

Simplifying and Increasing the Efficiency of siRNA Screening with a Novel Tip Cleaning Technology

Erica Stec ¹, Kevin Huff ¹, Stacey Szymanski ¹, Geoffrey Schwartz ²,Oleg Kornienko ¹, Berta Strulovici¹

¹ Automated Biotechnology, Merck & Co. ² Cerionx Inc.

Abstract

Plasma technology has been shown to be a reliable alternative to the traditional tip wash or replacement techniques currently used with automated liquid handling devices throughout the biopharmaceutical and diagnostic industries. The TipCharger™ System utilizes cold plasma to clean disposable polypropylene micropipette tips without the use of traditional wash solvents or waste generation. The use of a dry cleaning method eliminates the problems associated with contamination in wash solutions and delivers results comparable to or better than existing washes in less than half the time. Evaluation goals in the Automated Biotechnology Lab in North Wales included simplification of tip wash processes, reduction/elimination of biological waste generation, and cost savings through reduction in assay time and consumable usage. Following a comparative analysis of tips cleaned with DNase/RNase free water and TipCharger-generated plasma in siRNA assays, this new technology was implemented on a linear track robotic system for genome scale screening (~22K genes). Evaluation and production results are presented.

Evaluation Goals and Results

The initial evaluation was designed to assess how well the TipCharger System eliminates carryover when compared to historical (DNase/RNase free water) wash protocols via a Bodipy fluorescent dye-based assay and a proprietary Merck siRNA cell-based assay.

When tips are introduced into the TipCharger™ Cleaning station, an electric field is produced, ionizing the room air, resulting in a continuous plasma field around each pipette tip. The result is that solvents and organic molecules are reduced into individual atoms eliminating carryover, tip washing or tip replacement.

Comparison of the Traditional Tip Wash with Plasma Cleaning
The ability of the TipCharger System to reduce Bodipy detection is shown in Figures 2 and 3. Fluorescence was reduced to pre-read detection levels after tips were exposed to plasma for 77 seconds. Figure 2 shows that a 1µL volume of Bodipy dye, at a concentration of 2.5µM, is reduced by 24,000-fold signal-to-background ratio on average. Further, Figure 3 shows that at similar concentration but higher volumes (5µL at 2.5µM), plasma cleaning reduced Bodipy signal by 6,500-fold on average. These values are comparable to historical water based protocols.

Precision, Accuracy and Carryover of Plasma Cleaning
Further precision and accuracy studies using dye showed that the CyBio tips were capable of withstanding upwards of 80 exposures to the TipCharger cleaning station without compromising data quality or tip integrity (data not shown).

Biological Assay Background

- The development of highly sensitive cell based assays are critical to the HTS and HCS environments
- siRNA assays are both sensitive and robust and have become relatively inexpensive through automation and miniaturization
- Reducing screening timelines and costs continues to be a priority
- The recurring costs associated with purchasing and using polypropylene pipette tips are significant
- There is an opportunity to substitute electrically-generated atmospheric pressure plasma for current tip washing techniques and thereby increase operational efficiency

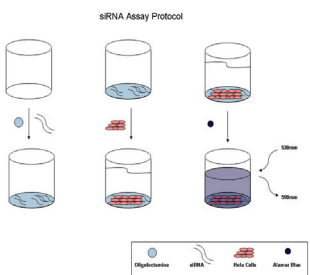
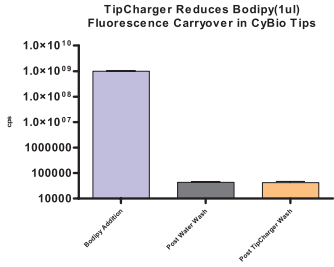
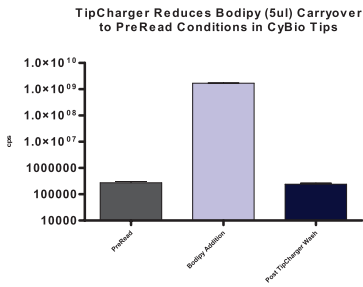


Figure 1. siRNA Forward Transfection Protocol. Details the transfection protocol used to transfect HeLa cells. siRNA and transfection lipid are added to pre-plated HeLa Cells. 72 hours later, an Alamar Blue assay is executed to determine percent viability of cells, and overall transfection efficiency.



Figures 2 and 3. Detail carryover data from both 1µL and 5µL transfers using TipCharger Plasma Technology

Performance of the TipCharger System in siRNA Viability Assay
The siRNA assay used to evaluate the performance of the TipCharger System was a cell viability assay in which a known toxic gene was used to induce knockdown and cell death. An Alamar blue assay can be carried out 72 hours later to measure live cells. Figure 4 details the results for a spiked gene plate followed by a water plate. It shows that the TipCharger system effectively removes siRNA from the pipetting tips while maintaining good transfection efficiency of control siRNA.

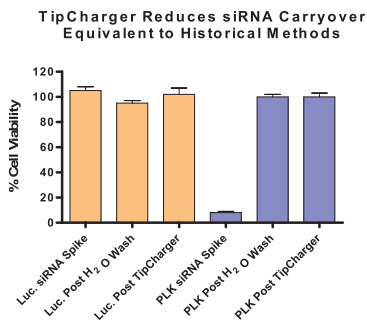


Figure 4. Shows effective knock-down of control siRNA (PLK) with both historical water wash and TipCharger plasma wash technology. siRNA is effectively cleaned from tips in both types of wash cycles.

TipCharger System In Production

The successful evaluation of the TipCharger system for cleaning siRNA from CyBio pipette tips led to the installation of three TipCharger systems on a linear track robotic system used in the automated siRNA screening environment. Each TipCharger system was installed on the CyBi Well pipetting stations used to transfer siRNA and siRNA/transfection lipid complex. Following installation, a screen designed to elucidate genes sensitive to particular compounds commenced.



Figure 7. Photographs of linear track robotic system used for automated siRNA screening. Included in the system are 3 CyBi Well pipetting stations and 1 CyBi Disk pipetting station. The pictures below depict the easy installation and set up of the TipCharger system on the CyBi Disk.

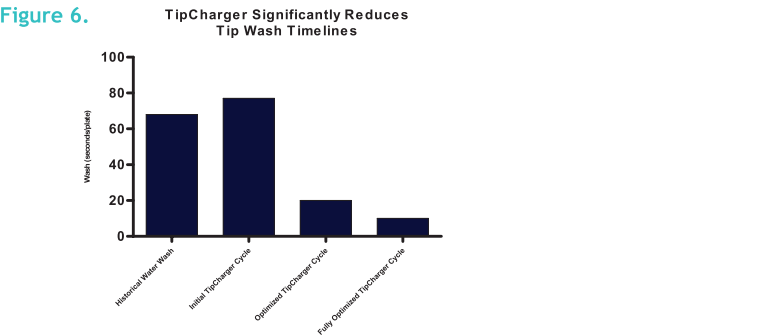
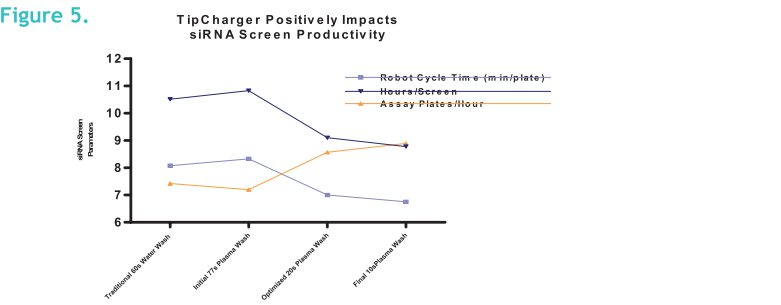
Effect of TipCharger Plasma Technology on Assay Productivity
Through further optimization, we were able to decrease the total TipCharger wash cycle time from an initial 77 seconds to 15 seconds. This is a significant improvement from the historical water wash time of 90 seconds. Since transfection times in siRNA assay has always proved to be a bottleneck on this particular robotic system, by lowering the cycle time of the wash, we are also able to lower the robotics cycle time by 20% and increase the throughput of source plates on the robotic system.

Production Screen Data

Following installation, a screen designed to elucidate genes sensitive to particular compounds was initiated. Using the same control siRNAs (PLK and Luciferase) as in the evaluation, cell viability acted as a proxy for transfection efficiency in the production screen. The percent viability for PLK control wells determined whether or not data from a particular assay plate was accepted or rejected. Specifically, cell viability values of greater than 20% for PLK wells required that an assay plate be re-screened. Figure 8 demonstrates that over the course of an 83 plate screen, only 5 plates were rejected based on the PLK, cell viability criteria. Overall, the median percent viability for PLK siRNA controls was 9.6% +/- 4.7. Further, similar data quality was achieved throughout the screen for other controls and corresponding siRNA library unknowns.

Conclusions

The results demonstrate that substituting the TipCharger System for traditional tip washing measurable improves throughput, assay run times, and direct and indirect costs. Our laboratory will save approximately 1 minute/tip wash cycle, thereby improving our daily assay run time by 20%. The purchase of DNase/RNase free water alone saves more than \$1,000 per screen. Eliminating the direct as well as indirect costs of using DNase/RNase free water and hazardous material disposal associated with everything in contact with the cells used for siRNA screening significantly reduces our costs and improves our lab operation. Finally, the ability of the TipCharger System to not only clean tips, but to sterilize them, eliminates concerns of bacterial or fungal contamination and the accompanying workflow issues associated with contamination remediation. Using this System significantly reduces our expense while at the same time improves the overall reliability and robustness of our siRNA screening campaigns.



Effect of TipCharger Plasma Technology on Direct and Indirect Costs
A typical siRNA screen in our lab utilizes approximately 88L of DNase/RNase free water to wash disposable pipette tips, at a direct cost of \$1,080. In an average year, many genome-scale screens will be run. In addition, the indirect costs associated with purchasing, transporting, disposing and managing this quantity of water are substantial as are the risks associated with contamination.

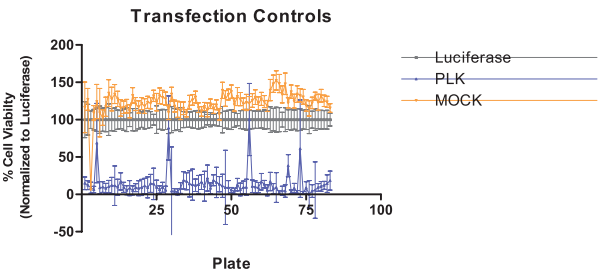


Figure 8. Comparative siRNA results. Similar results were achieved when the TipCharger System was applied to cleaning and sterilizing pi-