BioResearch

Lonza

Nucleofection™ Citation List

Selected Publications for
Nucleofector™ Technology

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Immunology Research

HIV-1 Nef Down-Modulates C-C and C-X-C Chemokine Receptors via Ubiquitin and Ubiquitin-Independent Mechanism

Chandrasekaran P *et al.* (2014) PLoS ONE 9(1): e86998. doi:10.1371/journal.pone.0086998

Nef is an HIV protein that is involved in the pathogenesis of HIV infections by dysregulating the trafficking of immune cell receptors. In this comprehensive study, the authors examined how Nef modulates various chemokine receptors, e.g. CXCR4. For this purpose they transfected various primary cell types (human T cells and monocytes) and T cell lines (CEM, Jurkat, K562) using Nucleofection™ with plasmids or siRNA to overexpress or down-regulate potentially involved components of the suggested signaling pathway. For easy-to-transfect cell lines (e.g. HEK293) a lipid reagent was used.

Optimization of Adult Sensory Neuron Electroporation to Study Mechanisms of Neurite Growth

McCall J *et al.* (2012) Frontiers in Molecular Neuroscience 5(11)

Primary adult dorsal root ganglia neurons (DRGs) are an ideal model for studying many neuronal functions because they survive in culture in an adult stage while most other mammalian neuron types that can be kept in culture for longer time are isolated from embryonic or early postnatal brain. Aim of this study was to find an electroporation protocol for adult DRGs that provides efficient transfection of larger plasmids to study neurite outgrowth, limits the number of required cells and minimizes the use of serum. For that purpose the authors first compared Invitrogen's Neon Transfection System with Lonza's 4D-Nucleofector™ System for transfection of a large plasmid (approx. 10 kb) into primary rat DRGs. After testing several transfection parameters on each device, the authors found that the 4D-Nucleofector™ X Unit was at least 3 times better than the Neon in terms of transfection efficiency (39-42%) and gave slightly better cell viability. Subsequent experiments with 4D-Nucleofector™ X Unit also showed that cell numbers can be reduced to $3x10^5$ cells (in 20 µl) with 2 µg DNA and cells can be kept serum-free post transfection.

Neurobiology Research

Neuronal Hypoxia Induces Hsp40-Mediated Nuclear Import of Type 3 Deiodinase As an Adaptive Mechanism to Reduce Cellular Metabolism

Jo S *et al.* (2012) Journal of Neuroscience 32(25):8491– 8500

The authors present a study of induced hypoxia in rat hippocampal neurons. The main idea is that hypoxia in neurons leads to the nuclear import of type 3 deiodinase (D3). To understand the mechamism of nuclear import and involved proteins, one step was to study the effect of overexpression or down-regulation of Hsp40. Therefore, the human neuroblastoma cell line SK-N-AS was transfected with Hsp40-expressing plasmid or Hsp40 shRNA vector using Lonza's 4D-Nucleofector™ System with solution SF and program DN-100. Overall the authors found that the Hsp40-mediated import of D3 into the nucleus of the cells is an adaptive mechanism which decreases thyroid hormone signaling and thus minimizes cell metabolism and damage caused by hypoxia.

The Long Non-coding RNA Gomafu is Acutely Regulated in Response to Neuronal Activation and Involved in Schizophrenia-associated Alternative Splicing

Barry G *et al.* (2013) Mol Psychiatry, 1–9

The author studied the involvement of a long non-coding RNA (lncRNA) called "Gomafu" in alternative splicing which is known to play a role in development of schizophrenia. As part of their study they wanted to investigate whether their findings in mouse neurons can be confirmed in human neurons. For this purpose they differentiated human induced pluripotent stem cells (iPSCs) into neurons. Those iPSC-derived neurons were transfected with a plasmid encoding "Gomafu" after 2-5 days in culture using the 4D-Nucleofector™ Y Unit. Transfection efficiencies of 50% and more were achieved. They were able to show that that lncRNAs Gomafu is associated with activation and pathological schizophrenia-related alternative splicing.

Comparison of Ectopic Gene Expression Methods in Rat Neural Stem Cells

Kim W *et al.* (2013) Korean J Physiol Pharmacol 17: 23-30

The authors compared lipofection, electroporation, Nucleofection™ and retroviral transduction in order to identify the most efficient method to transfect rat neural stem cells (NSC). With about 35% transfection efficiency, the 4D-Nucleofector™ System outperformed lipofection (3-4%), standard electroporation using the Nepagene Nepa21 system (about 25%) and retroviral transduction (about 30%).

Reprogramming and Genome Editing

Transcriptome Comparison of Human Neurons Generated Using Induced Pluripotent Stem Cells Derived from Dental Pulp and Skin Fibroblasts.

Chen J *et al.* (2013) PLoS One 8(10):1371

This group is using induced pluripotent stem cells (iPSCs) for *in vitro* disease modeling in a variety of neuro-psychiatric disorders. The iPSC technology is providing the opportunity to generate disease patient specific stem cells which can be differentiated to neurons to study the disease specific gene expression pattern. Dental pulp cells derived from deciduous teeth are a potential source of somatic cells. From a biological perspective, dental pulp could prove to be a better source of iPSCs for disease modeling neuropsychiatric disorders because of its developmental origins. The reprogramming was carried out using the 4D-Nucleofector™ System for transfection of non-integrating plasmids containing the genes OCT4, SOX2, KLF4, L-MYC, LIN28, and a p53 shRNA vector. The group described the expression profiles from fibroblast and dental pulp iPSC derived neurons.

Generation of Human Induced Pluripotent Stem Cells – Using Epigenetic Regulators Reveals a Germ Cell-Like Identity in Partially Reprogrammed Colonies

Goyal A *et al.* (2013) PLoS ONE 8(12): e82838. doi:10.1371/journal.pone.0082838

In this study alternative approaches were investigated to increase pluripotency of iPSCs derived from fibroblasts. First the authors identified potential new reprogramming factors by comparing expression profiles of human ESCs, iPSCs and fibroblasts. Then they analyzed the candidates for their efficacy by reprogramming fibroblasts. They tested overexpression of different factors in combination with morpholino-based silencing. Due to the proven suitability of the Nucleofector™ Technology for transfecting morpholinos, they either used the 4D-Nucleofector™ X Unit or the 96-well Shuttle™ Add-On. Their results suggest that the induction of DNMT3B, PRMT5 and AURKB expression and silencing of SETD7 expression in human fibroblasts might enhance the reprogramming of fibroblasts into iPSCs.

Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity

Ran FA *et al.* (2013) Cell 154:1380–1389

The authors tested modifications of the CRISPR/Cas9 genome editing system with the aim to reduce off-target effects. They evaluated the modified components in HEK293FT cells and the human embryonic stem cell line HUES62. While using a lipid reagent for transfection of HEK293FT, the 4D-Nucleofector™ System was used for HUES62.

Genome Engineering Using the CRISPR-Cas9 System

Ran A *et al.* (2013), Nat Prot 8(11): 2281–2308

This publication from the group of Feng Zhang (Broad Institute of Massachusetts Institute of Technology and Harvard, USA) gives comprehensive background information about CRISPR technology and provides a detailed protocol how to use Lonza's 4D-Nucleofector™ X Unit for CRISPR-based genome editing in HUES9 (a human stem cell line) and HEK293 cells. It also includes protocols for functional analyses, tips for minimizing off-target effects and FAQs.

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