MicroRNA prediction

Mert Cihan

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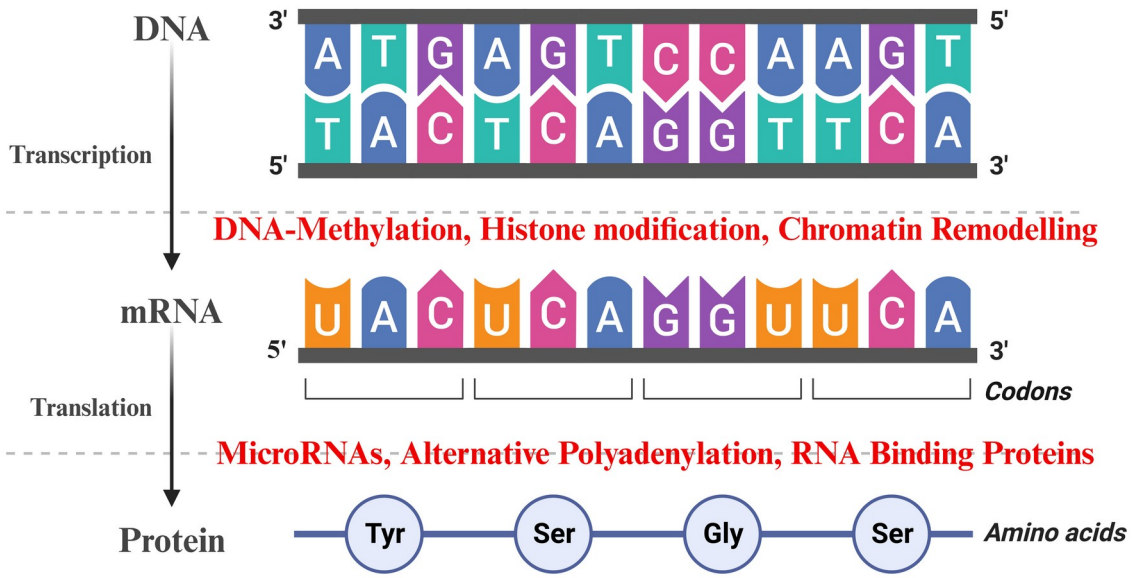
Supervisor: Prof. Miguel Andrade

Gene Expression And Regulation



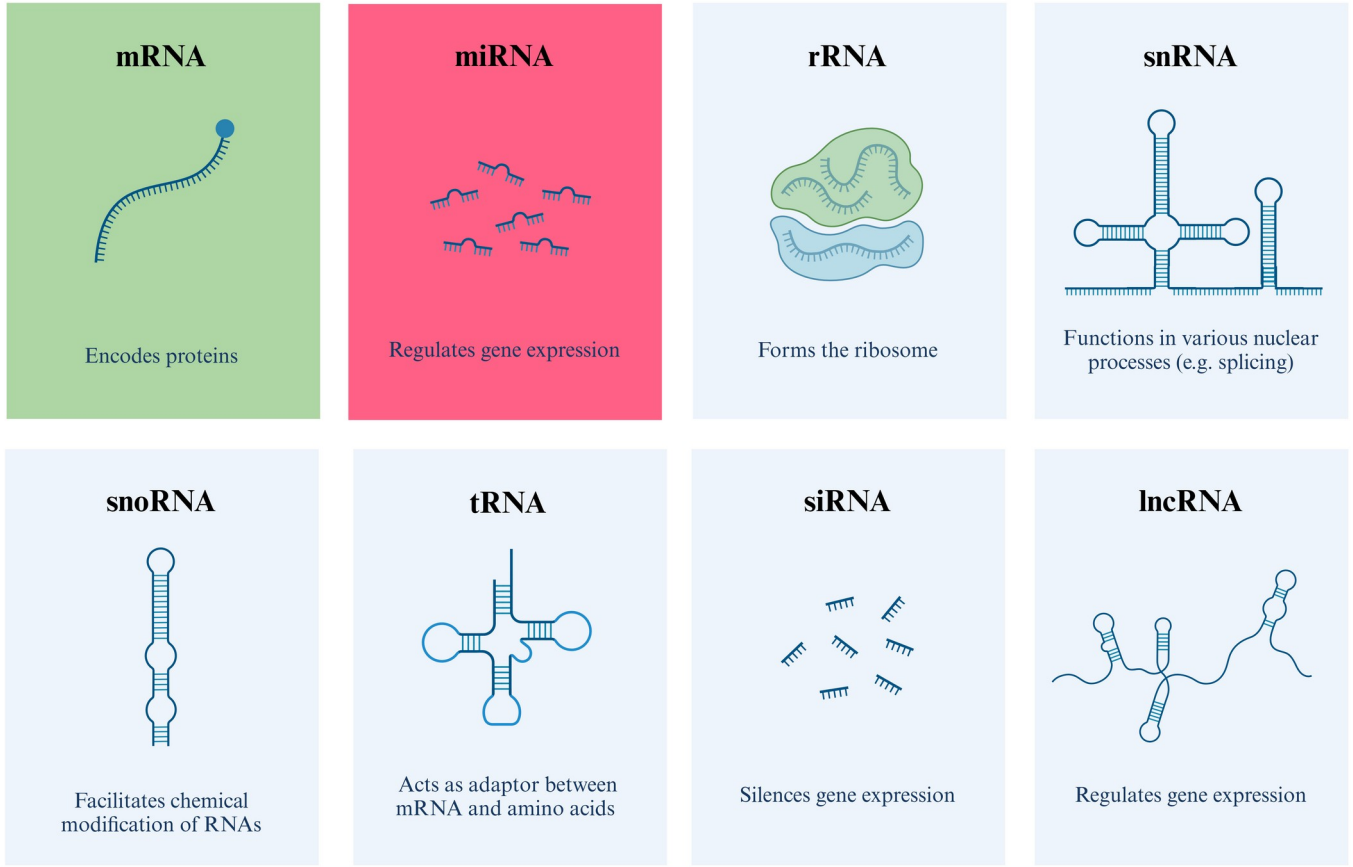
Pre-Transcriptional

- **DNA Methylation:** Addition of methyl groups to DNA, silencing gene expression.
- **Histone Modifications:** Chemical changes to histone proteins, influencing chromatin structure.
- **Transcription Factor Binding:** Activation or repression of gene transcription by specific proteins.

(Post)Transcriptional

- **RNA Splicing:** Removal of introns and joining of exons to generate diverse mRNA isoforms.
- **mRNA Stability:** Regulation of mRNA half-life influences translation.
- **MicroRNAs (miRNAs):** Small RNAs bind to mRNA 3' UTR, leading to degradation or translation inhibition.

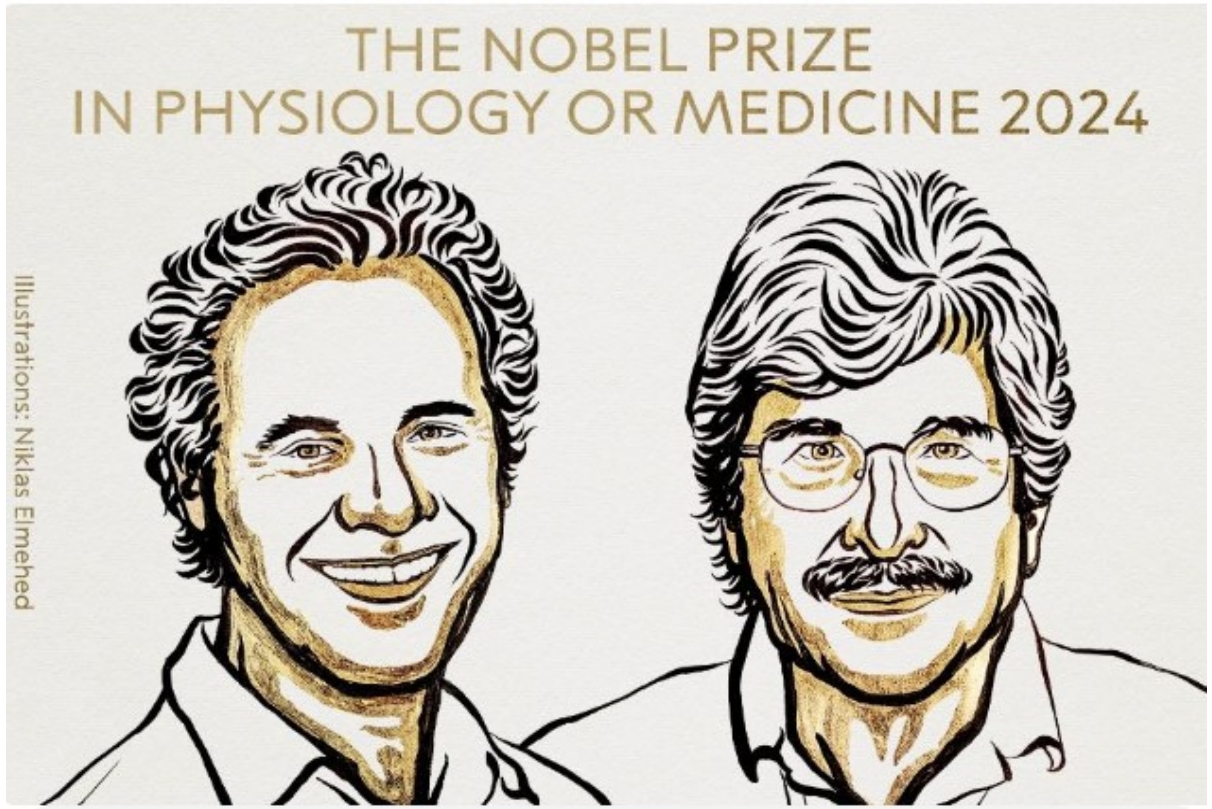
Transcriptome



- Transcriptome is a vast collection of RNA molecules transcribed from DNA

- Significant proportion of the transcriptome consists of non-coding RNAs (ncRNAs, up to 90 %), which do not code for proteins but play pivotal regulatory roles

- Non-coding RNAs add intricate layers of gene regulation and contribute significantly to the complexity of cellular processes.



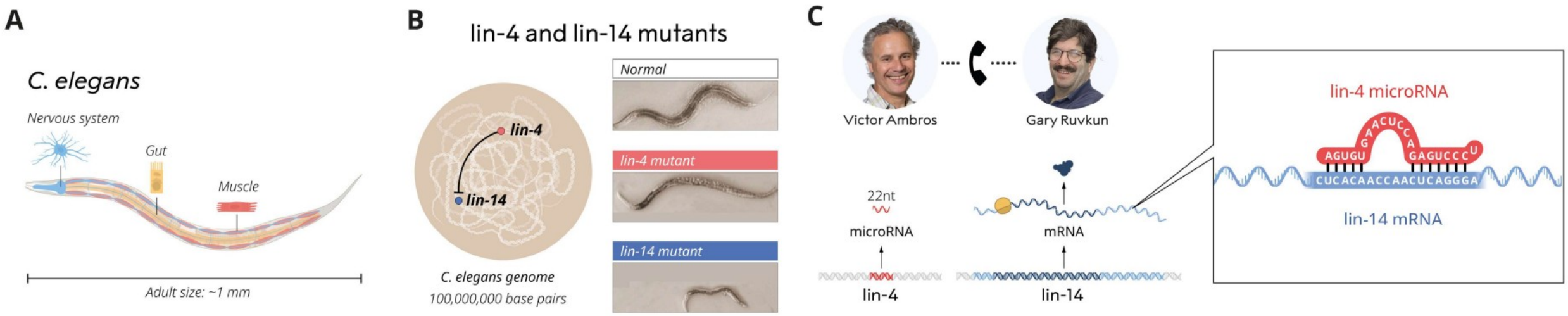
'The Nobel Assembly at the Karolinska Institutet has today decided to award the

2024 Nobel Prize in Physiology or Medicine

jointly to **Victor Ambros and Gary Ruvkun**

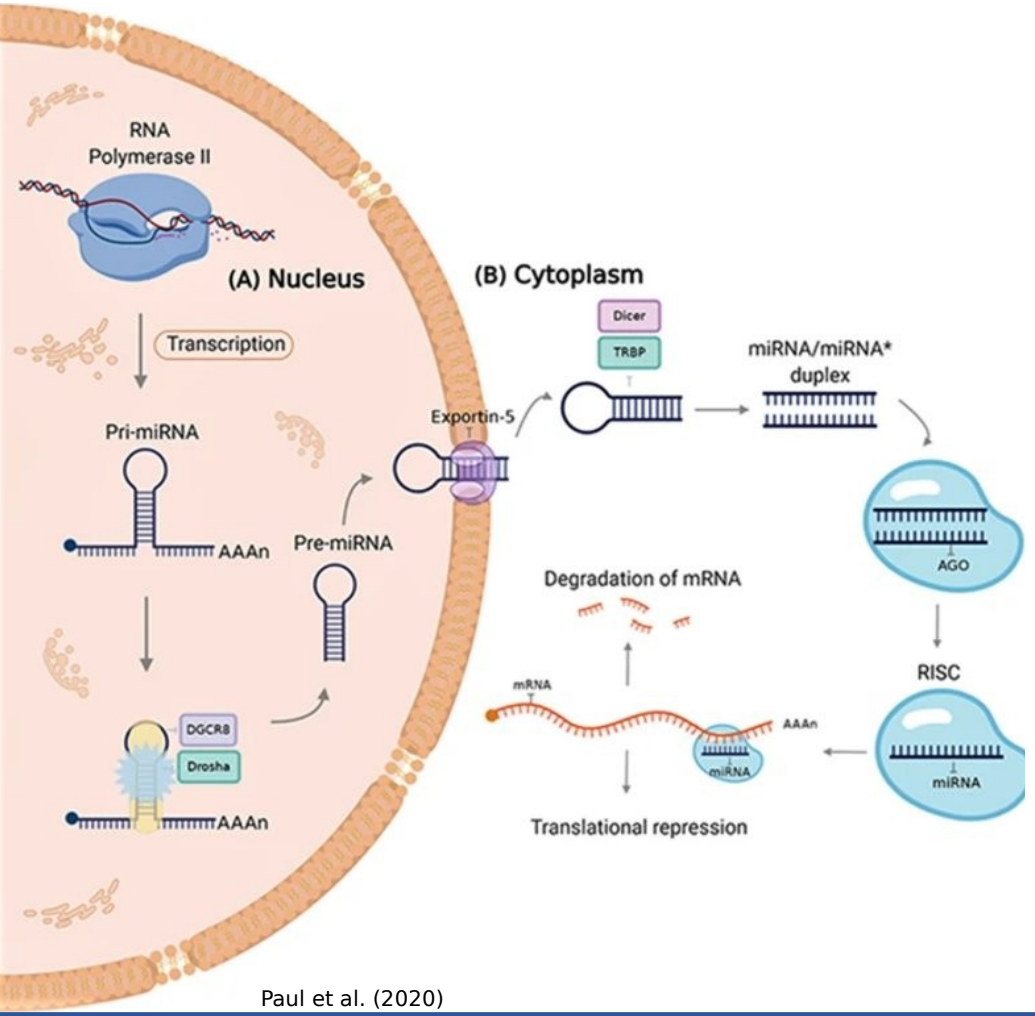
for the discovery of microRNA and

Nobel Price 2024



Discovery of microRNAs (lin-4): Ambros and Ruvkun found that the lin-4 gene in *C. elegans* produces a tiny RNA molecule (microRNA) that binds to complementary sequences in the lin-14 mRNA, blocking protein production and ensuring proper timing of developmental stages.

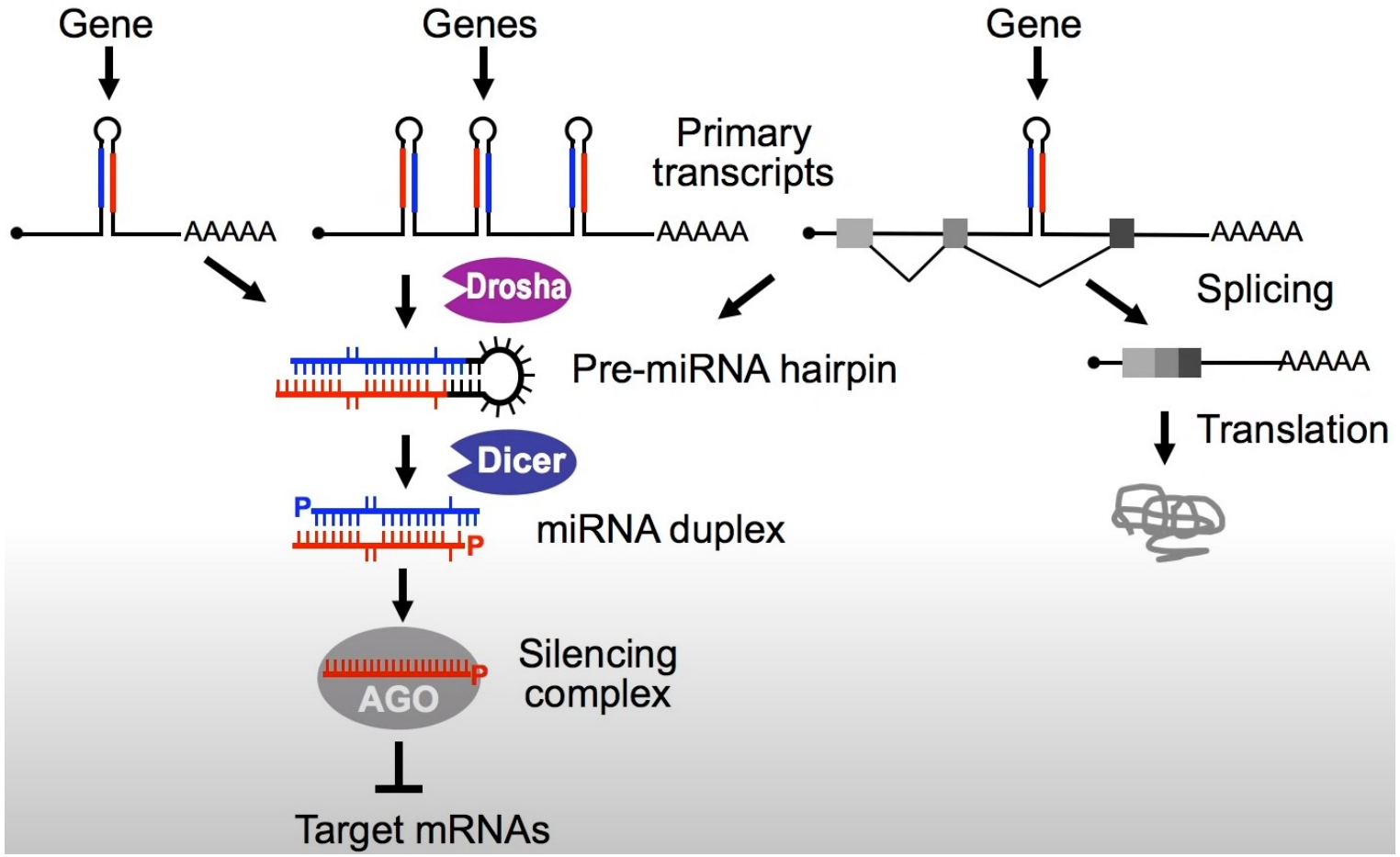
microRNA regulation



Paul et al. (2020)

- microRNAs are small non-coding RNAs (22nt)
- Approximately 2500 human microRNAs
- Key-proteins: Drosha, Dicer, Argonaute
- **Binding of microRNA seed in silencing complex to complementary 3' untranslated region of mRNA**
- Lead to translational repression/ mRNA degradation
- microRNA families often enriched in targets of transcription factor s (redundant functions)

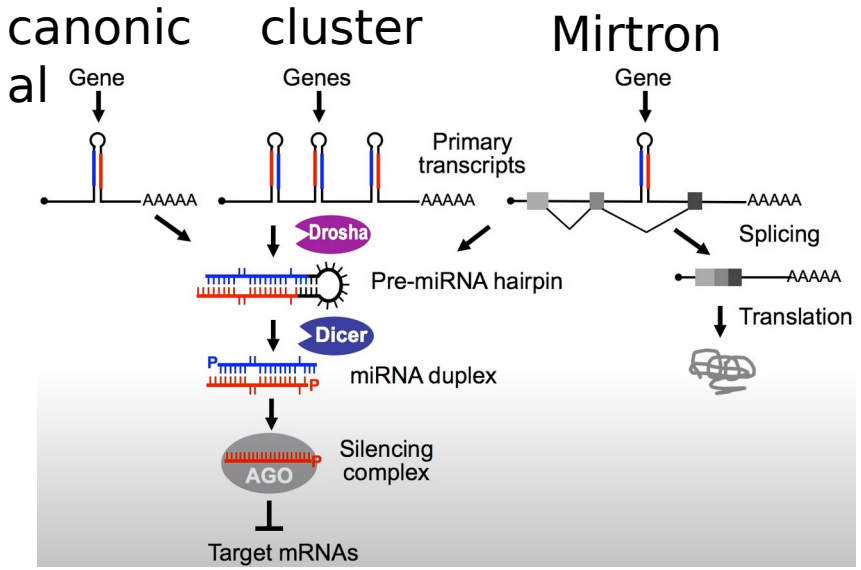
microRNA regulation



Paul et al. (2020)

- Approximately 2300 microRNA genes
- Different primary transcripts (up to 6)
- Multiple microRNAs can be within one ORF
- Multifunctional transcripts (MIRTRONS)
- Non-concial biogenesis can avoid Drosha/Dicer dependency

Ex.1 Explore microRNA genes



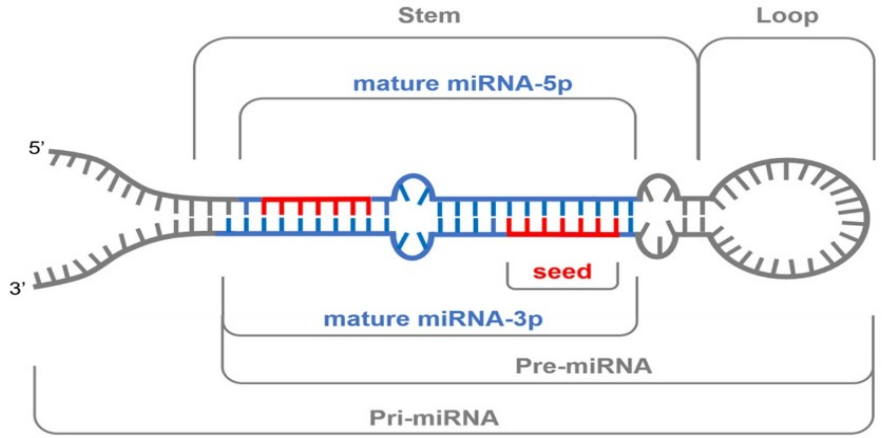
Use the UCSC genome browser (https://genome.ucsc.edu/) to explore human **MIR17HG, MIR1224 and MIR155 and** answer the following questions:

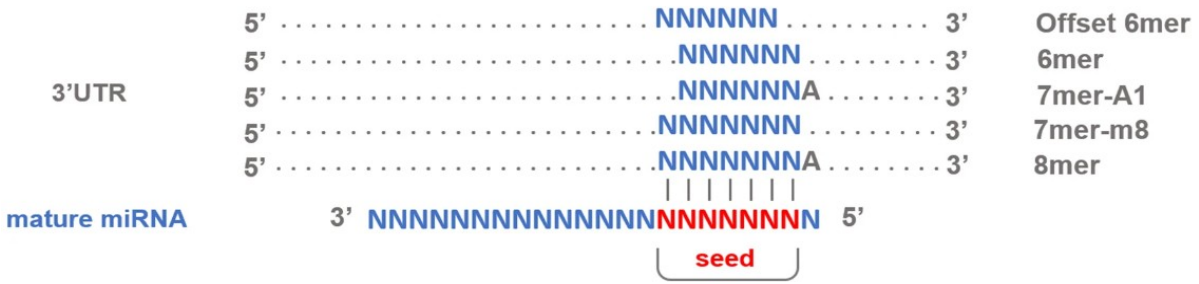
Which one is the 'canonical' microRNA gene?

Which one is the Mirtron and whats the host gene?

Which one is the microRNA cluster gene and how many hairpins does it have?

microRNA regulation

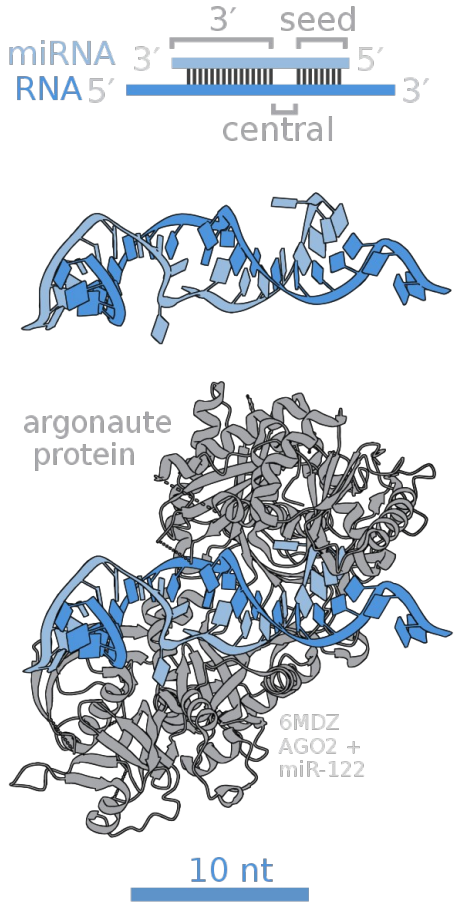




Marmol-Sanchez et al. (2020)

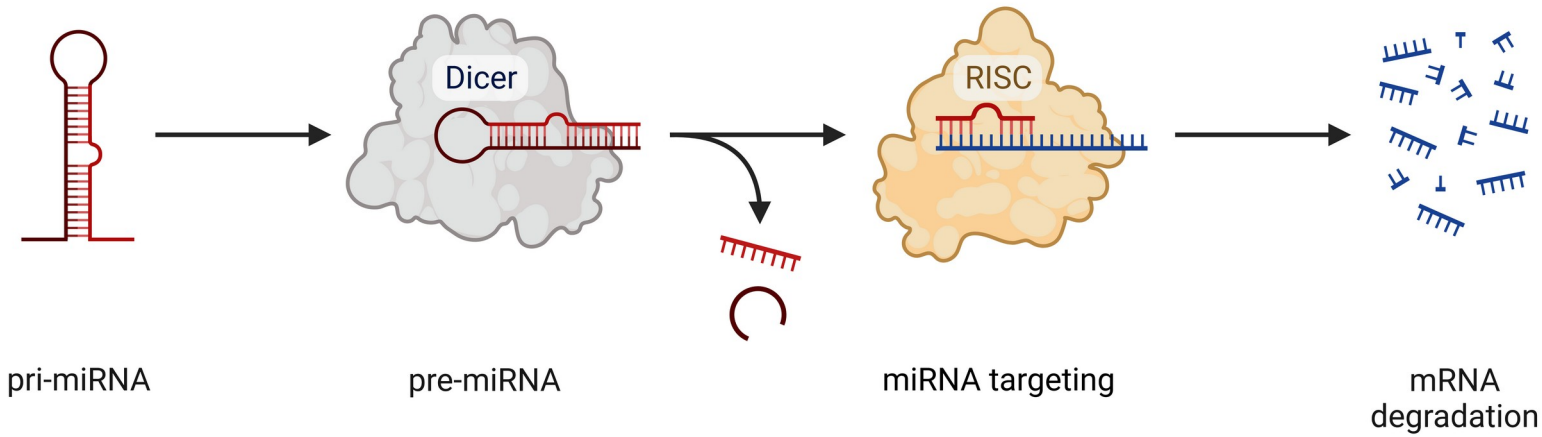
- **Drosha 3' cut leaves a 2 nt overlap and Dicer 5' cut leaves a 2nt overlap**
- Strong propensity for one of both mature strands
- Many microRNA binding sites in 3'UTRs are broadly conserved
- Multiple microRNA binding sites for the same microRNA family
- >60% of protein coding genes are targeted

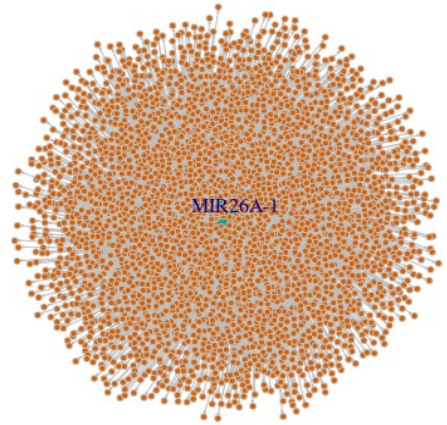
microRNA regulation



- Mature microRNA is loaded into argonaute protein
- **microRNA seed (nt 2-8 from 5' end in mature microRNA)** binds to complementary 3'UTR sequence of the gene
- 3'UTRs of mRNAs can be really long; on average 800 nt, but up to 10,000 nt
- Searching only complementary sequences leads to many false positives
- Integrate further information for scoring of miRNA-mRNA interactions

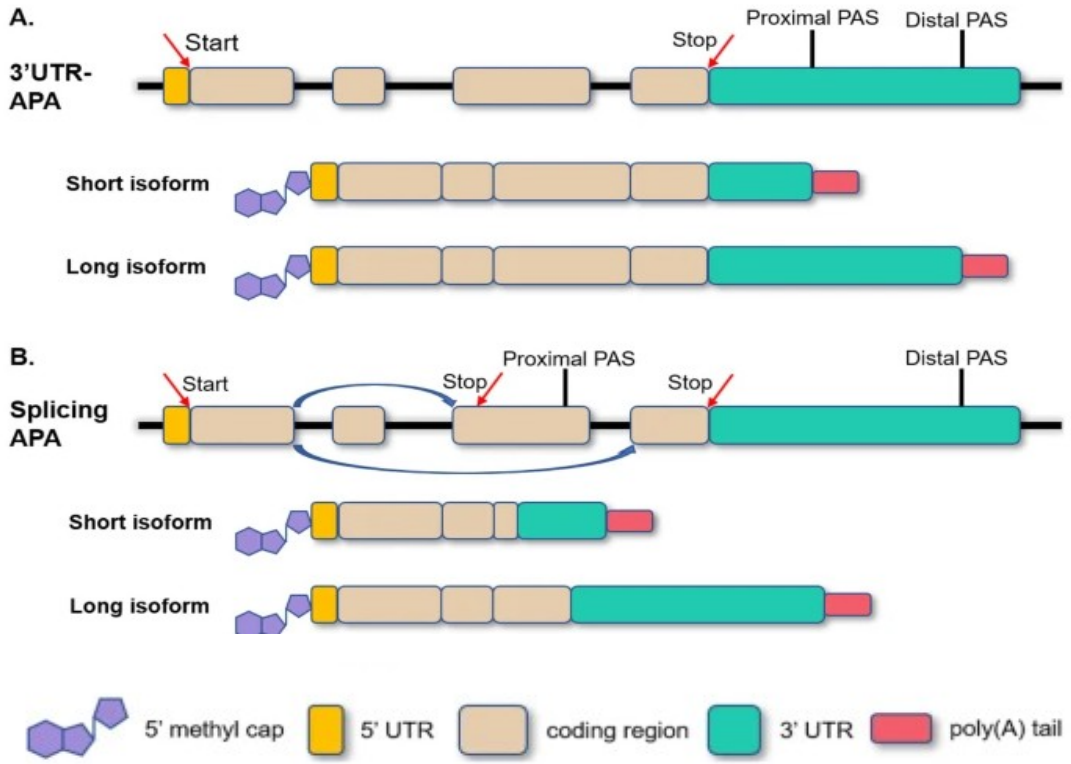
microRNA Regulation





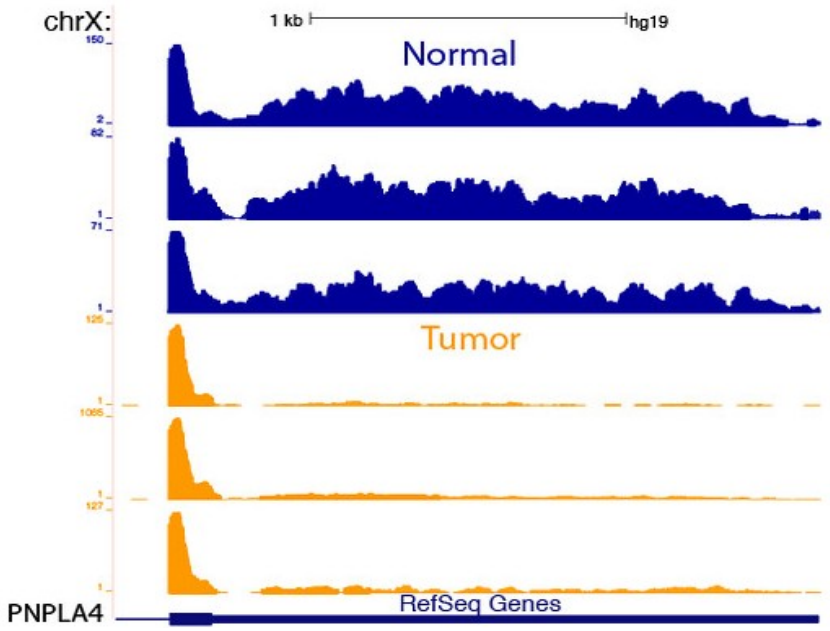
- **Diverse Binding**: MiRNAs can target multiple mRNAs, and one mRNA can have multiple miRNA binding sites due to imperfect complementarity.
- **Complex Prediction**: Predicting miRNA binding sites is challenging because it involves various factors and is not solely based on sequence matching.
- **Experimental Validation**: Accurate validation of miRNA-mRNA interactions often requires experimental techniques in addition to computational predictions.

Alternative Polyadenylation regulates microRNAs



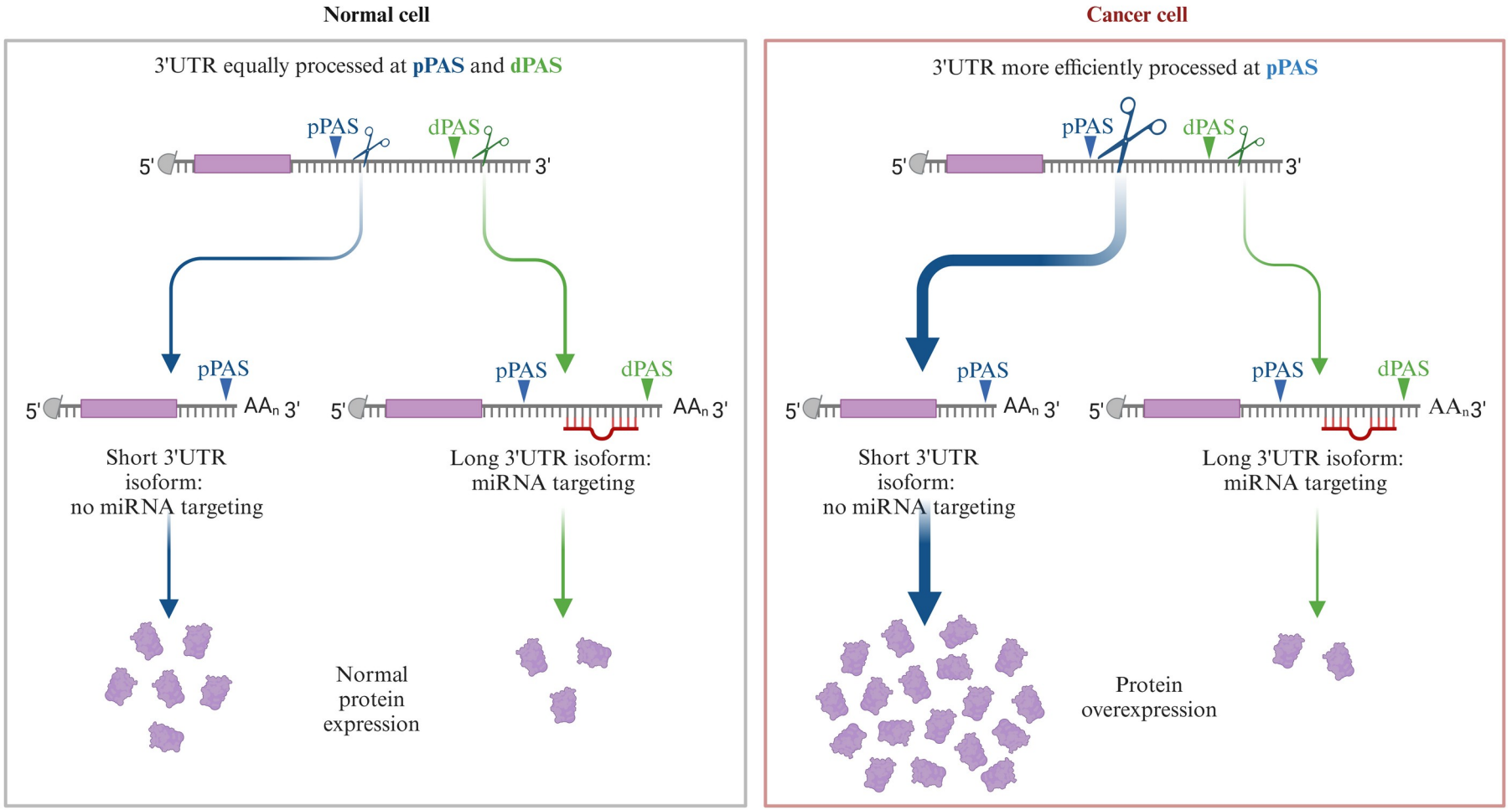
adapted from Zhang et al. (2021)

DaPars Algorithm / TC3A database



Xia et al. (2014)

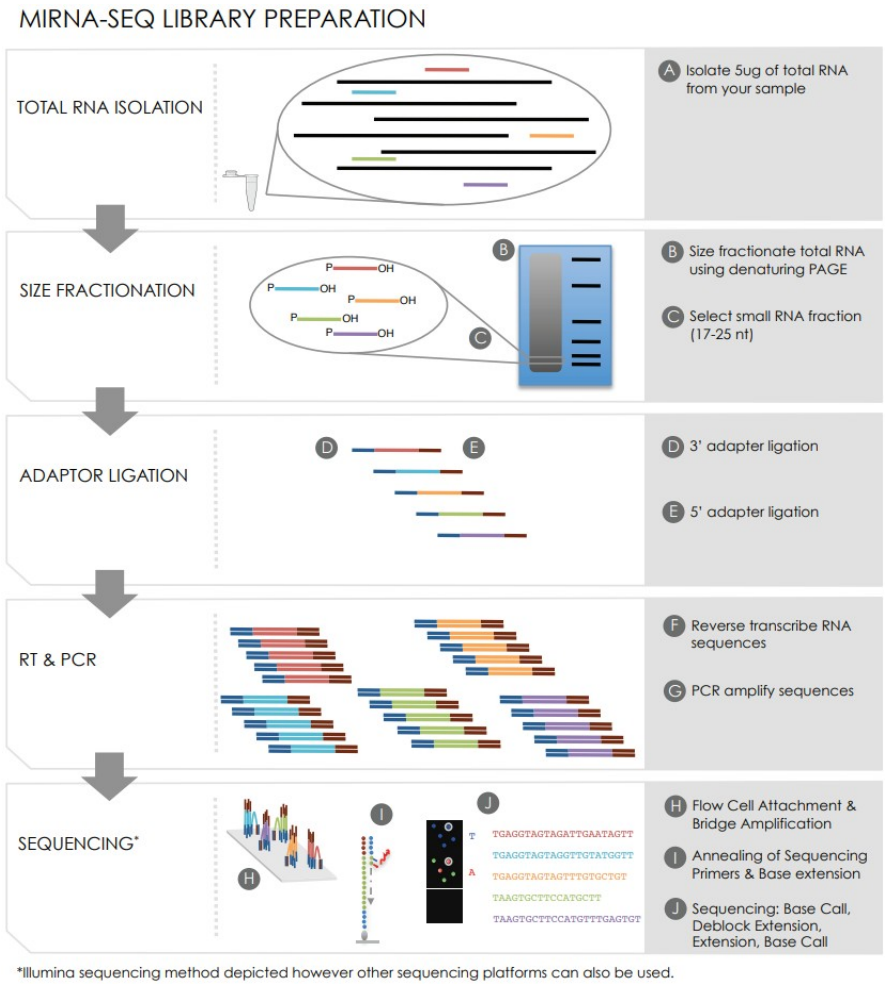
Alternative Polyadenylation



Experimental microRNA detection

METHOD	DISADVANTAGES	ADVANTAGES
Northern blotting	Low-throughput-Low sensitivity-Laborious and very time consuming	High specificity-Readily available and easy-to-perform
In situ hybridization	Low-throughput/Semi-quantitative	Monitor cellular and sub-cellular distributions/spatiotemporal expression profile
Reverse transcription(RT-qPCR)	Cannot identify novel miRNAs	High sensitivity and specificity - Can be used for absolute quantification
Microarray	Low-sensitivity and specificity/ Cannot identify novel miRNAs	Comparing the relative abundance of specific miRNAs/low cost
Next generation sequencing	Substantial computational work required	Very high sensitivity-High accuracy in distinguishing variants of miRNAs

Experimental microRNA detection



- A profile of all small RNAs and miRNAs in the transcriptome
- Small RNA targets are enriched through size selection using size-exclusion gels or commercially developed kits
- Follow RNA-seq procedure
- Bias because of degradated RNA fragments

Ex.2 Annotate microRNAs

Exercise 1: Find the (hsa-) microRNA sequence from the sequences_mirna.fa-file using blast *(https://blast.ncbi.nlm.nih.gov/Blast.cgi)*

>sequence_1

ACATTTACCTAGCAGAAGAAAAATCGTGTTTACGAAGGTGGTTTTCGCAGGGCGAAGCTAATTCGTGCAACTTCCCCAAATGTGG
GAAGCTCGACTGCATAATTTGTGGTAGTGGGAGACTGCGTTCGCTCTTTTCCCCCG

>sequence_2

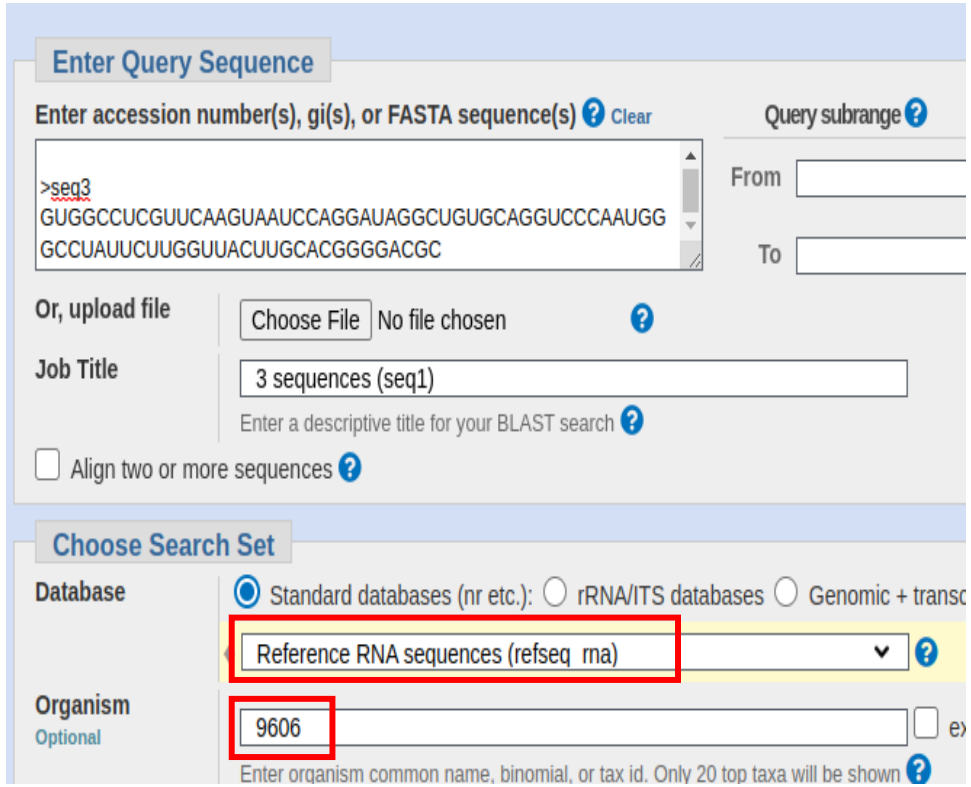
TCCAAACAGACACTGATGGCACCTTCTGCCATTTAGGAATTTGTTTTAAAACAGACATTTGTCTAGATATTTCCTTTGTGGCCTCCT
CCCCATCAAAAGTCAATCAAACATCG

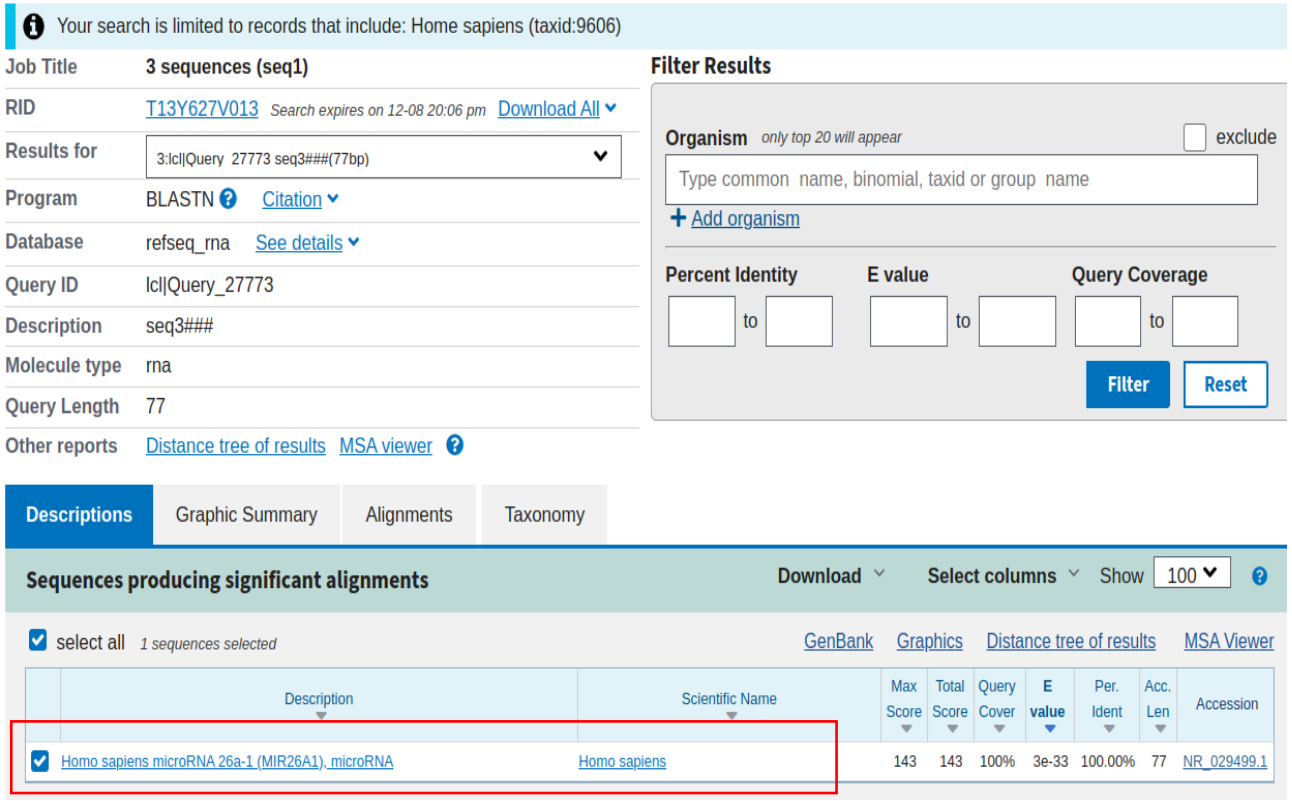
>sequence_3

GTAGAGGAGATGGCGCAGGGGACACGGGCAAAGACTTGGGGGTTCCTGGGACCCTCAGACGTGTGTCCTCTTCTCCCTCCTCC
CAG

and so on..

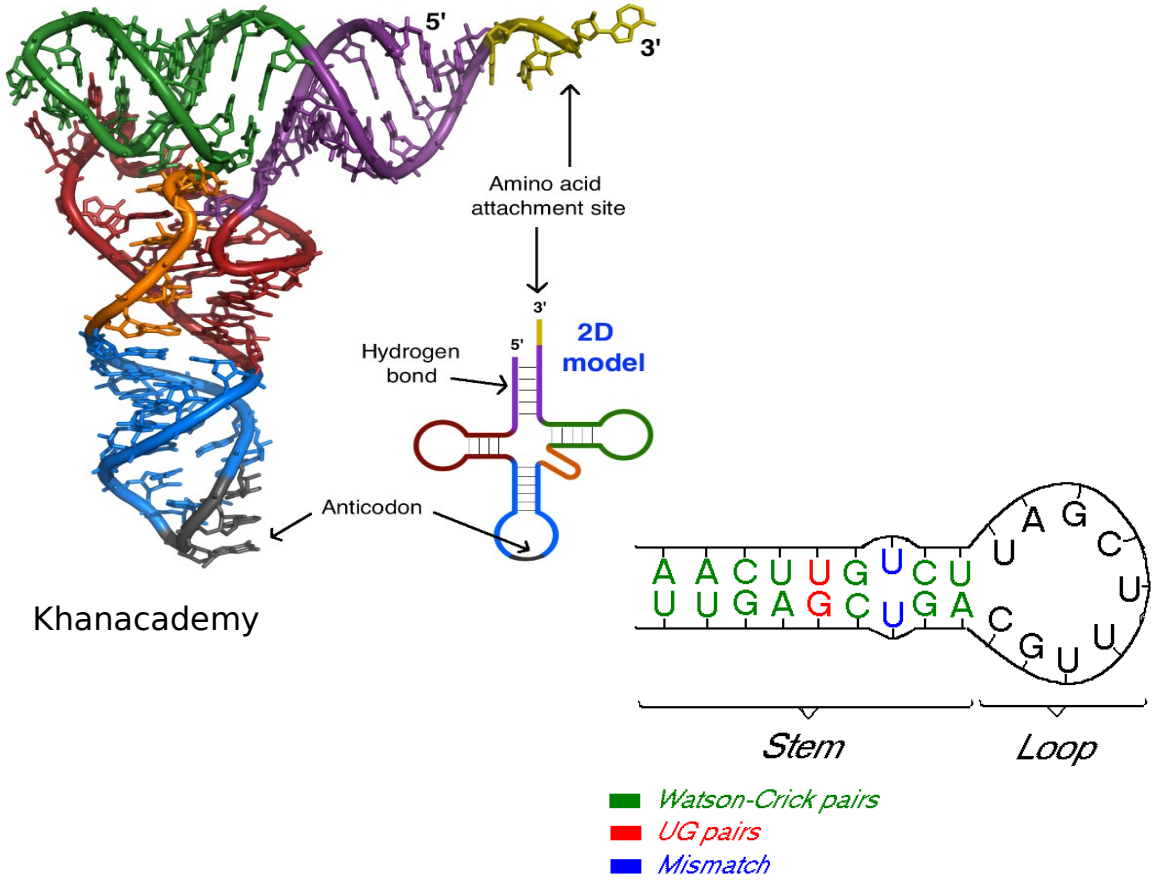
Sol. 2 Annotate microRNAs





RNA Secondary Structure

3D-structure of tRNA



Khanacademy

- Not only proteins and DNA have secondary structures
- Structured RNA is involved in all aspects of gene expression
- mRNAs are often structured in terminal regions
- Watson-Crick-pairing is preferred, but not as prominent as in DNA
- GU pairing creates wobbles (distinct hydrogen bonds)
- Mismatches can have similar stability

RNA Secondary Structure

Example:

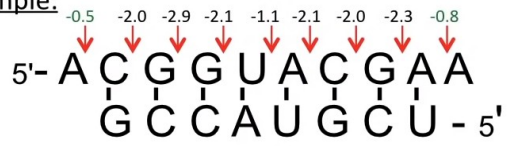
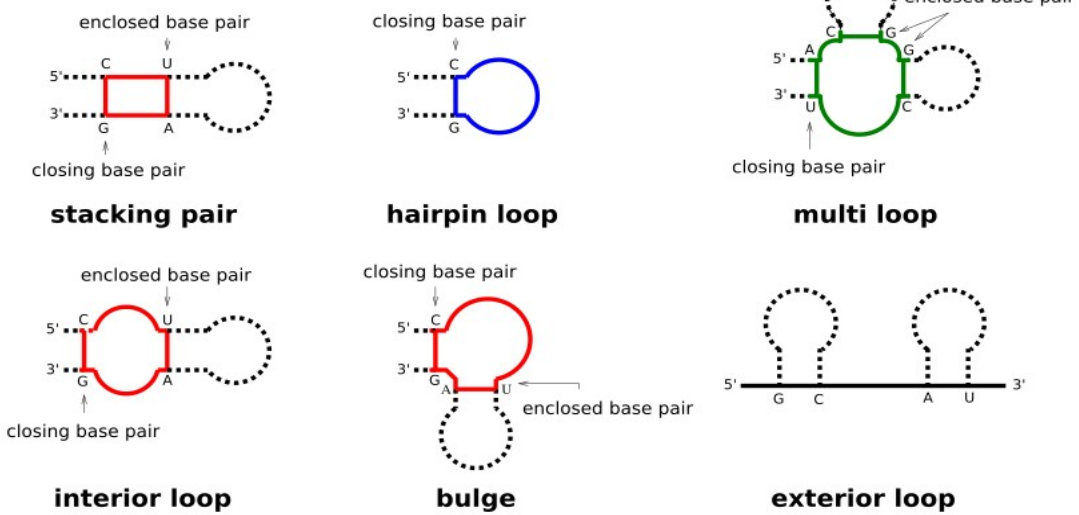
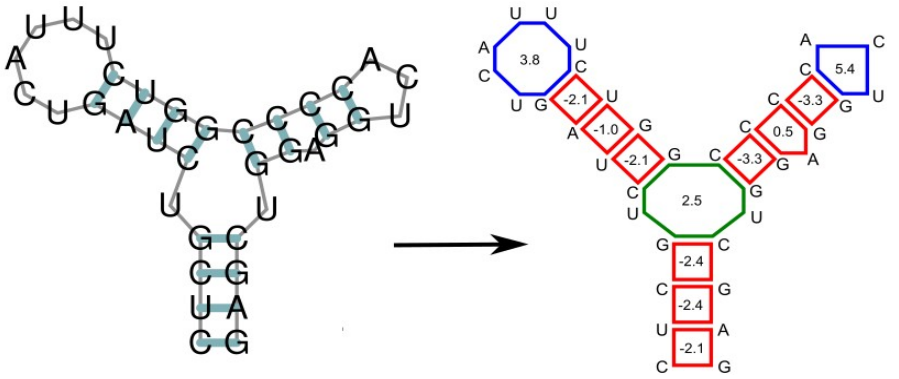


Table 2. Thermodynamic parameters for RNA helix initiation and propagation in 1 M NaCl

Propagation sequence	$\Delta H°$, kcal/mol	$\Delta S°$, eu	$\Delta G°_{37}$, kcal/mol
$\overrightarrow{AA}$ / $\underleftarrow{UU}$	−6.6	−18.4	−0.9
$\overrightarrow{AU}$ / $\underleftarrow{UA}$	−5.7	−15.5	−0.9
$\overrightarrow{UA}$ / $\underleftarrow{AU}$	−8.1	−22.6	−1.1
$\overrightarrow{CA}$ / $\underleftarrow{GU}$	−10.5	−27.8	−1.8
$\overrightarrow{CU}$ / $\underleftarrow{GA}$	−7.6	−19.2	−1.7
$\overrightarrow{GA}$ / $\underleftarrow{CU}$	−13.3	−35.5	−2.3
$\overrightarrow{GU}$ / $\underleftarrow{CA}$	−10.2	−26.2	−2.1
$\overrightarrow{CG}$ / $\underleftarrow{GC}$	−8.0	−19.4	−2.0
$\overrightarrow{GC}$ / $\underleftarrow{CG}$	−14.2	−34.9	−3.4
$\overrightarrow{GG}$ / $\underleftarrow{CC}$	−12.2	−29.7	−2.9
Initiation	(0)	−10.8	3.4
Symmetry correction (self-complementary)	0	−1.4	0.4
Symmetry correction (non-self-complementary)	0	0	0

Freier et al. (1986)

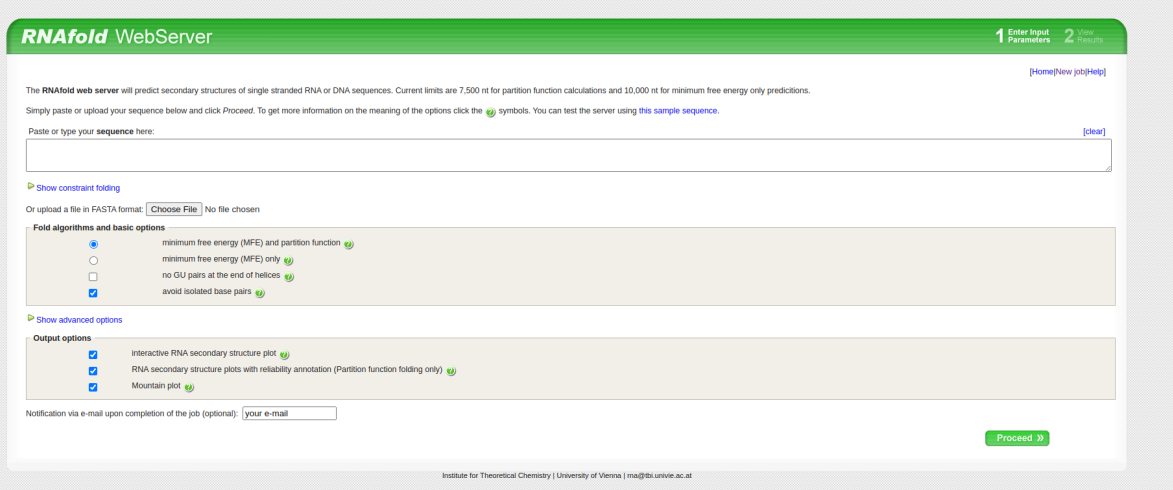


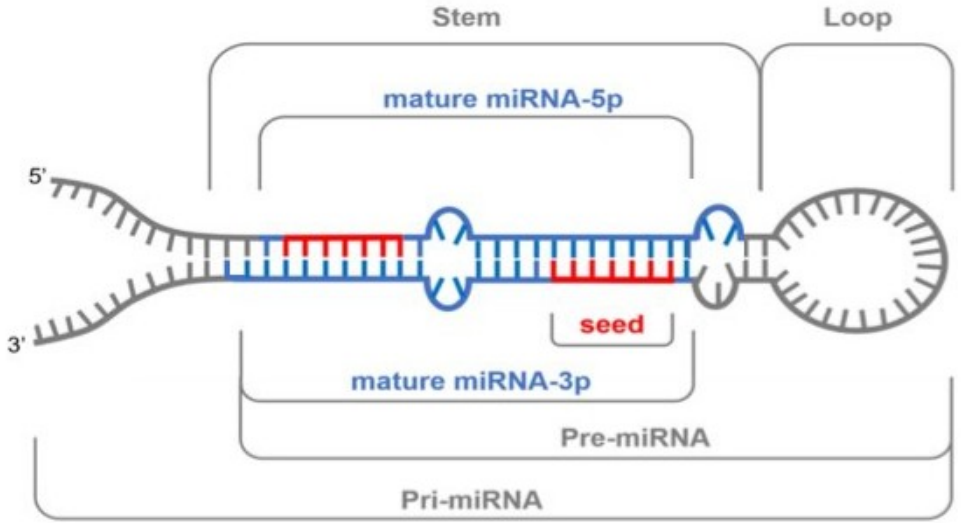


- Turner 1999 RNA parameters
- Mathews 1999 DNA parameters
- Andronescu 2007 RNA parameters
- Mathews 2004 DNA parameters

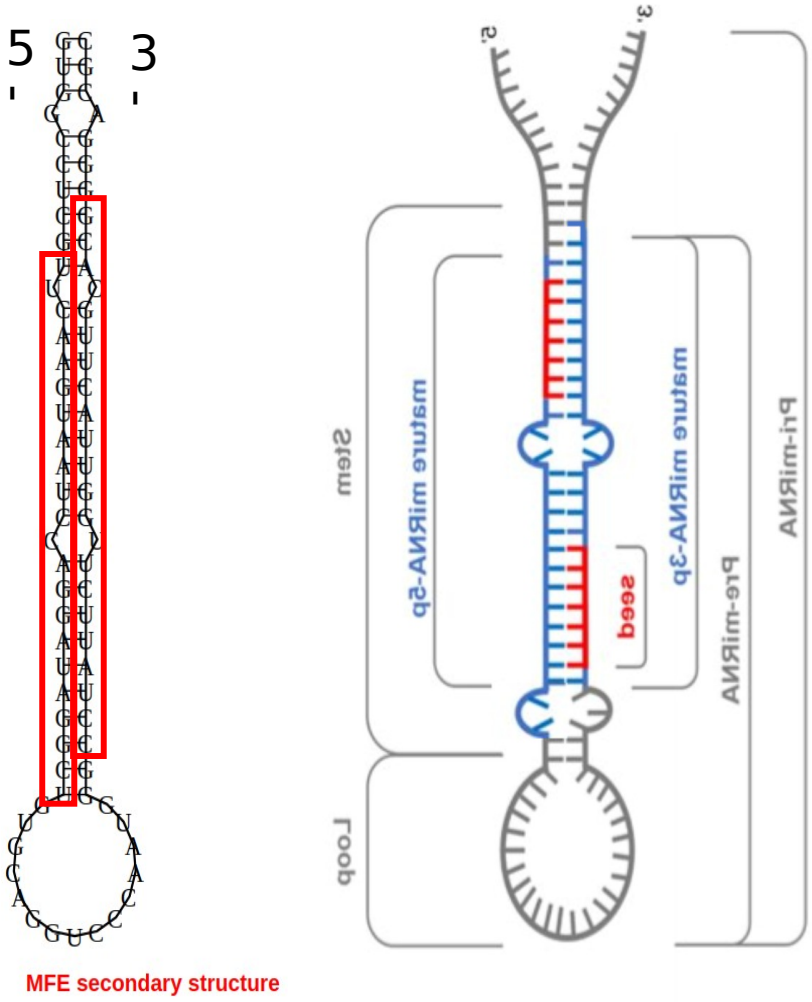
Ex.3 microRNA folding

1. Use the *RNAfold webserver* to compute the secondary structure of prior annotated microRNA sequence. **microRNA 26a-1**
2. Find the 22 nt mature microRNA-3p and -5p sequences. (*Tip: Dicer 5' cuts closing basepairs of loop*)



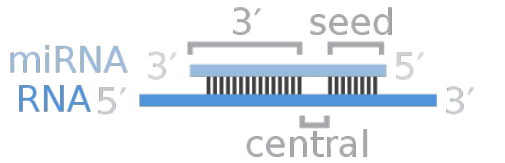


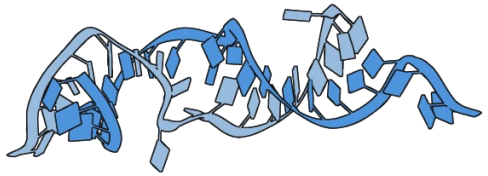
Sol.3 microRNA folding

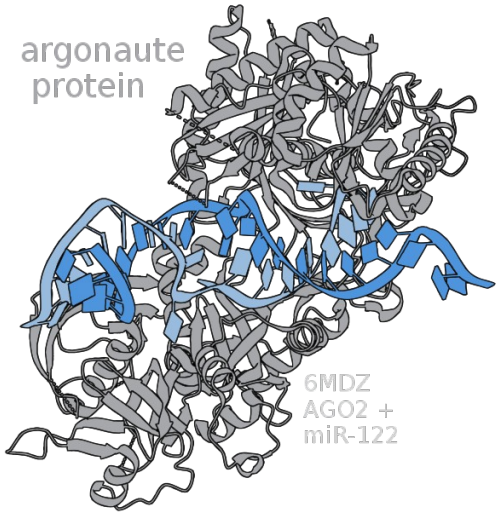


Check further Information on miR
Search for hsa-mir-26a-5p

Ex. 4 microRNA seed







1. How many human genes are potentially targeted by previously identified mature microRNA (hsa- miR-26-5p)? (www.targetscan.org/vert_80/)

2. Which gene has the most conserved 8-mer binding sites?

3. Find the microRNA seed of previously identified mature microRNA. Does it match the positions in the mature microRNA?

Sol. 4 microRNA seed

1045 transcripts with conserved sites, containing a total of **1209** conserved sites and **579** poorly conserved sites.

Genes with only poorly conserved sites are not shown. [View top predicted targets, irrespective of site conservation]

Table sorted by cumulative weighted context++ score [Sort table by predicted occupancy] [Sort table by aggregate P_{CT}]

The table shows at most one transcript per gene, selected for being the most prevalent, based on 3P-seq tags. [Download table]

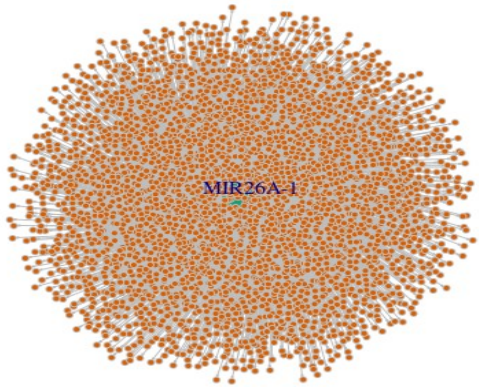
Target gene	Represen-tative transcript ENST0000...	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Conserved sites				Poorly conserved sites				6mer sites	Representative miRNA	Predicted occupancy			Cumulative weighted context++ score	Total context++ score	Aggregate P_{CT}	Previous TargetScan publica-tion(s)
					total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1			mod miRNA	high miRNA	trans-fected miRNA				
		polymerase (RNA) III (DNA directed) polypeptide G																			2009, '11
PTEN	0371953.3	phosphatase and tensin homolog	118	Sites in UTR	4	3	0	1	0	0	0	0	0	hsa-miR-26b-5p	0.1153	0.6550	2.1149	-0.55	-0.60	> 0.99	2011, '15

Position 1261-1268 of PTEN 3' UTR	5' ...ACUGUUAGGGAAUUUUACUUGAA...	8mer
hsa-miR-26a-5p	3' UCGGAUAGGACCUAAUGAACUU	

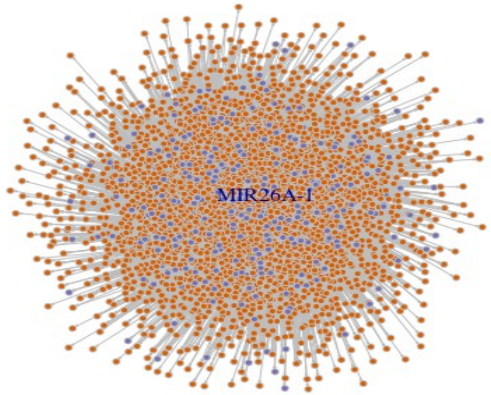
Mature sequence hsa-miR-26a-5p

Accession	MIMAT0000082
Previous IDs	hsa-miR-26a
Sequence	10 - ucaaguaauccaggauaggcu - 31
	Get sequence
Deep sequencing	28542742 reads, 159 experiments

microRNA target prediction



all interactions MIR26A (7897)



conserved sites MIR26A (3196)

- microRNA-mRNA interactions often false-positive
- Experimental validation often indirect from high-throughput
- Integration of further information can help ensuring accuracy:
  - Site type
  - Supplementary pairing
  - Local AU
  - Minimum distance
  - 3' UTR length
  - TA (target site abundance)
  - SPS (seed-pairing stability)
  - Conservation of 3'UTR
  - Conservation of microRNA family
  - Thermodynamic stability of precursor microRNA