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White Paper

The 96-well Shuttle® System – Nucleofection® of Cell Lines and Primary Cells in an Automated Framework

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Screening of siRNA or cDNA libraries requires an automated high throughput transfection process. Besides mere throughput considerations, the main drivers for automation are the standardization and robustness of the process. The Nucleofector® Technology in general has proven to deliver results of the highest statistical accuracy. However, in an automated transfection framework, the consistant physiological state of the cells during the whole process chain is most critical. Stability of all cell parameters is the key to statistically relevant data and valid results. Therefore, we analyzed the stability of transfection results of different cell types during a time span typically needed to screen a focused siRNA library using Nucleofection®. The transfection results generated demonstrate the robustness of the automated Nucleofection® Process.

Introduction

A robust process from cell dispensing to analysis is an indispensable basis for automated transfection. The quality and the behavior of the cells have to remain stable during the time span needed to complete the whole workflow. To reduce the number of parameters influencing the final result, the cells are typically introduced into the process in one single batch. One critical issue in doing so is the stability of the cells in bulk solution prior to mixing with substrate and Nucleofection®. Screening a focused siRNA library targeting roughly 800 genes (pools in triplicates or three different siRNAs per gene) requires a process time of approximately 3 hours. To assess cell behavior, we monitored the transfection efficiency (pmaxGFP® Vector) and viability of different cells over a process time of 4 hours.

Nucleofection® of HUVEC and Human T Cells

HUVEC (human umbilical vein endothelial cells), and unstimulated and stimulated human T cells were resuspended in the appropriate 96-well Nucleofector® Solution and supplied in one batch. Samples from this batch were submitted to 96-well Nucleofection® directly and after 4 hours of pre-incubation. Transfection efficiency and the percentage of viable cells were analyzed 24 hours after transfection (see Figure 1). The largest variation monitored was an increase in transfection efficiency of ~20% for the unstimulated T cells. All other parameters show low CVs of 1 – 8% (x-mean, number of transfected cells, number of viable cells). The 3 cell types analyzed can therefore be handled in an automated setup using the standard Amaxa® Optimized Protocol.

Developing Automation Optimized Protocols for Cell Lines

For cell lines, which did not meet the self imposed criteria using the standard Amaxa® Optimized Protocol (Neuro2a, HeLaS3, Jurkat), special automation protocols and kits have been developed. Unlike the standard protocols, the automation protocols are based on two 96-well Nucleofector® Solutions which have to be mixed prior to the experiment. In general, the cells were supplied in one batch. Samples from the batches were submitted to Nucleofection® directly and after 2, 4 and 6 hours (alt.: 1, 2, 4 hours) of pre-incubation. After cultivating the cells for 24 hours, transfection efficiency and the percentage of viable cells were analyzed. The results (Figures 2 – 4) demonstrate that transfection efficiency and viability of the cells remains constant over 4 hours (Jurkat: 6 hours).

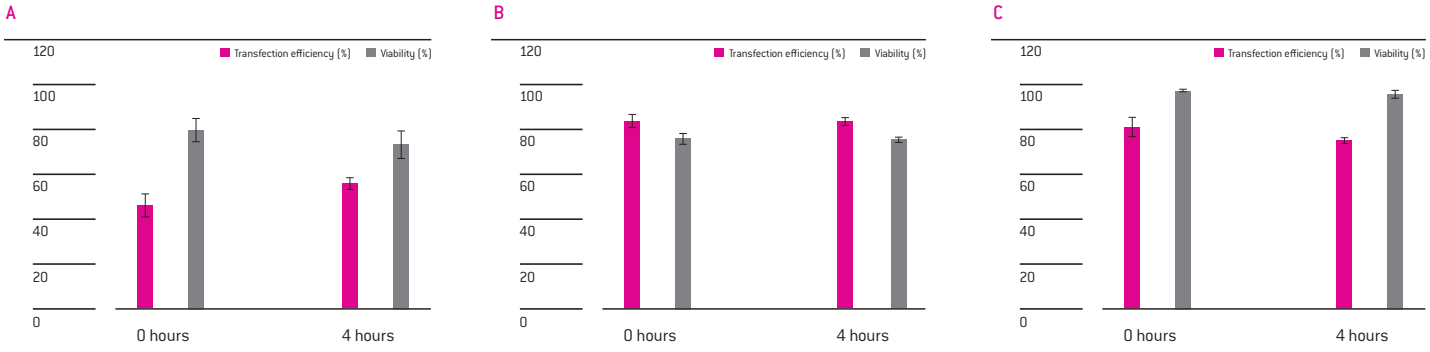

Figure 1: Influence of pre-incubation using standard 96-well Nucleofection® Protocols. Human unstimulated T cells (A), stimulated T cells (B) or HUVEC cells (C) were pre-incubated for 0 or 4 hours in 96-well Nucleofector® Solution and then transfected with 1 µg pmaxGFP® Vector according to their standard 96-well protocol. 48 hours post Nucleofection®, the cells were analyzed on a BD FACSCalibur™ (BD Biosciences) with HTS option. Cell viability was determined using PI-staining

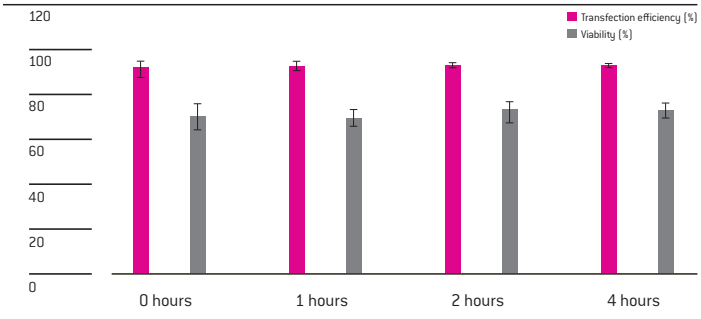

Figure 2: Transfection efficiency and viability of HeLaS3 after 0, 1, 2 and 4 hours of pre-incubation in 96-well Nucleofector® Solution. The cells were transfected with Program 96-DS-150 and 1 µg of pmaxGFP® Vector. 48 hours post Nucleofection®, the cells were analyzed on a BD FACSCalibur™ (BD Biosciences) with HTS option. Cell viability was determined using PI-staining.

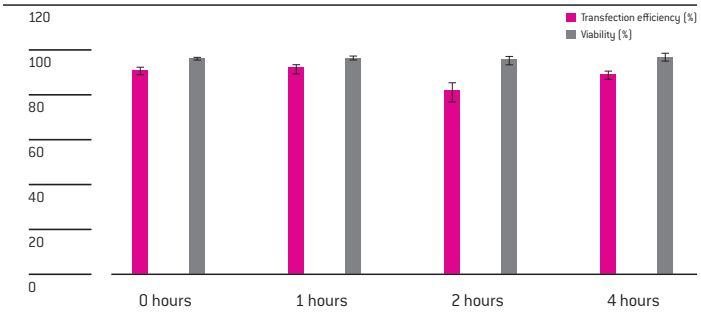

Figure 3: Transfection efficiency and viability of Neuro2a cells after 0, 1, 2 and 4 hours of pre-incubation in 96-well Nucleofector® Solution. The cells were transfected with 96-well Nucleofector® Program 96-DS-137 and 1 µg of pmaxGFP® Vector. 48 hours post Nucleofection®, the cells were analyzed on a BD FACSCalibur™ (BD Biosciences) with HTS option. Cell viability was determined using PI-staining.

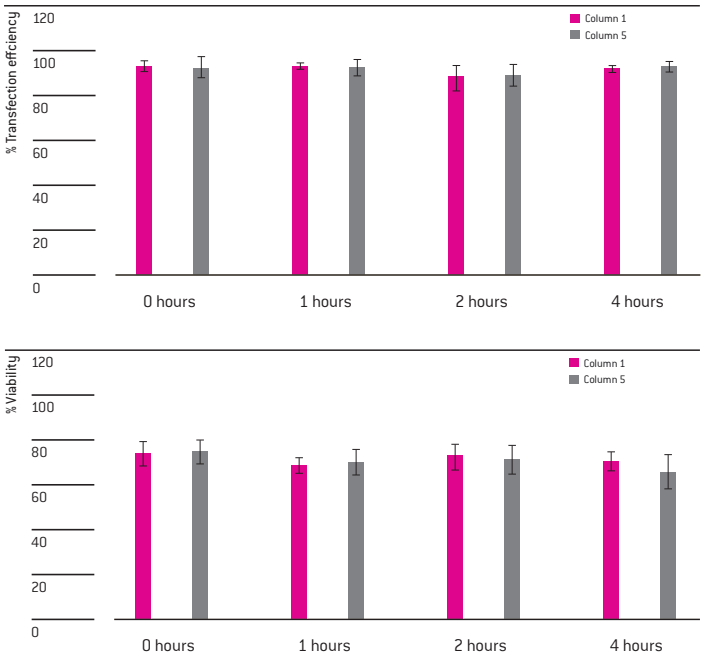

Figure 4: Transfection efficiency and viability of Jurkat cells after 0, 2, 4 and 6 hours of pre-incubation in 96-well Nucleofector® Solution. Jurkat cells (clone E6-1, ATCC® TIB 152™) were transfected with 96-well Nucleofector® Program 96-CM-137 and 1 µg of pmaxGFP® Vector. 48 hours post Nucleofection®, cells were analyzed on a BDFACSCalibur™ (BD Biosciences) with HTS option. Cell viability was determined using the PI-staining. Data from two columns (1,5) of a Nucleocuvette® Plate are shown.

Accuracy and Reproducibility

For Jurkat cells, we analyzed the variation related to the different pre-incubation times and in addition the intra-plate variation between the first and the last module of the Nucleocuvette® Plate. Analysis of the parameter variations during the time course yields a CV of ~6% for the viability and less than 2% for the transfection efficiency. The comparison between individual modules revealed a CV of ~3% for the viability and less than 1% for the transfection efficiency. That means using the automation protocol a batch of Jurkat cells can be used for a period of 6 hours without causing significant variations or drifts of the experimental parameter values.

Automated siRNA Mediated Knockdown in Jurkat Cells

To further validate the new automation protocol for Jurkat cells and to test its suitability for RNAi approaches, the siRNA mediated knockdown of vimentin in Jurkat cells was analyzed against the pre-incubation times (0, 2, 4 hours). For vimentin knockdown siGENOME® SMARTpool® siRNA (Thermo Fisher Scientific, Dharmacon Products) was used. 2.5 x 10^5 Jurkat cells/sample were resuspended in 96-well Nucleofector® Solution and mixed with a vimentin siRNA stock solution immediately prior to Nucleofection®. RNAi mediated gene silencing of vimentin was measured 24 hours post-transfection by the QuantiGene® Branched-DNA Assay (Panomics) (see Figure 5).

The data clearly emphasize the suitability of the automation protocol for RNAi experiments. In addition, detailed siRNA titration experiments were performed at a pre-incubation time of 4 hours. The resulting siRNA dose response curve is shown in Figure 6.

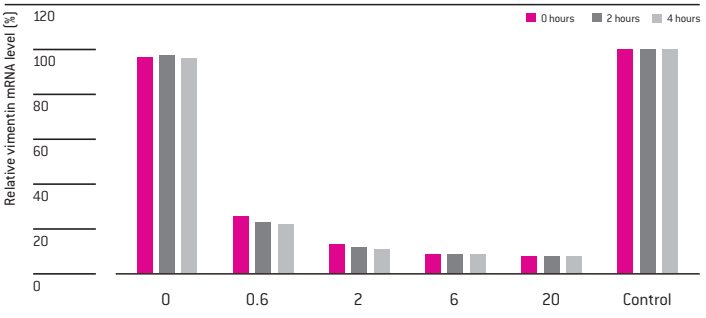

Figure 5: Time Dependence – siRNA mediated knockdown of vimentin in Jurkat cells. After different pre-incubation times (0, 2 and 4 hours) in 96-well Nucleofector® Solution, Jurkat cells (clone E6-1, ATCC® TIB 152™) (2.5 x 10^5 cells per sample) were transfected with siGENOME® SMARTpool® siRNA Reagent directed against endogenous vimentin. 24 hours post-transfection, vimentin mRNA levels were analyzed by the QuantiGene® Branched-DNA Assay. Data are plotted as relative expression levels compared to samples with control siRNA.
(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products.)

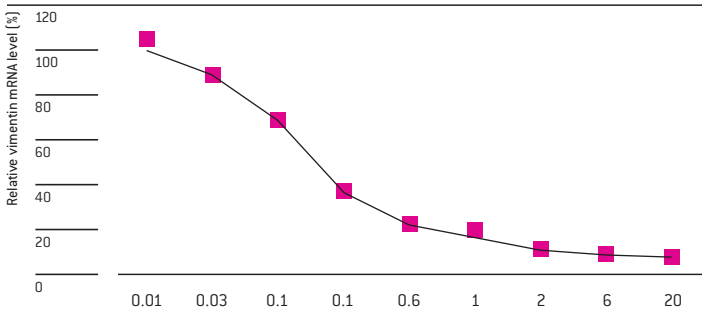

Figure 6: Concentration Dependence – siRNA mediated knockdown of vimentin in Jurkat cells. Jurkat cells (clone E6-1, ATCC® TIB 152™) (2.5 x 10^5 cells per sample) were transfected with siGENOME® SMARTpool® siRNA Reagent directed against endogenous vimentin after 4 hours pre-incubation in 96-well Nucleofector® Solution. 24 hours post transfection, vimentin mRNA levels were analyzed by the QuantiGene® Branched-DNA Assay. Data are plotted as relative expression levels compared to samples transfected with control siRNA (controls not shown).
(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products.)

Summary

Analysis of the data clearly demonstrates that T cells and HUVEC can be handled in an automated setup using the corresponding standard Amaxa® Optimized Protocol. The resulting parameter values, such as viability and transfection efficiency, showed sufficient stability during the incubation time of 4 hours.
For Jurkat, HeLaS3 and Neuro2a cells, new protocols were developed allowing for process times of up to 4 hours (Jurkat: 6 hours). Statistical data analysis revealed extremely low standard deviations for the transfection of DNA. These findings were supported by siRNA knockdown experiments demonstrating the suitability of the newly developed protocol for automated RNAi approaches. As shown here, the Nucleofector® 96-well Shuttle® System can efficiently be used in a fully automated transfection setup. That means siRNA- or cDNA library screening based approaches e.g., for target identification with a clear demand for high throughput transfection can now be performed in primary and other difficult-to-transfect cells.

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WTA-1005_2009-01-28