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 conserative 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 wvelentgh 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]

Name	Function	Sequence	Length
Luz	catalyzes light reaction of the luciferin	ATGCGCATTAACATTAGCCTCTCGTCTCTCTTCGAACGTCTCTCCAAACTTAGCAGTCGCAGCATAGCGATT ACATGTGGAGTTGTTCTCGCCTCCGCAATCGCCTTTCCCATCATCCGCAGAGACTACCAGACTTTCCTAGAA GTGGGACCCTCGTACGCTCCGCAGAACTTTAGAGGATACATCATCGTCTGTGTCCTCTCGCTATTCCGCCAA GAGCAGAAAGGGCTCGCCATCTATGATCGTCTTCCCGAGAAACGCAGGTGGTTGGCCGACCTTCCCTTTCG TGAAGGAACCAGACCCAGCATTACCAGCCATATCATTCAGCGACAGCGCACTCAACTGGTCGATCAGGAGT TTGCCACCAGGGAGCTCATAGACAAGGTCATCCCTCGCGTGCAAGCACGACACACCGACAAAACGTTCCTC AGCACATCAAAGTTCGAGTTTCATGCGAAGGCCATATTTCTCTTGCCTTCTATCCCAATCAACGACCCTCTGA ATATCCCTAGCCACGACACTGTCCGCCGAACGAAGCGCGAGATTGCACATATGCATGATTATCATGATTGCA CACTTCATCTTGCTCTCGCTGCGCAGGATGGAAAGGAGGTGCTGAAGAAAGGTTGGGGACAACGACATCCT TTGGCTGGTCCTGGAGTTCCTGGTCCACCAACGGAATGGACTTTTCTTTATGCGCCTCGCAACGAAGAAGA GGCTCGAGTAGTGGAGATGATCGTTGAGGCTTCCATAGGGTATATGACGAACGATCCTGCAGGAAAGATTG TAGAAAACGCCAAG	837
HispS1	catalyzes first step of luciferin synthesis from caffeic acid - fragment 1/2	AATAAGCGACCATGGGAAATAGCAGTAAGAATCCACCATCAACCCTCCTCGACGTGTTTCTCGATACAGCCA GAAACTTGGATACCGCTCTGAGAAATGTCCTTGAATGCGGGGAGCATAGATGGTCATATAGGgaaCTCGACA CCGTTAGCTCCGCTCTTGCTCAGCATTTGCGATATACCGTAGGGTTATCACCAACAGTGGCTgtcATAtctgagA ATCATCCTTATATTTTGGCACTTATGTTGGCCGTGTGGAAGTTAGGCGGCACTTTTGCTCCAATCGATGTGC ACTCTCCTGCCGAATTAGTGGCTGGAATGCTGAACATCGTGTCACCATCATGTCTTGTCATACCATCTAGCG ATGTAACAAATCAGACCCTTGCCTGTGATTTGAACATACCCGTAGTTGCTTTCCATCCTCATCAGTCTACTAT CCCAGAGCTGAATaagAAGTACCTTACAGACAGTCAGattTCAcctGATCTTCCCttcTCTGACCCAAACCGTCCA GCACTTTACTTATTCACCAGCAGTGCCACAAGCAGAAGTAATCTGAAATGCGTGCCATTGACTCATACATTC ATTCTTCGTAACAGCCTTTCTAAGAGGGCTTGGTGTAAACGTATGCGACCTGAGACTGACTTTGATggtATCC GTGTCCTGGGCTGGGCTcctTGGAGTCATGTGTTAGCCcacATGCAGGATATAGGGCCATTAACACTCTTGAA TGCTGGCTGCTATGTCTTCGCCACTACTCCATCAACTTATCCCACTGAACTCAAAGACGATAGGGACTTGAT TAGTTGCGCAGCAAACGCCATTATGTACAAGGGTGTGAAATCATTCGCATGTCTTCCCTTTGTTTTAGGAGG GTTAAAGGCCCTCTGTGAAAGCGAACCTAGTGTCAAGGCCCATCTCCAAGTTGAAGAGAGAGCACAGTTAT TGAAGTCACTCCAAcacATGGAGATTTTAGAGTGCGGTGGAGCTATGTTAgagGCTTCTGTTGCCAGCTGGGC TATCGAAAACTGCATACCCATCAGCATCGGTATAGGCATGACAGAGACTGGCGGTGCATTGTTTGCAGGTC CCGTCCAAGCCATCAAAACAGGCTTTAGCTCCGAGGATAAGTTTATTGAGGATGCTACCTACCTCCTTGTTA AAGATGATCATGAATCACATGCAGAGGAAGATATTAACGAAGGGGAACTTGTAGTGAAATCCAAAATGCTCC CTAGAGGGTACTTGGGCTACTCTGACCCATCATTTTCCGTTGATGACGCCGGATGGTAACTTTTCGTACAG GTGACCGTTACTCTGTGACACCCGATGGTAAGTTTAGTTGGTTGGGTAGGAATACAGACTTCATACAGATGA CCTCCGGAGAGACActtGATCCACGTCCTATCGAAAGCAGCCTGTGTGAGTCATCCTTGATTTCTAGGgctTGC GTAATCGGAGACAAGTTCCTCAATGGGCCCGCAGCTGCCGTGTGCGCCATAATCGAACTCGAACCTACAGC TGTGGAGAAAGGGCAAGCCCACTCACGTGAGATAGCAAGGGTGTTTgctCCCATAAATagggatCTTCCAcctCC ATTGAGAATCGCTTGGTCCCATGTACTTGTGTTGCAGCCCTCTGAGAAGATTCCTATGACAAAGAAAGGCAC TATTTTCAGAAAGAAAATCGAACAGGTATTCGGGAGCGCTCTTggtGGTAGTTCAGGAGACAATAGCCAAGCC ACTGCCGATgctGGTGTGGTACGTAGGGATGAACTCAGTAATACTGTCAAGCACATTATAtccagaGTACTTGGC GTGTCTGACGACGAGCTTTTGTGGACACTTAGCTTTGCCGAGTTAGGGATGACATCTGCCTTGGCCACAAG AATCGCAAACGAGCTTAACGAAGTACTTGTAGGGGTTAATCTTCCCATTAACGCCTGTTATATTCATGTCGAT TTAcctAGCTTAAGCAATGCAGTCTACGCCAAGctgGCACACCTGAAACTCCCAGATCGAACACCAGAGCCAC GTCAGGCTCCTGTCGAAAACAGCGGAGGCAAGGAGATAGTCGTAGTAGGCCAAGCATTTCGATTACCTGGA AGCATCAATGATGTGGCATCATTGCGTGACGCCTTCCTGGCCAGACAAGCCTCATCTATTATTACCGAAATT CCATCTGATAGGTGGGATCATGCTAGTttcTATCCAAAGGACATCAGGTTTAACAAGGCTGGGCTGGTTGACA TTGCAAACTACGACCATAGCTTCTTTGGTCTGACTGCTACCGAGGCATTGTACTTGTCCCCTACCATGAGAC TGGCACTTGAAGTATCTTTTGAAGCTCTCGAAAATGCAAACATCCCAGTGAGTCAGCTGAAAGGATCTCAAA CAGCCGTCTATGTCGCAACTACTGATGATGGGTTCGAAACACTTTTGAATGCAGAAGCAGGCTATGACGCAT ATACTAGGTTCTATGGTACTGGCAGGGCCGCCTCTACTGCTTCCGGCCGTATTTCTTGTCTGTTAGATgtgCA TGGCCCTAGTATTACAGTCGATACCGCTTGCAGTggtGGAGCCGTATGTATTGACCAAGCCATTGATTATCTC CAAAGCTCATCAGCCGCCGACACTGCCATCATCTGCGCCAGTAACACTCACTGTTGGCCAggtTCCTTTAGG TTTCTTtctGCTCAGGGAATGGTGAGTcctggtggtagatgtGCCACTTTTACAACTGACGCTGATGGATACGTACCTA GCGAGggtGCCGTCGCATTTATACTTAAAACTCGTGAGGCCGCAATGAGAGATAAAGACACAATATTAGCCAC CATAAAAGCCACTCAGATAAGCCATAATGGAAGGTCCCAGGGACTTGTGGCACCTAACGTGAACAGCCAGG CTGATCTTCACCGAAGCCTCctccagAAAGCTGGCCTTAGTCCCGCAGACATTAGGTTTATCGAGGCACACGG AactGGTactTCATTAGGCGATCTGAGCGAGATACAGGCCATAAATGATGCTTATACCAGTAGTCAGCCACGAA CTACTGGACCATTAATTGTGTCCGCAAGCAAGACAGTAATCGGGCATACCGAGCCAgctggtcctCTTGTTGGGA TGTTGTCCGTGCTTAACAGCTTTAAGGAAGGCGCAGTACCTGGGTTGGCTCATCTTACAGCTGACAACTTAA ACCCCTCCCTGGATTGCAGTtctGTACCATTACTCATCCCATACCAACCTGTACACTTAGCTGCACCCAAGCC ACATCGAGCCGCTGTCagatctTATG	3300
HispS2 Needs to be split up	catalyzes first step of luciferin synthesis from caffeic acid - fragment 2/2	CGCTGTCagatctTATGGTTTCTCAGGAACTCTTGGAGGTATCGTCctgGAGGCTCCCGACGAAGAGAGACTTG AGGAAGAATTGCCCAATGACAAACCAATGCTGTTTGTGGTTTCAGCTAAAACTCATACAGCCCTCATTGAGT ACTTGGGCAGATACCTGgagttcTTGCTGcaaGCTAATCCACAAGACTTTTGTGACATCTGCTATaccAGTTGCGT CGGTAGAGAGCACTATaggTATCGTTACGCATGTGTCGCTAATGACATGGAGGACTTAATTGGACAACTGCA AAAGAGGCTCGGTAGCAAGgttCCACCAAAGCCTTCTTACAAACGTGGCGCCCTCGCCTTCGCATTCTCAGG CCAGGGTACACAGTTTCGAGGCATGGCTACTGAGCTGGCTAAAGCCTATTCCGGTTTTCGAAAAATCGTATC AGACCTTGCTAAGCGTGCCTCTGAACTTTCAGGCCACGCCATTGATAGGTTCTTACTGGCATACGACATTGG AGCCGAAAATGTAGCACCTGACAGTGAAGCTGACCAAATCTGTATCTTTGTTTACCAGTGTTCAGTTTTACGT TGGCTTCAAACTATGGGAATCAGACCCTCTgctGTTATAGGACACTCCTTGGGAGAAATCTCAGCTTCCGTCG CTgctGGTGCTCTTTCACTCGACAGCGCACTCGATCTTGTCATATCAAGGGCCAGGTTATTAAGGAGTTCTGC AAGCGCCCCAGCTGGGATGGCTGCTATGTCTGCTTCCCAAGATGAGGTGGTAGAATTGATTGGCAAACTCG ATCTGGATAAAGCCAATAGCCTGAGCGTGAGTGTTATCAACggtcctCAAAATACCGTTGTCAGCGGCTCTTCC GCCGCTATAGAGTCAATAGTTGCTCTGGCCAAGGGGCGAAAGATAaagGCTTCTGCTCTGAATATTAACCAA GCATTCCACTCCCCTTATGTGGATTCTGCCGTTCCAGGACTGCGTGCATGGAGCGAGAAACACATTAGTTCT GCCagaCCTCTCCAAATTCCTCTCTATTCCACATTACTGGGTGCCCAGattTCTGAGggtGAAATGTTAAATCCT GACCATTGGGTCgatCACGCTAGGAAACCCGTTCAGTTCGCCCAGGCAGCTACCACAATGAAGGAGTCATTT ACAGGTGTCATCATAGACATTGGGCCACAAGTCGTAGCATGGTCACTTTTGCTGTCTAATGGCCTTACCTCT GTAACAGCTCTCGCTGCCAAGCGAGGAAGAAGCCAGCAGGTAGCCttcTTATCTGCATTAGCTGATCTGTATC AGGATTATGGAGTCGTACCTGATTTCGTGGGCTTGTATGCACAGCAAGAGGACGCCTCAAGGCTGAAGAAG ACTgacatcTTAACCTATCCCTTCCAAAGAGGTGAGGAGACTTTATCATCCGGTAGCAGTACTCCTACCCTTGA AAATACAGACTTGGACTCCGGCAAGGAGCTGTTAATGggtCCCACACGTGGGCTCTTGAGAGCTGACGACCT TAGGGACAGCATAGTCTCTTCCGTGAAAGATGTCCTCGAATTAaagAGTAACGAGGATTTGGACTTAAGCGA GAGTCTTAATGCCTTGGGAATGGACTCCATCATGTTTGCCCAGTTACGTAAGAGAATTGGTGAAGGCTTGGG TCTGAACGTCcctATGGTCTTTCTGAGTGATGCATTCTCCATTggtGAGATGGTATCTAACTTAGTAGAGCAGG CTGAGGCTtctGAAGACAACTAGgGAGCTCagatatgaagat	1787
H3H	catalyzes second step of luciferin synthesis	ATGGCATCGTTTGAGAATTCTCTAAGCGTTTTGATTGTCGGGGCCGGACTTGGTGGGCTTGCTGCTGCCAT CGCGCTGCGTCGCCAAGGGCATGTCGTGAAAATATACGACTCCTCTAGCTTCAAAGCCGAACTTGGTGCGG GACTCGCTGTGCCGCCTAACACCTTGCGCAGTCTACAGCAACTTGGTTGCAATACCGAGAACCTCAATGGT GTGGATAATCTTTGCTTCACTGCGATGGGGTATGACGGGAGTGTAGGGATGATGAACAACATGACTGACTA TCGAGAGGCATACGGTACTTCTTGGATCATGGTCCACCGCGTTGACTTGCATAACGAGCTGATGCGCGTAG CACTTGATCCAGGTGGGCTCGGACCTCCTGCGACACTCCATCTTAATCATCGTGTCACATTCTGCGATGTCG ACGCTTGCACCGTGACATTCACCAACGGGACCACTCAATCAGCTGATCTCATCGTTGGTGCAGACGGTATA CGCTCTACCATTCGGCGGTTTGTCTTAGAAGAAGACGTGACTGTGCCTGCGTCAGGAATCGTCGGGTTTCG ATGGCTTGTACAAGCTGACGCGCTGGACCCATATCCTGAACTCGACTGGATTGTTAAAAAGCCTCCTCTAGG CGCGCGACTGATCTCCACTCCTCAGAATCCACAGTCTGGTGTTGGCTTGGCTGACAGGCGCACTATCATCA TCTACGCATGTCGTGGCGGCACCATGGTCAATGTCCTTGCAGTGCATGATGACGAACGTGACCAGAACACC GCAGATTGGAGTGTACCGGCTTCCAAAGACGATCTATTTCGTGTTTTCCACGATTACCATCCACGCTTTCGG	1299

	from hispidin	CGGCTTTTAGAGCTTGCGCAGGATATTAATCTCTGGCAAATGCGTGTTGTACCTGTTTTGAAAAATGGGTTA ACAAGCGGGTTTGCTTGTTAGGAGATGCTGCCGCACGCTTCTTTACCGACGTTGGGTCAAGGTTTTGGTATG GGTCTGGAAGATGCCGTAGCACTTGGTACACTCCTTCCAAAGGGTACCACTGCATCTCAGATCGAGACTCG ACTTGCGGTGTACGAACAGCTACGTAAGGATCGTGCGGAATTTGTTGCGGCTGAATCATATGAAGAGCAAT ATGTTCCTGAAATGCGGGGGACTTTATCTGAGGTCAAAGGAACTGCGTGATAGAGTCATGGGTTATGATATCA AAGTGGAGAGCGAGAAGGTTCTCGAGACGCTCCTAAGAAGTTCTAATTCTGCC	
Vector Luz	provides transcription signals for Luz, has restriction sites to make it fit into whole construct	ggatccaatCCAACACTCGAATCCCCACCCGTTGGACCAAACCCGGCTCATTAAGC GTCGGTTCAGATTTATTTCCTTTATTTAAAAAAAGGAAAGGGTAAAAAATAGAA AATTGGAAACAGTTAAAGCCCAAAATTGTAATTTACCGAGAATTGTAAATTTACC TGAAAACCCTACGCTATAGTTTCGACTATAAATACCAAACTTAGGACCTCACTTC AGAATCCCCTCGTCGCTGCGTCTCTCCCGCAACCTTCGATTTTCGTTTATTC GCATCCATCGGAGAGAGAAACAATCAATAAGCGACCATGggtaactaacttAAGtaag GAGCTCagatatgaagatgaagatgaaatatttggtgtgtcaaataaaaagcttgtgtgcttaagtttgtttttttctt ggcttgtgtgttatgaatttgtggctttttctaatattaaatgaatgtaagatctcattataatgaataaacaaatgtttctata atccatttgtgaatgttttgtggatctcttctgcagcatataactactgtatgtgctatggtatggactatggaatatgattaaa gataagtctaga	598
Vector HispS	provides transcription signals for Luz, has restriction sites to make it fit into whole construct	tgaattcaGATATcaatCCAACACTCGAATCCCCACCCGTTGGACCAAACCCGGCTC ATTAAGCGTCGGTTCAGATTTATTTCCTTTATTTAAAAAAAGGAAAGGGTAAAA AATAGAAAATTGGAAACAGTTAAAGCCCAAAATTGTAATTTACCGAGAATTGTAA ATTTACCTGAAAACCCTACGCTATAGTTTCGACTATAAATACCAAACTTAGGACC TCACTTCAGAATCCCCTCGTCGCTGCGTCTCTCCCGCAACCTTCGATTTTCG TTTATTCGCATCCATCGGAGAGAGAAAACAATCAATAAGCGACCATGggtaactaac ttAAGtaagGAGCTCagatatgaagatgaagatgaaatatttggtgtgtcaaataaaaagcttgtgtgcttaagttt gtgttttttctttggcttgtgttatgaatttgtggctttttctaatattaaatgaatgtaagatctcattataatgaataaacaaatgtttctata atccatttgtgaatgttttgtggatctcttctgcagcatataactactgtatgtgctatggtatggactatggaatatgattaaa gataagtctaga	607
Vector H3H	provides transcription signals for Luz, has restriction sites to make it fit into whole construct	ggtacccaatCCAACACTCGAATCCCCACCCGTTGGACCAAACCCGGCTCATTAAG CGTCGGTTCAGATTTATTTCCTTTATTTAAAAAAAGGAAAGGGTAAAAAATAGA AAATTGGAAACAGTTAAAGCCCAAAATTGTAATTTACCGAGAATTGTAAATTTAC CTGAAAACCCTACGCTATAGTTTCGACTATAAATACCAAACTTAGGACCTCACTT CAGAATCCCCTCGTCGCTGCGTCTCTCCCGCAACCTTCGATTTTCGTTTATT CGCATCCATCGGAGAGAGAAAACAATCAATAAGCGACCATGggtaactaacttAAGta ggGAGCTCagatatgaagatgaagatgaaatatttggtgtgtcaaataaaaagcttgtgtgcttaagtttgtgttttttt cttggcttgtgttatgaatttgtggctttttctaatattaaatgaatgtaagatctcattataatgaataaacaaatgtttcta taatccatttgtgaatgttttgtggatctcttctgcagcatataactactgtatgtgctatggtatggactatggaatatgatta aagataaggatcaatggatcc	606
Luz-RFP	catalyzes light reaction of the luciferin, emitting red-shifted photons	AATAAGCGACCATGGGAagaATAAACATCTCCCTTTCAAGCCTGTTCGAGAGGC TGTCTAAGCTCTCTTCCAGGAGCATAGCTATCACAtgtggtGTGGTCCTTGCATCC GCCATAGCTTTTCCAATAATCAGACGAGATTATCAGACAttcTTAGAGGTTGGACC TtcttatgctCCTCAGAACTTTagaGGGTACATCATCGTATGTGTTCTGTCACTGTTCA GGCAGGAGCAGAAAGGCTTAGCTATTTATGATAGATTGcctGAGAAGCGAAGAT GGCTTGCTGACTTACCTTTTAGAGAGGGTACAAGACCTTCTATAACTTCCCACA TCATCCAAAGACAACGTACTCAACTCGTCGATCAGgagtttGCTACCAGAgaaCTCA TCGATAAGGTTATCccAGAGTCCAGGCCAGACATACTGACAAAACCTTCCTGTC AACAAGTAAGTTTGAGTTCCACGCCAAAGCCATATTGCTGCTTCCTTCTATCCCA ATTAACGATCCTCTTAATATCCCTTCACACGACACCGTTCGACGAACCAAGAGA GAAATTGCACACATGCACGATTACCATGATTGTACTCTGCACCTTGCATTAGCT GCCCAAGATGGAAAAGAGGTGCTTAAGAAGGGCTGGggtCAAAGACATCCCTTA gctGGCCCTGGCGTTCCAGGTCCTCCTACTGAGTGGACATTCTTGtatGCCcaagg AATGAAAGAGGAAGCACGTGTAGTGGAGATGATAGTTGAGGCTAGTATAggtTAC ATGACCAATGACCCAGCAGGAAAGATTGTCGAGAACGCTAAGgatcggtggtggatc tggtATGGCCAGTTCAGAGGACGTAATCAAAGAATTTATGCGTTTCAAGGTTAGG ATGGAGGGGAGCGTAAATGGGCATGAGTTTGAAATAGAGGGCGAGGGAGAAG GAAGACCTTACGAAGGCACCCAAACCGCCAAGTTGAAAGTAACAAAAGGTGGA CCACTACCTTTTGCATGGGATATTTTAAGTCCTCAGTTTCAATACGGGAGCAAA GCATACGTGAAACACCCCGCTGACATACCCGACTATCTAAAATTATCTTTCCCT GAGGGCTTCAAATGGGAGAGGGTTATGAATTTCGAGGATGGAGGAGTGGTTAC CGTAACACAGGATTCATCCTTACAAGATGGAGAGTTCATTTACAAAGTCAAGCT AAGGGGCACTAATTTTCCCTCTGACGGACCCGTCATGCAAAAGAAAACTATGG GGTGGGAGGCATCCACAGAAAGGATGTATCCAGAAGACGGAGCCCTAAAGGG AGAAATAAAGATGAGATTAAAGCTCAAAGACGGCGGCCATTACGACGCAGAAG TAAAGACTACATATATGGCCAAAAGCCAGTCCAACTCCCCGGAGCCTATAAAA CTGATATAAAATTAGATATTACCAGCCACAATGAAGATTACACTATTGTAGAGCA ATACGAGCGAGCAGAAGGGAGGCACAGTACCGGCGCTGCTAGCCACCACCAC CACCACCACGTGTGAgGAGCTCagatatgaagat	1560
fungal Luz homologue	see if it catalyzes same reaction, random mutagenesis	AATAAGCGACCATGggaAATGCTGTTTCTTCTAAGGCTCAATCTTTTTGACTAG AGCTTTTACTTCTTTGCCTGGATCTGCTAGAGAAAGAATGTTGGCTGTTGCTTGT ACTGCTTGTATTGCTACTACTGCTTTTTTTTATCCTGCTATTAGAAAGGATTATCa gGCTTATATGTCTTTGGGACCTGGAGGATTTCCTCATAATGTTGTTGGATGGTTG GCTTCTTGTTTTTCTGGATTGTTTGGAAGAGAAACTACTTCTGTTGAATGTTATG ATGCTATGCCTGAAAAGAGACAATGGTTGACTTCTCCTTTGCCTCAAAGAGTTG GAGAAAGACCTAAGACTGGAAGCATTGTATTCCTCATAGACAAATTGATCAAT TTGTTTCTTCTGAATTTGCTCAATCTCAATTGCATCCTATTATTCCTAGAGTTCAT GCTAAGTATTCTTCTATTACTACTTGTAAGAGAtccCAACATGAAAGAAGAACTGA TGCTTTGTTTTTGGCTCCTGATGTTCCTGCTCATAGAACTGCTAGAAAGGCTTG GTTGGAAATTGCTcacATGCATGAAACTGCTGAATATTCTTTGCATGTTGTTTTGG CTCCTAGAGATTGTATTGAAGTTATTTCTAAGGGATGGGGAGAAAGACATCCTG TTTCTGGATTTGCTTTGCCTGTTGAATATTTGTTTATTTATACTCCTAGAACTGAA GAAGAATTGCTGTTGTTGAAAGAATTATTGAAGCTGCTGTTGGATTTATGGCTA ATTTGCCTTGCCTGCTGGAGCTACTTCTGAATTTGTTCAAACTTCTATTCCTAC TAAGGATACTGATGGAACTGTTACTCCTGTTCCTCAATAAgGAGCTCagatatgaag at	885
Y233F	pocket mutant	AATAAGCGACCATGggtagaATAAACATCTCCCTTTCAAGCCTGTTCGAGAGGCTGTCT AAGCTCTCTTCCAGGAGCATAGCTATCACAtgtggtGTGGTCCTTGCATCCGCCATAGCT TTTCCAATAATCAGACGAGATTATCAGACAttcTTAGAGGTTGGACCTtcttatgctCCTCA GAACTTTagaGGGTACATCATCGTATGTGTTCTGTCACTGTTCAGGCAGGAGCAGAAA GGCTTAGCTATTTATGATAGATTGcctGAGAAGCGAAGATGGCTTGCTGACTTACCTTT TAGAGAGGGTACAAGACCTTCTATAACTTCCCACATCATCCAAAGACAACGTACTCAA CTCGTCGATCAGgagtttGCTACCAGAgaaCTCATCGATAAGGTTATCcctAGAGTCCAGG CCAGACATACTGACAAAACCTTCCTGTCAACAAGTAAGTTTGAGTTCCACGCCAAAGC CATATTCCTGCTTCCTTCTATCCCAATTAACGATCCTCTTAATATCCCTTCACACGACA CCGTTCGACGAACCAAGAGAGAAATTGCACACATGCACGATTtcCATGATTGTACTCTG CACCTTGCATTAGCTGCCCAAGATGGAAAAGAGGTGCTTAAGAAGGGCTGGggtCAAA GACATCCCTTAgctGGCCCTGGCGTTCCAGGTCCTCCTACTGAGTGGACATTCTTGtatG CCcaaggAATGAAGAGGAAGCACGTGTAGTGGAGATGATAGTTGAGGCTAGTATAggt TACATGACCAATGACCCAGCAGGAAAGATTGTCGAGAACGCTAAGTAAgGAGCTCaga tatgaagat	837
Y189F	pocket mutant	AATAAGCGACCATGggtagaATAAACATCTCCCTTTCAAGCCTGTTCGAGAGGCTGTCT AAGCTCTCTTCCAGGAGCATAGCTATCACAtgtggtGTGGTCCTTGCATCCGCCATAGCT TTTCCAATAATCAGACGAGATTATCAGACAttcTTAGAGGTTGGACCTtcttatgctCCTCA GAACTTTagaGGGTACATCATCGTATGTGTTCTGTCACTGTTCAGGCAGGAGCAGAAA GGCTTAGCTATTTATGATAGATTGcctGAGAAGCGAAGATGGCTTGCTGACTTACCTTT TAGAGAGGGTACAAGACCTTCTATAACTTCCCACATCATCCAAAGACAACGTACTCAA CTCGTCGATCAGgagtttGCTACCAGAgaaCTCATCGATAAGGTTATCcctAGAGTCCAGG CCAGACATACTGACAAAACCTTCCTGTCAACAAGTAAGTTTGAGTTCCACGCCAAAGC CATATTCCTGCTTCCTTCTATCCCAATTAACGATCCTCTTAATATCCCTTCACACGACA CCGTTCGACGAACCAAGAGAGAAATTGCACACATGCACGATTccCATGATTGTACTCTG CACCTTGCATTAGCTGCCCAAGATGGAAAAGAGGTGCTTAAGAAGGGCTGGggtCAAA GACATCCCTTAgctGGCCCTGGCGTTCCAGGTCCTCCTACTGAGTGGACATTCTTGtatG CCcaaggAATGAAGAGGAAGCACGTGTAGTGGAGATGATAGTTGAGGCTAGTATAggt TACATGACCAATGACCCAGCAGGAAAGATTGTCGAGAACGCTAAGTAAgGAGCTCaga tatgaagat	837
Fungal Luciferase - of	different luciferase for	AATAAGCGACCATGggtTTGCCTGCTTTTATTTATAAGCCTAGATTGGTTATTACTTGT GTTTTGTTTTGGCTTCTGCTTTGGCTTTTCCTTTTATTAGAAAGGATTATCAAACTTT TTTGGAAGTTGGACCTTCTTATGCTCCTCAAAATTTGCAAGGATATATTATTGTTTGTG	819

Omphalotus olearius	random mutation	TTTTGTCTTTGTTTAGACAAGAACAAAAGGATGTTGCTATTTATGATAGATTGCCTGA AAAGAGAAGATGGTTGGGAGATTTGCCTTTTAGAGAAGGACCTAGACCTTCTATTACT TCTCATATTATTCAAAGACAAAGAACTcagTTGGCTGATGCTGAATTTGCTACTAAGGA ATTGATTGGAAAGATTATTCCTAGAGTTCAAGCTAGACATACTAATACTACTTTTTTGT CTACTTCTAAGTTTGAATTTCATGCTCAAGCTATTTTTTTGTTGCCTTCTATTCCTATT AATGATCCTCAAAATATTCCTTCTCATGATACTGTTAGAAGAACTAAGAGAGAAATTG CTcacATGCATGATTATCATGATTGTACTTTGCATTTGGCTTTGGCTGCTCAAGATGGA AAGGAAGTTTTGGAAAAGGGATGGGGACAAAGACATCCTTTGGCTGGACCTGGAGTT CCTGGACCTCCTACTGAATGGACTTTTTTGTATGCTcccaggTCTGAAGAAGAAGTTAG AGTTGTTGAAATGATTGTTgagGCTTCTGTTGTTTATATGACTAATGATCCTGCTGATA AGATTGTTGAAGCTACTGTTCAAGGAACTGAAGAAtaagGAGCTCagatatgaagat	
Fungal Luciferase of Mycena citricolor	different luciferase for random mutation	AATAAGCGACCATGGCTTATCAAttaACTTGGATTCAAACTTTGGTTTTGGGAGCTTTG GTTGCTATGGCTGTTGCTTTCCTTTATTAAGAAGGATTATGAAACTTTTTTGAAGGG AGGACCTTCTTATGCTCCTCAAAATGTTAGAGGATATATTATTGTTTGGTTTTGGCTT TGTTTAGACAAGAAcagTTGGGATTGGAAATTTATGATAGAATGCCTGAAAAGAGAAG ATGGTTGGCTAATTTGCCTCAAAGAGAAGGACCTAGACCTAAGACTACTTCTCATATT ATTCAAAGAcagTTGTCTCAACATACTGATCCTGCTTTTGGAGCTGCTTATTTGAAGGA TACTGTTATTCCTAGAGTTCAAGCTAGACATGCTGCTAATACTCATATTGCTAGAtccA CTTTTGAATTTCATGCTGCTGCTATTTTTTTGAATGCTGATGTTCCTTTGCCTGAAGGA TTGCCTGCTTCTGAAACTGTTAGAAGAACTAAGGGAGAAATTGCTcacATTGCATGATT ATCATGATTTTACTTTGCATTTGGCTTTGGCTGCTGCTGATGGAAAGGAAGTTGTTGG AAAGGGATGGGGACAAAGACATCCTTTGGCTGGACCTGGAGTTCCTGGACCTCCTAA TGAATGGACTTTTGTTTATGCTCCTAGAAATGAAGAAGAAATGGGAGTTGTTGAACAA ATTGTTGAAGCTGCTATTGGATATATGTCTAATGTTCCTGCTTTGGAAtaagGAGCTCa gatatgaagat	771

[1] https://www.ncbi.nlm.nih.gov/pubmed/30478037 Kotlobay AA, Sarkisyan KS, Mokrushina YA, Marcet-Houben M, Serebrovskaya EO, Markina NM, Gonzalez Somermeyer L, Gorokhovatsky AY, Vvedensky A, Purtov KV, Petushkov VN, Rodionova NS, Chepurnyh TV, Fakhranurova LI, Guglya EB, Ziganshin R, Tsarkova AS, Kaskova ZM, Shender V, Abakumov M, Abakumova TO, Povolotskaya IS, Eroshkin FM, Zaraisky AG, Mishin AS, Dolgov SV, Mitiouchkina TY, Kopantzev EP, Waldenmaier HE, Oliveira AG, Oba Y, Barsova E, Bogdanova EA, Gabaldón T, Stevani CV, Lukyanov S, Smirnov IV, Gitelson JI, Kondrashov FA, Yampolsky IV. Genetically encodable bioluminescent system from fungi. Proceedings of the National Academy of Sciences of the United States of America 2018;115(50):12728-32.

[2] https://www.ncbi.nlm.nih.gov/pubmed/25410250 Han YJ, Kim YM, Hwang OJ, Kim JI. Characterization of a small constitutive promoter from Arabidopsis translationally controlled tumor protein (AtTCTP) gene for plant transformation. Plant cell reports 2015;34(2):265-75.

[3] https://www.nature.com/articles/srep02490 Wang, Y., Akiyama, H., Terakado, K. et al. Impact of Site-Directed Mutant Luciferase on Quantitative Green and Orange/Red Emission Intensities in Firefly Bioluminescence. Sci Rep 3, 2490 (2013). https://doi.org/10.1038/srep02490

[4] https://link.springer.com/article/10.1007/s13258-016-0417-3 Ning Li, Yuanyuan Li, Chengchao Zheng, Jinguang Huang & Shizhong Zhang. Genome-wide comparative analysis of the codon usage patterns in plants. Genes & Genomics volume 38, pages723–731(2016)

[5] https://link.springer.com/article/10.1007/s11103-006-0041-8 Liangjiang Wang & Marilyn J. Roossinck. Comparative analysis of expressed sequences reveals a conserved pattern of optimal codon usage in plants. Plant Molecular Biology volume 61, pages699–710(2006)