Tech Note

Accuracy and Precision When Using Different Volumes in qPCR Applications

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Thermo Scientific Solaris qPCR Gene Expression Assays and Solaris™ qPCR Gene Expression Master Mixes offer optimal relative quantification of complementary DNA (cDNA) using hybridization probe-based detection chemistry. In this Technical Note, Solaris qPCR Gene Expression reagents were used together with Thermo Scientific PikoReal 24 Real-Time PCR System and Thermo Scientific Finnpipette Novus Electronic Pipettes to demonstrate comparable results with different reaction volumes for quantitative real-time PCR (qPCR). In addition, the same reaction setup was prepared by using Finnpipette F2 Manual Pipettes as a comparison to using electronic pipettes.

Introduction

The reverse transcription quantitative real-time PCR (RT-qPCR) workflow for relative gene expression analysis requires many steps. In addition to multiple enzymatic reactions, using a high-quality real-time PCR instrument along with accurate and precise pipetting is essential for robust experimental results. Accuracy refers to the deviation of a measurement from a standard or true value of the quantity being measured while precision is how close a group of measurements are to one another. Therefore, proper experimental design and implementation as well as delivery of accurate and precise volumes are critical in multistep gene expression experiments[1].

Solaris qPCR Gene Expression Assays and Master Mixes offer optimal relative quantification of cDNA with great sensitivity and repeatability. Experimental design is facilitated by using pre-designed Solaris Assays containing gene-specific probe and primer pairs. Pipetting steps are minimized by using Solaris Master Mix, which contains a mixture of the necessary components for qPCR.

Finnpipette Novus Electronic pipettes offer performance with simple operation and reduced stress for comfortable, productive pipetting. The natural index finger operation allows the thumb to relax while pipetting. Manual Finnpipettes are lightweight, enabling longer pipetting periods without fatigue. Equipped with the soft-touch tip ejector the tip ejection forces are reduced by up to 50%. The pipettes have been designed to enhance pipetting ergonomics and, thus, to reduce the risk of pipetting stress.

The qPCR reaction volumes can be easily scaled down to save precious sample material and reagents. Here we present an example of qPCR with different reaction volumes using Solaris qPCR Gene Expression reagents with PikoReal® 24 Real-Time PCR System and Finnpipette Novus Electronic Pipettes. In addition, the same reaction setup was prepared by using Finnpipette manual pipettes.

Materials and Methods

- Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR (#K1641)
- Human HeLa Cell total RNA (Clontech-Takara Bio Europe #CB0767909)
- Solaris qPCR Gene Expression Master Mix, plus ROX vial (#AB-4350)
- Solaris qPCR Gene Expression Assay for ALDOA gene (Thermo Scientific #AX-010376-00-0100)
- PikoReal 24 Real-Time PCR System (#TCR0024)
- Thermo Scientific 24-well Piko PCR Plate (#SPL0241)
- Thermo Scientific Clear Seal Diamond Heat Sealing Film (#AB-0812)
- 4s2™ Thermal Sealer (4Titude #4ti-0650)
- Finnpipette Novus Electronic Pipette (single channel)
  - 5-50 µL (#46200300)
  - 30-300 µL (#46200500)
  - 100-1000 µL (#46200600)
- Finnpipette F2 Manual Pipette (single channel)
  - 2-20 µL (#4642060)
  - 20-200 µL (#4642080)
  - 100-1000 µL (#4642090)

cDNA synthesis

Human Hela Cell total RNA (500 ng) was reverse transcribed using Maxima® First Strand cDNA Synthesis Kit following the manufacturer's protocol containing a mixture of oligo(dT)18 and random hexamer primers) in 20 µL reaction volume. Total RNA equivalents (5, 7.5 or 12.5 ng) were used as templates in qPCR in 10, 15 or 25 µL reaction volumes.

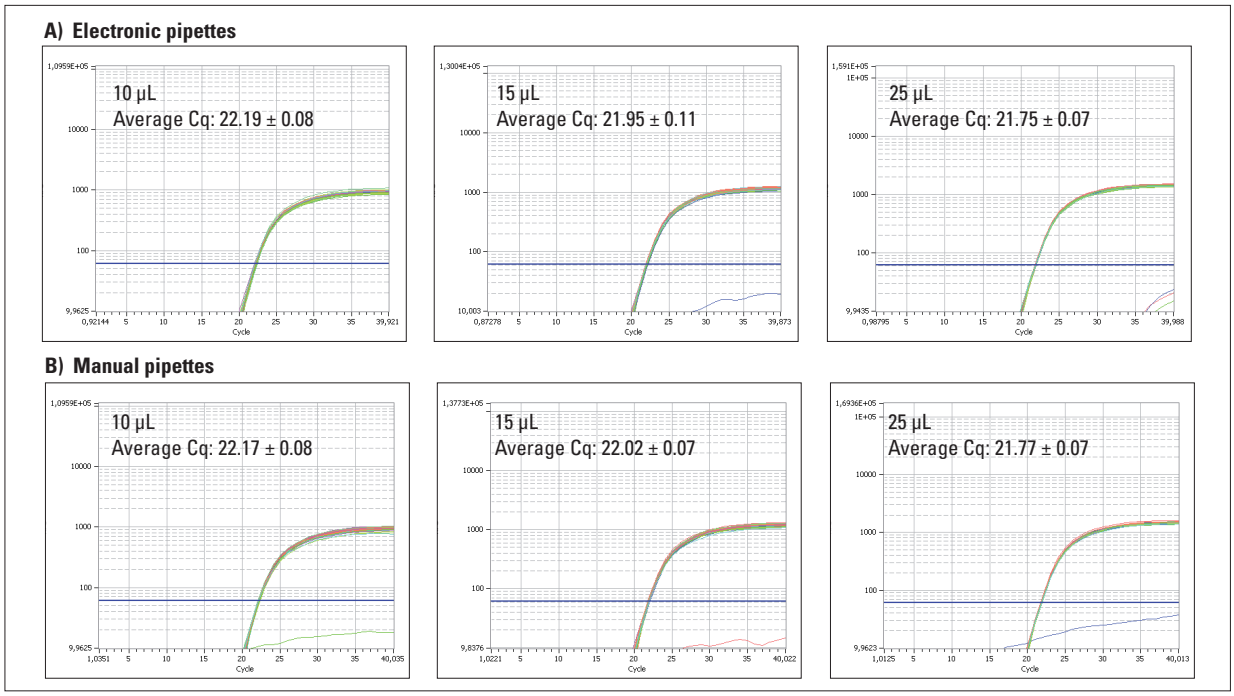

Figure 1. Replicate reactions (21) amplifying *ALDOA* from HeLa cDNA (5, 7.5 or 12.5 ng of total RNA equivalents) using Thermo Scientific Solaris qPCR Gene Expression reagents either with 10, 15 or 25 µL qPCR reaction volumes. The pipetting was prepared with Finnpipette Novus Electronic pipettes (A) or with Finnipipette Manual pipettes (B). Three NTC reactions were included in all test setups and did not result in amplification as expected (at RFUs 5-20).

qPCR

Replicate reactions (21) amplifying *ALDOA* from HeLa cDNA using Solaris qPCR Gene Expression Assay and Master Mix. The qPCR volume was either 10, 15 or 25 µL. The pipetting was prepared either with electronic or manual pipettes (single channel). Triplicate no template control (NTC) reactions without cDNA template were included in all experiments. Cycling conditions were: DNA polymerase activation at 95°C for 15 min; denaturation at 95°C for 15 sec, annealing/extension at 60°C for 1 min × 40 cycles.

Table 1. Comparison of qPCR results at different reaction volumes and between Thermo Scientific Finnpipette Novus Electronic pipettes and Manual pipettes.

Component	Electronic			Manual		
qPCR volume	10 µL	15 µL	25 µL	10 µL	15 µL	25 µL
Average Cq value	22.19	21.95	21.75	22.17	22.02	21.77
Standard Deviation	0.08	0.11	0.07	0.08	0.07	0.07
ΔCq (Max-Min)	0.24	0.33	0.25	0.30	0.29	0.24

Results and Discussion

In this application note we present an example of using Solaris qPCR Gene Expression reagents together with PikoReal 24 Real-Time PCR System using different qPCR reaction volumes. First, we demonstrated that the qPCR results are comparable with different qPCR reaction volumes by performing pipetting with Finnpipette Novus Electronic pipettes. The average Cq for each volume ranged from 21.75 ± 0.07 to 22.19 ± 0.08, demonstrating good accuracy and precision (Table 1). The endpoint RFU values were slightly higher with the larger qPCR reaction volumes (Figure 1A).

After assessing the reproducibility with different qPCR reaction volumes, we repeated the test setup by using Finnpipette manual pipettes (Figure 1B). The average Cq values were in the range of 21.77 ± 0.07 to 22.17 ± 0.08 (Table 1). The results show that the high accuracy and precision were comparable to using electronic pipettes. This enables the user to switch between electronic and manual pipettes and, thereby, to optimize pipetting ergonomics.

Reference

Taylor, S. *et al.* (2010) A practical approach to RT-qPCR –Publishing data that conform to the MIQE guidelines. Methods. 50:S1-S5.

Troubleshooting

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