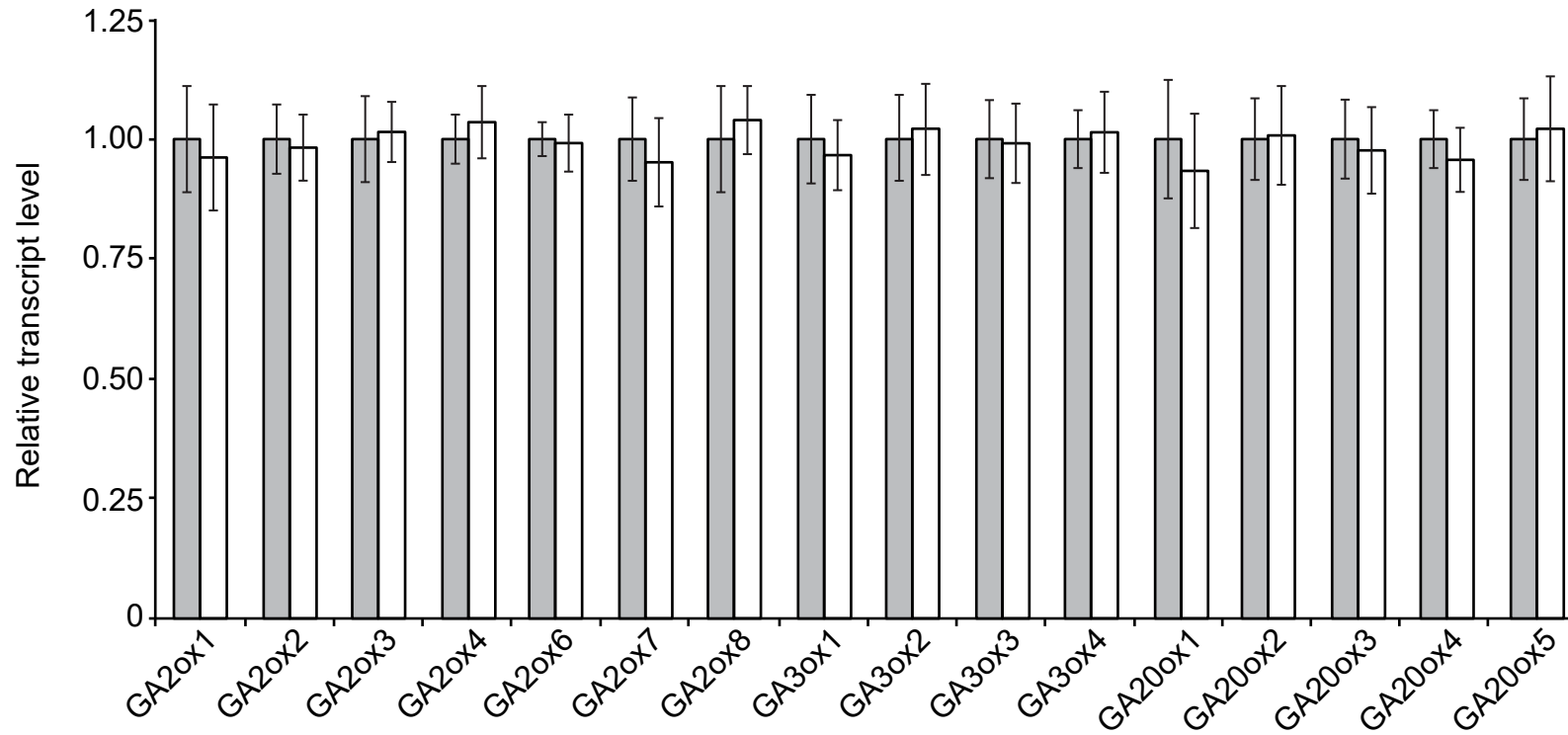
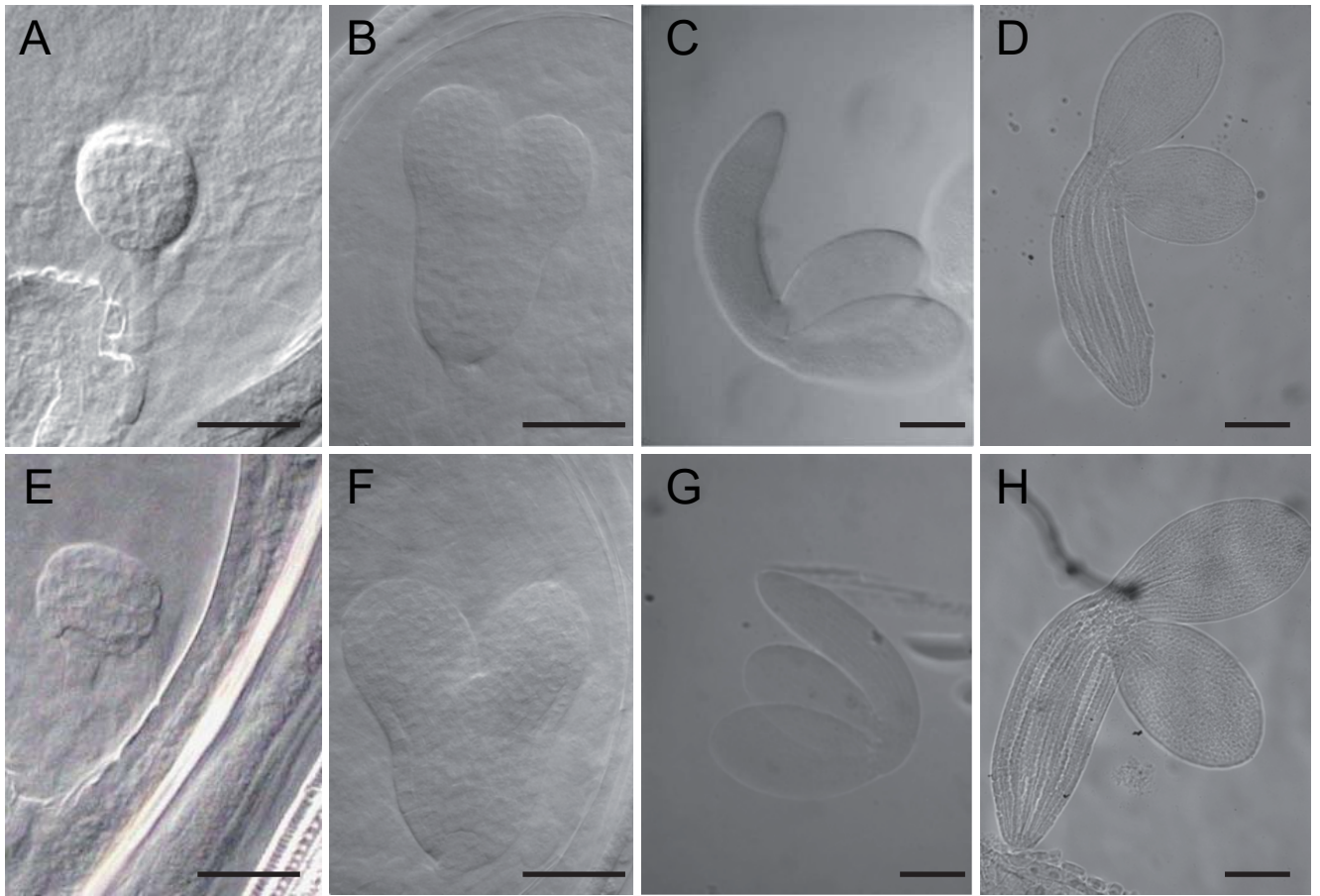


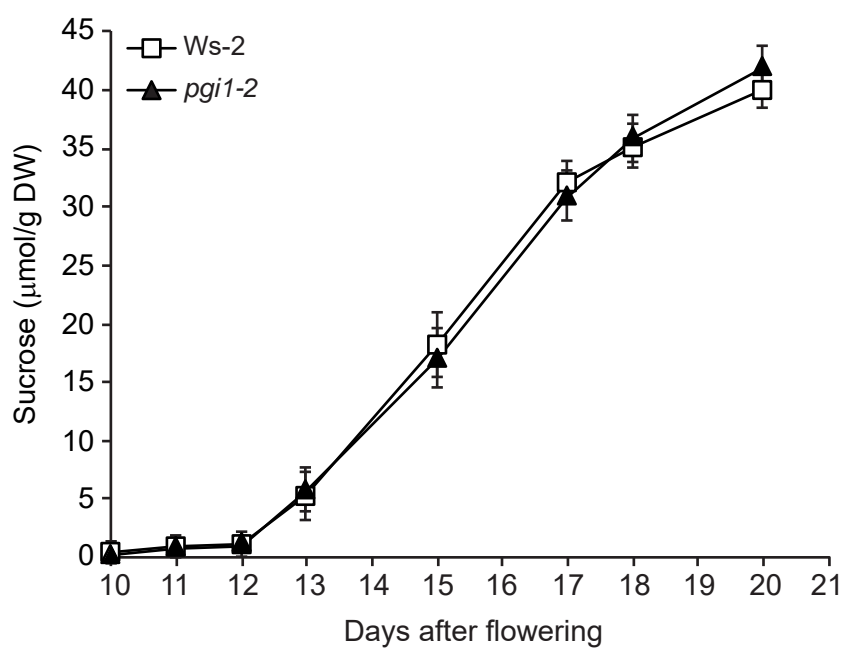
Supplemental Figure 1: Starch content in mature leaves of *Ws-2*, *pgi1-2* and *promPGI1:PGI1(1)* plants. Values represent the means \pm SE of four biological replicates obtained from four independent experiments, each biological replicate being a pool of mature leaves from four plants. Leaves were harvested after 12 h of illumination. The asterisk indicates significant differences from WT leaves according to Student's t-tests ($p < 0.05$) (**Supports Figure 1**).



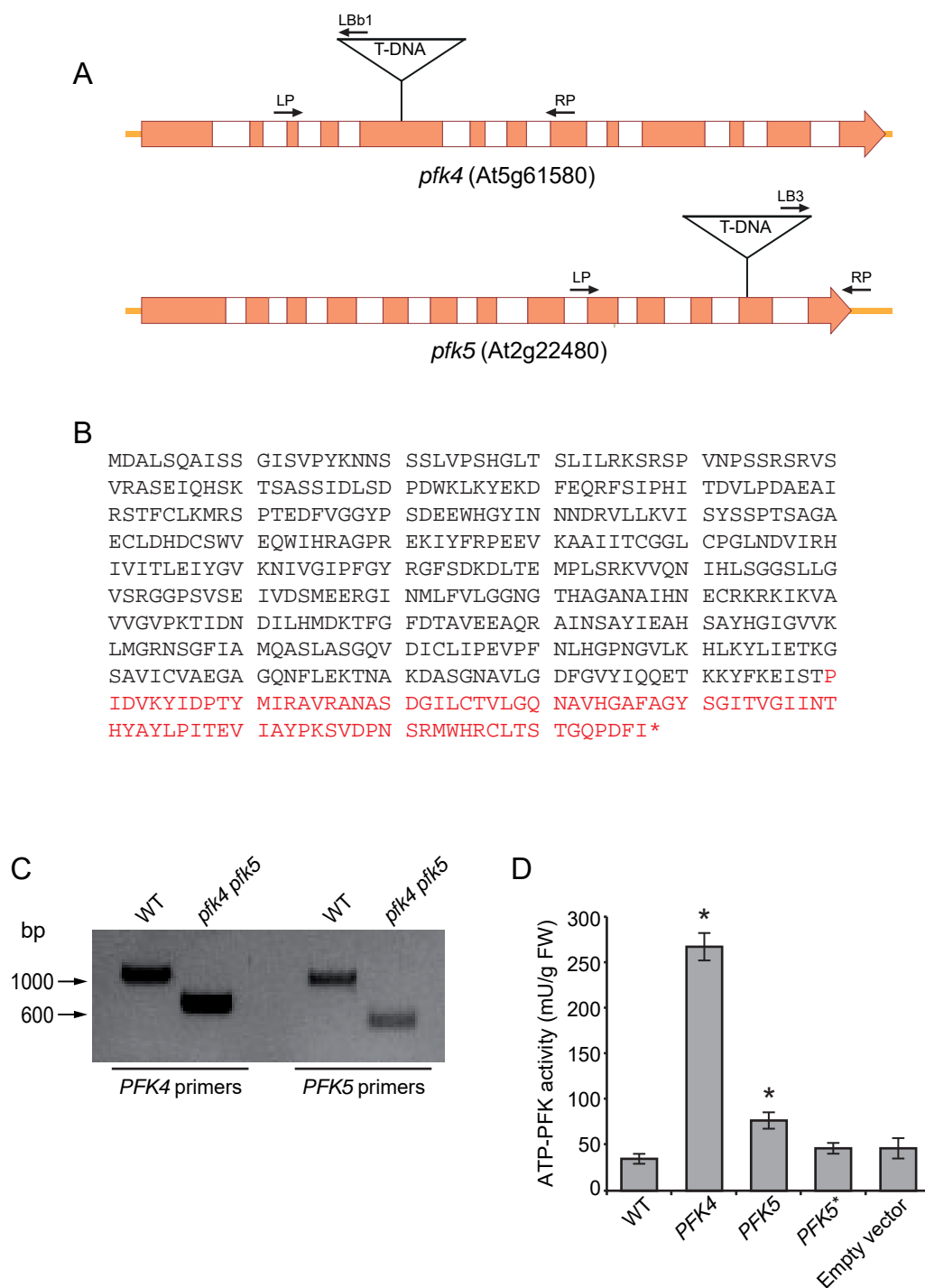
Supplemental Figure 2: Expression levels of different *GA2ox*, *GA3ox* and *GA20ox* genes in WT (*Ws-2*) and *pgi1-2* shoots (gray and white bars, respectively). mRNA levels are set to 1. Values represent the means \pm SE of three independent experiments, each consisting of four biological replicates corresponding to a pool of four shoots. Leaves were harvested after 12 h of illumination (**Supports Figure 3**).



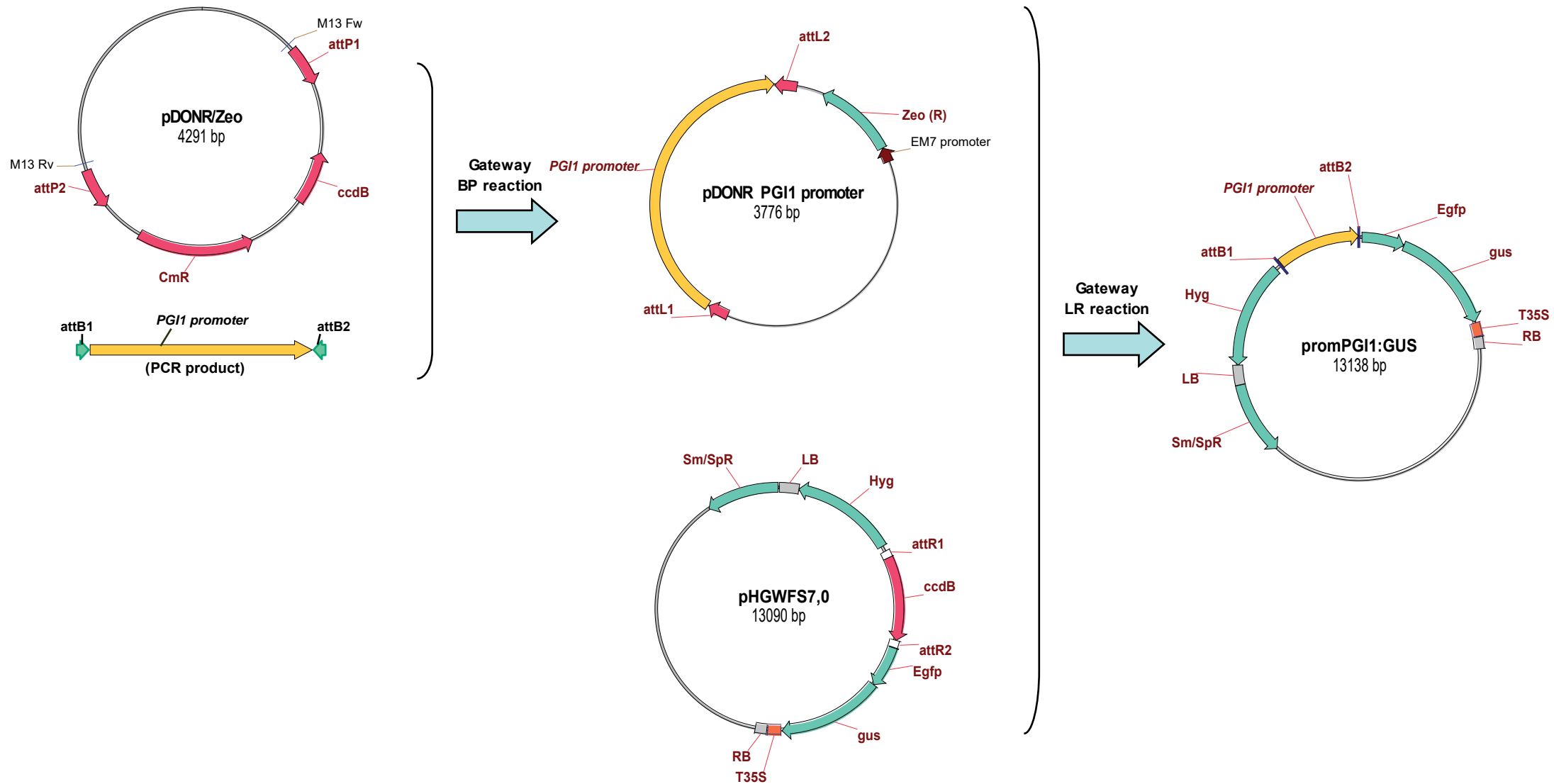
Supplemental Figure 3: Embryo development in WT (Ws-2) (A–D) and *pgi1-2* (E–H) embryos at: A and E, globular embryo stage; B and F, heart stage; C and G, mid-torpedo stage; D and H, mature embryo stage. Bars = 10 µm in A and E; 20 µm in B–D and F–H (Supports Figure 4).



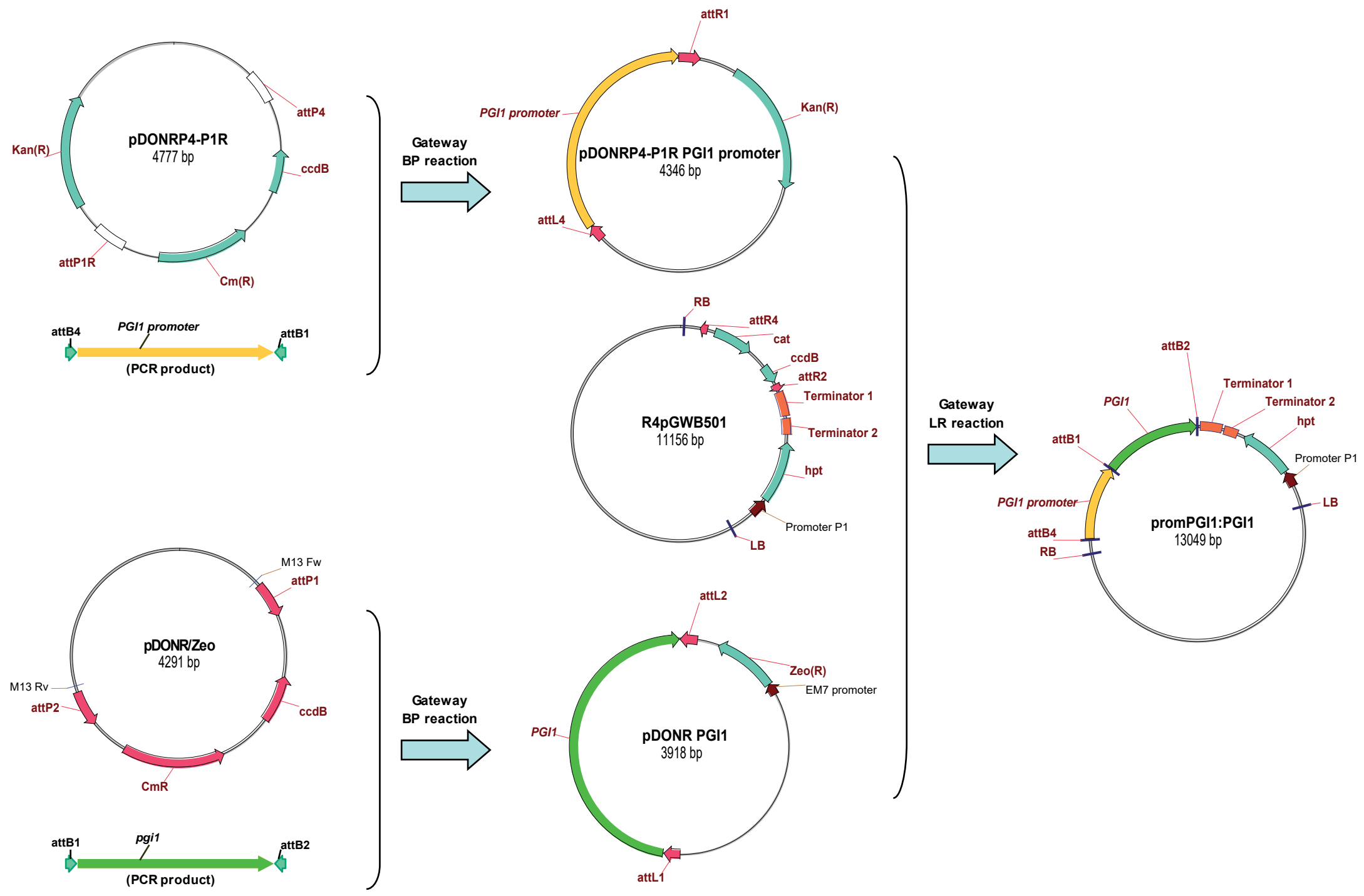
Supplemental Figure 4: Time-course of sucrose content in developing Ws-2 and *pgi1-2* seeds. Values represent the means \pm SE of three biological replicates obtained from three independent experiments, each biological replicate being a pool of 50 seeds from four plants (**Supports Figure 4**).

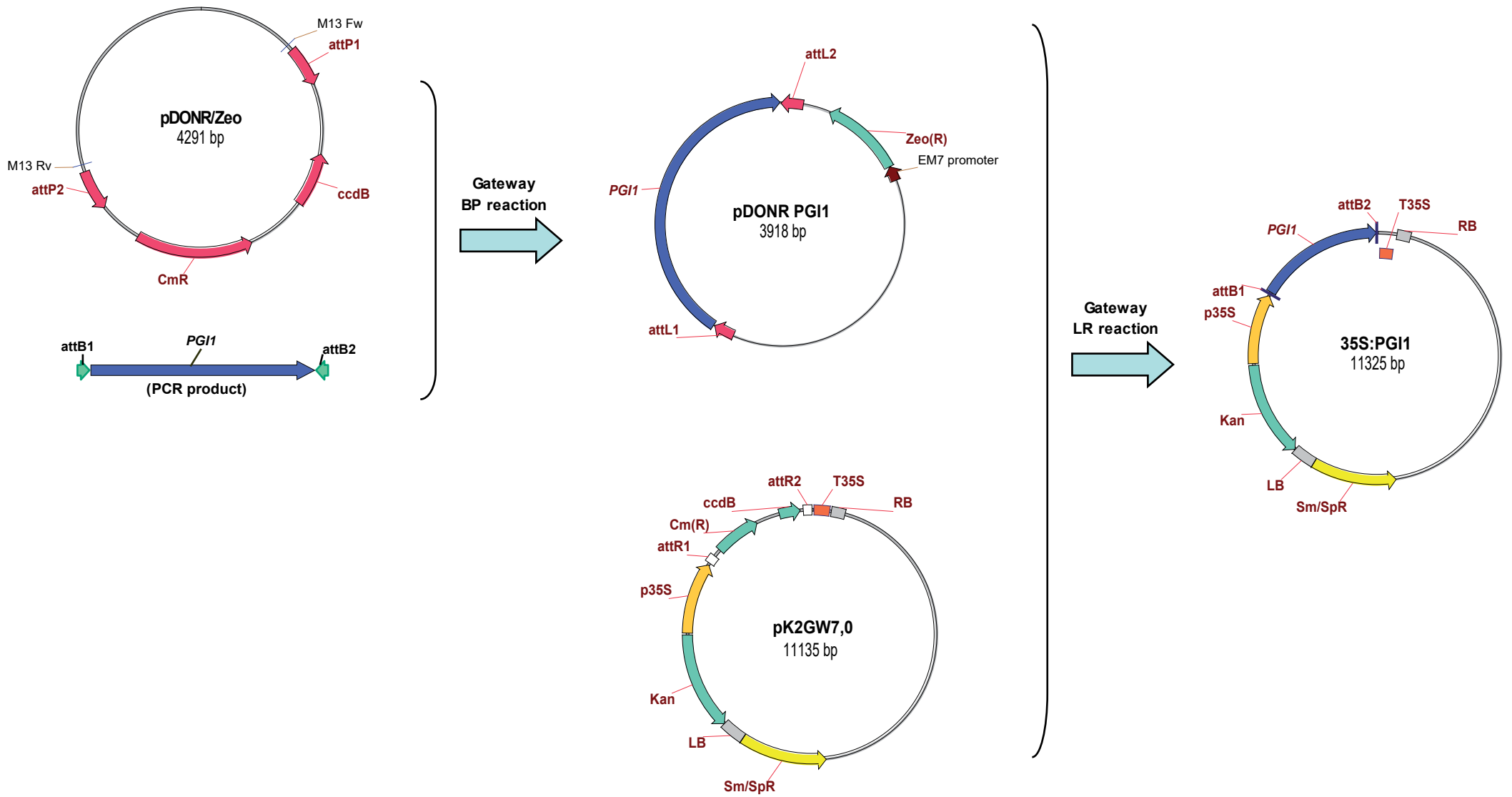


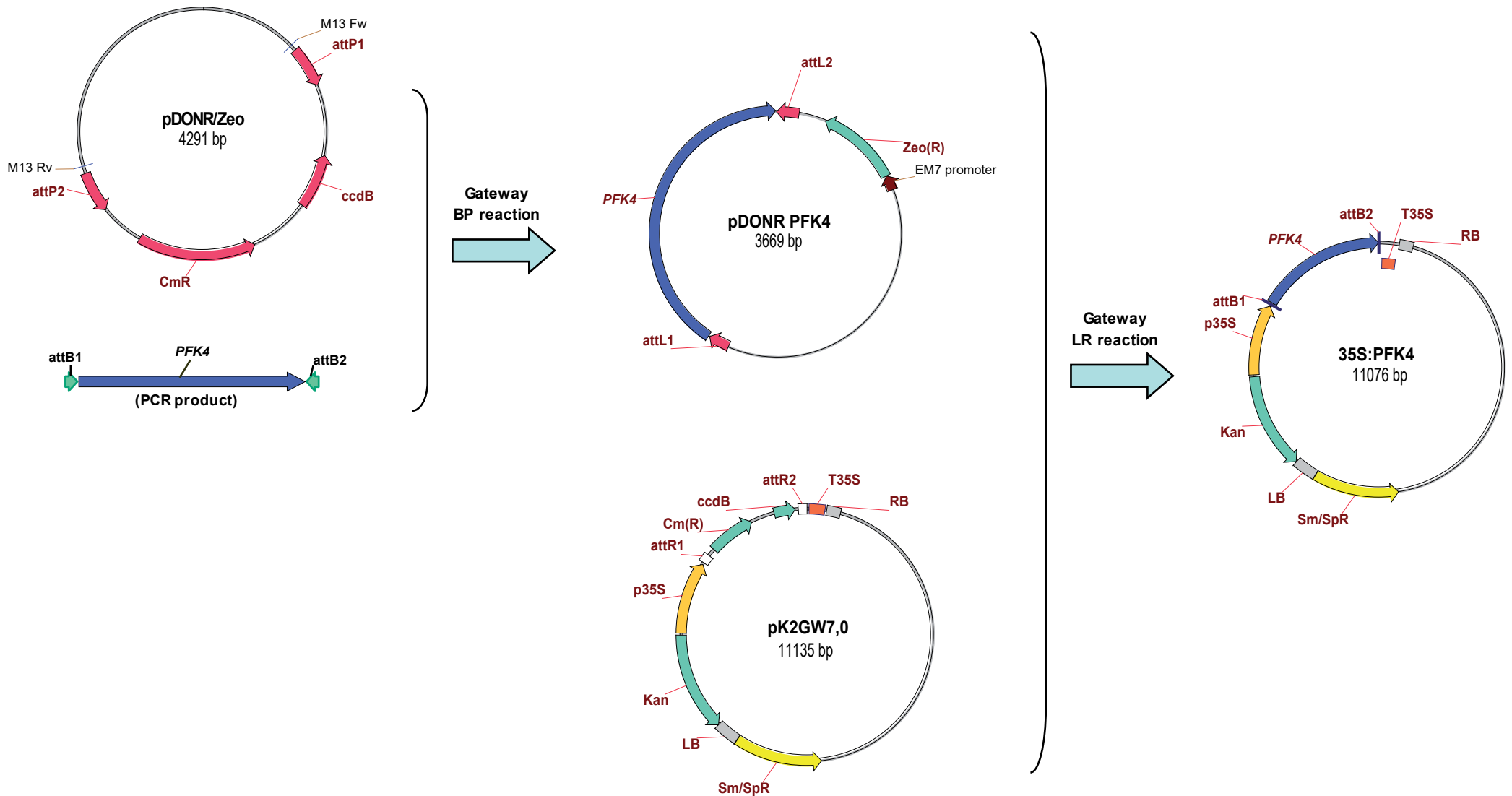
Supplemental Figure 5: Confirmation of the knock-out status of the *pfk4 pfk5* mutant. **(A)** Schematic illustration of the sites of T-DNA insertion in the *pfk4* (SALK_012602) and *pfk5* (SAIL_297_F05) alleles. **(B)** Deduced amino acid sequence of PFK5. The sequence that is not expressed in *pfk5* is highlighted in red. **(C)** PCR analyses of the *pfk4/pfk5* mutant. PFK LP and RP specific primers, and T-DNA specific primers used are listed in **Supplemental Table 1**. Annealing positions of PFK LP and RP specific primers, and T-DNA specific primers are shown in panel A. **(D)** ATP-PFK activities of tobacco leaves transiently expressing PFK4, PFK5 or PFK5*. Values represent the means \pm SE obtained from three independent experiments, each consisting of three biological replicates corresponding to a pool of 4 agro-infiltrated tobacco leaves. Asterisks indicate significant differences from WT leaves according to Student's t-tests ($p < 0.05$). Note that PFK5 has weak activity, relative to PFK4, in accordance with findings by Mustroph et al. (2007). Note also that the truncated PFK5* form totally lacks ATP-PFK activity (**Supports Figure 8**).



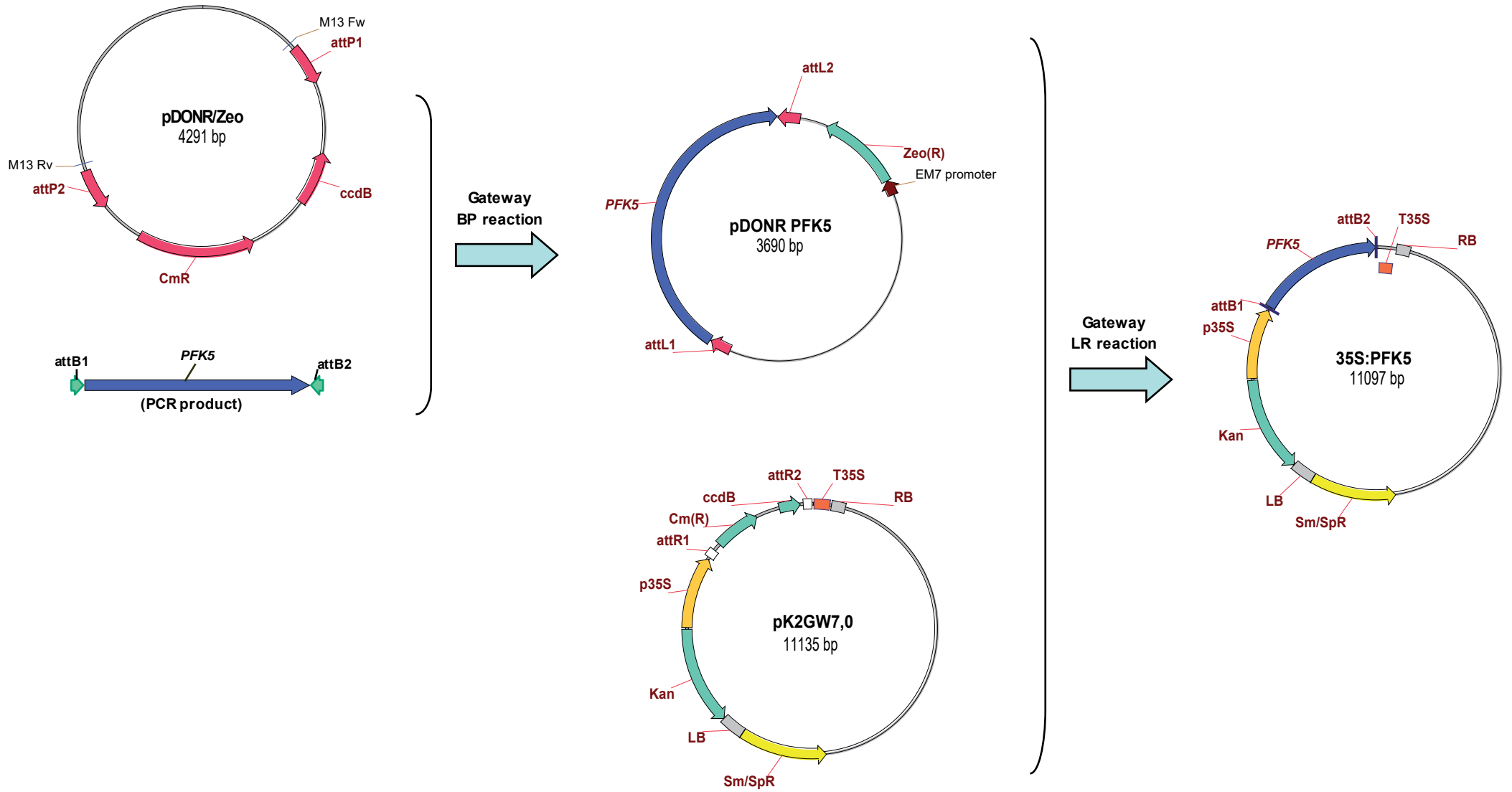
Supplemental Figure 6: Stages in construction of the *promPGI1:GUS*, *35S:PGI1* and *promPGI1:PGI1* plasmids used to produce *promPGI1:GUS*-, *35S:PGI1*- and *promPGI1:PGI1*-expressing plants. Plasmid constructs were produced using Gateway technology and confirmed by sequencing. Complete *PGI1*-encoding cDNA was obtained from the RIKEN Arabidopsis cDNA collection (Seki et al., 1998; 2002). To construct *promPGI1-GUS* a 1.7-kb fragment of the *PGI1* upstream region was amplified by PCR and subcloned in front of the *GUS* reporter gene. Primers used for PCR amplification of *PGI1* cDNA, *GUS* and the *PGI1* promoter are listed in **Supplemental Table 2 (Supports Figure 1)**.

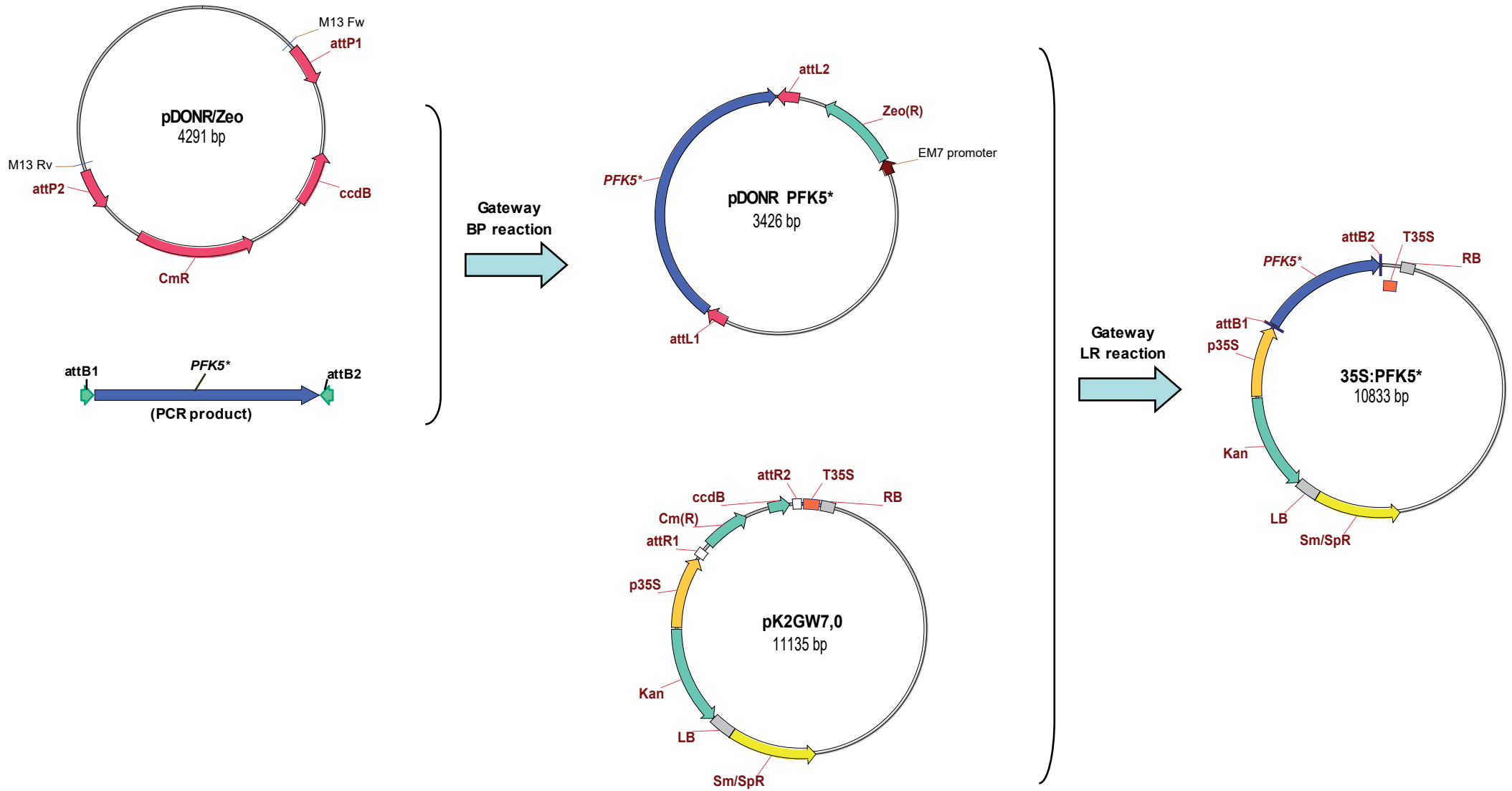






Supplemental Figure 7: Stages in construction of the 35S:PFK4, 35S:PFK5 and 35S:PFK5* plasmids used for transient expression in tobacco leaves. Plasmid constructs were produced using Gateway technology and confirmed by sequencing. Complete PFK4- and PFK5-encoding cDNAs were obtained from the RIKEN Arabidopsis cDNA collection (Seki et al., 1998; 2002). Primers used for PCR amplification of *PFK4*, *PFK5* and *PFK5** are listed in **Supplemental Table 2 (Supports Figure 8)**.





Supplemental Table 1. Primers used for PCR screening of the *pfk4 pfk5* mutant.

Mutant	Primer	Sequence
<i>pfk4</i> (SALK_012602)	RP (<i>pfk4</i>)	5' AGGCGAACTTTTGTTCAGTTCC 3'
	LP (<i>pfk4</i>)	5' ACCACTGTATCTGCCCATGAG 3'
	LBb1 (T-DNA)	5' GCGTGGACCGCTTGCTGCAACT 3'
<i>pfk5</i> (SAIL_297_F05)	RP (<i>pfk5</i>)	5' TTTTAGATGAAATCGGGTTGG 3'
	LP (<i>pfk5</i>)	5' TTTGGGCCTTCTGTATTAGGC 3'
	LB3 (T-DNA)	5' TAGCATCTGAATTTTCATAACCAATCTCGATACAC 3'

Supplemental Table 2. Primers used to produce the promPGI1:GUS, promPGI1:PGI1, 35S:PGI1, 35S:PFK4, 35S:PFK5 and 35S:PFK5* plasmids. Primer sequences for attB sites (see **Supplemental Figures 6 and 7**) are indicated in bold.

Primer	Sequence
promPGI1:GUS	
attB1 <i>PGI1</i> promoter	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT ACTTTATATGATCCGATTCAATCTAAAC 3'
attB2 <i>PGI1</i> promoter	5' GGGGACCACTTTGTACAAGAAAGCTGGGT AAAAATCTTGGCTCATTGAGAAGG 3'
promPGI1:PGI1	
attB4 <i>PGI1</i> promoter	5' GGGGACA ACTTTGTATAGAAAAGTTGCTCTTTATATGATCCGATTCAATCTAAAC 3'
attB1R <i>PGI1</i> promoter	5' GGGGACTGCTTTTTTGTACAA ACTTGCAAATCTTGGCTCATTGAGAAGG 3'
attB1 <i>PGI1</i>	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT AATGGCCTCTCTCTCAGGC 3'
attB2 <i>PGI1</i>	5' GGGGACCACTTTGTACAAGAAAGCTGGGT ATTATGCGTACAGGTCATCCAC 3'
35S:PGI1	
attB1 <i>PGI1</i>	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT AATGGCCTCTCTCTCAGGC 3'
attB2 <i>PGI1</i>	5' GGGGACCACTTTGTACAAGAAAGCTGGGT ATTATGCGTACAGGTCATCCAC 3'
35S:PFK4	
attB1 <i>PFK4</i>	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT AATGGAAGCTTCGATTTTCGTTTC 3'
attB2 <i>PFK4</i>	5' GGGGACCACTTTGTACAAGAAAGCTGGGT ATTAGATAGAAGAGATCTTCATGTT 3'
35S:PFK5	
attB1 <i>PFK5</i>	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT AATGGATGCTCTTTCTCAGGCG 3'
attB2 <i>PFK5</i>	5' GGGGACCACTTTGTACAAGAAAGCTGGGT ATTAGATGAAATCGGGTTGGCC 3'
35S:PFK5*	
attB1 <i>PFK5</i>	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTT AATGGATGCTCTTTCTCAGGCG 3'
attB2 <i>PFK5*</i>	5' GGGGACCACTTTGTACAAGAAAGCTGGGT ATTAAGTACTTATTTCTTTGAAATACTTC 3'

Supplemental Table 3. Primers used in qRT-PCR analyses

Gene	Direction	Sequence
EF-1 α RNA At1g07940	Forward	5' TTCTTGACAACACCGACAGC 3'
	Reverse	5' AAGCCCATGGTTGTTGAGAC 3'
<i>PGI1</i> At4g24620	Forward	5' GCTGCGTTTAAGGCTATGGA 3'
	Reverse	5' GGCTTAGGTGCGAGCTTAGA 3'
GA2ox1 At1g78440	Forward	5' CCAAGTCTTCTCAAAAGCCCG 3'
	Reverse	5' GTA CTCTTCCAATGCGTTTCTGAA 3'
GA2ox2 At1g30040	Forward	5' GGTTCGGTTTCTCACTTCCC 3'
	Reverse	5' GGATCGGCTAGGTTGACGAC 3'
GA2ox3 At2g34555	Forward	5' AGCCAGCCAGTTTGTAGTAGCA 3'
	Reverse	5' GCGGTTTGCATTTTGGATTAAC 3'
GA2ox4 At1g47990	Forward	5' CTTTGCTAAACCGGCTCACG 3'
	Reverse	5' GGCTGGTTAACTGGTCGGAC 3'
GA2ox6 At1g02400	Forward	5' GATCCTTTCAAGTTCAGCTCGG 3'
	Reverse	5' TCTAACCGTGCGTATGTAATCATTC 3'
GA2ox7 At1g50960	Forward	5' ATGGACAATGGATCAGCGTAAA 3'
	Reverse	5' TGTTGACTGTAAGGGCTTCCAA 3'
GA2ox8 At4g21200	Forward	5' CATGGAGCAATGGCATGTACA 3'
	Reverse	5' GGTTCGTATCACACGGTGTT 3'
GA3ox1 At1g15550	Forward	5' CCATTCACCTCCCACACTCT 3'
	Reverse	5' AGCGGAGAAGAGGAGATCGT 3'
GA3ox2 At1g80340	Forward	5' CTGCCGCTCATCGACCTC 3'
	Reverse	5' AGCATGGCCCACAAGAGTG 3'
GA3ox3 At4g21690	Forward	5' CGCTACACTCTTATGGCCCG 3'
	Reverse	5' TCCATCACATTGCAGAACTCG 3'
GA3ox4 At1g80330	Forward	5' GATCACACCAAGTACTGCGGTATAA 3'
	Reverse	5' TTCCATTTCGTCCACGTATTCTT 3'
GA20ox1	Forward	5' CTTCCATCAACGTTCTCGAGC 3'

At4g25420	Reverse	5' GGTTTTGAAGGTCGATGAGAGG 3'
<i>GA20ox2</i>	Forward	5' AGAAACCTTCCATTGACATTCCA 3'
At5g51810	Reverse	5' AGAGATCGATGAACGGGACG 3'
<i>GA20ox3</i>	Forward	5' ACTCGTCTCAAAGGCTGCAAC 3'
At5g07200	Reverse	5' GAGGCTCTCATCGACACCATG 3'
<i>GA20ox4</i>	Forward	5' CTATCCAAAATGCAAGCAACCA 3'
At1g60980	Reverse	5' CAGTGAGGCCCGTACCTAGT 3'
<i>GA20ox5</i>	Forward	5' GCCACCCCATGTTGTTGAAG 3'
At1g44090	Reverse	5' CGATGGTTGCCTAGCCTTGA 3'

Supplemental Table 4. ANOVA Tables

Figure 3

df = degrees of freedom; Mean Sq = Mean Squares; Sum Sq = Sum of Squares

GA1

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	5.33571	5.33571	50.51	0.0004***
Residual	6	0.6338	0.10563		
Total	7	5.9695			

GA4

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.23823	0.23823	9.09	0.0236*
Residual	6	0.15729	0.02621		
Total	7	0.39552			

GA5

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0	0	5.41e-05	0.9944
Residual	6	0.03864	0.00644		
Total	7	0.03864			

GA6

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.00122	0.00122	0.08	0.7932
Residual	6	0.09726	0.01621		
Total	7	0.09847			

GA7

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.00002	0.00002	0.01	0.907
Residual	6	0.00833	0.00139		
Total	7	0.00835			

GA8

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	8.4912	8.49122	18.49	0.0051**
Residual	6	2.7557	0.45919		
Total	7	11.2464			

GA9

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.09103	0.09103	0.09	0.7721
Residual	6	5.94785	0.99131		
Total	7	6.03888			

GA15

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.06504	0.06504	14.01	0.0096**
Residual	6	0.02785	0.00464		
Total	7	0.09289			

GA19

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.01987	0.01987	0.05	0.8354
Residual	6	2.5335	0.42225		
Total	7	2.55338			

GA20

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	1.82745	1.82745	6.54	0.0431*
Residual	6	1.67781	0.27964		
Total	7	3.50527			

GA24

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	1.30947	1.30947	1.1	0.3344
Residual	6	7.13448	1.18908		
Total	7	8.44395			

GA29

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	50.9593	50.9593	68.12	0.0002***
Residual	6	4.4886	0.7481		
Total	7	55.4479			

GA34

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.00161	0.00161	0.69	0.4371
Residual	6	0.01393	0.00232		
Total	7	0.01553			

GA44

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.20416	0.20416	0.56	0.481
Residual	6	2.17092	0.36182		
Total	7	2.37508			

GA51

	df	Sum Sq	Mean Sq	F-value	P-value
Treatment	1	0.90201	0.90201	3.55	0.1086
Residual	6	1.52578	0.2543		
Total	7	2.42779			

SUPPLEMENTAL REFERENCES

Mustroph, A., Sonnewald, U., and Biemelt, S. (2007). Characterization of the ATP-dependent phosphofructokinase gene family from *Arabidopsis thaliana*. FEBS Lett. 581: 2401-2410.

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