

# 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 next generation sequencing (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 DNA clean-up automated protocols show high reproducibility, along with top-notch yield and purity values.

### Key benefits:

- Automated DNA clean-up with Zymo's DCC on the ASSIST PLUS pipetting robot makes DNA clean-up a cakewalk. Manual intervention is needed only when moving labware on and off the magnetic separator and shaker.
- The optimized liquid handling parameters on 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 speed 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 automated 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 thanks to our innovative labware accessories. Reservoirs with SureFlo™ anti-sealing array allows 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



## Step-by-step procedure

The ASSIST PLUS pipetting robot is used together with the 8 channel 300 µl VOYAGER automated tip spacing electronic pipette and 300 µl sterile, filter, low retention 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:

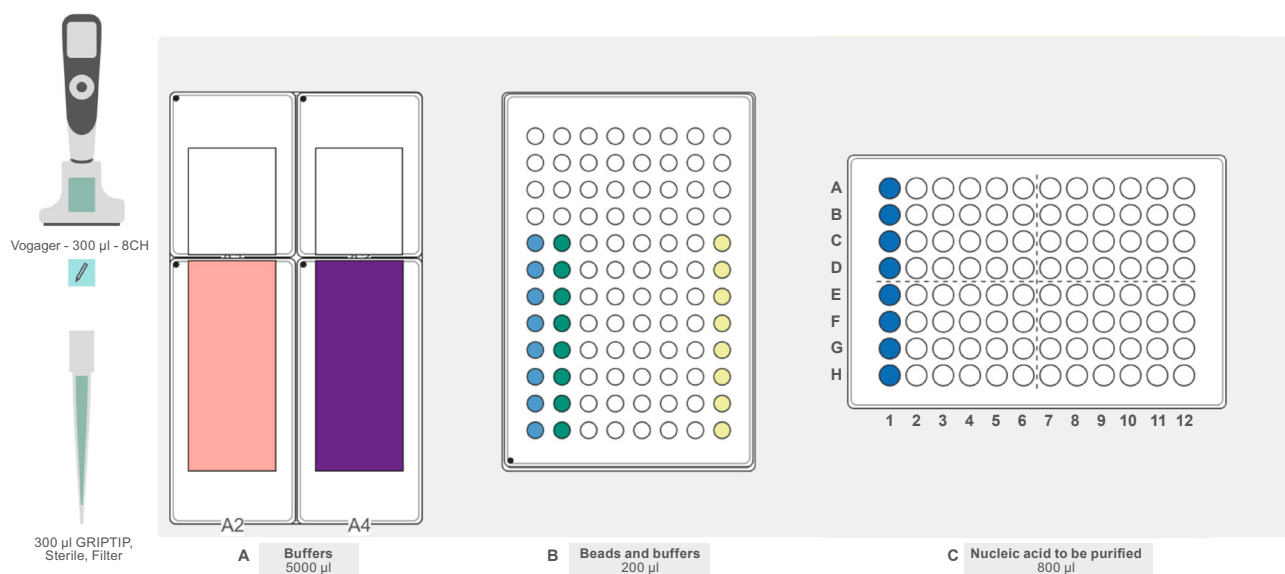
1. Binding
2. Washing
3. Elution

## 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

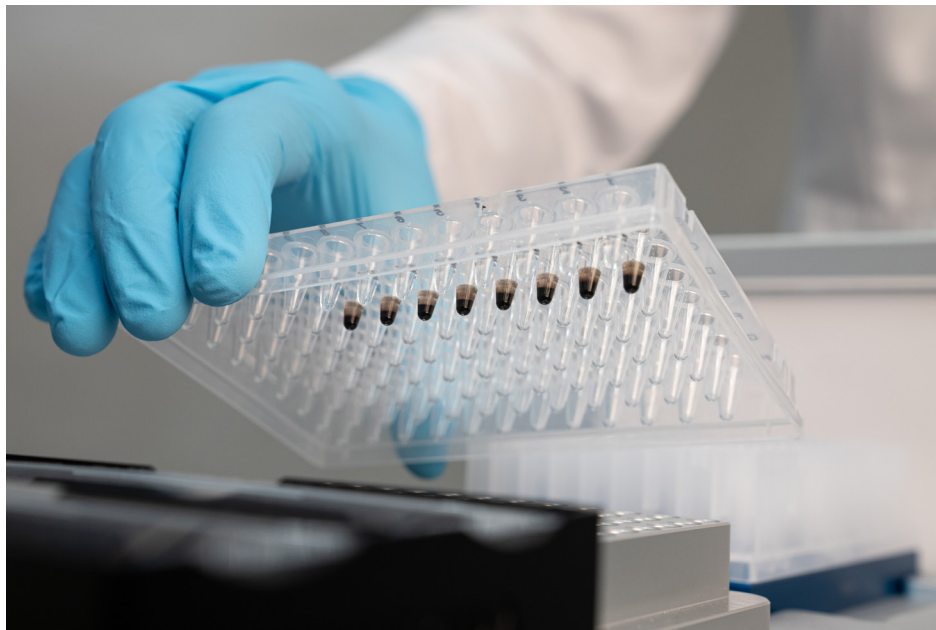


**Figure 2:** Deck set-up for the binding step. **Position A:** dual reservoir adapter with 2 divided reservoirs with DNA MagBinding Buffer (A2 – pink) and DNA Wash Buffer (A4 – lilac). **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue), MagBinding Beads (green) and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

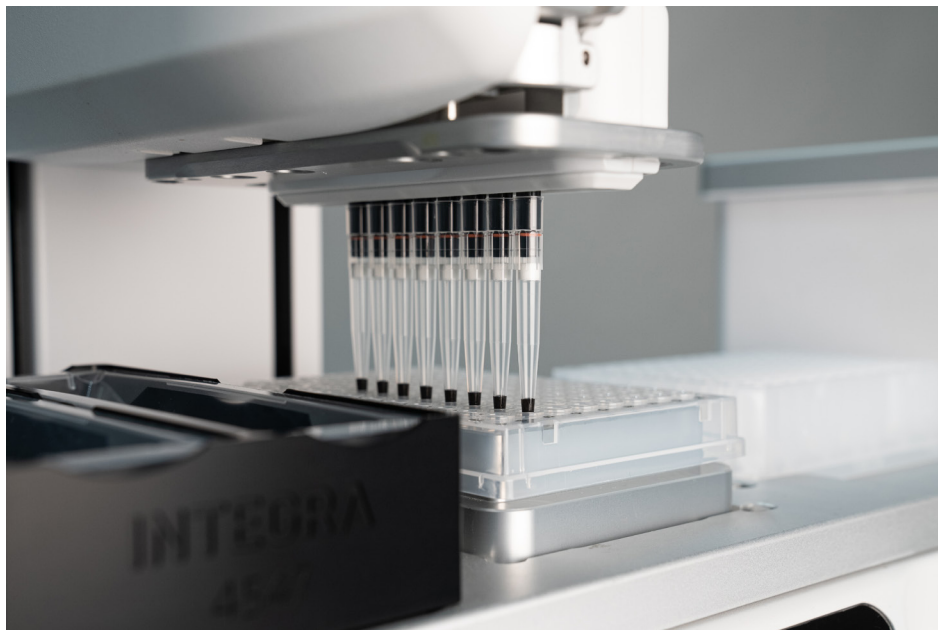
**HOW TO:** Place the dual reservoir adapter at deck position A. Place 2 divided reservoirs onto the deck, fill 1 of the 10 ml compartments with 2.2 ml of DNA MagBinding Buffer (**Figure 2**, pink) and the other with 9 ml of DNA Wash Buffer (**Figure 2**, lilac). Next, place a 96 well PCR plate with 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  $\mu$ l samples (**Figure 2**, dark blue) at deck position C.

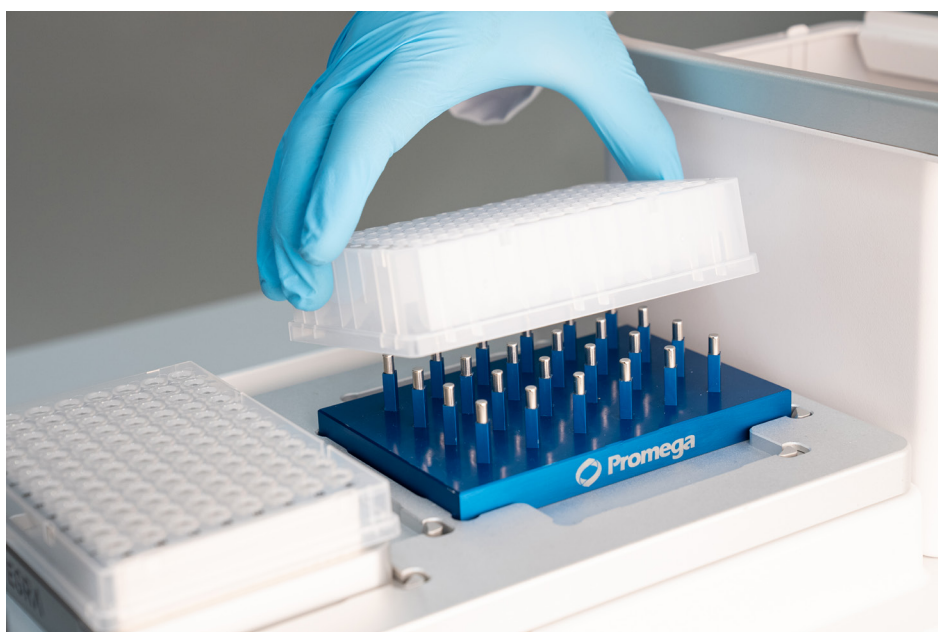
Select and run the VIALAB program 'Zymo DCC'. The VOYAGER automated tip spacing electronic pipette will transfer 20  $\mu$ l of nuclease-free water (with a 5  $\mu$ l pre- and post-dispense) into the samples, so that the starting volume of the nucleic acid to be purified is 50  $\mu$ l, as suggested by the DCC kit manufacturer. Next, the correct volume of DNA MagBinding Buffer – corresponding to 4 times the volume of input DNA so, in this case,  $4 \times 50 \mu\text{l} = 200 \mu\text{l}$  – 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  $\mu$ l 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.

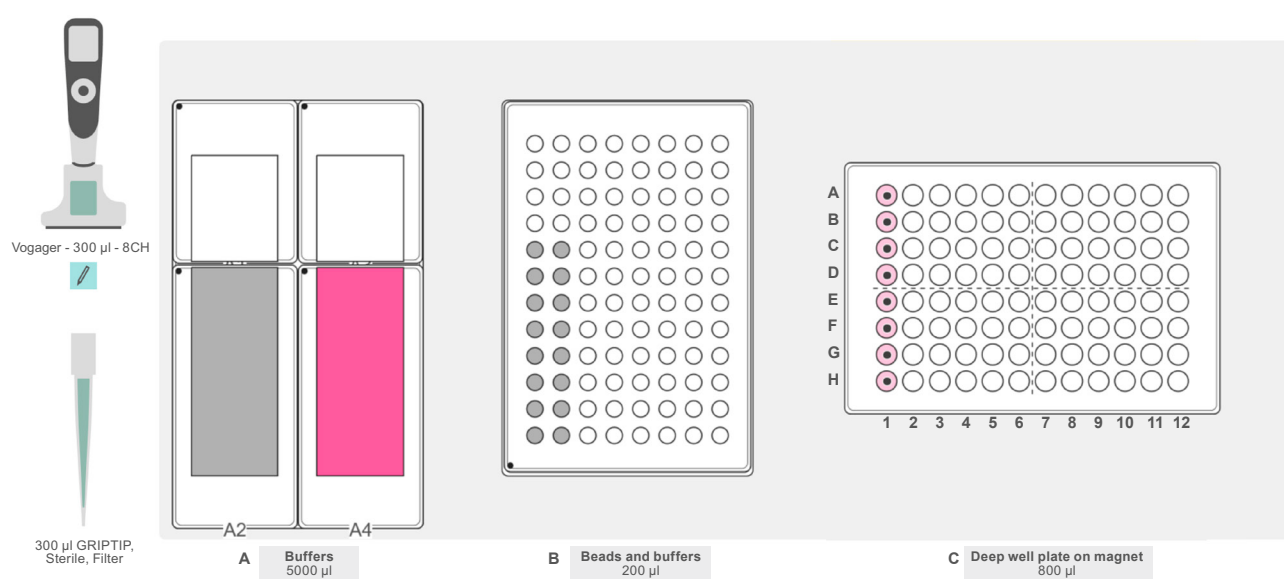
**2. Washing**

**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 at 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 DNA Wash Buffer (A4 – magenta). **Position C:** 0.8 ml DeepWell plate with nucleic acid bound to the magnetic beads (pink).

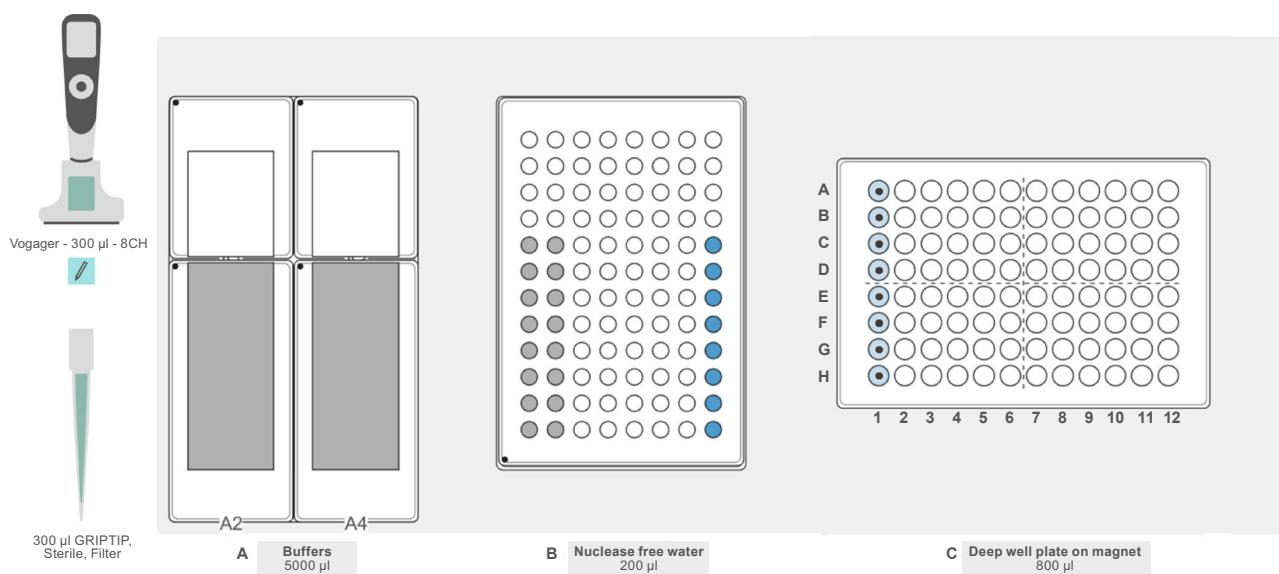
## 3. Elution

**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. ASSIST PLUS will add 50  $\mu$ l 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  $\mu$ l of eluted DNA to the new 96 well plate.

**TIP:**

- The eluted DNA can be used immediately or stored at  $-20^{\circ}\text{C}$  until further use.

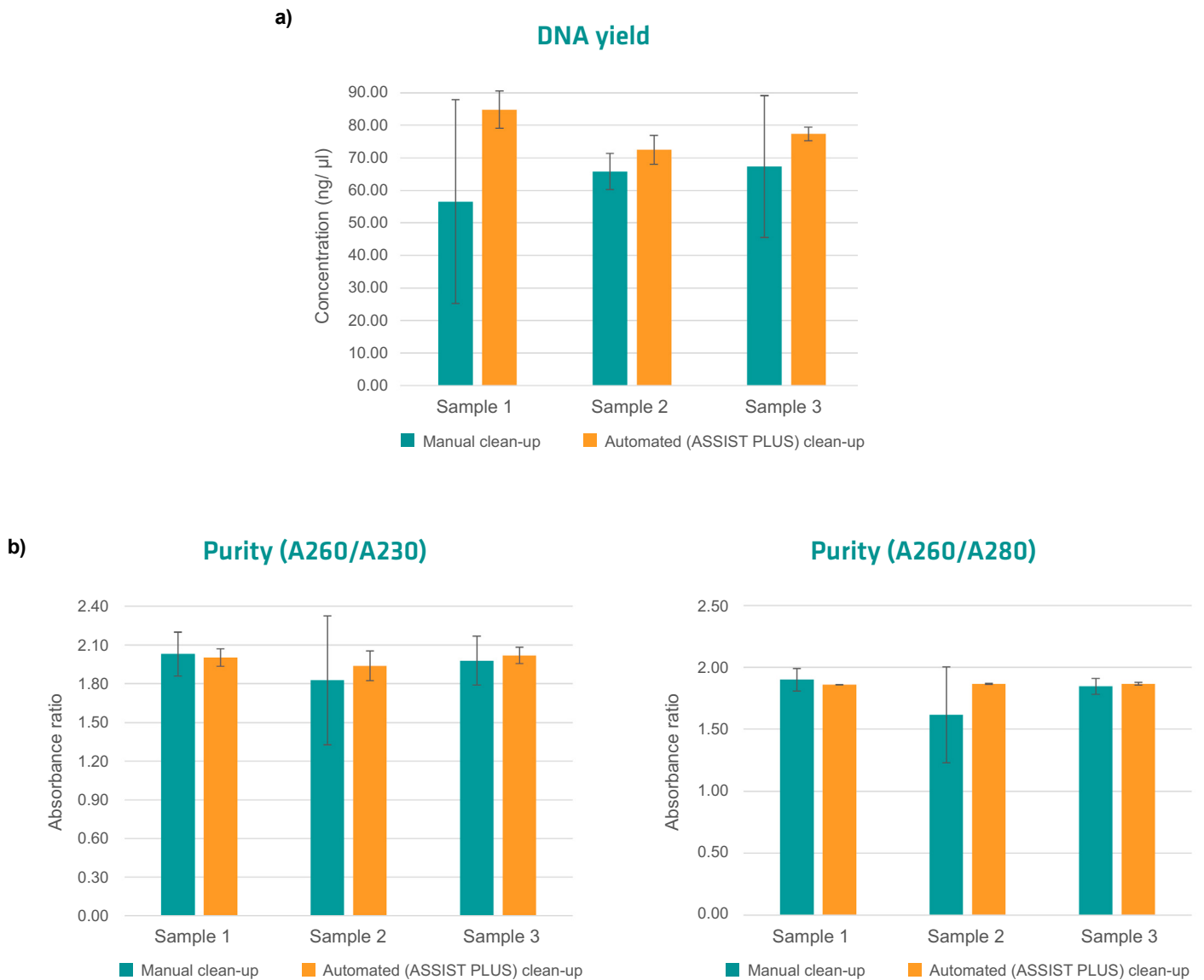


**Figure 7:** Deck set-up for the elution step. **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue). **Position C:** 0.8 ml DeepWell plate with the purified nucleic acid bound to the magnetic beads (light blue).



## Results

The automated DCC MagBead protocol on 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 (A260/A280 and A260/A230 ratios) and higher reproducibility (lower SD values).



**Figure 8:** DNA from a PCR reaction was purified using the automated protocol on ASSIST PLUS and manually. 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 error, generating highly reproducible and reliable results.
- The optimized liquid handling parameters on ASSIST PLUS enable precision pipetting, resulting in high DNA yields and purity.
- Wash buffers normally contain ethanol, but no dripping occurs in the washing steps in this protocol, thanks to INTEGRA's special low retention GRIPTIPS.
- The whole 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 DNA clean-up process over manual clean-up.



## Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
INTEGRA Biosciences	4723	VOYAGER 8 channel 300 µl electronic pipette	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager">https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager</a>
INTEGRA Biosciences	4221	Pipette Communication Module for INTEGRA electronic pipettes	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
INTEGRA Biosciences	6535	300 µl Sterile, Filter, Low retention, GRIPTIPS®	<a href="https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide">https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide</a>
INTEGRA Biosciences	4547	Dual reservoir adapter	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
INTEGRA Biosciences	4356	25 ml divided reservoir, sterile, polypropylene	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/divided-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/divided-reagent-reservoirs</a>
INTEGRA Biosciences	6250	PCR 96 well cooling block	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
Zymo Research	D4012	DNA Clean & Concentrator™ MagBead Kit	<a href="https://zymoresearch.eu/products/dna-clean-concentrator-magbead-kit">https://zymoresearch.eu/products/dna-clean-concentrator-magbead-kit</a>
Promega	V3031	Deep Well MagnaBot® 96 Magnetic Separation Device	<a href="https://worldwide.promega.com/products/biochemicals-and-labware/tips-and-accessories/deep-well-magnabot-96-magnetic-separation-device/?catNum=V3031">https://worldwide.promega.com/products/biochemicals-and-labware/tips-and-accessories/deep-well-magnabot-96-magnetic-separation-device/?catNum=V3031</a>
Thermo Fisher Scientific	AB0859	Abgene™ 96 Well 0.8 ml Polypropylene DeepWell™ Sample Processing & Storage Plate for Genomics and NGS library preparation	<a href="https://www.thermofisher.com/order/catalog/product/AB0859">https://www.thermofisher.com/order/catalog/product/AB0859</a>
Greiner Bio-One International	652270	Sapphire microplate, 96 well, PP, for PCR	<a href="https://shop.gbo.com/en/switzerland/products/bioscience/molecular-biology/pcr-microplates/652270.html">https://shop.gbo.com/en/switzerland/products/bioscience/molecular-biology/pcr-microplates/652270.html</a>

**INTEGRA Biosciences AG**  
7205 Zizers, Switzerland  
T +41 81 286 95 30  
F +41 81 286 95 33  
info@integra-biosciences.com

**INTEGRA Biosciences Corp.**  
Hudson, NH 03051, USA  
T +1 603 578 5800  
F +1 603 577 5529  
info-us@integra-biosciences.com

**INTEGRA Biosciences Deutschland GmbH**  
35444 Biebertal, Deutschland  
T +49 6409 81 999 15  
F +49 6409 81 999 68  
info-de@integra-biosciences.com

**INTEGRA Biosciences SAS**  
95062 Cergy-Pontoise Cedex 1, France  
T +33 (0)1 34 30 76 76  
F +33 (0)1 34 30 76 79  
info-fr@integra-biosciences.com

**INTEGRA Biosciences Ltd.**  
Thatcham, Berks RG19 4EP, UK  
T: +44 1635 797000  
F: +44 1635 797001  
info-uk@integra-biosciences.com

**INTEGRA Biosciences Nordic ApS**  
Vallensbækvej 22A 3TV  
Brøndby 2605, Denmark  
T + 45 3173 5373  
info-nordic@integra-biosciences.com

**INTEGRA Biosciences (Shanghai) Co., Ltd.**  
中国上海自由贸易试验区环科路515号1110室  
邮编: 201315  
电话: +86 21 5844 7203  
info-cn@integra-biosciences.com

インテグラ・バイオサイエンス株式会社  
〒101-0031  
東京都千代田区東神田 1-5-6  
東神田MK第五ビル 3階  
T 03-5962-4936  
F 03-5962-4937  
info-jp@integra-biosciences.com