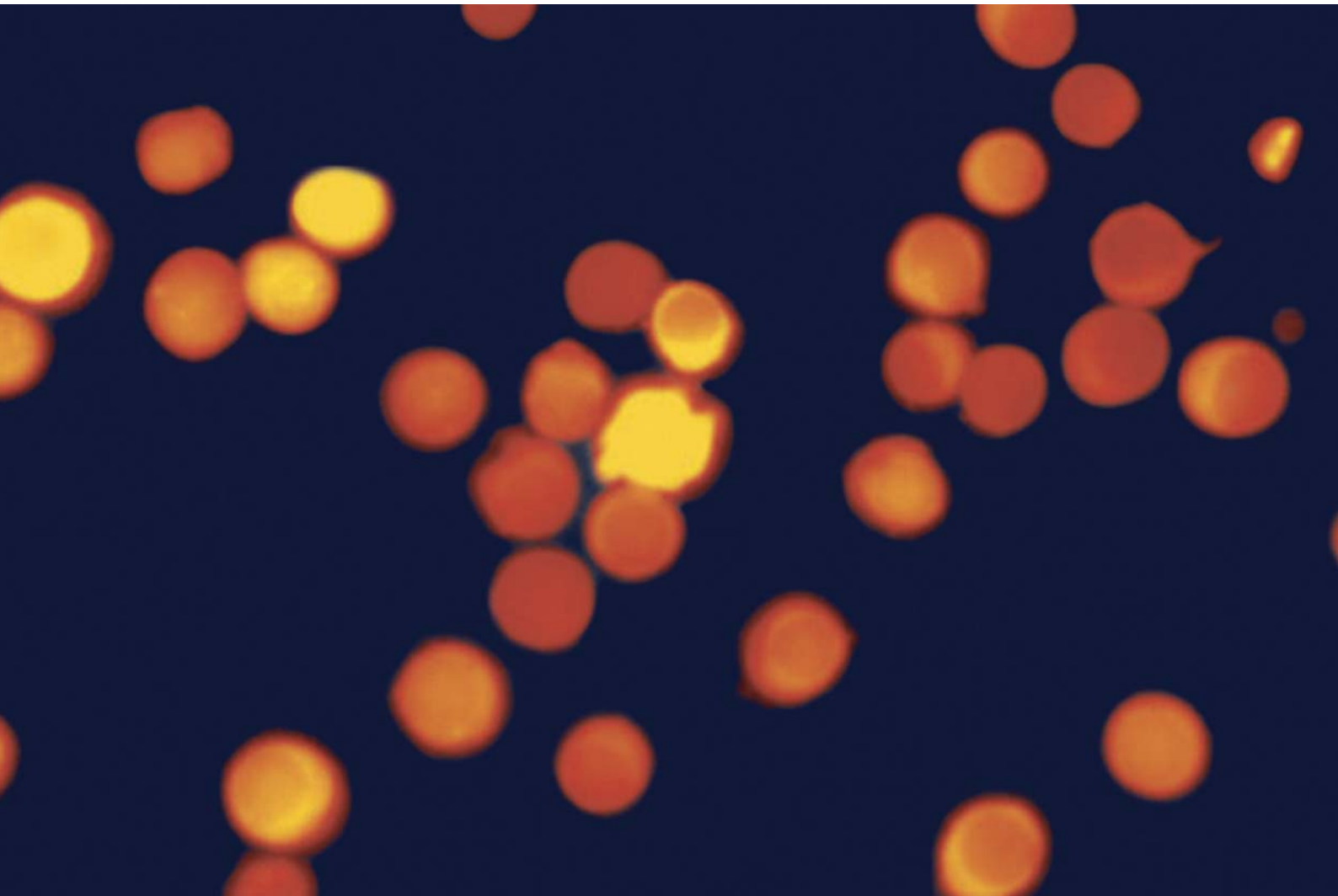


Amaxa[®] Nucleofector[®] Technology and RNAi



Nucleofection® of RNAi Substrates

Gene silencing by RNA interference (RNAi) emerged as a powerful technology for assessing gene function in mammalian cells and has become a valuable tool in functional genomics, target discovery and validation. The two most critical factors determining the effectiveness of RNAi experiments are the ability of the siRNA sequence to specifically silence the target mRNA, and the efficiency with which siRNA (or the siRNA-expressing plasmid) can be transfected into the cells of interest. With over 400 RNAi publications, Nucleofection® has proven to be the delivery method of choice for any RNAi substrate. The unique versatility of the technology allows for a wide range of research applications from basic research studies such as analyzing the mechanisms of microRNA, to functional studies via RNAi-mediated gene knockdown. In addition, the Nucleofector® 96-well Shuttle® System now offers the possibility to perform RNAi library screenings using siRNA or shRNA in more relevant cell types such as primary cells and difficult-to-transfect cell lines such as Jurkats.

■ RNAi using the Amaxa® Nucleofector® Technology adds:

- Substrate versatility – deliver siRNA oligos, DNA vectors, or any combination using the same protocol
- Up to 99% delivery – even to the most physiologically relevant primary cells
- Excellent functionality – without residual reagent toxicity or off-target effects
- Reproducible transfection – using single samples or in 96-well plate format
- Ease-of-use – ready-to-use protocols eliminate optimization

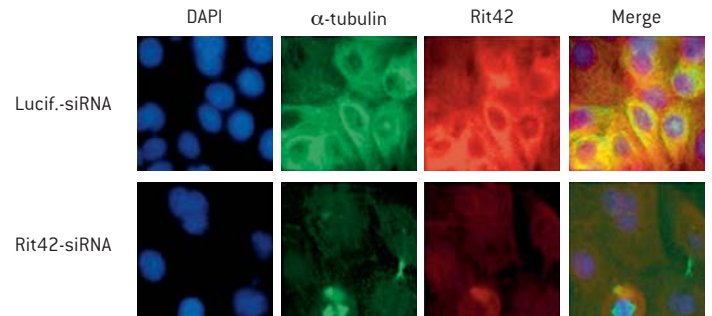


Figure 1. siRNA in primary human epithelial cells — efficient downregulation of Rit42 leads to decreased accumulation of acetylated α -tubulin and disrupts spindle fiber formation. Cells were transfected with Rit42 or control (luciferase) siRNA. 48 hours after transfection, cells were co-stained with DAPI (blue), anti- α -tubulin monoclonal antibody (green), and anti-Rit42 polyclonal antibodies (red). Merged images are shown in the last column.
[Courtesy of Kyung-tae Kim, Hematology and Oncology Division, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA.]

Proven Performance

The unique combination of optimized Nucleofector® Protocols and cell type specific Nucleofector® Solutions allows efficient transfection of even non-dividing cells, such as neurons.

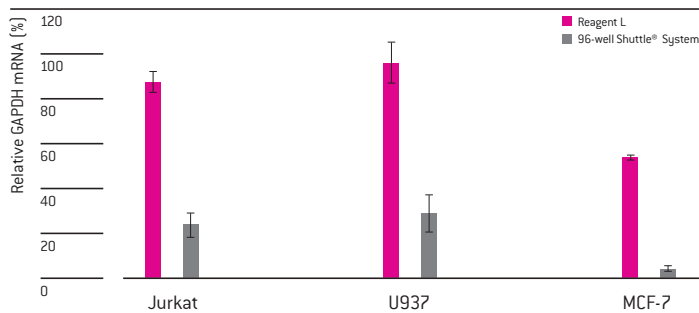


Figure 2. Nucleofection® outperforms lipofection for effective GAPDH mRNA knockdown in difficult-to-transfect cell types. Cells were transfected with 5 pmol SMARTpool® siRNA Reagent targeting GAPDH (Dharmacon) using the 96-well Shuttle System (according to the respective Amaxa® Optimized Protocol) or reagent L (after titration of optimal reagent amount). Negative control samples were transfected with 5 pmol siGENOME® Non-targeting siRNA #1 (Dharmacon). 24 hours post-transfection, cells were analyzed for mRNA expression by QuantiGene® Branched DNA Assay (Panomics). GAPDH mRNA levels were normalized to cyclophilin B and to negative siRNA control samples.

(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products).

Now Available for 1 to 96 Transfections per Experiment

The Nucleofector® 96-well Shuttle® System opens a new dimension for RNAi applications.

10⁴ to 10⁶ cells per well can now be transfected in a high-throughput format. Thereby, applications such as target identification and validation can now for the first time be performed in primary cells, hence drastically increasing result significance.

Furthermore, the Nucleofector® 96-well Shuttle® System can be used for transfection of virtually any type of cell line such as commonly-used cells or difficult-to-transfect cell lines. High transfection efficiencies combined with high cell viability and low well-to-well variances make it the ideal tool for siRNA and shRNA library screening.

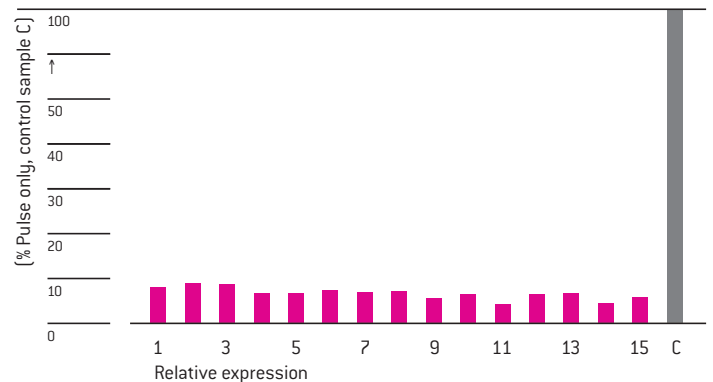


Figure 3. siRNA-mediated depletion of vimentin in human T cells. Knockdown on mRNA level measured by qRT-PCR. 15 samples compared to control [C] set to 100%.

(Data kindly provided by C. Merz, Bayer Schering Pharma AG, Berlin.)

Efficient Delivery and Knockdown Using siRNA Oligonucleotides

More than 300 publications in top ranking journals have reported successful target gene knockdown using Nucleofection® for delivery of siRNA oligonucleotides. The Nucleofector® Technology enables researchers to perform siRNA experiments in primary cells and difficult-to-transfect cell lines such as suspension cells. Depending on cell type and target, efficient knockdown can be observed at siRNA concentrations lower than 10 nM.

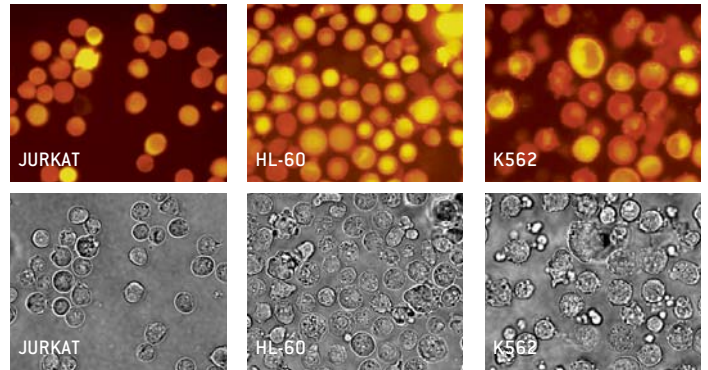


Figure 4. Up to 99% delivery of siRNA duplexes. Different suspension cell lines were transfected using the appropriate Nucleofector® Kit and rhodamine-labeled siRNA duplexes. 3 hours after delivery, cells were analyzed by light and fluorescence microscopy.

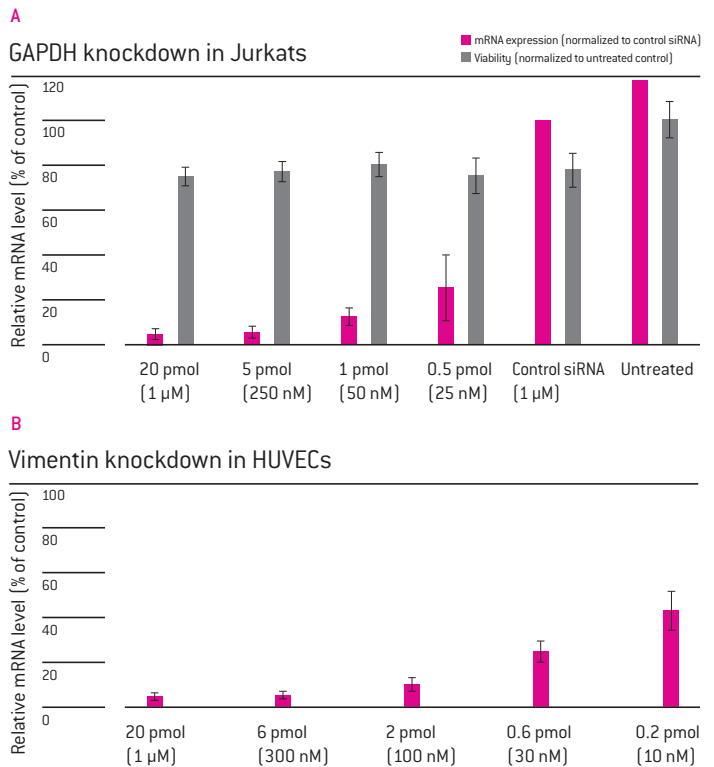


Figure 5. Efficient mRNA downregulation with less than 1 pmol siRNA in suspension cell lines or primary cells. Using the 96-well Shuttle® System, Jurkat clone E6-1 [ATCC® TIB-152™] (A) or HUVECs (B) were transfected with various amounts of SMARTpool® siRNA Reagents (Dharmacon) targeting GAPDH (A) or vimentin (B). The control sample is a Dharmacon siGENOME® Non-Targeting siRNA pool, while "untreated" samples are cells that received neither siRNA nor Nucleofection®. mRNA levels were analyzed 24 hours post-transfection by the QuantiGene® Branched-DNA Assay (Panomics) and normalized to negative siRNA control sample. Cell viability (only shown in A) was determined 48 hours post-transfection by the CellTiter-Blue® Assay (Promega) and normalized to untreated cells. [Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products].

Reliable siRNA Screening in Difficult-to-Transfect Cell Types

Most siRNA library screens have been limited to easy-to-transfect adherent cell lines. With the Nucleofector® 96-well Shuttle® System such screens are now possible in more biologically relevant cell types that are often difficult to transfect such as suspension cell lines or primary cells.

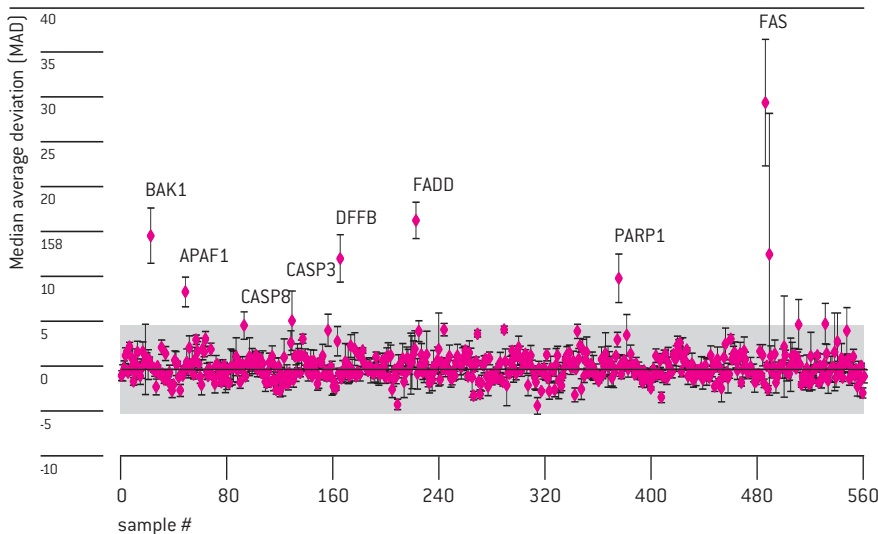


Figure 6. Hits of primary screen for genes involved in FAS-induced apoptosis in Jurkat T cells. Jurkat cells (clone E6-1, ATCC® TIB-152™) were transfected in three independent experiments with the Human ON-TARGETplus® siRNA Library – Apoptosis (targeting 558 genes). Apoptosis was induced by adding 10 ng FAS-L to the cells 48 hours post Nucleofection®. Cell viability was analyzed after 2 hours. The mean of robust Z-scores of cell viability measures was calculated for three independent experiments. Targets with an |MAD| of at least 5 are marked as potential hits. [Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products].

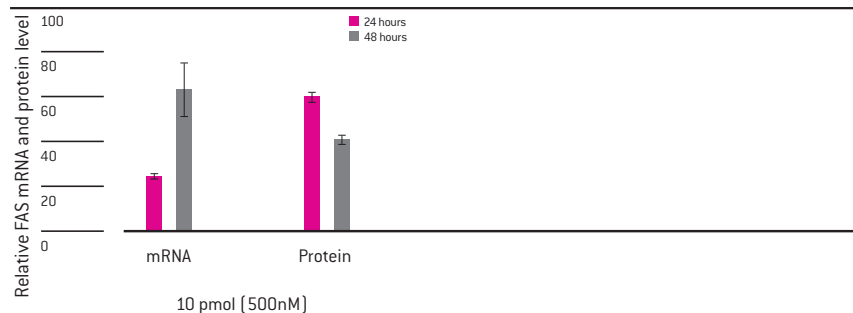


Figure 7. Different kinetics for effective knockdown of mRNA or protein. Using the 96-well Shuttle® System, Jurkat clone E6-1 (ATCC® TIB-152™) was transfected with 10 pmol (500 nM) of SMARTpool® siRNA Reagent (Dharmacon) targeting FAS. The control sample (not shown) is a Dharmacon siGENOME® Non-Targeting siRNA pool. 24 and 48 hours post-transfection mRNA levels were analyzed by the QuantiGene® Branched-DNA Assay (Panomics) and protein levels were determined by flow cytometry after antibody staining. Values were normalized to negative siRNA control sample. [Data generated in collaboration with Thermo Fisher Scientific, Dharmacon products].

Supplementary Information

White Paper	Critical Parameters for Successful siRNA Experiments
White Paper	siRNA Library Screening in HUVEC and Jurkat Cells

Related Products

MycoAlert® Mycoplasma Detection Kits

www.lonza.com/mycoalert

ViaLight® Plus Cell Proliferation and Cytotoxicity BioAssay Kits

www.lonza.com/vialight

ToxiLight® Non-Destructive Cytotoxicity BioAssay Kits

www.lonza.com/toxilight

ApoGlow® Rapid Apoptosis Screening Kit

www.lonza.com/apoglow

StellARay™ Gene Expression System

www.lonza.com/arrays

Efficient Delivery and Knockdown Using shRNA Vectors

In addition, or as an alternative to siRNA duplexes, shRNA vectors are frequently used for RNAi applications. Vectors expressing shRNA offer an advantage over siRNA duplexes in that they permit a longer transient knockdown and can be stably integrated. Nucleofection® offers the possibility to easily switch from siRNA duplexes to shRNA vectors and back.

The ability of the Nucleofector® Technology to non-virally transfect a wide range of cells with high efficiencies, significantly increases the range of cell types in which plasmid-based RNAi systems can be used, such as primary neurons and Jurkats. This makes it an ideal alternative to circumvent laborious viral transduction methods.

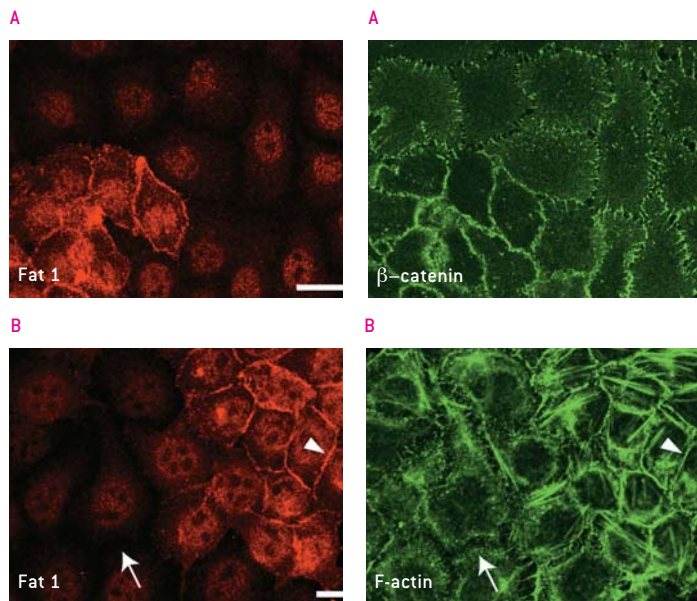


Figure 8. Efficient knockdown using shRNA vectors. PAM212 cells were transfected with Fat1 RNAi plasmid and doubly immunostained for Fat1 (red) and (A) beta-catenin or (B) F-actin (green) 2 days after transfection. More than 95% of the cells were transfected and showed a significant reduction in the level of Fat1 protein. Transfected areas lacking Fat1 show looser cell-cell associations (A) and a disrupted actin organisation (B, arrows; arrowheads: cell junctions in Fat1-positive cells). [Tanoue *et al.*, reproduced from The Journal of Cell Biology, 2004, 165(4), 517 by copyright permission of The Rockefeller University Press and by permission of the authors.]

Nucleofection® Covers All RNAi Related Approaches

MicroRNAs (miRNAs), endogenous, small, non-coding RNA molecules, are key regulators of gene expression at the level of translation. They are differentially expressed in tissues, critical in the development of organisms, involved in viral infection, and associated with oncogenesis. Their functionality is analyzed by overexpression using transfection of synthetic miRNAs or miRNA-expressing plasmids or by downregulation using transfection of miRNA inhibitors. The Nucleofector® Technology allows studying this class of genetic elements in difficult-to-transfect suspension cell lines and primary cells.

- More than 90% transfection efficiency for both, RNA oligonucleotides or DNA vectors even in suspension cell lines and primary cells
- 96-well Shuttle® System enabling miRNA library screening

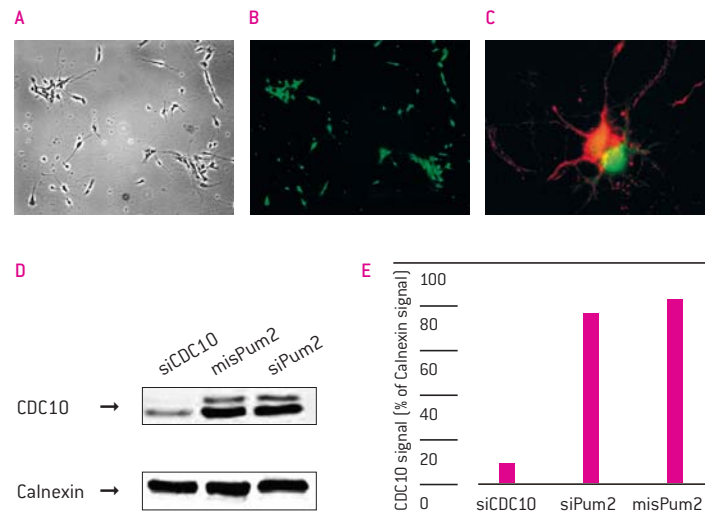


Figure 9. Gene silencing in primary neurons by transfection of an shRNA vector – quantitative downregulation of CDC10 protein. Rat hippocampal neurons [E17] were transfected with the shRNA vector pSuperior targeting CDC10 using the 96-well Shuttle® System. A and B: Efficient Nucleofection® of pSuperior is shown by eGFP expression after 1 day *in vitro*. C: Immunostaining of CDC10 (red fluorescence) after 4 days *in vitro* compared to untransfected cells (red). Western blot analysis (D) and quantification (E) of CDC10 downregulation. [Data courtesy of Prof. Kiebler, Medical University of Vienna, Vienna, Austria.]

Nucleofection® Passes the Co-Transfection Challenge

Some approaches require co-transfection of RNAi oligonucleotides together with DNA vectors, e.g., rescue experiments or labeling of transfected cells (transfection control). Nucleofection® overcomes the limitations of reagent-based methods which require differing conditions for different substrate types.

For more detailed information about Nucleofection® and RNAi, please see:

■ Overview	www.lonza.com/rnai
■ miRNA	www.lonza.com/mirnaA
■ siRNA	www.lonza.com/sirna
■ Screening	www.lonza.com/rnai-screening
■ shRNA	www.lonza.com/shrna

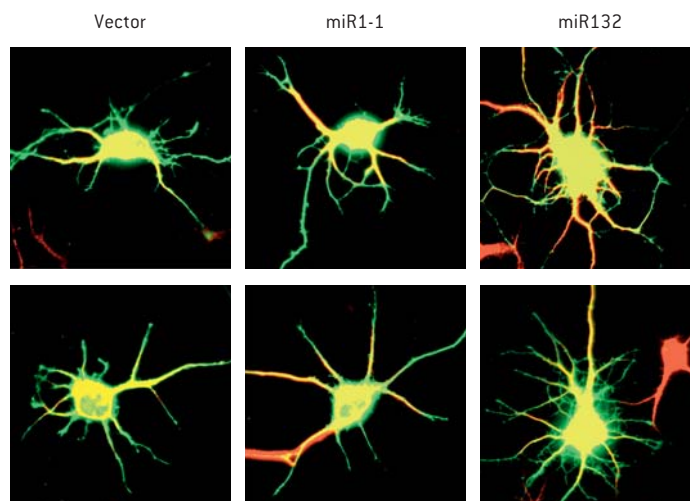


Figure 10. Co-transfection of neurons, maintenance of functionality – expression of miR132 induces neurite sprouting by targeting a protein that represses neurite outgrowth (p250GAP). Rat neonatal cortical neurons were transfected with a GFP reporter (green) and co-transfected with vector control, or expression constructs for premiR1-1 and premiR132 using Rat Neuron Nucleofector® Kit and Nucleofector® Device. Cells were immunostained for the neuronal marker MAP2 (red). Only cells transfected with premiR132 show neurite sprouting. [Vo *et al.*, reproduced from the Proc Natl Acad Sci USA, 102(45): 16429 by copyright of the National Academy of Science and by permission of the authors.]

Ordering Information

Cat. No.	Description	
AAD-1001S	Nucleofector® II Device	
AAM-1001S	96-well Shuttle® Device*	
	Standard and 96-well Nucleofector® Kits for Primary Cells**	For an up-to-date list of all standard and 96-well Nucleofector® Kits for Primary Cells please visit www.lonza.com/nucleofection-for-primary-cells
	Standard and 96-well Nucleofector® Kits for Cell Lines**	For an up-to-date list of all standard and 96-well Nucleofector® Kits for Cell Lines please visit www.lonza.com/nucleofection-for-cell-lines
VSC-1001	siRNA Test Kit**	For easy establishment of siRNA experiments. Contains 9 ml siRNA Suspension Buffer, 75 µg siRNA*** against maxGFP® Reporter Protein and 100 µg pmaxGFP® Vector.

* 96-well Shuttle® Device includes Laptop with Nucleofector® 96-well Shuttle® Software. For using the 96-well Shuttle® Device the Nucleofector® II Device is required.
 ** These kits contain a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as positive control with these Lonza products only. Any use of proprietary nucleic acid or fluorescent protein other than as positive control with this Lonza product is strictly prohibited. Use in any other application requires license from Evrogen. To obtain such a license, please contact Evrogen at license@evrogen.com.
 The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.
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