Electronic Supplementary Material (ESI) for Molecular BioSystems


# A red light-controlled synthetic gene expression switch for plant systems

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| **Supplementary Table 1** | Expression vectors and oligonucleotides designed and used in this study. |
| **Supplementary Figure 1** | Effect of clarithromycin on constitutive gene expression in *N. tabacum* |
| **Supplementary Figure 2** | Spectrum of the white light source |

# Supplementary Table 1

**Expression vectors and oligonucleotides designed and used in this study.**

| Plasmid | Description | Ref. or source |
|---|---|---|
| auxin sensor | Plasmid encoding $P_{CaMV35S}$-controlled optimized auxin sensor ($P_{CaMV35S}$-RLuc-p2A-SM[L2min17]-FLuc-pA) | [1] |
| pKM002 | Vector encoding SEAP under control of a modified $P_{Tet}$ ($tetO_{13}$-394bp-$P_{hCMVmin}$-SEAP-pA) | [2] |
| pKM006 | Vector encoding SEAP under the control of a modified $P_{Tet}$ ($tetO_{13}$-422bp-$P_{hCMVmin}$-SEAP-pA) | [2] |
| pKM022 | Bicistronic vector encoding PhyB(1-650)-VP16-NLS and TetR-PIF6(1-100)-HA under control of $P_{SV40}$ ($P_{SV40}$-PhyB(1-650)-VP16-NLS-$IRES_{PV}$-TetR-PIF6(1-100)-HA-pA) | [2] |
| pKM033 | Vector encoding $VEGF_{121}$ under control of a modified $P_{Tet}$ ($tetO_{13}$-422bp-$P_{hCMVmin}$-$VEGF_{121}$-pA) | [2] |
| pKM081 | Vector encoding SEAP under control of a modified $P_{ETR}$ ($etr_8$-$P_{CMVmin}$-SEAP-pA) | [3] |
| pKM082 | Vector encoding SEAP under control of a modified $P_{ETR}$ ($etr_8$-386bp-$P_{hCMVmin}$-SEAP-pA)<br>A 372 bp fragment was amplified from *CFP* using oligos oKM090 (5'-caagtagctagcCCCTGAAGTTCATCTGCACC-3') and oKM003 (5'-caagtcgctagcTCTTGAAGTTGGCCTTGATGC-3'), digested (*Nhe*I) and ligated (*Nhe*I) into pKM081. | This work |
| pKM271 | Vector for $P_{CaMV35S}$-controlled expression of PiP-VP16-NLS ($P_{CaMV35S}$-PiP-VP16-NLS-pA)<br>PiP-VP16-NLS was amplified from pMF156 using oligos oKM373 (5'-caagtcaccggtcatatggccaccATGAGTCGAGGAGAGGTGCG-3') and oKM374 (5'-caagtcgaattcctacaccttcctcttcttctttggcCCACCGTACTCGTCAATTCC-3'), digested (*Nde*I/*Eco*RI) and ligated (*Nde*I/*Eco*RI) into pMZ824. | This work |
| pKM272 | Vector encoding FLuc under control of a modified $P_{PTR}$ ($PIR_3$-$P_{HSP70min}$-FLuc-pA)<br>FLuc was excised (*Eco*RI/*Hin*dIII) from pMZ836 and ligated (*Eco*RI/*Hin*dIII) into pMF199. | This work |
| pKM295 | Vector encoding $VEGF_{121}$ under the control of a modified $P_{ETR}$ ($etr_8$-$P_{hCMVmin}$-$VEGF_{121}$-pA)<br>$VEGF_{121}$ was excised (*Eco*RI/*Not*I) from pKM033 and ligated (*Eco*RI/*Not*I) into pMZ836. | This work |
| pKM300 | Bicistronic vector encoding PhyB(1-650)-VP16-NLS and E-PIF6(1-100)-HA under control of $P_{SV40}$ ($P_{SV40}$-PhyB(1-650)-VP16-NLS-$IRES_{PV}$-E-PIF6(1-100)-HA-pA)<br>$IRES_{PV}$ was amplified from pKM022 using oligos oKM400 (5'-ACCCACCCCAGAGGCCC-3') and oKM401 (5'-gatatcGCCGCAATCCAATTCGCTTTATG-3'), while PIF6(1–100)-HA was amplified from pKM022 using oligos oKM402 (5'-ATGATGTTCTTACCAACCGATTATTGTTG-3') and oKM403 (5'-AAAAACCTCCCACACCTCCCC -3'). E was amplified from pWW043 using oligos oKM404 (5'-atcacagattgttatcataaagcgaattggattgcggcgatatcgCCACCATGCCCCGCCCC-3') and oKM405 (5'-tgatcgcttaacctgcaacaataatcggttggtaagaacatcataccagcactaccagcactaccagcactgttaattaaGCTGTACGCGGACGCATGTG-3') and the three fragments were cloned into *Kpn*I/*Mfe*I-digested pKM022 by Gibson cloning. | This work |
| pKM301 | Bicistronic vector encoding PhyB(1-650)-VP16-NLS and PiP-PIF6(1-100)-HA under control of $P_{SV40}$ ($P_{SV40}$-PhyB(1-650)-VP16-NLS-$IRES_{PV}$-PiP-PIF6(1-100)-HA-pA)<br>$IRES_{PV}$ and PIF6(1–100) were amplified as described for pKM300. PiP was amplified from pMF150 using oligos oKM406 (5'-atcacagattgttatcataaagcgaattggattgcggcgatatcgCCACCATGAGTCGAGGAGAG-3') and oKM407 (5'-tgatcgcttaacctgcaacaataatcggttggtaagaacatcataccagcactaccagcactaccagcactgttaattaaGGCCTGTTCGACCATCGC-3') and all fragments were cloned into *Kpn*I/*Mfe*I-digested pKM022 by Gibson cloning. | This work |
| pMF150 | Vector encoding PiP under control of $P_{hCMV}$ ($P_{hCMV}$-PiP-pA) | [4] |
| pMF156 | Vector encoding PiP-VP16 under control of $P_{SV40}$ ($P_{SV40}$-PiP-VP16-pA) | [4] |
| pMF199 | Vector encoding SEAP under control of a modified $P_{PTR}$ ($PIR_3$-$P_{HSP70min}$-SEAP-pA) | [4] |
| pMK052 | Vector encoding $P_{EF1\alpha}$-controlled TIR1 ($P_{EF1\alpha}$-TIR1-pA) | [5] |
| pMZ802 | Vector encoding FLuc under control of a modified $P_{Tet}$ ($tetO_{13}$-$P_{hCMVmin}$-FLuc-pA)<br>FLuc was amplified from pSW209 using oligos oMZ807 (5'-tcagcttccgctcaattggctGCGCTGGCGCTAGCATGGAAG-3') and oMZ808 (5'-gggaccacgacgccatggacgGGTTTACACGGCGATCTTTCCGCC-3'), while the backbone of pKM002 was amplified using oMZ809 (5'-gtgtaaacccgtccatggcgtCGTGGTCCCCGCGTTGCTTC-3') and oMZ810 (5'-cgccagcgcagccaattgagcGGAAGCTGACTCTAGAGGATCCCC-3'). Finally, both fragments were fused by Gibson cloning. | This work |
| pMZ824 | Vector for $P_{CaMV35S}$-controlled expression of E-VP16-NLS ($P_{CaMV35S}$-E-VP16-NLS-pA)<br>E-VP16-NLS was amplified from pWW035 using oligos oMZ809 (5'- | This work |

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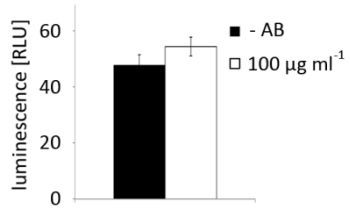
| | gtgtaaacccgtccatggcgtCGTGGTCCCCGCGTTGCTTC-3') and oMZ8123 (5'-tcacgtcgtgctagccgttgcctacaccttcctcttcttctttggCCCACCGTACTCGTCAATTCCAAG-3'), while the backbone of pSW209 was amplified using oligos oMZ873 (5'-gcaacggctagcacgacgtgaTGCGGCAGCGGCCGAATTCC-3') and oMZ874 (5'-tgttgtcatatgcgtcgttcaGGTGGTACCAAGCTTACCTAGCC-3'). Finally, both fragments were fused by Gibson cloning. | |
| pMZ827 | Vector encoding $P_{CaMV35S}$-controlled nuclear-targeted E-PIF6(1-100) ($P_{CaMV35S}$-E-PIF6(1-100)-NLS-pA)<br>E-Pif6 was amplified from pKM300 using oligos oMZ895 (5'-tgaacgacgcatatgacaacaATGCCCCGCCCCAAGCTCAAG-3') and oMZ8127 (5'-tcacgtcgtgctagccgttgcctacaccttcctcttcttctttggGTCAACATGTTTATTGCTTTCCAACATGTTTG-3'), while the backbone of pSW209 was amplified using oligos oMZ873 and oMZ874. Finally, both fragments were fused by Gibson cloning. | This work |
| pMZ828 | Vector encoding $P_{CaMV35S}$-controlled nuclear-targeted PhyB(1-650)-VP16 ($P_{CaMV35S}$-PhyB(1-650)-VP16-NLS-pA)<br>PhyB-VP16-NLS was amplified from pKM300 using oligos oMZ856 (5'-gccatggtgagcacgGTCGACTCTAGATCACACCTTCCG-3' and oMZ8123 (5'-tcacgtcgtgctagccgttgcctacaccttcctcttcttctttggCCCACCGTACTCGTCAATTCCAAG-3'), while the backbone of pSW209 was amplified using oligos oMZ873 and oMZ874. Finally, both fragments were fused by Gibson cloning. | This work |
| pMZ833 | Vector for $P_{CaMV35S}$-controlled expression of TetR-VP16-NLS ($P_{CaMV35S}$-TetR-VP16-NLS-pA)<br>Tet-VP16 was amplified from pSAM200 using oligos oMZ891 (5'-tgaacgacgcatatgacaacaCGGCCGCCACCATGTCTAGATTAG-3') and oMZ8123 (5'-tcacgtcgtgctagccgttgcctacaccttcctcttcttctttggCCCACCGTACTCGTCAATTCCAAG-3'), while the backbone of pSW209 was amplified using oligos oMZ873 and oMZ874. Finally, both fragments were fused by Gibson cloning. | This work |
| pMZ836 | Vector encoding FLuc under control of a modified $P_{ETR}$ ($etr_8$-$P_{hCMVmin}$-FLuc-pA)<br>FLuc was amplified from pSW209 using oligos oMZ807 and oMZ808, while the backbone of pKM081 was amplified using oligos oMZ809 (5'-gtgtaaacccgtccatggcgtCGTGGTCCCCGCGTTGCTTC-3') and oMZ810 (5'-cgccagcgcagccaattgagcGGAAGCTGACTCTAGAGGATCCCC-3'). Finally, both fragments were fused by Gibson cloning. | This work |
| pMZ837 | Vector encoding $P_{CaMV35S}$-driven expression of $miRNA_{TIR1}$ ($P_{CaMV35S}$-$miRNA_{TIR1}$-pA)<br>For the design of an miRNA targeting *N. tabacum* TIR1the online tool CentroidFold[6] was used and miRNA cloning was performed by a modification of a previously described protocol.[7] First, the 5'-stem sequence was amplified from pRS300[7] using oligos oMZ880 (5'-tgaacgacgcatatgacaacaGAGGTCGACGGTATCGATAAGCTTG-3') and oMZ8163 (5'-cggtagacaaattggatcattgattctctttggtggtcaactgactggattgtCTCTCTCTTTTGTATTCCAATTTTCTT-3'. In doing so, the miRNA that was contained in the 5'-overhang of oMZ1861 was introduced. In the same way, the 3'-stem sequence was amplified from pRS300 using oligos oMZ883 (5'-tcacgtcgtgctagccgttgcGCGGATAACAATTTCACACAGGAAACAG-3') and oMZ8164 (5'-gaagctaattgaatcatatcacgacctgtgagtgctcaactgacaggattgaTCTACATATATATTCCTAAAACATCAAA-3'). Next, both PCR-products were extended by overlapping loop sequences by amplification with oMZ880 and oMZ882 (5'-cgagtctagtttgaattttggcgactcggtatttggatgaatgagtcgGAAGCTAATTGAATCATATCACGACCTGTGAG-3)' or oMZ883 and oMZ885 (5'-cgagtcgccaaaattcaaactagactcgttaaatgaatgaatgatgCGGTAGACAAATTGGATCATTGATTCTCTTTG-3'), respectively. Finally, both fragments were fused and introduced into the oMZ873/oMZ874-amplified backbone of pSW209 by Gibson cloning. | This work |
| pMZ839 | Vector encoding $miRNA_{TIR1}$ under control of a modified $P_{ETR}$ ($etr_8$-$P_{hCMVmin}$-$miRNA_{TIR1}$-pA)<br>$miRNA_{TIR1}$ was amplified from pMZ837 using oligos oMZ8118 (5'-gctcaattggctgcgctggcgCCACCTGAACGACGCATATGACAAC-3') and oMZ8119 (5'-acgccatggacgggtttacacCATCACGTCGTGCTAGCCGTTGC-3') and fused with the oMZ809/oMZ810-amplified backbone of pKM081 by Gibson cloning. | This work |
| pMZ841 | Vector encoding TIR1 under control of a modified $P_{ETR}$ ($etr_8$-$P_{hCMVmin}$-TIR1-pA)<br>TIR1 was excised (*Eco*RI/*Xba*I) from pMK052 and ligated (*Eco*RI/*Spe*I) into pKM081. | This work |
| pSW209 | Vector encoding firefly luciferase and renilla luciferase separated by a 2A-peptide under control of $P_{CaMV35S}$ ($P_{CaMV35S}$-FLuc-p2A-RLuc-pA). | [1] |
| pWW035 | Vector encoding $P_{SV40}$-driven expression of E-VP16 ($P_{SV40}$-E-VP16-pA) | [8] |
| pWW043 | Vector encoding $P_{SV40}$-driven expression of E-KRAB ($P_{SV40}$-E-KRAB-pA) | [8] |

E, macrolide-responsive repressor protein; etr, operator sequence binding E; FLuc, firefly luciferase; HA, human influenza hemagglutinin-derived epitope tag; $IRES_{PV}$, polioviral internal ribosome entry site; KRAB, transcriptional repressor domain from human Kox1; NLS, nuclear localization signal from simian virus 40 large T antigen; pA, polyadenylation signal; p2A; foot-and-mouth disease virus-derived self-processing 2A peptide; $P_{CaMV35S}$, cauliflower mosaic virus 35S promoter; PiP, pristinamycin-induced protein, $P_{hCMV,}$ human cytomegalovirus immediate early promoter; $P_{hCMVmin}$, minimal human cytomegalovirus immediate early promoter; $P_{HSP70min}$, minimal heat-shock protein 70 promoter from Drosophila; PhyB, Phytochrome B; PhyB(1-650), N-terminus of Phytochrome B with amino acids 1-650; PIF6, Phytochrome-interacting-factor 6; PIF6(1-100), N-terminus of Phytochrome-interacting-factor 6 with amino acids 1-100; $P_{SV40}$, simian virus 40 early promoter; PIR, operator sequence binding PiP; $P_{Tet}$, tetracycline-responsive promoter; RLuc, renilla luciferase; SEAP, human placental secreted alkaline phosphatase; SM, auxin sensor module; tetO, operator sequence binding TetR; TetR, tetracycline repressor protein; TIR1, auxin receptor transport inhibitor reponse 1; $VEGF_{121}$, 121 amino acids splice variant of human vascular endothelial growth factor; VP16, *Herpes simplex* virus-derived transactivation domain.

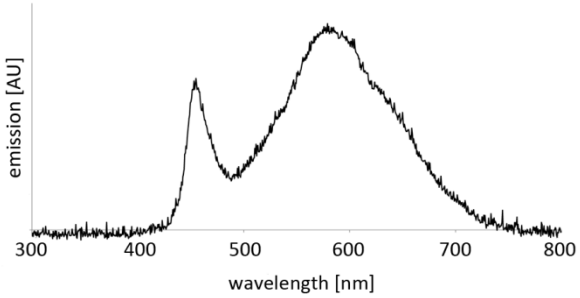
Uppercase in oligos, annealing sequence; underlined sequence, restriction site.

## Supplementary Figure 1




**Effect of clarithromycin on constitutive gene expression in *N. tabacum*.** 125,000 protoplasts were transformed for constitutive firefly luciferase expression. After incubation for 24 h in the absence (-AB) or presence of 100 µg $ml^{-1}$ clarithromycin, the firefly luciferase luminescence was quantified. Data are means ± SEM (n=12).

## Supplementary Figure 2




**Spectrum of the white light source.** The light spectrum between 300 nm and 800 nm was recordeded using an Avaspec-ULS2048 spectroradiometer (Avatec).

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