Optimization of qPCR Master Mix Dispensing with PIPETMAX®

APPLICATION NOTE TRANS0815

qPCR master mixes contain components that may impact liquid handling. These reagents, including buffers, surfactants, and detergents, can negatively influence accuracy and precision, thereby increasing sample variability and decreasing data reliability. In this application note, we used the PIPETMAX® automated liquid handler to test whether the number of times a tip was pre-wet before transferring master mix affected qPCR performance. Increasing the number of pre-wet steps resulted in improved qPCR sample replicate uniformity, demonstrating the importance of proper pipetting technique according to the characteristics of the liquid. The flexibility and consistency of the PIPETMAX increases data reliability, enabling verifiable science through easily integrated automation.

INTRODUCTION

A successful qPCR assay begins with the precise dispensing of the sample and master mix preparations. To achieve accurate and consistent quantitative PCR results, the PIPETMAX, an automated liquid handling platform, was used to modify the final plate preparation process (Figure 1). PIPETMAX methods were optimized by including multiple tip pre-wetting steps and by modifying the pipetting speed when transferring the master mix and samples.

Master mix solutions are often comprised of surfactants and detergents such as glycerol and Tween™ 20. cDNA samples used in qPCR which are processed from cell lysates without purification or sample clean-up will also contain many of these problematic liquids. These liquids are known to negatively impact pipetting accuracy, often demanding use of positive displacement pipetting when 100% solutions are used[1]. qPCR reactions contain much lower percentages of these liquids, allowing standard air displacement pipettes to be used.

Figure 1
PIPETMAX system with tablet. The PIPETMAX was used to prepare a 96 well plate for qPCR analysis.

The addition of a pre-wet step increases accuracy and precision, by fully coating the inner surface of the pipette tip with the solution to be transferred. With volatile solutions, such as acetone, alcohols, and hexane, the pre-wet step also saturates and equilibrates the dead volume of the pipette. Standard pre-wetting includes aspirating the sample from the source and then dispensing it back into the source or waste prior to the desired transfer step (Figure 2).

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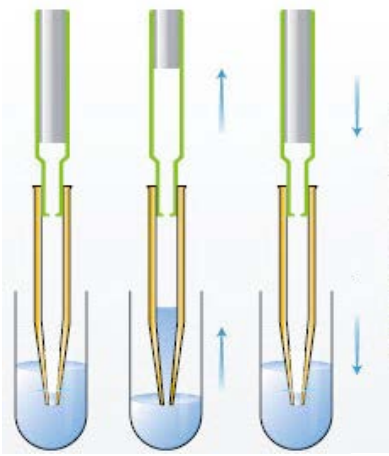


Figure 2
Pre-wet step. The pipette tip enters the source vessel, aspirates and then dispenses the liquid.

MATERIALS & METHODS

Sample Preparation

cDNA templates were manually prepared from Silkworm BmN4 (*Bombyx mori* ovary derived) cell lysates using the *Power* SYBR® Green Cellsto- CT™ Kit.

qPCR Assay

The *Power* SYBR® Green Cells-to-CT™ Kit was used to set up the qPCR reaction according to the manufacturers specifications. Master mix solutions contained primers targeting the tubulin gene. cDNA solution from 8 independent samples and eight aliquots of the master mix were placed into 8-strip PCR tubes for multichannel dispensing on the PIPETMAX (Figure 3). First, the master mix was dispensed into the 96 well qPCR plate, followed by four technical replicates of each sample. The qPCR plate was then transferred to the Roche LightCycler® 96 Real-Time PCR System for thermal cycling and data analysis.

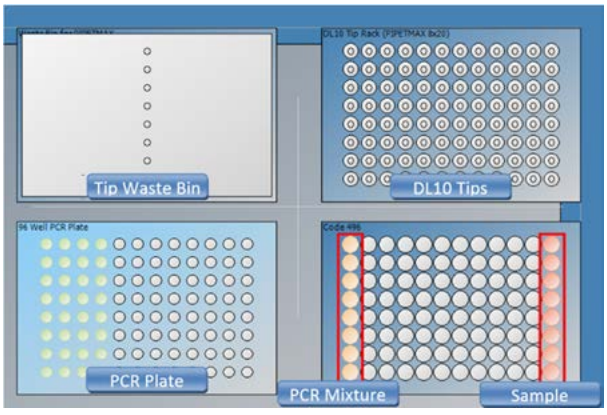


Figure 3
Bed Layout for the qPCR plate preparation protocol. DL10 (small volume) tips were used to deliver master mix and sample from 0.2mL PCR strip tubes to the final PCR plate.

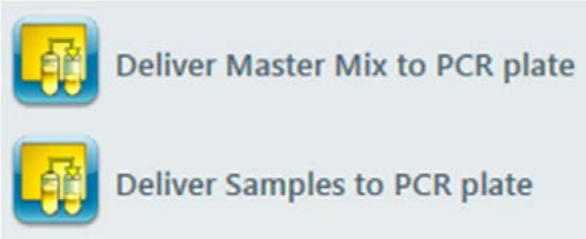
PIPETMAX Protocol Optimization

The PIPETMAX automated protocol used consists of two transfer tasks that deliver the master mix and the samples. Each task has numerous parameters that can be modified to optimize pipetting, such as pipetting speed, volume, equilibration time and pre-wet options (Figure 4).

To optimize pipetting on the PIPETMAX, preliminary experiments were performed using a representative solution containing 2% dish washing detergent and 8% glycerol. This protocol was created and tested with the representative solution in order to identify parameters that produced a visually acceptable transfer.

Two PIPETMAX protocols were created which utilized either two or four pre-wet steps to deliver the final qPCR components. The final protocol also utilized an extra volume of 0.5 per dispense instead of a final purge step.

A.



B.

Use Prewet	True
Prewet Cycles	4
Prewet Volume	*Prewet_Vol

Figure 4
Protocol for delivering master mix and sample to the qPCR plate. A) The two tasks, which deliver the master mix and samples, contain multiple parameters that can be modified to optimize pipetting. B) Prewet can be turned on or off, and the number of cycles and volumes be easily adjusted.

RESULTS AND DISCUSSION

The two cycle pre-wet protocol produced qPCR results with Tubulin Cq values in samples 1-8 from 18.11-19.44 (+/-0.08-0.26) with a coefficient of variation from 0.44-1.35% (Figure 5). It was noted however that the first replicate from each sample produced a higher Cq value than each subsequent replicate (Figure 6).

The four cycle pre-wet protocol produced qPCR results with Tubulin Cq values in samples 1-8 from 18.06-19.52 (+/-0.02-0.11) with a coefficient of variation from 0.10-0.61% (Figure 7). The higher Cq

value in the first replicate was not observed with the four cycle pre-wet protocol (Figure 8).

Increasing the tip pre-wetting step from two to four strokes when aspirating the problematic PCR mixture and replacing the purge stroke by including extra volume in the tip improved the reproducibility of the qPCR reaction. This was due to the problematic properties of the qPCR solutions inaccurately dispensing the first replicate for each sample. To overcome this, the PIPETMAX protocol was modified to include four cycles of pre-wetting with the PCR mixture.

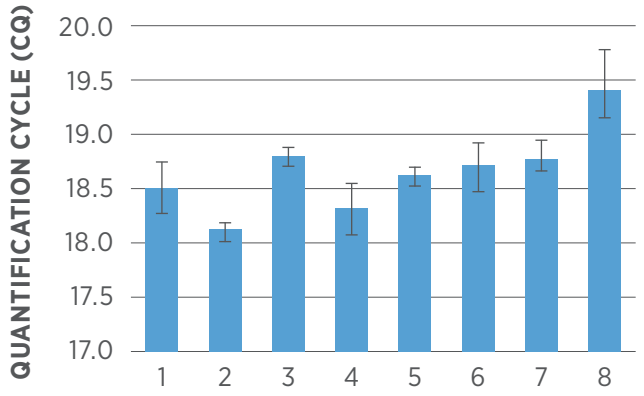


Figure 5
Average Tubulin expression (Cq) in 8 independent silkworm BmN4 cell lysate samples (n=4). qPCR mixtures dispensed following two pre-wet steps.

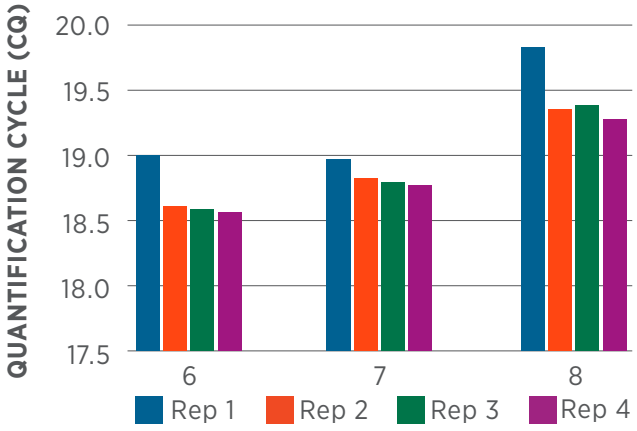


Figure 6
Tubulin expression (Cq) from three independent silkworm BmN4 cell lysate samples (samples 6, 7, 8). qPCR mixtures dispensed following two pre-wet steps.

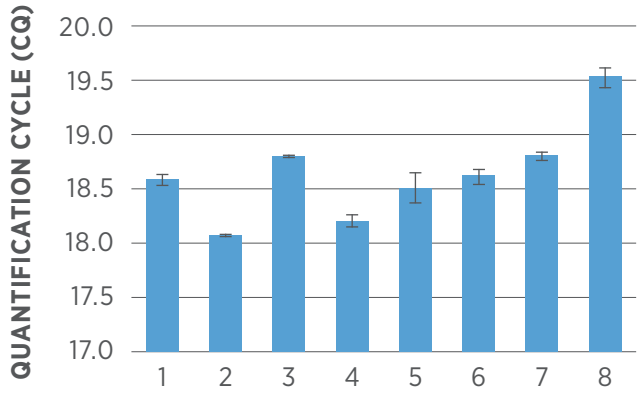


Figure 7
Average Tubulin expression (Cq) in 8 independent silkworm BmN4 cell lysate samples (n=4). qPCR mixtures dispensed following four pre-wet steps.

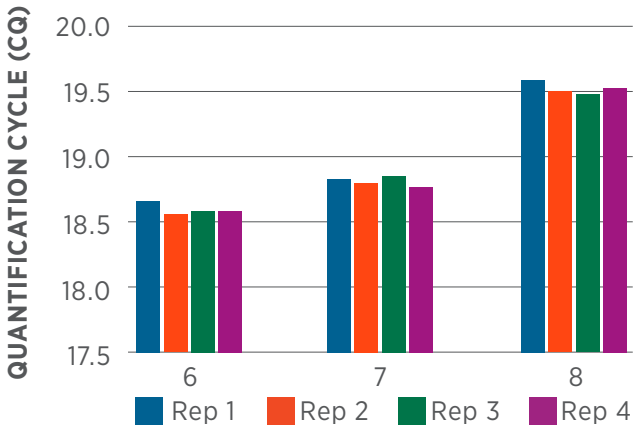


Figure 8
Tubulin expression (Cq) from three independent silkworm BmN4 cell lysate samples (samples 6, 7, 8). qPCR mixtures dispensed following two pre-wet steps.

SUMMARY OR CONCLUSIONS

- Increasing the pre-wet cycles from two to four using the PIPETMAX automated liquid handler reduced standard error between sample replicates, increasing data confidence.
- A pre-wet step coats the tip with solution prior to transfer, creating a more accurate dispense.
- Problematic liquids (viscous, volatile) require optimization of liquid handling parameters compared to standard pipetting techniques in order to increase accuracy.

REFERENCES

1. Gilson guide to pipetting. 3rd ed. Villiers-le- Bel, France: 2014. Print.

ACKNOWLEDGEMENTS

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