

Dorsal Root Ganglion in Culture

Cell types:
DRG's from
embryonic rat

System:
Cellaxess ACE

Data courtesy of Uppsala University, Department of Neuroscience

Introduction

The introduction of plasmid DNA into cells with the aim to manipulate the protein expression is a widely used method in cell biology research. Several methods are available, although all methods does not suit all cellular systems. Specifically, many primary cell types, such as neurons, has proven to be notoriously difficult to transfect with high efficiency and viability.

Primary neurons can be studied directly after isolation, but are typically cultured for several days, which allows for attachment to the surface and development of networks before experiments. Neurons in networks enable the study of signaling processes, secretion and other cellular processes in live neurons.

Below we present the Cellaxess method for transfection of primary neurons in 96 well microtitreplates (MTPs). The method results in high transfection efficiency and excellent viability.

Results

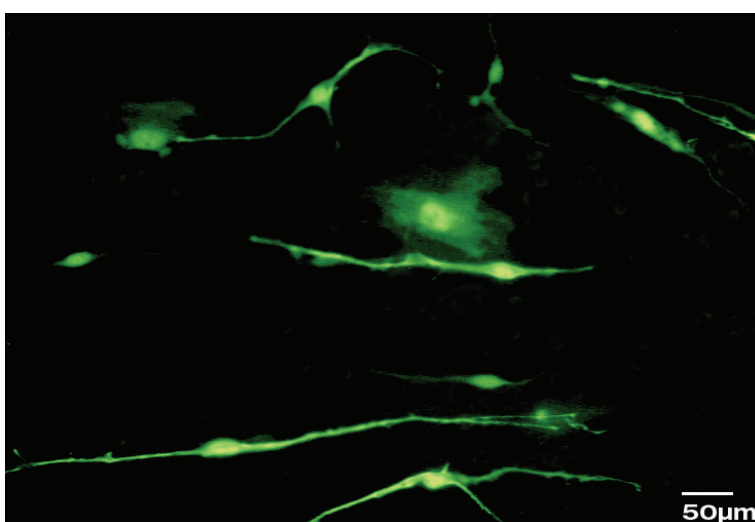
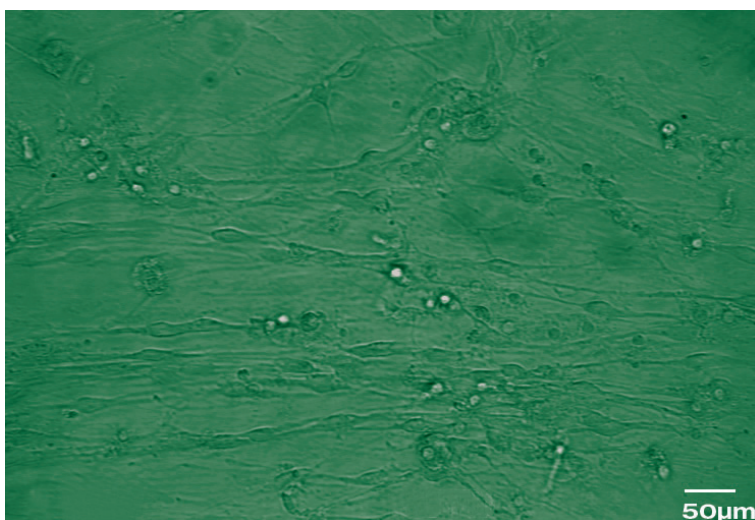
Dorsal root ganglion (DRG) primary cultures from rat E15 was plated in 96 well MTPs, and transfected with the Cellaxess ACE system. Transfection was performed at day 7-8 after plating, when the cells had developed axonal networks in culture. The cultures were dissociated mixed primary cultures, containing DRG neurons and Schwann cells (approximately 1:10). The transfection efficiency of the cells evaluated at 24 h after transfection was 25-35% for neurons and approximately 50% for Schwann cells and the reproducibility of the efficiency and viability was generally very high. The transfected cells showed no morphological difference compared to non-transfected cells. Cell viability evaluated with propidium iodide (PI) staining was 60-80%.

Transfection of developed neuronal networks

Schwann cells transfection efficiency 50%

DRG transfection efficiency 25-35%

Figure 1



Transfection with the Cellaxess ACE system

The plate containing the cells to be transfected was removed from the incubator. The transfection solution containing the pCopGFP vector (Eurogen) at 50-100 ng/ μ l final concentration was pipetted into the ACE capillary by means of a standard p100 micropipette. The dish was positioned under the ACE module, and the capillary was lowered into the dish. After an approximated 15 second wait time, the pre-programmed transfection protocol was executed. The capillary was raised, the dish was temporarily removed, and remaining solution was drained by blowing through the capillary using a p1000 micropipette. After this, the capillary was once again filled with plasmid solution, and the next transfection was carried out. Once the desired number of transfections had been carried out, the dish or plate was returned to the incubator.

Cell culture

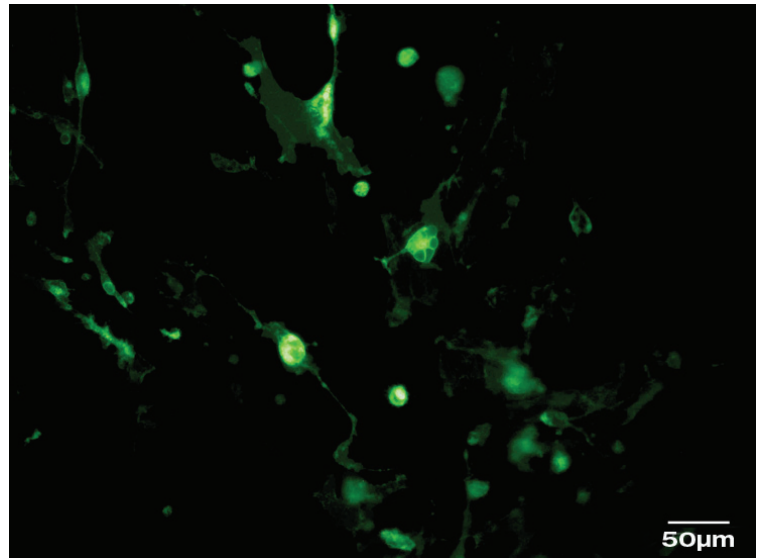
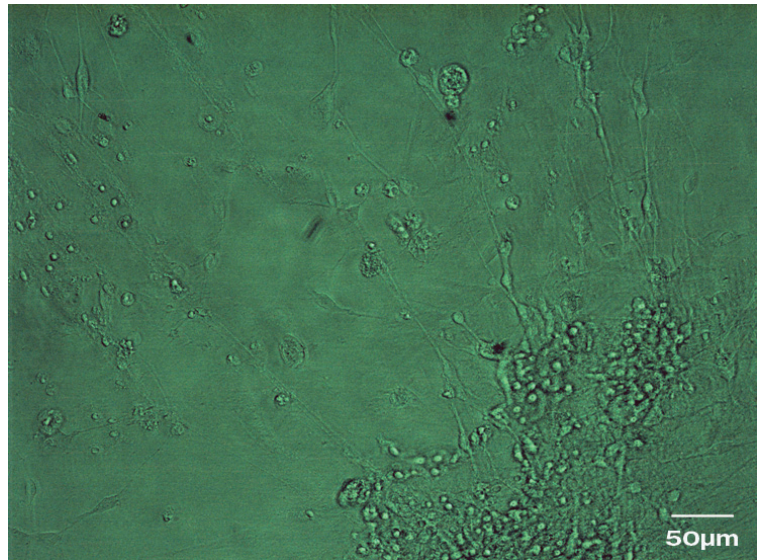
DRGs from embryonic rat (Sprague Dawley, E15) were dissected according to the (Kleitman, Wood et al. 1998) method, modified as described previously (Svenningsen, Shan et al. 2003). After dissection the DRGs were treated with 0,25% trypsin at 37°C for 15min and then dissociated mechanically. Dissociated cells were washed with L15 medium, spun down and re-suspended in Neurobasal medium containing 2% B27, 0,3% L-glutamine and 100ng/ml nerve growth factor. The cells were plated in 96 well plates pre-coated with poly-L-lysine at a density of approximately $1-5 \times 10^4$ cells/ml. The cultures were maintained in incubator (37°C, 5%CO₂ and 92% humidity) for 6-10 days before transfection. The cultures were then 60-100% confluent with DRG neurons and Schwann cells in about 1:10.

References

Kleitman, N., Wood, P. N. et al. (1998). Tissue culture methods for the study of myelination. *Culturing nerve cells*. G. Baker and K. Goslin. Cambridge, The MIT Press. 1: 545-594.

Svenningsen, A. F., Shan, W. S. et al. (2003). "Rapid method for culturing embryonic neuron-glia cell cocultures." *J Neurosci Res* 72(5): 565-73.

Figure 2



Contact us for more information:

EU: sales-eu@cellectricon.com

US: sales-us@cellectricon.com

www.cellectricon.com