Miniaturizing qPCR assays and simplifying standard curve preparations

Key words: Multidrop Pico 8, Digital Dispenser, qPCR, RT-PCR, Liquid dispensing, Miniaturization, QuantStudio, direct mixing, standard curve, low volume qPCR reaction

Abstract

This study demonstrates and executes a successfully miniaturized qPCR assay with the Thermo Scientific™ Multidrop™ Pico 8 Digital Dispenser and PicoIT 8 software. In three simple steps, the PicoIT 8 software enables the setup of a qPCR assay plate including titrations of reagents needed for a standard curve. Then, with the Multidrop Pico 8 Digital Dispenser, the reagents for a miniaturized volume (5 µL reaction) in a 384-well qPCR assay plate are dispensed, mixed, and ready to be placed on the Applied Biosystems™ QuantStudio™ Real-Time PCR System for amplification. The results show how the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software allows users to easily set up a miniaturized TaqMan qPCR assay plate with successful amplification of the input cDNA.

Introduction

The Multidrop Pico 8 Digital Dispenser is a non-contact liquid dispenser that enables assay miniaturization and liquid dispensing down to the picoliter range. The Multidrop Pico 8 Digital Dispenser precisely delivers fluids with %CV values of ≤ 8% for volumes as low as 11 pL into any well of a microplate including 96, 384, and 1,536 well-plate formats. The non-contact dispense technology removes the risk of contamination of the reagents. Additionally, the Multidrop Pico 8 Digital Dispenser is capable of direct mixing of the dispensed reagents to ensure a homogenous reaction.

The PicoIT 8 software included with the Multidrop Pico 8 Digital Dispenser provides an easy to use user interface. PicoIT 8 software includes pre-set protocol templates made for qPCR layouts or the user can simply create a custom protocol using the PCR function. With the pre-set protocol qPCR templates, the software guides you through 3 steps: opening a pre-set template, customizing the protocol template to your specific assay, and finally running your newly developed protocol with the Multidrop Pico 8 Digital Dispenser. In addition, the software automatically generates associated reports in the appropriate format (.DA.csv) to be imported into the QuantStudio Real-Time PCR System. The direct importing of the plate layout with the sample information eliminates the additional steps of recreating the plate set-up in the QuantStudio allowing the user to start the qPCR run instantly.

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A common hurdle in setting up TaqMan qPCR assays is ensuring pipetting precision at small reaction volumes. In many cases of qPCR assay development, the scientists settle for a larger reaction volume such as 20 µL because of pipetting errors. Decreasing the qPCR reaction volume while maintaining precision improves cost-effectiveness as well as increases throughput.

In this study, we highlight the use of the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software to set up a 5 µL reaction qPCR assay in a 384-well plate. The %CV of Ct values from each technical replicate in this study provides evidence of the Pico 8 dispenser precision when creating low volume TaqMan reactions. The plate layout including sample information is transferred directly to a QuantStudio 5 for thermal cycling steps. The purpose of this study is to demonstrate the ability to miniaturize and successfully execute a qPCR standard curve using the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software. It is important to note that this is an example of a qPCR workflow and can be used as a guide. However, the sample type, reagents, and conditions will differ depending on experimental design.

Materials

Table 1: Equipment, reagents, and consumables used in this example qPCR workflow.

Equipment	Cat. No.
Multidrop Pico 8 Digital Dispenser	Thermo Scientific, 5840600
QuantStudio 5, 384-well heat-block	Applied Biosystems, A28575
E1-ClipTip Electronic Single Channel Pipettes	Thermo Scientific, 4670000BT, 4670020BT, 4670040BT
Reagents and consumables	
TaqMan Gene Expression Master Mix (2x)	Applied Biosystems, 4370048
RT-PCR grade, Nuclease-free water	Invitrogen, AM9935
Triton X100, 1%	Invitrogen, HFH10
TaqMan Assay A (20x, custom) FAM-MGB	Applied Biosystems, custom
TaqMan Assay (20x) RNaseP, VIC-TAMRA	Applied Biosystems, custom
cDNA (34 ng/µL)	Generated from Total RNA Control (human), Applied Biosystems, 4307281
384-well MicroAmp EnduraPlate with barcode	Applied Biosystems, 4483285
MicroAmp Optical Adhesive Film	Applied Biosystems, 4360954
ClipTip filter-barrier tips (various sizes)	Thermo Scientific, 94420043, 94420318, 94420813
200 µL 4-channel Cartridges for Pico 8	Thermo Scientific, LTR0003
20 µL 8-channel Cartridges for Pico 8	Thermo Scientific, LTR0004

Methods

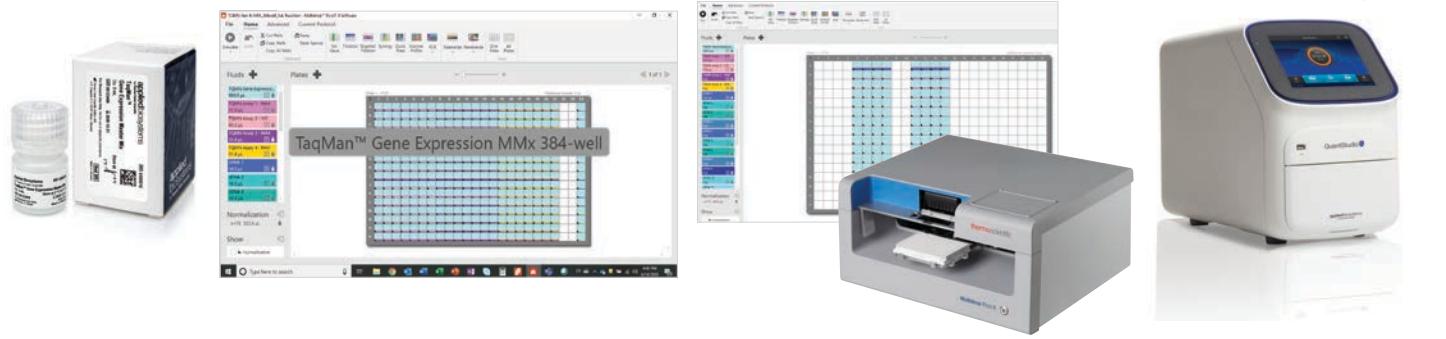
First, the PCR reaction components were prepared. Using E1-ClipTip pipettes, the TaqMan Assay reagent was prepared and cDNA template was diluted in nuclease-free water. Triton X100 was added to both mixtures at a final concentration of 0.1% to facilitate dispensing by Multidrop Pico 8 Dispenser (1). Table 2 shows the volume of each reaction component for a 5 µL reaction volume of a TaqMan qPCR assay.

Table 2: PCR reaction components in each well (5 µL total volume).

TaqMan Gene Expression Master Mix (2x)	2.5 µL
TaqMan Assay (20x) + Triton X100 (1%)	0.275 µL (0.1% Triton X100)
cDNA Template + Nuclease free water + Triton X100 (1%)	2.225 µL (0.1% Triton X100)
Total Volume	**5 µL**

3-steps used to prepare the plate for this qPCR assay:

1	2	3	
Select a pre-programmed protocol template	Customize and save as a protocol (adjust variables including standards)	Run the dispense protocol and follow the software prompts	Plate ready for qPCR



Step 1:
Open a protocol template from the PicoIT 8 software Click File > Open Template > TaqMan Gene Expression MMx 384-well (Figures 1 and 2).

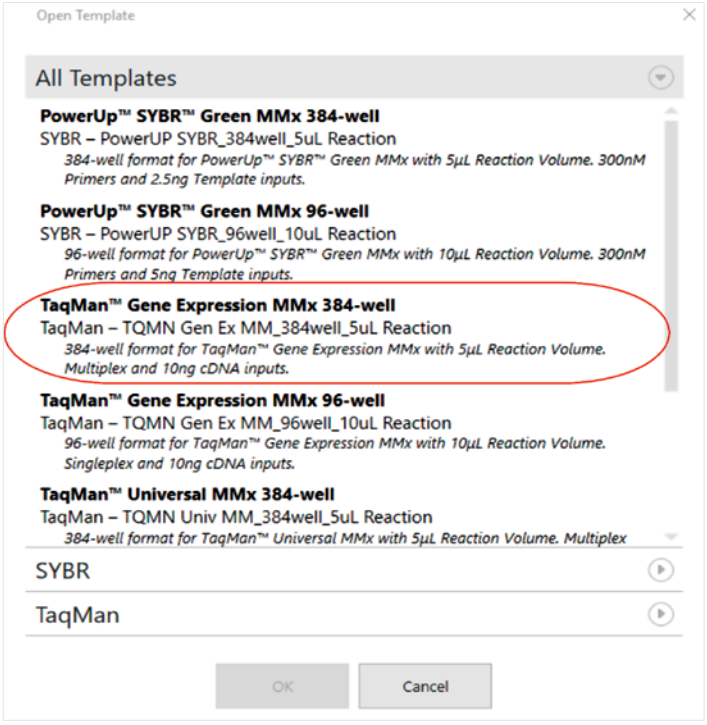


Figure 1: Screenshot of Multidrop PicoIT 8 software, the protocol template option for TaqMan Gene Expression Master Mix, 384-well 5 µL reaction volume, and Multiplex highlighted in red.

Table 3: Volume dispensed by Pico 8 for each titration.

Level	cDNA volume
1	1.9 µL
2	0.95 µL
3	0.475 µL
4	0.238 µL
5	0.119 µL
6	59.4 nL
7	29.7 nL
8	14.8 nL
9	7.42 nL
10	3.71 nL
11	1.86 nL
12	0.928 nL
13	464 pL
14	232 pL
15	116 pL

Step 2:

Customize the protocol template with the PCR function within the PicoIT software. First, select PCR function from the ribbon (red circle Figure 2), then proceed according to the PCR function: Clicked Plate > Fluids > Unknown Groups > Standards/NTCs > Layout > Summary to set up the parameters specific to the experimental design (Figures 3, 4, and 5). Customization, such as sample numbers or replicates, can also be done manually. In this study, the PCR function was used to create a set of titration curves (15 points, linear titration of 1:2 starting from cDNA 1.9 µL, 4 technical replicates) with 4 NTC (Table 3). All reactions are normalized to 5 µL.

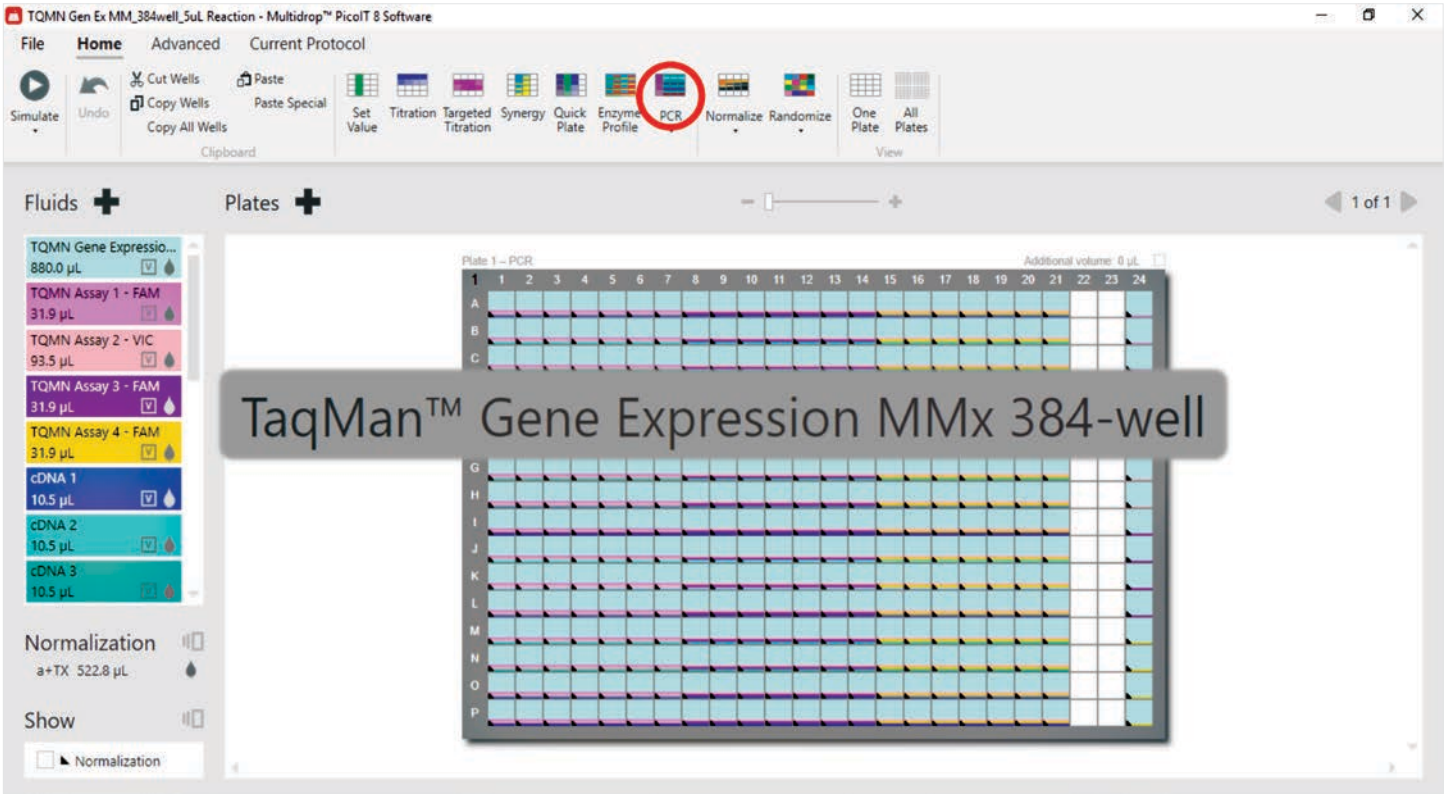


Figure 2. Screenshot of Multidrop PicoIT 8 software showing the TaqMan Gene Expression MMx 384-well protocol template. Red circle indicates the PCR function icon.

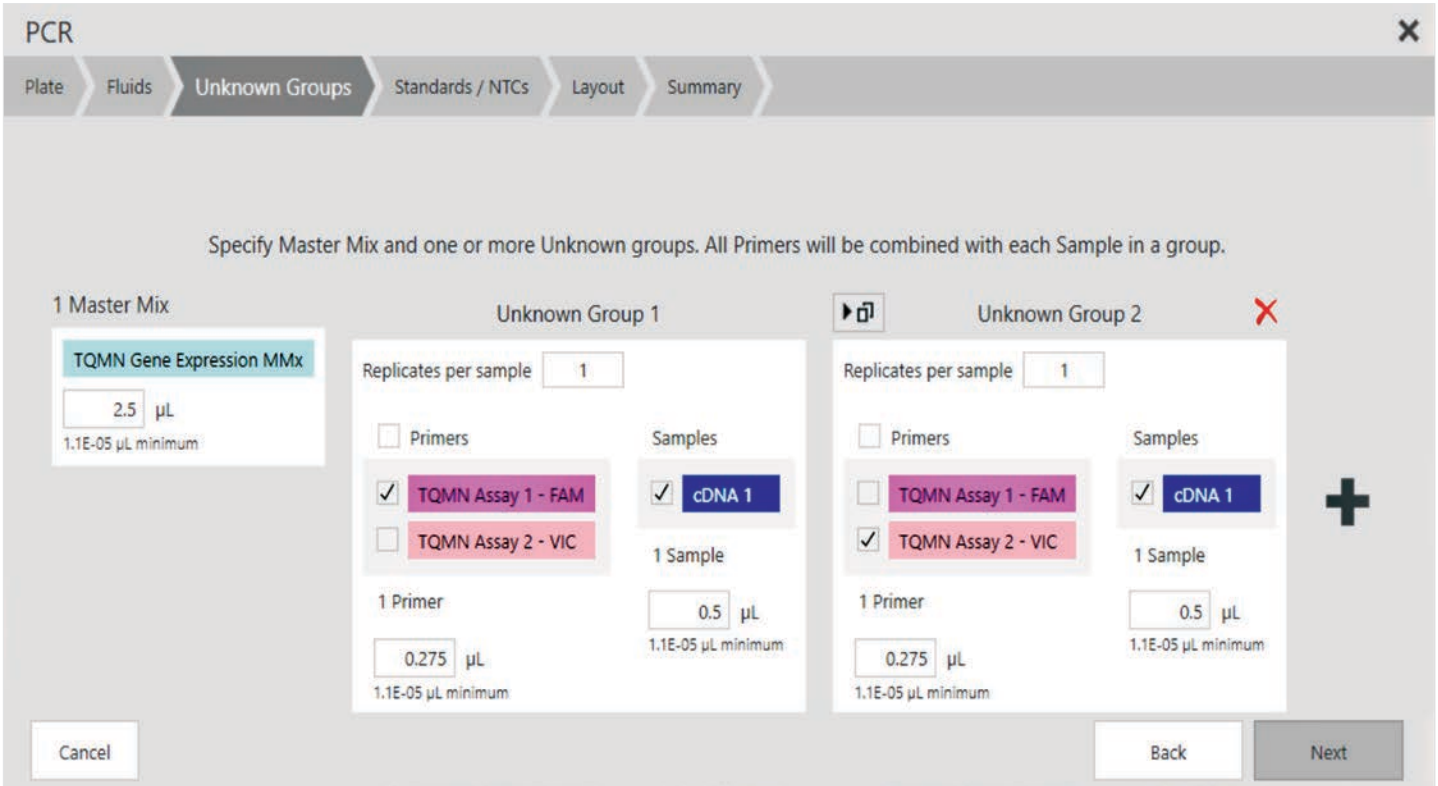


Figure 3. Screenshot of PCR function window and the parameters set for this study in the Unknown Groups tab.

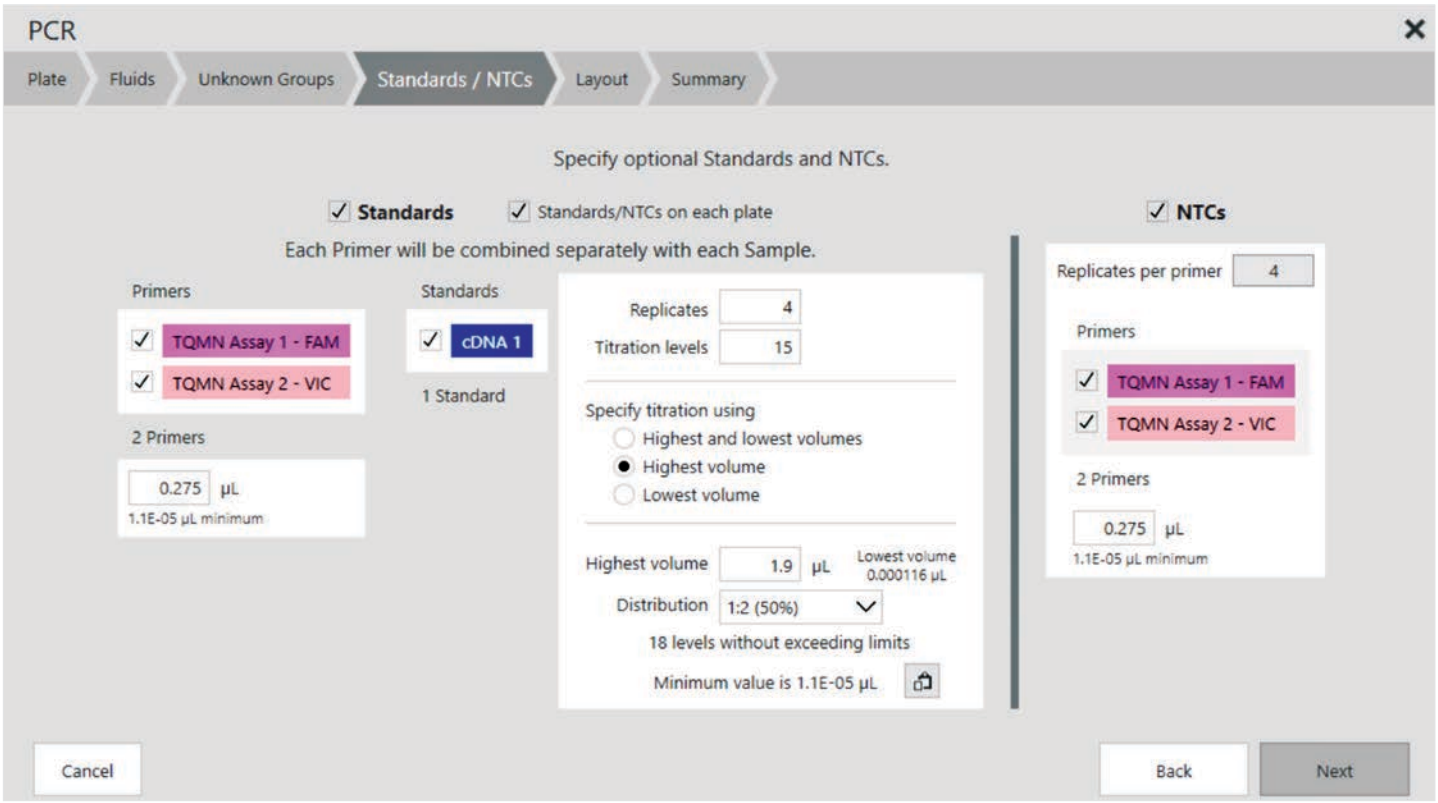


Figure 4. Screenshot of PCR function window with the parameters set for this study in the Standards/NTCs tab.

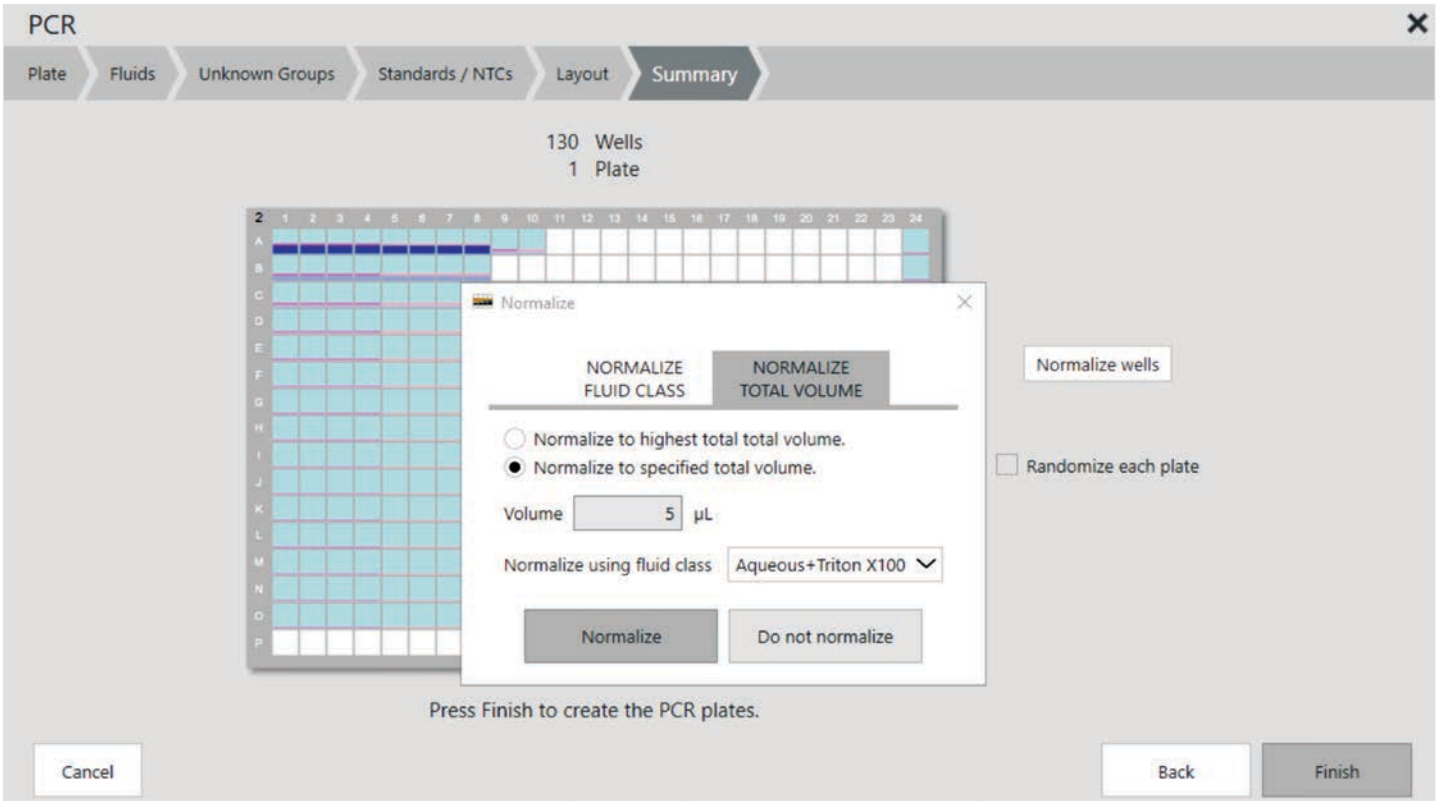


Figure 5. Screenshot of PCR function Summary window with the normalization criteria set for this study.

Step 3:

Click Run to start the dispense protocol. Using E1-ClipTip, load reagents and sample, and dispense into a 384-well microplate with the Multidrop Pico 8 Digital Dispenser (red circle Figure 6). See Table 2 for specific volumes dispensed for each well. After the run completes, the PicoIT 8 software automatically generates the report that includes a .DA.csv file. This file was then imported into QuantStudio Design and Analysis software v1.5.1 for the qPCR run.

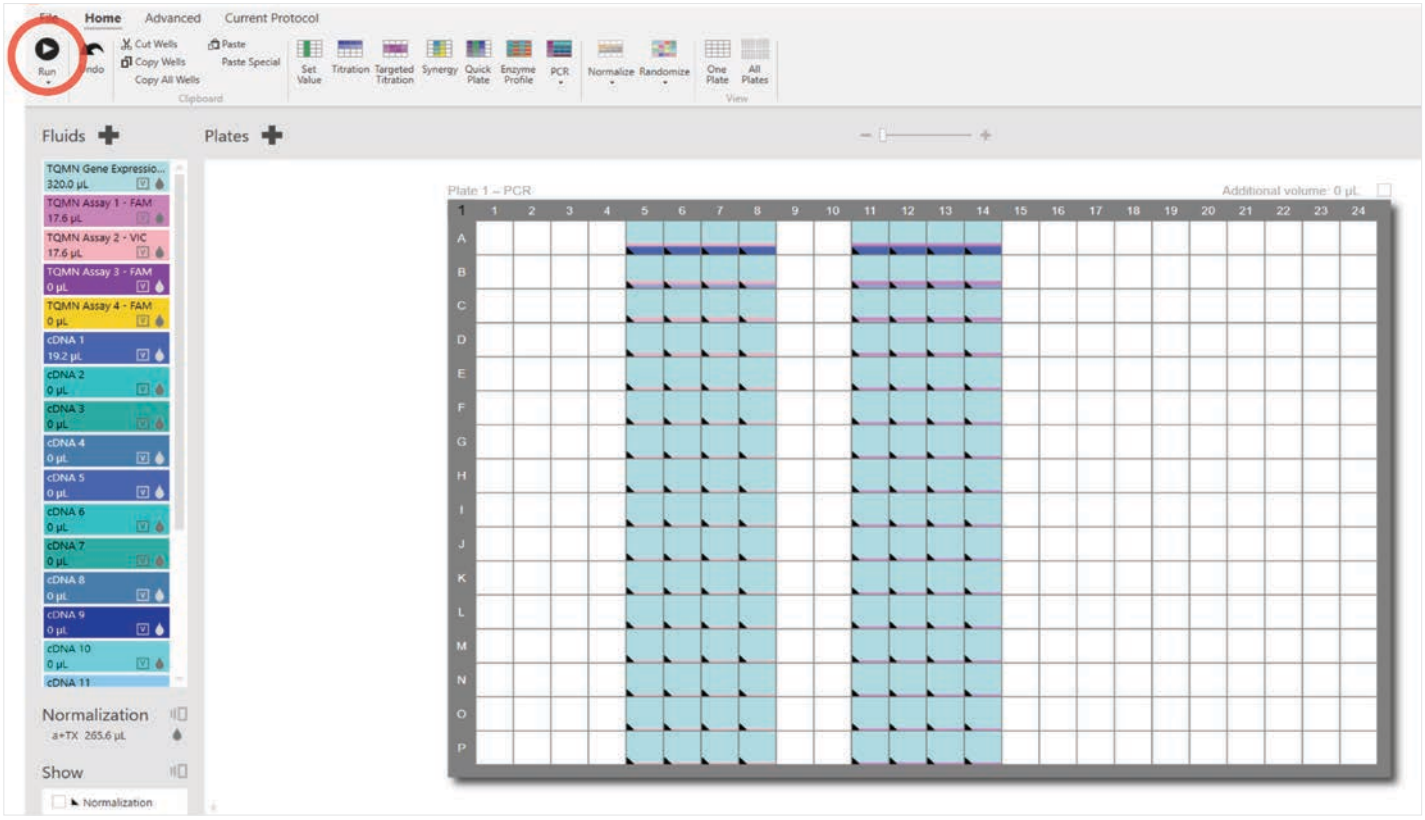


Figure 6. Screenshot of the customized protocol and the parameters set for this study. Red circle indicates the Run command.

After the qPCR assay plate was prepared with the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software, the plate was run on standard mode for qPCR with the QuantStudio 5 system. To import the .DA.csv file, click File > Import Plate Setup in the QuantStudio Design and Analysis software v1.5.1 (Figure 7).

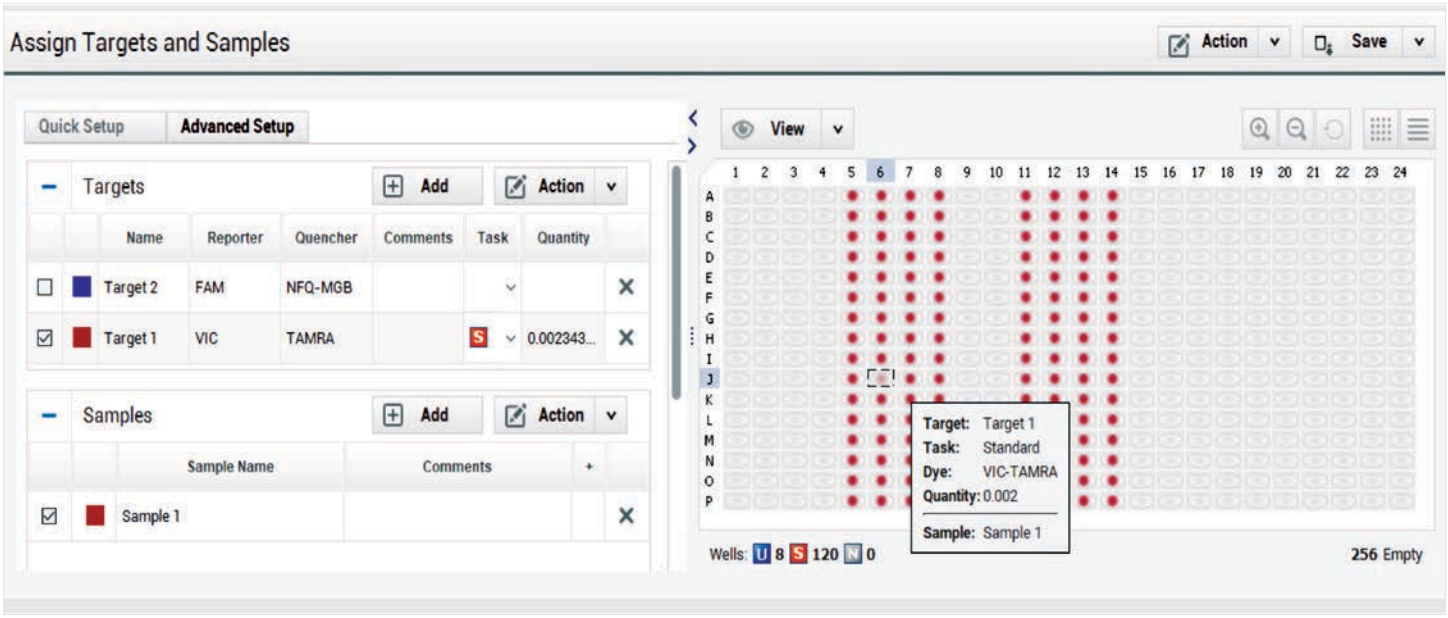


Figure 7. Screenshot of the plate setup in QuantStudio Design and Analysis software v1.5.1 and the parameters set for this study.

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Results

The Multidrop Pico 8 Digital Dispenser and PicoIT 8 software were successfully used to set up a standard curve qPCR assay plate as seen in the amplification of the cDNA (Figure 8). The run report indicated that the Pico 8 dispensed cDNA for each titration level (Table 3). The analyzed data in QuantStudio Design and Analysis software showed the standard curve (VIC) with a slope of -3.325 and primer efficiency of 99.9%. More specifically, R^2 value was 0.996 indicating the accurate dispensing of reagents. The %CV of the Ct values from technical replicates (4) of cDNA at each dilution point ranged from 0.11 – 0.61% and the average of Ct %CV for all points was 0.27%, revealing outstanding precision.

Amplification Plot **Standard Curve**

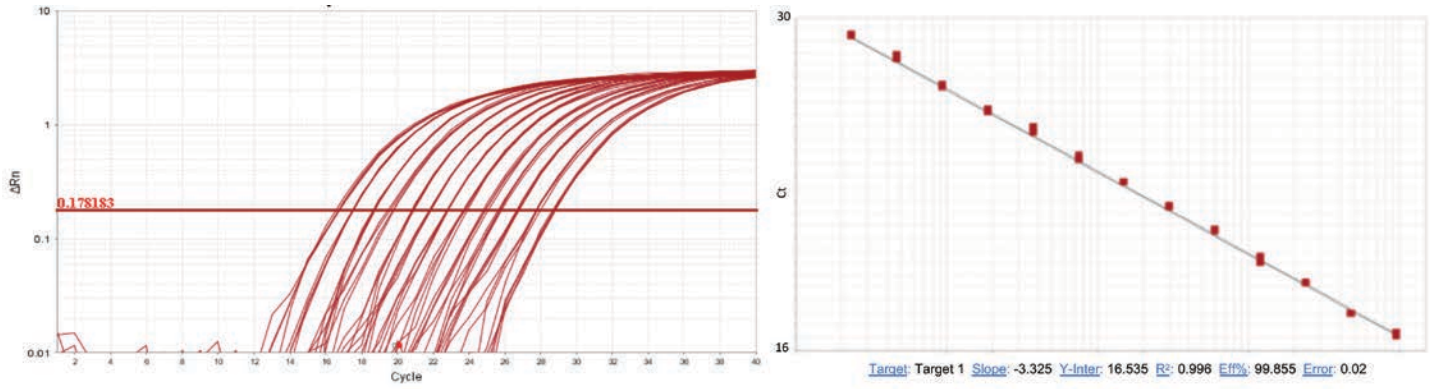

Figure 8: Screenshot of QuantStudio Design and Analysis software shows the amplification plot and standard curve from this study.

Conclusions

This study demonstrates that the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software is an excellent solution to prepare a miniaturized qPCR assay. Setting up a qPCR assay plate can be done in three easy steps when using the PicoIT 8 pre-programed protocol template and PCR function features. The results show that with the Multidrop Pico 8 Digital Dispenser, it is possible to miniaturize the TaqMan qPCR assay reaction total volume to 5 µL without compromising assay results. The Multidrop Pico 8 Digital Dispenser and PicoIT 8 software also titrates the reagents needed for a standard curve and eliminates manual pipetting for serial dilution. The titration is beneficial to accelerating the standard curve preparation process in relative quantitative PCR assays, gene expression assays, primer efficiency validations, or PCR efficiency validation.

Furthermore, each dilution is generated directly in an individual well with non-contact dispensing technology. This feature reduces the risk of contamination and technical errors that are typically introduced in manual pipetting and serial dilution.

Taken together, the Multidrop Pico 8 Digital Dispenser and PicoIT 8 software provide increased productivity in assay development. They facilitate an accelerated and miniaturized qPCR assay with decreased manual pipetting, reduced reagent amounts, and precise results.

References

1. Weyent RS, Edmonds P, Swaminathan B. (1990) Effect of ionic and nonionic detergents on the *Taq* polymerase. Biotechniques Volume 9. Issue 3. Pages 308-309.

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