Introduction

Quantitative reverse transcriptase PCR (RT-qPCR) offers unmatched accuracy and quantification of gene expression. Combining the one step RT-qPCR assay with a streamlined workflow upstream for generation of template from cellular material has enabled the use of higher density 384- and 1536-well microplates in high throughput methods employing fewer reagents. To successfully miniaturize RT-qPCR assay preparation for high-throughput workflows, one must overcome high risks for cross contamination, lower precision, and poor accuracy. The Access workstation combines the benefits of the Echo liquid handler with novel reagent chemistries and qPCR analysis into a single walk-away platform for high throughput qPCR. The Echo liquid handler overcomes the challenges of assay miniaturization by enabling tipless, touchless transfer of reagents to deliver the precision and accuracy needed for high quality qPCR. This poster examines the ability to automate qPCR assay preparation from lysis to analysis utilizing the RealTime ready Cell Lysis Kit and LightCycler® 1536 system from Roche on the Access workstation—increasing throughput and reducing overall reaction volumes to as little as 500 nL.

Echo liquid handler

The Labcyte Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo 500 series allows for assay miniaturization to previously unattainable volumes. Echo liquid handlers transfer 2.5 nL droplets repeatedly, so precision and accuracy are consistent over a larger volume range. Large volume transfer is achieved by transferring several hundred droplets per second. Transfer is non-contact and tipless, with increased cost savings from elimination of tip costs and washing fluids.

Miniaturization with the Echo liquid handler retains high assay performance, allowing quantitative results at higher assay well densities. The Echo liquid handler can be used to transfer from any source well position to any destination well position. These can be simple fluids (media for growing cells, buffers, DMSO) or viscous solutions (lysis buffers, antibodies with glycerol, or transfection reagents).

Results

One-Step Gene Expression

The accelerated workflow for two-step RT-qPCR analysis of cell cultures single-step cell lysis, fast cDNA synthesis combined with qPCR transfers. qPCR results in half the time required for gene-expression qPCR as compared to the conventional workflow (multistep column-based RNA isolation, standard cDNA synthesis, qPCR) cuts in half the time required for gene-expression analysis as compared to the conventional workflow.

The AcceleratedWorkflow

HT1080 cells were seeded in 96 wells of a 1536-well, Echo® qualified tissue culture treated plate (Labcyte Inc.) at a concentration of 1000 cells/well and grown overnight. The media was removed, cells were washed with PBS and lysed with the RealTime ready Cell Lysis Buffer (Roche) for 5 minutes at room temperature. The lysate was then treated two ways (see below) after which assays were run on a LightCycler®480 384-well real-time qPCR instrument (Roche).

Figure 3A and 3B. HT1080 cells were harvested at a final concentration of 4K cells/well and lysed with the RealTime ready Cell Lysis Buffer (Roche) for 5 minutes at room temperature. The lysate was then treated two ways (see below) after which assays were run on a LightCycler® 480 384-well real-time qPCR instrument (Roche).

Figure 5. In one day, >10K reactions in 384-well assays or >40K reactions in 1536-well assays can be processed on an Access workstation (Labcyte Inc.)

One-Step Protocol

HT1080 cells were seeded in 96 wells of a 1536-well, Echo® qualified tissue culture treated plate (Labcyte Inc.) at a concentration of 1000 cells/well and grown overnight. The media was removed, cells were washed with PBS and lysed with the RealTime ready Cell Lysis Buffer (4 µL/well, Roche) for 5 minutes at room temperature. The Echo liquid handler was used to transfer 5 µL lystate into a 1536 qPCR plate predispensed with the LC480 RNA Master Hydrolysis Probes (Roche) along with primer (500 nM) and UPL probe (200 nM) against the UPL reference gene assay for GAPDH (Roche). The cDNA generated was then dispensed as template using the Echo 555 (Labcyte Inc.) into a 384-well qPCR plate predispensed with the LC480 RNA Master Hydrolysis Probes (Roche) along with primer (500 nM) and UPL probe (200 nM) against the UPL reference gene assay for GAPDH (Roche).

Automated Workflow

The Access workstation combines any Echo liquid handler with a modular robotic platform, offering a range of options for high-throughput workflows. In a simple compact system, the Echo liquid handler and two LightCycler® 1536 systems are integrated with plate handling devices for sealing, peeling, centrifugation, bulk dispensing, barcode reading and labware management. The Access workstation includes Tempo™ automation control software, which instantly adds scheduling power to protocols created by Echo software applications. Instead of requiring complex scheduling logic, Tempo software automatically prioritizes plate handling, liquid handling, and data analysis requirements using a run setup wizard. In this configuration the Access workstation enables miniaturization of qPCR experiments and simplification of the entire workflow from cell lystate to qPCR results, for a walk-away high throughput qPCR solution.

Summary

- Accelerated qPCR gene expression can be achieved by utilizing the RealTime ready Cell Lysis Kit from Roche.
- Increased throughput into 384- and 1536-well formats with volumes as low as 500 nL can be attained by incorporating the Echo liquid handler.
- Automated, miniaturized assay preparation for the entire workflow can be performed using the Access Workstation.