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KAPA HyperCap Workflow v3.4

Instructions For Use with:

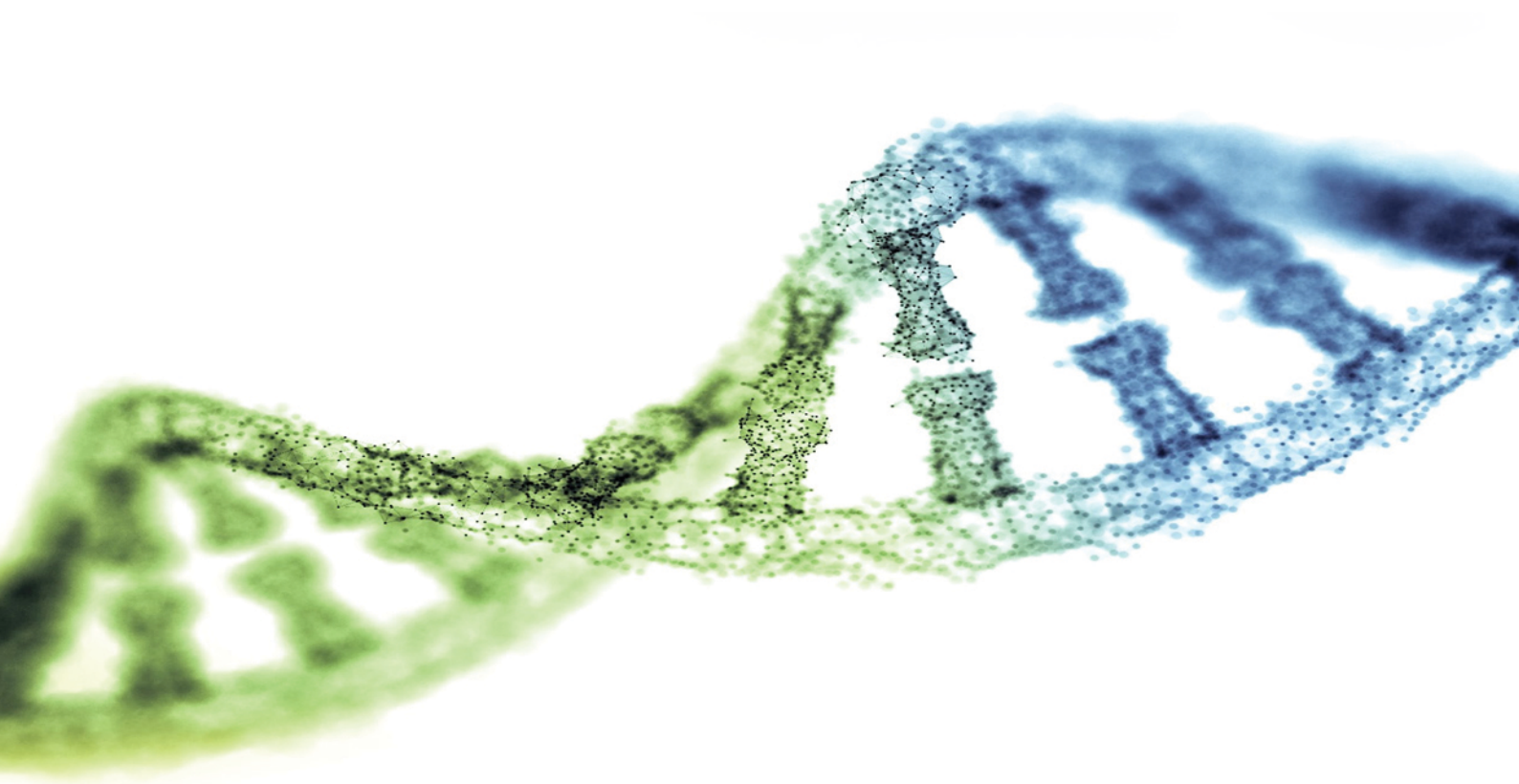
KAPA HyperExome Probes,

KAPA HyperExome V2 Probes,

KAPA HyperCap Heredity Panel,

KAPA HyperChoice, and

KAPA HyperExplore Probes



Contents

The KAPA HyperCap Target Enrichment Probes package contains:

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

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 Probes, KAPA HyperCap Fixed Panels, KAPA HyperChoice Probes, and KAPA HyperExplore Probes.

Storage and Stability

- KAPA HyperCap Target Enrichment 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 HyperExome Portfolio enables targeted enrichment of human coding exons. The KAPA HyperExome Portfolio utilizes empirically optimized designs which deliver high-performance enrichment in terms of coverage and uniformity. Refer to [design-specific documentation and design files](#) for more details on each specific exome design. KAPA HyperExome Probes is a whole exome sequencing design that targets the GRCh38/hg38 human genome assembly. It covers 20859 genes across CCDS, RefSeq, Ensembl, GENCODE, ClinVar (pathogenic, likely pathogenic and variants of unknown significance) in a capture target of 43 Mb. KAPA HyperExome Probes also enable accurate, convenient sample tracking by intrinsically targeting 387 carefully selected SNPs to improve the reliability and confidence of the results.

The KAPA HyperExome V2 Probes deliver exceptional coverage of the recent versions of ACMGv3.1, RefSeq (June 29, 2022), CCDS, ClinVar (June 29, 2022), Ensembl v106 and COSMIC genomic databases across 20626 genes within a compact capture target of 43.2 Mb and with low sequencing requirements. The new T2T (telomere-to-telomere) genome assembly was utilized as part of the design process to identify potentially problematic regions not apparent in the GRCh38 genome assembly. The number of sample tracking SNPs has increased to 529.

The KAPA HyperCap Heredity panel is a 10 Mb capture target panel covering 3332 genes strongly associated with hereditary disease, plus the ClinVar pathogenic and likely pathogenic variants content. It provides an optimal balance between relevant content carefully selected by the Roche Sequencing Scientists and sequencing efficiency driven from exceptional uniformity and very low duplication rates.

KAPA HyperChoice Probes enable 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 product is intended for capture of human target regions up to 200 Mb.

KAPA HyperExplore product 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 design-specific documentation and design files for more details on specific designs at www.hyperdesign.com.

Warnings and Precautions

- Wear appropriate personal protective equipment, such as gloves and safety glasses, to avoid direct contact while handling the reagents.
- Use good laboratory practices to avoid contamination when working with the reagents.
- Do not eat, drink or smoke in laboratory area.
- 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 and included information about the KAPA HyperExome V2 Probes and related kits.

Ordering Information

For complete overview of Roche Sequencing products, including those used in the KAPA HyperCap Workflow v3.4 go to sequencing.roche.com/products.

Trademarks

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Contact and Support

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Preface

Regulatory Disclaimer

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Contact Information

Technical Support



If you have questions, contact your local Roche Technical Support.
Go to sequencing.roche.com/support 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.
<i>Italic type, blue</i>	Highlights a resource in a different area of this manual or on a web site.
<i>Italic type</i>	Identifies the external general resources or names.
Bold type	Identifies names of paragraphs, sections or emphasized words.

Chapter 1. Before You Begin

These Instructions for Use describe the process of using KAPA HyperCap Target Enrichment Probes to enrich individual or multiplexed genomic DNA (gDNA) sample libraries. Prior to target enrichment, the sample libraries are prepared by using the KAPA HyperPrep, or the KAPA HyperPlus or the KAPA EvoPlus Kits by following the protocol that is outlined in [Figure 1](#). Modification of certain workflow steps may be appropriate for individual experimental needs (contact your local support). The output of this protocol are enriched gDNA libraries that can be sequenced using an Illumina sequencing instrument.

The KAPA HyperCap Target Enrichment portfolio supported from the KAPA HyperCap Workflow v3.4 provides:

- High performance based on proprietary probe design algorithms and optimized kits and reagents:
 - Enhanced capture uniformity
 - Fewer PCR duplicates
 - Deeper target coverage
 - Higher sensitivity in SNP detection
- 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 targeted NGS sample preparation:
 - KAPA HyperPrep and KAPA HyperPlus and KAPA EvoPlus Kits
 - KAPA Universal Adapter and 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.

Overview of the KAPA HyperCap Workflow v3.4

The KAPA HyperCap Workflow v3.4 involves:

Workflow Step	Processing Time
Prepare the Sample Library: Mechanical Shearing (KAPA HyperPrep Kit) or Enzymatic Fragmentation (KAPA HyperPlus Kit or KAPA EvoPlus Kit)	2.5 h
↓	
Amplify and Purify the Sample Library Using the KAPA UDI Primer Mixes and KAPA HyperPure Beads*	0.75 h
↓	
Prepare the Multiplex DNA Sample Library Pool (if Multiplexing Several Samples)	0.5 h
↓	
Hybridize the Amplified Sample Library to KAPA HyperCap Target Enrichment Probes	0.5 h
↓	16 - 20 h incubation
Wash and Recover Captured Singleplex or Multiplex DNA Sample Library	0.75 h
↓	
Amplify and Purify the Enriched Singleplex or Multiplex DNA Sample Library*	1 h
↓	
Illumina Sequencing	

Figure 1: KAPA HyperCap Workflow v3.4, with mechanical or enzymatic DNA fragmentation. Where applicable, incubation times are indicated between steps. Processing time may vary based on the number of samples processed and multiplexing levels.

* Safe stopping points, see more details in the following Instructions for Use.

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 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 selecting 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 genomic DNA by fragmentation and ligation of sequencing platform-specific adapters. In the context of this document, this is the sample library before amplification and prior to capture.

Amplified Sample Library: The sample library after amplification by adapter ligation-mediated PCR but before capture.

UDI Primer Mixes: Unique Dual-Indexed Primer Mixes.

Prepare the Following Equipment and Reagents

Thermocyclers should be programmed with the following:

KAPA HyperPlus Kit

- Fragmentation program ([Chapter 3, Prepare the Sample Library, Step 2a.4](#))
- End Repair and A-tailing program ([Chapter 3, Prepare the Sample Library, Step 2a.6.e](#))
- Adapter Ligation program ([Chapter 3, Prepare the Sample Library, Step 2a.7.e](#))

KAPA HyperPrep Kit

- End Repair and A-tailing program ([Chapter 3, Prepare the Sample Library, Step 2b.5.e](#))
- Adapter Ligation program ([Chapter 3, Prepare the Sample Library, Step 2b.6.e](#))

KAPA EvoPlus Kit

- Fragmentation and A-tailing program ([Chapter 3, Prepare the Sample Library, Step 2c.5](#))
- Adapter Ligation program ([Chapter 3, Prepare the Sample Library, Step 2c.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 Libraries to KAPA HyperCap Target Enrichment Probes, Step 4.7](#))

Post-Capture PCR program ([Chapter 7, Amplify Enriched Singleplex or Multiplex DNA Sample, Step 3.1](#))



It is recommended to use a thermocycler with a programmable heated lid. For guidance on lid temperatures, please follow the recommended lid temperatures in these Instructions for Use. If further guidance is needed, please contact [Roche Technical Support](#).

Take the following steps 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 the Post-Capture PCR Oligos ([Chapter 7, Amplify Enriched Singleplex or Multiplex DNA Sample, Step 1](#))



To verify you are using the most up-to-date version of these Instructions For Use, go to [Roche Technical Support](#).

Required Equipment, Labware & Consumables

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

Laboratory Equipment

Equipment	Supplier	Catalog No.
Covaris Ultra Sonicator (optional)	Covaris	Multiple models (e.g. S220, E220)
DynaMag-2 Magnet (16 x 2.0 mL tube holder) (optional) 0.2 mL PCR Strip Magnetic Separator Rack	Thermo Fisher Permagen	12321D MSR812
DynaMag-96 Side Magnet	Thermo Fisher	12331D
Microcentrifuge (16,000 x g capability)	<i>Multiple Vendors</i>	
Qubit Fluorometer	Thermo Fisher	Multiple models
Bioanalyzer 2100	Agilent	
Thermocycler (capable of maintaining +55°C for 16 - 20 hours), with programmable heated lid	<i>Multiple Vendors</i>	
Vortex mixer	<i>Multiple Vendors</i>	
Plate Centrifuge which can reach at least 280 x g	<i>Multiple Vendors</i>	

Consumables Available from Roche

Component	Package Size/Contents	Catalog No.
KAPA Universal Adapter	96 reactions 384 reactions*	09 063 781 001 09 063 790 001
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 HyperPrep Kit	8 reactions 24 reactions 96 reactions	07 962 312 001 07 962 347 001 07 962 363 001
KAPA HyperPlus Kit	8 reactions 24 reactions 96 reactions	07 962 380 001 07 962 401 001 07 962 428 001
KAPA EvoPlus Kit	24 reactions 96 reactions 384 reactions*	09 420 037 001 09 420 053 001 09 420 339 001

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

Chapter 1. Before You Begin

Component	Package Size/Contents	Catalog No.
KAPA HyperPure Beads	5 mL	08 963 835 001
	30 mL	08 963 843 001
	60 mL	08 963 851 001
	4 x 60 mL	08 963 878 001
	450 mL	08 963 860 001
KAPA Hybrid Enhancer Reagent ¹	1 mL	09 075 763 001
KAPA Probes Resuspension Buffer	1 mL	09 075 879 001
	5 mL	09 075 887 001
KAPA HyperCap Target Enrichment Probes	Multiple	Multiple, Please see Appendix C.
KAPA HyperExome Prep Kit ²	For 192 samples, contains: 2 x KAPA HyperPrep Kit 96 rxn – 07 962 363 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome Probes, 24 rxn – 09 062 556 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 107 592 001
KAPA HyperExome Plus Kit ²	For 192 samples, contains: 2 x KAPA HyperPlus Kit 96 rxn – 07 962 428 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome Probes, 24 rxn – 09 062 556 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 107 606 001
KAPA HyperExome Evo Kit ²	For 192 samples, contains: 2 x KAPA EvoPlus Kit 96 rxn – 09 420 053 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome Probes, 24 rxn – 09 062 556 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 844 848 001

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

1. To replace COT Human DNA in hybridizations with libraries generated from non-human samples.

2. Virtual Kit, contains required reagents for 192 samples, does not include the KAPA UDI Primer Mixes, which have to be ordered separately.

Chapter 1. Before You Begin

Component	Package Size/Contents	Catalog No.
KAPA HyperExome V2 Prep Kit ¹	For 192 samples, contains: 2 x KAPA HyperPrep Kit 96 rxn – 07 962 363 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome V2 Probes, 24 rxn – 09 718 648 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 983 759 001
KAPA HyperExome V2 Plus Kit ¹	For 192 samples, contains: 2 x KAPA HyperPlus Kit 96 rxn – 07 962 428 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome V2 Probes, 24 rxn – 09 718 648 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 983 775 001
KAPA HyperExome V2 Evo Kit ¹	For 192 samples, contains: 2 x KAPA EvoPlus Kit 96 rxn – 09 420 053 001 2 x KAPA Universal Adapter, 15 µM 960 µL – 09 063 781 001 1 x KAPA HyperPure Beads Kit (30 ml) – 08 963 843 001 1 x KAPA HyperPure Beads Kit (5 ml) – 08 963 835 001 1 x KAPA HyperExome V2 Probes, 24 rxn – 09 718 648 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24 rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24 rxn – 09 075 780 001	09 983 783 001

1. Virtual Kit, contains required reagents for 192 samples, does not include the KAPA UDI Primer Mixes, which have to be ordered separately.

Consumables Purchased from Other Vendors

Component	Supplier	Package Size	Catalog No.
Agilent High Sensitivity DNA Kit (recommended)	Agilent	1 kit	5067-4626
Agilent DNA 1000 Kit (optional)	Agilent	1 kit	5067-1504
10 mM Tris-HCl, pH 8.0	<i>Multiple Vendors</i>		
Ethanol, 200 proof (absolute), for molecular biology	Sigma-Aldrich	500 mL	E7023-500ML
Covaris microTUBEs (required only if using KAPA HyperPrep Kit)	Covaris, Inc.	1 package of tubes, strips or plates	520166, 520167, 520174, 520168
TE Buffer (low EDTA): 10 mM Tris-HCl, 0.1 mM EDTA pH 8.0, (required only if using KAPA HyperPrep Kit)	<i>Multiple Vendors</i>	100 mL	
Qubit dsDNA HS Assay Kit	Thermo Fisher	1 kit	Q32851
Qubit Assay Tubes	Thermo Fisher	1 package of 500 tubes	Q32856
Tubes: • 0.2 mL PCR tubes or strip-tubes, (preferably low-bind) • 1.5 mL microcentrifuge tubes (optional)	<i>Multiple Vendors</i>		
PCR grade water	Sigma-Aldrich	1 x 25 mL 25 x 1 mL 4 x 25 mL	03 315 959 001 03 315 932 001 03 315 843 001



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 Universal Adapter
- KAPA HyperPure Beads
- KAPA HyperPrep Kit or KAPA HyperPlus Kit or KAPA EvoPlus Kit
- KAPA HyperCapture Bead Kit
- KAPA UDI Primer Mixes
- KAPA Probes Resuspension Buffer
- KAPA HyperCapture Reagent Kit
- KAPA HyperCap Target Enrichment Probes

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 Adapter	-15°C to -25°C
KAPA UDI Primer Mixes or KAPA UDI Primer Mixes (resuspended)	+2°C to +8°C or -15°C to -25°C
KAPA 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 Hybrid Enhancer Reagent (if using KAPA HyperExplore Probes with non-human targets)	-15°C to -25°C
KAPA HyperPrep Kit or KAPA HyperPlus Kit or KAPA EvoPlus 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 at 10,000 x g for 30 seconds to ensure the contents are at the bottom of the tube.
3. Add the recommended volume of fresh KAPA Probes Resuspension Buffer indicated 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) into 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 hybridization portion of 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:

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



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

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



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

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



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

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



Ensure aliquoted KAPA UDI Primer Mixes are correctly labeled.

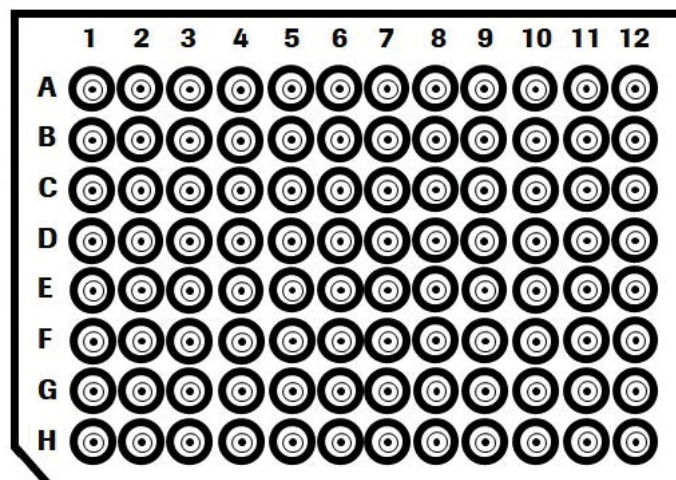


Figure 2: KAPA UDI Primer Mixes plate layout.

Chapter 3. Prepare the Sample Library

This chapter describes the three alternative sample library preparation methods. If mechanically fragmenting the gDNA with a Covaris focused-ultrasonicator, use the KAPA HyperPrep Kit. The KAPA HyperPlus Kit and the KAPA EvoPlus Kit are used for enzymatically fragmenting gDNA. This chapter requires use of components from the following kits:

- KAPA HyperPlus Kit, KAPA HyperPrep Kit or KAPA EvoPlus Kit
- KAPA Universal Adapter
- KAPA UDI Primer Mixes
- KAPA HyperPure Beads

Ensure that the following are available:

- TE Buffer (low EDTA): 10 mM Tris-HCl, 0.1 mM EDTA pH 8.0 (KAPA HyperPrep Kit only)
- PCR grade water
- Freshly-prepared 80% ethanol
- 10 mM Tris-HCl, pH 8.0

References

- *Covaris Focused-ultrasonicator User's Guide*
- *Thermocycler Manual*





Sample Requirements

This workflow was validated with 100 ng of high quality gDNA for sample library preparation. The gDNA should be quantified by using the Qubit dsDNA HS Assay Kit. Lower input amounts and sample quality may not yield equivalent results. For guidance on lower input amounts or sample quality, contact us at sequencing.roche.com/contactus.

Step 1. Prepare the Sample Library

- With the KAPA HyperPlus Kit – integrated enzymatic fragmentation, or
- With the KAPA HyperPrep Kit – mechanical fragmentation (skip Step 2a below)
- With the KAPA EvoPlus Kit – integrated enzymatic fragmentation and A-tailing (skip Steps 2a and 2b below)

Step 2a. If using the KAPA HyperPlus Kit

-  Make sure the 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 gDNA so that the average DNA fragment size is 180 – 220 bp.
-  **Enzymatic fragmentation is very sensitive to the presence of EDTA, which must be removed or neutralized prior to fragmentation.**
Please refer to the [KAPA HyperPlus Kit Technical Data Sheet](#) for further guidance on fragmentation optimization and EDTA removal from samples.

Chapter 3. Prepare the Sample Library

1. Dilute 100 ng of gDNA with 10 mM Tris-HCl, pH 8.0 to a total volume of 35 μL into a 0.2 mL PCR tube or well of a PCR plate.
2. Assemble each Fragmentation Reaction on ice by adding the components in the order shown:

Component	Volume
100 ng gDNA	35 μL
KAPA Frag Buffer (10x)	5 μL
KAPA Frag Enzyme	10 μL
Total	50 μL



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

3. Mix the Fragmentation Reaction thoroughly and return the plate/tube(s) on ice. Proceed immediately to the next step.
4. Incubate in a thermocycler, pre-cooled to +4°C and programmed as outlined below. Set the lid temperature to +50°C:
 - a. Pre-cool block: +4°C
 - b. Fragmentation: 25 minutes at +37°C
 - c. Hold: +4°C
5. Quickly transfer the reaction on ice and proceed immediately to the next step.
6. Perform End Repair and A-Tailing Reaction as follows:
 - a. Prepare a master mix of the following reagents:

End Repair & A-Tailing Master Mix	Per Individual Sample
KAPA 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 KAPA 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 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:
 - Step 1: 30 minutes at +65°C
 - Step 2: Hold at +4°C
 - f. Following the 30 minute incubation, proceed immediately to the next step.
7. Proceed with the Adapter Ligation Reaction:
 - a. Prepare the Ligation Master Mix of the following

Ligation Master Mix	Per Individual Sample
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.

Chapter 3. Prepare the Sample Library

- b. Add 10 μL of KAPA Universal Adapter to wells containing a sample from the previous [step \(6.f\)](#).



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



The KAPA Universal UMI Adapter may be used instead of the KAPA Universal Adapter in somatic oncology research applications. Follow the [Instructions For Use](#) of the KAPA HyperCap FFPE DNA Workflow or the KAPA HyperCap cfDNA Workflow, instead.

- c. Add 40 μL of the Ligation Master Mix to each well containing sample and KAPA Universal 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 15 minutes with lid temperature set to +50°C.
- f. Following the incubation, proceed immediately to the next step.
8. Perform the Post-ligation Cleanup as follows:
- a. To each Ligation Reaction, add 88 μL of room temperature KAPA HyperPure Beads that have been thoroughly resuspended.

Post-Ligation Cleanup	Per Individual Sample
Ligation Reaction	110 μL
KAPA HyperPure Beads	88 μL
Total	198 μL

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



Ensure that the solution is thoroughly mixed and appears homogenous. Insufficient mixing may compromise recovery and size selection.

- c. Incubate at room temperature for 5 minutes to allow the DNA to bind to the beads.
- d. Place the tube(s) on a magnet to collect the beads. Incubate until the liquid 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. Remove residual ethanol without disturbing the beads.
- l. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.



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

- m. Remove the tube(s) from the magnet.
- n. Thoroughly resuspend the beads in 22 μL of 10 mM Tris-HCl, pH 8.0.
- 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 collect the beads. Incubate until the liquid is clear.
- q. Transfer 20 μL of the eluate 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.

Step 2b. If using the KAPA HyperPrep Kit



Make sure the 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).

1. Transfer 100 ng of the gDNA sample into a 0.2 mL PCR tube.
2. Adjust the volume to a total of 55 μ L using TE Buffer (low EDTA) and transfer to a Covaris microTUBE-50 for fragmentation. For other Covaris shearing consumables, please consult the *Covaris user manual* for guidance.
3. Fragment the gDNA to achieve an average DNA fragment size between 180 – 220 bp. Consult *manufacturer's instructions* for appropriate parameters to achieve this size distribution.
4. Transfer 50 μ L of the fragmented DNA to a 0.2 mL PCR tube or well of a PCR plate. Proceed immediately to the next step.
5. Perform the End Repair and A-Tailing Reaction as follows:
 - a. Prepare a master mix of the following reagents:

End Repair & A-Tailing Master Mix	Per Individual Sample
KAPA End Repair & A-Tailing Buffer	7 μ L
KAPA HyperPrep End Repair & A-Tailing Enzyme Mix (purple cap color)	3 μ L
Total	10 μL

- b. Add 10 μ L of End Repair and A-Tailing Master Mix to the fragmented DNA sample to obtain a total volume of 60 μ L.
 - c. Mix the End Repair and A-Tailing Reaction thoroughly.
 - 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.
 - Step 1: 30 minutes at +20°C
 - Step 2: 30 minutes at +65°C
 - Step 3: Hold at +4°C
 - f. Following the 60 minute incubation proceed immediately to the next step.
6. Perform the Adapter Ligation Reaction as follows:
 - a. Prepare a master mix of the following reagents and set aside:

Ligation Master Mix	Per Individual Sample
KAPA Ligation Buffer	30 μ L
KAPA DNA Ligase	10 μ L
Total	40 μL



The KAPA Ligation Buffer is very viscous and requires special attention during pipetting.

- b. First, add 10 μ L of the KAPA Universal Adapter to each well containing a sample from the previous [step \(5.f\)](#).



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



The KAPA Universal UMI Adapter may be used instead of the KAPA Universal Adapter in somatic oncology research applications. Follow the *Instructions For Use* of the KAPA HyperCap FFPET DNA Workflow or the KAPA HyperCap cfDNA Workflow, instead.

Chapter 3. Prepare the Sample Library

- c. Add 40 μL of Ligation Master Mix to each well containing a sample and KAPA Universal 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 15 minutes with the lid temperature set to +50°C.
 - f. Following the incubation, proceed immediately to the next step.
7. Perform the Post-ligation Cleanup as follows:
- a. To each Ligation Reaction, add 88 μL room temperature, thoroughly resuspended, KAPA HyperPure Beads.

Post-Ligation Cleanup	Per Individual Sample
Ligation Reaction	110 μL
KAPA HyperPure Beads	88 μL
Total	198 μL

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



Ensure that the solution is thoroughly mixed and appears homogenous. Insufficient mixing may compromise recovery and size selection.

- c. Incubate at room temperature for 5 minutes to allow the DNA to bind to the beads.
- d. Place the tube(s) on the magnet to collect the beads. Incubate until the liquid 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. Remove residual ethanol without disturbing the beads.
- l. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.



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

- m. Remove the tube(s) from the magnet.
- n. Thoroughly resuspend the beads in 22 μL of 10 mM Tris-HCl, pH 8.0.
- 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 collect the beads. Incubate until the liquid is clear.
- q. Transfer 20 μL of the eluate 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.

Step 2c. If using the KAPA EvoPlus Kit



Make sure the 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.



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 gDNA so that the average DNA fragment size is 180 - 220 bp.



It is important to the success of the library preparation workflow to ensure that KAPA EvoPlus Kit components have been fully thawed and thoroughly mixed before use. Specifically, the FragTail ReadyMix contains a high concentration of PEG 6000 and glycerol, and is very viscous, so ensure this solution is sufficiently mixed. Pipette-mix at least 10X or vortex mix for 10 - 20 seconds. In some cases there may be small PEG 6000 droplets visible in the FragTail ReadyMix. Ensure the buffer is thoroughly vortexed until the droplets have been resuspended.

Please refer to the [KAPA EvoPlus Kit Instructions For Use](#) for further guidance on fragmentation optimization.

1. Dilute 100 ng of gDNA with 10 mM Tris-HCl, pH 8.0 to a total volume of 35 μ L into a 0.2 mL PCR tube or well of a PCR plate.
2. Mix the KAPA FragTail ReadyMix thoroughly by pulse vortexing and briefly spin down.
3. Assemble each Fragmentation and A-tailing Reaction as follows:

FragTail Reaction	Per Individual Sample
100 ng gDNA	35 μ L
KAPA FragTail ReadyMix	25 μ L
Total	60 μL



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

4. Mix the FragTail Reaction thoroughly and return the plate/tube(s) on ice. Proceed immediately to the next step.
5. Incubate in a thermocycler, pre-cooled to +4°C. Set the lid temperature to +65°C and program as detailed below:
 - a. Pre-cool block: +4°C
 - b. Fragmentation: 18 minutes at +37°C
 - c. A-tailing: 30 minutes at +55°C
 - d. Hold: +4°C
6. Quickly transfer the reaction on ice and proceed immediately to the next step.
7. Proceed with the Adapter Ligation Reaction:
 - a. Keeping on ice and in the same well(s)/tube(s) in which the FragTail Reaction was performed, assemble the Ligation Reaction by adding the components in the following order:
 - b. Add 5 μ L of the KAPA Universal Adapter to each tube/well. Ensure that you add only 5 μ L of the KAPA Universal Adapter when using the KAPA EvoPlus Kit.



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

- c. Add 10 μ L of the Ligation ReadyMix to each tube/well containing 60 μ L sample and 5 μ L KAPA Universal Adapter, resulting in a total volume of 75 μ L.

Ligation Reaction	Per Individual Sample
FragTail Reaction Product	60 μ L
KAPA Universal Adapter	5 μ L
Ligation ReadyMix	10 μ L
Total	75 μL



The KAPA Ligation ReadyMix is very viscous and requires special attention during pipetting.

- d. Mix the Ligation Reaction thoroughly and perform a quick spin.
 - e. Incubate the Ligation Reaction at +20°C on a thermocycler for 15 minutes with lid temperature set to +50°C.
 - f. Following the incubation, proceed immediately to the next step.
8. Perform the Post-Ligation Cleanup as follows:
- a. To each Ligation Reaction, add 60 μ L of room temperature KAPA HyperPure Beads that have been thoroughly resuspended.

Post-Ligation Cleanup	Per Individual Sample
Ligation Reaction	75 μ L
KAPA HyperPure Beads	60 μ L
Total	135 μL

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



Ensure that the solution is thoroughly mixed and appears homogenous. Insufficient mixing may compromise recovery and size selection.

- c. Incubate at room temperature for 5 minutes to allow the DNA to bind to the beads.
- d. Place the tube(s) on a magnet to collect the beads. Incubate until the liquid is clear.
- e. Carefully remove and discard the supernatant.
- f. Keeping the tube(s) on the magnet, add 200 μ L of freshly-prepared 80% ethanol.
- g. Incubate at room temperature for \geq 30 seconds.
- h. Carefully remove and discard the ethanol.
- i. Keeping the tube(s) on the magnet, add 200 μ L of freshly-prepared 80% ethanol.
- j. Incubate at room temperature for \geq 30 seconds.
- k. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
- l. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate.



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

- m. Remove the tube(s) from the magnet.
- n. Thoroughly resuspend the beads in 22 μ L of 10 mM Tris-HCl, pH 8.0.
- 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 collect the beads. Incubate until the liquid is clear.
- q. Transfer 20 μ L of the eluate 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 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 HyperPrep Kit, or KAPA HyperPlus Kit, or KAPA EvoPlus 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 (pre-capture and/or post-capture), please refer to the [KAPA UDI Primer Mixes Instructions for Use](#).



Make sure the 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 μ L of a KAPA UDI Primer Mix to each individual Sample Library.
5. Add 25 μ L of KAPA HiFi HotStart ReadyMix to each mixture of Sample Library and KAPA UDI Primer Mixes.
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

- Place the sample in the thermocycler and amplify using 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, Variable (see table below for recommendation)
 - Step 6: 1 minute at +72°C
 - Step 7: Hold at +4°C

Library Preparation Kit	Go to Step 2:
KAPA HyperPrep Kit	7 times (8 total cycles)
KAPA HyperPlus Kit	5 times (6 total cycles)
KAPA EvoPlus Kit	6 times (7 total cycles)

- Proceed immediately to the next step.

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

- Add 70 µL of room temperature, thoroughly resuspended, KAPA HyperPure Beads to each Amplified Sample Library.
- Mix the Amplified Sample Library and KAPA HyperPure Beads thoroughly and perform a quick spin.



Ensure that the solution is thoroughly mixed and appears homogenous. Insufficient mixing may compromise recovery and size selection.

- Incubate the tube(s) at room temperature for 5 minutes to allow the DNA to bind to the beads.
- Place the tube(s) on a magnet to collect the beads. Incubate until the liquid is clear.
- Carefully remove and discard the supernatant.
- Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
- Incubate at room temperature for ≥30 seconds.
- Carefully remove and discard the ethanol.
- Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
- Incubate at room temperature for ≥30 seconds.
- Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
- Allow the beads to dry at room temperature, sufficiently for all of the ethanol to evaporate.



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

- Remove the tube(s) from the magnet.
- Thoroughly resuspend the beads in 32 µL of PCR grade water.
- Incubate at room temperature for 2 minutes to allow the DNA to elute off the beads.
- Place the tube(s) on the magnet to collect the beads. Incubate until the liquid is clear.
- Transfer 30 µL of the supernatant to a new tube/well.
- 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

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



Accurate quantification is essential when working with samples that will be pooled for hybridization (i.e., pre-capture multiplexing). 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.

- The undiluted Amplified Sample Library should contain ≥ 1000 ng of total DNA. If the library contains <1000 ng of total DNA, please refer to the [troubleshooting section](#) for guidance.
 - The negative control yield should be <1 ng/ μL .
3. Use 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, refer to the *user manual* for guidance.
 - Amplified Sample Libraries (pre-capture libraries) should have an average fragment size distribution at ~ 320 bp ([Figure 3](#), [Figure 4](#), [Figure 5](#)) with a range setting at 150 to 1000 bp on the Bioanalyzer. Sharp peaks may be visible in the region <150 bp. These peaks correspond to unincorporated primers, primer-dimers or carryover adapter-dimers and will not interfere with the capture process.
 - 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.
 4. If the Amplified Sample Library meets requirements, proceed to [Chapter 5](#). If the Amplified Sample Library does not meet these requirements, repeat the library preparation.

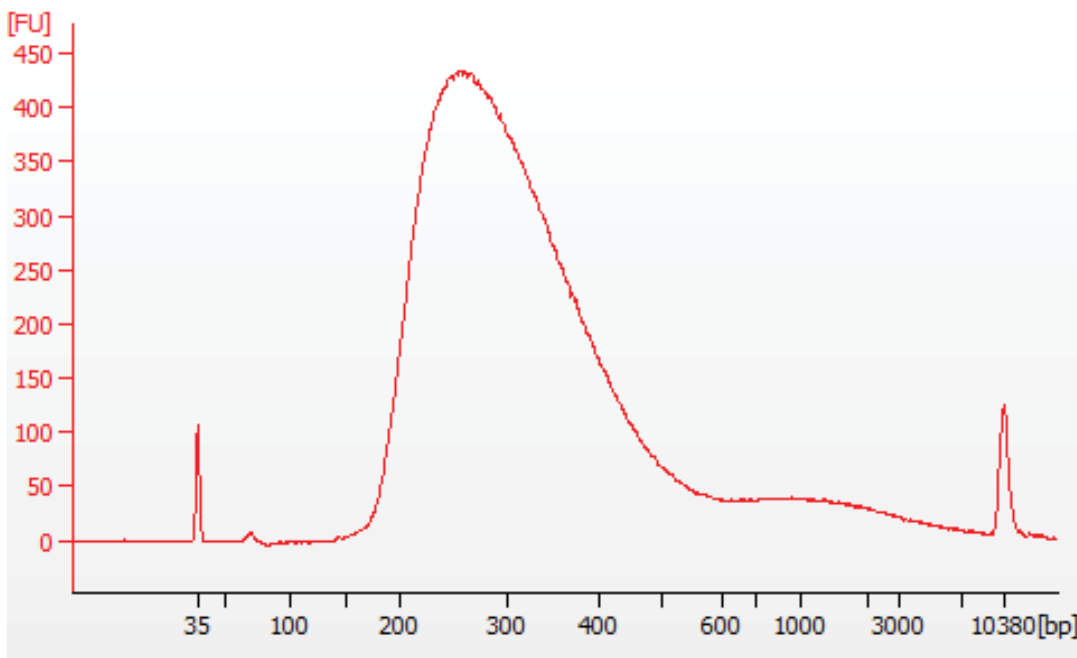


Figure 3: Example of an amplified KAPA HyperPlus sample library analyzed using the Agilent Bioanalyzer High Sensitivity DNA assay.

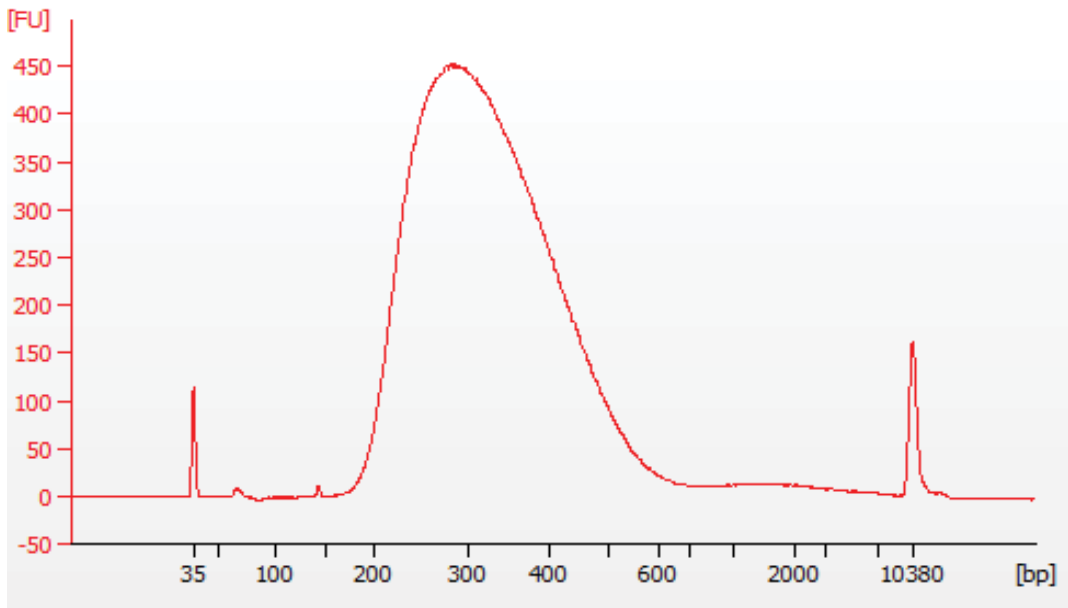


Figure 4: Example of an amplified KAPA HyperPrep sample library analyzed using the Agilent Bioanalyzer High Sensitivity DNA assay.

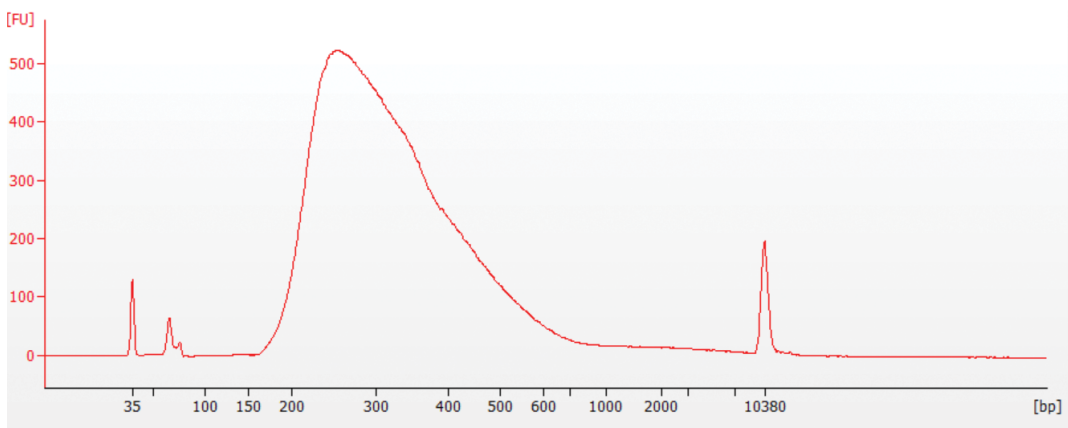


Figure 5: Example of an amplified KAPA EvoPlus sample library analyzed using the Agilent Bioanalyzer High Sensitivity DNA assay.

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

This chapter describes the hybridization of the Amplified Sample Libraries to the KAPA HyperCap Target Enrichment Probes. The following protocol provides instructions based on capture target size (found in coverage_summary.txt design deliverable file) and categorized as <40 Mb or ≥40 Mb panel capture target size. For optimal performance, it is recommended to review the panel design in [HyperDesign Tool](#) and determine the hybridization conditions to follow before proceeding. This chapter requires the use of the 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.



In this chapter we use the term ‘Multiplex DNA Sample Library Pool’, however a single DNA sample library may be enriched using similar instructions. It is not required to capture more than one library at a time.

Step 1. Prepare for Hybridization

1. Remove the appropriate number of 4 µL KAPA HyperCap Target Enrichment Probe aliquots (one per hybridization) from the -15°C to -25°C storage and allow them to thaw on ice.

Step 2. Prepare the Singleplex or Multiplex DNA Sample Library Pool

1. Thaw on ice the Amplified DNA Sample Libraries that will be included in the capture experiment (generated in [Chapter 4](#)).
2. Prepare the Singleplex / Multiplex DNA Sample Library by doing the following:
 - a. For multiplex samples, mix together equal amounts (by mass) of each uniquely indexed Amplified DNA Sample Libraries to obtain a combined DNA mass of 1.5 µg (*i.e.*, for a two-plex Amplified DNA Sample Library Pool, mix together 750 ng of each uniquely indexed Amplified DNA Sample Library for a total mass of 1.5 µg). This mixture will subsequently be referred to as the ‘Multiplex DNA Sample Library Pool’.



To obtain equal numbers of sequencing reads from libraries in the Multiplex DNA Sample Library Pool, it is very important to combine identical mass of each independently Amplified DNA Sample Library at this step. Accurate quantification and pipetting are critical.

- b. If capturing a single sample, utilize 1000 ng of a uniquely indexed Amplified DNA Sample Library.
3. Add PCR grade water to achieve a final volume 45 µL.



If the Multiplex DNA Sample Library Pool exceeds 45 µL, please refer to the [troubleshooting section](#) for further guidance.

Step 3. Prepare the Sample for Hybridization

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



Make sure the 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 working with non-human gDNA, consider using the KAPA Hybrid Enhancer Reagent (catalog number 09 075 763 001) in place of COT Human DNA. Optimization is needed when using the KAPA Hybrid Enhancer Reagent, but a good starting point is to add 20 μL of this reagent to each hybridization instead of COT Human DNA.

1. Add 20 μL of COT Human DNA to the Amplified DNA Sample Library (or Pool), for a total volume of 65 μL .
2. Add 130 μL of KAPA HyperPure Beads to each tube/well containing the Amplified Sample Library and COT Human DNA.
3. Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
4. Incubate at room temperature for 10 minutes to ensure the Amplified Sample Library and COT Human DNA bind to the beads.
5. Place the tube(s) on the magnet to collect the beads. Incubate until the liquid is clear.
6. Carefully remove and discard the supernatant.
7. Keeping the tube(s) on the magnet, add 200 μL of freshly-prepared 80% ethanol.
8. Incubate at room temperature for ≥ 30 seconds.
9. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads.
10. 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.

11. Add 13.4 μL of the Universal Enhancing Oligos to the bead-bound DNA Sample.
12. Remove the tube(s) from the magnet and mix thoroughly by vortexing. It is important that sufficient mixing is performed to result in a homogenous mixture.

Step 4. Prepare the Hybridization Reaction

1. Prepare the Hybridization Master Mix following the tables below according to the capture target size.
 - a. For a KAPA HyperCap Target Enrichment design < 40 Mb in capture target size, prepare a master mix of the following reagents:

< 40 Mb 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

- b. For a KAPA HyperCap Target Enrichment design ≥ 40 Mb in capture target size, prepare a master mix of the following reagents:

>= 40 Mb Capture Target Size – Hybridization Master Mix	Per Individual Capture
Hybridization Buffer	28 µL
Hybridization Component H	9 µL
PCR grade water	6 µL
Total	43 µL

2. Add 43 µL of the Hybridization Master Mix to the tube(s) from [step 3.12](#) above.
3. Mix thoroughly and perform a quick spin. Incubate at room temperature for 2 minutes.
4. Place the tube(s) on the magnet to collect the beads. Incubate until the liquid is clear.
5. Transfer 56.4 µL of the eluate (entire volume) into a new 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.

6. Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
7. Perform the hybridization incubation in a thermocycler using the following program with the lid temperature set to +105°C:
 - a. +95°C for 5 minutes
 - b. +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](#).

8. Continue to [Chapter 6](#). Wash and Recover Captured Singleplex or Multiplex DNA Sample.

Chapter 6. Wash and Recover Captured Singleplex or Multiplex DNA Sample

This chapter describes the process of the washing and recovery of the captured Singleplex or Multiplex DNA Sample after hybridization to the KAPA HyperCap Target Enrichment Probes. Refer to the [HyperDesign Tool](#) to determine 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 are not precipitated or 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 captures are processed.**



It is expected that excess volume of 1X Bead Wash Buffer will remain when following the < 40 Mb Capture Target Size Capture Bead preparation protocol.

3. To pre-warm the 1X Stringent Wash Buffer, make two aliquots of 200 µL in 0.2 mL PCR tubes and place the tubes into 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 PCR tube and place the tube into 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 hybridization incubation step.

Step 2. Prepare the Capture Beads

For < 40 Mb Capture Target Size

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.

Chapter 6. Wash and Recover Captured Singleplex or Multiplex DNA Sample

3. Aliquot 50 μL of beads per capture reaction into a 0.2 mL PCR or 1.5 mL tube (i.e., for one capture use 50 μL beads and for two captures use 100 μL beads). Beads for up to two captures can be prepared in a single 0.2 mL PCR 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 collect 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).
7. Remove the tube(s) from the magnet and mix thoroughly by vortexing. Perform a quick spin.
8. Place the tube(s) on the magnet to collect 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 buffer and for two captures use 200 μL buffer).
11. Remove the tube(s) from the magnet and mix thoroughly by vortexing. Perform a quick spin.
12. Place the tube(s) on the magnet to collect 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. Perform a quick spin.
16. If preparing Capture Beads for more than one capture in a single tube, aliquot 50 μL of resuspended beads into a new 0.2 mL PCR tube/well for each capture.
17. Place the tube(s) on a magnet to collect 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 by the Capture Beads.

For ≥ 40 Mb Capture Target Size

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 100 μL of beads per capture reaction into a 0.2 mL PCR or 1.5 mL tube (i.e., for one capture use 100 μL beads and for two captures use 200 μL beads). Beads for up to two captures can be prepared in a single 0.2 mL PCR 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 collect 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 1X the initial beads' volume of 1X Bead Wash Buffer (e.g., for one capture use 100 μL buffer and for two captures use 200 μL buffer).
7. Remove the tube(s) from the magnet and mix thoroughly by vortexing. Perform a quick spin.
8. Place the tube(s) on the magnet to collect 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 1X the initial beads' volume of 1X Bead Wash Buffer (e.g., for one capture use 100 μL buffer and for two captures use 200 μL buffer).
11. Remove the tube(s) from the magnet and mix thoroughly by vortexing. Perform a quick spin.
12. Place the tube(s) on the magnet to collect 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., 100 μL buffer per capture).
15. Remove the tube(s) from the magnet and mix thoroughly by vortexing for 10 seconds.

Chapter 6. Wash and Recover Captured Singleplex or Multiplex DNA Sample

16. If preparing Capture Beads for more than one capture in a single tube, aliquot 100 μL of resuspended beads into a new 0.2 mL PCR tube/well for each capture.
17. Place the tube(s) on the magnet to collect the beads. Incubate until the liquid is clear.
18. 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 by the Capture Beads.

Step 3. Bind Hybridized DNA to the Capture Beads

1. Transfer each hybridization sample into 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. Avoid 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 hybridization incubation step.

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

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



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 collect 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 tube.
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 the thermocycler pre-heated to +55°C, close the 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 collect 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 the tube(s) in the thermocycler pre-heated to +55°C, close the lid (set to +105°C) and incubate for 5 minutes.
15. After the 5 minutes incubation, remove the tube(s) from the thermocycler and place on the magnet to collect 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.

Chapter 6. Wash and Recover Captured Singleplex or Multiplex DNA Sample

19. Incubate at room temperature for 1 minute.
20. Place the tube(s) on a magnet to collect the beads. Incubate until the liquid is clear.
21. Remove and discard the supernatant being careful not to disturb the beads.
22. Add 200 μ L 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 transfer the contents to a new tube.



Transferring the samples to a new tube is essential.

24. Incubate at room temperature for 1 minute.
25. Place the tube(s) on the magnet to collect the beads. Incubate until the liquid is clear.
26. Remove and discard the supernatant being careful not to disturb the beads.
27. Add 200 μ L 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 collect 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 μ L PCR grade water to each tube/plate well and mix thoroughly. Perform a quick spin.
33. Proceed to [Chapter 7](#). Amplify Enriched Singleplex or Multiplex 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 Singleplex or Multiplex DNA Sample

This chapter describes the amplification of the Enriched Singleplex or Multiplex 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
- 10 mM Tris-HCl, pH 8.0, optional
- PCR grade water

References

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

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 μ L 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 μ L
Post-Capture PCR Oligos	5 μ L
Total	30 μL

* The Post-Capture PCR Oligos and the KAPA HiFi HotStart ReadyMix (2X) are components of the KAPA HyperCapture Reagent Kit.

2. Add 30 μ L of Post-Capture PCR Master Mix to a 0.2 mL PCR tube or well of a PCR plate.
3. Retrieve the bead-bound DNA Sample from [Chapter 6, Step 4.33](#) and mix thoroughly by vortexing for 10 seconds. Perform a quick spin. Avoid pellet formation.
4. Transfer 20 μ L of the bead-bound DNA Sample as template into the tube/well with the 30 μ L Post-Capture PCR Master Mix (if performing a negative control, add 20 μ L 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

- Place the sample in the thermocycler and amplify using 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, Variable (see table below for recommendation)
 - Step 6: 1 minute at +72°C
 - Step 7: Hold at +4°C

Capture Target Size	Total Number of Post-Capture PCR Cycles
< 100 kb	18
100 kb – 2 Mb	16
> 2 Mb – 40 Mb	10
> 40 Mb	8



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

- Proceed immediately to the next step.

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

- Retrieve KAPA HyperPure Beads. Ensure the beads are equilibrated to room temperature and a homogenous mixture.
- Mix the Amplified Enriched DNA Sample Library from Step 3 of this chapter by vortexing and place on the magnet to collect the beads. Incubate until the liquid is clear.
- Carefully transfer the supernatant to a new tube.



Do not discard the supernatant. The supernatant contains the Amplified Enriched DNA Sample Library.

- Add 70 µL of KAPA HyperPure Beads to the 50 µL Amplified Enriched Singleplex or Multiplex DNA Sample Library.
- Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous and perform a quick spin.
- Incubate at room temperature for 5 minutes to allow the Amplified Enriched DNA Sample Library to bind to the beads.
- Place the tube(s) containing the bead-bound DNA Sample on a magnet to collect the beads. Incubate until the liquid is clear.
- Remove and discard the supernatant being careful not to disturb the beads.
- Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
- Incubate at room temperature for ≥30 seconds.
- Remove and discard the ethanol.
- Keeping the tube(s) on the magnet, add 200 µL of freshly-prepared 80% ethanol.
- Incubate the tube(s) at room temperature for ≥30 seconds.
- Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads. Allow the beads to dry at room temperature with the tube lid open.



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

15. Remove the tube(s) from the magnet.
16. Resuspend the bead pellet using 22 μL of PCR grade water.
17. Vortex for at least 10 seconds to ensure that all of the beads are resuspended. Perform a quick spin.
18. Incubate at room temperature for 2 minutes to allow the sample to elute off the beads.
19. Briefly spin and place the tube(s) back on the magnet and allow the liquid to clear.
20. 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 Singleplex or Multiplex DNA Sample Library

1. Measure the concentration of the Amplified Enriched and Purified DNA Sample Library using a Qubit dsDNA HS Assay Kit:
 - 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 and Purified DNA Sample Library yield should be ≥ 100 ng.
 - If the negative control yields show significant amplification then analyze using an Agilent Bioanalyzer DNA High Sensitivity assay, as this could indicate 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.
 - The average fragment length should be between 150 to 500 bp with an average peak of ~ 320 bp ([Figures 6, 7, 8](#)).

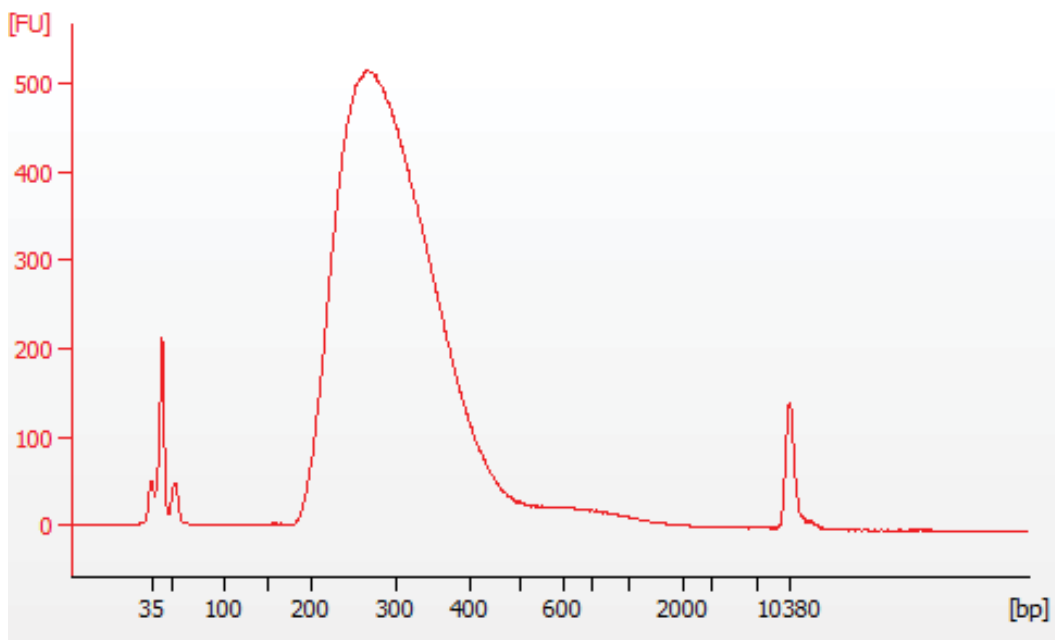


Figure 6: Example of a successfully amplified enriched Multiplex DNA Sample using the KAPA HyperPlus Kit and analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

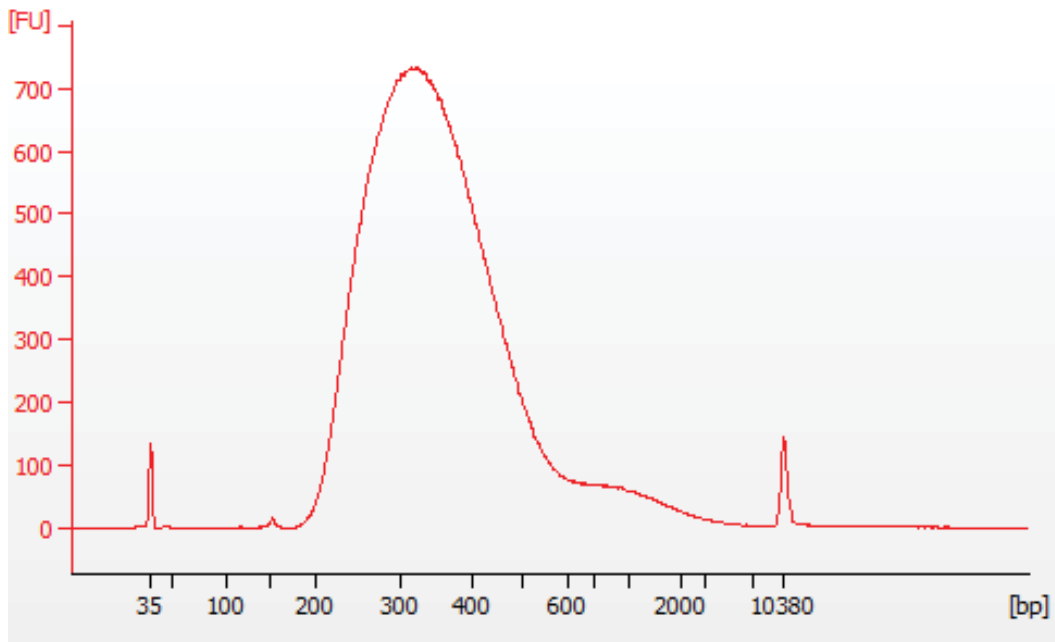


Figure 7: Example of a successfully amplified enriched Multiplex DNA Sample Library using the KAPA HyperPrep Kit and analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

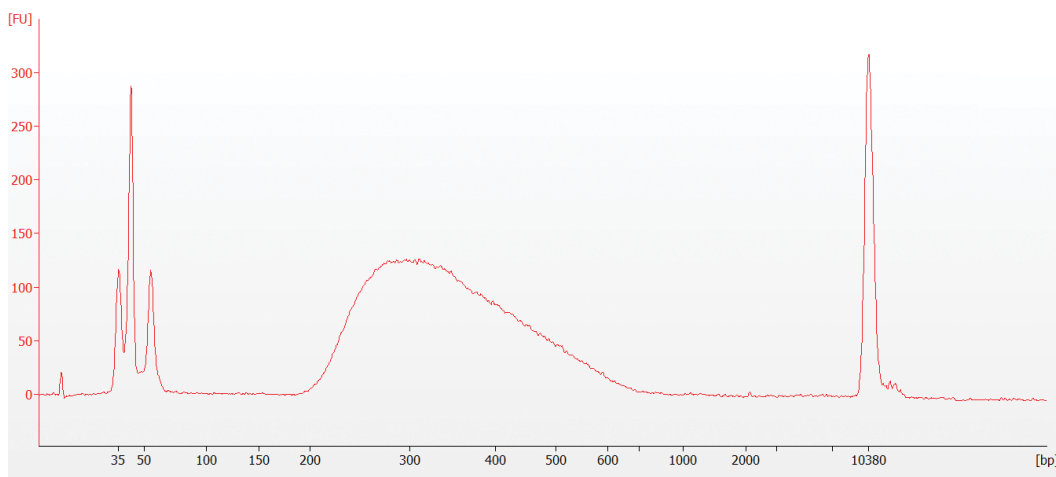


Figure 8: Example of a successfully amplified enriched Multiplex DNA Sample Library using the KAPA EvoPlus Kit and analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

3. The Amplified Enriched Singleplex or Multiplex DNA Sample Library is ready for sequencing. Refer to the *Illumina guide* on instructions for sequencing.

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 for contact information.

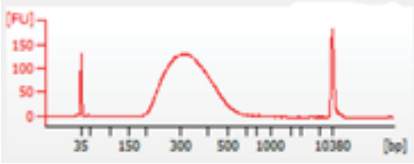
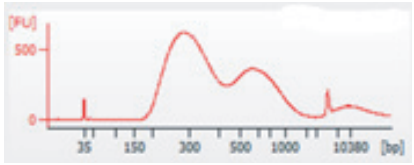


The Illumina sequencing workflow is not supported by Roche Technical Support.

Observation	Cause(s) / Recommendation(s)
Sample Library Preparation	
Less than 100 ng of input DNA is available for library preparation.	<p>Libraries generated using <100 ng of input gDNA can produce high quality capture results; however, several adjustments summarized below, will increase the probability of success.</p> <ul style="list-style-type: none"> ● Adjust the adapter concentration to preserve the adapter: insert molar ratio in order to maintain high ligation efficiency. For more information, contact Roche. ● Increase the number of PCR cycles during Pre-Capture PCR by 1 – 3 cycles, depending on starting gDNA amount. Performance of these cycle number recommendations may vary for your particular sample. <p>Note: There is a possibility that these steps will not lead to success with lower input amounts. For the most current guidance on working with lower input amounts, contact Roche.</p>
DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is used for library preparation.	<p>DNA extracted from FFPE tissue is highly variable in quality due to chemical damage and fragmentation, and often is available only in small amounts.</p> <ul style="list-style-type: none"> ● To increase probability for success, follow recommendations for library construction using less than 100 ng of input DNA and perform enzymatic DNA fragmentation according to 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 samples should not be used. Instead, fragmentation parameters should be optimized using non-precious DNA samples that are representative of the actual sample to be processed. <p>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 review the KAPA HyperCap FFPE DNA Workflow user's guide and/or contact Roche.</p>
Amplified Sample Library (Pre-Capture PCR Product)	
Yield is <1 µg	<p>Possible error occurred during library preparation or compromised reagents were used.</p> <p>Use a previously processed DNA sample as a positive control for library construction and or an evaluated sample library as a positive control for PCR reagents.</p>
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.
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.

Appendix A. Troubleshooting

Observation	Cause(s) / Recommendation(s)
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 genomic 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 Sample Library. Please refer to the manufacturer's user manual for further guidance.
Prepare the Multiplexing Sample Library Pool and KAPA HyperCap Target Enrichment Probes	
The Multiplex DNA Sample Library volume is greater than 45 μL.	<p>For KAPA HyperPure bead based library concentration, 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 3, point 3, on page 24, section "Step: Prepare the Sample for Hybridization."</p> <p>For vacuum concentration (if the required bead volume is very large) of the Multiplex DNA Sample Library, use the following recommendations:</p> <ol style="list-style-type: none"> 1) Mix the Multiplex DNA Sample Library with 20 μL of COT DNA. 2) Concentrate the sample with a vacuum concentrator to a volume of 65 μL. 3) Once concentrated proceed with step 3, point 2, on page 27, section "Step 3. Prepare the Sample for Hybridization."
Amplified enriched Multiplex DNA Sample (Post-Capture PCR Product)	
Yield is <100 ng (yield should be ≥100 ng).	<p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>
Fragment distribution (analyzed using an 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.

Observation	Cause(s) / Recommendation(s)
<p>Fragment distribution (analyzed using an Agilent HS DNA chip) is bimodal, with a larger set of fragments observed in addition to (Fig B), the expected set of fragments (Fig A):</p> <p>A. </p> <p>B. </p>	<p>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 larger products on an Agilent HS DNA chip than their actual length in base pairs. The artifact can be resolved by increasing primer concentration or reducing cycle number in the PCR reaction, however the products themselves are perfectly acceptable for use in sequence capture and sequencing, and this artifact will not affect capture performance. Consider using the KAPA Library Quantification Kit for proper quantification of libraries containing heteroduplexes (which Qubit may fail to measure).</p> <p>The Agilent HS DNA chip traces shown in Fig. A and Fig. B, show the result of amplification of the same enriched gDNA sample library following Post-Capture PCR amplification for 14 or 20-cycles, respectively. The same artifact can appear in Pre-Capture PCR amplification.</p>
<p>Only the Agilent Bioanalyzer DNA 1000 chip is available for qualifying sample following Post-Capture PCR amplification and purification.</p>	<p>The Agilent Bioanalyzer DNA 1000 chip can be used to analyze the Post-Capture Sample Library. Please refer to the manufacturer's user manual for further guidance.</p>
Sequencing Performance Metrics	
<p>High Duplicate rates</p>	<p>Reduction in Pre-Capture and/or Post-Capture PCR cycles may reduce duplicate rates. Take the following points into consideration when altering cycle numbers.</p> <ul style="list-style-type: none"> ● Enough material is present to accurately quantify after PCR clean-up. ● For the Pre-Capture PCR, enough amplified library is produced for at least 1 µg or 1.5 µg respectively for singleplex or multiplex hybridization. <p>Increasing input into hybridization may improve duplicate rates. Take the following points into consideration when adjusting sample input into hybridization.</p> <ul style="list-style-type: none"> ● Enough material is produced from the Pre-Capture PCR reaction(s) to increase sample input into hybridization. <p>Note: There is a possibility that these steps will not lead to success in reducing duplicate rates. For the most current guidance, contact Roche.</p>

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 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

Appendix C. Products Overview

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 HyperExome Probes	
KAPA HyperChoice MAX 20Mb, 1536 rxn	09053280001	KAPA HyperExome Probes, 12 rxn	09062548001
KAPA HyperChoice MAX 20Mb, 4000 rxn	09053301001	KAPA HyperExome Probes, 24 rxn	09062556001
KAPA HyperChoice MAX 20Mb, 10000 rxn	09053310001	KAPA HyperExome Probes, 48 rxn	09062564001
KAPA HyperChoice MAX 20Mb, 20000 rxn	09053328001	KAPA HyperExome Probes, 96 rxn	09062572001
KAPA HyperChoice MAX 20Mb, 50000 rxn	09053336001	KAPA HyperExome Probes, 192 rxn	09062599001
KAPA HyperChoice MAX 20Mb, 100000 rxn	09053344001	KAPA HyperExome Probes, 384 rxn	09062602001
KAPA HyperChoice MAX 40Mb, 24 rxn	09053352001	KAPA HyperExome Probes, 768 rxn	09062629001
KAPA HyperChoice MAX 40Mb, 48 rxn	09053379001	KAPA HyperExome Probes, 1152 rxn	09062637001
KAPA HyperChoice MAX 40Mb, 96 rxn	09053387001	KAPA HyperExome Probes, 1536 rxn	09062645001
KAPA HyperChoice MAX 40Mb, 192 rxn	09053395001	KAPA HyperExome Probes, 4000 rxn	09062653001
KAPA HyperChoice MAX 40Mb, 384 rxn	09053409001	KAPA HyperExome Probes, 10000 rxn	09062661001
KAPA HyperChoice MAX 40Mb, 768 rxn	09053417001	KAPA HyperExome Probes, 20000 rxn	09062670001
KAPA HyperChoice MAX 40Mb, 1152 rxn	09053425001	KAPA HyperExome Probes, 50000 rxn	09062688001
KAPA HyperChoice MAX 40Mb, 1536 rxn	09053433001	KAPA HyperExome Probes, 100000 rxn	09062696001
KAPA HyperChoice MAX 40Mb, 4000 rxn	09053441001	KAPA HyperExome V2 Probes	
KAPA HyperChoice MAX 40Mb, 10000 rxn	09053450001	KAPA HyperExome V2 Probes, 12 rxn	09718630001
KAPA HyperChoice MAX 40Mb, 20000 rxn	09053468001	KAPA HyperExome V2 Probes, 24 rxn	09718648001
KAPA HyperChoice MAX 40Mb, 50000 rxn	09053476001	KAPA HyperExome V2 Probes, 48 rxn	09718656001
KAPA HyperChoice MAX 40Mb, 100000 rxn	09053484001	KAPA HyperExome V2 Probes, 96 rxn	09718664001
KAPA HyperChoice MAX 60Mb, 24 rxn	09053492001	KAPA HyperExome V2 Probes, 192 rxn	09718672001
KAPA HyperChoice MAX 60Mb, 48 rxn	09053506001	KAPA HyperExome V2 Probes, 384 rxn	09718699001
KAPA HyperChoice MAX 60Mb, 96 rxn	09053514001	KAPA HyperExome V2 Probes, 768 rxn	09718702001
KAPA HyperChoice MAX 60Mb, 192 rxn	09053522001	KAPA HyperExome V2 Probes, 1152 rxn	09718729001
KAPA HyperChoice MAX 60Mb, 384 rxn	09053549001	KAPA HyperExome V2 Probes, 1536 rxn	09718737001
KAPA HyperChoice MAX 60Mb, 768 rxn	09053557001	KAPA HyperExplore Probes	
KAPA HyperChoice MAX 60Mb, 1152 rxn	09062335001	KAPA HyperExplore MAX 0.5Mb T1, 12 rxn	09062700001
KAPA HyperChoice MAX 60Mb, 1536 rxn	09062343001	KAPA HyperExplore MAX 0.5Mb T1, 24 rxn	09062718001
KAPA HyperChoice MAX 60Mb, 4000 rxn	09062351001	KAPA HyperExplore MAX 0.5Mb T1, 48 rxn	09062726001
KAPA HyperChoice MAX 60Mb, 10000 rxn	09062360001	KAPA HyperExplore MAX 0.5Mb T1, 96 rxn	09062734001
KAPA HyperChoice MAX 60Mb, 20000 rxn	09062378001	KAPA HyperExplore MAX 0.5Mb T1, 192 rxn	09062742001
KAPA HyperChoice MAX 60Mb, 50000 rxn	09062386001	KAPA HyperExplore MAX 0.5Mb T1, 384 rxn	09062769001
KAPA HyperChoice MAX 60Mb, 100000 rxn	09062394001	KAPA HyperExplore MAX 0.5Mb T1, 768 rxn	09062777001
KAPA HyperChoice MAX 200Mb, 24 rxn	09062408001	KAPA HyperExplore MAX 0.5Mb T1, 1152 rxn	09062785001

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Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperExplore MAX 0.5Mb T1, 1536 rxn	09062793001	KAPA HyperExplore MAX 3Mb T2, 12 rxn	09063307001
KAPA HyperExplore MAX 0.5Mb T1, 4000 rxn	09062807001	KAPA HyperExplore MAX 3Mb T2, 24 rxn	09063315001
KAPA HyperExplore MAX 0.5Mb T2, 12 rxn	09062815001	KAPA HyperExplore MAX 3Mb T2, 48 rxn	09063323001
KAPA HyperExplore MAX 0.5Mb T2, 24 rxn	09062823001	KAPA HyperExplore MAX 3Mb T2, 96 rxn	09063331001
KAPA HyperExplore MAX 0.5Mb T2, 48 rxn	09062831001	KAPA HyperExplore MAX 3Mb T2, 192 rxn	09063340001
KAPA HyperExplore MAX 0.5Mb T2, 96 rxn	09062840001	KAPA HyperExplore MAX 3Mb T2, 384 rxn	09063358001
KAPA HyperExplore MAX 0.5Mb T2, 192 rxn	09062858001	KAPA HyperExplore MAX 3Mb T2, 768 rxn	09063366001
KAPA HyperExplore MAX 0.5Mb T2, 384 rxn	09062866001	KAPA HyperExplore MAX 3Mb T2, 1152 rxn	09063374001
KAPA HyperExplore MAX 0.5Mb T2, 768 rxn	09062874001	KAPA HyperExplore MAX 3Mb T2, 1536 rxn	09063382001
KAPA HyperExplore MAX 0.5Mb T2, 1152 rxn	09062882001	KAPA HyperExplore MAX 3Mb T2, 4000 rxn	09063404001
KAPA HyperExplore MAX 0.5Mb T2, 1536 rxn	09062904001	KAPA HyperExplore MAX 3Mb T2, 10000 rxn	09063412001
KAPA HyperExplore MAX 0.5Mb T2, 4000 rxn	09062912001	KAPA HyperExplore MAX 3Mb T2, 20000 rxn	09063439001
KAPA HyperExplore MAX 0.5Mb T3, 12 rxn	09062939001	KAPA HyperExplore MAX 3Mb T2, 50000 rxn	09063447001
KAPA HyperExplore MAX 0.5Mb T3, 24 rxn	09062947001	KAPA HyperExplore MAX 3Mb T2, 100000 rxn	09063455001
KAPA HyperExplore MAX 0.5Mb T3, 48 rxn	09062955001	KAPA HyperExplore MAX 3Mb T3, 12 rxn	09063463001
KAPA HyperExplore MAX 0.5Mb T3, 96 rxn	09062963001	KAPA HyperExplore MAX 3Mb T3, 24 rxn	09063471001
KAPA HyperExplore MAX 0.5Mb T3, 192 rxn	09062971001	KAPA HyperExplore MAX 3Mb T3, 48 rxn	09063480001
KAPA HyperExplore MAX 0.5Mb T3, 384 rxn	09062980001	KAPA HyperExplore MAX 3Mb T3, 96 rxn	09063498001
KAPA HyperExplore MAX 0.5Mb T3, 768 rxn	09062998001	KAPA HyperExplore MAX 3Mb T3, 192 rxn	09063501001
KAPA HyperExplore MAX 0.5Mb T3, 1152 rxn	09063005001	KAPA HyperExplore MAX 3Mb T3, 384 rxn	09063510001
KAPA HyperExplore MAX 0.5Mb T3, 1536 rxn	09063013001	KAPA HyperExplore MAX 3Mb T3, 768 rxn	09063528001
KAPA HyperExplore MAX 0.5Mb T3, 4000 rxn	09063021001	KAPA HyperExplore MAX 3Mb T3, 1152 rxn	09063536001
KAPA HyperExplore MAX 0.5Mb T4, 12 rxn	09063030001	KAPA HyperExplore MAX 3Mb T3, 1536 rxn	09063544001
KAPA HyperExplore MAX 0.5Mb T4, 24 rxn	09063048001	KAPA HyperExplore MAX 3Mb T3, 4000 rxn	09063552001
KAPA HyperExplore MAX 0.5Mb T4, 48 rxn	09063056001	KAPA HyperExplore MAX 3Mb T3, 10000 rxn	09063579001
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

Appendix C. Products Overview

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperExplore MAX 20Mb, 192 rxn	09068449001	KAPA HyperExplore MAX 200Mb, 4000 rxn	09068937001
KAPA HyperExplore MAX 20Mb, 384 rxn	09068457001	KAPA HyperExplore MAX 200Mb, 10000 rxn	09068945001
KAPA HyperExplore MAX 20Mb, 768 rxn	09068465001	KAPA HyperExplore MAX 200Mb, 20000 rxn	09068953001
KAPA HyperExplore MAX 20Mb, 1152 rxn	09068473001	KAPA HyperExplore MAX 200Mb, 50000 rxn	09068961001
KAPA HyperExplore MAX 20Mb, 1536 rxn	09068481001	KAPA HyperExplore MAX 200Mb, 100000 rxn	09068970001
KAPA HyperExplore MAX 20Mb, 4000 rxn	09068490001	KAPA Target Enrichment, reagents and kits	
KAPA HyperExplore MAX 20Mb, 10000 rxn	09068503001	KAPA Universal Adapter, 15uM 960 uL	09063781001
KAPA HyperExplore MAX 20Mb, 20000 rxn	09068511001	KAPA Universal Adapter, 15uM 4x960 uL	09063790001
KAPA HyperExplore MAX 20Mb, 50000 rxn	09068520001	KAPA Universal UMI Adapter, 960 uL	09329862001
KAPA HyperExplore MAX 20Mb, 100000 rxn	09068538001	KAPA Universal UMI Adapter, 4x960 uL VK	09329889001
KAPA HyperExplore MAX 40Mb, 24 rxn	09068546001	KAPA UDI Primer Mixes, 1-96, 96 rxn	09134336001
KAPA HyperExplore MAX 40Mb, 48 rxn	09068554001	KAPA UDI Primer Mixes, 97-192, 96 rxn	09053603001
KAPA HyperExplore MAX 40Mb, 96 rxn	09068562001	KAPA UDI Primer Mixes, 193-288, 96 rxn	09053611001
KAPA HyperExplore MAX 40Mb, 192 rxn	09068589001	KAPA UDI Primer Mixes, 289-384, 96 rxn	09053620001
KAPA HyperExplore MAX 40Mb, 384 rxn	09068597001	KAPA HyperCapture Reagent kit, 24 rxn	09075810001
KAPA HyperExplore MAX 40Mb, 768 rxn	09068619001	KAPA HyperCapture Reagent kit, 96 rxn	09075828001
KAPA HyperExplore MAX 40Mb, 1152 rxn	09068627001	KAPA HyperCapture Reagent kit, 384 rx VK	09075917001
KAPA HyperExplore MAX 40Mb, 1536 rxn	09068635001	KAPA HyperCapture Bead kit, 24 rxn	09075780001
KAPA HyperExplore MAX 40Mb, 4000 rxn	09068643001	KAPA HyperCapture Bead kit, 96 rxn	09075798001
KAPA HyperExplore MAX 40Mb, 10000 rxn	09068651001	KAPA HyperCapture Bead kit, 384 rx VK	09075909001
KAPA HyperExplore MAX 40Mb, 20000 rxn	09068660001	KAPA Universal Enhancing Oligos, 24 rxn	09075836001
KAPA HyperExplore MAX 40Mb, 50000 rxn	09068678001	KAPA Universal Enhancing Oligos, 96 rxn	09075852001
KAPA HyperExplore MAX 40Mb, 100000 rxn	09068686001	KAPA Universal Enhancing Oligos 384 rx VK	09075895001
KAPA HyperExplore MAX 60Mb, 24 rxn	09068694001	KAPA Hybrid Enhancer Reagent, 1 mL	09075763001
KAPA HyperExplore MAX 60Mb, 48 rxn	09068708001	KAPA Probes Resuspension Buffer, 1 mL	09075879001
KAPA HyperExplore MAX 60Mb, 96 rxn	09068716001	KAPA Probes Resuspension Buffer, 5 mL	09075887001
KAPA HyperExplore MAX 60Mb, 192 rxn	09068724001	KAPA HyperExome Kits*	
KAPA HyperExplore MAX 60Mb, 384 rxn	09068732001	KAPA HyperExome Prep Kit, 192 samples	09107592001
KAPA HyperExplore MAX 60Mb, 768 rxn	09068759001	KAPA HyperExome Plus Kit, 192 samples	09107606001
KAPA HyperExplore MAX 60Mb, 1152 rxn	09068767001	KAPA HyperExome Evo Kit, 192 samples	09844848001
KAPA HyperExplore MAX 60Mb, 1536 rxn	09068775001	KAPA HyperExome V2 Kits*	
KAPA HyperExplore MAX 60Mb, 4000 rxn	09068783001	KAPA HyperExome V2 Prep Kit, 192 samples	09983759001
KAPA HyperExplore MAX 60Mb, 10000 rxn	09068791001	KAPA HyperExome V2 Plus Kit, 192 samples	09983775001
KAPA HyperExplore MAX 60Mb, 20000 rxn	09068805001	KAPA HyperExome V2 Evo Kit, 192 samples	09983783001
KAPA HyperExplore MAX 60Mb, 50000 rxn	09068813001	KAPA HyperCap Oncology panel	
KAPA HyperExplore MAX 60Mb, 100000 rxn	09068821001	KAPA HyperCap Oncology panel, 12 rxn	09462384001
KAPA HyperExplore MAX 200Mb, 24 rxn	09068830001	KAPA HyperCap Oncology panel, 24 rxn	09462457001
KAPA HyperExplore MAX 200Mb, 48 rxn	09068848001	KAPA HyperCap Oncology panel, 48 rxn	09462465001
KAPA HyperExplore MAX 200Mb, 96 rxn	09068856001	KAPA HyperCap Heredity panel	
KAPA HyperExplore MAX 200Mb, 192 rxn	09068864001	KAPA HyperCap Heredity panel, 12 rxn	09462473001
KAPA HyperExplore MAX 200Mb, 384 rxn	09068872001	KAPA HyperCap Heredity panel, 24 rxn	09462481001
KAPA HyperExplore MAX 200Mb, 768 rxn	09068899001	KAPA HyperCap Heredity panel, 48 rxn	09462490001
KAPA HyperExplore MAX 200Mb, 1152 rxn	09068902001	KAPA HyperCap Heredity panel, 96 rxn	09462503001
KAPA HyperExplore MAX 200Mb, 1536 rxn	09068929001	KAPA HyperCap Heredity panel, 192 rxn	09462511001

VK: Virtual Kit, consists of 4 x 96 reaction kits.

*: Virtual Kit that contains required reagents for 192 samples, does not include the KAPA UDI Primer Mixes, which have to be ordered separately.

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