

Supplementary information

Cloning and expression of new microRNAs from zebrafish

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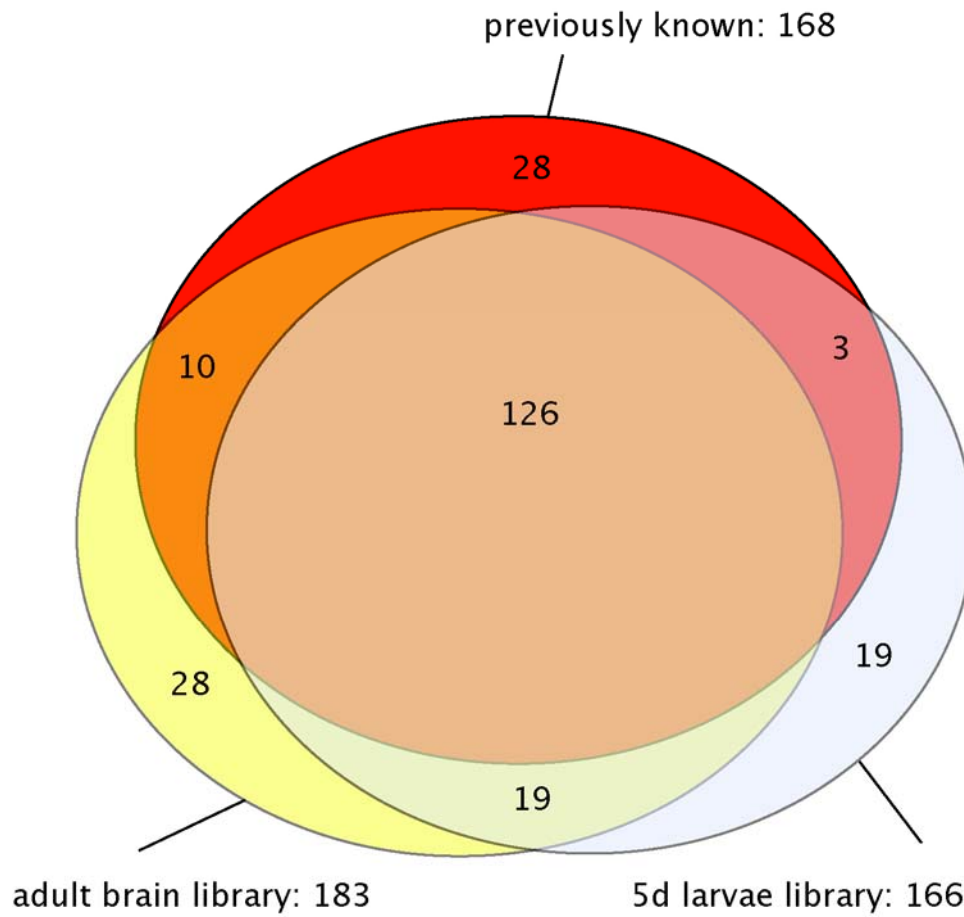


Figure S1. Venn-diagram of known zebrafish miRNAs from the miRNA registry, miRNAs cloned from the zf-brain library and miRNAs cloned from the zf-embryo library.

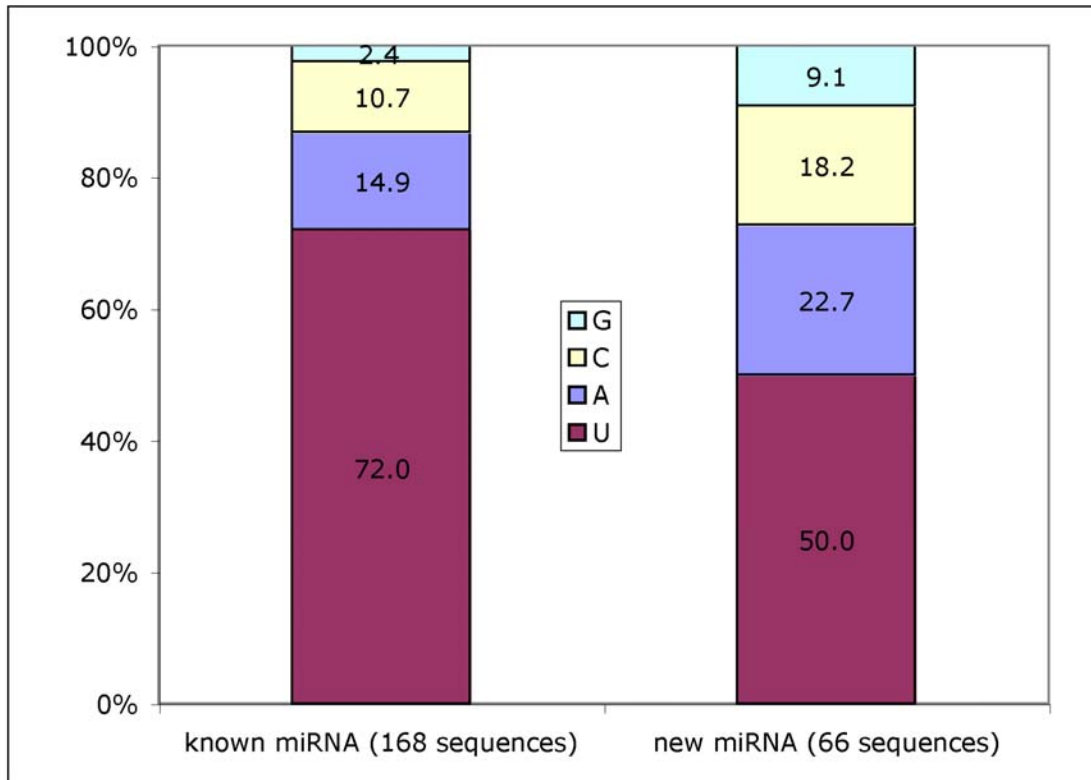


Figure S2. Bar diagram, showing the frequency of the base identity of the first base of known miRNAs (from the miRNA registry) and novel miRNAs cloned in this study.

Table S1. LNA probe sequences

new ID	probe sequence
dre-miR-499	AAACATCACTGCAAGTCTTAAC
ZF_nl_384	CCCAATAAGGTTCCCAGGGA
dre-miR-731	CGATCCGGGAGAAAACGTGCATT
ZF_nl_21	CAGTGTCGTTACTCCCAATCGTG
dre-miR-723-5p ¹	AAAGTAACATCATTAAAACTGTC
dre-miR-726-3p	CCGAGTTCTGCTAGTAGTGAA
dre-miR-489	AGCAGCCGTACATATGATGTCAC
ZF_nl_149	GTACCCGAGTGATGTCTGAAACCA
ZF_nl_11	GAATCAGCGGAGTGGGGAGA
dre-miR-15c	GAAAACCATGACGCGCTGCTT
dre-miR-730-5p	ACACACAGCATGCACAATGAGGA
dre-miR-733	AACCACTGAGCTAAACCAACGCA
dre-miR-34c-5p	GTAATCAACTAACTGCACTGCCT
dre-miR-739	AACCCTTCTCCACTTCGGCCT
ZF_nl_236	ACCAATCCACCAGCTGTGCCCA
ZF_nl_157	TGCCAGGGATTGTAGGTGTGA
ZF_nl_286	CGCAGGCAACTTAAAAACAAGCC
dre-miR-727-3p	TAAGTCTTCAACTCGCCTCAAC
dre-miR-725	ACTACTAGAAACAATGACTGAA
dre-miR-735-3p	GTCAAAGTTTAGCGGTGGGAGAG
dre-miR-737-3p	TATTTTCTTTAGGTTTTGATT
dre-miR-734	CGGTACGATTCTGCAGCATTAC
dre-miR-727-5p ¹	GCTGGGAGGAATTGAAGACTGA
dre-miR-723-3p	AGCACAGATTTAATTGATGTCTT
dre-miR-730-3p ¹	CCTCCACATTGCAGGGCCTGTG
dre-miR-34c-3p ¹	CCTGGTAGTGAGGTTAGTGATT
dre-miR-732	ACCGAGAGTTCTCTGCTTTGAG
dre-miR-729	AACCCAGGTCGTATCATACCCATG
ZF_nl_263	GACGTCAATTAGCGACCCGA
dre-miR-724-5p	CTTAAAAGGAAGGTGTGGCTG
ZF_nl_51	ACTGACAGAATCAGCGGAGCCG
ZF_nl_264	GAATGTACACTGGATGCAGACA
dre-miR-728	GAAAACGTAGTGTACTTAGTAT
ZF_nl_139	AATGGCAATATGGAGGCATAGCA
dre-miR-722	AATCTGAAACGTTTCTGCAAAAAA
dre-miR-736	AAAACTTTTGTTCGCTTAC
dre-miR-190b	CAACCGAATATCAACATATCA
dre-miR-726-5p ¹	AGTTCAGAACTAGCGGAATTCC
dre-miR-738	GAGGTCCCAGCGCGGCCGTAGC
ZF_nl_33	AAACTCTGCTAAAACAGGACTCA
dre-miR-724-3p ¹	AAACAGTCGCAAATCCCTTTAA
dre-miR-130c	ATGCCCTTTTAAATTGCACTG
dre-miR-451	AACTCAGTAATGGTAACGGTTT
dre-miR-455	TGTATATGCCCATGGACTGCAT
dre-miR-92b	GGAGGCCGGGACGAGTGCAATAT
dre-miR-429	ACGGCATTACCAGACAGTATTA
dre-miR-430a	CTACCCCAACAATAGCACTTA
dre-miR-130a	ATGCCCTTTTAACTTGCCTG
dre-miR-202	CAAAGAGGTATATGCATAGGAA
dre-miR-365	ATAAGGATTTTTAGGGGCATTA
dre-miR-101b	CTTCAGTTATCATAGTACTGTA
dre-miR-130b	ATGCCCTTTTCAATTGCACTG
dre-miR-135	CACATAGGAATAGAAAGCCATA
dre-miR-187	GTTCCACTGGCTGCAACACAAG
dre-miR-193b	AGCGGGACTTTGCGGGCCAGTT
dre-miR-27c	GCAGAACTTAACCACTGTGAA
dre-miR-301b	CAATGACAATACTATTGCACTG
dre-miR-363	TACAGATGGATACCGTGCAATT
dre-miR-454a	CCCTATTAGCAATATTGCACTA
dre-miR-454b	CCCTATAAGCAATATTGCACTA
dre-miR-430c	CTACCCCAAGAGAAGCACTTA
dre-miR-462	AGCTGCATTATGGGTTCCGTTA
dre-miR-460	CGCACAGTGTGACAATGCAGG
dre-miR-461	TTGGCATTTAGCCATTCTGTA
dre-miR-430i	CTACGCCAACAATAGCACTTA
dre-miR-430b	CTACCCCAACTTGATAGCACTTT
dre-miR-430j	TACCCCAATTTGATAGCACTTT
dre-miR-456	TGACAACCATCTAACCAGCCTG
dre-miR-457a	TGCCAATATTGATGTGCTGCTT
dre-miR-457b	CTCCAGTATTTATGTGCTGCTT

dre-miR-458 GCAGTACCATTCAAAGAGCTAT
 dre-miR-459 CAGGATGAATCCTTGTACTGA
 dre-miR-740 ACTGTACCATCCACTTTTAT

[†]probes for miRNA star sequences

Table S2. Cloning frequency and conservation of known miRNAs from zebrafish

mir ID	brain	larvae	total	conservation
dre-let-7a	155	13	168	zfish,fugu,todon,chicken,opossum,chimp,human,macaca,cow,dog,mouse,rat
dre-let-7b	107	4	111	zfish,fugu,todon,chicken,cow,dog,chimp,macaca,human,opossum,mouse,rat
dre-let-7c	39	4	43	zfish,chicken,chimp,cow,dog,human,macaca,mouse,rat
dre-let-7d	44	10	54	zfish,fugu,todon,chicken,chimp,cow,dog,human,macaca,mouse,rat
dre-let-7e	7	1	8	zfish,fugu,chicken,todon,cow
dre-let-7f	6	0	6	zfish,mouse,rat,chimp,cow,dog,human,macaca,opossum,chicken,todon
dre-let-7g	6	4	10	zfish,todon,fugu,chimp,dog,human,mouse,rat,macaca,opossum,cow,chicken
dre-let-7h	2	0	2	zfish,todon,fugu,cow,chimp,human,macaca,rat,mouse,dog
dre-let-7j	2	0	2	zfish,chimp,dog,macaca,mouse
dre-miR-1	18	22	40	zfish,todon,fugu,macaca,chicken,chimp,human,mouse,opossum,cow,dog,rat
dre-miR-100	298	41	339	zfish,fugu,todon,mouse,rat,chimp,human,macaca,opossum,dog,cow
dre-miR-101a	24	5	29	zfish,fugu,chimp,cow,dog,macaca,mouse,rat,todon,opossum,human
dre-miR-103	17	2	19	zfish,fugu,todon,chicken,opossum
dre-miR-107	6	6	12	zfish,fugu,todon,mouse,chimp,cow,human,macaca,rat,dog,chicken
dre-miR-10a	38	43	81	zfish,chimp,human,macaca,mouse,rat,cow,dog,opossum,chicken
dre-miR-10b	18	25	43	zfish,fugu,todon
dre-miR-10d	15	22	37	zfish,mouse,rat,chimp,human,macaca,opossum,chicken,todon,fugu,cow,dog
dre-miR-122	14	18	32	zfish,todon,fugu,chimp,human,dog,chicken
dre-miR-124	92	40	132	zfish,fugu,todon,mouse,opossum,rat,chimp,human,dog,macaca,chicken,cow
dre-miR-125a	776	73	849	zfish,fugu
dre-miR-125b	2357	201	2558	zfish,todon,fugu,opossum,dog,chimp,human,macaca,rat,mouse
dre-miR-125c	163	17	180	zfish
dre-miR-126	39	5	44	zfish,todon,fugu,mouse,dog
dre-miR-128	450	9	459	zfish
dre-miR-129	255	12	267	zfish,todon,fugu,chimp,human,dog,mouse,macaca,rat,opossum
dre-miR-130a	4	3	7	zfish,chicken
dre-miR-130b	10	8	18	zfish,dog,chimp,macaca,rat,cow,mouse
dre-miR-132	165	6	171	zfish,todon,fugu
dre-miR-133a	64	73	137	zfish,mouse,opossum,chicken,dog,cow,fugu,chimp,human,macaca,rat,todon
dre-miR-133b	33	38	71	zfish,todon,chimp,human,macaca,opossum,rat,chicken,mouse
dre-miR-133c	13	18	31	zfish,todon
dre-miR-135	61	31	92	zfish,fugu,todon
dre-miR-135b	16	17	33	zfish
dre-miR-137	48	28	76	zfish,fugu,opossum,chicken,chimp,cow,dog,human,macaca,mouse,rat,todon
dre-miR-138	365	41	406	zfish,fugu,todon,chicken,rat,dog,chimp,cow,human,macaca,mouse,opossum
dre-miR-140	14	19	33	zfish,dog,chicken,mouse,chimp,human,macaca,rat,opossum,todon,fugu
dre-miR-141	6	10	16	zfish
dre-miR-142a	6	2	8	zfish,todon,fugu,chimp,dog,human,macaca,rat,chicken,mouse,opossum
dre-miR-142b	5	2	7	zfish,chicken,chimp,dog,human,macaca,rat,fugu,todon,mouse,opossum
dre-miR-143	9	5	14	zfish,rat,mouse,fugu,dog,human
dre-miR-144	2	4	6	zfish,fugu,todon,chicken
dre-miR-145	21	7	28	zfish,fugu
dre-miR-146a	3	0	3	zfish,todon
dre-miR-146b	3	0	3	zfish
dre-miR-148	11	8	19	zfish,todon,fugu
dre-miR-150	18	2	20	zfish
dre-miR-152	15	13	28	zfish,cow,dog,mouse,rat,human,macaca,chimp
dre-miR-153a	4	0	4	zfish,chicken,opossum,chimp,cow,human,macaca,dog,fugu,rat,todon,mouse
dre-miR-153b	12	8	20	zfish,todon,fugu,opossum,cow,chimp,human,macaca,rat
dre-miR-15a	8	1	9	zfish,todon
dre-miR-15b	2	4	6	zfish
dre-miR-16a	15	4	19	zfish,fugu,todon,opossum,rat,cow,dog,chimp,chicken
dre-miR-16b	15	4	19	zfish,todon,fugu
dre-miR-16c	5	5	10	zfish
dre-miR-17a	18	10	28	zfish,chicken,opossum
dre-miR-181a	264	131	395	zfish,fugu,todon,chicken,cow,mouse,rat,opossum,chimp,human,macaca,dog
dre-miR-181b	91	59	150	zfish,mouse,rat,chicken,macaca,chimp,dog,human,opossum,cow,fugu,todon
dre-miR-181c	16	11	27	zfish
dre-miR-182	18	15	33	zfish,todon,fugu,opossum,mouse,macaca,dog,chimp,human
dre-miR-183	13	23	36	zfish,fugu,opossum,todon,chicken,chimp,human,cow,dog,macaca,rat
dre-miR-184	14	17	31	zfish,fugu,todon,opossum,macaca,chimp,human,mouse
dre-miR-187	46	4	50	zfish,todon,fugu
dre-miR-18a	5	13	18	zfish,fugu,todon,chicken,cow,mouse,rat,chimp,dog,human,macaca,opossum
dre-miR-18c	4	3	7	zfish
dre-miR-190	2	1	3	zfish,fugu,opossum,todon,chicken,chimp,cow,dog,human,macaca,mouse,rat
dre-miR-192	15	38	53	zfish,todon,fugu

dre-miR-193b	6	1	7	zfish
dre-miR-194a	43	57	100	zfish,todon
dre-miR-194b	2	0	2	zfish
dre-miR-196a	2	3	5	zfish,todon,fugu
dre-miR-196b	0	2	2	zfish
dre-miR-199	117	122	239	zfish,fugu,todon,chicken,cow,mouse,chimp,human,dog,opossum,macaca
dre-miR-19a	58	63	121	zfish,fugu,todon,chimp,cow,dog,human,macaca,opossum,rat,chicken,mouse
dre-miR-19b	153	130	283	zfish,fugu,chimp,dog,human,cow
dre-miR-19c	19	32	51	zfish,chicken,opossum
dre-miR-19d	21	26	47	zfish,todon
dre-miR-200a	39	46	85	zfish,fugu,todon,macaca,cow,mouse,rat,chicken
dre-miR-200b	11	7	18	zfish,fugu,todon,chicken,mouse,rat
dre-miR-200c	14	7	21	zfish,fugu,cow,chicken
dre-miR-203b	88	125	213	zfish,todon
dre-miR-204	14	9	23	zfish,chicken,opossum,todon,fugu
dre-miR-205	35	60	95	zfish,fugu,todon,opossum
dre-miR-20a	28	20	48	zfish,fugu,todon,opossum
dre-miR-20b	2	4	6	zfish
dre-miR-21	95	10	105	zfish,fugu,todon,chicken,mouse,opossum
dre-miR-210	55	25	80	zfish,todon,fugu
dre-miR-212	40	3	43	zfish,fugu,todon
dre-miR-214	4	2	6	zfish,fugu,todon,opossum,chimp,cow,dog,human,macaca,mouse,rat
dre-miR-216a	10	18	28	zfish
dre-miR-216b	13	17	30	zfish,todon,fugu
dre-miR-217	11	17	28	zfish,chicken,todon,chimp,human,macaca,fugu,dog,mouse,opossum,rat
dre-miR-218a	29	39	68	zfish,fugu,todon
dre-miR-218b	21	21	42	zfish,todon,fugu
dre-miR-219	206	129	335	zfish,todon,fugu,cow,rat
dre-miR-221	250	39	289	zfish,todon,fugu,cow,mouse,rat,chimp,human,macaca,dog,opossum,chicken
dre-miR-222	160	22	182	zfish,fugu,todon,chicken,rat,human,macaca,dog,mouse,opossum,cow
dre-miR-223	3	1	4	zfish,todon,fugu
dre-miR-22a	102	58	160	zfish,fugu,todon,chicken,dog,opossum,chimp,cow,human,mouse,rat
dre-miR-22b	4	1	5	zfish,todon,fugu
dre-miR-23a	6	7	13	zfish,fugu,todon
dre-miR-24	31	10	41	zfish,cow,chimp,dog,human,macaca,mouse,rat
dre-miR-25	9	12	21	zfish,fugu,todon,dog,opossum,human,macaca,mouse
dre-miR-26a	51	22	73	zfish,todon,fugu
dre-miR-27a	2	1	3	zfish,fugu,todon,opossum
dre-miR-27b	17	2	19	zfish,chicken,chimp,human,macaca,mouse,rat,cow,dog,fugu,todon,opossum
dre-miR-27c	1	1	2	zfish
dre-miR-27d	3	1	4	zfish,opossum,cow,chimp,human,macaca,mouse,rat,dog,chicken,fugu,todon
dre-miR-27e	4	1	5	zfish,todon,fugu
dre-miR-29a	19	4	23	zfish,todon,cow,fugu,chicken,opossum,dog,rat
dre-miR-29b	3	1	4	zfish
dre-miR-301a	13	4	17	zfish,rat,mouse,chicken,dog,chimp,human,macaca,opossum
dre-miR-301b	4	8	12	zfish,fugu,todon
dre-miR-301c	6	9	15	zfish,todon,fugu
dre-miR-30b	17	9	26	zfish,fugu,todon
dre-miR-30c	66	20	86	zfish
dre-miR-30d	38	6	44	zfish
dre-miR-30e	23	22	45	zfish,todon,fugu,chicken
dre-miR-338	64	3	67	zfish,fugu,todon
dre-miR-34	28	6	34	zfish,todon,chicken,macaca,chimp,human,opossum,dog,cow,mouse,rat
dre-miR-363	5	5	10	zfish,dog,human,macaca,rat,mouse,opossum
dre-miR-365	7	2	9	zfish,fugu,todon,chicken,cow,dog,human,macaca,mouse,rat,opossum,chimp
dre-miR-375	10	12	22	zfish,fugu,todon
dre-miR-429	0	4	4	zfish,fugu,todon,cow,mouse,rat,dog,human,chicken,chimp,opossum,macaca
dre-miR-454a	2	2	4	zfish,macaca,chicken,chimp,human,cow,dog,fugu
dre-miR-454b	7	9	16	zfish
dre-miR-455	5	7	12	zfish,todon,fugu,chicken,opossum,chimp,dog,human,macaca,rat,mouse
dre-miR-456	13	2	15	zfish
dre-miR-457a	2	0	2	zfish
dre-miR-457b	0	3	3	zfish
dre-miR-458	2	4	6	zfish,chicken,fugu
dre-miR-460	2	1	3	zfish,fugu
dre-miR-462	2	0	2	zfish
dre-miR-7b	35	9	44	zfish,fugu,macaca,mouse,opossum,chimp,human,rat,cow,dog,chicken
dre-miR-9	811	467	1278	zfish,fugu,todon,opossum,chimp,cow,dog,human,macaca,mouse,rat,chicken
dre-miR-92a	20	18	38	zfish,chimp,human,chicken,opossum,cow,dog,rat,fugu,todon
dre-miR-92b	7	0	7	zfish
dre-miR-93	38	32	70	zfish
dre-miR-96	6	11	17	zfish,todon,fugu,opossum,cow,chimp,human,mouse,dog,rat
dre-miR-99	166	34	200	zfish,dog,chimp,cow,human,macaca,rat,chicken,mouse
total	9607	3150	12757	

Table S3. Sequences, cloning frequency and conservation of new miRNA from zebrafish

miRNA	sequence	brain	larvae	cloned total	conservation
>ZF_nl_107	CTTCAGAGCTGAGGTGAG	1	0	1	zfish
>ZF_nl_108	ACAGTCTCTCGGAGCGCTCG	1	0	1	zfish
>ZF_nl_11	TCTCCCCACTCCGCTGATTTC	1	0	1	zfish
>dre-miR-724	TAAAGGGAATTTGCGACTGTT	21	0	21	zfish,fugu,todon
>dre-miR-725	TTCAGTCATTGTTTCTAGTAGT	5	7	12	zfish
>dre-miR-726	TTCACTACTAGCAGAACTCGG	4	1	5	zfish,todon,fugu
>ZF_nl_125	TAATACTGCCTGGTAATGCCAT	1	0	1	zfish
>ZF_nl_135	TTGCAGGTTCAATTCCTGTC	0	1	1	zfish
>ZF_nl_138	TAGCACCAATTTGAAATCGGTCCG	1	1	2	zfish,opossum
>ZF_nl_139	TGCTATGCCTCCATATTGCCATT	2	0	2	zfish
>ZF_nl_149	TGGTTTCAGACATCACTCGGGTAC	0	1	1	zfish
>ZF_nl_157	TCACACCTACAATCCCTGGCA	5	0	5	zfish
>ZF_nl_164	GAACCTGATGGATCTTCTCT	0	1	1	zfish
>dre-miR-727	GTTGAGCGGAGTTGAAGACTTA	13	4	17	zfish,fugu,todon
>dre-miR-728	ATACTAAGTACACTACGTTTTTC	0	2	2	zfish,todon
>ZF_nl_176	TATGGCTTTTTATTCCCTATGTG	5	2	7	zfish,todon
>dre-miR-729	CATGGGTATGATACGACCTGGGTT	1	3	4	zfish
>ZF_nl_21	CACGATTGGGAGTAACGACACTG	0	1	1	zfish
>ZF_nl_210	TGAGCGGCTCAGTGAGGTCTCGGATC	2	0	2	zfish
>ZF_nl_224	ACAGGCATGTGTGTGGGTAGAG	1	0	1	zfish
>dre-miR-190b	TGATATGTTTTGATATTCGGTTG	17	24	41	zfish,fugu,todon
>ZF_nl_230	CTGTGCCGAAAGACCTGGAACAAAC	1	0	1	zfish
>ZF_nl_231	ACTGAGCTGCTGATCCCG	0	1	1	zfish,cow,dog,chimp,human,mouse,macaca
>ZF_nl_236	TGGGCACAGCTGGTGGATTGGT	1	0	1	zfish
>ZF_nl_240	TATTGTTATTCTGCTGGTT	1	0	1	zfish
>dre-miR-34c	AGGCAGTGCAGTTAGTTGATTAC	0	2	2	zfish
>ZF_nl_249	TCAGTGCATTACAGAACTTTG	8	7	15	zfish
>dre-miR-722	TTTTTTGCAGAAACGTTTCAGATT	1	1	2	zfish,fugu,todon
>ZF_nl_254	TGAATCTGGTCCCTCTGG	0	1	1	zfish
>dre-miR-730	TCCTCATTGTGCATGCTGTGTGT	10	3	13	zfish,todon
>ZF_nl_263	TCGGGTCGCTAATGACGTC	1	0	1	zfish
>ZF_nl_264	TGTCTGCATCCAGTGTACATTC	1	0	1	zfish
>dre-miR-731	AATGACACGTTTTCTCCCGGATCG	3	1	4	zfish
>ZF_nl_286	GGCTTGTTTTAAGTTGCCTGCG	0	1	1	zfish,fugu,todon,chicken
>ZF_nl_29	CTCTGCACTTCATTAGTG	1	0	1	zfish
>ZF_nl_290	CGGTCCGGGGCGTCAGTGCC	0	1	1	zfish
>dre-miR-732	CTCAAAGCAGAGAACTCTCGGT	0	1	1	zfish
>ZF_nl_298	TGAGGTGGTTTTCTGTAC	0	1	1	zfish
>ZF_nl_300	GCCAAATGCCTCGTCATCTAAT	2	0	2	zfish,cow
>ZF_nl_302	CTCGTACCGTGAGTAATAGTGC	10	10	20	zfish,todon,fugu
>ZF_nl_304	CCTGAGACCCGGGTTTTGTT	1	0	1	zfish
>dre-miR-733	TGCGTTGGTTTAGCTCAGTGGTT	1	1	2	zfish
>dre-miR-15c	AAGCAGCGCGTCATGGTTTTTC	1	0	1	zfish
>ZF_nl_325	TCTACAGTGCATGTGTCTCCAGT	31	2	33	zfish,opossum,fugu,todon
>ZF_nl_33	TGAGTCTGTTTTAGCAGAGTTT	1	0	1	zfish
>dre-miR-734	GTAATGCTGCAGAAATCGTACCG	3	1	4	zfish
>ZF_nl_345	AAAATATCCTGAGCTGTTTTCT	1	0	1	zfish
>ZF_nl_350	ATGCAGTCCATGGGCATATACACT	9	7	16	zfish,fugu,chicken,dog,todon,opossum,chimp,human,macaca,rat,mouse
>ZF_nl_372	TCCCTCCGTCATTGAATTCCTG	1	0	1	zfish
>ZF_nl_376	ACGAGAGCTGGGGTCTTGCTGG	0	1	1	zfish
>dre-miR-735	CTCTCCCACCGCTAAACTTGAC	19	0	19	zfish
>ZF_nl_384	TCCCTGGGAACCTTATTGGG	1	0	1	zfish
>ZF_nl_39	TTTACAGGCTATGCTAATCTAT	1	0	1	zfish,fugu,todon
>dre-miR-736 ¹	GTAAGACGAACAAAAAGTTTT	N.D.	N.D.	N.D.	zfish,fugu
>dre-miR-737	AATCAAAACCTAAAGAAAATA	0	7	7	zfish
>dre-miR-738	GCTACGGCCCGCTCGGGACCTC	4	0	4	zfish
>dre-miR-739	AGGCCGAAGTGAGAGAAGGGTT	0	1	1	zfish,mouse,chicken
>ZF_nl_51	CGGCTCCGCTGATTCTGTCAGT	1	0	1	zfish
>ZF_nl_58	TCAGAAAAGTTACCACAGGGAT	0	1	1	zfish,rat,cow
>ZF_nl_63	CCGCAACACGAAACTGTCTT	0	1	1	zfish,fugu
>dre-miR-499	TTAAGACTTCAGTGATGTTTA	6	15	21	zfish,todon,fugu,chicken
>ZF_nl_68	AGACACTGAGGGGTGTAG	0	1	1	zfish
>dre-miR-723	AAGACATCAATTAATCTGTGCT	3	3	6	zfish
>ZF_nl_9	TGAACACGGTCTCTTTTT	1	0	1	zfish
>ZF_nl_91	TGTCTGTCTCCCGCTTTCT	1	0	1	zfish
>ZF_nl_94	AGGCGATGGGCATCATGAACCTGTT	0	1	1	zfish,dog,fugu,opossum
>dre-miR-489 ²	TGACATCATATGACGGCTGCT	9	1	10	zfish,todon,fugu,chicken
total		216	121	337	

N.D.=no data

¹cloned from human and conserved in zebrafish²predicted in zebrafish based on hsa-miR-489

Table S4. microRNA hairpins assigned for cloned sequences
miRNA hairpin IDs

miRNA hairpin IDs	miRNA sequence
ZF_nl_107 ZF_nl_110 ZF_nl_114 ZF_nl_116 ZF_nl_117 ZF_nl_119	CTTCAGAGCTGAGGTGAG
ZF_nl_108	ACAGTCTCTCGGAGCGCTCG
ZF_nl_11 ZF_nl_34 ZF_nl_55 ZF_nl_56 ZF_nl_65 ZF_nl_66 ZF_nl_235 ZF_nl_269 ZF_nl_292 ZF_nl_317 ZF_nl_324	TCTCCCACTCCGCTGATTCT
dre-miR-724-5p ZF_nl_272 ZF_nl_273	TTAAAGGGAATTTGCGACTGTT
dre-miR-724-3p ZF_nl_272 ZF_nl_273	CAGCCACACCTTCCTTTTAAG
dre-miR-725 ZF_nl_378	TTCAGTCAATTTTCTAGTAGT
dre-miR-726-5p ZF_nl_134	GGAATTCGCTAGTTCTGAACT
dre-miR-726-3p ZF_nl_134	TTCCTACTAGCAGAACTCGG
ZF_nl_125	TAATACTGCCTGGTAATGCCAT
ZF_nl_135	TTGCAGGTTCAATTCCTGTC
ZF_nl_138	TAGCACCATTTGAAATCGGTGCG
ZF_nl_139	TGCTATGCCTCCATATTGCCATT
ZF_nl_149	TGGTTTCAGACATCACTCGGGTAC
ZF_nl_157	TCACACCTACAACTCCCTGGCA
ZF_nl_164 ZF_nl_207 ZF_nl_348 ZF_nl_349 ZF_nl_360 ZF_nl_368 ZF_nl_369	GAACCTGATGGATCTTCTCT
dre-miR-727-5p	TCAGTCTTCAATTCCTCCAGC
dre-miR-727-3p	GTTGAGGCGAGTTGAAGACTTA
dre-miR-728	ATACTAAGTACACTACGTTTTTC
ZF_nl_176	TATGGCTTTTTATTCCTATTGTG
dre-miR-729	CATGGGTATGATACAGCTGGGTT
ZF_nl_21	CACGATGGGATTAACGACACTG
ZF_nl_210_5p ZF_nl_212 ZF_nl_215 ZF_nl_217	TGAGGCGCTCAGTGAGGCTCCGGATC
ZF_nl_210_3p ZF_nl_211 ZF_nl_212 ZF_nl_213 ZF_nl_214 ZF_nl_215 ZF_nl_216 ZF_nl_217 ZF_nl_342	TCGTAACAAGGTTTCCCGT
ZF_nl_224	ACAGGCATGTGTGGGTAGAG
dre-miR-190b	TGATATGTTTGATATTCCGTTG
ZF_nl_230	CTGTGCCGAAAGACCTGGAACAAAC
ZF_nl_231 ZF_nl_234 ZF_nl_362	ACTGAGCTGCTGATCCGC
ZF_nl_236 ZF_nl_379	TGGGCACAGCTGGTGGATTGGT
ZF_nl_240	TATTGTTATTCCTGCTGGTT
dre-miR-34c-5p ZF_nl_243	AGGCAGTGCAGTTAGTTGATTAC
dre-miR-34c-3p ZF_nl_243	AATCACTAACCTCACTACCAGG
ZF_nl_249	TCAGTGCATTACAGAATTTTG
dre-miR-722 ZF_nl_35 ZF_nl_185	TTTTTGCAGAAAGCTTTCAGATT
ZF_nl_254 ZF_nl_344	TGAATCTGGTCCCTCTGG
dre-miR-730-5p	TCCTCATTGTGCATGCTGTGT
dre-miR-730-3p	CACAGCGCTGCAATGTGGAGG
ZF_nl_263	TGGGTGCGTAAATGACGTC
ZF_nl_264	TGTCTGCATCCAGTGTACATTC
dre-miR-731 ZF_nl_330	AATGACAGTTTTCTCCCGGATCG
ZF_nl_286	GGCTTGTTTAAGTTGCCTGCG
ZF_nl_29	CTCTGCACTTCAATTAGTG
ZF_nl_290 ZF_nl_291	CGGTCCGGGGCGTCAGTGCC
dre-miR-732 ZF_nl_299	CTCAAAGCAGAACTCTCGGT
ZF_nl_298	TGAGGTGGTTTTCTGTGTCAC
ZF_nl_300 ZF_nl_336	GCCAAATGCCTCGTCATCTAAT
ZF_nl_302_5p	CATTATTACTTTTTGGTACGCG
ZF_nl_302_3p	CTCGTACCGTGAGTAATAGTGC
ZF_nl_304	CCTGAGACCCGGTTTTGTT
dre-miR-733	TGCGTTGGTTTTAGCTCAGTGGTT
dre-miR-15c	AAGCAGCGCGTCAATGTTTTTC
ZF_nl_325	TCTACAGTGCATGTGCTCCAGT
ZF_nl_33	TGAGTCTGTTTTAGCAGAGTTT
dre-miR-734	GTAATGCTGCAGAACTGACCG
ZF_nl_345	AAAATATCCTGAGCTGTTTTCT
ZF_nl_350_5p	TATGTGCCCTTGGACTACATTTG
ZF_nl_350_3p	ATGCAGTCCATGGGCATATACACT
ZF_nl_372	TCCCTCCGTCAATGAATTCCTG
ZF_nl_376	ACGAGAGCTGGGGTCTTGCTGG
dre-miR-735-5p	GGCTGGTCCGAAAGGCGGTGGTTAGTC
dre-miR-735-3p	CTCTCCACCGCTAAACTTGAC
ZF_nl_384	TCCCTGGGAACCTTATTGGG
ZF_nl_39	TTTACAGGCTATGCTAATCTAT
dre-miR-736 ¹	GTAAGACGAAACAAAAGTTTT
dre-miR-737-5p	GTTTTTTTAGGTTTTGATTTT
dre-miR-737-3p	AATCAAAACCTAAAGAAAATA
dre-miR-738	GCTACGGCCCGCTCGGGACCTC
dre-miR-739	AGGCCGAAGTGGAGAAGGGTT
ZF_nl_51 ZF_nl_89 ZF_nl_90 ZF_nl_343	CGGCTCCGCTGATTCTGTGAGT
ZF_nl_58	TCAGAAAAGTTACCACAGGGAT
ZF_nl_63	CCGCAACACGAAACTGTCTT
dre-miR-499 ZF_nl_233	TTAAGACTTGGAGTGATGTTTA
ZF_nl_68	AGACTGAGGGGTGTAG
dre-miR-723-5p	GACAGTTTTAATATGATTTACTTT
dre-miR-723-3p	AAGACATCAATTAATCTGTGCT
ZF_nl_9	TGAACACGGTCTCTTTTT
ZF_nl_91	TGTCTGTCTCCCGCTTTCT
ZF_nl_94 ZF_nl_363	AGGCGATGGGCATCATGAACCTGTT
miR-489 ²	TGACATCATATGTACGGCTGCT

¹cloned from human and conserved in zebrafish

²predicted in zebrafish based on hsa-miR-489

Table S5. miRNA and star sequences of known miRNAs

miRNA	sequence	number of clones
let-7b	TGAGGTAGTAGGTTGTGTGGT	109
let-7b*	CTATACAACCTACTGCCTTCCC	2
miR-1	TGGAATGTAAAGAAGTATGTATT	39
miR-1*	ACATACTTCTTTATGTGCCCAT	1
miR-100*	CAAGCTCGTGTCTATAGGTATG	2
miR-100	AACCCGTAGATCCGAACTTGT	337
miR-103	AGCAGCATTGTACAGGGCTATGA	17
miR-103*	AGCCTCTTACGGTGTGCCTTG	2
miR-107	AGCAGCATTGTACAGGGCTATCA	10
miR-107*	AGCTTCTTACAGTGTGTCTTG	2
miR-10b	TACCCGTAGAACCGAATTGTG	42
miR-10b*	CAAATACGTCTCTACAGGAAT	1
miR-124	TAAGGCACGCGGTGAATGCC	123
miR-124*	TGTGTTACAGTGGACCTTGATT	9
miR-125a	TCCCTGAGACCCTAACCTGTGAG	848
miR-125a*	ACAGGTGAGGTCTCAGGAAC	1
miR-125b	TCCCTGAGACCCTAACTTGTGA	2553
miR-125b*	ACGGGTTAGGTTCTTGGGAGCT	5
miR-126*	CATTACTTTTGGTACGCG	2
miR-126	TCGTACCGTGAGTAATAATGC	42
miR-128	TCACAGTGAACCGTCTCTTTT	458
miR-128*	CGGGCCGTGGCACTGTATGAG	1
miR-129*	AAGCCCTTACCCAAAAAGTAT	205
miR-129	CTTTTGGCGTCTGGGCTTGC	62
miR-130b*	ACTCTTCCCTGTTGCACTACTG	10
miR-130b	CAGTGAATAATGAAAGGGCAT	8
miR-132	TAACAGTCTACAGCCATGGTCC	169
miR-132*	ACCGTGGCTTTAGATTGTACT	2
miR-133a*	AGCTGGTAAAATGGAACCAAAT	1
miR-133a	TTTGGTCCCCTCAACCAGCTGT	136
miR-135	TATGGCTTCTATTCCTATGTG	91
miR-135*	ACATAGGGTTCAAAGCCATTGG	1
miR-135b*	TATAGGGATGGAAGCCATGCAG	5
miR-135b	TATGGCTTTTATTCCTATCTGA	28
miR-137	TTATTGCTAAGAATACGCGTAG	74
miR-137*	ACGGGTATTCTTGGGTGGATAAT	2
miR-138	AGCTGGTGTGTGAATCAGGCCG	401
miR-138*	GCTATTCACAACACCAGGGT	5
miR-140	CAGTGGTTTACCCTATGGT	26
miR-140*	TACCACAGGGTAGAACCCACGG	7
miR-142a-3p	TGTAGTGTTCCTACTTTATGG	6
miR-142a-5p	CATAAAGTAGAAAGCACTACT	2
miR-142b-3p	TGTAGTGTTCCTACTTTATGG	6
miR-142b-5p	CATAAAGTAGAGCACTACTA	1
miR-143*	GGTGCAGTGTCTCATCTCTGG	2
miR-143	TGAGATGAAGCACTGTAGCTC	12
miR-144*	GGATATCATCGTATACTGTAAGTT	1
miR-144	TACAGTATAGATGATGTACT	5
miR-145	GTCCAGTTTTCCAGGAATCCCTT	24
miR-145*	GGATTCTGGAATACTGTTCT	4
miR-148	TCAGTGCATTACAGAACTTTGT	18
miR-148*	AAGTCTGTGATACACTCCGAC	1
miR-153b	TTGCATAGTACAAAAATGAGC	18
miR-153b*	GTCATTTTGTGGT TTGCAGCT	2
miR-16c	TAGCAGCATGTAATATTGGAGT	7
miR-16c*	GCCTCCAATATTGCTCGTGCTG	3
miR-17a	CAAAGTGCTTACAGTGCAGGT	27
miR-17a*	ACTGCAGTGGAGGCACTTCAAG	1
miR-181a	AACATTCAACGCTGTCGGTGAGTT	391
miR-181a*	ACCATCGACCGTTGACTGTACC	4
miR-181b*	ACTCACTGATCAATGAATGC	2
miR-181b	AACATTCAATTGCTGTCGGTGGTT	148
miR-181c	CACATTCATTGCTGTCGGTGGG	26
miR-181c*	CTCGCCGGACAATGAATGAG	1
miR-182	TTTGGCAATGGTAGAACTCAC	30
miR-182*	TGGTTCTAGACTTGCCAACTA	3
miR-183	TATGGCACTGGTAGAATTCAC	35
miR-183*	TGAATTACCAAAGGGCCAT	1
miR-184	TGGACGGAGAAGTATAAGGG	30
miR-184*	TCCTTATCACTTTTCCAGCCCAG	1
miR-187	TCGTGTCTTGTGTGACGCCAG	48
miR-187*	GGCTGCAACACAGGACATGG	2
miR-18a*	ACTGCCCTAAGTGCTCCTCTG	1
miR-18a	TAAGGTGCATCTAGTGCAGATAG	17

miR-18c	TAAGGTGCATCTTGTGTAGTTAG	5
miR-18c*	TACTGCGCTAGATGTTCCTTTTG	2
miR-192	ATGACCTATGAATTGACAGCCA	49
miR-192*	CCTGTCAGTTCGTAGGCCACTG	4
miR-193b*	GACTTTGGGGGCGAGATG	1
miR-193b	AACTGGCCCGAAAAGTCCCGCT	6
miR-194b*	TGGAGAAGCTGTTACCTG	1
miR-194b	TGTAACAGCCGCTCCATGTGGA	1
miR-196a	TAGGTAGTTTCATGTTGTGGG	4
miR-196a*	CTGCAACGTGAAAAGTCTTAA	1
miR-199	CCCAGTGTTCAGACTACCTGTCA	197
miR-199*	ACAGTAGTCTGCACATTGGTT	42
miR-19d	TGTGCAAAACCCATGCAAACTGA	46
miR-19d*	AGCTTTGCGGGGTGGCAGTCAGC	1
miR-203b	TGAAATGTTCCAGGACCACTTG	194
miR-203b*	AGTGGTTCTCAACAGTTCAACAGT	19
miR-20a	TAAAGTGCTTATAGTGCAGGTAG	43
miR-20a*	ACTGCAGTGTGAGCACTTGAAG	5
miR-210	CTGTGCGTGTGACAGCGGCT	74
miR-210*	AGCCACTGACTAACGCACATTG	6
miR-212*	ACCTGGCTCTAGACTGCTTACT	35
miR-212	TAACAGTCTACAGTCATGGCTAC	8
miR-214	ACAGCAGGCACAGACAGGCAG	3
miR-214*	GCCTGTCTACACTTGCTGTGC	3
miR-216a	TAATCTCAGCTGGCAACTGTGA	27
miR-216a*	CACAGGCGCTGTGGGGTTCTG	1
miR-218a*	ATGGTTCCGTC AAGCACCAGG	1
miR-218a	TTGTGCTTGATCTAACCATGTG	67
miR-219	TGATTGTCCAAACGCAATCTTG	333
miR-219*	GGAGTTGTGGATGGACATCATG	2
miR-222	AGCTACATCTGGCTACTGGGTCTC	181
miR-222*	TGCTCAGTAGTCAGTGTAGAT	1
miR-22a*	AGTTCCTCACTGGCAAGCTTT	9
miR-22a	AAAGTGCCAGCTGAAGAAGCTG	151
miR-22b*	CGTTCCTCACTGGCTAGCTTT	1
miR-22b	AAGCTGCCAGTTGAAGAGCTG	4
miR-24	TGGCTCAGTTCAGCAGGAACAGG	40
miR-24*	GTGCCCTTCTGAGCTGATATCAG	1
miR-26a*	CCTATTCATGATTACTTGCACT	1
miR-26a	TTCAAGTAATCCAGGATAGGCT	72
miR-27d	TTCACAGTGGCTAAGTTCTTC	3
miR-27d*	CAGAGCTTGGCTGATTGGTG	1
miR-29b	TAGCACCATTTGAAATCAGTGT	3
miR-29b*	GCTGAATTCAGATGGTGCCATAG	1
miR-30c	TGTAAACATCCTACACTCTCAGCT	85
miR-30c*	CCGGGAGTGGGATGTTTTG	1
miR-30e*	CTTTCAGTCGGATGTTGCAGC	16
miR-30e	TGTAAACATCCTTGACTGGAAGCT	29
miR-455*	ATGCAGTCCATGGGCATATACAC	8
miR-455	TATGTGCCCTTGACTACATCG	4
miR-460-3p	CACAGCGCATACAATGTGGATG	1
miR-460-5p	CCTGCATTGTACACACTGTGCG	2
miR-9	TCTTTGGTTATCTAGCTGTATG	1121
miR-9*	TAAAGCTAGATAACCGAAAGT	157
miR-92b*	AGGTGTGGGATGTTGTGCAGTGT	1
miR-92b	TATTGCACTCGTCCCGCCTCC	6

Table S6. miRNA and star sequences of new miRNAs

miRNA	sequence	brain	larvae	total clones	tested	detection	miRNA
>dre-miR-724-3p	CAGCCACACCTTCCTTTAAG	1	0	1	yes	no	
>dre-miR-724-5p	TTAAAGGGAATTTGCGACTGTT	20	0	20	yes	northern	5p
>dre-miR-726-3p	TTCACACTAGCAGAACTCGG	3	0	3	yes	northern	3p
>dre-miR-726-5p	GGAATCCGCTAGTTCTGAAGT	1	1	2	yes	no	
>dre-miR-727-3p	GTTGAGGCGAGTTGAAGACTTA	8	4	12	yes	northern	3p
>dre-miR-727-5p	TCAGTCTCAATTCCTCCAGC	5	0	5	yes	northern	
>ZF_nl_210_3p	TCGTAACAAGGTTCCGT	1	0	1	no		
>ZF_nl_210_5p	TGAGCGGCTCAGTGAGGTCCTCGGATC	1	0	1	no		5p
>dre-miR-34c-3p	AATCACTAACCTCACTACCAGG	0	1	1	yes	no	
>dre-miR-34c-5p	AGGCAGTGCAGTTAGTTGATTAC	0	1	1	yes	northern, in situ	5p
>dre-miR-730-3p	CACAGCGCCTGCAATGTGGAGG	1	0	1	yes	no	
>dre-miR-730-5p	TCCTCATTGTGCATGCTGTGTGT	9	3	12	yes	northern	5p
>ZF_nl_302_3p	CTCGTACCGTGAGTAATAGTGC	8	10	18	no		3p
>ZF_nl_302_5p	CATTATTACTTTTGGTACGCG	2	0	2	no		
>ZF_nl_350_3p	ATGCAGTCCATGGGCATATACACT	3	6	9	no		3p
>ZF_nl_350_5p	TATGTGCCCTTGGACTACATTGT	6	1	7	no		
>dre-miR-735-3p	CTCTCCCACCGCTAAACTTGAC	1	0	1	yes	northern, in situ	3p
>dre-miR-735-5p	GGCTGGTCCGAAGCGGTGGGTTAGTC	18	0	18	no		
>dre-miR-723-3p	AAGACATCAATTAATCTGTGCT	3	2	5	yes	northern	3p
>dre-miR-723-5p	GACAGTTTTAAATGATGTTACTTT	0	1	1	yes	northern	
>dre-miR-737-3p	AATCAAACCTAAAGAAAATA	0	1	1	yes	northern	3p
>dre-miR-737-5p	GTTTTTTAGGTTTTGATTTT	0	6	6	no		

Table S7. Overview of miRNA cloning and expression

	known	new	
miRNAs in registry	168		
miRNA genes in registry	369		
miRNAs cloned	139	66	
star sequences cloned	65	11	
miRNA genes	255	116	
analyzed miRNAs	32	35	
analyzed star sequences	0	6	
miRNAs detected in situ	24	4	
star sequences detected in situ	0	0	
miRNAs detected on Northern	30	23	
star sequences detected on Northern	0	2	
	brain	larvae	total
known miRNAs cloned	136	129	139
of which found in both libraries			126
new miRNAs cloned	47	38	66
of which found in both libraries			19