LABCYTE™

Application Note G104 | Echo 525 Liquid Handler

High-throughput Miniaturized Quantitative PCR with the Echo® 525 Liquid Handler

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Abstract

Quantitative PCR (qPCR) is a prevalent tool spanning many phases of drug discovery. Advances in qPCR detection have incentivized researchers to miniaturize qPCR assays as a means to offset the costs of increasing throughput. To significantly reduce qPCR volumes and maintain data quality, the liquid handling methods employed for such low-volume transfers must be precise and accurate. Tipless, touchless acoustic droplet ejection with the Echo liquid handler eliminates the cost of disposable tips or tip-wash cycles and simplifies assay setup by eliminating dilution steps. This study utilized the Echo 525 liquid handler to assemble low-volume qPCR assays at speeds that keep pace with high-throughput demands. Precision for the resulting quantification curves across 384-well plates was excellent with standard deviations less than 0.25 and CVs less than 2.0%. The results demonstrated the Echo 525 liquid handler effectively miniaturized reaction volumes for high-throughput qPCR in 384-well formats.

Introduction

The Labcyte® Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo 525 liquid handler is designed for rapid transfer of biochemical and genomics reagents for assay assembly. The Echo 525 platform transfers 25 nL droplets of most biochemical reagents. These can be simple fluids (media for growing cells, buffer) or viscous solutions (lysis buffer, antibodies with glycerol, or transfection reagents). Microliter-scale volumes are rapidly transferred by repeating 25 nL transfers hundreds of times per second. With any-well to any-well tranfer at any volume, the Echo 525 platform enables contamination-free reagent transfer to precisely and accurately build assays. Miniaturization with the Echo 525 liquid handler retains high assay performance, allowing quantitative results at higher densities.

Experiment 1

Echo liquid handler dispensing precision and accuracy

Prior to PCR testing, drop volume precision was evaluated by doping master mix solution (Roche Applied Science, 05 502 381 001) with fluorescein. Sodium fluorescein was added to master mix at 150 µM concentration. The fluorescein master mix solution was added to an Echo® qualified polypropylene 384-well microplate at a volume of 40 µL per well. 100 nL was transferred from each well to the corresponding well of a Greiner Bio-One black microplate. The microplate was filled with 50 µL of 10 mM NaOH, pH 12, and the fluorescence was measured on a BioTek Synergy™ H4 fluorescence reader. The fluorescence level was compared to a calibrated standard curve to determine actual dispensed volumes, and volume measurements across the 384 wells were compared for precision and accuracy. Over the entire plate a CV of 1.59% was achieved. With a target volume of 100 nL, an average volume of 98.88 nL was measured.

Experiment 2

Quantitative PCR using the Echo 525 liquid handler

The capability of the Echo 525 liquid handler to transfer all qPCR assay components was determined by using the Echo 525 platform to setup qPCR experiments for three reference genes at a 5 µL volume in a 384-well format.

Materials

- Universal Probe Library (UPL) Reference Gene Assays (Roche Applied Science)
  - Human G6PD Gene Assay (05 046 246 001)
  - Human GAPDH Gene Assay (05 190 541 001)
  - Human HPRT Gene Assay (05 046 157 001)
- RealTime ready DNA Probes Master mix (Roche Applied Science, 05 502 381 001)
- LightCycler® 480 Multiwell plate 384, White (Roche Applied Science, 04 729 749 001)
- Echo qualified 384-well polypropylene microplates (Labcyte Inc., P-05525)

Methods

Three reference genes ranging from high copy number to low copy number were chosen for testing (GAPDH, G6PD and HPRT). Five microliter solutions consisting of 600 nM primer, 300 nM probe and master mix were transferred from a 384-well Echo qualified polypropylene microplate to each well of a 384-well qPCR microplate. Subsequently cDNA at a concentration of 1 ng per reaction (25 nL) was transferred to each well of the 384-well qPCR microplates with the Echo 525 liquid handler. The final PCR volume was 5.025 µL.

Figure 1 ▸
Full plate amplification curve for glucose-6 phosphate dehydrogenase (G6PD).

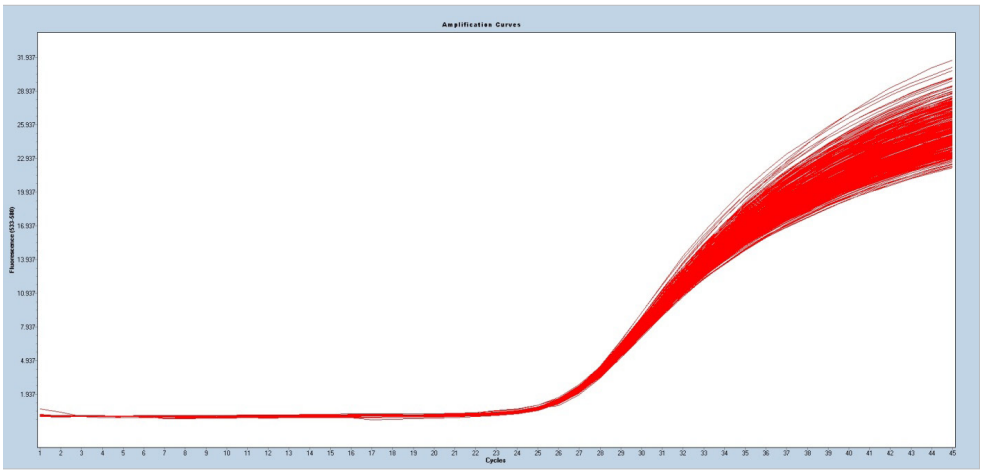


Table 1 ▸
Amplification results for three reference genes.

Gene	*G6PD*	*GAPDH*	*HPRT*
Cp Min	26.05	21.09	26.04
Cp Max	26.93	22.49	27.84
Cp Average	26.39	21.87	26.89
SD	0.18	0.30	0.46
N	383	381	384
CV	0.67%	1.36%	1.71%

Results

Table 1 shows qPCR results for the three reference genes. Cp values ranged from 21 to 28 with standard deviations from 0.18 to 0.46 and CVs of less than 1.8%. All amplification curves across a full microplate show uniform and precise crossing point values.

Experiment 3

qPCR assay assembly using the Echo 525 liquid handler

To evaluate the ability to create qPCR experiments using the Echo 525 liquid handler, the amplification values were measured for a range of total qPCR assay volumes from 3-10 µL.

Methods

A reaction mixture consisting of 600 nM G6PD primer set and 300 nM G6PD probe set, 1X RealTime ready DNA Probes master mix, and 1 ng human reference cDNA was transferred into an Echo qualified microplate. The Echo 525 liquid handler was then used to transfer 3.0, 5.0, 7.0, and 10.0 µL of the reaction mixture to each quadrant of a 384-well qPCR microplate. The qPCR microplate was then cycled in the LightCycler® 480 system. Reactions were thermal cycled at 95°C for 60 seconds, followed by 45 cycles at 95°C for 10 seconds and 60°C for 30 seconds, with a cool down at 42°C for 30 seconds. Final results were determined using the LightCycler® 480 software.

Results

Measured Cp standard deviations were compared for each volume ranging from 0.43 for the 3.0 µL quadrant to 0.18 for the 10 µL quadrant (Table 2 and Figure 2). The average CV for the Cp expression values was consistently below 2%.

Figure 2 ▸
G6PD amplification curves from qPCR mixtures transferred with the Echo 525 liquid handler.

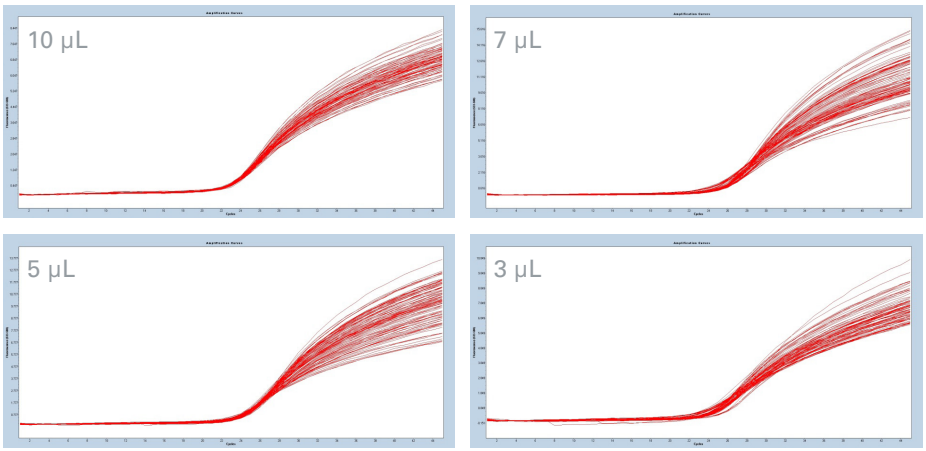


Table 2 ▸
qPCR miniaturization results for 3-10 µL

Volume	*3 µL*	*5 µL*	*7 µL*	*10 µL*
Cp Min	24.25	23.46	23.57	22.59
Cp Max	26.23	25.69	24.68	23.22
Cp Average	25.41	24.21	24.08	22.90
SD	0.43	0.48	0.30	0.18
CV	1.71%	1.99%	1.23%	0.81%

Experiment 4

cDNA dilution series using the Echo liquid handler to assemble 384-well qPCR assays

To evaluate the ability of the Echo 525 liquid handler to create a cDNA dilution series without pre-dilution of cDNA, increasing volumes of concentrated stock cDNA were transferred directly into a 384-well qPCR destination microplate to create a dilution series.

Methods

Using a two-step process, the Echo 525 liquid handler dispensed master mix with premixed primers and probes into a qPCR microplate, followed by transfers of human reference cDNA in incremental volumes. Source microplates containing 50 µL of premixed master mix, primers and probes (at a concentration of 600 nM primers and 300 nM probes) were pipetted into an Echo qualified 384-well polypropylene source microplate. The Echo 525 liquid handler was used to transfer 5 µL of master mix, followed by a second transfer of human reference cDNA at a concentration of 0.05 ng/µL to create a dilution series from 1.25 pg to 15 pg cDNA (25 to 300 nL). Reference genes G6PD, GAPDH, HPRT, and PBGD (UPL 05 046 157 001) were studied.

Results

Clear delineation in Cp can be detected for small changes in cDNA amounts (Figure 3). Figure 4 shows the dilutional linearity for the four reference genes. All reference genes were tested at low cDNA amounts ranging from 1.25 pg to 15 pg (y-axis) and the experimental data shows dilutional linearity for both low copy number (HPRT) and high copy number genes (GAPDH).

Figure 3 ▸
cDNA titration for glucose-6 phosphate dehydrogenase (G6PD)

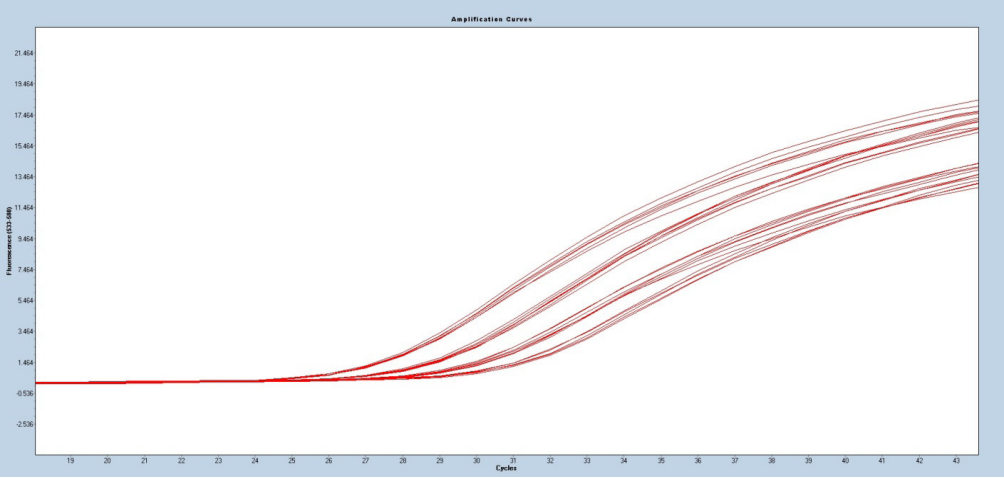
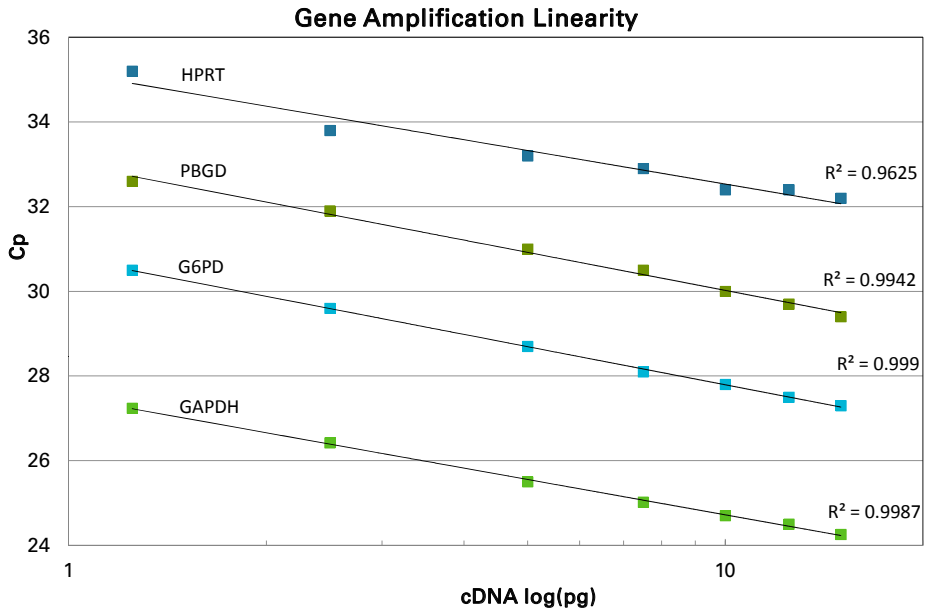


Figure 4 ▸
Linearity of dilution series results for reference gene assays



Summary

The Labcyte Echo 525 liquid handling enables exciting new capabilities for 384-well qPCR experimental setup in the 3-10 µL range. The low-volume transfer increment enables scientists to explore qPCR miniaturization without sacrificing data quality that may come with imprecise or inaccurate liquid transfer at low volumes. Superior volumetric precision ensures excellent cycle quantification even with very little target DNA in very low reaction volumes. This tool enables scientists to fully explore the capabilities of miniaturized PCR and other genomics applications, while reducing reagent consumption and eliminating tip costs, thereby reducing operational running costs.