

Table S1. Expression of 6 *de novo* miRNAs in testis across 9 lines of *D. melanogaster*.

miRNA	Precursor	Normalized expression(RPM) in different Genetic Background								Total read count	Definition	
		Oregon R-1	Oregon R-2	Oregon R-3	hs-Penelope A1	yw67c23(2)	A2	Canton S	Z56			
dme-miR-973-3p	dme-mir-973	17.41	11.96	61.19	8.14	11.07	15.03	18.94	61.62	34.04	239.4	miR*
dme-miR-973-5p		672.6	358.49	71.83	696.07	411.05	945.55	389.57	36.54	29.7	3611.4	mature
dme-miR-975-3p	dme-mir-975	107.55	101.13	21.28	19.77	11.07	37.58	22.24	245.03	283.21	848.86	miR*
dme-miR-975-5p		66.24	67.85	5419.02	20.35	29.06	66.14	40.36	547.38	717.81	6974.21	mature
dme-miR-977-3p	dme-mir-977	7510.57	4871.74	20024.05	1141.51	1853.17	1649.83	1375.45	481.46	507.03	39414.81	mature
dme-miR-977-5p		80.92	63.95	436.29	25.59	261.58	75.16	259.44	68.78	39.11	1310.82	miR*
dme-miR-978-3p	dme-mir-978	647.33	535.27	20766.27	1346.78	1461.5	2178.23	1360.62	133.98	187.6	28617.58	mature
dme-miR-978-5p		123.94	106.59	396.38	71.53	127.33	157.09	157.31	5.02	5.79	1150.98	miR*
dme-miR-983-5p	dme-mir-983	868.92	649.91	803.41	172.13	770.89	489.31	500.76	3578.72	2741.58	10575.63	miR*
dme-miR-983-3p		2073.1	1322.96	16520.44	753.64	1125.19	1241.7	935.63	81.68	82.57	24136.91	mature
dme-miR-984-3p	dme-mir-984	1541.17	730.24	101.09	881.57	1008.93	1077.09	1056.71	56.6	25.35	6478.75	miR*
dme-miR-984-5p		14159.05	8872.6	5506.81	8969.26	2111.98	10800.95	2350.61	7038.51	8155.2	67964.97	mature

Note: Small RNA-seq data are retrieved from the GEO database, accession numbers: GSM909277 for Oregon R-1,GSM 909278 for Oregon R-2, GSM 280085 for Oregon R-3,GSM 548591 for hs-Penlope, GSM 548589 for A1,GSM 548584 for yw67c23(2) , GSM 548582 for A2, GSM2562978 for Canton S, GSM2562975 for Z56.