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

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4D-Nucleofector™ Y Unit

Introduction

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 testing[1-10].

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 imaged[3].

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.

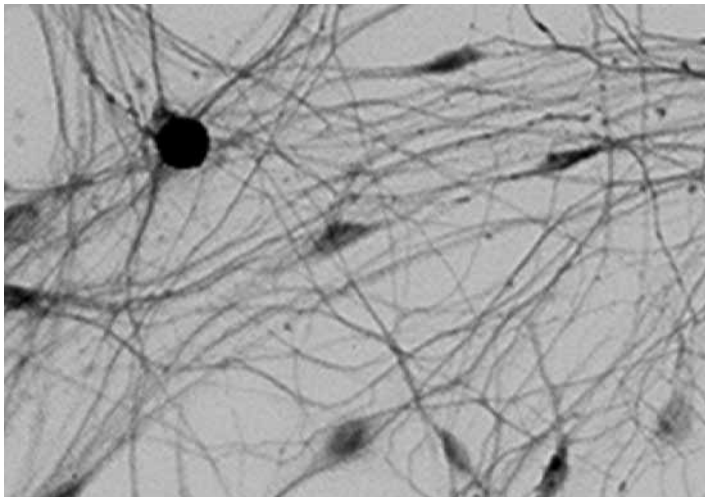

Figure 1. Cryopreserved Rat DRG cells, thawed and cultured 4 days in 96-well plates were stained using neuron specific anti-juvenile ß-tubulin antibody (personal gift W. Staines) and Schwann cell specific anti-S-100 antibody (Chemicon). The DRG cells in culture display abundant outgrowth and round cell soma. Schwann cells are more elongate.

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 summated 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.

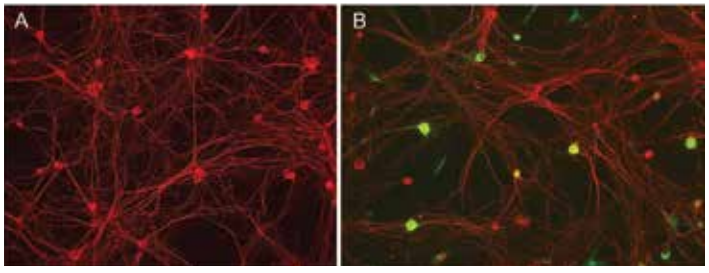

Figure 2. Cryopreserved dissociated rat DRG thawed and cultured in 96-well Nucloecuvette™ AD Plates. (**A**). Control (non-transfected) culture 3 DIV. (**B**). Culture transfected using 96-well Shuttle™ Device at 2 DIV and fixed 24 hours post Nucleofection™ (program CM-129). Neuronal networks are stained using anti Tuj-1 antibody (red; personal gift W. Staines). Transfected cells can be seen in green (maxGFP™ Protein). In (**B**), round neuronal cell soma can be distinguished from the more elongate soma of Schwann cells.

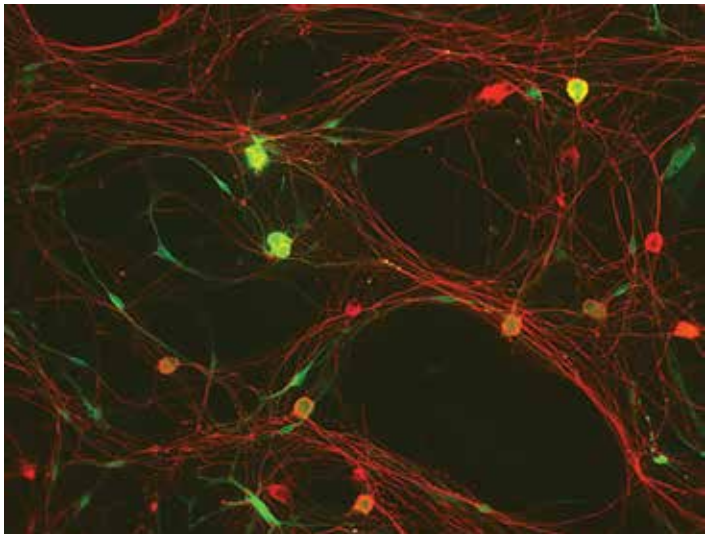

Figure 3. High magnification overlayed image of cryopreserved DRG cells in culture, transfected at 2 DIV (96-well Shuttle™ Program CP-125) and fixed 24 hours post Nucleofection™. Transfected neurons and Schwann cells can be easily observed (maxGFP™ Protein, green).

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.

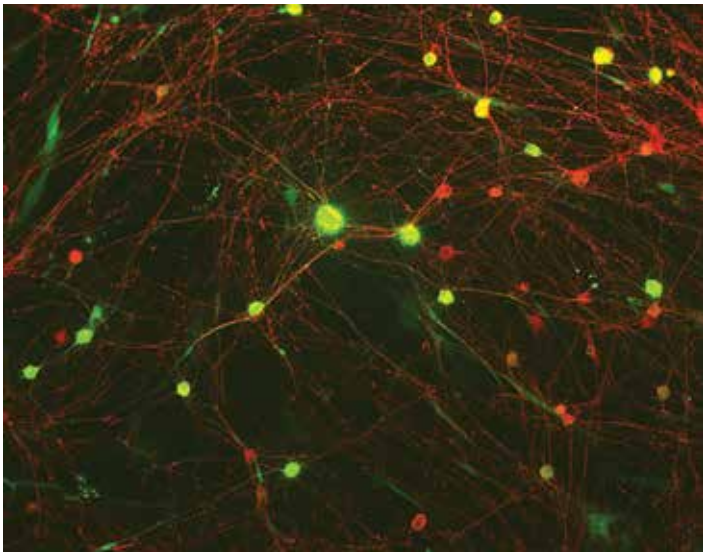

Figure 4. Cryopreserved dissociated rat DRG cells were thawed and cultured in 24-well plates for Nucleofection™ using 4D-Nucleofector™ Y Unit. DRG cell culture was transfected at 2 DIV and fixed 24 hours post Nucleofection™ (program EH-166). Neuronal networks are stained using anti Tuj-1 antibody (red; personal gift W. Staines). Transfected neurons and Schwann cells can be seen in green (maxGFP™ Protein).

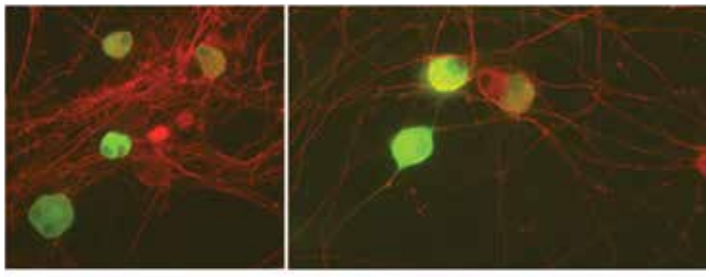

Figure 5. High magnification overlayed images of cryopreserved DRG cells in culture after transfection with 4D-Nucleofector Y (maxGFP™, green). The neural networks are immunostained using anti Tuj-1 antibody (red; personal gift W. Staines).

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