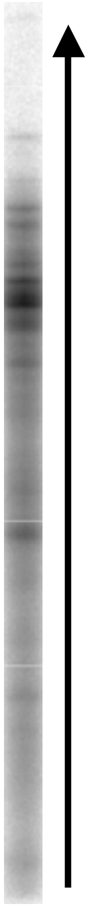
# "(micro)RNomics: Identification and expression analysis of small regulatory RNAs

Mihaela Zavolan
Biozentrum, Basel
Swiss Institute of Bioinformatics

☐ **1:** EMBO J. 2001 Jun 1;20(11):2943-53.

**RNomics: an experimental approach that identifies 201 candidates for novel, small, non-messenger RNAs in mouse.**

Huttenhofer A, Kiefmann M, Meier-Ewert S, O'Brien J, Lehrach H, Bachellerie JP, Brosius J.



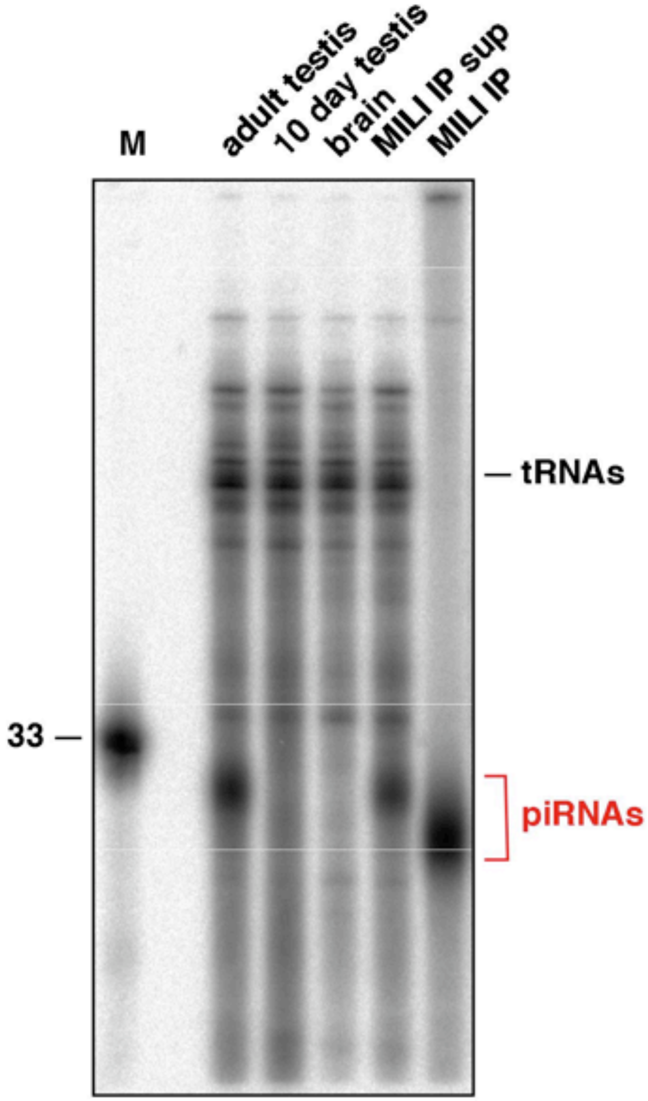
~120 H/ACA box snoRNA

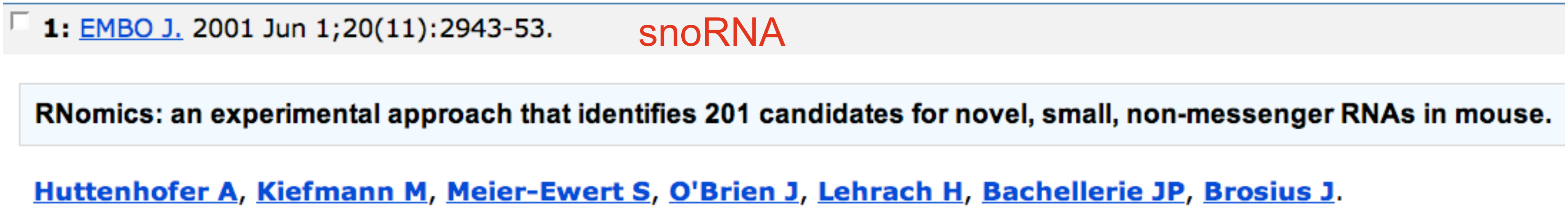
65-75 tRNA

50-70 C/D box snoRNA

21-23 miRNA

Length (nucleotides)




☐ **1:** EMBO J. 2001 Jun 1;20(11):2943-53. snoRNA

**RNomics: an experimental approach that identifies 201 candidates for novel, small, non-messenger RNAs in mouse.**

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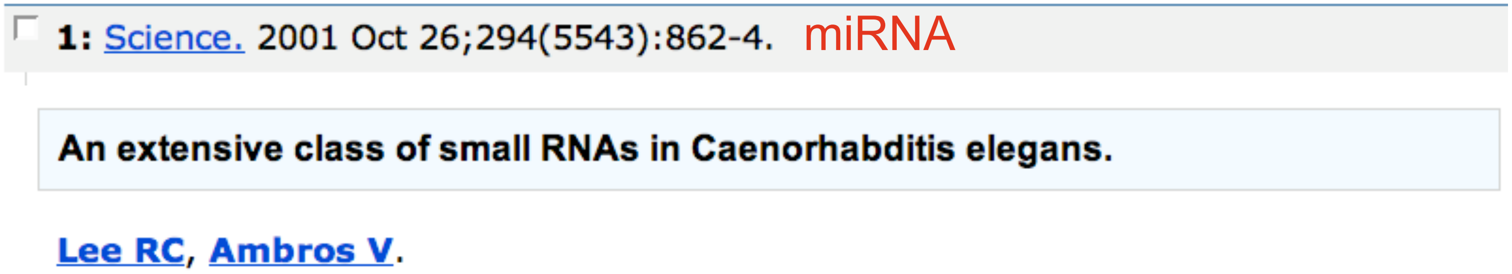
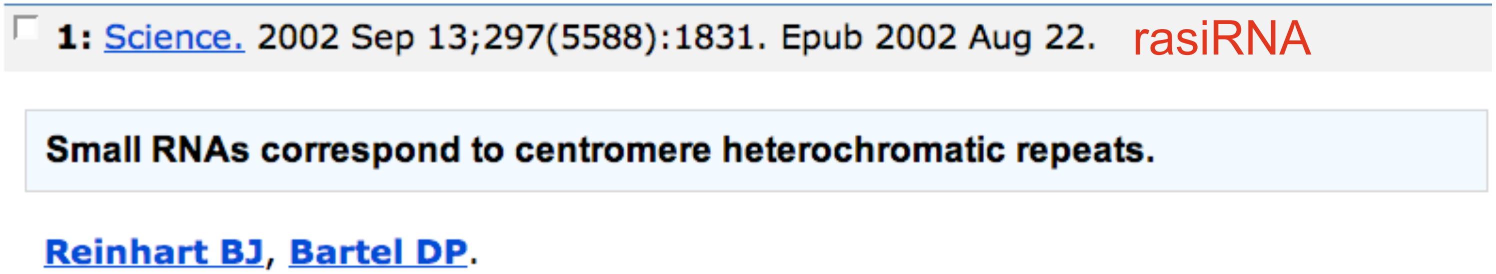

☐ **1:** Science. 2001 Oct 26;294(5543):862-4. miRNA

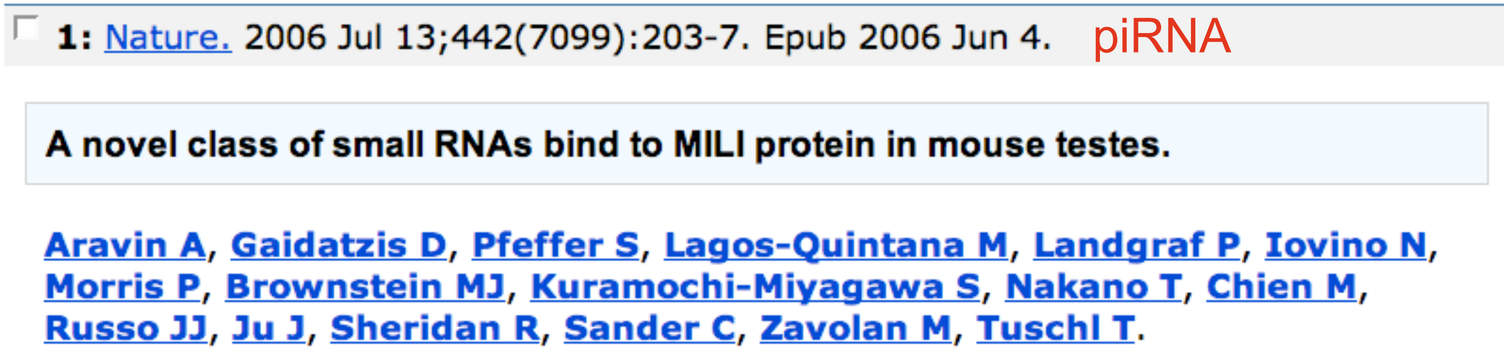
**An extensive class of small RNAs in Caenorhabditis elegans.**

**Lee RC**, **Ambros V**.


☐ **1:** Science. 2002 Sep 13;297(5588):1831. Epub 2002 Aug 22. rasiRNA

**Small RNAs correspond to centromere heterochromatic repeats.**

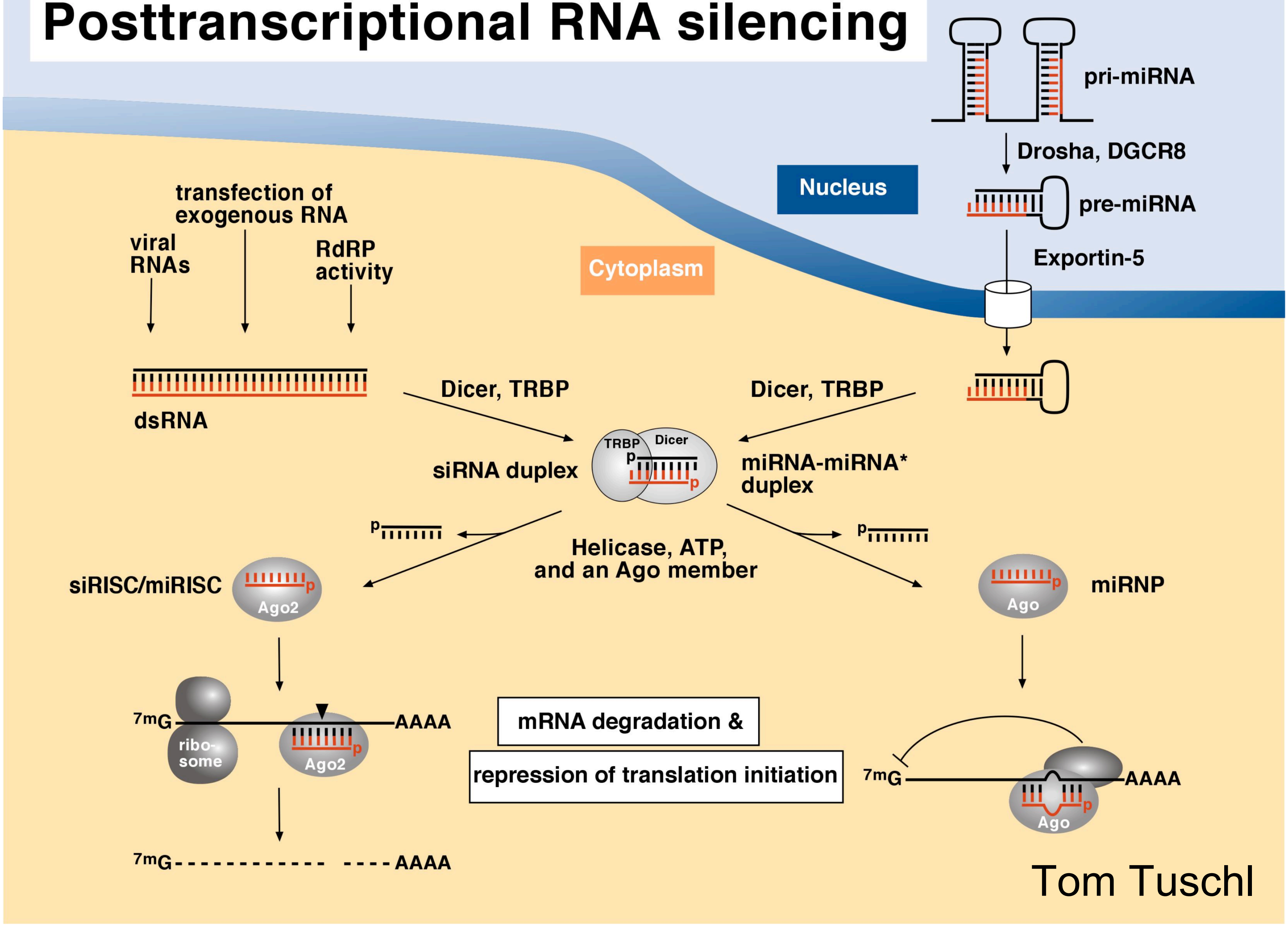
**Reinhart BJ**, **Bartel DP**.


☐ **1:** Nature. 2006 Jul 13;442(7099):203-7. Epub 2006 Jun 4. piRNA

**A novel class of small RNAs bind to MILI protein in mouse testes.**

**Aravin A**, **Gaidatzis D**, **Pfeffer S**, **Lagos-Quintana M**, **Landgraf P**, **Iovino N**, **Morris P**, **Brownstein MJ**, **Kuramochi-Miyagawa S**, **Nakano T**, **Chien M**, **Russo JJ**, **Ju J**, **Sheridan R**, **Sander C**, **Zavolan M**, **Tuschl T**.

# Posttranscriptional RNA silencing



pri-miRNA

Drosha, DGCR8

Nucleus

pre-miRNA

Exportin-5

Cytoplasm

transfection of exogenous RNA

viral RNAs

RdRP activity

dsRNA

Dicer, TRBP

Dicer, TRBP

siRNA duplex

miRNA-miRNA* duplex

Helicase, ATP, and an Ago member

siRISC/miRISC

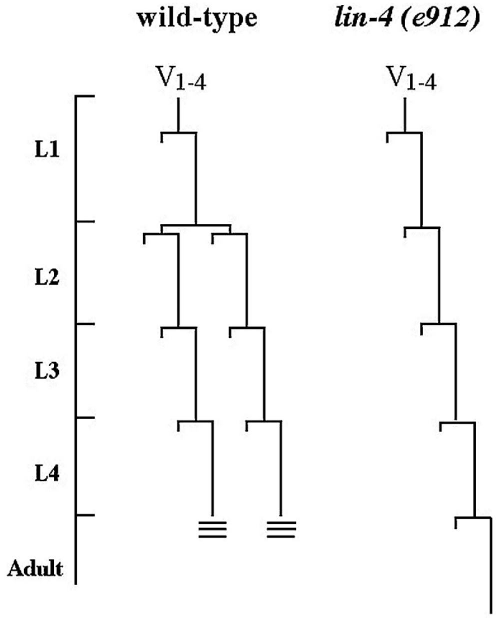
miRNP

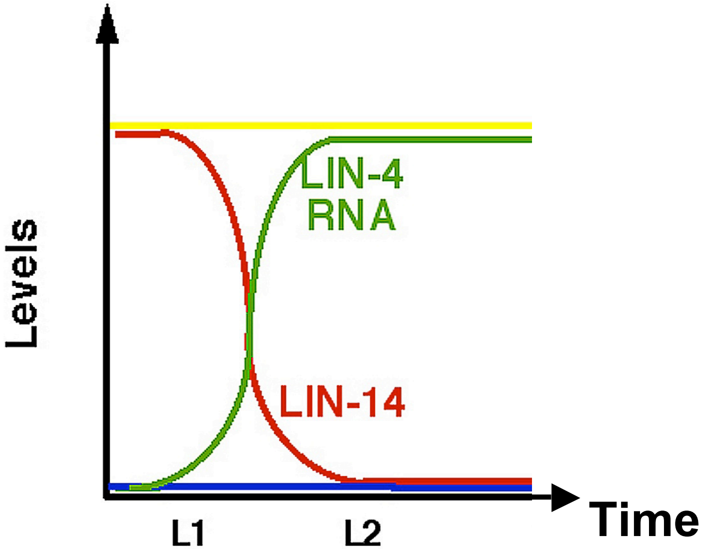
mRNA degradation &

repression of translation initiation

Tom Tuschl

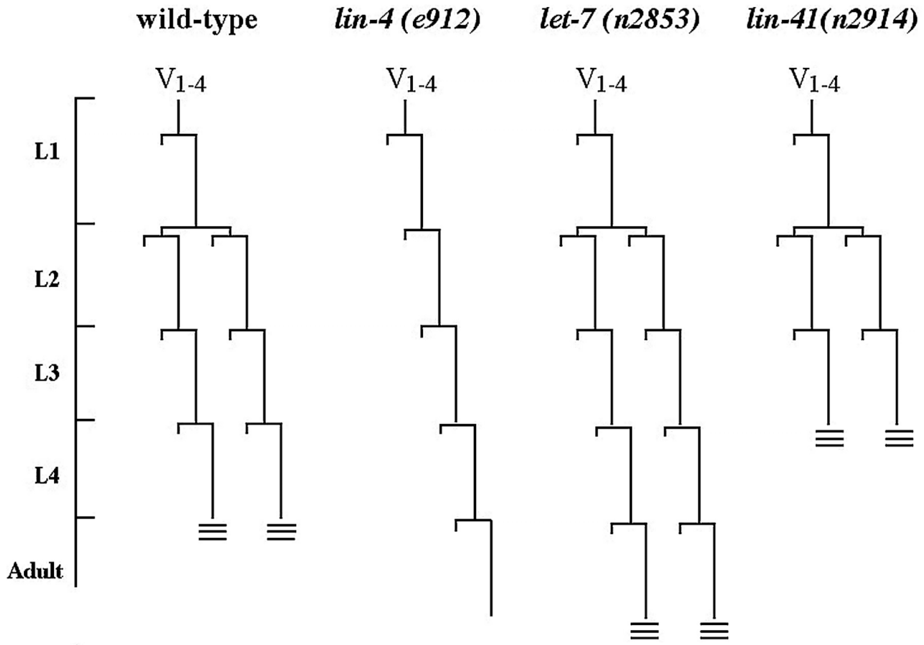
# miRNA gene discovery

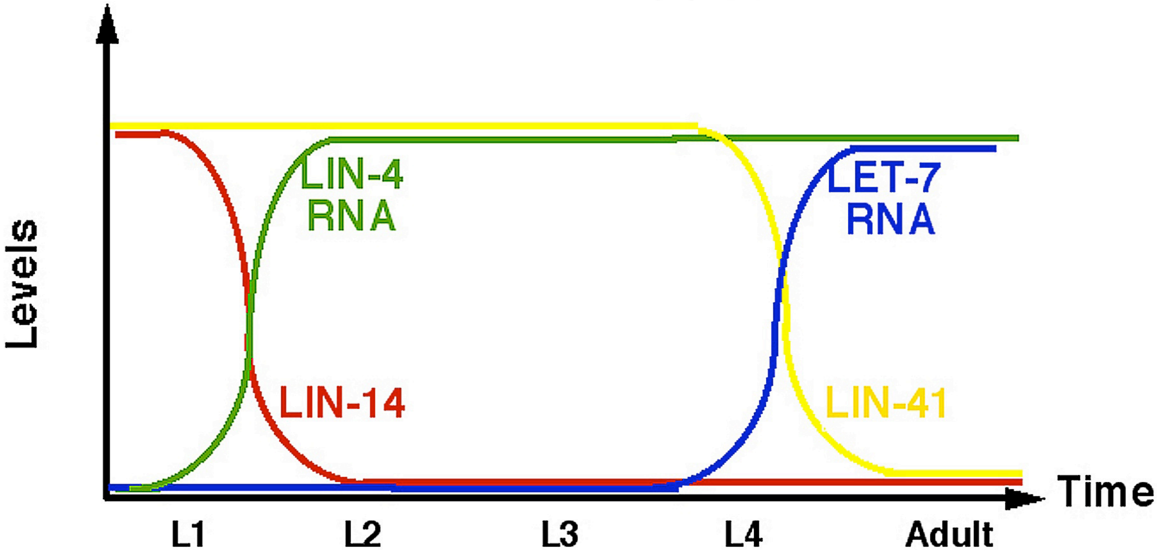




Vella & Slack
http://www.wormbook.org

# miRNA gene discovery





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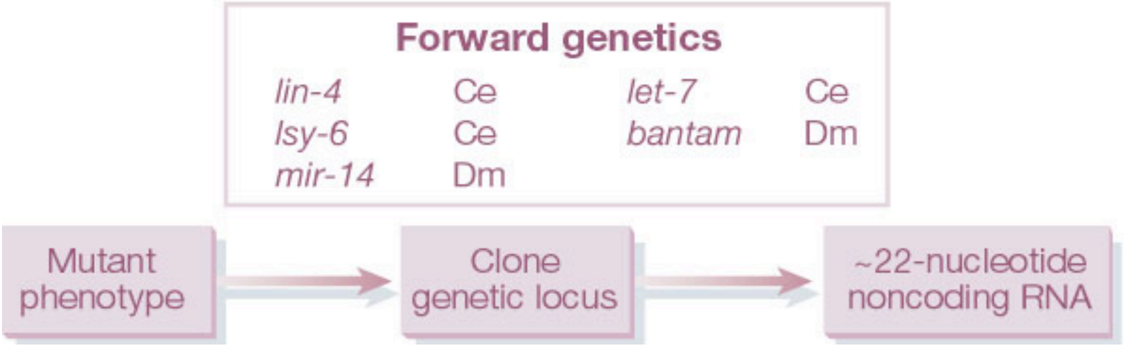
# miRNA gene discovery

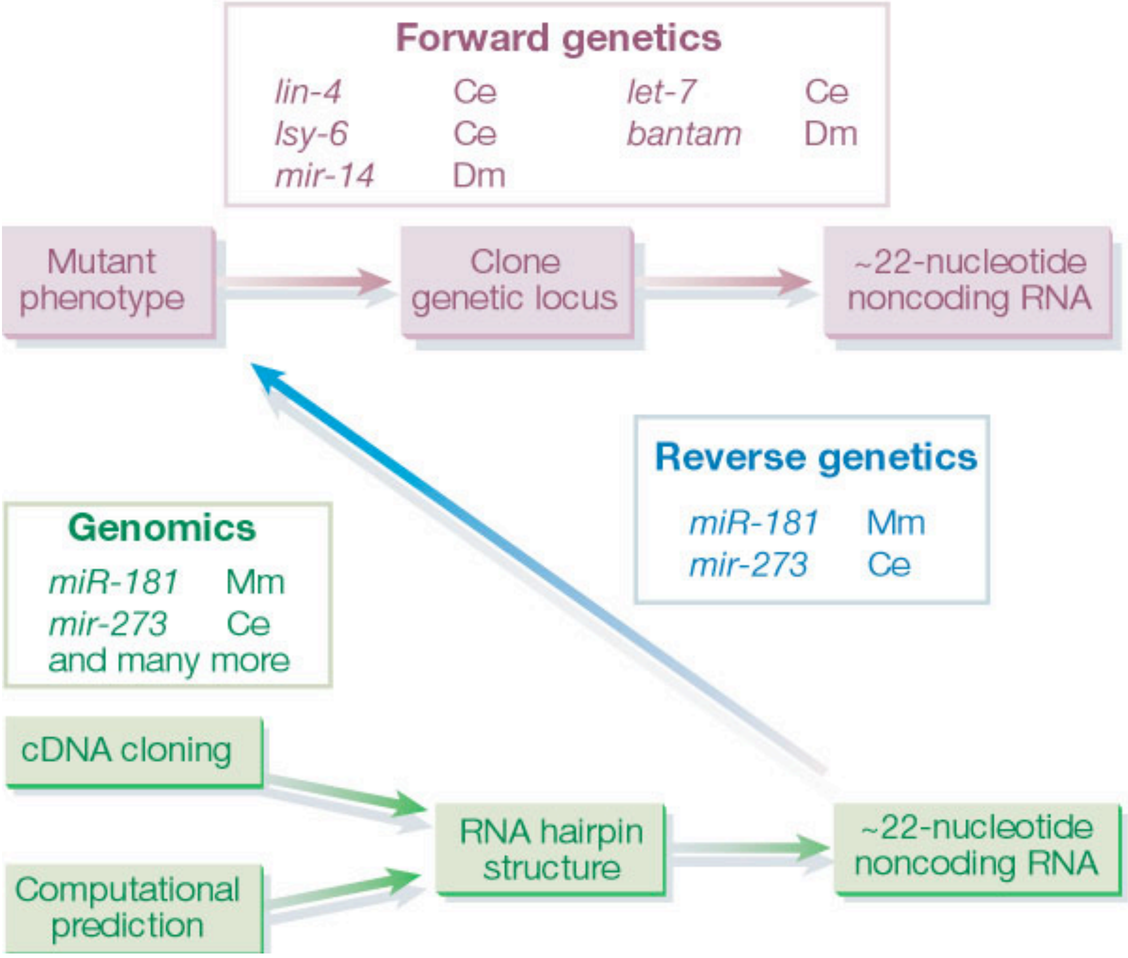


The functions of animal microRNAs
Victor Ambros Nature 341:350 (2004)

# Let-7 is a strongly conserved miRNA

```
           ((((..(((.(((.(((((((((((((............(((......)))))))))))))))).))).)))..)))).............
                TGAGGTAGTAGGTTGTATAGT
human      CAGGTTGAGGTAGTAGGTTGTATAGTTTAG-AAT-T-ACATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGTCTT-GCACT
chimp      CAGGTTGAGGTAGTAGGTTGTATAGTTTAG-AAT-T-ACATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGTCTT-GCACT
dog        CAGGTTGAGGTAGTAGGTTGTATAGTTTAG-AAT-T-ACATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGTCTT-GCAC-
mouse      CAGGTTGAGGTAGTAGGTTGTATAGTTTAG-AGT-T-ACATCAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGACTT-GCAC-
rat        CAGGCTGAGGTAGTAGGTTGTATAGTTTAG-AGT-T-ACAACAAGGGAGATAACTGTACAGCCTCCTAGCTTTCCTTGGGACTT-GCAC-
chicken    CAGGTTGAGGTAGTAGGTTGTATAGTTTAG-AAT-T-ACACCAAGGGAGATAACTGTACAACCTCCTAGCTTTCCTTGGGTCTT-GCAC-
zebrafish  CAGGCTGAGGTAGTAGGTTGTATAGTTTAG-AAT-A-ACATCACTGGAGATAACTGTACAACCTCCTAGCTTTCCCTGAG----------
fugu       CAGGTTGAGGTAGTAGGTTGTATAGTTGAG-AGT-G-ACACCACAGGAGATGACTGTACAGCCTCCTAGCTTTCCCTGAG----------
pufferfish -AGGTTGAGGTAGTAGGTTGTATAGTTGAG-AGT-T-ACACCCCCAGAGATAACTGTACAGCCTCCTAGCTTTCCCTGAG-CTACGCAC-
frog       CAGGCTGAGGTAGTAGGTTGTATAGTTGAG-GAT-A-ACACCAAAGGAGATAACTGTACAGCCTCCTATCTTTCCCTGGGGCTT------
D.mel      CAAATTGAGGTAGTAGGTTGTATAGT--AGTAAT-T-ACA-CATC----ATA-CTATACAATGTGCTAGCTTTCTTTG------------
D.pse      CAAATTGAGGTAGTAGGTTGTATAGT--AG-----T-ACTTCA-GATCG-TA-CTATACAATGTGCTAGCTTTCTTTG------------
D.vir      CAAATTGAGGTAGTAGGTTGTATAGT--AG-TAT-T-T-AT-A-GAGC-ATA-CTATACAGCGTGCTAGCTTTCTTTG------------
D.moj      CAAATTGAGGTAGTAGGTTGTATAGT--AG-TAC-T-T-AT--AGACC-ATA-CTATACAGCGTGCTAGCTTTCTTTG------------
D.ana      CAAATTGAGGTAGTAGGTTGTATAGT--AG-CA----AC-T-AAGAATCGTA-CTATACAATGTGCTAGCTTTCTTTG------------
D.yak      CAAATTGAGGTAGTAGGTTGTATAGT--AGTAAT-T-ACA-CATC----ATA-CTATACAATGTGCTAGCTTTCTTTG------------
C.ele      ---GGTGAGGTAGTAGGTTGTATAGTTTGG-AAT-A-TTACCACCGGTGA-A-CTATGCAATTTTCTACCTTACC---------------
C.bri      -ACGGTGAGGTAGTAGGTTGTATAGTTTAG-AATATTAC-TCTCGGT-GA-A-CTATGCAAGTTTCTACCTCACC---------------
```

# miRNA gene discovery



**Forward genetics**

| | | | |
|---|---|---|---|
| *lin-4* | Ce | *let-7* | Ce |
| *lsy-6* | Ce | *bantam* | Dm |
| *mir-14* | Dm | | |

Mutant phenotype → Clone genetic locus → ~22-nucleotide noncoding RNA

**Reverse genetics**

| | |
|---|---|
| *miR-181* | Mm |
| *mir-273* | Ce |

**Genomics**

| | |
|---|---|
| *miR-181* | Mm |
| *mir-273* | Ce |

and many more

Experiment-driven: cDNA cloning → RNA hairpin structure

Computation-driven: Computational prediction → RNA hairpin structure

RNA hairpin structure → ~22-nucleotide noncoding RNA

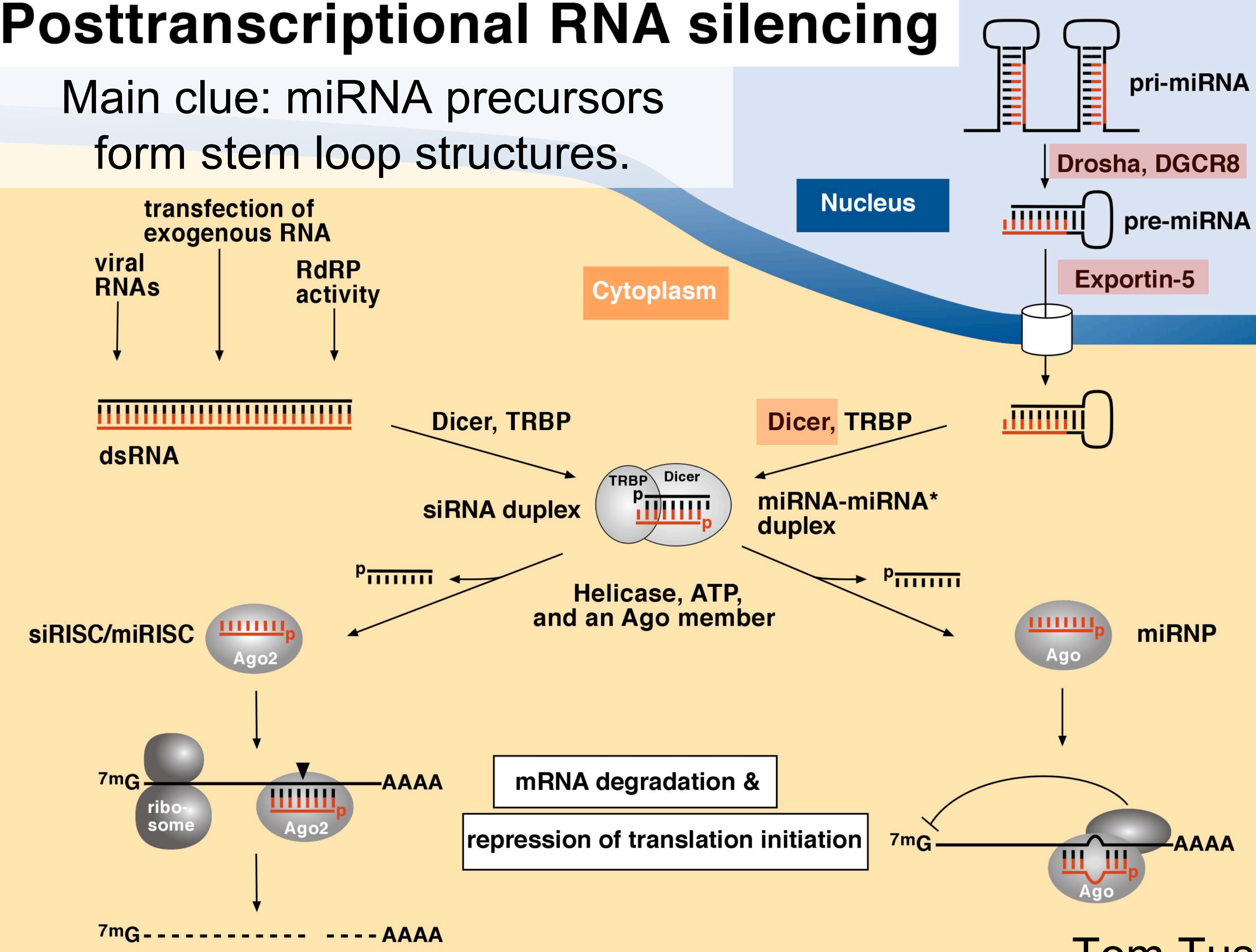
PubMed: miRNA & 2001 [dp] - 4
2006 - 598

The functions of animal microRNAs
Victor Ambros Nature 341:350 (2004)

# Computation-driven miRNA discovery

# Posttranscriptional RNA silencing

Main clue: miRNA precursors form stem loop structures.



pri-miRNA

Drosha, DGCR8

Nucleus

pre-miRNA

Exportin-5

Cytoplasm

transfection of exogenous RNA

viral RNAs

RdRP activity

dsRNA

Dicer, TRBP

Dicer, TRBP

siRNA duplex

TRBP

Dicer

miRNA-miRNA* duplex

Helicase, ATP, and an Ago member

siRISC/miRISC

Ago2

miRNP

Ago

$^{7m}$G

AAAA

ribosome

Ago2

mRNA degradation &

repression of translation initiation

$^{7m}$G

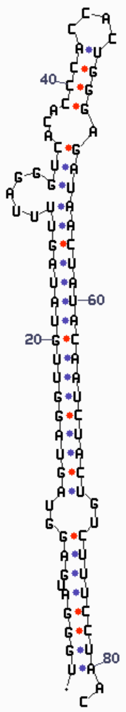
AAAA

Ago

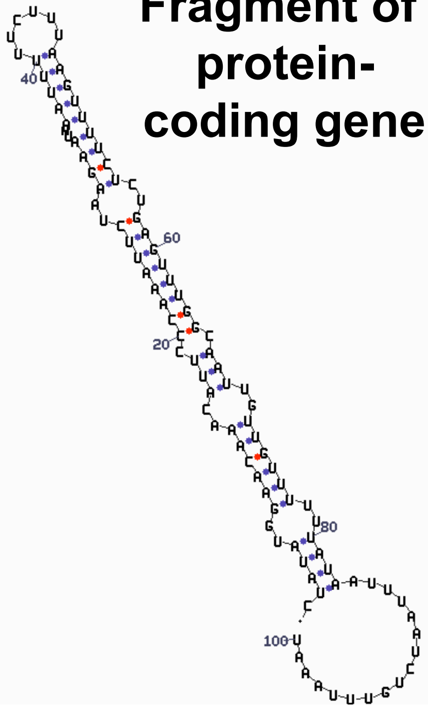
$^{7m}$G - - - - - - - - - - - -   - - - - AAAA

Tom Tuschl

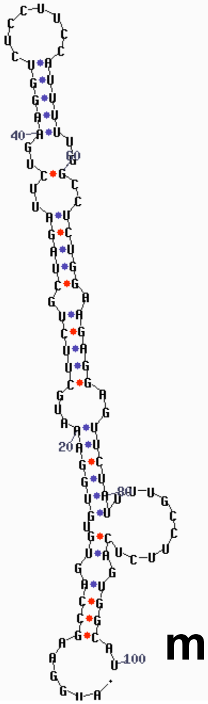
# ... so do many other genomic regions



**let-7a**



**Fragment of protein-coding gene**



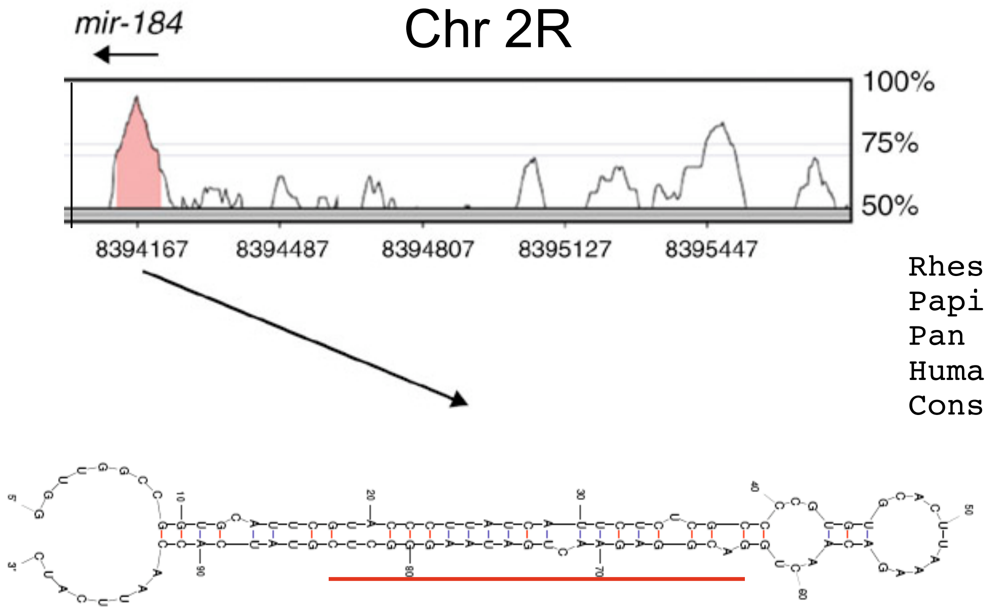
**mir-147**

RNA secondary structures predicted using Mfold
http://www.bioinfo.rpi.edu/applications/mfold

# miRNA gene prediction

Use evolutionary information.

Whole-genome alignment of D.melanogaster and D.pseudoobscura

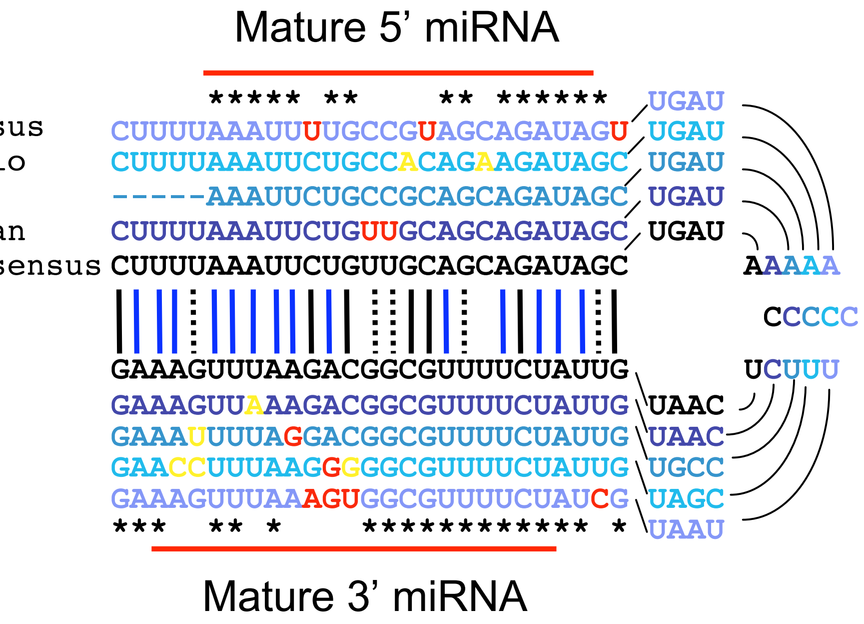


**UGGACGGAGAACUGAUAAGGGC**

Lai et al. Genome Biology 2003

Viral miRNAs are poorly conserved.

ebv-mir-BHRF1-2

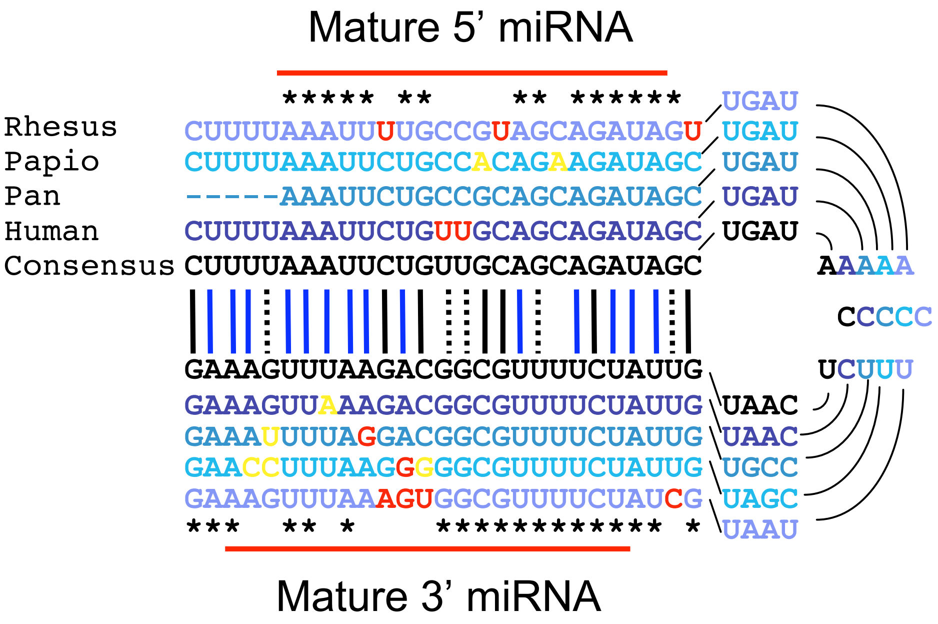


# miRNA gene prediction

Need for a prediction method that does not require the miRNA to be conserved across species. ⇐ Viral miRNAs are poorly conserved.

1. Identify robust stems in viral genomes.
2. Build a model from positive and negative examples.
3. Classify the stems using the model.

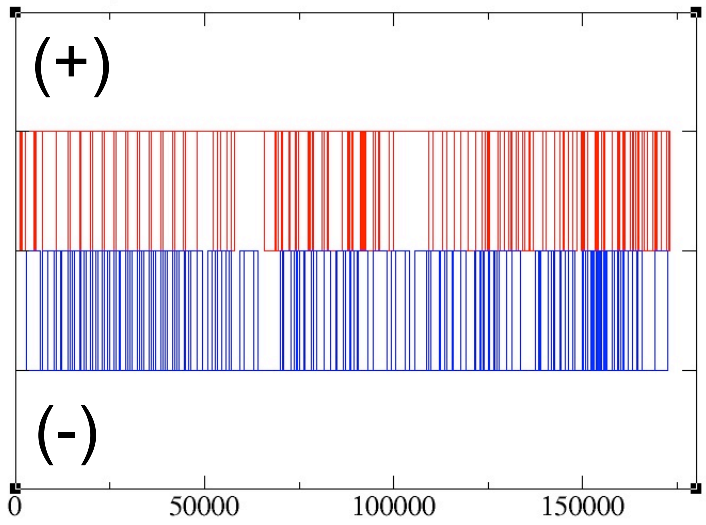
ebv-mir-BHRF1-2



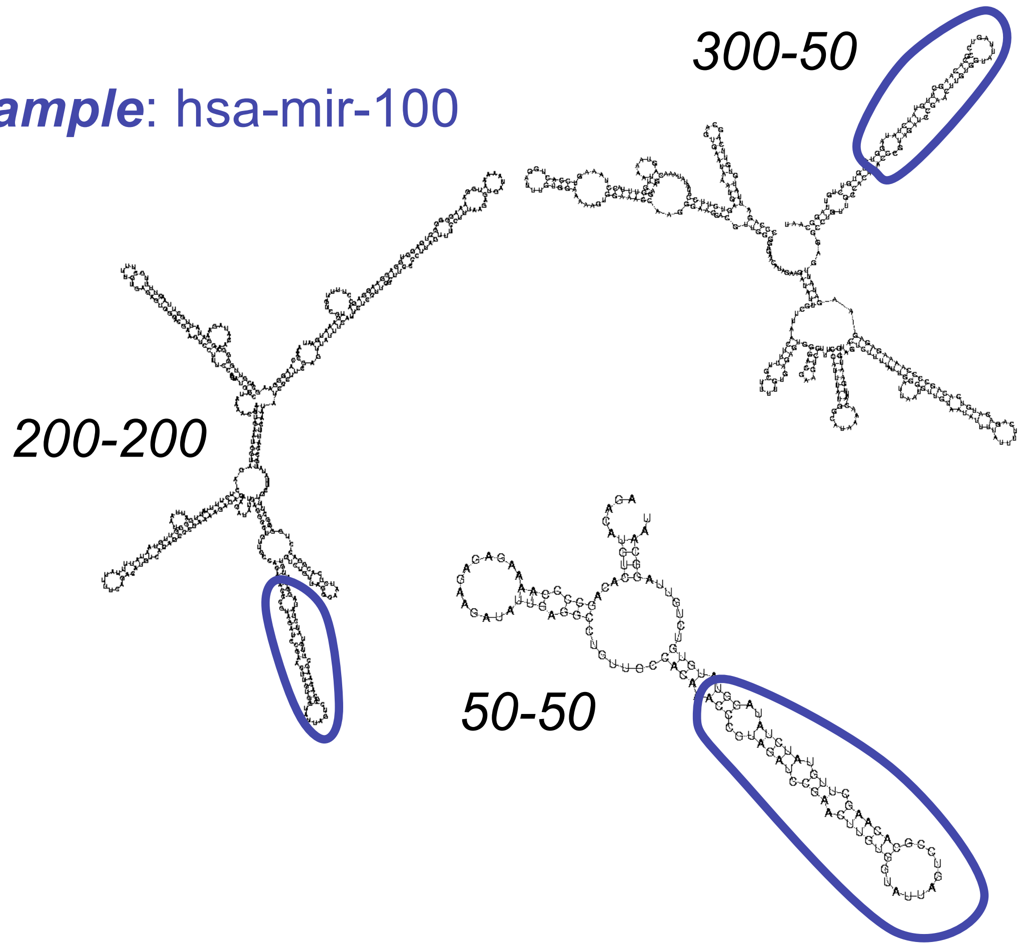
# Detection of robust stems in whole genome sequences

Search for stems whose secondary structure remains the same irrespective of their flanking sequences.

Location of robust stems in the EBV genome

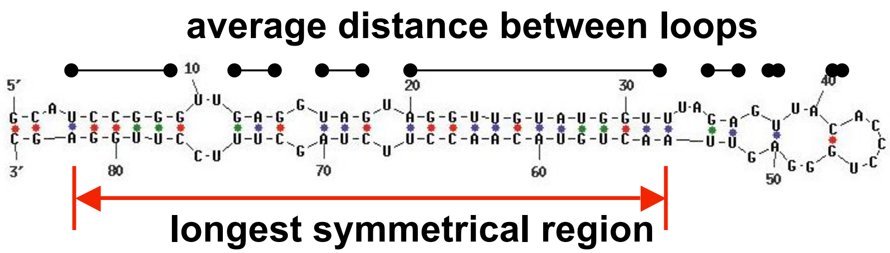


***example***: hsa-mir-100



# SVM model for ab initio miRNA gene prediction

hsa-let-7c

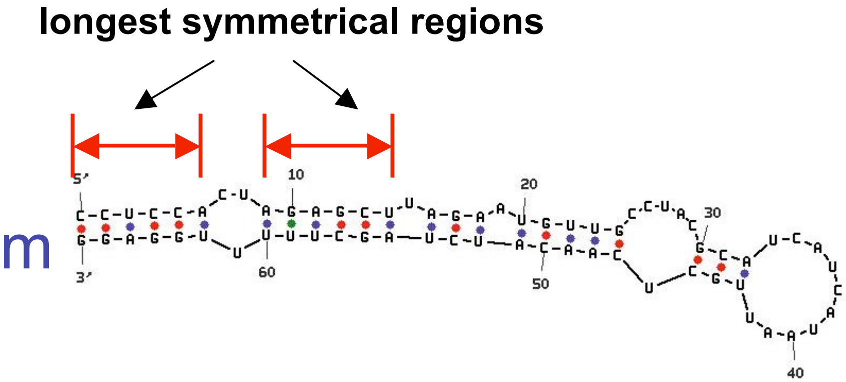


average distance between loops

longest symmetrical region

**Positives:**
**human genomic regions containing known miRNAs**

negative stem



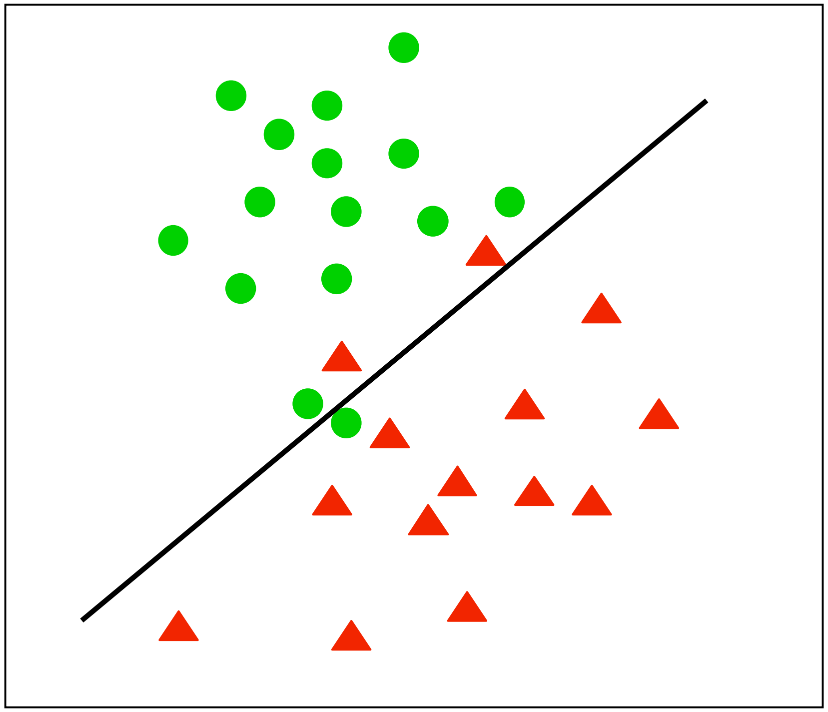
longest symmetrical regions

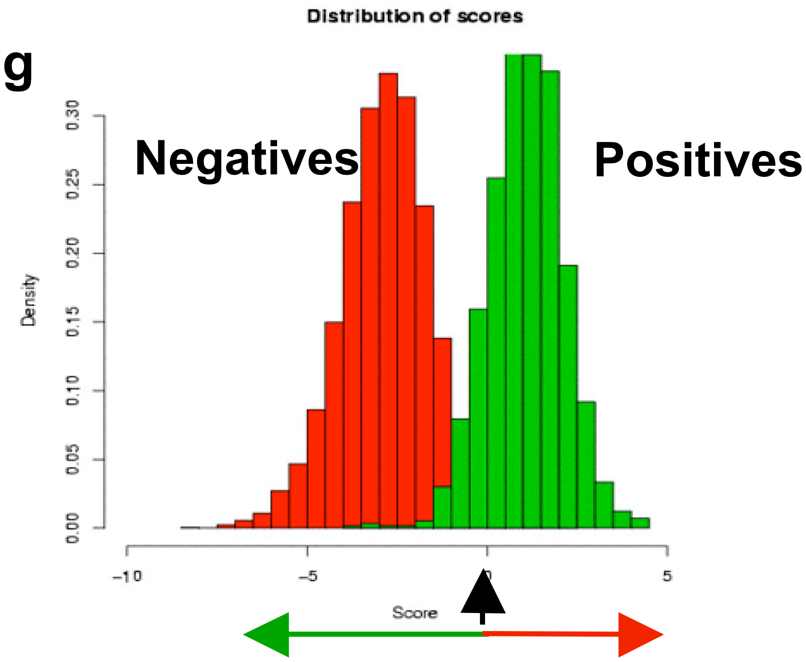
**Negatives:**
**mRNAs, rRNAs, tRNAs,**
**viral stem loops**

# SVM model for miRNA gene prediction

**Negatives:**
**mRNAs, rRNAs, tRNAs,**
**viral stem loops**

**Positives:**
**human genomic regions containing known miRNAs**





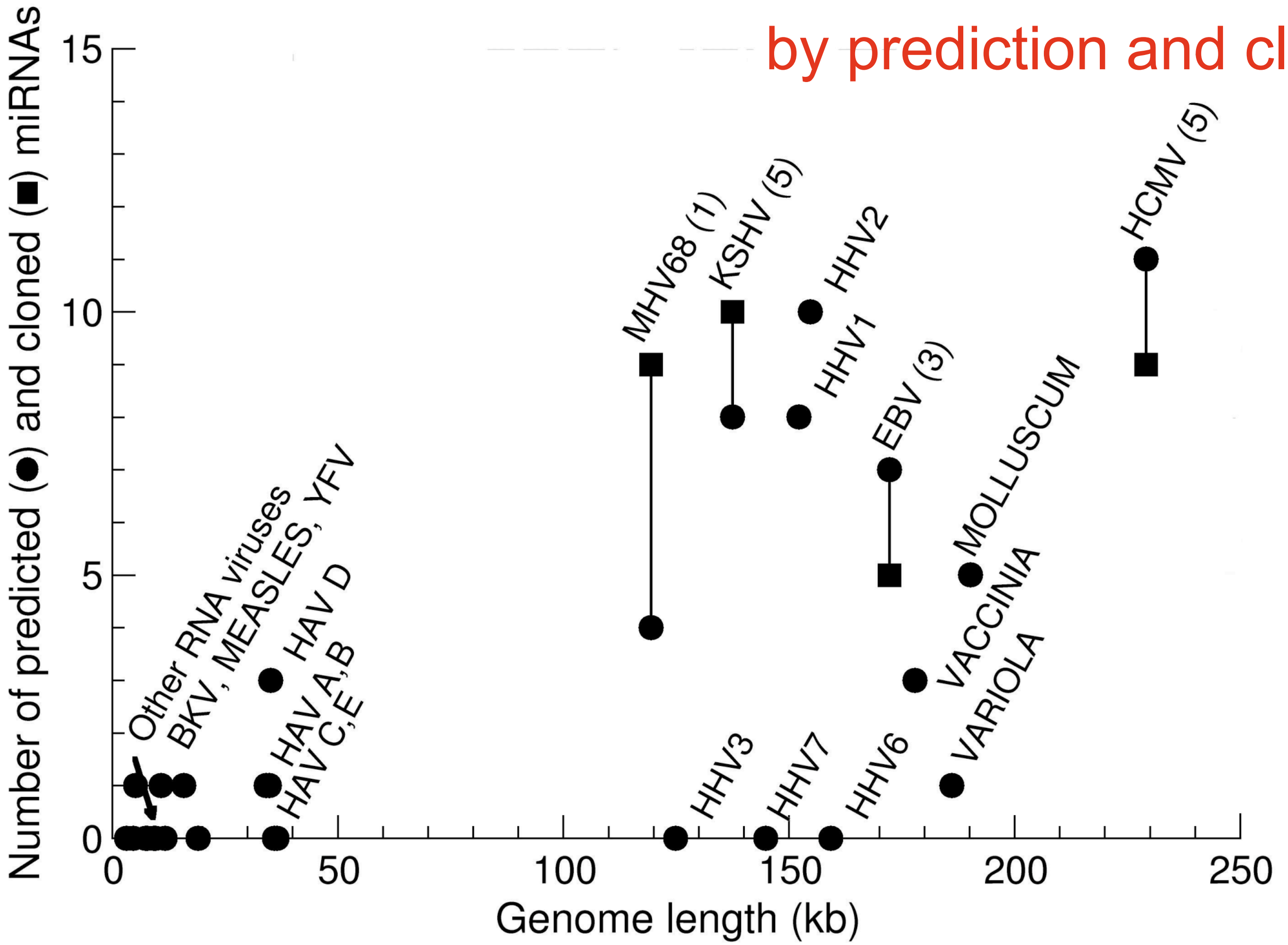
**29% false negatives**

**3% false positives**

Used SVMlight http://svmlight.joachims.org/

# Application: miRNA gene prediction in viruses

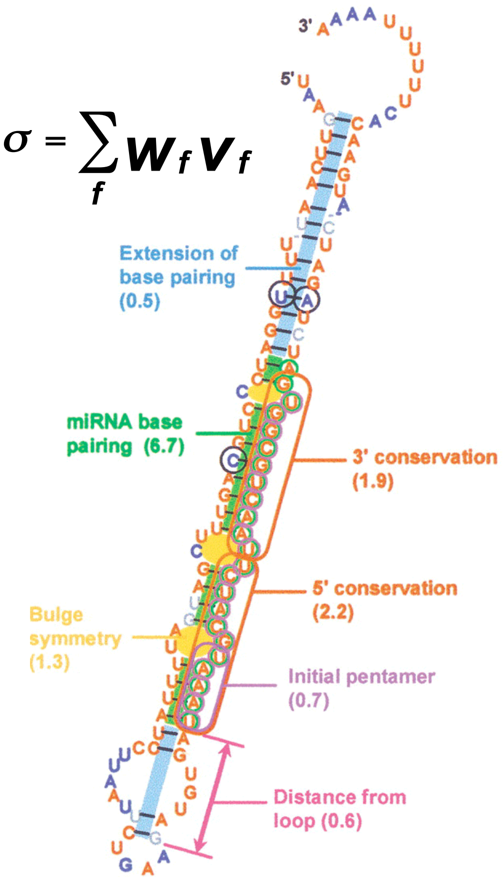
33 viral miRNAs identified by prediction and cloning

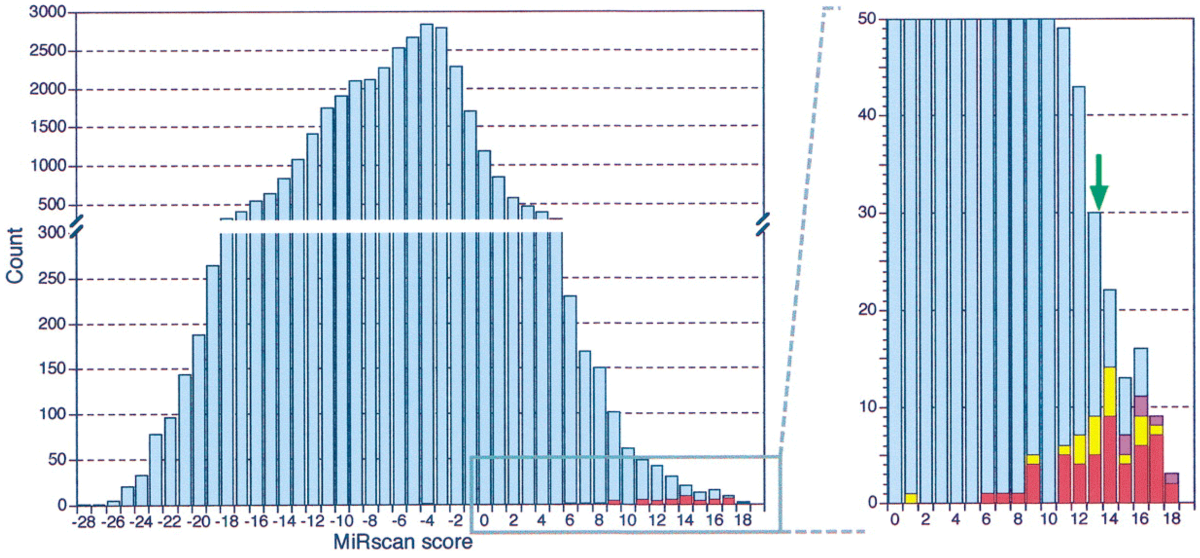


Pfeffer, Sewer et al. Nature Methods (2005)

# Variations on miRNA gene prediction

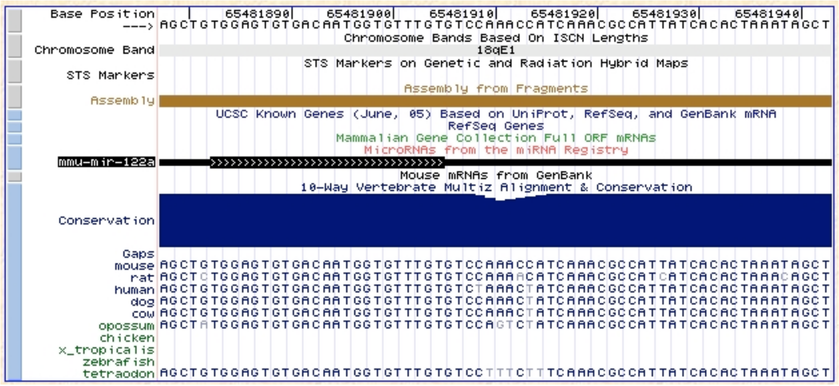
$$\sigma = \sum_f w_f v_f$$





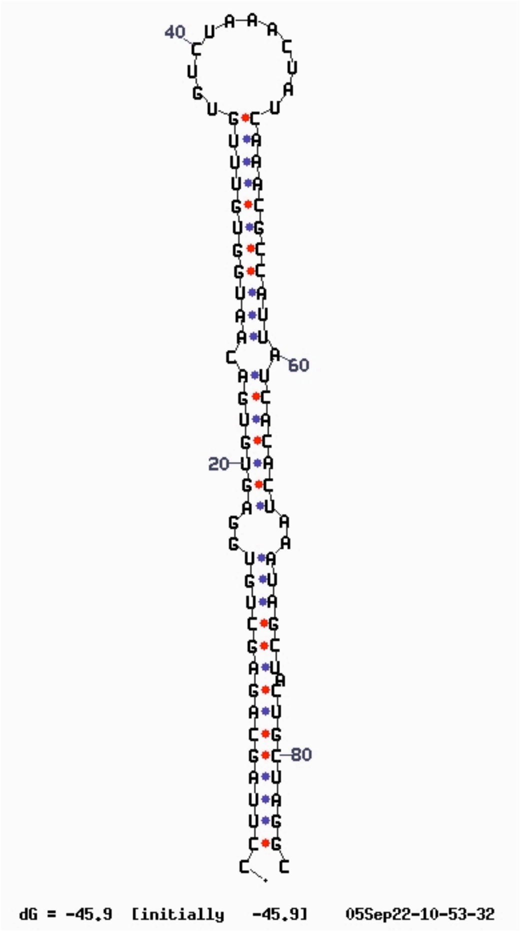
Lim, L. P. et *al*. (2003) *Genes & Dev*. **17**:991

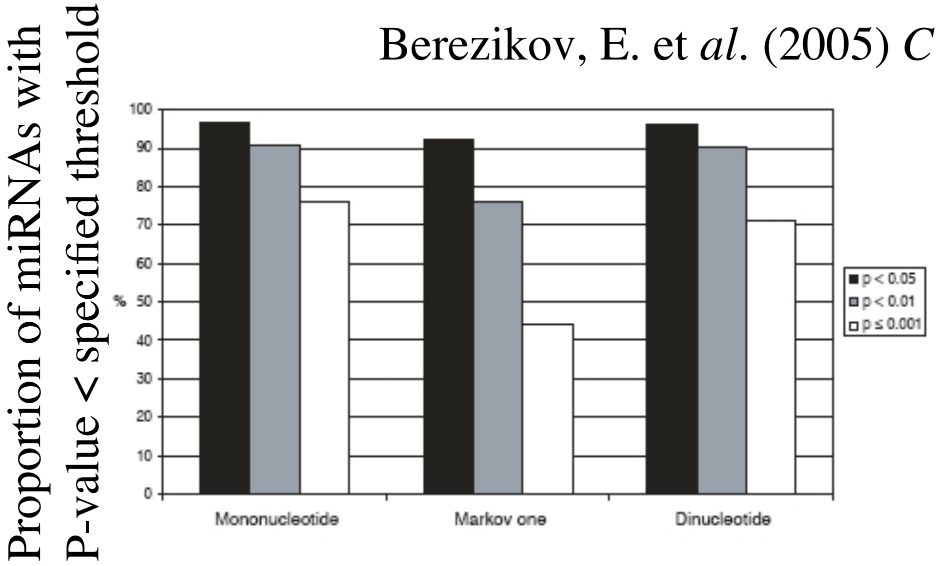
# Variations on miRNA gene prediction



```
Base Position      |    65481890|    65481900|    65481910|    65481920|    65481930|    65481940|
         ---> AGCTGTGGAGTGTGACAATGGTGTTTGTGTCCAAACCATCAAACGCCATTATCACACTAAATAGCT
                          Chromosome Bands Based On ISCN Lengths
Chromosome Band                         18qE1
                          STS Markers on Genetic and Radiation Hybrid Maps
  STS Markers
                                   Assembly from Fragments
     Assembly
                  UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
                                        RefSeq Genes
                          Mammalian Gene Collection Full ORF mRNAs
                              MicroRNAs from the miRNA Registry
 mmu-mir-122a       >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                   Mouse mRNAs from GenBank
                          10-Way Vertebrate Multiz Alignment & Conservation

 Conservation

         Gaps
        mouse AGCTGTGGAGTGTGACAATGGTGTTTGTGTCCAAACCATCAAACGCCATTATCACACTAAATAGCT
          rat AGCTCTGGAGTGTGACAATGGTGTTTGTGTCCAAAACATCAAACGCCATCATCACACTAAACAGCT
        human AGCTGTGGAGTGTGACAATGGTGTTTGTGTCTAAACTATCAAACGCCATTATCACACTAAATAGCT
          dog AGCTGTGGAGTGTGACAATGGTGTTTGTGTCCAAACTATCAAACGCCATTATCACACTAAATAGCT
          cow AGCTGTGGAGTGTGACAATGGTGTTTGTGTCCAAACTATCAAACGCCATTATCACACTAAATAGCT
      opossum AGCTATGGAGTGTGACAATGGTGTTTGTGTCCAGTCTATCAAACGCCATTATCACACTAAATAGCT
      chicken
 x_tropicalis
    zebrafish
    tetraodon AGCTGTGGAGTGTGACAATGGTGTTTGTGTCCTTTCTTTCAAACGCCATTATCACACTAAATAGCT
```

Berezikov, E. et *al*. (2005) *Cell* **120**:21



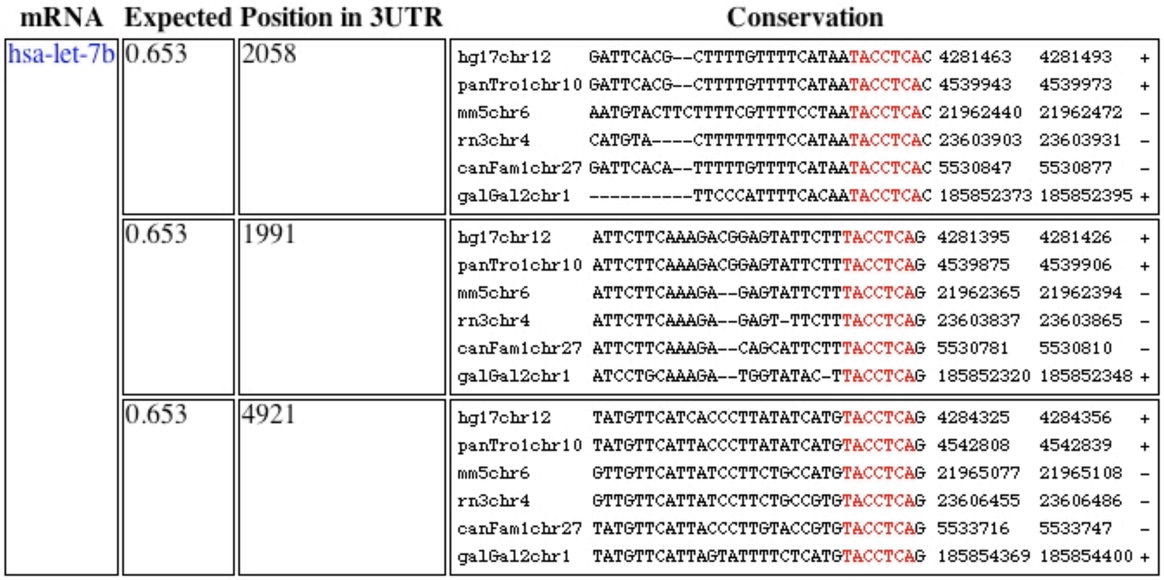


miRNA stems are signficantly more stable than randomized counterparts.

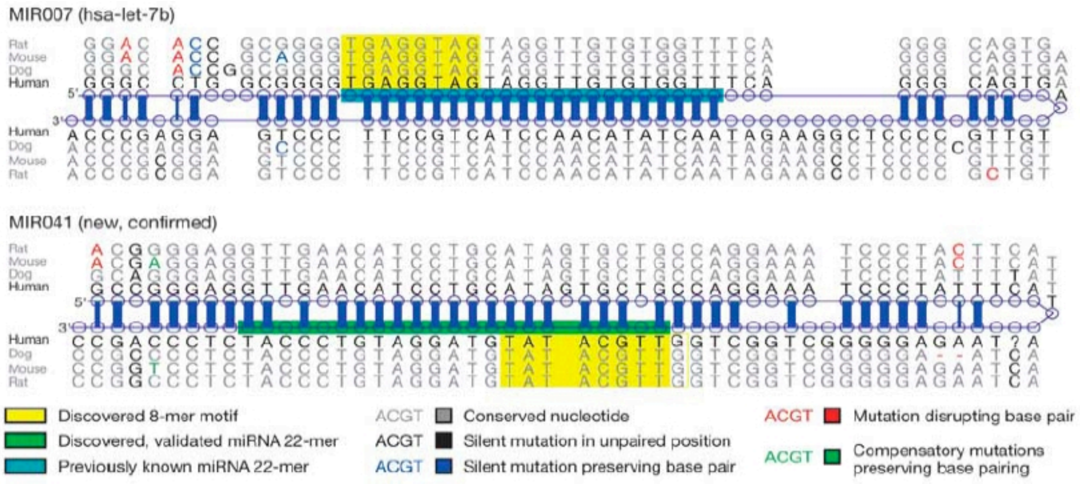
Bonnet et *al*. (2004) *Bioinformatics* **20**:2911

# Variations on miRNA gene prediction

```
C. Elegans

COG-1A 5'       C   CA              A 3'
                 GU   CUUAUACAAAA
                 CG   GAGUAUGUUUU
lsy-6   3' GCUUUA   CA               5'
```



| mRNA | Expected | Position in 3UTR | Conservation |
|---|---|---|---|
| hsa-let-7b | 0.653 | 2058 | hg17chr12 GATTCACG--CTTTTGTTTTCATAATACCTCAC 4281463 4281493 +<br>panTro1chr10 GATTCACG--CTTTTGTTTTCATAATACCTCAC 4539943 4539973 +<br>mm5chr6 AATGTACTTCTTTTCGTTTTCCTAATACCTCAC 21962440 21962472 -<br>rn3chr4 CATGTA----CTTTTTTTTCCATAATACCTCAC 23603903 23603931 -<br>canFam1chr27 GATTCACA--TTTTTGTTTTCATAATACCTCAC 5530847 5530877 -<br>galGal2chr1 -----------TTCCCATTTTCACAATACCTCAC 185852373 185852395 + |
| | 0.653 | 1991 | hg17chr12 ATTCTTCAAAGACGGAGTATTCTTTACCTCAG 4281395 4281426 +<br>panTro1chr10 ATTCTTCAAAGACGGAGTATTCTTTACCTCAG 4539875 4539906 +<br>mm5chr6 ATTCTTCAAAGA--GAGTATTCTTTACCTCAG 21962365 21962394 -<br>rn3chr4 ATTCTTCAAAGA--GAGT-TTCTTTACCTCAG 23603837 23603865 -<br>canFam1chr27 ATTCTTCAAAGA--CAGCATTCTTTACCTCAG 5530781 5530810 -<br>galGal2chr1 ATCCTGCAAAGA--TGGTATAC-TTACCTCAG 185852320 185852348 + |
| | 0.653 | 4921 | hg17chr12 TATGTTCATCACCCTTATATCATGTACCTCAG 4284325 4284356 +<br>panTro1chr10 TATGTTCATTACCCTTATATCATGTACCTCAG 4542808 4542839 +<br>mm5chr6 GTTGTTCATTATCCTTCTGCCATGTACCTCAG 21965077 21965108 -<br>rn3chr4 GTTGTTCATTATCCTTCTGCCGTGTACCTCAG 23606455 23606486 -<br>canFam1chr27 TATGTTCATTACCCTTGTACCGTGTACCTCAG 5533716 5533747 -<br>galGal2chr1 TATGTTCATTAGTATTTTCTCATGTACCTCAG 185854369 185854400 + |

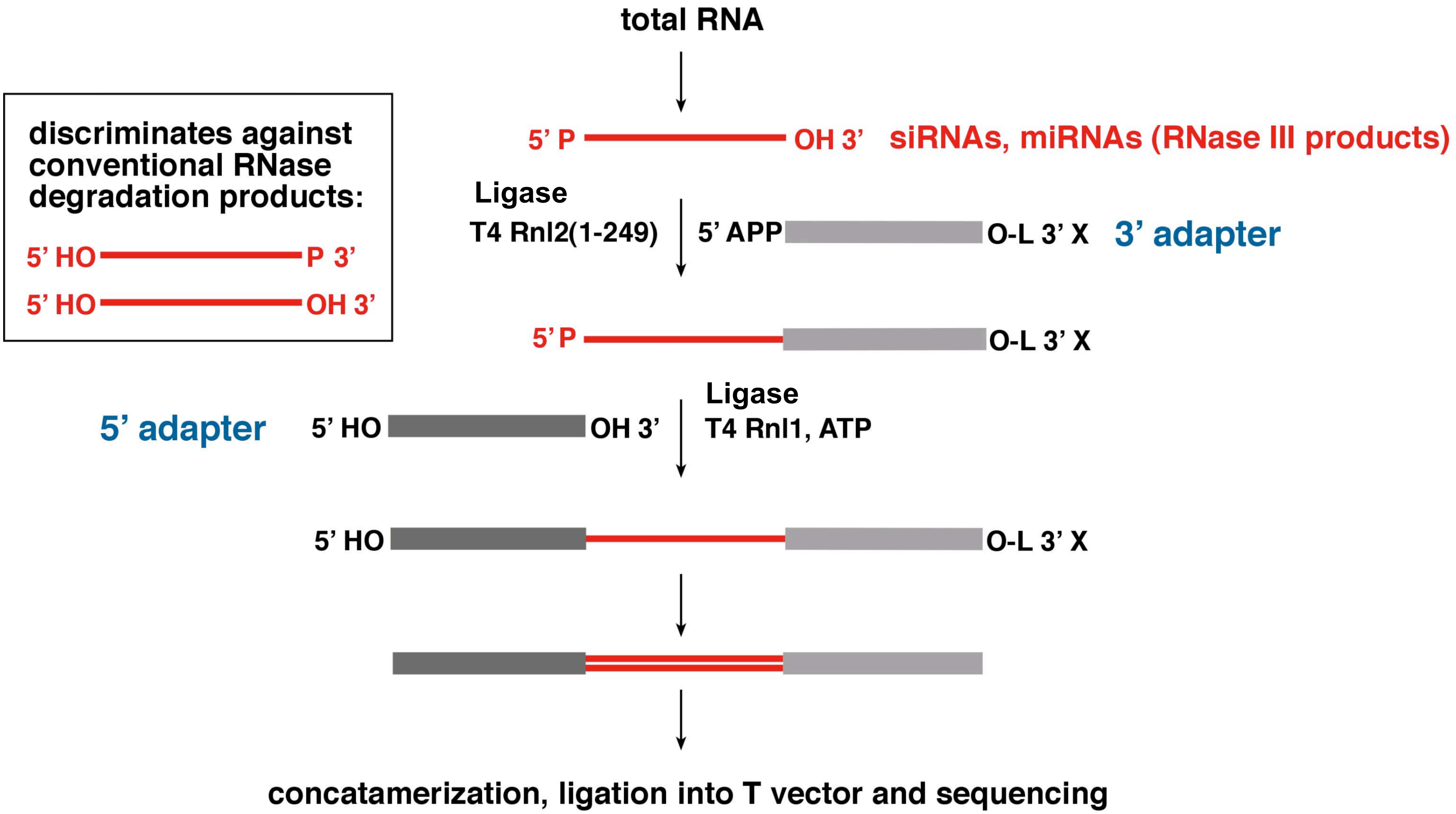


… and many more, particularly SVMs.

Xie, X. et *al*. (2004) *Nature* **434**:338

# Experiment-driven miRNA discovery

# Cloning protocol for small RNAs



total RNA

5’ P OH 3’ siRNAs, miRNAs (RNase III products)

Ligase

T4 Rnl2(1-249)

5’ APP O-L 3’ X **3’ adapter**

5’ P O-L 3’ X

Ligase

T4 Rnl1, ATP

**5’ adapter** 5’ HO OH 3’

5’ HO O-L 3’ X

concatamerization, ligation into T vector and sequencing

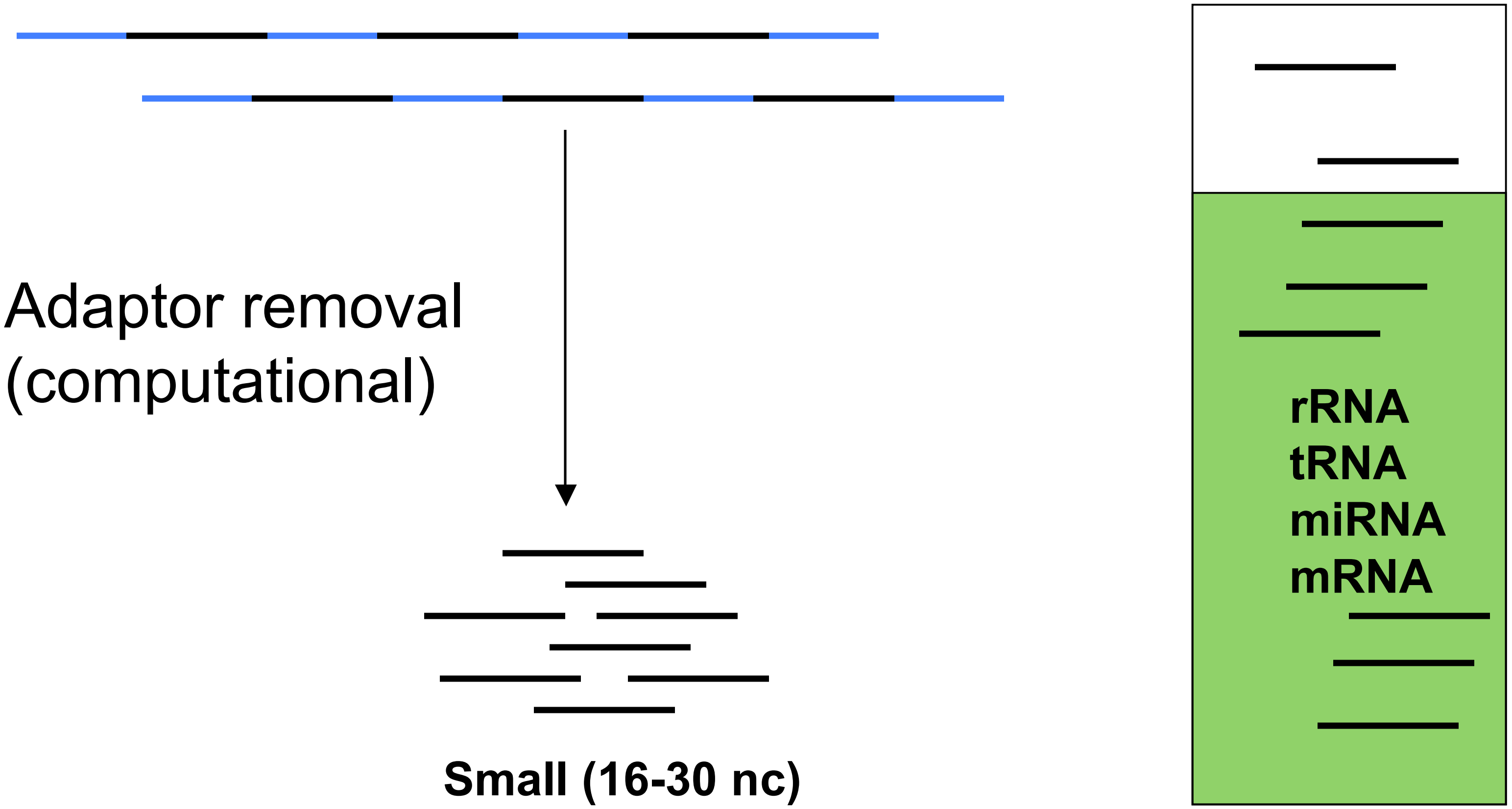
discriminates against conventional RNase degradation products:

5’ HO P 3’

5’ HO OH 3’

Tom Tuschl

# Functional annotation of small RNAs



Adaptor removal
(computational)

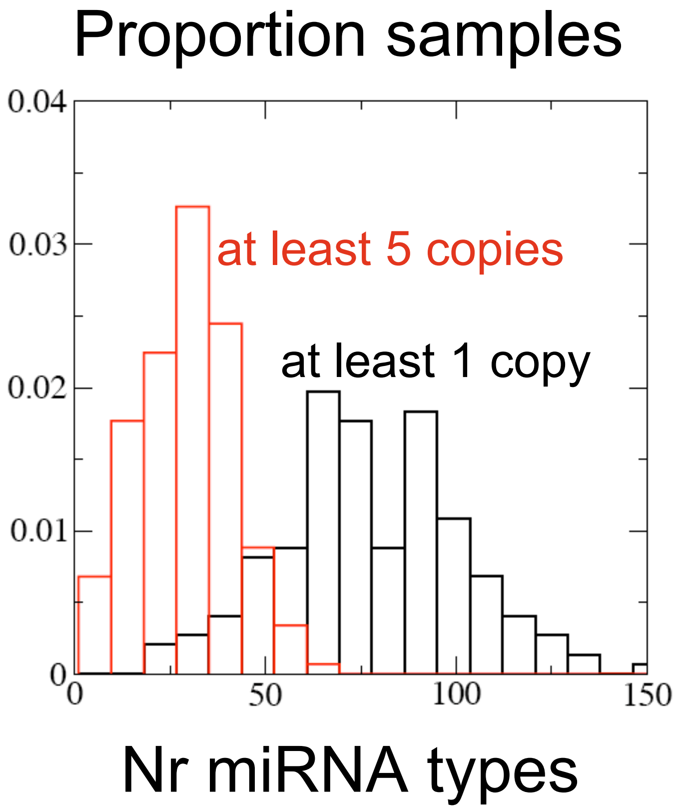
**Small (16-30 nc)
cloned RNAs**

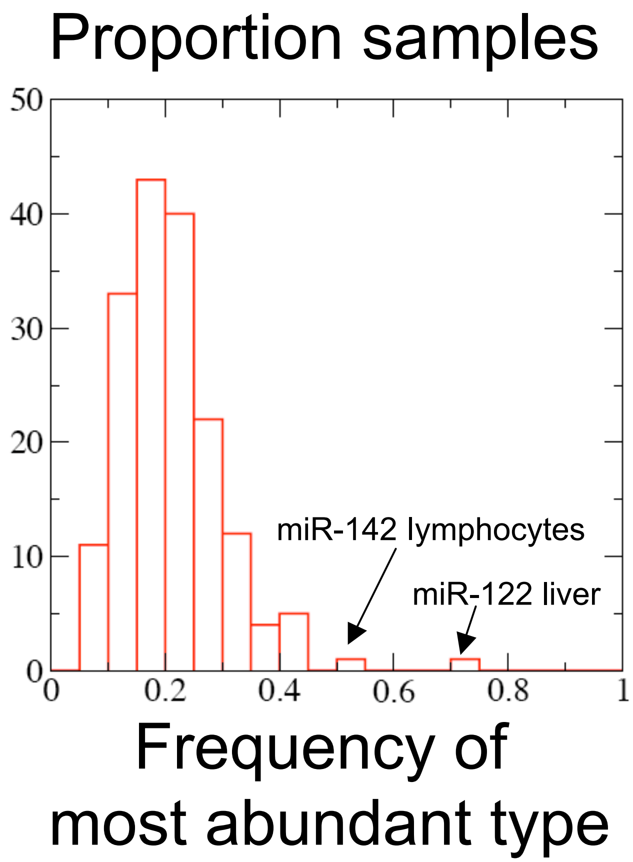
**rRNA
tRNA
miRNA
mRNA**

Match to sequences
of known function

# Characterization of mature miRNA sequences

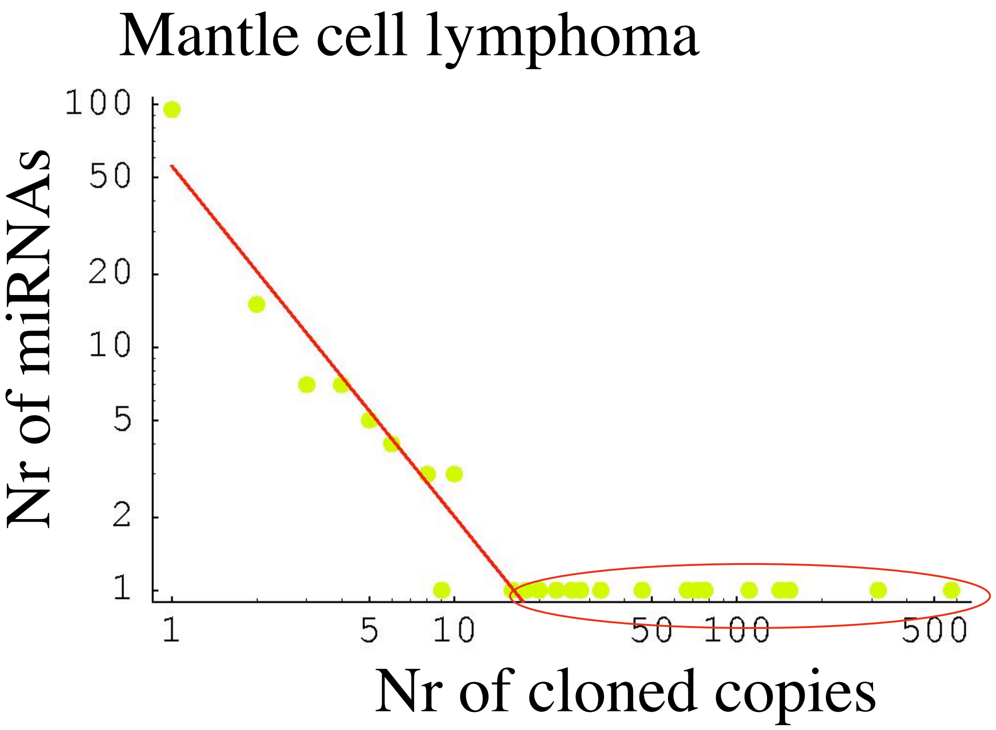
# How many miRNA types in a cell/tissue?





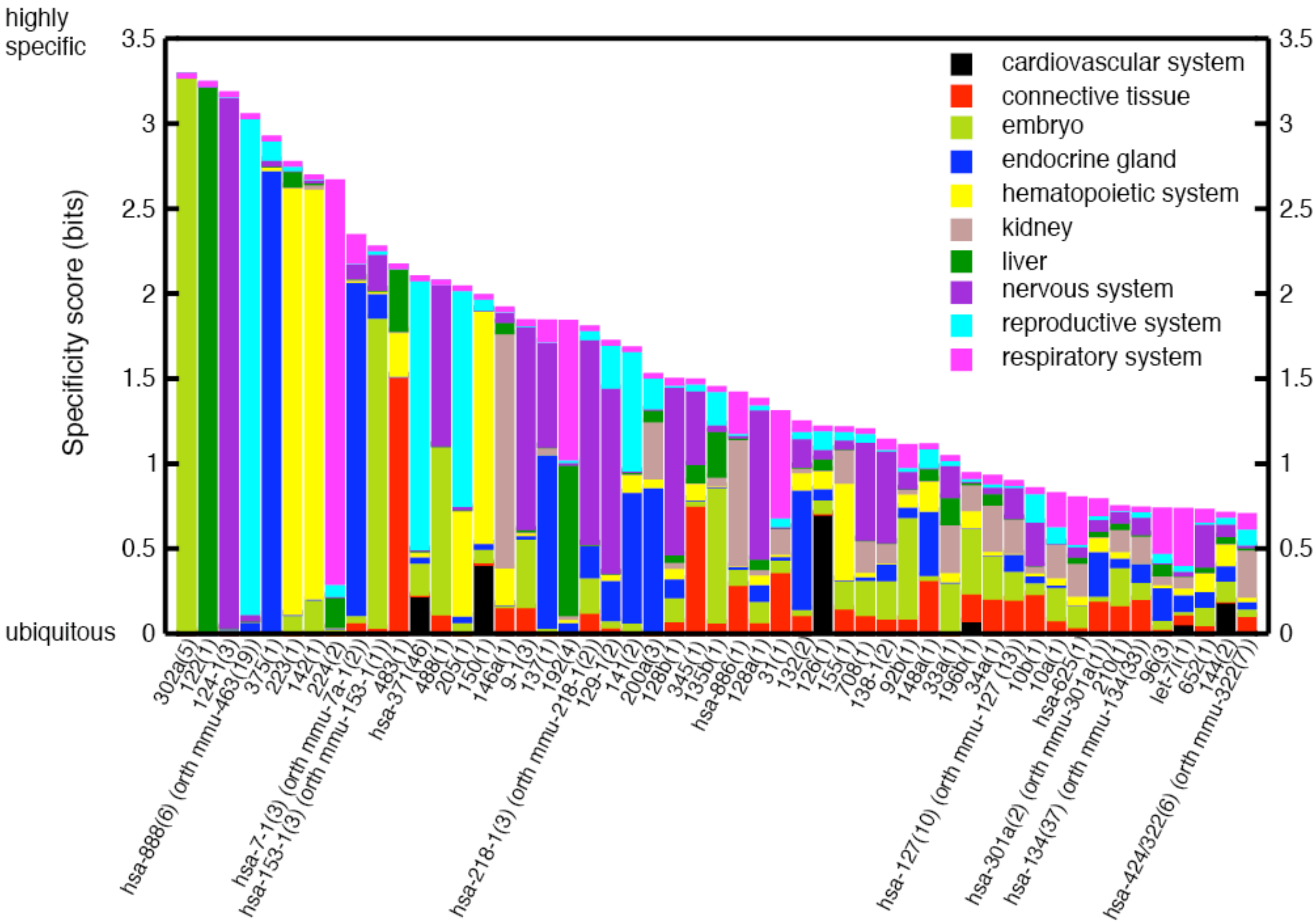
No corrections for
- Non-uniform sampling of tissues
- Polycistronic precursors

# Relative frequency of miRNA types in a cell/tissue?

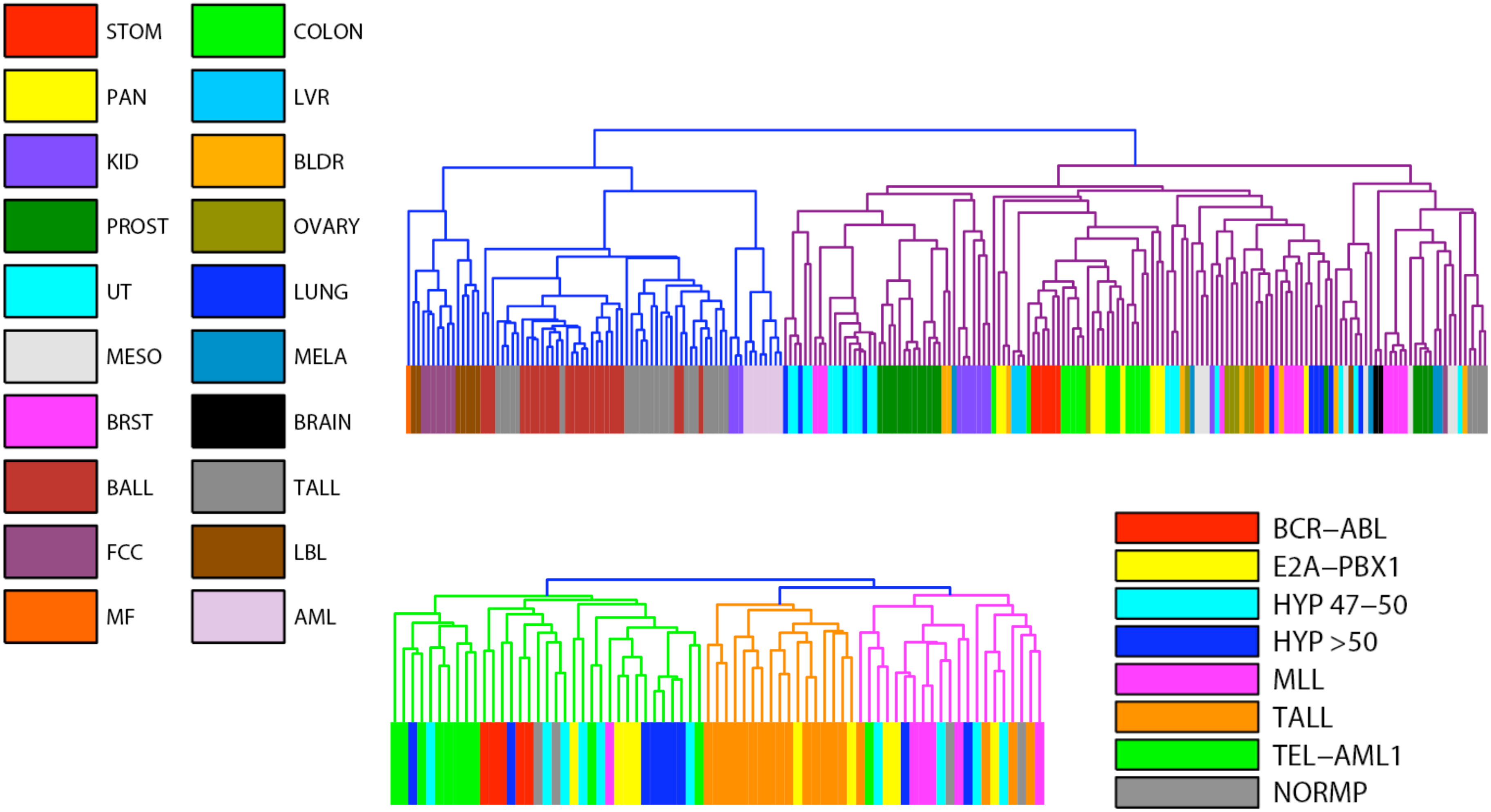


Perhaps not all “detectable” miRNAs are functionally relevant.

# How specific is miRNA expression?

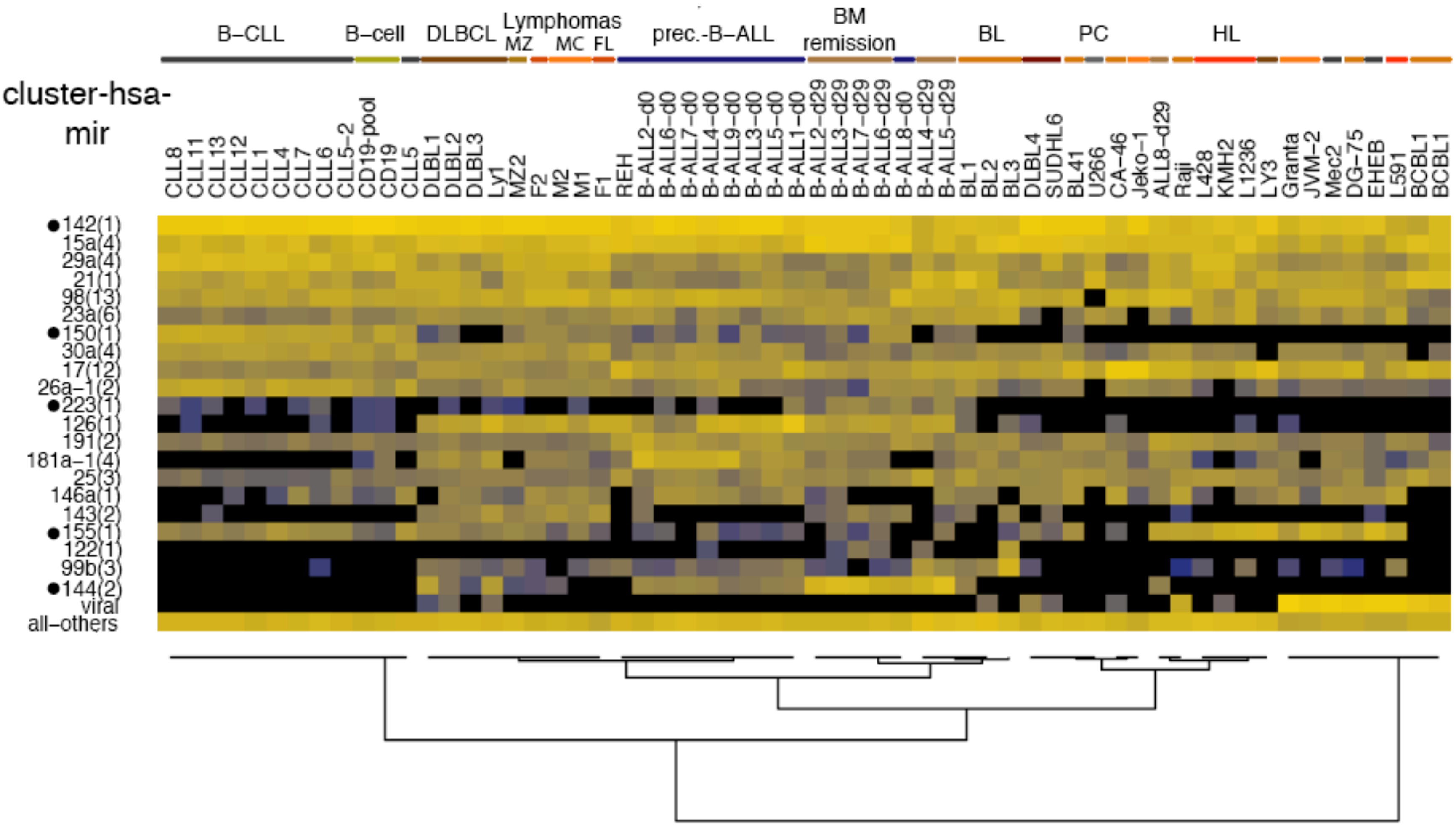


# Cancer classification using miRNAs

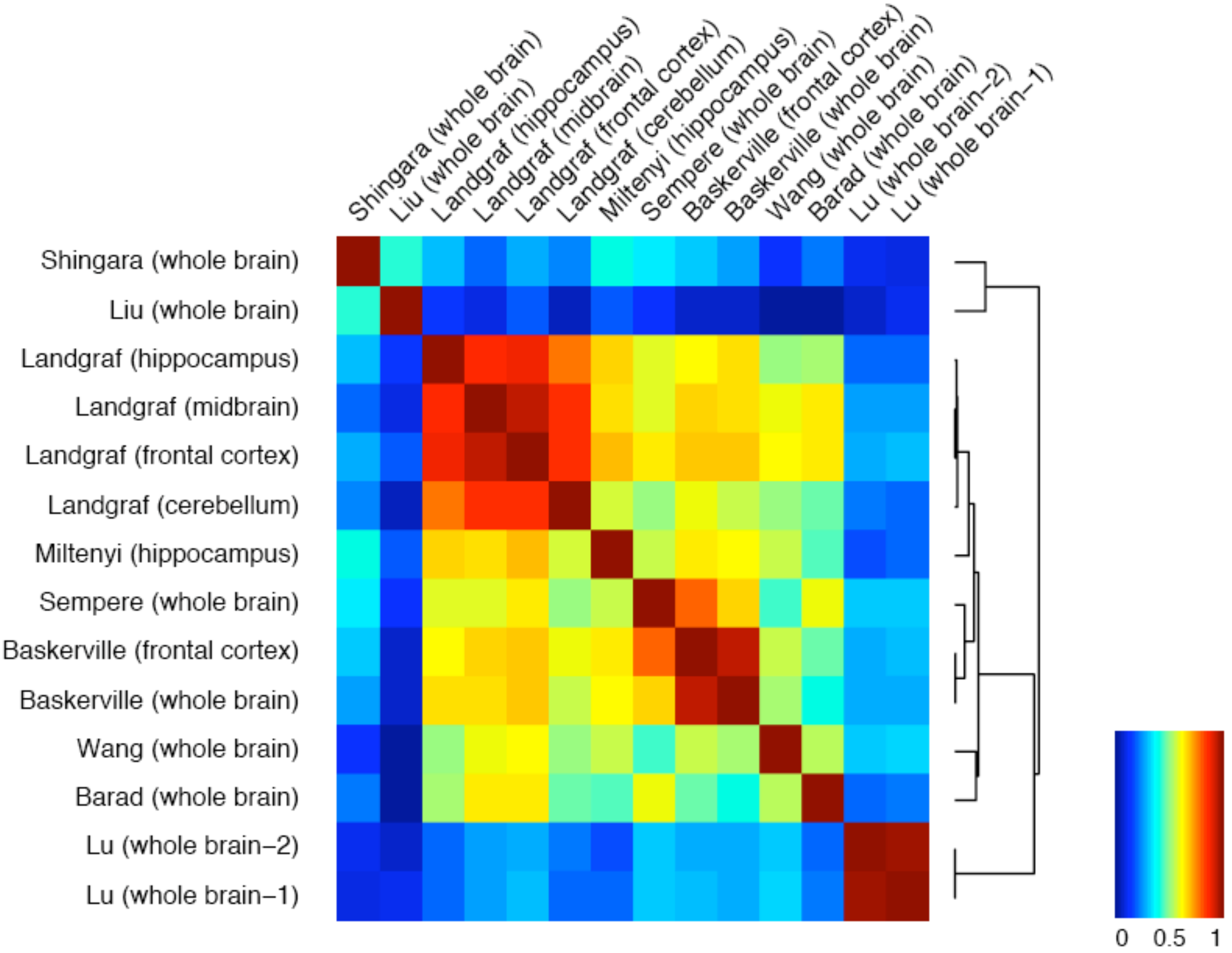


Lu et al. Nature 435:834 (2005)

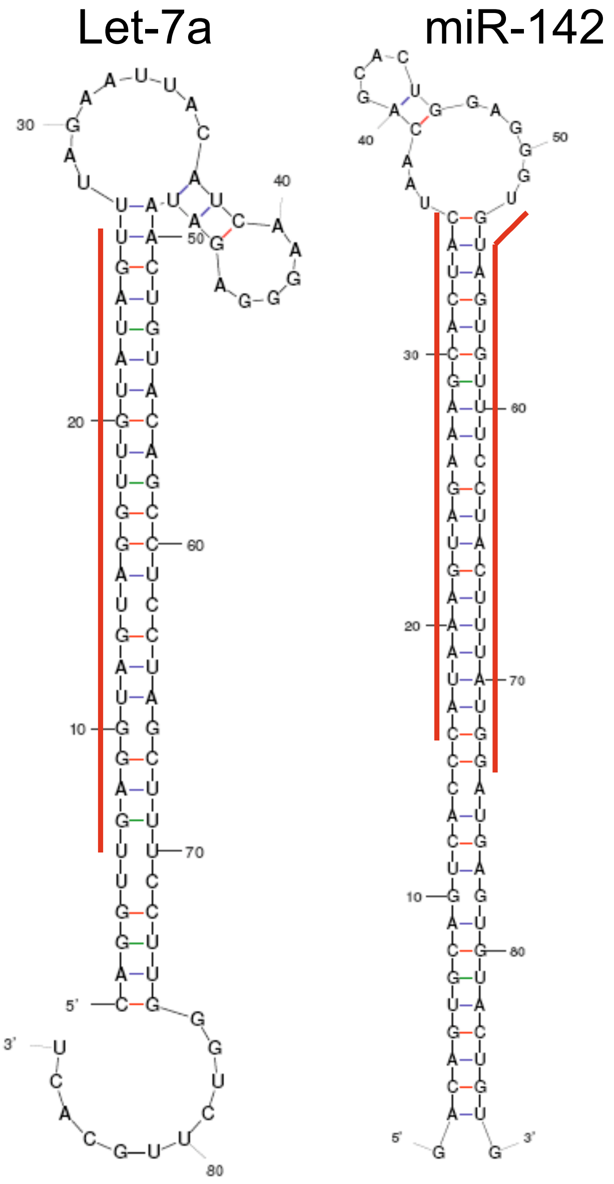
# miRNA profiling of related cells



# Accurate methods for miRNA profiling are still needed

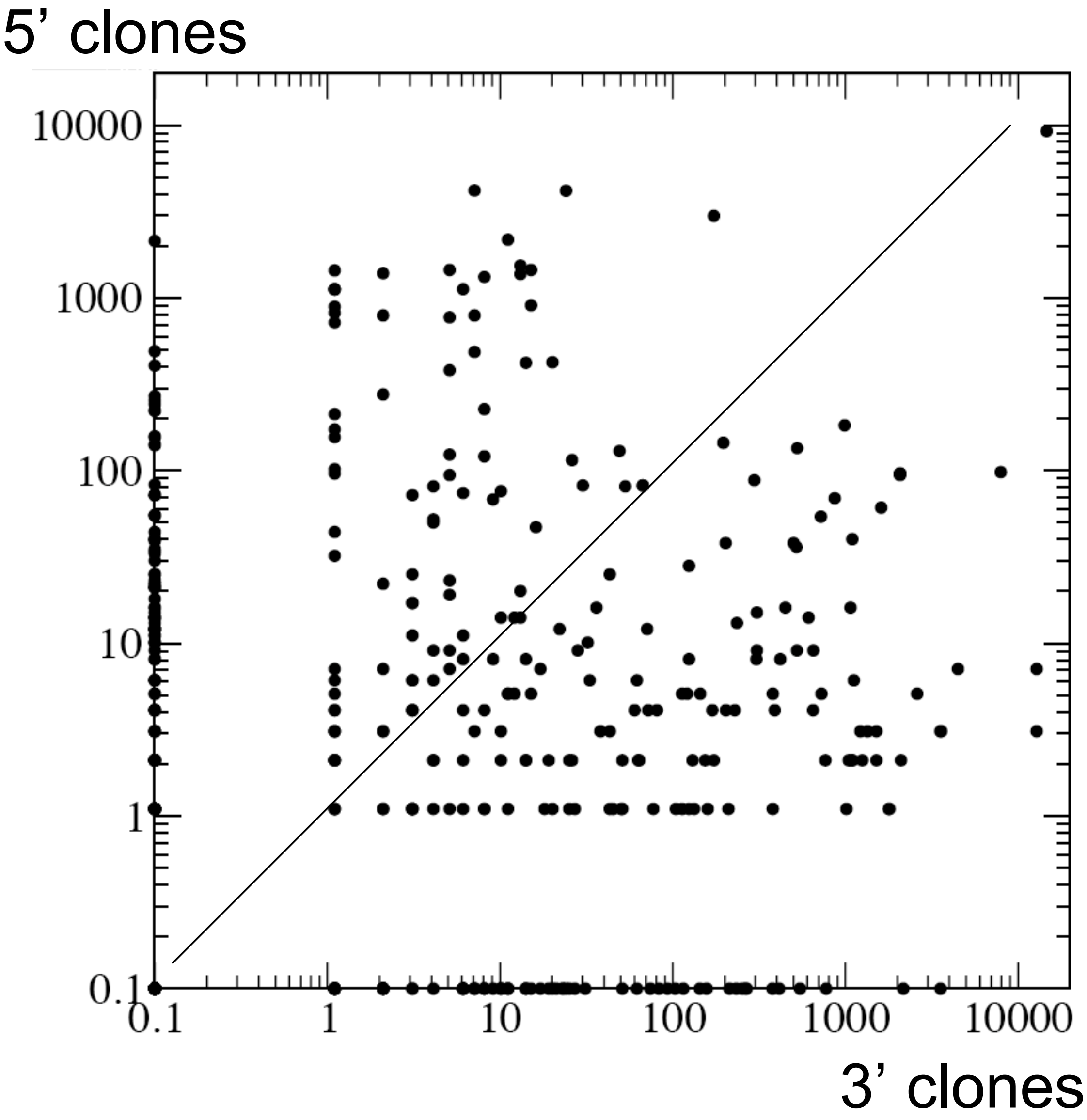


# 5’/3’ asymmetry

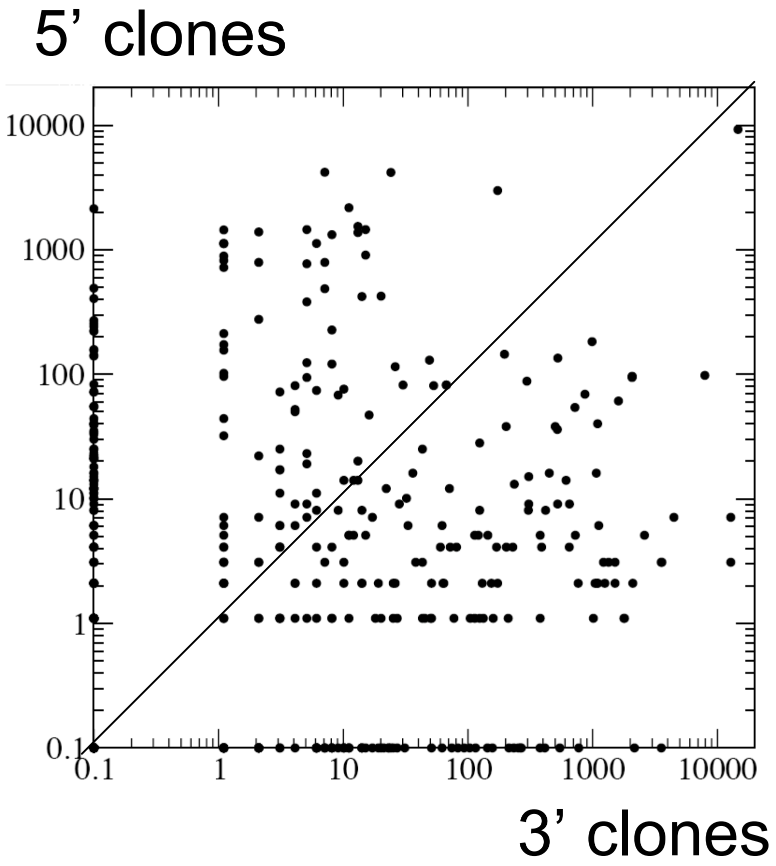

Let-7a

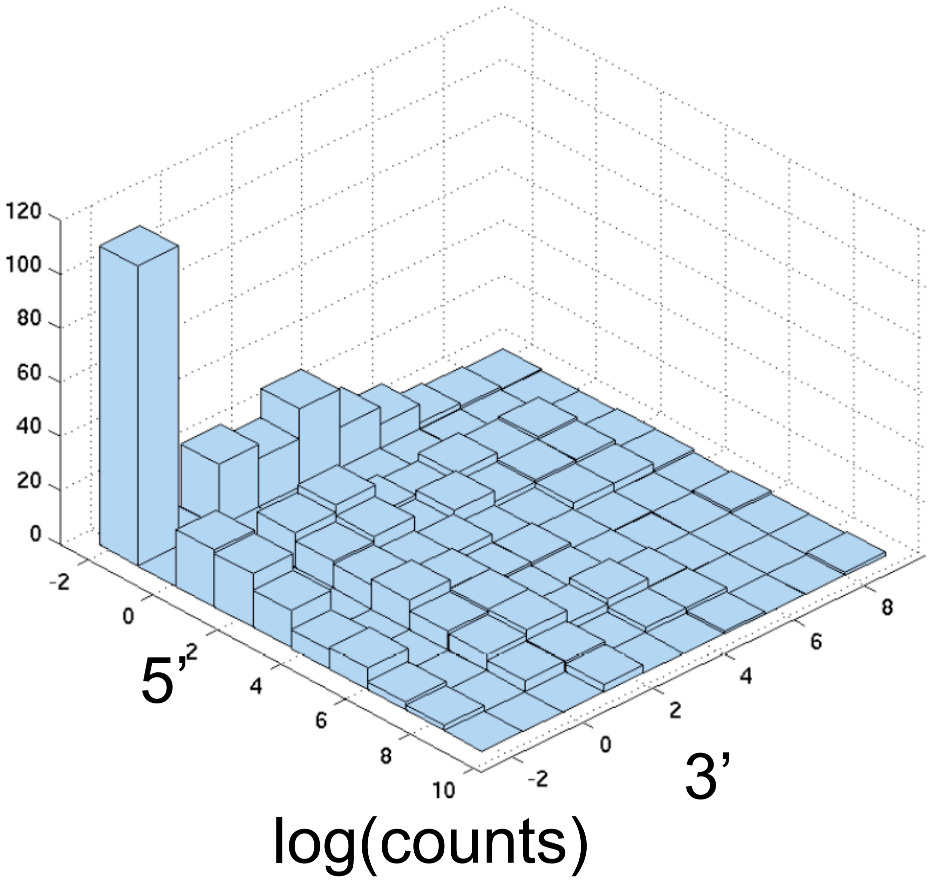
miR-142

Mfold (M. Zucker)

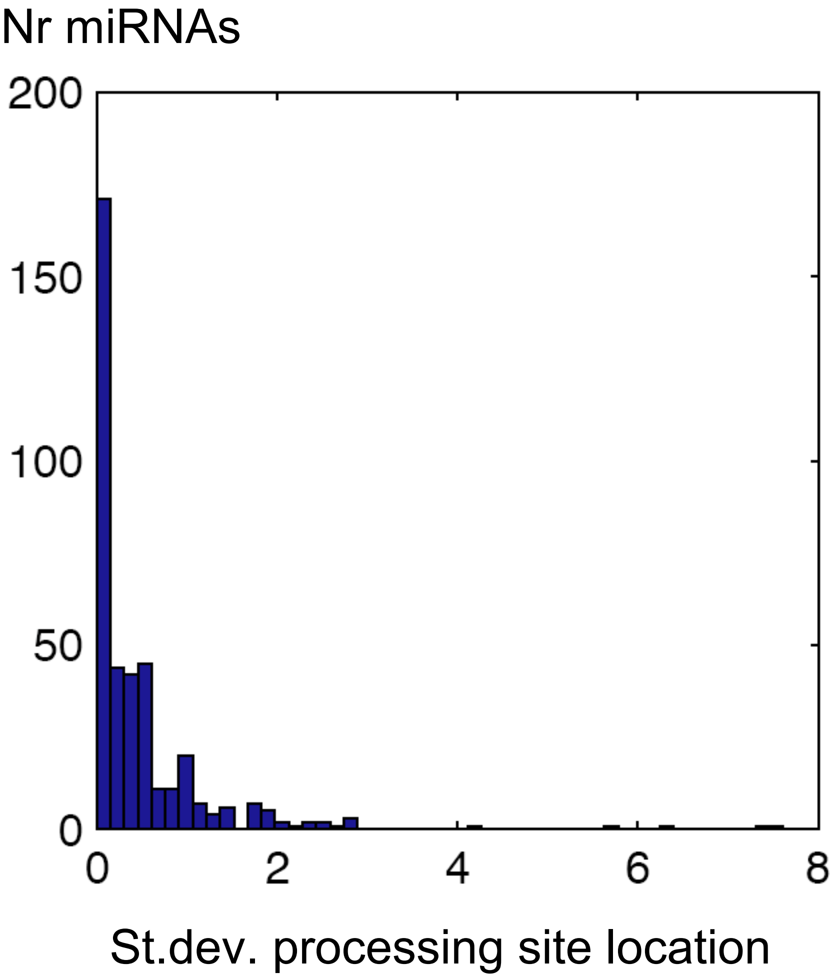


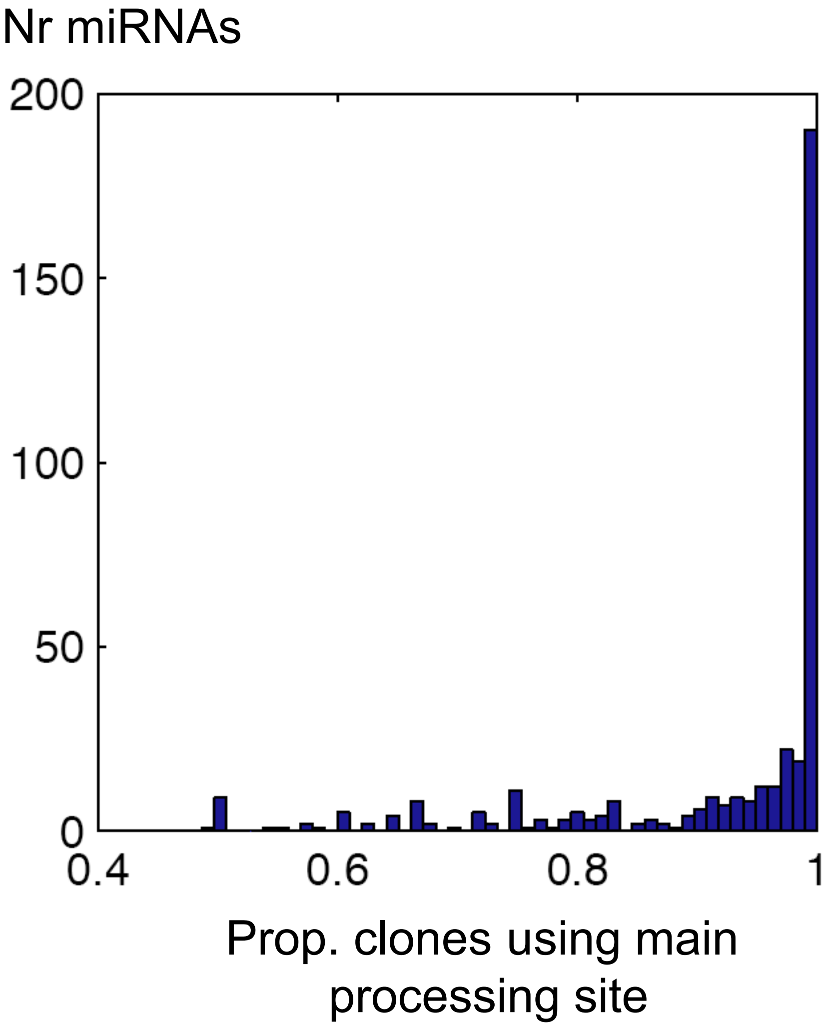
# 5'/3' asymmetry





# Processing precision





# Processing precision

## Precise processing of hsa-let-7a-2

```
preMir   GCUCCCAGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCCUUGGGUCUUG
fold     ...((((((..(((.(((.(((((((((((((.....................))))))))))))).))).)))..)))))).....
1958     ----------UGAGGUAGUAGGUUGUAUAGUU-------------------------------------------------------
1054     ----------UGAGGUAGUAGGUUGUAUAGU--------------------------------------------------------
389      ----------UGAGGUAGUAGGUUGUAUAG---------------------------------------------------------
73       ----------UGAGGUAGUAGGUUGUAUAGUUU------------------------------------------------------
33       ----------UGAGGUAGUAGGUUGUAUA----------------------------------------------------------
10       ----------UGAGGUAGUAGGUUGUA------------------------------------------------------------
5        ----------UGAGGUAGUAGGUUGUAUAGUUUA-----------------------------------------------------
5        ----------UGAGGUAGUAGGUUGU-------------------------------------------------------------
4        ----------UGAGGUAGUAGGUUGUAU-----------------------------------------------------------
4        -----------GAGGUAGUAGGUUGUAUAGUU-------------------------------------------------------
4        -----------GAGGUAGUAGGUUGUAUAGU--------------------------------------------------------
3        ---------UUGAGGUAGUAGGUUGUAUAGUU-------------------------------------------------------
3        ---------------UAGUAGGUUGUAUAGUU-------------------------------------------------------
1        ---------UUGAGGUAGUAGGUUGUAUAGU--------------------------------------------------------
1        --------------GUAGUAGGUUGUAUAGUU-------------------------------------------------------
1        --------------GUAGUAGGUUGUAUAG---------------------------------------------------------
1        -------------GGUAGUAGGUUGUAUAGUU-------------------------------------------------------
1        -------------GGUAGUAGGUUGUAUAGU--------------------------------------------------------
1        -----------GAGGUAGUAGGUUGUAUAG---------------------------------------------------------
1        ----------------AGUAGGUUGUAUAGUU-------------------------------------------------------
```

# Processing precision

## Less precise processing of hsa-mir-302a

```
preMir  GCUCCCCACCACUUAAACGUGGAUGUACUUGCUUUGAAACUAAAGAAGUAAGUGCUUCCAUGUUUUGGUGAUGGUAAGUCU
fold    (((..(((.((((.(((((((((.(((((((((((.........))))))))))).))))))))).)))).)))..)))..
243     ------------------------------------------------UAAGUGCUUCCAUGUUUUGGUGA----------
108     ------------------------------------------------UAAGUGCUUCCAUGUUUUGGUG-----------
34      -------------------------------------------------------UUCCAUGUUUUGGUGA----------
10      ------------------------------------------------UAAGUGCUUCCAUGUUUUGGU------------
10      -------------------------------------------------AAGUGCUUCCAUGUUUUGGUGA----------
5       ------------------------------------------------------CUUCCAUGUUUUGGUGA----------
3       ------------------------------------------------------CUUCCAUGUUUUGGUG-----------
3       ----------ACUUAAACGUGGAUGUACUUGCU------------------------------------------------
2       ------------------------------------------------UAAGUGCUUCCAUGUUUUGGUGAU---------
2       ------------------------------------------------UAAGUGCUUCCAUGUUUUGG-------------
2       -------------UAAACGUGGAUGUACUUGCUUUG---------------------------------------------
2       -------------UAAACGUGGAUGUACUUGCUU-----------------------------------------------
2       -------------UAAACGUGGAUGUACUUGCU------------------------------------------------
2       -------------------------------------------------AAGUGCUUCCAUGUUUUGGUG-----------
1       -------------UAAACGUGGAUGUACUUGCUUUGA--------------------------------------------
1       -------------UAAACGUGGAUGUACUUGCUUU----------------------------------------------
1       -------------UAAACGUGGAUGUACUUGC-------------------------------------------------
1       -----------------------------------------------------GCUUCCAUGUUUUGGUGA----------
1       -----------CUUAAACGUGGAUGUACUUGCU------------------------------------------------
1       --------------------------------------------------AGUGCUUCCAUGUUUUGGUGA----------
1       ----------ACUUAAACGUGGAUGUACUUGC-------------------------------------------------
```

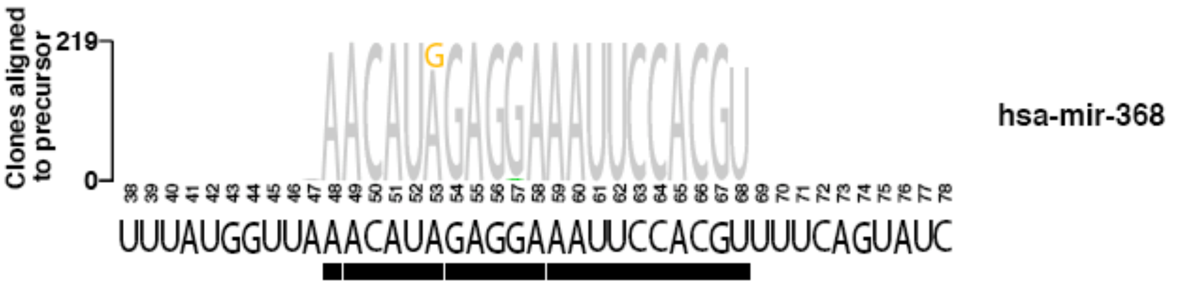
# Post-processing modifications

Total of 3-4% of cloned sequences have mutations relative to genome sequence.

A additions

U additions

A-to-I editing



Rob Sheridan, Nick Socci

# How many miRNAs are there?

“Our analysis can be used to calculate an upper bound on the number of human miRNA genes. If all 188 candidates were authentic miRNA genes and these represented 74% of the total miRNA genes, then there are no more than 255 miRNA genes in the genome.” Lim et al. Science 299:1540 (2003). Conservation up to zebrafish.

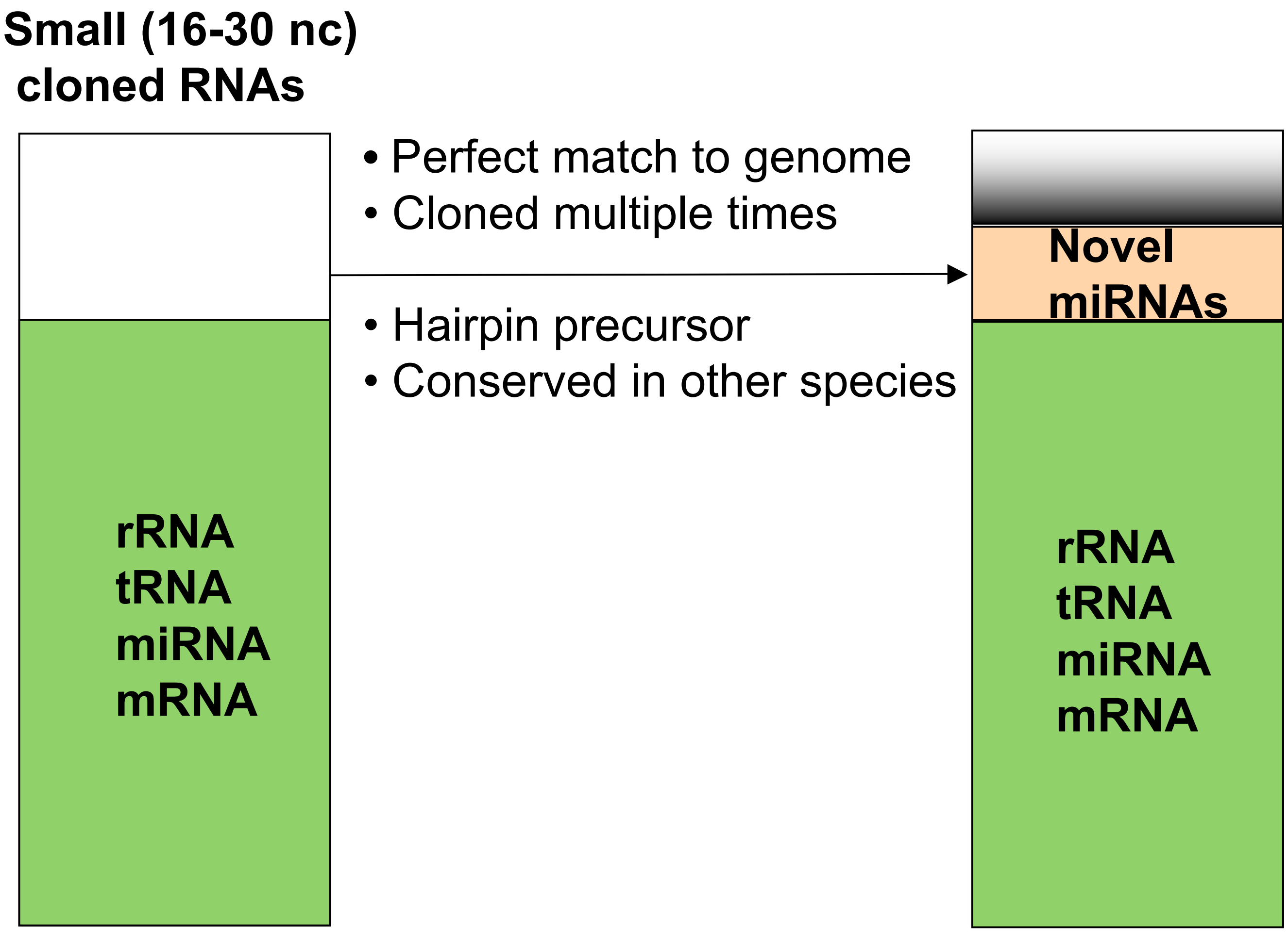
“Here we report the use of this approach to clone and sequence 89 new human microRNAs (nearly doubling the current number of sequenced human microRNAs), 53 of which are not conserved beyond primates. These findings suggest that the total number of human microRNAs is at least 800”. Bentwich et al. Nat Genet. 37:766 (2005). Conservation in primates.

“Our studies suggest that the number of microRNA precursors in mammalian genomes likely ranges in the tens of thousands.” Miranda et al. 126:1203 (2006).

In our data, more than 97% clones originate from less than 300 prototypical miRNA precursors.

# What else can we find in small RNA cloning data?

# Functional annotation of small RNAs



**Small (16-30 nc) cloned RNAs**

- Perfect match to genome
- Cloned multiple times

- Hairpin precursor
- Conserved in other species

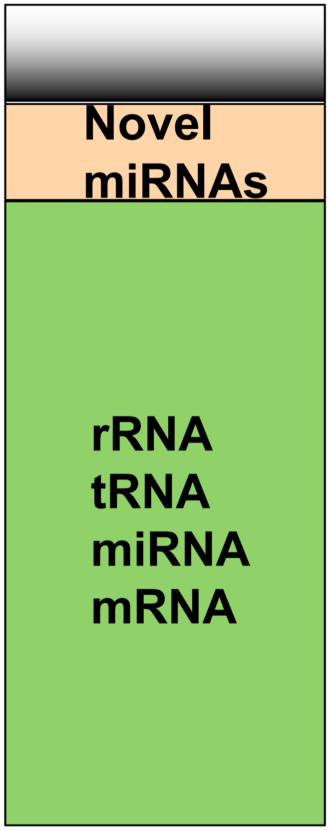
**Novel miRNAs**

**rRNA**
**tRNA**
**miRNA**
**mRNA**

**rRNA**
**tRNA**
**miRNA**
**mRNA**

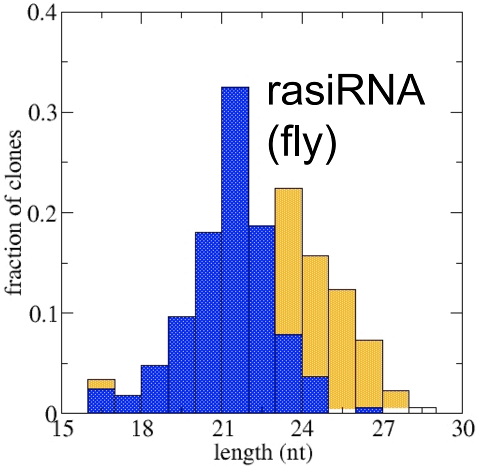
Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T. A uniform system for microRNA annotation. RNA. 2003 Mar;9(3):277-9.

# Functional annotation of small RNAs



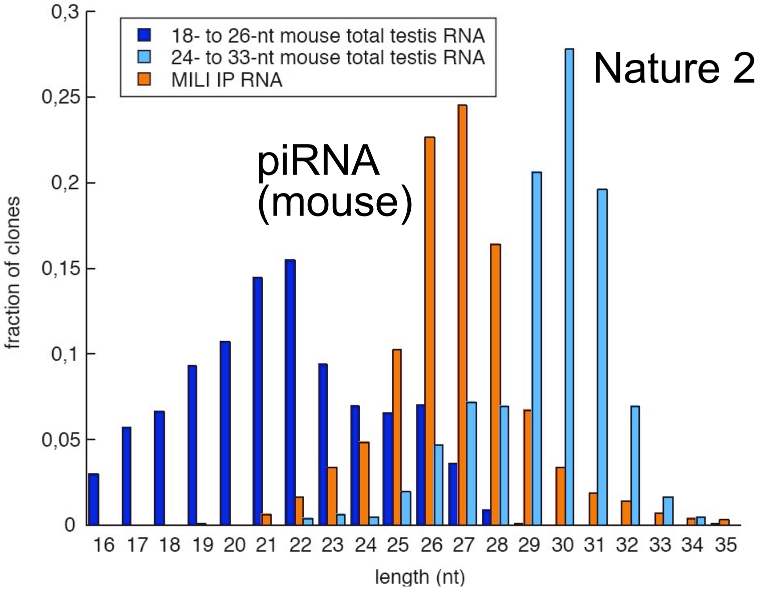
**Novel miRNAs**

**rRNA**
**tRNA**
**miRNA**
**mRNA**



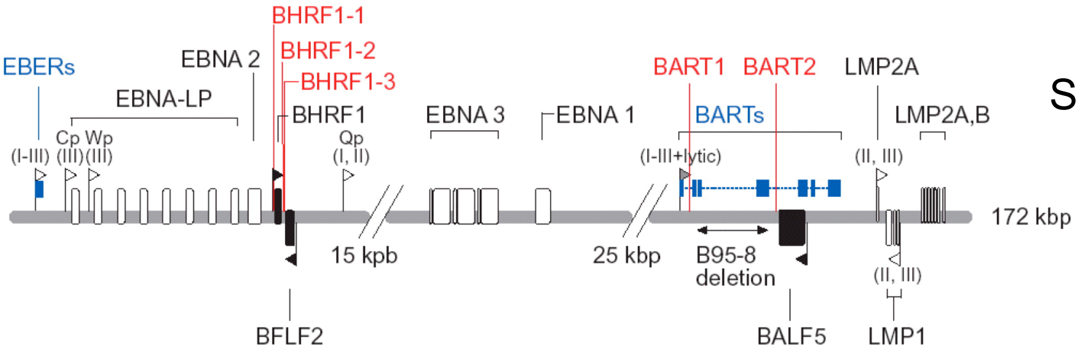
Dev. Cell 2003

Collaborations
with Tuschl Lab



Nature 2006

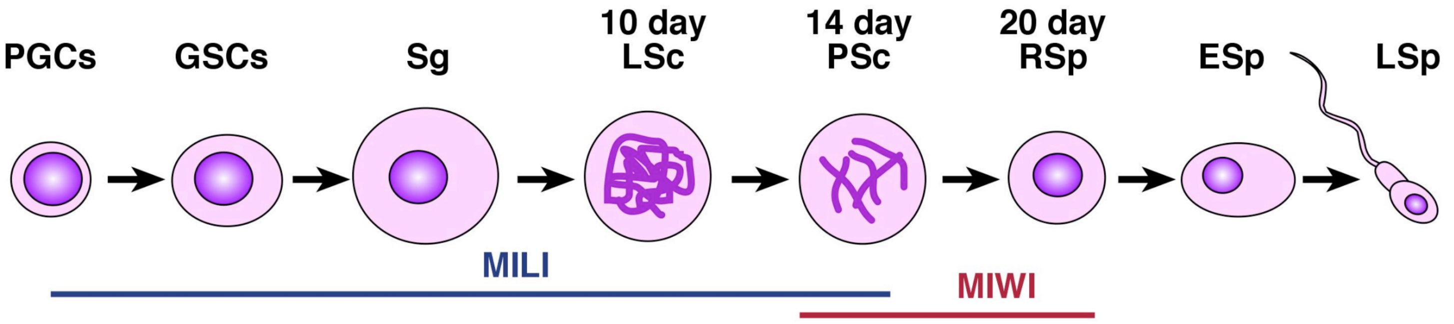
viral miRNA

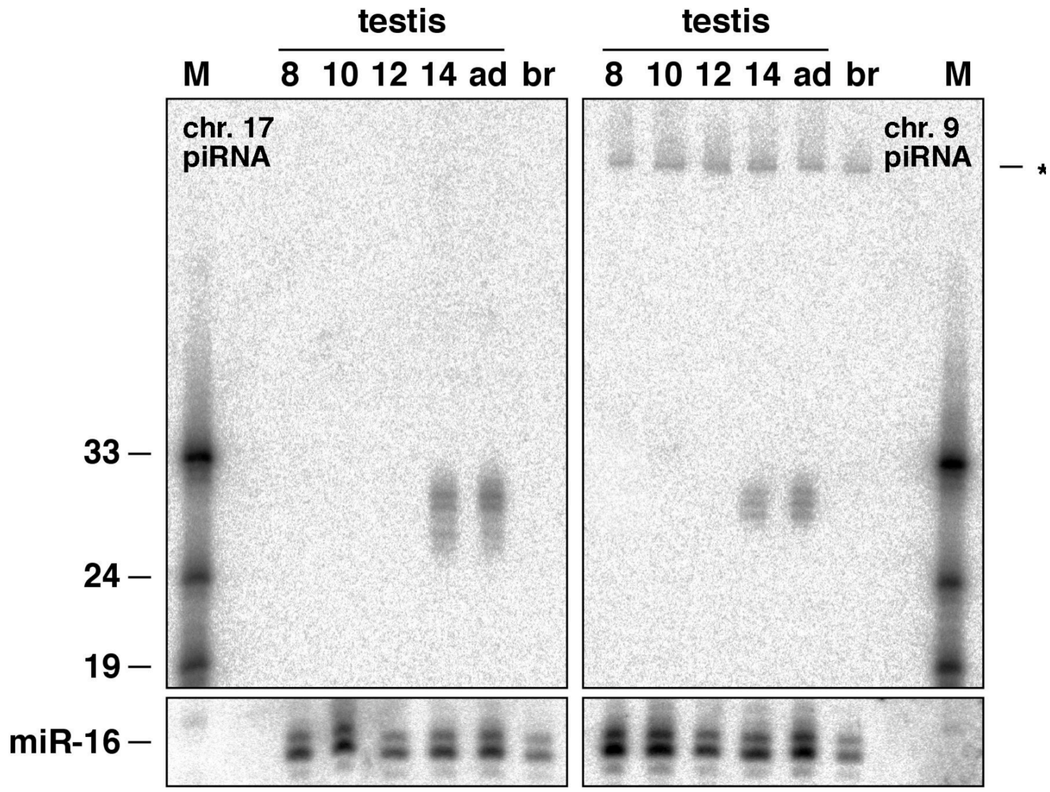


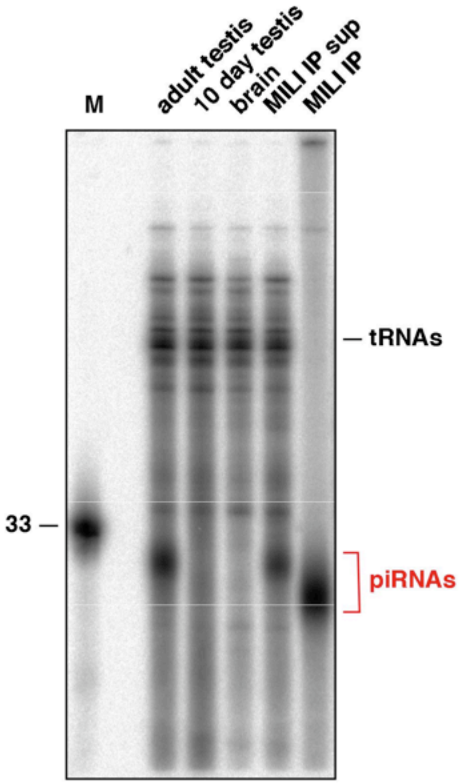
Science 2004

# A few words about piRNAs

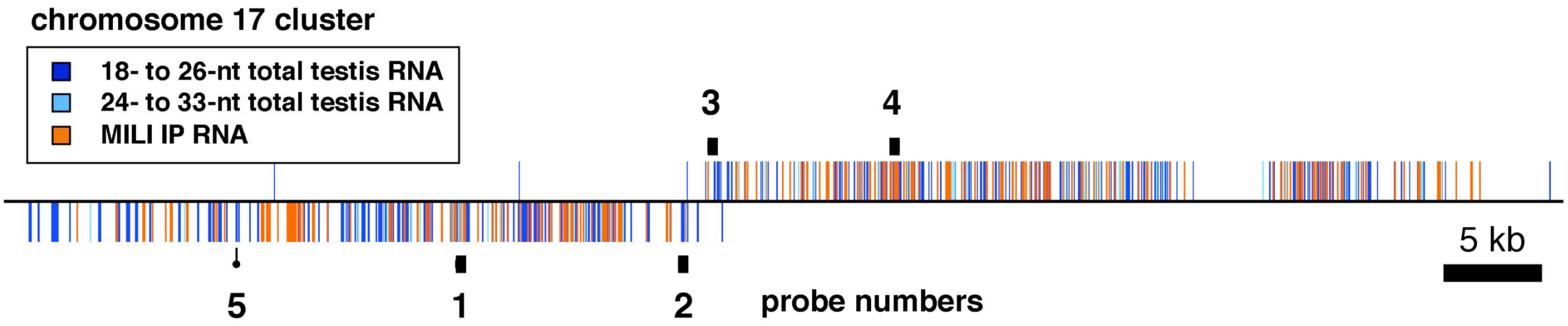
# piRNAs appear at the onset of mitosis in maturing mice

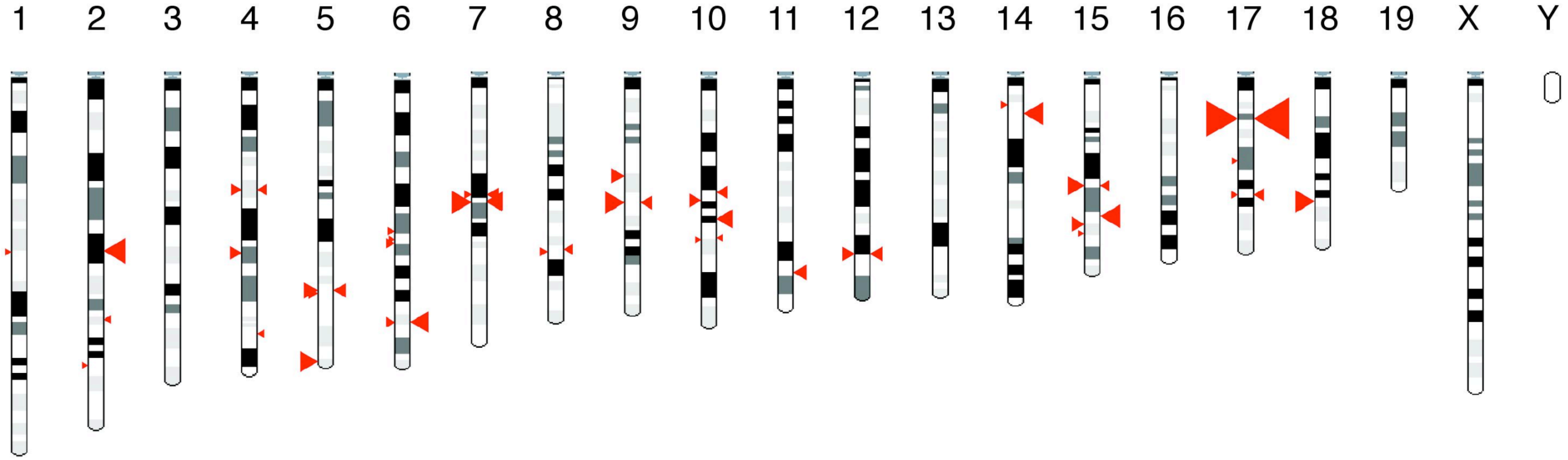






# piRNAs are clustered and processed from longer transcripts

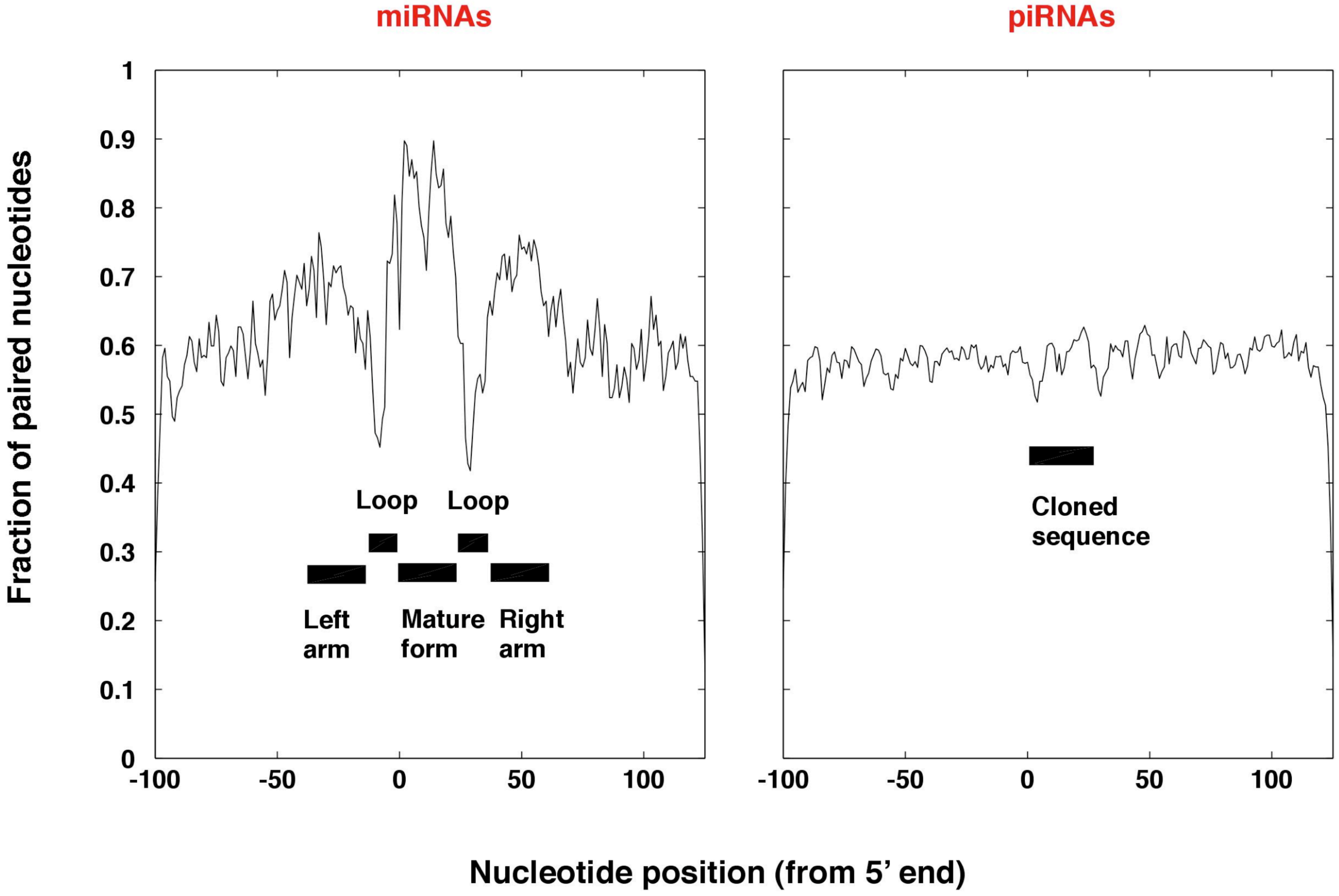




# piRNA primary transcript processing

```
chr17|+|25131742|25131774      UGAAUCUGGAAUGGUCUUUCUGGAAUGAUGGGG

25131742|25131767|n=1          UGAAUCUGGAAUGGUCUUUCUGGAAU-------
25131742|25131764|n=1          UGAAUCUGGAGUGGUCUUUCUGG----------
25131748|25131773|n=1          ------UGGAAUGGUCUUUCUGGAAUGAUGGGU
25131748|25131772|n=1          ------UGGAAUGGCCUUUCUGGAAUGAUGG--
25131748|25131772|n=3          ------UGGAAUGGUCUUUCUGGAAUGAUGG--
25131748|25131774|n=2          ------UGGAAUGGUCUUUCUGGAGUGAUGGGG
25131748|25131773|n=4          ------UGGAAUGGUCUUUCUGGAAUGAUGGG-
25131748|25131770|n=1          ------UGGAAUGGUCUUUCUGGAAUGAU----
25131749|25131773|n=1          -------GGAAUGGUCUUUCUGGAAUGAUGGGU

chr17|+|25159577|25159606      UGUGUUAAAAUGCUUGUUCCCAGCUUGCCU

25159577|25159601|n=2          UGUGUUAAAAUGCUUGUUCCCAGCU-----
25159577|25159600|n=5          UGUGUUAAAAUGCUUGUUCCCAGC------
25159577|25159606|n=1          UGUGUUAAAAUGCUUGUUCCCAGCUUGCCU
25159577|25159602|n=1          UGUGUUAAAAUGCUUGUUCCCUGCUU----
25159577|25159602|n=4          UGUGUUAAAAUGCUUGUUCCCAGCUU----

chr17|-|25100375|25100430      UAGAGUGGUUUUGCGUUUAUCUGAUGCCCUAGAGGCGUCAACCUGUUCAUCAGCUC

25100375|25100401|n=1          -----------------------------UAGAGGCGUCAACCUGUUCAUCAGCUC
25100376|25100401|n=1          -----------------------------UAGAGGCGUCAACCUGUUCAUCAGCUU
25100377|25100404|n=1          --------------------------CCCUAGAGGCGUCAACCUGUUCAUCAGC--
25100377|25100401|n=1          -----------------------------UAGAGGCGUCAACCUGUUCAUCAGC--
25100392|25100419|n=1          -----------UGCGUUUAUCUGAUGCCCUAGAGGCGUC-----------------
25100402|25100430|n=1          UAGAGUGGUUUUGCGUUUAUCUGAUGCCC---------------------------
25100402|25100425|n=1          -----UGGUUUUGCGUUUAUCUGAUGCCC---------------------------
25100404|25100430|n=1          UAGAGUGGUUUUGCGUUUAUCUGAUGC-----------------------------
25100406|25100430|n=1          UAGAGUGGUUUUGCGUUUAUCUGAU-------------------------------
25100407|25100430|n=1          UAGAGUGGUUUUGCGUUUAUCUGA--------------------------------
```

# Analysis of base-pairing around piRNAs and miRNAs

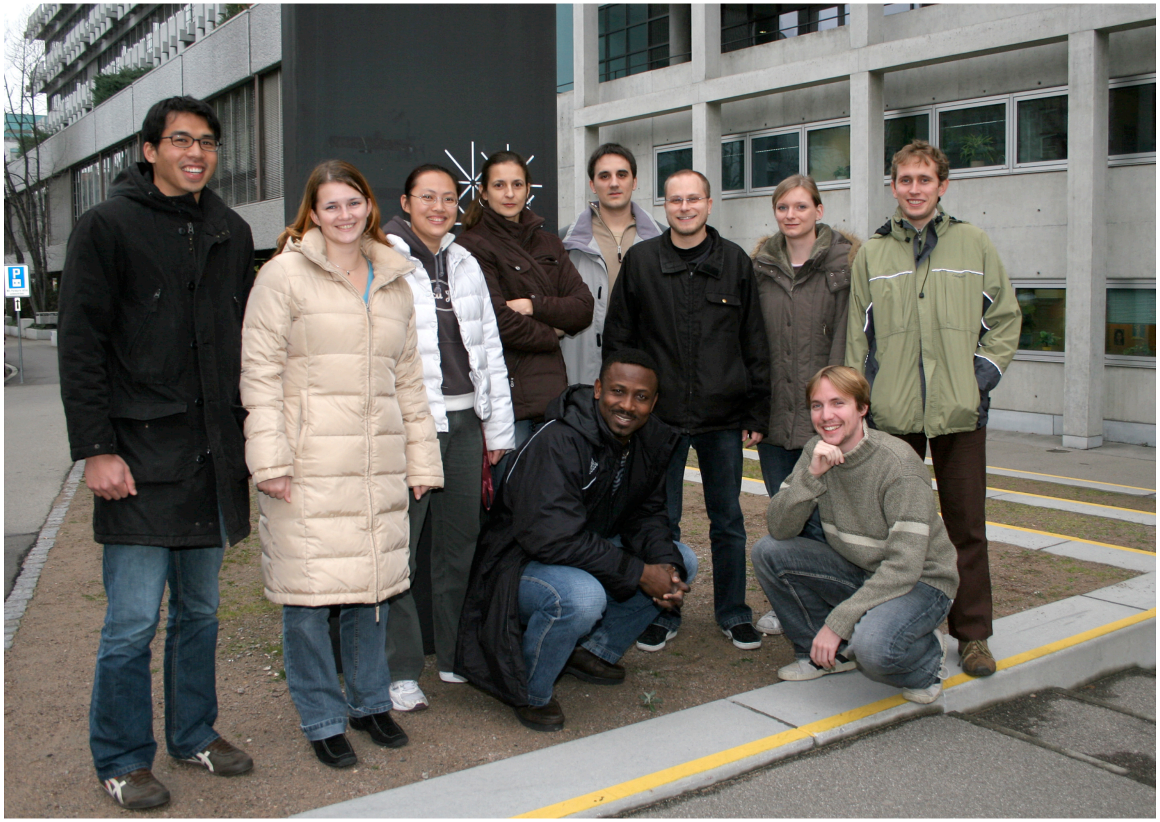


*The Rockefeller University*
Tom Tuschl
Alexei Aravin
Pablo Landgraf

*Memorial Sloan-Kettering Cancer Center*
Chris Sander
Rob Sheridan
Nick Socci

*Institut de Biologie Moléculaire des Plantes*
Sébastien Pfeffer

*Zavolan Group*
*Biozentrum Basel*
Dimos Gaidatzis
Alain Sewer
Jean Hausser
Philipp Berninger
Piotr Balwierz
Mirabela Rusu
Tzu-Ming Chern
Nicodeme Paul
Erik van Nimwegen



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