BioResearch

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Efficient Transfection of Cancer Cell Lines Using the 4D-Nucleofector™ System

By Jenny Schroeder[1], Ludger Altrogge[1], Elke Lorbach[1], Srinivasan Kokatam[2], Sabine Schaepermeier[1], Meike Weigel[1], Gina Andretta-Beu[1], Stefanie Buesch[1], Tamara Grabeck[1], Alexandra Krumnow[1], Sonja Spicker[1], Sampada Kallol[2], Preeti Kapoor[2] and Andrea Toell[1]

[1]Lonza Cologne GmbH, Koeln, Germany; [2]Lonza India PVT Ltd., Hyderabad, India

Introduction

Cell lines isolated from tumors are an important tool for studying cancer *in vitro*. They can be used for drug development as well as for understanding the basic mechanisms underlying cancer. Transfection of cancer cell lines with different molecules such as plasmid DNA, siRNA or mRNA is often an integral part of this kind of research.

Lonza's 4D-Nucleofector™ System (Figure 1A) is a modular system for the efficient transfection of primary cells and cell lines with a variety of substrates including plasmid DNA and siRNA. The 4D-Nucleofector™ X Unit supports Nucleofection™ in two different formats. The aluminum-free 20 µL Nucleocuvette™ Strip (Figure 1B) allows the transfection of low cell numbers down to 2 x 10^4 cells per reaction. As 16 reactions can be performed in parallel, it is well suited for optimizing Nucleofection™ Conditions for cells lacking a ready-to-use Optimized Protocol. For higher cell numbers of up to 2 x 10^7 cells per reaction, the same Nucleofection™ Conditions can be applied in the 100 µL single Nucleocuvette™ Vessel (Figure 1C). For higher throughput needs, the 96-well Shuttle™ Add-on can be connected to the 4D-Nucleofector™ System (Figure 1A). With this add-on, six 20 µL Nucleocuvette™ Strips can be processed in parallel, allowing for screening applications or accelerating the optimization of transfection parameters for many cell types.

In this study, we used the 4D-Nucleofector™ System in combination with the 96-well Shuttle™ Add-on for optimizing transfection conditions for more than 30 cancer cell lines. An exemplary optimization process is depicted for the human prostate carcinoma cell line DU 145 and the human colorectal adenocarcinoma cell line COLO 205.

Figure 1. 4D-Nucleofector™ System. (A) 4D-Nucleofector™ Core- and X Unit with 96-well Shuttle™ Add-on. (B) 20 µL Nucleocuvette™ Strip. (C) 100 µL single Nucleocuvette™.

Materials and Methods

Initial Optimization of Nucleofection™ Conditions

All cell lines were cultured according to standard protocols. Adherent cell lines were harvested using trypsin (Lonza, cat. no. 17-161E). Dependent on the cell type, 1–5 x 10^5 cells per sample were resuspended in 20 µL of the respective Nucleofector™ Solution SE, SF or SG containing 0.4 µg of pmaxGFP™ Vector. Samples were transferred into a 96-well Nucleocuvette™ Plate and processed in parallel with 31 different programs and a no program control in the 96-well Shuttle™ Add-on. After transfection, 80 µL of equilibrated medium was added to each sample. Dependent on cell type, 2.5–10 x 10^4 cells were seeded into a standard 96-well cell culture plate.

Further Fine-tuning of Nucleofection™ Conditions (Optional)

A) 10 minutes post-incubation: 2 x 10^5 cells per sample were resuspended in 20 µL of the selected Nucleofector™ Solution containing 0.4 µg pmaxGFP™ Vector and pulsed with the selected Nucleofector™ Program. Post Nucleofection™, samples were incubated for 10 minutes in Nucleofector™ Solution prior to adding 80 µL of equilibrated cell culture medium.

B) DNA Titration: 2 x 10^5 cells per sample were resuspended in 20 µL of the selected Nucleofector™ Solution containing 0.4 µg, 1 µg or 5 µg of pmaxGFP™ Vector. After transfection – if indicated by fine-tuning step A – samples were incubated for 10 minutes in Nucleofector™ Solution prior

to adding 80 µL of equilibrated cell culture medium.

Transfer to 100 µL Single Nucleocuvette™ Vessels

Based on the cell number used in the 20 µL format, a 5 fold increased cell number per sample was resuspended in 100 µL of the respective Nucleofector™ Solution SE, SF or SG containing five times the amount of pmaxGFP™ Vector. Samples were transferred into the 100 µL single Nucleocuvette™ Vessel and processed with the Nucleofector™ Program identified in the 20 µL Nucleocuvette™ Strip. After transfection, 400 µL of equilibrated medium was added to each sample. Dependent on cell type, 2.5–10 x 10^4 cells were seeded into a standard 96-well cell culture plate.

Analysis

24 hours after transfection, the percentage of maxGFP™-positive, propidium-iodide negative cells was determined using flow cytometry (FACSCalibur™, Becton Dickinson) according to standard procedures. Cell viability was determined using the ViaLight™ Plus BioAssay Kit (Lonza, cat. no. LT07-321) according to the protocol. Cell viability is expressed as % viability compared to the non-transfected no program controls.

Results

Using the 4D-Nucleofector™ System in combination with the 96-well Shuttle™ Add-on, we found that the human prostate carcinoma cell line DU 145 can be easily transfected. Best results were obtained with Nucleofector™ Solution SE and Program CA-137, resulting in a transfection efficiency of 78% (Figure 2A) with a cell viability of 55% (Figure 2B). These results show that efficient Nucleofection™ Conditions for cancer cell lines can often be easily determined in a single experiment.

For the human colorectal adenocarcinoma cell line COLO 205, the initial optimization experiment achieved maximal transfection efficiencies of ~30% (Figure 3A) with program-dependent cell viabilities (Figure 3B). Thus, we performed two further program fine-tuning rounds and identified Nucleofector™ Program DP-113, in combination with Nucleofector™ Solution SG and a cell seeding density of 1 x 10^5 cells in 96-well, as the best selections under the given experimental conditions (data not shown). However, transfection efficiency still remained below 30% with a cell viability of 50%.

In order to improve transfection efficiency and viability, further optimization experiments were performed. Incubation of COLO 205 cells for 10 minutes in Nucleofector™ Solution post transfection increased transfection efficiency and cell viability (Figure 4A). An additional increase of transfection efficiency was observed when increasing the amount of plasmid DNA per reaction. Cell viability remained above 60% compared to the no program control with DNA amounts up to 1 µg per reaction. With a higher DNA concentration, a drop in cell viability was observed (Figure 4B).

One important feature of the 4D-Nucleofector™ X Unit is the transferability of conditions from the 20 µL Nucleocuvette™ Strips (for low cell numbers and higher throughput) to the 100 µL single Nucleocuvette™ Vessels (for higher cell numbers). For the latter, the same Nucleofection™ Conditions can be applied using a five times higher cell number and substrate amount. This is shown for 32 different human cancer cell lines in Figure 5.

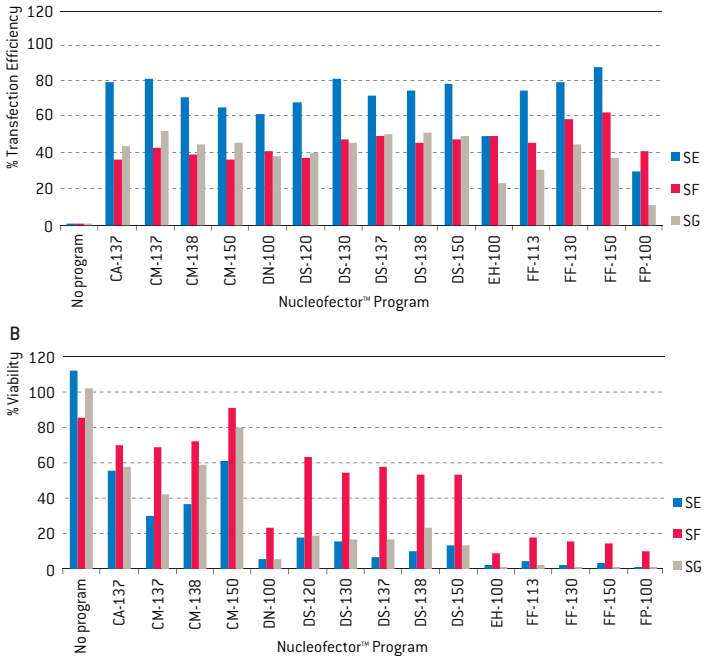

Figure 2
First optimization round for the human prostate carcinoma cell line DU 145 using the 4D-Nucleofector™ System in combination with the 96-well Shuttle™ Add-on. 2 x 10^5 cells were transfected with 0.4 µg pmaxGFP™ Vector using Nucleofector™ Solution SE, SF or SG and 31 different programs (15 of which are shown). 5 x 10^4 transfected cells were seeded and transfection efficiency was analyzed 24 hours post transfection by flow cytometry (A). Viability was determined with the ViaLight™ Plus BioAssay Kit (B). Cell viability is expressed as % viability compared to the non-transfected no program controls.

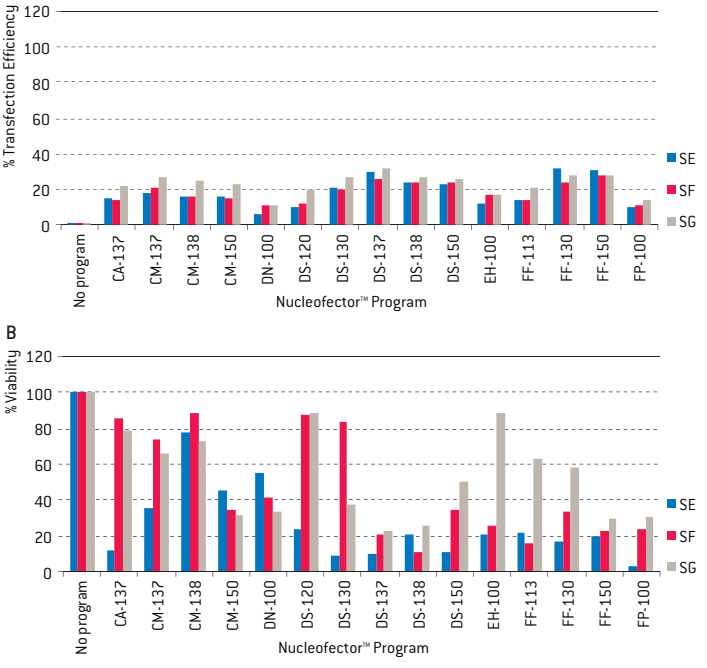

Figure 3
First optimization round for the human colorectal adenocarcinoma cell line COLO 205 using the 4D-Nucleofector™ in combination with the 96-well Shuttle™ Add-on. 2 x 10^5 cells were transfected with 0.4 µg pmaxGFP™ Vector using Nucleofector™ Solution SE, SF or SG and 31 different programs (15 of which are shown). 5 x 10^4 transfected cells were seeded and transfection efficiency was analyzed 24 hours post transfection by flow cytometry (A). Viability was determined with the ViaLight™ Plus BioAssay Kit (B). Cell viability is expressed as % viability compared to the non-transfected no program controls.

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Summary

Excellent transfection efficiencies of up to 99% combined with high cell viability can be obtained with the 4D-Nucleofector™ System for different adherent and suspension cancer cell lines. With the 96-well Shuttle™ Add-on, six 20 µL Nucleocuvette™ Strips can be processed in parallel enabling screening applications as well as offering a convenient, effective and time-saving approach when optimizing Nucleofection™ Conditions for multiple cells lacking a ready-to-use Optimized Protocol. In many cases, the optimal Nucleofection™ Conditions can already be determined during the first optimization round. The same protocol can be applied for 100 µL and 20 µL transfection volumes, allowing the transfection of variable cell numbers.

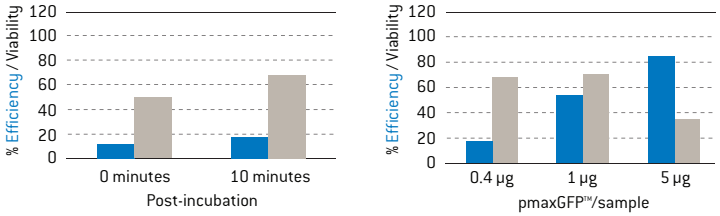

Figure 4

Further optimization of transfection conditions for the human colorectal adenocarcinoma cell line COLO 205. Cells were transfected using Nucleofector™ Solution SG and Nucleofector™ Program DP-113. (A) After transfection with 0.4 µg pmaxGFP™ Vector, 80 µL of equilibrated culture medium was added either directly to the samples (0 minutes) or after a 10-minute incubation in Nucleofector™ Solution. (B) Various amounts of pmaxGFP™ Vector (0.4 µg, 1 µg or 5 µg) were used per 20 µL sample for the transfection of COLO 205. Samples were incubated for 10 minutes in Nucleofector™ Solution prior to adding 80 µL of equilibrated cell culture medium.

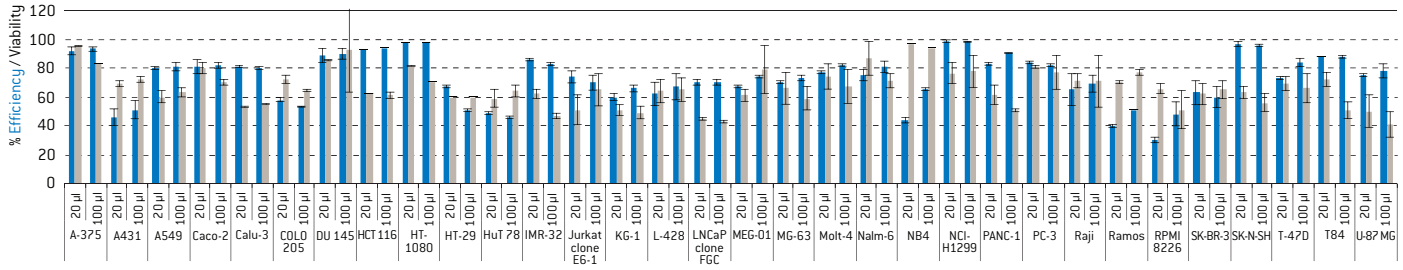

Figure 5

Transferability of Nucleofection™ Conditions from the 20 µL Nucleocuvette™ Strip to the 100 µL single Nucleocuvette™ Vessel for 32 cancer cell lines. Transfection parameters that have been optimized in the 20 µL Nucleocuvette™ Strip are directly applicable to the 100 µL single Nucleocuvette™ Format (n=3).

Contact Information

North America

Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe

Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International

Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

International Offices

Australia	+ 61 3 9550 0883
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 22 4342 4000
Japan	+ 81 3 6264 0660
Luxemburg	+32 87 321 611
Singapore	+ 65 6521 4379
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

Lonza Walkersville, Inc. – Walkersville, MD 21793