

Labcyte Application Highlights 8/2011

Echo Application Highlight: Miniaturized High-Throughput siRNA Workflows

POD Application Highlight: High-Throughput, Low Volume, siRNA Screening

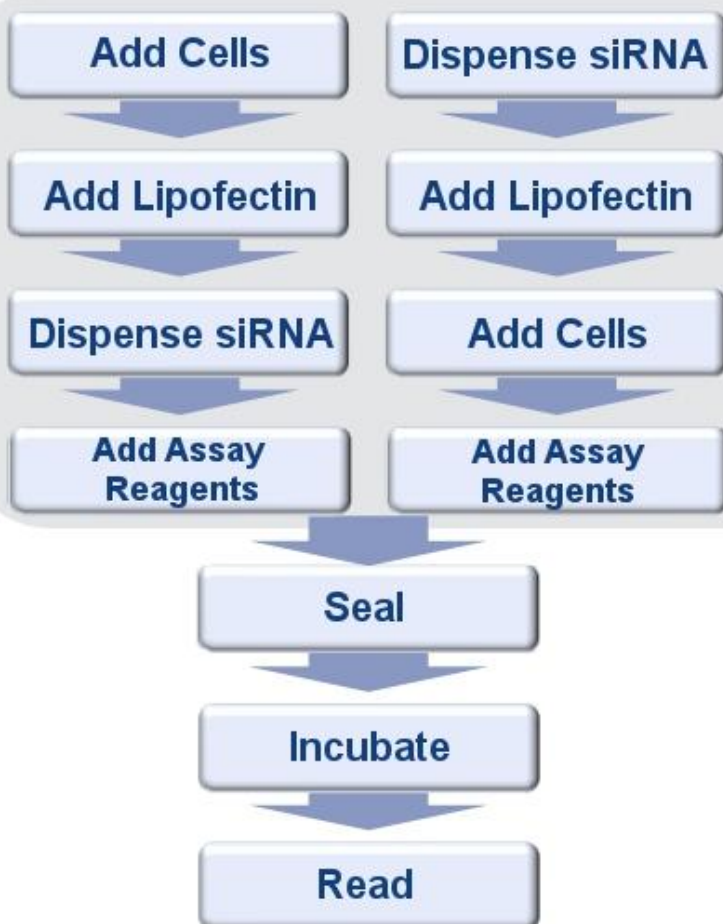
Echo Application Highlight Miniaturized High-Throughput siRNA Workflows

The Echo[®] liquid handler enables low volume delivery of siRNA into high density plates. Additionally, the tipless nature of acoustic dispensing ensures low volume assay plates avoid cross contamination. The Echo liquid handler enables the creation of assay-ready plates and reduces freeze thaw effects. siRNA samples can be diluted directly utilizing the Echo Plate Reformat Software, saving labor and repetitive error seen in serial dilutions. Valuable savings from the elimination of tip waste and reagent consumption makes siRNA screening a viable option for target identification and validation.

- Miniaturized siRNA delivery enables assay-ready plate creation
- Contamination-free transfer of samples and reagents
- Eliminate pipette tips and washing to save consumables cost and waste
- Direct dilution of siRNA reduces error propagation



siRNA Workflow

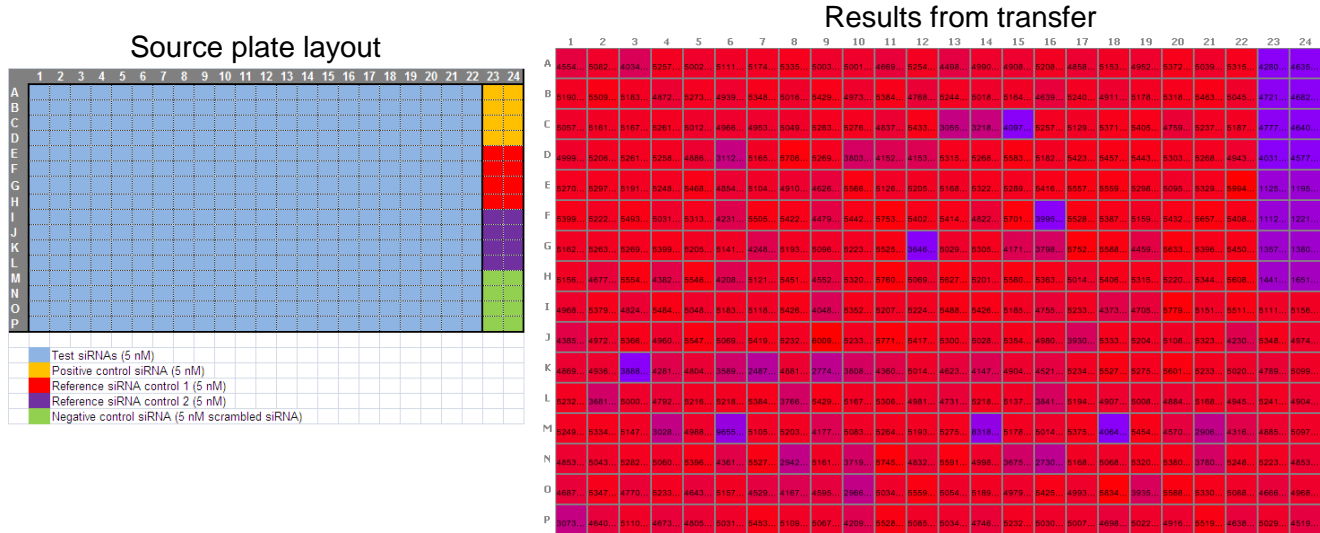


Any combination of these steps may be performed with the Echo liquid handler

Echo Application Highlight Miniaturized High-Throughput siRNA Workflows

siRNA Results Show Excellent Correlation

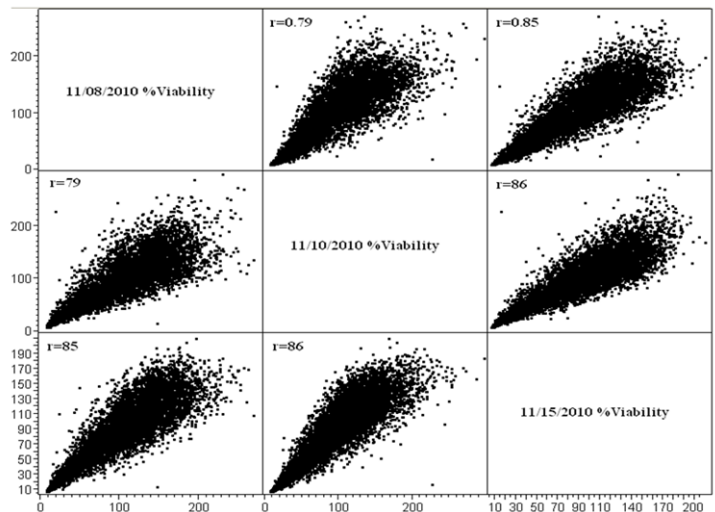
The source template below illustrates the layout of samples and controls. Controls are located on the right hand side of the plate. Samples are plated within the blue area as illustrated by the figure legend. The results on the right show a typical output read after detection reagent has been added. There are several hits (shown in purple) which indicate knock-down of the interested target by a siRNA sample.



Excellent correlation seen after screening 7K siRNA samples

Correlation data comparing the viability results after siRNA knock-down for three screens where siRNA samples were transfected using the Echo liquid handler and the workflow describe above. The screens show excellent correlation as depicted by the graphs with a Pearson's correlation of 0.79 or better.

		Pearson's Correlation	Spearman's Correlation
%Viability	11/08/10 vs. 11/10/10	0.79	0.79
	11/08/10 vs. 11/15/10	0.85	0.84
	11/10/10 vs. 11/15/10	0.86	0.85



(Data courtesy of Lynn Rasmussen,

Southern Research Institute, Birmingham, Alabama US.)

POD Application Highlight High-Throughput, low volume, siRNA Screening

The **POD™ automation platform** utilizes the revolutionary Echo® liquid handler and Echo® software applications to produce low volume assay plates for siRNA screening with unmatched precision and accuracy. Using lower quantities of siRNA and assay reagents makes the POD system the most cost-effective solution for target identification and validation. This highlight reviews the outcome of a study comparing the knock-down of gene expression after siRNA transfection followed by reporter gene and cell viability assays.

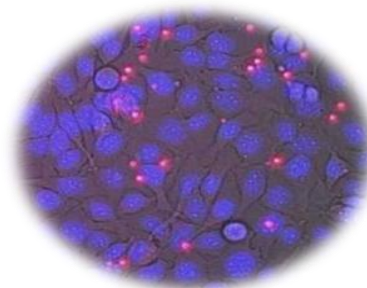
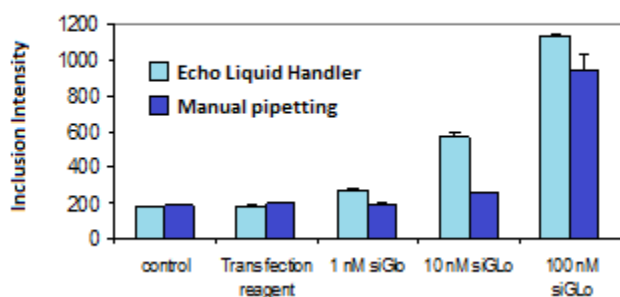


Automated siRNA Transfection

Researchers have used a variety of methods to transfect cells with siRNA. The Echo liquid handler can precisely transfer nanoliter volumes of siRNA transfection reagents to cells—volumes much lower than with tip-based liquid handlers. This provides researchers a powerful tool for miniaturizing both forward- and reverse transfection processes. With the added ability to seed cells, dispense media and store samples in controlled environments, the POD system is a complete solution for automating siRNA screening.

- Store up to 2016 plates in dry, incubated or ambient conditions with CO₂ control
- Bulk fill plates with up to four different reagents without intervention
- Eliminate risk for sample retention or cross contamination seen with tip-based liquid handling
- Improve quality to eliminate the need for additional replicates

siGlo Transfection Efficiency

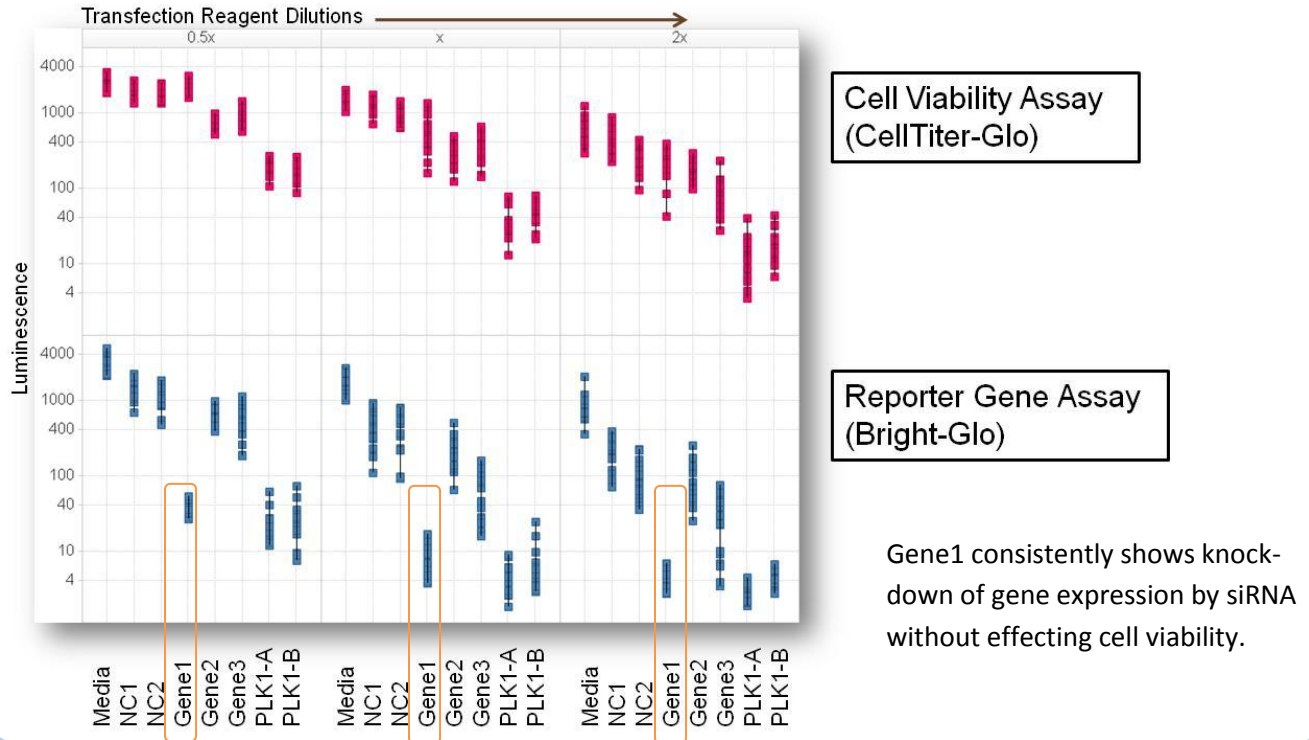


Transfection of siRNA in volumes as low as 5 nL with the Echo liquid handler have shown higher efficiency when compared to 10 μ L transfection by manual pipetting. Efficiency was determined visually with fluorescence imaging of KYSE cells, and directly by measuring the inclusion intensities of esophageal cancer cells.

(Data courtesy of Dr. Anthony Davies, Institute of Molecular Medicine, Trinity College, Dublin, Ireland. See Labcyte Application Note #400 for experimental details.)

Results

The graph below shows a comparison of assay plates generated from the POD system. Each reporter gene assay plate contains HEK293T cells transfected with 50 nL of siRNA and Bright-Glo and each cell viability assay plate contains the same cell line transfected with 50 nL of siRNA and CellTiter-Glo:



As demonstrated in the results above, the POD system accurately dispenses nanoliter quantities of siRNA and assay reagents for fully automated transfection and assay plate creation. By continuously maintaining the highest level of efficiency, the POD system provides incomparable throughput while offering tools to easily manage plate replication, cherry picking and dose-response processes. With integrated environmentally controlled storage, the POD system is the only out-of-the-box solution for miniaturized high throughput siRNA screening.