Transient luciferase expression in Ostreococcus (OTTH595, RCC809) and Bathycoccus

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Abstract

This protocol describes the quantification of luciferase activity in Ostreococcus and Bathycoccus cells that have been transformed with a firefly luciferase coding sequence upstream of the OTTH595 high affinity transporter promoter. Using this methods we were able to show that O. tauri promoters work in Ostreococcus RCC809 and Bathycoccus.

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Protocol

Step 1.

1) Prepare CCLR Buffer

Solution stock	Final concentration
Potassium phosphate 1 M, pH 7.8	100 mM
EDTA 500 mM, pH 8	1 mM
Triton X 100	1%
Glycérol 50%	10%

Cool the buffer on ice.

2) Start rom the 40 ml transformation (see protocol on Transient transformation). Transfer cells in a 50 ml falcon tube

3) Centrifuge at 8000g for 10 min at 4°C

4) Discard the supernatant and resuspend cells in 300 µl of CCLR in 2ml eppendorf

5) Add 2 tungsten beads.

6) Break the cells in a tissu Lyser (2 x 45 sec. at 30 Hz).

7) Centrifuge for 5 minutes à 4°C, 6000g.

8) Transfer the supernatant in a new Eppendorf

9) Centrifuge for 20 minutes at 4°C, 13000gu

10) Save the supernatant and Keep on ice.

Protein dosage using the bicinchoninic assay (BCA)

Step 2.

Principle : Proteins reduced Cu(II) to Cu(I) under alcaline conditions. Bicinchoninic acid is highly specific for Cu (I), which forms a purple complexe that can be colorimetrically quantified at a 562 nM. Absorbance is proportional to the amount of of proteins.

1) Mix 1 ml of bicinchoninic acid with 20 µl of copper sulfate at 4% (W/v) for each sample

2)For each sample, add 20 µl of protein extract to the 1m BCA reagent in 1.5 ml eppendof.

3) incubate tubes at 37°C for 30 min (you should include a BSA standard between 0 and 2 mg/ml).

4) Transfer samples in cuvettes for measurement by spectrophotometry of absorbance at 562 nm.

Luciferase assay

Step 3.

1) Prepare Luciferase assay reagent (LAR)

Stock solution	Final concentration
Tricine 1M pH 7.8	100mM
MgCl2 500mM	5 mM
EDTA 500mM pH8	100µM
DTT 1M	3.3mM
CoA sodium salt hydrate MM 767.53	270µM 2.07mg/10ml

D Luciférin 10mM	500µM
ATP dissodium salt hydrate MM 551 275.5 mg/ml	500µM

2) Keep LAR on ice in the dark until use

3) Transfer cell extracts in 96 wells white microplates (Nunc)

4) Place themicroplate in a luminoter (such as Berthold Centro)

5) Read the luminescence 5 secondes after automated injection of LAR

Quantification of luciferase expression

Step 4.

Normalize the luminescence value (RLU) by the amount of protein. You should include a background control (no cell extract) .