Increasing the Efficiency of siRNA Screening using Plasma-based Pipette Tip Cleaning

Introduction

Highly-sensitive cell-based assays are critical to both HTS and HCS environments for the rapid identification of genomic and proteomic targets. The assays are also useful in determining small molecule efficacy and associated mechanisms of action. The use of small interfering RNAs (siRNAs), used to induce sequence-specific gene silencing in cultured cells, enable research and screening labs to rapidly elucidate gene function. Libraries of siRNAs targeting entire human gene classes can be used to identify genes with specific cellular functions and are critical for leveraging new genomic knowledge for drug discovery. Although siRNA cell-based assays are both sensitive and robust, they have recently become relatively inexpensive through automation and miniaturization. In addition, improvements in detection method sensitivity helped drive reductions in screening costs and timelines while improving data quality. However, these efforts alone are not enough to fully optimize most screening environments. The cost of consumables and the inefficiencies associated with consumable delivery to automated systems remain hurdles for most HTS campaigns. Specifically, the use of polypropylene pipette tips, through direct use and disposal or through time consuming tip-washing protocols, remains a significant source of cost in screening efforts. In this study, plasma-based pipette tip cleaning efficacy was demonstrated using a well-characterized and fully automated siRNA cell-based assay.

Plasma Cleaning of Pipette Tips

The TipCharger™ by IonField Systems™ is used for cleaning pipette tips specific to automated liquid handlers and provides pipette tip cleaning equivalent to tip replacement. Pipette tip cleaning of siRNA molecules and an associated transfection reagent complex was initially optimized during an evaluation period at a major multinational BioPharma company. Evaluation goals include simplification of tip wash processes, reduction of of biological waste generation and cost savings through reduction in assay time. The resulting optimized pipette tip cleaning protocols were then expanded into an siRNA-driven, drug sensitivity production screen. Resulting data was compared to historical methods regarding assay signal-to-noise ratio, throughput, and total screen cost.

TipCharger Integration

The TipCharger, provided in 8, 96 and 384-well plate densities, is easily integrated into most liquid handling platforms using standard SBS footprints. The TipCharger can be taught as either a device or consumable within the liquid handler software. In the production phase of this study, a CyBi®-Disk (CyBio AG) was used with the TipCharger TC-384 cleaning station taught as a conventional CyBio tip wash station.

Contaminants on the exterior of pipette tips exposed to TipCharger-generated plasma were immediately ionized. Both siRNA and siRNA/siLentfect complex retained inside the tips were ionized using a 6 second exposure to plasma incorporating several aspirating and dispensing steps.

Tissue Culture and Cell Plating

Hela S3 cells (ATCC Cat # CCL-2.2) were cultured in DMEM (Invitrogen Corporation, Carlsbad CA, Cat # 11965) with high glucose L-glutamine, containing 10% Fetal Bovine Serum, Certified, Heat-Inactivated US (Invitrogen Cat # 10082), and 1% Penicillin-Streptomycin (Invitrogen Cat # 15140). Cells were incubated at 5% CO_2, 37°C and 90% humidity on the SelecT Tissue Culture Robot (The Automation Partnership, UK) until approximately 80% confluent. Cells were dispensed in columns 2-24 in each 384-well plate (BD Falcon, Cat # 353962) at a density of $1.125x10^4$ cells/mL via a SelecT integrated multi- drop dispenser (Thermo Electron Corporation, Waltham MA). For control purposes, column 1 contained media only.

Transfection

All siRNA stocks were diluted to working concentrations of 5 µM in an intermediate plate containing siLentfect (BioRad Corporation, Bath, UK Cat # 170-3362) diluted in Optimem (Invitrogen Cat # 31985). Luciferase and PLK siRNAs, used as controls for measuring both transfection efficiency and cell viability, were added to wells I2-L2 and E2-H2 respectively. Wells A2-D2 and M2-P2 were used as mock controls. Finally, all other wells received the respective siRNA of interest and the complexes were then incubated for 40 minutes at room temperature (RT). The contents of each intermediate plate were distributed across 4 cell plates and incubated for 4 hours (Figure 1) under normal tissue culture conditions (5% CO_2, 37°C and 90% humidity).

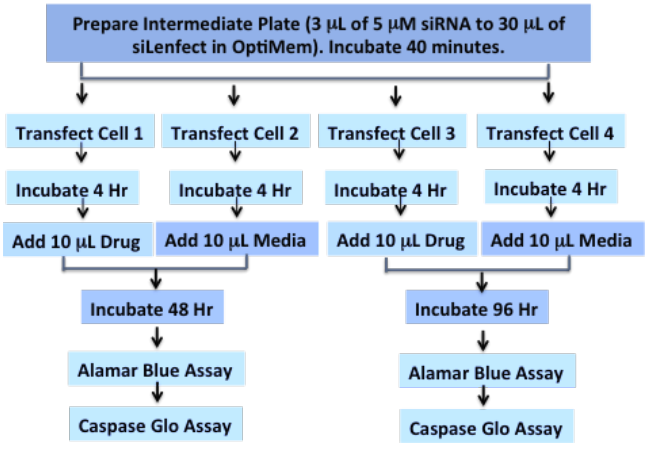


Figure 1: *Schematic of assay protocol.*

Post incubation, the 4 cell plates were divided into two groups of two plates; one received compound and the other received media in place of compound. In the first group, plates were incubated for 48 hours under normal tissue culture conditions, while the second group was incubated for a total of 96 hours under the same conditions. All 4 cell plates were assessed for cell viability using standard Alamar Blue (Invitrogen Cat # DAL 1025) and Caspace 3-7 reagents (Promega Corporation, Madison WI, Cat # 8092).

Results

Initial Performance of the TipCharger System Using a Cell Viability Assay

Early TipCharger optimization efforts using a commonly used dye showed pipette tip cleaning equivalent to historical DNAse/RNAse free water wash protocols. 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).

Similar results were achieved when the TipCharger was applied to cleaning pipette tips after exposure to siRNA for Luciferase (a non-toxic gene) and PLK (a known apoptitic gene) complexes (Figure 2).

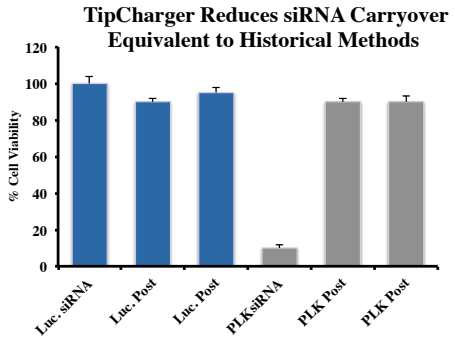


Figure 2. *Comparative siRNA carryover results.*

Although equivalent cleaning was observed the time required per tip wash using water (66 seconds) was reduced to 10 seconds using the TipCharger system (Figure 3).

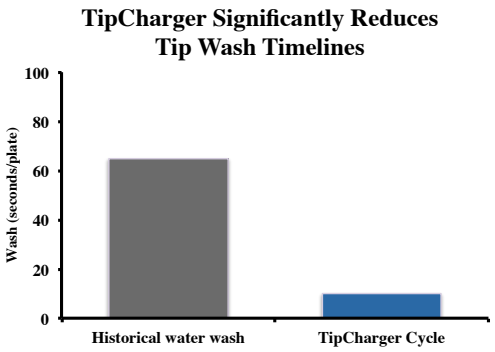


Figure 3. *Comparative tip washing protocol results.*

Overall, the data shows that the TipCharger effectively and efficiently removes siRNA from the pipette tips while maintaining good transfection efficiency of control siRNA.

Incorporation of the TipCharger System in an siRNA Production Screen

The successful evaluation detailed in this study led to the incorporation of three TipCharger systems into a fully automated siRNA screening environment. A screen to elucidate genes sensitive to particular compounds was implemented post-installation. Cell viability using PLK and Luciferase siRNAs served 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. Cell viability values of greater than 20% for PLK wells required that an assay plate be re-screened. Figure 4 demonstrates that over an 83 plate screen, only 5 plates were rejected based on the PLK cell viability criteria. Poor viability was attributed to cell culture issues, rather than to the TipCharger.

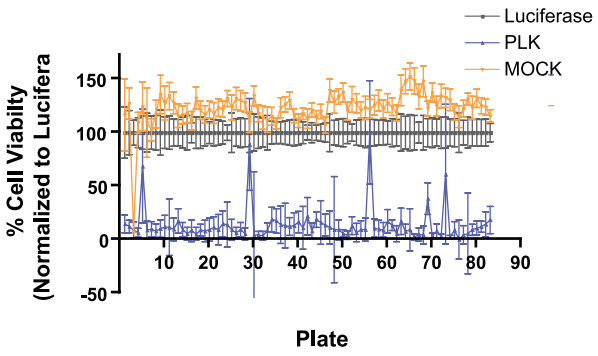


Figure 4. *Cell viability as a function of transfection efficiency.*

Overall, the median percent cell viability for PLK siRNA controls was 9.6% +/- 4.7. Similar data quality was achieved throughout the screen for other controls and corresponding siRNA library unknowns.

Summary

Substituting the TipCharger for traditional tip washing significantly increases throughput, eliminates the requirement for DNAse / RNAse free water and improves the overall reliability and robustness of siRNA screening campaigns. The automated biotechnology laboratory participating in the study described in this note saved approximately 1 minute/tip wash cycle, thereby improving daily assay run time by 20% (from the historic 10.5 hours to 8.5 hours).

Since transfection times in siRNA assays have always proved to be a bottleneck when used on the specific robotic system implemented in this lab, lowering the cycle time of the wash, reduced the overall robotic cycle time by 20%, and increased the throughput of source plates on the robotic system.

Both direct and indirect costs associated with purchasing, storing, handling and disposing of costly DNAse/RNAse free water as well as the costs and procedures surrounding the disposal of pipette tips in contact with the cells used for siRNA screening were significantly reduced with the implementation of the TipCharger.

Finally, the ability of the TipCharger to clean tips in situ, eliminated concerns of bacterial or fungal carryover contamination, and streamlined workflows associated with contamination remediation practices.

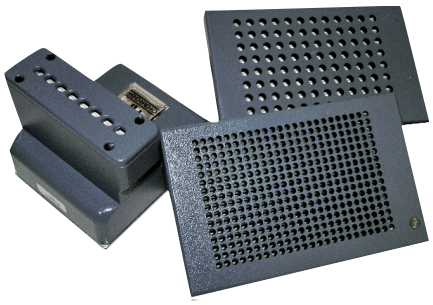
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Confidence TipCharger cleans better than any other washing technology - in most applications the TipCharger will clean to background, so there is no difference between plasma cleaning and a new tip.

Cost Benefit TipCharger can save up to 98% on the cost of the disposable tips and extends the life of fixed tips.

Speed Incorporating the TipCharger System can result in a time savings of 10-30 seconds for every microplate processed or rack of tips cleaned.

Convenience Clear away the clutter and save time: Integrating the TipCharger System eliminates the need to store cases of new pipette tips and dispose of racks of hazardous used tips.



TipCharger Plasma Cleaning Stations
Available in 8, 96 and 384 channel versions

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About the TipCharger™

The TipCharger Cleaning System replaces existing wash stations and easily integrates with most existing and new automation platforms. The system utilizes a low temperature, atmospheric pressure plasma process that cleans metal and plastic pipette tips and pin tools. Treated surfaces are clean, dry and have uniform surface properties.

The TipCharger cleaning process reduces the incidence of micro-bubble formation and other random surface effects that degrade liquid handling precision and accuracy, even with new disposable tips.

IonField Systems' TipCharger improves the reproducibility of process results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The overall result is increased confidence in results and a more effective and productive laboratory operation.

About IonField Systems™

IonField Systems is an advanced technology company focused on the development of state-of-the-art pipette tip cleaning for life science laboratory research applications. IonField Systems provides on-site technical support services to assist laboratories in rapidly integrating the system into day-to-day operations.