Application Note

INTEGRA

Automated DNA size selection for flexible NGS workflow integration

Introduction

DNA size selection with magnetic beads plays a significant role in molecular biology, employing specific ratios to capture and separate fragments by size. Single-sided DNA size selection used after polymerase chain reaction (PCR) removes primer dimers with a high magnetic bead ratio during PCR product purification. DNA double-sided size selection uses 2 ratios to effectively remove small and large fragments, resulting in the purification of targeted 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 workflow using MAGFLO™ NGS – our magnetic beads for NGS size selection – and fully automated DNA size selection protocols.

Additionally, the RNAse-free MAGFLO NGS production enables RNA size selection. 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 the accurate handling of magnetic beads for reproducible PCR product purification and DNA size selection. Testing MAGFLO NGS and AMPure XP magnetic beads (Beckman Coulter Life Sciences) confirmed the interchangeability of the 2 reagents as well as the performance of the automated size selection protocols. MAGFLO NGS, as a cost-effective alternative to the gold standard, offers the same reliable results.

Key benefits:

- Effective automated magnetic bead handling when using the MAG module for magnetic separation. MAG captures and releases magnetic beads with vertical 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 deep well plates (DWP), or 96 and 384 well PCR plates.
- Efficient and reproducible PCR product purification for NGS uses our magnetic beads – MAGFLO NGS – for size selection, to remove fragments below 100 bp.
- Reduce processing costs and boost your NGS library preparation reproducibility with double-sided DNA size selection using MAGFLO NGS.
- Gain additional hands-free time with ASSIST PLUS and MAG and effortlessly adapt to various DNA/RNA size selection protocols. VIALAB's user-friendly programming makes adjusting magnetic bead ratios easy.

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

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In this application note, we demonstrate the fully automated DNA size selection of 48 samples with MAGFLO NGS – magnetic beads for NGS size selection – 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

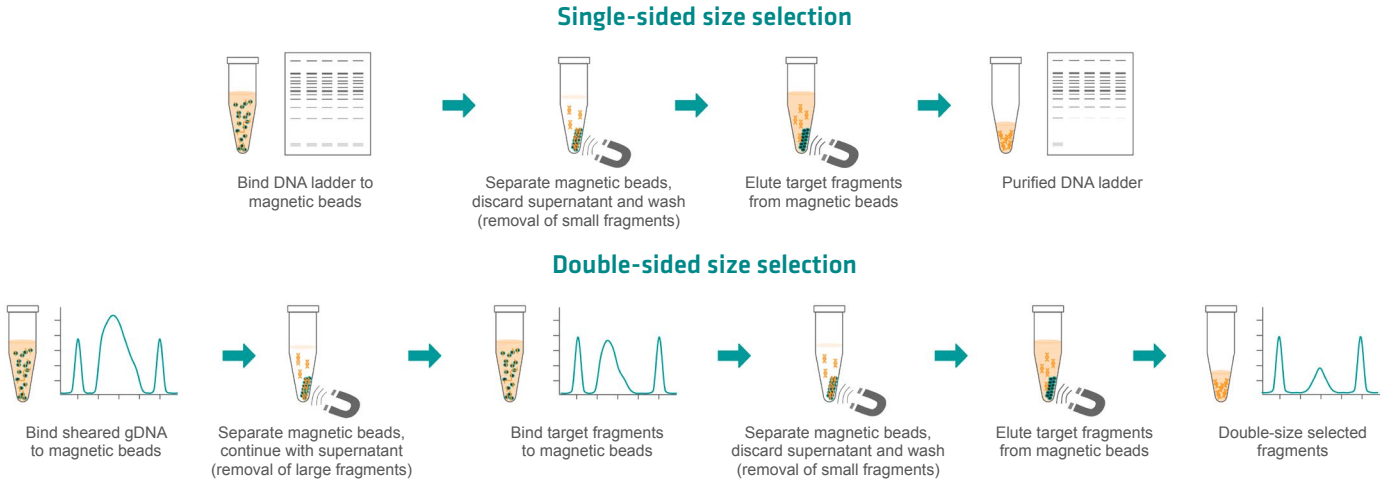

Figure 1: Step-by-step procedure of single- and double-sided DNA size selection.

Experimental setup:

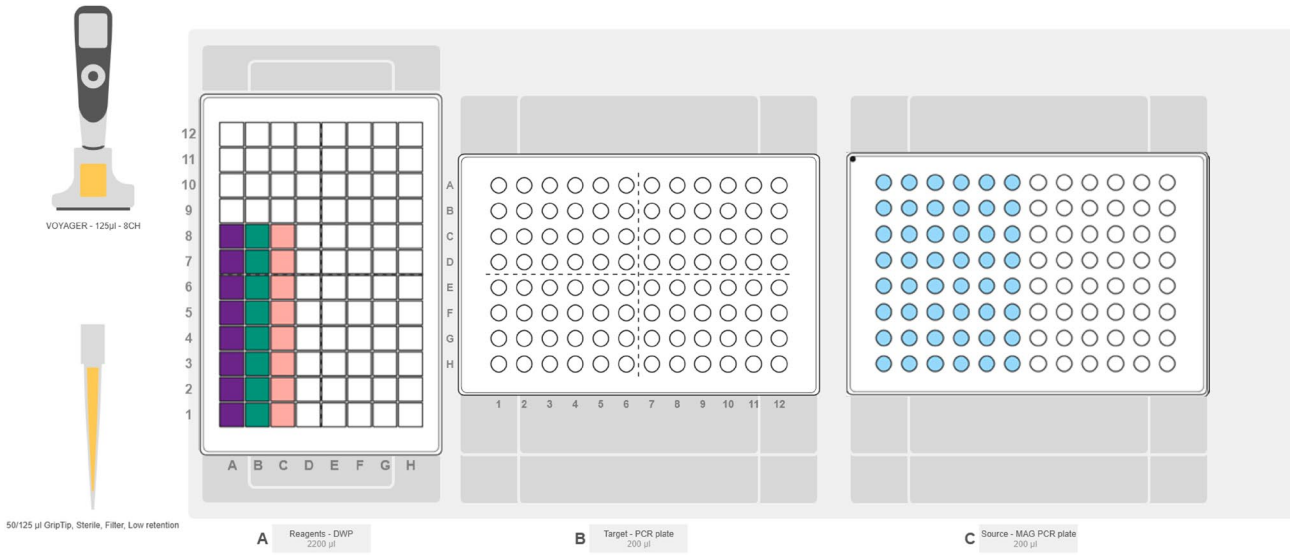

Figure 2: Deck set-up for automated DNA size selection on the MAG module. Position A: Reagents – INTEGRA DWP (lilac: MAGFLO NGS; green: 80 % ethanol; pink: molecular grade water). Position B: Target – 96 well Bio-Rad Hard-Shell® PCR plate. Position C: Source – MAG module with 96 well Bio-Rad Hard-Shell PCR plate with samples (blue).

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 MAGFLO NGS to room temperature (RT). Place the 96 well INTEGRA DWP in portrait orientation on position A with 450 µl of MAGFLO NGS in wells A1-A8 (**Figure 2**, lilac), 1.6 ml of 80 % ethanol in wells B1-B8 (**Figure 2**, green) and 320 µl of molecular grade water in wells C1-C8 (**Figure 2**, pink). Place 1 empty 96 well Bio-Rad Hard-Shell PCR plate on position B and 1 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**, blue).

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**, lilac) of the INTEGRA DWP on position A (**Figure 3a**) to every well of the first half of the PCR plate on position C (**Figure 3b**). Magnetic beads will be mixed 10 times before aspiration, and 15 times after dispensing into samples, guaranteeing a homogeneous magnetic bead mixture. GRIPTIPS will be changed automatically between samples. With the magnet array disengaged in position home (**Figure 4a**, Pos. Home, 0 mm), VOYAGER will initiate an incubation of 5 minutes at RT to bind the PCR fragments to the magnetic beads (**Figure 4b**).

a) b)

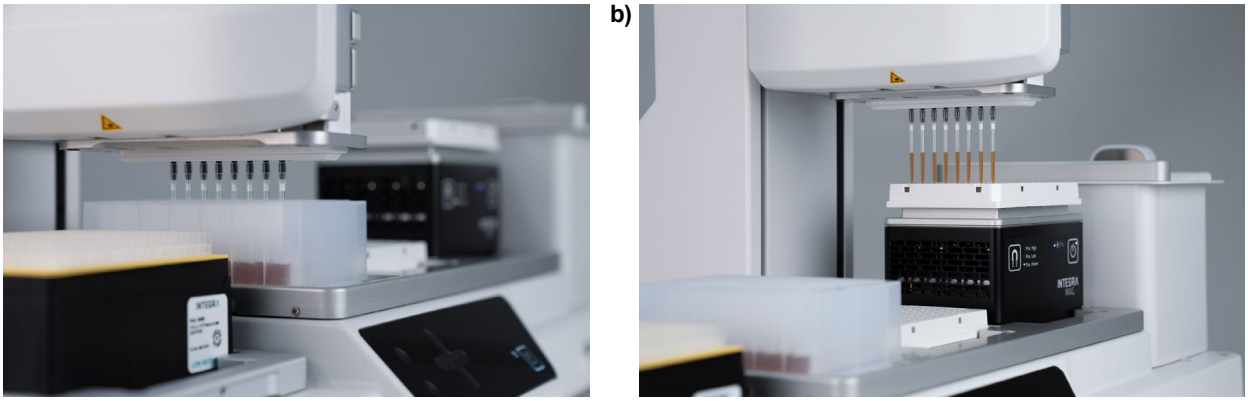

Figure 3: VOYAGER on ASSIST PLUS transfers MAGFLO NGS from (a) the 96 well INTEGRA DWP to (b) a 96 well Bio-Rad Hard-Shell PCR plate on MAG with 96 well PCR plate adapter.

a) b)

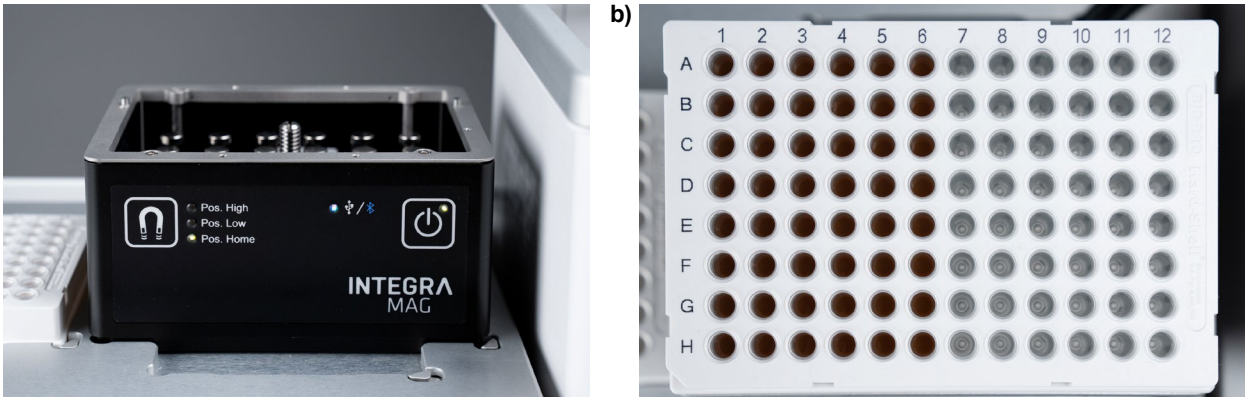

Figure 4: MAG on ASSIST PLUS (a) without PCR plate adapter showing disengaged magnet array (Pos. Home, 0 mm) and (b) with 96 well PCR plate adapter and 96 well Bio-Rad Hard-Shell PCR plate showing uncaptured magnetic beads.

STEP: Removal of small fragments and washing.

HOW TO: MAG will automatically lift the magnet array from Pos. Home (**Figure 4b**) to position high (Pos. 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 the targeted capturing of magnetic beads at the well surface.

VOYAGER – using fresh GRIPTIPS for each sample – will remove the supernatant while the magnet array remains engaged (**Figure 5a**). The pipette will then transfer the supernatant into columns F-H of the INTEGRA DWP on position A. Slow aspiration (speed 1) and precise height settings prevent magnetic bead loss during washing. Magnetic beads will then be washed twice with 125 µl of 80 % ethanol from column B of the INTEGRA DWP on position A (**Figure 2**, green). VOYAGER will aspirate an additional time to ensure the complete removal of ethanol from each well. MAG will lower the magnet array by 5 mm to position low (Pos. 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.
- The drying condition has been optimized to 3 minutes but may vary in different lab conditions.

a)

b)

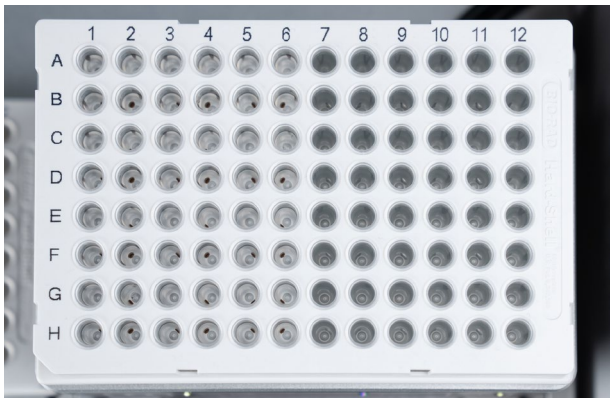

Figure 5: MAG on ASSIST PLUS (a) without 96 well PCR plate adapter showing engaged magnet array (Pos. High, 29 mm), and (b) with 96 well PCR plate adapter and 96 well Bio-Rad Hard-Shell PCR plate showing captured magnetic beads after 3 minutes.

	STEP: Elution of single-sided size selected fragments.	**HOW TO:** 40 µl of molecular grade water will be transferred from column C of the INTEGRA 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 will transfer 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 MAG.
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 MAGFLO NGS (**Figure 2**, lilac), 350 µl of molecular grade water (**Figure 2**, pink), and 55 µl of sample in each well of the first half of a Bio-Rad Hard-Shell 96 well PCR plate (**Figure 2**, blue). Select and run the VIALAB program 'MAG_DNA_double_size_selection'. VOYAGER will follow the steps described in PCR product purification but will transfer 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 0.79x magnetic bead ratio, and removal of small fragments (0.79x, left size selection) during the washing process.	**HOW TO:** Following the same procedure as the right size selection, MAG will lower the magnet array back to Pos. Home (**Figure 4a**) and VOYAGER will transfer 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 beads. 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 the equivalent performance of MAGFLO NGS – our magnetic bead for NGS size selection – 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 MAGFLO NGS 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 (MAGFLO NGS 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 (MAGFLO NGS) and row E (AMPure XP) of the 96 well plate when performing single-sided DNA size selection with 30-fold diluted 100 bp DNA ladder and a 1.8x magnetic bead ratio. Both reagents 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.

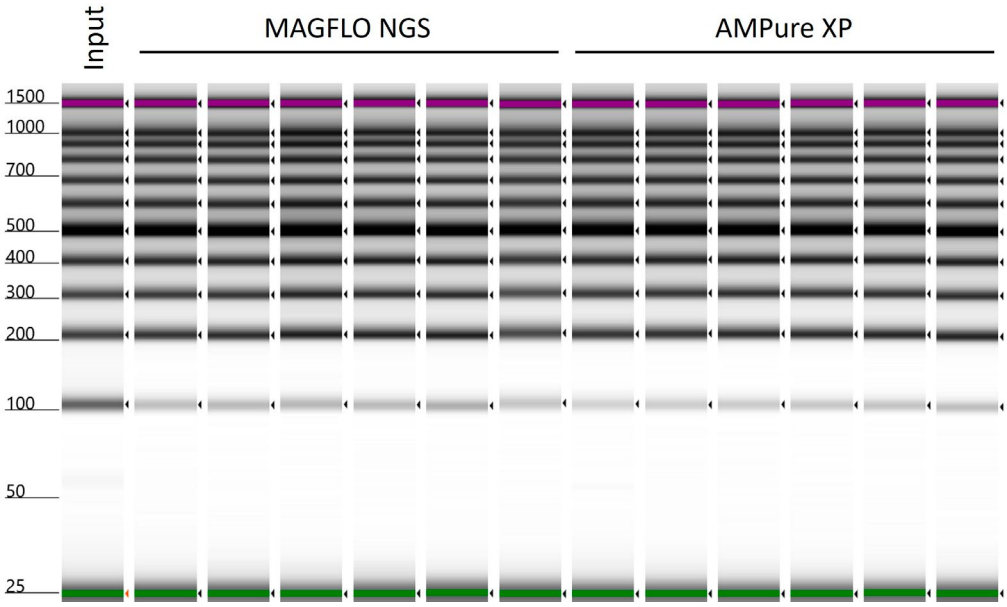

Figure 6: Single-sided DNA size selection with MAGFLO NGS guarantees automated PCR product purification. Results of fragment analysis using a 4150 TapeStation showing a gel with 30-fold diluted 100 bp PCR ladder before (input) and after single-sided DNA size selection using a 1.8x ratio of MAGFLO NGS (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 MAGFLO NGS (left) and AMPure XP (right) when performing double-sided DNA size selection of sheared gDNA using 0.79x-0.7x (left-right) magnetic bead ratios. Both reagents achieved a similar recovery of over 12 % (n=22) of 330 ng sheared gDNA. Average fragment sizes were 372 bp with MAGFLO NGS and 396 bp with AMPure XP magnetic beads, while the overall size variation for each reagent was below 5 %.

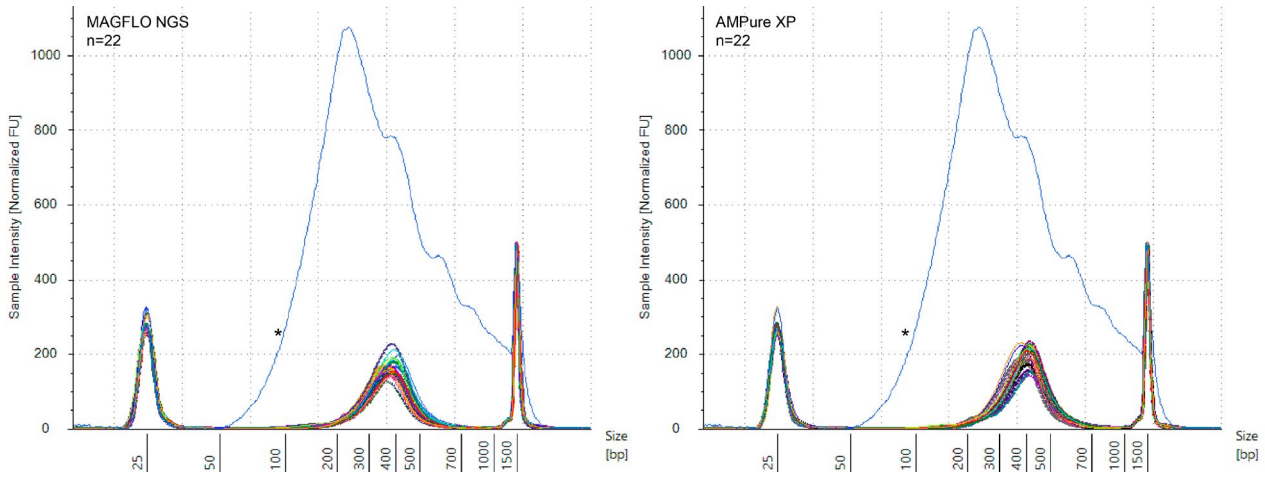

Figure 7: Efficient automated double-sided DNA size selection with MAGFLO NGS. Results of fragment analysis using a 4150 TapeStation showing an EPG of sheared gDNA before (*) and after double-sided DNA size selection using a 0.79x-0.7x ratio (left-right) of MAGFLO NGS (left, n=22) or AMPure XP (right, n=22).

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 for flexible NGS integration 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 ratio of MAGFLO NGS magnetic beads for NGS size selection, ensuring reproducible recovery of precious fragments above 100 bp.
- Double-sided DNA size selection with MAGFLO NGS is game-changing in reducing experimental costs. Fragments of 340 bp to 390 bp are successfully selected with 12 % recovery when using a 0.79x-0.7x (left-right) ratio. MAGFLO NGS 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, magnet height settings or sample counts.

INTEGRA

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	4900	MAG module for magnetic separation	https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag
INTEGRA Biosciences	4906	Adapter for 96 well PCR plates (MAG / HEATMAG)	https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag
INTEGRA Biosciences	6565	125 µl sterile, filter, low retention GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6353	INTEGRA DWP	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	7000 7002 7004	MAGFLO NGS	TBD
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://worldwide.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101
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 (%)
MFL-SS-01	37	36.5	32.5	11.6	1660	1690	107	4
MFL-SS-02	39.2				1670			
MFL-SS-03	41.9				1860			
MFL-SS-04	38.9				1720			
MFL-SS-05	46				1670			
MFL-SS-06	35.6				1590			
MFL-SS-07	31.9				1800			
MFL-SS-08	34.1				1720			
MFL-SS-09	33.4				1740			
MFL-SS-10	43.2				1840			
MFL-SS-11	38.8				1720			
MFL-SS-12	35.8				1730			
MFL-SS-13	33.7				1650			
MFL-SS-14	32.9				1660			
MFL-SS-15	30.8				1650			
MFL-SS-16	33.1				1710			
MFL-SS-17	33.3				1660			
MFL-SS-18	37.9				1630			
MFL-SS-19	42.8				1650			
MFL-SS-20	42.7				1710			
MFL-SS-21	36.1				1670			
MFL-SS-22	39.5				1700			
MFL-SS-23	43.2				1830			
MFL-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 (%)
MFL-DS-01	362	372	14	0.644	0.7	0.12	12
MFL-DS-02	375			0.616			
MFL-DS-03	383			0.623			
MFL-DS-04	388			0.833			
MFL-DS-05	365			0.808			
MFL-DS-06	354			0.627			
MFL-DS-07	378			0.605			
MFL-DS-08	380			0.766			
MFL-DS-09	359			0.756			
MFL-DS-10	372			0.623			
MFL-DS-11	367			1.03			
MFL-DS-12	353			0.713			
MFL-DS-13	349			0.57			
MFL-DS-14	376			0.621			
MFL-DS-15	389			0.803			
MFL-DS-16	-			-			
MFL-DS-17	377			0.674			
MFL-DS-18	-			-			
MFL-DS-19	348			0.767			
MFL-DS-20	372			0.699			
MFL-DS-21	354			0.741			
MFL-DS-22	390			0.694			
MFL-DS-23	374			0.746			
MFL-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			