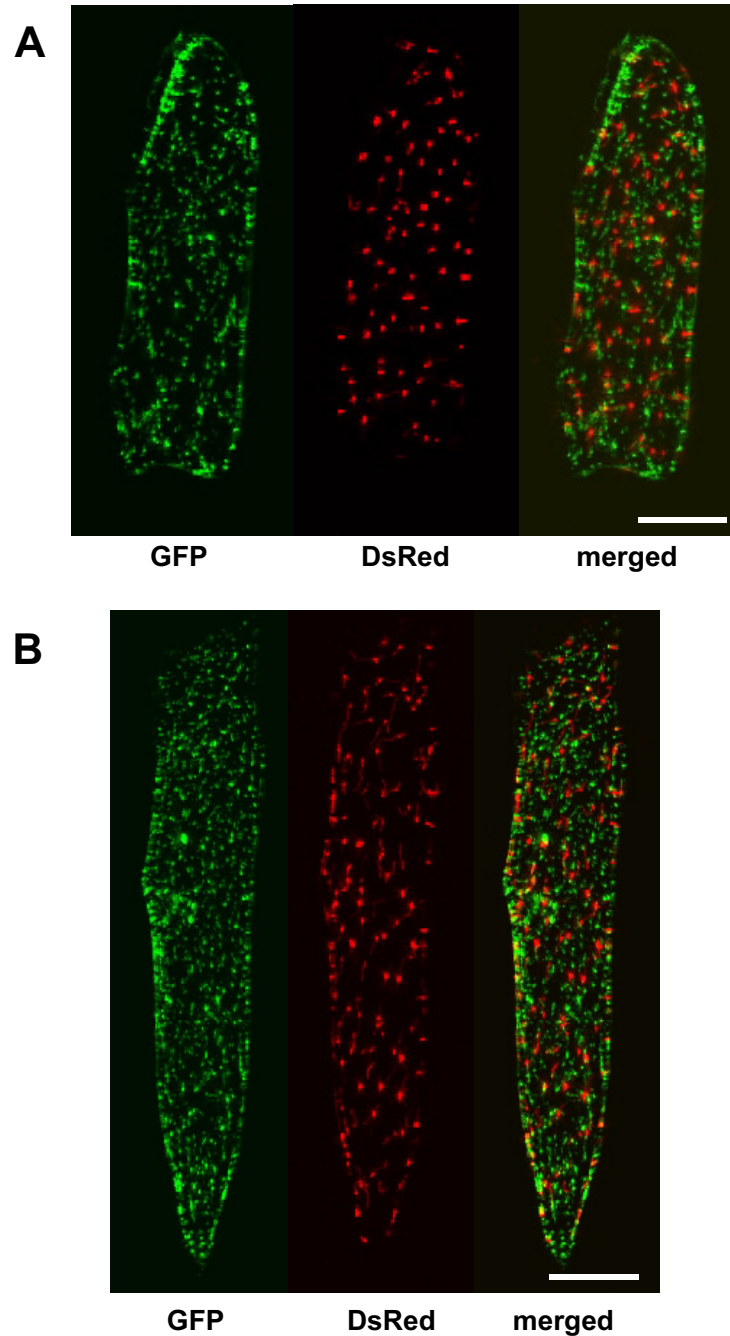


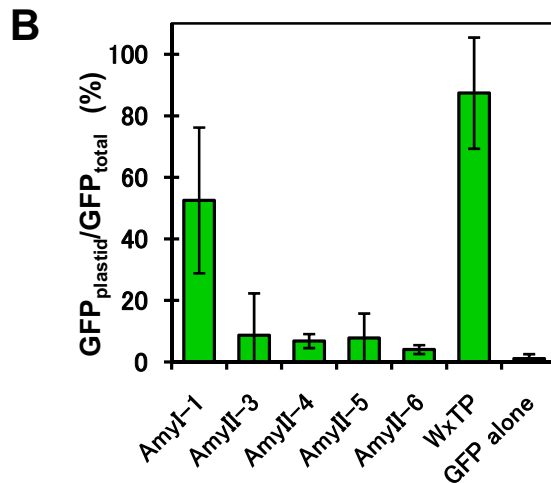
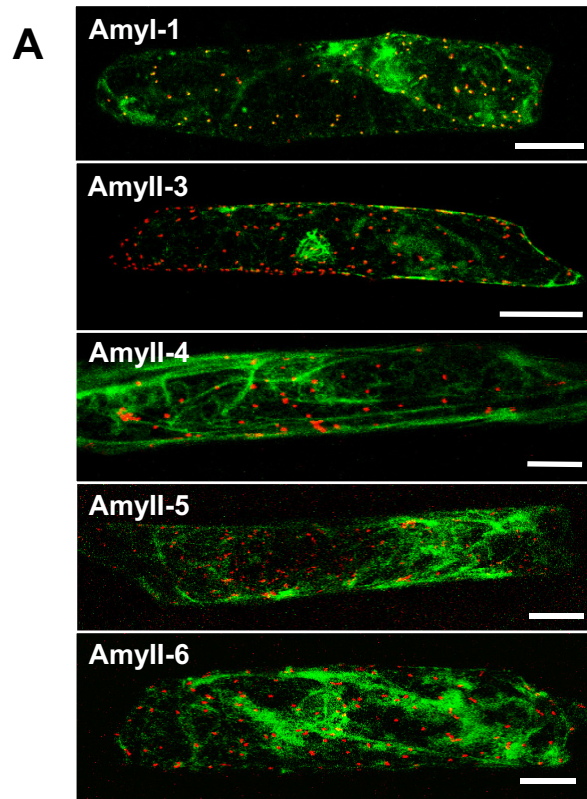
**Supplemental Figure 1.** Fluorescence images of onion epidermal cells expressing either GFP, DsRed, WxTP-GFP or WxTP-DsRed.

**(A)** *GFP*- and *WxTP-GFP*- bombarded cells. **(B)** *DsRed*- and *WxTP-DsRed*- bombarded cells. The epidermal cells were incubated on 0.6% gelrite with 2,4-D-free Murashige-Skoog (MS) medium for 24 h at 25°C in darkness. Images of GFP and DsRed were observed using a BX-61 microscope (Olympus, Tokyo, Japan) and a cooled CoolSnap-fx CCD camera (Photometrics). An Hg lamp was used to excite the fluorescent proteins. GFP fluorescence was obtained by 470 to 490-nm excitation with 510 to 550-nm detection, and that of DsRed was by 520 to 550-nm excitation with  $\geq$  580-nm detection. Deconvolution was carried out using Lumina Vision imaging software (Mitani, Tokyo, Japan). In a whole cell, 15 to 20 images per cell, from the top to middle of the cell, every 1 to 2  $\mu$ m, were taken and combined into one image. All of the images presented are stacks. The right-hand panels represent the fluorescent plastids in close-up. Plastids with stromules were similarly visualized using *WxTP-GFP* and *WxTP-DsRed*. Left panels, bars = 100  $\mu$ m; Right panels, bars = 10  $\mu$ m.



**Supplemental Figure 2.** Fluorescence images of onion cells simultaneously expressing WxTP-DsRed and per-GFP (the peroxisomal targeting signal 2 fused with GFP) (A) or mt-GFP (the presequence of the  $\gamma$  subunit of *Arabidopsis* F1-ATPase fused with GFP) (B).

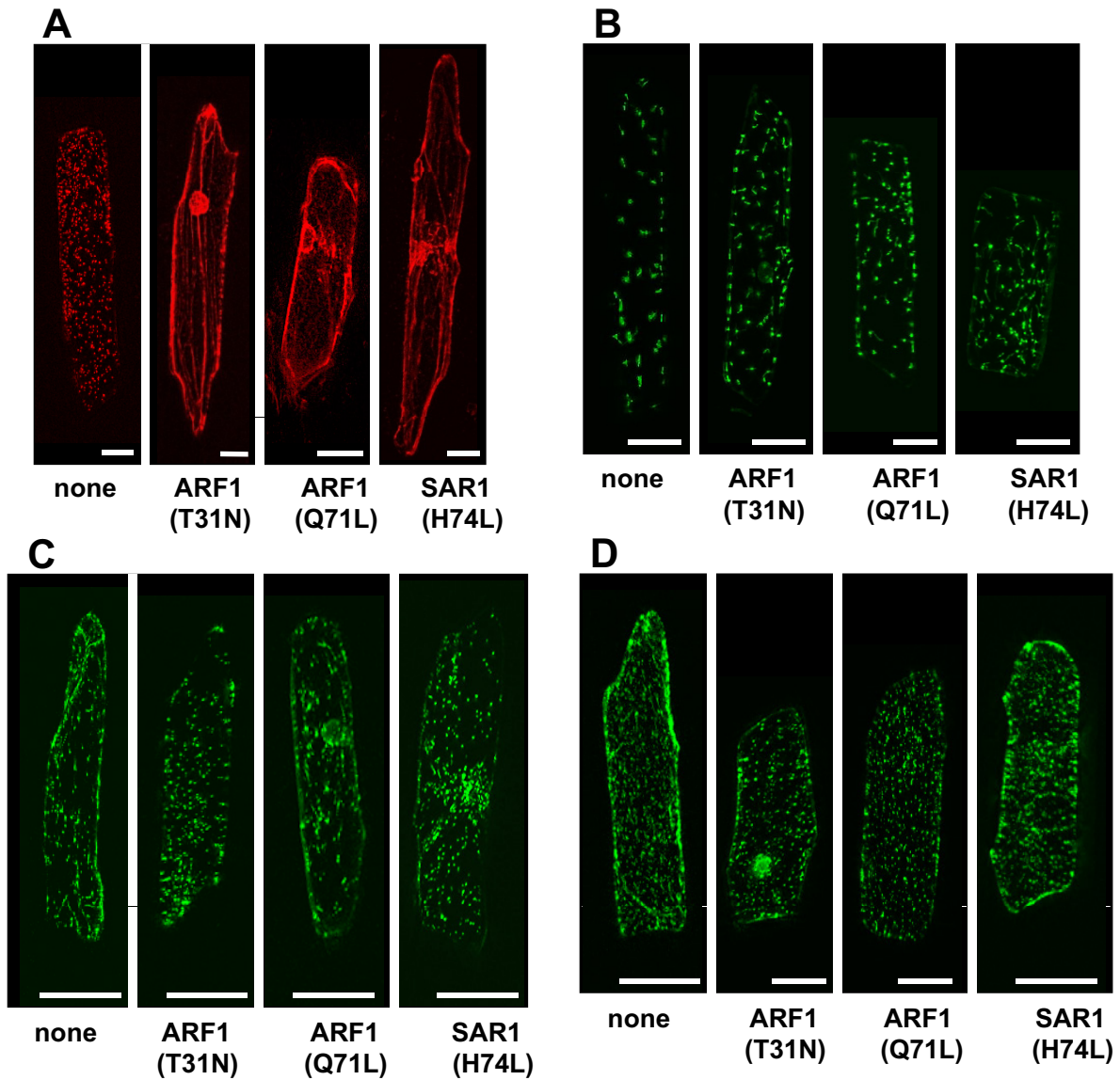
Images were obtained as described in the legend for Supplemental Figure 1. The results show that the distribution of WxTP-DsRed is distinguishable from those of per-GFP and mt-GFP, with the peroxisomal and mitochondrial markers not overlapping with the plastid marker. Bars = 100  $\mu$ m



**Supplemental Figure 3.** Distinct localization of various Amyl isoforms in onion epidermal cells.

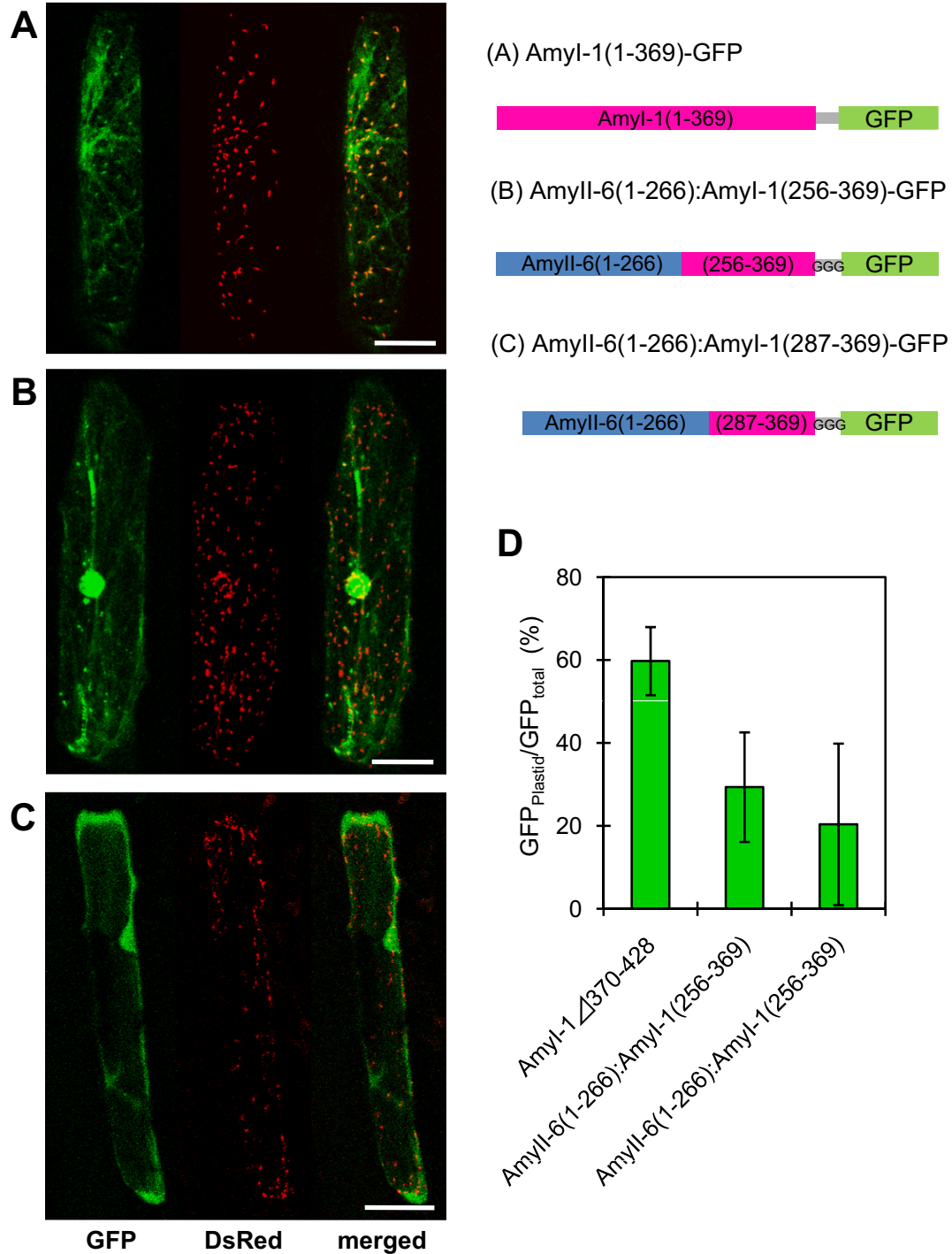
**(A)** Simultaneous expression of WxTP-DsRed and either Amyl-1-, AmyII-3-, AmyII-4-, AmyII-5- or AmyII-6-GFP was carried out. The merged images were constructed as described in Figure 3. Bars = 100  $\mu$ m.

**(B)** Statistical evaluation of the plastidial localization of different Amyl isoforms. Ratios of the fluorescence intensity of GFP in the plastidial area to GFP in the whole cell ( $\text{GFP}_{\text{plastid}}/\text{GFP}_{\text{total}}$ ) (%) were determined. Values show the means  $\pm$  s.d. ( $n = 4$ ).



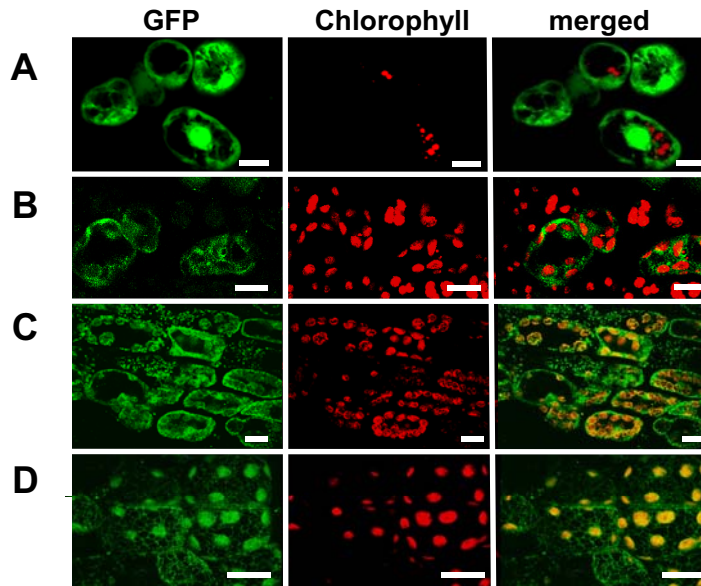
**Supplemental Figure 4.** Effects of ARF1(T31N), ARF1(Q71L) and SAR1(H74L) on the localization of various organelle markers.

Simultaneous expression of the organelle marker and either ARF1(T31N), ARF1(Q71L) or SAR1(H74L) was carried out in onion cells. **(A)** trans-Golgi marker (ST-mRFP), **(B)** plastidial marker (WxTP-GFP), **(C)** peroxisomal marker (per-GFP), **(D)** mitochondrial marker (mt-GFP). Twenty to thirty images per cell, from the top to middle of the cell, every 1–2  $\mu\text{m}$ , were taken and combined into one image. Bars = 100  $\mu\text{m}$ .



**Supplemental Figure 5.** Plastid targeting abilities of Amyll-6: Amyl-1 chimera proteins.

The Amyll-6: Amyl-1 chimera proteins showed little targeting to the plastids in onion cells. The merged images were constructed as described in Figure 3. **(A)** Amyl-1(1-369)-GFP. **(B)** Amyll-6(1-266):Amyl-1(256-369)-GFP. **(C)** Amyll-6(1-266):Amyl-1(287-369)-GFP. Bars = 100  $\mu$ m. **(D)** Quantitative results. Ratios of the fluorescence intensity of GFP in the plastidial area to GFP in the whole cell ( $GFP_{\text{plastid}}/GFP_{\text{total}}$ ) (%) were determined. Values show the means  $\pm$  s.d. ( $n = 4$ ).



**Supplemental Figure 6.** Plastid targeting abilities of Amyl-1( $\Delta$ 101–428)- and Amyl-1( $\Delta$ 370–428)-GFP in cells of transgenic rice plants.

The stable transformant cells were sectioned with a vibratome to 25  $\mu$ m thickness, and immediately observed by means of confocal laser scanning microscopy. **(A)** GFP alone, **(B)** Amyl-1( $\Delta$ 101–428)–GFP, **(C)** Amyl-1 ( $\Delta$ 370–428)–GFP and **(D)** Amyl-1-GFP. Left, GFP fluorescence; middle, chlorophyll autofluorescence; right, merged image. Bars = 5  $\mu$ m.

**Supplemental Table 1: Primer sequences for PCR-amplification**

Amplified DNA (Plasmid constructed)	DNA template	Primer sequences
WxTP	pWCW	5'-ctggatccatgctcggtctcaccacg-3'
(pWxTP-GFP, pWxTP-DsRed)		5'-tatggatccggcagggggaggccaccgag-3'
Amyl-1	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1-GFP)		5'-aaggatccgctttctccagattgctg-3'
Amyl-3	pAmyl-3	5'-aaggatccatgggcaagcaccatgtcac-3'
(pAmyl-3-GFP)		5'-ttgtaccatagtgctttctaccggca-3'
Amyl-4	pAmyl-4	5'-atcgggatccatgaagaacaccagcagc-3'
(pAmyl-4-GFP)		5'-aaggatcctaggtgccgccccgccggac-3'
Amyl-5	pAmyl-5	5'-aaggatccatggcaagcgcatagcctc-3'
(pAmyl-5-GFP)		5'-ttggatccatagtggtgccgcccctgcagg-3'
Amyl-6	pAmyl-6	5'-taggatccatggcaagcattccaccac-3'
(pAmyl-6-GFP)		5'-atggatccgtggcggcctctggaac-3'
Amyl-1(Δ34-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ34-428)-GFP)		5'-aaggatccgttgaatccctgaacaggac-3'
Amyl-1(Δ101-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ101-428)-GFP)		5'-aaggatccctcgatcagcgactgagct-3'
Amyl-1(Δ201-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ201-428)-GFP)		5'-aaggatccccacgctcgaagccgatgctc-3'
Amyl-1(Δ301-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ301-428)-GFP)		5'-taggatccccgatcatgccggcgccctt-3'
Amyl-1(Δ304-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ304-428)-GFP)		5'-aaggatccccggcaccaccggatgacc-3'
Amyl-1(Δ351-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ351-428)-GFP)		5'-aaggatcccaaatggtcgtagaagatgca-3'
Amyl-1(Δ370-428)	pAmyl-1	5'-atcgggatccatggtgaacaacacttctt-3'
(pAmyl-1(Δ370-428)-GFP)		5'-aaggatccctgccggttctgattgaca-3'
Amyl-1(W302A)	pAmyl-1	5'-gccccggcatgatcgggtggcgccggccaagcgacgacc-3'
(pAmyl-1(W302A)-GFP)		5'-ggtcgtcgcttggcggcgcccaccggatcatgccggggcgc-3'
Amyl-1(W302L)	pAmyl-1	5'-atgatcgggtgctgctccggccaagggcg-3'
(pAmyl-1(W302L)-GFP)		5'-cgcttggcggcagccaccggatcat-3'
Amyl-1(T307V)	pAmyl-1	5'-ccggccaagcggtgacctcgtcgac-3'
(pAmyl-1(T307V)-GFP)		5'-gtcgacgaagtcaccgcttggccgg-3'
Amyl-1(G354N)	pAmyl-1	5'-ttcttcgattggaatcctcaagtggtg-3'
(pAmyl-1(G354N)-GFP)		5'-ctcctccttgagattccaatcgaagaa-3'
Amyl-1(256-369)(Gly) <sub>4</sub>	pAmyl-1	5'-aaggatccgtcgatcgtcggcggcgcc-3'
(pAmyl-1(256-369)-GFP)		5'-ataggtacccccggccccctgcccgttctgattga-3'
Amyl-1(256-369)(Gly) <sub>4</sub>	pAmyl-1	5'-aaggatccctccggcgaggacggcaag-3'
(pAmyl-1(287-369)-GFP)		5'-ataggtacccccggccccctgcccgttctgattga-3'
35S-(BamHI to Apa1)-Amyl-6	pAmyl-6-GFP	5'-ggggactctagagggccatggcaagcat-3'
(p35S-(BamHI to Apa1)-Amyl-6-GFP)		5'-atgcttggatgggcccctctagatcccc-3'
35S-(BamHI to Apa1)-Amyl-6(1-266)	p35S-(BamHI to Apa1)-Amyl-6-GFP	5'-gcgcaagcttagattagcctttcaalttc-3'
(pAmyl-6(1-266)/Amyl-1(256-369)GFP)		5'-taaggatcccgatcaggggcaccga-3'
35S-(BamHI to Apa1)-Amyl-6(1-266)	p35S-(BamHI to Apa1)-Amyl-6-GFP	5'-gcgcaagcttagattagcctttcaalttc-3'
(pAmyl-6(1-266)/Amyl-1(287-369)GFP)		5'-taaggatcccgatcaggggcaccga-3'