Application Note 400

Labcyte Echo® 500 series liquid handler

# Miniaturizing siRNA Transfection Using Acoustic Droplet Ejection

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## Introduction

Large-scale, high-throughput siRNA experiments require a transfer system that can facilitate reliable and reproducible transfections in high-density well-plate formats, as well as effective delivery of the siRNA molecule. This study demonstrates superior performance of the Echo liquid handler compared to more traditional liquid handling methods for standard, reverse and lipid-free transfection of siRNA molecules into mammalian cells.

Results of the study clearly demonstrate the ability of the Echo liquid handler to deliver comparable transfection efficiency, knock-down and cell morphology to reactions prepared using traditional pipeting methods. In fact, reactions prepared with the Echo liquid handler were miniaturized to use significantly less volume of siRNA and other reagents, and still yielded more representative inclusion intensities than traditional manual pipetting techniques. Such miniaturization can result in significant cost savings and greater confidence in the results from siRNA screening studies.

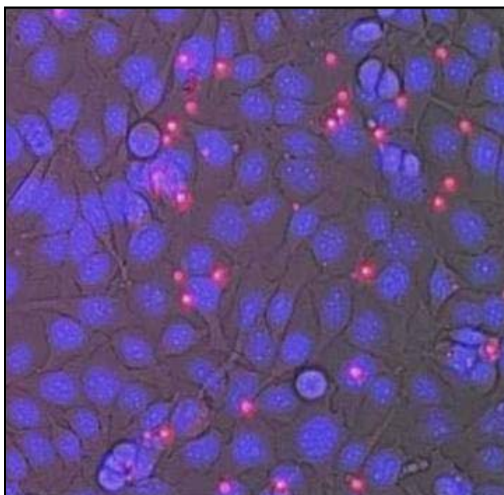

**Figure 1.** Fluorescence image reveals KYSE cells successfully transfected with siGLO (pink), transferred using the Echo 555 liquid handler.

## Example 1: Standard transfection using siGLO® siRNA

This example compares the transfection efficiencies of cell-based assays that were prepared by acoustic droplet ejection (ADE) technology using the Echo 555 liquid handler versus manual pipette dispensing. Since siGLO (Thermo Scientific) transfection indicators localize to the nucleus, they are often used to determine optimal siRNA transfection conditions or to monitor relative efficiency of delivery when co-transfected with siRNA. In this experiment we compare the transfection efficiencies of siGLO transfection indictor when it is transferred manually by pipette and using the Echo 555 liquid handler.

## Methods

**ADE method:** The Echo 555 liquid handler transferred volumes between 10 nL and 1000 nL of siGLO and DharmaFECT3 (Thermo Scientific) transfection reagent into a 96-well flat-bottomed microplate. The plate was then backfilled with 40 µL of serum-free RPMI medium.



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**Manual pipetting method:** Dilutions of siGLO and transfection reagent were prepared in 20 µL volumes,and then each dilution was backfilled with an additional 20 µL of serum-free RPMI medium to the same final concentrations of siRNA as above.

Both methods were performed in triplicate for each concentration of siRNA and transfection reagent. The siRNA and transfection reagent mixture was incubated at room temperature for 20 mins before 160 µL KYSE cells were added to the wells. After 24 hrs incubation, the inclusion intensities of the cells were measured using an IN Cell Analyzer 1000 (GE Healthcare). Cells transfected with siGLO were also stained with nuclear stain Hoechst 33358 and imaged using fluorescence microscopy (Figure 1).

## Results

The following data shows that transfections prepared using the Echo 555 liquid handler to transfer the siRNAs yielded stronger inclusion intensities and at lower concentrations than manually transferred siRNAs (Figure 2). Inclusion intensities in samples transferred using the Echo liquid handler are also more directly proportional to the amount of siGLO added than in manually transferred samples.

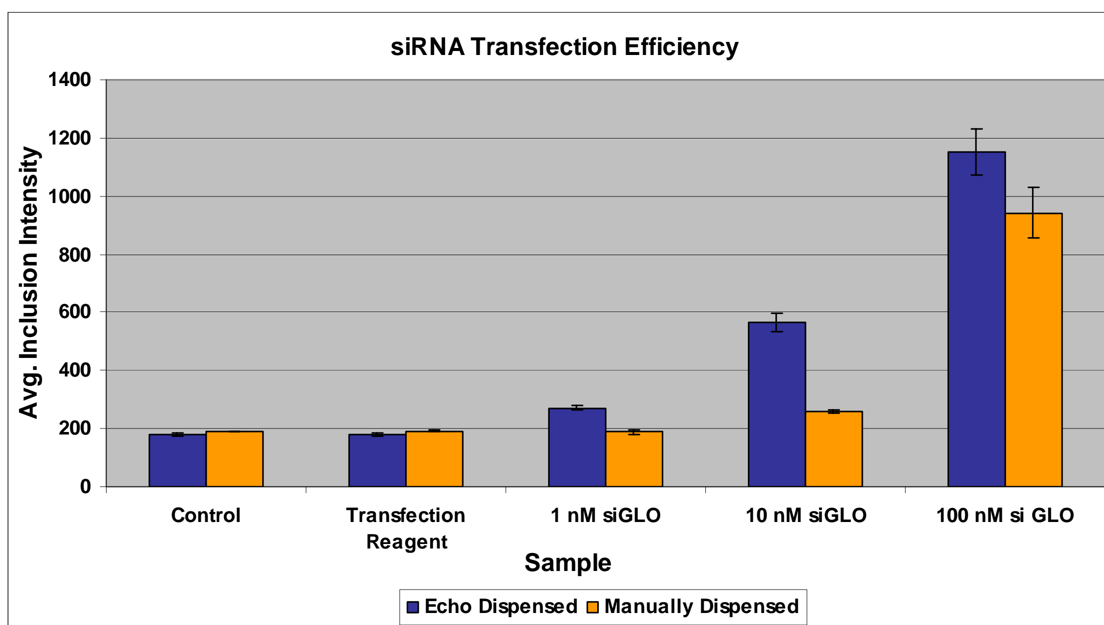


**Figure 2.** Inclusion intensities of esphogeal cancer cells transfected using the Echo 555 liquid handler vs. manual transfer. Error bars indicate standard deviations.

## Example 2: Reverse transfection using Bcl-xl siRNA

This example uses varying concentrations of Bcl-xl siRNAs and non-homologous scrambled nucleotide controls, followed by a cell proliferation (MTT) assay, to measure the effectiveness of the knockout of B-cell lymphoma-extra large (Bcl-xl; a mitochondrial transmembrane protein implicated in cancer cell survival).

## Methods

**ADE method:** The Echo 555 liquid handler transferred volumes between 5 nL and 500 nL of siRNA and DharmaFECT3 transfection reagent (Thermo Scientific) into a 96-well flat-bottomed microplate. The plate was then backfilled with 20 µL of serum-free RPMI media.
**Manual pipetting method:** Dilutions of siRNA and transfection reagent were prepared in 10 µL volumes, and then each dilution was backfilled with an additional 10 µL of serum-free RPMI medium to the same final concentrations of siRNA as above.

Both methods were performed in six replicates for each concentration of siRNA and transfection reagent, with and without deoxycholic acid (DCA; induces apoptosis). The siRNA and transfection reagent were incubated at room temperature for 20 mins before 80 µL SKGT4 cells ($2.0\times10^4$) were added to the wells. The plates were incubated (72 hrs, 37°C, 5% $CO_2$), and then half of the wells were treated with 500 µM of DCA at 64 hrs incubation. The MTT assay was performed and absorbance was read at 570 nm.



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## Results

The overall cell proliferation responses were comparable in both transfer methods, exhibiting Bcl-xl knock-down with Bcl-xl siRNAs but not with the scrambled controls, and inhibition of cell proliferation with DCA (Figure 3).

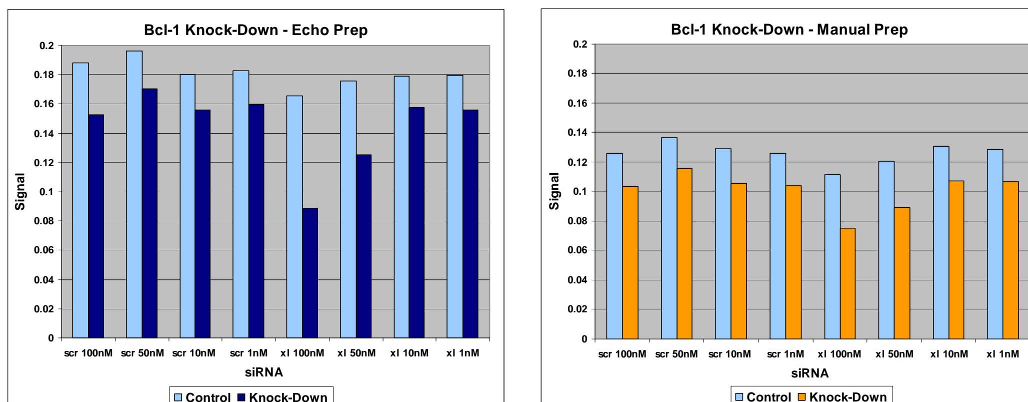


**Figure 3.** Knock-down of Bcl-xl inhibition of SKGT4 cell proliferation by Bcl-xl siRNAs (xl) compared to scrambled (scr) controls, with and without DCA, plotted as optical density ±standard deviation. Transfections were prepared using the Echo 555 liquid handler (left) and a manual pipettor (right).

A dose-response curve of the knock-down response was prepared to show the percent of viable cells treated with DCA relative to untreated controls, normalized against the corresponding scrambled control (Figure 4). The dose-response curve from samples transferred using the Echo 555 liquid handler more accurately reflects the amount of siRNA added than the curve from samples that were transferred manually.

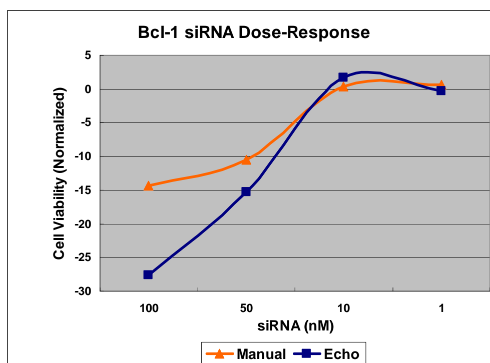


**Figure 4.** Dose-response for Bcl-xl siRNA knock-down of inhibition of SKGT4 cell proliferation, plotted as cell viability relative to the controls.

## Example 3: Acoustic transfer for lipid-free transfection

This example evaluates the performance of ADE for lipid-free transfection. We used Accell siRNAs (Thermo Scientific), which are chemically modified to promote cellular uptake and eliminate the need for conventional lipid transfection reagents.

## Methods

Accell siRNA plates were reconstituted with siRNA buffer and delivered into daughter plates (pre-coated with goat anti-mouse IgG and anti-LFA-1 to enable cell migration), using a multichannel pipette and the Echo 555 liquid handler. Peripheral blood T-lymphocytes in Accell siRNA delivery medium were added to each well, and the plates were incubated for 72 hrs at 37°C, 5% $CO_2$. The cells were fixed, permeabilized, and stained with Hoechst 33358 and phalloidin TRITC. Acquisition and analysis was performed on the IN Cell Analyzer 1000. Data were normalized and scored using HITs, a modified version of CELL HTS software created at Trinity College (Boutros et al. *Science* **303**:832, 2004).

## Results

To compare the impact of the siRNA transfections on cell morphology, five morphological factors were scored using HITs:

- Cell 1/form factor: a measure of roundness that incorporates cell area and perimeter complexity.
- Cell area: the two-dimensional surface area enclosed within a boundary.
- Nuclear displacement: the position of the nucleus relative to the centroid of the cell.



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- Cell gyration radius: the spread of a cell, defined as the square root of the mean squared distance between the cell periphery and its center of gravity.
- Cell elongation: length versus breadth of the cell. Both ADE and manual transfer methods successfully identified siRNAs that target proteins that are known to influence T-cell migration.

Both methods of delivery successfully identify siRNAs that target proteins that are known to influence T-cell migration (Figure 5).

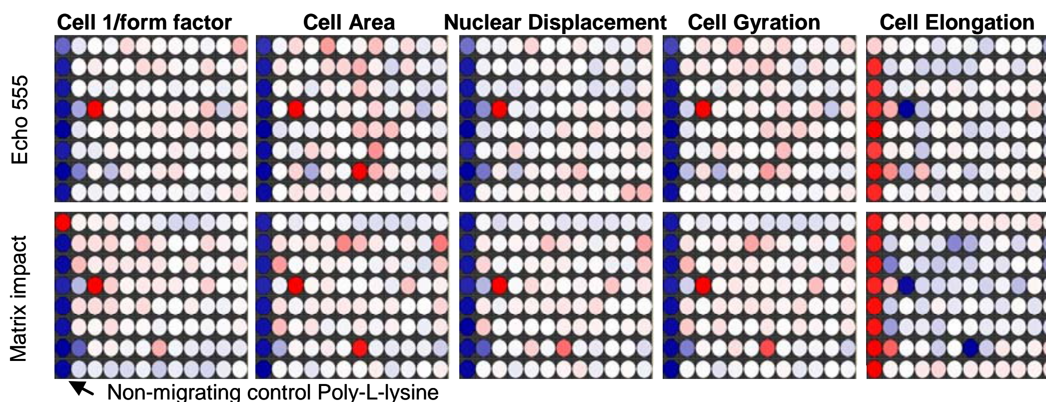


**Figure 5.** Comparison of five cell morphology factors in transfections prepared using the Echo 555 liquid handler (top) and a manual pipettor (bottom). Red indicates activation of the given morphology factor as a result of siRNA transfection; blue indicates repression.

Figure 6 shows the scored results for cell 1/form factor for both dispensing methods.

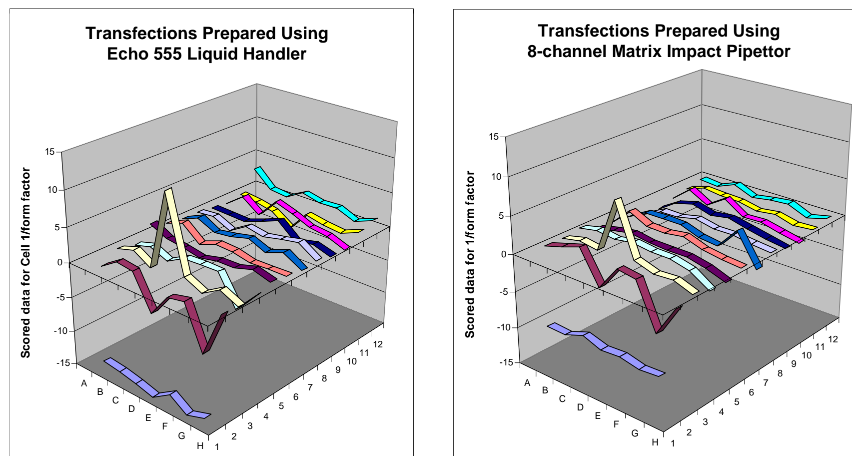


**Figure 6.** Scored results for cell 1/form factor in 80 wells from each dispense method, depicted in Figure 5. Echo 555 liquid handler (left) and a manual pipettor (right). Row 1 is non-migrating poly-L-lysine negative control.

These results demonstrate that siRNA transfections prepared using the Echo 555 liquid handler show no significant change in morphological behavior compared to transfections prepared using manual methods.

## Conclusions

Labcyte ADE technology presents exciting new options for assay miniaturization of RNAi studies. In standard siRNAs to Bcl-xl, ADE transfer had no detrimental effects on the siRNA transfection or knock-down. In lipid-mediated transfection, ADE transfer yielded more accurate inclusion intensities and dose-response curves than manual transfer. In lipid-free transfection, ADE transfer enabled successful identification of siRNAs that were known to influence T-cell migration, comparable to manual pipette transfer.



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