KAPABIOSYSTEMS

Technical Data Sheet

KAPA Pure Beads

KR1245 – v3.16

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

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Kapa/*Roche* Kit Codes and Components		
KK8000 *07983271001*	KAPA Pure Beads	5 mL
KK8001 *07983280001*	KAPA Pure Beads	30 mL
KK8002 *07983298001*	KAPA Pure Beads	60 mL

Quick Notes

- KAPA Pure Beads is designed for the rapid and efficient purification or size selection of DNA, for inputs ranging from 1 ng – 5 µg in a single reaction.
- KAPA Pure Beads is recommended for use with all KAPA NGS library preparation kits.
- Protocols are compatible with manual processing and automated liquid handling.
- KAPA Pure 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 Pure 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 Pure 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 Pure 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 Pure Beads buffer includes PEG/NaCl—a crowding agent designed to drive DNA molecules to the beads for binding. The volumetric ratio of KAPA Pure 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 Applications

KAPA Pure Beads is ideally suited for:

- fast and efficient reaction cleanups to remove adapters, adapter-dimers, primers, primer-dimers, nucleotides, salts, and enzymes in NGS library preparation, PCR, and qPCR workflows,
- size selection of fragmented input DNA, adapter-ligated library molecules, or amplified libraries in NGS library preparation workflows, and
- general manipulation of DNA samples, e.g., sample concentration and buffer exchange (e.g., from salts, buffer components, enzymes, etc.).

Product Specifications

Shipping and Storage

KAPA Pure Beads is 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 at -20°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 Pure Beads will retain full functionality until the expiry date indicated on the kit label.

Handling

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

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 **kapabiosystems.com/support** for more information.

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 Pure 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 Pure Beads.***
- 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 Pure Beads. This is recommended for starting volumes <50 µL, 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 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 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 Pure 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., for target capture) 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.
- Elution time and temperature may impact DNA recovery, particularly for high-molecular weight genomic DNA. ***For optimal results with genomic DNA, elution may be performed at an elevated temperature;*** 37°C for 10 min is recommended. Please refer to **Protocols: Genomic DNA Purification (Cleanup)** (p. 4) for details.
- Purified DNA in elution buffer should be stable at 4°C for 1 – 2 weeks, or for at least 1 month at -20°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 Pure Beads-to-sample volumetric ratio of 3X is recommended. Expected recoveries are 50 – 90%, depending upon sample quality and quantity. 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 Pure 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 Pure Beads added to the DNA sample. To increase the recovery of smaller DNA fragments, increase the volume (ratio) of KAPA Pure 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 a dedicated size selection procedure, you might consider decreasing the volume (ratio) of KAPA Pure Beads in one or more of the cleanup reactions.
- As indicated above, a heated elution is recommended to ensure optimal recovery of high-quality genomic DNA. This is not a requirement for the cleanup of other DNA types.

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:
  - before or after DNA fragmentation;
  - after individual enzymatic reactions;
  - after the first post-ligation cleanup; or
  - 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 Pure Beads used for the first cut. To decrease the upper size limit of the selected fragments, increase the volume of KAPA Pure Beads use in the first cut (Table 1).
- To increase the lower size limit of the selected fragments, reduce the volume of KAPA Pure Beads added in the second cut. To decrease the lower size limit of the size selected fragments, increase the volume of KAPA Pure 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 Pure Beads. ***Please note the volume of the KAPA Pure 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 Pure 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** 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 Pure 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. Please pay special attention to steps 1.15 and 1.16 (elution of DNA off beads). To ensure optimal recovery, ***these steps may be performed at an elevated temperature: 37°C for 10 min.*** Elution buffer may be pre-heated for this step and/or the elution performed in a thermocycler or heating block. The heated elution is not required for the cleanup, purification, or buffer exchange of other DNA types, e.g., fragmented DNA, NGS libraries or amplicons.

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

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

1.2 Add 300 µL of KAPA Pure 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 10 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 pre-heated elution buffer at 37°C and/or perform the elution incubation (step 1.16) in a thermocycler or heating block set to 37°C. 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 10 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, or store DNA at 4°C for 1 – 2 weeks, or at -20°C.

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 Pure 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 Pure Beads added to the DNA sample. For fragmented DNA, NGS libraries, and amplicons, recommendations for KAPA Pure Beads-to-sample volumetric ratios based upon desired fragment lengths to be retained are provided in Table 2 (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 Pure Beads has been equilibrated to room temperature and that the beads are fully resuspended before proceeding.

2.2 Add 80 µL of KAPA Pure 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 – 15 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. The elution time may be extended up to 10 min if necessary to improve DNA recovery.

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, or store DNA at 4ºC for 1 – 2 weeks, or at -20ºC.

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

Fragments to be retained	Recommended KAPA Pure Beads-to-sample volumetric ratio
≥1 kb	0.5X
≥450 bp	0.6X
≥350 bp	0.7X
≥300 bp	0.8X
≥250 bp	0.9X
≥150 bp	1.5X
≥100 bp	2.2X – 3X

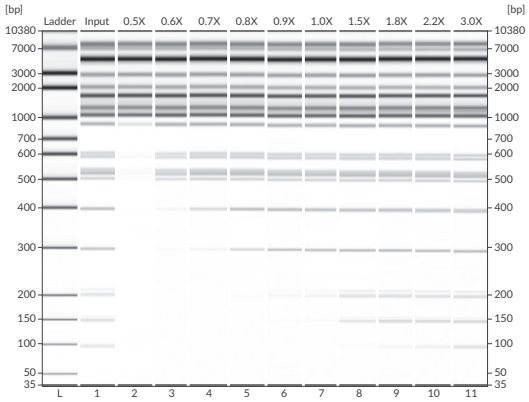

Figure 1. Effect of KAPA Pure Beads-to-sample ratio on the recovery of dsDNA fragments

A defined mixture of dsDNA fragments (ranging from 100 bp to 10 kb) was processed using various KAPA Pure 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.

3. Size Selection in NGS Workflows

Size selection requirements vary widely for different sequencing applications. KAPA Pure 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 Pure 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 library size distribution of 250 – 450 bp. The first 0.6X cut is designed to exclude molecules >450 bp from the library-containing supernatant retained for the second cut. The additional 0.2 volumes of KAPA Pure Beads results in the binding of all molecules >250 bp (but <450 bp) to the beads. DNA fragments <250 bp are discarded with the supernatant during the bead washes.

3.1 Ensure that KAPA Pure 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 Pure 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 – 15 min to bind large library molecules (>450 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 (<450 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 ~450 bp are bound.

3.8 Perform the second size cut (0.8X) by adding 10 µL of KAPA Pure 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 >250 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 smaller than >250 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. The elution time may be extended up to 10 min if necessary to improve DNA recovery.

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, or store DNA at 4ºC for 1 – 2 weeks, or at -20ºC.

Table 3: Guidelines for size selection with KAPA Pure Beads

Input DNA type	Recommended KAPA Pure Beads ratios to achieve different size ranges[1,2]		
	200 – 350 bp	250 – 450 bp	300 – 750 bp
Fragmented dsDNA	0.8X – 1.0X	0.7X – 0.9X	0.6X – 0.8X
Adapter-ligated library[3]	0.7X – 0.9X	0.6X – 0.8X	0.5X – 0.7X
Amplified library	0.7X – 0.9X	0.6X – 0.8X	0.5X – 0.7X

[1]Adapter ligation increases the length of DNA fragments. To achieve the same final fragment size, a lower ratio of KAPA Pure Beads is therefore required for the first cut when performing size selection after ligation or library amplification, as opposed to after fragmentation. Please refer to Figure 2 for more details.

[2]These size selection parameters were optimized for libraries prepared with single-indexed, Illumina® TruSeq®-style adapters. Parameters have to be optimized for libraries prepared with dual-indexed adapters, shorter or custom adapter designs.

[3]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 (double-sided cleanups) if not removed.

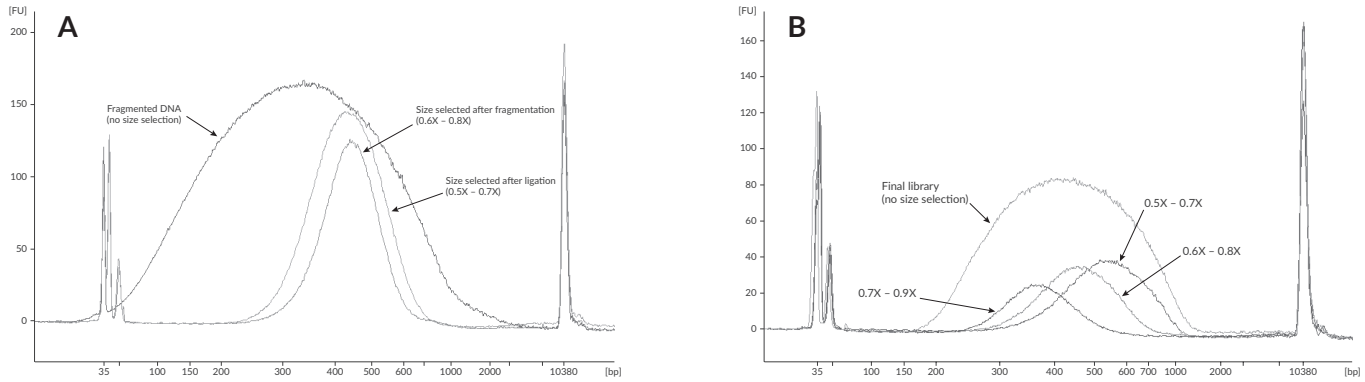

Figure 2. Examples of input DNA and NGS libraries subjected to bead-based size selection using KAPA Pure Beads at different stages of the library construction process

A. Size distributions of fragmented input DNA (no size selection), vs. final, amplified libraries prepared from the same DNA, but size selected after fragmentation or after ligation, respectively. Since the mode fragment size of input DNA increases after adapter ligation, the KAPA Pure Beads-to-sample volumetric ratio used for the first cut during post-ligation size selection was lower than for post-fragmentation size selection to target the same final mode library size distribution (300 – 750 bp).

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).

All libraries were prepared with the KAPA Hyper Prep 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 350 – 450 bp. 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.

Troubleshooting

Problem	Possible Cause	Possible Solution
Poor recovery of purified DNA	Bead solution not equilibrated to room temperature	Allow sufficient time for KAPA Pure 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	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 Pure Beads at 4ºC protected from light; do not store at -20ºC.
	Low recovery of gDNA due to room temperature elution	To ensure optimal gDNA recovery, the elution of DNA off the beads should be performed at 37ºC for 10 min.
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 the volume or ratio of KAPA Pure 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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For information on product use limitations and licenses: **www.kapabiosystems.com/company/terms-conditions**

This product has been manufactured under an ISO 13485:2003 quality management system.

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