

Supplemental Data. Ruckle et al. (2007) Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in Arabidopsis

EMS mutagenesis of an Arabidopsis line that harbors an *Lhcb:luc+* reporter gene



Screen for M₂ seedlings that exhibit derepression of *Lhcb:luc+* when grown on media that contains Norflurazon (i.e., a *gun* phenotype)



Propagate putative mutants



Identify M₃ progeny that exhibit derepression of both the reporter gene and the endogenous *Lhcb* genes when grown on media that contains Norflurazon



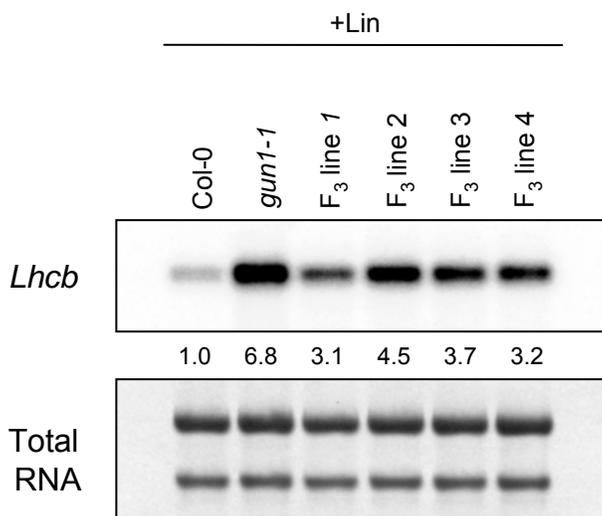
Screen M₃ seedlings that exhibit a *gun* phenotype with both the reporter gene and the endogenous *Lhcb* genes for seedlings that exhibit derepression of the endogenous *Lhcb* genes when grown on media that contains lincomycin

Supplemental Figure 1. The *gun* mutant screen procedure. Ethyl methanesulfonate (EMS); other abbreviations are defined in the text.

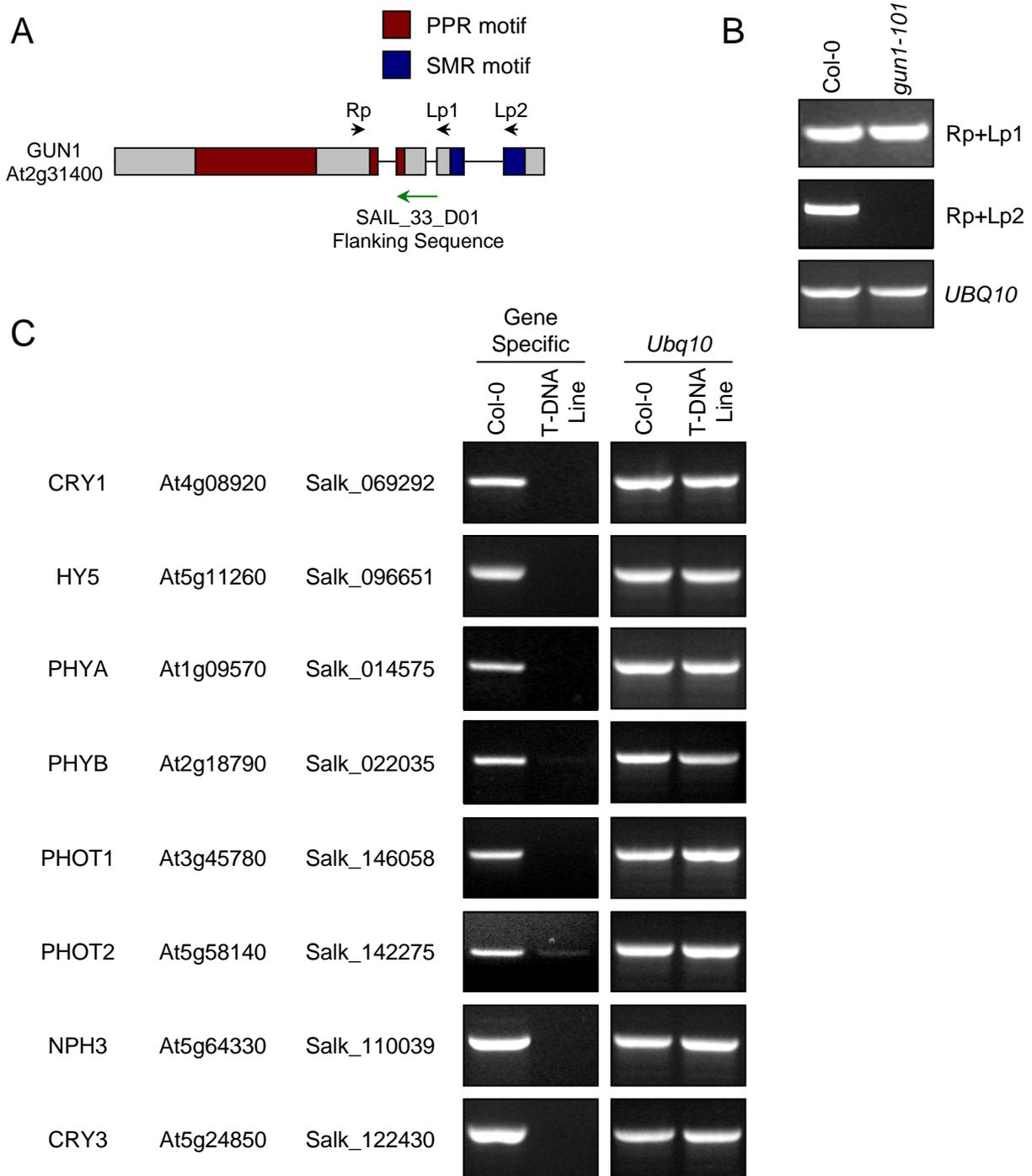
Supplemental Table 1. Segregation of the long hypocotyl phenotype in 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light in F_3 seedlings

1	2	3	4
1	58	58	100%
2	49	51	96%
3	56	56	100%
4	52	53	98%

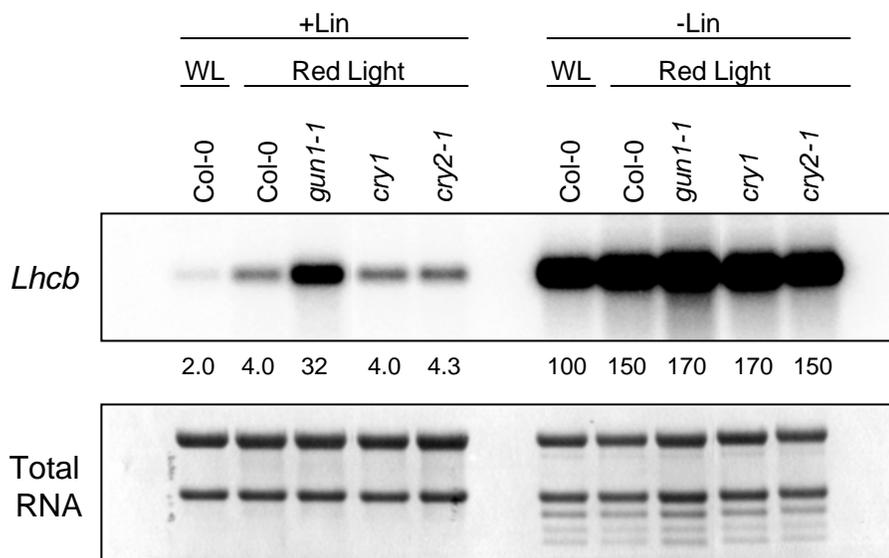
1. F_3 mutant line, 2. Number of seedlings with long hypocotyls, 3. Total number of seedlings, 4. Percentage of seedlings with long hypocotyls. Less than 100% long hypocotyls in some lines may be caused by unlinked mutations.



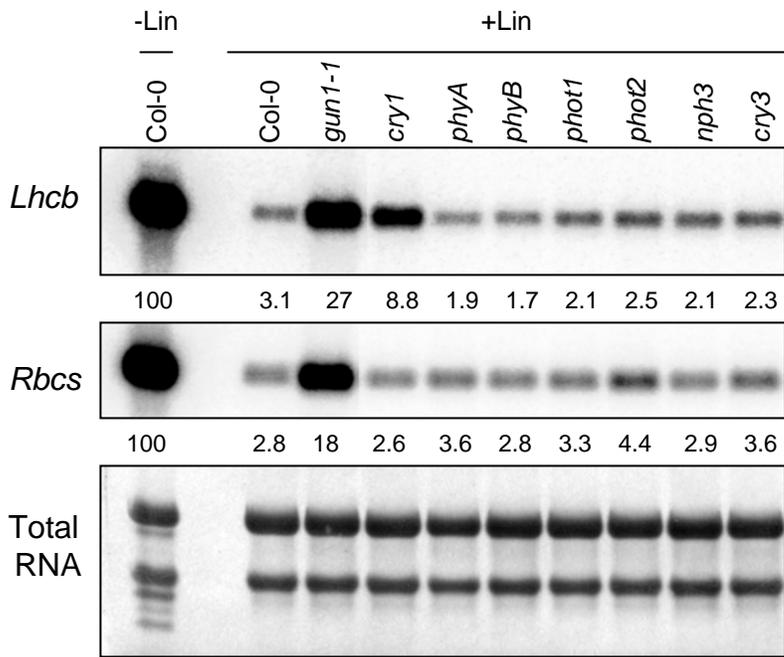
Supplemental Figure 2. Cosegregation analysis of long hypocotyl in blue light and *gun* phenotypes. *cry1-403* was backcrossed and four F_2 seedlings that displayed long hypocotyls in blue light were propagated for segregation analysis. F_3 seedlings were grown in 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ blue light on media that contained lincomycin (+Lin). *Lhcb* expression was analyzed in four F_3 lines in which the long hypocotyl phenotype did not segregate. Quantitation of *Lhcb* mRNA levels was as described in figure 1B except that numbers below each lane indicate fold increase relative to wild type.



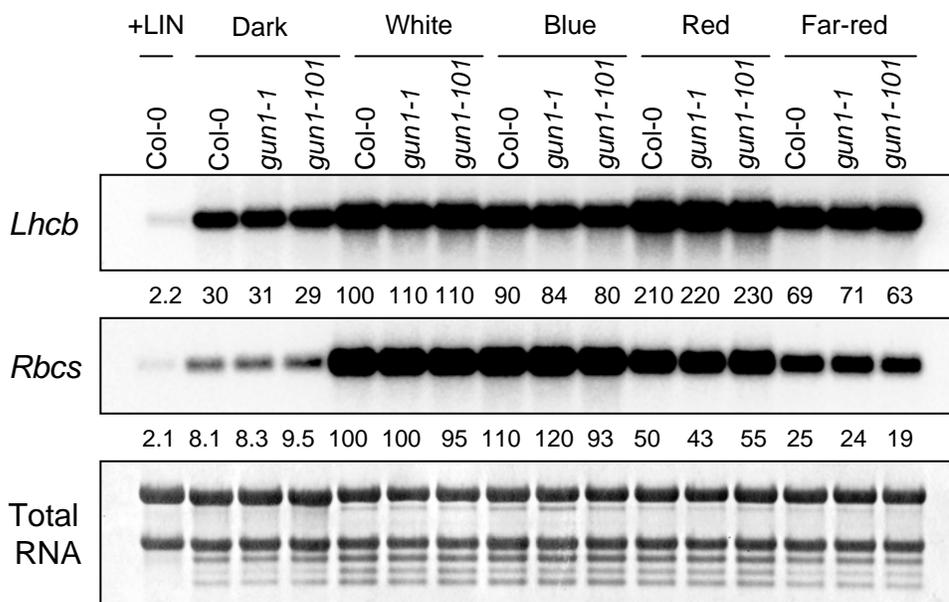
Supplemental Figure 3. Analysis of T-DNA alleles. (A) Map of the *GUN1* gene. The T-DNA flanking sequence is represented as a green arrow. RT-PCR primer locations are represented by black arrows. (B) Truncation of *GUN1* mRNA in *gun1-101*. RT-PCR analysis was performed with gene-specific primers that either target the gene product upstream of the predicted T-DNA insertion site (Rp+Lp1) or span the insertion site (Rp+Lp2). The *UBQ10* transcript was used as a control. (C) The effects of T-DNA insertions on the expression of *CRY1*, *HY5*, *PHYA*, *PHYB*, *PHOT1*, *PHOT2*, *NPH3* and *CRY3*. RT-PCR analysis was performed using primers that span the T-DNA insertion site in their respective genes. The *UBQ10* transcript was used as a control. PPR, pentatricopeptide repeat; SMR, small mutS-related domain



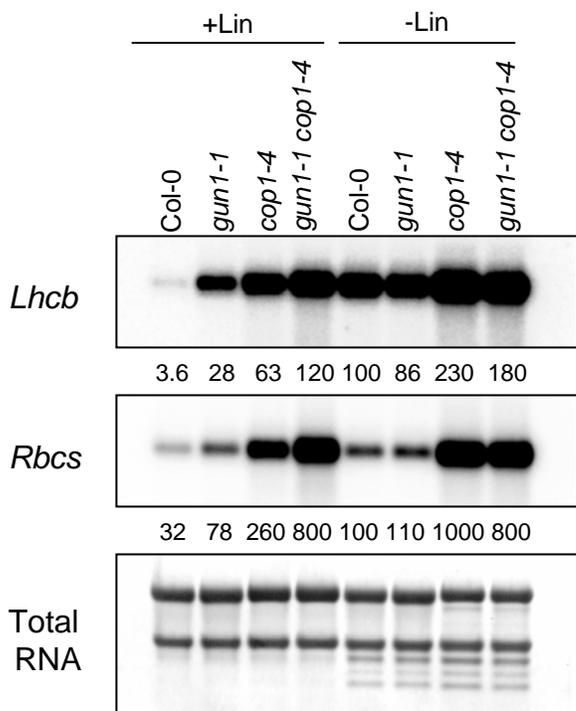
Supplemental Figure 4. Expression of *Lhcb* in *cry1* after chloroplast biogenesis was blocked in red light. Seedlings were grown in $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ red light in the presence of lincomycin (+ Lin) and the absence of Lincomycin (-Lin). RNA was analyzed as described in Figure 1B.



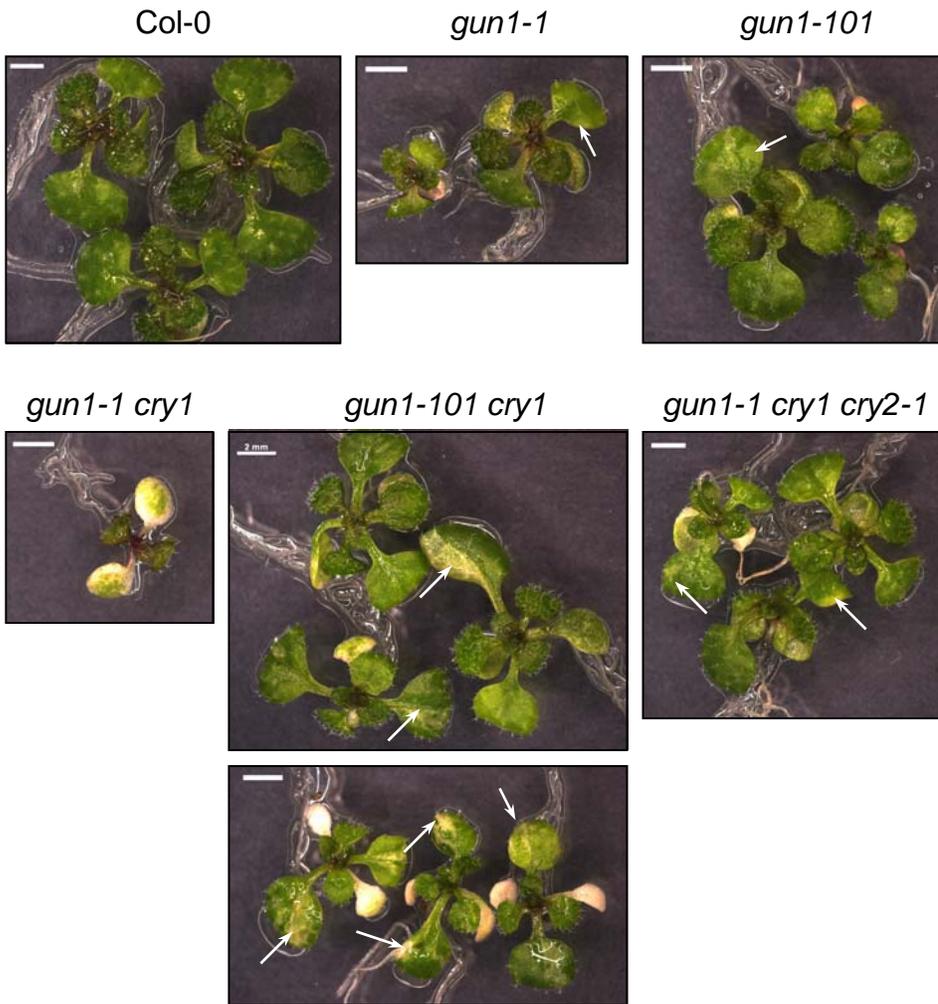
Supplemental Figure 5. *Lhcb* and *Rbcs* expression in *gun1-1* and various photoreceptor mutants after chloroplast biogenesis was blocked in blue light. Seedlings were grown on media that contained lincomycin (+Lin) or lacked lincomycin (-Lin) under $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue light. RNA was analyzed as described in Figure 1B.



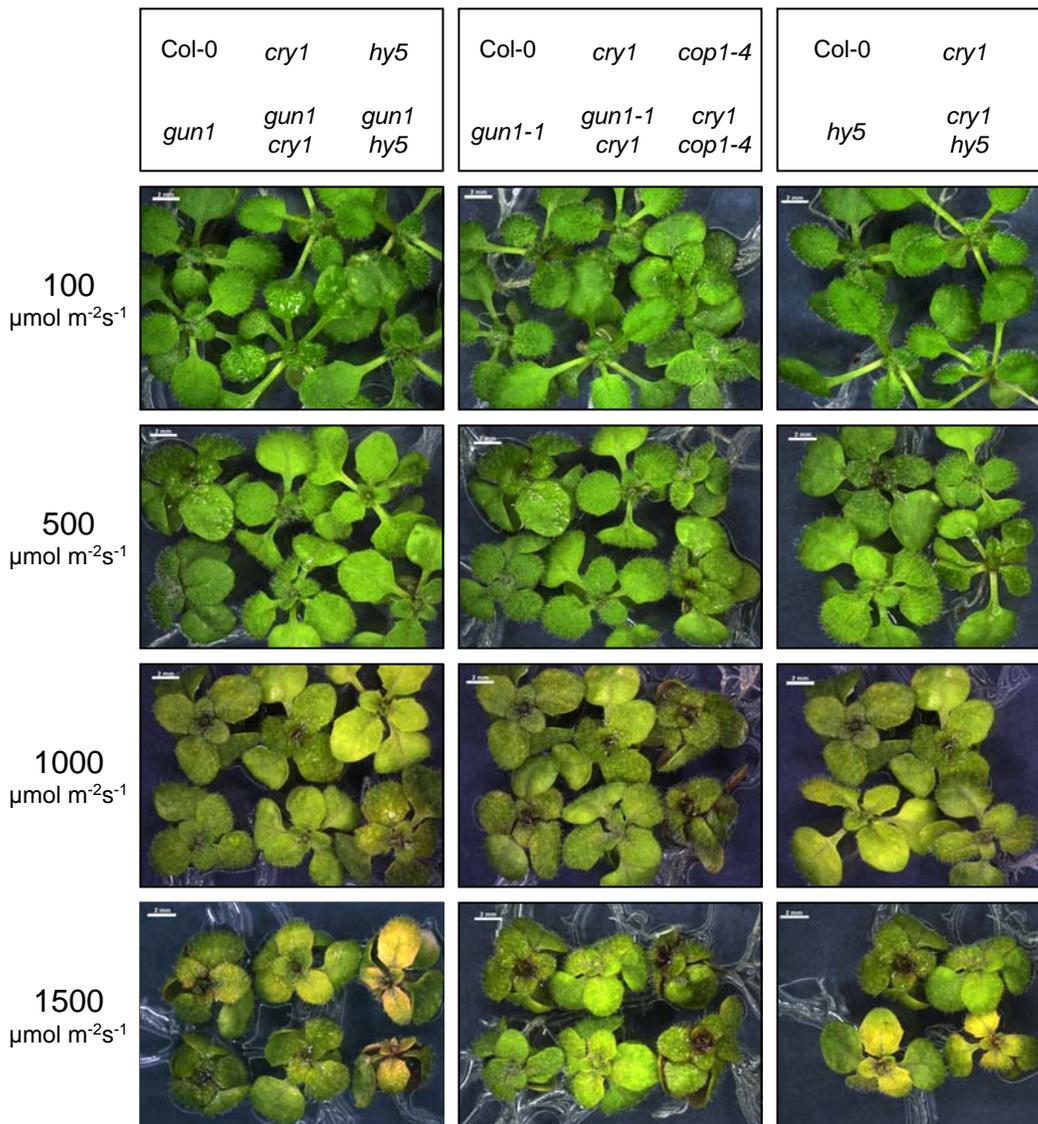
Supplemental Figure 6. *Lhcb* and *Rbcs* mRNA levels in wild type and *gun1* mutants grown in darkness and various light qualities without inhibitors of chloroplast biogenesis. Seedlings were grown in the indicated light conditions on media that did not contain an inhibitor of chloroplast biogenesis. Fluence rates for each light quality were the same as in Figure 5 in the text. RNA was analyzed as described in Figure 5 in the text. Numbers indicate percent of wild type grown in white light.



Supplemental Figure 7. Analysis of *det/copl/fus* phenotypes in *gun1-1* and *cop1-4*. Seedlings were grown in the dark on media that contained lincomycin (+Lin) or lacked lincomycin (-Lin). RNA was analyzed as described in Figure 1B.



Supplemental Figure 8. Chlorophyll-deficient leaves in *gun1* and *gun1cry1* double mutants. Seedlings were grown in $125 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light. Two-week-old seedlings with both green and partially chlorophyll-deficient true leaves are shown. Arrows indicate some of these chlorophyll deficiencies. Bars = 2 mm.



Supplemental Figure 9. Phenotypes of *gun1* and light signaling mutants following HL incubations. Green plants were transferred to HL as described in the text. *gun1-101* is indicated as *gun1* for clarity. Bars = 2 mm.

Supplemental Table 2. SSLP markers used for rough mapping *cry1-401*

1	2	3	4
nda22	GAAGTTATCTTGAGGCAGTTGGTCAC	GAAAGTGTTGCTTGATAGAGCATCTG	174/114
smd1	TCGGCAGAAAATTCCAGCAACTC	TTGTGTGGCTCACCCATCTAAACA	238/223

1. Marker name, 2. Primer sequence, 3. Primer sequence, 4. PCR product length (Col/Ler)

Supplemental Table 3. Primers used for T-DNA genotyping and RT-PCR

1	2	3	4
<i>CRY1</i>	Salk_069292	CGACAGACTGGATACATCATCC/ TCATGCCACTTGGTTAGACC	CCAAGTCACTGCTATGATTCC/ CAAATGTACTCGGTTTACAGTCC
<i>CRY3</i>	Salk_122430	AGAGATACACCCAAAGGCAAGC/ CGTGTAGTGAGGAGGTAGACC	CTGATGTCTACACTCAGTTTTCG/ GATAAAACGAGGAGAGATACACC
<i>GUN1</i>	SAIL_33_D01	TGCTACTAGAGGAGCTCAGGC/ CCCTCAAAGAACTTCAACCG	ACTGAGAGTAACAACCGAACG/ CCCTCAAAGAACTTCAACCG
<i>HY5</i>	Salk_096651	AGAGAATATGCGAGTGAATGACC/ GCAAGCTCTTACCATCAAGC	TGAAAGCGATGAGGAGATACG/ GATCAAAGGCTTGCATCAGC
<i>NPH3</i>	Salk_110039	GAGCTTGCATCCAAATTCTGC/ TCTTTCTGTGGAGGGATTATGC	GAGCTTGCATCCAAATTCTGC/ TCTTTCTGTGGAGGGATTATGC
<i>PHOT1</i>	Salk_146058	GCATCAGGAAGTTCTCGAACC/ TCATCACTGATCCTAGGCTTCC	GCATCAGGAAGTTCTCGAACC/ TCATCACTGATCCTAGGCTTCC
<i>PHOT2</i>	Salk_142275	TGCTACTTCAACCTGCATCC/ CGAACCTACAGTTGTTGTGTCTGC	TGGTGGAACTGAGAATGATCC/ CCAACCACTTTGCACATATCC
<i>PHYA</i>	Salk_014575	CTTTGACCTTACCTTGTGTGGC/ TGTTCCAACCATTAACCAAGTCC	CTGAGCTGACTGGTCTTTTCG/ AGACGACAACATGACTTCTGC
<i>PHYB</i>	Salk_022035	CAACATACAAGGAGATTACAAGGC/ /CGAATCCATCAGCCATTTGC	GACCAGATAAGGATTCAACAGC/ CATCATCAGCATCATGTCACC

1. Gene, 2. Insertion, 3. Primers for T-DNA genotyping (RP/LP), 4. Primers used for semi-quantitative PCR (RP/LP)

Supplemental Table 4. CAPS and dCAPS markers for genotyping *gun1-1* and *cop1-4*

1	2	3	4	5
At2g31400	<i>gun1-1</i>	AGTGCGATAAAGCTGTTGGC/ CCTTCTCCCATTAAGCGC	HpyCH4III	(212) 169 + 43/ 142 + 43 + 27
At2g32950	<i>cop1-4</i>	GATGCGCTGAGTGGGCCA/ TGCCATTGTCTTTTACCATTTCAGC	MwoI	(139) 120/129

1. AGI locus identifier, 2. Allele, 3. Oligonucleotide sequences (RP/LP), 4. Restriction endonucleases, 5. PCR product lengths for (undigested) and digested PCR products derived from wild type/mutant in base pairs.