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Intronic microRNA precursors that bypass Drosha processing

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Figure S1 – Mirtrons accumulate as lariats after splicing and require debranching enzyme (Ldbr) for conversion into functional pre-miRNAs. Page 2

Figure S2 – Confirmation of RNAi knockdowns. Page 3

Table S1 – Mirtrons of *Drosophila melanogaster*. Page 4

Table S2 – Mirtrons of *Caenorhabditis elegans*. Page 11

Table S3 – Quantification of signals from RNA blots of Figure 2c and 2d. Page 12

Table S4 – DNA construct sequences. Page 13

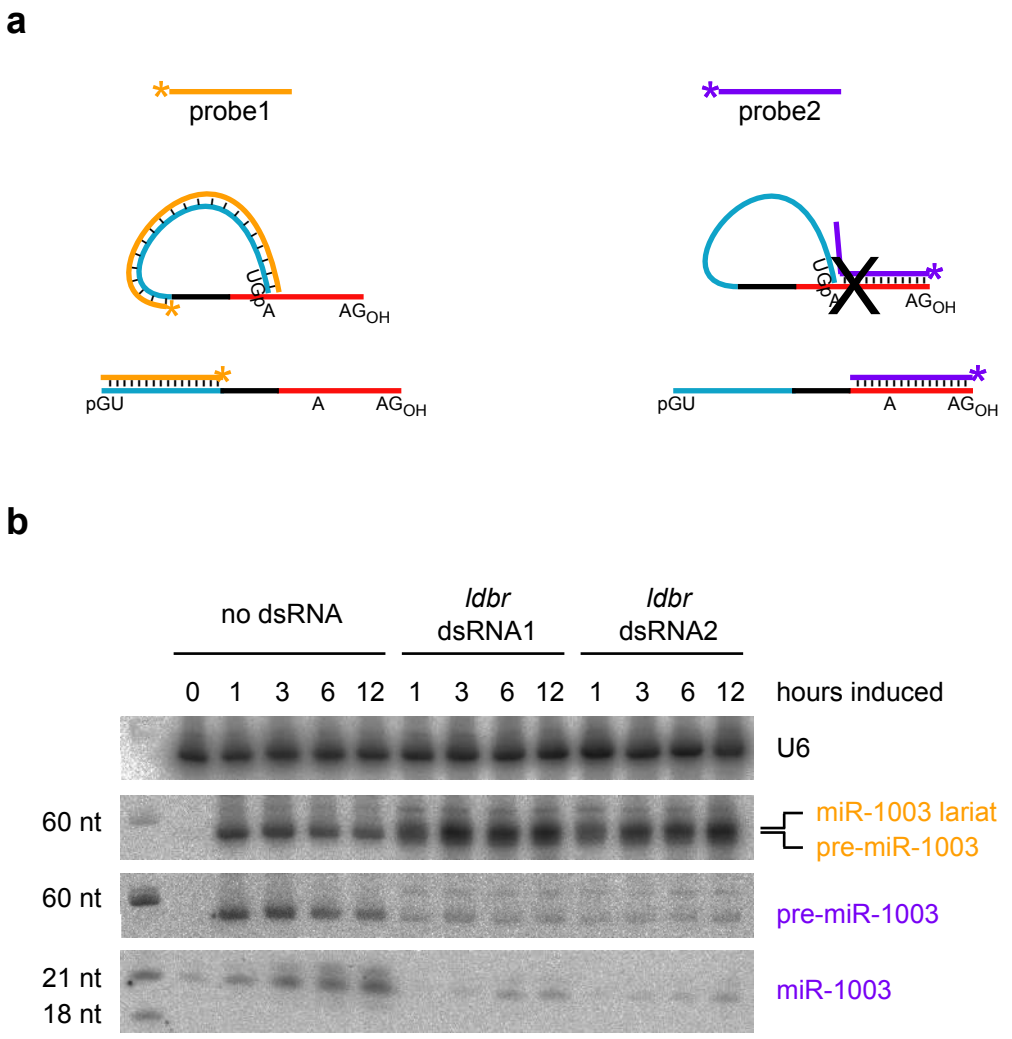


Figure S1. Mirtrons accumulate as lariats after splicing and require debranching enzyme (Ldbr) for conversion into functional pre-miRNAs. a, Left, hybridization of probe1 to miR-1003 intron lariat or linear pre-miR-1003. Right, stable hybridization of probe2 occurs only with linear pre-miR-1003, and 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 *ldbr* (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.

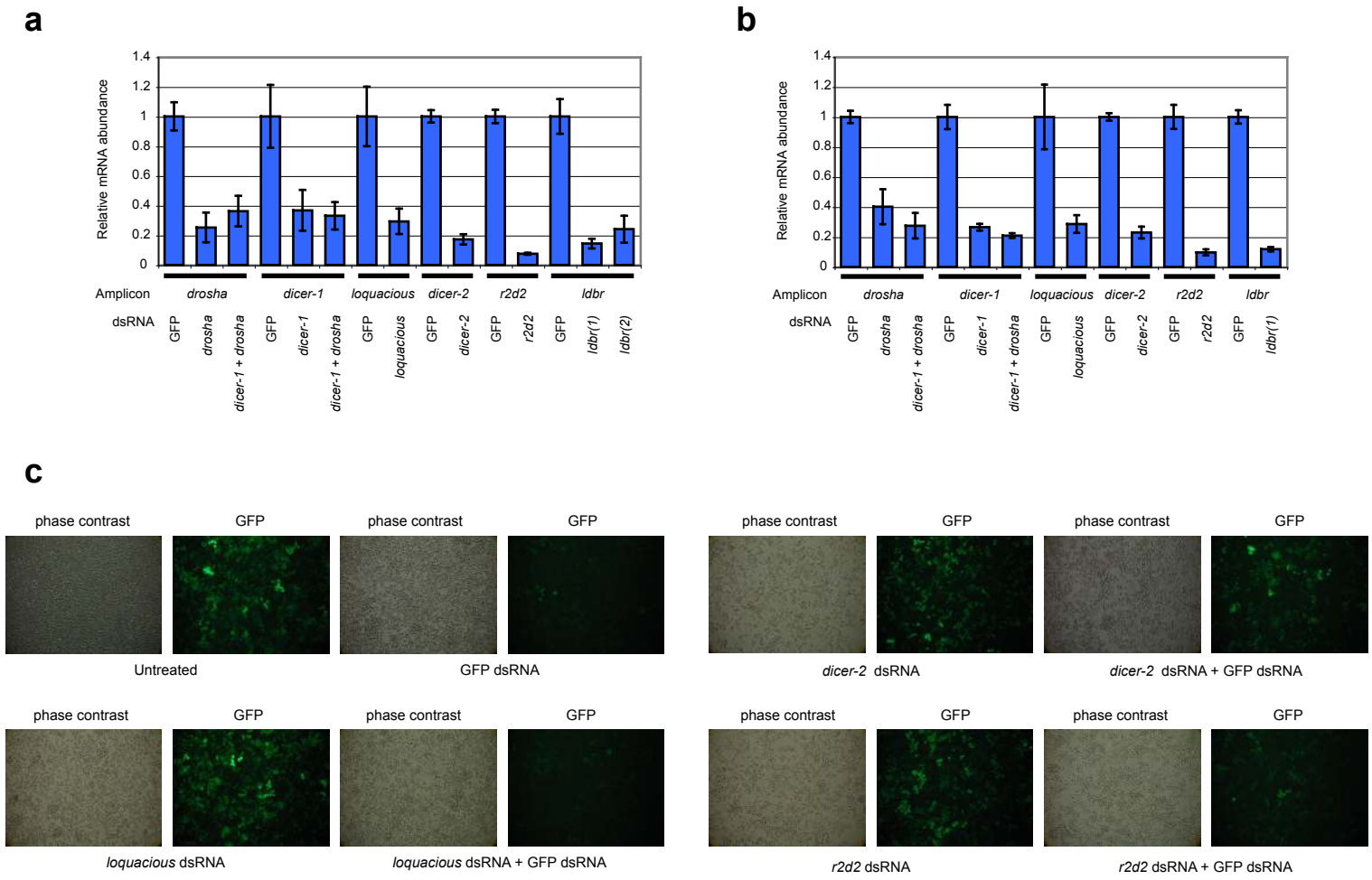


Figure S2. Confirmation of RNAi knockdowns. a, Quantitative RT-PCR analysis of samples from Fig. 2c. Relative abundance was measured using the ΔΔCt method, normalizing to *actin 5c* (ΔCt), and then to samples soaked in GFP dsRNA (ΔΔCt). Values are reported as geometric mean ± s.d. (n=3). **b,** Analysis 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-1003

 # reads: 352
Most abundant read: UCUCACAUUUACAUAUUCACAG
Host gene: CG6695-RA, CG6695-RB
Intron coordinates: chr3R:20484326-20484382(+)

	# reads	# loci
GUGGGUAUCUGGAUGUGGUUGGCUCUGGCGGUCCUCUCACAUUUACAUAUUCACAG		
((((((((.((((((((((..((..(....)..))..)))))))))).))))))))..		
GUGGGUAUCUGGAUGUGGUUG.....	6	1
GUGGGUAUCUGGAUGUGGUUGG.....	9	1
GUGGGUAUCUGGAUGUGGUUGGC.....	1	1
.....UCUCACAUUUACAUAUUCA...	3	1
.....UCUCACAUUUACAUAUUCAC..	15	1
.....UCUCACAUUUACAUAUUCACA.	84	1
.....UCUCACAUUUACAUAUUCACAG	233	1
.....CUCACAUUUACAUAUUCACAG	1	1

```
D.melanogaster  GTGGGTATC-TGGATGTGGTTGGCTCT------GGCGGTCCTCTCACATTTACATATTCACAG
D.simulans      GTGGGTATC-TGGATGTGGTTGGCTCT------GGCGGTCCTCTCACATTTACATATTCACAG
D.yakuba        GTGGGTATC-TGGATGTGGTTGGCTCT------GGCGGTCCTCTCACATTTACATATTCACAG
D.ananassae     GTGAGTATAGTGGATGTGGGTGGCTCTTATTTGGCCGGTCCTCTCACATCTCCATATTCACAG
D.pseudoobscura GTGGGTATCGTGCTTGTGGGTGGCTCT----TGGTCAGTCCTCTCACATCTCTATATTCACAG
D.virilis       GTGAGTAAT-CAGTTGTGGGTGGCTTT----TTGAAAGCCCTCTCACATCTCTTTATTCACAG
D.mojavensis    GTGAGTAAT-CAGTTGTGGGTGGCTCT----AGTGAAGCCCTCTCACATCTCTTTATTCACAG
```

mir-1004

 # reads: 50
Most abundant read: UCUCACAUCACUUCCCUCACAG
Host gene: CG31772-RA
Intron coordinates: chr2L:3767620-3767688(+)

	# reads	# loci
GUUGGGGGACAUUGAUCUCGGAGACGGCGGUUUAACUGAUCCAUUCUCUCACAUCACUUCCCUCACAG		
..(((((((...((((.(.(((((.(((((.....)))..))..))))))).).)))))..)))))))....		
.....UCUCACAUCACUUCCCUCACA.	4	1
.....UCUCACAUCACUUCCCUCACAG	46	1

```
D.melanogaster  GT-TGGGGGACAT----------TGATCTCGGAG------ACGGCGGTTTAACTGATCCAT--TCTCTCACATC-ACT---TCCC---------TCACAG
D.simulans      GT-TGGGGGACAT----------TGATCTCGGAG------ACGGCGGTTTAACTGATCCAT--TCTCTCACATC-ACT---TCCC---------TCACAG
D.yakuba        GT-TGGGGGACAT----------TGATCTCGGAG------ACGGCGGTTTAACTGATCCAT--TCTCTCACATC-ACT---TCCC---------TCACAG
D.ananassae     GT-GAGATAACACGTTCTAACCAACTGATATGAAACTACGAATGTTTTTATCG------CTGTCGATTCGAACGA-------TCTCTCATATA-ACCGTACCTA---------TTACAG
D.pseudoobscura GTGTGTGGGATAC----------TGATTTTAGAGAAAAAAAAAACCATTAACGTGAGGCTTCGTTTCTCACATC-ATTTT-CCCC---------TCACAG
D.virilis       GT-TGGGGCACAT----------TGATCTTCAAGAAACTCACAGCCAGCTCATTTACTCAC--TCTCTCTCTCT---TCTCTCCC---------TCACAG
D.mojavensis    GT--------AAT----------TGATC--------ACTCcta------tctctgtctctc--tttctctttctgactctctcccactctttATTTGTAG
```

Table S1

mir-1005

 # reads: 8
Most abundant read: UCUGGAAUCUUUAAUUCGCAG
Host gene: CG2969-RA CG2969-RB
Intron coordinates: chr2L:4343695-4343756(+)

	# reads	# loci
GUGAGUUGAUCGAUUUCGAGGUUUUGGCACACGAAUAUAAUCUGGAAUCUUUAAUUCGCAG		
(((((((((..(((((((.(((((((((.....))).....))))))))))).)))))))))..		
.....UCUGGAAUCUUUAAUUCGCA.	1	1
.....UCUGGAAUCUUUAAUUCGCAG	7	1

```
D.melanogaster  GTGAGT-------TGATCGATTTCGAGGTTTTGGCA-------------------------CACGA-------ATATAATCTGGAATCTTTAA-------TTCGCAG
D.simulans      GTGAGT-------TGATCGATTTCGAGTTTTTGGC-------------------------CACAA-------ATATAATCTGGAATCTTTAA-------TTCGCAG
D.yakuba        GTGAGT-------TGATCGATTTCGAGGTTTTGGCA-------------------------GCCAA-------AATAATCTGGAATCTTTAA-------TTCGCAG
D.ananassae     GTAAGT-------ACATTGTGGATATTGTTTATAC-------------------------------------TACAGCCTCTAATCTTATACTATATTTTTGCAG
D.pseudoobscura GTAAGTG------TCCATATCCTCGAGGCTCctgcaatgcactgcaatgcactgcaatgaccgcaatgcTGACGTATTATTTATGTTCTCCGA-------TCCCCAG
D.virilis       GTAAGGGCTGA--ATTTTAAAATTG-AATTT-----------------------------AACAAGTATACAACAATATTATAATCC----------CACACAG
D.mojavensis    GTAAGCATAGAGCAGATCAGATTTATGATTT-----------------------------CACATATTCTCAATACTGCTTCGATCC----------TCCACAG
```

mir-1006

 # reads: 57
Most abundant read: UAAAUUCGAUUUCUUAUUCAUAG
Host gene: CG17332-RA CG17332-RB CG17332-RD
Intron coordinates: chr2L:16720723-16720787(-)

	# reads	# loci
GUGAGUUUGAAAUUGAAAUGCGUAAAUUGUUUGGUACAAUUUAAAUUCGAUUUCUUAUUCAUAG		
((((((..((((((((((.....(((((((((.....)))))))))..))))))))))..))))))..		
.....UAAAUUCGAUUUCUUAUUCA...	1	1
.....UAAAUUCGAUUUCUUAUUCAU..	12	1
.....UAAAUUCGAUUUCUUAUUCAUA.	8	1
.....UAAAUUCGAUUUCUUAUUCAUAG	35	1
.....AAAUUCGAUUUCUUAUUCAUAG	1	1

```
D.melanogaster  GTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
D.simulans      GTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
D.yakuba        GTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
D.ananassae     GTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
D.pseudoobscura GTGAGTTTGAAATTGAAATGTGTAAATTGTTTGTGCCAATTTAAATTCGATTTCTTATTCATAG
D.virilis       GTGAGTTTGAAATTGAAATATGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
D.mojavensis    GTGAGTTTGAAATTGAAATGTGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAG
```

Table S1

mir-1007

 # reads: 9
Most abundant read: UAAGCUCAAUUAACUGUUUGCA
Host gene: CG1718-RA
Intron coordinates: chrX:21107060-21107125(-)

```
                                                                    # reads        # loci
GUAAGCAGUGUUUGAACUCGAUCUUGGUUCUUGGACUCUUGAUAAGCUCAAUUAACUGUUUGCAG
(((((((((..((((.((..(((..((((....))).)..))).)).))))...))))))))).. 
.........................................UAAGCUCAAUUAACUGUUUGC..          2             1
.........................................UAAGCUCAAUUAACUGUUUGCA.          6             1
.........................................UAAGCUCAAUUAACUGUUUGCAG          1             1

D.melanogaster  GTAAGCAGTGTTTGAACTCGATC--TTGGTTC----TTG---GACTCT-------TGATAAGCTCAATTAACTGTTTGCAG
D.simulans      GTAAGCAGTGTTTGAACTCGATC--TTGGTTC----TTG---GACTCT-------TGATAAGCTCAATTAACTGTTTGCAG
D.yakuba        GTAAGCAGTGTTTGAACTCGATC--TAGGATC----TTG---GACTCT-------TGATAAGCTCAATTAACTGTTTGCAG
D.ananassae     GTAAGCAGTGTTTGAACTCGATC--TTGGAAT----------AGCTCC-------CGATAAGCTCAATTAACTGTTTGCAG
D.pseudoobscura GTAAGCAGCGATTGA--TCAATCaattgaatc----------gaatcgaatcgaatGATAAACTCCATTAACTGTTTGCAG
D.virilis       GTAAGCAGTGCTTGAGCTTATTC--TCTGGCTTCATTTGACCATTTTC-------TGATAAGCTCAATTAACTGTTTGCAG
D.mojavensis    GTAAGCAGTGTTTGAACTAAATC--TCTGGCT--ACTTGGCCGTATAT-------TGATAAGCTCAACTAACTGTTTGCAG
```

mir-1008

 # reads: 46
Most abundant read: UCACAGCUUUUUGUGUUUACA
Host gene: CG18004-RA CG18004-RB
Intron coordinates: chr2R:6401439-6401496(+)

```
                                                                    # reads        # loci
GUAAAUAUCUAAAGUUGAACUUGGCCAAUGGCAAGUCACAGCUUUUUGUGUUUACAG
((((((((..(((((((.(((((.(....).)))))..)))))))..))))))))..
GUAAAUAUCUAAAGUUGAACU...................................          1             1
...................................UCACAGCUUUUUGUGU......          1             1
...................................UCACAGCUUUUUGUGUUU....          1             1
...................................UCACAGCUUUUUGUGUUUAC..          6             1
...................................UCACAGCUUUUUGUGUUUACA.         22             1
...................................UCACAGCUUUUUGUGUUUACAG         14             1
....................................CAGCUUUUUGUGUUUACAG          1             1

D.melanogaster  GTAAATAT----CTAAAGTTGAAC---TTGGCCAATGGCAAGTCACA----GCTTTTTGTGTTTACAG
D.simulans      GTAAATAT----CTAAAGTTGAAC---TTGGCCAACGGCAAGTCACA----GCTTTTTGTGTTTACAG
D.yakuba        GTAAATAT----CTAAAGTTGAAC---TTGGCCAACGGCAAGTCACA----GCTTTTTGTGTTTACAG
D.ananassae     GTAAGGAA----CTCAATTTTTAC--ATTAAACCGAAGCAATTTAAC----ACGTTTCTTATTT-CAG
D.pseudoobscura GTAAGGGATCGGCGAGAGTTTTTCCCACGGAATATCATTATATTATA----TTGTTATGTGCCTGCAG
D.virilis       GTAAGTGA----TGAT--GCGTCC---ATTGGGAATATCATTTAATT----------TGTGTTGGTAG
D.mojavensis    GTAAGTAG----TAATAGGTGTTT---GTAGACATATTCAGTTAATTTTCGCATTTGTGTATTGGCAG
```

Table S1

mir-1009

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

	# reads	# loci
GUAAGUGUAAGACUUUCUUGAGUUACCCGCGAUGAGUAUCUCAAAAAUUGUUACAUUUCAG		
(.(((((((((.(.(((.((((((.(((.(.....).))).)))))))).).).))))))))..		
.UAAGUGUAAGACUUUCUUGAGU.....	1	1
.....UCUCAAAAAUUGUUACAUUUCA.	3	1
.....UCUCAAAAAUUGUUACAUUUCAG	10	1

```
D.melanogaster  GTAAGTGTAAGACTTTCT------TGAGTT----ACCCGCGATGAGTATCTCAAAAATTGT--TACATTTCAG
D.simulans      GTAAGTGTAAGACTTTCT------TGAGTT----ACCCGCGATGAGTATCTCAAAAATTGT--TACATTTCAG
D.yakuba        GTAAGTGTAAGACTTTCT------TGAGTT----ACCCGCGAGGAGTATCTCAAAAATTAT--TACATTTCAG
D.ananassae     GTAAGTTTTGAATACTTC------TACTCT----ATCTTGGA-ATGTCTCTCATAATTGTC--CATCTTCTAG
D.pseudoobscura GTAAGTTCGAGAATCCCAAACACATAAGTT----CTTTTTTA-----ACATCCAAA-------TATTTTGTAG
D.mojavensis    GTAAGGACCAA---TTGG------TAAAGTGAGAAATTGGAAGAAATTTATTGAAATTTATACTCGTGTTTAG
```

mir-1010

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

	# reads	# loci
GUAAGUGGUGUAGAUGAAACAAAUUUACCAACAAUUUUGUUGGAUUGUUUCACCUAUCGUUCCAUUUGCAG		
(((((((.((((.(((((((((.....(((((((....)))))))..)))))))).)))).....))))))))..		
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
.....UUCACCUAUCGUUCCAUUUGC..	3	1
.....UUCACCUAUCGUUCCAUUUGCA.	1	1
.....UUCACCUAUCGUUCCAUUUGCAG	1	1

```
D.melanogaster  GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AAT----TTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.simulans      GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AATA-TTTTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.yakuba        GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AATATTTTTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.ananassae     GTAAGTGGTGTAGATGAAACAAATTTACCAAC-AATA-TTTTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.pseudoobscura GTAAGTGGTGTAGATGAAACAAATTAACCAAC-CTTT-ATTGTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.virilis       GTAAGTGGTGTAGATGAAACAAATTTCACAACAAAT--TTTTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
D.mojavensis    GTAAGTGGTGTAGATGAAACAAATTTCACAAC-AAT--TTTTTGTTGGATTGTTTCACCTATCGTTCCATTTGCAG
```

Table S1

mir-1011

 # reads: 2
Most abundant read: UUAUUGGUUCAAAUCGCUCGCAG
Host gene: CG17274-RA CG17274-RB
Intron coordinates: chr3R:16679026-16679080(-)

```
                                                                  # reads        # loci
GUGAGUUUUUGAGCCAGGAAUAUAGUUCUUAUUAUUGGUUCAAAUCGCUCGCAG
((((((.(((((((((((.((((.......)))).))))))))))..))))))..
..............................UUAUUGGUUCAAAUCGCUCGCAG              2             1

D.melanogaster  GTGAGTTTTTGAGCCAGG-----AATATAGTT--------CTTAT-----TAT-TGGTTCAAATCGCTCGCAG
D.simulans      GTGAGTTTTTGAGCCAGG-----AATATAGTT--------CTTAT-----TAT-TGGTTCAAATCGCTCGCAG
D.yakuba        GTGAGTTTTTGAGCCAGG-----AATATAATT--------CTTAT-----TAT-TGGTTCAAATCGCTCGCAG
D.ananassae     GTGAGTCTTTGAACCAGG-----AATATAATT--------TGTAT----ATAT-TGGTTCAAATCGCTCGTAG
D.pseudoobscura GTGAGATTTTGAATCTAATATATAATATAATC--------CGTACGTGTATATATGGTTCAAATTACTCGTAG
D.virilis       GTGAGTCATTGAACCAGG-----AATATATGTATGTAATTCTTAT----ATAT-TGGTTCAAATTTCTCGCAG
D.mojavensis    GTGAGTCTTTGAGCCAGG-----AATATATGTTCAT----CTTAT-----TAT-TGGTTCAAATCTCTCGTAG
```

mir-1012

 # reads: 101
Most abundant read: UUAGUCAAAGAUUUUCCCCAUAG
Host gene: CG31072-RA CG31072-RB
Intron coordinates: chr3R:22687070-22687129(-)

```
                                                                  # reads        # loci
GUGGGUAGAACUUUGAUUAAUAUUGCUUGAAAAAUAUUAGUCAAAGAUUUUCCCCAUAG
(((((.(((((((((((((((((((.........)))))))))))))).)))).)))))..
GUGGGUAGAACUUUGAUUA.......................................          1             1
GUGGGUAGAACUUUGAUUAA......................................          5             1
GUGGGUAGAACUUUGAUUAAU.....................................         20             1
GUGGGUAGAACUUUGAUUAAUA....................................         16             1
GUGGGUAGAACUUUGAUUAAUAU...................................          1             1
...................................UUAGUCAAAGAUUUUCCCCAUA.          2             1
...................................UUAGUCAAAGAUUUUCCCCAUAG         56             1

D.melanogaster  GTGGGTAGAACTTTGATTAAT-----------------------ATTGCTTGAAAAAT-------ATTAGTCAA----AGATTTT-C------CCCATAG
D.simulans      GTGGGTAGAACTTTGATTAAT-----------------------ATTGCTTGAGAA-T-------ATTAGTCAA----AGATTTT-C------CCCATAG
D.yakuba        GTGGGTAGAACTTTGATTAAT-----------------------ATTGCTTGCAAGAT-------ATTAGTCAA----AGGTTTTTC------CCCATAG
D.ananassae     GTAGGT-----TTCAACCAAA-----------------------TTTCCTTTGAGAGT--------TCAGTTAACTTTATATATT-C------TTTTTAG
D.pseudoobscura GTGGGTAGT-CTCTCATATAT-----------------------AGTTATAAAAGAACGAACACCAGTGGTTAA-GCAATGCATT-T------CTTGTAG
D.virilis       GT---------ACGGATTGTTTATTTA-----------------AATGCTTTATATAT--------TTATCTAT----AAGCTAT-CTTTTTGTTTGCAG
D.mojavensis    GTGTGTAAA-TATGGATTATT-ATTTATAAATTATCGAAAACTTAACTTCTAATGTTT--------TTATATTT----ATATTTT-CAACATACTCTCAG
```

Table S1

mir-1013

 # reads: 17
Most abundant read: AUAAAAGUAUGCCGAACUCG
Host gene: CG12072-RA
Intron coordinates: chr3R:26617357-26617418(-)

	# reads	# loci
GUGAGUUUCGUACACUUAAUUAAUAGGAUCGGCCGUUAAUAAAAGUAUGCCGAACUCGCAG		
((((((((.(((.((((.(((((((.((.....)))))))).)))).)))).))))))))..		
.....UAAUAGGAUCGGCCGUUAAU.....	2	1
.....AUAAAAGUAUGCCGAACUCG...	4	1
.....AUAAAAGUAUGCCGAACUCGC..	4	1
.....AUAAAAGUAUGCCGAACUCGCA.	2	1
.....AUAAAAGUAUGCCGAACUCGCAG	4	1
.....UAAAAGUAUGCCGAACUCGCAG	1	1

D.melanogaster	GTGAGTT------TCGTACACTTAATTAATAGGATCGGCCGTTAATAAAAGTATGCC---GAACTCGCAG
D.simulans	GTGAGTT------TCGTACACTTAATTAATAGGATCGGCCGTTAATAAAAGTATGCC---GAACTCGCAG
D.yakuba	GTGAGTT------TCGTACACTTAATTAATTGGGACGGCCGTTAATAAAAGTATGCC---GAACTCGCAG
D.ananassae	GTAATCT------TTGAATAATTATCTGTGAGTTGTGGCATCTAATGATTGT--------TATCTTCCAG
D.pseudoobscura	GTAAGTCCATGAATTGCATCCCCCTTTGAT-----TATTCTTTAATCTGGAAATCCCTGTGATCCCATAG

mir-1014

 # reads: 3
Most abundant read: AAAAUUCAUUUUCAUUUGCAG
Host gene: CG2196-RA
Intron coordinates: chr3R:27579245-27579313(-)

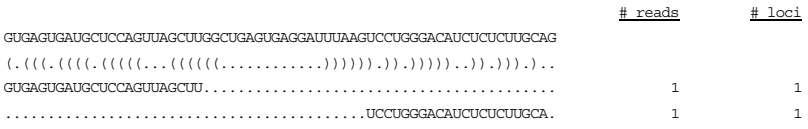
	# reads	# loci
GUAUAAUGGAAAUAGAUUUUAAUCGCAGGCGCGUCAGUGGUUGAAUUAAAAAUUCAUUUUCAUUUGCAG		
(((.((((((((((..((((((((..(((.(((....))).)))).)))))))))..))))))))))))..		
.....UAAAAUUCAUUUUCAUUUGCAG	1	1
.....AAAAUUCAUUUUCAUUUGCAG	2	1

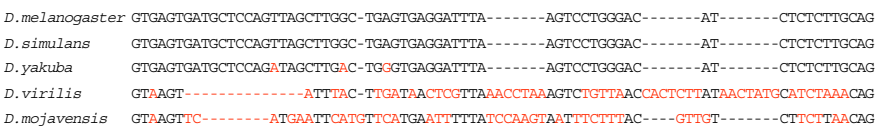
D.melanogaster	GTATAATGGAAATAGATTTTAATCGCAGGCGCGTCAGTGGTTGAATTAAAATTCATTTTCATTTGCAG
D.simulans	GTATAATGGAAATAGATTTTAATCGCTGGCGCGTCAGTGGTTGAATTAAAATTCATTTTCATTTGCAG
D.yakuba	GTATAATGGAAATAGATTTTAATCGCAGGCGCGTCAGTGGTTGAATTAAAATTCATTTTCATTTGCAG
D.ananassae	GTATAATGAAAATTGATTTTAATCACACGGATCGGAGTGGCAAAATTAAAATTCATTTTCATTTGCAG
D.pseudoobscura	GTACAATGGAAATAGATTTTAATCGGGTTTCGTTTGGCGGTGAAATTAAAATTCATTTTCATTTACAG

Table S1

mir-1015

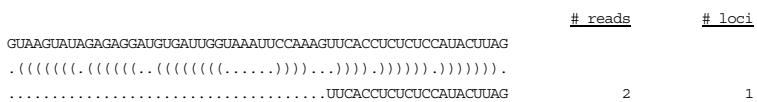
 # reads: 8
Most abundant read: UCCUGGGACAUCUCUCUUGCAG
Host gene: CG6432-RA
Intron coordinates: chr3R:20164953-20165017(+)





mir-1016

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



mir-1017

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

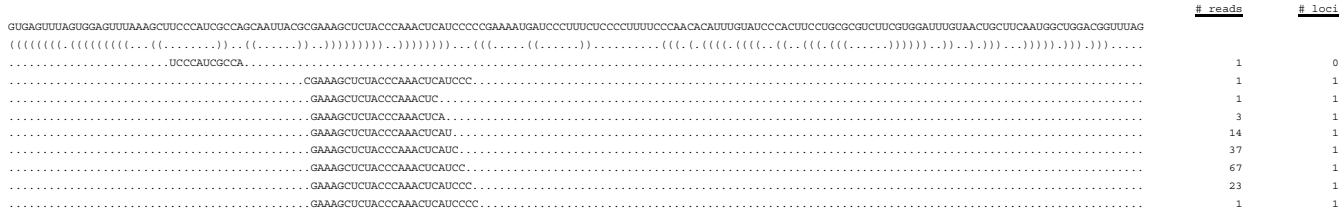


Table S2

mir-62

 # reads: 1071
Most abundant read: UGAUAUGUAAUCUAGCUUACAG
Host gene: T07C5.1b T07C5.1c
Intron coordinates: chrX:12692524-12692582(+)

	# reads	# loci
GUGAGUUAGAUCUCAUAUCCUUCCGCAAAAUGGAAAUGAUAUGUAAUCUAGCUUACAG		
((((((((((..((((((.((((((.....)))))..))))))..))))))))))..		
.....AUGAUAUGUAAUCUAGCUUACAG	2	1
.....UGAUAUGUAAUCUAGCUUAC..	2	1
.....UGAUAUGUAAUCUAGCUUACA.	58	1
.....UGAUAUGUAAUCUAGCUUACAG	1008	1
.....UAUGUAAUCUAGCUUACAG	1	1

C.elegans GTGAGTTAGATCTCATATCCTTCCGCAAAATGGAAATGATATGTAATCTAGCTTACAG
C.briggsae GTGGGTTAGATCCCATATCCTTCCGCTTGATGGAAATGATATGTAATCTAGCTTACAG

mir-1018

 # reads: 2
Most abundant read: AGAGAGAUCAUUGGACUUACAG
Host gene: Y59E1B.1
Intron coordinates: chrX:1879451-1879507(+)

	# reads	# loci
GUAAGUUCAUGAUUUCUCCCAUAUAUUUUUCAUGAGAGAGAUCAUUGGACUUACAG		
((((((((((((((((((.(((.........))).)))))))))).))))))))..		
.....AGAGAGAUCAUUGGACUUACAG	2	1

mir-1019

 # reads: 2
Most abundant read: GUGAGCAUUGUUCGAGUUUCAUUU
Host gene: M04C9.5
Intron coordinates: chrI:9369650-9369719(+)

	# reads	# loci
GUGAGCAUUGUUCGAGUUUCAUUUUUAAUAAAAUUUAUUUAAAAACUGUAAUUCCACAUUGCUUUCCAG		
..((((((.(((..((((((.((((((((((.........)))))))).)).)))))).))).))))))....		
GUGAGCAUUGUUCGAGUUUCAUUU.....	1	1
.....CUGUAAUUCCACAUUGCUUUCCAG	1	1

C.elegans GTGAGCATTGTTCG-AGTTTCatttttaataaaatttatttaaaaaCTGTAATTCCACATTGCTTTCCAG
C.briggsae GTTTGCATTCTTTGAAGTGTCACAGTT----------CTATAACGTCGCA----GTTCCAG

mir-1020

 # reads: 2
Most abundant read: GUAAGUGUUACAGAAUAAUCU
Host gene: T16G12.1
Intron coordinates: chrIII:10047630-10047700(-)

	# reads	# loci
GUAAGUGUUACAGAAUAAUCUUAGACAAAACAACUAAAAUUAAUGAAAAAUUAUUCUGUGACACUUUCAG		
(.((((((((((((((((((.....)))))))))))))))))).))..		
GUAAGUGUUACAGAAUAAUCU.....	1	1
.....AUUAUUCUGUGACACUUUCAG	1	1

C.elegans GTAAGTGTTACAGAATAATCTTAGACAAAACAACTAAAATTAATGAAAAATTATTCTGTGACACTTTCAG
C.briggsae GTGAGGAACACATACAAATGTTGGAT----------TTTATTC----GAATTTTCAG

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	*drosha*	*dicer-1*	*loquacious*	*dicer-2*	*r2d2*	*drosha* + *dicer-1*	*ldbr*(1)	*ldbr*(2)
pre-*let-7* miRNA	1.0	0.03	3.54	0.90	0.51	0.60	0.14	0.48	0.91
let-7 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	*drosha*	*dicer-1*	*loquacious*	*dicer-2*	*r2d2*	*drosha* + *dicer-1*	*ldbr*(1)
pre-*let-7* miRNA	1.0	b.d. (<.05)	4.56	2.19	1.15	1.51	0.15	1.05
let-7 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)
actagtAACAACGAATACATCCAGTCGGTGGCCCGGTGCCTGCAGATGATGCTCCGTGTGGATGAGTATCGATTTGCCTTTGTGGGAGTCGACGGAATCAGCACTCTGATCCGTATCTTGTCGAC
CCGTGTCAACTTCCAGGTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAGGTGCAATACCAGTTGATCTTTTGCCTGTGGGTGCTGACCTTCAAT
CCCCTGCTGGCCGCCAAGATGAATAAGTTCAGCGTGATCCCCATCCTGGCTGATATCCTCAGCGATTGTGCCAAGGAGAAGGTGACACGCATTATCCTCGCGGTCTTCCGCAATCTGATCGAGAA
GCCGGAGGATTCATCGGTGGCCAAGGACCATTGCATCGCCATGGTCCAGTGCAAGGTGCTGAAGCAGCTATCCATCCTGGAGCAGCGTCGCTTCGACGACGAGGACATTACCGCCGACGTAGAGT
ACCTGAGCGAGAAGCTCCAGAATTCGGTGCAAGACTTGAGCTCCTTTGATGAGTACGCCACAGAGGTGCGCAGCGGTCGCTTGGAATGGTCGCCTGTGCACAAGTCGGCCAAGTTCTGGCGCGAG
AATGCCCAGCGCCTAAACGAAAAGAACTACGAGTTGCTGCGCATCCTCGTCCACCTCCTGGAAACCTCAAAAGATGCCATCATCCTTTCCGTCGCCTGCTTCGACATCGGGGAGTATGTGCGCCA
CTATCCCCGCGGCAAGCAgcggccgc

>pCJ20 (pMT-puro_miR-1003)
actagtATAAAGCCGATAAGCGTGCGGAAATCGAAAAGACCGAGCGTGAACGACTGCAACAGCAGGAACGCGAGGATGAGATGCGCGAACTGGCCCTCAAGCTGCGCAGAAGGTGGGTATCTGGA
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AGCCAGCTGGCAAAGTCGTCGACGCGTCGCAGTCGCAGCAGATCGGAGAGTCGTAGGCGGTCGCCACCGCCGGAAACACAGCACAGCAGACGGAGCAGCAGCCGAACGGAAAGGAGGAGACGCAC
AAGGACGgcggccgc

>pCJ24 (pMT-puro_let-7)
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ATATGTAATATGAAAACCATCGATAATAAATCGTAAAACTAAATTAAACTAAATATCCAACGTCATATAAATACAACTCAAAACGTCATAATAAGAAAACTGATATGGTATAACAAACTCAAGTT
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>pCJ30 (p2032_miR-1003)
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TGTGGTTGGCTCTGGCGGTCCTCTCACATTTACATATTCACAGGCGCCGTGAGCTGCGTCACAAATATGGAACGCCCTCGAGTGGAAAGCTCTCGGACAGCGATGCCGAATCTGTGGCATCGGAA
AGCCAGCTGGCAAAGTCGTCGACGCGTCGCAGTCGCAGCAGATCGGAGAGTCGTAGGCGGTCGCCACCGCCGGAAACACAGCACAGCAGACGGAGCAGCAGCCGAACGGAAAGGAGGAGACGCAC
AAGGACGgcggccgc

>pCJ31 (p2032_miR-1006)
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CCGTGTCAACTTCCAGGTGAGTTTGAAATTGAAATGCGTAAATTGTTTGGTACAATTTAAATTCGATTTCTTATTCATAGGTGCAATACCAGTTGATCTTTTGCCTGTGGGTGCTGACCTTCAAT
CCCCTGCTGGCCGCCAAGATGAATAAGTTCAGCGTGATCCCCATCCTGGCTGATATCCTCAGCGATTGTGCCAAGGAGAAGGTGACACGCATTATCCTCGCGGTCTTCCGCAATCTGATCGAGAA
GCCGGAGGATTCATCGGTGGCCAAGGACCATTGCATCGCCATGGTCCAGTGCAAGGTGCTGAAGCAGCTATCCATCCTGGAGCAGCGTCGCTTCGACGACGAGGACATTACCGCCGACGTAGAGT
ACCTGAGCGAGAAGCTCCAGAATTCGGTGCAAGACTTGAGCTCCTTTGATGAGTACGCCACAGAGGTGCGCAGCGGTCGCTTGGAATGGTCGCCTGTGCACAAGTCGGCCAAGTTCTGGCGCGAG
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CTATCCCCGCGGCAAGCAgcggccgc

>pCJ32 (p2032_Let-7)
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TACAAAAGTTGGTGGTGCAACAAATTTTGATTGTGGACAACAAAGAAAGTGTTCTGAGCCAAACTATTGTTAAATATCATTACGAATGCCAAAGTATGTAAATGCAACCGGGCATATGTAAATAT
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>Drosha dsRNA
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AACACATTAGTGAGCATTATGT

>Dicer-1 dsRNA
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TGTCTATCTCAGTTGCGAGGTTGGCACCAGCACGGAACCATGCTCCATCTACACGATGCTCACCCACTTGACTGACCTGCGGGTGTGGCAGGAGCAGCCGGATATGCAAATTCCCTTTGATCATT
GCTGGACGGACTATCACGTTTCCATCCTACGGCCAGAGGGATTTCTTTATCTGCTCGAAACTCGCGAGCTGCTGCTGAGCAGCGTCGAACTGATCGTGCTGGAAGATTGTCATGACAGCGCCGTT
TATCAGAGGATAAGGCCTCTGTTCGAGAATCACATTATGCCAGCGCCACCGGCGGACAGGCCACGGATTCTCGGACTCGCTGGACCGCTGCACAGCGCCGGATGTGAGCTGCAGCAACTGAGCGC
CATGCTGGCCACCCTGGAGCAGAGTGTGCTTTGCCAGATCGAGACGGCCAGTGATATTGTCACCGTGTTGCGTTACTGTTCCCGACCGCACGAATACATCGTACAGTGCGCCCCCTTCGAGATGG
ACGAACTG

>Loquacious dsRNA
ATGGACCAGGAGAATTTCCACGGCTCCAGCTTGCCGCAGCAGCTACAGAACCTCCACATCCAGCCGCAGCAGGCGTCCCCCAATCCTGTCCAGACGGGATTTGCTCCACGGCGGCACTATAATAA
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CAGGTCAGCTGCAGCTGTCAGATGTTGGTGATCCTGCCTTGGCGGGCGGATCGGGCTTACAAGGTGGAGTCGGCCTTATGGGCGTAATATTGCCCAGCGACGAGGCC

>Dicer-2 dsRNA
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CGGAATGTCGACCCCTTGGGCAATGTCATACCAACTGAGGATATCGAGGAGCCGAAATCCCTTTTAGAGCCAATGCCCACAAAGTCCATTGAGGCGTCCGTGGCCAATCTTGAAATAACAGAATT
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>R2D2 dsRNA
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CTGCCCTGCATTGAGGTAGTGCAGCAAAGCGGCACCCCGAGCGCCCCGGAATTCGTGGCCTGTTGCTCCGTGGCCTCCATAGTACGCTACGGAAAGTCGGACAAAAAGAAGGATGCCCGTCAGCG
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AGGCATTGCGCAGAAAGAAATTTACCA

>CG7942[1](Debranching enzyme) dsRNA
GTGGCGAGCTGGTAGCACCAGTGCTGACCATATTCATTGGCGGCAACCATGAGGCCTCCAATTACCTGCAGGAGCTCCCATACGGCGGTTGGGTGGCTCCAAATATTTACTACCTTGGTTATGCC
GGCGTCGTCAATGTAAACGGTGTTCGGATAGCTGGAATAAGCGGAATCTTCAAGGGTCACGACTTTTTGCGCGGCCATCACGAATTCCCTCCGTATACCGATTCCACGTGTCGCAGTGTCTACCA
TGTGCGGCAGCTAGAAGTCTTTCGGCTGAAACAAATTTCCGGGCGAGTTGATATTTTCCTGTCCCACGACTGGCCCACCGGCATCTATGAATACGGAAACAAGGCGCAACTGCTCCGCAAGAAAC
CATTTTTTGCTGCAGACATGGAAAGCGGGAAGCTGGGTAGCCAGCCACTGGAGGAGTTACTGAAAGCGGTCCAACCGGCCTACTGGTTTGCTGCCCATTTGCATTGCAAGTTTGCCGCTTTGGTG
CCGCACAATCACAGCCAGAAGCTAGGAGATGCTGAATC

>CG7942[2](Debranching enzyme)
GCAGCAGTGAAGATGAAGACGAGGAAAGGGAGAAGGTAAAGAAAGCTGCTCCTGTACCTCCACCATCCAAATCTGTTCCCGTGACCAAGTTTCTGGCTCTCGACAAATGCCTGCCACGTCGTGCT
TTCCTGCAAGTGGTAGAGGTACCCAGTGACCCCATCGAAGGCACTCCCCGCCTGGAATACGACGCAGAGTGGCTAGCCATCTTGCACAGTACAAATCACTTGATTTCAGTGAAGGAGAATTATTA
TTACCTGCCCGGAAAAAAGGCGGGAGAGTTTACAGAGCGATCAAACTTTACCCCCACTGAAGAAGAACTAGAAGCAGTGACCGCAAAGTTTCAGAAACTTCAAGTCCCCGAGAACTTTGAGCGCA
CAGTGCCAGCTTTCGATCCCGCGGAGCAGTCTGATTATAAGCACATGTTTGTGGATCAACCCAAGGTTCAACTAAACCCCCAGAGCAATACGTTCTGTGCCACTCTGGGTATAGACGATCCGCTG
TGCTTAGTTTTGTTGGCGAATGGCCTAGATCTGCCTGC

Table S4

>GFP dsRNA
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CTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTG

>UTR insert CG11094
actagtTGATAATTTTTCATTAACTAGAGTAACGAATACTACTTTGCCCCGATATTTATTATTGTTCAGCATCACATATTAGCTTAATGCTTCGGTGAAATCGCGCGAATTTAACTTTTATAACT
TAGAGTTGAGTAACTTAGAGTTTTATGGAGCAAAACCTCTGTAAATAAATCGAATTTATCGGTAAACTAAAGCGCGACTTGGACTATCTTCAATCAACAAGCCAAATATGTCGATGTGTGACAGC
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AATCgcggccgc

>UTR insert CG11094-mutant
actagtTGATAATTTTTCATTAACTAGAGTAACGAATACTACTTTGCCCCGATATTTATTATTGTTCAGCATCACATATTAGCTTAATGCTTCGGTGAAATCGCGCGCAGTGAACTTTTATAACT
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AATCgcggccgc

>UTR insert CG1849
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>UTR insert CG1849-mutant
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>UTR insert CG5166a
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cggccgc

>UTR insert CG5166a-mutant
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cggccgc

>UTR insert CG6551
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>UTR insert CG6551-mutant
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