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Accelerated loading of Fluidigm's 192.24 Dynamic Array[™] IFC for genotyping with the ASSIST PLUS pipetting robot

Introduction

Genotyping is widely used in disease associated studies, molecular epidemiology, agricultural research (trait selection, modern selective breeding), forensics, the development of novel markers in pharmaceutical and biotechnology research, population genetics, and more. It enables the detection of small genetic differences across individual organisms, which may be single nucleotide polymorphisms (SNPs), copy number variants, or large structural changes in DNA. Fluidigm has developed a flexible, affordable, high throughput tool - the 192.24 Dynamic Array IFC (integrated fluidic circuit) - using

Key benefits:

- · Manually pipetting to a complex microplate format like the 192.24 Dynamic Array IFC is highly error prone and tedious. Pipetting errors are generally not recognized immediately but influence the results; whole experiments may need to be repeated. Automation on the ASSIST PLUS helps to overcome these limitations.
- Samples are stored in 96 well plates and need to be • transferred onto an IFC. The sample sequence can be easily copied onto the 192.24 Dynamic Array IFC with the VOYAGER adjustable tip spacing pipette.

Overview: How to load the Fluidigm 192.24 Dynamic Array IFC

Step-by-step procedure:

The ASSIST PLUS is equipped with a VOYAGER 8 channel 12.5 µl electronic pipette using 12.5 µl LONG, Sterile, Filter, Low Retention GripTips to automate the pipetting steps of sample and assay loading on the 192.24 Dynamic Array IFC. The pipette guides the user whenever manual intervention is needed during the process. The sample and assay inlets are shown in Figure 1.

nanoliter sample volumes for SNP genotyping, allowing genotyping of 192 samples against 24 assays to provide a total of 4608 reactions in a single run.

Sample and assay loading requires precise pipetting of small volumes, which can be easily achieved using the ASSIST PLUS pipetting robot together with a VOYAGER adjustable tip spacing pipette. It takes less than 15 minutes to load the 192.24 Dynamic Array IFC with 192 samples and 24 assays using the ASSIST PLUS pipetting robot.

- · It can be challenging to maintain a constant pipetting height when working with multichannel pipettes. This task is more reproducible with the ASSIST PLUS pipetting robot than when performed manually.
- Bubble-free transfer of liquids is required during IFC loading. The liquid handling parameters can be adapted to achieve optimal transfer of samples and assays. This process only needs to be done once. Thereafter, it is performed reliably by the ASSIST PLUS.



ASSIST PLUS

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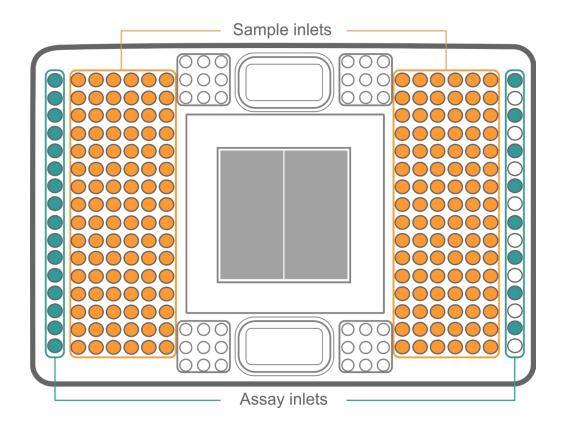


Figure 1: Pipetting positions of the samples (orange) and assays (green) on the 192.24 Dynamic Array IFC.

The 192.24 Dynamic Array IFC should be freshly prepared according to the Fluidigm Quick Reference Guide: Genotyping with the 192.24 IFC Using SNP Type Assays (see downloads) prior to loading the samples and assays. The assay mixes and sample premixes should be prepared following the Fluidigm Quick Reference Guide, then mixed and spun down before loading onto the IFC.

IMPORTANT: Once opened, the 192.24 Dynamic Array IFC should be used withing 24 hours.

The protocol is divided into two programs:

Program 1: IFC_Sample
Program 2: IFC_Assay

Experimental set-up – Program 1

Deck position A: Empty.Deck position B: 192.24 Dynamic Array IFC.Deck position C: Armadillo 96 well PCR plate (Thermo Fisher Scientific) containing the samples.



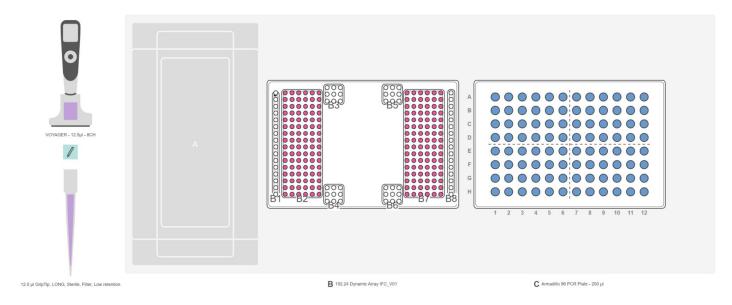


Figure 2: Deck set-up for sample loading onto the IFC. **Position A:** Empty. **Position B:** 192.24 Dynamic Array IFC (magenta). **Position C:** Armadillo 96 well PCR plate containing the samples (blue).

1. IFC sample loading	STEP: Loading the IFC with the samples	HOW TO: Place the 192.24 Dynamic Array IFC on position B and the 96 well PCR plate containing the samples on position C (Figure 2). Select and run the custom program 'IFC_Sample' on the VOYAGER pipette. The pipette transfers 3 µl of sample from the 96 well PCR plate to the left sample inlet of the 192.24 Dynamic Array IFC in 'Repeat Dispense' steps. Once the pipetting steps are complete, the pipette informs the user to change the sample plate. The process is repeated, using 'Repeat Dispense' steps to transfer samples from the second 96 well PCR plate to the right sample inlet of the 192.24 Dynamic Array IFC. Pre- and post-dispensing are used at each pipetting step for improved accuracy and precision during liquid handling.
		 Tip: Low aspiration and dispense speeds (set at 2 and 4, respectively), together with the use of Low Retention GripTips,

respectively), together with the use of Low Retention GripTips, prevent bubble formation when pipetting the samples. It is also possible to program a tip touch onto the wall of the well or the surface of the liquid, removing droplets that may stick to the point of the pipette tip and increasing pipetting precision.

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Figure 3: The ASSIST PLUS pipetting robot equipped with an 8 channel VOYAGER electronic pipette loads the first samples onto the 192.24 Dynamic Array IFC.

Experimental set-up – Program 2

Deck position A: Empty.Deck position B: 192.24 Dynamic Array IFC.Deck position C: Armadillo 96 well PCR plate (Thermo Fisher Scientific) containing the assays.

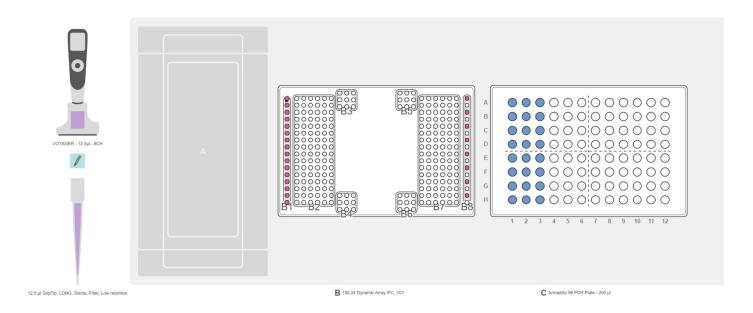


Figure 4: Set-up for assay loading onto the IFC. **Position A:** Empty. **Position B:** 192.24 Dynamic Array IFC (magenta). **Position C:** Armadillo 96 well PCR plate containing the assays (blue).

Application Note

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· · · · · ·	STEP: Loading the IFC with the assays		 HOW TO: Keep the 192.24 Dynamic Array IFC on position B and place the 96 well PCR plate containing the assays on position C (Figure 4). Select the custom program 'IFC_Assay' and press 'Run' on the VOYAGER electronic pipette. The ASSIST PLUS pipetting robot transfers 3 µl of each assay from the first column of the 96 well PCR plate to every odd-numbered position of the left assay inlet on the IFC (Figure 5, 1). At the second pipetting step, every odd-numbered position of the PCR plate (Figure 5, 2), and at the final pipetting step, assays from the third column are transferred to every even-numbered position of the left assay inlet on the IFC (Figure 5, 3). As in the previous program, pre- and post-dispensing are programmed at every step for precise pipetting results. Tips: To be able to pipette to every odd-numbered position of the assay inlet, the user should click on the tip spacing icon in VIALAB when editing the target positions. It is very important to place the 192.24 Dynamic Array IFC on position B, as this is the only deck position where the right inlets are available to the ASSIST PLUS pipetting robot. 		
1) Sample inlets		2)	Sample inlets	3)	

Figure 5: The sequence of the assay loading.



Figure 6: The first eight assay are loaded onto the inlets of the 192.24 Dynamic Array IFC.

Application Note



The user should pipette the pressure fluids and blot the carrier surface with a dry, lint-free cloth after loading the assays onto the Dynamic Array IFC. The 192.24 Dynamic Array IFC is then ready to run.

Remark

VIALAB software: The VIALAB programs can be easily adapted to the user's specific labware and protocols.

Partial plates: Programs can be adapted to a different number of samples at any time, giving laboratories total flexibility to meet current and future demands.

Conclusion

- The ASSIST PLUS pipetting robot, together with the Fluidigm 192.24 Dynamic Array IFC, offers a ready-to-use, high throughput automated solution for SNP genotyping.
- Fast, efficient, precise and reproducible sample and assay loading is easily achieved using the ASSIST PLUS pipetting robot.
- Extended pipetting processes may cause repetitive strain injuries (RSIs), which can be avoided by automating the IFC loading steps with the ASSIST PLUS pipetting robot.
- The VOYAGER electronic pipette loaded onto the ASSIST PLUS informs the user whenever manual intervention is need, reducing human errors.

- Precise pipetting of viscous or low surface tension liquids can be challenging. Using low retention GripTips can overcome this hurdle.
- Fluidigm offers a wide range of IFCs for different sample/ assay combinations for PCR-based or NGS workflows, depending on the needs of the customer. Labware files can be created for each IFC in VIALAB software. This function is included in the VIALAB software package for no extra cost.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus
INTEGRA Biosciences	4721	VOYAGER 8 channel 12.5 µl electronic pipette	https://www.integra-biosciences.com/global/en/ pipetting-robots/voyager
INTEGRA Biosciences	6505	12.5 µl Low Retention, Sterile, Filter Grip Tips	https://www.integra-biosciences.com/global/en/pipette- tips/griptip-selector-guide
Fluidigm	SKU BMK-M- 192.24GT	192.24 Dynamic Array IFC for SNP Genotyping	https://store.fluidigm.com/Genomics/ ApplicationsGenomics/Genotyping/192-24%20 Dynamic%20Array%E2%84%A2%20IFC%20for%20 SNP%20Genotyping?cclcl=en_US
Thermo Fisher Scientific	AB2396	Armadillo PCR Plate, 96- well, clear, clear wells	https://www.thermofisher.com/order/catalog/product/ AB2396

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