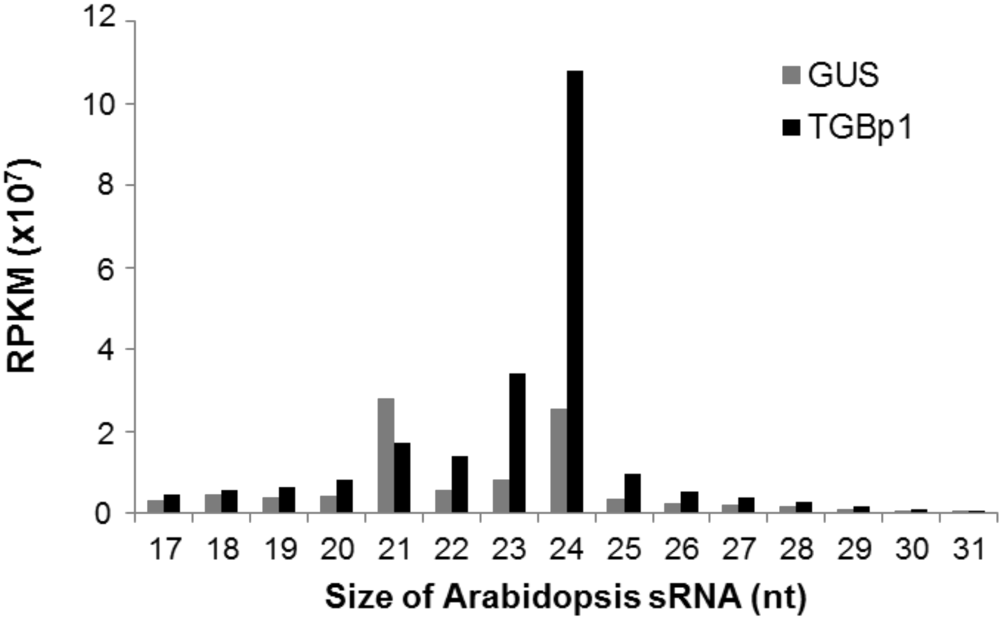
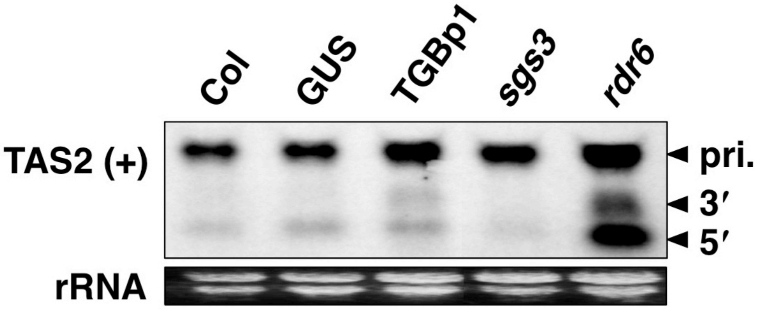


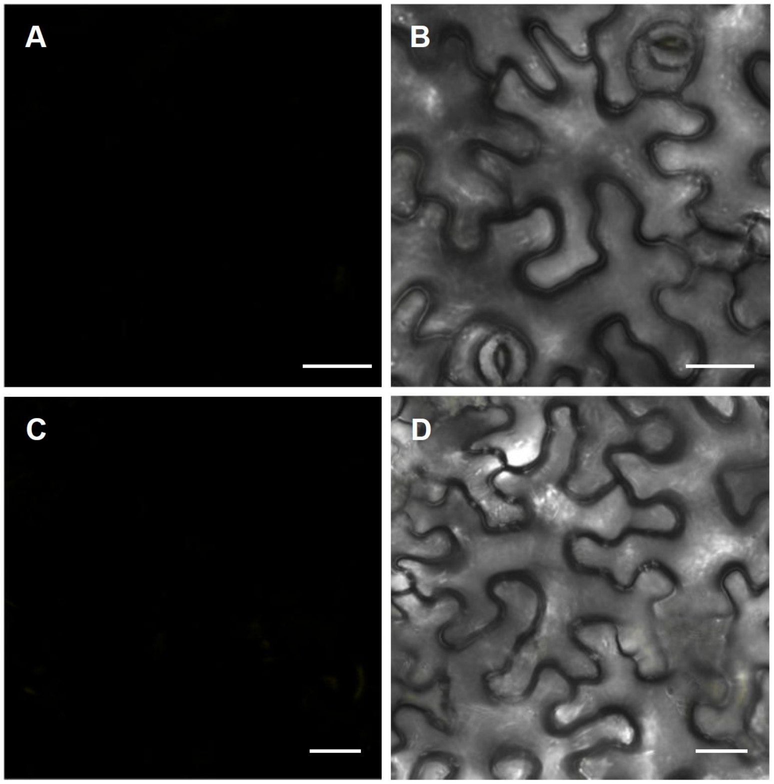
Supplemental Figure 1. Size distribution of *Arabidopsis* sRNAs in the GUS and the TGBp1 transformants.

The number of the sequencing reads annotated as known sRNAs recovered from the GUS (gray bars) and the TGBp1 transformants (black bars) sRNA library is expressed as reads per kilobase of exon model per million mapped reads (RPKM).



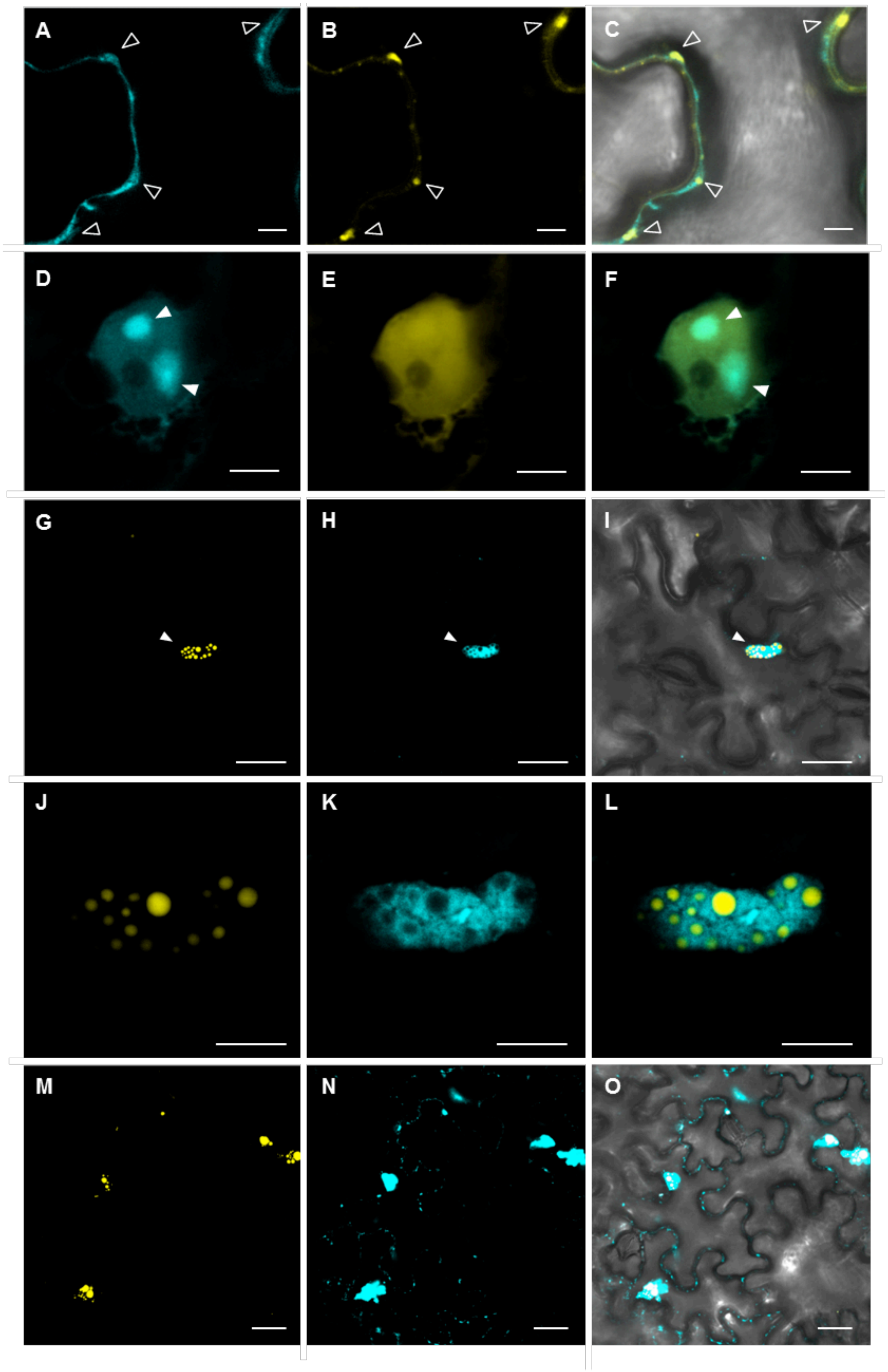
Supplemental Figure 2. RNA gel blot analysis to detect the primary TAS2 transcript and its cleavage products.

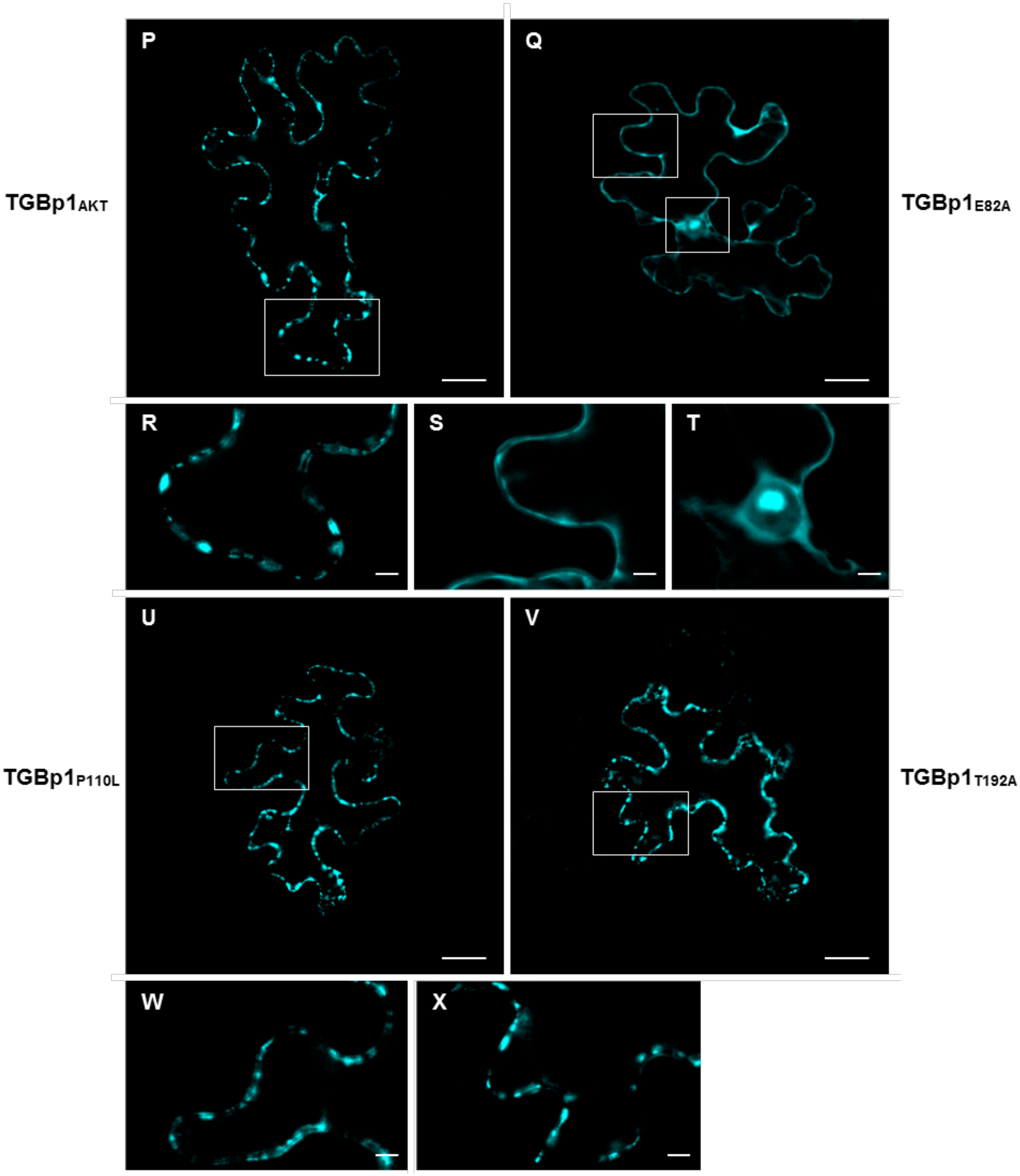
RNA gel blot analysis performed on 10 µg of total RNA from plants at the reproductive stage to detect the primary TAS2 transcript (pri.) and its 5′- and 3′- cleavage products. Relative gel loadings are shown by ethidium bromide staining of rRNA.



Supplemental Figure 3. Confocal laser scanning microscopy observation of RDR6.

(A and B) BiFC assay between TGBp1 and RDR6. TGBp1-YFP^N and RDR6-YFP^C were co-expressed by agroinfiltration in leaf epidermal cells of *N. benthamiana* plants. **(A)** YFP fluorescence image. **(B)** Overlay of bright-field and fluorescence image of (A). **(C and D)** Confocal sections of *N. benthamiana* leaves expressing RDR6-YFP alone. **(C)** YFP fluorescence image. **(D)** Overlay of bright-field and fluorescence image of (C).Bars: 25 µm.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O


P
Q
TGBp1$_{\mathrm{AKT}}$
TGBp1$_{\mathrm{E82A}}$
R
S
T
U
V
TGBp1$_{\mathrm{P110L}}$
TGBp1$_{\mathrm{T192A}}$
W
X

Supplemental Figure 4. Subcellular localizations of SGS3, TGBp1 and TGBp1 mutants.

(A-C) Confocal sections of *N. benthamiana* leaves co-expressing TGBp1-CFP and PDLP1-YFP. Images show cell periphery of a single cell. The cyan and yellow signals of the same plane and the overlay of bright-field and fluorescence images are presented. TGBp1-CFP co-localized with PDLP1-YFP at the cell periphery (open arrowheads). **(D-F)** Confocal sections of *N. benthamiana* leaves co-expressing TGBp1-CFP and YFP. The cyan and yellow signals of the same plane and the overlay of the fluorescence images are presented. TGBp1-CFP was localized throughout the entire nucleus with punctate structures (filled arrowheads). **(G-O)** Confocal sections of *N. benthamiana* leaves expressing both SGS-YFP and TGBp1-CFP. **(G-I)** Images show a single cell different from the cell observed in Figure 4 **(F-H)**. The yellow and cyan signals of the same plane and the overlay of bright-field and fluorescence images are presented. **(J-L)** Higher-magnification views of region near arrowheads in **(G-I)**. The yellow and cyan signals of the same plane and overlay of the fluorescence images are presented. **(M-O)** Images show the same cells observed in Figure 4 **(F-H)** with a longer exposure. **(P and R)** Confocal sections of *N. benthamiana* leaves expressing TGBp1$_{AKT}$-CFP. **(R)** Higher-magnification view of the boxed region in **(P)**. **(Q, S and T)** Confocal sections of *N. benthamiana* leaves expressing TGBp1$_{E82A}$-CFP. **(S and T)** Higher-magnification views of the upper left and lower right boxed region in **(Q)**, respectively. **(U and W)** Confocal sections of *N. benthamiana* leaves expressing TGBp1$_{P110L}$-CFP. **(W)** Higher-magnification view of the boxed region in **(U)**. **(V and X)** Confocal sections of *N. benthamiana* leaves expressing TGBp1$_{T192A}$-CFP. **(X)** Higher-magnification view of the boxed region in **(V)**. Bars: 25 µm except **(D-F)**, **(J-L)**, **(R)**, **(S)**, **(T)**, **(W)** and **(X)**: 5 µm

Supplemental Table 1. RPKM value of sRNA reads mapped to the *Arabidopsis* genome.

	GUS		**TGBp1**	
Type	**Unique reads**	**Total reads**	**Unique reads**	**Total reads**
miRNA	22,067,259	23,600,352	7,903,754	10,674,912
mature	22,033,542	23,561,989	7,777,525	10,531,731
hairpin	33,716	38,363	126,230	143,180
rRNA	2,779	1,891,893	5,825	2,833,327
tRNA	182,057	1,141,088	688,125	2,329,724
snRNA	612	891	460	704
snoRNA	3,447	3,806	7,626	9,014
miscellaneous RNA	20,296	21,830	16,787	21,880

Supplemental Table 2. Comparison of RPKM value of tasiRNAs from the GUS and TGBp1 transformant sRNA libraries.

Gene	**Gene family**	**GUS**	**TGBp1**	**$\log_2$ (TGBp1/GUS)**
tasiRNA loci				
At2g27400	TAS1a	3137.10	167.22	-4.23
At1g50055	TAS1b	917.94	87.54	-3.39
At2g39675	TAS1c	2534.72	389.38	-2.70
At2g39681	TAS2	7171.20	209.10	-5.10
At3g17185	TAS3a	488.45	70.64	-2.79
At5g49615	TAS3b	163.23	2.83	-5.85
At5g57735	TAS3c	3.52	2.89	-0.28
At3g25795	TAS4	10.20	2.63	-1.96

Supplemental Table 3. Characteristics of TGBp1 mutants used in this study.

	Suppression of RNA silencing	Interaction with SGS3	Co-aggregation with SGS3	Formation of homo-oligomers
TGBp1	+	+	+	+
TGBp1$_{AKT}$	-	-	-	-
TGBp1$_{E82A}$	+	+	+	+
TGBp1$_{p110L}$	-	-	-	-
TGBp1$_{T192A}$	±	+	-	-

Supplemental Table 4. Primers used in this study.

Primer	Sequence (5' to 3')
PlTGBp1AKT-F	TAGCTGGCGCGGCGAAAACCACACT
PlTGBp1AKT-R	GGTTTTCGCCGCGCCAGCTACAGCG
PlTGBp1E82A-F	TCCTCGACGCGTACTCCGCCCTCCA
PlTGBp1E82A-R	GGCGGAGTACGCGTCGAGGAGGTTG
PlTGBp1P110L-F	CTCGCGCTCCGACTCCACTTC
PlTGBp1P110L-R	ACTTTACGAAGTGGAGTCGGAG
PlTGBp1T192A-F	AGTTTGACACGGTCGCCGTCG
PlTGBp1T192A -R	CGGAGACGACGGCGACCGTG
ENTA-GUS-F	AATTCAGTCGACTGGATCATGTTACGTCCTGTAGAAACC
ENTA-GUS-R	AAGCTGGGTCTAGATATCTTTGTTTGCCTCCCTGCTGCG
ENTA-TGBp1-F	AATTCAGTCGACTGGATCATGGACATAGTCATCTCAGCC
ENTA-TGBp1-R	AAGCTGGGTCTAGATAGTAGGTGGGGTGAGGTGG
ENTA-SGS3-F	AATTCAGTCGACTGGATCATGAGTTCTAGGGCTGGTCC
ENTA-SGS3-R	AAGCTGGGTCTAGATAATCATCTTCATTGTGAAGGCCATG
ENTA-RDR6-F	AATTCAGTCGACTGGATCATGGGGTCAGAGGGAAATATGAAGAAGTCGG
ENTA-RDR6-R	AAGCTGGGTCTAGATAGAGACGCTGAGCAAGAAACTTAGCCAAAG
ENTA-PDLP-F	CTGGATCCGGATGAAACTCACCTATCAATTC
ENTA-PDLP-R	TATCTCGAGTGATAAGCATCATATTTATTACTCTTC
PPR-F	GGAATAGGATTTCAGATGCCGTATCTCTTG
PPR-R	CACCTCTCTTACATAATCCATTTACTACAA
ARF3-F	TAAGGTGGGACGACATTGTGGC
ARF3-R	TCAACACTTGTTCGGATGGTGG
Actin2-F	GCACCCTGTTCTTCTTACCG
Actin2-R	AACCCTCGTAGATTGGCACA
TAS2-767R	TTGCATATCCATGAATATGTTTAG
TAS3-931R	CTCCATCTTGTATCCCACTGAAA
TAS2-725R	ATGAATCTTATATGTTTACTAAAACA
TAS3-910R	CATACATCAATAACAAACAAAAGATATATG
TAS2-450F	GAATGATGATACTTAAACTATTC
TAS2-570R	ATGTATATCCCATTTCTACCATC
TAS3-704F	TTGTAAGGCCTTTTCTTGACC
TAS3-841R	ATGACTCGAACGAATTAGAGG