

Supplemental Files for:

The microRNA expression profile of mouse Müller glia *in vivo* and *in vitro*.

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Supplemental Figures 1-3

Supplemental Tables 1-6

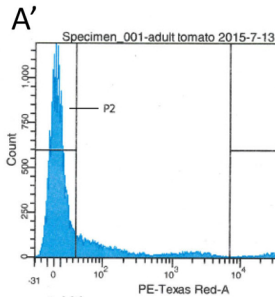
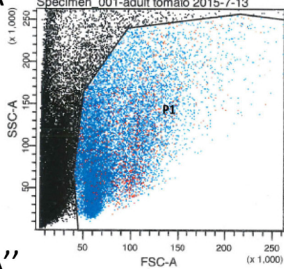
Supplement Figure 1. Purity of FAC-sorted cells from adult R1bp-CreER: Stop^{f/f}-tdTomato mice. A-A''': Gating for FACS and proportions of sorted cells for adult retinas. A: all cells in gate P1 (blue dots) have been sorted based on their size (forward scattered, FSC) and fluorescence/granularity (side scattered, SSC). Very small cells and debris (black dots) have been excluded. A': tdTomato⁺ cells were found in gate P3 (brightest red fluorescence), while the vast majority of tdTomato⁻ cells were found in gate P2 (no red fluorescence). The percentage of the P2 and P3 events of either P1 or total events or are shown in A''. A''' shows the fraction of the sorted tdTomato⁺ cells in the SSC-A and FSC-A scheme. B, D: Immunofluorescent labeling with antibodies against tdTomato, Sox9 (B), Sox2 (D) and DAPI nuclear staining of the MG-enriched tdTomato⁺ FAC-sorted fractions, plated on a feeder layer of young glia, after 8 days *in vitro* (DIV). C, E: Percentage of tdTomato⁺Sox9⁺ (C) or tdTomato⁺Sox2⁺ (E) cells found in the P3-gated (tdTomato⁺ MG) and P2-gated (tdTomato⁻ neuronal) FAC-sorted fractions of 2 mice. F-H: Immunofluorescent labeling with antibodies against tdTomato and Sox2 and DAPI nuclear staining of a periphery of a retinal cross section (adult). I: Retinal cross section of a R1bp-CreER-tdTomato: Nrl-GFP mouse. J-K: Live images of plated tdTomato⁺ Nrl-GFP⁻ cells (MG, C, C') and tdTomato⁻ Nrl-GFP⁺ cells (neurons/rods, D, D') after FACS. ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layers, RPE: retinal pigment epithelium, CE: ciliary epithelium.

Supplement Figure 2. Purity of FAC-sorted cells from P11 R1bp-CreER: Stop^{f/f}-tdTomato mice. A-A'': Gating for FACS and proportions of sorted cells from P11 retinas: all cells in gate P1 (blue dots) have been sorted based on their size (forward scattered, FSC) and fluorescence/granularity (side scattered, SSC). Very small cells and debris (black dots) have been excluded. tdTomato⁺ cells were found in gate P3 (brightest fluorescence), while the vast majority of tdTomato⁻ cells were found in gate P2 (no fluorescence, A'). The

percentage of the P2- and P3-gated events of either P1 or total events or are shown in A''. A''' shows the fraction of the sorted tdTomato⁺ cells in the SSC and FSC-A scheme. **B-E:** Live images of cultured P11 FAC-sorted tdTomato⁺ (MG, B, B', D, D') and tdTomato⁻ cells (neurons, C, C', E, E') after the sort (B, C) and after 5 DIV (D, E). **F:** Number of tdTomato⁺ cells per field found in the tdTomato⁺ (MG) and tdTomato⁻ (neurons) cultured fractions 5 days after FACS. **G, H:** Immunofluorescent labeling of tdTomato⁺ cells with Id1 and DAPI (G) and Sox2 and DAPI (H) to confirm glial identity (from 2 independence sorts, total n = 6 mice). **I:** Percentage of tdTomato⁺Id1⁺ cells of total Id1⁺ or DAPI⁺ cells. **J:** Percentage of tdTomato⁺Sox2⁺ cells of total Sox2⁺ or DAPI⁺ cells. Significant differences are indicated, **: p > 0.01, Student's t-test for independent samples and Levene's test for equality of variances.

Supplement Figure 3: Most MG miRNAs decline in the early culture period. **A.** Scatter plot of Log2 counts of the miRNAs expressed in P11 MG and 8 DIV MG. The red dots represent miRNAs expressed higher in P11 MG *in vivo* than in 8 DIV MG, the black dots miRNAs higher expressed in 8 DIV MG than P11 MG *in vivo*. **B, C:** Bar graphs of 4 mGliomiRs which expression levels decreased after 8 DIV as compared to P11 MG *in vivo* (B) and of 3 mGliomiRs which expression levels increased after 8 DIV as compared to P11 MG *in vivo* (C). **D, E:** Bar graphs of 10 shared miRs in which expression levels decreased after 8 DIV as compared to P11 MG *in vivo* (D) and of 3 shared miRs in which expression levels increased after 8 DIV as compared to P11 MG *in vivo* (E). Bar graphs cutoff 20,000 counts.

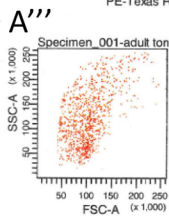
A Specimen_001-adult tomato 2015-7-13



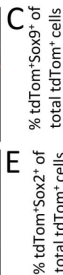
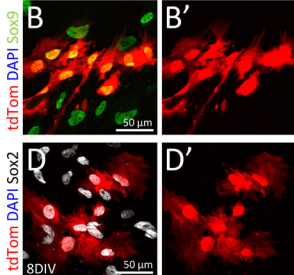
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Tube: adult tomato 2015-7-13

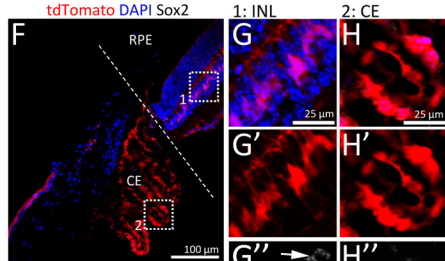
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All Events	63,656	####	100.0
P1	20,000	31.4	31.4
P2	14,602	73.0	22.9
P3	934	4.7	1.5
P4			



positives (Müller glia)

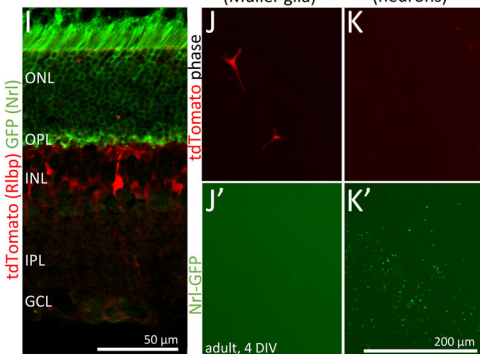


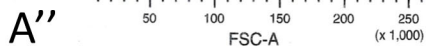
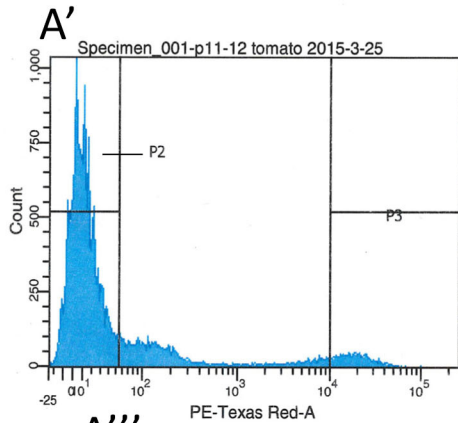
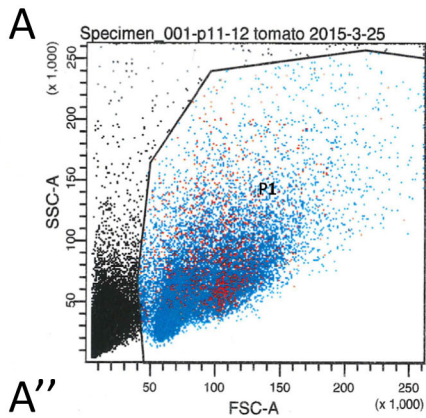
tdTomato DAPI Sox2



positives (Müller glia)

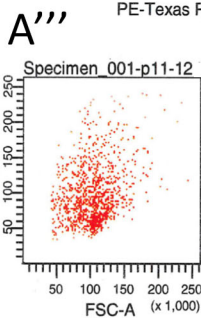
negatives (neurons)





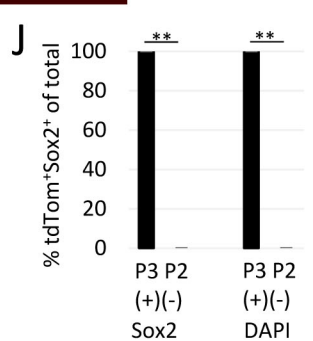
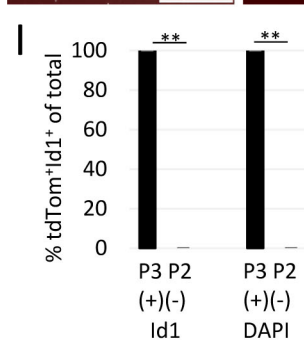
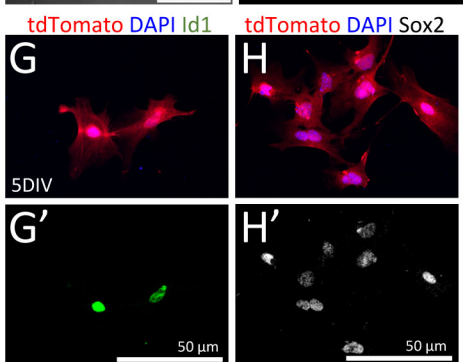
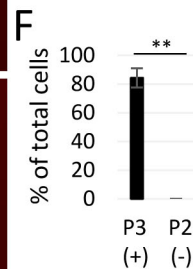
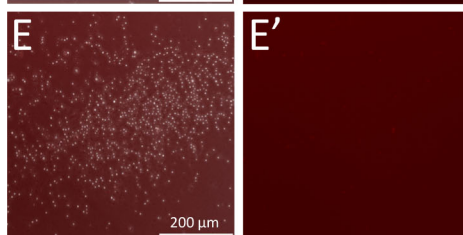
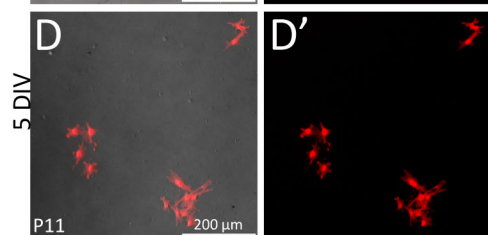
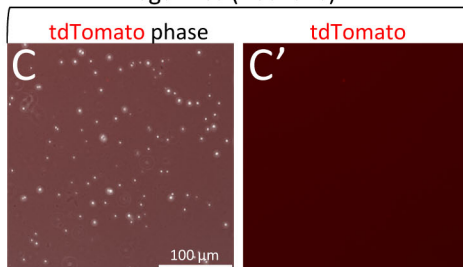
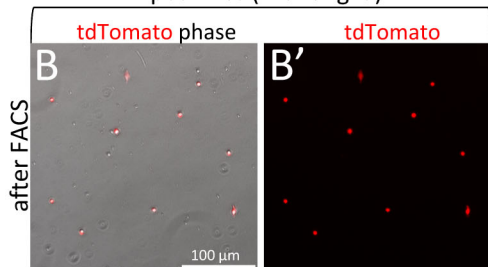
Tube: p11-12 tomato 2015-3-25

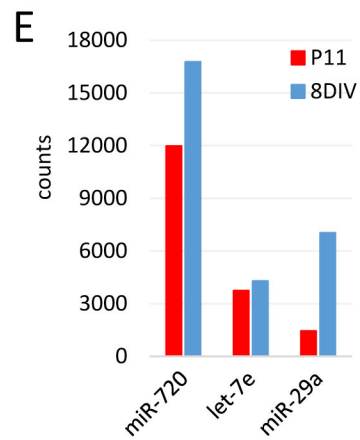
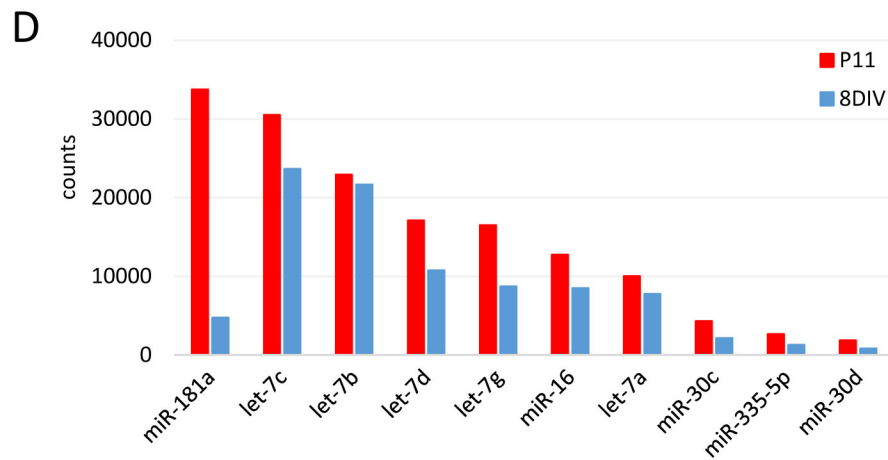
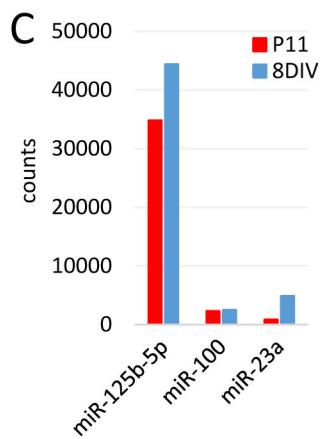
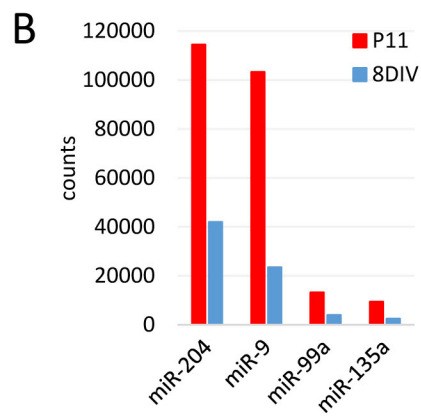
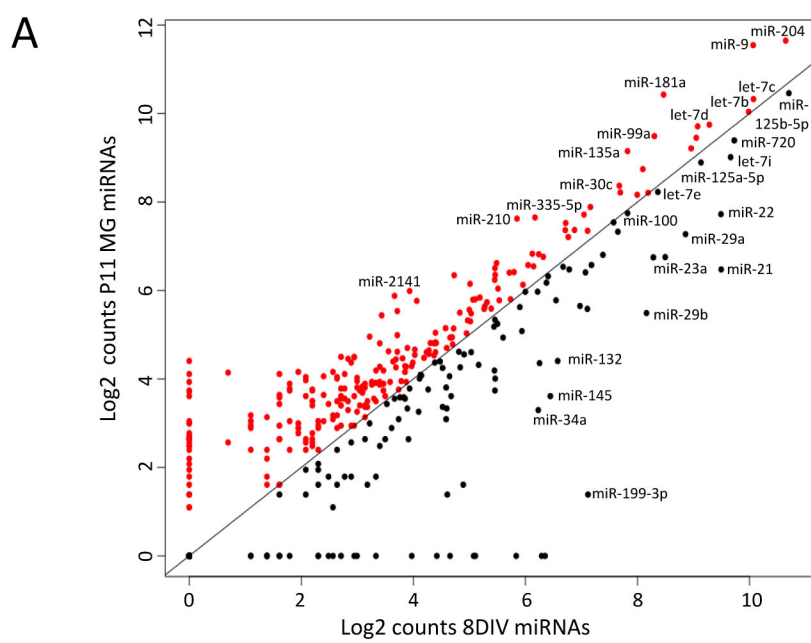
Population	#Events	%Parent	%Total
All Events	56,168	####	100.0
P1	20,000	35.6	35.6
P2	15,156	75.8	27.0
P3	1,095	5.5	1.9
P4			
P5			



positives (Müller glia)

negatives (neurons)





Supplemental Table 1: FACS data - input and purity.

sample	# of sorts	# of retinas	# of cells in suspension "positives"	postsort purity "positives"	% of total P1 events	% of total events	# of cells in suspension "negatives"	postsort purity "negatives"
adult	6	40	4444839	96%	5%	1.8%	56.5 x 10 ⁶	0%
P11/12	5	32	3378620	95%	4%	2.1%	29.7 x 10 ⁶	0%

Supplemental Table 2: Expression levels of the highly expressed 23 miRNAs (cutoff 2000 counts) in adult Müller glia compared to adult neurons and young Müller glia (mGliomiRs are bold).

miRNA	Müller glia (MG) adult (counts)	Neurons adult (counts)	adult MG vs. neurons (fold)	adult neurons vs. MG (fold)	Müller glia P11/12 (counts)	% increase P11 to adult
mmu-miR-204	213460	11740	18	0	62946	71
mmu-miR-125b-5p	81882	9291	9	0	19159	77
mmu-miR-9	56346	8338	7	0	56806	-1
mmu-miR-181a	36482	69753	1	2	18556	49
mmu-let-7c	19558	11497	2	1	16782	14
mmu-miR-720	17859	15686	1	1	6585	63
mmu-miR-99a	14804	2394	6	0	7259	51
mmu-let-7b	13480	12275	1	1	12600	7
mmu-let-7g	11687	41497	0	4	9065	22
mmu-miR-30c	10425	22609	0	2	2367	77
mmu-let-7d	9932	26367	0	3	9405	5
mmu-miR-135a	9588	1148	8	0	5173	46
mmu-miR-29a	6511	4391	1	1	792	88
mmu-let-7a	6073	11665	1	2	5513	9
mmu-miR-16	5424	27745	0	5	7007	-29
mmu-miR-125a-5p	4213	7019	1	2	4001	5
mmu-miR-100	4203	2	2102	0	1272	70
mmu-miR-1944	3753	32472	0	9	3448	8
mmu-miR-22	3672	7435	0	2	1244	66
mmu-miR-30d	3493	3976	1	1	1019	71
mmu-let-7e	2831	5004	1	2	2057	27
mmu-miR-335-5p	2487	2568	1	1	1464	41
mmu-miR-23a	2485	374	7	0	472	81

Supplemental Table 3: Expression levels of the highly expressed 46 neuronal miRNAs (cutoff 2000 counts) in adult neurons compared to adult Müller glia and young neurons (17 miRNAs with expression levels in MG <20% are bold).

miRNA	Neurons adult (counts)	MG glia adult (counts)	MG fold vs neurons (adult)	Neurons fold vs MG (adult)	% expression in adult MG	Neurons young (counts)	% increase from P11 to adult in neurons
mmu-miR-124	308141	1011	0	305	0.3	39060	87
mmu-miR-183	101370	271	0	374	0.3	12473	88
mmu-miR-96	87840	212	0	414	0.2	16891	81
mmu-miR-181a	69753	36482	1	2	52	40225	42
mmu-let-7g	41497	11687	0	4	28	15320	63
mmu-miR-1944	32472	3753	0	9	12	2813	91
mmu-miR-16	27745	5424	0	5	20	7497	73
mmu-let-7d	26367	9932	0	3	38	9005	66
mmu-miR-30c	22609	10425	0	2	46	4928	78
mmu-miR-29c	19530	1600	0	12	8	949	95
mmu-miR-720	15686	17859	1	1	114	4077	74
mmu-let-7b	12275	13480	1	1	110	5918	52
mmu-miR-204	11740	213460	18	0	1818	7275	38
mmu-let-7a	11665	6073	1	2	52	5499	53
mmu-let-7c	11497	19558	2	1	170	7258	37
mmu-miR-211	11181	250	0	45	2	2732	76
mmu-miR-125b-5p	9291	81882	9	0	881	3047	67
mmu-miR-129-3p	8723	236	0	37	3	357	96
mmu-miR-9	8338	56346	7	0	676	15876	-90
mmu-miR-25	8203	1761	0	5	21	5420	34
mmu-miR-22	7435	3672	0	2	49	1343	82
mmu-miR-342-3p	7103	1827	0	4	26	1606	77
mmu-miR-125a-5p	7019	4213	1	2	60	5689	19

mmu-let-7f	5850	1718	0	3	29	2539	57
mmu-miR-151-5p	5273	942	0	6	18	748	86
mmu-miR-1186	5130	175	0	29	3	638	88
mmu-let-7e	5004	2831	1	2	57	1865	63
mmu-miR-182	4959	1	0	4959	0.02	1039	79
mmu-miR-451	4684	1	0	4684	0.02	340	93
mmu-miR-29a	4391	6511	1	1	148	127	97
mmu-miR-30d	3976	3493	1	1	88	835	79
mmu-miR-210	3767	566	0	7	15	858	77
mmu-let-7i	3516	1820	1	2	52	3958	-13
mmu-miR-500	3073	1	0	3073	0.03	534	83
mmu-miR-15b	3001	697	0	4	23	2815	6
mmu-miR-1224	2828	129	0	22	5	338	88
mmu-miR-301a	2750	534	0	5	19	895	67
mmu-miR-1937a+b	2681	817	0	3	30	361	87
mmu-miR-15a	2571	376	0	7	15	2011	22
mmu-miR-335-5p	2568	2487	1	1	97	773	70
mmu-miR-191	2538	671	0	4	26	337	87
mmu-miR-99a	2394	14804	6	0	618	1691	29
mmu-miR-26b	2320	466	0	5	20	346	85
mmu-miR-30a	2320	1612	1	1	69	318	86
mmu-miR-132	2018	88	0	23	4	257	87
mmu-miR-148a	2015	1598	1	1	79	685	66

Supplemental Table 4: genotyping primers

Gene name	Forward sequence (5' to 3')	Reverse sequence (3' to 5')
<i>Rbp1Cre transgene</i>	CAAGTGTGAGAGACAGCATTG	TCCTTAGCGCCGTAAATCAA
<i>tdTomato wildtype</i>	AAG GGA GCT GCA GTG GAG TA	CCG AAA ATC TGT GGG AAG TC
<i>tdTomato mutant</i>	CTG TTC CTG TAC GGC ATG G	GGC ATT AAA GCA GCG TAT CC
<i>Nrl-GFP</i>	TGG AAA CCA AGC TCA CAA CA	GAA CTT CAG GGT CAG CTT GC

Supplemental Table 5: primary antibodies

antibody	concentration	Company, Catalog #
rat anti RFP (tdTomato)	1:500	Antibodies online, ABIN334653
mouse anti glutamine synthetase (GS)	1:200	Millipore, MAB 302
rabbit anti Sox9	1:1000	Millipore, AB5535
goat anti Sox2 (Y-17)	1:100	Santa Cruz, sc-17320
Rabbit anti Id1	1:1000	BioCheck, BCH-1/37-2
chicken anti GFP	1: 500	Abcam, ab13970

Supplemental Table 6: RT-qPCR primers for miRNAs

miRNA	Forward sequence (5' to 3')	Reverse sequence (3' to 5')	Stem loop RT primer (3' to 5')
miR-204	CGC GCT TCC CTT TGT CAT CC	TCG GAG CGC CAA GTG TAC AG	TGA GCA GCG CTC GGA GCG CCA AGT GTA CAG GCG CTG CTC AAG GCA TAG
miR-125b-5p	CCG CTC CCT GAG ACC CTA A	CGA AGG AAC TTG GGA TAT GAC G	ATA GGG GCG CGA AGG AAC TTG GGA TAT GAC GCG CCC CTA TTC ACA AGT
miR-9	GCC GGT CTT TGG TTA TCT AGC	TTT CCT CGA CCG CAC CAC TC	AAA TCG CAG CTT TCC TCG ACC GCA CCA CTC GCT GCG ATT TTC ATA CAG
miR-181b	GCG CCA ACA TTC ATT GCT GTC	GAT CAA GGG GCA GCA CGT AG	GGC TAG CAG CGA TCA AGG GGC AGC ACG TAG GCT GCT AGC CAC CCA CCG
miR-9	GCC GGT CTT TGG TTA TCT AGC	TTT CCT CGA CCG CAC CAC TC	AAA TCG CAG CTT TCC TCG ACC GCA CCA CTC GCT GCG ATT TTC ATA CAG
let-7c	CGG CCT TGA GGT AGT AGG TT	GCT TCG CTC TTA TTT CCT GAT GG	CGC TAC AGG CGC TTC GCT CTT ATT TCC TGA TGG GCG CGA TGT CAA CCA TAC
5s	GCC ATA CCA CCC TGA ACG	TGC AGG GTC CGA GGT ATT CG	ACT GCT GCG CTG CAG GGT CCG AGG TAT TCG GCG CAG CAG TAA AGC CTA