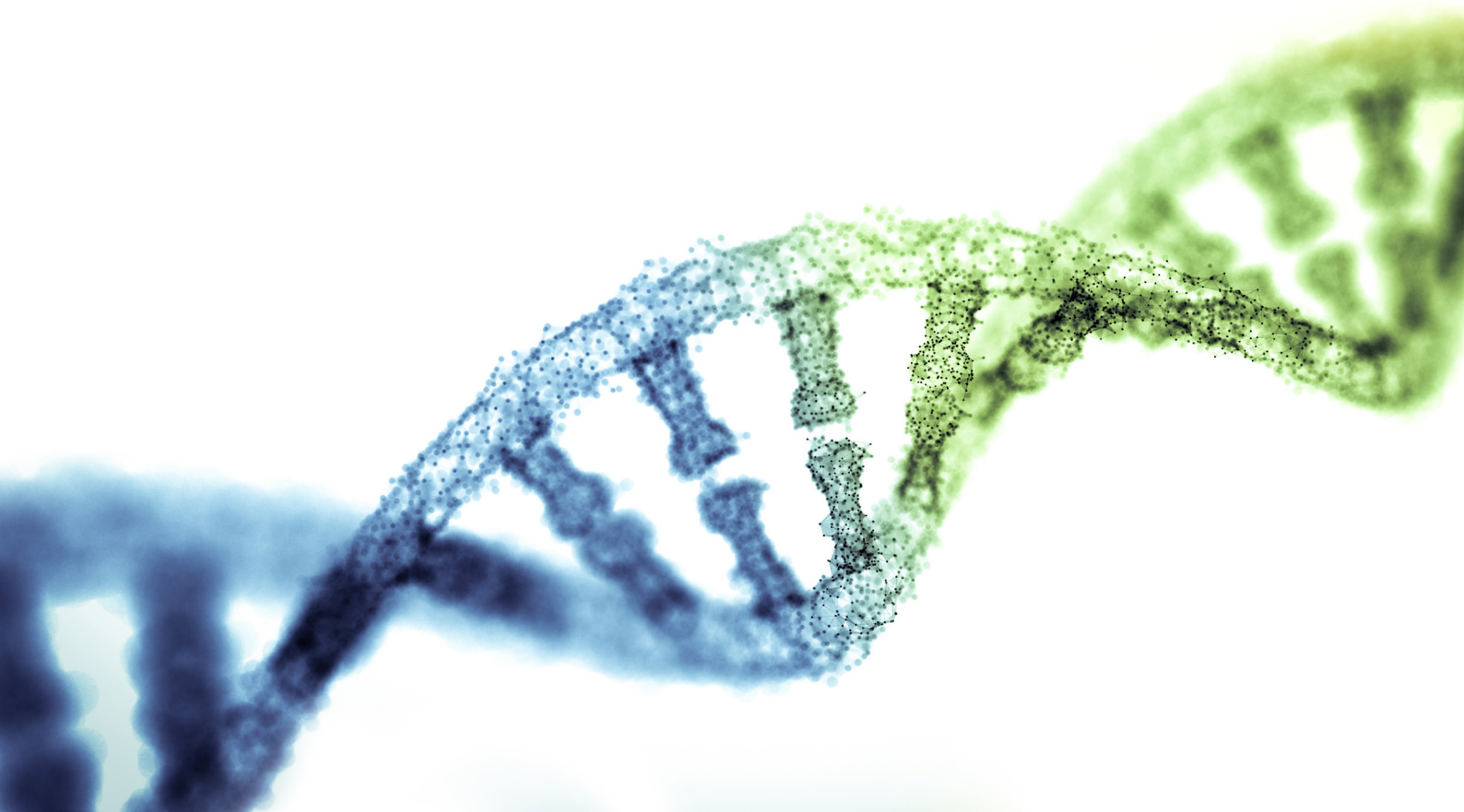
INSTRUCTIONS FOR USE OF

KAPA HyperPlus Kit

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

March 2024, Version 10.0



Contents

The KAPA HyperPlus Kits contain:

Kit Material Number	Description	Volume
KK8510 07962380001 **KK8511*** 07962398001	**KAPA HyperPlus Kit (8 rxn)**	
	KAPA Frag Enzyme	100 µL
	KAPA Frag Buffer (10X)	50 µL
	Conditioning Solution	580 µL
	End Repair & A-Tailing Buffer	70 µL
	HyperPrep ERAT Enzyme Mix	30 µL
	HyperPlus ERAT Enzyme Mix**	30 µL
	Ligation Buffer	300 µL
	DNA Ligase	100 µL
	KAPA HiFi HotStart ReadyMix (2X)*	250 µL
	Library Amplification Primer Mix (10X)*	50 µL
KK8512 07962401001 **KK8513*** 07962410001	**KAPA HyperPlus Kit (24 rxn)**	
	KAPA Frag Enzyme	270 µL
	KAPA Frag Buffer (10X)	140 µL
	Conditioning Solution	580 µL
	End Repair & A-Tailing Buffer	210 µL
	HyperPrep ERAT Enzyme Mix	90 µL
	HyperPlus ERAT Enzyme Mix**	90 µL
	Ligation Buffer	900 µL
	DNA Ligase	300 µL
	KAPA HiFi HotStart ReadyMix (2X)*	690 µL
	Library Amplification Primer Mix (10X)*	138 µL
KK8514 07962428001 **KK8515*** 07962436001	**KAPA HyperPlus Kit (96 rxn)**	
	KAPA Frag Enzyme	1.27 mL
	KAPA Frag Buffer (10X)	640 µL
	Conditioning Solution	580 µL
	End Repair & A-Tailing Buffer	930 µL
	HyperPrep ERAT Enzyme Mix	400 µL
	HyperPlus ERAT Enzyme Mix**	400 µL
	Ligation Buffer	3.8 mL
	DNA Ligase	1.26 mL
	KAPA HiFi HotStart ReadyMix (2X)*	3.0 mL
	Library Amplification Primer Mix (10X)*	600 µL

*07962398001, 07962410001, and 07962436001 are available for PCR-free workflows, and do not contain library amplification reagents (KAPA HiFi HotStart ReadyMix & Library Amplification Primer Mix).
**KAPA HyperPlus End Repair & A-Tailing (ERAT) Enzyme Mix is only compatible with the KAPA HyperPlus Workflow.
Note: Accessory kits (KAPA cleanup beads, KAPA Universal Adapter & KAPA UDI Primer Mixes and KAPA Unique Dual-Indexed Adapters) are sold separately.

Shipping, Storage and Stability

- KAPA HyperPlus Kits are shipped on dry ice or ice packs, depending on the destination country.
- Upon receipt, immediately store kit contents at -15°C to -25°C in a constant-temperature freezer.
- When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Applications

KAPA HyperPlus Kits are ideally suited for low- and high-throughput Next-Generation Sequencing (NGS) library construction workflows that require DNA fragmentation, A-tailing, adapter ligation and library amplification (optional). Kits are designed for library construction from a wide range of sample types and inputs (1 ng – 1 µg), and are compatible with complex, genomic DNA; low-complexity samples such as small viral genomes, plasmids, cDNA and long amplicons; and low-quality DNA such as that extracted from formalin-fixed, paraffin-embedded tissue (FFPET) samples.

This kit is ideally suited for germline mutation detection. It is automation-friendly and may be used for the following workflow applications:

- Whole-genome sequencing (WGS)
- Whole exome (WES) or targeted sequencing, using hybridization capture methods
- RNA-seq (starting with cDNA)

The KAPA HyperPlus Kits contain an enzymatic fragmentation module that is NOT validated for use in Methyl-Seq applications. The enzymatic fragmentation chemistry involves DNA repair mechanisms that may affect methylation patterns.

Warnings and Precautions

- Wear the appropriate personal protective equipment, such as gloves and safety glasses, to avoid direct contact while handling the reagents. Wash hands thoroughly after handling samples and reagents.
- Use good laboratory practices to avoid contamination when working with the reagents.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- 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.
- 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 HyperPlus Kits, go to ***sequencing.roche.com/products***.

Trademarks

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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

Version 3.16, October 2016; Version 4.17, September 2017; Version 5.19, July 2019; Version 6.20, March 2020; Version 7.20, December 2020; Version 8.21, April 2021; Version 9.23, June 2023; Version 10.0, March 2024

Changes to Previous Versions

Workflow modifications when using KAPA HyperPlex Adapter Kits (KAPA Universal Adapter & KAPA UDI Primer Mixes) added. Branding changes from legacy Kapa Biosystems to Roche Sequencing Solutions.

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Preface

Regulatory Disclaimer

For Research Use Only.

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 Dia Cape Town, South Africa
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.

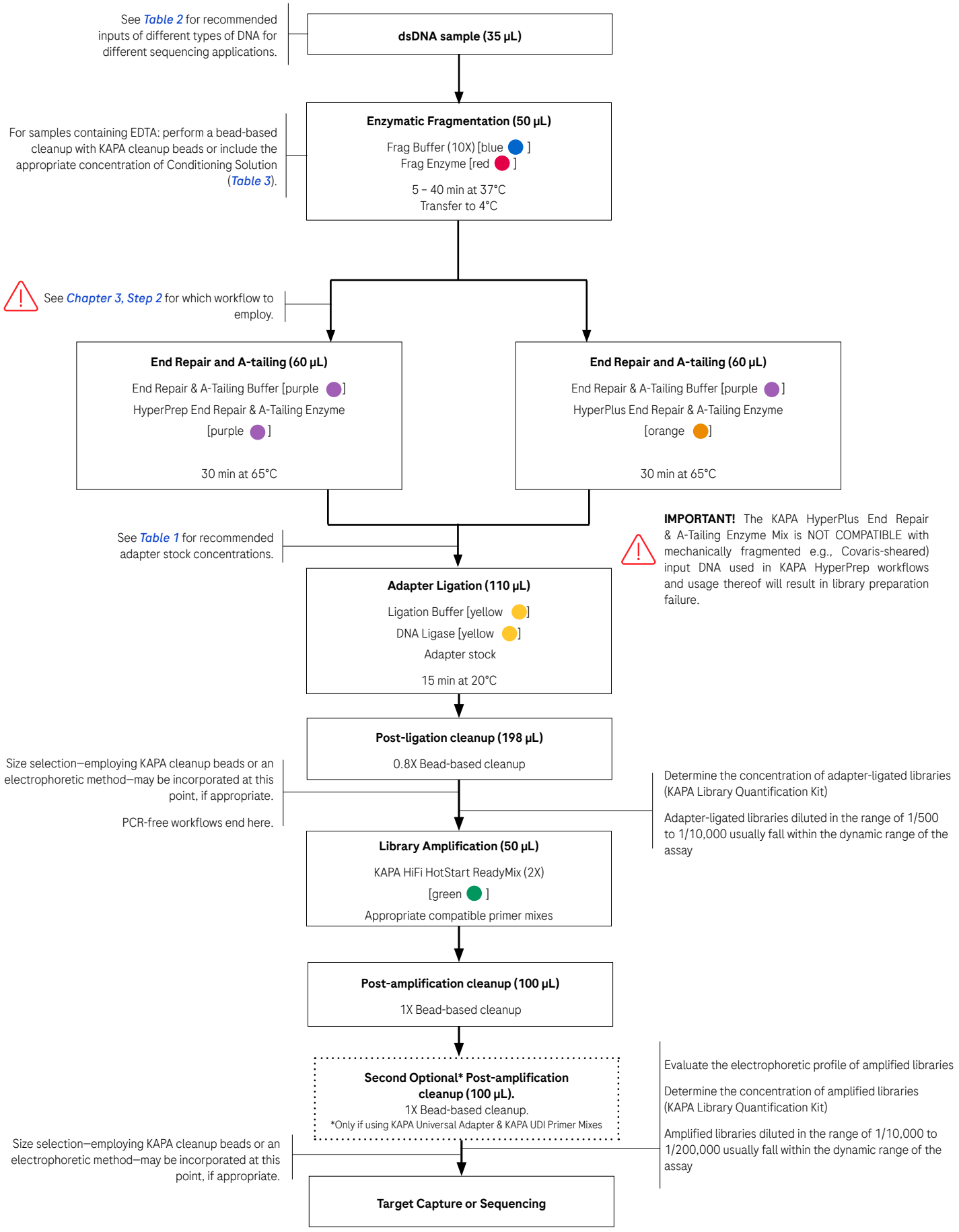
Chapter 1. Before You Begin

These Instructions for Use describe the process for a streamlined DNA fragmentation and library construction protocol for the rapid preparation of libraries for Illumina sequencing. Specifically, these Instructions for Use provide a protocol for the workflow outlined in ***Figure 1*** using the KAPA HyperPlus Kit.

The KAPA HyperPlus Workflow provides:

- An easy to use, streamlined, and automation-friendly workflow with minimal resource requirements.
- 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

Figure 1 - KAPA HyperPlus Quick Guide



The KAPA HyperPlus Kits contain an enzymatic fragmentation module that is NOT validated for use in Methyl-Seq applications. The enzymatic fragmentation chemistry involves DNA repair mechanisms that may affect methylation patterns.

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.
- 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 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.

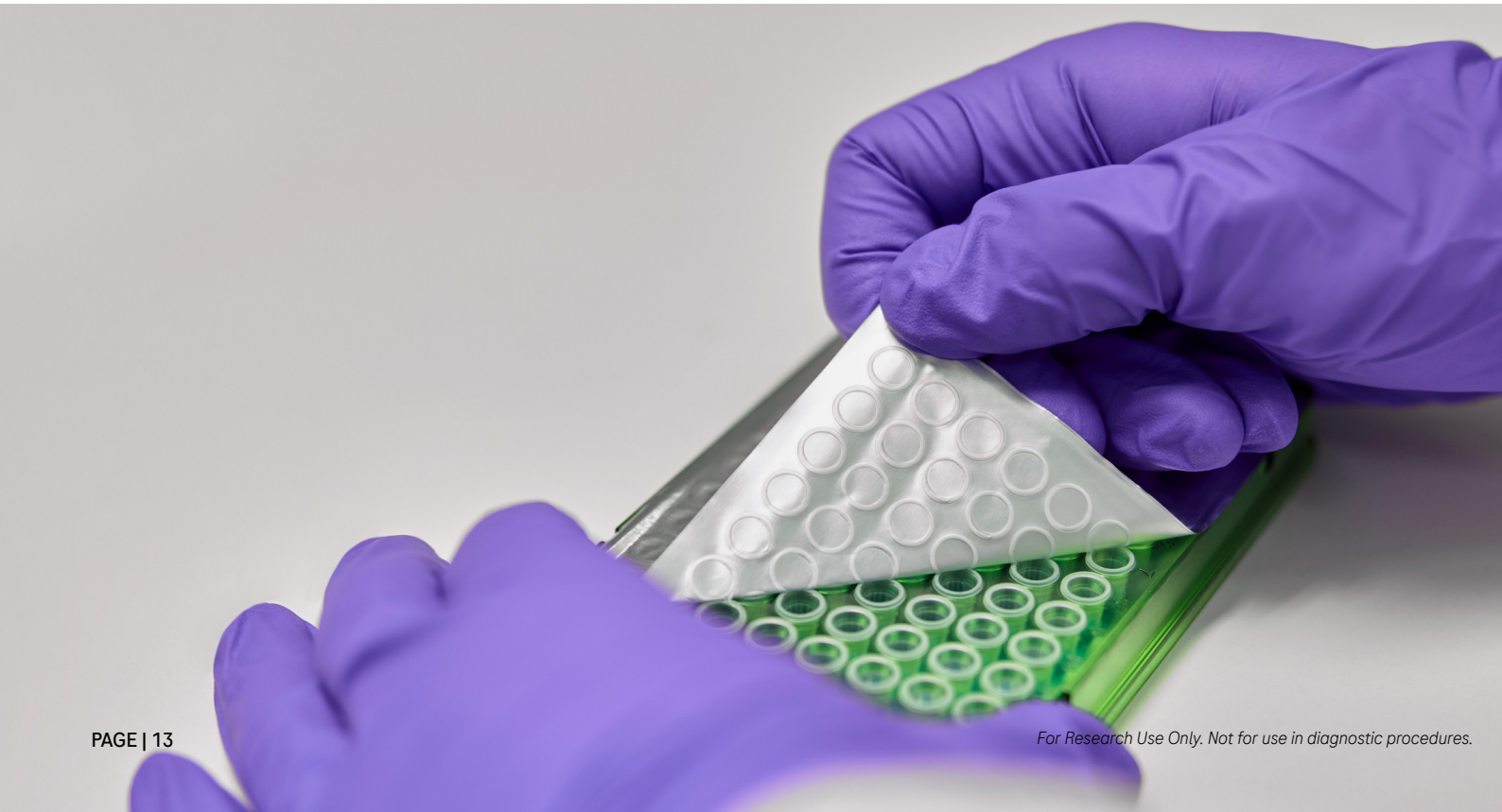
Prepare the Following Equipment

- Thermocyclers should be programmed with the following:
  - Fragmentation (Chapter 3, *Prepare the Sample Library, Step 1*)
  - End Repair & A-tailing program (Chapter 3, Prepare the Sample Library, Step 2)
  - Adapter Ligation program (Chapter 3, *Prepare the Sample Library, Step 3*)
  - Library Amplification program (Chapter 4, *Amplify the Sample Library, Step 1*)

  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 & 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
DynaMag-96 Side Magnet	Thermo Fisher
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 Available from Roche

Description	Package Size	Material Number
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 kits - consist of 4 x 96 reaction kits

Consumables Purchased from Other Vendors

Component	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

Chapter 2.

Store and Prepare the Reagents

Step 1. Store the Reagent Kits

Reagent Kit	Storage Temperature
KAPA HyperPlus Kit	-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 Mix	-15°C to -25°C

*The KAPA HyperPure Beads kit must not be frozen.

Step 2. Prepare KAPA Adapters and 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.

Step 2a. Dilute the KAPA Unique Dual-Indexed Adapters (if required)

1. Retrieve the KAPA UDI Adapter plate from storage (-15°C to -25°C) and thaw at room temperature.
2. Centrifuge the KAPA UDI Adapter plate at room temperature (280 x *g* for at least 1 min) to ensure the liquid is collected at the bottom of the wells.

   Do not vortex the adapter plate as it could result in cross-contamination of the KAPA UDI Adapters. Pipette-mix individual adapters prior to use.
3. Before removing the foil cover, please ensure the plate is in the correct orientation. Please refer to the *KAPA UDI Adapter Technical Data Sheet* (Document number KR1736, available ***online*** on eLabDoc) for additional handling instructions.
4. Upon first use, carefully remove the foil cover of the plate to avoid cross contamination.

   Discard the original foil cover. Do not reuse.

   Maintain good lab practices when removing the foil seal. E.g., refrain from contact with the underside of the seal. If contact is made, change gloves before handling the adapter or sample plates.

   A new pipette tip must be used for each well to avoid cross contamination. If you are not using the entire contents of the KAPA UDI Adapter plate at this time, apply a new adhesive foil seal provided in the kit. Make sure that the foil is properly aligned and fully covers all 96 wells. Use a roller or other appropriate tool to ensure that the foil is evenly applied.
5. If applicable, dilute adapters to the required concentration, see Table 1 below, using the KAPA Adapter Dilution Buffer.

Step 2b. Dilute the KAPA Universal Adapters (if required)

1. Retrieve the KAPA Universal Adapters tube from storage (-15°C to -25°C) and thaw at room temperature or on ice.
2. Mix thoroughly before first use.
3. Centrifuge the KAPA Universal Adapters at room temperature (280 x *g* for at least 1 min) to ensure the liquid is collected at the bottom of the tube.
4. If applicable, dilute KAPA Universal Adapters to the required concentration, see Table 1 below, using 10 mM Tris-HCl, pH 8.0 – 8.5.

Table 1. Recommended KAPA Adapter concentrations for libraries constructed from 1 ng – 1 µg input DNA

Input DNA	Adapter stock concentration*	Adapter:insert molar ratio**	Input DNA	Adapter stock concentration*	Adapter:insert molar ratio**
1 µg	15 µM	10:1	25 ng	7.5 µM	200:1
500 ng	15 µM	20:1	10 ng	3 µM	200:1
250 ng	15 µM	40:1	5 ng	1.5 µM	200:1
100 ng	15 µM	100:1	2.5 ng	750 nM	200:1
50 ng	15 µM	200:1	1 ng	300 nM	200:1

*Concentration required when using both KAPA UDI Adapter (full length) and KAPA Universal Adapter (truncated). Both KAPA UDI Adapter and KAPA Universal Adapter are provided at 15 µM.
**Adapter:insert molar ratio calculations are based on a mode DNA fragment length of 200 bp, and will be higher for longer DNA fragments, or slightly lower for DNA fragmented to a mode size <200 bp. The lower adapter:insert molar ratios recommended for inputs >100 ng represent a fair compromise between library construction efficiency and cost; higher library yields will be achieved if a higher adapter concentration is used.

Step 2c. Preparation of the KAPA UDI Primer Mixes

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 room temperature (280 x *g* for at least 1 min) 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 2*.
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 room temperature (280 x *g* for at least 1 min) 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.

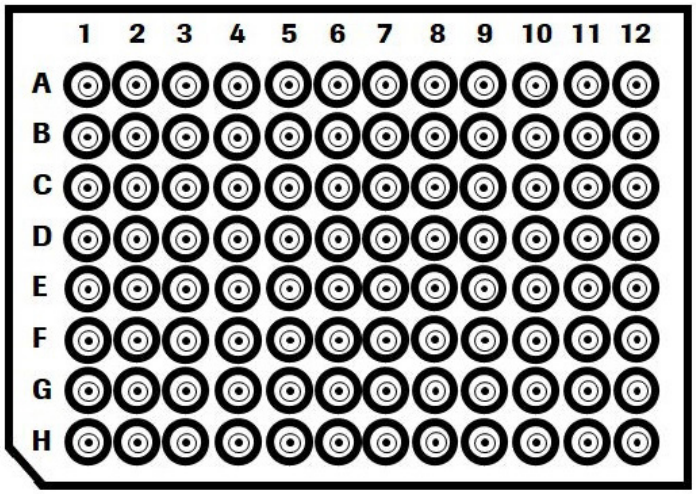


Figure 2: KAPA UDI Primer Mixes plate layout.

Considerations for Adapter Design and Concentration

- Adapter concentration affects ligation efficiency, as well as adapter and adapter-dimer carry-over during the post-ligation cleanup. The optimal adapter concentration for your workflow represents a compromise between the above factors and cost.
- Ligation efficiency is robust for adapter:insert molar ratios ranging from 10:1 to >200:1, making it unnecessary to adjust adapter stock concentrations to accommmodate moderate variations in DNA input or fragment length.
- High adapter:insert molar ratios (>200:1) are beneficial for low-input applications. When optimizing workflows for DNA inputs ≤25 ng, two or three adapter concentrations should be evaluated: try the recommended adapter concentration, as well as one or two additional concentrations in a range that is 2 – 10 times higher than the recommended concentration.
- Adapter quality has an impact on the effective concentration of adapter available for ligation. Always source the highest quality adapters from a reliable supplier, dilute and store adapters in a buffered solution with the requisite ionic strength, and avoid excessive freezing and thawing of adapter stock solutions.
- To accommodate different adapter concentrations within a batch of samples processed together, it is best to vary the concentrations of adapter stock solutions, and dispense a fixed volume (5 µL) of each adapter. The alternative (using a single stock solution, and dispensing variable volumes of adapter into ligation reactions) is not recommended.

Chapter 3.

Prepare the Sample Library

In this chapter the KAPA HyperPlus Kit is used to perform enzymatic fragmentation and indexed libraries are prepared. The workflow requires the use of components from the following kits:

- KAPA HyperPlus Kit
- KAPA Unique Dual-Indexed Adapter Kit & KAPA Library Amplification Primers OR*
- KAPA Universal Adapter** & KAPA UDI Primer Mixes
- KAPA HyperPure Beads***

Ensure that the following are available:

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

*KAPA Unique Dual-Indexed (UDI) Adapters are recommended for use with the KAPA HyperPlus Kit. However, the kit is also compatible with KAPA Universal Adapter & KAPA UDI Primer Mixes as well as other full-length or truncated adapter designs from 3rd party suppliers.

The KAPA Universal UMI Adapter (Catalog # 09329862001, and provided at 33 µM), may also be considered for this workflow. The KAPA Universal UMI Adapter with Unique Molecular Identifiers (UMI) allows for molecular barcoding within each sample, enabling proper molecule counting for low-frequency variant detection. However, the KAPA Universal UMI Adapter has only been validated within specific KAPA Target Enrichment workflows and may not confer the same computational benefits for non-target enrichment workflows (such as whole-genome sequencing). Contact your local Roche Technical Support at ***sequencing.roche.com/support* for guidance and important considerations.

***KAPA HyperPure Beads are recommended for use with the KAPA HyperPlus Kit. However, the kit is also compatible with KAPA Pure Beads. Conditions for DNA binding and size selection may differ if other beads are used.

Sample Requirements

This workflow was validated with 1 ng - 1 µg of high quality gDNA, and is compatible with ≥50 ng low quality DNA extracted from FFPET for sample library preparation. The DNA should be quantified using the Qubit dsDNA HS Assay Kit. Lower input amounts and sample quality may not yield equivalent results. For guidance on lower input amounts or sample quality, please see the table below or contact ***sequencing.roche.com/support***.

Table 2. Recommended inputs into library construction

Application	Sample Type	Recommended Input
WGS	Complex gDNA (high quality)	50 ng – 1 µg
Target capture (WES, custom panels)	Complex gDNA (high quality)	10 ng – 1 µg
WGS, target capture	FFPE DNA*	≥50 ng (quality dependent)
WGS	Microbial DNA	1 ng – 1 µg
WGS (PCR-free)	High-quality DNA	≥50 ng (no SS)** ≥200 ng (w/SS)**
Targeted sequencing	Long amplicons	≥1 ng
RNA-seq	Full-length/unfragmented cDNA	≥1 ng

*Reach out to ***Technical Support*** for possible workflow modifications when using this sample type.

**SS = double-sided size selection; a requirement when performing WGS on patterned flow cells but may result in sample losses of 60 – 95%, irrespective of whether a bead- or gel-based technique is used. For PCR-free workflows, due to the inherent sample losses, performing double-sided size selection with inputs <200 ng is not recommended.

⚠ For best results, it is suggested that users do not exceed a maximum input of 500 ng when planning to use KAPA Universal Adapter & KAPA UDI Primer Mixes. When using incomplete, or truncated adapters, in conjunction with indexed PCR primers, a minimum number of amplification cycles (3) are required to complete adapter sequences for the next step in the process (target capture or sequencing). With inputs >500 ng, there is a risk of overamplification when using a minimum of 3 amplification cycles.

Overamplification is a result of primer depletion during the library amplification reaction resulting in single stranded amplification products. These products can anneal to each other via adapter homology on both ends of the fragments to form heteroduplexes, and migrate as larger products on electrophoresis-based fragment analyzers than their actual length in base pairs. The artifact can be resolved by increasing primer concentration or reducing cycle number in the PCR reaction, however the products themselves are perfectly acceptable for use in sequence capture and sequencing.

The KAPA HyperPrep/Plus End Repair & A-Tailing Buffer and KAPA HyperPrep Ligation Buffer may contain white precipitates when thawed at +2°C to +8°C. These buffers must be thawed at room temperature and vortexed thoroughly until the precipitate is completely resuspended before use.

Always ensure that KAPA HyperPlus Kit components have been fully thawed and thoroughly mixed before use. Keep all components on ice as long as possible during handling and preparation.

The KAPA HyperPrep Ligation Buffer contains a high concentration of PEG 6000 and is very viscous. Small PEG 6000 droplets may be visible when thawed. Ensure the buffer is thoroughly vortexed until the droplets have been resuspended. Gentle heating at +37°C for 5 –10 min, followed by vortexing, is suggested in cases where vortexing alone does not suffice.

The fragmentation parameters in this Instructions for Use are provided as a starting point and may require optimization for your specific sample type. Please refer to ***Appendix C*** for Fragmentation Optimization Guidelines.

Make sure KAPA HyperPure Beads are removed from storage at least 30 minutes prior to starting the workflow and fully equilibrated to room temperature. For best performance, store the beads protected from light when not in use.

Handling of DNA Samples Containing EDTA

The enzymatic fragmentation reaction is very sensitive to the presence of EDTA, which must be removed or neutralized prior to fragmentation.

- This may be achieved by means of a 3X bead-based cleanup with KAPA cleanup beads. Please refer to the relevant Technical Data Sheet (Document number KR1705 or KR1245, available ***online*** on eLabDoc) for a detailed DNA cleanup protocol. For optimal fragmentation results, elute DNA in 10 mM Tris- HCl, pH 8.0 - 8.5 after the cleanup.
- DNA isolated from blood samples has been reported to contain inhibitors, which can affect the efficiency of fragmentation. Performing a 3X bead-based cleanup prior to fragmentation is recommended.
- Bead-based cleanups to remove EDTA from FFPE DNA samples may not yield comparable results. Recovery of FFPE DNA may be low, and not always proportional to DNA quality. For FFPE DNA, neutralization of EDTA with the Conditioning Solution (see below) is recommended as a first approach.
- If a DNA cleanup is not feasible, the inhibitory effect of the EDTA can be mitigated by the inclusion of Conditioning Solution at the appropriate final concentration in the fragmentation reaction.
- To facilitate reaction setup, the Conditioning Solution is pre-diluted to the appropriate working concentration as outlined in ***Table 3***, and a fixed volume (5 µL) is included in the fragmentation reaction. Please note that dilution of the Conditioning Solution is based on the final concentration of EDTA in the fragmentation reaction (once input DNA has been diluted in a volume of 50 µL), and not on the EDTA concentration in the DNA preparation.
- Prepare a minimum of 100 µL of diluted Conditioning Solution (as indicated in ***Table 3***), or calculate the volume needed using the following formula: (number of reactions x 5 µL) + 10% excess

The addition of Conditioning Solution to fragmentation reactions will lead to suboptimal results if your DNA does not contain EDTA, or if the final concentration of the Conditioning Solution is not matched to the final EDTA concentration in the reaction.

Table 3. Conditioning Solution dilutions for DNA samples containing EDTA

Final EDTA concentration in 50 µL rxn	Dilution factor	Volume of Conditioning Solution (per 100 µL)	Volume of PCR-grade water (per 100 µL)
0.02 – 0.05 mM	32.0	3.1 µL	96.9 µL
0.1 mM	15.4	6.5 µL	93.5 µL
0.2 mM	7.4	13.5 µL	86.5 µL
0.3 mM	4.8	21.0 µL	79.0 µL
0.4 mM	3.3	30.0 µL	70.0 µL
0.5 mM	2.6	38.8 µL	61.2 µL
0.6 mM	2.2	46.5 µL	53.5 µL
0.7 mM	1.8	56.0 µL	44.0 µL
0.8 mM	1.6	64.0 µL	36.0 µL
0.9 mM	1.4	72.0 µL	28.0 µL
1.0 mM	1.3	80.0 µL	20.0 µL

Step 1. Enzymatic Fragmentation

1. Dilute 1 ng – 1000 ng of DNA with 10 mM Tris-HCl, pH 8.0 – 8.5 (recommended) to a total volume of 35 µL in a 0.2 mL tube or well of a PCR plate.
   - If the DNA preparation does not contain EDTA, dilute in 10 mM Tris-HCl (pH 8.0 – 8.5) in a total of 35 µL.
   - If the DNA preparation does contain EDTA, dilute in the EDTA-containing buffer in which samples are currently suspended, in a total of 30 µL. To each reaction with 30 µL of EDTA-containing DNA, add 5 µL of diluted Conditioning Solution.

2. Assemble each Fragmentation reaction on ice as per the table below:

Component	Volume Per Individual Sample
1 ng – 1000 ng DNA (with Conditioning Solution, if needed)	35 µL
KAPA Frag Buffer (10X)*	5 µL
KAPA Frag Enzyme*	10 µL
Total volume	**50 µL**

*The KAPA Frag Buffer and KAPA Frag Enzyme may be pre-mixed and kept on ice prior to reaction setup and dispensed as a single solution. Please note that the volume of the buffer is less than the volume of the enzyme in this reaction.

3. Mix the Fragmentation reaction thoroughly and centrifuge briefly. Return the plate/tube(s) on ice and proceed immediately to the next step.

⚠ If the Fragmentation reaction is not mixed properly, it can result in increased fragment size.

4. Incubate in a thermocycler, pre-cooled to +4°C and programmed as outlined below. Set the lid temperature to ~ +65°C (if possible):

    a. Pre-cool block: +4°C

    b. Fragmentation: +37°C - See table below

    c. Hold: +4°C

Mode fragment length	Incubation time at +37°C*	Optimization range
600 bp	5 min	3 – 10 min
350 bp	10 min	5 – 20 min
200 bp	20 min	10 – 25 min
150 bp	30 min	20 – 40 min

*These parameters are a good starting point for high-quality genomic DNA. Please refer to ***Appendix A*** for guidelines on how to optimize fragmentation time and temperature. If incubation times longer than the recommended range are needed, samples likely contain inhibitors which impact the fragmentation efficiency. Bead-based DNA cleanup, prior to fragmentation, is recommended over longer fragmentation times.

This is not a validated safe stopping point. Proceed directly to ***Step 2: End Repair and A-tailing***.

Step 2: End Repair and A-tailing

- This protocol has been validated for use with either the KAPA Hyper**Prep** End Repair & A-Tailing Enzyme Mix (purple cap) or the KAPA Hyper**Plus** End Repair & A-Tailing Enzyme Mix (orange cap).
- The KAPA HyperPlus workflow does not change depending on the End Repair & A-Tailing Enzyme Mix selected. Either option can be used in the workflow.
- For all new, or sensitive applications and assays, it is recommended to use the KAPA HyperPlus End Repair & A-tailing Enzyme Mix. For existing validated workflows, it is recommended to perform a side-by-side comparison using the two enzyme mixes and determine which one is most suitable for the specific application.

The KAPA HyperPrep/Plus End Repair & A-Tailing Buffer may contain white precipitates when thawed. Ensure the buffer is thoroughly vortexed until the precipitate has been resuspended. Heat at +37°C for 5 – 10 min, if indicated.

The KAPA HyperPlus End Repair & A-Tailing Enzyme Mix is NOT COMPATIBLE with mechanically fragmented (e.g., Covaris-sheared) input DNA used in KAPA HyperPrep workflows and usage thereof will result in library preparation failure.

1. In the same plate/tube(s) in which enzymatic fragmentation was performed, assemble each End Repair and A-Tailing reaction as per table below:

Component	Volume Per Individual Sample
Fragmented, double-stranded DNA	50 µL
End Repair & A-Tailing Buffer*	7 µL
End Repair & A-Tailing Enzyme Mix**	3 µL
Total volume	**60 µL**

*The buffer and enzyme mix should preferably be pre-mixed and added in a single pipetting step. Premixes are stable for ≤24 hrs at room temperature, for ≤3 days at +2°C to +8°C, and for ≤4 weeks at -15°C to -25°C.
**Use either the HyperPrep End Repair & A-Tailing Enzyme Mix (purple cap) or the HyperPlus End Repair & A-Tailing Enzyme Mix (orange cap).

2. Mix the End repair and A-tailing reaction thoroughly and centrifuge briefly. Return the reaction plate/tube(s) to ice. Proceed immediately to the next step.

3. Incubate in a thermocycler programmed as outlined below. A heated lid is required for this step. If possible, set the temperature of the heated lid to ~ +85°C (instead of the usual +105°C).

Step	Temperature	Time
End repair and A-tailing	+65°C*	30 min
Hold	+4°C**	∞

*Both the fragmentation and end repair enzymes are inactivated at +65°C. When reactions are set up according to recommendations, additional fragmentation should be negligible. The brief period of end repair is sufficient for enzymatically fragmented DNA.

**If proceeding to the Adapter Ligation reaction setup without any delay, the reaction may be cooled to +20°C instead of +4°C.

⚠ This is not a validated safe stopping point. Proceed directly to ***Step 3: Adapter Ligation***.

Step 3. Adapter Ligation

KAPA UDI Adapter and KAPA Universal Adapter, unless specified, will hereafter be referred to as **KAPA Adapters**.

⚠ The KAPA HyperPrep Ligation Buffer contains a high concentration of PEG 6000 and is very viscous. Small PEG 6000 droplets may be visible when thawed and require special attention during pipetting. Ensure the buffer is thoroughly vortexed until the droplets have been resuspended. Heat at +37°C for 5 – 10 min, if indicated.

⚠ KAPA Adapters must be added to each tube/well individually prior to addition of the Ligation reagents.

⚠ If using KAPA Universal Adapter, sample indexes are incorporated during the Library Amplification step. Precautions should be taken to avoid sample cross contamination.

1. Transfer the reaction from the thermocycler to ice.
2. In the same plate/tube(s) in which End repair and A-tailing was performed, assemble each Adapter Ligation reaction on ice as per the table below:

Component	Volume Per Individual Sample
End repair and A-tailing reaction product	60 µL
KAPA Adapters (Chapter 2)	5 µL
PCR-grade water*	5 µL
Ligation Buffer*	30 µL
DNA Ligase*	10 µL
Total volume	**110 µL**

*The water, buffer and ligase enzyme should preferably be premixed and added in a single pipetting step. Premixes are stable for ≤24 hrs at room temperature, for ≤3 days at +2°C to +8°C, and for ≤4 weeks at -15°C to -25°C.

3. Mix the Adapter Ligation reaction thoroughly and centrifuge.
4. Incubate the Adapter Ligation reaction at +20°C on a thermocycler for 15 minutes.
5. Following the incubation, proceed immediately to the next step.

To achieve higher conversion rates and library yields, particularly for low-input samples, consider increasing the ligation time to a maximum of 4 hrs at +20°C or overnight at +2°C to +8°C. Please note that longer ligation times may lead to increased levels of adapter-dimer. Adapter concentrations may have to be optimized if ligation times are extended significantly.

Step 4. Purify the Sample Library using KAPA HyperPure Beads

1. To each Adapter Ligation reaction, add 88 µL of room temperature KAPA HyperPure Beads that have been thoroughly resuspended.

Component	Volume
Ligation reaction product	110 µL
KAPA HyperPure Beads	88 µL
Total volume	**198 µL**

2. Once added, mix thoroughly and centrifuge briefly to collect all droplets. Do NOT allow beads to pellet.

   It is important at this step to ensure that the solution is thoroughly mixed and appears homogeneous.

   Insufficient mixing may compromise recovery and result in size selection.

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 the liquid is clear.
5. Carefully remove and discard the supernatant.
6. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
7. Incubate the sample at room temperature for ≥30 seconds.
8. Carefully remove and discard the ethanol.
9. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
10. Incubate the sample at room temperature for ≥30 seconds.
11. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
12. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.

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

13. Remove the sample from the magnet.
14. Thoroughly resuspend the beads:

    14.1 in 25 µL of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5) to proceed with Library Amplification *(Chapter 4)*, or

    14.2 in 55 µL of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5) to proceed with Double-sided Size Selection *(Appendix B)*.

15. Incubate the sample at room temperature for 2 minutes to allow the sample library to elute off the beads.
16. Place the sample on the magnet to collect the beads. Incubate until the liquid is clear.
17. Transfer an appropriate volume of the clear supernatant/eluate to a fresh tube/well:

    17.1 to proceed with Library Amplification *(Chapter 4)*, transfer 20 µL of supernatant, or

    17.2 to proceed with Double-sided Size Selection *(Appendix B)*, transfer 50 µL of supernatant.

The remaining 5 µL can be used for quality control purposes e.g., quantification using the KAPA Library Quantification Kit.

18. Proceed to ***Chapter 4. Amplify The Sample Library*** (optional for sample inputs of ≥50 ng but mandatory if using KAPA Universal Adapter) or ***Chapter 5. Quality Control***, if performing a PCR-free workflow (not applicable if using KAPA Universal Adapter).

Safe stopping point – If necessary, this is a safe stopping point. Purified, adapter-ligated library may be stored at +2°C to +8°C for 1 – 2 weeks or at -15°C to – 25°C for ≤1 month before amplification and/or sequencing. To avoid degradation, always store DNA in a buffered solution (10 mM Tris-HCl, pH 8.0 – 8.5) when possible, and minimize the number of freeze-thaw cycles.

Chapter 4.

Amplify the Sample Library

This chapter describes how to amplify the adapter-ligated library, if necessary, using 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 (provided with the kit) is used for library amplification.

⚠ If KAPA Universal Adapters were used for adapter ligation, amplification is mandatory. Ensure a unique KAPA UDI Primer Mix 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

Library Amplification Considerations

- KAPA HiFi HotStart, the enzyme provided in the KAPA HiFi HotStart ReadyMix, is an antibody-based hotstart formulation of KAPA HiFi DNA Polymerase, a B-family DNA polymerase engineered for increased processivity and high fidelity. KAPA HiFi HotStart has 5' --> 3' polymerase and 3' --> 5' exonuclease (proofreading) activity, but no 5' --> 3' exonuclease activity. The error rate of KAPA HiFi HotStart is 2.8 x 10^{-7} errors/base, equivalent to 1 error in 3.5 x 10^{6} nucleotides incorporated.
- The KAPA Library Amplification Primer Mix (10X) is designed to eliminate or delay primer depletion during library amplification reactions performed with KAPA HiFi HotStart ReadyMix. The primer mix is suitable for the amplification of all Illumina libraries flanked by the P5 and P7 flow cell sequences. Primers are supplied at a 10X concentration of 20 µM each, and have been formulated as described below. User-supplied primer mixes may be used in combination with incomplete or custom adapters. Please contact Technical Support at ***sequencing.roche.com/support*** for guidelines on the formulation of user-supplied library amplification primers.
- To achieve the highest amplification efficiency and avoid primer depletion, it is critical to use an optimal concentration of high quality primers. Primers should be used at a final concentration of 0.5 – 4 µM each. For libraries constructed from ≥100 ng input DNA, a final concentration of at least 2 µM of each primer is recommended.
- Library amplification 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 HotStart DNA Polymerase). Always store and dilute primers in a buffered solution (e.g.,10 mM Tris-HCl, pH 8.0 – 8.5), and limit the number of freeze-thaw cycles. To achieve the latter, store primers at +2°C to +8°C for short-term use, or as single-use aliquots at -15°C to -25°C.
- In library amplification reactions (set up according to the recommended protocol), 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 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 typically comprise library molecules of the desired length, which are individualized during denaturation prior to cluster amplification or probe hybridization. Since these heteroduplexes contain significant portions of single-stranded DNA, overamplification leads to the under-quantification of library molecules with assays employing dsDNA-binding dyes. qPCR-based library quantifications methods, such as the KAPA Library Quantification assay, quantify DNA by denaturation and amplification, thereby providing an accurate measure of the amount of adapter-ligated molecules in a library—even if the library was over-amplified.
- Excessive library amplification can result in other unwanted artifacts such as amplification bias, PCR duplicates, chimeric library inserts and nucleotide substitutions. The extent of library amplification should therefore be limited as much as possible, while ensuring that sufficient material is generated for QC and downstream processing.
- If cycled to completion (not recommended), one 50 µL library amplification can produce 8 – 10 µg of amplified library. To minimize overamplification and its associated, undesired artifacts, the number of amplification cycles should be tailored to produce the optimal amount of final library required for downstream processes. This is typically in the range of 250 ng – 1.5 µg.

Step 1. Prepare the Library Amplification Reaction

Ensure the KAPA HyperPure Beads 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 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 2c*. Centrifuge the plate at 280 x *g* for 30 seconds to collect the contents to the bottom of the wells 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.

Keep all components on ice as long as possible during handling.

1. Assemble each Library Amplification reaction as per table below:

Component	Volume per Individual Library
KAPA HiFi HotStart ReadyMix (2X)	25 µL
KAPA Library Amplification Primer Mix* OR KAPA UDI Primer Mix**	5 µL
Adapter-ligated library	20 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 HiFi HotStart 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. Immediately proceed to the next step.

Step 2. Perform the Library Amplification

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

Step	Temperature	Duration	Cycles
Initial denaturation	+98°C	45 sec	1
Denaturation	+98°C	15 sec	Variable, see Table 4 or Table 5 below for cycle numbers tailored to the KAPA Adapter that was used during Adapter Ligation
Annealing	+60°C	30 sec	
Extension	+72°C	30 sec	
Final extension	+72°C	1 min	1
Hold	+4°C	∞	1

Table 4. Recommended cycle numbers to generate 100 ng or 1 µg of amplified DNA when using KAPA UDI Adapters

Input into library construction	Number of cycles required to generate	
	100 ng library	1 µg library
1 µg	0*	0 – 1*
500 ng	0*	2 – 3
250 ng	0 – 1*	3 – 5
100 ng	0 – 2*	5 – 6
50 ng	3 – 5	7 – 8
25 ng	5 – 6	8 – 10
10 ng	7 – 9	11 – 13
5 ng	9 – 11	13 – 14
2.5 ng	11 – 13	14 – 16
1 ng	13 – 15	17 – 19

*When using incomplete adapters from 3rd party vendors a minimum number of amplification cycles (3) is required to complete adapter sequences for the next step in the process (target capture or sequencing), irrespective of whether a sufficient amount of library is available after ligation. The number of cycles needed depends on the specific adapter and amplification primer design.

Table 5. Recommended number of amplification cycles to generate 4 nM of amplified DNA when using KAPA Universal Adapter & KAPA UDI Primer Mixes**

Input amount	Amplification cycle number
50 – 500 ng*	3 – 4
10 ng	3 – 5
1 ng	6 – 8

*For best results, it is suggested that users do not exceed a maximum input of 500 ng when planning to use KAPA Universal Adapter & KAPA UDI Primer Mixes. When using incomplete, or truncated, adapters in conjunction with indexed PCR primers, a minimum number of amplification cycles (3) are required to complete adapter sequences for the next step in the process (target capture or sequencing). With inputs >500 ng, there is a risk of overamplification when using a minimum of 3 amplification cycles. Overamplification is a result of primer depletion during the library amplification reaction resulting in single stranded amplification products. These products can anneal to each other via adapter homology on both ends of the fragments to form heteroduplexes, and migrate as larger products on electrophoresis-based fragment analyzers than their actual length in base pairs. The artifact can be resolved by increasing primer concentration or reducing cycle number in the PCR reaction, however the products themselves are perfectly acceptable for use in sequence capture and sequencing.

**Based on sequencing recommendations, 4 nM is the minimum starting concentration to proceed with sequencing. 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.

2. Proceed immediately to the next step.

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

If KAPA UDI Adapters and KAPA Library Amplification Primer Mix were used for library construction – proceed to Step 3a or,

If KAPA Universal Adapter and KAPA UDI Primer Mixes were used for library construction – proceed to Step 3b.

Step 3a. Purify the Amplified Sample Library constructed using KAPA UDI Adapter & KAPA Library Amplification Primer Mix

1. Add 50 µL 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 amplified sample library to bind to the beads.
4. Place the sample on a magnet to capture the beads. Incubate until the liquid is clear.
5. Carefully remove and discard the supernatant.
6. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
7. Incubate the sample at room temperature for ≥30 seconds.
8. Carefully remove and discard the ethanol.
9. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
10. Incubate the sample at room temperature for ≥30 seconds.
11. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
12. 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 of the bead pellet. 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. Do NOT allow beads to pellet.

    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 allow the amplified sample library to elute off the beads.
16. Place the sample on the magnet to capture the beads. Incubate until the liquid is clear.
17. Transfer an appropriate volume of the clear supernatant to a fresh tube(s)/well and proceed with double-sided size selection (refer to ***Appendix B***), library QC, target capture or sequencing.
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.

Step 3b. 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 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..
3. Incubate the sample at room temperature for 5 minutes to allow the amplified sample library to bind to the beads.
4. Place the sample on a magnet to capture the beads. Incubate until the 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.
7. Add 50 µL of room temperature, thoroughly resuspended, of KAPA HyperPure Beads to each sample.
8. Mix thoroughly by pipetting or vortexing, and centrifuge briefly to collect all droplets.
9. Incubate the sample at room temperature for 5 minutes to allow the amplified 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 of the bead pellet. 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. Centrifuge briefly to collect all droplets. Do NOT allow beads to pellet.

If proceeding with double-sided size selection (***Appendix B***), resuspend the beads in 55 µL of elution buffer.

21. Incubate the sample at room temperature for 2 minutes to allow the sample library to elute off the beads.
22. Place the sample on the 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 (refer to ***Appendix B***), library QC, target capture or sequencing.
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.

Chapter 5.

Quality Control

This chapter describes how to determine the concentration and size distribution of the sample library. Your specific library construction workflow should be tailored and optimized to yield a sufficient amount of adapter-ligated molecules of the desired size distribution for the next step in the process (e.g., target capture or sequencing), as well as for library QC and archiving purposes.

Quantification

KAPA Library Quantification Kits for Illumina platforms are recommended for qPCR-based quantification of libraries generated using the KAPA HyperPlus workflow. Libraries may also be quantified using a Qubit Fluorometer and the Qubit dsDNA HS Assay Kit.

The proportion of fragmented DNA that is successfully converted to adapter-ligated molecules decreases as input is reduced. Table 6 summarizes the expected conversion rates for different DNA input ranges. These figures apply to high-quality DNA, and may be lower for DNA of lower quality, e.g., FFPE samples. Workflows with additional cleanups or size selection prior to adapter ligation are also likely to result in a lower yield of adapter-ligated molecules.

Table 6. Expected conversion rates for DNA input ranges

DNA Input	Expected Conversion Rate
1 – 10 ng	5 – 20%
11 – 100 ng	10 – 50%
>100 ng	50 – 100%

Sizing

The size distribution of the final libraries should be confirmed with an electrophoretic method. A LabChip GX, GXII, or GX Touch (PerkinElmer), Bioanalyzer, TapeStation (Agilent Technologies), Fragment Analyzer (Advanced Analytical) system or similar instruments are recommended over conventional gels. KAPA HyperPlus Kit libraries may require dilution prior to electrophoretic assessment. Refer to the respective instrument and assay user manuals for guidance on how to dilute your sample library so as not to exceed the detection limit of the specific assay.

While it is possible to remove aliquots of the fragmentation reaction product (i.e. after enzymatic fragmentation, but prior to End repair & A-tailing) for analysis in the integrated fragmentation/library construction workflow, it is most productive to assess the outcome of fragmentation once the entire KAPA HyperPlus workflow has been completed for the following reasons:

- It is difficult and disruptive to process low-volume aliquots in a way that is fully representative of the final library.
- Fragmentation profiles for low-input samples (1 – 10 ng into fragmentation) may not be informative, even when high-sensitivity assays are used.
- The final size distribution of libraries prepared from FFPE samples is typically smaller than expected based on the size distribution after fragmentation and adapter length. This is a common phenomenon, attributable to the inability of high-fidelity DNA polymerases used in library amplification to efficiently amplify damaged DNA, particularly templates that contain deaminated or oxidized bases.

Typical electrophoretic profiles for libraries prepared with the KAPA HyperPlus Kit are given in ***Figure 3.***

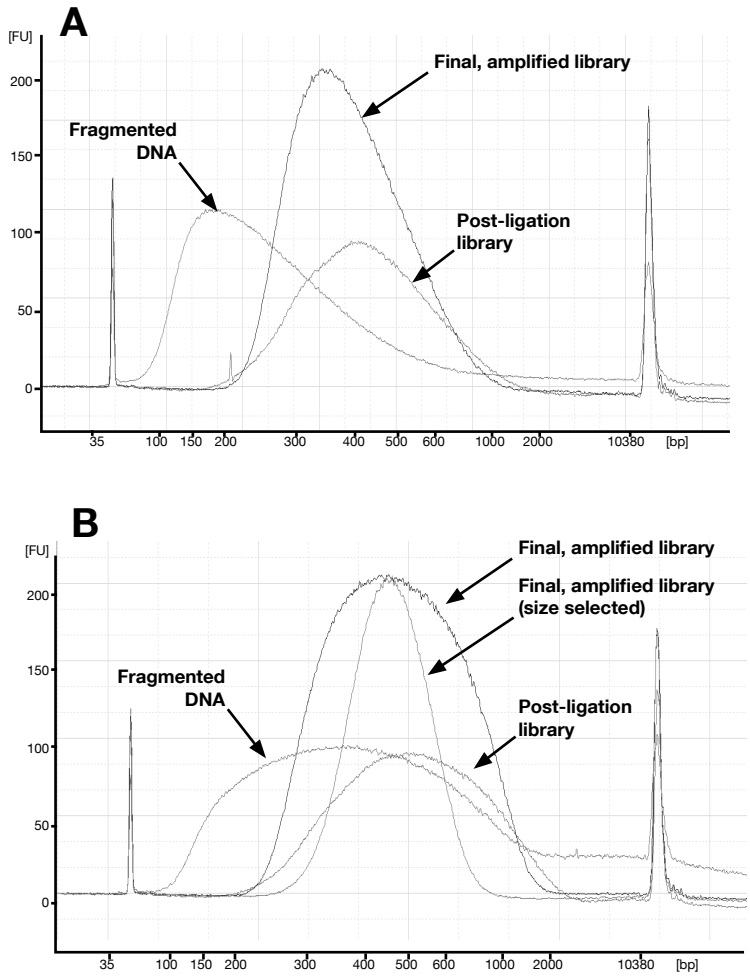


Figure 3. Examples of libraries prepared with the KAPA HyperPlus Kit
Input DNA (100 ng high-quality human genomic DNA) was fragmented at +37°C for 30 min (A) or 10 min (B), to achieve a mode fragment length of ~150 bp or ~350 bp, respectively. Libraries were prepared as described in the Library Construction Protocol (Chapter 3 & 4 of this Instructions for Use), using the recommended adapter:insert molar ratio. Larger-insert libraries (B) were prepared in duplicate. One library was subjected to double-sided size selection after the Post-ligation cleanup, as described in ***Appendix B: Size Selection***, whereas the other was not. Electropherograms were generated with a Bioanalyzer 2100 High Sensitivity DNA Kit. DNA concentrations were normalized prior to analysis and are not reflective of the actual DNA concentrations at different stages of the process. Electrophoretic profiles for fragmented DNA were generated in a "standalone" workflow whereby the protocol was terminated after fragmentation, and reaction products purified using a 2X bead-based cleanup.

Please note that libraries prepared with "forked" or Y-adapters in PCR-free workflows will appear to have a longer than expected mode fragment length, and/or may display a broad or bimodal size distribution when analyzed electrophoretically. The difference in overall appearance and fragment size distribution of an unamplified versus the corresponding amplified library varies, and depends on the adapter design and electrophoretic system used. To accurately determine the size distribution of an unamplified library, an aliquot of the library may be subjected to a few cycles of amplification prior to electrophoretic analysis, to ensure that all adapter-ligated molecules are fully double-stranded (e.g., amplify 1 µL adapter-ligated library for 4 amplification cycles followed by a bead cleanup).

Appendices

Appendix A: Optimization of Fragmentation Parameters

Optimization of Fragmentation Time

The fragmentation guidelines in ***Chapter 3, Step 1***, are a good starting point for high-quality genomic DNA.

When evaluating the KAPA HyperPlus Kit for the first time, it is recommended that you proceed as follows:

- Set up at least three replicate reactions with the desired input of a non-precious, bulk sample that is representative of the actual samples to be processed.
- Select the most appropriate fragmentation time (for the desired mode fragment length) from the table in ***Chapter 3, Step 1***. Perform one reaction with that time, and one reaction each with either a slightly shorter or slightly longer fragmentation time within the optimization range. Increments of 3 – 5 min are recommended.
- Complete the library construction process, and evaluate the size distribution of the final libraries electrophoretically.
  - If the mode fragment length is too long, increase the fragmentation time in increments of 2 – 5 min until the optimal final library distribution is achieved.
  - If the mode fragment length is too short, reduce the fragmentation time in increments of 2 – 5 min until the optimal final library distribution is achieved.
- Further fine-tuning (plus or minus 1 – 2 min) may be necessary if the fragmentation time is relatively short (10 min or less). If this is the case, consider optimizing the fragmentation temperature (see below).

A similar strategy may be employed to optimize the fragmentation time for FFPE samples, remembering that lower quality samples may benefit from slightly longer fragmentation times. For FFPE samples:

- Set up 4 – 5 replicate reactions with the desired input of a non-precious, bulk sample that is representative of the actual samples to be processed. This sample may have to be generated by pooling a few individual samples.
- Select the fragmentation time corresponding to the desired mode fragment length from the table in ***Chapter 3, Step 1*** Use that as the minimum fragmentation time, and increase the incubation time at +37°C by 5 min for each additional replicate.
- Complete the library construction process, evaluate the size distribution of the final libraries, and fine-tune fragmentation time if needed, as described above.

With respect to FFPE samples, please note the following:

- Electrophoretic profiles of FFPE samples, generated during sample QC prior to fragmentation, are not always good predictors of library and sequence quality. Samples that appear to consist of high-molecular weight DNA may not yield libraries of significantly better quality than samples that appear to be degraded.
- The mode fragment length of an amplified FFPE library is typically shorter than than expected based on the size distribution after fragmentation and adapter length. This is a common phenomenon, attributable to the inability of high-fidelity DNA polymerases used in library amplification to efficiently amplify damaged DNA, particularly templates that contain deaminated or oxidized bases. For this reason, it is not productive to try and optimize fragmentation parameters independently of the rest of the library construction process when using the KAPA HyperPlus workflow.

Optimization of Fragmentation Temperature

The standard fragmentation temperature is +37°C. If you are fragmenting high-quality genomic DNA, any other high-complexity DNA sample, or FFPE DNA to a mode fragment length <500 bp, it is unlikely that you will have to change or optimize the fragmentation temperature.

Low-complexity samples (e.g., small viral genomes, plasmids, long amplicons and cDNA) may, however, be over-fragmented at +37°C, even with short incubation times. The likelihood of over-fragmentation depends on the nature, molecular weight/length of the input DNA, the desired size distribution after fragmentation and, to a lesser degree, the DNA input into fragmentation. For example, 100 ng of a 1.8 kb PCR product will yield a similar mode fragment length (~300 bp) as 100 ng *E. coli* or human genomic DNA when fragmented at +37°C for 10 min, whereas 1 ng of a 1 kb PCR product will be fragmented to a mode size <250 bp using the same parameters.

To determine the optimal fragmentation parameters for low-complexity samples, or high-complexity samples when the desired mode fragment length is >500 bp:

- Set up four replicate reactions with a non-precious, bulk sample that is representative of the actual samples to be processed.
- Fragment two of the samples at +37°C, for 5 min and 10 min, respectively. Repeat these fragmentations with the other two samples, but at +30°C.
- Complete the library construction process, and evaluate the size distribution of the final libraries electrophoretically.
  - If the mode fragment length obtained with a 10 min incubation at +37°C is too long, continue optimizing (increasing) the fragmentation time at +37°C.
  - If the mode fragment length obtained with a 10 min incubation at +30°C is too long, but 5 min at +37°C resulted in over-fragmentation, continue optimizing (increasing) the fragmentation time at +30°C.
  - If a 5 min incubation at +30°C resulted in over-fragmentation, perform a second set of reactions (e.g., for 5 min, 10 min, 15 min, and 20 min) at +25°C, and fine-tune the fragmentation time if needed.

Appendix B. 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 KAPA HyperPlus 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.

The Ligation Buffer contains high concentrations of PEG 6000, which will interfere with efficient size selection and can affect the efficiency of other size selection techniques if not removed. If size selection is performed after ligation, it is important to perform at least one bead-based cleanup prior to performing bead- or electrophoresis-based size selection.

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.

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.

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 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 – 600 bp if fragmenting high quality DNA for ~15 minutes using KAPA Frag. The protocol will need to be modified if truncated adapters were used for library construction. Contact *Technical Support* for guidance if needed yo 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 volume	**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 volume	**80 µL**

*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. 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 10 µL 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. Do NOT allow beads to pellet.
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 of the bead pellet. The bead pellet should have a matte appearance when sufficiently dried.

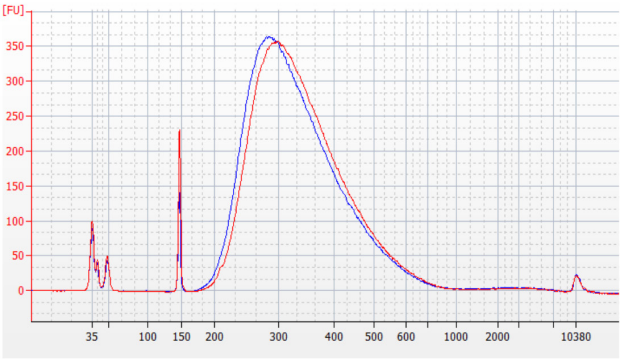
18. Remove the sample from the magnet.
19. Thoroughly resuspend the beads in 25 µL of 10 mM Tris-HCl, pH 8.0 – 8.5. Centrifuge briefly to collect all droplets. Do NOT allow beads to pellet.
20. Incubate the sample at room temperature for 2 minutes to allow the sample library to elute off the beads.
21. 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 C. Troubleshooting

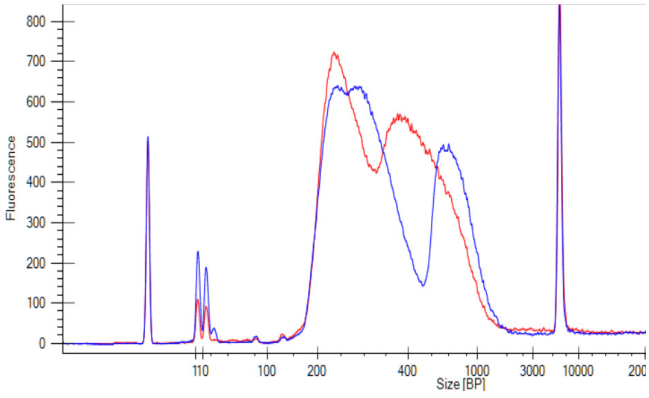
This appendix provides guidance for interpreting unexpected results and recommendations for implementing corrective action if problems occur. For technical questions, contact your local Roche Technical Support.
Go to **sequencing.roche.com/support** for contact information.

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

Observation	Cause(s) / Recommendation(s)
Low library yields	Input DNA was not quantified correctly. If less DNA is added to the reaction, expected yields will be impacted.
	Low quality DNA (typically with a DIN of <7) may require additional workflow modifications compared to intact DNA, such as increasing the input into library construction.
	Adapter quality was compromised. Ensure high quality adapters are used with minimal freeze/thaw cycles (F/T). E.g ., KAPA UDI Adapters are stable up to 10 F/T cycles.
	Adapter concentration too low. Ensure the correct adapter concentration is used.
	Improper bead cleanup practises followed. Ensure best practices are used during bead cleanups. ▪ Equilibrate KAPA HyperPure Beads to room temperature prior to use. ▪ 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.
	Double-sided size selection performed. Size selection will result in significant sample loss and should only be performed if absolutely necessary.
	Ensure that the KAPA HyperPlus End Repair & A-Tailing Enzyme Mix (orange lid) is NOT used in the KAPA HyperPrep Workflow. The KAPA HyperPlus End Repair & A-Tailing Enzyme Mix is NOT COMPATIBLE with mechanically fragmented (e.g., Covaris-sheared) input DNA used in KAPA HyperPrep workflows and usage thereof will result in library preparation failure.
	Libraries stored incorrectly. Libraries may degrade over time if stored incorrectly.

Fragment distribution (analyzed using an Agilent Bioanalyzer 2100 High Sensitivity DNA assay displays a sharp peak at ~150 bp (adapter-dimer) Generally, another 0.8X or 1X bead cleanup will remove the adapter-dimer contamination. A second cleanup is recommended if the adapter-dimer contamination is >5% of the total library concentration. The percentage adapter-dimer can be calculated by performing a smear analysis using e.g., the Bioanalyzer software. Adapter-dimers are to be avoided at all cost if sequencing on patterned flow cells such as those utilized by the Illumina NovaSeq and HiSeq X.  	Input DNA was not quantified correctly and the incorrect adapter concentration used as a result.
	Degraded or FFPET-derived DNA may not support efficient ligation, resulting in adapter-dimer formation. Reduce the adapter concentration for degraded DNA. Titrate until a compromise is achieved between yield and adapter-dimer carryover.
	Adapter quality was compromised. Ensure high quality adapters are used with minimal freeze/thaw cycles (F/T). E.g KAPA UDI Adapters are stable up to 10 F/T cycles.
	Molar ratio of adapter:insert will affect adapter-dimer formation. If the concentration of adapter is too high it may result in adapter-dimer carryover.
	Improper bead cleanup practises followed. Ensure best practises are used during bead cleanups. ▪ KAPA HyperPure Beads need to be equilibrated to room temperature prior to use. ▪ 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 overdry beads.
	Incorrect bead:sample ratio used. This will result in retention of adapter dimers instead of removal.

Fragment distribution (analyzed using a LabChip GX Touch and NGS 3K Assay) is bimodal, with a larger set of fragments observed in addition to the expected set of fragments



A. A PCR-free library was subjected to electrophoretic analysis.

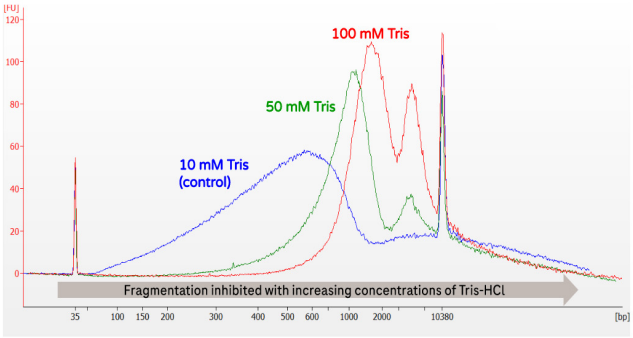
In PCR-free workflows it is difficult to obtain accurate average fragment sizes from electrophoretic systems, as molecules flanked by adapters with long single-stranded terminals migrate anomalously in gel matrices, thereby appearing to be longer than they truly are. Easy workarounds for this problem include the following:

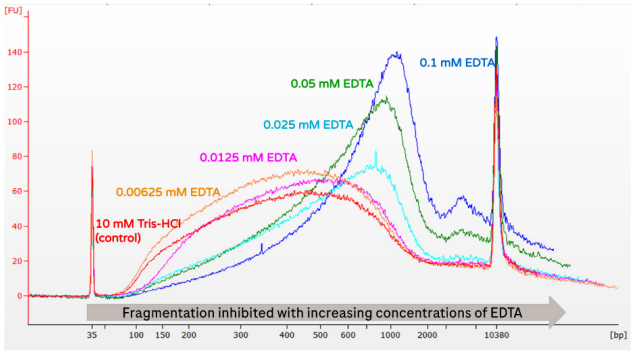
- Use the average length of the fragmented DNA plus the total length of the two adapters (usually ~140 bp) as an estimate for the average library fragment size in concentration calculations. This approach is only feasible if no size selection was performed, or if the size selection parameters were optimized to preserve the size distribution of the fragmented DNA.
- Amplify a small aliquot of the PCR-free library for 2 – 5 cycles (followed by a 1X bead cleanup) prior to electrophoretic analysis. Amplification will render all molecules fully double-stranded, and yield a reliable size determination from the electrophoretic assay.

B. Primer depletion

Primer depletion due to overamplification of the sample library relative to the amount of primers available in the reaction results in single stranded amplification products. These products can anneal to each other via adapter homology on both ends of the fragments to form heteroduplexes, and migrate as larger products on a fragment analyzer than their actual length in base pairs. The artifact can be resolved by increasing primer concentration or reducing cycle number in the PCR reaction, however the products themselves are perfectly acceptable for use in sequence capture and sequencing.

Fragment distribution shows that the average amplified fragment is not within the expected size range and high molecular artefacts are visible.





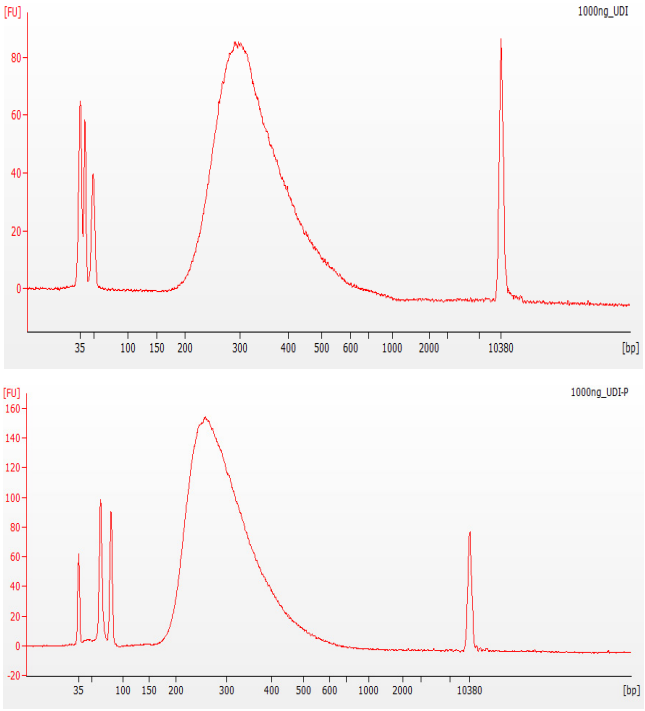
The enzymatic fragmentation reaction is very sensitive to the presence of the following inhibitors:

1) Sodium Azide
A preservative present in e.g., Buffer ATE (Qiagen). Inhibition is rescued/mitigated with addition of Conditioning Solution (provided in kit). Please contact your local Roche Technical Support for optimization guidelines.

2) Buffer EB (Qiagen)
Delayed fragmentation of DNA eluted in Qiagen EB has been observed. If DNA is already in EB, we would recommend performing a 3X bead cleanup and then elute in 10 mM Tris-HCl (pH 8.0 – 8.5).

3) High Tris-HCl (salts)
Fragmentation is delayed with increasing concentrations of Tris-HCl (>10 mM). Inhibition is rescued/mitigated with addition of Conditioning Solution (provided in kit). Please contact your local Roche Technical Support for optimization guidelines.

4) EDTA
From blood collection tubes and or DNA extraction elution buffers.
The effect of final EDTA concentrations less than 0.02 mM on the enzymatic activity of KAPA Frag is deemed negligible. As a result, it is not necessary to add Conditioning Solution to the fragmentation reaction where final EDTA concentrations are <0.02 mM.

Fragment distribution (analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip) displays sharp peaks at 44 & 45 bp or ~ 47 & 49 bp (primer carryover)  **Top image:** primer carryover for libraries constructed using KAPA UDI Adapter and amplified with KAPA Library Amplification Primer Mix. **Bottom image:** indexed primer carryover for libraries constructed using KAPA Universal Adapter and amplified using KAPA UDI Primer Mixes.	Primer carryover is typically seen in amplified libraries and not completely removed by the post-amplification cleanup. Carryover of standard KAPA Library Amplification Primers (used to amplify libraries constructed using full length, KAPA UDI Adapter) will not interfere with sequencing, and may be safely ignored. However, when using KAPA UDI Primer Mixes (in conjunction with KAPA Universal Adapter), carryover indexed primers may exacerbate index hopping on patterned flow-cells (e.g., Illumina NovaSeq, HiSeq X instruments). Generally another 1X bead cleanup after amplification will remove the primer carryover and is highly recommended when using KAPA UDI Primer Mixes.

Appendix D. Limited Warranty

1. Limited Warranty

A. Products: Roche Sequencing Solutions, Inc. (“Roche”) warrants that its Products conform to its published specifications and are free from defects in material or workmanship. Customer’s sole and exclusive remedy (and Roche’s sole and exclusive liability) under this limited warranty shall be to either (a) replace the defective Products, or (b) provide Customer with a refund, as solely determined by Roche.

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Evidence of original purchase is required. It is important to save your sales receipt or packaging slip to verify purchase.

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