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. During polymerase chain reaction (PCR) cleanup, single-sided DNA size selection uses one high magnetic bead ratio to remove primer dimers, ensuring efficient downstream sequencing. In contrast, DNA double-sided size selection uses two ratios to effectively remove small and large fragments, resulting in the purification of specific 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 any DNA size selection workflow with CleanNGS magnetic beads (CleanNA) by automating all liquid handling steps with the VOYAGER adjustable tip spacing pipette and ASSIST PLUS pipetting robot. The automated protocols provided demonstrate accurate handling of magnetic beads and reproducible right- and left-side selection. Using CleanNGS and AMPure XP magnetic beads (Beckman Coulter Life Sciences) confirmed their comparability for DNA size selection, establishing CleanNGS as a cost-effective alternative offering reliable results.

Key benefits:

- Efficient and reproducible single-sided DNA size selection for downstream sequencing uses CleanNGS magnetic beads and removes fragments below 100 bp.
- Gain additional hands-free time with the ASSIST PLUS pipetting robot and effortlessly adapt to various DNA size selection protocols. VIALAB's user-friendly programming makes adjusting magnetic bead ratios easy.
- Reduce the processing costs and boost your NGS library preparation reproducibility for double-sided DNA size selection with CleanNGS magnetic beads.
- Fail-proof liquid handling of magnetic beads with the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS, thanks to optimized pipetting height and speed settings in VIALAB.

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

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In this application note, we demonstrate automated DNA size selection of 48 samples with CleanNGS magnetic beads using the 8 channel 125 µl VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot.

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

- Single-sided DNA size selection
- Double-sided DNA size selection

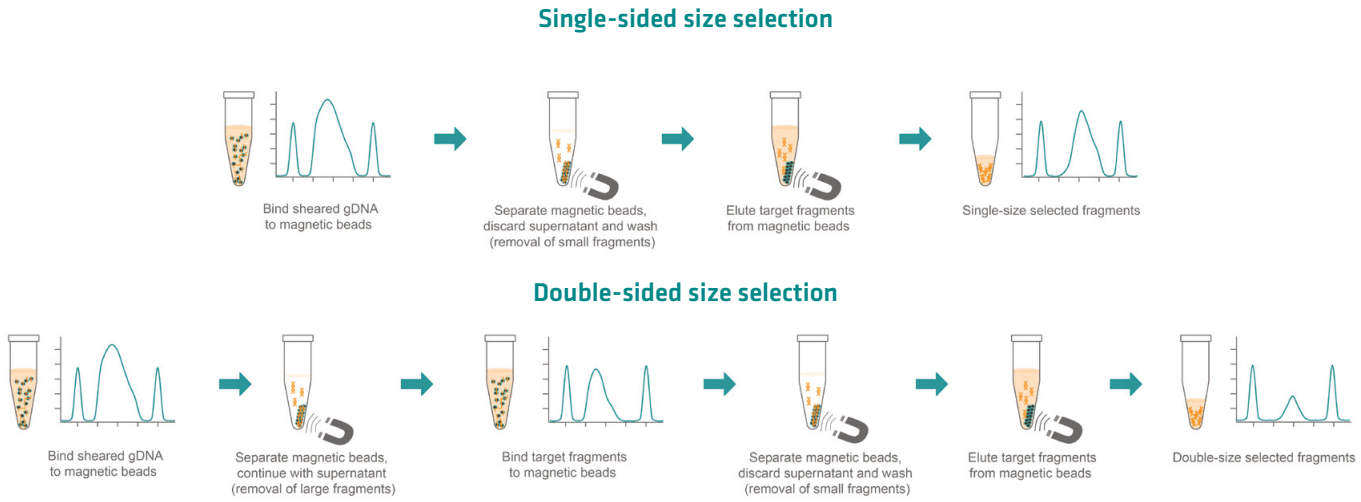


Figure 1: Step-by-step procedure of single- and double-sided DNA size selection.

Step by step procedure:

1. DNA single-sided size selection

STEP: Bind sheared gDNA 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 deep well plate (DWP) in portrait orientation on position A with 450 µl of CleanNGS magnetic beads in wells A1-A8 (**Figure 2**, light blue), 1.2 ml of 80 % ethanol in wells B1-B8 and C1-C8 (**Figure 2**, blue) and 320 µl of molecular grade water in wells D1-D8 (**Figure 2**, pink). Place a 96 well skirted, low profile PCR plate with 40 µl of samples in each well of the first half in landscape orientation on position B (**Figure 2**, yellow) and the CleanNA magnet plate on position C.

Select and run the VIALAB program 'DNA_single_size_selection'. With 125 µl sterile, filter, low retention GRIPTIPS®, the 8 channel 125 µl VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot transfers 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 B (**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. Afterwards, the VOYAGER will initiate an incubation delay of 5 minutes at RT, so that DNA can bind to the magnetic beads.

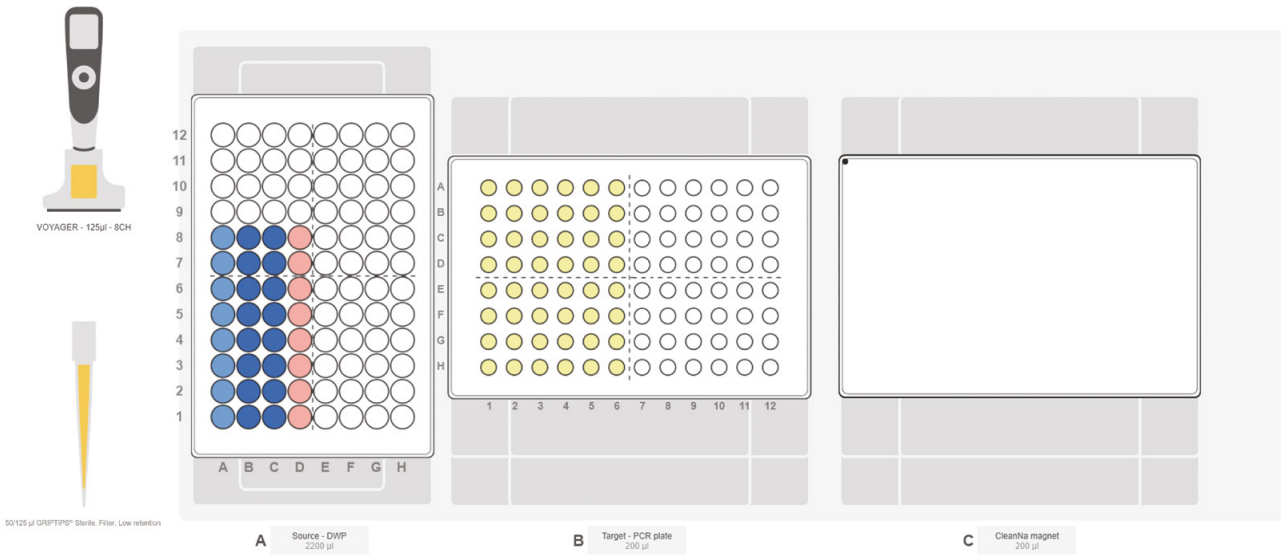
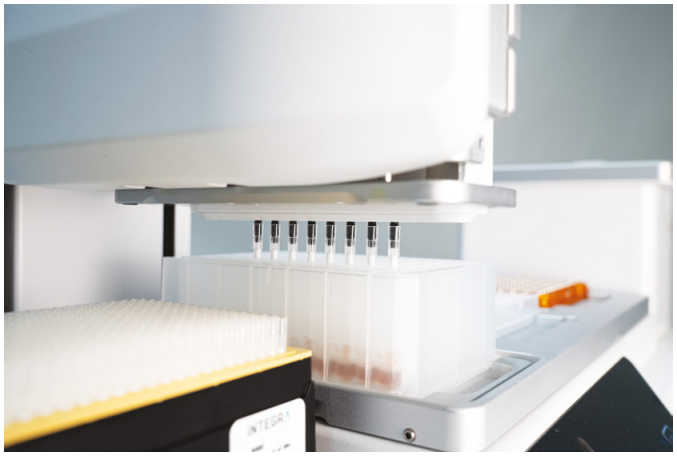


Figure 2: Deck set-up for DNA size selection with CleanNGS magnetic beads. **Position A:** Source – deep well plate with reagents (light blue: CleanNGS magnetic beads; blue: 80 % ethanol; pink: molecular grade water). **Position B:** Target – skirted, low profile 96 well PCR plate with samples in every well of the first half (yellow). **Position C:** Clean magnet plate.

a)



b)

Figure 3: The VOYAGER on the ASSIST PLUS transfers CleanNGS magnetic beads from (a) a 96 well DWP to (b) a 96 well PCR plate.

STEP: Removal of small fragments and washing.

HOW TO: The VOYAGER prompts the operator to place the PCR plate onto the magnet plate on Position C (**Figure 4**), followed by a 3 minute incubation to capture all magnetic beads with the ring magnet (**Figure 5**). When placing the PCR plate onto the magnet plate, the operator must ensure that the plate sits tight and straight by pressing down at the corners.

The VOYAGER – using fresh GRIPTIPS for each sample – removes the supernatant while the PCR plate remains on the magnet plate (Position C). The pipette transfers the supernatant into columns F-H of the DWP on position A. A slow aspiration (speed 2) and precise height settings prevent magnetic bead loss during washing. Magnetic beads are washed two times with 180 µl of 80 % ethanol from columns B and C of the DWP on position A (**Figure 2**, blue). The VOYAGER then aspirates the supernatant from each well again to ensure complete ethanol removal, followed by air drying for 3 minutes at RT.

TIP:

- Worry-free liquid handling of ethanol uses a 10 µl air gap after aspiration to prevent dripping. Dispensing is performed at a slow speed, followed by a tip touch at the side of the well to guarantee droplet removal.

STEP: Elution of single-sided size selected fragments.

HOW TO: The operator is instructed to transfer the PCR plate from position C to B. 40 µl of molecular grade water is then transferred from column D of the DWP on position A (**Figure 2**, pink) to every well of the first half of the PCR plate on Position B (**Figure 2**, yellow). Mixing 15 times ensures proper re-suspension of the magnetic beads, followed by a 5 minute RT incubation for elution. The VOYAGER will then prompt the operator to place the PCR plate back onto the magnet plate on position C (**Figure 4**), and to place a new 96 well PCR plate on position B. After a 3 minute delay to capture magnetic beads with the ring magnet (**Figure 5**), the VOYAGER transfers 35 µl of eluate to the new 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 informed to store the PCR plate from position B, and remove the plate from the magnetic plate.

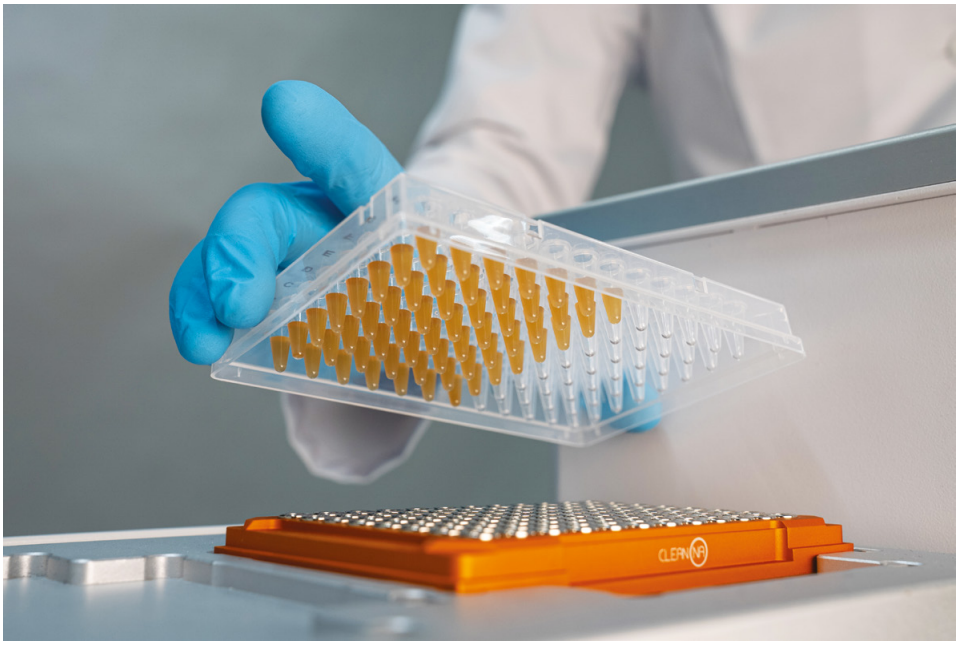


Figure 4: Manual transfer of the 96 well PCR plate onto the Clean magnet plate.

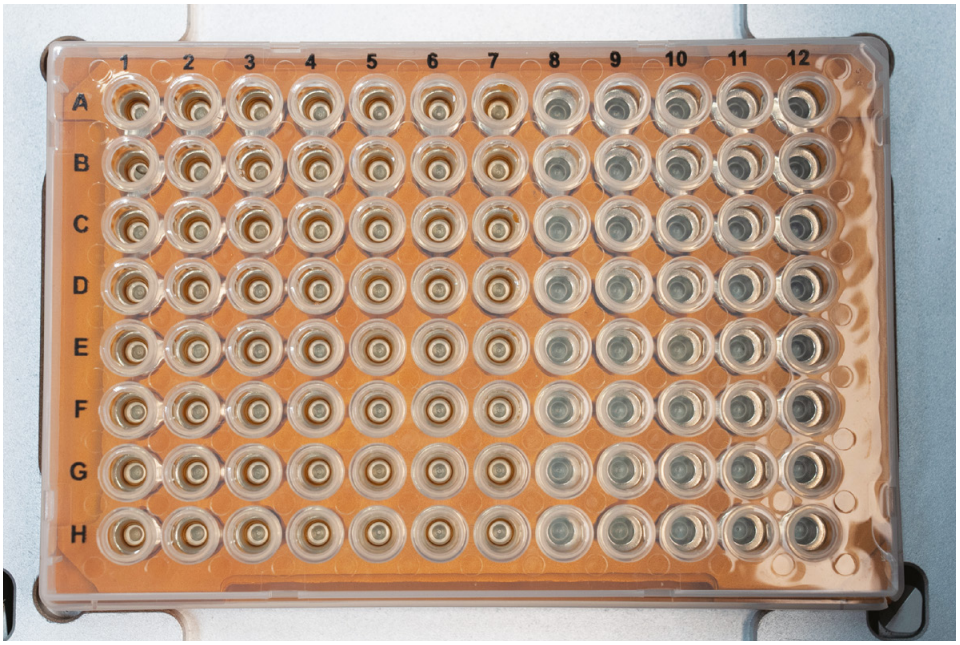


Figure 5: Magnetic beads after incubation on the Clean magnet plate.

2. DNA double-sided size selection	**STEP:** Binding sheared gDNA to a 0.7x magnetic bead ratio.	**HOW TO:** The deck set-up for DNA double-sided size selection is similar to single-sided DNA 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 'DNA_double_size_selection'. The VOYAGER will follow the steps described in single-sided DNA size selection, but transfers 38.5 µl of magnetic beads to each well containing samples (**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) with the ring magnet (**Figure 5**), the VOYAGER prompts the operator to place a new HardShell 96 well PCR plate on position B. It then transfers 85 µl of supernatant from each well in position C to the corresponding well in the first half of the new PCR plate. Before starting the second DNA size selection, the VOYAGER informs the operator to remove the plate from the Clean magnet plate.
	STEP: Binding target fragments to a 0.8x magnetic bead ratio, removal of small fragments (left size selection) during the washing process.	**HOW TO:** Following the same procedure as the right size selection, the VOYAGER transfers 5 µl of magnetic beads to the supernatant of the first size selection. A 5 µl pre-dispense guarantees accurate pipetting of small volumes of magnetic bead. The subsequent washing procedure mirrors single-sided DNA size selection, with a 5 minute delay to capture magnetic beads and prevent any bead loss.
	STEP: Elution of double-sided size selected fragments.	**HOW TO:** The VOYAGER follows the same procedure as the DNA single-sided size selection but transferring 50 µl of molecular grade water before elution, and 45 µl after capturing magnetic beads with the ring magnet. **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 higher reproducibility and equivalent performance of CleanNGS magnetic beads in DNA size selection, showing the interchangeability while reducing the experimental costs. Therefore, we performed single- and double-sided DNA size selection of sheared gDNA with CleanNGS magnetic beads while simultaneously comparing them to AMPure XP magnetic beads using the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot.

We processed 48 replicates using AMPure XP magnetic beads in rows A to D and CleanNGS magnetic beads in rows E to H. The size-selected fragments (CleanNGS vs. AMPure XP) were analyzed and compared using the 4150 TapeStation System (Agilent, complete data can be found in the appendix). **Figure 6** illustrates the electropherograms of (a) single-sided and (b) double-sided DNA size selection for row A (AMPure XP) and row E (CleanNGS).

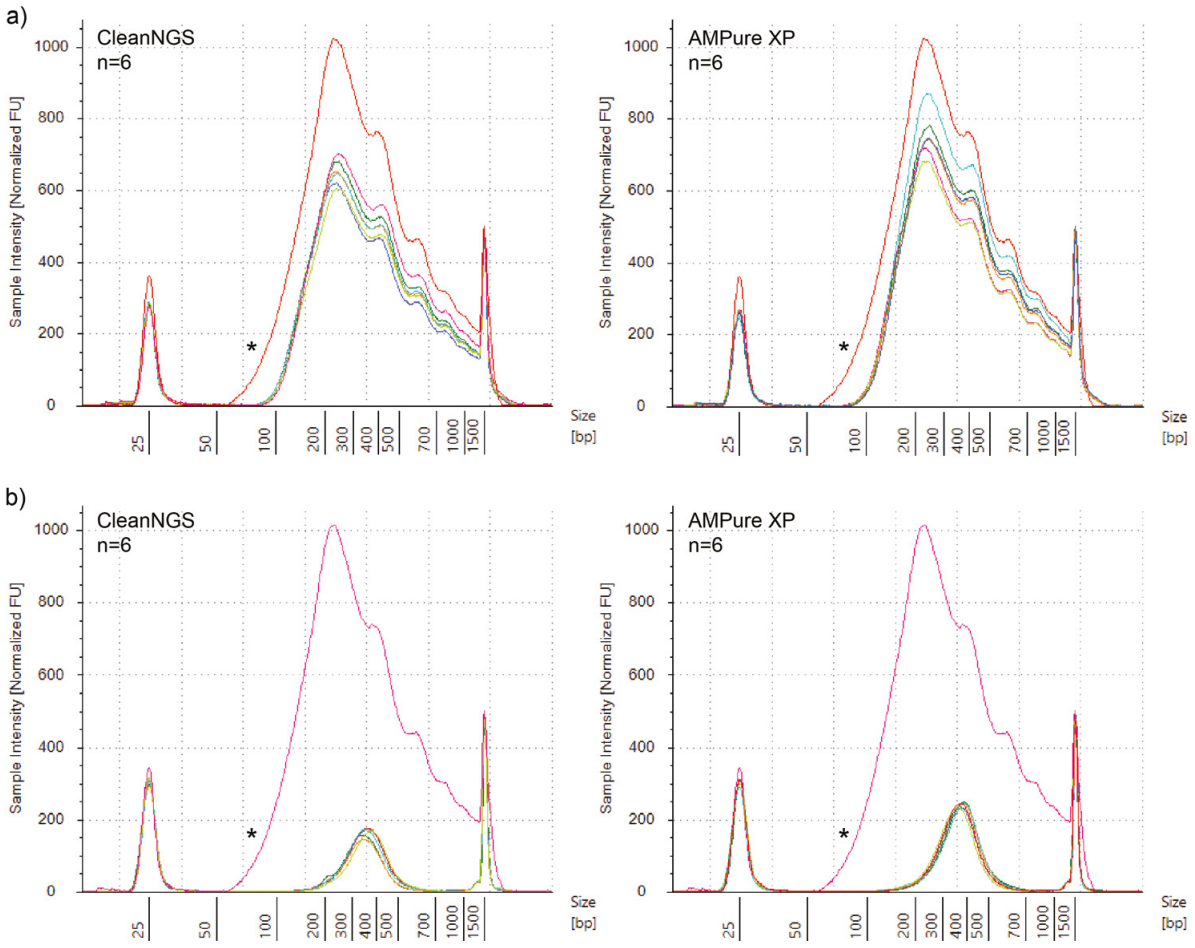


Figure 6: Efficient single- and double-sided DNA size selection with CleanNGS magnetic beads. Electropherogram results from fragment analysis using a 4150 TapeStation for (a) single-sided DNA size selection with 1.8x ratio and (b) double-sided DNA size selection with 0.8-0.7x ratio (left-right) of sheared gDNA input before (*) and after CleanNGS (left; row E; n=6) or AMPure XP (right; row A; n=6) magnetic bead processing.

Both reagents effectively removed fragments smaller than 100 bp during single-sided size selection using a 1.8x magnetic bead ratio. The average yield of sheared gDNA processed with CleanNGS magnetic beads was 5.5 ng/µl (±0.25) and 6.0 ng/µl (±0.45) with AMPure XP magnetic beads (**Figure 6a**; 6 out of 24 replicates shown per magnetic bead; left = Row E CleanNGS; right = Row A AMPure XP).

Double-sided DNA size selection with 0.8x-0.7x (left-right) magnetic bead ratios yielded average fragment sizes of 358 bp (±9.9) with CleanNGS and 371 bp (±10.4) with AMPure XP magnetic beads. Both reagents achieved a recovery of over 12 %. (**Figure 6b**; 6 out of 24 replicates shown per magnetic bead; left = Row E CleanNGS; right = Row A AMPure XP).

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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.

Conclusion

- Small fragment removal is stress-free when performing single-sided DNA size selection with a 1.8x CleanNGS magnetic bead ratio, ensuring reproducible recovery of precious fragments above 100 bp.
- Automated DNA size selection on the ASSIST PLUS pipetting robot with the VOYAGER adjustable tip spacing pipette further increases the reproducibility of results by maintaining identical liquid handling steps without disturbing the magnetic beads.
- 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 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
CleanNA	RN50	Clean Magnet Plate 96-well	https://www.cleanna.com/96-well-magnet-plates/
Irish Life Science	2.2S96-011V	2.2 ml Square well Pyramid KingFisher plate-type	https://irishlifesciences.com/product/2-2ml-96-square-well-v-bottom
BioRad	HSP9631	Hard-Shell 96-well PCR plate, low profile, thin wall, skirted	https://www.bio-rad.com/en-ch/product/low-profile-96-well-pcr-plates?ID=OC0OBU4VY
CleanNA	CNGS-0050	CleanNGS	https://www.cleanna.com/cleanngs/
AMPure XP	A63881	AMPure XP Reagent	https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/pcr

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Appendix

Table 1: Data single-sided DNA size selection

sample name	average size	average	SD	concentration (ng/µl)	average (ng/µl)	SD	recovery (%)
AMP-SS-01	411	405	8.65	5.94	6.03	0.44	92
AMP-SS-02	406			5.97			
AMP-SS-03	408			6.27			
AMP-SS-04	409			6.9			
AMP-SS-05	399			5.68			
AMP-SS-06	395			5.85			
AMP-SS-07	412			5.98			
AMP-SS-08	408			5.86			
AMP-SS-09	406			5.5			
AMP-SS-10	412			6.88			
AMP-SS-11	400			5.73			
AMP-SS-12	397			5.99			
AMP-SS-13	411			5.91			
AMP-SS-14	406			6.08			
AMP-SS-15	403			5.78			
AMP-SS-16	418			6.83			
AMP-SS-17	400			5.77			
AMP-SS-18	393			5.57			
AMP-SS-19	433			6.56			
AMP-SS-20	403			5.95			
AMP-SS-21	402			5.95			
AMP-SS-22	401			6.59			
AMP-SS-23	399			5.93			
AMP-SS-24	395			5.22			
CLN-SS-01	416	407	5.96	5.79	5.46	0.25	83
CLN-SS-02	410			5.13			
CLN-SS-03	406			5.48			
CLN-SS-04	406			5.58			
CLN-SS-05	401			5.57			
CLN-SS-06	396			5.44			
CLN-SS-07	417			5.9			
CLN-SS-08	408			5.14			
CLN-SS-09	404			4.97			
CLN-SS-10	405			5.56			
CLN-SS-11	400			5.34			
CLN-SS-12	397			5.59			
CLN-SS-13	417			5.57			
CLN-SS-14	415			5.5			
CLN-SS-15	408			5.02			
CLN-SS-16	412			5.56			
CLN-SS-17	407			5.6			
CLN-SS-18	403			5.24			
CLN-SS-19	414			5.78			
CLN-SS-20	410			5.51			
CLN-SS-21	406			5.15			
CLN-SS-22	408			5.71			
CLN-SS-23	405			5.5			
CLN-SS-24	401			5.32			
INPUT sheared gDNA	376	373	2.45	6.43	6.5725	0.15	
INPUT sheared gDNA	373			6.48			
INPUT sheared gDNA	373			6.75			
INPUT sheared gDNA	370			6.63			
t-test	0.19			0.00			

Table 2: Data double-sided DNA size selection

sample name	average size	average	SD	concentration (ng/µl)	average (ng/µl)	SD	recovery (%)
AMP-DS-01	382	371	10.44	1.04	1.01	0.09	16
AMP-DS-02	368			0.976			
AMP-DS-03	375			1.04			
AMP-DS-04	368			1.05			
AMP-DS-05	378			1.08			
AMP-DS-06	354			0.995			
AMP-DS-07	392			1.08			
AMP-DS-08	351			0.673			
AMP-DS-09	380			1.07			
AMP-DS-10	367			0.917			
AMP-DS-11	377			1.1			
AMP-DS-12	359			0.953			
AMP-DS-13	355			0.888			
AMP-DS-14	370			1.04			
AMP-DS-15	378			1.06			
AMP-DS-16	370			1.05			
AMP-DS-17	377			1.09			
AMP-DS-18	369			1.12			
AMP-DS-19	375			1.02			
AMP-DS-20	363			1.02			
AMP-DS-21	389			1.07			
AMP-DS-22	374			1.02			
AMP-DS-23	375			1			
AMP-DS-24	360			0.991			
CLN-DS-01	375	357	9.85	0.699	0.74	0.10	12
CLN-DS-02	356			0.733			
CLN-DS-03	362			0.776			
CLN-DS-04	347			0.635			
CLN-DS-05	361			0.758			
CLN-DS-06	343			0.704			
CLN-DS-07	354			0.759			
CLN-DS-08	356			0.69			
CLN-DS-09	364			0.708			
CLN-DS-10	352			0.569			
CLN-DS-11	362			0.659			
CLN-DS-12	343			0.496			
CLN-DS-13	359			0.76			
CLN-DS-14	345			0.72			
CLN-DS-15	377			0.91			
CLN-DS-16	359			0.769			
CLN-DS-17	362			0.736			
CLN-DS-18	376			0.954			
CLN-DS-19	360			0.883			
CLN-DS-20	347			0.775			
CLN-DS-21	352			0.832			
CLN-DS-22	348			0.762			
CLN-DS-23	351			0.817			
CLN-DS-24	348			0.76			
INPUT sheared gDNA	374	373	2.94	6.25	6.3725	0.14	
INPUT sheared gDNA	369			6.51			
INPUT sheared gDNA	376			6.48			
INPUT sheared gDNA	373			6.25			
t-test	0.00			0.00			