Bioinformatics Resources for microRNAs: Tools, Databases, and Platforms

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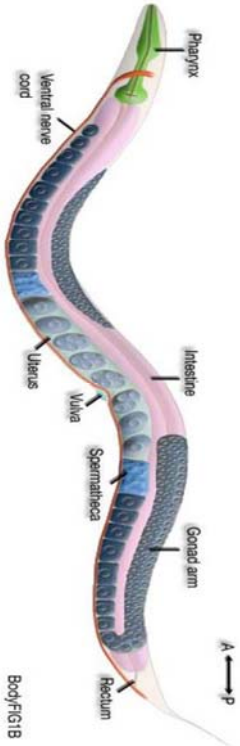
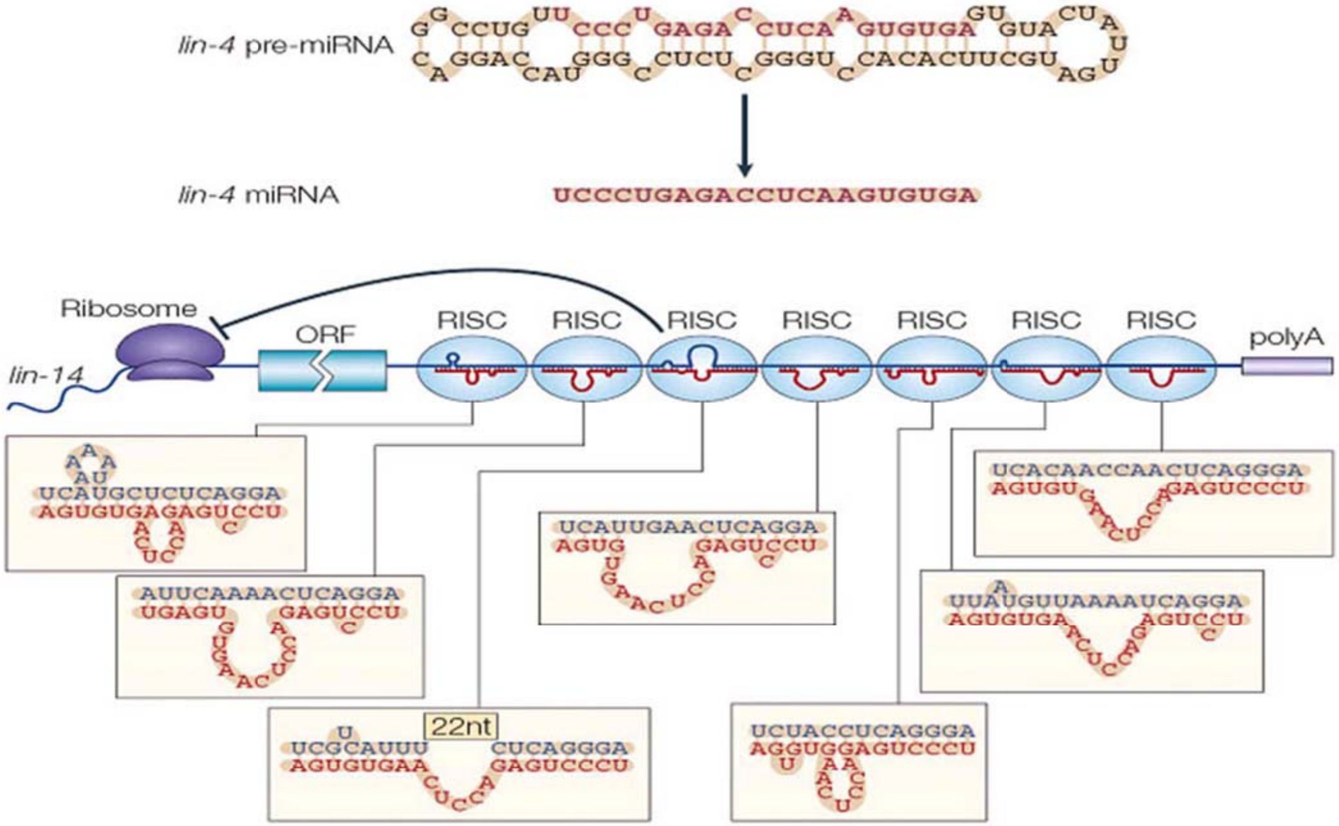
Outline

- **Background**
    - What is microRNA?
    - miRNA functions
- **Databases for miRNAs and miRNA-target interactions**
    - miRBase
    - miRNAMap
    - miRTarBase
- **Tools for identifying miRNA-target interactions**
    - miRTar
    - miRTarCLIP
    - miRGator
    - TargetScan
    - microRNA.org

The first discovered miRNA: *lin-4*

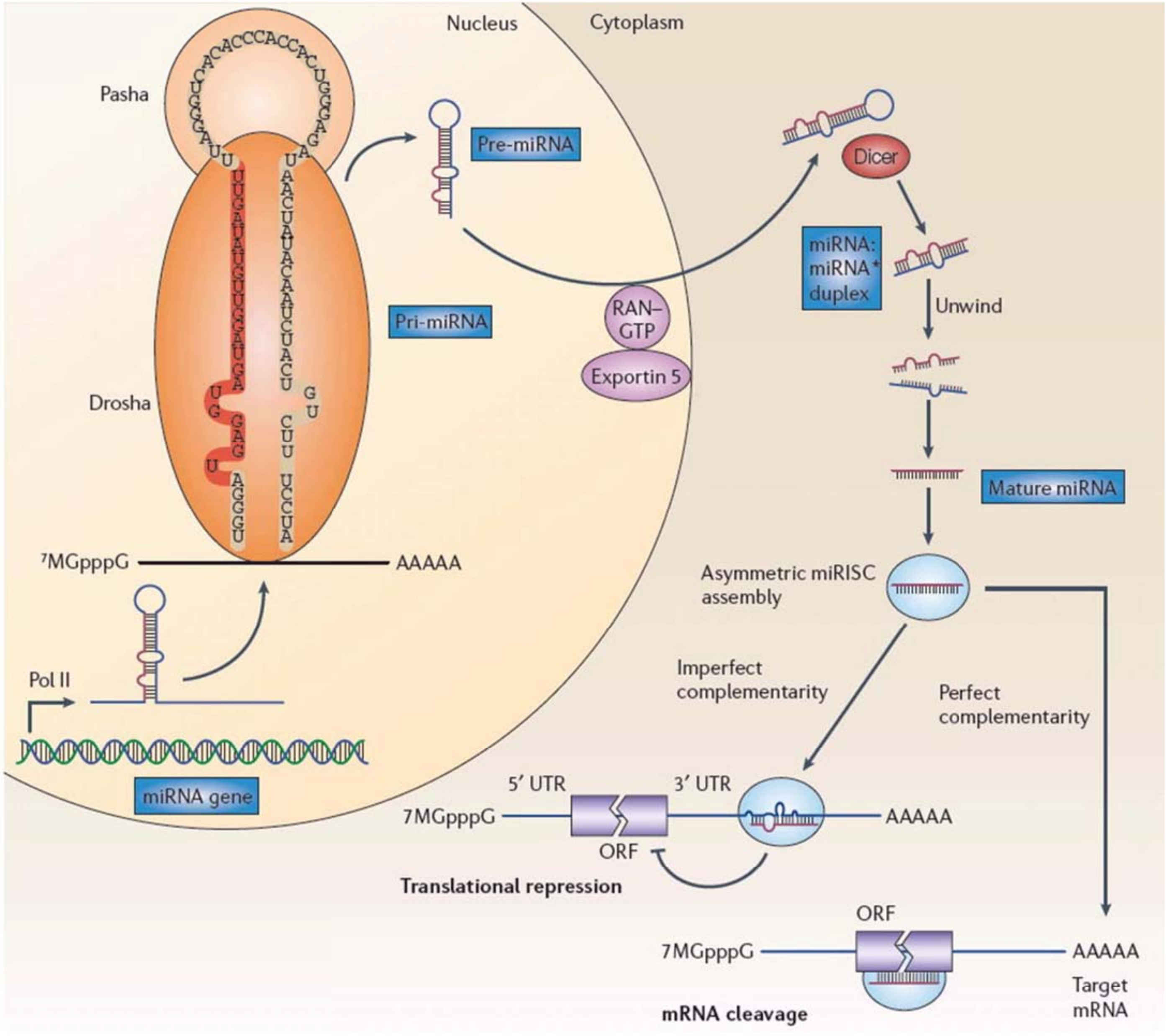




Ruvkun G, Wightman B, Ha I. The 20 years it took to recognize the importance of tiny RNAs. Cell. 2004 Jan 23;116 (2 Suppl):S93-6.

Lee R, Feinbaum R, Ambros V. A short history of a short RNA. Cell. 2004 Jan 23;116 (2 Suppl):S89-92

The biogenesis of microRNAs



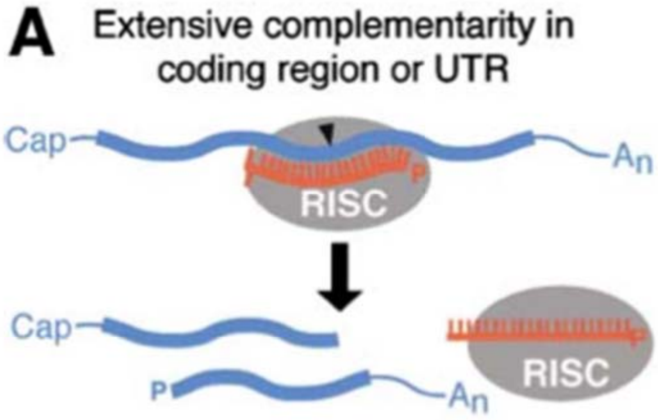
(Esquela-Kerscher and Slack, 2006)

MicroRNAs:

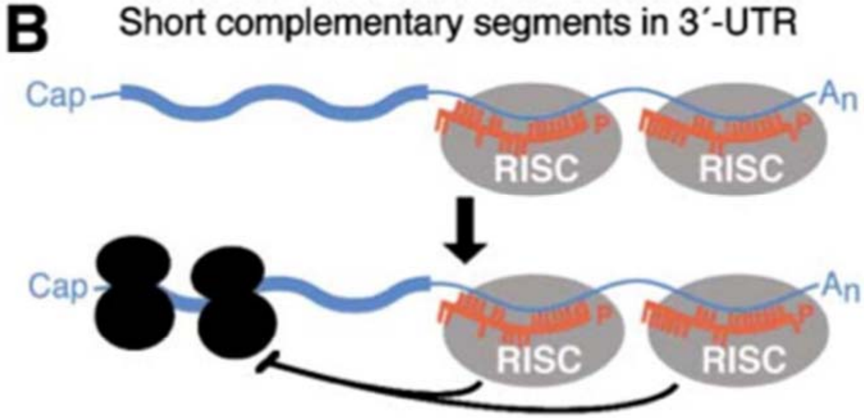
Gene Regulation at the Post-transcriptional Level

MicroRNAs are small (17 to 25 nt.) RNA molecules which regulate gene expression by degrading mRNAs of certain genes or interfering with translational machinery of mRNAs.

mRNA Degradation



mRNA Suppression

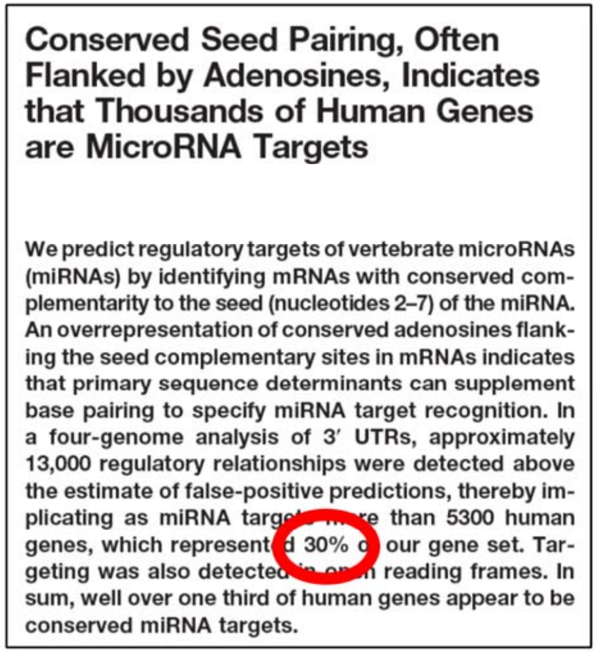


RISC - RNA induced silencing complex

UTR - untranslated region of an mRNA

Images from Bartel. (2004) Cell, Vol 116: 281-297

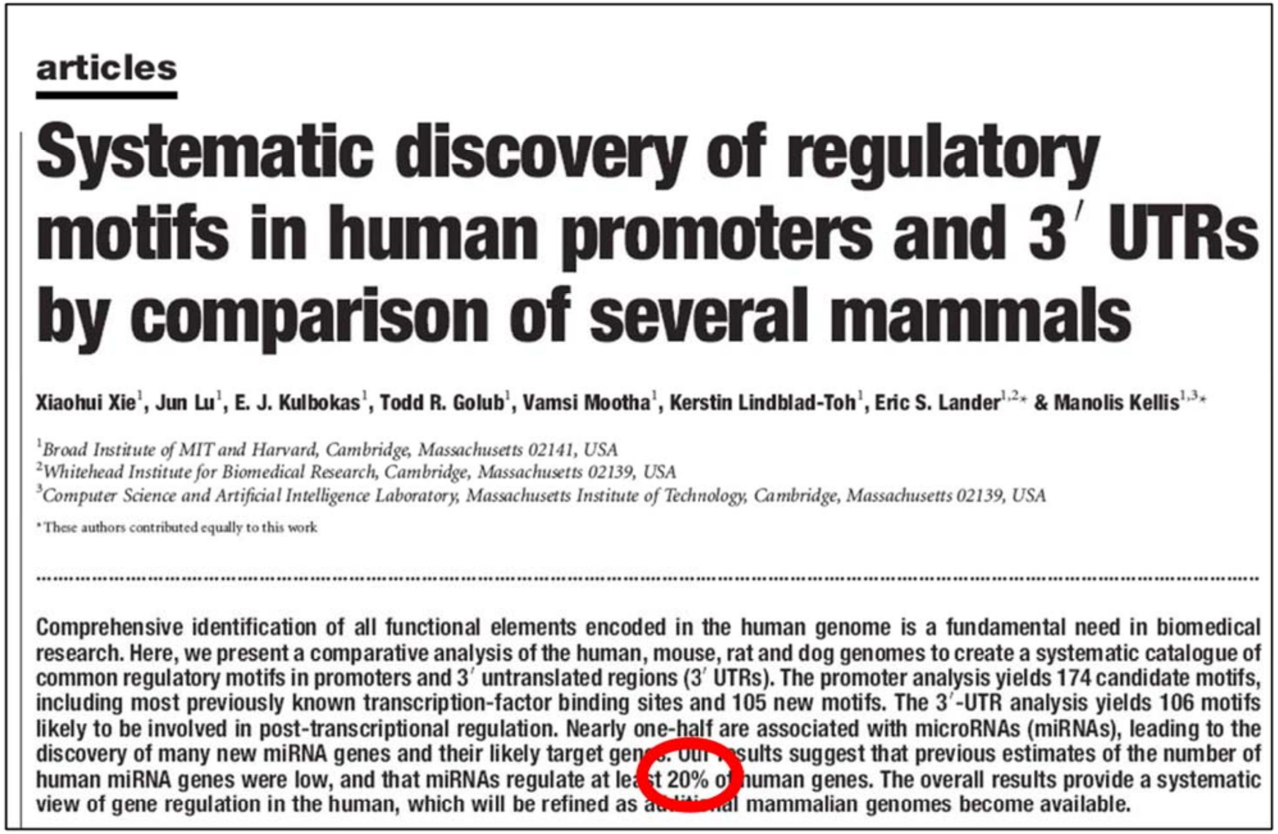
Are All RNAs Regulated by miRNAs?



Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA Targets

We predict regulatory targets of vertebrate microRNAs (miRNAs) by identifying mRNAs with conserved complementarity to the seed (nucleotides 2–7) of the miRNA. An overrepresentation of conserved adenosines flanking the seed complementary sites in mRNAs indicates that primary sequence determinants can supplement base pairing to specify miRNA target recognition. In a four-genome analysis of 3′ UTRs, approximately 13,000 regulatory relationships were detected above the estimate of false-positive predictions, thereby implicating as miRNA targets more than 5300 human genes, which represented 30% of our gene set. Targeting was also detected in open reading frames. In sum, well over one third of human genes appear to be conserved miRNA targets.

Adapter from Lewis BP et. al., ***Cell*** 2005.



articles

Systematic discovery of regulatory motifs in human promoters and 3′ UTRs by comparison of several mammals

Xiaohui Xie[1], Jun Lu[1], E. J. Kulbokas[1], Todd R. Golub[1], Vamsi Mootha[1], Kerstin Lindblad-Toh[1], Eric S. Lander[1,2*] & Manolis Kellis[1,3*]

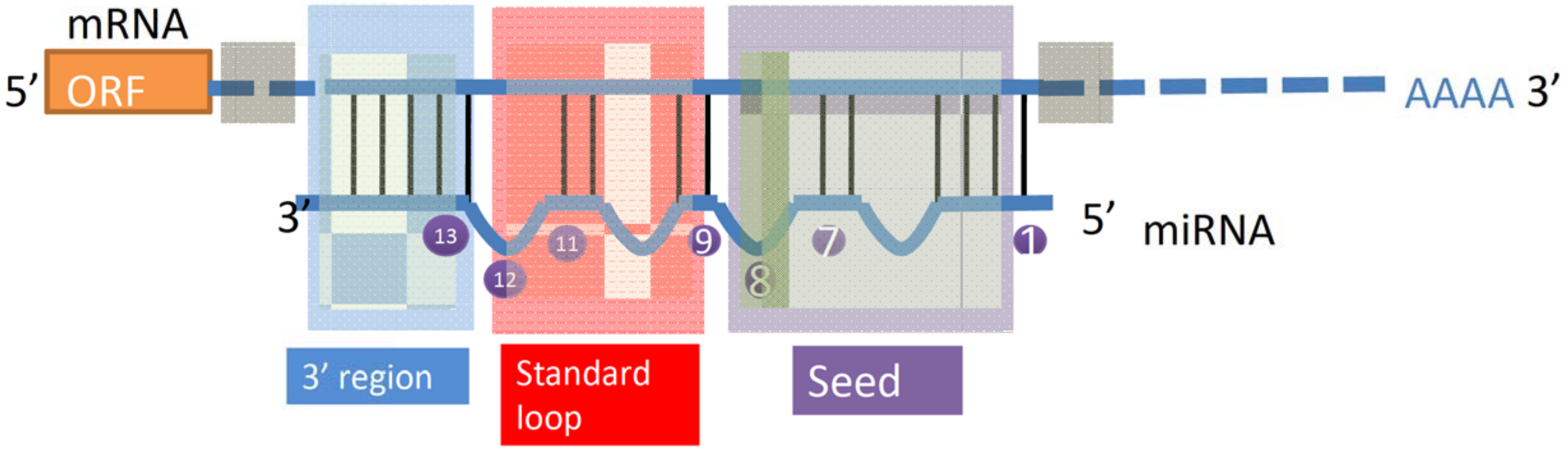
[1]*Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02141, USA*
[2]*Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02139, USA*
[3]*Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

*These authors contributed equally to this work

Comprehensive identification of all functional elements encoded in the human genome is a fundamental need in biomedical research. Here, we present a comparative analysis of the human, mouse, rat and dog genomes to create a systematic catalogue of common regulatory motifs in promoters and 3′ untranslated regions (3′ UTRs). The promoter analysis yields 174 candidate motifs, including most previously known transcription-factor binding sites and 105 new motifs. The 3′-UTR analysis yields 106 motifs likely to be involved in post-transcriptional regulation. Nearly one-half are associated with microRNAs (miRNAs), leading to the discovery of many new miRNA genes and their likely target genes. Our results suggest that previous estimates of the number of human miRNA genes were low, and that miRNAs regulate at least 20% of human genes. The overall results provide a systematic view of gene regulation in the human, which will be refined as additional mammalian genomes become available.

Adapter from Xie X et. al., ***Nature*** 2005.

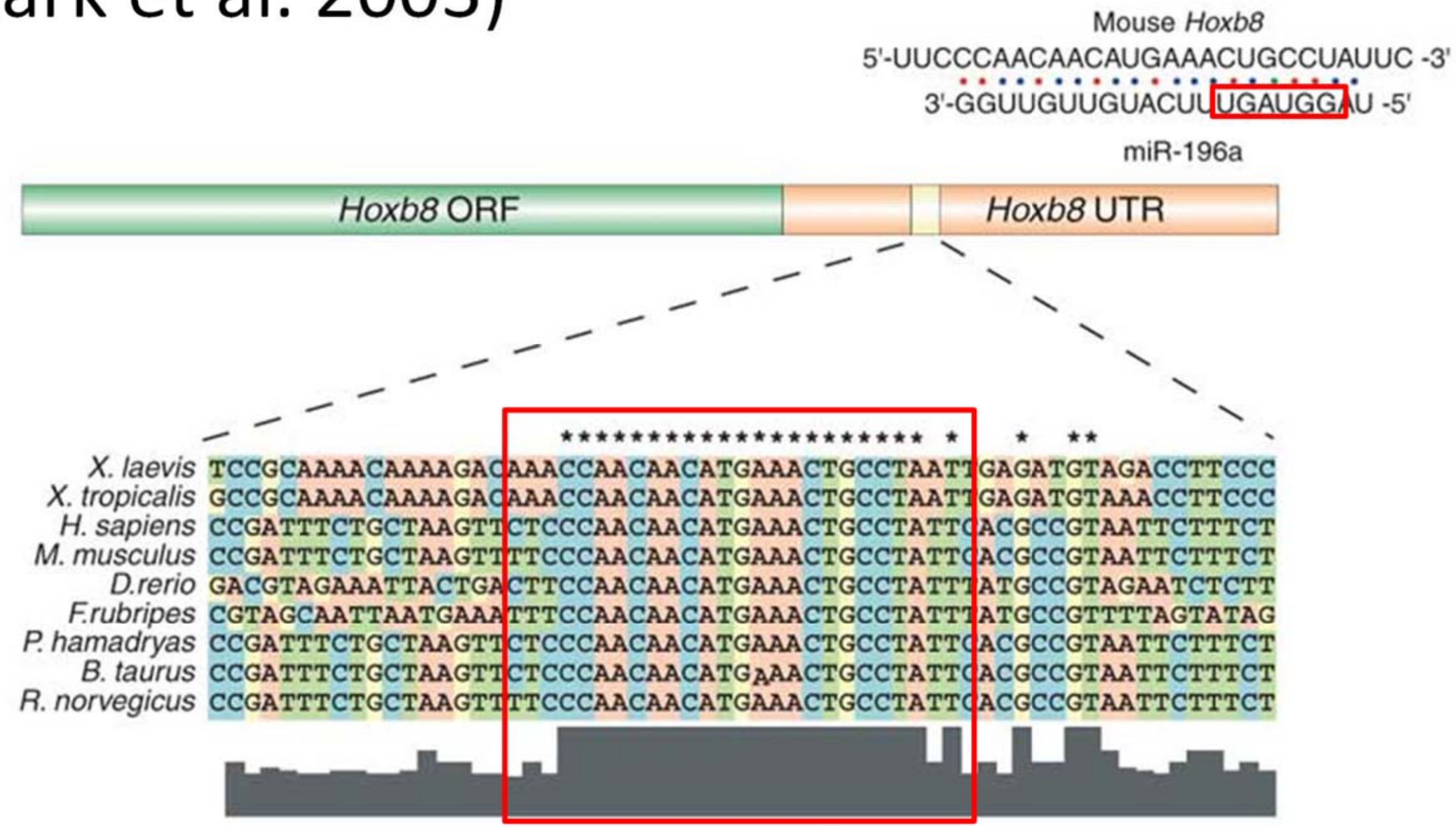
Target Prediction is often based on…



- Perfect pairing of miRNA-mRNA seed match
- Seed match conservation across different species
- Number of predicted sites for the same miRNA on a certain 3′UTR
- Thermodynamics of miRNA-target pair
- Target site accessibility

Cross Species Conserved Seed Match

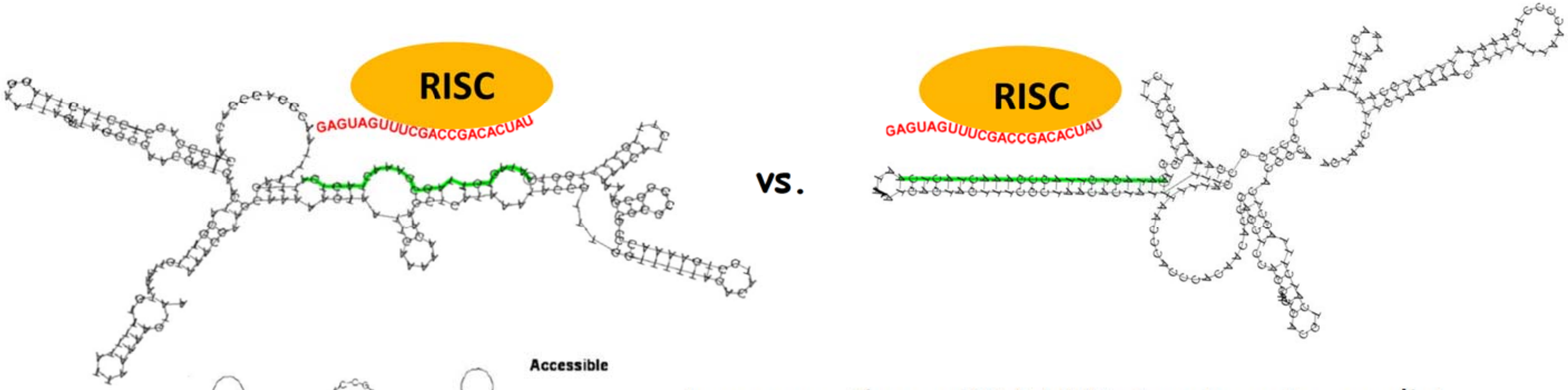
- MicroRNA targets conserve across species. (Stark et al. 2003)

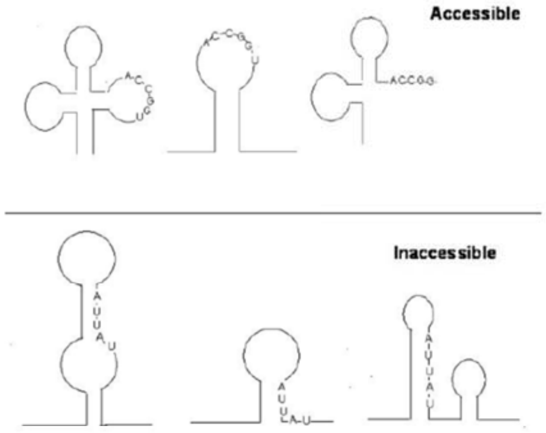


Mansfield, 2004, Nat Genet; MicroRNA-responsive 'sensor' transgenes uncover Hox-like and other developmentally regulated patterns of vertebrate microRNA expression.

Accessibility: The Missing Component

What about target accessibility?



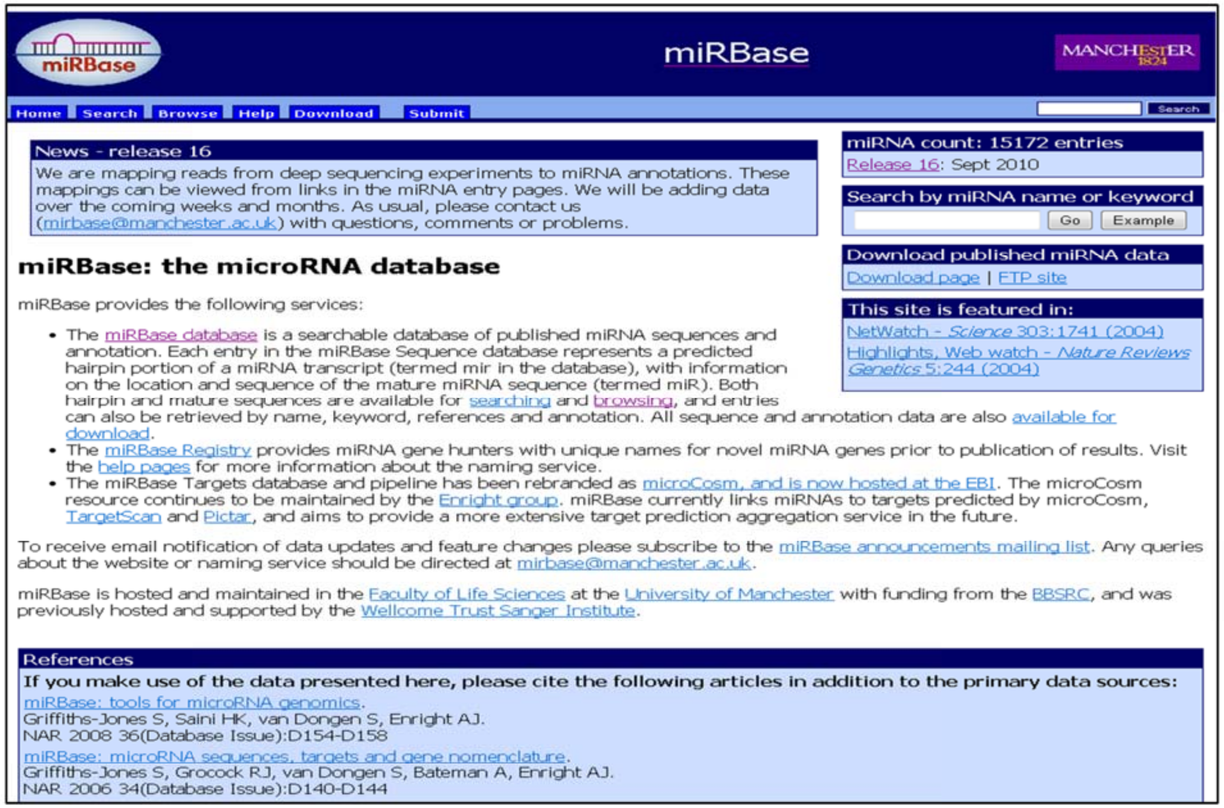


Incorporating mRNA UTR structure to predict microRNA targets (Robins et al. 2005)

- Make sure the predicted target "accessible".
- Not forming basing pairing its self.

Databases for miRNAs and miRNA-target interactions

- **miRBase**
- **miRNAMap**
- **miRTarBase**



News - release 16

We are mapping reads from deep sequencing experiments to miRNA annotations. These mappings can be viewed from links in the miRNA entry pages. We will be adding data over the coming weeks and months. As usual, please contact us (mirbase@manchester.ac.uk) with questions, comments or problems.

miRNA count: 15172 entries

Release 16: Sept 2010

Search by miRNA name or keyword

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

miRBase provides the following services:

- The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.
- The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.
- The miRBase Targets database and pipeline has been rebranded as microCosm, and is now hosted at the EBI. The microCosm resource continues to be maintained by the Enright group. miRBase currently links miRNAs to targets predicted by microCosm, TargetScan and Pictar, and aims to provide a more extensive target prediction aggregation service in the future.

To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.

References

If you make use of the data presented here, please cite the following articles in addition to the primary data sources:

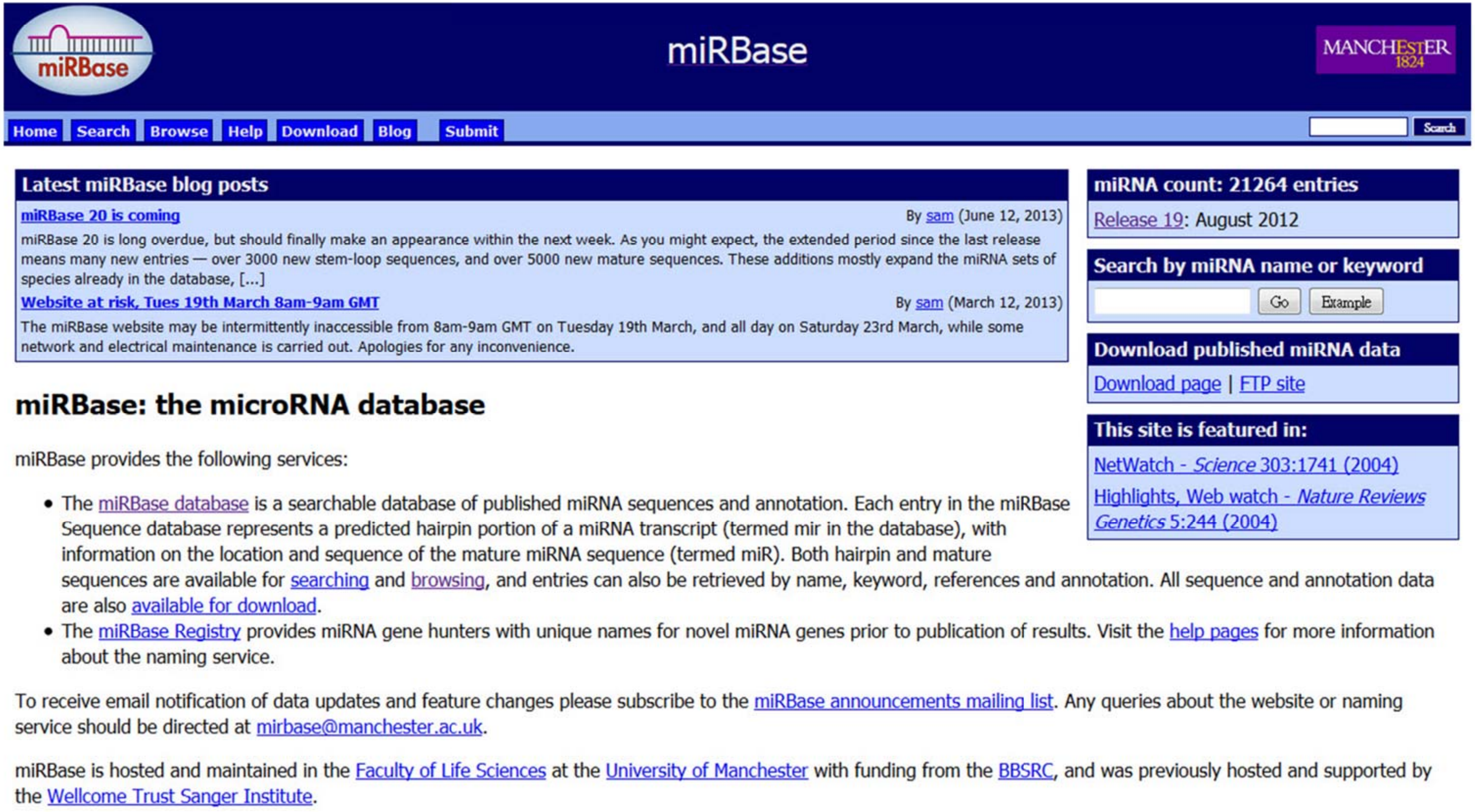
miRBase: tools for microRNA genomics.
Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ.
NAR 2008 36(Database Issue):D154-D158

miRBase: microRNA sequences, targets and gene nomenclature.
Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ.
NAR 2006 34(Database Issue):D140-D144

miRBase Database

The microRNA database

http://www.mirbase.org/



Latest miRBase blog posts

miRBase 20 is coming By sam (June 12, 2013)

miRBase 20 is long overdue, but should finally make an appearance within the next week. As you might expect, the extended period since the last release means many new entries — over 3000 new stem-loop sequences, and over 5000 new mature sequences. These additions mostly expand the miRNA sets of species already in the database, [...]

Website at risk, Tues 19th March 8am-9am GMT By sam (March 12, 2013)

The miRBase website may be intermittently inaccessible from 8am-9am GMT on Tuesday 19th March, and all day on Saturday 23rd March, while some network and electrical maintenance is carried out. Apologies for any inconvenience.

miRNA count: 21264 entries

Release 19: August 2012

Search by miRNA name or keyword

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

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miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.

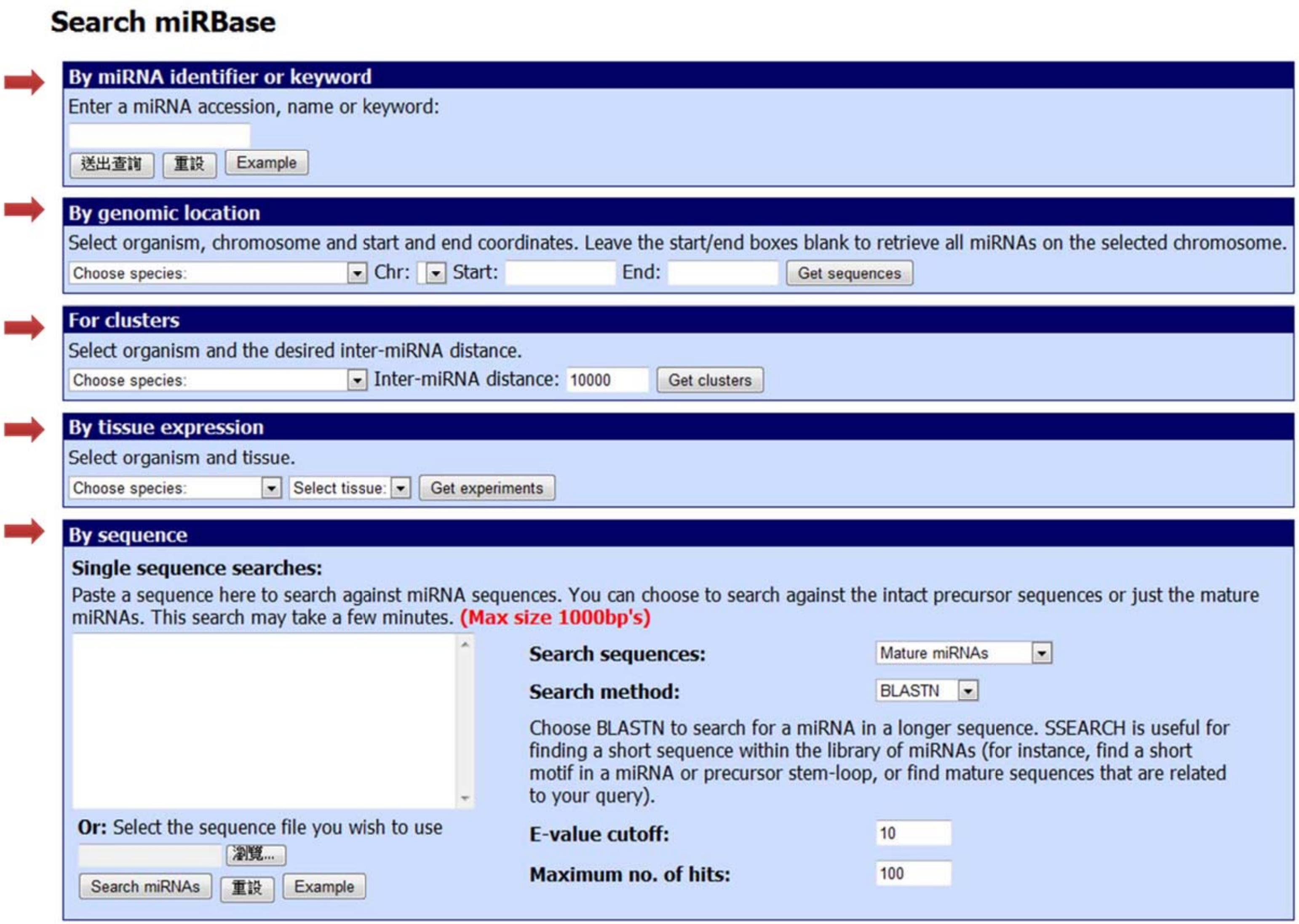
miRNA nomenclature

Pre-miRNA	Mature miRNA	Note
xxx-bantam	xxx-bantam	insect
xxx-lin-4	xxx-lin-4	nematode
xxx-mir-yyy	xxx-miR-yyy	
xxx-MIRyyy	xxx-miRyyy	Plant

xxx: abbreviations of species
yyy: three or four digital numbers and one alphabet

Pre-miRNA	Genomic location	Mature miRNA
rno-mir-103-1	chr10: 20695027-20695112 [+]	rno-miR-103, rno-miR-103-1*
rno-mir-103-2	chr3: 118996602-118996687 [+]	rno-miR-103, rno-miR-103-2*
has-let-7a-1	chr9: 96938239-96938318 [+]	hsa-let-7a-5p, hsa-let-7a-3p
hsa-let-7b	chr22: 46509566-46509648 [+]	hsa-let-7b-5p, hsa-let-7b-3p

Search page



Search miRBase

By miRNA identifier or keyword

Enter a miRNA accession, name or keyword:

By genomic location

Select organism, chromosome and start and end coordinates. Leave the start/end boxes blank to retrieve all miRNAs on the selected chromosome.

Choose species: Chr: Start: End:

For clusters

Select organism and the desired inter-miRNA distance.

Choose species: Inter-miRNA distance: 10000

By tissue expression

Select organism and tissue.

Choose species: Select tissue:

By sequence

Single sequence searches:

Paste a sequence here to search against miRNA sequences. You can choose to search against the intact precursor sequences or just the mature miRNAs. This search may take a few minutes. **(Max size 1000bp's)**

Or: Select the sequence file you wish to use

Search sequences: Mature miRNAs

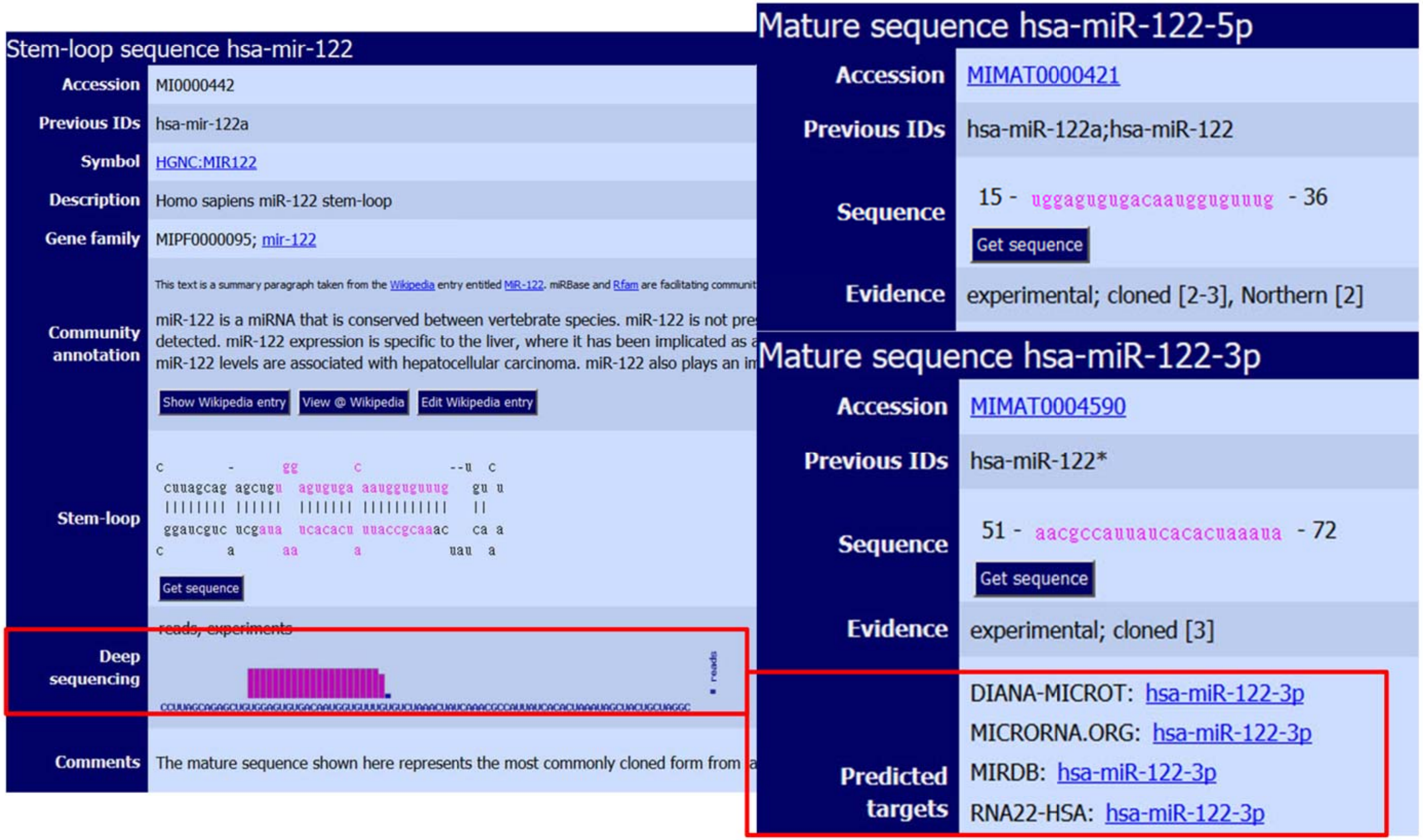
Search method: BLASTN

Choose BLASTN to search for a miRNA in a longer sequence. SSEARCH is useful for finding a short sequence within the library of miRNAs (for instance, find a short motif in a miRNA or precursor stem-loop, or find mature sequences that are related to your query).

E-value cutoff: 10

Maximum no. of hits: 100

hsa-mir-122



Stem-loop sequence hsa-mir-122

Accession	MI0000442
Previous IDs	hsa-mir-122a
Symbol	HGNC:MIR122
Description	Homo sapiens miR-122 stem-loop
Gene family	MIPF0000095; mir-122
Community annotation	This text is a summary paragraph taken from the Wikipedia entry entitled MiR-122. miRBase and Rfam are facilitating communit miR-122 is a miRNA that is conserved between vertebrate species. miR-122 is not pre detected. miR-122 expression is specific to the liver, where it has been implicated as a miR-122 levels are associated with hepatocellular carcinoma. miR-122 also plays an in
Stem-loop	
Deep sequencing	reads, experiments CCUUAGCAGAGCUGUGGAGUGUGACAAUGGUGUUUGUGUCUAAACUAUCAAACGCCAUUAUCACACUAAAUAGCUACUGCUAGGC
Comments	The mature sequence shown here represents the most commonly cloned form from a

Stem-loop

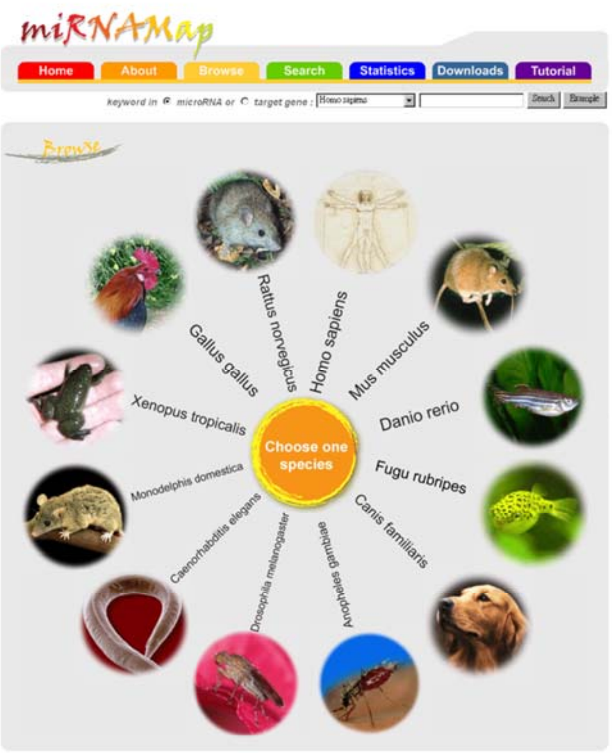
```
c        -      gg       c            --u  c
 cuuagcag agcugu  aguguga aauggguguuug   gu u
 |||||||| ||||||  ||||||| ||||||||||||   ||
 ggaucguc ucgaua  ucacacu uuaccgcaaac   ca a
c        a      aa       a            uau  a
```

Mature sequence hsa-miR-122-5p

Accession	MIMAT0000421
Previous IDs	hsa-miR-122a;hsa-miR-122
Sequence	15 - uggagugugacaaugguguuug - 36
Evidence	experimental; cloned [2-3], Northern [2]

Mature sequence hsa-miR-122-3p

Accession	MIMAT0004590
Previous IDs	hsa-miR-122*
Sequence	51 - aacgccauuaucacacuaaaua - 72
Evidence	experimental; cloned [3]
Predicted targets	DIANA-MICROT: hsa-miR-122-3p MICRORNA.ORG: hsa-miR-122-3p MIRDB: hsa-miR-122-3p RNA22-HSA: hsa-miR-122-3p



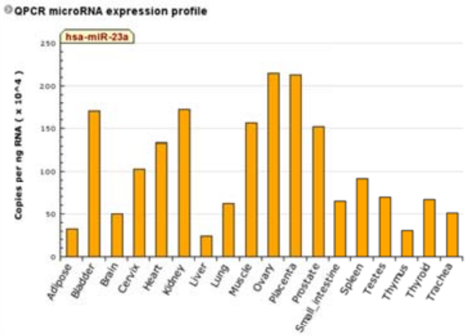
Known miRNAs

Known miRNA targets

Putative miRNA targets

miRNA expression profiles

Filtering criteria for putative miRNA targets



miRNAMap Database

Nucl Acids Res, Database Issue, 2006

Nucl Acids Res, Database Issue, 2008

An integrated resource to collect experimentally verified microRNAs and both known and putative miRNA target genes in human, mouse, rat and other metazoan genomes.

http://miRNAMap.mbc.nctu.edu.tw/

Homepage of miRNAMap 2.0

keyword in ● microRNA or ○ target gene : Homo sapiens

Welcome to miRNAMap!

What's new?

- More species
- More prediction tools
- Gene group search
- miRNA target accessibility
- faster and More comprehensive

Release 2.0 : July 2007

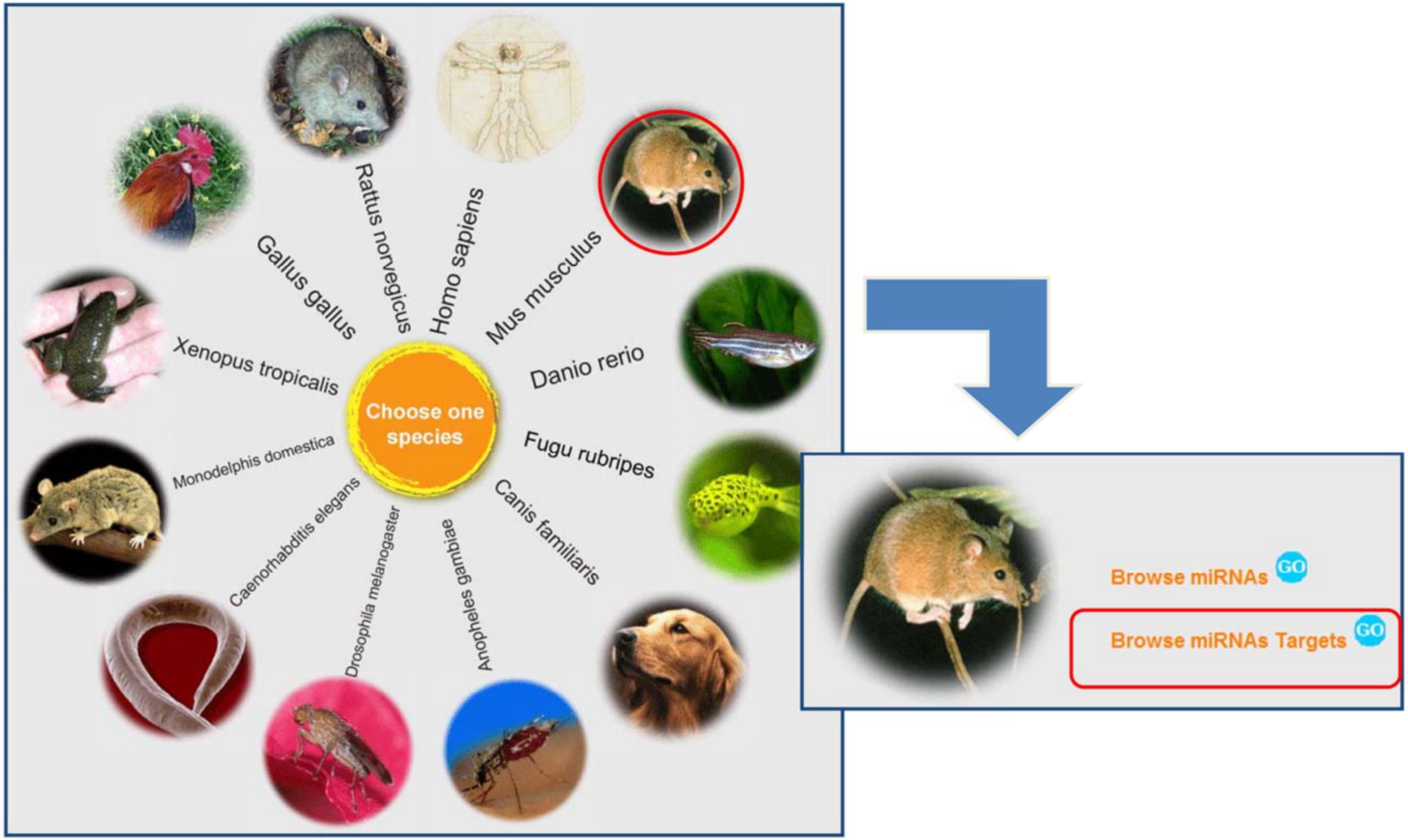
miRNAMap Previous version - miRNAMap 1.0

mature miRNA count:

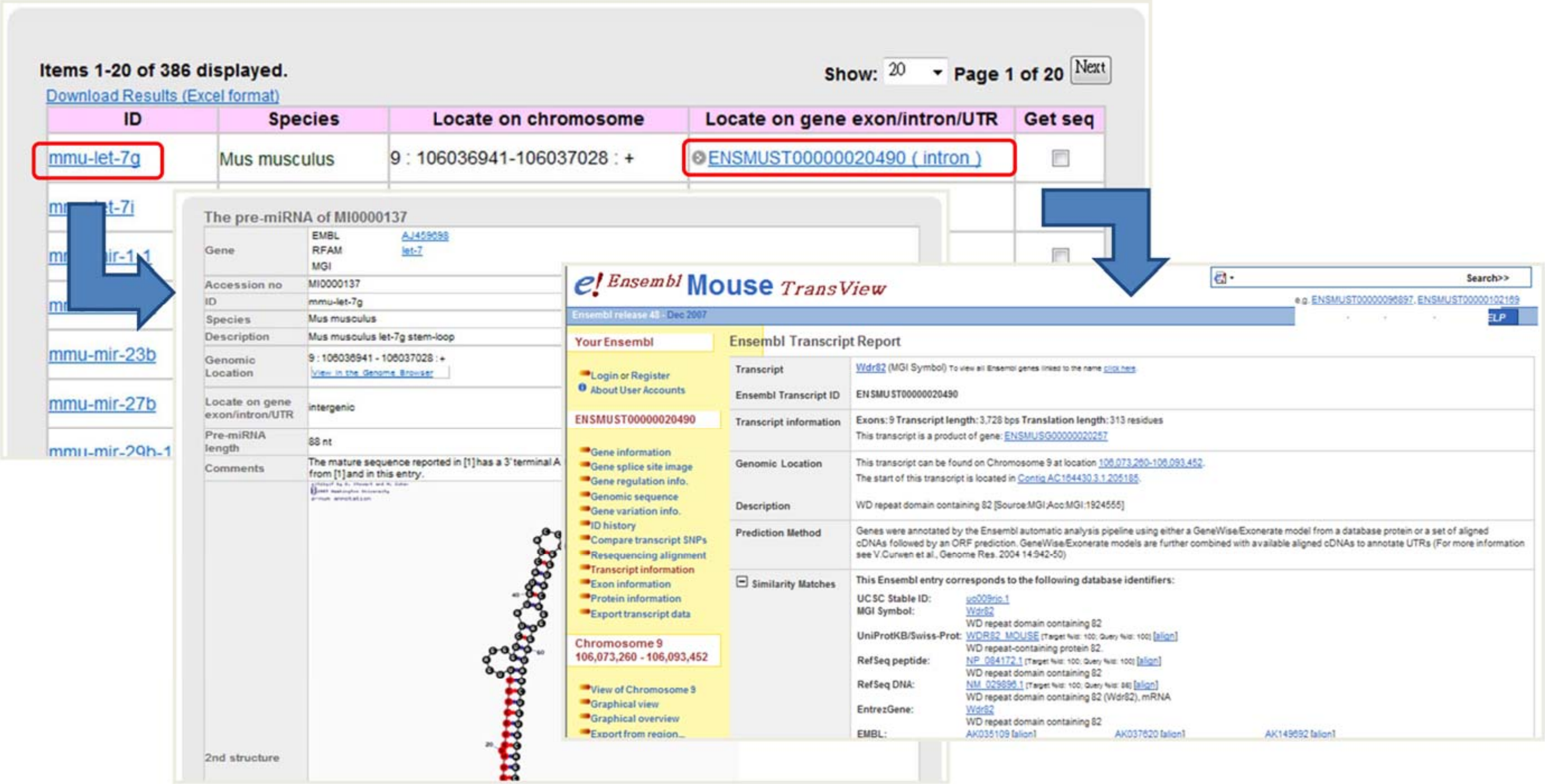
Homo sapiens	542
Mus musculus	424
Danio rerio	371
Rattus norvegicus	261
Xenopus tropicalis	196
Gallus gallus	162
Caenorhabditis elegans	135
Fugu rubripes	133
Monodelphis domestica	111
Drosophila melanogaster	85
Anopheles gambiae	38
Canis familiaris	6

Department of Biological Science and Technology,
Institute of Bioinformatics National Chiao Tung University, Hsinchu, Taiwan
Contact with: bryan@mail.NCTU.edu.tw

Web interface - Browse



Web interface - Browse



Items 1-20 of 386 displayed.

Download Results (Excel format)

ID	Species	Locate on chromosome	Locate on gene exon/intron/UTR	Get seq
mmu-let-7g	Mus musculus	9 : 106036941-106037028 : +	ENSMUST00000020490 (intron)	☐
mmu-mir-23b				
mmu-mir-27b				
mmu-mir-29b-1				

The pre-miRNA of MI0000137

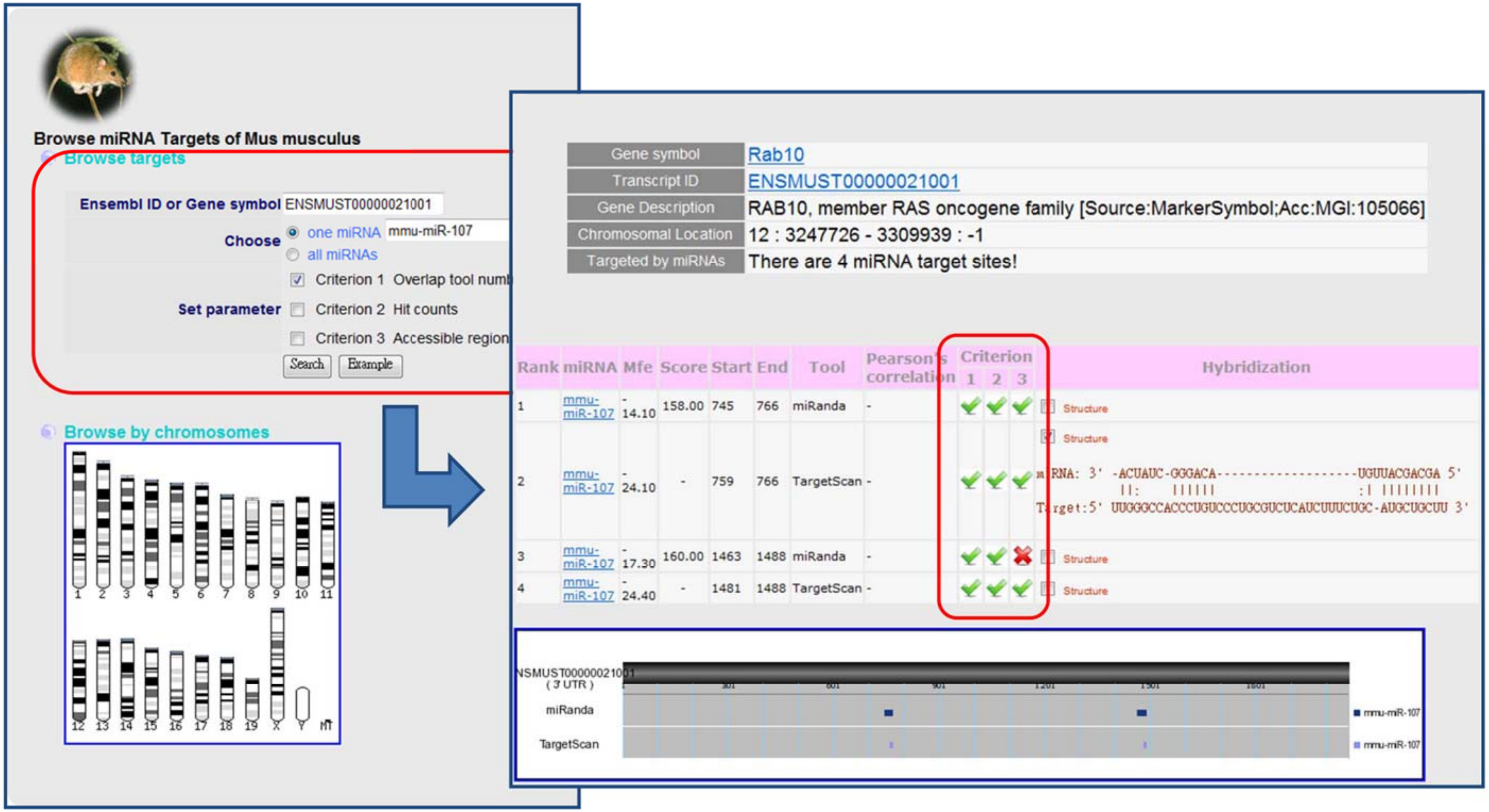
Gene	EMBL RFAM MGI	AJ459698 let-7
Accession no	MI0000137	
ID	mmu-let-7g	
Species	Mus musculus	
Description	Mus musculus let-7g stem-loop	
Genomic Location	9 : 106036941 - 106037028 : + View in the Genome Browser	
Locate on gene exon/intron/UTR	intergenic	
Pre-miRNA length	88 nt	
Comments	The mature sequence reported in [1] has a 3' terminal A from [1] and in this entry.	
2nd structure		

Ensembl Transcript Report

Transcript	Wdr82 (MGI Symbol) To view all Ensembl genes linked to the name click here.
Ensembl Transcript ID	ENSMUST00000020490
Transcript information	Exons: 9 Transcript length: 3,728 bps Translation length: 313 residues This transcript is a product of gene: ENSMUSG00000020257
Genomic Location	This transcript can be found on Chromosome 9 at location 106,073,260-106,093,452. The start of this transcript is located in Contig AC164430.3.1.205185.
Description	WD repeat domain containing 82 [Source:MGI;Acc:MGI:1924555]
Prediction Method	Genes were annotated by the Ensembl automatic analysis pipeline using either a GeneWise/Exonerate model from a database protein or a set of aligned cDNAs followed by an ORF prediction. GeneWise/Exonerate models are further combined with available aligned cDNAs to annotate UTRs (For more information see V.Curwen et al., Genome Res. 2004 14:942-50)
⊟ Similarity Matches	This Ensembl entry corresponds to the following database identifiers:

UCSC Stable ID:	uc009rio.1		
MGI Symbol:	Wdr82 WD repeat domain containing 82		
UniProtKB/Swiss-Prot:	WDR82_MOUSE [Target %id: 100; Query %id: 100] [align] WD repeat-containing protein 82.		
RefSeq peptide:	NP_084172.1 [Target %id: 100; Query %id: 100] [align] WD repeat domain containing 82		
RefSeq DNA:	NM_029896.1 [Target %id: 100; Query %id: 86] [align] WD repeat domain containing 82 (Wdr82), mRNA		
EntrezGene:	Wdr82 WD repeat domain containing 82		
EMBL:	AK035109 [align]	AK037620 [align]	AK149692 [align]

Web interface - Search



Browse miRNA Targets of Mus musculus

Browse targets

Ensembl ID or Gene symbol ENSMUST00000021001

Choose ● one miRNA mmu-miR-107 ○ all miRNAs

Set parameter
☑ Criterion 1 Overlap tool numb
☐ Criterion 2 Hit counts
☐ Criterion 3 Accessible region

Browse by chromosomes

Gene symbol	Rab10
Transcript ID	ENSMUST00000021001
Gene Description	RAB10, member RAS oncogene family [Source:MarkerSymbol;Acc:MGI:105066]
Chromosomal Location	12 : 3247726 - 3309939 : -1
Targeted by miRNAs	There are 4 miRNA target sites!

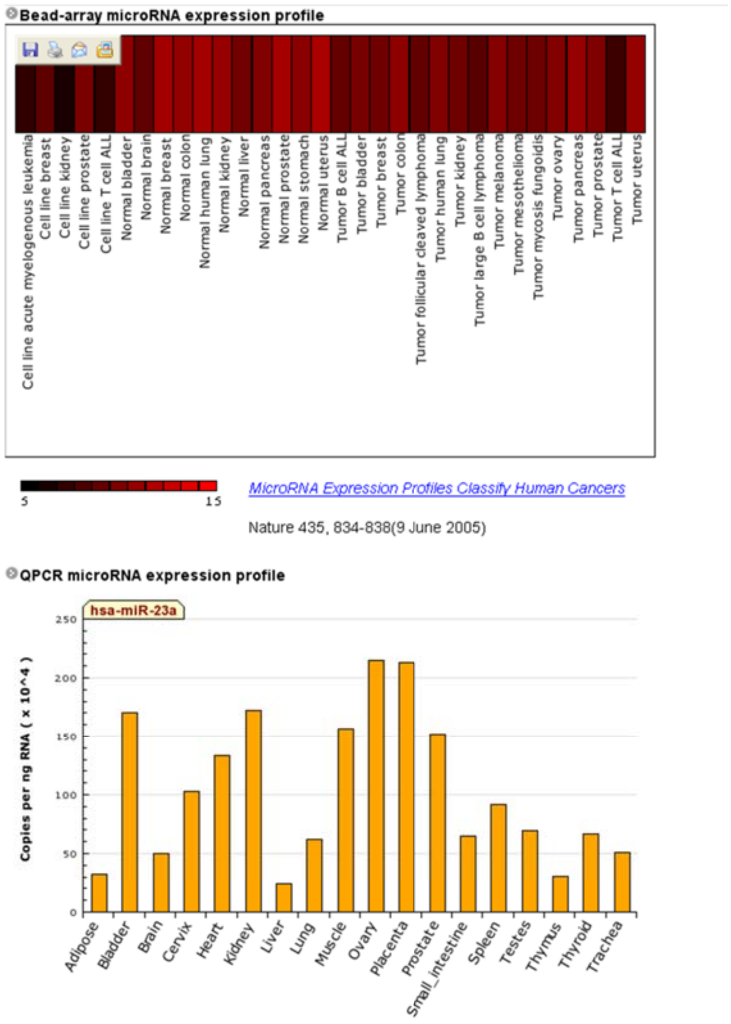
Rank	miRNA	Mfe	Score	Start	End	Tool	Pearson's correlation	Criterion 1	Criterion 2	Criterion 3	Hybridization
1	mmu-miR-107	-14.10	158.00	745	766	miRanda	-	✓	✓	✓	☐ Structure
2	mmu-miR-107	-24.10	-	759	766	TargetScan	-	✓	✓	✓	☑ Structure
3	mmu-miR-107	-17.30	160.00	1463	1488	miRanda	-	✓	✓	✗	☐ Structure
4	mmu-miR-107	-24.40	-	1481	1488	TargetScan	-	✓	✓	✓	☐ Structure

```
m RNA: 3' -ACUAUC-GGGACA------------------UGUUACGACGA 5'
            ||:    ||||||                  :| ||||||||
T rget:5' UUGGGCCACCCUGUCCCUGCGUCUCAUCUUUCUGC-AUGCUGCUU 3'
```

miRNA expression profiles

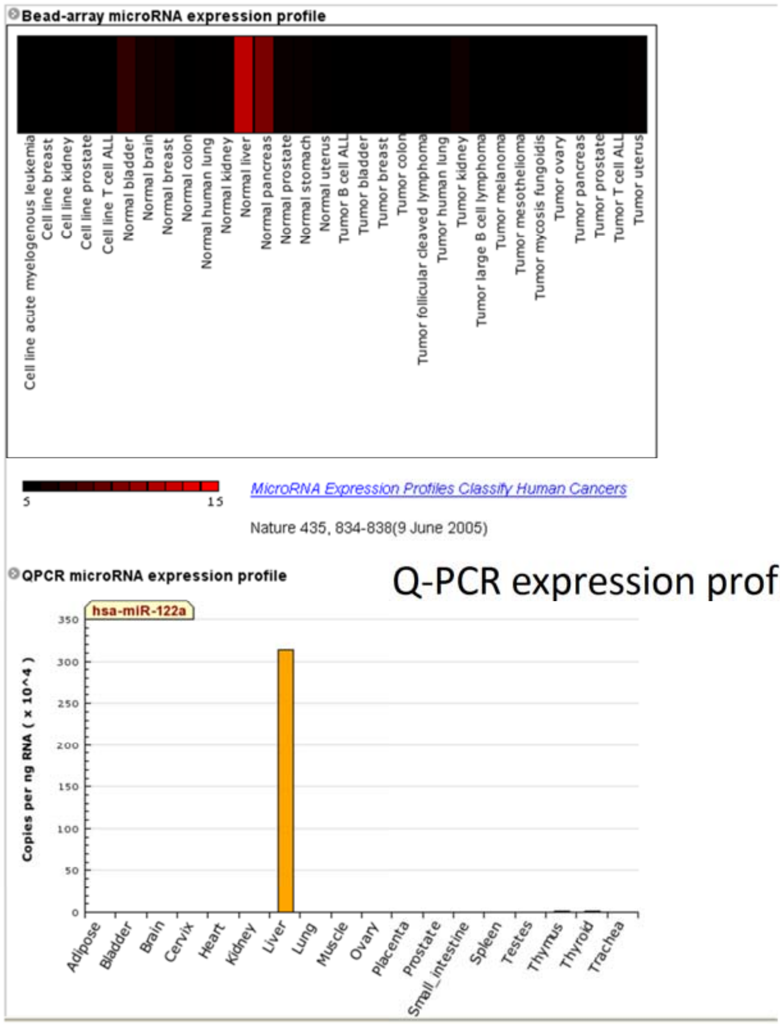
hsa-miR-23a

hsa-miR-122a (liver-specific miRNA)



MicroRNA Expression Profiles Classify Human Cancers

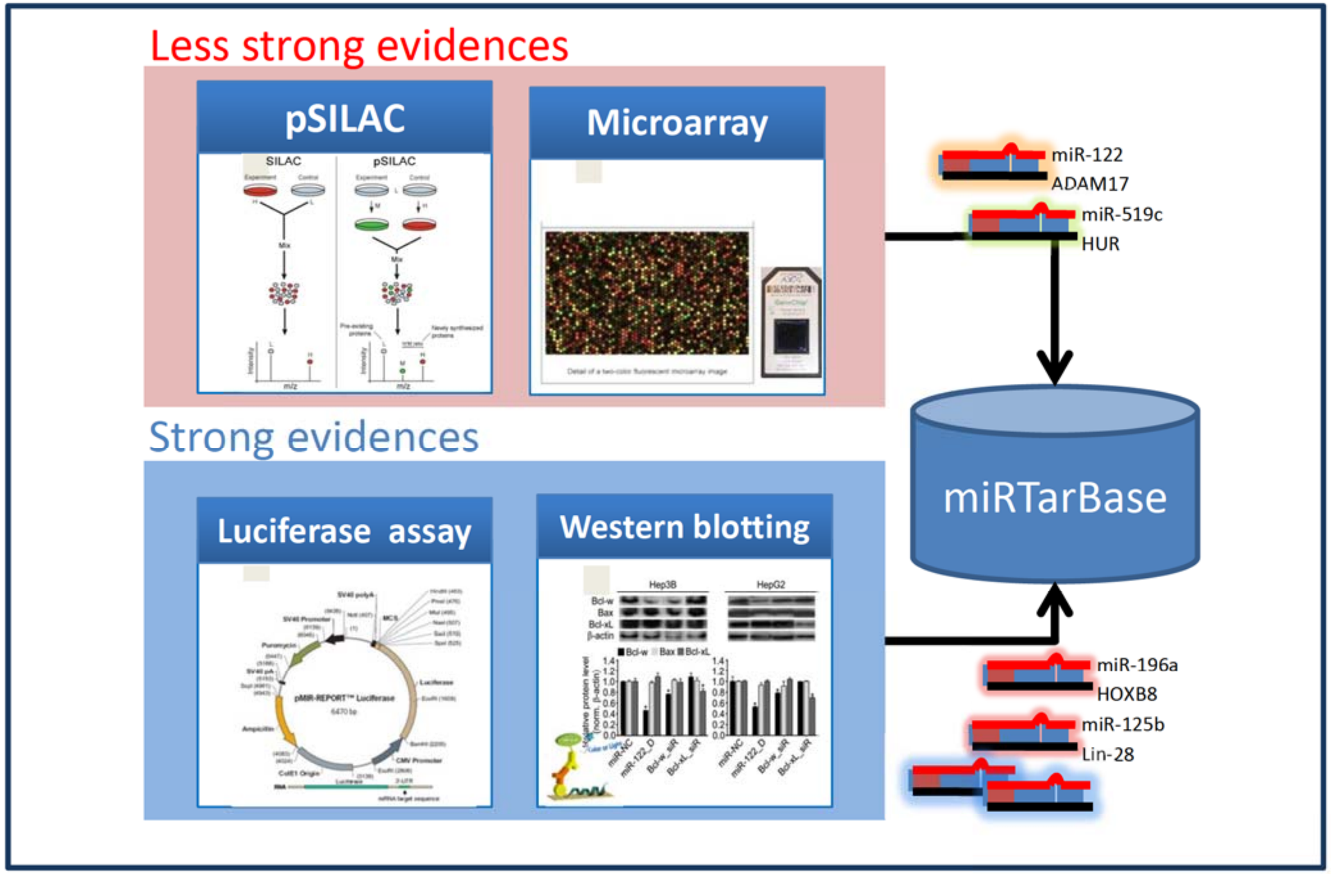
Nature 435, 834-838(9 June 2005)



MicroRNA Expression Profiles Classify Human Cancers

Nature 435, 834-838(9 June 2005)

Q-PCR expression profiles



Less strong evidences

pSILAC

Microarray

Strong evidences

Luciferase assay

Western blotting

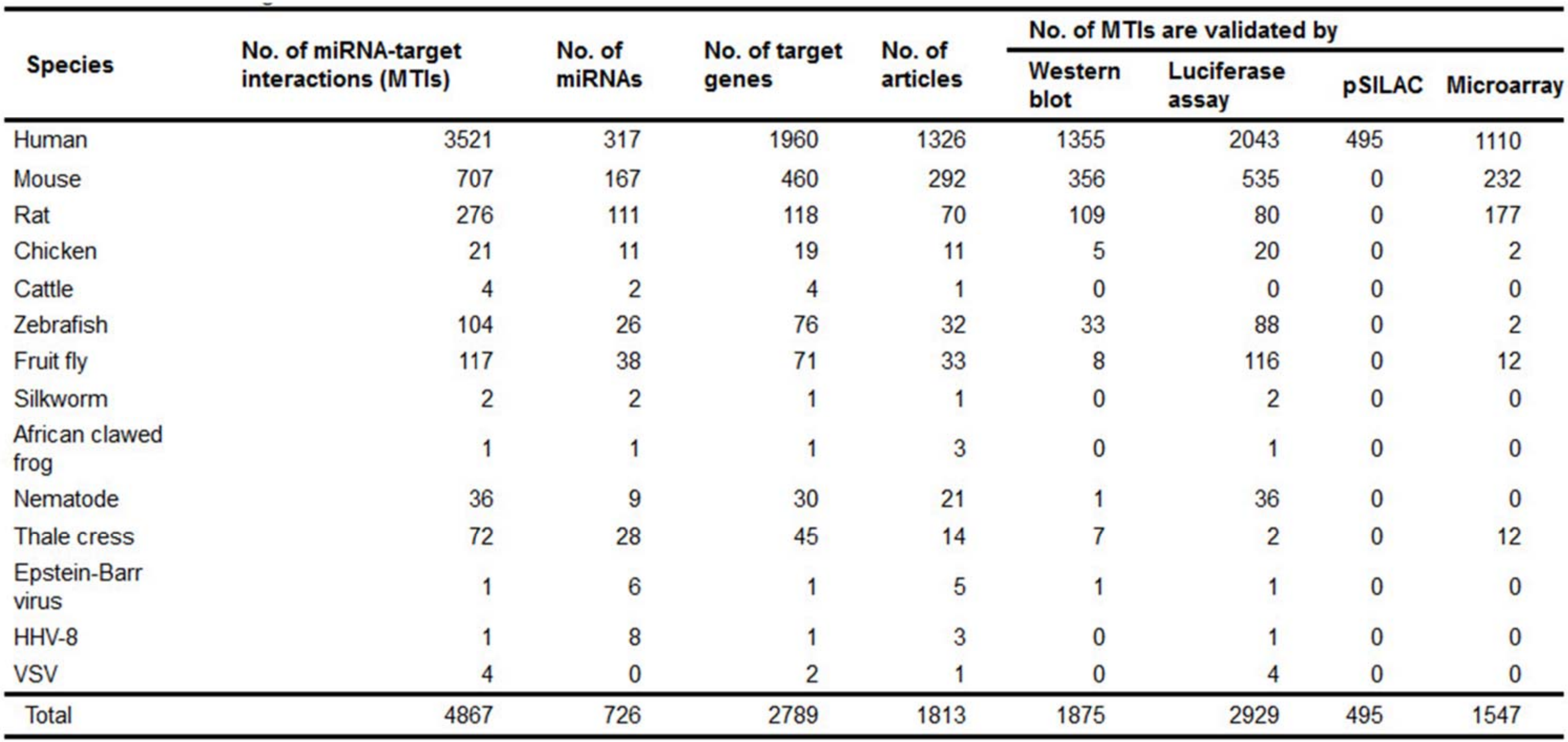
miRTarBase

miRTarBase

A database curates experimentally validated microRNA-target interactions

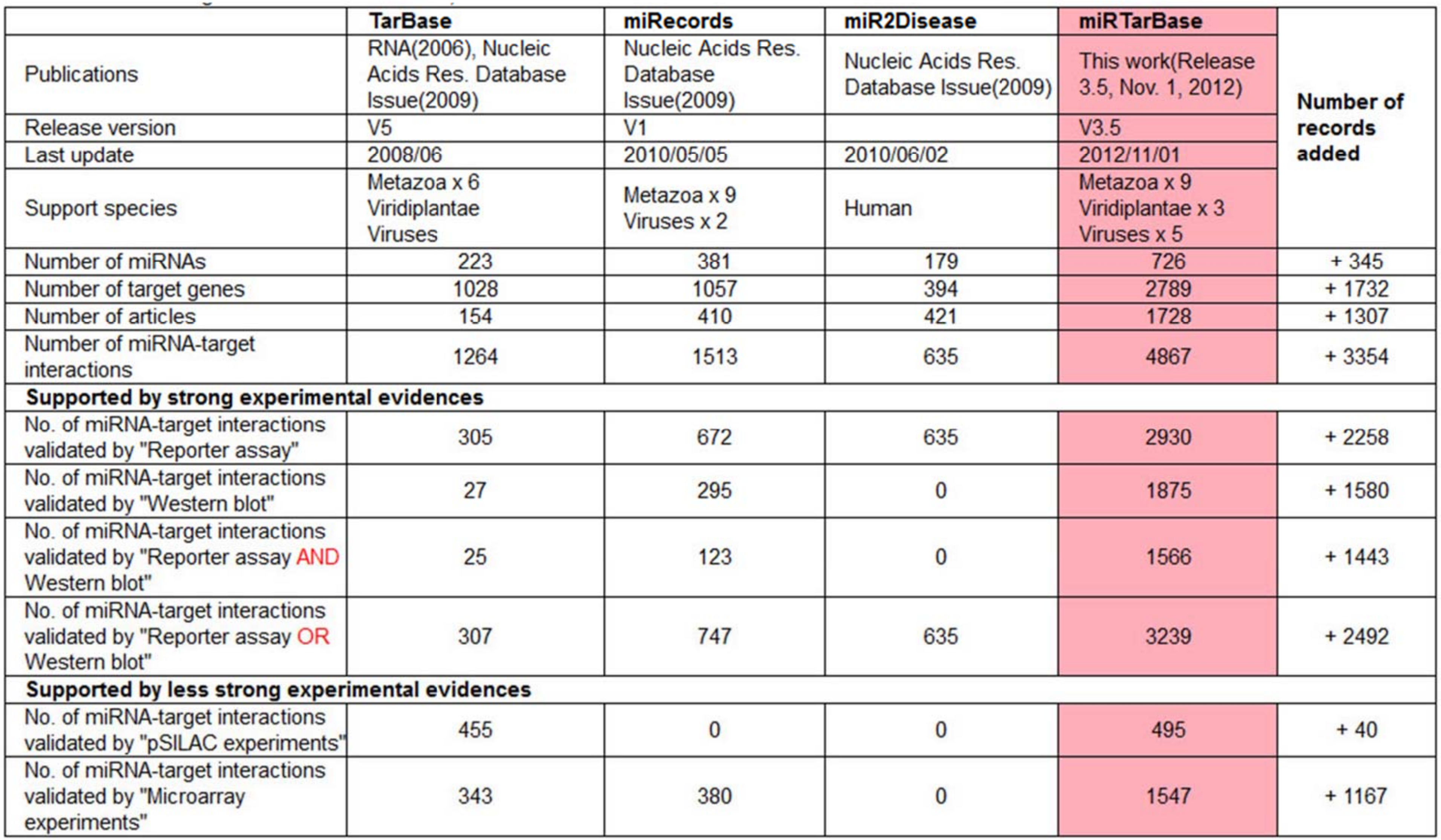
http://miRTarBase.mbc.nctu.edu.tw/

The statistics of miRTarBase



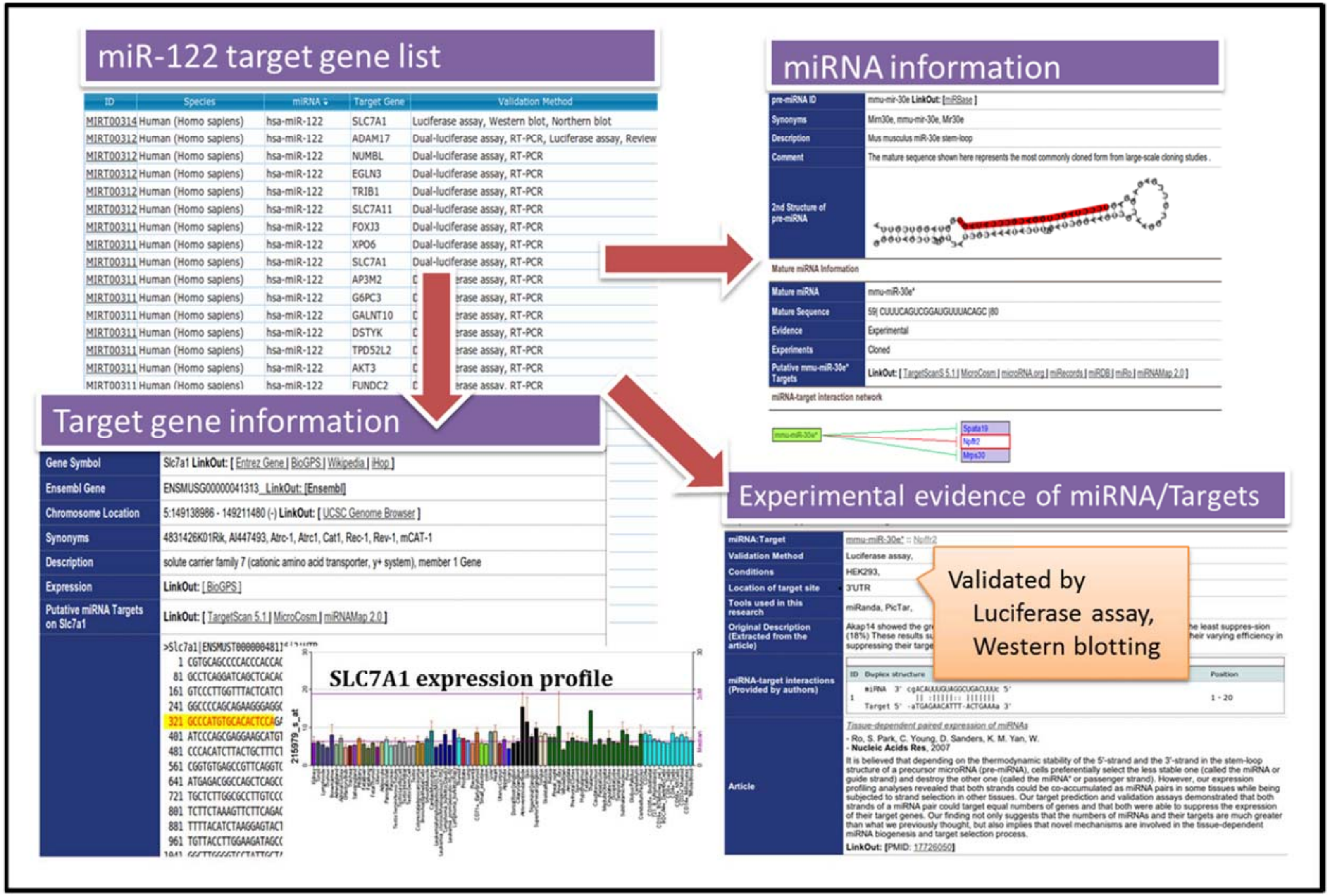
Species	No. of miRNA-target interactions (MTIs)	No. of miRNAs	No. of target genes	No. of articles	No. of MTIs are validated by			
					Western blot	Luciferase assay	pSILAC	Microarray
Human	3521	317	1960	1326	1355	2043	495	1110
Mouse	707	167	460	292	356	535	0	232
Rat	276	111	118	70	109	80	0	177
Chicken	21	11	19	11	5	20	0	2
Cattle	4	2	4	1	0	0	0	0
Zebrafish	104	26	76	32	33	88	0	2
Fruit fly	117	38	71	33	8	116	0	12
Silkworm	2	2	1	1	0	2	0	0
African clawed frog	1	1	1	3	0	1	0	0
Nematode	36	9	30	21	1	36	0	0
Thale cress	72	28	45	14	7	2	0	12
Epstein-Barr virus	1	6	1	5	1	1	0	0
HHV-8	1	8	1	3	0	1	0	0
VSV	4	0	2	1	0	4	0	0
Total	4867	726	2789	1813	1875	2929	495	1547

Comparison of miRTarBase with other MTI databases



	TarBase	**miRecords**	**miR2Disease**	**miRTarBase**	**Number of records added**
Publications	RNA(2006), Nucleic Acids Res. Database Issue(2009)	Nucleic Acids Res. Database Issue(2009)	Nucleic Acids Res. Database Issue(2009)	This work(Release 3.5, Nov. 1, 2012)	
Release version	V5	V1		V3.5	
Last update	2008/06	2010/05/05	2010/06/02	2012/11/01	
Support species	Metazoa x 6 Viridiplantae Viruses	Metazoa x 9 Viruses x 2	Human	Metazoa x 9 Viridiplantae x 3 Viruses x 5	
Number of miRNAs	223	381	179	726	+ 345
Number of target genes	1028	1057	394	2789	+ 1732
Number of articles	154	410	421	1728	+ 1307
Number of miRNA-target interactions	1264	1513	635	4867	+ 3354
Supported by strong experimental evidences					
No. of miRNA-target interactions validated by "Reporter assay"	305	672	635	2930	+ 2258
No. of miRNA-target interactions validated by "Western blot"	27	295	0	1875	+ 1580
No. of miRNA-target interactions validated by "Reporter assay AND Western blot"	25	123	0	1566	+ 1443
No. of miRNA-target interactions validated by "Reporter assay OR Western blot"	307	747	635	3239	+ 2492
Supported by less strong experimental evidences					
No. of miRNA-target interactions validated by "pSILAC experiments"	455	0	0	495	+ 40
No. of miRNA-target interactions validated by "Microarray experiments"	343	380	0	1547	+ 1167

Experimentally verified miR-122 targets



miR-122 target gene list

ID	Species	miRNA	Target Gene	Validation Method
MIRT00314	Human (Homo sapiens)	hsa-miR-122	SLC7A1	Luciferase assay, Western blot, Northern blot
MIRT00312	Human (Homo sapiens)	hsa-miR-122	ADAM17	Dual-luciferase assay, RT-PCR, Luciferase assay, Review
MIRT00312	Human (Homo sapiens)	hsa-miR-122	NUMBL	Dual-luciferase assay, RT-PCR
MIRT00312	Human (Homo sapiens)	hsa-miR-122	EGLN3	Dual-luciferase assay, RT-PCR
MIRT00312	Human (Homo sapiens)	hsa-miR-122	TRIB1	Dual-luciferase assay, RT-PCR
MIRT00312	Human (Homo sapiens)	hsa-miR-122	SLC7A11	Dual-luciferase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	FOXJ3	Dual-luciferase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	XPO6	Dual-luciferase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	SLC7A1	Dual-luciferase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	AP3M2	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	G6PC3	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	GALNT10	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	DSTYK	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	TPD52L2	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	AKT3	D erase assay, RT-PCR
MIRT00311	Human (Homo sapiens)	hsa-miR-122	FUNDC2	D erase assay, RT-PCR

miRNA information

pre-miRNA ID	mmu-mir-30e **LinkOut:** [miRBase]
Synonyms	Mirn30e, mmu-mir-30e, Mir30e
Description	Mus musculus miR-30e stem-loop
Comment	The mature sequence shown here represents the most commonly cloned form from large-scale cloning studies .
2nd Structure of pre-miRNA	

Mature miRNA Information

Mature miRNA	mmu-miR-30e*
Mature Sequence	59| CUUUCAGUCGGAUGUUUACAGC |80
Evidence	Experimental
Experiments	Cloned
Putative mmu-miR-30e* Targets	**LinkOut:** [TargetScanS 5.1 | MicroCosm | microRNA.org | miRecords | miRDB | miRo | miRNAMap 2.0]

miRNA-target interaction network

Target gene information

Gene Symbol	Slc7a1 **LinkOut:** [Entrez Gene | BioGPS | Wikipedia | iHop]
Ensembl Gene	ENSMUSG00000041313 LinkOut: [Ensembl]
Chromosome Location	5:149138986 - 149211480 (-) **LinkOut:** [UCSC Genome Browser]
Synonyms	4831426K01Rik, AI447493, Atrc-1, Atrc1, Cat1, Rec-1, Rev-1, mCAT-1
Description	solute carrier family 7 (cationic amino acid transporter, y+ system), member 1 Gene
Expression	**LinkOut:** [BioGPS]
Putative miRNA Targets on Slc7a1	**LinkOut:** [TargetScan 5.1 | MicroCosm | miRNAMap 2.0]

```
>Slc7a1|ENSMUST00000048111
   1 CGTGCAGCCCCACCCACCA
  81 GCCTCAGGATCAGCTCACA
 161 GTCCCTTGGTTTACTCATC
 241 GGCCCCAGCAGAAGGGAGG
 321 GCCCATGTGCACACTCCAG
 401 ATCCCAGCGAGGAAGCATG
 481 CCCACATCTTACTGCTTTC
 561 CGGTGTGAGCCGTTCAGGT
 641 ATGAGACGGCCAGCTCAGC
 721 TGCTCTTGGCGCCTTGTCC
 801 TCTTCTAAAGTTCTTCAGA
 881 TTTTACATCTAAGGAGTAC
 961 TGTTACCTTGGAAGATAGC
```

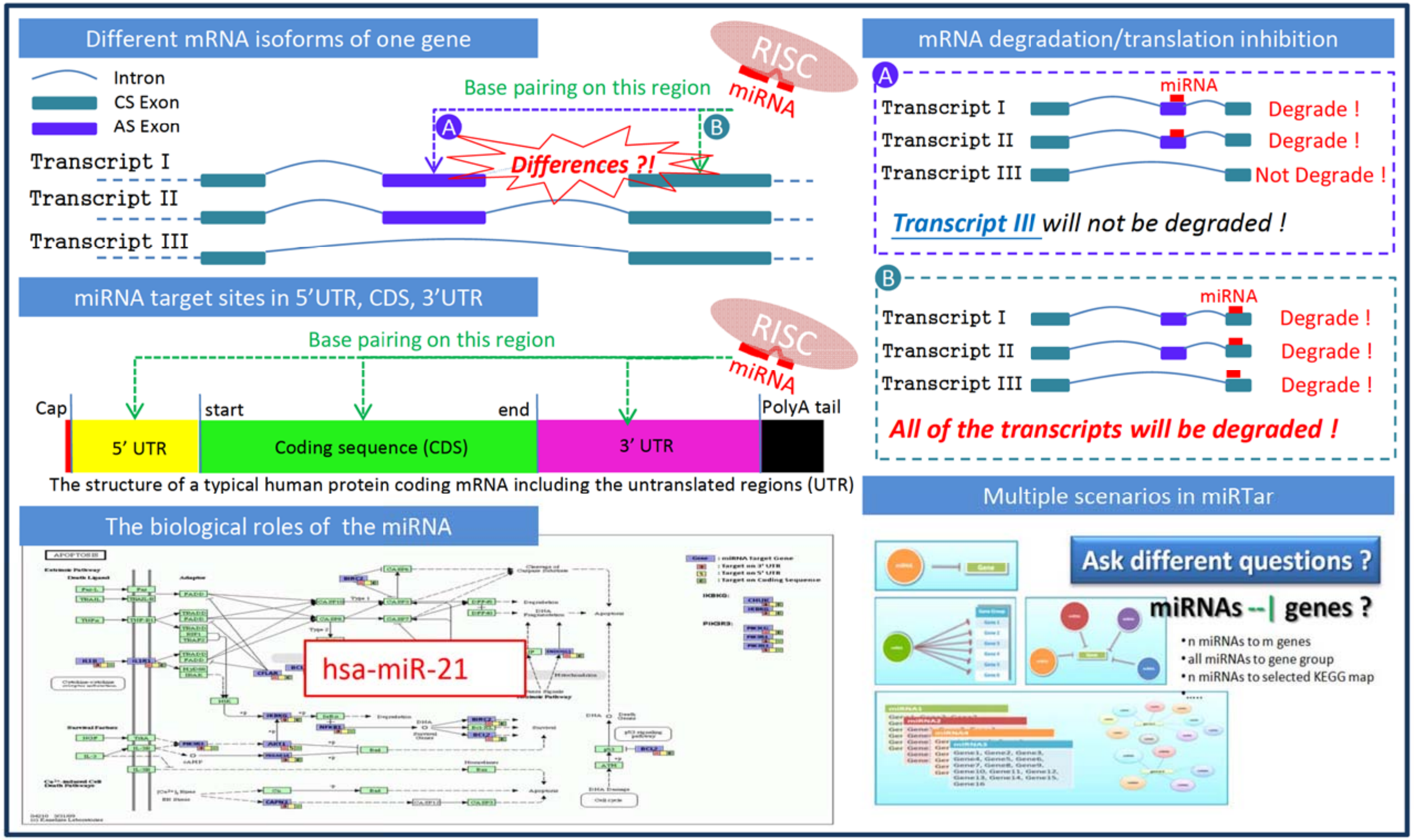
Experimental evidence of miRNA/Targets

miRNA:Target	mmu-miR-30e* :: Npffr2
Validation Method	Luciferase assay,
Conditions	HEK293,
Location of target site	3'UTR
Tools used in this research	miRanda, PicTar,
Original Description (Extracted from the article)	Akap14 showed the gr … he least suppres-sion (18%) These results su … heir varying efficiency in suppressing their targe
miRNA-target interactions (Provided by authors)	ID 1; Duplex structure: miRNA 3' cgACAUUUGUAGGCUGACUUUc 5' / Target 5' -aTGAGAACATTT-ACTGAAAa 3'; Position 1 - 20
Article	*Tissue-dependent paired expression of miRNAs* - Ro, S. Park, C. Young, D. Sanders, K. M. Yan, W. - **Nucleic Acids Res**, 2007 It is believed that depending on the thermodynamic stability of the 5'-strand and the 3'-strand in the stem-loop structure of a precursor microRNA (pre-miRNA), cells preferentially select the less stable one (called the miRNA or guide strand) and destroy the other one (called the miRNA* or passenger strand). However, our expression profiling analyses revealed that both strands could be co-accumulated as miRNA pairs in some tissues while being subjected to strand selection in other tissues. Our target prediction and validation assays demonstrated that both strands of a miRNA pair could target equal numbers of genes and that both were able to suppress the expression of their target genes. Our finding not only suggests that the numbers of miRNAs and their targets are much greater than what we previously thought, but also implies that novel mechanisms are involved in the tissue-dependent miRNA biogenesis and target selection process. **LinkOut:** [PMID: 17726050]

Validated by
Luciferase assay,
Western blotting

Tools for identifying miRNA-target interactions

-miRTar
-miRTarCLIP
-miRGator
-TargetScan
-microRNA.org



Different mRNA isoforms of one gene

miRNA target sites in 5'UTR, CDS, 3'UTR

The structure of a typical human protein coding mRNA including the untranslated regions (UTR)

The biological roles of the miRNA

mRNA degradation/translation inhibition

Transcript III will not be degraded !

All of the transcripts will be degraded !

Multiple scenarios in miRTar

Ask different questions ?

miRNAs --| genes ?

- n miRNAs to m genes
- all miRNAs to gene group
- n miRNAs to selected KEGG map

miRTar

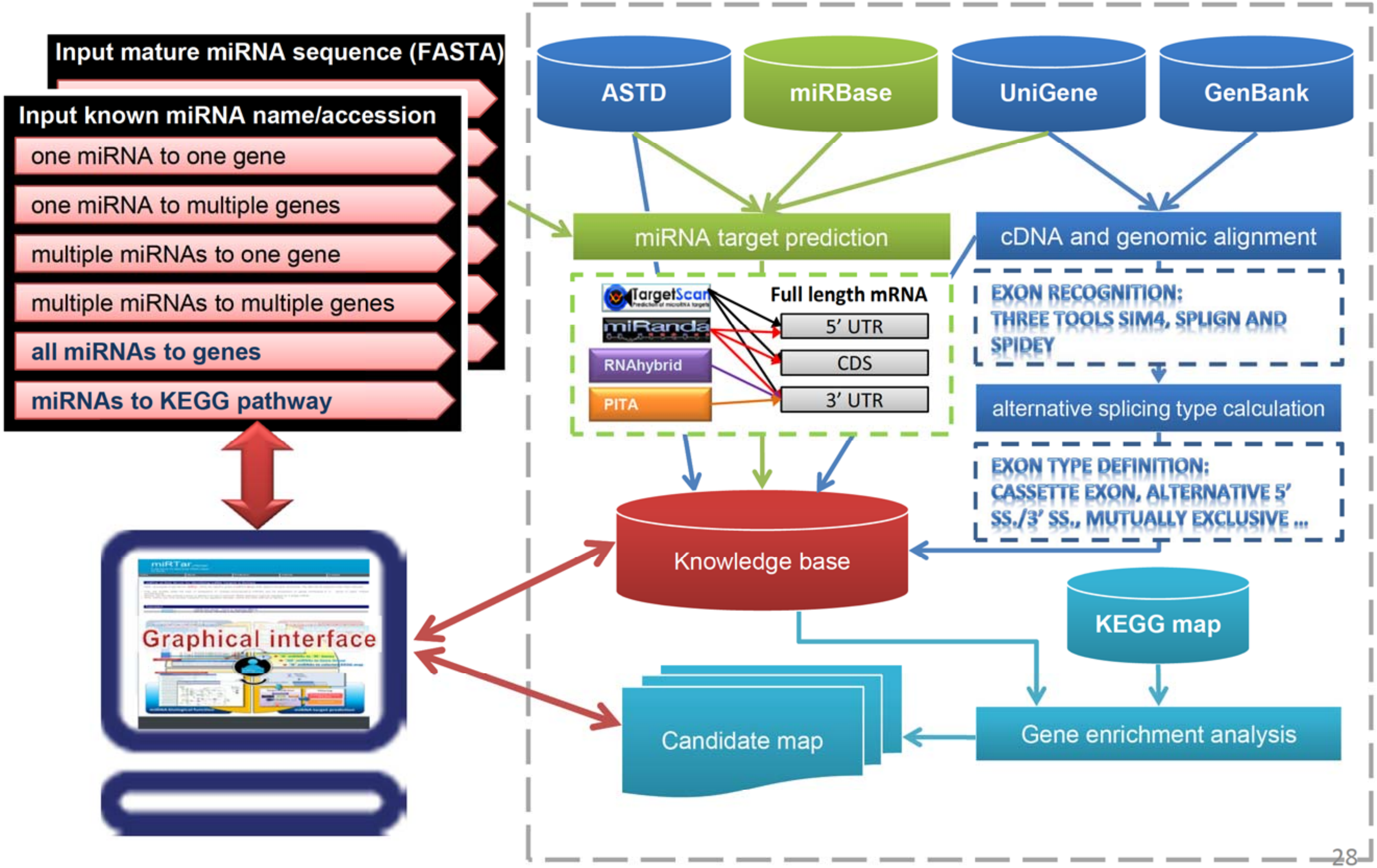
A web server for identifying miRNA targets for human

miRTar.Human
A web server for identifying miRNA targets for human

http://mirtar.mbc.nctu.edu.tw

BMC Bioinformatics, 2011

The system flow of miRTar



Input mature miRNA sequence (FASTA)

Input known miRNA name/accession

- one miRNA to one gene
- one miRNA to multiple genes
- multiple miRNAs to one gene
- multiple miRNAs to multiple genes
- **all miRNAs to genes**
- **miRNAs to KEGG pathway**

Graphical interface

ASTD

miRBase

UniGene

GenBank

miRNA target prediction

TargetScan

miRanda

RNAhybrid

PITA

Full length mRNA

5′ UTR

CDS

3′ UTR

cDNA and genomic alignment

EXON RECOGNITION:
THREE TOOLS SIM4, SPLIGN AND SPIDEY

alternative splicing type calculation

EXON TYPE DEFINITION:
CASSETTE EXON, ALTERNATIVE 5′ SS./3′ SS., MUTUALLY EXCLUSIVE ...

Knowledge base

KEGG map

Gene enrichment analysis

Candidate map

Multiple scenarios of miRTar

- Single miRNA to single gene (1:1)
- Single miRNA to multiple genes (1:N)
- Multiple miRNAs to single gene (N:1)
- Multiple miRNAs to multiple genes (N:M)
  - The miRNAs in one of the pathway
  - All miRNAs to one/multiple genes

The biological roles of miRNA in metabolic pathway

- An interesting gene group will be targeted by a single miRNA
- These genes will be involved in different metabolic pathway
- Using gene enrichment analysis of metabolic pathway

Sort by P-Value

Metabolic Pathway

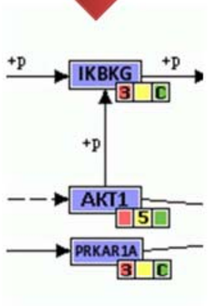
miRNA: hsa-miR-21

S/N	Title [ID]	P-Value	#matched genes	#genes in pathway
1	Apoptosis - Homo sapiens (human)[hsa04210] ENDOGL1; TRAF2; PRKAR1A; PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IL1R1; IL1B; IKBKG; EXOG; ENDOGL1; CHUK; CFLAR; CAPN2; BIRC2; BCL2; AKT3; AKT1	0.00000e-1	20	89
2	Small cell lung cancer - Homo sapiens (human)[hsa05222] TRAF2; PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BIRC2; BCL2; AKT3; AKT1	1.18578e-21	13	86
3	Acute myeloid leukemia - Homo sapiens (human)[hsa05221] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1	2.41585e-17	10	59
4	Prostate cancer - Homo sapiens (human)[hsa05215] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BCL2; AKT3; AKT1	2.86204e-17	11	88
5	Pancreatic cancer - Homo sapiens (human)[hsa05212] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BCL2; AKT3; AKT1	3.30356e-17	11	89
6	Toll-like receptor signaling pathway - Homo sapiens (human)[hsa04620] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IL1B; IKBKG; CHUK; AKT3; AKT1	1.84709e-16	11	102
7	B cell receptor signaling pathway - Homo sapiens (human)[hsa04662] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1	4.12520e-16	10	75
8	Chronic myeloid leukemia - Homo sapiens (human)[hsa05220] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1	4.12520e-16	10	75
9	T cell receptor signaling pathway - Homo sapiens (human)[hsa04660] PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; AKT3; AKT1	3.40123e-14	10	110
10	Pathways in cancer - Homo sapiens (human)[hsa05200] TRAF2; PIK3R5; PIK3R3; PIK3R2; PIK3R1; PIK3CG; NFKB1; IKBKG; CHUK; BIRC2; BCL2; AKT3; AKT1	3.50114e-13	13	332

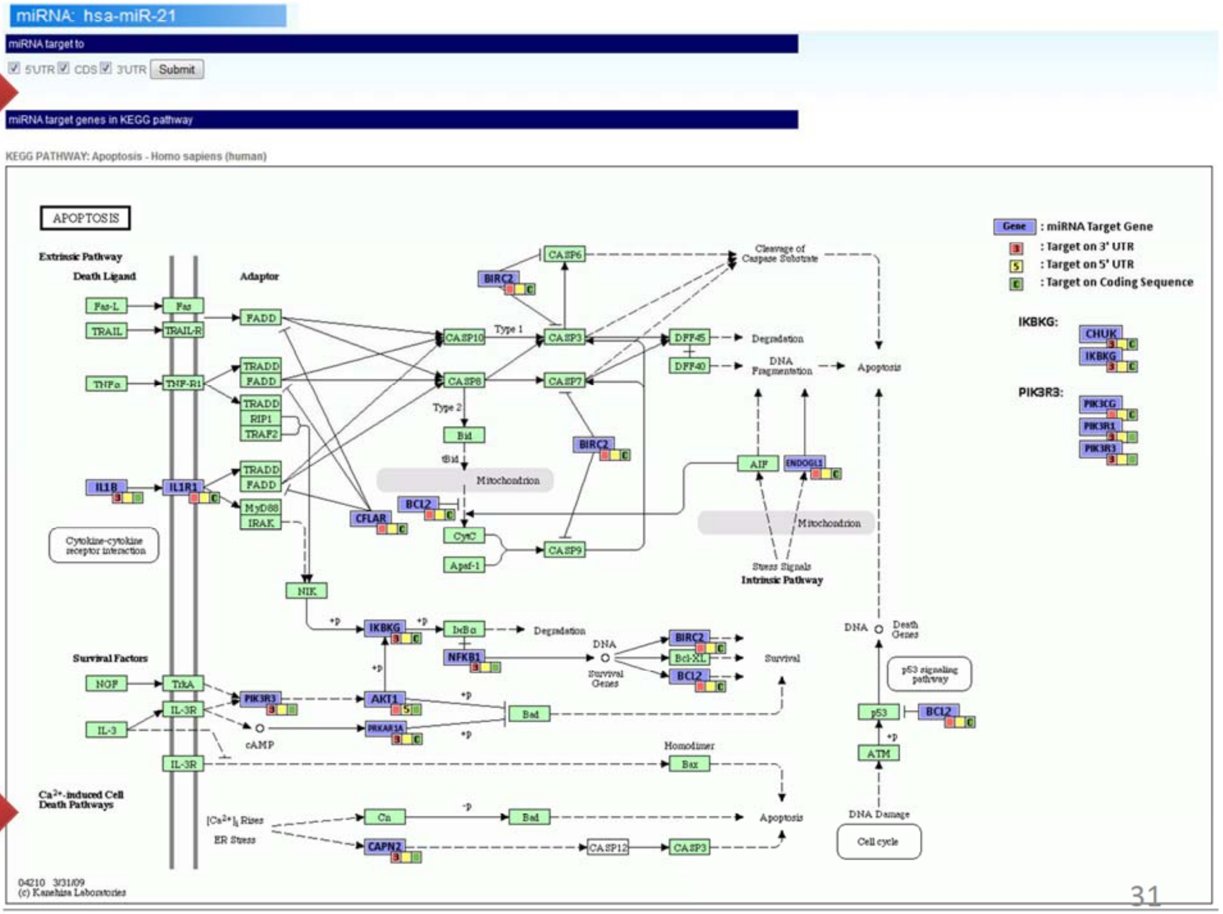
The biological roles of miR-21 in metabolic pathway

- hsa-miR-21 might target in 5’UTR, CDS or 3’UTR of genes’ transcripts.

Focus on different target region of the transcripts



hsa-miR-21 target genes in KEGG Map


miRNA: hsa-miR-21

miRNA target to

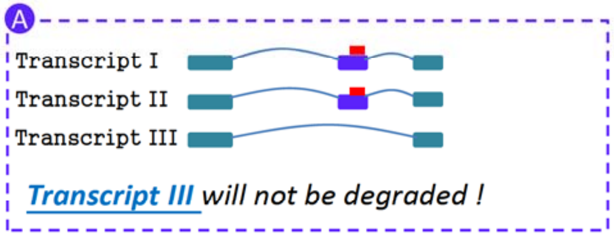
☑ 5'UTR ☑ CDS ☑ 3'UTR Submit

miRNA target genes in KEGG pathway

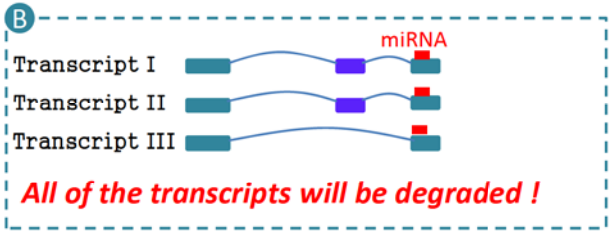
KEGG PATHWAY: Apoptosis - Homo sapiens (human)

miRNA target on alternatively spliced exons

Assumption: one gene has three different transcripts

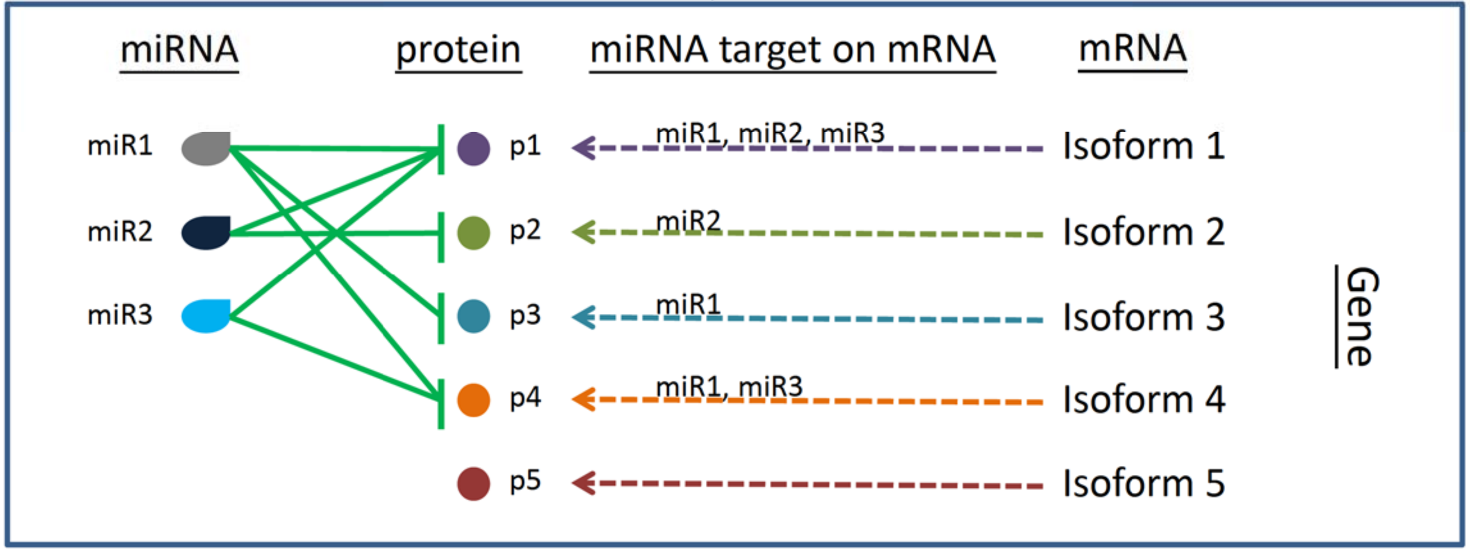


Transcript III *will not be degraded !*



All of the transcripts will be degraded !

- miRNA base pairing on the purple exon
  - Transcript I and II will degradation/translation inhibition
  - Transcript III will produce the protein product
- miRNA base pairing on the green exon
  - None protein products will produce from the gene

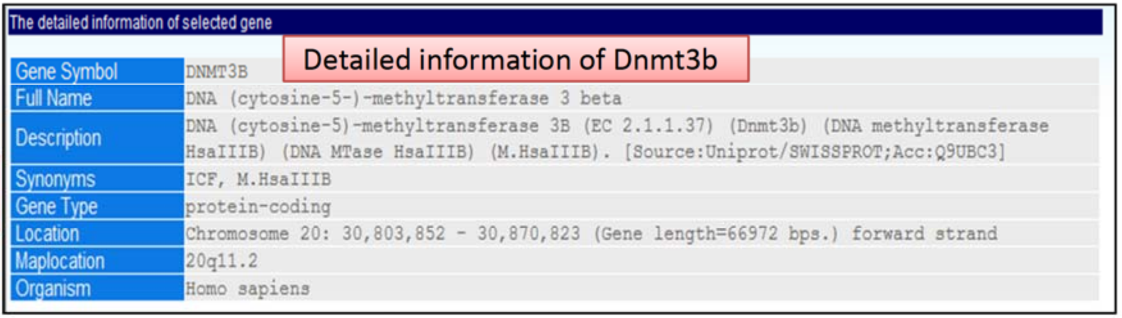


Case study of miRNA:target duplex on alternatively spliced exon

Dnmt3b will be regulated by hsa-miR-148a

• The detailed gene or miRNA information please click gene symbol or miRNA name, respectively.

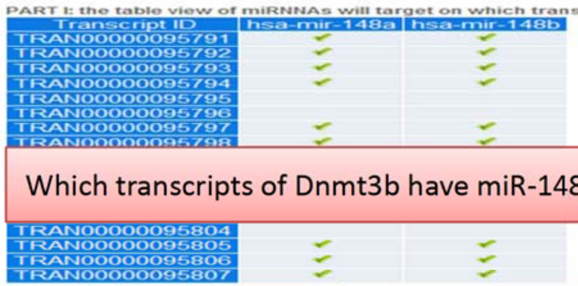
hsa-miR-148 indeed will base pairing on the transcripts of Dnmt3b



The detailed information of selected gene

Gene Symbol	DNMT3B
Full Name	DNA (cytosine-5-)-methyltransferase 3 beta
Description	DNA (cytosine-5)-methyltransferase 3B (EC 2.1.1.37) (Dnmt3b) (DNA methyltransferase HsaIIIB) (DNA MTase HsaIIIB) (M.HsaIIIB). [Source:Uniprot/SWISSPROT;Acc:Q9UBC3]
Synonyms	ICF, M.HsaIIIB
Gene Type	protein-coding
Location	Chromosome 20: 30,803,852 - 30,870,823 (Gene length=66972 bps.) forward strand
Maplocation	20q11.2
Organism	Homo sapiens

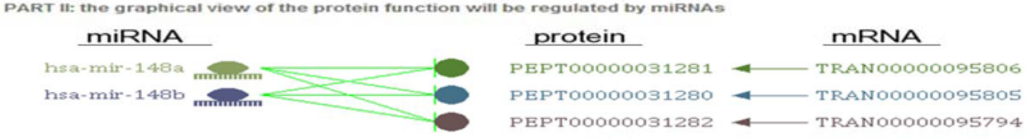
Detailed information of Dnmt3b



PART I: the table view of miRNNAs will target on which transcripts

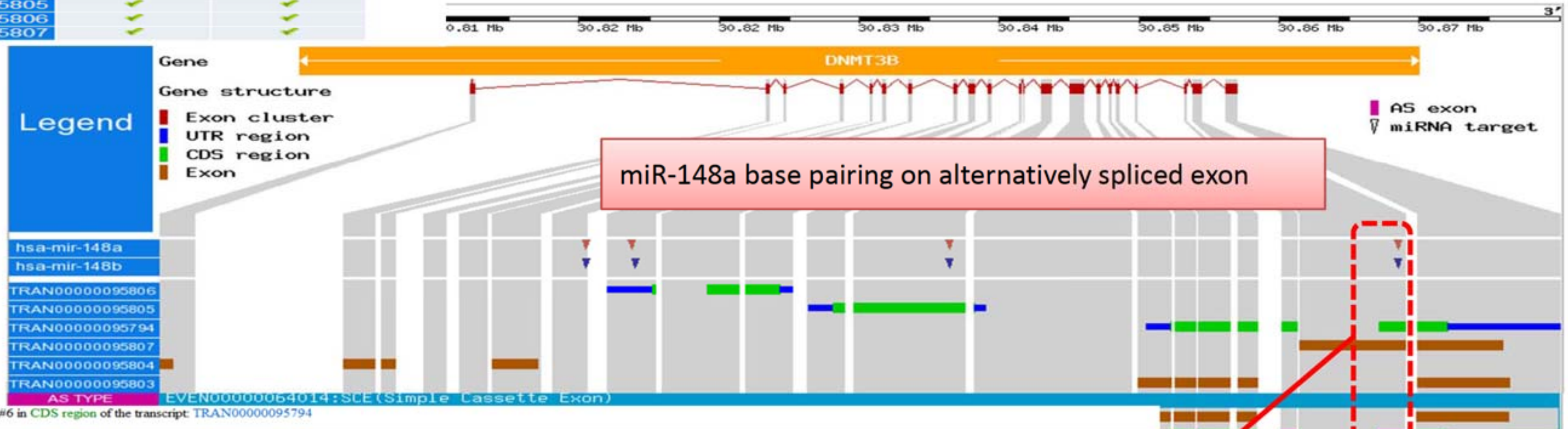
Transcript ID	hsa-mir-148a	hsa-mir-148b
TRAN00000095791	✓	✓
TRAN00000095792	✓	✓
TRAN00000095793	✓	✓
TRAN00000095794	✓	✓
TRAN00000095795		
TRAN00000095796		
TRAN00000095797	✓	✓
TRAN00000095798	✓	✓
TRAN00000095804		
TRAN00000095805	✓	✓
TRAN00000095806	✓	✓
TRAN00000095807	✓	✓

Which transcripts of Dnmt3b have miR-148 target sites



PART II: the graphical view of the protein function will be regulated by miRNAs

Which protein function of Dnmt3b might be regulated by miR-148

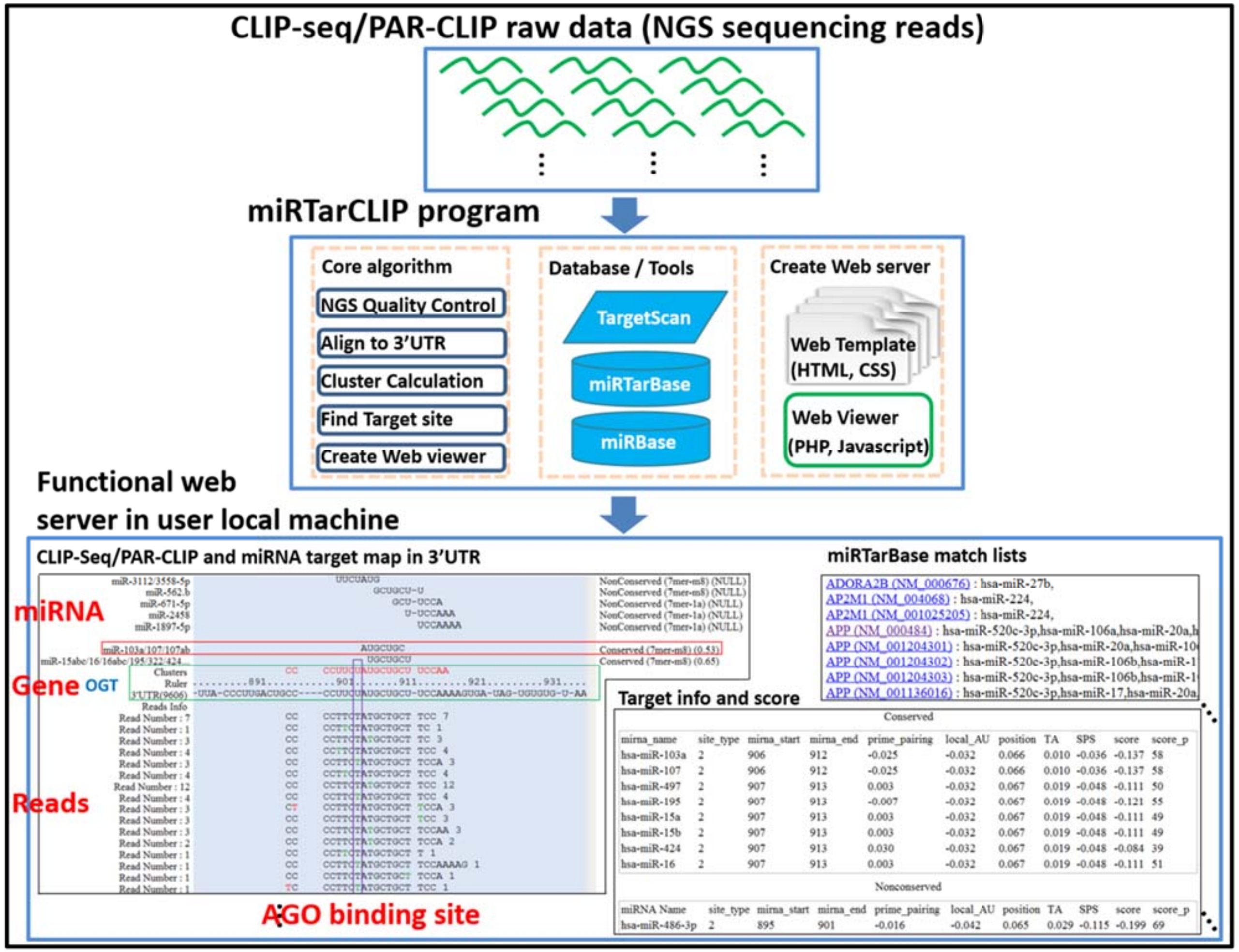


miR-148a base pairing on alternatively spliced exon

miRNA target on Exon #6 in CDS region of the transcript: TRAN00000095794

<table>
<tr><th rowspan="2">miRNA</th><th rowspan="2">Pos. on Gene</th><th rowspan="2">Transcript (Tx.)</th><th rowspan="2">Pos. on Tx.</th><th colspan="3">Tx. Region</th><th colspan="2">Exon Type of Tx.</th><th rowspan="2">Conserved Region</th><th rowspan="2">Target Accessibility</th><th rowspan="2">MFE</th><th rowspan="2">Alignment Score</th><th rowspan="2">Alignment</th><th rowspan="2">Seed Type</th><th rowspan="2">Seed Accessibility</th></tr>
<tr><th>5'UTR</th><th>CDS</th><th>3'UTR</th><th>CS</th><th>AS</th></tr>
<tr><td>hsa-miR-148a</td><td>53913 - 53934</td><td>TRAN00000095794</td><td>603 - 624</td><td></td><td>√</td><td></td><td></td><td>√</td><td>cons.</td><td>0.027</td><td>-25.60</td><td>180.00</td><td>miRNA 3'-ugUUUCAAGACAUCACGUGACu-5'
:| |||:|||:||||||||
mRNA 5'-aaGATGTTTTGTGGTGCACTGa-3'</td><td>8mer</td><td>0.000</td></tr>
<tr><td>hsa-miR-148b</td><td>53913 - 53934</td><td>TRAN00000095794</td><td>603 - 624</td><td></td><td>√</td><td></td><td></td><td>√</td><td>cons.</td><td>0.027</td><td>-25.90</td><td>180.00</td><td>miRNA 3'-ugUUUCAAGACACUACGUGACu-5'
:| |||:||||:|||||||
mRNA 5'-aaGATGTTTTGTGGTGCACTGa-3'</td><td>8mer</td><td>0.000</td></tr>
</table>

The duplex structure



miRTarBase match lists

ADORA2B (NM_000676) : hsa-miR-27b,
AP2M1 (NM_004068) : hsa-miR-224,
AP2M1 (NM_001025205) : hsa-miR-224,
APP (NM_000484) : hsa-miR-520c-3p,hsa-miR-106a,hsa-miR-20a,
APP (NM_001204301) : hsa-miR-520c-3p,hsa-miR-20a,hsa-miR-10
APP (NM_001204302) : hsa-miR-520c-3p,hsa-miR-106b,hsa-miR-1
APP (NM_001204303) : hsa-miR-520c-3p,hsa-miR-106b,hsa-miR-1
APP (NM_001136016) : hsa-miR-520c-3p,hsa-miR-17,hsa-miR-20a,

Target info and score

Conserved

mirna_name	site_type	mirna_start	mirna_end	prime_pairing	local_AU	position	TA	SPS	score	score_p
hsa-miR-103a	2	906	912	-0.025	-0.032	0.066	0.010	-0.036	-0.137	58
hsa-miR-107	2	906	912	-0.025	-0.032	0.066	0.010	-0.036	-0.137	58
hsa-miR-497	2	907	913	0.003	-0.032	0.067	0.019	-0.048	-0.111	50
hsa-miR-195	2	907	913	-0.007	-0.032	0.067	0.019	-0.048	-0.121	55
hsa-miR-15a	2	907	913	0.003	-0.032	0.067	0.019	-0.048	-0.111	49
hsa-miR-15b	2	907	913	0.003	-0.032	0.067	0.019	-0.048	-0.111	49
hsa-miR-424	2	907	913	0.030	-0.032	0.067	0.019	-0.048	-0.084	39
hsa-miR-16	2	907	913	0.003	-0.032	0.067	0.019	-0.048	-0.111	51

Nonconserved

miRNA Name	site_type	mirna_start	mirna_end	prime_pairing	local_AU	position	TA	SPS	score	score_p
hsa-miR-486-3p	2	895	901	-0.016	-0.042	0.065	0.029	-0.115	-0.199	69

miRTarCLIP

miRNA-Target Interactions Analysis in CLIP and PAR-CLIP Sequencing Data

http://mirtarclip.mbc.nctu.edu.tw/ miRTarCLIP

BMC Genomics, 2013

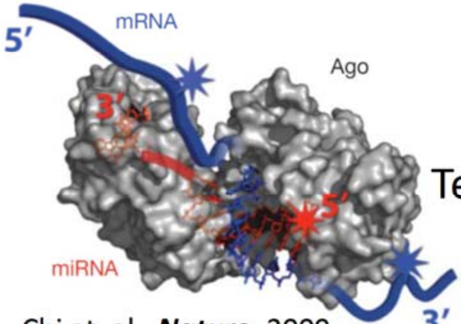
Major Features

- The conventionally approaches to verify MTIs such as the reporter assay are still time consuming and incapable of handling the large-scale screening.
- A large amount of miRNA-target interactions (MTIs) have been identified by the crosslinking and immunoprecipitation (CLIP) and the photoactivatable-ribonucleoside-enhanced CLIP (PAR-CLIP) along with the next-generation sequencing (NGS).

miRTarCLIP

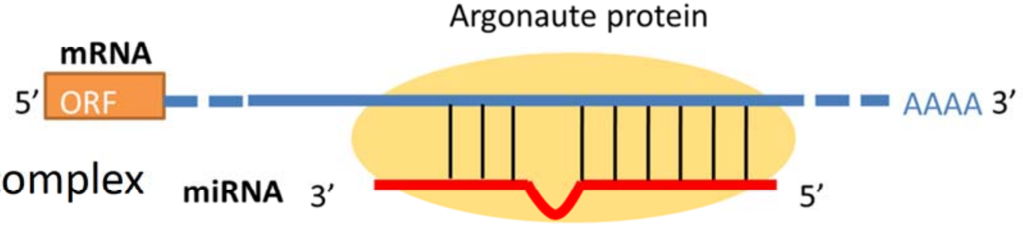
Crosslinking immunoprecipitation sequencing

CLIP-seq



Chi et. al., ***Nature***, 2009

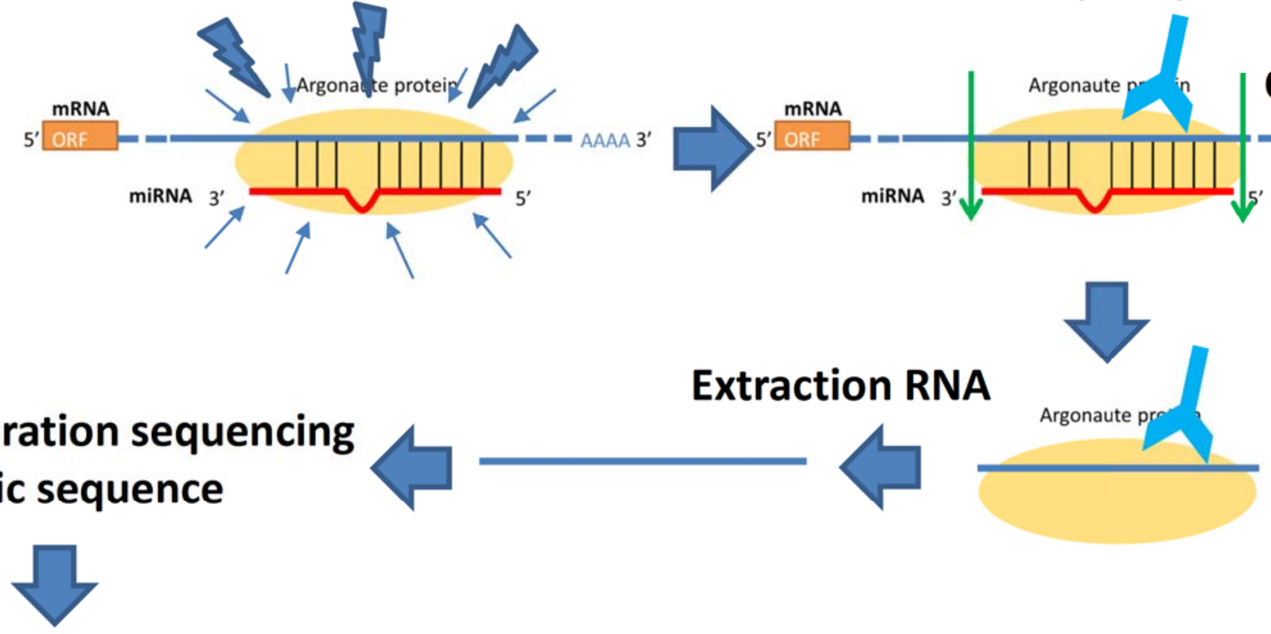
Ternary complex



UV Crosslink

immunoprecipitation

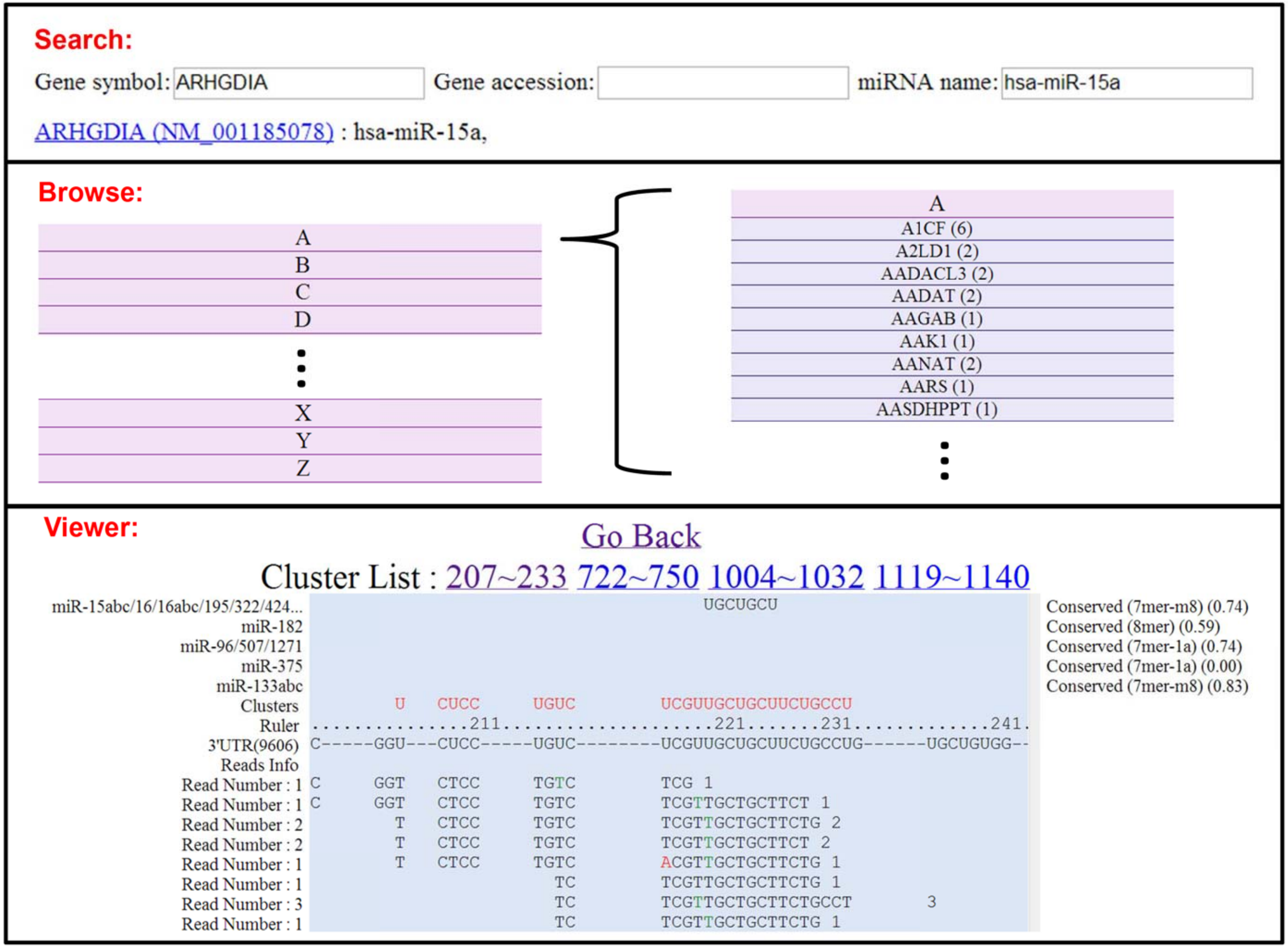
Cleavage by RNAse



Extraction RNA

Next generation sequencing the specific sequence

Identify miRNA Target Site



Search:

Gene symbol: ARHGDIA Gene accession: miRNA name: hsa-miR-15a

ARHGDIA (NM_001185078) : hsa-miR-15a,

Browse:

- A
- B
- C
- D
- ⋮
- X
- Y
- Z

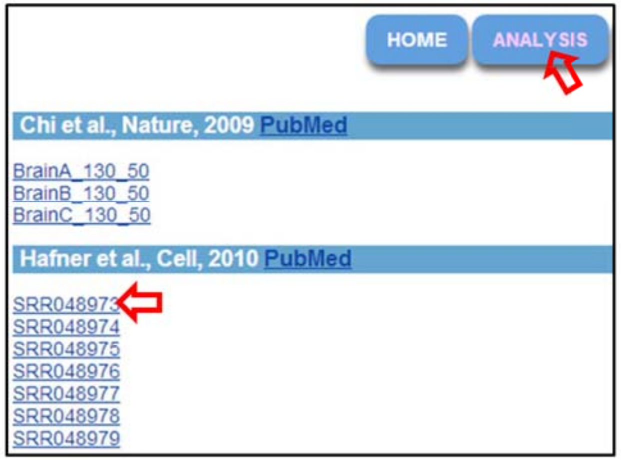
A
A1CF (6)
A2LD1 (2)
AADACL3 (2)
AADAT (2)
AAGAB (1)
AAK1 (1)
AANAT (2)
AARS (1)
AASDHPPT (1)
⋮

Viewer:

Go Back

Cluster List : 207~233 722~750 1004~1032 1119~1140

```
miR-15abc/16/16abc/195/322/424...                                       UGCUGCU                           Conserved (7mer-m8) (0.74)
                         miR-182                                                                          Conserved (8mer) (0.59)
                    miR-96/507/1271                                                                       Conserved (7mer-1a) (0.74)
                         miR-375                                                                          Conserved (7mer-1a) (0.00)
                      miR-133abc                                                                          Conserved (7mer-m8) (0.83)
                        Clusters         U   CUCC     UGUC        UCGUUGCUGCUUCUGCCU
                           Ruler ...............211....................221.......231.............241.
                   3'UTR(9606) C-----GGU---CUCC-----UGUC--------UCGUUGCUGCUUCUGCCUG------UGCUGUGG--
                      Reads Info
                 Read Number : 1 C     GGT   CTCC     TGTC        TCG 1
                 Read Number : 1 C     GGT   CTCC     TGTC        TCGTTGCTGCTTCT 1
                 Read Number : 2         T   CTCC     TGTC        TCGTTGCTGCTTCTG 2
                 Read Number : 2         T   CTCC     TGTC        TCGTTGCTGCTTCT 2
                 Read Number : 1         T   CTCC     TGTC        ACGTTGCTGCTTCTG 1
                 Read Number : 1                        TC        TCGTTGCTGCTTCTG 1
                 Read Number : 3                        TC        TCGTTGCTGCTTCTGCCT       3
                 Read Number : 1                        TC        TCGTTGCTGCTTCTG 1
```

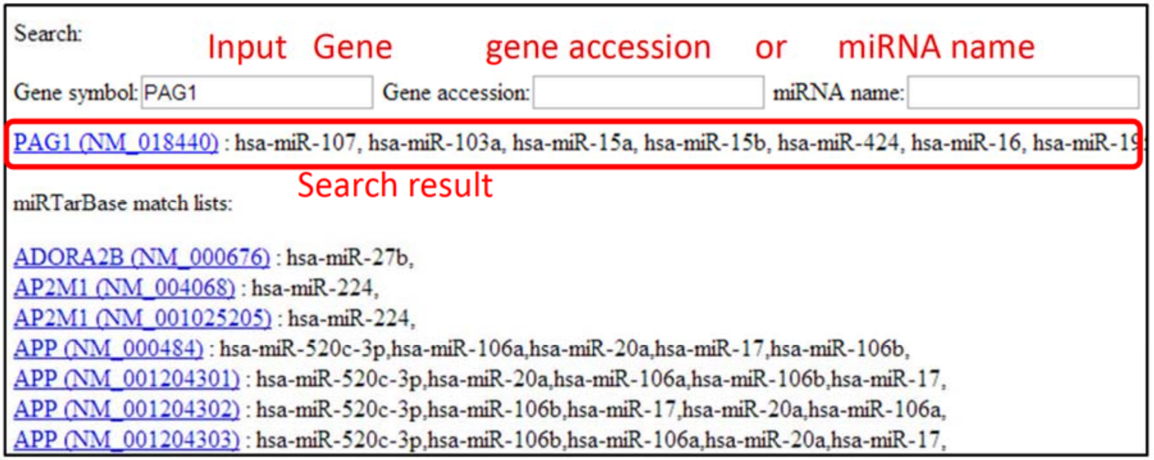


Chi et al., Nature, 2009 PubMed

BrainA_130_50
BrainB_130_50
BrainC_130_50

Hafner et al., Cell, 2010 PubMed

SRR048973
SRR048974
SRR048975
SRR048976
SRR048977
SRR048978
SRR048979



Search: Input Gene gene accession or miRNA name

Gene symbol: PAG1 Gene accession: miRNA name:

PAG1 (NM_018440) : hsa-miR-107, hsa-miR-103a, hsa-miR-15a, hsa-miR-15b, hsa-miR-424, hsa-miR-16, hsa-miR-19

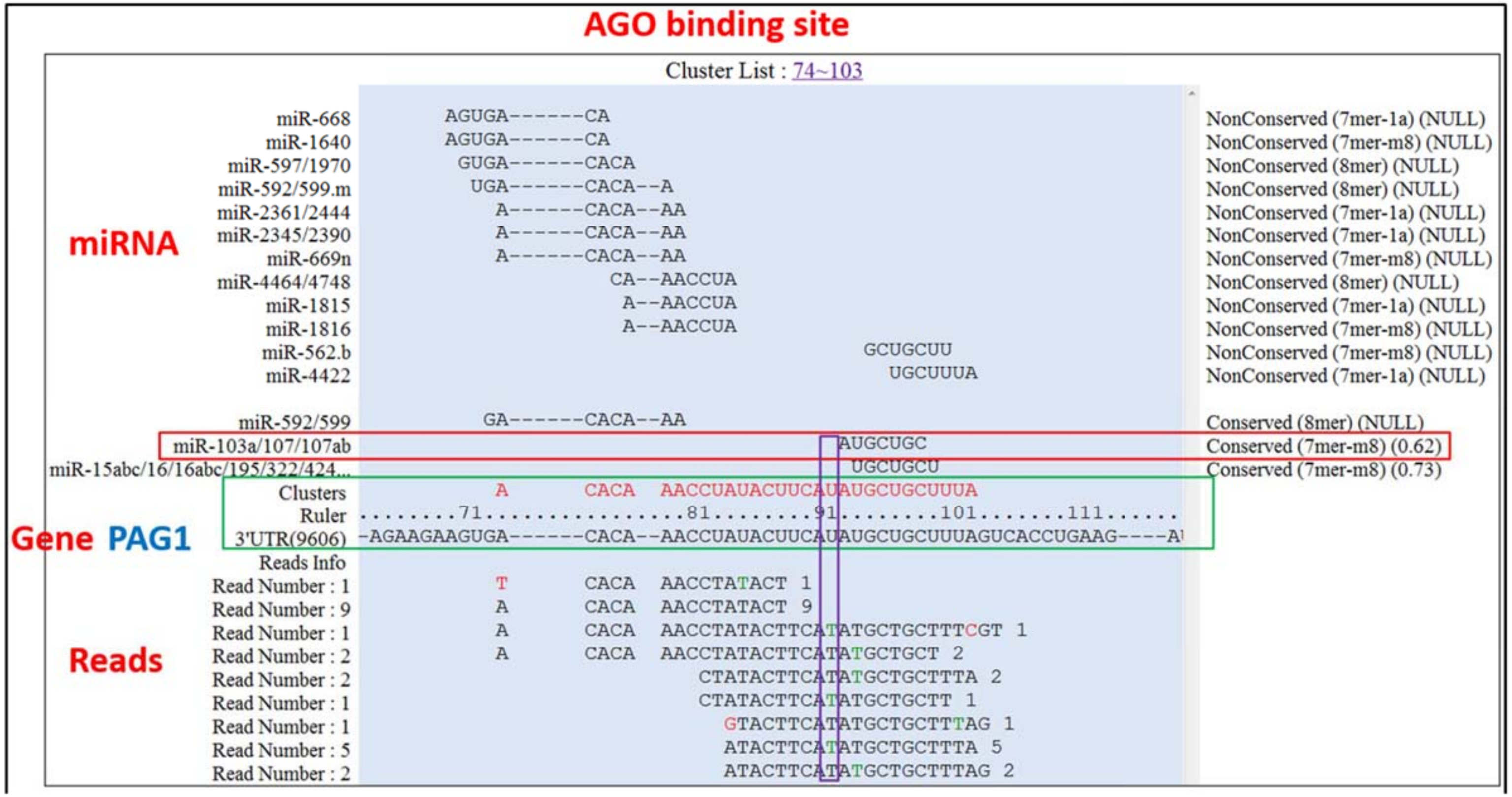
Search result

miRTarBase match lists:

ADORA2B (NM_000676) : hsa-miR-27b,
AP2M1 (NM_004068) : hsa-miR-224,
AP2M1 (NM_001025205) : hsa-miR-224,
APP (NM_000484) : hsa-miR-520c-3p,hsa-miR-106a,hsa-miR-20a,hsa-miR-17,hsa-miR-106b,
APP (NM_001204301) : hsa-miR-520c-3p,hsa-miR-20a,hsa-miR-106a,hsa-miR-106b,hsa-miR-17,
APP (NM_001204302) : hsa-miR-520c-3p,hsa-miR-106b,hsa-miR-17,hsa-miR-20a,hsa-miR-106a,
APP (NM_001204303) : hsa-miR-520c-3p,hsa-miR-106b,hsa-miR-106a,hsa-miR-20a,hsa-miR-17,

Go Back
Cluster List : 74~103 816~831 1582~1597 2021~2036 5234~5253 8250~8266 8529~8543 8649~8663

Select location

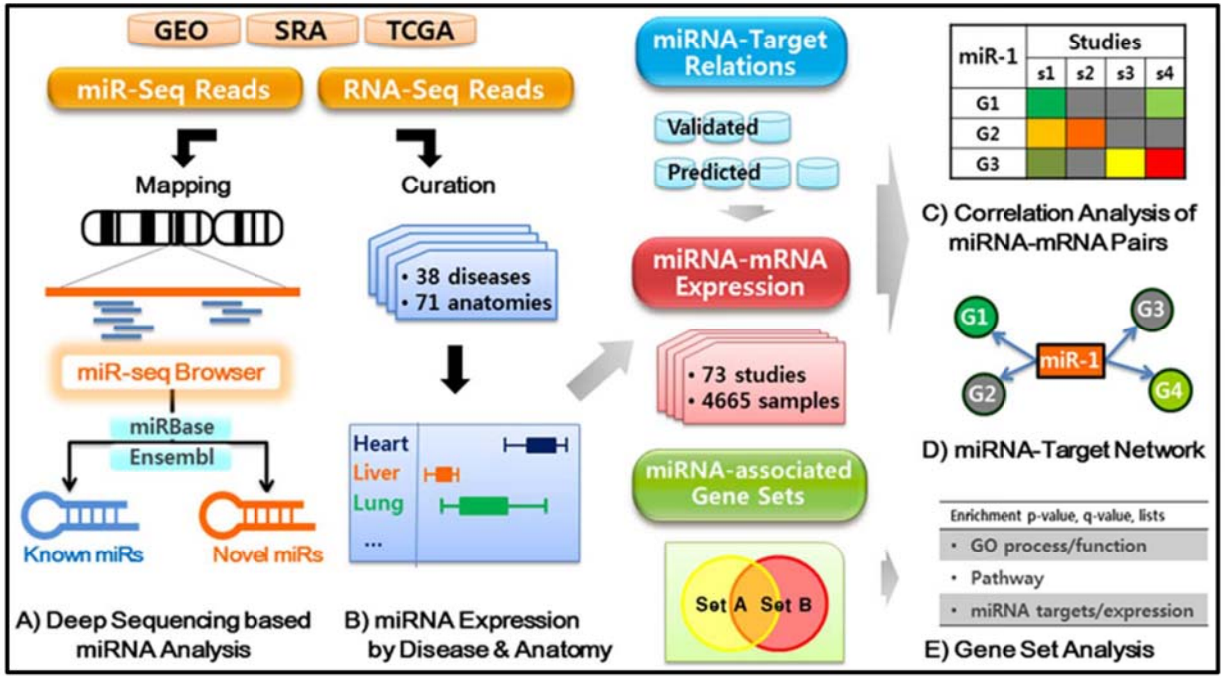


AGO binding site

Cluster List : 74~103

```
                          miR-668        AGUGA------CA                                NonConserved (7mer-1a) (NULL)
                         miR-1640        AGUGA------CA                                NonConserved (7mer-m8) (NULL)
                     miR-597/1970         GUGA------CACA                              NonConserved (8mer) (NULL)
                    miR-592/599.m          UGA------CACA--A                           NonConserved (8mer) (NULL)
                    miR-2361/2444            A------CACA--AA                          NonConserved (7mer-1a) (NULL)
                    miR-2345/2390            A------CACA--AA                          NonConserved (7mer-1a) (NULL)
                         miR-669n            A------CACA--AA                          NonConserved (7mer-m8) (NULL)
                    miR-4464/4748                     CA--AACCUA                      NonConserved (8mer) (NULL)
                         miR-1815                      A--AACCUA                      NonConserved (7mer-1a) (NULL)
                         miR-1816                      A--AACCUA                      NonConserved (7mer-m8) (NULL)
                        miR-562.b                                         GCUGCUU                   NonConserved (7mer-m8) (NULL)
                         miR-4422                                           UGCUUUA                 NonConserved (7mer-1a) (NULL)

                      miR-592/599           GA------CACA--AA                          Conserved (8mer) (NULL)
               miR-103a/107/107ab                                       AUGCUGC                     Conserved (7mer-m8) (0.62)
miR-15abc/16/16abc/195/322/424...                                        UGCUGCU                    Conserved (7mer-m8) (0.73)
                         Clusters            A      CACA  AACCUAUACUUCAUAUGCUGCUUUA
                            Ruler ........71................81........91........101.......111......
                      3'UTR(9606) -AGAAGAAGUGA------CACA--AACCUAUACUUCAUAUGCUGCUUUAGUCACCUGAAG----A
                       Reads Info
                  Read Number : 1            T      CACA  AACCTATACT 1
                  Read Number : 9            A      CACA  AACCTATACT 9
                  Read Number : 1            A      CACA  AACCTATACTTCATATGCTGCTTTCGT 1
                  Read Number : 2            A      CACA  AACCTATACTTCATATGCTGCT 2
                  Read Number : 2                            CTATACTTCATATGCTGCTTTA 2
                  Read Number : 1                            CTATACTTCATATGCTGCTT 1
                  Read Number : 1                              GTACTTCATATGCTGCTTTAG 1
                  Read Number : 5                              ATACTTCATATGCTGCTTTA 5
                  Read Number : 2                              ATACTTCATATGCTGCTTTAG 2
```



miRGator

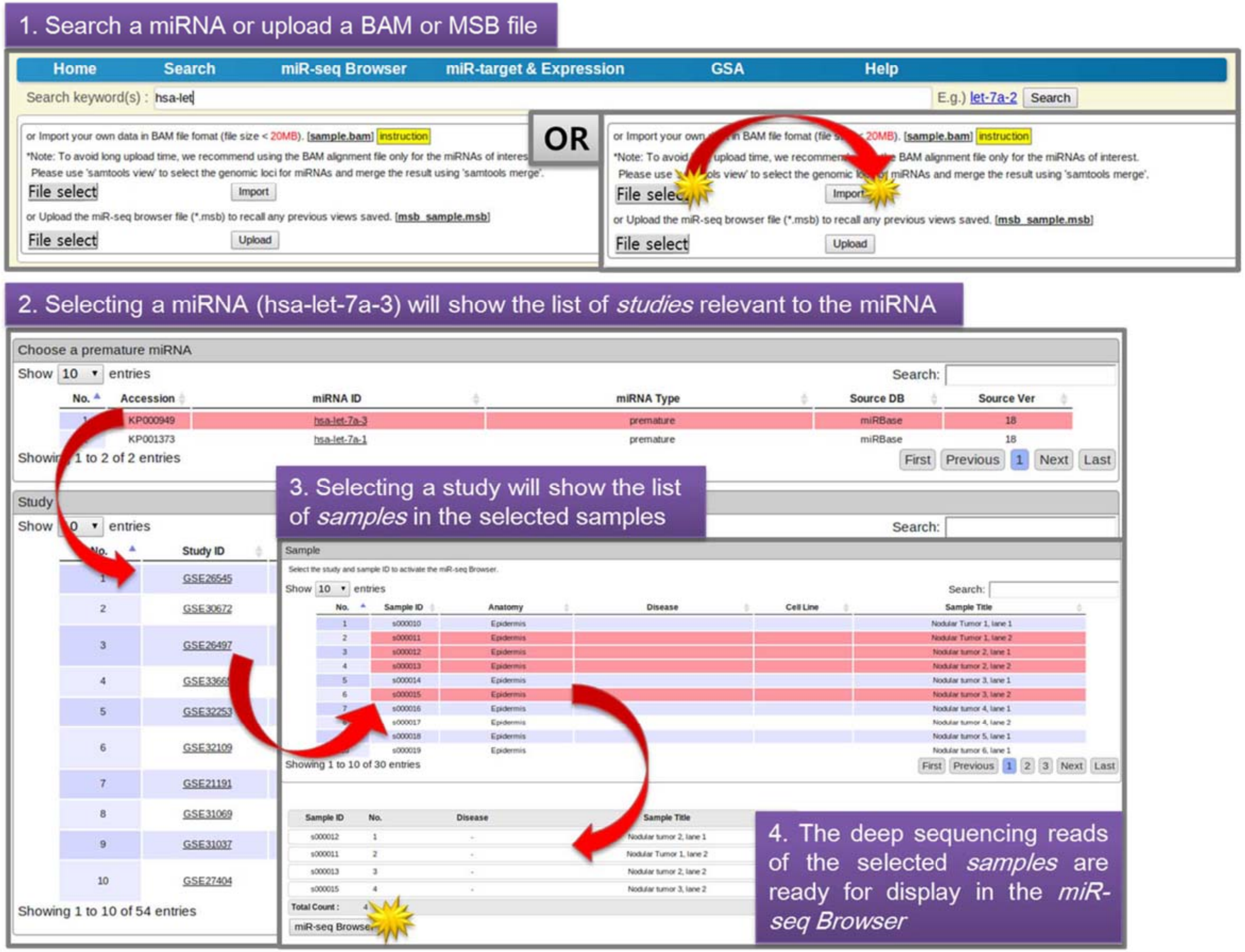
A microRNA portal in deep sequencing era

http://mirgator.kobic.re.kr

Major Features

- miR-seq Browser
- miR-target & Expression
  - Correlation Heat Map
  - Network Viewer
- Gene set analysis (GSA)

miR-seq Browser



1. Search a miRNA or upload a BAM or MSB file

Search keyword(s) : hsa-let

E.g.) let-7a-2 Search

or Import your own data in BAM file fomat (file size < 20MB). [sample.bam] instruction

*Note: To avoid long upload time, we recommend using the BAM alignment file only for the miRNAs of interes

Please use 'samtools view' to select the genomic loci for miRNAs and merge the result using 'samtools merge'.

or Upload the miR-seq browser file (*.msb) to recall any previous views saved. [msb_sample.msb]

2. Selecting a miRNA (hsa-let-7a-3) will show the list of *studies* relevant to the miRNA

Choose a premature miRNA

Show 10 entries Search:

No.	Accession	miRNA ID	miRNA Type	Source DB	Source Ver
1	KP000949	hsa-let-7a-3	premature	miRBase	18
	KP001373	hsa-let-7a-1	premature	miRBase	18

Showing 1 to 2 of 2 entries

3. Selecting a study will show the list of *samples* in the selected samples

Study

Show 0 entries Search:

No.	Study ID
1	GSE26545
2	GSE30672
3	GSE26497
4	GSE3366
5	GSE32253
6	GSE32109
7	GSE21191
8	GSE31069
9	GSE31037
10	GSE27404

Showing 1 to 10 of 54 entries

Sample

Select the study and sample ID to activate the miR-seq Browser.

Show 10 entries Search:

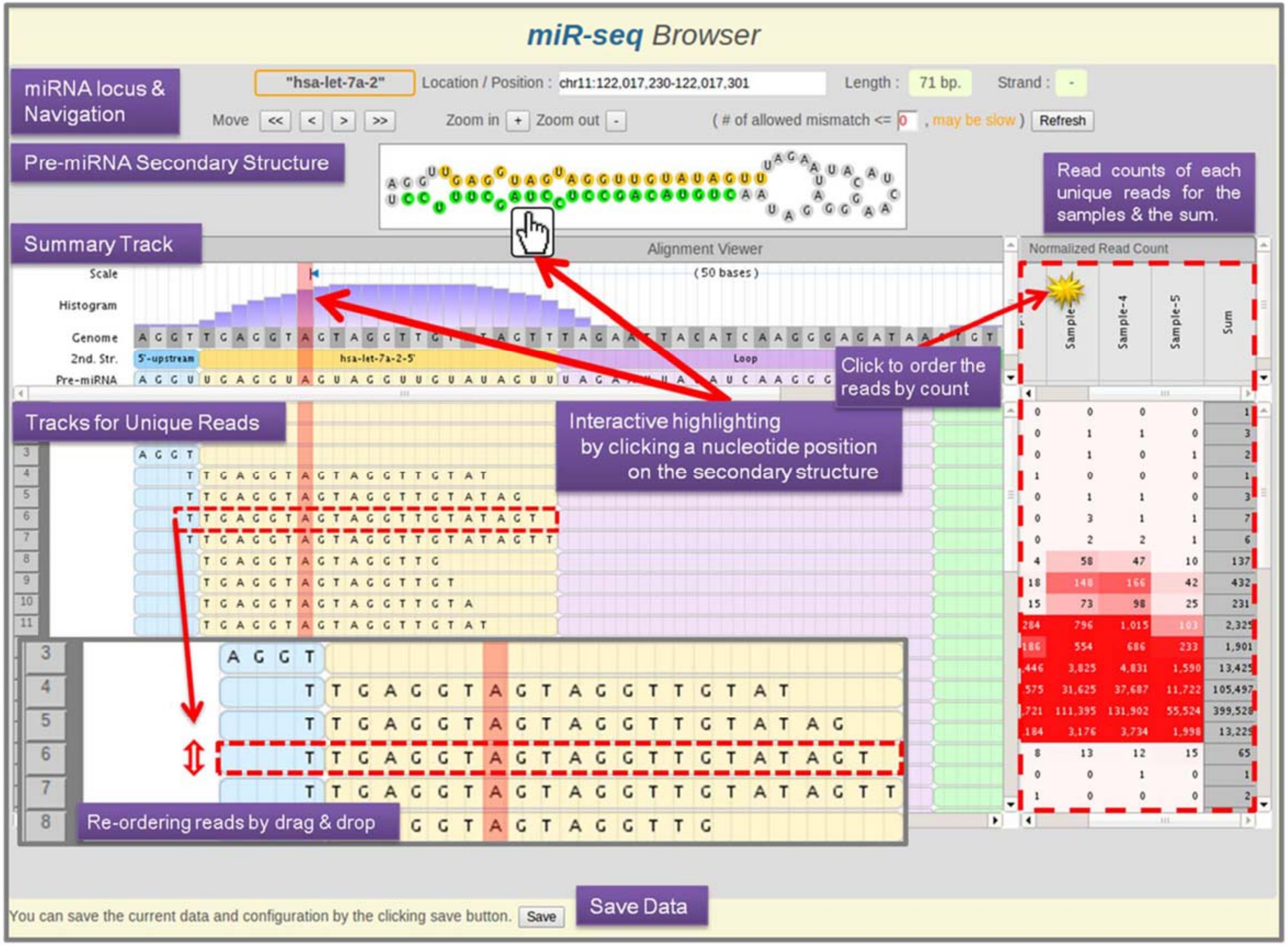
No.	Sample ID	Anatomy	Disease	Cell Line	Sample Title
1	s000010	Epidermis			Nodular Tumor 1, lane 1
2	s000011	Epidermis			Nodular Tumor 1, lane 2
3	s000012	Epidermis			Nodular tumor 2, lane 1
4	s000013	Epidermis			Nodular tumor 2, lane 2
5	s000014	Epidermis			Nodular tumor 3, lane 1
6	s000015	Epidermis			Nodular tumor 3, lane 2
7	s000016	Epidermis			Nodular tumor 4, lane 1
	s000017	Epidermis			Nodular tumor 4, lane 2
	s000018	Epidermis			Nodular tumor 5, lane 1
	s000019	Epidermis			Nodular tumor 6, lane 1

Showing 1 to 10 of 30 entries

Sample ID	No.	Disease	Sample Title
s000012	1	-	Nodular tumor 2, lane 1
s000011	2	-	Nodular Tumor 1, lane 2
s000013	3	-	Nodular tumor 2, lane 2
s000015	4	-	Nodular tumor 3, lane 2
Total Count :	4		

4. The deep sequencing reads of the selected *samples* are ready for display in the *miR-seq Browser*

miR-seq Browser



miR-seq Browser

miRNA locus & Navigation

"hsa-let-7a-2" Location / Position : chr11:122,017,230-122,017,301 Length : 71 bp. Strand : -

Move << < > >> Zoom in + Zoom out - (# of allowed mismatch <= 0 , may be slow) Refresh

Pre-miRNA Secondary Structure

Read counts of each unique reads for the samples & the sum.

Summary Track

Click to order the reads by count

Tracks for Unique Reads

Interactive highlighting by clicking a nucleotide position on the secondary structure

Re-ordering reads by drag & drop

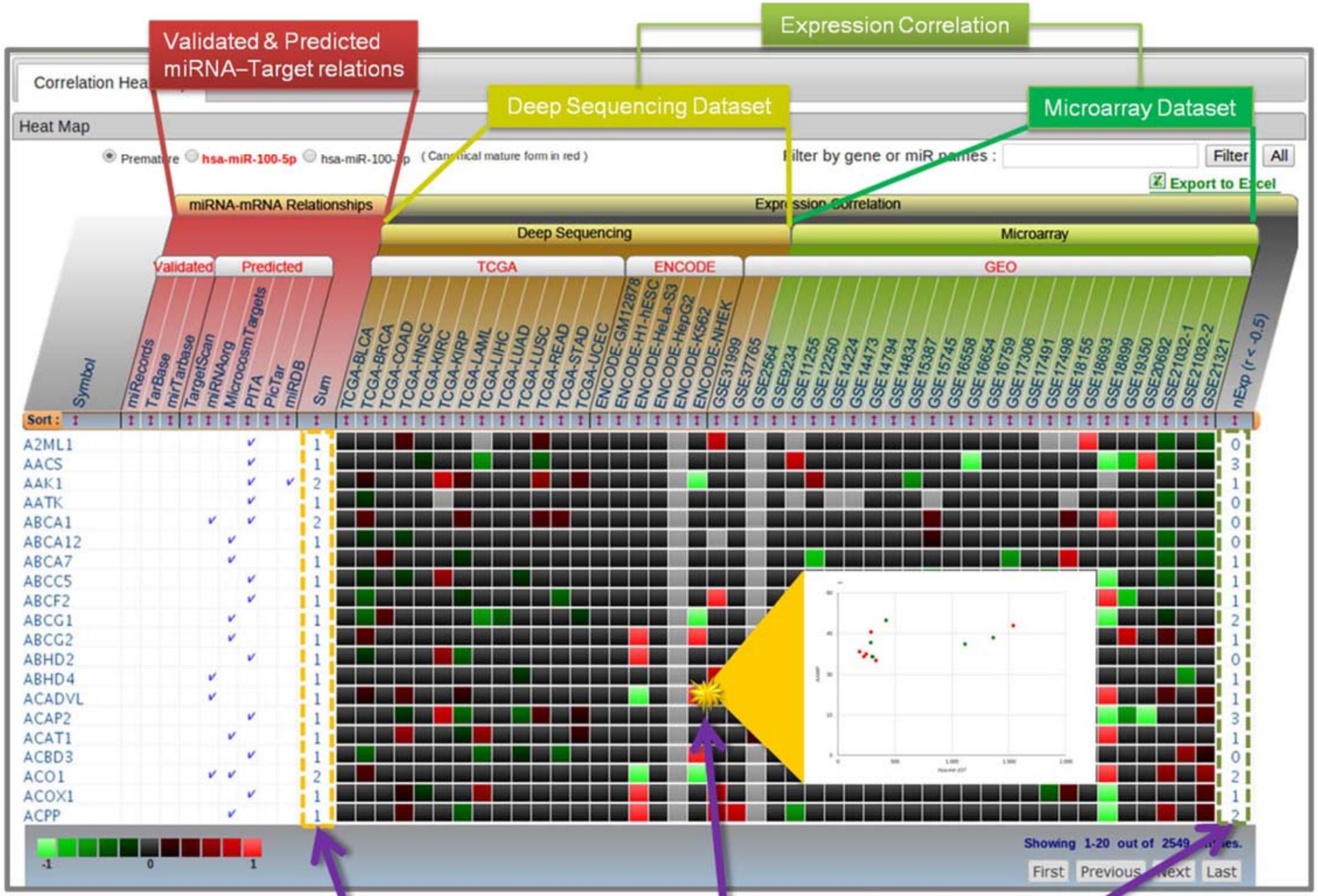
Normalized Read Count

Sample-	Sample-4	Sample-5	Sum
0	0	0	0	1
0	1	1	0	3
0	1	0	1	2
1	0	0	0	1
0	1	1	0	3
0	3	1	1	7
0	2	2	1	6
4	58	47	10	137
18	148	166	42	432
15	73	98	25	231
284	796	1,015	103	2,325
186	554	686	233	1,901
,446	3,825	4,831	1,590	13,425
,575	31,625	37,687	11,722	105,497
,721	111,395	131,902	55,524	399,528
,184	3,176	3,734	1,998	13,229
8	13	12	15	65
0	0	1	0	1
1	0	0	0	2

Save Data

You can save the current data and configuration by the clicking save button. Save

miR-target and Expression
- Correlation Heat Map



Validated & Predicted miRNA–Target relations

Expression Correlation

Deep Sequencing Dataset

Microarray Dataset

Symbol	miRecords	TarBase	mirTarbase	TargetScan	miRNAorg	MicrocosmTargets	PITA	PicTar	miRDB	Sum	nExp (r < -0.5)
A2ML1							✓			1	0
AACS							✓			1	3
AAK1							✓		✓	2	1
AATK							✓			1	0
ABCA1					✓		✓			2	0
ABCA12						✓				1	0
ABCA7						✓				1	1
ABCC5							✓			1	1
ABCF2							✓			1	1
ABCG1						✓				1	2
ABCG2						✓				1	1
ABHD2							✓			1	0
ABHD4					✓					1	1
ACADVL					✓					1	1
ACAP2							✓			1	3
ACAT1						✓				1	1
ACBD3							✓			1	0
ACO1					✓	✓				2	2
ACOX1							✓			1	1
ACPP						✓				1	2

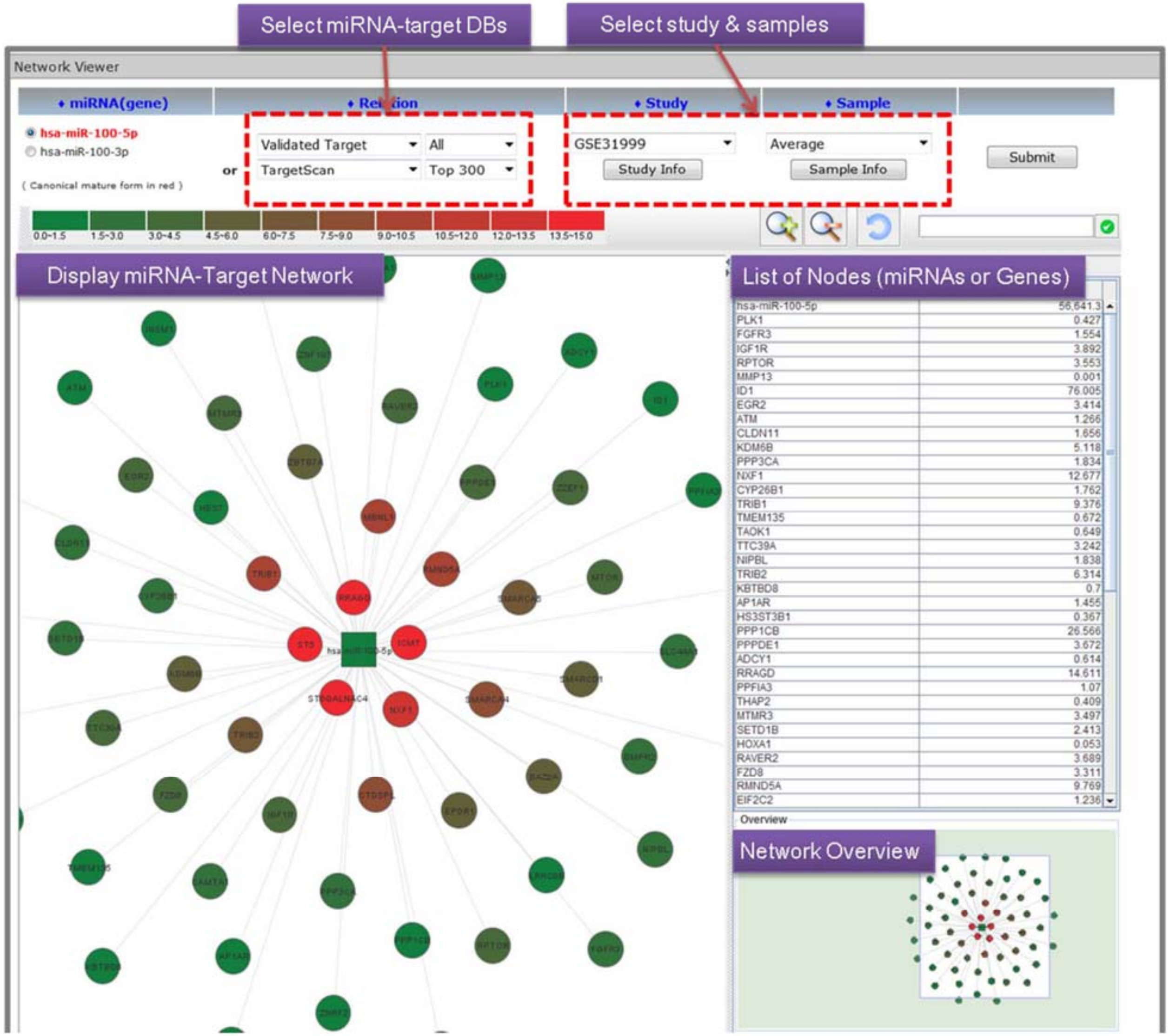
Showing 1-20 out of 2549 entries.

The degree of consensus among validated/predicted miRNA-target DBs

Click a cell shows the scatter plot of miRNA & mRNA expressions

The number of datasets showing strong negative correlation (< -0.5)

miR-target and Expression
- Nework viewer



♦ miRNA(gene)

● hsa-miR-100-5p
○ hsa-miR-100-3p
(Canonical mature form in red)

♦ Relation

Validated Target ▾ All ▾
or TargetScan ▾ Top 300 ▾

♦ Study

GSE31999 ▾

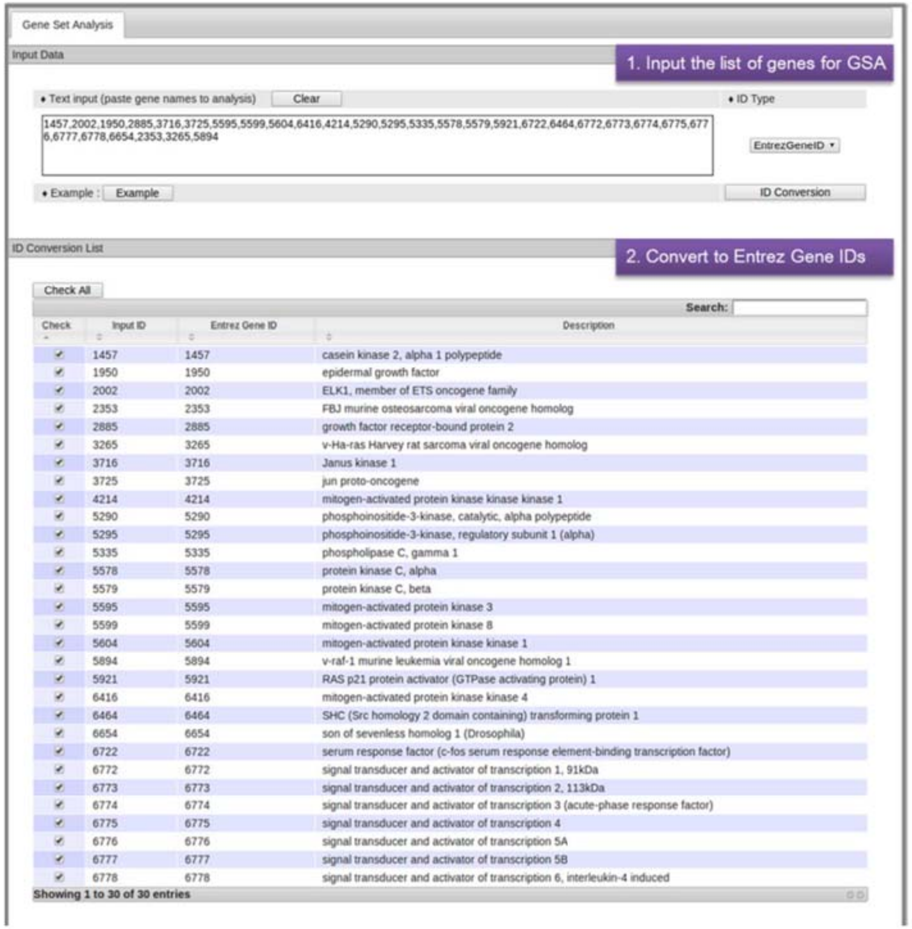
♦ Sample

Average ▾

🟩	🟩	🟩	🟫	🟫	🟫	🟥	🟥	🟥	🟥
0.0~1.5	1.5~3.0	3.0~4.5	4.5~6.0	6.0~7.5	7.5~9.0	9.0~10.5	10.5~12.0	12.0~13.5	13.5~15.0

hsa-miR-100-5p	56,641.3
PLK1	0.427
FGFR3	1.554
IGF1R	3.892
RPTOR	3.553
MMP13	0.001
ID1	76.005
EGR2	3.414
ATM	1.266
CLDN11	1.656
KDM6B	5.118
PPP3CA	1.834
NXF1	12.677
CYP26B1	1.762
TRIB1	9.376
TMEM135	0.672
TAOK1	0.649
TTC39A	3.242
NIPBL	1.838
TRIB2	6.314
KBTBD8	0.7
AP1AR	1.455
HS3ST3B1	0.367
PPP1CB	26.566
PPPDE1	3.672
ADCY1	0.614
RRAGD	14.611
PPFIA3	1.07
THAP2	0.409
MTMR3	3.497
SETD1B	2.413
HOXA1	0.053
RAVER2	3.689
FZD8	3.311
RMND5A	9.769
EIF2C2	1.236

Gene Set Analysis (GSA)



1. Input the list of genes for GSA

• Text input (paste gene names to analysis)

1457,2002,1950,2885,3716,3725,5595,5599,5604,6416,4214,5290,5295,5335,5578,5579,5921,6722,6464,6772,6773,6774,6775,6776,6777,6778,6654,2353,3265,5894

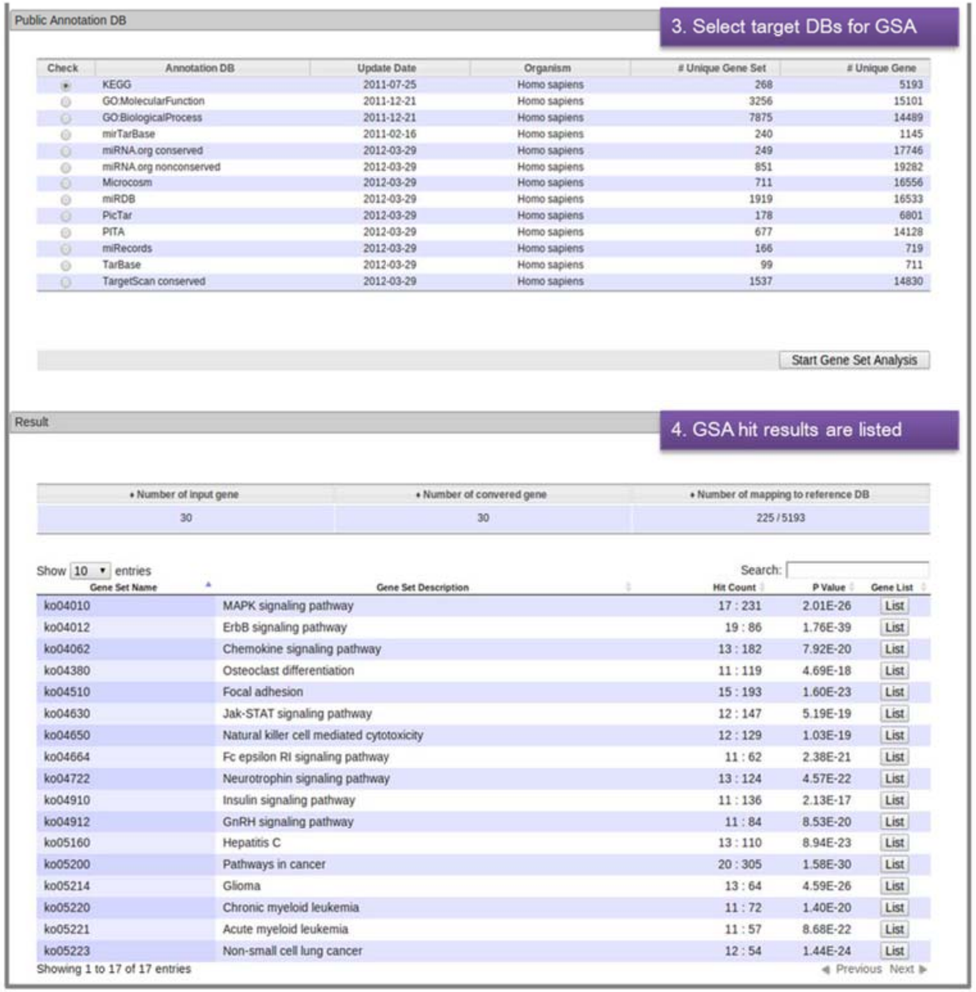
• ID Type: EntrezGeneID

• Example :

2. Convert to Entrez Gene IDs

Check	Input ID	Entrez Gene ID	Description
☑	1457	1457	casein kinase 2, alpha 1 polypeptide
☑	1950	1950	epidermal growth factor
☑	2002	2002	ELK1, member of ETS oncogene family
☑	2353	2353	FBJ murine osteosarcoma viral oncogene homolog
☑	2885	2885	growth factor receptor-bound protein 2
☑	3265	3265	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
☑	3716	3716	Janus kinase 1
☑	3725	3725	jun proto-oncogene
☑	4214	4214	mitogen-activated protein kinase kinase kinase 1
☑	5290	5290	phosphoinositide-3-kinase, catalytic, alpha polypeptide
☑	5295	5295	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
☑	5335	5335	phospholipase C, gamma 1
☑	5578	5578	protein kinase C, alpha
☑	5579	5579	protein kinase C, beta
☑	5595	5595	mitogen-activated protein kinase 3
☑	5599	5599	mitogen-activated protein kinase 8
☑	5604	5604	mitogen-activated protein kinase kinase 1
☑	5894	5894	v-raf-1 murine leukemia viral oncogene homolog 1
☑	5921	5921	RAS p21 protein activator (GTPase activating protein) 1
☑	6416	6416	mitogen-activated protein kinase kinase 4
☑	6464	6464	SHC (Src homology 2 domain containing) transforming protein 1
☑	6654	6654	son of sevenless homolog 1 (Drosophila)
☑	6722	6722	serum response factor (c-fos serum response element-binding transcription factor)
☑	6772	6772	signal transducer and activator of transcription 1, 91kDa
☑	6773	6773	signal transducer and activator of transcription 2, 113kDa
☑	6774	6774	signal transducer and activator of transcription 3 (acute-phase response factor)
☑	6775	6775	signal transducer and activator of transcription 4
☑	6776	6776	signal transducer and activator of transcription 5A
☑	6777	6777	signal transducer and activator of transcription 5B
☑	6778	6778	signal transducer and activator of transcription 6, interleukin-4 induced

Showing 1 to 30 of 30 entries



3. Select target DBs for GSA

Check	Annotation DB	Update Date	Organism	# Unique Gene Set	# Unique Gene
●	KEGG	2011-07-25	Homo sapiens	268	5193
○	GO:MolecularFunction	2011-12-21	Homo sapiens	3256	15101
○	GO:BiologicalProcess	2011-12-21	Homo sapiens	7875	14489
○	mirTarBase	2011-02-16	Homo sapiens	240	1145
○	miRNA.org conserved	2012-03-29	Homo sapiens	249	17746
○	miRNA.org nonconserved	2012-03-29	Homo sapiens	851	19282
○	Microcosm	2012-03-29	Homo sapiens	711	16556
○	miRDB	2012-03-29	Homo sapiens	1919	16533
○	PicTar	2012-03-29	Homo sapiens	178	6801
○	PITA	2012-03-29	Homo sapiens	677	14128
○	miRecords	2012-03-29	Homo sapiens	166	719
○	TarBase	2012-03-29	Homo sapiens	99	711
○	TargetScan conserved	2012-03-29	Homo sapiens	1537	14830

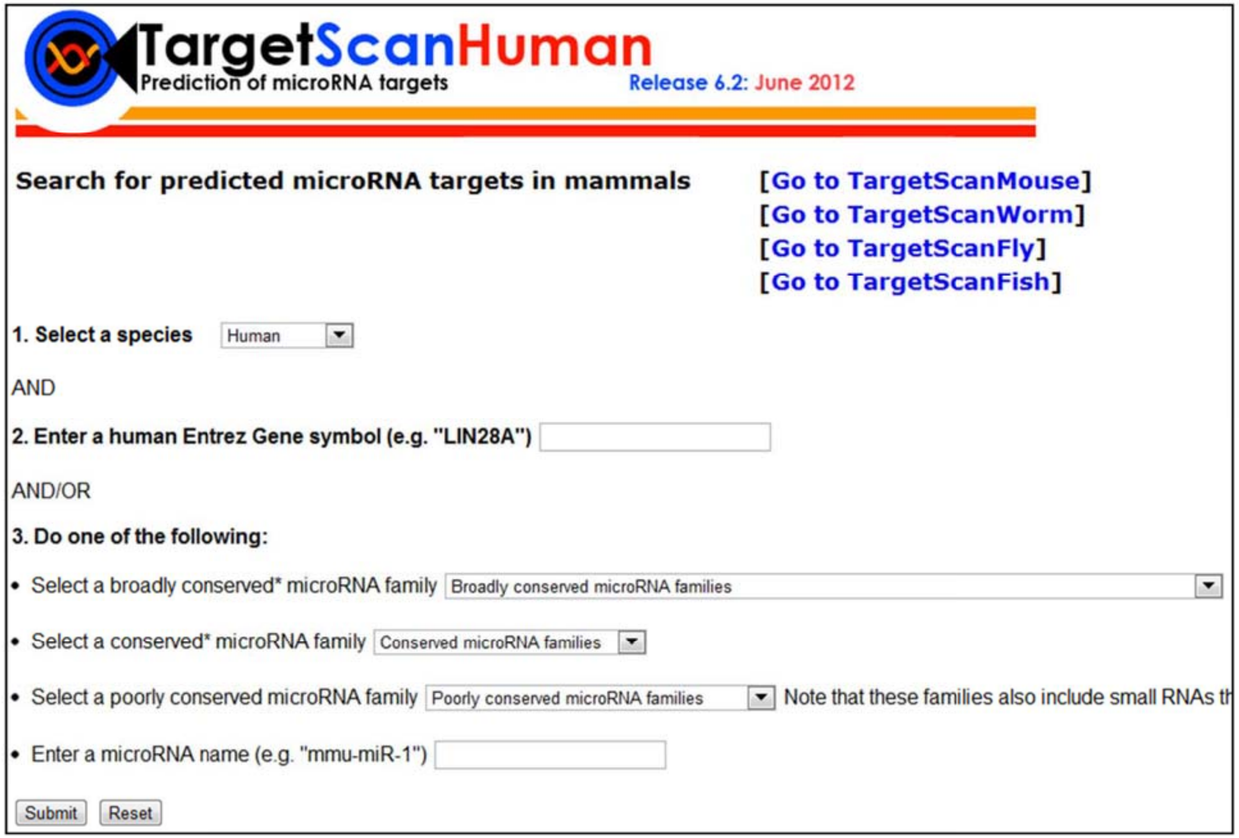
4. GSA hit results are listed

• Number of input gene	• Number of convered gene	• Number of mapping to reference DB
30	30	225 / 5193

Show 10 entries

Gene Set Name	Gene Set Description	Hit Count	P Value	Gene List
ko04010	MAPK signaling pathway	17 : 231	2.01E-26	List
ko04012	ErbB signaling pathway	19 : 86	1.76E-39	List
ko04062	Chemokine signaling pathway	13 : 182	7.92E-20	List
ko04380	Osteoclast differentiation	11 : 119	4.69E-18	List
ko04510	Focal adhesion	15 : 193	1.60E-23	List
ko04630	Jak-STAT signaling pathway	12 : 147	5.19E-19	List
ko04650	Natural killer cell mediated cytotoxicity	12 : 129	1.03E-19	List
ko04664	Fc epsilon RI signaling pathway	11 : 62	2.38E-21	List
ko04722	Neurotrophin signaling pathway	13 : 124	4.57E-22	List
ko04910	Insulin signaling pathway	11 : 136	2.13E-17	List
ko04912	GnRH signaling pathway	11 : 84	8.53E-20	List
ko05160	Hepatitis C	13 : 110	8.94E-23	List
ko05200	Pathways in cancer	20 : 305	1.58E-30	List
ko05214	Glioma	13 : 64	4.59E-26	List
ko05220	Chronic myeloid leukemia	11 : 72	1.40E-20	List
ko05221	Acute myeloid leukemia	11 : 57	8.68E-22	List
ko05223	Non-small cell lung cancer	12 : 54	1.44E-24	List

Showing 1 to 17 of 17 entries



Search for predicted microRNA targets in mammals

[Go to TargetScanMouse]
[Go to TargetScanWorm]
[Go to TargetScanFly]
[Go to TargetScanFish]

1. Select a species Human

AND

2. Enter a human Entrez Gene symbol (e.g. "LIN28A")

AND/OR

3. Do one of the following:

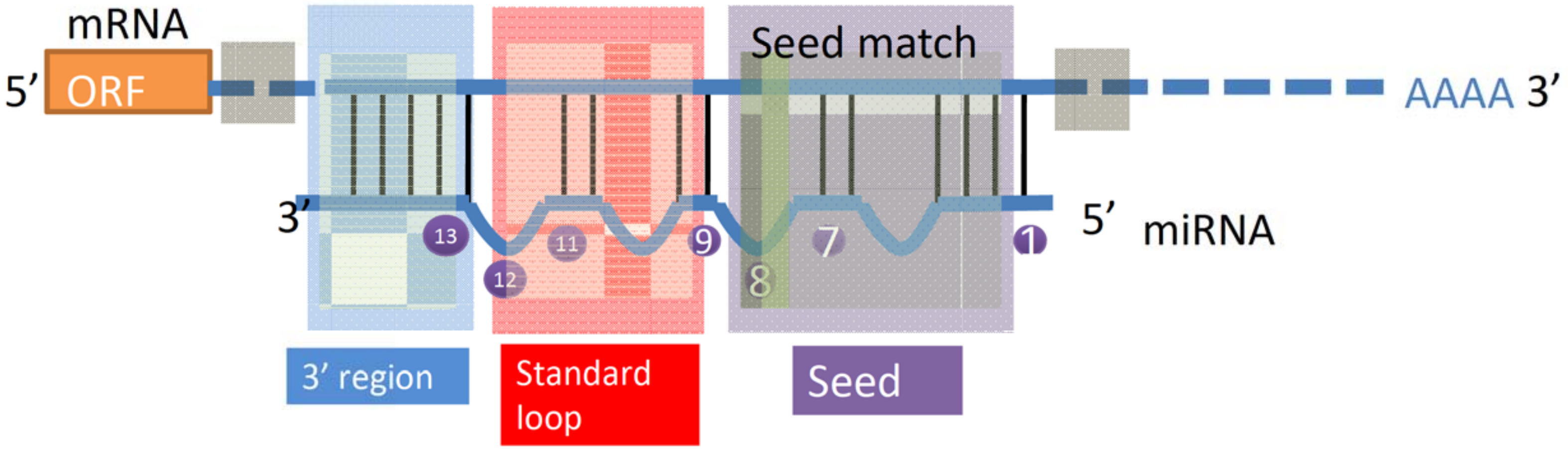
- Select a broadly conserved* microRNA family Broadly conserved microRNA families
- Select a conserved* microRNA family Conserved microRNA families
- Select a poorly conserved microRNA family Poorly conserved microRNA families Note that these families also include small RNAs th
- Enter a microRNA name (e.g. "mmu-miR-1")

TargetScan

Prediction of microRNA targets

http://www.targetscan.org/

MicroRNA Target Prediction



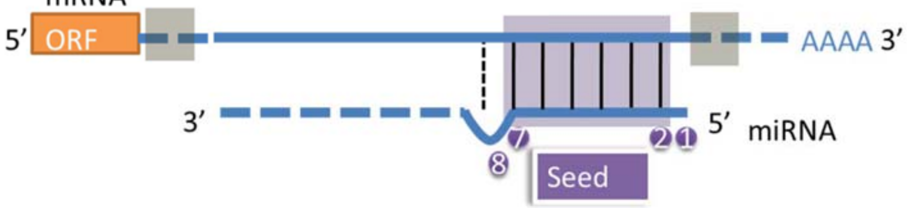
- Perfect pairing of miRNA-mRNA seed match (2-8mer).
- Thermodynamic stability of miRNA::mRNA regulation element duplex.
- Conserved miRNA targets.
- Target site accessibility.
- Order of target proficiency in different mRNA region is that:
  - 3′UTR > ORF >> 5′UTR.

TargetScan: Context+ scores

- Seed-type contribution
  - 8mer >> 7mer-m8 > 7mer-A1 >> 6mer > no site.
- 3' pairing contribution
  - additional Watson-Crick pairing at nucleotides 12–17 enhances miRNA targeting.
- Local AU contribution
  - Out of seed region is prefect AU rich sequence.
- Position contribution
  - two ends of long UTRs generally were more effective than those near the center.
- TA and SPS contribution
  - TA: **t**arget-site **a**bundance
  - SPS: **s**eed-**p**airing **s**tability
- Context+ score is sum of these contribution.

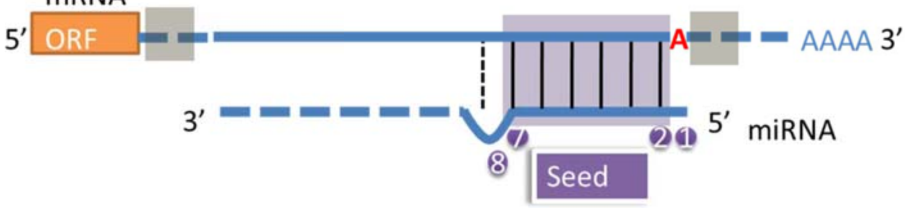
Seed-type contribution

6mer



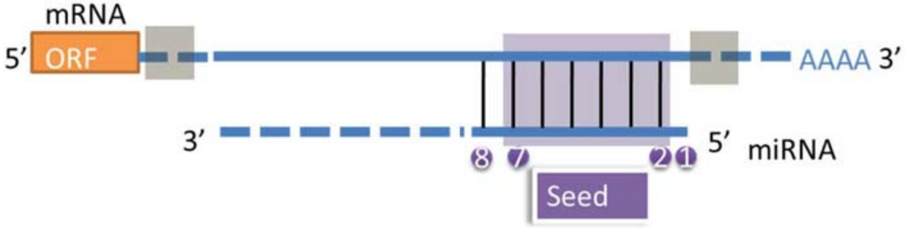
2 to 7 mer perfect match

7mer-A1



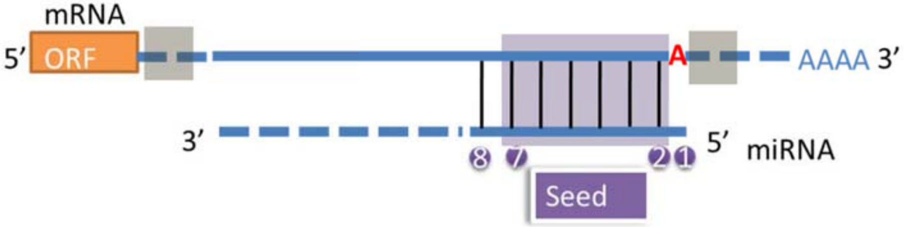
2 to 7 mer perfect match and position 1 in mRNA must be nucleotide A

7mer-m8



2 to 8 mer perfect match

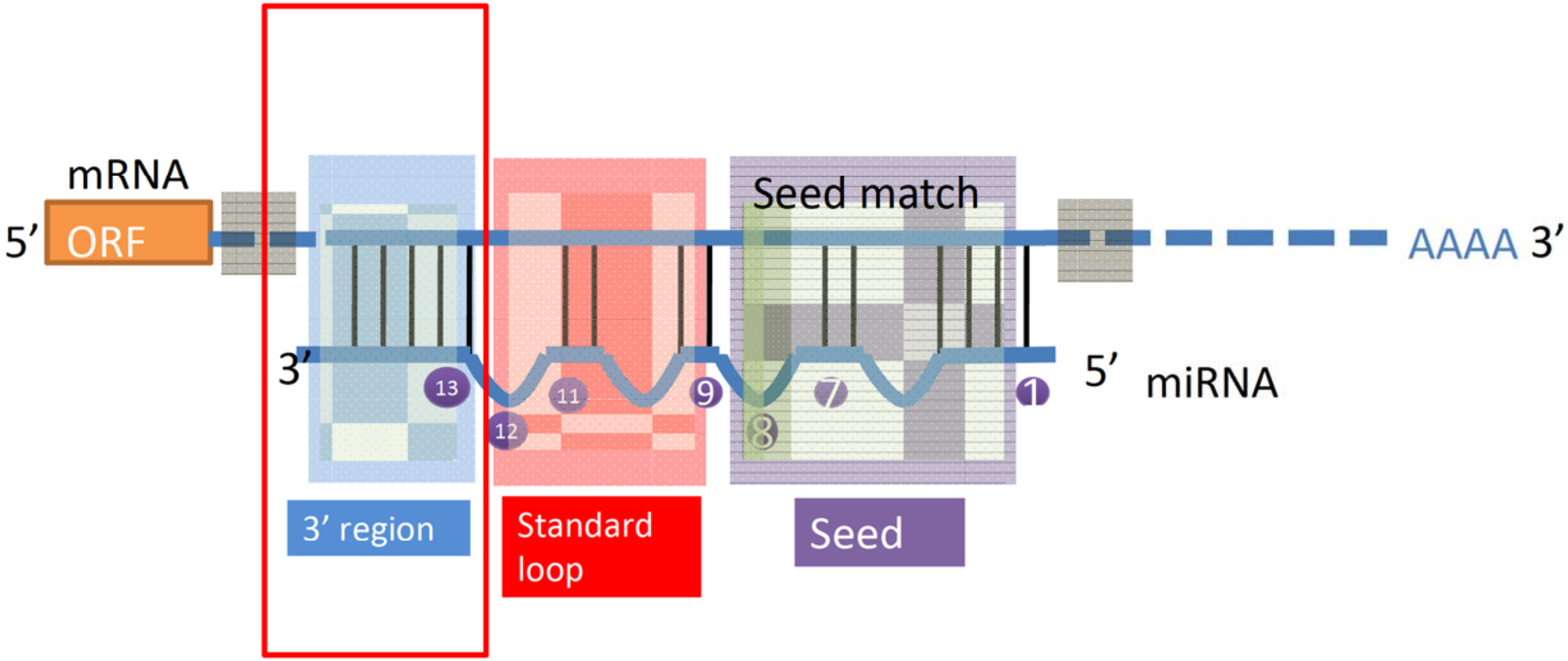
8mer



2 to 8 mer perfect match and position 1 in mRNA must be nucleotide A

8mer >> 7mer-m8 > 7mer-A1 >> 6mer > no site.

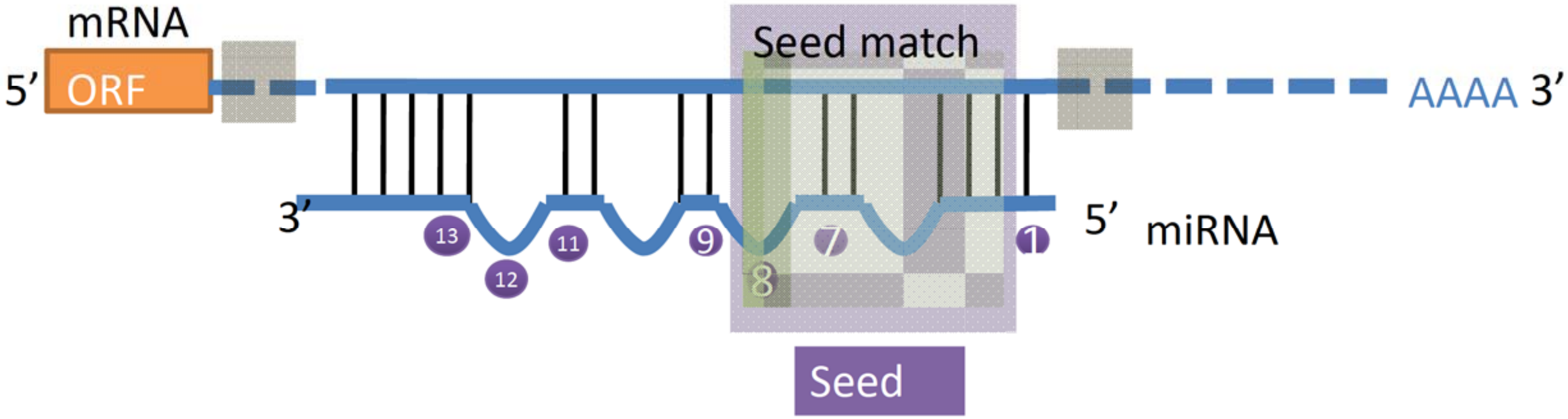
3' pairing contribution



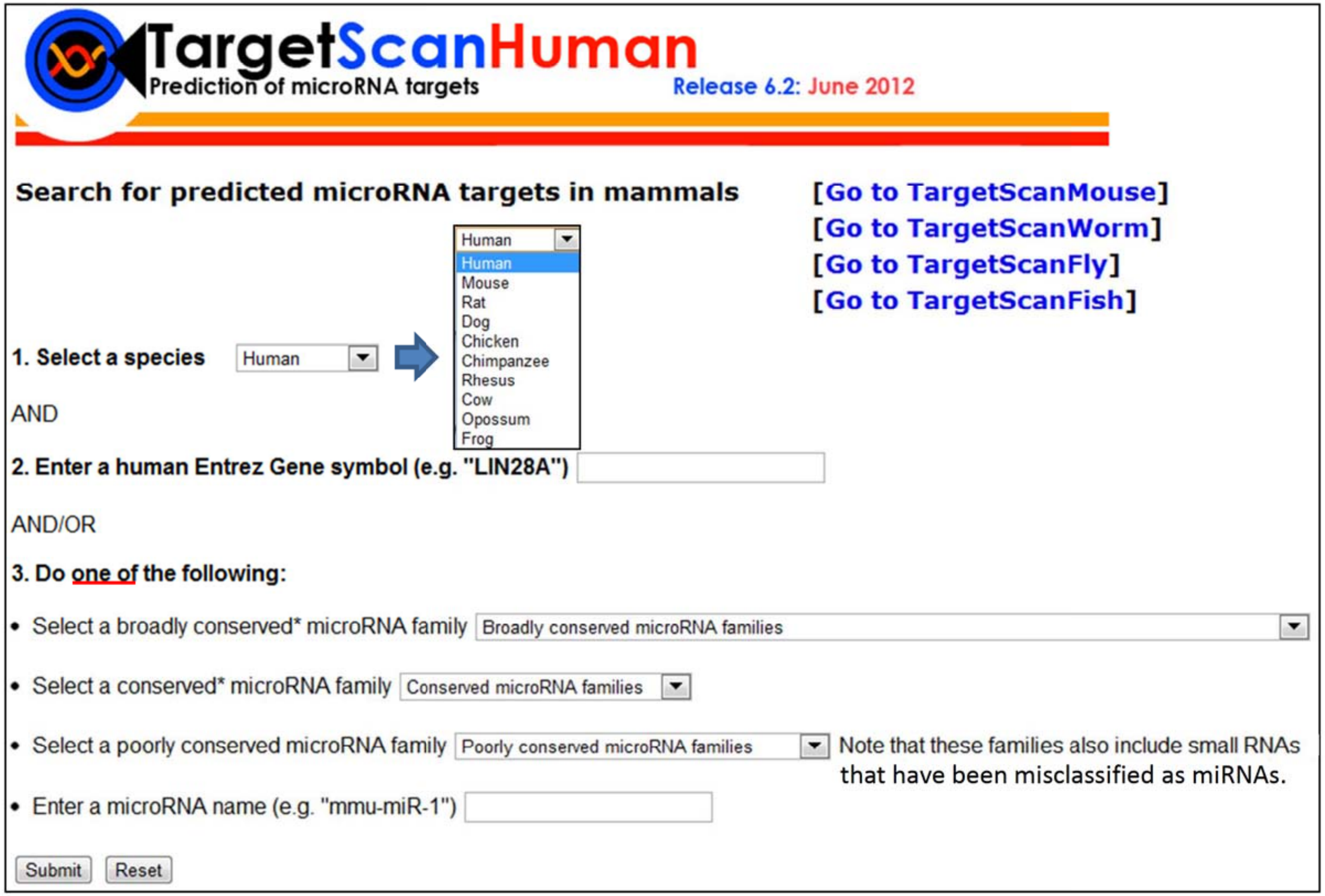
additional Watson-Crick pairing at nucleotides 12–17 enhances miRNA targeting

Local AU & Position contribution

- Local AU contribution
  - Out of seed region is prefer AU rich sequence.
- Position contribution
  - Two ends of long UTRs generally were more effective than those near the center.



Targetscan web interface



Search for predicted microRNA targets in mammals

[Go to TargetScanMouse]
[Go to TargetScanWorm]
[Go to TargetScanFly]
[Go to TargetScanFish]

1. Select a species Human

AND

2. Enter a human Entrez Gene symbol (e.g. "LIN28A")

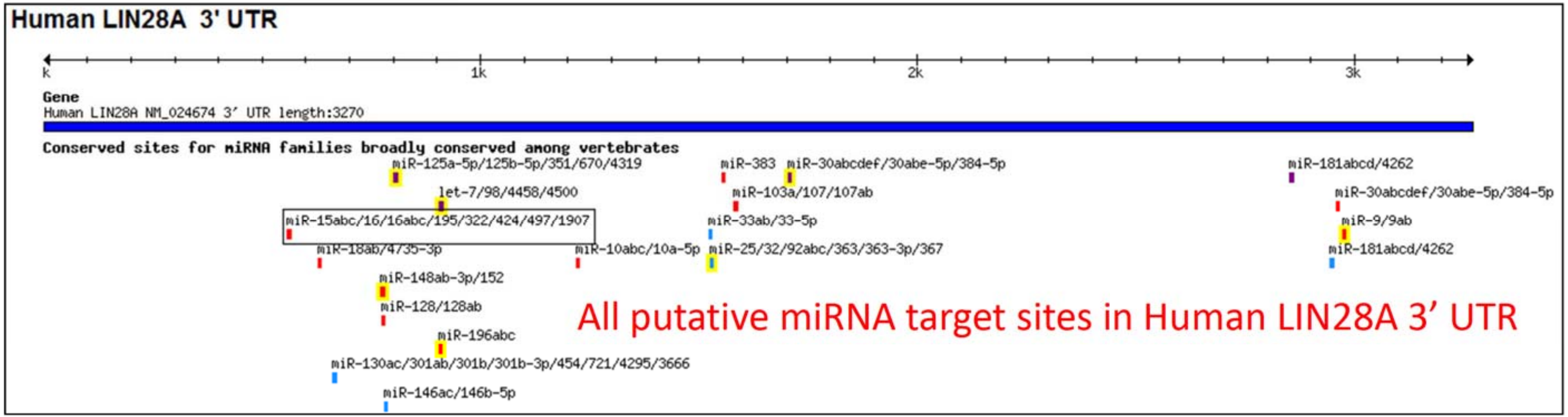
AND/OR

3. Do one of the following:

- Select a broadly conserved* microRNA family: Broadly conserved microRNA families
- Select a conserved* microRNA family: Conserved microRNA families
- Select a poorly conserved microRNA family: Poorly conserved microRNA families — Note that these families also include small RNAs that have been misclassified as miRNAs.
- Enter a microRNA name (e.g. "mmu-miR-1")

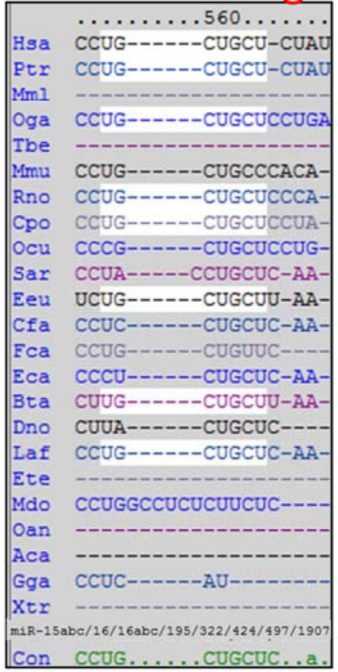
broadly conserved = conserved across most vertebrates, usually to zebrafish.
conserved = conserved across most mammals, but usually not beyond placental mammals.

Targetscan search results



All putative miRNA target sites in Human LIN28A 3' UTR

Conserved Target sites in different species



```
     ...........560.......
Hsa  CCUG------CUGCU-CUAU
Ptr  CCUG------CUGCU-CUAU
Mml  --------------------
Oga  CCUG------CUGCUCCUGA
Tbe  --------------------
Mmu  CCUG------CUGCCCACA-
Rno  CCUG------CUGCUCCCA-
Cpo  CCUG------CUGCUCCUA-
Ocu  CCCG------CUGCUCCUG-
Sar  CCUA-----CCUGCUC-AA-
Eeu  UCUG------CUGCUU-AA-
Cfa  CCUC------CUGCUC-AA-
Fca  CCUG------CUGUUC----
Eca  CCCU------CUGCUC-AA-
Bta  CUUG------CUGCUU-AA-
Dno  CUUA------CUGCUC----
Laf  CCUG------CUGCUC-AA-
Ete  --------------------
Mdo  CCUGGCCUCUCUUCUC----
Oan  --------------------
Aca  --------------------
Gga  CCUC------AU--------
Xtr  --------------------
miR-15abc/16/16abc/195/322/424/497/1907
Con  CCUG......CUGCUC..a.
```

Target prediction rank and others parameter

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contri-bution	3' pairing contri-bution	local AU contri-bution	position contri-bution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	P_{CT}
Position 558-564 of LIN28A 3' UTR hsa-miR-424	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' AAGUUUUGUACUUAACGACGAC	7mer-m8	-0.120	0.003	0.075	0.017	0.019	-0.048	-0.05	28	1.324	0.46
Position 558-564 of LIN28A 3' UTR hsa-miR-497	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' UGUUUGGUGUCACACGACGAC	7mer-m8	-0.120	0.003	0.075	0.017	0.019	-0.048	-0.05	27	1.324	0.46
Position 558-564 of LIN28A 3' UTR hsa-miR-15b	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' ACAUUUGGUACUACACGACGAU	7mer-m8	-0.120	0.003	0.075	0.017	0.019	-0.048	-0.05	27	1.324	0.46
Position 558-564 of LIN28A 3' UTR hsa-miR-15a	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' GUGUUUGGUAAUACACGACGAU	7mer-m8	-0.120	0.003	0.075	0.017	0.019	-0.048	-0.05	27	1.324	0.46
Position 558-564 of LIN28A 3' UTR hsa-miR-195	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' CGGUUAUAAAGACACGACGAU	7mer-m8	-0.120	0.021	0.075	0.017	0.019	-0.048	-0.04	22	1.324	0.46
Position 558-564 of LIN28A 3' UTR hsa-miR-16	5' ...GAACAGCUGCAGACCUGCUGCUC... ||||||| 3' GCGGUUAUAAAUGCACGACGAU	7mer-m8	-0.120	0.021	0.075	0.017	0.019	-0.048	-0.04	22	1.324	0.46

Appendix: other miRNA resources

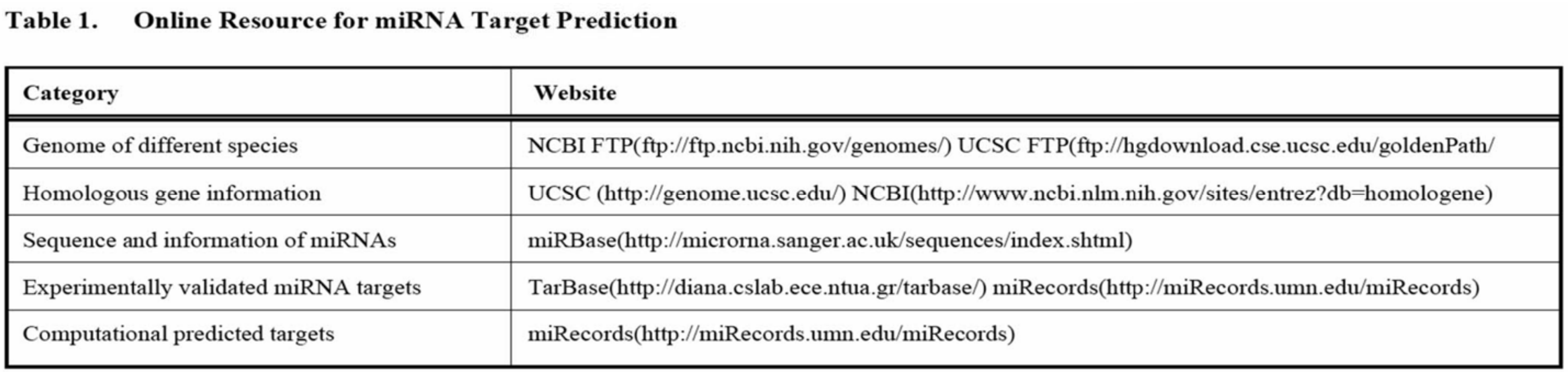


Table 1. **Online Resource for miRNA Target Prediction**

Category	Website
Genome of different species	NCBI FTP(ftp://ftp.ncbi.nih.gov/genomes/) UCSC FTP(ftp://hgdownload.cse.ucsc.edu/goldenPath/
Homologous gene information	UCSC (http://genome.ucsc.edu/) NCBI(http://www.ncbi.nlm.nih.gov/sites/entrez?db=homologene)
Sequence and information of miRNAs	miRBase(http://microrna.sanger.ac.uk/sequences/index.shtml)
Experimentally validated miRNA targets	TarBase(http://diana.cslab.ece.ntua.gr/tarbase/) miRecords(http://miRecords.umn.edu/miRecords)
Computational predicted targets	miRecords(http://miRecords.umn.edu/miRecords)

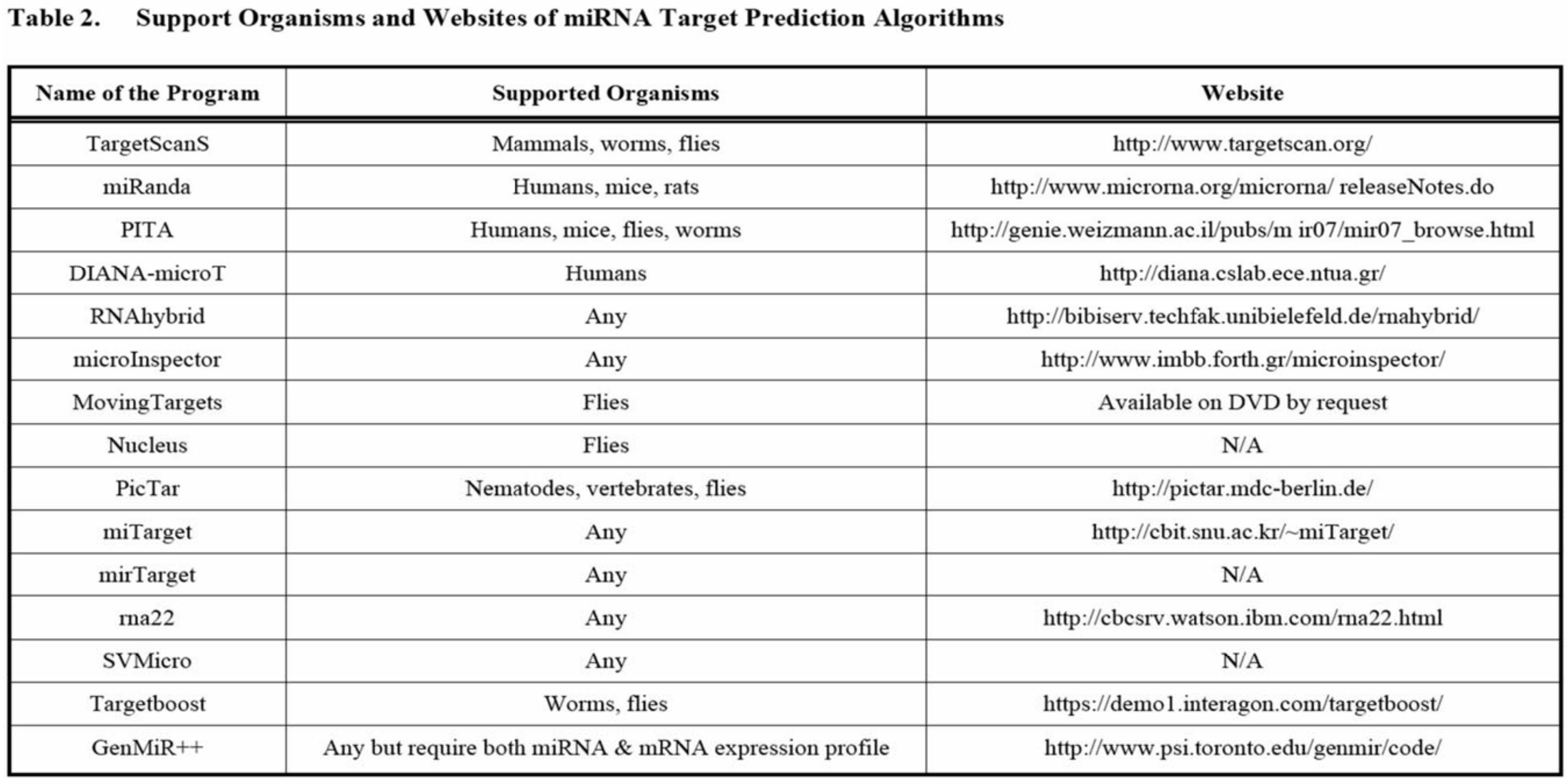


Table 2. **Support Organisms and Websites of miRNA Target Prediction Algorithms**

Name of the Program	Supported Organisms	Website
TargetScanS	Mammals, worms, flies	http://www.targetscan.org/
miRanda	Humans, mice, rats	http://www.microrna.org/microrna/ releaseNotes.do
PITA	Humans, mice, flies, worms	http://genie.weizmann.ac.il/pubs/m ir07/mir07_browse.html
DIANA-microT	Humans	http://diana.cslab.ece.ntua.gr/
RNAhybrid	Any	http://bibiserv.techfak.unibielefeld.de/rnahybrid/
microInspector	Any	http://www.imbb.forth.gr/microinspector/
MovingTargets	Flies	Available on DVD by request
Nucleus	Flies	N/A
PicTar	Nematodes, vertebrates, flies	http://pictar.mdc-berlin.de/
miTarget	Any	http://cbit.snu.ac.kr/~miTarget/
mirTarget	Any	N/A
rna22	Any	http://cbcsrv.watson.ibm.com/rna22.html
SVMicro	Any	N/A
Targetboost	Worms, flies	https://demo1.interagon.com/targetboost/
GenMiR++	Any but require both miRNA & mRNA expression profile	http://www.psi.toronto.edu/genmir/code/

Thanks for your attention

Useful resource for miRNA research

miRNA database	
miRBase	http://www.mirbase.org
miRNAMap	http://miRNAMap.mbc.nctu.edu.tw
miRTarBase	http://miRTarBase.mbc.nctu.edu.tw
miRNA target prediction tools	
TargetScan	http://www.targetscan.org
miRTar	http://mirtar.mbc.nctu.edu.tw
microRNA.org	http://www.microrna.org/microrna/home.do
miRTarCLIP	http://miRTarCLIP.mbc.nctu.edu.tw
miRGator	http://mirgator.kobic.re.kr
MicroCosm	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5
RNA secondary structure folding tools	
mfold	http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form
RNAfold	http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
Example of this class	
Example	http://mirtarbase.mbc.nctu.edu.tw/example.txt