Table S1. The parameters selected for the first prediction strategy.

No	Parameter	Parameter description
1	minimum free energy	Calculated using RNAfold for the free energy of the ensemble of all secondary structures of the candidate pre-microRNA structure
2	GC content	GC content of the candidate pre-microRNA sequence
3-12	bulge in sub-region 1-10 (left arm)	For single-stem microRNAs, the left arm of the hairpin structure was divided into 10 parts, and the bulge ratio in each part was calculated
13-22	bulge in sub-region 1-10 (right arm)	For single-stem microRNAs, the right arm of the hairpin structure was divided into 10 parts, and the bulge ratio in each part was calculated
23	the ratio of long arm to short arm in the hairpin	Ratio of the length of long arm over that of short arm in the hairpin
24	the ratio of long arm to short arm in stem region	Ratio of the length of long arm over that of short arm in the stem region
25	loop length	Loop length
26	the ratio of loop length to hairpin length	Ratio of loop length to the total hairpin length
27	the ratio of paired nucleotide to unpaired nucleotide	Ratio of paired nucleotide to unpaired nucleotide in the hairpin
28-59	triplet elements	Extract triplet elements of the pre-microRNA sequence as previously described by Xue et al. (1)

Table S2. Primers used for the validation of predicted novel and known microRNAs.

Name	Chromsome position	Forward primer	Reverse primer	Sequence length	Length of pre-miRNA	Enzyme used for double digestion	Note
novel-001	4:139228118:139228596:-1	TAGGATCCTACCTGGATGTTTTCTCCACT	CGCGAATTCATCATCCATCCATTCGTTAATTCC	177bp	79bp	BamHI&EcoRI	Single-stem
novel-002	9:20410950:20411431:-1	GTAGGATCCTTGTTTGGACTGTCTCCTACC	CGCCTCGAGACACACAACTAGGACAATGT	223bp	82bp	BamHI&XhoI	Single-stem
novel-003	8:6602487:6602962:1	GTAGGATCCAAAGAGTGCACCCAATATCCC	CGCCTCGAGAGCTATAGACTTAGTTATTTCAG	276bp	76bp	BamHI&XhoI	Single-stem
novel-004	X:12940571:12941064:-1	GTAGGATCCGGTCAACTGCTAAGATGGTC	CGCCTCGAGCACTTTGGCCATATTCTAT	234bp	94bp	BamHI&XhoI	Single-stem
novel-005	18:3885153:3885632:1	GCGGGATCCTGTGACCAAGAGCATCATTAG	ATACTCGAGGGCCTGAAAGGCAATTTCC	306bp	80bp	BamHI&XhoI	Single-stem
novel-006	8:92217512:92217989:1	ATAAAGCTTGCTTAGGACATGGAGGCTCAC	ATGCGGCCGCAAAGGACATTGTGAGAAA	337bp	58bp	HindⅢ&NotI	Single-stem
novel-007	1:25349795:25350275:1	GCTGGATCCAGATAAACTGCGGAACACAC	CGCCTCGAGAGGTTTGAATCTCACTTTGC	264bp	81bp	BamHI&XhoI	Single-stem
novel-008	15:89154876:89155356:-1	TATGGATCCGAACACGTGGAAGGATAGCCA	ATACTCGAGAATAGGAGCGGAGACAACGC	264bp	81bp	BamHI&XhoI	Single-stem
novel-009	9:35607883:35608363:1	ATGGATCCGACTAGCTGGCTCATAACCAAG	ATACTCGAGAAGGAGTGTGAGGCTCTTACG	223bp	81bp	BamHI&XhoI	Single-stem
novel-010	17:67095503:67095978:-1	ATGGATCCCAGAAAGTGGAACCAGAGGAGT	ATACTCGAGATGTCTTTCTGAGCAAACCCAGAG	184bp	76bp	BamHI&XhoI	Single-stem
novel-011	12:48139193:48139688:1	ATCGGATCCTGCAGCTCTCAACTCCAGTAG	GTCTCGAGTGATGCTGCACCTCATTCAACC	229bp	96bp	BamHI&XhoI	Multi-stem
novel-012	20:3898001:3898497:1	ATCAAGCTTGAAGTCACGGAAGCTGGGAGA	GTGCGGCCGCCTAAAGAATAACTTCTTA	280bp	97bp	HindⅢ&NotI	Multi-stem
novel-013	6:28918619:28919103:1	GCCGGATCCTGGACGATATCAAGTTTCTGC	CTCCTCGAGGGTTTAAAATTTGGGGTTCTA	273bp	85bp	BamHI&XhoI	Multi-stem
novel-014	9:131154703:131155186:1	ATCGGATCCAGAGAAGGAAGAAGGACGAGG	ATCTCGAGGCTTTTTAGGAGCGAAGGG	311bp	84bp	BamHI&XhoI	Multi-stem
novel-015	7:100465453:100465950:1	GTCAAGCTTATCTGAGCAGTGTTACCAGGC	ATGCGGCCGCTCTGAGAGTCCAGAGAA	388bp	98bp	HindⅢ&NotI	Multi-stem
novel-016	19:56906528:56907028:1	ATAGGATCCACAGGTACAAAGCCAGCCATC	GCCTCGAGATACTCTTAAAAGGAAGAAACTGG	280bp	101bp	BamHI&XhoI	Multi-stem
miR-376a	14:101507119-101507186:1	GCAGGATCCCCTGGATCGAGTTGTTCTTTG	GCCTCGAGATACTCTTAAAAGGAAGAAACTGG	120bp	68bp	BamHI&EcoRI	Single-stem

Table S3. Primers used for qRT-PCR.

I. For novel miRNAs

Name	Reverse transcript stem-loop primer	Gene specific primer for real time-PCR	Universal Primer	Sequence of mature miRNAs
novel-001	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTGTGTGT	CAACCTAGCAGTCTCAGGAC	CAGTGCGTGTCGTGGAGT	CCTAGCAGTCTCAGGACACACA
novel-002	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCTTAGT	GCCCTTGTAATGGAGAACAC	CAGTGCGTGTCGTGGAGT	CTTGTAATGGAGAACACTAAGC
novel-003	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTTGCCAT	GCCTTTCTTCTTAGACATG	CAGTGCGTGTCGTGGAGT	TTTCTTCTTAGACATGGCAA
novel-004	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTGGTCAT	GCCAAGGGAAAATAGTTGAT	CAGTGCGTGTCGTGGAGT	AAGGGAAAATAGTTGATGACCA
novel-005	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCATAAGC	GTCTAGTGGTCAGAGGGC	CAGTGCGTGTCGTGGAGT	TAGTGGTCAGAGGGCTTATG
novel-006	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGTCAGGA	GCCAACTAGCTCTGTGGATC	CAGTGCGTGTCGTGGAGT	AACTAGCTCTGTGGATCCTGAC
novel-007	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAATGGTC	GCCTTGGGATTCAGCAGG	CAGTGCGTGTCGTGGAGT	TTGGGATTCAGCAGGACCATT
novel-008	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACAACA	GCCAGGTAGACTGGGATTT	CAGTGCGTGTCGTGGAGT	AGGTAGACTGGGATTTGTTGTT
novel-009	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTTCTCCT	GCTTACTGGGGAGCAGAA	CAGTGCGTGTCGTGGAGT	ACTGGGGAGCAGAAGGAGAA
novel-010	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTGAGACA	GCCATAGCAGCATGAACCTG	CAGTGCGTGTCGTGGAGT	ATAGCAGCATGAACCTGTCTCA
novel-011	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGAAGAGC	ATTCTGCACTGGAGTTGGC	CAGTGCGTGTCGTGGAGT	TCTGCACTGGAGTTGGCTCTTC
novel-012	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGGCAGCC	GGCAAGAACCAAGAATGGG	CAGTGCGTGTCGTGGAGT	AAGAACCAAGAATGGGCTGCC
novel-013	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAAAAGCC	ATTCGGGCGGGAGTGGT	CAGTGCGTGTCGTGGAGT	TCGGGCGGGAGTGGTGGCTTTT
novel-014	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCTGATTG	GCCAGAATTGCGTTTGGAC	CAGTGCGTGTCGTGGAGT	AGAATTGCGTTTGGACAATCAG
novel-015	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCTCCTG	GTTGAGGGACCCAGGACA	CAGTGCGTGTCGTGGAGT	TGAGGGACCCAGGACAGGAGA
novel-016	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAGAAGTG	GCTGAGGATGATGAAATGGCCA	CAGTGCGTGTCGTGGAGT	GAGGATGATGAAATGGCCACTTCT

II. For known miRNAs

Name	Reverse transcript stem-loop primer	Gene specific primer for real time- PCR	Universal primer	Sequence of mature miRNAs
has-mir-376a	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACGTGGA	GCCATCATAGAGGAAAATCC	CAGTGCGTGTCGTGGAGT	ATCATAGAGGAAAATCCACGT
hsa-miR-200a*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCCAGCA	CATCATCTTACCGGACAGTG	CAGTGCGTGTCGTGGAGT	CATCTTACCGGACAGTGCTGGA
hsa-miR-34b*	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCAATCAG	CCATAGGCAGTGTCATTAGCT	CAGTGCGTGTCGTGGAGT	TAGGCAGTGTCATTAGCTGATTG
hsa-miR-320b	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTTGCCCT	CCTAAAAGCTGGGTTGAGAG	CAGTGCGTGTCGTGGAGT	AAAAGCTGGGTTGAGAGGGCAA
hsa-miR-448	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACATGGGAC	CCGTTGCATATGTAGGATGTC	CAGTGCGTGTCGTGGAGT	TTGCATATGTAGGATGTCCCAT
hsa-miR-625	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGGACTAT	CCGAGGGGGAAAGTTCTA	CAGTGCGTGTCGTGGAGT	AGGGGGAAAGTTCTATAGTCC
hsa-miR-449c	GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACAGCCG	CCATAGGCAGTGTATTGCTAG	CAGTGCGTGTCGTGGAGT	TAGGCAGTGTATTGCTAGCGGCTGT

Table S4.The statistics of multi-stem pre-microRNAs in different species.

Species	Number of multi-stem pre-microRNAs	Total number of pre-microRNAs	Percentage of multi-stem pre-microRNAs (%)
H. sapiens (GRCh37)	95	940	10.1
M. musculus (NCBIM37)	59	590	10.0
B. mori (SILKDB2.0)	144	487	29.6
C. elegans(WormBase WS200)	12	175	6.9
A. thaliana (TAIR9)	86	199	43.2
Others	1806	11783	15.3

Table S5. Performance of different strategies in predicting both multi-stem and single-stem pre-microRNAs.

Stem type	Strategy	*Sn*	*Sp*	*AC*	*MCC*
Multi-stem	**Strategy A**	0.819	0.932	88.50%	0.763
Single-stem	**Strategy B**	0.901	0.972	92.30%	0.837
	Strategy A	**0.903**	0.979	92.60%	0.844
	Strategy fusion	**0.903**	**0.986**	**92.90%**	**0.851**

Table S6. Comparison of miRD with different methods using test dataset I.

Stem type	Strategy	*Sn*	*Sp*	*AC*	*MCC*
Single-stem	**Strategy B**	0.916	0.98	93.60%	0.863
	Strategy A	0.918	0.983	93.80%	0.867
	Strategy fusion	**0.92**	**0.985**	**94.00%**	**0.872**
	tripleSVM	0.819	0.88	83.80%	0.661
	MIReNA	0.826	0.922	85.50%	0.703
Multi-stem	**Strategy A**	**0.932**	0.819	**88.50%**	**0.763**
	tripleSVM	0.236	0.105	15.80%	-0.669
	MIReNA	0.586	**0.97**	81.40%	0.631

Table S7. Comparison of miRD with different methods using test dataset II.

Stem type	Strategy	Arabidopsis lyrata %*	Homo sapiens %	Mus musculus %	Rattus norvegicus %	Other species %
Single-stem	tripleSVM	87.3 (110/126)	66.1 (74/112)	83.2 (84/101)	55.6 (40/72)	85.5 (365/427)
	MIReNA	92.9 (117/126)	80.4 (90/112)	91.1 (92/101)	77.8 (56/72)	80.1 (342/427)
	Strategy B	**100** (126/126)	86.6 (97/112)	95.0 (96/101)	**94.4** (68/72)	94.6 (404/427)
	Strategy A	99.2 (125/126)	**89.3** (100/112)	**98.0** (99/101)	88.9 (64/72)	**95.6** (408/427)
Multi-stem	tripleSVM	17.3 (13/75)	38.5 (5/13)	0.0 (0/2)	46.7 (7/15)	16.7 (24/144)
	MIReNA	81.3 (61/75)	69.2 (9/13)	**50.0** (1/2)	66.7 (10/15)	62.5 (90/144)
	Strategy A	**98.7** (74/75)	**84.6** (11/13)	0.0 (0/2)	**86.7** (13/15)	**84.7** (122/144)

***: percentage of predicted microRNAs over known microRNAs**

Table S8. The candidate novel pre-microRNAs that predicated by miRD.

Type	ID	Chromosome	Start site	End site	Strand	Probability	Validated
Single-stem	1	chr4	139228318	139228396	-	0.999999578	novel-001
Single-stem	2	chr9	20411150	20411231	-	0.999994724	novel-002
Single-stem	3	chr8	6602687	6602762	+	0.999990098	novel-003
Single-stem	4	chrX	12940771	12940864	-	0.999986749	novel-004
Single-stem	5	chr18	3885353	3885432	+	0.999986569	novel-005
Single-stem	6	chr8	92217712	92217789	+	0.999969701	novel-006
Single-stem	7	chr1	25349995	25350075	+	0.999962718	novel-007
Single-stem	8	chr15	89155076	89155156	-	0.999394151	novel-008
Single-stem	9	chr9	35608083	35608163	+	0.999222437	novel-009
Single-stem	10	chr17	67095701	67095776	-	0.999029928	novel-010
Single-stem	11	chr19	6416436	6416508	-	0.997227239	Not tested
Single-stem	12	chr8	79679467	79679541	+	0.997001507	Not tested
Single-stem	13	chr13	67805410	67805488	-	0.990079216	Not tested
Single-stem	14	chrY	1362810	1362886	+	0.985628982	Not tested
Single-stem	15	chr18	21901636	21901711	+	0.985250107	Not tested
Single-stem	16	chrX	53228057	53228151	-	0.976337949	Not tested
Single-stem	17	chr13	26104410	26104481	+	0.975062966	Not tested
Single-stem	18	chr21	40818927	40819001	+	0.973879018	Not tested
Single-stem	19	chr1	98510819	98510895	-	0.971168341	Not tested
Single-stem	20	chr7	102111909	102111985	+	0.947991805	Not tested
Single-stem	21	chr11	16984502	16984582	-	0.944592888	Not tested
Single-stem	22	chrY	1362507	1362583	+	0.927036211	Not tested
Single-stem	23	chr3	127294107	127294179	-	0.923680787	Not tested
Single-stem	24	chr2	219206629	219206708	+	0.921611988	Not tested
Single-stem	25	chr1	161196975	161197054	+	0.834420076	Not tested
Single-stem	26	chr19	50357840	50357919	+	0.824947219	Not tested
Single-stem	27	chr12	128778640	128778719	+	0.804705948	Not tested
Single-stem	28	chr4	10293865	10293938	+	0.757517385	Not tested
Single-stem	29	chr6	290997	291067	+	0.606604033	Not tested
Single-stem	30	chr6	253423	253493	+	0.606588051	Not tested
Single-stem	31	chr6	253429	253499	+	0.606556086	Not tested
Single-stem	32	chr6	468829	468899	+	0.606540102	Not tested
Single-stem	33	chr6	253437	253507	+	0.606540102	Not tested
Single-stem	34	chr6	253428	253498	+	0.606524118	Not tested
Single-stem	35	chr6	28950026	28950096	+	0.606492149	Not tested
Single-stem	36	chr1	28573675	28573749	-	0.581052841	Not tested
Single-stem	37	chr6	33665919	33666003	+	0.505237593	Not tested
Single-stem	38	chr4	184426548	184426626	-	0.44581839	Not tested
Single-stem	39	chr1	228745188	228745288	-	0.386939993	Not tested
Single-stem	40	chr1	228747429	228747529	-	0.386939993	Not tested
Single-stem	41	chr1	228749670	228749770	-	0.386939993	Not tested
Single-stem	42	chr1	228751911	228752011	-	0.386939993	Not tested
Single-stem	43	chr1	228754152	228754252	-	0.386939993	Not tested
Single-stem	44	chr1	228758587	228758687	-	0.386939993	Not tested
Single-stem	45	chr1	228760829	228760929	-	0.386939993	Not tested
Single-stem	46	chr1	228763068	228763168	-	0.386939993	Not tested
Single-stem	47	chr1	228765310	228765410	-	0.386939993	Not tested
Single-stem	48	chr1	228767551	228767651	-	0.386939993	Not tested
Single-stem	49	chr1	228769791	228769891	-	0.386939993	Not tested
Single-stem	50	chr1	228774257	228774357	-	0.386939993	Not tested
Single-stem	51	chr1	228776488	228776588	-	0.386939993	Not tested
Single-stem	52	chr1	228778729	228778829	-	0.386939993	Not tested
Single-stem	53	chr1	228780960	228781060	-	0.386939993	Not tested
Single-stem	54	chr5	56247966	56248047	-	0.308126184	Not tested
Single-stem	55	chr15	51481832	51481912	-	0.276076176	Not tested
Single-stem	56	chr9	135927378	135927447	+	0.221102653	Not tested
Single-stem	57	chr22	50862834	50862911	+	0.173427747	Not tested
Single-stem	58	chr17	25748614	25748706	-	0.156808792	Not tested
Single-stem	59	chr4	10349759	10349852	+	0.144199799	Not tested
Single-stem	60	chr1	227680460	227680535	-	0.083136752	Not tested
Single-stem	61	chr16	32127853	32127936	-	0.073566426	Not tested
Single-stem	62	chr16	33071024	33071107	-	0.073564191	Not tested
Single-stem	63	chr17	20171902	20171995	+	0.060583292	Not tested
Single-stem	64	chr4	78236543	78236626	+	0.052836348	Not tested
Single-stem	65	chr17	62856912	62856999	+	0.044356329	Not tested
Single-stem	66	chr17	28882878	28882965	-	0.026019691	Not tested
Multi-stem	67	chr12	48139393	48139488	+	0.997944	novel-011
Multi-stem	68	chr20	3898201	3898297	+	0.75444631	novel-012
Multi-stem	69	chr6	28918819	28918903	+	0.74080956	novel-013
Multi-stem	70	chr6	437555	437639	+	0.74080956	novel-014
Multi-stem	71	chr6	222224	222308	+	0.74080956	novel-015
Multi-stem	72	chr6	222372	222456	+	0.74080956	novel-016
Multi-stem	73	chr6	222219	222303	+	0.74080956	Not tested
Multi-stem	74	chr6	259726	259810	+	0.74080956	Not tested
Multi-stem	75	chr9	131154903	131154986	+	0.68614679	Not tested
Multi-stem	76	chr7	100465653	100465750	+	0.55670966	Not tested
Multi-stem	77	chr19	56906728	56906828	+	0.51762136	Not tested
Multi-stem	78	chr19	35836433	35836529	+	0.4478419	Not tested
Multi-stem	79	chr15	70814457	70814550	-	0.43371836	Not tested
Multi-stem	80	chr22	31556037	31556127	-	0.41668504	Not tested
Multi-stem	81	chr6	1390549	1390646	-	0.35415736	Not tested
Multi-stem	82	chr9	139565908	139566001	+	0.26283868	Not tested
Multi-stem	83	chr10	135064595	135064666	-	0.20896448	Not tested
Multi-stem	84	chr9	35710651	35710743	-	0.19576325	Not tested
Multi-stem	85	chr17	76136813	76136903	+	0.15279782	Not tested
Multi-stem	86	chrX	125606788	125606872	-	0.10787042	Not tested
Multi-stem	87	chr22	32143439	32143520	-	0.10017895	Not tested
Multi-stem	88	chr15	45493361	45493452	+	0.09164062	Not tested
Multi-stem	89	chr19	47777535	47777606	+	0.06852491	Not tested
Multi-stem	90	chr20	61483950	61484031	-	0.05971561	Not tested
Multi-stem	91	chrX	53172514	53172599	+	0.04228261	Not tested
Multi-stem	92	chr20	17486149	17486232	+	0.02371282	Not tested