Video Article

Transfecting Human Neural Stem Cells with the Amaxa Nucleofector

Steven Marchenko, Lisa Flanagan
Department of Pathology, University of California, Irvine

Correspondence to: Steven Marchenko at smarchen@uci.edu

URL: http://www.jove.com/index/Details.stp?ID=240

DOI: 10.3791/240

Citation: Marchenko S., Flanagan L. (2007). Transfecting Human Neural Stem Cells with the Amaxa Nucleofector. JoVE. 6. http://www.jove.com/index/Details.stp?ID=240, doi: 10.3791/240

Abstract

Transfection of primary mammalian neural cells, such as human neural stem/precursor cells (hNSPCs), with commonly used cationic lipid transfection reagents has often resulted in poor cell viability and low transfection efficiency. Other mechanical methods of introducing a gene of interest, such as a “gene gun” or microinjection, are also limited by poor cell viability and low numbers of transfected cells. The strategy of using viral constructs to introduce an exogenous gene into primary cells has been constrained by both the amount of time and labor required to create viral vectors and potential safety concerns. We describe here a step-by-step protocol for transfecting hNSPCs using Amaxa's Nucleofector device and technology with electrical current parameters and buffer solutions specifically optimized for transfecting neural stem cells. Using this protocol, we have achieved initial transfection efficiencies of ~35% and ~70% after stable transfection. The protocol entails combining a high number of hNSPCs with the DNA to be transfected in the appropriate buffer followed by electroporation with the Nucleofector device.

Protocol

Note: Refer to the **Passaging Human Neural Stem Cells** article (http://www.jove.com/index/Details.stp?ID=263) and the **Counting Human Neural Stem Cells** article (http://www.jove.com/index/Details.stp?ID=262) to learn how to resuspend and count hNSPCs.

Preparation

1. Make sure that there are plenty of cells available for transfection (several million cells for each DNA to be transfected).
2. Coat a tissue culture dish, into which the transfected cells will be seeded, with 10 µg/ml human fibronectin the night before transfection. Right before starting the transfection protocol, remove the fibronectin, rinse the dish with PBS, and place culture media in the dish. Leave the dish in the 37°C tissue culture incubator to warm the media. Also, place 1 ml of culture media in the incubator to pre-warm to 37°C.
3. Place the DNA(s) to be transfected, and transfection solution (from Amaxa), on ice.

Transfection

1. Wash a defined number of cells with PBS (we usually use ~ 5 x 106 cells per transfection). Pellet the cells by centrifugation (1000 rpm (~200xg) for 5 minutes), remove as much PBS as possible, and resuspend the cell pellet in 100 µl of the transfection solution provided with the transfection kit. Transfer the cell suspension to a 1.7 ml eppendorf tube.
2. Add 5 µl of the DNA (~5-10 µg of DNA per transfection) to the cell suspension and mix gently.
3. Using the Amaxa mini-pipette, transfer the cell-DNA mix into the Amaxa transfection cuvette. Make sure you have 1 ml of pre-warmed culture media for the next step. Also, remove the cell dish with culture media from the incubator and place it in the tissue culture hood.
4. Place the cuvette into the Nucleofector, rotate the wheel clockwise, and select program "A-033". Press *Enter*.
5. A successful transfection is indicated by the presence of a very dense layer of bubbles on top of the solution in the cuvette. Add 500 µl warm media into the cuvette, and using the Amaxa mini-pipette, transfer the transfected cells into the dish with pre-warmed media. Rinse the cuvette with more warm media and add the rinse to the same dish. Place the dish with cells in the 37°C tissue culture incubator.

Discussion

This protocol describes a relatively rapid and efficient transfection procedure for hNSPCs. Using this procedure, we have obtained initial transfection efficiencies of ~35%. Cells transfected by this procedure can be used for short-term studies (transiently transfected cells) or for longer-term studies if a selection agent is used to generate a stably transfected population. We have generated stably transfected cells in which ~70% of the cells express the exogenous protein. In transiently transfected hNSPCs, we have observed continued expression of the exogenous protein for ~7 days.

Acknowledgements

The authors would like to acknowledge Dr. Philip H. Schwartz of the National Human Neural Stem Cell Resource at the Children s Hospital, Orange County Research Institute for providing hNSPC cultures.

References

1. Gresch O, Engel FB, Nesic D, Tran TT, England HM, Hickman ES, K rner I, Gan L, Chen S, Castro-Obregon S, Hammermann R, Wolf J, M ller-Hartmann H, Nix M, Siebenkotten G, Kraus G, Lun K. New non-viral method for gene transfer into primary cells. Methods 33, 151-163, (2004).

2. Marchenko, S., Flanagan, L.A. Passaging Human Neural Stem Cells. Journal of Visualized Experiments, (7). http://www.jove.com/index/Details.stp?ID=263 (22 August 2007).

3. Marchenko, S., Flanagan, L.A. Counting Human Neural Stem Cells. Journal of Visualized Experiments, (7). http://www.jove.com/index/Details.stp?ID=262 (22 August 2007).

