INTEGR

Efficient and automated 384 well qPCR set-up with the ASSIST PLUS pipetting robot

Introduction

Setting up a qPCR is a tedious process consisting of multiple pipetting steps. One particularly challenging task is reformatting from microcentrifuge tubes into a 384 well plate, which is time consuming and requires a lot of concentration. Another common problem is the loss of valuable and expensive substances, such as Mastermix and precious samples, due to the reservoir dead volume. The ASSIST PLUS pipetting robot, in combination with the VIAFLO and VOYAGER electronic pipettes, streamlines the workflow and increases the throughput and the reproducibility of qPCR set-ups, with minimal manual input. The loss of expensive substances or valuable samples due to reformatting errors is eliminated. The unique design of the ASSIST PLUS pipetting robot, together with the intuitive VIALAB software, offers exceptional flexibility and straightforward implementation.

Key benefits:

- Automating the qPCR set-up with the VIAFLO 16 channel electronic pipette and the ASSIST PLUS pipetting robot allows considerably faster sample preparation, freeing up time for scientists to focus on other experiments.
- Automation of VOYAGER adjustable tip spacing pipettes with the ASSIST PLUS offers a reliable pipetting method that requires minimal manual intervention and eliminates the risk of reformatting errors.

Step-by-step procedure:

The ASSIST PLUS pipetting robot is used to set up a 384 well format qPCR by pipetting 64 samples in triplicate with two different Mastermixes for the detection of two genes of interest (GOI 1 and GOI 2).

The protocol is divided into two programs that guide the user through all the steps of the qPCR set-up.

Program 1: Mastermix_qPCR
Program 2: Samples_qPCR

The ASSIST PLUS pipetting robot operates a VIAFLO 16 channel 125 μ I electronic pipette with 125 μ I Sterile, Filter, Low Retention GripTips for **Program 1** and a VOYAGER 8 channel 12.5 μ I electronic pipette with 12.5 μ I Sterile, Filter, Low Retention GripTips for **Program 2**.

- The use of Low Retention GripTips with heightened hydrophobic properties and SureFlo[™] low dead volume reservoirs with an anti-sealing array helps to save precious samples and Mastermix. Combined with the high pipetting accuracy and precision of the ASSIST PLUS pipetting robot, this enables exceptionally low dead volumes to be achieved.
- The ASSIST PLUS pipetting robot, in combination with the intuitive VIALAB software, is quick to set up and easy to use.



Application Note

INTEGR

Program 1 – Mastermix transfer (Mastermix_qPCR)

Prepare the pipetting robot deck as follows (Figure 1):

Deck position A: Dual reservoir adapter – 2 x 10 ml reagent reservoir with SureFlo anti-sealing array (**Figure 2**) containing Mastermix 1 and 2.

Deck position B: 384 well PCR plate, placed on an INTEGRA cooling block in the landscape position.



Figure 1: Set-up for the Mastermix transfer. **Position A:** Dual reservoir adapter with 2 x 10 ml reagent reservoirs with SureFlo anti-sealing array. **Position B:** 384 well PCR placed on an INTEGRA cooling block.

1. Transfer Mastermixes into the 384 well plate

STEP: Add Mastermixes 1 and 2 into the left and right sides of the 384 well PCR plate, respectively.



Figure 2: The INTEGRA dual reservoir adapter accommodates both 10 ml reagent reservoirs on one deck position.

HOW TO: Use an EVOLVE 5000 μ I manual pipette with 5000 μ I Sterile, Filter, Low Retention GripTips to fill the left 10 ml reagent reservoir with SureFlo anti-sealing array with 1.6 ml of Mastermix 1 and the right reservoir with 1.6 ml of Mastermix 2 (**Position A**). Select and run the VIALAB program 'Mastermix_qPCR' on the VIAFLO 16 channel 125 μ I electronic pipette with 125 μ I Sterile, Filter, Low Retention GripTips. The ASSIST PLUS pipetting robot automatically transfers 7.5 μ I of Mastermix 2 (blue) into the right half (**Figure 3**) using the Repeat Dispense mode with a tip touch on the surface of the liquid to increase pipetting precision. **Figure 4** shows the pipetting robot transferring the Mastermix into a 384 well plate.

Tips:

- A pre- and post-dispense step is recommended to increase the accuracy and precision of pipetting. The pre- and postdispense volume should be between 3 and 5 % of the nominal volume of the pipette.
- The Low Retention GripTips are made from a unique polypropylene blend with heightened hydrophobic properties for superior accuracy and precision while pipetting viscous and low surface tension liquids.
- The reservoirs' SureFlo anti-sealing array and a unique surface treatment that spreads liquid evenly enable the pipette tips to sit on the bottom and still aspirate liquids accurately, reducing dead volumes.

Application Note



Figure 3: Pipetting scheme for Mastermixes 1 (pink) and 2 (blue).



Figure 4: Example of the ASSIST PLUS pipetting robot transferring a Mastermix into a 384 well PCR plate.

Program 2 – Sample transfer (Samples_qPCR)

Prepare the pipetting robot deck as follows (Figure 5):

Deck position B: 384 well PCR plate, placed on an INTEGRA cooling block. **Deck position C**: INTEGRA 1.5 ml microcentrifuge tube rack, with tubes containing samples 1-32.



Figure 5: Set-up for the sample transfer protocol. **Position B:** 384 well PCR plate, placed on an INTEGRA cooling block. **Position C:** INTEGRA 1.5 ml microcentrifuge tube rack, with tubes containing samples 1-32 (Figure 6).

INTEGR





Figure 6: Example of the ASSIST PLUS pipetting samples from the INTEGRA microcentrifuge tube rack into a 96 well plate.



1. Sample transfer into the 384 well plate

STEP: Add the 64 samples in triplicate to the Mastermixes.

How To: Place samples 1-32 in an INTEGRA 1.5 ml microcentrifuge tube rack on **Position C**. Run the VIALAB program 'Samples_qPCR' on a VOYAGER 8 channel 12.5 μ l electronic pipette to start the sample transfer. The ASSIST PLUS transfers 2.5 μ l of the first 32 samples in triplicate into Mastermixes 1 and 2 (**Figure 7**, yellow/brown), using the Repeat Dispense mode with a tip touch on the side of the well to make sure that no droplets adhere to the GripTips. After this step, a prompt informs the user to place the second series of samples (33-64) on **Position C**. The ASSIST PLUS pipetting robot continues by transferring 2.5 μ l of the samples in triplicate into the other half of Mastermixes 1 and 2 (**Figure 7**, green).

Tip:

• Use Sterile, Filter, Low Retention GripTips for optimal liquid recovery of precious solutions, such as Mastermix and samples.

Remarks

VIALAB software: The VIALAB program can easily be adapted to fit the user's demands, especially if specific labware, incubation times or protocols are needed.

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

Application Note

INTEGR

Conclusion

- The time required for a 384 well qPCR set-up can be reduced from 1.5 hours using a single channel pipette to 12 minutes using the ASSIST PLUS pipetting robot in combination with VIAFLO 16 channel and VOYAGER 8 channel pipettes.
- The ASSIST PLUS, together with the VOYAGER adjustable tip spacing pipette, guarantees perfectly reproducible test results and eliminates all risks of reformatting errors when transferring samples from microcentrifuge tubes into a 384 well plate.
- INTEGRA's Low Retention GripTips increase pipetting precision for viscous or low surface tension liquids. The reagent reservoirs with SureFlo anti-sealing array reduce the dead volume of costly reagents and precious samples.
- The intuitive VIALAB qPCR program is quick to set up and easy to use or adapt to other pipetting protocols.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4221	Communication module for INTEGRA electronic pipettes	https://www.integra-biosciences.com/switzerland/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4540	Rack for 1.5 / 2 ml microcentrifuge tubes	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6255	PCR 384 well cooling block	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4721	VOYAGER 8 channel pipette 12.5 µl	https://www.integra-biosciences.com/global/en/ electronic-pipettes/voyager#parts-and-numbers
INTEGRA Biosciences	4642	VIAFLO 16 channel pipette 125 µl	https://www.integra-biosciences.com/global/en/ electronic-pipettes/viaflo#parts-and-numbers
INTEGRA Biosciences	3019	EVOLVE manual pipette 5000 µl	https://www.integra-biosciences.com/global/en/ manual-pipettes/evolve
INTEGRA Biosciences	4371	10 ml multichannel reagent reservoirs, sterile, SureFlo anti-sealing array	https://www.integra-biosciences.com/global/ en/reagent-reservoirs/multichannel-reagent- reservoirs#parts-and-numbers
INTEGRA Biosciences	6555	12.5 µl GripTips Sterile, Filter, Low Retention	https://www.integra-biosciences.com/global/en/ griptip-selector-guide
INTEGRA Biosciences	6565	125 μl GripTips Sterile, Filter, Low Retention	https://www.integra-biosciences.com/global/en/ griptip-selector-guide
INTEGRA Biosciences	6545	1250 μl GripTips Sterile, Filter, Low Retention	https://www.integra-biosciences.com/global/en/ griptip-selector-guide
Greiner Bio-one	785201	Sapphire microplate, 384 well, PP, PCR, with skirt, natural	https://shop.gbo.com/en/usa/products/bioscience/ molecular-biology/pcr-microplates/bs-384-well- polypropylene-microplates/785201.html

INTEGRA Biosciences AG 7205 Zizers, Switzerland T +41 81 286 95 30 F +41 81 286 95 33 info@integra-biosciences.com INTEGRA Biosciences Corp. Hudson, NH 03051, USA T +1 603 578 5800 F +1 603 577 5529 info-us@integra-biosciences.com INTEGRA Biosciences Deutschland GmbH 35444 Biebertal, Deutschland T +49 6409 81 999 15 F +49 6409 81 999 68 info-de@integra-biosciences.com INTEGRA Biosciences SAS 95062 Cergy-Pontoise Cedex 1, France T +33 (0)1 34 30 76 76 F +33 (0)1 34 30 76 79 info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd. Egham, Surrey TW20 9EY, UK info-uk@integra-biosciences.com