Transient genetic transformation of Bodo caudatus using square wave electroporation system and pUB-GFP plasmid

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Abstract

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Protocol

Step 1.

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

2- Cells at logarithmic growth phase, approximately 1x107 cells of B. 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 ddH2O) and 10 to 20 µg of circular plasmid DNA (pUB-GFP, Addgene 11155) in 2mm cuvette.

3- The NEPA21 Electro-Kinetic Transfection System (Bulldog Bio), utilizing square wave pulses was used for electroporation of B. 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 B. caudutus using the electroporation parameters described in (table 1).

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 signal was detected under fluorescence microscopy using the FITC

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filter set (Figure2)

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