Application Note

Automated DNA clean-up using the Zymo Research DNA Clean & Concentrator® MagBead Kit

Introduction

DNA clean-up methods are used to purify samples of DNA by removing any unwanted components. This is crucial for the success of downstream processes in genomics, biotechnology, molecular biology, clinical research and other fields of biology.

Zymo Research's DNA Clean & Concentrator (DCC) MagBead Kit offers a magnetic bead-based DNA clean-up for PCR and NGS. Its single buffer system can recover DNA from enzymatic reactions, impure extractions, library preparations, and other sources. The kit can be used with the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot to automate DNA purification and concentration, enabling high throughput processing.

The results of the automated DNA clean-up protocols show high reproducibility, excellent yield and purity values..

Key benefits:

- Automated DNA clean-up with Zymo's DCC on the ASSIST PLUS pipetting robot makes DNA clean-up simple. Manual intervention is needed only when moving labware on and off the magnetic separator and shaker.
- The optimized liquid handling parameters on the ASSIST PLUS allow efficient DNA clean-up, without bead or buffer carryover. Using the pipetting robot guarantees that pipetting is always performed from and to the same position, ensuring consistent results every time. Accurate pipetting height, pre- and post-dispense, and ideal pipetting speeds have been refined for careful handling of liquids and magnetic beads, helping to achieve the desired results.
- The VIALAB software provides easy programming and includes a labware change feature for overcoming the hurdle of limited deck space.
- Using the VOYAGER adjustable tip spacing electronic pipette gives unlimited flexibility and easy transfers between different labware formats.
- GRIPTIPS® pipette tips are designed to create a perfect seal with the VOYAGER pipette, ensuring that they never leak or fall off. Using low retention GRIPTIPS guarantees precise pipetting of volatile solutions, such as ethanol.
- The DNA clean-up protocol can be simplified by using innovative labware accessories. Reservoirs with SureFlo™ anti-sealing array allow the lowest possible dead volume for valuable liquids. The dual reservoir adapter accommodates 2 divided reagent reservoirs with compartments for 4 different reagents. The 96 well cooling block keeps precious reagents cold and serves as a storage place for the PCR plate on the deck.

Overview: How to get ultra pure DNA with Zymo's DCC kit

ASSIST PLUS

Step-by-step procedure

The ASSIST PLUS pipetting robot is used together with the 300 µl 8 channel VOYAGER adjustable tip spacing electronic pipette and 300 µl low retention, sterile, filter GRIPTIPS in this semi-automated workflow to purify nucleic acid (**Figure 1**).

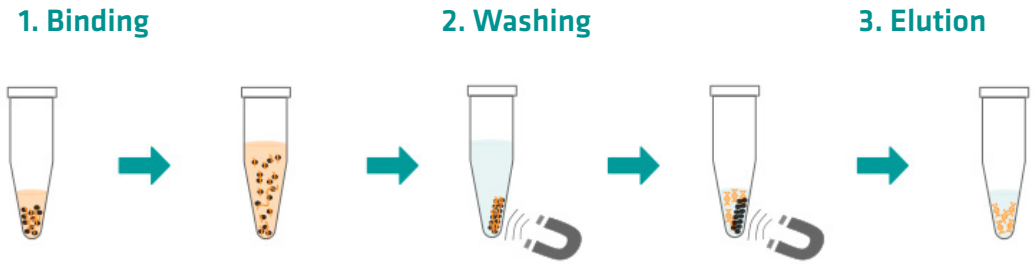

Figure 1: The total DNA clean-up workflow.

The total DNA clean-up workflow is composed of 3 main steps:

1. Binding
2. Washing
3. Elution

Experimental set-up

Deck Position A: dual reservoir adapter with 2 divided reservoirs
Deck Position B: Sapphire 96 well PCR plate (Greiner Bio-one) on a PCR cooling block
Deck Position C: Thermo Scientific™ Abgene™ 96 well 0.8 ml polypropylene DeepWell™ plate

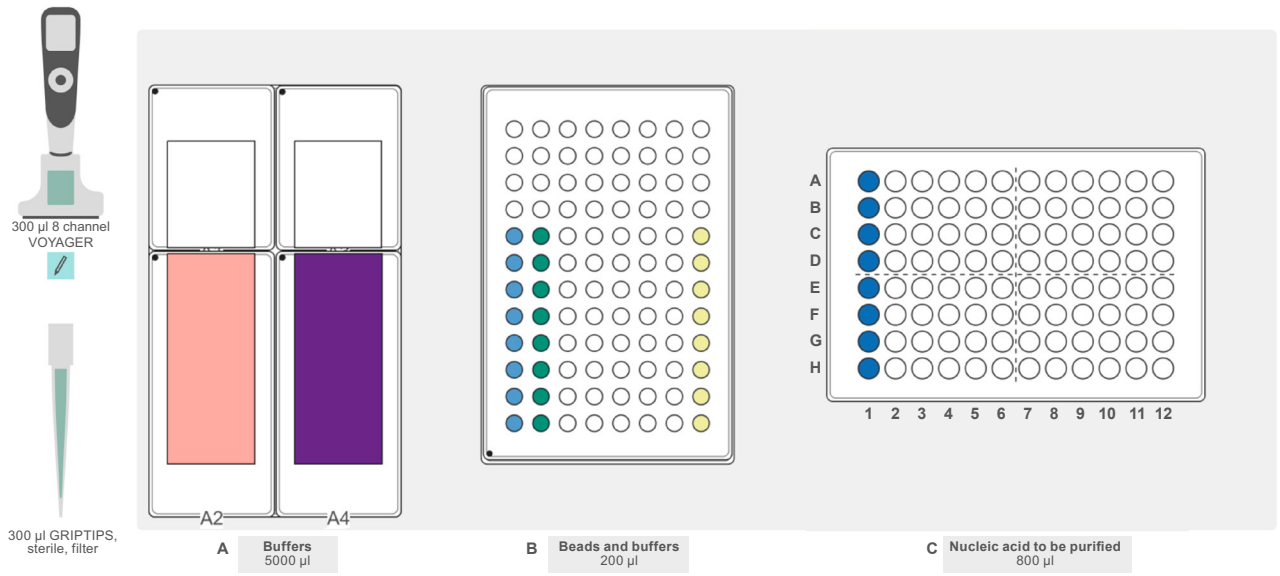

Figure 2: Deck set-up for the binding step. **Position A:** dual reservoir adapter with 2 divided reservoirs with DNA MagBinding Buffer (A2 – pink) and DNA Wash Buffer (A4 – lilac). **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue), MagBinding Beads (green) and DNA Elution Buffer (yellow). **Position C:** 0.8 ml DeepWell plate with the nucleic acid to be purified (dark blue).

1. Binding

STEP: Preparation of samples and addition of buffer and magnetic beads.

HOW TO: Place the dual reservoir adapter at deck Position A. Place 2 divided reservoirs onto the deck, fill 1 of the 10 ml compartments with 2.2 ml of DNA MagBinding Buffer (**Figure 2**, pink) and the other with 9 ml of DNA Wash Buffer (**Figure 2**, lilac). Next, place a 96 well PCR plate with nuclease-free water, MagBinding Beads and DNA Elution Buffer (**Figure 2**, blue, green and yellow, respectively) at Position B (**Figure 3**).

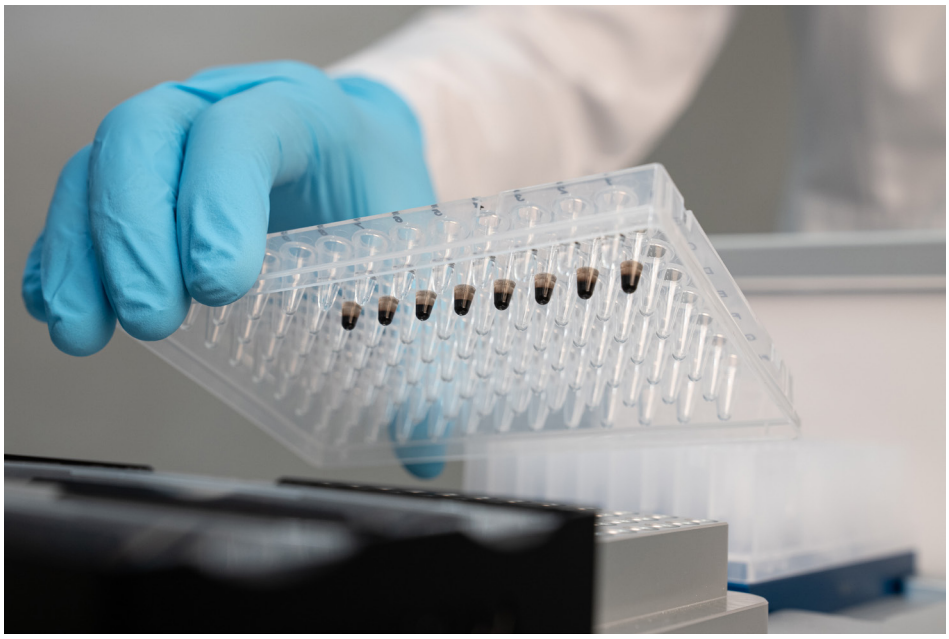

Figure 3: Placing the 96 well PCR plate onto the PCR cooling block.

Lastly, place the DeepWell plate containing the 30 µl samples (**Figure 2**, dark blue) at deck Position C.

Select and run the VIALAB program 'Zymo DCC'. The VOYAGER adjustable tip spacing electronic pipette will transfer 20 µl of nuclease-free water (with a 5 µl pre- and post-dispense) into the samples, so that the starting volume of the nucleic acid to be purified is 50 µl, as suggested by the DCC kit manufacturer. Next, the correct volume of DNA MagBinding Buffer – corresponding to 4 times the volume of input DNA in this case, 4x50 µl = 200 µl – is added to each sample, followed by 10 cycles of accurate mixing at speed 5. The MagBinding Beads are mixed thoroughly over 10 cycles to ensure they are kept in suspension, and 20 µl of beads are added to the samples (**Figure 4**). The magnetic beads are then mixed with the nucleic acid to be purified over 15 cycles.

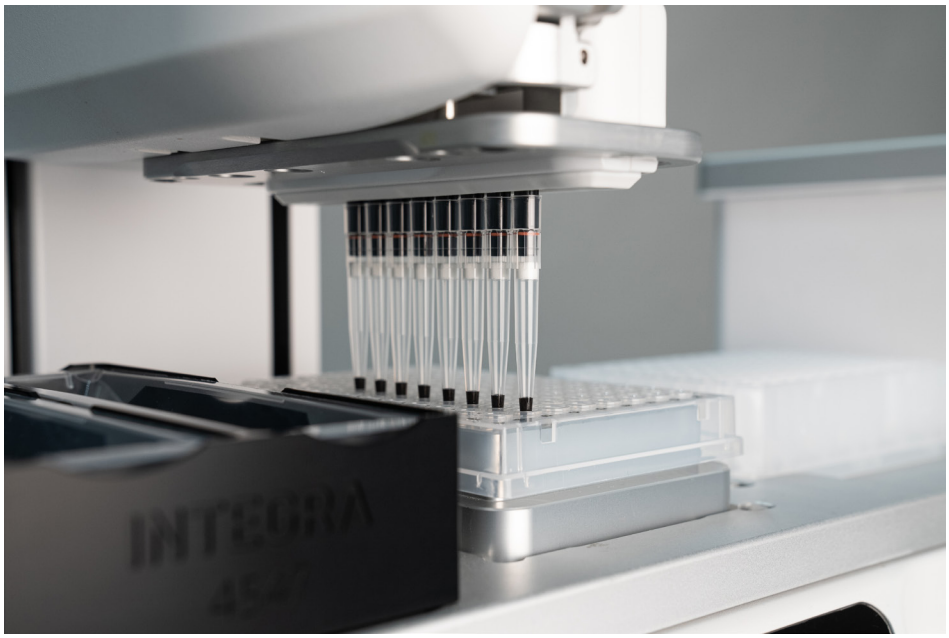

Figure 4: Addition of the MagBinding Beads to the samples to be cleaned up.

In the next step, the pipetting robot instructs the user to put the DeepWell plate onto the shaker for 10 minutes at 1300 rpm. This step improves the binding of the nucleic acid to the magnetic beads.

After shaking, a message on the pipette will tell the user to put an empty reservoir at deck Position A (A2) for waste and to place the DeepWell plate with the sample and magnetic beads onto the magnetic separation device at deck Position C (**Figure 5**).

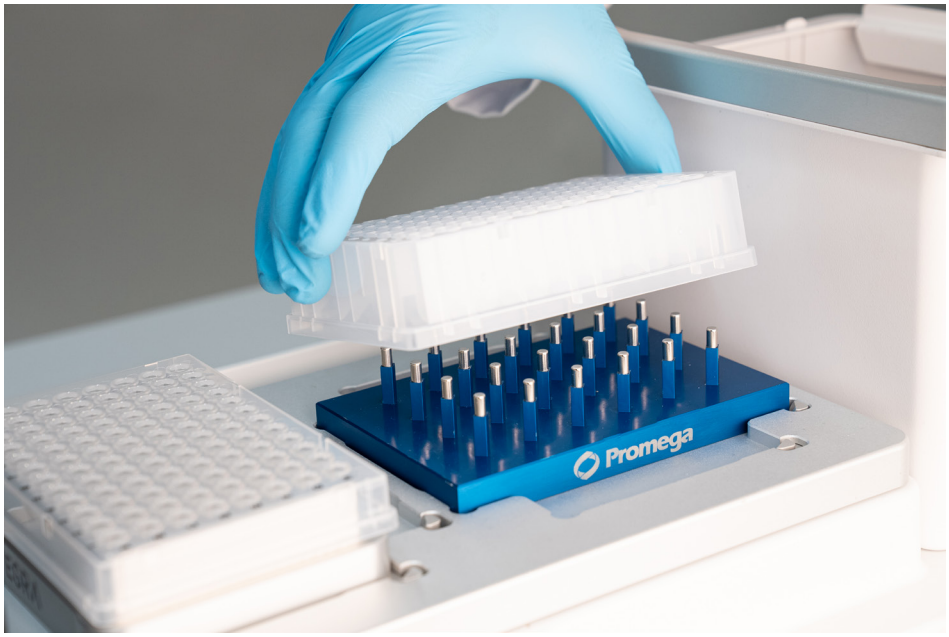

Figure 5: Placing the DeepWell plate onto the magnetic separation device.

After pressing ‘OK’, a 2-minute incubation will follow to allow magnetic bead capture. The pipetting robot will then automatically move on to the next step, where it will remove the supernatant from the plate on the magnetic separation device.

TIPS:

- Pre- and post-dispense steps can be used in liquid transfers throughout this protocol to guarantee precise pipetting.
- Use slow aspiration speeds of 1 or 2 during supernatant removal to avoid magnetic bead loss.

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2. Washing

STEP: Purification with DNA Wash Buffer.

HOW TO: The pipette directs the user to remove the magnetic separation device and place the DeepWell plate back on Position C. In the first purification step, 500 µl of DNA Wash Buffer is added in 2 transfer steps and mixed with the magnetic beads (**Figure 6**). Then, a message on the pipette tells the operator to place the DeepWell plate back onto the magnetic separation device at Position C and incubate it for 2 minutes. After incubation, the pipetting robot goes directly to the next step and removes the supernatant. The first washing step is repeated, and then the leftover wash buffer is carefully removed. The pipette informs the user that a 10-minute incubation time is now required to dry the magnetic beads; this incubation step is needed to ensure that residual buffer does not inhibit downstream applications.

TIP:

- The wash buffer contains ethanol. Make sure you use the correct pipetting speed, air gap and low retention tips for precise pipetting without dripping.

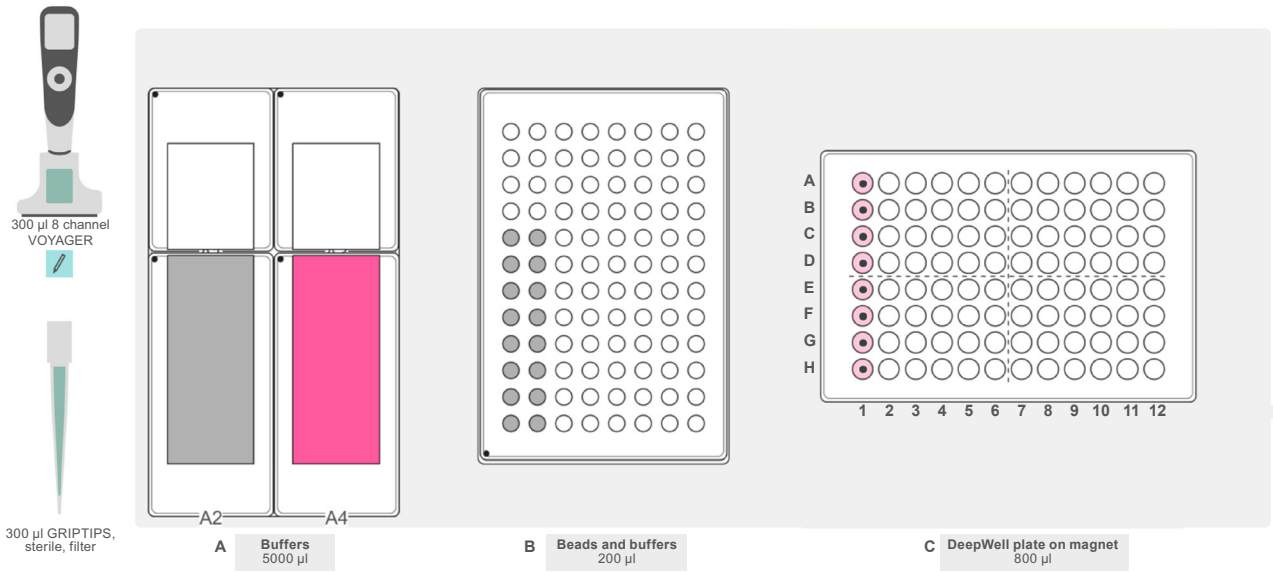

Figure 6: Deck set-up for the washing steps. **Position A:** Dual reservoir adapter with 2 divided reservoirs containing dead volume form previous steps (A2 – gray) and Wash Buffer (A4 – magenta). **Position C:** 0.8 ml DeepWell plate with nucleic acid bound to the magnetic beads (pink).

3. Elution

STEP: Eluting the samples in DNA Elution Buffer.

HOW TO: After the drying step, the operator should remove the magnetic separation device from below the DeepWell plate. The ASSIST PLUS will add 50 µl of DNA Elution Buffer and then perform 10 mixing cycles (**Figure 7**). A message will pop up, telling the user that the DeepWell plate should be placed onto the shaker for 5 minutes. This guarantees that all the DNA is eluted from the magnetic beads into the buffer. After shaking, the DeepWell plate should be placed onto the magnetic separation device at Position C, where the magnetic beads will be captured during a 2-minute incubation. The pipetting robot will then inform the user that a new 96 well plate for the eluted DNA should be placed at deck Position B in landscape orientation. In the last step, the pipette transfers 45 µl of eluted DNA to the new 96 well plate.

TIP:

- The eluted DNA can be used immediately or stored at -20 °C until further use.

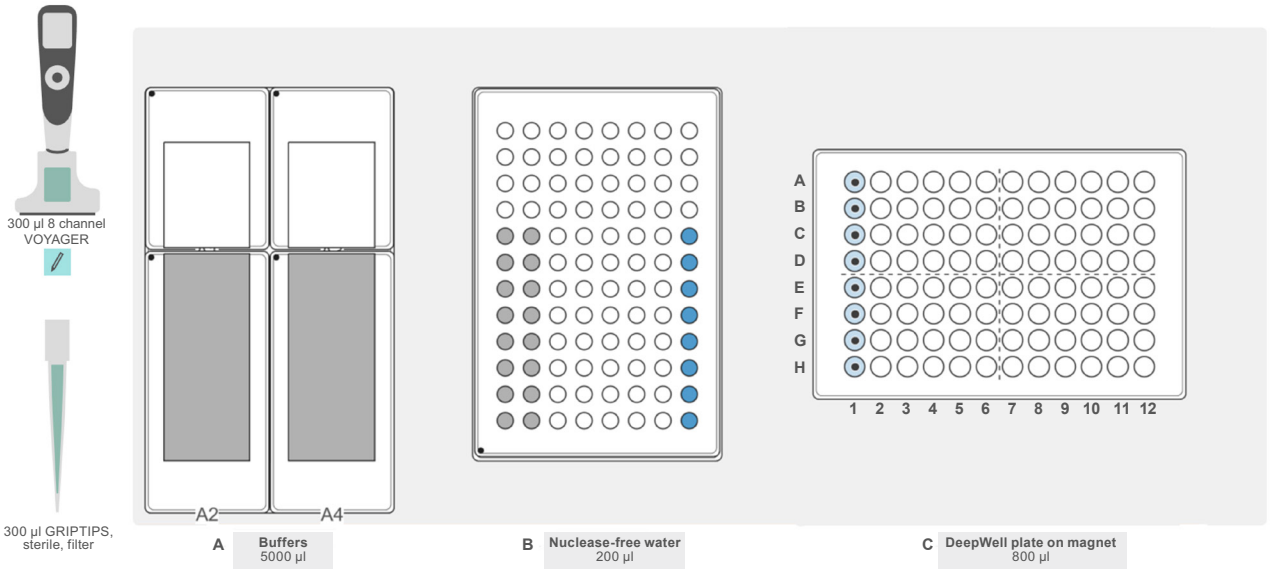

Figure 7: Position A: Dual reservoir adapter with 2 divided reservoirs containing dead volume from previous steps (A2, A4 – gray). Deck set-up for the elution step. **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue). **Position C:** 0.8 ml DeepWell plate with the purified nucleic acid bound to the magnetic beads (light blue).

Results

The automated DCC MagBead protocol on the ASSIST PLUS shows high DNA recovery from PCR, that is ready to use in any downstream application. The automated workflow resulted in greater DNA yield, better purity (A_{260}/A_{280} and A_{260}/A_{230} ratios) and higher reproducibility (lower SD values).

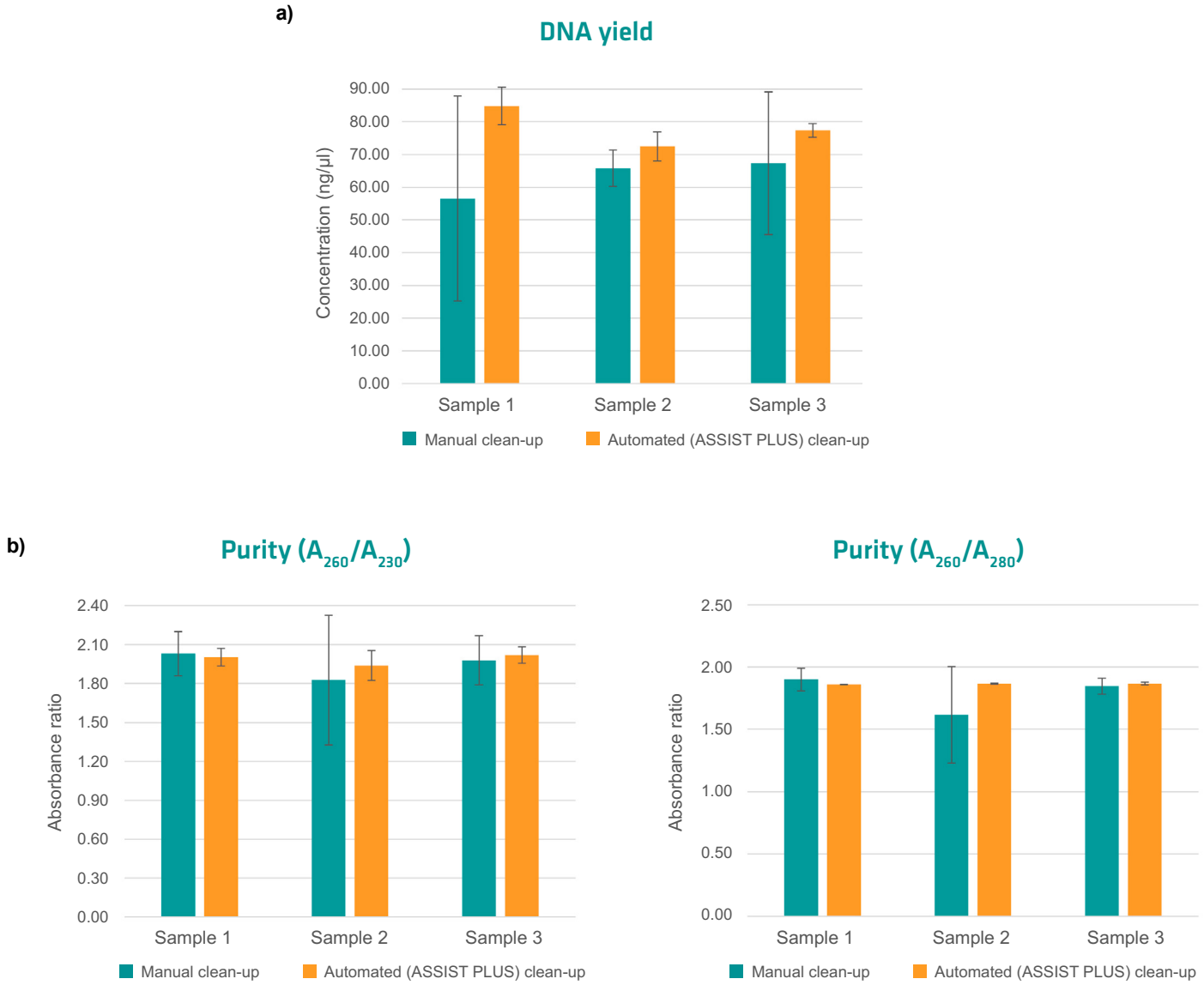

Figure 8: DNA from a PCR reaction was purified manually and using the automated protocol on the ASSIST PLUS. DNA a) yield and b) purity were determined by UV spectroscopy. N=3 for each sample.

Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to the user's specific labware and protocols.
- **Run report:** If the ASSIST PLUS pipetting robot is connected to the PC with VIALAB, programs can be started directly from the PC. A report is automatically generated after a run, documenting details such as the start/end time, user, calculated volumes and any errors that occurred. This offers a convenient way to fulfill regulatory requirements.

Conclusion

- The ASSIST PLUS pipetting robot in combination with the VOYAGER electronic pipette offers a seamless and efficient DNA clean-up solution using the Zymo Research DNA Clean & Concentrator MagBead Kit for low to high throughput sample batches.
- Automated pipetting steps avoid the introduction of human errors, generating highly reproducible and reliable results.
- The optimized liquid handling parameters on the ASSIST PLUS enable precision pipetting, resulting in high DNA yields and purity.
- Wash buffers normally contain ethanol, and the use of low retention GRIPTIPS prevents dripping from occurring in the washing steps.
- The entire semi-automated DNA clean-up workflow can be programmed as a single VIALAB program using the labware change function.
- The results of the semi-automated protocol were comparable to the manual protocol in terms of DNA yield and purity. The ASSIST PLUS protocol helped to reduce standard deviations, indicating improved consistency and reproducibility of the automated DNA clean-up process over manual clean-up.

INTEGRA

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	4723	300 µl 8 channel VOYAGER electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/voyager
INTEGRA Biosciences	4221	Pipette Communication Module for INTEGRA electronic pipettes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6535	300 µl low retention, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4356	25 ml divided reservoir, sterile, polypropylene	https://www.integra-biosciences.com/en/reagent-reservoirs/divided-reagent-reservoirs
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
Zymo Research	D4012	DNA Clean & Concentrator MagBead Kit	https://zymoresearch.eu/products/dna-clean-concentrator-magbead-kit
Promega	V3031	Deep Well MagnaBot® 96 Magnetic Separation Device	https://worldwide.promega.com/products/biochemicals-and-labware/tips-and-accessories/deep-well-magnabot-96-magnetic-separation-device/?catNum=V3031
Thermo Fisher Scientific	AB0859	Abgene 96 well 0.8 ml polypropylene DeepWell sample processing & storage plate for genomics and NGS library preparation	https://www.thermofisher.com/order/catalog/product/AB0859
Greiner Bio-One International	652270	Sapphire microplate, 96 well, polypropylene, for PCR	https://shop.gbo.com/en/switzerland/products/bioscience/molecular-biology/pcr-microplates/652270.html

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