

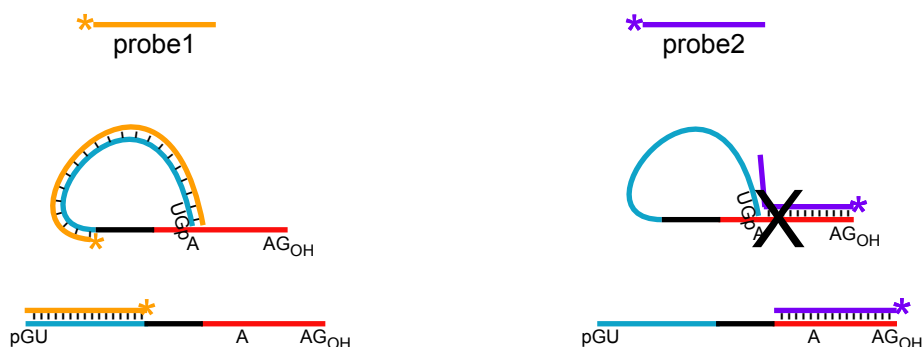
Intronic microRNA precursors that bypass Drosha processing

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a



b

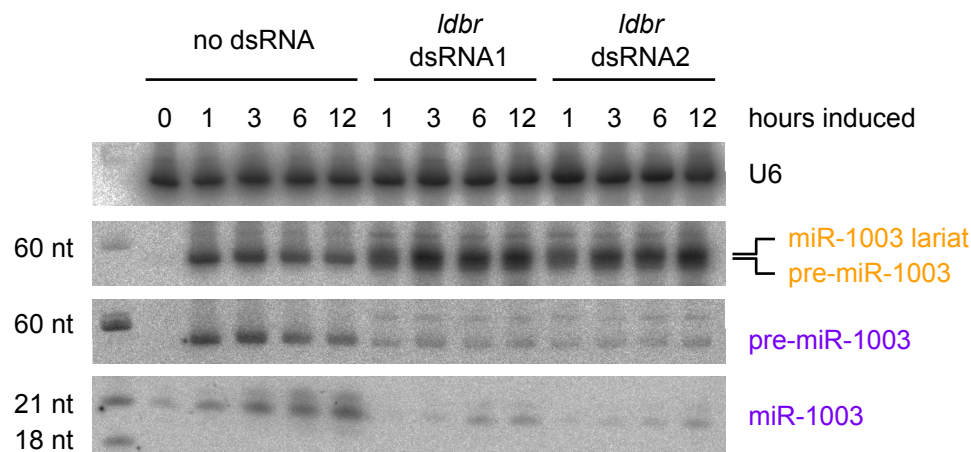


Figure S1. Mirtrons accumulate as lariats after splicing and require debranching enzyme (Lbr) for conversion into functional pre-miRNAs. **a**, Left, hybridization of probe1 to miR-1003 intron lariat or linear pre-miR-1003. Right, stable hybridization of probe2 occurs only with linear pre-miR-1003, and is inhibited by the presence of the branch-point adenosine in the lariat. **b**, Northern blotting was used to analyze miR-1003 maturation in a time course after induction of mini-gene expression. Prior to induction, cells were soaked with either of two dsRNAs targeting *lbr* (CG7942) or left untreated. RNA was resolved on a denaturing 15% acrylamide gel. Under these conditions, the lariat runs slightly above the pre-miRNA hairpin. In DBR dsRNA lanes, the major band detected by probe1 is absent when the blot is hybridized to probe2, indicating the presence of a lariat in these samples. When separated on a 17% gel, the lariat runs significantly higher (Fig. 2c). Changes in relative mobility in gels with different polyacrylamide densities are characteristic of non-linear RNA species.

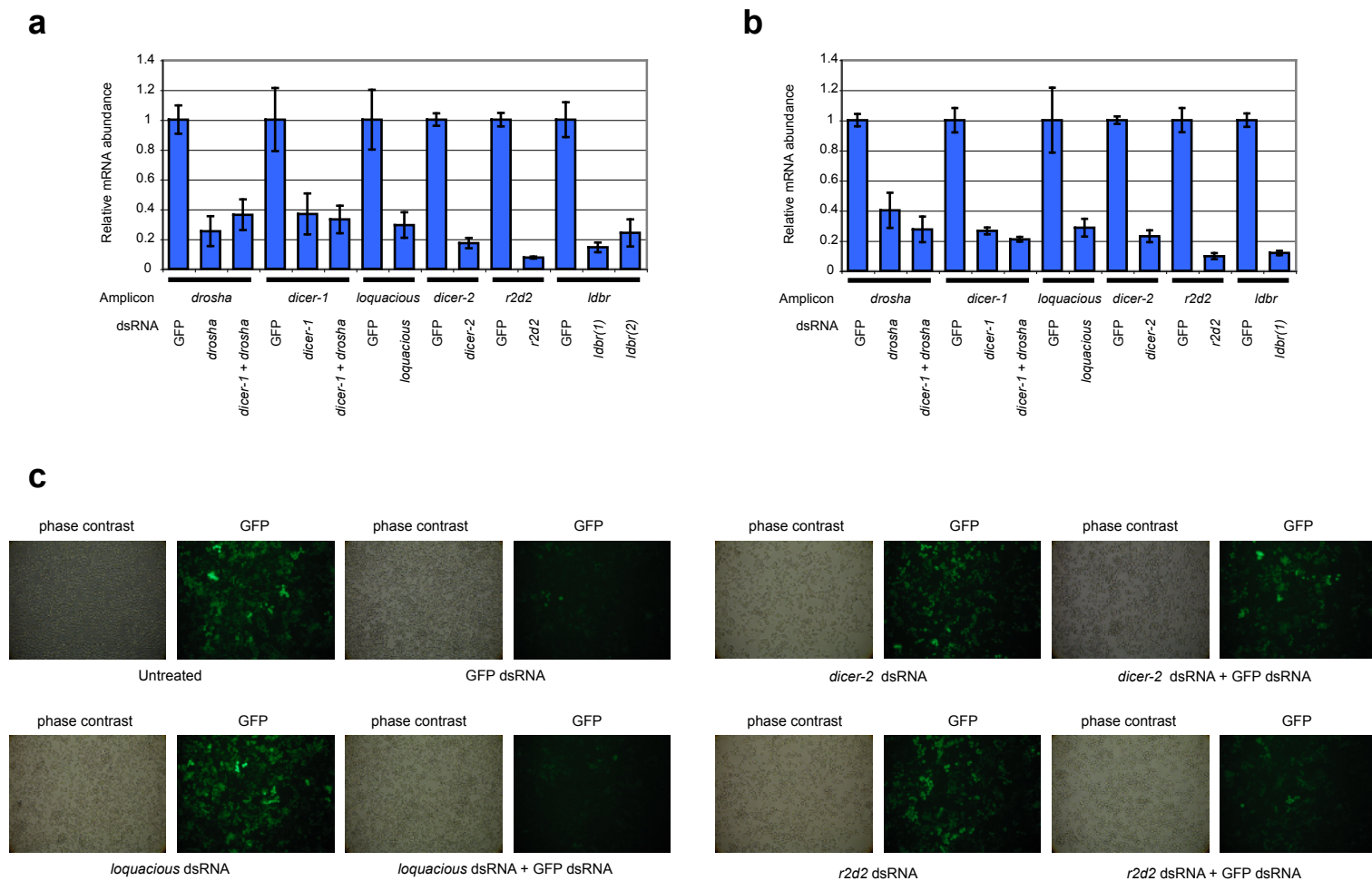


Figure S2. Confirmation of RNAi knockdowns. **a**, Quantitative RT-PCR analysis of samples from Fig. 2c. Relative abundance was measured using the $\Delta\Delta C_t$ method, normalizing to *actin 5c* (ΔC_t), and then to samples soaked in GFP dsRNA ($\Delta\Delta C_t$). Values are reported as geometric mean \pm s.d. ($n=3$). **b**, Analysis as in (a), using samples from Fig. 2d. **c**, Functional analysis of *dicer-2* and *r2d2* knockdown by fluorescence microscopy. Cells stably expressing GFP were soaked in dsRNAs targeting *loquacious*, *dicer-2*, or *r2d2*. After 4 days, dsRNA targeting GFP was added. Depletion of Dicer-2 or R2D2 reduces the ability of GFP dsRNA to silence GFP. Depletion of Loquacious serves as a negative control. Functional efficacy of the other dsRNAs was assessed by northern blot analysis of miRNA or intron processing (Fig. 2).

Table S1

mir-1009

reads: 14
Most abundant read: UCUCAAAAUUGUUACAUUUCAG
Host gene: CG3860-RA
Intron coordinates: chr2R:19500653-19500714(-)

| | <u># reads</u> | <u># loci</u> |
|--|----------------|---------------|
| GUAAGUGUAAGACUUUCUUGAGUUACCCGCGAUGAGUAUCUCAAAAAUUGUUACAUUUCAG (.(((((((.(.(((.((((.((((.(.....).)))).)))))).).))))))))).. | | |
| .UAAGUGUAAGACUUUCUUGAGU..... | 1 | 1 |
|UCUCAAAAUUGUUACAUUUCAG. | 3 | 1 |
|UCUCAAAAUUGUUACAUUUCAG | 10 | 1 |
| <i>D. melanogaster</i> GTAAGTGTAAAGACTTTCT-----TGAGTT---ACCCGCGATGAGTATCTCAAAAATTGT--TACATTTTCAG | | |
| <i>D. simulans</i> GTAAGTGTAAAGACTTTCT-----TGAGTT---ACCCGCGATGAGTATCTCAAAAATTGT--TACATTTTCAG | | |
| <i>D. yakuba</i> GTAAGTGTAAAGACTTTCT-----TGAGTT---ACCCGCGAGGAGTATCTCAAAAATTAT--TACATTTTCAG | | |
| <i>D. ananassae</i> GTAAGT TTTGAATACTTC ----- TACTCT --- ATCTTGGA-ATGTCTCTCAATAATGTC--CATCTTCTAG | | |
| <i>D. pseudoobscura</i> GTAAGT TCGAGAATCCAAACACA TAAGTT--- CTTTTTTA ----- ACATCCAAA ----- TATTTTGTAG | | |
| <i>D. mojavensis</i> GTAAG GACCA --- TTGG ----- TAAAGTGAGAAATTGGAAGAAATTATTTGAAATTTACTCGTGT TTAG | | |

mir-1010

reads: 193
Most abundant read: UUUCACCUAUCGUUCCAUUUGCAG
Host gene: CG31163-RA CG31163-RB CG31163-RC
Intron coordinates: chr3R:18118600-18118671(+)

| | <u># reads</u> | <u># loci</u> |
|--|----------------|---------------|
| GUAAGUGGUGUAGAUGAAACAAUUUACCAACAAUUUUGUUGAUUGUUUACCUAUCGUUCCAUUUGCAG ((((((((.(.(((.((((.((((.(.....).)))).)))))).).))))))))).. | | |
| GUAAGUGGUGUAGAUGA..... | 1 | 1 |
| GUAAGUGGUGUAGAUGAAA..... | 2 | 1 |
| GUAAGUGGUGUAGAUGAAAC..... | 3 | 1 |
| GUAAGUGGUGUAGAUGAAACA..... | 30 | 1 |
| GUAAGUGGUGUAGAUGAAACAA..... | 1 | 1 |
|UUUCACCUAUCGUUCCAUUUG..... | 10 | 1 |
|UUUCACCUAUCGUUCCAUUUGC..... | 39 | 1 |
|UUUCACCUAUCGUUCCAUUUGCA..... | 38 | 1 |
|UUUCACCUAUCGUUCCAUUUGCAG..... | 64 | 1 |
|UUUCACCUAUCGUUCCAUUUGC..... | 3 | 1 |
|UUUCACCUAUCGUUCCAUUUGCA..... | 1 | 1 |
|UUUCACCUAUCGUUCCAUUUGCAG..... | 1 | 1 |
| <i>D. melanogaster</i> GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AAT---TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. simulans</i> GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AATA- TTTT TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. yakuba</i> GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AAT TTTT TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. ananassae</i> GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AATA- TTTT TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. pseudoobscura</i> GTAAGTGGTGTAGATGAAACAAATTT ACCAAC-CTTT-AT TGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. virilis</i> GTAAGTGGTGTAGATGAAACAAATTT CACAACA AAT-- TTTT TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |
| <i>D. mojavensis</i> GTAAGTGGTGTAGATGAAACAAATTT CACAAC -AAT-- TTTT TTTGTGGATTGTTTCACCTATCGTTCCATTTGCAG | | |

Table S1

mir-1015

reads: 8
Most abundant read: UCCUGGGACAUCUCUCUUGCAG
Host gene: CG6432-RA
Intron coordinates: chr3R:20164953-20165017(+)

Table with 3 columns: sequence, # reads, # loci. Shows sequence alignment for mir-1015 with 1 read and 1 locus.

D.melanogaster GTGAGTGATGCTCCAGTTAGCTTGGC-TGAGTGAGGATTTA-----AGTCCTGGGAC-----AT-----CTCTCTTGCAG
D.simulans GTGAGTGATGCTCCAGTTAGCTTGGC-TGAGTGAGGATTTA-----AGTCCTGGGAC-----AT-----CTCTCTTGCAG
D.yakuba GTGAGTGATGCTCCAGTATAGCTTGGC-TGGGTGAGGATTTA-----AGTCCTGGGAC-----AT-----CTCTCTTGCAG
D.virilis GTAAGT-----ATTAC-TTGATACTCGTTAAACCTAAAGTCTGTTAACTACTCTTATAACTATGCATCTAAACAG
D.mojavensis GTAAGTTC-----ATGAATTCATGTTCAATGATTTTATCCAAAGTAAATTCCTTTAC---GTTGT-----CTTCTTAAACAG

mir-1016

reads: 2
Most abundant read: UUCACCUCUCUCCAUCUAG
Host gene: CG8479-RA CG8479-RB
Intron coordinates: chr2R:9747992-9748050(-)

Table with 3 columns: sequence, # reads, # loci. Shows sequence alignment for mir-1016 with 2 reads and 1 locus.

D.melanogaster GTAAGTATAGAGAGGAT--GTGATGGTAAAT-----TCCAAAGTTCACCTCTCTCCACTTAA-----G
D.simulans GTAAGTACAGAGATGAT--GTAATGGTAAAT-----TCCAAAGTTCACCTCTCTCCACTTAA-----G
D.yakuba GTAAGTATAGAGAGGAT--GTGATGGTAAAT-----TCCAAAGTTCACCTCTCTCCACTTAA-----G
D.ananassae GTGAGTACTTGTAAATAT--A-AATCCATAAAT-----CAAACCTCACITTT-TCCA-ACTTA-----G
D.pseudoboscra GTGAGTACACAATTCAA--TTCTCCGCAAGG-CTATCGTCTGAAACTAATTTCCCTTCA--CTTATCCCTGCCCTGCAG
D.virilis GTAAGTAGCAGGTGC---TGAGTGCATGATT--GTCCTATATCTTGATATCTTTG---CA-----G
D.mojavensis GTAAGTAGAGCACGATTTCCAGTACCACATATTCGTACTATATCTGTACAAITTTG---TA-----G

mir-1017

reads: 148
Most abundant read: GAAAGCUCUACCCAAACUCAUCC
Host gene: CG6844-RA CG6844-RB
Intron coordinates: chr3R:20314333-20314502(+)

Table with 3 columns: sequence, # reads, # loci. Shows sequence alignment for mir-1017 with 148 reads and 1 locus.

D.melanogaster GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----TGATCCCTT-----TCTCCCTTTCCGAGACAT--TTGATGCC-----ACTTCTGCGGCTTT--CTGGAATTTGAACTCTCTGAAATGCG--TGAGC-----GTTT-TAG
D.simulans GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----ATATCCCTT-----TCTCCCTTTCCGAGACAT--TTGATGCC-----ACTTCTGCGGCTTT--CTGGAATTTGAACTCTCTGAAATGCG--TGAGC-----GTTT-TAG
D.yakuba GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----ATATCCCTT-----TCTCCCTTTTCCGAGACAT--TTGATGCC-----ACTTCTGCGGCTTT--CTGGAATTTGAACTCTCTGAAATGCG--TGAGC-----GTTT-TAG
D.ananassae GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---CC--TTTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----T-----CCTTTTTCCTCCCAATTT--TCTCCCTT-----ACTTCTGCGGCTTT--CTGGAATTTGAACTCTCTGAAATGCG--TGAGC-----GTTT-TAG
D.pseudoboscra GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----T-----TCTTTCTCTCCCAACAATA--ATTCCTCT-----AC--CCTGTAAATGAC--TATGAGGCTGCTCTGATATGCGCT--TGAGC-----GTTT-TAG
D.virilis GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----T-----TCTTTCTCTCCCAACAATA--ATTCCTCT-----AC--CCTGTAAATGAC--TATGAGGCTGCTCTGATATGCGCT--TGAGC-----GTTT-TAG
D.mojavensis GTGAGTTAGTGAAGTTAAAGT-----TCCATGCGCAGC---AA--TTACGCGAAAGCTCTACCCAACTCATCCCGG-AAAA-----T-----TCTTTCTCTCCCAACAATA--ATTCCTCT-----AC--CCTGTAAATGAC--TATGAGGCTGCTCTGATATGCGCT--TGAGC-----GTTT-TAG

Table S3. Quantification of signals from RNA blots of Figure 2c and 2d. Signals were first normalized to that of the loading control (U6), then to that of the control dsRNA (GFP). When signal was below detection (b.d.), the upper bound of the value, based on the normalized detection limit, is shown for relevant lanes.

Fig. 2c Quantification

| | dsRNA | | | | | | | | |
|-------------------------|-------|----------------|----------------|-------------------|----------------|-------------|--------------------------|----------------|----------------|
| | GFP | <i>droscha</i> | <i>dicer-1</i> | <i>loquacious</i> | <i>dicer-2</i> | <i>r2d2</i> | <i>droscha + dicer-1</i> | <i>Idbr(1)</i> | <i>Idbr(2)</i> |
| pre- <i>let-7</i> miRNA | 1.0 | 0.03 | 3.54 | 0.90 | 0.51 | 0.60 | 0.14 | 0.48 | 0.91 |
| <i>let-7</i> miRNA | 1.0 | 0.45 | 1.36 | 1.58 | 1.37 | 2.02 | 0.23 | 1.90 | 3.80 |
| pre-miR-1003 probe1 | 1.0 | 0.12 | 0.57 | 0.65 | 0.32 | 0.29 | 0.35 | 0.06 | 0.08 |
| pre-miR-1003 lariat | b.d. | b.d. | b.d. | b.d. | b.d. | b.d. | b.d. | 0.36 | 0.57 |
| pre-miR-1003 probe2 | 1.0 | 0.10 | 0.51 | 0.68 | 0.36 | 0.32 | 0.31 | 0.03 | 0.03 |
| miR-1003 | 1.0 | 0.92 | 0.08 | 0.09 | 0.81 | 0.31 | 0.10 | b.d. (<.04) | b.d. (<.04) |

Fig. 2d Quantification

| | dsRNA | | | | | | | |
|-------------------------|-------|----------------|----------------|-------------------|----------------|-------------|--------------------------|----------------|
| | GFP | <i>droscha</i> | <i>dicer-1</i> | <i>loquacious</i> | <i>dicer-2</i> | <i>r2d2</i> | <i>droscha + dicer-1</i> | <i>Idbr(1)</i> |
| pre- <i>let-7</i> miRNA | 1.0 | b.d. (<.05) | 4.56 | 2.19 | 1.15 | 1.51 | 0.15 | 1.05 |
| <i>let-7</i> miRNA | 1.0 | 0.17 | 0.85 | 1.61 | 1.21 | 0.41 | 0.21 | 0.91 |
| pre-miR-1006 probe1 | 1.0 | 0.36 | 1.37 | 1.33 | 0.92 | 0.73 | 1.15 | 0.46 |
| pre-miR-1006 lariat | b.d. | b.d. | b.d. | b.d. | b.d. | b.d. | b.d. | 0.18 |
| pre-miR-1006 probe2 | 1.0 | 0.34 | 1.37 | 1.41 | 1.10 | 0.86 | 1.28 | 0.53 |
| miR-1006 | 1.0 | 0.73 | 0.14 | 0.15 | 0.56 | 0.31 | 0.37 | 0.37 |

Table S4

>pCJ19 (pMT-puro miR-1006)
 actagtAACACGAATACATCCAGTCCGGTGGCCCGGTGCCTGCAGATGATGCTCCGTGTGGATGAGTATCGATTTGCCCTTTGTGGGAGTGCAGCGAATCAGCACTCTGATCCGATCTTGTTCGAC
 CCGTGTCAACTCCAGGTGAGTTTGAATTTGAAATCGCTAAATTTGTTGGTACAATTTAAATTCGATTTCTTATTATCATAGGTGCAATACCAGTGTATCTTTTGCCTGTGGGTGCTGACCTTCAAT
 CCCCCTGTGGCCGCAAGATGAATAAGTTTCAGCGTGCATCCCCATCCTGGCTGATATCCTCAGCGATTTGTGCCAAGGAGAGAGTGCACGCATTTATCCTCGCGCTCTTCCCAATCTGATCGAGAA
 GCCGGAGGATTCATCGGTGGCCCAAGGACCATAGCATCGCCATGTTCCAGTGCAGGTGCTGAAAGCAGCTATCCATCCTGGAGCAGCGTCCGTTTCGACGACGAGGACATTAACCGCCGACGTAGAGT
 ACCTGAGCGAGAAGCTCCAGAATTCGGTCCAGACTTGAGCTCCTTTGATGAGTACGCCACAGAGGTGCAGCGGTCCGTTGGAATGGTTCGCTGTCACAACTCGGCCGAGTTCGGCCGAG
 AATGCCACGCGCTTAAACGAAAAGAACTACGAGTTGCTGCGCATCCTCGTCCACCTCCTGGAACCTCAAAGATGCCATCATCTTTCCGTCGCTGCTTCGACATCGGGGAGTATGTGCGCCA
 CTATCCCCGGCGAAGCgagggcgc

>pCJ20 (pMT-puro miR-1003)
 actagtATAAAGCCGATAAGCTGCGGAAATCGAAAAGACCGAGCGTGAACGACTGCAACAGCAGGAACGCGAGGATGAGATGCGCGAACTGGCCCTCAAGCTGCGCAGAAGTGGGTATCTGGA
 TGTGGTTGGCTCTGGCGTCTCTCACATTTACATATTCACAGGCGCGGTGAGCTGCGTCAAAATATGGAACGCGCTCGATGGAAGACTCTCGGACAGCGATGCCAATCTGTGGCATCGGAA
 AGCCAGTGGCAAAGTCTGACGCGTTCGACGAGTTCGAGAGTCTGAGCGGTGCCACCGCCGAAACACAGCACAGCAGCAGGAGCAGCAGCCGAAACGGAAGGAGGAGACGCAC
 AAGGACGgagggcgc

>pCJ24 (pMT-puro let-7)
 actagtGACAAATGGCGGAGTAAAGGATAAAAAGTCCAGTCAAAAACCGGATTAATACGAAATAAATAACTACTAAAATAACTAAATAGAAGATCAACAGCGATCCATTAACAAAATAAACAATA
 ATATGTAATAGAAAACCATCGATAAATAAATCGTAAACTAAATTAACCTAAATATCCAACGTCATATAAATAACAACCTCAAAACGTCATAATAAGAAAACCTGATATGGTATAAACAACCTCAAGTT
 TAAGTTTGAATAACACAAAGTAATTTACTTAAATATCTCATCTTATGATATTTTATTTAAACATTCAAATGTAACCTTCAAAGCATTTTAAATATGATTTCTCCGATATTTTCTTCTG
 TTTGCCATCATCGTTTCAACAAAAACCGAACCAATGATATCCAGAAGATCCTTTAAATACCAAAACCACTAGCAAAAAGGACTACACCAAGGACCTTTTCTCTGCGCAATTTAGGTAGTAG
 GTTGTATAGTAAATAACACATCTACTATACAATGTGCTAGCTTTCTTTGGTTGACTACAAAGCGCATTTGATAAAAAGAAATCCAAATCGAACTGCACCACTTAATAAAACCAATCCCCGACCA
 TACAAAAGTTGTTGGTGCACAAATTTTGTATTTGGACAAACAAAGAAAGTGTCTGAGCCAACTATTGTTAAATATCATTACGAATGCCAAAGTATGTAATGCAACCGGGCATATGTAATAT
 TGGCATTGTGACATGTGCAAAATGTTTGTATGGCTGATTCCTGAGACCTAACTTGTGACTTTTAAATACCAGTTTCACAAGTTTGTATCTCCGGTATTGGACGCAAACTTGTCTGgagggcgc

>pCJ30 (p2032 miR-1003)
 ggtaccATAAAGCCGATAAGCTGTCGGAATCGAAAAGACCGAGCGTGAACGACTGCAACAGCAGGAACGCGAGGATGAGATGCGCGAACTGGCCCTCAAGCTGCGCAGAAGTGGGTATCTGGA
 TGTGGTTGGCTCTGGCGTCTCTCACATTTACATATTCACAGGCGCGGTGAGCTGCGTCAAAATATGGAACGCGCTCGATGGAAGACTCTCGGACAGCGATGCCAATCTGTGGCATCGGAA
 AGCCAGTGGCAAAGTCTGACGCGTTCGACGAGTTCGAGAGTCTGAGCGGTGCCACCGCCGAAACACAGCACAGCAGCAGGAGCAGCAGCCGAAACGGAAGGAGGAGACGCAC
 AAGGACGgagggcgc

>pCJ31 (p2032 miR-1006)
 ggtaccAACACGAATACATCCAGTCCGGTGGCCCGGTGCCTGCAGATGATGCTCCGTGTGGATGAGTATCGATTTGCCCTTTGTGGGAGTGCAGCGAATCAGCACTCTGATCCGATCTTGTTCGAC
 CCGTGTCAACTCCAGGTGAGTTTGAATTTGAAATCGCTAAATTTGTTGGTACAATTTAAATTCGATTTCTTATTATCATAGGTGCAATACCAGTGTATCTTTTGCCTGTGGGTGCTGACCTTCAAT
 CCCCCTGTGGCCGCAAGATGAATAAGTTTCAGCGTGCATCCCCATCCTGGCTGATATCCTCAGCGATTTGTGCCAAGGAGAAAGTGCACGCATTTATCCTCGCGTCTTCCGCAATCTGATCGAGAA
 GCCGGAGGATTCATCGGTGGCCCAAGGACCATAGCATCGCCATGTTCCAGTGCAGGTCGATGAAGCAGCTATCCATCCTGGAGCAGCGTCCGTTTCGACGACGAGGACATTAACCGCCGACGTAGAGT
 ACCTGAGCGAGAAGCTCCAGAATTCGGTGCAGACTTGAGCTCCTTTGATGAGTACGCCACAGAGGTGCAGCGGTCCGTTGGAATGGTTCGCTGTCACAACTCGGCCGAGTTCGGCCGAG
 AATGCCACGCGCTTAAACGAAAAGAACTACGAGTTGCTGCGCATCCTCGTCCACCTCCTGGAACCTCAAAGATGCCATCATCTTTCCGTCGCTGCTTCGACATCGGGGAGTATGTGCGCCA
 CTATCCCCGGCGAAGCgagggcgc

>pCJ32 (p2032 Let-7)
 ggtaccGACAAATGGCGGAGTAAAGGATAAAAAGTCCAGTCAAAAACCGGATTAATACGAAATAAATAACTACTAAAATAACTAAATAGAAGATCAACAGCGATCCATTAACAAAATAAACAATA
 ATATGTAATAGAAAACCATCGATAAATAAATCGTAAACTAAATTAACCTAAATATCCAACGTCATATAAATAACAACCTCAAAACGTCATAATAAGAAAACCTGATATGGTATAAACAACCTCAAGTT
 TAAGTTTGAATAACACAAAGTAATTTACTTAAATATCTCATCTTATGATATTTTATTTAAACATTCAAATGTAACCTTCAAAGCATTTTAAATATGATTTCTCCGATATTTTCTTCTG
 TTTGCCATCATCGTTTCAACAAAAACCGAACCAATGATATCCAGAAGATCCTTTAAATACCAAAACCACTAGCAAAAAGGACTACACCAAGGACCTTTTCTCTGCGCAATTTAGGTAGTAG
 GTTGTATAGTAAATAACACATCTACTATACAATGTGCTAGCTTTCTTTGGTTGACTACAAAGCGCATTTGATAAAAAGAAATCCAAATCGAACTGCACCACTTAATAAAACCAATCCCCGACCA
 TACAAAAGTTGTTGGTGCACAAATTTTGTATTTGGACAAACAAAGAAAGTGTCTGAGCCAACTATTGTTAAATATCATTACGAATGCCAAAGTATGTAATGCAACCGGGCATATGTAATAT
 TGGCATTGTGACATGTGCAAAATGTTTGTATGGCTGATTCCTGAGACCTAACTTGTGACTTTTAAATACCAGTTTCACAAGTTTGTATCTCCGGTATTGGACGCAAACTTGTCTGgagggcgc

>Drosha dsRNA
 GGTCCACAGCCGGTTATAAGCCGTGTTGCTACGCGTTGACCAATTTGGACAGGAATAAATCCGATTTGCCCGAGTGCCTGATCGCGAGACTGGAATCTCACATCCAGCAATCGTGCACCTTTG
 CAGTTTGTCTCATCTCAGCTTAAGTACGCTGGAATCCAGAGTACCAAGAGCGGTGGCGAGAGTACGTTAAGTACCGTCTATGATGGCCAAATGTCGAAGCCCTCTTCAAGGATAAGCGCAAG
 CTAGTGAAGAGGAGCAACGCTCTCCAGGAGATGCGAACTCAGGGGCGCATGAAACGAAATATCACAGCTGGCGATCAGCTCGAATCGGATCCGATTCGACCGCATTATGTCGACCGTTGTGCAGCA
 TGCCATGTTGATTCCTGTCTAACTGGTCACTTCGCTTTCACAAGTCTGCTGGACCTGCTAGAGGAGAGATATCGGGTACCGCTTTAAAATAACCGTACCTTCTCAAATTTGGCGTGCAGCATCCCT
 CATACAGGAGAACTACGAACTCCGATCACGCCGTAATTCGCTGACTAATTCGCGAATTCGTCAGCCGGAGTACGGAGATCGCAAGATCCATTTACATGAACACAGCAAGCGGGGTATC
 AACACATTAGTGACATTATGT

>Dicer-1 dsRNA
 CTACTGGCCACCGCTACGAGCGGAACAGATTTATTTGCTGGGCCATCGAAGTTCCAAGGAGTTTATAGCCCTCAAGCTGCTCCAGGAGCTGTGCGCTCGAGCACGCCGACATGGTCTGTCTCAG
 TGTCTATCTCAGTTGCGAGTTGGCCACCGAGCAGGACCATGCTCCATCTACACGATCTCACCCACTTGACTGACCTGCGGGTGTGGCAGGAGCAGCCGGATATGCAAAATCCCTTTGATCATT
 GCTGGACGACTATCAGCTTTCCATCCTACGCGCAGAGGATTTCTTTATCTGCTCGAACTCGCGAGCTGCTGTGAGCAGCGTCCGATGATCGTGTGGAAGATTTGATGACAGCGCGCTT
 TATCAGAGGATAAGCCCTGTGTTTCAGAAATCACATTTGCGAGCGCCACCGGCGGACAGGCAAGCTTTCTCGGATCTGCTGGACTGCGACAGCGCCGGATGTGAGCTGACGAACTGAGCGC
 CATGCTGGCCACCTGGAGCAGAGTGTGCTTTGCCAGATCGAGACGCGCAGTGTATTTGTCACCGTGTGCGTTACTGTTCCCGACCGCACGAATACATCGTACAGTGCGCCCTTCGAGATGG
 ACGAACTG

>Loquacious dsRNA
 ATGGACACGAGGAAATTTCCACGCGTCCAGCTTGGCCGAGCAGCTACAGAACCTCCACATCCAGCCGAGCAGGCGTCCCCCAATCTGTCCAGACGGGATTTGCTCCAGCGGGCACTATAATAA
 CCTGTGTCGCGCTGGGCAATGGAATGCGCTCAGTGTGATTCGGTGAAGGTTGCTCCGCTGGGGCAGCCGATGTGAAGCTCAAGAGGAGAAAGATATCCGCCAGGTTGCGCAGCTGTCTCAGC
 CAGGTGAGCTGCAGCTGTGATGTTGTTGATCTCTGCTTTGGCGGGCGGATCGGGCTTACAAGTGGAGTCCGCTTATGGCGTAATATTGCCACGACGAGGCGC

>Dicer-2 dsRNA
 AAAAGTTCAACGCGAAGAGCAAGGCTAAGATGAAAGTTATTTTGTATCCGGAGCTAGCTTTCAATTTTAACTTTCTGGGGATTTATGGTCTAAGTTGATCTTCTCCTACCAGCATTTTAAACCGCA
 GTACTTCTCTTCCACGAGAGGCTTACGTAAGCGATTTAATACGATTTTAAACCTCCATCTGCTGCTTTTAAATGGAATGATTCATGACGCCAGCACTAGAAATTTGATTTCTGCTAAAG
 CGGAATGTCGACCCCTTTGGCAATGTCATACCAACTGAGGATATCGAGGAGCCGAAATCCCTTTTAGAGCCTAAGTCCCAAAAGTCCATTTAGGCGCTCGGTGCCCAATCTTGAATAACAGAAAT
 CGAAAATCCCTGGCAAAATGATATGAGCAGCGTTGATCTGTCGCGAAATCTTTGAGTACGATATCCCGTAGAGCTGGACTACTATCATTTTAGCGTTGGTAAATGATGTGAGATGAATGAGA
 TGGATTTTGAAGATAAGGAATACTGGGCAAAAATCAGT

>R2D2 dsRNA
 TGCTGCCCGCATACACGCTTGTGAAAGGATTCGACTGTGGGTGATCTGGATGAGGAACTGACTAACTCAACCGGGACATGGTGAAGGAGTGCCTGACTACTGCTCCGCGCGAGATGCCA
 CTGCCCTGCAATGAGGTAGTGCAGCAAGCCGCGCACCCGAGCGCCCGGAAATTCGTGGCTGTGCTCCGTTGGCTTCAATAGTACGCTACGGAAGTCCGCAAAAAGAGGATGCCCTGACGG
 AGCCGCCATTTGAATGCTGGCTTTTACTCTCCAGCAATTCGCAAAATTCGCTCCGATCAAATGCAAGTAGCGAGCACAAAGCAAAATGAAAGTGTGATATGGAAGAATCTATGAGGAAATTTGG
 AGCATTGGCGAAGAAATTTAC

>CG7942 [1] (Debranching enzyme) dsRNA
 GTGGCGAGCTGGTAGCACCAGTGTGACCATATTCATTGGCGGCAACCATGAGGCTCCAAATTTACCTGCGAGGACTCCCATACGCGCGGTTGGGTGGCTCCAAATTTACTACCTTGGTTATGCG
 GGCTGCTGCTAAACCGTGTTCGGAATAGAGGGAATCTTCAAGGTTACGACTTTTGGCGGCGCATACGAAATCCCTTTCGAGCCTAAGTCCCAAAAGTCCCAAGTTCAGTGCAGCTGTCTACCA
 TGTGCGCGAGCTAGAAGTCTTTCGGCTGAAACAAATTTCCGGCGAGTTGATATTTCTGCTCCACGACTGGCCACCAGGATCTATGATAACGAAACAAAGCGCAACTGCTCCGCAAGAAAC
 CATTTTTGCTGCGAGACATGGAAGGCGGAAGCTGGTATGAGCCAGCCACTGAGGAGTACTGAAAGCGGTCCAAACCGGCTACTGTTTGTGCTCCATTTGCATTGCAAGTTTGGCGCTTTGGT
 CCGCAAACTCACAGCAGAAAGCTAGGATGCTGAAAT

>CG7942 [2] (Debranching enzyme)
 GCACAGTGAAGATGAAGACGAGGAAAGGAGGAAAGTAAAGAAAGCTGCTCCCTGACTCCACCATCAAATCTGTTCCGTTGACCAAGTTTCTGGCTCTCGCAAACTGCTGCCAGCTGCTGCTGCT
 TTCTCCAGTGGTAGAGTACCCAGTCCCACTCGAAGCCACTCCCGCTGGAATACGACGAGTGGTGGTCCATCTTGACAGTACAACTCACTTGAATTTAGTGAAGGAGATTTATTA
 TTACTGCTCCGGAATAAAGCGGAGAGTTTACAGAGCGATCAAACCTTACCCCACTGAAGAAAGAACTAGAAGCAGTACCGCAAGTTTTCAGAACTTCAAGTCCCGAGAACTTTGAGCGCA
 CAGTCCAGCTTTGATCCCGCGGACGATCTGATTTAAGCACATGTTTGTGGATCAACCAAGGTTCAACTAAACCCCGAGCAATACGTTTGTGCCACTCTGGGATATGACGATCCGCTG
 TGCTTATGTTTGTGGCAATGCTAGATGCTG

Table S4

>GFP dsRNA
GATCACATGGTCCCTGCTGGAGTTCGTGACCGCCGCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCGCGACTCTAGATCATAAATCAGCCATACCAATTTGTAGAGGTTTTA
CTTGCTTTAAAAAACCTCCCACACCTCCCCGTAACCTG

>UTR insert CG11094
actagtTGATAAATTTTCATTAAGTACGAGTAAACGAATACTACTTTGCCCGGATATTTATTATTGTTTCAGCATCACATATTAGCTTAATGCTTCGGTGAATTCGCGCAATTTAACTTTTATAACT
TAGAGTTGAGTAACTTAGAGTTTTATGGAGCAAAACCTCTGTAATAAATCGAATTTATCGGTAAACTAAAGCGCGACTTGGACTATCTTCAATCAACAAGCCAAATATGTCGATGTGTGACAGC
CGTTCTACCGGTACAGCTTTCTTCAATCAACATTACCCCGTGTGAGATGTCTGGCCCTCAATGTTAATAATCTCAATCTACAATCAACATTTCTTTCTTCAATCAACAATCCGCAAAACGGATCT
AATGcgggcgcg

>UTR insert CG11094-mutant
actagtTGATAAATTTTCATTAAGTACGAGTAAACGAATACTACTTTGCCCGGATATTTATTATTGTTTCAGCATCACATATTAGCTTAATGCTTCGGTGAATTCGCGCAGTGAACCTTTTATAACT
TAGAGTTGAGTAACTTAGAGTTTTATGGAGCAAAACCTCTGTAATAAATCGCAGTGATCGGTAAACTAAAGCGCGACTTGGACTATCTTCAATCAACAAGCCAAATATGTCGATGTGTGACAGC
CGTTCTACCGGTACAGCTTTCTTCAATCAACATTACCCCGTGTGAGATGTCTGGCCCTCAATGTTAATAATCTCAATCTACAATCAACATTTCTTTCTTCAATCAACAATCCGCAAAACGGATCT
AATGcgggcgcg

>UTR insert CG1849
actagtCCTGGAAATCAGACTCCGGCGAAGTTTTATGCTCGGACTCATAAAATCGTGCAGAGTTTGAATCAACAGGCCCTCGATTTTACCAGGATTTTTTACAAATCCAGCAGAAAAACCGA
AAACTCAAAAACCTCAGCCAAAAAGAAAATACCAAGAAAGCAAACCTTAGTTCAATTTCAATTTCAACACAAAAACAACAACAACAATTTGTACATAGCTAACTAGTTGTAACACTCATAACTTTT
TTTTTTTTGAGAACCTATTTTTTCGATGGATAATATGCGAATTTAGCTATTTTTAATCATTAGTTTAACTAGTCTAAGCGAGAAAAACAATTTTTTTGTCTAGCCATAAGTTTTTAGCGCGA
AAAGAGATCTAACACAAAAATCGAATTTGAAACAAAACCAATAAAAAACAAAAATCACACAAAAAgcgccgcg

>UTR insert CG1849-mutant
actagtCCTGGAAATCAGACTCCGGCGAAGTTTTATGCTCGGACTCATAAAATCGTGCAGAGTTTGAATCAACAGGCCCTCGATTTTACCAGGATTTTTTACAAATCCAGCAGAAAAACCGA
AAACTCAAAAACCTCAGCCAAAAAGAAAATACCAAGAAAGCAAACCTTAGTTCAATTTCAATTTCAACACAAAAACAACAACAACAATTTGTACATAGCTAACTAGTTGTAACACTCATAACTTTT
TTTTTTTTGAGAACCTATTTTTTCGATGGATAATATGCGCAGTGAAGCTATTTTTAATCATTAGTTTAACTAGTCTAAGCGAGAAAAACAATTTTTTTGTCTAGCCATAAGTTTTTAGCGCGA
AAAGAGATCTAACACAAAAATCGCAGTGGAAACAAAACCAATAAAAAACAAAAATCACACAAAAAgcgccgcg

>UTR insert CG5166a
actagtGACACCAGAAACCCAAAGTCATCATTCCAAGTTAGTTTTCCACCAGCGCAAGGAAAGGGCCGCGCTTCATCCAGCATTCCGATTGTAAACTTACTTAGCATATAATGTGAACTCGGTTT
GGAAGGAGCTGATCGCTGATCGCTGATCGAAGCTGCAAGCTGGATGGAAGCTCTTTGCTTGCCCTGCGGGAAATGAAAAACGAATGTGAGATTTAGAGAGCTTCAAATTTATTCGTTTCCTTTT
CGAAATTCGGTAGAACTAAATTAATTTTTGTTTAAATGAAATTTGTTGCCACTTCTCCGCCTCTTCTTACACATTATTCCGAGCATTACAGAAAATGTAATGACATCGATATATAAATGATTG
TTTTGACGTTTCTCGGAGAAATTTCTTGTAGCTTTACAGGCAGAAAGCTAATGTGAGAGCAAGAGCTTGAGTCAGGCTTCTTTGGGTTTTAGTGCCTCCGTTGTCTCCGAATTAATGAAAAAT
TAAACAAGAACAAATCCGTATTACTTCTTTGCCCGTCATAAATCGGTTTGGTTATATTTTCTGATGATCTAGAAGCATCTGTTGTGGTCTGTTTTGTTTTGTAAACCTTCAAGTTTCTTAAATGAAG
cgccgcg

>UTR insert CG5166a-mutant
actagtGACACCAGAAACCCAAAGTCATCATTCCAAGTTAGTTTTCCACCAGCGCAAGGAAAGGGCCGCGCTTCATCCAGCATTCCGATTGTAAACTTACTTAGCATATAATGTGAACTCGGTTT
GGAAGGAGCTGATCGCTGATCGCTGATCGAAGCTGCAAGCTGGATGGAAGCTCTTTGCTTGCCCTGCGGGAAATGAAAAACGAATCTCACATTTTAGAGAGCTTCAAATTTATTCGTTTCCTTTT
CGAAATTCGGTAGAACTAAATTAATTTTTGTTTAAATGAAATTTGTTGCCACTTCTCCGCCTCTTCTTACACATTATTCCGAGCATTACAGAAAATGTAATGACATCGATATATAAATGATTG
TTTTGACGTTTCTCGGAGAAATTTCTTGTAGCTTTACAGGCAGAAAGCTAATGTGAGAGCAAGAGCTTGAGTCAGGCTTCTTTGGGTTTTAGTGCCTCCGTTGTCTCCGAATTAATGAAAAAT
TAAACAAGAACAAATCCGTATTACTTCTTTGCCCGTCATAAATCGGTTTGGTTATATTTTCTGATGATCTAGAAGCATCTGTTGTGGTCTGTTTTGTTTTGTAAACCTTCAAGTTTCTTAAATGAAG
cgccgcg

>UTR insert CG6551
actagtTGATATCCACCCGATTCAAACCACAGCATCAGCATCCGCATCTATATTTCGCATCAGCAACAGGAAACCTCTTGCCATGCTACCCACACATCTGAGGACACTGATTGTTAGCTCAAGAC
AACCAACTGAAATCGAAACGCATTGAATTTAGATCAAATTCGAGCTGGTATCGAATATTAAACCATACAACAAACATAAACAAAAGGCTCCCTAAATGATTTAAATATTGGTCTGGTCCCTTTA
AGATTTAAAAATATCAATTAGTTTTTATGGAAATAGTTAGTTTCAATCGTAATAGGCATTTAAAAACATTTTACCCTAATTGAGTTTTTAAATCTCCAGAGGATTTCAACGCACCAATATTTTG
TACACAACACACATTTGTTAAATTTAAATTTTCACTCGAATTTCAAGTATTCTATTTTTGCAAAAATTTTGTGTAAATCTCGcgccgcg

>UTR insert CG6551-mutant
actagtTGATATCCACCCGATTCAAACCACAGCATCAGCATCCGCATCTATATTTCGCATCAGCAACAGGAAACCTCTTGCCATGCTACCCACACATCTGAGGACACTGATTGTTAGCTCAAGAC
AACCAACTGAAATCGAAACGCATTGAATTTAGATCAAATTCGAGCTGGTATCGAATATTAAACCATACAACAAACATAAACAAAAGGCTCCCTAAATGATTTAAATATTGGTCTGGTCCCTTTA
AGATTTAAAAATATCAATTAGTTTTTATGGAAATAGTTAGTTTCAATCGTAATAGGCATTTAAAAACATTTTACCCTAATTGAGTTTTTAAATCTCCAGAGGATTTCAACGCACCAATATTTTG
TACACAACACACATTTGTTAAATTTAAATTTTCACTCGCAGTGAAGTATTCTATTTTTGCAAAAATTTTGTGTAAATCTCGcgccgcg