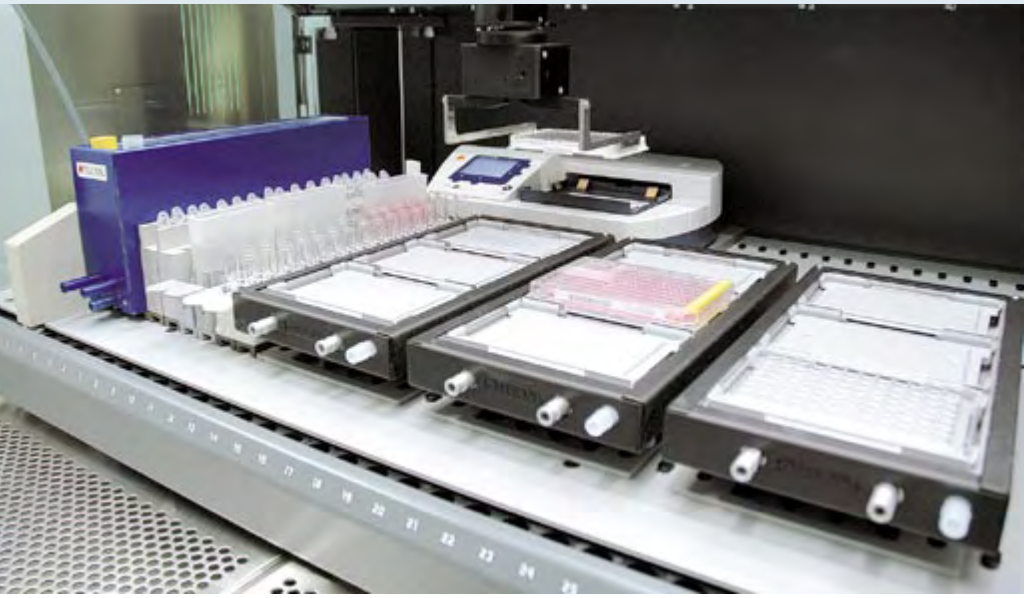
High throughput nucleofection® with the **Freedom EVO®** workstation and **amaxa 96-well Shuttle®**

Tecan and amaxa GmbH have successfully integrated the amaxa Nucleofector® 96-well Shuttle® System with the Tecan Freedom EVO® liquid handling workstation, allowing fully automated, high throughput transfection of primary cells and difficult-to-transfect cell lines, including non-dividing neurons and T cells, with siRNA, DNA and other substrates. The collaboration was originally instigated for Dr Claudia Merz at Bayer Schering Pharma AG, Germany, who needed fully automated, high throughput transfection of immune system cells for RNAi-based screening experiments.

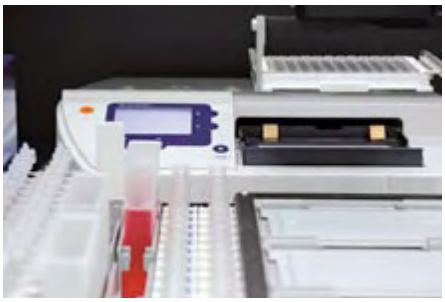
Scientists routinely use transfection techniques to insert DNA or RNA into cells and modify the expression levels of a certain gene or genes. This approach is often used for functional studies of genes, such as RNA interference (RNAi) to silence gene expression; for cell-based manufacturing of therapeutic products (eg. antibodies, hormones or vaccines); and for structural studies of proteins and amplification of DNA or RNA in cells for purification. Many of these applications require high throughput transfection in combination with primary cells or non-dividing cell lines that are notoriously difficult to transfect with standard lipofection approaches.

The Nucleofector® technology from amaxa, a German biotechnology company, has been specifically designed for difficult-to-transfect cell lines and primary cells, including stem cells, T lymphocytes and non-dividing neurons. The technology uses electroporation instead of lipofection, where electrical currents cause the gene of interest to be transferred directly into the cell and even the cell's nucleus. This quick and simple approach is the only non-viral method to achieve very high transfection efficiencies while maintaining high survival rates in such cells, with up to 80% efficiencies in human T cells, for example, compared with 1-3% or fewer usually transfected by lipofection. As nucleofection causes the DNA to directly enter the nucleus, this



method does not require cell division, and expression of the transfected gene can be analyzed shortly after nucleofection. For some cell types this can be within as little as two hours, rather than the standard 24 to 48 hours.

Integrated cooling and heating elements.



Transportation of amaxa's Nucleocuvette™ plate to the 96well Shuttle.

Tecan and amaxa have teamed up to integrate amaxa's Nucleofector® 96-well Shuttle® System with Tecan's Freedom EVO® liquid handling workstation. All necessary steps for transfection can be automated, including cell harvesting, diluting and plating; DNA/RNA normalization; preparation of reagent mixes; resuspension of cells and substrates in Nucleofector® solution; the nucleofection process; and analysis of transfection results. Nucleofection occurs in disposable, 96-well Nucleocuvette™ modules, which consist of an innovative conductive polymer electrode material that ensures no metal ions are released into the cell suspension during transfection. The system uses identical transfection parameters for any nucleic acid substrate, so that DNA vectors, such as expression plasmids, and RNAi vectors, including short hairpin (sh)RNA vectors or short interfering (si)RNA duplexes, can be transfected using the same protocol.

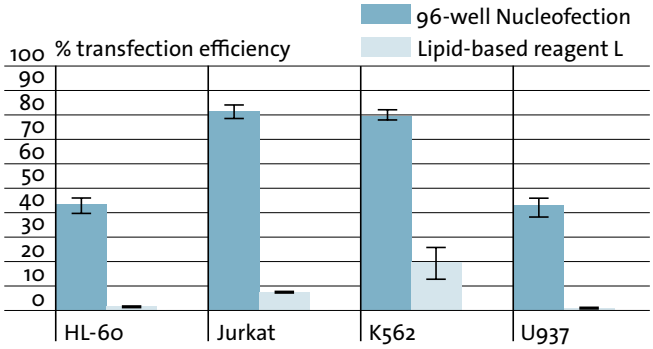
The entire nucleofection process is controlled by the Freedom EVOware® software, using an interface provided by amaxa. Nucleofection conditions for the 96-well Shuttle® are defined in the amaxa software and the resulting parameter files are uploaded from Freedom EVOware and executed on the 96-well Shuttle®.

"Many easily-transfected cell lines are quite different from primary cells of the human body so are not applicable for most research and drug development studies," explained Andreas Schroers, Product Manager at amaxa. "The integration enables, for the first time, transfection of medically relevant primary cells in a fully automated environment, providing a valuable tool for research and drug discovery studies."

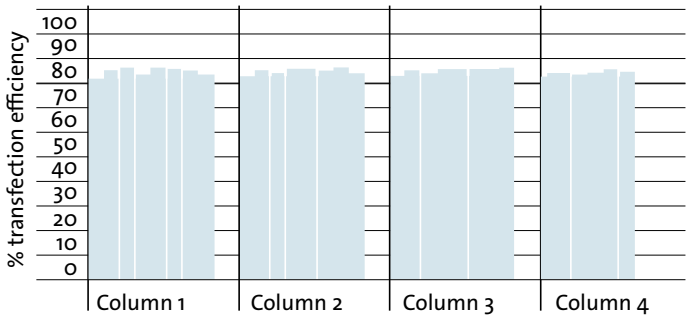
The collaboration originally began in response to a request from Dr Claudia Merz, scientist in Target Discovery at Bayer Schering Pharma AG in Berlin, Germany. Dr Merz had been performing lipofection using a Freedom EVO workstation for some time, and needed to expand the set-up for large scale, automated nucleofection of human T lymphocytes and the Jurkat lymphocyte cell line using focused siRNA libraries. These small libraries have between 200 and 800 siRNAs that are applied in triplicate, requiring multiple 96-well Nucleocuvette™ plates to be completed each day. Dr Merz's integrated system automates the complete plate preparation, nucleofection and replating of nucleofected cells for one 96-well plate in about 40 minutes, and twelve 96-well plates can be processed in one day, equating to 1,152 samples. A Freedom EVO 200 workstation is used for all additional steps of the RNAi studies, such as mRNA knockdown analysis and analysis of phenotypic changes of the knocked down cells.

"We are very excited and pleased that the final installation of the integrated system into our laboratory was achieved so smoothly, including setting up the software protocols to run the 96-well transfections," said Dr Merz. "Our protocols for manual transfection of primary human T cells or Jurkat cells, for example, have been fully adapted for high throughput nucleofection on the Freedom EVO workstation. We can now run RNAi screens using difficult-to-transfect cells, primary cells or suspension cells in an automated fashion, which will enhance and facilitate our target discovery efforts in dermatology or immunology, for example."

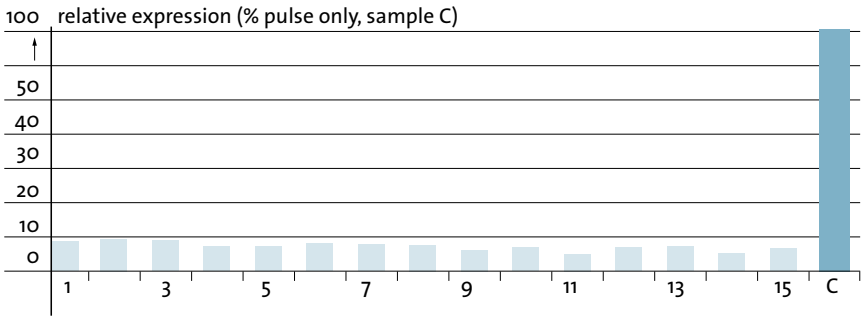
Come and see the amaxa integration on show on Tecan's booth 311 at the Society for Biomolecular Screening exhibition in Montreal, Canada, April 2007.



Nucleofection versus lipofection of suspension cells
Cells were transfected with (pmaxGFP™). The efficiency was measured on a BD FACSCalibur™ 24h post transfection.



Reproducible intra-plate transfection efficiency in nucleofected Jurkat E6-1 cells (ATCC® TIB-152™)
Analysis was performed on a BD FACSCalibur™ 24h post Nucleofection. The transfection efficiency of each well is shown per column of a 96-well Nucleocuvette Module. Column 4 contained two control samples (no pulse, no plasmid). (Data kindly provided by C. Merz. Bayer Schering Pharma AG, Berlin)



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)

Not for clinical use or blood and plasma screening. The Nucleofector 96-well Shuttle System and the 96-well Nucleocuvette plates and modules are covered by patent and/or patent-pending rights owned by amaxa. PmaxGFP is a trademark of amaxa.
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