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KAPA HyperCap cfDNA Workflow v1.1

Instructions For Use with

- KAPA HyperChoice,**
- KAPA HyperExplore, and**
- KAPA HyperCap Oncology Panel**



Contents

The package contains:

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

Storage and Stability

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

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

Warnings and Precaution

- § Wear the appropriate personal protective equipment, such as gloves, lab coat, and safety glasses, to avoid direct contact while handling the reagents.
- § Use good laboratory practices to avoid contamination when using the reagents.
- § In the event of a spill, clean up the solution with absorbent pads, allow pads to dry, and dispose of pads. Observe all national, regional, and local regulations for waste disposal and management.

Changes to Previous Version

Formatting changes.

Ordering Information

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

Notice to Purchaser

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

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Contact and Support

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

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Table of Contents

Preface	6
Regulatory Disclaimer	6
KAPA HyperCap Target Enrichment Probes	6
Contact Information	6
Roche Technical Support.....	6
Manufacturer and Distribution	6
Conventions Used in This Manual	6
Symbols	6
Text.....	6
Chapter 1: Before you Begin	7
Overview of the KAPA HyperCap cfDNA Workflow v1.1	7
Protocol Information & Safety.....	9
Terminology.....	9
Prepare the Following Equipment and Reagents	9
Required Equipment, Labware & Consumables	10
Laboratory Equipment.....	10
Consumables Available from Roche	10
Consumables Purchased from Other Vendors.....	11
Chapter 2: Prepare and store the KAPA HyperCap Reagents	12
Step 1. Store the Reagent Kits.....	12
Step 2. Resuspend and Aliquot the KAPA HyperCap Target Enrichment Probe Pool.....	12
Step 3. Preparation of the KAPA UDI Primer Mixes	13
Chapter 3: Prepare the Sample Library	15
Sample Requirements	15
Step 1. Prepare the Sample Library.....	15
Chapter 4: Amplify the Sample Library Using the KAPA UDI Primer Mixes	18
References.....	18
Step 1. Prepare the Pre-Capture PCR Reaction	18
Step 2. Prepare the Pre-Capture PCR Amplification	19
Step 3. Purify the Amplified Sample Library using KAPA HyperPure Beads.....	19
Step 4. Qualify the Amplified Sample Library	20
Chapter 5: Hybridize the Sample and KAPA HyperCap Target Enrichment Probes	22
Step 1. Prepare the Sample for Hybridization.....	22
Chapter 6: Wash and Recover Captured DNA Sample	24
Step 1. Prepare the Wash Buffers.....	24
Step 2. Prepare the Capture Beads	25
Step 3. Bind DNA to the Capture Beads.....	25
Step 4. Wash the Capture Beads Plus Bead-Bound DNA.....	26
Chapter 7: Amplify Enriched DNA Sample	28
References.....	28

Table of Contents

Step 1. Resuspend the Post-Capture PCR Oligos	28
Step 2. Prepare the Post-Capture PCR Master Mix	28
Step 3. Perform the Post-Capture PCR Amplification	29
Step 4. Purify the Amplified Enriched Multiplex DNA Sample using KAPA HyperPure Beads	29
Step 5. Qualify the Amplified Enriched Multiplex DNA Sample Library	30
Step 6. Sequencing Guidance for Pooling of enriched libraries from cfDNA Samples	31
Appendix A. Troubleshooting	33
Appendix B. Limited Warranty	38
Appendix C. Products Overview	39

Preface

Regulatory Disclaimer

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KAPA HyperCap Target Enrichment Probes

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

Contact Information

Roche Technical Support



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

Manufacturer and Distribution

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

Conventions Used in This Manual

Symbols

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

Text

Conventions	Description
Numbered listing	Indicates steps in a procedure that must be performed in the order listed.
<i>Italic type, blue</i>	Identifies a resource in a different area of this manual or on a web site.
<i>Italic type</i>	Identifies the names of dialog boxes, windows, tabs, panels, views, or message boxes in the software.
Bold type	Identifies names of menus and controls (buttons, checkboxes, <i>etc.</i>) in the software.

Chapter 1. Before You Begin

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

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

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

This specific version of the workflow has optimizations designed for limited input oncology samples, specifically cell free DNA (cfDNA) samples. For the cfDNA workflow, optimizations were targeted toward maximizing molecule recovery and ensuring some molecule duplication to enable error removal using unique molecular indexes (UMIs).

Overview of the KAPA HyperCap cfDNA Workflow v1.1

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

Workflow Step	Processing Time
Prepare the Sample Library: KAPA HyperPrep Kit, KAPA Universal UMI Adapter	20 h for cfDNA
Amplify the Sample Library Using the KAPA UDI Primer Mixes	0.75 h
Prepare the Amplified Sample Library for hybridization	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 DNA Sample	0.75 h
Amplify Enriched DNA Sample	1 h
Illumina Sequencing	

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

Protocol Information & Safety

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

Terminology

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

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

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

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

UDI Primer Mixes: Unique Dual-Indexed Primer Mixes.

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

Prepare the Following Equipment and Reagents

- Thermocyclers should be programmed with the following:
 - End Repair and A-tailing program ([Chapter 3](#). Prepare the Sample Library, Step 1.2.e)
 - Adapter Ligation program ([Chapter 3](#). Prepare the Sample Library, Step 1.3.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 and KAPA HyperCap Target Enrichment Probes, Step 1.22)
 - Post-Capture PCR program ([Chapter 7](#). Amplify Enriched DNA Sample, Step 3.1)



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

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



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

Chapter 1. Before You Begin

Required Equipment, Labware & Consumables

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

Laboratory Equipment

Equipment	Supplier	Catalog No.
DynaMag-2 Magnet (16 x 2.0 mL tube holder) (optional)	Thermo Fisher	12321D
DynaMag-96 Side Magnet	Thermo Fisher	12331D
0.2 mL PCR Strip Magnetic Separator Rack	Permagen	MSR812
Microcentrifuge (16,000 x g capability)	<i>Multiple Vendors</i>	
Qubit	ThermoFisher	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 UMI Adapter	96 reactions	09 329 862 001
	384 reactions*	09 329 889 001
KAPA UDI Primer Mixes, 1-96	96 reactions	09 134 336 001
KAPA UDI Primer Mixes, 97-192	96 reactions	09 329 838 001
KAPA UDI Primer Mixes, 193-288	96 reactions	09 329 846 001
KAPA UDI Primer Mixes, 289-384	96 reactions	09 329 854 001
KAPA HyperCapture Bead Kit	24 reactions	09 075 780 001
	96 reactions	09 075 798 001
	384 reactions*	09 075 909 001
KAPA HyperCapture Reagent Kit	24 reactions	09 075 810 001
	96 reactions	09 075 828 001
	384 reactions*	09 075 917 001
KAPA HyperPrep Kit	8 reactions	07 962 312 001
	24 reactions	07 962 347 001
	96 reactions	07 962 363 001
KAPA Universal Enhancing Oligos	24 reactions	09 075 836 001
	96 reactions	09 075 852 001
	384 reactions*	09 075 895 001
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 Probes Resuspension Buffer	1 mL	09 075 879 001
	5 mL	09 075 887 001
KAPA HyperCap Target Enrichment Probes	Multiple	Multiple, see Appendix C

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

Chapter 1. Before You Begin

Consumables Purchased from Other Vendors

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



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

Chapter 2. Prepare and store the KAPA HyperCap Reagents

This chapter describes the storage conditions for the following kits:

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

Step 1. Store the Reagent Kits

Reagent Kit	Storage Temperature
KAPA HyperCapture Bead Kit	+2°C to +8°C
KAPA HyperPure Beads	+2°C to +8°C
KAPA HyperCapture Reagent Kit	-15°C to -25°C
KAPA Universal UMI Adapter	-15°C to -25°C
KAPA UDI Primer Mixes or KAPA UDI Primer Mixes (resuspended)	+2°C to +8°C 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 HyperPrep Kit	-15°C to -25°C



The KAPA HyperCapture Bead Kit must not be frozen.

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

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

1. Review the KAPA HyperCap Target Enrichment Probes tube label to verify the probe reaction number and resuspension volume.
2. Spin the KAPA HyperCap Target Enrichment Probes tube 10,000 x *g* for 30 seconds to ensure the contents are at the bottom of the tube.
3. Add the recommended volume of KAPA Probes Resuspension Buffer provided on the tube label of the KAPA HyperCap Target Enrichment Probes tube.
4. Vortex the tube for 1 minute to resuspend the probe pool.

Chapter 2. Prepare and Store the KAPA HyperCap Reagents

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



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

Step 3. Preparation of the KAPA UDI Primer Mixes

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



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

1. Before removing the foil cover, ensure the plate is in the correct orientation before proceeding. In order to have well position A1 on the top left corner, the notched corner must be facing the user on the bottom left, as shown in [Figure 2](#).
2. Carefully remove the foil cover on the plate ensuring to avoid cross contamination. Discard the original foil cover.
3. Using a multichannel pipette, add 10 μL of PCR Grade water directly to the bottom of each well and discard tips after dispensing.



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

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



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

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



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

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



Ensure aliquoted KAPA UDI Primer Mixes are correctly labeled.

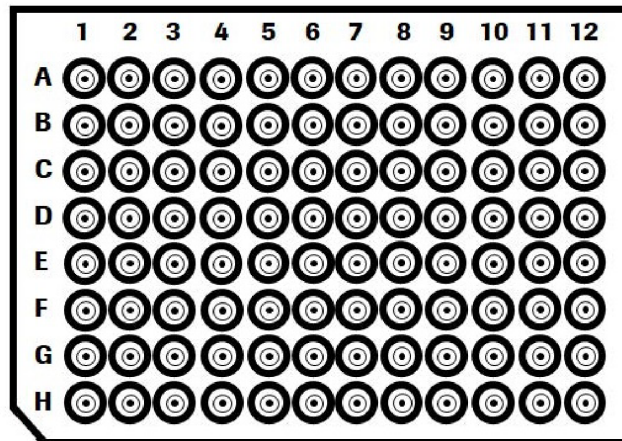


Figure 2: KAPA UDI Primer Mixes Plate layout.

Chapter 3. Prepare the Sample Library

Chapter 3 describes the sample library preparation from cell free DNA (cfDNA) as input by using the KAPA HyperPrep Kit. This chapter requires use of components from the following kits:

- KAPA HyperPrep Kit
- KAPA Universal UMI Adapter
- KAPA UDI Primer Mixes
- KAPA HyperPure Beads

Ensure that the following are available:

- PCR Grade water
- Freshly-prepared 80% ethanol

References

- *Thermocycler Manual*

Sample Requirements




The workflow has been tested with 10-50 ng of cfDNA input. Successful sequencing libraries were made for these inputs, though results will depend on input mass and sample quality. For optimal performance, 50 ng of cfDNA extracted from plasma samples is recommended for sample library preparation. Lower input amounts may not yield equivalent results. For guidance on lower input amounts or sample quality, contact [Roche Technical Support](#).

The user is responsible for proper blood collection, careful plasma sample handling, cfDNA extraction and accurate QC of the sample before starting the KAPA HyperCap cfDNA Workflow. The user must ensure that the selected blood collection tube is used according to its manufacturer's protocol. Ensure that the blood collection tube contains the anticoagulant EDTA. The anticoagulant heparin should not be used as it will interfere with the library preparation. To minimize cellular lysis which releases high molecular weight genomic DNA that can interfere with library generation, separate and collect the plasma soon after blood collection. It is critical that the plasma layer is collected with minimal leukocyte contamination. Ensure that the cfDNA is free of high-molecular weight genomic DNA by analyzing a small diluted (< 2ng/μL) aliquot with an Agilent Bioanalyzer using a High Sensitivity DNA Chip. Follow the manufacturer's suggested protocol.

Step 1. Prepare the Sample Library

The sample library should be prepared according to the following workflow:

KAPA HyperPrep Kit

-  Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.
 -  When assembling a master mix for processing multiple samples, always prepare an appropriate excess (5 to 10% for manual workflows and ~20% for automated liquid handling).
1. Dilute 50 ng of the cfDNA sample with PCR Grade water to a total volume of 50 μL into a 0.2 mL tube or well of a PCR plate.
-  If 50 ng of cfDNA is not available, add the maximum amount, down to 10 ng, and a maximum volume of 50 μL. cfDNA is naturally at the correct size for library preparation and does not need to be sheared.

Chapter 3. Prepare the Sample Library

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

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

End Repair and A-Tailing Master Mix	Per Individual Sample Library
KAPA HyperPrep End Repair & A-Tailing Buffer	7 μ L
KAPA HyperPrep End Repair & A-Tailing Enzyme Mix	3 μ L
Total	10 μL

- b. Add 10 μ L of End Repair and A-Tailing Master Mix to the DNA sample to obtain a total volume of 60 μ L.
- c. Mix the End Repair and A-Tailing reaction thoroughly and perform a quick spin.
- d. Place on ice and immediately proceed to the next step.
- e. Perform the End Repair and A-Tailing incubation in a thermocycler using the following program with the lid temperature set to +85°C:
 1. Step 1: 30 minutes at +20°C
 2. Step 2: 30 minutes at +65°C
 3. Step 3: Hold at +4°C
- f. Proceed immediately to the next step following the 60 minutes incubation.

3. Perform the Adapter Ligation Reaction as follows:

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

Ligation Master Mix	Per Individual Sample Library
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 and additional excess of the Ligation Master Mix may be required.

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



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



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

- c. Add 40 μ L of the Adapter Ligation Master Mix to each sample and KAPA Universal UMI Adapter, resulting in a total volume of 110 μ L.
- d. Mix the Ligation Reaction thoroughly and perform a quick spin.
- e. Incubate the Ligation Reaction at +16°C for 16-18 hours with the lid temperature set to +50°C.
- f. Following the overnight incubation, proceed immediately to the next step.

Chapter 3. Prepare the Sample Library

4. 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 Library
Ligation Reaction	110 μL
KAPA HyperPure Beads	88 μL
Total	198 μL

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



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

- c. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.
- d. Place the tube(s) on a magnet to capture the beads. Incubate for 5 minutes until the solution is clear.
- e. Carefully remove and discard the supernatant.
- f. Keeping the tube(s) on the magnet, add 200 μL of freshly-prepared 80% ethanol.
- g. Incubate at room temperature for ≥ 30 seconds.
- h. Carefully remove and discard the ethanol.
- i. Keeping the tube(s) on the magnet, add 200 μL of freshly-prepared 80% ethanol.
- j. Incubate at room temperature for ≥ 30 seconds.
- k. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
- l. Allow the beads to dry at room temperature, sufficiently for all the ethanol to evaporate, ~ 3 minutes.



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

- m. Remove the tube(s) from the magnet.
- n. Thoroughly resuspend the beads in 20 μL of PCR Grade water.
- o. Incubate at room temperature for 2 minutes to allow the DNA to elute off the beads.
- p. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
- q. Transfer 20 μL of supernatant to a fresh tube/well.
- r. Proceed immediately to [Chapter 4](#). Amplify the Sample Library Using the KAPA UDI Primer Mixes.



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

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

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

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





Ensure that the following is available:




- Freshly-prepared 80% ethanol
- PCR Grade water

References

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

Step 1. Prepare the Pre-Capture PCR Reaction


-  We recommend the inclusion of negative (water) and positive (previously amplified library) controls in the Pre-Capture PCR step.
-  For guidance on sample multiplexing, refer to the [KAPA UDI Primer Mixes Instructions for Use](#).
-  Make sure KAPA HyperPure Beads are removed from storage to allow time for proper equilibration to room temperature. For best performance, store the beads protected from light when not in use.
-  Ensure to record the well position of the KAPA UDI Primer Mix used for each sample.

1. Retrieve and thaw the KAPA UDI Primer Mixes plate prepared in [Chapter 2](#), Step 3.
2. Spin the plate at 280 x *g* for 30 seconds to collect the contents to the bottom of the wells.
3. Peel off or pierce the foil seal for the appropriate number of wells needed.
 If piercing the foil seal, avoid cross contamination by using a new pipette tip for every well.
4. Add 5 µL of a KAPA UDI Primer Mix to each individual Sample Library.
5. Add 25 µL of KAPA HiFi HotStart ReadyMix to each mixture of Sample Library and KAPA UDI Primer 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

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

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

1. Add 50 µL room temperature, thoroughly resuspended, KAPA HyperPure Beads to each Amplified Sample Library.
2. Mix the Amplified Sample Library and KAPA HyperPure Beads thoroughly and perform a quick spin.
 Ensure that the solution is thoroughly mixed and appears homogeneous. Insufficient mixing may compromise recovery and size selection.
3. Incubate at room temperature for 10 minutes to allow the DNA to bind to the beads.
4. Place the tube(s) on a magnet to capture the beads. Incubate for 3 minutes.
5. Carefully remove and discard the supernatant.
6. Remove the tube(s) from the magnet. Resuspend the beads in 50 µL of PCR Grade water.

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

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



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

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

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



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

18. Remove the tube(s) from the magnet.
19. Thoroughly resuspend the beads in 65 μL of PCR Grade water.



Resuspending and eluting in 65 μL provides enough volume for two hybridization reactions. Since limited input DNA mass is typically available for cfDNA, having sufficient Amplified Sample Library enables re-capture if the initial capture fails.

20. Incubate at room temperature for 2 minutes to allow the DNA to elute off the beads.
21. Place the tube(s) on a magnet to capture the beads. Incubate until the liquid is clear, ~ 3 minutes.
22. Transfer 65 μL of supernatant to a fresh tube/well.
23. Purified, Amplified Sample Libraries can be stored at $+2^{\circ}\text{C}$ to 8°C for 1-2 weeks or at -15°C to -25°C for up to one month.

Step 4. Qualify the Amplified Sample Library

For this limited input cfDNA protocol, singleplex capture with 30 μL of amplified sample library is recommended.

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

1. Make a 10-fold dilution of the Amplified Sample Library by combining 2 μL of the 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.



Unlike hereditary genetics workflows, which are used to identify phenotype associated genetic variants and genomic regions, the somatic oncology workflows are designed to detect low level allele frequencies. The somatic oncology workflows do not have an input into capture requirement. This is in order to maximize the amount of Pre-Capture Amplified Sample Library going into capture and increase the odds of detecting low level variants. The negative control yield should be $< 1 \text{ ng}/\mu\text{L}$. Table 1 below is showing typical yield ranges from high quality cfDNA inputs.

Input Mass (ng)	Yield Range (ng) Per Individual Capture
10 ng	975 - 2990 ng
30 ng	1820 - 6825 ng
50 ng	3120 - 7800 ng

Table 1: Amplified Sample Library yield range (ng) per cfDNA input mass (ng) into library preparation.

- Analyze $1 \mu\text{L}$ of the diluted Amplified Sample Library (and any controls) with an Agilent Bioanalyzer DNA High Sensitivity Kit. If using the Agilent Bioanalyzer DNA 1000 Kit, diluting the Amplified Sample Library is not necessary.
 - Amplified Sample Libraries (Pre-Capture libraries) should have an average fragment size distribution at $\sim 320 \text{ bp}$ (Figure 3) with a range setting at 150 to 1000 bp on the Bioanalyzer. Sharp peaks may be visible in the region $< 150 \text{ bp}$. 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.
- For best results we strongly recommend that the Amplified Sample Library meets the yield and size distribution requirements, before proceeding to [Chapter 5](#). Hybridize the Sample and KAPA HyperCap Target Enrichment Probes. During protocol development it was observed that the libraries meeting these conditions have delivered satisfactory results. If the Amplified Sample Library does not meet the yield and size distribution requirements, consider preparing a new sample library if possible.

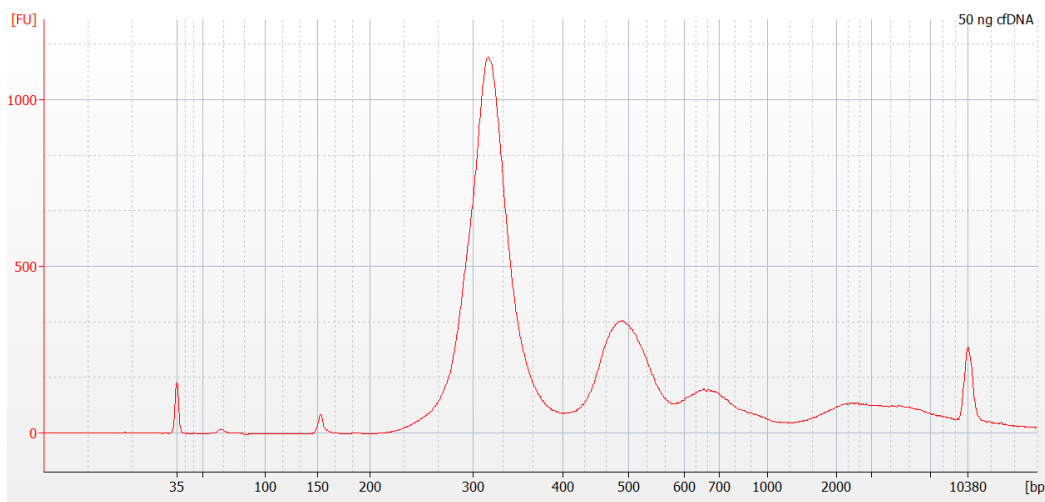


Figure 3: Example of a Pre-Capture amplified cfDNA sample library analyzed using an Agilent Bioanalyzer High Sensitivity DNA assay.

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

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

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

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

Ensure that the following are available:

- Freshly-prepared 80% ethanol
- PCR Grade water



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



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

Step 1. Prepare the Sample for Hybridization



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



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

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

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

- Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
- Incubate at room temperature for 10 minutes to ensure the Amplified Sample Library and COT Human DNA bind to the beads.
- Place the tube(s) on the magnet to capture 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. Try to remove all residual ethanol without disturbing the beads.
- 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.

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

Hybridization Master Mix *	Per Individual Capture
Hybridization Buffer 28 μL	
Hybridization Component H	12 μL
PCR Grade water	3 μL
Total	43 μL

* The above Hybridization Master Mix was tested with panels of capture targets ranging in size from ~ 90 kb to ~ 300 kb.

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



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

- Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
- Perform the hybridization incubation in a thermocycler using the following program with the lid temperature set to $+105^\circ\text{C}$:

- +95 $^\circ\text{C}$ for 5 minutes
- +55 $^\circ\text{C}$ for 16 to 20 hours



The hybridization must remain at $+55^\circ\text{C}$ until it is transferred to the Capture Beads in [Chapter 6](#). Wash and Recover Captured DNA Sample, Step 3.

- Continue to [Chapter 6](#). Wash and Recover Captured DNA Sample.

Chapter 6. Wash and Recover Captured DNA Sample

Chapter 6 describes the process for the washing and recovery of the captured DNA Sample after hybridization to the KAPA HyperCap Target Enrichment Probes.

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, completely thaw the Hybridization Wash Buffers.



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

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

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

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



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

3. To pre-warm the 1X Stringent Wash Buffer, make two aliquots of 200 µL in 0.2 mL 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 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 probe hybridization incubation step.


Step 2. Prepare the Capture Beads

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



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

Step 3. Bind Hybridized DNA to the Capture Beads

1. Transfer each hybridization sample to a single tube/well with prepared Capture Beads from the previous step.
2. Mix thoroughly by vortexing for 10 seconds and perform a quick spin, avoiding pellet formation.
3. Incubate the capture reaction by placing the tube in a thermocycler set to +55°C for 15 minutes, with the thermocycler lid temperature set to +105°C.
 This step can be performed in the same thermocycler used in the probe hybridization incubation step.
4. Following the 15 minutes incubation, proceed immediately to the next step.

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



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



Transferring each sample to a new tube is essential.

24. Incubate at room temperature for 1 minute.
25. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.
26. Remove and discard the supernatant being careful not to disturb the beads.
27. Add 200 µL of room temperature 1X Wash Buffer III.
28. Mix thoroughly by vortexing for 10 seconds, ensuring that the mixture is homogeneous. Perform a quick spin.
29. Incubate at room temperature for 1 minute.
30. Place the tube(s) on the magnet to capture the beads. Incubate until the liquid is clear.

Chapter 6. Wash and Recover Captured DNA Sample

- 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.
- 34.** Proceed to [Chapter 7](#). Amplify Enriched DNA Sample.



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

Chapter 7. Amplify Enriched DNA Sample

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

- KAPA HyperCapture Reagent Kit
- KAPA HyperCapture Bead Kit

In addition, ensure that the following are available:

- Freshly-prepared 80% ethanol
- PCR Grade water

References

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

Step 1. Resuspend the Post-Capture PCR Oligos

1. Briefly spin the lyophilized Post-Capture PCR Oligos, from 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 $\sim 5\%$ to allow for complete pipetting (automated liquid handling systems may require an excess of $\sim 20\%$).



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.

Chapter 7. Amplify Enriched DNA Sample

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

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

2. Add 30 μ L Post-Capture PCR Master Mix to 0.2 mL tube or well of a PCR plate.
3. Retrieve the bead-bound DNA from [Chapter 6](#) step 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 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

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



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

2. Proceed immediately to the next step.

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

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



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

4. Vortex the KAPA HyperPure Beads for 10 seconds before use to ensure a homogeneous mixture of beads.
5. Add 50 μ L KAPA HyperPure Beads to the 50 μ L Amplified Enriched DNA Sample Library.
6. Mix thoroughly by vortexing for 10 seconds, ensuring that the mixture is homogeneous. Perform a quick spin.

Chapter 7. Amplify Enriched DNA Sample

7. Incubate at room temperature for 10 minutes to allow the Amplified Enriched DNA Sample Library to bind to the beads.
8. Place the tube(s) containing the bead-bound DNA on a magnet to capture the beads. Incubate until the liquid is clear.
9. Remove and discard the supernatant being careful not to disturb the beads.
10. Keeping the tube(s) on the magnet, add 200 μ L of freshly-prepared 80% ethanol.
11. Incubate at room temperature for \geq 30 seconds.
12. Remove and discard the 80% ethanol.
13. Keeping the tube(s) on the magnet, add 200 μ L of freshly-prepared 80% ethanol.
14. Incubate the tube(s) at room temperature for \geq 30 seconds.
15. Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
16. Allow the beads to dry at room temperature with the tube lid open for 3 minutes (or until dry).
17. Remove the tube(s) from the magnet.
18. Resuspend the bead pellet using 22 μ L of PCR Grade water.
19. Vortex at least 10 seconds to ensure that all of the beads are resuspended.
20. Incubate at room temperature for 2 minutes to allow the sample to elute off the beads.
21. Briefly spin and place the tube back on the magnet and allow the solution to clear.
22. Transfer 20 μ L of the eluate to a new tube/well.



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



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

Step 5. Qualify the Amplified Enriched DNA Sample Library

1. Measure the concentration of the Amplified Enriched DNA Sample Library using a Qubit Fluorometer:
 - a) Make a 10-fold dilution by combining 2 μ L of the sample with 18 μ L of PCR Grade water.
 - b) Utilize 5 μ L 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 μ L of the diluted sample to be analyzed using the Agilent Bioanalyzer DNA High Sensitivity Kit.
 - A cfDNA input of 10 ng into Library Prep and captured with a \sim 220 kb panel will yield at least 400 ng per sample. A 50 ng cfDNA input and a panel size of \sim 220 kb will typically yield $>$ 1000 ng per sample.
 - The negative control yield should not show significant amplification which if present, could be indicative of contamination.
2. Analyze 1 μ L of each diluted sample (and any controls) using an Agilent Bioanalyzer DNA High Sensitivity assay according to manufacturer's instructions. If using the Agilent Bioanalyzer DNA 1000 Kit, diluting the Amplified Enriched Sample Library may not be necessary.

- The ideal peak size of the library is ~320 bp. There should be minimal to no adapter dimers present.

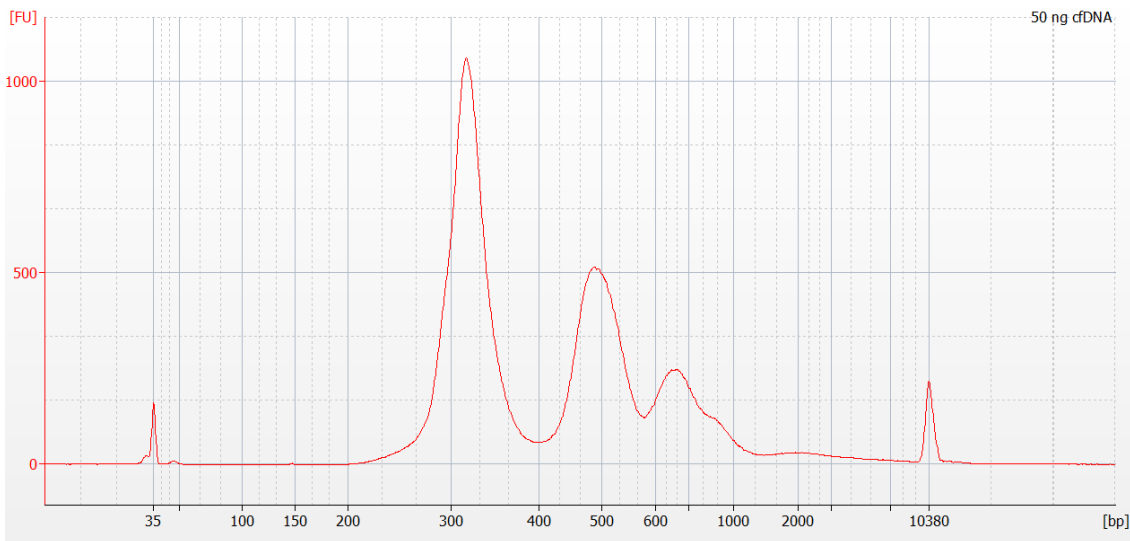


Figure 4: Example of successfully Amplified Enriched cfDNA Sample Library following the KAPA HyperCap cfDNA Workflow v1.1. Sample was analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

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

Step 6. Sequencing Guidance for Pooling of enriched libraries from cfDNA Samples



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

The table below provides recommendations that can be a starting point for pooling prior to sequencing, targeting sufficient unique depth (~3000x) for variant calling from 50 ng cfDNA input.

Table 3: Recommended pooling of amplified enriched libraries prior to sequencing

Recommended Pooling for Sequencing			
Panel Size	# Clusters Desired	Samples to Pool on NextSeq High Output Flow Cell	Samples to Pool on NovaSeq S1 Flow Cell
~90 kb	20 million clusters	16	64
~220 kb	30 million clusters	10	40
~300 kb	40 million clusters	8	32

Chapter 7. Amplify Enriched DNA Sample



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



Variant calling performance may be further enhanced by pooling fewer samples to reach a higher unique sequencing depth and/or more copies per molecule. To properly leverage the error reduction benefits of UMIs, multiple copies of a given molecule are necessary, so sequencing to target at least 2 copies per molecule is recommended.



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

1. Using the concentration determined in [Step 5. Qualify the Amplified Enriched DNA Sample](#), pool together an appropriate volume of each library, so that there is equal mass of each library in the pool. Prepare enough total volume sufficient for Illumina's denaturing protocol. Each pool will have a different tube:



Pooling by mass calculation:

$$\text{Volume } (\mu\text{L}) \text{ of Library X added to the pool} = \frac{\text{Mass (ng) in 1 } \mu\text{L of the most concentrated library's Post-Capture PCR}}{\text{Post-Capture PCR Concentration (ng/}\mu\text{L) of Library X}}$$

2. Vortex and spin down pooled library briefly.
3. Measure the concentration of the pooled library using the Qubit dsDNA HS Assay Kit according to the manufacturer's instructions.
4. Determine the average fragment size of the pool by diluting the pool to the 1-5 ng/ μL range and analyzing it on the Bioanalyzer instrument with an Agilent High Sensitivity DNA Kit following the manufacturer's instructions.
5. Calculate the molarity of the pooled library using the concentration and the average fragment size of the pooled library as follows:

Pooled library concentration = value from Qubit (ng/ μL)

Average fragment size = determined from Bioanalyzer (bp)

Molecular weight (g/mol) = (Average fragment size (bp) x 607.4 g/mol) + 157.9 g/mol

Molarity (nM) = [pooled library concentration (ng/ μL) x 1×10^6 $\mu\text{L/L}$] / molecular weight (g/mol)

Example calculation:

Pooled library concentration = 10.0 ng/ μL

Average fragment size = 300 bp

Molecular weight = (300 bp x 607.4 g/mol) + 157.9 g/mol = 182377.9 g/mol

Molarity (nM) = [10 ng/ μL x 1×10^6 $\mu\text{L/L}$] / 182377.9 g/mol = 54.8 nM

6. Proceed to sequencing or store at -20°C for up to 2 weeks.

Appendix A. Troubleshooting

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



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

Observation	Cause(s) / Recommendation(s)
Sample Library Preparation	
DNA Extracted from plasma is used for library preparation.	To increase probability for success, follow recommendations for library construction using 10 - 50 ng of input DNA.
Amplified Sample Library (Pre-Capture PCR Product)	
Yield is <1 µg with 50 ng input (yield should be ≥1 µg).	<p>Possible error occurred during library preparation or compromised reagents were used.</p> <ol style="list-style-type: none"> Poor ligation efficiency. <ul style="list-style-type: none"> Ensure that the proper amount of input DNA and Universal UMI Adapter is used. Ensure proper ligation time and temperature are used. Poor PCR amplification. <ul style="list-style-type: none"> High number of adapter dimers can inhibit the PCR reaction. Follow proper cleanup steps before PCR. Ensure that the KAPA UDI Primer Mixes are fully resuspended. <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 a Agilent HS DNA chip) shows that the average amplified library fragment size is not within the size range of 150 to 500 bp.	High molecular weight contamination in cfDNA sample.
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 and/or contact Roche Technical Support .
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 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 cfDNA.
If only the Agilent Bioanalyzer DNA 1000 chip is available for qualifying samples following Pre-Capture PCR amplification and purification.	The Agilent Bioanalyzer DNA 1000 chip can be used to analyze the Pre-Capture Amplified Sample Library. Refer to the manufacturer’s user manual for further guidance.

Multiplexing the Sample Library and KAPA HyperCap Target Enrichment Probes

General guidance for Multiplex DNA Sample Library

Warning: for optimal performance it is highly recommended to only perform a singleplex capture. However, for multiplex sample capture we recommend, mixing together equal amounts (by mass) of each uniquely indexed DNA sample libraries to obtain a combined DNA mass of 1.5 µg (i.e. for two-plex DNA Sample Library, mix together 750 ng of each uniquely indexed amplified DNA sample library for a total mass of 1.5 µg). Four samples is the recommended maximum number per multiplex capture. This mixture will subsequently be referred to as the 'Multiplex DNA Sample Library Pool'. The maximum volume of the Multiplex DNA Sample Library Pool should be 45 µL, if needed add PCR Grade water to achieve this volume.

Note: To obtain equal numbers of sequencing reads from each component library in the Multiplex DNA Sample Library Pool upon completion of the experiment, it is very important to combine identical mass from each independently amplified DNA sample library at this step. Accurate quantification and pipetting are critical. Slight differences in the mass of each sample combined to form the Multiplex DNA Sample Library Pool will result in variations in the sequencing reads obtained for each sample in the library pool.

Continue with Chapter 5: Hybridize the Sample to KAPA HyperCap Target Enrichment Probes , Step 3 Prepare the Hybridization Sample.

The Multiplex DNA Sample Library volume is greater than 45 µL.

Transfer the Multiplex DNA Sample Library to a 1.5 mL tube and add 20 µL of COT DNA. Add 2X KAPA HyperPure Beads to the mixture and proceed with Step 1, point 6, on page 23, section "Step 1: Prepare the the Sample for Hybridization".

If the KAPA HyperPure Beads volume that is required is very large and a vacuum concentrator is preferred, use the following recommendations.

- 1) Add 20 µL of COT DNA to the Multiplex DNA Sample Library.
 - 2) Concentrate the sample with a vacuum concentrator to a volume of 65 µL.
 - 3) Once concentrated proceed with Step 1, point 5, on page 22, section "Step 1: Prepare the Sample for Hybridization".
-

Appendix A. Troubleshooting

Amplified enriched DNA Sample (Post-Capture PCR Product)

Low yield of the Post-Capture enriched library	<p>Possible errors that may have occurred:</p> <ul style="list-style-type: none">§ Poor binding with the Capture Beads.<ul style="list-style-type: none">○ Ensure thorough washing of the Capture Beads.§ Sample loss.<ul style="list-style-type: none">○ Ensure that the DNA is not accidentally discarded during the enrichment procedure. Ensure that the Post-Capture PCR uses as a template the Capture Beads, which have the enriched library bound to them.§ Poor PCR amplification.<ul style="list-style-type: none">○ Ensure that the final PCR reaction is set up properly.
Yield is < 100 ng (yield should be ≥ 100 ng).	<p>Assuming library construction was successful (based on Pre-Capture library QC):</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 cfDNA (<i>i.e.</i> 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 a Agilent HS DNA chip) shows that the average amplified fragment size is not within the size range of 150 to 500 bp.	High molecular weight DNA contamination. QC of extracted material is recommended before proceeding into library preparation.
Only the Agilent Bioanalyzer DNA 1000 chip is available for qualifying sample following Post-Capture PCR amplification and purification.	The Agilent Bioanalyzer DNA 1000 chip can be used to analyze the Post-Capture Sample Library. Refer to the manufacturer's user manual for further guidance.

Appendix A. Troubleshooting

Sequencing Performance Metrics

High Duplicate rates

Reduction in Pre-Capture and/or Post-Capture PCR cycles may reduce duplicate rates. Take the following points into consideration when altering cycle numbers.

- § Enough material is present to accurately quantify after PCR clean-up.
- § For the Pre-Capture PCR, at least 1 µg total library, from 50 ng input into the workflow, is produced when single libraries are hybridized.

Increasing input into hybridization may improve duplicate rates. Take the following points into consideration when adjusting sample input into hybridization.

- § Enough material is produced from the Pre-Capture PCR reaction(s) to increase sample input into hybridization.

Note: There is a possibility that these steps will not lead to success in reducing duplicate rates. For the most current guidance, contact [Roche Technical Support](#).

Poor On-Target Rate

Possible errors that may have occurred:

Wrong panel used.

- Confirm panel name before addition into the hybridization reaction.

Poor post-hybridization washes.

- Ensure all wash buffers are fully thawed and resuspended before preparing 1X wash buffers. Ensure thorough washing by carefully following the protocol. Ensure that fresh tubes are used during post-hybridization washes when indicated in the user guide. Avoid long delays or bead drying during the post-hybridization wash steps.

Improper hybridization temperature.

- Confirm thermocycler temperature and program before addition of samples.

UEO reagent not added.

- Ensure that the KAPA HyperPure Beads with bead-bound DNA are resuspended with the UEO reagent.

High Error Rate

Possible errors that may have occurred:

Contamination.

- Always use best laboratory practices when handling samples to minimize risk of contamination.

Improper hybridization set up and washes.

- Do not deviate from the steps listed in the protocol. Ensure the temperature of the heated buffer washes remain at 55°C.

Long Fragment Length

Possible error that may have occurred:

cfDNA: Presence of high molecular weight DNA.

- QC of the input samples is recommended before beginning the workflow.

Appendix A. Troubleshooting

Low Deduped Depth

Possible errors that may have occurred:

- § Poor sequencing run.
 - Refer to the Illumina Sequencing Guide for troubleshooting.
 - § Poor on-target rate.
 - Do not deviate from the steps and conditions listed in the protocol.
 - Confirm the correct Target Enrichment Probe Panel is used.
 - § Poor ligation efficiency.
 - Do not deviate from the steps and conditions listed in the protocol.
-

Unable to detect unique molecules

Possible error that may have occurred:

- Universal Adapter used instead of Universal UMI adapter.
 - Confirm tube label before addition of reagent to the Ligation Master Mix.
-

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 customers and not to third parties.

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

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

2. FURTHER LIABILITY LIMITATION

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

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

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

Appendix C. Products Overview

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

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 HyperExplore Probes	
KAPA HyperChoice MAX 20Mb, 1536 rxn	09053280001	KAPA HyperExplore MAX 0.5Mb T1, 12 rxn	09062700001
KAPA HyperChoice MAX 20Mb, 4000 rxn	09053301001	KAPA HyperExplore MAX 0.5Mb T1, 24 rxn	09062718001
KAPA HyperChoice MAX 20Mb, 10000 rxn	09053310001	KAPA HyperExplore MAX 0.5Mb T1, 48 rxn	09062726001
KAPA HyperChoice MAX 20Mb, 20000 rxn	09053328001	KAPA HyperExplore MAX 0.5Mb T1, 96 rxn	09062734001
KAPA HyperChoice MAX 20Mb, 50000 rxn	09053336001	KAPA HyperExplore MAX 0.5Mb T1, 192 rxn	09062742001
KAPA HyperChoice MAX 20Mb, 100000 rxn	09053344001	KAPA HyperExplore MAX 0.5Mb T1, 384 rxn	09062769001
KAPA HyperChoice MAX 40Mb, 24 rxn	09053352001	KAPA HyperExplore MAX 0.5Mb T1, 768 rxn	09062777001
KAPA HyperChoice MAX 40Mb, 48 rxn	09053379001	KAPA HyperExplore MAX 0.5Mb T1, 1152 rxn	09062785001
KAPA HyperChoice MAX 40Mb, 96 rxn	09053387001	KAPA HyperExplore MAX 0.5Mb T1, 1536 rxn	09062793001
KAPA HyperChoice MAX 40Mb, 192 rxn	09053395001	KAPA HyperExplore MAX 0.5Mb T1, 4000 rxn	09062807001
KAPA HyperChoice MAX 40Mb, 384 rxn	09053409001	KAPA HyperExplore MAX 0.5Mb T2, 12 rxn	09062815001
KAPA HyperChoice MAX 40Mb, 768 rxn	09053417001	KAPA HyperExplore MAX 0.5Mb T2, 24 rxn	09062823001
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KAPA HyperChoice MAX 40Mb, 1536 rxn	09053433001	KAPA HyperExplore MAX 0.5Mb T2, 96 rxn	09062840001
KAPA HyperChoice MAX 40Mb, 4000 rxn	09053441001	KAPA HyperExplore MAX 0.5Mb T2, 192 rxn	09062858001
KAPA HyperChoice MAX 40Mb, 10000 rxn	09053450001	KAPA HyperExplore MAX 0.5Mb T2, 384 rxn	09062866001
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KAPA HyperChoice MAX 40Mb, 50000 rxn	09053476001	KAPA HyperExplore MAX 0.5Mb T2, 1152 rxn	09062882001
KAPA HyperChoice MAX 40Mb, 100000 rxn	09053484001	KAPA HyperExplore MAX 0.5Mb T2, 1536 rxn	09062904001
KAPA HyperChoice MAX 60Mb, 24 rxn	09053492001	KAPA HyperExplore MAX 0.5Mb T2, 4000 rxn	09062912001
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KAPA HyperChoice MAX 60Mb, 10000 rxn	09062360001	KAPA HyperExplore MAX 0.5Mb T3, 1536 rxn	09063013001
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KAPA HyperChoice MAX 60Mb, 100000 rxn	09062394001	KAPA HyperExplore MAX 0.5Mb T4, 24 rxn	09063048001
KAPA HyperChoice MAX 200Mb, 24 rxn	09062408001	KAPA HyperExplore MAX 0.5Mb T4, 48 rxn	09063056001

Appendix C. Products Overview

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

Appendix C. Products Overview

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

VK: Virtual kits, consist of 4 x 96 reaction kits.

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