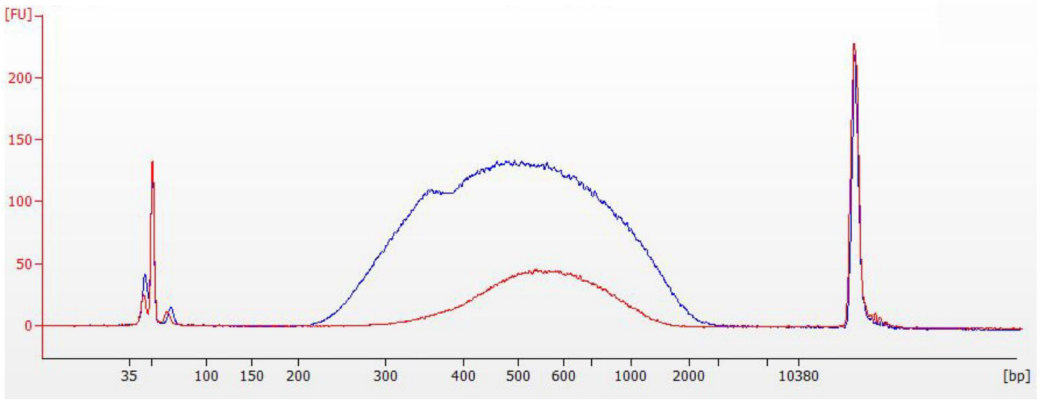


Figure 2: Example library subjected to double-sided size selection. Size selection inevitably leads to a loss of sample material, and can be dramatic (60 - 95%). Blue trace: library before double-sided size selection. Red trace: library after double-sided size selection. 500 ng of high-quality human genomic DNA was Covaris-sheared to ~350 bp and used to prepare libraries with KAPA UDI Adapters. These libraries were subjected to 0.6X - 0.8X double-sided size selection post-ligation using KAPA HyperPure Beads (libraries were amplified for visualization). Electropherograms were generated with a Bioanalyzer 2100 High Sensitivity DNA Kit.

A double-sided size selection consists of a first and second “cut”, performed with different bead-to-sample volume ratios. The first cut determines the upper size limit of the size-selected sample library, whereas the second cut determines the lower size limit.

To increase the upper size limit of the selected fragments, reduce the volume of KAPA HyperPure Beads used for the first cut. To decrease the upper size limit of the selected fragments, increase the volume of KAPA HyperPure Beads used in the first cut.

To increase the lower size limit of the selected fragments, reduce the volume of KAPA HyperPure Beads added in the second cut. To decrease the lower size limit of the size selected fragments, increase the volume of KAPA HyperPure Beads added in the second cut.

⚠ Please note that the volume of KAPA HyperPure Beads needed for the second cut is calculated relative to the volume of the sample library at the start of the size selection procedure, not the volume of the sample containing supernatant transferred after the first cut.

The second size cut should be performed with at least 0.2 volumes of original input of KAPA HyperPure Beads. Sample recovery is dramatically reduced if the difference between first and second cuts is less than ~0.2 volumes. To increase the amount of sample recovered, >0.2 volumes of KAPA HyperPure Beads may be used for the second cut, but note that this may result in the recovery of smaller library fragments and/or a broader size distribution.

The potential advantages of one or more size selection steps in a library construction workflow should be weighed against the potential loss of library complexity, especially when input DNA is limited. A well-optimized fragmentation protocol, especially for shorter insert libraries and/or read lengths, may eliminate the need for size selection, thereby simplifying the library construction process and limiting sample losses.

The double-sided size selection protocol outlined in this appendix (0.5X – 0.7X) is designed for the selection of library molecules (inclusive of a full length adapter such as KAPA UDI Adapter) in the range of 300 bp – 600 bp.

The protocol will need to be modified if truncated adapters were used for library construction. Contact ***Technical Support*** for guidance if needed.

To obtain a population of shorter or longer molecules, the protocol may be modified as follows:

Upper size limit of captured fragments	Modification	Lower size limit of captured fragments	Modification
Increase	Decrease the ratio of the first cut (e.g., 0.4X or 0.45X)	Increase	Decrease the ratio of the second cut (e.g., 0.6X or 0.65X)
Decrease	Increase the ratio of the first cut (e.g., 0.6X or 0.65X)	Decrease	Increase the ratio of the second cut (e.g., 0.8X or 0.85X)

1. Perform the first (0.5X) size cut (to bind and exclude library molecules larger than ~600 bp) by combining the following:

Component	Volume per Individual Sample
Library to be size selected	50 µL
KAPA HyperPure Beads	25 µL
Total	75 µL

2. Mix the sample library and KAPA HyperPure Beads thoroughly and centrifuge briefly to collect all droplets. Do NOT allow beads to pellet.
3. Incubate the sample at room temperature for 5 minutes to allow the library molecules larger than ~600 bp to bind to the beads.
4. Place the sample on the magnet to capture the beads. Incubate until the liquid is clear.
5. Carefully transfer ~70 µL of supernatant containing library molecules smaller than ~600 bp to a new plate/tube. It is critical that no beads are transferred with the supernatant. Discard the plate/tube(s) with the beads to which library molecules larger than ~600 bp were bound.
6. Perform the second size cut (0.7X), to retain library molecules > 300 bp by combining the following:

Component	Volume per Individual Sample
Supernatant from first size cut	70 µL
KAPA HyperPure Beads	10 µL*
Total	80 µL

*The volume of KAPA HyperPure Beads needed for the second cut is calculated relative to the volume of the sample library at the start of the size selection procedure, not the volume of the sample containing supernatant transferred after the first cut. A volume of 10 µL of KAPA HyperPure Beads is added during the second cut. This is not an error. The supernatant from Step 5 contains PEG/NaCl from the initial 0.5X volume of KAPA HyperPure Beads, and is carried over from the first cut into the second cut. This volume of PEG/NaCl (the crowding reagent) is the critical functional component. The 0.7X ratio required for the second cut is thus a cumulative total ratio. It is the sum of the original 0.5X ratio that is retained from the first cut plus the 0.2X added during the second cut for a total ratio of 0.7X (0.5X + 0.2X): 25 µL volume KAPA HyperPure Beads = 0.5X of the original 50 µL library sample PLUS 10µL volume of KAPA HyperPure Beads = 0.2X of the original 50 µL library sample.

7. Mix the supernatant from the first size cut and KAPA HyperPure Beads thoroughly and centrifuge briefly to collect all droplets.
8. Incubate the sample at room temperature for 5 minutes to allow the library molecules larger than >300 bp to bind to the beads.
9. Place the sample on the magnet to capture the beads. Incubate until the liquid is clear.
10. Carefully remove and discard the supernatant.
11. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
12. Incubate the sample at room temperature for ≥30 seconds.
13. Carefully remove and discard the ethanol.

⚠ The low bead volume used for the second cut results in a small bead pellet that is easily disturbed and may also dry out considerably faster than during other reaction cleanups.

14. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
15. Incubate the sample at room temperature for ≥30 seconds.
16. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
17. Allow the beads to dry at room temperature, sufficiently for all of the ethanol to evaporate.

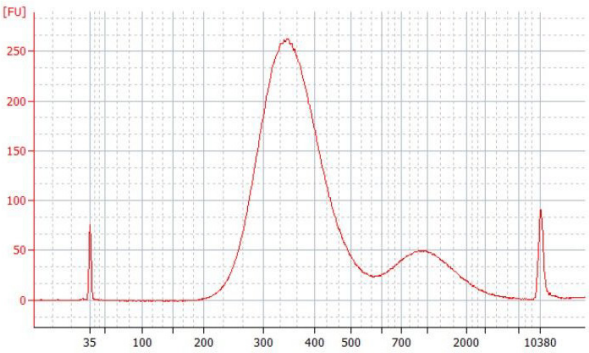
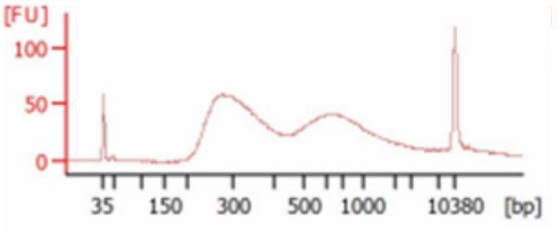
⚠ Over-drying the beads may result in dramatic yield loss. Over-drying is indicated by a dry, cracked appearance on the surface of the bead pellet. The surface of the bead pellet should have a matteappearance when sufficiently dried.

18. Remove the sample from the magnet.
19. Thoroughly resuspend the beads in 25 µL of 10 mM Tris-HCl, pH 8.0 – 8.5. Centrifuge briefly to collect all droplets. Do NOT allow beads to pellet.
20. Incubate the sample at room temperature for 2 minutes to allow the sample library to elute off the beads.
21. Place the sample on the magnet to capture the beads. Incubate until the liquid is clear.
22. Transfer 20 µL of the eluate to a new tube/well.
23. Purified libraries can be stored as follows:
    - 23.1. post-ligation libraries: +2°C to +8°C for 1 – 2 weeks or at -15°C to -25°C for up to 1 month.
    - 23.2. post-amplification libraries: +2°C to +8°C for 1 – 2 weeks or at -15°C to -25°C for up to 3 months.

Appendix D. Troubleshooting

This appendix provides guidance for interpreting unexpected results and recommendations for implementing corrective action if problems occur. For technical questions, contact your local Roche Technical Support. Go to *sequencing.roche.com/support* for contact information.

⚠ The Illumina sequencing workflow is not supported by Roche Technical Support.

Observation	Cause(s)/Recommendation(s)
Second peak after expected library peak on fragment analyzer   	Possible cause is library overamplification. In library amplification reactions, primers are typically depleted before dNTPs. When DNA synthesis can no longer take place due to substrate 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 results in the formation of so-called “daisy chains”, 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. Ensure correct number of cycles are used for specific sample input amount. If possible take a small aliquot of the amplified library and perform a an additional cycle of amplification using fresh reagents, followed by a bead cleanup to help release the heteroduplexed molecules that cause anomalous migration on the fragment analyser. Reach out to technical support for additional support at *sequencing.roche.com/support*

Observation	Cause(s)/Recommendation(s)
Lower than expected post-amplification yields	Inefficient handling of the amplification ReadyMix. • Ensure the KAPA EvoAmp ReadyMix or KAPA HiFi HotStart ReadyMix is thawed sufficiently prior to use. • Ensure sufficient mixing is performed. KAPA EvoAmp ReadyMix or KAPA HiFi HotStart ReadyMix can be viscous and insufficient mixing may result in inefficient amplification of libraries. Pipette-mix at least 10X or vortex for 10 – 20 seconds.
	Libraries may degrade over time if stored incorrectly.
	Improper bead cleanup practises followed. Ensure best practices are used during bead cleanups. • Equilibrate KAPA HyperPure Beads to room temperature prior to use. • Equilibrate elution buffer, 10 mM Tris-HCl (pH 8.0 – 8.5) to room temperature prior to eluting amplified libraries off the beads. • Always prepare fresh 80% ethanol for bead cleanups. Long term storage of 80% ethanol will result in evaporation, resulting in a lower ethanol percentage being used and subsequent sample loss. • Do not freeze/thaw KAPA HyperPure Beads. Beads will be damaged if stored at -20°C. • Protect KAPA HyperPure Beads from light during long term storage. • Do not over-dry beads.

Appendix E. Low GC Library Amplification parameters

This appendix should be used for amplifying libraries with GC content < 20%. Improved GC coverage was shown when KAPA EvoAmp ReadyMix was used with these PCR parameters:

1. Place the sample in the thermocycler and amplify the adapter-ligated sample library using the following Library Amplification program with the lid temperature set to +105°C:

Step	Temperature	Time	Cycles
Hold	+4°C	∞	1
Initial denaturation	+98°C	45 sec	1
Denaturation	+98°C	15 sec	Variable, see the *Table 1* depending on application/sample type**
Annealing & Extension	+65°C*	90 sec	
Final extension	+72°C	1 min	1
Hold	+4°C	∞	1

* Lowered combined Annealing & Extension temperature profile compared to KAPA EvoAmp ReadyMix amplification
** PCR parameters specifically adapted to ultra-low GC libraries.

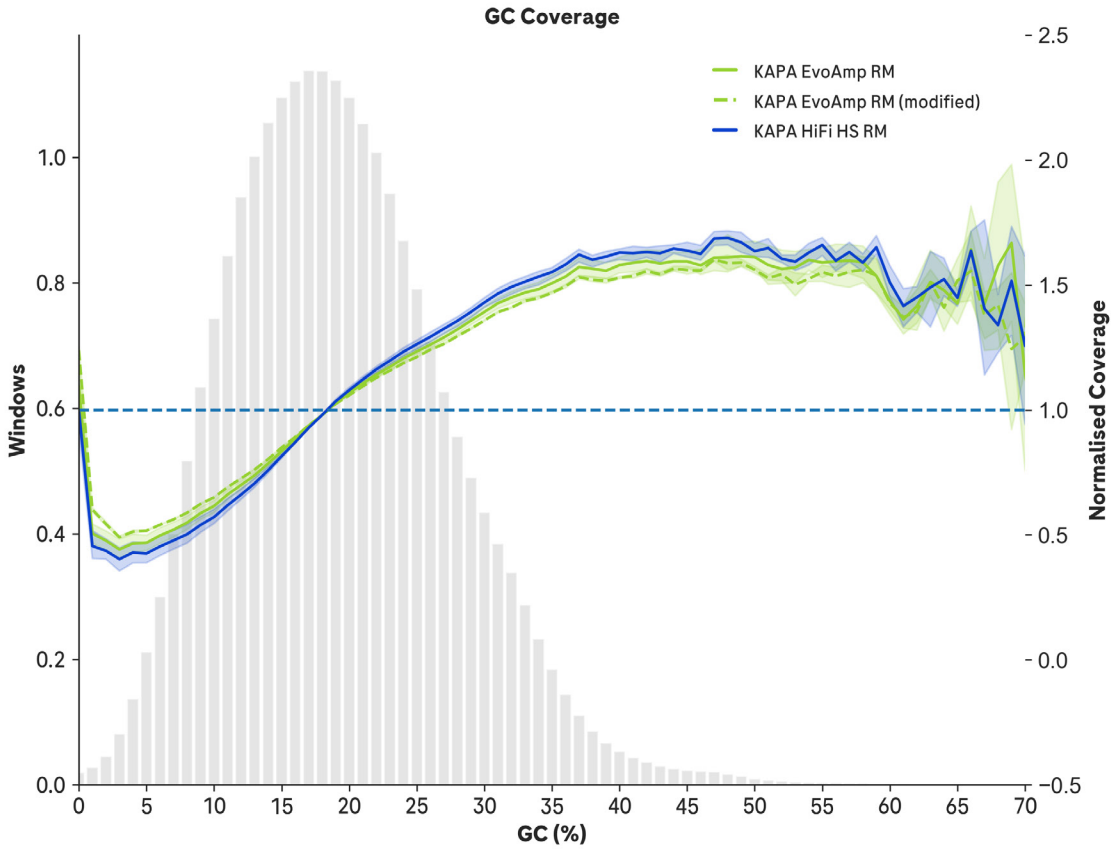


Figure 3: Impact of using KAPA EvoAmp ReadyMix with different library amplification PCR parameters for libraries with low GC content. GC Coverage plot detailing how different PCR parameters impact GC coverage of libraries with low-GC content. Libraries generated using 1 ng of *P. falciparum* and KAPA EvoPrep library prep kit. These libraries were amplified with either KAPA HiFi HotStart ReadyMix (using KAPA HiFi HotStart PCR parameters), KAPA EvoAmp ReadyMix (using KAPA EvoAmp PCR parameters) or KAPA EvoAmp ReadyMix (using modified PCR for low parameters GC libraries).

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Limited Warranty

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