Bioinformatics Analysis of microRNA Regulation and Function

應用生物資訊方法分析microRNA的調控與功能

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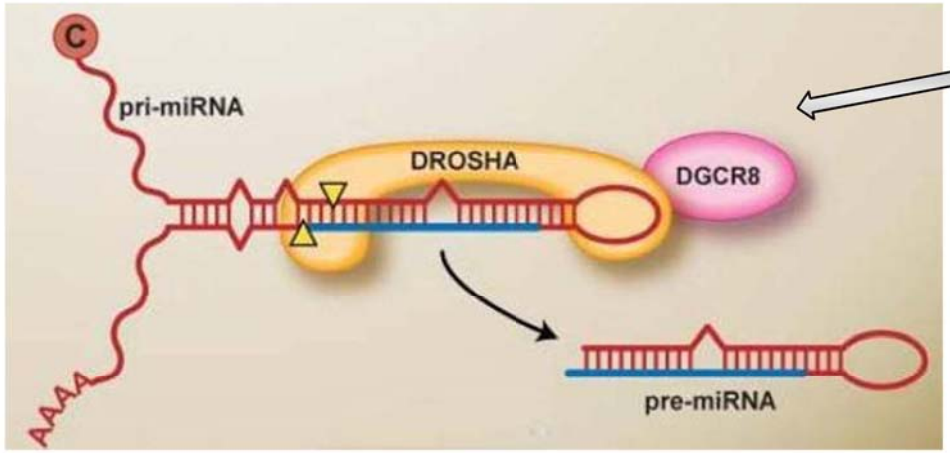
MicroRNA Biogenesis

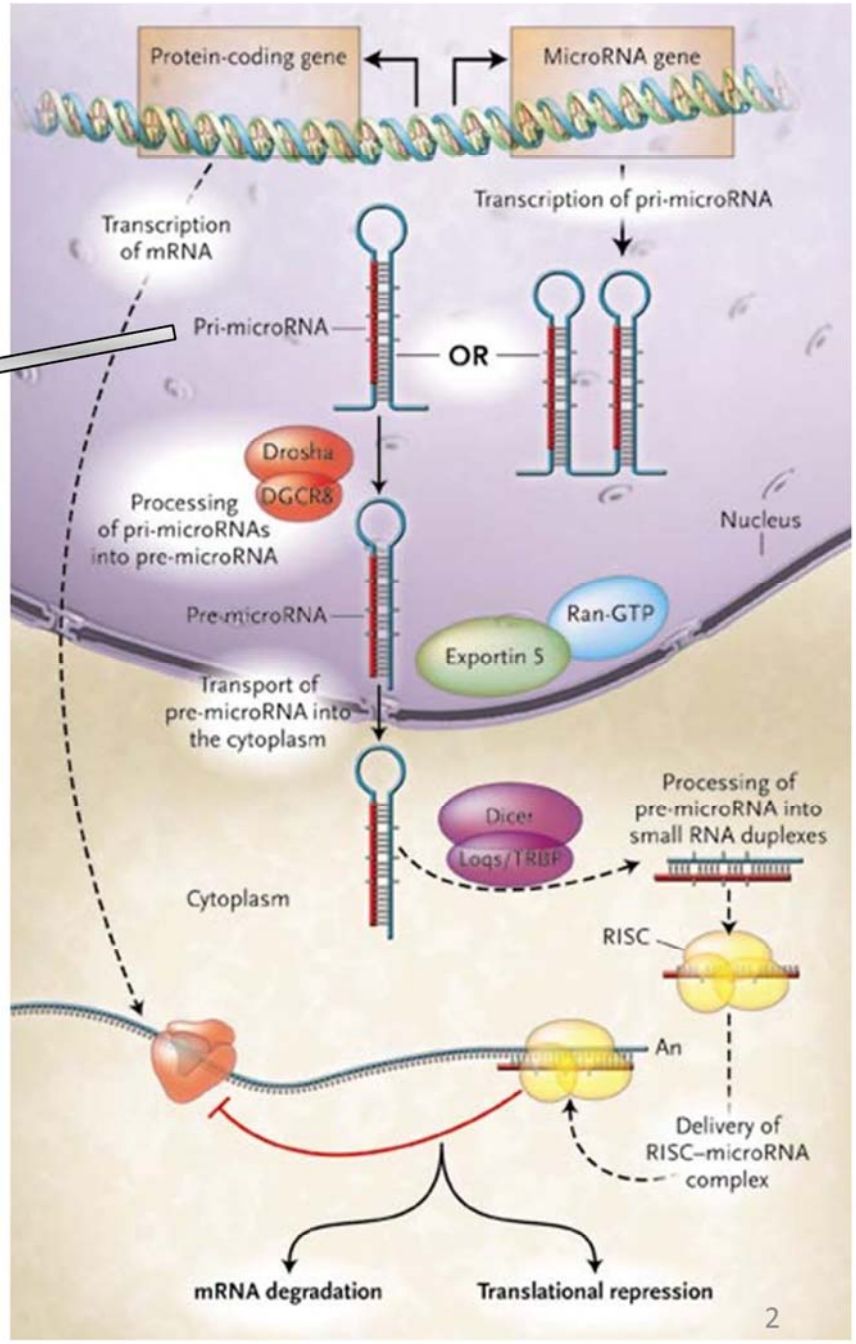


Key Steps in miRNA Biogenesis (Cullen BR., 2004)

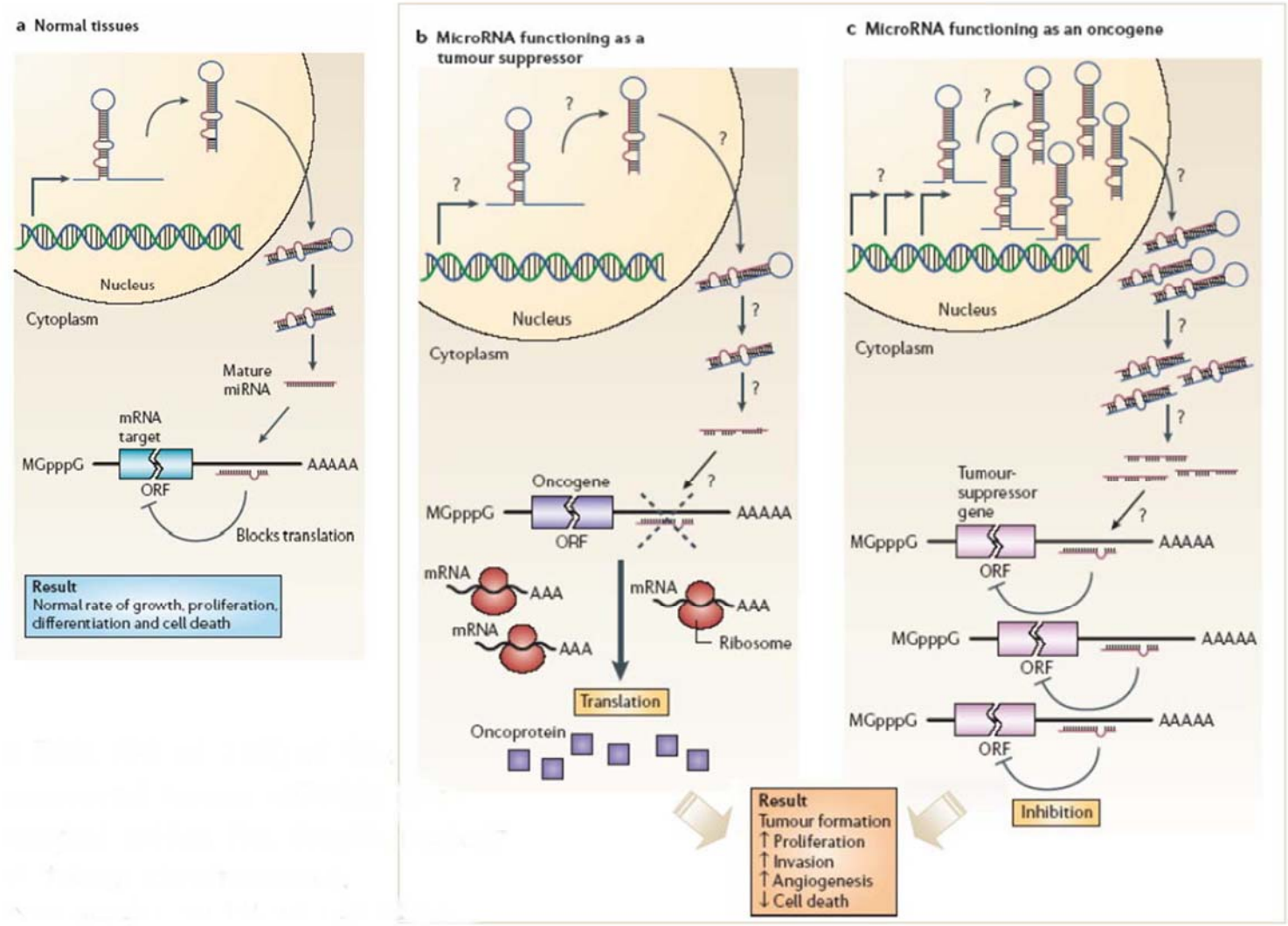
MicroRNAs (miRNAs) are important genetic regulators of development, differentiation, growth, and metabolism

The mammalian genome encodes ≈500 known miRNA genes

Approximately 50% are expressed from non-protein-coding transcripts, whereas the rest are located mostly in the introns of coding genes



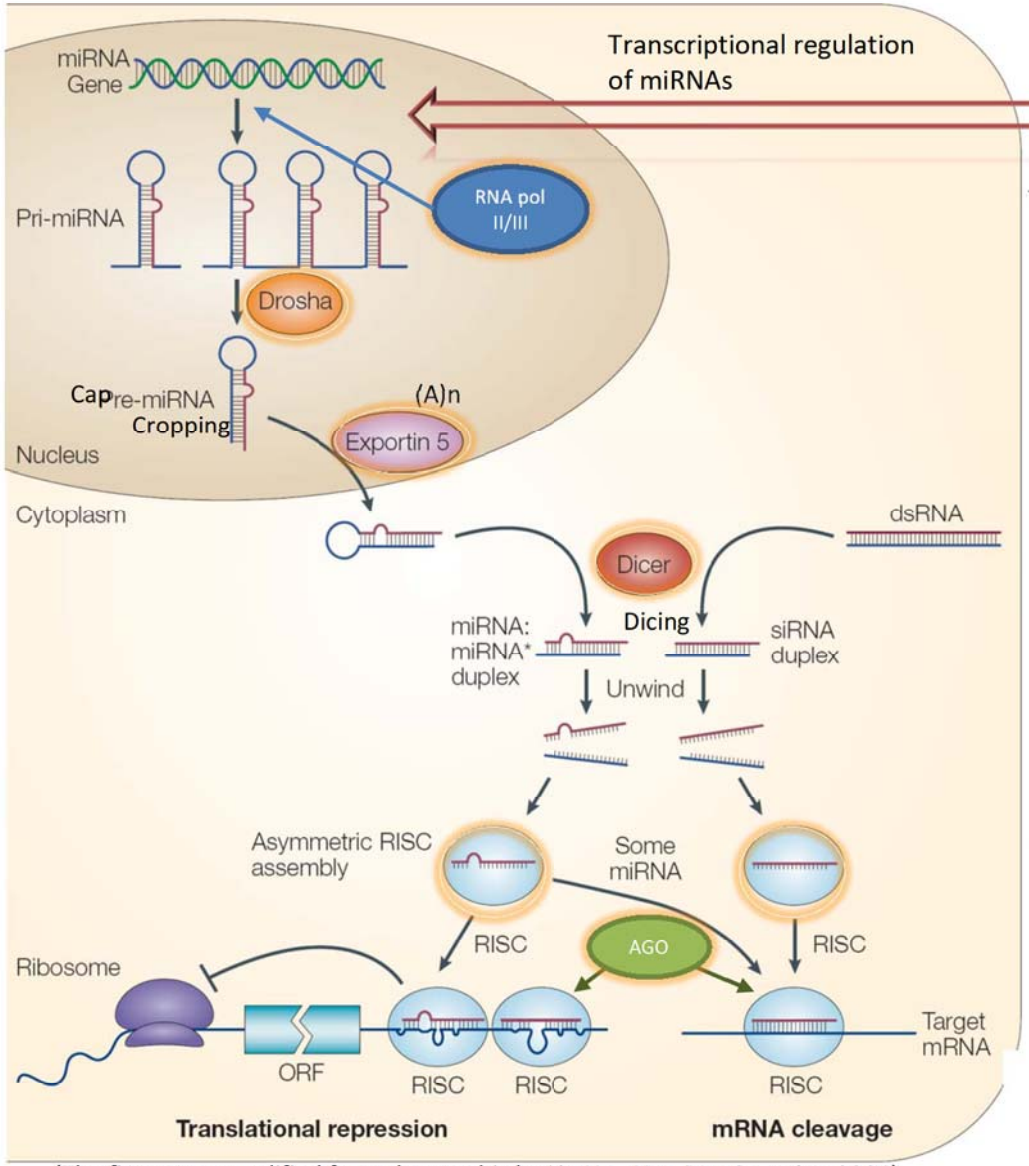
Oncomir: miRNA functions as a tumor suppressor or an oncogene



Nature Reviews 6: 259-267, 2006.

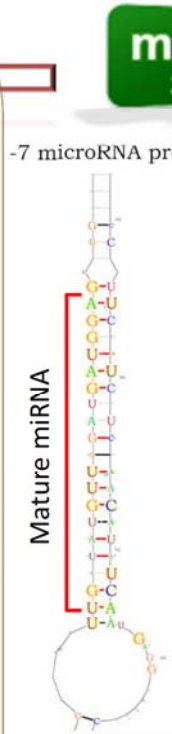
transcription

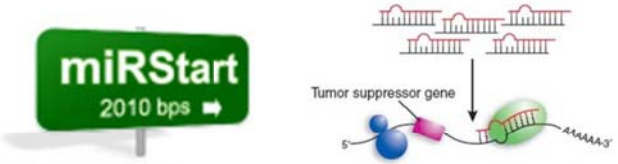
miRNA gene ------> pri-miRNA -> pre-miRNA -> miRNA ----down-regulation-----> target genes



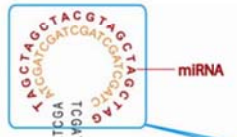
(The figure was modified from the graphic by Lin He, Nat Rev Genetics, 2004)

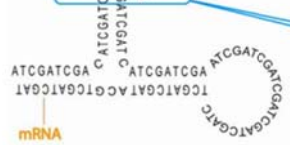
Bioinformatics Research in MicroRNA Regulation: Databases and Tools

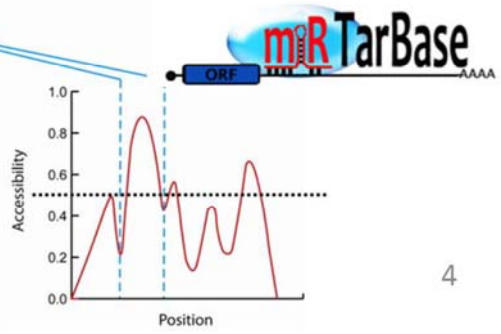




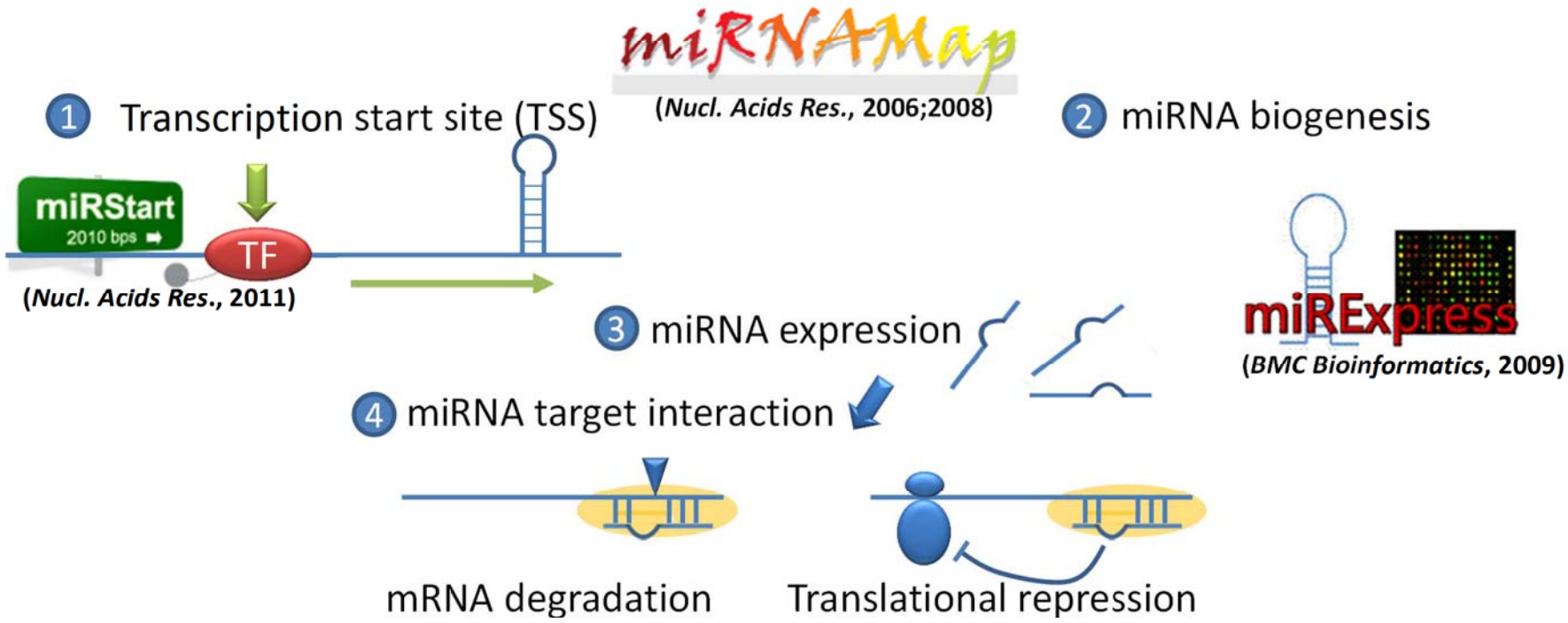
Identifying miRNA targets







MicroRNA Repository



(Nucl. Acids Res., 2011)

(Nucl. Acids Res., 2006)

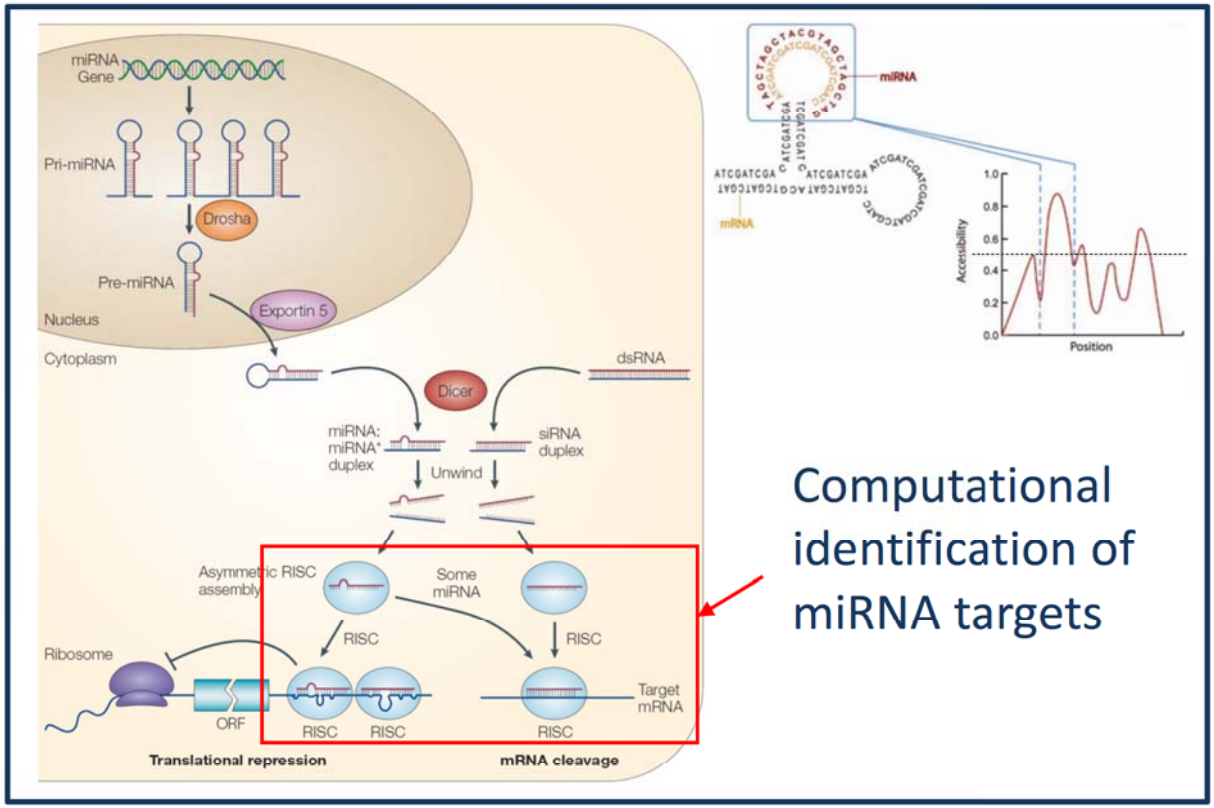
(BMC Bioinformatics, 2011)

(Nucl. Acids Res., 2007)

(BMC Genomics, 2013)

Bioinformatics Resources for MicroRNA Research

- Gene prediction and annotation:
  - Identification of miRNA genes (intergenic and intragenic miRNAs)
    - miRNAMap (*NAR, 2006, 2008*), miRExpress (*BMC Bioinformatics, 2009*)
  - Comparative analysis of miRNAs
    - RNALogo (*NAR, 2009*)
- Functions of miRNAs:
  - Identification of miRNA targets
    - miRNAMap (*NAR, 2006, 2008*), miRTarBase (*NAR, 2011*), miRTar (*BMC Bioinformatics, 2011*) and ViTa (*NAR, 2009*), miRTarCLIP (*BMC Genomics*, 2013)
  - Tumor suppressors or oncogenes
- Regulation of miRNAs:
  - Transcriptional regulation of miRNA
    - miRStart (*NAR, 2011*)
  - Expression profiles of miRNAs
    - miRExpress (*BMC Bioinformatics, 2009*)

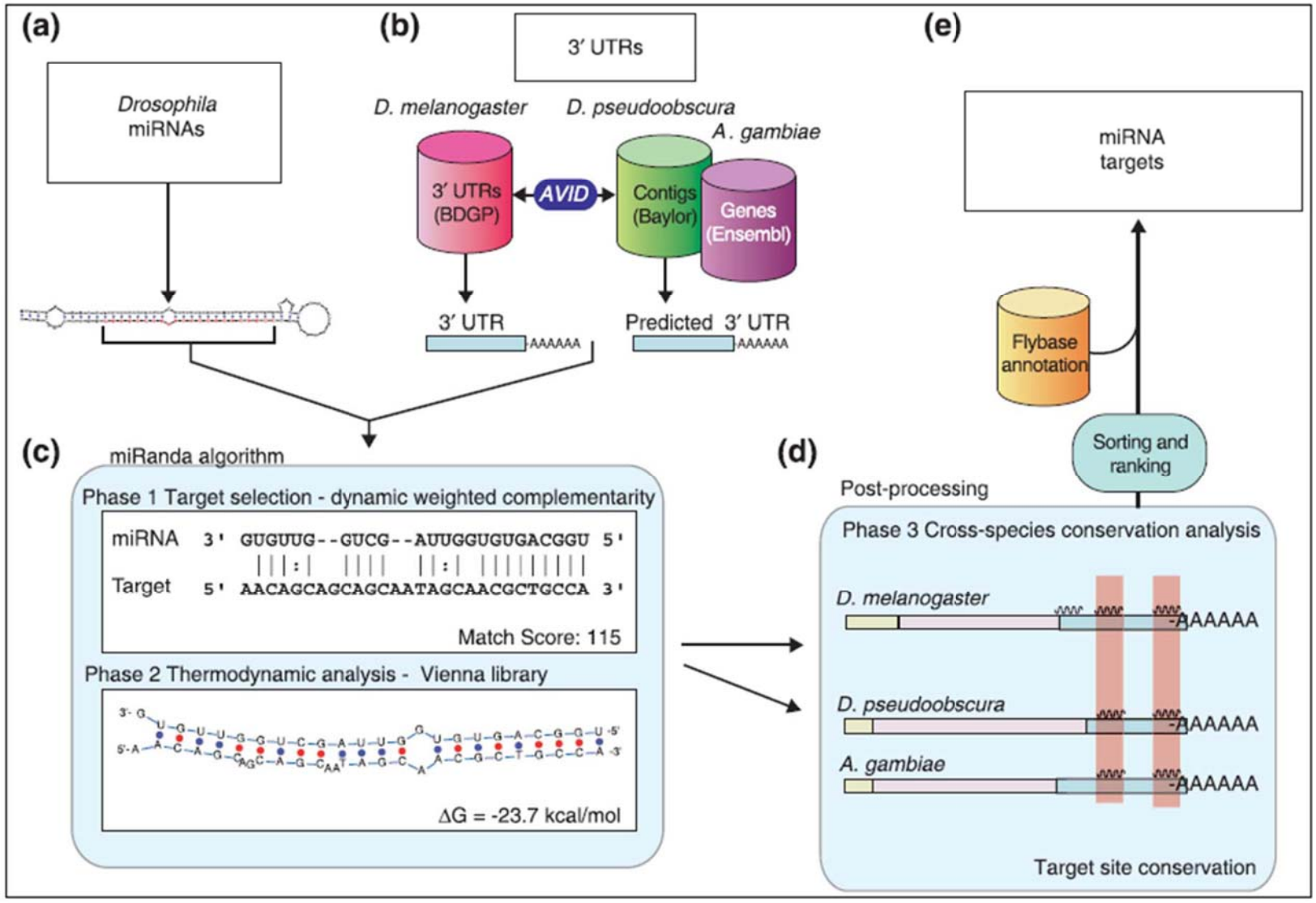


Tools for identifying miRNA targets

miRanda

- microRNA sequences (in 2003) (*Genome Biol. 5*, R1, 2003)
  - miRBase
  - 73 unique miRNA sequences
- 3'UTR sequences
  - BDGP (Berkeley *Drosophila* Genome Project)
- Looking for high-complementarity regions on the 3'UTRs.
  - dynamic programming algorithm
- Evaluate thermodynamic of potential binding sites
  - Vienna RNA folding package

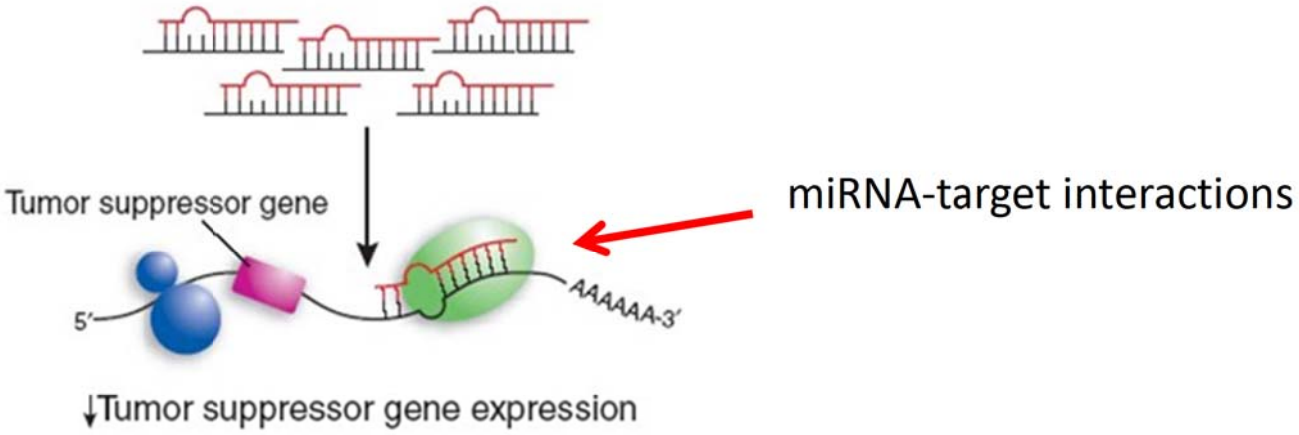
miRanda - Algorithms




ISBLAB Dr. Hsien-Da Huang, professor
miRTar.Mouse miRTar.Dog
miRTar.Human An integrated web server for identifying miRNA-target interactions in human
Home About Prediction Tutorial Contact Us
miRTar
MicroRNA Target prediction (miRTar) is a tool that enables biologists easily to identify the biological functions and regulatory relationships between a group of known/putative miRNAs and protein coding genes. It also provides perspective of information on the miRNA targets on alternatively spliced transcripts.
miRTar supports four major features:
Identifying miRNA Targets in Human
Multiple scenarios
Viewpoint on the regulation between miRNA and RNA alternative splicing
Gene enrichment in KEGG pathway maps
Examples
1 Mature miRNA sequence
Targets To
2 Target Gene
Submit
Reset

miRTar provides multiple functions for identifying miRNA-target interactions

- 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:N)



Input

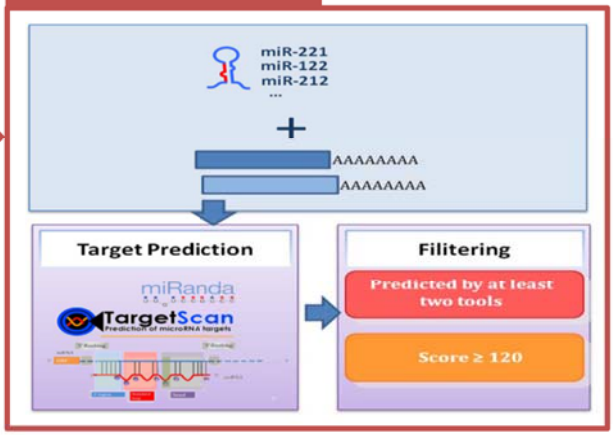
miRNA(s)

has-miR-34a; has-miR-34a*; has-miR-34b; ...

a group of genes

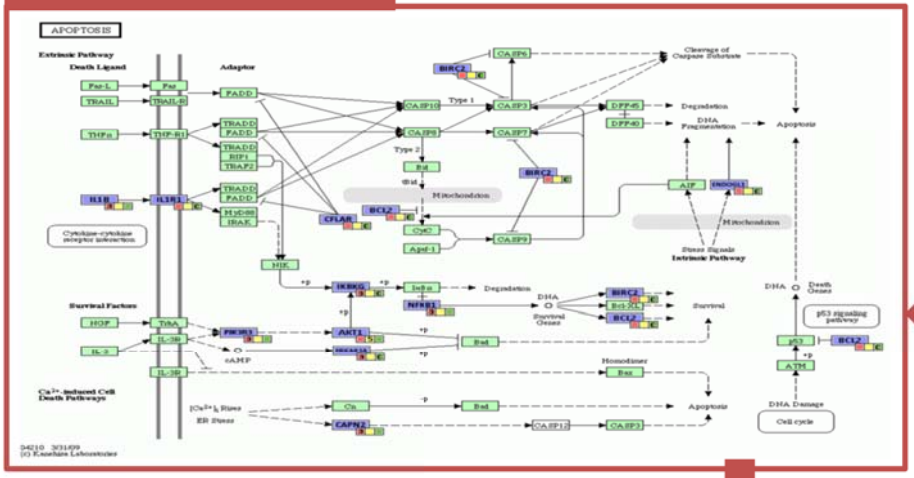
BCL2; CCNE2; MET; CDK4; NOTCH1;SIRT1

miRNA target prediction

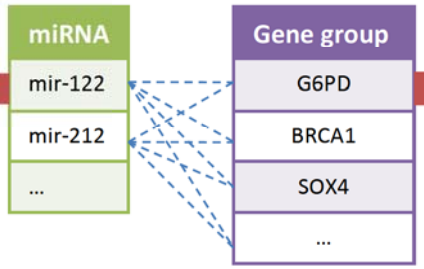


The pathway of the target gene

1 miRNA vs. m genes /3'(5')UTR|CDS



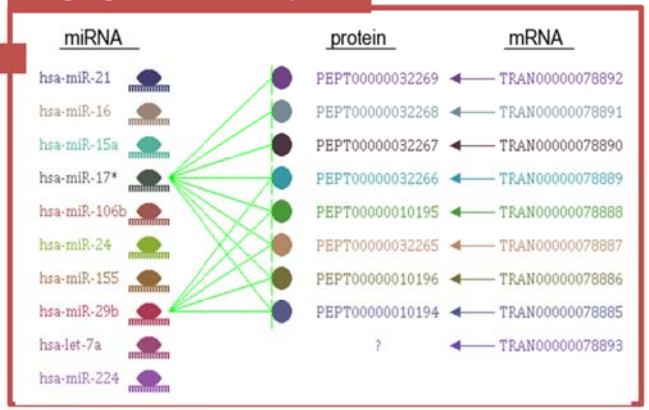
Possible relation between miRNA and gene group



miRNA target gene info. and structure

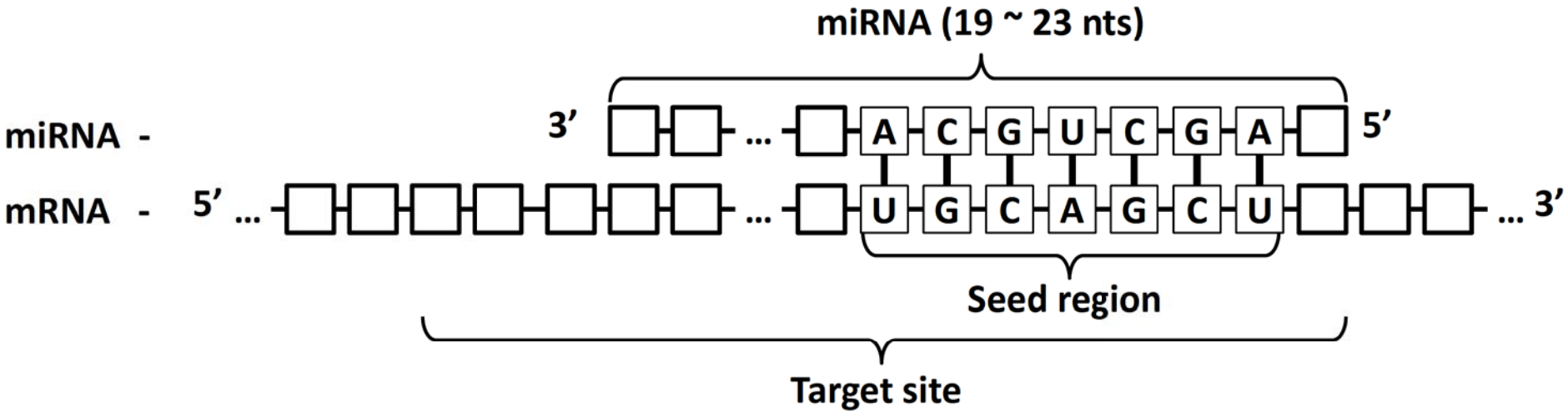
Target gene and AS analysis

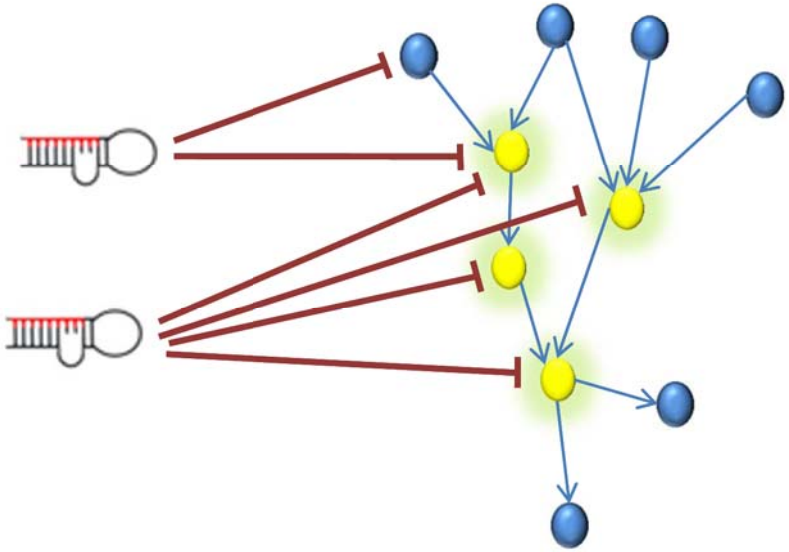
n miRNA vs. 1 gene

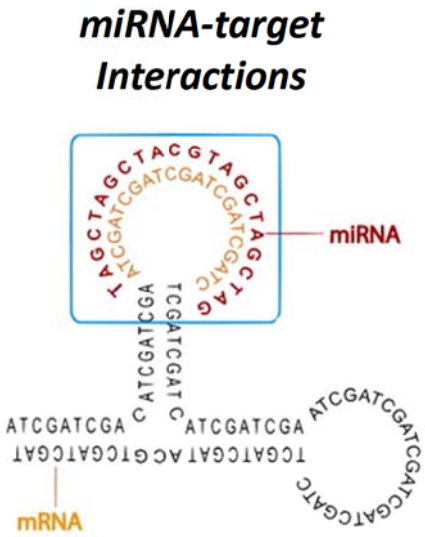


*AS: alternative splicing

miRNA-target interactions (MTIs)



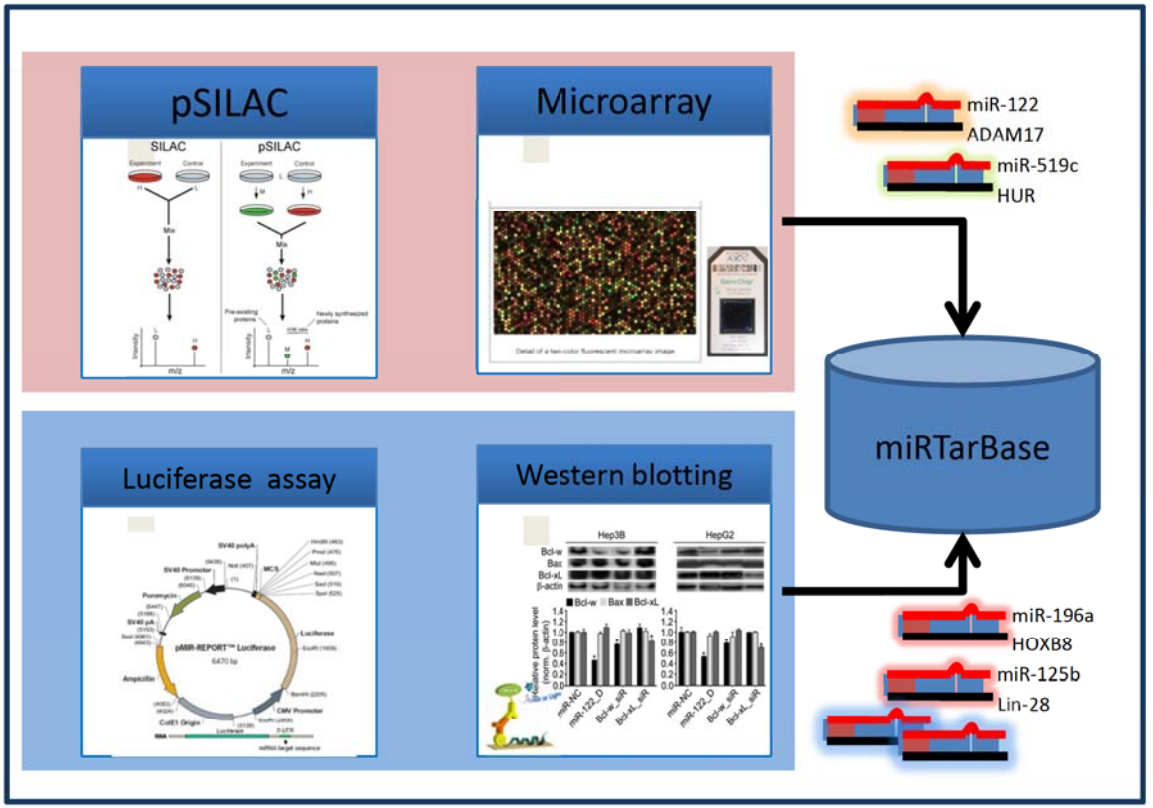


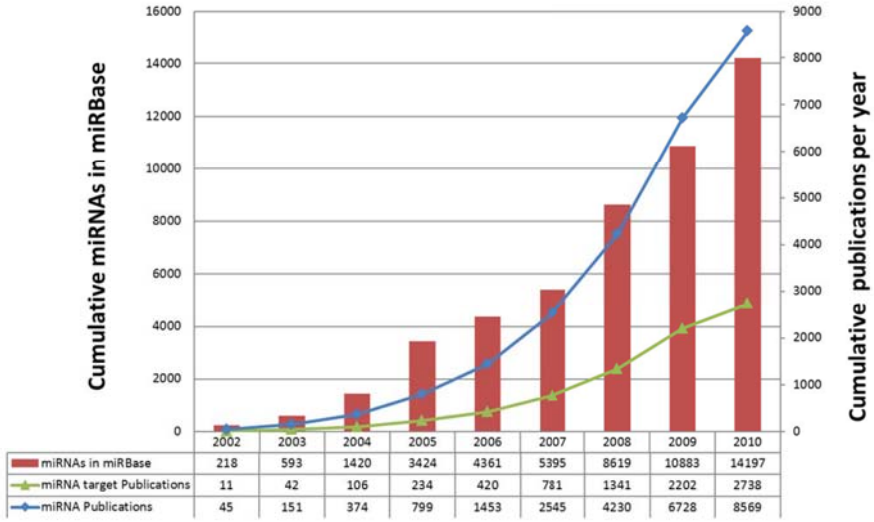


miRTarBase

a database curates experimentally validated miRNA-target interactions (NAR database issue, 2011)

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

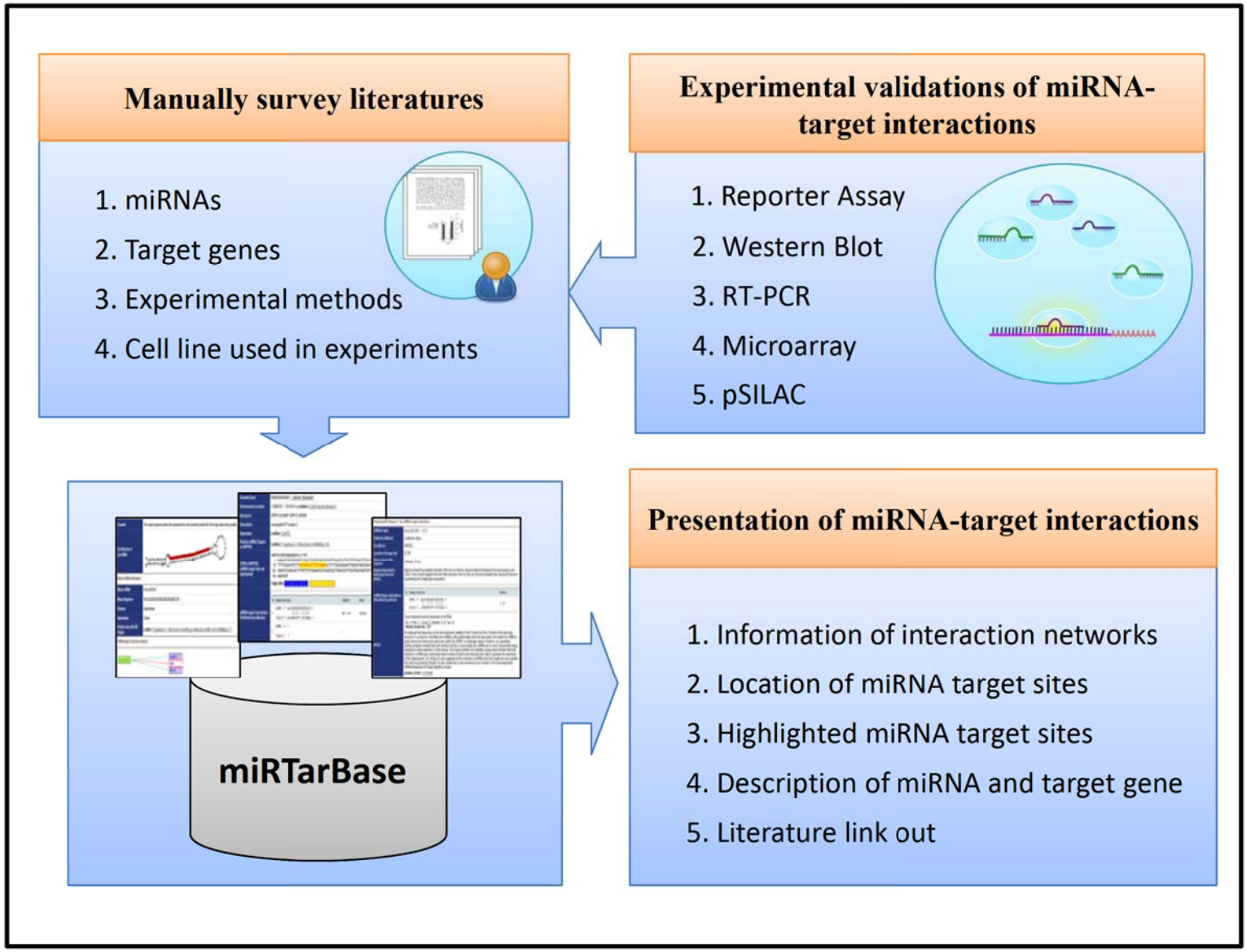




	2002	2003	2004	2005	2006	2007	2008	2009	2010
miRNAs in miRBase	218	593	1420	3424	4361	5395	8619	10883	14197
miRNA target Publications	11	42	106	234	420	781	1341	2202	2738
miRNA Publications	45	151	374	799	1453	2545	4230	6728	8569

1. By surveying literature
2. To collect updated information of miRNA-target interactions
3. _4270 entries now, will be more than 10,000 entries in 2013, expectedly
4. Biggest collection now
4. Discover miRNA-target interactions in mouse based on the extension of human miRNA-target interaction (Evolutionary conservation)

System flow of miRTarBase

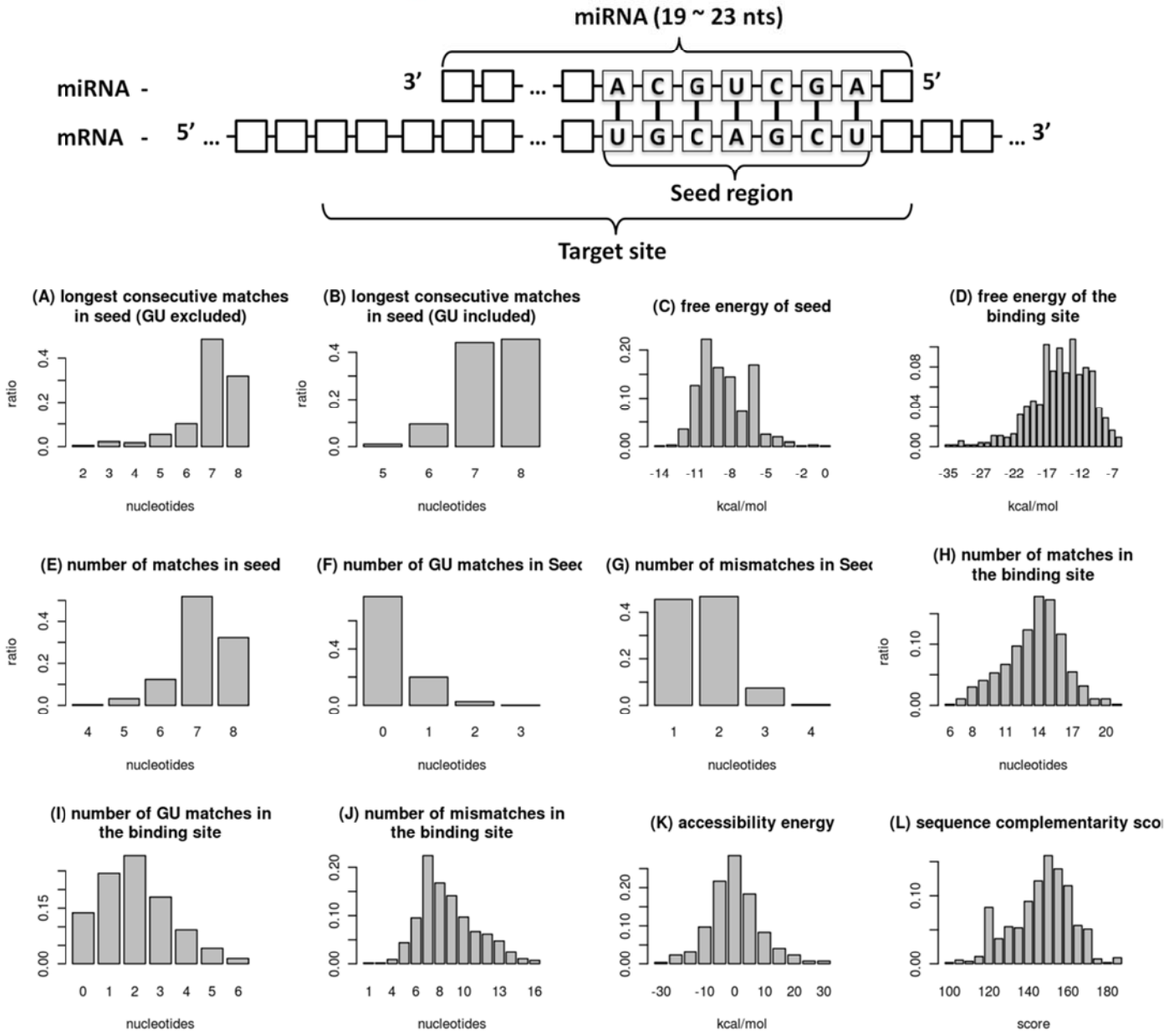


Comparison of miRTarBase with other MTI databases

	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

Histogram of various features of experimental proven miRNA-target sites

Data were obtained from 527 human miRNA-target interactions curated in miRTarBase



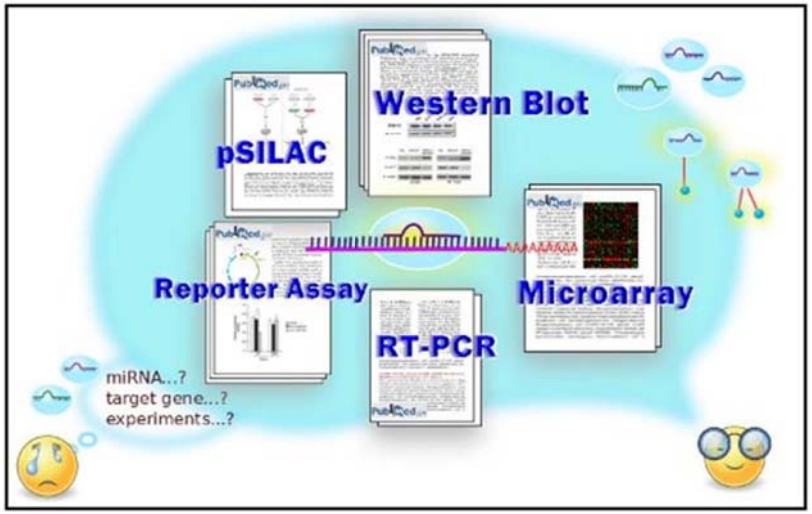
As a database, miRTarBase has accumulated more than three thousand **miRNA-target interactions (MTIs)**, which are collected by manually surveying pertinent literature after data mining of the text systematically to filter research articles related to functional studies of miRNAs. Generally, the collected MTIs are validated experimentally by reporter assay, western blot, or microarray experiments with overexpression or knockdown of miRNAs. miRTarBase currently curates **3,576 experimentally verified MTIs between 657 miRNAs and 2,297 target genes** among 17 species. While containing the largest amount of validated MTIs, the miRTarBase provides the most updated collection by comparing with other similar, previously developed databases.

Go to browse the database !!

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

miRTarBase: a database curates experimentally validated microRNA-target interactions

Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, Chien CH, Wu MC, Huang CY, Tsou AP, Huang HD. (2011) Nucleic acids research. [PUBMED]



Current curation

- Number of miRNA-target interactions: **3,576**
- Number of miRNAs:**657**
- Number of target genes: **2,297**
- Number of species: **17**
- Number of articles: **985**
- Release 1.0: Oct. 15, 2010

How to donate your data?

- Suggest an article
- Submit miRNA-target interactions
- Report errors

Quick links for popular miRNAs or target genes

- Example 1: *hsa-miR-122* is a liver-specific microRNA which is significantly down-regulated in liver cancers.
- Example 2: *hsa-miR-1* - microRNA-1-1 (miR-1-1) and miR-1-2 are specifically expressed in cardiac and skeletal muscle precursor cells.
- Example 3: *CDKN1A* - Cyclin-dependent kinase inhibitor 1A (CDKN1A), also known as p21Cip1/Waf1, is a master downstream effector of tumor suppressors.
- Example 4: *HIF1A* - Hypoxia-inducible factor-1alpha (HIF-1alpha) is widely considered to be one of the key regulators of tumor angiogenesis.
- Example 5: *HMGA2* is an important regulator of cell growth, differentiation, apoptosis, and transformation.

Development plans and sponsors

- **We are a dedicated group of people, who aim to create a leading repository for miRNA-target interactions.**
- Funded by National Science Council, Taiwan
- Funded by National Chiao Tung University

Search... SEARCH EXAMPLE

Home | Search | Browse | Statistics | Help | Submit | Contact Us

Search the experimentally verified miRNA-target interactions with miRNA like ' %mir-122% '

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ID	Species	miRNA	Target Gene	Validation Method
MIRT000012	Human (Homo sapiens)	hsa-miR-122	CYP7A1	qRT-PCR, Luciferase assay
MIRT000364	Human (Homo sapiens)	hsa-miR-122	Igf1R	Luciferase assay
MIRT000365	Human (Homo sapiens)	hsa-miR-122	SRF	Luciferase assay
MIRT000366	Human (Homo sapiens)	hsa-miR-122	ADAM-10	Luciferase assay
MIRT000663	Human (Homo sapiens)	hsa-miR-122	RAC1	Luciferase assay, Review
MIRT000717	Human (Homo sapiens)	hsa-miR-122	RHOA	Luciferase assay, Review
MIRT000943	Human (Homo sapiens)	hsa-miR-122	Bcl-w	Luciferase assay, RT-PCR, Western blot
MIRT003006	Human (Homo sapiens)	hsa-miR-122	CCNG1	Reporter assay, Luciferase assay
MIRT003079	Human (Homo sapiens)	hsa-miR-122	GTF2B	RT-PCR, real time RT-PCR
MIRT003080	Human (Homo sapiens)	hsa-miR-122	GYS1	RT-PCR, Western blot, Northern blot, real time RT-PCR
MIRT003081	Human (Homo sapiens)	hsa-miR-122	ANK2	Dual-luciferase assay, RT-PCR
MIRT003082	Human (Homo sapiens)	hsa-miR-122	NFATC2IP	Dual-luciferase assay, RT-PCR
MIRT003083	Human (Homo sapiens)	hsa-miR-122	ENTPD4	Dual-luciferase assay, RT-PCR
MIRT003084	Human (Homo sapiens)	hsa-miR-122	ANXA11	Dual-luciferase assay, RT-PCR
MIRT003085	Human (Homo sapiens)	hsa-miR-122	ALDOA	Dual-luciferase assay, RT-PCR, Northern blot
MIRT003086	Human (Homo sapiens)	hsa-miR-122	RAB6B	Dual-luciferase assay, RT-PCR
MIRT003087	Human (Homo sapiens)	hsa-miR-122	RAB11FIP1	Dual-luciferase assay, RT-PCR
MIRT003088	Human (Homo sapiens)	hsa-miR-122	FOXP1	Dual-luciferase assay, RT-PCR
MIRT003089	Human (Homo sapiens)	hsa-miR-122	MECP2	Dual-luciferase assay, RT-PCR
MIRT003090	Human (Homo sapiens)	hsa-miR-122	NCAM1	Dual-luciferase assay, RT-PCR
MIRT003091	Human (Homo sapiens)	hsa-miR-122	UBAP2	Dual-luciferase assay, RT-PCR
MIRT003092	Human (Homo sapiens)	hsa-miR-122	TBX19	Dual-luciferase assay, RT-PCR
MIRT003093	Human (Homo sapiens)	hsa-miR-122	AACS	Dual-luciferase assay, RT-PCR
MIRT003094	Human (Homo sapiens)	hsa-miR-122	DUSP2	Dual-luciferase assay, RT-PCR
MIRT003095	Human (Homo sapiens)	hsa-miR-122	ATP1A2	Dual-luciferase assay, RT-PCR
MIRT003096	Human (Homo sapiens)	hsa-miR-122	ALS2CR13	Dual-luciferase assay, RT-PCR
MIRT003097	Human (Homo sapiens)	hsa-miR-122	MAPK11	Dual-luciferase assay, RT-PCR
MIRT003098	Human (Homo sapiens)	hsa-miR-122	FUNDC2	Dual-luciferase assay, RT-PCR
MIRT003099	Human (Homo sapiens)	hsa-miR-122	AKT3	Dual-luciferase assay, RT-PCR
MIRT003100	Human (Homo sapiens)	hsa-miR-122	TPD52L2	Dual-luciferase assay, RT-PCR

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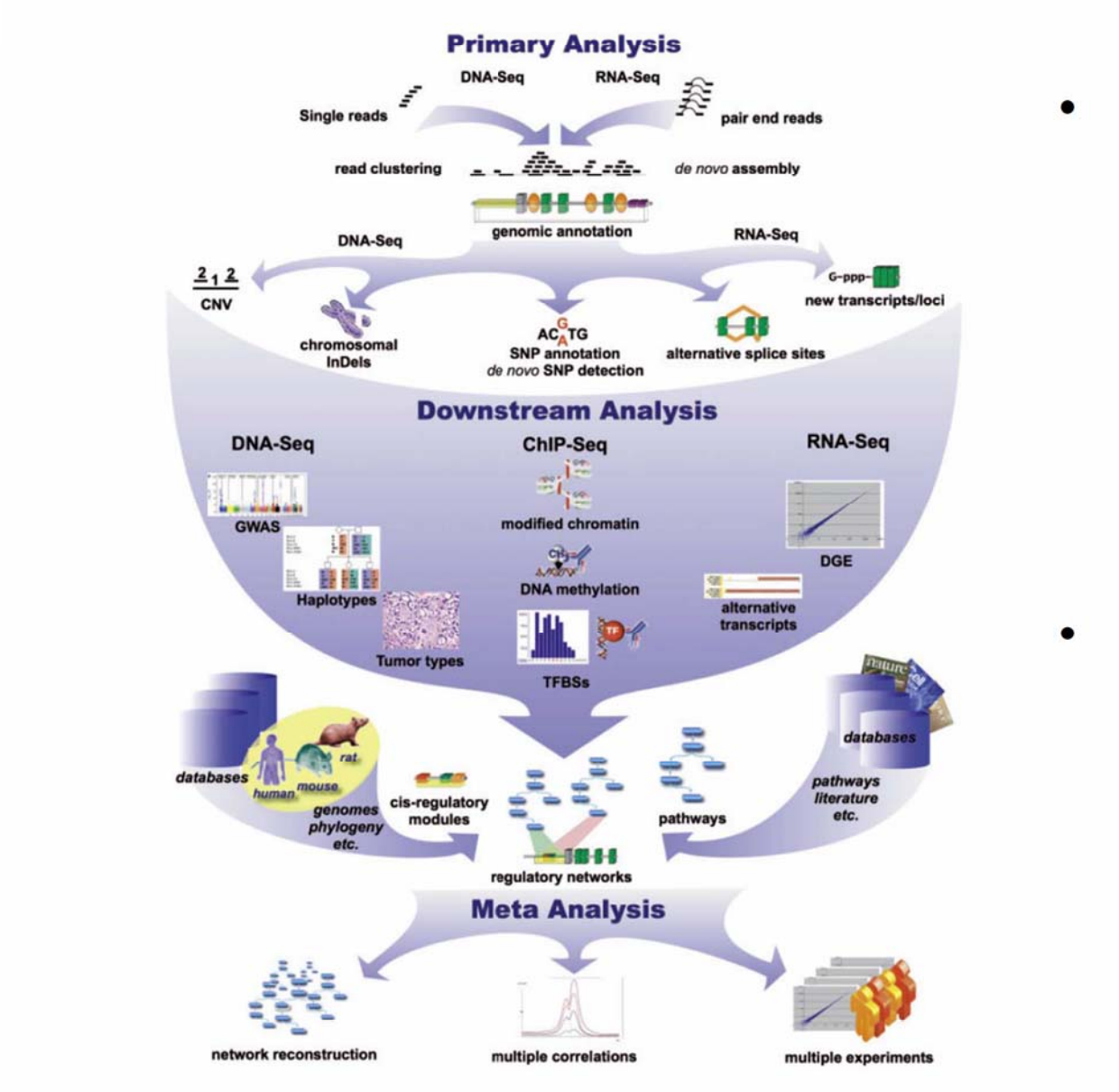
Next Generation Sequencing (NGS) in Functional Genomics



- Pipeline ready
  - RNA-seq
  - **Small RNA-seq**
  - ChIP-seq
  - Genome sequencing
  - Human resequencing
  - DNA methylation sequencing
- In developing
  - Amplicons sequencing
  - Metagenomics
    - Ribosomal RNAs sequencing only
    - Multiple genome sequencing

Figure from Briefings in Bioinformatics, 2010, Vol. 11, No.5, pp. 499-511

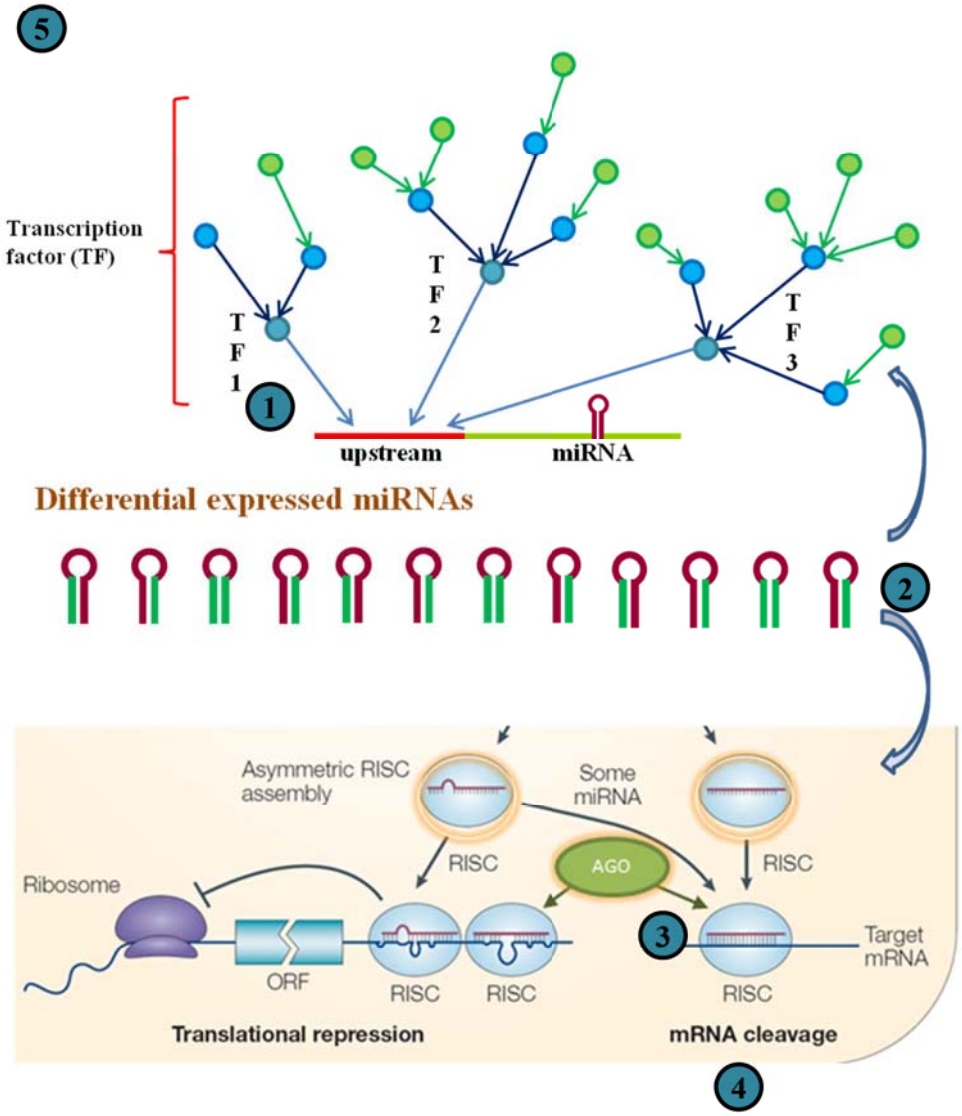
Next-Generation Sequencing (NGS) - small RNA sequencing

- Identifying TFs which regulate miRNAs

- Identification of miRNAs and miRNA expression profiling
- Identification of miRNAs-targets interaction

miRTarCLIP

Using NGS for analyzing small non-coding RNAs



1. **Identifying TFs which regulate miRNAs**

   Chip (chromatin immunoprecipitation)-seq

2. **Profiling miRNA expression**

   Small RNA NGS sequencing

3. **Identifying miRNA target interaction**

   CLIP (crosslinking immunoprecipitation)-seq

   Degradome-seq

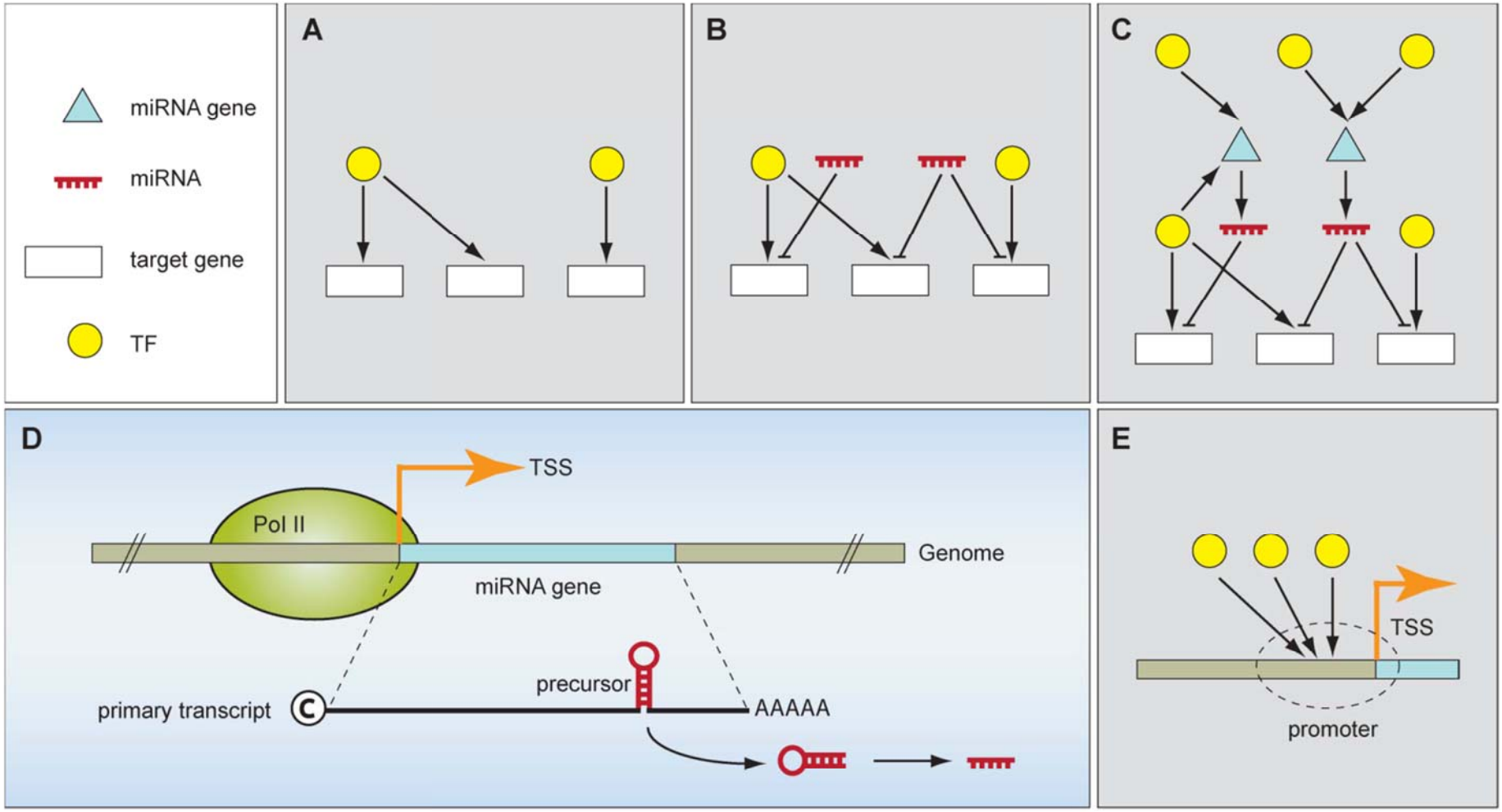
4. **mRNA expression**

   RNA-seq

5. **DNA methylation**

   BS (bisulfate)-seq
   MeDIP (methylated DNA immunoprecipitation)-seq
   MBD (methyl-binding protein)-seq

Transcriptional regulation of miRNA genes

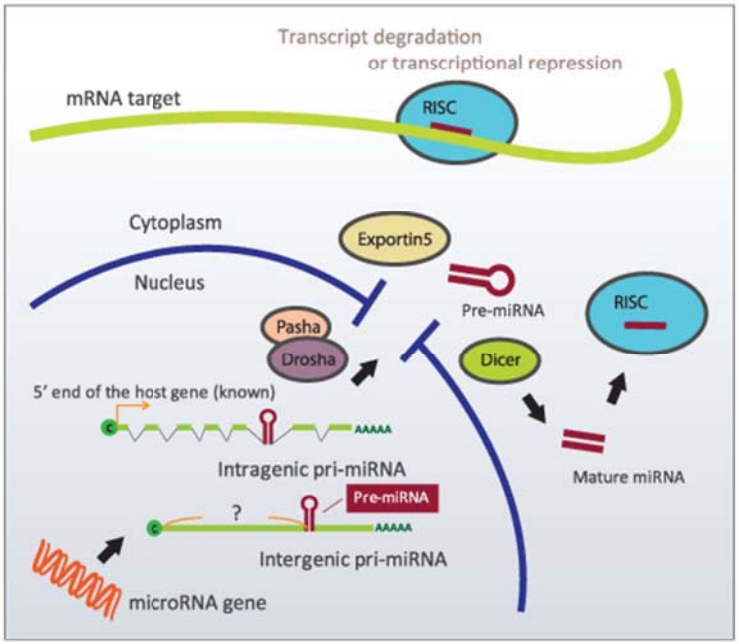


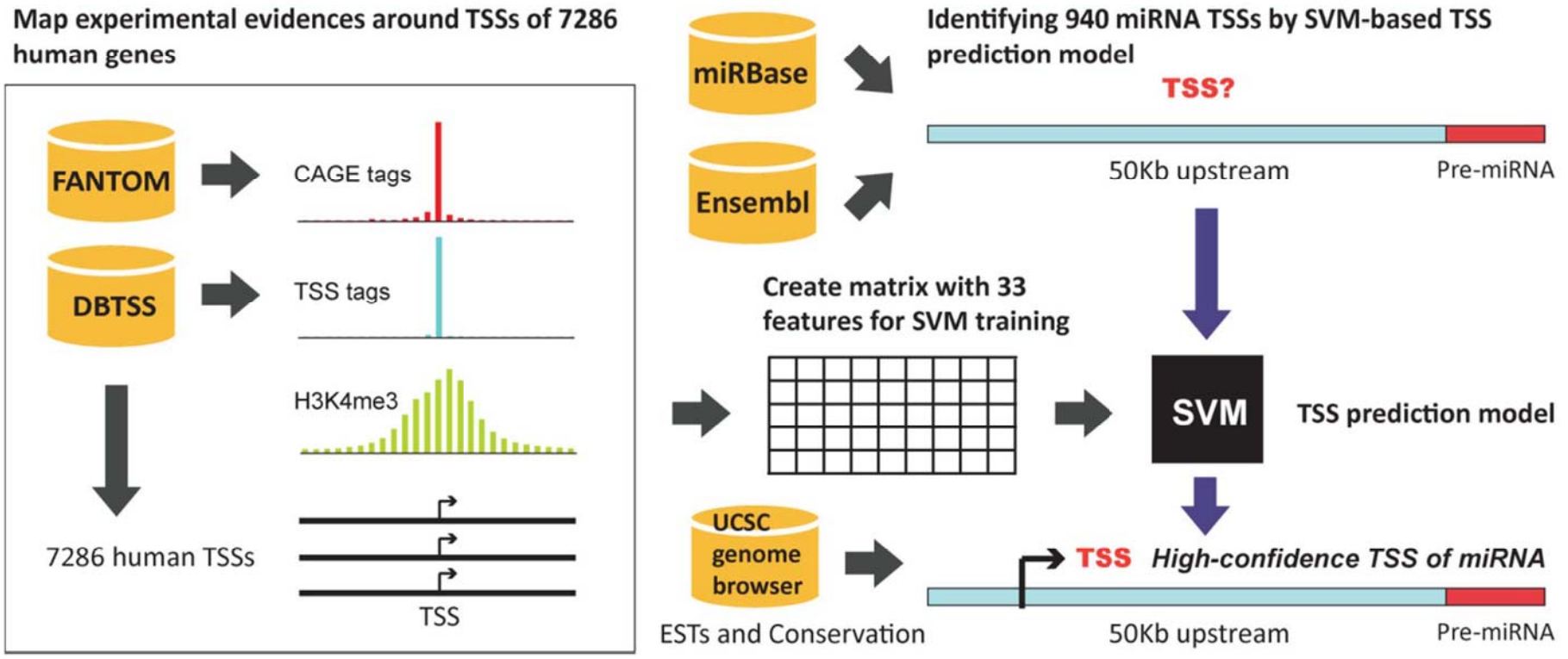
Deciphering transcriptional regulation of miRNAs

miRStart: An experimental-based resource defines transcriptional start sites of human microRNAs

(Nucleic Acids Research, 2011)

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



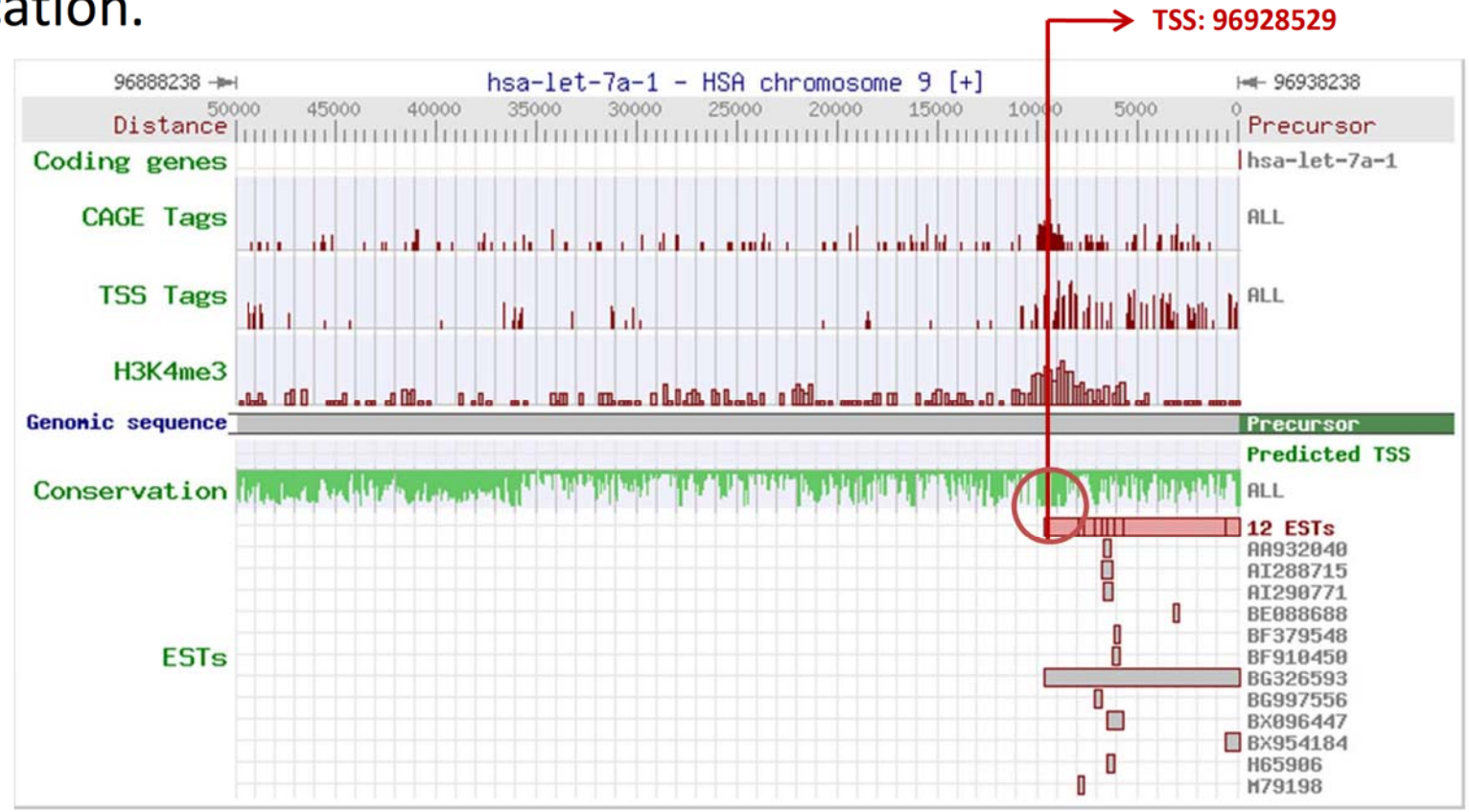


An example

5-fold cross-validation:

Sensitivity = 90.36%, Specificity = 90.05%, Accuracy = 90.21% and Precision = 90.08%, using promoters of protein-coding genes as training data

The human miRNA let-7a-1 demonstrates a good example of TSS identification.



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miRStart 2010 bps

A Database of microRNA Transcription Start Sites

AAAAAA

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Explore a human miRNA now:

hsa-mir-122

GO

What's new?

July 21, 2010
miRStart is online for evaluation...

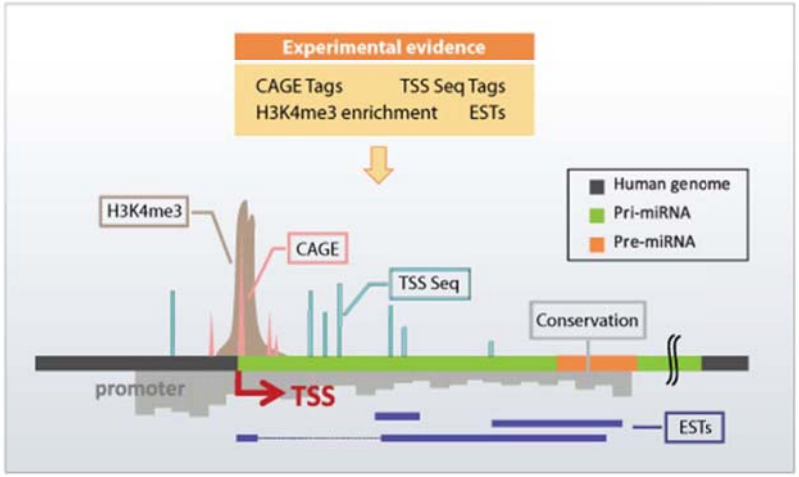
miRStart, a novel resource of human microRNA TSSs (transcription start sites), systematically incorporates significant datasets derived from TSS-relevant experiments to identify transcription start sites of microRNAs. The distribution patterns of these experimental features within 50 k upstream region of microRNA precursors provides an insight into determining reliable microRNA TSSs. In general, a high-confidence TSS is recommended for each microRNA based on a SVM training model. However, users can customize their preferable microRNA TSSs according to the straightforward display of experimental TSS signals.

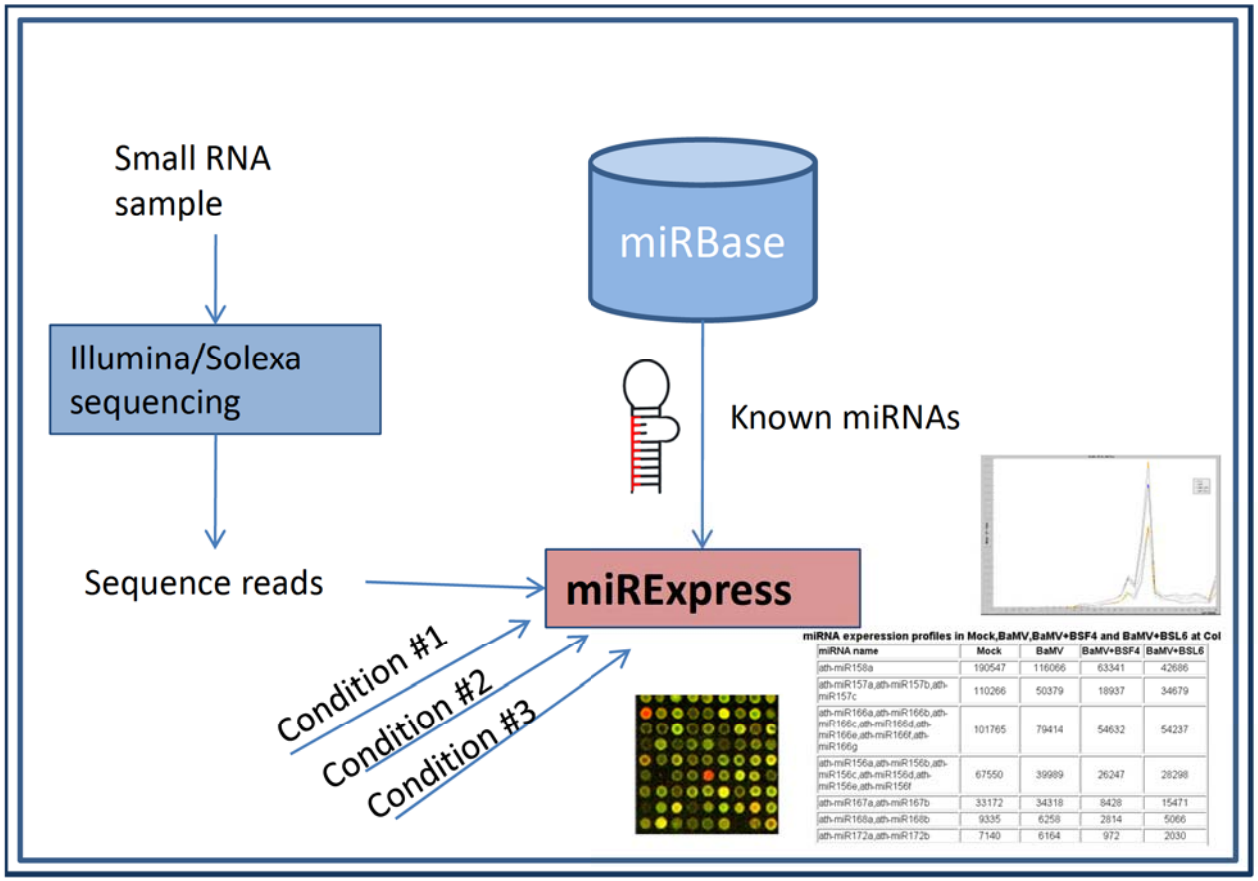
Published experimental evidences used in miRStart are described as follows:

(1) CAGE (Cap Analysis of Gene Expression) tags: Recognize 5'-end of a gene
(2) TSS Seq tags: More than 300 million 5'-end sequences of human and mouse cDNAs by combining oligo-capping method and Solexa sequencing technology
(3) H3K4me3 enrichment (histone H3 is trimethylated at its lysine 4 residue): Enriched surrounding TSSs

Moreover, ESTs and comparative genomics around putative miRNA TSSs are used to provide strong supports for reconfirmation.

The following figure demonstrates the concept of miRStart.





miRExpress

An effective tool to generate **miR**NA **Express**ion profiles from high-throughput sequencing data (***BMC Bioinformatics, 2009***)

a. High-throughput
b. Sequencing short sequences
c. Inexpensive

Next-generation Sequencing Technology

(Elaine R. Mardis 2008)

	Platform		
	Roche(454)	**Illumina**	**SOLiD**
Sequencing chemistry	Pyrosequencing	Polymerase-based sequencing-by-synthesis	Ligation-based sequencing
Amplification approach	Emulsion PCR	Bridge amplification	Emulsion PCR
Paired ends/separation	Yes/3 kb	yes/200 bp	Yes/3 kb
Mb/run	100 Mb	1300 Mb	3000 Mb
Time/run (paired ends)	7 h	4 days	5 days
Read length	250 bp	32–40 bp	35 bp
Cost per run (total direct[a])	$8439	$8950	$17 447
Cost per Mb	$84.39	$5.97	$5.81

The example of next-generation sequencing (NGS) for miRNAs

Table 1. Top 20 miRNAs differentially expressed between the hESC and EB libraries

microRNA ID	Pre-miRNA arm (5-p or 3-p)	Most abundant sequence (isomiR)	hESC count	EB count	*P*-value	Fold change
miR-199a	3-p	ACAGTAGTCTGCACATTGGTTA	1110	13,163	0.00	11.86
miR-372	3-p	AAAGTGCTGCGACATTTGAGCGT	1388	13,653	0.00	9.84
miR-122	5-p	TGGAGTGTGACAATGGTGTTTG	436	2565	0.00	5.88
miR-152	3-p	TCAGTGCATGACAGAACTTGG	622	3028	0.00	4.87
miR-10a	5-p	TACCCTGTAGATCCGAATTTGT	948	3887	0.00	4.10
let-7a	5-p	TGAGGTAGTAGGTTGTATAGTT	11,902	2951	0.00	4.03
miR-302a	5-p	TAAACGTGGATGTACTTGCTTT	36,800	9917	0.00	3.71
miR-222	3-p	AGCTACATCTGGCTACTGGGTCTC	4719	1331	0.00	3.55
miR-340	5-p	TTATAAAGCAATGAGACTGATT	2247	7198	0.00	3.20
miR-363	3-p	AATTGCACGGTATCCATCTGTA	5775	17,912	0.00	3.10
miR-21	5-p	TAGCTTATCAGACTGATGTTGAC	39,818	21,003	0.00	1.90
miR-221	3-p	AGCTACATTGTCTGCTGGGTTTC	16,275	8716	0.00	1.87
miR-26a	5-p	TTCAAGTAATCCAGGATAGGCT	4892	8530	0.00	1.74
miR-26b	5-p	TTCAAGTAATTCAGGATAGGTT	1003	2957	1.39×10^{-278}	2.95
miR-130a	3-p	CAGTGCAATGTTAAAAGGGCAT	2334	4798	2.20×10^{-265}	2.06
miR-594	5-p	ATGGATAAGGCATTGGC	1717	211	1.96×10^{-253}	8.14
miR-302b	3-p	TAAGTGCTTCCATGTTTTAGTAG	15,169	8855	1.39×10^{-213}	1.71
miR-744	5-p	TGCGGGGCTAGGGCTAACAGCA	4166	1516	4.17×10^{-213}	2.75
miR-30d	5-p	TGTAAACATCCCCGACTGGAAGCT	2798	4988	3.29×10^{-205}	1.78
miR-146b	5-p	TGAGAACTGAATTCCATAGGCTGT	703	2075	2.27×10^{-196}	2.95

Morin, R.D., et al., *Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells.* Genome Res, 2008. 18(4): p. 610-21.

Solexa reads with adaptor

```
Read 1  TGACAGAAGAAAGAGAGCACTCGTATGCCGTCTT
Read 2  TGACAGAAGAAAGAGAGCACCTTCGTATGCCGTC
Read 3  TCGTATGCCGTCTTCTGCTTGTCTTACCCCGTCT
Read 4  TGACAGAAGTCGTATGCCGTCTTCTGCTTGAAAG
```

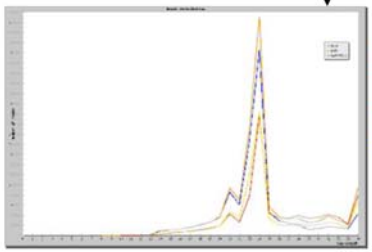
Removing adaptors

Adaptor sequence may appear in the start or end or middle of Solexa reads

```
Read 1  TGACAGAAGAAAGAGAGCACTCGTATGCCGTCTT
Read 2  TGACAGAAGAAAGAGAGCACCTTCGTATGCCGTC
Read 3  TCGTATGCCGTCTTCTGCTTGTCTTACCCCGTCT
Read 4  TGACAGAAGTCGTATGCCGTCTTCTGCTTGAAAG
```

Solexa reads without adaptor

```
Read 1  TGACAGAAGAAAGAGAGCAC
Read 2  TGACAGAAGAAAGAGAGCACC
Read 3  TCTTACCCCGTCT
```



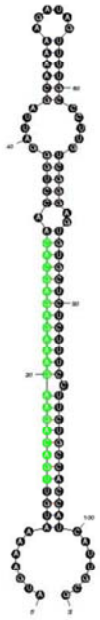
Distribution of solexa read number and count according to read length

Each reads are aligned to known miRNAs

Each reads align with all known miRNAs and is assigned to the highest alignment score miRNA

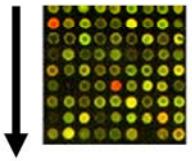
http://microrna.sanger.ac.uk/sequences/

```
ath-miR156h  ..AAAAUGUUGACAGAAGAAAGAGAGCACAACCUGG..
Reads 1            TGACAGAAGAAAGAGAGCAC
Reads 2            TGACAGAATAAAGAGAGCAC
Reads 3            TGACAGCAGAAAGAGAGCAC
```



Each known miRNA compute total solexa reads counts and build miRNA expression profile

miRNA expression profile



miRNA name	Mock	BaMV	BaMV+BSF4	BaMV+BSL6
ath-miR158a	193319	144993	72515	135784
ath-miR158b	150	111	61	93
ath-miR159a	580	876	170	619
ath-miR159b	124	240	48	138

Discovery of novel miRNAs in Human

Step 1

To cluster sequencing reads which can't be mapped to known miRNAs

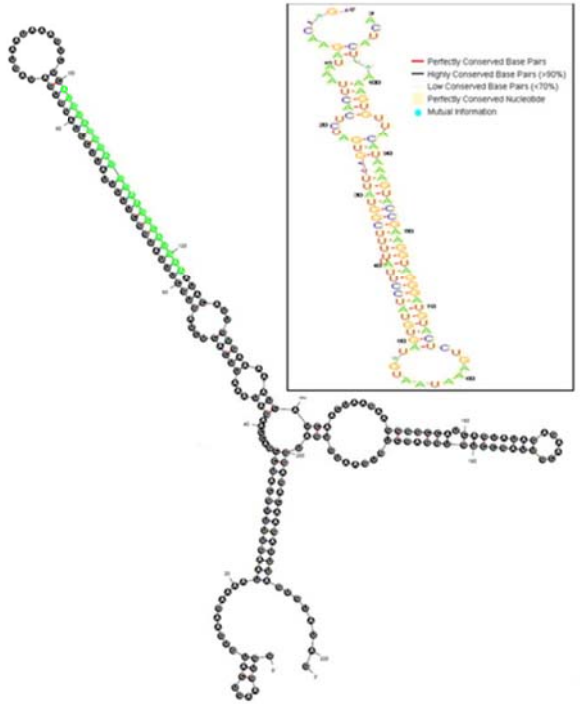
Constraints of miRNAs

Step 2

To map sequencing read to genome and to extract its flanking upstream and downstream

Step 3

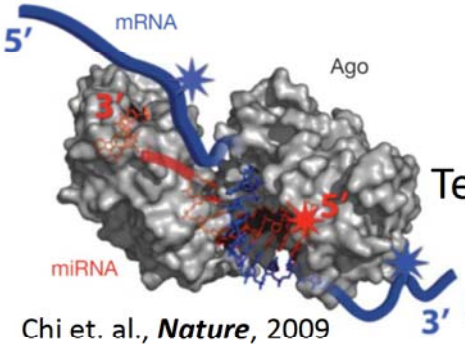
To fold the RNA secondary structure of the candidate
[mfold]
To observe the conservation of the RNA secondary structure
[UCSC genome browser, RNALogo]



miRTarCLIP

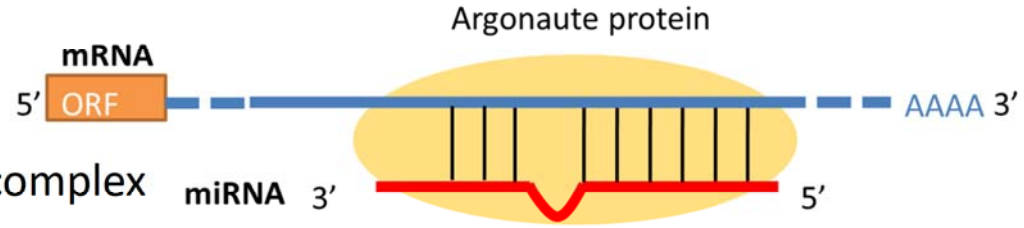
Crosslinking immunoprecipitation sequencing

CLIP-seq



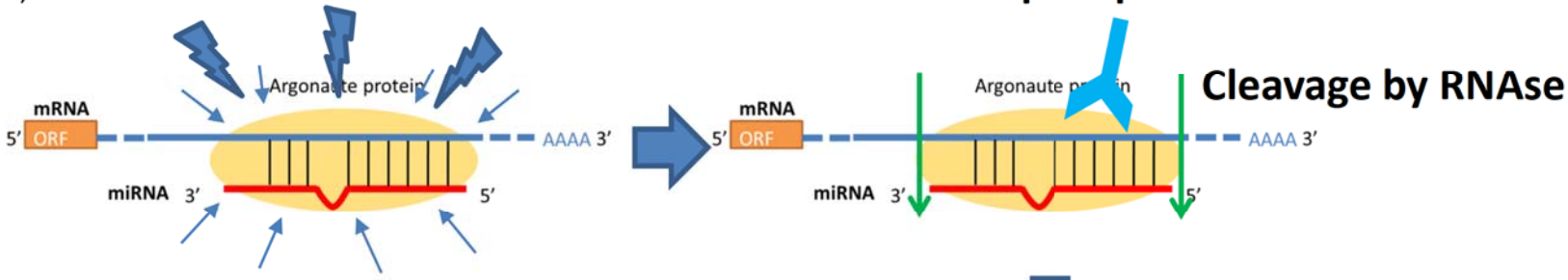
Chi et. al., ***Nature***, 2009

Ternary complex

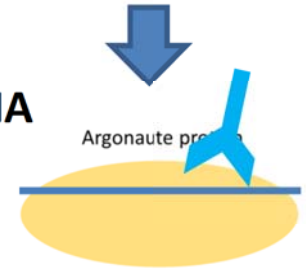


UV Crosslink

immunoprecipitation



Cleavage by RNAse

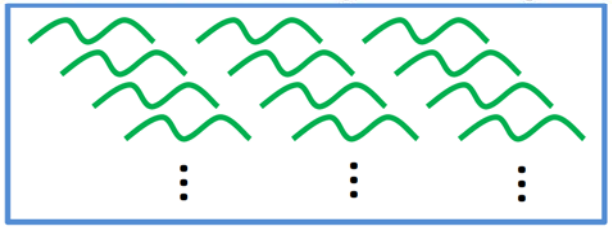


Extraction RNA

Next generation sequencing the specific sequence

Identify miRNA Target Site

CLIP-seq/PAR-CLIP raw data (NGS sequencing reads)



miRTarCLIP program

Core algorithm

- NGS Quality Control
- Align to 3'UTR
- Cluster Calculation
- Find Target site
- Create Web viewer

Database / Tools

- TargetScan
- miRTarBase
- miRBase

Create Web server

- Web Template (HTML, CSS)
- Web Viewer (PHP, Javascript)

Functional web server in user local machine

CLIP-Seq/PAR-CLIP and miRNA target map in 3'UTR

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,h
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

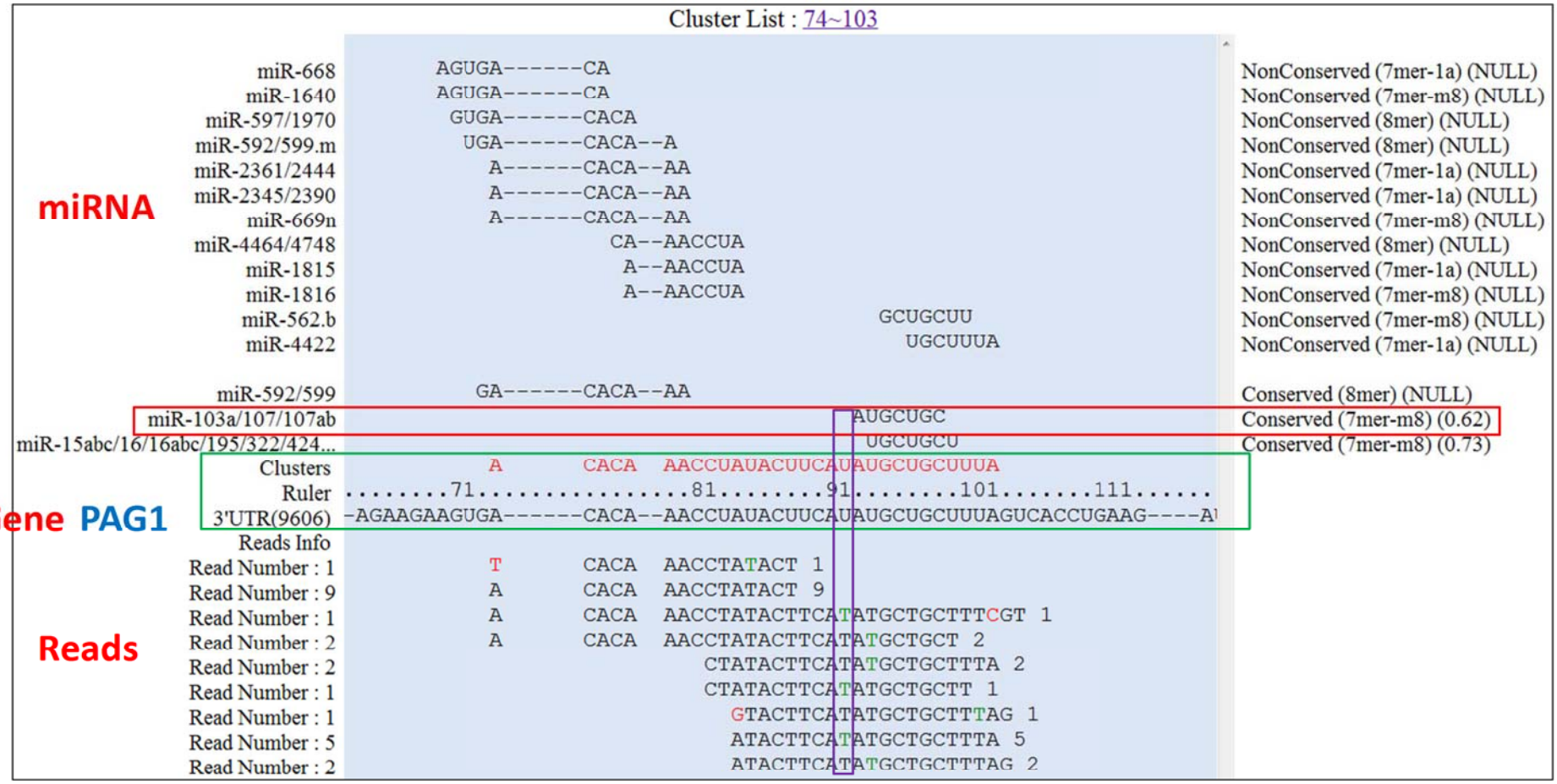
Conserved

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

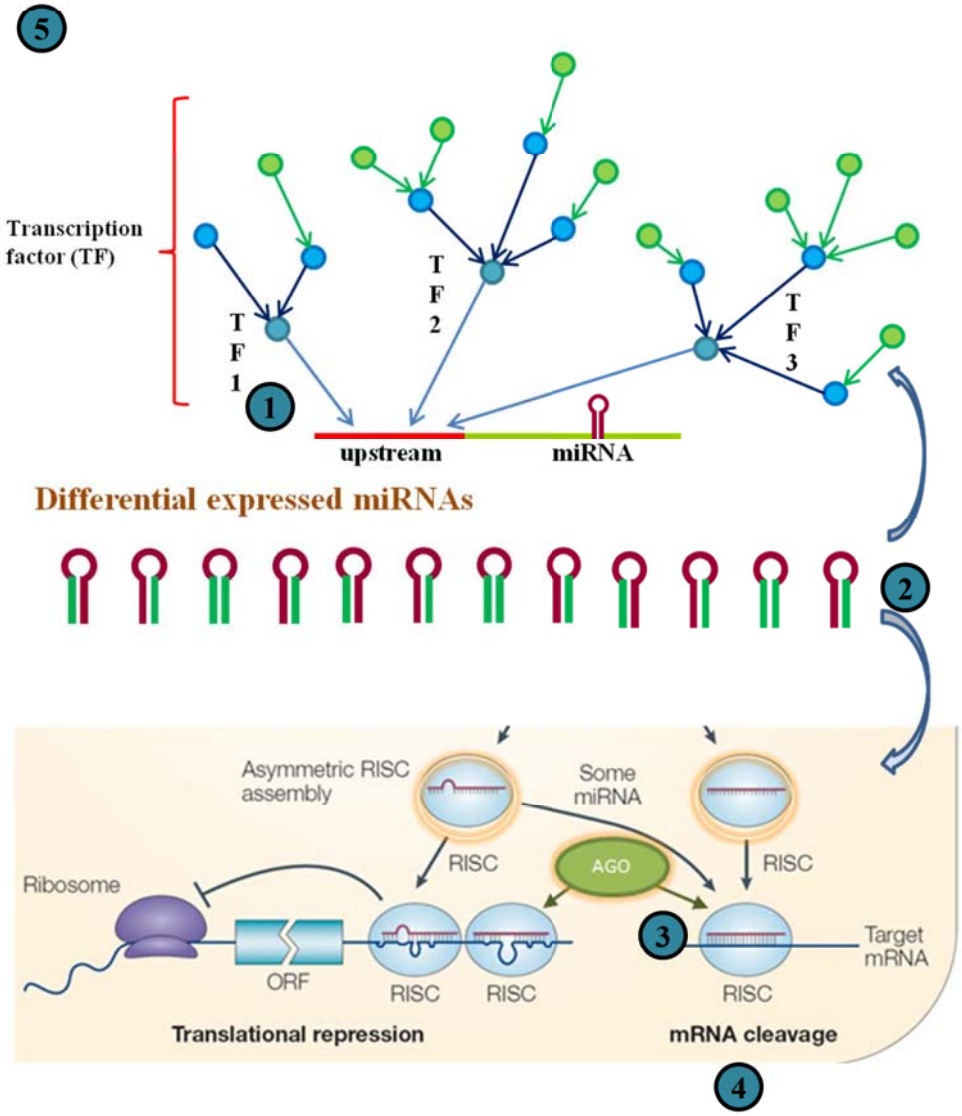
AGO binding site



Conserved

miRNA Name	seed match	mirna start	mirna end	3' pairing contribution	local AU contribution	position contribution	TA contribution	SPS contribution	context+ score	context+ score percentile
hsa-miR-107	2	93	99	0.003	-0.071	-0.049	0.010	-0.036	-0.263	90
hsa-miR-103a	2	93	99	0.003	-0.071	-0.049	0.010	-0.036	-0.263	90
hsa-miR-15a	2	94	100	-0.007	-0.071	-0.049	0.019	-0.048	-0.276	91
hsa-miR-15b	2	94	100	-0.007	-0.071	-0.049	0.019	-0.048	-0.276	92
hsa-miR-424	2	94	100	-0.007	-0.071	-0.049	0.019	-0.048	-0.276	92
hsa-miR-16	2	94	100	-0.007	-0.071	-0.049	0.019	-0.048	-0.276	91
hsa-miR-195	2	94	100	-0.007	-0.071	-0.049	0.019	-0.048	-0.276	91

Using NGS for analyzing small non-coding RNAs



1. **Identifying TFs which regulate miRNAs**

   Chip (chromatin immunoprecipitation)-seq

2. **Profiling miRNA expression**

   Small RNA NGS sequencing

3. **Identifying miRNA target interaction**

   CLIP (crosslinking immunoprecipitation)-seq

   Degradome-seq

4. **mRNA expression**

   RNA-seq

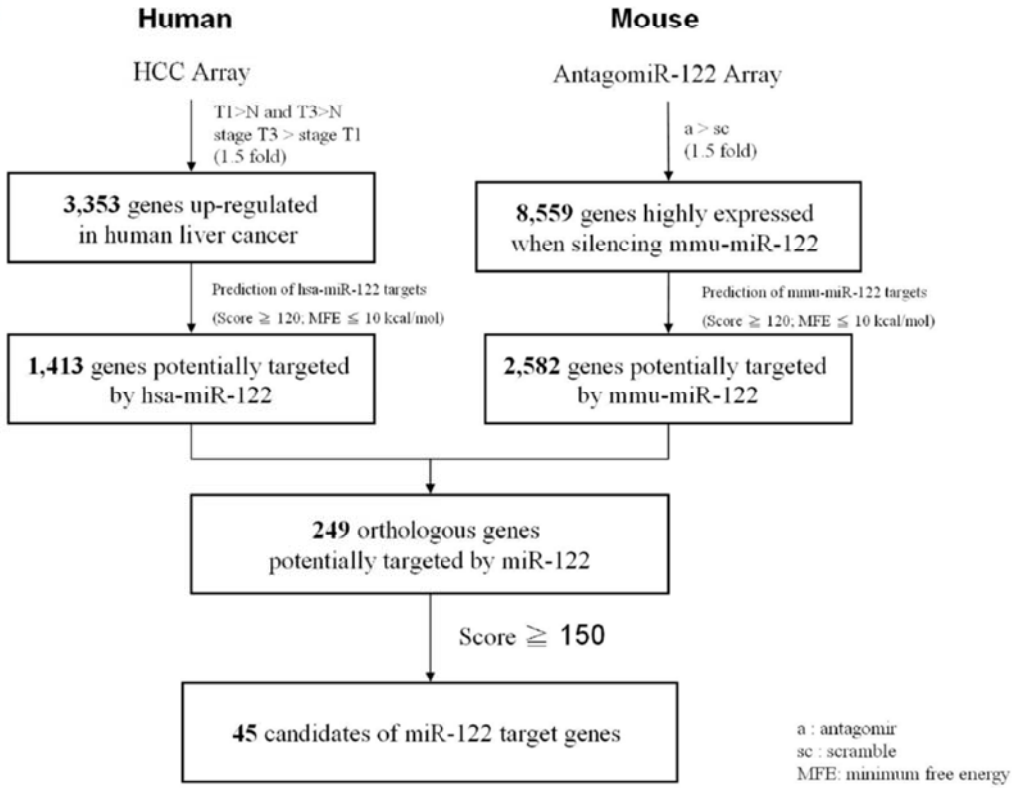
5. **DNA methylation**

   BS (bisulfate)-seq
   MeDIP (methylated DNA immunoprecipitation)-seq
   MBD (methyl-binding protein)-seq

An example of utilizing our miRNA databases and tools

Investigation roles of miR-122a in human hepatocellular carcinoma (HCC)

W.C. Tsai *et al.* (2009) "**MicoRNA-122, a tumor suppressor microRNA that regulates intra-hepatic metastasis of hepatocellular carcinoma**" ***Hepatology,*** Vol. 49, No. 5, pp. 1571-1582. (SCI IF=10.885, Rank = 2/72, GASTROENTEROLOGY & HEPATOLOGY)



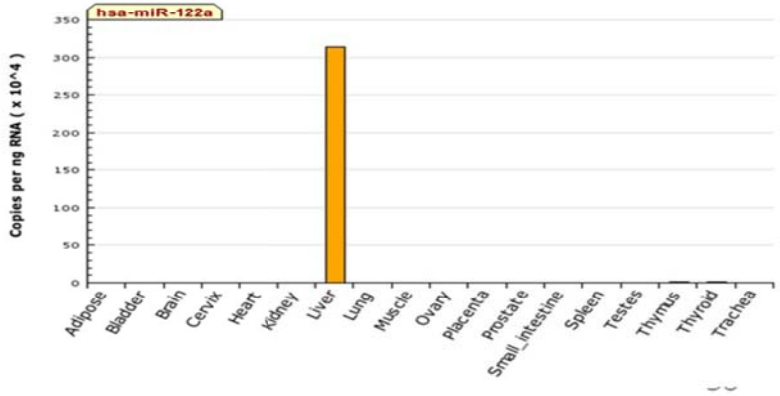
Collaborations with Prof. A.P. Tsou, National Yang Ming University

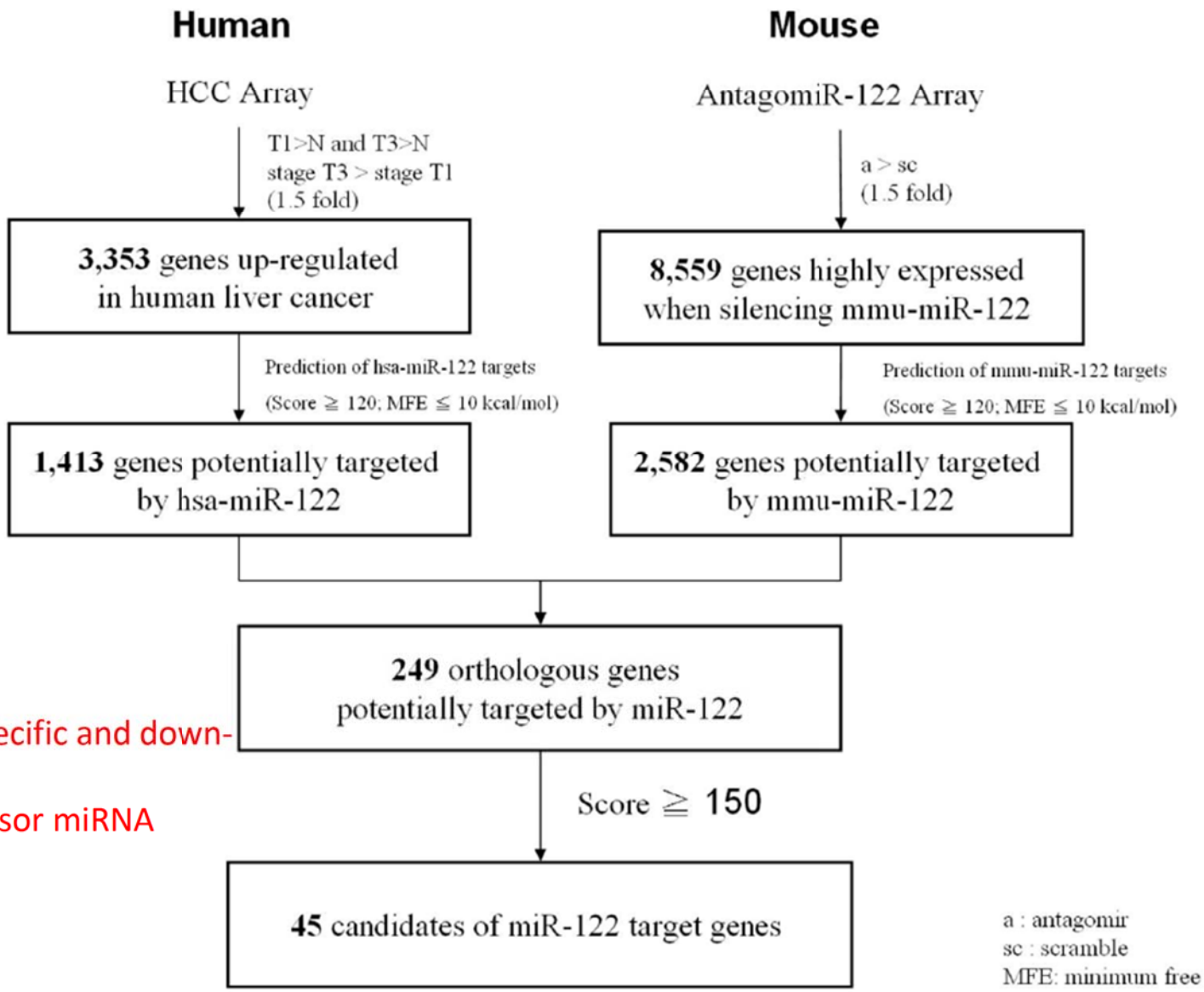
SCI Citations ~ 190

Highly-cited paper selected by Essential Science Indicator

Identifying miR-122 targets in human hepatocellular carcinoma

- miR-122 is liver-specific and down-regulated in HCC
- As a tumor suppressor microRNA
- Genes, which are potentially down-regulated by miR-122, were derived from HCC microarray and AntigomiR-122 microarray experiments
  - Hepatocellular carcinoma (HCC) microarray - 3353 up-regulated genes in liver cancer. (1.5 fold change) human
  - AntigomiR-122 microarray – 8859 genes up-regulated genes when silencing mmu-mir-122. (1.5 fold change) mouse

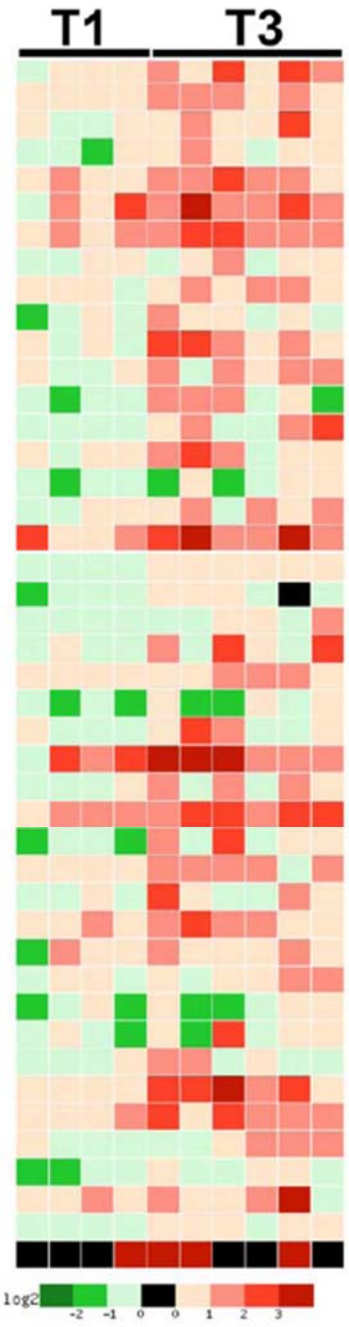




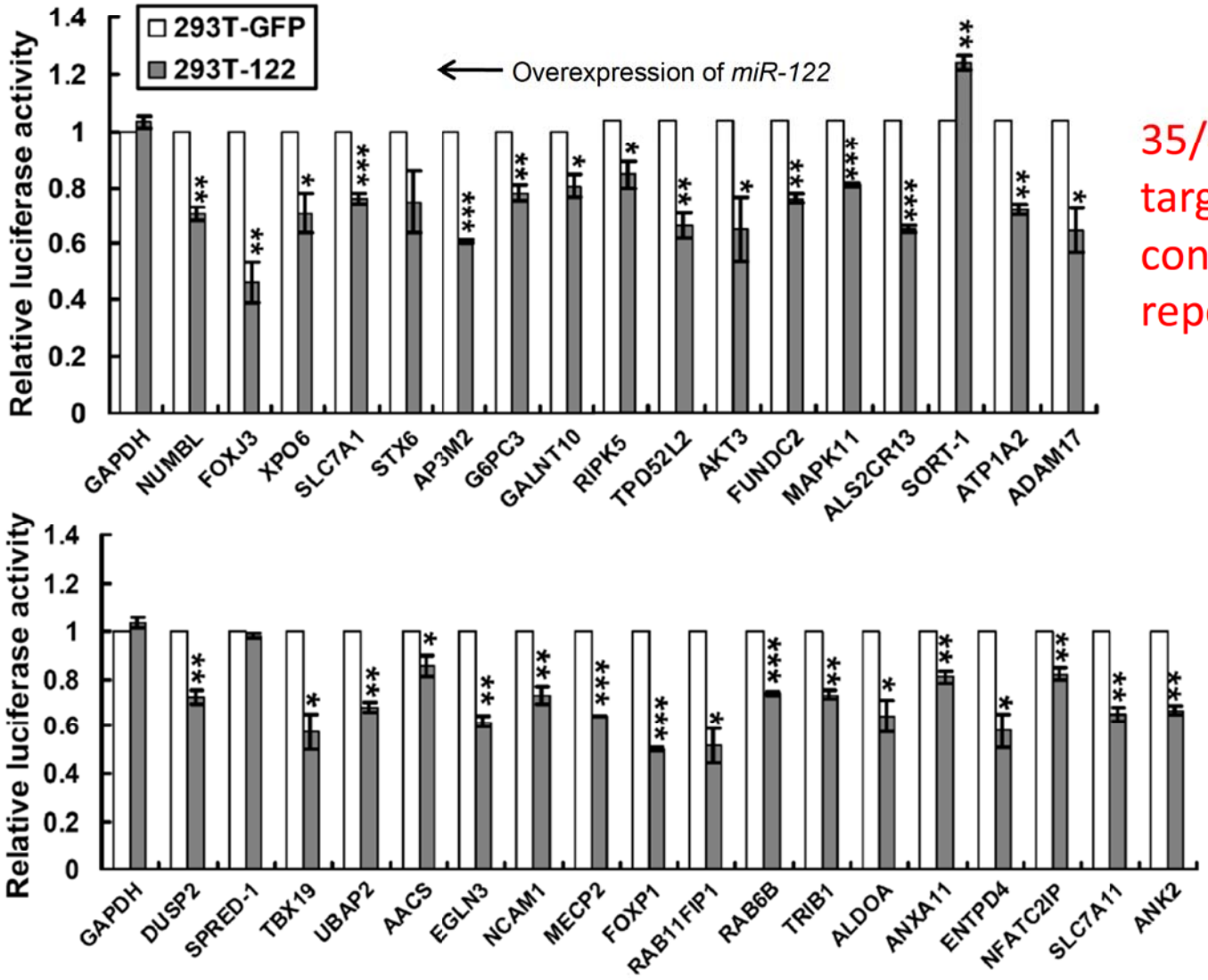
- miR-122 is liver-specific and down-regulated in HCC
- As a tumor suppressor miRNA

Candidate target genes of *miR-122*.

Relative gene expression detected in the microarray assays is shown as mean ratios of T3/T1 for HCC samples and an/c for mice treated with antagomiR-122 (an) compared to control mm-antagomiR122 (c).



	Human			Mouse				
Gene	T3/T1	Sc-H	MFE-H	an/c	Sc-H	MFE-M	Molecular Function	# Binding sites
NUMBL	3.1	181	-20.5	4.5	131	-14.5	Protein binding	7
FOXJ3	1.6	176	-21	1.7	133	-10.6	Transcription factor	3
XPO6	2.2	174	-22.3	1.7	125	-16.4	Nuclear protein transport	2
SLC7A1	2.6	173	-22.4	1.7	173	-23.6	Amino acid transport	4
STX6	1.6	169	-20.9	2.5	135	-17.2	Protein transport	3
AP3M2	1.6	168	-19.9	3.0	129	-15.9	Protein trafficking	3
G6PC3	2.1	167	-18.4	6.1	158	-13.1	Glucose-6-phospgatase	1
GALNT10	1.6	167	-20.4	1.9	152	-20.5	Calcium binding	6
ARHGAP19	1.7	166	-21.5	1.7	143	-17.2	GTPase	4
RIPK5	1.8	166	-18	1.6	150	-16.6	Kinase activity	4
TPD52L2	3.0	166	-23.8	16.3	140	-16.5	Cell proliferation	6
AKT3	2.0	165	-18.7	2.1	162	-15.6	Cell proliferation, apoptosis	2
FUNDC2	2.5	165	-18	2.1	161	-15.8	HCV core binding protein	4
MAPK11	3.2	165	-20.2	1.6	123	-19.1	MAPK activity	2
ALS2CR13	2.0	162	-17.3	6.7	163	-17	Unknown	3
BACH2	1.8	162	-15	2.7	140	-13.5	Transcription factor	3
ATP11A	2.0	161	-12.9	1.9	162	-18.1	Transport ions	7
SORT1	2.2	161	-16.2	2.0	154	-15.3	Cell differentiation	2
ATP1A2	3.5	160	-17	1.5	132	-11.7	Ion concentration balance	7
ADAM17	1.7	159	-19	2.0	122	-17	Cell cell interaction	1
DUSP2	1.8	159	-13.4	1.6	123	-11.1	MAPK phosphatase	2
OSMR	2.5	159	-15.8	2.9	142	-15.5	Cell proliferation	4
RABIF	1.7	159	-20.1	2.4	139	-15.1	Small GPT regulator activity	2
PALM	2.3	156	-15.9	16.1	161	-18.7	Cell mobility and cell shape	3
SPRED1	2.2	156	-21.2	1.6	145	-19.8	Activate MAPK kinase	1
AACS	1.8	155	-13.8	1.8	163	-17.8	isoprenoid biogenesis	2
TBX19	2.1	155	-17.2	4.3	131	-13.1	Transcription factor	5
UBAP2	2.2	155	-18.8	2.7	131	-23.3	Ubiquitin associated protein	2
EGLN3	3.9	154	-17	1.6	137	-15.7	Apoptosis	1
NCAM1	1.9	154	-15.2	3.8	154	-15.2	Cell differentiation	4
MECP2	2.2	153	-12.1	3.1	160	-26.2	Transcription	6
CS	1.6	152	-12.6	1.8	148	-11.9	Catalyze synthesis of citrate	3
FOXP1	1.6	152	-10.8	1.8	167	-17.7	Transcription factor	1
RAB11FIP1	1.5	152	-20.1	1.9	133	-13.3	Protein transport	4
RAB6B	1.5	152	-13.3	1.6	164	-19.6	GTPase	5
TRIB1	2.1	152	-17.4	1.8	134	-12.8	Kinase activity	4
TTYH3	1.8	152	-17.1	1.9	160	-16.6	Chlorode anion channel	2
ALDOA	3.7	151	-13.2	2.2	157	-16.7	Amino acid transport	2
ANXA11	2.0	151	-18.6	4.3	139	-17.2	Calcium binding	7
CLDN18	1.7	151	-18.5	1.9	130	-15.1	Cell and cell adhesion	4
ENTPD4	2.0	151	-18.5	2.0	131	-13.5	Calcium binding	2
NFATC2IP	2.7	151	-19.9	2.2	135	-15.2	Protein modification	4
ANK2	2.1	150	-12	3.1	137	-17.9	Cell proliferation	5
MEP1A	4.3	150	-17.8	1.6	132	-12.8	Peptidase	3
NFATC1	1.8	150	-13.1	2.3	148	-14.9	Transcription factor	3
SLC7A11	9.5	150	-19.5	1.6	164	-14.4	Amino acid transport	4



35/45 (78%) miR-122 target genes were confirmed by luciferase reporter assay.

Confirmation of *miR-122* target genes. *A,* The 3'UTR reporter assay. Putative *miR-122* binding sites in the 3'UTR of the predicted target genes were sublconed downstream of the luciferase gene in pGL3-Control vector. Target reporter assay was carried out in 293T cells overexpressing *miR-122* from lenti-122 virus infection (293T-122).

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ID	Species	miRNA	Target Gene	Validation Method
MIRT003131	Human (Homo sapiens)	hsa-miR-122	SLC7A1	Luciferase assay, Western blot, Northern blot
MIRT003112	Human (Homo sapiens)	hsa-miR-122	ADAM17	Dual-luciferase assay, RT-PCR, Luciferase assay
MIRT003111	Human (Homo sapiens)	hsa-miR-122	NUMBL	Dual-luciferase assay, RT-PCR
MIRT003110	Human (Homo sapiens)	hsa-miR-122	EGLN3	Dual-luciferase assay, RT-PCR
MIRT003109	Human (Homo sapiens)	hsa-miR-122	TRIB1	Dual-luciferase assay, RT-PCR
MIRT003108	Human (Homo sapiens)	hsa-miR-122	SLC7A11	Dual-luciferase assay, RT-PCR
MIRT003107	Human (Homo sapiens)	hsa-miR-122	FOXJ3	Dual-luciferase assay, RT-PCR
MIRT003106	Human (Homo sapiens)	hsa-miR-122	XPO6	Dual-luciferase assay, RT-PCR
MIRT003105	Human (Homo sapiens)	hsa-miR-122	SLC7A1	Dual-luciferase assay, RT-PCR
MIRT003104	Human (Homo sapiens)	hsa-miR-122	AP3M2	Dual-luciferase assay, RT-PCR
MIRT003103	Human (Homo sapiens)	hsa-miR-122	G6PC3	Dual-luciferase assay, RT-PCR
MIRT003102	Human (Homo sapiens)	hsa-miR-122	GALNT10	Dual-luciferase assay, RT-PCR
MIRT003101	Human (Homo sapiens)	hsa-miR-122	DSTYK	Dual-luciferase assay, RT-PCR
MIRT003100	Human (Homo sapiens)	hsa-miR-122	TPD52L2	Dual-luciferase assay, RT-PCR
MIRT003099	Human (Homo sapiens)	hsa-miR-122	AKT3	Dual-luciferase assay, RT-PCR
MIRT003098	Human (Homo sapiens)	hsa-miR-122	FUNDC2	Dual-luciferase assay, RT-PCR
MIRT003097	Human (Homo sapiens)	hsa-miR-122	MAPK11	Dual-luciferase assay, RT-PCR
MIRT003096	Human (Homo sapiens)	hsa-miR-122	FAM117B	Dual-luciferase assay, RT-PCR
MIRT003095	Human (Homo sapiens)	hsa-miR-122	ATP1A2	Dual-luciferase assay, RT-PCR
MIRT003094	Human (Homo sapiens)	hsa-miR-122	DUSP2	Dual-luciferase assay, RT-PCR
MIRT003093	Human (Homo sapiens)	hsa-miR-122	AACS	Dual-luciferase assay, RT-PCR
MIRT003092	Human (Homo sapiens)	hsa-miR-122	TBX19	Dual-luciferase assay, RT-PCR
MIRT003091	Human (Homo sapiens)	hsa-miR-122	UBAP2	Dual-luciferase assay, RT-PCR
MIRT003090	Human (Homo sapiens)	hsa-miR-122	NCAM1	Dual-luciferase assay, RT-PCR
MIRT003089	Human (Homo sapiens)	hsa-miR-122	MECP2	Dual-luciferase assay, RT-PCR
MIRT003088	Human (Homo sapiens)	hsa-miR-122	FOXP1	Dual-luciferase assay, RT-PCR
MIRT003087	Human (Homo sapiens)	hsa-miR-122	RAB11FIP1	Dual-luciferase assay, RT-PCR
MIRT003086	Human (Homo sapiens)	hsa-miR-122	RAB6B	Dual-luciferase assay, RT-PCR
MIRT003085	Human (Homo sapiens)	hsa-miR-122	ALDOA	Dual-luciferase assay, RT-PCR, Northern blot
MIRT003084	Human (Homo sapiens)	hsa-miR-122	ANXA11	Dual-luciferase assay, RT-PCR

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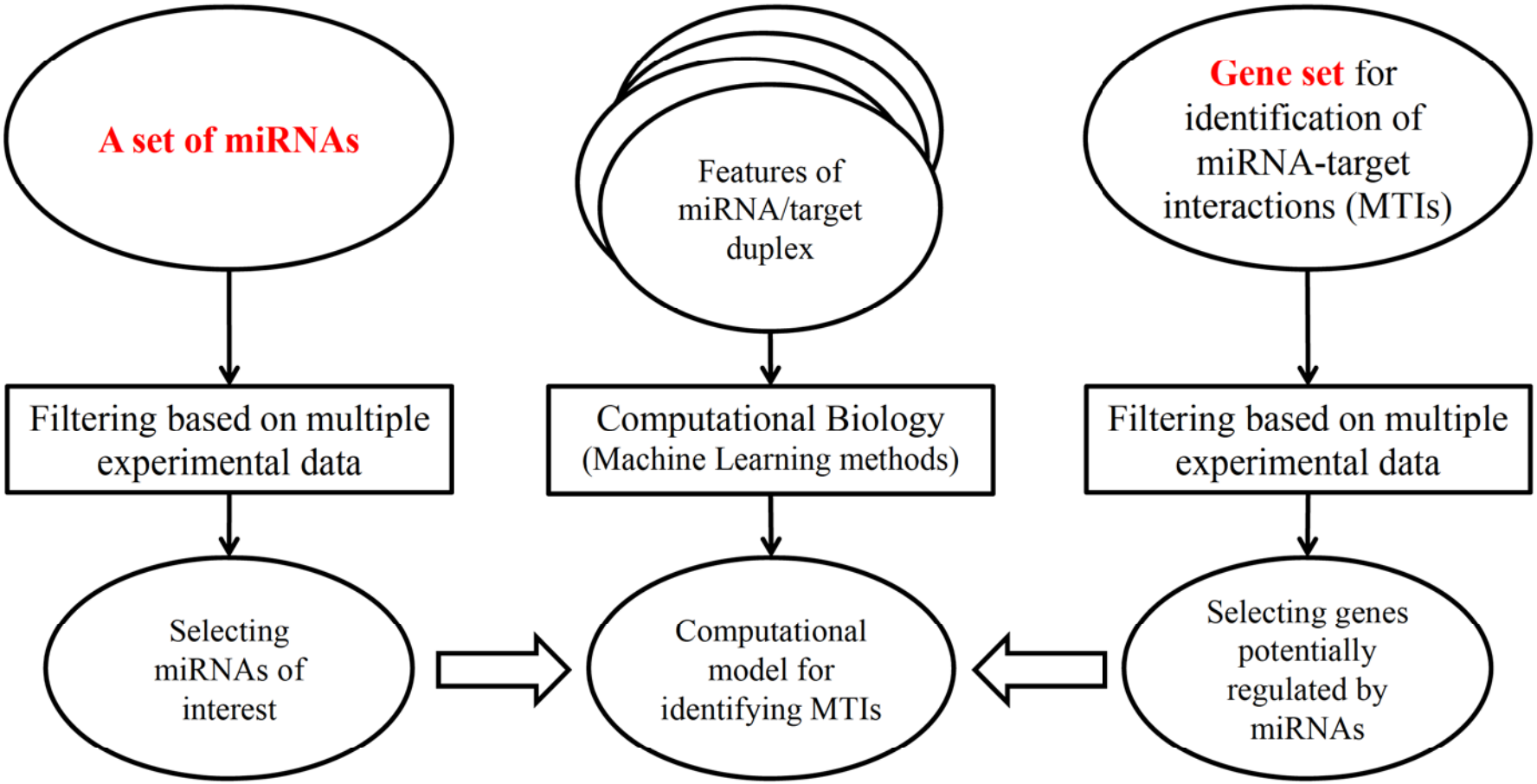
Strategies to achieve both high sensitivity and high specificity



Ways to achieve higher prediction specificity (lower false positive rate)

- More specific miRNAs
- More experimental data
- Selecting miRNAs by biologists' knowledge

- Species dependent
- More positive samples
- More reasonable negative samples
- More effective biological features or properties

- More experimental data
- Multi-dimensional data
- Multiple screening criteria
- Selecting genes by biologists' knowledge
- Smaller gene set

http://www.cc.nctu.edu.tw/~bryan

Or Google Search by "Hsien-Da Huang"

Thank you for your attention!!