LIFE

Leipzig Research Center
for Civilization Diseases

UNIVERSITÄT LEIPZIG

Faculty of Medicine

What Would DICER Do?

by dem Kommissar

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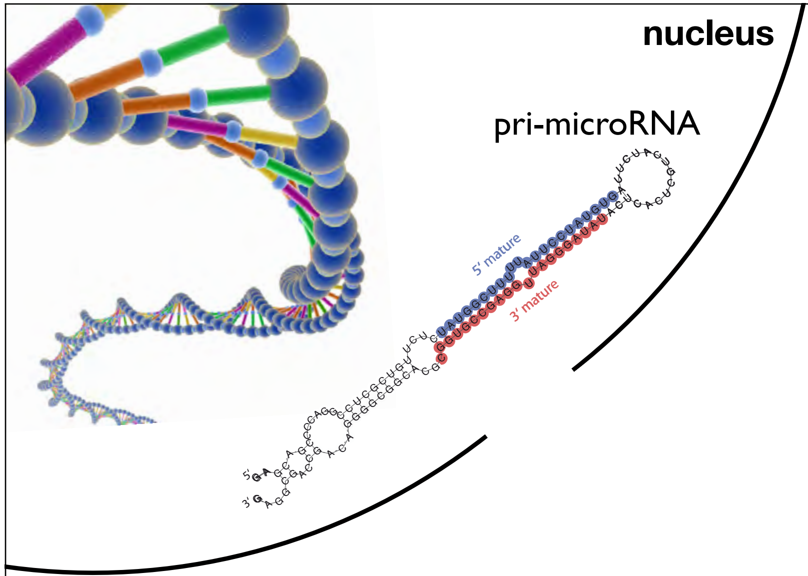
Europa fördert Sachsen.
ESF
Europäischer Sozialfonds

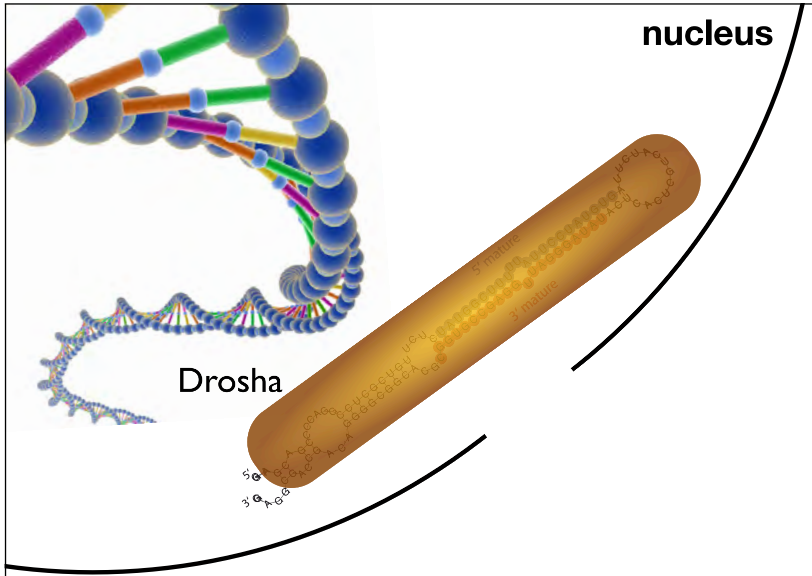
Freistaat Sachsen

microRNA pathway



nucleus / **cytoplasm**

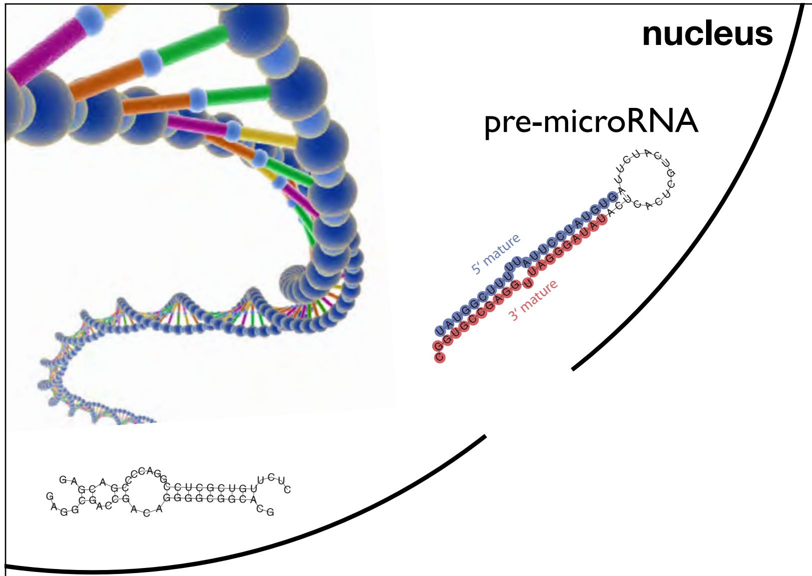
pri-microRNA



nucleus

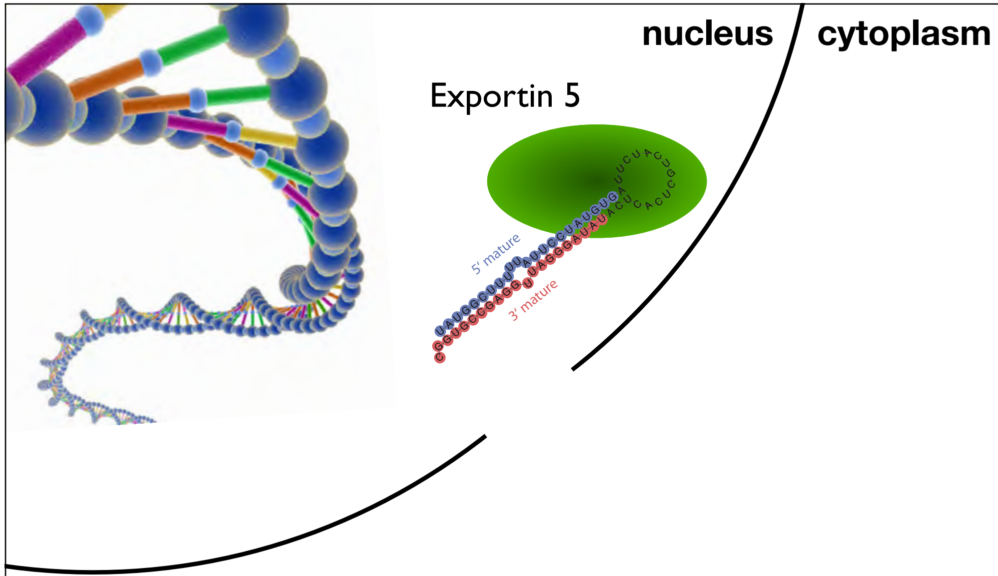
cytoplasm

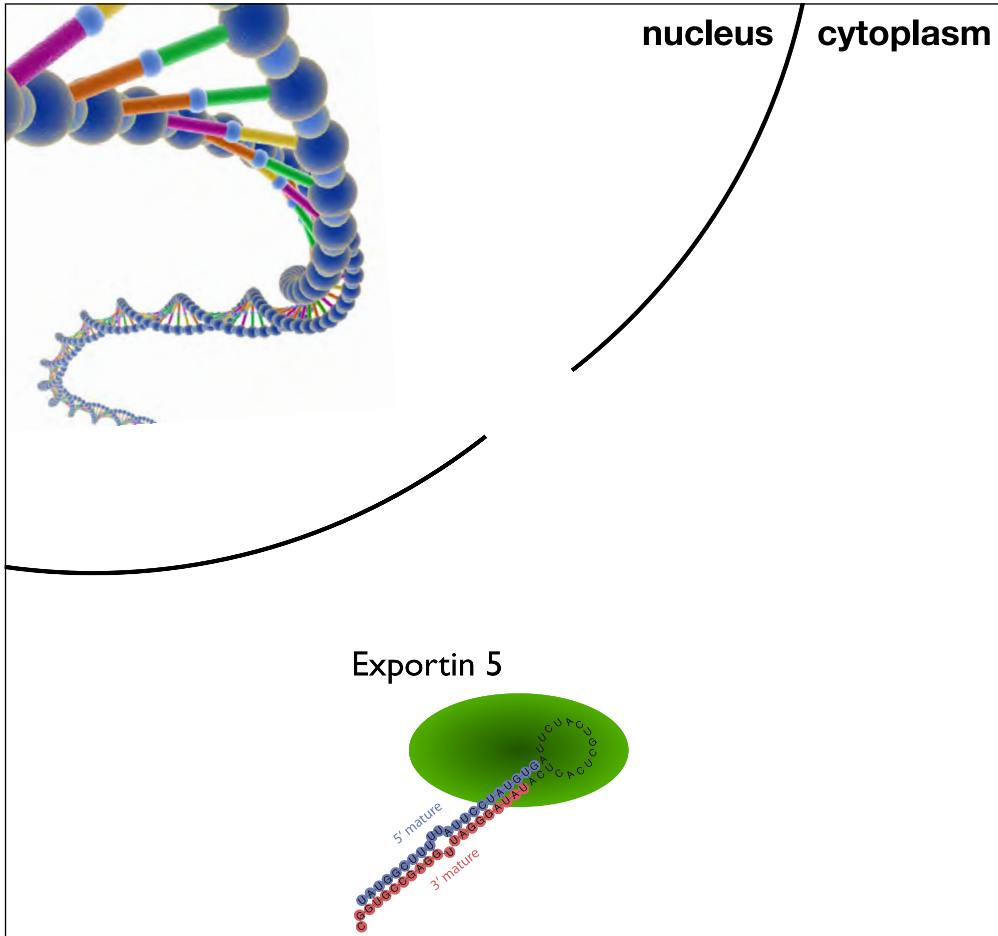
Drosha

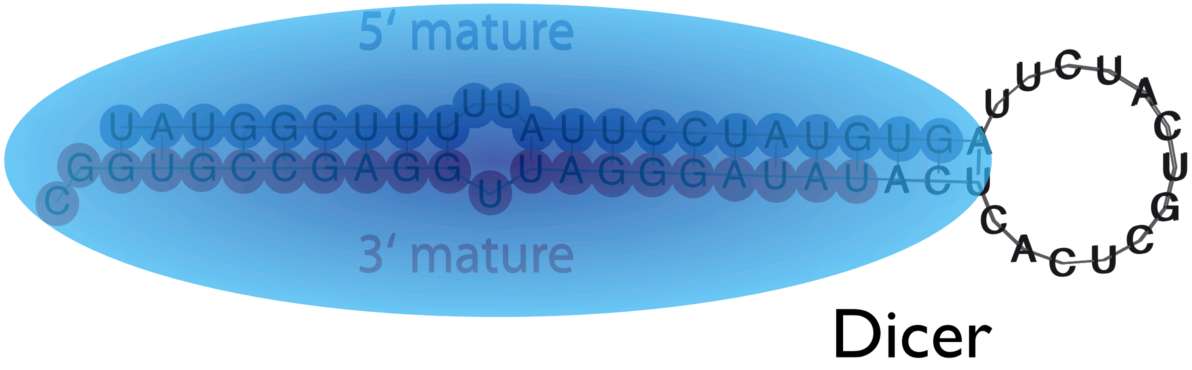


nucleus / **cytoplasm**

pre-microRNA

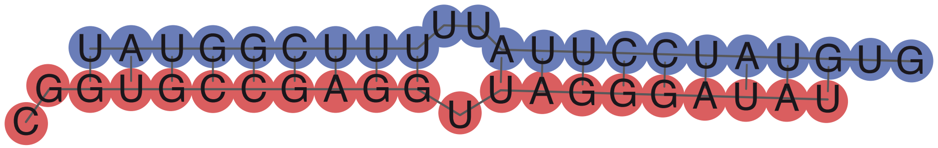

nucleus
cytoplasm
Exportin 5
5′ mature
3′ mature


nucleus
cytoplasm
Exportin 5
5′ mature
3′ mature

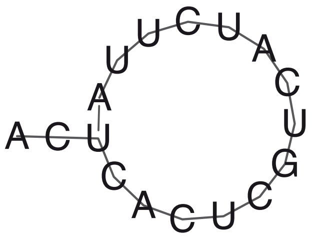

5′ mature
UAUGGCUUUUUAUUCCUAUGUGA
UUCUACUGCUCAC
CGGUGCCGAGGUUAGGGAUAUACU
3′ mature
Dicer

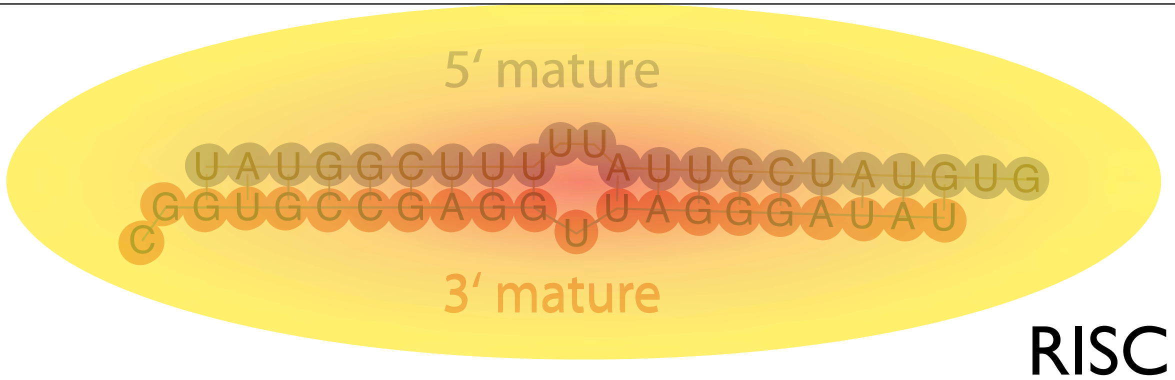
mature microRNA

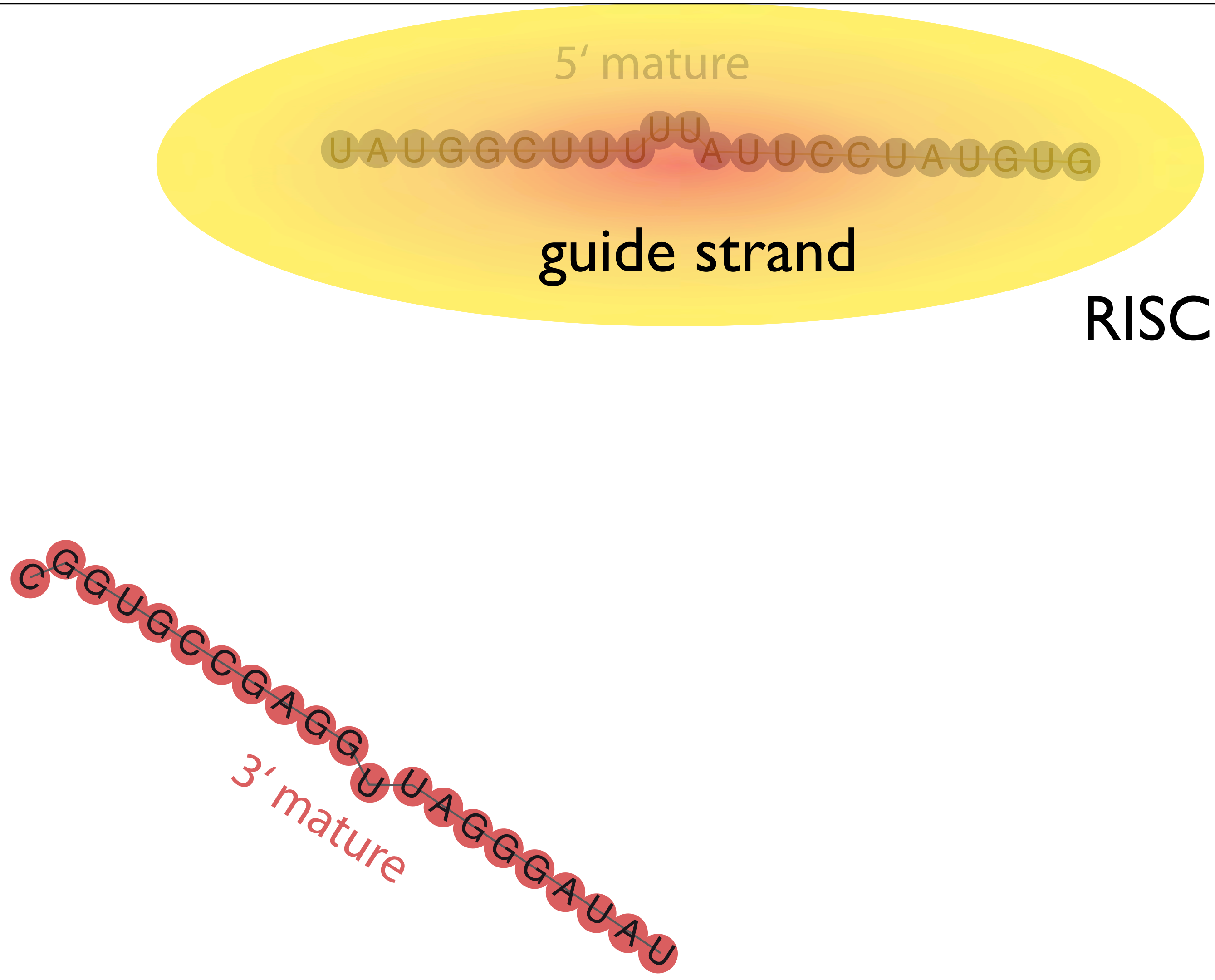
5ʻ mature



3ʻ mature



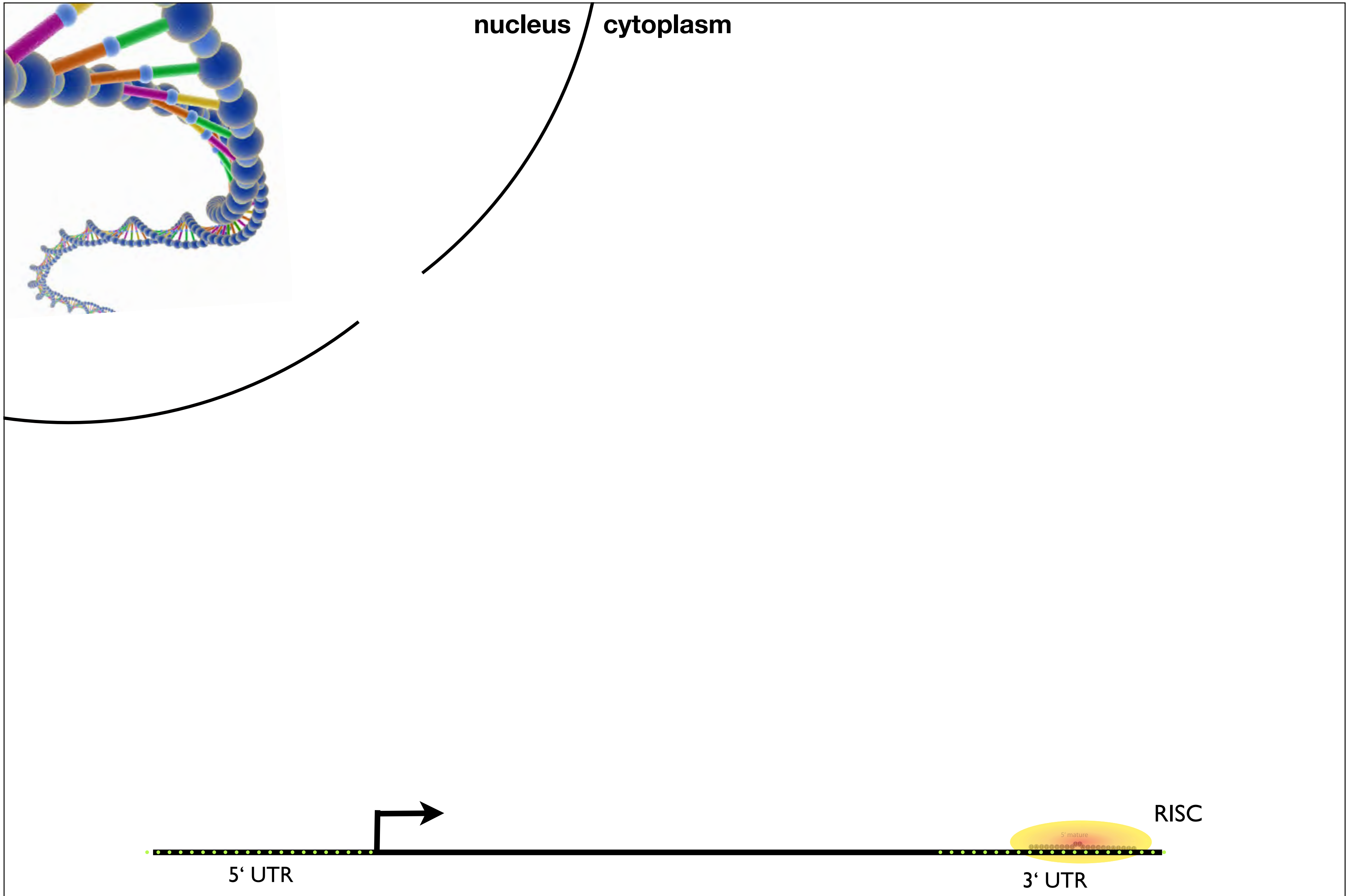

5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature
RISC



guide strand

RISC

pessenger strand



nucleus **cytoplasm**

RISC

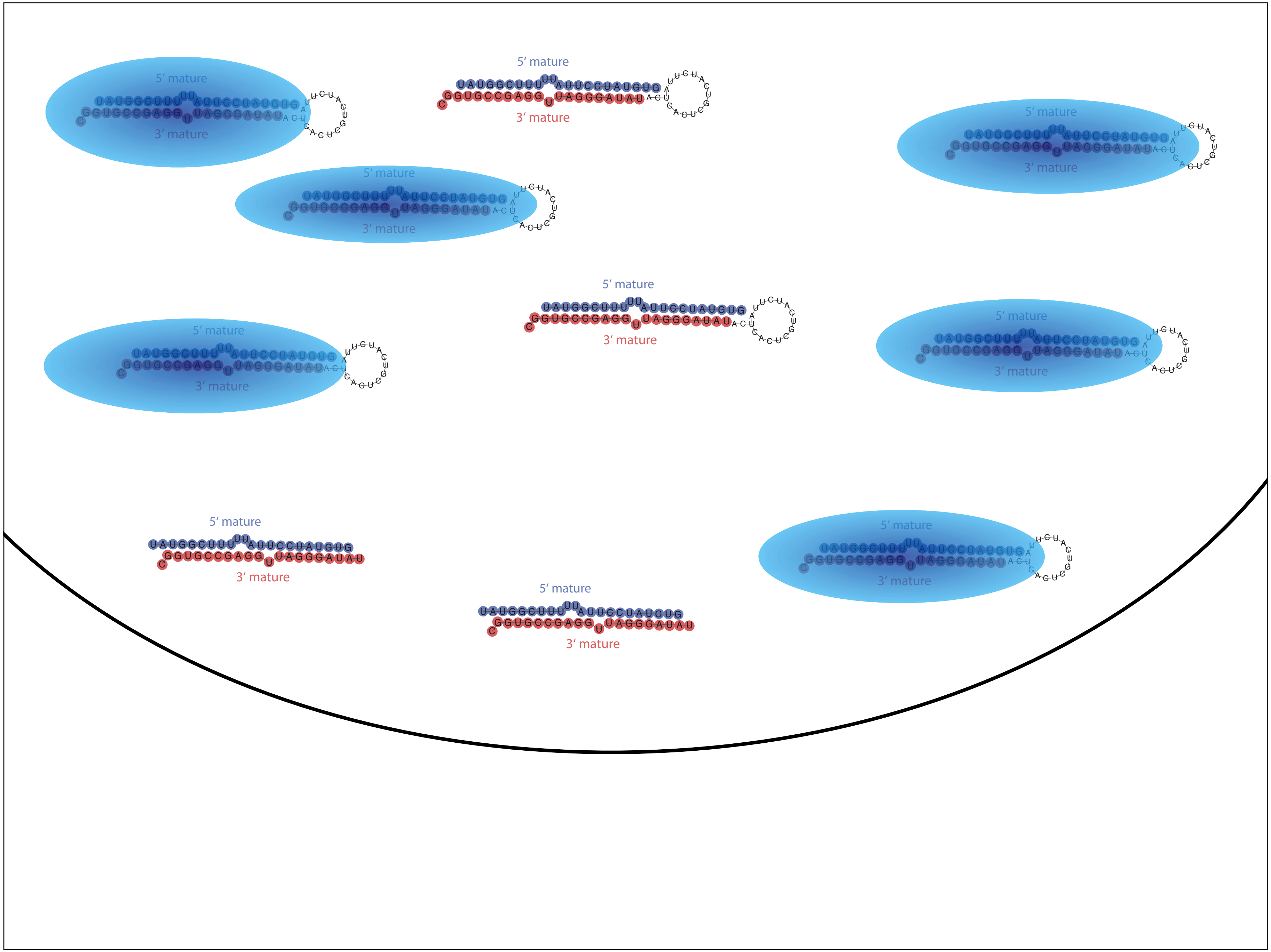
5‘ UTR

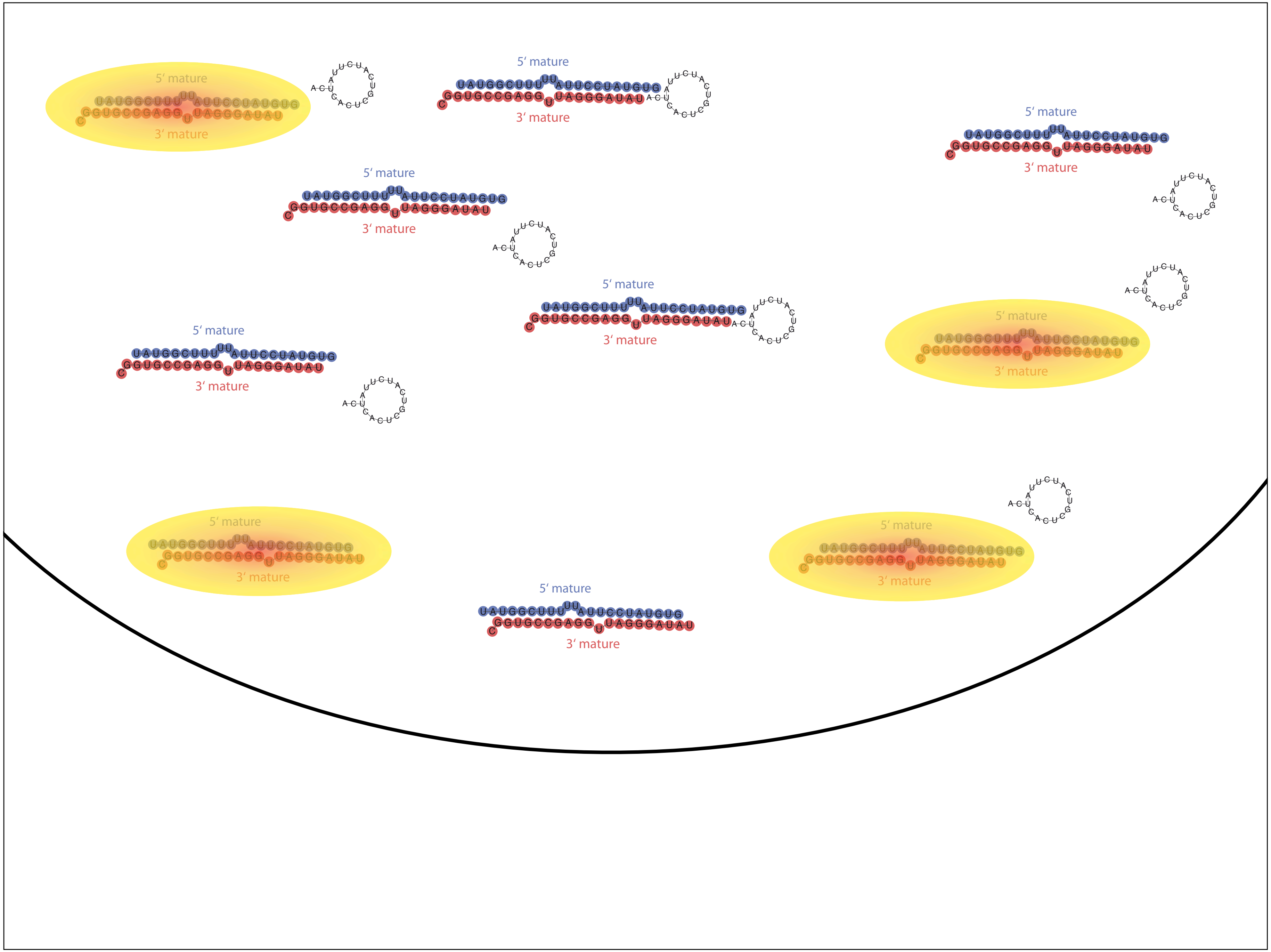
3‘ UTR

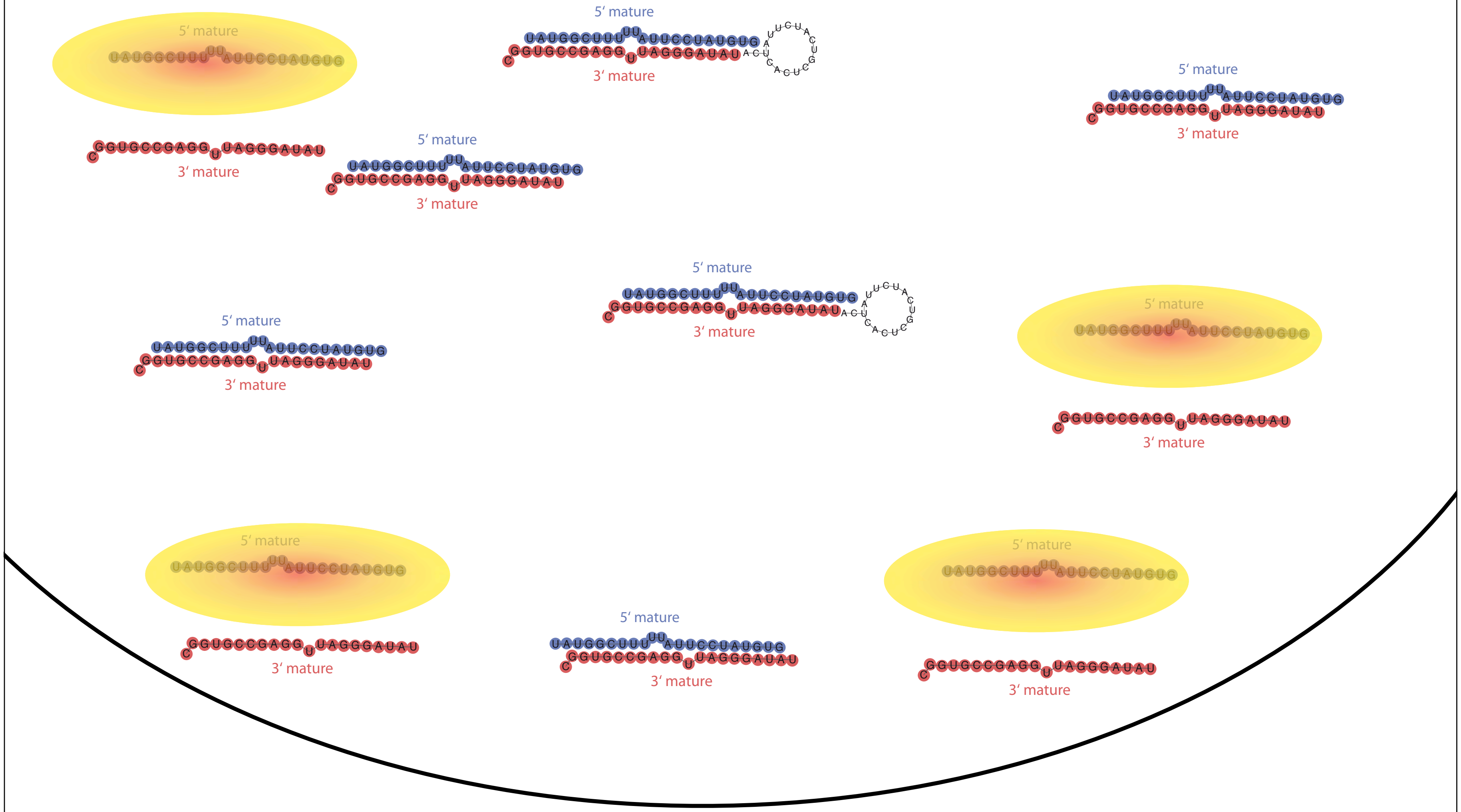
regulation

mRNA

short RNA-seq

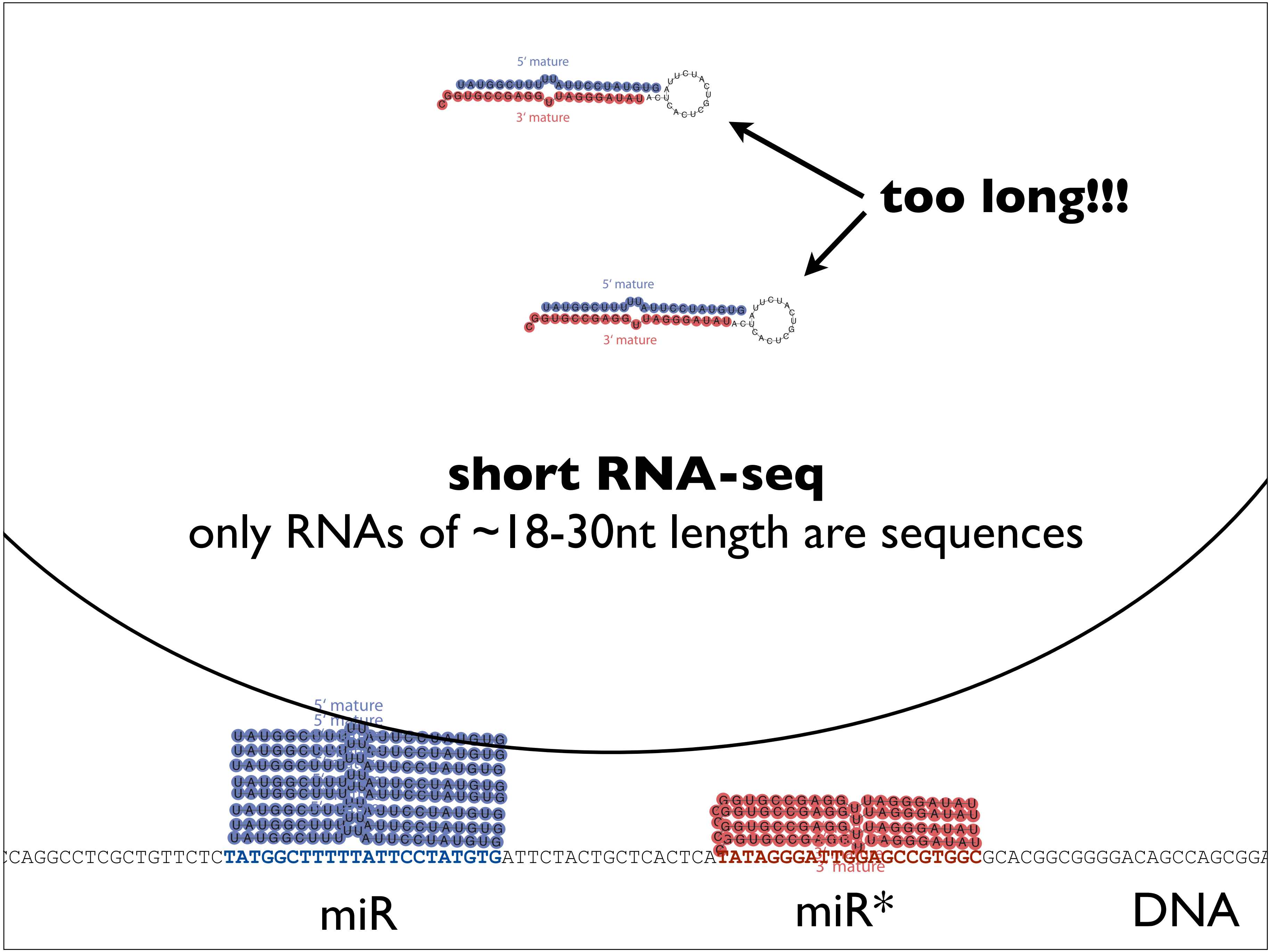

5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature
5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature
5′ mature
3′ mature
5′ mature
UAUGGCUUUUUAUUCCUAUGUG
CGGUGCCGAGGUUAGGGAUAU
3′ mature


5′ mature
UAUGGCUUU
UU
AUUCCUAUGUG
C
GGUGCCGAGG
U
UAGGGAUAU
3′ mature
A-C-U
A
U
U-C-U
A
C
U
G
G
C-U-C
A
C



CAGGCCTCGCTGTTCTC**TATGGCTTTTTATTCCTATGTG**ATTCTACTGCTCACTCA**TATAGGGATTGGAGCCGTGGC**GCACGGCGGGGACAGCCAGCGGA

DNA



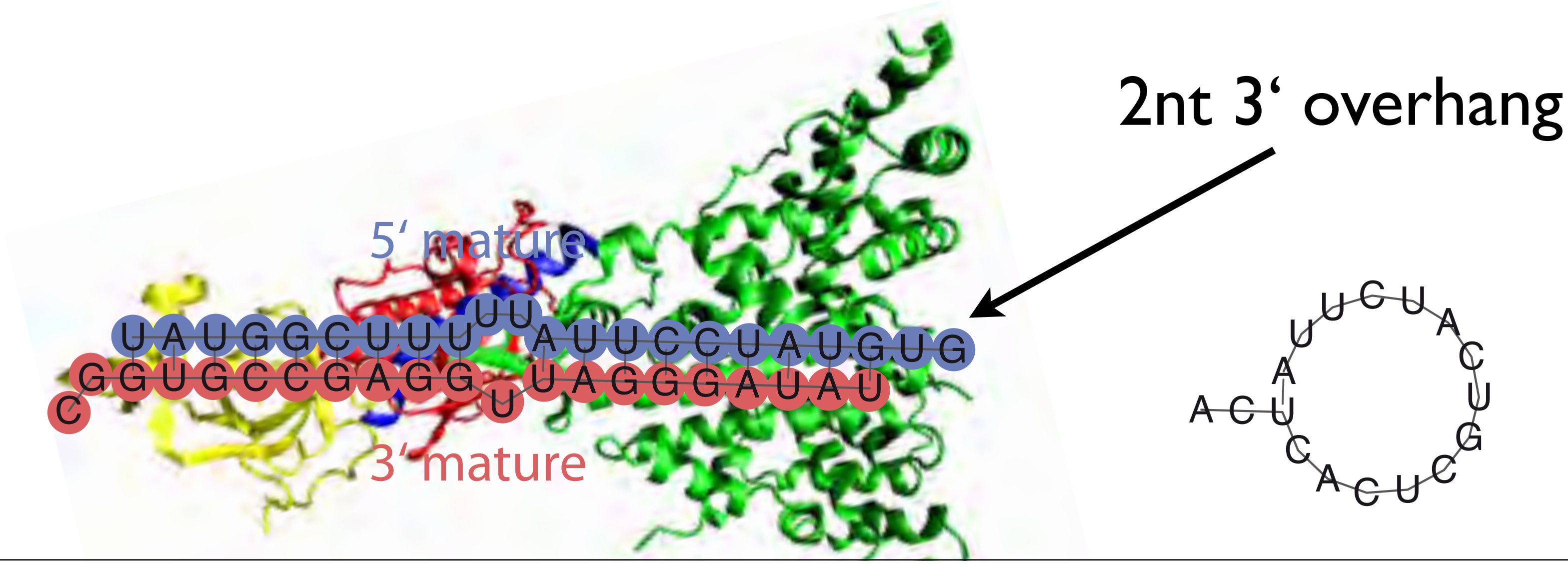
too long!!!

short RNA-seq

only RNAs of ~18-30nt length are sequences

DICER

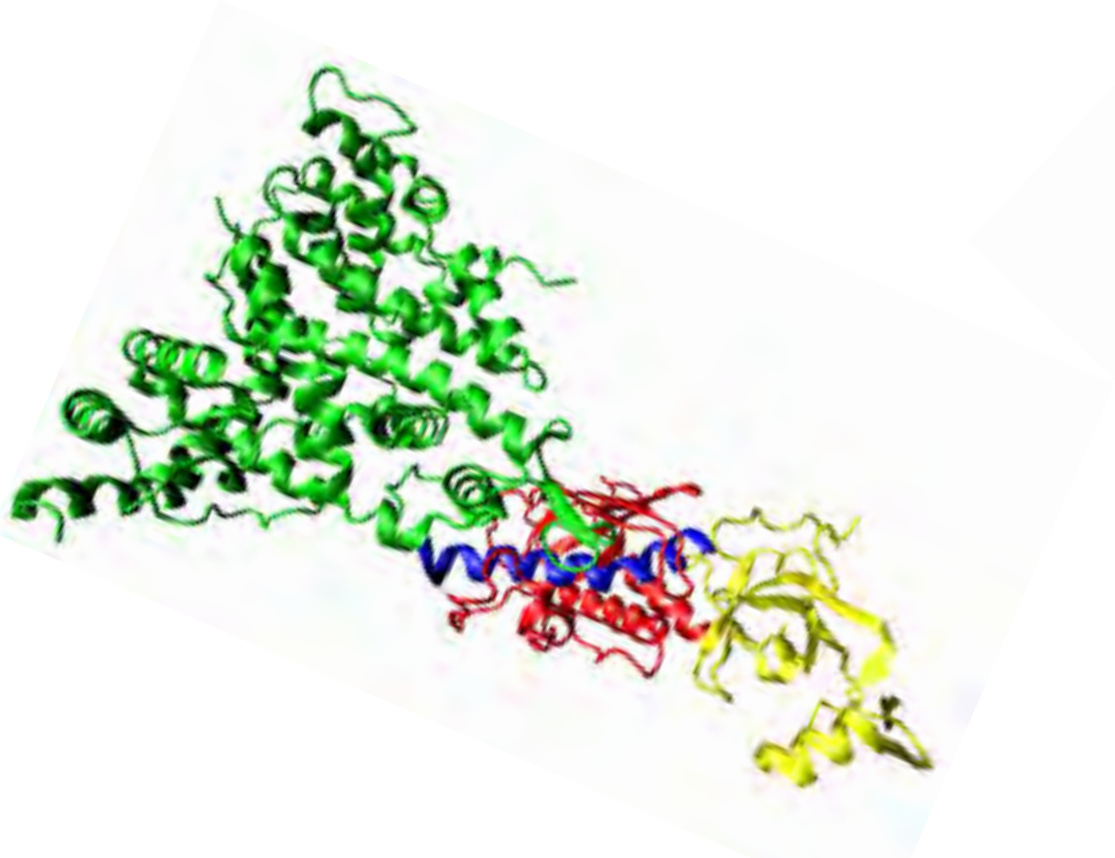
DICER cleavage



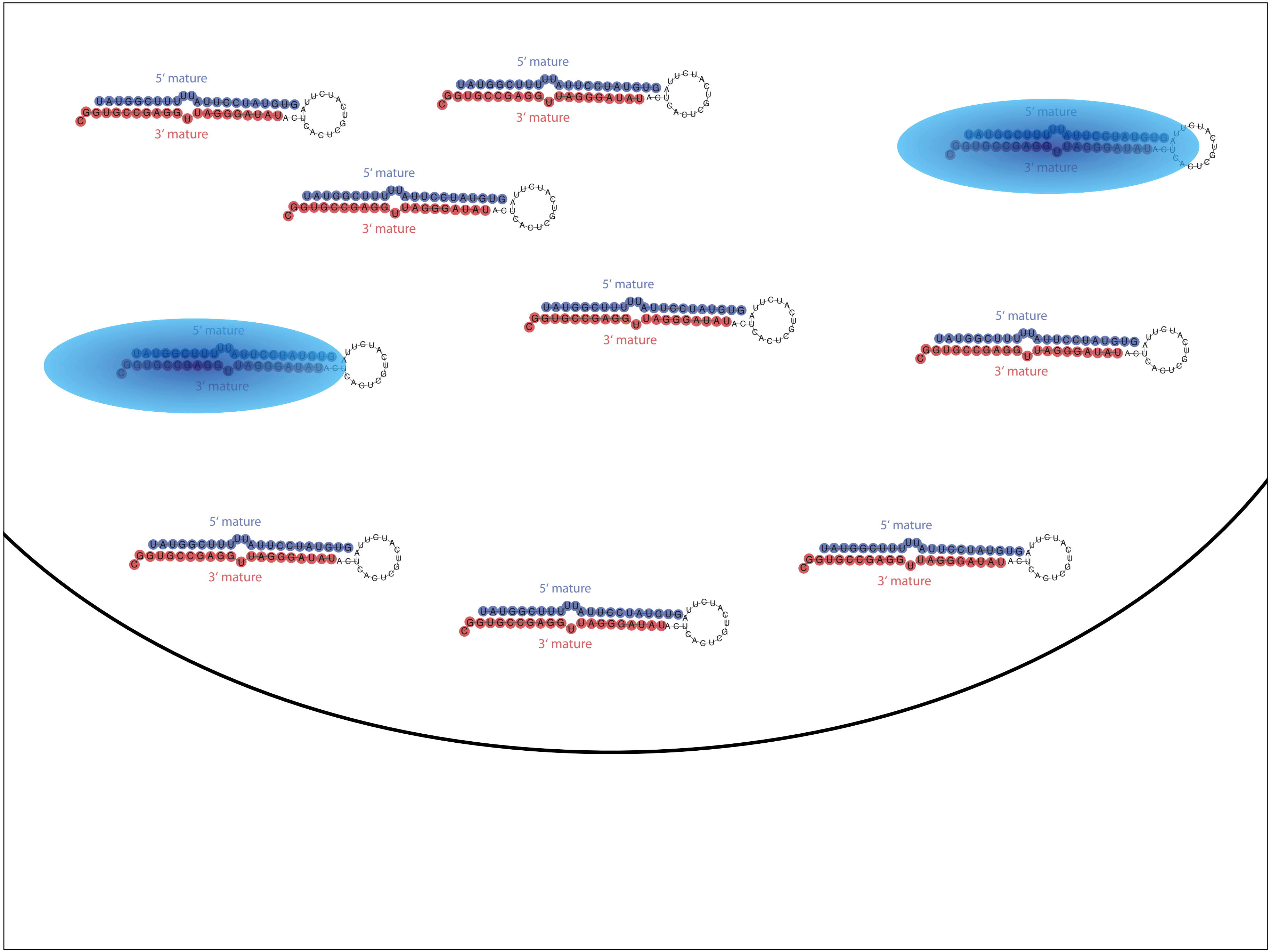
2nt 3' overhang

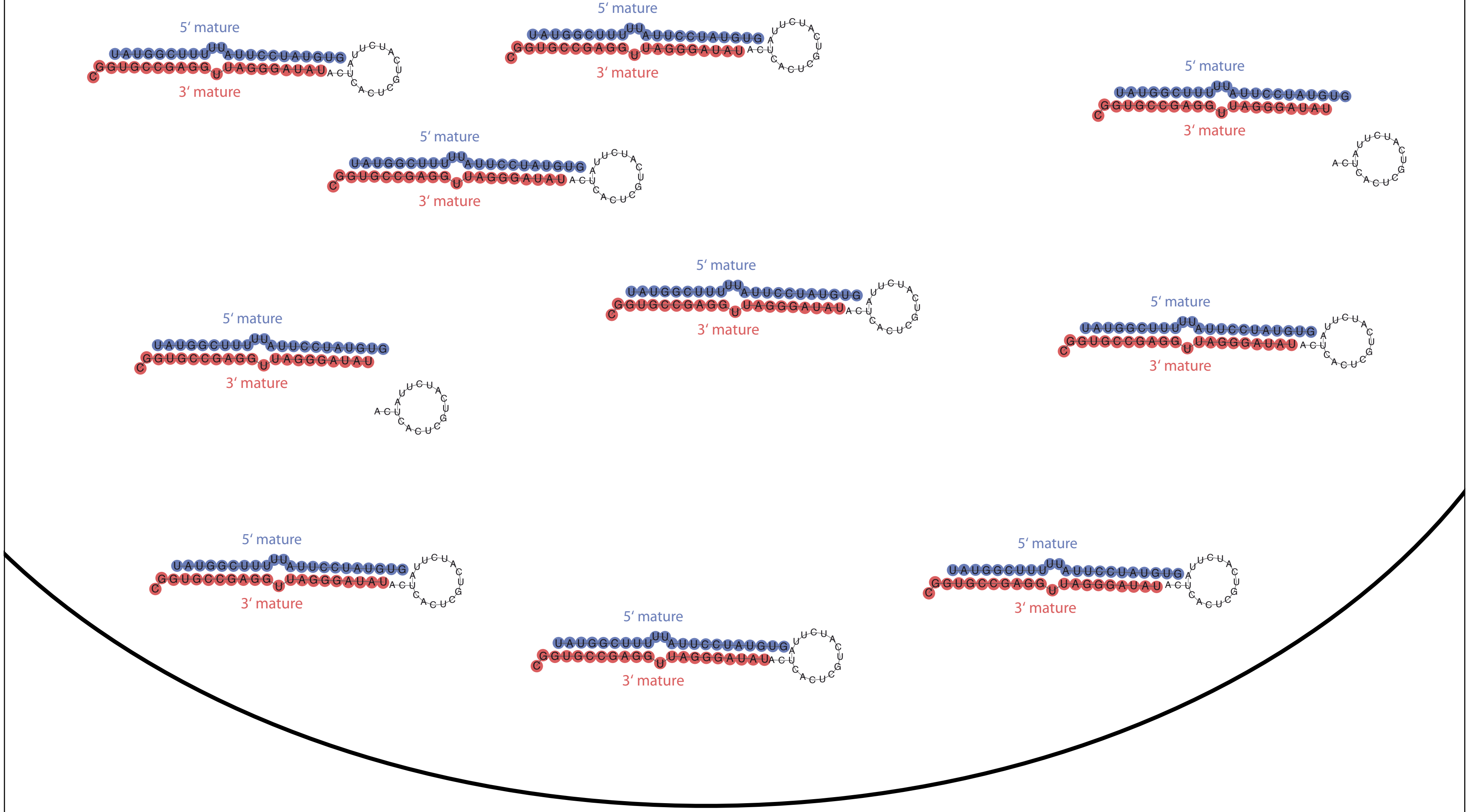
DICER knockdown

DOWN
KNOCKO~~UT~~



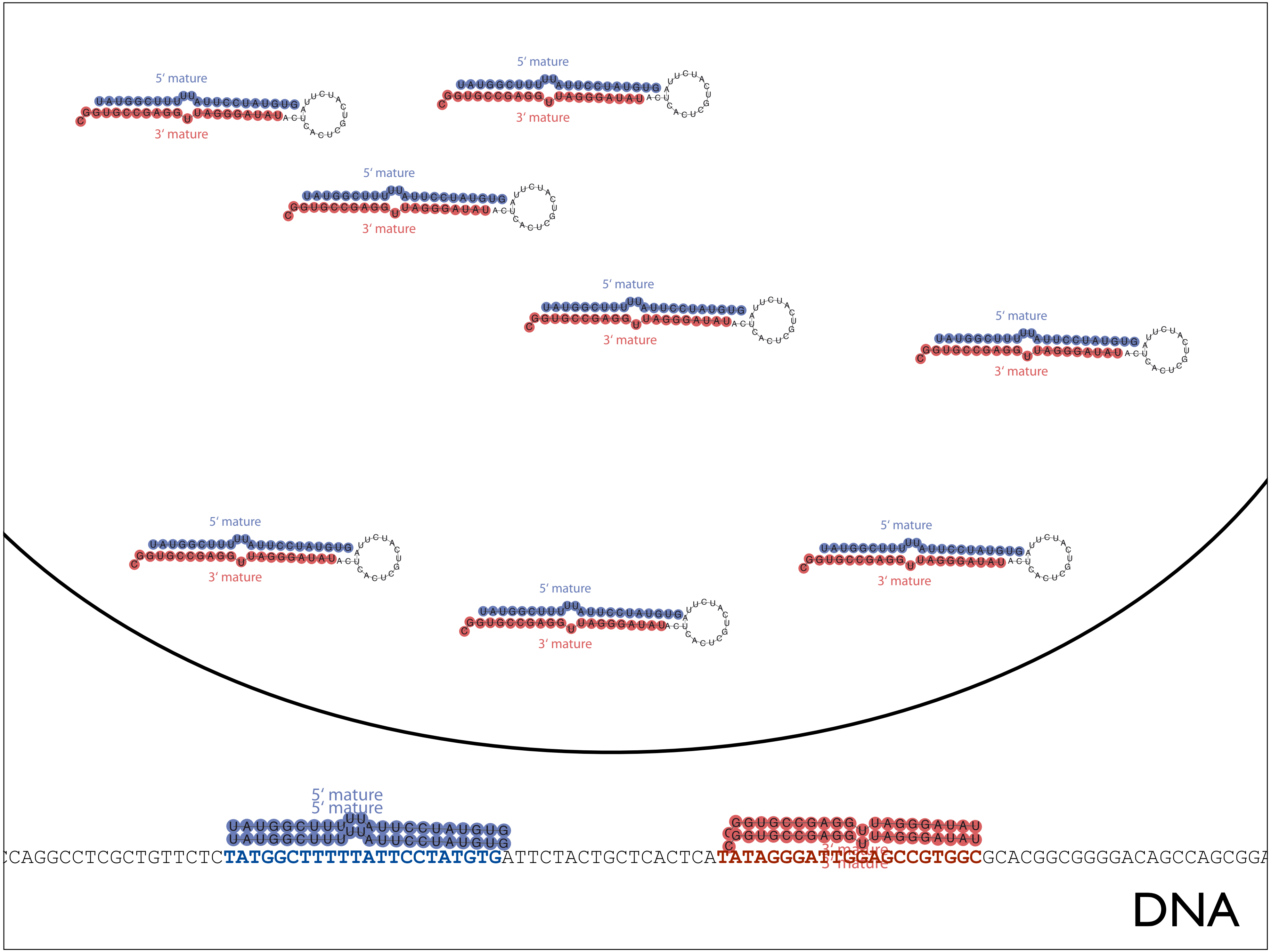
Friedlander, M. R., Mackowiak, S. D., Li, N., Chen, W., & Rajewsky, N. (2011). miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic acids research*. doi:10.1093/nar/gkr688


5′ mature
UAUGGCUUUUUAUUCCUAUGUG
GGUGCCGAGGUUAGGGAUAU
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature
5′ mature
3′ mature

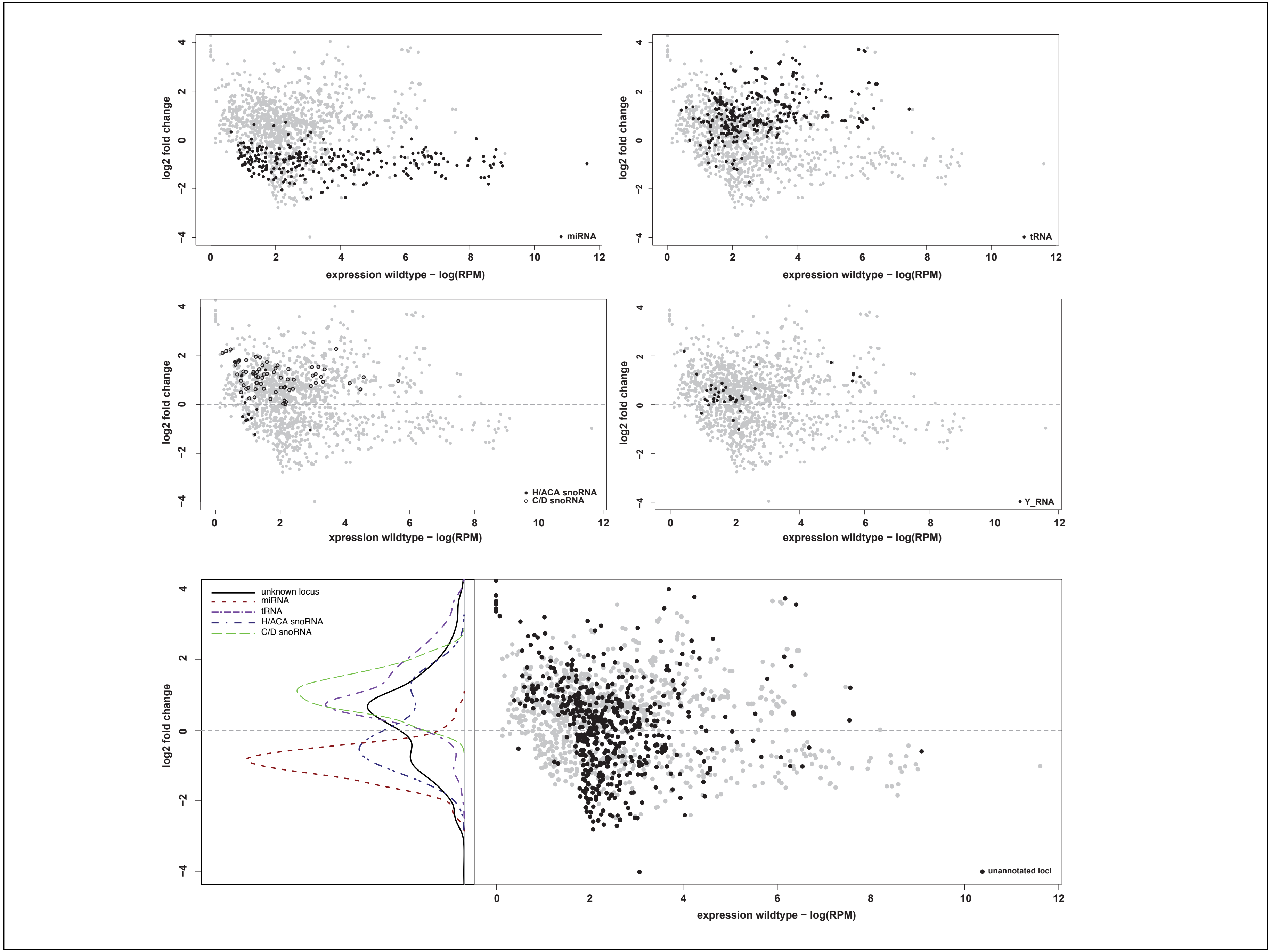


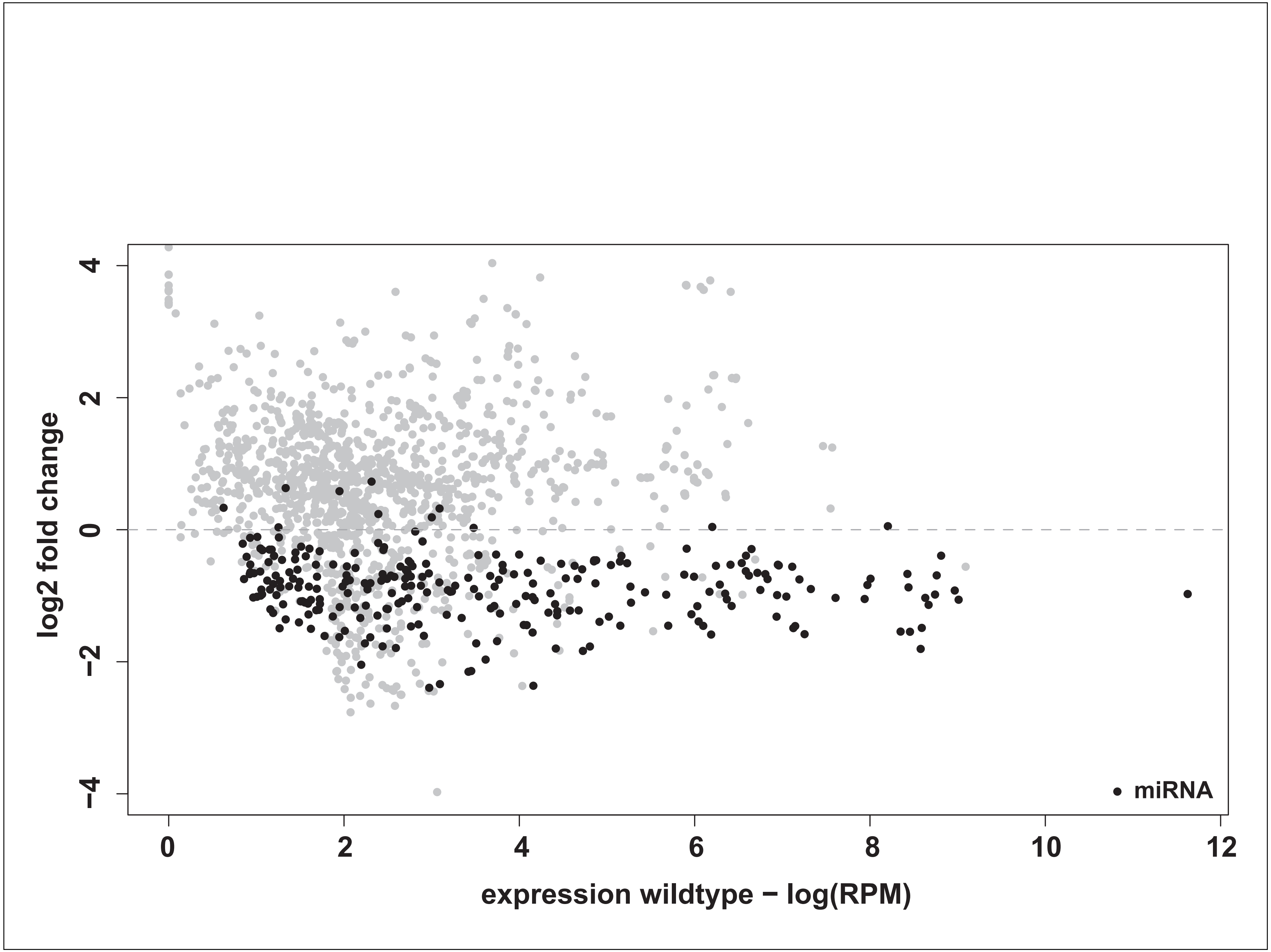
CAGGCCTCGCTGTTCTC**TATGGCTTTTTATTCCTATGTG**ATTCTACTGCTCACTCA**TATAGGGATTGGAGCCGTGGC**GCACGGCGGGGACAGCCAGCGG

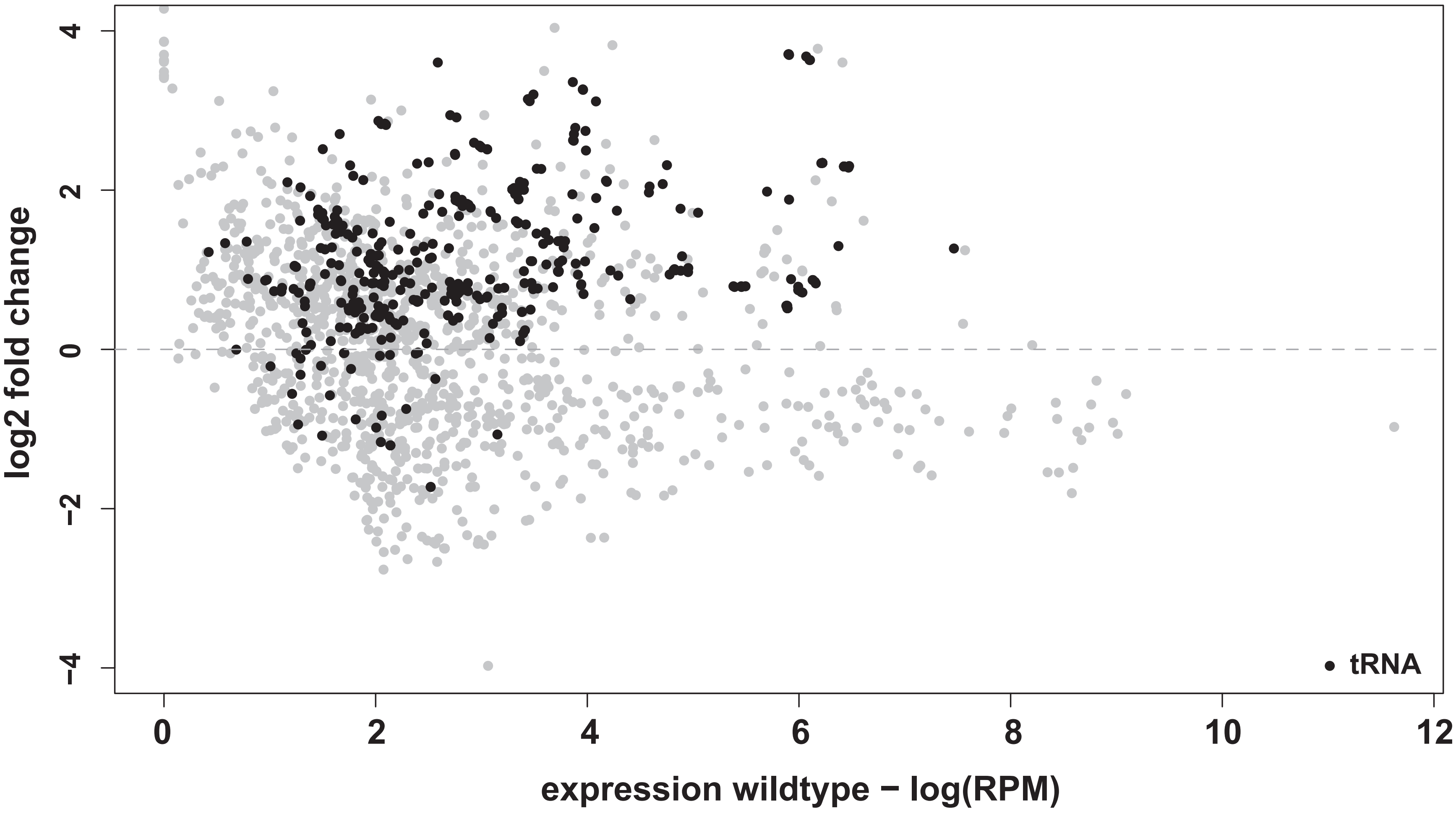
DNA


5′ mature
UAUGGCUUUUUAUUCCUAUGUG
GGUGCCGAGGUUAGGGAUAU
3′ mature
CAGGCCTCGCTGTTCTCTATGGCTTTTTATTCCTATGTGATTCTACTGCTCACTCATATAGGATTGGAGCCGTGGCGCACGGCGGGACAGCCAGCGGA
DNA

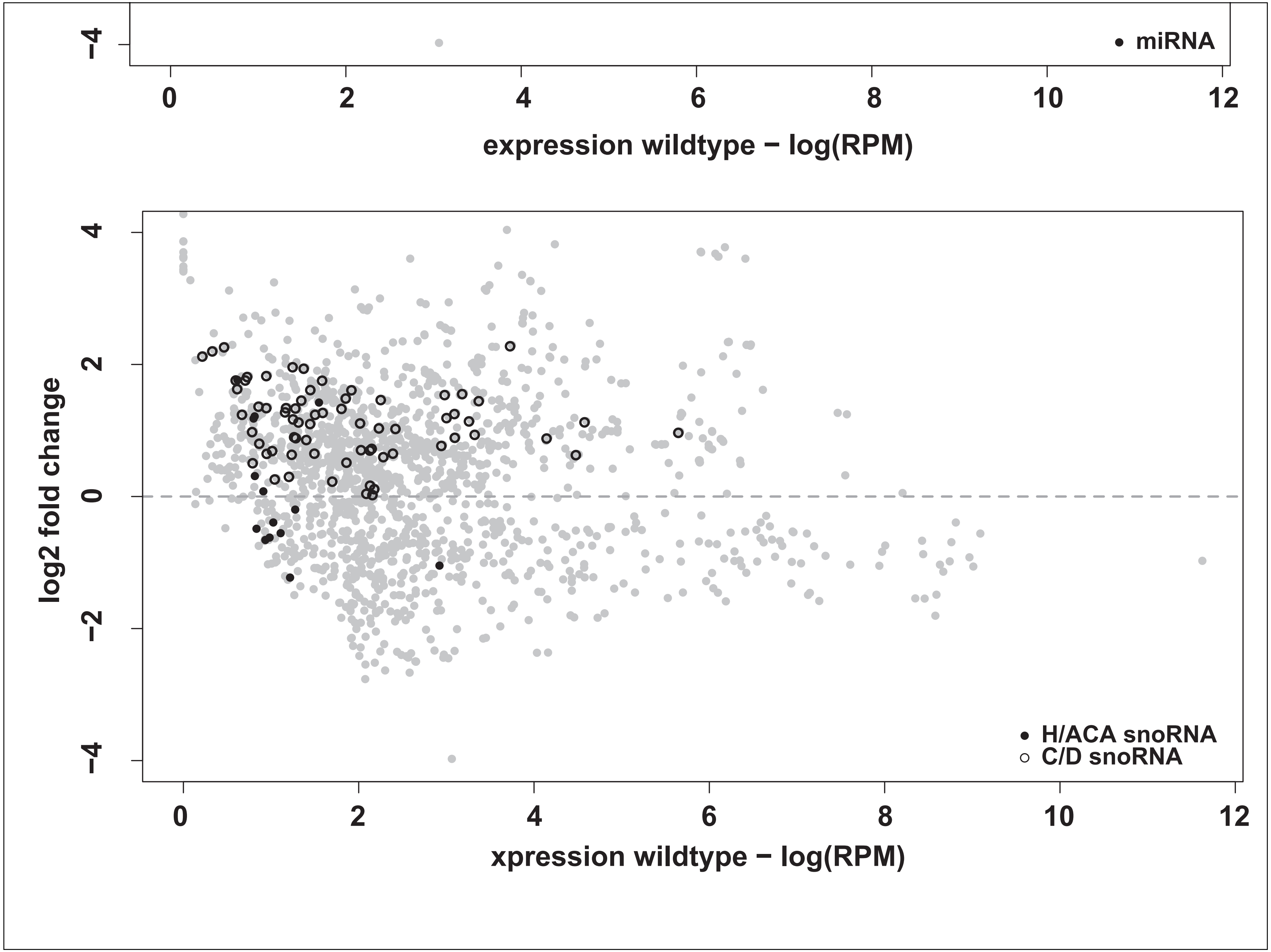
What is cleaved by DICER?

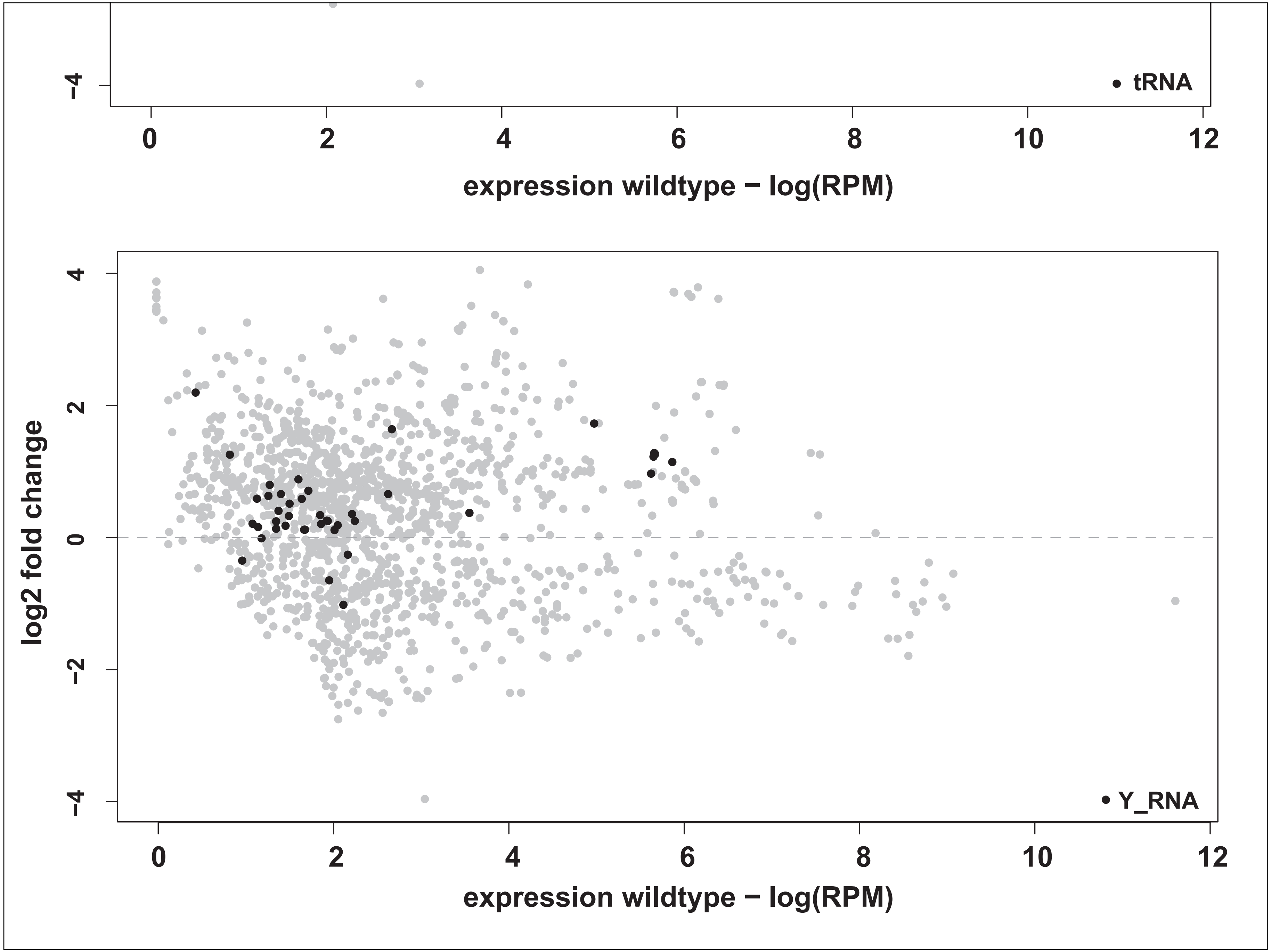

log2 fold change
expression wildtype − log(RPM)
● miRNA
log2 fold change
expression wildtype − log(RPM)
● tRNA
log2 fold change
xpression wildtype − log(RPM)
● H/ACA snoRNA
○ C/D snoRNA
log2 fold change
expression wildtype − log(RPM)
● Y_RNA
log2 fold change
unknown locus
miRNA
tRNA
H/ACA snoRNA
C/D snoRNA
● unannotated loci
expression wildtype − log(RPM)
−4
−2
0
2
4
0
2
4
6
8
10
12

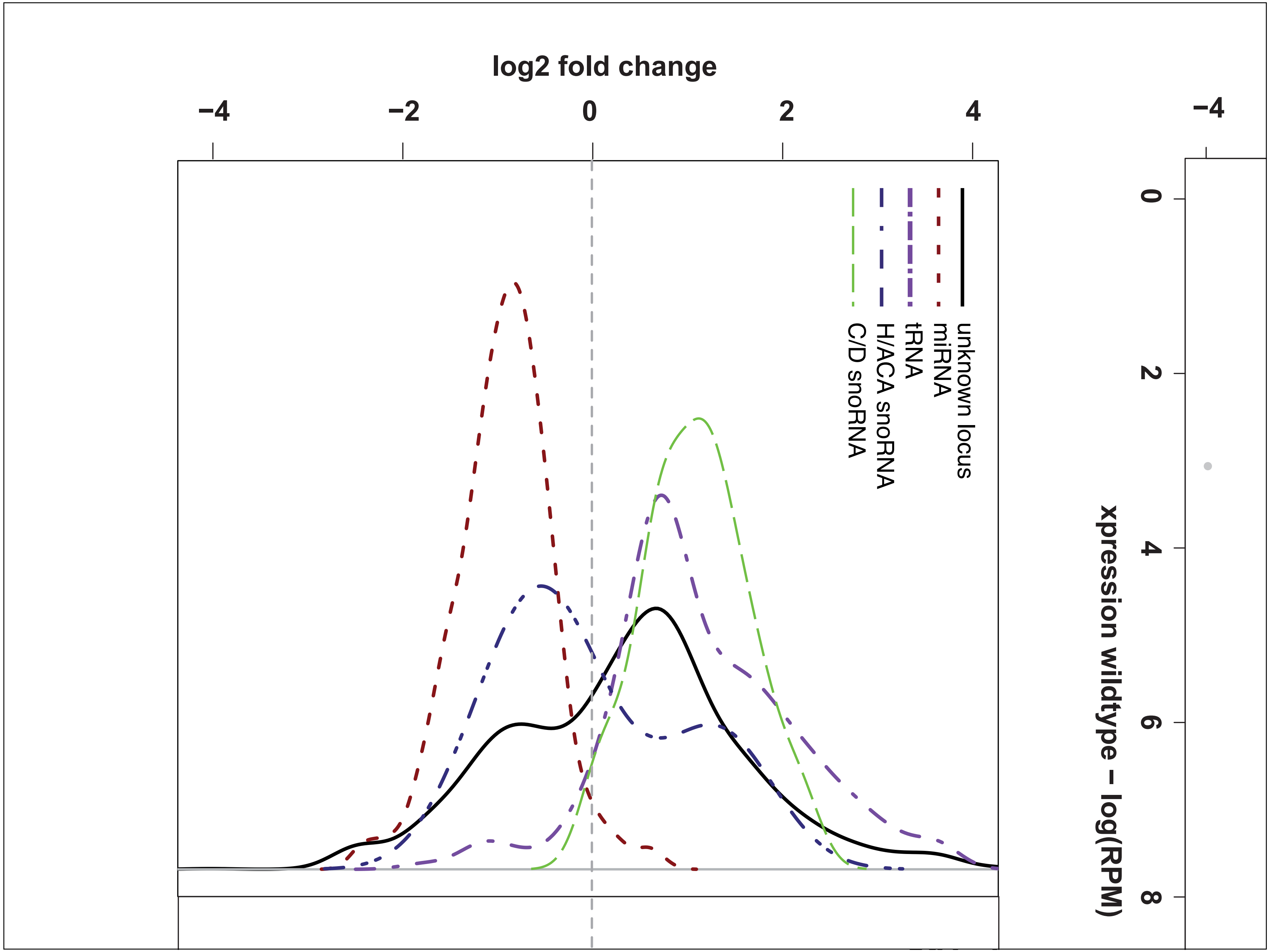

log2 fold change
4
2
0
−2
−4
0
2
4
6
8
10
12
expression wildtype − log(RPM)
● miRNA

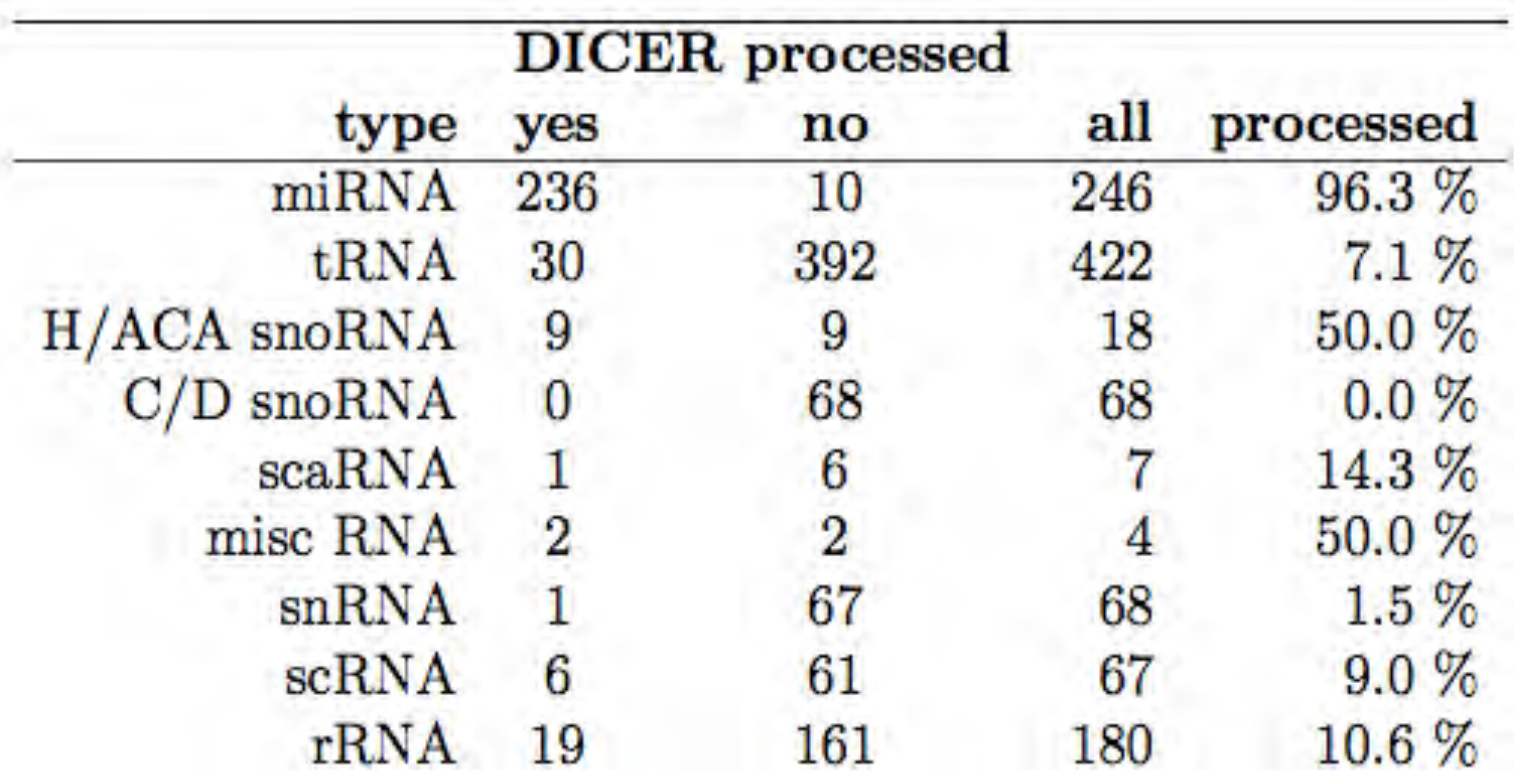


log2 fold change
4
2
0
−2
−4
0
2
4
6
8
10
12
expression wildtype − log(RPM)
● tRNA


−4
● miRNA
0
2
4
6
8
10
12
expression wildtype − log(RPM)
4
2
0
−2
−4
log2 fold change
● H/ACA snoRNA
○ C/D snoRNA
0
2
4
6
8
10
12
xpression wildtype − log(RPM)

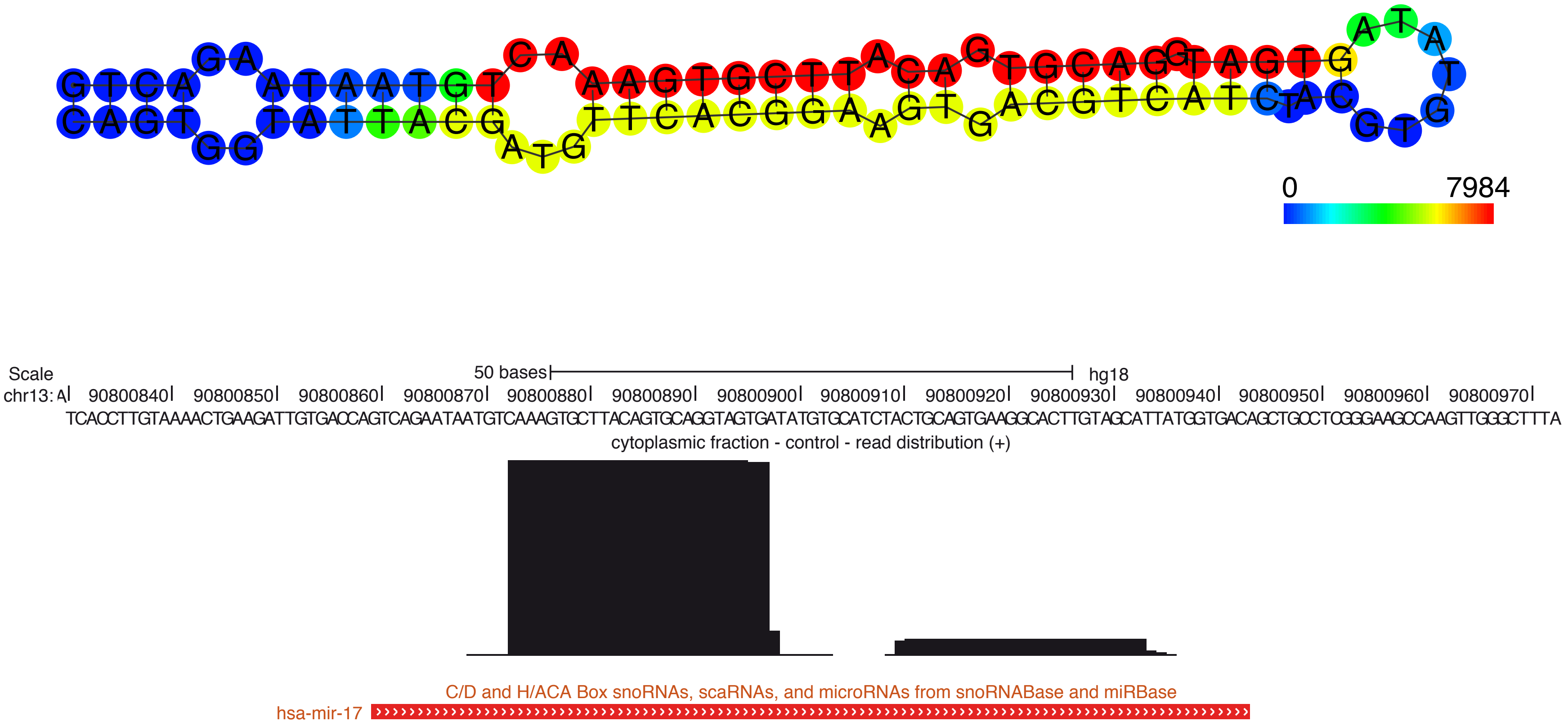

−4
0
2
4
6
8
10
12
expression wildtype − log(RPM)
● tRNA
log2 fold change
4
2
0
−2
−4
0
2
4
6
8
10
12
expression wildtype − log(RPM)
● Y_RNA


log2 fold change
−4
−2
0
2
4
−4
unknown locus
miRNA
tRNA
H/ACA snoRNA
C/D snoRNA
xpression wildtype – log(RPM)
0
2
4
6
8

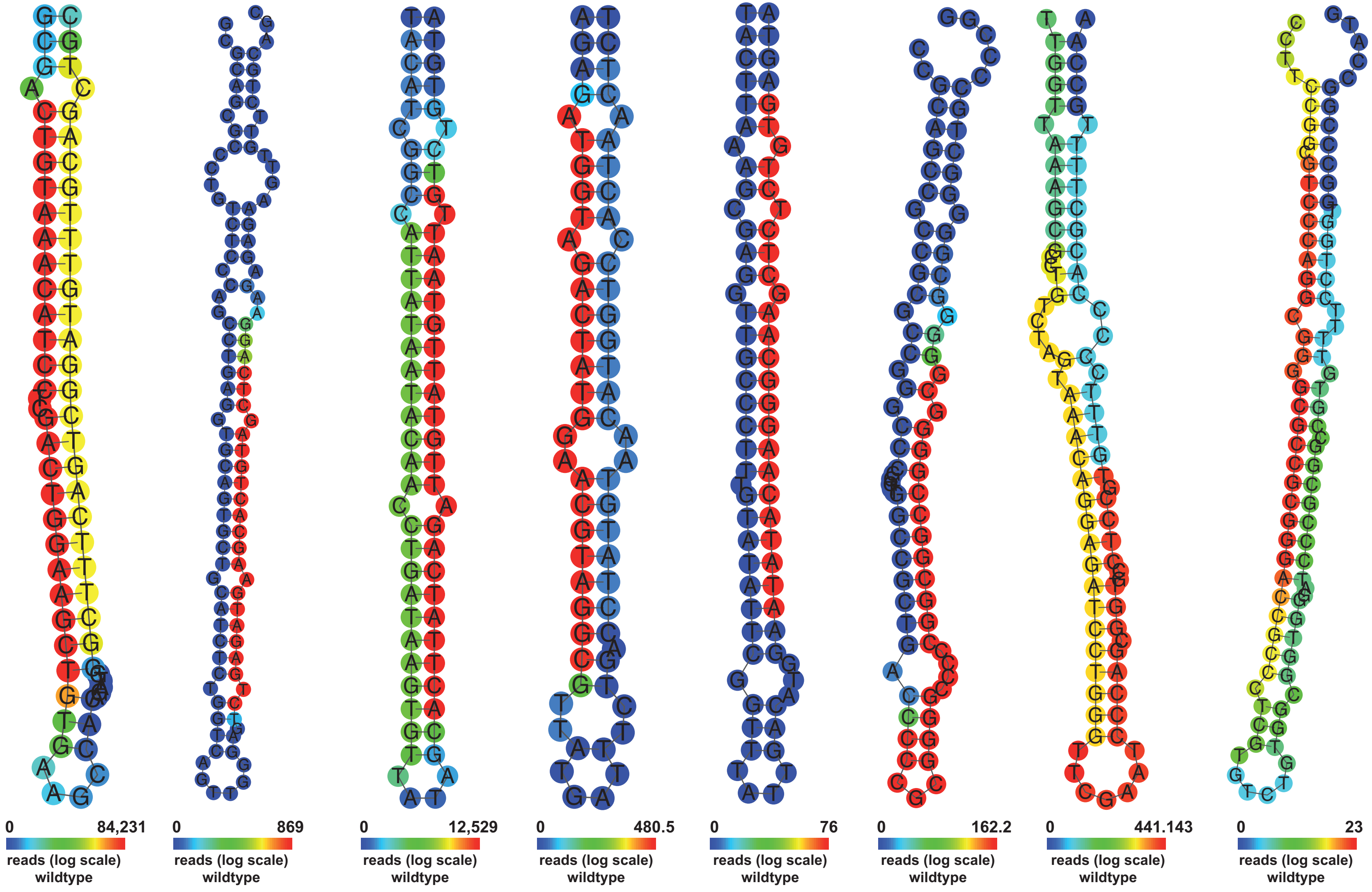


	DICER processed			
type	**yes**	**no**	**all**	**processed**
miRNA	236	10	246	96.3 %
tRNA	30	392	422	7.1 %
H/ACA snoRNA	9	9	18	50.0 %
C/D snoRNA	0	68	68	0.0 %
scaRNA	1	6	7	14.3 %
misc RNA	2	2	4	50.0 %
snRNA	1	67	68	1.5 %
scRNA	6	61	67	9.0 %
rRNA	19	161	180	10.6 %

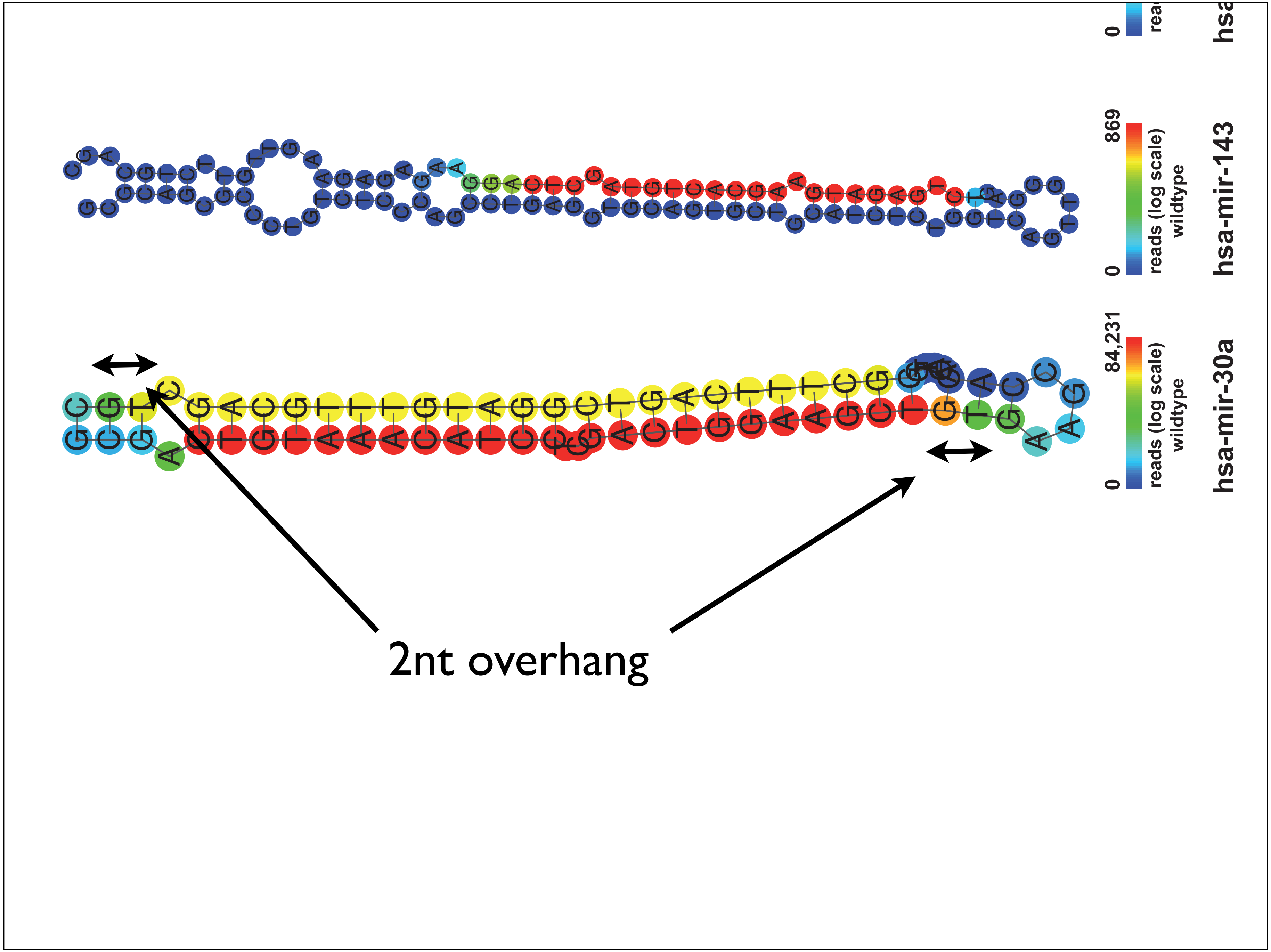
RNAfold expression coloring


0
7984
Scale
50 bases
hg18
chr13:
90800840
90800850
90800860
90800870
90800880
90800890
90800900
90800910
90800920
90800930
90800940
90800950
90800960
90800970
TCACCTTGTAAAACTGAAGATTGTGACCAGTCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGATATGTGCATCTACTGCAGTGAAGGCACTTGTAGCATTATGGTGACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTA
cytoplasmic fraction - control - read distribution (+)
C/D and H/ACA Box snoRNAs, scaRNAs, and microRNAs from snoRNABase and miRBase
hsa-mir-17

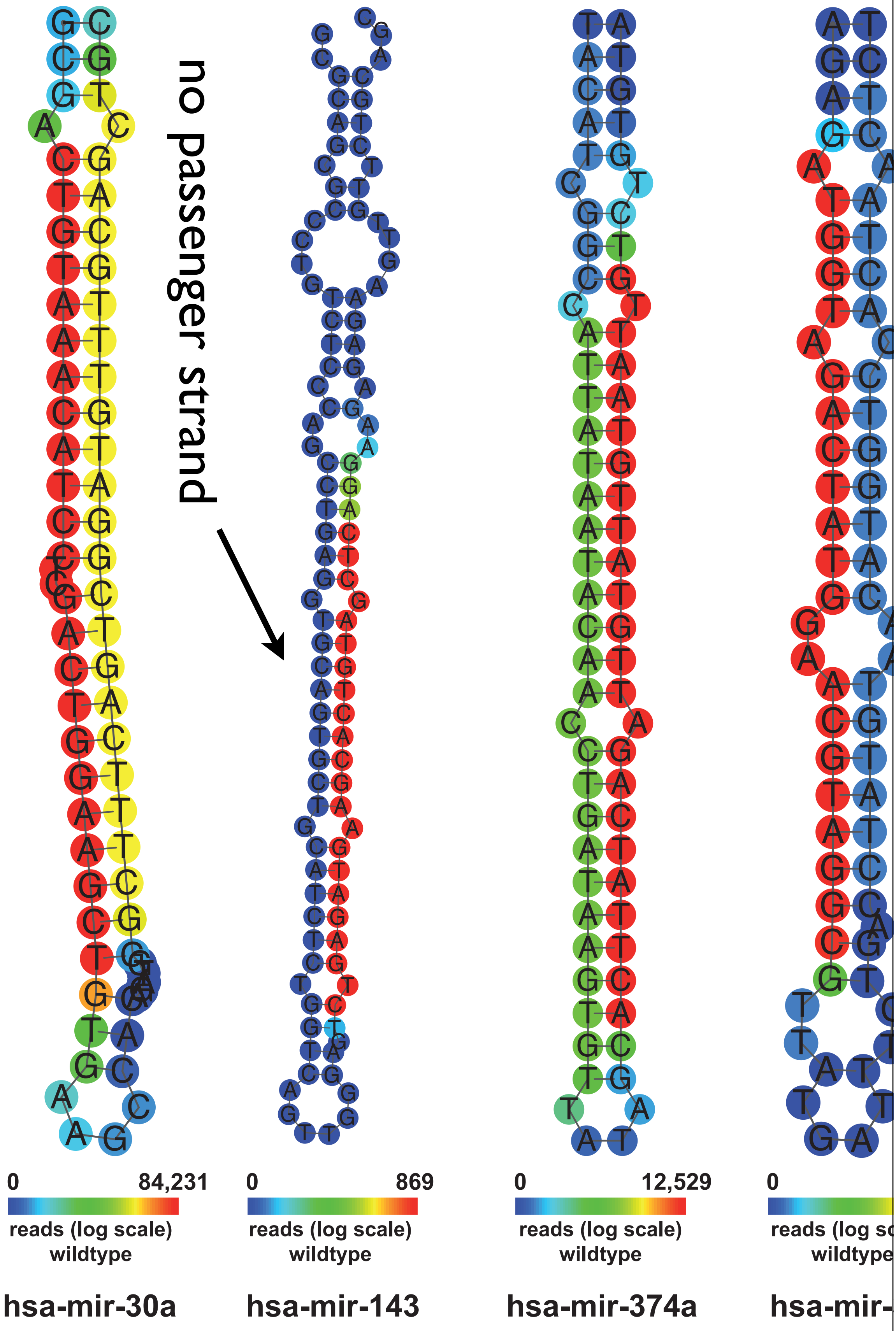
Are all miRBase microRNAs cut by DICER?



hsa-mir-30a **hsa-mir-143** **hsa-mir-374a** **hsa-mir-379** **hsa-mir-381** **hsa-mir-3195** **hsa-mir-3676** **hsa-mir-663a**

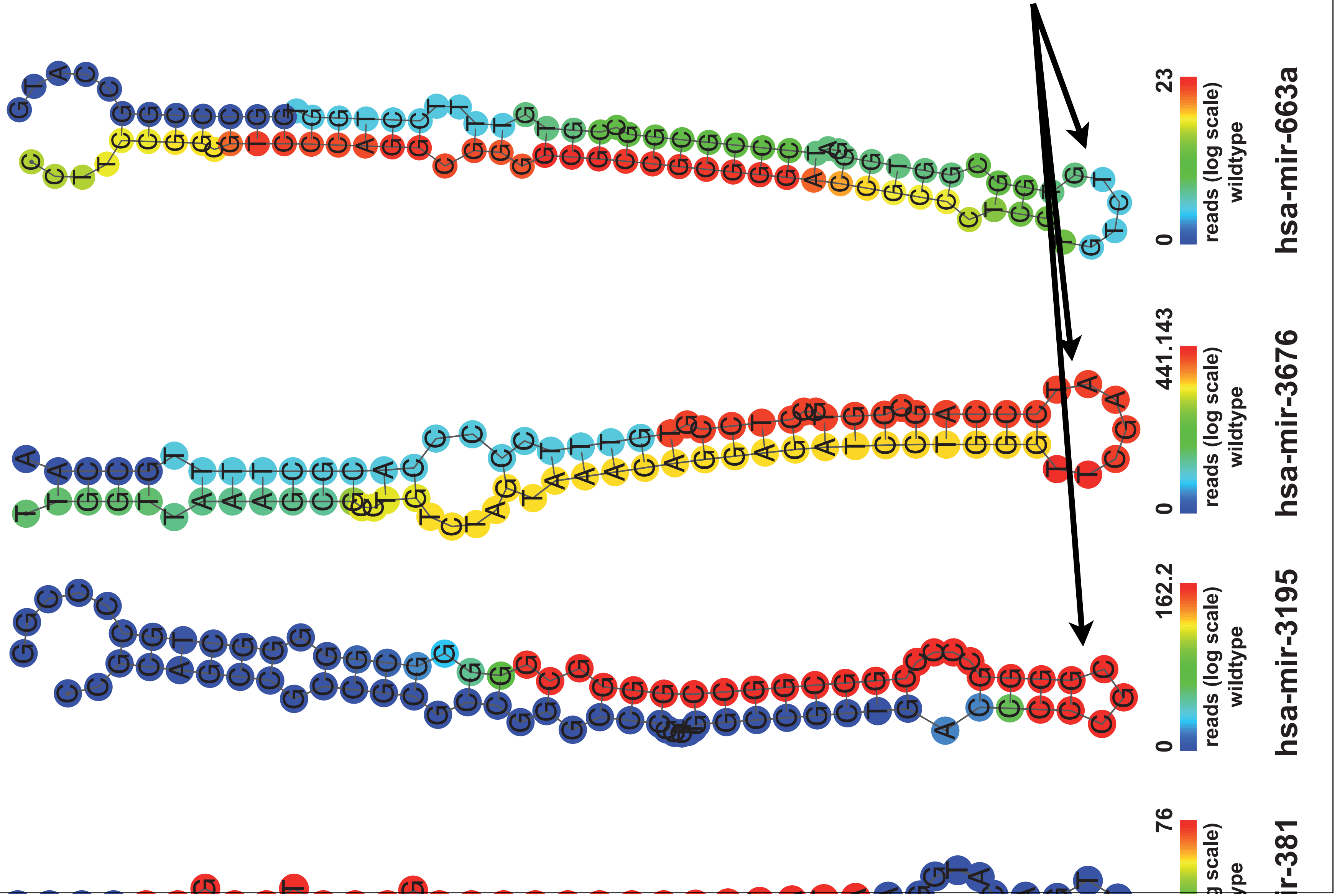


2nt overhang

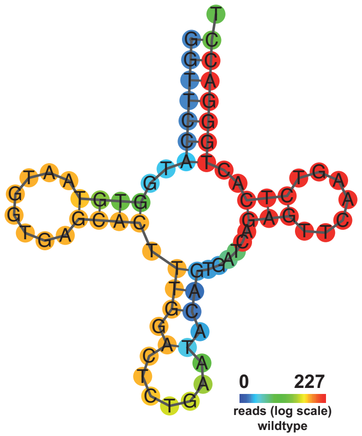


no passenger strand

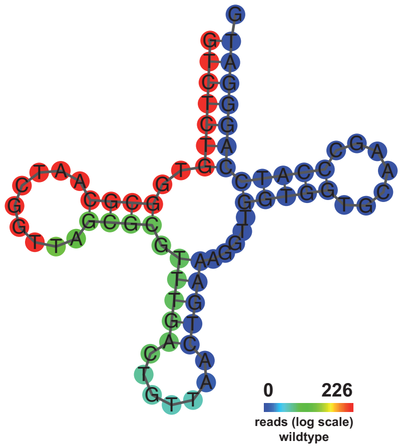
reads completely in the loop region



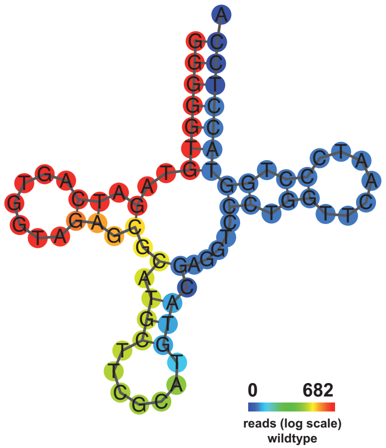
What tRNAs are processed by DICER?



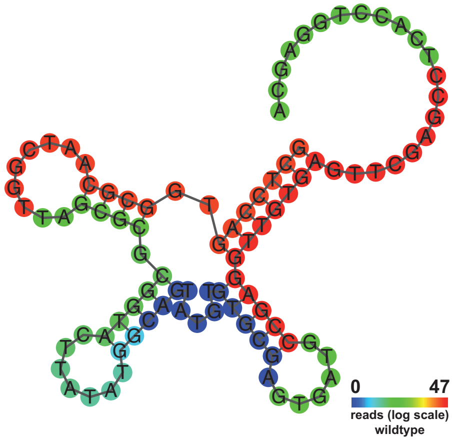
tRNA$^{\mathrm{Gln/CTG}}$
log2 fold change: -1.73



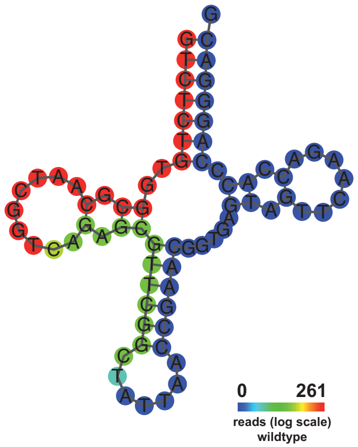
tRNA$^{\mathrm{Asn/GTT}}$
log2 fold change: -1.21



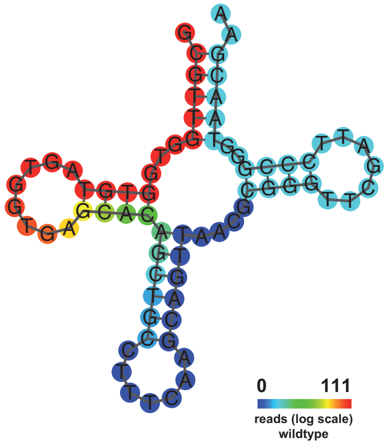
tRNA$^{\mathrm{Ala/CGC}}$
log2 fold change: -1.07



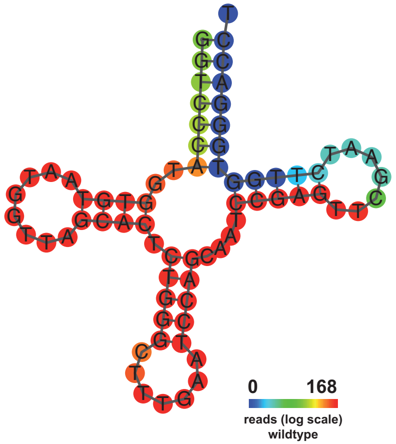
tRNA$^{\mathrm{Ile/TAT}}$
log2 fold change: -0.88



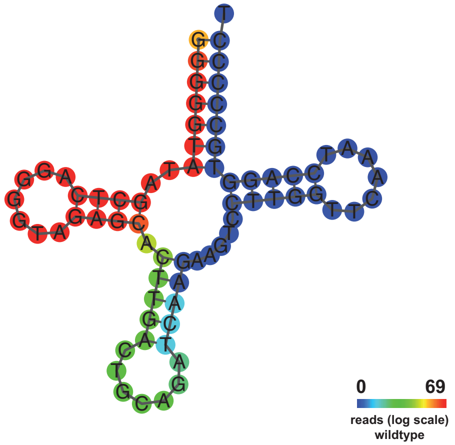
tRNA$^{\mathrm{Asn/ATT}}$
log2 fold change: -0.75



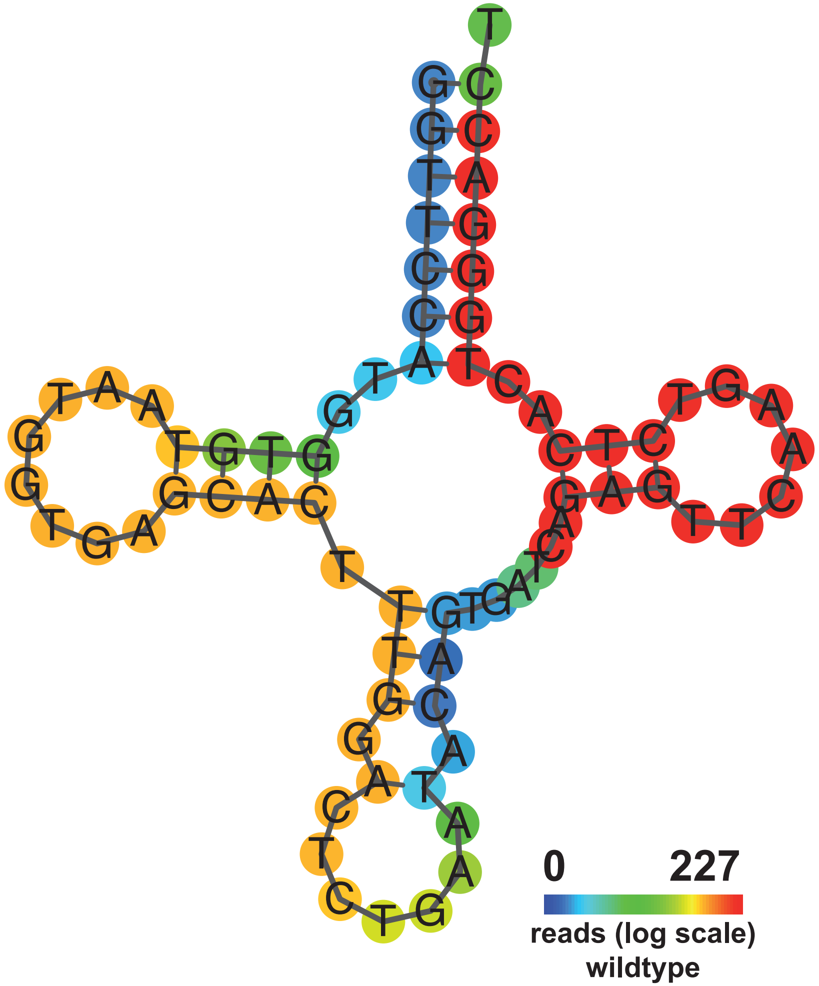
tRNA$^{\mathrm{Glu/TTC}}$
log2 fold change: -0.58



tRNA$^{\mathrm{Gln/TTG}}$
log2 fold change: -0.37

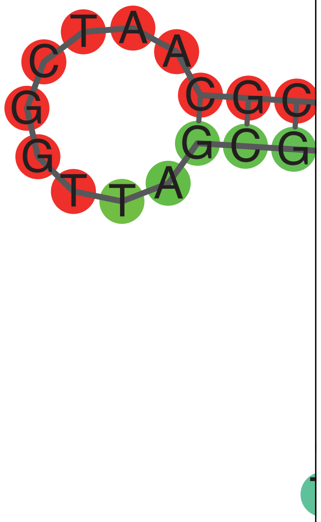


tRNA$^{\mathrm{Cys/GCA}}$
log2 fold change: -0.32



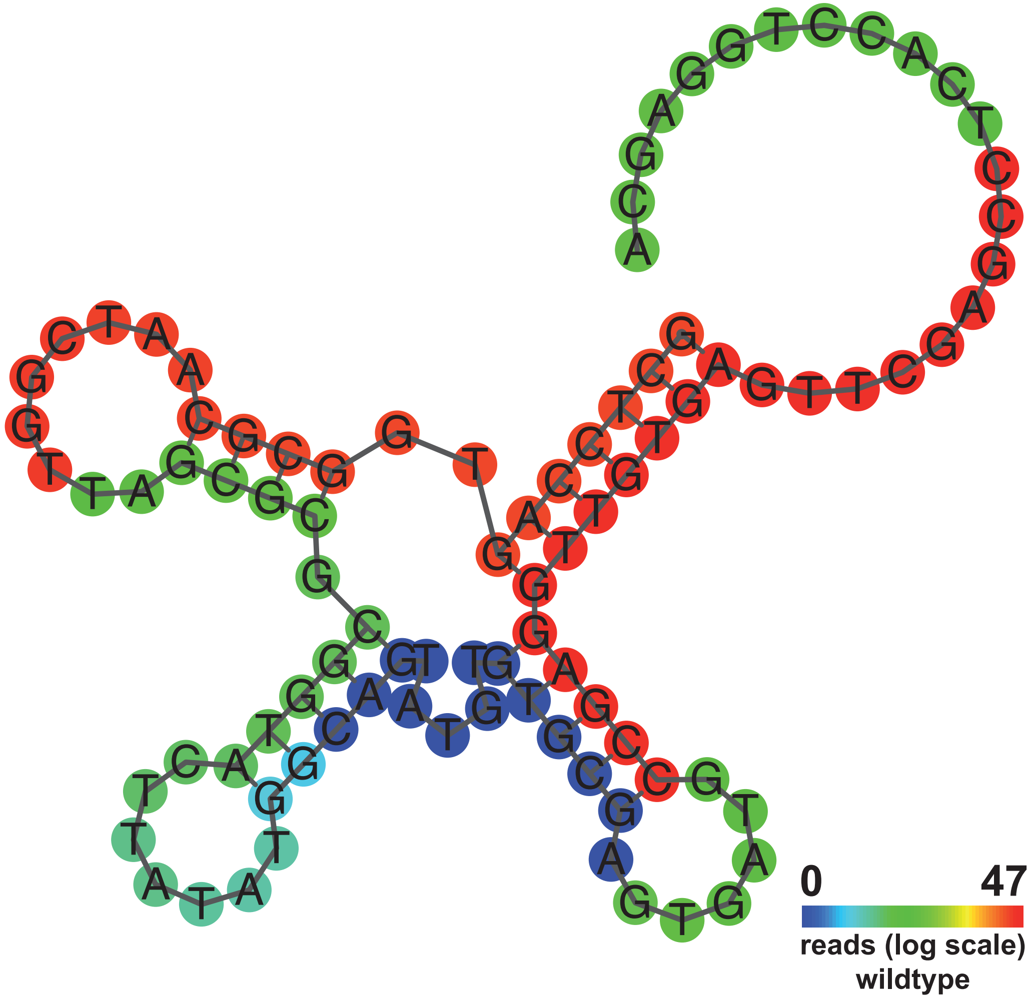
tRNA$^{\mathrm{Gln/CTG}}$

log2 fold change: -1.73



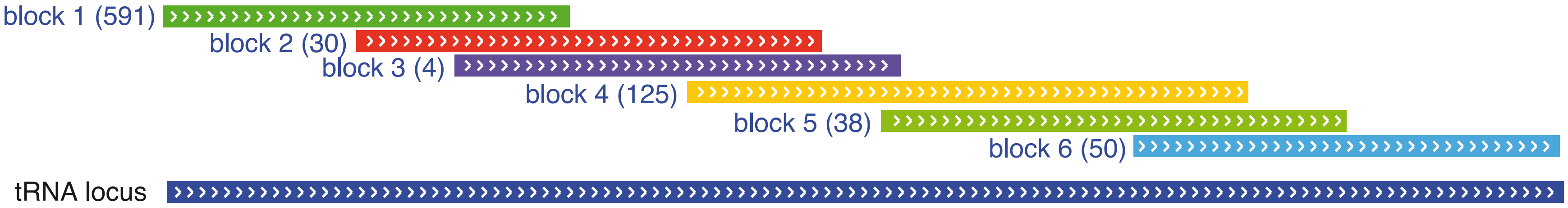
tR

log2 f



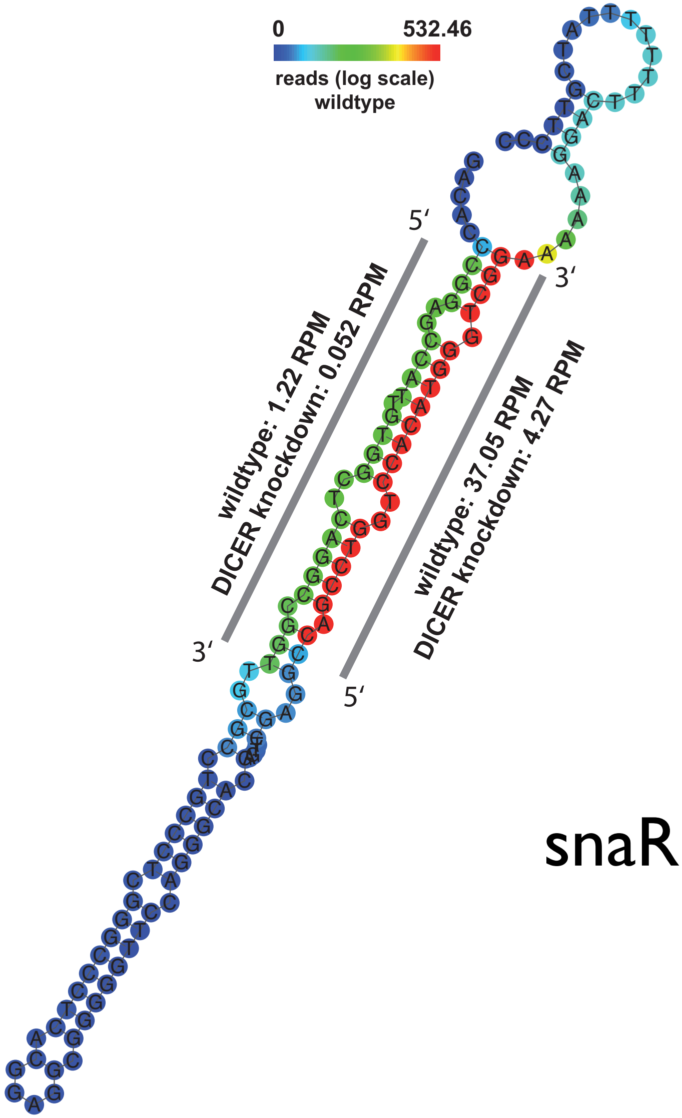
tRNA$^{\mathrm{Ile/TAT}}$

log2 fold change: -0.88



no block is favored for DICER cleavage

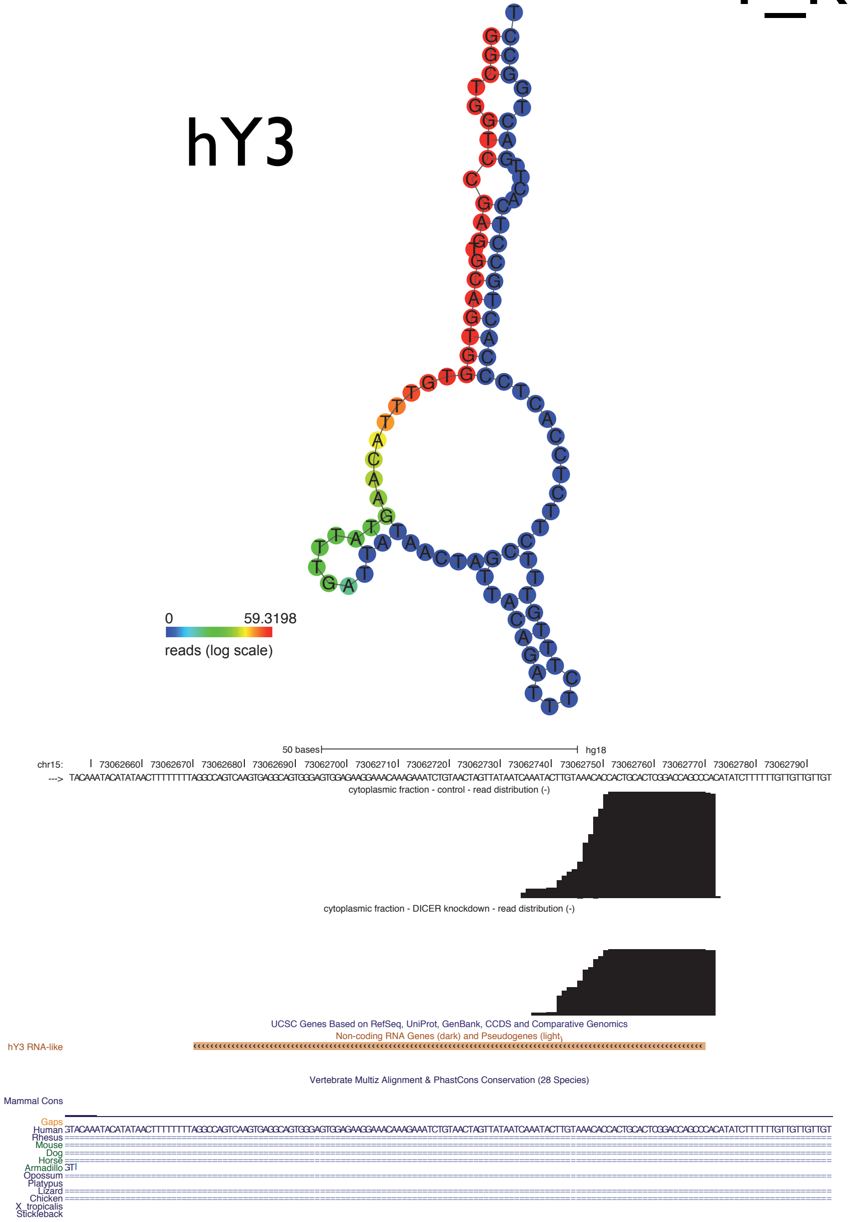
Are there any new ncRNA types cut by DICER?


0
532.46
reads (log scale)
wildtype
5′
3′
wildtype: 1.22 RPM
DICER knockdown: 0.052 RPM
3′
5′
wildtype: 37.05 RPM
DICER knockdown: 4.27 RPM

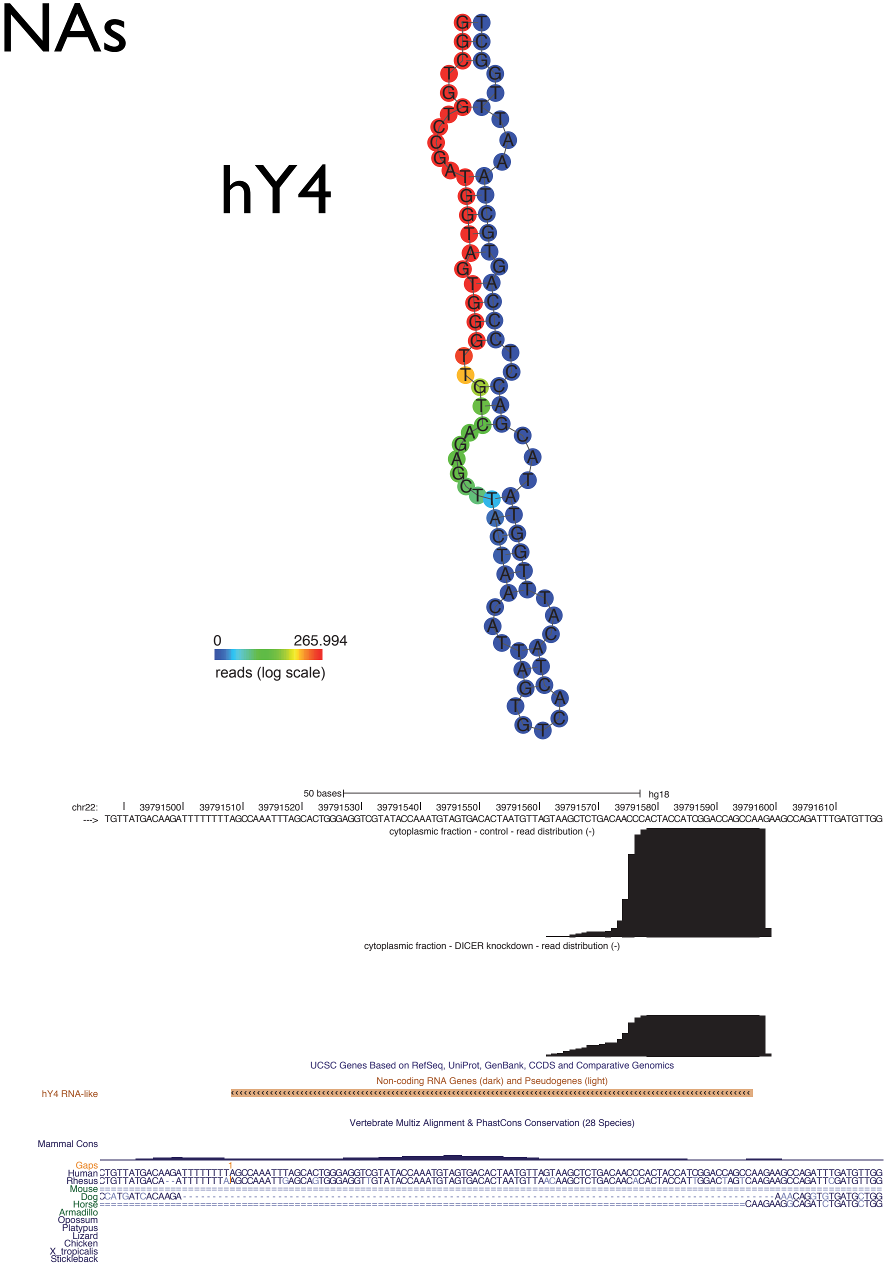
snaRs

Y_RNAs

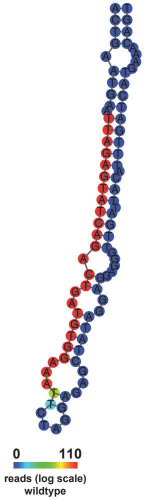
hY3



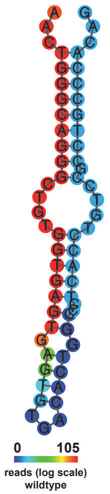
hY4



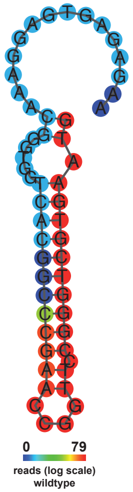
Is it possible to find new microRNAs?



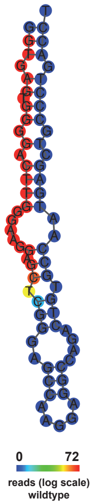
chr2:81,100,049-81,100,134(+)
log2 fold change: - 2.37



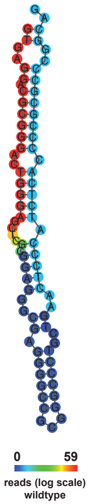
chr11:66,569,729-66,569,790(+)
log2 fold change: - 2.35



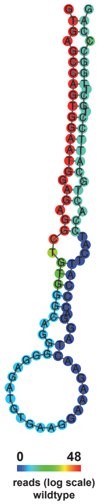
chr7:150,394,782-150,394,835(+)
log2 fold change: - 2.35



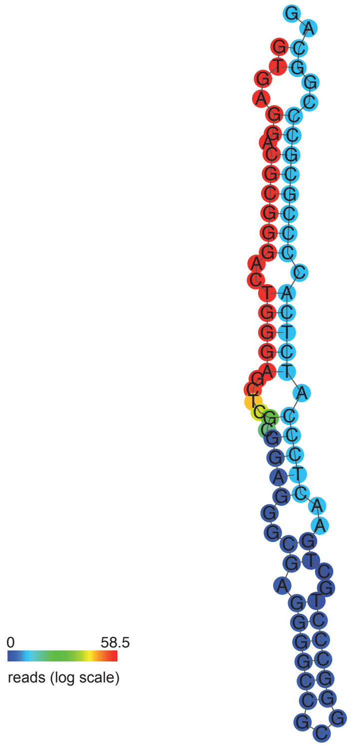
chrX:153,235,873-153,235,943(-)
log2 fold change: - 1.93

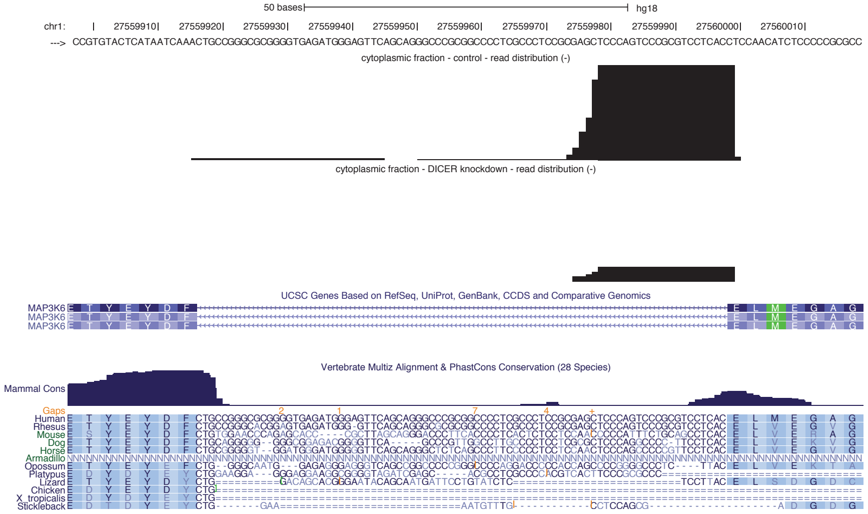


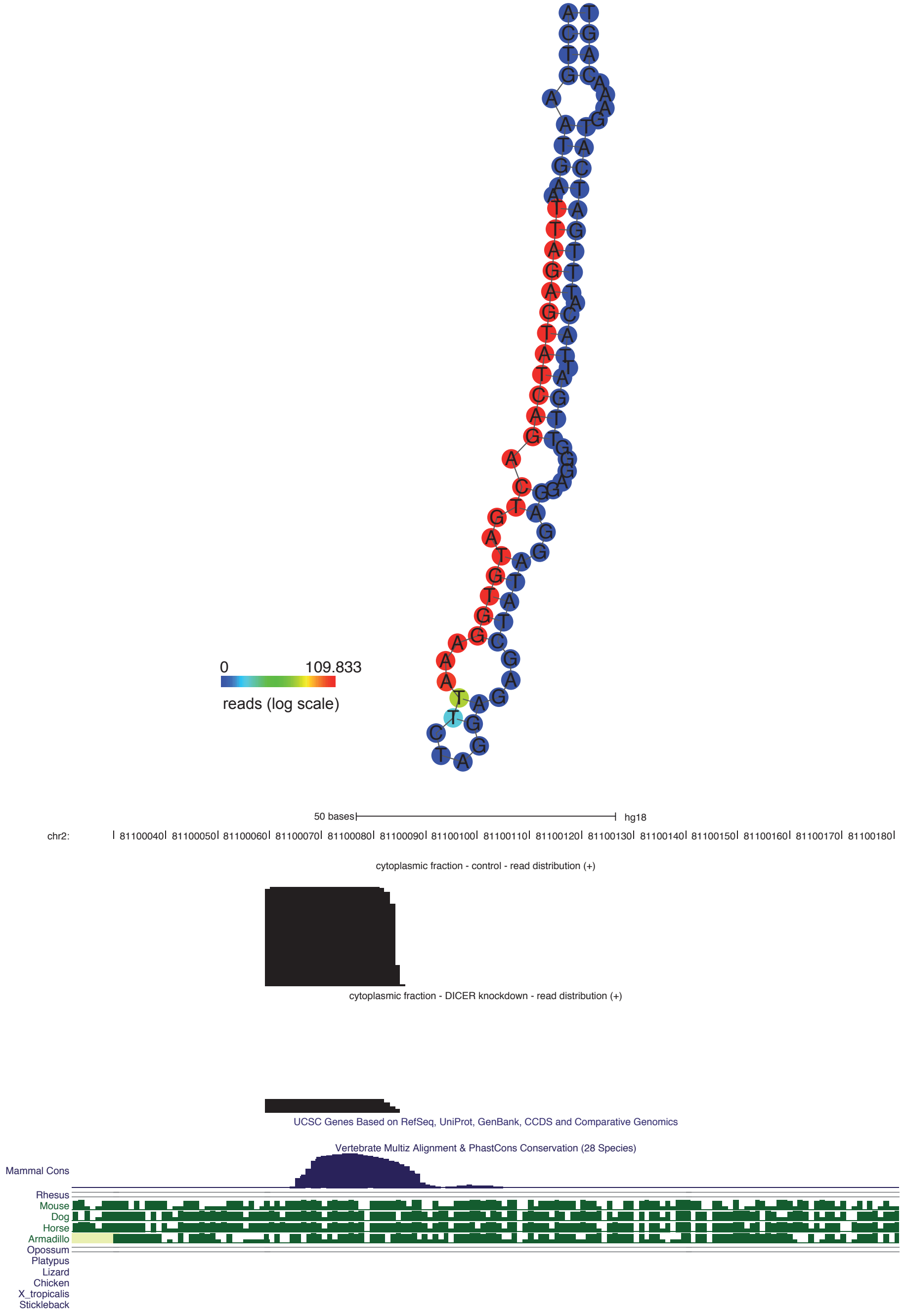
chr1:27,559,917-27,559,998(-)
log2 fold change: - 1.86



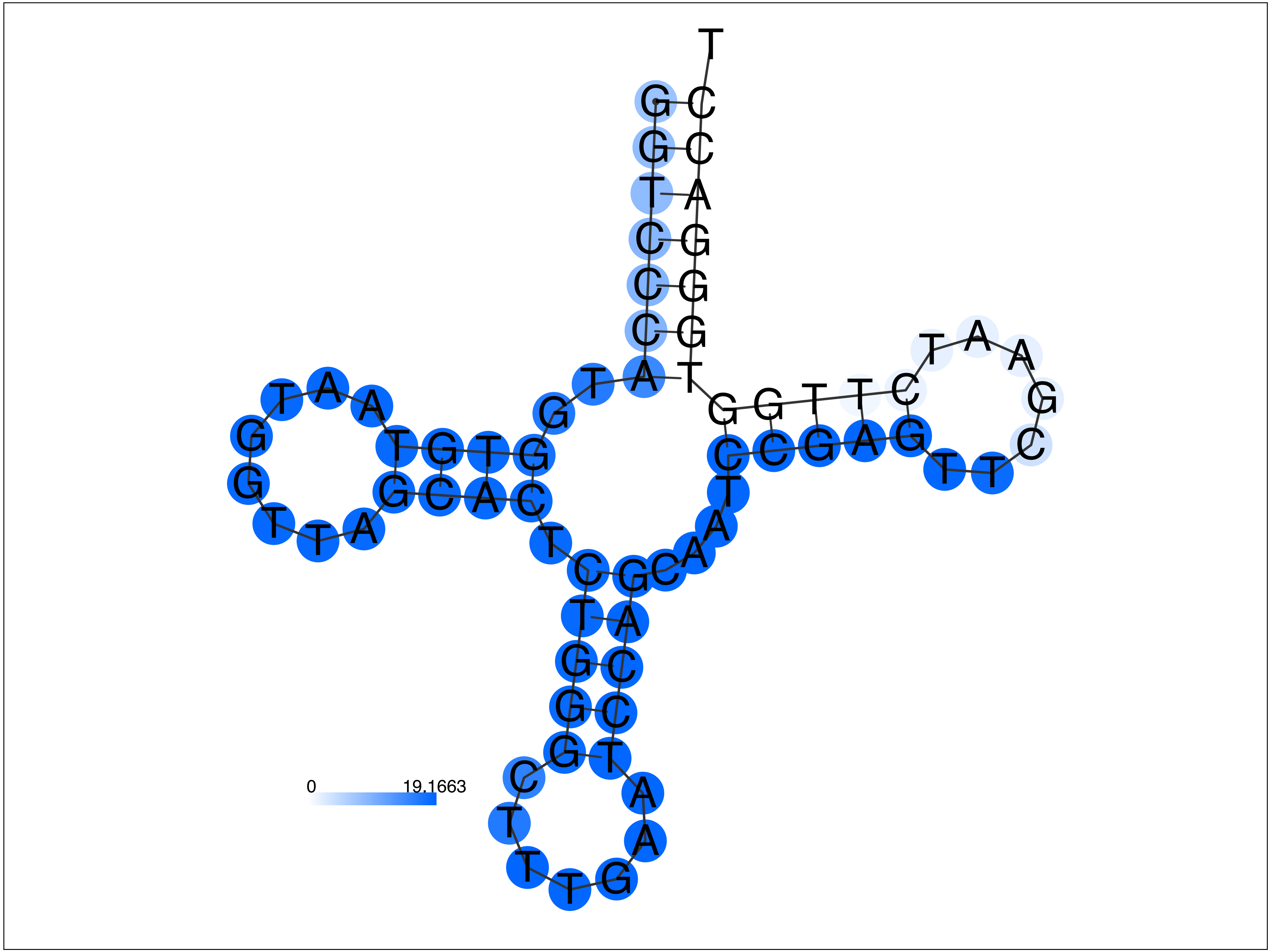
chr19:63,753,464-63,753,555(+)
log2 fold change: - 1.61


0
58.5
reads (log scale)


50 bases
hg18
chr1:
27559910
27559920
27559930
27559940
27559950
27559960
27559970
27559980
27559990
27560000
27560010
---> CCGTGTACTCATAATCAAACTGCCGGGCGCGGGGTGAGATGGGAGTTCAGCAGGGCCCGCGGCCCCTCGCCCTCCGCGAGCTCCCAGTCCCGCGTCCTCACCTCCAACATCTCCCCCGCGCC
cytoplasmic fraction - control - read distribution (-)
cytoplasmic fraction - DICER knockdown - read distribution (-)
UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics
MAP3K6
MAP3K6
MAP3K6
Vertebrate Multiz Alignment & PhastCons Conservation (28 Species)
Mammal Cons
Gaps
Human
Rhesus
Mouse
Dog
Horse
Armadillo
Opossum
Platypus
Lizard
Chicken
X_tropicalis
Stickleback


0
109.833
reads (log scale)
50 bases
hg18
chr2:
81100040
81100050
81100060
81100070
81100080
81100090
81100100
81100110
81100120
81100130
81100140
81100150
81100160
81100170
81100180
cytoplasmic fraction - control - read distribution (+)
cytoplasmic fraction - DICER knockdown - read distribution (+)
UCSC Genes Based on RefSeq, UniProt, GenBank, CCDS and Comparative Genomics
Vertebrate Multiz Alignment & PhastCons Conservation (28 Species)
Mammal Cons
Rhesus
Mouse
Dog
Horse
Armadillo
Opossum
Platypus
Lizard
Chicken
X_tropicalis
Stickleback

Usefull script?


0
19.1663

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