

# 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 our handheld electronic pipettes – VIAFLO 96 and VIAFLO 384 – simultaneously processes 96 samples, including automated magnetic bead handling on the MAG module for magnetic separation. Here, we demonstrate 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 – our 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 uses our PCR purification protocol for the MAG module on VIAFLO 96 or VIAFLO 384 handheld electronic pipettes equipped with a 10-300 µl 96 channel pipetting head.
- Interchangeable pipetting heads for VIAFLO 96 or VIAFLO 384 allow performing the PCR purification protocol with the 10-300 µl or 5-125 µl 96 channel pipetting heads.
- MAGFLO PCR – magnetic beads for PCR purification – guarantee the high recovery of the targeted fragments and effectively remove fragments below 100 bp for downstream applications such as sequencing.
- Capture and release magnetic beads without plate transfer using MAG's automated vertical magnetic movement, minimizing the risk of liquid spillage.

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



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

### Experimental set-up:

Equip 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, 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 – bind, wash and elute – shown in **Figure 1**, with a preliminary transfer of MAGFLO PCR 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 precious MAGFLO PCR.
- You can directly transfer MAGFLO PCR with any of our electronic pipettes from a multichannel reservoir. 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

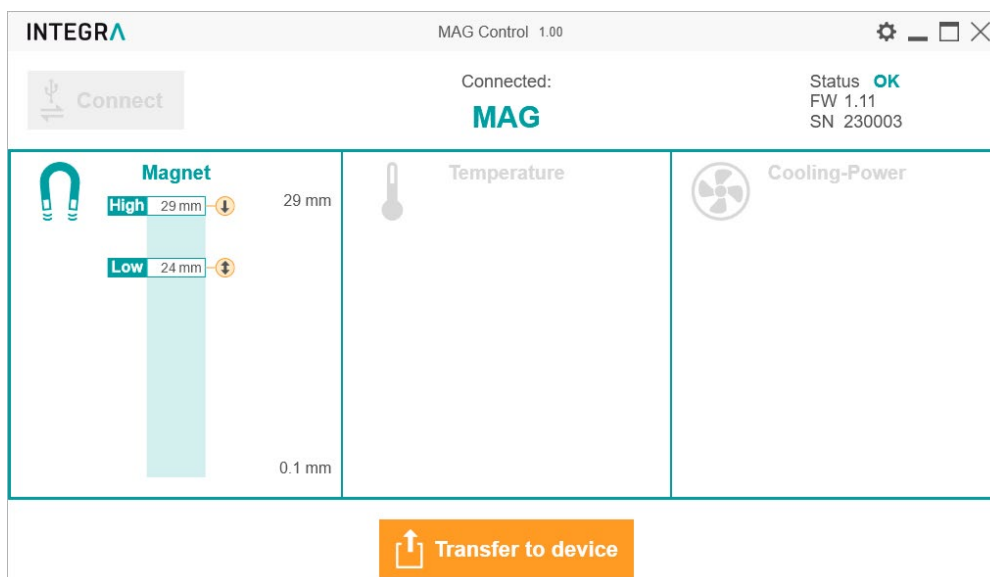
**Program 2:** Bind

**Program 3:** Wash

**Program 4:** Elute

**1. MAG Control** **STEP:** Define magnet heights for positions low (Pos. Low) and high (Pos. High).

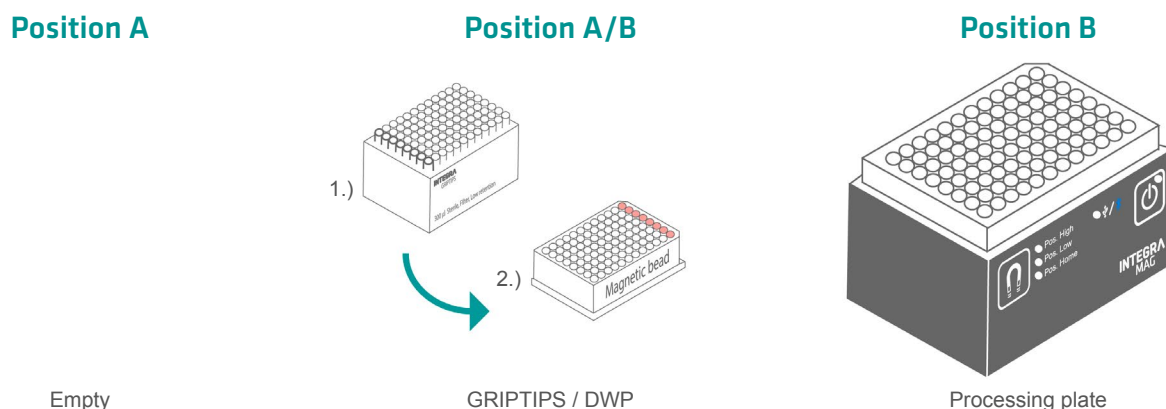
**HOW TO:** Open the MAG Control software or MAG Control app and connect to MAG. Set 24 mm magnet height for Pos. Low, 29 mm for Pos. High and transfer the settings to the device (**Figure 2**).



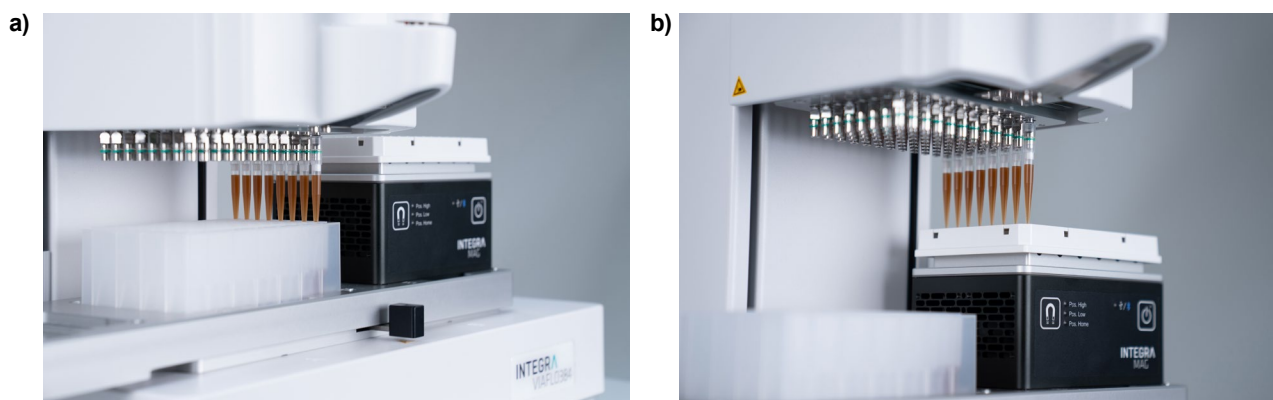
**Figure 2:** Using MAG Control software to change the height settings of the magnet.

**2. Transfer** **STEP:** Transfer magnetic beads to the processing plate.

Prepare the INTEGRA DWP containing at least 900  $\mu$ l MAGFLO PCR 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  $\mu$ l sterile, filter, low retention GRIPTIPS on A/B-B, and MAG with 96 well PCR plate adapter and empty processing plate on 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, VIAFLO 96 or VIAFLO 384 will prompt the user to move the stage to the left (A/B-A) and indicate Set-up 2. Exchange the GRIPTIPS box on 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 MAGFLO PCR 10 times at Speed 5, then aspirate 308  $\mu$ l (extended volume setting), including 10  $\mu$ l 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 4x 72  $\mu$ l 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 following mixing is reduced to 5 cycles. Transfer the final post-dispense back into the 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  $\mu$ l sterile, filter, low retention GRIPTIPS (partial tip loading, bold); Set-up 2 – INTEGRA DWP containing magnetic beads (column 12, red). Position B: MAG module with 96 well PCR plate adapter and empty Bio-Rad Hard-Shell 96 well PCR plate (processing plate).



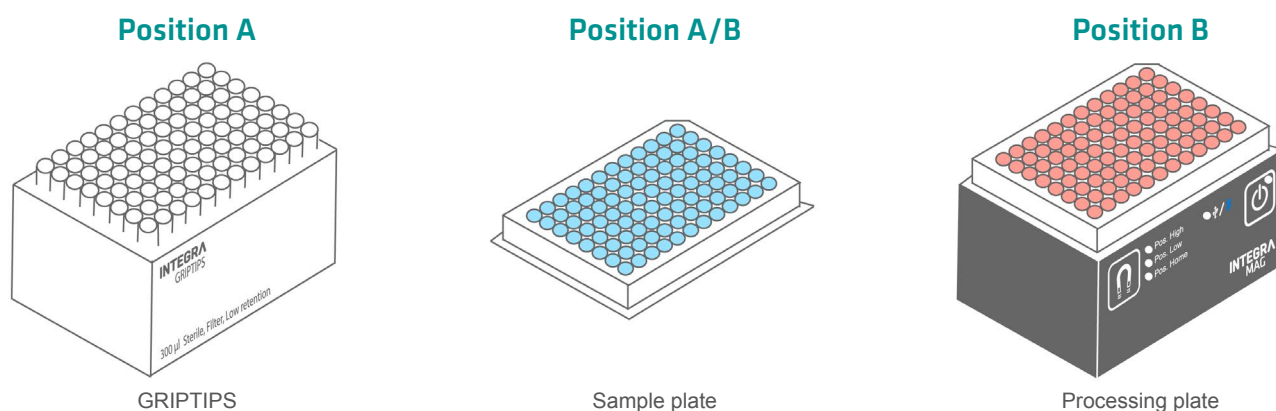
**Figure 4:** VIAFLO 384 with partial tip loading transferring MAGFLO PCR from (a) column 12 of the DWP on position A/B-A to (b) column 1 of the processing plate on MAG position B.

### 3. Bind

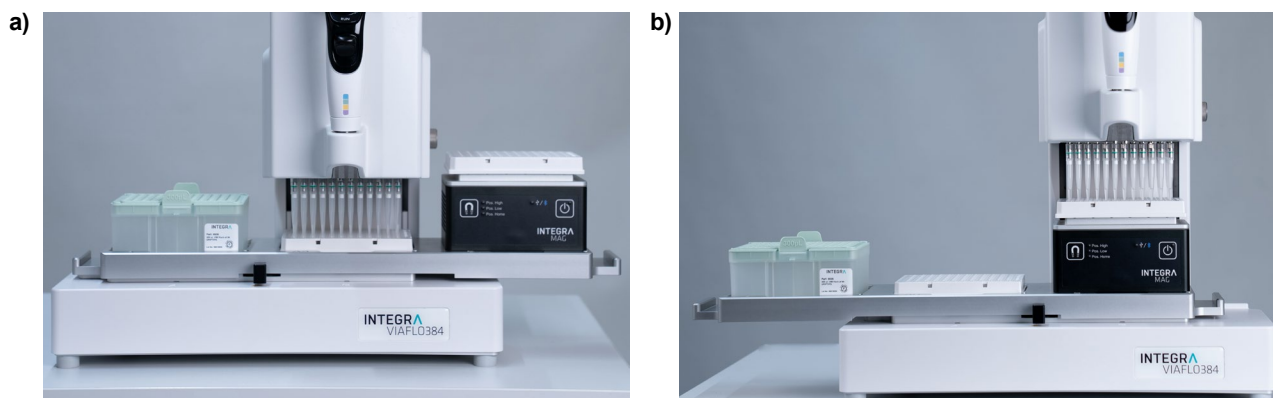
**STEP:** Transfer samples from the sample plate to the processing plate and let them bind to magnetic beads.

**HOW TO:** The processing plate containing MAGFLO PCR stays on MAG (position B). Stage position A will be dedicated to the GRIPTIPS box, meaning that the stage must be slid to the right (A/B-B) when starting the protocol to load GRIPTIPS from position A. Select and run the program '300-BIND-M'. 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  $\mu$ l of PCR product per well on A/B-B (**Figure 5**, blue). The magnet will be at Pos. Home and the pipette will request GRIPTIPS loading on position A, followed by the aspiration of PCR products on A/B-B. After tip loading, move the head to A/B-B and lower it slowly into the wells of the sample plate (**Figure 5**, blue).

The pipette will then aspirate 40  $\mu$ l of PCR products (**Figure 6a**). Perform a liquid tip touch, elevate the pipetting head, and move the stage to the left (A/B-A). Lower the pipetting head slowly into the wells of the processing plate containing magnetic beads on MAG 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  $\mu$ l sterile, filter, low retention 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).

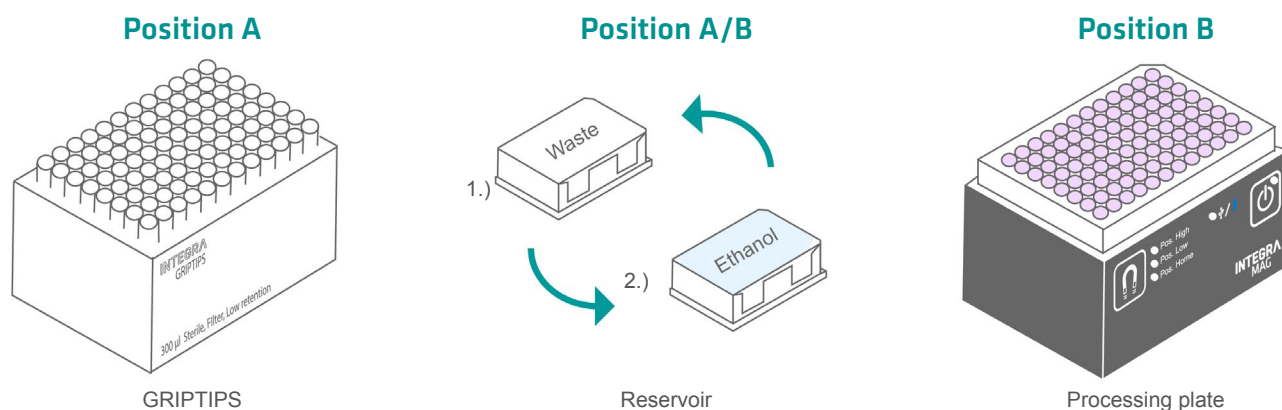


**Figure 6:** VIAFLO 384 simultaneously transferring 96 PCR products from (a) the sample plate on position A/B-B to (b) the processing plate on MAG position B.

## 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 (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 A/B-B (**Figure 7**). When prompted by the pipette, push the magnet button on MAG twice to select Pos. High (29 mm, engaged). VIAFLO 96 or VIAFLO 384 will initialize an 8-minute incubation to capture MAGFLO PCR. Afterwards, the pipette will request GRIPTIPS loading at position A, followed by the moving of the stage to the left (A/B-A) to aspirate the supernatant from the processing plate on MAG position B. Lower the pipetting head slowly into the wells of the processing plate. The pipette will aspirate 112  $\mu$ l 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  $\mu$ l sterile, filter, low retention GRIPTIPS. Position A/B: Set-up 1 – 300 ml of automation-friendly reservoir for waste; Set-up 2 – 300 ml of 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).

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 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  $\mu$ l (**Figure 8a**). Elevate the pipetting head, move the deck to the left (A/B-A), and lower the head into the wells of the processing plate on MAG position B to dispense the ethanol when prompted (**Figure 8b**).

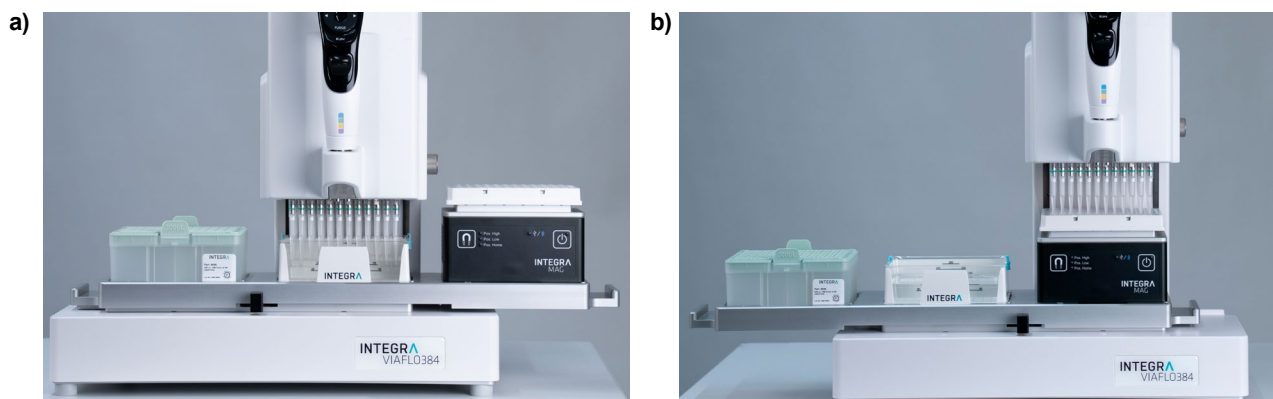
Perform a liquid tip touch to remove residual droplets. Slightly elevate the pipetting head when prompted to remove the GRIPTIPS from the liquid. The pipette will indicate Set-up 1 (**Figure 7**) to exchange the ethanol reservoir on position A/B-A with the waste reservoir. The pipetting head should remain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

After incubation, 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 MAG position B. Set the magnet to Pos. Low (24 mm, engaged) and air dry MAGFLO PCR 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 MAG to dry magnetic beads closer to the well bottom.
- The drying condition was optimized to 5 minutes but may vary for lab conditions.

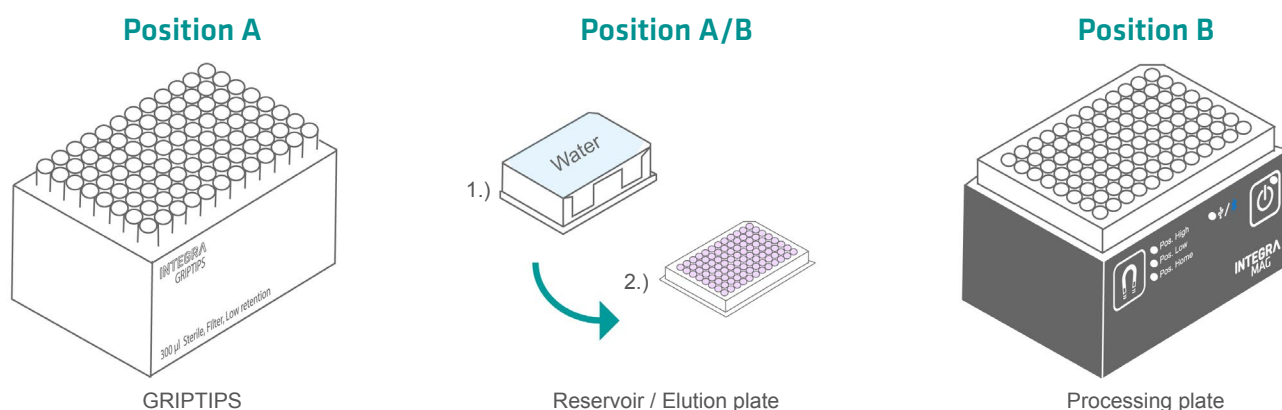


**Figure 8:** VIAFLO 384 transferring 70 % ethanol from (a) the 300 ml automation-friendly reagent reservoir on position A/B-B to (b) the processing plate on MAG position B.

## 5. Elute

**STEP:** Elute samples from 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 (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 A/B-B (**Figure 9**). Disengage MAG 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  $\mu$ l, elevate the pipetting head, move the stage to the left (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 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. VIAFLO 96 or VIAFLO 384 will inform the user to slide the stage to the right (A/B-B) to exchange GRIPTIPS and then slide the stage back to the left (A/B-A) to aspirate from position B. Slowly lower the pipetting head to the wells of the processing plate without disturbing the pellet and aspirate 35  $\mu$ l 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. Next, seal and store the elution plate, or continue with downstream applications. Finally, remove the GRIPTIPS and processing plate.



**Figure 9:** Set-up of the VIAFLO 96 or VIAFLO 384 3-position stage during elution. Position A: 300  $\mu$ l sterile, filter, low retention GRIPTIPS. Position A/B: Set-up 1 – 300 ml automation-friendly reservoir for molecular-grade water; 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).

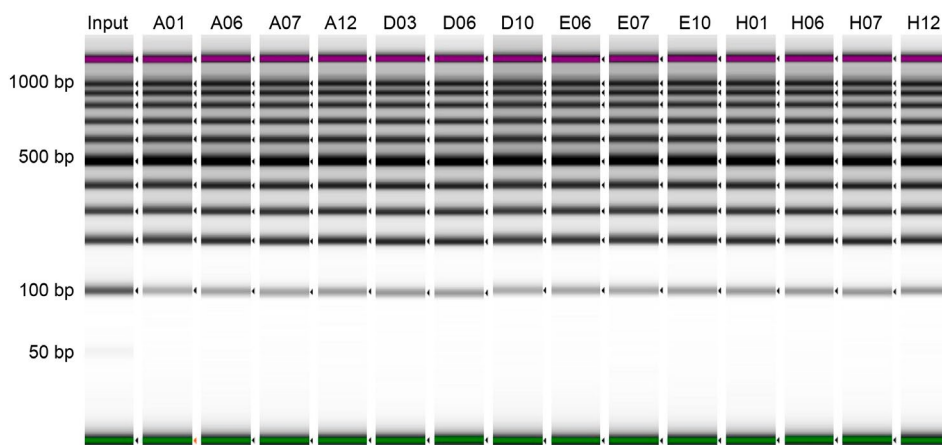


## Results

PCR purification with magnetic beads plays a significant role in downstream applications of molecular biology such as sequencing. Here we demonstrate high throughput PCR product purification on the VIAFLO 96 or VIAFLO 384 handheld electronic pipettes using our magnetic beads for PCR purification – MAGFLO PCR – 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 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, the data of all runs 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. 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 (A) workflow differs slightly from the manual (M) workflow. It is designed to reduce hands-on time for the operator, including stage movement directly after loading or exchanging GRIPTIPS; direct starting of incubation time 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

- Our PCR purification workflow using the VIAFLO 96 or VIAFLO 384 handheld electronic pipettes with MAG and MAGFLO PCR – our module for magnetic separation and magnetic beads for PCR purification – 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 (M) and automatic (A) modes, providing flexibility without influencing the results.
- Fast and efficient high throughput PCR product purification with the VIAFLO 96 or VIAFLO 384 and MAG reduces the time-to-result by simultaneously processing 96 samples with identical liquid handling parameters.
- Establish an even greater throughput of the PCR purification process and downscale the protocol in VIALINK for the VIAFLO 384's 0.5-12.5 µl 384 channel pipetting head. Furthermore, MAG's DWP adapter enables high volume PCR purification when scaling up to the 50-1250 µl 96 channel head.

## Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6031	VIAFLO 384 24, 96 and 384 channel handheld electronic pipettes	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA Biosciences	6103	96 channel pipetting head 10-300 µl	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA Biosciences	4900	MAG module for magnetic separation	<a href="https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag">https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag</a>
INTEGRA Biosciences	4906	Adapter for 96 well PCR plates	<a href="https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag">https://www.integra-biosciences.com/global/en/modules/mag-and-heatmag</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	6348	300 ml polypropylene reservoir	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
INTEGRA Biosciences	6305	300 ml reservoir base	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
INTEGRA Biosciences	6353	INTEGRA DWP	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
INTEGRA Biosciences	7010 7012 7014	MAGFLO™ PCR	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
Bio-Rad	HSP9601	Low-profile 96-well PCR plates	<a href="https://www.bio-rad.com/en-ch/product/low-profile-96-well-pcr-plates?ID=OC00BU4VY">https://www.bio-rad.com/en-ch/product/low-profile-96-well-pcr-plates?ID=OC00BU4VY</a>
Promega	G2101	100 bp DNA ladder	<a href="https://ch.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101">https://ch.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101</a>

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