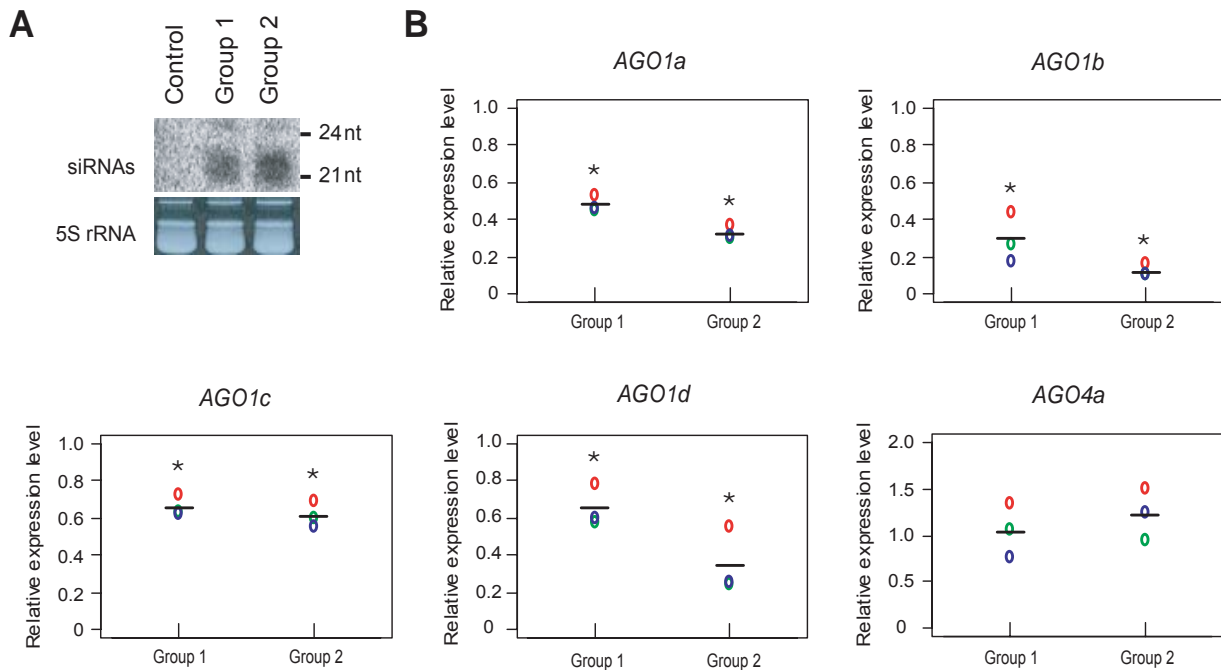


**Supplemental Figure 1. Phylogenetic relationships of Arabidopsis and rice AGO proteins.**

Alignments of full-length AGO protein sequences were produced by CLUSTALW, and used for phylogenetic analysis. The midpoint-rooted phylogenetic tree was constructed by MEGA program using Neighbor-Joining method with bootstrap values from 1000 trials. The alignment file is included in Supplemental Dataset 1.



**Supplemental Figure 2. Molecular characterization of *AGO1* RNAi lines.**

**(A)** siRNAs derived from the *AGO1* IR construct were detected by RNA gel blot in both weak and strong RNAi lines, not in control rice plants. **(B)** Relative expression levels of four *AGO1*s and *AGO4a* in the leaves of the two *AGO1* RNAi lines. The gene expression levels were normalized using the signal from the *GAPDH* gene. Values (colored circles) from three technical repeats of quantitative reverse-transcription PCR are shown. Horizontal bars represent the average values. Asterisks show where differences between RNAi lines and the Control line were significant ( $P \leq 0.05$  from t test).

**AGO1a**Nominal mass (M<sub>r</sub>): 120373; Calculated pI value: 9.46

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **43%**Matched peptides shown in **Bold Red**

1 MAFQLDNGYY SHQALAMMRK KKTEPR**NAGE** **SSGTQQATGA** **PGR**GPSQRPE  
 51 RAQQHGGGGW QPANPQYAQQ AGRGGGQHQG RGGRYQGRGG PTSHQPGGGP  
 101 VEYQAHEEYRG RGVQRQGGMP QHR**SGSGGHG** **VPASPSRTVP** **ELHQASQDQY**  
 151 **QATVVAPSPS** **RTGPSSLPVE** ASSEEVQHQF QELAIQGGSP TSQAIQPAPP  
 201 SSKSVRFPMR PGKGTFGDRC IVK**ANHFFAE** **LPDKDLHQYD** **VSITPEVPSR**  
 251 GVNRA**AVIGEI** **VTQYRQSHLG** GRLPVYDGRK **SLYTAGPLPF** **TSRTFDVILQ**  
 301 **DEESLAVGQ** **GAQR**RERPFK VVIKFAARAD **LHHLAMFLAG** **RQADAPQEAL**  
 351 **QVL**DIVLREL PTARYSPVAR **SFYS**PNLGRR **QQLGEGLESW** RGFYQSIRPT  
 401 QMGLSLNIDM SSTAFIEPLP VIDFVAQLLN **RDISVRPLSD** **ADRVKIKKAL**  
 451 RGVK**VEVTHR** GNMRRKYRIS **GLTSQATREL** **SFPIDNHGTV** **KTVVQYFQET**  
 501 **YGFNIK**HTTL PCLQVGNQQR PNYLPMEVCK IVEGQRYSKR LNEK**QITALL**  
 551 **KVTCQR**PQER **ELDILQTVHH** **NAYHQDPYAQ** **EFGIRIDERL** **ASVEARVLPP**  
 601 **PWLK**YHDSGR **EKDVLPR**IGQ WNMNKKMVN GGRVNNWTCI NFSRHVQDNA  
 651 ARSFCRELAI MCQISGMDFS IDPVVPLVTA RPEHVERALK ARYQEAMNIL  
 701 KPQGELDLL IAILPDNNGS LYGDLKRICE TDLGLVSQCC LTKHVFKMSK  
 751 **QYLANVALKI** NVKVGGR**NTV** **LVDALTRRIP** LVSDRPTIIF GADVTHPHPG  
 801 EDSSPSIAAV VASQDWPEVT **KYAGLVSAQA** **HRQELIQDLF** **KVWKDPQRGT**  
 851 **VSGGMIRELL** **ISFKRATGQK** PQRIIFYRDG **VSEGQFYQVL** **FYELDAIRKA**  
 901 CASLEADYQP PVTFFVVQKR HHTR**LFANNH** **KDQRTVDRSG** **NILPGTVVDS**  
 951 **KICHPTEFDF** YLCSHAGIQG TSRPAHYHVL WDENKFTADG LQTLTNNLCY  
 1001 TYARCTR**SVS** **IVPPAYY AHL** **AAFRA**RYME **PDTSDSGSMA** **SGAHTRGGGP**  
 1051 **LPGARSTKPA** **GNVAVRPLPD** **LKENVKRVMF** YC

**AGO1b**Nominal mass (M<sub>r</sub>): 121557; Calculated pI value: 9.55

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: **34%**Matched peptides shown in **Bold Red**

1 MVKKKR**TGSG** **STGESSGEAP** **GAPGHGSSQR** AERGPQQHGG GRGWVPQHGG  
 51 RGGGQYQGRG GHYQGRGGQG SHHPGGGPPE YQGRGGPGSH HPGGGPPDYQ  
 101 GRGGSGSHHP GGGPPEYQPR DYQGRGGPRP RGGMPQPYG GPRGSGGRSV  
 151 PSGSSRTVPE LHQAPHVQYQ APMVSPTPSG AGSSSQPAAE VSSGQVQQQF  
 201 QQLATR**DQSS** **TSQAIQIAPP** **SSKSVR**FPLR **PGKGT**YGDRC IVK**ANHFFAE**  
 251 **LPDKDLHQYD** **VSITPEVTSR** GVNRA**VMFEL** **VTLYR**YSHLG GRLPAYDGRK

(continued)

301 **SLYTAGPLPF ASRTFEITLQ DEEDSLGGGQ GTQRRERLFR** VVIKFAARAD  
351 **LHHLAMFLAG RQADAPQEAL QVLDIVLREL** PTTRYSPVGR **SFYSPNLGRR**  
401 **QQLGEGLESW RGFYQSIRPT** QMGLSLNIDM SSTA FIEPLP VIDFVAQLLN  
451 **RDISVRPLSD SDRVKIKKAL** RGVKVEVTHR GNMRRKYRIS **GLTSQATREL**  
501 **SFPVDDR**GTV KTVVQYFLET YGFSIQHTTL PCLQVGNQQR PNYLPMEVCK  
551 IVEGQRYSKR LNEKQITALL KVTCQRPQER ELDILR**TVSH NAYHEDQYAO**  
601 **EFGIK**IDERL ASVEARVLPP PRLKYHDSGR **EKDVLPRVGQ WNMNKKMVN**  
651 GGR**VNNWACI NFSRN**VQDSA ARGFCHELAI MCQISGMDFAL EPVLPPLTA  
701 RPEHVERALK **ARYQDAMNML RPQGRE**LDLL IVILPDNNGS LYGDLKRICE  
751 TDLGLVSQCC LTKHVFKMSK **QYLANVALKI** NVKVGGRNTV **LVDALTRRIP**  
801 LVSDRPTIIF GADVTHPHPG EDSSPSIAAV VASQDWPEVT **KYAGLVSAQA**  
851 **HRQELIQDLF KVVQDPHRGT VTGGMIKELL ISFKRATGQK** PQRIIFYRDG  
901 **VSEGQFYQVL LYELDAIRKA** CASLEPNYQP PVTFFVVQKR **HHTRLFANNH**  
951 **NDQRTVDRSG NILPGTVVDS KICHPTEFDF** YLCSHAGIQG TSRPAHYHVL  
1001 WDENKFTADE LQTLTNNLCY TYARCTRSVS IVPPAYY AHL AAFRRARFYME  
1051 PETS DSGSMA SGAATSRGLP PGVRSARVAG **NVAVRPLPAL KENVKRVMFY**  
1101 C

### AGO1c

Nominal mass ( $M_r$ ): 113073; Calculated pI value: 9.56

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 28%

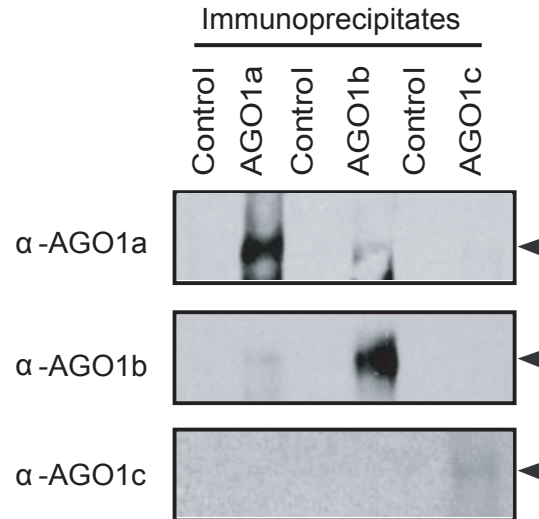
Matched peptides shown in **Bold Red**

1 MASRRP HRH | HTEAPDPGGR GRGRGRAAR**Y AQPQPQ**QQQ **QQQQGR**GCRA  
51 **RGASPPPP**PQ **QQQQQQ**PRST PTRATTVTVA SSSSTTATAS SSPLAPELRQ  
101 AIMEAPRPSE LAQPSPTPPQ EQPVDAATTT PHHIPSSSKS IRFPLRPGKG  
151 TIGTRCMVKA NHFFAHLPNK **DLHHYDVSIT PEVTSRIVNR** AVIK**ELVNLY**  
201 **KASYLGGRLP** AYDGRKSLYT AGPLPFTSQE FQITLLDDDD GSGSERRQRT  
251 FRVVIKFAAR ADLHR**LELFL AGRHAEAPQE ALQVLDIVLR** ELPSARYAPF  
301 GR**SFFSPYLG RRQPLGEGLE** **SWR**GFYQSIR PTQMGLSLNI DMSATAFIEP  
351 LPVIDFVAQL LNSDIHSRPL SDAERVKIKK ALRGVKVEVT HRGNMRRKYR  
401 **ISGLTIQPTR ELTFPVDEGG TVKSVVQYFQ** ETYGFAIQHT YLPCLTVQRL  
451 NYLPMEVCKI VEGQRYSKRL NQNQIRALLE ETCQHPRDRE RDI IKMVK**HN**  
501 **AYQDDPYAKE** FGIKISDRLA SVEARILPAP RLKYNETGRE KDCLPR**VGQW**  
551 **NMNK**KMVNG GKVRSWMCVN FARNVQESV RGFCHALM CQASGMDFAP  
601 EPILPPLNAH PDQVERALKA **RYHDAMNVLG PQRRE**LDLLI GILPDNNGSL  
651 YGDLKRVCEI DLGIVSQCC TKQVFKMN**KQ ILANLALKIN** VKVGGRNTVL  
701 VDAVSRRIPL VTDRPTIIFG ADVTHPHPG E DSSPSIAAVV ASQDWPEVTK  
751 **YAGLVSAQAH RQELIEDLYK IWQDPQRGTV SGGMIRELLI SFKR**STGEKP  
801 QR IIFYR**DGV SEGQFYQVLL YELNAIRKAC** ASLETNYQPK **VTFIVVQKR**H  
851 HTR**LF**AHNH **DQNSVDRSGN ILPGTVVDSK** ICHPTEFDFY LCSHAGIKGT

(continued)

901 SRPAHYHVLW DENNFTADAL QILTNNLCYT YARCTRSVSI VPPAYYAHLA  
951 AFRARFYMEP DTSDSSSVVS GPGVR**GPLSG SSTSRTRAPG GAAVKLPAL**  
1001 **K**DSVKRVMFY C

**Supplemental Figure 3. Sequencing of purified rice AGO1a, AGO1b, and AGO1c by mass spectrometry.**

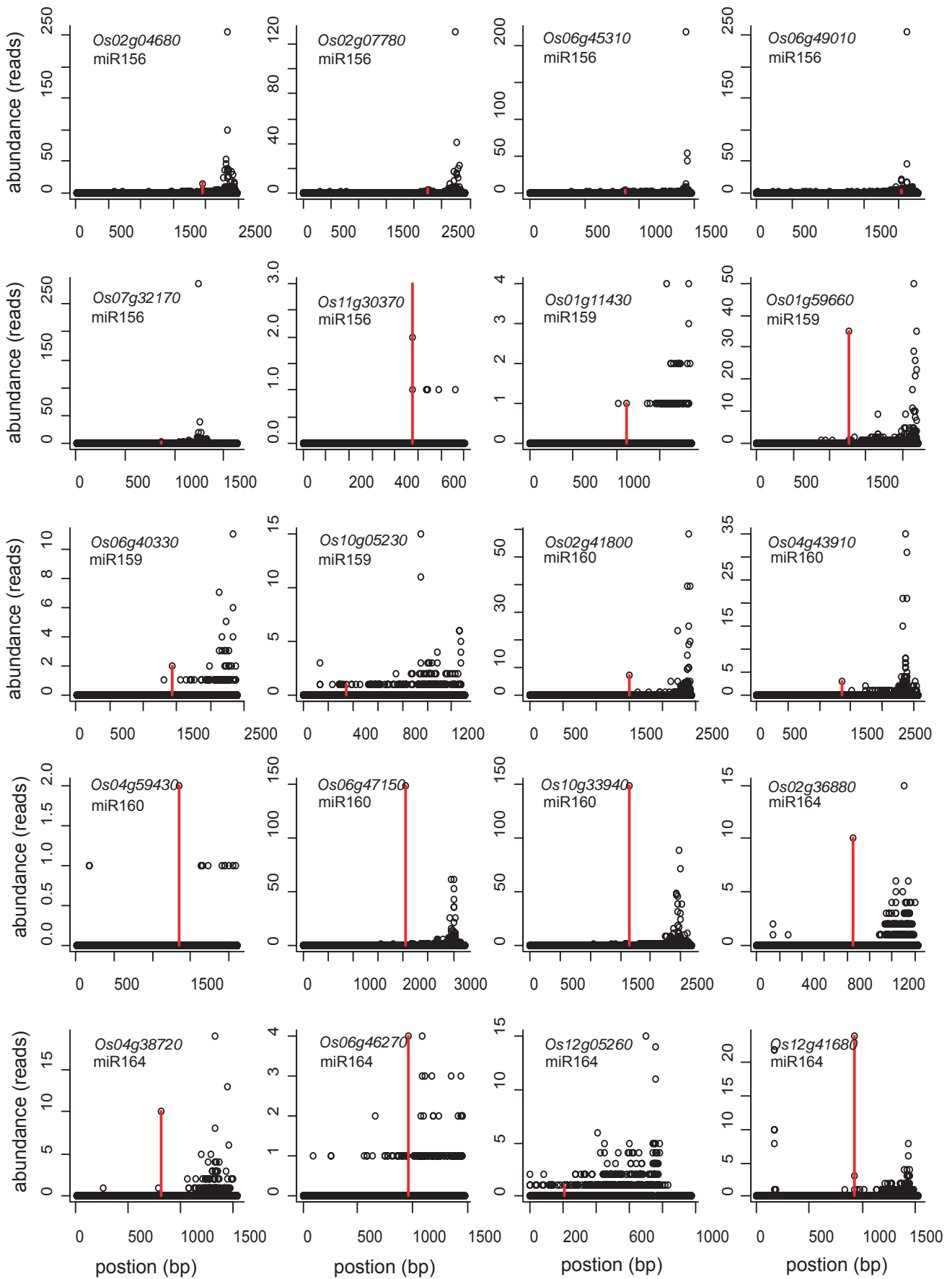


**Supplemental Figure 4. Immunoblot analysis with AGO1 immunoprecipitates.**

AGO1a, AGO1b, and AGO1c were immunopurified from rice seedling extracts. Pre-immune sera were used for control immunoprecipitation (Control). Immunoprecipitates were separated on 10% SDS-PAGE, transferred to Hybond-ECL membranes, and subjected to immunoblotting using indicated antibodies. Positions of the AGO1 proteins are indicated by the arrows.

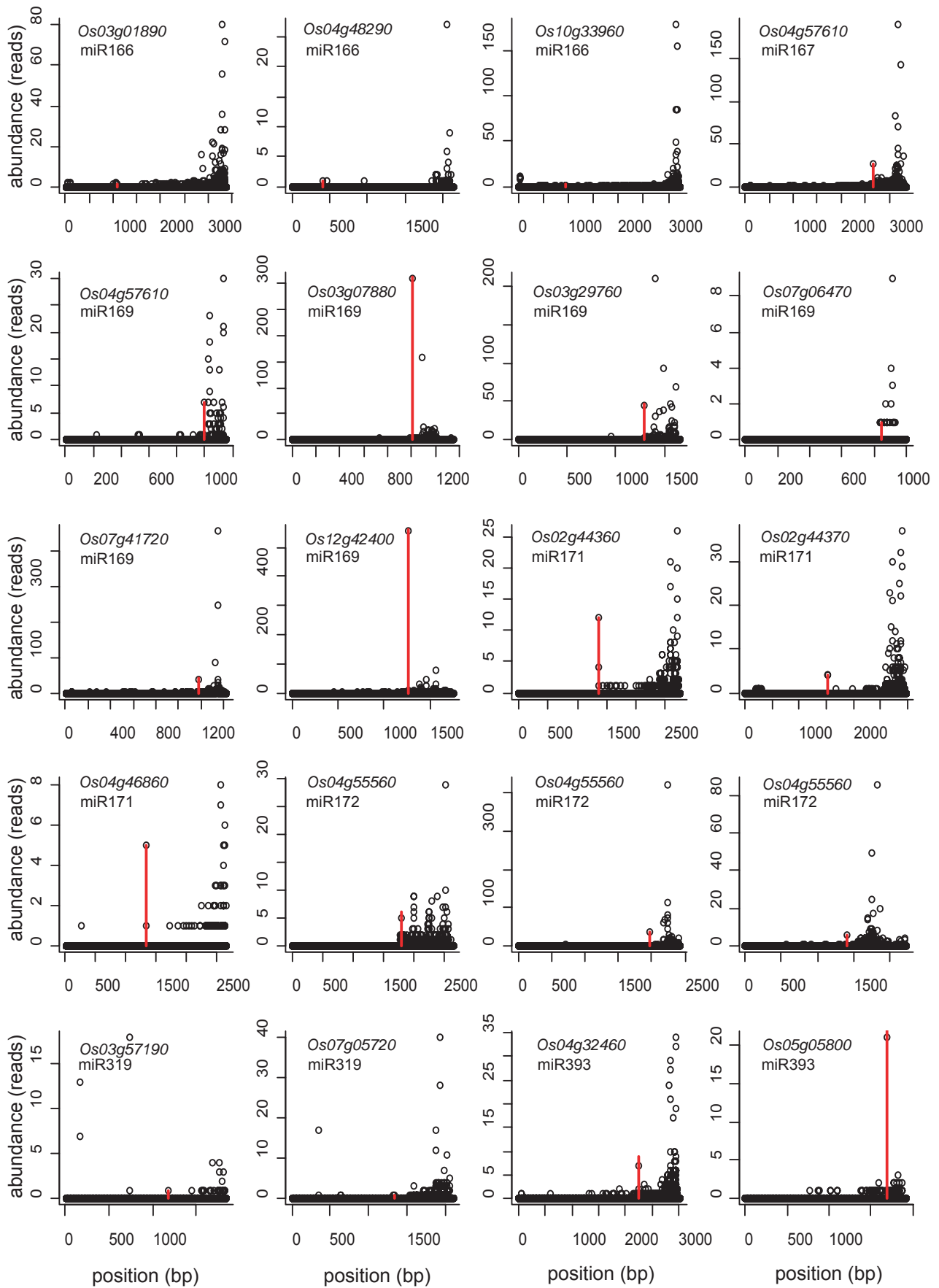


Supplemental Figure 6, Wu et al.

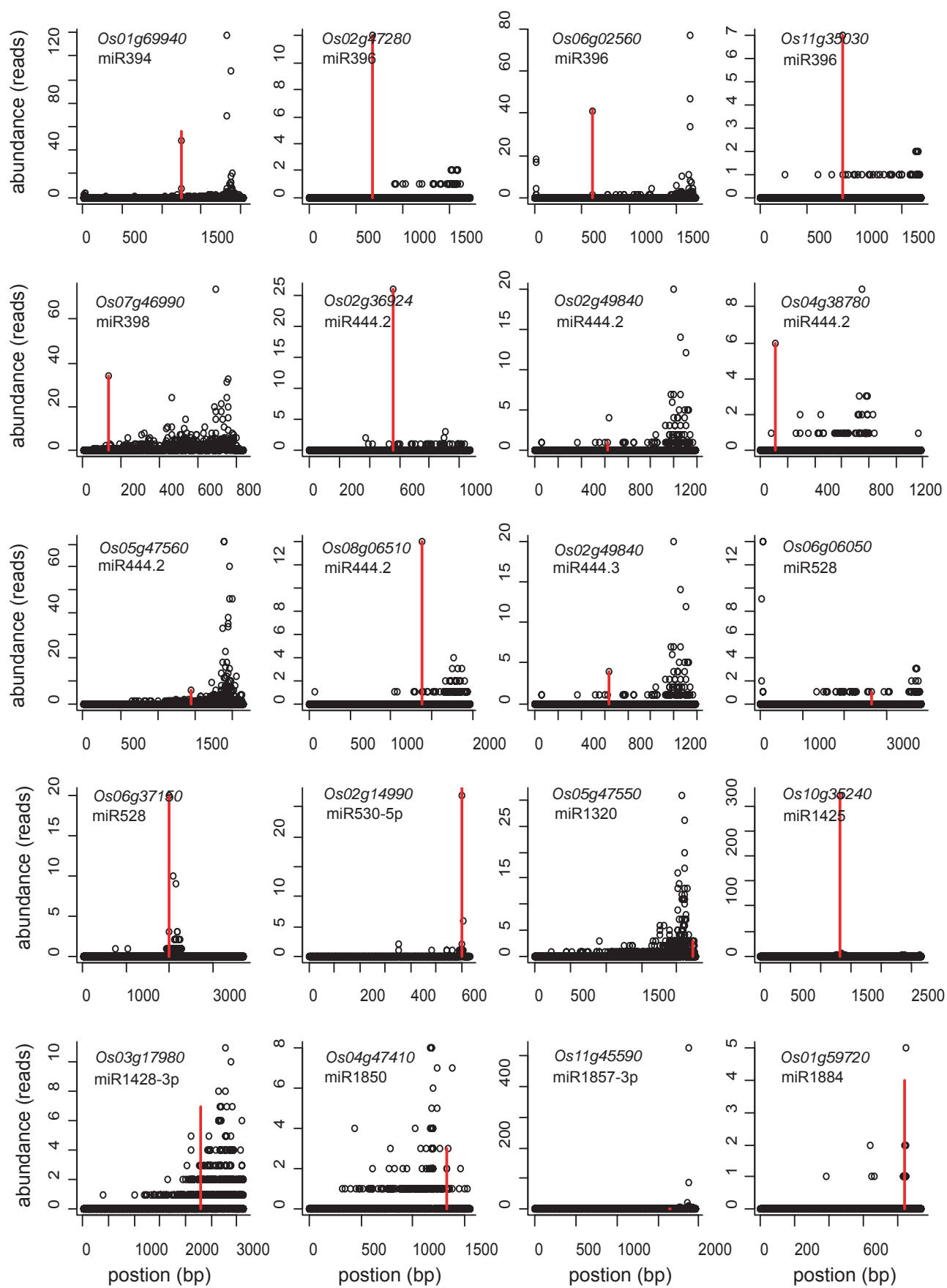




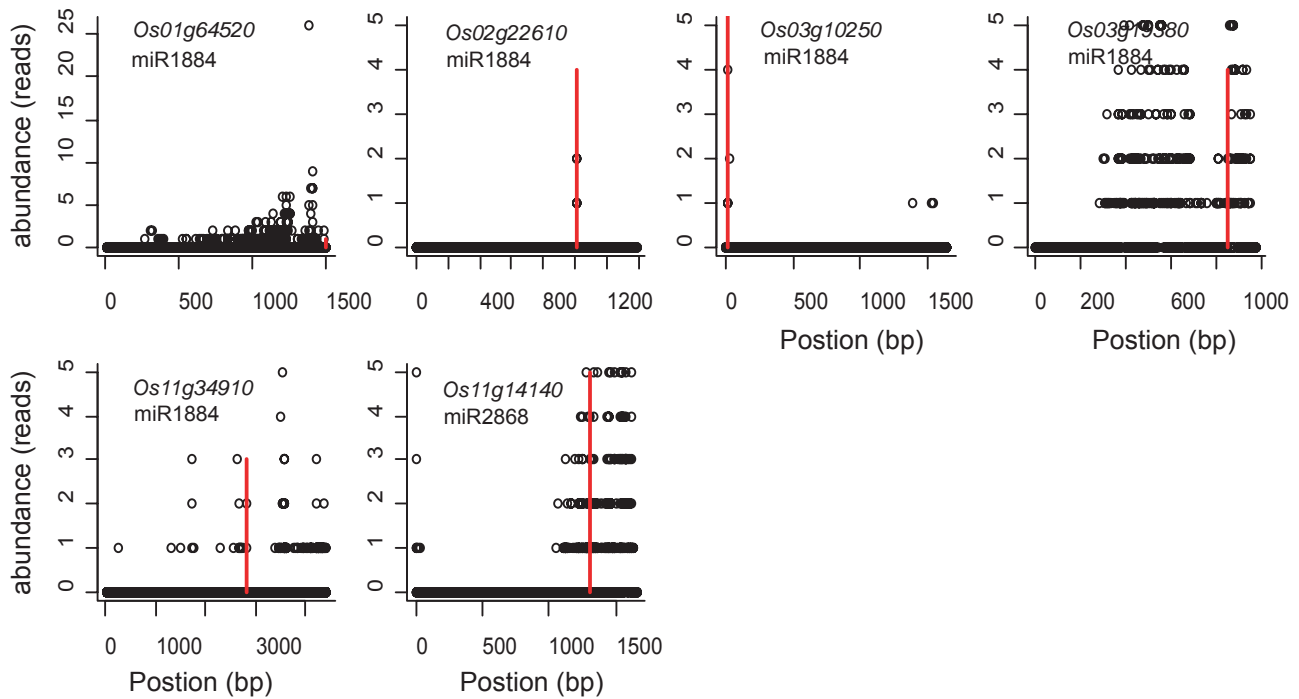
(continued)



(continued)



(continued)



### Supplemental Figure 6. Target plots of validated rice miRNA targets.

The abundance of each signature from degradome sequencing is plotted as a function of its position in the transcript. The signatures matching  $\pm 1$  positions of the expected miRNA cleavage site are combined and shown in red.

**Supplemental Table 1. Summary of sequenced small RNAs in rice total extract and AGO1 complexes**

Category	Total Extract		AGO1a		AGO1b		AGO1c	
	Unique	Reads	Unique	Reads	Unique	Reads	Unique	Reads
<b>miRNAs</b>	9798(0.492%) 364108(9.036% )	698(3.090%)	3449107(90.981%)	264 ( 5.376%)	978003 ( 79.469%)	624(3.894%) 3383266(86.93 6%)		
<b>miRNA<sup>a</sup></b>	944(0.047%) 280338(6.957% )	273(1.209%)	3419816(90.209%)	163 ( 3.319%)	968846 ( 78.725%)	279(1.741%) 3344958(85.95 1%)		
<b>miRNA<sup>*a</sup></b>	783(0.039%) 21708(0.539% )	54(0.239%)	7113(0.188%)	21 ( 0.428%)	2596 ( 0.211%)	47(0.293%) 7886(0.203% )		
<b>Other<sup>b</sup></b>	8458(0.425%) 74055(1.838% )	411(1.820%)	35102(0.926%)	98 ( 1.996%)	14697 ( 1.194%)	330(2.059%) 44170(1.135% )		
<b>Ta-siRNAs</b>	494(0.025%) 3785(0.094% )	82(0.363%)	10759(0.284%)	29 ( 0.591%)	2386 ( 0.194%)	66(0.412%) 5721(0.147% )		
<b>TAS3a1</b>	117(0.006%) 603(0.015% )	16(0.071%)	566(0.015%)	4 ( 0.081%)	215 ( 0.017%)	13(0.081%) 1173(0.030% )		
<b>TAS3a2</b>	98(0.005%) 594(0.015% )	7(0.031%)	186(0.005%)	3 ( 0.061%)	3 ( 0.0001%)	7(0.044%) 198(0.005% )		
<b>TAS3b1</b>	239(0.012%) 2480(0.062% )	53(0.235%)	9896(0.261%)	21 ( 0.428%)	2005(0.163%) 41(0.256% )	4187(0.108%)		
<b>TAS3b2</b>	46(0.002%) 187(0.005% )	7(0.031%)	124(0.003%)	1 ( 0.020%)	163(0.013%) 5(0.031% )	163(0.004%)		
<b>Nat-siRNAs<sup>c</sup></b>	19378(0.97%)	73980(1.84%)	499 (2%)	68566 (2%)	155 (3%)	18562 (2%)	367 (2%)	23413 (0.6%)
<b>Ra-siRNAs<sup>d</sup></b>	1325425(66.54%)	2662041(66.06%)	16845 (75%)	538179 (14%)	3473 (71%)	243675 (20%)	12120 (76%)	602906 (15%)
<b>Dispersed repeat</b>	1250628(62.78%)	2369705(58.81%)	14990 (66%)	195662 (5%)	3040 (62%)	115772 (9%)	10643 (66%)	273782 (7%)
<b>Tandem repeat</b>	115735(5.81%)	263508(6.54%)	2306 (10%)	36817 (1%)	465 (9%)	11381 (1%)	1648 (10%)	54661 (1%)
<b>Inverted repeat</b>	512499(25.73%)	1184107(29.39%)	10069 (45%)	463929 (12%)	1971 (40%)	163000 (13%)	7525 (47%)	501307 (13%)
<b>Non-coding RNAs<sup>e</sup></b>	9225(0.46%)	152138(3.78%)	146 (0.6%)	9195 (0.2%)	101 (2%)	54426 (4%)	136 (0.8%)	18458 (0.5%)
<b>rRNA</b>	1098(0.055%)	9913(0.25%)	13 (0.06%)	174 (0.005%)	13 (0.3%)	914 (0.07%)	12 (0.07%)	420 (0.01%)
<b>tRNA</b>	2112(0.106%)	137952(3.42%)	56 (0.2%)	7784 (0.2%)	51 (1%)	50574 (4%)	52 (0.3%)	15401 (0.4%)
<b>snoRNA</b>	1017(0.051%)	1882(0.047%)	68 (0.3%)	1115 (0.03%)	33 (0.7%)	2852 (0.2%)	63 (0.4%)	2392 (0.06%)
<b>snRNA</b>	842(0.042%)	1342(0.033%)	9 (0.04%)	122 (0.003%)	3 (0.06%)	85 (0.007%)	5 (0.03%)	80 (0.002%)
<b>Protein-coding genes</b>	218638(10.98%) 543423(13.49% )	3206 (14.19%)	82897(2.19%)	857(17.45%)	88748(7.2%)	2391(14.92%)	138233(3.55%)	
<b>Others<sup>f</sup></b>	150666(7.56%)	261939(6.50%)	3969 (18%)	61303 (2%)	876 (18%)	46935 (4%)	2579 (16%)	90260 (2%)
<b>Chloroplast</b>	39410(1.98%)	278808(6.92%)	508 (2%)	12753 (0.3%)	351 (7%)	69861 (6%)	462 (3%)	19505 (0.5%)
<b>Mitochondrial</b>	9171(0.46%)	37931(0.94%)	114 (0.5%)	1655 (0.04%)	60 (1%)	4486 (0.4%)	142 (0.9%)	3665 (0.09%)
<b>Total<sup>g</sup></b>	1991942 4029462 2		2588	3791013	4911	1230668	16024	3891687

<sup>a</sup>Reads encompass the defined miRNA/miRNA\* sequence $\pm$ 2 nts on each side.

<sup>b</sup>Other small RNAs that are produced from miRNA precursors.

<sup>c</sup>Antisense transcripts were annotated as described (Osato et al., *Genome Biology* 2003, 5: R5)

<sup>d</sup>Dispersed repeats were annotated by RepeatMasker, Tandem repeats were annotated by tandem repeat finder program, and Inverted repeat were annotated by inverted repeat finder program

<sup>e</sup>rfam annotated ncRNAs.

<sup>f</sup>sRNA mapped to the TIGR annotated intergenic regions that do not encode miRNAs, ta-siRNAs, Nat-siRNAs, ra-siRNAs and ncRNAs.

<sup>g</sup>Some small RNAs can match more than one category of the sequences listed so the sum of the numbers is bigger than the input total number.

**Supplemental Table 2.** Distribution of miRNAs in Rice AGO1 Complexes

miRNA family	miRNA reads <sup>a</sup>				Fold enrichment (log <sub>2</sub> scale) <sup>b</sup>		
	Total	AGO1a	AGO1b	AGO1c	AGO1a	AGO1b	AGO1c
osa-mir156	1779	14735	9056	1208	3.05	2.35	-0.56
osa-mir159	10704	357262	221326	466378	5.06	4.37	5.45
osa-mir160	71	183	77	238	1.37	0.12	1.75
osa-mir162	983	4167	4759	3619	2.08	2.28	1.88
osa-mir164	478	63	167	352	-2.92	-1.52	-0.44
osa-mir166	2314	16778	16538	31230	2.86	2.84	3.75
osa-mir167	15595	9282	3263	24053	-0.75	-2.26	0.63
osa-mir168	7690	8314	7041	705	0.11	-0.13	-3.45
osa-mir169	207	537	574	289	1.38	1.47	0.48
osa-mir171	508	328	2255	2154	-0.63	2.15	2.08
osa-mir172	176	9	0	38	-4.15	-7.47	-2.18
osa-mir319	24	10	100	0	-1.18	2.01	-4.64
osa-mir390	10	0	0	0	-3.46	-3.46	-3.46
osa-mir393	815	26	189	62	-4.97	-2.11	-3.72
osa-mir393b.3p	2115	162	1448	1462	-3.70	-0.55	-0.53
osa-mir394	31	23	361	167	-0.43	3.54	2.43
osa-mir395	55	150	161	157	1.45	1.55	1.51
osa-mir395a.2	8	3	1	38	-1.15	-2.34	2.10
osa-mir396	22379	483132	510450	319560	4.43	4.51	3.84
osa-mir397	130	761	1127	1248	2.55	3.12	3.26
osa-mir397a.2	266	296	3016	3192	0.15	3.50	3.58
osa-mir397b.2	1116	360	3798	3483	-1.63	1.77	1.64
osa-mir398	17	219	249	229	3.69	3.87	3.75
osa-mir399	8	33	171	52	2.04	4.42	2.70
osa-mir408	13	2305	2014	2279	7.47	7.28	7.45
osa-mir435	31	47	2	92	0.60	-3.95	1.57
osa-mir437	2	0	1	0	-1.58	-0.58	-1.58
osa-mir437-3p.2	71	0	1	0	-6.17	-5.31	-6.17
osa-mir437-3p.3	141	0	0	0	-7.15	-7.15	-7.15
osa-mir437-5p	62	1	1	5	-5.29	-5.12	-3.49
osa-mir438	0	0	0	0	0.00	0.00	0.00
osa-mir440	6	0	0	0	-2.81	-2.81	-2.81
osa-mir444a.1	2	4	0	0	0.74	-1.58	-1.58
osa-mir444a.2	23	39	80	53	0.76	1.80	1.20
osa-mir444b.1	900	1827	3547	1909	1.02	1.98	1.08
osa-mir444b.2	388	797	1949	799	1.04	2.33	1.04
osa-mir444c.1	900	1827	3547	1909	1.02	1.98	1.08
osa-mir444c.2	388	797	1949	799	1.04	2.33	1.04
osa-mir444d.1	2	4	0	0	0.74	-1.58	-1.58
osa-mir444d.2	22	39	80	53	0.83	1.86	1.27
osa-mir444d.3	34	20	0	74	-0.74	-5.13	1.10

osa-mir444	29	47	83	58	0.70	1.52	1.00
osa-mir528	31	93	1	1457	1.58	-4.95	5.55
osa-mir529	9	0	0	0	-3.32	-3.32	-3.32
osa-mir530-3p	4	0	0	0	-2.32	-2.32	-2.32
osa-mir530-5p	2	0	0	20	-1.58	-1.58	2.81
osa-mir531	4	0	0	0	-2.32	-2.32	-2.32
osa-mir535	149	448	651	184	1.59	2.13	0.30
osa-mir820	89	42	172	8	-1.08	0.95	-3.48
osa-miR820-5p.1	59	5	0	0	-3.26	-5.91	-5.91
osa-miR820-5p.2	51	0	0	0	-5.70	-5.70	-5.70
osa-mir827	0	0	0	0	0.00	0.00	0.00
osa-mir1317	17	0	0	0	-4.17	-4.17	-4.17
osa-mir1317.3p	193	0	0	0	-7.60	-7.60	-7.60
osa-mir1317.5p.2	172	4	89	11	-5.09	-0.94	-3.88
osa-mir1318	169	9	0	3	-4.09	-7.41	-5.41
osa-mir1320	3	0	0	0	-2.00	-2.00	-2.00
osa-mir1320.3p	24	1	2	0	-3.42	-3.23	-4.62
osa-mir1423	13	4	0	5	-1.49	-3.81	-1.22
osa-miR1423-5p.1	1	0	0	0	-1	-1	-1
osa-miR1423-5p.2	19	0	0	0	-4	-4	-4
osa-miR1423-3p.2	12	0	0	0	-4	-4	-4
osa-mir1424	0	0	0	0	0.00	0.00	0.00
osa-mir1425	277	286	310	336	0.05	0.16	0.28
osa-mir1426	0	0	0	0	0.00	0.00	0.00
osa-mir1427	18	0	0	0	-4.25	-4.25	-4.25
osa-mir1428a-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1428a-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1428e-3p	18	0	0	0	-4.25	-4.25	-4.25
osa-mir1428e-5p	3	0	0	0	-2.00	-2.00	-2.00
osa-mir1428	0	0	0	0	0.00	0.00	0.00
osa-mir1429	3	11	174	5	1.87	5.86	0.74
osa-mir1429-5p.2	82	0	0	0	-6.38	-6.38	-6.38
osa-mir1429-3p.2	55	0	0	0	-6	-6	-6
osa-mir1430	8	0	0	0	-3.17	-3.17	-3.17
osa-mir1431	5	1	0	0	-1.58	-2.58	-2.58
osa-mir1432	169	9	0	3	-4.09	-7.41	-5.41
osa-mir1433	0	0	0	5	0.00	0.00	2.58
osa-mir1435	0	0	0	0	0.00	0.00	0.00
osa-mir1846a-3p	0	0	46	6	0.00	5.55	2.81
osa-mir1846a-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1846b-3p	0	0	46	6	0.00	5.55	2.81
osa-mir1846b-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1846c-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1846c-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1846d-3p	1	0	0	0	-1.00	-1.00	-1.00

osa-mir1846d-5p	4	15	0	3	1.68	-2.32	-0.32
osa-mir1848	0	0	0	0	0.00	0.00	0.00
osa-mir1849	1	13	1	7	3.70	0.00	2.81
osa-mir1849.2	35	0	1	0	-5.16	-4.30	-4.83
osa-mir1850	23	0	0	5	-4.58	-4.58	-2.00
osa-mir1850.2	42	0	0	0	-5.43	-5.43	-5.43
osa-mir1851	1	0	0	0	-1.00	-1.00	-1.00
osa-mir1852	0	0	0	0	0.00	0.00	0.00
osa-mir1853-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1853-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1854-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1854-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1855	0	0	0	0	0.00	0.00	0.00
osa-mir1856	50	119	2	100	1.25	-4.64	1.00
osa-mir1857-3p	5	25	48	173	2.32	3.26	5.11
osa-mir1857-5p	0	3	0	0	2.00	0.00	0.00
osa-mir1857.5p.2	0	0	0	0	-0.58	-0.58	-0.58
osa-mir1858	0	0	0	0	0.00	0.00	0.00
osa-mir1859	0	4	0	0	2.32	0.00	0.00
osa-mir1860-3p	14	0	0	0	-3.91	-3.91	-3.91
osa-mir1860-5p	2	0	0	0	-1.58	-1.58	-1.58
osa-mir1861	6	0	0	0	-2.81	-2.81	-2.81
osa-mir1862	193	17	67	43	-3.50	-1.53	-2.17
osa-mir1863	1362	6	2	37	-7.83	-9.41	-5.20
osa-mir1863b	8	0	0	0	-3.16	-3.16	-3.16
osa-mir1863c	2	0	0	0	-1.45	-1.45	-1.45
osa-mir1864	1	0	0	0	-1.00	-1.00	-1.00
osa-mir1865-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1865-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1866	0	0	0	0	0.00	0.00	0.00
osa-mir1867	232	22	0	25	-3.34	-7.86	-3.16
osa-mir1868	1	0	0	0	-1.00	-1.00	-1.00
osa-mir1868.2	364	0	0	0	-17.02	-8.51	-8.51
osa-mir1869	0	0	0	0	0.00	0.00	0.00
osa-mir1870	11	0	0	0	-3.58	-3.58	-3.58
osa-mir1870-3p	39	0	0	0	-5.32	-5.32	-5.32
osa-mir1871	96	0	1	0	-6.60	-5.60	-6.60
osa-mir1872	0	0	0	0	0.00	0.00	0.00
osa-mir1873	24	30	2	78	0.32	-3.58	1.70
osa-mir1873.2	8	0	0	0	-3.17	-3.17	-3.17
osa-mir1874-3p	0	0	0	0	0.00	0.00	0.00
osa-mir1874-5p	0	0	0	0	0.00	0.00	0.00
osa-mir1875	3	0	0	0	-2.00	-2.00	-2.00
osa-mir1876	34	0	1	0	-5.13	-4.13	-5.13
osa-mir1877	0	0	0	0	0.00	0.00	0.00



osa-mir1878	15	0	1	0	-4.00	-3.00	-4.00
osa-mir1879	7	4	0	10	-0.68	-3.00	0.46
osa-mir1880	1	0	0	0	-1.00	-1.00	-1.00
osa-mir1881	0	0	0	0	0.00	0.00	0.00
osa-mir1882	172	41	89	11	-2.07	-0.95	-3.97
osa-mir1883	9	11	0	11	0.26	-3.32	0.26
osa-mir1884	125	188	5	159	0.59	-4.64	0.35
osa-miR2862	1	0	84	11	-0.24	5.60	2.82
osa-miR2863a	1	2	0	0	0.47	-1.32	-1.32
osa-miR2863b	3	0	0	7	-1.43	-1.99	1.00
osa-miR2864.1	5	1	0	39	-1.33	-2.63	2.69
osa-miR2864.2	4	2	0	26	-1.00	-2.38	2.35
osa-miR2865	1	0	0	0	-0.74	-1.16	-1.16
osa-miR2866	1	0	0	0	-0.29	-0.80	-0.80
osa-miR2867	0	0	0	0	0.13	-0.32	-0.32
osa-miR2868	0	0	0	5	-0.32	-0.32	2.30
osa-miR2869	8	0	0	0	-2.56	-3.12	-3.12
osa-miR2870	3	1	1	0	-1.07	-1.13	-1.99
osa-miR2871a	53	5	1	31	-3.27	-4.91	-0.75
osa-miR2871b	53	5	1	31	-3.27	-4.91	-0.75
osa-miR2872	16	0	1	7	-3.54	-3.22	-1.00
osa-miR2873	92	5	2	52	-4.02	-4.75	-0.81
osa-miR2874	84	0	0	0	-6.04	-6.41	-6.41
osa-miR2875	9	0	0	0	-3.28	-3.28	-3.28
osa-miR2876.1	3	2	0	24	-0.43	-1.99	2.63
osa-miR2876.2	7	3	0	41	-1.17	-3.08	2.32
osa-miR2877	5	0	0	13	-2.51	-2.51	1.30
osa-miR2878-5p	54	1	0	3	-4.87	-5.79	-3.76
osa-miR2878-3p	107	0	0	0	-6.54	-6.75	-6.75
osa-miR2879	23	0	0	0	-4.59	-4.59	-4.59
osa-miR2880	3	0	0	0	-1.99	-1.99	-1.99
osa-miR2905	8	0	0	8	-3.12	-3.12	0.12

<sup>a</sup>Normalized number, which divided the reads of each miRNA by total small RNA reads in each database, and multiplied by 10<sup>6</sup>.

<sup>b</sup>For the miRNAs that had no reads in the Total or AGO1s databases, fold-enrichment was calculated using normalized reads+1.

**Supplemental Table 3.** Prediction and Validation of Rice miRNA Targets

miRNA family	Predicted targets	Mispair score	Cleavage site	Confirmation of targets	Annotation of targets
<b>osa-miR156</b>	Os01g69830	1	1163	-	teosinte glume architecture 1, putative, expressed
	Os02g04680	2	1961	1_3_13(II)	squamosa promoter-binding-like protein 10, putative, expressed
	Os02g07780	1	2231	0_1_2(II)	squamosa promoter-binding-like protein 10, putative, expressed
	Os04g46580	1	828	-	SBP domain containing protein, expressed
	Os06g45310	1	864	0_3_1(II)	squamosa promoter-binding-like protein 11, putative, expressed
	Os06g49010	1	2028	0_2_1(II)	squamosa promoter-binding-like protein 11, putative, expressed
	Os07g32170	2	862	0_2_0(II)	SBP domain containing protein, expressed
	Os08g39890	1	1002	-	squamosa promoter-binding-like protein 9, putative, expressed
	Os08g41940	1	1064	-	teosinte glume architecture 1, putative, expressed
	Os09g31438	1	819	-	squamosa promoter-binding-like protein 9, putative, expressed
	Os09g32944	1	1044	-	teosinte glume architecture 1, putative, expressed
	Os11g30370	1	477	0_2_1(I)	teosinte glume architecture 1, putative, expressed
	<b>osa-miR159</b>	Os01g11430	3.5	1123	0_1_0(III)
Os01g12700		3	814	-	myb-like DNA-binding domain containing protein
Os01g59660		3.5	1271	0_35_0(II)	transcription factor GAMYB, putative, expressed
Os03g21380		4	1527	-	calcium-binding protein CAST, putative, expressed
Os03g38210		4	949	-	myb-like DNA-binding domain containing protein, expressed
Os04g46384		4	171	-	DUO1, putative
Os05g41166		3	1079	-	transcription factor GAMYB, putative, expressed
Os05g42240		3	637	-	hypothetical protein
Os06g40330		3.5	1429	0_2_0(II)	myb-like DNA-binding domain containing protein, expressed
Os09g36650		3.5	710	-	expressed protein
Os10g05230		4	346	0_1_0(III)	protein binding protein, putative, expressed
<b>osa-miR160</b>	Os02g41800	1	1495	0_7_0(II)	auxin response factor 16, putative, expressed
	Os04g43910	1	1355	0_3_0(II)	auxin response factor 16, putative, expressed
	Os04g59430	2	1345	0_2_0(I)	B3 DNA binding domain containing protein, expressed
	Os06g47150	1	2053	0_149_0(I)	auxin response factor 16, putative, expressed
	Os09g29160	2.5	1462	-	expressed protein
	Os10g33940	1	1646	0_149_0(I)	auxin response factor 16, putative, expressed
<b>osa-miR162</b>	Os03g02970	2	2989	-	endoribonuclease Dicer, putative, expressed
<b>osa-miR164</b>	Os02g36880	3	852	0_10_0(I)	NAC domain protein NAC5, putative, expressed
	Os04g38720	3	811	0_10_0(II)	NAC domain protein NAC5, putative, expressed
	Os06g23650	2	805	-	CUC2, putative, expressed
	Os06g46270	2	965	0_4_0(I)	NAC domain-containing protein 21/22, putative, expressed
	Os12g05260	3	213	0_1_0(III)	phytosulfokines 5 precursor, putative, expressed
	Os12g41680	2	922	0_24_0(I)	NAC domain-containing protein 21/22, putative, expressed
<b>osa-miR166</b>	Os01g33740	2.5	1565	-	retrotransposon protein, putative, unclassified
	Os02g45380	3	188	-	antiporter/ drug transporter/ transporter, putative, expressed

	Os02g49670	1	2599	-	zinc knuckle family protein, expressed
	Os03g01890	3	1100	0_1_0(III)	rolled leaf1, putative, expressed
	Os03g10290	3	1052	-	hypothetical protein
	Os03g34270	4	2015	-	retrotransposon protein, putative, unclassified
	Os03g35380	4	2015	-	retrotransposon protein, putative, unclassified
	Os03g43930	3	966	-	class III HD-Zip protein 4, putative, expressed
	Os03g56920	3.5	1544	-	conserved hypothetical protein
	Os04g48290	3	409	0_1_0(III)	antiporter/ drug transporter/ transporter, putative, expressed
	Os04g49300	4	344	-	retrotransposon protein, putative, unclassified
	Os05g14610	4	1622	-	retrotransposon protein, putative, unclassified
	Os06g40000	4	1853	-	retrotransposon protein, putative, unclassified
	Os07g33120	4	2161	-	expressed protein
	Os09g06140	4	350	-	retrotransposon protein, putative, unclassified
	Os10g33960	3	935	0_2_1(II)	HB1, putative, expressed
	Os11g29890	4	1829	-	retrotransposon protein, putative, unclassified
	Os11g45899	4	203	-	retrotransposon protein, putative, unclassified
	Os12g41860	3	888	-	class III HD-Zip protein 4, putative, expressed
	Os12g43900	3	1340	-	retrotransposon protein, putative, LINE subclass
<b>osa-miR167</b>	Os04g57610	4	2669	0_26_0(II)	auxin response factor 8, putative, expressed
	Os07g29820	4	2880	-	NBS-LRR disease resistance protein, putative, expressed
<b>osa-miR168</b>	Os02g45070	0	574	-	PINHEAD protein, putative, expressed
	Os04g40750	2	396	-	expressed protein
	Os04g47870	1	678	-	PINHEAD protein, putative, expressed
	Os06g42210	2	396	-	hypothetical protein
	Os07g49000	3	687	-	heat shock protein binding protein, putative, expressed
<b>osa-miR169</b>	Os02g53620	3	999	0_7_0(II)	nuclear transcription factor Y subunit A-3, putative, expressed
	Os03g07880	3.5	1004	0_311_1(I)	nuclear transcription factor Y subunit A-3, putative, expressed
	Os03g29760	3.5	1295	3_44_0(II)	nuclear transcription factor Y subunit A-8, putative, expressed
	Os07g06470	2.5	848	0_1_0(III)	nuclear transcription factor Y subunit A-10, putative, expressed
	Os07g41720	3.5	1180	0_39_2(II)	nuclear transcription factor Y subunit A-3, putative, expressed
	Os12g42400	3	1267	0_556_7(I)	nuclear transcription factor Y subunit A-2, putative, expressed
<b>osa-miR171</b>	Os02g44360	0	1362	0_12_0(II)	SCARECROW gene regulator, putative, expressed
	Os02g44370	0	1537	0_4_0(II)	nodulation signaling pathway 2 protein, putative, expressed
	Os03g04300	2	2090	-	ankyrin repeat protein, chloroplast precursor, putative, expressed
	Os04g46860	0	1335	0_5_0(II)	scarecrow-like 6, putative, expressed
	Os06g01620	0	467	-	scarecrow-like 6, putative, expressed
	Os10g40390	1	179	-	scarecrow-like 6, putative
	Os05g34460	4	1968	-	protease Do-like 4, mitochondrial precursor, putative, expressed
	Os03g15680	1.5	473	-	nodulation signaling pathway 2 protein, putative
	Os12g18080	3.5	3390	-	retrotransposon protein, putative, unclassified
<b>osa-miR172</b>	Os03g60430	2	1241	-	floral homeotic protein, putative, expressed

	Os04g55560	4	1788	0_5_1(II)	AP2 domain containing protein, expressed
	Os05g03040	2	1977	0_37_1(II)	floral homeotic protein APETALA2, putative, expressed
	Os06g43220	3	1425	-	floral homeotic protein APETALA2, putative, expressed
	Os07g13170	2	1415	0_6_0(II)	floral homeotic protein, putative, expressed
<b>osa-miR319</b>	Os01g59660	4	1358	-	transcription factor GAMYB, putative, expressed
	Os03g57190	2.5	1189	0_1_0(III)	TCP family transcription factor containing protein, expressed
	Os07g05720	2.5	1339	0_1_0(III)	TCP family transcription factor containing protein, expressed
	Os12g42190	3.5	857	-	transposon protein, putative, unclassified, expressed
<b>osa-miR390</b>	Os01g33110	4	1675	-	receptor-like protein kinase 5 precursor, putative, expressed
	Os02g10100	2	2181	-	leucine-rich repeat receptor protein kinase EXS precursor, putative, expressed
	Os08g34650	4	872	-	receptor-like protein kinase precursor, putative, expressed
	Os11g36140	2.5	1044	-	receptor-like protein kinase precursor, putative, expressed
<b>osa-miR393</b>	Os03g36080	3.5	732	-	expressed protein
	Os04g32460	2.5	2235	0_7_2(II)	transport inhibitor response 1 protein, putative, expressed
	Os04g58734	2.5	296	-	expressed protein
	Os05g05800	2.5	1694	0_21_1(I)	transport inhibitor response 1 protein, putative, expressed
<b>osa-miR394</b>	Os01g69940	0	1251	1_48_7(II)	F-box domain containing protein, expressed
	Os05g51150	3	1405	-	RNA polymerase sigma factor rpoD, putative, expressed
<b>osa-miR395</b>	Os03g09930	4	122	-	sulfate transporter 2.1, putative, expressed
	Os03g09940	3	329	-	low affinity sulphate transporter 3, putative, expressed
	Os03g53230	1	606	-	bifunctional 3-phosphoadenosine 5-phosphosulfate synthetase, putative, expressed
	Os06g46480	3.5	1115	-	expressed protein
	Os10g35870	4	663	-	membrane steroid-binding protein 1, putative, expressed
<b>osa-miR395a.2</b>	Os03g09940	2	328	-	low affinity sulphate transporter 3, putative, expressed
	Os03g53230	1	605	-	bifunctional 3-phosphoadenosine 5-phosphosulfate synthetase, putative, expressed
	Os03g09930	3	121	-	sulfate transporter 2.1, putative, expressed
<b>osa-miR396</b>	Os02g45570	1	640	-	growth-regulating factor, putative, expressed
	Os02g47280	1	678	0_12_0(I)	growth-regulating factor, putative, expressed
	Os02g53690	1	581	-	atGRF5, putative, expressed
	Os03g47140	1	958	-	atGRF2, putative, expressed
	Os03g51970	3	430	-	growth-regulating factor 1, putative, expressed
	Os04g24190	3	838	-	growth-regulating factor 1, putative
	Os04g48510	3	869	-	transcription activator, putative, expressed
	Os04g51190	1	546	-	growth-regulating factor, putative, expressed
	Os06g02560	1	616	0_41_1(II)	growth-regulating factor, putative, expressed
	Os06g10310	1	293	-	growth-regulating factor 1, putative
	Os11g35030	3.5	880	0_7_0(I)	expressed protein
	Os12g29980	3	743	-	atGRF2, putative, expressed
<b>osa-miR397</b>	Os01g44330	4	692	-	L-ascorbate oxidase precursor, putative, expressed
	Os01g62490	1	772	-	L-ascorbate oxidase precursor, putative, expressed

	Os01g63200	3	677	-	L-ascorbate oxidase precursor, putative, expressed
	Os03g16610	4	792	-	L-ascorbate oxidase precursor, putative, expressed
	Os05g38410	2	758	-	L-ascorbate oxidase precursor, putative, expressed
	Os05g38420	2	758	-	L-ascorbate oxidase precursor, putative, expressed
	Os11g48060	2	1169	-	monocopper oxidase-like protein SKS1 precursor, putative, expressed
	Os01g63180	4	689	-	L-ascorbate oxidase precursor, putative, expressed
	Os12g15680	4	758	-	L-ascorbate oxidase precursor, putative, expressed
<b>osa-miR397a.2</b>	Os01g44330	3	689	-	L-ascorbate oxidase precursor, putative, expressed
<b>osa-miR397b.2</b>	Os01g61160	2	791	-	L-ascorbate oxidase precursor, putative, expressed
	Os01g62490	2	769	-	L-ascorbate oxidase precursor, putative, expressed
	Os01g63180	2	686	-	L-ascorbate oxidase precursor, putative, expressed
	Os01g63190	3	762	-	L-ascorbate oxidase precursor, putative, expressed
	Os03g16610	3	789	-	L-ascorbate oxidase precursor, putative, expressed
	Os05g38410	1	755	-	L-ascorbate oxidase precursor, putative, expressed
	Os05g38420	1	755	-	L-ascorbate oxidase precursor, putative, expressed
	Os11g48060	3	1166	-	monocopper oxidase-like protein SKS1 precursor, putative, expressed
	Os12g15680	2	755	-	L-ascorbate oxidase precursor, putative, expressed
<b>osa-miR398</b>	Os07g46990	3	135	0_34_0(II)	superoxide dismutase 2, putative, expressed
	Os11g09780	4	511	-	hypothetical protein
<b>osa-miR399</b>	Os04g33860	3.5	505	-	expressed protein
	Os05g45350	3.5	1121	-	electron transporter/ heat shock protein binding protein, putative, expressed
	Os05g48390	2.5	832	-	ubiquitin conjugating enzyme, putative, expressed
	Os08g45000	4	303	-	inorganic phosphate transporter 1-7, putative, expressed
	Os10g31864	4	4094	-	conserved hypothetical protein
<b>osa-miR408</b>	Os01g03530	1.5	1390	-	copper ion binding protein, putative, expressed
	Os03g15340	1	96	-	chemocyanin precursor, putative, expressed
	Os06g15600	1.5	31	-	chemocyanin precursor, putative
<b>osa-miR437</b>	Os01g38190	4	1463	-	expressed protein
	Os02g42350	0.5	1542	-	nitrilase 4, putative, expressed
	Os02g48900	4	281	-	aspartic proteinase nepenthesin-1 precursor, putative, expressed
	Os06g46670	1	3866	-	glutamate receptor 3.4 precursor, putative, expressed
<b>osa-miR437-3p.</b>	Os06g46670	1	3532	-	glutamate receptor 3.4 precursor, putative, expressed
	Os02g42350	1.5	1542	-	nitrilase 4, putative, expressed
<b>osa-miR437-3p.</b>	Os06g46670	3	3524	-	glutamate receptor 3.4 precursor, putative, expressed
	Os02g42350	3	1534	-	nitrilase 4, putative, expressed
<b>osa-miR439</b>	Os01g23940	3.5	117	-	hypothetical protein
	Os01g26340	0	579	-	dirigent-like protein, putative
	Os01g36270	3.5	117	-	hypothetical protein
	Os06g19250	2	590	-	conserved hypothetical protein
<b>osa-miR444.1</b>	Os02g36924	4	551	-	MADS-box transcription factor 27, putative, expressed

	Os02g49840	0	619	-	MADS-box transcription factor 57, putative, expressed
	Os04g38780	4	103	-	MADS-box transcription factor 27, putative, expressed
	Os08g33488	0	317	-	MADS-box transcription factor 23, putative, expressed
<b>osa-miR444.2</b>	Os02g36924	4	556	0_26_0(I)	MADS-box transcription factor 27, putative, expressed
	Os02g49840	0	624	0_1_0(III)	MADS-box transcription factor 57, putative, expressed
	Os04g38780	4	108	0_6_0(II)	MADS-box transcription factor 27, putative, expressed
	Os05g47560	4	1447	0_6_0(II)	serine/threonine-protein kinase SNT7, chloroplast precursor, putative, expressed
	Os08g06510	1.5	1382	0_14_0(I)	zinc finger, C3HC4 type family protein, expressed
	Os08g33479	1	487	-	expressed protein
	Os08g33488	0	322	-	MADS-box transcription factor 23, putative, expressed
<b>osa-miR444.3</b>	Os02g49840	0	645	0_4_0(II)	MADS-box transcription factor 57, putative, expressed
<b>osa-miR528</b>	Os01g62600	4	50	-	laccase, putative
	Os06g06050	3.5	2659	0_1_0(III)	F-box/LRR-repeat MAX2, putative, expressed
	Os06g37150	3	2009	0_20_0(I)	L-ascorbate oxidase precursor, putative, expressed
	Os07g38290	2	538	-	copper ion binding protein, putative, expressed
	Os10g24090	3	588	-	expressed protein
<b>osa-miR529</b>	Os08g39890	1	998	-	squamosa promoter-binding-like protein 9, putative, expressed
	Os09g31438	1.5	815	-	squamosa promoter-binding-like protein 9, putative, expressed
<b>osa-miR530-5p</b>	Os01g56780	2	440	-	plus-3 domain containing protein, expressed
	Os02g14990	2.5	599	0_27_2(I)	zinc finger, C3HC4 type family protein, expressed
	Os12g44340	4	920	-	ATMAP70-2, putative, expressed
<b>osa-miR531</b>	Os08g04990	0	28	-	retrotransposon protein, putative, unclassified
<b>osa-miR535</b>	Os02g09080	4	49	-	expressed protein
	Os03g14880	3.5	1745	-	expressed protein
<b>osa-miR820</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os12g16350	4	93	-	CHY1, putative, expressed
<b>osa-miR820a-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR820a-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR820b-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR820b-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR820c-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR820c-5p</b>	Os03g02010	2.5	315	-	DNA cytosine methyltransferase Zmet3, putative, expressed
	Os11g03310	3	140	-	NAC domain-containing protein 77, putative
<b>osa-miR827</b>	Os02g43314	3	649	-	expressed protein
<b>osa-miR1317</b>	Os03g02010	4	387	-	DNA cytosine methyltransferase Zmet3, putative, expressed
<b>osa-miR1317-3p</b>	Os03g02010	3.5	411	-	DNA cytosine methyltransferase Zmet3, putative, expressed
<b>osa-miR1318</b>	Os03g59770	1	127	-	calcium-binding allergen Ole e 8, putative, expressed

	Os03g59790	1	55	-	calcium-binding protein, putative
	Os03g59870	1	322	-	calmodulin-like protein, putative, expressed
	Os04g51610	1	140	-	calcium-transporting ATPase 9, plasma membrane-type, putative, expressed
<b>osa-miR1320</b>	Os01g64100	2.5	1081	-	acidic endochitinase precursor, putative, expressed
	Os05g47550	2.5	2234	1_1_1(II)	clathrin assembly protein, putative, expressed
	Os06g10980	3.5	1816	-	fucosyltransferase 7, putative, expressed
<b>osa-miR1320-3p</b>	Os03g13800	2.5	845	-	NHP2-like protein 1, putative, expressed
	Os06g10980	3.5	1768	-	fucosyltransferase 7, putative, expressed
	Os05g31230	3.5	707	-	N-acetyltransferase ESCO1, putative, expressed
<b>osa-miR1423</b>	Os11g28540	4	55	-	expressed protein
	Os06g09560	3	826	-	dnaJ protein, putative, expressed
<b>osa-miR1423-5p</b>	Os06g09560	3	826	-	dnaJ protein, putative, expressed
<b>osa-miR1424</b>	Os04g33940	4	1721	-	expressed protein
<b>osa-miR1425</b>	Os08g01640	3.5	1464	-	rf1 protein, mitochondrial precursor, putative, expressed
	Os08g01650	3	1336	-	rf1 protein, mitochondrial precursor, putative, expressed
	Os08g17970	1.5	125	-	hypothetical protein
	Os10g35230	3.5	1448	-	rf1 protein, mitochondrial precursor, putative, expressed
	Os10g35240	3.5	1307	1_322_2(I)	rf1 protein, mitochondrial precursor, putative, expressed
	Os10g35436	3	1319	-	rf1 protein, mitochondrial precursor, putative, expressed
	Os10g35640	2.5	1387	-	rf1 protein, mitochondrial precursor, putative, expressed
<b>osa-miR1427</b>	Os06g49340	2.5	305	-	F-box domain containing protein, expressed
<b>osa-miR1428-3p</b>	Os03g17980	4	2288	2_2_3(II)	carbon catabolite derepressing protein kinase, putative, expressed
<b>osa-miR1429</b>	Os04g01340	4	861	-	retrotransposon protein, putative, LINE subclass
<b>osa-miR1432</b>	Os03g59770	1	128	-	calcium-binding allergen Ole e 8, putative, expressed
	Os03g59790	1	56	-	calcium-binding protein, putative
	Os03g59870	1	323	-	calmodulin-like protein, putative, expressed
	Os04g51610	3	141	-	calcium-transporting ATPase 9, plasma membrane-type, putative, expressed
<b>osa-miR1433</b>	Os05g43070	2.5	20	-	hypothetical protein
<b>osa-miR1435</b>	Os04g44354	2.5	1380	-	UDP-glucuronosyl and UDP-glucosyl transferase family protein, expressed
<b>osa-miR1438</b>	Os06g09230	2.5	2873	-	serine threonine kinase, putative, expressed
<b>osa-miR1846-3p</b>	Os02g43950	2	58	-	expressed protein
	Os02g43950	0.5	87	-	expressed protein
	Os10g12320	0.5	87	-	hypothetical protein
	Os02g43950	2.5	58	-	expressed protein
	Os10g12320	2	58	-	hypothetical protein
<b>osa-miR1850</b>	Os04g33510	3	914	-	expressed protein
	Os04g47410	3	1326	0_1_2(II)	metal ion binding protein, putative, expressed
	Os10g36650	2.5	14	-	actin-2, putative, expressed
<b>osa-miR1851</b>	Os01g61520	1	381	-	hypothetical protein
	Os03g52030	4	53	-	hypothetical protein

<b>osa-miR1854-3p</b>	Os11g34680	4	271	-	RNA recognition motif containing protein, putative, expressed
<b>osa-miR1855</b>	Os01g14860	4	2079	-	glycogen synthase kinase-3 homolog MsK-3, putative, expressed
<b>osa-miR1857-3p</b>	Os01g35880	4	293	-	hypothetical protein
	Os11g45590	3	1647	0_1_0(III)	splicing factor U2AF 65 kDa subunit, putative, expressed
<b>osa-miR1857-5p</b>	Os12g16250	3	338	-	isoflavone reductase, putative, expressed
<b>osa-miR1859</b>	Os02g43780	4	393	-	protein dimerization, putative
<b>osa-miR1860-3p</b>	Os09g36100	3.5	1166	-	expressed protein
<b>osa-miR1860-5p</b>	Os02g58390	3.5	1317	-	receptor-kinase isolog, putative, expressed
	Os12g13950	2.5	34	-	DNA polymerase alpha subunit B, putative, expressed
<b>osa-miR1861</b>	Os01g63810	3.5	676	-	starch binding domain containing protein, expressed
	Os05g51790	3.5	545	-	ATP binding protein, putative, expressed
	Os02g52560	3	1694	-	galactoside 2-alpha-L-fucosyltransferase, putative, expressed
	Os03g03710	3	854	-	exosome complex exonuclease RRP44, putative, expressed
<b>osa-miR1862</b>	Os02g37694	3.5	1104	-	expressed protein
	Os07g28260	4	2175	-	expressed protein
	Os02g30730	3.5	2798	-	SART-1 family protein, expressed
	Os04g31120	4	2476	-	influenza virus NS1A binding protein isoform 3, putative, expressed
	Os05g37910	4	1391	-	expressed protein
<b>osa-miR1863</b>	Os05g13804	4	49	-	expressed protein
<b>osa-miR1863b</b>	Os06g38480	3	28	-	retrotransposon protein, putative, unclassified, expressed
<b>osa-miR1864</b>	Os01g14020	4	288	-	expressed protein
<b>osa-miR1866</b>	Os04g33310	4	1352	-	expressed protein
<b>osa-miR1867</b>	Os10g30550	4	2337	-	phosphoglycerate kinase, chloroplast precursor, putative, expressed
<b>osa-miR1871</b>	Os06g19610	3.5	731	-	steroid dehydrogenase SPM2, putative
	Os08g02870	4	309	-	hypothetical protein
<b>osa-miR1872</b>	Os06g46910	3.5	221	-	nucleic acid binding protein, putative, expressed
	Os12g14230	4	242	-	hypothetical protein
<b>osa-miR1873</b>	Os05g01790	2	1125	-	OsWRKY69 - Superfamily of rice TFs having WRKY and zinc finger domains
<b>osa-miR1873.2</b>	Os08g29660	4	2274	-	expressed protein
<b>osa-miR1874-3p</b>	Os02g20950	3	460	-	expressed protein
<b>osa-miR1876</b>	Os02g05890	3.5	796	-	expressed protein
	Os07g41090	4.5	2252	-	histone deacetylase 6, putative, expressed
<b>osa-miR1879</b>	Os05g28110	3.5	2219	-	retrotransposon protein, putative, unclassified
	Os05g33060	3	443	-	hypothetical protein
	Os09g15389	4	825	-	expressed protein
<b>osa-miR1882</b>	Os03g02010	3.5	411	-	DNA cytosine methyltransferase Zmet3, putative, expressed
<b>osa-miR1883</b>	Os12g15260	3	314	-	hypothetical protein
<b>osa-miR1884</b>	Os01g59720	2	845	2_1_1(II)	expressed protein
	Os01g64520	3	1505	0_1_0(III)	uricase, putative, expressed
	Os01g73250	4	1127	-	bundle sheath cell specific protein 1, putative, expressed



	Os02g22610	2.5	1005	2_1_1(I)	transposon protein, putative, CACTA, En/Spm sub-class
	Os02g49870	4	1146	-	expressed protein
	Os03g10250	4	18	1_4_1(I)	expressed protein
	Os03g19380	2	851	2_1_1(II)	CP12-2, putative, expressed
	Os03g62330	3	665	-	expressed protein
	Os04g09900	3.5	364	-	ent-kaurene synthase A, chloroplast precursor, putative, expressed
	Os04g58730	4	375	-	DNA binding protein, putative, expressed
	Os06g48240	1	1673	-	ATPase protein, putative, expressed
	Os07g35870	4	772	-	bHLH transcription factor, putative, expressed
	Os09g30454	4	1197	-	OsWAK87 - OsWAK receptor-like protein kinase, expressed
	Os09g33710	2	1802	-	beta-glucosidase homolog precursor, putative, expressed
	Os11g34460	4	1602	-	adagio protein 1, putative, expressed
	Os11g34910	4	2822	2_1_0(II)	expressed protein
<b>osa-miR2862</b>	Os11g42420	3	372	-	nuclear pore protein 84 / 107 containing protein, expressed
<b>osa-miR2864.1</b>	Os09g07154	3	278	-	expressed protein
<b>osa-miR2864.2</b>	Os02g16270	2	898	-	xa1-like protein, putative, expressed
	Os12g41430	2	1493	-	expressed protein
<b>osa-miR2865</b>	Os01g70850	2.5		-	esterase PIR7B, putative, expressed
<b>osa-miR2868</b>	Os11g14140	3	1302	1_3_1(II)	protein kinase Kelch repeat:Kelch, putative, expressed
	Os11g29370	3	21	-	hydrolase, putative, expressed
<b>osa-miR2870</b>	Os02g28400	3.5	3005	-	retrotransposon protein, putative, Ty3-gypsy subclass
<b>osa-miR2905</b>	Os08g30780	1.5	1910	-	ATATH3,, putative
	Os06g39840	1	4256	-	retrotransposon protein, putative, unclassified
	Os01g14100	2.5	1485	-	folate/biopterin transporter family protein, expressed
	Os05g40170	3.5	1330	-	expressed protein
	Os02g24134	3	1311	-	vacuolar protein-sorting protein 45, putative, expressed
<b>osa-miR2872</b>	Os07g28050	3	294	-	ribosome biogenesis protein RLP24, putative, expressed
<b>osa-miR2875</b>	Os08g42850	2.5	805	-	FKBP-type peptidyl-prolyl cis-trans isomerase 2, chloroplast precursor, putative, expressed
<b>osa-miR2877</b>	Os03g12950	3.5	634	-	protein AINTEGUMENTA, putative, expressed
<b>osa-miR2880</b>	Os03g40920	3.5	1347	-	expressed protein

**Supplemental Table 4. Primers and probes**

<b>Primer</b>	<b>Sequence (5'-3')</b>
<b>Primers for generating <i>Os AGO1</i> inverted repeat</b>	
XhoI-OsAGO1-F	GGC ctcgag ATCGTATATC TGGACTCACT T
BamHI-OsAGO1-R	ATT ggatcc CCTGCGGTCT GAGCATGTTC A
<b>Primers for quantitative RT-PCR</b>	
AGO1a-F	CTGTTATCTAAGACGGAACGACCT
AGO1a-R	TGATTTGAGTAGCCACACTGAAAG
AGO1b-F	GCTGTTGGACTATTCTGGGTATTG
AGO1b-R	TCTTCAAGCACAACAAAGGTCC
AGO1c-F	AAATCCCATAATAATCTGCCAGC
AGO1c-R	GATGAAATGCTCAAGCCCAG
AGO1d-F	CTACTACTGCTTCGCTTGGCTACT
AGO1d-R	GAAATAGAATGGTGTGTCCCAGA
AGO4a-F	TACAGGGCTTCAGTCCGTTT
AGO4a-R	TCAACTAATAACTCCCGAATGAGG
Os06g49010-F	AGCAGCGTAAGGAATGCATGG
Os06g49010-R	GTGCCACAGGATTGCCATCAA
Os03g01890-F	TTGATATGAA AACAGATGGTGCA
Os03g01890-R	GTCAGAACAGATCGCAAGTTACA
Os04g57610-F	CGTCTTCCCTCCTTTGGATTTCA
Os04g57610-R	GTTAGCAGGTGTGCTTTGGGT
Os06g47150-F	CATTCTCGCCTCCACGGAAG
Os06g47150-R	CGGGAAATAGCACATAGGACCAA
Os03g07880-F	CAACACAAAGCAGCAGCCTG
Os03g07880-R	TTCGCACGACGATGATGGAGA
<b>Primers for generating miRNA target templates for <i>in vitro</i> transcription</b>	
T7-Os06g49010-F	TTGTAATACGACTCACTATAGGGCAACACATACAGCGGCCAAGG
Os06g49010-R	GGCTGCAGCCTGGTTGTGGCT
T7-Os06g43910-F	TTGTAATACGACTCACTATAGGGAATCCAATGGCGCACGACCA
Os06g43910-F	TAGAGCATCAGATTCCGGCTG C
T7-Os12g41680-F	TTGTAATACGACTCACTATAGGGAGCTCCAGGCCTCCATGAACA
Os12g41680-R	CCAAATTCAGGCAGGCCATGA
<b>Probes for small RNA gel blot</b>	
AGO1-1	GTCTCCAGAAAATATTGCACCACAGTCTTCACAGTACCAC
AGO1-2	TGCCCACTTGAAGGCAAGGCAAAGTGGTGTGCTGAATACT
AGO1-3	CTCAACGATC TTACAAACCT CCATAGGCAGATAATTGGGC
AGO1-4	AATAGCGCAGTAATCTGTTTCTCGTTAAGCCGCTTCGAGT
miR156	GTGCTCACTCTCTTCTGTCA
miR160	TGGCATAACAGGGAGCCAGGCA
miR166	GGGGAATGAAGCCTGGTCCGA
miR167	TAGATCATGCTGGCAGCTTCA
miR168	GTCCCGATCTGCACCAAGCGA
miR171	GATATTGGCGCGGCTCAATCA

miR528

CTCCTCTGCATGCCCTTCCA

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Supplemental Data, Wu et al., (2009) Rice MicroRNA Effector Complexes and Targets.

### **Supplemental Method. Solexa-based small RNA cloning protocol**

**(modified from a Hannon Lab protocol)**

#### ***Required oligos:***

##### **Size markers:**

Pme18: GUACGGUUUAAACUUCGA

Pme28: AUCGAUAAAAGUUUAAACGAGCUUCCCG

##### **3' ligation:**

Modban: AMP-5'p=5'pCTGTAGGCACCATCAATdideoxyC-3'  
IDT – Product name: miRNA cloning linker 1

##### **5' ligation:**

Solexa linker: 5' GUU CAG AGU UCU ACA GUC CGA CGA UC 3'  
IDT – Custom oligo – HPLC purified

##### **RT:**

SBS3: 5'-CAAGCAGAAGACGGCATAACGATTGATGGTGCCT  
ACAG-3'  
IDT – Custom oligo – HPLC purified

##### **PCR:**

SBS5: 5'AATGATACGGCGACCACCGACAGGTTTCAGAGTTC  
TACAGTCCGA 3'  
IDT – Custom oligo – Desalt

SBS3: 5'-CAAGCAGAAGACGGCATAACGATTGATGGTGCCT  
ACAG-3'  
IDT – Custom oligo – HPLC purified

### ***Section 0. RNA Preparation***

For each sample, you will need approximately 10-30ug of enriched small RNAs.

#### Total RNA extraction protocol (for large amounts of RNAs):

Trizol

Add chloroform (spin, take top layer)

Precipitate

Resuspend in desired amount of H<sub>2</sub>O

PEG-precipitation protocol (Dalmay et al.2000 Plant Cell)

Add 30% PEG to 5% final concentration.

Add NaCl to 0.5 M final concentration.

42 ul 5 M NaCl

70 ul 30% PEG8000

308 ul total RNA (~1 mg)

20 min on ice.

Centrifuge 10 min at 13000 rpm (this pellets large RNAs)

Add ~2.5 volumes of EtOH (1 ml) to supernatant

30 min at -20

Centrifuge 15 min at 13000 rpm (this pellets small RNAs)

Discard supernatant

Wash pellet twice with 70% ethanol

Dry the pellet at RT (no visible water allowed)

Dissolve the pellet in 30 ul nuclease-free water

***Section I: Gel purification of RNA sample***

- 1 Prepare a 15% polyacrylamide/urea gel (Sequagel, National Diagnostics)
- 2 Load 10ng of <sup>32</sup>P-labeled decade marker (Ambion) as a size marker
- 3 Spike all samples with <sup>32</sup>P-labeled 18bp and 28bp RNA oligos (~10,000 counts per second)
- 4 Heat total RNA at 95°C for 5 min, then chill for 5 min
- 5 Load the sample. Run the gel at constant 10W for 1-2 hrs (until first dye front reaches the middle of the gel)
- 6 Expose the gel for 1 hr
- 7 Excise the band corresponding to the desired size of small RNA (~19-24 nt) into Eppendorf tubes, including the radio-labeled oligos
- 8 Spin briefly in a centrifuge at max speed.
- 9 Add 400µL of 0.4M NaCl
- 10 Freeze rapidly in dry ice. Incubate (thaw) overnight at room temperature with agitation. Allow overnight incubation to maximize RNA elution
- 11 (Optional) Spin gel slice homogenate through micropore filter (Ultrafree-MC, 0.22µm, Millipore) for 1 min at RT
- 12 Add 20µg glycogen and 2 volume of 100% EtOH (RNase-free)
- 13 Incubate at -20°C 3-6 hours
- 14 Spin at 4°C for 30 min
- 15 Wash with 70% EtOH. Remove all EtOH
- 16 Air dry (5-10 min). Resuspend in 13µL DEPC MQ

## ***Section II: Linker ligation***

- 1 Set up the following reaction:

Gel purified RNA (in water)	13 $\mu$ L
ATP-free T4 RNA ligase buffer (10X)	2 $\mu$ L
DMSO	2 $\mu$ L
Modban oligo (50 $\mu$ M)	1 $\mu$ L
T4 RLN2 (NEB)	2 $\mu$ L
<u>Total</u>	<u>20<math>\mu</math>L</u>
- 2 Incubate at RT for 1 hr
- 3 Add 20 $\mu$ L RNA loading dye
- 4 Heat inactivate at 95°C for 5 min
- 5 Proceed to section III

\*\*\*

10X ATP-free T4 RNA ligase buffer:

500mM Tris-HCl (pH 7.5-7.6), 100mM MgCl<sub>2</sub>, 100mM DTT, 600 $\mu$ g/mL BSA

\*\*\*

## ***Section III: Gel purification of ligated RNA product***

- 1 Prepare a 15% polyacrylamide/urea gel (Sequagel, National Diagnostics)
- 2 Load 10ng of <sup>32</sup>P-labeled decade marker (Ambion) as a size marker
- 3 Heat samples at 95°C for 5 min, then chill for 5 min
- 4 Load the sample. Run the gel at constant 10W for 1.5-2 hrs (until first dye front reaches the bottom)
- 5 Expose the gel for 1 hr
- 6 Excise the band corresponding to the desired size of small RNA (~36-41 nt) into Eppendorf tubes. Include the ligated radio-labeled oligos
- 7 Spin briefly in a centrifuge at max speed.
- 8 Add 400 $\mu$ L of 0.4M NaCl
- 9 Freeze rapidly in dry ice. Incubate (thaw) overnight at room temperature with agitation. Allow overnight incubation to maximize RNA elution
- 10 (Optional) Spin gel slice homogenate through micropore filter (Ultrafree-MC, 0.22 $\mu$ m, Millipore) for 1 min at RT
- 11 Add 20 $\mu$ g glycogen and 2.5 volume of 100% EtOH (RNase-free)
- 12 Incubate at -20°C 3-6 hours

- 13 Spin at 4°C for 30 min
- 14 Wash with 70% EtOH. Remove all EtOH
- 15 Air dry (2 min). Resuspend in 13µL DEPC MQ

#### ***Section IV: 5' Ligation***

- 1 Set up the following reaction:
 

Ligated product (in water)	13µL
T4 RNA ligase buffer (10X) (Ambion)	2µL
DMSO	2µL
Solexa linker (50µM)	1µL
T4 RNA ligase (Ambion)	2µL
<u>Total</u>	<u>20µL</u>
- 2 Incubate at 37°C for 1 hr
- 3 Add 20ul loading dye
- 4 Heat inactivate at 95°C for 5 min
- 5 Proceed to section III

#### ***Section V: Gel purification of ligated RNA product***

- 1 Prepare a 15% polyacrylamide/urea gel (Sequagel, National Diagnostics)
- 2 Load 10ng of <sup>32</sup>P-labeled decade marker (Ambion) as a size marker
- 3 Heat samples at 95°C for 5 min, then chill for 5 min
- 4 Load the sample. Run the gel at constant 10W for 1.5-2 hrs (until first dye front reaches the bottom)
- 5 Expose the gel for 1 hr
- 6 Excise the band corresponding to the desired size of small RNA (~68-73 nt) into Eppendorf tubes. Include the ligated radio-labeled oligos
- 7 Spin briefly in a centrifuge at max speed.
- 8 Add 400µL of 0.4M NaCl
- 9 Freeze rapidly in dry ice. Incubate (thaw) overnight at room temperature with agitation. Allow overnight incubation to maximize RNA elution
- 10 (Optional) Spin gel slice homogenate through micropore filter (Ultrafree-MC, 0.22µm, Millipore) for 1 min at RT
- 11 Add 20µg glycogen and 2.5 volumes of 100% EtOH (RNase-free)
- 12 Incubate at -20°C 3-6 hours
- 13 Spin at 4°C for 30 min

- 14 Wash with 70% EtOH. Remove all EtOH
- 15 Air dry (2 min). Resuspend in 6.3 $\mu$ L DEPC MQ

### ***Section VI: Reverse transcription***

- 1 Set up the following
 

Ligated RNA product (in water)	6.3 $\mu$ L
BanOne primer (5 $\mu$ M)	4.2 $\mu$ L
- 2 Incubate at 72°C for 2 min
- 3 Centrifuge at RT for 1 min
- 4 Cool on ice for 2 min
- 5 Add 8.4 $\mu$ L RT Mix
 

RT Mix stock:	30 $\mu$ L 5X first strand buffer, 15 $\mu$ L dNTP (10mM each)
	3 $\mu$ L 0.1M DTT, 12 $\mu$ L MQ
- 6 Split into two tubes (9 $\mu$ L each)
- 7 Add either 1 $\mu$ L Superscript III RT (Invitrogen) (+RT) or DEPC MQ (-RT)
- 8 Incubate at 50°C for 1 hr. Heat to 70°C for 15 min. Store at -20°C until use

### ***Section VII: PCR amplification of cDNA***

- 1 Set up the following reaction:
 

First strand cDNA (+RT) or control (-RT)	5 $\mu$ L
PCR buffer (10X)	10 $\mu$ L
dNTP mix (10 mM each)	2 $\mu$ L
Sol_5_SBS3 (100 $\mu$ M)	1 $\mu$ L
Sol_3_Modban (100 $\mu$ M)	1 $\mu$ L
MQ	80 $\mu$ L
Taq Polymerase (Roche, 5U/ $\mu$ L)	1 $\mu$ L
<u>Total</u>	<u>100<math>\mu</math>L</u>
- 2 Place in a thermal cycler
- 3 Run the following PCR program:
 

94°C for 2 min	}	X 5 cycles
94°C for 15 sec		
54°C for 30 sec		
72°C for 30 sec		
94°C for 15 sec	}	X 17 cycles
60°C for 30 sec		
72°C for 30 sec		



72°C for 2 min  
4°C

- 4 Transfer +RT samples to fresh eppendorf tubes.
- 5 Add 200ul 0.5M NaCl
- 6 Add 300μL phenol:chloroform. Mix well.
- 7 Spin at max speed for 5 min
- 8 Transfer aqueous layer to 300μL chloroform. Mix well
- 9 Spin at max speed for 5 min
- 10 Transfer aqueous layer to 900ul (or 2.5-3 volumes) 100% EtOH and (optional) 20μg glycogen.
- 11 Incubate at -20°C 3-6 hrs
- 12 Spin at 4°C for 30 min at max speeds
- 13 Wash with 70% EtOH. Remove all EtOH
- 14 Air dry. Resuspend in 23.7μL water

### ***Section VIII: PmeI digestion of radiolabeled oligos***

- 1 Set up the following reaction:

PCR products	23.7μL
10x NEB buffer 4	3μL
Pme I (NEB)	3μL
100x BSA	0.3
<u>Total</u>	<u>30μL</u>

- 2 Incubate at 37°C for 3 hrs
- 3 Repeat phenol:chloroform extraction/EtOH precipitation
- 4 Proceed to section IX

### ***Section IX: Gel purification of amplified cDNA***

- 1 Prepare a 2% low-melt agarose gel (SeaKem) according to the manufacturer's instructions
- 2 Load 1μL of 1kb DNA ladder, amplified cDNA (+RT) and control (-RT)
- 3 Run gel at constant 100V until bromophenol blue band (~35nt in TAE) is at 2/3 of the gel length
- 4 View the fragment with a long wave UV source
- 5 Excise the DNA band with a clean scalpel or razor blade

- 6 Transfer the gel slice into a pre-weighed eppendorf
- 7 Add 0.4-0.5M NaCl to a final concentration of not less than 0.3M and no more than 400ul total volume. Melt agarose in solution at 70 degrees C for 10 minutes, flicking tube every couple of minutes.
- 8 Add 1 volume of phenol (equilibrated, pH 8), vortex 15-30 sec then spin at max for 5 minutes.
- 9 Transfer aqueous layer to 1 volume phenol:chloroform, vortex and spin.
- 10 Transfer aqueous layer to 1 volume chloroform and repeat as above.
- 11 Transfer aqueous layer to 2.5 volumes of 100% EtOH.
- 12 Incubate at -20°C overnight.
- 13 Spin at 4°C for 15 min at max speed.
- 14 Wash with 70% EtOH. Remove all EtOH
- 15 Air dry. Resuspend in 20µL water
- 16 Nanodrop and dilute sample to 10nM.