Critical factors for successful RNAi experiments in primary cells and hard to transfect cell lines

Markus Zumbansen[1], Nicole Spottke[1], Sheila Offizier[1], Allison St. Amand[2], Devin Leake[2], Ludger Altrogge[1], Meike Weigel[1], Sandra Domzalski[1], Dietmar Lenz, and Herbert Müller-Hartmann[1]

[1] amaxa AG, Cologne, Germany, D-50829
[2] Dharmacon Inc., Thermo Fisher Scientific, Lafayette, CO 80026

Abstract

The amaxa Nucleofector® Technology is a well established method for effective, non-viral transfection of any nucleic acid substrate into hard-to-transfect cells, especially suspension and primary cells. With the Nucleofector® 96-well Shuttle® system high throughput applications such as siRNA-library screenings have become amenable for the first time in these cell types. This renders target validation and identification possible in cell types highly relevant for biomedical research.
Here we discuss critical factors for setting up RNAi experiments using Nucleofection®. We focused on relevant cells poorly accessible with reagent-based transfection methods and present data showing the efficient RNAi-mediated gene knockdown in Jurkat cells T-cells.

Introduction

siRNA-mediated gene knockdown is a powerful tool that has been used to identify gene function and elucidate biological pathways. Successful siRNA experiments involving knockdown of individual genes or collections of gene targets require efficient delivery of highly functional and specific siRNA molecules into appropriate cells. While reagent-mediated transfection is a common approach for siRNA delivery, many cell types, including suspension cell lines and primary cells, are not compatible with this technology.
With the combination of Dharmacon's highly specific siRNA reagents and amaxa's transfection technology we are able to perform RNAi experiments in highly relevant cell types. The Nucleofector® 96-well Shuttle® System (Fig. 2) now expands this opportunity to a high-throughput format.
Using the guidelines shown in Fig. 1 we set up experiments showing the efficient knock-down of several genes in primary and hard-to-transfect cell types and developed strategies for the efficient impairment of anti- and proapoptotic signalling pathways in Jurkat T-cells.
Jurkat cells are derived from a human acute T-cell leukemia and are used extensively in the study of T-cell signaling and cancer drug development.

Results and Conclusions

- Jurkat cells are hard to transfect with DNA and siRNA using lipid-based transfection methods (Fig. 3)
- Knock-down of target genes is siRNA concentration-dependent (Fig. 4A, 5A)
- Targeted knock-down of PLK-1 mRNA results in increased Caspase 3 activity and cell death (Fig. 4)
- Targeted knock-down of FAS mRNA protects from FAS-ligand induced apoptosis (Fig.5)
- Established assay systems can now be used for screening approaches targeting pro- and anti-apoptotic pathways as they provide good screening windows (Z'-factor > 0.2) in Jurkat cells.

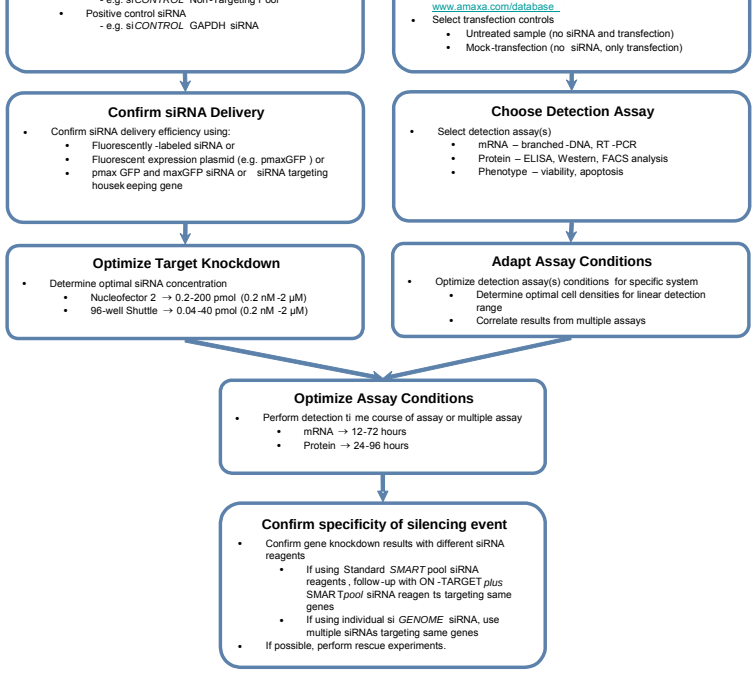

Figure 1: Guideline for setting up successful siRNA experiments

Figure 2: The Nucleofector® 96-well Shuttle® System

The Nucleofector® 96-well Shuttle® System enables the Nucleofection® of up to 96 samples in one experiment 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. 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. The system works as an add-on to the Nucleofector II Device.

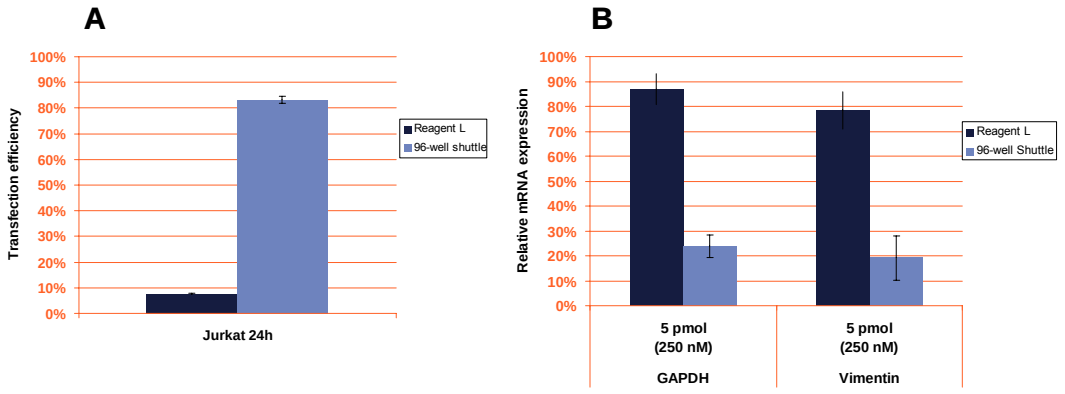

Figure 3: Hard-to-transfect Jurkat T-Cells show very low RNA interference effects using lipid based methods

(A) Jurkat clone E6-1 (ATCC® TIB-152™) were transfected with the plasmid pmaxGFP® 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). 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.

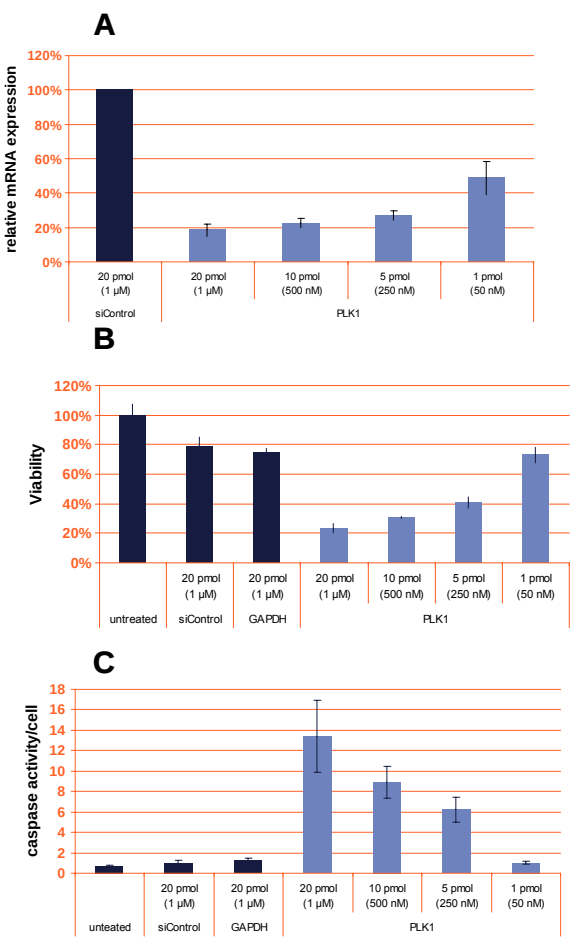

Figure 4: Targeted knock-down of PLK-1 mRNA results in increased Caspase 3 activity and cell death

Polo-like kinase 1 (PLK-1) is a key regulator of mitotic progression in mammalian cells and the knockdown of PLK-1 is known to induce apoptosis in cancer cells. As such, downregulation of PLK-1 is expected to decrease cell viability and increase caspase 3/7 activity. Jurkat clone E6-1 (ATCC® TIB-152™) was transfected with a *SMART*pool® siRNA reagent targeting PLK-1 (Dharmacon) using the 96-well Shuttle®.
(A) Jurkat cells show a dose-dependent decrease in transcript levels 24h post transfection. PLK mRNA levels were measured with QuantiGene® branched-DNA assay (Panomics).
Cell viability (**B**) and caspase 3/7 (**C**) 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.

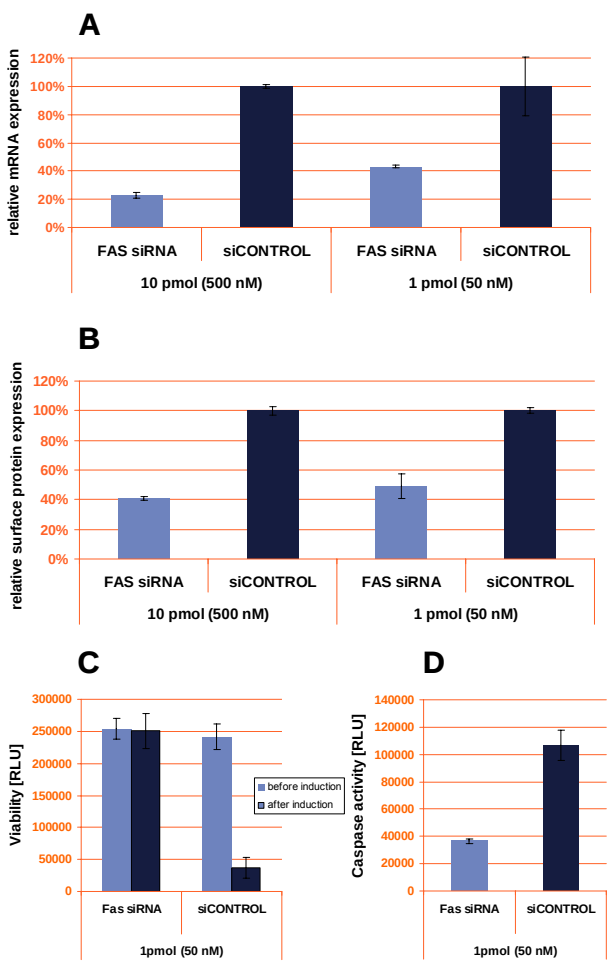

Figure 5: Targeted knock-down of FAS mRNA results in decreased sensitivity to FAS-induced apoptosis

FAS-induced apoptosis is a key apoptosis pathway in T-lymphocytes. FAS is a member of the TNF-Receptor superfamily and is highly expressed on the surface of immune cells. It contributes to peripheral depletion of lymphoid cells, maintenance of self-tolerance, down-regulation of the immune response and the homeostasis of the immune system.
Jurkat clone E6-1 (ATCC® TIB-152™) was transfected with a *SMART*pool® siRNA reagent targeting FAS (Dharmacon) using the 96-well Shuttle®.
(A) FAS mRNA levels were measured with QuantiGene® branched-DNA assay (Panomics) 24h post Nucleofection®.
(B) FAS surface protein levels were measured by FACS after antibody staining of extracellular FAS protein 48h post transfection.
Protein and mRNA level decrease dose-dependently.
48 h post transfection cells were stimulated for 4h with recombinant FAS-L to induce apoptosis. **(C)** Cell viability and Caspase 3 (**D**) activation was measured with CellTiter Glo® assay (Promega) and Caspase Glo™ assay (Promega), respectively.
About 50% reduction of the FAS protein protects Jurkat cells from FAS-ligand induced Caspase 3 activation and cell death.