Acoustic Droplet Ejection Enables Miniaturized Real-Time qPCR in 1536-Well Plate Format

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1. ABSTRACT

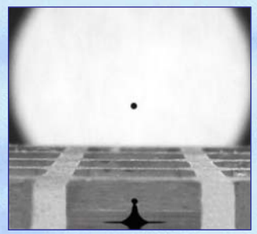
Advances in the miniaturization of thermal cycler technologies have enabled PCR in a higher density format with lower total assay reaction volumes. By utilizing newly developed 1536-well plates (IT-IS International Ltd.) for PCR one can save on reagent costs and increase throughput. However, the liquid handling employed in such low-volume PCR reactions must be robust in accuracy and precision. Using tipless, touchless acoustic droplet ejection (ADE) technology for low-volume liquid handling eliminates the cost of disposable tips and wash cycles. For the ADE we used the Labcyte Echo® 555 liquid handler (Labcyte Inc.) to transfer primers and probes from a 384-well source plate into a 1536-well plate. Sample volumes at 50 nL or 100 nL were transferred from each source well. PCR master mix (Hoffman La Roche) was transferred at 1 µl per well from a single well source reservoir to an entire 1536-well plate. With the ability to transfer from any well of the source plate to any well of the destination plate, setup can be formatted to a wide array of different combinations of primers and probes with each template DNA. We demonstrate that ADE technology dispenses precisely and accurately into 1536-well PCR plates to miniaturize real-time quantitative PCR (qPCR) gene expression reactions, yielding reproducible results in low-volume reactions.

2. Quantitative PCR Assay

Gene expression analysis using real time qPCR can be scaled down in volume and performed in high density 1536-well plates. Reagent savings and increased throughput are primary factors driving the need for fast, accurate low-volume liquid handlers for qPCR.

3. Low-Volume Liquid Handlers

The Labcyte Echo 555 liquid handler (right) was used to dispense primers and probes at 100 nL into 1536-well PCR plates. Using the Echo Plate Reformat application software, 100 nl was distributed from source wells into the 1536-well PCR plates. The Echo 555 uses acoustic droplet ejection (ADE), i.e. focused acoustic energy, to touchlessly transfer droplets of reagents in 2.5 nL increments directly from source plates to PCR plates. Tipless transfer eliminates washing steps.



Stroboscopic image of acoustic droplet ejection (2.5 nl droplet).

Dispensing Precision and Accuracy

Dispensing precision and accuracy were tested with the MVS volume verification system (Artel). Premixed primers and probes solution was mixed with MVS dyes, and 10 µL was loaded into a Labcyte 384LDV Echo Qualified (Labcyte) source plate, 100 nl volumes were transferred from the source plate to each well of an empty (dry) 384-well destination plate (Artel) using the Labcyte Echo 555 liquid handler, 55 µl of diluent was added, and then the plate was centrifuged, mixed, and absorbance measurement taken. Table 1 shows the obtained volume, relative inaccuracy, standard deviation, and CV for each target volume. The transfer volume accuracy and precision were excellent, with accuracy above 96% and precision below 4% CV.

Echo Precision and Accuracy

Target Volume (µL)	0.1	0.1	1
Dispense type	Dry	Dry	Dry
Number of data points per channel	384	384	384
Mean volume for all Channels (µL)	0.104	0.1041	1.036
Relative Inaccuracy for all Channels	4.00%	4.10%	3.60%
Standard Deviation for all Channels (µL)	0.0036	0.0029	0.025
Coefficient of Variation for all Channels	3.46%	2.79%	2.41%

4. qPCR Assay Results

Table 2 shows the Echo liquid handler transfer precision results for four plates at 1 µL total assay volume per well in 1536-well PCR plates. The plates were thermal cycled in the LightCycler 1536 system (Hoffman La Roche). C_P values were collected and analyzed. C_P precision for all four plates ranged from 0.96% to 1.28% CV.

CP Values

	Average	Stdev	CV	Min	Max	Range	N
Plate 1	22.73	0.29	1.28	20.61	24.86	4.25	1536
Plate 2	22.56	0.25	1.09	21.92	24.39	2.47	1536
Plate 3	22.5	0.24	1.07	21.91	26.19	4.28	1536
Plate 4	22.46	0.22	0.96	20.55	25.42	4.87	1536

5. Conclusions

- The Echo liquid handler delivers from nanoliter to microliter volumes of valuable reagents in one system for qPCR setup.
- ADE enables miniaturization of qPCR assays in 1536-well plates with excellent CVs.
- ADE offers a tipless, touchless solution to assay setup, saving costs and reducing waste.
- With one well to many wells, and any well to any well dispensing, the Echo liquid handler provides flexible and easily modified assay setup layout.

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