High Throughput Miniaturized Quantitative PCR using 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 to enable 384- and 1536-well microplate formats 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- and 1536-well plates was excellent with standard deviations less than 0.25 and CVs less than 2.0%. The results confirm the advantages of using the Echo 525 liquid handler to miniaturize reaction volumes for high-throughput qPCR in both 384- and 1536-well formats.

The Echo 525 Liquid Handler

The Labcyte® Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo 525 liquid handler, the newest model of Echo liquid handlers, is designed for rapid transfer of biochemical and genomics reagents for assay assembly. The Echo 525 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 transferred rapidly by repeating 25 nL transfers hundreds of times per second. The Echo 525 system 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. The Echo liquid handler can be used to transfer any volume to any well.

qPCR assay assembly using the Echo 525 liquid handler

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

Materials

- Universal Probe Library Reference Gene Assays (Roche Applied Science)
- Human G6PD Gene Assay (05 046 246 001)
- RealTime ready DNA Probes Master mix (Roche Applied Science, 05 502 381 001)
- LightCycler® 480 Multiwell plate 384, White (Roche Applied Science, 04 79 749 001)
- Echo® qualified 384-well polypropylene microplates (Labcyte Inc., P-05525)

The Echo 525 liquid handler

- No tips or nozzles to clog
- No calibration required
- Rapid assay assembly
- Rapid assay optimization

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 source microplate. The Echo 525 liquid handler was then used to add 3, 5, 7 and 10 µL of the reaction mix 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 of 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

Figure 1. G6PD amplification curves from qPCR mixtures transferred with the Echo 525 liquid handler.

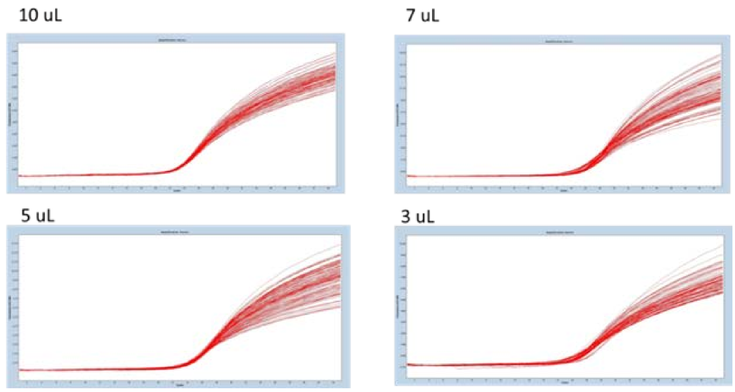


Table 1. qPCR miniaturization results for 3-10 µL.

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%

Measured Cp standard deviations were compared for each volume ranging from 0.43 for the 3 µL quadrant to 0.18 for the 10 µL quadrant (Table 1 and Figure 1). The average CV for the Cp expression values was consistently below 2%.

cDNA dilution series with the Echo 525 liquid handler

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 probe) were pipetted into an Echo qualified 384-well polypropylene source microplate. The Echo 525 liquid handler was used to transfer 5 µL master mix, followed by a second transfer of human reference cDNA at a concentration of 0.05 ng/mL to create a dilution series from 1.25 pg to 15 pg cDNA (25-300 nL). Reference genes G6PD, GAPDH (UPL cat. 05 190 541 001), PBGD (UPL cat 05 046 157 001) and HPRT (UPL probe cat. 05 046 157 001) were studied.

Results

Figure 2. cDNA titration for glucose-6-phophate dehydrogenase (G6PD)

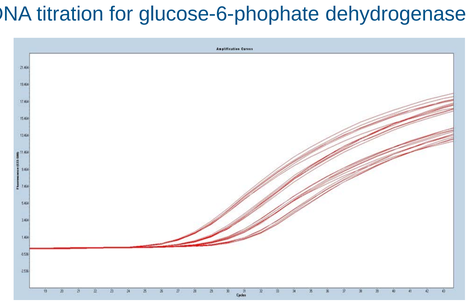
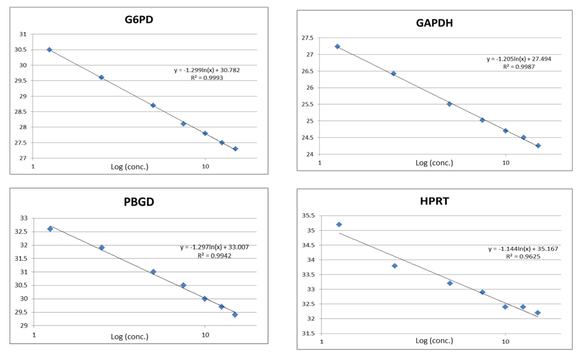


Figure 3. Dilutional linearity results for four reference gene assays



Clear delineation in Cp can be detected for small changes in cDNA amounts (Figure 2). The dilutional linearity for the four reference genes (Figure 3) showed excellent dilutional linearity for low copy number genes (HPRT) and high copy number genes (GAPDH).

Full plate data for three reference gene assays

The capability of the Echo 525 liquid handler to precisely transfer all qPCR assay components across a whole 384-well microplate was assessed. Three reference genes ranging from high copy number to low copy number (GAPDH, G6PD, and HPRT) were tested. 5 µL solutions of master mix, 600 nM primer, 300 nM probe were transferred for each gene from a 384-well Echo qualified source 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. The final PCR volume was 5.025 µL.

Results

Figure 4. Full plate amplification curve for G6PD

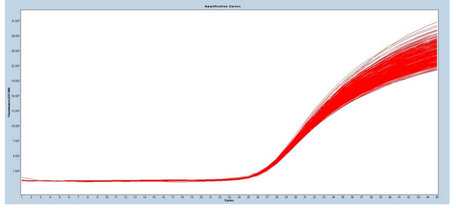


Table 2. Full plate amplification results for three reference genes

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%

Table 2 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.

Summary

- The Labcyte Echo 525 liquid handler enables total qPCR assay assembly from 3-10 µL.
- Superior volumetric precision ensures excellent cycle quantification even with very little target DNA in very low reaction volumes.
- This technology enables scientists to rapidly explore the capabilities of miniaturized PCR and other genomics applications, while reducing reagent consumption and eliminating tip costs, and therefore reducing operational running costs.