Experimental check of DNA delivery into E. gymnastica by electroporation

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Abstract

This protocol was used to check, whether the DNA can be delivered to Eutreptiella gymnastica.

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Protocol

DNA labeling

Step 1.

90 µg of EutreptiellaPACLUC plasmid was labelled with tetramethylrhodamine using Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit and the instructions in the kit's manual: 900µl reaction containing 64 µl DNA (concentration 1.4 µg/µl), 45 µl IT reagent and 90 µl labeling buffer was incubated on heating block at 37°C for 1 hour. The DNA was then precipitated with 96% ethanol and 5M NaCl to discard the excess labeling reagents. The labeled, concentrated plasmid DNA resuspended in distilled water was stored in -20°C and dark.

Cell cultivation and preparation

Step 2.

E. gymnastica culture was grown in F2 media with 3% seawater in 200ml tissue flask under 12h light cycle and 20°C. Prior to the experiment, the cells were harvested by spinning down on 2000 g for 10 minutes and resuspended in mannitol buffer (0.77M mannitol) to simulate the osmolarity of seawater while using non-conductive chemical. The cells were counted using Bürker chamber and diluted to the final concentration of 1.3 × 107 cells/ml.

Electroporation

Step 3.

The culture was transferred to 0.2 or 0.4 cm micropulser electroporation cuvettes (400 µl of suspension, that is 5 × 106 cells, per cuvette) along with 30 µl of labeled DNA, incubated briefly on ice and then electroporated in Gene Pulser Xcell™ (BioRad) electroporator using following settings:

- EP1: exponential protocol, 1.5 kV, 25 µF + 0.4cm cuvettes
- EP2: exponential protocol, 0.5 kV, 200 µF + 0.2cm cuvettes

The cells were transferred to fresh F2 media immediately after electroporation and kept in dark for 15

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minutes. Non-electroporated cells which were otherwise handled the same way were used as a negative control.

Fixation and slide preparation

Step 4.

The cells were then fixated in 1% formaldehyde for 30 minutes, spinned down and resuspended in small volume of media. The concentrated suspension of fixated cells was used for making microscopic slides which were then kept overnight in wet chamber and dark in 4°C.

Fluorescent microscopy

Step 5.

The slides were checked under Leica SP8 confocal microscope. The overlapping signals of rhodamine and chlorophyll autofluorescence were distinguished by setting the spectral detector specifically to the emission wavelengths of TM-rhodamine. The successful distinction between the two signals was also tested by FLIM (fluorescence lifetime imaging) as chlorophyll autofluorescence decays rapidly in comparison to rhodamine.

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