Openly Glowing Plant #1 SynMemo
by Andreas Stürmer, Justin Atkin and Sebastian Cocioba

This is a follow up to Openly Glowing Plant #1 memo. We propose synthesis of 13 DNA products in the range of 0.5kb-5kb. All pass Twist's gene checks.

The DNA will be transformed into plants by a true master in the field. Sebastian Cocioba runs the educational non-profit Binomica labs, as well as New York Botanics. In his work, he has done nuclear and plastid transformation of various plants such as tobacco, petunia, arabidopsis and wheat. Most frequently he employs agrobacterium, but he also possesses a functioning gene gun. He also has skills in protoplast transformation.

The research builds upon the recent discovery of the fungal metabolic pathway for light emission through the caffeic acid cycle.

7 different DNA fragments are luciferases. 3 are wild type to compare function between source species. 2 are rationally designed luciferase mutants to attempt to shift the color. 1 is a closely related (found through BLAST) fungal enzyme that will be checked for luciferase activity and finally one is an RFP-tagged Luciferase again to attempt a color shift [1]. One of the two luciferin synthesis enzymes is too big to be synthesized as a 5000 bp brick so it will be split up into two parts and will be ligated by BglII digestion.

3 of the fragments are expression cassettes that contain a promoter and terminator and will fit into the pCambia2300 multiple cloning site. The promoter and terminator in these fragments will be AtTCT Promoter and HSP-Terminator of Arabidopsis. [2]

The wt luciferase and the two luciferin biosynthesis enzymes will be cloned into the respective expression cassette to produce the final plasmids before transformation into a selection of plants (primarily tobacco to start) to assay for expression, function, and glow.

Once the wild type and rationally designed variants are assayed, new alternative luciferases will be produced by mutating the wt enzymes (using manganese PCR) and then re-subcloned into the vectors and checked for kinetic properties and shifted colour emission.

We designed mutants versions of the luciferase, which change the putative binding pocket and believe they can shift the light emission wavelength. The pockets were found by aligning the known fungal luciferases from the paper and looking for conserved domains. We adapted the reasoning from a firefly luciferase color-shift paper, where Y->F [3] is a relatively conservative mutation and causes the enzyme to alter its structure by just a little bit. Besides side-chain size
of the amino acid, other alterations such as “disturbances in the hydrogen-bond network or changes in the rigidity of the active pocket due to substitution” could just slightly push the wavelength of the emitted photons.

All coding sequences meant for inducible expression have been codon optimized for Nicotiana tabaccum because this model organism is very easy to work with and genetically transform.

Another reason why we codon optimized the sequences for tobacco is because the glowing plant paper did it in tobacco, and it worked. Furthermore, it grows fast and is easy to transform. Also, it appears to us that codon usage of tobacco should work decently in any plant. Sebastian is an expert in the field of plant expression of foreign genes and recommended this route. The codon usage adaption was done by IDT codon analyser because Sebastian has had great experience with it. The optimized codons have further been manually checked with the tool Kazusa. Besides empirical experience of Sebastian in transforming plants with codon-optimized genes, also the literature shows that the same codons should work consistently across higher plants [4].

“Interestingly, the use of optimal codons appears to be well conserved between eudicots and monocots, and to a lesser degree between the higher plants and C. reinhardtii.” [5]

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luz</td>
<td>catalyzes light reaction of the luciferin</td>
<td></td>
<td>837</td>
</tr>
<tr>
<td>HispS1</td>
<td>catalyzes first step of luciferin synthesis from caffeic acid - fragment 1/2</td>
<td></td>
<td>3300</td>
</tr>
<tr>
<td>HispS2</td>
<td>catalyzes first step of luciferin synthesis from caffeic acid - fragment 2/2</td>
<td></td>
<td>1787</td>
</tr>
<tr>
<td>Needs to be split up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3H</td>
<td>catalyzes second step of luciferin synthesis</td>
<td></td>
<td>1299</td>
</tr>
</tbody>
</table>
from hispidin

Vector Luz
provides transcription signals for Luz, has restriction sites to make it fit into whole construct

Vector HisS
provides transcription signals for Luz, has restriction sites to make it fit into whole construct

Vector H3H
provides transcription signals for Luz, has restriction sites to make it fit into whole construct

Luz-RFP
catalyzes light reaction of the luciferin, emitting red-shifted photons

fungal Luz homologue
see if it catalyzes same reaction, random mutagenesis

Y233F
pocket mutant

Y189F
pocket mutant

Fungal Luciferase - of different luciferase for

598

607

606

1560

885

837

837

819
Omphalotus olearius
random mutation

Fungal Luciferase of Mycena citricolor
different luciferase for random mutation


