

PCR purification with Beckman Coulter AMPure XP magnetic beads and the ASSIST PLUS

Introduction

Agencourt AMPure XP beads (Beckman Coulter) are used for DNA purification in a variety of applications, including PCR, NGS, cloning and microarrays. The ASSIST PLUS pipetting robot provides a solution for optimal bead separation and maximized recovery of precious samples. User guidance throughout the entire protocol ensures an error-free pipetting procedure. Careful and accurate

handling of the magnetic beads by the ASSIST PLUS leads to superior reproducibility and consistency during the experiment. Taken together, the ASSIST PLUS provides researchers with an easy and highly efficient way to purify DNA from PCR reactions using AMPure XP magnetic beads.

Key benefits:

- The VIAFLO and VOYAGER electronic pipettes, in combination with ASSIST PLUS, provide unmatched pipetting ergonomics.
- Optimal pipette settings, including tip immersion depth, pipetting speeds and angles, maximize reproducibility and sample recovery.
- Exact positioning of the pipette tips in the sample wells avoids the risk of disturbing the ring of magnetic beads or bead carryover.
- The ASSIST PLUS automates many steps of a magnetic bead purification protocol and guides the user through the remaining manual operations to ensure an error-free process.

Step-by-step procedure:

The ASSIST PLUS is used to purify DNA samples using AMPure XP beads (Beckman Coulter). The pipetting robot runs a VOYAGER 8 channel 125 µl electronic pipette with 125 µl Sterile, Filter, Low Retention GripTips. The use of Low Retention GripTips guarantees optimal liquid handling of viscous (AMPure XP buffer) and volatile (70 % ethanol) solutions.

Below is an example set-up for 24 samples, preparing 10 µl DNA samples (**position B**) with 18 µl of AMPure XP beads (**position A**). The pipetting programs were prepared according to the manufacturer's protocol (AMPure XP, Beckman Coulter) using VIALAB software.

The protocol is divided into two programs that guide the user through every step of the PCR purification process.

Program 1: Binding (AMP_BINDING)

Program 2: Washing and elution (AMP_WASH_ELUTE)

ASSIST PLUS



Program 1 – binding

Experimental set-up

Deck position A: PCR 8 tube strip containing the AMPure XP beads (**Figure 1**, blue), placed onto a cooling block from INTEGRA. Note: the cooling block is just used as a support in this instance, and not for cooling down the samples.

Deck position B: 96 well plate with 24 DNA samples for purification (**Figure 1**, green).

Deck position C: 96 well plate ring magnet.

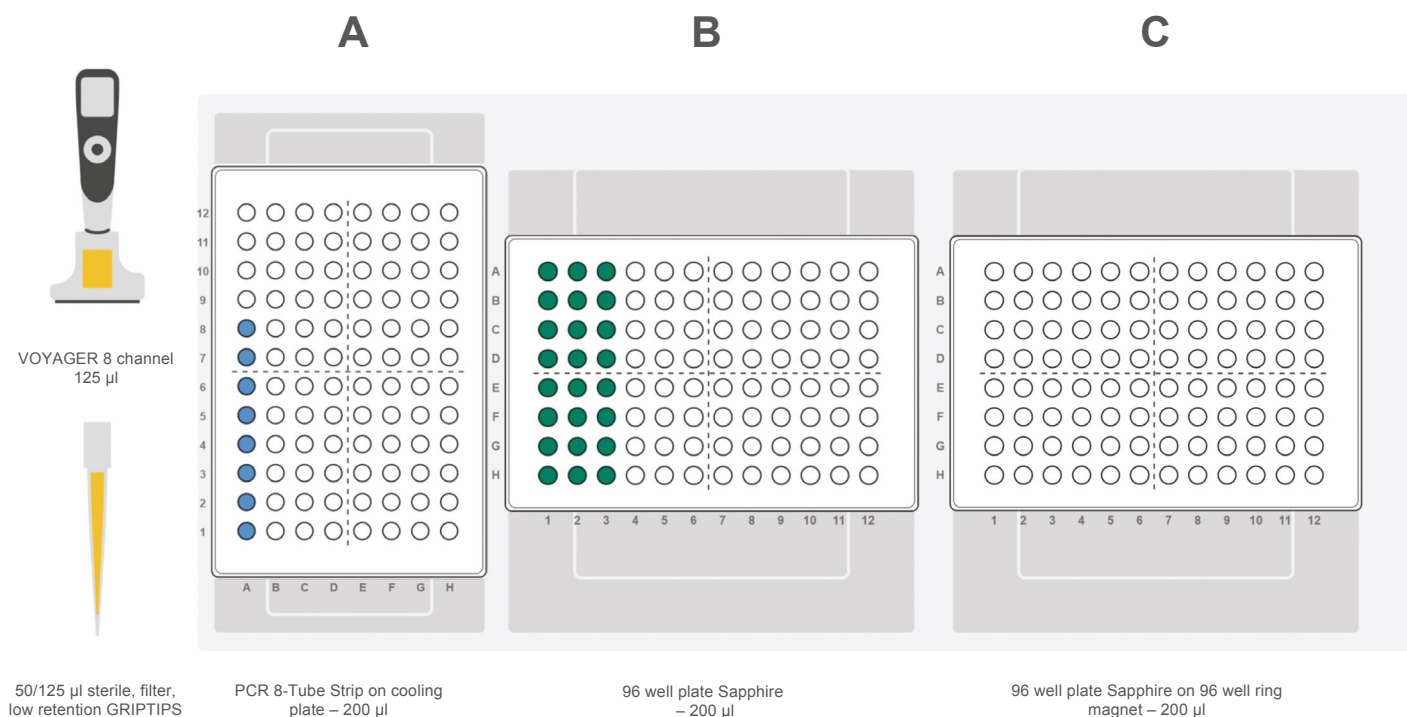


Figure 1: The set-up for program 1

1. AMPure XP bead transfer

STEP: Transferring AMPure XP beads from an 8 tube PCR strip to a 96 well plate containing the DNA samples.

HOW TO: Select and run the AMP_BINDING program on the VOYAGER electronic pipette. The ASSIST PLUS pipetting robot immediately starts the protocol. To ensure the AMPure XP buffer is homogenous, the beads are resuspended by pipetting up and down 10 times before being transferred to the samples. The beads and DNA fragments are thoroughly mixed together before the pipette automatically starts the timer for a five minute incubation period, ensuring optimal conditions for the DNA strands to bind onto the magnetic beads.

Tip: Using Low Retention GripTips rather than regular GripTips prevents the loss of AMPure XP beads during the pipetting steps (see **Figure 2**).

2. Magnetic separation of the AMPure XP beads

STEP: Separating the magnetic beads from the PCR samples.

HOW TO: A message instructs the user to move the plate (**position B**) onto the magnet (**position C**). Continue the program to start the timer. After a two minute incubation on the magnet the beads form a ring in the sample well and the solution becomes clear. The program resumes automatically, and the supernatant is removed. On completion of this step, the pipette prompts the user to continue with the AMP_WASH_ELUTE program and to replace the labware on **position A** with the 8 row polypropylene (PP) reagent reservoir containing the ethanol and elution buffer.

Tip: The supernatant is aspirated slowly using the Tip Travel feature of the ASSIST PLUS to avoid disturbing the ring of beads. The Tip Travel feature keeps the tip immersion depth constant during aspiration and dispensing. 5 µl of supernatant remain in the plate to prevent beads being drawn out during aspiration.

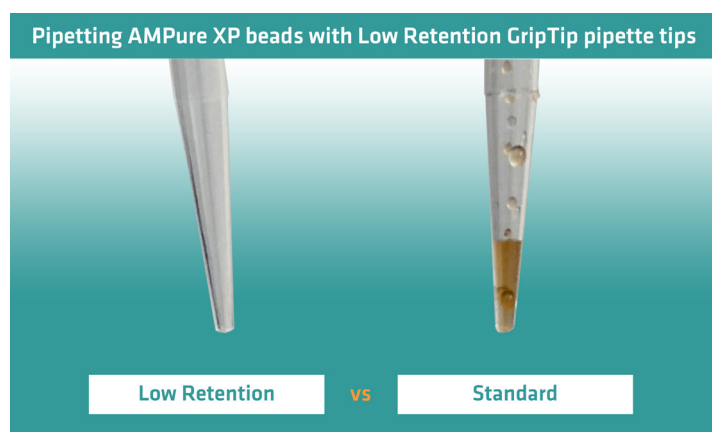


Figure 2: The image highlights the advantages of using Low Retention GripTips versus regular GripTips when pipetting AMPure XP beads



Figure 3: The ASSIST PLUS settings allow removal of the supernatant without any bead carryover

Program 2 – washing and elution

Deck position A: The 96 well PCR cooling block is replaced by an 8 row polypropylene (PP) reagent reservoir filled with 70 % ethanol in row 1 (blue) and elution buffer in row 2 (orange). Row 8 is used for waste (purple).

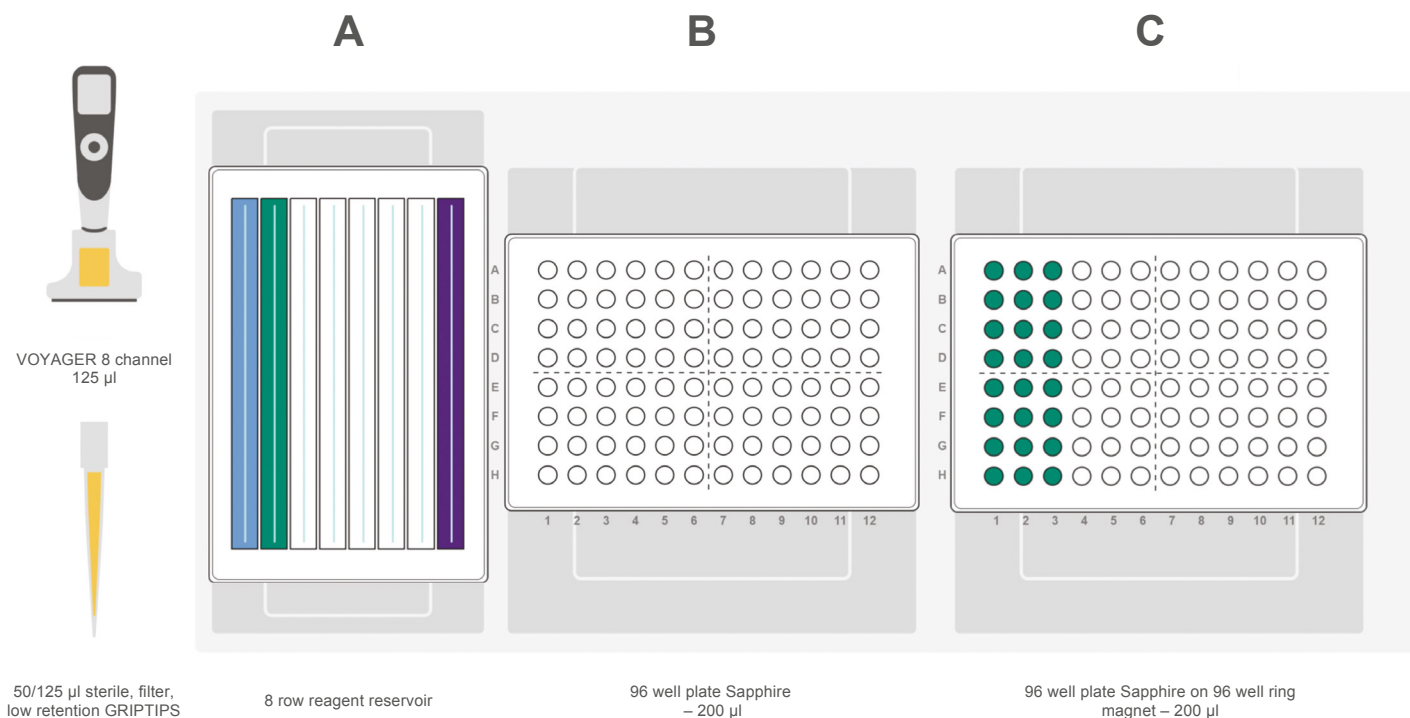


Figure 4: The set-up for program 2

3. Magnetic bead clean-up

STEP: Washing the magnetic beads twice with 70 % ethanol.

HOW TO: Start the AMP_WASH_ELUTE program on the VOYAGER electronic pipette. The ASSIST PLUS washes the beads twice by automatically adding and removing ethanol.

Tip: The programmed pipette settings allow the beads to be washed without disturbing the bead ring. At the end of the second washing step, all the ethanol is removed. If necessary, an additional drying time can easily be added using VIALAB software. The use of Low Retention GripTips prevents ethanol from dripping while traveling from **position A** to **position C**.

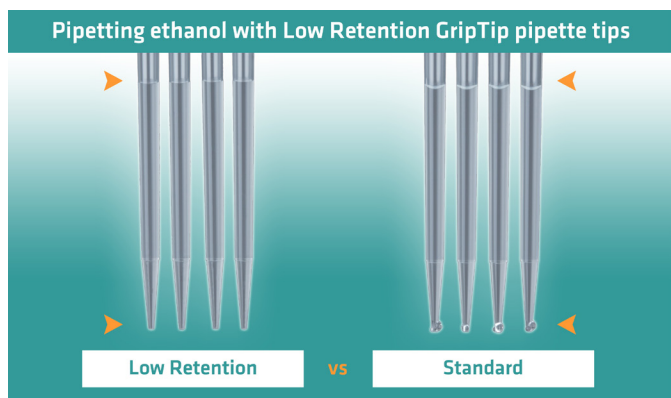


Figure 5: The image highlights the advantages of using Low Retention GripTips (left) versus regular GripTips (right) when pipetting ethanol.

4. Elute samples from the magnetic beads

STEP: Eluting the samples from the magnetic beads by adding an elution buffer.

HOW TO: The pipette prompts the user to move the reaction plate from the magnet (**position C**) to **position B**. Continuing the protocol, the ASSIST PLUS transfers the elution buffer to the DNA samples bound to the magnetic beads (**position B**, orange). After mixing carefully and thoroughly 10 times, the pipette prompts the user to place the 96 well plate on the magnet (**position C**).

5. Transfer the sample eluates

STEP: Transferring the sample eluates into a new 96 well plate.

HOW TO: As indicated by the pipette, place a new 96 well plate onto **position B** and continue the program. The sample eluates are then transferred into the new plate automatically.

Tip: Optimized pipette settings (aspiration speed, volume, height, tip travel and tip touch) allow the volume of eluate transferred to be maximized without carryover of beads (see **Figure 6**). A tip touch after the transfer removes droplets that may still cling to the end of the pipette tips. Pipetting heights on the ASSIST PLUS can be fine-tuned at any time. Performing a test run with water before implementing any new assay is an ideal way to optimize pipette settings.

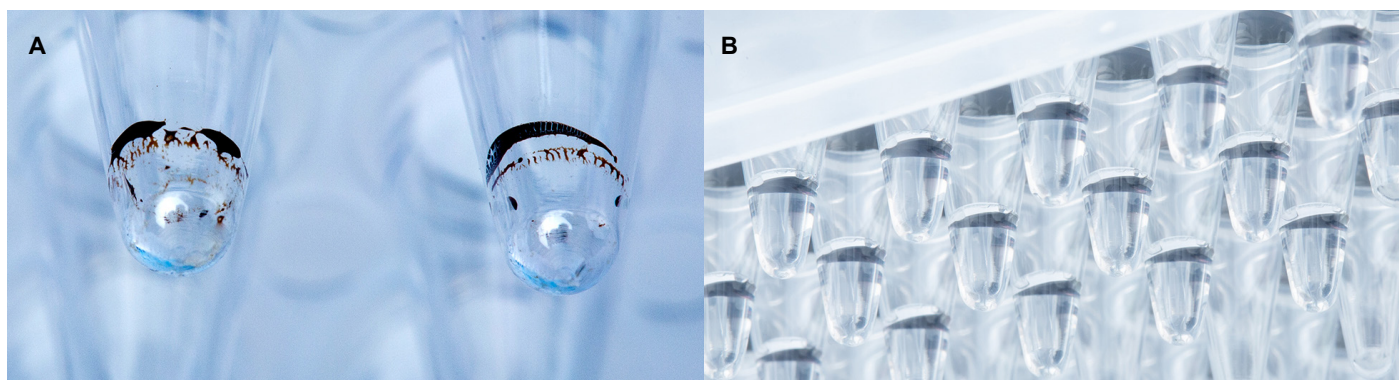


Figure 6: Magnetic beads are clearly visible in the 96 well plate with no supernatant remaining (A). No carryover of beads is observed in the eluate (B).

Conclusion

- Magnetic bead purifications can be easily automated on the ASSIST PLUS pipetting robot.
- Optimized tip immersion and pipette settings together with the use of Low Retention GripTips allow maximum sample recovery at the end of the purification protocol.
- The pipette loaded onto the ASSIST PLUS prompts the user when needed, eliminating the risk of human errors.
- VIALAB programs can be easily adapted to specific labware.
- Prolonged pipetting tasks lead to repetitive strain injury. This can be avoided by automating these steps with the ASSIST PLUS.

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/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6565	125 µl Sterile, Filter, Low retention GripTips	https://www.integra-biosciences.com/global/en/grip-tip-selector-guide
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6371	8 Row Polypropylene Reservoirs	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
BECKMAN COULTER Life Sciences	A63880 A63881 A63882	Agencourt AMPure XP	https://www.beckman.ch/reagents/genomic/clean-up-and-size-selection/pcr
ALPAQUA Accelerating Genomic Discovery	A001322	96S Super Magnet Plate	https://www.alpaqua.com/Products/Magnet-Plates/Selection-Guide
Greiner Bio-One	669285	96 Well Polypropylene Microplates	https://www.gbo.com/en_GB/suche.html?id=110&L=15&q=Sapphire+96+well
CORNING	3741	Corning® Thermowell® GOLD 0.2 ml 8-Well PCR tube strips	https://ecatalog.corning.com/life-sciences/b2b/US/en/Genomics-&-Molecular-Biology/PCR-Consumables/PCR-Tubes-and-Strip-Tubes/Corning%C2%AE-Thermowell%E2%84%A2-GOLD-and-Thermowell-8-well-PCR-Tube-Strips/p/corningThermowellGOLDAndThermowell8WellPCR-TubeStrips

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