# Automated High Throughput Nucleofection®

## RNAi in Primary Cells and Difficult-to-Transfect Cell Lines

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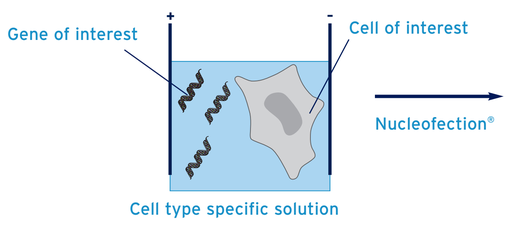
## Introduction

Using primary cells for RNAi based applications such as target identification or – validation, requires a highly efficient transfection technology in combination with a reliable and robust automation system. To accomplish these requirements we integrated the amaxa 96-well Shuttle® in a Tecan Freedom EVO® cell transfection workstation which is based on Tecan's Freedom EVO® liquid handling platform and include all the necessary components and features for unattended cell transfection.

## Nucleofector® Technology

The 96-well Shuttle® combines high-throughput compatibility with the Nucleofector® Technology, which is a non-viral transfection method ideally suited for primary cells and hard-to-transfect cell lines based on a combination of buffers and electrical parameters.



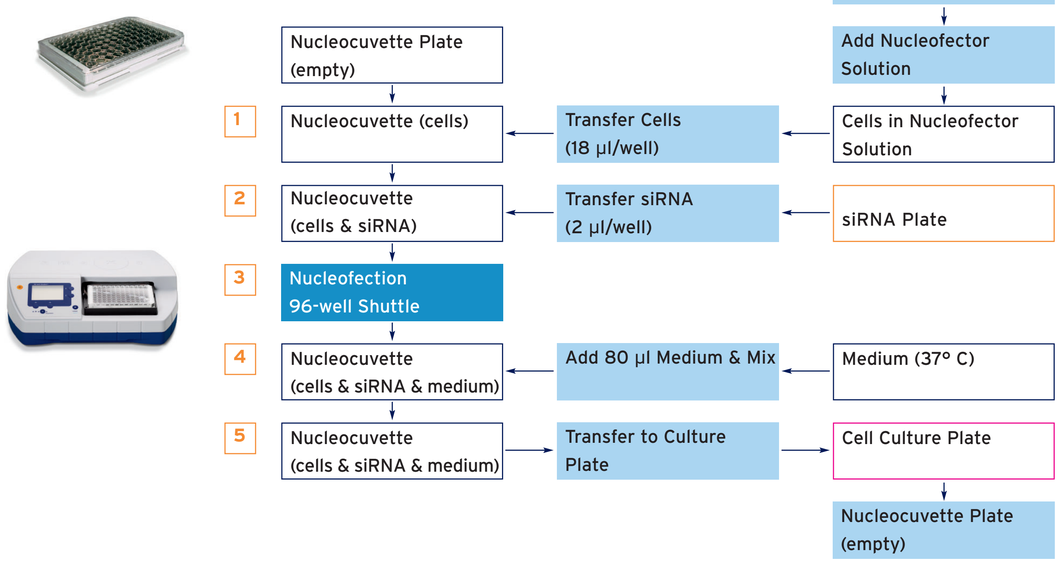

**The basic principle and benefits of the Nucleofector® Technology**

› Enables highly-efficient transfection of difficult-to-transfect cell lines
› Fast onset of gene expression

## Materials & Methods - Workflow

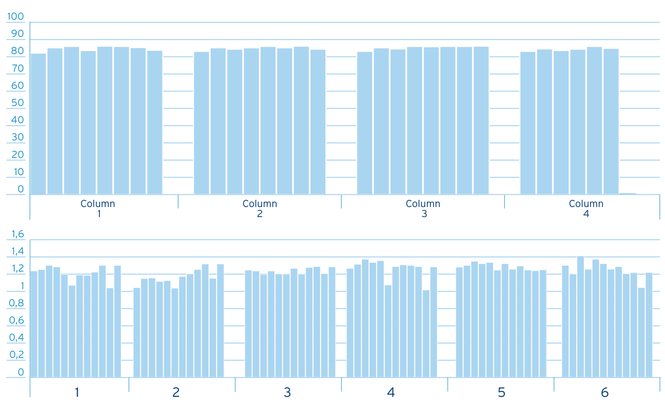
displaying the essential steps of the automated Nucleofector® Process:

1. Transfer of the cells to the Nucleocuvette™ plate,
2. Addition of the siRNA, (Steps 1 and 2 could be exchanged),
3. Nucleofection® process,
4. Addition of medium,
5. Transfer of transfected cells to cell culture plate for incubation prior to analysis.




## Results 1 - Reproducibility

Jurkat E.6-1 cells (ATCC® TIB-152™) were either transfected with pmaxGFP™ or a plasmid encoding secreted alkaline phosphatase (SEAP). The results shown reflect the excellent data quality and the absence of technical artifacts such as plate- or edge effects, demonstrating a high reproducibility.



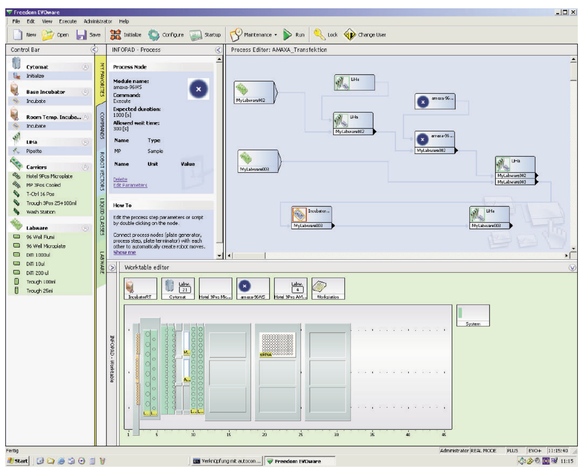

**maxGFP™ expression** - The analysis was performed on a BD FACSCalibur™ 24h post Nucleofection®. The transfection efficiency of each well is shown per well of of a 96-well Nucleocuvette™ Module. Column 4 contained two control samples receiving either no pulse or no plasmid. The standard deviations for the single columns were 1.26 / 0.95 / 0.88 / 0.94% with an over-all standard deviation of 1.04% for all samples.

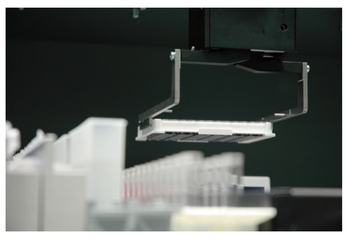
**SEAP expression** - Cells were transfected with 1 µg plasmid encoding secreted alkaline phosphatase (SEAP). 24 h post Nucleofection® SEAP activity was analyzed by a colorimetric alkaline phosphatase assay. From the assay a standard deviation of 0.08 (mean value = 1.24) was calculated, resulting in an excellent coefficient of variation (CV) of 6.9%.

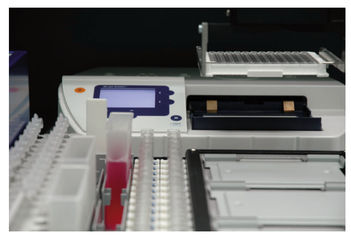
## Materials & Methods - Process Control

The entire process is controlled by Tecan's Freedom EVOware® software, which allows customized programming of your specific protocols. All of the necessary steps for transfection are automated, including:

- Plating of cells to the desired density
- Overnight incubation and cell washing
- Re-suspension of cells and substrates prior to Nucleofection®
- Culture of transfected cells for further analysis.

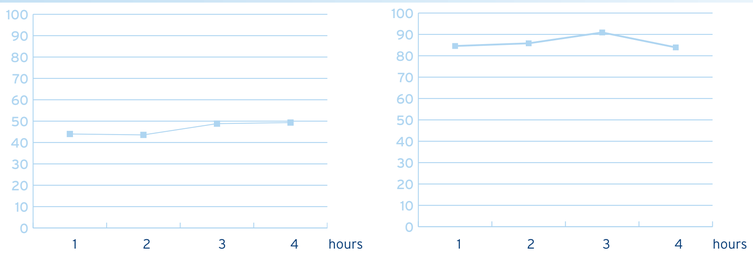






## Results 2 - Robustness

During the screening process the cells have to be stored in Nucleofector® solution for up to two hours prior to dispensing. The data shown, demonstrate the robustness of the main readout parameters efficiency and viability over time.

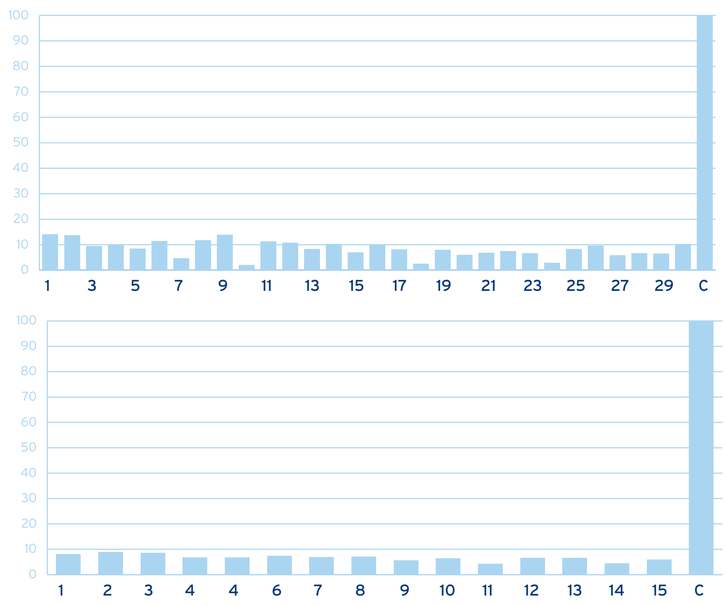



**Transfection Efficiency** (left, maxGFP™ expression) and **Viability** (right) in human T-cells after pre-incubation of the cells in Nucleofector® solution for up to four hours. The analysis was performed on a BD FACSCalibur™ 24 h post Nucleofection®. The mean efficiency and viability from two Nucleocuvette™ modules is shown.

## Results 3 - Highly Efficient Knockdown

Efficient knockdown is demonstrated by data showing siRNA-mediated depletion of vimentin-mRNA in Jurkat and primary human T-cells. Cells were transfected with a siRNA duplex directed against endogenous vimentin. 24h post transfection vimentin mRNA levels were analyzed by RealTime PCR. Relative expressions compared to untreated control sample are shown.

% relative expression (% pulse only, sample 31




**Jurkat E6-1**
(ATCC®), TIB-152™ Relative expressions compared to untreated control sample (C, set to 100%) are shown.

**Human T-cells**
15 samples compared to control (C, set to 100%) are shown.

## Summary & Conclusions

Combining Tecan's Freedom EVO® liquid handling workstation with amaxa's Nucleofector® Technology allows for fully automated, reproducible and efficient transfection of difficult-to-transfect cell lines and primary cells, including T-cells. The system is ideal for large-scale studies that involve high throughput transfection, such as RNAi-based screening for target identification and validation, or screening of cDNA libraries.

## Additional Benefits

| | |
|---|---|
| Ease of use | Optimized 96-well Nucleofector® Kits and protocols are available for many primary cells and difficult-to-transfect cell lines. |
| Flexibility | The technology allows for identical transfection conditions for various substrates including DNA, siRNA and shRNA. |
| Safety | The unique conductive polymer electrodes of the Nucleocuvette™ plates prevent metal ion release, and disposable plates minimize the risk of cross contamination. |

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Cologne, June 2007