

## Introduction

To facilitate large-scale, high-throughput functional genomics studies using RNAi, we have developed acoustic droplet ejection (ADE) technology to meet the current demands of a successful siRNA library based screen. High-throughput applications require reliable and reproducible transfections to be performed in high density well plate formats. The success of an siRNA experiment relies on the effective delivery of the siRNA molecule.

Using ADE technology, a unique tipless and touchless liquid transfer technology used in the Echo® liquid handlers (Labcyte Inc.), we have successfully transfected esophageal cancer cells using a liposome-mediated transfection method. We have also demonstrated the use of ADE technology for reverse transfection. Here, ADE was used to deposit siRNAs in a transfection matrix onto glass slides, then overlaid with a monolayer of adherent cells and the slides incubated to allow reverse transfection. The effects of gene silencing were then assessed by digital image analysis at a single cell level.

### Acoustic Droplet Ejection: Move Liquids with Sound™

Acoustic Droplet Ejection (ADE) uses focused ultrasonic energy to eject small droplets from a liquid (Fig. 1.) The technology can be used to eject droplets smaller than one picoliter and as large as 10 µL. Larger volumes can be transferred as multiple drops. There is no contact between the ejection mechanism and the ejected sample. This technology is especially well suited to biological applications in which volume and/or positional precision are critical, and in which cross-contamination of samples can interfere with interpretation of the results. The acoustic mechanism eliminates the need for pipette tips, pin tools or nozzles. See [www.labcyte.com](http://www.labcyte.com) for additional information on ADE.



Figure 1. Stroboscopic image of ADE

## Gene silencing using microplates

The Echo series of liquid handlers have been successfully used in the area of high content screening (HCS). Using the same technology we are investigating the application of the Echo 555 liquid handler in siRNA library screening.

Approximately 10,000 KYSE esophageal cancer cells contained in RPMI media were transfected with the transfection indicator siGLO using Dharmacon's transfection reagent (No. 3). Inclusion intensities (after 24 hours) of cells were measured using an In Cell Analyzer 1000 and the data for manually and ADE transferred siRNA and transfection reagents were compared.

The results in Figure 2 indicate that the ADE technology produced more consistent inclusion intensities even at the lower concentrations of siRNA.

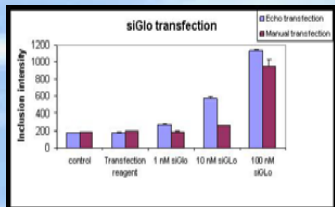


Figure 2. Inclusion intensities of esophageal cancer cells transfected using manual and ADE transfer.

Fluorescent cells containing the siGLO were observed using fluorescence microscopy. The image was then further enhanced by overlaying bright field images when the cells were stained with a nuclear stain.

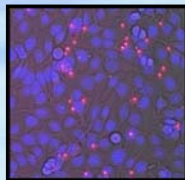


Figure 3. Transfected esophageal cancer cells with siRNA transferred with an Echo liquid handler.

## Gene silencing using microarrays

There is currently much interest in lab-on-a-chip devices which allow cell-based assays to be performed in nanoliter drops. These devices significantly reduce experimental costs whilst enabling experimentation at the single cell level.

ADE technology has been used to transfer a wide variety of biological solutions at volumes as low as 1 pL (12 microns diameter) and as large as 525 nL (1000 microns diameter).

Using the Echo liquid handler we transferred 10 nL of THB cells ( $8 \times 10^6$  cells/mL) into an array format on a microscope slide with 1000-micron spacing. The transferred cells were then viewed using an In Cell Analyzer 1000.



Figure 4. 10 nL THB cell array on a microscope slide

By controlling the transfer volume of cells we were able to accurately and reproducibly define the number of cells per spot.

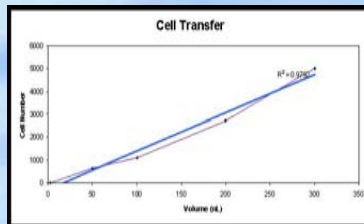


Figure 5. Number of THB cells transferred by ADE technology

Previous experiments have demonstrated the positional accuracy of the Echo. These studies also revealed that the coefficient of variation (CV) of spot diameters using 50% glycerol was 1.78%.

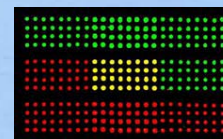


Figure 6. 2.5 nL of Cy3 (red) and Cy5 (green) spotted in 4x16 arrays with the centre spots overlapping.



Figure 7. 2.5 nL siRNA array on a microscope slide

Figure 7 shows siRNA transferred in phosphate buffered saline with 0.15 M fluorescein (to aid visualization) and arrayed onto a microscope slide using the same method and spacing as the cells in Figure 6. Ongoing studies will investigate the feasibility of overlaying the cells with compounds or gene silencing reagents.

## Conclusions

ADE can be used to transfer siRNA molecules into various plate formats for liposome mediated transfection.

The results indicate that ADE transferred siRNAs perform more constantly and give stronger inclusion intensities especially at lower concentrations.

ADE can be used to further reduce the assay volume by creating cell-based microarrays on glass slides.

By transferring low volumes of cells without effecting viability it is possible to analyze results at the single cell level.

ADE technology provides an all-around solution to performing low-cost, high-throughput siRNA library screens.