Highly Efficient High-Throughput Transfection of Hard-to-Transfect Cell Types for RNAi Based Applications

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Abstract and Introduction

Successful RNAi experiments and large-scale siRNA screens require efficient delivery of highly functional and specific nucleic acids including siRNA oligonucleotides, shRNA vectors, or micro RNAs into an appropriate cell system. Cell types relevant for immunological research, such as primary T cells and several suspension cell lines, are poorly accessible using reagent-based transfection approaches.

Nucleofection® is an established method for the effective, non-viral transfection of nucleic acids into difficult-to-transfect cell types including primary cells. With the expansion of the technology to a 96-well format (Fig. 1) high-throughput applications, such as siRNA or shRNA library screenings can now be performed in these cell types rendering target validation and identification possible in cell types highly relevant for medical research. Integration of the 96-well Shuttle® into a liquid handling workstation allows for a fully automated screening approach.

Using the powerful combination of highly functional Dharmacon si*GENOME*® siRNA reagents and 96-well nucleofection®, we here present data showing the efficient siRNA-mediated gene knockdown in various cell lines and primary cells. Our studies focus on Jurkat T-cells which are derived from a human acute T-cell leukemia and are extensively used in the study of T-cell signaling and cancer drug development.

Results and Conclusions

- Highly efficient siRNA-mediated knock-down using the Nucleofector® 96-well Shuttle® System can be performed in different primary and hard-to transfect cell types (Fig 3). The same siRNA concentration results in different knock-down efficiencies depending on cell type, suggesting the need of titration of every siRNA for every cell type (Fig. 2).
- Jurkat cells are hard-to-transfect with DNA or siRNA using lipid-based reagents while Nucleofection® achieves up to 80% transfection efficiency and strong target gene knockdown with low siRNA amounts (Fig. 3).
- Targeted knock-down GAPDH mRNA did not alter cell viability (Fig. 4a). In contrast knock-down of polo-like kinase 1 (PLK-1) mRNA results in increased apoptotic activity and cell death in dose-dependent manner (Fig. 4). This confirms effects described in the literature.
- The established assay system can now be used for screening approaches targeting pro- and anti-apoptotic pathways as they provide good screening windows (Z'-factor > 0.5) in Jurkat cells.

Figure 1: Nucleofector® 96-well Shuttle®

The 96-well Shuttle® works as an computer-controlled add-on to the Nucleofector® II Device **(A)**. It enables the Nucleofection® of up to 96 samples at once in a convenient standard 96-well format. Cells can be transfected in small volumes (20 µl per sample) which also leads to a reduction in required cell numbers ($2x10^4$ to $1x10^6$). In addition the system is capable of applying a different program to each individual well. It can therefore be used for a wide spectrum of applications from optimization of Nucleofection® conditions to reproducible high-throughput Nucleofection® with a single program for the complete plate. It is capable of integration into liquid handling workstations **(B)** allowing for an easy and straight-forward workflow for automated siRNA screenings **(C)**.

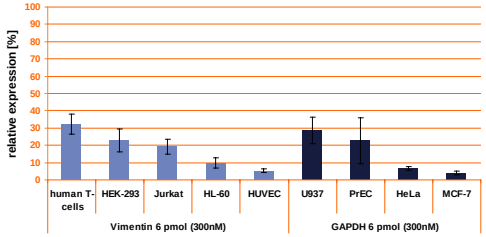

Figure 2: siRNA mediated knockdown in different cell types after 96-well nucleofection®

Various cell types were transfected with 6 pmol (300 nM), *SMART*pool® siRNA reagents (Dharmacon) against vimentin or GAPDH, respectively. 24 h post transfection endogenous mRNA level were determined by the QuantiGene® branched-DNA assay (Panomics) and normalized to si*CONTROL*® non-targeting siRNA *SMART*pool®.

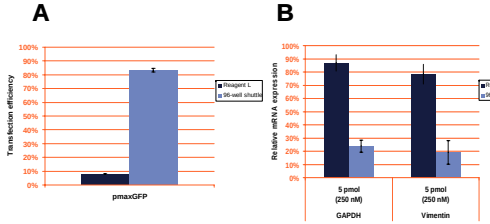

Figure 3: Compared to lipid-based transfection Nucleofection® results in high transfection efficiencies and mRNA knockdown in hard-to-transfect Jurkat T-Cells

(A) Jurkat clone E6-1 (ATCC® TIB-152™) was transfected with the plasmid pmaxGFP® (1µg for Nucleofection®; 1.6 µg for Lipofection) and analyzed after 24h by FACS. Transfected cells show high transfection efficiency of > 80%.
(B) Targeted knock-down of endogenous GAPDH and Vimentin mRNA with 5 pmol *SMART*pool® siRNA reagents (Dharmacon). Jurkat cells were analyzed 24 h post transfection by the QuantiGene® branched-DNA assay (Panomics) and normalized to si*CONTROL*® Non-targeting siRNA *SMART*pool®. With Nucleofection® we reached > 75% reduction of mRNA levels.

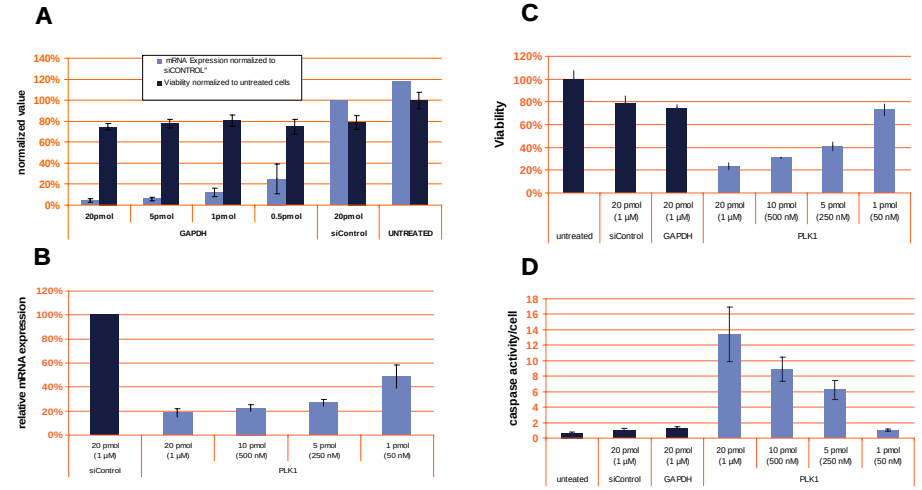

Figure 4: Targeted knock-down of PLK-1 mRNA results in increased Caspase 3 activity and cell death

Jurkat clone E6-1 (ATCC® TIB-152™) was tansfected with *SMART*pool® siRNA reagents targeting GAPDH or PLK-1, respectively (Dharmacon) using the 96-well Shuttle®.
(A) Jurkat cells show a dose dependent decrease in GAPDH mRNA levels 24 h post transfection, Viability of the cells was unimpaired. **(B)** Jurkat cells show a dose-dependent decrease in transcript levels 24 h post transfection. mRNA levels were measured with QuantiGene® branched-DNA assay (Panomics) 24h post Nucleofection®. Cell viability **(C)** and caspase 3/7 **(D)** activity were measured at 48 h post Nucleofection® with the CellTiter Blue™ assay (Promega) and the Apo-ONE® assay (Promega), respectively. 5 pmol (250 nM) of siRNA is sufficient to silence ~75% of PLK-1 mRNA and demonstrate an increase of apoptotic activity and cell death. Cells transfected with non-related GAPDH siRNA (20 pmol, 1 µM) showed similar viability and cellular caspase activity as compared to si*CONTROL*® treated cells.