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

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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**).

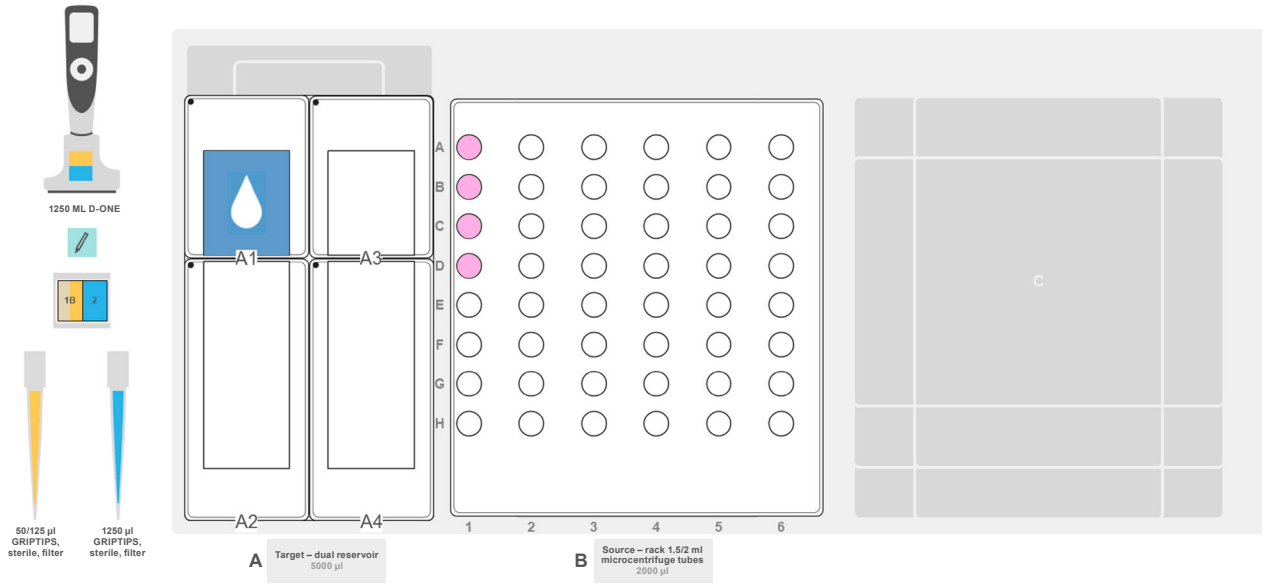

Figure 1: Deck set-up for qPCR master mix preparation in a divided reagent reservoir. **Position A:** Target – INTEGRA dual reservoir adapter with divided reagent reservoir (blue). **Position B:** Source – INTEGRA tube rack for 2 ml tubes with screw caps and 1.5 ml microcentrifuge tubes (pink). **Position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

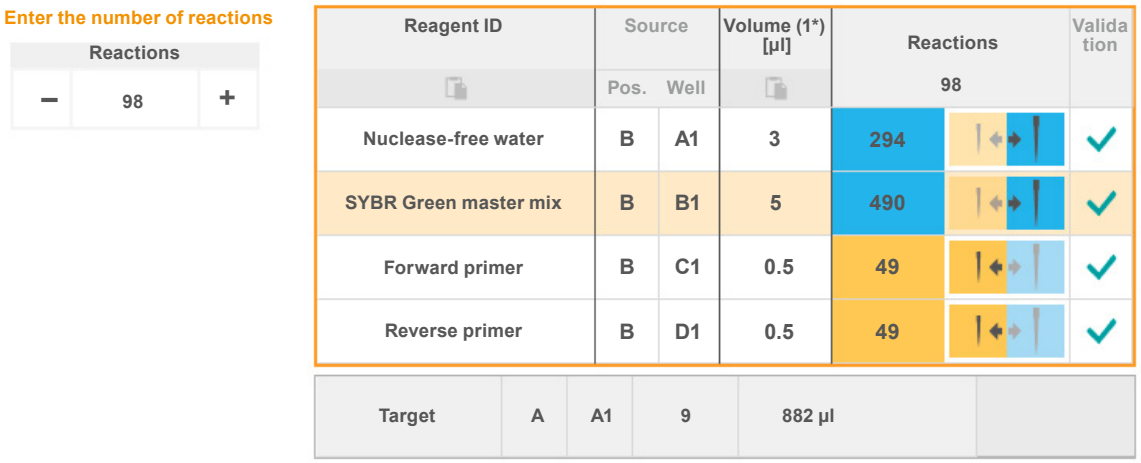

Figure 2: VIALAB calculates the volume of qPCR master mix components according to the reaction count in the master mix step.

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.

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.

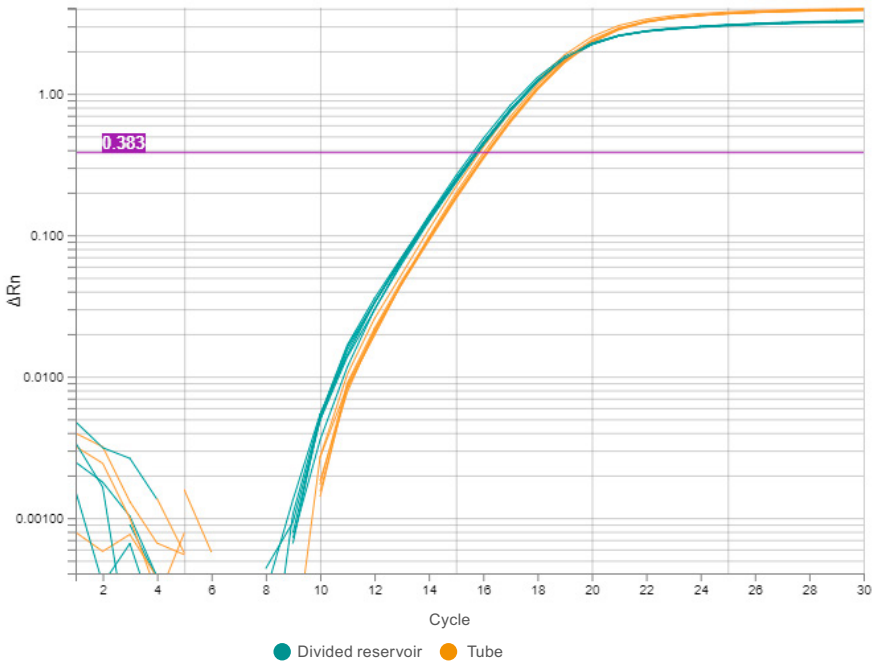

Figure 3: Amplification plot of a 250 bp fragment of the fifth variable region of the bacterial 16S rRNA gene showing a section of 6 replicates.

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.

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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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