KAPABIOSYSTEMS

Technical Data Sheet

KAPA HyperPure Beads

KR1705 – v3.23

This Technical Data Sheet provides product information and detailed protocols for the implementation of KAPA HyperPure Beads in NGS workflows.

This document applies to KAPA HyperPure Beads 08963835001, 08963843001, 08963851001, 08963860001 and 08963878001.

Kapa/*Roche* Kit Codes and Components		
KK8007 *08963835001*	KAPA HyperPure Beads	5 mL
KK8008 *08963843001*	KAPA HyperPure Beads	30 mL
KK8009 *08963851001*	KAPA HyperPure Beads	60 mL
KK8010 *08963860001*	KAPA HyperPure Beads	450 mL
KK8011 *08963878001*	KAPA HyperPure Beads	4 x 60 mL

Contents

Quick Notes

- KAPA HyperPure Beads is designed for the rapid and efficient purification of DNA, for inputs ranging from 1 ng – 5 µg in a single reaction.
- KAPA HyperPure Beads is **NOT** compatible with KAPA library preparation kits that implement a "with-bead" protocol. Please refer to the section **Compatibility with KAPA Library Preparation Kits** (p. 4) for validated workflows.
- KAPA HyperPure Beads are compatible with manual processing and automated liquid handling.
- KAPA HyperPure Beads may be shipped on dry ice which does not affect product performance. Upon receipt the product must be stored at 2°C to 8°C, and protected from light when not in use.
- For optimal performance, always ensure that the KAPA HyperPure Beads is equilibrated to room temperature, that the beads are fully resuspended before use, and that the DNA and bead solutions are thoroughly mixed prior to the binding incubation.
- Several factors may impact the efficiency of DNA recovery and exclusion. Please refer to the **Important Parameters** section for details.

Product Description

KAPA HyperPure Beads is a suspension of paramagnetic beads in a buffer optimized for the purification or size selection of single- and double-stranded DNA in next-generation sequencing and other molecular biology workflows. The product is designed for fast and reliable purification or size selection of 1 ng to 5 µg DNA in a single reaction. KAPA HyperPure Beads is compatible with manual processing or automated liquid handling and enables efficient recovery of input DNA in both formats.

For purification and size selection of DNA, the KAPA HyperPure Beads buffer includes an optimized concentration of PEG/NaCl—a crowding agent designed to drive DNA molecules to the beads for binding. The volumetric ratio of KAPA HyperPure Beads to sample is the critical factor in determining the size distribution of DNA fragments retained by the beads. The volume (ratio) may be modified/optimized based upon the specific application and/or point in the library construction workflow where a cleanup or size selection is employed.

Product Specifications

Shipping and Storage

KAPA HyperPure Beads may be shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store at 2°C to 8°C in a constant-temperature refrigerator; **DO NOT** store the product at -15°C to -25°C. The product is light-sensitive and must be stored protected from light when not in use. When stored under these conditions and handled correctly, KAPA HyperPure Beads will retain full functionality until the expiry date indicated on the kit label.

Handling

Always ensure that KAPA HyperPure Beads is fully equilibrated to room temperature and thoroughly resuspended before use.

Disposal

Properly discard KAPA HyperPure Beads as biohazardous waste.

Quality Control

All kit components are subjected to stringent functional quality control and meet strict requirements with respect to DNA contamination. Please contact Technical Support at sequencing.roche.com/support for more information.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online (eLabDoc) or upon request from the local Roche office.

Compatibility with KAPA Library Preparation Kits

KAPA HyperPure Beads is compatible with, and validated for use in combination with, the following library construction kits and workflows:

» KAPA HyperPrep Kits

» KAPA HyperPlus Kits

» KAPA EvoPlus Kits

» KAPA RiboErase (HMR) Kits*

Please refer to the Technical Data Sheet included with your library preparation kit for additional detailed information and protocols.

*Standalone kit only (07962266001 and 07962274001).

KAPA HyperPure Beads is **NOT** compatible with the following library construction kits and workflows that implement a “with-bead” protocol:

» KAPA HTP and LTP Library Preparation Kits

» KAPA RNA HyperPrep Kits with RiboErase (HMR), or with RiboErase (HMR) Globin

» KAPA RNA and mRNA HyperPrep Kits

» KAPA Stranded RNA-Seq Kit with RiboErase (HMR), or with RiboErase (HMR) Globin

» KAPA Stranded RNA-Seq and mRNA-Seq Kits

These kits are provided with a PEG/NaCl solution required for “with-bead” reaction cleanups that is optimized for use with KAPA Pure Beads only.

Important Parameters

Factors that Impact Overall Performance

- The temperature at which the binding of DNA to beads is performed is critical for optimal recovery of desired DNA fragment lengths. ***Always ensure that KAPA HyperPure Beads is fully equilibrated to room temperature before use.***
- Beads will settle gradually over time. ***Always ensure that the solution is fully homogeneous before aspirating KAPA HyperPure Beads. Pay special attention if working with larger volumes (e.g. 450 mL).***
- The volume in which cleanups and size selection are performed may impact performance. DNA samples may be diluted in 10 mM Tris-HCl (pH 8.0 – 8.5) or PCR-grade water to increase the working volume prior to the addition of KAPA HyperPure Beads. This is recommended for starting volumes <50 µL, or for DNA preparations that are viscous and/or contain a high concentration of salts or PEG.
- ***Complete mixing of DNA and beads prior to DNA binding is a critical factor affecting DNA recovery; mixing can be performed either by vortexing or by extensive up-and-down pipetting.***
- The incubation times provided in the following protocols are guidelines only. In order to maximize efficiency and throughput, incubation times should be modified/optimized according to your current protocols, previous experience, specific equipment, and samples.
- The time required for complete capture of beads on the magnet varies according to the plate/tube(s), solution volume, sample viscosity, and magnet used. It is important not to discard or transfer any beads with the removal or transfer of supernatant. Capture times should be optimized accordingly.
- It is important to prepare fresh 80% ethanol prior to use, since ethanol absorbs atmospheric water.
- The volume of freshly-prepared 80% ethanol used for bead washes may be adjusted to accommodate different plate/tube volumes and/or limited pipetting capacity, but it is important that the beads are entirely submerged during the wash steps. Where possible, use a wash volume that is equal to the volume of sample plus KAPA HyperPure Beads.
- It is important to remove all ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, and may result in a dramatic loss of DNA. Drying of beads for 3 – 5 min at room temperature should be sufficient. ***Drying of beads at 37ºC is not recommended.***
- Where appropriate, DNA should be eluted from beads in elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5). Elution of DNA in PCR-grade water is not recommended as DNA is unstable in unbuffered solutions. However, certain applications and protocols (e.g., hybridization target capture that requires drying of DNA prior to probe hybridization) may require elution and storage of purified DNA in PCR-grade water. Always ensure that the volume in which DNA is recovered (elution volume) is sufficient to completely submerge captured beads.
- Purified DNA in elution buffer should be stable at 2°C to 8°C for 1 – 2 weeks, or for at least 1 month at -15°C to -25°C. The long-term stability of library DNA at -20°C depends on a number of factors, including library concentration. Always use low DNA-binding tubes for long-term storage and avoid excessive freezing and thawing.

DNA Purification (Cleanup)

- The considerations outlined below apply to general protocols for the purification (cleanup), concentration, or buffer exchange of double-stranded DNA.
- For maximum recovery of the original DNA sample during cleanup, buffer exchange, and/or concentration of genomic DNA, fragmented DNA for NGS library construction, PCR or qPCR products—regardless of quality—a KAPA HyperPure Beads-to-sample volumetric ratio of 3X is recommended. If a lower ratio is used, it may result in lower yields and exclusion of smaller fragments.
- To increase the recovery of DNA at a specific stage in an NGS library construction protocol, the volume of KAPA HyperPure Beads used in the cleanup may be increased, but this will most likely result in the retention of smaller DNA fragments.
- DNA cleanups may also be used to intentionally exclude a portion of the DNA sample, smaller than a specific target size. The size range of DNA fragments recovered during single-sided bead-based cleanups is dependent on the volume of KAPA HyperPure Beads added to the DNA sample. To increase the recovery of smaller DNA fragments, increase the volume (ratio) of KAPA HyperPure Beads, and *vice versa*.
- If the DNA input for NGS library construction is not limiting and/or you prefer to eliminate smaller fragments during reaction cleanup(s) instead of using adedicatedsize

selection procedure, you might consider decreasing the volume (ratio) of KAPA HyperPure Beads in one or more of the cleanup reactions.

Size Selection in NGS Workflows

- Size selection requirements vary widely for different NGS applications. Depending on your specific application, sample type, and input and library construction chemistry, size selection may be performed at the following points during the overall library construction process:
  - after DNA fragmentation
  - after individual enzymatic reactions
  - after the post-ligation cleanup
  - after library amplification.
- Bead-based size selection, or "double-sided size selection", consists of a "first" and "second" cut, represented as ratios of beads-to-sample volumes. The first cut determines the upper size limit of the size-selected DNA, whereas the second cut determines the lower size limit.
- To increase the upper size limit of the selected fragments, reduce the volume of KAPA HyperPure Beads used for the first cut. To decrease the upper size limit of the selected fragments, increase the volume of KAPA HyperPure Beads use in the first cut (Table 1).
- To increase the lower size limit of the selected fragments, reduce the volume of KAPA HyperPure Beads added in the second cut. To decrease the lower size limit of the size selected fragments, increase the volume of KAPA HyperPure Beads added in the second cut (Table 1).

Table 1: Guidelines for the modification of size selection parameters

Upper size limit	Modification
Increase	Decrease the ratio of the first cut
Decrease	Increase the ratio of the first cut
Lower size limit	**Modification**
Increase	Decrease the ratio of the second cut
Decrease	Increase the ratio of the second cut

- The second size cut should be performed with at least 0.2 volumes of KAPA HyperPure Beads. ***Please note the volume of the KAPA HyperPure Beads needed for the second cut is calculated relative to the volume of the sample at the start of the size selection procedure, not the volume of the DNA-containing supernatant transferred after the first cut.*** Please refer to **Protocols: Size Selection in NGS Workflows** (p. 5) for an example. DNA recovery is dramatically reduced if the difference between the first and second cuts is less than ~0.2 volumes. To increase the amount of DNA recovered, more than 0.2 volumes of KAPA HyperPure Beads may be used for the second cut, but note that this may result in the recovery of smaller library fragments and a broader size distribution.
- Size selection inevitably leads to a loss of sample material, and can be dramatic (>80%). This may have a significant impact on final library concentrations in PCR-free workflows or increase the number of amplification cycles needed to generate sufficient material for target capture or sequencing. The potential advantages of one or more size selection steps in a library construction workflow should be weighed against the potential loss of library complexity, especially when DNA input is limited.
- In some instances, a single-sided cleanup may suffice to remove unwanted, small, or large library fragments, as unwanted fragments can be or may have been removed with standard or modified cleanups at an earlier stage in the library construction process.
- NGS library size selection is sensitive to multiple factors that are beyond the scope of this document. The **KAPA NGS Library Preparation Technical Guide** (available from Technical Support at sequencing.roche.com/support) contains additional guidelines for the optimization of size selection parameters. Any size selection protocol should be carefully optimized and validated before it is used for precious samples.

Protocols

1. Genomic DNA Purification (Cleanup)

Prior to library construction in NGS workflows, it may be beneficial to perform an upfront genomic DNA cleanup. For cleanup, buffer exchange, and/or concentration of high-quality genomic DNA prior to library construction, a KAPA HyperPure Beads-to-sample volumetric ratio of 3X is recommended.

The detailed protocol below is an example of a 3X cleanup of genomic DNA in 100 µL.

Ensure that the plate/tube(s) selected for the cleanup can accommodate the DNA sample plus the appropriate volume of KAPA HyperPure Beads, and that it is compatible with your magnet and heating device.

1.1 Ensure that KAPA HyperPure Beads has been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

1.2 Add 300 µL of KAPA HyperPure Beads to the 100 µL genomic DNA sample.

1.3 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

1.4 Incubate the plate/tube(s) at room temperature for 5 min to bind the DNA to the beads.

1.5 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

1.6 Carefully remove and discard the supernatant.

1.7 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

1.8 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

1.9 Carefully remove and discard the ethanol.

1.10 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

1.11 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

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

1.13 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

1.14 Remove the plate/tube(s) from the magnet.

1.15 Resuspend the beads in an appropriate volume of elution buffer. The appropriate elution buffer may be either 10 mM Tris-HCl, (pH 8.0 – 8.5) or PCR-grade water, depending on the downstream application.

1.16 Incubate the plate/tube(s) for 2 min to elute the DNA off the beads.

1.17 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

1.18 Transfer the clear supernatant to a new plate/tube(s). Proceed with your downstream application. DNA may be stored for 1 – 2 weeks at 2°C to 8°C, or at -15°C to -25°C for a minimum of 1 month.

Notes on RNA Purification (Cleanup)

KAPA HyperPure Beads have been validated for use with RNA **prior to** RNA enrichment/RNA library preparation workflows. E.g. in some cases, inhibitors may be present in a total RNA sample that could limit the efficiency of the rRNA and/or globin mRNA depletion. If the presence of inhibitors is unknown or suspected, an optional, up-front 3X bead purification may be used.

Follow the protocol as outlined in “Genomic DNA Purification” with the following modifications:

- Elute purified RNA in RNase-free water for subsequent processing.
- To mix samples containing RNA, gently pipette the reaction mixture several times.

2. Cleanup of Fragmented DNA in NGS Workflows

In NGS library construction workflows, the appropriate bead-to-sample ratio depends on the point in the workflow at which the cleanup is performed (e.g., after fragmentation, adapter ligation, or library amplification), and the desired fragment sizes to be retained/excluded. KAPA HyperPure Beads may be employed for the effective cleanup of fragmented DNA at various stages of NGS library preparation workflows.

The size range of DNA fragments recovered during a single-sided bead-based cleanup is dependent on the volume (ratio) of KAPA HyperPure Beads added to the DNA sample. For fragmented DNA, NGS libraries, and amplicons, recommendations for KAPA HyperPure Beads-to-sample volumetric ratios based upon desired fragment lengths to be retained are provided in Table 2 (and shown in Figure 1), and should be used as a guideline.

The detailed protocol outlined below is an example of a 0.8X cleanup of a 100 µL fragmented DNA sample.

2.1 Ensure that KAPA HyperPure Beads has been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

2.2 Add 80 µL of KAPA HyperPure Beads to the 100 µL fragmented DNA sample.

2.3 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

2.4 Incubate the plate/tube(s) at room temperature for 5 min to bind the DNA to the beads.

2.5 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

2.6 Carefully remove and discard the supernatant.

2.7 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

2.8 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

2.9 Carefully remove and discard the ethanol.

2.10 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

2.11 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

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

2.13 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

2.14 Remove the plate/tube(s) from the magnet.

2.15 Resuspend the beads in an appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5) or PCR-grade water, depending on the downstream application.

2.16 Incubate the plate/tube(s) at room temperature for 2 min to elute the DNA off the beads.

2.17 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

2.18 Transfer the clear supernatant to a new plate/tube(s). Proceed with your downstream application. DNA may be stored for 1 – 2 weeks at 2°C to 8°C, or at -15°C to -25°C for a minimum of 1 month.

Table 2: Guidelines for the purification (cleanup) of fragmented DNA, NGS libraries, and amplicons with KAPA HyperPure Beads

Fragments to be retained	Recommended KAPA HyperPure Beads-to-sample volumetric ratio
≥600 bp	0.5X
≥400 bp	0.6X
≥300 bp	0.7X / 0.8X
≥200 bp	0.9X
≥150 bp	1.5X
≥100 bp	2.2X – 3X

3. Size Selection in NGS Workflows

Size selection requirements vary widely for different sequencing applications. KAPA HyperPure Beads may be integrated into most DNA library construction workflows, and size selection can be carried out at various points in the overall workflow (e.g., after fragmentation, post-ligation cleanup, or library amplification).

Guidelines for size selection of Illumina libraries with KAPA HyperPure Beads are given in Table 3, and representative traces of size-selected input DNA and libraries are given in Figure 2. These parameters are provided as guidelines only and may require additional optimization.

The following detailed protocol is an example of size selection of adapter-ligated library in a 50 µL volume. As per Table 3, a 0.6X – 0.8X size selection is used to target a final average library size distribution of 400 bp. The first 0.6X cut is designed to exclude molecules >600 bp from the library-containing supernatant retained for the second cut. The additional 0.2 volumes of KAPA HyperPure Beads results in the binding of all molecules >300 bp (but <600 bp) to the beads. DNA fragments <300 bp are discarded with the supernatant during the bead washes.

3.1 Ensure that KAPA HyperPure Beads has been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

3.2 Perform the first size cut (0.6X, to exclude large library fragments) by adding 30 µL of KAPA HyperPure Beads to 50 µL of adapter-ligated library (0.6 x 50 µL = 30 µL).

3.3 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

3.4 Incubate the plate/tube(s) at room temperature for 5 min to bind large library molecules (>600 bp) to the beads.

3.5 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

3.6 Carefully transfer the supernatant containing the smaller library molecules (<600 bp) to a new plate/tube(s). ***It is critical that no beads are transferred with the supernatant.***

3.7 Discard the plate/tube(s) containing the beads to which library fragments larger than ~600 bp are bound.

3.8 Perform the second size cut (0.8X) by adding 10 µL of KAPA HyperPure Beads to the supernatant. This volume is calculated relative to the **original** sample volume of 50 µL, e.g., (0.8 – 0.6) x 50 µL = 10 µL.

3.9 Mix thoroughly by vortexing and/or pipetting up and down multiple times.

3.10 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind library molecules >300 bp to the beads.

3.11 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear. Carefully remove and discard the supernatant which contains library molecules <300 bp.

3.12 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

3.13 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

3.14 Carefully remove and discard the ethanol.

3.15 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

3.16 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

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

3.18 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

3.19 Remove the plate/tube(s) from the magnet.

3.20 Thoroughly resuspend the beads in the required volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5), or PCR-grade water, depending on the downstream application.

3.21 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.

3.22 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

3.23 Transfer the clear supernatant with size-selected DNA to a new plate/tube(s). Proceed with your downstream application. DNA may be stored for 1 – 2 weeks at 2°C to 8°C, or at -15°C to -25°C for a minimum of 1 month.

Table 3: Guidelines for size selection with KAPA HyperPure Beads

Input DNA type	Size ranges achieved when using different KAPA HyperPure Beads ratios[1,2]			
	0.8X – 1.0X	0.7X – 0.9X	0.6X – 0.8X	0.5X – 0.7X
Fragmented dsDNA[3]	150 – 350 bp	200 – 400 bp	250 – 550 bp	300 – 1000 bp
Average fragment size[3]	250 bp	300 bp	350 bp	500 bp
Adapter-ligated library[4,5]	200 – 400 bp	250 – 500 bp	300 – 600 bp	350 – 1200 bp
Average fragment size	300 bp	350 bp	400 bp	550 bp
Amplified library	200 – 400 bp	250 – 500 bp	300 – 600 bp	350 – 1200 bp
Average fragment size	300 bp	350 bp	400 bp	525 bp

[1]High quality human genomic DNA was fragmented with a Covaris E220 Focused Ultrasonicator using conditions optimized to yield a peak mode fragment length in the range of 250 – 400 bp.

[2]These size selection parameters were optimized for libraries prepared with KAPA Unique Dual-Indexed Adapters. Parameters have to be optimized for libraries prepared with single-indexed adapters, shorter or custom adapter designs.

[3]The reported size range refers to the size of *fragmented DNA* prior to library preparation. The *library* size will be ~120 bp longer once adapter ligation has taken place.

[4]When performing size selection after ligation, it is important to perform at least one post-ligation cleanup, as KAPA Ligation Buffers contain high concentrations of PEG 6000, which will interfere with size selection if not removed.

[5]The reported size range refers to the size of *adapter-ligated libraries*. Adapter ligation increases the length of DNA fragments. To achieve the same final mode library size distribution, different ratios of KAPA HyperPure Beads is therefore required for the first cut when performing size selection after ligation or library amplification, as opposed to after fragmentation.

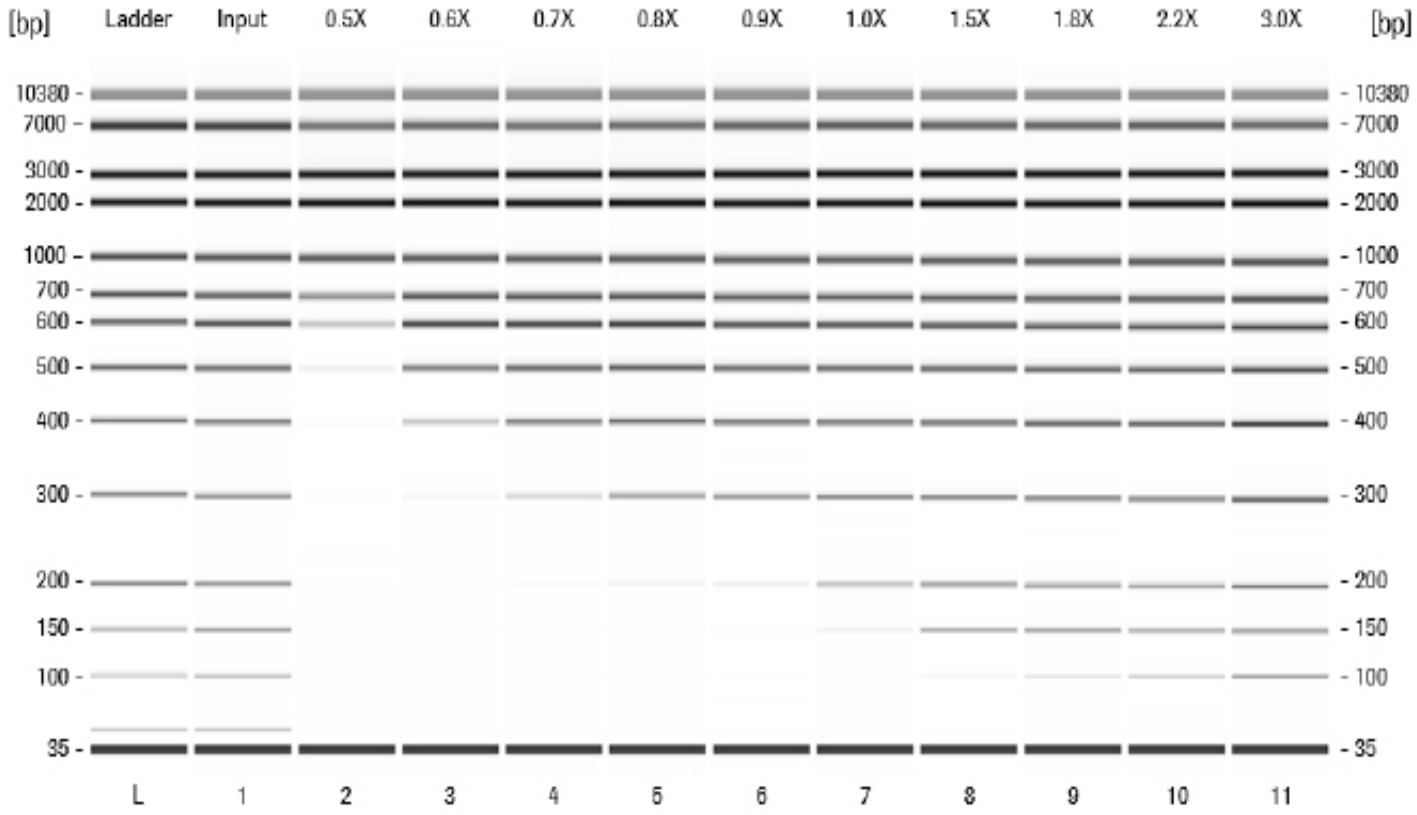

Figure 1. Effect of KAPA HyperPure Beads-to-sample ratio on the recovery of dsDNA fragments

A defined mixture of dsDNA fragments (ranging from 35 bp to 10 kb) was processed using various KAPA HyperPure Beads-to-sample ratios. The impact on DNA fragment size retention was assessed with an Agilent Bioanalyzer 2100 High Sensitivity DNA Kit. Retained DNA fragment lengths are inversely proportional to the bead-to-sample ratio; a greater volume or ratio of beads is required to retain lower molecular weight fragments.

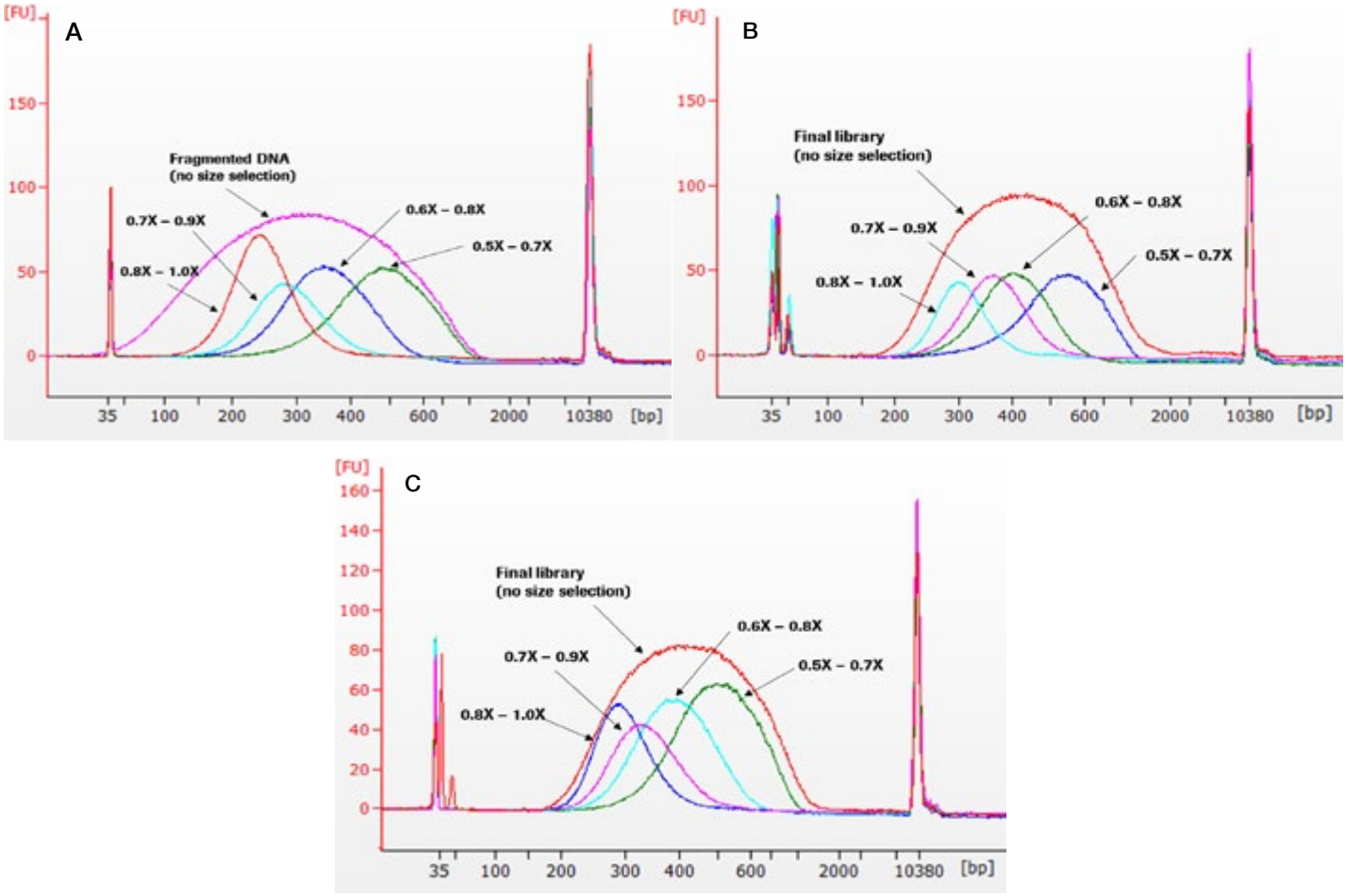

Figure 2. Examples of input DNA and NGS libraries subjected to bead-based size selection using KAPA HyperPure Beads at different stages of the library construction process

A. Size distributions of fragmented input DNA subjected to size selection after fragmentation with different parameters to achieve different final size distributions (as outlined in Table 2). DNA was mechanically fragmented with a Covaris E220 Focused Ultrasonicator.

B. Size distributions for final, amplified libraries that were not size selected vs. libraries prepared from the same input DNA, but size selected with different parameters after the post-ligation cleanup to achieve different final size distributions (as outlined in Table 2).

C. Size distributions for final, amplified libraries that were not size selected vs. libraries prepared from the same input DNA, but size selected with different parameters after the post-amplification cleanup to achieve different final size distributions (as outlined in Table 2).

All libraries were prepared with the KAPA HyperPrep Kit, from 100 ng high-quality human genomic DNA, fragmented with a Covaris E220 Focused Ultrasonicator using conditions optimized to yield a mode fragment length in the range of 250 – 400 bp. Libraries were prepared using 15 µM KAPA Unique Dual-Indexed Adapters. Electropherograms were generated with a Bioanalyzer 2100 High Sensitivity DNA Kit. DNA concentrations were normalized prior to analysis and are not reflective of actual concentrations at different stages of the library preparation workflow.

Appendix – Troubleshooting

Problem	Possible Cause	Possible Solution
Poor recovery of purified DNA	Bead solution not equilibrated to room temperature	Allow sufficient time for KAPA HyperPure Beads to equilibrate to room temperature; time may vary depending on volume of beads.
	Bead solution not homogeneous prior to use	Gently vortex to ensure beads are fully resuspended.
	Inappropriate reaction volume used	Increase sample volume before cleanup or size selection by diluting the DNA in 10 mM Tris-HCl (pH 8.0 – 8.5) or PCR-grade water. Ensure that reaction volume is compatible with magnet. Volume in which DNA is eluted must be large enough to completely submerge captured beads.
	Insufficient mixing of bead solution and sample	Mix thoroughly by vortexing for at least 30 sec at high speed or pipetting up and down at least 20 times.
	Insufficient binding of DNA to beads	Incubate DNA/bead solution for at least 5 min at room temperature to allow DNA to bind to beads.
	Effective concentration of ethanol is <80%	Prepare fresh 80% ethanol daily prior to use.
	Over-drying of beads during wash steps	Reduce the amount of time allotted for drying; do not dry the beads at 37ºC. Beads should not have a cracked appearance after drying.
	Insufficient elution time	Allow at least 2 min for DNA to elute from the beads.
	Incorrect storage of kit	Always store KAPA HyperPure Beads at 2°C to 8°C protected from light; do not store at -15°C to -25°C.
Beads present in purified/size selected DNA	Insufficient incubation time on magnet	Capture beads on magnet until supernatant is completely clear. Plate/tube(s), volume, sample viscosity, and magnet may affect time needed for bead capture.
	Pipetting error	Care should be taken not to aspirate beads when transferring solution.
Range of size selected DNA is not as expected	Incorrect volume of beads added to the reaction	Ensure that the correct volume or ratio of KAPA HyperPure Beads solution is used for the first cut (refer to Tables 1 and 2). For size selection, the volume of beads needed for the second cut is calculated relative to the volume of the DNA at the **start** of the size selection procedure, not the volume of the DNA-containing supernatant transfer after the first cut.
	Did not perform a post-ligation cleanup prior to size selection	KAPA Ligation Buffers contain high concentrations of PEG 6000, which will interfere with size selection if a post-ligation cleanup is not performed.

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