Application Note

INTEGRA

PCR purification with Beckman Coulter AMPure XP magnetic beads and the VIAFLO 96

Introduction

Agencourt AMPure XP magnetic beads (Beckman Coulter) are an efficient way to clean up samples for PCR, NGS, cloning and microarrays. The kit provides a solution for medium to high throughput requirements when carried out in a 96 well plate, but the protocol involves many washing and transfer steps that make it tedious to perform manually. With the VIAFLO 96, a handheld 96 channel electronic pipette, multistep protocols such as PCR clean-up and DNA purification can be performed quickly and efficiently, increasing throughput tremendously by transferring samples and reagents to all 96 wells at once. Thanks to its unique operating concept, the VIAFLO 96 remains as easy to use as a traditional handheld pipette and can even provide critical information (user-defined prompts) about the protocol steps.

Key benefits:

- The VIAFLO 96 enables transfer of samples, reagents and wash solutions to 96 wells at once, increasing the throughput of magnetic bead-based DNA purification methods.
- The partial tip loading of the VIAFLO 96 allows purification of fewer than 96 DNA samples if necessary; 8, 16, 24, 32, 40 or 48 GripTips can be loaded for easy purification of different numbers of samples.
- The optimal immersion depth for removing supernatant or adding liquid right onto the samples is guaranteed by defining the z-height of the VIAFLO 96.
- The Tip Align setting of the VIAFLO 96 automatically positions the tips in the center of the wells of a 96 well plate, avoiding any disturbance of the beads.

Step-by-step procedure:

Experimental set-up

The VIAFLO 96 handheld electronic pipette with a three position stage (see **Figure 1**) is used to purify DNA with AMPure XP beads from Beckman Coulter. The following protocol is an example of a set-up for 96 samples, where each well of a 96 well plate is filled with 10 µl of DNA sample and 18 µl of AMPure XP beads, then further processed with the VIAFLO 96. The PCR purification can be performed manually or semi-automated using the VIALINK programs are provided. The VIALINK programs are set up according to the manufacturer's protocol (AMPure XP Beckman Coulter).

VIAFLO 96/384

Three position stage

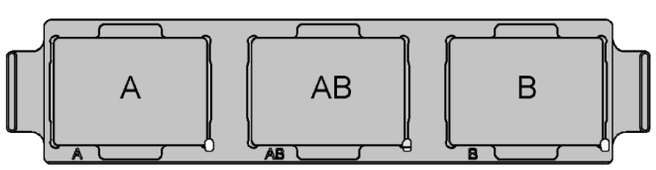

Figure 1: VIAFLO 96/384 with a three position stage

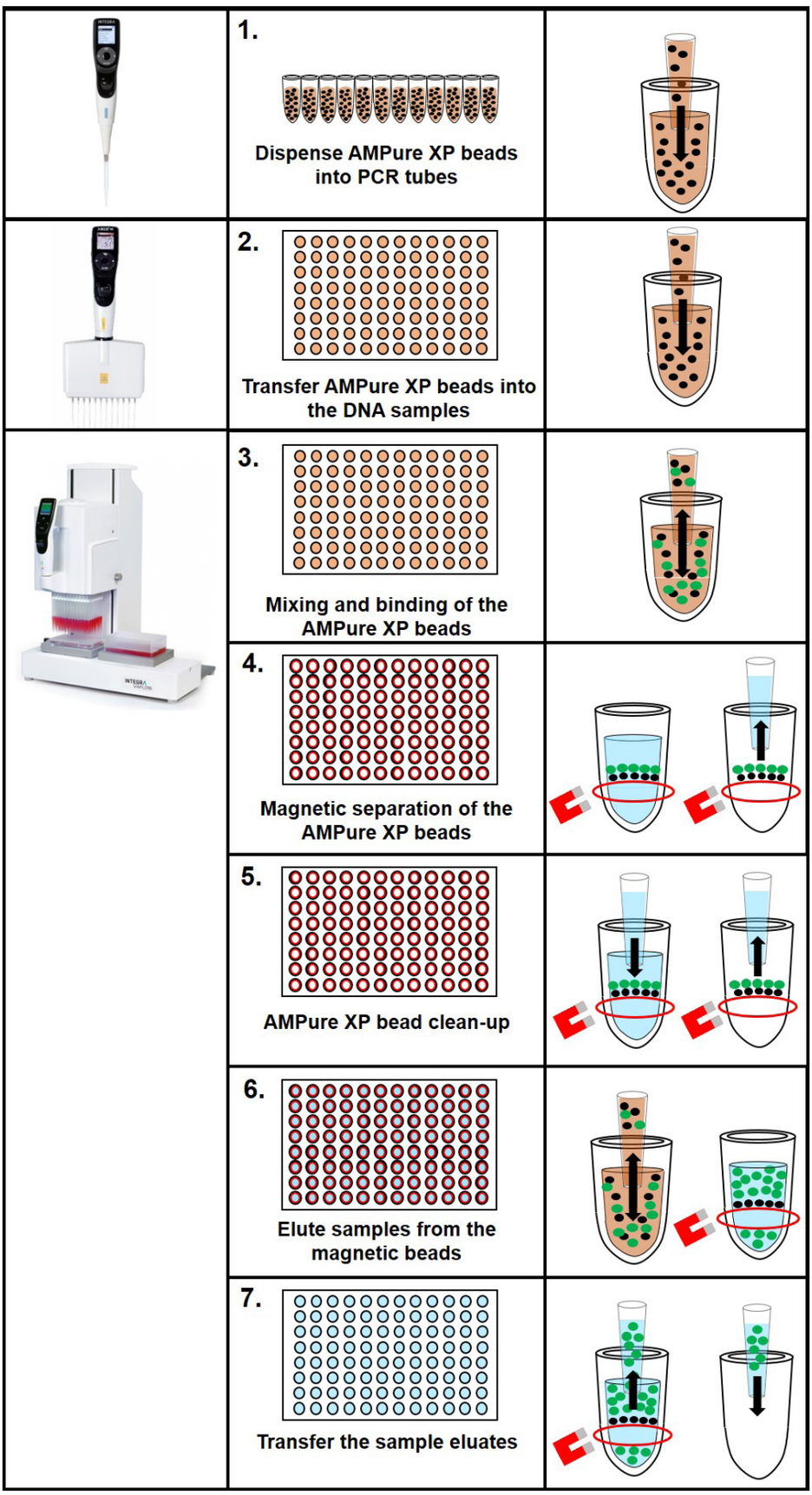

Figure 2: Schematic drawing of the AMPure XP magnetic bead purification procedure

1. Dispense AMPure XP beads into PCR tubes	**STEP:** Transfer AMPure XP beads from the stock solution into 12 PCR tubes placed in a cooling block from INTEGRA. **Note:** The cooling block is just used as a support in this instance, not for cooling down the samples.	**HOW TO:** To ensure a homogenous stock solution, beads are thoroughly mixed by shaking/inverting until the solution appears consistent in color. The beads are transferred into 12 PCR tubes using the Repeat Dispense mode of a VIAFLO single channel 1250 µl electronic pipette. A customized VIALINK program (*AMP_Transfer1*) is available to aid bead transfer. **Tip:** For optimal pipetting, ensure beads are thoroughly mixed before each transfer. Mixing steps can be defined by the number of cycles and the pipetting speed. Both influence the efficiency of mixing and thus the quality of the clean-up. Saving these parameters in the pipetting program ensures that mixing is always carried out as defined, yielding consistent results. Insert a pre- and post-dispense step to enhance accuracy and precision while pipetting precious reagents, such as AMPure XP beads. The use of Sterile, Filter, Low Retention GripTips ensures that every dispense is as accurate as possible, with no loss of beads or sample.
2. Transfer AMPure XP beads into the DNA samples	**STEP:** Transfer AMPure XP beads from the PCR tubes into a 96 well plate preloaded with DNA samples.	**HOW TO:** Pipette the beads from the PCR tubes into the 96 well plate using a VIAFLO 12 channel 50 µl electronic pipette. For optimal pipetting, make sure the tips are exchanged, and mix the beads thoroughly before each transfer. A customized VIALINK program (*AMP_Transfer2*) is provided for this step. **Tip:** Use Low Retention GripTips to minimize loss of beads adhering to the tip wall.
3. Mixing and binding of the AMPure XP beads	**STEP:** Mixing and binding of the magnetic beads to the PCR samples.	**HOW TO:** Load GripTips (**position A**) then select and run the *AMPure_XP_M* program on the VIAFLO 96. The samples are now mixed 10 times by pipetting up and down on **position B**. A five minute wait time follows, timed by the VIAFLO 96, to allow the DNA to bind to the beads. **Tip**: Use the z-height setting of the VIAFLO 96 to define the optimal tip immersion depth. This prevents air entering the tip during mixing and avoids the pipette tip touching the bottom of the plate. Setting the Tip Align support strength to 3 for **positions A** and **B** makes it more comfortable to use the VIAFLO 96. These settings can be incorporated into the program so that they are not forgotten.

4. Magnetic separation of the AMPure XP beads	**STEP:** Separating the magnetic beads from the PCR samples. **Note:** Make sure new GripTips are loaded before continuing the protocol to ensure removal of the supernatant without bead carryover.	**HOW TO:** A prompt on the pipette screen reminds the user to move the sample plate from **position AB** onto the 96 well magnet (**position B**) and place an automation friendly reagent reservoir for waste collection on **position AB**. After a two minute incubation time the beads form a ring-shaped structure and the solution becomes clear. Load new GripTips before continuing the procedure to ensure accurate removal of the supernatant without bead carryover. Follow the instructions on the pipette and aspirate the supernatant slowly from the sample, dispensing it into the waste reagent reservoir (**position AB**). **Tip:** To avoid disturbing the ring of beads the supernatant is aspirated slowly at speed 1. Leave 5 µl of supernatant in the plate to prevent beads being drawn out during aspiration. The z-height limit is again used to ensure that the beads are not disturbed during pipetting.
5. AMPure XP bead clean-up	**STEP:** Wash the magnetic beads twice with 70 % ethanol.	**HOW TO:** Place an automation friendly reagent reservoir containing 70 % ethanol on **position A** and change the GripTips before continuing with the wash step. Follow the prompts on the pipette. Pre-wet the GripTips with 70 % ethanol. Then wash the samples with 70 % ethanol. Repeat the washing step again as indicated by the pipette. **Tip:** Pre-wetting the GripTips with 70 % ethanol ensures equilibration of the humidity and the temperature between the air in the pipette/tips and the sample/liquid. In-house testing has shown that Low Retention GripTips prevent ethanol from dripping while traveling from one pipetting position to another.
6. Elute samples from the magnetic beads	**STEP:** Elute the purified samples from the magnetic beads by adding the elution buffer.	**HOW TO:** As indicated by the pipette, replace the 70 % ethanol reagent reservoir on **position A** with an elution buffer reagent reservoir and move the sample plate from the magnet (**position B**) to **position AB**. Load new GripTips before continuing with the protocol. After transferring and thoroughly mixing the elution buffer with the beads, the pipette prompts the user to place the sample plate back onto the magnet (**position B**). During the one minute incubation time, place a new 96 well plate on **position AB**.
7. Transfer the sample eluates	**STEP:** Transfer the sample eluates into the new 96 well plate. **Note:** Load new GripTips to ensure a clean eluate transfer without bead carryover.	**HOW TO:** Continue with the same program, slowly and carefully transferring the eluates from **position B** into the new plate (**position AB)**. **Tip:** Optimizing pipette settings (aspiration speed, volume and height) allows the volume of the transferred eluate to be maximized without carryover of beads. These settings can be easily tweaked at any time. Performing a test run with water before implementing any new assay is an ideal way to optimize pipette settings.

Remarks

Automatic mode: The VIAFLO 96 can also operate on its own, enabling less user interaction which in turn improves ergonomics and reproducibility. This is also making is even more ideal for use in tight spaces, such as under a laminar flow cabinet.

Partial tip load: If you are not working with a full set of 96 samples, the VIAFLO 96 is able to work with any number of tips loaded, allowing purification of smaller numbers of samples.

Conclusion

- The VIAFLO 96 is perfectly suited to magnetic bead purification in a 96 well format. An entire plate with 96 samples can be purified in a fraction of the time it would take with a traditional pipette.
- Optimized tip immersion and pipette settings in combination with the use of Low Retention GripTips allow maximum sample recovery at the end of the purification protocol.
- The VIAFLO 96 can guide the user through the entire protocol step by step, ensuring the correct workflow and enhancing the reproducibility of results.
- The optional automatic mode of the VIAFLO 96 enables the instrument to operate on its own to minimize pipetting errors, making it even more ideal for use under a laminar flow cabinet.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6001/6031	VIAFLO 96 or 384 handheld electronic pipette (base unit)	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#downloads
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6102	96 channel pipetting head 125 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	4014	VIAFLO single channel 1250 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#downloads
INTEGRA Biosciences	4636	VIAFLO 12 channel 50 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo#parts-and-numbers
INTEGRA Biosciences	6250	PCR 96 well cooling block	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	6307, 6305, 6327	300 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs#parts-and-numbers

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
ThermoFisher Scientific	AB-2396	Armadillo high performance 96-well PCR plate, Thermo Scientific	https://www.thermofisher.com/order/catalog/product/AB2396#/AB2396
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/corningThermowellGOLDAndThermowell8WellPCRTubeStrips

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