Supplementary information for

Orthogonal control of gene expression using synthetic promoters and CRISPR-based transcription factors

Shaunak Kar[1,2,#], Yogendra Bordiya[1,4,#], Nestor Rodriguez[1], Junghyun Kim[1], Elizabeth C. Gardner[1,2], Jimmy Gollihar[2], Sibum Sung[1,*] and Andrew D. Ellington[1,2,*]

[1] Department of Molecular Biosciences, University of Texas at Austin, Austin TX, USA
[2] Center for Systems and Synthetic Biology, University of Texas at Austin, Austin, TX, USA
[3] US Army Research Laboratories−South, Austin, Texas, USA
[4] Present address: Life Sciences Solutions group, Thermo Fisher Scientific, Austin, TX, USA

[#] Authors contributed equally
[*] Corresponding authors

Sibum Sung: sbsung@austin.utexas.edu, Andrew D. Ellington: ellingtonlab@gmail.com

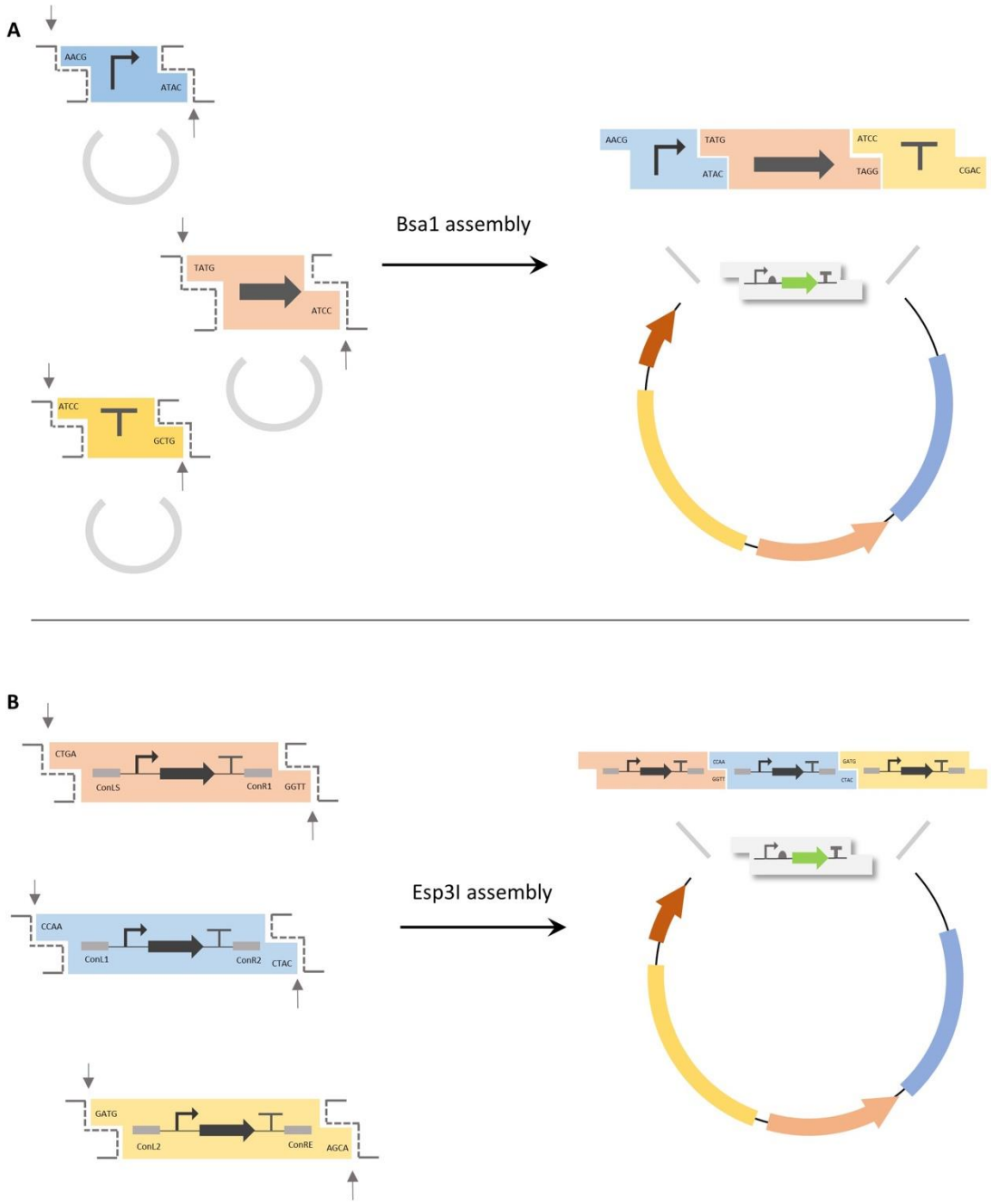
Figure S1: Workflow describing the assembly of single and multiple transcriptional units (TUs) in a plant expression vector

Figure S2: Western blot to analyze the expression of dCas9:VP64 in OCS constructs – OCS1-1 and OCS 1-5

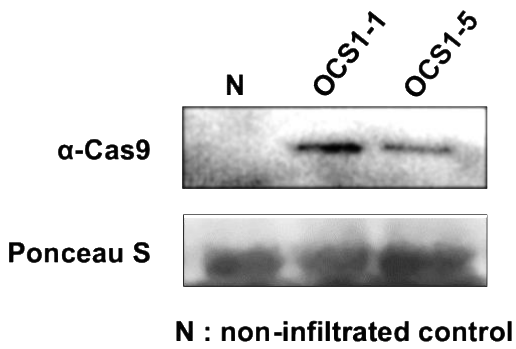
Table S1: List of all genetic parts used for the construction of OCS constructs

Table S2: List of all OCS constructs

Table S3: List of all Addgene plasmids used in this work



Supplementary figure 1: Schematic demonstrating the assembly of single (A) or multiple (B) transcriptional units into a plant expression vector. A single transcription unit consists of a promoter, gene and a terminator parts while for multiple transcriptional units, each TU is flanked by appropriate connector sequences. The arrows depict the restriction sites for Bsa1 (A) and Esp3I (B). B) Schematic showing the assembly of multiple TUs into plant expression vector where each TU is encoded in separate plasmids flanked by appropriate connector sequences.



Supplementary figure 2: The expression of hdCas9 for both OCS 1-1 and OCS 1-5 was confirmed via Western blot analysis (see Methods).

Table S1: List of genetic elements used.

Highlighted regions in red indicate BsaI recognition sites and the corresponding overhangs are highlighted in blue.

S1.1 Promoters

Name	Description	Sequence
P1	35S promoter	GGTCTCAAACGCGTCAACATGGTGGAGCACGACAC TCTGGTCTACTCCAAAAATGTCAAAGATACAGTCTCA GAAGATCAAAGGGCTATTGAGACTTTTCAACAAAGG ATAATTTCGGGAAACCTCCTCGGATTCCATTGCCCA GCTATCTGTCACTTCATCGAAAGGACAGTAGAAAAG GAAGGTGGCTCCTACAAATGCCATCATTGCGATAAA GGAAAGGCTATCATTCAAGATCTCTCTGCCGACAGT GGTCCCAAAGATGGACCCCCACCCACGAGGAGCAT CGTGGAAAAAGAAGAGGTTCCAACCACGTCTACAAA GCAAGTGGATTGATGTGACATCTCCACTGACGTAAG GGATGACGCACAATCCCACTATCCTTCGCAAGACCC TTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGG ACACGCGTTTATTTACAAGAGCGTACGGTTCAATCC CTGCCTCCCCTGTAAAACTACCCTTTGAAAACCTCTC TTTCTTAATCTTTTCTTTGTAATTCCAGATCTATGTGA GACC
P2	Mas promoter	GGTCTCAAACGCGGAGATTTTTCAAATCAGTGCGCA AGACGTGACGTAAGTATCCGAGTCAGTTTTTATTTTT CTACTAATTTGGTCGTTTATTTCGGCGTGTAGGACAT GGCAACCGGGCCTGAATTTCGCGGGTATTCTGTTTC TATTCCAACTTTTTCTTGATCCGCAGCCATTAACGAC TTTTGAATAGATACGCTGACACGCCAAGCCTCGCTA GTCAAAAGTGTACCAAACAACGCTTTACAGCAAGAA CGGAATGCGCGTGACGCTCGCGGTGACGCCATTTC GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCCTAT TATATCTTCCCAAATTACCAATACATTACACTAGCAT CTGAATTTCATAACCAATCTCGATACACCAAATCGAG ATCTATGTGAGACC
P4	EBS promoter	GGTCTCAAACGCATTACGAATTCCCGGGGATCCTCA TGATCAAAGGGGGGATGCACTATTTAAGATCCTCAT GATCAAAGGGGGGATGCACTATTTAAGATCCTCATG ATCAAAGGGGGGATGCACTATTTAAGATCCTCATGA TCAAAGGGGGGATGCACTATTTAAGATCCTCATGAT CAAAGGGGGGATGCACTATTTAAGATCCTTCGCAAG ACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGA GAGGACAGGGTATCAAGCTTGGCACTGGCCGTCGT TTTACAACGTCGTGACTGGGAAAACCCTGGCGTTAC

		CCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGC CAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATC GCCCTTCCCAACAGTTGCGCAGCCTGAAGCCTAGG GAGGAGTCCACTCTATGTGAGACC
pATF-1	Artificial promoter 1 (3 gRNA repeats)	GGTCTCAAACGCCAAACCACAATTTGCACACCCTGG CATCTCAAACCACAATTTGCACACCCTGGCATCTCA AACCACAATTTGCACACCCTGGCATCTCCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGA GGACACGCGTTTATTTACAAGAGCGTACGGTTCAAT CCCTGCCTCCCCTGTAAAACTACCCTTTGAAAACCT CTCTTTCTTAATCTTTTCTTTGTAATTCCAGATCTATG TGAGACC
pATF-3	Artificial promoter 3 (3 gRNA repeats)	GGTCTCAAACGCCTGACTCACAGTCCTATCGAGTGG CATCTCTGACTCACAGTCCTATCGAGTGGCATCTCT GACTCACAGTCCTATCGAGTGGCATCTCCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGA GGACACGCGTTTATTTACAAGAGCGTACGGTTCAAT CCCTGCCTCCCCTGTAAAACTACCCTTTGAAAACCT CTCTTTCTTAATCTTTTCTTTGTAATTCCAGATCTATG TGAGACC
pATF-4	Artificial promoter 4 (3 gRNA repeats)	GGTCTCAAACGCCATTGCGCACCATTCCACTAGTGG CATCTCATTGCGCACCATTCCACTAGTGGCATCTCA TTGCGCACCATTCCACTAGTGGCATCTCCGCAAGAC CCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGA GGACACGCGTTTATTTACAAGAGCGTACGGTTCAAT CCCTGCCTCCCCTGTAAAACTACCCTTTGAAAACCT CTCTTTCTTAATCTTTTCTTTGTAATTCCAGATCTATG TGAGACC

S1.2 Terminators

Name	Description	Sequence
T1	35S Terminator	GGTCTCAATCCTAACTCGAGCTCTAGCTAGAGTCGA TCGACAAGCTCGAGTTTCTCCATAATAATGTGTGAGT AGTTCCCAGATAAGGGAATTAGGGTTCCTATAGGGT TTCGCTCATGTGTTGAGCATATAAGAAACCCTTAGTA TGTATTTGTATTTGTAAAATACTTCTATCAATAAAATT TCTAATTCCTAAAACCAAAATCCAGTACTAAAATCCA GATGCTGTGAGACC

S1.3 Coding sequences

Name	Description	Sequence

G1	YFP	GGTCTCATATGGTATCCAAAGGAGAAGAATTGTTTA CAGGGGTAGTCCCCATATTGGTCGAGCTCGATGGG GATGTAAACGGGCACAAGTTCTCAGTGTCTGGCGAA GGCGAAGGGGACGCCACATACGGAAAGTTGACTCT GAAGTTCATCTGCACAACCGGTAAACTACCCGTACC CTGGCCAACACTGGTGACGACCTTTGGGTACGGGC TACAATGTTTCGCTCGATATCCTGACCACATGAAGC AACACGATTTCTTCAAAAGCGCCATGCCTGAGGGGT ATGTACAGGAGCGTACCATATTTTTCAAAGACGATG GAAACTATAAGACCCGAGCTGAAGTGAAATTTGAAG GAGATACGTTAGTCAATCGAATAGAACTAAAGGGAA TTGATTTCAAGGAGGATGGGAATATTCTGGGCCATA AGCTGGAGTACAATTACAATAGTCACAACGTCTATAT AATGGCAGACAAGCAGAAGAATGGAATAAAGGTTAA TTTCAAGATAAGGCATAACATTGAAGACGGAAGCGT TCAGCTAGCCGATCATTACCAACAGAATACACCGAT AGGTGATGGACCCGTCCTGCTCCCCGACAACCATTA CTTGTCTTACCAGAGTGCACTTTCTAAAGATCCAAAT GAGAAAAGAGATCATATGGTTTTATTAGAATTTGTTA CCGCAGCCGGCATAACTTTGGGAATGGACGAACTG TACAAATGAGGATCCTGAGACC
G2	RFP	GGTCTCATATGAGCGAGCTGATTAAGGAGAACATGC ACATGAAGCTGTACATGGAGGGCACCGTGAACAAC CACCACTTCAAGTGCACATCCGAGGGCGAAGGCAA GCCCTACGAGGGCACCCAGACCATGAGAATCAAGG TGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGAC ATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACC TTCATCAACCACACCCAGGGCATCCCCGACTTCTTT AAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAG AGTCACCACATACGAAGACGGGGGCGTGCTGACCG CTACCCAGGACACCAGCCTCCAGGACGGCTGCCTC ATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCA TCCAACGGCCCTGTGATGCAGAAGAAAACACTCGG CTGGGAGGCCAACACCGAGATGCTGTACCCCGCTG ACGGCGGCCTGGAAGGCAGAAGCGACATGGCCCTG AAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTT CAAGACCACATACAGATCCAAGAAACCCGCTAAGAA CCTCAAGATGCCCGGCGTCTACTATGTGGACCACAG ACTGGAAAGAATCAAGGAGGCCGACAAAGAAACTTA CGTCGAGCAGCATGAGGTTGCTGTGGCCAGATACT GCGACCTCCCTAGCAAACTGGGGCACAAGTGAGGA TCCTGAGACC

G10	BFP	GGTCTCATATGAGCGAAGAACTAATCAAGGAAAATA TGCACATGAAACTCTACATGGAGGGTACGGTTGACA ATCATCATTTCAAATGTACCGCAGAGGGCGAAGGTA AACCCTATGAGGGCACCCAGACTATGCGAATCAAGG TTGTGGAGGGCGGGCCATTGCCCTTCGCTTTTGACA TCTTAGCTACTAGTTTCTTATATGGGAGCAAGACCTT TATAGATCACACACAGGGTATCCCGGACTTCTTTAAA CAGAGCTTTCCAGAAGGGTTCACCTGGGAAAGGGT GACAACCTATGAAGATGGCGGAGTGCTTACAGCGA CACAAGACACCTCCCTACAGGACGGCACCCTAATAT ATAATGTCAAGATTCGTGGCGTAGATTTCCTCAGCA ATGGCCCTGTTATGCAGAAGAAGACACTTGGATGGG AGGCTTTCACTGAAACCCTCTACCCGGCGGATGGA GGCTTAGAGGGGAGAAACGATATGGCGTTAAAGCT GGTCGGGGGATCACACTTGATCGCGCATGCAAAAA CTACGTACAGGTCCAAGAAACCAGCAAAGAATCTCA AGATGCCAGGTGTATACTATGTGGATTACCGACTCG AGCGTATTAAGGAAGCAAACGACGAAACGTACGTCG AACAACACGAAGTTGCAGTAGCAAGGTATAGCGACC TTCCCTCCAAGCTAGGACATAAGCTGAATGGGAGCG GATAAGGATCCTGAGACC
G17	Luciferase	GGTCTCATATGGAAGATGCAAAGAATATCAAAAAAG GCCCAGCGCCCTTCTACCCATTAGAAGATGGAACCG CAGGAGAGCAACTTCACAAGGCGATGAAACGATATG CTCTTGTCCCGGGAACCATCGCTTTTACGGACGCAC ACATAGAGGTTAACATTACCTATGCGGAATATTTTGA AATGTCAGTCAGATTAGCAGAAGCAATGAAACGTTA TGGGCTCAACACTAACCATCGTATTGTTGTATGTAG CGAAAACAGCCTGCAGTTTTTTATGCCGGTGCTCGG TGCGCTGTTCATCGGTGTAGCGGTTGCTCCCGCAAA CGACATTTACAACGAGAGAGAACTGCTTAACAGCAT GAATATCAGCCAACCGACCGTCGTGTTTGTCTCAAA AAAGGGACTACAAAAAATTCTAAATGTCCAAAAGAAG TTACCTATTATCCAGAAAATTATTATTATGGATAGCAA GACGGATTATCAAGGATTCCAATCTATGTACACATTT GTTACGAGCCACTTACCTCCAGGTTTTAACGAATAT GATTTTGTGCCTGAGAGCTTTGATCGAGATAAGACC ATCGCGTTAATTATGAATAGTTCCGGCTCTACGGGG CTCCCAAAGGGAGTCGCACTACCACATCGAACTGC GTGCGTTAGATTTTCACATGCCAGAGATCCTATCTTC GGGAATCAGATTATTCCGGACACTGCAATACTGAGT GTGGTTCCGTTTCATCACGGGTTCGGGATGTTCACG ACACTCGGTTACCTCATATGCGGATTTCGTGTGGTG CTGATGTATAGGTTTGAAGAAGAGTTGTTCCTAAGAT CCTTGCAGGATTACAAAATTCAGTCCGCCCTGTTAG TTCCTACCTTATTTTCCTTTTTCGCCAAGTCAACGTT

		AATTGATAAATATGACCTATCCAACCTCCACGAAATT GCCAGTGGTGGGGCCCCCTTGTCCAAAGAAGTTGG TGAGGCAGTCGCTAAGAGGTTCCACCTGCCGGGTA TCCGTCAAGGCTACGGACTTACCGAAACAACCTCCG CTATTCTTATTACACCTGAAGGCGATGATAAGCCGG GTGCTGTCGGTAAGGTGGTGCCTTTTTTTGAGGCGA AGGTAGTGGACTTAGATACTGGCAAGACGCTCGGA GTTAATCAACGAGGCGAGCTCTGTGTCCGTGGTCCC ATGATAATGAGCGGATACGTCAACAATCCTGAGGCA ACCAACGCATTAATTGATAAAGACGGCTGGTTGCAT AGCGGGGATATCGCGTATTGGGATGAGGACGAACA CTTCTTTATAGTCGATAGGTTAAAGTCACTTATTAAG TATAAAGGTTATCAAGTCGCTCCCGCCGAACTGGAG AGTATTCTTCTTCAGCATCCGAATATCTTTGACGCCG GTGTTGCAGGTCTTCCTGACGACGACGCCGGAGAA CTACCTGCAGCGGTCGTGGTGCTCGAACATGGAAA GACAATGACCGAGAAGGAGATTGTAGATTACGTAGC TTCACAGGTCACCACCGCTAAAAAACTAAGAGGTGG TGTTGTCTTCGTAGATGAAGTGCCTAAAGGACTTAC CGGTAAACTCGACGCCAGGAAGATAAGAGAAATCCT GATCAAAGCGAAGAAGGGTGGTAAGTCTAAGCTGTA AGGATCCTGAGACC
G15	dCas9:VP64	GGTCTCATATGCCCAAGAAGAAGAGGAAGGTGGAC AAGAAGTACTCCATTGGGCTCGCTATCGGCACAAAC AGCGTCGGCTGGGCCGTCATTACGGACGAGTACAA GGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATAC CGATCGCCACAGCATAAAGAAGAACCTCATTGGCGC CCTCCTGTTCGACTCCGGGGAAACGGCCGAAGCCA CGCGGCTCAAAAGAACAGCACGGCGCAGATATACC CGCAGAAAGAATCGGATCTGCTACCTGCAGGAGATC TTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCT TCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAG GATAAAAAGCACGAGCGCCACCCAATCTTTGGCAAT ATCGTGGACGAGGTGGCGTACCATGAAAAGTACCC AACCATATATCATCTGAGGAAGAAGCTTGTAGACAG TACTGATAAGGCTGACTTGCGGTTGATCTATCTCGC GCTGGCGCATATGATCAAATTTCGGGGACACTTCCT CATCGAGGGGGACCTGAACCCAGACAACAGCGATG TCGACAAACTCTTTATCCAACTGGTTCAGACTTACAA TCAGCTTTTCGAAGAGAACCCGATCAACGCATCCGG AGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTC CAAATCCCGGCGGCTCGAAAACCTCATCGCACAGCT CCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCT TATCGCCCTGTCACTCGGGCTGACCCCCAACTTTAA ATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCA ACTGAGCAAAGACACCTACGATGATGATCTCGACAA

		TCTGCTGGCCCAGATCGGCGACCAGTACGCAGACC TTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTC TGCTGAGTGATATTCTGCGAGTGAACACGGAGATCA CCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCT ATGATGAGCACCACCAAGACTTGACTTTGCTGAAGG CCCTTGTCAGACAGCAACTGCCTGAGAAGTACAAGG AAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCGG ATACATTGACGGCGGAGCAAGCCAGGAGGAATTTTA CAAATTTATTAAGCCCATCTTGGAAAAAATGGACGG CACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAG ATCTGTTGCGCAAACAGCGCACTTTCGACAATGGAA GCATCCCCCACCAGATTCACCTGGGCGAACTGCAC GCTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTT TTGAAAGATAACAGGGAAAAGATTGAGAAAATCCTC ACATTTCGGATACCCTACTATGTAGGCCCCCTCGCC CGGGGAAATTCCAGATTCGCGTGGATGACTCGCAAA TCAGAAGAGACTATCACTCCCTGGAACTTCGAGGAA GTCGTGGATAAGGGGGCCTCTGCCCAGTCCTTCAT CGAAAGGATGACTAACTTTGATAAAAATCTGCCTAAC GAAAAGGTGCTTCCTAAACACTCTCTGCTGTACGAG TACTTCACAGTTTATAACGAGCTCACCAAGGTCAAAT ACGTCACAGAAGGGATGAGAAAGCCAGCATTCCTGT CTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCT TCAAGACGAACCGGAAAGTTACCGTGAAACAGCTCA AAGAAGATTATTTCAAAAAGATTGAATGTTTCGACTC TGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGC ATCCCTGGGAACGTATCACGATCTCCTGAAAATCAT TAAAGACAAGGACTTCCTGGACAATGAGGAGAACGA GGACATTCTTGAGGACATTGTCCTCACCCTTACGTT GTTTGAAGATAGGGAGATGATTGAAGAACGCTTGAA AACTTACGCTCATCTCTTCGACGACAAAGTCATGAAA CAGCTCAAGAGGCGCCGATATACAGGATGGGGGCG GCTGTCAAGAAAACTGATCAATGGGATCCGAGACAA GCAGAGTGGAAAGACAATCCTGGATTTTCTTAAGTC CGATGGATTTGCCAACCGGAACTTCATGCAGTTGAT CCATGATGACTCTCTCACCTTTAAGGAGGACATCCA GAAAGCACAAGTTTCTGGCCAGGGGGACAGTCTCC ACGAGCACATCGCTAATCTTGCAGGTAGCCCAGCTA TCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGG ATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCG AGAATATCGTTATCGAGATGGCCCGAGAGAACCAAA CTACCCAGAAGGGACAGAAGAACAGTAGGGAAAGG ATGAAGAGGATTGAAGAGGGTATAAAAGAACTGGGG TCCCAAATCCTTAAGGAACACCCAGTTGAAAACACC CAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTG CAGAACGGCAGGGACATGTACGTGGATCAGGAACT

		GGACATCAATCGGCTCTCCGACTACGACGTGGATG CCATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTAT TGATAATAAAGTGTTGACAAGATCCGATAAAAATAGA GGGAAGAGTGATAACGTCCCCTCAGAAGAAGTTGTC AAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAAC GCCAAACTGATCACACAACGGAAGTTCGATAATCTG ACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGAT AAAGCCGGCTTCATCAAAAGGCAGCTTGTTGAGACA CGCCAGATCACCAAGCACGTGGCCCAAATTCTCGAT TCACGCATGAACACCAAGTACGATGAAAATGACAAA CTGATTCGAGAGGTGAAAGTTATTACTCTGAAGTCTA AGCTGGTTTCAGATTTCAGAAAGGACTTTCAGTTTTA TAAGGTGAGAGAGATCAACAATTACCACCATGCGCA TGATGCCTACCTGAATGCAGTGGTAGGCACTGCACT TATCAAAAAATATCCCAAGCTTGAATCTGAATTTGTT TACGGAGACTATAAAGTGTACGATGTTAGGAAAATG ATCGCAAAGTCTGAGCAGGAAATAGGCAAGGCCAC CGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTT TCAAGACCGAGATTACACTGGCCAATGGAGAGATTC GGAAGCGACCACTTATCGAAACAAACGGAGAAACAG GAGAAATCGTGTGGGACAAGGGTAGGGATTTCGCG ACAGTCCGGAAGGTCCTGTCCATGCCGCAGGTGAA CATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTT CTCCAAGGAAAGTATCCTCCCGAAAAGGAACAGCGA CAAGCTGATCGCACGCAAAAAAGATTGGGACCCCAA GAAATACGGCGGATTCGATTCTCCTACAGTCGCTTA CAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGA AGTCTAAAAAACTCAAAAGCGTCAAGGAACTGCTGG GCATCACAATCATGGAGCGATCAAGCTTCGAAAAAA ACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAG AGGTCAAAAAAGACCTCATCATTAAGCTTCCCAAGTA CTCTCTCTTTGAGCTTGAAAACGGCCGGAAACGAAT GCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACG AGCTGGCACTGCCCTCTAAATACGTTAATTTCTTGTA TCTGGCCAGCCACTATGAAAAGCTCAAAGGATCTCC CGAAGATAATGAGCAGAAGCAGCTGTTCGTGGAACA ACACAAACACTACCTTGATGAGATCATCGAGCAAAT AAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGC TAACCTCGATAAGGTGCTTTCTGCTTACAATAAGCAC AGGGATAAGCCCATCAGGGAGCAGGCAGAAAACAT TATCCACTTGTTTACTCTGACCAACTTGGGCGCGCC TGCAGCCTTCAAGTACTTCGACACCACCATAGACAG AAAGCGGTACACCTCTACAAAGGAGGTCCTGGACG CCACACTGATTCATCAGTCAATTACGGGGCTCTATG AAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACA GCAGGGCTGATTCGGACCCAAAAAAGAAGCGTAAG

		GTCGATCCCAAGAAGAAGAGAAAGGTAGATCCTAAG AAGAAGAGAAAGGTAGACGCATTGGATGACTTCGAC TTAGACATGCTTGGGTCAGATGCTTTGGACGACTTT GACCTCGACATGTTGGGTTCTGACGCGCTAGATGAC TTCGACTTGGACATGTTGGGCTCCGACGCTTTGGAC GACTTTGATCTGGACATGTTATGAGGATCCTGAGAC C

133
134
135 **S1.4 gRNA expression cassettes**
136

Name	Description	Sequence
U6-gRNA-1	U6 promoter driving gRNA-1 expression	GGTCTCAAACGTTCGACGTAAAGCCTGTAGAAGAGG TTTCTAGCGAACGACACGAGTTTGAGCCTCATGAAG CTTCGTTGAACAACGGAAACTCGACTTGCCTTCCGC ACAATACATCATTTCTTCTTAGCTTTTTTTCTTCTTCT TCGTTCATACAGTTTTTTTTTGTTTATCAGCTTACATT TTCTTGAACCGTAGCTTTCGTTTTCTTCTTTTTAACTT TCCATTCGGAGTTTTTGTATCTTGTTTCATAGTTTGT CCCAGGATTAGAATGATTAGGCATCGAACCTTCAAG AATTTGATTGAATAAAACATCTTCATTCTTAAGATATG AAGATAATCTTCAAAAGGCCCCTGGGAATCTGAAAG AAGAGAAGCAGGCCCATTTATATGGGAAAGAACAAT AGTATTTCTTATATAGGCCCATTTAAGTTGAAAACAA TCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGA AGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGA TTAAACCACAATTTGCACACCCGTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGCAAA ATTTTCCAGATCGATTTCTTCTTCCTCTGTTCTTCGG CGTTCAATTTCTGGGTTTTTCTCTTCGTTTTCTGTAAC TGAAACCTAAAATTTGACCTAAAAAAAATCTCAAATA ATATGATTCAGTGGTTTTGTACTTTTCAGTTAGTTGA GTTTTGCAGTTCCGATGAGATAAACCAATAACTTTGC TTAGATCTAATTCATTCCGTTACACCTCTGATGGAGA TGGAAGGTTCTTAATAATGATGCCATTTTTTGGGTAA TAATTTTGAATTAGAATCAAGGGTATAAGATTCATAA TTAACATCACTTAAGCAAAGTTCGTAATATACGACCA CAGGATATAATTTTTGGTACGCTGTGAGACC

U6-gRNA-3	U6 promoter driving gRNA-3 expression	GGTCTCAAACGTTCGACGTAAAGCCTGTAGAAGAGG TTTCTAGCGAACGACACGAGTTTGAGCCTCATGAAG CTTCGTTGAACAACGGAAACTCGACTTGCCTTCCGC ACAATACATCATTTCTTCTTAGCTTTTTTTCTTCTTCT TCGTTCATACAGTTTTTTTTTGTTTATCAGCTTACATT TTCTTGAACCGTAGCTTTCGTTTTCTTCTTTTTAACTT TCCATTCGGAGTTTTTGTATCTTGTTTCATAGTTTGT CCCAGGATTAGAATGATTAGGCATCGAACCTTCAAG AATTTGATTGAATAAAACATCTTCATTCTTAAGATATG AAGATAATCTTCAAAAGGCCCCTGGGAATCTGAAAG AAGAGAAGCAGGCCCATTTATATGGGAAAGAACAAT AGTATTTCTTATATAGGCCCATTTAAGTTGAAAACAA TCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGA AGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGA TTTGACTCACAGTCCTATCGAGGTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGCAAA ATTTTCCAGATCGATTTCTTCTTCCTCTGTTCTTCGG CGTTCAATTTCTGGGTTTTTCTCTTCGTTTTCTGTAAC TGAAACCTAAAATTTGACCTAAAAAAAATCTCAAATA ATATGATTCAGTGGTTTTGTACTTTTCAGTTAGTTGA GTTTTGCAGTTCCGATGAGATAAACCAATAACTTTGC TTAGATCTAATTCATTCCGTTACACCTCTGATGGAGA TGGAAGGTTCTTAATAATGATGCCATTTTTTGGGTAA TAATTTTGAATTAGAATCAAGGGTATAAGATTCATAA TTAACATCACTTAAGCAAAGTTCGTAATATACGACCA CAGGATATAATTTTTGGTACGCTGTGAGACC
U6-gRNA-4	U6 promoter driving gRNA-4 expression	GGTCTCAAACGTTCGACGTAAAGCCTGTAGAAGAGG TTTCTAGCGAACGACACGAGTTTGAGCCTCATGAAG CTTCGTTGAACAACGGAAACTCGACTTGCCTTCCGC ACAATACATCATTTCTTCTTAGCTTTTTTTCTTCTTCT TCGTTCATACAGTTTTTTTTTGTTTATCAGCTTACATT TTCTTGAACCGTAGCTTTCGTTTTCTTCTTTTTAACTT TCCATTCGGAGTTTTTGTATCTTGTTTCATAGTTTGT CCCAGGATTAGAATGATTAGGCATCGAACCTTCAAG AATTTGATTGAATAAAACATCTTCATTCTTAAGATATG AAGATAATCTTCAAAAGGCCCCTGGGAATCTGAAAG AAGAGAAGCAGGCCCATTTATATGGGAAAGAACAAT AGTATTTCTTATATAGGCCCATTTAAGTTGAAAACAA TCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGA AGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGA TTATTGCGCACCATTCCACTAGGTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT GAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGCAAA ATTTTCCAGATCGATTTCTTCTTCCTCTGTTCTTCGG CGTTCAATTTCTGGGTTTTTCTCTTCGTTTTCTGTAAC

		TGAAACCTAAAATTTGACCTAAAAAAAATCTCAAATA ATATGATTCAGTGGTTTTGTACTTTTCAGTTAGTTGA GTTTTGCAGTTCCGATGAGATAAACCAATAACTTTGC TTAGATCTAATTCATTCCGTTACACCTCTGATGGAGA TGGAAGGTTCTTAATAATGATGCCATTTTTTGGGTAA TAATTTTGAATTAGAATCAAGGGTATAAGATTCATAA TTAACATCACTTAAGCAAAGTTCGTAATATACGACCA CAGGATATAATTTTTGGTACGCTGTGAGACC
HHR-gRNA-1	gRNA-1 expression flanked by 5' HHR and 3' HDV	GGTCTCATATGCGACTACTGATGAGTCCGTGAGGAC GAAACGAGTAAGCTCGTCAAACCACAATTTGCACAC CCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCG CTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG AATGGGACGGATCCTGAGACC
HHR-gRNA-3	gRNA-3 expression flanked by 5' HHR and 3' HDV	GGTCTCATATGGAGTCACTGATGAGTCCGTGAGGAC GAAACGAGTAAGCTCGTCTGACTCACAGTCCTATCG AGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCG CTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG AATGGGACGGATCCTGAGACC
HHR-gRNA-4	gRNA-4 expression flanked by 5' HHR and 3' HDV	GGTCTCATATGCGCAATCTGATGAGTCCGTGAGGAC GAAACGAGTAAGCTCGTCATTGCGCACCATTCCACT AGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCG CTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG AATGGGACGGATCCTGAGACC

S1.5 Plant expression vector backbone elements

Name	Description	Sequence
Pnos	Nos promoter driving the plant resistance cassette	AGCGGAGAATTAAGGGAGTCACGTTATGACCCCC GCCGATGACGCGGGACAAGCCGTTTTACGTTTGG AACTGACAGAACCGCAACGTTGAAGGAGCCACTCA GCCGCGGGTTTCTGGAGTTTAATGAGCTAAGCACA TACGTCAGAAACCATTATTGCGCGTTCAAAAGTCG CCTAAGGTCACTATCAGCTAGCAAATATTTCTTGTC AAAAATGCTCCACTGACGTTCCATAAATTCCCCTC

		GGTATCCAATTAGAGTCTCATATTCACTCTCAATCC AAATAATCTGCACCGGATCT
KanR (Plant)	Kanamycin coding sequence (plant)	ATGATTGAACAAGATGGATTGCACGCAGGTTCTCC GGCCGCTTGGGTGGAGAGGCTATTCGGCTATGAC TGGGCACAACAGACAATCGGCTGCTCTGATGCCG CCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGT TCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGA ATGAACTCCAGGACGAGGCAGCGCGGCTATCGTG GCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTG CTCGACGTTGTCACTGAAGCGGGAAGGGACTGGC TGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCT GTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCA TCATGGCTGATGCAATGCGGCGGCTGCATACGCTT GATCCGGCTACCTGCCCATTCGACCACCAAGCGA AACATCGCATCGAGCGAGCACGTACTCGGATGGA AGCCGGTCTTGTCGATCAGGATGATCTGGACGAA GAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCG CCAGGCTCAAGGCGCGTATGCCCGACGGCGAGGA TCTCGTCGTGACTCATGGCGATGCCTGCTTGCCGA ATATCATGGTGGAAAATGGCCGCTTTTCTGGATTC ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCT ATCAGGACATAGCGTTGGCTACCCGTGATATTGCT GAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCC TCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAG CGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTT C
Tnos	Nos terminator	CTAGAGTCAAGCAGATCGTTCAAACATTTGGCAAT AAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTG CGATGATTATCATATAATTTCTGTTGAATTACGTTAA GCATGTAATAATTAACATGTAATGCATGACGTTATT TATGAGATGGGTTTTTATGATTAGAGTCCCGCAATT ATACATTTAATACGCGATAGAAAACAAAATATAGCG CGCAAACTAGGATAAATTATCGCGCGCGGTGTCAT CTATGTTACTAGATCGA
pVS1 replicon	OriV; RepA ; StaA for propagation in Agrobacterium	CGTGCGGCTGCATGAAATCCTGGCCGGTTTGTCT GATGCCAAGCTGGCGGCCTGGCCGGCCAGCTTGG CCGCTGAAGAAACCGAGCGCCGCCGTCTAAAAAG GTGATGTGTATTTGAGTAAAACAGCTTGCGTCATG CGGTCGCTGCGTATATGATGCGATGAGTAAATAAA CAAATACGCAAGGGGAACGCATGAAGGTTATCGCT GTACTTAACCAGAAAGGCGGGTCAGGCAAGACGA CCATCGCAACCCATCTAGCCCGCGCCCTGCAACT

		CGCCGGGGCCGATGTTCTGTTAGTCGATTCCGATC CCCAGGGCAGTGCCCGCGATTGGGCGGCCGTGC GGGAAGATCAACCGCTAACCGTTGTCGGCATCGA CCGCCCGACGATTGACCGCGACGTGAAGGCCATC GGCCGGCGCGACTTCGTAGTGATCGACGGAGCGC CCCAGGCGGCGGACTTGGCTGTGTCCGCGATCAA GGCAGCCGACTTCGTGCTGATTCCGGTGCAGCCA AGCCCTTACGACATATGGGCCACCGCCGACCTGG TGGAGCTGGTTAAGCAGCGCATTGAGGTCACGGA TGGAAGGCTACAAGCGGCCTTTGTCGTGTCGCGG GCGATCAAAGGCACGCGCATCGGCGGTGAGGTTG CCGAGGCGCTGGCCGGGTACGAGCTGCCCATTCT TGAGTCCCGTATCACGCAGCGCGTGAGCTACCCA GGCACTGCCGCCGCCGGCACAACCGTTCTTGAAT CAGAACCCGAGGGCGACGCTGCCCGCGAGGTCC AGGCGCTGGCCGCTGAAATTAAATCAAAACTCATT TGAGTTAATGAGGTAAAGAGAAAATGAGCAAAAGC ACAAACACGCTAAGTGCCGGCCGTCCGAGCGCAC GCAGCAGCAAGGCTGCAACGTTGGCCAGCCTGGC AGACACGCCAGCCATGAAGCGGGTCAACTTTCAGT TGCCGGCGGAGGATCACACCAAGCTGAAGATGTA CGCGGTACGCCAAGGCAAGACCATTACCGAGCTG CTATCTGAATACATCGCGCAGCTACCAGAGTAAAT GAGCAAATGAATAAATGAGTAGATGAATTTTAGCG GCTAAAGGAGGCGGCATGGAAAATCAAGAACAAC CAGGCACCGACGCCGTGGAATGCCCCATGTGTGG AGGAACGGGCGGTTGGCCAGGCGTAAGCGGCTG GGTTGTCTGCCGGCCCTGCAATGGCACTGGAACC CCCAAGCCCGAGGAATCGGCGTGACGGTCGCAAA CCATCCGGCCCGGTACAAATCGGCGCGGCGCTGG GTGATGACCTGGTGGAGAAGTTGAAGGCCGCGCA GGCCGCCCAGCGGCAACGCATCGAGGCAGAAGC ACGCCCCGGTGAATCGTGGCAAGCGGCCGCTGAT CGAATCCGCAAAGAATCCCGGCAACCGCCGGCAG CCGGTGCGCCGTCGATTAGGAAGCCGCCCAAGGG CGACGAGCAACCAGATTTTTTCGTTCCGATGCTCT ATGACGTGGGCACCCGCGATAGTCGCAGCATCAT GGACGTGGCCGTTTTCCGTCTGTCGAAGCGTGAC CGACGAGCTGGCGAGGTGATCCGCTACGAGCTTC CAGACGGGCACGTAGAGGTTTCCGCAGGGCCGGC CGGCATGGCCAGTGTGTGGGATTACGACCTGGTA CTGATGGCGGTTTCCCATCTAACCGAATCCATGAA CCGATACCGGGAAGGGAAGGGAGACAAGCCCGG CCGCGTGTTCCGTCCACACGTTGCGGACGTACTC AAGTTCTGCCGGCGAGCCGATGGCGGAAAGCAGA AAGACGACCTGGTAGAAACCTGCATTCGGTTAAAC

		ACCACGCACGTTGCCATGCAGCGTACGAAGAAGG CCAAGAACGGCCGCCTGGTGACGGTATCCGAGGG TGAAGCCTTGATTAGCCGCTACAAGATCGTAAAGA GCGAAACCGGGCGGCCGGAGTACATCGAGATCGA GCTAGCTGATTGGATGTACCGCGAGATCACAGAAG GCAAGAACCCGGACGTGCTGACGGTTCACCCCGA TTACTTTTTGATCGATCCCGGCATCGGCCGTTTTCT CTACCGCCTGGCACGCCGCGCCGCAGGCAAGGC AGAAGCCAGATGGTTGTTCAAGACGATCTACGAAC GCAGTGGCAGCGCCGGAGAGTTCAAGAAGTTCTG TTTCACCGTGCGCAAGCTGATCGGGTCAAATGACC TGCCGGAGTACGATTTGAAGGAGGAGGCGGGGCA GGCTGGCCCGATCCTAGTCATGCGCTACCGCAAC CTGATCGAGGGCGAAGCATCCGCCGGTTCCTAAT GTACGGAGCAGATGCTAGGGCAAATTGCCCTAGC AGGGGAAAAAGGTCGAAAAAGCTTCTTTCCTGTGG ATAGCACGTACATTGGGAACCCAAAGCCGTACATT GGGAACCGGAACCCGTACATTGGGAACCCAAAGC CGTACATTGGGAACCGGTCACACATGTAAGTGACT GATATAAAAGAGAAAAAAGGCGATTTTTCCGCCTA AAACTCTTTAAAACTTATTAAAACTCTTAAAACCCG CCTGGCCTGTGCATAACTGTCTGGCCAGCGCACA GCCGAAGAGCTGCAAAAAGCGCCTACCCTTCGGT CGCTGCGCTCCCTACGCCCCGCCGCTTCGCGTCG GCCTATCGCGGCCGCTGGCCGCTCAAAAATGGCT GGCCTACGGCCAGGCAATCTACCAGGGCGCGGAC AAGCCGCGCCGTCGCCACTCGACCGCCGGCGCC CACATCAAGGCACC
pMB1 origin	Origin of replication for propagation in E.coli	AAAGGATCTTCCTGAGATCCTTTTTTTCTGCGCGTA ATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC AGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAA CTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTA GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGC CTACATACCTCGCTCTGCTAATCCTGTTACCAGTG GCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCG GGTTGGACTCAAGACGATAGTTACCGGATAAGGC GCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC ACAGCCCAGCTTGGAGCGAACGACCTACACCGAA CTGAGATACCTACAGCGTGAGCTATGAGAAAGCGC CACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT CCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATC TTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTT GAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGC GGAGCCTATGGAAAAACGCCAGCAACGCG

KanR (bacterial)	Kanamycin expression cassette for bacterial selection	GCCAATTCGTGCGCGGAACCCCTATTTGTTTATTTT TCTAAATACATTCAAATATGTATCCGCTCATGAGAC AATAACCCTGATAAATGCTTCAATAATATTGAAAAA GGAAGAGTATGGCTAAAATGAGAATATCACCGGAA TTGAAAAAACTGATCGAAAAATACCGCTGCGTAAA AGATACGGAAGGAATGTCTCCTGCTAAGGTATATA AGCTGGTGGGAGAAAATGAAAACCTATATTTAAAA ATGACGGACAGCCGGTATAAAGGGACCACCTATG ATGTGGAACGGGAAAAGGACATGATGCTATGGCT GGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACT TTGAACGGCATGATGGCTGGAGCAATCTGCTCATG AGTGAGGCCGATGGCGTCCTTTGCTCGGAAGAGT ATGAAGATGAACAAAGCCCTGAAAAGATTATCGAG CTGTATGCGGAGTGCATCAGGCTCTTTCACTCCAT CGACATATCGGATTGTCCCTATACGAATAGCTTAG ACAGCCGCTTAGCCGAATTGGATTACTTACTGAAT AACGATCTGGCCGATGTGGATTGCGAAAACTGGG AAGAGGACACTCCATTTAAAGATCCGCGCGAGCTG TATGATTTTTTAAAGACGGAAAAGCCCGAAGAGGA ACTTGTCTTTTCCCACGGCGACCTGGGAGACAGCA ACATCTTTGTGAAAGATGGCAAAGTAAGTGGCTTT ATTGATCTTGGGAGAAGCGGCAGGGCGGACAAGT GGTATGACATTGCCTTCTGCGTCCGGTCGATCAGG GAGGATATCGGGGAAGAACAGTATGTCGAGCTATT TTTTGACTTACTGGGGATCAAGCCTGATTGGGAGA AAATAAAATATTATATTTTACTGGATGAATTGTTTTA GCTGTCAGACCAAGTTTACTCATATATACTTTAGAT TGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAG GTGAAGATCCTTTTTGATAATC
LB	Left border repeat	CTGATGGGCTGCCTGTATCGAGTGGTGATTTTGTG CCGAGCTGCCGGTCGGGGAGCTGTTGGCTGGCTG GTGGCAGGATATATTGTGGTGTAAACAAATTGACG CTTAGACAACTTAATAACACATTGCGGACGTTTTTA ATGTACTG
RB	Right border repeat	TGGTTGGCACATACAAATGGACGAACGGATAAACC TTTTCACGCCCTTTTAAATATCCGATTATTCTAATAA ACGCTCTTTTCTCTTAGGTTTACCCGCCAATATATC CTGTCAAACACTGATAGTTT
GFP DO	GFP expression cassette drop-out flanked by connector sequences	ACTGATGAGACGTGGTAGAGCCACAAACAGCCGG TACAAGCAACGATCTCCAGGACCATCTGAATCATG CGCGGATGACACGAACTCACGACGGCGATCACAG ACATTAACCCACAGTACAGACACTGCGACAACGTG GCAATTCGTCGCAATACAACGTGAGACCGAAAGTG AAACGTGATTTCATGCGTCATTTTGAACATTTTGTA AATCTTATTTAATAATGTGTGCGGCAATTCACATTT AATTTATGAATGTTTTCTTAACATCGCGGCAACTCA

		AGAAACGGCAGGTTCGGATCTTAGCTACTAGAGAA AGAGGAGAAATACTAGATGCGTAAAGGCGAAGAG CTGTTCACTGGTGTCGTCCCTATTCTGGTGGAACT GGATGGTGATGTCAACGGTCATAAGTTTTCCGTGC GTGGCGAGGGTGAAGGTGACGCAACTAATGGTAA ACTGACGCTGAAGTTCATCTGTACTACTGGTAAAC TGCCGGTTCCTTGGCCGACTCTGGTAACGACGCT GACTTATGGTGTTCAGTGCTTTGCTCGTTATCCGG ACCATATGAAGCAGCATGACTTCTTCAAGTCCGCC ATGCCGGAAGGCTATGTGCAGGAACGCACGATTT CCTTTAAGGATGACGGCACGTACAAAACGCGTGC GGAAGTGAAATTTGAAGGCGATACCCTGGTAAACC GCATTGAGCTGAAAGGCATTGACTTTAAAGAGGAC GGCAATATCCTGGGCCATAAGCTGGAATACAATTT TAACAGCCACAATGTTTACATCACCGCCGATAAAC AAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCC ATAACGTTGAGGATGGCAGCGTGCAGCTGGCTGA TCACTACCAGCAAAACACTCCAATCGGTGATGGTC CTGTTCTGCTGCCAGACAATCACTATCTGAGCACG CAAAGCGTTCTGTCTAAAGATCCGAACGAGAAACG CGATCATATGGTTCTGCTGGAGTTCGTAACCGCAG CGGGCATCACGCATGGTATGGATGAACTGTACAAA TGACCAGGCATCAAATAAAACGAAAGGCTCAGTCG AAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCG GTGAACGCTCTCTACTAGAGTCACACTGGCTCACC TTCGGGTGGGCCTTTCTGCGTTTATAGGTCTCAGC TGGAAATCTGCTCGTCAGTGGTGCTCACACTGACG AATCATGTACAGATCATACCGATGACTGCCTGGCG ACTCACAACTAAGCAAGACAGCCGGAACCAGCGC CGGCGAACACCACTGCATATATGGCATATCACAAC AGTCCACGTCTCAAGCAGTTACAGAGATGTTACGA ACCG

146 **Table S2 : List of OCS constructs used**

147

Name	Description	Transcriptional Units
OCS 1-1 (13,694bp)	U6 driven constitutive expression of gRNA-1 leading to constitutive expression of YFP under the control of pATF-1 promoter	TU1 : U6 :gRNA-1 (1013 bp) TU2 : pATF-1: YFP: T35S (1231 bp) TU3E : P35S : dCas9:VP64: T35S (5162 bp)

OCS 1-5 (13,685bp)	35S driven expression of gRNA-1 (flanked by ribozymes) leading to constitutive expression of YFP under the control of pATF-1 promoter	TU1 : P35S: HHR-gRNA-1-HDV:T35S (1001 bp) TU2 : pATF-1 : YFP: T35S (1231 bp) TU3E : P35S : dCas9:VP64: T35S (5162 bp)
OCS 1-9 (13,592bp)	Ethylene inducible expression of gRNA-1 under the control of EBS promoter. YFP under the control of pATF-1 promoter	TU1 : EBS: HHR-gRNA-1-HDV:T35S (917 bp) TU2 : pATF-1 : YFP: T35S (1231 bp) TU3E : P35S : dCas9:VP64: T35S (5162 bp)
OCS 4-1 (14,627bp)	U6 driven constitutive expression of gRNA-1 leading to constitutive expression of Luc under the control of pATF-1 promoter	TU1 : U6 :gRNA-1 (1013 bp) TU2 : pATF-1 : Luc: T35S (2150 bp) TU3E : P35S : dCas9:VP64: T35S (5162 bp)
OCS 3-5 (16,989bp)	Ratiometric circuit where YFP under the control of pATF-1 inducible by ethylene, while RFP and BFP under the control of Patf-3 are constitutively expressed	TU1 : EBS: HHR-gRNA-1-HDV:T35S (917 bp) TU2 : pATF-1 : YFP: T35S (1231 bp) TU3: pATF-3 : BFP : T35S (1228 bp) TU4: U6 :gRNA3 (1013 bp) TU5: pATF-3 : RFP : T35S (1214 bp) TU6E : P35S : dCas9:VP64: T35S (5162 bp)

Table S3: List of Addgene plasmids used in this study

Name	Description
pICH86966 Addgene #48075	Used as the backbone of the shuttle vector; Contains the pVS1 and pMB1 replicons, KanR (bacterial) as well as a plant KanR under pNos.

pYTK001 Addgene #65108	Backbone for cloning all the parts listed in Table S1
pYTK002 Addgene #65109	Contains the connector LS; Used for the construction of TU1
pYTK003 Addgene #65110	Contains the connector L1; Used for the construction of TU2
pYTK004 Addgene #65111	Contains the connector L2; Used for the construction of TU3
pYTK005 Addgene #65112	Contains the connector L3; Used for the construction of TU4
pYTK006 Addgene #65113	Contains the connector L4; Used for the construction of TU5
pYTK007 Addgene #65114	Contains the connector L5; Used for the construction of TU6
pYTK067 Addgene #65174	Contains the connector R1; Used for the construction of TU1
pYTK068 Addgene #65175	Contains the connector R2; Used for the construction of TU2

pYTK069 Addgene #65176	Contains the connector R3; Used for the construction of TU3
pYTK070 Addgene #65177	Contains the connector R4; Used for the construction of TU4
pYTK071 Addgene #65178	Contains the connector R5; Used for the construction of TU5
pYTK072 Addgene #65179	Contains the connector RE; Used for the construction of TU6
pYTK095 Addgene #65202	Used as the backbone for the construction of any transcriptional unit.