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Tech-Trends Application Note Volume 4, Series 7

96-well electroporation assay for neurons

ABSTRACT
Following an injury, central nervous system (CNS) neurons show a very limited regenerative response which results in their failure to successfully form functional connections with their original target. This is due in part to the reduced intrinsic growth state of CNS neurons, which is characterized by their failure to express key regeneration associated genes (RAGs) and by the presence of growth inhibitory molecules in CNS environment that form a molecular and physical barrier to regeneration. Hutson, T. et al, has optimized a 96 well electroporation and neurite outgrowth assay for post natal rat cerebellar granule neurons (CGNs) cultured upon an inhibitory cellular substrate expressing myelin-associated glycoprotein or a mixture of growth inhibitory chondroitin sulfate proteo glycans. Optimal electroporation parameters resulted in 28% transfection efficiency and 51% viability for post natal rat CGNs..

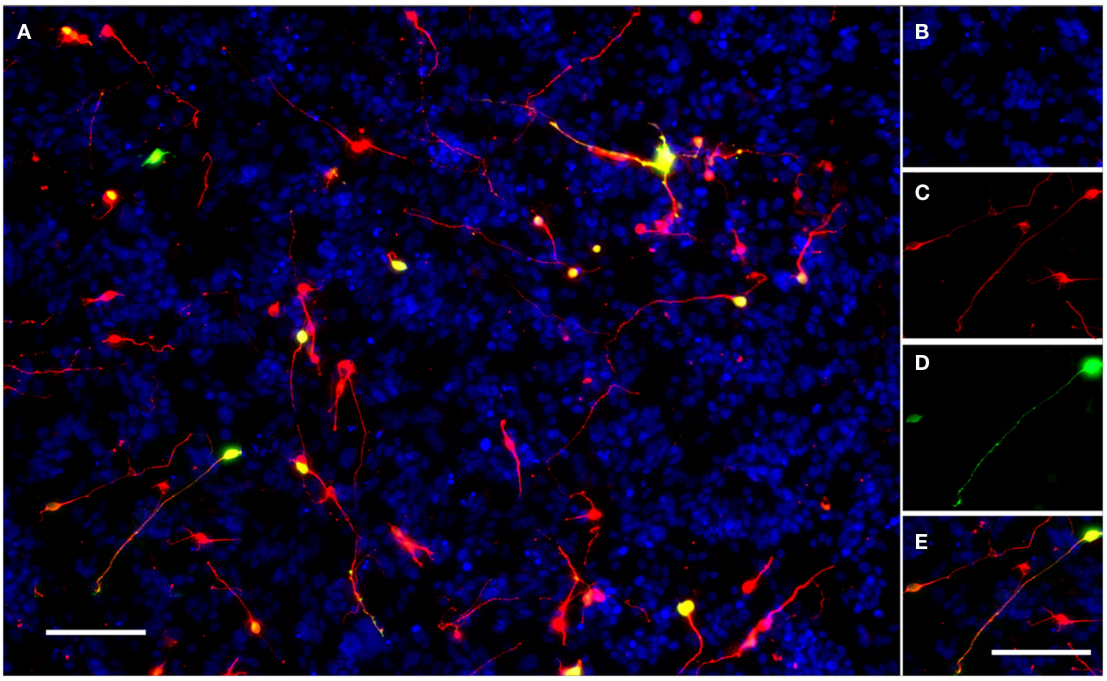

FIGURE 1 Atypical field of view showing electroporated CGNs cultured on a monolayer of growth-permissive CHO-R2 cells. (A) A typical field of view taken using the IN Cell Analyzer 1000 semi-automated cell imager with a 10× Nikon Apo Plan objective. **(B)** DAPI stained nuclei of CHO-R2 cells and CGNs. **(C)** CGNs stained for beta-III-tubulin. **(D)** eGFP positive,transfected CGNs expressing GFP. **(E T**he merged image; transfected neurons appear yellow. Scalebars: 100µm.

INTRODUCTION

Most non-viral methods of transfection are inefficient at transfecting primary neurons, resulting in low transfection efficiencies (1–10%) and high cytotoxicity (Washbourne and McAllister, 2002). In contrast, when optimized, electroporation can provide an efficient method of transfecting primary neurons with reported transfection efficiencies of up to 50% (Washbourne and McAllister, 2002; Zeitelhofer et al., 2007). The recent production of 96-well plate electroporators allows the rapid, efficient, and relatively inexpensive screening of large numbers of genes in primary neurons (Buchser et al.,2010).

METHODS

The desired amount of DNA was added to 30µl internal neuronal buffer (INB) containing 135 mM KCl, 0.2 mM $CaCl_2$, 2 mM $MgCl_2$, 10mM HEPES, 5mM ethylene glycol tetra acetic acid (EGTA), in sterile water, pH7.3 (Buchser et al., 2006), and pipetted into the wells of the 96-well electroporation plate. The 250,000 CGNs/well were resuspended in 35µl INB/well and then added to the 96-well electroporation plate wells, which already contained the DNA/INB solution and had a gap size of 2mm (BTX, Holliston, MA, USA). The 96-well electroporation plate was then placed in the HT-200 plate handler (BTX) which was connected to a ECM830 square-wave pulse generator (BTX Harvard Apparatus) that generates and delivers the specified electric pulse. The ECM 830 square-wave pulse generator was connected to a TDS1002 oscilloscope (BTX) to monitor the delivered pulse parameters. For CGN electroporation optimization the ECM 830 square-wave pulse generator was set to deliver a range of parameters. For voltage optimization CGNs were electroporated with a single pulse with a duration of 1ms and 1 of 11 different voltages (0,200,220, 240, 260,280,300,320,340,360,or 380V). For pulse length optimization CGNs were electroporated with a single 300V pulse at a pulse length of either 0 (non-electroporated), 0.1,0.2,0.3,0.5, 0.7, 0.9,1,or 2ms. Pulse number optimization was assessed using 300V for 1ms with either 0 (non-electroporated),1,2,or 3 pulses. In all further electroporation experiments the optimized electroporation pulse parameters (1 pulse at 300V, 1ms pulse length) were used. It has previously been shown that lower temperatures, e.g., 4˚C or 21˚C at the time of electroporation followed by immediate warming to 37˚C increases transfection efficiency and cell viability (Rols et al.,1994). Therefore in all experiments, solutions were kept on ice or at room temperature and immediately following electroporation, 80µl of Hibernate E (BrainBits, Springfield, IL, USA), pre-warmed to 37˚C was added per well. Electroporated CGNs were then plated at a density of 3.5×10^4 CGNs/well in a 96-well assay plate.

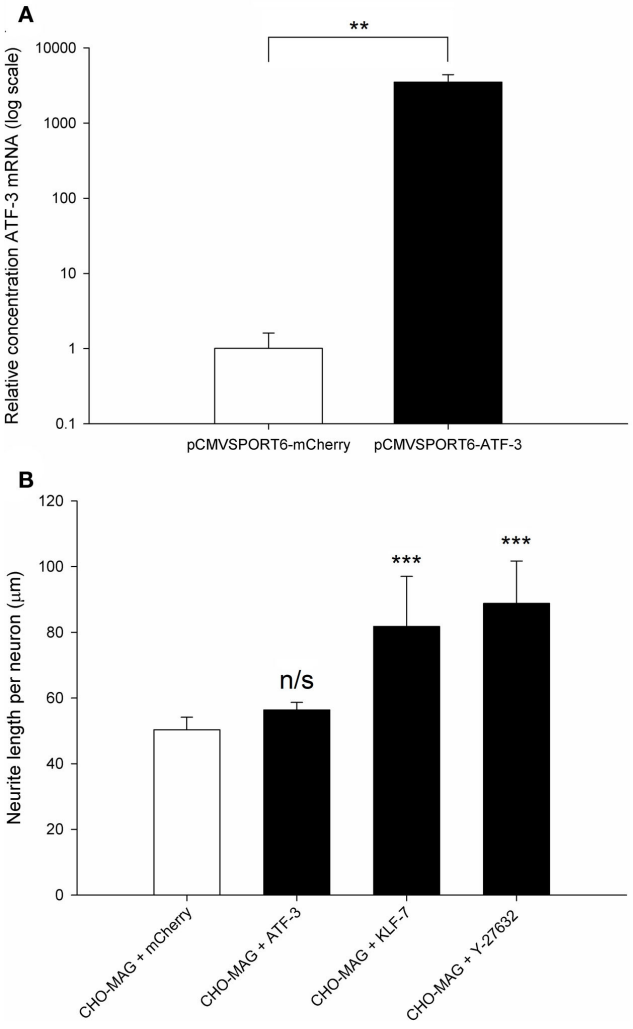

FIG 6 Regeneration-associated genes are successfully over-expressed following electroporation, and over-expression of KLF-7 but not ATF-3 was able to enhance neurite outgrowth on an inhibitory MAG substrate.(A) Quantification of the relative level of ATF-3 mRNA inelectroporated CGNs. Significantly higher levels(2700-fold) of ATF-3 were detected in CGNs transfected with the ATF-3 plasmid compared to CGNs transfected with the mCherry plasmid. Values represent mean and SD; analysis was performed using an independent sample sStudent's *t* -test (***P* <0.01), *n*=4. **(B)** Quantification of the mean neurite length per transfected neuron of CGNs cultured on CHO-MAG cells. The CHO-MAG inhibition could be partially reversed by over-expressingKLF-7 or addition of 20µ MY-27632 but not by over-expressingATF-3.Values represent mean and SD; analysis was performed using one way ANOVA with Dunnett's *post hoc* tests comparing to CHO-MAG cells (****P* <0.001), *n*=8.

Tech-Trends Application Note

Volume 4, Series 7

DISCUSSION

Successful transfection of a range of primary neurons has been reported using several different electroporation systems. Using a BTX 96-well electroporation system identical to the one in the present study, identical buffers and similar parameters (340V, 900µs, 1 pulse), post natal mouse CGNs have been previously transfected with an average efficiency of 26.8% and a viability of 37% (Buchser et al., 2006). These values are consistent with our results although a slightly higher cell viability (50%) was observed in the present study, which is probably a result of the lower voltage used. CGNs have also previously been transfected using the Bio-Rad electroporation system, which is not a medium-throughput system and requires larger numbers of neurons (Mertz et al., 2002). This study reported a transfection efficiency of 10% and cell viability of 44%.The transfection efficiency is considerably lower than what was observed in the present study. The Lonza single cuvette electroporation system has been demonstrated to successfully electroporate several neuronal types. Postnatal CGNs have been electroporated with an average transfection efficiency of 20% (Gartner et al., 2006), which is consistent with the results presented in this study. Embryonic hippocampal and cortical neurons have been successfully electroporated, obtaining an average transfection efficiency of 50% (Dityateva et al., 2003; Gartner et al., 2006). RGCs and DRG neurons have also been electroporated with reported transfection efficiencies of 28 and 20% respectively (Leclere et al., 2005). These studies demonstrate that different electroporation systems, buffers and protocols can be used to successfully transfect a range of neurons to study gene function. However, the number of cells required for the single cuvette electroporation systems is inappropriate for medium-throughput screening.

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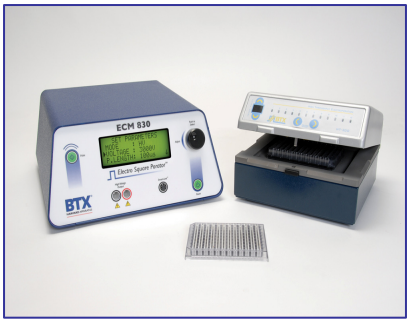

CONCLUSION

In the present study a 96-well medium-throughput electroporation system from BTX was chosen as it allows complete control of the electroporation parameters delivered to the cells, unlike the predetermined programs of the 96-well Lonza system. In addition, the recent optimization of an effective electroporation buffer (INB) composed of inexpensive benchtop reagents removed the requirement to use the expensive proprietary electroporation buffers (Buchser et al., 2006).

Hutson, T. et al., Optimization of a 96-well electroporation assay for postnatal rat CNS neurons suitable for cost-effective medium-throughput screening of genes that promote neurite growth.
***Frontiers in Molecular Neuroscience*, 2011, (4) 55;1**