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

KAPA HyperCap FFPET DNA Workflow v1.1

Instructions For Use with
– KAPA HyperChoice,
– KAPA HyperExplore,
– KAPA HyperCap Oncology Panel

Contents

The package contains:

Component	Description
KAPA HyperCap Target Enrichment Probes	Biotinylated capture oligos designed against target regions in the genome.

Storage and Stability

- KAPA HyperCap Target Enrichement Probes are provided lyophilized and will need to be resuspended prior to first use, with the KAPA Probes Resuspension Buffer (sold separately). It is recommended to aliquot the probes into single-use volumes and freeze at –15°C to –25°C.
- KAPA HyperCap Target Enrichment Probes are stable at –15°C to –25°C until the expiration date printed on the label, lyophilized or resuspended.

Application

KAPA HyperChoice products enable solution-phase targeted enrichment of customer-defined regions of the human genome. Proprietary design algorithms improve capture uniformity and reduce the amount of sequencing needed to efficiently identify sequence variants. KAPA HyperChoice Probes are intended for capture of human target regions up to 200 Mb. KAPA HyperExplore is intended for capture of up to 200 Mb of non-human genomic targets, non-standard (including repetitive and mitochondrial) human sequences or when the user defines the probe replication in the target regions.

Refer to the protocol below for the target size ranges that this protocol is validated with. Refer to design-specific documentation and design files for more details on specific designs at *www.hyperdesign.com*.

Warnings and Precaution

- Wear the appropriate personal protective equipment, such as gloves, lab coat and safety glasses, to avoid direct contact while handling the reagents.
- Use good laboratory practices to avoid contamination when using 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.

Changes to Previous Version

Formatting changes.

Ordering Information

For a complete overview of Roche Sequencing products, including those used in the KAPA HyperCap FFPET DNA Workflow v1.1, go to *sequencing.roche.com/products*.

Notice to Purchaser

1. **Limited Warranty**
2. **Further Liability Limitation**

Trademarks

Contact and Support

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

Copyright

Roche Sequencing Solutions, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588 USA

Editions

Version 1.0, July 2021; Version 1.1, September 2021

Restrictions and Liabilities

This document is provided “as is” and Roche Sequencing Solutions, Inc. (Roche) assumes no responsibility for any typographical, technical, or other inaccuracies in this document. Roche reserves the right to periodically change information that is contained in this document; however, Roche makes no commitment to provide any such changes, updates, enhancements, or other additions to this document to you in a timely manner or at all.

OTHER THAN THE LIMITED WARRANTY CONTAINED IN THIS INSTRUCTIONS FOR USE, ROCHE MAKES NO REPRESENTATIONS, WARRANTIES, CONDITIONS OR COVENANTS, EITHER EXPRESS OR IMPLIED (INCLUDING WITHOUT LIMITATION, ANY EXPRESS OR IMPLIED WARRANTIES OR CONDITIONS OF FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, MERCHANTABILITY, DURABILITY, TITLE, OR RELATED TO THE PERFORMANCE OR NON-PERFORMANCE OF ANY PRODUCT REFERENCED HEREIN OR PERFORMANCE OF ANY SERVICES REFERENCED HEREIN).

This document might contain references to third party sources of information, hardware or software, products, or services and/or third party web sites (collectively the “Third-Party Information”). Roche does not control, and is not responsible for, any Third-Party Information, including, without limitation the content, accuracy, copyright compliance, compatibility, performance, trustworthiness, legality, decency, links, or any other aspect of Third-Party Information. The inclusion of Third-Party Information in this document does not imply endorsement by Roche of the Third-Party Information or the third party in any way.

Roche does not in any way guarantee or represent that you will obtain satisfactory results from using Roche products as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of Roche products.

Roche is not responsible nor will be liable in any way for your use of any software or equipment that is not supplied by Roche in connection with your use of Roche products.

Conditions of Use

You are responsible for understanding and performing the protocols described within. Roche does not guarantee any results you may achieve. These protocols are provided as Roche s recommendations based on its use and experience with Roche products.

User Restrictions

For patent license limitations for individual products, refer to: *www.technical-support.roche.com*.

Table of Contents

Table of Contents

Preface

Regulatory Disclaimer

For Research Use Only.

Not for use in diagnostic procedures.

KAPA HyperCap Target Enrichment Probes

KAPA HyperCap Target Enrichment Probes is a solution-based capture reagent that enables enrichment of the whole exome or customer-defined regions of interest in a single tube. Throughout this document, 'KAPA HyperCap Target Enrichment Probes' refers to KAPA HyperExome, KAPA HyperChoice, KAPA HyperExplore, KAPA HyperCap Heredity, and KAPA HyperCap Oncology products.

Contact Information

Roche Technical Support

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

Manufacturer and Distribution

Manufacturer	Roche Sequencing Solutions, Inc. Santa Clara, CA USA
Distribution	Roche Diagnostics GmbH Mannheim, Germany
Distribution in USA	Roche Diagnostics Corporation Indianapolis, IN USA

Conventions Used in This Manual

Symbols

Symbol	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	Identifies a resource in a different area of this manual or on a web site.
Italic type	Identifies the names of dialog boxes, windows, tabs, panels, views, or message boxes in the software.
Bold type	Identifies names of menus and controls (buttons, checkboxes, *etc.*) in the software.

Chapter 1. Before You Begin

This Instructions for Use document describes the process for enrichment of Formalin Fixed Paraffin Embedded Tissue (FFPET) DNA sample libraries using KAPA HyperCap Target Enrichment Probes and the amplification of these sample libraries by ligation-mediated PCR. Specifically, these Instructions for Use provides a protocol for the workflow outlined in *Figure 1* using the KAPA HyperPlus Kit. Modification of certain workflow steps may be appropriate for individual experimental needs. The following protocol provides instructions that have been tested for capture target sizes between 90 kb and 300 kb. The capture target size can be found in the coverage_summary.txt design deliverable file. For larger capture target sizes additional optimization and sequencing may be needed. The output of this protocol consists of enriched gDNA fragments that can be sequenced directly using an Illumina sequencing instrument.

The KAPA Target Enrichment portfolio supported from the KAPA HyperCap FFPET DNA Workflow v1.1 provides:

- An easy to use streamlined and automation friendly workflow with minimal resource requirements:
  - Moderate hybridization and wash temperatures
  - Independent of a vacuum concentrator
  - Streamlined washes
- Single vendor service and support for NGS sample preparation:
  - KAPA HyperPlus Kits
  - KAPA Universal UMI Adapter
  - KAPA UDI Primer Mixes 1-384
  - KAPA HyperPure Beads
  - KAPA HyperCapture Reagent and Bead Kits
  - KAPA HyperCap Target Enrichment Probes
- Catalog off-the-shelf, as well as customizable content through the HyperDesign Tool, an on-line custom design interface and a team of expert designers.

This specific version of the workflow has optimizations designed for limited input oncology samples, specifically FFPET DNA samples. For the FFPET workflow, optimizations were targeted toward ensuring sufficient coverage to enable somatic variant calling across the panel regions for a wide range of sample quality and enabling error removal using unique molecular indexes (UMIs).

Overview of the KAPA HyperCap FFPET DNA Workflow v1.1

The KAPA HyperCap FFPET DNA Workflow v1.1 (*Figure 1*) involves:

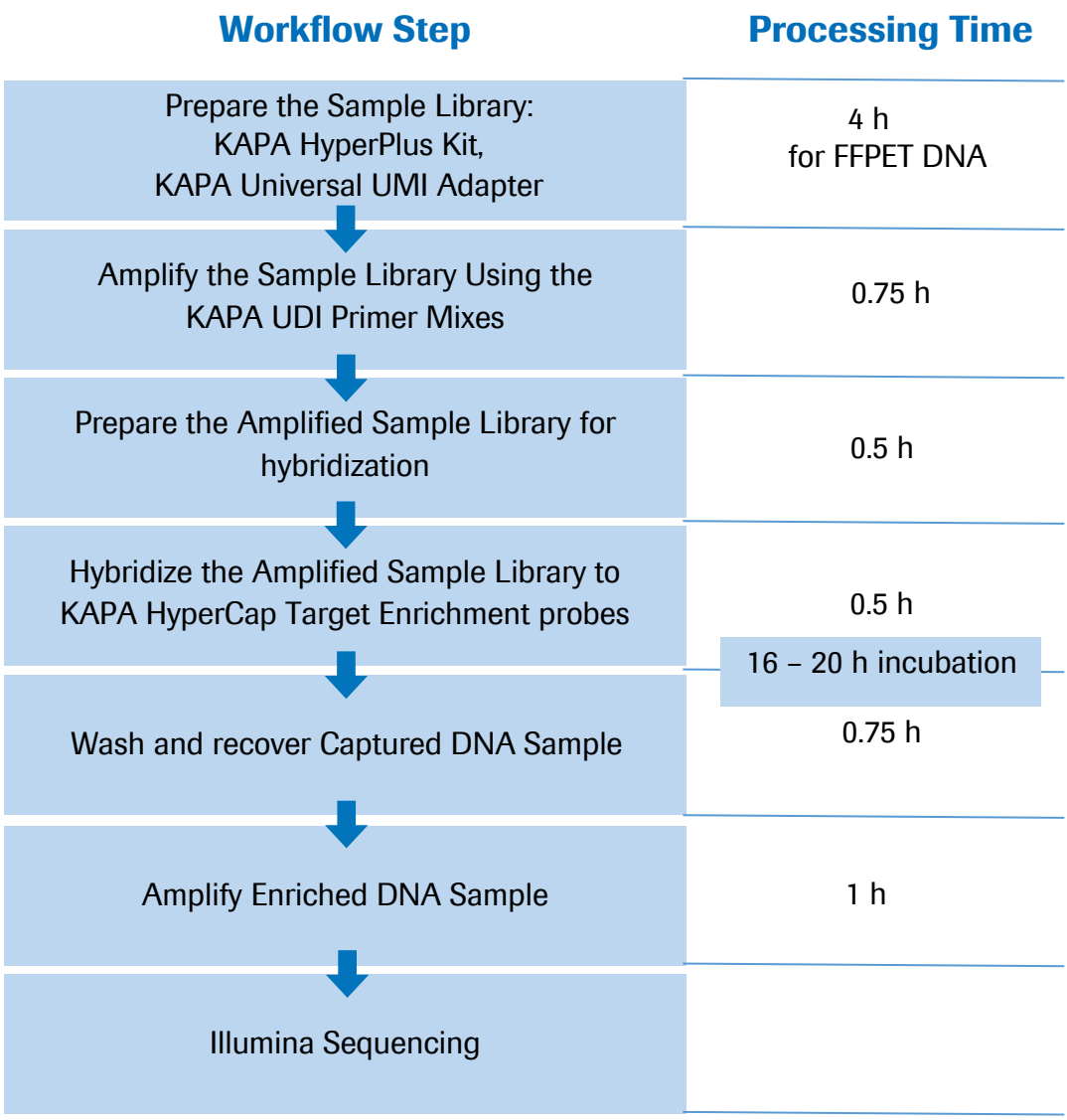


Figure 1: KAPA HyperCap FFPET DNA Workflow v1.1, with enzymatic DNA fragmentation. Where applicable, incubation times are indicated between steps. Processing time may vary based on the number of samples processed.

Protocol Information & Safety

- Wear gloves, lab coat, safety glasses and take precautions to avoid sample contamination.
- Perform all centrifugations at room temperature (+15°C to +25°C) unless indicated otherwise.
- Unless otherwise specified, all mixing steps are listed as 'mix thoroughly' and indicate that mixing should be performed by either vortexing for 10 seconds or pipetting up and down 10 times.
- If liquid has collected in a tube's cap after mixing, gently tap or briefly spin the sample to collect the liquid into the tube's bottom, ensuring that the mixture remains homogeneous before progressing to the next step.
- It is recommended to perform thermocycler incubations using a thermocycler with a programmable heated lid set to the provided temperature.

Terminology

Target Enrichment (or Capture): The process of enriching targeted regions from genomic DNA. In the context of this document, the hybridization of the Amplified Sample Library to the KAPA HyperCap Target Enrichment Probes, and subsequent washing steps.

KAPA HyperCap Target Enrichment Probes: The complete set of biotinylated 120 bp oligonucleotide probes (KAPA HyperExome, KAPA HyperCap Fixed Panels, KAPA HyperChoice or HyperExplore) provided by Roche for target enrichment.

Sample Library: The initial shotgun library generated from FFPET DNA by fragmentation and ligation of sequencing platform-specific adapters. In the context of this document, it is the sample library before amplification by PCR and before capture.

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

UDI Primer Mixes: Unique Dual-Indexed Primer Mixes.

UMI Adapter: Universal sample adapter containing a double stranded Unique Molecular Index (UMI).

Prepare the Following Equipment and Reagents

- Thermocyclers should be programmed with the following:
  - DNA Polishing program and Fragmentation program (*Chapter 3.* Prepare the Sample Library, Step 1.4)
  - End Repair and A-tailing program (*Chapter 3.* Prepare the Sample Library, Step 1.6.d)
  - Adapter Ligation program (*Chapter 3.* Prepare the Sample Library, Step 1.7.e)
  - Pre-Capture PCR program (*Chapter 4.* Amplify the Sample Library Using the KAPA UDI Primer Mixes, Step 2.1)
  - Hybridization incubation program (*Chapter 5.* Hybridize the Sample to KAPA HyperCap Target Enrichment Probes , Step 1.22)
  - Post-Capture PCR program (*Chapter 7*, Step 3.1)

It is recommended to use a thermocycler with a programmable heated lid. For guidance on lid temperatures follow the recommended lid temperatures in these Instruction For Use. If further guidance is needed, contact *Roche Technical Support*.

- The following steps should be taken before beginning the workflow:
  - Resuspend and aliquot the KAPA HyperCap Target Enrichment Probes (*Chapter 2.* Prepare and store the KAPA HyperCap Reagents, Step 2)
  - Preparation of the KAPA UDI Primer Mixes (*Chapter 2*. Prepare and store the KAPA HyperCap Reagents, Step 3)
  - Resuspend Post-Capture PCR Oligos (*Chapter 7.* Amplify Enriched DNA Sample, Step 1)

To verify you are using the most up-to-date version of these *Instructions For Use* to process your captures, go to *sequencing.roche.com/support.html*.

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	Catalog No.
DynaMag-2 Magnet (16 x 2.0 mL tube holder) (optional)	Thermo Fisher	12321D
DynaMag-96 Side Magnet 0.2 mL PCR Strip Magnetic Separator Rack	Thermo Fisher Permagen	12331D MSR812
Microcentrifuge (16,000 x *g* capability)	*Multiple Vendors*	
Qubit	ThermoFisher	Multiple models
Bioanalyzer 2100	Agilent	
Thermocycler (capable of maintaining +55°C for 16 - 20 hours), with programmable heated lid	*Multiple Vendors*	
Vortex mixer	*Multiple Vendors*	
Plate Centrifuge which can reach at least 280 x *g*	*Multiple Vendors*	

Consumables Available from Roche

Component	Package Size/Contents	Catalog No.
KAPA Universal UMI Adapter	96 reactions 384 reactions*	09 329 862 001 09 329 889 001
KAPA UDI Primer Mixes, 1-96 KAPA UDI Primer Mixes, 97-192 KAPA UDI Primer Mixes, 193-288 KAPA UDI Primer Mixes, 289-384	96 reactions 96 reactions 96 reactions 96 reactions	09 134 336 001 09 329 838 001 09 329 846 001 09 329 854 001
KAPA HyperCapture Bead Kit	24 reactions 96 reactions 384 reactions*	09 075 780 001 09 075 798 001 09 075 909 001
KAPA HyperCapture Reagent Kit	24 reactions 96 reactions 384 reactions*	09 075 810 001 09 075 828 001 09 075 917 001
KAPA HyperPlus Kit	8 reactions 24 reactions 96 reactions	07 962 380 001 07 962 401 001 07 962 428 001
KAPA Universal Enhancing Oligos	24 reactions 96 reactions 384 reactions*	09 075 836 001 09 075 852 001 09 075 895 001
KAPA HyperPure Beads	5 mL 30 mL 60 mL 4 x 60 mL 450 mL	08 963 835 001 08 963 843 001 08 963 851 001 08 963 878 001 08 963 860 001
KAPA Probes Resuspension Buffer	1 mL 5 mL	09 075 879 001 09 075 887 001
LightCycler® Uracil-DNA Glycosylase	50 µL	03 539 806 001
KAPA HyperCap Target Enrichment Probes	Multiple	Multiple, see *Appendix C*

*** Virtual kits, consist of 4 x 96 reaction kits.**

Consumables Purchased from Other Vendors

Component	Supplier	Package Size	Catalog No.
Agilent DNA 1000 Kit (optional)	Agilent	1 kit	5067-1504
Agilent High Sensitivity DNA Kit (recommended)	Agilent	1 kit	5067-4626
Ethanol, 200 proof (absolute), for molecular biology	Sigma-Aldrich	500 mL	E7023-500ML
Qubit dsDNA HS Assay Kit	ThermoFisher	1 kit	Q32851
Qubit Assay Tubes	ThermoFisher	1 package of 500 tubes	Q32856
Tubes: § 0.2 mL PCR tubes or strip-tubes, (preferably low-bind) § 1.5 mL microcentrifuge tubes (optional)	*Multiple Vendors*		
Water, PCR Grade	Sigma-Aldrich	1 x 25 mL 25 x 1 mL 4 x 25 mL	3315959001 3315932001 3315843001

Use nuclease-free, PCR Grade water for all described protocol steps. Working with a liquid handler system may require excess volumes from various reagents.

Chapter 2. Prepare and store the KAPA HyperCap Reagents

This chapter describes the storage conditions for the following kits:

- KAPA HyperCapture Bead Kit
- KAPA HyperPure Beads
- KAPA HyperCapture Reagent Kit
- KAPA Universal UMI Adapter
- KAPA UDI Primer Mix
- KAPA Probes Resuspension Buffer
- KAPA Probes Resuspension Buffer
- KAPA HyperPlus Kit

Step 1. Store the Reagent Kits

Reagent Kit	Storage Temperature
KAPA HyperCapture Bead Kit	+2°C to +8°C
KAPA HyperPure Beads	+2°C to +8°C
KAPA HyperCapture Reagent Kit	-15°C to -25°C
KAPA Universal UMI Adapter	-15°C to -25°C
KAPA UDI Primer Mixes or KAPA UDI Primer Mixes (resuspended)	+2°C to +8°C -15°C to -25°C
KAPA Probes Resuspension Buffer	-15°C to -25°C
KAPA HyperCap Target Enrichment Probes or KAPA HyperCap Target Enrichment Probes (resuspended)	-15°C to -25°C
KAPA HyperPlus Kit	-15°C to -25°C

The KAPA HyperCapture Bead Kit must not be frozen.

Step 2. Resuspend and Aliquot the KAPA HyperCap Target Enrichment Probes

Store the KAPA HyperCap Target Enrichment Probes in their lyophilized form at -15°C to -25°C until ready to use. Before first use of the KAPA HyperCap Target Enrichment Probes, undertake the following steps to ensure the highest performance and to avoid multiple freeze/thaw cycles or potential accidental contamination:

1. Review the KAPA HyperCap Target Enrichment Probes tube label to verify the probe reaction number and resuspension volume.
2. Spin the KAPA HyperCap Target Enrichment Probes tube 10,000 x *g* for 30 seconds to ensure the contents are at the bottom of the tube.

3. Add the recommended volume of KAPA Probes Resuspension Buffer provided on the tube label to the KAPA HyperCap Target Enrichment Probes tube.
4. Vortex the tube for 1 minute to resuspend the probe pool.
5. Centrifuge the tube at 10,000 x *g* for 30 seconds to ensure that the liquid is at the bottom of the tube before removing the cap.
6. Aliquot the KAPA HyperCap Target Enrichment Probes into single-use aliquots (4 µL/capture) in 0.2 mL PCR tubes and store at -15°C to -25°C until use. The presence of some residual volume after dispensing all single-use aliquots is normal.
7. When ready to perform the experiment, thaw the required number of single-use probe pool aliquots on ice.

The KAPA HyperCap Target Enrichment Probes should not undergo freeze/thaw cycles after aliquoting. Ensure that you properly label and record the expiration date of the aliquoted probes.

Step 3: Preparation of the KAPA UDI Primer Mixes

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

Take out the sample primer plate from storage (+2°C to 8°C), and spin down plate for 1 minute at 280 x *g* to ensure that the contents are at the bottom of the wells.

1. Before removing the foil cover, 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*.
2. Carefully remove the foil cover on the plate ensuring to avoid cross contamination. Discard the original foil cover.
3. Using a multichannel pipette, add 10 µL of 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.

4. Ensure every well contains 10 µL of 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.

5. Use a roller or appropriate tool to ensure the foil seal is evenly applied.
6. Spin the plate at 280 x *g* for 30 seconds to ensure the dispensed 10 µL is at the bottom of the well.
7. 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.

8. Spin the plate at 280 x *g* for 1 minute to ensure the contents are collected at the bottom of the wells.
9. The KAPA UDI Primer Mixes plate is now ready for use in the Pre-Capture PCR step.
10. Store any unused but already resuspended KAPA UDI Primer Mixes at -15°C to -25°C. To avoid repeated freeze/thaw cycles of the primer plate, you may transfer the resuspended primers to separate tubes or tube strips for storage.

Ensure aliquoted KAPA UDI Primer Mixes are correctly labeled.

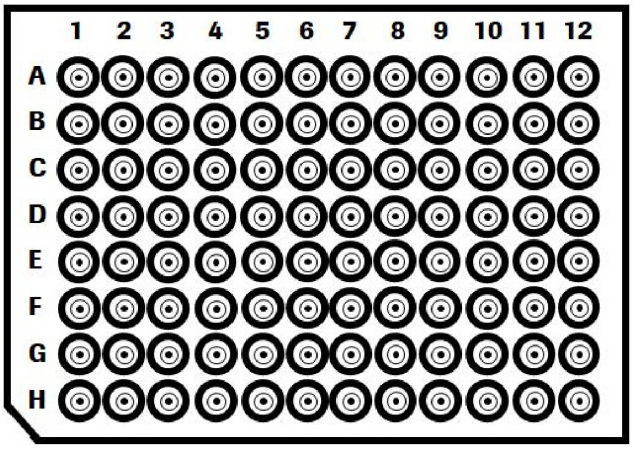


Figure 2: KAPA UDI Primer Mixes Plate layout.

Chapter 3. Prepare the Sample Library

Chapter 3 describes the sample library preparation. The KAPA HyperPlus Kit is used for enzymatically fragmenting gDNA and DNA extracted from Formalin-Fixed Paraffin-Embedded Tissue (FFPET). This chapter requires use of components from the following kits:

- KAPA HyperPlus Kit
- LightCycler® Uracil-DNA Glycosylase (referred to as UNG Enzyme in this document)
- KAPA Universal UMI Adapter
- KAPA UDI Primer Mixes
- KAPA HyperPure Beads

Ensure that the following are available:

- PCR Grade water
- Freshly-prepared 80% ethanol

References

- *Thermocycler Manual*

Sample Requirements

Workflow performance was confirmed with 300 ng of DNA extracted from FFPET for sample library preparation. Successful sequencing libraries were made for these inputs, though results will depend on input mass and sample quality. Lower input amounts may be possible but may not yield equivalent results. For guidance on lower input amounts or sample quality, contact *Roche Technical Support*.

Step 1. Prepare the Sample Library

The sample library should be prepared using the following kit:

- KAPA HyperPlus Kit

Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.

When assembling a master mix for processing multiple samples, always prepare an appropriate excess (5% to 10% for manual workflows and ~20% for automated liquid handling).

The fragmentation parameters in these Instructions For Use are provided as a starting point and may not result in the optimal size distribution for libraries prepared for your specific DNA samples. Fragment the DNA so that the average DNA fragment size for high quality DNA is 180 – 220 bp. Lower quality FFPET samples are expected to have shorter average DNA fragment size.

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

Refer to the *KAPA HyperPlus Kit Technical Data Sheet* for further guidance on fragmentation optimization and EDTA removal from samples.

1. Dilute 300 ng of FFPET extracted DNA with PCR Grade water to a total volume of 30.5 µL into a 0.2 mL tube or well of PCR plate.

The recommended input amount for FFPET library preparation is 300 ng of DNA if the quality of the sample is unknown. If the DNA is known to be high quality (e.g. control cell line DNA), 50 ng of DNA input is recommended.

2. Prepare the DNA Polishing master mix on ice:

DNA Polishing Master Mix	Per Individual Sample Library
UNG Enzyme (2U/µL)	1 µL
KAPA Frag Buffer (10x)	3.5 µL
Total	**4.5 µL**

3. Add 4.5 µL of the DNA Polishing master mix to each sample, for a total volume of 35 µL. Mix thoroughly and perform a quick spin. Proceed immediately to the next step.
4. Incubate in a thermocycler, pre-cooled to 4°C and programmed as outlined below. A heated lid is not required for this step. If used, set the lid temperature to ≤ 50°C:
    1. Pre-cool block: +4°C
    2. 30 minutes at +37°C
    3. Hold: +4°C
5. Transfer reaction to ice and proceed immediately to the next step.
6. Perform Fragmentation Reaction as follows:
    a. Prepare a master mix of the following reagents on ice:

Fragmentation Master Mix	Per Individual Sample Library
PCR Grade Water	3.5 µL
KAPA Frag Buffer (10x)	1.5 µL
KAPA Frag Enzyme	10 µL
Total	**15 µL**

Note that the volume of the buffer is less than the volume of the enzyme in this reaction. The Fragmentation Master Mix is highly viscous and additional excess should be made.

    b. Add 15 µL of the fragmentation master mix to each sample. Total volume should be 50 µL. Mix thoroughly and perform a quick spin.

When handling multiple strip tubes, it is recommended to add the master mix to the side of the tubes and not directly into the samples. Once all the master mix has been dispensed, close the caps, vortex the strips quickly, and spin down briefly. This starts the fragmentation reaction relatively at the same time for all samples.

c. Return the plate/tube(s) to ice. Proceed immediately to the next step.

d. Incubate in a thermocycler, pre-cooled to 4°C and programmed as outlined below. A heated lid is not required for this step. If used, set the lid temperature to ≤ 50°C:

1. Pre-cool block: +4°C
2. 30 minutes at +37°C
3. Hold: +4°C

7. Transfer reaction to ice and proceed immediately to the next step.

8. Perform End Repair and A-Tailing Reaction as follows:

a. Prepare a master mix of the following reagents on ice:

End Repair and A-Tailing Master Mix	Per Individual Sample Library
KAPA HyperPrep End Repair & A-Tailing Buffer	7 µL
KAPA Hyper**Plus** End Repair & A-Tailing Enzyme Mix (orange cap color)	3 µL
Total	**10 µL**

Ensure the HyperPlus End Repair & A-Tailing Enzyme Mix is used for best performance.

b. Add 10 µL of End Repair and A-Tailing Master Mix to the fragmented DNA sample to obtain a total volume of 60 µL.

c. Mix the End Repair and A-Tailing reaction thoroughly and perform a quick spin.

d. Place on ice and immediately proceed to the next step.

e. Perform the End Repair and A-Tailing incubation in a thermocycler using the following program with the lid temperature set to +85°C:

1. Step 1: 30 minutes at +65°C
2. Step 2: Hold at +4°C

f. Following the 30 minutes incubation, proceed immediately to the next step.

9. Proceed with the Adapter Ligation Reaction:

a. Prepare a master mix of the following reagents:

Ligation Master Mix	Per Individual Sample Library
KAPA Ligation Buffer	30 µL
KAPA DNA Ligase	10 µL
Total	**40 µL**

The KAPA Ligation Buffer is very viscous and requires special attention during pipetting and additional excess of the Ligation Master Mix may be required.

b. Add 10 µL of KAPA Universal UMI Adapter to samples from the previous step. Mix the reaction thoroughly and perform a quick spin.

The KAPA Universal UMI Adapter must be added to each sample individually prior to addition of the Ligation Master Mix. Addition of the adapter to the Ligation Master Mix may cause formation of adapter dimers.

The Ligation Master Mix is very viscous and requires special attention during pipetting.

c. Add 40 µL of the Ligation Master Mix to each sample and KAPA Universal UMI Adapter, resulting in a total volume of 110 µL.

d. Mix the Ligation Reaction thoroughly and perform a quick spin.

e. Incubate the Ligation Reaction at +20°C for 2 hours with lid temperature set to +50°C.

f. Following the incubation, proceed immediately to the next step.

10. Perform the Post-Ligation Cleanup as follows:

a. To each Ligation Reaction, add 110 µL room temperature KAPA HyperPure Beads that have been thoroughly resuspended.

Post-Ligation Cleanup	Per Individual Sample Library
Ligation Reaction	110 µL
KAPA HyperPure Beads	110 µL
Total	**220 µL**

b. Mix the Ligation Reaction and KAPA HyperPure Beads thoroughly and perform a quick spin.

It is important at this step to ensure that the solution is thoroughly mixed and appears homogeneous. Insufficient mixing may compromise recovery and size selection.

The total volume will be 220 µL in each tube, take care to avoid spillover during mixing.

c. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.

d. Place the tube(s) on a magnet to capture the beads. Incubate at room temperature for 5 minutes until the solution is clear.

e. Carefully remove and discard the supernatant.

f. Keeping the tube(s) on the magnet add 200 µL of freshly-prepared 80% ethanol.

g. Incubate at room temperature for ≥30 seconds.

h. Carefully remove and discard the ethanol.

i. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.

j. Incubate at room temperature for ≥30 seconds.

k. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

l. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate, ~ 3 minutes.

Caution: Over-drying the beads may result in dramatic yield loss.

m. Remove the tube(s) from the magnet.

n. Thoroughly resuspend the beads in 20 µL of PCR Grade water.

o. Incubate at room temperature for 2 minutes to allow the DNA to elute off the beads.

p. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.

q. Transfer 20 µL of supernatant to a fresh tube/well.

r. Proceed immediately to *Chapter 4*. Amplify the Sample Library Using the KAPA UDI Primer Mixes.

Sample indexes are incorporated in the Pre-Capture Amplification step.
Precautions should be taken to avoid sample cross contamination.

Chapter 4. Amplify the Sample Library Using the KAPA UDI Primer Mixes

This chapter describes how to amplify the sample library (prepared in *Chapter 3.* Prepare the Sample Library) using the KAPA UDI Primer Mixes in preparation for hybridization to the KAPA HyperCap Target Enrichment Probes. This chapter requires the use of the components from the following kits:

- KAPA HyperPlus Kit
- KAPA UDI Primer Mixes
- KAPA HyperPure Beads

Ensure that the following is available:

- Freshly-prepared 80% ethanol
- PCR Grade water

References

- *Thermocycler Manual*
- *Qubit Fluorometer Manual*
- *Qubit dsDNA HS Assay Kit Guide*
- *Agilent 2100 Bioanalyzer Instrument Manual*
- *Agilent Bioanalyzer DNA Kits Guide*

Step 1. Prepare the Pre-Capture PCR Reaction

We recommend the inclusion of negative (water) and positive (previously amplified library) controls in the Pre-Capture PCR step.

For guidance on sample multiplexing, refer to the *KAPA UDI Primer Mixes Instructions for Use*.

Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.

Ensure to record the well position of the KAPA UDI Primer Mix used for each sample.

1. Retrieve and thaw the KAPA UDI Primer Mixes plate prepared in *Chapter 2,* Step 3.
2. Spin the plate at 280 x *g* for 30 seconds to collect the contents to the bottom of the wells.
3. Peel off or pierce the foil seal for the appropriate number of wells needed.

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

4. Add 5 µL of a KAPA UDI Primer Mix to each individual Sample Library.
5. Add 25 µL of KAPA HiFi HotStart ReadyMix to each mixture of Sample Library and KAPA UDI Primer Mix.
6. Mix thoroughly and perform a quick spin. Immediately proceed to amplification.

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.

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

Step 2. Perform the Pre-Capture PCR Amplification

1. Place the tube(s) in the thermocycler and run the following Pre-Capture PCR program with the lid temperature set to +105°C:
   - Step 1: 45 seconds at +98°C
   - Step 2: 15 seconds at +98°C
   - Step 3: 30 seconds at +60°C
   - Step 4: 30 seconds at +72°C
   - Step 5: Go to Step 2, 7 times (8 cycles total)
   - Step 6: 1 minute at +72°C
   - Step 7: Hold at +4°C
2. Proceed immediately to the next step.

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

1. Add 50 µL room temperature, thoroughly resuspended, KAPA HyperPure Beads to each Amplified Sample Library.
2. Mix the Amplified Sample Library and KAPA HyperPure Beads thoroughly and perform a quick spin.

   It is important at this step to ensure that the solution is thoroughly mixed and appears homogeneous. Insufficient mixing may compromise recovery and size selection.
3. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.
4. Place the tube(s) on a magnet to capture the beads. Incubate for 3 minutes.
5. Carefully remove and discard the supernatant.
6. Remove the tube(s) from the magnet. Resuspend the beads in 50 µL of PCR Grade water.

7. Add 50 µL room temperature, thoroughly resuspended, KAPA HyperPure Beads to each Amplified Sample Library and mix thoroughly.

   It is important at this step to ensure that the solution is thoroughly mixed and appears homogeneous. Insufficient mixing may compromise recovery.

   This is a double bead cleanup process – it has been optimized to minimize the amount of adapter dimers present in the Amplified Sample Libraries.

8. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.
9. Place the tube(s) on the magnet to capture the beads. Incubate for 3 minutes.
10. Carefully remove and discard the supernatant.
11. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
12. Incubate at room temperature for ≥30 seconds.
13. Carefully remove and discard the ethanol.
14. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
15. Incubate at room temperature for ≥30 seconds.
16. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
17. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.

    Over-drying of the beads can result in yield loss.

18. Remove the tube(s) from the magnet.
19. Thoroughly resuspend the beads in 40 µL of PCR Grade water.
20. Incubate at room temperature for 2 minutes to allow the DNA to elute off the beads.
21. Place the tube(s) on a magnet to capture the beads. Incubate until the liquid is clear, ~ 3 minutes. Transfer 40 µL of supernatant to a fresh tube/well.
22. 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 one month.

Step 4. Qualify the Amplified Sample Library

For this limited input FFPET DNA protocol, singleplex capture with 30 µL of Amplified Sample Library is recommended.

To support potential troubleshooting, it is recommended to check the quality of the libraries before capture.

Make a 10-fold dilution of the Amplified Sample Library by combining 2 µL of the library with 18 µL of PCR Grade water.

1. Utilize 5 µL of the diluted library with the Qubit dsDNA HS Assay Kit to obtain the concentration of the diluted library. Multiply by 10 to obtain results for the undiluted Amplified Sample Library.

   When working with samples that will be pooled for hybridization (*i.e.* pre-capture multiplexing), accurate quantitation is **essential**. Slight differences in the mass of each sample combined to form the 'Multiplex DNA Sample Library Pool' will result in variations in the sequencing reads obtained for each sample in the library pool.

   a. The undiluted Amplified Sample Library should contain ≥ 1.0 µg of total DNA. If the library contains <1.0 µg of total DNA, refer to the troubleshooting section for guidance.
   b. The negative control yield should be <1 ng/µL.

2. Utilize 1 µL of the diluted Amplified Sample Library (and any controls) with an Agilent Bioanalyzer DNA High Sensitivity Kit. If using the Agilent Bioanalyzer DNA 1000 Kit, diluting the Amplified Sample Library is not necessary.

   a. Amplified Sample Libraries (Pre-Capture libraries) should display a fragment size distribution in the range of 150 to 500 bp, with an average size of ~320 bp for high quality gDNA or FFPET samples *(Figure 3)*. For lower quality FFPET samples, the average size may be lower (*Figure 4)*. Sharp peaks may be visible in the region <150 bp. These peaks correspond to unincorporated primers, primer-dimers or carry-over adapter-dimer and will not interfere with the capture process.
   b. The negative control should not show any signal above baseline within the 150 to 500 bp size range, which could indicate contamination between Amplified Sample Libraries, but it may exhibit sharp peaks visible below 150 bp. If the negative control reaction shows a positive signal by the Qubit measurement, but the Bioanalyzer trace indicates only the presence of a sharp peak below 150 bp, then the negative control should not be considered contaminated.

3. For best results we strongly recommend that the Amplified Sample Library meets these requirements, before proceeding to *Chapter 5*. Hybridize the Sample and KAPA HyperCap Target Enrichment Probes. During protocol development it was observed that the libraries meeting these conditions have delivered satisfactory results. If the Amplified Sample Library does not meet the yield and size distribution requirements, consider preparing a new sample library if possible.

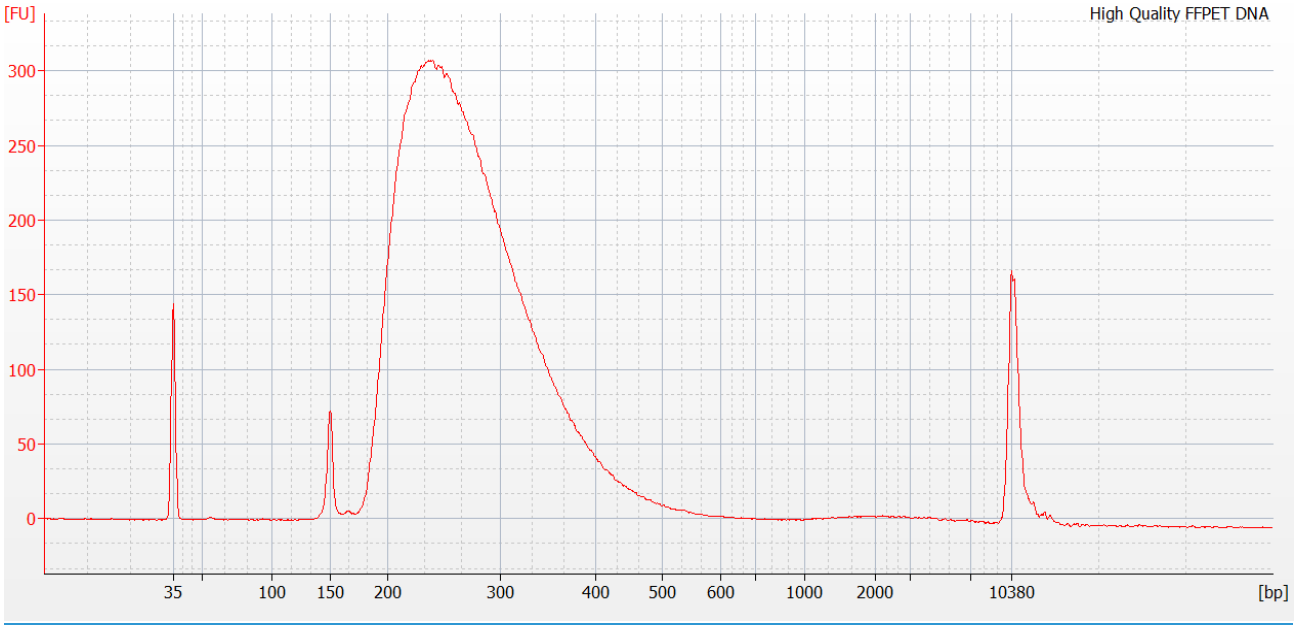


Figure 3: Example of a Pre-Capture amplified high quality FFPET sample library analyzed using an Agilent Bioanalyzer High Sensitivity DNA assay.

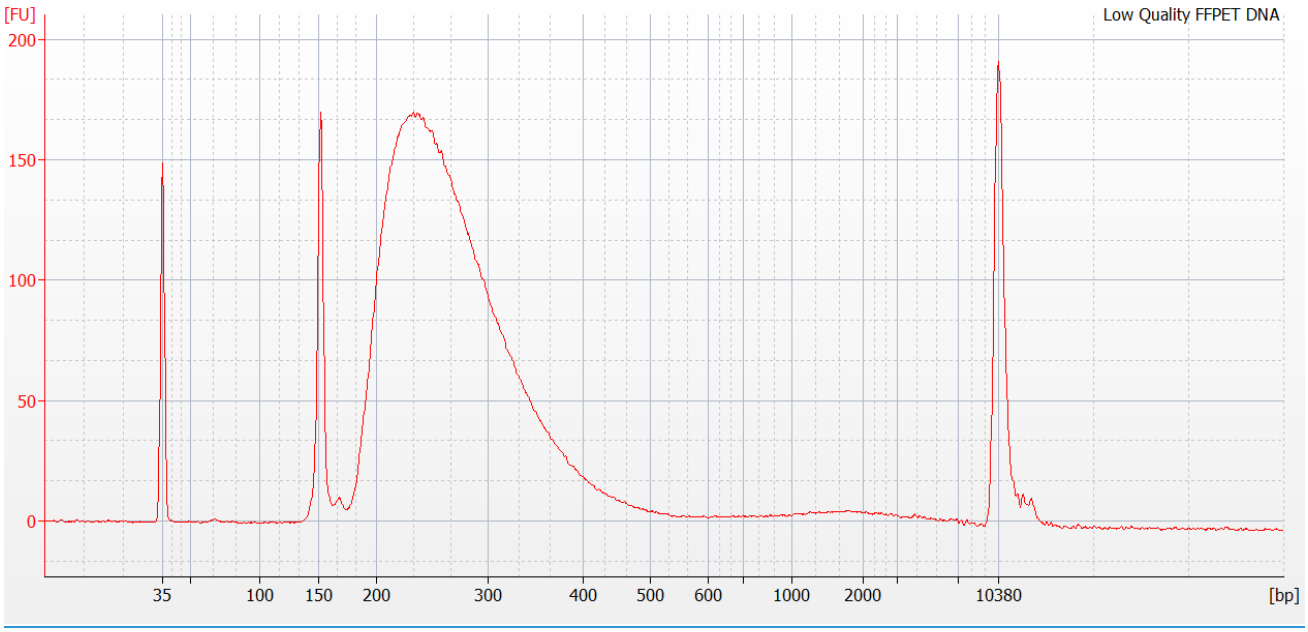


Figure 4: Example of a Pre-Capture amplified low quality FFPET sample library analyzed using an Agilent Bioanalyzer High Sensitivity DNA assay.

Chapter 5. Hybridize the Sample to KAPA HyperCap Target Enrichment Probes

Chapter 5 describes the hybridization of the Amplified Sample Libraries to the KAPA HyperCap Target Enrichment Probes . The following protocol provides instructions that have been tested for capture target sizes between 90 kb and 300 kb. The capture target size can be found in the coverage_summary.txt design deliverable file. For larger capture target sizes additional optimization (for example Hybridization Mix, Capture Bead volume, Post-Capture PCR cycle number) and sequencing may be needed.

This chapter requires the use of components from the following kits:

- KAPA HyperCap Target Enrichment Probes
- KAPA HyperCapture Reagent Kit
- KAPA HyperCapture Bead Kit

Ensure that the following are available:

- Freshly-prepared 80% ethanol
- PCR Grade water

The hybridization protocol requires a thermocycler capable of maintaining +55°C for 16 to 20 hours. A programmable heated lid is required.

Note: For limited input oncology samples, it is recommended to capture single Amplified DNA Sample Libraries separately. However, for multiplex sample capture see the recommendations and limitations in the *Troubleshooting Appendix*.

Step 1. Prepare the Sample for Hybridization

This step outlines how to prepare the sample for hybridization, by using KAPA HyperPure Beads.

Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.

1. Remove the appropriate number of 4 µL KAPA HyperCap Target Enrichment Probes aliquots (one per hybridization) from the -15°C to -25°C sotrage and allow them to thaw on ice.
2. Thaw on ice each of the uniquely indexed Amplified DNA Sample Libraries that will be included in the capture experiment (generated in *Chapter 4*).
3. Transfer 30 µl of the Amplified DNA Sample Library (generated in *Chapter 4)* into a fresh tube.
4. Add 20 µL of COT Human DNA to the Amplified DNA Sample Library, resulting in a total volume of 50 µL.
5. Add 130 µL of KAPA HyperPure Beads to each tube/well containing the Amplified DNA Sample Library and COT Human DNA mixture.

6. Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
7. Incubate at room temperature for 10 minutes to ensure the Amplified Sample Library and COT Human DNA bind to the beads.
8. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
9. Carefully remove and discard the supernatant.
10. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
11. Incubate at room temperature for ≥30 seconds.
12. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
13. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.

Caution: Over-drying the beads may result in dramatic yield loss.

14. Add 13.4 µL of the Universal Enhancing Oligos to the bead-bound DNA sample.
15. Remove the tube(s) from the magnet and mix thoroughly by vortexing. It is important that sufficient mixing is performed to result in a homogeneous mixture.
16. Prepare the Hybridization Master Mix following the table below.

< 40 Mbp Capture Target Size – Hybridization Master Mix	Per Individual Capture
Hybridization Buffer	28 µL
Hybridization Component H	12 µL
PCR Grade water	3 µL
Total	**43 µL**

17. Add 43 µL of the Hybridization Master Mix to the tube(s) from step 15 above.
18. Mix thoroughly and perform a quick spin. Incubate at room temperature for 2 minutes.
19. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
20. Transfer 56.4 µL of supernatant (entire volume) to a tube/well containing 4 µl of the KAPA HyperCap Target Enrichment Probes.

Slight bead carryover may be observed when transferring the supernatant. This is unlikely to impact results.

21. Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
22. Perform the hybridization incubation in a thermocycler using the following program with the lid temperature set to +105°C:

- +95°C for 5 minutes
- +55°C for 16 to 20 hours

The hybridization must remain at +55°C until it is transferred to the capture beads in *Chapter 6.* Wash and Recover Captured DNA Sample, Step 3.

23. Continue to *Chapter 6.* Wash and Recover Captured DNA Sample.

Chapter 6. Wash and Recover Captured DNA Sample

Chapter 6 describes the process for the washing and recovery of the captured DNA Sample after hybridization to the KAPA HyperCap Target Enrichment Probes. Check the capture target size for the following procedure.

This chapter requires the use of components from the following kits:

- KAPA HyperCapture Reagent Kit
- KAPA HyperCapture Bead Kit

Ensure that the following is available:

- Additional PCR Grade water for buffer preparation and elution

Step 1. Prepare Wash Buffers

Volumes for an individual capture are shown here. When preparing 1X buffers for processing multiple reactions, prepare an excess volume of ~5% (automated liquid handling systems may require an excess of ~20%).

1. Before completion of the hybridization incubation, thaw the Hybridization Wash Buffers.

   Ensure that stock wash buffers do not contain precipitates or are cloudy. Allow sufficient time for the buffers to thaw. Thoroughly vortex and warm cloudy buffers at 37°C until buffers are completely clear.

2. Dilute 10X Wash Buffers (I, II, III and Stringent) and 2.5X Bead Wash Buffer, contained in the KAPA HyperCapture Reagent Kit, to create 1X working solutions. Volumes listed below are sufficient for one capture.

Concentrated Buffer	Volume of Concentrated Buffer	Volume of PCR Grade Water	Total Volume of 1X Buffer*	Temperature
10X Stringent Wash Buffer	40 µL	360 µL	400 µL	+55°C
10X Wash Buffer I	10 µL	90 µL	100 µL	+55°C
	20 µL	180 µL	200 µL	Room temp.
10X Wash Buffer II	20 µL	180 µL	200 µL	Room temp.
10X Wash Buffer III	20 µL	180 µL	200 µL	Room temp.
2.5X Bead Wash Buffer	120 µL	180 µL	300 µL	Room temp.

*Store working solutions at room temperature (+15°C to +25°C) for up to 2 weeks. The volumes in this table are calculated for a single experiment; scale up accordingly if multiple capture reactions are processed.

It is expected that excess volume of 1X Bead Wash Buffer will remain after the following Capture Bead preparation protocol.

3. To pre-warm the 1X Stringent Wash Buffer, make two aliquots of 200 µL into 0.2 mL tubes and place the tubes in a thermocycler set to +55°C.
4. To pre-warm the 1X Wash Buffer I, make one aliquot of 100 µL into a 0.2 mL tube and place the tube in a thermocycler set to +55°C.

5. Pre-warm the buffers for a minimum of 15 minutes.

Pre-warming buffers can be performed in the same thermocycler used in the probe hybridization incubation step.

Step 2. Prepare the Capture Beads

1. Allow the Capture Beads to equilibrate to room temperature prior to use.
2. Vortex the Capture Beads for 15 seconds before immediate use to ensure a homogeneous mixture.
3. Aliquot 50 µL of beads per capture reaction into a 0.2 mL or 1.5 mL tube (*i.e.* for one capture use 50 µL beads and for two captures use 100 µL beads, *etc.*). Beads for up to two captures can be prepared in a single 0.2 mL tube or up to twelve captures can be prepared in a single 1.5 mL tube.
4. Place the tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
5. Remove and discard the supernatant being careful not to disturb the beads.
6. Keeping the tube(s) on the magnet, add 2X the initial beads' volume of 1X Bead Wash Buffer (*e.g.* for one capture use 100 µL of buffer and for two captures use 200 µL buffer, *etc.*).
7. Remove the tube(s) from the magnet and mix thoroughly by vortexing and perform a quick spin.
8. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
9. Remove and discard the supernatant being careful not to disturb the beads.
10. Keeping the tube(s) on the magnet perform a second wash by adding 2X the initial beads' volume of 1X Bead Wash Buffer (*e.g.* for one capture use 100 µL of buffer and for two captures use 200 µL buffer, *etc.*).
11. Remove the tube(s) from the magnet and mix thoroughly by vortexing and perform a quick spin.
12. Place the tube(s) on the magnet to bind the beads. Incubate until the liquid is clear.
13. Remove and discard the supernatant being careful not to disturb the beads.
14. Add 1X the initial beads' volume of 1X Bead Wash Buffer (*i.e.* 50 µL buffer per capture).
15. Remove the tube(s) from the magnet and mix thoroughly by vortexing for 10 seconds.
16. If preparing Capture Beads for more than one capture in a single tube, aliquot 50 µL of resuspended beads into a new tube/well for each capture.
17. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
18. Carefully remove and discard the supernatant.
19. The Capture Beads are now ready to bind the hybridized DNA. Proceed immediately to the next step.

Do not allow the Capture Beads to dry out. Small amounts of residual 1X Bead Wash Buffer will not interfere with binding of DNA to the Capture Beads.

Step 3. Bind Hybridized DNA to the Capture Beads

1. Transfer each hybridization sample to a single tube/well with prepared Capture Beads from the previous step.
2. Mix thoroughly by vortexing for 10 seconds and perform a quick spin, avoiding pellet formation.

3. Incubate the capture reaction by placing the tube in a thermocycler set to +55°C for 15 minutes, with the thermocycler lid temperature set to +105°C.

   This step can be performed in the same thermocycler used in the probe hybridization incubation step.

4. Following the 15 minutes incubation, proceed immediately to the next step.

Step 4. Wash the Capture Beads Plus Bead-Bound DNA

The thermocycler should remain at +55°C with the heated lid set to +105°C for the following steps.

1. Add 100 µL of pre-warmed 1X Wash Buffer I to the 60.4 µL of Capture Beads-bound DNA.
2. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
3. Place the tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
4. Remove and discard the supernatant without disturbing the beads.
5. Add 200 µL of pre-warmed 1X Stringent Wash Buffer to each sample.
6. Remove the tube(s) from the magnet.
7. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
8. Place the tube(s) in a thermocycler pre-heated to +55°C, close lid (set to +105°C) and incubate for 5 minutes.
9. After the 5 minutes incubation, remove the tube(s) from the thermocycler and place on the magnet to capture the beads. Incubate until the liquid is clear.
10. Remove and discard the supernatant being careful not to disturb the beads.
11. Add 200 µL of pre-warmed 1X Stringent Wash Buffer to each tube.
12. Remove the tube(s) from the magnet.
13. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
14. Place in a thermocycler pre-heated to +55°C, close lid (set to +105°C) and incubate for 5 minutes.
15. After incubating 5 minutes, remove the tube(s) from the thermocycler and place on the magnet to capture the beads. Incubate until the liquid is clear.
16. Remove and discard the supernatant being careful not to disturb the beads.
17. Add 200 µL of room temperature 1X Wash Buffer I.
18. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
19. Incubate at room temperature for 1 minute.
20. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
21. Remove and discard the supernatant being careful not to disturb the beads.
22. Add 200 µL of room temperature 1X Wash Buffer II.

23. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin and carefully transfer contents to a new tube.

Transferring each sample to a new tube is essential.

24. Incubate at room temperature for 1 minute.
25. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
26. Remove and discard the supernatant being careful not to disturb the beads.
27. Add 200 µL of room temperature 1X Wash Buffer III.
28. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
29. Incubate at room temperature for 1 minute.
30. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
31. Remove and discard the supernatant being careful not to disturb the beads.
32. Remove the tube(s) from the magnet.
33. Add 20 µL PCR Grade water to each tube/plate well and mix thoroughly. Perform a quick spin.
34. Proceed to *Chapter 7*. Amplify Enriched DNA Sample.

No elution is performed at this step. The enriched DNA sample library remains bound to the Capture Beads and will be used as the template in the PCR as described in *Chapter 7*.

Chapter 7. Amplify Enriched DNA Sample

Chapter 7 describes the amplification of the enriched DNA sample, bound to the Capture Beads. This chapter requires the use of components from the following kits:

- KAPA HyperCapture Reagent Kit
- KAPA HyperCapture Bead Kit

In addition, ensure that the following are available:

- Freshly-prepared 80% ethanol
- PCR Grade Water

References

- *Thermocycler Manual*
- *Qubit Fluorometer Manual*
- *Qubit dsDNA HS Assay Kit Guide*
- *Agilent 2100 Bioanalyzer Instrument Manual*
- *Agilent Bioanalyzer DNA Kits Guide*

Step 1. Resuspend the Post-Capture PCR Oligos

1. Briefly spin the lyophilized Post-Capture PCR Oligos, contained in the KAPA HyperCapture Reagent Kit, to allow the contents to pellet at the bottom of the tube. Note that both oligos are contained within a single tube.
2. Add 480 µL PCR Grade water to the tube of centrifuged oligos.
3. Briefly vortex the resuspended oligos.
4. Spin down the tube to collect the contents.
5. Store resuspended oligos at -15°C to -25°C.

Step 2. Prepare the Post-Capture PCR Master Mix

Instructions for preparing individual PCR reactions are shown here. When assembling a master mix for processing multiple samples, prepare an excess volume of ~5% to allow for complete pipetting (automated liquid handling systems may require an excess of ~20%).

Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.

1. Prepare a master mix of the following reagents.

Post-Capture PCR Master Mix*	Per Individual PCR Reaction
KAPA HiFi HotStart ReadyMix (2X)	25 µL
Post-Capture PCR Oligos	5 µL
Total	**30 µL**

* Note: The Post-Capture PCR Oligos and the KAPA HiFi HotStart ReadyMix (2X) are contained in the KAPA HyperCapture Reagent Kit.

2. Add 30 µL Post-Capture PCR Master Mix to a 0.2 mL tube or well of a PCR plate.
3. Retrieve the bead-bound DNA sample from *Chapter 6* step 33 and mix thoroughly by vortexing for 10 seconds. Perform a quick spin. Avoid pellet formation.
4. Transfer 20 µL of the bead-bound DNA as template into the tube/well with the 30 µL Post-Capture PCR Master Mix. (If performing a negative control, add 20 µL PCR Grade water to this tube/well).
5. Mix thoroughly by pipetting up and down several times.

Step 3. Perform the Post-Capture PCR Amplification

1. Place the tube(s) in the thermocyclerand run the following Post-Capture PCR program with the lid temperature set to +105°C:
   - Step 1: 45 seconds at +98°C
   - Step 2: 15 seconds at +98°C
   - Step 3: 30 seconds at +60°C
   - Step 4: 30 seconds at +72°C
   - Step 5: Go to Step 2, 15 times (16 total cycles)
   - Step 6: 1 minute at +72°C
   - Step 7: Hold at +4°C

   The Post-Capture PCR cycling conditions are recommendations and can be adjusted to individual experimental needs.

2. Proceed immediately to the next step.

Step 4. Purify the Amplified Enriched DNA Sample using KAPA HyperPure Beads

1. Mix the Amplified Enriched DNA Sample from Step 3 by vortexing. Perform a quick spin. Avoid pellet formation.
2. Place the sample tube on a magnet to collect the capture beads. Incubate until the liquid is clear.
3. Carefully transfer the supernatant to a new tube.

   Do not discard the supernatant. The supernatant contains the PCR-amplified enriched library.

4. Vortex the KAPA HyperPure Beads for 10 seconds before use to ensure a homogenous mixture of beads.

5. Add 50 µL KAPA HyperPure Beads to the 50 µL Amplified Enriched DNA Sample library.
6. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
7. Incubate at room temperature for 10 minutes to allow the Amplified Enriched DNA Sample Library to bind to the beads.
8. Place the tube(s) containing the bead-bound DNA on a magnet to capture the beads. Incubate until the liquid is clear.
9. Remove and discard the supernatant being careful not to disturb the beads.
10. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
11. Incubate at room temperature for ≥30 seconds.
12. Remove and discard the 80% ethanol.
13. Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
14. Incubate the tube(s) at room temperature for ≥30 seconds.
15. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
16. Allow the beads to dry at room temperature with the tube lid open for 3 minutes (or until dry).

Over-drying of the beads can result in yield loss.

17. Remove the tube(s) from the magnet.
18. Resuspend the bead pellet using 22 µL of PCR Grade water.
19. Vortex at least 10 seconds to ensure that all of the beads are resuspended.
20. Incubate at room temperature for 2 minutes to allow the sample to elute off the beads.
21. Brifely spin and place the tube(s) back on the magnet and allow the solution to clear.
22. Transfer 20 µL of the eluate to a new tube/well.

This tube contains the Amplified Enriched and purified DNA Sample Library.

Step 5. Qualify the Amplified Enriched DNA Sample Library

1. Measure the concentration of the Amplified Enriched DNA Sample Library using a Qubit Fluorometer:
   a. Make a 10-fold dilution by combining 2 µL of the sample with 18 µL of PCR Grade water.
   b. Utilize 5 µL of the diluted sample using the Qubit dsDNA HS Assay Kit to obtain the concentration of the diluted sample. Multiply by 10 to obtain results for the undiluted sample. Reserve at least 1 µL of the diluted sample to be analyzed using the Agilent Bioanalyzer DNA High Sensitivity Kit.

- The Amplified Enriched DNA Sample library yield should be ≥100ng. The yield will vary depending on DNA quality (FFPET DNA), DNA input and the size of the panel used for capture. A larger panel will produce higher yields.
  - For FFPET DNA, 300 ng of low quality DNA input into Library Prep and captured with a ~220kb panel will typically yield >500 ng per sample.
- The negative control yield should not show significant amplification which could be indicative of contamination.

2. Analyze 1 µL of each diluted sample (and any controls) using an Agilent Bioanalyzer DNA High Sensitivity assay according to manufacturer's instructions. If using the Agilent Bioanalyzer DNA 1000 Kit, diluting the Amplified Enriched Sample Library is not necessary.

- The ideal peak size of the library is ~300 bp. There should be minimal to no adapter dimers present.

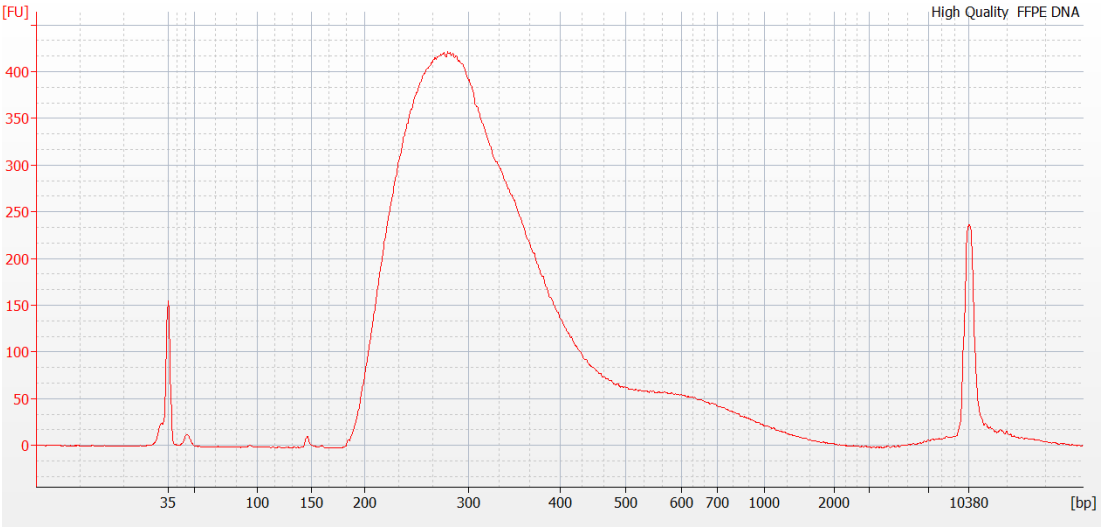


Figure 5: Example of successfully amplified enriched high quality FFPET DNA Sample library following the KAPA HyperCap FFPET DNA Workflow v1.1. Sample was analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

3. The Amplified Enriched DNA Sample Library is ready for sequencing.

Step 6. Guidance for Multiplex Pooling for Sequencing

After QC of the enriched libraries, the samples are ready for pooling for sequencing.

Libraries prepared from FFPET DNA samples

The number of samples pooled per lane differs between sequencing platforms as it depends on the panel size and the number of reads required for analysis. The performance of the protocol depends on the FFPET DNA input mass, the panel size, and the number of sequencing reads required.

The table below has some recommendations that can be a starting point for pooling prior to sequencing, targeting sufficient unique depth for variant calling from input FFPET DNA ranges recommended in this protocol:

Recommended Multiplexing for Sequencing			
Panel Size	# Clusters Desired	Samples to Multiplex on NextSeq High Output Flowcell	Samples to Multiplex on NovaSeq S1 Flowcell
~220 kb	10M clusters	24	96
2 Mb	30M clusters	8	32

Ensure that each Library was prepared with a unique KAPA UDI Primer Mix.

Variant calling performance may be further enhanced by pooling fewer samples to reach a greater sequencing depth.

It is important to pool equal moles per sample, to ensure that each sample receives approximately equal number of sequencing reads for data analysis.

1. Use the concentration and the average library size determined in *Step 5.* Qualify the Amplified Enriched DNA Sample Library, to calculate the molarity of each sample in nM using the following equation:
2. Library Molarity in nM = (library concentration in ng/µL) / ((average library size in bp x 607.4)+157.9) $x10^6$
3. For example, if library concentration = 10 ng/µL and average library size is 300 bp, then the Library Molarity in nM = 10/((300 x 607.4)+157.9) x 10^6=54.83
4. Pool an appropriate volume of each library so that there are equal moles of each library in the pool. Prepare enough total volume sufficient for Illumina's suggested denaturing protocol.
5. Vortex and spin-down pooled libraries briefly.
6. Measure the concentration of the pooled library using the Qubit dsDNA HS Assay Kit according to the manufacturer's instructions.
7. Assess the size of the pool by diluting the pool to the 1–5 ng/µL range and analyzing it on the Bioanalyzer instrument with an Agilent High Sensitivity DNA Kit following the manufacturer's instructions.
8. Calculate the molarity of the pooled library as outlined in point 2 above.
9. Proceed to sequencing or store at -20°C for up to two weeks.

Appendix A. Troubleshooting

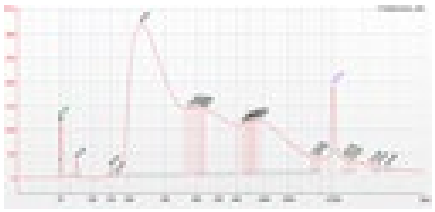
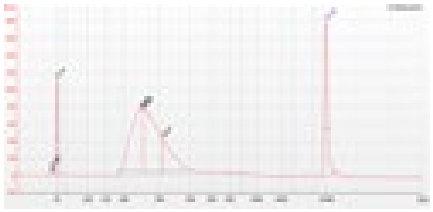
This appendix provides guidance for interpreting unexpected results and recommendations for implementing corrective action if problems occur. For technical questions, contact your local Roche Technical Support. Go to *sequencing.roche.com/support.html* for contact information.

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

Observation	Cause(s) / Recommendation(s)
Sample Library Preparation	
DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is used for library preparation.	DNA extracted from FFPE tissue is highly variable in quality due to chemical damage and fragmentation, and often is available only in small amounts. § To increase probability for success, follow recommendations for library construction using 300 ng of input DNA and perform enzymatic DNA fragmentation following the KAPA HyperPlus workflow. This can help to remove chemically-damaged termini that will interfere with adapter ligation. § KAPA HyperPlus library preparation is recommended since it improves sample complexity through increased adapter ligation efficiency. § If fragmentation optimization is required, precious sample should not be used. Instead, fragmentation parameters should be optimized using non-precious DNA sample that is representative of the actual sample to be processed. **Note:** There is a possibility that these steps will not lead to success with DNA extracted from FFPE samples. For the most current guidance on working with FFPE samples, contact Roche Technical Support.

Amplified Sample Library (Pre-Capture PCR Product)	
Yield is <1 µg with 300 ng input (yield should be ≥1 µg).	Possible error occurred during library preparation or compromised reagents were used. § Poor ligation efficiency o Ensure that the proper amount of input DNA and Universal UMI adapter is used. Ensure proper ligation time and temperature are used. § Poor PCR amplification o High number of adapter dimers can inhibit the PCR reaction. Follow proper cleanup steps before PCR. Ensure that the KAPA UDI Primer Mixes are fully resuspended. Use a previously processed DNA sample as a positive control for library construction and or an evaluated sample library as a positive
High adapter dimers in the pre-enrichment library after the first PCR	§ Insufficient or poor quality DNA used in the library prep o Ensure proper quantification of the input DNA. Follow recommended DNA amount according to the Instructions for Use. § Poor clean-up o Ensure proper volumes are used in the clean-up. Repeat clean-up, maintaining the sample to beads ratio. Note that this additional cleanup may result in some sample loss and lower Deduped Depth.
Fragment distribution (analyzed using an Agilent HS DNA chip) shows that the average amplified library fragment size is not within the size range of 150 to 500 bp.	Poor fragmentation occurred. Repeat library preparation. § Ensure that the Fragmentation Buffer is fully thawed and resuspended. § Ensure proper mixing of viscous Fragmentation Enzyme in the master mix and in the fragmentation reaction with the samples.
Fragment distribution (analyzed using an Agilent HS DNA chip) is bimodal, with a larger set of fragments observed in addition to, or instead of, the expected set of fragments.	See the section entitled "Amplified Enriched Multiplex DNA Sample (Post-Capture PCR Product)" in this appendix.
The negative control yield measured by the Qubit is >1 ng/µL.	The measurement may be high due to the presence of adapter dimers carried over from previous steps/PCR. This carryover will be apparent as one or more sharp peaks visible less than 150 bp in size when examining the data from the Agilent Bioanalyzer HS DNA chip. This carryover is not a sign of contamination.
The Agilent Bioanalyzer HS DNA chip indicates one or more visible sharp peaks that are <150 bp in size.	These peaks, which represent primers, primer-dimers or adapter-dimers will not interfere with the capture process but could lead to overestimation of the amplified library yield when interpreting the data from Qubit. Repeat the PCR cleanup.
The Agilent Bioanalyzer HS DNA chip indicates that the average amplified material is 150 to 500 bp in size in the negative control for sample library amplification.	This material could represent cross contamination between amplified sample libraries. Test reagents for contamination and replace if necessary. Repeat library construction using fresh DNA.
Only the Agilent Bioanalyzer DNA 1000 chip is available for qualifying sample following Pre-capture PCR amplification and purification.	The Agilent Bioanalyzer DNA 1000 chip can be used to analyze the Pre-Capture Amplified Sample Library. Refer to the manufacturer's user manual for further guidance.

Multiplexing the Sample Library and KAPA HyperCap Target Enrichment Probes	
General guidance for Multiplex DNA Sample Library	Warning: for optimal performance it is highly recommended to only perform a singleplex capture. However, for multiplex sample capture we recommend, mixing together equal amounts (by mass) of each uniquely indexed DNA sample libraries to obtain a combined DNA mass of 1.5 µg (i.e. for two-plex DNA Sample Library, mix together 750 ng of each uniquely indexed amplified DNA sample library for a total mass of 1.5 µg). Four samples is the recommended maximum number per multiplex capture. This mixture will subsequently be referred to as the 'Multiplex DNA Sample Library Pool. The maximum volume of the Multiplex DNA Sample Library Pool should be 45 µL, if needed add PCR Grade water to achieve this volume. Note: To obtain equal numbers of sequencing reads from each component library in the Multiplex DNA Sample Library Pool upon completion of the experiment, it is very important to combine identical mass from each independently amplified DNA sample library at this step. Accurate quantification and pipetting are critical. Slight differences in the mass of each sample combined to form the Multiplex DNA Sample Library Pool will result in variations in the sequencing reads obtained for each sample in the library pool. Continue with Chapter 5: Hybridize the Sample to KAPA HyperCap Target Enrichment Probes , Step 3 Prepare the Hybridization Sample.
The Multiplex DNA Sample Library volume is greater than 45 µL.	Transfer the Multiplex DNA Sample Library to a 1.5 mL tube and add 20 µL of COT DNA. Add 2X KAPA HyperPure Beads to the mixture and proceed with Step 1, point 6, on page 27, section "Step 1. Prepare the Sample for Hybridization". If the KAPA HyperPure Beads volume that is required is very large and a vacuum concentrator is preferred, use the following recommendations. 1) Add 20 µL of COT DNA to the Multiplex DNA Sample Library. 2) Concentrate the sample with a vacuum concentrator to a volume of 65 µL. 3) Once concentrated proceed with Step 1, point 5, on page 26, section "Step 1. Prepare the Sample for Hybridization."

Amplified Enriched DNA Sample (Post-Capture PCR Product)	
Yield is <100 ng (yield should be ≥100 ng).	Library construction or Pre-Capture PCR failed. Pre-Capture PCR yield should be ≥ 1 µg. Repeat with a DNA sample that was previously processed with success. Incorrect hybridization or wash temperatures were used. Make sure the correct hybridization and wash temperatures were used. If temperatures were not correct, repeat the experiment starting from *Chapter 5*. PCR reagents are compromised. Verify that the positive control worked. If the positive control did not work, repeat hybridization starting from Chapter 5 and re-amplify using fresh PCR reagents. **Note:** Experiments designed to capture less genomic DNA (*i.e.* a smaller cumulative target size) may be successful even though they can generate lower PCR yields than experiments designed to capture larger targets. Target size should be taken into consideration when evaluating low Post-Capture PCR yield.
Fragment distribution (analyzed using a Agilent HS DNA chip) shows that the average amplified fragment size is not within the size range of 150 to 500 bp.	Poor fragmentation occurred. Repeat library preparation.
Fragment distribution (analyzed using an Agilent HS DNA chip) is bimodal. A:  B: 	Primer depletion due to over-amplification of 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 apparently much larger products on an Agilent HS DNA chip than their actual length in base pairs. The pre-capture PCR product is still useable for capture and sequencing. *The Agilent HS DNA chip traces shown in Fig. A and Fig. B, show the pre and Post-Capture PCR libraries of a high quality FFPET sample with 300 ng of DNA input* into the library prep.
Only the Agilent Bioanalyzer DNA 1000 chip is available for qualifying sample following Post-capture PCR amplification and purification.	The Agilent Bioanalyzer DNA 1000 chip can be used to analyze the Post-Capture Sample Library. Refer to the manufacturer's user manual for further guidance.

Sequencing Performance Metrics	
High Duplicate rates	Reduction in Pre-Capture and/or Post-Capture PCR cycles may reduce duplicate rates. Take the following points into consideration when altering cycle numbers. § Enough material is present to accurately quantify after PCR clean-up. § For the pre-capture PCR, at least 1 µg total library is produced when single libraries are hybridized. § Increasing input into hybridization may improve duplicate rates. Take the following points into consideration when adjusting sample input into hybridization. § Enough material is produced from the Pre-Capture PCR reaction(s) to increase sample input into hybridization. **Note:** There is a possibility that these steps will not lead to success in reducing duplicate rates. For the most current guidance, contact *Roche Technical Support*.
Poor On-Target Rate	Possible errors that may have occurred: § Wrong panel used. o Confirm panel name before addition into the hybridization reaction. § Poor post-hybridization washes. o Ensure all wash buffers are fully thawed and resuspended before preparing 1X wash buffers. Ensure thorough washing by carefully following the protocol. Ensure that fresh tubes are used during post-hybridization washes when indicated in the user guide. Avoid long delays or bead drying during the post-hybridization wash steps. § Improper hybridization temperature. o Confirm thermocycler temperature and program before addition of samples. § UEO reagent not added. o Ensure that the KAPA HyperPure Beads with bead-bound DNA are resuspended with the UEO reagent.
High Error Rate	Possible errors that may have occurred: § Contamination. o Always use best laboratory practices when handling samples to minimize risk of contamination. § Improper hybridization set up and washes. o Do not deviate from the steps listed in the protocol. Ensure the temperature of the heated buffer washes remain at +55°C.

Low Deduped Depth	Possible errors that may have occurred: § Poor sequencing run. o Refer to the Illumina Sequencing Guide for troubleshooting. § Poor on-target rate. o Do not deviate from the steps and conditions listed in the protocol. o Confirm the correct Target Enrichment Probe Panel is used. § Poor ligation efficiency. o Do not deviate from the steps and conditions listed in the protocol.
Unable to detect unique molecules	Possible errors that may have occurred: § Universal Adapter used instead of Universal UMI adapter

Appendix B. 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.

B. Under no circumstances shall Roche's liability to Customer exceed the amount paid by Customer for the Services and Products to Roche. Roche will bear all reasonable shipping costs if service is re-performed at Roche or the Products are replaced. This warranty does not apply to any defect or nonconformance caused by (i) the failure by Customer to provide a suitable storage, use, or operating environment for the Materials or Customer's submission of substandard quality Materials or contaminated or degraded Materials to Roche, (ii) Customer's use of non-recommended reagents, (iii) Customer's use of the Products, Materials or Data for a purpose or in a manner other than that for which they were designed, (iv) the failure by Customer to follow Roche's published protocols; or (v) as a result of any other abuse, misuse or neglect of the Products, Materials or Data by Customer. This warranty applies only to Customer and not to third parties.

C. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, ROCHE DISCLAIMS ALL OTHER REPRESENTATIONS, AND WARRANTIES, EXPRESS OR IMPLIED, WITH RESPECT TO THE PRODUCTS, SERVICES AND DATA, INCLUDING BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT. CUSTOMER'S SOLE REMEDY FOR BREACH OF WARRANTY IS STATED ABOVE.

D. Any action by Customer against Roche for Roche's breach of this warranty must be commenced within 12 months following the date of such breach. Notwithstanding such 12-month period, within twenty (20) days of the delivery of Data and/or Products to Customer, Customer must notify Roche in writing of any nonconformity of the Services and Products, describing the nonconformity in detail; otherwise all Services and Products shall be conclusively deemed accepted without qualification.

2. FURTHER LIABILITY LIMITATION

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, ROCHE SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF ROCHE HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. CUSTOMER UNDERSTANDS THAT ANY RISKS OF LOSS HEREUNDER ARE REFLECTED IN THE PRICE OF THE SERVICES AND PRODUCTS AND THAT THESE TERMS WOULD HAVE BEEN DIFFERENT IF THERE HAD BEEN A DIFFERENT ALLOCATION OF RISK.

If you have any questions concerning service of this product, contact your local Roche Technical Support. Go to *sequencing.roche.com/support.html* for contact information.

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

Appendix C. Products Overview

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperChoice Probes		KAPA HyperChoice MAX 3Mb T1, 384 rxn	09052631001
KAPA HyperChoice MAX 0.5Mb T1, 12 rxn	09052143001	KAPA HyperChoice MAX 3Mb T1, 768 rxn	09052640001
KAPA HyperChoice MAX 0.5Mb T1, 24 rxn	09052151001	KAPA HyperChoice MAX 3Mb T1, 1152 rxn	09052658001
KAPA HyperChoice MAX 0.5Mb T1, 48 rxn	09052160001	KAPA HyperChoice MAX 3Mb T1, 1536 rxn	09052666001
KAPA HyperChoice MAX 0.5Mb T1, 96 rxn	09052178001	KAPA HyperChoice MAX 3Mb T1, 4000 rxn	09052674001
KAPA HyperChoice MAX 0.5Mb T1, 192 rxn	09052186001	KAPA HyperChoice MAX 3Mb T1, 10000 rxn	09052682001
KAPA HyperChoice MAX 0.5Mb T1, 384 rxn	09052194001	KAPA HyperChoice MAX 3Mb T1, 20000 rxn	09052704001
KAPA HyperChoice MAX 0.5Mb T1, 768 rxn	09052208001	KAPA HyperChoice MAX 3Mb T1, 50000 rxn	09052712001
KAPA HyperChoice MAX 0.5Mb T1, 1152 rxn	09052216001	KAPA HyperChoice MAX 3Mb T1, 100000 rxn	09052739001
KAPA HyperChoice MAX 0.5Mb T1, 1536 rxn	09052224001	KAPA HyperChoice MAX 3Mb T2, 12 rxn	09052747001
KAPA HyperChoice MAX 0.5Mb T1, 4000 rxn	09052232001	KAPA HyperChoice MAX 3Mb T2, 24 rxn	09052755001
KAPA HyperChoice MAX 0.5Mb T2, 12 rxn	09052259001	KAPA HyperChoice MAX 3Mb T2, 48 rxn	09052763001
KAPA HyperChoice MAX 0.5Mb T2, 24 rxn	09052267001	KAPA HyperChoice MAX 3Mb T2, 96 rxn	09052771001
KAPA HyperChoice MAX 0.5Mb T2, 48 rxn	09052275001	KAPA HyperChoice MAX 3Mb T2, 192 rxn	09052780001
KAPA HyperChoice MAX 0.5Mb T2, 96 rxn	09052283001	KAPA HyperChoice MAX 3Mb T2, 384 rxn	09052798001
KAPA HyperChoice MAX 0.5Mb T2, 192 rxn	09052291001	KAPA HyperChoice MAX 3Mb T2, 768 rxn	09052801001
KAPA HyperChoice MAX 0.5Mb T2, 384 rxn	09052305001	KAPA HyperChoice MAX 3Mb T2, 1152 rxn	09052810001
KAPA HyperChoice MAX 0.5Mb T2, 768 rxn	09052313001	KAPA HyperChoice MAX 3Mb T2, 1536 rxn	09052828001
KAPA HyperChoice MAX 0.5Mb T2, 1152 rxn	09052321001	KAPA HyperChoice MAX 3Mb T2, 4000 rxn	09052836001
KAPA HyperChoice MAX 0.5Mb T2, 1536 rxn	09052330001	KAPA HyperChoice MAX 3Mb T2, 10000 rxn	09052844001
KAPA HyperChoice MAX 0.5Mb T2, 4000 rxn	09052348001	KAPA HyperChoice MAX 3Mb T2, 20000 rxn	09052852001
KAPA HyperChoice MAX 0.5Mb T3, 12 rxn	09052356001	KAPA HyperChoice MAX 3Mb T2, 50000 rxn	09052879001
KAPA HyperChoice MAX 0.5Mb T3, 24 rxn	09052364001	KAPA HyperChoice MAX 3Mb T2, 100000 rxn	09052887001
KAPA HyperChoice MAX 0.5Mb T3, 48 rxn	09052372001	KAPA HyperChoice MAX 3Mb T3, 12 rxn	09052895001
KAPA HyperChoice MAX 0.5Mb T3, 96 rxn	09052399001	KAPA HyperChoice MAX 3Mb T3, 24 rxn	09052909001
KAPA HyperChoice MAX 0.5Mb T3, 192 rxn	09052402001	KAPA HyperChoice MAX 3Mb T3, 48 rxn	09052917001
KAPA HyperChoice MAX 0.5Mb T3, 384 rxn	09052429001	KAPA HyperChoice MAX 3Mb T3, 96 rxn	09052925001
KAPA HyperChoice MAX 0.5Mb T3, 768 rxn	09052437001	KAPA HyperChoice MAX 3Mb T3, 192 rxn	09052933001
KAPA HyperChoice MAX 0.5Mb T3, 1152 rxn	09052445001	KAPA HyperChoice MAX 3Mb T3, 384 rxn	09052941001
KAPA HyperChoice MAX 0.5Mb T3, 1536 rxn	09052453001	KAPA HyperChoice MAX 3Mb T3, 768 rxn	09052950001
KAPA HyperChoice MAX 0.5Mb T3, 4000 rxn	09052461001	KAPA HyperChoice MAX 3Mb T3, 1152 rxn	09052968001
KAPA HyperChoice MAX 0.5Mb T4, 12 rxn	09052470001	KAPA HyperChoice MAX 3Mb T3, 1536 rxn	09052976001
KAPA HyperChoice MAX 0.5Mb T4, 24 rxn	09052488001	KAPA HyperChoice MAX 3Mb T3, 4000 rxn	09052984001
KAPA HyperChoice MAX 0.5Mb T4, 48 rxn	09052496001	KAPA HyperChoice MAX 3Mb T3, 10000 rxn	09052992001
KAPA HyperChoice MAX 0.5Mb T4, 96 rxn	09052500001	KAPA HyperChoice MAX 3Mb T3, 20000 rxn	09053000001
KAPA HyperChoice MAX 0.5Mb T4, 192 rxn	09052518001	KAPA HyperChoice MAX 3Mb T3, 50000 rxn	09053018001
KAPA HyperChoice MAX 0.5Mb T4, 384 rxn	09052526001	KAPA HyperChoice MAX 3Mb T3, 100000 rxn	09053026001
KAPA HyperChoice MAX 0.5Mb T4, 768 rxn	09052534001	KAPA HyperChoice MAX 5Mb, 12 rxn	09053034001
KAPA HyperChoice MAX 0.5Mb T4, 1152 rxn	09052542001	KAPA HyperChoice MAX 5Mb, 24 rxn	09053042001
KAPA HyperChoice MAX 0.5Mb T4, 1536 rxn	09052569001	KAPA HyperChoice MAX 5Mb, 48 rxn	09053069001
KAPA HyperChoice MAX 0.5Mb T4, 4000 rxn	09052577001	KAPA HyperChoice MAX 5Mb, 96 rxn	09053077001
KAPA HyperChoice MAX 3Mb T1, 12 rxn	09052585001	KAPA HyperChoice MAX 5Mb, 192 rxn	09053085001
KAPA HyperChoice MAX 3Mb T1, 24 rxn	09052593001	KAPA HyperChoice MAX 5Mb, 384 rxn	09053093001
KAPA HyperChoice MAX 3Mb T1, 48 rxn	09052607001	KAPA HyperChoice MAX 5Mb, 768 rxn	09053107001
KAPA HyperChoice MAX 3Mb T1, 96 rxn	09052615001	KAPA HyperChoice MAX 5Mb, 1152 rxn	09053115001
KAPA HyperChoice MAX 3Mb T1, 192 rxn	09052623001	KAPA HyperChoice MAX 5Mb, 1536 rxn	09053123001

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperChoice MAX 5Mb, 4000 rxn	09053131001	KAPA HyperChoice MAX 200Mb, 48 rxn	09062416001
KAPA HyperChoice MAX 5Mb, 10000 rxn	09053140001	KAPA HyperChoice MAX 200Mb, 96 rxn	09062424001
KAPA HyperChoice MAX 5Mb, 20000 rxn	09053158001	KAPA HyperChoice MAX 200Mb, 192 rxn	09062432001
KAPA HyperChoice MAX 5Mb, 50000 rxn	09053166001	KAPA HyperChoice MAX 200Mb, 384 rxn	09062459001
KAPA HyperChoice MAX 5Mb, 100000 rxn	09053174001	KAPA HyperChoice MAX 200Mb, 768 rxn	09062467001
KAPA HyperChoice MAX 20Mb, 12 rxn	09053182001	KAPA HyperChoice MAX 200Mb, 1152 rxn	09062475001
KAPA HyperChoice MAX 20Mb, 24 rxn	09053204001	KAPA HyperChoice MAX 200Mb, 1536 rxn	09062483001
KAPA HyperChoice MAX 20Mb, 48 rxn	09053212001	KAPA HyperChoice MAX 200Mb, 4000 rxn	09062491001
KAPA HyperChoice MAX 20Mb, 96 rxn	09053239001	KAPA HyperChoice MAX 200Mb, 10000 rxn	09062505001
KAPA HyperChoice MAX 20Mb, 192 rxn	09053247001	KAPA HyperChoice MAX 200Mb, 20000 rxn	09062513001
KAPA HyperChoice MAX 20Mb, 384 rxn	09053255001	KAPA HyperChoice MAX 200Mb, 50000 rxn	09062521001
KAPA HyperChoice MAX 20Mb, 768 rxn	09053263001	KAPA HyperChoice MAX 200Mb, 100000 rxn	09062530001
KAPA HyperChoice MAX 20Mb, 1152 rxn	09053271001	**KAPA HyperExplore Probes**	
KAPA HyperChoice MAX 20Mb, 1536 rxn	09053280001	KAPA HyperExplore MAX 0.5Mb T1, 12 rxn	09062700001
KAPA HyperChoice MAX 20Mb, 4000 rxn	09053301001	KAPA HyperExplore MAX 0.5Mb T1, 24 rxn	09062718001
KAPA HyperChoice MAX 20Mb, 10000 rxn	09053310001	KAPA HyperExplore MAX 0.5Mb T1, 48 rxn	09062726001
KAPA HyperChoice MAX 20Mb, 20000 rxn	09053328001	KAPA HyperExplore MAX 0.5Mb T1, 96 rxn	09062734001
KAPA HyperChoice MAX 20Mb, 50000 rxn	09053336001	KAPA HyperExplore MAX 0.5Mb T1, 192 rxn	09062742001
KAPA HyperChoice MAX 20Mb, 100000 rxn	09053344001	KAPA HyperExplore MAX 0.5Mb T1, 384 rxn	09062769001
KAPA HyperChoice MAX 40Mb, 24 rxn	09053352001	KAPA HyperExplore MAX 0.5Mb T1, 768 rxn	09062777001
KAPA HyperChoice MAX 40Mb, 48 rxn	09053379001	KAPA HyperExplore MAX 0.5Mb T1, 1152 rxn	09062785001
KAPA HyperChoice MAX 40Mb, 96 rxn	09053387001	KAPA HyperExplore MAX 0.5Mb T1, 1536 rxn	09062793001
KAPA HyperChoice MAX 40Mb, 192 rxn	09053395001	KAPA HyperExplore MAX 0.5Mb T1, 4000 rxn	09062807001
KAPA HyperChoice MAX 40Mb, 384 rxn	09053409001	KAPA HyperExplore MAX 0.5Mb T2, 12 rxn	09062815001
KAPA HyperChoice MAX 40Mb, 768 rxn	09053417001	KAPA HyperExplore MAX 0.5Mb T2, 24 rxn	09062823001
KAPA HyperChoice MAX 40Mb, 1152 rxn	09053425001	KAPA HyperExplore MAX 0.5Mb T2, 48 rxn	09062831001
KAPA HyperChoice MAX 40Mb, 1536 rxn	09053433001	KAPA HyperExplore MAX 0.5Mb T2, 96 rxn	09062840001
KAPA HyperChoice MAX 40Mb, 4000 rxn	09053441001	KAPA HyperExplore MAX 0.5Mb T2, 192 rxn	09062858001
KAPA HyperChoice MAX 40Mb, 10000 rxn	09053450001	KAPA HyperExplore MAX 0.5Mb T2, 384 rxn	09062866001
KAPA HyperChoice MAX 40Mb, 20000 rxn	09053468001	KAPA HyperExplore MAX 0.5Mb T2, 768 rxn	09062874001
KAPA HyperChoice MAX 40Mb, 50000 rxn	09053476001	KAPA HyperExplore MAX 0.5Mb T2, 1152 rxn	09062882001
KAPA HyperChoice MAX 40Mb, 100000 rxn	09053484001	KAPA HyperExplore MAX 0.5Mb T2, 1536 rxn	09062904001
KAPA HyperChoice MAX 60Mb, 24 rxn	09053492001	KAPA HyperExplore MAX 0.5Mb T2, 4000 rxn	09062912001
KAPA HyperChoice MAX 60Mb, 48 rxn	09053506001	KAPA HyperExplore MAX 0.5Mb T3, 12 rxn	09062939001
KAPA HyperChoice MAX 60Mb, 96 rxn	09053514001	KAPA HyperExplore MAX 0.5Mb T3, 24 rxn	09062947001
KAPA HyperChoice MAX 60Mb, 192 rxn	09053522001	KAPA HyperExplore MAX 0.5Mb T3, 48 rxn	09062955001
KAPA HyperChoice MAX 60Mb, 384 rxn	09053549001	KAPA HyperExplore MAX 0.5Mb T3, 96 rxn	09062963001
KAPA HyperChoice MAX 60Mb, 768 rxn	09053557001	KAPA HyperExplore MAX 0.5Mb T3, 192 rxn	09062971001
KAPA HyperChoice MAX 60Mb, 1152 rxn	09062335001	KAPA HyperExplore MAX 0.5Mb T3, 384 rxn	09062980001
KAPA HyperChoice MAX 60Mb, 1536 rxn	09062343001	KAPA HyperExplore MAX 0.5Mb T3, 768 rxn	09062998001
KAPA HyperChoice MAX 60Mb, 4000 rxn	09062351001	KAPA HyperExplore MAX 0.5Mb T3, 1152 rxn	09063005001
KAPA HyperChoice MAX 60Mb, 10000 rxn	09062360001	KAPA HyperExplore MAX 0.5Mb T3, 1536 rxn	09063013001
KAPA HyperChoice MAX 60Mb, 20000 rxn	09062378001	KAPA HyperExplore MAX 0.5Mb T3, 4000 rxn	09063021001
KAPA HyperChoice MAX 60Mb, 50000 rxn	09062386001	KAPA HyperExplore MAX 0.5Mb T4, 12 rxn	09063030001
KAPA HyperChoice MAX 60Mb, 100000 rxn	09062394001	KAPA HyperExplore MAX 0.5Mb T4, 24 rxn	09063048001
KAPA HyperChoice MAX 200Mb, 24 rxn	09062408001	KAPA HyperExplore MAX 0.5Mb T4, 48 rxn	09063056001

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperExplore MAX 0.5Mb T4, 96 rxn	09063064001	KAPA HyperExplore MAX 3Mb T3, 20000 rxn	09063587001
KAPA HyperExplore MAX 0.5Mb T4, 192 rxn	09063072001	KAPA HyperExplore MAX 3Mb T3, 50000 rxn	09063595001
KAPA HyperExplore MAX 0.5Mb T4, 384 rxn	09063099001	KAPA HyperExplore MAX 3Mb T3, 100000 rxn	09063609001
KAPA HyperExplore MAX 0.5Mb T4, 768 rxn	09063102001	KAPA HyperExplore MAX 5Mb, 12 rxn	09063617001
KAPA HyperExplore MAX 0.5Mb T4, 1152 rxn	09063129001	KAPA HyperExplore MAX 5Mb, 24 rxn	09063625001
KAPA HyperExplore MAX 0.5Mb T4, 1536 rxn	09063137001	KAPA HyperExplore MAX 5Mb, 48 rxn	09063633001
KAPA HyperExplore MAX 0.5Mb T4, 4000 rxn	09063145001	KAPA HyperExplore MAX 5Mb, 96 rxn	09063641001
KAPA HyperExplore MAX 3Mb T1, 12 rxn	09063153001	KAPA HyperExplore MAX 5Mb, 192 rxn	09063650001
KAPA HyperExplore MAX 3Mb T1, 24 rxn	09063161001	KAPA HyperExplore MAX 5Mb, 384 rxn	09063668001
KAPA HyperExplore MAX 3Mb T1, 48 rxn	09063170001	KAPA HyperExplore MAX 5Mb, 768 rxn	09063676001
KAPA HyperExplore MAX 3Mb T1, 96 rxn	09063188001	KAPA HyperExplore MAX 5Mb, 1152 rxn	09063684001
KAPA HyperExplore MAX 3Mb T1, 192 rxn	09063196001	KAPA HyperExplore MAX 5Mb, 1536 rxn	09063692001
KAPA HyperExplore MAX 3Mb T1, 384 rxn	09063200001	KAPA HyperExplore MAX 5Mb, 4000 rxn	09063706001
KAPA HyperExplore MAX 3Mb T1, 768 rxn	09063218001	KAPA HyperExplore MAX 5Mb, 10000 rxn	09063714001
KAPA HyperExplore MAX 3Mb T1, 1152 rxn	09063226001	KAPA HyperExplore MAX 5Mb, 20000 rxn	09063722001
KAPA HyperExplore MAX 3Mb T1, 1536 rxn	09063234001	KAPA HyperExplore MAX 5Mb, 50000 rxn	09063749001
KAPA HyperExplore MAX 3Mb T1, 4000 rxn	09063242001	KAPA HyperExplore MAX 5Mb, 100000 rxn	09063757001
KAPA HyperExplore MAX 3Mb T1, 10000 rxn	09063269001	KAPA HyperExplore MAX 20Mb, 12 rxn	09063765001
KAPA HyperExplore MAX 3Mb T1, 20000 rxn	09063277001	KAPA HyperExplore MAX 20Mb, 24 rxn	09063773001
KAPA HyperExplore MAX 3Mb T1, 50000 rxn	09063285001	KAPA HyperExplore MAX 20Mb, 48 rxn	09068414001
KAPA HyperExplore MAX 3Mb T1, 100000 rxn	09063293001	KAPA HyperExplore MAX 20Mb, 96 rxn	09068422001
KAPA HyperExplore MAX 3Mb T2, 12 rxn	09063307001	KAPA HyperExplore MAX 20Mb, 192 rxn	09068449001
KAPA HyperExplore MAX 3Mb T2, 24 rxn	09063315001	KAPA HyperExplore MAX 20Mb, 384 rxn	09068457001
KAPA HyperExplore MAX 3Mb T2, 48 rxn	09063323001	KAPA HyperExplore MAX 20Mb, 768 rxn	09068465001
KAPA HyperExplore MAX 3Mb T2, 96 rxn	09063331001	KAPA HyperExplore MAX 20Mb, 1152 rxn	09068473001
KAPA HyperExplore MAX 3Mb T2, 192 rxn	09063340001	KAPA HyperExplore MAX 20Mb, 1536 rxn	09068481001
KAPA HyperExplore MAX 3Mb T2, 384 rxn	09063358001	KAPA HyperExplore MAX 20Mb, 4000 rxn	09068490001
KAPA HyperExplore MAX 3Mb T2, 768 rxn	09063366001	KAPA HyperExplore MAX 20Mb, 10000 rxn	09068503001
KAPA HyperExplore MAX 3Mb T2, 1152 rxn	09063374001	KAPA HyperExplore MAX 20Mb, 20000 rxn	09068511001
KAPA HyperExplore MAX 3Mb T2, 1536 rxn	09063382001	KAPA HyperExplore MAX 20Mb, 50000 rxn	09068520001
KAPA HyperExplore MAX 3Mb T2, 4000 rxn	09063404001	KAPA HyperExplore MAX 20Mb, 100000 rxn	09068538001
KAPA HyperExplore MAX 3Mb T2, 10000 rxn	09063412001	KAPA HyperExplore MAX 40Mb, 24 rxn	09068546001
KAPA HyperExplore MAX 3Mb T2, 20000 rxn	09063439001	KAPA HyperExplore MAX 40Mb, 48 rxn	09068554001
KAPA HyperExplore MAX 3Mb T2, 50000 rxn	09063447001	KAPA HyperExplore MAX 40Mb, 96 rxn	09068562001
KAPA HyperExplore MAX 3Mb T2, 100000 rxn	09063455001	KAPA HyperExplore MAX 40Mb, 192 rxn	09068589001
KAPA HyperExplore MAX 3Mb T3, 12 rxn	09063463001	KAPA HyperExplore MAX 40Mb, 384 rxn	09068597001
KAPA HyperExplore MAX 3Mb T3, 24 rxn	09063471001	KAPA HyperExplore MAX 40Mb, 768 rxn	09068619001
KAPA HyperExplore MAX 3Mb T3, 48 rxn	09063480001	KAPA HyperExplore MAX 40Mb, 1152 rxn	09068627001
KAPA HyperExplore MAX 3Mb T3, 96 rxn	09063498001	KAPA HyperExplore MAX 40Mb, 1536 rxn	09068635001
KAPA HyperExplore MAX 3Mb T3, 192 rxn	09063501001	KAPA HyperExplore MAX 40Mb, 4000 rxn	09068643001
KAPA HyperExplore MAX 3Mb T3, 384 rxn	09063510001	KAPA HyperExplore MAX 40Mb, 10000 rxn	09068651001
KAPA HyperExplore MAX 3Mb T3, 768 rxn	09063528001	KAPA HyperExplore MAX 40Mb, 20000 rxn	09068660001
KAPA HyperExplore MAX 3Mb T3, 1152 rxn	09063536001	KAPA HyperExplore MAX 40Mb, 50000 rxn	09068678001
KAPA HyperExplore MAX 3Mb T3, 1536 rxn	09063544001	KAPA HyperExplore MAX 40Mb, 100000 rxn	09068686001
KAPA HyperExplore MAX 3Mb T3, 4000 rxn	09063552001	KAPA HyperExplore MAX 60Mb, 24 rxn	09068694001
KAPA HyperExplore MAX 3Mb T3, 10000 rxn	09063579001	KAPA HyperExplore MAX 60Mb, 48 rxn	09068708001

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperExplore MAX 60Mb, 96 rxn	09068716001	KAPA HyperCap Oncology panel	
KAPA HyperExplore MAX 60Mb, 192 rxn	09068724001	KAPA HyperCap Oncology panel, 12 rxn	09462384001
KAPA HyperExplore MAX 60Mb, 384 rxn	09068732001	KAPA HyperCap Oncology panel, 24 rxn	09462457001
KAPA HyperExplore MAX 60Mb, 768 rxn	09068759001	KAPA HyperCap Oncology panel, 48 rxn	09462465001
KAPA HyperExplore MAX 60Mb, 1152 rxn	09068767001	KAPA HyperCap Heredity panel	
KAPA HyperExplore MAX 60Mb, 1536 rxn	09068775001	KAPA HyperCap Heredity panel, 12 rxn	09462473001
KAPA HyperExplore MAX 60Mb, 4000 rxn	09068783001	KAPA HyperCap Heredity panel, 24 rxn	09462481001
KAPA HyperExplore MAX 60Mb, 10000 rxn	09068791001	KAPA HyperCap Heredity panel, 48 rxn	09462490001
KAPA HyperExplore MAX 60Mb, 20000 rxn	09068805001	KAPA HyperCap Heredity panel, 96 rxn	09462503001
KAPA HyperExplore MAX 60Mb, 50000 rxn	09068813001	KAPA HyperCap Heredity panel, 192 rxn	09462511001
KAPA HyperExplore MAX 60Mb, 100000 rxn	09068821001	Other reagents	
KAPA HyperExplore MAX 200Mb, 24 rxn	09068830001	LightCycler® Uracil-DNA Glycosylase	03539806001
KAPA HyperExplore MAX 200Mb, 48 rxn	09068848001		
KAPA HyperExplore MAX 200Mb, 96 rxn	09068856001		
KAPA HyperExplore MAX 200Mb, 192 rxn	09068864001		
KAPA HyperExplore MAX 200Mb, 384 rxn	09068872001		
KAPA HyperExplore MAX 200Mb, 768 rxn	09068899001		
KAPA HyperExplore MAX 200Mb, 1152 rxn	09068902001		
KAPA HyperExplore MAX 200Mb, 1536 rxn	09068929001		
KAPA HyperExplore MAX 200Mb, 4000 rxn	09068937001		
KAPA HyperExplore MAX 200Mb, 10000 rxn	09068945001		
KAPA HyperExplore MAX 200Mb, 20000 rxn	09068953001		
KAPA HyperExplore MAX 200Mb, 50000 rxn	09068961001		
KAPA HyperExplore MAX 200Mb, 100000 rxn	09068970001		
KAPA Target Enrichment, reagent and kits			
KAPA UDI Primer Mixes, 1-96, 96 rxn	09134336001		
KAPA UDI Primer Mixes, 97-192, 96 rxn	09053603001		
KAPA UDI Primer Mixes, 193-288, 96 rxn	09053611001		
KAPA UDI Primer Mixes, 289-384, 96 rxn	09053620001		
KAPA Universal Adapter, 15uM 960 uL	09063781001		
KAPA Universal Adapter, 15uM 4x960 uL	09063790001		
KAPA Universal UMI Adapter, 960 uL	09329862001		
KAPA Universal UMI Adapter, 4x960 uL VK	09329889001		
KAPA HyperCapture Reagent kit, 24 rxn	09075810001		
KAPA HyperCapture Reagent kit, 96 rxn	09075828001		
KAPA HyperCapture Reagent kit, 384 rx VK	09075917001		
KAPA HyperCapture Bead kit, 24 rxn	09075780001		
KAPA HyperCapture Bead kit, 96 rxn	09075798001		
KAPA HyperCapture Bead kit, 384 rxn VK	09075909001		
KAPA Universal Enhancing Oligos, 24 rxn	09075836001		
KAPA Universal Enhancing Oligos, 96 rxn	09075852001		
KAPA Universal Enhancing Oligos 384rx VK	09075895001		
KAPA Hybrid Enhancer Reagent, 1 mL	09075763001		
KAPA Probes resuspension buffer, 1 mL	09075879001		
KAPA Probes resuspension buffer, 5 mL	09075887001		

sequencing.roche.com

Roche Sequencing Solutions, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588 USA

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