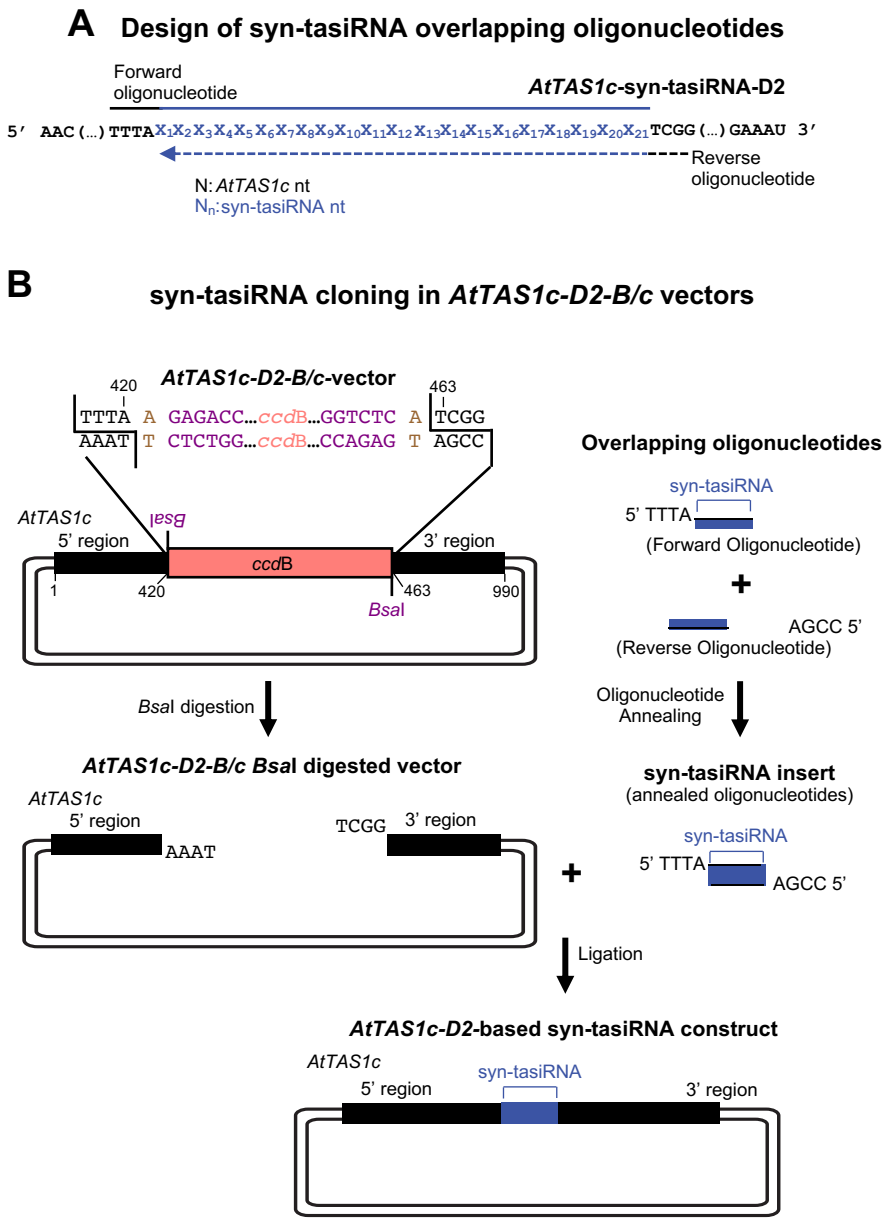
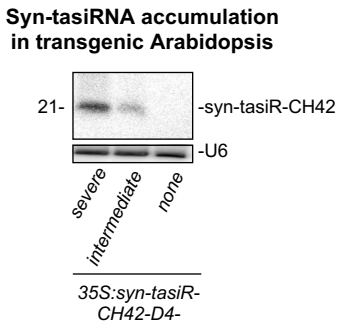
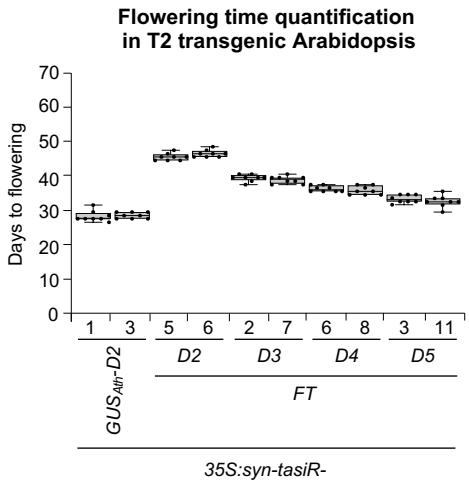


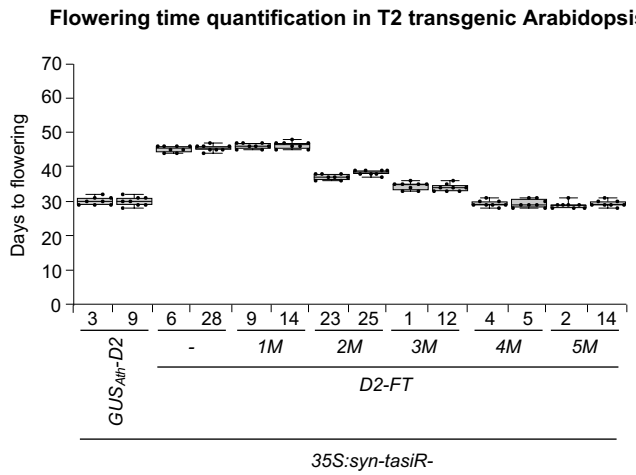
Supplementary Figure S1. Direct syn-tasiRNA cloning in *AtTAS1c-D2-B/c*-based vectors including a modified version of *AtTAS1c* with a *ccd*B cassette flanked by two *Bsa*I sites (*Bsa*I/*ccd*B or "B/c" vectors).
(A) Design of two overlapping oligonucleotides for syn-tasiRNA cloning. Sequence covered by the forward and reverse oligonucleotides are represented with continuous or dotted lines, respectively.
(B) Diagram of the steps for syn-tasiRNA cloning in *AtTAS1c-D2-B/c* vectors. The syn-tasiRNA insert obtained after annealing the two overlapping oligonucleotides has 5' TTTA and 5'-CCGA overhangs and is directly inserted into the *Bsa*I-linearized *AtTAS1c-D2-B/c* vector. Nucleotides of the *Bsa*I sites and arbitrary nucleotides used as spacers between the *Bsa*I recognition site and the *AtTAS1c* sequence are in purple and light brown, respectively. Other details are as in Panel A.



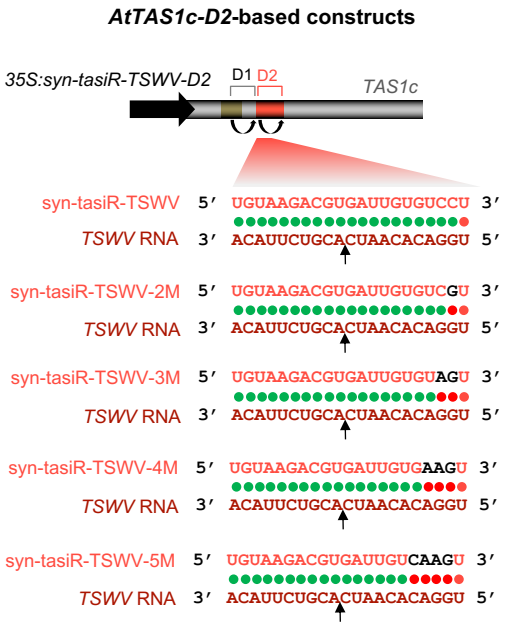
Supplementary Figure S2. Northern blot detection of syn-tasiR-CH42 in Arabidopsis T1 *35S:syn-tasiR-CH42-D4* transgenic lines. Each sample corresponds to a pool of at least 3 independent lines showing either severe, intermediate or no phenotype. The U6 RNA blot is shown as a loading control.



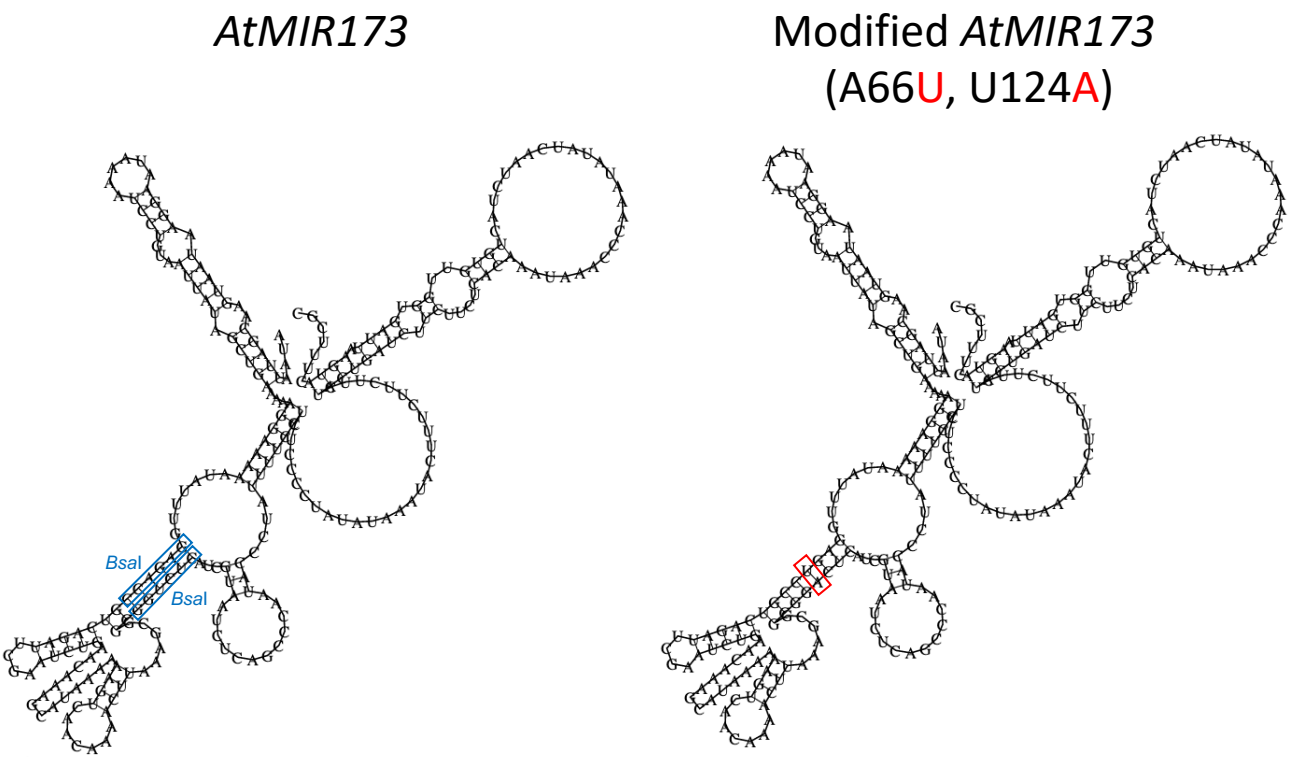
Supplementary Figure S3. Jitter box plot representing the mean flowering time (days to flowering) of Arabidopsis T2 transgenic lines expressing syn-tasiRNA constructs. Selected T2 lines originated from representative T1 lines with a flowering time similar to the mean of the corresponding group.



Supplementary Figure S4. Jitter box plot representing the mean flowering time (days to flowering) of Arabidopsis T2 transgenic lines expressing syn-tasiRNA constructs. Selected T2 lines originated from representative T1 lines with a flowering time similar to the mean of the corresponding group.



Supplementary Figure S5. Diagram of the syn-tasiRNA constructs. Base-pairing and mismatches between syn-tasiRNA and target RNA nucleotides are shown with green and red circles, respectively. Mutated nucleotides are shown in black. Other details are as in Figures 1A and 3A.



Supplementary Figure S6. M-fold predicted secondary structures of wild-type and modified *AtMIR173* precursors. Functional and disrupted *Bsa*I sites are highlighted in blue and red, respectively.

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Supplementary Protocol S1

Protocol to design and clone syn-tasiRNAs downstream the 3'D1[+] position in *AtTAS1c-D2-Bsa*I/*ccd*B-based ('B/c') vectors containing *AtTAS1c* precursor.

1. Selection of the syn-tasiRNA sequence(s)

Use the Syn-tasiRNA Designer app from the P-SAMS webtool at http://p-sams.carringtonlab.org/syntasi/designer.

2. Design of syn-tasiRNA oligonucleotides

Use the Syn-tasiRNA Designer app from the P-SAMS webtool at http://p-sams.carringtonlab.org/syntasi/designer.

2.2.1 Sequence of the *AtTAS1c* cassette containing the syntasiRNA(s)

The following FASTA sequence includes two syn-tasiRNA sequences inserted in the *AtTAS1c* precursor sequence downstream position 3'D1[+]:

>syn-tasiRNA-1 and syn-tasiRNA-2 in *AtTAS1c*

AAACCTAAACCTAAACGGCTAAGCCCGACGTCAAATACCAAAAAGAGAAAAACAAGAGCGCCGT

CAAGCTCTGCAAATACGATCTGTAAGTCCATCTTAACACAAAAGTGAGATGGGTTCTTAGATCA

TGTTCCGCCGTTAGATCGAGTCATGGTCTTGTCTCATAGAAAGGTACTTTCGTTTACTTCTTTT

GAGTATCGAGTAGAGCGTCGTCTATAGTTAGTTTGAGATTGCGTTTGTCAGAAGTTAGGTTCAA

TGTCCCGGTCCAATTTTCACCAGCCATGTGTCAGTTTCGTTCCTTCCCGTCCTCTTCTTTGATT

TCGTTGGGTTACGGATGTTTTCGAGATGAAACAGCATTGTTTTGTTGTGATTTTTCTCTACAAG

CGAATAGACCA**TTTA**X1X2X3X4X5X6X7X8X9X10X11X12X13X14X15X16X17X18X19X20X21X1X2X3X4

X5X6X7X8X9X10X11X12X13X14X15X16X17X18X19X20X21**TCGG**TGGATCTTAGAAAATTATTCTAAG

TCCAACATAGCGTATTCTAAGTTCAACATATCGACGAACTAGAAAAGACATTGGACATATTCCA

GGATATGCAAAAGAAAACAATGAATATTGTTTTGAATGTGTTCAAGTAAATGAGATTTTCAAGT

CGTCTAAAGAACAGTTGCTAATACAGTTACTTATTTCAATAAATAATTGGTTCTAATAATACAA

AACATATTCGAGGATATGCAGAAAAAAAGATGTTTGTTATTTTGAAAAGCTTGAGTAGTTTCTC

TCCGAGGTGTAGCGAAGAAGCATCATCTACTTTGTAATGTAATTTTCTTTATGTTTTCACTTTG

TAATTTTATTTGTGTTAATGTACCATGGCCGATATCGGTTTTATTGAAAGAAAATTTATGTTAC

TTCTGTTTTGGCTTTGCAATCAGTTATGCTAGTTTTCTTATACCCTTTCGTAAGCTTCCTAAGG
AATCGTTCATTGATTTCCACTGCTTCATTGTATATTAAAACTTTACAACTGTATCGACCATCAT
ATAATTCTGGGTCAAGAGATGAAAATAGAACACCACATCGTAAAGTGAAAT

Where:

-**X** is a DNA base of the syn-tasiRNA-1 sequence, and the subscript number is the base position in the syn-tasiRNA-1 21-mer

-**X** is a DNA base of the syn-tasiRNA-2 sequence, and the subscript number is the base position in the syn-tasiRNA-2 21-mer

-**X** is a DNA base of the *AtTAS1c* precursor included in the oligonucleotides required to clone the syn-tasiRNA insert in B/c vectors

-X is a DNA base of the *AtTAS1c* precursor

Note that in general, X_1=T and X_1=T for syn-tasiRNA association with AGO1.

In the sequence above, replace the sequences $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ by the sequences of syn-tasiRNA_1 and syn-tasiRNA_2, respectively.

2.2.2. Sequence of the syn-tasiRNA oligonucleotides

The sequences of the two syn-tasiRNA oligonucleotides are:

-Sense oligonucleotide (46 b):

TTTA$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$

-Antisense oligonucleotide (46 b):

CCGA$Y_{21}Y_{20}Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1Y_{21}Y_{20}Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1$

Where:

-$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$=syn-tasiRNA-1 sequence

-$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$=syn-tasiRNA-2 sequence

-$Y_{21}Y_{20}Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1$=syn-tasiRNA-1 reverse-complement sequence

-$Y_{21}Y_{20}Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1$=syn-tasiRNA-2 reverse-complement sequence

Example

The sequences of the two oligonucleotides to clone syn-tasiRNAs 'syn-tasiR-Trich' (**TCCCATTCGATACTGCTCGCC**) and 'syn-tasiR-Ft' (**TTGGTTATAAAGGAAGAGGCC**) in positions 3'D3[+] and 3'D4[+] of *AtTAS1c,* respectively, are:

-Sense oligonucleotide (46 b):

TTTATCCCATTCGATACTGCTCGCCTTGGTTATAAAGGAAGAGGCC

-Antisense oligonucleotide (46 b):

CCGAGGCCTCTTCCTTTATAACCAAGGCGAGCAGTATCGAATGGGA

3. Cloning of the syn-tasiRNA sequence(s) in AtTAS1c-D2-B/c-based vectors

Notes:

-Available AtTAS1c-D2-B/c vectors are listed in Table I at the end of the section.

-AtTAS1c-D2-B/c-*based vectors must be propagated in a* ccd*B resistant* E. coli *strain such as DB3.1.*

-Alternatively, Bsa*I digestion of the B/c vector and subsequent ligation of the amiRNA oligonucleotide insert can be done in separate reactions*

3.1. Oligonucleotide annealing

-Dilute sense oligonucleotide and antisense oligonucleotide in sterile H_2O to a final concentration of 100 µM.

-Prepare Oligo Annealing Buffer:

60 mM Tris-HCl (pH 7.5)
500 mM NaCl
60 mM $MgCl_2$
10 mM DTT

Note: *Prepare 1 ml aliquots of Oligo Annealing Buffer and store at -20ºC.*

-Assemble the annealing reaction in a PCR tube as described below:

Forward oligonucleotide (100 µM)	2 µL
Reverse oligonucleotide (100 µM)	2 µL
Oligo Annealing Buffer	46 µL
Total volume	50 µL

The final concentration of each oligonucleotide is 4 µM.

-Use a thermocycler to heat the annealing reaction 5 min at 94°C and then cool down (0.05ºC/sec) to 20°C.

-Dilute the annealed oligonucleotides just prior to assembling the digestion-ligation reaction as described below:

Annealed oligonucleotides	3 µL
dH_2O	37 µL
Total volume	40 µL

The final concentration of each oligonucleotide is 0.15 µM.

Note*: Do not store the diluted oligonucleotides.*

3.2. Digestion-ligation reaction

- Assemble the digestion-ligation reaction as described below:

B/c vector (x ug/uL)	Y µL (50 ng)
Diluted annealed oligonucleotides	1 µL
10x T4 DNA ligase buffer	1 µL
T4 DNA ligase (400 U/µL)	1 µL
*Bsa*I (10U/ µL, NEB)	1 µL
dH_2O	to 10 µL

Total volume	10 µL

Prepare a negative control reaction lacking *Bsa*I.

-Mix the reactions by pipetting. Incubate the reactions at room temperature for 5 minutes at 37°C.

3.3. *E.coli* transformation and analysis of transformants

-Transform 1-5 ul of the digestion-ligation reaction into an *E. coli* strain that doesn't have *ccd*B resistance (e.g. DH10B, TOP10, …) to do counter-selection.

-Pick two colonies/construct, grow LB-Kan (100 mg/ml) cultures and purify plasmids.

-Sequence with appropriate primers: M13-F (CCCAGTCACGACGTTGTAAAACGACGG) and M13-R (CAGAGCTGCCAGGAAACAGCTATGACC) for *pENTR*-based vectors; attB1 (ACAAGTTTGTACAAAAAAGCAGGCT) and attB2 (ACCACTTTGTACAAGAAAGCTGGGT) primers for *pMDC32B*-based vectors).

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Table I: *Bsa*I/*ccd*B-based ('B/c') vectors for direct cloning of syn-tasiRNAs downstream position 3'D1[+] in *AtTAS1c* precursor.

Vector	Small RNA expressed	Bacterial antibiotic resistance	Plant antibiotic resistance	GATEWAY use	Backbone	Promoter of syn-tasiRNA cassette	Terminator of syn-tasiRNA cassette	Plant species tested
pENTR-AtTAS1c-D2-B/c	–	Kanamycin	–	Donor	*pENTR*	–	–	–
pMDC32B-AtTAS1c-D2-B/c	syn-tasiRNA(s)	Kanamycin Hygromycin	Hygromycin	–	*pMDC32*	*CaMV* 2x35S	*Nos*	*A. thaliana* *N. benthamiana*
pMDC32B-AtTAS1c-D2-B/c-AtMIR173	syn-tasiRNA(s) miR173	Kanamycin Hygromycin	Hygromycin	–	*pMDC32*	*CaMV* 2x35S	*nos*	*A. thaliana* *N. benthamiana*

Supplementary Table S1: Phenotypic penetrance of syn-tasiRNAs expressed in Arabidopsis Col-0 T1 transgenic plants

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:syn-tasiR-GUS-D2&D3	39	0% FT 0% Trich
35S:syn-tasiR-FT-D2-Trich-D3	16	100% FT 71% Trich
35S:syn-tasiR-Trich-D2-FT-D3	18	100% FT 82% Trich
35S:syn-tasiR-FT-D3-Trich-D4	48	100% FT 61% Trich
35S:syn-tasiR-Trich-D3-FT-D4	34	100% FT 76% Trich

[a] The Ft phenotype was defined as a higher 'days to flowering' value when compared to the average 'days to flowering' value of the *35S:syn-tasiR-GUS-D2&D3* control set.
The Trich phenotype was defined as a higher number of trichomes when compared to transformants of the 3*5S:syn-tasiR-GUS-D2&D3* control set.

Supplementary Table S2: Phenotypic penetrance of syn-tasiRNAs expressed in *A. thaliana* Col-0 T1 transgenic plants

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtTAS1c-GUS$_{Ath}$-D2	77	0%
35S:AtTAS1c-FT-D2	38	100%
35S:AtTAS1c-FT-D3	33	100%
35S:TAS1c-FT-D4	66	100%
35S:TAS1c FT-D5	40	100%

[a] The Ft phenotype was defined as a higher 'days to flowering' value when compared to the average 'days to flowering' value of the *35S:AtTAS1c-GUS$_{Ath}$-D2* control set.

Supplementary Table S3: Phenotypic penetrance of syn-tasiRNAs expressed in Arabidopsis Col-0 T1 transgenic seedlings.

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtTAS1c-GUS$_{Ath}$-D2	147	0%
35S:AtTAS1c-CH42-D2	106	100% 1% weak 39% intermediate 60 % severe
35S:AtTAS1c-CH42-D3	126	96% 26% weak 38% intermediate 32% severe
35S:TAS1c-CH42-D4	171	90% 30% weak 43% intermediate 17% severe
35S:TAS1c CH42-D5	65	94% 29% weak 51% intermediate 14% severe

[a] Ch42 phenotype is scored in 10 days-old seedling and is considered 'weak', 'intermediate' or 'severe' if seedlings have >2 leaves, exactly 2 leaves or no leaves (only 2 cotyledons), respectively.

Supplementary Table S4. Summary of results obtained from symptom and DAS-ELISA analyses in *Nicotiana benthamiana* bioassays.

	Analysis at 10 dpi		**Analysis at 20 dpi**	
Sample	**Symptomatic plants/Total**	**DAS-ELISA positive/Total**	**Symptomatic plants/Total**	**DAS-ELISA positive/Total**
35S:GUS	0/6	0/6	0/6	0/6
35S:syn-tasiR-GUS + *35S:MIR173a* + TSWV	6/6	6/6	6/6	6/6
35S:syn-tasiR-TSWV-D2 + *35S:MIR173a* + TSWV	0/6	0/6	06	0/6
35S:syn-tasiR-TSWV-D3 + *35S:MIR173a* + TSWV	0/6	1/6	1/6	1/6
35S:syn-tasiR-TSWV-D4 + *35S:MIR173a* + TSWV	0/6	1/6	3/6	3/6
35S:syn-tasiR-TSWV-D5 + *35S:MIR173a* + TSWV	6/6	6/6	6/6	6/6

Supplementary Table S5: Phenotypic penetrance of syn-tasiRNAs expressed in Arabidopsis Col-0 T1 transgenic plants

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtTAS1c-GUS$_{Ath}$-D2	48	0%
35S:AtTAS1c-FT-D2	59	100%
35S:AtTAS1c-FT-D2-1M	77	100%
35S:AtTAS1c-FT-D2-2M	79	97%
35S:AtTAS1c FT-D2-3M	45	89%
35S:AtTAS1c-FT-D2-4M	92	59%
35S:AtTAS1c FT-D2-5M	39	8%

[a] The Ft phenotype was defined as a higher 'days to flowering' value when compared to the average 'days to flowering' value of the *35S:AtTAS1c-GUS$_{Ath}$-D2* control set.

Supplementary Table S6. Summary of results obtained from symptom and DAS-ELISA analyses in *Nicotiana benthamiana* bioassays.

	Analysis at 10 dpi		**Analysis at 20 dpi**	
Sample	**Symptomatic plants/Total**	**DAS-ELISA positive/Total**	**Symptomatic plants/Total**	**DAS-ELISA positive/Total**
35S:GUS	0/6	0/6	0/6	0/6
35S:syn-tasiR-GUS + 35S:MIR173a + TSWV	6/6	6/6	6/6	6/6
35S:syn-tasiR-TSWV-D2 + 35S:MIR173a + TSWV	0/6	0/6	0/6	0/6
35S:syn-tasiR-TSWV-D2-2M + 35S:MIR173a + TSWV	2/6	3/6	6/6	6/6
35S:syn-tasiR-TSWV-D2-3M + 35S:MIR173a + TSWV	6/6	6/6	6/6	6/6
35S:syn-tasiR-TSWV-D2-4M + 35S:MIR173a + TSWV	6/6	6/6	6/6	6/6
35S:syn-tasiR-TSWV-D2-5M + 35S:MIR173a + TSWV	6/6	6/6	6/6	6/6

Supplementary Table S7. Name, sequence and use of DNA oligonucleotides used in this study.

Oligonucleotide	Sequence	Construct/Aim
AC-14	ATCGCGCGCGGTGTCATCTATGTTACTGAATTCAAGCTTGGCGTGCCTGCA	*pMDC32B-AtTAS1c-D2-B/c-MIR173* (Gibsoon assembly)
AC-15	GGAAACAGCTATGACCATGATTACGAATTCGAATTCAGTAACATAGATGACACCGCG	
AC-49	AGGACACAATCACGTCTTACA	Probe for syn-tasiR-TSWV/syn-tasiR-TSWV-1M detection
AC-55	AGGGGCCATGCTAATCTTCTC	Probe for snoU6 detection
AC-82	TTTATGCGCTTGCTGAGTTTCCCCC	*35S:syn-tasiR-GUS$_{Ath}$-D2*
AC-83	CCGAGGGGGAAACTCAGCAAGCGCA	
AC-86	TTTATTGGTTATAAAGGAAGAGGCC	*35S:syn-tasiR-FT-D2*
AC-87	CCGAGGCCTCTTCCTTTATAACCAA	
AC-88	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACATAGCGTATTGGTTATAAAGGAAGAGGCC	*35S:syn-tasiR-FT-D4*
AC-89	CCGAGGCCTCTTCCTTTATAACCAATACGCTATGTTGGACTTAGAATAATTTTCTAAGATCCACCGA	
AC-90	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACATAGCGTATTCTAAGTTCAACATATCGACTTGGTTATAAAGGAAGAGGCC	*35S:syn-tasiR-FT-D5*
AC-91	CCGAGGCCTCTTCCTTTATAACCAAGTCGATATGTTGAACTTAGAATACGCTATGTTGGACTTAGAATAATTTTCTAAGATCCACCGA	
AC-92	TTTATTAAGTGTCACGGAAATCCCT	*35S:syn-tasiR-CH42-D2*
AC-93	CCGAAGGGATTTCCGTGACACTTAA	
AC-94	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACATAGCGTATTAAGTGTCACGGAAATCCCT	*35S:syn-tasiR-CH42-D4*
AC-95	CCGAAGGGATTTCCGTGACACTTAATACGCTATGTTGGACTTAGAATAATTTTCTAAGATCCACCGA	
AC-96	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACATAGCGTATTCTAAGTTCAACATATCGACTTAAGTGTCACGGAAATCCCT	*35S:syn-tasiR-CH42-D5*
AC-97	CCGAAGGGATTTCCGTGACACTTAAGTCGATATGTTGAACTTAGAATACGCTATGTTGGACTTAGAATAATTTTCTAAGATCCACCGA	
AC-98	TTTATTGGTTATAAAGGAAGAGGCCTCCCATTCGATACTGCTCGCC	*35S:syn-tasiR-FT-D2-Trich-D3*
AC-99	CCGAGGCGAGCAGTATCGAATGGGAGGCCTCTTCCTTTATAACCAA	
AC-100	TTTATCCCATTCGATACTGCTCGCCTTGGTTATAAAGGAAGAGGCC	*35S:syn-tasiR-Trich-D2-FT-D3*
AC-101	CCGAGGCCTCTTCCTTTATAACCAAGGCGAGCAGTATCGAATGGGA	
AC-102	TTTATGTAAGACGTGATTGTGTCCT	*35S:syn-tasiR-TSWV-D2*
AC-103	CCGAAGGACACAATCACGTCTTACA	
AC-104	TTTATCGGTGGATCTTAGAAAATTATGTAAGACGTGATTGTGTCCT	*35S:syn-tasiR-TSWV-D3*
AC-105	CCGAAGGACACAATCACGTCTTACATAATTTTCTAAGATCCACCGA	
AC-106	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACATAGCGTATGTAAGACGTGATTGTGTCCT	*35S:syn-tasiR-TSWV-D4*
AC-107	CCGAAGGACACAATCACGTCTTACATACGCTATGTTGGACTTAGAATAATTTTCTAAGATCCACCGA	

AC-108	TTTATCGGTGGATCTTAGAAAATTATTCTAAGTCCAACA TAGCGTATTCTAAGTTCAACATATCGACTGTAAGACGTG ATTGTGTCCT	*35S:syn-tasiR-TSWV-D5*
AC-109	CCGAAGGACACAATCACGTCTTACAGTCGATATGTTGAA CTTAGAATACGCTATGTTGGACTTAGAATAATTTTCTAA GATCCACCGA	
AC-114	TTTATCGGTGGATCTTAGAAAATTATTGGTTATAAAGGA AGAGGCC	*35S:syn-tasiR-FT-D3*
AC-115	CCGAGGCCTCTTCCTTTATAACCAATAATTTTCTAAGAT CCACCGA	
AC-116	TTTATCGGTGGATCTTAGAAAATTATTAAGTGTCACGGA AATCCCT	*35S:syn-tasiR-CH42-D3*
AC-117	CCGAAGGGATTTCCGTGACACTTAATAATTTTCTAAGAT CCACCGA	
AC-124	TTTATTGGTTATAAAGGAAGAGGCG	*35S:syn-tasiR-FT-D2-1M*
AC-125	CCGACGCCTCTTCCTTTATAACCAA	
AC-126	TTTATTGGTTATAAAGGAAGAGGGG	*35S:syn-tasiR-FT-D2-2M*
AC-127	CCGACCCCTCTTCCTTTATAACCAA	
AC-128	TTTATTGGTTATAAAGGAAGAGCGG	*35S:syn-tasiR-FT-D2-3M*
AC-129	CCGACCGCTCTTCCTTTATAACCAA	
AC-130	TTTATTGGTTATAAAGGAAGACCGG	*35S:syn-tasiR-FT-D2-4M*
AC-131	CCGACCGGTCTTCCTTTATAACCAA	
AC-132	TTTATTGGTTATAAAGGAAGTCCGG	*35S:syn-tasiR-FT-D2-5M*
AC-133	CCGACCGGACTTCCTTTATAACCAA	
AC-138	TTTATGTAAGACGTGATTGTGTCGT	*35S:syn-tasiR-TSWV-D2-2M*
AC-139	CCGAACGACACAATCACGTCTTACA	
AC-140	TTTATGTAAGACGTGATTGTGTAGT	*35S:syn-tasiR-TSWV-D2-3M*
AC-141	CCGAACTACACAATCACGTCTTACA	
AC-142	TTTATGTAAGACGTGATTGTGAAGT	*35S:syn-tasiR-TSWV-D2-4M*
AC-143	CCGAACTTCACAATCACGTCTTACA	
AC-144	TTTATGTAAGACGTGATTGTCAAGT	*35S:syn-tasiR-TSWV-D2-5M*
AC-145	CCGAACTTGACAATCACGTCTTACA	
AC-156	GGCGAGCAGTATCGAATGGGA	syn-tasiR-Trich probe
AC-158	AGGGATTTCCGTGACACTTAA	Probe for syn-tasiR-Ch42 detection
AC-157	GGCCTCTTCCTTTATAACCAA	Probe for syn-tasiR-FT detection
AC-190	CGCCTCTTCCTTTATAACCAA	Probe for syn-tasiR-FT-1M detection
AC-191	CCCCTCTTCCTTTATAACCAA	Probe for syn-tasiR-FT-2M detection
AC-192	CCGCTCTTCCTTTATAACCAA	Probe for syn-tasiR-FT-3M detection
AC-193	CCGGTCTTCCTTTATAACCAA	Probe for syn-tasiR-FT-4M detection
AC-194	CCGGACTTCCTTTATAACCAA	Probe for syn-tasiR-FT-5M detection
AC-197	ACGACACAATCACGTCTTACA	Probe for syn-tasiR-TSWV-2M detection
AC-198	ACTACACAATCACGTCTTACA	Probe for syn-tasiR-TSWV-3M detection
AC-199	ACTTCACAATCACGTCTTACA	Probe for syn-tasiR-TSWV-4M detection
AC-200	ACTTGACAATCACGTCTTACA	Probe for syn-tasiR-TSWV-5M detection
AC-211	TTTATATTGACCCACACTTTGCCGA	*35S:syn-tasiR-GUS$_{Sly}$-D2*
AC-212	CCGATCGGCAAAGTGTGGGTCAATA	
AC-213	TTTATGCGCTTGCTGAGTTTCCCCCTGCGCTTGCTGAGT TTCCCCC	

AC-214	CCGAGGGGGAAACTCAGCAAGCGCAGGGGGAAACTCAGCAAGCGCA	*35S:syn-tasiR-GUS$_{Ath}$-D2&D3*
AC-267	GCGGGAAGTCCACCACGGTTA	Probe for syn-tasiR-Su detection
AC-278	AAAAAGTCAACAAAACTTAAAGCGGCGGACTCATCGTAATCTCA	*Bsa*I mutagenesis in *MIR173*
AC-284	TATGCTTTGTTCAGATTCGAATCTGACGGACTCCAAATATTTTTC	
AC-288	TTTATAACCGTGGTGGACTTCCCGC	*35S:syn-tasiR-Su, 35S:syn-tasiR-Su/MIR173*
AC-289	CCGAGCGGGAAGTCCACCACGGTTA	
AC-333	TTTATCTTGTAACGCGCTTTCCCAG	*35S:syn-tasiR-GUS$_{Nbe}$-D2*
AC-334	CCGACTGGGAAAGCGCGTTACAAGA	
D-2042	GGCGGGTCTCATCGGTGGATCTTAGAAAATTATTCT	*pENTR-AtTAS1c-B/c-D2*
D-2043	GGCGGGTCTCTTAAATGGTCTATTCGCTTGTAGAGA	
D-2698	GTGATTTCTCTCTGCAAGCGAA	Probe for miR173 detection

Supplementary Text S1. DNA sequence in FASTA format of all *AtTAS1c*-based constructs used to express and analyze syn-tasiRNAs. Sequence corresponding to syn-tasiRNA-1 (position 3'D3[+]) and syn-tasiRNA-2 (position 3'D4[+]) is highlighted in blue and green, respectively. Sequence corresponding miR173 target site, and to tasiRNA D2, D3 and D4 are highlighted in blue, green, dark red and light pink, respectively. All the other sequences from Arabidopsis *TAS1c* gene are highlighted in black.

>AtTAS1c

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CTGTATCGACCATCATATAATTCTGGGTCAAGAGATGAAAATAGAACACCACATCGTAAAGTGAAAT

GTGATTTTTCTCTACAAGCGAA miR173 target site
TCGGTGGATCTTAGAAAATTA tasiRNA D2
TTCTAAGTCCAACATAGCGTA tasiRNA D3
TTCTAAGTTCAACATATCGAC tasiRNA D4

>AtTAS1c-FT-D2-Trich-D3

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AAGAGATGAAAATAGAACACCACATCGTAAAGTGAAAT

Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC
Syn-tasiR-Trich: TCCCATTCGATACTGCTCGCC

>AtTAS1c-Trich-D2-FT-D3

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CTTCTGTTTTGGCTTTGCAATCAGTTATGCTAGTTTTCTTATACCCTTTCGTAAGCTTCCTAAGGAATCGT
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Syn-tasiR-Trich: TCCCATTCGATACTGCTCGCC

Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-Trich-D3-FT-D4

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Syn-tasiR-Trich: TCCCATTCGATACTGCTCGCC

Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-Ft-D3-Trich-D4

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Syn-tasiR-Trich: TCCCATTCGATACTGCTCGCC

Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-GUS$_{Ath}$-D2&D3

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Syn-tasiR-GUS$_{Ath}$: TGCGCTTGCTGAGTTTCCCCC

>AtTAS1c-FT-D2

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Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-FT-D3

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Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-FT-D4

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ACCATCATATAATTCTGGGTCAAGAGATGAAAATAGAACACCACATCGTAAAGTGAAAT
Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-FT-D5

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Syn-tasiR-FT: TTGGTTATAAAGGAAGAGGCC

>AtTAS1c-CH42-D2

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Syn-tasiR-AtCH42: TTAAGTGTCACGGAAATCCCT

>AtTAS1c-CH42-D3

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Syn-tasiR-CH42: TTAAGTGTCACGGAAATCCCT

>AtTAS1c-CH42-D4

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Syn-tasiR-CH42: TTAAGTGTCACGGAAATCCCT

>AtTAS1c-CH42-D5

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>AtTAS1c-GUS$_{Sly}$-D2

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Syn-tasiR-GUS$_{Sly}$: TATTGACCCACACTTTGCCGA

>AtTAS1c-TSWV-D2

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>AtTAS1c-FT-D2-3M

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Syn-tasiR-FT-5M: TTGGTTATAAAGGAAGTCCGG

>AtTAS1c-TSWV-D2-2M

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Syn-tasiR-TSWV-2M: TGTAAGACGTGATTGTGTCGT

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Syn-tasiR-TSWV-3M: TGTAAGACGTGATTGTGTAGT

>AtTAS1c-TSWV-D2-4M

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CTATAGTTAGTTTGAGATTGCGTTTGTCAGAAGTTAGGTTCAATGTCCCGGTCCAATTTTCACCAGCCATG
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CAGTTATGCTAGTTTTCTTATACCCTTTCGTAAGCTTCCTAAGGAATCGTTCATTGATTTCCACTGCTTCA
TTGTATATTAAAACTTTACAACTGTATCGACCATCATATAATTCTGGGTCAAGAGATGAAAATAGAACACC
ACATCGTAAAGTGAAAT

Syn-tasiR-GUS$_{Nb}$: TCTTGTAACGCGCTTTCCCAG

>AtTAS1c-Su-D2

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CAGTTATGCTAGTTTTCTTATACCCTTTCGTAAGCTTCCTAAGGAATCGTTCATTGATTTCCACTGCTTCA
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ACATCGTAAAGTGAAAT

Syn-tasiR-Su: TAACCGTGGTGGACTTCCCGC

Supplementary Text S2. DNA sequence of *Bsa*I-*ccd*B-based (B/c) vectors used for direct cloning of syn-tasiRNAs downstream of 3'D1[+] position in *AtTAS1c*.

>pENTR-AtTAS1c-D2-B/c **(5031 bp)**

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CGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGC
CTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAG
TGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCG
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CAACAGATAAAACGAAAGGCCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCT
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TCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAACAAATTGATGAGCAATG
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ACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

PURPLE/UPPERCASE: M13-F binding site

orange/lowercase: attL1

BLUE/UPPERCASE: *AtTAS1c 5'* region

RED/UPPERCASE: *Bsa*I site

red/lowercase: inverted *Bsa*I site

magenta/lowercase: Chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

blue/lowercase: *AtTAS1c* 3' region

orange/lowercase/underlined: attL2

PURPLE/UPPERCASE/UNDERLINED: M13-R binding site

brown/lowercase: Kanamycin resistance gene

>pMDC32B-AtTAS1c-D2-B/c **(12592 bp)**

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brown/lowercase: kanamycin resistance gene

CYAN/UPPERCASE/UNDERLINED: C->A transversion to block vector's BsaI site

cyan/lowercase: T-DNA right border

GREEN/UPPERCASE: 2x35S CaMV promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *AtTAS1c* 5' region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *Bsa*I site

blue/lowercase: *AtTAS1c* 3' region

ORANGE/UPPERCASE/UNDERLINED: attB2

GREY/UPPERCASE/UNDERLINED: Nos terminator

green/lowercase: CaMV promoter

BROWN/UPPERCASE: hygromycin resistance gene

green/lowercase/underlined: CaMV terminator

CYAN/UPPERCASE: T-DNA left border

>pMDC32B-AtTAS1c-D2-B/c-AtMIR173 **(14339 bp)**

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GCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCTGCCA

brown/lowercase: kanamycin resistance gene

CYAN/UPPERCASE/UNDERLINED: C->A transversion to block vector's BsaI site

cyan/lowercase: T-DNA right border

GREEN/UPPERCASE: 2x35S CaMV promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *AtTAS1c* 5' region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *Bsa*I site

blue/lowercase: *AtTAS1c* 3' region

orange/lowercase: *AtMIR173*

ORANGE/UPPERCASE/UNDERLINED: attB2

GREY/UPPERCASE/UNDERLINED: Nos terminator

green/lowercase: CaMV promoter

BROWN/UPPERCASE: hygromycin resistance gene

green/lowercase/underlined: CaMV terminator

CYAN/UPPERCASE: T-DNA left border