

**Supplementary Information for**  
**Identification and functional analysis of Dicer and Argonaute genes involved in**  
**RNA interference in the entomopathogenic fungus *Metarhizium robertsii***

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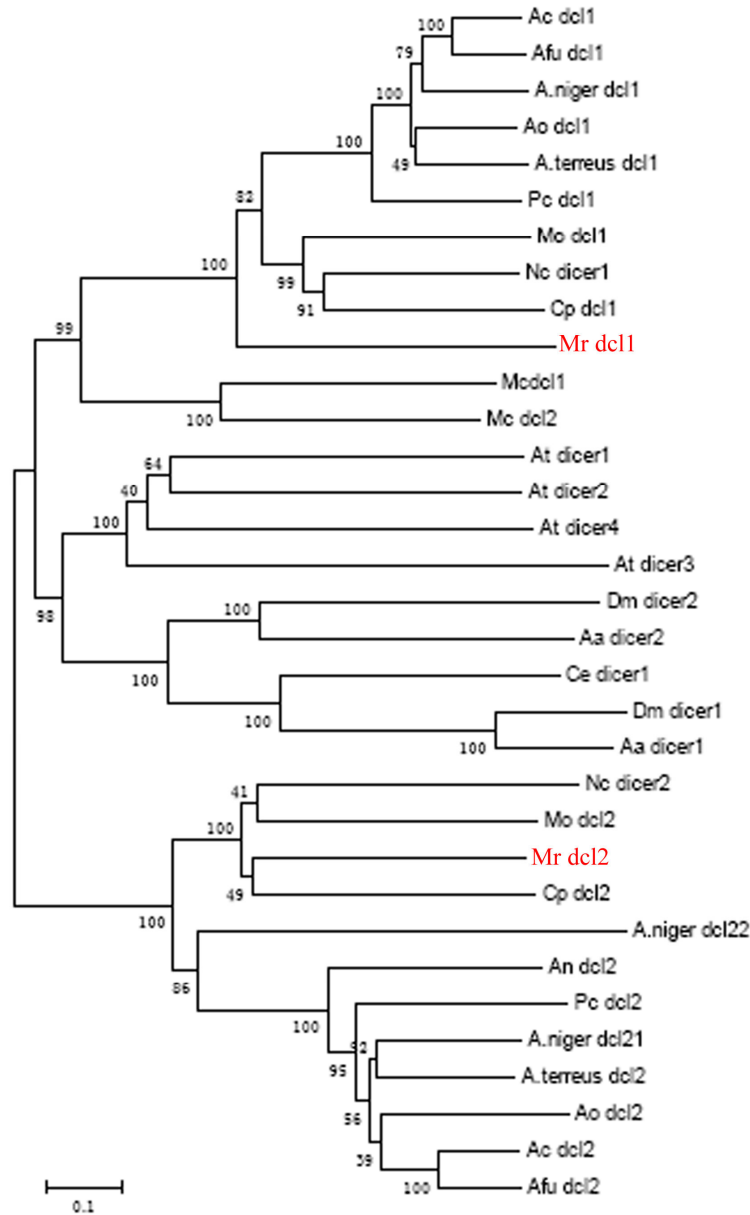


Fig.S1 Sequence alignment and phylogenetic tree of MaDCLs. Phylogenetic tree of Dicer proteins from plants, animals, and fungi.

*D.melanogaster*(Dm) Dicer1 (NP\_524453.1), Dicer2 (NP\_523778.2); *C. elegans*(Ce) Dicer1 (NP\_498761.1); *A.aegypti*(Aa) Dicer1 (XP\_001659747.1), Dicer2 (AAW48725.1); *A. thaliana*(At) DCL1 (NP\_171612.1), DCL2 (NP\_001189798.1), DCL3 (NP\_001154662.2), DCL4 (NP\_197532.3); *N. crassa*(Nc)

DCL1 (Q7S8J7.1), DCL2(Q7SCC1.3); *C. parasitica*(Cp) DCL1(Q2VF19.1), DCL2 (Q2VF18.1); *A. nidulans*(An) DCL2(P0C5H7.1); *M. circinelloides*(Mc) DCL1 (CAK32533.1), DCL2(CAZ65730.1); *P. chrysogenum*(Pc) DCL1 (XP\_002567731.1), DCL2 (XP\_002558177.1); *M. oryzae*(Mo) DCL1(A4RKC3.2), DCL2(A4RHU9.3); *A. fumigatus*(Afu) DCL1(Q4WVE3.3), DCL2(Q4WA22.2); *A. clavatus*(Ac) DCL1(A1CBC9.2), DCL2(A1C9M6.1); *A. niger*(*A. niger*) DCL1(A2RAF3.2), DCL2(A2R345.2), DCL2(A2QX45.2); *A. oryzae*(Ao) DCL1(XP\_001824024.2), DCL2(XP\_001818742.1); *A. terreus*(*A. terreus*) DCL1(Q0CW42.2), DCL2(Q0CEI2.2).

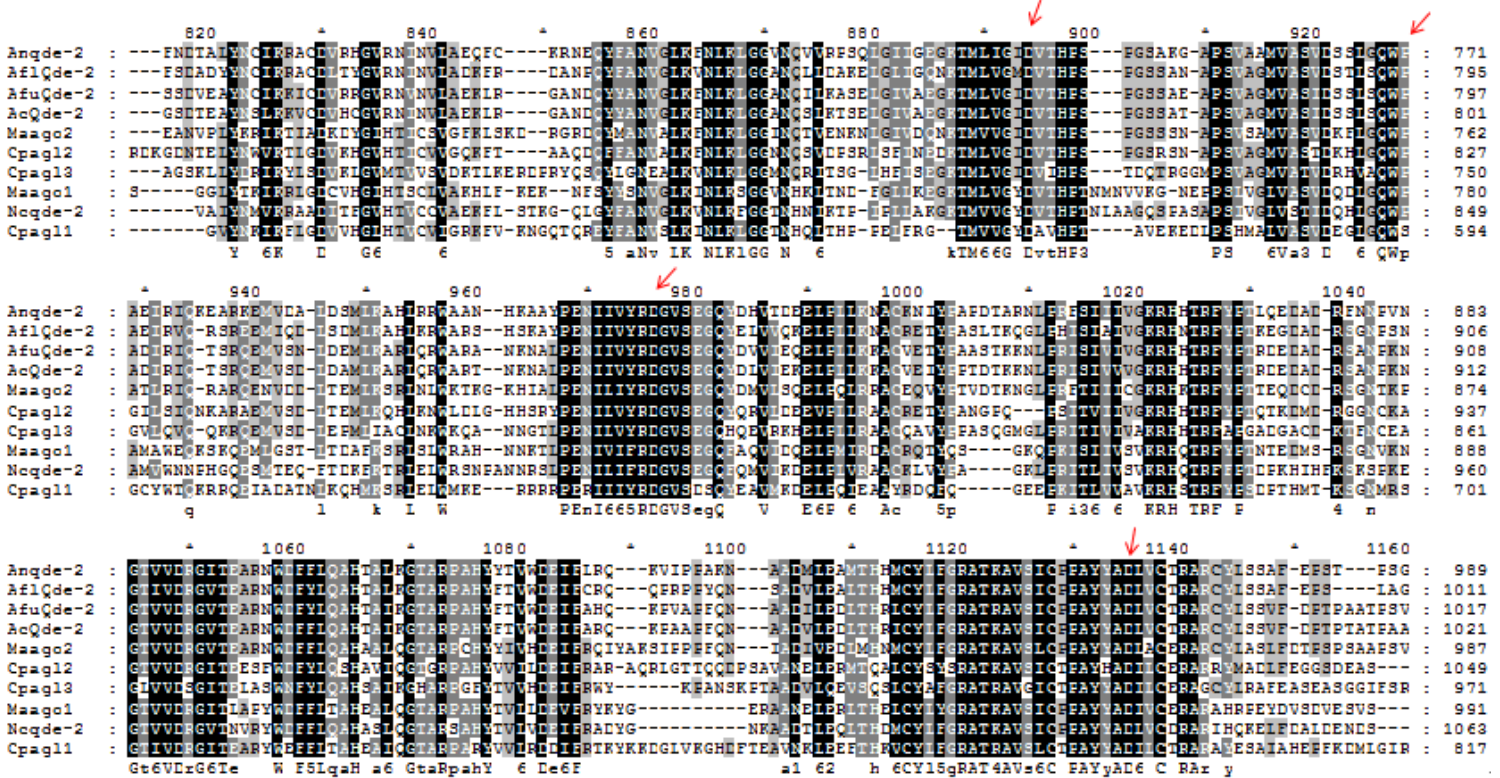


Fig.S2 Alignment profile of PIWI domain amino acids of *M. robertsii*. and other fungi AGO proteins. The beginning and end positions of PIWI domains in each protein are marked. Arrows indicate the conserved DDH triad residues and the conserved H residue.

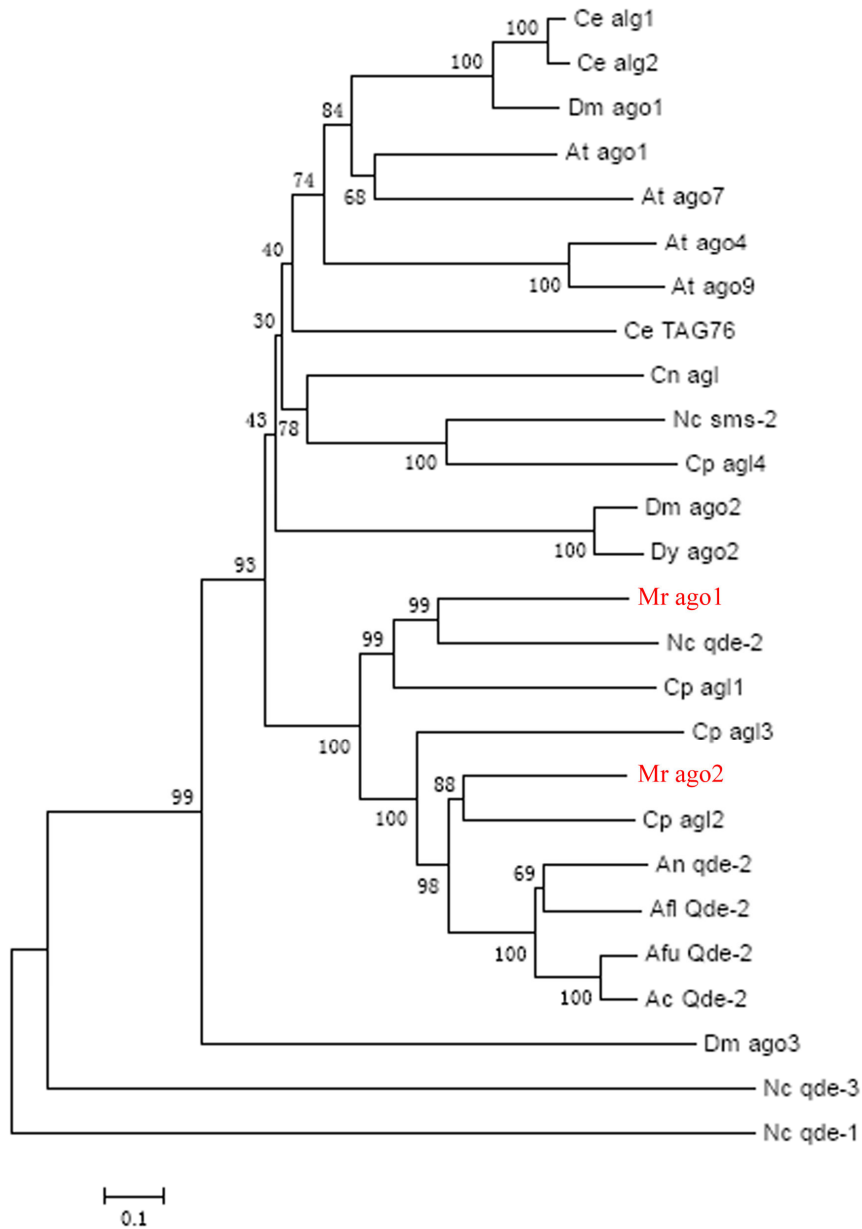


Fig.S3 Sequence alignment and phylogenetic tree of MaAGOs. Phylogenetic tree of Argonaute proteins from plants, animals, and fungi.

*D. melanogaster*(Dm) AGO1 (NP\_725341), AGO2 (NP\_730054.1), AGO3 (NP\_001036628.2); *D.yakuba* (Dy) AGO2(XP\_002095171.1); *C. elegans*(Ce) ALG1 (NP\_510322.2), ALG2 (NP\_493837.1), TAG76 (NP\_499192.1); *A. thaliana*(At) AGO1 (NP\_001185169.1), AGO4 (NP\_565633.1), AGO7 (NP\_177103.1), AGO9 (NP\_197613.2); *N. crassa*(Nc) SMS-2(XP\_958586.1), QDE-1 (Q9Y7G6),

QDE-2(XP\_960365.2), QDE-3(Q9P8U6); *C. parasitica*(Cp) AGL1(ACY36939.1), AGL2(ACY36940.1), AGL3(ACY36941.1), AGL4(ACY36942.1); *A. nidulans*(An) QDE-2(XP\_659123.1); *A. fumigatus*(Afu) QDE-2(XP\_747330.1); *A. flavus*(Afl) QDE-2(XP\_002372786.1); *A. clavatus*(Ac) QDE-2(XP\_001276635.1); *C. neoformans*(Cn) AGL(XP\_567314.1).

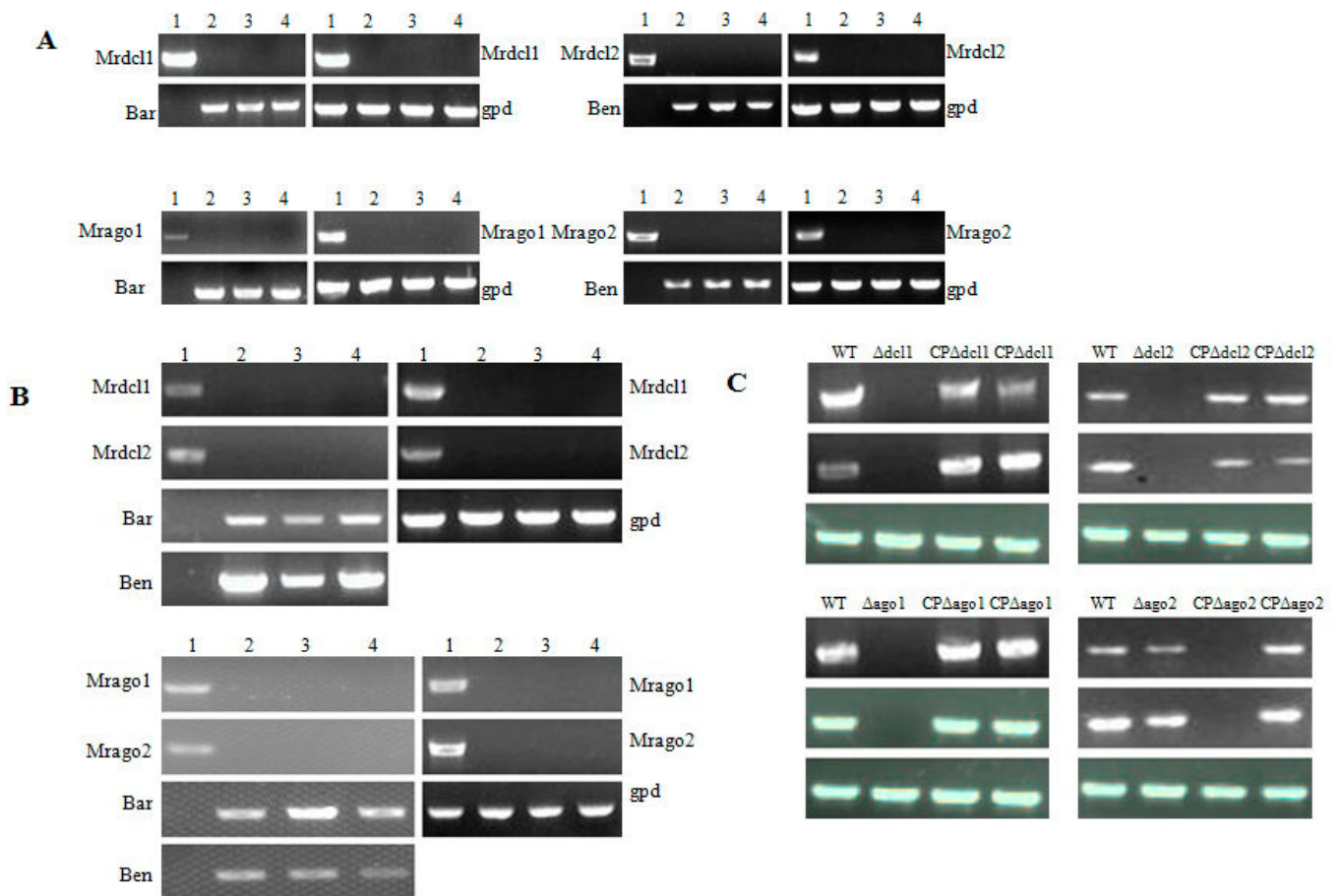


Fig.S4 Identification of knockout mutants and complemented strains by PCR and RT-PCR. (A) Identification of  $\Delta dcl1$ ,  $\Delta dcl2$ ,  $\Delta ago1$  and  $\Delta ago2$  by PCR and RT-PCR. 1: WT; 2-4 three knockout transformants. (B) Identification of  $\Delta dcl1\Delta dcl2$  and  $\Delta ago1\Delta ago2$  by PCR and RT-PCR. 1: WT; 2-4 three double-knockout transformants. (C) Identification of  $cp\Delta dcl1$ ,  $cp\Delta dcl2$ ,  $cp\Delta ago1$  and  $cp\Delta ago2$  by PCR and RT-PCR.

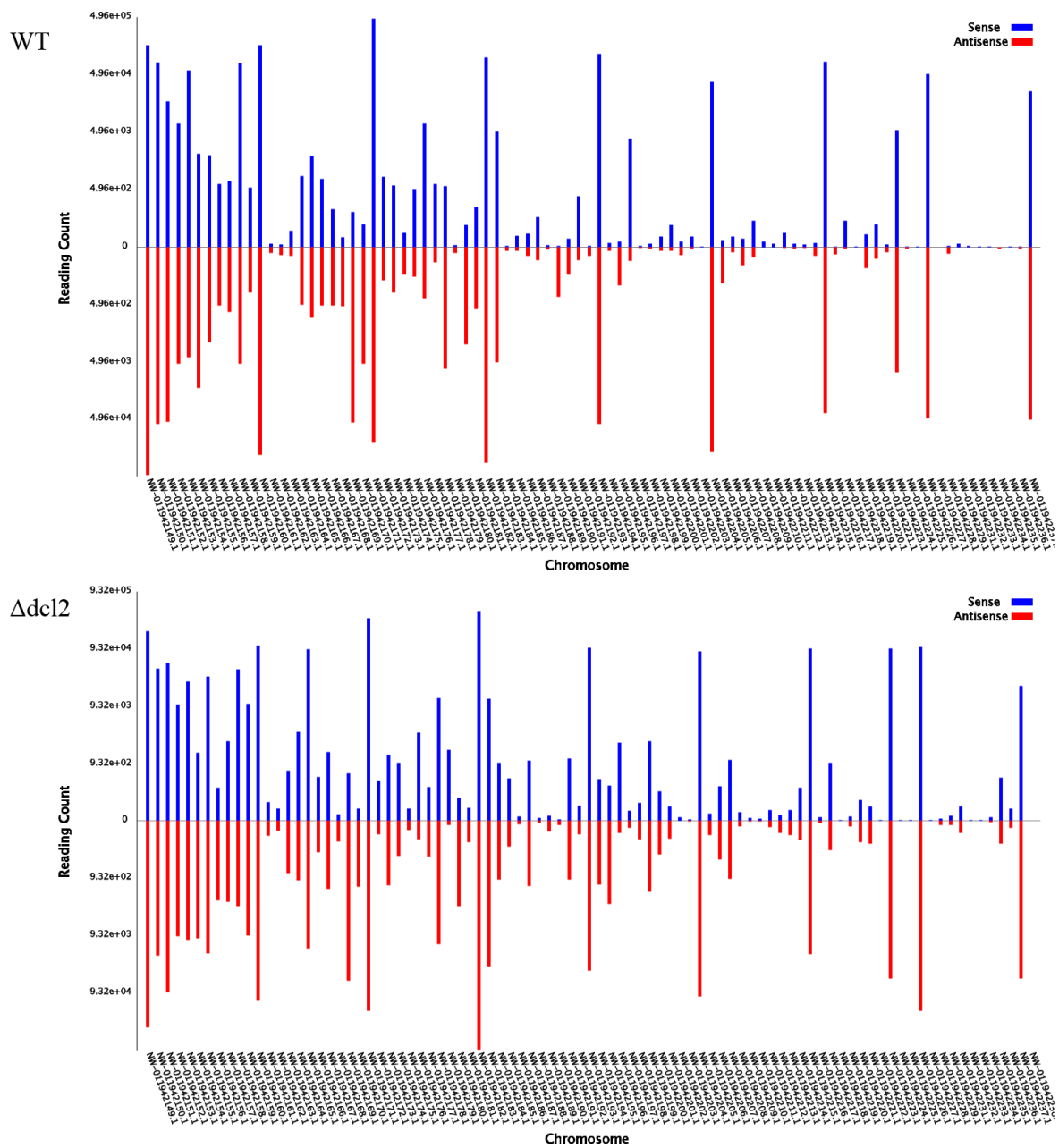


Fig.S5 sRNA distribution across different chromosomes. The horizontal coordinates are chromosomes and the vertical coordinates are count of tags. Above 0:count of tags with chromosomes positive chain. Under 0:count of tags with chromosomes negative chain.

**Table S4 Mapping statistical analysis of small RNA libraries in WT and  $\Delta dcl2$  strain**

type	Total reads	Clean reads	Percent % (Total)	Unique sRNAs	Mapping to genome	Percent % (Unique)
$\Delta dcl2$	13684922	12622473	93.68%	1767138	917527	51.92%
WT	12597803	11404976	91.95%	1715070	757695	44.18%

**Table S5 The summary distribution of sRNAs among different categories in WT and  $\Delta dcl2$** 

sRNA category	$\Delta dcl2$				WT			
	Unique sRNA	Percent (%)	Total sRNA	Percent (%)	Unique sRNA	Percent (%)	Total sRNA	Percent (%)
rRNA	100484	5.6863	3307157	26.2005	68844	4.0141	1914470	16.7863
tRNA	10550	0.5970	277385	2.1975	6078	0.3544	86668	0.7599
snRNA	9313	0.5270	210425	1.6671	5566	0.3245	159043	1.3945
snoRNA	2152	0.1218	24560	0.1946	1198	0.0699	9521	0.0835
Other <sup>a</sup>	1644639	93.0679	8802946	69.7403	1633384	95.2372	9235274	80.9758
Total	1767138	100	12622473	100	1715070	100	11404976	100

<sup>a</sup> Other sRNAs indicate sequences that do not match any nonprotein-coding RNAs, or nonannotated sRNAs.

**Table S6 MiRNAs identified by deep sequencing in WT and  $\Delta dcl2$** 

milRNA	milRNA sequence(5'-3')	Length (nt)	WT expression	$\Delta dcl2$ expression	$\log_2(\Delta dcl2 / WT)$	p-value	MFE (kcal/ mol)
<b>novel miRNA</b>							
novel_mir_11	TAGCACAACATGACAATCGG	20	36125	16	-11.287	0	-52.70
novel_mir_33	TAACAAAATAATGGCACCTCT	21	889	0	-12.9283	1.9E-288	-62.3
novel_mir_37	TTGAAGGGTTGAAAAACAGG	21	28	0	-7.93964	8.25E-10	-64.3
novel_mir_39	TGGACCGATTGCATGGAAGGC	21	25	0	-7.7761	7.71E-09	-26.7
novel_mir_41	TGTTTGCAGCCGGGCGGTGCTT	23	18	0	-7.30223	1.42E-06	-37.3
novel_mir_26	TAGTTGGTGTATTACGTTATTC	22	8	0	-6.1323	0.0018238	-60.8
novel_mir_21	TAGATGCGGAATGGCTTGTG	20	7	0	-5.93966	1.25E-05	-65
novel_mir_3	TACCCGTTGGCAACTAAGTG	20	0	7	5.793311	0.0004193	-33.1/-31.1/-49.1
novel_mir_43	TCATCAAGTCGTGCTCGCCCAT	22	6	0	-5.71727	4.94E-06	-30.2
novel_mir_6	TTCATCTGTCTATATATTATTT	22	0	6	5.570918	1.18E-05	-28.20
novel_mir_29	TTTGTTCGAGCCCTTCTG	21	5	0	-5.45423	8.46E-06	-36.40
novel_mir_4	TTTGTGTGAGGACTGAGCCGTG	22	0	5	5.307884	3.11E-05	-29.90
novel_mir_10	GAGAAGCAAGCGCTGGGCATG	21	0	5	5.307884	7.66E-08	-32.20
<b>known miRNA</b>							
man-milR-10	AGGGAUCUAGAAAAGAAGGCUU	22	182	1	-7.65455	1.17E-57	-100.00
man-milR-13	CGACGACUCUGGCGAGGACAA	21	9	25	1.327658	0.014191	-28.30
man-milR-14	UUAAAGAUGUGGAAAAGAAGGC	22	207	0	-10.8258	9.76E-68	-105.32
man-milR-15	GAUGUCGAAUCGUGCCGGGGCUC	23	534	0	-12.193	1.5E-173	-102.70
man-milR-2	UACAAGGGCACGAGCAAGGU	20	90	1	-6.63862	3.47E-28	-66.60
man-milR-3	UUGUCGAGGCAUACCACUAUU	21	131	0	-10.1657	3.84E-43	-41.00/-36.40
man-milR-5	UUAACAAGGCGUCGAGGGAUA	21	10422	7	-10.6862	0	-55.30
man-milR-6	UUUGGAGAGGCUGCUGUGUUU	21	56	0	-8.93961	7.16E-19	-31.10
man-milR-7	GCGGGUGCUGAGAAAGCGUUUA	22	562	1307	1.071287	6.51E-53	-49.69
man-milR-9	UGCCUAGGCAGGUAGAUCAG	21	16530	7	-11.3517	0	-72.90
man-milR-1	UAUCUUGUGGACUAAUAGGUA	21	4628	9004	0.813466	1.38E-07	-26.60
man-milR-8	UUGCAUGAUGAGACUUAUUU	20	258	529	0.889186	1.21E-05	-20.03
man-milR-12	AGACCUCGUUGAUGCUGGCAUU	22	0	4	4.9855	4.10E-07	-37.40
man-milR-4	UCGAGGAGCAGAAGCUGAUCUU	22	8	1	-3.14671	4.79E-07	-31.60