Application Note

INTEGRA

Automated RT-PCR set-up for COVID-19 testing

Introduction

The emergence and outbreak of the novel coronavirus SARS-CoV-2 (COVID-19) has placed unprecedented demands on laboratories testing for COVID-19, leaving scientific staff to contend with a spiraling influx of patient samples and a rapid, continuous growth in workload.

Laboratories need additional automated liquid handling instruments for viral nucleic acid extraction and RT-PCR set-up – which are the most labor-intensive processes in the diagnostic testing workflow – to increase the sample throughput capacity, reduce manual intervention by laboratory analysts and fast track the development of COVID-19 assays.

The ASSIST PLUS pipetting robot together with a VOYAGER adjustable tip spacing pipette, Low Retention GripTips and SureFlo™ 10 ml Reagent Reservoirs were successfully used for RT-PCR set-up in COVID-19 testing laboratories.

Key benefits:

- The full automation capability of the ASSIST PLUS reduces manual intervention and frees highly valuable time for laboratory personnel in this critical COVID-19 pandemic.
- The compact and easy-to-use ASSIST PLUS pipetting robot allows fast set-up regarding installation and programming, allowing labs to immediately increase their sample processing capacity and fast track assay development for COVID-19 sample testing.
- VOYAGER adjustable tip spacing pipettes in combination with the ASSIST PLUS provide unmatched pipetting ergonomics by automatically reformatting patient samples from tube racks into 384 well plates.
- Optimal pipette settings, including tip immersion depth, pipetting speeds and angles, deliver reproducible, precise and accurate results, with no contamination observed in controls or patient samples.
- The use of INTEGRA's low dead volume, SureFlo 10 ml reagent reservoirs, together with Low Retention GripTips, demonstrated excellent results, enabling efficient handling of the precious and expensive One-step RT-PCR Mastermix used for patient testing.

Step-by-step procedure:

Experimental set-up

The ASSIST PLUS pipetting robot is used to automate testing of suspected COVID-19 positive cases in a 384 well plate. The pipetting robot operates a VOYAGER 12 channel 50 µl electronic pipette with 125 µl Sterile, Filter, Low Retention GripTips.

To double the available testing capacity and, concurrently, decrease the cost per test of expensive One-Step RT-PCR reagents of dwindling availability, the total PCR reaction volume was miniaturized, reducing it to 10 µl – inclusive of 7.5 µl One-step RT-PCR Mastermix and 2.5 µl of nucleic acid template. The templates were extracted from combined nasopharyngeal/ oropharyngeal flocked swabs or sputum samples.

The following procedure is based on the protocol used by the Microbiology and Molecular Pathology Department at Sullivan Nicolaides Pathology (SNP) – part of the Sonic Healthcare Group – in Brisbane, Australia.

ASSIST PLUS

The protocol is divided into two pipetting programs.

Overview of the pipetting steps and corresponding programs:

Program 1: Add the Mastermix (1-COVID-19)
Program 2: Add the nucleic acid template (2-COVID-19)

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1. Add the Mastermix

STEP: Fill the 384 well plate with the One-step RT-PCR Mastermix.

HOW TO: Place the One-step RT-PCR Mastermix in a 10 ml sterile, polystyrene reagent reservoir with INTEGRA's SureFlo anti-sealing array. Set up the deck with the required labware, as indicated in **Figure 1**. Select the VIALAB program 1-COVID-19. The VOYAGER pipette automatically transfers the Mastermix from the reservoir into the 384 well plate (LightCycler® 480 Multiwell Plate, Roche) using the Repeat Dispense Mode with tip touch. Each well of the plate is filled with 7.5 µl of Mastermix.

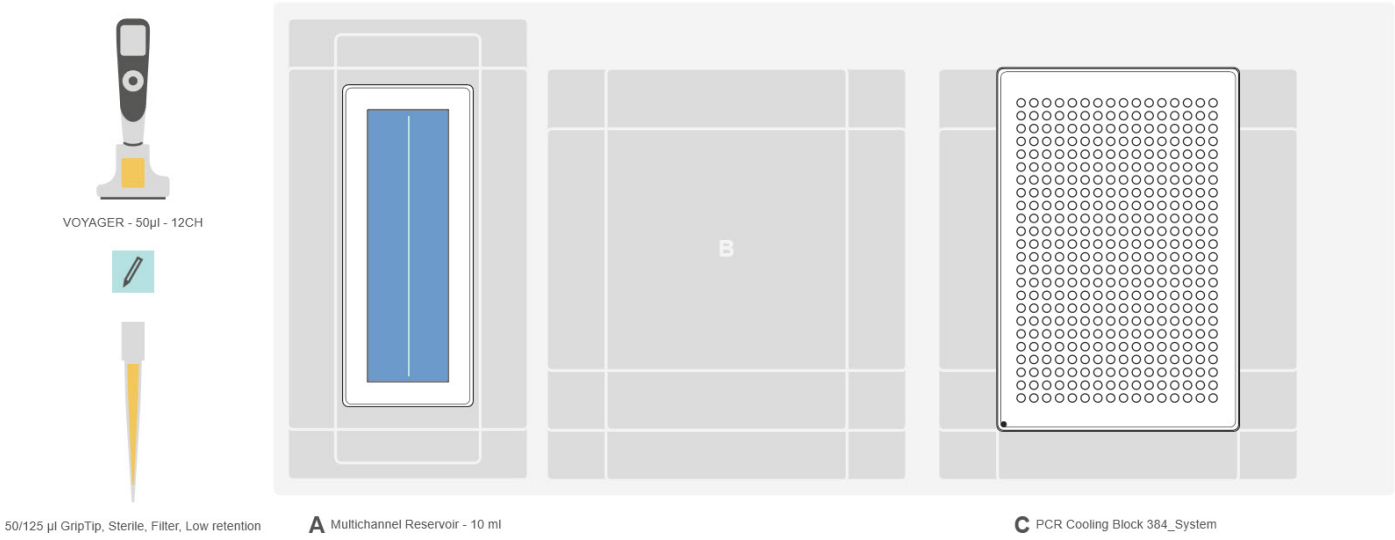


Figure 1: The set-up for program 1-COVID-19. **Position A:** 10 ml reagent reservoir with SureFlo anti-sealing array containing 3 ml of One-step RT-PCR Mastermix. **Position C:** 384 well plate placed on a PCR 384 well cooling block, allowing Mastermix and samples to be kept cold, and enabling exact positioning of the PCR plate on the deck.

Tips:

- Using a 10 ml reagent reservoir with SureFlo anti-sealing array allows a very low dead volume (<20 µl) to minimize the loss of expensive reagent of dwindling availability (see **Figure 2**).
- The combination of a low pipetting speed – set at 2 – and Low Retention GripTips shows excellent results when pipetting the viscous and foamy Mastermix.
- Pre- and post-dispense settings, together with the tip touch option, guarantee reproducible, precise and accurate pipetting results (see **Figure 2**).
- The PCR cooling block is used as a support to fix the position of the 384 well plate on the deck, ensuring exact tip positioning when pipetting. The cooling block also helps to keep samples and reagents cool if required by the protocol.

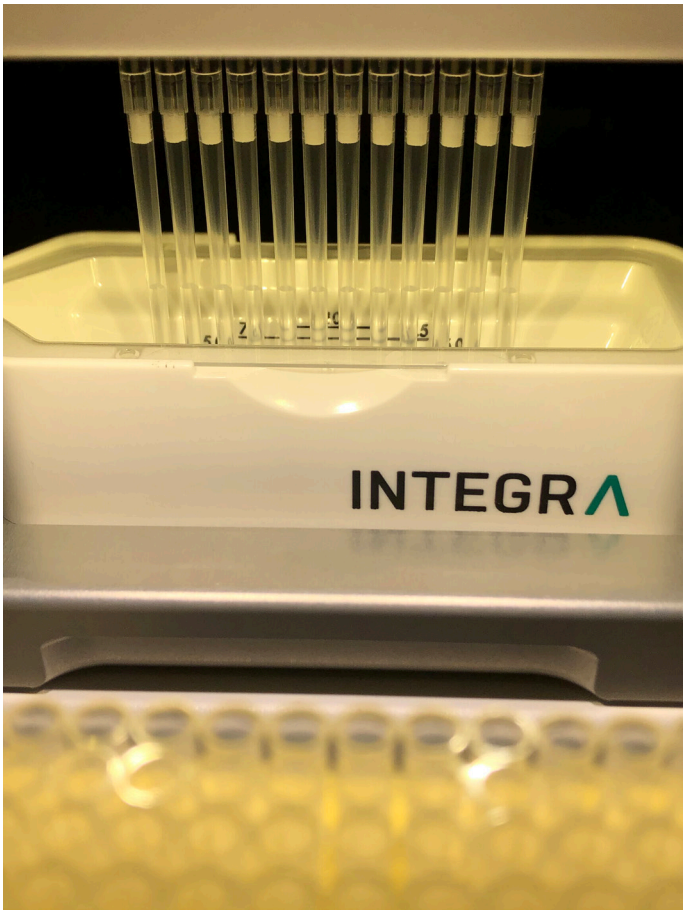


Figure 2: Precise and accurate dispensing of One-step RT-PCR Mastermix from the low dead volume reagent reservoir to the 384 well plate.

2. Add the nucleic acid templates

STEP: Transfer the samples from four 96-format tube racks to the 384 well plate.

HOW TO: Nucleic acid templates extracted from combined nasopharyngeal/oropharyngeal flocked swabs or sputum samples are stored in FluidX Cluster 0.7 ml tubes placed in a 96-format rack. The VOYAGER pipette transfers 2.5 µl of template from the tubes to the 384 well plate, automatically changing the GripTip pipette tips after each dispense. Both **Position A** and **B** are used to house the samples on the deck (see Figure 3). The pipette prompts the user when it is time to replace the tube racks on the deck. After user confirmation, the VOYAGER pipette continues reformatting the samples from tubes to the plate.

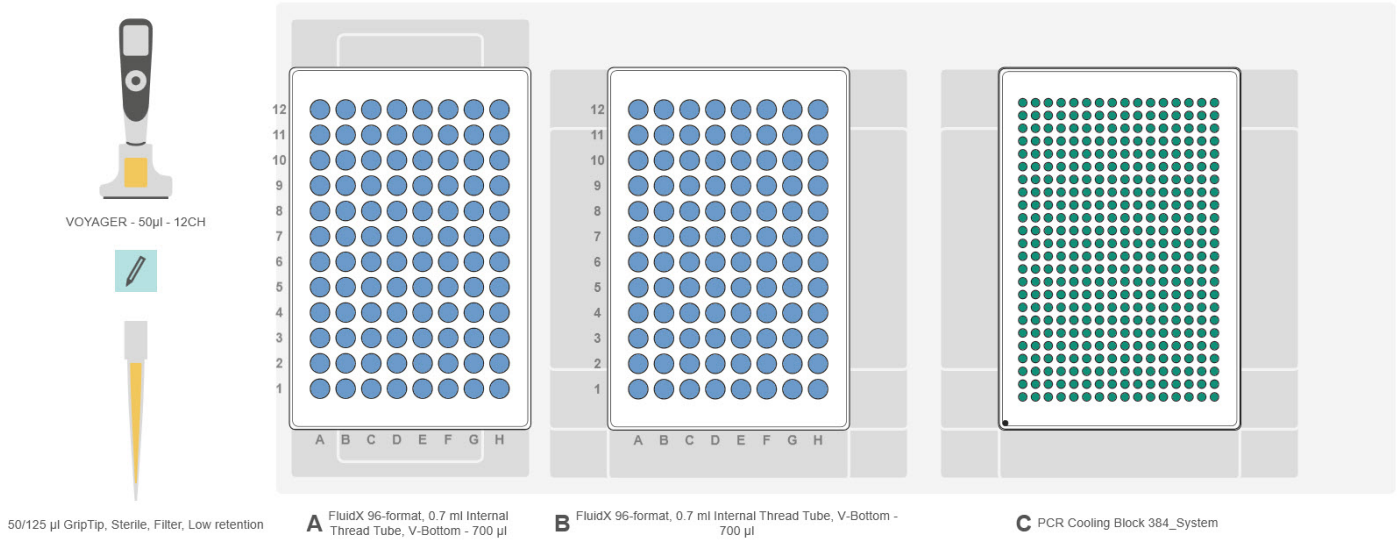
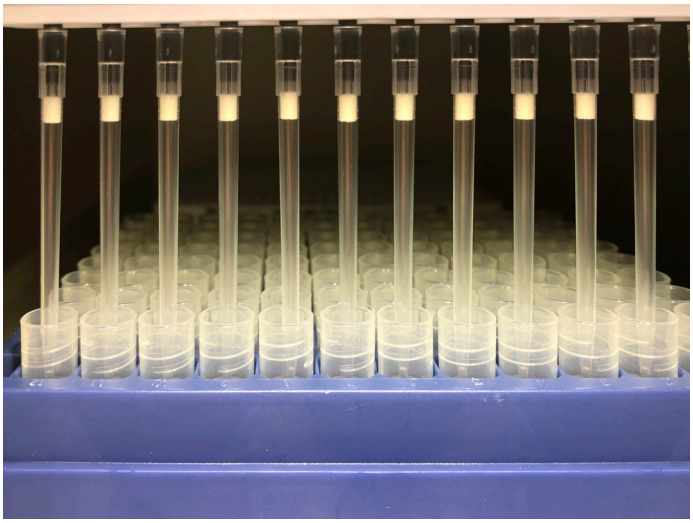


Figure 3: The set-up for program 2-COVID-19. **Position A** and **B**: FluidX Cluster 0.7 ml tubes containing the nucleic acid templates. The tubes are stored in a 96-format rack. A total of four sample racks are used for the protocol (two on **Position A** and two on **Position B**). **Position C**: 384 well plate placed on a PCR 384 well cooling block.

Tips:

- The VOYAGER pipette's tip spacing capability combined with automatic Tip Change ensures easy and rapid sample transfer without risk of contamination or reformatting errors.
- Using an air gap of 1.5 µl when aspirating the viral nucleic acid template eliminates the risk of contamination risk during pipette tip travel.



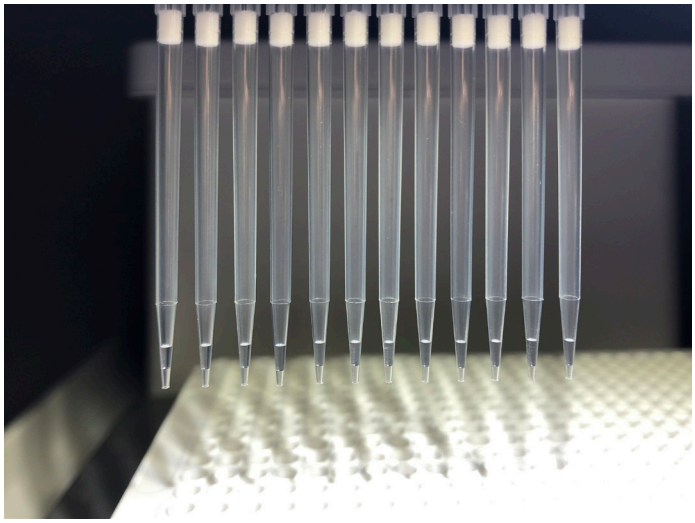


Figure 4: Easy and rapid transfer of patient nucleic acid templates from the tube rack to the 384 well plate using the VOYAGER adjustable tip spacing pipette together with the ASSIST PLUS pipetting robot.

Note: Automated RT-PCR testing for COVID-19 with the ASSIST PLUS can also be performed using a VOYAGER 8 channel 50 µl electronic pipette (see **Figure 5**).

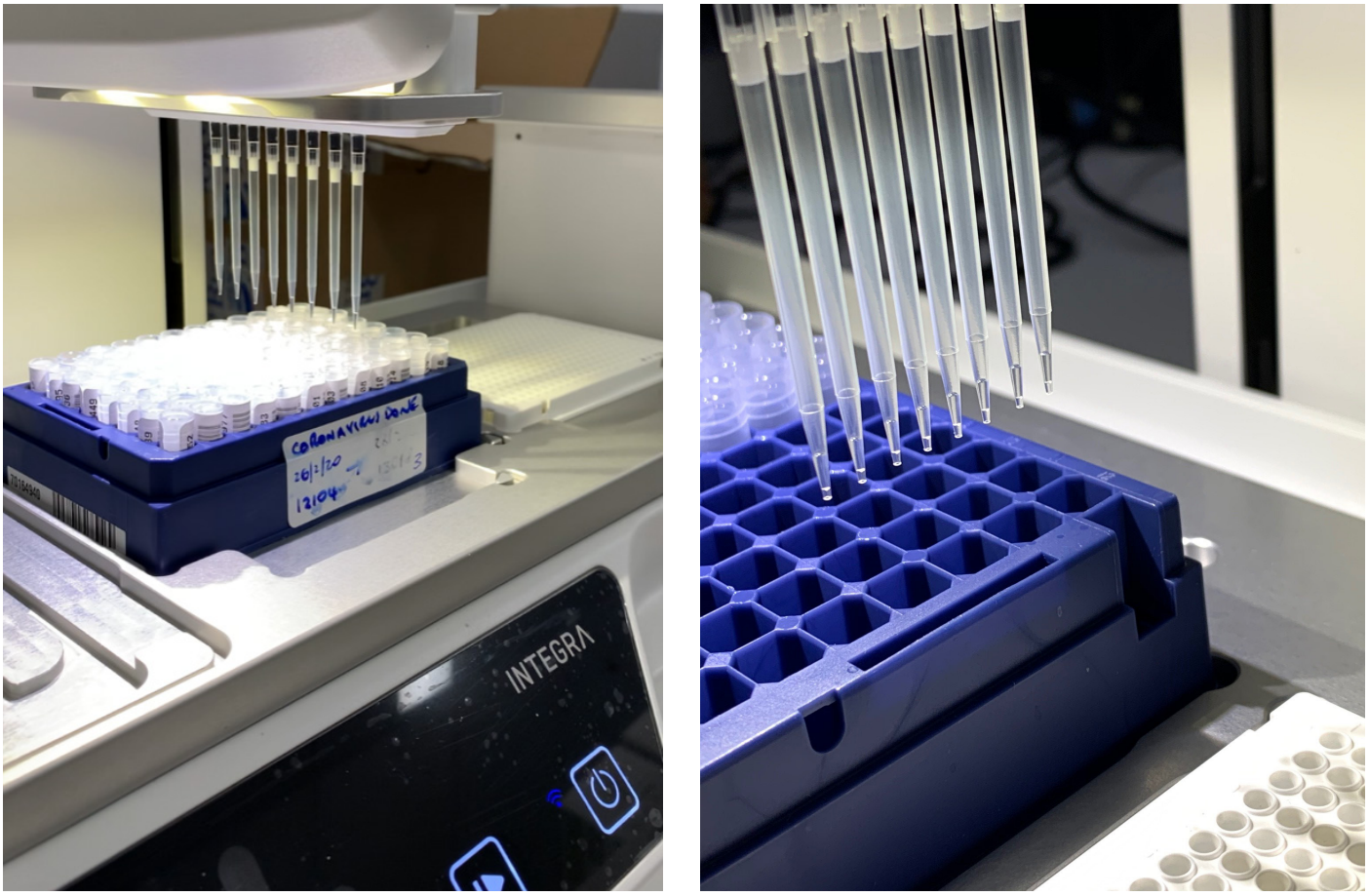


Figure 5: Automated RT-PCR testing for COVID-19 using the ASSIST PLUS pipetting robot together with a VOYAGER 8 channel adjustable tip spacing pipette, as performed in the Microbiology and Molecular Pathology Department at SNP.

Remarks

4 Position Portrait Deck: If your process allows, the protocol can be compiled in one simple program using the 4 Position Portrait Deck option of the ASSIST PLUS (see **Figure 6**).

96 well plates: The protocol can be readily adapted to 96 well format.

VIALAB software: The VIALAB programs can be easily adapted to your specific labware and protocols.

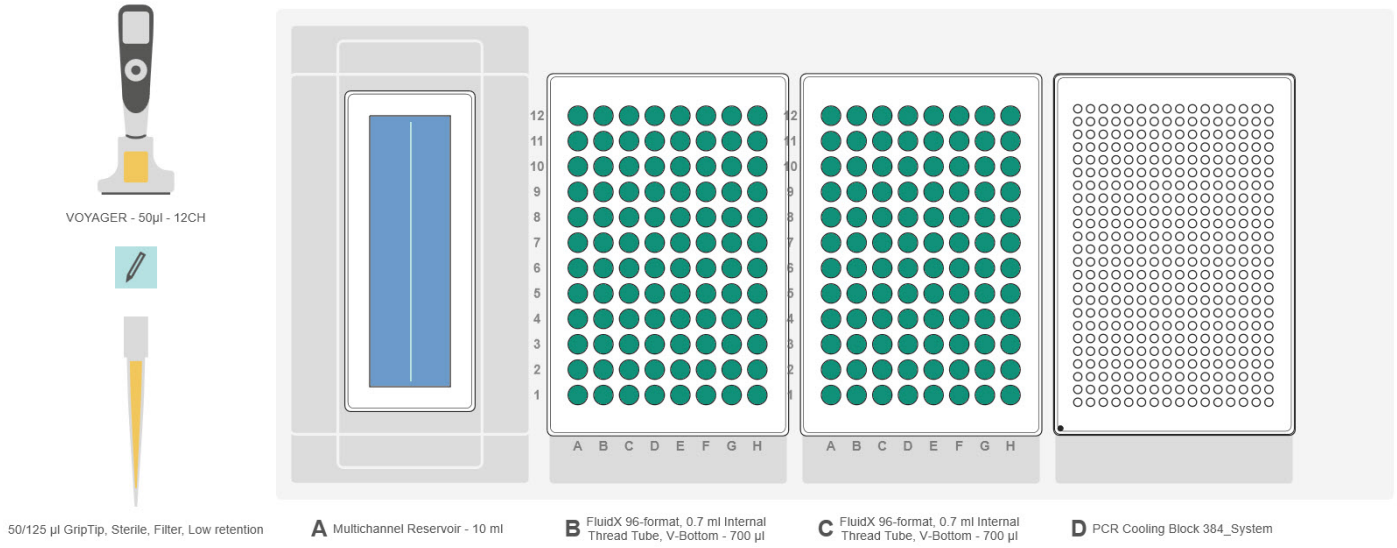


Figure 6: Example set-up of the 4 Position Portrait Deck when combining programs 1-COVID-19 and 2-COVID-19 in one program.

Conclusion

In the context of a global pandemic where laboratories are under increasing pressure to analyze more and more patient specimens to confirm COVID-19 cases, testing labs can rapidly benefit from the advantages of the ASSIST PLUS pipetting robot, allowing them to increase their sample processing capacity.

- Pipetting results were reproducible, precise and accurate, with no contamination observed in controls or patient samples.
- The ASSIST PLUS pipetting robot, together with the VOYAGER adjustable tip spacing pipette, increases sample processing capacity, reduces the need for manual intervention by laboratory personnel and fast tracks assay development for COVID-19 testing.
- Low Retention GripTips and a low dead volume SureFlo Reagent Reservoir allow the use of costly reagents, such as One-step RT-PCR Mastermix, to be reduced.
- The simple and fast ASSIST PLUS pipetting robot combined with the easy-to-use VIALAB software, offers immediate help for testing labs.
- While the current protocol uses 384 well plates, it can be readily adapted to 96 well format to meet future needs.
- Thanks to the VIALAB software, the pipetting programs can be easily adapted to any specific protocols and labware.

For more information about the use of the ASSIST PLUS for COVID-19 sample testing and RT-PCR set-up at the Microbiology and Molecular Pathology Department at Sullivan Nicolaides Pathology, please click here.

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	4736	VOYAGER 12 channel 50 µl pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4221	Bluetooth module for VIAFLO/ VOYAGER pipette	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6565	125 µl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	4371, 4372	10 ml Reagent Reservoir, Sterile, Polystyrene, SureFlo anti-sealing array	https://www.integra-biosciences.com/switzerland/en/reagent-reservoirs/multichannel-reagent-reservoirs#parts-and-numbers
INTEGRA Biosciences	6255	PCR 384 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4521	4 Position Portrait Deck	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
Roche	04729749001	LightCycler® 480 Multiwell Plate 384, white	https://lifescience.roche.com/global_en/products/lightcycler14301-480-multiwell-plate-384-white.html
Brooks Life Sciences	SP-1064 (Base Part No: 66-32034)	FluidX 96-format, 0.7 ml Internal Thread, Non-coded Tube, V-Bottom, Uncapped, HighBase Rack, Blue rack base, Clear Lid with White Latches	https://www.brookslifesciences.com/
Brooks Life Sciences	66-32033	FluidX 96-format, 0.7 ml Internal Thread, Non-coded Tube, V-Bottom, Uncapped	https://www.brookslifesciences.com/

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