The Nucleofector®
96-well Shuttle® System

A solid tool for the rapid and efficient transfection of primary cells and difficult-to-transfect cell lines in a high-throughput framework.

Andreas Schroers¹, Claudia Merz²
¹ amaxa GmbH, 50829 Cologne, Germany
² Department of Genomics & Bioinformatics, Enabling Technologies, Schering AG, Müllerstraße 178, Berlin, Germany
Correspondence should be addressed to A. Schroers (andreas.schroers@amaxa.com)

With the 96-well Shuttle® System amaxa creates a new benchmark in transfection of primary cells and difficult-to-transfect cell lines. RNAi based high-throughput screening approaches like target identification and validation can now be performed in cells of the highest medical relevance.

- Excellent transfection efficiencies – up to 99% with siRNA / 98% with DNA
- True high throughput – more than 1400 transfections per hour possible
- Unsurpassed reproducibility – intra plate SD ~ 1%

Introduction
Transfection of cells, i.e. the transfer of DNA or siRNA into these cells, is a key technique in cell biology. With this approach, genes can be added or specifically knocked down in order to study their functions in the cell. The Nucleofector Technology enables efficient and reproducible transfer of nucleic acids into cells so far considered difficult or even impossible to transfect. Besides primary cells also most suspension cell lines fall into that category. Primary cells are freshly isolated from body tissue. Unlike many standard cell lines that have often been cultured for decades and meanwhile even might significantly differ from their origin primary cells are unchanged, closely resembling the in-vivo situation, and are therefore of particular relevance for medical research and drug discovery purposes.

Summary
We show here that the Nucleofector® 96-well Shuttle® System can be efficiently used for the reproducible transfection of a multitude of cell types. RNAi library screening based approaches to target identification with a clear demand for high-throughput transfection can now be performed in primary and other difficult-to-transfect cells.

High Throughput Nucleofection®
Based on the Nucleofector device established in research labs worldwide, amaxa has expanded the technology to 96-well format and has concomitantly adapted it to small volumes (20 µl) and low cell numbers (down to 10⁴ cells). Such a format provides the prerequisites for the analysis of gene functions and the identification and
validation of novel drug targets by performing experiments such as RNAi and cDNA library screens or certain cell-based assays directly in primary cells and cell lines including many difficult-to-transfect cells (see figure 1 +2).

Besides a high efficiency the accuracy and robustness of a technology employed in the drug development process is of highest importance. Using the 96-well Shuttle, Nucleofection is performed in a special disposable plate with conductive polymer electrodes that rule out any metal ion contamination. In this Nucleocuvette™ plate the 96 samples are processed sequentially within three to four minutes, assuring a reproducible homogeneous electrical conditions in each sample, thereby excluding plate effects and parameter drifts. For a detailed analysis of the accuracy Jurkat E.6-1 cells (ATCC®) were transfected with pmaxGFP™. Two wells in column 4 were used for control samples either receiving no pulse or no plasmid. The results shown not only reflect the outstanding data quality but also the absence of technical artifacts mentioned above. The standard deviation was determined to 1.04 % for all samples, demonstrating an excellent reproducibility.

**Accuracy and Reproducibility**

Besides a high efficiency the accuracy and robustness of a technology employed in the drug development process is of highest importance. Using the 96-well Shuttle, Nucleofection is performed in a special disposable plate with conductive polymer electrodes that rule out any metal ion contamination. In this Nucleocuvette™ plate the 96 samples are processed sequentially within three to four minutes, assuring a reproducible homogeneous electrical conditions in each sample, thereby excluding plate effects and parameter drifts. For a detailed analysis of the accuracy Jurkat E.6-1 cells (ATCC®) were transfected with pmaxGFP™. Two wells in column 4 were used for control samples either receiving no pulse or no plasmid. The results shown not only reflect the outstanding data quality but also the absence of technical artifacts mentioned above. The standard deviation was determined to 1.04 % for all samples, demonstrating an excellent reproducibility.
siRNA Transfection in Suspension Cell Lines

RNA interference (RNAi) is a powerful and versatile tool to down-regulate genes in a rapid and gene-specific manner. Especially high-throughput transfection using siRNA libraries has become an indispensable tool in target identification. However, such screenings have so far mostly been limited to easy-to-transfect adherent cell lines. The Nucleofector® 96-well Shuttle® System extends these approaches also to primary and hard-to-transfect cells. The efficiency of siRNA delivery into the cell must be as high as possible, as limits in the effectiveness of delivery inevitably lead to a decrease in knockdown. Using the Nucleofector Technology siRNA delivery is accomplished with up to 99% efficiency even in suspension cell lines (see figure 4).

siRNA knockdown in Jurkat cells

For vimentin knockdown the functional siRNA sequence GAA UGG UAC AAA UCC AAG UdTdT (synthesized by Dharmacon, Inc., Lafayette) was used. 2.5 x 10⁵ Jurkat cells/sample were resuspended in 96-well Nucleofector® Solution and mixed with a vimentin-siRNA stock solution. Sample 31 served as control: pulse only without siRNA. RNAi-mediated gene silencing of vimentin was measured 24h post-transfection by qRT-PCR (figure 5).