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Transient transformation of Ostreococcus species (OTTH595, RCC809 and RCC802) and Bathycoccus V.3

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1 Works for me dx.doi.org/10.17504/protocols.io.83uhynw

Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

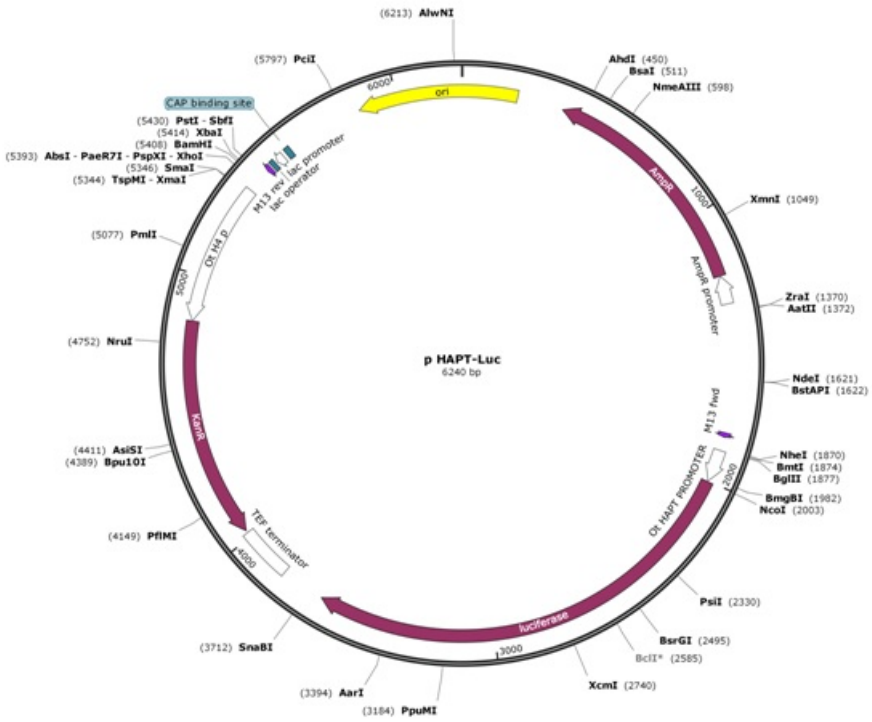
This protocol describes the preparation of cells and introduction of DNA into the cells by electroporation. For selection of stable transformants or measure of transient gene expression see related protocols.

MATERIALS TEXT

Ostreococcus lucimarinus (RCC802)

pHAPT:Luc vector (Djouani Tahri et al., PLOS ONE 2011 https://doi.org/10.1371/journal.pone.0028471)

pHAPT:Luc map



PHAPT:Luc sequence

O. tauri pHAPT:Luc6240 bp ds-DNAcircular
DEFINITIONsynthetic circular DNA

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Validation of *O. lucimarinus* transgenic lines by PCR (Figure 4A)

Forward primer: 1GAGCGCAACGGTACCCGGGCGGTACCTGTGCG
Reverse primer: CTGGCGACGCTGACGGCGTTACTTCACGT

Bathycoccus prasinos (RCC4222)

pH4:KanMx pHAPT:Luc transgene (PCR product)

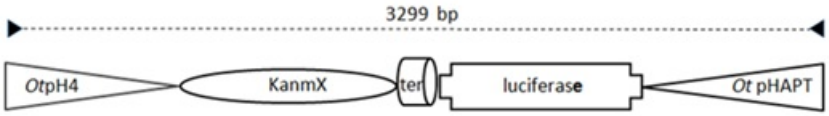
Construction ofthe transgene by PCR :

The transgene was generated by fusion PCR as described in Shevchuk et al. (2004) using DNA templates described below and oligonucleotides mapped on the sequence below.
Promoters of Histone H4 and high affinity phosphate (HAPT) transporters were amplified using (Fas1/Ras1) and (Fas4/Ras4)oligonucucleotides respectively. KanMx and Luciferase sequences were amplified from pHAPT:luc plasmid template using oligonucleotides (Fas2/Ras2) and (Fas3/Ras3). Final PCR was done on amplified fragments using (Fas5/Ras5) oligonucleotides.

Oligonucleotide sequences:
Fas1:CCGGCTTCGTGATGCCTTGGATGTTGTCTC
Ras1: TCGAAACGTGAGTCTTTTCCTTACCcatTGTGTTTGATTTATAATGAGGTTTTCTT
Fas2: AAGAAAACCTCATTATAAATCAAACACAatgGGTAAGGAAAAGACTCACGTTTCGA
Ras2: CCAAGAAGGGCGGAAAGATCGCCGTGTAAGAAATACCGTCTATCATCGATGAATTCGA
Fas3: CGAATTCATCGATGATAGACGGTATTTCTTACACGGCGATCTTTCCGCCCTTCTTGG
Ras3: AAGCATATATACATACACACACATACAAAATGGAAGACGCCAAAAACATAAAGAAAGG
Fas4: CCTTTCTTTATGTTTTTGGCGTCTTCCATTTTGTATGTGTGTGTATGTATATATGCTT
Ras4: AAGTGTACACGACCAAACGCGCGTCGAC
Fas5: CCACCTTTACCTCTGCCGGACATTGTGA
Ras5: GCGCGGTAATATCTACGAGGTAGCACGAG

Note that transformants were checked by PCR using (to amplify Kanx

Map of the transgene:



Sequence of the transgene:

4020 bp ds-DNAlinear

REFERENCE1(bases 1 to 4020)
AUTHORSFY Bouget and JC Lozano

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1621 agtactgaca ataaaaagat tcttgttttc aagaacttgt catttgtata gttttttttat
1681 attgtagttg ttctatttta atcaaatgtt agcgtgattt atatttttttt tcgcctcgac
1741 atcatctgcc cagatgcgaa gttaagtgcg cagaaagtaa tatcatgcgt caatcgtatg
1801 tgaatgctgg tcgctatact gctgtcgatt cgatactaac gccgccatcc agtgtcgaaa
1861 acgagctcga attcatcgat gatagacggt atttcttaca cggcgatctt tccgcccttc
1921 ttggccttta tgaggatctc tctgattttt cttgcgtcga gttttccggt aagacctttc
1981 ggtacttcgt ccacaaacac aactcctccg cgcaactttt tcgcggttgt tacttgactg
2041 gcgacgtaat ccacgatctc tttttccgtc atcgtctttc cgtgctccaa aacaacaacg

2101 gcggcgggaa gttcaccggc gtcatcgtcg ggaagacctg cgacacctgc gtcgaagatg
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2221 ttaatcagag acttcaggcg gtcaacgatg aagaagtgtt cgtcttcgtc ccagtaagct
2281 atgtctccag aatgtagcca tccatccttg tcaatcaagg cgttggtcgc ttccggattg
2341 tttacataac cggacataat cataggacct ctcacacaca gttcgcctct ttgattaacg
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2461 ccgaccgcgc ccggtttatc atccccctcg ggtgtaatca gaatagctga tgtagtctca
2521 gtgagcccat atccttgcct gatacctggc agatggaacc tcttggcaac cgcttccccg
2581 acttccttag agaggggagc gccaccagaa gcaatttcgt gtaaattaga taaatcgtat
2641 ttgtcaatca gagtgctttt ggcgaagaag gagaataggg ttggcaccag cagcgcactt
2701 tgaatcttgt aatcctgaag gctcctcaga aacagctctt cttcaaatct atacattaag
2761 acgactcgaa atccacatat caaatatccg agtgtagtaa acattccaaa accgtgatgg
2821 aatggaacaa cacttaaaat cgcagtatcc ggaatgattt gattgccaaa aataggatct
2881 ctggcatgcg agaatctcac gcaggcagtt ctatgaggca gagcgacacc tttaggcaga
2941 ccagtagatc cagaggagtt catgatcagt gcaattgtct tgtccctatc gaaggactct
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3061 gactgaaatc cctggtaatc cgttttagaa tccatgataa taatttttg gatgattggg
3121 agctttttt gcacgttcaa aatttttgc aacccctttt tggaaacgaa caccacggta
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3241 gcaactgcaa ctccgataaa taacgcgccc aacaccggca taaagaattg aagagagttt
3301 tcactgcata cgacgattct gtgatttgta ttcagcccat atcgtttcat agcttctgcc
3361 aaccgaacgg acatttcgaa gtactcagcg taagtgatgt ccacctcgat atgtgcatct
3421 gtaaaagcaa ttgttccagg aaccagggcg tatctcttca tagccttatg cagttgctct
3481 ccagcggttc catcttccag cggatagaat ggcgccgggc ctttctttat gtttttggcg
3541 tcttccattt tgtatgtgtg tgtatgtata tatgctttgg gaatatatgt tcacagaatg
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3661 gctttttgct ttttttcaac cacccggatt tttgcttttt ttcaaaacaa cccaccgacc
3721 gtaaatgttg tgtgttcttg tttctgttgg ggctgctttc ttttagagga gggaggatgc
3781 attcagagtt aatatattat atgtgctccg agatctgtgg tatacgagga gttggtgttg
3841 gctttttaat acacaaaata cgcctaaacg cgaggaggcg tcgttgaaac gtaaaggtac
3901 tattactcgt gctacctcgt agatattacc gcgcgtaatt agaagtcgtg ggagttgttg
3961 tcgttgtcgt tgtattcgtc gacgcgcgtt tggtcgtgta cactttactc gcgccgcgac
//

Validation of *B. prasinos* transgenic lines by PCR (Figure 4b)

Oligonucleotide sequences

1(Fas5) CCACCTTTACCTCTGCCGGACATTGTGA

2TCGAAACGTGAGTCTTTTCCTTACC

3 TCGCCTCGACATCATCTGCCCAGATGC

4 (Ras5)GCGCGGTAATATCTACGAGGTAGCACGAG

Reference:

Nikolai A. Shevchuk, Anton V. Bryksin, Yevgeniya A. Nusinovich, Felipe C. Cabello, Margaret Sutherland, Stephan Ladisch /Nucleic Acids Research/, Volume 32, Issue 2, 16 January 2004, Page e19, https://doi.org/10.1093/nar/gnh014

Cell preparation

1 1) Starting from a culture of Ostreococcus tauri, RCC809 or Bathycoccus in stationary phase, innoculate cultures at 1 million cells/ml as determined by flow cytometry (Accuri C6 BD) in 200 ml plastic flasks in Artificial Seawater supplemented with Keller medium supplement (trace metals, vitamins, nitrate and Phosphate as described in Djouani Tahri et al., PloS ONE 2011).For each transformation (including control), you should plan on using 50 ml de culture in exponential phase.

2) Grow cells for 4 to 5 days depending on the light conditions, until they reach densities of 30 to 40.10^6 cells/ml.
3) Count cells by flow cytometry. Check by SyBR Green II straining that bacterial contamination is below 2%.
4) Transfer lcultures to 50 ml Falcon tubes.
5) Centrifuge at 8000g for 10 min at 4°C.
6) Remove the supenatant, resuspend the cell pellet in 1 ml de sorbitol 1M (pH 7.5) in H_2O MQ, at 4°C.
7) Transférer the cell suspension to 1.5 ml eppendorf.
8) Centrifuge at 8000g for 10 min at 4°C.
9) Remove 900 µl of supernatant
10) R**esuspend cells by gently pipeting.**

Electroporation of the transgene

2 1) Add 5µg of transgene DNA to cell suspension. Keep on ice for 5 minutes. The transgene consist of the high affinity phosphate promoter fused to the firefly luciferase (see Djouani Tahri et al., PloS one 2011).
2) Transfer cells to a 2 mm electroporation cuvette (Biorad).
3) Apply an electric field
For Ostreococcus tauri (OTTH595) : **capacitance:** 25µF, **resistance** 600 Ω, **voltage** 1.35KV.
For Ostreococcus sp RCC809 : **capacitance:** 25µF, **resistance** 600 Ω, **voltage** 1.4KV.
For Bathycoccus (RC4222) : **capacitance:** 25µF, **resistance** 600 Ω, **voltage** 1.5KV.
For Ostreococcus lucimarinus RCC802 : **capacitance:** 25µF, **resistance** 600 Ω, **voltage** 1.2KV.
4) Add 1ml of fresh culture Medium to resuspend the cells.
5) Add 40 ml of culture medium and transfer to a culture flask.
6) Incubate at 20°C overnight in a light incubator.

At this stage, transient transgene expression can me measured or stable transformants can be selected (see relevant protocols).