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 bead-to-sample ratios to capture and separate fragments by size. Single-sided size selection using a high magnetic bead ratio removes primer dimers during polymerase chain reaction (PCR) product purification. Double-sided DNA size selection uses 2 distinct ratios to effectively remove small and large fragments, resulting in the purification of targeted average fragment sizes. Both single- and double-sided DNA size selection with magnetic beads are integral to next generation sequencing (NGS) library preparation.

MAGFLO™ NGS magnetic beads for NGS size selection offer an effective solution for NGS and PCR product purification. These beads are suitable for both single- and double-sided DNA size selection methods, and manufactured under RNase-free conditions to enable the purification of both RNA and DNA.

MAGFLO NGS beads are compatible with fully automated DNA size selection protocols. The VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot automates all liquid handling steps, while the MAG module ensures precise automated magnetic bead handling.

The protocols provided here demonstrate the accurate handling of MAGFLO NGS beads for reproducible PCR product purification and DNA size selection. Automated testing of MAGFLO NGS beads and AMPure XP bead-based reagent (Beckman Coulter Life Sciences) confirmed the interchangeability of the products, demonstrating that INTEGRA's cost-effective alternative delivers the same reliable results as the gold standard.

Key benefits:

- Using MAGFLO NGS beads for library preparation steps reduces processing costs, while increasing the reproducibility of double-sided DNA size selection.
- MAGFLO NGS beads enable efficient and reproducible PCR product purification by removing fragments below 100 bp.
- The MAG module captures and releases beads using vertical magnet movements, eliminating the need for manual intervention and minimizing the risk of spillage during plate transfers.
- The VOYAGER on the ASSIST PLUS guarantees fail-proof liquid handling of magnetic beads with optimized pipetting height and speed settings.
- With the ASSIST PLUS and the MAG module, protocols can be effortlessly adapted to various DNA/RNA size selection protocols. VIALAB's user-friendly programming makes adjusting magnetic bead ratios easy.
- INTEGRA provides MAG module adapters to enable automated magnetic bead handling in a range of labware formats, including microcentrifuge tubes, deep well plates (DWPs) and 96 or 384 well PCR plates.

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

ASSIST PLUS

Application Note

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This application note describes the fully automated DNA size selection of 48 samples with MAGFLO NGS beads on the ASSIST PLUS pipetting robot. An 8 channel 125 µl VOYAGER automates the liquid handling steps, and the MAG module automates the magnetic bead handling steps.

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

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

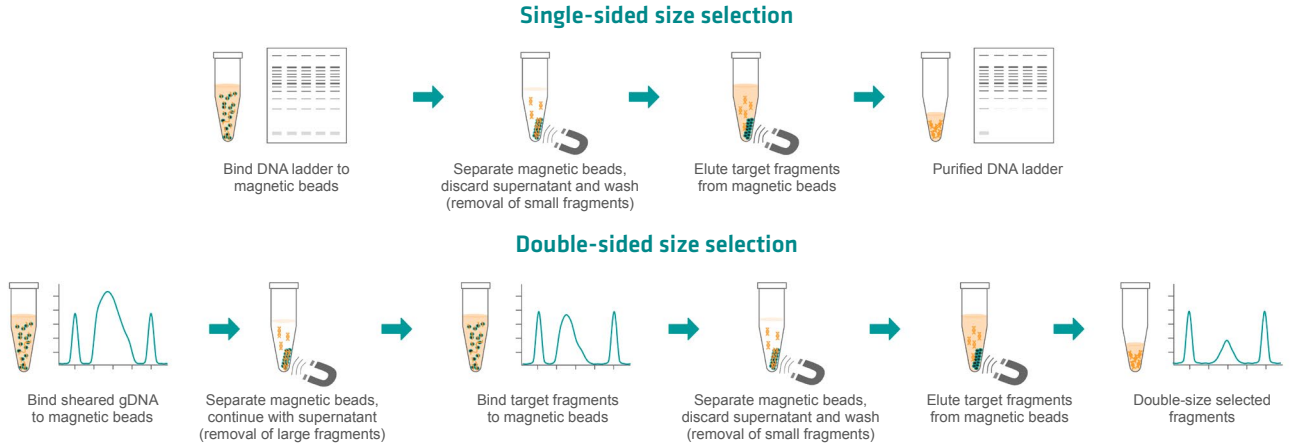

Figure 1: Step-by-step procedure of single- and double-sided DNA size selection.

Experimental set-up:

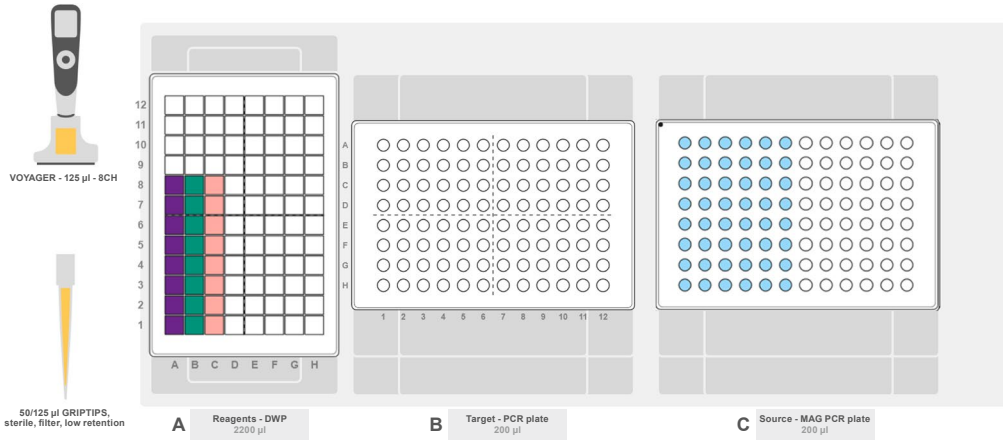

Figure 2: The ASSIST PLUS deck set-up for automated DNA size selection with the MAG module. **Position A:** Reagents – INTEGRA DWP (lilac: MAGFLO NGS beads; green: 80 % ethanol; pink: molecular-grade water). **Position B:** Target – 96 well Bio-Rad Hard-Shell® PCR plate. **Position C:** Source – MAG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

Step-by-step procedure:

1. Single-sided DNA size selection (PCR product purification)

STEP: Binding the PCR product using a 1.8x magnetic bead ratio.

HOW TO: Prepare fresh 80 % ethanol and bring MAGFLO NGS beads to room temperature (RT). Place a 96 well INTEGRA DWP on Position A of the ASSIST PLUS in portrait orientation, with 450 µl of MAGFLO NGS beads 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 one empty 96 well Bio-Rad Hard-Shell PCR plate on Position B. Place a second 96 well plate containing 40 µl of sample in each well in the first half (**Figure 2**, blue), in landscape orientation on the 96 well adapter of the MAG module on Position C.

Select and run the VIALAB program 'MAG_PCR_product_purification'. Using 125 µl sterile, filter, low retention GRIPTIPS®, the VOYAGER on the 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 each well in 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, to guarantee 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), the VOYAGER will initiate an incubation of 5 minutes at RT, binding the PCR fragments to the magnetic beads (**Figure 4b**).

a) b)

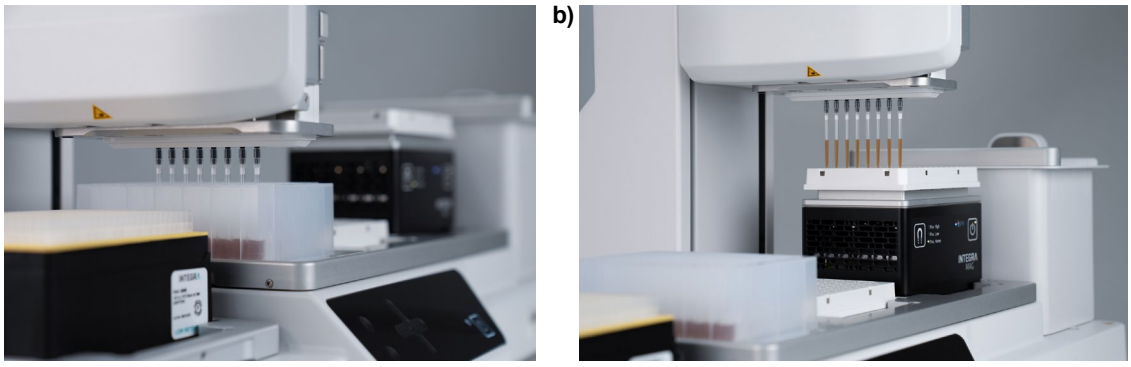

Figure 3: The VOYAGER on the ASSIST PLUS transfers MAGFLO NGS beads from (a) the 96 well INTEGRA DWP to (b) a 96 well Bio-Rad Hard-Shell PCR plate on the MAG module.

a) b)

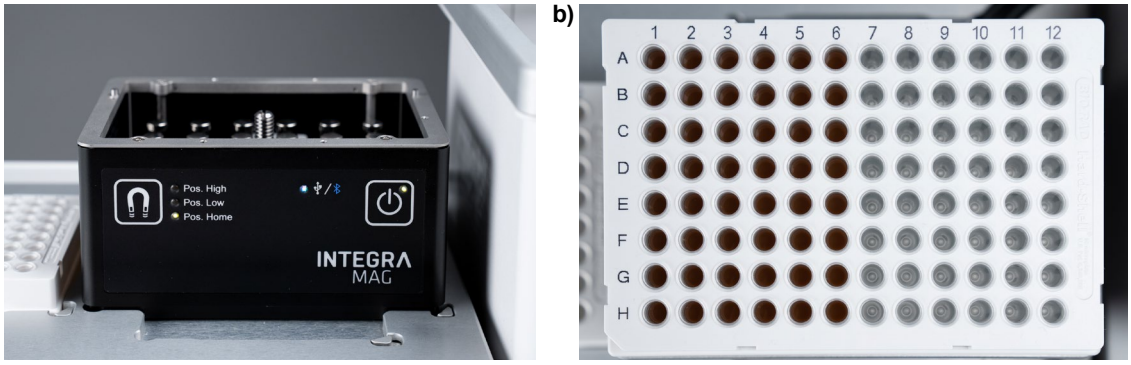

Figure 4: The MAG module on the ASSIST PLUS (a) without a PCR plate adapter, showing a disengaged magnet array (Pos. Home, 0 mm), and (b) with a 96 well PCR plate adapter and a 96 well Bio-Rad Hard-Shell PCR plate, showing uncaptured magnetic beads.

STEP: Removal of small fragments and washing.

HOW TO: The MAG module will automatically lift the magnet array from Pos. Home (**Figure 4b**) to Position High (Pos. High) at 29 mm (**Figure 5a**) for 3 minutes to capture the magnetic beads (**Figure 5b**). The PCR plate adapter for the MAG module has small holes to visually confirm the targeted capture of magnetic beads at the well surface.

The 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. Next, magnetic beads will be washed twice with 125 µl of 80 % ethanol from column B of the INTEGRA DWP on Position A (**Figure 2**, green). The VOYAGER will aspirate an additional time to ensure the complete removal of ethanol from each well. The MAG module will then 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 bottom of the wells, 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.
- When handling ethanol, fast aspiration and slow dispensing with a tip touch are required to prevent droplet formation.
- The drying condition has been optimized to 3 minutes, but may vary in different lab conditions.

a)

b)

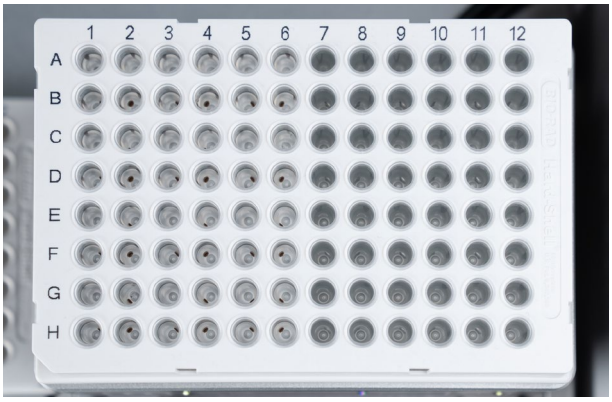

Figure 5: The MAG module on the ASSIST PLUS (a) without a PCR plate adapter, showing an engaged magnet array (Pos. High, 29 mm), and (b) with a 96 well PCR plate adapter and a 96 well Bio-Rad Hard-Shell PCR plate, showing magnetic beads captured after 3 minutes.

	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 in the first half of the PCR plate on Position C. Mixing 25 times ensures proper resuspension of the magnetic beads, regardless of volume. This is followed by an incubation at RT for 5 minutes. Again, the MAG module will then automatically lift the magnet array to 29 mm (**Figure 5a**) for 3 minutes to capture the magnetic beads (**Figure 5b**). Afterwards, the 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 the MAG module.
2. Double-sided DNA size selection	**STEP:** Binding sheared genomic DNA (gDNA) using a 0.7x magnetic bead ratio.	**HOW TO:** The deck set-up for double-sided DNA size selection is similar to single-sided size selection, but with 320 µl of MAGFLO NGS beads (**Figure 2**, lilac), 350 µl of molecular-grade water (**Figure 2**, pink), and 55 µl of sample in each well in 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'. The 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 – and using new GRIPTIPS before each 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**), the VOYAGER will transfer 85 µl of supernatant from each well in the first half of the PCR plate to the corresponding well in the second half of the same plate on Position C.
	STEP: Binding target fragments using a 0.8x magnetic bead ratio, and removing small fragments (left size selection) during the washing process.	**HOW TO:** Following the same procedure as the right size selection, the MAG module will lower the magnet array back to Pos. Home (**Figure 4a**), then the 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 single-sided DNA size selection.
	STEP: Elution of double-sided size selected fragments.	**HOW TO:** The MAG module and the VOYAGER will follow the same procedure used for single-sided DNA size selection, but 50 µl of molecular-grade water will be transferred before elution, and 45 µl after capturing the magnetic beads (**Figure 5b**). **Tip** • VIALAB software enables operators to switch to different fragment sizes as needed. Simply calculate the magnetic bead volume for any ratio, and update it in VIALAB.

Results

Most providers of reagent kits for NGS library preparation recommend AMPure XP magnetic beads. This application note demonstrates the equivalent performance of MAGFLO NGS magnetic beads during automated 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.

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

Figure 6 shows the gel picture of row A (MAGFLO NGS beads) and row E (AMPure XP beads) of the 96 well plate for single-sided DNA size selection with a 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.

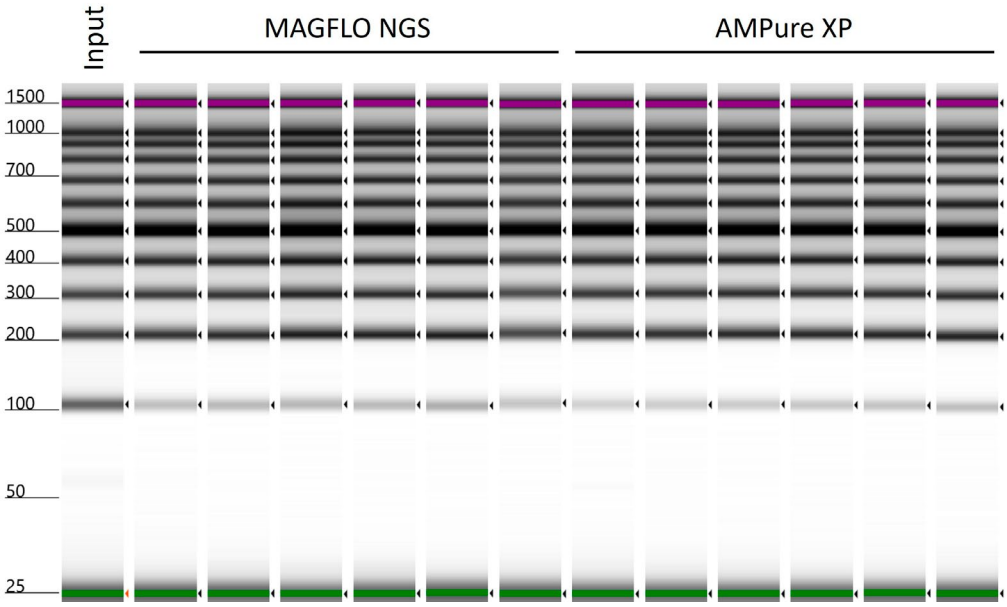

Figure 6: Single-sided DNA size selection with MAGFLO NGS beads guarantees automated PCR product purification. Results of fragment analysis using a 4150 TapeStation, showing a gel with 30-fold diluted 100 bp DNA ladder before (input) and after single-sided DNA size selection. The analysis shows the results for a 1.8x ratio of MAGFLO NGS (left, row A, n=6) or AMPure XP (right, row E, n=6) beads.

Figure 7 depicts an electropherogram (EPG) showing 22 out of 24 replicates (outliers excluded) for MAGFLO NGS (left) and AMPure XP (right) beads during double-sided DNA size selection of sheared gDNA. Magnetic bead ratios of 0.8x and 0.7x were used for left and right size selection, respectively. Both reagents achieved similar recovery rates, exceeding 12 % (n=22) of 330 ng sheared gDNA. Average fragment sizes were 372 bp with MAGFLO NGS beads, and 396 bp with AMPure XP beads, with overall size variation for each reagent remaining below 5 %.

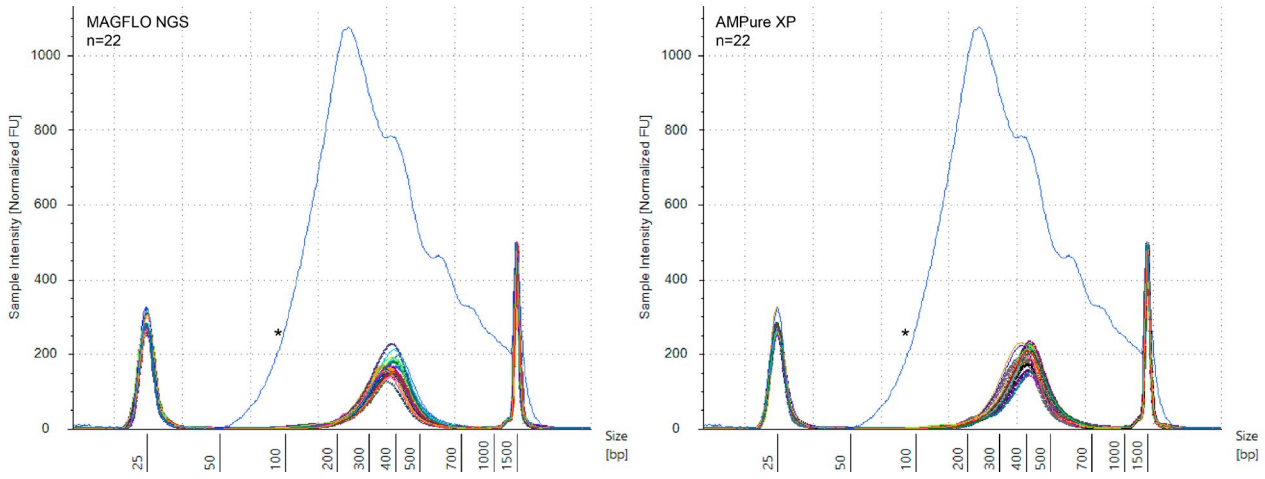

Figure 7: Efficient automated double-sided DNA size selection with MAGFLO NGS. Results of fragment analysis using a 4150 TapeStation, showing an EPG of sheared gDNA before (*) and after double-sided DNA size selection. The analysis compares magnetic bead ratios of 0.8x (left) to 0.7x (right) for MAGFLO NGS (n=22) and AMPure XP (n=22) beads.

Remarks

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

Conclusion

- Fully automated DNA size selection and PCR product purification can be effectively achieved using the MAG module for magnetic bead handling and the VOYAGER on the ASSIST PLUS for precise liquid handling, allowing flexible NGS workflow integration.
- A 1.8x ratio of MAGFLO NGS beads enables the removal of small fragments during PCR product purification, consistently recovering valuable fragments larger than 100 bp.
- Double-sided DNA size selection with MAGFLO NGS beads reduces experimental costs. Magnetic bead ratios of of 0.8x (left) and 0.7x (right) successfully select fragments between 340 bp and 390 bp, with a 12 % recovery rate. MAGFLO NGS beads provide comparable performance to AMPure XP beads at a lower cost.
- VIALAB‘s programming features allow easy adjustments to different protocols, enabling changes to magnetic bead volumes, magnet height settings or sample counts as needed.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/voyager
INTEGRA Biosciences	4900	MAG module for magnetic separation	https://www.integra-biosciences.com/en/modules/mag-and-heatmag
INTEGRA Biosciences	4906	Adapter for 96 well PCR plates (MAG / HEATMAG)	https://www.integra-biosciences.com/en/modules/mag-and-heat-mag
INTEGRA Biosciences	6565	125 µl sterile, filter, low retention GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6353	INTEGRA DWP	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	7000 7002 7004	MAGFLO NGS	https://www.integra-biosciences.com/en/ngspcr-purification/magflotm-ngs
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

Contact us:

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			