Application Note

INTEGRA

Reliable time-to-result with a high throughput PCR purification protocol using magnetic beads

Introduction

Polymerase chain reaction (PCR) is a fundamental molecular biology technique used to amplify specific DNA sequences or fragments exponentially. PCR purification with magnetic beads is a streamlined product purification approach to selectively bind fragments while removing primer dimers and other unwanted components from the PCR reaction. The PCR purification process enables the isolation of high quality DNA fragments, free from contaminants, enabling reliable downstream applications such as sequencing, cloning, genotyping and restriction enzyme digestion.

The PCR purification protocol for INTEGRA's handheld electronic pipettes – the VIAFLO 96 and VIAFLO 384 – simultaneously processes 96 samples, including automated magnetic bead handling on the MAG module for magnetic separation. This application note demonstrates an efficient PCR purification workflow with the 10-300 µl 96 channel pipetting head and 3-position stage, using a 1.8x ratio of MAGFLO™ PCR magnetic beads for PCR purification on a 100 bp DNA ladder. The results show 100 % recovery of fragments larger than 100 bp, and removal of >50 % of 100 bp and 100 % of 50 bp fragments.

Key benefits:

- High throughput PCR product purification using INTEGRA's PCR purification protocol for the MAG module on a VIAFLO 96 or VIAFLO 384 equipped with a 10-300 µl 96 channel pipetting head.
- Interchangeable pipetting heads for the VIAFLO 96 or VIAFLO 384 enable the PCR purification protocol to be performed either with the 10-300 µl or the 5-125 µl 96 channel pipetting head.
- MAGFLO PCR beads guarantee the high recovery of the targeted fragments and effectively remove fragments below 100 bp for downstream applications such as sequencing.
- Magnetic beads are captured and released without transferring the plate. The MAG module's automated vertical magnet movement minimizes the risk of liquid spillage.

Overview: How to perform PCR purification with MAGFLO PCR beads using the MAG module on the VIAFLO 96 or VIAFLO 384

VIAFLO 384

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This application note showcases the protocol for PCR purification of 40 µl of 30-fold diluted DNA ladder (Promega) with a 1.8x ratio of MAGFLO PCR beads, using the MAG module on the VIAFLO 96 or VIAFLO 384.

Experimental set-up:

Equip the VIAFLO 96 or VIAFLO 384 with the 10-300 µl 96 channel pipetting head and the 3-position stage (Positions A, A/B and B).

Using the 3-position stage:

Position A: Empty, or 300 µl sterile, filter, low retention GRIPTIPS® pipette tips
Position A/B: Labware exchange zone
A/B-A: Stage moved to the left, A/B on device Position A
A/B-B: Stage moved to the right, A/B on device Position B
Position B: MAG module, 96 well PCR adapter, processing plate (Bio-Rad Hard-Shell® 96 well PCR plate)

Step-by-step procedure:

The PCR purification protocol consists of 3 main steps – binding, washing and elution – shown in **Figure 1**, with a preliminary transfer of MAGFLO PCR beads to the processing plate.

Tips:
- Performing the preliminary transfer from a single deep well plate (DWP) column with partial tip loading reduces the overall dead volume of MAGFLO PCR beads.
- The MAGFLO PCR beads can be transferred directly from a multichannel reservoir with any INTEGRA electronic pipette. The repeat dispense function speeds up the process.

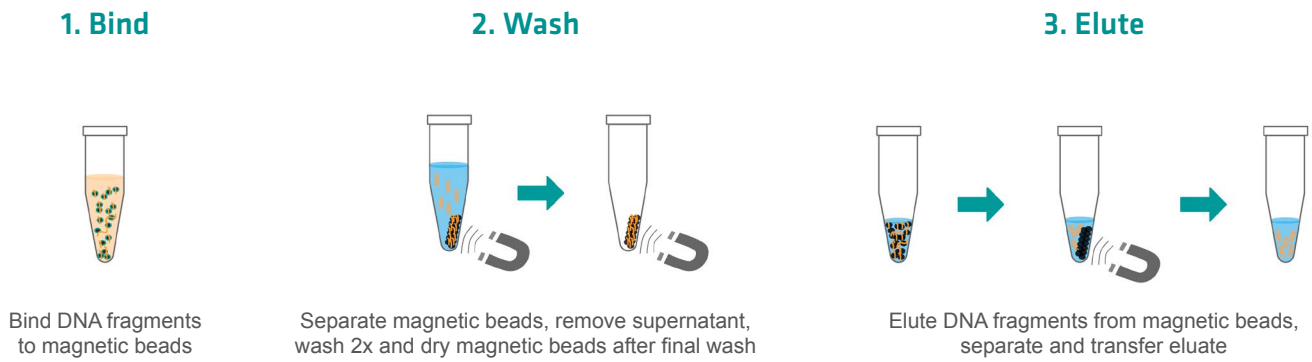

Figure 1: Step-by-step procedure of the MAGFLO PCR purification protocol.

The following customized VIALINK programs are provided:

Program 1: Transfer
Program 2: Bind
Program 3: Wash
Program 4: Elute

1. MAG Control

STEP: Define the magnet heights for low (Pos. Low) and high (Pos. High) positions.

HOW TO: Open the MAG Control software or MAG Control app and connect to a MAG module. Set a 24 mm magnet height for Pos. Low, 29 mm for Pos. High, and transfer the settings to the device (**Figure 2**).

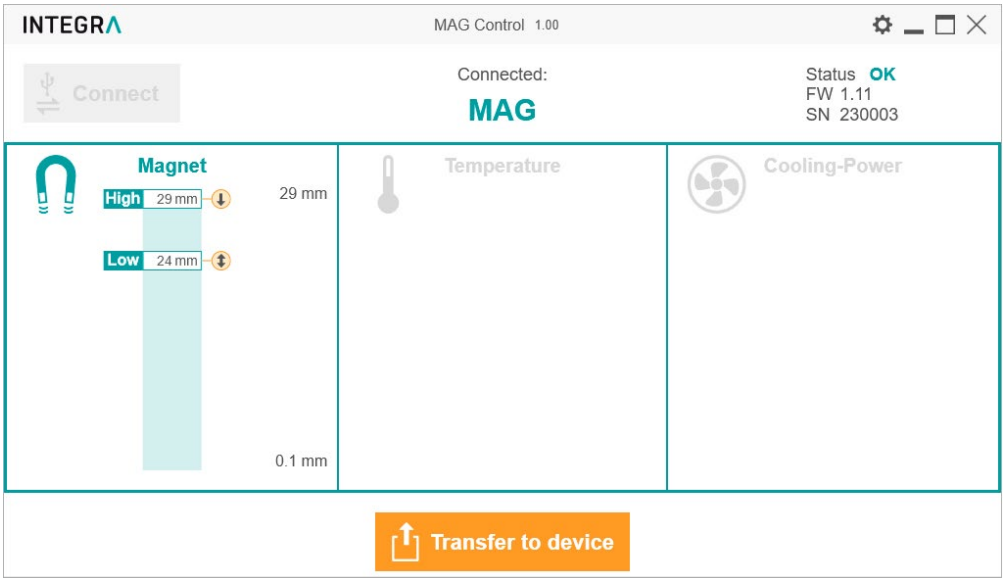

Figure 2: Using the MAG Control software to change the magnet height settings.

2. Transfer

STEP: Transfer magnetic beads to the processing plate.

HOW TO: Prepare an INTEGRA DWP containing at least 900 µl of MAGFLO PCR beads in every well of column 12 (**Figure 3**, red). Slide the 3-position stage to the right so that stage Position A/B is on device Position B (A/B-B). Select and run the program '300-TRANSFER-M'. The pipette will indicate stage Set-up 1 shown in **Figure 3**. Place a box of 300 µl low retention, sterile, filter GRIPTIPS on Position A/B-B, and a MAG module with a 96 well PCR plate adapter and empty processing plate on Position B (**Figure 3**). The magnet will be at Position Home by default (Pos. Home, 0 mm, disengaged). Perform a partial tip loading with column 12 of the pipetting head in column 1 of the GRIPTIPS box on stage Position A/B-B (**Figure 3**, bold). Afterwards, the VIAFLO 96 or VIAFLO 384 will prompt the user to move the stage to the left (Position A/B-A) and indicate Set-up 2. Exchange the GRIPTIPS box on Position A/B-A with the INTEGRA DWP containing magnetic beads in column 12 (**Figure 3**, red) and lower the pipetting head. The pipette will mix the MAGFLO PCR beads 10 times at Speed 5, then aspirate 308 µl (extended volume setting), including 10 µl pre- and post-dispense (**Figure 4a**). Elevate the pipetting head slowly from the liquid, perform the pre-dispense and a tip touch into the liquid. The pipette will instruct the user to dispense magnetic beads into the first 4 columns on Position B. Move the pipetting head to Position B and perform 4x72 µl dispenses of magnetic beads into columns 1 to 4 (**Figure 4b**). Perform a liquid tip touch to ensure accurate liquid transfer after each dispense. Repeat the procedure twice to fill columns 5 to 8 and 9 to 12. After the initial mixing of magnetic beads, the mixing steps that follow are reduced to 5 cycles. Transfer the final post-dispense back into the INTEGRA DWP source wells when prompted by the pipette, and discard the used GRIPTIPS. Remove the INTEGRA DWP and continue with the program '300-BIND-M'.

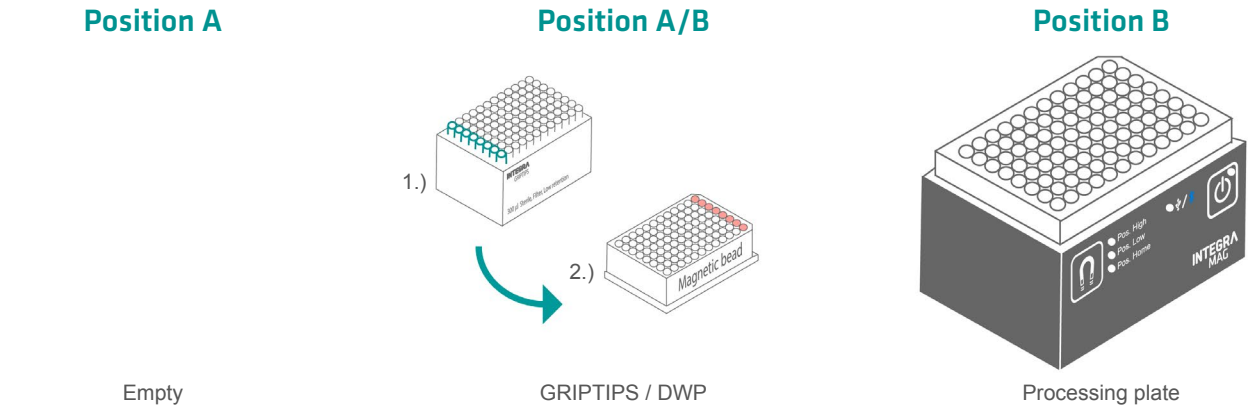

Figure 3: Set-up for the VIAFLO 96 or VIAFLO 384 3-position stage during magnetic bead transfer with partial tip loading. **Position A:** empty. **Position A/B:** Set-up 1 – 300 µl low retention, sterile, filter GRIPTIPS (partial tip loading, green); Set-up 2 – INTEGRA DWP containing magnetic beads (column 12, red). **Position B:** The MAG module with 96 well PCR plate adapter and empty Bio-Rad Hard-Shell 96 well PCR plate (processing plate).

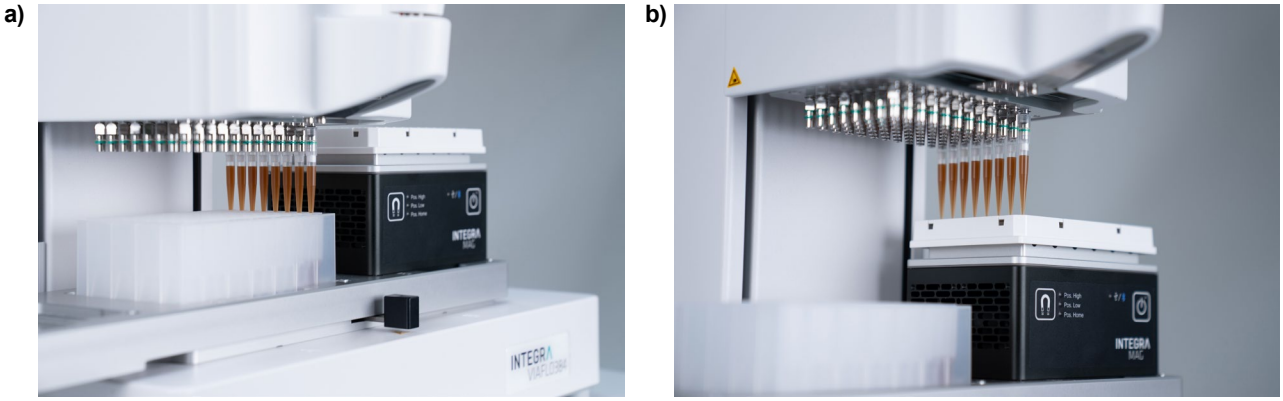

Figure 4: The VIAFLO 384 with partial tip loading transferring MAGFLO PCR beads from (a) column 12 of the INTEGRA DWP on Position A/B-A to (b) column 1 of the processing plate on the MAG module (Position B).

3. Bind	**STEP:** Transfer samples from the sample plate to the processing plate and let them bind to magnetic beads.	**HOW TO:** The processing plate containing the MAGFLO PCR beads stays on the MAG module (Position B). Stage Position A is dedicated to the GRIPTIPS box, meaning that the stage must be slid to the right (Position A/B-B) when starting the protocol to load GRIPTIPS from Position A. Select and run the program '300-BIND-M'. The VIAFLO 96 or VIAFLO 384 will indicate the set-up shown in **Figure 5**. Place a full GRIPTIPS box on Position A and the 96 well PCR sample plate containing more than 40 µl of PCR product per well on Position A/B-B (**Figure 5**, blue). The magnet will be at Pos. Home and the pipette will request that GRIPTIPS are loaded on Position A, followed by the aspiration of PCR products on Position A/B-B. After tip loading, move the head to Position A/B-B and lower it slowly into the wells of the sample plate (**Figure 5**, blue).

The pipette will then aspirate 40 µl of PCR products (**Figure 6a**). Perform a liquid tip touch, elevate the pipetting head, and move the stage to the left (Position A/B-A). Lower the pipetting head slowly into the wells of the processing plate containing magnetic beads on the MAG module (Position B). Dispense the PCR product and mix 15 times at Speed 5 (**Figure 6b**). After mixing, remove the GRIPTIPS slowly from the liquid and perform a liquid tip touch. The pipette will then initialize a 5-minute incubation (binding). Discard the used GRIPTIPS and remove the tip box and sample plate from the 3-position stage. Continue with the program '300-WASH-M'.

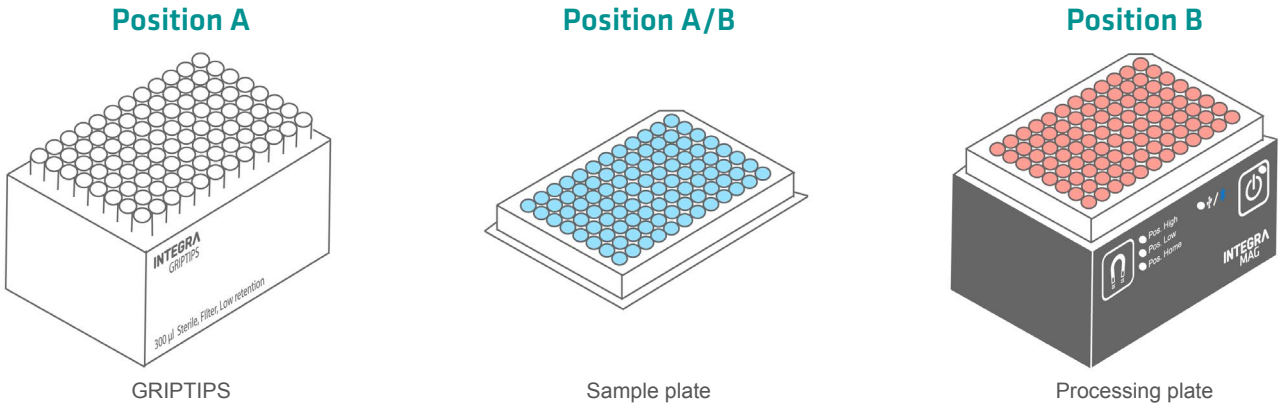

Figure 5: Set-up for the VIAFLO 96 or VIAFLO 384 3-position stage during sample transfer and binding. **Position A:** 300 µl low retention, sterile, filter GRIPTIPS. **Position A/B:** Bio-Rad Hard-Shell PCR plate containing PCR products (sample plate, blue). **Position B:** MAG module with 96 well PCR plate adapter and Bio-Rad Hard-Shell 96 well PCR plate containing magnetic beads (processing plate, red).

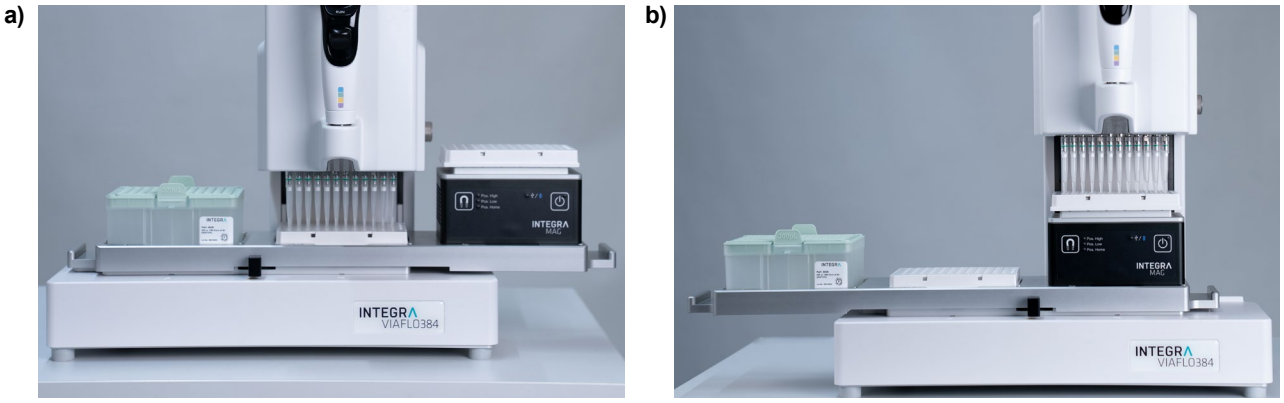

Figure 6: The VIAFLO 384 simultaneously transferring 96 PCR products from (a) the sample plate on Position A/B-B to (b) the processing plate on the MAG module (Position B).

4. Wash

STEP: Remove supernatant and wash magnetic beads twice with 70 % ethanol.

HOW TO: Start with the 3-position stage slid to the right (Position A/B-B). Select and run the program '300-WASH-M'. The pipette will indicate stage Set-up 1 (**Figure 7**). Place a full GRIPTIPS box on Position A and an empty automation friendly reservoir on Position A/B-B. (**Figure 7**). When prompted by the pipette, push the magnet button on the MAG module twice to select Pos. High. The VIAFLO 96 or VIAFLO 384 will initialize an 8-minute incubation to capture the MAGFLO PCR beads. Afterwards, the pipette will request that GRIPTIPS are loaded at Position A, followed by moving the stage to the left (Position A/B-A) to aspirate the supernatant from the processing plate on the MAG module (Position B). Lower the pipetting head slowly into the wells of the processing plate. The pipette will aspirate 112 µl of supernatant at Speed 1 without disturbing the pellet. Move the GRIPTIPS out of the wells and purge the supernatant in the waste reservoir on Position A/B-A. Perform a tip touch to remove droplets from the GRIPTIPS, then lift the pipetting head.

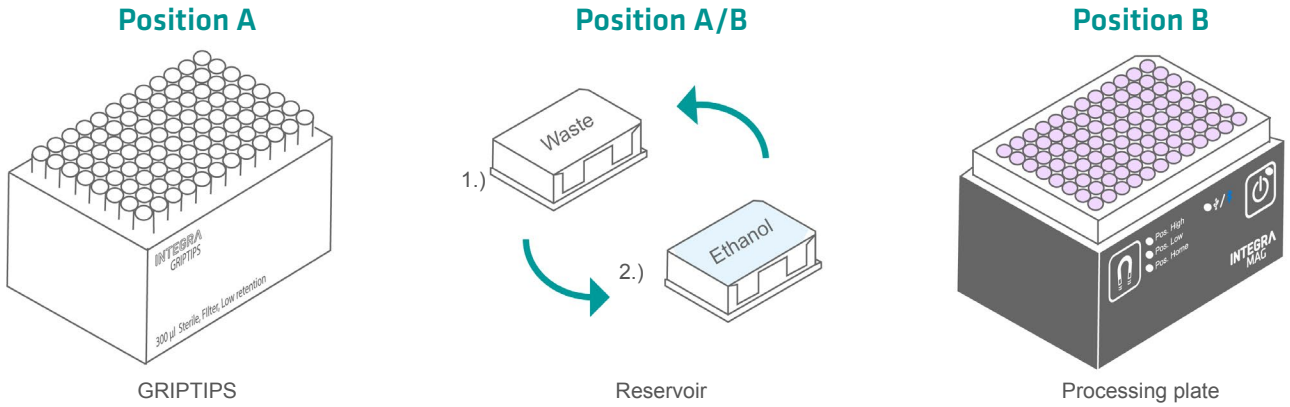

Figure 7: Set-up of the VIAFLO 96 or VIAFLO 384 3-position stage during washing. **Position A:** 300 µl low retention, sterile, filter GRIPTIPS. **Position A/B:** Set-up 1 – 300 ml automation friendly reservoir for waste; Set-up 2 – 300 ml automation friendly reservoir with 70 % ethanol (blue). **Position B:** MAG module with 96 well PCR plate adapter and Bio-Rad Hard-Shell 96 well PCR plate containing PCR products bound to magnetic beads (processing plate, lilac).

The VIAFLO 96 or VIAFLO 384 will show Set-up 2 (**Figure 7**). Exchange the waste reservoir with a 300 ml automation friendly reservoir containing fresh 70 % ethanol on Position A/B-B and slide the stage to the right. The pipette will request GRIPTIPS to be exchanged on Position A, followed by aspirating ethanol from Position A/B-B. Move the pipetting head with fresh GRIPTIPS to Position A/B-B and lower them into the ethanol. Perform a pre-wetting step and aspirate 125 µl (**Figure 8a**). Elevate the pipetting head, move the deck to the left (Position A/B-A), and lower the head into the wells of the processing plate on the MAG module (Position B) to dispense the ethanol when prompted (**Figure 8b**).

Perform a liquid tip touch to remove residual droplets. Slightly elevate the pipetting head when prompted to remove the GRIPTIPS from the liquid. The pipette will indicate Set-up 1 (**Figure 7**) to exchange the ethanol reservoir on Position A/B-A with the waste reservoir. The pipetting head should remain in position while incubating for 1 minute.

After incubation, the VIAFLO 96 or VIAFLO 384 will inform the operator to aspirate the ethanol again from Position B. Slowly lower the GRIPTIPS back into the wells of the processing plate and aspirate the supernatant at Speed 1. When requested, move the pipetting head to Position A/B-A and purge the supernatant into the waste reservoir using a liquid tip touch, if necessary.

The pipette will request the same procedure for the second wash with new GRIPTIPS. After purging the supernatant from the second wash into the waste, the pipette will request another exchange of GRIPTIPS for final liquid removal from the wells of the processing plate on the MAG module (Position B). Set the magnet to Pos. Low and air dry the MAGFLO PCR beads for 5 minutes when prompted. Afterwards, remove the GRIPTIPS and waste reservoir from the deck, and continue with the program '300-ELUTE-M'.

Tips:

- Pre-wetting the GRIPTIPS prevents dripping when working with volatile liquids, and a slow dispensing speed with a liquid tip touch removes any remaining droplets after dispensing.
- Ease elution by using the Pos. Low function of the MAG module to dry magnetic beads closer to the well bottom.
- The drying condition was optimized to 5 minutes but may vary for different lab conditions.

a) b)

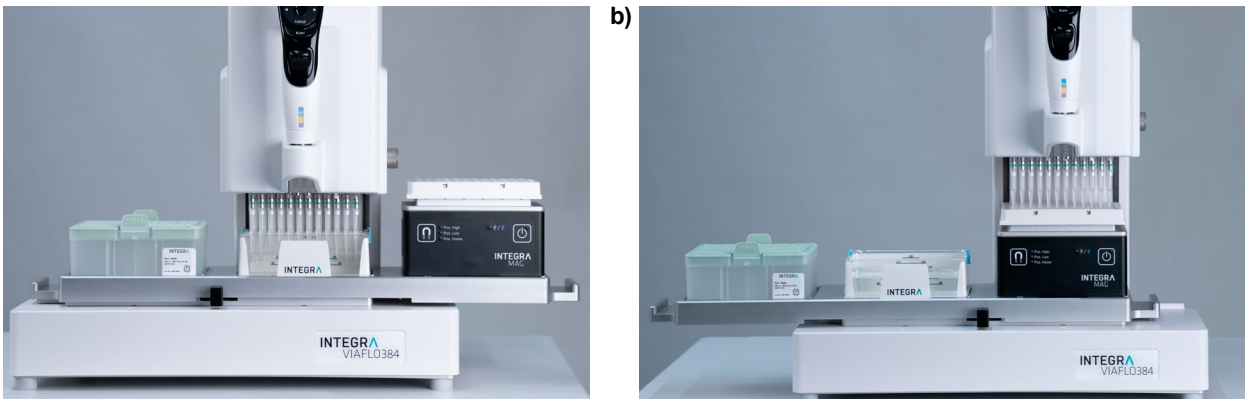

Figure 8: The VIAFLO 384 transferring 70 % ethanol from (a) the 300 ml automation friendly reagent reservoir on Position A/B-B to (b) the processing plate on the MAG module (Position B).

5. Elute

STEP: Elute samples from the magnetic beads and transfer them to the elution plate.

HOW TO: Select and run the program '300-ELUTE-M' with the 3-position stage slid to the right (Position A/B-B). The pipette will indicate Set-up 1, as shown in **Figure 9**. Place a full GRIPTIPS box on Position A and a 300 ml automation friendly reservoir with molecular-grade water on Position A/B-B (**Figure 9**). Disengage the MAG module by selecting Pos. Home when requested, then load new GRIPTIPS on Position A. Like the ethanol transfer, move the pipetting head to the reservoir containing molecular-grade water on Position A/B-B. When requested, aspirate 40 µl, elevate the pipetting head, move the stage to the left (Position A/B-A), and dispense into the wells of the processing plate. The pipette will mix 25 times at Speed 5 to resuspend the pellets and indicate deck Set-up 2 with the elution plate (any 96 well PCR plate) on Position A/B-A, followed by a 5-minute incubation. Engage the magnet by selecting Pos. High when requested, then wait 8 minutes until the magnetic beads are captured. The VIAFLO 96 or VIAFLO 384 will inform the user to slide the stage to the right (Position A/B-B) to exchange the GRIPTIPS and then slide the stage back to the left (Position A/B-A) to aspirate from Position B. Slowly lower the pipetting head into the wells of the processing plate without disturbing the pellet and aspirate 35 µl of eluate at Speed 1. Move the pipetting head to Position A/B-A and dispense the purified PCR product into the wells of the new 96 well PCR elution plate. If necessary, perform a liquid tip touch and discard the used GRIPTIPS on Position A. Seal and store the elution plate, or continue with downstream applications. Finally, remove the GRIPTIPS and the processing plate.

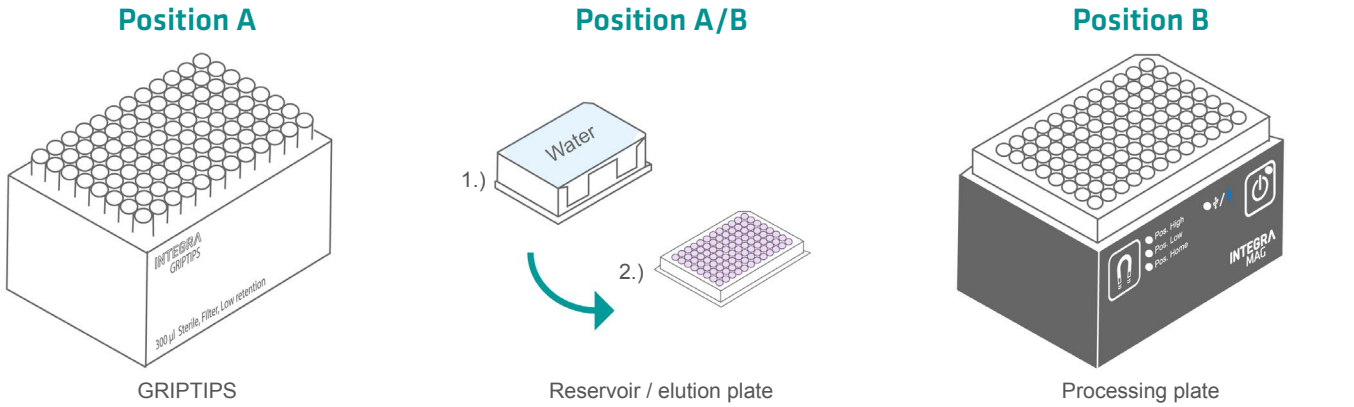

Figure 9: Set-up of the VIAFLO 96 or VIAFLO 384 3-position stage during elution. **Position A:** 300 µl low retention, sterile, filter GRIPTIPS. **Position A/B:** Set-up 1 – 300 ml automation friendly reservoir for molecular-grade water (blue); Set-up 2 – elution plate (lilac). **Position B:** MAG module with 96 well PCR plate adapter and Bio-Rad Hard-Shell 96 well PCR plate containing PCR product bound to magnetic beads (processing plate).

Methods and results

PCR purification with magnetic beads plays a significant role in downstream applications of molecular biology such as sequencing. The results below demonstrate high throughput PCR product purification on the VIAFLO 96 or VIAFLO 384 using INTEGRA's MAGFLO PCR beads and the MAG module for automated magnetic bead handling.

40 µl of 30-fold diluted 100 bp DNA ladder (Promega) was purified using the 4 custom programs of the MAGFLO PCR purification protocol for the VIAFLO 384 equipped with a 300 µl 96 channel pipetting head. Afterwards, 14 out of 96 wells were analyzed on an Agilent 4150 TapeStation System (**Figure 10**). The PCR purification workflow was repeated in 3 individual runs (n=3, full data can be found in the appendix).

The MAGFLO PCR purification protocol removes all fragments smaller than 100 bp and >50 % of 100 bp fragments, while fully recovering all fragments larger than 100 bp (**Figure 10**).

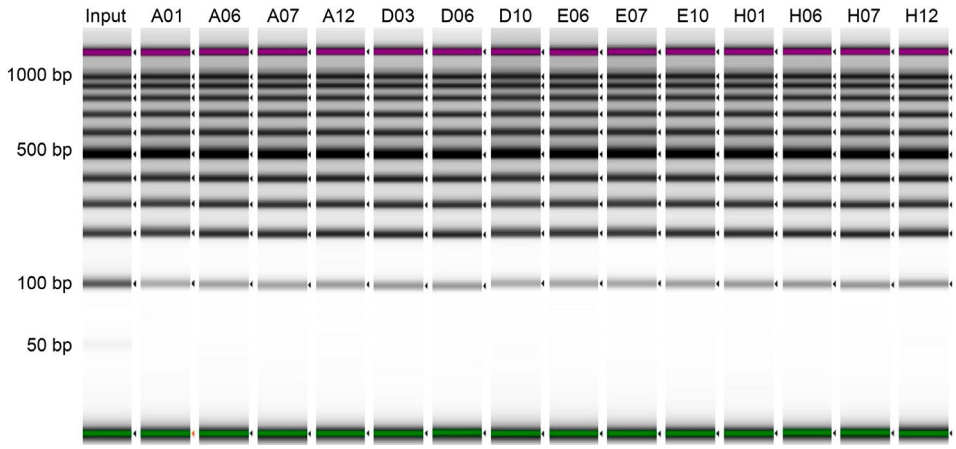

Figure 10: Efficient PCR purification using MAGFLO PCR beads. Results of fragment analysis using a 4150 TapeStation showing a gel picture with a 30-fold diluted 100 bp PCR ladder before (input) and after PCR purification of 14 wells from 1 individual run using a 1.8x ratio.

Remarks

- **Automatic mode:** The automatic workflow differs slightly from the manual workflow. It is designed to reduce hands-on time for the operator, including stage movement directly after loading or exchanging GRIPTIPS, directly starting the incubation timer after pipetting without a prompt message, and loading GRIPTIPS before selecting the magnet position.

- **5-125 µl 96 channel head:** The operator can load GRIPTIPS freely from full or pre-used boxes, and will be informed when the stage slider must be set back into the neutral (middle) position.

Conclusion

- The PCR purification workflow using the VIAFLO 96 or VIAFLO 384 with the MAG module and MAGFLO PCR beads recovered 100 % of >100 bp fragments, and removed >50 % of 100 bp and 100 % of <100 bp fragments.
- The PCR purification protocol provided can be used with 5-125 µl or 10-300 µl 96 channel pipetting heads in both manual and automatic modes, providing flexibility without influencing the results.
- Fast and efficient high throughput PCR product purification with the VIAFLO 96 or VIAFLO 384 and MAG module reduces the time-to-result by simultaneously processing 96 samples with identical liquid handling parameters.
- The protocol can be adapted in VIALINK to increase throughput and downscale the PCR purification process for the VIAFLO 384 0.5-12.5 µl 384 channel pipetting head.
- The MAG module's DWP adapter enables high volume PCR purification when scaling up to the 50-1250 µl 96 channel head.

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6031	VIAFLO 384 24, 96 and 384 channel handheld electronic pipettes	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6103	96 channel pipetting head 10-300 µl	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6230	3-position stage for 96 and 384 well plates	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	4900	MAG module for magnetic separation	https://www.integra-biosciences.com/en/modules/mag-and-heatmag
INTEGRA Biosciences	4906	Adapter for 96 well PCR plates	https://www.integra-biosciences.com/en/modules/mag-and-heatmag
INTEGRA Biosciences	6535	300 µl low retention, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6348	300 ml polypropylene reservoir	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	6305	300 ml reservoir base	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	6353	INTEGRA DWP	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	7010 7012 7014	MAGFLO PCR magnetic beads for PCR purification	https://www.integra-biosciences.com/en/ngspcr-purification/magflotm-pcr
Bio-Rad	HSP9601	Low profile 96-well PCR plates	https://www.bio-rad.com/en-ch/product/low-profile-96-well-pcr-plates?ID=OC0OBU4VY
Promega	G2101	100 bp DNA ladder	https://ch.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101

Contact us: