Application Note

Automated size selection with CleanNGS – the cost-effective alternative to AMPure XP

Introduction

DNA size selection with magnetic beads plays a significant role in molecular biology, employing specific ratios to capture and separate fragments by size. After the polymerase chain reaction (PCR), single-sided DNA size selection uses 1 high magnetic bead ratio to remove primer dimers, ensuring PCR product purification for efficient downstream sequencing. In contrast, DNA double-sided size selection uses 2 ratios to effectively remove small and large fragments, resulting in the purification of specific target average fragment sizes. Both single- and double-sided DNA size selections with magnetic beads are integral to next generation sequencing (NGS) library preparation.

Level up your PCR clean-up protocol and DNA size selection workflow using magnetic beads with our fully automated size selection protocols. The VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot automates all liquid handling steps and the MAG module ensures precise automated magnetic bead handling. The protocols provided demonstrate accurate handling of magnetic beads for reproducible PCR product purification and DNA size selection. Using CleanNGS and AMPure XP magnetic beads (Beckman Coulter Life Sciences) confirmed their interchangeability, establishing CleanNGS as a cost-effective alternative offering reliable results.

Key benefits:

- Efficient automated magnetic bead handling when using the MAG module – our module for magnetic separation. MAG captures and releases magnetic beads with vertical back and forth magnet movement to the plate without user interference, avoiding possible spillage during manual plate transfer.
- Fail-proof liquid handling of magnetic beads with the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot, thanks to optimized pipetting height and speed settings in VIALAB.
- Flexibility is key! No matter what labware you are using, MAG's adapter selection provides optimized solutions for automated magnetic bead handling in microcentrifuge tubes, 96 and 384 well PCR plates.
- Efficient and reproducible PCR product purification for downstream sequencing uses CleanNGS magnetic beads and removes fragments below 100 bp.
- Reduce the processing costs and boost your NGS library preparation reproducibility for double-sided DNA size selection with CleanNGS magnetic beads.
- Gain additional hands-free time with ASSIST PLUS and MAG and effortlessly adapt to various DNA size selection protocols. VIALAB's user-friendly programming makes adjusting magnetic bead ratios easy.

Overview: How to automate DNA size selection with ASSIST PLUS and MAG

ASSIST PLUS

Application Note

In this application note, we demonstrate fully automated DNA size selection of 48 samples with CleanNGS magnetic beads on the ASSIST PLUS pipetting robot. The 8 channel 125 µl VOYAGER adjustable tip spacing pipette automates the liquid handling steps and the MAG module automates the magnetic bead handling steps in this protocol.

Figure 1 illustrates the step-by-step procedure of the provided size selection protocols for:

- Single-sided DNA size selection (PCR product purification)
- Double-sided DNA size selection

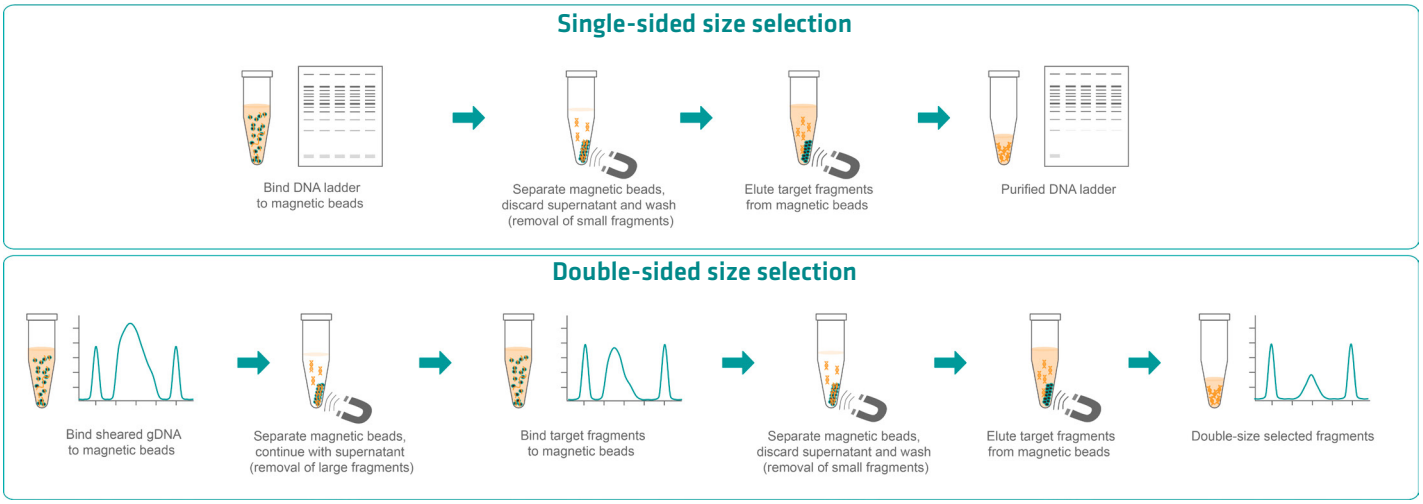

Figure 1: Step-by-step procedure of single- and double-sided DNA size selection.

Experimental setup:

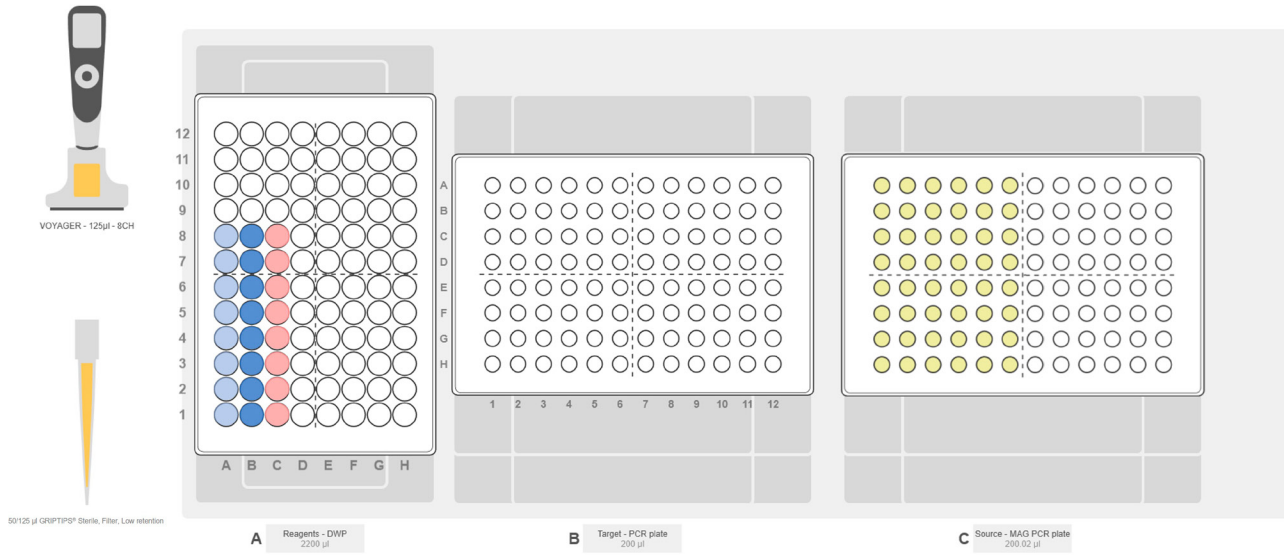

Figure 2: Deck set-up for automated PCR product purification on the MAG module. **Position A:** Reagents – deep well plate (DWP) (light blue: CleanNGS magnetic beads; blue: 80 % ethanol; pink: molecular grade water). **Position B:** Target – 96 well HardShell® PCR plate. **Position C:** Source – MAG module with 96 well HardShell PCR plate with samples (yellow).

Step by step procedure:

1. DNA single-sided size selection (PCR product purification)	**STEP:** Bind PCR product to a 1.8x magnetic bead ratio.	**HOW TO:** Prepare fresh 80 % ethanol, and bring CleanNGS magnetic beads up to room temperature (RT). Place a 96 well DWP in portrait orientation on position A with 450 µl of CleanNGS magnetic beads in wells A1-A8 (**Figure 2**, light blue), 1.6 ml of 80 % ethanol in wells B1-B8 (**Figure 2**, blue) and 320 µl of molecular grade water in wells C1-C8 (**Figure 2**, pink). Place one empty 96 well HardShell PCR plate on position B and one with 40 µl of sample in each well of the first half in landscape orientation on the MAG module with the 96 well adapter on position C (**Figure 2**, yellow). Select and run the VIALAB program 'MAG_PCR_product_purification'. With 125 µl sterile, filter, low retention GRIPTIPS®, VOYAGER on ASSIST PLUS will transfer 72 µl of magnetic beads from column A (**Figure 2**, light blue) of the DWP on position A (**Figure 3a**) to every well of the first half of the PCR plate on position C (**Figure 3b**). Magnetic beads are mixed 10 times before aspiration, and 15 times after dispensing into samples, guaranteeing a homogenous magnetic bead mixture. GRIPTIPS are changed automatically between samples. With the magnet array disengaged (**Figure 4a**, position home, 0 mm), VOYAGER will initiate an incubation of 5 minutes at RT to bind the PCR fragments to the magnetic beads (**Figure 4b**).
	STEP: Removal of small fragments and washing.	**HOW TO:** MAG will automatically lift the magnet array from position home (**Figure 4b**) to position high at 29 mm (**Figure 5a**) and capture magnetic beads within 3 minutes (**Figure 5b**). The PCR plate adapter for MAG has small holes to visually confirm targeted capturing of magnetic beads at the well surface. VOYAGER – using fresh GRIPTIPS for each sample – removes the supernatant while the magnet array remains engaged (**Figure 5a**). The pipette transfers the supernatant into columns F-H of the DWP on position A. Slow aspiration (speed 1) and precise height settings prevent magnetic bead loss during washing. Magnetic beads are washed twice with 125 µl of 80 % ethanol from columns B of the DWP on position A (**Figure 2**, blue). VOYAGER aspirates an additional time to ensure complete removal of ethanol from each well. MAG will lower the magnet array by 5 mm (position low, 24 mm) followed by air drying for 3 minutes at RT. Lowering the magnet array before air drying will move the pellets closer to the well bottom, allowing easier elution and smaller volumes. **TIPS:** • The magnet step in VIALAB provides total control of the magnet array, by setting customized heights anywhere between 0 and 29 mm. • Worry-free liquid handling of ethanol uses fast aspiration and slow dispensing with a tip touch to prevent droplet formation.
	STEP: Elution of single-sided size selected fragments.	**HOW TO:** 40 µl of molecular grade water is then transferred from column C of the DWP on position A (**Figure 2**, pink) to every well of the first half of the PCR plate on position C. Mixing 25 times ensures proper resuspension of the magnetic beads, independently of the volume, followed by a 5-minute RT incubation for elution. Again, MAG will automatically lift the magnet array to 29 mm (**Figure 5a**) and capture magnetic beads within 3 minutes (**Figure 5b**). Afterwards, VOYAGER transfers 35 µl of eluate to the unused PCR plate on position B, leaving 5 µl in the plate at position C to prevent magnetic bead carryover. At the end of the run, the user is prompted to store the PCR plate from position B, and remove the plate from the MAG.

a) b)

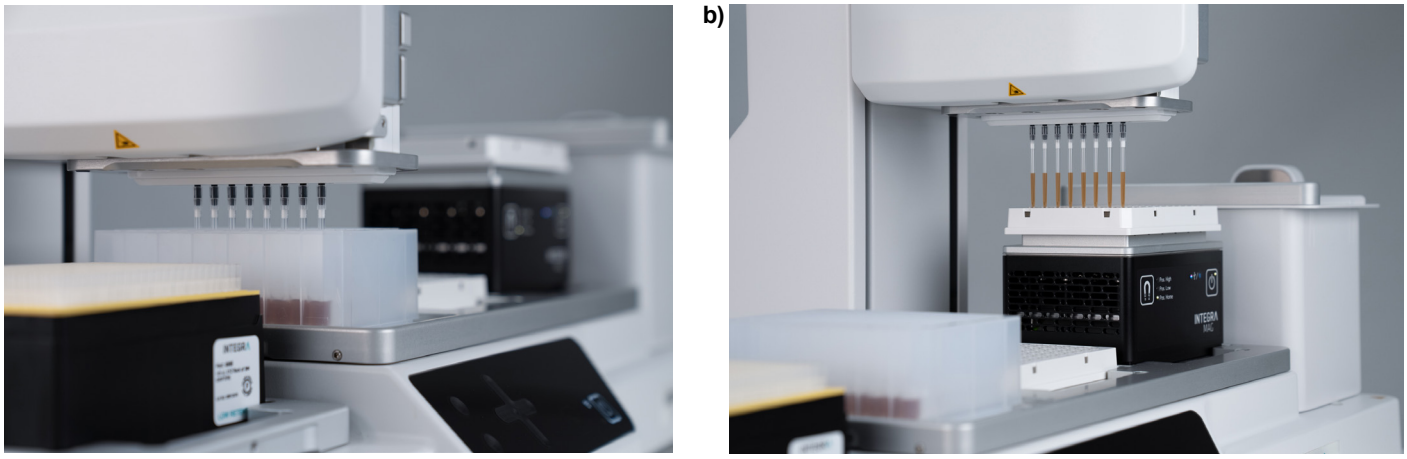

Figure 3: VOYAGER on ASSIST PLUS transfers CleanNGS magnetic beads from (a) a 96 well DWP to (b) a 96 well HardShell PCR plate on MAG with 96 well PCR plate adapter.

a) b)

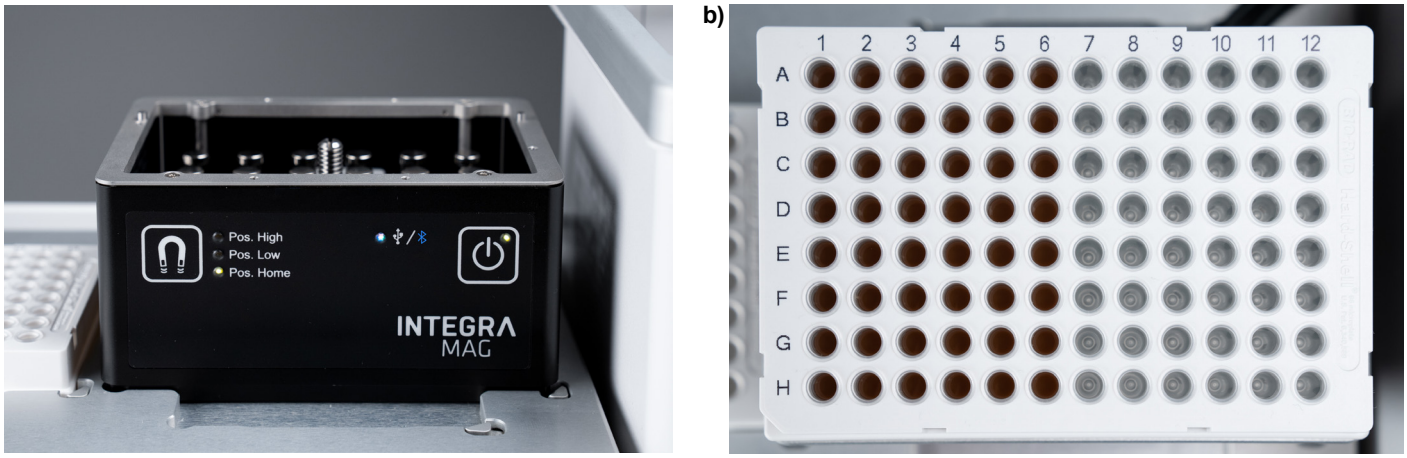

Figure 4: MAG on ASSIST PLUS **(a)** without PCR plate adapter showing disengaged magnet array (position home, 0 mm) and **(b)** with 96 well PCR plate adapter and 96 well HardShell PCR plate showing uncaptured magnetic beads.

a) b)

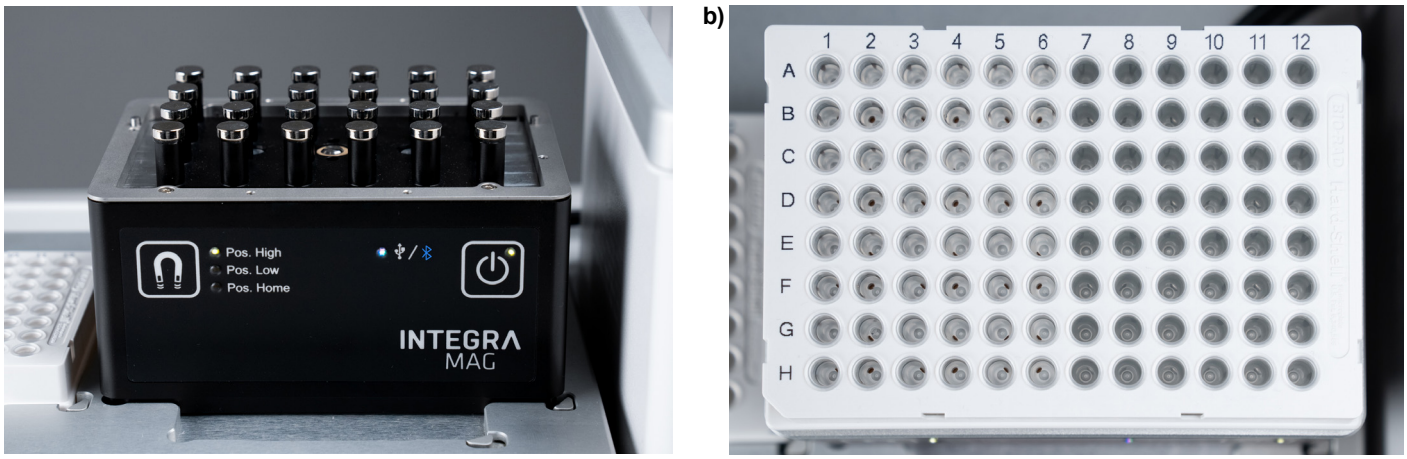

Figure 5: MAG on ASSIST PLUS (a) without 96 well PCR plate adapter showing engaged magnet array (position high, 29 mm), and (b) with 96 well PCR plate adapter and 96 well HardShell PCR plate showing captured magnetic beads after 3 minutes.

2. DNA double-sided size selection	**STEP:** Binding sheared genomic DNA (gDNA) to a 0.7x magnetic bead ratio.	**HOW TO:** The deck set-up for DNA double-sided size selection is similar to DNA single-sided size selection, but with 320 µl of CleanNGS magnetic beads (**Figure 2**, light blue), 350 µl of molecular grade water (**Figure 2**, pink), and 55 µl of sample in each well of the first half of a HardShell 96 well PCR plate (**Figure 2**, yellow). Select and run the VIALAB program 'MAG_DNA_double_size_selection'. VOYAGER will follow the steps described in PCR product clean-up but transfers 38.5 µl of magnetic beads to each well containing a sample (**Figure 3b**). Mixing 10 times before every other aspiration using new GRIPTIPS before aspiration guarantees precise, low volume pipetting of magnetic beads.
	STEP: Removal of large fragments (right size selection).	**HOW TO:** After capturing large fragments bound to magnetic beads (right size selection) (**Figure 5b**), VOYAGER will transfer 85 µl of supernatant from each well of the first half of the PCR plate to the corresponding well of the second half of the same plate on position C.
	STEP: Binding target fragments to a magnetic bead ratio, and removal of small fragments (0.8x, left size selection) during the washing process.	**HOW TO:** Following the same procedure as the right size selection, MAG lowers the magnet array back to position home (**Figure 4a**) and VOYAGER transfers 5 µl of magnetic beads to the supernatant of the first size selection in the second half of the PCR plate (position C). A 5 µl pre-dispense guarantees accurate pipetting of small volumes of magnetic bead. The subsequent washing procedure mirrors the DNA single-sided size selection.
	STEP: Elution of double-sided size selected fragments.	**HOW TO:** MAG and VOYAGER will follow the same procedure used for DNA single-sided size selection but transfer 50 µl of molecular grade water before elution, and 45 µl after capturing magnetic beads with MAG (**Figure 5b**). **TIP:** • Changing to different fragment sizes is trouble free, as the operator can calculate the magnetic bead volume for any ratio, and simply update it in VIALAB.

Results

Most reagent kit providers for library preparation recommend AMPure XP magnetic beads for NGS. Here, we demonstrate equivalent performance of CleanNGS magnetic beads during full automation of single-sided DNA size selection using a 100 bp DNA ladder (Promega) to mimic PCR product purification and double-sided DNA size selection using sheared gDNA.

With the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot, 48 replicates were processed using CleanNGS magnetic beads in rows A to D, and AMPure XP magnetic beads in rows E to H. Automated magnetic bead handling with optimized magnet array heights was ensured with the MAG module. The size-selected fragments (CleanNGS vs AMPure XP) were analyzed and compared using the 4150 TapeStation System (Agilent, complete data set can be found in the appendix).

Figure 6 illustrates the gel picture of row A (CleanNGS) and row E (AMPure XP) of the 96 well plate when performing single-sided DNA size selection with 100 bp DNA ladder and a 1.8x magnetic bead ratio. Both magnetic beads purified all fragments of PCR ladder smaller than 100 bp and ~70 % of 4 ng 100 bp fragments while recovering ~100 % of 65 ng fragments ranging from 200 bp to 1500 bp.

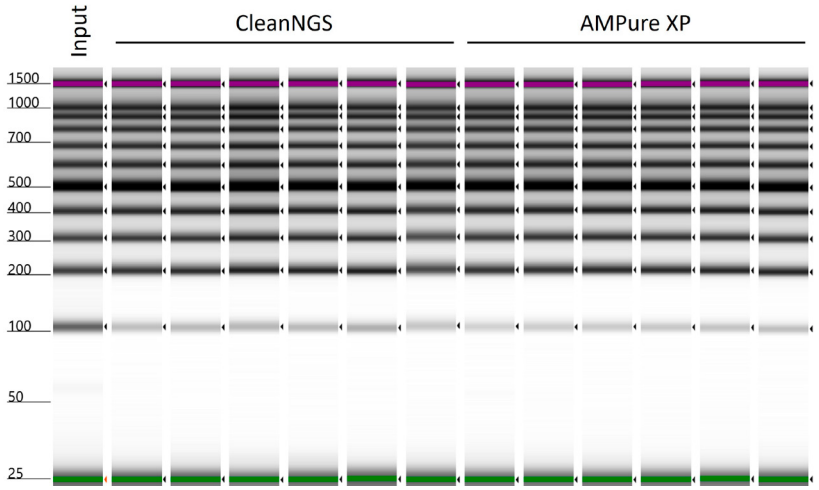

Figure 6: Single-sided DNA size selection with CleanNGS magnetic beads guarantees PCR product purification. Results of fragment analysis using a 4150 TapeStation showing a gel with 100 bp PCR ladder before (input) and after single-sided DNA size selection using a 1.8x ratio of CleanNGS (left, row A, n=6) or AMPure XP (right, row E, n=6).

Figure 7 illustrates an electropherogram (EPG) with 22 out of 24 replicates (outliers excluded) of CleanNGS (left) and AMPure XP (right) when performing double-sided DNA size selection of sheared gDNA using 0.8x-0.7x (left-right) magnetic bead ratios. Both magnetic beads achieved a similar recovery of over 12 % (n=22) of 330 ng sheared gDNA. Average fragment sizes were 372 bp with CleanNGS and 396 bp with AMPure XP magnetic beads, while overall size variation for each reagent was below 5 %.

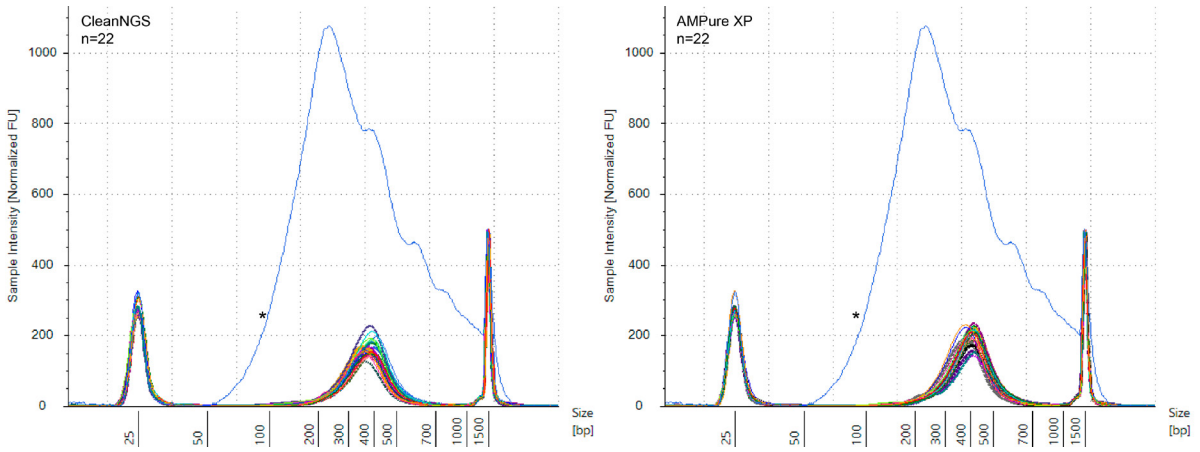

Figure 7: Efficient double-sided DNA size selection with CleanNGS magnetic beads. Results of fragment analysis using a 4150 TapeStation showing an EPG of sheared gDNA before (*) and after double-sided DNA size selection using a 0.8x-0.7x ratio (left-right) of CleanNGS (left, n=22) or AMPure XP (right, n=22).

Application Note

INTEGRA

Remarks

- **VIALAB software:** The VIALAB programs can be easily adapted to your specific pipette, labware and protocols.
- **Partial plates:** The pre-set programs offer laboratories complete flexibility to accommodate varying sample sizes, ensuring they can meet both current and future demands.
- **Semi-automation:** There is a protocol that uses a magnet plate for a semi-automated workflow in the appendix.

Conclusion

- Fully automated DNA size selection and PCR product purification uses the MAG module for reliable magnetic bead handling and the VOYAGER adjustable tip spacing pipette for precise liquid handling on the ASSIST PLUS pipetting robot.
- Small fragment removal is stress free when performing PCR product purification with a 1.8x CleanNGS magnetic bead ratio, ensuring reproducible recovery of precious fragments above 100 bp.
- Double-sided DNA size selection with CleanNGS magnetic beads is game-changing by reducing experimental costs. Fragments of 340 bp to 390 bp are successfully selected with 12 % recovery when using a 0.8x-0.7x (left-right) ratio. On top, CleanNGS results are comparable to AMPure XP, making them the cost-effective alternative.
- Easy adjustment to different protocols with VIALAB's simple programming allows seamless adaptation to changing workloads by modifying magnetic bead volumes and magnet height settings, or sample counts.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager
INTEGRA Biosciences	6565	125 µl Sterile, Filter Low retention GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4900	MAG module for magnetic separation	
INTEGRA Biosciences	4906	Adapter for 96 well PCR plates (MAG / HEATMAG)	
Irish Life Sciences	2.2S96-011V	2.2 ml Square well Pyramid bottom plate	https://irishlifesciences.com/product/2-2ml-96-square-well-v-bottom
Bio-Rad	HSP9601	Hard-Shell 96-well PCR plate, low profile, thin wall, skirted	https://www.bio-rad.com/en-ch/sku/HSP9601-hard-shell-96-well-pcr-plates-low-profile-thin-wall-skirted-white-clear?ID=HSP9601
Promega	G2101	100 bp DNA Ladder	https://ch.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101
CleanNA	CNGS-0050	CleanNGS	https://www.cleanna.com/CleanNGS/
Beckman Coulter Life Sciences	A63881	AMPure XP Reagent	https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/pcr

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Appendix

Table 1: Data from single-sided DNA size selection

	100 bp				>100 bp			
sample name	concentration (pg/µl)	average (pg/µl)	recovery (%)	CV (%)	concentration (pg/µl)	average (pg/µl)	recovery (%)	CV (%)
CLN-SS-01	37	36.5	32.5	11.6	1660	1690	107	4
CLN-SS-02	39.2				1670			
CLN-SS-03	41.9				1860			
CLN-SS-04	38.9				1720			
CLN-SS-05	46				1670			
CLN-SS-06	35.6				1590			
CLN-SS-07	31.9				1800			
CLN-SS-08	34.1				1720			
CLN-SS-09	33.4				1740			
CLN-SS-10	43.2				1840			
CLN-SS-11	38.8				1720			
CLN-SS-12	35.8				1730			
CLN-SS-13	33.7				1650			
CLN-SS-14	32.9				1660			
CLN-SS-15	30.8				1650			
CLN-SS-16	33.1				1710			
CLN-SS-17	33.3				1660			
CLN-SS-18	37.9				1630			
CLN-SS-19	42.8				1650			
CLN-SS-20	42.7				1710			
CLN-SS-21	36.1				1670			
CLN-SS-22	39.5				1700			
CLN-SS-23	43.2				1830			
CLN-SS-24	38.7				1700			
AMP-SS-01	25.8	30.5	27.3	11	1700	1695	107	2
AMP-SS-02	28.6				1740			
AMP-SS-03	28.9				1760			
AMP-SS-04	30.4				1720			
AMP-SS-05	32.7				1740			
AMP-SS-06	32.5				1680			
AMP-SS-07	34.4				1690			
AMP-SS-08	27.1				1680			
AMP-SS-09	27.4				1700			
AMP-SS-10	30.6				1680			
AMP-SS-11	29.4				1700			
AMP-SS-12	29.6				1700			
AMP-SS-13	37.2				1670			
AMP-SS-14	33.5				1730			
AMP-SS-15	33.8				1730			
AMP-SS-16	31				1690			
AMP-SS-17	29.2				1710			
AMP-SS-18	27.7				1660			
AMP-SS-19	36.6				1760			
AMP-SS-20	35.5				1730			
AMP-SS-21	28.5				1700			
AMP-SS-22	35.8				1750			
AMP-SS-23	33.6				1750			
AMP-SS-24	35.2				1680			
INPUT DNA ladder	118	111.7	100	4	1610	1580	100	2
INPUT DNA ladder	108				1560			
INPUT DNA ladder	111				1590			
INPUT DNA ladder	114				1620			

Table 2: Data from double-sided DNA size selection

sample name	average size (bp)	average (bp)	SD (bp)	concentration (ng/µl)	average (ng/µl)	SD (ng/µl)	recovery (%)
CLN-DS-01	362	372	14	0.644	0.7	0.12	12
CLN-DS-02	375			0.616			
CLN-DS-03	383			0.623			
CLN-DS-04	388			0.833			
CLN-DS-05	365			0.808			
CLN-DS-06	354			0.627			
CLN-DS-07	378			0.605			
CLN-DS-08	380			0.766			
CLN-DS-09	359			0.756			
CLN-DS-10	372			0.623			
CLN-DS-11	367			1.03			
CLN-DS-12	353			0.713			
CLN-DS-13	349			0.57			
CLN-DS-14	376			0.621			
CLN-DS-15	389			0.803			
CLN-DS-16	-			-			
CLN-DS-17	377			0.674			
CLN-DS-18	-			-			
CLN-DS-19	348			0.767			
CLN-DS-20	372			0.699			
CLN-DS-21	354			0.741			
CLN-DS-22	390			0.694			
CLN-DS-23	374			0.746			
CLN-DS-24	387			0.821			
AMP-DS-01	378	396	13	0.938	0.8	0.13	13
AMP-DS-02	417			0.812			
AMP-DS-03	399			0.835			
AMP-DS-04	409			0.87			
AMP-DS-05	385			0.911			
AMP-DS-06	377			0.839			
AMP-DS-07	379			0.867			
AMP-DS-08	405			0.61			
AMP-DS-09	399			0.583			
AMP-DS-10	394			0.774			
AMP-DS-11	415			0.771			
AMP-DS-12	-			-			
AMP-DS-13	400			0.855			
AMP-DS-14	403			0.91			
AMP-DS-15	389			0.685			
AMP-DS-16	392			0.924			
AMP-DS-17	397			0.84			
AMP-DS-18	365			0.809			
AMP-DS-19	408			0.88			
AMP-DS-20	401			0.745			
AMP-DS-21	396			0.62			
AMP-DS-22	397			0.878			
AMP-DS-23	399			0.911			
AMP-DS-24	-			-			
INPUT sheared gDNA	373	376	7.5	6.81	6	0.81	
INPUT sheared gDNA	366			6.51			
INPUT sheared gDNA	382			5.44			
INPUT sheared gDNA	381			5.13			