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**Application Note G101 | Echo Liquid Handler**

# Miniaturized Quantitative PCR in a 1536-well Format Using the Echo® Liquid Handler

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## Abstract

Miniaturizing quantitative PCR (qPCR) assays into 1536-well plates holds great promise for increased throughput and reagent savings. However, delivering reagents into such high-density plates can be challenging for conventional tip-based liquid handling systems, leading to inaccurate assay volumes and contamination errors across wells. The Labcyte Echo 500 series liquid handlers are completely touchless—they use no tips or nozzles, and the dispensing mechanism never touches the reagents in the wells, thereby eliminating well-to-well cross contamination. This study utilized the Echo 555 liquid handler to perform miniaturized qPCR with zero cross contamination, high precision and high accuracy.

# Experiment 1

## Echo liquid handler dispensing precision and accuracy

To measure transfer volume performance of the Echo liquid handler for PCR reagents, dispensing precision and accuracy were tested using dual-dye ratiometric photometry.

### Methods

Dispensing precision and accuracy were tested with the MVS (Multi-channel Verification System; Artel). Solutions of premixed primers and probes were combined with MVS dyes. Aliquots of 10 µL volumes were loaded into a 384LDV (low-dead-volume) Echo® qualified source plate (LP-0200; Labcyte Inc.). Subsequently, 0.1, 0.5 and 1.0 µL volumes were transferred from the source plate to each well of an empty (dry) 384- well destination plate (Artel) using the Echo 555 liquid handler. Finally, 55 µL of diluent (Artel) was added. The plate was centrifuged, incubated at room temperature for 30 minutes, and an absorbance measurement was taken.

### Results

Table 1 shows the obtained volume, relative accuracy, standard deviation, and coefficient of variation (CV) for each target volume. The transfer volume accuracy and precision were excellent, with accuracy above 96% and precision below 4% CV.

**Table 1 ▸**
Echo liquid handler volumetric precision and accuracy

| *Target volume (µL)* | *0.100* | *0.500* | *1.000* |
|---|---|---|---|
| **Mean volume for all wells (µL)** | 0.104 | 0.511 | 1.036 |
| **Relative accuracy for all wells** | 96.0% | 97.8% | 96.4% |
| **Standard deviation for all wells (µL) (SD)** | 0.004 | 0.011 | 0.025 |
| **Coefficient of variation for all wells** | 3.46% | 2.15% | 2.41% |

# Experiment 2

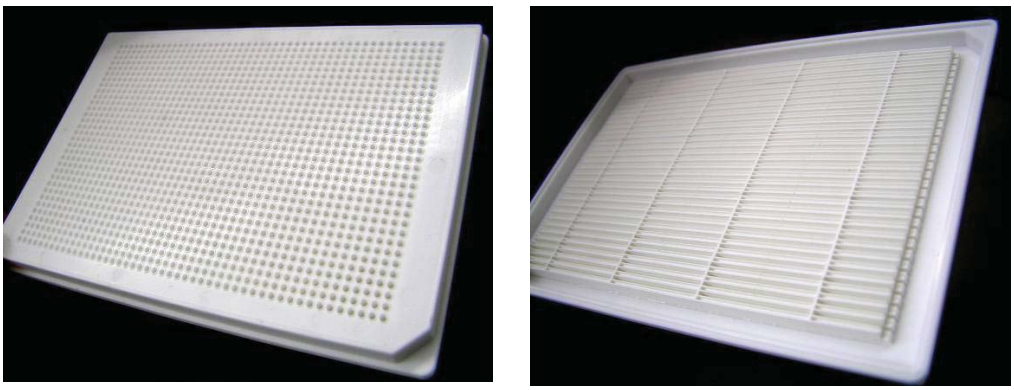
## Plate-to-plate amplification reproducibility

Plate-to-plate amplification reproducibility was evaluated by using the Echo 555 liquid handler to transfer qPCR reagents into four individual 1536-well qPCR plates (Figure 1). Reproducibility was evaluated by comparing standard deviation and CV for amplification (crossing point; Cp) values generated by each plate.

### Methods

The Echo 555 liquid handler was used to transfer 1.0 µL one-step reaction mix (Roche Applied Science) from a 384-well polypropylene plate (P-05525, Labcyte Inc.) into four separate 1536-well qPCR plates (05 546 338 001, Roche Applied Science, Figure 1). The plates were subsequently thermal cycled in the LightCycler® 1536 system (Roche Applied Science). The amplification results were calculated using the LightCycler® 1536 software. In an amplification reaction, the cycle at which the fluorescence of a sample rises above the background fluorescence is called the “crossing point” (Cp) of the sample. A sample with a lower initial concentration of target DNA requires more amplification cycles to reach the crossing point than a sample with a higher concentration. The LightCycler® 1536 software uses a predefined fluorescence threshold value to calculate the crossing point of a sample (LightCycler® 1536 Manual, Reference).

**Figure 1 ▸**
1536-well qPCR plates (Roche Applied Science); top view (left) and bottom view (right).



## Results

Table 2 shows qPCR performance results for four replicate plates at 1.0 µL total assay volume per well in 1536-well PCR plates using the Echo 555 liquid handler. The CV of the amplification value (Cp) was calculated for each plate. Cp precision for all four plates ranged from 0.95% to 1.09% CV.

**Table 2 ▸**
Plate-to-plate amplification reproducibility

| | *Plate 1* | *Plate 2* | *Plate 3* | *Plate 4* |
|---|---|---|---|---|
| **Average crossing value (Cp)** | 22.46 | 22.56 | 22.50 | 22.46 |
| **Cp Standard deviation (SD)** | 0.21 | 0.25 | 0.24 | 0.22 |
| **Coefficient of variation (CV)** | 0.95% | 1.09% | 1.07% | 0.96% |
| **Minimum Cp** | 21.64 | 21.92 | 21.91 | 20.55 |
| **Maximum Cp** | 24.86 | 24.39 | 26.19 | 25.42 |

# Experiment 3

## Miniaturization of qPCR assays using the Echo liquid handler

To evaluate qPCR assays miniaturized by the Echo 555 liquid handler, the amplification values were tested for a range of total qPCR volumes from 0.25 to 1 µL.

## Methods

Reaction mix containing 500 pg/µL of cDNA, 500 nM GAPDH primer, 1X probe 60, 1X master mix and 1X DABCYL quencher was added to 96 wells of a 384-well polypropylene Echo qualified source plate. The Echo 555 liquid handler was then used to add 0.25, 0.50 and 1.0 µL of the reaction mix to each well of three separate 1536-well qPCR plates. Reactions were thermal cycled at 95°C for 60 seconds, followed by 45 cycles of 95°C for 0 seconds and 60°C for 60 seconds. Amplification results were quantified using the LightCycler® 1536 software.

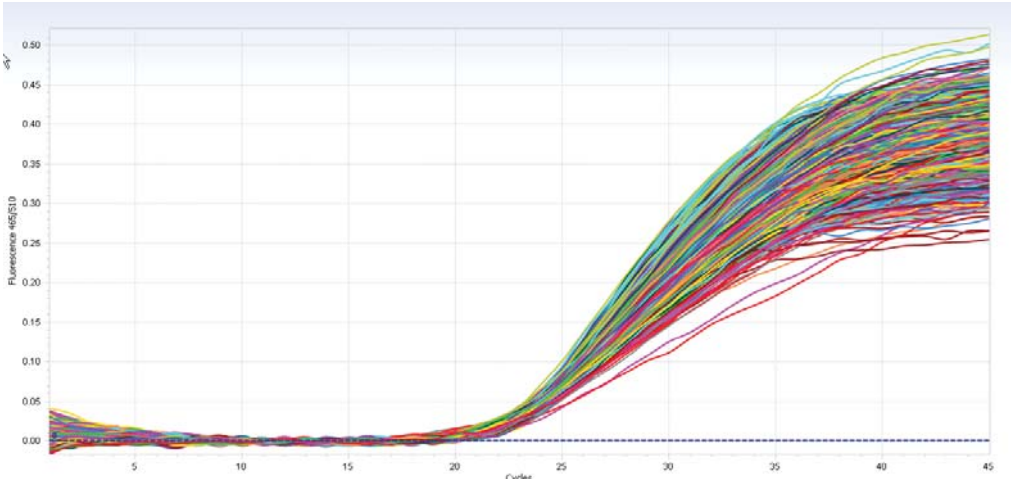
**Table 3 ▸**
Amplification reproducibility in miniaturized reaction volumes

| *Total reaction volume* | *0.25 µL* | *0.50 µL* | *1.0 µL* |
|---|---|---|---|
| **Average crossing point value (Cp)** | 22.92 | 22.38 | 22.03 |
| **Cp standard deviation (SD)** | 0.25 | 0.21 | 0.21 |
| **Coefficient of variation (CV)** | 1.10% | 0.94% | 0.97% |
| **Cp minimum** | 21.97 | 21.61 | 20.98 |
| **Cp maximum** | 24.05 | 23.09 | 22.53 |
| **Cp range** | 2.08 | 1.48 | 1.54 |

## Results

Comparable standard deviations were seen for each plate ranging from 0.211 for the 0.5 µL plate to 0.251 for the 0.25 µL plate (Table 3 and Figure 2). The average CV for the Cp expression values was consistent at approximately 1% (Table 3).

**Figure 2 ▸**
Cp amplification curves for a 0.25 µL qPCR assay

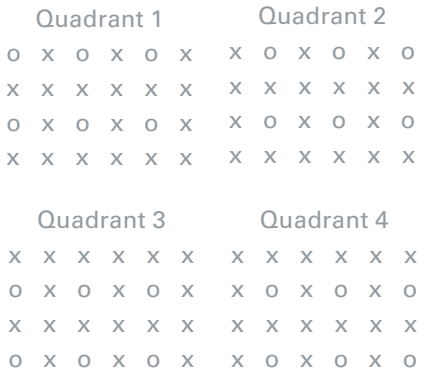



# Experiment 4

## Zero cross contamination in 1536-well transfer using the Echo liquid handler

Cross contamination was evaluated using a checkerboard pattern (Figure 3) that alternated the position of the positive control in each quadrant of a 1536-well plate. Positive samples (O) and negative samples (X) were transferred from a 384-well polypropylene Echo qualified source plate into a 1536-well qPCR plate using the Echo 555 liquid handler. This experiment was designed to show that a positive well would not contaminate any of the eight surrounding negative sample wells, regardless of dispensing order or their relative positions in the grid. Each quadrant was duplicated 16 times in each corner of the 1536-well destination plate.

**Figure 3 ▸**
Cross contamination plate set-up




1536-well plate

| | |
|---|---|
| Quadrant 1 | Quadrant 2 |
| Quadrant 3 | Quadrant 4 |

## Methods

The Echo liquid handler transferred 0.5 µL of a one-step reaction from the 384-well polypropylene plate to the qPCR plate. Positive samples consisted of 500 nM HPRT primer set (UPL Probe Library, Roche Applied Science), 300 nM probe (UPL Probe Library, Roche Applied Science), 1X Real Time Ready DNA Probes Master (Roche Applied Science), and 500 pg cDNA (qPCR Human Reference cDNA, random primed, Clontech). Negative samples consisted of 500 nM primers, 300 nM probe, and 1X Real Time Ready DNA Probes Master. The qPCR plate was then cycled in the LightCycler® 1536 system. Reactions were thermal cycled at 95°C for 60 seconds, followed by 45 cycles of 95°C for 0 seconds and 60°C for 60 seconds. Final results were determined using the LightCycler® 1536 software.

## Results

On the heat map shown in Figure 4, green circles are positives. A reaction is considered positive if the amplification crosses a certain threshold as determined by the LightCycler® 1536 software algorithm. Red circles are negatives since there was no cDNA present. Negative samples all showed fluorescence values of less than 0.03 fluorescence units. There were no false positives, showing zero cross contamination. Figure 5 shows the Cp expression curves for the 384 positives on the 1536-well plate. The negative reaction results had values below the background fluorescent noise of the plate (data not shown). Table 4 shows the data analysis of the positives with standard deviation below 0.35 and CV of the Cp of less than 1.5%. The LightCycler® 1536 software did not show any results for the negatives because they were not detected by the software as being above the minimum threshold.

**Figure 4 ▸**
Heat map results from qPCR cross contamination test.

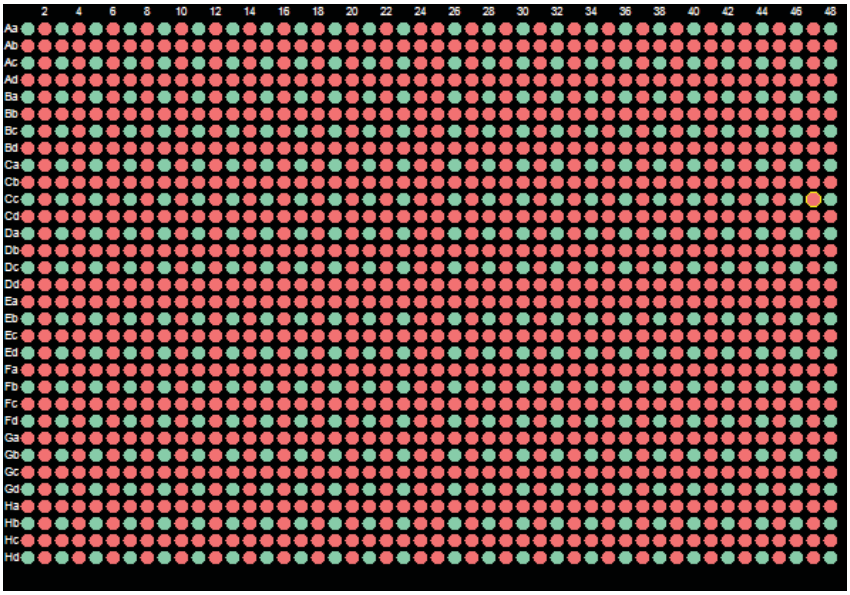


**Figure 5 ▸**
Cp amplification curves for cross contamination results.

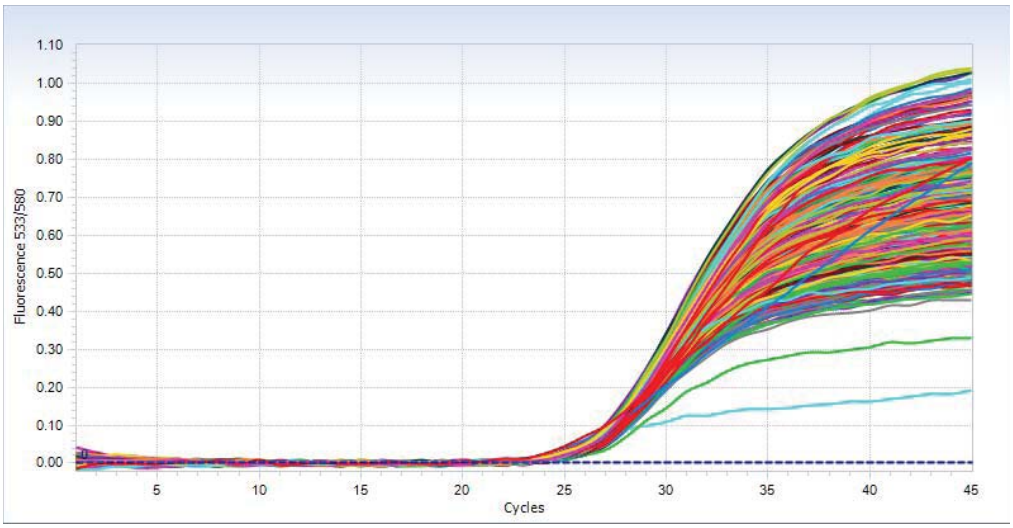



**Table 4 ▸**
Cross contamination results.

| | |
|---|---|
| **Average crossing point value (Cp)** | 25.63 |
| **Cp minimum** | 24.28 |
| **Cp maximum** | 26.66 |
| **Delta (Cp max - Cp min)** | 2.38 |
| **Cp standard deviation (SD)** | 0.32 |
| **Coefficient of variation (CV)** | 1.25% |
| **Number of positive reactions** | 384 |

## Summary

The ability to miniaturize assays and produce accurate and precise results is limited by the capabilities of the liquid transfer instruments among other factors. The Echo liquid handler enables quantitative 1536-well real-time PCR with as little as 0.25 µL final reaction volume in the LightCycler® 1536 system. Echo liquid handler dispensing precision and accuracy was demonstrated for experiments with Cp standard deviation less than 0.26 and Cp coefficient of variation less than 1.5%. The Echo 555 liquid handler successfully transfers nanoliter quantities of qPCR reagents from a 384-well plate into a 1536-well qPCR plate with zero cross contamination. The technology also enables the user to save on tips and extra washing steps by utilizing the tipless nature of acoustic dispensing.

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