

Table S1. Michaelis-Menten parameters for target cleavage by *Drosophila* Ago2-RISC (mean \pm S.D.; $n \geq 3$ except $n = 2$ for mismatch positions marked with an asterisk).

<i>let-7</i> -programmed Ago2-RISC							
Mismatch position	Mismatched target		Fully complementary target		$\frac{\text{relative } k_{cat}}{\text{relative } K_M}$	Single-turnover relative v_0 , $\frac{\text{mismatched}}{\text{fully complementary}}$	$\frac{\text{relative } k_{cat}}{\text{relative } v_0}$
	K_M (nM)	V_{max} (nMs ⁻¹)	K_M (nM)	V_{max} (nMs ⁻¹)			
g1 (C:A)	17 \pm 8	0.22 \pm 0.09	17 \pm 5	0.23 \pm 0.08	0.99 \pm 0.34	NA	NA
g1-g2 (CU:AC)	42 \pm 5	0.80 \pm 0.10	26 \pm 12	0.63 \pm 0.20	0.72 \pm 0.37	NA	NA
g2-g3 (UC:CU)	250 \pm 10	0.48 \pm 0.12	23 \pm 10	0.24 \pm 0.05	0.17 \pm 0.08	NA	NA
g3-g4 (UC:UC)	320 \pm 40	0.35 \pm 0.24	31 \pm 8	0.21 \pm 0.14	0.15 \pm 0.06	NA	NA
g4-g5 (UC:CC)	2400 \pm 300	0.63 \pm 0.15	32 \pm 11	0.23 \pm 0.04	0.035 \pm 0.015	NA	NA
g5-g6 (UC:CA)	1100 \pm 100	0.27 \pm 0.02	51 \pm 20	0.28 \pm 0.02	0.041 \pm 0.012	NA	NA
g6-g7 (CU:AU)	390 \pm 70	0.55 \pm 0.03	15 \pm 3	0.18 \pm 0.03	0.11 \pm 0.03	NA	NA
g7-g8 (UC:UC)	88 \pm 60	0.023 \pm 0.008	24 \pm 8	0.27 \pm 0.08	0.024 \pm 0.009	0.0035 \pm 0.0011	23 \pm 7
g8 (U:C)	74 \pm 15	0.43 \pm 0.10	49 \pm 1	0.47 \pm 0.05	0.59 \pm 0.14	0.61 \pm 0.06	1.5 \pm 0.3
g9 (C:A)	30 \pm 10	0.15 \pm 0.04	16 \pm 5	0.079 \pm 0.016	0.84 \pm 0.64	0.80 \pm 0.01	2.4 \pm 0.7
g8-g9 (UC:CA)	6.0 \pm 1.6	0.0018 \pm 0.0006	15 \pm 2	0.23 \pm 0.03	0.025 \pm 0.015	0.0052 \pm 0.0017	2.1 \pm 1.3
g9-g10 (CU:AU)	17 \pm 6	0.021 \pm 0.005	20 \pm 7	0.11 \pm 0.04	0.23 \pm 0.08	0.052 \pm 0.008	3.8 \pm 1.1
g10-g11 (UC:UC)	8.3 \pm 1.2	0.0069 \pm 0.0003	8 \pm 1	0.11 \pm 0.00	0.062 \pm 0.005	0.016 \pm 0.001	4.0 \pm 0.4
g12-g13 (UC:CA)	34 \pm 18	0.017 \pm 0.007	20 \pm 4	0.28 \pm 0.06	0.037 \pm 0.014	0.0068 \pm 0.0013	9.2 \pm 2.6
g13-g14 (CC:AA)	68 \pm 23	0.23 \pm 0.05	19 \pm 3	0.14 \pm 0.02	0.47 \pm 0.16	0.25 \pm 0.02	6.9 \pm 1.0
g15 (U:C)	12 \pm 4	0.11 \pm 0.02	7 \pm 1	0.17 \pm 0.03	0.36 \pm 0.09	NA	NA
g16 (G:G)*	13 \pm 7	0.24 \pm 0.06	4.3 \pm 1.6	0.15 \pm 0.03	0.60 \pm 0.11	NA	NA
g16 (G:A)*	14 \pm 1	0.21 \pm 0.01	4.3 \pm 1.6	0.15 \pm 0.03	0.38 \pm 0.16	NA	NA
g16 (C:A)	7.0 \pm 2.8	0.15 \pm 0.01	3.4 \pm 1.7	0.12 \pm 0.02	0.54 \pm 0.12	NA	NA
g14-g15 (CU:AC)	36 \pm 10	0.018 \pm 0.002	10 \pm 6	0.10 \pm 0.01	0.042 \pm 0.020	0.0071 \pm 0.0011	25 \pm 4
g15-g16 (UC:CA)	20 \pm 9	0.011 \pm 0.004	6.7 \pm 6.1	0.11 \pm 0.04	0.027 \pm 0.016	0.0045 \pm 0.0010	24 \pm 9

g15-g16 (AG:CA)	20 ± 14	0.064 ± 0.024	5.4 ± 1.2	0.17 ± 0.02	0.11 ± 0.07	0.033 ± 0.000	12 ± 5
g17 (U:U)	23 ± 4	0.25 ± 0.08	12 ± 4	0.19 ± 0.06	0.67 ± 0.32	NA	NA
g16-g17 (CU:AU)	22 ± 10	0.049 ± 0.006	6.9 ± 5.1	0.15 ± 0.01	0.086 ± 0.030	0.031 ± 0.001	10 ± 2
g17-g18 (UC:UA)	19 ± 8	0.053 ± 0.019	12 ± 6	0.11 ± 0.03	0.27 ± 0.06	0.037 ± 0.007	12 ± 3
g18-g19 (CU:AU)	33 ± 16	0.17 ± 0.03	22 ± 12	0.21 ± 0.05	0.45 ± 0.40	NA	NA
g19-g20 (UC:UC)	19 ± 9	0.45 ± 0.26	13 ± 8	0.35 ± 0.20	0.79 ± 0.32	0.73 ± 0.03	1.8 ± 0.2
g20-g21 (UC:CA)	29 ± 1	0.52 ± 0.03	16 ± 3	0.33 ± 0.05	0.82 ± 0.22	NA	NA
g17-g21 (UCUCC:UAUCA)	15 ± 7	0.053 ± 0.016	11 ± 5	0.10 ± 0.03	0.34 ± 0.19	NA	NA
Parental <i>let-7</i>	NA	NA	25 ± 6	0.19 ± 0.04	NA	NA	NA

<i>Renilla reniformis</i> luciferase siRNA-programmed Ago2-RISC							
g4 (G:A)	1700 ± 800	0.27 ± 0.11	55 ± 20	0.39 ± 0.08	0.021 ± 0.019	0.021 ± 0.007	30 ± 21
g8g9 (UA:CG)	31 ± 15	0.068 ± 0.029	55 ± 20	0.39 ± 0.08	0.31 ± 0.38	0.023 ± 0.015	6.8 ± 6.9
g15g16 (AU:GC)	660 ± 430	0.058 ± 0.023	55 ± 20	0.39 ± 0.08	0.011 ± 0.015	0.0021 ± 0.0013	62 ± 65

Table S2A, related to Figure 1. Synthetic siRNAs used in this study.

Description	Sequence (seed, guide, passenger, altered sequences)
<i>let-7</i> guide strand sequence. Completely complementary to perfect target	pUGAGGUAGUAGGUUGUAUAGU
Passenger strand to <i>let-7</i> guide and creates frayed siRNA with UU mismatch at g1 of preceding guide strand	pUAUACAACCUACUACCUCCUU
Passenger strand to <i>let-7</i> guide and creates frayed siRNA with UC mismatch at g1 of preceding guide strand	pUAUACAACCUACUACCUUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g1g2:t1t2 when paired to <i>let-7</i> perfect target	pCUAGGUAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACUACCUAUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g2g3:t2t3 when paired to <i>let-7</i> perfect target	pUUCGGUAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACUACCGAUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g3g4:t3t4 when paired to <i>let-7</i> perfect target	pUGUCGUAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACUACGACUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g4g5:t4t5 when paired to <i>let-7</i> perfect target	pUGAUCUAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACUAGAUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g5g6:t5t6 when paired to <i>let-7</i> perfect target	pUGAGUCAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACUGACUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g6g7:t6t7 when paired to <i>let-7</i> perfect target	pUGAGGCUGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUACAGCCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g7g8:t7t8 when paired to <i>let-7</i> perfect target	pUGAGGUUCUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUAGAACCUUUU

Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g8g9:t8t9 when paired to <i>let-7</i> perfect target	pUGAGGUAUCAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCCUGAUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g9g10:t9t10 when paired to <i>let-7</i> perfect target	pUGAGGUAGCUGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCAGCUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g9g10:t9t10 when paired to <i>let-7</i> perfect target	pUGAGGUAGGCGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCGCCUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	pUGAGGUAGUUCGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACGACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	pUGAGGUAGUCAGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACUGACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAAUUGUAUAGU
Passenger strand to preceding guide	pUAUACAAUUUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAUUUUGUAUAGU
Passenger strand to preceding guide	pUAUACAAAUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAUAUUGUAUAGU
Passenger strand to preceding guide	pUAUACAAUAUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAUCUUGUAUAGU
Passenger strand to preceding guide	pUAUACAAGAUACUACCUCUUU

Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g12g13:t12t13 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGUCUGUAUAGU
Passenger strand to preceding guide	pUAUACAGACUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g13g14:t13t14 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGCCGUAUAGU
Passenger strand to preceding guide	pUAUACGGCCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g14g15:t14t15 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUCUUAUAGU
Passenger strand to preceding guide	pUAUAAGACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUUCUAUAGU
Passenger strand to preceding guide	pUAUGAAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUAGAUAUAGU
Passenger strand to preceding guide	pUAUCUAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g16g17:t16t17 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGCUUAGU
Passenger strand to preceding guide	pUAAGCAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g17g18:t17t18 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUUCAGU
Passenger strand to preceding guide	pUGACAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g18g19:t18t19 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUACUGU
Passenger strand to preceding guide	pAGUACAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g19g20:t19t20 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUAUUCU
Passenger strand to preceding guide	pAAUACAACCUACUACCUCUUU

Modified <i>let-7</i> guide strand that creates 2 nt mismatches at g20g21:t20t21 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUAUAUC
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g1:t1 when paired to <i>let-7</i> perfect target	pCGAGGUAGUAGGUUGUAUAGU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g14:t14 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACGACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUUUAUAGU
Passenger strand to preceding guide	pUAUAACAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUAUAUAGU
Passenger strand to preceding guide	pUAUAUAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g16:t16 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGGAUAGU
Passenger strand to preceding guide	pUAUCCAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g16:t16 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGCAUAGU
Passenger strand to preceding guide	pUAUJCAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g17:t17 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUUAGU
Passenger strand to preceding guide	pUAACAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g9:t9 when paired to <i>let-7</i> perfect target	pUGAGGUAGCAGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCUJGUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g10:t10 when paired to <i>let-7</i> perfect target	pUGAGGUAGUUGGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACCAACUACCUCUUU

Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g11:t11 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAAGUUGUAUAGU
Passenger strand to preceding guide	pUAUACAACUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 1 nt mismatch at g12:t12 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGAUUGUAUAGU
Passenger strand to preceding guide	pUAUACAAUCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 3 nt mismatches at g15–g17:t15–t17 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUUCUAGU
Passenger strand to preceding guide	pUAAGAAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 4 nt mismatches at g14–g17:t14–t17 when paired to <i>let-7</i> target	pUGAGGUAGUAGGUCUCUAGU
Passenger strand to preceding guide	pUAAGAGACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 5 nt mismatches at g17–g21:t17–t21 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGUUCUCC
Passenger strand to preceding guide	pAGACAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 6 nt mismatches at g16–g21:t16–t21 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUGCUCUCC
Passenger strand to preceding guide	pAGAGCAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 7 nt mismatches at g15–g21:t15–t21 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUUUCUCUCC
Passenger strand to preceding guide	pAGAGAAACCUACUACCUCUUU
Modified <i>let-7</i> guide strand that creates 8 nt mismatches at g14–g21:t14–t21 when paired to <i>let-7</i> perfect target	pUGAGGUAGUAGGUCUCUCUCC
Passenger strand to preceding guide	pAGAGAGACCUACUACCUCUUU
Renilla reniformis luciferase derived siRNA guide strand	pAUAGCUAUAUGAAAUGCCUU
Passenger strand to preceding guide	pGGCAUUUCAUUUAGCUACUU

RNA that is exactly complementary to <i>let-7</i> guide used as Northern probe	pACU <u>AUACAACCU</u> AGU <u>ACCUC</u> A
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Description	Sequence
7 nt target strand for RNA melt	pCU <u>ACCUC</u>
9 nt target strand for RNA melt	pU <u>ACUACCUC</u>
10 nt target strand for RNA melt	pCU <u>ACUACCUC</u>

Description	Sequence (m, 2'-O-methyl ribose; ps; phosphorothioate; complementary to guide; mismatch)
Complete complementary target to <i>let-7</i>	pGAU <u>ACU<u>AUACAAC</u>mCpsmU<u>ACUACCUC</u>AACCU</u>
Complete complementary target to <i>let-7</i> (unmodified and cleavable)	pGAU <u>ACU<u>AUACAACCU</u>ACU<u>ACCUC</u>AACCU</u>
21 nt complete complementary target to <i>let-7</i>	pACU <u>AUACAACmCpsmU<u>ACUACCUC</u>A</u>
Target complementary to <i>let-7</i> with g9–g11:t9–t11 central internal loop	pGAU <u>ACU<u>AUACAAC</u>AGUCU<u>ACCUC</u>AACCU</u>
Target to <i>let-7</i> with g2–g21 complementarity	pGAU <u>ACU<u>AUACAACmCpsmU<u>ACUACCUC</u></u>UACCU</u>
Target to <i>let-7</i> with g1–g19 complementarity	pGAU <u>U<u>AUACAACmCpsmU<u>ACUACCUC</u></u>AACCU</u>
Target to <i>let-7</i> with g4g5:t4t5 seed internal loop	pGAU <u>ACU<u>AUACAACmCpsmU<u>ACU</u>AA<u>UCAACCU</u></u></u>
Target to <i>let-7</i> with g8g9:t8t9 central internal loop	pGAU <u>ACU<u>AUACAACmCpsmU<u>UAUACCUC</u></u>AACCU</u>

Target to <i>let-7</i> with g10g11:t10t11 central internal loop	pGAUACU <u>AUACAAC</u> mGpsmACU <u>ACCUCA</u> ACCU
Target to <i>let-7</i> with g15g16:t15t16 3' supplementary region internal loop	pGAUACU <u>AUCGAA</u> CmCpsmUACU <u>ACCUCA</u> ACCU
Target to <i>let-7</i> with g2–g16:t2–t16 complementarity	pGAUUAGCC <u>ACAAC</u> CmCpsmUACU <u>ACCUCA</u> ACCU
Target to <i>let-7</i> with g9–g21:t9–t21 complementarity	pGAUACU <u>AUACAAC</u> CmCpsmUAG <u>AUGGAG</u> AAAAU
Target to <i>let-7</i> with seed only pairing (g2–g8:t2–t8)	pGAAAA <u>AAAAAAAA</u> mApsmAU <u>CUACCUCU</u> AAAAU
Target to <i>let-7</i> with seed + 3' supplementary pairing (g2–g8:t2–t8; g13–g16:t13–t16)	pGAAAA <u>AAACAAA</u> mApsmAU <u>CUACCUCU</u> AAAAU
Target to <i>let-7</i> with extended seed pairing (g2–g10:t2–t10)	pGAAAA <u>AAAAUU</u> A mApsmU <u>ACUACCUCU</u> AAAAU
Target to <i>let-7</i> with seed + extended 3' supplementary pairing (g2–g8:t2–t8; g12–g17:t12–t17)	pGAAAA <u>UUACAAC</u> CmApsmAU <u>CUACCUCU</u> AAAAU
Target to <i>let-7</i> with centered pairing (g4–g15:t4–t15)	pGAU <u>GGAAACCA</u> CmCpsmU <u>ACUACCAGU</u> ACCU
Perfect target to <i>Renilla reniformis</i> luciferase	pGAUAAGGCA <u>UUUC</u> mApsmU <u>UAUAGCU</u> AUACCU
Target to <i>let-7</i> with 1 GU wobble in the seed at g4	pGAUACU <u>AUACAAC</u> CmCpsmUACU <u>ACUUCA</u> ACCU
Target to <i>let-7</i> with 1 GU wobble in the 3' supplementary region at g15	pGAUACU <u>AUUAAC</u> CmCpsmUACU <u>ACCUCA</u> ACCU
Target to <i>let-7</i> with 2 GU wobble in the seed at g2 and g8	pGAUACU <u>AUACAAC</u> CmCpsmU <u>AUUACCU</u> UAACCU
Target to <i>let-7</i> with 2 GU wobble in the seed at g4 and g5	pGAUACU <u>AUACAAC</u> CmCpsmUACU <u>AUUUCA</u> ACCU
Target to <i>let-7</i> with 4 GU wobble in the seed at g2, g4, g5 and g8	pGAUACU <u>AUACAAC</u> CmCpsmU <u>AUUUUUA</u> ACCU

Table S2B, related to Figure 1. DNA oligonucleotides used in this study.

Description	Sequence (siRNA pairing site, change from <i>let-7</i> complementary target)
Forward primer containing T7 promoter consensus sequence for making DNA template used in transcription of <i>let-7</i> based targets.	GCG TAA TAC GAC TCA CTA TAG GGT CAC ATC TCA TCT ACC TCC
Reverse primer for making <i>let-7</i> guide perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making <i>let-7</i> passenger strand perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTA TAC AAC CTA CTA CCT CTT TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g1g2:t1t2 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TCT AGG TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g2g3:t2t3 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTT CGG TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g3g4:t3t4 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG TCG TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g4g5:t4t5 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG ATC TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g5g6:t5t6 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGT CAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G

Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g6g7:t6t7 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG CTG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g7g8:t7t8 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TTC TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g8g9:t8t9 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAT CAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making first compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g9g10:t9t10 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG CTG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making second compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g9g10:t9t10 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG GCG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making first compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TTC GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making second compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TCA GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making first compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAA ATT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for second complementary target to p21- modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAT TTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making third compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAT ATT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G

Reverse primer for making fourth compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAT CTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g12g13:t12t13 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG TCT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g13g14:t13t14 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GCC GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g14g15:t14t15 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTC TTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making first compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT TCA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making second compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT AGA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g16g17:t16t17 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GCT TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g17g18:t17t18 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTT CAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g18g19:t18t19 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTA CTG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g19g20:t19t20 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTA TTC TAT CCA GAG GAA TTC ATT ATC AGT G

Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 2 nt mismatches at g20g21:t20t21 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTA TAT CAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g1:t1 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG T CG AGG TAG TAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g14:t14 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTC GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making first compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT ITA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making second compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT ATA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for first compensatory target to p21-976 modified <i>let-7</i> guide strand that creates 1 nt mismatch at g16:t16 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GGA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making second compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g16:t16 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GCA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g17:t17 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTT TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g9:t9 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG CAG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G

Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g10:t10 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TTG GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g11:t11 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAA GTT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 1 nt mismatch at g12:t12 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG ATT GTA TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 3 nt mismatches at g15–g17:t15–t17 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT TCT TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 4 nt mismatches at g14–g17:t14–t17 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTC TCT TAG TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 5 nt mismatches at g17–g21:t17–t21 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GTT CTC CAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 6 nt mismatches at g16–g21:t16–t21 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT GCT CTC CAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 7 nt mismatches at g15–g21:t15–t21 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTT TCT CTC CAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making compensatory target to modified <i>let-7</i> guide strand that creates 8 nt mismatches at g14–g21:t14–t21 when paired to <i>let-7</i> perfect target	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TTG AGG TAG TAG GTC TCT CTC CAT CCA GAG GAA TTC ATT ATC AGT G

Reverse primer for making complementary target to <i>Renilla reniformis</i> luciferase derived siRNA guide strand	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TAT AGC TAT AAT GAA ATG CCT TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making target to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 1 nt mismatch at g4:t4	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TAT A <u>T</u> C TAT AAT GAA ATG CCT TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making target to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 2 nt mismatches at g8g9:t8t9	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TAT AGC TAG <u>C</u> A <u>T</u> GAA ATG CCT TAT CCA GAG GAA TTC ATT ATC AGT G
Reverse primer for making target to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 2 nt mismatches at g15g16:t15t16	CCC ATT TAG GTG ACA CTA TAG ATT TAC ATC GCG TTG AGT GTA GAA CGG TTG TAT AAA AGG TAT AGC TAT AAT GAA <u>C</u> G <u>G</u> CCT TAT CCA GAG GAA TTC ATT ATC AGT G

Table S2C, related to Figure 1. RNA transcripts used in this study.

Description	Sequence (siRNA pairing site, change from <i>let-7</i> complement)
Transcript with perfect target site to <i>let-7</i> guide strand	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with perfect target site to <i>let-7</i> passenger strand	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AAA GAG GUA GUA GGU UGU AUA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g1g2:t1t2 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU ACC UAG ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g2g3:t2t3 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU ACC GAA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g3g4:t3t4 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU ACG ACA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g4g5:t4t5 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUCCCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU AGA UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g5g6:t5t6 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU GAC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g6g7:t6t7 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACA GCC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g7g8:t7t8 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU AGA ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g8g9:t8t9 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU GAU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g9g10:t9t10 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCA GCU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
First transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCG CCU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Second transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CGA ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Third transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g10g11:t10t11 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CUG ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
First transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA UUU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

Second transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA AAU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Third transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA UAU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Fourth transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g11g12:t11t12 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA GAU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g12g13:t12t13 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAG ACU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g13g14:t13t14 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CGG CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g14g15:t14t15 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA AGA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
First transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUG AAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Second transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g15g16:t15t16 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUC UAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g16g17:t16t17 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AAG CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g17g18:t17t18 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU GAA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g18g19:t18t19 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACA GUA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g19g20:t19t20 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AGA AUA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 2 nt mismatches at g20g21:t20t21 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU GAU AUA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g1:t1 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU ACU ACC UCG ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g14:t14 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CGA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
First transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA AAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

Second transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g15:t15 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA UAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
First transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g16:t16 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUC CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Second transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatches at g16:t16 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUG CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g17:t17 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AAA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g9:t9 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCU GCU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g10:t10 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CCA ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g11:t11 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA CUU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 1 nt mismatch at g12:t12 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AUA CAA UCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

Transcript with compensatory target site to <i>let-7</i> guide strand that creates 3 nt mismatches at g15–g17:t15–t17 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AAG AAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 4 nt mismatches at g14–g17:t14–t17 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU ACU AAG AGA CCU AAG AGC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 5 nt mismatches at g17–g21:t17–t21 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UG AUU CCU CUG GAU GGA GAA CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 6 nt mismatches at g16–g21:t16–t21 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU GGA GAG CAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 7 nt mismatches at g15–g21:t15–t21 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU GGA GAG AAA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with compensatory target site to <i>let-7</i> guide strand that creates 8 nt mismatches at g14–g21:t14–t21 when paired to <i>let-7</i> perfect target	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU GGA GAG AGA CCU ACU ACC UCA ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with complementary target site to <i>Renilla reniformis</i> luciferase derived siRNA guide strand	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AAG GCA UUU CAU UAU AGC UAU ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G
Transcript with target site to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 1 nt mismatch at g4:t4	GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AAG GCA UUU CAU UAU AGA UAU ACC UUU UAU ACA ACC GUU CUA CAC UCA ACG CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G

<p>Transcript with target site to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 2 nt mismatches at g8g9:t8t9</p>	<p>GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AAG GCA UUU CAU GCU AGC UAU ACC UUU UAU ACA ACC GUU CUA CAC UCA <u>ACG</u> CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G</p>
<p>Transcript with target site to <i>Renilla reniformis</i> luciferase derived siRNA guide strand with 2 nt mismatches at g15g16:t15t16</p>	<p>GGG UCA CAU CUC AUC UAC CUC CCG GUU UUA AUG AAU ACG AUU UUG UAC CAG AGU CCU UUG AUC GUG ACA AAA CAA UUG CAC UGA UAA UGA AUU CCU CUG GAU AAG GCC GUU CAU UAU AGC UAU ACC UUU UAU ACA ACC GUU CUA CAC UCA <u>ACG</u> CGA UGU AAA UCU AUA GUG UCA CCU AAA UGG G</p>

Table S3, Related to Figure 3. Thermodynamic parameters from optical melts of RNA duplexes. T_M values are for 1.0×10^{-4} M total single-strand concentration.

Guide:Target Duplex	T_M^{-1} versus $\ln(C_T/4)$				Average of Individual Fits				Nearest Neighbor Prediction			
	$\Delta G_{25^\circ\text{C}}$ (kcal mol ⁻¹)	ΔH (kcal mol ⁻¹)	ΔS (cal K ⁻¹ mol ⁻¹)	T_M (°C)	$\Delta G_{25^\circ\text{C}}$ (kcal mol ⁻¹)	ΔH (kcal mol ⁻¹)	ΔS (cal K ⁻¹ mol ⁻¹)	T_M (°C)	$\Delta G_{25^\circ\text{C}}$ (kcal mol ⁻¹)	ΔH (kcal mol ⁻¹)	ΔS (cal K ⁻¹ mol ⁻¹)	T_M (°C)
 CUCCAUCp	-8.7 ± 0.3	-50 ± 8	-150 ± 30	39 ± 0	-8.8 ± 0.3	-54 ± 6	-150 ± 20	40 ± 1	-8.5	-48	-130	39
 CUCCAUCp	-12 ± 1	-71 ± 7	-200 ± 20	52 ± 0	-12 ± 1	-62 ± 7	-170 ± 20	53 ± 1	-12	-66	-180	55
 CUCCAUCAUp	-9.8 ± 0	-73 ± 1	-210 ± 0	40 ± 0	-10 ± 0	-64 ± 0	-180 ± 0	42 ± 0	-8.3	-50	-140	37
 CUCCAUCAUCp	-15 ± 0	-100 ± 1	-300 ± 0	51 ± 0	-14 ± 0	-99 ± 2	-280 ± 10	51 ± 0	-13	-77	-220	52
 CUCCAUCAUp	-14 ± 0	-82 ± 2	-230 ± 10	56 ± 0	-14 ± 0	-76 ± 1	-210 ± 0	57 ± 0	-13	-69	-190	58
 CUCCAUCAUp	-18 ± 0	-106 ± 3	-290 ± 10	61 ± 0	-17 ± 0	-93 ± 1	-260 ± 0	62 ± 0	-16	-84	-230	64

Table S4, Related to Figure 5 and Figure 7. Equilibrium competition parameters for fly Ago2-RISC and mouse AGO2-RISC (mean \pm S.D.; $n = 3$).

<i>let-7</i>-programmed, affinity-purified <i>Drosophila melanogaster</i> Ago2-RISC			
Target	K_C (pM)	K_{rel}	$K_{D(adjusted)}$ (pM)
28 nt complete complementarity	10 ± 1	1.0 ± 0.2	4.0 ± 1.0
21 nt complete complementarity	9.0 ± 0	1.0 ± 0.1	4.0 ± 1.0
28 nt unmodified g9–g11 central internal loop	9.0 ± 2.0	1.0 ± 0.2	3.0 ± 1.0
g2–g21 complementary	8.7 ± 0.8	1.0 ± 0.1	4.0 ± 1.0
g4g5 mismatches in seed	$(5.6 \pm 0.8) \times 10^3$	600 ± 100	$(2.3 \pm 0.7) \times 10^3$
g8g9 central internal loop	13 ± 1	1.3 ± 0.2	5.0 ± 2.0
g10g11 central internal loop	7.6 ± 0.4	1.0 ± 0.1	3.1 ± 0.9
g15g16 mismatches in 3' supplementary region	$(2.4 \pm 0.3) \times 10^3$	250 ± 40	$(1.0 \pm 0.3) \times 10^3$
g2–g16 complementary	$(1.1 \pm 0.1) \times 10^2$	11 ± 2	40 ± 10
g9–g21 complementary	$(2.3 \pm 0.2) \times 10^3$	240 ± 40	$(1.0 \pm 0.3) \times 10^3$
Seed only (g2–g8)	$(7.3 \pm 0.9) \times 10^2$	100 ± 10	$(3.0 \pm 0.9) \times 10^2$

Seed plus g13–g16 3' supplementary	$(4.1 \pm 0.4) \times 10^2$	43 ± 7	$(1.7 \pm 0.5) \times 10^2$
Extended seed (g2–g10)	$(5.1 \pm 0.6) \times 10^2$	53 ± 9	$(2.1 \pm 0.6) \times 10^2$
Seed plus extended g12–g17 3' supplementary	19 ± 1	2.0 ± 0.2	8.0 ± 2.0
6 nt seed (g2–g7)	$(1.6 \pm 0.2) \times 10^3$	170 ± 30	$(7 \pm 2) \times 10^2$
Non-complementary luciferase target	$(1.5 \pm 0.1) \times 10^4$	1600 ± 200	$(6.0 \pm 2.0) \times 10^3$
1 GU wobble (g4) in seed	$(2.9 \pm 0.8) \times 10^2$	30 ± 9	$(1.2 \pm 0.5) \times 10^2$
1 GU wobble (g15) in 3' supplementary region	60 ± 5	6.3 ± 0.9	24 ± 7
2 GU wobbles (g2, g8) in seed	$(3.8 \pm 0.3) \times 10^2$	40 ± 5	$(1.5 \pm 0.5) \times 10^2$
2 GU wobbles (g4, g5) in seed	$(3.5 \pm 0.5) \times 10^3$	370 ± 70	$(1.4 \pm 0.5) \times 10^3$
4 GU wobbles (g2, g4, g5, g8) in seed	$(4.5 \pm 0.1) \times 10^3$	470 ± 60	$(1.8 \pm 0.5) \times 10^3$
<i>let-7</i>-programmed, affinity-purified <i>Mus musculus</i> AGO2-RISC			
Target	K_c (pM)	K_{rel}	$K_{D(adjusted)}$ (pM)
28 nt complete complementarity	36 ± 5	1.0 ± 0.2	20 ± 10
g10g11 central internal loop	80 ± 30	2.2 ± 0.8	50 ± 30

g15g16 mismatches in 3' supplementary region	50 ± 20	1.4 ± 0.6	30 ± 20
g1–g19 complementary	70 ± 10	2.0 ± 0.4	40 ± 20
Seed only (g2–g8)	50 ± 10	1.5 ± 0.3	30 ± 20
Seed plus g13–g16 3' supplementary	34 ± 5	1.0 ± 0.2	20 ± 10
g4g5 mismatches in seed	$(1.3 \pm 0.7) \times 10^3$	40 ± 20	$(1.0 \pm 0.6) \times 10^3$
Non-complementary luciferase target	$(3.2 \pm 0.8) \times 10^3$	100 ± 30	$(2.0 \pm 1.0) \times 10^3$