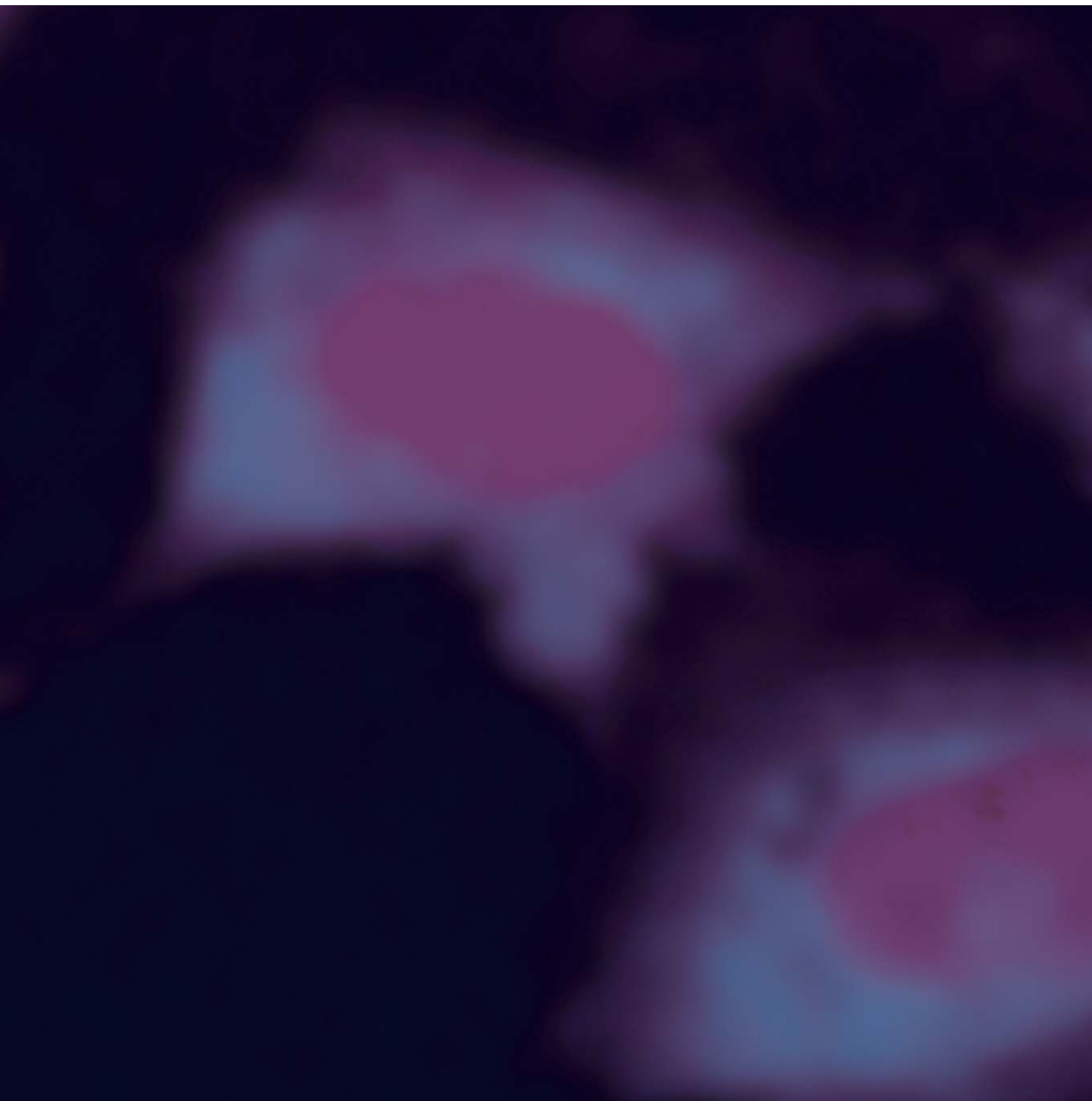
Lonza

Amaxa® Nucleofector® Technology



The Amaxa® Nucleofector® Technology

The application of systems biology and multidisciplinary approaches require that cells and model systems display *in vivo* like cellular functionality. This means that the future of cell transfection is in using primary cell types, and that transfecting these physiologically relevant cell types presents researchers with a series of technical challenges. The most physiologically relevant cell types are typically the most difficult to transfect using traditional methods. In contrast, when using relevant cell lines as model systems, the critical issues are to achieve reproducibly efficient transfection with high levels of viability while matching throughput capability with the number of transfections required at each project phase – from proof of concept, through to scale-up and screening-like approaches.

With Amaxa® Optimized Protocols for more than 200 primary cells and cell lines, Nucleofector® Technology is the technology of choice for leaders in the field of cell biology.

Nucleofector® Technology delivers:

Rapid and reliable transfection of primary cells
- Simply select the cell type-specific Nucleofector® Kit and Amaxa® Optimized Protocol for accelerated startup and deliver your substrate straight to the nuclei of even non-dividing primary cells

High efficiency transfection of cell lines and primary cells
- Get up to 99% transfection efficiency with the unparalleled viability and unaltered functionality that make for truly informative assay

Multiple savings in cell and reagent useage
- 20 µl transfection volumes in 96-well format and specialized Small Cell Number (SCN) Nucleofector® Kits maximize savings by allowing as few as 1.5 x 10^4 cells and 200 ng DNA per transfection

Your choice of throughput format
- Choose the modular devices you need for transfection in single cuvettes or in 96-well plates

The freedom to expand your research
- Explore complex systems by using the same conditions to deliver DNA, RNA, oligonucleotides, PNA, peptides, or proteins to your choice of cells or automate for high throughput

Nucleofector® Technology – two modular devices

Nucleofector® Device

96-well Shuttle® Device

Nucleofection® – Your Unique Advantage

Nucleofection® is a technology based on the momentary creation of small pores in cell membranes by applying an electrical pulse. The comprehensive way in which Nucleofector® Programs and cell type-specific solutions are developed ensures that nucleic acid substrates are delivered not only to the cytoplasm, but also through the nuclear membrane and into the nucleus. Transfected cells retain excellent viability and the function of intracellular systems is highly conserved. Whatever your application, Amaxa® Transfection Specialists are available to assist you in rapidly optimizing your transfection workflow.

Nucleic acid delivery direct to the site of action – efficiency and fast expression

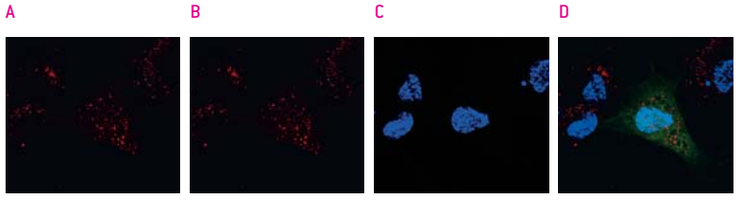


Figure 1: Normal human dermal fibroblasts (neonatal) were transfected with 2.5 µg TMR-labeled plasmid DNA encoding eGFP. After 2 hours, cells were fixed with 3.5% PFA and analyzed by confocal microscopy. TMR label is shown in (A), GFP fluorescence in (B), DAPI nuclear staining in (C) and a merge of all three fluorescent labels in (D).

Nucleofector® Technology – the superior non-viral method

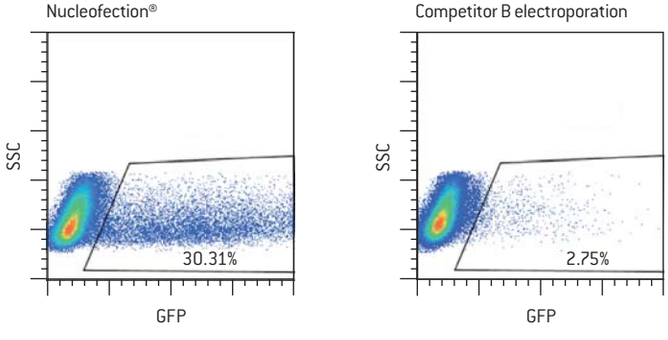


Figure 2: Transfection of the human natural killer cell line NKL using tradional electroporation and Nucleofection®. 5 x 10^6 NKL cells were transfected with 2.5 µg of pmaxGFP® Vector. Nucleofection®: Nucleofector® Solution V; Program O-017. Competitor B electroporation: 25 mV, 96 µF. Transfection efficiency was monitored by flow cytometry after 24 hours. Cells transfected by Nucleofection® show a significantly better transfection efficiency compared to cells transfected by tradional electroporation. Cell viability, as measured 18 hours after transfection was also superior using Nucleofection®.
(Data courtesy of Dr. John Coligan, Laboratory of Immunogenetics, NIH/NIAID, Rockville, MD, USA. J Immunol Methods [2004] 284: 133-140.)

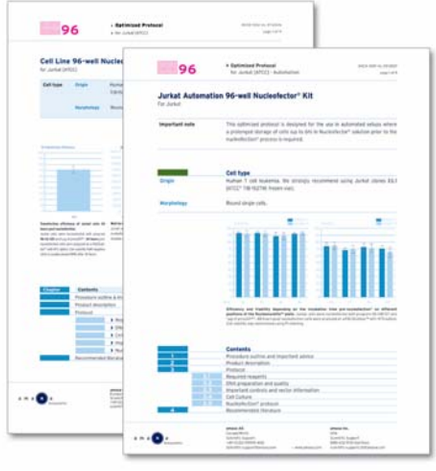
One Technology – the Broadest Choice of Cell Types

Using the Nucleofector® Technology, cell lines, as well as primary cells and stem cells, can be reliably transfected at high efficiency. Delivery of nucleic acid substrates directly into both the nucleus and cytoplasm ensures transfection efficiencies of up to 99%. More than 200 ready-to-use Amaxa® Optimized Protocols for both the Nucleofector® Device and the 96-well Shuttle® System contain cell type-specific guidance and Lonza's Amaxa® Cell Database contains user-developed protocols and data for more than 1300 cell types.

Proven Technology – Unparalleled Resources and Online Support

Accessing service and support for Nucleofector® Technology is easy and immediate, whether you wish to request a Nucleofector® Demonstration, upgrade your warranty, order a custom protocol, or ask for guidance from one of our PhD-level scientists.

Amaxa® Optimized Protocols offer comprehensive guidance, including tips for cell sourcing, passage number, growth conditions and media, and post-transfection culture.



- www.lonza.com/cell-database
- www.lonza.com/nucleofection-citations

Two Amaxa® Nucleofector® Platforms – Fulfilling Your Choice of Throughput

Using Nucleofector® Technology allows your transfection capabilities to grow in pace with your group, to adapt to changing applications that require different levels of sample throughput, to accelerate the pace of your research, or to transfect only the number of cells that fits your unique application.

The Components of the Nucleofector® Technology

Proven – in Leading Labs

The Nucleofector® Technology is an established and trusted transfection method in opinion-leading labs worldwide. Several thousand peer-reviewed publications illustrate the importance of Nucleofection® in hundreds of leading edge research applications using primary cells, such as neurons and stem cells, as well as difficult-to-transfect and standard cell lines.

The Nucleofector® II Device and 96-well Shuttle® System

Both the Nucleofector® Device and 96-well Shuttle® System deliver unique electrical parameters. Electrical settings are pre-programmed for each optimized cell type and can be selected using the Nucleofector® Device or through the laptop PC and software supplied as part of the Nucleofector® 96-well Shuttle® System.

Nucleofector® Kits and Nucleofector® 96-well Shuttle® Kits

Kits for Nucleofector® Technology contain specified plasticware, pipettes, pmaxGFP® Vector, Nucleofector® Solution and Supplement. Each solution and supplement is individually developed for every primary cell type. For cell lines, a set of different Nucleofector® Solutions is available. All solutions provide a protective environment that ensures the highest transfection efficiency and cell viability, while helping maintain physiologically relevant cellular functions.
Cell Line Optimization Nucleofector® Kits provide the ideal tools to conveniently determine the optimal Nucleofection® Parameters for your cell line of interest within a single experiment.

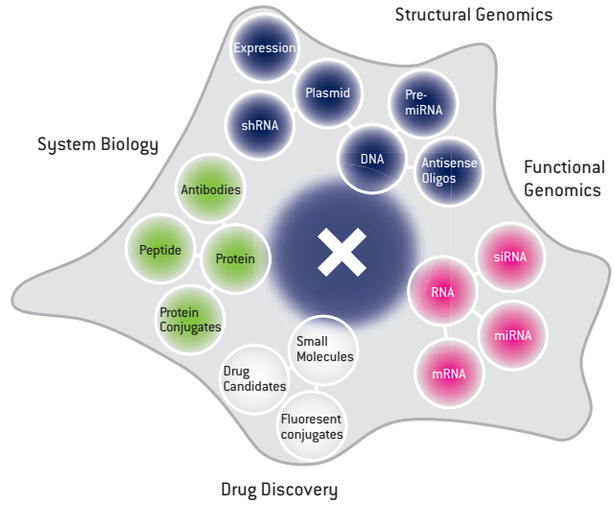
	Low throughput	Low to high throughput
Device	Nucleofector® Device	96-well Shuttle® System
		
Samples per run	1	1 – 96
Reaction volume	100 µl	20 µl
Cell number	2×10^5 to 2×10^7	5×10^4 to 1×10^6
Substrate amount	Oligonucleotides: 0.2 – 200 pmol (2 nM – 2 µM)	Oligonucleotides: 0.04 – 40 pmol (2 nM – 2 µM)
	Vector DNA: 1 – 5 µg	Vector DNA: 0.2 – 1 µg

Substrate Flexibility – Explore the Limits of Your Imagination

The flexibility that Nucleofector® Technology allows in planning experimental projects stems from the proven capability to transfect the widest range of substrate molecules into the widest range of cell types. Imagine the flexibility to first overexpress genes of interest using DNA vectors, then to explore the regulation of the gene or genes of interest using multiple or single shRNA or siRNA substrates using the same Nucleofector® Kit or 96-well Shuttle® Kit and Conditions.

Reflecting the focus of molecular biologists, the flexibility of Nucleofection® now extends beyond nucleic acid substrates, to include peptides, proteins, and antibodies. This allows yet further characterization and validation of the cellular functions that interest your research group, e.g., allowing researchers to identify and characterize related signaling or protein modification pathways using inhibitory peptides, before tracking active protein trafficking, or localization of active target molecules using antibody-conjugated nano-fluorophores or quantum-dots.

Nucleofector® Technology – delivers the widest range of substrates



siRNA knockdown of vimentin mRNA in human T-cells

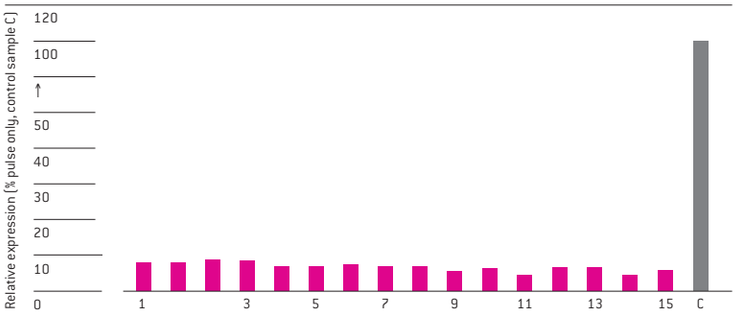


Figure 3: Knockdown on mRNA level measured by qRT-PCR. 15 samples compared to control (C): Nucleofection pulse only set to 100%.
(Data kindly provided by C. Merz, Bayer Schering Pharma AG, Berlin.)

Efficient transfection of peptides

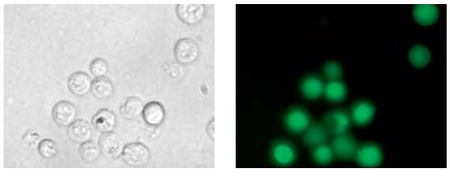


Figure 4: Jurkat cells were transfected in the presence of the Amaxa® Peptide Transfection Control 30 uM, a FAM-labeled octapeptide. Images were captured 2 hours post-transfection.
(Data courtesy of Prof. Brock, NCMLS, University of Nijmegen, Netherlands.)

Knockdown of protein expression in primary neurons using shRNA

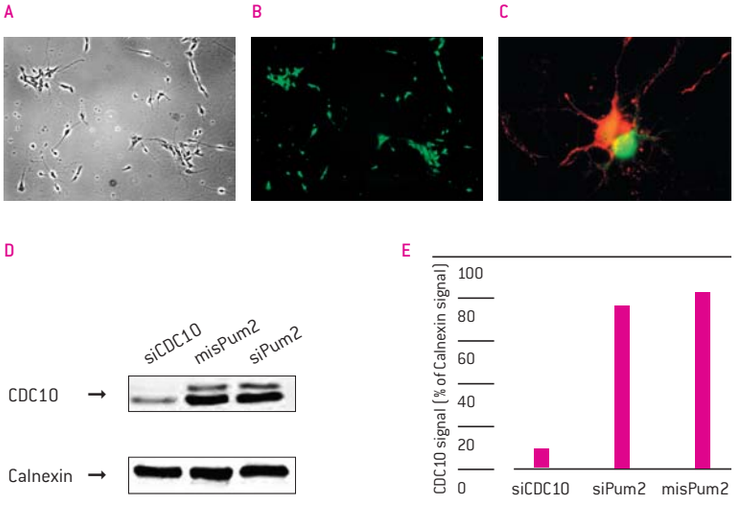


Figure 5: Quantitive downregulation of CDC10 protein. Rat hippocampal neurons (E17) were transfected with the shRNA vector pSuperior targeting CDC10 using the 96-well Shuttle® System. (A) and (B) Efficient Nucleofection® of pSuperior is shown by eGFP expression after 1 day *in vitro*. (C) Immunostaining of CDC10 (red fluorescence) shows reduced endogenous CDC10 protein levels in transfected neurons (green) 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.)

Post Transfection Functionality – Examine the Real Cellular Phenotype

Allowing cells to express the true *in vivo* phenotype is key to generating biologically relevant data. Where many transfection methods compromise cellular functionality through their innate toxicity or by inducing immune responses, Nucleofector® Technology delivers substrates efficiently to the nucleus and cytoplasm without disturbing cellular processes – there is no reagent incorporated into the cell membrane or any delivery particles accumulating in the cytoplasm.

Nucleofector® Technology has the potential to improve your transfection applications, whether trying to uncover the functionality of cancers that cause pathology by proliferation, metastasis, or vascularisation or by transfecting stem cells for developmental or therapeutic research. Nucleofector® Technology is proven as a valuable tool to explore cellular functionality at the DNA, RNA, and protein levels.

Conserving functionality – the first step to meaningful analysis

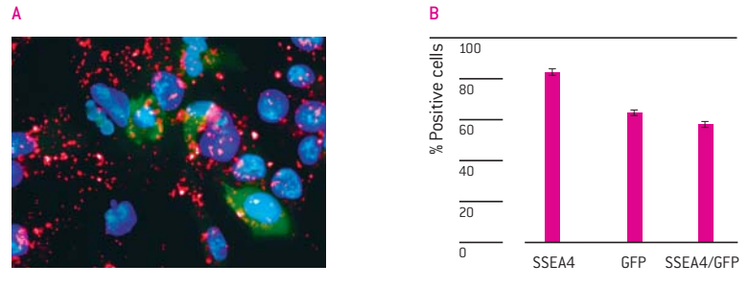


Figure 6: Human H9 ES cells preserve pluripotency post Nucleofection®. H9 cells were transfected by Nucleofection® with the pmaxGFP® Vector. (A) Cells analyzed after 24 hours show expression of GFP (green) as well as of the pluripotency markers SSEA4 (red) and Oct4 (purple). The blue signals refer to nuclear staining by DAPI. (B) The percentage of double-positive cells (GFP/SSEA) was analyzed by flow cytometry.
(Data kindly provided by Jennifer Moore, Rutgers University, Piscataway, USA.)

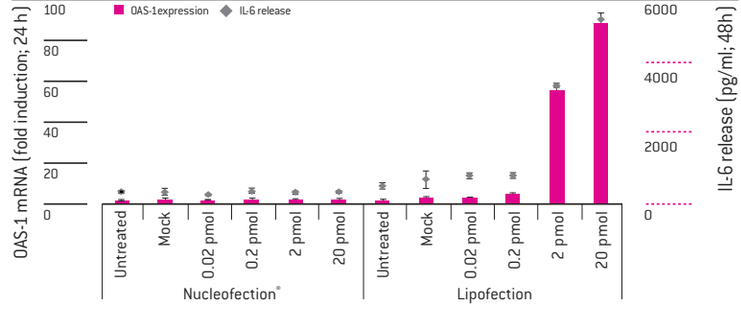


Figure 7: Induction of interferon responses is related to the method of substrate delivery. HeLaS3 (ATCC® CCL-2.2™) were transfected with different amounts of 29-mer siRNA targeting DBI (Dharmacon) – a described trigger of interferon responses (Reynolds A *et al.*, (2006), RNA 12:988-993) – using the 96-well Shuttle® Device or a lipid reagent. Interferon response was measured by expression of OAS-1 mRNA, determined 24 hours post transfection by QuantiGene® branched DNA assay (Panomics; normalized to GAPDH mRNA), and IL-6 release analyzed 48 hours post transfection by human IL-6 ELISA (Biosource).
(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products.)

Nucleofector® 96-well Shuttle® System – Flexibility and Value for Money

The Nucleofector® 96-well Shuttle® System utilizes a unique 96-well disposable Nucleocuvette® Plate. Each Nucleocuvette® Plate comprises 6 modules, each with 2 columns of 8 wells. Each Nucleocuvette® Well may be individually addressed with your pre-optimized program of choice through contacts to the conductive polymer electrodes.

The transfection volume of 20 µl reduces the required number of cells by 80% compared with the standard 100 µl transfection volume of the Nucleofector® Device and as few as 1 sample at a time may be easily transfected. Should optimization for your unique cell type be required, up to 96 individual programs may be tested within a single plate in less than five minutes and using as few as 2 x 10^6 cells in total.

The 96-well Shuttle® System delivers flexible throughput combined with economical processing, speed, and pre-optimized protocols for a range of both primary cells and cell lines. A choice of Service Contracts offering levels of service to suit your needs is available to further reduce risks of project 'downtime'.

Unparalleled performance and economy – with primary cells and cell lines

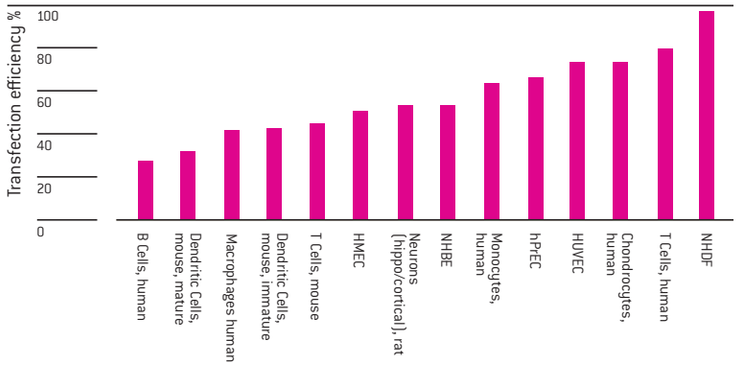


Figure 8A: Example for the transfection of primary cells using the Nucleofector® 96-well Shuttle®. Cells were transfected with 400 ng pmaxGFP® Vector and analyzed for maxGFP® Reporter Protein expression on a BD FACSCalibur™ (BD Biosciences) with HTS option 24 hours post Nucleofection®. Cell viabilities were in the range between 30% and 92% depending on the cell type.

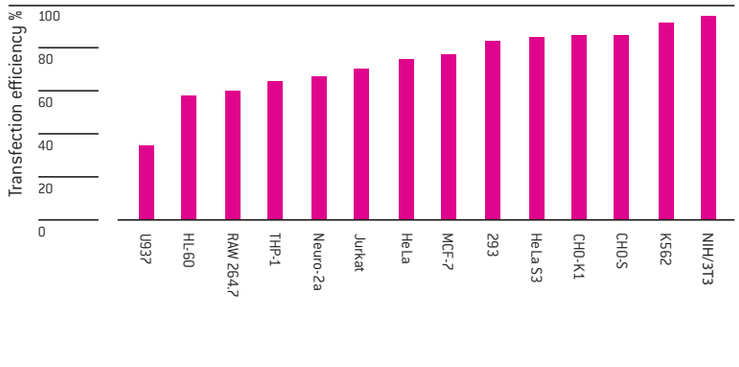


Figure 8B: Highly efficient transfection of various cell lines using the Nucleofector® 96-well Shuttle®. Cell lines (ATCC®) were transfected with 400 ng pmaxGFP® Vector and analyzed for maxGFP® Reproter Protein expression on a BD FACSCalibur™ (BD Biosciences) with HTS option 24 hours post Nucleofection®. Cell viabilities were in the range between 50% and 97% depending on the cell line.

	Cell Data	Nucleofector® 96-well Shuttle® Data			
Cell Type	**Lonza Clonetics® Primary Cell Catalogue Number**	**Cells per 96-well Shuttle® Nucleofection® (single well)**	**Transfection Efficiency**	**Viability**	**96-well Shuttle® Kit Catalogue Number**
Smooth muscle cells (AoSMC), aortic, human	CC-2571	5 x 10^4	74%	92%	VHPI-1004
Smooth muscle cells, pulmonary artery (PASMC), human	CC-2581	5 x 10^4	67%	86%	VHPI-1004
Smooth muscle cells, coronary artery (CASMC), human	CC-2583	5 x 10^4	81%	67%	VHPI-1004
Fibroblasts, Dermal (NHDF), human (adult)	CC-2511	1 x 10^5	96%	92%	VHPD-1001
Epithelial Cells, bronchial (NHBE), human	CC-2542	7.5 x 10^4	54%	53%	VHPK-1001
Dendritic Cells (DC), Mouse, Balb-c (immat.)	–	5 x 10^4	43%	74%	VHPA-1011
Epithelial cells, prostate (PrEC), human	CC-2555	1 x 10^5	67%	48%	VHPK-1003
Chondrocytes, human	–	2 x 10^5	74%	84%	VHPF-1001
Hepatocytes, human	CC-2591	1 x 10^5	54%	69%	VHPL-1001
Embryonic stem cell line (H9), human	–	2 x 10^5	64%	78%	VHPH-5003
Embryonic stem cells, mouse	–	5 x 10^4	90%	80%	VHPH-1001

96-well Shuttle® System – Reliable High Throughput Transfection

In a high throughput transfection framework, the manipulation of cells during the whole process chain is a critical issue. Stability of cell parameters such as transfection efficiency and viability is key to statistically relevant data and valid results.

Reproducibility – The Key to Success for High Throughput Transfection

During the workflow of cell-based screening, cells are often subject to environmental changes such as oxygen partial pressure, temperature, or agitation. Each step of such demanding workflows must be validated to minimize the sources of error introduced at each step. In the following data, Nucleofector® 96-well Shuttle® Technology was used to first examine the impact of extended incubation in Nucleofection® Solution at room temperature and, secondly, to analyse siRNA screening assay data reproducibility.

Robust transfection – suited to every workflow

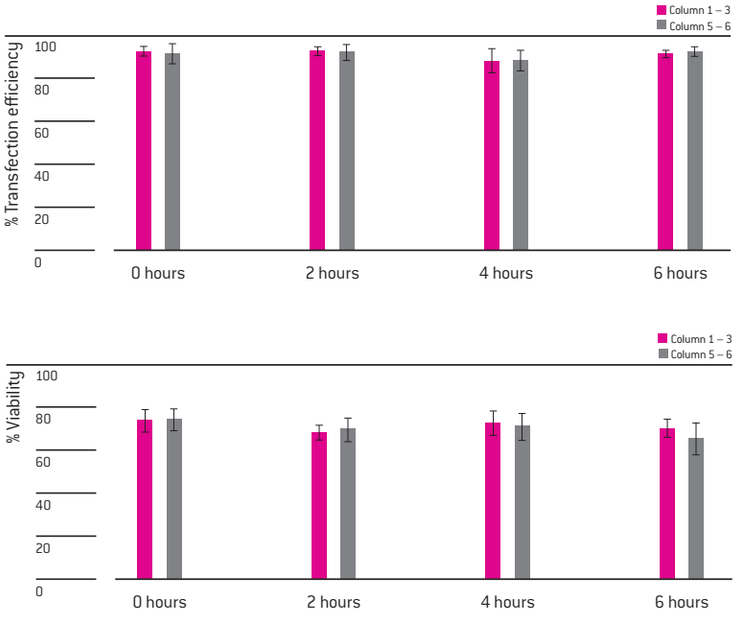
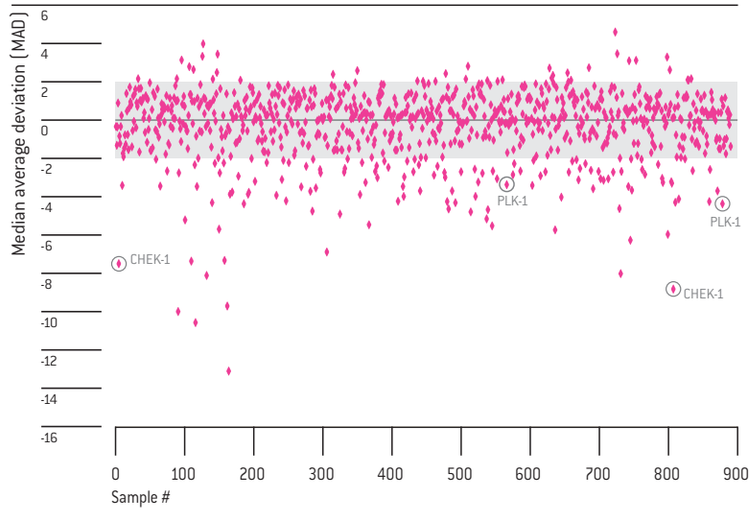


Figure 9: Jurkat E6-1 (ATCC® TIB 152™) cells were incubated in room temperature 96-well Nucleofector® Solution for 0, 2, 4, and 6 hours prior to transfection with 1 µg of pmaxGFP® Vector. Cells were analyzed 48 hours post Nucleofection®, using a BD FACSCalibur™ (BD Biosciences) with HTS option. Cell viability was determined using Propidium Iodide (PI) staining.

Reproducible transfection – making the most of your assays

A Result from 1 screening experiment



B Reproducible hits in 3 screens

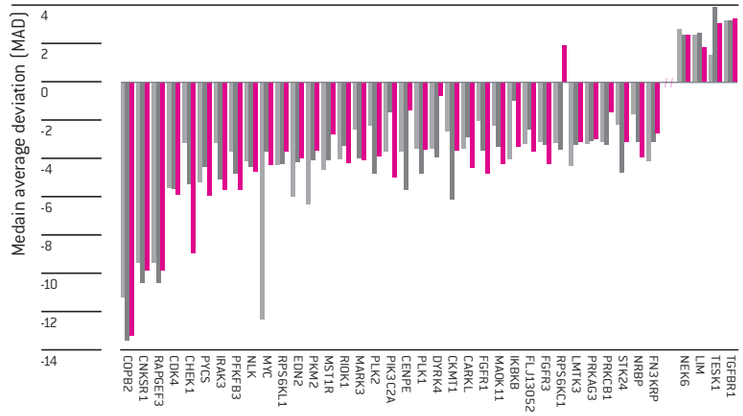


Figure 10: Primary Screen HUVEC cells were transfected with 20 pmol of the combined Human siGENOME® siRNA Libraries for Kinases (targeting 779 genes) and Cell Cycle Regulators (targeting 111 genes). Cell viability was analyzed 72 hours post Nucleofection®. (A) Representation of robust Z-scores of cell viability measures from 1 screening experiment. (B) Robust Z-scores of the top 37 primary hits (with an |MAD| > 2) from three independent experiments.
(Data generated in collaboration with Thermo Fisher Scientific, Dharmacon Products.)

Contact Lonza today regarding your unique application or to arrange to test the Nucleofector® Technology with your cells and in your lab.

Ordering Information

Cat. No.	Description
AAD-1001S	Nucleofector® Device
AAM-1001S	96-well Shuttle® Device (includes the Nucleofector® Device, notebook PC and software)
SBA-1001	96-well Shuttle® Automation Package
AXA-1001	96-well Shuttle® Rack
	Nucleofector® Kits for primary cells
VPA-1001	Human B Cell Nucleofector® Kit
VPA-1002	Human T Cell Nucleofector® Kit
VPA-1003	Human CD34$^+$ Cell Nucleofector® Kit
VPA-1005	Human Natural Killer Cell Nucleofector® Kit
VPA-1006	Mouse T Cell Nucleofector® Kit
VPA-1007	Human Monocyte Nucleofector® Kit
VPA-1008	Human Macrophage Nucleofector® Kit
VPA-1009	Mouse Macrophage Nucleofector® Kit
VPC-1001	Human Aortic Smooth Muscle Cell Nucleofector® Kit
VPD-1003	NHEM-Neo (Normal Human Epidermal Melanocyte-Neo) Nucleofector® Kit
VPE-1001	Human MSC (Mesenchymal Stem Cell) Nucleofector® Kit
VPE-1002	Rat Cardiomyocyte – Neonatal Nucleofector® Kit
VPG-1001	Mouse Neuron Nucleofector® Kit
VPG-1002	Chicken Neuron Nucleofector® Kit
VPG-1003	Rat Neuron Nucleofector® Kit
VPG-1004	Mouse NSC (Neural Stem Cell) Nucleofector® Kit
VPG-1005	Rat NSC (Neural Stem Cell) Nucleofector® Kit
VPG-1006	Mouse Astrocyte Nucleofector® Kit
VPG-1007	Rat Astrocyte Nucleofector® Kit
VPG-1009	Rat Oligodendrocyte Nucleofector® Kit
	Basic Nucleofector® Kits for primary cells
VPI-1001	Basic Nucleofector® Kit for Primary Mammalian Endothelial Cells
VPI-1002	Basic Nucleofector® Kit for Primary Mammalian Fibroblasts
VPI-1003	Basic Nucleofector® Kit for Primary Mammalian Neurons
VPI-1004	Basic Nucleofector® Kit for Primary Smooth Muscle Cells
VPI-1005	Basic Nucleofector® Kit for Primary Mammalian Epithelial Cells
	Nucleofector® Kits for cell lines
VCA-1001	Cell Line Nucleofector® Kit R
VCA-1002	Cell Line Nucleofector® Kit T
VCA-1003	Cell Line Nucleofector® Kit V
VCA-1004	Cell Line Nucleofector® Kit C
VCA-1005	Cell Line Nucleofector® Kit L
VCO-1001N	Cell Line Optimization Nucleofector® Kit

Ordering Information

Cat.No. 96 reactions	Cat.No. 960 reactions	Description
		96-well Nucleofector® Kits for primary cells
VHPA-1001	VHPA-2001	Human B Cell Nucleofector® Kits
VHPA-1002	VHPA-2002	Human T Cell 96-well Nucleofector® Kits
VHPA-1006	VHPA-2006	Mouse T Cell 96-well Nucleofector® Kits
VHPA-1011	VHPA-2011	Mouse Dendritic Cell (Immature) 96-well Nucleofector® Kits
VHPA-1012	VHPA-2012	Mouse Dendritic Cell (Mature) 96-well Nucleofector® Kits
VHPB-1002	VHPB-2002	HUVEC 96-well Nucleofector® Kits
VHPF-1001	VHPF-2001	Human Chondrocyte 96-well Nucleofector® Kits
VHPG-1003	VHPG-2003	Rat Neuron 96-well Nucleofector® Kits
VHPH-1001	VHPH-2002	Mouse ES Cell 96-well Nucleofector® Kits
VHPK-1002	VHPK-2002	Human Mammary Epithelial Cell (HMEC) 96-well Nucleofector® Kits
VHPK-1003	VHPK-2003	Human Prostate Epithelial Cell (hPrEC) 96-well Nucleofector® Kits
		Basic 96-well Nucleofector® Kits for primary cells
VHPI-1002		Basic 96-well Nucleofector® Starter Kit for Primary Mammalian Fibroblasts (64 reactions)
VHPI-1003	VHPI-2003	Basic 96-well Nucleofector® Kits for Primary Mammalian Neurons
VHPI-1004	VHPI-2004	Basic 96-well Nucleofector® Kits for Primary Smooth Muscle Cells
VHPI-1005		Basic 96-well Nucleofector® Starter Kit for Primary Mammalian Epithelial Cells (64 reactions)
VHPH-5002		Basic 96-well Nucleofector® Starter Kit for Human Stem Cells (64 reactions)
		96-well Nucleofector® Kits for cell lines
VHCA-1001	VHCA-2001	Cell Line 96-well Nucleofector® Kits SE
VHCA-1002	VHCA-2002	Cell Line 96-well Nucleofector® Kits SF
VHCA-1003	VHCA-2003	Cell Line 96-well Nucleofector® Kits SG
VHCO-1001		Cell Line Optimization 96-well Nucleofector® Kit

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
StellARray™ Gene Expression System	www.lonza.com/arrays

For an up-to-date overview or more detailed information about Amaxa® Products please visit www.lonza.com/amaxa-productlist.

Lonza

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Fax:	301-845-8291
E-mail:	scientific.support@lonza.com

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Italy	0039 0363 45710
Japan	81 3 5566 0612
Poland	48 22 833 87 45
Singapore	65 64914214
Spain	34 902 531 366
Sweden	020 140 4410
Switzerland	0800 83 86 20
The Netherlands	0800 022 4525
United Kingdom	44 118 979 5234

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