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Application Note

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Benefits of Electronic Pipette Use for Pipetting PCR Master Mix

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Abstract

Sensitivity of Quantitative Polymerase Chain Reaction (qPCR) based assays is one of the most important reasons for their success and abundant use in scientific laboratories. However, many factors including pipetting technique can influence qPCR assay results. PCR Master mix is routinely used during qPCR set-up but can be challenging to pipette accurately. In this study, we tested the use of electronic pipettes for pipetting Master mix for qPCR. We demonstrate that Master mix can be pipetted to obtain good precision and accuracy using electronic pipette and low retention filter tips or standard filter tips. Use of electronic pipettes ensured both speed and reproducibility of the results. We conclude that for pipetting Master mix, use of electronic pipette ensures reproducible and reliable results when performing PCR-based assays.

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Introduction

PCR-based applications have become pivotal in biopharmaceutical process, clinical diagnostics, and academic research. However, variability in assay results can be a problem when performing Quantitative PCR, qPCR.[1] Pipetting errors are one of the sources of variability that is important to focus on. Master mix is a challenging reagent to pipette during qPCR set-up. Typically, Master mixes contain polymerase, dNTPs, $MgCl_2$ in buffers that may contain Tween and glycerol.[2] Nowadays, Master mixes are commercially available as ready-to-use solutions. They are slightly viscous and cold since they must be kept on ice. These properties make it difficult to pipette correct volumes.

The goal of this application note is to test the reliability of using electronic pipette for pipetting Master mix for PCR based applications. Electronic pipette was used with low retention filter tips in pipetting the Master mix in quantitative PCR set-up, and the experimental results (cycle of quantification, Cq) was evaluated for:

- Accuracy and Precision (Reproducibility)

Methods

Pipettes and Pipette Tips

Sartorius Picus® electronic pipettes, Sartorius Safetyspace Low Retention filter tips, and Sartorius Safetyspace filter tips were used. Multi-dispensing pipetting mode on Picus® was used. Pipetting parameters for the electronic pipette were set at speed setting of 1 when pipetting Master mix.

qPCR Setup

Sartorius Picus® electronic pipette, Sartorius Safetyspace filter tips and Low Retention filter tips were used for qPCR set-up. Lo-Bind EP tubes (Eppendorf) were used for DNA sample preparation and Master mix preparation. A stock PCR Master mix for all tests was prepared using Maxima SYBR Green qPCR Master mix (without ROX) (Thermo Fisher Scientific), primers for *E. coli uidA* gene and nuclease-free water.

PCR primers UAL 5'–TGGTAAT-TACCGACGAAAACGGC (Sigma-Aldrich) and UAR 5'–ACGCGTGGTTACAGTCTTGCG (Sigma-Aldrich) amplify a 147 bp segment of the *uidA* gene in genomic *E. coli* DNA. *E. coli* strains contain a single copy of the *uidA* gene.[4] Eight 15 µL replicates of Master mix were pipetted into wells of the PCR plate for each condition tested. Non-template control (NTC) samples did not contain *E. coli* genomic DNA and received 5 µL of nuclease-free water. Serially diluted standards containing 5 µL of *E. coli* gDNA containing 1×10^6, 1×10^5, 1×10^4, 1×10^3 and 1×10^2 copies | reaction were pipetted similarly.

Each test well contained 5 µL of *E. coli* gDNA containing 1×10^3 copies | reaction. All DNA samples were added to the PCR tubes in the same manner using multi-dispensing program on a Picus® electronic pipette and Low Retention filter tips. qPCR was performed using LightCycler® 480 qPCR instrument (Applied Biosystems, Foster City, CA). The cycling parameters were as follows: pre-incubation at 95° C for 10 min, 40 cycles of 95° C for 10 sec, 55° C for 10 sec and 75° C for 15 sec, extension at 75° C for 10 sec.

SYBR green fluorescence emission was quantified in standards, controls, and samples. Cycle of quantification (Cq) values and actual copy numbers were determined using LightCycler® 480 software and MS Excel was used to analyze the results.

Data Analysis

Percent systemic error (%S) of Cq values reflects the error in Cq value of the instrument system (pipette and tip system) for handling Master mix. Random error during pipetting is a measure of the precision of the results, and reflects the variance between replicates in the experiment. Percent random error (%R) of Cq values reflects the reproducibility of the results and could be influenced by the experimenter's pipetting.

Results

Electronic pipette use for pipetting of Master mix was tested using Sartorius Picus® electronic pipette, and Low Retention filter tips. The multi-dispensing mode of the electronic pipette was used. As shown in Figure 1, when Low Retention filter tips were used to pipette Master mix, multi-dispensing mode of electronic pipette gave Cq = 24.54 ± 0.09, %Systemic error of Cq = 0.12, %Random error of Cq = 0.37 for low retention filter tips compared to standard filter tips (Cq = 24.52 ± 0.16, %Systemic error of Cq = 0.04, %Random error of Cq = 0.67). In both cases, the use of electronic pipette gave good reproducibility of the results (%Random error) and kept the %Systemic error in Cq values at a low level. The multi-dispensing mode of the electronic pipette ensured that with one aspiration, Master mix was dispensed into all eight replicate wells sequentially, increasing the speed of pipetting significantly as well as reducing the number of pipette tips used, making it more ecologically friendly and reducing the tip-to-tip variance.

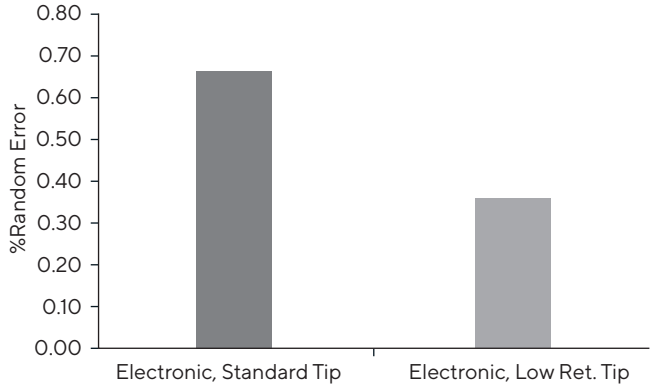

Figure 1: %Random Error of Cq (cycle of quantification) for multi-dispensing of Master mix. For each data point, n = 8. Use of electronic pipette for Master mix gives low percent errors of Cq values. The Low Retention filter tips gave the lowest random error compared to standard tips. Low Retention Tip—Sartorius Safetyspace Low Retention Filter Tips.

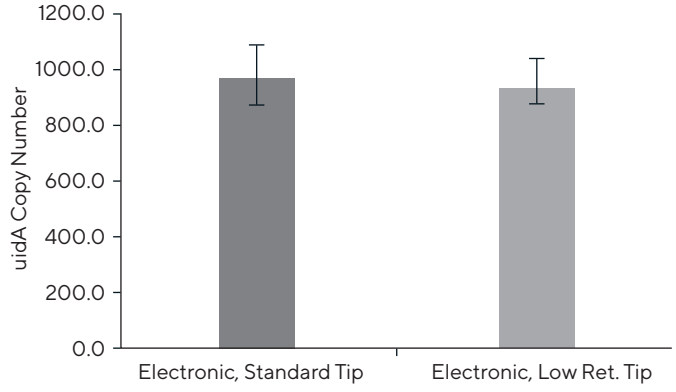

Figure 2: Quantified *E. coli uidA* copy number. For each data point, *n = 8*. Low Retention Tip—Sartorius Safetyspace Low Retention Filter Tips.

Discussion

In this study, the pipetting of Master mix, an important component of PCR, was investigated to determine the accuracy and precision of the results obtained using the electronic pipette. Here, we have demonstrated that use of electronic pipette gives excellent results when used in PCR setup, giving low percent error in the results. Multi-dispensing mode of electronic pipette using Low Retention filter tips gave the best results compared to standard filter tips. For laboratories which still use standard pipette tips for pipetting Master mixes, multi-dispensing using standard filter tips gave the next best reproducibility (precision) of results.

Conclusion

We conclude that for pipetting Master mix for PCR-based assays, electronic pipettes ensured high accuracy and precision. Additionally, it increased the speed to complete the assay, making it a more ergonomic option since it reduces the amount of time spent pipetting. Therefore, use of electronic pipette would reduce the chances of Repetitive Strain Injury (RSI) for the laboratory worker, make the experiment less error prone, and it is also the more environmentally friendly option since it used less pipette tips for the same experiment.

References

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