Adherent Nucleofection™ of Cryopreserved Primary Rat DRG Cells - Simply Thaw, Culture and Transfect

Anthony Krantis1, PhD; Susan VanderHoek1; Barbro Tinner-Staines1; Paul Shock1; Kevin Grady, PhD2
1QBM Cell Science, Inc., Ottawa, Canada; 2Lonza Walkersville, Inc., Walkersville, MD, USA

Rat dorsal root ganglia (DRG) cells isolated and dissociated using standard procedures and then cryopreserved (non-purified), are available from Lonza. Batch tested, ready-to-use neonatal DRG cells have been proven to be ideal for in vitro study of axonal outgrowth, path finding, neuropathy, nerve re-growth, sensory receptor physiology and drug testing1-10.

The cryopreserved DRG cells can be thawed and cultured on poly-D-lysine (PDL) coated multiwell plates with demonstrated survival to 28+ days in vitro. This represents a significant step for multiwell DRG cell testing and an opportunity for automated screening. When thawed and cultured, the cryopreserved dissociated rat DRG cells show the same morphologies and neurochemical type distribution as freshly dissociated rat DRG cells. By day 3 in culture, there is an abundance of long, loosely bundled axons within the cultures as well as support (Schwann) cells. The DRG neurons display extensive neurite outgrowth and the interconnected round cell soma of the neurons are easily distinguished from the more elongate Schwann cells (Figure 1). The quality of DRG cell axon outgrowth and development can be followed with plasmid transfections of GFP where the outgrowth of fluorescent processes can be readily imaged3.

We have now examined the application of cultured neonatal cryopreserved rat DRG cells for high throughput transfection using the two different multiwell adherent electroporation formats of the Nucleofector™ Technology, the 96-well Shuttle™ Device and the 4D-Nucleofector™ Y Unit, which offers a combination of high efficiency and high throughput.

Methods

Cryopreserved neonatal DRG cells [Lonza, Cat. No. R-DRG-505] - isolated and dissociated at P2-3 [non-purified] - were thawed and cultured in the multiwell formats as directed by the optimized Nucleofection™ Protocols provided. Thawed cells were immediately aliquoted into the PDL-coated plates at 50,000 cells/ml. Transfection of the dissociated DRG cells was undertaken by Nucleofection™ with pmaxGFP™ Control Vector using either the 96-well Shuttle™ Device or the 4D-Nucleofector™ Y Unit at 2 days in vitro (DIV) according to the protocols and cultures examined up to 24 hours post transfection.
Results

**Adherent Nucleofection™ using the 96-well Shuttle™ Device**

Figure 2A shows a typical control DRG cell culture 3 DIV (cultured in 96-well Nucleocuvette™ AD Plates) that was not transfected, immunostained for neuron specific anti-β-Tubulin (Tuj-1). This can be compared with a culture 3 DIV, 24 hours post Nucleofection™ using program CM-129 (figure 2B). Program CM-129 gave optimal neuron transfection. Typically, non-neuronal cells were also transfected and these could be distinguished by their elongate Schwann cell morphology. The relative number of Schwann cell transfection/well and intensity of transfection varied with the Nucleofection™ Program used. Figure 3 shows intense transfection of Schwann cells using program CP-125. Neuronal transfection efficiency was estimated from the microscopic analysis of a series of images summed for each well, immunostained for the specific neural marker PGP9.5. The number of DRG neurons was compared to the number of neurons which were also fluorescent for maxGFP™. Maximum transfection efficiency (34%) in the 96-well Shuttle™ Device was achieved using program CM-129.

**Adherent Nucleofection™ using the 4D-Nucleofector™ Y Unit**

Cryopreserved DRG cells were cultured in 24-well culture plates and transfected in the 4D-Nucleofector™ Y Unit – configured for 24-well adherence culture formats - using AD1 4D Nucleofector Y Kit with pmaxGFP™ Vector. Cells displayed similar high quality neural networks and cell morphology but with even higher neuron transfection efficiency (55%, program EH-166, figure 4). Program ED-158 gave similar transfection efficiency and quality (not shown).

The typical pseudo unipolar morphology of the DRG neurons was not impaired by transfection and can be seen in examples of transfected DRG neurons presented in Figure 5.

**Conclusion**

The simplicity and robust output of this combination of ready-to-use cryopreserved primary cells and Nucleofector™-based neuronal transfection will help speed the research and screening applications of genetically modified dissociated DRG cells.
References

1. Anesti et al., 2008 Efficient delivery of RNA Interference to peripheral neurons in vivo using herpes simplex virus. Nucleic Acids Research 36:1–12
2. Eibl et al., 2010 Multi-potent Neurotrophin Antagonist Targets Brain-Derived Neurotrophic Factor and Nerve Growth Factor. J. Exp. Therapeutics 332:446-454
7. Tominaña et al., 2011 Matrix Metalloproteinase-8 Is Involved in Dermal Nerve Growth:Implications for Possible Application to Pruritus from In Vitro Models. J. Investigative Dermatology 131:
8. Riera et al., 2009 Compounds from Sichuan and Melegueta peppers activate, covalently and noncovalently, TRPA1 and TRPV1 channels. B. J. Pharmacology 157:1398–1409
9. Yoshinori T., 2009 Phosphorylation of Nogo Receptors Suppresses Nogo Signaling, Allowing Neurite Regeneration. Science Signaling 2:

www.lonza.com/research

Lonza Cologne GmbH – 50829 Cologne Germany

For research use only. Not for use in diagnostic procedures.
The Nucleofector™ Technology is covered by patent and/or patent pending rights owned by the Lonza Group Ltd or its affiliates.
Alexa Fluor is a registered trademark of Miliapore
Unless otherwise noted, all trademarks herein are marks of the Lonza Group or its affiliates. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party’s intellectual property rights.

© Copyright 2012, Lonza Cologne GmbH. All rights reserved.
WP-NucDRG 09/12 CD-SP003