Direct identification of human cellular microRNAs by nano-flow liquid chromatography–high-resolution tandem mass spectrometry and database searching

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Table S-1. Analysis of ath-miR-173: RNA amount *versus* signal intensity

RNA amount (fmol)	Signal intensity (counts)
0.3	4.5×10^3
1	1.5×10^4
3	5.8×10^4
10	2.2×10^5
30	7.8×10^5
100	2.9×10^6
300	1.0×10^7

Table S-2. Product ion peaks picked up from a MS/MS spectrum of mmu-miR-335 by SpiceCmd program and their assignments by Ariadne

mgf		SpiceCmd			
m/z	Intensity	*m/z*	Charge	Manual inspection	Assignment by Ariadne
305.02	62185	305.02	1		c1
323.03	303969	323.03	1		w1
344.04	36413	344.04	1		i(G)
384.98	17018	384.98	1		i(U+p)
419.05	53404	419.05	1		NA
424.00	9568	424.00	1		i(G+p)
442.08	10892	442.08	1		NA
498.03	31870	498.03	1		NA
530.09	23735	530.09	1		a2
546.92	67104	272.96	2	1-	NA
570.10	10004	570.10	1		
588.11	558899	588.11	1		y2
610.06	678867	610.06	1		c2
633.08	38132	633.08	1		i(AC)
634.07	77787	634.07	1		i(AU)
639.08	11153	639.08	1		
649.08	10550	649.08	1		
650.06	37330	650.06	1		i(UG)
657.10	108331	657.10	1		i(AA)
673.09	68844	673.09	1		i(AG)
713.05	21451	713.05	1		
724.09	75519	724.09	1		NA
737.07	62263	737.07	1		i(AA+p)
753.06	25477	753.06	1		
771.13	15830	771.13	1		
787.12	31181	787.12	1		
804.06	16807	804.06	1		
827.08	10776	827.08	1		
851.09	32647	851.09	1		

867.09	17550	867.09	1		
876.12	10926	876.12	1		
894.13	554698	894.13	1		y3
939.10	340513	939.10	1		c3
945.11	14075	945.11	1		
956.09	16586	956.09	1		
957.12	27164	957.12	1		
962.14	65054	962.14	1		i(AAC)
963.12	116005	963.12	1		i(AAU)
974.10	218253	974.10	1		w2
979.12	18491	979.12	1		
986.15	80322	986.15	1		i(AAA)
1002.14	76005	1002.14	1		i(AAG)
1043.09	55516	1043.09	1		i(AAU+p)
1053.14	78622	1053.14	1		NA
1058.10	16557	1058.10	1		
1066.11	39839	1066.11	1		i(AAA+p)
1072.13	33703	1072.13	1		NA
1133.11	37250	1133.11	1		NA
1157.12	37371	1157.12	1		NA
1194.17	11480	1194.17	1		
1205.17	26359	1205.17	1		NA
1223.18	289126	1223.18	1		y4
1268.16	329524	1268.16	1		c4
1269.68	192937	1269.68	1	2-	NA
1286.17	88660	1286.17	1		NA
1292.17	69917	1292.17	1		i(AAAU)
1303.14	111354	1303.14	1		w3
1348.13	11605	1348.13	1		
1372.14	14807	1372.14	1		
1382.20	72680	1382.20	1		NA
1395.16	16319	1395.16	1		
1461.19	31209	1461.19	1	not monoisotopic, overlap	NA
1481.14	5348	1481.14	1	2-	NA
1481.69	25370	1481.69	1	2-	NA
1534.23	13179	1534.23	1		NA

1552.26	162242	1552.26	1		y5
1613.21	92631	1613.21	1		c5
1621.21	15074	1621.21	1		i(AAAAU)
1630.22	2378	1630.22	1		NA
1676.21	12591	1676.21	1		i(AAAGG)
1680.20	9541	1680.20	1		NA
1717.21	5025	1717.21	1		
1726.22	14377	1726.22	1	not monoisotopic	
1740.74	53124	1740.74	1		NA
1789.24	68598	1789.24	1	M-2B(A,C/U), 4-	NA
1812.24	67122	1812.24	1	M-B(A)-H_2O, 4-	NA
1823.50	188032	1823.50	1	M-B(C/U), 4-	NA
1840.25	42610	1840.25	1		NA
1866.25	3174	1866.25	1		
1874.25	6386	1874.25	1		
1882.30	79622	940.65	2		NA
1923.27	36246	1923.27	1		NA
1923.77	22461	1923.77	1		NA
1933.25	12703	1933.25	1		
1942.26	33790	970.62	2		c6
1960.26	10273	1960.26	1		
1961.26	110814	980.13	2		w6
1970.57	20223	1970.57	1		
1976.25	13507	1976.25	1		
2056.29	98868	1027.64	2		NA
2075.29	11133	1037.14	2		NA
2136.25	10455	1067.62	2		NA
2155.30	51828	1077.14	2		NA
2210.37	148992	1104.68	2		y7
2287.31	48150	1143.15	2		c7
2404.34	26050	1201.66	2		NA
2468.31	11185	1233.65	2		NA
2484.30	53168	1241.65	2		NA
2521.39	10053	1260.19	2		NA
2592.32	82408	1295.66	2		c8
2601.31	4226	1300.15	2		NA

2619.35	132473	1309.17	2		w8
2706.37	13059	1352.68	2		NA
2733.38	74059	1366.19	2		NA
2749.37	40843	1374.18	2		NA
2813.37	12518	1406.18	2	not monoisotopic	NA
2866.42	16240	1432.71	2		NA
2884.47	107109	1441.73	2		y9
2921.38	51224	1460.19	2		c9
3189.47	74783	1594.23	2		y10
3250.43	20330	1624.71	2		c10
3270.44	45139	1634.72	2	not monoisotopic, overlap	NA
3405.40	14444	1702.20	2		NA
3422.41	2895	1710.70	2		NA
3445.42	13900	1722.21	2		NA
3518.52	42558	1758.76	2		y11
3599.43	4053	1799.21	2	4-	NA
3659.47	9724	1829.23	2		i(CAAGAGCAAUA+p)
3885.55	41333	1942.27	2		c12
5043.66	3041	1680.55	3		
5063.69	14362	1687.23	3		
5098.94	15330	1698.98	3		
5108.61	14444	1702.20	3		
5126.65	53098	1708.21	3		NA
5158.16	27203	1718.72	3		NA
5174.68	20818	1724.22	3		NA
5193.57	2353	1730.52	3	4-	
5196.66	31598	1731.55	3		w16
5212.95	83969	1736.98	3	M-3B(G,G,G), 4-	NA
5222.68	10223	1740.22	3		
5227.93	201050	1741.97	3	M-3B(A,G,G), 4-	NA
5239.93	288351	1745.97	3	M-3B(A,A,G), 4-	NA
5252.66	107023	1750.22	3		NA
5257.92	99752	1751.97	3	M-3B(C/U,G,G), 4-	NA
5269.95	73227	1755.98	3	M-3B(A,C/U,G), 4-	NA
5309.71	48043	1769.23	3	4-	NA
5314.95	76496	1770.98	3	M-2B(G,G)-H_2O, 4-	NA

5327.71	451833	1775.23	3	M-2B(G,G), 4-	NA
5336.72	43188	1778.24	3	NA, 4-	
5341.22	1116377	1779.74	3	M-2B(A,G), 4-	NA
5353.22	618807	1783.73	3	M-2B(C/U,G), 4-	NA
5387.84	3511	1795.27	3	4-	
5407.74	10894	1801.91	3	4-	
5414.74	31895	1804.24	3	4-	
5428.99	401010	1808.99	3	M-B(G)-H_2O, 4-	NA
5442.49	1722070	1813.49	3	M-B(G), 4-	NA
5454.49	1976636	1817.49	3	M-B(A), 4-	NA
5461.80	6362	1819.93	3	M-B(C/U)-H_2O, 4-	
5506.79	13340	1834.93	3		
5522.75	42610	1840.25	3		c17
5542.30	690156	1846.76	3	M-H_2O, 4-	NA
5555.78	2886586	1851.25	3	precursor, 4-	NA
5565.69	3571	1854.56	3		
5583.69	2955	1860.56	3		
5637.79	15001	1878.59	3		NA
5655.79	3426	1884.59	3		NA
5700.77	13623	1899.58	3		NA
5717.74	61346	1905.24	3		NA
5735.75	7681	1911.24	3		NA
5791.85	73528	1929.94	3		NA
5834.79	101087	1944.26	3		NA
5849.80	3353	1949.26	3		NA
5870.78	14564	1956.25	3		w18
5896.76	10169	1964.91	3		NA
5965.85	3765	1987.94	3		NA

Red letters represent the incorrectly picked peaks by SpiceCmd program. i(N…+p) in a "Assignment by Ariadne" column denotes an internal fragment generated by multiple cleavage of the molecular ion during CID, where N is nucleotide residue and p is terminal phosphate. NA: not assigned

Table S-2. Calculated mass values of product ions derived from mmu-miR-335.

Sequence		a	c		w	y
5':OH						
U	1	225.051	305.017	23		7405.081
C	2	530.092	610.059	22	7179.023	7099.056
A	3	859.145	939.111	21	6873.981	6794.015
A	4	1188.197	1268.164	20	6544.929	6464.962
G	5	1533.245	1613.211	19	6215.876	6135.910
A	6	1862.297	1942.264	18	5870.829	5790.862
G	7	2207.345	2287.311	17	5541.776	5461.810
C	8	2512.386	2592.352	16	5196.729	5116.762
A	9	2841.439	2921.405	15	4891.688	4811.721
A	10	3170.491	3250.458	14	4562.635	4482.669
U	11	3476.517	3556.483	13	4233.582	4153.616
A	12	3805.569	3885.535	12	3927.557	3847.591
A	13	4134.622	4214.588	11	3598.505	3518.538
C	14	4439.663	4519.629	10	3269.452	3189.486
G	15	4784.710	4864.677	9	2964.411	2884.444
A	16	5113.763	5193.729	8	2619.363	2539.397
A	17	5442.815	5522.782	7	2290.311	2210.345
A	18	5771.868	5851.834	6	1961.258	1881.292
A	19	6100.920	6180.887	5	1632.206	1552.239
A	20	6429.973	6509.939	4	1303.153	1223.187
U	21	6735.998	6815.965	3	974.101	894.134
G	22	7081.046	7161.012	2	668.075	588.109
U	23	7387.071	7467.037	1	323.028	243.062
3':OH						

Table S-3. Observed product ions derived from mmu-miR-335 (100 fmol).

Sequence		a	c		w	y
5':OH						
U	1		305.017	23		
C	2	530.090	610.057	22		
A	3		939.102	21		
A	4		1268.161	20		
G	5		1613.213	19		
A	6		1942.257	18	5870.776	
G	7		2287.310	17		
C	8		2592.321	16	5196.658	
A	9		2921.384	15		
A	10		3250.431	14		
U	11			13		
A	12		3885.552	12		
A	13			11		3518.522
C	14			10		3189.474
G	15			9		2884.473
A	16			8	2619.350	
A	17		5522.753	7		2210.367
A	18			6	1961.263	
A	19			5		1552.258
A	20			4	1303.145	1223.182
U	21			3	974.097	894.134
G	22			2		588.112
U	23			1	323.027	
3':OH						

Table S-4. Observed product ions of 30 fmol of mmu-miR-335.

Sequence		A	c		w	y
5':OH						
U	1		305.029	23		
C	2		610.064	22		
A	3		939.119	21		
A	4		1268.173	20		
G	5		1613.220	19		6135.940
A	6			18		
G	7		2287.329	17		
C	8			16		
A	9			15		
A	10			14		
U	11			13		
A	12			12		
A	13			11		
C	14			10		
G	15			9		2884.466
A	16			8	2619.383	
A	17	5442.565		7	2290.329	2210.361
A	18			6	1961.275	
A	19		6180.920	5		1552.250
A	20		6509.995	4	1303.163	1223.196
U	21			3	974.109	894.142
G	22			2		588.114
U	23			1		
3':OH						

Table S-5. Observed product ions derived from mmu-miR-335 (10 fmol).

Sequence		a	c		W	y
5':OH						
U	1		305.020	23		
C	2		610.064	22		
A	3			21		
A	4		1268.173	20		
G	5		1613.220	19		
A	6			18		
G	7			17		
C	8			16		
A	9			15		
A	10			14		
U	11			13		
A	12			12		
A	13			11		
C	14			10		
G	15			9		
A	16			8	2619.386	
A	17			7		2210.356
A	18		5851.865	6		
A	19			5		1552.250
A	20			4		1223.195
U	21			3		894.142
G	22			2		588.114
U	23			1		
3':OH						

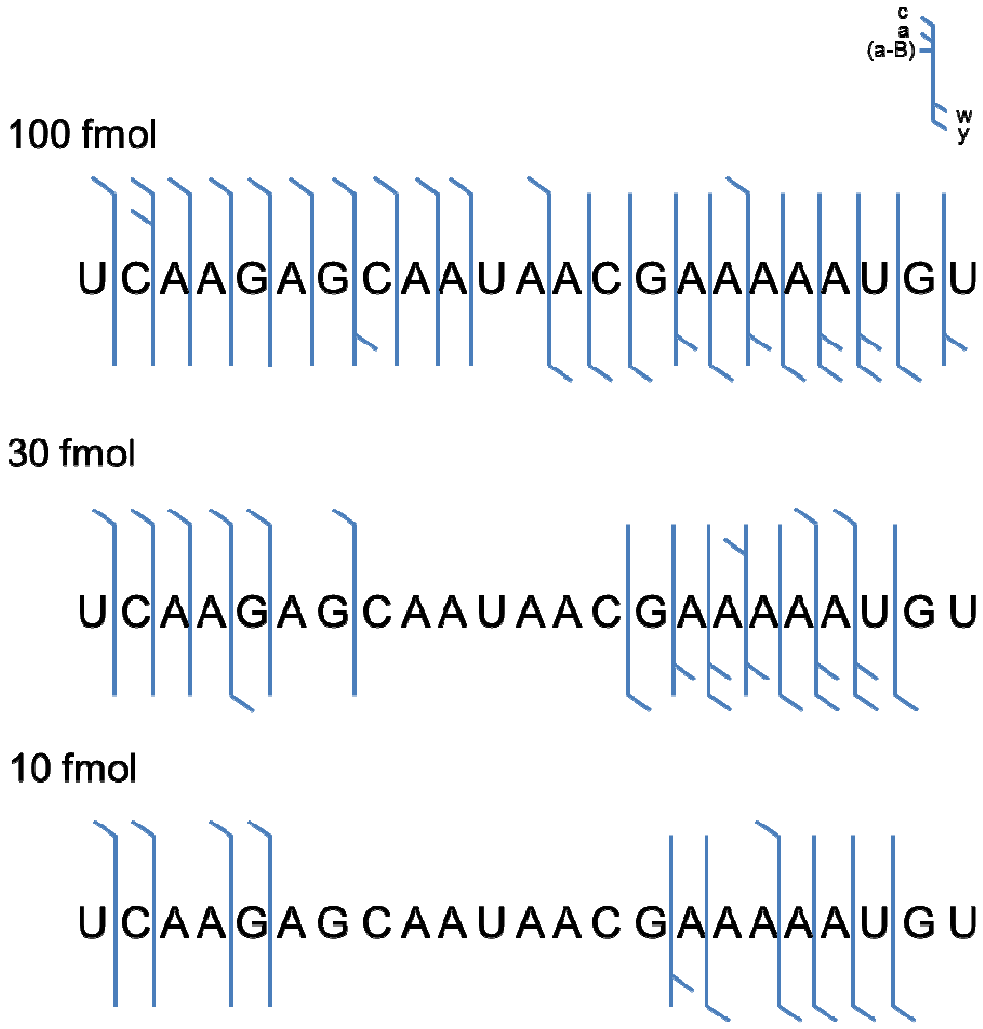

Figure S-1. Summary of product ion assignments for mmu-miR-335.