Genetic transformation of Parabodo caudatus using square wave electroporation system and pUB-GFP plasmid Version 2

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Abstract

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Protocol

Step 1.

1- *Parabodo caudatus* (ATCC50361) was grown in ATCC Medium 1525: Seawater 802 medium.

2- Cells at logarithmic growth phase, approximately $1x10^7$ cells of *P. caudatus*, were harvested by centrifugation at 5000 x g for 30 seconds and re-suspended in 200 µl of 100 % cytomix, or 50 % cytomix (diluted with DI H_2O) and 10 to 20 µg of circular plasmid DNA (pUB-GFP, Addgene 11155, Matsuda 2014) in 2-mm cuvette.

3- The NEPA21 Electro-Kinetic Transfection System (Bulldog Bio), utilizing square wave pulses was used for electroporation of *P. caudatus* cells. The NEPA21 system uses two sets of square pulses to achieve transfection (Figure 1). The first sets of 'poring' pulses are high in amplitude and short in duration in order to electroporate the cells in a reversible manner. The second set of 'transfer' pulses then facilitates electrophoretic transport of charges species such as DNA into the cell cytoplasm.

4- We initially used the same electroporation parameters that have been applied for diatoms transformation (see Miyahara et al., 2013). However, we did not establish successful transformation based on these parameters.

5- We successful transformed *P. caudutus* using the electroporation parameters described in (Table 2).

6- It is important to note that successful electroporation conditions may vary among the organisms, the utilized buffers, and the size of the plasmid DNA.

7- The green fluorescent protein (GFP) signal was detected under fluorescence microscopy using the

FITC filter set for several days (Figure 4)

Figures and Tables are uploaded in the 'MORE' folder

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.

Figure 4: Exponential decay and square wave transfection of *Bodo caudatus*. Fluorescence imaging confirmation of *P. caudatus* after a) transient transfection with pEF-GFP using the MicroPulser™ exponential decay electroporator, after b) transient transfection with pEYFP-Mitotrap using 800 V (E_{max} = 4,000 V/cm) in the exponential decay electroporator, and after c) transient transfection with pUB-GFP using the NEPA21 square-wave transfection system at 99 V (E_{max} = 500 V/cm). Panel d) shows merged fluorescence image from c) with the brightfield image.

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