Biolistic transformation experiment on Eutreptiella gymnastica, Pyramimonas parkeae and Bigelowiella natans- 1250 psi gene gun

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Abstract

The protocol describes our attempt to transform three marine protists by biolistic.

Citation: Anna Vanclová and Vladimír Hampl Biolistic transformation experiment on Eutreptiella gymnastica, Pyramimonas parkeae and Bigelowiella natans- 1250 psi gene gun. **protocols.io**
dx.doi.org/10.17504/protocols.io.ibvcan6
Published: 06 Jun 2017

Protocol

Step 1.

Approximately 1-5 × 10^6 cells of *E. gymnastica* and *P. parkeae* and 1-5 × 10^8 cells of *B. natans* were used for the experiment

Step 2.

Thick cell suspensions were layered on agar plates just prior to the experiment.

Step 3.

Each sample was bombarded by gold particles coated with 5 µg of plasmid DNA with organism-specific upstream and downstream region, antibiotic resistance gene and gene for firefly lucipherase (Eutre-PACLUC, Pyr-G418LUC, Bigelo-G418LUC) labelled by fluorescein using *Label*IT system (Mirus).

Step 4.

Two separate experiments were performed on *E. gymnastica* and *P. parkeae* using 1 µm and 0.6 µm particles, respectively. Only 0.6 µm particles were used on *B. natans* because of its small size.

Step 5.

Particles were delivered by PDS-1000/He (Bio-Rad) on 1250 psi, with 6 cm distance between the macrocarrier and the plate.

Step 6.

Cells were transferred to fresh media immediately after the particle bombardment.

Step 7.

DNA, DNA-coated particles and DNA-treated cells were kept in dark as much as possible to minimize the loss of fluorescence.

Step 8.

The cultures were left undisturbed for several hours before the addition of antibiotics (10 µg/ml puromycin in case of *E. gymnastica*, 400 µg/ml geneticin in case of *P. parkeae* and *B. natans*).

Step 9.

1 ml worth of cells from each sample was fixated by 1% formaldehyde and mounted onto microscopic slide with Vectashield medium.

Step 10.

After this step, the cells were cultivated for 14 days in normal conditions (18-20°C, 12 hour light cycle).