

Supplementary materials for

Benchmarking intrinsic promoters and terminators for plant synthetic biology research

Chenfei Tian^{1,2}, Yixin Zhang³, Jianhua Li^{1*}, Yong Wang^{1*}

¹ CAS-Key Laboratory of Synthetic Biology, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

² University of Chinese Academy of Sciences, Beijing 100039, China

³ College of Life Science, Jilin Agricultural University, Changchun 130118, PR China

* Corresponding author

Email address: yongwang@cemps.ac.cn (Y. Wang); jhli@cemps.ac.cn (J. Li).

Tel/Fax: 86-21-54924295

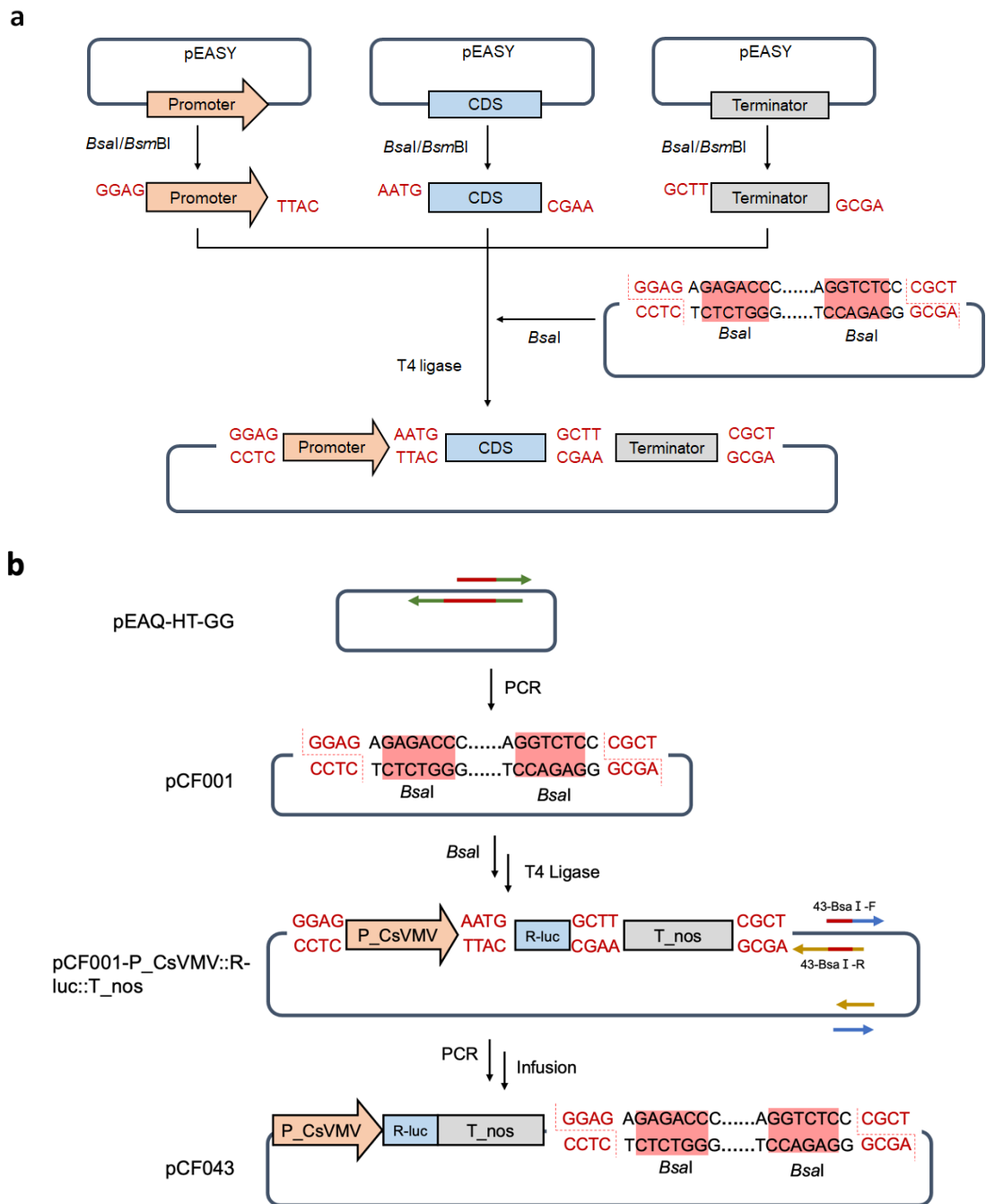


FIGURE S1. Type IIS assembly strategy for rapid plasmid construction. (a) Schematic diagram of plasmid construction. Standard genetic parts were inserted into the pEASY vector (TRANSGEN) with a standard restriction site. After digestion with *BsaI/BsmBI*, the genetic part segments with standardized 4-bp sticky ends could be sequentially linked by T4 ligase. The recognition sites of the Type II restriction enzymes *BsaI/BsmBI* were designed on the outside of both ends of the target fragment, so that the fragments recovered after digestion did not have the recognition site for digestion.

(b) GG3-*BsaI*-F/GG3-*BsaI*-R was used to PCR-amplify the pEAQ-HT-GG plasmid, adding the standard *BsaI* restriction sites with GGAG and CGCT 4-bp overhangs to pEAQ-HT-GG to obtain the basic vector pCF001. For dual-luciferase assays, the contrasting reporter gene REN was expressed under the P_CsVMV promoter and the T_nos terminator, and this cassette was constructed according to the method in (a). Two pairs of primers were used to PCR-amplify the pCF001-P_CsVMV::REN-luc::T_nos plasmid to append the standard *BsaI* restriction sites and obtain the vector pCF043 for dual-luciferase plasmid construction. The primers used for plasmid construction are listed in Supplementary Table 1.

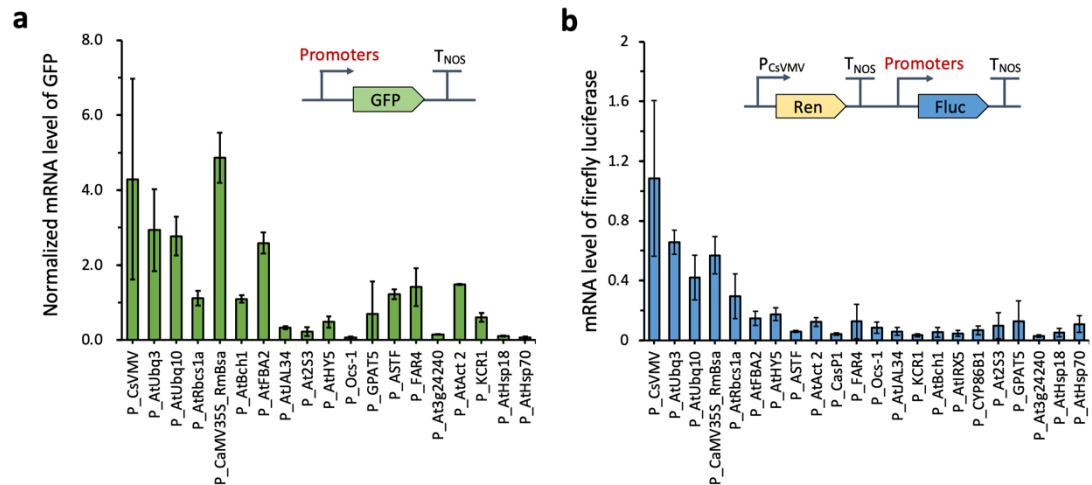


FIGURE S2. mRNA levels of reporter genes driven by different promoters. (a) normalized mRNA levels of GFP driven by different promoters. The internal reference used for qPCR was the actin gene. (b) normalized mRNA levels of firefly luciferase driven by different promoters. The internal reference used for qPCR was the *Renilla* luciferase gene in the plasmid. The mRNA expression levels were detected by real-time quantitative reverse transcription PCR. Data are presented as the mean \pm standard error of three independent replicates.

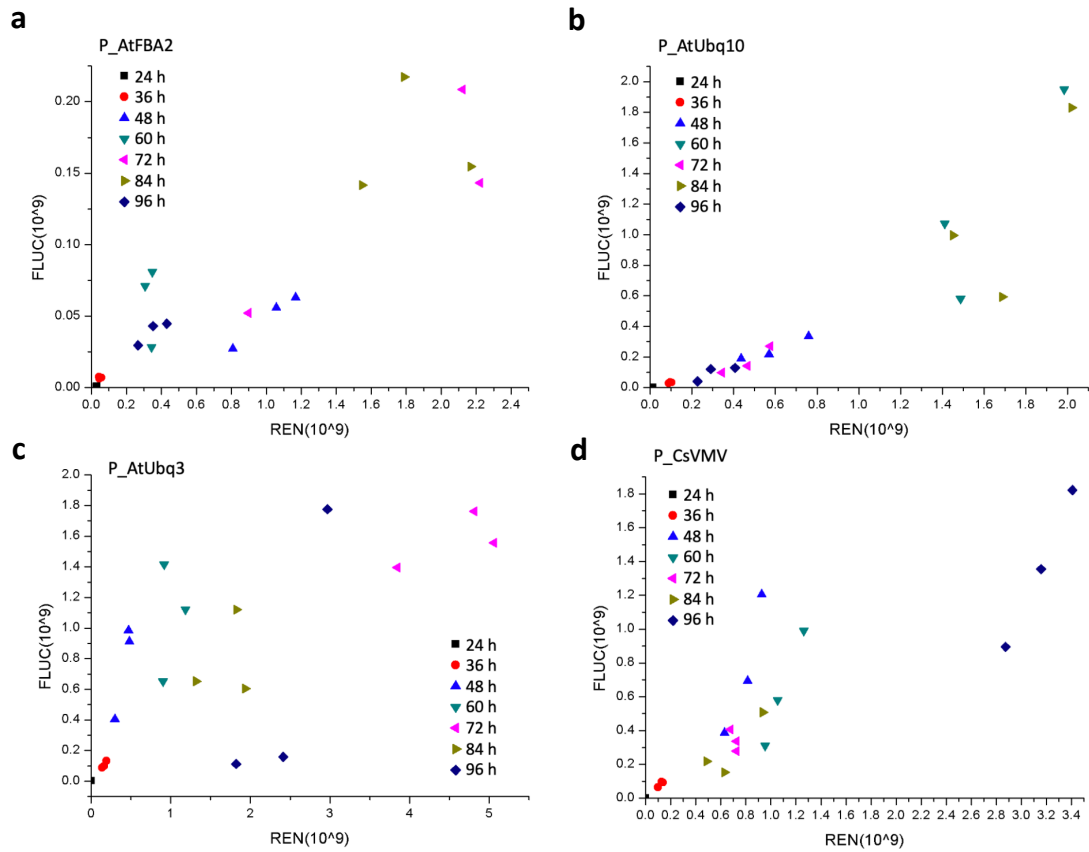


FIGURE S3. Dual-luciferase activity measurements with four representative promoters at different sampling times. Dual-luciferase activities with $P_{CsVMV}::R-luc::T_{nos}$ and $P_{AtFBA2}::F-luc::T_{nos}$ (a), $P_{AtUbq10}::F-luc::T_{nos}$ (b), $P_{AtUbq3}::F-luc::T_{nos}$ (c), and $P_{CsVMV}::F-luc::T_{nos}$ (d).

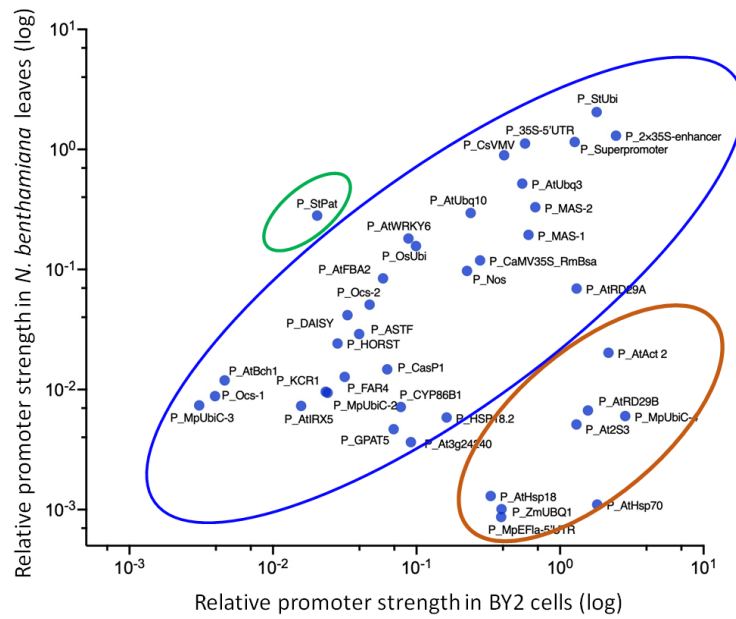


FIGURE S4. Comparison of relative transient expression levels of individual promoters in *N.benthamiana* leaves and BY2 cells. The relative promoter strengths (R-luc/F-luc activities) were monitored on a log scale. The scatter plots correspond to the relative strengths of the promoters. The plots located in the blue circle represent that the luminescence intensity is consistent within *N. benthamiana* and BY2 cells. The plots in the brown circle indicate represent that performance of promoters tested in BY2 cells are better than tested in *N.benthamiana*. The promoter P_StPat in the green circle shows a higher expression level in *N. benthamiana* than in BY2 cells.

326-fold

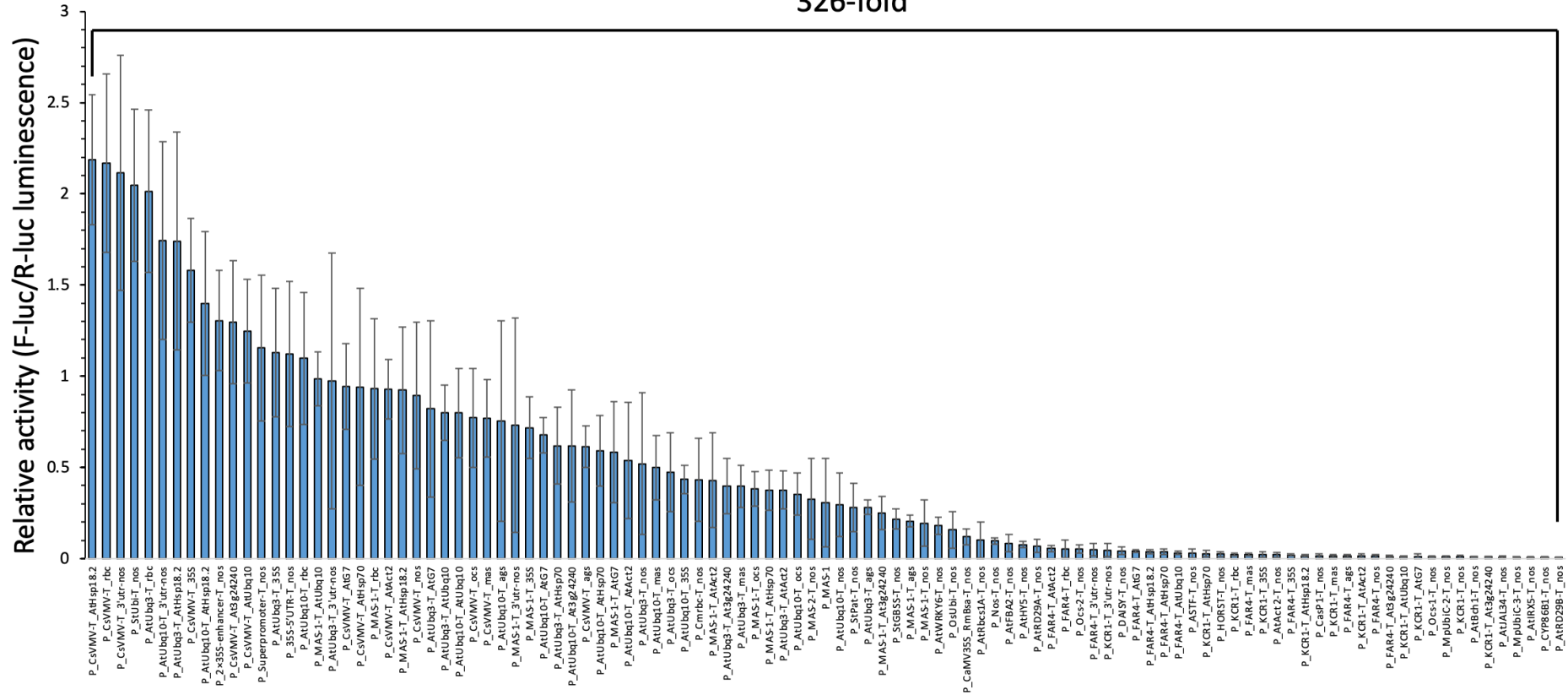


FIGURE S5. Expression cassette strength library obtained by integration of terminator elements. Expression cassette strength is presented as the ratio of firefly luciferase to *Renilla* luciferase luminescence intensity in *N. benthamiana*. These data are also shown in Figure 3 and Figure 5. Here,

we integrated different regulatory elements (promoters and terminators) to obtain more precise control of gene expression than that obtained by promoters alone.

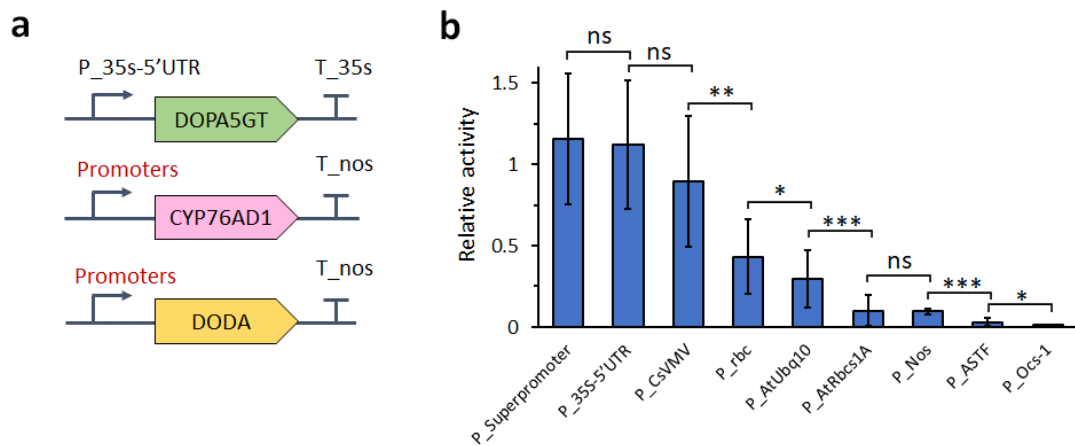


FIGURE S6. Different strengths of promoters used in betalain synthesis. (a) Schematic diagram of constructs used for betalain synthesis. DODA: DOPA 4,5-dioxygenase; DOPA5GT: cyclo-DOPA-5-O-glucosyltransferase. (b) Different strengths of promoters selected for use in betalain synthesis. The data are the same as the strengths shown in Figure 6.

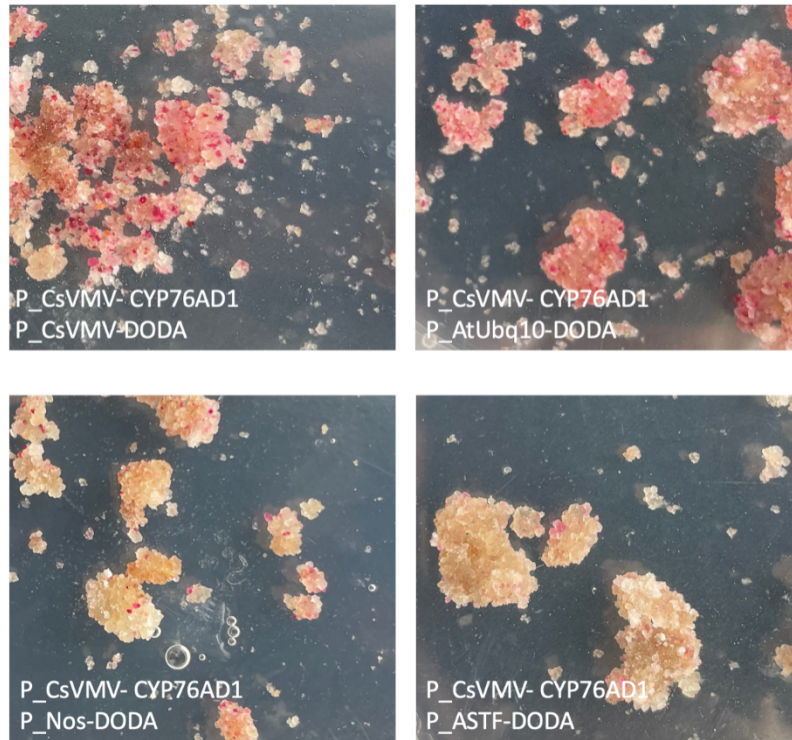


FIGURE S7. BY2-cells transformed with betalain synthesis genes. The cell-color phenotypes of BY2 cells co-infiltrated with *Agrobacterium* harboring plasmids for the expression of DOPA5GT, CYP76AD1, and DODA. DOPA5GT was controlled by the combination of P_{35S-5'UTR} and T_{nos}. CYP76AD1 and DODA were under the control of various promoters and T_{nos}.

Supplementary Table 1. Detailed information on the promoters used in this study

Element	Species	Length	Reference
P_CaMV35S_R mBsa	virus	315 bp	Peyret, H., et al., (2013). Plant Mol Biol 83:51-58.
GGAAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGAGAAGATAGTG GAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGT TGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAGCA TCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGAT ATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCC TCTATATAAGGAAGTTCATTTCAATTTGGAGAGG			
P_CsVMV	virus	520 bp	
CCAGAAGGTAATTATCCAAGATGTAGCATCAAGAATCCAATGTTTACGGGAAAAAC TATGGAAGTATTATGTGAGCTCAGCAAGAAGCAGATCAATATGCGGCACATATGCA ACCTATGTTCAAAAATGAAGAATGTACAGATACAAGATCCTATACTGCCAGAATAC GAAGAAGAATACGTAGAAATTGAAAAAGAAGAACAGGCGAAGAAAAAGAATCTTG AAGACGTAAGCACTGACGACAACAATGAAAAGAAGAAGATAAGGTCGGTGATTGT GAAAGAGACATAGAGGACACATGTAAGGTGGAAAAATGTAAGGGCGGAAAAGTAACC TTATCACAAGGAATCTTATCCCCACTACTTATCCTTTTATATTTTTCCGTGTCATT TTTGCCCTTGAGTTTTCTATATAAGGAACCAAGTTCGGCATTGTGAAAACAAGAA AAAATTTGGTGTAAGCTATTTTCTTTGAAGTACTGAGGATACAACCTTCAGAGAAATT TGTAAGTTTGT			
P_2×35S- enhancer	virus	884 bp	Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.
GTGGAGCACGACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGA CCAAAGGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGAT TCCATTGCCAGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCT CCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCC GACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAGCATCGTGGAAAAAGAAG ACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATAACATGGTGGAGCAC GACACACTTGTCTACTCCAAAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGC AATTGAGACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCC AGCTATCTGTCACTTTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAAT GCCATCATTGCGATAAAGGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGT CCCAAAGATGGACCCCAACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAA CCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC GCACAATCCCACTATCCTTCGCAAGACCCTTCTCTATATAAGGAAGTTCATTTCAT TTGGAGAGGACGTCGAGAGTTCTCAACACAACATATACAAAACAAACGAATCTCAA GCAATCAAGCATTCTACTTCTATTGCAGCAATTTAAATCATTTCTTTTAAAGCAAAA GCAATTTTCTGAAAATTTTCACCATTACGAACGATAG			
P_35S-5'UTR	virus	830 bp	Peyret, H., et al., (2013). Plant Mol Biol 83:51-58.
GGAAACCTCCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGAGAAGATAGTG GAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCCATCGT TGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCAACCCACGAGGAGCA TCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGAT			

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TATTTTCTTGGAAACAGAGTTTTCCCGTGGTTTTTCGAACTTGAGAAAAGATTGTTAA
GCTTCTGTATATTCTGCCAAATTCGCG

P_MAS-1 *Agrobacterium* 385 bp Langridge, W.H., et al., (1989). Proc Natl Acad Sci USA
86:3219-3223.

TTTTCAAATCAGTGCGCAAGACGTGACGTAAGTATCCGAGTCAGTTTTTATTTTTCT
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AGCATCTGAATTCATAACCAATCTCGATACACCAAATCG

P_MAS-2 *Agrobacterium* 392 bp Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.

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TTTCGCGGGTATTCTGTTTCTATTCCAACCTTTTTCTTGATCCGCAGCCATTAACGACT
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GCTTTACAGCAAGAACGGAATGCGCGTGACGCTCGCGGTGACGCCATTTTCGCCTTTT
CAGAAATGGATAAATAGCCTTGCTTCTTATTATATCTTCCCAAATTACCAATACATT
ACACTAGCATCTGAATTCATAACCAATCTCGATACACCAAATCGA

P_Ocs-1 *Agrobacterium* 235 bp

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TGATGGTAGGCAGAAAGTTAAAGGATTATCGCAAGTCAATACTTGCCATTATTG
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CCAA

P_Ocs-2 *Agrobacterium* 365 bp Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.

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TATATTTGCAATCTACCAA

P_Nos *Agrobacterium* 289 bp Chung, S.M., et al., (2005). Trends in Plant Science 10:357-361.

GATCATGAGCGGAGAATTAAGGGAGTCACGTTATGACCCCCGCCGATGACCGGGGA
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CCACTGACGTTCCATAAATCCCCTCGGTATCCAATTAGAGTCTCATATTTACTCTC
AAT

P_Superpromoter

Agrobacterium 1145 bp Ni, M., et al.,(1995). Plant J 7:661-676.

ACGGTATCGATAAGCTCGCGGATCCCTGAAAGCGACGTTGGATGTTAACATCTACA
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P_AtAct 2

Arabidopsis 791 bp Sarrion-Perdigones, A., et al., (2013). Plant Physiol 162:1618-1631.

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P_AtFBA2

Arabidopsis 1111 bp Lu, W., et al., (2012). Gene 503:65-74.

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P_AtRbc1a

Arabidopsis 1183 bp Farhi, M., et al. (2011). Nat Biotechnol 29:1072-1074

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P_AtUbq10 *Arabidopsis* 643 bp Sarrion-Perdigones, A., et al., (2013). Plant Physiol 162:1618-1631.

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ACT

P_AtHsp18 *Arabidopsis* 838 bp Farhi, M., et al. (2011). Nat Biotechnol 29:1072-1074

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P_AtHsp70 *Arabidopsis* 1982 bp Sung, D.Y., et al., (2001). Plant Physiol 126:789-800.

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CATTAGAAAGGACCAGTGAATTGTTTTGATAGAGATCTGCATAAGATGAGAAGGT
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ACCAGCATTAAAGGAGCCTCATTTTTAATCTGGACAACCTGAAAAGTCCAGATTTACA

ATCAACATTTCCACAATTAGAAAGAAAATAAAAAGATATAGAGAAAAGAAGATCTTAG
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GCAACCAGGTTATGAAAGAAAGTTTTAGTACCCATAGGAGACGCAGAGTGAGAGTT
GGATCAGAAATGAGATCGACAGAGTATTTGTTACGGACCACGTGAAATCCGAAGAT
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ACAAAAGAAATCGAAAAACCTCACTTCCAATTTCAATTCAATTACTGAAGCTTTTTTT
TAGCA

P_At2S3

Arabidopsis 350 bp Emami, S., et al., (2013). Front Plant Sci 4:339.

GAAACCAAATTAACATAGGGTTTTTATTTAAATAAAAAGTTTAACTTCTTTTTAAAA
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AAAAC

P_AtBch1

Arabidopsis 1985 bp Shih, P.M., et al., (2016). Nat Commun 7:13215.

TGCCTTAATCTTGTCTGCAATGTAGGGTATTGATGTCTTGAACGGCATAGTGACAAG
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P_AtUbq3 *Arabidopsis* 1970 bp Sarrion-Perdigones, A., et al., (2013). Plant Physiol 162:1618-1631.

CAGAGCTAAATTCCAAAAAATATGATCTTTGAACTTGCATCAATAACTAACGA
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P_At3g24240 *Arabidopsis* 1628 bp Shinohara, H., et al., (2016). Proc Natl Acad Sci USA 113:3897-3902.

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P_AtJAL34 *Arabidopsis* 1471 bp Yamada, K., et al., (2011). Plant Cell Physiol 52:2039-2049.
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ATGGTTATCACAGATAGATAAAAAGAAGTCAACAACG

P_AtWRKY6 *Arabidopsis* 1718 bp Robatzek, S., et al., (2002). Genes Dev 16:1139-1149.
GTCGGTGACAAGCAACGAAACATAAATAATCGACTTTGTCAAGATATGTATAAAAA
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TTTCTATAT

P_AtHY5

Arabidopsis 1700 bp Chattopadhyay, S., et al., (1998). Plant Cell 10:673-683.

GAGCATTGGGTGTTGGTGACGATGTTGATGACATCGACTTGTTTGGTTCAGTCACTG
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P_CasP1

Arabidopsis

2088 bp

Naseer, S., et al., (2012). Proc Natl Acad Sci USA 109:10101-10106.

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P_DAI5Y

Arabidopsis

2047 bp

Naseer, S., et al., (2012). Proc Natl Acad Sci USA 109:10101-10106.

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CC

P_KCR1

Arabidopsis

2223 bp

Naseer, S., et al., (2012). Proc Natl Acad Sci USA 109:10101-10106.

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TCTTCTCCTTAAAACTCTAACTTTACTCATCTCATCTCCAAAGTCTCAACCTTCT
TCTCT

P_HORST

Arabidopsis 2055 bp Shih, P.M., et al., (2016). Nat Commun 7:13215.

GGAGTATGCATCGATGATGATTCTGCCTCACTTAGAAGAATCAAAAAGCTAAAAGG
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ATATGCCTCTTAACAATCTCTCCACAGAACAAAAGCAAAAAGCCTAAACCGGGAT

P_GPAT5

Arabidopsis 2153 bp Shih, P.M., et al., (2016). Nat Commun 7:13215.

TGATCGCAAACGTCAATGGTCTATATCCATCTCTTGGGGATACACCATTGAGATATA
CACTTACTTCTTGACTGCTACGGAGCTGACTACGCCGTTGCAGACCTTCAAAAATTG
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GAGCATACTGATTAATAAGTGAAGAGGATATTGGTGACTTCAATGAAGACGGA
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CATGTATACAAATACGGTAGGTGCAATACTGATAAACTGCCATTTCCATTGCATTAG
ACGCTAGTCAATCCAAACCTTATAACATAAAAAGTTATAGTCCATTTCTTTTCATATC
CAATCGAGTCAAATAATATTCGAGCAAAAAACAAAAGA

P_ASTF

Arabidopsis 2081 bp Shih, P.M., et al., (2016). Nat Commun 7:13215.

GGAGCAGGGTCTTCATCATTCACATGAAGATCAGCAGCAGAGTGCAGAGGAA
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CATTATAGTTTATTTTGGCTCGACCTTTCTTTAATCAATAGCTTTGATGTACTGATTA
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GCCATTGTGCTGTTTTCTCCATTTGGATCAA

P_FAR4

Arabidopsis 2065 bp Shih, P.M., et al., (2016). Nat Commun 7:13215.

GGAGAGTTCTAACCCCGACATAAGGGATAGGGCAGCGGTGATGGACCTCATATTT
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TCTTC

P_AtIRX5

Arabidopsis

1990 bp

Gondolf, V.M., et al., (2014). BMC Plant Biol 14:344.

GGAGATGAAGCCATCCTCTACCTCGGAAAACTTGTGCGAGAAGAAGACATGCGA
TGGCATGGATGCTTGGATCTTTGACATTGATGACACTCTTCTCTCAACCATTCTTAC
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P_CYP86B1

Arabidopsis

2163 bp

Naseer, S., et al., (2012). Proc Natl Acad Sci USA 109:10101-10106.

GGAGCTAAACACAACAAGTTTTTCATGAGCACAAATAGTGGATGTATATACAAGAAT
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ATTCTTATTAATCTCTCTCAATTCTTCATTTCTGAGATTCATCGCTCTCTCTCTTT
GTCAC

P_CmRbc *Chrysanthemum morifolium* 1007 bp Chung, S.M., et al., (2005). Trends Plant Sci10:357-361.
CTTAGACAAACACCCCTTGTTATACAAAGAATTTTCGCTTTACAAAATCAAATTCGAG
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TGGATTCATAGAAGTCCATTCCTCCTAAGTATCTAGAAA

P_HSP18.2 *Arabidopsis* 915 bp Takahashi, T., et al., (1989). Mol Gen Genet 219:365-372.
AAGCTTGCTGCAGCTTTGACGACAAGTAGGTTTGTTCATTTAGTAGCATCTTTACAA
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P_AtRD29A *Arabidopsis* 1525 bp Bihmidine, S., et al., (2013). Planta 237:55-64.

GGAGAGATTGGGGTTTTGCTTTTGATGTTTGTGTTTTTGTATGATGCCTCTGTTTGTG
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P_AtRD29B *Arabidopsis* 649 bp Bihmidine, S., et al., (2013). *Planta* 237:55-64.

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P_MpUbiC-4 *Marchantia*
polymorpha 2500 bp

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P_MpUbiC-2	<i>Marchantia</i>	2494 bp
	<i>polymorpha</i>	

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P_MpUbiC-3 *Marchantia* 1500 bp
 polymorpha

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P_MpEFla- *Marchantia* 1729 bp
5'UTR *polymorpha*

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P_StGBSS *Solanum* 857 bp Ni, M., et al, (1995). Plant J 7:661-676.
tuberosum

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P_StUbi *Solanum* 1197 bp Ni, M., et al., (1995). Plant J 7:661-676.
tuberosum

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P_StPat

Solanum

1737 bp Ni, M., et al., (1995). Plant J 7:661-676.

tuberosum

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P_ZmUBQ1

Zea mays

1814 bp

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AATTATTT

P_OsUbi

rice

1484 bp

Ma, X., et al. (2015). Mol Plant 8:1274-1284.

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ACTACCAAAATATACCATAGCCATCACAACCTTTATTAATTTTGGTAGCTTAAGATGGT
ATATATAATAACCAATTAACAACCTGATTCTAATTTTACTACGGCCCAGTATCTACCAA
TACAAAACAACGAGTATGTTTTCTCCGTCGTAATCGTACACAGTACAAAAAACCTG
GCCAGCCTTTCTTGGGCTGGGGCTCTCTTTGCAAAGGTCACAAAACGTACACGGCAGT
AACGCCGCTTCGCTGCGTGTTAACGGCCACCAACCCCGCCGTGAGCAAACGGCATCA
GCTTTCCACCTCCTCGATATCTCCGCGGCGCCGTCTGGACCCGCCCCCTTCCGTTCCCT
TTCTTTCTCTCGCGTTTGCCTGGTGGGGACGGACTCCCCAAACCGCCTCTCCCTCTC
TTTATTTGTCTATATTCTCACTGGGCCCCACCCACCGCACCCCTGGGCCCACTCACGA

GTCCCCCTCCCCACCTATAAAATACCCACCCCCTCCTCGCCTCTTCTCCATCAATC
GAATCCCCAAAATCGCAGAGAAAAAAAATCTCCCCTCGAAGCGAAGCGTCGAATCG
CCTTCTCAAGGTATGCGATTTTCTGATCCTCTCCGTTCTCGCGTTTGATTTGATTTCCC
GGCCTGTTTCGTGATTGTGAGATGTTGTGGTTAGTCTCCGTTTTGCGATCTGTGGTAGAT
TTGAACAGGGTTAGATGGGGTTCGCGTGGTATGCTGGATCTGTGATTATGAGCGATGC
TGTTTCGTGGTCCAAGTATTGATTGGTTCGGATCTAGAAGTAGAACTGTGCTAGGGTTG
TGATTTGTTCCGATCTGTTCAATCAGTAGGATTTAGTCTCTGTTTTTCTCGTTGATCCA
AGTAGCAGCTTCAGGTATATTTGCTTAGGTTGTTTTGATTTCAGTCCCTCTAGTTGCA
TAGATTCTACTCTGTTTCATGTTTAATCTAAGGGCTGCGTCTTGTTGATTAGTGATTACA
TAGCATAGCTTTCAGGATATTTTACTTGCTTATGCCTATCTTATCAACTGTTGCACCTG
TAAATTCTAGCCTATGTTAATTAACCTGCCTTATGTGCTCTCGGGATAGTGCTAGTAGT
TATTGAATCAGTTTGCCGATGGAATTCTAGTAGTTCATAGACCTGCAGATTATTTTTGT
GAACTCGAGCACGGTTCGTCTCTATTTGTTAGGTCAGTGTGGTGTGATAGGTA
CACTGATGTTATTGTGGTTTAGATCGTGTATCTAACATATTGGAATAATTTGATTGACT
GATTTCTGCTGTACTTGCTTGGTATTGTTATAATTTTCATGTTTCATAGTTGCTGACCATG
CTTCGGTAATTGTGTGTGCAG

Supplementary Table 2. Detailed information on the terminators used in this study

Terminator	Length	Reference
T_AtUbq10	636 bp	
ATCTCGTCTCTGTTATGCTTAAGAAGTTCAATGTTTCGTTTCATGTAAAACCTTGGT GGTTTGTGTTTTGGGGCCTTGATAATCCCTGATGAATAAGTGTCTACTATGTTTC CGTTCCTGTTATCTCTTTCTTCTAATGACAAGTCGAACTTCTTCTTTATCATCGCT TCGTTTTTATTATCTGTGCTTCTTTGTTAATACGCCTGCAAAGTGACTCGACTCT GTTTAGTGCAGTTCTGCGAACTTGAAATAGTCCAATTGTTGGCCTCTAGTAATA GATGTAGCGAAAGTGTGAGCTGTTGGGTTCTAAGGATGGCTTGAACATGTAAAT CTTTTAGGTTCTGAGTATGATGAACATTCGTTGTTGCTAAGAAATGCCTGTAATGT CCCACAAATGTAGAAAATGGTTCGTACCTTTGTCCAAGCATTGATATGTCTGATGA GAGGAACTGCAAGATACTGAGCTTGGTTAACGAAGGAGAGGCAGTTTCTTCT TCCAAAGCATTTCATTTGACAATGCCTTGATCATCTTAAGTAGAGTTTCTGTTGTG GAAAGTTTGAACTTTGAAGAAACGACTCTCAAGTAAATTGATGATCACAAGTGA AAGTGATGTTACATAAGT		
T_AtHsp70	607 bp	Sung, D.Y., et al., (2001). <i>Plant Physiol</i> 126:789-800.
GCCTTTTGGCTTTTGTACTCTGTTGCTTGAGATTCTAGTTGGTTTCTTGTCTTA GTTTTATCTTCTATGTCACTCTGAAACTGGTGTGTGATCATTTTGATGCTTTAAGA ATTTAGCTTTACCGTTTTTAAACTCGCTCTGACCTATGAAAGACGACTGGGCATA ATACTCTACACGAAATATAGTAGACAAAGTGAAAGAAGTTAAGTTCATGGTTTAA GGAATATGATCTTAGCACTTGTATTTATAATTAACCATCCAATATGAATCCAAGA TCTCTTGTATACATATTAAGAAAATTTCTTCTTATTTCAATATAGTTTATCTGA AAAACCGTACAAATTGTAGTGGGATTCCATATTCTATATATGAGATGTAAGATTC ATTTGTTGTTGTCCAAAAAAAAAATGATTTCAATTTGTTAAAGAGTCTTCCATTGA ATGTTGTCACATATTGCGTAACACAAATCAAGCATATGAACAAATAAGGAATTA AGAGGTTAAAGAACACAATGAAATGGAGTAAATATGATTAATATGAATAATC CAATGATACATGAACAATAAAATTTCTTTAAGAAGTTATGCGC		
T_AtHsp18.2	238 bp	Farhi, M., et al. (2011). <i>Nat Biotechnol</i> 29:1072-1074
ATATGAAGATGAAGATGAAATATTTGGTGTGCAAATAAAAAGCTTGTGTGCTTA AGTTTGTGTTTTTTCTTGGCTTGTGTGTTATGAATTTGTGGCTTTTTCTAATATTA AATGAATGTAAGATCTCATTATAATGAATAAAACAAATGTTTCTATAATCCATTGTG AATGTTTTGTTGGATCTCTTCTGCAGCATATAACTACTGTATGTGCTATGGTATGG ACTATGGAA		
T_AtAct2	662 bp	Sarrion-Perdigones, A., et al., (2013). <i>Plant Physiol</i> 162:1618-1631.
GCTCTCAAGATCAAAGGCTTAAAAAGCTGGGGTTTTATGAATGGGATCAAAGTTT CTTTTTTCTTTATATTTGCTTCTCCATTTGTTTGTTCATTTCCCTTTTTGTTTTCG TTTCTATGATGCACTTGTGTGTGACAACTCTCTGGGTTTTTACTTACGTCTGCGTT TCAAAAAAAAAAACCGCTTTCGTTTTGCGTTTTAGTCCCATTGTTTTGTAGCTCTG AGTGATCGAATTGATGCCTCTTATTCCTTTTGTCCCTATAATTTCTTTCAAACCT CAGAAGAAAAACCTTGAAACTCTTGAATGTTAATATAAGTATTGTATAAGATTT TTATTGATTTGGTTATTAGTCTTACTTTTGCTACCTCCATCTTCACTTGGAACTGAT ATTCTGAATAGTTAAAGCGTTACATGTCTTCCATTCACAAATGAACTTAAACTAGC ACAAAGTCAGATATTTAAGATCGCACCATTTTATATAACCCCAATCGTCAATTCT ACTGTTTCAAGTTTTACACCAAAAACAATTACGAGGTGTATCTATTCGTTACTCTTTT		

CGCTTATTAATCCTTTTACATGATGCAAAATATAACAAATTAATTTCAAATTGTCC
TGCACATTTGGTTTATAACATCTTGATTCCAAAAAGT

T_AtG7 298 bp Butaye, K.M., et al., (2004). *Plant J* 39:440-449.
GCTTACTGACTAACTAGGATGAGCTAAGCTAGCTATATCATCAATTTATGTATTAC
ACATAATATCGCACTCAGTCTTTCATCTACGGCAATGTACCAGCTGATATAATCAG
TTATTGAAATATTTCTGAATTTAACTTGCATCAATAAAATTTATGTTTTTGCTTGG
CTATAATACCTGACTTGTATTTTATCAATAAAATTTAACTATATTTCTTTCAAG
ATGGGAATTAACATCTACAAATTGCCTTTTCTTATCGACCATGTACCCCGGGTACC
AAGCTTCTCGAG

T_At3g24240 722 bp Shinohara, H., et al., (2016). *Proc Natl Acad Sci USA* 113:3897-3902.
TTTTAAGGTTTGAAGGTTACGTGAGAAGAAAGCTTTTGTGTGTTGTTGAGAAAA
GGCCTTTTTTTTTCATTTATCATTTTAAGTCTTTATATATGTTATGTATAAAAGAAG
AAGAATAGAATGAGAGAAGTGAGTCAGTAATGTTATGGAATTGTGATTAGTAAAG
GGTGACATGATATTGTACCTTTTTGGGCGGTTTGAATCAATAATTTCTCTAGTTTT
CTCTCCAAGTGTGACTTTAGATTGAATGTAGTCCTTTATAGTTTTCACTCATAAAC
AGTACTATATATACACCTTCCAGAAACGTATTAATAATAATATTGAATTATTGAT
GCATAAAACAATTCTTCAAAAATGATATTTTCATGGGGCTCTTGAATCAGTAAGCC
ACATAATATTGACCATGTTTTGGTTTACATGCAACATTATATTGCATACCGTCCG
GCCAATTGAGTTGGTCTCTATTTTCTTTCTTCGTGTGGCAATTTTGGCACAATGAA
AAAGCATGCATTTGACCTTTTTCATTTTTTAAACCTTGAGCCTTTCATATATTCTTA
ATCTTACTATAATTGTTGATCACCAAATACATTTTGGAGAGCTAGAGCTGGTAAAT
ACGTTTAGCTCTATTTCCATTCATTATAATTTGGAAAAATTGGATATTAAGGATCA
TTTTTCAAAAATTTGTCCATCTATACCTTAGTTTGTAAATTGT

T_nos 257 bp Farhi, M., et al. (2011). *Nat Biotechnol* 29:1072-1074
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGC
GATGATTATCATATAATTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGT
AATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATAC
ATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAACCTAGGATAAATTATCGC
GCGCGGTGTCATCTATGTTACTAGATCGGG

T_ocs 730 bp Farhi, M., et al. (2011). *Nat Biotechnol* 29:1072-1074
ACTAGTCCCTAGAGTCCTGCTTAAATGAGATATGCGAGACGCCATGATCGCATG
ATATTTGCTTTCAATTCTGTTGTGCACGTTGTAAAAACCTGAGCATGTGTAGCTC
AGATCCTTACCGCCGTTTCGGTTCATTCTAATGAATATATCACCCGTTACTATCG
TATTTTTATGAATAATATTCTCCGTTCAATTTACTGATTGTACCCTACTACTTATAT
GTACAATATTTAAATGAAAACAATATATTGTGCTGAATAGGTTTATAGCGACATC
TATGATAGAGCGCCACAATAACAAAACAATTGCGTTTTATTATTACAAATCCAATTT
TAAAAAAAGCGGCAGAACCGGTCAAACCTAAAAGACTGATTACATAAATCTTATT
CAAATTTCAAAGTGCCCCAGGGGCTAGTATCTACGACACACCGAGCGGCGAACT
AATAACGCTCACTGAAGGGAACCTCCGGTTCCTCCGCGCGCATGGGTGAGATT
CCTTGAAGTTGAGTATTGGCCGTCCGCTCTACCGAAAGTTACGGGCACCATTCAAC
CCGGTCCAGCACGGCGGCCGGTAACCGACTTGCTGCCCGAGAATTATGCAGCA
TTTTTTTGGTGTATGTGGGCCCCAAATGAAGTGCAGGTCAAACCTTGACAGTGACG
ACAAATCGTTGGGCGGGTCCAGGGCGAATTTTGCACAACATGTCGAGGCTCAGC
AGGA

T_mas	265 bp	Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.
AATCTTGGACTCCCATGTTGGCAAAGGCAACCAAAACAACAAATGAATGATCCGCT CCTGCATATGGGGCGGTTTGAGTATTTCAACTGCCATTTGGGCTGAATTGAAGACA TGCTCCTGTCAGAAATCCGTGATCTTACTCAATATTCAGTAATCTCGGCCAATAT CCTAAATGTGCGTGGCTTTATCTGTCTTTGTATTGTTTCATCAATTCATGTAACGTT TGCTTTTCTTATGAATTTTCAAATAAATTATCAGATC		
T_35S	215 bp	Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.
GTCCGCAAAAATCACCAGTCTCTCTCTACAAATCTATCTCTCTATTTTTCTCCAG AATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTCTTATAGGGTTTCGCT CATGTGTTGAGCATATAAGAAACCCCTTAGTATGTATTTGTATTTGTAAAATACTTC TATCAATAAAAATTTCTAATTCCTAAAACCAAAATCCAGTGAC		
T_rbc	922 bp	Chung, S.M., et al., (2005). Trends Plant Sci 10:357-361.
TCATAAGCCCGATGGCTACTAAGTTTACTATTTACCAAGACTTTTGAATATTAAC CTTCTTGTAACGAGTCGGTTAAATTTGATTGTTTAGGGTTTTGTATTATTTTTTTTT GGTCTTTTAATTCATCACTTTAATTCCTAATTGTCTGTTCAATTCGTTGTTTGTTC CGGATCGATAATGAAATGTAAGAGATATCATATATAAATAATAAATTGTCGTTTC ATATTTGCAATCTTTTTTTTACAAACCTTTAATCGTTGTATGTATGACATTTTCTTC TTGTTATATTAGGGGAAATAATGTTAAATAAAAAGTACAAAATAAACTACAGTAC ATCGTACTGAATAAATTACCTAGCCAAAAAGTACACCTTTCCATATACTTCCTACA TGAAGGCATTTTCAACATTTTCAAATAAGGAATGCTACAACCGCATAATAACATC CACAAATTTTTTTATAAAAATAACATGTCAGACAGTGATTGAAAAGATTTTATTATAG TTTCGTTATCTTCTTTTCTCATTAAAGCGAATCACTACCTAACACGTCATTTTGTGAA ATATTTTTTTGAATGTTTTTATATAGTTGTAGCATTCTCTTTTCAAATTAGGGTTTG TTTGAGATAGCATTTCAGCCGGTTCATACAACCTTAAAAGCATACTCTAATGCTGGA AAAAAGACTAAAAAATCTTGTAAGTTAGCGCAGAATATTGACCCAAATTATATAC ACACATGACCCCATATAGAGACTAATTACCTTTTAAACCAATAAATTATTACTG TATTATAACATCTACTAATTAACCTTGAGTGTGAGTTTTTGTAGAATTATTATCATATAT ACTAAAAGGCAGGAACGCAAACATTGCCCGGTACTGTAGCAACTACGGTAGAC GCATTAATTGTCTATAGTGGA		
T_ags	406 bp	Farhi, M., et al., (2011). Nat Biotechnol 29:1072-1074
GAATTAACAGAGGTGGATGGACAGACCCGTTCTTACACCGGACTGGGCGCGGGAT AGGATATTCAGATTGGGATGGGATTGAGCTTAAAGCCGGCGCTGAGACCATGCTC AAGGTAGGCAATGTCCTCAGCGTCGAGCCCGGCATCTATGTCGAGGGCATTGGTG GAGCGCGCTTCGGGGATACCGTGCTTGTAAGTGAAGCCGGATATGAGGCCCTCAC TCCGCTTGATCTTGCAAAGATATTTGACGCATTTATTAGTATGTGTTAATTTTCAT TTGCAGTGCAGTATTTCTATTTCGATCTTTATGTAATTCGTTACAATTAATAAATAT TCAAATCAGATTATTGACTGTCATTTGTATCAAATCGTGTAAATGGATATTTTTAT TATAATATTGATGAT		
T_3'utr-nos	495 bp	Peyret, H., et al., (2013). Plant Mol Biol 83:51-58.
TTAACTCTGGTTTCATTAATTTTCTTTAGTTTGAATTTACTGTTATTCGGTGTGCA TTTCTATGTTTGGTGAGCGGTTTTCTGTGCTCAGAGTGTGTTTATTTTATGTAATTT AATTTCTTTGTGAGCTCCTGTTTAGCAGGTCGTCCCTTCAGCAAGGACACAAAAAG ATTTTAATTTTATTAATAAAAAAAAAAAAAAAAAAAGACCGGGAATTCGATATCAAG CTTATCGACCTGCAGATCGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAAT		

CCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCA
TGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTA
GAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGCAAA
CTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTCT

Supplementary Table 3. Primers used in this study.

Primer	Sequence (5'→3')
GG3-BsaI-F	AGTGAATTGTTAATGGAGAGAGACCCCGATGGAAACGTTTAAGCAGG TCTCCCGCTCGTAATCATGGTCATA
GG3-BsaI-R	TATGACCATGATTACGAGCGGGAGACCTGCTTAAACGTTTCCATCGGG GTCTCTCTCCATTAACAATCACT
43-BsaI-F	CTTTTAAGAAGTTATGCGCCGCTGGAGAGAGACCCCGATGGAAAC
43-inter-R	CAAGCTGGAAACCTTCCGC
43-inter-F	GCGGAAGGTTTCCAGCTTG
43-BsaI-R	CAGCTATGACCATGATTACGAGCGGGAGACCTGCTTAAACGTTTCCAT CGGGGTCTCTCTCCAGCGGCATAACTTCTT
GFP208-F	TGCAGTGCTTCAGCCGCTA
GFP494-R	AGTTCACCTTGATGCCGTTT
qP19-F	GACTGAGTGGCGGCTACAT
qP19-R	TCCTCCAGAAACGGTGATAC
Fluc-F-635	ATAGAAGTGCCTGCGTGAG
Fluc-R-886	CGAAGAAGGAGAATAGGGTT
REN-F-194	CACATATTGAGCCAGTAGCG
REN-R-476	ATATCAGGCCATTCATCCC
P_CaMV35S_RmBsa-F	GGAATTCGGTCTCAGGAGGGAAACCTCCTCGGATTCC
P_CaMV35S_RmBsa-R	CGGGATCCGGTCTCACATTCTCTCCAAATGAAATGAAC
P_CsVMV-F	GGAATTCGGTCTCAGGAGCCAGAAGGTAATTATCCAAG
P_CsVMV-R	CGGGATCCGGTCTCACATTACAACTTACAAATTTCTCTG
P_35S-5'UTR-F	CGGTCTCAGGAGGGAAACCTCCTCGGATTCCAT
P_35S-5'UTR-R	CGGTCTCACATTTCGCAATTTGGGCAGAATATACAG
P_MAS-1-F	GGAATTCGGTCTCAGGAGTTTTCAAATCAGTGCGCAAG
P_MAS-1-R	CGGGATCCGGTCTCACATTCGATTTGGTGTATCGAGATTGG
P_Ocs-1-F	GGAATTCGGTCTCAGGAGATCATTAATTTCCACCTTAC
P_Ocs-1-R	CGGGATCCGGTCTCACATTTTGGTAGATTGCAAATATAATG
P_AtAct 2-F	GGAATTCGGTCTCAGGAGTCGACAAAATTTAGAACGAAC
P_AtAct 2-R	CGGGATCCGGTCTCACATTTTCAAAGCGGAGAGGAAAATATATG
P_AtFBA2-F	GGAATTCGGTCTCAGGAGCCTACACATCCACTTGCC
P_AtFBA2-R	CGGGATCCGGTCTCACATTTTTTTTTTGTGTGTGTTATC
P_AtRbcs1a-F	GGAATTCGGTCTCAGGAGAACATTCATACGTTTTTCAATTTG
P_AtRbcs1a-R	CGGGATCCGGTCTCACATTTTGTCTTCTTACTCTTTGTG
P_AtUbq10-F	GGAATTCGGTCTCAGGAGTACCCGACGAGTCAGTAATAAAC
P_AtUbq10-R	CGGGATCCGGTCTCACATTAGTGTTAATCAGAAAACTC
P_AtHsp18-F	GGAATTCGGTCTCAGGAGAAGCTTTTCTTTCATTTCTC
P_AtHsp18-R	CGGGATCCGGTCTCACATTGACTTTTGGTTCTTGATTTTCC
P_AtHsp70-F	GGAATTCGGTCTCAGGAGGAAGTGCAGAAAAAGGGAGC
P_AtHsp70-R	CGGGATCCGGTCTCACATTTGCTAAAAAAGCTTCAGTAATTG
P_At2S3-F	GGAATTCGGTCTCAGGAGGAAACCAAATTAACATAGG
P_At2S3-R	CGGGATCCGGTCTCACATTGTTTTGCTATTTGTGTATGTTTTT
P_AtBch1-F	GGAATTCGGTCTCAGGAGTGCCTTAATCTTGTCTGCAATG

P_AtBch1-R	CGGGATCCGGTCTCACATTCTAATGGAAGGAGGAGCTAC
P_AtUbq3-F	ATGGTCTCAGGAGCAGAGCTAAATCCAAAAAATATG
P_AtUbq3-R	ATGGTCTCACATTCTGAAATAAAACAATAGAACAAG
P_At3g24240-F	ATGGTCTCAGGAGGAAAAAGTGATGGAAACTCGG
P_At3g24240-R	ATGGTCTCACATTTTGCCTCAAATACGAAAAGAAG
P_AtJAL34-F	GGAATTCGGTCTCAGGAGGCTAATGACATTAGATCG
P_AtJAL34-R	CGGGATCCGGTCTCACATTCTGTTGACTTCTTTTATC
P_AtWRKY6-F	GGAATTCGGTCTCAGGAGGTCGGTGACAAGCAACGAAAC
P_AtWRKY6-R	CGGGATCCGGTCTCACATTATATAGAAAAAGAAAGAGATCACG
P_AtHY5-F	GGAATTCGGTCTCAGGAGGAGCATTGGGTGTTGGTG
P_AtHY5-R	CGGGATTCGGTCTCACATTTTTCTTACTCTTTGAAGATCG
P_CasP1-F	GGAATTCGGTCTCAGGAGAATAACGCATGCATTGTATTTGTATTC
P_CasP1-R	CGGGATTCGGTCTCACATTTTCTTGTCAATTGGGGTTTAAAG
P_DAISSY-F	GGAATTCGGTCTCAGGAGATTAATGCTCGCTTTGATTG
P_DAISSY-R	CGGGATCCGGTCTCACATTGGTAGGTTTTTGGTTTTAAATG
P_KCR1-F	GGAATTCGGTCTCAGGAGCCAAGAAAGTTGGAAGAGGAAG
P_KCR1-R	CGGGATCCGGTCTCACATTAGAGAAGAAAGTTGAGACTTTGG
P_HORST-F	GGAATTCGGTCTCAGGAGTATGCATCGATGATGATTCTGCC
P_HORST-R	CGGGATCCGGTCTCACATTATCCCGGTTTAGGCTTTTTGC
P_GPAT5-F	GGAATTCGGTCTCAGGAGTGATCGCAAACGTCAATG
P_GPAT5-R	CGGGATCCGGTCTCACATTTCTTTGTTTTTGTCTCG
P_ASTF-F	GGAATTCGGTCTCAGGAGCAGGTTCTTCATCATCATTAC
P_ASTF-R	CGGGATCCGGTCTCACATTTTGATCCAAATGGAGAAAACAGC
P_FAR4-F	GGAATTCGGTCTCAGGAGAGTTCTAACCCCGACATAAGG
P_FAR4-R	CGGGATCCGGTCTCACATTGAAGAACTTATATCTATCC
P_AtIRX5-F	GGAATTCGGTCTCAGGAGATGAAGCCATCCTCTACCTCGG
P_AtIRX5-R	CGGGATCCGGTCTCACATTGGCGAGGTACACTGAGCTC
P_CYP86B1-F	GGAATTCGGTCTCAGGAGCTAAACACAACAAGTTTTCATGAG
P_CYP86B1-R	CGGGATCCGGTCTCACATTGTGACAAAGAGAAGAGAGAGCG
P_AtRD29A-F	ATTGGTCTCGGGAGGAGATTTGGGGTTTTGCTTTTG
P_AtRD29A-R	ATTGGTCTCACATTCCAAAGATTTTTTCTTTCC
P_AtRD29B-F	ATTGGTCTCGGGAGGCGTAATTTCTAGATCCGTCTTGG
P_AtRD29B-R	ATTGGTCTCACATTCCAAAGCTGTGTTTTCTC
P_MpUbiC-4-F	ATTGGTCTCGGGAGGGTATGAATCATTGTAGAGAC
P_MpUbiC-4-R	ATTGGTCTCACATTGATCCTTCTTCTCACTCAC
P_MpUbiC-2-F	ATTGGTCTCGGGAGGTCGCTGTCCAGAGACACTTTTAC
P_MpUbiC-2-R	ATTGGTCTCACATTCTTCTCCCGACGACAACCG
P_MpUbiC-3-F	ATTGGTCTCGGGAGGATAGAGTTGATCGAGAGATTG
P_MpUbiC-3-R	ATTGGTCTCACATTCTTGGCGGAGCGCACCCGG
P_MpEFla-5'UTR-F	ATTGGTCTCGGGAGGCAAATGAGTCACACACATTG
P_MpEFla-5'UTR-R	ATTGGTCTCACATTCAACCTTCTGCAGGCAC
P_StGBSS-F	ATTGGTCTCGGGAGAAGCTTTAACGAGATAGAAAATTATATTAC
P_StGBSS-R	ATTGGTCTCACATTTGCATGAAATCAGAAATAATTGGAG
P_StUbi-F	ATTGGTCTCGGGAGGGAATCTAATACTTACCTCTTAG

P_StUbi-R	ATTGGTCTCACATTCTGCAAATTCATAAAAAACAAC
P_StPat-F	ATTGGTCTCGGGAGAAGCTTATGTTGCCATATAGAG
P_StPat-R	ATTGGTCTCACATTTTTGCAAATGTTCAAAGTGTTTTAAATTTTG
P_ZmUBQ1-F	ATTGGTCTCGGGAGGTAATGAGCATTGCATGTCTAAGTTATAAAAAAT TACC
P_ZmUBQ1-R	ATTGGTCTCACATTAATAATTATAAAAACATACTTGTTTATTATAATA GATAGGTACTC
P_OsUbi-F	ATTGGTCTCGGGAGAGCCCCATTACCACATTTG
P_OsUbi-R	ATTGGTCTCACATTCTGCACACACAATTACC
T_AtUbq10-F	GGAATTCGGTCTCAGCTTATCTCGTCTCTGTTATGCT
T_AtUbq10-R	CGGGATCCGGTCTCAAGCGCACTTATGTAACATACACTTTC
T_AtHsp70-F	GGAATTCGGTCTCAGCTTGCCTTTTGGCTTTTGTTFCTC
T_AtHsp70-R	CGGGATCCGGTCTCAAGCGGCGCATAACTTCTTAAAAG
T_AtHsp18.2-F	GGAATTCGGTCTCAGCTTATATGAAGATGAAGATGAAATATTTG
T_AtHsp18.2-R	CGGGATCCGGTCTCAAGCGTTCATAGTCCATACCATAGCAC
T_AtAct2-F	GGAATTCGGTCTCAGCTTGCTCTCAAGATCAAAGGCTTAAAAAG
T_AtAct2-R	CGGGATCCGGTCTCAAGCGACTTTTTGGAATCAAGATGTATAAAC
T_AtG7-F	GGAATTCGGTCTCAGCTTACTGACTAACTAGGATGAGCTAAG
T_AtG7-R	CGGGATCCGGTCTCAAGCGCTCGAGAAGCTTGGTACCCG
T_At3g24240-F	GGAATTCGGTCTCAGCTTTTTTAAGGTTTGAAGGTTACGTG
T_At3g24240-R	CGGGATCCGGTCTCAAGCGACAATTTACAAACTAAGGTATAG
T_nos-F	GGAATTCGGTCTCAGCTTCGTTCAAACATTTGGCAATAAAG
T_nos-R	CGGGATCCGGTCTCAAGCGCCCGATCTAGTAACATAGATGAC
T_3'utr-nos-F	TTCGGTCTCAGCTTTTAACTCTGGTTTCATTAAATTTTC
T_3'utr-nos-R	TCCGGTCTCAAGCGAGAGATCTAGTAACATAGATG