Supporting information: Highly specific gene silencing in a monocot species by artificial microRNAs derived from chimeric *MIRNA* precursors

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Figure S9. Base-pairing of amiRNAs and Arabidopsis target transcripts.

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Data S1A. Differential gene expression analysis between 35S:GUS and 35S:OsMIR390-AtL-Bri1 Brachypodium samples.

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Data S3A. amiR-BdBri1 predicted off-targets differentially underexpressed in 35S:OsMIR390-AtL-Bri1 transgenic Brachypodium plants.

Data S3B. amiR-BdCad1 predicted off-targets differentially underexpressed in 35S:OsMIR390-AtL-Cad1 transgenic Brachypodium plants.

Data S3C. amiR-BdCao predicted off-targets differentially underexpressed in 35S:OsMIR390-AtL-Cao transgenic Brachypodium plants.

Data S3D. amiR-BdSpl11 predicted off-targets differentially underexpressed in 35S:OsMIR390-AtL-Spl11 transgenic Brachypodium plants.

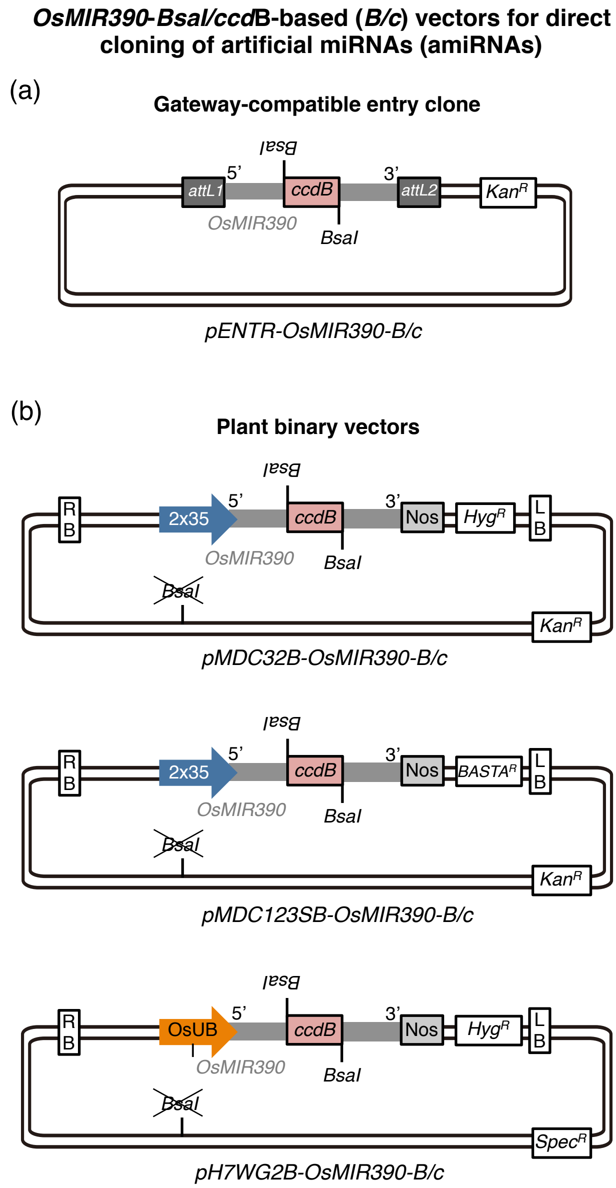


Figure S1. *OsMIR390-B/c* vectors for direct cloning of amiRNAs.
(a) Diagram of an *OsMIR390-B/c* Gateway-compatible entry vector (*pENTR-OsMIR390-B/c)*.
(b) Diagrams of *OsMIR390-B/c*-based binary vectors for expression of amiRNAs in monocot species (*pMDC32B-OsMIR390-B/c*, *pMDC123SB-OsMIR390-B/c* and *pH7WG2B-OsMIR390-B/c*). RB: right border; 35S: *Cauliflower mosaic virus* promoter; OsUbi: *Oryza sativa* ubiquitin 2 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.

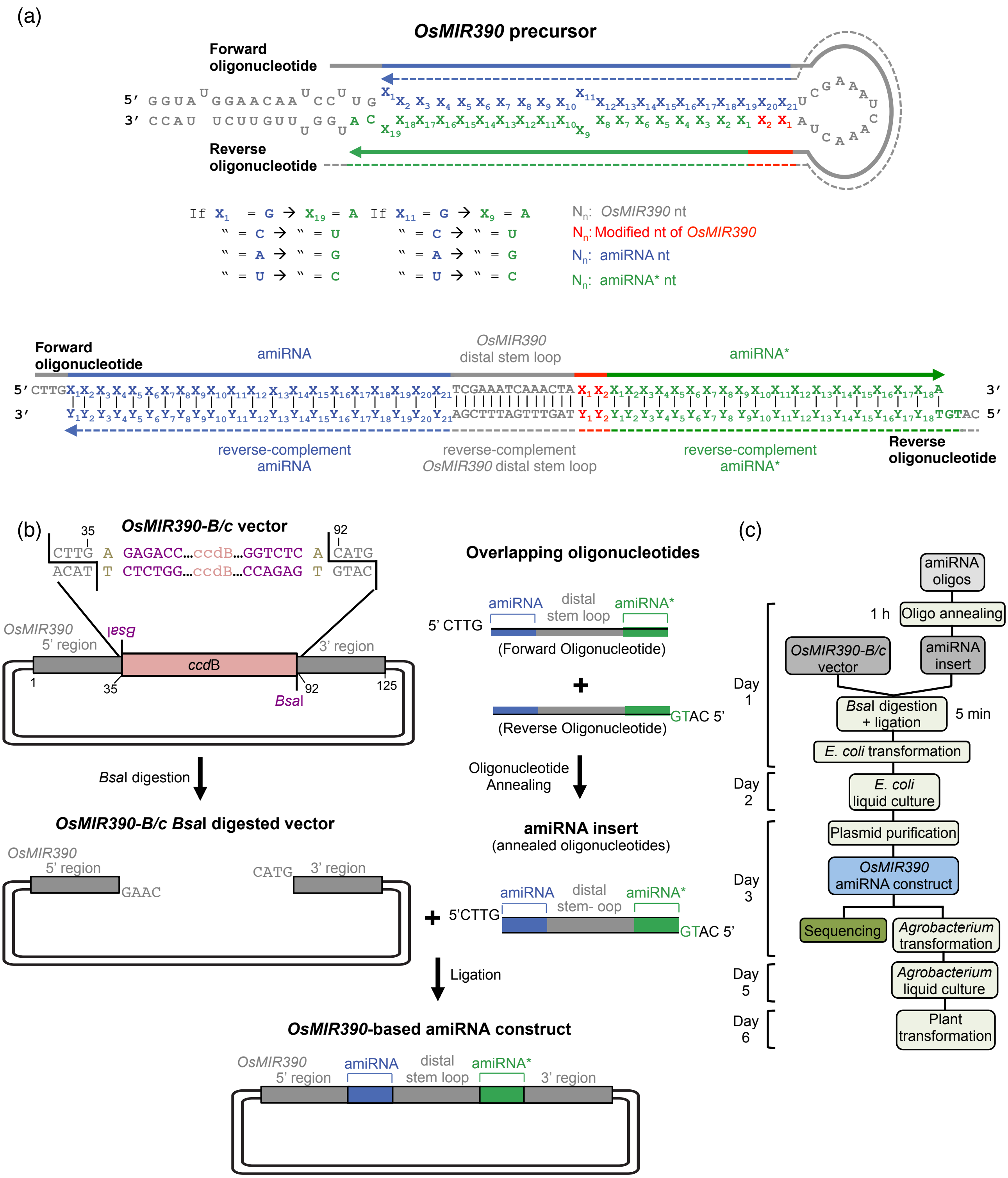


Figure S2. Generation of constructs to express amiRNAs from authentic *OsMIR390* precursors.
(a) Design of the two overlapping oligonucleotides required for amiRNA cloning into *OsMIR390*-based vectors. Sequences covered by the forward and reverse oligonucleotides are represented with solid and dotted lines, respectively. Nucleotides of *OsMIR390* precursor, amiRNA guide strand, and amiRNA* strand are in grey, blue, and green respectively. Other *OsMIR390* nucleotides that may be modified for preserving authentic *OsMIR390* precursor secondary structure are in red. Rules for assigning identity to positions 1 and 9 of amiRNA* are indicated.

Figure S2 (cont.) (b) Diagram of the steps for amiRNA cloning in *OsMIR390* precursors. The amiRNA insert obtained after annealing the two overlapping oligonucleotides has 5'CTTG and 5'CATG overhangs and is directly inserted in a directional manner into an *OsMIR390-B/c* vector previously linearized with *Bsa*I. Nucleotides of the *Bsa*I sites and those arbitrarily chosen and used as spacers between the *Bsa*I recognition sites and the *OsMIR390* sequence are in purple and light brown, respectively. Other details are as described in A. C, flow chart of the steps from amiRNA construct generation to plant transformation.

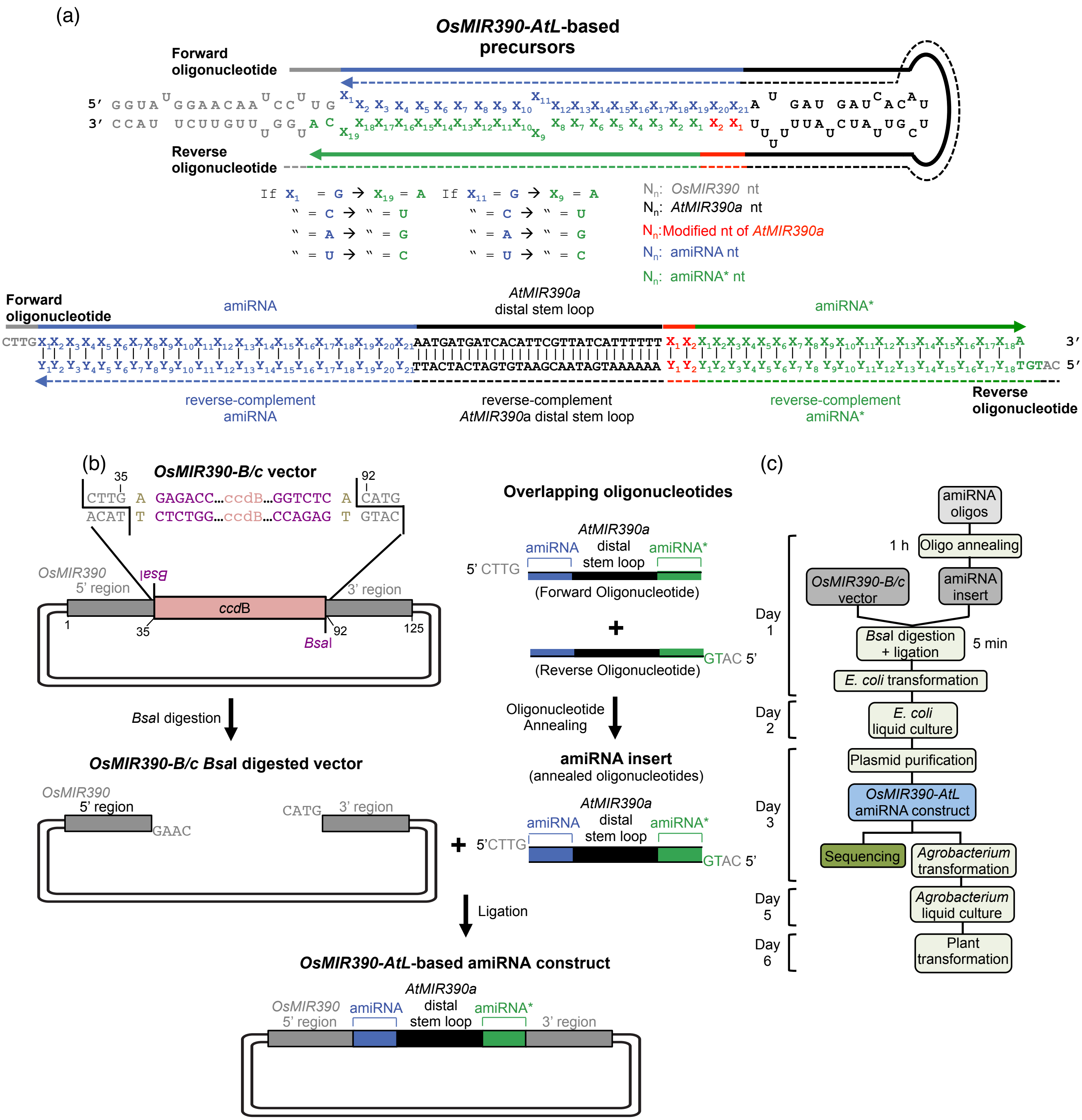


Figure S3. Generation of constructs to express amiRNAs from chimeric *OsMIR390-AtL* precursors.

(a) Design of the two overlapping oligonucleotides containing *OsMIR390aa* and *AtMIR390a* basal stem and distal stem loop sequences, respectively. Sequences covered by the forward and reverse oligonucleotides are represented with solid and dotted lines, respectively. Nucleotides of *AtMIR390a* and *OsMIR390* precursors are in black and grey, respectively. Nucleotides of the amiRNA guide strand, and amiRNA* strand are in blue, and green respectively. Other *OsMIR390* nucleotides that may be modified for preserving authentic *OsMIR390* precursor secondary structure are in red. Rules for assigning identity to positions 1 and 9 of amiRNA* are indicated.

Figure S3 (Cont.) (b) Diagram of the steps for generating constructs for expressing amiRNAs from chimeric *OsMIR390-AtL* precursors. The amiRNA insert obtained after annealing the two overlapping oligonucleotides has 5'CTTG and 5'CATG overhangs and is directly inserted in a directional manner into an *OsMIR390-B/c* vector previously linearized with *Bsa*I. Nucleotides of the *Bsa*I sites and those arbitrarily chosen and used as spacers between the *Bsa*I recognition sites and the *OsMIR390* sequence are in purple and light brown, respectively. Other details are as described in (a).

(c) Flow chart of the steps from amiRNA construct generation to plant transformation.

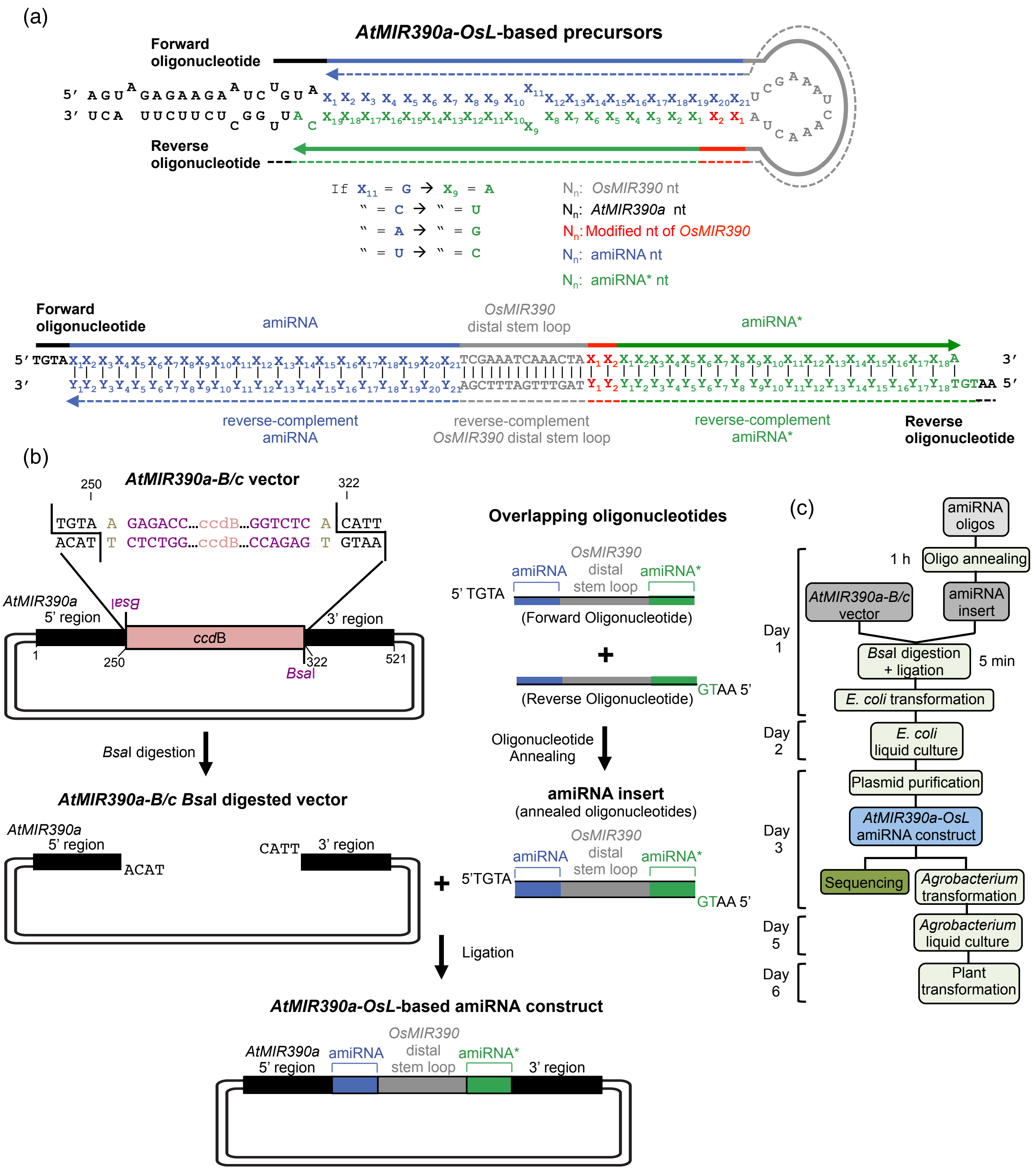


Figure S4. Generation of constructs to express amiRNAs from chimeric *AtMIR390a-OsL* precursors.

(a) Design of the two overlapping oligonucleotides containing *AtMIR390a* and *OsMIR390* basal stem and distal stem loop sequences, respectively. Sequences covered by the forward and reverse oligonucleotides are represented with solid and dotted lines, respectively. Nucleotides of *AtMIR390a* and *OsMIR390* precursors are in black and grey, respectively. Nucleotides of the amiRNA guide strand, and amiRNA* strand are in blue, and green respectively. Other *AtMIR390a* nucleotides that may be modified for preserving authentic *AtMIR390a* precursor secondary structure are in red. Rules for assigning identity to position 9 of amiRNA* are indicated.

Figure S4 (Cont.) (b) Diagram of the steps for generating constructs for expressing amiRNAs from chimeric *AtMIR390a-OsL* precursors. The amiRNA insert obtained after annealing the two overlapping oligonucleotides has 5'TGTA and 5'AATG overhangs and is directly inserted in a directional manner into an *AtMIR390a-B/c* vector previously linearized with *Bsa*I. Nucleotides of the *Bsa*I sites and those arbitrarily chosen and used as spacers between the *Bsa*I recognition sites and the *AtMIR390a* sequence are in purple and light brown, respectively. Other details are as described in (a).
(c) Flow chart of the steps from miRNA construct generation to plant transformation.

```
     amiR-BdBri1  5'  TCGCAATCTTCCGCCTTGCTC  3'
                      |||||||||||||||||||||
          BdBRI1  3'  AGCGTTAGAAGGCGGAACGAC  5'
  (Bradi2g48280)

     amiR-BdCad1  5'  TCGATCTGAGAAGTAAGCCCA  3'
                      |||||||||||||||||||||
          BdCAD1  3'  AGCTAGACTCTTCATTCGGGA  5'
  (Bradi3g06480)

      amiR-BdCao  5'  TCTGCATGGATTGTAAACCCA  3'
                      |||||||||||||||||||||
           BdCAO  3'  AGACGTACCTAACATTTGGGA  5'
  (Bradi2g61500)

    amiR-BdSpl11  5'  TTAGCAACACTACAAGGGCAC  3'
                      |||||||||||||||||||||
         BdSPL11  3'  AATCGTTGTGATGTTCCCGTC  5'
  (Bradi4g04270)
```

Figure S5. Base-pairing of amiRNAs and Brachypodium target mRNAs. amiRNA and mRNA target nucleotides are in blue and brown, respectively.

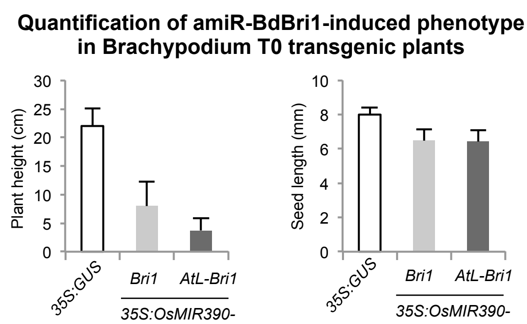
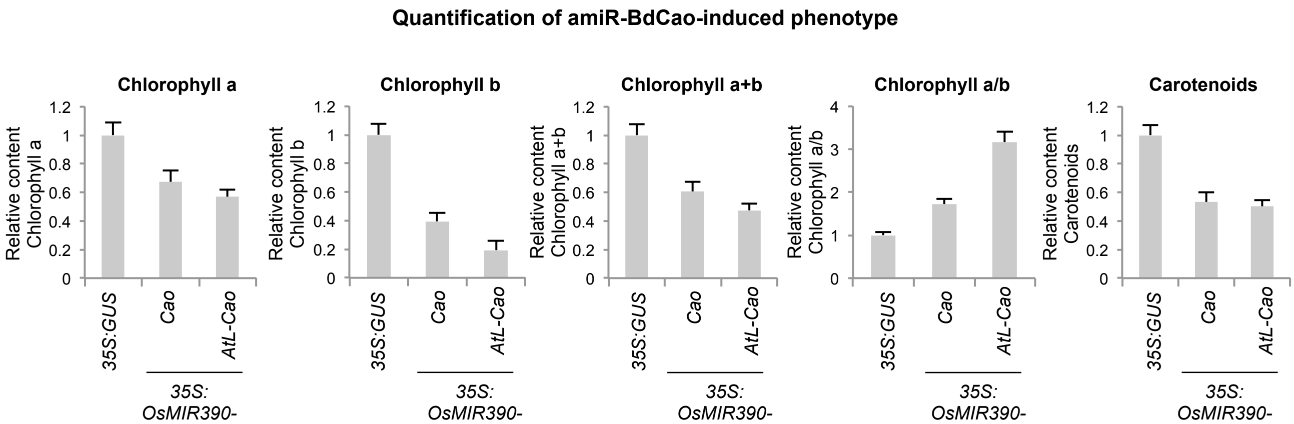


Figure S6. Plant height and seed length analyses in *Brachypodium distachyon* T0 transgenic plants expressing amiR-BdBri1 from authentic *OsMIR390* or chimeric *OsMIR390-AtL* precursors.

(a)



(b)

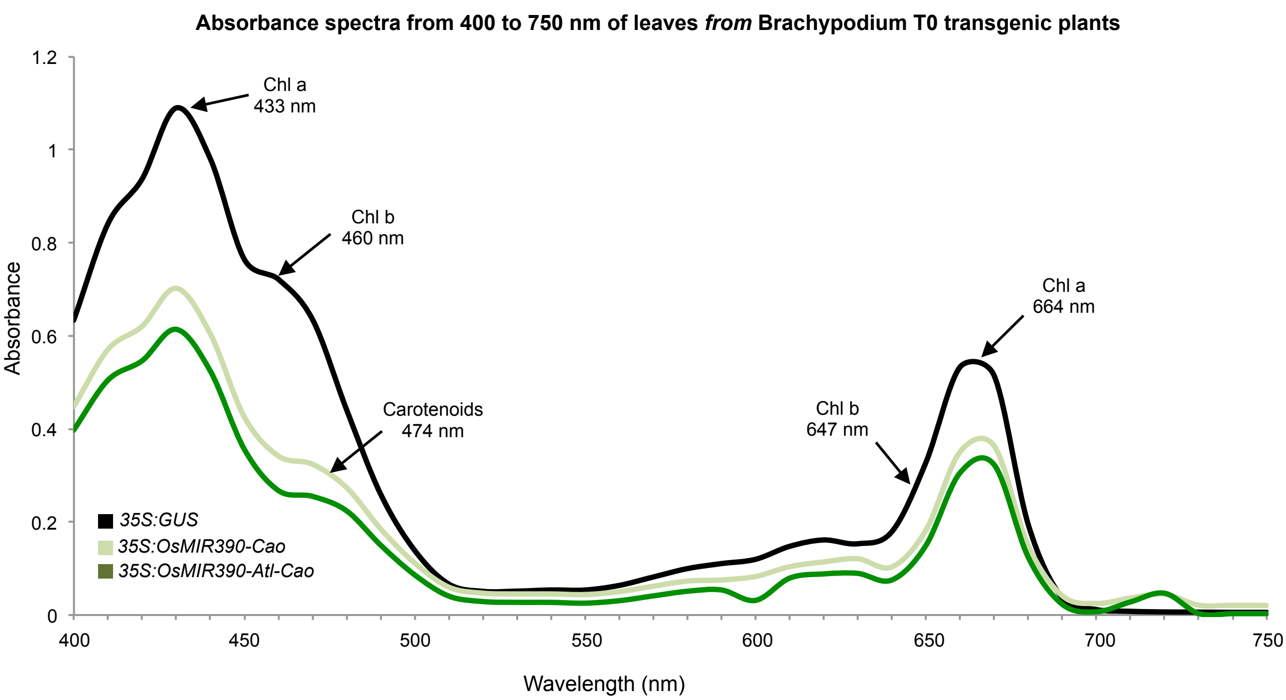


Figure S7. Quantification of amiR-BdCao-induced phenotype in *Brachypodium distachyon 35S:OsMIR390-AtL-Cao*, *35S:OsMIR390-Cao* and *35S:GUS* T0 transgenic lines.

(a) Quantification of chlorophyll a, chlorophyll b, chlorophyll a+b, chlorophyll a/b, and carotenoid content.

(b) Absorbance spectra from 400 to 750 nm of leaves from Brachypodium transgenic lines. Arrows indicate absorbance wavelengths of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids.

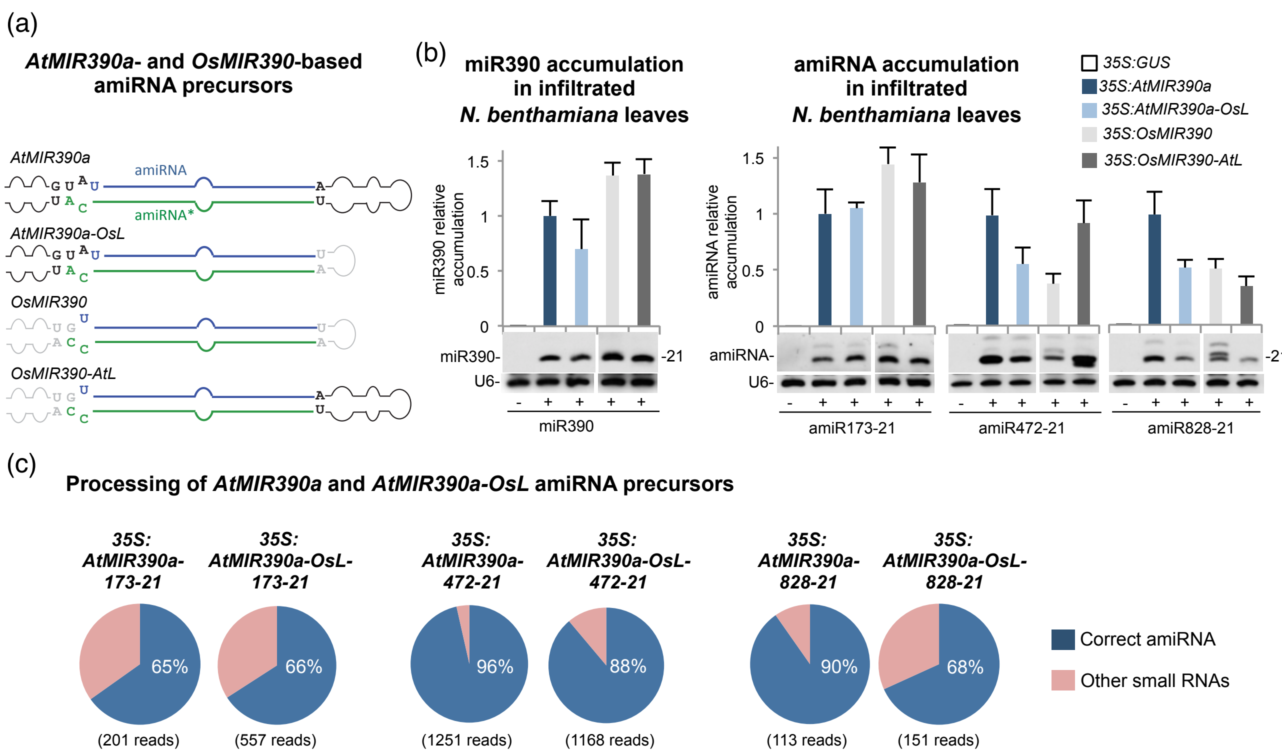


Figure S8. Comparative analysis of the accumulation and processing of several amiRNAs produced from *AtMIR390a*, *AtMIR390a-OsL*, *OsMIR390* and *OsMIR390-AtL* based precursors in *Nicotiana benthamiana* leaves.

(a) Diagrams of *AtMIR390a*, *AtMIR390a-OsL*, *OsMIR390* and *OsMIR390a-AtL* precursors. Nucleotides corresponding to the miRNA guide strand are in blue, and nucleotides of the miRNA* strand are in green. Other nucleotides from the *AtMIR390a* and *OsMIR390* precursors are in black and grey, respectively. Shapes of the *AtMIR390a* and *OsMIR390* precursors are in black and grey, respectively.

(b) Accumulation of miR390 (left) and of several 21-nucleotide amiRNAs (right) expressed from the *AtMIR390a*, *AtMIR390a-OsL*, *OsMIR390* or *OsMIR390-AtL* precursors in *N. benthamiana* leaves. Mean (n=3) relative amiRNA levels + s.d. when expressed from the *AtMIR390a* (dark blue, amiRNA level =1.0). Only one blot from three biological replicates is shown. U6 RNA blot is shown as loading control.

(c) Processing analysis of *AtMIR390a* and *AtMIR390-OsL* amiRNA precursors. Pie charts show the percentage of reads corresponding to accurately processed 21-nt mature amiRNAs (blue sectors) or to other small RNAs (pink sectors).

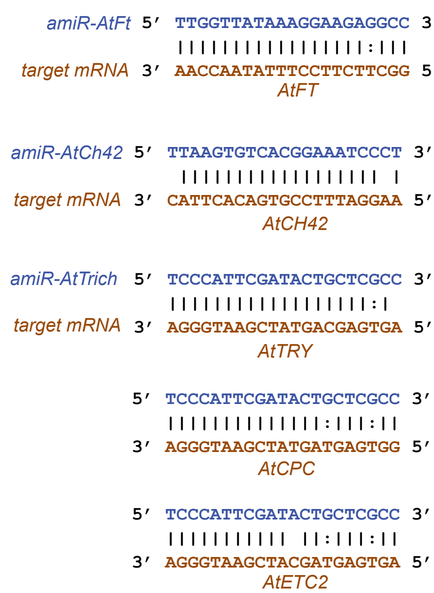
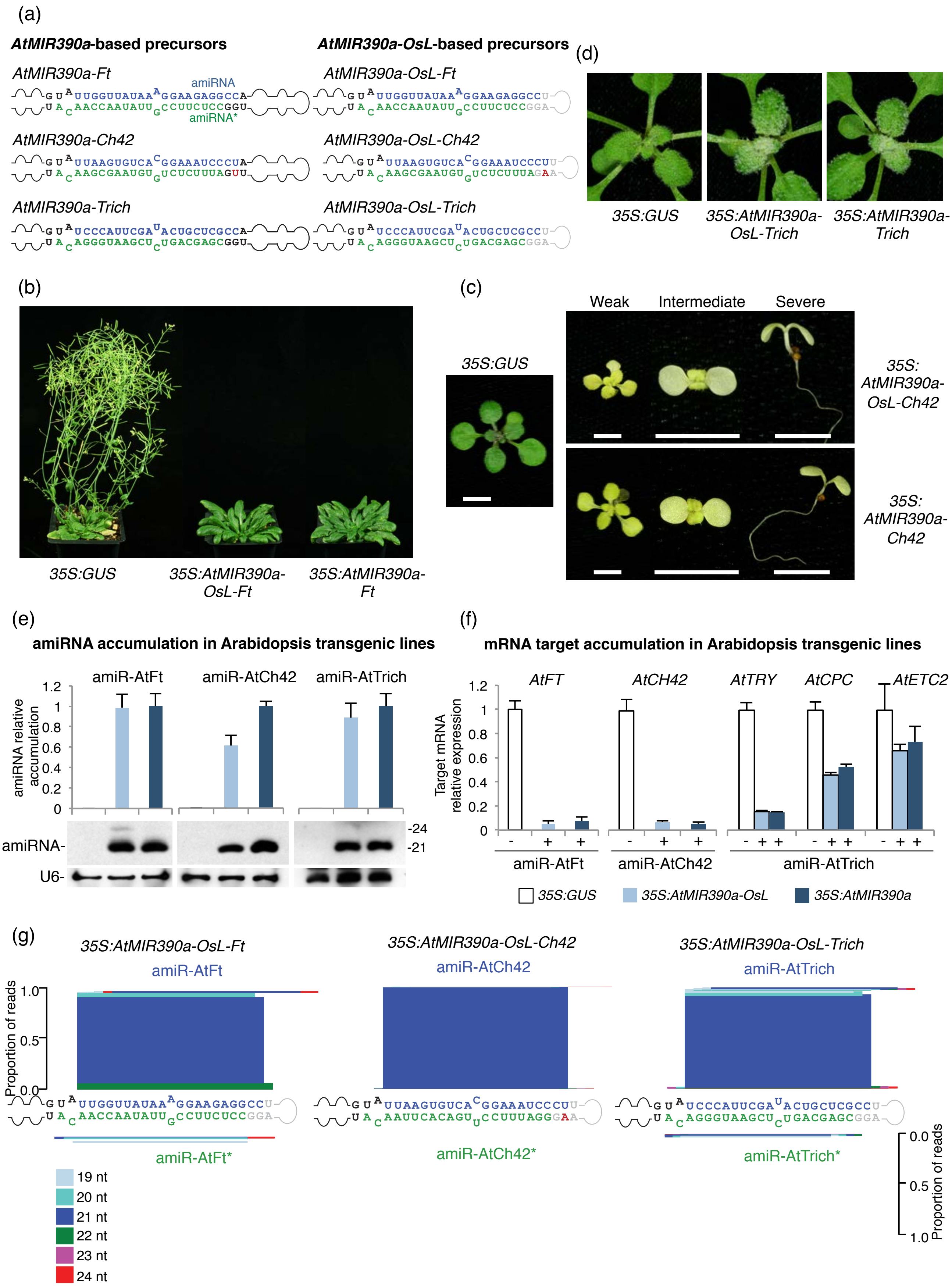


Figure S9. Base-pairing of amiRNAs and Arabidopsis target mRNAs. amiRNA and mRNA target nucleotides are in blue and brown, respectively.



(a)
AtMIR390a-based precursors
AtMIR390a-OsL-based precursors
AtMIR390a-Ft
amiRNA
amiRNA*
AtMIR390a-Ch42
AtMIR390a-Trich
AtMIR390a-OsL-Ft
AtMIR390a-OsL-Ch42
AtMIR390a-OsL-Trich
(d)
35S:GUS
35S:AtMIR390a-OsL-Trich
35S:AtMIR390a-Trich
(b)
35S:GUS
35S:AtMIR390a-OsL-Ft
35S:AtMIR390a-Ft
(c)
Weak
Intermediate
Severe
35S:GUS
35S:AtMIR390a-OsL-Ch42
35S:AtMIR390a-Ch42
(e)
amiRNA accumulation in Arabidopsis transgenic lines
amiR-AtFt
amiR-AtCh42
amiR-AtTrich
amiRNA relative accumulation
amiRNA-
U6-
-24
-21
(f)
mRNA target accumulation in Arabidopsis transgenic lines
AtFT
AtCH42
AtTRY
AtCPC
AtETC2
Target mRNA relative expression
amiR-AtFt
amiR-AtCh42
amiR-AtTrich
35S:GUS
35S:AtMIR390a-OsL
35S:AtMIR390a
(g)
35S:AtMIR390a-OsL-Ft
35S:AtMIR390a-OsL-Ch42
35S:AtMIR390a-OsL-Trich
amiR-AtFt
amiR-AtCh42
amiR-AtTrich
Proportion of reads
amiR-AtFt*
amiR-AtCh42*
amiR-AtTrich*
19 nt
20 nt
21 nt
22 nt
23 nt
24 nt

Figure S10. Functionality in Arabidopsis T1 transgenic plants of amiRNAs derived from *AtMIR390a*-based chimeric precursors containing *Oryza sativa* distal stem-loop sequences (*AtMIR390a-OsL*).

(a) *AtMIR390a*- and *AtMIR390a-OsL*-based precursors containing Ft-, Ch42- and Trich-amiRNAs. Nucleotides corresponding to the miRNA guide and miRNA* strands are in blue and green, respectively; nucleotides from the *AtMIR390a* or *OsMIR390* precursors are in black or grey, respectively, except those that were modified to preserve authentic *AtMIR390a* or *OsMIR390* precursor secondary structures that are in red.

(b-d) Representative images of plants expressing amiRNAs from *AtMIR390a-OsL* or *AtMIR390a-OsL* precursors.

(b) Adult control plant (*35S:GUS*) or plants expressing *35S:AtMIR390a-Ft-OsL* or *35S:AtMIR390a-Ft* plant with a delayed flowering phenotype.

(c) Ten days-old seedlings expressing *35S:AtMIR390a-OsL-Ch42* or *35S:AtMIR390a-Ch42* and showing bleaching phenotypes.

(d) Fifteen days-old control seedling (*35S:GUS*), or seedling expressing *35S:AtMIR390a-OsL-Trich* or *35S:AtMIR390a-Trich* with increased number of trichomes.

(e) Accumulation of amiRNAs in transgenic plants. One blot from three biological replicates is shown. Each biological replicate is a pool of at least 8 independent plants. U6 RNA blot is shown as a loading control.

(f) Mean relative level +/- s.e. of *A. thaliana FT*, *CH42*, *TRY*, *CPC* and *ETC2* mRNAs after normalization to *ACT2*, *CPB20*, *SAND* and *UBQ10*, as determined by quantitative real-time RT-PCR (*35S:GUS* = 1.0 in all comparisons).

(g) Mapping of amiRNA reads from *AtMIR390a-OsL* precursors expressed in transgenic plants. Analysis of amiRNA and amiRNA* reads in plants expressing amiR-AtFt (left), amiR-AtCh42 (center) and amiR-AtTrich (right), respectively. amiRNA guide and amiRNA* strands are highlighted in blue and green, respectively. Nucleotides from *AtMIR390a* or *OsMIR390* precursors are in black and grey, respectively, except those that were modified to preserve the corresponding authentic precursor secondary structure that are in red. Proportion of small RNA reads are plotted as stacked bar graphs. Small RNAs are color-coded by size

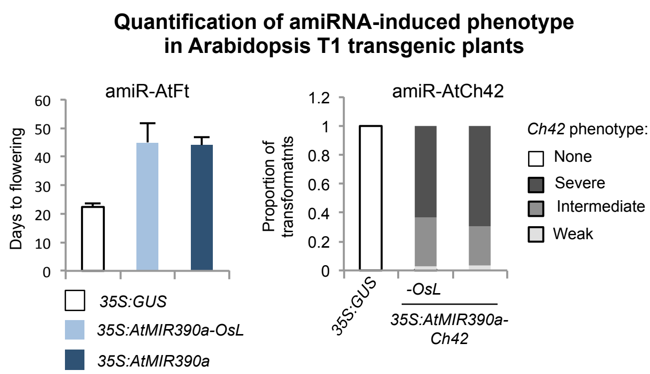


Figure S11. Quantification of amiRNA-induced phenotypes in Arabidopsis transgenic plants expressing amiR-AtFt (left) and amiR-AtCh42 (right) from *AtMIR390a* or chimeric *AtMIR390a-OsL* precursors.

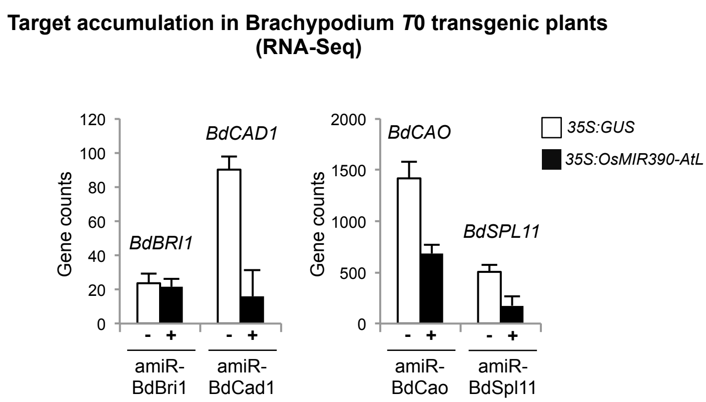


Figure S12. Target accumulation determined by RNA-Seq analysis in transgenic Brachypodium plants including *35S:OsMIR390-AtL*-based or *35S:GUS* constructs.

Table S1. MiRbase locus identifiers of *Orzya sativa* conserved *MIRNA* precursors.	
MIRNA precursor	Locus Identifier
osa-MIR156a	MI0000653
osa-MIR156b	MI0000654
osa-MIR156c	MI0000655
osa-MIR156d	MI0000656
osa-MIR156e	MI0000657
osa-MIR156f	MI0000658
osa-MIR156g	MI0000659
osa-MIR156h	MI0000660
osa-MIR156i	MI0000661
osa-MIR156j	MI0000662
osa-MIR156k	MI0001090
osa-MIR156l	MI0001091
osa-MIR159a.1	MIMAT0001022
osa-MIR159b	MI0001093
osa-MIR159c	MI0001094
osa-MIR159d	MI0001095
osa-MIR159e	MI0001096
osa-MIR159f	MI0001097
osa-MIR160a	MI0000663
osa-MIR160b	MI0000664
osa-MIR160c	MI0000665
osa-MIR160d	MI0000666
osa-MIR160e	MI0001100
osa-MIR160f	MI0001101
osa-MIR162a	MI0000667
osa-MIR162b	MI0001102
osa-MIR164a	MI0000668
osa-MIR164b	MI0000669
osa-MIR164c	MI0001103
osa-MIR164d	MI0001104
osa-MIR164e	MI0001105
osa-MIR164f	MI0001159
osa-MIR166a	MI0000670
osa-MIR166b	MI0000671
osa-MIR166c	MI0000672
osa-MIR166d	MI0000673
osa-MIR166e	MI0000674
osa-MIR166f	MI0000675

MIRNA precursor	Locus Identifier
osa-MIR166g	MI0001142
osa-MIR166h	MI0001143
osa-MIR166i	MI0001144
osa-MIR166j	MI0001158
osa-MIR166k	MI0001107
osa-MIR166l	MI0001108
osa-MIR166m	MI0001157
osa-MIR166n	MIMAT0001088
osa-MIR167a	MI0000676
osa-MIR167b	MI0000677
osa-MIR167c	MI0000678
osa-MIR167d	MI0001109
osa-MIR167e	MI0001110
osa-MIR167f	MI0001111
osa-MIR167g	MI0001112
osa-MIR167h	MI0001113
osa-MIR167i	MI0001114
osa-MIR167j	MI0001156
osa-MIR168a	MI0001115
osa-MIR169a	MI0000679
osa-MIR169b	MI0001117
osa-MIR169c	MI0001118
osa-MIR169d	MI0001119
osa-MIR169e	MI0001120
osa-MIR169f	MI0001121
osa-MIR169g	MI0001122
osa-MIR169h	MI0001123
osa-MIR169i	MI0001124
osa-MIR169j	MI0001125
osa-MIR169k	MI0001126
osa-MIR169l	MI0001127
osa-MIR169m	MI0001128
osa-MIR169n	MI0001129
osa-MIR169o	MI0001130
osa-MIR169p	MI0001131
osa-MIR169q	MI0001132
osa-MIR171a	MI0000680
osa-MIR171b	MI0001133
osa-MIR171c	MI0001134
osa-MIR171d	MI0001135

MIRNA precursor	Locus Identifier
osa-MIR171e	MI0001136
osa-MIR171f	MI0001137
osa-MIR171g	MI0001138
osa-MIR171h	MI0001147
osa-MIR171i	MI0001155
osa-MIR172a	MI0001139
osa-MIR172b	MI0001140
osa-MIR172c	MI0001141
osa-MIR172d	MI0001154
osa-MIR319a	MI0001098
osa-MIR319b	MI0001099
osa-MIR390	MI0001690
osa-MIR393	MI0001026
osa-MIR393b	MI0001148
osa-MIR394	MI0001027
osa-MIR395a	MI0001042
osa-MIR395b	MI0001028
osa-MIR395c	MI0001041
osa-MIR395d	MI0001029
osa-MIR395e	MI0001030
osa-MIR395f	MI0001043
osa-MIR395g	MI0001031
osa-MIR395h	MI0001032
osa-MIR395i	MI0001033
osa-MIR395j	MI0001034
osa-MIR395k	MI0001035
osa-MIR395l	MI0001036
osa-MIR395m	MI0005084
osa-MIR395n	MI0005085
osa-MIR395o	MI0005086
osa-MIR395p	MI0005087
osa-MIR395q	MI0005088
osa-MIR395r	MI0005092
osa-MIR395s	MI0001037
osa-MIR395t	MI0001038
osa-MIR395u	MI0001044
osa-MIR395v	MI0005090
osa-MIR395w	MI0005091
osa-MIR396a	MI0001046
osa-MIR396b	MI0001047

MIRNA precursor	Locus Identifier
osa-MIR396c	MI0001048
osa-MIR396d	MI0013049
osa-MIR396e	MI0001703
osa-MIR396f	MI0010563
osa-MIR396h	MI0013048
osa-MIR397a	MI0001049
osa-MIR397b	MI0001050
osa-MIR398a	MI0001051
osa-MIR398b	MI0001052
osa-MIR399a	MI0001053
osa-MIR399b	MI0001054
osa-MIR399c	MI0001055
osa-MIR399d	MI0001056
osa-MIR399e	MI0001057
osa-MIR399f	MI0001058
osa-MIR399g	MI0001059
osa-MIR399h	MI0001060
osa-MIR399i	MI0001061
osa-MIR399j	MI0001062
osa-MIR399k	MI0001063
osa-MIR408	MI0001149
osa-MIR528	MI0003201
osa-MIR827	MI0010490

Table S2. MiRbase locus identifiers of plant *MIR390* precursors.

MIRNA precursor	Locus Identifier
aly-MIR390a	MI0014569
aly-MIR390b	MI0014570
ath-MIR390a	MI0001000
ath-MIR390b	MI0001001
bna-MIR390a	MI0006447
bna-MIR390b	MI0006448
bna-MIR390c	MI0006449
cca-MIR390	MI0021077
cme-MIR390a	MI0023238
cme-MIR390b	MI0018164
cme-MIR390c	MI0023239
cme-MIR390d	MI0023237
csi-MIR390	MI0013317
ghr-MIR390a	MI0005647
ghr-MIR390b	MI0005648
ghr-MIR390c	MI0005649
gma-MIR390a	MI0007214
gma-MIR390b	MI0007215
gma-MIR390c	MI0017845
gma-MIR390d	MI0021700
gma-MIR390e	MI0021701
gma-MIR390f	MI0021702
gma-MIR390g	MI0021703
hex-MIR390a	MI0022249
hex-MIR390b	MI0022250
mdm-MIR390a	MI0023073
mdm-MIR390b	MI0023074
mdm-MIR390c	MI0023075
mdm-MIR390d	MI0023076
mdm-MIR390e	MI0023077
mdm-MIR390f	MI0023078
mtr-MIR390	MI0005586
nta-MIR390a	MI0021391
nta-MIR390b	MI0021392
nta-MIR390c	MI0021393
pde-MIR390	MI0022095
pta-MIR390	MI0005787
ptc-MIR390a	MI0002305

MIRNA precursor	Locus Identifier
ptc-MIR390b	MI0002306
ptc-MIR390c	MI0002307
ptc-MIR390d	MI0002308
rco-MIR390a	MI0013410
rco-MIR390b	MI0013411
tcc-MIR390a	MI0017503
tcc-MIR390b	MI0017504
vvi-MIR390	MI0006552

Table S3: AmiRNA phenotypic penetrance in Brachypodium T0 transgenic plants.

Construct	T0 analyzed	Phenotypic penetrance[a]
35S:OsMIR390-Bri1	11	64%
35S:OsMIR390-AtL-Bri1	20	80%
UBI:OsMIR390-AtL-Bri1	22	32%
35S:OsMIR390-Cad1	52	94%
35S:OsMIR390-AtL-Cad1	27	100%
35S:OsMIR390-Cao	12	100%
35S:OsMIR390-AtL-Cao	27	100%
UBI:OsMIR390-AtL-Cao	32	53%
35S:OsMIR390-Spl11	22	95%
35S:OsMIR390-AtL-Spl11	43	91%
UBI:OsMIR390-AtL-Spl11	13	61%

[a]The Bri1 phenotype was defined as a shorter height and presence of splindly leaves in amiR-Bri1 transformants when compared to transformants of the *35S:GUS* control set.
The Cad1 phenotype was defined as the presence of brown to red colorations in stems and nodes in amiR-Cad transformants.
The Cao phenotype was defined as a lighter green color amiR-Cao1 transformants when compared to transformants of the *35S:GUS* control set.
The Spl11 phenotype was defined as the presence of necrotic areas in leaves from amiR-Spl11 transformants.

Table S4: AmiRNA phenotypic penetrance in Brachypodium T1 transgenic plants.

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:OsMIR390-Bri1	1	100%
35S:OsMIR390-AtL-Bri1	2	50%
35S:OsMIR390-AtL-Cad1	6	100%
35S:OsMIR390-AtL-Cao	2	100%
35S:OsMIR390-AtL-Spl11	4	100%
UBI:OsMIR390-AtL-Spl11	4	100%

[a]The Bri1 phenotype was defined as a shorter height and presence of splindly leaves in amiR-Bri1 transformants when compared to transformants of the *35S:GUS* control set.
The Cao1 phenotype was defined as a lighter green color amiR-Cao1 transformants when compared to transformants of the *35S:GUS* control set.
The Cad phenotype was defined as the presence of brown to red colorations in stems and nodes in amiR-Cad transformants.
The Spl11 phenotype was defined as the presence of necrotic areas in leaves from amiR-Spl11 transformants.

Table S5: AmiRNA phenotypic penetrance in Arabidopsis T1 transgenic plants.

Construct	T1 analyzed	Phenotypic penetrance[a]
35S:AtMIR390a-Ft	64	100%
35S:AtMIR390a-OsL-Ft	44	100%
35S:AtMIR390a-Ch42	406	100% 3% weak 28% intermediate 69% severe
35S:AtMIR390a-OsL-Ch42	267	98% 3% weak 33% intermediate 64% severe
35S:AtMIR390a-Trich	45	93% 12% *try cpc* type
35S:AtMIR390a-OsL-Trich	69	99% 9% *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 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.

Table S6: AmiRNA phenotypic penetrance in Arabidopsis T2 transgenic plants.

Construct	T2 analyzed	Phenotypic penetrance[a]
35S:AtMIR390a-Ft	5	100%
35S:AtMIR390a-OsL-Ft	5	100%
35S:AtMIR390a-Trich	10	90%
35S:AtMIR390a-OsL-Trich	10	90%

[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 *35S:GUS* control set.

Table S7. DNA, LNA and RNA oligonucleotides[1].

Oligonucleotide Name	Sequence
3'PCR primer i1	CAAGCAGAAGACGGCATACGAACATCGATTGATGGTGCCTACAG
3'PCR primer i2	CAAGCAGAAGACGGCATACGAGTGATCATTGATGGTGCCTACAG
3'PCR primer i3	CAAGCAGAAGACGGCATACGACATCTGATTGATGGTGCCTACAG
3'PCR primer i4	CAAGCAGAAGACGGCATACGAAACGTAATTGATGGTGCCTACAG
3'PCR primer i5	CAAGCAGAAGACGGCATACGATGGTAAATTGATGGTGCCTACAG
3'PCR primer i6	CAAGCAGAAGACGGCATACGATACAGTATTGATGGTGCCTACAG
3'PCR primer i7	CAAGCAGAAGACGGCATACGACGTGATATTGATGGTGCCTACAG
3'PCR primer i8	CAAGCAGAAGACGGCATACGAACAAGTATTGATGGTGCCTACAG
3'PCR primer i10	CAAGCAGAAGACGGCATACGACTAGCAATTGATGGTGCCTACAG
3'PCR primer i11	CAAGCAGAAGACGGCATACGATACAAGATTGATGGTGCCTACAG
5'PCR primer P5	AATGATACGGCGACCACCGACAGGTTCAGAGTTCTACAGTCCGA
Adaptor 1	ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
Adaptor 2	/5Phos/G*ATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
AtMIR390a-OsL-F	TGTAAAGCTCAGGAGGGATAGCGCCTCGAAATCAAACTAGGCGCTATCCATCCTGAGTTT
AtMIR390a-OsL-R	AATGAAACTCAGGATGGATAGCGCCTAGTTTGATTTCGAGGCGCTATCCCTCCTGAGCTT
AtMIR390a-OsL-173-21-F	TGTATTCGCTTGCAGAGAGAAATCATCGAAATCAAACTATGATTTCTCTGTGTAAGCGAA
AtMIR390a-OsL-173-21-R	AATGTTCGCTTACACAGAGAAATCATAGTTTGATTTCGATGATTTCTCTCTGCAAGCGAA
AtMIR390a-OsL-472-21-F	TGTATTTTTCCTACTCCGCCCATACTCGAAATCAAACTAGTATGGGCGGCGTAGGAAAAA
AtMIR390a-OsL-472-21-R	AATGTTTTTCCTACGCCGCCCATACTAGTTTGATTTCGAGTATGGGCGGAGTAGGAAAAA
AtMIR390a-OsL-828-21-F	TGTATCTTGCTTAAATGAGTATTCCTCGAAATCAAACTAGGAATACTCAGTTAAGCAAGA
AtMIR390a-OsL-828-21-R	AATGTCTTGCTTAACTGAGTATTCCTAGTTTGATTTCGAGGAATACTCATTTAAGCAAGA
AtMIR390a-OsL-AtCh42-F	TGTATTAAGTGTCACGGAAATCCCTTCGAAATCAAACTAAGGGATTTCCTTGACACTTAA
AtMIR390a-OsL-AtCh42-R	AATGTTAAGTGTCAAGGAAATCCCTTAGTTTGATTTCGAAGGGATTTCCGTGACACTTAA
AtMIR390a-OsL-AtFt-F	TGTATTGGTTATAAAGGAAGAGGCCTCGAAATCAAACTAGGCCTCTTCCGTTATAACCAA
AtMIR390a-OsL-AtFt-R	AATGTTGGTTATAACGGAAGAGGCCTAGTTTGATTTCGAGGCCTCTTCCTTTATAACCAA
AtMIR390a-OsL-AtTrich-F	TGTATCCCATTCGATACTGCTCGCCTCGAAATCAAACTAGGCGAGCAGTCTCGAATGGGA
AtMIR390a-OsL-AtTrich-R	AATGTCCCATTCGAGACTGCTCGCCTAGTTTGATTTCGAGGCGAGCAGTATCGAATGGGA
Bradi1g30690-510-F	ACCAAAATTACCGAGACGAGCAGCAG
Bradi1g30690-666-R	AGGCCTGTCATGTGATGGTTCTTGC
Bradi1g41825-987-F	CCGTGCTAAAACACTTGCAAGGAAGC
Bradi1g41825-1180-R	CCTCACCAGGTGCCAACGATACATT
Bradi1g54680-821-F	TCTCATCATCATCCTGTCGGTGTGC
Bradi1g54680-1010-R	CACGACATTAGGACACCCGGATCA
Bradi1g61790-2634-F	GAACTTCTCCGCCATCGTGGAGTCT
Bradi1g61790-2876-R	CATTGATGGGCAACTCCCTGTCTCTC
Bradi1g62572-1091-F	ACGACTGCCCGCCCTCATCTACT
Bradi1g62572-1221-R	CAGCAAAGGAAGCCCGCTGAATTAGT
Bradi1g72485-602-F	AACGAAGGAGAAGGGTCTGCGTCTG
Bradi1g72485-847-R	CTGCACCTCCTCCCTCACCATCTC
Bradi2g48280-2698-F	GGGGTAAAACTGAACTGGCCAGCAA
Bradi2g48280-2884-R	CCACACTCATCATCCTCGCCATACC
Bradi2g61500-1136-F	CCATCCCTTCTCTGCTGCCTCCTT
Bradi2g61500-1335-R	CCCTTGGAGCCCAGAAGTAGGTGTC
Bradi3g06480-1047-F	TGCGTCGAGAAAGGGCTTACTTCTCA
Bradi3g06480-1248-R	CACGCACGCACGCACTCTACCTA
Bradi3g07850-1195-F	TGTGCAGATACAATGGTGGGTGACAG
Bradi3g07850-1334-R	GAGCTGTCCAGACCGGTGGAGATTT
Bradi4g04270-1581-F	TGATTATCGGGGGAACAGGGGCTAT
Bradi4g04270-1750-R	CACCAGACCCATGATTAGTGGCACA
Bradi4g09648-1378-F	GATGGCTTGTCTCAGCTCCCATGTTT
Bradi4g09648-1579-R	CTTGCTCCTCCCACTCCCACTCTTC
Bradi4g17230-1460-F	GTTGCAAGCTGCTGGTGAAGTCGAT
Bradi4g17230-1581-R	CACGGACGTACGACGACACATACAAA
Bradi4g21000-201-F	TCCGTATCCAGAAAGCCAAAGCTCAC
Bradi4g21000-490-R	TTGCTGAACTGGAGGAGGAAGACGA
BsaI-OsMIR390-F	CACCGAGCTCGAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGAGAGACCGGTCTCACATGGTTTGTTCTTACC ACACGACCAATTAAATCGAGCTC
BsaI-OsMIR390-R	GAGCTCGATTTAATTGGTCGTGTGGTAAGAACAAACCATGTGAGACCGGTCTCTCAAGGATTGTTCCATACCCTTC CTCAAAACATCTCGAGCTCGGTG
GeneRacer 3' Primer	GGACACTGACATGGACTGAAGGAGTA
GeneRacer 5' Nested Primer	GGACACTGACATGGACTGAAGGAGTA
GeneRacer 5' primer	CGACTGGAGCACGAGGACACTGA

Oligonucleotide Name	Sequence
GeneRacer Oligo dT Primer	GCTGTCAACGATACGCTACGTAACGGCATGACAGTG(T)$_{24}$
GeneRacer RNA Oligo	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA
OsMIR390-F	CTTGAAGCTCAGGAGGGATAGCGCCTCGAAATCAAACTAGGCGCTATCTATCCTGAGCTC
OsMIR390-R	CATGGAGCTCAGGATAGATAGCGCCTAGTTTGATTTCGAGGCGCTATCCCTCCTGAGCTT
OsMIR390-AtL-F	CTTGAAGCTCAGGAGGGATAGCGCCATGATGATCACATTCGTTATCTATTTTTTGGCGCTATCTATCCTGAGCTC
OsMIR390-AtL-R	CATGGAGCTCAGGATAGATAGCGCCAAAAAATAGATAACGAATGTGATCATCATGGCGCTATCCCTCCTGAGCTT
OsMIR390-173-21-F	CTTGTTCGCTTGCAGAGAGAAATCATCGAAATCAAACTATGATTTCTCTGTGTAAGCGAC
OsMIR390-173-21-R	CATGGTCGCTTACACAGAGAAATCATAGTTTGATTTCGATGATTTCTCTCTGCAAGCGAA
OsMIR390-AtL-173-21-F	CTTGTTCGCTTGCAGAGAGAAATCAATGATGATCACATTCGTTATCTATTTTTTTGATTTCTCTGTGTAAGCGAC
OsMIR390-AtL-173-21-R	CATGGTCGCTTACACAGAGAAATCAAAAAAATAGATAACGAATGTGATCATCATTGATTTCTCTCTGCAAGCGAA
OsMIR390-472-21-F	CTTGTTTTTCCTACTCCGCCCATACTCGAAATCAAACTAGTATGGGCGGCGTAGGAAAAC
OsMIR390-472-21-R	CATGGTTTTCCTACGCCGCCCATACTAGTTTGATTTCGAGTATGGGCGGAGTAGGAAAAA
OsMIR390-AtL-472-21-F	CTTGTTTTTCCTACTCCGCCCATACATGATGATCACATTCGTTATCTATTTTTTGTATGGGCGGCGTAGGAAAAC
OsMIR390-AtL-472-21-R	CATGGTTTTCCTACGCCGCCCATACAAAAAATAGATAACGAATGTGATCATCATGTATGGGCGGAGTAGGAAAAA
OsMIR390-828-21-F	CTTGTCTTGCTTAAATGAGTATTCCTCGAAATCAAACTAGGAATACTCAGTTAAGCAAGC
OsMIR390-828-21-R	CATGGCTTGCTTAACTGAGTATTCCTAGTTTGATTTCGAGGAATACTCATTTAAGCAAGA
OsMIR390-AtL-828-21-F	CTTGTCTTGCTTAAATGAGTATTCCATGATGATCACATTCGTTATCTATTTTTTGGAATACTCAGTTAAGCAAGC
OsMIR390-AtL-828-21-R	CATGGCTTGCTTAACTGAGTATTCCAAAAAATAGATAACGAATGTGATCATCATGGAATACTCATTTAAGCAAGA
OsMIR390-AtL-BdBri1-F	CTTGTCGCAATCTTCCGCCTTGCTCATGATGATCACATTCGTTATCTATTTTTTGAGCAAGGCGTAAGATTGCGC
OsMIR390-AtL-BdBri1-R	CATGGCGCAATCTTACGCCTTGCTCAAAAAATAGATAACGAATGTGATCATCATGAGCAAGGCGGAAGATTGCGA
OsMIR390-AtL-BdCad1-F	CTTGTCGATCTGAGAAGTAAGCCCAATGATGATCACATTCGTTATCTATTTTTTTGGGCTTACTGCTCAGATCGC
OsMIR390-AtL-BdCad1-R	CATGGCGATCTGAGCAGTAAGCCCAAAAAAATAGATAACGAATGTGATCATCATTGGGCTTACTTCTCAGATCGA
OsMIR390-AtL-BdCao-F	CTTGTCTGCATGGATTGTAAACCCAATGATGATCACATTCGTTATCTATTTTTTTGGGTTTACACTCCATGCAGC
OsMIR390-AtL-BdCao-R	CATGGCTGCATGGAGTGTAAACCCAAAAAAATAGATAACGAATGTGATCATCATTGGGTTTACAATCCATGCAGA
OsMIR390-AtL-BdSpl11-F	CTTGTTAGCAACACTACAAGGGCACATGATGATCACATTCGTTATCTATTTTTTGTGCCCTTGTCGTGTTGCTAC
OsMIR390-AtL-BdSpl11-R	CATGGTAGCAACACGACAAGGGCACAAAAAATAGATAACGAATGTGATCATCATGTGCCCTTGTAGTGTTGCTAA
OsMIR390-BdBri1-F	CTTGTCGCAATCTTCCGCCTTGCTCTCGAAATCAAACTAGAGCAAGGCGTAAGATTGCGC
OsMIR390-BdBri1-R	CATGGCGCAATCTTACGCCTTGCTCTAGTTTGATTTCGAGAGCAAGGCGGAAGATTGCGA
OsMIR390-BdCad1-F	CTTGTCGATCTGAGAAGTAAGCCCATCGAAATCAAACTATGGGCTTACTGCTCAGATCGC
OsMIR390-BdCad1-R	CATGGCGATCTGAGCAGTAAGCCCATAGTTTGATTTCGATGGGCTTACTTCTCAGATCGA
OsMIR390-BdCao-F	CTTGTCTGCATGGATTGTAAACCCATCGAAATCAAACTATGGGTTTACACTCCATGCAGC
OsMIR390-BdCao-R	CATGGCTGCATGGAGTGTAAACCCATAGTTTGATTTCGATGGGTTTACAATCCATGCAGA
OsMIR390-BdSpl11-F	CTTGTTAGCAACACTACAAGGGCACTCGAAATCAAACTAGTGCCCTTGTCGTGTTGCTAC
OsMIR390-BdSpl11-R	CATGGTAGCAACACGACAAGGGCACTAGTTTGATTTCGAGTGCCCTTGTAGTGTTGCTAA
PE Primer-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
PE-Primer-R-N701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N705	CAAGCAGAAGACGGCATACGAGATGGACTCCTGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N706	CAAGCAGAAGACGGCATACGAGATTAGGCATGGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N707	CAAGCAGAAGACGGCATACGAGATCTCTCTACGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N708	CAAGCAGAAGACGGCATACGAGATCAGAGAGGGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N709	CAAGCAGAAGACGGCATACGAGATGCTACGCTGTGACTGGAGTTCAGACGTGT
PE-Primer-R-N710	CAAGCAGAAGACGGCATACGAGATCGAGGCTGGTGACTGGAGTTCAGACGTGT
Probe-amiR-173	GTGATTTCTCTCTGCAAGCGAA
Probe-amiR-828	T+GGA+ATA+CTC+ATT+TAA+GCA+AGA
Probe-amiR-BdBri1	G+AGC+AAG+GCG+GAA+GAT+TGC+GA
Probe-amiR-BdCad1	TGGGCTTACTTCTCAGATCGA
Probe-amiR-BdCao	T+GGG+TTT+ACA+ATC+CAT+GCA+GA
Probe-amiR-AtCh42	AGGGATTTCCGTGACACTTAA
Probe-amiR-AtFt	GGCCTCTTCCTTTATAACCAA
Probe-amiR-BdSpl11	GTGCCCTTGTAGTGTTGCTAA
Probe-amiR-AtTrich	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

Oligonucleotide Name	Sequence
qAtETC2-CS-F	GCGGTCCCAGTCTTAGGCA
qAtETC2-CS-R	TTCGATGCTACTCACTTCTTCAGAGT
qAtFT-F	TGGAACAACCTTTGGCAATG
qAtFT-R	CGACACGATGAATTCCTGCA
qAtSAND-F	CTCAAAGATTGCAGGGTACGC
qAtSAND-R	TCTTCAACACGCATTCCACCT
qAtTRY-CS-F	ACACAAAATCGCCCTCCATG
qAtTRY-CS-R	TCAAATCCCACCTATCACCGA
qAtUBQ10-F	CGCCTGCAAAGTGACTCGA
qAtUBQ10-R	CCAACAGCTCAACACTTTCGC
qBdBRI1-F	TGCACGACCGGAAAAAGATC
qBdBRI1-R	TGGAGAAATGCCAATCCTCG
qBdCAD1-CS-F	CGGAGGAGGTGCTCAAGTTC
qBdCAD1-CS-R	GAGCGCCTCGTTGAGGTAGT
qBdCAO-F	TCATGGGTGGGAGTATTCGAC
qBdCAO-R	TGCGCACATTGAGCATCTTT
qBdSAMDC-F	TGTACGAAGCTCCCCTCGG
qBdSAMDC-R	GCAGTTCGAGTACGCAGCAG
qBd-SPL11-F	AGACGTACGAGCGGACATGC
qBdSPL11-R	GTGTCAATGTCGTGTTCGCC
qBdUBC-F	CATTATCCCATGGAGGCACCT
qBdUBC-R	GCGGGTGACCAGGAGTCATA
qBdUBI4-F	GCTGTTGGAACTGCTGCTATACCT
qBdUBI4-R	TTGCACCAAACCAACACACACCAG
qBdUBI10-F	TGGACTTGCTTCTGTCTGGGTTCA
qBdUBI10-R	TGGTACACAGGCATAACACTGACG

[1] * - Phosphorothioate bond;
/5Phos/ - 5' phosphorylation

Table S8. Sequences and predicted targets for all amiRNAs analyzed.

amiRNA name	amiRNA sequence (5'->3')	Predicted target(s)	Plant specie	Reference
amiR173-21	UUCGCUUGCAGAGAGAAAUCA	*TAS1a, TAS1b, TAS1c, TAS2*	*Arabidopsis thaliana*	Cuperus *et al.*, 2010
amiR472-21	UUUUUCCUACUCCGCCCAUAC	*RFL1, RPS5, CC-NBS-LRR, NBS*	*Arabidopsis thaliana*	Cuperus *et al.*, 2010
amiR828-21	UCUUGCUUAAAUGAGUAUUCC	*MYB113, MYB82, TAS4*	*Arabidopsis thaliana*	Cuperus *et al.*, 2010
amiR-AtCh42	UUAAGUGUCACGGAAAUCCCU	*CH42*	*Arabidopsis thaliana*	Felippes and Weigel, 2009 Carbonell *et al.*, 2014
amiR-AtFt	UUGGUUAUAAAGGAAGAGGCC	*FT*	*Arabidopsis thaliana*	Schwabb *et al.*, 2006 Carbonell *et al.*, 2014
amiR-AtTrich	UCCCAUUCGAUACUGCUCGCC	*TRY, CPC, ETC2*	*Arabidopsis thaliana*	Schwabb *et al.*, 2006 Carbonell *et al.*, 2014
amiR-BdBri1	UCGCAAUCUUCCGCCUUGCUC	*BRI1*	*Brachypodium distachyon*	This work
amiR-BdCad1	UCGAUCUGAGAAGUAAGCCCA	*CAD1*	*Brachypodium distachyon*	This work
amiR-BdCao	UCUGCAUGGAUUGUAAACCCA	*CAO*	*Brachypodium distachyon*	This work
amiR-BdSpl11	UUAGCAACACUACAAGGGCAC	*SPL11*	*Brachypodium distachyon*	This work

Table S9. High-throughput small RNA libraries from Arabidopsis, Brachypodium or *Nicotiana benthamiana* plants.

Sample ID	Construct	Species	Tissue	3'PCR primer	Barcode Sequence	Adaptor-parsed reads	SRA Identifier
1	*35S:AtMIR390a-173-21*	*N. benthamiana*	Leaf	i1	CGATGT	25,652,072	SRR1771846
2	*35S:AtMIR390a-472-21*	*N. benthamiana*	Leaf	i3	CAGATG	23,512,059	SRR1771847
3	*35S:AtMIR390a-828-21*	*N. benthamiana*	Leaf	i5	TTACCA	26,746,930	SRR1771848
4	*35S:AtMIR390a-OsL-173-21*	*N. benthamiana*	Leaf	i1	CGATGT	42,522,405	SRR1771851
5	*35S:AtMIR390a-OsL-472-21*	*N. benthamiana*	Leaf	i2	GATCAC	47,332,026	SRR1771852
6	*35S:AtMIR390a-OsL-828-21*	*N. benthamiana*	Leaf	i3	CAGATG	52,048,606	SRR1771853
7	*35S:OsMIR390-173-21*	*B. distachyon*	Callus	i1	CGATGT	14,756,652	SRR1771445
8	*35S:OsMIR390-472-21*	*B. distachyon*	Callus	i3	CAGATG	69,380,781	SRR1771511
9	*35S:OsMIR390-828-21*	*B. distachyon*	Callus	i5	TTACCA	60,437,057	SRR1771523
10	*35S:OsMIR390-AtL-173-21*	*B. distachyon*	Callus	i2	GATCAC	17,972,261	SRR1771539
11	*35S:OsMIR390a-AtL-472-21*	*B. distachyon*	Callus	i4	TACGTT	25,830,535	SRR1771545
12	*35S:OsMIR390a-AtL-828-21*	*B. distachyon*	Callus	i6	ACTGTA	25,129,002	SRR1771546
13	*35S:AtMIR390a-OsL-AtCh42*	*A. thaliana*	Seedling	i10	TGCTAG	10,429,854	SRR1842772
14	*35S:AtMIR390a-OsL-AtFt*	*A. thaliana*	Inflorescence	i11	CTTGTA	32,295,617	SRR1842774
15	*35S:AtMIR390a-OsL-AtTrich*	*A. thaliana*	Inflorescence	i4	TACGTT	51,516,926	SRR1842775
16	*35S:OsMIR390-BdBri1*	*B. distachyon*	Leaf	i1	CGATGT	19,319,670	SRR1771782
17	*35S:OsMIR390-AtL-BdBri1*	*B. distachyon*	Leaf	i2	GATCAC	20,856,916	SRR1771775
18	*35S:OsMIR390-BdCad1*	*B. distachyon*	Leaf	i5	TTACCA	21,308,138	SRR1771776
19	*35S:OsMIR390-AtL-BdCad1*	*B. distachyon*	Leaf	I6	ACTGTA	22,929,175	SRR1771777
20	*35S:OsMIR390-BdCao*	*B. distachyon*	Leaf	i3	CAGATG	21,930,111	SRR1771778
21	*35S:OsMIR390-AtL-BdCao*	*B. distachyon*	Leaf	i4	TACGTT	22,199,088	SRR1771779
22	*35S:OsMIR390-BdSpl11*	*B. distachyon*	Leaf	i7	ATCACG	21,231,525	SRR1771780
23	*35S:OsMIR390-AtL-BdSpl11*	*B. distachyon*	Leaf	i8	ACTTGT	24,735,881	SRR1771781

Table S10. High-throughput strand-specific transcript RNA libraries from independent Brachypodium T0 transgenic lines.

Sample ID	Construct	PE Primer-R Index	Index Sequence	Adaptor-parsed reads	SRA Identifier
1	*35S:GUS*	N707	GTAGAGA	16,779,027	SRR1850587
2	*35S:GUS*	N708	CCTCTCT	20,182,946	SRR1850670
3	*35S:GUS*	N709	AGCGTAG	19,472,243	SRR1850671
4	*35S:GUS*	N710	CAGCCTC	19,128,516	SRR1850716
5	*35S:OsMIR390-AtL-BdBri1*	N701	TAAGGCG	17,265,195	SRR1772223
6	*35S:OsMIR390-AtL-BdBri1*	N702	CGTACTA	16,300,588	SRR1772224
7	*35S:OsMIR390-AtL-BdBri1*	N703	AGGCAGA	15,724,668	SRR1772225
8	*35S:OsMIR390-AtL-BdBri1*	N704	TCCTGAG	18,807,736	SRR1772226
9	*35S:OsMIR390-AtL-BdCad1*	N709	AGCGTAG	22,853,726	SRR1772227
10	*35S:OsMIR390-AtL-BdCad1*	N710	CAGCCTC	22,562,039	SRR1772228
11	*35S:OsMIR390-AtL-BdCad1*	N701	TAAGGCG	16,877,134	SRR1772229
12	*35S:OsMIR390-AtL-BdCad1*	N702	CGTACTA	17,142,684	SRR1772230
13	*35S:OsMIR390-AtL-BdCao*	N705	AGGAGTC	18,778,386	SRR1772231
14	*35S:OsMIR390-AtL-BdCao*	N706	CATGCCT	19,333,658	SRR1772232
15	*35S:OsMIR390-AtL-BdCao*	N707	GTAGAGA	19,648,254	SRR1772233
16	*35S:OsMIR390-AtL-BdCao*	N708	CCTCTCT	20,379,073	SRR1772234
17	*35S:OsMIR390-AtL-BdSpl11*	N703	AGGCAGA	16,234,590	SRR1772235
18	*35S:OsMIR390-AtL-BdSpl11*	N704	TCCTGAG	15,407,203	SRR1772236
19	*35S:OsMIR390-AtL-BdSpl11*	N705	AGGAGTC	21,167,509	SRR1772237
20	*35S:OsMIR390-AtL-BdSpl11*	N706	CATGCCT	19,068,045	SRR1772238

Appendix S1. Characterization of *AtMIR390a-OsL*-based amiRNAs in eudicots

Accumulation and processing of amiRNAs produced from *AtMIR390a*- or *OsMIR390*-based precursors in *Nicotiana benthamiana*

A key feature of the *AtMIR390a-B/c*-based cloning system to produce amiRNA constructs for eudicots is that the amiRNA insert can be synthesized by annealing two relatively short 75 bases-long oligonucleotides (Carbonell *et al.*, 2014). Because the oligonucleotides containing *OsMIR390* distal stem-loop sequences are even shorter (60 bases), we first tested if amiRNAs derived from precursors including *OsMIR390* distal stem-loop sequences could be expressed efficiently in eudicot species. This would reduce the synthesis cost of the oligonucleotides required for generating *AtMIR390a*-based amiRNA constructs, and benefit the generation of large amiRNA construct libraries for gene knockdown in eudicots such as those reported recently (Hauser *et al.*, 2013; Jover-Gil *et al.*, 2014).

To test the functionality of authentic *OsMIR390* precursors to produce high levels of accurately processed small RNAs, miR390 and three different amiRNA sequences (amiR173-21, amiR472-21 and amiR828-21) (Cuperus *et al.*, 2010) were directly cloned into *pMDC32B-OsMIR390-B/c* (Figure S1, Table I) and expressed transiently in *N. benthamiana* leaves (Figure S5). The same small RNA sequences were also expressed from the chimeric *AtMIR390a-OsL* precursor including *AtMIR390a* basal stem and *OsMIR390* distal stem-loop sequences (Figure S4, Figure S8a). For comparative purposes, the same small RNA sequences were expressed from the authentic *AtMIR390a* precursor or from a chimeric precursor including *OsMIR390* basal stem and *AtMIR390a* stem-loop sequences (*OsMIR390-AtL*) (Figure S3, Figure S8a). Samples expressing the ß-glucuronidase transcript from the *35S:GUS* construct were used as negative controls.

MiR390 accumulated to similar levels when expressed from each of the different precursors (Figure S8b). In each case, amiRNAs expressed from *AtMIR390a-OsL* precursors did not accumulate to significantly different levels than did the corresponding amiRNAs produced from authentic *AtMIR390a* precursors (P> 0.11 for all pairwise *t*-test comparisons) (Figure S8b). *AtMIR390a-OsL*-derived amiRNAs accumulated predominantly to 21 nt species, suggesting that the chimeric amiRNA precursors were likely processed accurately (Figure S8b). Finally, amiRNAs produced from either authentic *OsMIR390* or chimeric *OsMIR390-AtL* precursors did not always accumulated as 21 nt species (e.g. miR828-21 and amiR472-21 from *OsMIR390* or *OsMIR390-AtL* precursors, respectively) (Figure S8b). Therefore, further analyses focused on characterizing *AtMIR390a-OsL*-based amiRNAs.

To more accurately assess processing of the amiRNA populations produced from *AtMIR390a-OsL* precursors, small RNA libraries were prepared and sequenced. For comparative purposes, small RNA libraries from samples containing *AtMIR390a*-derived amiRNAs were also analyzed. In each case, the majority of reads from either the chimeric *AtMIR390a-OsL* or authentic *AtMIR390a* precursors corresponded to correctly processed, 21 nt amiRNA (Figure S8c).

Gene Silencing in Arabidopsis by amiRNAs derived from chimeric precursors

To test the functionality of *AtMIR390a-OsL* based amiRNAs in repressing target transcripts, three different amiRNA constructs were introduced into *A. thaliana* Col-0 plants. For comparative purposes, the same three amiRNA sequences were also expressed from authentic *AtMIR390a* precursors as reported before (Carbonell *et al.*, 2014). In particular, amiR-AtFt, and amiR-AtCh42 each targeted a single gene transcript [*FLOWERING LOCUS T* (*FT)* and *CHLORINA 42* (*CH42*), respectively], and amiR-AtTrich targeted three *MYB* transcripts [*TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*) and *ENHANCER OF TRIPTYCHON AND CAPRICE2* (*ETC2*)] (Figure S9). Plants including *35S:GUS* were used as negative controls. Plant phenotypes, amiRNA accumulation, mapping of amiRNA reads in *AtMIR390a-OsL* precursors and target mRNA accumulation were measured in Arabidopsis T1 transgenic lines.

Each of the 44 transformants containing *35S:AtMIR390a-OsL-Ft* was significantly delayed in flowering time compared to control plants not expressing the amiRNA ($P < 0.01$ two sample *t*-test, Figure S10b, Figure S11, Table S5), as previously observed in amiRNA knockdown lines (Schwab *et al.*, 2006; Liang *et al.*, 2012; Carbonell *et al.*, 2014) and *ft* mutants (Koornneef *et al.*, 1991). Two hundred and sixty-six out of 267 transgenic lines containing *35S:AtMIR390a-OsL-Ch42* were smaller than controls and had bleached leaves and cotyledons (Figure S10c, Figure S11, Table S5), as consequence of defective chlorophyll biosynthesis and loss of Ch42 magnesium chelatase (Koncz *et al.*, 1990; Felippes and Weigel, 2009). One hundred and seventy of these plants had a severe bleached phenotype with a lack of visible true leaves at 14 days after plating (Figure S10c, Figure S11, Table S5). Finally, 68 out of 69 lines containing *35S:AtMIR390a-OsL-Trich* had increased number of trichomes in rosette leaves; six lines had highly clustered trichomes on leaf blades like *try cpc* double mutants (Schellmann *et al.*, 2002) or other amiR-Trich overexpressor transgenic lines (Schwab *et al.*, 2006; Liang *et al.*, 2012; Carbonell *et al.*, 2014) (Figure S10d, Table S5). The delayed flowering and trichome phenotypes were maintained in the Arabidopsis T2 progeny expressing amiR-Ft and amiR-Trich, respectively, from chimeric *AtMIR390a-OsL* precursors (Table S6). No obvious phenotypic differences were observed between plants expressing the amiRNAs from the *AtMIR390a-OsL* or *AtMIR390a* precursors in either T1 or T2 generations (Figure S10b-d, Figure S11, Tables S5 and S6). In summary, *AtMIR390-OsL*-based amiRNAs conferred a high proportion of expected and heritable target-knockdown phenotypes in transgenic plants.

The accumulation of all three amiRNAs produced from chimeric *AtMIR390-OsL* or authentic *AtMIR390a* precursors was confirmed by RNA blot analysis in T1 transgenic lines showing amiRNA-induced phenotypes (Figure S10e). In all cases, *AtMIR390-OsL*- and *AtMIR390a*-derived amiRNAs accumulated to similarly high levels and as a single species of 21 nt (Figure S10e), suggesting that *AtMIR390a-OsL*-based amiRNAs were as accurately processed as *AtMIR390a*-based amiRNAs. To more precisely assess processing and accumulation of the *AtMIR390a-OsL*-based amiRNA populations, small RNA libraries from samples containing each of the *AtMIR390a-OsL*-based constructs were prepared. In each case, the majority of reads from *AtMIR390a-OsL* precursors corresponded to correctly processed, 21 nt amiRNA while reads from the amiRNA* strands were always relatively under-represented (Figure S10g) as observed before with the same amiRNAs expressed from *AtMIR390a* precursors (Carbonell *et al.*, 2014).

Finally, accumulation of target mRNAs in *A. thaliana* transgenic lines expressing *AtMIR390a-OsL*- or *AtMIR390a*-based amiRNAs was analyzed by quantitative real time RT-PCR assay. The expression of all target mRNAs was significantly reduced compared to control plants (P < 0.023 for all pairwise *t*-test comparisons, Figure S10f) when the specific amiRNA was expressed. No significant differences were observed in target mRNA expression between lines expressing *AtMIR390a-OsL*- or *AtMIR390a*-based amiRNAs.

Collectively, all these results indicate that amiRNAs produced from chimeric *AtMIR390a-OsL* precursors are highly expressed, accurately processed and highly effective in target gene knockdown. Therefore, the use of chimeric *AtMIR390a-OsL* precursors is an attractive alternative to express effective amiRNAs in eudicots in a cost-optimized manner.

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Appendix S2

DNA sequence of B/c vectors used for direct cloning of amiRNAs in zero-background vectors containing the *OsMIR390* sequence.

Index:

>pENTR-OsMIR390-B/c

>pMDC32B-OsMIR390-B/c

>pMDC123SB-OsMIR390-B/c

>pH7WG2B-OsMIR390-B/c

>pENTR-OsMIR390-B/c (4122 bp)

CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGC
CGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGC
CTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAG
TGAGCGCAACGCAATTAATACGCGTACCGCTAGCCAGGAAGAGTTTGTAGAAACGCAAAAAGGCCATCCG
TCAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGCGGGCGTCCTGCCCGCCACCCTCCGGG
CCGTTGCTTCACAACGTTCAAATCCGCTCCCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAA
CAACAGATAAAACGAAAGGCCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCT
ACTCTCGCGTTAACGCTAGCATGGATGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTCTTAAGC
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AAGCGGAAAATCAGGAAGGGATGGCTGAGGTCGCCCGGTTTATTGAAATGAACGGCTCTTTTGCTGACGA
GAACAGGGGCTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGT
GGATGTACAGAGTGATATTATTGACACGCCCGGCCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCTG
CTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGA
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AACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

PURPLE/UPPERCASE: M13-forward binding site

orange/lowercase: attL1

BLUE/UPPERCASE: *OsMIR390a* 5’ region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *BsaI* site

blue/lowercase: *OsMIR390a* 3' region

orange/lowercase/underlined: attL2

PURPLE/UPPERCASE/UNDERLINED: M13-reverse binding site

brown/lowercase: kanamycin resistance gene

>pMDC32B-OsMIR390-B/c (11675 bp)

CCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAAAATCACCACTCGATACAGGCAGCCCATCAG
TCCGGGACGGCGTCAGCGGGAGAGCCGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGG
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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: *OsMIR390* 5' region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *BsaI* site

blue/lowercase: *OsMIR390* 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-OsMIR390-B/c (11150 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: *OsMIR390* 5' region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *BsaI* site

blue/lowercase: *OsMIR390* 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

>pH7WG2B-OsMIR390-B/c (13122 bp)

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CCGGCGTGGTGGTGTCGGCCCACGGCTCTGCCAGGCTACGCAGGCCCGCGCCGGCCTCCTGGATGCGCTC
GGCAATGTCCAGTAGGTCGCGGGTGCTGCGGGCCAGGCGGTCTAGCCTGGTCACTGTCACAACGTCGCCA
GGGCGTAGGTGGTCAAGCATCCTGGCCAGCTCCGGGCGGTCGCGCCTGGTGCCGGTGATCTTCTCGGAAA
ACAGCTTGGTGCAGCCGGCCGCGTGCAGTTCGGCCCGTTGGTTGGTCAAGTCCTGGTCGTCGGTGCTGAC
GCGGGCATAGCCCAGCAGGCCAGCGGCGGCGCTCTTGTTCATGGCGTAATGTCTCCGGTTCTAGTCGCAA
GTATTCTACTTTATGCGACTAAAACACGCGACAAGAAAACGCCAGGAAAAGGGCAGGGCGGCAGCCTGTC
GCGTAACTTAGGACTTGTGCGACATGTCGTTTTCAGAAGACGGCTGCACTGAACGTCAGAAGCCGACTGC
ACTATAGCAGCGGAGGGGTTGGATCAAAGTAC

cyan/lowercase: T-DNA right border

grey/lowercase: OsUbi promoter

ORANGE/UPPERCASE: attB1

BLUE/UPPERCASE: *OsMIR390* 5' region

RED/UPPERCASE: *Bsa*I site

magenta/lowercase: chloramphenicol resistance gene

MAGENTA/UPPERCASE: *ccd*B gene

red/lowercase: inverted *BsaI* site

blue/lowercase: *OsMIR390* 3' region

ORANGE/UPPERCASE/UNDERLINED: attB2

green/lowercase/underlined: CaMV terminator

GREY/UPPERCASE: ZmUbi promoter

BROWN/UPPERCASE: hygromycin resistance gene

CYAN/UPPERCASE: T-DNA left border

brown/lowercase: spectinomycin resistance gene

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

Appendix S3. FASTA sequences of all amiRNA-producing *MIRNA* precursors analyzed.

(a) Sequences of *OsMIR390*-based amiRNA precursors

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-*OsMIR390* that had to be modified to preserve the authentic *OsMIR390* precursor structure are highlighted in red.

>**OsMIR390**
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGAAGCTCAGGAGGGATAGCGCCTCGAAATCAAACTAG
GCGCTATCTATCCTGAGCTCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGAAGCTCAGGAGGGATAGCGCCATGATGATCACATTC
GTTATCTATTTTTTGGCGCTATCTATCCTGAGCTCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-173-21
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTTCGCTTGCAGAGAGAAATCATCGAAATCAAACTAT
GATTTCTCTGTGTAAGCGAACATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-173-21
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTTCGCTTGCAGAGAGAAATCAATGATGATCACATTC
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OsMIR390-472-21
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTTTTTCCTACTCCGCCCATACTCGAAATCAAACTAG
TATGGGCGGCGTAGGAAAAACATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-472-21
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GTTATCTATTTTTTGTATGGGCGGCGTAGGAAAAACATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-828-21
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTCTTGCTTAAATGAGTATTCCTCGAAATCAAACTAG
GAATACTCAGTTAAGCAAGACATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-828-21
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>OsMIR390-Bri1
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AGCAAGGCGTAAGATTGCGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-Bri1
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GTTATCTATTTTTTGAGCAAGGCGTAAGATTGCGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-Cad1
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GGGCTTACTGCTCAGATCGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-Cad1
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GTTATCTATTTTTTTGGGCTTACTGCTCAGATCGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-Cao
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTCTGCATGGATTGTAAACCCATCGAAATCAAACTAT
GGGTTTACACTCCATGCAGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-Cao
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTCTGCATGGATTGTAAACCCAATGATGATCACATTC
GTTATCTATTTTTTTGGGTTTACACTCCATGCAGCCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-Spl11
GAGATGTTTTGAGGAAGGGTATGGAACAATCCTTGTTAGCAACACTACAAGGGCACTCGAAATCAAACTAG
TGCCCTTGTCGTGTTGCTACCATGGTTTGTTCTTACCACACGACCAATTAAATC

>OsMIR390-AtL-Spl11
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GTTATCTATTTTTTGTGCCCTTGTCGTGTTGCTACCATGGTTTGTTCTTACCACACGACCAATTAAATC

(b) Sequences of *AtMIR390a*-based amiRNA precursors

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* precursor structure are highlighted in red. Other details as in (a).

>AtMIR390a
TATAGGGGGGAAAAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAATGAATAATTTCAC
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GGCAAAACGCCTAAAATCACTTGAGAATCAATTCTTTTTACTGTCCATTTAAGCTATCTTTTATAAACGTG
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AGATTAGATCTCATCTTTAGTCTC

>AtMIR390a-OsL
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TTTAGTCTC

>AtMIR390a-173-21
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AGATTAGATCTCATCTTTAGTCTC

>AtMIR390a-OsL-173-21

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TTTAGTCTC

AtMIR390a-472-21

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AGATTAGATCTCATCTTTAGTCTC

AtMIR390a-OsL-472-21

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CTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATC
TTTAGTCTC

>AtMIR390a-828-21

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AGATTAGATCTCATCTTTAGTCTC

>AtMIR390a-OsL-828-21

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CTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATC
TTTAGTCTC

>AtMIR390a-Ch42

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TCTTATTTTCTATCTCTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAAC
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>AtMIR390a-OsL-Ch42

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CTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATC
TTTAGTCTC

>AtMIR390a-Ft

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GGCAAAACGCCTAAAATCACTTGAGAATCAATTCTTTTTACTGTCCATTTAAGCTATCTTTTATAAACGTG
TCTTATTTTCTATCTCTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAAC
AGATTAGATCTCATCTTTAGTCTC

>AtMIR390a-OsL-Ft

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CTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATC
TTTAGTCTC

>AtMIR390a-Trich

TATAGGGGGGAAAAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAATGAATAATTTCAC
GTTTAACGAAGAGGAGATGACGTGTGTTCCTTCGAACCCGAGTTTTGTTCGTCTATAAATAGCACCTTCTC
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GGCAAAACGCCTAAAATCACTTGAGAATCAATTCTTTTTACTGTCCATTTAAGCTATCTTTTATAAACGTG
TCTTATTTTCTATCTCTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAAC
AGATTAGATCTCATCTTTAGTCTC

>AtMIR390a-OsL-Trich

TATAGGGGGGAAAAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAATGAATAATTTCAC
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AGGCGAGCAGTCTCGAATGGGACATTGGCTCTTCTTACTACAATGAAAAAGGCCGAGGCAAAACGCCTAAA
ATCACTTGAGAATCAATTCTTTTTACTGTCCATTTAAGCTATCTTTTATAAACGTGTCTTATTTTCTATCT
CTTTTGTTTAAACTAAGAAACTATAGTATTTTGTCTAAAACAAAACATGAAAGAACAGATTAGATCTCATC
TTTAGTCTC

Appendix S4

Protocol to clone amiRNAs in *Bsa*I/*ccd*B-based ('B/c') vectors including the *OsMIR390* precursor.

Notes:

-Available OsMIR390 B/c vectors are listed in Table I at the end of this protocol.

-OsMIR390-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 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 *pH7WG2B*-based vectors).

Table 1: *OsMIR390-BsaI/ccdB* ('B/c') vectors for direct cloning of amiRNAs.

Vector	Bacterial antibiotic resistance	Plant antibiotic resistance	GATEWAY use	Backbone	Promoter	Terminator	Plant species tested	Addgene ID
pENTR-OsMIR390-B/c	Kanamycin	-	Donor	*pENTR*	-	-	-	61468
pMDC123SB-OsMIR390-B/c	Kanamycin	BASTA	-	*pMDC123*	*CaMV* 2x35S	*nos*	*Nicotiana benthamiana*	61466
pMDC32B-OsMIR390-B/c	Kanamycin Hygromycin	Hygromycin	-	*pMDC32*	*CaMV* 2x35S	*nos*	*Nicotiana benthamiana* *Brachypodium distachyon*	61467
pH7WG2B-OsMIR390-B/c	Spectinomycin	Hygromycin	-	*pH7WG2*	*Os Ubiquitin*	*CaMV*	*Brachypodium distachyon*	61465