Streamline liquid handling with automation

Maximize efficiency and precision

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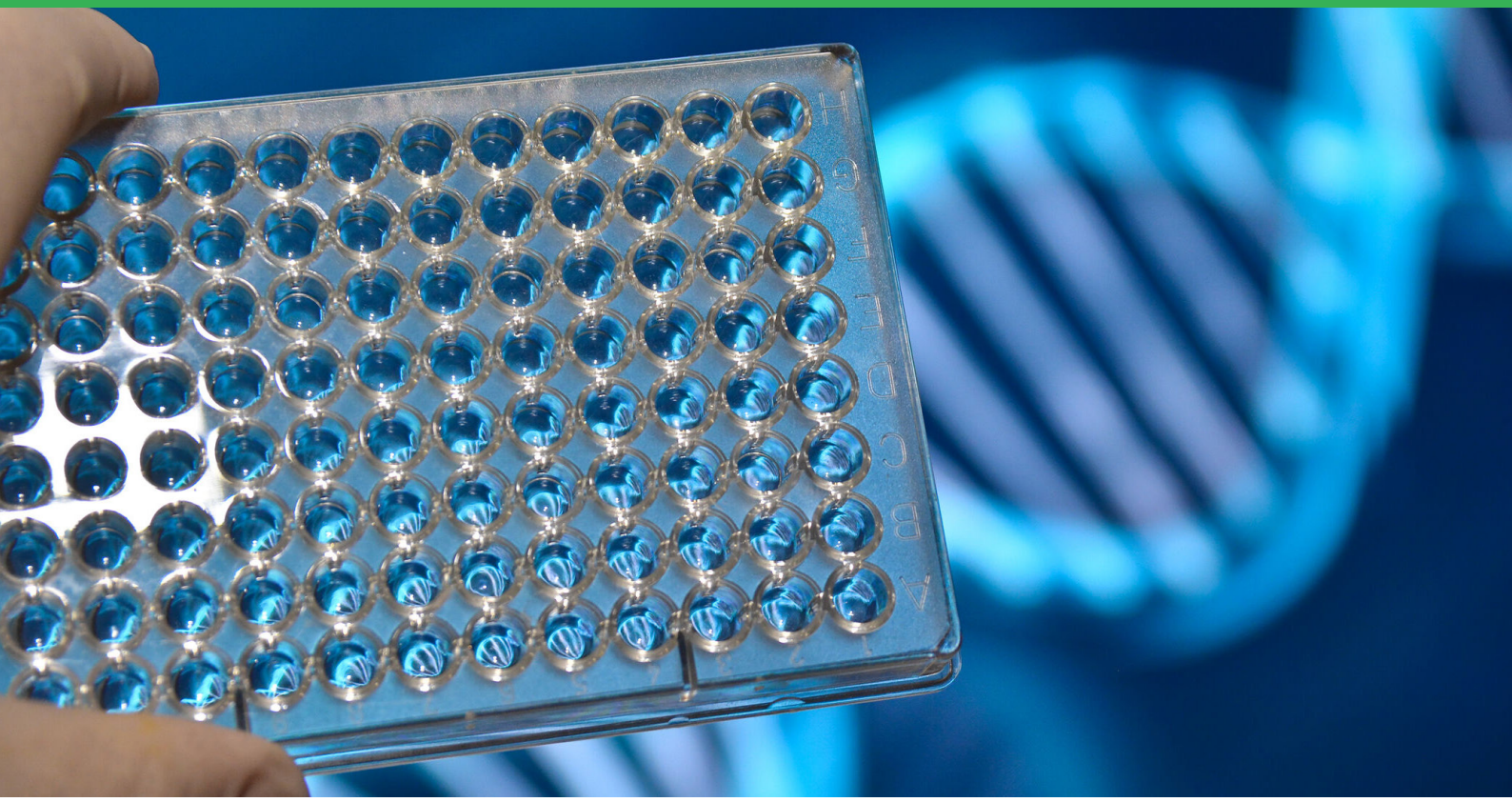

Introduction

Cutting-edge research in the life sciences industries is increasingly complex, demanding ever-greater precision. Automated liquid handling is a key driver of increased precision and efficiency in many laboratory processes.

There are some techniques where automated liquid handling is essential to not only save time and enhance laboratory efficiency, but also to improve reproducibility by avoiding errors. For example, serial dilution may be a simple technique, but it is often required at the start of much more complex workflows involving precise molecular biology techniques, meaning that researchers can ill afford liquid handling errors in the pursuit of reliable results.

Automation solutions help to simplify procedures that are error prone or tedious, enabling automation of the most complex protocols, such as next generation sequencing (NGS) library preparation.

In this eBook, we investigate several automated liquid handling solutions provided by INTEGRA Biosciences, such as its MIRO CANVAS compact digital microfluidics platform and ASSIST PLUS pipetting robot.

Contents

We also highlight 10 case studies to help you understand how these platforms can be used to streamline workflows and achieve consistent and reliable results in protocols ranging from DNA purification to DNA sequencing and general biological assay preparation.

DNA purification and qPCR
Purifying DNA is often the first step in precise molecular biology protocols. As such, qPCR master mix preparation, PCR clean-up and DNA size selection workflows can benefit from automated solutions offered by INTEGRA. The following 3 case studies outline how the company's pipetting robot can streamline these liquid handling tasks.

• INTEGRA's pipetting robot can accurately handle magnetic beads for reproducible PCR product purification and DNA size selection. The ASSIST PLUS equipped with the VOYAGER adjustable tip spacing pipette and MAG and HEATMAG modules for magnetic bead handling saves time and can be adapted effortlessly to various protocols.

• The pipetting robot and modules can automate the tedious process of qPCR master mix preparation. The ASSIST PLUS and D-ONE single channel pipetting module offer automated volume calculations, tip selection and liquid level detection, maximizing hands-free time and increasing reproducibility.

• Combining INTEGRA's ASSIST PLUS with Zymo Research's DNA Clean and Concentrator magnetic bead kit results in ultrapure DNA. This technology combination offers a seamless and efficient DNA clean-up solution, resulting in high DNA yields and purity. The protocol helps to reduce standard deviations, demonstrating improved consistency and reproducibility over manual clean-up.

DNA sequencing
NGS library preparation is a complex, time-consuming process that can be reliably automated using INTEGRA's advanced digital microfluidics platform, the MIRO CANVAS.

The following 4 case studies demonstrate the integration of this platform with kits from various manufacturers.

• The MIRO CANVAS can be integrated with the PacBio® SMRTbell® Prep Kit to fully automate whole genome sequencing library preparation using 1-3 µg of high quality, high molecular weight input DNA. Total library quantities, peak sizes and primary sequencing metrics are indistinguishable from manually prepared libraries.

• The MIRO CANVAS can be used to fully automate long-read DNA library preparation with the Oxford Nanopore Ligation Sequencing Kit, reducing reagent volumes by 75 % and yielding results comparable with manual library preparation.

• Automation of the Twist Human Core Exome Kit for whole exome sequencing on the MIRO CANVAS using 50 ng DNA input for library preparation, and 1,500 ng for hybridization capture, was comparable with manually prepared libraries and reduced hands-on time by over 85 %.

• Short-read NGS library preparation can be successfully automated by integrating the MIRO CANVAS with the Illumina DNA PCR-free Prep Kit using 50-500 ng DNA inputs. Automation reduces the amount of hands-on time required for library preparation by more than 60 %.

Biological assay preparation
Assays underpin life sciences research, but many are labor intensive and time consuming. Researchers seek high quality, reproducible results that avoid the risks of costly and onerous retests. The following 3 case studies show how INTEGRA's pipetting robot can fully automate well-known high throughput assays.

• The first case study demonstrates full automation of Lonza's PyroGene Recombinant Factor C Endotoxin Detection Assay on the ASSIST PLUS using the D-ONE and the VOYAGER adjustable tip spacing pipette to add the working reagent.

• The next shows a fully automated western blotting set-up protocol using the ASSIST PLUS and the D-ONE. Plates for the Simple Western™ chemiluminescence detection (developed by ProteinSimple) of single target, multi-target or total protein assays can be filled in under 3 hours.

• Applying INTEGRA's solutions to serial dilution helps to avoid error propagation and accumulation in workflows. The ASSIST PLUS robot with the VOYAGER adjustable tip spacing pipette can automate 2-, 5- and 10-fold serial dilutions, creating additional hands-free time while also reducing the risk of repetitive strain injuries.

Application Note

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 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 automated DNA clean-up protocols show high reproducibility, excellent yield and purity values..

Key benefits:

- Automated DNA clean-up with Zymo's DCC on the ASSIST PLUS pipetting robot makes DNA clean-up simple. Manual intervention is needed only when moving labware on and off the magnetic separator and shaker.
- The optimized liquid handling parameters on the 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 speeds 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 adjustable 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 by using innovative labware accessories. Reservoirs with SureFlo™ anti-sealing array allow 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

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Application Note

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Step-by-step procedure

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

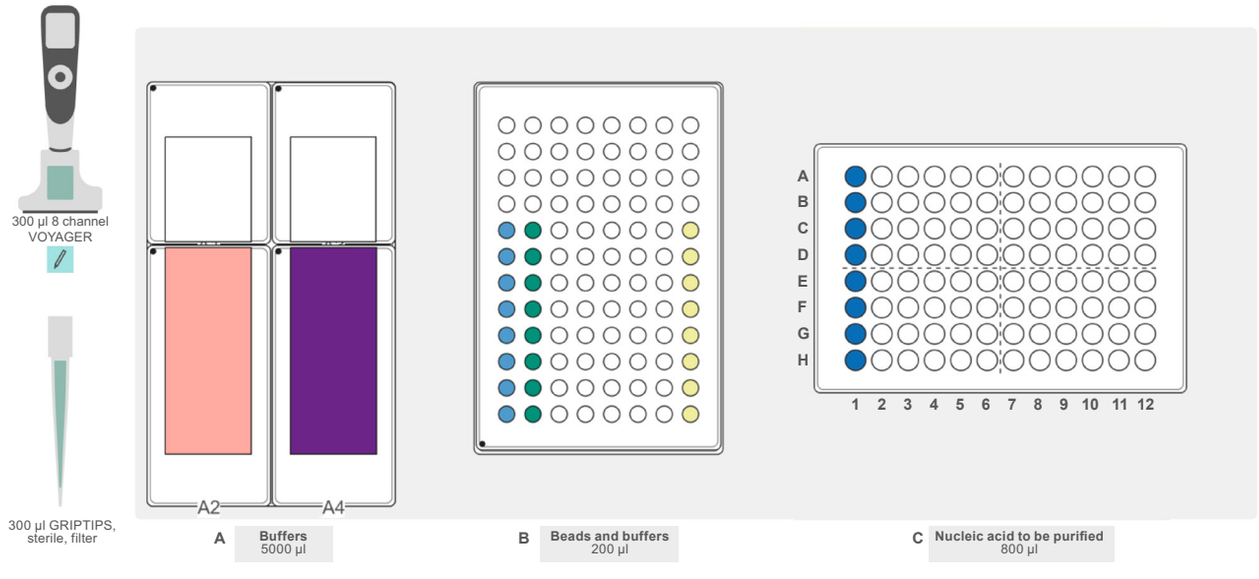

Figure 2: Deck set-up for the binding step. **Position A:** dual reservoir adapter with 2 divided reservoirs with DNA MagBinding Buffer (A2 – pink) and DNA Wash Buffer (A4 – lilac). **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue), MagBinding Beads (green) and DNA Elution Buffer (yellow). **Position C:** 0.8 ml DeepWell plate with the nucleic acid to be purified (dark blue).

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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**).

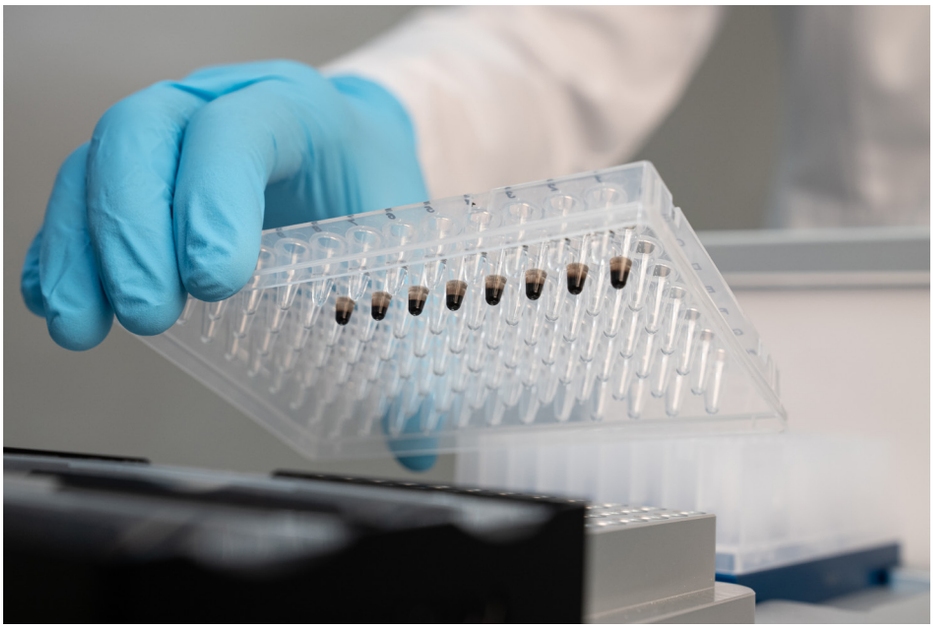

Figure 3: Placing the 96 well PCR plate onto the PCR cooling block.

Lastly, place the DeepWell plate containing the 30 µl samples (**Figure 2**, dark blue) at deck Position C.

Select and run the VIALAB program 'Zymo DCC'. The VOYAGER adjustable tip spacing electronic pipette will transfer 20 µl of nuclease-free water (with a 5 µl pre- and post-dispense) into the samples, so that the starting volume of the nucleic acid to be purified is 50 µl, as suggested by the DCC kit manufacturer. Next, the correct volume of DNA MagBinding Buffer – corresponding to 4 times the volume of input DNA in this case, 4x50 µl = 200 µl – 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 µl 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.

Application Note

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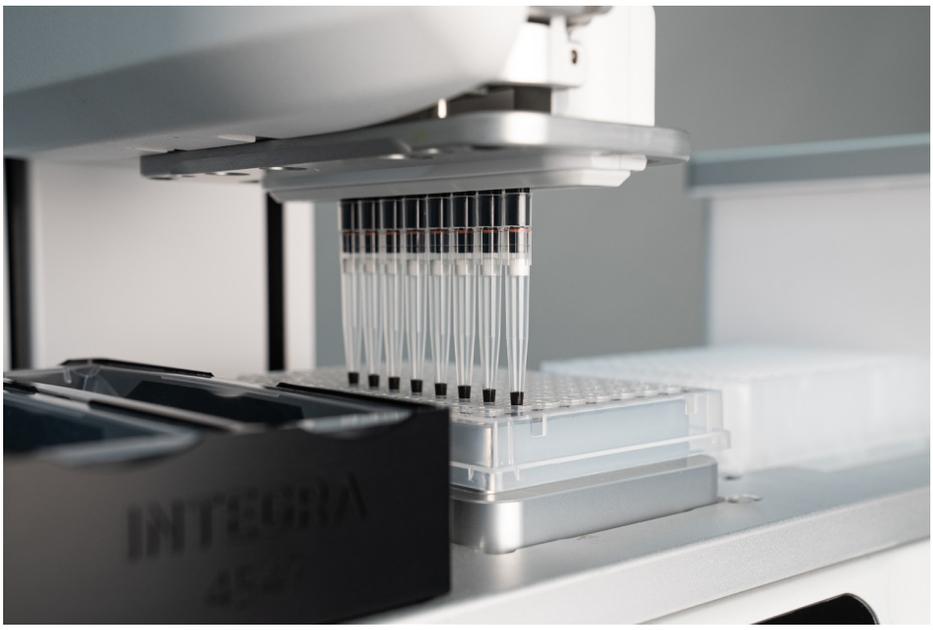

Figure 4: Addition of the MagBinding Beads to the samples to be cleaned up.

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**).

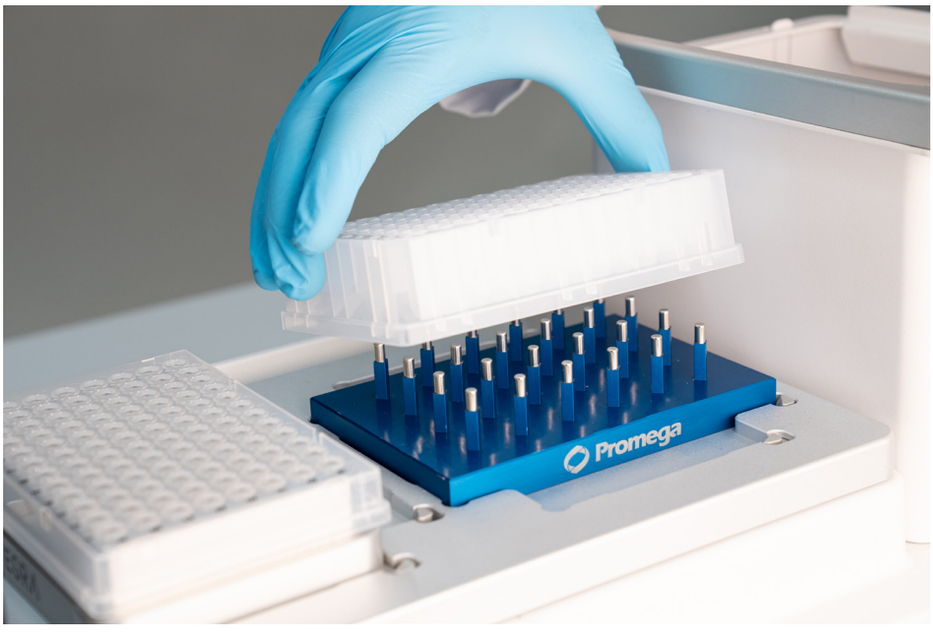

Figure 5: Placing the DeepWell plate onto the magnetic separation device.

Application Note

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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.

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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 on 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.

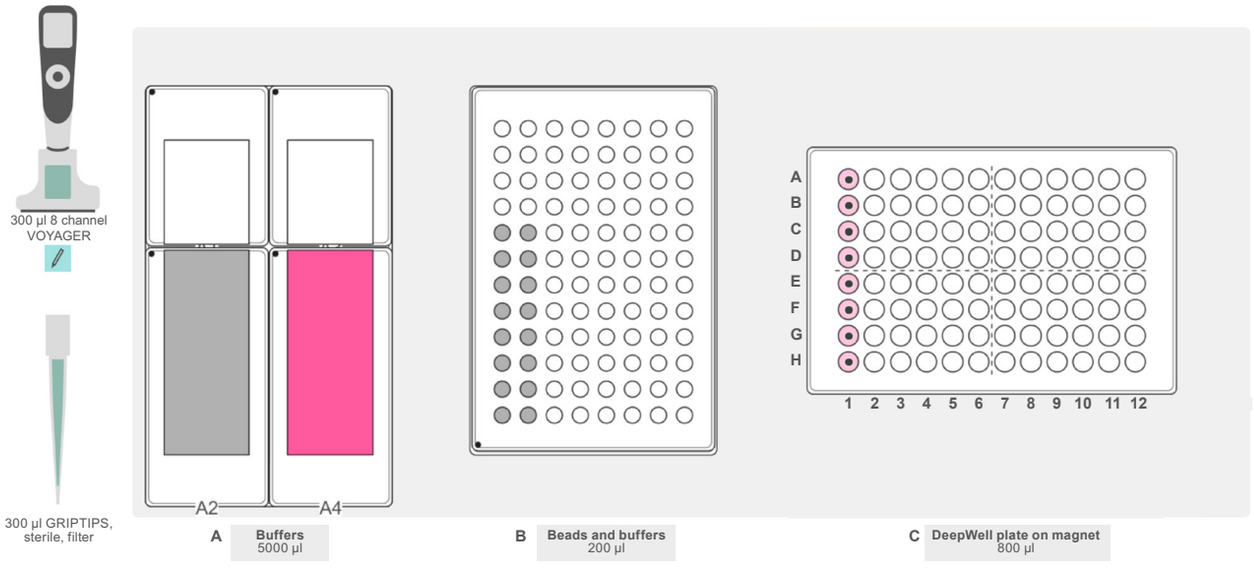

Figure 6: Deck set-up for the washing steps. **Position A:** Dual reservoir adapter with 2 divided reservoirs containing dead volume form previous steps (A2 – gray) and Wash Buffer (A4 – magenta). **Position C:** 0.8 ml DeepWell plate with nucleic acid bound to the magnetic beads (pink).

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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. The ASSIST PLUS will add 50 µl 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 µl of eluted DNA to the new 96 well plate.

TIP:

- The eluted DNA can be used immediately or stored at -20 °C until further use.

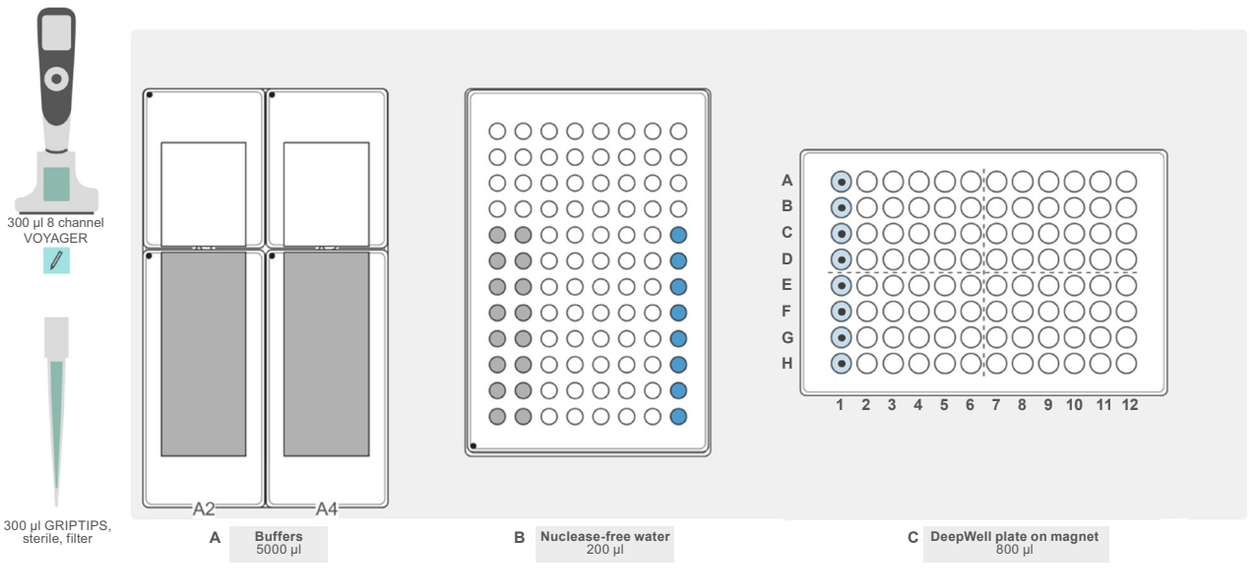

Figure 7: Position A: Dual reservoir adapter with 2 divided reservoirs containing dead volume from previous steps (A2, A4 – gray). Deck set-up for the elution step. **Position B:** 96 well PCR plate on a cooling block with nuclease-free water (blue). **Position C:** 0.8 ml DeepWell plate with the purified nucleic acid bound to the magnetic beads (light blue).

Application Note

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Results

The automated DCC MagBead protocol on the 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 (A_{260}/A_{280} and A_{260}/A_{230} ratios) and higher reproducibility (lower SD values).

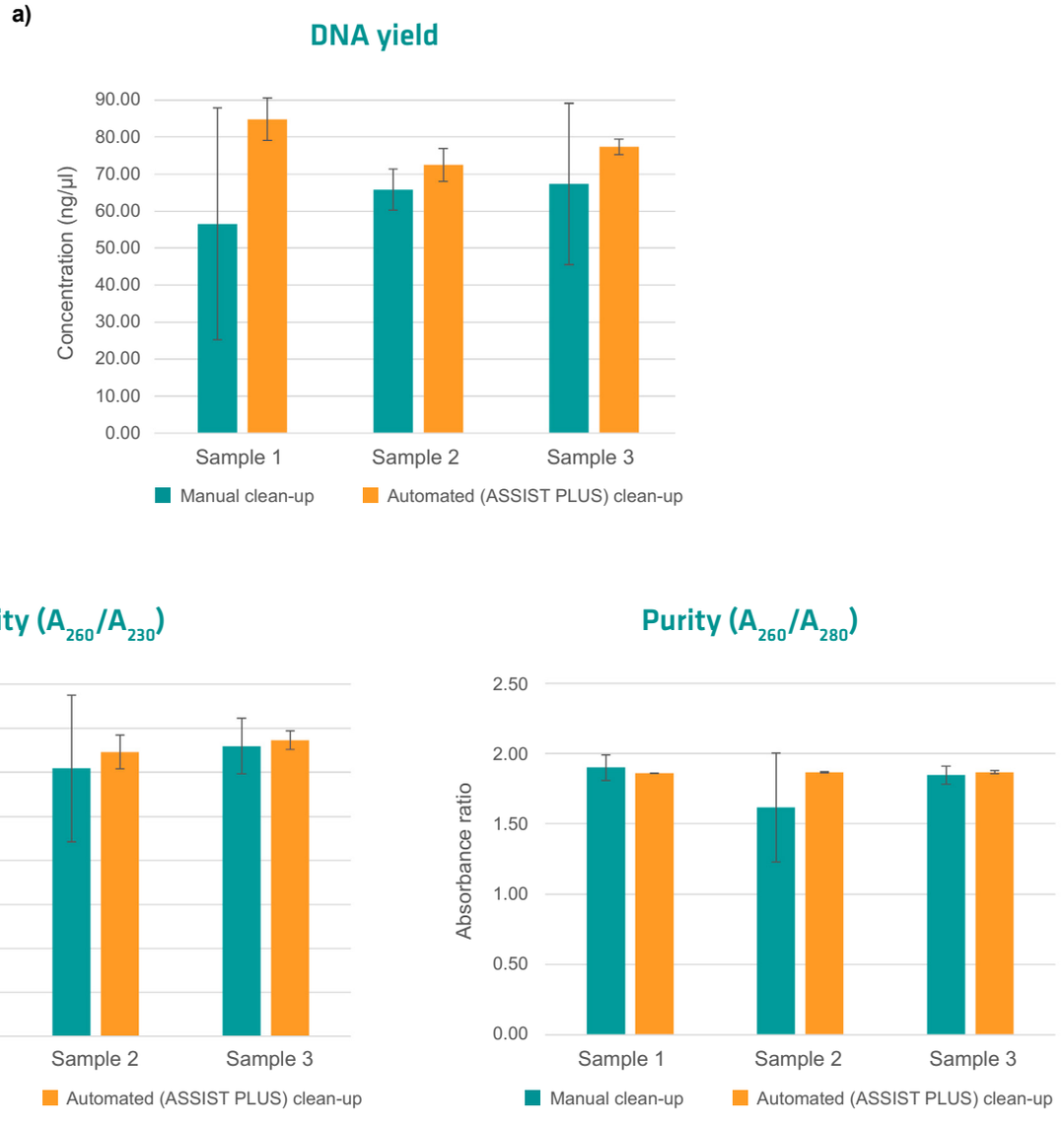

Figure 8: DNA from a PCR reaction was purified manually and using the automated protocol on the ASSIST PLUS. DNA a) yield and b) purity were determined by UV spectroscopy. N=3 for each sample.

Application Note

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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 errors, generating highly reproducible and reliable results.
- The optimized liquid handling parameters on the ASSIST PLUS enable precision pipetting, resulting in high DNA yields and purity.
- Wash buffers normally contain ethanol, and the use of low retention GRIPTIPS prevents dripping from occurring in the washing steps.
- The entire 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 automated DNA clean-up process over manual clean-up.

Application Note

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Materials

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

Contact us:

Application Note

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Automated qPCR master mix preparation with the D-ONE single channel pipetting module and the ASSIST PLUS pipetting robot

Introduction

Automating qPCR master mix preparation is often considered extremely tedious, due to the need to constantly change the program set-up based on varying master mix volumes and sample numbers. However, the D-ONE single channel pipetting module for the ASSIST PLUS pipetting robot simplifies the programming of automated qPCR master mix preparation protocols down to a single step. The D-ONE individually reaches tubes of different qPCR master mix components, while the VIALAB software takes care of all calculations and provides automatic tip selection.

This solution streamlines qPCR master mix preparation by enabling efficient pipetting into any tube or reservoir, and subsequent distribution to plates with multichannel pipettes to speed up the process. This application note verifies a SYBR® Green qPCR protocol, prepared directly in a divided reservoir, using the D-ONE on the ASSIST PLUS.

Key benefits:

- Fully automated liquid handling of various qPCR master mix components on the ASSIST PLUS with the D-ONE ensures reproducible results.
- qPCR master mix components and volumes can be easily adjusted in VIALAB to perform different qPCR protocols. The final calculation is automatically done by VIALAB according to the selected reaction count.
- The D-ONE offers automatic liquid level detection, allowing the use of reagents with different aliquot volumes and informing the user if there is insufficient liquid. Furthermore, automated tip selection guarantees high precision when pipetting different volumes.
- qPCR master mixes can be pipetted into various target vessels, including INTEGRA's multichannel reagent reservoirs – with a very low dead volume – to speed up the plating of master mixes using a multichannel pipette.

Overview: How to automate protocols for SYBR Green qPCR master mix preparation

ASSIST PLUS

Application Note

INTEGRA

The 5-1250 µl D-ONE, with 125 and 1250 µl sterile, filter GRIPTIPS® pipette tips, is mounted on the ASSIST PLUS. An INTEGRA divided polypropylene reagent reservoir with SureFlo™ anti-sealing array is placed onto the dual reservoir adapter on deck Position A. An INTEGRA tube rack with nuclease-free water (A1), SYBR Green master mix reagent (B1), forward primer (C1) and reverse primer (D1) is placed on Position B (**Figure 1**).

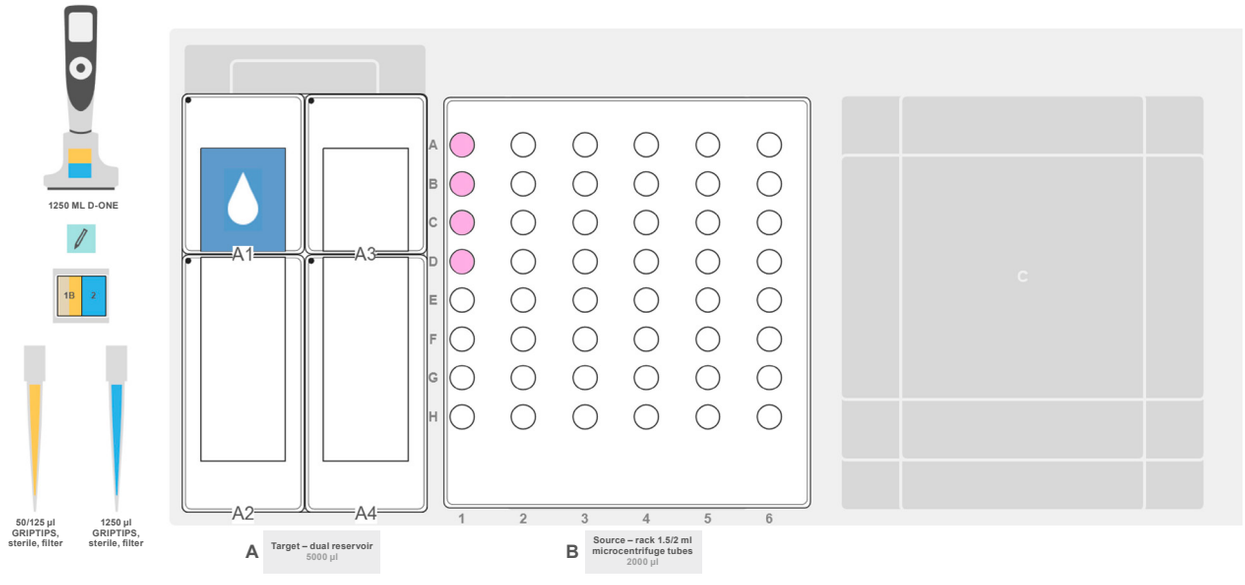

Figure 1: Deck set-up for qPCR master mix preparation in a divided reagent reservoir. **Position A:** Target – INTEGRA dual reservoir adapter with divided reagent reservoir (blue). **Position B:** Source – INTEGRA tube rack for 2 ml tubes with screw caps and 1.5 ml microcentrifuge tubes (pink). **Position C:** empty.

Step-by-step procedure:

1. Calculation of qPCR master mix components

STEP: Adjusting the number of reactions in VIALAB.

The reaction count is predefined to 98 reactions to fill a full 96 well qPCR plate (9 µl reaction volume, 10 µl dead volume). The user defines the reagent ID and its volume per reaction in VIALAB, which can be changed according to the specific qPCR protocol. By adjusting the reaction count, the transfer volume of all qPCR master mix components is calculated automatically, as well as the selection of the appropriate GRIPTIPS (**Figure 2**).

TIPS:

- Once a program with a master mix step is set up, it can be reopened and only the number of reactions needs to be changed prior to the start of the run. No reprogramming is needed, saving time for the operator.
- Depending on the final volume calculated by VIALAB, the tip type (125/1250 µl) is automatically chosen, further alleviating the need for user input.

Application Note

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Enter the number of reactions

Reactions		
−	98	+

Reagent ID	Source		Volume (1*) [µl]	Reactions	Validation
	Pos.	Well		98	
Nuclease-free water	B	A1	3	294	✓
SYBR Green master mix	B	B1	5	490	✓
Forward primer	B	C1	0.5	49	✓
Reverse primer	B	D1	0.5	49	✓
Target	A	A1	9	882 µl	

Figure 2: VIALAB calculates the volume of qPCR master mix components according to the reaction count in the master mix step.

2. Transfer of qPCR master mix components

STEP: The different components of qPCR master mix are added one by one.

HOW TO: Select and run the VIALAB program 'Master_mix_prep_in_reservoir'. The 5-1250 µl D-ONE, with 1250 µl sterile, filter GRIPTIPS, aspirates 294 µl of nuclease-free water from a 2 ml tube (Position B – A1), and dispenses it into the 5 ml compartment of the divided reservoir (Position A – A1). The D-ONE then changes tips automatically between different reagents, and transfers 490 µl of SYBR Green master mix from Position B – B1 to Position A – A1. With 12.5 µl sterile, filter GRIPTIPS, the D-ONE then transfers 49 µl each of the forward primer (Position B – C1) and reverse primer (Position B – D1) into the divided reservoir (Position A – A1). With new 1250 µl sterile, filter GRIPTIPS, the D-ONE mixes 600 µl of SYBR Green master mix 3 times, in 3 different offsets within the 5 ml compartment of the divided reservoir (Position A – A1).

TIP:

- Different mixing offsets in the reagent reservoir improve the homogeneity of the qPCR master mix components. The mixing volume can be adjusted easily when reducing the reaction count, while mixing cycles can be increased to produce even higher volumes of qPCR master mix.

Application Note

INTEGRA

Results

A SYBR Green qPCR protocol was used to amplify a 250 bp fragment from the fifth variable region of the bacterial 16S rRNA gene. A QuantStudio™ Real-Time PCR System 3 (Thermo Fisher Scientific) was used to demonstrate proper homogeneity when performing qPCR master mix preparation in divided reagent reservoirs. The master mix was prepared both manually in tubes and with the ASSIST PLUS and the D-ONE in the divided reservoir.

Figure 3 represents the amplification of a section of 6 replicates from a qPCR plate using SYBR Green master mix prepared with the ASSIST PLUS and the D-ONE in a divided reservoir (green), compared to 6 replicates using master mix produced manually in tubes (orange). Both qPCR master mix preparations show valid amplification, demonstrating the proper homogeneity of qPCR master mix components.

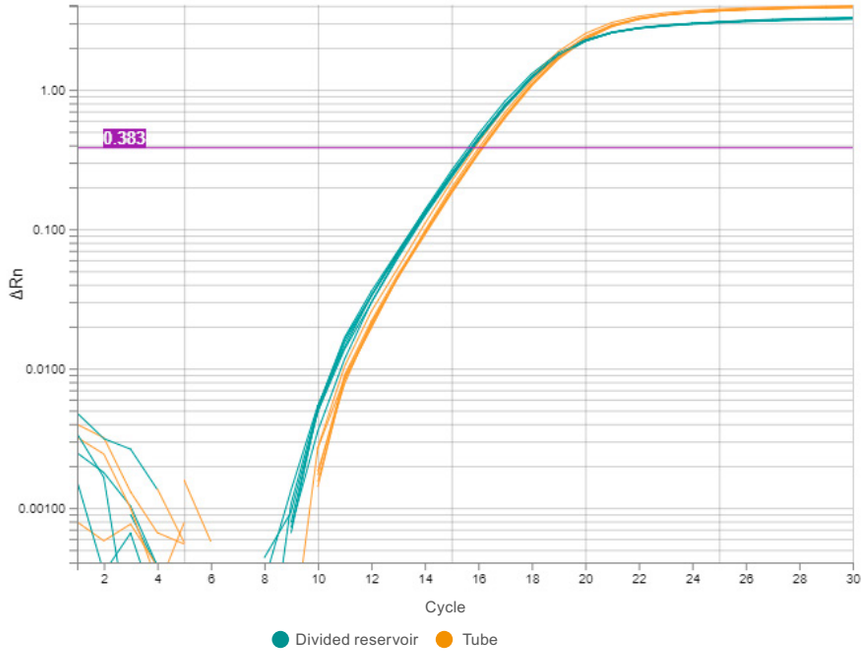

Figure 3: Amplification plot of a 250 bp fragment of the fifth variable region of the bacterial 16S rRNA gene showing a section of 6 replicates.

Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to your specific labware and protocols, such as when partial plates are needed.
- **Partial plates:** Programs can be adapted at any time to varying sample numbers, giving laboratories total flexibility to meet current and future demands.

Application Note

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Conclusion

- The ASSIST PLUS pipetting robot and D-ONE single channel pipetting module, together with the VIALAB master mix step, enable the easy and reliable automation of qPCR master mix preparation, including automatic volume calculations, tip selection and liquid level detection.
- The ASSIST PLUS automates qPCR master mix preparation, set-up and sample addition, maximizing hands-free time and increasing reproducibility by eliminating any variability introduced by operators.
- The small footprint of the ASSIST PLUS allows it to fit into biosafety cabinets, ensuring sterile conditions while pipetting precious qPCR master mix components.
- The D-ONE can individually transfer various qPCR master mix components, and effectively mix them in divided reservoirs for large-scale qPCR master mix preparation.
- The homogeneity of qPCR master mix components can be guaranteed by adjusting mixing volumes and cycles, setting different mixing offsets, and the unique surface treatment of the SureFlo polypropylene divided reagent reservoir.
- The divided reagent reservoir can be easily accessed using an 8 channel 12.5 µl VOYAGER adjustable tip spacing pipette to quickly set up qPCR plates.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4532	D-ONE pipetting module	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4535	D-ONE tip deck	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4540	Rack for 1.5/2 ml microcentrifuge tubes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4356	25 ml divided reservoir, polypropylene	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6465	125 µl sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/grip-tip-selector-guide
INTEGRA Biosciences	6445	1250 µl sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/grip-tip-selector-guide

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Application Note

INTEGRA

Automated DNA size selection for flexible NGS workflow integration

Introduction

DNA size selection with magnetic beads plays a significant role in molecular biology, employing specific bead-to-sample ratios to capture and separate fragments by size. Single-sided size selection using a high magnetic bead ratio removes primer dimers during polymerase chain reaction (PCR) product purification. Double-sided DNA size selection uses 2 distinct ratios to effectively remove small and large fragments, resulting in the purification of targeted average fragment sizes. Both single- and double-sided DNA size selection with magnetic beads are integral to next generation sequencing (NGS) library preparation.

MAGFLO™ NGS magnetic beads for NGS size selection offer an effective solution for NGS and PCR product purification. These beads are suitable for both single- and double-sided DNA size selection methods, and manufactured under RNase-free conditions to enable the purification of both RNA and DNA.

MAGFLO NGS beads are compatible with fully automated DNA size selection protocols. The VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot automates all liquid handling steps, while the MAG module ensures precise automated magnetic bead handling.

The protocols provided here demonstrate the accurate handling of MAGFLO NGS beads for reproducible PCR product purification and DNA size selection. Automated testing of MAGFLO NGS beads and AMPure XP bead-based reagent (Beckman Coulter Life Sciences) confirmed the interchangeability of the products, demonstrating that INTEGRA's cost-effective alternative delivers the same reliable results as the gold standard.

Key benefits:

- Using MAGFLO NGS beads for library preparation steps reduces processing costs, while increasing the reproducibility of double-sided DNA size selection.
- MAGFLO NGS beads enable efficient and reproducible PCR product purification by removing fragments below 100 bp.
- The MAG module captures and releases beads using vertical magnet movements, eliminating the need for manual intervention and minimizing the risk of spillage during plate transfers.
- The VOYAGER on the ASSIST PLUS guarantees fail-proof liquid handling of magnetic beads with optimized pipetting height and speed settings.
- With the ASSIST PLUS and the MAG module, protocols can be effortlessly adapted to various DNA/RNA size selection protocols. VIALAB's user-friendly programming makes adjusting magnetic bead ratios easy.
- INTEGRA provides MAG module adapters to enable automated magnetic bead handling in a range of labware formats, including microcentrifuge tubes, deep well plates (DWPs) and 96 or 384 well PCR plates.

Overview: How to automate DNA size selection with the ASSIST PLUS and MAG module

ASSIST PLUS

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This application note describes the fully automated DNA size selection of 48 samples with MAGFLO NGS beads on the ASSIST PLUS pipetting robot. An 8 channel 125 µl VOYAGER automates the liquid handling steps, and the MAG module automates the magnetic bead handling steps.

Figure 1 illustrates the step-by-step procedure of the provided NGS size selection protocols for:

- Single-sided DNA size selection (PCR product purification)
- Double-sided DNA size selection

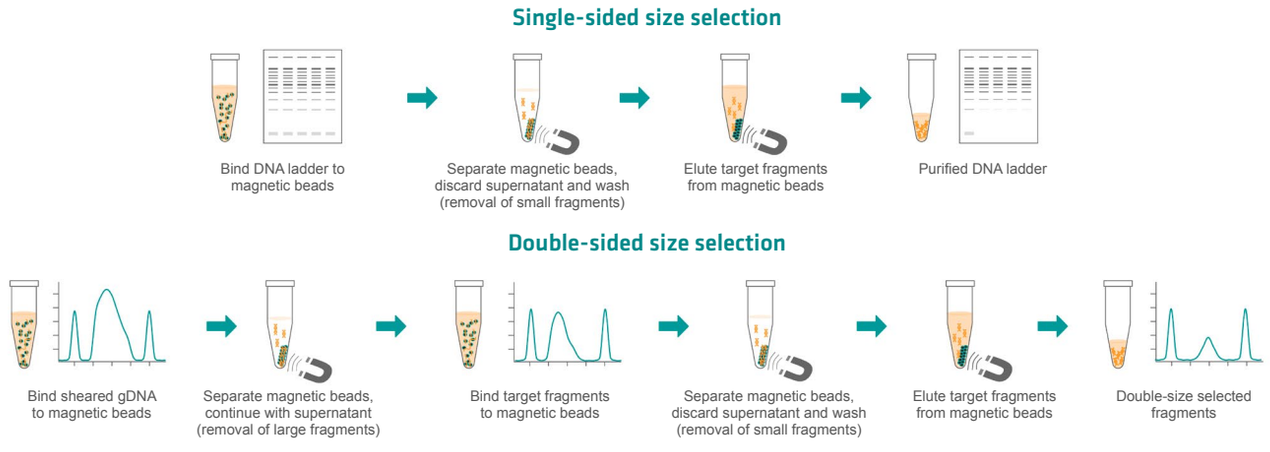

Figure 1: Step-by-step procedure of single- and double-sided DNA size selection.

Experimental set-up:

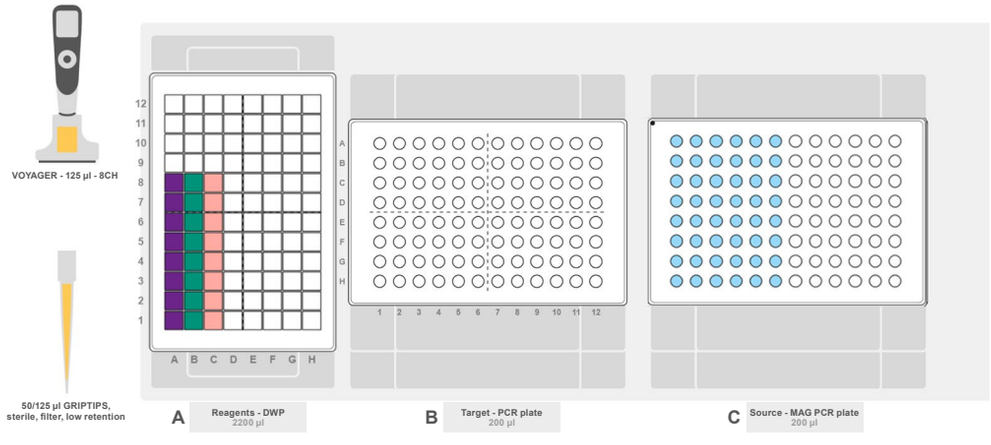

Figure 2: The ASSIST PLUS deck set-up for automated DNA size selection with the MAG module. **Position A:** Reagents – INTEGRA DWP (lilac: MAGFLO NGS beads; green: 80 % ethanol; pink: molecular-grade water). **Position B:** Target – 96 well Bio-Rad Hard-Shell® PCR plate. **Position C:** Source – MAG module with 96 well Bio-Rad Hard-Shell PCR plate containing samples (blue).

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Step-by-step procedure:

1. Single-sided DNA size selection (PCR product purification)

STEP: Binding the PCR product using a 1.8x magnetic bead ratio.

HOW TO: Prepare fresh 80 % ethanol and bring MAGFLO NGS beads to room temperature (RT). Place a 96 well INTEGRA DWP on Position A of the ASSIST PLUS in portrait orientation, with 450 µl of MAGFLO NGS beads in wells A1-A8 (**Figure 2**, lilac), 1.6 ml of 80 % ethanol in wells B1-B8 (**Figure 2**, green) and 320 µl of molecular-grade water in wells C1-C8 (**Figure 2**, pink). Place one empty 96 well Bio-Rad Hard-Shell PCR plate on Position B. Place a second 96 well plate containing 40 µl of sample in each well in the first half (**Figure 2**, blue), in landscape orientation on the 96 well adapter of the MAG module on Position C.

Select and run the VIALAB program 'MAG_PCR_product_purification'. Using 125 µl sterile, filter, low retention GRIPTIPS®, the VOYAGER on the ASSIST PLUS will transfer 72 µl of magnetic beads from column A (**Figure 2**, lilac) of the INTEGRA DWP on Position A (**Figure 3a**) to each well in the first half of the PCR plate on Position C (**Figure 3b**). Magnetic beads will be mixed 10 times before aspiration, and 15 times after dispensing into samples, to guarantee a homogeneous magnetic bead mixture. GRIPTIPS will be changed automatically between samples. With the magnet array disengaged in Position Home (**Figure 4a**, Pos. Home, 0 mm), the VOYAGER will initiate an incubation of 5 minutes at RT, binding the PCR fragments to the magnetic beads (**Figure 4b**).

a) b)

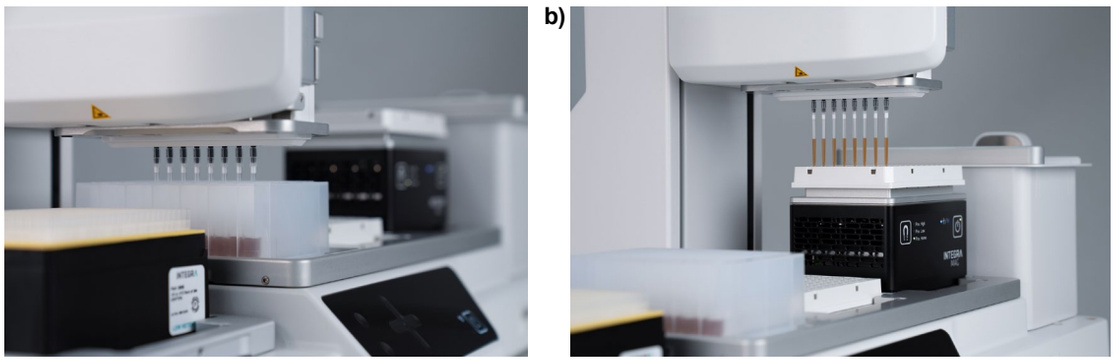

Figure 3: The VOYAGER on the ASSIST PLUS transfers MAGFLO NGS beads from (a) the 96 well INTEGRA DWP to (b) a 96 well Bio-Rad Hard-Shell PCR plate on the MAG module.

a) b)

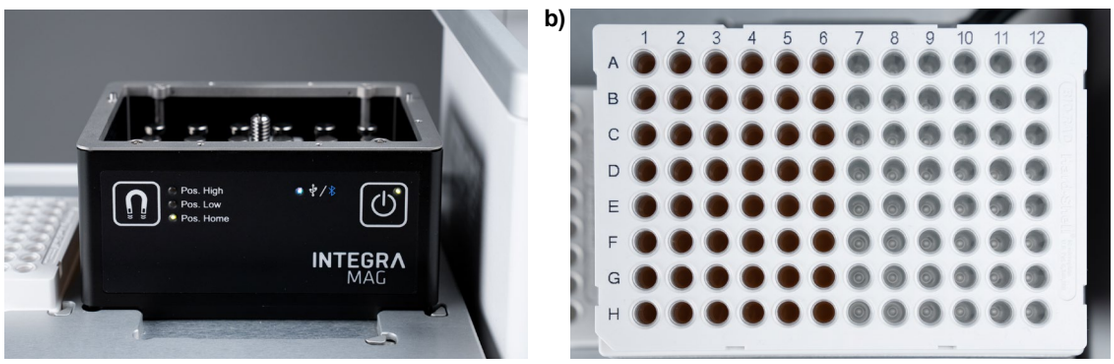

Figure 4: The MAG module on the ASSIST PLUS (a) without a PCR plate adapter, showing a disengaged magnet array (Pos. Home, 0 mm), and (b) with a 96 well PCR plate adapter and a 96 well Bio-Rad Hard-Shell PCR plate, showing uncaptured magnetic beads.

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STEP: Removal of small fragments and washing.

HOW TO: The MAG module will automatically lift the magnet array from Pos. Home (**Figure 4b**) to Position High (Pos. High) at 29 mm (**Figure 5a**) for 3 minutes to capture the magnetic beads (**Figure 5b**). The PCR plate adapter for the MAG module has small holes to visually confirm the targeted capture of magnetic beads at the well surface.

The VOYAGER – using fresh GRIPTIPS for each sample – will remove the supernatant while the magnet array remains engaged (**Figure 5a**). The pipette will then transfer the supernatant into columns F-H of the INTEGRA DWP on Position A. Slow aspiration (Speed 1) and precise height settings prevent magnetic bead loss. Next, magnetic beads will be washed twice with 125 µl of 80 % ethanol from column B of the INTEGRA DWP on Position A (**Figure 2**, green). The VOYAGER will aspirate an additional time to ensure the complete removal of ethanol from each well. The MAG module will then lower the magnet array by 5 mm to Position Low (Pos. Low, 24 mm) followed by air drying for 3 minutes at RT. Lowering the magnet array before air drying will move the pellets closer to the bottom of the wells, allowing easier elution and smaller volumes.

Tips

- The magnet step in VIALAB provides total control of the magnet array by setting customized heights anywhere between 0 and 29 mm.
- When handling ethanol, fast aspiration and slow dispensing with a tip touch are required to prevent droplet formation.
- The drying condition has been optimized to 3 minutes, but may vary in different lab conditions.

a) b)

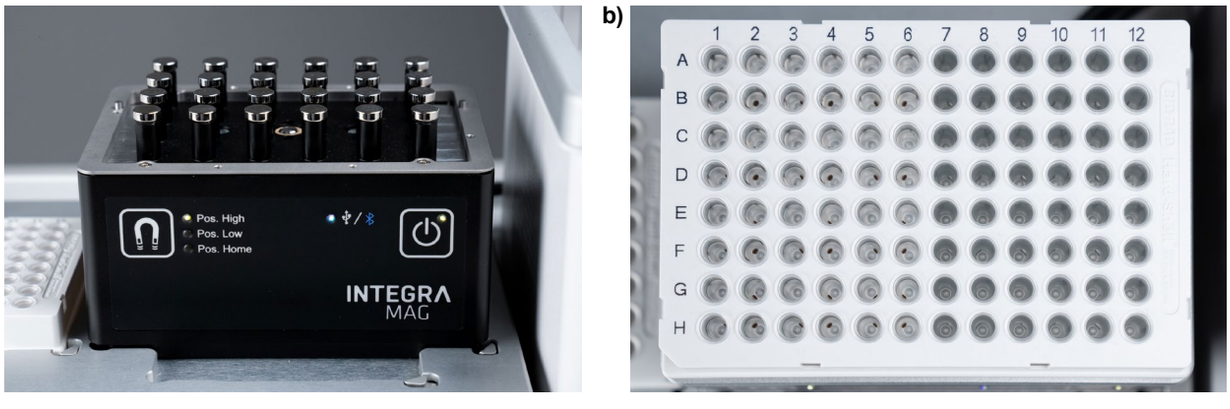

Figure 5: The MAG module on the ASSIST PLUS (a) without a PCR plate adapter, showing an engaged magnet array (Pos. High, 29 mm), and (b) with a 96 well PCR plate adapter and a 96 well Bio-Rad Hard-Shell PCR plate, showing magnetic beads captured after 3 minutes.

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	STEP: Elution of single-sided size selected fragments.	**HOW TO:** 40 µl of molecular-grade water will be transferred from column C of the INTEGRA DWP on Position A (**Figure 2**, pink) to every well in the first half of the PCR plate on Position C. Mixing 25 times ensures proper resuspension of the magnetic beads, regardless of volume. This is followed by an incubation at RT for 5 minutes. Again, the MAG module will then automatically lift the magnet array to 29 mm (**Figure 5a**) for 3 minutes to capture the magnetic beads (**Figure 5b**). Afterwards, the VOYAGER will transfer 35 µl of eluate to the unused PCR plate on Position B, leaving 5 µl in the plate at Position C to prevent magnetic bead carryover. At the end of the run, the user is prompted to store the PCR plate from Position B, and remove the plate from the MAG module.
2. Double-sided DNA size selection	**STEP:** Binding sheared genomic DNA (gDNA) using a 0.7x magnetic bead ratio.	**HOW TO:** The deck set-up for double-sided DNA size selection is similar to single-sided size selection, but with 320 µl of MAGFLO NGS beads (**Figure 2**, lilac), 350 µl of molecular-grade water (**Figure 2**, pink), and 55 µl of sample in each well in the first half of a Bio-Rad Hard-Shell 96 well PCR plate (**Figure 2**, blue). Select and run the VIALAB program 'MAG_DNA_double_size_selection'. The VOYAGER will follow the steps described in PCR product purification, but will transfer 38.5 µl of magnetic beads to each well containing a sample (**Figure 3b**). Mixing 10 times before every other aspiration – and using new GRIPTIPS before each aspiration – guarantees precise, low volume pipetting of magnetic beads.
	STEP: Removal of large fragments (right size selection).	**HOW TO:** After capturing large fragments bound to magnetic beads (right size selection) (**Figure 5b**), the VOYAGER will transfer 85 µl of supernatant from each well in the first half of the PCR plate to the corresponding well in the second half of the same plate on Position C.
	STEP: Binding target fragments using a 0.8x magnetic bead ratio, and removing small fragments (left size selection) during the washing process.	**HOW TO:** Following the same procedure as the right size selection, the MAG module will lower the magnet array back to Pos. Home (**Figure 4a**), then the VOYAGER will transfer 5 µl of magnetic beads to the supernatant of the first size selection in the second half of the PCR plate (Position C). A 5 µl pre-dispense guarantees accurate pipetting of small volumes of magnetic beads. The subsequent washing procedure mirrors the single-sided DNA size selection.
	STEP: Elution of double-sided size selected fragments.	**HOW TO:** The MAG module and the VOYAGER will follow the same procedure used for single-sided DNA size selection, but 50 µl of molecular-grade water will be transferred before elution, and 45 µl after capturing the magnetic beads (**Figure 5b**). **Tip** • VIALAB software enables operators to switch to different fragment sizes as needed. Simply calculate the magnetic bead volume for any ratio, and update it in VIALAB.

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Results

Most providers of reagent kits for NGS library preparation recommend AMPure XP magnetic beads. This application note demonstrates the equivalent performance of MAGFLO NGS magnetic beads during automated single-sided DNA size selection – using a 100 bp DNA ladder (Promega) to mimic PCR product purification – and double-sided DNA size selection, using sheared gDNA.

Using the VOYAGER on the ASSIST PLUS, 48 replicates were processed with MAGFLO NGS beads in rows A to D, and AMPure XP beads in rows E to H. Automated magnetic bead handling with optimized magnet array heights was ensured by using the MAG module. The size-selected fragments were analyzed and compared using the 4150 TapeStation System (Agilent, complete data set can be found in the appendix).

Figure 6 shows the gel picture of row A (MAGFLO NGS beads) and row E (AMPure XP beads) of the 96 well plate for single-sided DNA size selection with a 30-fold diluted 100 bp DNA ladder and a 1.8x magnetic bead ratio. Both reagents purified all fragments of PCR ladder smaller than 100 bp and ~70 % of 4 ng 100 bp fragments, while recovering ~100 % of 65 ng fragments ranging from 200 bp to 1500 bp.

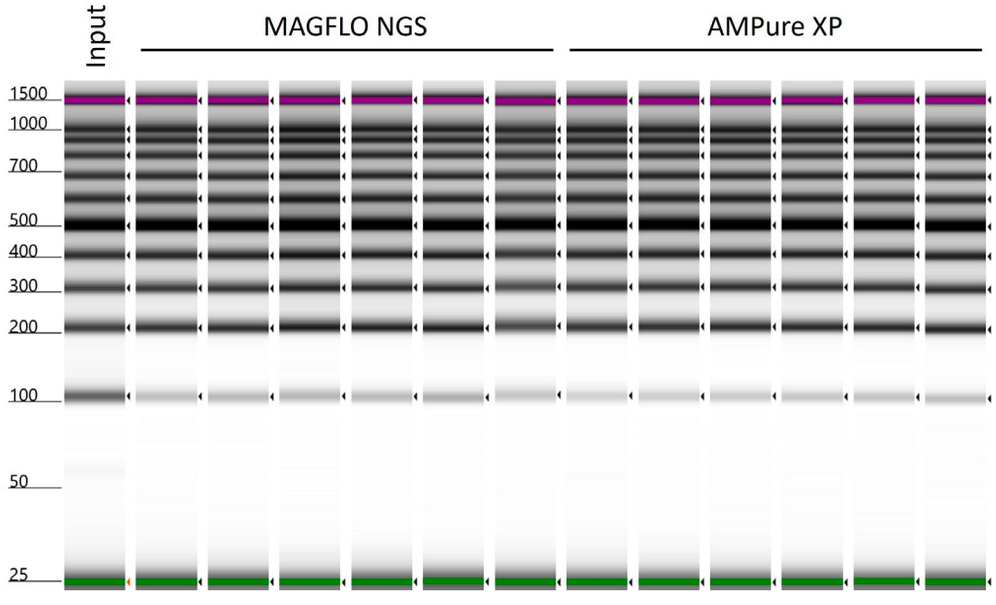

Figure 6: Single-sided DNA size selection with MAGFLO NGS beads guarantees automated PCR product purification. Results of fragment analysis using a 4150 TapeStation, showing a gel with 30-fold diluted 100 bp DNA ladder before (input) and after single-sided DNA size selection. The analysis shows the results for a 1.8x ratio of MAGFLO NGS (left, row A, n=6) or AMPure XP (right, row E, n=6) beads.

Application Note

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Figure 7 depicts an electropherogram (EPG) showing 22 out of 24 replicates (outliers excluded) for MAGFLO NGS (left) and AMPure XP (right) beads during double-sided DNA size selection of sheared gDNA. Magnetic bead ratios of 0.8x and 0.7x were used for left and right size selection, respectively. Both reagents achieved similar recovery rates, exceeding 12 % (n=22) of 330 ng sheared gDNA. Average fragment sizes were 372 bp with MAGFLO NGS beads, and 396 bp with AMPure XP beads, with overall size variation for each reagent remaining below 5 %.

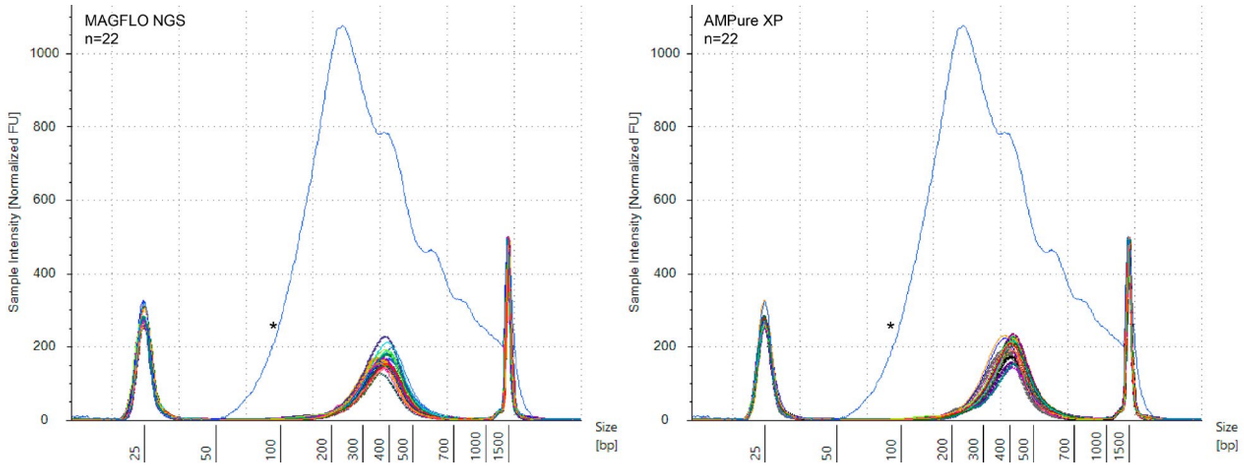

Figure 7: Efficient automated double-sided DNA size selection with MAGFLO NGS. Results of fragment analysis using a 4150 TapeStation, showing an EPG of sheared gDNA before (*) and after double-sided DNA size selection. The analysis compares magnetic bead ratios of 0.8x (left) to 0.7x (right) for MAGFLO NGS (n=22) and AMPure XP (n=22) beads.

Remarks

- **VIALAB software:** The VIALAB programs can be easily adapted for specific pipettes, labware and protocols.
- **Partial plates:** Pre-set programs offer laboratories complete flexibility to accommodate varying sample sizes, ensuring they can meet both current and future demands.
- **Semi-automation:** The appendix includes a protocol that uses a magnet plate for a semi-automated workflow.

Conclusion

- Fully automated DNA size selection and PCR product purification can be effectively achieved using the MAG module for magnetic bead handling and the VOYAGER on the ASSIST PLUS for precise liquid handling, allowing flexible NGS workflow integration.
- A 1.8x ratio of MAGFLO NGS beads enables the removal of small fragments during PCR product purification, consistently recovering valuable fragments larger than 100 bp.
- Double-sided DNA size selection with MAGFLO NGS beads reduces experimental costs. Magnetic bead ratios of of 0.8x (left) and 0.7x (right) successfully select fragments between 340 bp and 390 bp, with a 12 % recovery rate. MAGFLO NGS beads provide comparable performance to AMPure XP beads at a lower cost.
- VIALAB‘s programming features allow easy adjustments to different protocols, enabling changes to magnetic bead volumes, magnet height settings or sample counts as needed.

Application Note

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/voyager
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 (MAG / HEATMAG)	https://www.integra-biosciences.com/en/modules/mag-and-heat-mag
INTEGRA Biosciences	6565	125 µl sterile, filter, low retention GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6353	INTEGRA DWP	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	7000 7002 7004	MAGFLO NGS	https://www.integra-biosciences.com/en/ngspcr-purification/magflotm-ngs
Bio-Rad	HSP9601	Hard-Shell 96-well PCR plate, low profile, thin wall, skirted	https://www.bio-rad.com/en-ch/sku/HSP9601-hard-shell-96-well-pcr-plates-low-profile-thin-wall-skirted-white-clear?ID=HSP9601
Promega	G2101	100 bp DNA Ladder	https://worldwide.promega.com/products/cloning-and-dna-markers/dna-ladder-rna-ladder/100bp-dna-ladder/?catNum=G2101
Beckman Coulter Life Sciences	A63881	AMPure XP Reagent	https://www.beckman.com/reagents/genomic/cleanup-and-size-selection/pcr

Contact us:

Application Note

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Appendix

Table 1: Data from single-sided DNA size selection

	100 bp				>100 bp			
Sample name	Concentration (pg/µl)	Average (pg/µl)	Recovery (%)	CV (%)	Concentration (pg/µl)	Average (pg/µl)	Recovery (%)	CV (%)
MFL-SS-01	37	36.5	32.5	11.6	1660	1690	107	4
MFL-SS-02	39.2				1670			
MFL-SS-03	41.9				1860			
MFL-SS-04	38.9				1720			
MFL-SS-05	46				1670			
MFL-SS-06	35.6				1590			
MFL-SS-07	31.9				1800			
MFL-SS-08	34.1				1720			
MFL-SS-09	33.4				1740			
MFL-SS-10	43.2				1840			
MFL-SS-11	38.8				1720			
MFL-SS-12	35.8				1730			
MFL-SS-13	33.7				1650			
MFL-SS-14	32.9				1660			
MFL-SS-15	30.8				1650			
MFL-SS-16	33.1				1710			
MFL-SS-17	33.3				1660			
MFL-SS-18	37.9				1630			
MFL-SS-19	42.8				1650			
MFL-SS-20	42.7				1710			
MFL-SS-21	36.1				1670			
MFL-SS-22	39.5				1700			
MFL-SS-23	43.2				1830			
MFL-SS-24	38.7				1700			
AMP-SS-01	25.8	30.5	27.3	11	1700	1695	107	2
AMP-SS-02	28.6				1740			
AMP-SS-03	28.9				1760			
AMP-SS-04	30.4				1720			
AMP-SS-05	32.7				1740			
AMP-SS-06	32.5				1680			
AMP-SS-07	34.4				1690			
AMP-SS-08	27.1				1680			
AMP-SS-09	27.4				1700			
AMP-SS-10	30.6				1680			
AMP-SS-11	29.4				1700			
AMP-SS-12	29.6				1700			
AMP-SS-13	37.2				1670			
AMP-SS-14	33.5				1730			
AMP-SS-15	33.8				1730			
AMP-SS-16	31				1690			
AMP-SS-17	29.2				1710			
AMP-SS-18	27.7				1660			
AMP-SS-19	36.6				1760			
AMP-SS-20	35.5				1730			
AMP-SS-21	28.5				1700			
AMP-SS-22	35.8				1750			
AMP-SS-23	33.6				1750			
AMP-SS-24	35.2				1680			
INPUT DNA ladder	118	111.7	100	4	1610	1580	100	2
INPUT DNA ladder	108				1560			
INPUT DNA ladder	111				1590			
INPUT DNA ladder	114				1620			

Application Note

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Table 2: Data from double-sided DNA size selection

Sample name	Average size (bp)	Average (bp)	SD (bp)	Concentration (ng/µl)	Average (ng/µl)	SD (ng/µl)	Recovery (%)
MFL-DS-01	362	372	14	0.644	0.7	0.12	12
MFL-DS-02	375			0.616			
MFL-DS-03	383			0.623			
MFL-DS-04	388			0.833			
MFL-DS-05	365			0.808			
MFL-DS-06	354			0.627			
MFL-DS-07	378			0.605			
MFL-DS-08	380			0.766			
MFL-DS-09	359			0.756			
MFL-DS-10	372			0.623			
MFL-DS-11	367			1.03			
MFL-DS-12	353			0.713			
MFL-DS-13	349			0.57			
MFL-DS-14	376			0.621			
MFL-DS-15	389			0.803			
MFL-DS-16	-			-			
MFL-DS-17	377			0.674			
MFL-DS-18	-			-			
MFL-DS-19	348			0.767			
MFL-DS-20	372			0.699			
MFL-DS-21	354			0.741			
MFL-DS-22	390			0.694			
MFL-DS-23	374			0.746			
MFL-DS-24	387			0.821			
AMP-DS-01	378	396	13	0.938	0.8	0.13	13
AMP-DS-02	417			0.812			
AMP-DS-03	399			0.835			
AMP-DS-04	409			0.87			
AMP-DS-05	385			0.911			
AMP-DS-06	377			0.839			
AMP-DS-07	379			0.867			
AMP-DS-08	405			0.61			
AMP-DS-09	399			0.583			
AMP-DS-10	394			0.774			
AMP-DS-11	415			0.771			
AMP-DS-12	-			-			
AMP-DS-13	400			0.855			
AMP-DS-14	403			0.91			
AMP-DS-15	389			0.685			
AMP-DS-16	392			0.924			
AMP-DS-17	397			0.84			
AMP-DS-18	365			0.809			
AMP-DS-19	408			0.88			
AMP-DS-20	401			0.745			
AMP-DS-21	396			0.62			
AMP-DS-22	397			0.878			
AMP-DS-23	399			0.911			
AMP-DS-24	-			-			
INPUT sheared gDNA	373	376	7.5	6.81	6	0.81	
INPUT sheared gDNA	366			6.51			
INPUT sheared gDNA	382			5.44			
INPUT sheared gDNA	381			5.13			

Application Note

Automating PacBio® SMRTbell® whole genome sequencing library prep on the MIRO CANVAS

Introduction

Long-read sequencing plays an important role in generating contiguous, high quality genomes for haplotype phasing, structural variant detection, and *de novo* assemblies.[1] Additionally, long-read libraries that are prepared without PCR amplification avoid a common source of base composition bias in sequencing data.[2]

Many long-read library prep workflows have traditionally used gel-based size selection to efficiently remove small molecules from the library. However, this type of size selection generally requires large DNA inputs and is not automatable. The PacBio SMRTbell Prep Kit 3.0 combines the advantages of PCR-free long-read sequencing with a streamlined protocol and fast bead-based size selection for easily automated long-read library preparation.

The MIRO CANVAS NGS prep system is a digital microfluidics (DMF) platform that allows low throughput workflow automation for complex protocols, such as NGS library preparation. The system is compatible with a wide range of reagents. This application note describes the results that can be expected when using the SMRTbell Prep Kit 3.0 in a protocol developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using the PacBio sequencing systems.

Key benefits:

- Whole genome sequencing (WGS) library preparation with PacBio's SMRTbell Prep Kit 3.0 is fully automated on the MIRO CANVAS using 1-3 µg of high quality, high molecular weight input DNA.
- This protocol offers the flexibility to choose automated, fast, bead-based size selection, or a more stringent gel-based size selection.
- Using the SMRTbell Prep Kit 3.0 protocol, MIRO CANVAS total library quantities, peak sizes and primary sequencing metrics are indistinguishable from manually prepared libraries.

Overview: How to automate PacBio SMRTbell WGS on the MIRO CANVAS

MIRO CANVAS NGS PREP SYSTEM

Application Note

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Experimental set-up

The SMRTbell Prep Kit 3.0 protocol was designed with automated systems – such as the MIRO CANVAS – in mind, and has been tested using high quality, high molecular weight 1-3 µg DNA inputs. Before beginning, DNA should be fragmented to 15-18 kb using a Megaruptor®, and quantified before and/or after fragmentation using a Q32850 Qubit™ dsDNA Quantification Assay Kit, Broad Range, or similar. Post-shearing clean-up, repair and A-tailing, adapter ligation, post-ligation clean-up, nuclease treatment and bead-based size selection for the SMRTbell Prep Kit 3.0 are all automated on the MIRO CANVAS (**Figure 1**), resulting in a ready-to-sequence library.

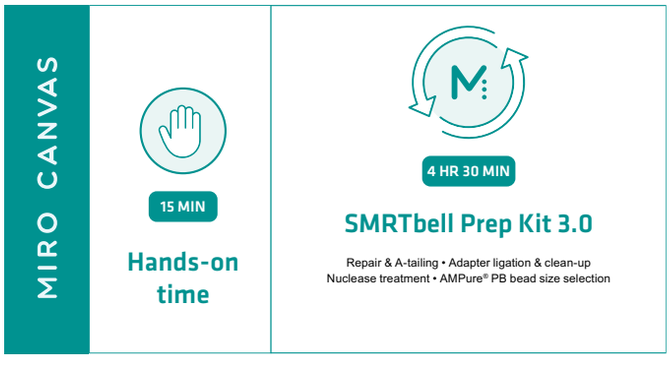

Figure 1: Experimental set-up. The MIRO CANVAS automates all of the steps for the SMRTbell Prep Kit 3.0 following reaction set-up.

Method and results

SMRTbell libraries were constructed with 1 µg of high quality, high molecular weight NA24385 (HG002)* DNA, manually and using the MIRO CANVAS. Final library quantities were assessed using broad range or high sensitivity Qubit kits. The MIRO CANVAS produced comparable libraries to manual preparation for each of the kits evaluated (**Table 1**).

* NA24385 DNA was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

Table 1: SMRTbell Prep Kit 3.0 libraries generated with the MIRO CANVAS were comparable to manually prepared libraries. Total library quantity shown as average +/- standard deviation (n=6).

		Total library (ng)	
	Input DNA	Manual	MIRO CANVAS
SMRTbell Prep Kit 3.0	1 µg	182 ±16	159 ±16

Application Note

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Efficient removal of small molecules from libraries using diluted bead-based size selection with the SMRTbell Prep Kit 3.0

Diluted bead-based size selection offers many advantages over traditional gel-based size selection of long-read libraries, including automatability, reduced workflow times and lower input requirements. Performing the SMRTbell Prep Kit 3.0 protocol on the MIRO CANVAS enabled construction of libraries from just 1 µg of input material, and efficiently removed small molecules from the library with automated bead-based size selection (**Figure 2**).

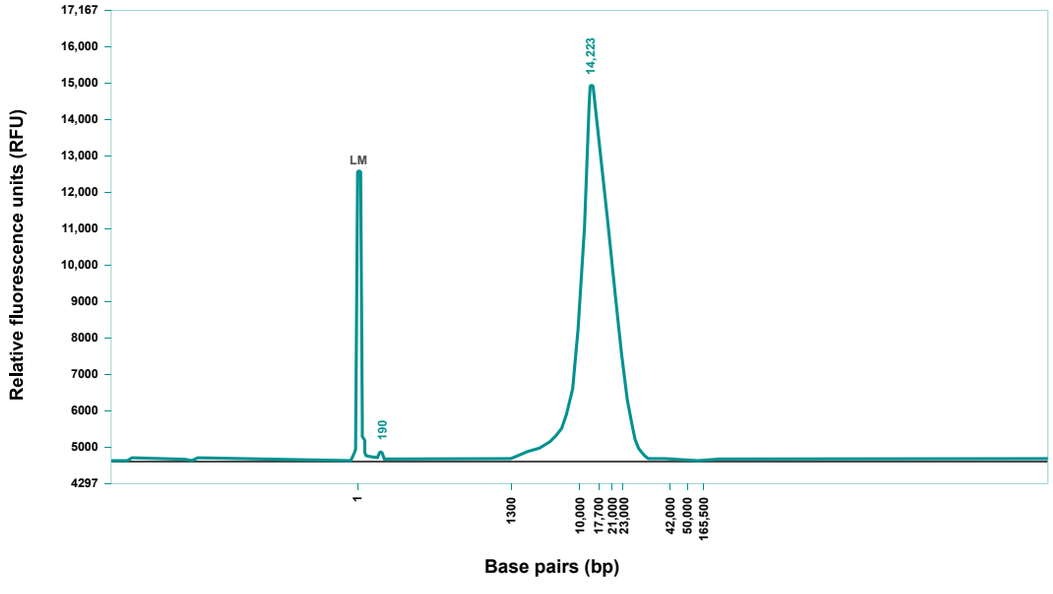

Figure 2: SMRTbell Prep Kit 3.0 library size distribution. Following automated preparation on the MIRO CANVAS, including bead-based size selection, libraries were examined with a Femto Pulse (Agilent) to demonstrate efficient removal of small library molecules. LM=lower marker.

SMRTbell libraries were sequenced on a Sequel® II System using binding kit 2.2, sequencing kit 2.0 (PacBio), and 30-hour movies. This demonstrated equivalency across manual and automated library preps. Of particular note, the new SMRTbell Prep Kit 3.0 not only requires less input material and eliminates the need for cumbersome gel-based size selection methods, it also results in libraries with excellent sequencing performance (**Figure 3**) and structural variant detection (**Figure 4**).

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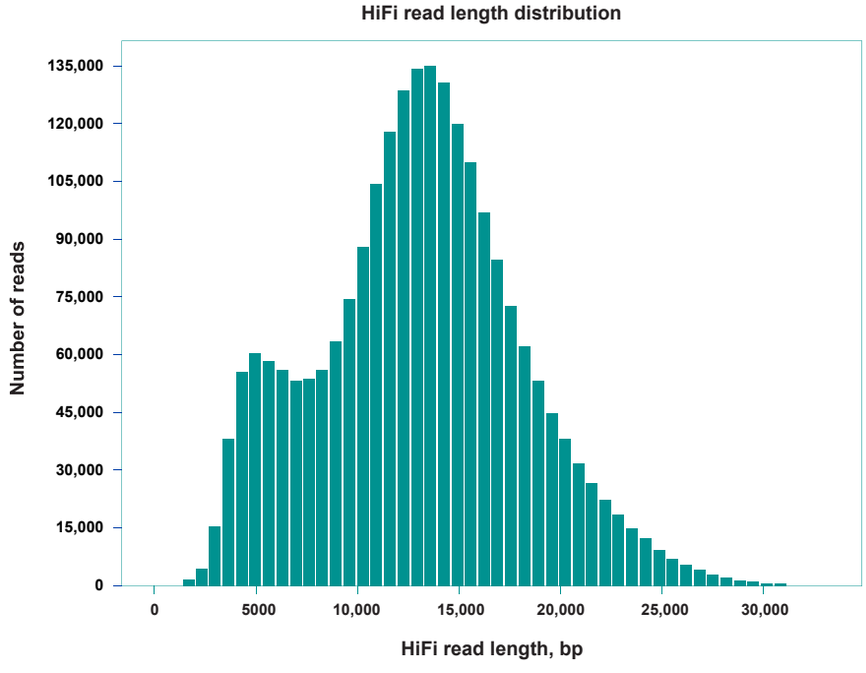
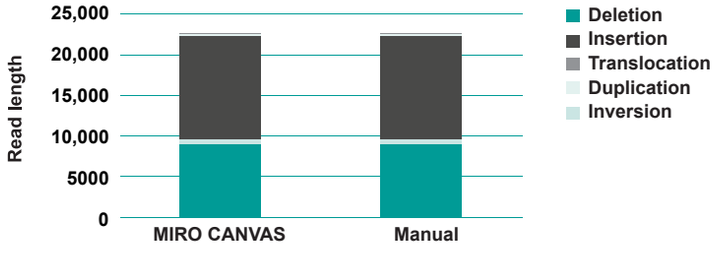


Value	Analysis metrics
2,268,270	HiFi reads
29,567,143,518	HiFi yield (bp)
13,035	HiFi read length (mean, bp)
Q37	HiFi read quality (median)

Figure 3: SMRTbell Prep Kit 3.0 sequencing metrics. MIRO CANVAS yield, read length and read quality metrics are all equivalent to manually prepared libraries.



	MIRO CANVAS	Manual
Deletion	9070	9174
Duplication	446	442
Insertion	12,586	12,437
Inversion	88	94
Translocation	122	162
Total	22,312	22,309

Figure 4: SMRTbell Prep Kit 3.0 structural variant detection. MIRO CANVAS detection of deletions, duplications, insertions, inversions and translocations in an NA24385 (HG002) DNA sample are all comparable to manually prepared libraries.

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Conclusion

- The MIRO CANVAS is an advanced DMF platform that can be used to automate library preparation with the PacBio SMRTbell Prep Kit 3.0.
- When using the SMRTbell Prep Kit 3.0 on the MIRO CANVAS, the protocol is fully automated from post-shear clean-up to elution.
- Both MIRO CANVAS and manual library preparation yield high quality libraries with comparable sequencing performance and structural variant detection.

References

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
PacBio	102-141-700	SMRTbell prep kit 3.0	https://www.pacb.com/wp-content/uploads/Insert-SMRTbell-prep-kit-3.0.pdf
Coriell Institute for Medical Research	NA24385 (HG002)	High molecular weight DNA	https://catalog.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA24385&Product=DNA

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Application Note

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Automating the Oxford Nanopore Ligation Sequencing Kit on the MIRO CANVAS

Introduction

Long-read sequencing is particularly well suited to the detection of large genomic mutations, coverage of long repeat regions that confound short-read assemblies,[1] and the identification of signatures that can be lost due to PCR amplification (including relative abundance in metagenomic samples[2] and nucleotide modifications present on original DNA).[3]

Oxford Nanopore Technologies (ONT) long-read sequencing of single-stranded DNA and RNA moving through nanoscale pores has been a major technological achievement in genomic research.[3,4] Its advantages include the use of a small, portable sequencer that can be deployed in the laboratory or the field, low capital cost requirements, rapid turnaround times, and a user-friendly bioinformatics pipeline that allows real-time analysis during sequencing.[2]

The MIRO CANVAS NGS prep system is a digital microfluidics (DMF) platform that allows low throughput workflow automation for complex protocols, such as NGS library preparation. The system is compatible with a wide range of reagents. This application note describes the results that can be expected when using the ONT Ligation Sequencing kit V14 (LSK-114) in a protocol developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using ONT sequencing platforms.

Key benefits:

- Library preparation using the ONT Ligation Sequencing kit is fully automated on the MIRO CANVAS.
- 75 % reduction in reaction volumes compared to manual library preparation.
- This protocol has been demonstrated on the MIRO CANVAS using 1 µg of high quality, high molecular weight input DNA.
- 2 hr 30 min run time.
- N50 comparable to manual library prep.

Overview: How to automate the ONT Ligation Sequencing kit on the MIRO CANVAS

MIRO CANVAS

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Experimental set-up

The protocol was designed for fully automated use on the MIRO CANVAS, and has been tested using 1 µg of high quality, high molecular weight (HMW) input DNA. Before beginning, DNA should be quantified using a Qubit™ dsDNA Quantification Assay Kit, Broad Range or similar. DNA repair and end prep, post-repair bead clean-up, adapter ligation and library clean-up are all automated on the MIRO CANVAS (**Figure 1**). Downstream quantification requires additional hands-on time.

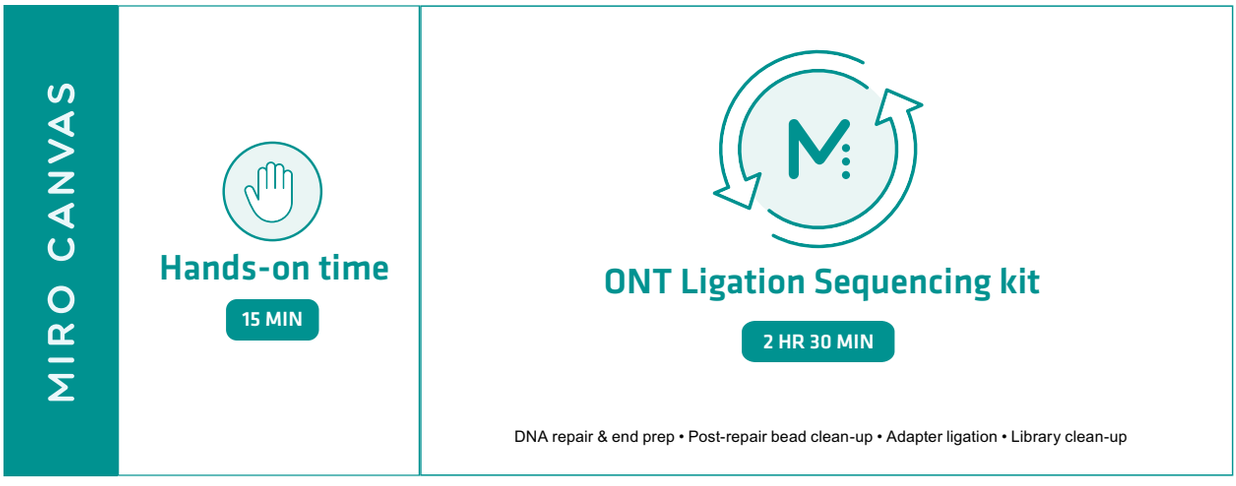

Figure 1: Experimental set-up. The MIRO CANVAS automates all the steps following reaction set-up, including DNA repair and end prep, post-repair bead clean-up, adapter ligation and library clean-up.

Results

1 µg of ZymoBIOMICS HMW DNA Standard was used as input for both manual library preparation and libraries prepared on the MIRO CANVAS, and the volumes listed in the ONT Ligation Sequencing Protocol were reduced by 75 %. Each library prepared was loaded into a MinION flow cell and sequenced for up to 3 hours. Libraries prepared using the automated workflow on the MIRO CANVAS produced read length distributions (**Figure 2**) and N50 read lengths (**Table 1**) comparable to those prepared using the manual technique.

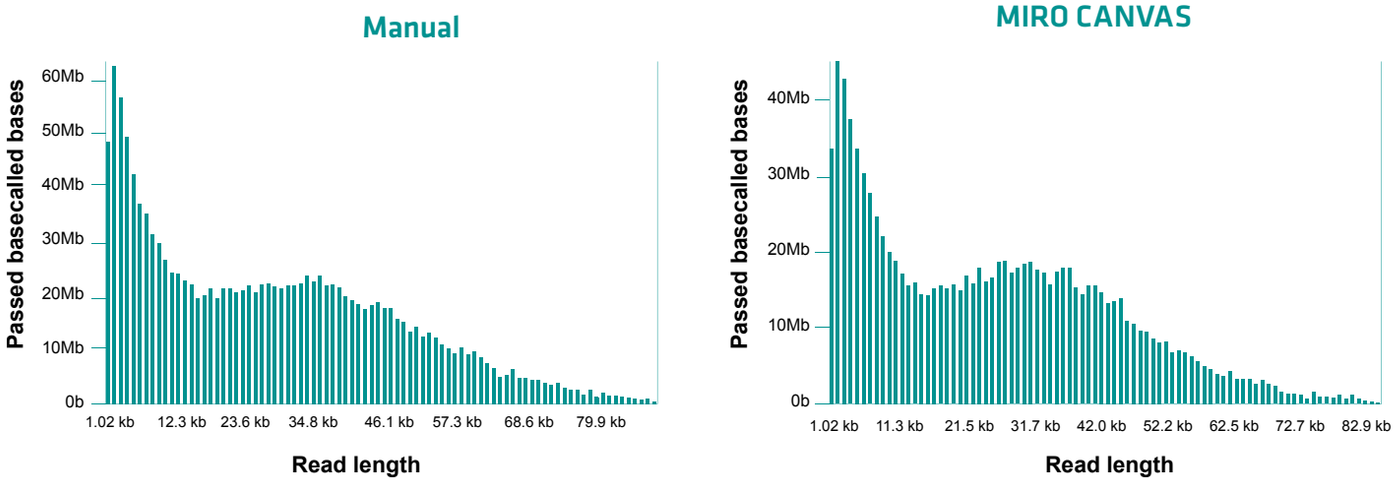

Figure 2: Read length distribution of libraries prepared manually and using the MIRO CANVAS. Representative read length histogram shows a similar distribution for libraries prepared both manually and using the MIRO CANVAS.

Application Note

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Representative sequencing metrics for libraries prepared manually and using the MIRO CANVAS are shown below. Read length and quality statistics are comparable between the MIRO CANVAS and manual preparation.

Table 1: Summary of the sequencing metrics for libraries prepared manually and using the MIRO CANVAS.

SEQUENCING METRICS	MANUAL	MIRO CANVAS
Mean read length	6051	5981
Mean read quality	10.8	9.7
Median read length	1663	1756
Median read quality	11.4	10.1
Numbers of reads	253,463	217,098
Read length N50	23.71 kb	23.06 kb
Total bases	1.55 Gb	1.31 Gb

The 5 longest sequenced reads in the MIRO CANVAS library were all longer than 120 kb, and of similar length to the 5 longest ranked reads from the manually prepared library. Additionally, the longest read from the MIRO CANVAS library exceeded the length of the longest read from the manually prepared library, and had a higher mean call base quality score (**Figure 3**).

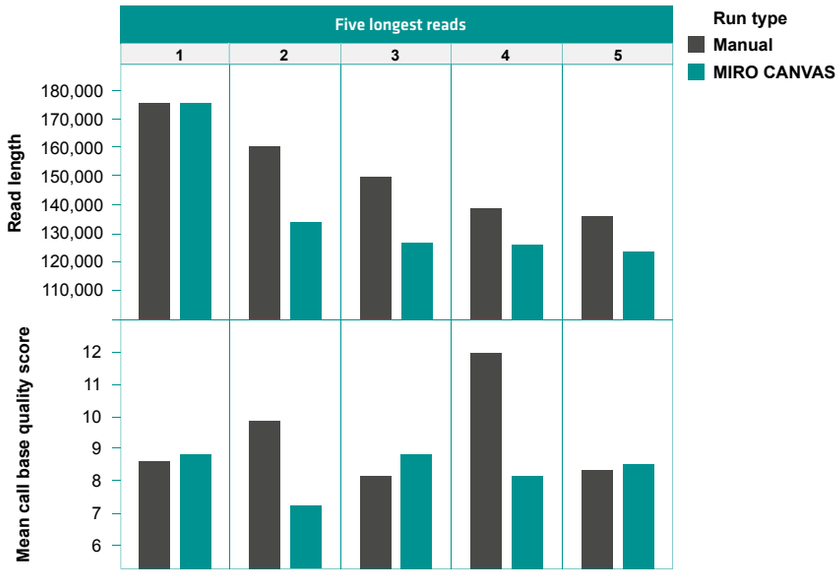

Figure 3: Read length and mean call base quality score for the 5 longest reads sequenced.

The ZymoBIOMICS HMW DNA Standard is composed of genomic DNA from 7 bacterial and 1 yeast species. All of the 8 expected species were identified through nanopore sequencing of libraries prepared both manually and using the MIRO CANVAS. **Figure 4** shows how the cumulative reads of each species compare between manual and MIRO CANVAS runs.

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Manual		MIRO CANVAS	
Salmonella	83,067	*Salmonella*	51,982
Escherichia	52,641	*Escherichia*	35,669
Enterococcus	20,011	*Enterococcus*	19,242
Staphylococcus	11,849	*Staphylococcus*	11,629
Pseudomonas	8362	*Pseudomonas*	8821
Listeria	8015	*Listeria*	8816
Bacillus	6334	*Bacillus*	7732
Shigella	2134	*Shigella*	1508
Saccharomyces	1413	*Saccharomyces*	1459

Figure 4: Cumulative read numbers for a representative mock community using libraries prepared both manually and with the MIRO CANVAS.

The compact nature, simple set-up and minimal infrastructure requirements (a 120 V adapter) of the MIRO CANVAS enable scientists to use it outside of the laboratory and aid collaboration between working groups. The MIRO CANVAS has been tested after air travel in carry-on baggage and in a backpack (**Figure 5**).

Figure 5: The MIRO CANVAS's compact dimensions (20.2 x 40.6 x 17.6 cm, w x d x h) make it easy to pack for travel, providing the ideal companion for ONT's portable sequencers.

Application Note

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Conclusion

- The MIRO CANVAS is an advanced DMF platform that can be used to automate library preparation with the ONT Ligation Sequencing kit.
- The ONT Ligation Sequencing Protocol for the MIRO CANVAS is fully automated – from DNA repair to elution – can reduce reagent volumes by 75 %, and yields results comparable to manual library preparation.
- The MIRO CANVAS's portability and compatibility with standard electrical sockets make it an ideal companion for highly portable ONT sequencers, offering library preparation and sequencing beyond the walls of the traditional laboratory.

References

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas

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Application Note

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Implementing the Twist Human Core Exome Kit on the MIRO CANVAS

Introduction

Whole exome sequencing (WES) provides a unique opportunity to dive deeply into the coding regions of the genome. It plays an important role in generating data for research and, in some cases, clinical applications.[1] Strong WES analyses rely on even coverage of these regions and, ultimately, on high quality capture reactions.[2] Variability in coverage can be minimized by ensuring that reagents are high quality, and by automating laboratory processes that have traditionally been performed manually.[3]

The Twist Human Core Exome Kit undergoes thorough quality control testing to ensure that all probes in the probe pools are present at the appropriate levels in order to limit wasted reads.[4] The uniformity of these reagents reduces costs and improves coverage of reads in singleplex and 8-plex pools.[4] Automating this kit on the MIRO CANVAS reduces the potential for contamination and variability.

The MIRO CANVAS also provides full walk-away automation, giving users the flexibility to perform other tasks while maintaining high quality results.

The MIRO CANVAS NGS prep system is a digital microfluidics platform that allows customized, low throughput workflow automation for complex protocols, such as NGS library preparation and hybridization capture. The system is compatible with a wide range of reagents.[5] This application note describes the results that can be expected when using the Twist Human Core Exome Kit in protocols developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using Illumina sequencing platforms.

Key benefits

- Library preparation and hybridization capture using the Twist Human Core Exome Kit are automated on the MIRO CANVAS.
- These protocols have been developed using 50 ng DNA input for library prep and 1500 ng for hybridization capture.
- Depth of coverage, quality scores and other key metrics are comparable between manually prepared libraries and those run on the MIRO CANVAS.
- Automating library preparation and hybridization capture on the MIRO CANVAS reduces hands-on time by over 85 %.

Overview: How to implement the Twist Human Core Exome Kit on the MIRO CANVAS

MIRO CANVAS NGS prep system

Application Note

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Experimental set-up

The fully automated Twist Universal Library Prep protocol has been tested using 50 ng of high molecular weight DNA. DNA should be quantified using the Qubit™ dsDNA Broad Range Quantification Assay or similar before starting.[6] Fragmentation, end repair, adapter ligation, amplification and purification steps are all automated in this protocol. The Twist Fast Hybridization Target Enrichment protocol has been tested using single samples and 8-plex pools, and the volume of each sample library used depends on their respective concentrations.[7] Minimal hands-on sample preparation is required at the beginning of this protocol, leaving most steps automated on the MIRO CANVAS, including hybridizing probes with pools, binding targets to beads, post-capture amplification and purification.

Figure 1: Experimental workflows. Both the MIRO Universal Library Prep and Twist Fast Hybridization Target Enrichment protocols are fully automated after reaction set-up.

Methods and results

Library preparation

The MIRO Universal Library Prep (Twist) protocol has been tested using 50 ng of NA12878* gDNA. Hybridization capture was then performed manually to assess the quality of the library preparation protocol alone on the MIRO CANVAS. Replicates of the libraries prepared on the MIRO CANVAS and manually (n=4, 8 in total) were sequenced on an Illumina NextSeq 500 or 550 platform, 75 Paired-End. Key metrics, such as depth of coverage and fold-80 scores, were comparable between the methods. Additionally, both methods yielded a similar percentage of duplicated reads and reads on target (**Figure 2**).

*NA12878 DNA was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

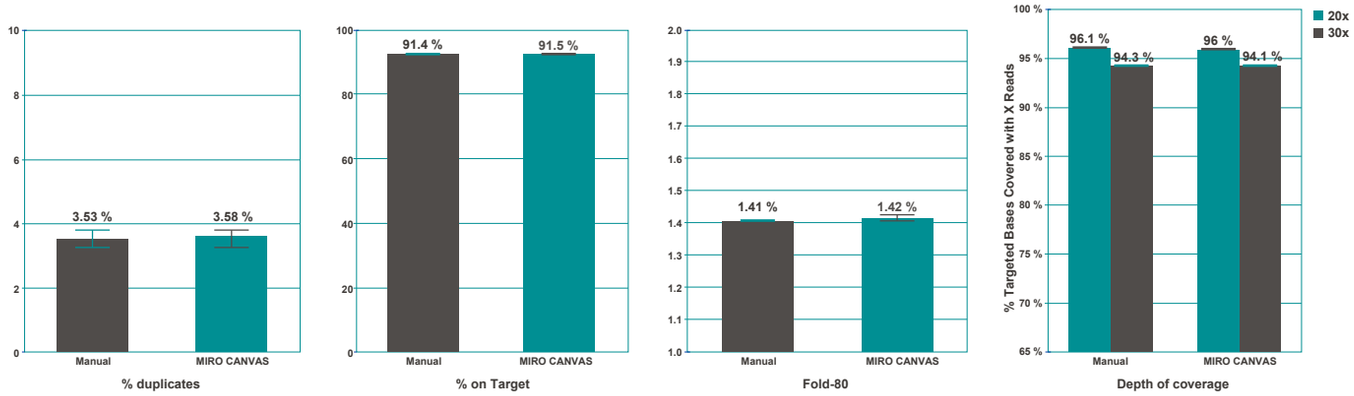

Figure 2: Multiple metrics were used to evaluate the sequencing data generated using both manual and Twist Universal Library Prep protocols. Hybridization capture was performed manually. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

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Target enrichment

The Twist Fast Hybridization Target Enrichment protocol has been tested using inputs of 1500 ng of mixed DNA for 8-plex pools (multiplex, 187.5 ng per library) and 500 ng for individual libraries (singleplex). Samples run on the MIRO CANVAS for hybridization capture previously underwent manual library preparation. Sequencing was performed using the NextSeq High Output 75PE platform. Singleplex and 8-plex pools run on the MIRO CANVAS were compared to singleplex samples that had been enriched manually. Coverage of target bases at 20x and 30x was comparable between manual and automated protocols for single samples and 8-plex pools. Fold-80 scores were also similar between methods, with median scores varying by no more than 0.02 (**Figure 3**).

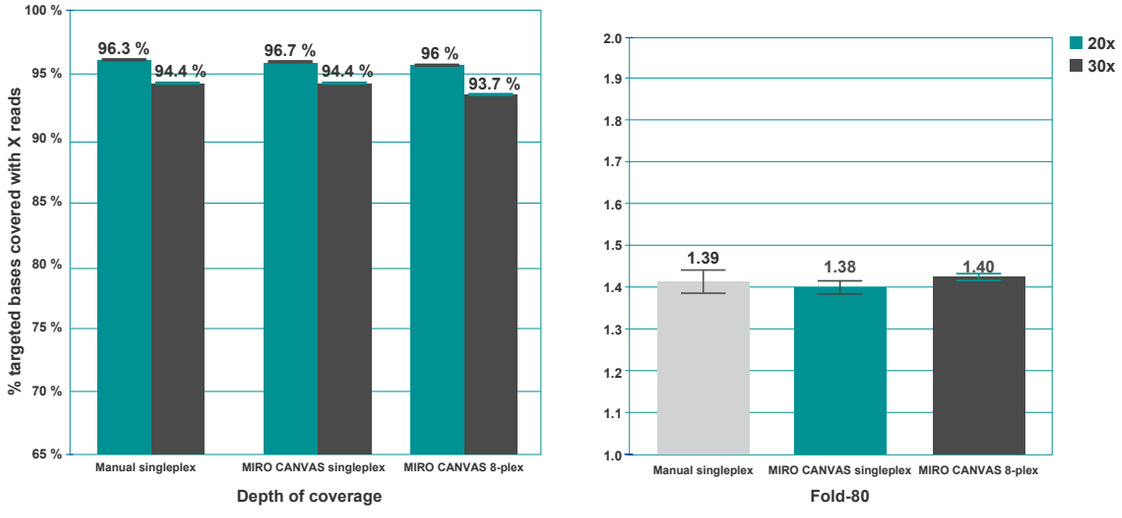

Figure 3: There was no significant difference between manual singleplex, MIRO CANVAS singleplex, and MIRO CANVAS 8-plex coverage of targets and fold-80 scores. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

Additional sequencing was completed using 2 x 8-plex pools, one prepared manually and the other on the MIRO CANVAS. Targets covered at 30x and fold-80 scores for each pool were comparable (**Table 1**). Reads for these runs were assessed using the MIRO CANVAS Integrative Genomics Viewer (IGV). The IGV outputs displayed confident mapping of target genes across multiple exons from pools enriched using the MIRO CANVAS (**Figure 4**).

Table 1: Key metrics were comparable for 8-plex pools enriched manually and on the MIRO CANVAS. All samples were subsampled to 150x raw sequencing coverage (70 M reads, 5.3 GB of data per sample).

METRIC	MANUAL 8-PLEX	MIRO CANVAS 8-PLEX
Fold-80	1.37	1.4
Off-bait	7 %	9.30 %
Mean coverage	58.4	72.4
Hs-library size	571 M	443 M
30x coverage	93 %	93.70 %
Zero coverage	1.10 %	1.30 %
AT dropout	6.88	5.71
GC dropout	0.64	0.74
Median insert size	248	200

Application Note

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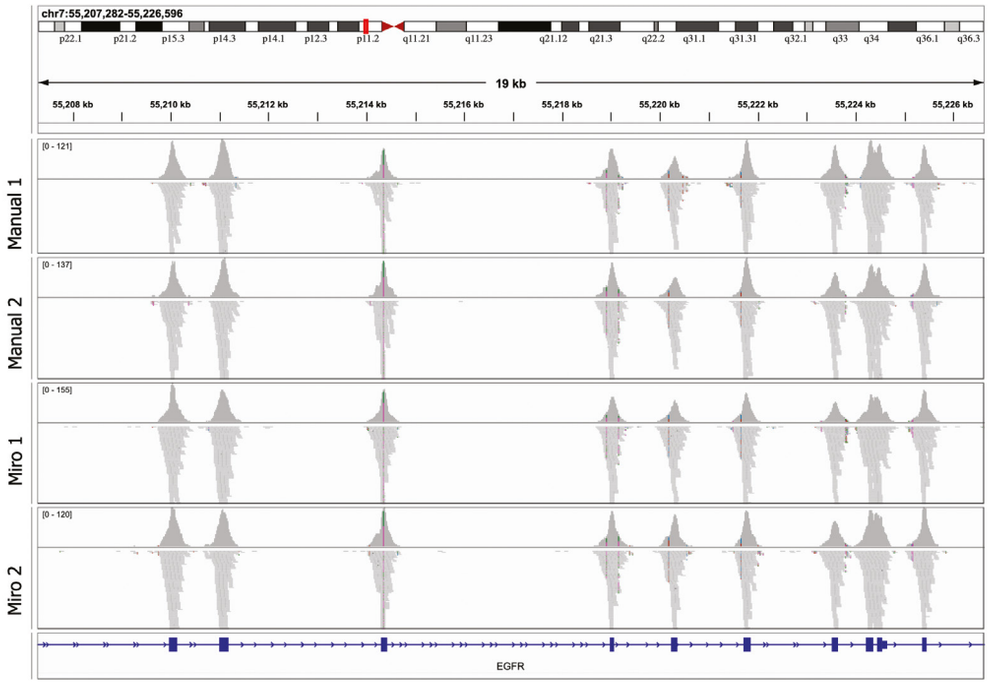

Figure 4: Representative IGV browser tracks of EGFR exons 2-10 from core exome-enriched libraries prepared manually and on the MIRO CANVAS.

Time savings

While the overall time taken between the library preparation and hybridization capture protocols does not vary significantly between manual preparation and MIRO CANVAS automation, the hands-on time is far less for the automated applications. Manual library preparation requires about 3 hours of hands-on time, while the Twist Universal Library Prep requires about 15 minutes to set up for a fully automated run of 3 hours and 20 minutes.

The Twist Fast Hybridization Target Enrichment protocol begins with several steps that cannot be automated, such as preparing pools and hybridization mix, and therefore requires about an hour of benchtop work. The steps that follow are fully automated on the MIRO CANVAS, taking about 5.5 hours to complete. This hour of hands-on time pales in comparison to the time taken by the manual protocol, which requires as much as 7.5 hours.

Application Note

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Conclusion

The MIRO CANVAS is an advanced digital microfluidics platform that can be used to automate library preparation and hybridization capture with the Twist Bioscience Human Core Exome Kit. Both the Twist Universal Library Prep and Twist Fast Hybridization Target Enrichment protocols are fully automated from fragmentation to elution and hybridization to elution, respectively. These automated protocols and their manual counterparts yield comparable results, but the greatly reduced hands-on time required by the MIRO CANVAS makes it a valuable tool for any laboratory.

References

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7. Miroculus, Inc. Miro Human Exome Hyb Capture (Twist) Protocol. Available at: https://www.integra-biosciences.com/global/en/ngs-automation/miro-canvas. Accessed: Feb 2022.

Materials

Manufacturer	Part Number	Description	Link
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INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
Twist Biosciences	102026	Twist Human Core Exome Kit, 12 Reactions	https://www.twistbioscience.com/products/ngs/fixed-panels/human-core-exome?tab=overview
Coriell Institute for Medical Research	NA12878	Genomic DNA from LCL	https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA12878&Product=DNA

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Application Note

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Enabling the Illumina DNA PCR-Free Library Prep kit on the MIRO CANVAS NGS prep system

Introduction

There is an increasing demand for NGS library preparation protocols that do not include PCR to avoid the introduction of PCR bias into the pool of DNA for sequencing.[1]

The Illumina DNA PCR-Free Prep kit follows a PCR-free workflow and is being increasingly used in sensitive applications such as whole genome sequencing (WGS) because it is both flexible and easy to automate.[2] Its on-bead tagmentation step is especially important for reducing library preparation time and sample input requirements. These features are of great interest for clinical applications such as tumor evaluations and newborn diagnostics, and are also important for research uses.

The MIRO CANVAS is a digital microfluidics (DMF) platform that allows custom low throughput workflow automation for complex protocols such as NGS library preparation. The system is compatible with a wide range of reagents.[3]

This application note describes the results that can be expected when using the Illumina DNA PCR-Free Prep kit in a protocol developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using Illumina platforms.

Key benefits

- Library preparation with the Illumina DNA PCR-Free Prep kit is fully automated on the MIRO CANVAS.
- This protocol has been demonstrated on the MIRO CANVAS using 50-500 ng DNA inputs.
- Sequencing metrics of libraries prepared on the MIRO CANVAS using this protocol are comparable to those of manually prepared libraries.
- Automation on the MIRO CANVAS reduces the amount of hands-on time required for library preparation by >60 % when using this protocol.

Overview: How to enable the Illumina DNA PCR-Free Library Prep kit on the MIRO CANVAS

MIRO CANVAS NGS prep system

Application Note

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Experimental set-up

The Illumina DNA PCR-free library prep protocol was designed for fully automated use on the MIRO CANVAS and has been tested using high quality DNA inputs in the 50-500 ng range. DNA should be quantified using a Qubit™ dsDNA Quantification Assay Kit, Broad Range, or similar before starting. Libraries are quantified using a Qubit ssDNA Assay Kit or qPCR. Tagmentation, post-tagmentation clean-up, ligation and library clean-up steps are all automated on the MIRO CANVAS (**Figure 1**). Downstream normalization and pooling require hands-on time.

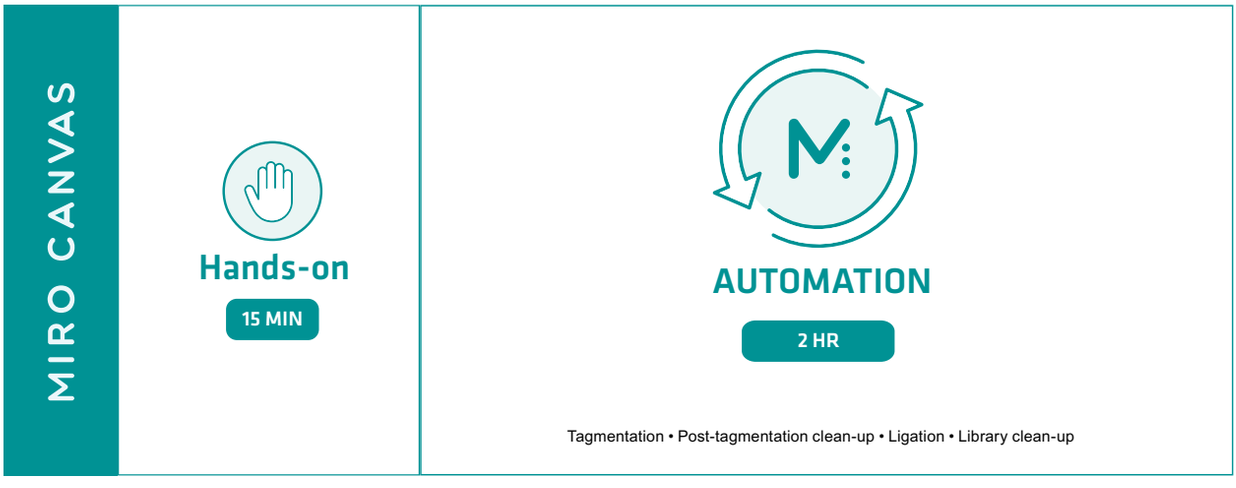

Figure 1: Experimental set-up. The MIRO CANVAS automates all the steps following reaction set-up, including tagmentation, post-tagmentation clean-up, ligation and library clean-up.

Results

Automating the experimental workflow on the MIRO CANVAS produces library yields and insert sizes that result in quality sequencing metrics. The Illumina DNA PCR-Free Prep protocol for standard inputs has been modified and tested using 50-500 ng NA12878 gDNA* on the MIRO CANVAS. In this modified version (**Table 1**), combining the standard input protocol volumes of DNA and bead-linked transposomes PCR-free (BLT-PF) with low input single-sided bead purification (1.8x ratio) was determined to be optimal for obtaining libraries of an ideal size and with sufficient quantity for sequencing (**Table 2**). Libraries prepared with as low as 50 ng of input gDNA were sequenced on a NovaSeq 6000 S4. The 1.8x ratio resulted in the kit's expected insert size of ~450 bp for >300 ng input. BLT-PF and DNA input volumes will need to be further adjusted for 50 ng input to achieve the expected insert size in both manual preparation and the automated workflow on the MIRO CANVAS.

*NA12878 gDNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

Table 1: Conditions for sample purification bead addition and insert size selection across the different DNA inputs tested. IPB=Illumina Purification Beads

	DNA VOLUME INPUT	BLT VOLUME INPUT	VOLUME INTO FIRST CLEAN-UP	FOLD FIRST CLEAN-UP	VOLUME FIRST IPB	TOTAL VOLUME INTO SECOND CLEAN-UP	FOLD SECOND CLEAN-UP	VOLUME SECOND IPB
Standard input	25 µl	15 µl	45 µl	0.8	36 µl	76 µl	1.8	42 µl
Low input	30 µl	10 µl	45 µl	1.8	81 µl	N/A	N/A	N/A
Modified version	25 µl	15 µl	45 µl	1.8	81 µl	N/A	N/A	N/A

Application Note

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Table 2: Library insert sizes and yields generated from different inputs of unsheared NA12878 DNA.

	MANUAL			MIRO CANVAS		
TOTAL WORKFLOW TIME	1 HR 45 MIN			1 HR 55 MIN		
DNA input amount (ng)	500	300	50	500	300	50
Mean yield (nM)	34.4	26.7	5.3	26.2	17.8	5.3
Median insert size (bp)	496	475	364	454	447	285

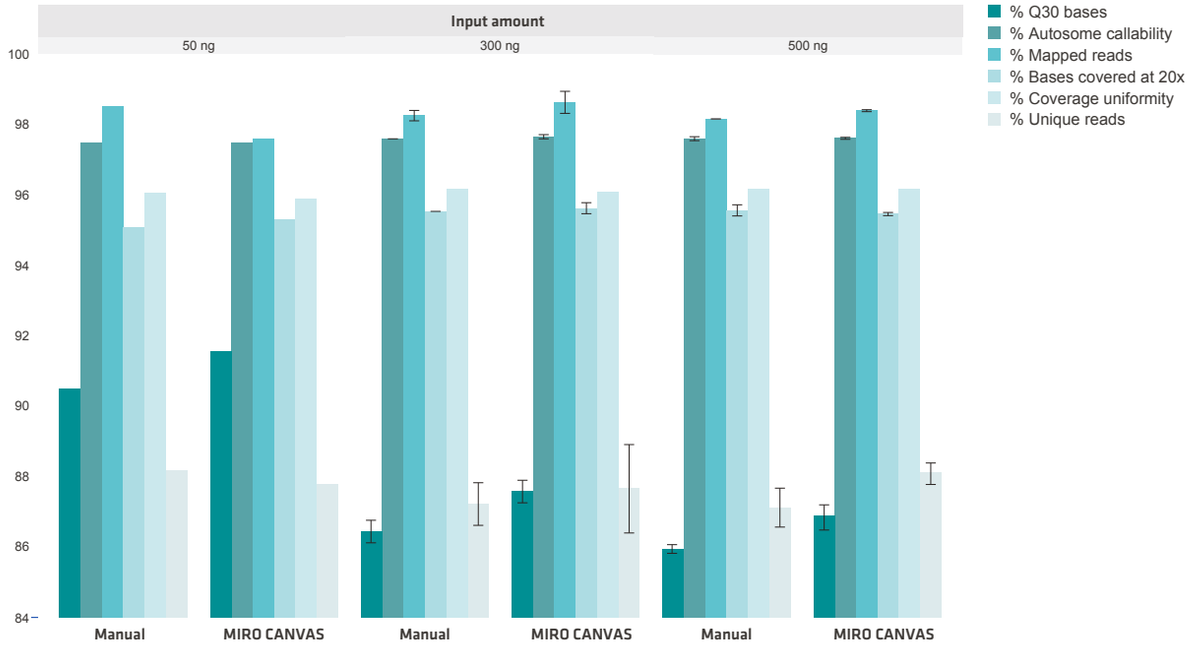

Figure 2: Sequencing metrics performance across a range of DNA inputs.

Illumina DNA PCR-free libraries prepared from a range of DNA inputs using either manual or MIRO CANVAS preparation methods demonstrate comparable % Q30 score, autosome callability, % mapped reads, % of bases covered at 20x, coverage uniformity and % unique reads.

The resulting sequencing metrics are comparable between manually prepared libraries and those generated using the automated workflow on the MIRO CANVAS (**Figure 2**). For DNA input amounts >300 ng, MIRO CANVAS libraries match or exceed the sequencing metrics for manual libraries, including base call accuracy, passing genotype calls in autosomal chromosomes, reads that confidently map to the reference genome, the % of bases covered at 20x, uniformity of coverage and duplication rates. Quality control (QC) metrics used in applications aimed at variant detection were additionally examined after subsampling to 40x sequencing coverage (**Table 3**). MIRO CANVAS libraries presented equal or better F1 scores for both SNVs and INDELs, as well as % bases covered at 20x, across all input ranges of tested DNA.

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Table 3: The performance of QC metrics relevant for variant calling across a range of input DNA. All samples were subsampled to 40x sequencing coverage.

SAMPLE ID	TOTAL NUMBER OF BASES SEQUENCED (GB)	AVERAGE AUTOSOMAL COVERAGE	% BASES COVERED AT 20x	AVERAGE MITOCHONDRIAL COVERAGE	TOTAL NUMBER SNVs	SNVs F1 SCORE	TOTAL NUMBER INDELs	INDELs F1 SCORE
Manual 500 ng	127	35.29	94.75	7527.37	4,052,452	99.90 %	25,511	99.55 %
MIRO CANVAS 500 ng	126	35.54	94.86	6023.38	4,055,818	99.90 %	25,565	99.59 %
Manual 300 ng	127	35.31	94.87	6667.97	4,051,961	99.90 %	25,473	99.57 %
MIRO CANVAS 300 ng	127	36.21	95.03	6947.82	4,054,773	99.91 %	25,563	99.59 %
Manual 50 ng	122	34.34	94.18	9902.86	4,053,721	99.88 %	25,772	99.50 %
MIRO CANVAS 50 ng	123	34.68	94.56	7956.68	4,054,906	99.89 %	25,643	99.54 %

MIRO CANVAS walk-away automation reduces hands-on time

The total time required for library preparation with the Illumina DNA PCR-Free Prep kit is 25 minutes greater when automated on the MIRO CANVAS, but the hands-on time is considerably less than for manual preparation workflows (**Table 1**). Automation with the MIRO CANVAS reduces hands-on time to zero for the tagmentation, post-tagmentation clean-up, ligation and library clean-up steps.

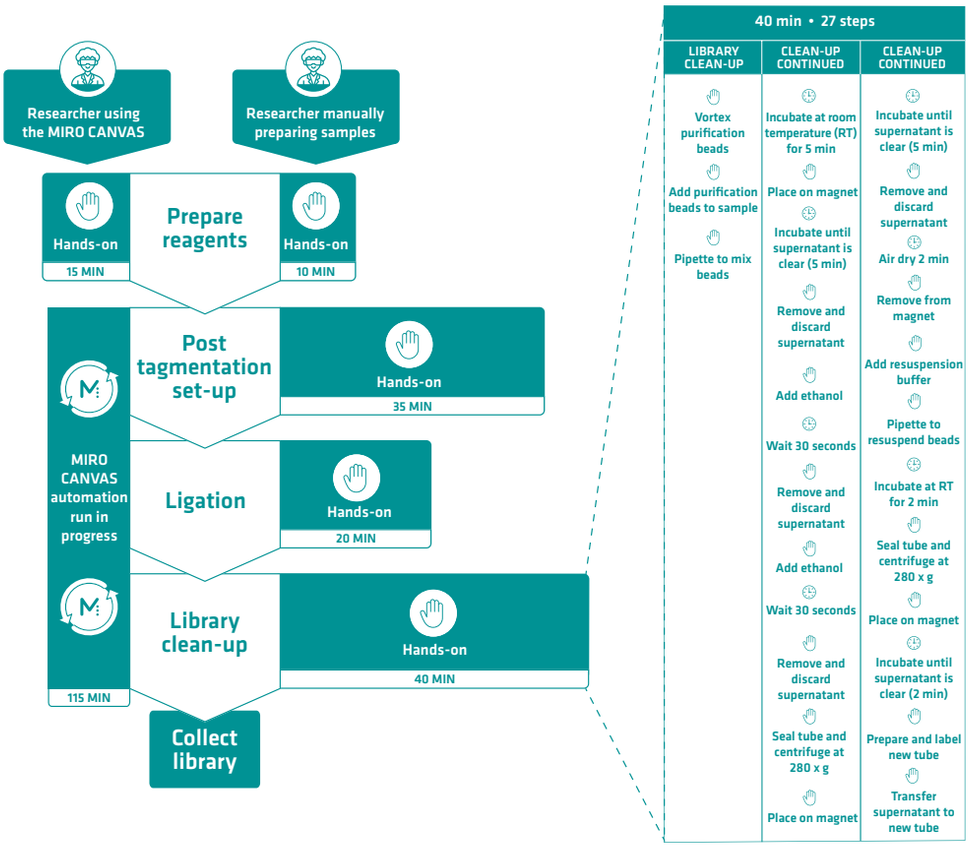

Figure 3: Average time requirements when manually preparing libraries or automating library preparation on the MIRO CANVAS with the Illumina DNA PCR-Free Prep kit.

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Conclusion

The MIRO CANVAS is an advanced DMF platform that can be used to automate library preparation with the Illumina DNA PCR-Free Prep kit. When using the Illumina DNA PCR-free library prep protocol for the MIRO CANVAS, the process is fully automated from the tagmentation incubation step to elution, and can be used with DNA inputs ranging from 50-500 ng. Both the MIRO CANVAS and manual library preparation yield comparable results, but the true walk-away automation and minimal hands-on time provided by the MIRO CANVAS makes it a valuable addition to any laboratory.

References

1. Kebschull JM *et al. Nucleic Acids Res* 2015; 43 (21): e143.
2. Yoo J *et al.* Poster 32 presented at the Association of Biomolecular Resources Facilities (ABRF) 2021 Virtual Annual Meeting; 7–11 March 2021.
3. Illumina DNA PCR-Free Prep. Available at: https://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html accessed April 2021.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
Illumina	20041794	Illumina DNA PCR-Free Prep kit	https://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html
Coriell Institute for Medical Research	NA12878	DNA standard	https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA12878&Product=DNA

Contact us:

Application Note

Lonza INTEGRA

Automated set-up of the Lonza PyroGene® Recombinant Factor C (rFC) Assay for endotoxin detection on the ASSIST PLUS

Introduction

The Lonza PyroGene rFC Assay is an alternative to the traditional limulus amebocyte lysate (LAL) assay, which is widely used to screen for bacterial endotoxin contamination in human and animal parenteral pharmaceuticals and medical devices. The rFC test is used in both high and low throughput laboratories and, unlike the LAL assay, is not derived from horseshoe crab blood.

Setting up the test requires preparation of 10-fold diluted standards from the endotoxin stock solution supplied. Standards and samples are tested in duplicate in a 96 well plate. To check for product inhibition, positive product controls (PPCs) – samples spiked with a known concentration of endotoxin – are tested alongside the samples. Following the initial plating of the standards, samples and PPCs, a 10-minute pre-incubation is performed, during which the user prepares a working solution consisting of the fluorogenic substrate, assay buffer and rFC enzyme.

This application note demonstrates that the preparation of the standards, samples, PPCs and blanks – and their addition to the 96 well plate – can be easily automated on the ASSIST PLUS pipetting robot using a D-ONE single channel pipetting module. Addition of the working reagent is then completed using a VOYAGER adjustable tip spacing multichannel pipette. Automation of all the pipetting steps reduces the opportunity for pipetting errors, and ensures assay robustness and reproducibility. The key quality indicators in this assay are the correlation coefficient of the standard curve and coefficient of sample variation (CV).

Key benefits:

- Automated preparation of standard dilutions eliminates pipetting errors that could invalidate entire runs.
- Users can perform testing of full or partial plates by using the D-ONE single channel pipetting module in combination with the ASSIST PLUS.
- Error-free pipetting ensures replicate samples with tight CV values, reducing the likelihood of repeat testing.
- On-screen prompts guide the user through instrument set-up.

Overview: How to perform the PyroGene rFC Assay

ASSIST PLUS

Application Note

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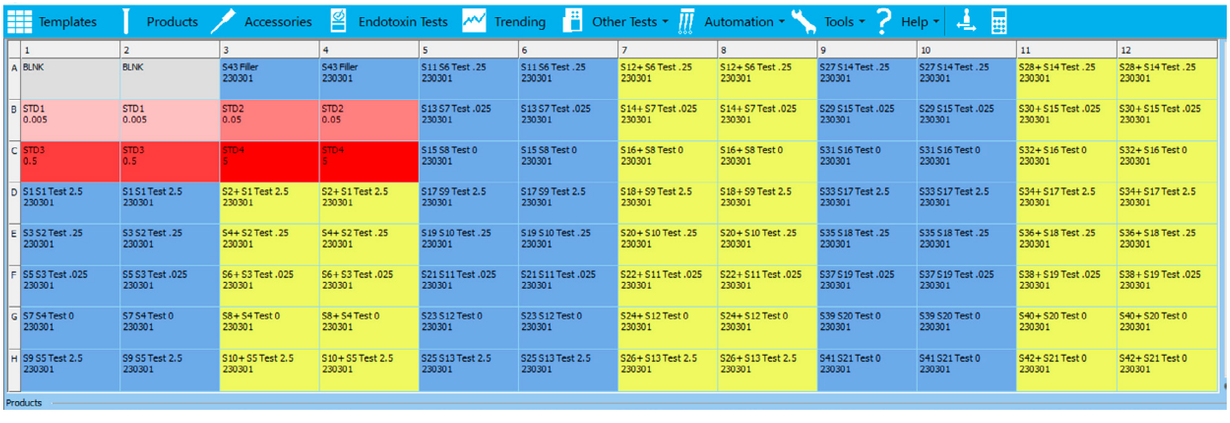
The PyroGene rFC Assay is a fluorogenic assay that requires a fluorescence microplate reader – such as the PyroWave® XM Fluorescence Reader, paired with the WinKQCL® Endotoxin Detection & Analysis Software – to measure endotoxin values. Prior to setting up the plate, a template is prepared in WinKQCL software. **Figure 1** shows the WinKQCL software template that was developed for use with the ASSIST PLUS. This template allows up to 21 samples to be tested in duplicate on one plate, with paired PPCs. It is designed to provide optimal flexibility for varying numbers of samples, while still allowing use of an 8 channel pipette to deliver the rFC working solution to the plate, as described in the assay instructions for use.



	1	2	3	4	5	6	7	8	9	10	11	12
A	BLNK	BLNK	S43 Filler 230301	S43 Filler 230301	S11 S6 Test .25 230301	S11 S6 Test .25 230301	S12+ S6 Test .25 230301	S12+ S6 Test .25 230301	S27 S14 Test .25 230301	S27 S14 Test .25 230301	S28+ S14 Test .25 230301	S28+ S14 Test .25 230301
B	STD1 0.005	STD1 0.005	STD2 0.05	STD2 0.05	S13 S7 Test .025 230301	S13 S7 Test .025 230301	S14+ S7 Test .025 230301	S14+ S7 Test .025 230301	S29 S15 Test .025 230301	S29 S15 Test .025 230301	S30+ S15 Test .025 230301	S30+ S15 Test .025 230301
C	STD3 0.5	STD3 0.5	STD4 5	STD4 5	S15 S8 Test 0 230301	S15 S8 Test 0 230301	S16+ S8 Test 0 230301	S16+ S8 Test 0 230301	S31 S16 Test 0 230301	S31 S16 Test 0 230301	S32+ S16 Test 0 230301	S32+ S16 Test 0 230301
D	S1 S1 Test 2.5 230301	S1 S1 Test 2.5 230301	S2+ S1 Test 2.5 230301	S2+ S1 Test 2.5 230301	S17 S9 Test 2.5 230301	S17 S9 Test 2.5 230301	S18+ S9 Test 2.5 230301	S18+ S9 Test 2.5 230301	S33 S17 Test 2.5 230301	S33 S17 Test 2.5 230301	S34+ S17 Test 2.5 230301	S34+ S17 Test 2.5 230301
E	S3 S2 Test .25 230301	S3 S2 Test .25 230301	S4+ S2 Test .25 230301	S4+ S2 Test .25 230301	S19 S10 Test .25 230301	S19 S10 Test .25 230301	S20+ S10 Test .25 230301	S20+ S10 Test .25 230301	S35 S18 Test .25 230301	S35 S18 Test .25 230301	S36+ S18 Test .25 230301	S36+ S18 Test .25 230301
F	S5 S3 Test .025 230301	S5 S3 Test .025 230301	S6+ S3 Test .025 230301	S6+ S3 Test .025 230301	S21 S11 Test .025 230301	S21 S11 Test .025 230301	S22+ S11 Test .025 230301	S22+ S11 Test .025 230301	S37 S19 Test .025 230301	S37 S19 Test .025 230301	S38+ S19 Test .025 230301	S38+ S19 Test .025 230301
G	S7 S4 Test 0 230301	S7 S4 Test 0 230301	S8+ S4 Test 0 230301	S8+ S4 Test 0 230301	S23 S12 Test 0 230301	S23 S12 Test 0 230301	S24+ S12 Test 0 230301	S24+ S12 Test 0 230301	S39 S20 Test 0 230301	S39 S20 Test 0 230301	S40+ S20 Test 0 230301	S40+ S20 Test 0 230301
H	S9 S5 Test 2.5 230301	S9 S5 Test 2.5 230301	S10+ S5 Test 2.5 230301	S10+ S5 Test 2.5 230301	S25 S13 Test 2.5 230301	S25 S13 Test 2.5 230301	S26+ S13 Test 2.5 230301	S26+ S13 Test 2.5 230301	S41 S21 Test 0 230301	S41 S21 Test 0 230301	S42+ S21 Test 0 230301	S42+ S21 Test 0 230301

Figure 1: Lonza rFC template in WinKQCL software. Red wells: standards; blue wells: samples in duplicate (wells A3 and A4 are empty); yellow wells: PPCs tested in duplicate.

A 5-300 µl D-ONE single channel pipetting module is first used to prepare standard dilutions. Following preparation of the standards, PPC is added to the designated wells using repeat dispense mode. Next, standards and samples are added to the plate in duplicate according to the above template, with samples added to both clean and PPC-spiked wells. Once all samples are added, the plate is pre-incubated at 37 °C for 10 minutes. During the incubation, the user prepares the working reagent in an INTEGRA 10 ml SureFlo™ reagent reservoir, which is added to the plate at the end of the pre-incubation period using a 300 µl VOYAGER 8 channel pipette on the ASSIST PLUS.

TIPS:

- Use pyrogen-free certified GRIPTIPS® pipette tips in combination with SureFlo reservoirs to ensure accurate results. 10 ml SureFlo reservoirs require a dead volume of less than 30 µl.
- 300 µl long GRIPTIPS can access sample volumes of below 1 ml in 13x100 mm Lonza pyrogen-free test tubes, and will never leak or fall off.
- PPCs and samples can be dispensed using repeat dispense mode to save time and money.
- Plate layout of the blank, standards and samples in the WinKQCL software template is designed to offer the most flexibility for running full or partial plates.

Experimental set-up

Deck Position A: LAL water – 10 ml multichannel reagent reservoir
Deck Position B: PyroGene rFC Assay plate – 96 well flat bottom plate (Corning)
Deck Position C: Standards, samples and PPC – INTEGRA tube rack

Application Note

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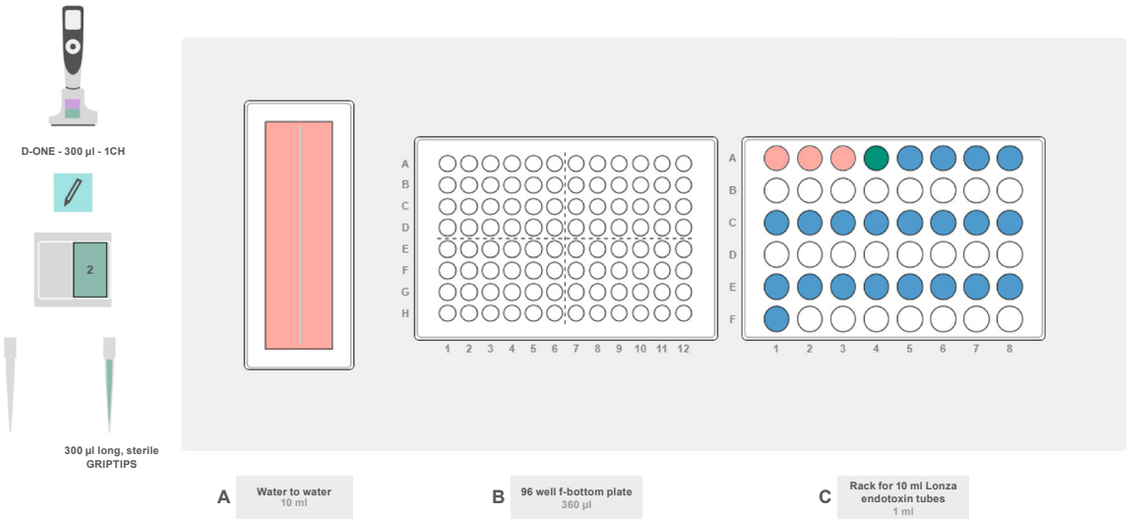

Figure 2: Set-up of the ASSIST PLUS for assay sample and standard addition. Position A: LAL water in 10 ml SureFlo reservoir. **Position B:** 96 well assay plate. Position C: Tube rack with Lonza pyrogen-free tubes (empty, magenta), 250 µl of 20 EU/ml endotoxin standard (green) and 1 ml of sample (blue).

1. PyroGene rFC Assay plate set-up

STEP: Standards, samples and PPC-spiked samples are added to the plate.

HOW TO: Pair the 0.5-300 µl D-ONE single channel pipetting module with the ASSIST PLUS pipetting robot. Place pyrogen-free test tubes containing 1 ml sample in the tube rack on deck Position C (blue tubes in **Figure 2**). Place three empty tubes in Positions A1-A3 within the rack. These are used to create the standard dilutions (magenta tubes in **Figure 2**). Place a tube holding 250 µl of 20 EU/ml endotoxin standard in A4 in the rack (green tube in **Figure 2**). Place a 10 ml SureFlo reservoir holding LAL water on deck Position A, and a 96 well flat bottom plate on deck Position B.

When the 'Lonza PyroGene Assay plate set-up' program is started, the pipette first dispenses 900 µl of LAL water into each of the 3 empty dilution tubes (**Figure 3**). Next, 750 µl of LAL water is dispensed into the tube holding 250 µl of 20 EU/ml endotoxin standard, creating a 5 EU/ml standard. Following package insert instructions, a message on the pipette instructs the user to vortex the 5 EU/ml standard for 1 minute. The ASSIST PLUS pauses for the user to perform this step, then restarts when the user acknowledges the message. The next standard is created by transfer of 100 µl of the 5 EU/ml standard to the adjacent tube holding 900 µl of LAL water, followed by vortexing. The remaining 2 standards are created in a similar manner.

Application Note

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Figure 3: LAL water is dispensed by the D-ONE single channel pipetting module.

Once all the standards have been created, 10 µl of the 5 EU/ml standard is added to each well of the plate designated as a PPC (**Figure 4**). This serves as a control to monitor for sample inhibition of endotoxin detection. Each blank, standard and sample is then added to the appropriate wells in duplicate. Duplicate samples are also added to the PPC wells, creating the 0.5 EU/ml PPC-spiked samples. When all standards and samples have been added to the plate, the plate is pre-incubated at 37 °C for 10 minutes.

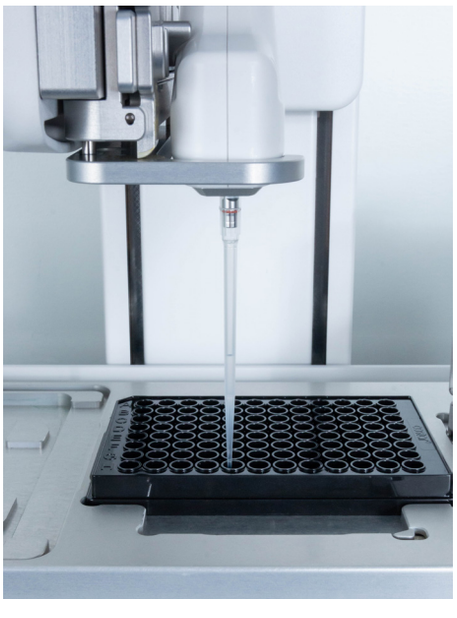

Figure 4: PPC is added by the D-ONE single channel pipetting module.

Application Note

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2. PyroGene rFC Assay reagent addition

STEP: Add 100 µl working reagent to each well of the plate.

HOW TO: While the plate is pre-incubating, manually prepare the working reagent in a 10 ml SureFlo reservoir by combining fluorogenic substrate, rFC assay buffer and rFC enzyme solution in a 5:4:1 ratio. Place the working reagent in a clean 10 ml SureFlo reservoir on deck Position A (**Figure 5**). Pair a 300 µl 8 channel VOYAGER pipette with the ASSIST PLUS, and exchange the D-ONE tip deck for a standard tip deck. At the end of the 10 minute pre-incubation, place the plate on deck Position B. Initiate the VIALAB program 'Lonza PyroGene Assay reagent addition' to add 100 µl reagent to each well of the plate. When reagent addition is complete, place the plate in the fluorescence microplate reader to complete the assay.

TIPS:

- VIALAB programs can be adapted to accommodate different numbers of samples, providing flexibility to meet current and future testing demands.
- For simplicity, 300 µl long GRIPTIPS are used in all steps of this assay.

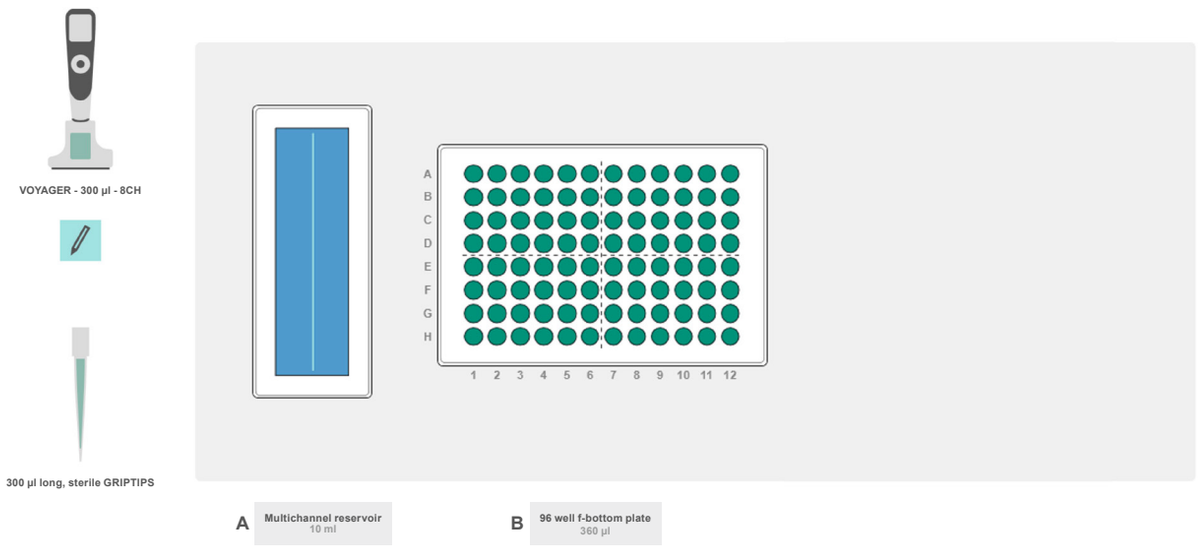

Figure 5: Deck set-up of the ASSIST PLUS for reagent addition. **Position A:** working endotoxin reagent in a 10 ml SureFlo reservoir. **Position B:** 96 well plate containing pre-incubated standards and samples.

Application Note

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Assay verification

Three runs of 21 samples plus standards were set up on the ASSIST PLUS, and tested according to the PyroGene rFC Assay instructions for use. Samples consisted of LAL water spiked with known concentrations of endotoxin standard. Five samples at each concentration were run on each plate, except for the 0 EU/ml sample, which was run 6 times per plate. Data analysis was performed using WinKQCL software. **Table 1** shows the concentrations of standards and samples.

Table 1: Endotoxin concentrations of standards and spiked samples.

Standards (EU/ml)	Samples of LAL water spiked with endotoxin (EU/ml)
5	2.5
0.5	0.25
0.05	0.025
0.005	0

Results

The results are displayed in **Tables 2** and **3**. The standard curve for all runs displayed good linearity, and all curves were within the quality parameters as defined in the instructions for use (**Table 2**). Samples spiked with each concentration of endotoxin were detected (**Figure 6**). All unspiked samples remained undetectable at <0.005 EU/ml, which is the cut-off for acceptable endotoxin concentrations in pharmaceuticals and medical devices. All replicates of standards, samples and PPCs displayed a CV within the acceptable limit of less than 25 % (**Table 3**).

Table 2: Standard curve results with quality specifications for runs 1-3.

	Correlation coefficient (0.980-1.000)	Slope (0.760-1.110)	Y-intercept (2.500-5.000)
Run 1	1.000	0.930	4.112
Run 2	0.999	0.903	4.258
Run 3	0.998	0.909	4.267

Application Note

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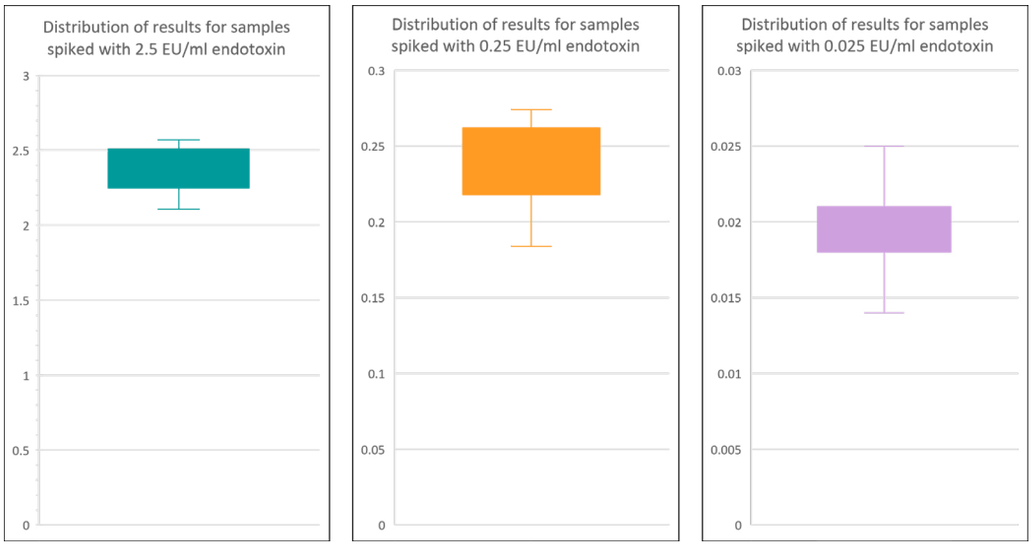

Figure 6: Distribution of results for samples spiked at endotoxin concentrations of 2.5, 0.25 and 0.025 EU/ml.

Table 3: Mean CV per plate for paired samples and PPCs.

	Mean paired sample %CV	Mean PPC %CV	QC fail %CV per plate
Run 1	4.19	4.37	0
Run 2	1.84	4.03	0
Run 3	3.66	3.87	0
Overall mean	3.23	4.09	0

Remarks

- **Partial plates:** The supplied VIALAB programs can be adapted for partial plates, or for running samples in triplicate.
- **Run report:** VIALAB programs can be started directly from a PC connected to the ASSIST PLUS pipetting robot. A report is automatically generated after the run, documenting details such as start and end times, user identification, calculated volumes and any errors that occurred. This offers a convenient way to fulfill regulatory requirements.

Conclusion

- The ASSIST PLUS provides an affordable, easy-to-use automation solution for low to medium throughput users of the Lonza PyroGene rFC Assay.
- High quality, reproducible results can be achieved with the ASSIST PLUS, eliminating the risk of costly and time-consuming retests.
- The ease and flexibility of VIALAB software allows users to customize plate layouts or set up partial plates using the same labware defined in this application note.

Application Note

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4531	0.5-300 µl D-ONE single channel pipetting module	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4723	300 µl 8 channel VOYAGER electronic pipette	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbersrs
INTEGRA Biosciences	4535	Tip deck for D-ONE on ASSIST PLUS	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4552	6 x 8 tube rack	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	6484, specific lots	300 µl long, sterile (certified endotoxin free) GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/grip-tip-selector-guide
INTEGRA Biosciences	4370, 4372, Specific lots	10 ml sterile polystyrene SureFlo Reagent Reservoir, certified endotoxin free	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
Lonza	50-658U, 50-658NV	PyroGene Recombinant Factor C (rFC) Assay	https://bioscience.lonza.com/lonza_bs/CH/en/recombinant-factor-c-assay
Lonza	25-345S	Pyrowave XM Fluorescence Reader	https://bioscience.lonza.com/lonza_bs/US/en/Endotoxin-Detection/p/000000000000213090/PyroWave™-XM-Fluorescence-Reader
Lonza	25-611	WinKQCL Software	https://bioscience.lonza.com/lonza_bs/US/en/winkqcl-endotoxin-detection-and-analysis-software
Lonza	N207	Pyrogen-free Dilution Tubes	https://www.lonzabioscience.com.au/product/pyrogen-free-dilution-tubes-13x100mm-without-cap/
Lonza	W50-640	LAL Reagent Water	https://bioscience.lonza.com/lonza_bs/CH/en/Endotoxin-Detection/p/000000000000187031/LAL-Reagent-Water
Corning	3603	96 well, flat, clear bottom, black polystyrene, TC-treated microplate	https://ecatalog.corning.com/life-sciences/b2b/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Corning®-96-well-Black-Clear-and-White-Clear-Bottom-Polystyrene-Microplates/p/3603
IKA		Vortex 2	https://www.ika.com/en/Products-LabEq/Shakers-pg179/Vortex-2-25000258/

Contact us:

Application Note

INTEGRA

Western blot protocol automation with the ASSIST PLUS pipetting robot and Simple Western™

Introduction

Automated western blotting is a cutting-edge technology revolutionizing traditional protein detection and analysis. Employing robotic systems, microscale separation, and advanced imaging technologies streamlines the labor-intensive and time-consuming process of western blotting. Automating the multiple workflow steps increases efficiency, making it an invaluable tool in various fields of biological research.

Simple Western – developed by ProteinSimple, a Bio-Techne brand – is the only fully automated western blotting solution on the market. The advanced, capillary-based technology enables efficient and accurate high throughput protein separation, detection and quantification, with all assay reagents and samples in 1 plate. Simple Western assays are advancing research and development in many applications, including cancer and immuno-oncology, cell and gene therapy, regenerative medicine and targeted protein degradation.

The ASSIST PLUS pipetting robot and D-ONE single channel pipetting module effortlessly fill plates specifically designed for Simple Western instruments – like Jess™ or Abby™ – allowing 100 % hands-free time without worrying about tedious liquid handling. INTEGRA protocols automate the Simple Western plate set-up for chemiluminescence detection of single target, multi-target (using RePlex™) or total protein assays. This application note describes fully automated liquid handling of all 3 assay types by performing chemiluminescence detection and total protein analysis in 1 RePlex normalization assay. The results show equivalent performance to manual plate filling, with comparable on-deck sample and reagent stability.

Key benefits:

- Full walk-away western blot protocol automation combines the ASSIST PLUS pipetting robot with the D-ONE single channel pipetting module and the Simple Western Jess. On top of that, the operator benefits from VIALAB's flexibility to create sample preparation protocols for the ASSIST PLUS.
- Foolproof liquid handling and plate filling with D-ONE's liquid level detection (LLD) and automated GRIPTIPS® pipette tip selection, ensuring precision and accuracy for both low and high volume transfers.
- VIALAB's labware tool simplifies the labware definition for unique plates, and the D-ONE module can even accommodate irregular well distributions.
- Simple Western advanced capillary electrophoresis immunoassay technology enables reliable, high throughput, automated western blot analysis of up to 24 samples per run, with results ready in as little as 3 hours.
- Simple Western chemiluminescence and NIR/IR fluorescence detection provide flexible multiplex analysis capabilities, and ensure high sensitivity when working with precious samples or low abundance targets. RePlex enables 2 immunoassays in a single capillary, and even provides total protein detection to accurately normalize protein expression data.
- The small footprints of the ASSIST PLUS and Jess instruments save space, so they fit easily into any laboratory.

ASSIST PLUS

Overview: How to fill the Simple Western Jess plate with the ASSIST PLUS

Application Note

INTEGRA

The ASSIST PLUS pipetting robot and D-ONE single channel pipetting module, together with the Simple Western Jess, automate all the liquid handling steps required to analyze 24 samples, providing a complete walk-away solution for western blot protocol automation.

The whole workflow consists of 2 steps (**Figure 1**):

1. Simple Western Jess plate filling with the D-ONE and the ASSIST PLUS
2. Protein analysis with the Simple Western Jess

This application note provides Jess plate filling protocols for chemiluminescence detection of single targets, multiple targets (using RePlex) and total protein analysis of prepared samples and reagents for reliable downstream protein analysis using Simple Western Technology.

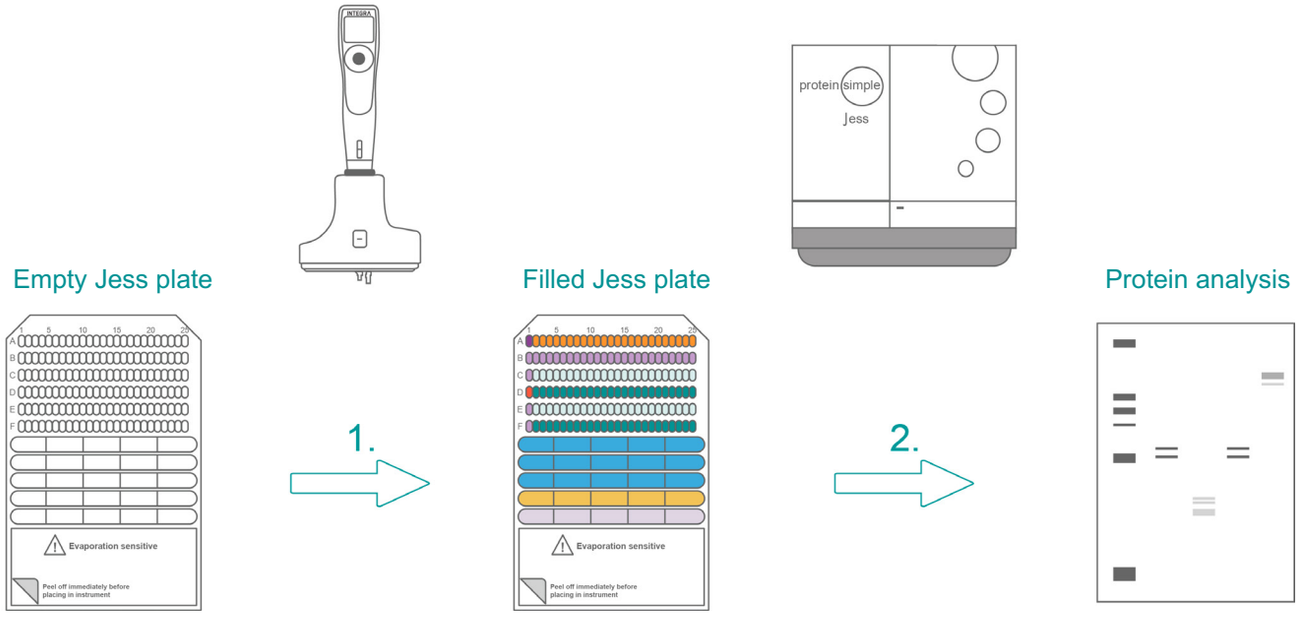

Figure 1: 2-step western blot automation protocol.

Application Note

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Plate set-up for RePlex chemiluminescence western blot protocol automation

Experimental set-up

Deck Position A: Wash buffer (blue)
Deck Position B: B1 – antibody diluent (lavender), streptavidin-HRP (red), luminol-peroxide mix (yellow), RePlex mix (pink); B2 – primary antibody probe 1 (light green-1), secondary antibody probe 1 (green-1), primary antibody probe 2 (light green-2), secondary antibody probe 2 (green-2); B3 – biotinylated ladder (violet); B4-B6 – prepared samples (orange, arrow indicates processing direction)

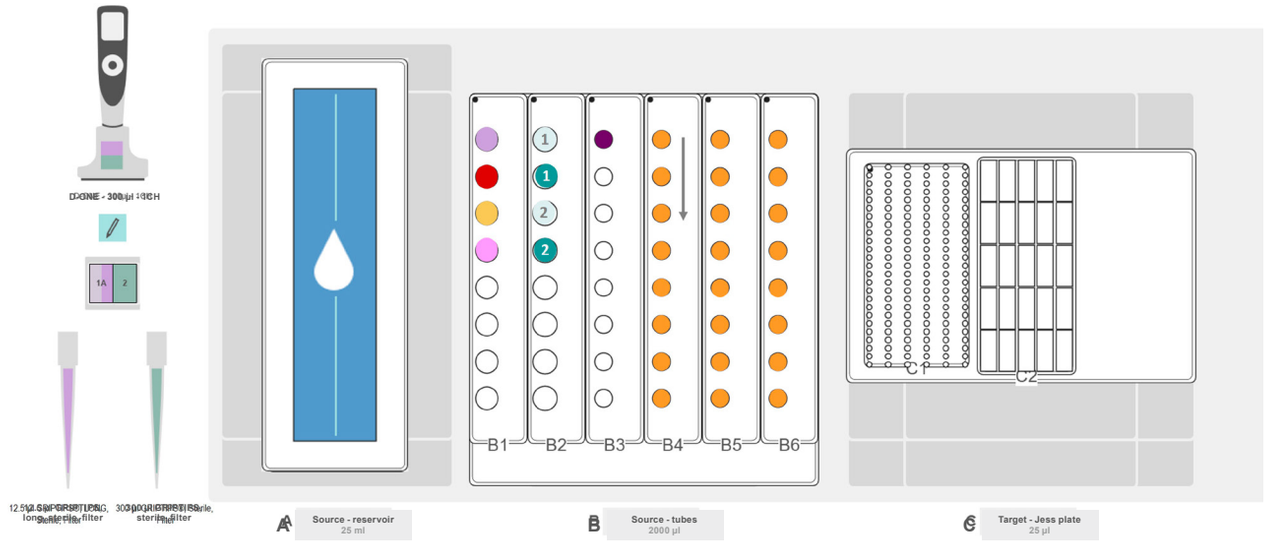

Figure 2: Deck set-up for Jess plate filling to perform RePlex chemiluminescence analysis. **Position A:** Source – 25 ml reservoir. **Position B:** Source – INTEGRA tube rack for 1.5/2 ml (B1-B2) and 0.5 ml (B3-B6) microcentrifuge tubes. **Position C:** Target – Simple Western Jess plate.

---

1. RePlex plate set-up

STEP: Jess plate set-up for RePlex.

HOW TO: Place a 25 ml reservoir on deck Position A and fill it with at least 8 ml of wash buffer. Place the INTEGRA tube rack on Position B with the specific reagent and sample tubes, as indicated in the experimental set-up. As shown in **Figure 2**, the Jess plate is in landscape orientation on Position C. LLD enables the use of different aliquot sizes, and the D-ONE informs the operator if the liquid volume is insufficient.

Application Note

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Equip the ASSIST PLUS with the 0.5-300 µl D-ONE, and run the VIALAB program 'Jess_plate_setup_RePlex'. The ASSIST PLUS and D-ONE with 12.5 µl long, sterile, filter GRIPTIPS transfer 10 µl of antibody diluent from a 2 ml tube in Position B1 (**Figure 2**, lavender) to wells B1-B25, C1, E1 and F1 of the Jess plate (**Figure 3**) in Position C. A 1 µl pre- and post-dispense guarantees precise pipetting while preventing bubble creation during dispensing. By automatically changing GRIPTIPS between different reagents or samples, the D-ONE transfers 10 µl of primary antibody probe 1 from a 1.5 ml microcentrifuge tube in B2 (**Figure 2**, light green-1) to wells C2-C25 (**Figure 3**) of the Jess plate in Position C (**Figure 4a**). The D-ONE transfers 10 µl of streptavidin-HRP/NIR from the second 2 ml screw cap vial in B1 (**Figure 2**, red) to well D1 (**Figure 3**). Wells D2 to D25 (**Figure 3**) are filled with 10 µl secondary antibody probe 1 from a 1.5 ml microcentrifuge tube in B2 (**Figure 2**, green-1). 10 µl primary antibody probe 2 is transferred from Position B2 (**Figure 2**, light green-2) to wells E2 to E25 (**Figure 3**), and 10 µl secondary antibody probe 2 is transferred from B2 (**Figure 2**, green-2) to wells F2 to F25 (**Figure 3**).

Using 300 µl sterile, filter GRIPTIPS, the D-ONE transfers 500 µl of wash buffer (**Figure 2**, blue) from the 25 ml reservoir on Position A to the Jess plate compartments in 2 steps (**Figure 4b**), as indicated in **Figure 3**. A slow speed (5) prevents bubble creation during buffer dispensing. Afterwards, 170 µl of luminol-peroxide mix is transferred from the 2 ml tube in B1 (**Figure 5**, yellow), and 300 µl of RePlex reagent mix from another 2 ml tube in B1 (**Figure 5**, pink) to the compartments indicated in **Figure 3**. The D-ONE then transfers the RePlex reagent mix in 2 steps, with a pre- and post-dispense of 10 µl to prevent bubble creation during dispensing.

5 µl of biotinylated ladder is transferred from a 0.5 ml microcentrifuge tube in Position B3 (**Figure 2**, violet) to well A1 (**Figure 3**). As indicated by the arrow in **Figure 2**, the D-ONE transfers 3 µl of prepared sample (**Figure 2**, orange) from each 0.5 ml microcentrifuge tube in Positions B4 to B6 (**Figure 2**, orange) to wells A2-A25 (**Figure 3**). Fast dispensing (speed 10) increases the accuracy for small volumes. The pipette then instructs the user to centrifuge the plate for 5 minutes at 2500 rpm, before proceeding with Simple Western Jess protein separation and immunodetection.

Tips:

- A prompt can be included in the protocol before starting the sample transfer to instruct the user to prepare the samples during reagent transfer.
- VIALAB's simplified programming allows plate set-up to be easily adjusted to perform western blot normalization by replacing the second chemiluminescence detection with a total protein analysis, as indicated in the kit insert.

Application Note

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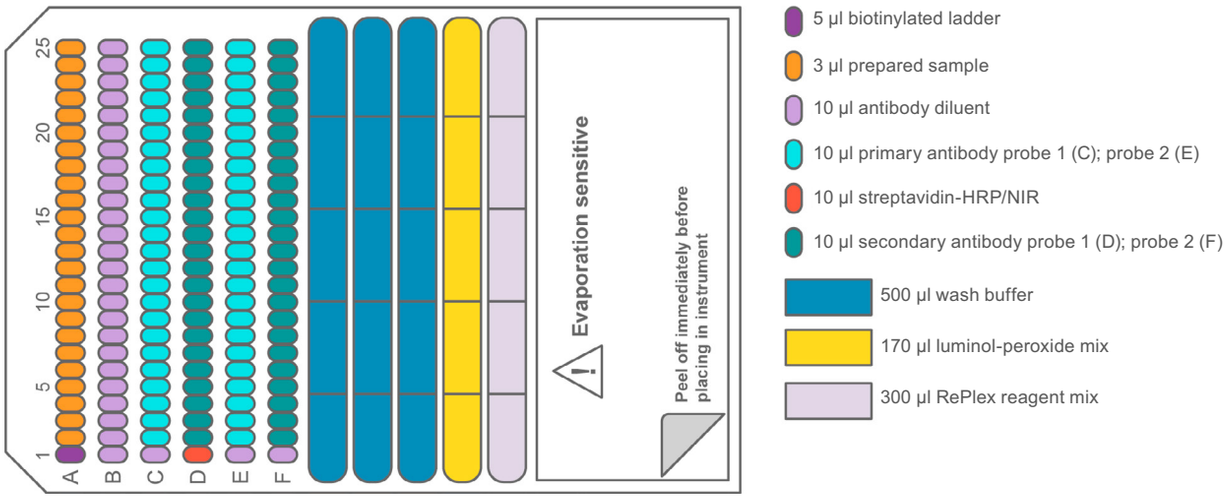

Figure 3: How to fill the Simple Western Jess plate for RePlex chemiluminescence analysis.

a)

b)

Figure 4: The D-ONE transfers a) the primary antibody and b) the wash buffer to the Simple Western Jess plate.

Application Note

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Plate set-up for chemiluminescence western blot protocol automation

Experimental set-up

Deck Position A: Wash buffer (blue)
Deck Position B: B1 – antibody diluent (lavender), streptavidin-HRP (red), luminol-peroxide mix (yellow); B2 – primary antibody (light green), secondary conjugate (green); B3 – biotinylated ladder (violet); B4-B6 – prepared samples (orange, arrow indicates processing direction)

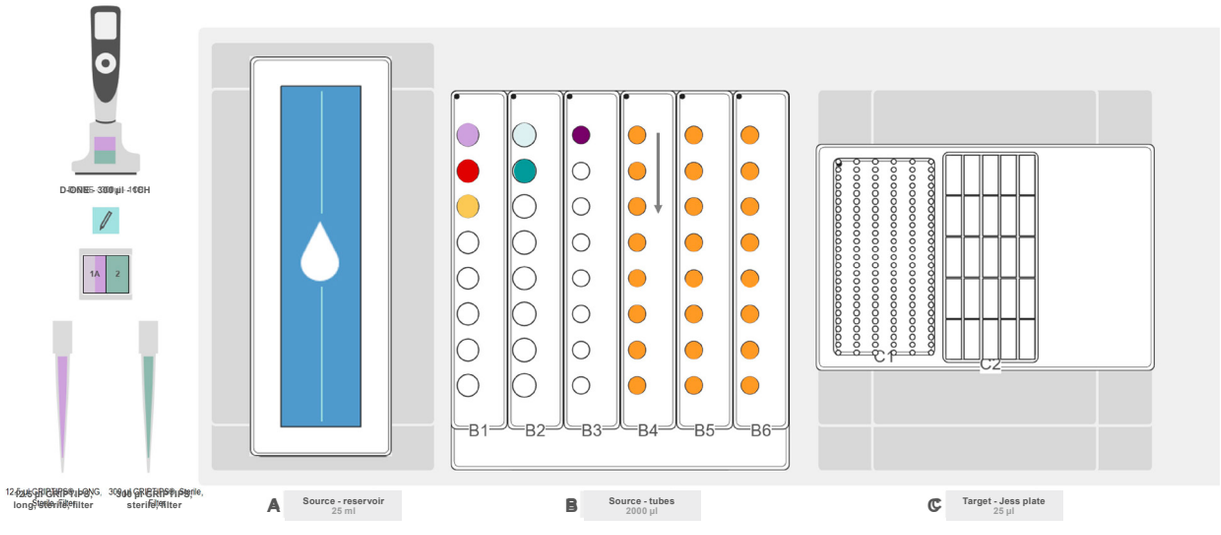

Figure 5: Deck set-up for Jess plate filling to perform chemiluminescence analysis. **Position A:** Source – 25 ml reservoir. **Position B:** Source – INTEGRA tube rack for 1.5/2 ml (B1-B2) and 0.5 ml (B3-B6) microcentrifuge tubes. **Position C:** Target – Simple Western Jess plate.

---

2. Chemiluminescence plate set-up

STEP: Jess plate set-up for chemiluminescence western blot.

HOW TO: Prepare a similar deck set-up to the RePlex western blot protocol, but without the RePlex reagent mix or the primary and secondary antibodies for probe 2 (**Figure 5**).

Select and run the VIALAB program 'Jess_plate_setup_chemiluminescence'. Following a similar procedure as for RePlex chemiluminescence detection, the D-ONE starts by transferring 10 µl of antibody diluent (**Figure 5**, lavender) to wells B1-B25 and C1 of the Jess plate (**Figure 6**) in Position C. 10 µl of primary antibody (**Figure 5**, light green) is then transferred to wells C2-C25, 10 µl of streptavidin-HRP/NIR (**Figure 5**, red) to well D1, 10 µl of secondary conjugate (**Figure 5**, green) to wells D2 to D25, and 15 µl of luminol-peroxide mix to all wells of row E, as indicated in **Figure 6**.

The D-ONE follows this with transfer of the wash buffer (**Figure 5**, blue), biotinylated ladder (**Figure 5**, violet) and samples (**Figure 5**, orange), as described for RePlex chemiluminescence detection, and instructs the operator to centrifuge the plate before proceeding with the automated western blot.

Application Note

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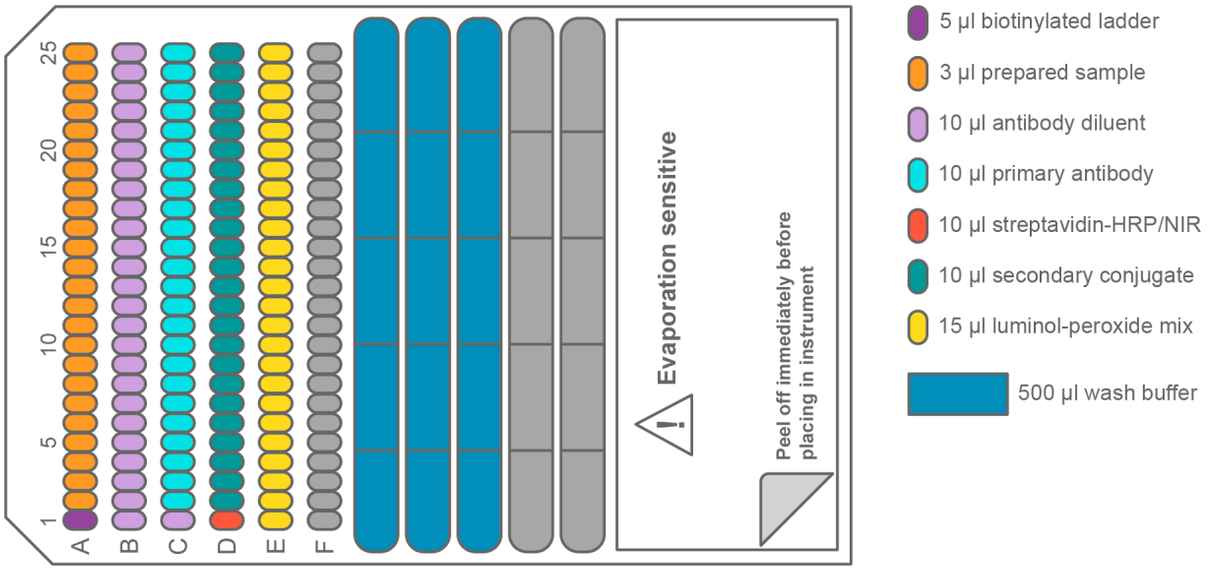

Figure 6: How to fill the Simple Western Jess plate for chemiluminescence analysis.

Application Note

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Plate set-up for total protein western blot protocol automation

Experimental set-up

Deck Position A: Wash buffer (blue)
Deck Position B: B1 – antibody diluent (lavender), total protein labeling reagent (light green), total protein streptavidin-HRP (green), luminol-peroxide mix (yellow); B3 – biotinylated ladder (violet); B4-B6 – prepared samples (orange, arrow indicates processing direction)

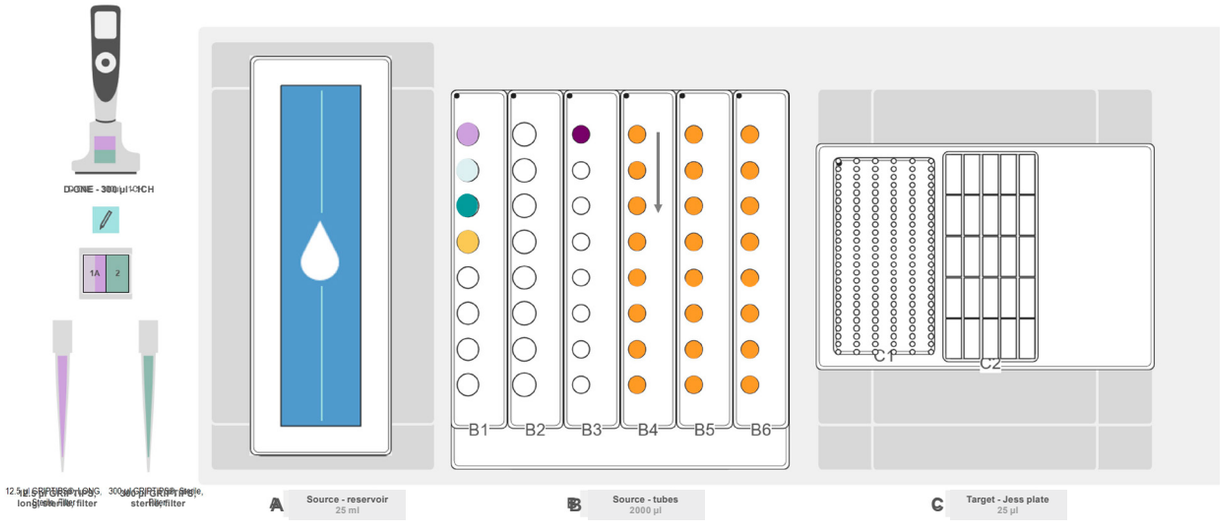

Figure 7: Deck set-up for Jess plate filling to perform total protein analysis. **Position A:** Source – 25 ml reservoir. **Position B:** Source – INTEGRA tube rack for 1.5/2 ml (B1-B2) and 0.5 ml (B3-B6) microcentrifuge tubes. **Position C:** Target – Simple Western Jess plate.

3. Total protein plate set-up

STEP: Jess plate set-up for total protein analysis.

HOW TO: Set up the ASSIST PLUS deck in a similar way to the chemiluminescence detection protocol, but with the reagents for total protein analysis (**Figure 7**).

Select and run the VIALAB program 'Jess_plate_setup_total_protein'. The D-ONE automatically selects GRIPTIPS, and transfers 10 µl of antibody diluent (**Figure 7**, lavender) into wells B1 and C1 to C25, 8 µl of total protein streptavidin-HRP (**Figure 7**, green) to row D, 15 µl of luminol-peroxide mix (**Figure 7**, yellow) to row E, and wash buffer from the reservoir to the compartment of the Jess plate, as indicated in **Figure 8**. Similar to the western blot protocol for chemiluminescence detection, 5 µl of biotinylated ladder (**Figure 7**, violet) and 3 µl of each sample (**Figure 7**, orange) are also transferred to the Jess plate in Position C. The run is completed by the transfer of 10 µl of total protein labeling reagent (**Figure 7**, light green) into wells B2 to B25, as shown in **Figure 8**. After finishing the liquid transfers, the instrument instructs the operator to centrifuge the plate, as indicated in the kit.

Application Note

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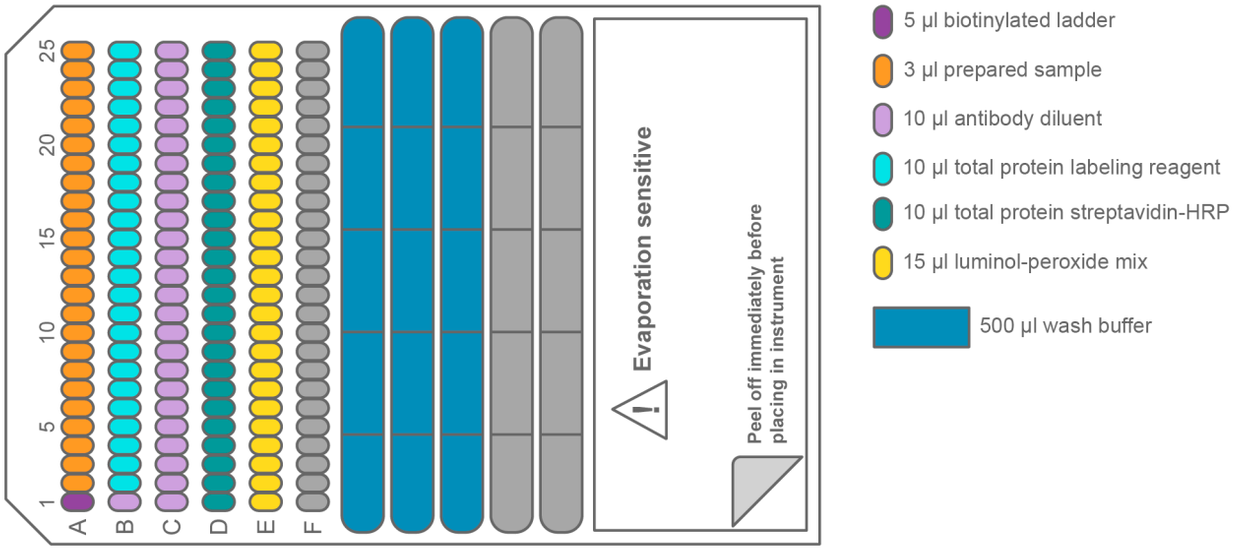

Figure 8: How to fill the Simple Western Jess plate for total protein analysis.

Application Note

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Methods and results

Western blotting can be very time consuming and prone to errors. This application note demonstrates a full, walk-away solution for reliable and high throughput automated western blotting, combining INTEGRA's ASSIST PLUS pipetting robot and D-ONE single channel pipetting module with ProteinSimple's Simple Western Jess.

With RePlex, chemiluminescence and total protein detection were combined in a single assay to prove accurate liquid handling for all reagents when setting up plates with the automated protocols. Reagents, HeLa and C2C12 lysates were prepared according to the protocol in the product insert, with lysate dilutions of 0.64, 0.32, 0.16 and 0.08 mg/ml, together with ready-to-use ERK1 primary and secondary antibodies. Each HeLa or C2C12 dilution was prepared in single tube triplicates as individual samples during plate filling.

Figure 9 shows fully automated western blot normalization of HeLa lysates (lanes 2-13), and C2C12 (lanes 14-25), in triplicate. After ERK1 detection (**Figure 9a**; left), the primary and secondary antibodies were removed with the RePlex reagent mix, to re-stain samples for total protein analysis (**Figure 9a**; right). The data sets generated were automatically analyzed, using the Simple Western software tool to visualize the uncorrected (**Figure 9b**; left) and corrected (**Figure 9b**; right) ERK1 peak target areas. All 3 replicates of each lysate showed great reproducibility while successfully normalizing ERK1 protein levels in 4 different concentrations, proving accurate liquid handling and confirming on-deck reagent/sample stability during plate set-up.

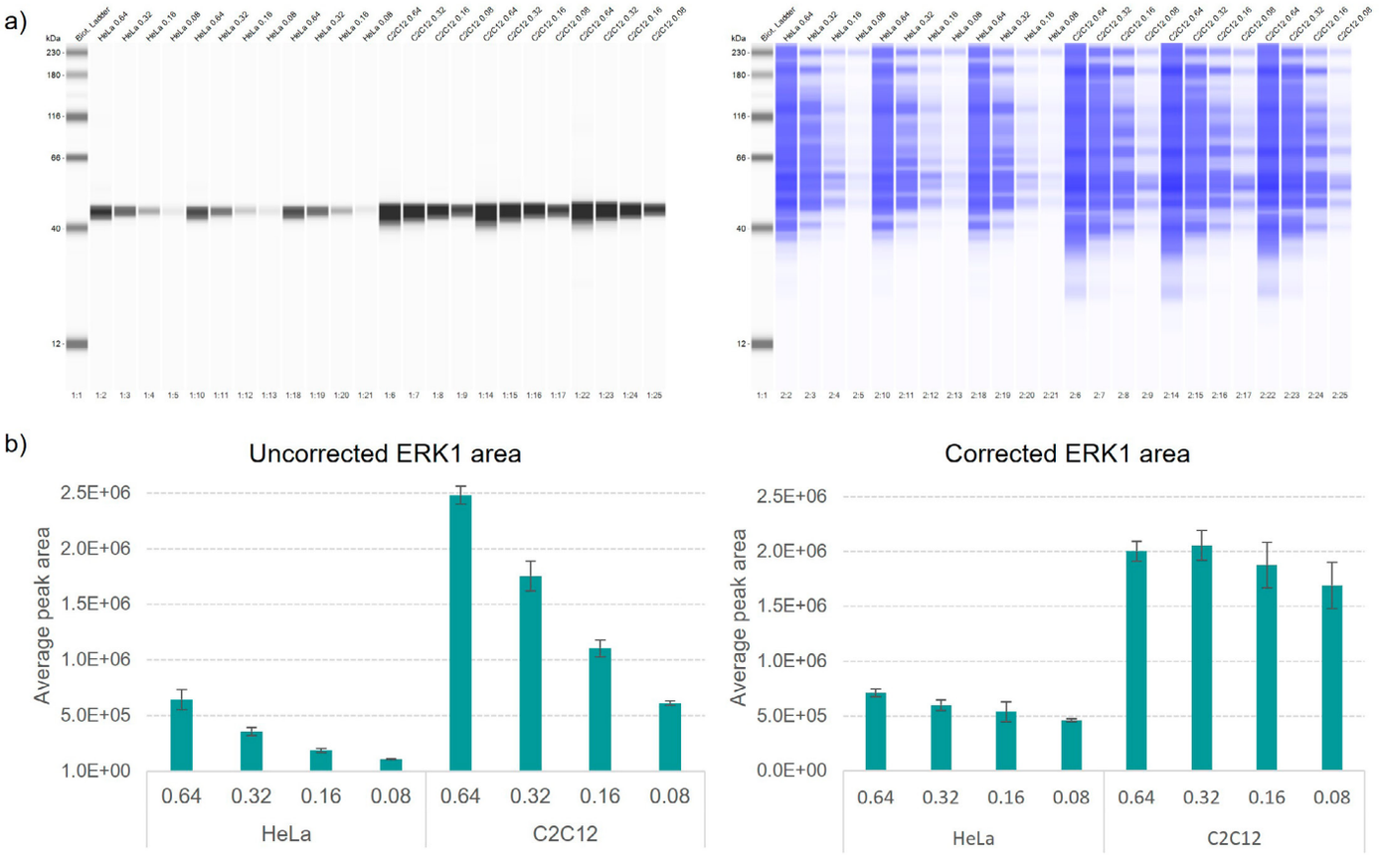

Figure 9: Full walk-away western blot normalization with the ASSIST PLUS, D-ONE and Jess using RePlex. HeLa (lanes 2-13) and C2C12 (lanes 14-25) lysates were each titrated to 0.64, 0.32, 0.16 and 0.08 mg/ml concentrations and run in triplicate. a) ERK1 detection (left) and total protein detection (right). b) Automatic quantification of uncorrected and corrected ERK1 peak area using the Simple Western software.

Application Note

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Furthermore, simple fluorescence-based detection was carried out to prove the equivalence in performance between automated and manual plate filling, by removing the luminol-peroxide mix from the VIALAB protocol for chemiluminescence detection (Page 6).

Again, reagents and HeLa lysates were prepared according to the protocol in the product insert, with sample dilutions of 0.64, 0.32, 0.16, 0.08, 0.04 and 0.00 mg/ml (0.1x sample buffer). The primary antibody was prepared by diluting 15 µl of HSP60 and 6 µl of ß-actin in 279 µl of milk-free antibody diluent. The secondary antibody was prepared by diluting 15 µl of anti-rabbit IR and 15 µl of anti-mouse NIR in 270 µl of milk-free antibody diluent.

Figure 10a illustrates the results of automated and manual Jess plate set-up (lane view). Wells 1-13 of rows A to D, as well as the wash buffer compartments, were filled using the D-ONE and ASSIST PLUS. Wells 14-25 of rows A to D were filled manually using a single channel pipette. Both filling methods produced reliable fluorescence data when processing the first 3 dilutions of the HeLa lysates in quadruplicate (0.64 and 0.32 mg/ml) and triplicate (0.16 mg/ml), with CVs within the instrument's specifications.

Figure 10b illustrates the results when performing fluorescent detection of all 6 HeLa dilutions, by preparing each dilution in 4 single tube replicates on the ASSIST PLUS deck (lane view and electropherogram showing 0.64 mg/ml replicates as representation). Again, CVs met the instrument specifications while showing high detection sensitivity for low abundance targets when automating plate filling with the D-ONE and ASSIST PLUS.

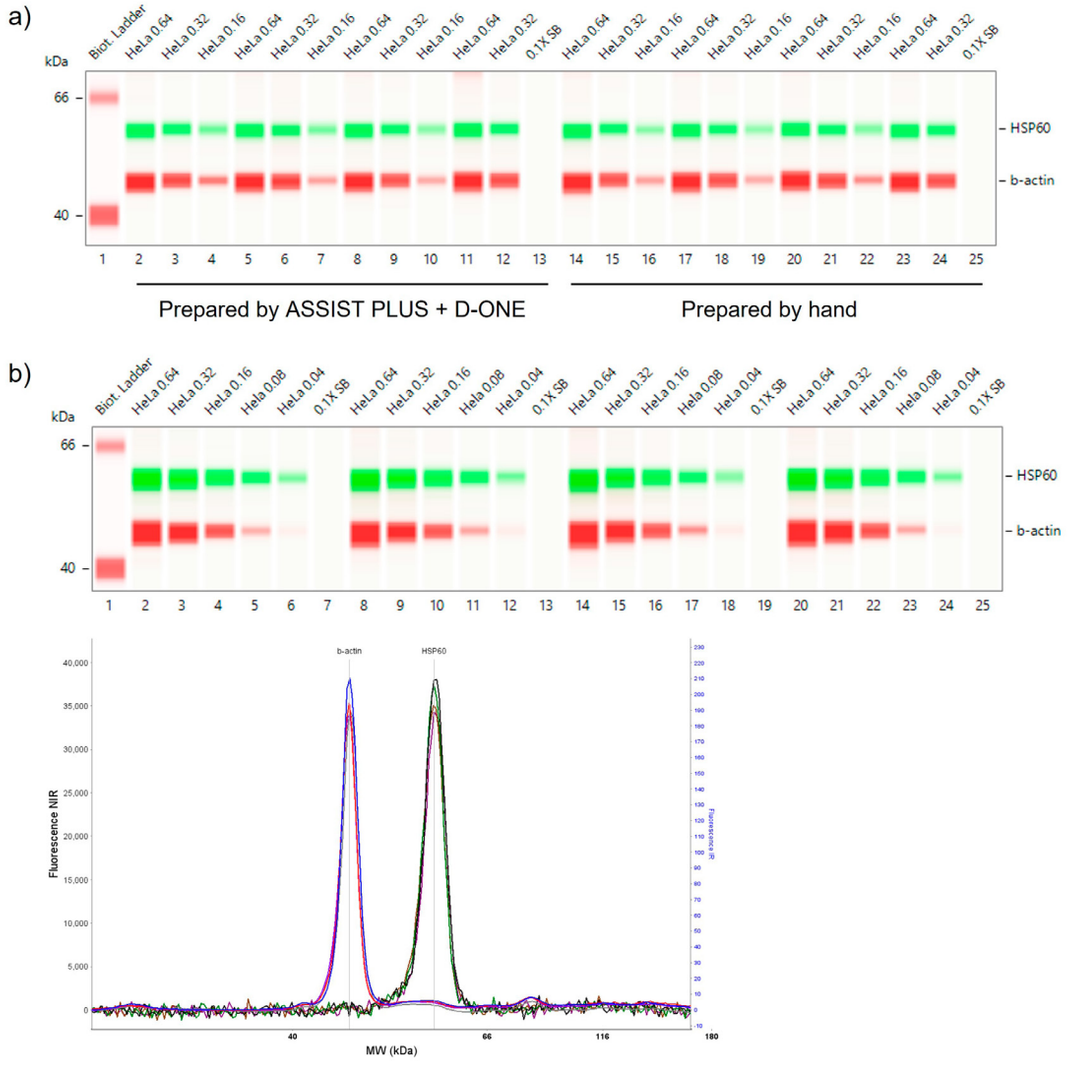

Figure 10: Fluorescence-based detection results demonstrate comparable Jess plate filling. a) Automated (2-13) and manual (14-25) set-up of a Jess plate with various dilutions of HeLa lysates in quadruplicate (0.64 and 0.32 mg/ml) and triplicate (0.16 mg/ml) (lane view). b) Fully automated set-up of 24 samples when processing 4 replicates of 0.64, 0.32, 0.16, 0.08, 0.04 and 0.0 mg/ml (0.1x sample buffer) HeLa lysates (lane view and electropherogram showing 0.64 mg/ml replicates as representation).

Application Note

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Remarks

- **Labware:** The simple labware creation tool in the VIALAB library makes the integration of special plates easier than ever.
- **VIALAB software:** VIALAB programs can be easily adapted to your specific pipette, labware and protocols.
- **Partial plates:** Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

Conclusion

- High throughput western blot protocol automation with the ASSIST PLUS pipetting robot, D-ONE single channel pipetting module and Simple Western Jess eliminates user error and demonstrates CVs below instrument specifications.
- The sensitivity of your western blots can be increased with Simple Western advanced capillary electrophoresis and immunodetection technology, and accurate and precise plate set-up can be performed with the D-ONE module for ASSIST PLUS.
- This method can decrease the time needed to run a
- western blot protocol from days to under 3 hours, by using the ASSIST PLUS and D-ONE to automate Simple Western Jess plate filling.
- Western blot workflows can be effortlessly accomplished with RePlex, and total protein normalization performed with ease, or multiplexed with simultaneous chemiluminescent and fluorescent assays on the same sample.

Application Note

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4531	D-ONE single channel pipetting module	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4535	D-ONE tip deck	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4540/4541	Tube rack for 1.5/2.0 ml and 0.5 ml microcentrifuge tubes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4304	25 ml reservoir base	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	4316	25 ml reservoir	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6405	12.5 µl long, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/griptipsr/automation-griptipsr
INTEGRA Biosciences	6435	300 µl standard, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/griptipsr/automation-griptipsr
INTEGRA Biosciences	4570	Waste bags	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
ProteinSimple	004-650	Simple Western Jess System	https://www.bio-techne.com/p/simple-western/jess_004-650
ProteinSimple	042-488	HeLa Lysate Controls	https://www.bio-techne.com/p/simple-western/hela-lysate-controls_042-488
Novus Biologicals (a Bio-Techne brand)	NBP2-10268	C2C12 Whole Cell Lysate	https://www.novusbio.com/products/c2c12-lysate_nbp2-10268
ProteinSimple	042-486	Erk 1 Primary Antibody for Size Assays	https://www.bio-techne.com/p/simple-western/erk-1-primary-antibody-for-size-assays_042-486
Novus Biologicals	MAB8929	Beta-Actin Antibody	https://www.novusbio.com/products/beta-actin-antibody-937215_mab8929
R&D Systems, Inc. (a Bio-Techne brand)	AF1800	HSP60 Antibody	https://www.bio-techne.com/p/antibodies/human-mouse-rat-hsp60-antibody_af1800
ProteinSimple	SM-W004	12-230 kDa Separation Module	https://www.bio-techne.com/p/simple-western/12-230-kda-separation-module_sm-w001
ProteinSimple	SM-FL004	12-230 kDa Fluorescence Separation Module	https://www.bio-techne.com/p/simple-western/12-230kda-fluorescence-separation-module_sm-fl001
ProteinSimple	DM-001	Anti-Rabbit Detection Module	https://www.bio-techne.com/p/simple-western/anti-rabbit-detection-module_dm-001
ProteinSimple	DM-008	Anti-Rabbit IR Detection Module	https://www.bio-techne.com/p/simple-western/anti-rabbit-ir-detection-module_dm-008

Application Note

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ProteinSimple	DM-009	Anti-Mouse NIR Detection Module	https://www.bio-techne.com/p/simple-western/anti-mouse-nir-detection-module_dm-009
ProteinSimple	DM-TP01	Total Protein Detection Module for Chemiluminescence based total protein assays	https://www.bio-techne.com/p/simple-western/total-protein-detection-module-for-chemiluminescence-based-total-protein-assays_dm-tp01
ProteinSimple	RP-001	RePlex Module	https://www.bio-techne.com/p/simple-western/replex-module_rp-001

Contact us:

Application Note

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3 simple and proven automation protocols for serial dilutions on the ASSIST PLUS pipetting robot

Introduction

Serial dilution – a reduction in concentration by a constant dilution factor – is a common approach for screening-related applications, such as determining minimum inhibitory concentrations (MIC) in drug discovery, calculating the most probable numbers (MPN) in microbiology, and performing general nucleic acid quantifications in molecular biology. Although it is a simple technique, poor liquid handling during interdependent dilution steps can cause error propagation and accumulation. Thorough mixing is therefore crucial, but this puts a lot of strain on the thumb, which increases the risk of repetitive strain injuries. In addition, performing serial dilutions regularly can be a time-consuming process.

This application note describes the simplest way to do serial dilutions, using the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot to gain more walk-away time. The protocols provided outline the optimal settings to ensure reliable results when diluting analytes in water. For further information about modifying key parameters to suit varying conditions, see INTEGRA's comprehensive guide to performing serial dilution.

Key benefits:

- Proven serial dilution protocols, with optimal settings for the VOYAGER on the ASSIST PLUS, guarantee uniform pipetting and mixing.
- The VOYAGER offers flexible automated serial dilutions across various tubes and plates, as well as the ability to switch pipette volumes seamlessly while maintaining the same protocol.
- INTEGRA's electronic pipettes prevent thumb strain during liquid handling steps and, together with the ASSIST PLUS, enable risk-free handling of hazardous samples.
- The ASSIST PLUS gives users additional hands-free time, eliminating time-consuming manual procedures.
- These efficient liquid handling solutions support 2-, 5- and 10-fold serial dilutions, with dynamic mixing volumes ensuring homogeneity of analytes.
- Simplified workflows are achieved with VIALAB's serial dilution protocol, which includes specific mixing parameters for managing poorly soluble analytes.

Overview: How to do serial dilution with the ASSIST PLUS

ASSIST PLUS

Application Note

INTEGRA

This application note demonstrates how to perform serial dilution of tartrazine in water with an 8 channel 125 µl VOYAGER on the ASSIST PLUS.

Experimental set-up

The ASSIST PLUS, together with the 125 µl 8 channel VOYAGER and 125 µl sterile, filter GRIPTIPS® pipette tips, automates complete serial dilutions in 1 program consisting of 3 steps (**Figure 1**):

1. Transfer diluent to target plate
2. Transfer analyte to target plate
3. Serial dilution of analyte within target plate

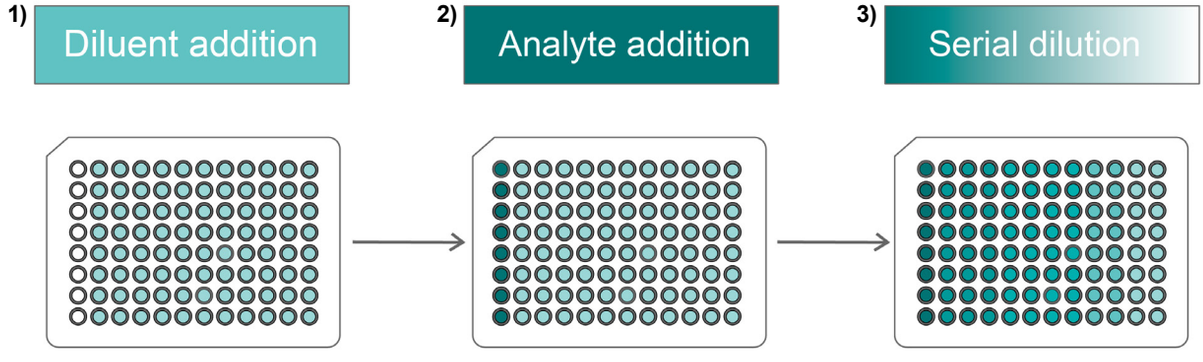

Figure 1: Experimental set-up for serial dilutions.

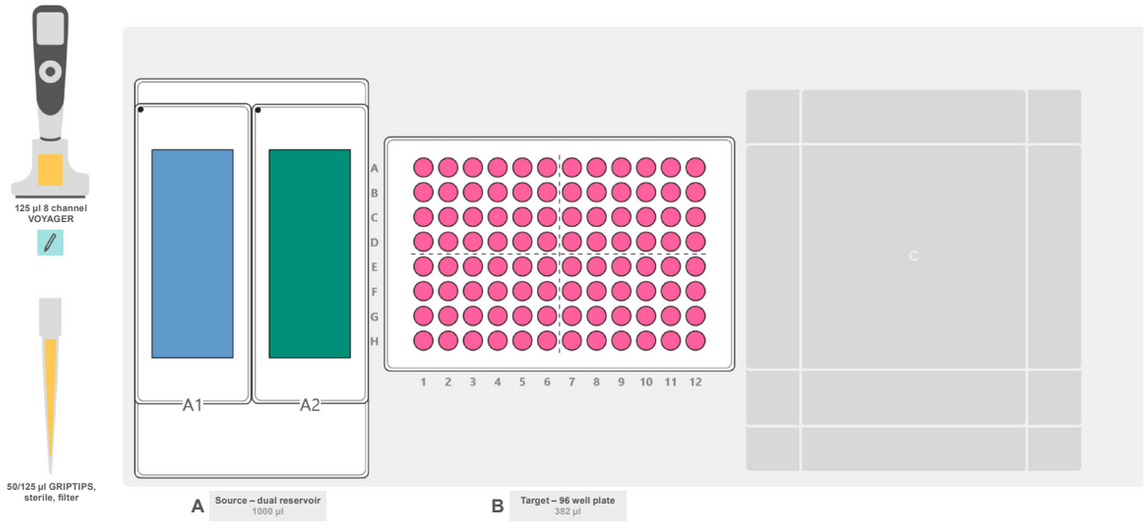

Figure 2: Deck set-up for performing serial dilutions. **Position A:** Source – dual reservoir adapter with 2x10 ml reservoirs; diluent in A1 (blue) and analyte in A2 (green). **Position B:** Target – 96 well flat bottom plate (pink). **Position C:** Empty.

Application Note

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Step-by-step procedure:

1. Serial dilution of an analyte

STEP: Serial dilution of an analyte.

HOW TO: The INTEGRA dual reservoir adapter, together with 2x10 ml reservoirs, is placed on deck Position A, with diluent (blue) in A1 and analyte (green) in A2 (**Figure 2**). A clear 96 well flat bottom plate (pink) is placed in landscape orientation on deck Position B (**Figure 2**).

Select and run one of the following VIALAB programs:

2-fold serial dilution → 125_VOYAGER_2_fold_serial_dilution
5-fold serial dilution → 125_VOYAGER_5_fold_serial_dilution
10-fold serial dilution → 125_VOYAGER_10_fold_serial_dilution

Specific volumes are handled by the VOYAGER (**Figure 3**). The diluent is transferred in multiple dispensing steps from the reservoir (Position A – A1) into each well of the 96 well flat bottom plate, starting with column 2 (Position B). To ensure precision during plate set-up, a pre- and post-dispense of 5 % of the transferred volume is used for the 10-fold serial dilution, and 10 % for the 2- and 5-fold serial dilutions.

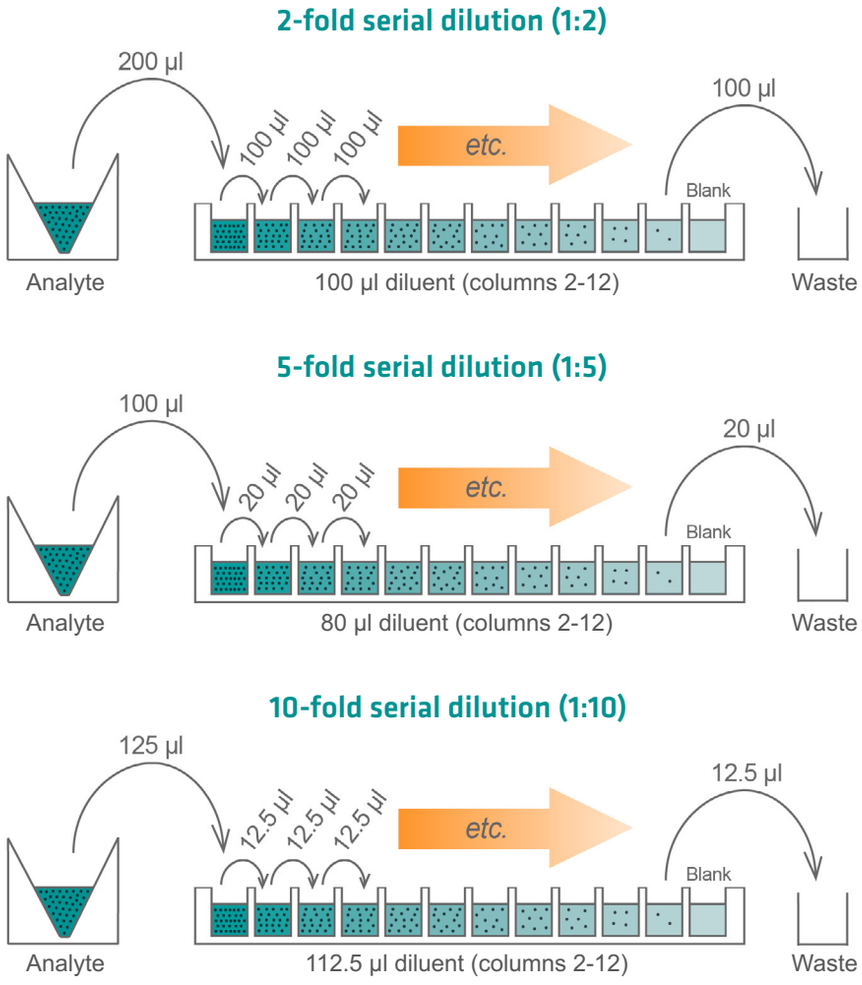

Figure 3: Serial dilutions in 96 well flat bottom plates with the 125 µl VOYAGER.

Application Note

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Using new GRIPTIPS, the VOYAGER aspirates the highest concentration of analyte from the reservoir (Position A – A2) and dispenses it into the first column of the 96 well flat bottom plate (Position B).

Without changing the GRIPTIPS, the VOYAGER begins the serial dilution by aspirating the specific volume (**Figure 3**) from column 1 of the 96 well flat bottom plate (Position B) and dispensing into the second column. The VOYAGER then mixes 100 µl (2- and 10-fold serial dilution) or 80 µl (5-fold serial dilutions) of the analyte/diluent 5 times at maximum speed (10). A blowout is performed to clear the tip of any remaining liquid before aspirating for the following dilution step. The procedure is repeated until column 11 is reached, where the last aspiration is discarded along with the GRIPTIPS (**Figure 4**). Column 12 only contains diluent, and functions as a blank for background noise elimination.

TIPS:

- Pre-wetting tips when pipetting aqueous liquids ensures excellent accuracy and precision.
- Using adjustable mixing cycles compensates for slower mixing speeds or poorly soluble analytes.

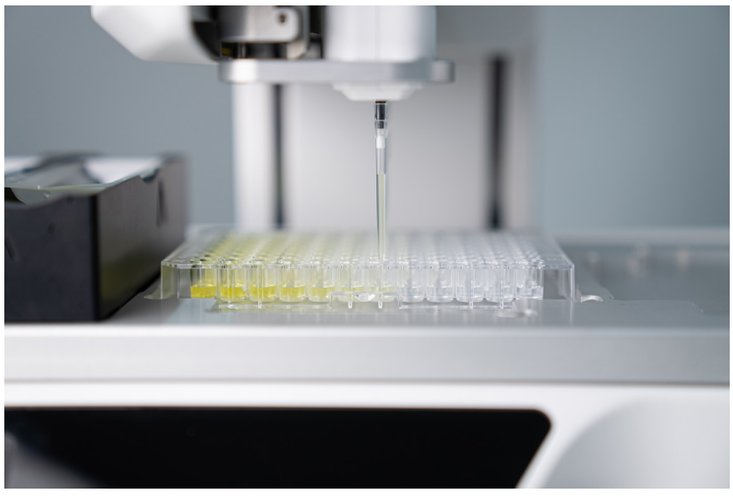

Figure 4: The ASSIST PLUS and VOYAGER performing serial dilution of tartrazine.

Application Note

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Results

The performance of the 8 channel 125 µl VOYAGER on the ASSIST PLUS during serial dilution of 0.36 mM tartrazine in water in 96 well flat bottom plates (**Figure 5**) was analyzed at 428 nm absorbance using the Tecan Infinite® M200 PRO. More detailed data is provided in INTEGRA's comprehensive guide to performing serial dilution.

Figure 5: 2-fold serial dilution of tartrazine in a 96 well flat bottom plate.

Figure 6 shows a representational, optimized calibration curve of a 2-fold serial dilution. Automating all liquid handling steps and mixing 100 µl of each dilution (>80 % GRIPTIPS volume) 5 times at maximum speed (10) led to reliable results in 3 independent runs. Furthermore, final values of less than 1 % were calculated for the accuracy and precision of the individual dilution steps.

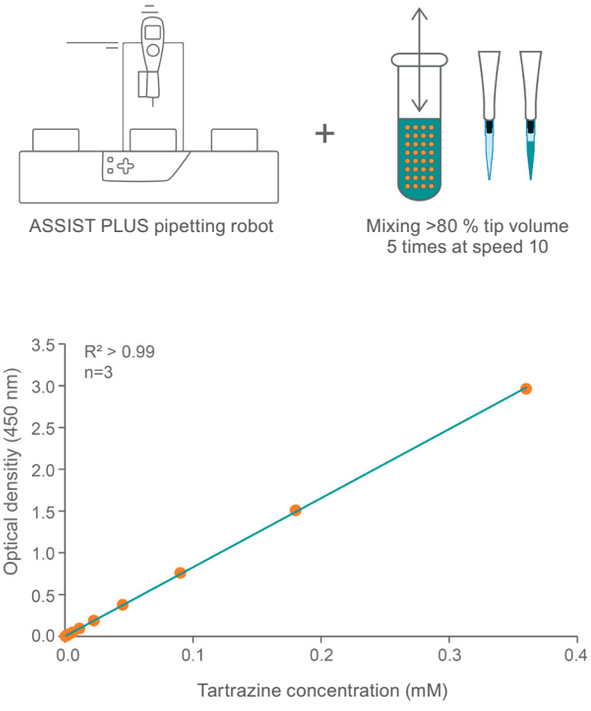

Figure 6: Result of a 2-fold serial dilution of tartrazine using optimized mixing settings on the ASSIST PLUS.

Application Note

INTEGRA

Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to your specific labware and protocols, such as when partial plates are needed.
- **Partial plates:** Programs can be adapted at any time to accommodate varying sample numbers, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Automated workflows on the ASSIST PLUS offer reproducible results and eliminate any operator influence on serial dilutions.
- INTEGRA's electronic pipettes ensure homogeneity of aqueous solutions with dynamic mixing of each dilution. This is achieved by aspirating and dispensing >80 % of the total GRIPTIPS or reaction volume, repeated 5 times at speed 10.
- Understanding how to perform serial dilutions is crucial to optimize workflows and prevent error propagation. The automated protocols on the ASSIST PLUS provide optimal liquid handling settings for 2-, 5- and 10-fold serial dilutions.
- The ASSIST PLUS has a compact footprint to enable risk-free dilution of hazardous compounds in a biosafety cabinet.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	125 µl 8 channel VOYAGER electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/voyager
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4372	10 ml divided reservoir, polystyrene SureFlo™	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6465	125 µl sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/grip-tip-selector-guide
Greiner Bio-One International	655161	96 well microplate, PS, F-bottom	https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161.html

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"Amazing results, minimizes error."

Ease of use: ★★★★★ **After-sales service:** ★★★★★ **Value for money:** ★★★★★

Rating: ★★★★★

Application area: Serial dilutions

"ASSIST PLUS has made our lives easy in the lab. It is fully automated and has a much lower error rate compared to manual serial dilutions. It is quick and consistent."

Aravindh Nagarajan, Texas A&M University

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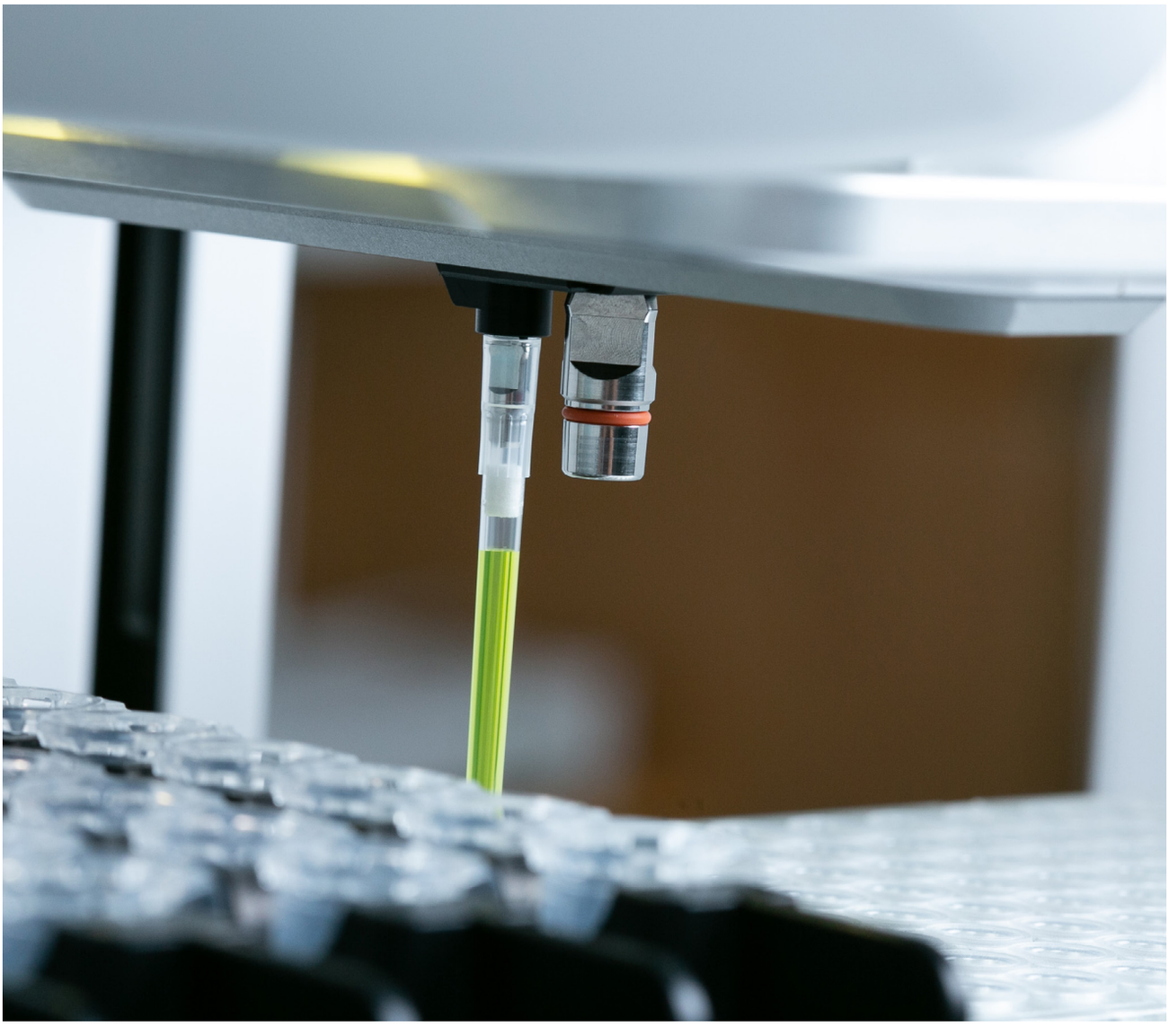
D-ONE single channel pipetting module by INTEGRA Biosciences



“User friendly and 'amplifies' your job effectively.”

Ease of use: ★★★★★ **After-sales service:** ★★★★★ **Value for money:** ★★★★★

Rating: ★★★★★

Application area: Compound plate reformatting, repeat dispenses, serial dilutions

“Functions as advertised. Very user friendly. My lab uses this for re-configuring 96 well to 384 well compound formats prior to screening and SAR work. Instrument is very cost efficient and perfect for space-saving automation and semi-automation needs. A plethora of holders (microfuge tubes, 15 ml tubes, 96 deepwell plates) allows for flexibility.”

Dave Solis, Boundless Bio

Learn more »

VOYAGER adjustable tip spacing pipette by INTEGRA Biosciences

"It's the best. I absolutely love it."

Ease of use: ★★★★★ **After-sales service:** ★★★★★ **Value for money:** ★★★★★

Rating: ★★★★★

Application area: Cell culture

"It's the best automated pipette, and the service post sale has been great."

Rajeshwar Nitiyanandan, SageMedic Corporation

Learn more »

GRIPTIPS® pipette tips for VIAFLO 96 and VIAFLO 384 handheld electronic pipettes by INTEGRA Biosciences

"Good value, good product."

Ease of use: ★★★★★ **After-sales service:** ★★★★★ **Value for money:** ★★★★★

Rating: ★★★★★

Application area: Analyzing proteasome activity of cell lysate and purified samples

"This product is very easy to use, very smooth, good quality and it is good value."

Xiaorong Wang, UC-Irvine

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