SUPPLEMENTAL MATERIAL

New Generation of Artificial MicroRNA and Synthetic *Trans*-Acting Small Interfering RNA Vectors for Efficient Gene Silencing in Arabidopsis

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Supplemental Figure S1. *AtMIR390a-B/c* vectors for direct cloning of amiRNAs.

Supplemental Figure S2. , Diagrams of *AtMIR319a*, *AtMIR319a-21* and *AtMIR390a* foldbacks used to express several amiRNAs in *N. benthamiana*.

Supplemental Figure S3. Base-pairing of amiRNAs and target mRNAs.

Supplemental Figure S4. *AtTAS1c-B/c* vectors for direct cloning of syn-tasiRNAs.

Supplemental Figure S5. Organization of syn-tasiRNA constructs.

Supplemental Figure S6. Flowering time analysis of Arabidopsis Col-0 T1 transgenic plants expressing amiRNAs or syn-tasiRNAs.

Supplemental Figure S7. Processing analyses of syn-tasiRNAs expressed in Arabidopsis Col-0 T1 transgenic lines *(35S:AtTAS1c-D3Trich-D4Ft* and *35S:AtTAS1c-D3Ft-D4Trich*).

Supplemental Figure S8. Processing and phasing analyses of endogenous *AtTAS1c*-tasiRNA in Arabidopsis Col-0 T1 transgenic lines expressing syn-tasiRNAs *(35S:AtTAS1c-D3Trich-D4Ft*, *35S:AtTAS1c-D3Ft-D4Trich* and *35S:GUS* control).

Supplemental Figure S9. Processing analyses of endogenous *AtTAS1c*-derived siRNAs in Arabidopsis Col-0 T1 transgenic plants expressing syn-tasiRNAs (*35S:AtTAS1c-D3Trich-D4Ft*, *35S:AtTAS1c-D3Ft-D4Trich* and *35S:GUS* control).

Supplemental Table SI. Phenotypic penetrance of amiRNAs expressed in *A. thaliana* Col-0 T1 transgenic plants.

Supplemental Table SII. Phenotypic penetrance of amiRNAs or syn-tasiRNAs expressed in *A. thaliana* Col-0 T1 transgenic plants.

Supplemental Table SIII. Phenotypic penetrance of amiRNAs or syn-tasiRNAs expressed in *A. thaliana* Col-0 T2 transgenic plants.

Supplemental Table SIV. DNA oligonucleotides used in this study.

Supplemental Table SV. Sequences and predicted targets for all the amiRNAs and syn-tasiRNAs used in this study.

Supplemental Table SVI. Summary of high-throughput small RNA libraries from *A. thaliana* transgenic lines.

Supplemental Table SVII. miRBase Locus Identifiers of the Arabidopsis conserved *MIRNA* precursors used in this study.

Supplemental Table SVIII. miRBase Locus Identifiers of those plant *MIRNA* precursors previously used for expressing amiRNAs.

Supplemental Protocol S1. Protocol to design and clone amiRNAs or syn-tasiRNAs in *Bsa*I/*ccd*B-based ('B/c') vectors containing the *AtMIR390a* or *AtTAS1c* precursors, respectively.

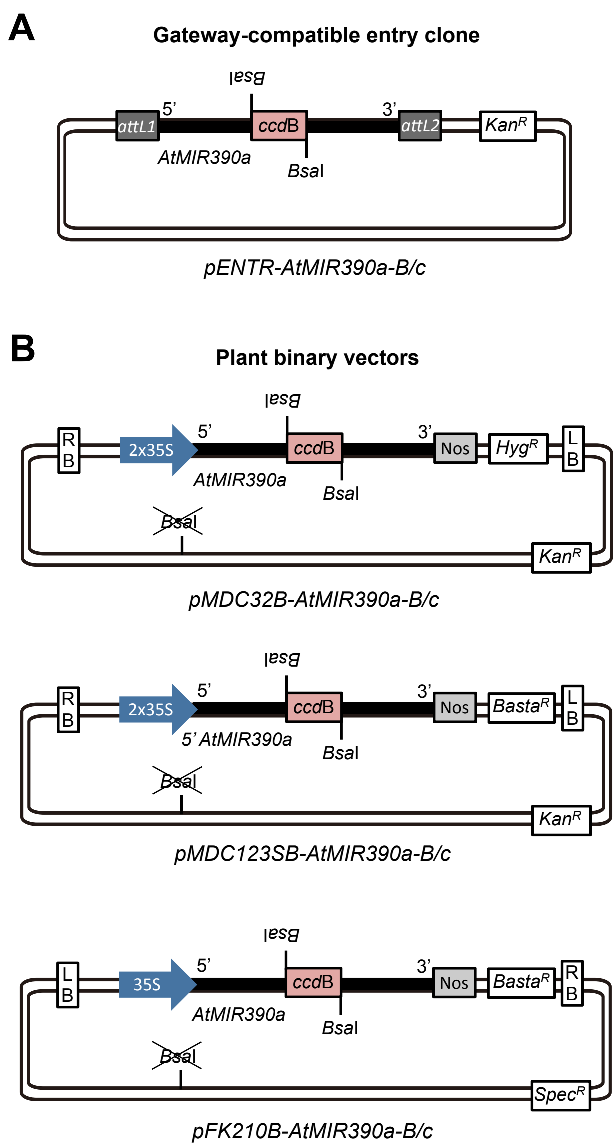
Supplemental Text S1. DNA sequence in FASTA format of all *MIRNA* foldbacks used in this study to express and analyze amiRNAs.

Supplemental Text S2. DNA sequence in FASTA format of all *AtTAS1c*-based constructs used to express and analyze syn-tasiRNAs.

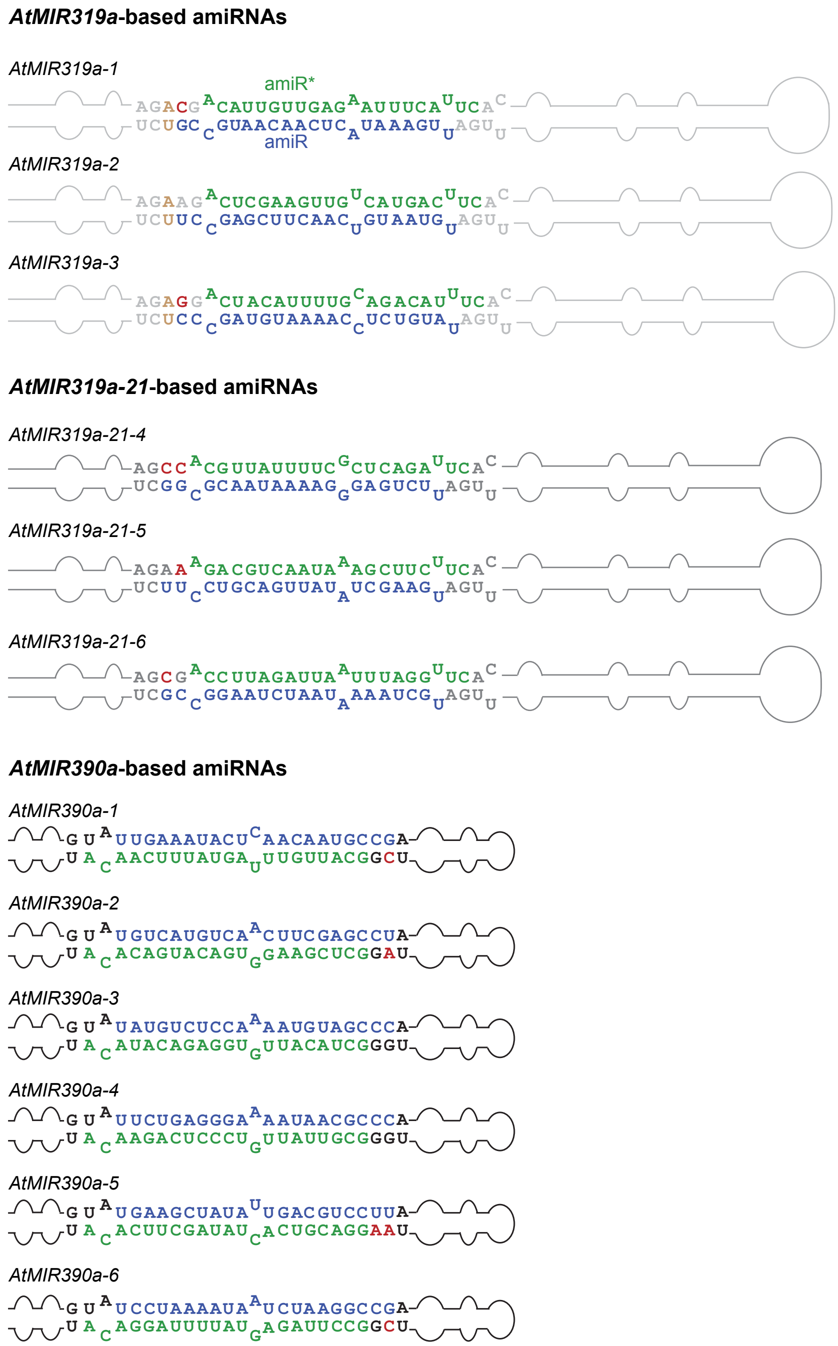
Supplemental Text S3. DNA sequence of *Bsa*I-*ccd*B-based (B/c) vectors used for direct cloning of amiRNAs or syn-tasiRNAs.

Supplemental References.

_AtMIR390a-BsaI/ccd_B-based (B/c) vectors for direct cloning of artificial miRNAs



Supplemental Figure S1. *AtMIR390a-B/c* vectors for direct cloning of amiRNAs. A, Diagram of an *AtMIR390a-B/c* Gateway-compatible entry vector (*pENTR-AtMIR390a-B/c)*. B, Diagrams of *AtMIR390a-B/c*-based binary vectors for expression of amiRNAs in plants (*pMDC32B-AtMIR390a-B/c*, *pMDC123SB-AtMIR390a-B/c* and *pFK210B-AtMIR390a-B/c*). RB: right border; 35S: *Cauliflower mosaic virus* promoter; *Bsa*I: *Bsa*I recognition site, *ccd*B: gene encoding the *ccd*B toxin; LB: left border; attL1 and attL2: gateway recombination sites. *Kan*^{R}: kanamycin resistance gene; *Hyg*^{R}: hygromycin resistance gene; *Basta*^{R}: glufosinate resistance gene; *Spec*^{R}: spectinomycin resistance gene. Undesired *Bsa*I sites removed from the plasmid are crossed out.



Supplemental Figure S2. , Diagrams of *AtMIR319a*, *AtMIR319a-21* and *AtMIR390a* foldbacks used to express several amiRNAs in *N. benthamiana*. Nucleotides corresponding to the miRNA guide and miRNA* are in blue and green, respectively. Other nucleotides from the *AtMIR319a*, *AtMIR319a-21* and *AtMIR390a* foldbacks are in light grey, dark grey, and black, respectively. Nucleotides that were added or modified that are in light brown and red, respectively. Shapes of the *AtMIR319a*, *AtMIR319a-21* and *AtMIR390a* foldbacks are in light grey, dark grey, and black, respectively.

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amiR-Ft      5′ TTGGTTATAAAGGAAGAGGCC 3′
                |||||||||||||||||:|||
target mRNA  3′ AACCAATATTTCCTTCTTCGG 5′
                         FT

amiR-Lfy     5′ TAACAGTGAACGTACTGTCGC 3′
                |||||||||||||||||||||
target mRNA  3′ ATTGTCACTTGCATCACAGCG 5′
                         LFY

amiR-Ch42    5′ TTAAGTGTCACGGAAATCCCT 3′
                 |||||||||||||||||| |
target mRNA  3′ CATTCACAGTGCCTTTAGGAA 5′
                         CH42

amiR-Trich   5′ TCCCATTCGATACTGCTCGCC 3′
                |||||||||||||||||||:|
target mRNA  3′ AGGGTAAGCTATGACGAGTGA 5′
                         TRY

             5′ TCCCATTCGATACTGCTCGCC 3′
                ||||||||||||||:|||:||
             3′ AGGGTAAGCTATGATGAGTGG 5′
                         CPC

             5′ TCCCATTCGATACTGCTCGCC 3′
                ||||||||||| ||:|||:||
             3′ AGGGTAAGCTACGATGAGTGA 5′
                         ETC2
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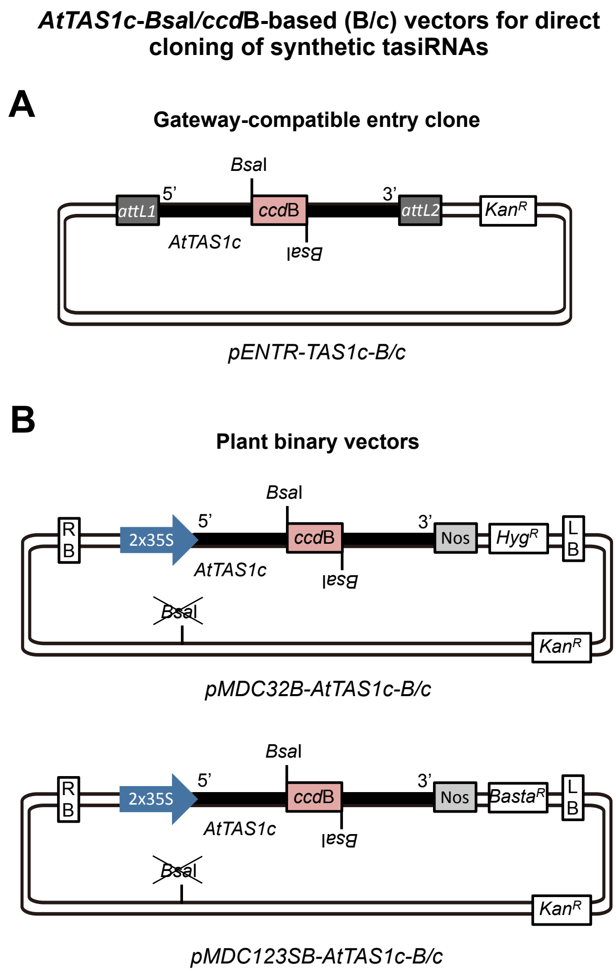
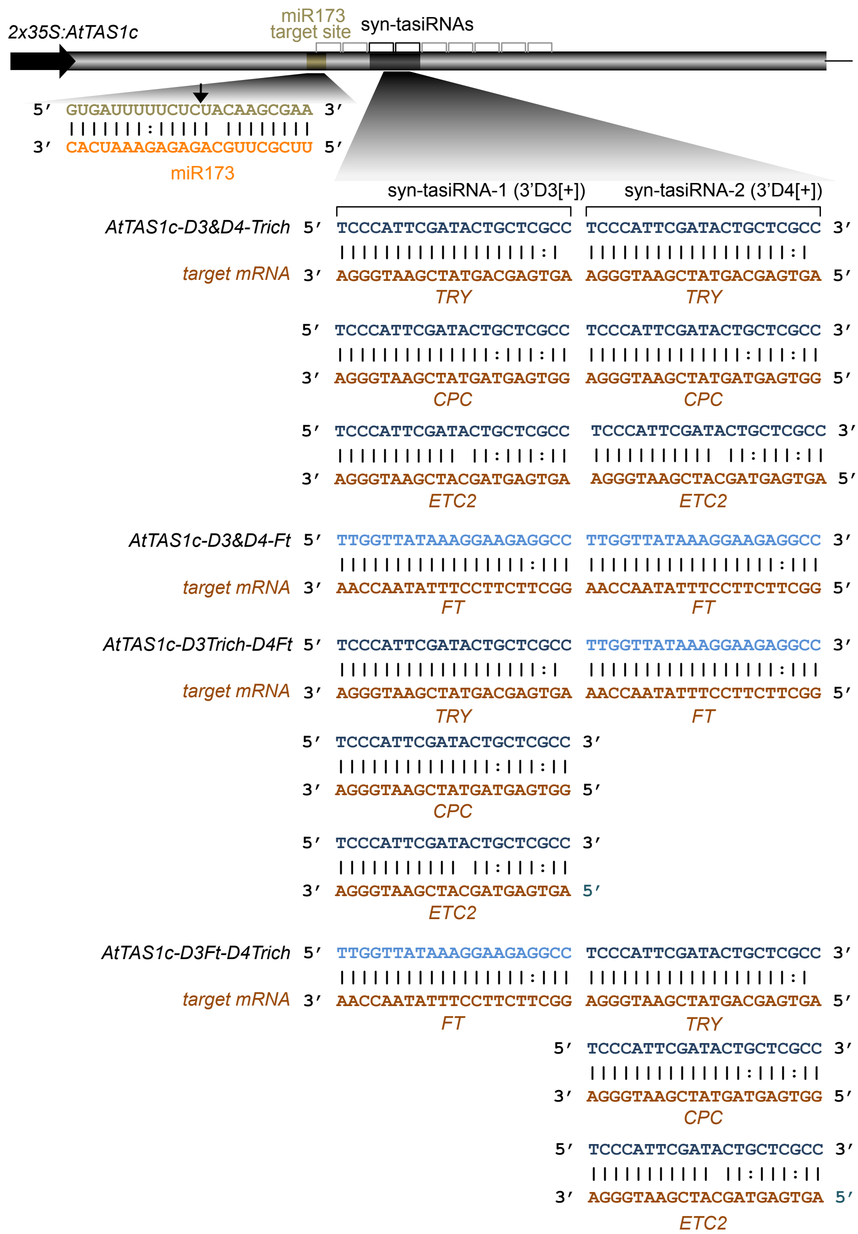
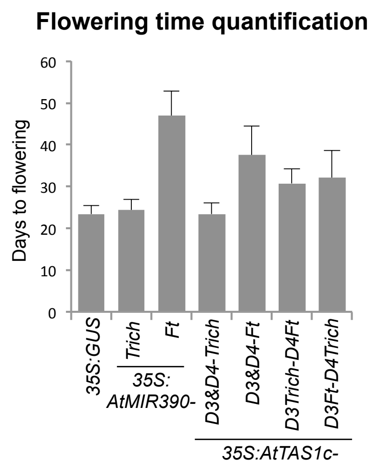
Supplemental Figure S3. Base-pairing of amiRNAs and target mRNAs. amiRNA and mRNA target nucleotides are in blue and brown, respectively.



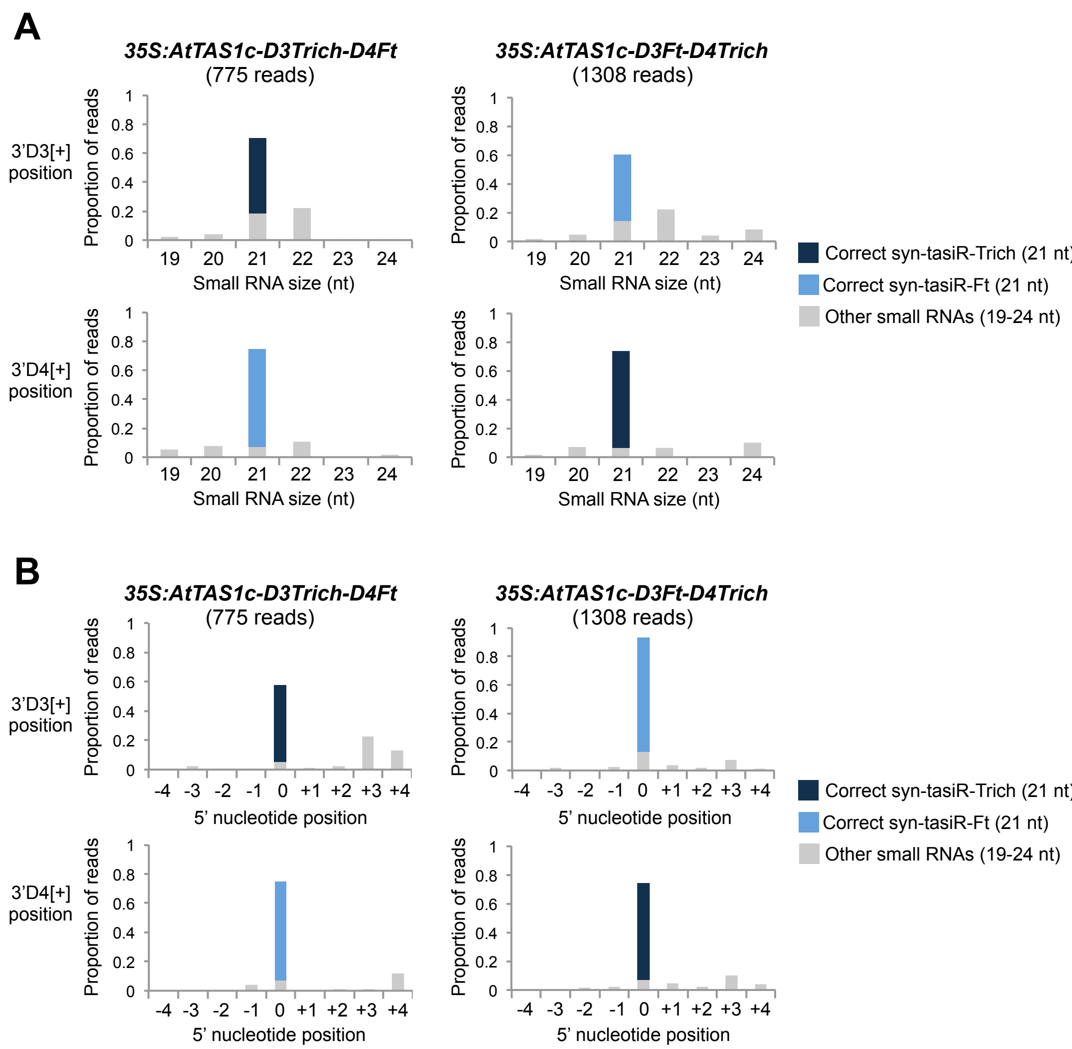
Supplemental Figure S4. *AtTAS1c-B/c* vectors for direct cloning of syn-tasiRNAs. A, Diagram of an *AtTAS1c-B/c* Gateway-compatible entry vector (*pENTR-AtTAS1c-B/c*). B, Diagrams of *AtTAS1c-B/c* binary vectors for expression of syn-tasiRNAs in plants (*pMDC32B-AtTAS1c-B/c*, *pMDC123SB-AtTAS1c-B/c* and *pFK210B-AtTAS1c-B/c*). RB: right border; 35S: *Cauliflower mosaic virus* promoter; *Bsa*I: *Bsa*I recognition site, *ccd*B: gene encoding the *ccd*B toxin; LB: left border; attL1 and attL2: GATEWAY recombination sites. Kan^R: kanamycin resistance gene; Hyg^R: hygromycin resistance gene; $Basta^R$: glufosinate resistance gene; $Spec^R$: spectinomycin resistance gene. Undesired *Bsa*I sites removed from the plasmid are crossed out.



Supplemental Figure S5. A, Organization of syn-tasiRNA constructs. Arrow indicates miR173-guided cleavage site. tasiRNA positions 3'D1[+] to 3'D10[+] are indicated by brackets, with positions 3'D3[+] and 3'D4[+] highlighted in black. The expected syn-tasiRNA-mRNA target interactions are represented. miR173, syn-tasiR-Trich and syn-tasiR-Ft sequences are in orange, dark blue and light blue, respectively. miR173 target site and syn-tasiRNA-mRNA target sequences are in light and dark brown, respectively.

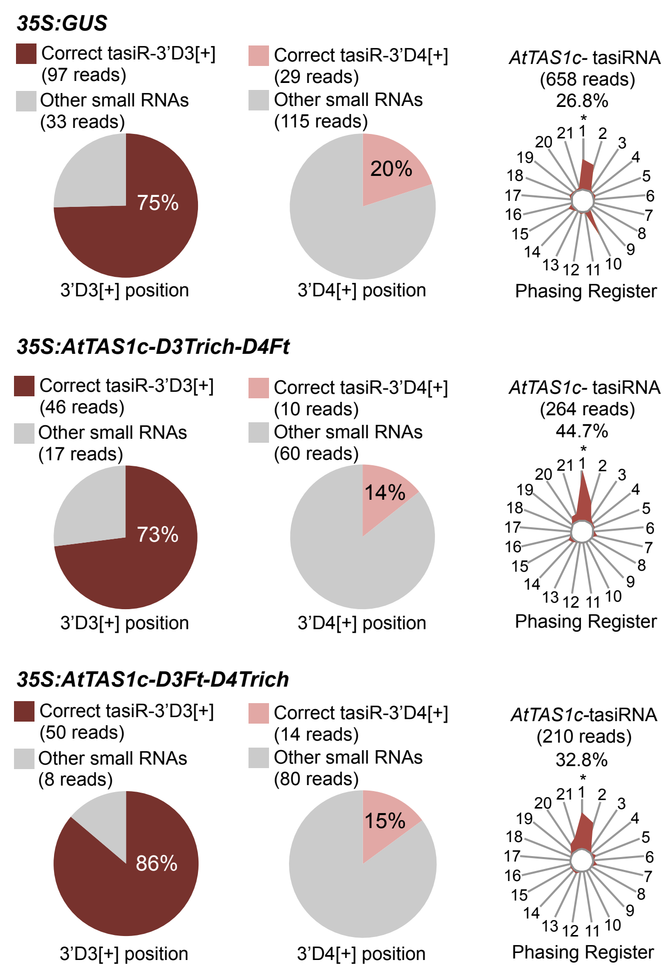


Supplemental Figure S6. Flowering time analysis of Arabidopsis Col-0 T1 transgenic plants expressing amiRNAs or syn-tasiRNAs. Mean (+ s.d.) days to flowering.

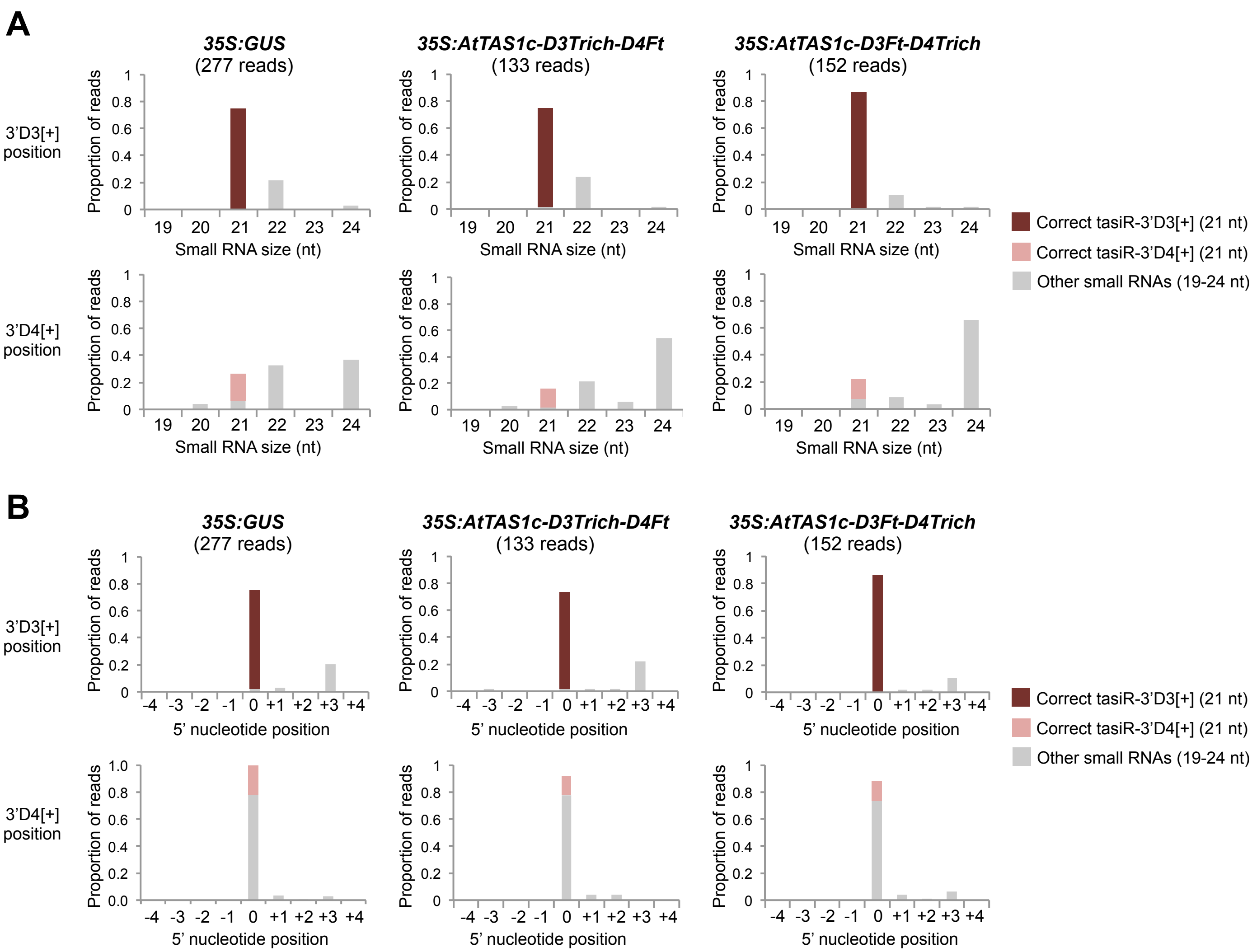


Supplemental Figure S7. Processing analyses of syn-tasiRNAs expressed in Arabidopsis Col-0 T1 transgenic lines *(35S:AtTAS1c-D3Trich-D4Ft* and *35S:AtTAS1c-D3Ft-D4Trich*). A, Small RNA size distribution of 19-24 nt siRNAs in both 3'D3[+] (up) and 3'D4[+] (bottom) positions in *35S:AtTAS1c-D3Trich-D4Ft* (left) and *35S:AtTAS1c-D3Ft-D4Trich* (right) transgenic plants. Correct syn-tasiR-Trich and syn-tasiR-Ft sequences are in dark and light blue, respectively. Other small RNA sequences are in grey. B, Distribution of small RNA reads (19-24 nt) having a 5' nucleotide within a -4/+4 region relative to the correct 5' nucleotide position of the syn-tasiRNA ('0' position). Other details as in panel A.

Endogenous *AtTAS1c*-tasiRNA processing and phasing analyses in Arabidopsis



Supplemental Figure S8. Processing and phasing analyses of endogenous *AtTAS1c*-tasiRNA in Arabidopsis Col-0 T1 transgenic lines expressing syn-tasiRNAs *(35S:AtTAS1c-D3Trich-D4Ft*, *35S:AtTAS1c-D3Ft-D4Trich* and *35S:GUS* control). Analyses of tasiR-3'D3[+] and tasiR-3'D4[+] (*AtTAS1c*-derived) siRNA sequences by high-throughput sequencing. Pie charts, percentage of 19-24 nt reads; radar plots, percentages of 21-nt reads corresponding to each register from *AtTAS1c* transcripts, with position 1 designated as immediately after the miR173-guided cleavage site.



Supplemental Figure S9. Processing analyses of endogenous *AtTAS1c*-derived siRNAs in Arabidopsis Col-0 T1 transgenic plants expressing syn-tasiRNAs (*35S:AtTAS1c-D3Trich-D4Ft*, *35S:AtTAS1c-D3Ft-D4Trich* and *35S:GUS* control). A, Small RNA size distribution of 19-24 nt siRNAs in both 3'D3[+] (up) and 3'D4[+] (bottom) positions in *35S:AtTAS1c-D3Trich-D4Ft* (left) and *35S:AtTAS1c-D3Ft-D4Trich* (right) transgenic plants. Correct tasiR-3'D3[+] and tasiR-3'D4[+] sequences are in dark and light pink, respectively. Other small RNA sequences are in grey. B, Distribution of small RNA reads (19-24 nt) having a 5' nucleotide within a -4/+4 region relative to the correct 5' nucleotide position of the endogenous tasiRNA ('0' position). Other details are as in panel A.

Supplemental Table SI: Phenotypic penetrance of amiRNAs expressed in *A. thaliana* Col-0 T1 transgenic plants

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtMIR390a-Ft	34	100%
35S:AtMIR390a-Lfy	67	34%
35S:AtMIR390a-Ch42	101	97%
		10% weak
		25% intermediate
		62% severe
35S:AtMIR390a-Trich	53	98%
		29% *try cpc* type

[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:GUS* control set.
The Lfy phenotype was defined as a higher 'number of secondary shoots' when compared to the average 'number of secondary shoots' value of the *35S:GUS* control set.
The Ch42 phenotype was scored in 10 days-old seedling and was considered 'weak', 'intermediate' or 'severe' if seedlings have >2 leaves, exactly 2 leaves or no leaves (only 2 cotyledons), respectively.
The Trich phenotype was defined as a higher number of trichomes when compared to transformants of the 3*5S:GUS* control set. Plants with a Trich phenotype were considered *'try cpc* type' if they resembled the Arabidopsis *try cpc* double mutant.

Supplemental Table SII: Phenotypic penetrance of amiRNAs or syn-tasiRNAs expressed in *A. thaliana* Col-0 T1 transgenic plants

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtMIR390-Trich	92	95% 20% *try cpc* type
35S:AtMIR390-Ft	95	95%
35S:TAS1c-D3&D4Trich	73	82% 0% *try cpc* type
35S:TAS1c-D3&D4Ft	47	100%
35S:TAS1c-D3Trich-D4Ft	43	74% Trich 0% *try cpc* type 98% Ft 73% Trich and Ft
35S:TAS1c-D3Ft-D4Trich	68	62% Trich 0% *try cpc* type 100% Ft 62% Trich and Ft

[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:GUS* control set.

The Trich phenotype was defined as a higher number of trichomes when compared to transformants of the 3*5S:GUS* control set. Plants with a Trich phenotype were considered *'try cpc* type' if they resembled the Arabidopsis *try cpc* double mutant.

Supplemental Table SIII: Phenotypic penetrance of amiRNAs or syn-tasiRNAs expressed in *A. thaliana* Col-0 T2 transgenic plants

Construct	T2 analyzed[a]	Phenotypic penetrance[b]
35S:AtMIR390-Trich	10	90% 100% *try cpc* type
35S:TAS1c-D3&D4Trich	10	80% 0% *try cpc* type
35S:TAS1c-D3Trich-D4Ft	10	90% 0% *try cpc* type
35S:TAS1c-D3Ft-D4Trich	10	90% 0% *try cpc* type

[a] 80-100 individuals for each T2 independent line were analyzed.
[b] The Trich phenotype was defined as a higher number of trichomes when compared to transformants of the 3*5S:GUS* control set. Plants with a Trich phenotype were considered *'try cpc* type' if they resembled the Arabidopsis *try cpc* double mutant.

Supplemental Table SIV. DNA oligonucleotides used.

Oligonucleotide Name	Sequence
3'PCR primer i1	CAAGCAGAAGACGGCATACGAACATCGATTGATGGTGCCTACAG
3'PCR primer i3	CAAGCAGAAGACGGCATACGACATCTGATTGATGGTGCCTACAG
3'PCR primer i4	CAAGCAGAAGACGGCATACGAAACGTAATTGATGGTGCCTACAG
3'PCR primer i5	CAAGCAGAAGACGGCATACGATGGTAAATTGATGGTGCCTACAG
3'PCR primer i9	CAAGCAGAAGACGGCATACGAATTGGCATTGATGGTGCCTACAG
5'PCR primer P5	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA
AtMIR319a-1-I	GATTGAAATACTCAACAATGCCGTCTCTCTTTTGTATTCC
AtMIR319a-1-II	GACGGCATTGTTGAGTATTTCAATCAAAGAGAATCAATGA
AtMIR319a-1-III	GACGACATTGTTGAGAATTTCATTCACAGGTCGTGATATG
AtMIR319a-1-IV	GAATGAAATTCTCAACAATGTCGTCTACATATATATTCCT
AtMIR319a-2-I	GATGTCATGTCAACTTCGAGCCTTCTCTCTTTTGTATTCC
AtMIR319a-2-II	GAAGGCTCGAAGTTGACATGACATCAAAGAGAATCAATGA
AtMIR319a-2-III	GAAGACTCGAAGTTGTCATGACTTCACAGGTCGTGATATG
AtMIR319a-2-IV	GAAGTCATGACAACTTCGAGTCTTCTACATATATATTCCT
AtMIR319a-3-I	GATATGTCTCCAAAATGTAGCCCTCTCTCTTTTGTATTCC
AtMIR319a-3-II	GAGGGCTACATTTTGGAGACATATCAAAGAGAATCAATGA
AtMIR319a-3-III	GAGGACTACATTTTGCAGACATTTCACAGGTCGTGATATG
AtMIR319a-3-IV	GAAATGTCTGCAAAATGTAGTCCTCTACATATATATTCCT
AtMIR319a-4-I	GATTCTGAGGGAAAATAACGCGGCTCTCTTTTGTATTCCAATT
AtMIR319a-4-II	GCCGCGTTATTTTCCCTCAGAATCAAAGAGAATCAATGATCC
AtMIR319a-4-III	GCCACGTTATTTTCGCTCAGATTCACAGGTCGTGATATGAT
AtMIR319a-4-IV	GAATCTGAGCGAAAATAACGTGGCTACATATATATTCCTAAAACG
AtMIR319a-5-I	GATGAAGCTATATTGACGTCCTTCTCTCTTTTGTATTCCAATT
AtMIR319a-5-II	GAAGGACGTCAATATAGCTTCATCAAAGAGAATCAATGATCC
AtMIR319a-5-III	GAAAGACGTCAATAAAGCTTCTTCACAGGTCGTGATATGAT
AtMIR319a-5-IV	GAAGAAGCTTTATTGACGTCTTTCTACATATATATTCCTAAAACG
AtMIR319a-6-I	GATCCTAAAATAATCTAAGGCCGCTCTCTTTTGTATTCCAATT
AtMIR319a-6-II	GCGGCCTTAGATTATTTTAGGATCAAAGAGAATCAATGATCC
AtMIR319a-6-III	GCGACCTTAGATTAATTTAGGTTCACAGGTCGTGATATGAT
AtMIR319a-6-IV	GAACCTAAATTAATCTAAGGTCGCTACATATATATTCCTAAAACG
AtMIR319a-F	CTGCAAGGCGATTAAGTTGGGTAAC
AtMIR319a-R	GCGGATAACAATTTCACACAGGAAACAG
AtMIR390a-F	CACCTATAGGGGGGAAAAAAAGGTAG
AtMIR390a-R	GAGACTAAAGATGAGATCTAATC
AtMIR390a-1-F	TGTATTGAAATACTCAACAATGCCGATGATGATCACATTCGTTATCTATTTTTTCGGCATTGTTTAGTATTTCAA
AtMIR390a-1-R	AATGTTGAAATACTAAACAATGCCGAAAAAATAGATAACGAATGTGATCATCATCGGCATTGTTGAGTATTTCAA
AtMIR390a-2-F	TGTATGTCATGTCAACTTCGAGCCTATGATGATCACATTCGTTATCTATTTTTTAGGCTCGAAGGTGACATGACA
AtMIR390a-2-R	AATGTGTCATGTCACCTTCGAGCCTAAAAAATAGATAACGAATGTGATCATCATAGGCTCGAAGTTGACATGACA
AtMIR390a-3-F	TGTATATGTCTCCAAAATGTAGCCCATGATGATCACATTCGTTATCTATTTTTTGGGCTACATTGTGGAGACATA
AtMIR390a-3-R	AATGTATGTCTCCACAATGTAGCCCAAAAAATAGATAACGAATGTGATCATCATGGGCTACATTTTGGAGACATA
AtMIR390a-4-F	TGTATTCTGAGGGAAAATAACGCGGATGATGATCACATTCGTTATCTATTTTTTCCGCGTTATTGTCCCTCAGAA
AtMIR390a-4-R	AATGTTCTGAGGGACAATAACGCGGAAAAAATAGATAACGAATGTGATCATCATCCGCGTTATTTTCCCTCAGAA
AtMIR390a-5-F	TGTATGAAGCTATATTGACGTCCTTATGATGATCACATTCGTTATCTATTTTTTAAGGACGTCACTATAGCTTCA
AtMIR390a-5-R	AATGTGAAGCTATAGTGACGTCCTTAAAAAATAGATAACGAATGTGATCATCATAAGGACGTCAATATAGCTTCA
AtMIR390a-6-F	TGTATCCTAAAATAATCTAAGGCCGATGATGATCACATTCGTTATCTATTTTTTCGGCCTTAGAGTATTTTAGGA
AtMIR390a-6-R	AATGTCCTAAAATACTCTAAGGCCGAAAAAATAGATAACGAATGTGATCATCATCGGCCTTAGATTATTTTAGGA
AtMIR390a-B/c-F	GTTGTTTGTAAGAGACCATTAGGCACCCCAGGCTTTACAC
AtMIR390a-B/c-R	GTTGTTAATGTGAGACCGTCGAGGTGCAGACTGGCTGTG
AtMIR390a-Ch42-F	TGTATTAAGTGTCACGGAAATCCCTATGATGATCACATTCGTTATCTATTTTTTAGGGATTTCCTTGACACTTAA
AtMIR390a-Ch42-R	AATGTTAAGTGTCAAGGAAATCCCTAAAAAATAGATAACGAATGTGATCATCATAGGGATTTCCGTGACACTTAA
AtMIR390a-Ft-F	TGTATTGGTTATAAAGGAAGAGGCCATGATGATCACATTCGTTATCTATTTTTTGGCCTCTTCCGTTATAACCAA
AtMIR390a-Ft-R	AATGTTGGTTATAACGGAAGAGGCCAAAAAATAGATAACGAATGTGATCATCATGGCCTCTTCCTTTATAACCAA
AtMIR390a-Lfy-F	TGTATAACAGTGAACGTACTGTCGCATGATGATCACATTCGTTATCTATTTTTTGCGACAGTACTTTCACTGTTA
AtMIR390a-Lfy-R	AATGTAACAGTGAAAGTACTGTCGCAAAAAATAGATAACGAATGTGATCATCATGCGACAGTACGTTCACTGTTA
AtMIR390a-Trich-F	TGTATCCCATTCGATACTGCTCGCCATGATGATCACATTCGTTATCTATTTTTTGGCGAGCAGTCTCGAATGGGA
AtMIR390a-Trich-R	AATGTCCCATTCGAGACTGCTCGCCAAAAAATAGATAACGAATGTGATCATCATGGCGAGCAGTATCGAATGGGA
AtTAS1c-F	CACCAAACCTAAACCTAAACGGCTAA
AtTAS1c-R	ATTTCACTTTACGATGTGGTGTT
AtTAS1c-D3&D4Ft-F	ATTATTGGTTATAAAGGAAGAGGCCTTGGTTATAAAGGAAGAGGCC
AtTAS1c-D3&D4Ft-R	GTTCGGCCTCTTCCTTTATAACCAAGGCCTCTTCCTTTATAACCAA
AtTAS1c-D3&D4Trich-F	ATTATCCCATTCGATACTGCTCGCCTCCCATTCGATACTGCTCGCC
AtTAS1c-D3&D4Trich-R	GTTCGGCGAGCAGTATCGAATGGGAGGCGAGCAGTATCGAATGGGA

Oligonucleotide Name	Sequence
AtTAS1c-D3Ft-D4Trich-F	ATTATTGGTTATAAAGGAAGAGGCCTCCCATTCGATACTGCTCGCC
AtTAS1c-D3Ft-d4Trich-R	GTTCGGCGAGCAGTATCGAATGGGAGGCCTCTTCCTTTATAACCAA
AtTAS1c-D3Trich-D4Ft-F	ATTATCCCATTCGATACTGCTCGCCTTGGTTATAAAGGAAGAGGCC
AtTAS1c-D3Trich-D4Ft-F	GTTCGGCCTCTTCCTTTATAACCAAGGCGAGCAGTATCGAATGGGA
BsaI-AtMIR390a-3'-F	ATCTGTAAGAGACCGTTGTTGGTCTCACATTGGCTCTTCTTACTACAATG
BsaI-AtMIR390a-5'-R	GAGCCAATGTGAGACCAACAACGGTCTCTTACAGATTCTTCTCTACTTTG
BsaI-AtTAS1c-3'-F	AAAATTAAGAGACCGTTGTTGGTCTCAGAACTAGAAAAGACATTGGACAT
BsaI-AtTAS1c-5'-R	TTCTAGTTCTGAGACCAACAACGGTCTCTTAATTTTCTAAGATCCACCGA
Probe-amiR-1	CGGCATTGTTGAGTATTTCAA
Probe-amiR-2	AGGCTCGAAGTTGACATGACA
Probe-amiR-3	GGGCTACATTTTGGAGACATA
Probe-amiR-4	CCGCGTTATTTTCCCTCAGAA
Probe-amiR-5	AAGGACGTCAATATAGCTTCA
Probe-amiR-6	CGGCCTTAGATTATTTTAGGA
Probe-amiR-Ch42	AGGGATTTCCGTGACACTTAA
Probe-amiR-Lfy	GCGACAGTACGTTCACTGTTA
Probe-amiR/syn-tasiR-Ft	GGCCTCTTCCTTTATAACCAA
Probe-amiR/syn-tasiR-Trich	GGCGAGCAGTATCGAATGGGA
Probe-U6	AGGGGCCATGCTAATCTTCTC
qAtACT2-F	AAAAATGGCTGAGGCTGATGA
qAtACT2-R	GAAAAACAGCCCTGGGAGC
qAtCBP20-F	AGCTGCGCCAACGAATTATG
qAtCBP20-R	TCCATGGCGATTTTGTCCTC
qAtCH42-CS-F	CATGCACAAGTAGGGACGGTT
qAtCH42-CS-R	GTCACGGAAATCCTTTGGGTT
qAtCPC-CS-F	TCGAATGGGAAGCTGTGAAGA
qAtCPC-CS-R	GCGATCAACTCCCACCTGTC
qAtETC2-CS-F	GCGGTCCCAGTCTTAGGCA
qAtETC2-CS-R	TTCGATGCTACTCACTTCTTCAGAGT
qAtFT-F	TGGAACAACCTTTGGCAATG
qAtFT-R	CGACACGATGAATTCCTGCA
qAtLFY-F	CCAAGGTGACGAACCAAGTATTC
qAtLFY-R	AGGCAGTGGAGAGCGTAACAG
qAtSAND-F	CTCAAAGATTGCAGGGTACGC
qAtSAND-R	TCTTCAACACGCATTCCACCT
qAtTRY-CS-F	ACACAAAATCGCCCTCCATG
qAtTRY-CS-R	TCAAATCCCACCTATCACCGA
qAtUBQ10-F	CGCCTGCAAAGTGACTCGA
qAtUBQ10-R	CCAACAGCTCAACACTTTCGC

Supplemental Table SV. Sequences and predicted targets for all the amiRNA and syn-tasiRNA sequences used in this study.

Cassette Name	small RNA name	small RNA class	Foldback/ Transcript	small RNA sequence (5'->3')	Reference	Predicted target(s)
AtMIR319-1	*amiR-1*	*amiRNA*	*AtMIR319a*	UUGAAAUACUCAACAAUGCCG	This work	*AGO2, AGO3*
AtMIR319-2	*amiR-2*	*amiRNA*	*AtMIR319a*	UGUCAUGUCAACUUCGAGCCU	This work	*RDR3*, *RDR4, RDR5*
AtMIR319-3	*amiR-3*	*amiRNA*	*AtMIR319a*	UAUGUCUCCAAAAUGUAGCCC	This work	*RDR3*, *RDR4, RDR5*
AtMIR319-4	*amiR-4*	*amiRNA*	*AtMIR319a-21*	UUCUGAGGGAAAAUAACGCGG	This work	*RDR6*
AtMIR319-5	*amiR-5*	*amiRNA*	*AtMIR319a-21*	UGAAGCUAUAUUGACGUCCUU	This work	*RDR6*
AtMIR319-6	*amiR-6*	*amiRNA*	*AtMIR319a-21*	UCCUAAAAUAAUCUAAGGCCG	This work	*RDR6*
AtMIR390a-1	*amiR-1*	*amiRNA*	*AtMIR390a*	UUGAAAUACUCAACAAUGCCG	This work	*AGO2, AGO3,*
AtMIR390a-2	*amiR-2*	*amiRNA*	*AtMIR390a*	UGUCAUGUCAACUUCGAGCCU	This work	*RDR3*, *RDR4, RDR5*
AtMIR390a-3	*amiR-3*	*amiRNA*	*AtMIR390a*	UAUGUCUCCAAAAUGUAGCCC	This work	*RDR3*, *RDR4, RDR5*
AtMIR390a-4	*amiR-4*	*amiRNA*	*AtMIR390a*	UUCUGAGGGAAAAUAACGCGG	This work	*RDR6*
AtMIR390a-5	*amiR-5*	*amiRNA*	*AtMIR390a*	UGAAGCUAUAUUGACGUCCUU	This work	*RDR6*
AtMIR390a-6	*amiR-6*	*amiRNA*	*AtMIR390a*	UCCUAAAAUAAUCUAAGGCCG	This work	*RDR6*
AtMIR390a-Ft	*amiR-Ft*	*amiRNA*	*AtMIR390a*	UUGGUUAUAAAGGAAGAGGCC	Schwabb et al., 2006	*FT*
AtMIR390a-Lfy	*amiR-Lfy*	*amiRNA*	*AtMIR390a*	UAACAGUGAACGUACUGUCGC	Schwabb et al., 2006	*LFY*
AtMIR390a-Ch42	*amiR-Ch42*	*amiRNA*	*AtMIR390a*	UUAAGUGUCACGGAAAUCCCU	Felippes and Weigel, 2009	*CH42*
AtMIR390a-Trich	*amiR-Trich*	*amiRNA*	*AtMIR390a*	UCCCAUUCGAUACUGCUCGCC	Schwabb et al., 2006	*TRY*, *CPC*, *ETC2*
AtTAS1c-d3&d4Trich	*syn-tasiR-Trich*	*syn-tasiRNA*	*AtTAS1c*	UCCCAUUCGAUACUGCUCGCC	Schwabb et al., 2006	*TRY*, *CPC*, *ETC2*
AtTAS1c-d3&d4Ft	*syn-tasiR-Ft*	*syn-tasiRNA*	*AtTAS1c*	UUGGUUAUAAAGGAAGAGGCC	Schwabb et al., 2006	*FT*
AtTAS1c-d3Trich-d4Ft	*syn-tasiR-Trich*	*syn-tasiRNA*	*AtTAS1c*	UCCCAUUCGAUACUGCUCGCC	Schwabb et al., 2006	*TRY*, *CPC*, *ETC2*
	syn-tasiR-Ft	*syn-tasiRNA*	*AtTAS1c*	UUGGUUAUAAAGGAAGAGGCC	Schwabb et al., 2006	*FT*
AtTAS1c-d3Ft-d4Trich	*syn-tasiR-Ft*	*syn-tasiRNA*	*AtTAS1c*	UUGGUUAUAAAGGAAGAGGCC	Schwabb et al., 2006	*FT*
	syn-tasiR-Trich	*syn-tasiRNA*	*AtTAS1c*	UCCCAUUCGAUACUGCUCGCC	Schwabb et al., 2006	*TRY*, *CPC*, *ETC2*

Supplemental Table SVI. Summary of high-throughput small RNA libraries from *A. thaliana* transgenic lines

Sample ID	Construct	3'PCR primer	Barcode Sequence	Adaptor-parsed reads
1	*35S:AtMIR390a-Ft*	i3	CAGATG	31,046,134
2	*35S:AtMIR390a-Lfy*	i5	TTACCA	33,795,367
3	*35S:AtMIR390a-Ch42*	i9	GCCAAT	19,417,667
4	*35S:AtMIR390a-Trich*	i1	CGATGT	30,544,221
5	*35S:GUS*	i1	CGATGT	17,503,977
6	*35S:AtTAS1c-D3Trich-D4Ft*	i4	TACGTT	25,061,705
7	*35S:AtTAS1c-D3Ft-D4Trich*	i5	TTACCA	25,777,455

Supplemental Table SVII. miRbase Locus Identifiers of the Arabidopsis conserved *MIRNA* precursors used in this study.	
MIRNA precursor	Locus Identifier
Ath-MIR156a	MI0000178
Ath-MIR156b	MI0000179
Ath-MIR156c	MI0000180
Ath-MIR156d	MI0000181
Ath-MIR156e	MI0000182
Ath-MIR156f	MI0000183
Ath-MIR156g	MI0001082
Ath-MIR156h	MI0001083
Ath-MIR157a	MI0000184
Ath-MIR157b	MI0000185
Ath-MIR157c	MI0000186
Ath-MIR157d	MI0000187
Ath-MIR159a	MI0000189
Ath-MIR159b	MI0000218
Ath-MIR159c	MI0001085
Ath-MIR160a	MI0000190
Ath-MIR160b	MI0000191
Ath-MIR160c	MI0000192
Ath-MIR162a	MI0000194
Ath-MIR162b	MI0000195
Ath-MIR164a	MI0000197
Ath-MIR164b	MI0000198
Ath-MIR164c	MI0001087
Ath-MIR165a	MI0000199
Ath-MIR165b	MI0000200
Ath-MIR166a	MI0000201
Ath-MIR166b	MI0000202
Ath-MIR166c	MI0000203
Ath-MIR166d	MI0000204
Ath-MIR166e	MI0000205
Ath-MIR166f	MI0000206
Ath-MIR166g	MI0000207
Ath-MIR167a	MI0000208
Ath-MIR167b	MI0000209
Ath-MIR167c	MI0001088
Ath-MIR167d	MI0000975
Ath-MIR168a	MI0000210
Ath-MIR168b	MI0000211
Ath-MIR169a	MI0000212
Ath-MIR169b	MI0000976
Ath-MIR169c	MI0000977
Ath-MIR169d	MI0000978
Ath-MIR169e	MI0000979
Ath-MIR169f	MI0000980
Ath-MIR169g	MI0000981
Ath-MIR169h	MI0000982
Ath-MIR169i	MI0000983
Ath-MIR169j	MI0000984
Ath-MIR169k	MI0000985
Ath-MIR169l	MI0000986
Ath-MIR169m	MI0000987
Ath-MIR169n	MI0000988
Ath-MIR170	MI0000213

MIRNA precursor	Locus Identifier
Ath-MIR171a	MI0000214
Ath-MIR171b	MI0000989
Ath-MIR171c	MI0000990
Ath-MIR172a	MI0000215
Ath-MIR172b	MI0000216
Ath-MIR172c	MI0000991
Ath-MIR172d	MI0000992
Ath-MIR172e	MI0001089
Ath-MIR173	MI0000217
Ath-MIR319a	MI0000544
Ath-MIR319b	MI0000545
Ath-MIR319c	MI0001086
Ath-MIR390a	MI0001000
Ath-MIR390b	MI0001001
Ath-MIR391	MI0001002
Ath-MIR393a	MI0001003
Ath-MIR393b	MI0001004
Ath-MIR394a	MI0001005
Ath-MIR394b	MI0001006
Ath-MIR395a	MI0001007
Ath-MIR395b	MI0001008
Ath-MIR395c	MI0001009
Ath-MIR395d	MI0001010
Ath-MIR395e	MI0001011
Ath-MIR395f	MI0001012
Ath-MIR396a	MI0001013
Ath-MIR396b	MI0001014
Ath-MIR397a	MI0001015
Ath-MIR397b	MI0001016
Ath-MIR398a	MI0001017
Ath-MIR398b	MI0001018
Ath-MIR398c	MI0001019
Ath-MIR399a	MI0001020
Ath-MIR399b	MI0001021
Ath-MIR399c	MI0001022
Ath-MIR399d	MI0001023
Ath-MIR399e	MI0001024
Ath-MIR399f	MI0001025
Ath-MIR408	MI0001080
Ath-MIR827	MI0005383

Supplemental Table SVIII. miRBase Locus Identifiers of those plant *MIRNA* precursors previously used for expressing amiRNAs.

MIRNA precursor	Plant Species	Locus Identifier	Original Reference
Ath-MIR159a	*Arabidopsis thaliana*	MI0000189	Niu et al. 2006
Ath-MIR159b	*Arabidopsis thaliana*	MI0000218	Eamens et al. 2011
Ath-MIR164a	*Arabidopsis thaliana*	MI0000197	Alvarez et al. 2006
Ath-MIR164b	*Arabidopsis thaliana*	MI0000198	Alvarez et al. 2006
Ath-MIR169d	*Arabidopsis thaliana*	MI0000978	Liu et al. 2010
Ath-MIR171a	*Arabidopsis thaliana*	MI0000214	Qu et al. 2007
Ath-MIR172a	*Arabidopsis thaliana*	MI0000215	Schwab et al. 2006
Ath-MIR319a	*Arabidopsis thaliana*	MI0000544	Schwab et al. 2006
Ath-MIR390a	*Arabidopsis thaliana*	MI0001000	Montgomery et al. 2008
Ath-MIR395a	*Arabidopsis thaliana*	MI0001007	Liang et al. 2012
Cre-MIR1157	*Chlamydomonas reinhardtii*	MI0006219	Zhao et al. 2009
Cre-MIR1162	*Chlamydomonas reinhardtii*	MI0006223	Molnar et al. 2009
Ghb-MIR169a	*Gossypium herbaceum*	MI0005646	Ali et al. 2013
Osa-MIR528	*Oryza sativa*	MI0003201	Warthmann et al. 2008
Ptc-MIR408	*Populus trichocarpa*	MI0002352	Shi et al. 2010
Sly-MIR159	*Solanum lycopersicum*	MI0009974	Vu et al. 2013
Sly-MIR168a	*Solanum lycopersicum*	MI0024352	Vu et al. 2013

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Supplemental Protocol S1

Protocol to design and clone amiRNAs or syn-tasiRNAs in *Bsa*I/*ccd*B-based ('B/c') vectors containing *AtMIR390a* or *AtTAS1c* precursors, respectively.

INDEX

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

A link to a web tool for automated design of the amiRNA or syn-tasiRNA sequence(s) will be available at http://p-sams.carringtonlab.org/

2. Design of amiRNA or syn-tasiRNA oligonucleotides

A link to a web tool for automated design of the amiRNA or syn-tasiRNA oligonucleotide sequences will be available at http://p-sams.carringtonlab.org/

2.1 Design of amiRNA oligonucleotides

2.1.1 Sequence of the *AtMIR390a* cassette containing the amiRNA

The following FASTA sequence includes the amiRNA sequence inserted in the *AtMIR390a* precursor sequence:

>amiRNA in *AtMIR390a* precursor

TATAGGGGGAAAAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAATGAATA

ATTTCACGTTTAACGAAGAGGAGATGACGTGTGTTCCTTCGAACCCGAGTTTTGTTCGTCTATA

AATAGCACCTTCTCTTCTCCTTCTTCCTCACTTCCATCTTTTTAGCTTCACTATCTCTCTATAA

TCGGTTTTATCTTTCTCTAAGTCACAACCCAAAAAAACAAA**GTAGAGAAGAATCTGTA**$X_1X_2X_3X_4$

$X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$**ATGATGATCACATTCGTTATCTATTTTT**

T$X_1X_2X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}$**CATTGGCTCTTCTTAC**TACAAT

GAAAAAGGCCGAGGCAAAACGCCTAAAATCACTTGAGAATCAATTCTTTTTACTGTCCATTTAA

GCTATCTTTTATAAACGTGTCTTATTTTCTATCTCTTTTGTTTAAACTAAGAAACTATAGTATT

TTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATCTTTAGTCTC

Where:

- -**X** is a DNA base of the amiRNA sequence, and the subscript number is the base position in the amiRNA 21-mer
- -**X** is a DNA base of the amiRNA* sequence, and the subscript number is the base position in the amiRNA* 21-mer
- -**X** is a DNA base of the *AtMIR390a* foldback
- -**X** is a DNA base of the *AtMIR390a* foldback included in the oligonucleotides required to clone the amiRNA insert in B/c vectors
- -**X** is a DNA base of the *AtMIR390a* foldback that may be modified to preserve the authentic *AtMIR390a* duplex structure

-X is a DNA base of the *AtMIR390a* precursor

In the sequence above:

-Insert the amiRNA sequence where you see

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

-Insert the amiRNA* sequence that has to verify the following base-pairing:

$X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7\ X_8\ X_9\ X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}\ X_{19}\ X_{20}X_{21}$

| | | | | | | | | | | | | | | | | | | |

$X_{19}X_{18}X_{17}X_{16}X_{15}X_{14}X_{13}X_{12}X_{11}X_{10}X_9\ X_8\ X_7\ X_6\ X_5\ X_4\ X_3\ X_2\ X_1\ X_2\ X_1$

Note that:

-In general, **X_1=T** for amiRNA association with AGO1. In this case, **X_{19}=A**

-Bases **X_{11}** and **X_9** DO NOT base-pair to preserve the central bulge of the authentic *AtMIR390a* duplex. The following base-pair rule applies:

-If X_{11}=G, then X_9=A

-If X_{11}=C, then X_9=T

-If X_{11}=A, then X_9=G

-If X_{11}=U, then X_9=C

2.1.2. Sequence of the amiRNA oligonucleotides

The sequences of the two amiRNA oligonucleotides are:

-Forward oligonucleotide (75 b),

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

TTCGTTATCTATTTTTT$X_1X_2X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}$

-Reverse oligonucleotide (75 b),

AATG$Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1Y_2Y_1$**AAAAAATGATAACG**

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

Where:

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

-$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}$=partial amiRNA* sequence

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

-**TG**$Y_{19}Y_{18}Y_{17}Y_{16}Y_{15}Y_{14}Y_{13}Y_{12}Y_{11}Y_{10}Y_9Y_8Y_7Y_6Y_5Y_4Y_3Y_2Y_1$=amiRNA* reverse-complement sequence

-X_1X_2 = *AtMIR390a* sequence that may be modified to preserve authentic *AtMIR390a* duplex structure.

-Y_2Y_1 = reverse-complement of X_1X_2

Example:

The sequences of the two oligonucleotides to clone the amiRNA 'amiR-Trich' (TCCCATTCGATACTGCTCGCC) are:

-Sense oligonucleotide (75 b),

TGTATCCCATTCGATACTGCTCGCCATGATGATCACATTCGTTATCTATTTTTTGGCG AGCAGTCTCGAATGGGA

-Antisense oligonucleotide (75 b),

AATGTCCCATTCGAGACTGCTCGCCAAAAAATAGATAACGAATGTGATCATCATGGCG AGCAGTATCGAATGGGA

Note: *the 75 b long oligonucleotides can be ordered PAGE-purified, although oligonucleotides of 'Standard Desalting' quality work well.*

2.2 Design of syn-tasiRNA oligonucleotides

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:

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

AAACCTAAACCTAAACGGCTAAGCCCGACGTCAAATACCAAAAAGAGAAAAACAAGAGCGCCGT
CAAGCTCTGCAAATACGATCTGTAAGTCCATCTTAACACAAAAGTGAGATGGGTTCTTAGATCA
TGTTCCGCCGTTAGATCGAGTCATGGTCTTGTCTCATAGAAAGGTACTTTCGTTTACTTCTTTT
GAGTATCGAGTAGAGCGTCGTCTATAGTTAGTTTGAGATTGCGTTTGTCAGAAGTTAGGTTCAA
TGTCCCGGTCCAATTTTCACCAGCCATGTGTCAGTTTCGTTCCTTCCCGTCCTCTTCTTTGATT
TCGTTGGGTTACGGATGTTTTCGAGATGAAACAGCATTGTTTTGTTGTGATTTTTCTCTACAAG
CGAATAGACCATTTATCGGTGGATCTTAGAAA**ATTA**$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$
$X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$**GAAC**TAGA
AAAGACATTGGACATATTCCAGGATATGCAAAAGAAAACAATGAATATTGTTTTGAATGTGTTC
AAGTAAATGAGATTTTCAAGTCGTCTAAAGAACAGTTGCTAATACAGTTACTTATTTCAATAAA

TAATTGGTTCTAATAATACAAAACATATTCGAGGATATGCAGAAAAAAAAGATGTTTGTTATTTT

GAAAAGCTTGAGTAGTTTCTCTCCGAGGTGTAGCGAAGAAGCATCATCTACTTTGTAATGTAAT

TTTCTTTATGTTTTCACTTTGTAATTTTATTTGTGTTAATGTACCATGGCCGATATCGGTTTTA

TTGAAAGAAAATTTATGTTACTTCTGTTTTGGCTTTGCAATCAGTTATGCTAGTTTTCTTATAC

CCTTTCGTAAGCTTCCTAAGGAATCGTTCATTGATTTCCACTGCTTCATTGTATATTAAAACTT

TACAACTGTATCGACCATCATATAATTCTGGGTCAAGAGATGAAAATAGAACACCACATCGTAA

AGTGAAAT

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.

<u>2.2.2. Sequence of the syn-tasiRNA oligonucleotides</u>

The sequences of the two syn-tasiRNA oligonucleotides are:

-<u>Sense oligonucleotide</u> (46 b):

ATTA$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_1X_2X_3X_4X_5X_6X_7$
$X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$

-<u>Antisense oligonucleotide</u> (46 b):

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

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):

ATTATCCCATTCGATACTGCTCGCCTTGGTTATAAAGGAAGAGGCC

-Antisense oligonucleotide (46 b):

GTTCGGCCTCTTCCTTTATAACCAAGGCGAGCAGTATCGAATGGGA

3. Cloning of the amiRNA/syn-tasiRNA sequences in *Bsa*I/*ccd*B (B/c) vectors

Notes:

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

-AtMIR390-B/c- *and* AtTAS1c-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 -20^{0}C.*

-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*-, *pMDC123SB*- or *pFK210B*-based vectors).

Table I: *Bsa*I/*ccd*B-based ('B/c') vectors for direct cloning of amiRNAs and syn-tasiRNAs.

Vector	Small RNA class	Bacterial antibiotic resistance	Plant antibiotic resistance	GATEWAY use	Backbone	Promoter	Terminator	Plant species tested
pENTR-AtMIR390a-B/c	amiRNA	Kanamycin	-	Donor	*pENTR*	-	-	-
pFK210B-AtMIR390a-B/c	amiRNA	Spectomycin	BASTA	-	*pGreen III*	*CaMV* 35S	*rbcS*	*A. thaliana*
pMDC123SB-AtMIR390a-B/c	amiRNA	Kanamycin	BASTA	-	*pMDC123*	*CaMV* 2x35S	-	*A. thaliana* *N. benthamiana*
pMDC32B-AtMIR390a-B/c	amiRNA	Kanamycin Hygromycin	Hygromycin	-	*pMDC32*	*CaMV* 2x35S	*nos*	*A. thaliana* *N. benthamiana*
pENTR-AtTAS1c-B/c	syn-tasiRNA	Kanamycin	-	Donor	*pENTR*	-	-	-
pMDC123SB-AtTAS1c-B/c	syn-tasiRNA	Kanamycin Hygromycin	BASTA	-	*pMDC123*	*CaMV* 2x35S	*nos*	*N. benthamiana*
pMDC32B-AtTAS1c-B/c	syn-tasiRNA	Kanamycin Hygromycin	Hygromycin	-	*pMDC32*	*CaMV* 2x35S	*nos*	*A. thaliana* *N. benthamiana*

Supplemental Text S1

(A)

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CTCATTCATCCAAATACCGAGTCGCCAAAATTCAAACTAGACTCGTTAAATGAATGAATGATGCGGTAGAC
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(B)

>AtMIR390a

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>AtMIR390a-Trich

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Supplemental Text S1. DNA sequence in FASTA format of all *MIRNA* foldbacks used in this study to express and analyze amiRNAs. (A) *AtMIR319a* foldbacks. Sequences unique to the pri-miRNA, pre-miRNA, miRNA/amiRNA guide strand and miRNA*/amiRNA* strand sequences are highlighted in grey, white, blue and green, respectively. Bases of the pre-*AtMIR319a* that had to be modified to preserve the authentic *AtMIR319a* foldback structure are highlighted in red. Extra bases do to WMD2 design are highlighted in light brown. (B) *AtMIR390a* foldbacks. Sequence unique to the pri-*AtMIR390a* sequence is highlighted in black. Bases of the pre-*AtMIR390a* that had to be modified to preserve the authentic *AtMIR390a* foldback structure are highlighted in red. Other details as in (A).

Supplemental Text S2

>AtTAS1c

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>AtTAS1c-D3&D4-Trich

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>AtTAS1c-D3&D4-Ft

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>AtTAS1c-D3Trich-D4Ft

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Supplemental Text S2. 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 to Arabidopsis tasiR-3'D[(+], tasiR-3'D4[+] is highlighted in dark and light pink, respectively. All the other sequences from Arabidopsis *TAS1c* gene are highlighted in black.

Supplemental Text S3

DNA sequence of *Bsa*I-*ccd*B-based (B/c) vectors used for direct cloning of amiRNAs or syn-tasiRNAs.

INDEX

1. amiRNA vectors

>*pENTR-AtMIR390a-B/c* (4491 bp)

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PURPLE/UPPERCASE: M13-F binding site

orange/lowercase: attL1

BLUE/UPPERCASE: *AtMIR390a* 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: *AtMIR390a* 3' region

orange/lowercase/underlined: attL2

PURPLE/UPPERCASE/UNDERLINED: M13-Reverse binding site

brown/lowercase: Kanamycin resistance gene

>*pMDC32B-AtMIR390-B/c* (12044 bp)

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ACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCT
GCCA

brown/lowercase: kanamycin resistance gene

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

cyan/lowercase: T-DNA right border

GREEN/UPPERCASE: 2x35S CaMV promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *AtMIR390a* 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: *AtMIR390a* 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

***>pMDC123SB-AtMIR390a-B/c* (11519 bp)**

CCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAAAATCACCACTCGATACAGGCAGCCCATCAG
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CTAAGCGTCAATTTGTTTACACCACAATATATCCTGCCA

brown/lowercase: kanamycin resistance gene

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

cyan/lowercase: T-DNA right border

GREEN/UPPERCASE: 2x35S CaMV promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *AtMIR390a* 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: *AtMIR390a* 3' region

ORANGE/UPPERCASE/UNDERLINED: attB2

GREY/UPPERCASE/UNDERLINED: Nos terminator

green/lowercase: CaMV promoter

BROWN/UPPERCASE/UNDERLINED: BASTA resistance gene

green/lowercase/underlined: CaMV terminator

CYAN/UPPERCASE: T-DNA left border

>*pFK210B-AtMIR390-B/c* (7916 bp)

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TTTTTGAAAAAGAAAAAGCCCGAAAGGCGGCAACCTCTCGGGCTTCTGGATTTCCGATCCCCGGAATTAG
AGATCT

brown/lowercase: spectinomycin resistance gene

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

CYAN/UPPERCASE: T-DNA left border

GREY/UPPERCASE/UNDERLINED: Nos terminator

BROWN/UPPERCASE/UNDERLINED: BASTA resistance gene

GREY/UPPERCASE: Nos promoter

CYAN/UPPERCASE/UNDERLINED: C->T transversion to block vector's *Bsa*I site

GREEN/UPPERCASE: 35S promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *AtMIR390a* 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: *AtMIR390a* 3' region

ORANGE/UPPERCASE/UNDERLINED: attB2

GREY/UPPERCASE/BOLD: Pea rbcs terminator

cyan/lowercase: T-DNA right border

2. syn-tasiRNA vectors

>*pENTR-AtTAS1c-B/c* (4989 bp)

CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGC
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TCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT

GGCCTTTTGCTCACATGTT

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-B/c* (12550 bp)

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CACCACAATATATCCTGCCA

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

>*pMDC123SB-AtTAS1c-B/c* (12017 bp)

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actaaaatccagatcCCCCGAATTAATTCGGCGTTAATTCAGTACATTAAAAACGTCCGCAATGTGTTAT
TAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCTGCCA

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/UNDERLINED: BASTA resistance gene

green/lowercase/underlined: CaMV terminator

CYAN/UPPERCASE: T-DNA left border

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