

**Supplementary Table 1. Cloning verification of miRNA and candidate miRNA families in rice**

Family	Os-loci	miRNA	Cloned Sequence: 5' -3' (Cloned Times)	Satisfied <i>Criteria</i> <sup>4</sup>
<b>Newly identified families</b>				
<i>OsMIR528</i>	1	OsmiR528	UGGAAGGGGCAUGCAGAGGAG(1)	A+B+C
<i>OsMIR529</i>	2	OsmiR529a,b	CUGUACCCUCUCUCUUCUUC(1)	A+B+C
<i>OsMIR530</i>	1	OsmiR530	AGGUGCAGAGGCAGAUGCAAC(2)	A+B+C
Candidate 1	3		UCCAUUGUCGUCUAGUCCGG(3)	B+C
Candidate 2	1		ACCGAAGAAGCCUGUGCUCAA(1)	B+C
Candidate 3	11		CACCCUAUCGAACAACCGUGG(1)	B+C
Candidate 4	1		GGCCGGAUGGUGAGCUUGAUGG(1)	B+C
Candidate 5	3		AUGUAACUACAAUGUAACUAC(1)	B+C
Candidate 6	2		UACUAUGGCCUACAUGUCAGUGA(1)	B+C
Candidate 7	2		UGGUGUGAACUAAACAGGGCCUAA(1)	B+C
Candidate 8	6		AACUUGUGUCACUGACAUGUGGAC(1)	B+C
Candidate 9	1		UAGAGGUAGAAUUGAUCUCC(4)	B+C
<b>Previous predicted and identified families</b>				
<i>OsMIR156</i> <sup>1,2</sup>	12	OsmiR156a-j	UGACAGAAGAGAGUGAGCAC(1)	A+B+C+D
<i>OsMIR166</i> <sup>1,2</sup>	12	OsmiR166a-f	UCGGACCAGGCUUCAUCCCC(1)	A+B+C+D
<i>OsMIR167</i> <sup>1,2</sup>	10	OsmiR167a,b,c	UGAAGCUGCCAGCAUGAUCUA(1)	A+B+C+D
		OsmiR167d-h,j	UGAAGCUGCCAGCAUGAUCUGA(1)	
<i>OsMIR168</i> <sup>2</sup>	2	OsmiR168a	UCGCUUGGUGCAGAUCCGGAC(25)	A+B+C+D
		OsmiR168*	CCCGCCUUGCACCAAGUGAAU(2)	
<i>OsMIR171</i> <sup>1,2,3</sup>	7	OsmiR171a-f	UGAUUGAGCCGUGCCAAUAUC(2)	B+C+D
<i>OsMIR319</i> <sup>2</sup>	3	OsmiR319a,b	UUGGACUGAAGGGUGCUCCC(1)	A+B+C+D
<i>OsMIR393</i> <sup>2,3</sup>	1	OsmiR393	UCCAAAGGGAUCGCAUUGAUC(2)	B+C+D
<i>OsMIR396</i> <sup>2</sup>	5	OsmiR396d,e	UCCACAGGCUUUCUUGAACUG(2)	A+B+C+D
<i>OsMIR397</i> <sup>2</sup>	2	OsmiR397a,b	UUGAGUGCAGCGUUGAUGAAC(1)	B+C+D

Notes:

1:Reinhart *et al.*, 2002; 2: Jones-Rhoades and Bartel, 2004; 3: Sunkar and Zhu ,2004; 4: Ambros *et al.*, 2003.

Satisfied *Criteria* are as following:

- Detection of a distinct ~22-nt RNA transcript by Northern blot.
- Identification of the ~22-nt sequence in a size-fractionated cDNAs library.
- Prediction of a potential fold-back precursor structure that contains the ~22-nt miRNA sequence within one arm of the hairpin.
- Phylogenetic conservation between species.
- Detection of increased precursor accumulation in organisms with reduced Dicer function.

**Supplementary table 2. Predicted targets of cloned OsmiRNAs**

miRNA Gene	Target Proteins	Target Genes(Mismatches Between miRNA and Target Gene)
<i>OsMIR156</i>	(SBP)-like Proteins	AK109469(1), AK107191(1), AK073179(1), AK068749(1), AK068702(1), AK063561(1),AK062581(1),
<i>OsMIR166</i>	Hox Proteins	AK103284(4), AK102603(4), AK102378(4), AK102183(4)
<i>OsMIR167</i>	OsARF Proteins	AK110044(4), AK100167(3), AK071455(3), AB071298(3), AK121703(4), AK103280(4), AK070569(4)
<i>OsMIR168</i>	ARGONAUTE Proteins	AK111996(1), AK111587(1), AK120815(4)
<i>OsMIR168*</i>	Putative Transposase	AK100608(4)
<i>OsMIR171</i>	Scl Proteins	AK106868(0), AK101035(0), AK100757(0), AK072900(0), AK106239(2)
<i>OsMIR319</i>	Unknown	Unknown
<i>OsMIR393</i>	Transport Inhibitor Response 1 (TIR1)	AK121600(3), AK072338 (3),
<i>OsMIR396</i>	Growth-Regulating Factors	AK108170(2), AK103508(2), AK063983(2), AK058659(2),
<i>OsMIR397</i>	Putative Laccase	AK068047(2), AK071929(1)
<i>OsMIR528</i>	Unknown	Unknown
<i>OsMIR529</i>	Unknown	XM_465610(4)
<i>OsMIR530</i>	Unknown	Unknown

Supplementary table 3. Cloned miRNAs and their Precursor genes.

miRNA gene	Precursor gene
<i>OsMIR156</i>	AK110797(156c), AK073452(156d), AK103769(156h)
<i>OsMIR166</i>	Unknown
<i>OsMIR167</i>	AK067588(167h), AK067077(167h)
<i>OsMIR168</i>	AK065610(168a), AK063802(168a), AK111553(168b)
<i>OsMIR171</i>	Unknown
<i>OsMIR319</i>	AK064418(319a)
<i>OsMIR393</i>	Unknown
<i>OsMIR396</i>	AK120940(396d), AK066292(396e)
<i>OsMIR397</i>	AK109752(397b)
<i>OsMIR528</i>	AK099390, AK073820, AK063857
<i>OsMIR529</i>	Unknown
<i>OsMIR530</i>	Unknown

**Supplementary Figure 1.** Predicted fold-back structures of the putative miRNA precursors from rice (see Supplementary Table 1).

Candidate 1a

```

      AUU      A- CC-      A
5`GAAUCC  GUCGUCU GU  GGUUAGG \
3`CUUGGG  CAGCGGA CA  UCGGUCC U
      CC-      CC CUU      A

```

Candidate 1b

```

      U AUU      A- CC-      GA
5`GA CC  GUCGUCU GU  GGUUAG \
3`CU GG  CAGCAGA CA  UCGGUC  U
      U GCC      CC CUU      UA

```

Candidate 1c

```

      U- AUU      A- CC-      A
5`UUG CC  GUCGUCU GU  GGUUAGG \
3`AAC GG  CAGCGGA CA  UCGGUCC U
      UU GCC      CC CUU      A

```

Candidate 2

```

      U  A              AAAA
5`UU ACCG AGAAGCCUGUGCUC  \
3`AA UGGU UUUUCGGACACGAG  U
      C  C              CUAA

```

Candidate 3a

```

      CC      AA G      CAA  C      AAC
5`GCAC UAUCGAAC CC UGGUUCGA  GGUA CUAUCGAAC  \
3`CGUG  AUAGCUUG  GG AUCAAGCU  CCAU GAUAGCUUG  C
      U-      AC  A      AUA  -      ACA

```

Candidate 3b

```

      C  A      A  UG      A      -      AAC
5`GCA CCU UCGAAC ACCG GUUCGACA  GGUACC UAUCGAAC  \
3`CGU  GGA  AGCUUG  UGGC  CAAGCUGU  CCAUGG AUAGCUUG  C
      -  C      A  GU      -      G      ACA

```

Candidate 3c

CC AA G G A A G  
5`GCAC UAUCGAAC CC UGG UCGAUA GGUA GUUCGAUAGG \  
3`CGUG AUAGCUUG GG AUC AGCUAU CCAU CAAGCUGUCC U  
U- AC A A C - A

Candidate 3d

AC A A UG A G  
5`GC CCU UCGAAC ACCG GUUCGACA G U  
3`CG GGA AGCUUG UGGC CAAGCUGU C A  
-- C A GU - C

Candidate 3e

- CUA A UG A -- AAC  
5`UUAG CACC UCGAAC ACCG GUUCGACA GGUACCU AUCGAAC \  
3`AAUU GUGG AGCUUG UGGC CAAGCUGU CCAUGGG UAGCUUG C  
A AC- A GU - GA ACA

Candidate 3f

- CUA A UG AA - AAC  
5`UUAG CACC UCGAAC ACCG GUUCGAUA GUA CCUAUCGAAC \  
3`AAUU GUGG AGCUUG UGGC CAAGCUGU CAU GGAUAGCUUG C  
A AC- A GU C- G ACA

Candidate 3g

CC A C G G  
5`UUAGCA CUGUCGAAC AC GU GUUCGACAGA G  
3`AGUCGU GACAGCUUG UG CA CAAGCUGUCU U  
-- A C A A

Candidate 3h

CC A C G G  
5`AGCA CUGUCGAAC AC GU GUUCGACAGA G  
3`UUGU GACAGCUUG UG CA CAAGCUGUCU U  
-- A C A A

Candidate 3i

5`UUA A                    A C G    GAC  
      GC CCCUGUCGAAC AC GU GUUU    \  
      CG GGGACAGCUUG UG CA CAAG    A  
3`GGA -                    G    C    A    GGG

Candidate 3j

A--    G                    GUU  
5`UUAGC ACCCUGUCGAAC    ACC UGGUUCGACAGGUGUA    C  
3`AGUUG UGGGACAGCUUG    UGG GUCAAGCUGUCCACGU    A  
                              A                    ACG    -                    ACA

Candidate 4

5`ATTCGG    AT    A            T            T            C  
              CCGG GGTG GCTTGA GGCGAA CCTT GGTC  
              GGTT CCAC CGGATT CCGTTT GGAA CCAG  
3`GACTTA    C-            G            -            C            T

105nt  
loop

Candidate 5a

                              G                    CUA                    A  
5`GTAGUUAUA UGUAGUUACA    UAGUUAC    \  
3`CAUCAAAUGU ACAUCAAAUGU    AUUAAUG    A  
                              A                    AAC                    U

Candidate 5b

5`UCC                    C                    A    A  
      AGUUACACU UAGUUACA UAU A  
      UCAAUGUGA AUCAAAUGU AUG U  
3`GCA                    C                    A    U

Candidate 5c

5`UA                    GUA                    CUA                    A    A    A  
      UAGUUAUA    UAGUUACA    UAGUUACA UGUA UUAC    \  
      AUCAAAUGU    AUCAAAUGU    AUCAAUGU GCAU AAUG A  
3`GG                    AAC                    AG-                    A    C    U

Candidate 6a

```

          A      CCACT
5`GAGTCACTGGCAT TGGGTC      T
3`CTCAGTGACTGTA ATCCGG      T
          C      TATCA
```

Candidate 6b

```

          G      CCACT
5`TGAG CACTGACATGTGGGTC      T
3`ACTC GTGACTGTACACCCGG      T
          A      TATCA
```

Candidate 7a

```

5`GGGUAGUU  --  C-  GG  A
      UGGU  GUGAA  UAAACA  GCCUA  A
      AUCA  UACUU  AUUUGU  UGGGU  C
3`AUUUUUUCA  UU  UC  G-  U
```

Candidate 7b

```

5`UGGU  C  A  -  C  G
      GUGAA  UAA  CAG  GG  CUAAAUU  \
      UACUU  GUU  GUC  UC  GGUUUA  G
3`UGAC  C  A  A  A  U
```

Candidate 8a

```

5`A  GU-  AUCUA
      ACUU  GUCACUGACAUGUGGACC  U
      UGAA  CAGUGAUUGUACGCCUGG  C
3`C  ACU  GUAAC
```

Candidate 8b

```

5`AAC  UA  --  UCUA
      UUG  UCACUGACAUGU  GGGUCC  U
      AAU  AGUGACUGUACA  CCCGGG  C
3`CGA  UC  GG  UAAC
```

Candidate 8c

```
5`A  U--      C A  C U UA
      ACUUG  GUCACUGA AU UGGA CC C \
      UGAAU  CAGUGAUU UA ACCU GG G C
3`A   GUC      A C  A C AC
```

Candidate 8d

```
5`A-  GUGC-      A C- UA
      ACUU      CACUGACAUGUGG CC UC \
      UGAA      GUGACUGUACACC GG AG C
3`AC  ACUCA      C  AU UU
```

Candidate 8e

```
5`A  GU-  A G AUA      U A
      ACUU  GUC CU AC  UGGACCC CU C
      UGAA  CAG GA UG  ACCUGGG GA U
3`C  ACU  C  A CAC      U U
```

Candidate 8f

```
5`AAC U      - UA
      UUG GUCACUGACAUGUGGGCCC CU \
      GAC CAGUGACUGUACACCCGGG GA C
3`ACU  U      C  UC
```

Candidate 9

```
5`--  AGA      AA  A  TT T
      TTGGT GGTAG TTG TCTCC TC TGTC
      GGCCG  CCGTC  AGC  GGAGG  GG ACGAG
3`GG  ---      GG  -  T- T
```

120nt  
loop