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Optimization of mouse neural stem/progenitor cell (NSC) transfection with the 4D-Nucleofector™ system

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Abstract

Transfection of neural cells comprising neurons and glial cells as well as their progenitor cells is limited by their general resistance to common used methods like lipofection or electroporation. The situation improved remarkably when Nucleofector™ technology became available, thus enabling transfection of neural cells with high efficiency and reasonable viability. However, limitations were still encountered due to the need of high cell numbers for each transfection sample. Here, we demonstrate that mouse NSCs can be transfected with high efficiency and remarkably improved survival rate by using the 4D-Nucleofector™ system. Compared to former systems the cell number used per sample has been reduced by three-fourths. This illustrates a clear improvement over existing transfection capabilities.

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Introduction

Scientific research currently uses a variety of in vitro methods to gain information about the functional importance of genes in a diverse set of culture systems. A widely used approach is the transfection of cells in order to over express or knockdown genes of interest. Regarding the generally more sensitive cells in murine culture systems, transfection of neural stem cells (NSCs) was already feasible, even though, accompanied by low transfection rates and low viability. The situation clearly improved with the availability of the Amaxa™ Nucleofector™ technology. However, two disadvantages persisted: the high amount of cells needed for each transfection and the rather high loss of sensitive NSCs caused by cell death during the procedure.

Here we investigated the performance of the new 4D-Nucleofector™ system in comparison to previous data obtained for embryonic cortical mouse neural stem cells. In comparison to the Nucleofector™ II device the new 4D-Nucleofector™ system promised advantages regarding device handling, reduction of the cell number per sample as well as ease of optimization. So far, a minimum of 3 – 5 x 10^6 cells was required for each transfection. The 4D-Nucelofector™ system now provides the option to use 16-well Nucleocuvette™ strips or 100µl Nucleocuvettes™ allowing transfections with cell numbers ranging from 2.5 x 10^5 cells up to 5 x 10^6 cells per cuvette.

In this study, we used the pEGFP-N1 plasmid to transfect 5 x 10^5 mouse NSCs. Transfected NSCs were grown as neurospheres overnight to recover from the procedure of DNA transfer and subsequently plated as single cells on coated dishes. The exact number of successfully transfected NSCs was determined by immunocytochemical staining's against EGFP, the fluorescent tracer protein of the construct. Based on an optimization matrix provided with the 4D-Nucleofector™ system we performed a two-step screening to gain optimal transfection parameters. As a result a transfection protocol derived enabling 88% GFP positive cortical NSCs with a corresponding viability of more than 70% (determined 48hours post transfection).

Materials and methods

Isolation and expansion of mouse NSC

Cortical cells were isolated from E13.5 mouse forebrains and grown as single cells in chemically defined neurosphere medium [F12 nutrient mix, DMEM (both Sigma), L-Glutamine (Fluka), Pen-Strep and B27 (Gibco)] supplemented with EGF and FGF2 as growth factors in an incubator set at 37°C and 6% CO_2 in a humidified atmosphere. Under these conditions NSCs are growing as free-floating cellular aggregates called neurospheres published previously (1, 2, and 3). After 5 days in vitro neurospheres were digested and triturated to obtain single NSC cell suspensions that were either expanded as secondary neurospheres under the same conditions as above or directly used for transfection.

Vectors

Our routinely used antibody against GFP excluded to use the maxGFP™ vector (Lonza) provided with the Nucleofector™ kit due to lower detection ability. In order to determine the transfection rates as accurately as possible we used the pEGFP-N1 plasmid (Clontech) instead.

Nucleofection™

For lower cell numbers we used 2,5 – 5,0 x 10^5 neurosphere-derived NSCs per sample and suspended them in 20µl P3 primary cell Nucleofector™ solution containing 0,5µg plasmid DNA according to the provided Lonza optimized protocol in the 16-well Nucleocuvette™ strips (20µl per sample). After transfection180µl warmed neurosphere medium was added to transfected NSCs in the cuvette. NSC were gently suspended in the cuvette and transferred into a sterile 1,5 ml tube. After centrifugation (80 x g, 5 minutes), the supernatant was discarded and the cell pellet re-suspended in 500µl neurosphere medium containing EGF and FGF2. For higher cell numbers (2,5 – 5 x 10^6) 100µl Nucleocuvettes™ were used for transfection and the neurosphere-derived NSCs were re-suspended in 100 µl P3 primary cell solution with 5µg plasmid DNA. The transfected NSCs were re-suspended in 500µl warmed neurosphere medium, transfered into a 15ml Falcon tube and centrifuged. The supernatant was discarded and the pellet was re-suspended in neurosphere medium containing growth factors and cultivated as neurospheres over night at 37°C.

Immunocytochemical stainings of transfected NSC

To determine the transfection efficiency of mouse NSCs overnight neurosphere cultures were shortly trypsinized, dissociated and 2 x 10^4 cells were plated on coated 4-well dishes containing neurosphere medium according to established protocols (1, 2, and 3). After 1 day the cultures were immunocytochemically stained for EGFP to detect NSCs expressing the pEGFP-N1 plasmid as a marker for successful transfection (4).

Results

Evaluation of the optimal Nucleofector™ program for mouse neural stem cells

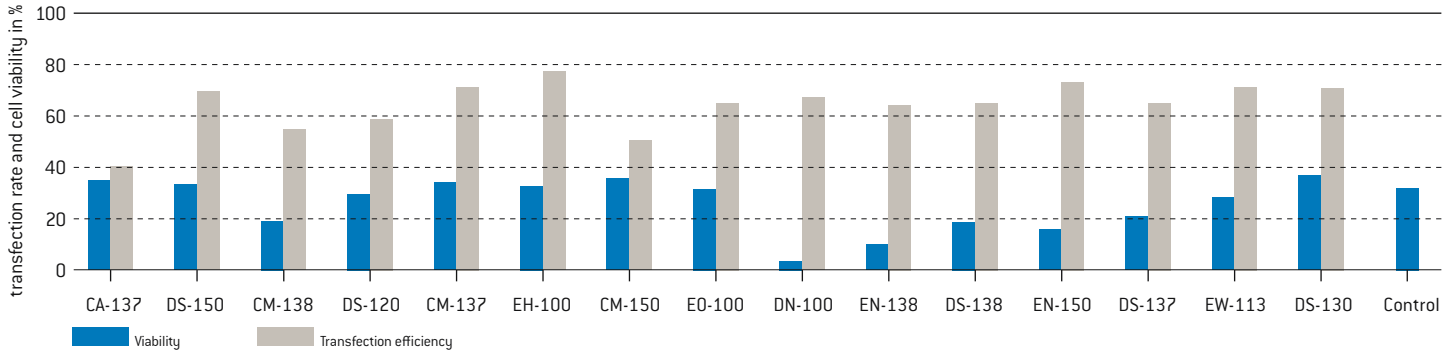
Lonza provided a primary cell optimization strategy comprising 15 different programs. These programs were initially tested in parallel on E13.5 mouse cortical neural stem cells with P3 primary cell solution using 16-well Nucleocuvette™ strips. Both, transfection efficiency and cell viability were chosen as parameters to evaluate the optimal program with respect to the low input number of just 5 x 10^5 embryonic cortical mouse NSCs. Figure 1 shows the relative transfection efficiency and cell viability of NSCs after application of 15 different progams based on the number of EGFP-positive and total cell recovery, respectively. Four programs (DS-150, EH-100, E0-100 and DS-130) showed the best compromise between cell viability and transfection efficiency and were selected for further experiments.

To increase the recovery after transfection, NSCs were cultivated as neurospheres overnight and plated as single cells on coated dishes 24hours after transfection (Figure 2). Direct expression of EGFP was used as a marker for transfection and detected by fluorescence microscopy after overnight recovery (Figure 2) as well as indirectly by immunocytochemical staining's for EGFP after 48 hours (Figure 3). Quantification of the two parameters revealed an increased transfection efficiency of 78 ± 22 % with the chosen programs, which is already improved compared to 50 – 60% obtained with the conventional Nucleofector™ II device. Relative to the viability no improvement could be detected with the four programs used, the viability yield with 31 ± 2,8 % was similar to the conventional Nucleofector™ II device.

Optimization of Nucleofector™ programs for mouse neural stem cells

In order to further improve viability of mouse NSC after transfection with the 4D-Nucleofector™ system, a second optimization round was started. Based on the four most promising programs of the first optimization,

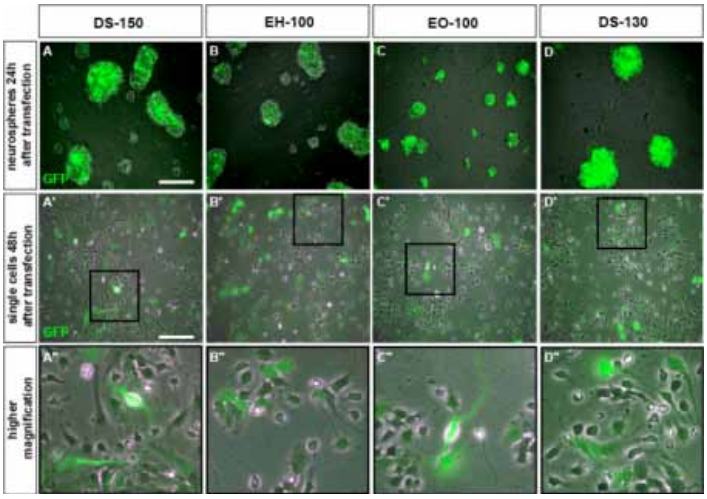
Figure 1 Cell line optimization strategy with 15 different programs



As indicated on the x-axis, 15 different Nucleofector™ programs were tested on mouse NSCs regarding the transfection efficiency (grey bars) and viability (blue bars). The programs DS-150, EH-100, E0-100 and DS-130 showed the best compromise between these parameters and were chosen for further experiments. n=1

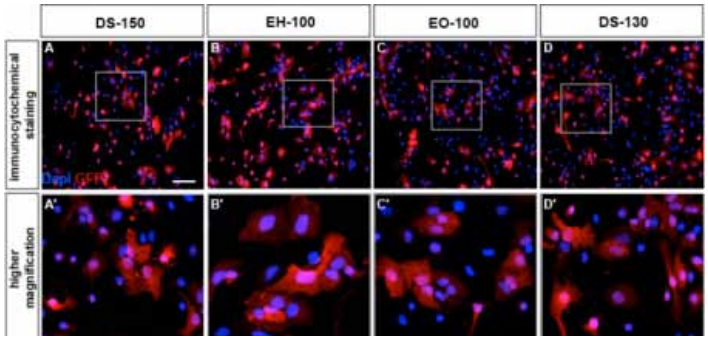
a new set of programs was selected and tested. Transfection with the pEGFP-N1 plasmid as well as analysis of data was accomplished according to the protocol as described above. Ten programs (DS-124, DS-120, DG-145, DS-146, DG-150, DG-154, DS-104, DS-109, DS-112 and DS-113) were applied to mouse NSCs and resulted in an increased cell viability and further improved the transfection efficiency. The application of program DS-112 or DS-113 increased the viability from 31 ± 2,8% to 70 ± 7,1 % (n=3). These programs also further improved the transfection efficiency to 88 ± 6,5 % (n=3; Figure 4.). Hence optimal programs could be evaluated for mouse NSCs.

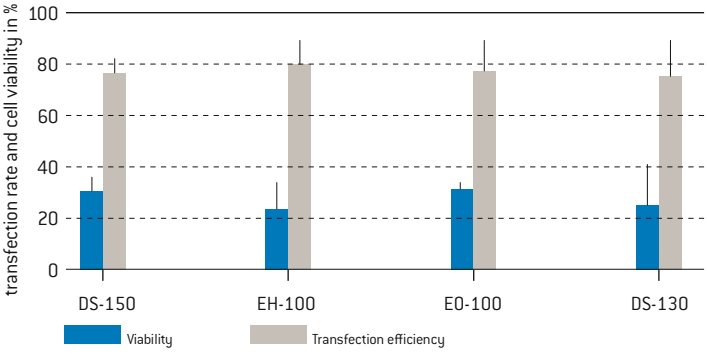
Figure 2 Transfected NSCs in neurosphere cultures and as single cells



Merged pictures of phase contrast and immunofluorescent EGFP images (green) of live cortical neurospheres cultures 24 hours post transfection of mouse NSCs with the plasmid pEGFP-N1 and the programs DS-150, EH-100, EO-100 or DS-130 are shown (A-D). Merged pictures of phase contrast and immunofluorescent EGFP images (green) of transfected, dissociated neurosphere-derived single cell cultures 24 hours after seeding (A'-D'). Pictures A''- D'' show higher magnifications of transfected single NSC cultures that are boxed in the middle panel. Scale bars are 100µm.

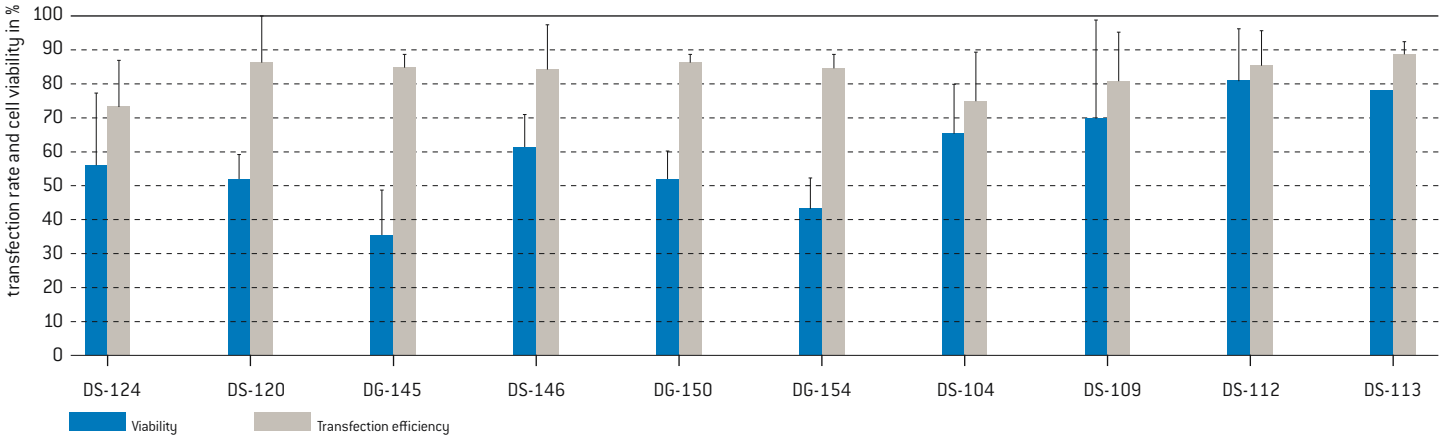
Figure 3 Immunocytochemical staining's against EGFP of transfected mouse NSCs





Merged pictures of immunocytochemical stained transfected NSCs are shown in A-D. EGFP is visualized with the fluorochrome Cy3 (red) and Hoechst-stained cell nuclei with the Dapi channel (blue). Images A'-D' show higher magnifications of transfected NSCs shown in boxes of A-D. Scale bars are 100µm. E) bar chart depicts the quantification of transfection efficiency and cell viability of mouse NSCs with the programs DS-150, EH-100, EO-100 and DS-130 as indicated. Data is expressed as mean ± SD (n=3).

Figure 4 Optimization strategy with 10 additional programs applied to mouse NSCs



The graph indicates quantification of transfection rate (grey bars) and cell viability (blue bars) of the programs applied to mouse NSCs shown on x-axis. Programs DS-112 and DS-113 showed the best compromise between the chosen parameters. The viability increased from 31 ± 2,8% up to 70 ± 7,1 % (n=3) and the transfection efficiency from 78% to 88 ± 6,5 %. Viability data is expressed as mean ± SD (n=3), transfection data is expressed as mean ± SEM (n=2).

Conclusion

The 4D-Nucleofector™ system represents a further improvement of this advanced transfection technology method suited to transfect routinely hard to transfect cells. Here, embryonic cortical mouse NSCs were tested and the data obtained clearly demonstrate that transfection of this sensitive cellular system is now possible with bona fide cell viability and high transfection rates. Due to its modularity the 4D-Nucleofector™ system is able to transfect low cell numbers (2.5×10^4) as well as high cell numbers (2.5×10^6) using the same conditions. Thus the 4D Nucleofector™ system contributes to a higher efficiency and quality regarding transfection of mouse NSCs.

References

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