BTX Applications

Tech-Trends
Volume 3, Series 11

Electroporation of polarized epithelial cells

Efficient Electroporation of DNA and Protein into Confluent and Differentiated Epithelial Cells in Culture

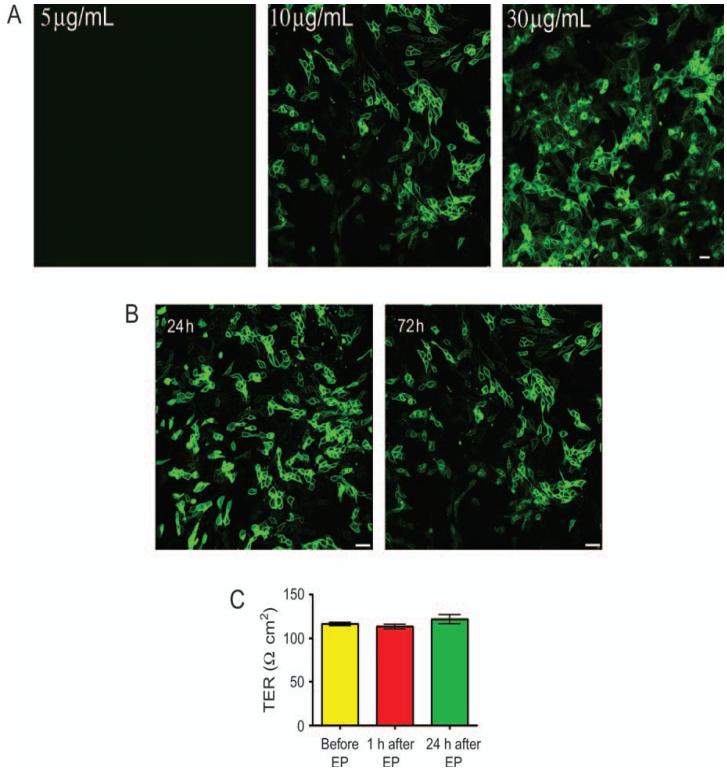
Electroporation-mediated delivery of molecules is a procedure widely used for transfecting complementary DNA in bacteria, mammalian and plant cells. This technique has proven very efficient for the introduction of macromolecules into cells in suspension culture and even into cells in their native tissue environment, e.g. retina and embryonic tissues. However, in spite of several attempts to date, there are no well-established procedures to electroporate polarized epithelial cells adhering to a tissue culture substrate (glass, plastic or filter).
This publication describes the development of a simple procedure using the BTX ECM 830 Square Wave System which works efficiently and reproducibly for a variety of epithelial cell lines in culture.

Electroporation Protocol

Cell Preparation: MDCK II cells were maintained in DMEM (Cellgro) supplemented with 10% FBS (ICN, Aurora,OH, USA), 1%glutamine and 1%penicillin–streptomycin at 37ºC in 95% air/5% CO_2 atmosphere. MDCK cells were plated on 12 mm polycarbonate or polyester transwell filter units (0.4-µm pore size) at a density of 250 000–300 000 cells per filter and cultured for 5 days to allow development of polarity. Medium was changed every other day. Filters exhibiting a TER around 80–100 Ω cm^2 were used for electroporation and immunofluorescence analysis. ARPE-19 cells (ATCC), a spontaneously arising human RPE cell line, were grown in Chee's essential medium containing 1% bovine retinal extract on laminin (BD Biosciences, CA, USA) coated transwell filters for 6 weeks to allow maximal development of polarity. The medium was changed twice a week. After 6 weeks, TER of ARPE-19 cells was 50 Ω cm^2.

Electroporation Settings:

Choose Mode:	LV
Voltage:	300 V
Pulse #:	1
Gap:	5 mm gap spacing
Field Strength:	600 V/cm
Temp:	Room Temperature
Transfectant:	5 – 30 µg
Post Treatment:	Media was replaced in both chambers and the cells were incubated at 37ºC.



Determination of optimal plasmid concentration and TER. A) MDCK cells were polarized for 5 days on polyester– polycarbonate filters and then subjected to electroporation using different concentration of CD147–GFP plasmid DNA. Concentration range of 5 – 30 mg/mL of DNA diluted in IEB was used. 10 to 30 mg/mL of DNA was highly effective concentration in yielding more than 60% cells positive around the electrode. Electroporation with the concentration of 5 µg/µL of CD147–GFP DNA did not result in any positive transfected cells. Images were obtained by laser scanning confocal microscope (LSCM) with 20 objective. Bar: 20 µm. B) Expression of CD147–GFP protein was robust after 24 h and was sustained till 72 h. Electroporation was performed with 10 µg/µL CD147–GFP plasmid DNA on polarized MDCK cells, and images were obtained by LSCM with 20 objective. Bar: 20 µm. C) TER was measured on MDCK monolayers on filters before, 1 h and 24 h after electroporation (EP) of various plasmids at a concentration of 10 – 15 µg/µL. No significant differences were observed in TER values before and after electroporation.

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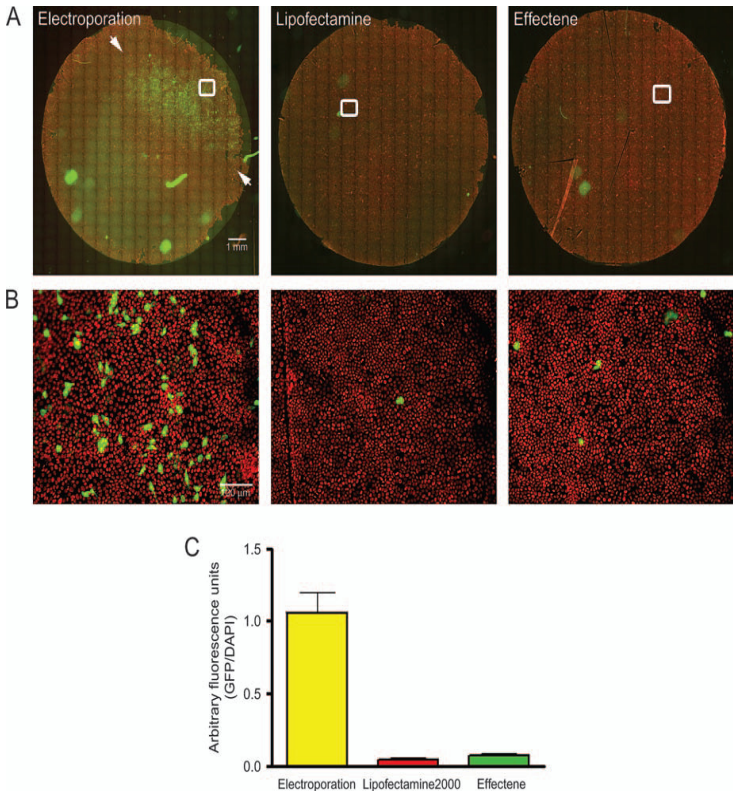
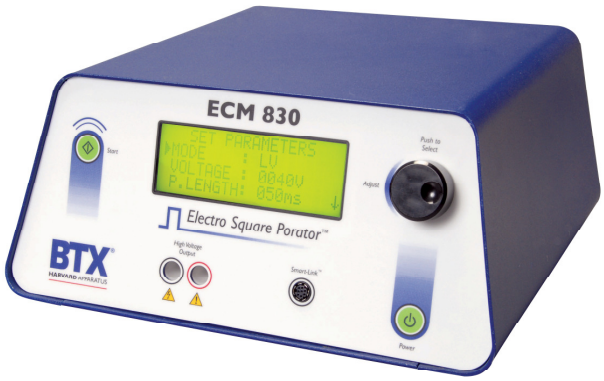
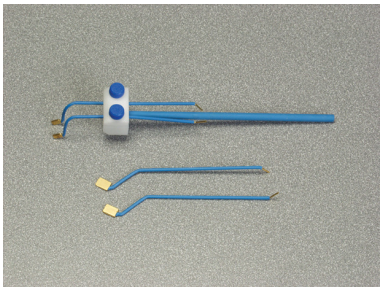


Comparison of efficacy of electroporation technique versus cationic lipid reagents. Fully polarized MDCK cells were electroporated with 10 mg/mL of CD147–GFP or transfected with lipid reagents, Lipofectamine and Effectene. After overnight incubation, filters were fixed and imaged. A) Entire 12-mm transwell filters were scanned to have an overview of the transfection in the epithelial monolayer. Bar: 1 mm. B) A magnified single field (white box from A) from the entire 12-mm transwell filter is shown. Bar: 100 mm. C) Individual images were acquired with a high-sensitivity camera for quantification purposes. Total GFP fluorescence from 10 positive fields were averaged and normalized to the number of nuclei as measured by DAPI fluorescence. Arbitrary fluorescence units (GFP/DAPI) obtained with different transfection methods are plotted in the graph.

ECM 830 Square Wave Generator (catalog 450052)



5 x 7 mm Genepaddle (catalog 450170)



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