Nucleofection of Pyramimonas parkeae, Chromera velia, Bigellowiella natans, Eutreptiella gymnastica, Neovalkampfia damariscottae

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Abstract

The protocol decribes the procedure and results of our attempts to transform several species of marine protists by nucleofection.

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Protocol

Material

Step 1.

Reagent kits for nucleofection were obtained from Lonza and experimental procedures were performed according to manufacturer's mannual on Amaxa Nucleofector® II device.

Lonza reagent kits used in the experiments:

- Human T Cell Nucleofector® Kit for stimulated human cells
- Basic Parasite Nucleofector® Kit 1 for parasitic protozoa
- Basic Parasite Nucleofector® Kit 2 for parasitic protozoa

Antibiotics

Step 2.

After nucleofection cells were placed in the growth media. Antibiotics (puromycin or geneticin) were added after 24h.

Measurement of Lucipherase activity

Step 3.

Luciferase activity was measured 1 week after growth in antibiotic selection. In case there were not enough cells in culture, the growth time was extended until enough viable cells could be obtained.

1. Break cells by beatbeater: 75-150 µm glass beads, 4800g (max), 1 min
2. Centrifuge at maximum speed.

3. Transfer 30 µl of supernatant to measuring tube. Use 30 µl of lysis buffer as blank sample.
4. Add 100 µl luciferin and measure activity immediately.