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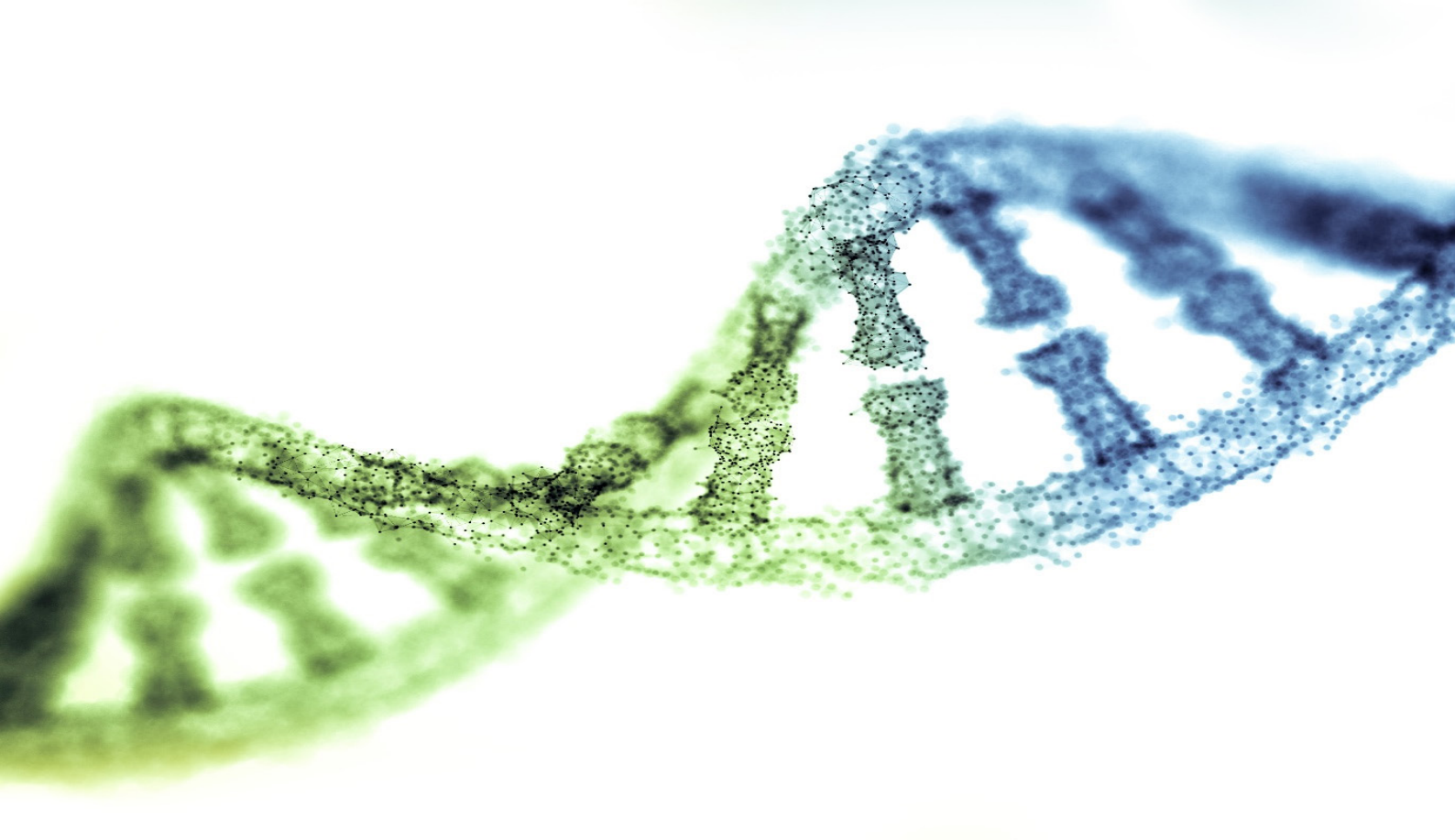
KAPA HyperCap Workflow v3.0

Instructions for use with
– KAPA HyperExome,
– KAPA HyperChoice, and
– KAPA HyperExplore Probes



Contents

The KAPA Target Enrichment Probes package contains:

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

Storage and Stability

- KAPA Target Enrichment Probes are stable at –15 to –25 °C until the expiration date printed on the label.
- Probes are provided lyophilized and will need to be resuspended prior to first use. It is recommended to aliquot the probes into single-use volumes and freeze at –15 to –25 °C.

Application

KAPA HyperExome probes enable targeted enrichment of human coding exons. Refer to design-specific documentation and design files for more details on the specific exome design. The KAPA HyperExome probes utilize an empirically optimized design and deliver high-performance enrichment in terms of coverage and uniformity.

KAPA HyperChoice products enable 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 is 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.

Warnings and Precautions

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

Changes to Previous Version

New version.

Ordering Information

For complete overview of Roche Sequencing products, including those used in the KAPA HyperCap Workflow v3.0 go to *sequencing.roche.com/products*.

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Contact and Support

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

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Preface

Regulatory Disclaimer

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KAPA Target Enrichment Probes

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

Contact Information

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. Pleasanton, CA USA
Distribution	Roche Diagnostics GmbH Mannheim, Germany
Distribution in USA	Roche Diagnostics Corporation Indianapolis, IN USA

Conventions Used in This Manual

Symbols

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

Text

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

Chapter 1. Before You Begin

These Instructions for Use describes the process for enrichment of individual or multiplexed genomic DNA (gDNA) sample libraries using any KAPA Target Enrichment Probes and the amplification of these sample libraries by ligation-mediated PCR. Specifically, this Instructions for Use provide a protocol for the workflow outlined in *Figure 1* using the KAPA HyperPrep Kit or KAPA HyperPlus Kit. 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 directly sequenced using an Illumina sequencing instrument.

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

- Improved performance over the previous versions, 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 NGS sample preparation:
  - KAPA HyperPrep and KAPA HyperPlus Kits
  - KAPA HyperPure Beads
  - KAPA HyperCapture Reagent and Bead Kits
- Catalog off-the-shelf, as well as customizable content through an on-line custom design interface and a team of expert designers.

Overview of the KAPA HyperCap Workflow v3.0

The KAPA HyperCap Workflow v3.0 involves:

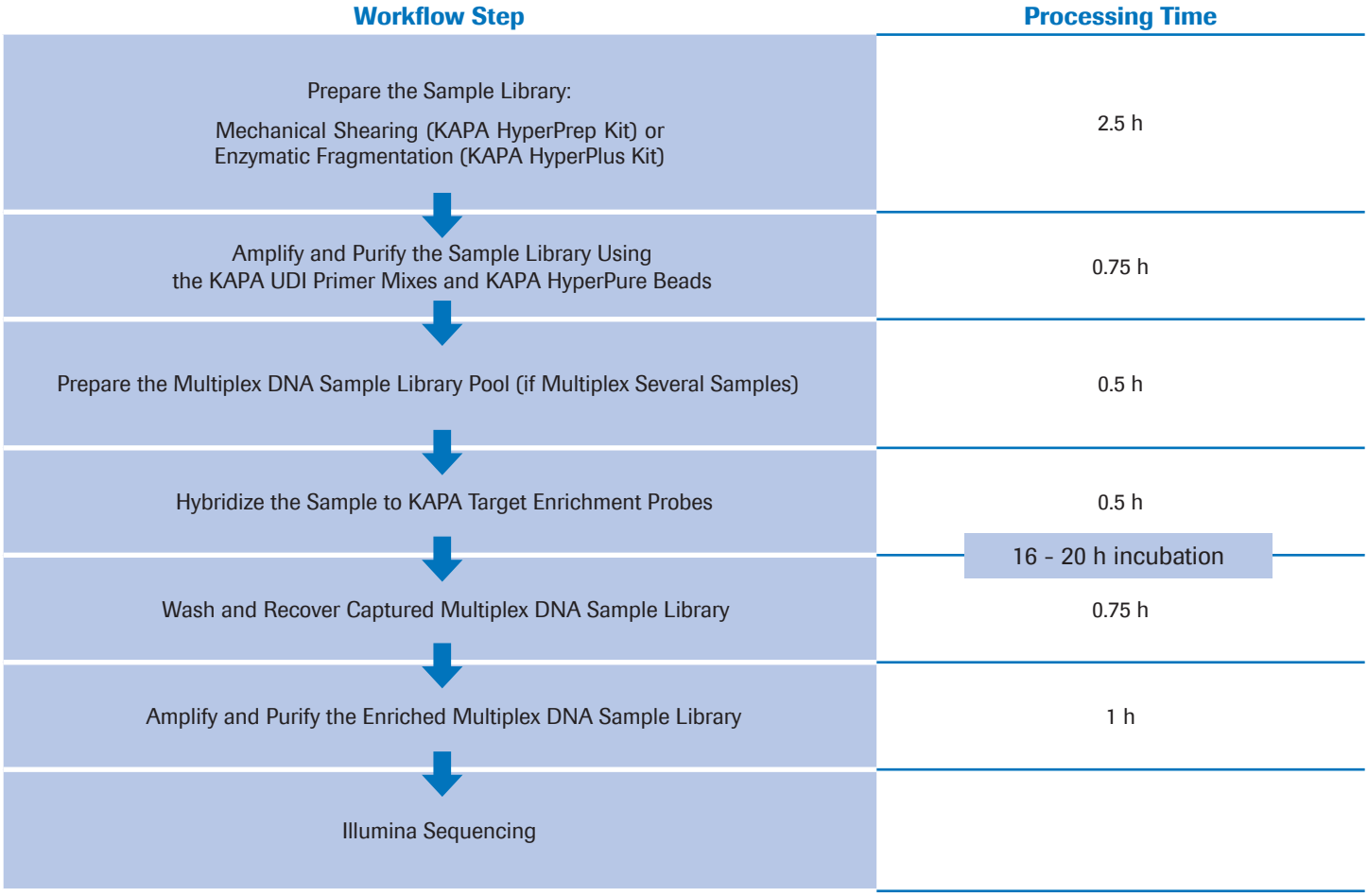


Figure 1: KAPA HyperCap Workflow, with mechanical or enzymatic DNA fragmentation. Where applicable, incubation times are indicated between steps. Processing time may vary based on number of samples processed and multiplexing levels.

Protocol Information & Safety

- Wear gloves and take precautions to avoid sample contamination.
- Perform all centrifugations at room temperature (+15 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 for incubations.

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 Target Enrichment Probes, and subsequent washing steps.

KAPA Target Enrichment Probes: The complete set of biotinylated 120 bp oligonucleotide probes (KAPA HyperExome, KAPA HyperChoice, and KAPA HyperExplore) provided by Roche to perform target enrichment.

Sample Library: The initial shotgun library generated from genomic DNA by fragmentation and ligation. 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 Adapter: Unique Dual-Indexed Adapter

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 3)
    - End Repair and A-tailing program (*Chapter 3*, Prepare the Sample Library, Step 6)
    - Adapter Ligation program (*Chapter 3*, Prepare the Sample Library, Step 7)
  - KAPA HyperPrep Kit
    - End Repair and A-tailing program (*Chapter 3*, Prepare the Sample Library, Step 5)
    - Adapter Ligation program (*Chapter 3*, Prepare the Sample Library, Step 6)
  - 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 Target Enrichment Probes, Step 3.19)
  - Post-Capture LM-PCR program (*Chapter 7*, Amplify Enriched 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 this Instruction for Use. If further guidance is needed, please contact Roche Technical Support.

- The following steps should be taken before beginning the workflow:
  - Resuspend the KAPA Target Enrichment Probes (*Chapter 2*, Store the HyperCap Reagents, Step 1)
  - Aliquot the KAPA Target Enrichment Probes (*Chapter 2*, Store the HyperCap Reagents, Step 1)
  - Resuspend the Post-Capture PCR Oligos (*Chapter 7*, Amplify Enriched Multiplex DNA Sample, Step 1)

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

Required Equipment, Labware & Consumables

You assume full responsibility when using the equipment, labware, and consumables described below. This protocol are 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)	Thermo Fisher	12321D
DynaMag-96 Side Magnet	Thermo Fisher	12331D
Microcentrifuge (16,000 x *g* capability)	*Multiple Vendors*	
Qubit Fluorometer	ThermoFisher	Multiple models
Bioanalyzer 2100	Agilent	
Thermocycler with programmable heated lid (capable of maintaining +55°C for 16 - 20 hours)	*Multiple Vendors*	
Vortex mixer	*Multiple Vendors*	
Plate Centrifuge	*Multiple Vendors*	

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	96 reactions	09 134 336 001
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 Universal Enhancing Oligos	24 reactions 96 reactions 384 reactions*	09 075 836 001 09 075 852 001 09 075 895 001
KAPA HyperPure Beads	5 mL 30 mL 60 mL 4 x 60 mL 450 mL	08 963 835 001 08 963 843 001 08 963 851 001 08 963 878 001 08 963 860 001
KAPA Hybrid Enhancer Reagent	1 mL	09 075 763 001
KAPA Probes Resuspension Buffer	1 mL 5 mL	09 075 879 001 09 075 887 001
KAPA Target Enrichment Probes	Multiple	Multiple, Please see Appendix C.

Component	Package Size/Contents	Catalog No.
KAPA HyperExome Prep Kit[1]	For 192 samples, contains: 2 x KAPA HyperPrep Kit 96rxn – 07 962 363 001 2 x KAPA Universal Adapter, 15µM 960 µL- 09 063 781 001 4 x KAPA HyperPure Beads 5 mL– 08 963 835 001 1 x KAPA HyperExome, 24rxn – 09 062 556 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24rxn – 09 075 780 001	09 107 592 001
KAPA HyperExome Plus Kit[1]	For 192 samples, contains: 2 x KAPA HyperPlus Kit 96rxn – 07 962 428 001 2 x KAPA Universal Adapter, 15µM 960 µL- 09 063 781 001 4 x KAPA HyperPure Beads 5 mL– 08 963 835 001 1 x KAPA HyperExome, 24rxn – 09 062 556 001 1 x KAPA Probes Resuspension Buffer – 09 075 879 001 1 x KAPA HyperCapture Reagent Kit 24rxn – 09 075 810 001 1 x KAPA HyperCapture Bead Kit 24rxn – 09 075 780 001	09 107 606 001

*** Virtual kits, consist of 4 x 96 reaction kits.**
[1] Virtual kit, contains required reagents for 192 samples, does not include the KAPA UDI Primer Mixes, which has to be ordered separately.

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
10 mM Tris-HCl, pH 8.0	*Multiple Vendors*		
Ethanol, 200 proof (absolute), for molecular biology	*Multiple Vendors*	500 mL	E7023-500ML
Covaris microTUBEs (required only if using KAPA HyperPrep Kit)	Covaris, Inc.	1 package of 25 tubes	520166, 520167, 52174, 520168
TE Buffer, 1X Solution, pH 8.0, 0.1 mM EDTA (required only if using KAPA HyperPrep Kit)	*Multiple Vendors*	100 mL	
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 ■ 1.5 mL microcentrifuge tubes (optional)	*Multiple Vendors*		
Water, PCR Grade	Sigma-Aldrich	1 x 25 mL 25 x 1 mL 4 x 25 mL	03315959001 03315932001 03315843001

Use nuclease-free, PCR Grade water for all described protocol steps.

Chapter 2. Prepare and Store the 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 Adapter
- KAPA UDI Primer Mixes
- KAPA Probes Resuspension Buffer
- KAPA HyperPrep Kit or KAPA HyperPlus Kit

Step 1. Store the Reagent Kits

Reagent Kit	Storage Temperature
KAPA HyperCapture Bead Kit	+2 to +8°C
KAPA HyperPure Beads	+2 to +8°C
KAPA HyperCapture Reagent Kit	-15 to -25°C
KAPA Universal Adapter	-15 to -25°C
KAPA UDI Primer Mixes or KAPA UDI Primer Mixes (resuspended)	+2 to +8°C or -15 to -25°C
KAPA Probes Resuspension Buffer	-15 to -25°C
KAPA Hybrid Enhancer Reagent (if using KAPA HyperExplore)	-15 to -25°C
KAPA HyperPrep Kit	-15 to -25°C
KAPA HyperPlus Kit	-15 to -25°C

The HyperCapture Bead kit must not be frozen.

Step 2. Resuspend & Aliquot the KAPA Target Enrichment Probe Pool

Upon receipt of the KAPA Target Enrichment Probes, undertake the following steps to ensure the highest performance of the KAPA Target Enrichment Probes and to avoid multiple freeze/thaw cycles or potential accidental contamination:

1. Review the KAPA Target Enrichment Probes tube label to verify the probe reaction number and resuspension volume.
2. Spin the KAPA 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 Resuspension Buffer provided on the tube label to the KAPA 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 Target Enrichment Probes into single-use aliquots (4 µL/capture) into 0.2 mL PCR tubes and store at -15 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.

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 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 PCR Grade water.

A new pipette tip should 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 is 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 to -25°C. To avoid repeated freeze/thaw cycles you may transfer the resuspended primers to separate tubes or tube strips for storage.

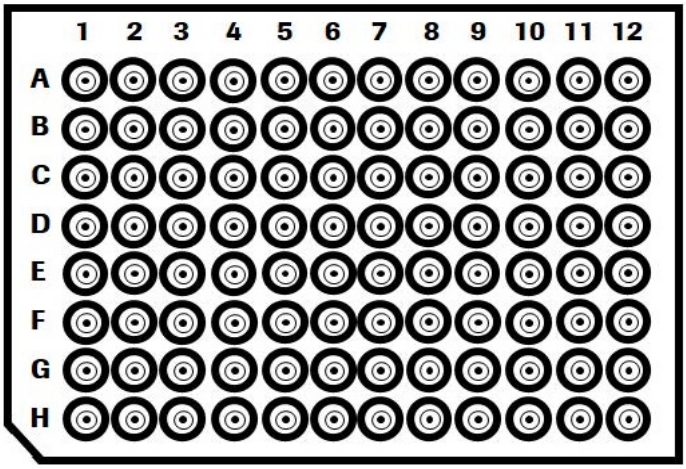


Figure 2: KAPA UDI Primer Mixes plate layout.

Chapter 3. Prepare the Sample Library

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

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

Ensure that the following are available:

- TE buffer, 1X solution pH 8.0, low EDTA (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*

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 technical support.

Step 1. Prepare the Sample Library

- KAPA HyperPlus Kit – enzymatic fragmentation
- KAPA HyperPrep Kit – mechanical fragmentation

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 this 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.

1. Dilute 100 ng of gDNA with 10 mM Tris-HCl, pH 8.0 to a total volume of 35 µL in to a 0.2 mL 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 the volume of buffer is less than the volume of enzyme in this reaction.

3. Mix 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:
    1. Pre-cool block: +4°C
    2. Fragmentation: 25 minutes at +37°C
    3. 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 HyperPlus End Repair & A-Tailing Enzyme Mix	3 µL
Total	**10 µL**

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

    b. Add 10 µL of End Repair and A-Tailing Master Mix to the fragmented DNA sample to obtain a total volume of 60 µL.
    c. Mix the End Repair and A-Tailing Reaction thoroughly and perform a quick spin.
    d. Place on ice and immediately proceed to next step.
    e. Perform the End Repair and A-Tailing incubation in a thermocycler using the following program with the lid temperature set to +85°C:
        1. Step 1: 30 minutes at +65°C
        2. Step 2: Hold at +4°C
    f. Following the 30 minute incubation, proceed immediately to the next step.
7. Proceed with the Adapter Ligation Reaction:
    a. Prepare a Ligation Master Mix of the following reagents:

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

    b. Add 10 µL of KAPA Universal Adapter to wells containing sample from the previous step.

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 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.

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

c. Incubate the sample at room temperature for 5 minutes to allow the DNA to bind to the beads.

d. Place the sample on a magnet to collect the beads. Incubate until the liquid is clear.

e. Carefully remove and discard the supernatant.

f. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.

g. Incubate the sample at room temperature for ≥30 seconds.

h. Carefully remove and discard the ethanol.

i. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.

j. Incubate the sample 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.

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

m. Remove the sample from the magnet.

n. Thoroughly resuspend the beads in 22 µL of 10 mM Tris-HCl, pH 8.0.

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

p. Place the sample 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.

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

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 tube.
2. Adjust the volume to a total of 55 µL using 1X TE buffer pH 8.0, 0.1 mM EDTA and transfer to a Covaris microTUBE-50 for fragmentation. For other Covaris shearing consumables, please consult the Covaris user manual for guidedance.
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 µL 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 and A-tailing Master Mix	Per Individual Sample
KAPA End Repair & A-Tailing Buffer	7 µL
KAPA End Repair & A-Tailing Enzyme Mix	3 µL
Total	**10 µL**

   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.
   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. Following the incubation proceed immediately to the next step following the the 60 minute incubation.
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 µL
KAPA DNA Ligase	10 µL
Total	**40 µL**

   b. First, add 10 µL of the KAPA Universal Adapter to each well containing the sample from the previous step.

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 40 µL of 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 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.

First 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..

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

c. Incubate the samples at room temperature for 5 minutes to allow the DNA to bind to the beads.

d. Place the sample on the magnet to collect the beads. Incubate until the liquid is clear.

e. Carefully remove and discard the supernatant.

f. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.

g. Incubate the sample at room temperature for ≥30 seconds.

h. Carefully remove and discard the ethanol.

i. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.

j. Incubate the sample 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.

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

m. Remove the sample from the magnet.

n. Thoroughly resuspend the beads in 22 µL of 10 mM Tris-HCl, pH 8.0.

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

p. Place the sample 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.

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 Target Enrichment Probes. This chapter requires the use of the components from the following kits:

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

Ensure that the following is available:

- Freshly-prepared 80% ethanol
- 10 mM Tris-HCl, pH 8.0
- 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 pre-capture and post-capture sample multiplexing, 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 Mixes 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 Mixes to each individual sample library.
5. Add 25 µL of KAPA HiFi Hotstart ReadyMix to each combined 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, 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 for utilization at a later date.

Step 2. Perform the Pre-Capture PCR Amplification

7. Place the sample in the thermocycler and amplify the sample library 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)

8. Proceed immediately to the next step.

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

1. Add 70 µL of room temperature, thoroughly resuspended, KAPA HyperPure Beads to each amplified sample library.
2. Mix the amplified sample library and KAPA HyperPure Beads thoroughly and perform a quick spin.

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

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

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

13. Remove the sample from the magnet.
14. Thoroughly resuspend the beads in 32 µL of 10 mM Tris-HCl, pH 8.0 or PCR Grade water.
15. Incubate the sample at room temperature for 2 minutes to allow the DNA to elute off the beads.
16. Place the sample on a magnet to collect the beads. Incubate until the liquid is clear.
17. Transfer 30 µL of the eluate to a new tube/well.
18. Purified, amplified libraries can be stored at +2 to +8°C for 1-2 weeks or at -15 to -25°C for long term storage.

Step 4. Determine the Concentration, Size Distribution, and Quality of 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 amplified library with the Qubit dsDNA HS Assay Kit to obtain the concentration of the diluted library sample. Multiply by 10 to obtain results.

   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 sample 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.
   - Pre-capture libraries should have an average fragment size distribution at ~320 bp (*Figure 3*, *Figure 4*) 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, but the Bioanalyzer trace indicates only the presence of a sharp peak below 150 bp in size, 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.

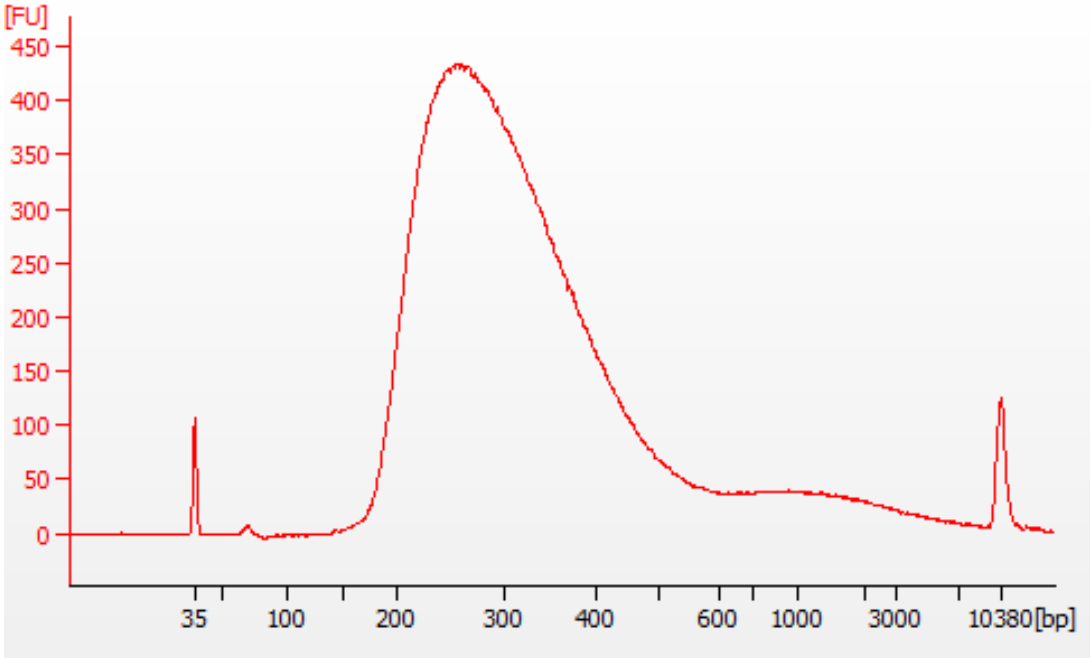


Figure 3: Example of an amplified HyperPlus sample library analyzed using the Agilent Bioanalyzer High Sensitivity DNA assay

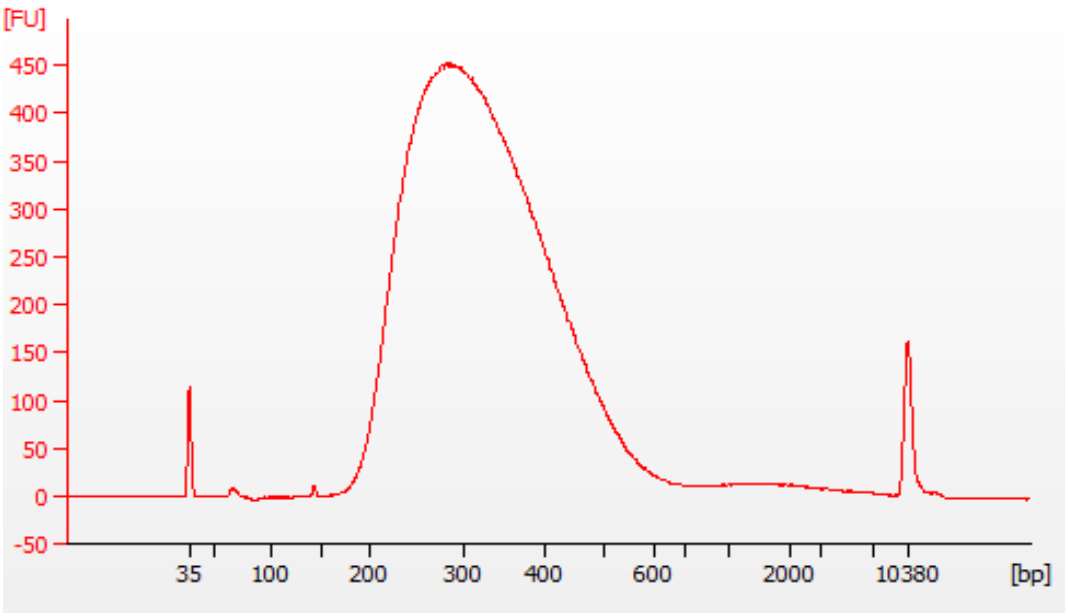


Figure 4: Example of an amplified HyperPrep sample library analyzed using the Agilent Bioanalyzer High Sensitivity DNA assay

Chapter 5. Hybridize the Sample to KAPA Target Enrichment Probes

This chapter describes the Roche protocol for the hybridization of the amplified sample libraries to the KAPA 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 Mbp or ≥40 Mbp panel capture target size. For optimal performance, it is recommended to review the panel design and determine the hybridization conditions to follow before proceeding. This chapter requires the use of the components from the following kits:

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

Ensure that the following is available:

- Freshly-prepared 80% ethanol

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

Note: 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 Target Enrichment Probe aliquots (one per hybridization) from the -15 to -25°C freezer and allow them to thaw on ice.

Step 2. Prepare the 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 Single / Multiplex DNA Sample Library by doing the following:

a. For multiplex samples, mix 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). 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 Hybridization Sample

This step outlines how to prepare the hybridization sample with 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.

Note: 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 (1 mg/mL) to the DNA Sample Library Pool, for a total volume of 65 µL.
2. Add 130 µL of KAPA HyperPure Beads to each tube/well containing the DNA Sample Library and COT Human DNA mixture.
3. Mix thoroughly by vortexing for 10 seconds and perform a quick spin.
4. Incubate at room temperature for 10 minutes to ensure the DNA Sample Library and COT Human DNA bind to the beads.
5. Place the sample on the magnet to collect the beads. Incubate until the liquid is clear.
6. Carefully remove and discard the supernatant.
7. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
8. Incubate the sample 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 sample from the magnet and mix thoroughly by vortexing. It is important that sufficient mixing is performed to result in a homogenous mixture.

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

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

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

≥ 40 Mbp 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 bead-bound DNA mixture resuspended in Universal Enhancing Oligos.
3. Mix thoroughly and perform a quick spin. Incubate at room temperature for 2 minutes.
4. Place the sample 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 Target Enrichment Probe.

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

   The sample 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 Multiplex DNA Sample For Washing and Recovery.

Chapter 6. Wash and Recover Captured Multiplex DNA Sample

This chapter describes the process of the washing and recovery of the captured Multiplex DNA Sample after hybridization to the KAPA Target Enrichment Probes. Refer to HyperDesign 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. If necessary, 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 to +25°C) for up to 2 weeks. The volumes in this table are calculated for a single experiment; scale up accordingly if multiple samples are processed.**

   Excess 1X Bead Wash Buffer should be expected for the < 40 Mbp 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 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

For <40 Mbp 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 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 four captures use 200 µL beads, etc.). Beads for four 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 sample 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 tubes on the magnet, add 2X the initial volume of beads of 1X Bead Wash Buffer (*e.g.* for one capture use 100 µL of buffer and for four captures use 400 µL buffer, *etc.*).
7. Remove tubes from the magnet and mix thoroughly by vortexing. Perform a quick spin.
8. Place the tubes 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 tubes on the magnet, perform a second wash by adding 2X the initial volume of beads of 1X Bead Wash Buffer (*e.g.* for one capture use 100 µL buffer and for four captures use 400 µL buffer, *etc.*).
11. Remove tubes from the magnet and mix thoroughly by vortexing. Perform a quick spin.
12. Place the tubes 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 volume of beads of 1X Bead Wash Buffer (*i.e.* 50 µL buffer per capture).
15. Remove tubes from magnet and mix thoroughly by vortexing for 10 seconds. Perform a quick spin.
16. Aliquot 50 µL of resuspended beads into a new tube/well for each capture.
17. Place the tubes 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 Bead Wash Buffer will not interfere with binding of DNA to the Capture Beads.

For ≥40 Mbp 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 or 1.5 mL tube (*i.e.* for one capture use 100 µL beads and for four captures use 400 µL beads, *etc.*). Beads for 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 tubes 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 tubes on the magnet, add 1X the initial volume of beads of 1X Bead Wash Buffer (*e.g.* for one capture use 100 µL buffer and for four captures use 400 µL buffer, *etc.*).
7. Remove tubes from the magnet and mix thoroughly by vortexing. Perform a quick spin.
8. Place the tubes 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 tubes on the magnet, perform a second wash by adding 1X the initial volume of beads of 1X Bead Was h Buffer (*e.g.* for one capture use 100 µL buffer and for four captures use 400 µL buffer, *etc.*).
11. Remove tubes from the magnet and mix thoroughly by vortexing. Perform a quick spin.
12. Place the tubes 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 volume of beads of 1X Bead Wash Buffer (*i.e.* 100 µL buffer per capture).
15. Remove tubes from magnet and mix thoroughly by vortexing for 10 seconds.

16. Aliquot 100 µL of resuspended beads into a new tube/well for each capture.
17. Place the tubes on a 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 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 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.
3. Incubate the hybridization reaction by placing the sample in a thermocycler set to +55°C for 15 minutes, with the thermocycler lid temperature set to +105°C.

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

4. Proceed immediately to the next step.

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

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 Hybridization reaction.
2. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
3. Place the sample 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 sample.
6. Remove the sample from the magnet.
7. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
8. Place the sample 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 sample from the thermocycler and place on a 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 sample.
12. Remove the sample from the magnet.
13. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
14. Place 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 sample from he thermocycler and place on a 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.

19. Incubate at room temperature for 1 minute.
20. Place the sample 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 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 transfer the contents to a new tube.
24. Incubate at room temperature for 1 minute.
25. Place the sample on a 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 µL of room temperature 1X Wash Buffer III.
28. Mix thoroughly by vortexing for 10 seconds, ensure that the mixture is homogeneous. Perform a quick spin.
29. Incubate at room temperature for 1 minute.
30. Place the sample on a 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 sample from the magnet.
33. Add 20 µL PCR Grade water to each tube/plate well and mix thoroughly, Perform a quick spin.
34. Proceed to *Chapter 7*. Amplify Enriched Multiplex DNA Sample.

No elution step is performed at this step. The beads contain the captured DNA libraries and will be used as the template in the PCR as described in *Chapter 7*.

Chapter 7. Amplify Enriched Multiplex DNA Sample

This chapter describes the amplification of the enriched Multiplex 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
- 10 mM Tris-HCl, pH 8.0, optional

References

- *Thermocycler Manual*
- *Agilent Bioanalyzer DNA Analysis Kit Guide*

Step 1. Resuspend the Post-Capture PCR Oligos

1. Briefly spin the lyophilized Post-Capture PCR Oligos, contained in the KAPA HyperCapture Reagent Kit, to allow the contents to pellet at the bottom of the tube. Note that both oligos are contained within a single tube.
2. Add 480 µL PCR Grade water to the tube of centrifuged oligos.
3. Vortex for at least 10 seconds to the resuspended oligos.
4. Spin down the tube to collect the contents.
5. The resuspended oligo tube should be stored at -15 to -25 °C.

Step 2. Prepare the Post-Capture PCR Master Mix

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

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

1. Prepare a master mix of the following reagents.

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

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

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

Total volume of sample with beads will be approximately 20 µL (15 µL of water with bead volume). If volume is <20 µL, add more water to achieve this volume.

5. Mix thoroughly by pipetting up and down several times.

Step 3. Perform the Post-Capture PCR Amplification

1. Place the sample in the thermocycler.
   - 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.

2. Proceed immediately to the next step.

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

1. Retrieve KAPA HyperPure Beads. Ensure the beads are equilibrated at room temperature and a homogenous mixture.
2. Place the sample containing the amplified enriched Multiplex DNA Sample on a magnet to collect the beads. Transfer the supernatant to a new tube.
3. Add 70 µL of KAPA HyperPure Beads to the 50 µL amplified enriched Multiplex DNA Sample library.
4. 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 sample to bind to the beads.
5. Place the sample containing the bead-bound DNA on a magnet to collect the beads. Incubate until the liquid is clear.
6. Remove and discard the supernatant being careful not to disturb the beads.
7. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol sample.
8. Incubate at room temperature for ≥30 seconds.
9. Remove and discard the ethanol.
10. Keeping the sample on the magnet, add 200 µL of freshly-prepared 80% ethanol.
11. Incubate the sample at room temperature for ≥30 seconds.
12. Carefully remove and discard the ethanol. Remove residual ethanol without disturbing the beads and allow the beads to dry at room temperature with the tube lid open.

Over drying of the beads can result in yield loss.

13. Remove the sample from the magnet.
14. Resuspend the bead pellet using 22 µL of 10 mM Tris-HCl, pH 8.0 or PCR Grade water.
15. Vortex for at least 10 seconds to ensure that all of the beads are resuspended. Perform a quick spin.
16. Incubate at room temperature for 2 minutes.

17. Place the sample back on the magnet and allow the liquid to clear.
18. Transfer 20 µL of the eluate to a new tube/well.

Step 5. Determine the Concentration, Size Distribution, and Quality of the Amplified Enriched Multiplex DNA Sample

1. Measure the concentration of the amplified enriched Multiplex DNA Sample using a Qubit dsDNA HS Assay Kit:
   a. Make a 10-fold dilution of the amplified enriched Multiplex DNA Sample by combining 2 µL of library with 18 µL of PCR Grade water.
   b. Use 5 µL of the diluted, amplified enriched Multiplex DNA Sample using the Qubit dsDNA HS Assay Kit to obtain the concentration of the diluted sample. Reserve at least 1 µL of the diluted, amplified enriched Multiplex DNA Sample to be analyzed using the Agilent Bioanalyzer DNA High Sensitivity Kit.
   - The amplified enriched Multiplex DNA Sample Library yield should be ≥ 100 ng.
   - If the negative control yields show significant amplification, this would indicate contamination.
2. Analyze 1 µL of each diluted, amplified enriched Multiplex DNA Sample (and any controls) using an Agilent Bioanalyze r DNA High Sensitivity assay according to manufacturer's instructions.
   - The average fragment size distribution should be ~320 bp (*Figure 5, Figure 6*) with a range setting of 150 to 1000 bp on the Bioanalyzer.

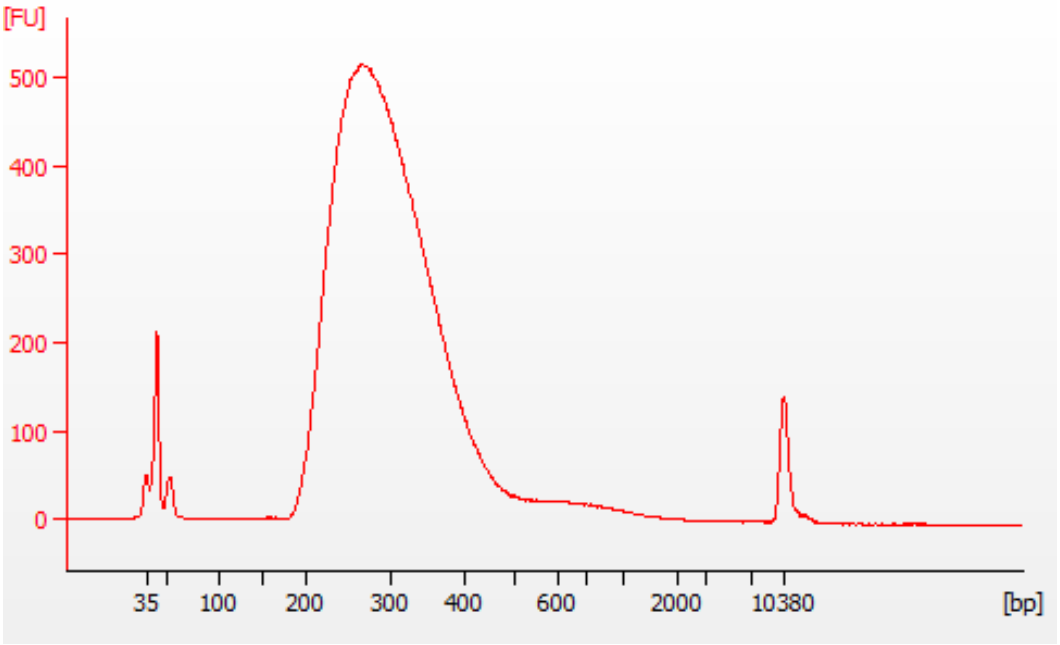


Figure 5: Example of a successfully amplified enriched Multiplex DNA Sample using the KAPA HyperPlus Kit and analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

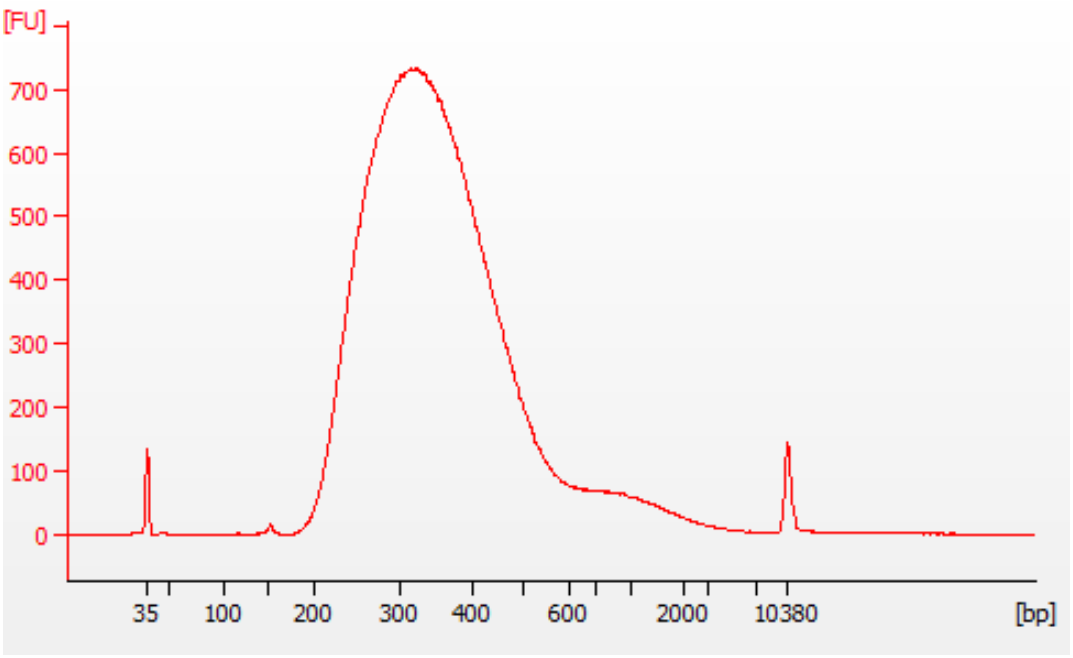


Figure 6: Example of a successfully amplified enriched Multiplex DNA Sample Library using the KAPA HyperPrep Kit and analyzed using an Agilent Bioanalyzer High Sensitivity DNA chip.

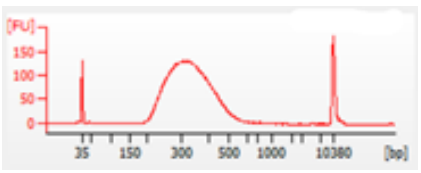
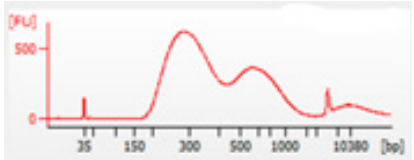
3. The amplified enriched Multiplex DNA Sample is ready for sequencing.

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.html* 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.	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. ■ Adjust the adapter concentration to preserve the adapter: insert molar ratio in order to maintain high ligation efficiency. For more information, contact Roche Technical Support. ■ 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. **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 Technical Support.
DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is used for library preparation.	DNA extracted from FFPE tissue is highly variable in quality due to chemical damage and fragmentation, and often is available only in small amounts. ■ To increase probability for success, follow recommendations for library construction using 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. **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 sample, contact Roche Technical Support.
Amplified Sample Library (Pre-Capture PCR Product)	
Yield is <1 µg for multiplex or <1.5 µg for singleplex hybridizations (yield should be ≥1 µg or ≥1.5 µg, respectively).	Possible error occurred during library preparation or compromised reagents were used. 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.
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.
Fragment distribution (analyzed using an Agilent DNA 1000 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.

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.
If 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 the Sample Library Pool and KAPA Target Enrichment Probes	
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 3 on page 23, section "Step: Prepare the Hybridization Sample.". If a vacuum concentrator is preferred, please use the following recommendations. 1) Incorporate the Multiplex DNA Sample Library with 20 µL of COT DNA. 2) Concentrate the sample with a vacuum concentrator. 3) Once concentrated proceed with step 3 on page 23, section "Step: Prepare the Hybridization Sample.".
Amplified enriched Multiplex DNA Sample (Post-Capture PCR Product)	
Yield is <100 ng (yield should be ≥100 ng).	Library construction or Pre-Capture PCR failed. Pre-Capture PCR yield should be ≥ 1 µg. Repeat with a DNA sample that was previously processed with success. Incorrect hybridization or wash temperatures were used. Make sure the correct hybridization and wash temperatures were used. If temperatures were not correct, repeat the experiment from hybridization. PCR reagents are compromised. Verify that the positive control worked. If the positive control did not work, repeat hybridization and re-amplify using fresh PCR reagents. **Note:** Experiments designed to capture less genomic DNA (*i.e.* a smaller cumulative target size) may be successful even though they can generate lower PCR yields than experiments designed to capture larger targets. Target size should be taken into consideration when evaluating low Post-Capture PCR yield.
Fragment distribution (analyzed using 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. Consider implementing the gel-cut size selection option.
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): A.  B. 	Primer depletion due to over-amplification of sample library relative to the amount of primers available in the reaction results in single stranded amplification products. These products can anneal to each other via adapter homology on both ends of the fragments to form heteroduplexes, and migrate as 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. Care should be taken to quantify the area under both peaks if quantification will be performed using the Bioanalyzer image. 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.

Observation	Cause(s) / Recommendation(s)
If 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 Pre-Capture Sample Library. Please refer to the manufacturer's user manual for further guidance.
Sequencing Performance Metrics	
High Duplicate rates	Reduction in Pre-Capture and/or Post-Capture PCR cycles may reduce duplicate rates. Take the following points into consideration when altering cycle numbers. ■ Enough material is present to accurately quantify after PCR clean-up. ■ For the Pre-Capture PCR, enough amplified library is produced for at least 1 ug or 1.5 ug respectively for multiplex or singleplex hybridization. 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.

Appendix B. Limited Warranty

1. Limited Warranty

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

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

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

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

2. FURTHER LIABILITY LIMITATION

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

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

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

Appendix C. Products Overview

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperChoice		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
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KAPA HyperChoice MAX 0.5Mb T3, 12 rxn	09052356001	KAPA HyperChoice MAX 3Mb T2, 50000 rxn	09052879001
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KAPA HyperChoice MAX 0.5Mb T3, 768 rxn	09052437001	KAPA HyperChoice MAX 3Mb T3, 192 rxn	09052933001
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KAPA HyperChoice MAX 0.5Mb T4, 4000 rxn	09052577001	KAPA HyperChoice MAX 5Mb, 96 rxn	09053077001
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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
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KAPA HyperChoice MAX 5Mb, 50000 rxn	09053166001	KAPA HyperChoice MAX 200Mb, 384 rxn	09062459001
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KAPA HyperChoice MAX 20Mb, 10000 rxn	09053310001	KAPA HyperExome, 48 rxn	09062564001
KAPA HyperChoice MAX 20Mb, 20000 rxn	09053328001	KAPA HyperExome, 96 rxn	09062572001
KAPA HyperChoice MAX 20Mb, 50000 rxn	09053336001	KAPA HyperExome, 192 rxn	09062599001
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KAPA HyperChoice MAX 40Mb, 4000 rxn	09053441001		
KAPA HyperChoice MAX 40Mb, 10000 rxn	09053450001	**KAPA HyperExplore**	
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Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
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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
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KAPA HyperExplore MAX 0.5Mb T3, 24 rxn	09062947001	KAPA HyperExplore MAX 3Mb T2, 100000 rxn	09063455001
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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
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

Product Name and Pack Size	Catalog #	Product Name and Pack Size	Catalog #
KAPA HyperExplore MAX 20Mb, 100000 rxn	09068538001	KAPA Target Enrichment, reagents and kits	
KAPA HyperExplore MAX 40Mb, 24 rxn	09068546001	KAPA Universal Adapter, 15uM 960 uL	09063781001
KAPA HyperExplore MAX 40Mb, 48 rxn	09068554001	KAPA Universal Adapter, 15uM 4x960 uL	09063790001
KAPA HyperExplore MAX 40Mb, 96 rxn	09068562001	KAPA UDI Primer Mixes, 1-96 , 96 rxn	09134336001
KAPA HyperExplore MAX 40Mb, 192 rxn	09068589001	KAPA HyperCapture Reagent kit, 24 rxn	09075810001
KAPA HyperExplore MAX 40Mb, 384 rxn	09068597001	KAPA HyperCapture Reagent kit, 96 rxn	09075828001
KAPA HyperExplore MAX 40Mb, 768 rxn	09068619001	KAPA HyperCapture Reagent kit, 384 rx VK	09075917001
KAPA HyperExplore MAX 40Mb, 1152 rxn	09068627001	KAPA HyperCapture Bead kit, 24 rxn	09075780001
KAPA HyperExplore MAX 40Mb, 1536 rxn	09068635001	KAPA HyperCapture Bead kit, 96 rxn	09075798001
KAPA HyperExplore MAX 40Mb, 4000 rxn	09068643001	KAPA HyperCapture Bead kit, 384 rxn VK	09075909001
KAPA HyperExplore MAX 40Mb, 10000 rxn	09068651001	KAPA Universal Enhancing Oligos, 24 rxn	09075836001
KAPA HyperExplore MAX 40Mb, 20000 rxn	09068660001	KAPA Universal Enhancing Oligos, 96 rxn	09075852001
KAPA HyperExplore MAX 40Mb, 50000 rxn	09068678001	KAPA Universal Enhancing Oligos 384rx VK	09075895001
KAPA HyperExplore MAX 40Mb, 100000 rxn	09068686001	KAPA Hybrid Enhancer Reagent, 1 mL	09075763001
KAPA HyperExplore MAX 60Mb, 24 rxn	09068694001	KAPA Probes resuspension buffer, 1 mL	09075879001
KAPA HyperExplore MAX 60Mb, 48 rxn	09068708001	KAPA Probes resuspension buffer, 5 mL	09075887001
KAPA HyperExplore MAX 60Mb, 96 rxn	09068716001	KAPA HyperExome kits	
KAPA HyperExplore MAX 60Mb, 192 rxn	09068724001	KAPA HyperExome Prep Kit, 192 samples	09107592001
KAPA HyperExplore MAX 60Mb, 384 rxn	09068732001	KAPA HyperExome Plus Kit, 192 samples	09107606001
KAPA HyperExplore MAX 60Mb, 768 rxn	09068759001		
KAPA HyperExplore MAX 60Mb, 1152 rxn	09068767001		
KAPA HyperExplore MAX 60Mb, 1536 rxn	09068775001		
KAPA HyperExplore MAX 60Mb, 4000 rxn	09068783001		
KAPA HyperExplore MAX 60Mb, 10000 rxn	09068791001		
KAPA HyperExplore MAX 60Mb, 20000 rxn	09068805001		
KAPA HyperExplore MAX 60Mb, 50000 rxn	09068813001		
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		

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