Protocols for mRNA electroporation

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Abstract

This protocol we have used for the electroporation of mRNA encoding reporter gene Lucipherase into following organisms: *Chromera velia*, *Alexandrium minutum*, *Euglena gracilis*, *Pyramimonas parkease*, *Pyramimonas orientalis, Eutreptiella gymnastica, Pseudonitzschia multiseries* and *Trichomonas vaginalis* (a control). In any case we have not detected a specific lucipharease activity.

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Protocol

PROTOCOL FOR MRNA ELECTROPORATION

Step 1.

Prepare luciferase mRNA using mMESSAGE mMACHINE® T7 Ultra Kit.

Step 2.

Clean mRNA using MEGAclear™ Kit.

Step 3.

Check the quality of mRNA by rabbit reticulocyte lysate translation and measurement of luciferase activity.

Step 4.

Prepare cells for electroporation to the final concentration 1x107cells/ml.

Step 5.

Place 4mm electroporation cuvettes on ice and pipet 300 µl of cells.

Step 6.

Add 2 µl of RNasin.

Step 7.

Add 1-5 µl of mRNA and mix well by pipetting.

Step 8.

Incubate on ice for 5 minutes.

Step 9.

Electroporate using various settings (see results).

Step 10.

After electroporation immediately place cells into the fresh media.

Step 11.

After 6-12-18-24 hours take samples for measurement of luciferase activity.

LUCIFERASE ACTIVITY MEASUREMENT

Step 12.

Break cells by beatbeater: 75-150 µm glass beads, 4800g (max), 1 min

Step 13.

Centrifuge at maximum speed.

Step 14.

Transfer 30 µl of supernatant to measuring tube. Use 30 µl of lysis buffer as blank sample.

Step 15.

Add 100 µl luciferin and measure activity immediately.