PRODUCT MANUAL

DINOMAG™ Clean-Up & Size Selection SPRI Magnetic Beads

SPRI Paramagnetic Beads, DNA Clean-Up, NGS Size Selection, PCR Purification, Manual or Automated Liquid Handling, Single-Tube, 96-Well, 384-Well Formats.

DESCRIPTION

DINOMAG™ is the gold standard for rapid, reliable and economical bead-based clean-up and size selection of DNA products for a wide range of genomic applications including but not limited to next-generation sequencing (NGS), PCR, qPCR, ddPCR, Sanger Sequencing, microarrays, and other enzymatic reactions.

DINOMAG™ utilizes SPRI technology which utilizes paramagnetic beads to selectively bind nucleic acids by type and size. The magnetic beads will directly attach DNA fragments to their surface, leaving unwanted substances like unincorporated dNTPs, enzymes, primers, primer dimers, salts, and contaminants in the solution. Afterward, the DNA fragments can be extracted using a low salt buffer or reagent-grade water. Importantly, the technology employed for binding DNA fragments to magnetic nanoparticles does not require the use of any harmful chaotropic buffers. The purification protocols have been fine-tuned to ensure a high yield and purity of the recovered DNA fragments.

DINOMAG™ allows either non-selective binding or size-targeted binding of double-stranded DNA fragments within the range of 100 – 1000 bp, utilizing specific reagent volume to sample volume ratios. The binding technology is designed to keep enzymes, salts, primers, and other small nucleotides in the solution. Increasing the volume of DINOMAG™ enhances the efficiency of capturing smaller fragments, while decreasing the volume reduces this efficiency. This feature allows users to selectively retain or discard undesired fragment sizes. Following the binding of the desired DNA fragment size, the beads undergo two washes with ethanol solutions. Any remaining traces of ethanol are eliminated through a brief drying step, after which the DNA fragments can be released from the beads using an Elution Buffer such as molecular biology grade water, TRIS (10 mM, pH 8.0), or TE-buffer.

SKU/CAT#	DN9004-5ML / DN9004-75ML / DN9004-500ML
UNIT SIZE	5mL, 75mL, 500mL
UOM	mL
PHYSICAL FORM	Liquid
APPEARENCE	Brown, Liquid (Suspension)
FORMAT	Single Tubes, 96-well, 384-well
BEAD RATIO	1.8x for Clean-Up
RECOVERY	Recovery for DNA within the range of 100 – 1000 bp typically ranges from 70-90%
INPUT	PCR products, fragmented DNA
OUTPUT	DNA
PREP DURATION	The entire preparation process, depending on the chosen protocol, takes approximately 20-30 minutes with minimal hands-on time required.
PROCESSING	Manual or automated liquid handling systems.
TECHNOLOGY	SPRI paramagnetic bead-based technology - Each SPRI bead, with a size of 1 µm ± 8%, features a buoyant polystyrene core surrounded by a thin layer of magnetite, making it paramagnetic and easily controllable using a magnetic field. Carboxyl molecules coating the surface of SPRI beads provide the necessary charge groups for DNA binding.
PRESERVATIVE	Sodium Azide (<0.1%), CAS#: 26628-22-8
STORAGE	Store at 2-8°C (KEEP REFRIGERATED)
SHELF LIFE	Stable up to 24-months from date of manufacture. Expiration dates will be indicated on the bottle.
SUPPLIES NOT INCLUDED	20µL/200µL multichannel pipettes, 96-well PCR plates, 384-well PCR plates, magnetic separator
INTENDED USE	For Research Use Only (RUO). Not intended or validated for use in the diagnosis of disease or other conditions.
APPLICATIONS	PCR Purification, DNA Clean-Up, DNA Size Selection, NGS Clean-Up, NGS Prep, PCR Clean-Up, qPCR, ddPCR, PCR, Post-PCR Clean-Up, Post-Enzymatic Reaction Clean-Up, DNA Cloning, Restriction Enzyme Digestion, Adapter Ligations, Microarrays, Sequencing, Fragment Analysis, Genotyping

KEY FEATURES

- One product for clean-up and size selection of successive enzymatic reactions in library preparation for NGS applications
- Efficient bind-wash-elute process with short processing time
- Easily adjustable protocol for clean-up or size selection using specific reagent-to-sample ratios
- The protocol is easily adaptable for a range of high-throughput automated liquid handling workstations, such as Hamilton, Beckman, Agilent, Caliper, Perkin Elmer, Tecan, and Eppendorf. It can seamlessly integrate with your current protocol or be utilized manually.
- Compatible with a wide range of standard protocols of common library preparation kits and instruments
- High recovery of PCR products
- Effective removal of enzymes, primers, oligonucleotides, polymerases, and other contaminants
- Allows either non-selective binding, or size-targeted binding of double-stranded DNA fragments ranging from 100 - 1,000 bp with specific reagent volume to sample volume ratios
- Ensures consistent sequencing results

SAFETY & DISCLAIMER

CAREFULLY READ ALL INSTRUCTIONS BEFORE PERFORMING ANY PROCEDURE. FOLLOW MANUFACTURER'S RECOMMENDATIONS AND PRODUCT LABELING. MATERIAL SAFETY DATA SHEET FOR THIS PRODUCT IS AVAILABLE UPON REQUEST.

THE INFECTIOUS POTENTIAL OF LIQUID WASTE RESULTING FROM THE USE OF THIS PRODUCT HAS NOT BEEN TESTED. ALTHOUGH CONTAMINATION OF WASTE WITH RESIDUAL INFECTIOUS MATERIAL IS UNLIKELY, COMPLETE EXCLUSION CANNOT BE GUARANTEED. THEREFORE, LIQUID WASTE SHOULD BE HANDLED AS POTENTIALLY INFECTIOUS AND DISCARDED ACCORDING TO LOCAL SAFETY REGULATIONS.

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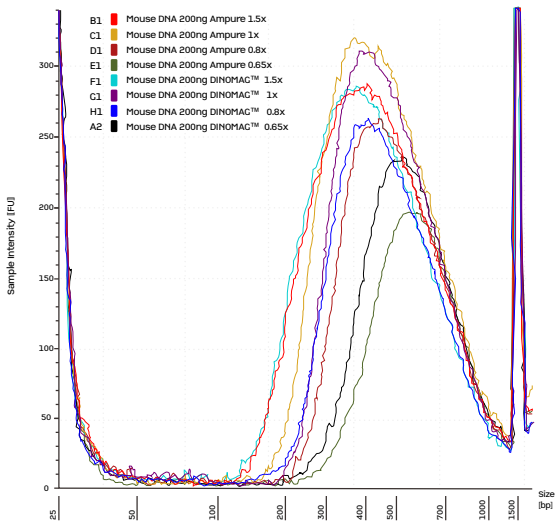
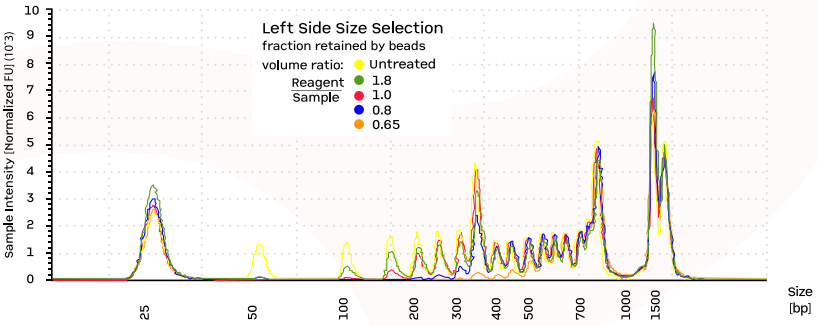


Figure 1: *DNA size distribution curves after left-sided size selection showing a direct comparison between DINOMAG™ and AMPure® XP. Four different bead-to-sample ratios were used.*



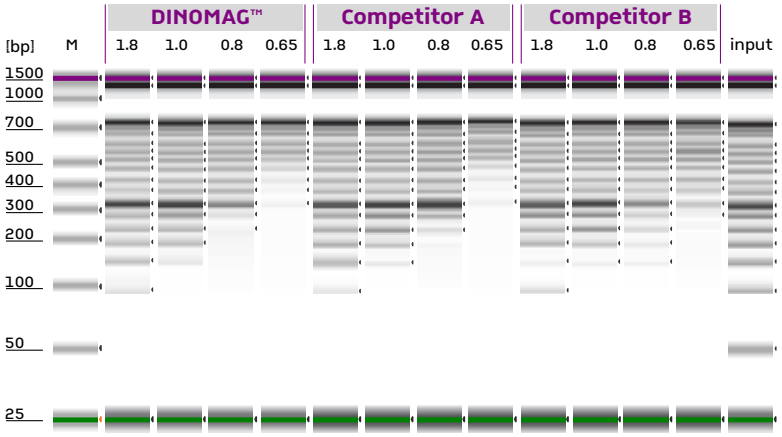


Figure 2 & 3: *Left side size selection fraction retained by beads with different reagent-to-sample volume ratios on TapeStation ScreenTape.*

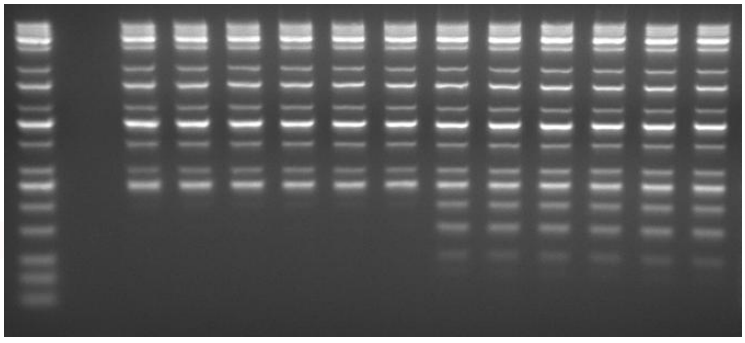


Figure 4: *Agarose gel showing 6 independent size selections from a DNA ladder, using ratios of 0.65x (left) and 1.0x (right)*

PREPARATION OF REAGENTS

- Prepare a fresh ethanol solution.
  - For protocol A, prepare a 70% ethanol solution.
  - For protocols B, C, D, prepare an 85% ethanol solution.
- Before use, intensely vortex DINOMAG™ into a homogeneous suspension.

PROTOCOL KEY

PROTOCOL A

GENERAL PURPOSE DNA CLEAN-UP

For the purification of DNA fragments (excluding size selection) after various DNA manipulation procedures*, DINOMAG™ Plus effectively captures and cleanses all double-stranded DNA fragments larger than approximately 100 base pairs. This protocol is suitable for:

- PCR amplification processes
- cDNA constructions
- DNA restriction digestions
- General DNA manipulation, concentration, and buffer exchange procedures

1. Vortex DINOMAG™ before use to fully resuspend the magnetic beads.
2. Add DINOMAG™ as per the provided table below; mix by pipetting until a uniform, homogeneous suspension is achieved. Incubate for 5-minutes to allow the beads to bind the DNA.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until obtaining a clear solution.
4. While the plate is on the magnet, carefully remove the cleared supernatant from the beads and discard it. Ensure minimal disturbance to the magnetic beads while removing the supernatant.
5. Add 180 µL of 70% EtOH and incubate for 30 seconds to facilitate the settling of beads to the magnet. This step can be executed with the plate on the magnet, and there's no need to resuspend the beads.
6. While the plate is on the magnet, discard the supernatant.
7. Repeat steps 5-6 for an additional washing cycle, making a total of 2 washing steps.
8. Allow the magnetic particles to air-dry for approximately 5 minutes. This step can be performed while the plate is on the magnet.
9. Remove the plate from the magnet, add 40 µL of Elution Buffer, mix by pipetting 10 times, and incubate for 2 minutes to elute.
10. Place the sample plate on the magnetic separator for 1 minute to gather the magnetic beads.
11. While the plate is on the magnet, transfer the supernatant to the final plate. Leave 5 µL of liquid behind to prevent the transfer of beads into the final plate.

REACTION VOLUME (µL)	DINOMAG™ VOLUME (µL)
10	18
15	27
20	36
25	45
50	90

For different reaction volumes, use the following equation: Volume of DINOMAG™ = 1.8x Reaction Volume

LEFT SIDE SIZE SELECTION

This protocol removes DNA fragments below a target size by binding the target DNA to beads, performing washing steps, and subsequently eluting the bound DNA. This protocol is applicable at different stages of NGS library preparation procedures.

1. Before usage, vortex DINOMAG™ thoroughly to ensure complete bead resuspension.
2. Add the required volume of DINOMAG™ to the sample for the desired ratio. Mix by pipetting the 10 times until achieving a homogeneous suspension, then incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect magnetic beads until a clear solution is achieved.
4. While the plate is on the magnet, carefully remove the cleared supernatant from the beads, ensuring minimal disturbance to the magnetic beads, and discard it.
5. Add 180 µL of 85% EtOH and incubate for 30 seconds to enable the beads to settle to the magnet again. This step can be carried out with the plate on the magnet.
6. While the plate remains on the magnet, discard the supernatant.
7. Repeat steps 5-6 for an additional washing cycle, completing a total of 2 washing steps.
8. Air-dry the magnetic particles for approximately 5 minutes. This step can be conducted with the plate on the magnet.
9. Remove the plate from the magnet, add 40 µL of Elution Buffer, mix by pipetting 10 times, and incubate for 2 minutes to elute.
10. Place the sample plate on the magnetic separator for 1 minute to collect the magnetic beads.
11. While the plate is on the magnet, transfer the eluate (size-selected sample) to the final plate. Leave 2-5 µL of liquid behind to prevent the transfer of beads into the final plate.

*Volume of Sample * Ratio = Volume of DINOMAG™ Example: 50 µL * 0.65 = 32.5 µL of DINOMAG™*

Note: Decreasing the ratio of DINOMAG™ Plus volume to sample volume for Left Side Selection will decrease the efficiency of binding smaller fragments (see Figure 5).

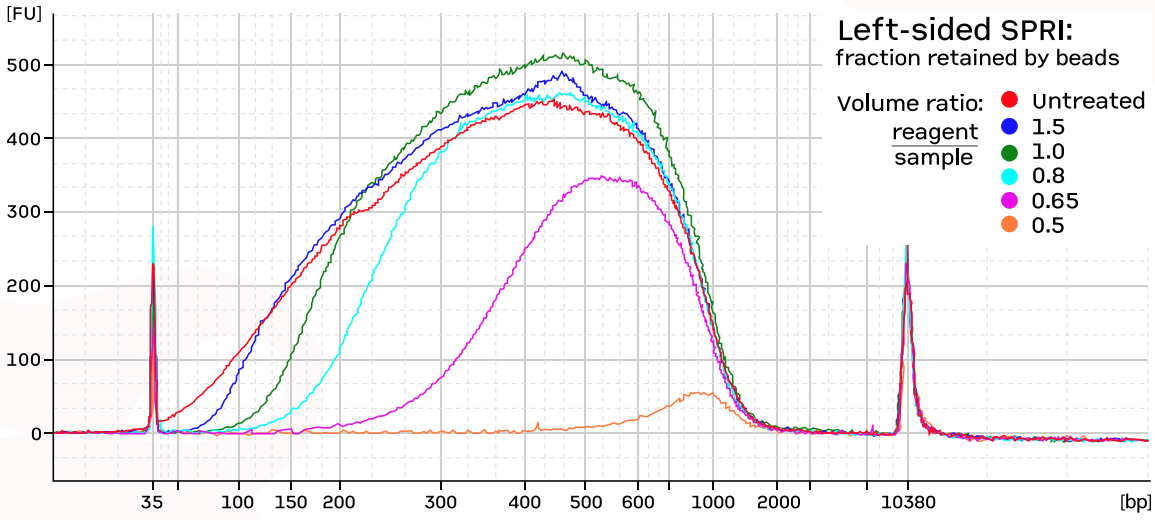


Figure 5: *Left Side Size Selection with given ratios of DINOMAG™ volume to sample volume*

RIGHT SIDE SIZE SELECTION

This protocol removes DNA fragments above a specified target size. DNA above the target size is bound by the beads, while the desired target DNA remains in the solution. In a subsequent binding phase, the target DNA is bound to the beads, undergoes washing, and is eluted.

1. Before usage, vortex DINOMAG™ thoroughly to ensure the beads are fully suspended.
2. Add the required volume of DINOMAG™ according to the desired ratio to the sample. Mix by pipetting 10 times until achieving a homogeneous suspension and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until achieving a clear solution.
4. Transfer the cleared supernatant from the beads to a new container, being cautious not to aspirate the beads containing larger undesired fragments.
5. Add the required volume of DINOMAG™ using the formula: ***Initial sample volume * (1.8 – Right Side ratio) = Volume of DINOMAG™***. Mix by pipetting 10 times until obtaining a homogeneous suspension and incubate for 5 minutes.
6. Place the sample plate on the magnetic separator to collect the magnetic beads until achieving a clear solution. Collection times may vary, with larger initial sample volumes, higher DINOMAG™ ratios, or a weaker magnet resulting in longer collection times.
7. Remove the cleared supernatant from the beads and discard it. This step must be performed while the plate is on the magnet. Remove the supernatant as much as possible, ensuring not to disrupt the magnetic beads.
8. Add 180 µL EtOH 85% and incubate for 30 seconds to enable the beads to settle to the magnet again. This step can be performed while the plate is placed on the magnet.
9. Discard the supernatant. This step must be performed while the plate is on the magnet.
10. Repeat steps 8-9 once more for a total of 2 washing steps.
11. Air-dry the magnetic particles for approximately 5 minutes. This step can be performed while the plate is placed on the magnet.
12. Remove the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10 times and incubate for 2 minutes to elute.
13. Place the sample plate on the magnetic separator for 1 minute to gather the magnetic beads.
14. Transfer the eluate (size-selected sample) to the final plate. This step must be executed while the plate is on the magnet. Leave 2-5 µL of liquid behind to prevent the transfer of beads into the final plate.

*Volume of sample * Ratio = Volume of DINOMAG™. Example: 50 µL * 0.65 = 32.5 µL of DINOMAG™*

Note: Increasing the ratio of DINOMAG™ Plus volume to sample volume for Right Side Selection will increase the efficiency of removing larger fragments (see Figure 6).

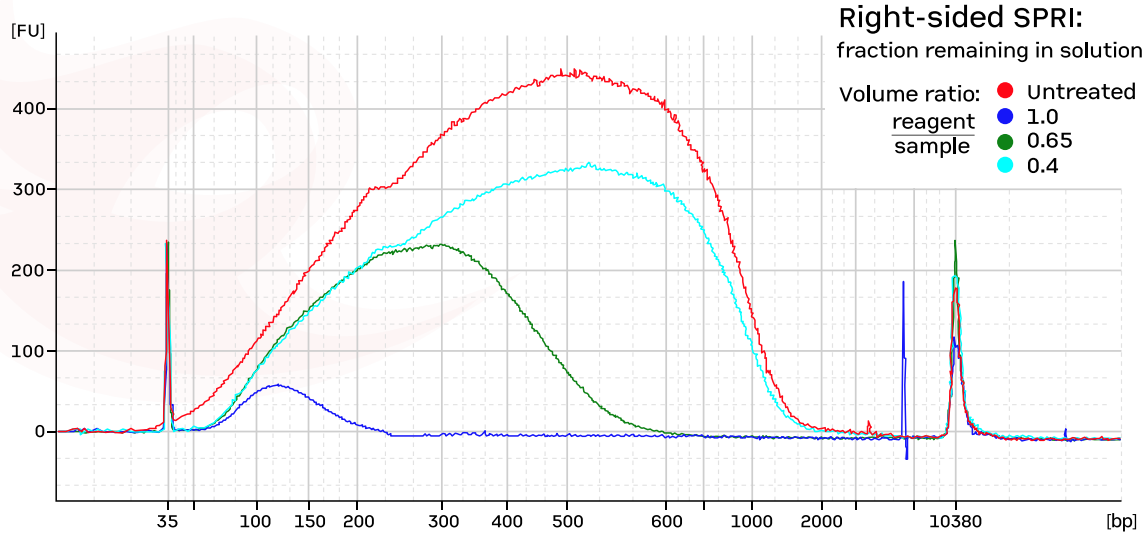


Figure 6: *Right Side Size Selection with given ratios of DINOMAG™ volume to sample volume*

DOUBLE SIDED SIZE SELECTION

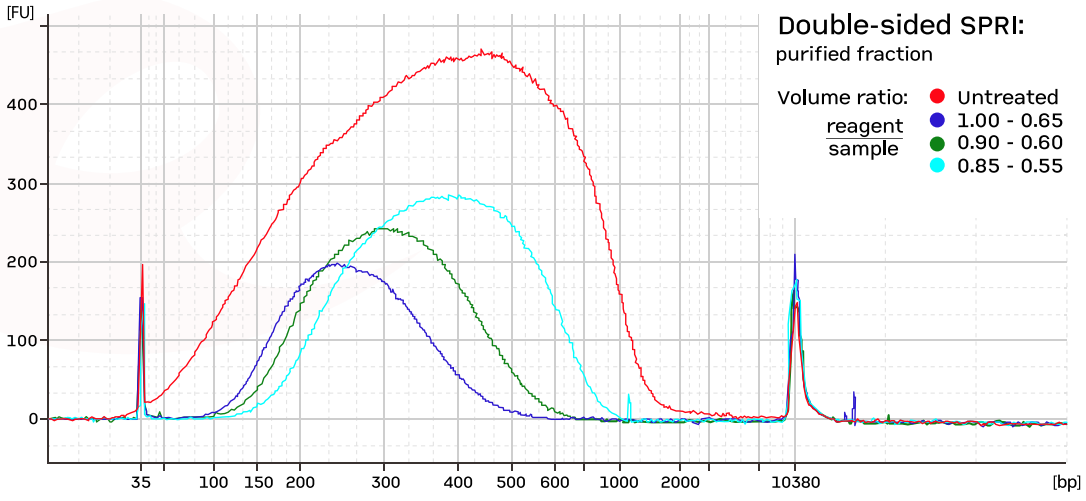
This protocol selectively removes DNA fragments both above and below the target size range. DNA above the target size binds to the beads, leaving the desired target DNA in solution. In a subsequent step, DNA below the target size is retained in the solution, while the target DNA is bound to the beads, washed, and eluted.

1. Prior to utilization, vortex DINOMAG™ to ensure thorough bead resuspension.
2. Add the required volume of DINOMAG™ for the desired Right Side Ratio into the sample. Mix by pipetting 10 times until achieving a homogeneous suspension and incubate for 5 minutes.
3. Place the sample plate on the magnetic separator for 2 minutes to collect the magnetic beads until obtaining a clear solution.
4. Transfer the cleared supernatant from the beads to a new container. Be careful not to aspirate beads, as they contain undesired larger fragment sizes.
5. Add the required DINOMAG™ volume using the formula: ***Initial sample volume * (Left Side ratio – Right Side ratio) = Volume of DINOMAG™***. Mix by pipetting 10 times until achieving a homogeneous suspension and incubate for 5 minutes.
6. Place the sample plate on the magnetic separator to collect the magnetic beads until obtaining a clear solution. Collection times may vary; a higher initial sample volume, higher DINOMAG™ ratio, or weaker magnet will result in longer collection times.
7. Remove the cleared supernatant from the beads and discard. Execute this step while the plate is on the magnet. Remove the supernatant as thoroughly as possible, ensuring not to disrupt the magnetic beads.
8. Add 180 µL EtOH 85% and incubate for 30 seconds to enable the beads to settle to the magnet again. This step can be executed while the plate is positioned on the magnet.
9. Discard the supernatant. Perform this step while the plate is on the magnet.
10. Repeat steps 8-9 once more for a total of 2 washing steps.
11. Air-dry the magnetic particles for approximately 5 minutes. This step can be executed while the plate is on the magnet.
12. Detach the plate from the magnet and add 40 µL Elution Buffer. Mix by pipetting 10 times and incubate for 2 minutes to elute.
13. Place the sample plate on the magnetic separator for 1 minute to gather the magnetic beads.
14. Transfer the eluate (size-selected sample) to the final plate. Execute this step while the plate is on the magnet. Leave 2-5 µL liquid behind to prevent the transfer of beads into the final plate.

*Volume of sample * Ratio = Volume of DINOMAG™. Example: 50 µL * 0.65 = 32.5 µL of DINOMAG™*

***Note:** The Left Side Selection ratio is always greater than the Right Side Selection ratio. In the first step (Right Side Selection), increasing the ratio of DINOMAG™ volume to sample volume will increase the efficiency of removing larger fragments. In the second step (Left Side Selection), decreasing the ratio of DINOMAG™ volume to sample volume will increase the efficiency of removing smaller fragments (see Figure 7).*

***Figure 7:** Double Sided Size Selection with given ratio's of DINOMAG™ volume to sample volume*



TROUBLESHOOTING

LOW RECOVERY OF DNA	INSUFFICIENT BINDING OF DNA	- Increase pipette mixing steps - Increase binding incubation time
	OVERDRYING OF BEADS	- Decrease drying time to a minimum for removal of traces of ethanol. Visually inspect for leftover liquid.
	INSUFFICIENT EtOH REMOVAL AFTER WASHING STEPS	- Make sure to discard all wash solution - - Increase drying time
	INSUFFICIENT ELUTION	- Increase pipette mixing steps - Increase elution incubation time
INSUFFICIENT REMOVAL OF UNWANTED REACTION PRODUCTS	UNWANTED PRODUCTS IN WELLS OR ON BEADS	- For washing, use the maximum working volume - Increase pipette mixing steps for washing
MAGNETIC BEADS IN FINAL ELUATE	CARRYOVER OF BEADS INTO FINAL CONTAINER	- Leave 5 µL of liquid behind to prevent beads from being aspirated - Decrease aspiration speed of pipetting
UNDESIRED FRAGMENT SIZES REMAINING AFTER SIZE SELECTION	INSUFFICIENT MIXING OF SAMPLE AND DINOMAG™	- After dispensing onto sample, immediately mix to prevent differences in buffer concentration within the sample - Increase mix steps after addition of DINOMAG™
	INSUFFICIENT BINDING EFFICIENCY IN REMOVAL OF UNDESIRED FRAGMENTS	- Increase incubation time for binding of DNA fragments
	CARRYOVER OF BEADS WITH UNDESIRED FRAGMENTS	- Increase incubation time for magnetic separation - Repeat magnetic separation and transfer of samples
	REAGENTS IN THE SAMPLE THAT AFFECT SIZE CUT-OFF (E.G. POLYETHYLENE GLYCOL, MgCl2)	- Adjust ratio for size selection - Remove reagents from sample with Protocol A, then proceed with size selection

HAVE QUESTIONS?

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HEADQUARTERS

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