SUPPLEMENTAL MATERIAL

GLABRA2-based selection efficiently enriches Cas9-generated non-chimeric *Arabidopsis* mutants in T1 generation

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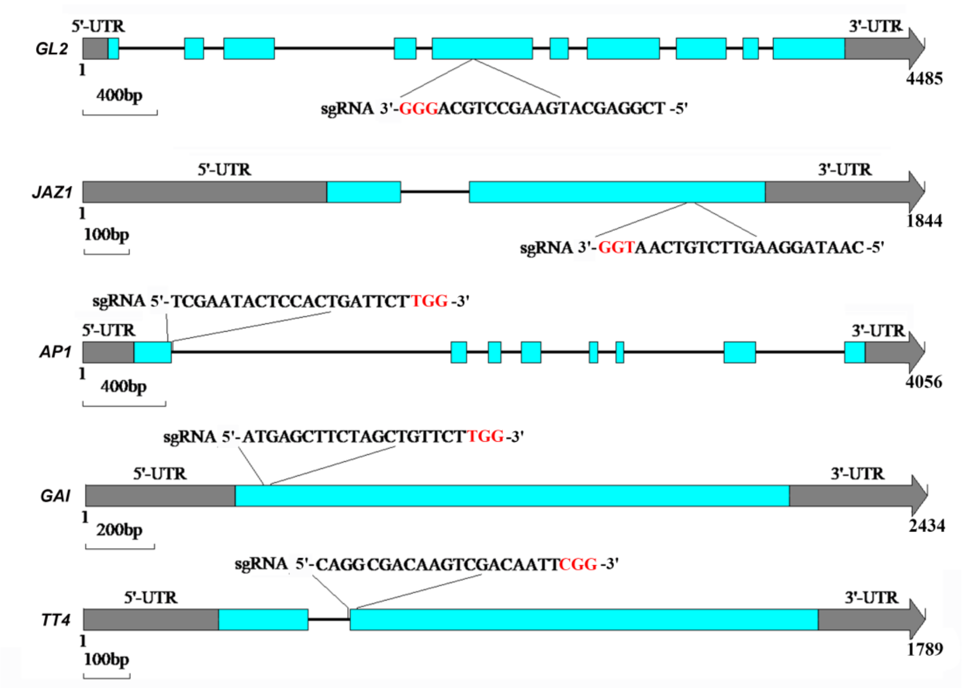
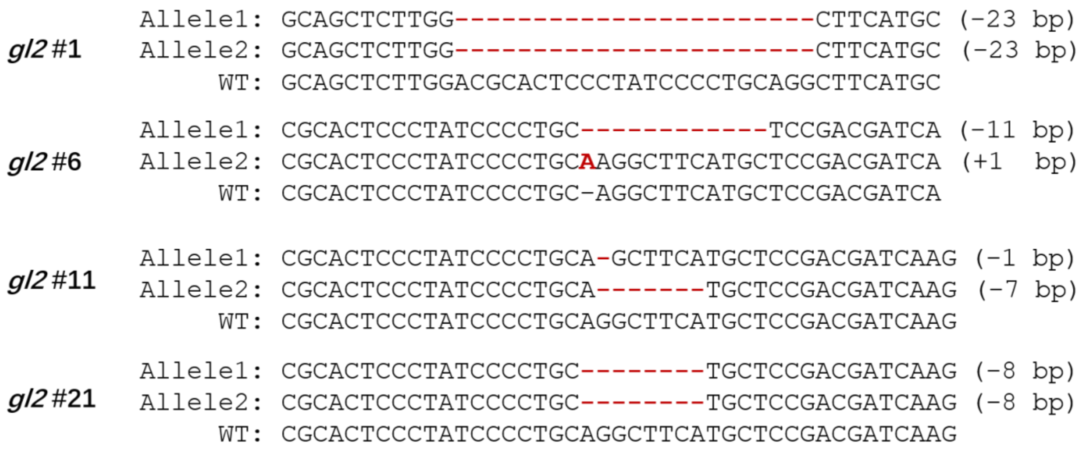


Supplemental Figure S1 The target sites of genes for monogenic mutation used in this study. The structures of *GL2*, *AP1*, *TT4*, *JAZ1*, and *GAI* are represented by exons (light blue bars), introns (black lines), and untranslated regions (UTRs) (gray bars). The sgRNA target sites are indicated and the protospacer adjacent motif (PAM) sites are highlighted in red.



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        Allele1: GCAGCTCTTGG-----------------------CTTCATGC (-23 bp)
gl2 #1  Allele2: GCAGCTCTTGG-----------------------CTTCATGC (-23 bp)
             WT: GCAGCTCTTGGACGCACTCCCTATCCCCTGCAGGCTTCATGC

        Allele1: CGCACTCCCTATCCCCTGC------------TCCGACGATCA (-11 bp)
gl2 #6  Allele2: CGCACTCCCTATCCCCTGCAAGGCTTCATGCTCCGACGATCA (+1  bp)
             WT: CGCACTCCCTATCCCCTGC-AGGCTTCATGCTCCGACGATCA

        Allele1: CGCACTCCCTATCCCCTGCA-GCTTCATGCTCCGACGATCAAG (-1 bp)
gl2 #11 Allele2: CGCACTCCCTATCCCCTGCA-------TGCTCCGACGATCAAG (-7 bp)
             WT: CGCACTCCCTATCCCCTGCAGGCTTCATGCTCCGACGATCAAG

        Allele1: CGCACTCCCTATCCCCTGC--------TGCTCCGACGATCAAG (-8 bp)
gl2 #21 Allele2: CGCACTCCCTATCCCCTGC--------TGCTCCGACGATCAAG (-8 bp)
             WT: CGCACTCCCTATCCCCTGCAGGCTTCATGCTCCGACGATCAAG
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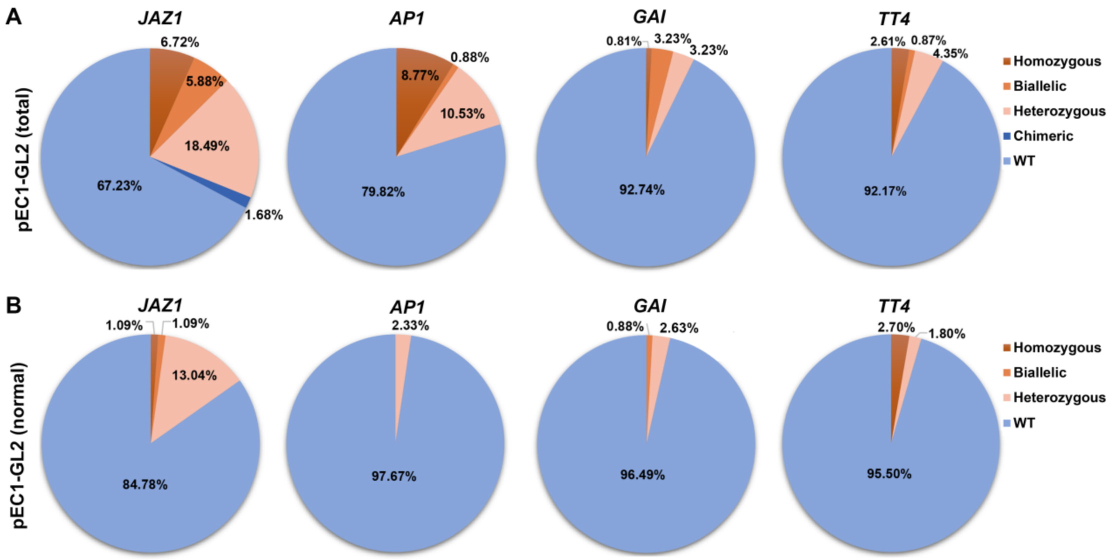
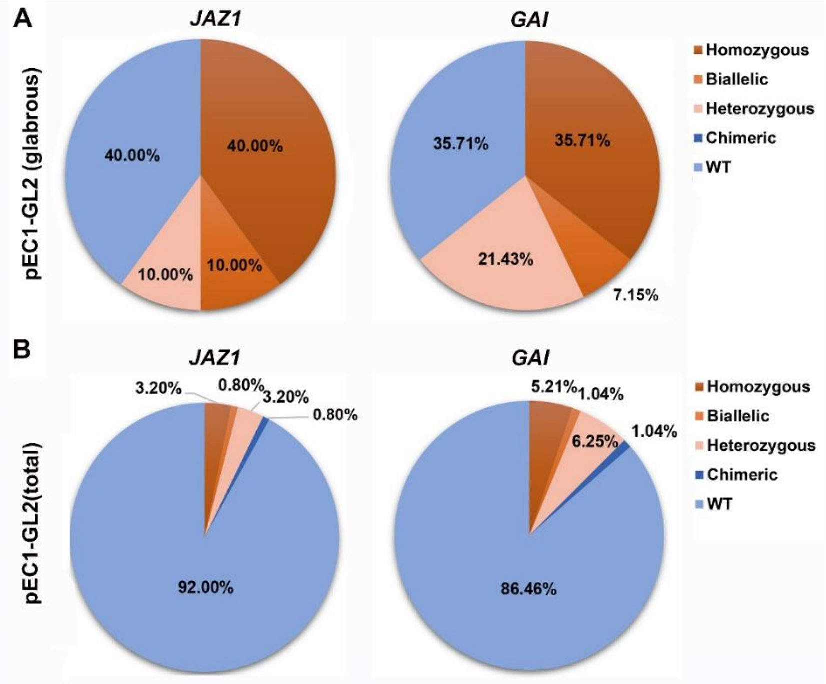
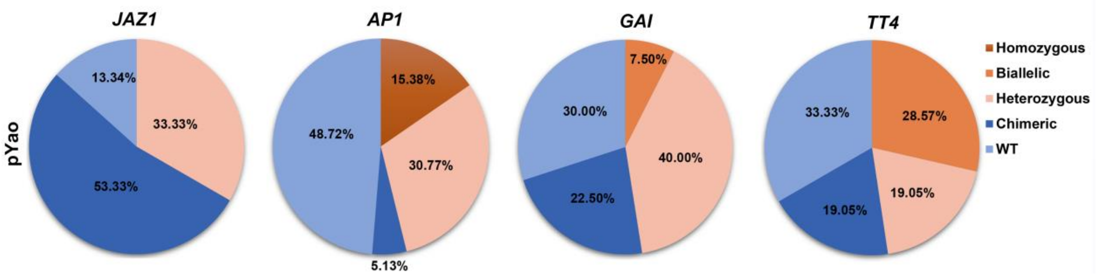
Supplemental Figure S2 DNA sequences of the *gl2* mutants. The reference sequence of WT is shown on the bottom. The indels in mutant alleles are depicted by a red dash (deletion) or red letter (insertion).



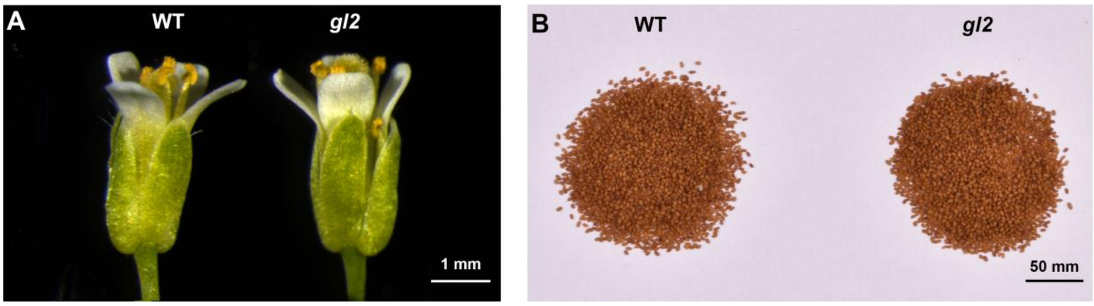
Supplemental Figure S3 Analyses of the frequencies of mutations for all transgenic T1 plants (total) and those with normal trichomes (normal) group obtained using the pEC1-GL2 vector. A, Percentages of WT and different types of mutation at four target sites among all T1 plants obtained using pEC1-GL2 vector. B, Percentages of WT and different types of mutation at four target sites among T1 plants with normal trichomes obtained using the pEC1-GL2 vector.



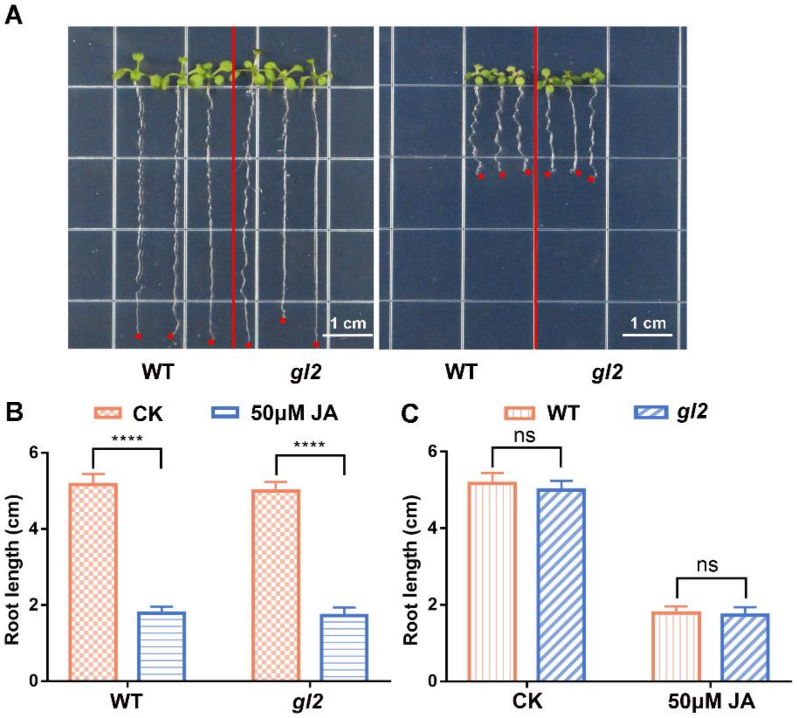
Supplemental Figure S4 Analyses of the frequencies of *JAZ1* and *GAI* mutations for all transgenic T1 plants (total) and glabrous (glabrous) group obtained using the pEC1-GL2 vector. A, Percentages of WT and different types of mutation at *JAZ1* and *GAI* target sites among glabrous T1 plants obtained using pEC1-GL2 vector. B, Percentages of WT and different types of mutation at *JAZ1* and *GAI* target sites among all T1 plants obtained using the pEC1-GL2 vector.



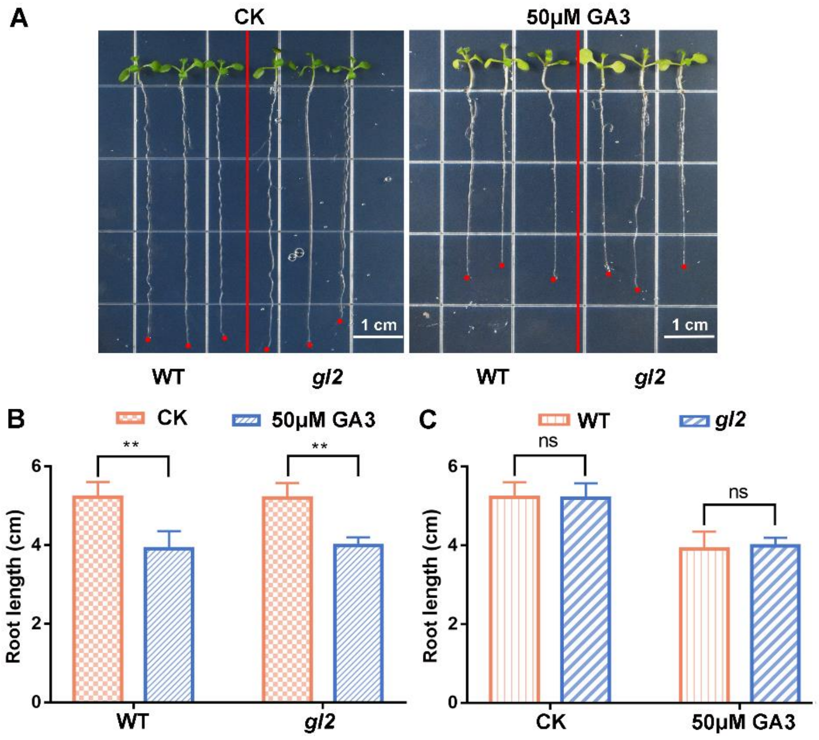
Supplemental Figure S5 Analyses of the frequencies of mutation in T1 plants obtained using the pYao vector. Percentages of WT and different types of mutation are shown for four target sites.



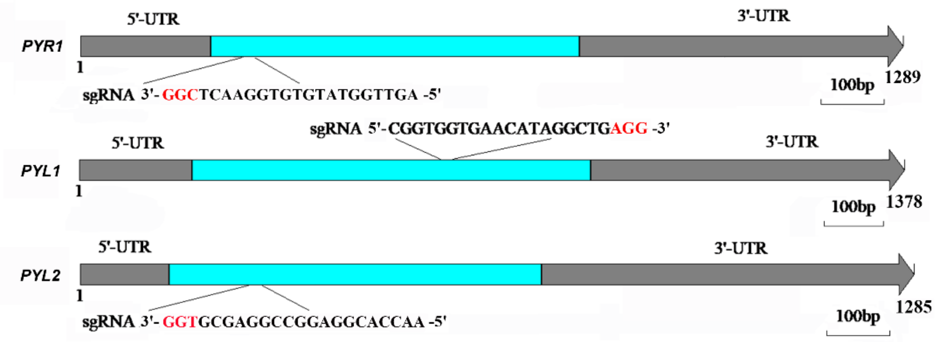
Supplemental Figure S6 Mutation of *GL2* does not affect flower development or seed color. A, Representative flowers from WT and the *gl2* mutant. B, Seeds of WT and the *gl2* mutant.



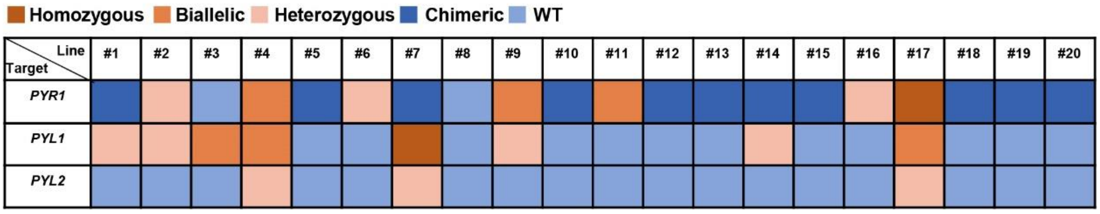
Supplemental Figure S7 Mutation of *GL2* does not affect plant response to JA. A, Representative T2 seedlings of WT and the *gl2* mutant grown on 1/2 MS plates with or without 50 μM JA. B, Analyses of primary root length to compare the JA response of WT and the *gl2* mutant. C, Analyses of primary root length to compare the growth of WT and the *gl2* mutant on 1/2 MS plates with or without 50 μM JA. Data are shown as mean ± SD (n = 15). ns: no significant difference; ****: $P<0.0001$ determined by Student's *t*-test.



Supplemental Figure S8 Mutation of *GL2* does not affect plant response to GA. A, Representative T2 seedlings of WT and the *gl2* mutant grown on 1/2 MS plates with or without 50 μM GA3. B, Analyses of primary root length to compare the GA response of WT and the *gl2* mutant. C, Analyses of primary root length to compare the growth of WT and the *gl2* mutant on 1/2 MS plates with or without 50 μM GA. Data are shown as mean ± SD (n = 15). ns: no significant difference; **: $P<0.01$ determined by Student's *t*-test.



Supplemental Figure S9 The target sites of genes for polygenic mutation used in this study. The structures of *PYR1*, *PYL1*, and *PYL2* are represented by exons (light blue bars), and untranslated regions (UTRs) (gray bars). The sgRNA target sites are indicated and the protospacer adjacent motif (PAM) sites are highlighted in red.



🟫 Homozygous 🟧 Biallelic 🟥 Heterozygous ■ Chimeric 🟦 WT

Line / Target	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
PYR1	■	🟥	🟦	🟧	■	🟥	■	🟦	🟧	■	🟧	■	■	■	■	🟥	🟫	■	■	■
PYL1	🟥	🟥	🟧	🟧	🟦	🟦	🟫	🟦	🟥	🟦	🟦	🟦	🟦	🟥	🟦	🟦	🟧	🟦	🟦	🟦
PYL2	🟦	🟦	🟦	🟥	🟦	🟦	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟥	🟦	🟦	🟦

Supplemental Figure S10 Mutation types at each target sites of multiplex edited T1 plants with normal trichomes. The types of mutation at *PYR1*, *PYL1* and *PYL2* loci in individual T1 plants with normal trichomes were analyzed.

Supplemental Table S1 Numbers of all transgenic T1 plants and glabrous T1 plants for each target gene obtained using the pEC-GL2 vector.

Target	**T1 (total)**	**T1 (glabrous)**	**glabrous /total**
JAZ1	119	27	22.69%
AP1	114	28	24.56%
GAI	124	10	8.06%
TT4	115	4	3.48%

Supplemental Table S2 Mutation frequencies of four target genes using different CRISPR-Cas9 systems.

	JAZ1	***AP1***	***GAI***	***TT4***
pYao	86.66%	51.28%	70.00%	66.67%
pEC1	35.91%	10.00%	14.64%	22.50%
pEC1-GL2	32.77%	20.18%	7.26%	7.83%
pEC1-GL2 (glabrous)	92.59%	75.00%	50.00%	100.00%

Supplemental Table S3 Primers used in this study

Primer name	Primer sequence
GL2-sgRNA-F	ATTGTCGGAGCATGAAGCCTGCA
GL2-sgRNA-R	AAACTGCAGGCTTCATGCTCCGA
AP1-sgRNA194-F	ATTGTCGAATACTCCACTGATTCT
AP1-sgRNA194-R	AAACAGAATCAGTGGAGTATTCGA
JAZ1-sgRNA1-F	ATTGCAATAGGAAGTTCTGTCAA
JAZ1-sgRNA1-R	AAACTTGACAGAACTTCCTATTG
TT4-sgRNA296-F	ATTGCAGGCGACAAGTCGACAATT
TT4-sgRNA296-R	AAACAATTGTCGACTTGTCGCCTG
GAI-sgRNA1-F	ATTGATGAGCTTCTAGCTGTTCT
GAI-sgRNA1-R	AAACAGAACAGCTAGAAGCTCAT
GL2-2Tar-F	TAGAGTCGAAGTAGTGATTGTGAGACCTGAATTCAGGTCTCAGTTTTAGAGCTAGAAATAGC
GL2-2Tar-R	GCTATTTCTAGCTCTAAAACTGCAGGCTTCATGCTCCGACAATCTCTTAGTCGACTCTA
YAOp-F	CAATACCATGGTTATACTAGTGATGGGAAATTCATTGAAAAC
YAOp-R	GGTCCTTGTAATCCATCTAGATTCTTCTCTCTCTCACTCCCTC
4DT-1F	TAGAGTCGAAGTAGTGATTGAGTTGGTATGTGTGGAACTGTTTTAGAGCTAGAAATAG
4DT-1R	CAGCCTATGTTCACCACCGCAATCTCTTAGTCGACTCTA
4DT-2F	GGTGGTGAACATAGGCTGGTTTTAGAGCTAGAAATAGC
4DT-2R	GCTATTTCTAGCTCTAAAACGGTACCCGCTCCGGCCTCCGTGGTTCAATCACTACTTCGTCTCTA
4DT-3F	ACCACGGAGGCCGGAGCGGTTTTAGAGCTAGAAATAGC
4DT-3R	GCTATTTCTAGCTCTAAAACTGCAGGCTTCATGCTCCGACAATCACTACTTCGACTCTA
GL2-IF	GTTCCAAAACCGCCGCA
GL2-IR	TAAACACAATCCCCGCATCTC
AP1-IF	TCTCCATAAATAAAGATCCCGAGAC
AP1-IR	CTGGCTAATCAAAAAATCCAAAAA
JAZ1-IF	TTGACTATATTCTACGCCGGGC
JAZ1-IR	GTAAACAAATGAAAACAAAACACACCT
TT4-IF	ATAATGGTGATGGCTGGTGCT
TT4-IR	TCAAGGTGGGTGTCAGAGGG
GAI-IF	CCCCATCACGAGCCTTTTTAT
GAI-IR	CGTCGCTGTAGTGGTTTCCAC
PYR1-IF	CATGTTTACCCTTCATCTTCCCT
PYR1-IR	ATCATCCTCCGAGTTACCTTCC
PYL1-IF	CCAACTCTCCCAATCAATCGCC
PYL1-IR	CCCCAAATTTCATCATTACCTAACC
PYL2-IF	ACAAACCCCCCAACCTCCAA
PYL2-IR	TCGACGAACTCAAGCCGCTC

Supplemental Data Set S1 Sequences for plasmids used in this paper.

pEC1-GL2 plasmid:
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