Parabodo caudatus post-electroporation cell viability quantification using exponential decay electroporation

Version 3

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Abstract

The process of developing transient transfection protocols in *P. caudatus* involved initial testing and determination of proper electroporation buffers and parameters (voltage strength, pulse duration and number) to maximize cell viability. Our results suggest that the type of electroporation buffer is critical for maintaining cell viability throughout the experiment and is essential for determining the optimum electric field range.

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Protocol

Growth conditions

Step 1.

Parabodo caudatus (ATCC 50361) was used in this study. Initially, *P. caudatus* was grown in 50 % ATCC seawater 802 media. Subsequently, seawater was replaced with distilled water in order to reduce the high electrical conductivity during the electroporation. Briefly, this is a cerophyl-based media enriched with 3.5 mM sodium phosphate dibasic (Na_2HPO_4) and with *Klebsiella pneumoniae* added as a food source. Cultures were incubated at 22°C and sub-cultured weekly in fresh T-25 vented tissue culture flasks (Falcon brand, Fisher Scientific) containing 30 ml of fresh media.

Electroporation Parameters

Step 2.

Prior to electroporation of *P. caudatus* cells in the MicroPulser Bio-Rad (CN 165-2100) exponential decay system (Fig. 1), cell pellets were re-suspended in 200 µl MilliQ water, 1 % seawater, 10 % seawater¸ 10 % cytomix, or 50 % cytomix and transferred to 2-mm gap cuvettes. The cells were electroporated with applied voltages of 300 V (E = 1,500 V/cm), 500 V (E = 2,500 V/cm), and 800 V (E = 4,000 V/cm). The pulse duration in milliseconds (ms) after each electroporation was recorded (Table S1). The cells were immediately transferred to a 1.5 ml Eppendorf tube containing 1 ml of fresh growth media (ATCC 802 medium prepared with distilled water) for recovery. To determine cell viability, aliquots (20-30 µl) of electroporated cells for each electric field applied were quantified as described above.

Figure 1: Electric field waveforms employed for transient and stable transfection of *Bodo caudatus*. Three independent electroporation systems were used for reproducible transfection including a) our microfluidic electroporation platform (Garcia et al., 2017), the NEPA21 square-wave transfection system (BulldogBio), and the MicroPulser™ exponential decay electroporator (Bio-Rad). Additionally, the b) signature waveforms for the NEPA21 square wave transfection system of 'poring' and 'transfer' pulses for electroporation are shown. Note: The time scale in Fig. 1a is a zoomed-in version of the red-dashed box from Fig. 1b.

Garcia, P.A., Ge, Z., Kelley, L.E., Holcomb, S.J., and Buie, C.R. (2017) High efficiency hydrodynamic bacterial electrotransformation. *Lab Chip* (**17**): 490-500.

Post-Electroporation Cell Viability Quantification

Step 3.

Using the exponential decay system, *P. caudatus* cells did not survive exposure to 1000 V in any of the above mentioned electroporation buffers (Table S1). In contrast, 40-50 % of cells were viable post-electroporation when a single exponentially decaying pulse was applied at 800 V (E = 4,000 V/cm) in all tested electroporation buffers. When the maximum voltage was limited to 500 V (E = 2,500 V/cm) cell viability increased to between 60-70 %. Applied voltages of 300 V (E = 1,500 V/cm) resulted in the highest cell viability of about 80-90 %.