# *GreenHardtii Project* Experiments

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## Lab Comission


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**Student Members:**

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# Methods

## *Chlamydomonas reinhardtii* growth

The strain that will be utilized is cell wall deficient, the used in [1] is the strain cw15-30-derived UVM4 C. The culture medium in which they grow are TAP (solid) and TAPS (liquid). Light stimulation will be required, for which a structure capable of covering the Erlenmeyer flasks or the petri dishes in which *C. reinhardtii* would be growing while uniformly illuminating with the whole visible light spectrum will be designed and built. In [1] the lamp that is put into use has the following characteristics: Nano Light, 11 Watts, Dennerle, Vinningen, Germany, which provided constant light with the following details: 2500 lux, eq. 72.5 µE/m2·s1.

For the construction of calibration curves the medium to be used is TAPS without acetate.

[1] Chávez, M. N., Schenck, T. L., Hopfner, U., Centeno-Cerdas, C., Somlai-Schweiger, I., Schwarz, C., ... & Nickelsen, J. (2016). Towards autotrophic tissue engineering: photosynthetic gene therapy for regeneration. Biomaterials, 75, 25-36.

*TAP (Tris-Acetate-Phosphate):*

Table I: Stock Solutions for 1 L of TAP medium (adjust final pH to 7.0).

| Solution/Compound | Volume (ml) |
|---|---|
| 1M Tris base (e.g. Trizma) | 20.0 |
| **Phosphate Buffer II*** | 1.0 |
| **Solution A*** | 10.0 |
| **Hutner´s trace elements*** | 1.0 |
| Glacial acetic acid | 1.0 |

Table II: Phosphate Buffer II (for 100 ml)

| Compound | Mass (g) |
|---|---|
| K2HPO4 | 10.8 |
| KH2PO4 | 5.6 |

Tabla III: Solution A (for 500 ml)

| Compound | Mass (g) |
|---|---|
| NH4Cl | 20.0 |
| MgSO4-7H2O | 5.0 |
| CaCl2-2H20 | 2.5 |

Source: http://www.chlamycollection.org/methods/media-recipes/tap-and-tris-minimal/

TableIV: Hutner´s trace elements. For a final mixture of 1 liter, dissolve each compound in the indicated water volume. EDTA must be dissolved in boiling water and FeSO4 must be prepared lastly to avoid oxidation.

| Compound | Mass (g) | Water volume (ml) |
|---|---|---|
| EDTA disodium salt | 50.00 | 250 |
| ZnSO4. 7 H2O | 22.00 | 100 |
| H3BO3 | 11.40 | 200 |
| MnCl2. 4 H2O | 5.06 | 50 |
| CoCl2. 6 H2O | 1.61 | 50 |
| CuSO4.5H2O | 1.57 | 50 |
| (NH4)6Mo7O24. 4 H2O | 1.10 | 50 |
| FeSO4. 7 H20 | 4.99 | 50 |

*Regarding Hutner´s trace elements:*

-Mix all solutions except EDTA. Bring to boil, then add the EDTA solution. The mixture must turn green. When all is dissolved, cool to 70°C.

-Keep temperature at 70°C, add 85 ml of hot KOH (20%).

-Cool to room temperature and bring to 1 liter of final volume.

-Generally, the solution will be initially green, but it will turn dark red or purple in the next days and will leave a redish brown precipitate. If no precipitate is formed or the solution remains green, check the pH. It must be around 6.7. If it is radically deactivated, try adding KOH or HCl to adjust it.

-The procedure requires to cover the flask with a cotton plug (to allow air exchange) and swhirl it once a day for 1 to 2 weeks. Or even better, use a 5 or 10 ml pipette (with a cotton filter on top) pushed through a foam plug and inject air through the solution for a week.

-Filter through two layers of filter paper Whatman #1. Repeat if necessary until the solution is clear.

-The final product should turn close to a burgundy color. However, even a subtle change in pH can cause a color shift.

-Store refrigerated or frozen in convenient aliquots.

-This can be done in bigger batches – 2 or 3 liters, if it is preferred.

Source: http://www.chlamycollection.org/methods/media-recipes/hutners-trace-elements/

*TAPS*

It's TAP medium supplemented with 1% w/v sorbitol.

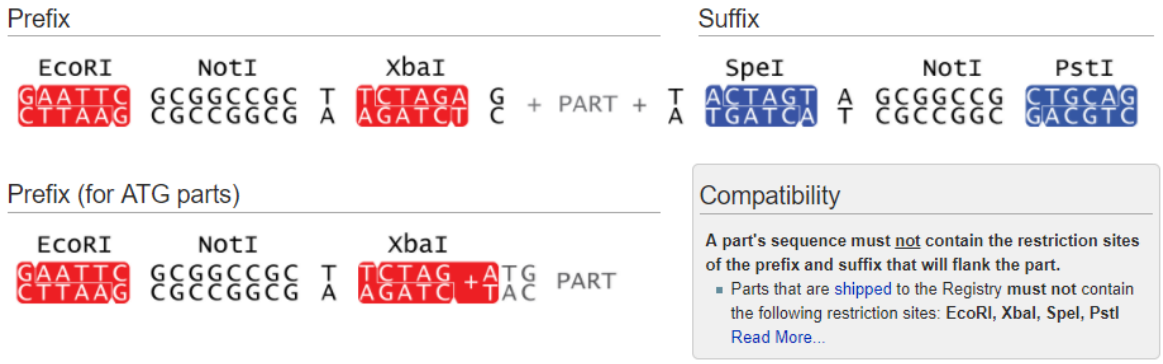
## Sequences

1. Design of the sequence of the fusion protein FBP/SBPase-GFP (cTP-FBP/SBPase-GFP and cTP-GFP-FBP/SBPase) is in Annex. Moreover, the control protein will be cTP-FBP/SBPase. The sequence will be obtained by DNA printing.

2. Design of the sequence of B12 responsive element-RFP is available in Annex (MetE-RFP operator). It will be obtained by DNA printing.

The sequences cTP-FBP/SBPase, cTP-FBP/SBPase-GFP, cTP-GFP-FBP/SBPase and METE-RFP operator will be adapted to the Codon bias of *Chlamydomonas reinhardtii* by the page https://www.idtdna.com/CodonOpt.

3. Design of the parts, incorporation of prefixes and suffixes. Prefix: ExoRI, NotI, Xbal. Suffix: SpeI, NotI, PstI. These are included in the BioBrick of the form:




Source: http://parts.igem.org/Help:Prefix-Suffix

## Construction of the transformation vector

1_Incorporation of sequences to pBC1 plasmid (Fig. 1) by Gibson Assembly. The following constructs will be obtained:

-pBC1- cTP-FBP/SBPase.

-pBC1- cTP-FBP/SBPase-EGFP.

- pBC1- cTP-EGFP-FBP/SBPase.

-pBC1-MetE Operator-RFP.

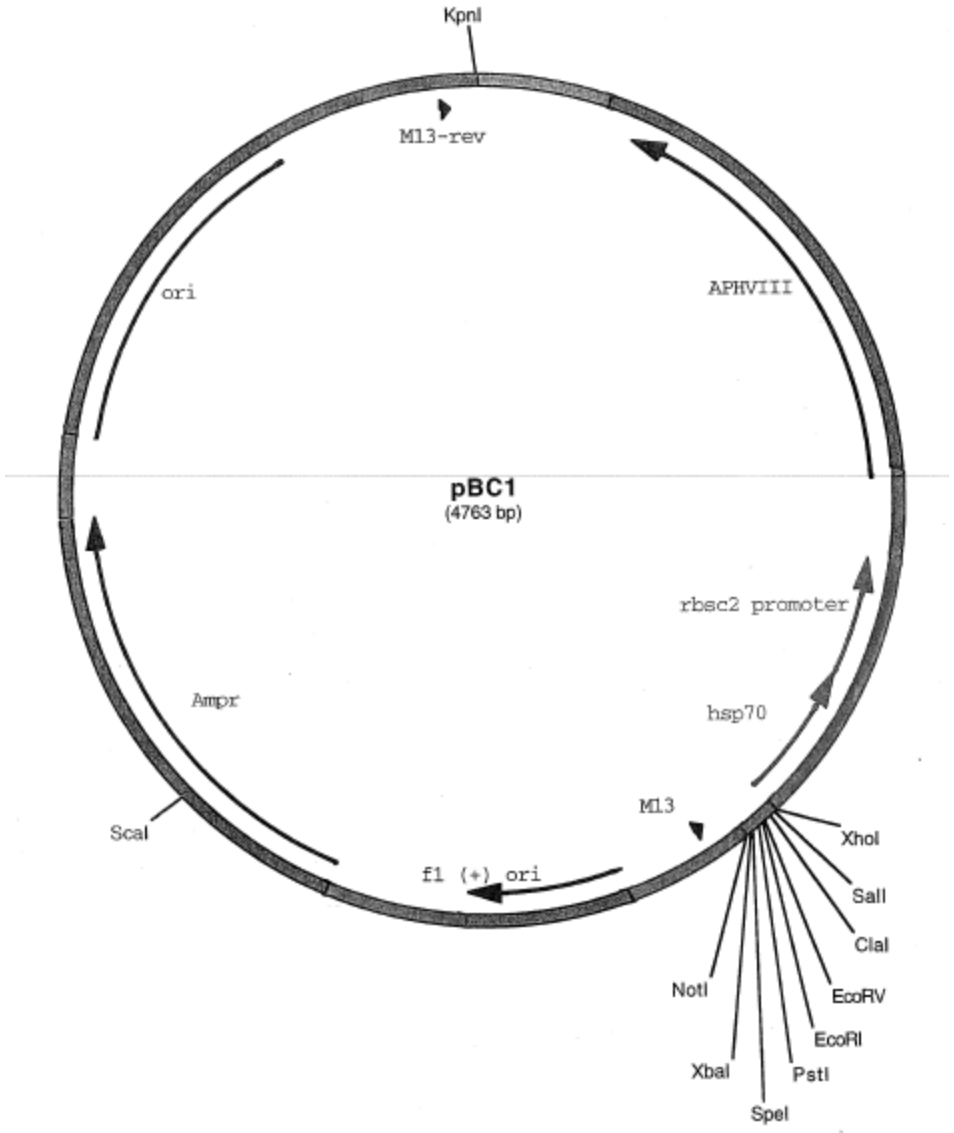



Fig. 1: pBC1 vector.

Source; http://www.chlamycollection.org/product/pbc1/

Verification of correct insertion by electrophoresis. A sample of each of the following must be run: Ladder, Gibson Assembly result without primers and Gibson Assembly result with primers.

3. DNA is recovered from gel by the Gene Clean method.

4. Verification of correct insertion by sequencing.

## Growth and transformation: *E. coli*

1. *E. coli* is maintained in LB medium.

2. Transformation by electroporation of strain DH5alpha for multiplication and maintenance of the construct.

3. Verification by growth in LB + Ampicillin.

4. Plasmid DNA recovery from *E. coli* DH5alpha.

## *Chlamydomonas reinhardtii* transformation

The transformation method will be by Glass-Bead according to the following:

$1*10^7$ UVM4 cells of *C. reinhardtii* are suspended in a volume of 300 µL and are vortexed by ø 0.5 mm of glass beads for 20 seconds in presence of 5 µg of plasmid DNA. Then, the transformants are seeded in the TAPS-liquid medium and are incubated for the night protected from light sourced and under continuous agitation. Afterwards, the algae are seeded on plaques of TAP-Agar that contain 10 µg/ml of paromomycin and are incubated for the first three days with weak light. After successful transformation, the new strains present an antibiotic resistance gene, so they are capable of growing selectively in presence of paromomycin. The plaques are then moved to a place under standard light (2500 lux) until the colonies are big enough to be punctured and plaqued in a new plaque. The clones are subsequently maintained in TAP-solid medium under selective conditions.

Source: [1]

The transformations to be done are:

1. *Chlamydomonas reinhardtii* with pBC1- cTP-FBP/SBPase.

2. *Chlamydomonas reinhardtii* with pBC1- cTP-FBP/SBPase-EGFP.

3. *Chlamydomonas reinhardtii* with pBC1- cTP-EGFP-FBP/SBPase.

4. *Chlamydomonas reinhardtii* with pBC1-Operador METE-RFP.

5. *Chlamydomonas reinhardtii* with pBC1-cTP-FBP/SBPase-EGFP or pBC1- cTP-EGFP-FBP/SBPase + pBC1-Operator METE-RFP. This strain will result from a successive transformation, where it will be transformed by integrating pBC1-Operator-RFP in the strain resulting from 2 or 3, depending on which of the two transformants present a greater optimization of $CO_2$ fixation.

## Verification of the transformation

1. Extraction of genomic DNA from *C. reinhardtii* cells using DNeasy Plant mini kit.

2. Western Blot.

3. PCR with specific primers.

## Determination of the number of copies inserted

The number of copies in the strains with the best performance resulting from transformations 1, 2 and 3 will be studied. This will be obtained by southern blot.

## Evaluation of $CO_2$ fixation optimization by growth rate

A comparision of the transformants 1, 2 and 3 with best performances and the wild-type would have to be made. For this, calibration curves of dry weight will be established as a function of optic density.

## Evaluation of correct localization of FBP/SBPase-GFP

Its position in the chloroplast will be checked by fluorescence microscopy by GFP excitation. This will be done with the strain that has greater growth rate.

## Pharmacological inhibition

To determine if the increase in growth rate of the obtained transformants is due to the presence of FBP/SBPase protein, strains with the best performance will be treated by adding the protein's antagonist: AMP 0.1 mM. Calibration curves will be established like the previous case for *Chlamydomonas* treated pharmacologically and they will be compared with wild-type and transformants without inhibition.

## Evaluation of the expression of optimized recombinant proteins

Measurement of fluorescence to evaluate expression of RFP.

# ANNEXO 1

## Sequences

To optimize sequences, this tool was used: https://www.idtdna.com/CodonOpt

### For the optimization of carbon dioxide fixation:

**Signal peptide (32 codons) optimized:**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG

http://onlinelibrary.wiley.com/doi/10.1046/j.1432-1327.1999.00701.x/full

**EGFP (239 codons) not optimized:**

ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG

http://www.algosome.com/resources/common-sequences.html#rfp

**FBP/SBPase (345 aminoacids) not optimized:**

MEKTIGLEIIEVVEQAAIASARLMGKGEKNEADRVAVEAMRVRMNQVEMLGRIVIGEGERDEAPMLYIGEEVGIYRDADKRA
GVPAGKLVEIDIAVDPCEGTNLCAYGQPGSMAVLAISEKGGLFAAPDFYMKKLAAPPAAKGKVDINKSATENLKILSECLDR
AIDELVVVVMDRPRHKELIQEIRQAGARVRLISDGDVSAAISCGFAGTNTHALMGIGAAPEGVISAAAMRCLGGHFQGQLIY
DPEVVKTGLIGESRESNIARLQEMGITDPDRVYDANELASGQEVLFAACGITPGLLMEGVRFFKGGARTQSLVISSQSRTA
RFVDTVHMFDDVKTVSLR

http://www.genome.jp/dbget-bin/www_bget?syf%3ASynpcc7942_0505

**Optimized (345 Codons):**

ATG GAG AAG ACC ATT GGG CTC GAG ATC ATC GAG GTC GTC GAG CAG GCG GCG ATC GCG TCG GCC CGC CTC ATG GGG AAG GGC GAG AAG AAC GAG GCC GAC CGC GTC GCT GTC GAG GCG ATG CGG GTC CGG ATG AAC CAG GTC GAG ATG CTC GGC CGG ATT GTC ATC GGC GAG GGC GAG CGG GAT GAG GCG CCC ATG CTC TAC ATT GGG GAG GAG GTC GGT ATC TAC CGC GAC GCG GAT AAG CGC GCC GGG GTG CCC GCT GGT AAG CTC GTC GAG ATT GAC ATT GCG GTG GAC CCT TGC GAG GGT ACC AAC CTG TGC GCG TAC GGT CAG CCC GGG TCG ATG GCT GTC CTC GCG ATT TCG GAG AAG GGT GGG CTC TTT GCC GCG CCT GAT TTT TAC ATG AAG AAG CTG GCT GCC CCT CCC GCT GCT AAG GGT AAG GTG GAT ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG ATC CTG AGC GAG TGC CTG GAT CGG GCT ATC GAT GAG CTG GTC GTC GTC GTG ATG GAC CGC CCC CGC CAT AAG GAG CTC ATT CAG GAG ATC CGG CAG GCT GGT GCT CGG GTC CGC CTC ATT AGC GAT GGG GAT GTC TCG GCC GCG ATC AGC TGC GGT TTC GCT GGG ACG AAC ACC CAT GCT CTG ATG GGG ATC GGG GCT GCT CCT GAG GGG GTG ATC TCC GCG GCT GCC ATG CGC TGC CTG GGT GGT CAC TTT CAG GGC CAG CTC ATT TAC GAC CCG GAG GTC GTC AAG ACC GGC CTC ATT GGG GAG TCC CGC GAG AGC AAC ATT GCT CGC CTC CAG GAG ATG GGT ATT ACG GAT CCC GAC CGC GTG TAC GAC GCT AAC GAG CTC GCC AGC GGC CAG GAG GTC CTG TTC GCT GCT TGC GGG ATC ACC CCT GGT CTG CTC ATG GAG GGT GTC CGG TTC TTT AAG GGT GGG GCT CGC ACG CAG TCG CTG GTC ATT AGC AGC CAG AGC CGG ACC GCT CGC TTC GTG GAC ACC GTG CAC ATG TTT GAT GAT GTG AAG ACC GTG AGC CTC CGC

**CTP- FBP/SBPase**

**Not optimized (377 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG ATG GAG AAG ACC ATT GGG CTC GAG ATC ATC GAG GTC GTC GAG CAG GCG GCG ATC GCG TCG GCC CGC CTC ATG GGG AAG GGC GAG AAG AAC GAG GCC GAC CGC GTC GCT GTC GAG GCG ATG CGG GTC CGG ATG AAC CAG GTC GAG ATG CTC GGC CGG ATT GTC ATC GGC GAG GGC GAG CGG GAT GAG GCG CCC ATG CTC TAC ATT GGG GAG GAG GTC GGT ATC TAC CGC GAC GCG GAT AAG CGC GCC GGG GTG CCC GCT GGT AAG CTC GTC GAG ATT GAC ATT GCG GTG GAC CCT TGC GAG GGT ACC AAC CTG TGC GCG TAC GGT CAG CCC GGG TCG ATG GCT GTC CTC GCG ATT TCG GAG AAG GGT GGG CTC TTT GCC GCG CCT GAT TTT TAC ATG AAG AAG CTG GCT GCC CCT CCC GCT GCT AAG GGT AAG GTG GAT ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG ATC CTG AGC GAG TGC CTG GAT CGG GCT ATC GAT GAG CTG GTC GTC GTC GTG ATG GAC CGC CCC CGC CAT AAG GAG CTC ATT CAG GAG ATC CGG CAG GCT GGT GCT CGG GTC CGC CTC ATT AGC GAT GGG GAT GTC TCG GCC GCG ATC AGC TGC GGT TTC GCT GGG ACG AAC ACC CAT GCT CTG ATG GGG ATC GGG GCT GCT CCT GAG GGG GTG ATC TCC GCG GCT GCC ATG CGC TGC CTG GGT GGT CAC TTT CAG GGC CAG CTC ATT TAC GAC CCG GAG GTC GTC AAG ACC GGC CTC ATT GGG GAG TCC CGC GAG AGC AAC ATT GCT CGC CTC CAG GAG ATG GGT ATT ACG GAT CCC GAC CGC GTG TAC GAC GCT AAC GAG CTC GCC AGC GGC CAG GAG GTC CTG TTC GCT GCT TGC GGG ATC ACC CCT GGT CTG CTC ATG GAG GGT GTC CGG TTC TTT AAG GGT GGG GCT CGC ACG CAG TCG CTG GTC ATT AGC AGC CAG AGC CGG ACC GCT CGC TTC GTG GAC ACC GTG CAC ATG TTT GAT GAT GTG AAG ACC GTG AGC CTC CGC

**Optimized (377 codons)**

ATG CAG GTG ACC ATG AAG TCC TCC GCC GTC TCG GGT CAG CGC GTC GGG GGC GCC CGG GTC GCT ACC CGC TCG GTC CGG CGG GCG CAG CTC CAG GTG ATG GAG AAG ACC ATC GGG CTG GAG ATT ATT GAG GTC GTG GAG CAG GCT GCG ATT GCG TCG GCC CGG CTG ATG GGC AAG GGT GAG AAG AAC GAG GCG GAT CGC GTG GCC GTC GAG GCG ATG CGC GTC CGC ATG AAC CAG GTG GAG ATG CTG GGG CGG ATC GTG ATC GGT GAG GGG GAG CGG GAC GAG GCT CCC ATG CTG TAC ATC GGG GAG GAG GTG GGC ATC TAC CGC GAC GCG GAC AAG CGG GCT GGC GTG CCG GCC GGC AAG CTG GTC GAG ATT GAC ATC GCT GTG GAC CCC TGC GAG GGG ACC AAC CTC TGC GCG TAC GGC CAG CCT GGC TCC ATG GCG GTG CTG GCG ATT TCG GAG AAG GGC GGG CTG TTT GCC GCC CCT GAC TTT TAC ATG AAG AAG CTC GCG GCG CCC CCC GCT GCT AAG GGT AAG GTG GAT ATC AAC AAG TCC GCT ACC GAG AAC CTC AAG ATT CTG AGC GAG TGC CTC GAT CGG GCG ATT GAC GAG CTC GTC GTG GTG GTC ATG GAT CGC CCT CGG CAT AAG GAG CTC ATC CAG GAG ATT CGC CAG GCG GGG GCT CGC GTG CGC CTG ATT TCC GAT GGG GAC GTG TCG GCG GCT ATC TCC TGC GGT TTT GCT GGC ACG AAC ACG CAT GCT CTC ATG GGT ATT GGG GCG GCT CCC GAG GGG GTG ATC TCC GCT GCG GCT ATG CGG TGC CTG GGC GGT CAC TTT CAG GGT CAG CTC ATT TAC GAT CCG GAG GTC GTG AAG ACC GGG CTG ATC GGG GAG TCG CGG GAG TCC AAC ATT GCC CGC CTG CAG GAG ATG GGG ATT ACC GAT CCC GAC CGC GTC TAC GAT GCC AAC GAG CTC GCG AGC GGT CAG GAG GTC CTG TTT GCG GCT TGC GGT ATT ACC CCT GGT CTC CTG ATG GAG GGG GTG CGC TTC TTT AAG GGG GGG GCT CGC ACC CAG TCG CTG GTG ATT TCC AGC CAG AGC CGC ACC GCC CGC TTT GTC GAT ACC GTG CAT ATG TTT GAC GAT GTC AAG ACG GTG AGC CTC CGC

**CTP-EGFP-FBP/SBPase**

**Not optimized (617 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG

GAC GAG CTG TAC AAG ATG GAG AAG ACC ATT GGG CTC GAG ATC ATC GAG GTC GTC GAG CAG GCG GCG ATC GCG TCG GCC CGC CTC ATG GGG AAG GGC GAG AAG AAC GAG GCC GAC CGC GTC GCT GTC GAG GCG ATG CGG GTC CGG ATG AAC CAG GTC GAG ATG CTC GGC CGG ATT GTC ATC GGC GAG GGC GAG CGG GAT GAG GCG CCC ATG CTC TAC ATT GGG GAG GAG GTC GGT ATC TAC CGC GAC GCG GAT AAG CGC GCC GGG GTG CCC GCT GGT AAG CTC GTC GAG ATT GAC ATT GCG GTG GAC CCT TGC GAG GGT ACC AAC CTG TGC GCG TAC GGT CAG CCC GGG TCG ATG GCT GTC CTC GCG ATT TCG GAG AAG GGT GGG CTC TTT GCC GCG CCT GAT TTT TAC ATG AAG AAG CTG GCT GCC CCT CCC GCT GCT AAG GGT AAG GTG GAT ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG ATC CTG AGC GAG TGC CTG GAT CGG GCT ATC GAT GAG CTG GTC GTC GTC GTG ATG GAC CGC CCC CGC CAT AAG GAG CTC ATT CAG GAG ATC CGG CAG GCT GGT GCT CGG GTC CGC CTC ATT AGC GAT GGG GAT GTC TCG GCC GCG ATC AGC TGC GGT TTC GCT GGG ACG AAC ACC CAT GCT CTG ATG GGG ATC GGG GCT GCT CCT GAG GGG GTG ATC TCC GCG GCT GCC ATG CGC TGC CTG GGT GGT CAC TTT CAG GGC CAG CTC ATT TAC GAC CCG GAG GTC GTC AAG ACC GGC CTC ATT GGG GAG TCC CGC GAG AGC AAC ATT GCT CGC CTC CAG GAG ATG GGT ATT ACG GAT CCC GAC CGC GTG TAC GAC GCT AAC GAG CTC GCC AGC GGC CAG GAG GTC CTG TTC GCT GCT TGC GGG ATC ACC CCT GGT CTG CTC ATG GAG GGT GTC CGG TTC TTT AAG GGT GGG GCT CGC ACG CAG TCG CTG GTC ATT AGC AGC CAG AGC CGG ACC GCT CGC TTC GTG GAC ACC GTG CAC ATG TTT GAT GAT GTG AAG ACC GTG AGC CTC CGC

**Optimized (616 codons):**

ATG CAG GTG ACC ATG AAG TCC TCC GCT GTC TCC GGC CAG CGG GTC GGG GGG GCC CGG GTG GCC ACC CGG TCC GTC CGC CGG GCT CAG CTG CAG GTC ATG GTC AGC AAG GGC GAG GAG CTG TTT ACC GGT GTC GTG CCC ATC CTG GTC GAG CTC GAT GGC GAC GTC AAC GGG CAT AAG TTT TCG GTC TCC GGG GAG GGG GAG GGG GAT GCG ACG TAC GGC AAG CTC ACG CTC AAG TTC ATT TGC ACG ACG GGC AAG CTC CCG GTC CCC TGG CCG ACG CTG GTC ACC ACG CTC ACG TAC GGC GTC CAG TGC TTC AGC CGG TAC CCG GAT CAC ATG AAG CAG CAC GAT TTT TTT AAG AGC GCG ATG CCT GAG GGT TAC GTG CAG GAG CGC ACG ATC TTC TTT AAG GAT GAC GGG AAC TAC AAG ACC CGG GCC GAG GTC AAG TTC GAG GGG GAC ACG CTG GTG AAC CGG ATC GAG CTC AAG GGC ATT GAC TTC AAG GAG GAC GGG AAC ATC CTC GGC CAC AAG CTG GAG TAC AAC TAC AAC AGC CAT AAC GTC TAC ATT ATG GCT GAT AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATT CGC CAT AAC ATC GAG GAT GGT AGC GTC CAG CTG GCT GAC CAC TAC CAG CAG AAC ACG CCC ATC GGG GAT GGC CCT GTC CTG CTG CCG GAC AAC CAC TAC CTC TCG ACG CAG TCC GCT CTG TCC AAG GAC CCT AAC GAG AAG CGG GAC CAC ATG GTG CTC CTC GAG TTC GTG ACG GCG GCC GGT ATC ACG CTC GGG ATG GAT GAG CTC TAC AAG ATG GAG AAG ACG ATC GGT CTC GAG ATT ATT GAG GTG GTG GAG CAG GCG GCG ATT GCT TCC GCC CGG CTG ATG GGT AAG GGG GAG AAG AAC GAG GCC GAT CGC GTC GCT GTG GAG GCT ATG CGC GTG CGG ATG AAC CAG GTC GAG ATG CTC GGC CGG ATT GTC ATT GGC GAG GGT GAG CGC GAT GAG GCG CCG ATG CTC TAC ATT GGG GAG GAG GTC GGT ATC TAC CGC GAC GCT GAC AAG CGG GCG GGC GTC CCC GCG GGC AAG CTC GTC GAG ATT GAC ATT GCT GTG GAT CCC TGC GAG GGC ACG AAC CTC TGC GCG TAC GGG CAG CCG GGG TCG ATG GCT GTG CTG GCT ATT TCC GAG AAG GGG GGG CTG TTT GCG GCG CCT GAC TTC TAC ATG AAG AAG CTG GCT GCC CCC CCC GCG GCG AAG GGC AAG GTC GAT ATC AAC AAG TCC GCC ACG GAG AAC CTC AAG ATC CTG TCG GAG TGC CTG GAC CGC GCC ATC GAT GAG CTC GTC GTC GTC GTG ATG GAT CGG CCT CGG CAC AAG GAG CTG ATC CAG GAG ATT CGG CAG GCC GGC GCG CGC GTG CGG CTG ATC AGC GAT GGC GAC GTG AGC GCT GCG ATT TCC TGC GGG TTT GCG GGT ACC AAC ACG CAC GCT CTC ATG GGT ATC GGG GCC GCC CCC GAG GGG GTC ATT TCC GCG GCG GCT ATG CGG TGC CTG GGT GGG CAC TTC CAG GGG CAG CTC ATT TAC GAC CCC GAG GTG GTG AAG ACC GGG CTC ATC GGC GAG TCC CGG GAG TCC AAC ATC GCT CGG CTC CAG GAG ATG GGT ATC ACC GAC CCT GAT CGG GTG TAC GAC GCT AAC GAG CTG GCC TCG GGT CAG GAG GTC CTG TTC GCC GCG TGC GGC ATT ACG CCT GGC CTG CTG ATG GAG GGG GTG CGC TTC TTT AAG GGC GGG GCT CGC ACG CAG TCG CTG GTC ATC AGC TCG CAG TCC CGC ACC GCT CGG TTT GTC GAC ACC GTG CAC ATG TTT GAT GAT GTG AAG ACG GTG AGC CTG CGC

**CTP-FBP/SBPase-EGFP**

**Not optimized (616 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG ATG GAG AAG ACC ATT GGG CTC GAG ATC ATC GAG GTC GTC GAG CAG GCG GCG ATC GCG TCG GCC CGC CTC ATG GGG AAG GGC GAG AAG AAC GAG GCC GAC CGC GTC GCT GTC GAG GCG ATG CGG GTC CGG ATG AAC CAG GTC GAG ATG CTC GGC CGG ATT GTC ATC GGC GAG GGC GAG CGG GAT GAG GCG CCC ATG CTC TAC ATT GGG GAG GAG GTC GGT ATC TAC CGC GAC GCG GAT AAG CGC GCC GGG GTG CCC GCT GGT AAG CTC GTC GAG ATT GAC ATT GCG GTG GAC CCT TGC GAG GGT ACC AAC CTG TGC GCG TAC GGT CAG CCC GGG TCG ATG GCT GTC CTC GCG ATT TCG GAG AAG GGT

GGG CTC TTT GCC GCG CCT GAT TTT TAC ATG AAG AAG CTG GCT GCC CCT CCC GCT GCT AAG GGT AAG GTG GAT ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG ATC CTG AGC GAG TGC CTG GAT CGG GCT ATC GAT GAG CTG GTC GTC GTC GTG ATG GAC CGC CCC CGC CAT AAG GAG CTC ATT CAG GAG ATC CGG CAG GCT GGT GCT CGG GTC CGC CTC ATT AGC GAT GGG GAT GTC TCG GCC GCG ATC AGC TGC GGT TTC GCT GGG ACG AAC ACC CAT GCT CTG ATG GGG ATC GGG GCT GCT CCT GAG GGG GTG ATC TCC GCG GCT GCC ATG CGC TGC CTG GGT GGT CAC TTT CAG GGC CAG CTC ATT TAC GAC CCG GAG GTC GTC AAG ACC GGC CTC ATT GGG GAG TCC CGC GAG AGC AAC ATT GCT CGC CTC CAG GAG ATG GGT ATT ACG GAT CCC GAC CGC GTG TAC GAC GCT AAC GAG CTC GCC AGC GGC CAG GAG GTC CTG TTC GCT GCT TGC GGG ATC ACC CCT GGT CTG CTC ATG GAG GGT GTC CGG TTC TTT AAG GGT GGG GCT CGC ACG CAG TCG CTG GTC ATT AGC AGC CAG AGC CGG ACC GCT CGC TTC GTG GAC ACC GTG CAC ATG TTT GAT GAT GTG AAG ACC GTG AGC CTC CGC ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG

**Optimized (616 codons):**

ATG CAG GTC ACG ATG AAG TCG AGC GCG GTC TCG GGT CAG CGG GTC GGG GGG GCT CGC GTC GCT ACC CGG TCG GTC CGG CGG GCT CAG CTG CAG GTG ATG GAG AAG ACC ATT GGT CTG GAG ATC ATT GAG GTG GTC GAG CAG GCC GCG ATC GCT TCC GCT CGG CTC ATG GGT AAG GGG GAG AAG AAC GAG GCC GAT CGG GTG GCC GTG GAG GCT ATG CGC GTG CGG ATG AAC CAG GTG GAG ATG CTG GGT CGC ATT GTC ATC GGT GAG GGT GAG CGG GAT GAG GCT CCG ATG CTC TAC ATC GGG GAG GAG GTC GGC ATC TAC CGG GAC GCC GAT AAG CGG GCG GGT GTC CCT GCT GGT AAG CTG GTG GAG ATT GAC ATC GCT GTG GAC CCC TGC GAG GGC ACC AAC CTC TGC GCG TAC GGC CAG CCT GGC TCC ATG GCG GTC CTC GCT ATC TCC GAG AAG GGT GGG CTC TTC GCC GCT CCC GAT TTT TAC ATG AAG AAG CTC GCT GCC CCT CCC GCC GCT AAG GGT AAG GTC GAC ATT AAC AAG TCG GCC ACG GAG AAC CTC AAG ATC CTG AGC GAG TGC CTC GAT CGG GCC ATC GAT GAG CTC GTG GTC GTC GTG ATG GAT CGC CCT CGC CAC AAG GAG CTG ATC CAG GAG ATT CGC CAG GCG GGT GCG CGG GTC CGG CTG ATC TCC GAT GGT GAC GTG TCG GCT GCT ATC AGC TGC GGC TTT GCG GGG ACG AAC ACG CAC GCG CTC ATG GGC ATT GGG GCC GCC CCC GAG GGC GTC ATT AGC GCT GCT GCC ATG CGG TGC CTG GGT GGC CAT TTC CAG GGT CAG CTG ATT TAC GAC CCG GAG GTC GTC AAG ACC GGG CTC ATT GGG GAG TCC CGC GAG AGC AAC ATC GCG CGG CTG CAG GAG ATG GGC ATC ACG GAT CCT GAT CGC GTG TAC GAT GCG AAC GAG CTG GCC AGC GGG CAG GAG GTC CTC TTT GCT GCT TGC GGG ATC ACG CCG GGC CTC CTC ATG GAG GGG GTG CGG TTT TTT AAG GGT GGT GCT CGG ACC CAG TCG CTC GTG ATC TCG TCG CAG TCG CGC ACC GCT CGC TTT GTG GAC ACG GTC CAT ATG TTC GAC GAT GTC AAG ACC GTG AGC CTG CGC ATG GTC AGC AAG GGT GAG GAG CTG TTC ACC GGG GTC GTC CCG ATC CTG GTC GAG CTC GAT GGT GAT GTG AAC GGC CAC AAG TTC AGC GTG TCC GGT GAG GGT GAG GGC GAT GCG ACC TAC GGT AAG CTG ACC CTC AAG TTT ATT TGC ACC ACC GGT AAG CTG CCG GTG CCT TGG CCC ACC CTG GTC ACC ACG CTC ACC TAC GGG GTC CAG TGC TTC TCC CGG TAC CCC GAT CAT ATG AAG CAG CAC GAT TTT TTC AAG TCC GCG ATG CCT GAG GGG TAC GTG CAG GAG CGC ACG ATC TTT TTT AAG GAC GAT GGG AAC TAC AAG ACG CGC GCG GAG GTG AAG TTT GAG GGG GAC ACG CTC GTC AAC CGG ATT GAG CTG AAG GGT ATT GAT TTT AAG GAG GAC GGC AAC ATC CTC GGC CAC AAG CTC GAG TAC AAC TAC AAC AGC CAC AAC GTC TAC ATT ATG GCT GAC AAG CAG AAG AAC GGG ATC AAG GTC AAC TTT AAG ATC CGG CAC AAC ATT GAG GAT GGC TCG GTC CAG CTC GCC GAT CAT TAC CAG CAG AAC ACC CCG ATC GGG GAC GGT CCT GTG CTG CTG CCT GAT AAC CAT TAC CTG AGC ACC CAG TCG GCG CTG TCC AAG GAT CCC AAC GAG AAG CGC GAC CAC ATG GTC CTG CTC GAG TTC GTC ACC GCC GCT GGT ATC ACC CTC GGG ATG GAC GAG CTG TAC AAG

## For the production of RFP:

**mCherry (RFP)**

**Not Optimized**

ATG GTG AGC AAG GGC GAG GAG GAT AAC ATG GCC ATC ATC AAG GAG TTC ATG CGC TTC AAG GTG CAC ATG GAG GGC TCC GTG AAC GGC CAC GAG TTC GAG ATC GAG GGC GAG GGC GAG GGC CGC CCC TAC GAG GGC ACC CAG ACC GCC AAG CTG AAG GTG ACC AAG GGT GGC CCC CTG CCC TTC GCC TGG GAC ATC CTG TCC CCT CAG TTC ATG TAC GGC TCC AAG GCC TAC GTG AAG CAC CCC GCC GAC ATC CCC GAC TAC TTG AAG CTG TCC TTC CCC GAG GGC TTC AAG TGG GAG CGC GTG ATG AAC TTC GAG GAC GGC GGC GTG GTG ACC GTG ACC CAG GAC TCC TCC CTG CAG GAC GGC GAG TTC ATC TAC AAG GTG AAG CTG CGC GGC ACC AAC TTC CCC TCC GAC GGC CCC GTA ATG CAG AAG AAG ACC ATG GGC TGG GAG GCC TCC TCC GAG CGG ATG TAC CCC GAG GAC GGC  GCC CTG AAG GGC GAG ATC AAG CAG AGG CTG AAG CTG AAG GAC GGC GGC CAC TAC GAC GCT GAG GTC AAG ACC ACC TAC AAG GCC AAG AAG CCC GTG CAG CTG CCC GGC GCC TAC AAC GTC AAC ATC AAG TTG GAC ATC ACC TCC CAC AAC GAG GAC TAC ACC ATC GTG GAA CAG TAC GAA CGC GCC GAG GGC CGC CAC TCC ACC GGC GGC ATG GAC GAG CTG TAC AAG TAA

http://www.algosome.com/resources/common-sequences.html#rfp

**Optimized**

ATG GTG TCC AAG GGG GAG GAG GAT AAC ATG GCG ATC ATC AAG GAG TTC ATG CGG TTT AAG GTC CAT ATG GAG GGC TCG GTC AAC GGG CAT GAG TTC GAG ATC GAG GGC GAG GGC GAG GGT CGG CCT TAC GAG GGC ACG CAG ACG GCG AAG CTC AAG GTC ACC AAG GGG GGC CCT CTC CCT TTT GCG TGG GAC ATT CTG AGC CCC CAG TTC ATG TAC GGC TCC AAG GCT TAC GTG AAG CAT CCG GCT GAT ATC CCC GAT TAC CTC AAG CTG AGC TTT CCG GAG GGT TTC AAG TGG GAG CGC GTG ATG AAC TTC GAG GAT GGT GGT GTC GTG ACC GTG ACC CAG GAT AGC TCC CTC CAG GAT GGC GAG TTC ATT TAC AAG GTG AAG CTC CGC GGC ACG AAC TTC CCC AGC GAT GGG CCT GTC ATG CAG AAG AAG ACG ATG GGT TGG GAG GCG TCC AGC GAG CGG ATG TAC CCC GAG GAC GGG GCG CTC AAG GGG GAG ATT AAG CAG CGG CTC AAG CTC AAG GAC GGC GGG CAT TAC GAC GCT GAG GTC AAG ACC ACG TAC AAG GCC AAG AAG CCT GTG CAG CTC CCC GGT GCT TAC AAC GTC AAC ATC AAG CTG GAC ATC ACC AGC CAC AAC GAG GAT TAC ACC ATT GTC GAG CAG TAC GAG CGC GCG GAG GGT CGG CAT TCC ACG GGC GGG ATG GAT GAG CTG TAC AAG TAA

**The sequence with promoter contains -574 to -1 region. Was supposed that it includes to MetE 5'UTR (574 nt), this corresponds to:**

TGTAGGTCAGGACCAGAGCCTACAACATTGTAAGGCCATGTATCCTCCAGTGCCATTTGCGACGTTACACGACGAGA
CAATCTGCGCTGTGTTCAAGCACACACATCCGGGACTCGTGTGATGTCTTAACATCTGCAAGGAAACATAAGGTGGC
CGGCGTCACGCAAGATGACGCGACGGACTCGAATGCTTGCTTCGTCCAGCGACAAATAAGGACAGGCAGGGCGCCT
GCATTGCGTGCGCTTAGAGGCGAGGCGCTCAAGACATTTCGGCAGCAATAATTGGTATTGAGGCACATTCTGCACCA
GGATGCCAAGAGGTGAACGTTGCTGCCTTAATGTATATCTGCACAGCTGGCCGGTTACTGCGAACCGGGTGCCTTTT
GGAGTCGCTCCTAAAAGACGTCACCGGCGCAACGTCGTCGTGCGGCCGTACAGGTATGCAAAATTGGCCCGGTTGC
GCGCAGGCCGATTATTTAAGTGACATTTGGTACCATGCATGAGCATCCGATTGTTGTTTTGCAATTCTGTCTGACTGC
ACAAGGAACCCGTCCTTGGGAATCAACATCTGTCAA

https://www.ncbi.nlm.nih.gov/gene/5728446

**The promoter sequence MetE-RFP (1285 nt) to optimize is:**

TGTAGGTCAGGACCAGAGCCTACAACATTGTAAGGCCATGTATCCTCCAGTGCCATTTGCGACGTTACACGACGAGA
CAATCTGCGCTGTGTTCAAGCACACACATCCGGGACTCGTGTGATGTCTTAACATCTGCAAGGAAACATAAGGTGGC
CGGCGTCACGCAAGATGACGCGACGGACTCGAATGCTTGCTTCGTCCAGCGACAAATAAGGACAGGCAGGGCGCCT
GCATTGCGTGCGCTTAGAGGCGAGGCGCTCAAGACATTTCGGCAGCAATAATTGGTATTGAGGCACATTCTGCACCA
GGATGCCAAGAGGTGAACGTTGCTGCCTTAATGTATATCTGCACAGCTGGCCGGTTACTGCGAACCGGGTGCCTTTT
GGAGTCGCTCCTAAAAGACGTCACCGGCGCAACGTCGTCGTGCGGCCGTACAGGTATGCAAAATTGGCCCGGTTGC
GCGCAGGCCGATTATTTAAGTGACATTTGGTACCATGCATGAGCATCCGATTGTTGTTTTGCAATTCTGTCTGACTGC
ACAAGGAACCCGTCCTTGGGAATCAACATCTGTCAA ATG GTG TCC AAG GGG GAG GAG GAT AAC ATG GCG ATC
ATC AAG GAG TTC ATG CGG TTT AAG GTC CAT ATG GAG GGC TCG GTC AAC GGG CAT GAG TTC GAG ATC GAG
GGC GAG GGC GAG GGT CGG CCT TAC GAG GGC ACG CAG ACG GCG AAG CTC AAG GTC ACC AAG GGG GGC
CCT CTC CCT TTT GCG TGG GAC ATT CTG AGC CCC CAG TTC ATG TAC GGC TCC AAG GCT TAC GTG AAG CAT

CCG GCT GAT ATC CCC GAT TAC CTC AAG CTG AGC TTT CCG GAG GGT TTC AAG TGG GAG CGC GTG ATG AAC TTC GAG GAT GGT GGT GTC GTG ACC GTG ACC CAG GAT AGC TCC CTC CAG GAT GGC GAG TTC ATT TAC AAG GTG AAG CTC CGC GGC ACG AAC TTC CCC AGC GAT GGG CCT GTC ATG CAG AAG AAG ACG ATG GGT TGG GAG GCG TCC AGC GAG CGG ATG TAC CCC GAG GAC GGG GCG CTC AAG GGG GAG ATT AAG CAG CGG CTC AAG CTC AAG GAC GGC GGG CAT TAC GAC GCT GAG GTC AAG ACC ACG TAC AAG GCC AAG AAG CCT GTG CAG CTC CCC GGT GCT TAC AAC GTC AAC ATC AAG CTG GAC ATC ACC AGC CAC AAC GAG GAT TAC ACC ATT GTC GAG CAG TAC GAG CGC GCG GAG GGT CGG CAT TCC ACG GGC GGG ATG GAT GAG CTG TAC AAG TAA

Primers for the PCR of FBP/SBP

5'-C**CATATG**GAGAAGACGATCGGTCT-3' NdeI

5'-AACTGCG**GGATCC**ATGAGGCCG-3' BamHI

## ANNEXO 2

## Sequences

To verify that each sequence optimization was accomplish correctly, was necessary make a second optimization of each one of them, with the purpose of compare obtained results. Differences in some codons that encode the same aminoacid in *Chlamydomonas reinhardtii* was found.

To optimize sequences, this tool was used: https://www.idtdna.com/CodonOpt

### For the optimization of carbon dioxide fixation:

**Signal peptide (32 codons) optimized:**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG

http://onlinelibrary.wiley.com/doi/10.1046/j.1432-1327.1999.00701.x/full

**EGFP (239 codons) not optimized:**

ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG

http://www.algosome.com/resources/common-sequences.html#rfp

**FBP/SBPase (345 aminoacids) not optimized:**

MEKTIGLEIIEVVEQAAIASARLMGKGEKNEADRVAVEAMRVRMNQVEMLGRIVIGEGERDEAPMLYIGEEVGIYRDADKRA
GVPAGKLVEIDIAVDPCEGTNLCAYGQPGSMAVLAISEKGGLFAAPDFYMKKLAAPPAAKGKVDINKSATENLKILSECLDR
AIDELVVVVMDRPRHKELIQEIRQAGARVRLISDGDVSAAISCGFAGTNTHALMGIGAAPEGVISAAAMRCLGGHFQGQLIY
DPEVVKTGLIGESRESNIARLQEMGITDPDRVYDANELASGQEVLFAACGITPGLLMEGVRFFKGGARTQSLVISSQSRTA
RFVDTVHMFDDVKTVSLR

http://www.genome.jp/dbget-bin/www_bget?syf%3ASynpcc7942_0505

**Optimized (345 Codons):**

ATG GAG AAG ACC ATT GGC CTG GAG ATC ATC GAG GTC GTC GAG CAG GCC GCG ATC GCC AGC GCT CGC C
TC ATG GGT AAG GGC GAG AAG AAC GAG GCC GACCGC GTC GCT GTC GAG GCG ATG CGC GTC CGC ATG AA
C CAG GTG GAG ATG CTG GGT CGC ATC GTC ATC GGG GAG GGC GAG CGC GAC GAG GCC CCC ATG CTCTAC

ATT GGG GAG GAG GTG GGG ATT TAC CGG GAC GCC GAT AAG CGG GCT GGT GTG CCG GCC GGG AAG CTG GTC GAG ATC GAC ATC GCG GTC GAT CCT TGCGAG GGT ACG AAC CTC TGC GCC TAC GGG CAG CCG GGC T CG ATG GCC GTC CTC GCT ATT TCC GAG AAG GGC GGT CTG TTT GCC GCG CCT GAC TTC TAC ATGAAG AAG CTC GCC GCC CCT CCT GCG GCT AAG GGG AAG GTC GAC ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG A TC CTG AGC GAG TGC CTC GAT CGG GCCATT GAC GAG CTC GTC GTG GTG GTG ATG GAT CGG CCG CGG CA C AAG GAG CTG ATC CAG GAG ATC CGC CAG GCG GGC GCT CGG GTC CGC CTC ATC TCC GATGGT GAT GTC AGC GCG GCT ATT TCG TGC GGC TTC GCT GGG ACC AAC ACG CAC GCC CTG ATG GGT ATT GGT GCT GCG C CC GAG GGT GTG ATT AGC GCT GCGGCG ATG CGC TGC CTG GGG GGT CAT TTC CAG GGC CAG CTC ATT TA C GAT CCC GAG GTC GTC AAG ACC GGG CTG ATC GGT GAG AGC CGC GAG TCC AAC ATCGCG CGC CTC CAG GAG ATG GGC ATT ACG GAC CCT GAC CGC GTC TAC GAC GCG AAC GAG CTG GCC TCC GGC CAG GAG GTG CTC TTT GCC GCG TGC GGT ATCACC CCC GGT CTC CTG ATG GAG GGT GTC CGG TTC TTC AAG GGC GGT GC G CGC ACG CAG TCG CTC GTC ATT AGC TCG CAG TCG CGG ACC GCG CGG TTC GTGGAC ACG GTC CAC ATG TTC GAC GAT GTC AAG ACG GTC TCG CTC CGC

**CTP- FBP/SBPase**

**Not optimized (377 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG
ATG GAG AAG ACC ATT GGC CTG GAG ATC ATC GAG GTC GTC GAG CAG GCC GCG ATC GCC AGC GCT CGC C TC ATG GGT AAG GGC GAG AAG AAC GAG GCC GACCGC GTC GCT GTC GAG GCG ATG CGC GTC CGC ATG AA C CAG GTG GAG ATG CTG GGT CGC ATC GTC ATC GGG GAG GGC GAG CGC GAC GAG GCC CCC ATG CTCTAC ATT GGG GAG GAG GTG GGG ATT TAC CGG GAC GCC GAT AAG CGG GCT GGT GTG CCG GCC GGG AAG CTG GTC GAG ATC GAC ATC GCG GTC GAT CCT TGCGAG GGT ACG AAC CTC TGC GCC TAC GGG CAG CCG GGC T CG ATG GCC GTC CTC GCT ATT TCC GAG AAG GGC GGT CTG TTT GCC GCG CCT GAC TTC TAC ATGAAG AAG CTC GCC GCC CCT CCT GCG GCT AAG GGG AAG GTC GAC ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG A TC CTG AGC GAG TGC CTC GAT CGG GCCATT GAC GAG CTC GTC GTG GTG GTG ATG GAT CGG CCG CGG CA C AAG GAG CTG ATC CAG GAG ATC CGC CAG GCG GGC GCT CGG GTC CGC CTC ATC TCC GATGGT GAT GTC AGC GCG GCT ATT TCG TGC GGC TTC GCT GGG ACC AAC ACG CAC GCC CTG ATG GGT ATT GGT GCT GCG C CC GAG GGT GTG ATT AGC GCT GCGGCG ATG CGC TGC CTG GGG GGT CAT TTC CAG GGC CAG CTC ATT TA C GAT CCC GAG GTC GTC AAG ACC GGG CTG ATC GGT GAG AGC CGC GAG TCC AAC ATCGCG CGC CTC CAG GAG ATG GGC ATT ACG GAC CCT GAC CGC GTC TAC GAC GCG AAC GAG CTG GCC TCC GGC CAG GAG GTG CTC TTT GCC GCG TGC GGT ATCACC CCC GGT CTC CTG ATG GAG GGT GTC CGG TTC TTC AAG GGC GGT GC G CGC ACG CAG TCG CTC GTC ATT AGC TCG CAG TCG CGG ACC GCG CGG TTC GTGGAC ACG GTC CAC ATG TTC GAC GAT GTC AAG ACG GTC TCG CTC CGC

**Optimized (377 codons):**

ATG CAG GTG ACG ATG AAG TCG TCC GCG GTC TCC GGT CAG CGG GTG GGG GGC GCT CGC GTG GCT ACC CGC AGC GTC CGC CGG GCT CAG CTC CAG GTC ATG GAG AAG ACC ATT GGG CTC GAG ATC ATT GAG GTC GTG GAG CAG GCT GCG ATC GCT TCG GCC CGG CTG ATG GGC AAG GGG GAG AAG AAC GAG GCT GAC CGG GTC GCG GTG GAG GCC ATG CGG GTC CGG ATG AAC CAG GTG GAG ATG CTG GGT CGG ATC GTG ATC GGG GAG GGG GAG CGG GAT GAG GCT CCG ATG CTC TAC ATT GGC GAG GAG GTG GGC ATT TAC CGG GAC GCT GAT AAG CGG GCG GGT GTC CCG GCC GGT AAG CTG GTC GAG ATC GAC ATC GCC GTC GAT CCC TGC GAG GGT ACC AAC CTC TGC GCG TAC GGT CAG CCT GGG TCG ATG GCG GTC CTC GCT ATC TCG GAG AAG GGC GGT CTG TTC GCG GCC CCT GAC TTC TAC ATG AAG AAG CTC GCG GCC CCT CCC GCC GCT AAG GGC AAG GTG GAC ATC AAC AAG TCC GCT ACC GAG AAC CTC AAG ATT CTC TCC GAG TGC CTC GAC CGC GCT ATC GAT GAG CTC GTG GTC GTG GTG ATG GAC CGC CCT CGC CAC AAG GAG CTG ATC CAG GAG ATT CGC CAG GCG GGG GCG CGC GTG CGC CTC ATC TCG GAC GGT GAT GTC TCG GCT GCG ATT TCG TGC GGG TTC GCC GGC ACG AAC ACG CAC GCT CTG ATG GGT ATC GGG GCC GCC CCT GAG GGT GTC ATT TCG GCG GCG GCT ATG CGC TGC CTG GGT GGG CAT TTT CAG GGT CAG CTG ATT TAC GAT CCG GAG GTC GTC AAG ACC GGT CTG ATC GGT GAG TCG CGG GAG AGC AAC ATT GCT CGC CTG CAG GAG ATG GGT ATT ACC GAT CCG GAC CGG GTC TAC GAT GCT AAC GAG CTC GCT AGC GGT CAG GAG GTC CTC TTC GCT GCG TGC GGT ATT ACG CCG GGT CTG CTC ATG GAG GGT GTC CGG TTT TTT AAG GGT GGG GCT CGC ACG CAG AGC CTG GTC ATT TCC TCC CAG AGC CGC ACC GCT CGC TTT GTG GAT ACC GTG CAT ATG TTC GAC GAC GTG AAG ACC GTC TCG CTC CGC

**CTP-EGFP-FBP/SBPase**

**Not optimized (616 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG
ATG GAG AAG ACC ATT GGC CTG GAG ATC ATC GAG GTC GTC GAG CAG GCC GCG ATC GCC AGC GCT CGC C TC ATG GGT AAG GGC GAG AAG AAC GAG GCC GACCGC GTC GCT GTC GAG GCG ATG CGC GTC CGC ATG AA C CAG GTG GAG ATG CTG GGT CGC ATC GTC ATC GGG GAG GGC GAG CGC GAC GAG GCC CCC ATG CTCTAC ATT GGG GAG GAG GTG GGG ATT TAC CGG GAC GCC GAT AAG CGG GCT GGT GTG CCG GCC GGG AAG CTG GTC GAG ATC GAC ATC GCG GTC GAT CCT TGCGAG GGT ACG AAC CTC TGC GCC TAC GGG CAG CCG GGC T CG ATG GCC GTC CTC GCT ATT TCC GAG AAG GGC GGT CTG TTT GCC GCG CCT GAC TTC TAC ATGAAG AAG CTC GCC GCC CCT CCT GCG GCT AAG GGG AAG GTC GAC ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG A TC CTG AGC GAG TGC CTC GAT CGG GCCATT GAC GAG CTC GTC GTG GTG GTG ATG GAT CGG CCG CGG CA C AAG GAG CTG ATC CAG GAG ATC CGC CAG GCG GGC GCT CGG GTC CGC CTC ATC TCC GATGGT GAT GTC AGC GCG GCT ATT TCG TGC GGC TTC GCT GGG ACC AAC ACG CAC GCC CTG ATG GGT ATT GGT GCT GCG C CC GAG GGT GTG ATT AGC GCT GCGGCG ATG CGC TGC CTG GGG GGT CAT TTC CAG GGC CAG CTC ATT TA C GAT CCC GAG GTC GTC AAG ACC GGG CTG ATC GGT GAG AGC CGC GAG TCC AAC ATCGCG CGC CTC CAG GAG ATG GGC ATT ACG GAC CCT GAC CGC GTC TAC GAC GCG AAC GAG CTG GCC TCC GGC CAG GAG GTG CTC TTT GCC GCG TGC GGT ATCACC CCC GGT CTC CTG ATG GAG GGT GTC CGG TTC TTC AAG GGC GGT GC G CGC ACG CAG TCG CTC GTC ATT AGC TCG CAG TCG CGG ACC GCG CGG TTC GTGGAC ACG GTC CAC ATG TTC GAC GAT GTC AAG ACG GTC TCG CTC CGC

**Optimized (616 codons):**

ATG CAG GTG ACC ATG AAG AGC TCG GCG GTC TCC GGC CAG CGG GTC GGC GGC GCC CGG GTC GCG ACC CGG AGC GTG CGC CGC GCG CAG CTC CAG GTC ATG GTG TCC AAG GGG GAG GAG CTG TTC ACG GGC GTC GTC CCT ATC CTC GTG GAG CTC GAC GGC GAC GTC AAC GGG CAC AAG TTC AGC GTC TCG GGT GAG GGG GAG GGG GAT GCT ACC TAC GGT AAG CTC ACG CTG AAG TTT ATC TGC ACC ACC GGC AAG CTC CCG GTC CCT TGG CCT ACG CTG GTG ACG ACC CTG ACC TAC GGT GTG CAG TGC TTT TCC CGC TAC CCG GAT CAT ATG AAG CAG CAT GAC TTC TTT AAG TCC GCC ATG CCT GAG GGC TAC GTC CAG GAG CGC ACC ATC TTT TTT AAG GAC GAT GGT AAC TAC AAG ACG CGG GCC GAG GTG AAG TTC GAG GGG GAT ACG CTC GTC AAC CGC ATC GAG CTC AAG GGC ATC GAC TTC AAG GAG GAC GGT AAC ATC CTG GGT CAC AAG CTC GAG TAC AAC TAC AAC AGC CAC AAC GTC TAC ATT ATG GCT GAT AAG CAG AAG AAC GGC ATT AAG GTG AAC TTT AAG ATT CGC CAC AAC ATC GAG GAC GGT TCC GTC CAG CTC GCT GAT CAC TAC CAG CAG AAC ACG CCG ATC GGG GAC GGT CCG GTC CTG CTC CCC GAC AAC CAC TAC CTG TCG ACG CAG AGC GCC CTG AGC AAG GAT CCG AAC GAG AAG CGG GAT CAT ATG GTG CTC CTC GAG TTT GTC ACG GCT GCC GGC ATT ACC CTG GGC ATG GAT GAG CTG TAC AAG ATG GAG AAG ACC ATC GGG CTC GAG ATC ATT GAG GTC GTG GAG CAG GCG GCC ATT GCC AGC GCT CGC CTC ATG GGC AAG GGT GAG AAG AAC GAG GCG GAT CGG GTG GCG GTG GAG GCC ATG CGC GTC CGG ATG AAC CAG GTG GAG ATG CTC GGG CGG ATC GTC ATC GGC GAG GGC GAG CGC GAC GAG GCG CCT ATG CTC TAC ATC GGT GAG GAG GTC GGT ATC TAC CGC GAT GCT GAC AAG CGG GCT GGT GTC CCG GCT GGC AAG CTC GTC GAG ATT GAC ATT GCG GTC GAC CCT TGC GAG GGG ACG AAC CTC TGC GCC TAC GGG CAG CCC GGG AGC ATG GCT GTC CTG GCT ATT AGC GAG AAG GGT GGT CTC TTT GCT GCC CCT GAT TTC TAC ATG AAG AAG CTG GCT GCG CCC CCG GCG GCT AAG GGG AAG GTC GAC ATT AAC AAG AGC GCC ACG GAG AAC CTG AAG ATC CTG TCG GAG TGC CTG GAC CGG GCC ATC GAC GAG CTG GTC GTC GTC GTG ATG GAC CGC CCG CGC CAC AAG GAG CTC ATC CAG GAG ATC CGC CAG GCC GGG GCC CGC GTG

CGG CTC ATT TCC GAC GGC GAT GTC TCC GCC GCG ATC TCC TGC GGG TTC GCG GGC ACC AAC ACG CAT GCC CTC ATG GGG ATC GGG GCT GCG CCG GAG GGG GTG ATT AGC GCT GCG GCC ATG CGC TGC CTG GGT GGC CAC TTT CAG GGT CAG CTC ATT TAC GAT CCT GAG GTG GTC AAG ACC GGC CTC ATT GGC GAG TCG CGC GAG AGC AAC ATC GCT CGG CTC CAG GAG ATG GGG ATT ACC GAC CCG GAT CGG GTC TAC GAT GCT AAC GAG CTC GCC TCG GGC CAG GAG GTG CTG TTT GCG GCC TGC GGT ATT ACC CCT GGC CTG CTC ATG GAG GGT GTC CGG TTC TTC AAG GGT GGT GCC CGG ACC CAG AGC CTG GTG ATC TCG TCG CAG TCC CGC ACC GCG CGC TTT GTG GAT ACC GTC CAT ATG TTC GAC GAT GTG AAG ACC GTC TCC CTG CGC

**CTP-FBP/SBPase-EGFP**

**Not optimized (616 codons):**

ATG CAG GTC ACC ATG AAG TCG AGC GCC GTC AGC GGC CAG CGG GTC GGC GGT GCT CGG GTC GCT ACG CGG AGC GTC CGG CGC GCG CAG CTC CAG GTG
ATG GAG AAG ACC ATT GGC CTG GAG ATC ATC GAG GTC GTC GAG CAG GCC GCG ATC GCC AGC GCT CGC C TC ATG GGT AAG GGC GAG AAG AAC GAG GCC GACCGC GTC GCT GTC GAG GCG ATG CGC GTC CGC ATG AA C CAG GTG GAG ATG CTG GGT CGC ATC GTC ATC GGG GAG GGC GAG CGC GAC GAG GCC CCC ATG CTCTAC ATT GGG GAG GAG GTG GGG ATT TAC CGG GAC GCC GAT AAG CGG GCT GGT GTG CCG GCC GGG AAG CTG GTC GAG ATC GAC ATC GCG GTC GAT CCT TGCGAG GGT ACG AAC CTC TGC GCC TAC GGG CAG CCG GGC T CG ATG GCC GTC CTC GCT ATT TCC GAG AAG GGC GGT CTG TTT GCC GCG CCT GAC TTC TAC ATGAAG AAG CTC GCC GCC CCT CCT GCG GCT AAG GGG AAG GTC GAC ATC AAC AAG TCG GCT ACG GAG AAC CTC AAG A TC CTG AGC GAG TGC CTC GAT CGG GCCATT GAC GAG CTC GTC GTG GTG GTG ATG GAT CGG CCG CGG CA C AAG GAG CTG ATC CAG GAG ATC CGC CAG GCG GGC GCT CGG GTC CGC CTC ATC TCC GATGGT GAT GTC AGC GCG GCT ATT TCG TGC GGC TTC GCT GGG ACC AAC ACG CAC GCC CTG ATG GGT ATT GGT GCT GCG C CC GAG GGT GTG ATT AGC GCT GCGGCG ATG CGC TGC CTG GGG GGT CAT TTC CAG GGC CAG CTC ATT TA C GAT CCC GAG GTC GTC AAG ACC GGG CTG ATC GGT GAG AGC CGC GAG TCC AAC ATCGCG CGC CTC CAG GAG ATG GGC ATT ACG GAC CCT GAC CGC GTC TAC GAC GCG AAC GAG CTG GCC TCC GGC CAG GAG GTG CTC TTT GCC GCG TGC GGT ATCACC CCC GGT CTC CTG ATG GAG GGT GTC CGG TTC TTC AAG GGC GGT GC G CGC ACG CAG TCG CTC GTC ATT AGC TCG CAG TCG CGG ACC GCG CGG TTC GTGGAC ACG GTC CAC ATG TTC GAC GAT GTC AAG ACG GTC TCG CTC CGC ATG GTG AGC AAG GGC GAG GAG CTG TTC ACC GGG GTG GTG CCC ATC CTG GTC GAG CTG GAC GGC GAC GTA AAC GGC CAC AAG TTC AGC GTG TCC GGC GAG GGC GAG GGC GAT GCC ACC TAC GGC AAG CTG ACC CTG AAG TTC ATC TGC ACC ACC GGC AAG CTG CCC GTG CCC TGG CCC ACC CTC GTG ACC ACC CTG ACC TAC GGC GTG CAG TGC TTC AGC CGC TAC CCC GAC CAC ATG AAG CAG CAC GAC TTC TTC AAG TCC GCC ATG CCC GAA GGC TAC GTC CAG GAG CGC ACC ATC TTC TTC AAG GAC GAC GGC AAC TAC AAG ACC CGC GCC GAG GTG AAG TTC GAG GGC GAC ACC CTG GTG AAC CGC ATC GAG CTG AAG GGC ATC GAC TTC AAG GAG GAC GGC AAC ATC CTG GGG CAC AAG CTG GAG TAC AAC TAC AAC AGC CAC AAC GTC TAT ATC ATG GCC GAC AAG CAG AAG AAC GGC ATC AAG GTG AAC TTC AAG ATC CGC CAC AAC ATC GAG GAC GGC AGC GTG CAG CTC GCC GAC CAC TAC CAG CAG AAC ACC CCC ATC GGC GAC GGC CCC GTG CTG CTG CCC GAC AAC CAC TAC CTG AGC ACC CAG TCC GCC CTG AGC AAA GAC CCC AAC GAG AAG CGC GAT CAC ATG GTC CTG CTG GAG TTC GTG ACC GCC GCC GGG ATC ACT CTC GGC ATG GAC GAG CTG TAC AAG

**Optimized (616 codons):**

ATG CAG GTG ACG ATG AAG TCG AGC GCT GTG TCG GGC CAG CGG GTG GGT GGT GCC CGG GTG GCG ACC CGC TCG GTC CGC CGC GCC CAG CTC CAG GTG ATG GAG AAG ACC ATT GGG CTG GAG ATT ATC GAG GTG GTC GAG CAG GCG GCC ATC GCC AGC GCC CGG CTG ATG GGG AAG GGT GAG AAG AAC GAG GCT GAT CGG GTC GCG GTC GAG GCG ATG CGC GTC CGC ATG AAC CAG GTC GAG ATG CTC GGG CGG ATT GTG ATT GGC GAG GGT GAG CGC GAC GAG GCT CCC ATG CTC TAC ATC GGC GAG GAG GTC GGT ATC TAC CGC GAC GCT GAT AAG CGG GCG GGT GTC CCT GCG GGG AAG CTC GTC GAG ATC GAC ATC GCC GTC GAT CCC TGC GAG GGG ACG AAC CTC TGC GCG TAC GGT CAG CCG GGG AGC ATG GCG GTG CTC GCT ATT TCC GAG AAG GGT GGC CTC TTC GCG GCC CCC GAC TTC TAC ATG AAG AAG CTG GCC GCC CCT CCC GCG GCC AAG GGT AAG GTC GAC ATT AAC AAG TCC GCC ACG GAG AAC CTC AAG ATT CTC TCG GAG TGC CTG GAC CGG GCT ATC GAT GAG CTG GTG GTC GTC GTC ATG GAT CGG CCT CGG CAC AAG GAG CTC ATT CAG GAG ATT CGC CAG GCG GGC GCG CGG GTG CGC CTC ATT AGC GAC GGT GAT GTC TCG GCC GCC ATT AGC TGC GGG TTC GCC GGC ACG AAC ACG CAT GCC CTG ATG GGC ATC GGG GCC GCC CCG GAG GGC GTC ATT TCC GCG GCG GCT ATG CGC TGC CTG GGG GGG CAC TTC CAG GGC CAG CTG ATT TAC GAT CCC GAG GTG GTC AAG ACG GGC CTC ATT GGG GAG AGC CGC GAG AGC AAC ATC GCG CGC CTC CAG GAG ATG GGT ATT ACC GAC CCG GAT

CGC GTC TAC GAC GCC AAC GAG CTG GCC TCG GGC CAG GAG GTG CTG TTC GCG GCT TGC GGT ATT ACC CCT GGT CTC CTC ATG GAG GGT GTG CGC TTT TTT AAG GGC GGG GCC CGG ACC CAG AGC CTG GTG ATT AGC TCC CAG TCC CGC ACG GCT CGG TTC GTC GAC ACC GTG CAT ATG TTT GAC GAT GTG AAG ACG GTC TCC CTC CGC ATG GTG TCG AAG GGC GAG GAG CTC TTC ACG GGC GTG GTC CCT ATC CTG GTC GAG CTC GAT GGC GAT GTG AAC GGT CAC AAG TTT AGC GTC TCC GGC GAG GGT GAG GGT GAC GCT ACC TAC GGG AAG CTC ACC CTG AAG TTT ATT TGC ACG ACG GGC AAG CTC CCT GTC CCT TGG CCC ACG CTC GTG ACG ACC CTG ACC TAC GGT GTC CAG TGC TTT TCG CGC TAC CCC GAC CAT ATG AAG CAG CAT GAT TTC TTC AAG AGC GCG ATG CCC GAG GGC TAC GTC CAG GAG CGC ACC ATC TTT TTC AAG GAC GAT GGG AAC TAC AAG ACG CGG GCT GAG GTC AAG TTC GAG GGT GAC ACC CTC GTG AAC CGG ATT GAG CTC AAG GGT ATT GAC TTT AAG GAG GAT GGT AAC ATT CTC GGT CAC AAG CTC GAG TAC AAC TAC AAC AGC CAC AAC GTG TAC ATT ATG GCT GAT AAG CAG AAG AAC GGT ATC AAG GTG AAC TTT AAG ATT CGC CAC AAC ATT GAG GAT GGC TCC GTG CAG CTG GCT GAC CAC TAC CAG CAG AAC ACG CCT ATT GGT GAT GGT CCT GTG CTC CTC CCT GAT AAC CAT TAC CTC TCG ACG CAG AGC GCC CTC TCC AAG GAC CCT AAC GAG AAG CGG GAT CAC ATG GTC CTC CTG GAG TTT GTC ACC GCT GCG GGC ATC ACC CTC GGT ATG GAT GAG CTG TAC AAG

## For the production of RFP:

**mCherry (RFP)**

**Not Optimized**

ATG GTG AGC AAG GGC GAG GAG GAT AAC ATG GCC ATC ATC AAG GAG TTC ATG CGC TTC AAG GTG CAC ATG GAG GGC TCC GTG AAC GGC CAC GAG TTC GAG ATC GAG GGC GAG GGC GAG GGC CGC CCC TAC GAG GGC ACC CAG ACC GCC AAG CTG AAG GTG ACC AAG GGT GGC CCC CTG CCC TTC GCC TGG GAC ATC CTG TCC CCT CAG TTC ATG TAC GGC TCC AAG GCC TAC GTG AAG CAC CCC GCC GAC ATC CCC GAC TAC TTG AAG CTG TCC TTC CCC GAG GGC TTC AAG TGG GAG CGC GTG ATG AAC TTC GAG GAC GGC GGC GTG GTG ACC GTG ACC CAG GAC TCC TCC CTG CAG GAC GGC GAG TTC ATC TAC AAG GTG AAG CTG CGC GGC ACC AAC TTC CCC TCC GAC GGC CCC GTA ATG CAG AAG AAG ACC ATG GGC TGG GAG GCC TCC TCC GAG CGG ATG TAC CCC GAG GAC GGC  GCC CTG AAG GGC GAG ATC AAG CAG AGG CTG AAG CTG AAG GAC GGC GGC CAC TAC GAC GCT GAG GTC AAG ACC ACC TAC AAG GCC AAG AAG CCC GTG CAG CTG CCC GGC GCC TAC AAC GTC AAC ATC AAG TTG GAC ATC ACC TCC CAC AAC GAG GAC TAC ACC ATC GTG GAA CAG TAC GAA CGC GCC GAG GGC CGC CAC TCC ACC GGC GGC ATG GAC GAG CTG TAC AAG TAA

http://www.algosome.com/resources/common-sequences.html#rfp

**Optimized**

ATG GTC TCC AAG GGC GAG GAG GAT AAC ATG GCT ATT ATT AAG GAG TTC ATG CGG TTC AAG GTG CAT ATG GAG GGG TCG GTG AAC GGT CAT GAG TTT GAG ATT GAG GGG GAG GGG GAG GGC CGC CCT TAC GAG GGG ACG CAG ACC GCG AAG CTG AAG GTG ACC AAG GGT GGT CCG CTG CCC TTC GCG TGG GAT ATT CTG TCC CCC CAG TTT ATG TAC GGC TCG AAG GCG TAC GTC AAG CAT CCG GCT GAC ATT CCG GAC TAC CTG AAG CTC AGC TTC CCT GAG GGG TTT AAG TGG GAG CGG GTG ATG AAC TTT GAG GAC GGT GGT GTC GTC ACG GTC ACC CAG GAT AGC AGC CTC CAG GAT GGG GAG TTT ATT TAC AAG GTG AAG CTC CGC GGG ACG AAC TTT CCG AGC GAT GGT CCG GTG ATG CAG AAG AAG ACC ATG GGC TGG GAG GCT TCG TCG GAG CGG ATG TAC CCG GAG GAC GGG GCT CTC AAG GGG GAG ATT AAG CAG CGG CTC AAG CTG AAG GAC GGC GGT CAC TAC GAT GCC GAG GTC AAG ACC ACC TAC AAG GCG AAG AAG CCT GTC CAG CTC CCG GGG GCG TAC AAC GTG AAC ATT AAG CTC GAC ATC ACG TCG CAT AAC GAG GAT TAC ACC ATT GTG GAG CAG TAC GAG CGG GCC GAG GGC CGG CAT AGC ACC GGG GGC ATG GAC GAG CTC TAC AAG TAA

**The promoter sequence MetE-RFP (1285 nt) to optimize is:**

TGTAGGTCAGGACCAGAGCCTACAACATTGTAAGGCCATGTATCCTCCAGTGCCATTTGCGACGTTACAC
GACGAGACAATCTGCGCTGTGTTCAAGCACACACATCCGGGACTCGTGTGATGTCTTAACATCTGCAAGG
AAACATAAGGTGGCCGGCGTCACGCAAGATGACGCGACGGACTCGAATGCTTGCTTCGTCCAGCGACAAA
TAAGGACAGGCAGGGCGCCTGCATTGCGTGCGCTTAGAGGCGAGGCGCTCAAGACATTTCGGCAGCAATA
ATTGGTATTGAGGCACATTCTGCACCAGGATGCCAAGAGGTGAACGTTGCTGCCTTAATGTATATCTGCA
CAGCTGGCCGGTTACTGCGAACCGGGTGCCTTTTGGAGTCGCTCCTAAAAGACGTCACCGGCGCAACGTC
GTCGTGCGGCCGTACAGGTATGCAAAATTGGCCCGGTTGCGCGCAGGCCGATTATTTAAGTGACATTTGG
TACCATGCATGAGCATCCGATTGTTGTTTTGCAATTCTGTCTGACTGCACAAGGAACCCGTCCTTGGGAA
TCAACATCTGTCAA ATG GTC TCC AAG GGC GAG GAG GAT AAC ATG GCT ATT ATT AAG GAG TTC ATG CGG TTC AAG GTG CAT ATG GAG GGG TCG GTG AAC GGT CAT GAG TTT GAG ATT GAG GGG GAG GGG GAG GGC

CGC CCT TAC GAG GGG ACG CAG ACC GCG AAG CTG AAG GTG ACC AAG GGT GGT CCG CTG CCC TTC GCG TGG GAT ATT CTG TCC CCC CAG TTT ATG TAC GGC TCG AAG GCG TAC GTC AAG CAT CCG GCT GAC ATT CCG GAC TAC CTG AAG CTC AGC TTC CCT GAG GGG TTT AAG TGG GAG CGG GTG ATG AAC TTT GAG GAC GGT GGT GTC GTC ACG GTC ACC CAG GAT AGC AGC CTC CAG GAT GGG GAG TTT ATT TAC AAG GTG AAG CTC CGC GGG ACG AAC TTT CCG AGC GAT GGT CCG GTG ATG CAG AAG AAG ACC ATG GGC TGG GAG GCT TCG TCG GAG CGG ATG TAC CCG GAG GAC GGG GCT CTC AAG GGG GAG ATT AAG CAG CGG CTC AAG CTG AAG GAC GGC GGT CAC TAC GAT GCC GAG GTC AAG ACC ACC TAC AAG GCG AAG AAG CCT GTC CAG CTC CCG GGG GCG TAC AAC GTG AAC ATT AAG CTC GAC ATC ACG TCG CAT AAC GAG GAT TAC ACC ATT GTG GAG CAG TAC GAG CGG GCC GAG GGC CGG CAT AGC ACC GGG GGC ATG GAC GAG CTC TAC AAG TAA

## Standardization

Two parts will be sent to iGEM. The first is FBP/SBPase protein, sequence that with prefix and suffix is shown:

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