BioResearch

Lonza

Transfection of Human Induced Pluripotent Stem Cells using Nucleofector™ Technology

Technical Reference Guide

Introduction

For studying differentiation pathways in human induced pluripotent stem cells (iPSCs) or modifying such pathways, it is important to find a transfection method which is efficient while maintaining pluripotency. The non-viral Nucleofector™ Technology has proven to be well suited for both iPSC generation[1-4] as well as iPSC transfection, e.g. for genome editing applications[5-11]. Here we present general guidelines for establishing Nucleofection™ Conditions for transfecting iPSCs. For more specific recommendations about genome editing please visit www.lonza.com/genome-editing.

1. Basic Protocol Concept

Similar to human embryonic stem cell (ESC) clones, generated iPSC clones may differ clone to clone regarding optimal Nucleofection™ Conditions. Thus, providing a ready-to-use Optimized Protocol with predefined conditions (i.e. one solution + one program) which are guaranteed to work with any clone is not possible. For each iPSC clone, it is recommended to first determine the optimal conditions using our pmaxGFP™ Control Vector following the „Basic Human Stem Cell Protocol" for the respective Nucleofection™ Platform*. A basic protocol describes how to easily define optimal Nucleofection™ Conditions by testing a predefined matrix of Nucleofector™ Programs and Solutions.

*For downloading the platform specific detailed protocol, please refer to www.lonza.com/protocols and filter for "stem cells".

2. Optimization Process

2.1 First Optimization Round

For 4D-Nucleofector™ X Unit**: When using the 4D-Nucleofector™ System the initial optimization experiment is comprised of 32 reactions. Seven different Nucleofector™ Programs plus a no program control are tested in duplicate with two 4D-Nucleofector™ Solutions (Table 1).

** The same applies for the 96-well Shuttle™ Add-on

Table 1. Optimization scheme for 4D-Nucleofector™ System

P3 Solution (Strip 1, n=2)		P4 Solution (Strip 2, n=2)	
CA-137	CA-137	CA-137	CA-137
CB-150	CB-150	CB-150	CB-150
CD-118	CD-118	CD-118	CD-118
CE-118	CE-118	CE-118	CE-118
CM-113	CM-113	CM-113	CM-113
DC-100	DC-100	DC-100	DC-100
DN-100	DN-100	DN-100	DN-100
No program	No program	No program	No program

For Nucleofector™ II/2b Device: When using the Nucleofector™ II/2b Device, the initial optimization experiment is comprised of 12 reactions. Five different Nucleofector™ Programs plus a no program control are tested in combination with two Nucleofector™ Solutions (Table 2).

Table 2. Optimization scheme for Nucleofector™ II/2b Device

HSC_1 Solution	HSC_2 Solution
No program	No program
A-012	A-012
A-013	A-013
A-023	A-023
A-027	A-027
B-016	B-016

The Nucleofector™ Program and Solution which turned out to be the most appropriate Nucleofection™ Condition should be used for all subsequent transfections.

2.2 Fine Tuning of Conditions

If results of the first optimization round are not fully satisfactory, our Scientific Support Team can provide fine-tuning suggestions.
Depending on the clone, transfection efficiencies up to 60% and viabilities between 50-75% can be achieved. For more details on results, you may check our cell transfection database (www.lonza.com/celldatabase) or the various publications[5-11].

3. Factors Influencing Nucleofection™ Process Results

3.1 Cell Handling Prior to Transfection

Single cell suspension: We recommend transfecting the cells in single cell suspension. Transfection of clumps leads to lower transfection efficiency and less reproducibility[12]. If single cell suspension passaging is not established, please perform some pre-experiments by testing Accutase[13, 14] and Trypsin[15] for detachment. Cultivate the cells afterwards and analyze which method led to highest viability and lowest differentiation.

Detachment of stem cells with feeder cell culture: There are three possibilities to remove feeder cells from your stem cell culture prior to Nucleofection™ Process:

- If stem cells are usually cultured on feeder cells, passage them once in matrix coated plates to remove the feeder cells (for details see reference 2). Then proceed as with feeder-free cultured cells.
- Cultivate the cells on feeder cells until the day of the experiment. Detach the stem cells with Collagenase. Then dissociate the clumps with Accutase into a single cell suspension.
- Cultivate the cells on feeder cells until the day of the experiment. Detach all cells with Accutase. Incubate the cells on an uncoated cell culture flask for 1 hour in a humidified 37°C/5% CO_2 incubator. The feeder cells will attach and the stem cells will stay in suspension. Harvest the cells in suspension.

Detachment of stem cells with feeder-free cultures: Prior to Nucleofection™ Process detach the cells from the matrix-coated plates by incubation with Accutase for 5 minutes at 37°C. Dissociate the cells into a single cell suspension by pipetting the suspension carefully up and down 4 – 6 times. Add medium to stop the Accutase reaction.

Apoptosis inhibitors: The use of apoptosis inhibitors like ROCK inhibitor[16] and neurotrophins[17] prior to and post-transfection have been reported to increase viability of human ESCs. For iPSCs, using apoptosis inhibitors seems to be advantageous as well[5-11].

3.2 Cell Handling Post-Transfection

Plating Density: The plating density post-transfection is a critical aspect for the viability of human pluripotent stem cells. Our experience shows that higher densities lead to better viability of the cells. Therefore, we recommend plating human pluripotent stem cells at densities from 4 - 6.5 x 10^5 cells per cm^2. These plating cell numbers and volumes produce optimal Nucleofection™ Results in most cases. However, an extended range of cell numbers might be tested depending on your specific needs. Because of the high plating density, a passaging step 48 hours post transfection using collagenase or dispase might be required.

Culture on matrix: When cells are cultured on matrix-coated plates the coating should be fresh. Storage of coated plates for more than 7 days leads to reduced attachment of the cells post-transfection. After cells have been transferred from the Nucleofection™ Vessel to the culture plates, the plates should be centrifuged for 3 minutes at 70xg (at room temperature).

www.lonza.com/research

References

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