RELIABLE qPCR PERFORMANCE FROM MANUAL PIPETTING TO AUTOMATION WITH PIPETMAX qPCR WORKSTATIONS

APPLICATION NOTE AN1060

BENEFITS

- Reliable qPCR plate preparation with consistent assay performance using PIPETMAX® automated workstations
- High-quality standard curves across multiple gene targets enabled by precise and repeatable liquid handling
- Scalable automation with PIPETMAX® 268 and PIPETMAX® 278 to increase application throughput
- Standardized workflows that reduce manual intervention and support consistent, repeatable results
- Automated setup to improve workflow efficiency, reduce hands-on time, and streamline laboratory operations

ADDRESSED ISSUES

- Variability in qPCR results due to inconsistent plate preparation and liquid handling in manual workflows
- Challenges in maintaining reliable amplification efficiency and standard curve quality across assays
- Risk of inconsistency across replicates and plates without standardized preparation approaches
- Need for scalable automation solutions to support both low- and mid-throughput qPCR workflows

THIS STUDY WAS PERFORMED BY JEREMY POULET, PhD, AUTOMATION PRODUCTS CATEGORY SPECIALIST

INTRODUCTION

Quantitative PCR (qPCR) is widely used for gene expression analysis and molecular diagnostics, as it is a versatile and sensitive method for DNA quantification. The accuracy and reliability of qPCR results depend not only on the amplification chemistry and instrument performance, but also on the quality of upstream samples, as well as sample and plate preparation through all the pipetting steps.

As qPCR continues to become the method of choice across a growing number of applications, laboratories are faced with increasing sample volumes and greater demands for efficiency. As a result, the need for standardized workflows has become more critical. Manual plate preparation can be time-consuming and introduce variability from user to user and from day to day, particularly across replicates and plates.

Automated liquid handling systems configured for qPCR applications, such as PIPETMAX® 268 and PIPETMAX® 278 (Figure 1), help laboratories increase throughput while maintaining consistent liquid handling performance. Both systems leverage Gilson's proven expertise in liquid handling and are designed to support different workflow requirements.

PIPETMAX 268 provides task-based automation solutions capable of fully autonomous processing of 96-well plate workflows. For 384-well applications, the system can be operated with user intervention for tip replenishment. PIPETMAX 278 expands automation capabilities through increased deck capacity, accommodating more samples, consumables, and tips. This enables autonomous processing of both 96- and 384-well plate workflows, making it well-suited for laboratories seeking greater productivity.

Figure 1
PIPETMAX® 268 qPCR and PIPETMAX® 278 qPCR

GILSON®

By standardizing pipetting steps and reducing hands-on intervention, both systems improve workflow efficiency while maintaining reliable assay performance.

This study compares the performance of PIPETMAX 268 and PIPETMAX 278 to manual pipetting for qPCR plate preparation across multiple housekeeping genes. The results demonstrate that qPCR workflows can be successfully transferred from manual pipetting to automation without compromising data quality. Together, these findings validate PIPETMAX systems as robust, precise, and easy-to-implement liquid handling solutions that help laboratories reduce repetitive manual pipetting while maintaining confidence in their qPCR results.

MATERIALS AND METHODS

Automated Liquid Handling Systems

A complete set of PIPETMAN® pipettes was used for master mix preparation following good pipetting practices.[1] Automated qPCR plate preparation was performed using PIPETMAX 268 and PIPETMAX 278 systems equipped with a MAX8X20 Pipette Head. All experiments were performed using the same sterile filtered PIPETMAN® DIAMOND Tips, enabling a seamless transition between manual and automated workflows. Both PIPETMAX 268 and PIPETMAX 278 were used to evaluate qPCR preparation across both instruments.

qPCR Workflow

qPCR experiments were performed on an Agilent AriaMX using a FAM filter. The qPCR Brilliant II SYBR master mix from Agilent was prepared according to the manufacturer's instructions, with a final reaction volume of 20 µL (17 µL master mix and 3 µL DNA).

Primers were purchased from Merck MilliporeSigma, resuspended and desalted to a concentration of 100 µM. Sequences are given in Table 1. Genomic DNA (gDNA) was extracted from HeLa cells using a QIAGEN blood and cell culture DNA kit and quantified by UV spectrophotometry at 260 nm (Denovix DS-11).

PRIMER NAME	SEQUENCE (5'–3')
ActB_Ex4_1_FWD	AGCTTCTCCTTAATGTCACGCA
ActB_Ex4_1_REV	GGACCTGACTGACTACCTCATG
B2-MIC_Ex2_2_FWD	TGGGTTTCATCCATCCGACATT
B2-MIC_Ex2_2_REV	GACAAGTCTGAATGCTCCACTT
GUSB_Ex10_1_FWD	CGCTCTGAATAATGGGCTTCTG
GUSB_Ex10_1_REV	GCTACTACTCTTGGTATCACGACT
PGK1_Ex3_2_FWD	AGTCGGTAGTCCTTATGAGCC
PGK1_Ex3_2_REV	GCAGAGATTTGAGTTCTACAGCA
TBP_Ex2_2_FWD	CACAGCTCTTCCACTCACAGA
TBP_Ex2_2_REV	AATCCCAGAACTCTCCGAAGC
TFRC_Ex17_1_FWD	TGTATTGGTTCAGATCCCTCACA
TFRC_Ex17_1_REV	TGAGAGGTACAACAGCCAACT

Table 1
Primer sequences

qPCR experiments were performed using genomic DNA from HeLa cells. Four concentration points were prepared by serial dilution and dispensed in triplicate, starting from 2 ng. Six Primer sets targeting housekeeping gene exons were analyzed in parallel on 96-well plates (ACT-Beta, B2-Mic, GUSB, PGK1, TBP and TFRC). Non-template controls (NTC) were included in each run to confirm absence of contamination and demonstrate clean and controlled workflow execution (Figure 2).

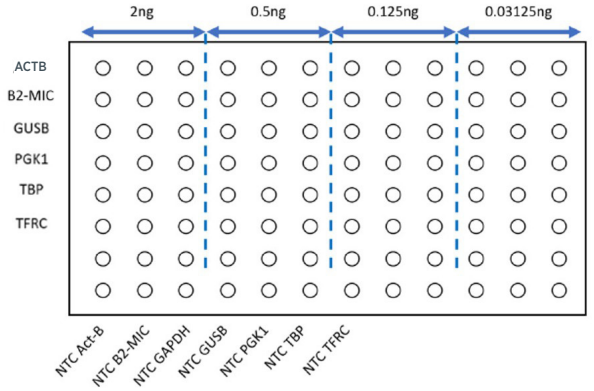


Figure 2
qPCR plate layout

RESULTS AND DISCUSSION

Seamless Transition from Manual to Automated qPCR Workflows with Consistent Assay Performance

Before assessing PIPETMAX pipetting precision during qPCR experiments, primer set and experimental design were validated manually. As illustrated in Table 2, all primer sets display an efficiency range between 90% and 110%, signifying proper amplification and primer design as described in the MIQE guidelines.[2] In addition, all manual R^2 values were above 0.99, illustrating the linearity of the standard curve. These results validate the qPCR experimental design and protocol and can then be used as a reference.

The qPCR workflow was then replicated using PIPETMAX 268 and PIPETMAX 278 automated workstations. Similar to the manual experiments, amplification efficiencies for all primer sets remained within the accepted MIQE guideline range of 90–110%, while R^2 values were consistently above 0.99 (Table 2). For most primer sets, including ACTB, B2-Mic, GUSB, PGK1, and TBP, amplification efficiencies were highly consistent between PIPETMAX 268 and PIPETMAX 278. Only the TFRC primer set showed a higher efficiency with PIPETMAX 278 (106.7%) compared to manual pipetting (90.2%) and PIPETMAX 268 (90.6%). However, this value remains within the acceptable efficiency range and was observed only for the TFRC primer set, suggesting that the difference is more likely related to the primer itself, rather than to the master mix preparation or PIPETMAX.

Taken together, these results demonstrate that qPCR workflows can be successfully transferred from manual pipetting to PIPETMAX 268 and PIPETMAX 278 without compromising assay quality, amplification efficiency, or overall qPCR performance.

PRIMER SET			
ACT-B			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	105.3%	106.4%	108.1%
R^2	0.9993	0.9999	0.9963
GUSB			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	102.2%	102.0%	106.8%
R^2	0.9986	0.9990	0.9996
TBP			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	97.6%	99.9%	90.1%
R^2	0.9965	0.9978	0.9972

B2-MIC			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	96.4%	98.9%	94.2%
R^2	0.9996	0.9969	0.9980
PGK1			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	105.4%	101.6%	101.5%
R^2	0.9971	0.9996	0.9993
TFRC			
INSTRUMENT	MANUAL	PIPETMAX 268	PIPETMAX 278
EFFICIENCY	90.2%	90.6%	106.7%
R^2	0.9905	0.9944	0.9997

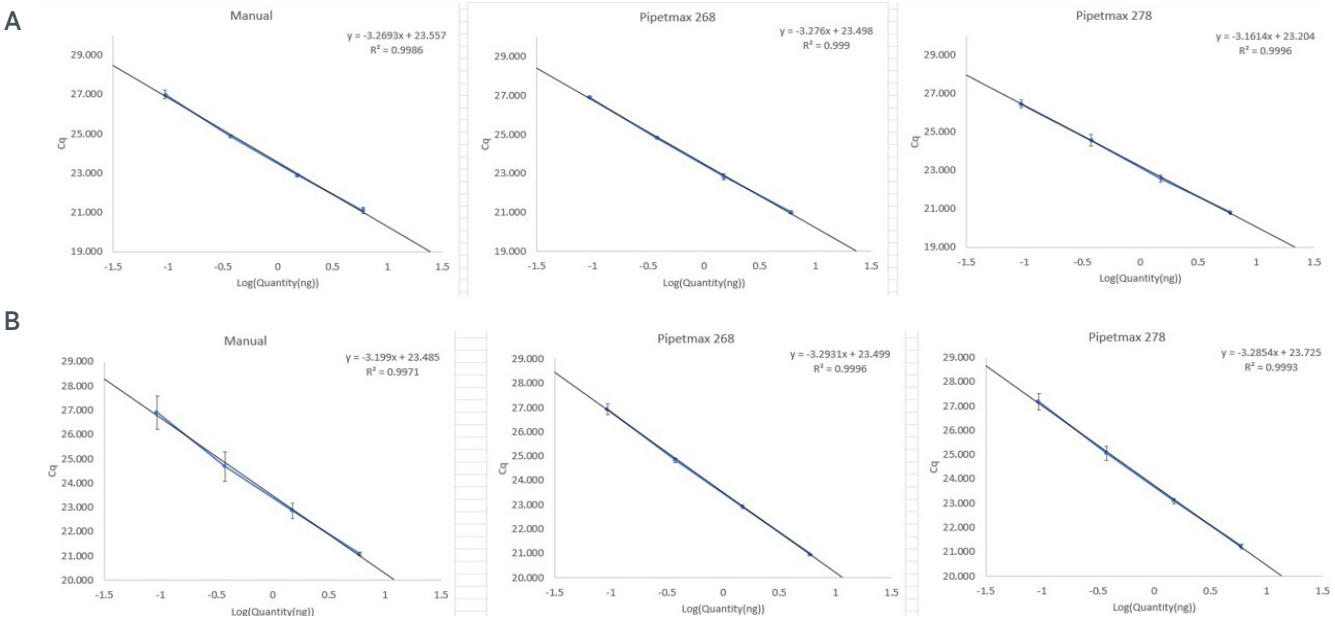
Table 2

Efficiency and R^2 summary for each primer and methods

Precise and Reproducible Liquid Handling by PIPETMAX Systems for qPCR Plate Preparation

Because qPCR relies on exponential amplification, it is particularly sensitive to pipetting variability. To assess liquid handling precision, all standard curve concentrations were dispensed in triplicate and quantification cycle (Cq) values were plotted against Log(Quantity). Error bars represent 2× the standard deviation (2SD) of triplicate measurements, giving a 95% confidence interval.

As described previously, all standard curves exhibited strong linearity across manual pipetting, PIPETMAX 268, and PIPETMAX 278 workflows. For the GUSB primer set, error bars remained consistent across all concentrations and liquid handling methods, including the lowest concentrations, which are typically more susceptible to fluctuations (Figure 3A). In addition, results obtained with the PGK1 primer set demonstrated improved precision across replicates for both PIPETMAX systems compared to manual pipetting, as illustrated by the consistently small 2SD error bars across concentrations (Figure 3B).



Figures 3A and 3B

qPCR standard curves
Quantification cycles (Cq) plotted against Log(Quantity(ng))
Error bars are 2SD
Standard curve equations and R^2 are displayed on the graph

3A. GUSB primer and **3B**. PGK1 primer

GUSB					
	Log(quantity(ng))	0.78	0.18	-0.43	-1.03
2SD	MANUAL	0.14	0.08	0.05	0.21
	PIPETMAX 268	0.08	0.16	0.01	0.05
	PIPETMAX 278	0.07	0.19	0.31	0.18

PGK1					
	Log(quantity(ng))	0.78	0.18	-0.43	-1.03
2SD	MANUAL	0.09	0.32	0.61	0.67
	PIPETMAX 268	0.01	0.05	0.09	0.23
	PIPETMAX 278	0.10	0.11	0.29	0.34

Table 3

Data for Figures 3A and 3B

To further assess consistency between workflows, Cq values for each concentration were plotted on the same chart. Figure 4 shows results for the PGK1 primer set, where similar Cq values were observed across manual pipetting, PIPETMAX 268, and PIPETMAX 278. These results indicate that automated and manual workflows generate comparable qPCR performance across concentrations.

Taken together, these findings demonstrate the precision and consistency of liquid handling by PIPETMAX 268 and PIPETMAX 278 during qPCR plate preparation, supporting reliable assay execution across replicates and concentration ranges.

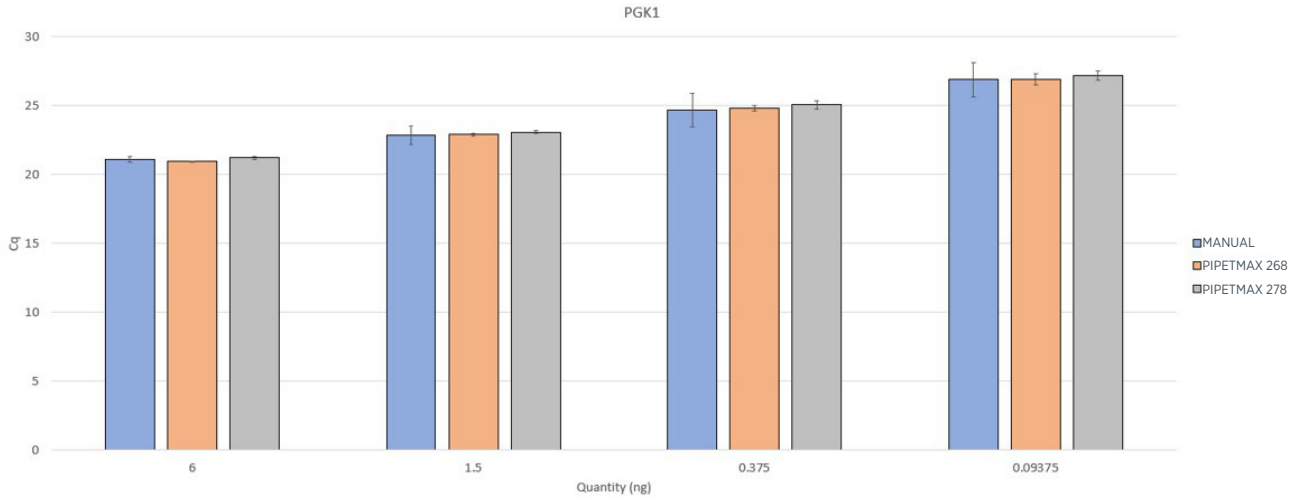


Figure 4

qPCR standard curve histogram of PGK1

Histogram of quantification cycles (Cq) plotted against Quantity(ng)
Error bars are 2SD

No Detectable Contamination in Manual and Automated qPCR Plate Preparation

As qPCR is also sensitive to cross-contamination, it is of great importance to ensure a reliable and clean environment during pipetting. Non-template controls (NTCs) were used to monitor potential contamination during qPCR plate preparation. From the experiments it appears that no contamination between primers were observed (Figure 5). These findings indicate that no detectable contamination was introduced during qPCR plate preparation using manual pipetting, PIPETMAX 268, or PIPETMAX 278.

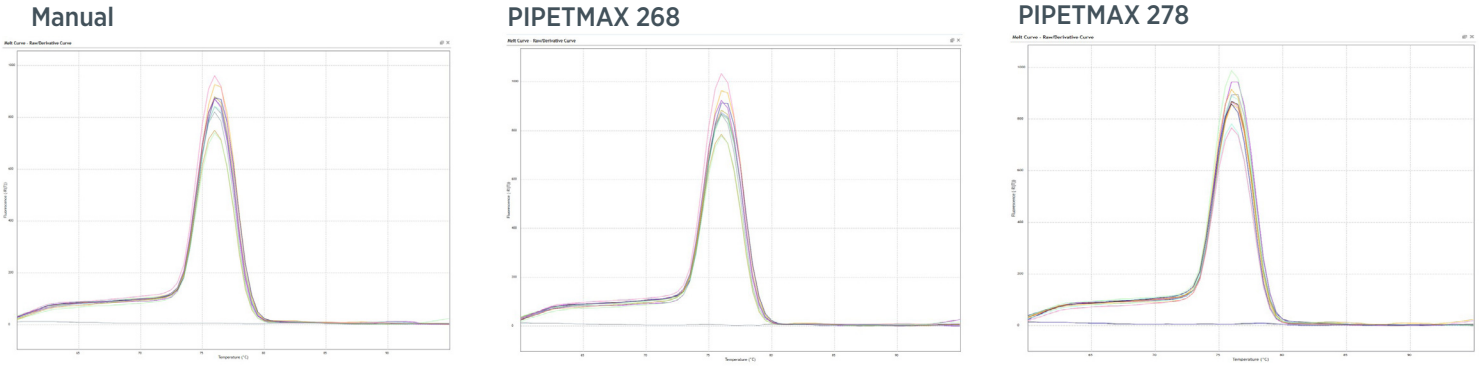


Figure 5

B2-MIC amplicon melting curves

Fluorescence (-R'(T)) plotted against temperatures (°C) for each experiment

Scalable qPCR Automation with PIPETMAX Systems for Increased Throughput and Consistent Results

In addition to maintaining assay performance, automation improves workflow efficiency by reducing manual pipetting steps and minimizing hands-on time during plate preparation.

The differing deck capacities of PIPETMAX 268 and PIPETMAX 278 allow laboratories to select an automation solution that best matches their throughput requirements. PIPETMAX 268 provides an accessible solution for smaller-scale workflows, supporting up to three 96-well plates per run. PIPETMAX 278 expands throughput capabilities by accommodating up to five 96-well plates or autonomous processing of 384-well plate workflows, making it well suited for laboratories handling larger sample volumes. Together, these systems offer a scalable approach to qPCR automation, enabling laboratories to increase efficiency while maintaining consistent and reliable results.

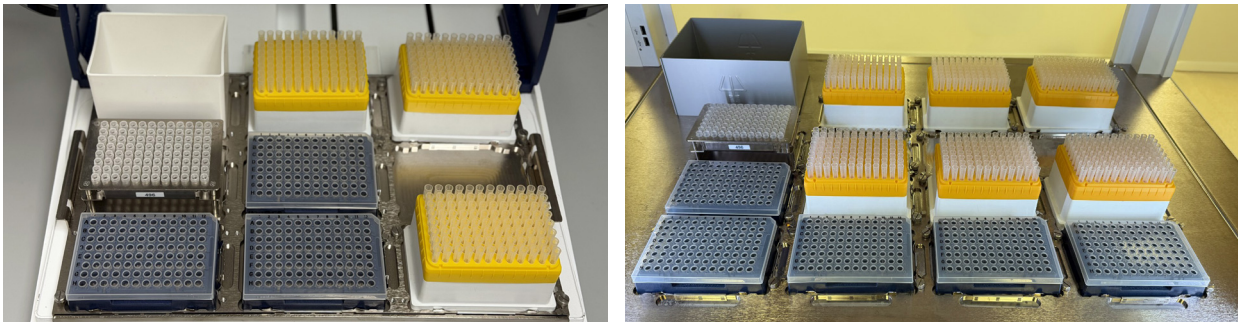


Figure 6

Scalable qPCR bed layouts, proposed deck organization for: PIPETMAX 268 (9 position deck, left) and PIPETMAX 278 (16 position deck, right)

CONCLUSIONS AND BENEFITS

PIPETMAX 268 qPCR and PIPETMAX 278 qPCR systems enable automated qPCR plate preparation while maintaining consistent assay performance across multiple gene targets. Leveraging Gilson's expertise in liquid handling, PIPETMAX workstations deliver precise and consistent pipetting performance, resulting in reliable qPCR results with no observable cross-contamination.

Consistent amplification behavior across all evaluated housekeeping genes demonstrates that PIPETMAX systems support reliable qPCR workflows across a range of assay conditions.

These results demonstrate that PIPETMAX qPCR systems:

- Support reliable amplification efficiency and standard curve quality
- Deliver results comparable to manual pipetting without compromising data quality
- Enable standardized and repeatable qPCR workflows
- Provide scalable automation solutions for both lower and higher-throughput applications

By combining reliable assay performance with workflow efficiency, PIPETMAX 268 qPCR and PIPETMAX 278 qPCR workstations provide laboratories with a flexible and dependable solution for qPCR setup, supporting both data quality and operational productivity.

References

1. Gilson, INC. Guide to pipetting, Third edition, LT800550/H. (2024).
2. Bustin, S. A. et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622 (2009).