Υπολογίζοντας την λειτουργία μη κωδικών γονιδίων.

Artemis G. Hatzigeorgiou

Professor of Bioinformatics

Department of Electrical and
Computer Engineering
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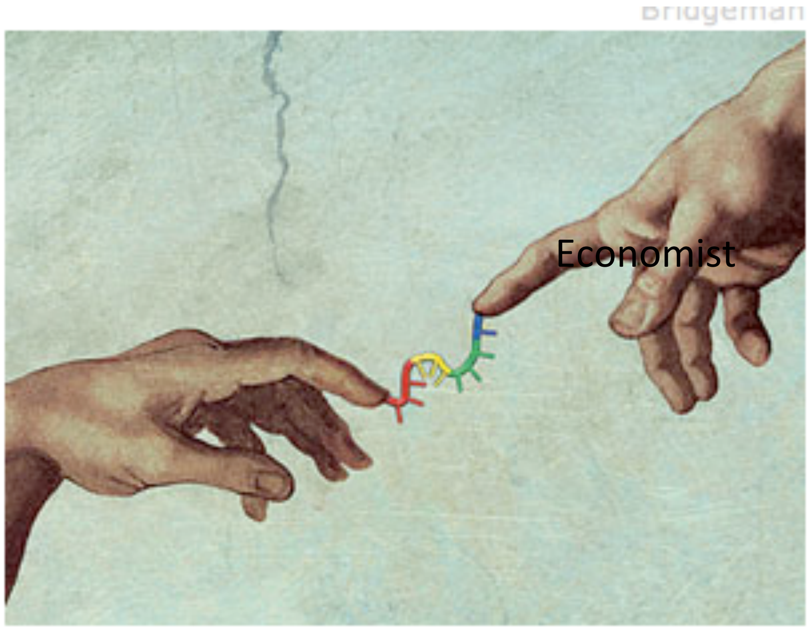
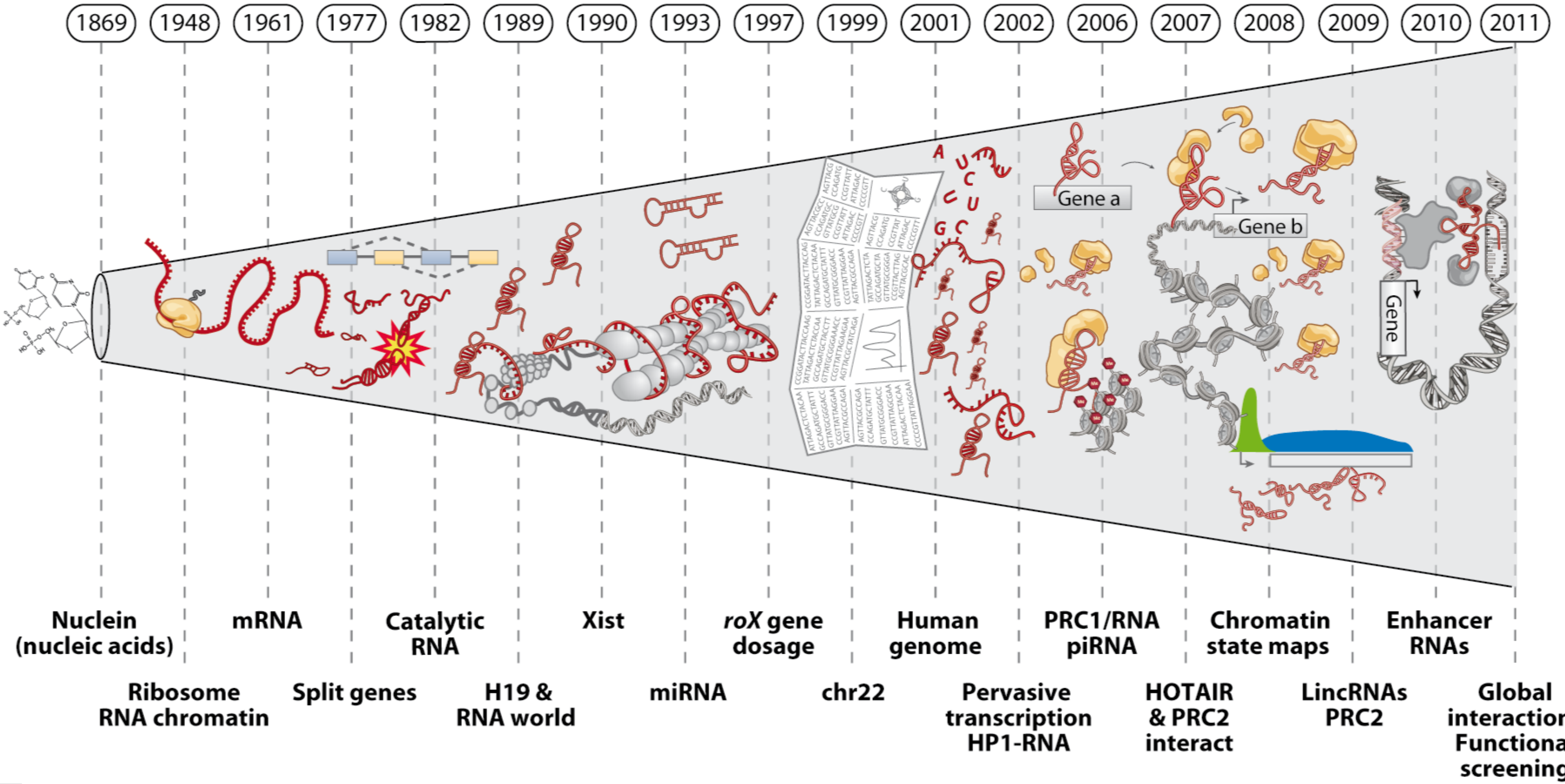
The RNA revolution: Biology's Big Bang



Jun 14th 2007 From The Economist :

What physics was to the 20th century, biology will be to the 21st

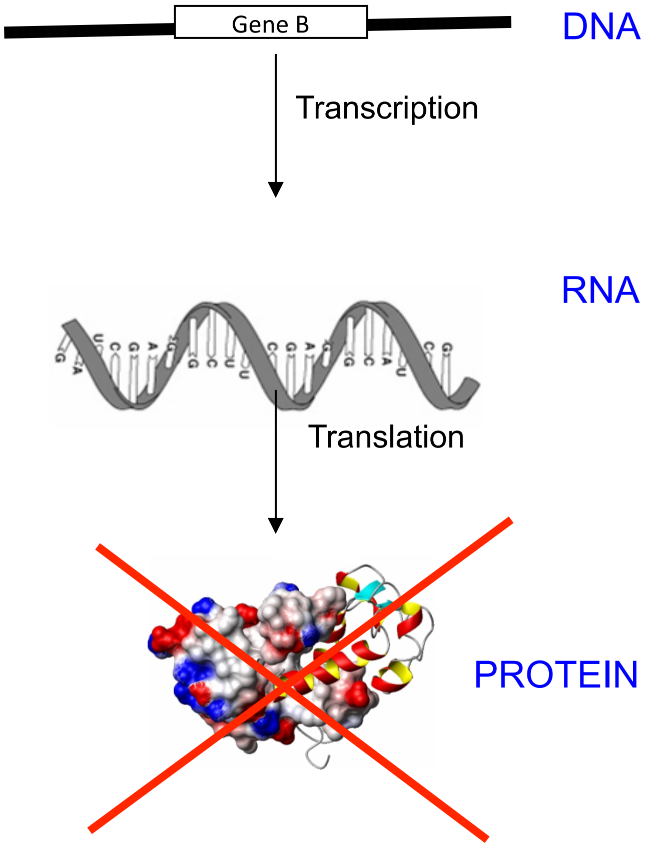
—and RNA will be a vital part of it



Rinn JL, Chang HY. 2012.
Annu. Rev. Biochem. 81:145–66

Annual Reviews

What are microRNAs (miRNAs)?



Gene B

DNA

Transcription

RNA

Translation

PROTEIN

miRNAs are about 22 nt long RNAs.

They post-transcriptionally regulate *protein coding* gene expression

MicroRNAs are involved in …

Development stem cell proliferation

Division Differentiation

regulation of innate & adaptive immunity

apoptosis cell signaling metabolism

human pathologies

Cancer viral infections cardiovascular diseases

metabolic disorders neurological pathologies

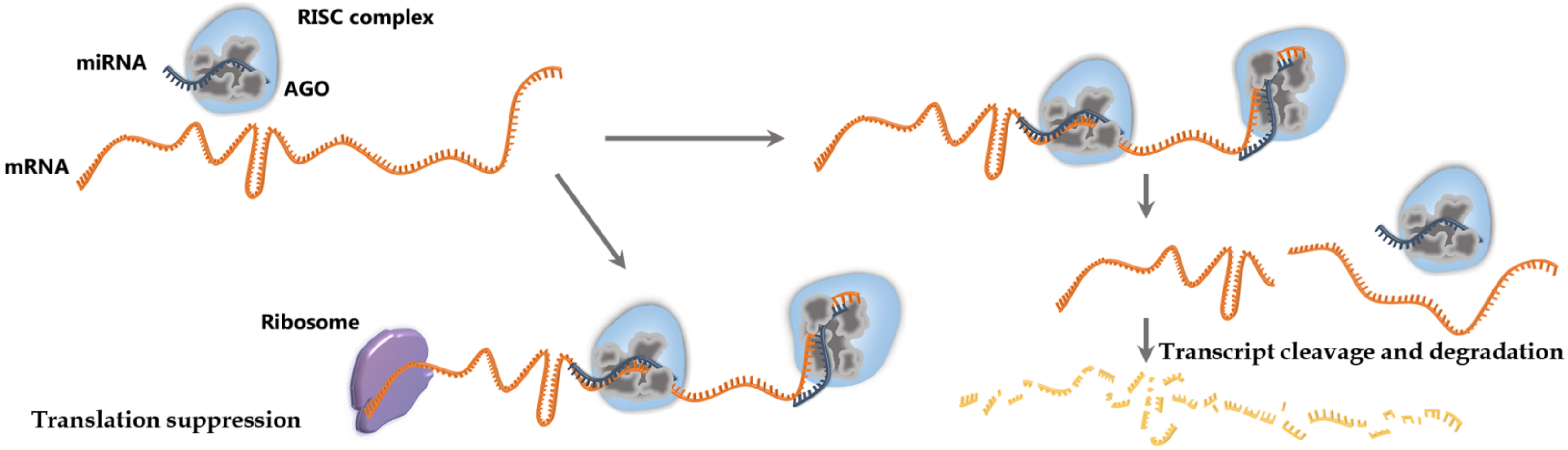
psychiatric disorders renal disease hepatological conditions

autoimmune diseases gastroenterological conditions

obesity reproductive disorders

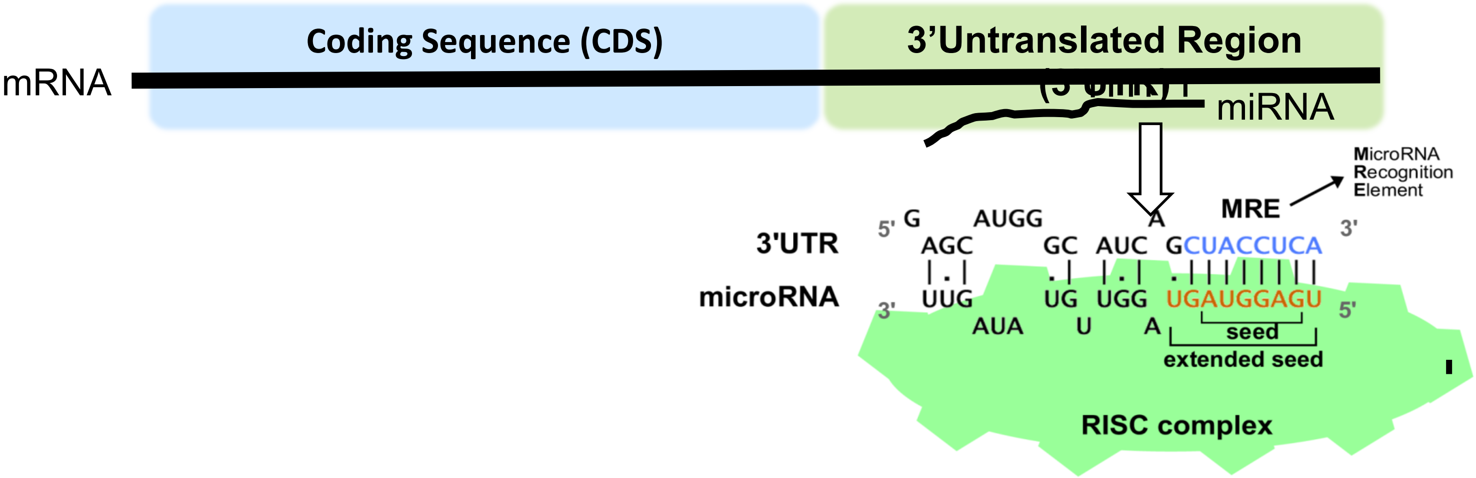
musculoskeletal disorders periodontal pathologies

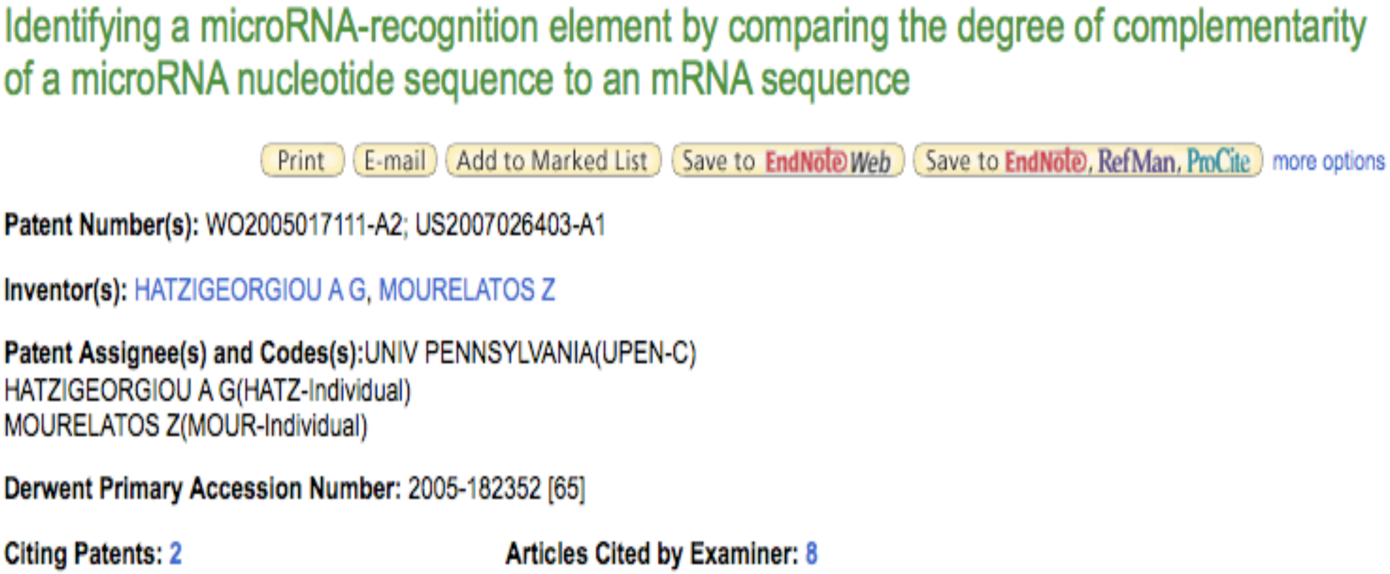
microRNAs



- loaded into Agronaute (AGO) to induce target cleavage, degradation and translational suppression
- Identification of miRNA targets
  - Computational methods
  - Experimental methods - Essential to identify genuine miRNA:targets

First DIANA microT algorithm





Identifying a microRNA-recognition element by comparing the degree of complementarity of a microRNA nucleotide sequence to an mRNA sequence

Patent Number(s): WO2005017111-A2; US2007026403-A1

Inventor(s): HATZIGEORGIOU A G, MOURELATOS Z

Patent Assignee(s) and Codes(s): UNIV PENNSYLVANIA(UPEN-C)
HATZIGEORGIOU A G(HATZ-Individual)
MOURELATOS Z(MOUR-Individual)

Derwent Primary Accession Number: 2005-182352 [65]

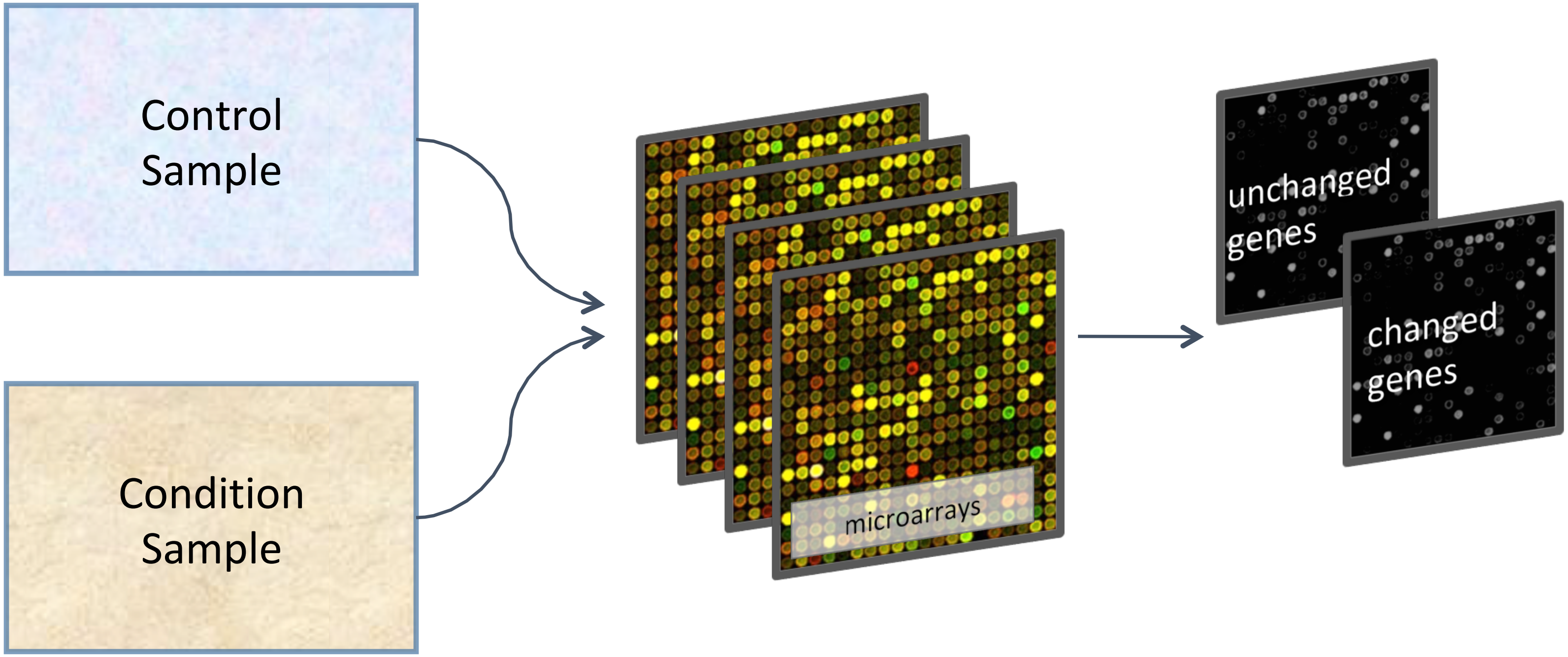
Citing Patents: 2 **Articles Cited by Examiner:** 8

Kiriakidou et al.,
Gen. & Dev. , 2004

Identifying biomarkers

High-throughput Expression Data

What can we learn about miRNA function only from gene expression?



microRNAs and Epithelial Ovarian Cancer

Identify cause / markers for ovarian cancer progression and malignancy

mRNA expression by microarray

microRNA expression by microarray

PNAS

Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer

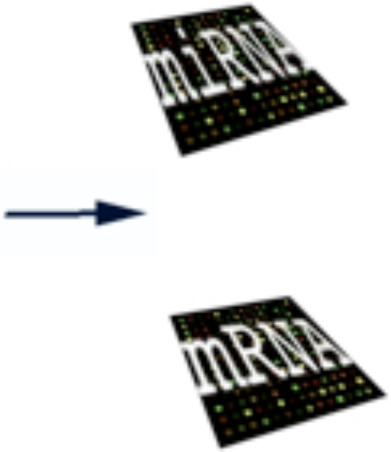
Lin Zhang[a,b,c], Stefano Volinia[d], Tomas Bonome[e], George Adrian Calin[d], Joel Greshock[f,g], Nuo Yang[a], Chang-Gong Liu[d], Antonis Giannakakis[a,h], Pangiotis Alexiou[i], Kosei Hasegawa[a], Cameron N. Johnstone[j], Molly S. Megraw[k], Sarah Adams[a,b], Heini Lassus[l], Jia Huang[f], Sippy Kaur[l], Shun Liang[a], Praveen Sethupathy[k], Arto Leminen[l], Victor A. Simossis[i], Raphael Sandaltzopoulos[h], Yoshio Naomoto[m], Dionyssios Katsaros[n], Phyllis A. Gimotty[o], Angela DeMichele[j], Qihong Huang[p], Ralf Bützow[l], Anil K. Rustgi[j], Barbara L. Weber[f,g], Michael J. Birrer[e], Artemis G. Hatzigeorgiou[c,f,i,k], Carlo M. Croce[c,d], and George Coukos[a,b,c,f]

Numerous miRNAs and protein coding genes are downregulated in late stage ovarian cancer.

Early EOC cells

vs

Late EOC cells



44 miRNAs
Downregulated
in late EOC

948 vs 15212
UP vs OTHER

MiRNA downregulation affects mRNA transcripts?

(miRNA = DOWN & targets = UP)

Calculating the hexamer distribution in the UTR's of genes that gain expression(UP) and genes that do not change(OTHER)

AAAAAA : 0,0,1,5,2,0,1 ...
AAAAAT : 1,1,3,0,0,1,2 ...

...

CCCCCG : 1,3,2,0,0,0,2 ...
CCCCCC : 1,0,0,0,1,0,2 ...

Normalize
divide by 3'UTR length

AAAAAA : 0.02343,0,0,0,0.00021,0,0.007462 ...
AAAAAT : 0,0.0001207,0.00072,0.1,0,0 ...

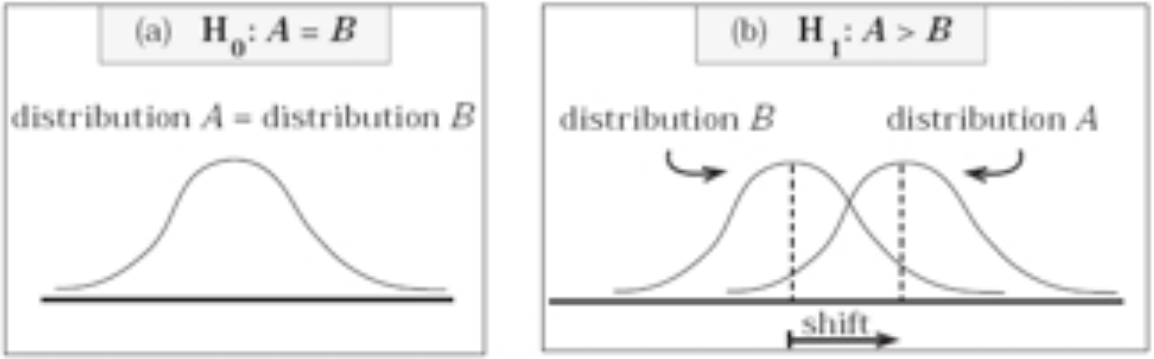
...

CCCCCG : 0.004,0.0667,0,0.1,0,0.0004 ...
CCCCCC : 0.12,0,0,0.00031,0.21109,0.005301 ...

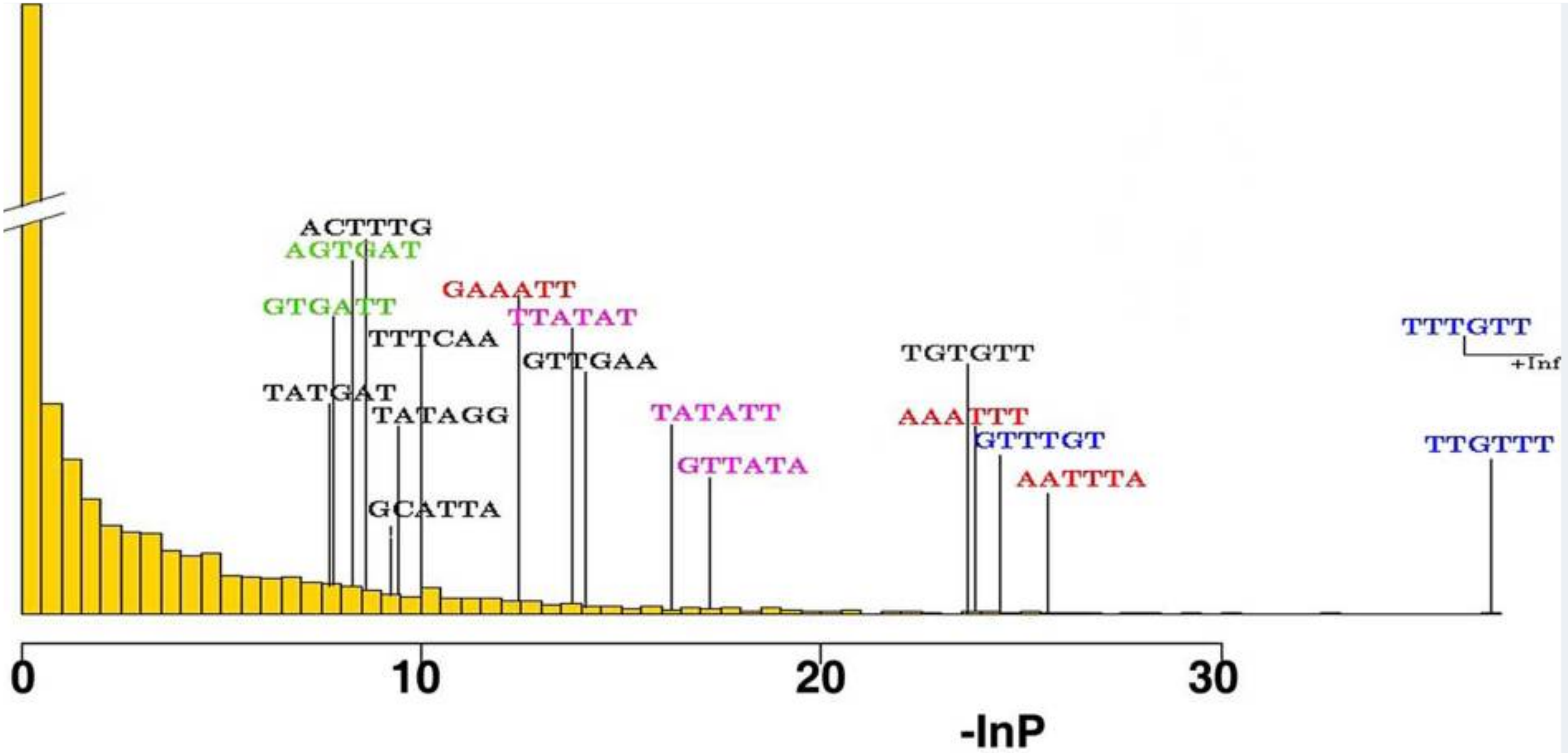
UP values	OTHER values
0.02401	0.34251
0.00054	0.00543
0.00022	0.00432
0.00322	0.00935
0.71533	0.00043
0.00640	0.04540
0.06422	0.07462
0.00001	0.00432
0.00242	0.00245
0.00535	0.05540
	0.60432
	0.04355
	0.05343
	0.04320
	0.01162
	0.00112
	0.02450
	0.01333

p - value

Calculating P-Values of hexamer distribution in two groups of genes (UP vs OTHER) through Wilcoxon Rank Sum test.

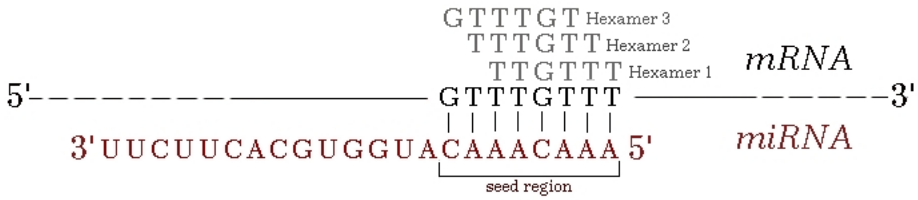


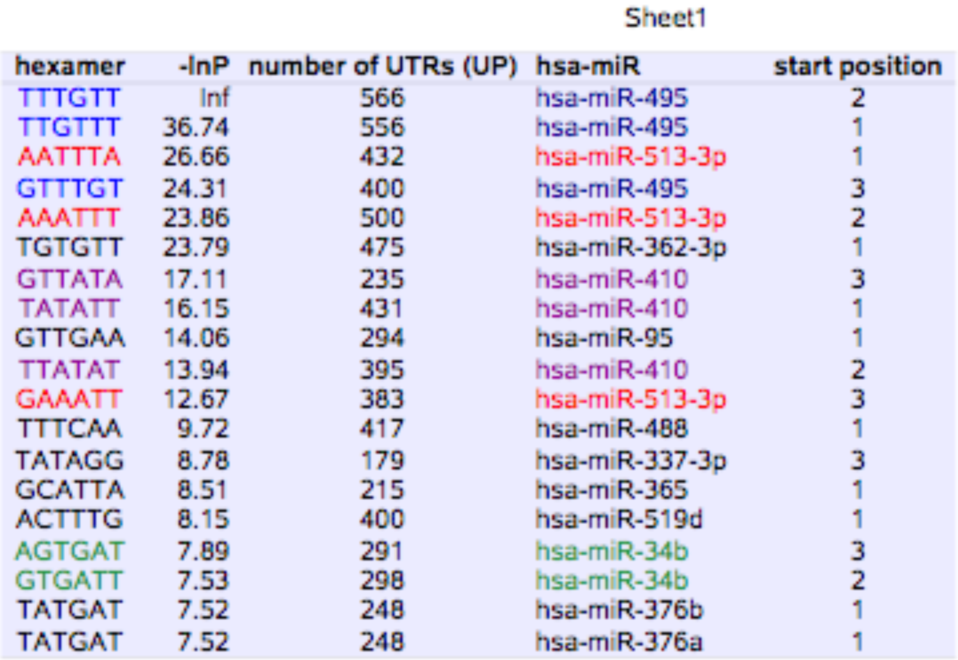
microRNA και Επιθηλιακός Καρκίνος των Ωοθηκών



Zhang et al, PNAS 2008

Linking hexamers to downregulated miRNAs.

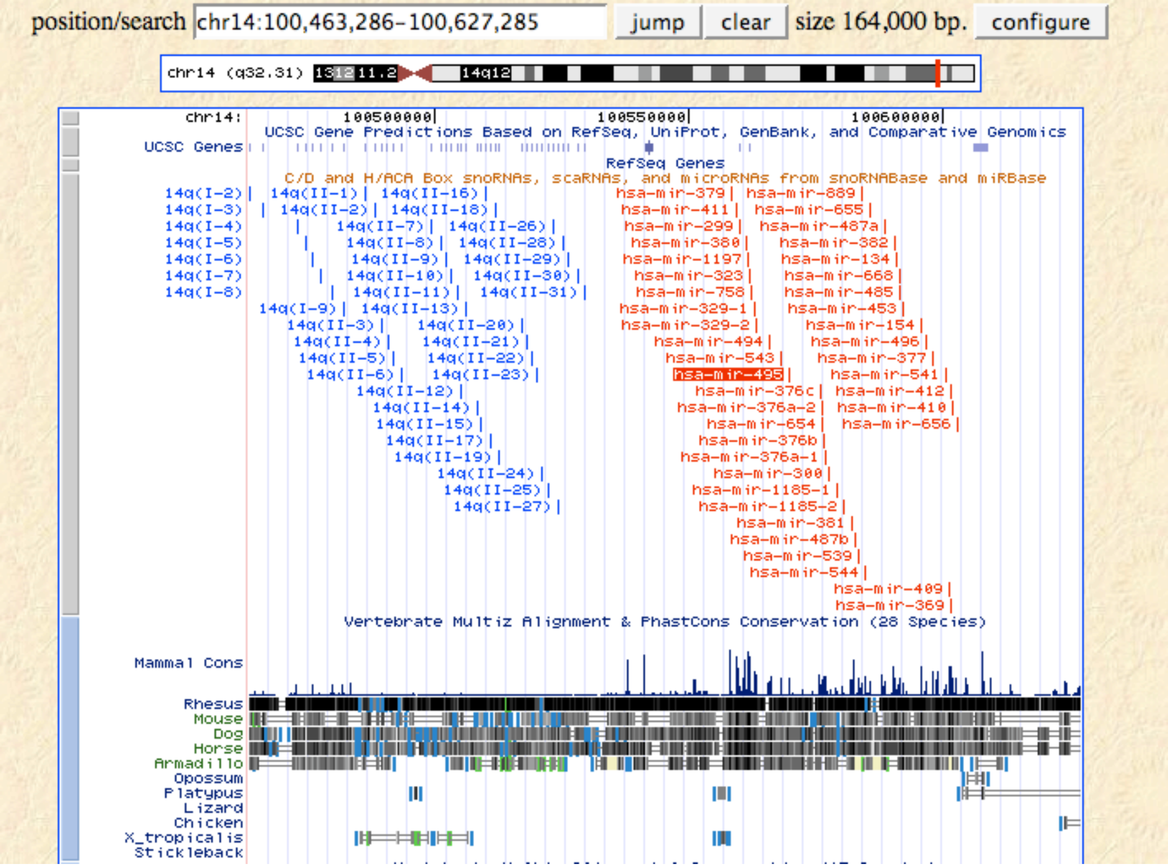




Sheet1

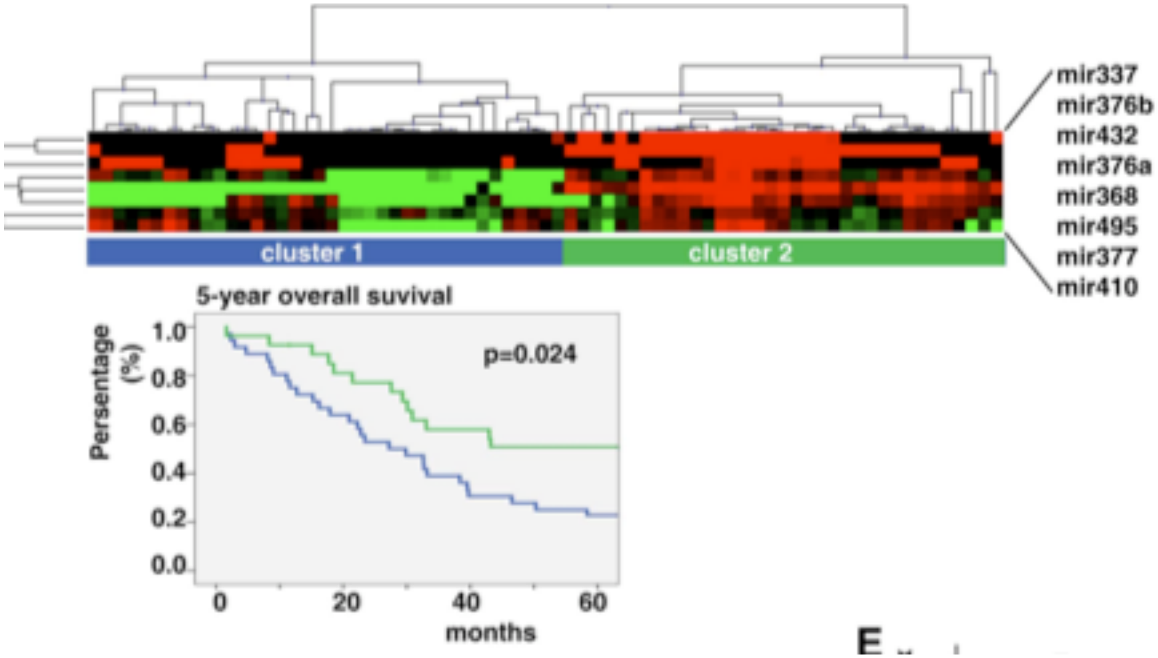
hexamer	-lnP	number of UTRs (UP)	hsa-miR	start position
TTTGTT	Inf	566	hsa-miR-495	2
TTGTTT	36.74	556	hsa-miR-495	1
AATTTA	26.66	432	hsa-miR-513-3p	1
GTTTGT	24.31	400	hsa-miR-495	3
AAATTT	23.86	500	hsa-miR-513-3p	2
TGTGTT	23.79	475	hsa-miR-362-3p	1
GTTATA	17.11	235	hsa-miR-410	3
TATATT	16.15	431	hsa-miR-410	1
GTTGAA	14.06	294	hsa-miR-95	1
TTATAT	13.94	395	hsa-miR-410	2
GAAATT	12.67	383	hsa-miR-513-3p	3
TTTCAA	9.72	417	hsa-miR-488	1
TATAGG	8.78	179	hsa-miR-337-3p	3
GCATTA	8.51	215	hsa-miR-365	1
ACTTTG	8.15	400	hsa-miR-519d	1
AGTGAT	7.89	291	hsa-miR-34b	3
GTGATT	7.53	298	hsa-miR-34b	2
TATGAT	7.52	248	hsa-miR-376b	1
TATGAT	7.52	248	hsa-miR-376a	1

The majority of the downregulated miRNAs are located at a big miRNA cluster (< 36) at the Dlk1-Gtl2 domain of chr. 14.



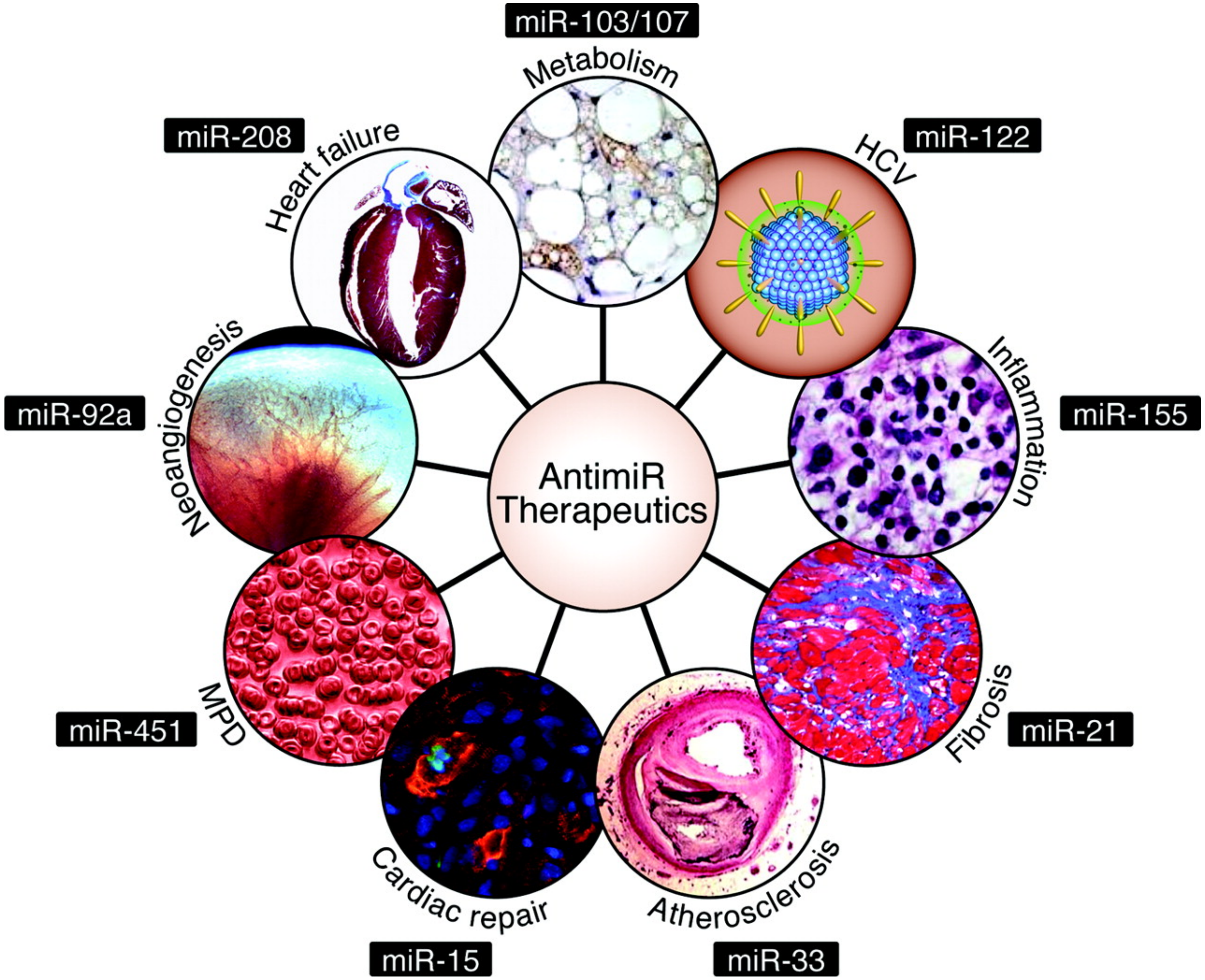
position/search chr14:100,463,286-100,627,285 size 164,000 bp.

Downregulation of miRNA cluster at *DLK1-GTL2* domain is associated with poor survival.



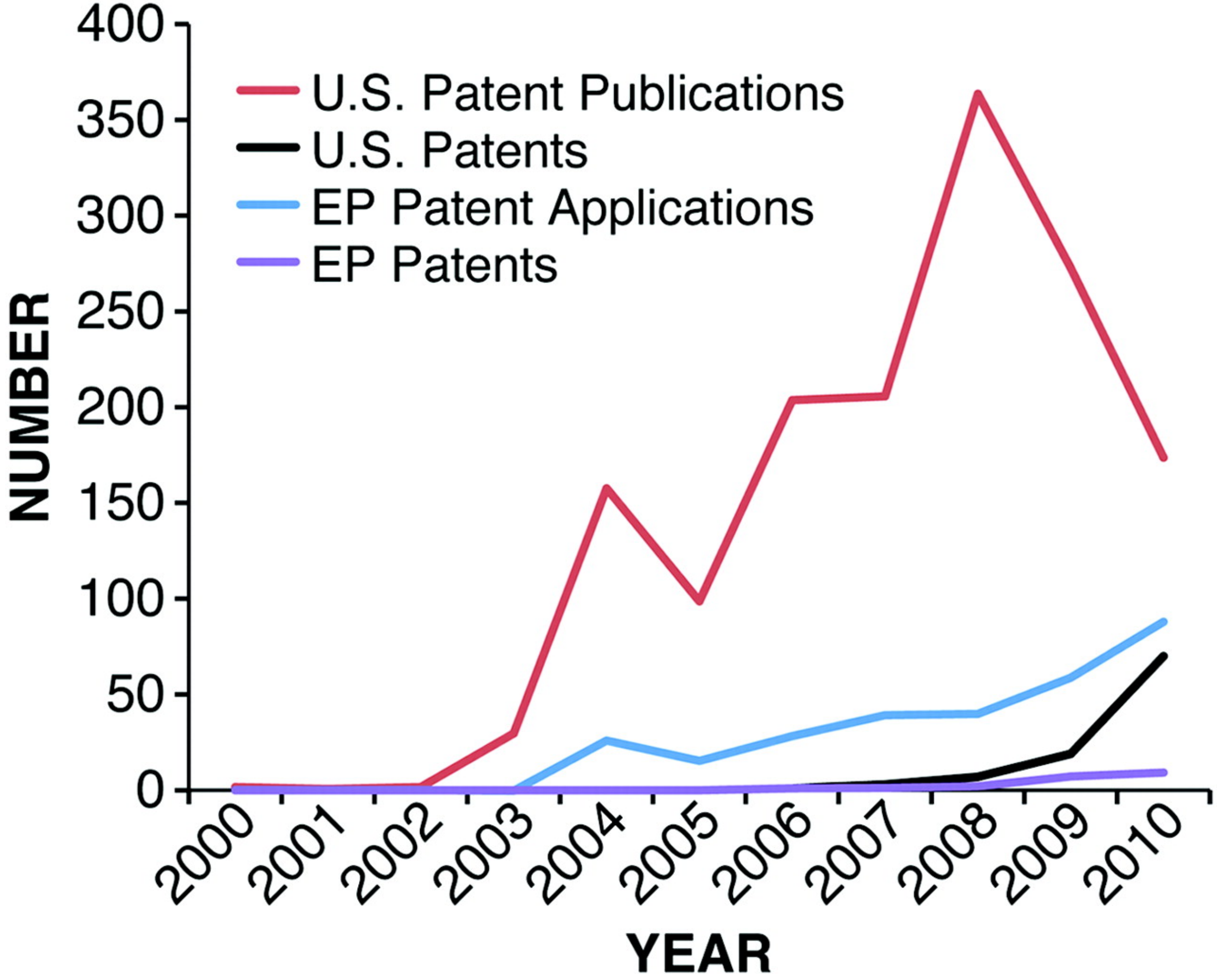
L. Zhang, S. Volinia, T. Bonome, G.A. Calin, J. Greshock, N. Yang , C. Liu, A. Giannakakis, P. Alexiou, K. Hasegawa, C. N. Johnstone, M. S. Megraw, S. Adams, H. Lassus, J. Huang, S. Kaur, S. Liang, P. Sethupathy, A. Leminen, V.A. Simossis, R. Sandaltzopoulos, Y. Naomoto, D. Katsaros, P. A. Gimotty, A. DeMichele, Q. Huang, R. Bützow, A.K. Rustgi, B.L. Weber, M.J. Birrer, A.G. Hatzigeorgiou, C.M. Croce and G. Coukos (2008) Genomic and Epigenetic Alterations Deregulate microRNA Expression in Human Epithelial Ovarian Cancer, ***PNAS*** May 13;105(19):7004-9.

Specific miRNAs that are currently being pursued as clinical candidates.



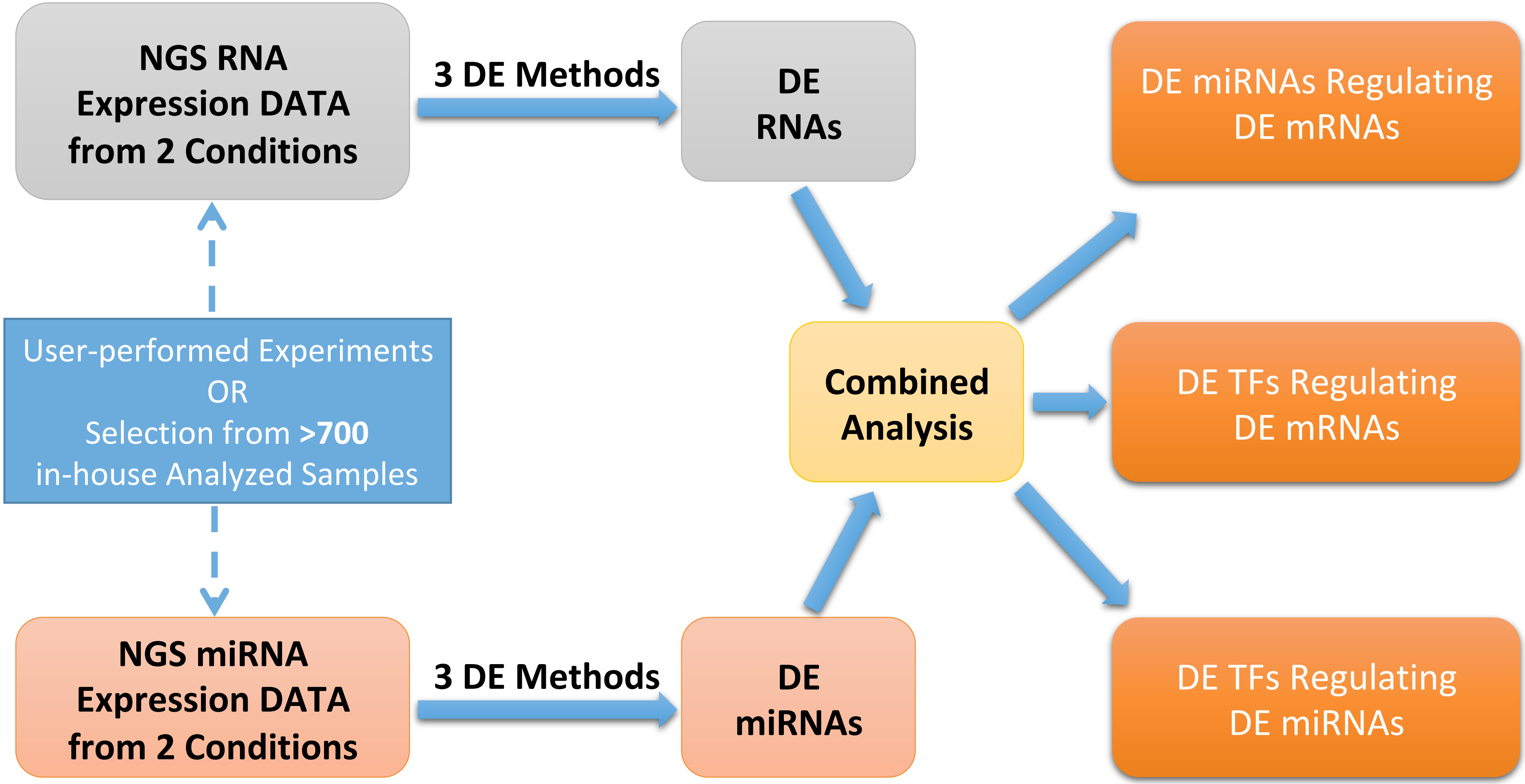
Eva van Rooij et al. Circ Res. 2012;110:496-507

Annual number of US and European patents related to miRNAs and their applications.



Eva van Rooij et al. Circ Res. 2012;110:496-507

miRNA - biomarkers:
combined analysis of mRNA and miRNA with miRExtra V2.0



NGS RNA Expression DATA from 2 Conditions

3 DE Methods

DE RNAs

User-performed Experiments
OR
Selection from **>700**
in-house Analyzed Samples

NGS miRNA Expression DATA from 2 Conditions

3 DE Methods

DE miRNAs

Combined Analysis

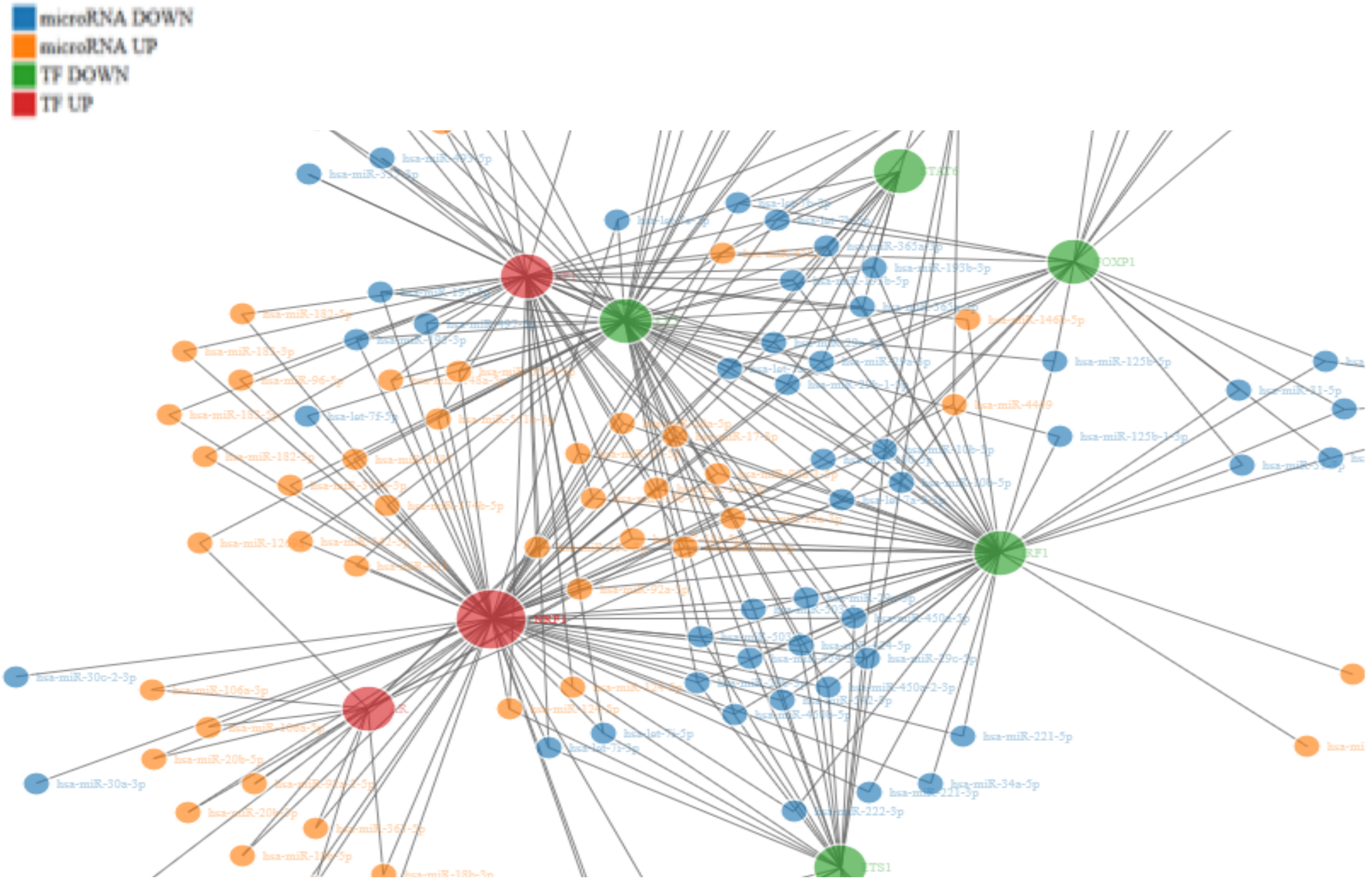
DE miRNAs Regulating DE mRNAs

DE TFs Regulating DE mRNAs

DE TFs Regulating DE miRNAs

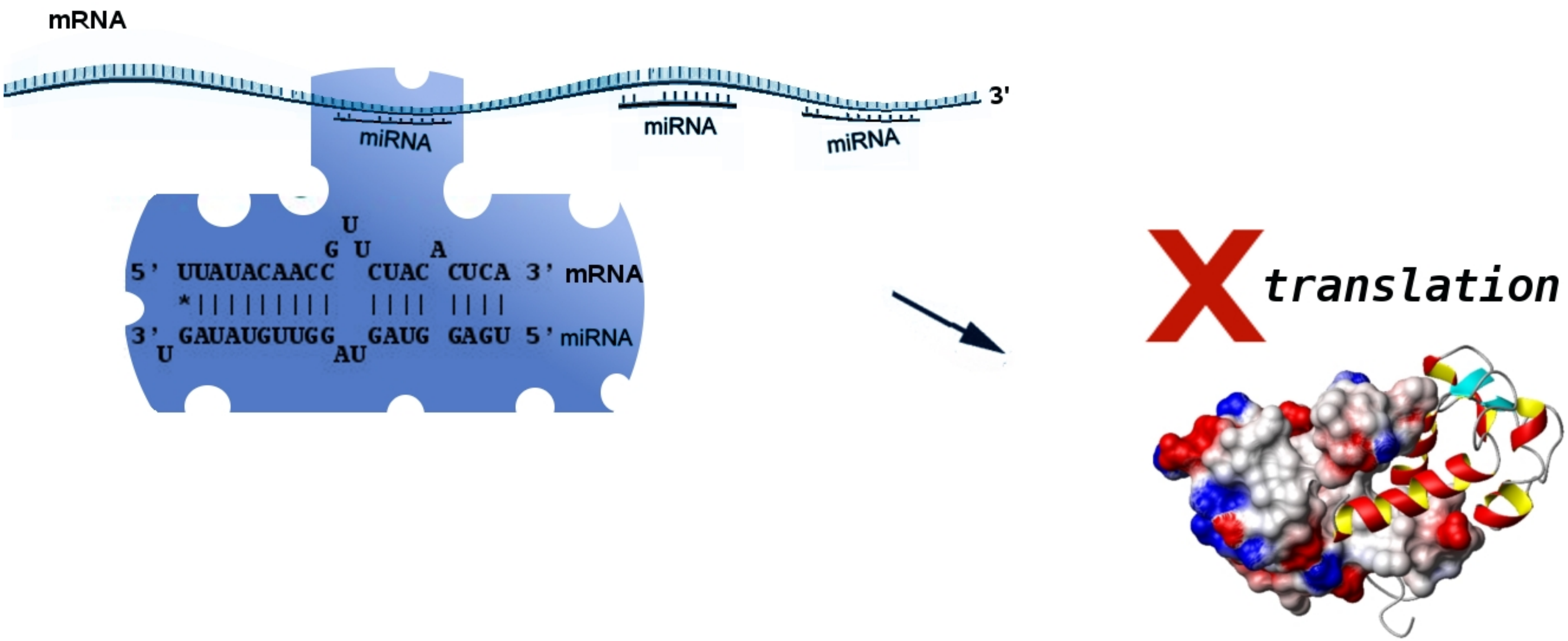
Vlachos et al. NAR 2016

miRNA - biomarkers:
combined analysis of mRNA and miRNA with miRExtra V2.0



Vlachos et al. NAR 2016

Computational identification of microRNA targets .



microRNAs bind the 3'UTR of mRNAs and repress translation

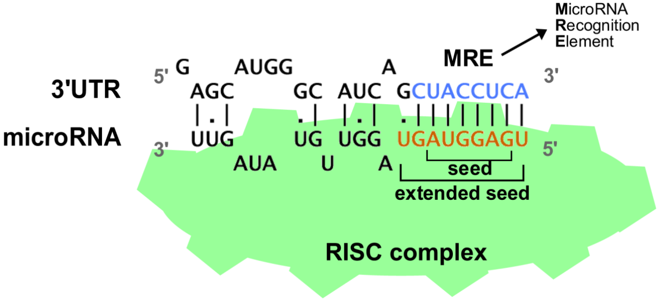
miRNA/ Target interaction sites (C.elegans)

```
             U
            G U    A
5' UUAUACAACC  CUAC CUCA 3' lin-41
   *|||||||||  |||| ||||
3' GAUAUGUUGG  GAUG GAGU 5' let-7
  U          AU
```

mRNA target

miRNA

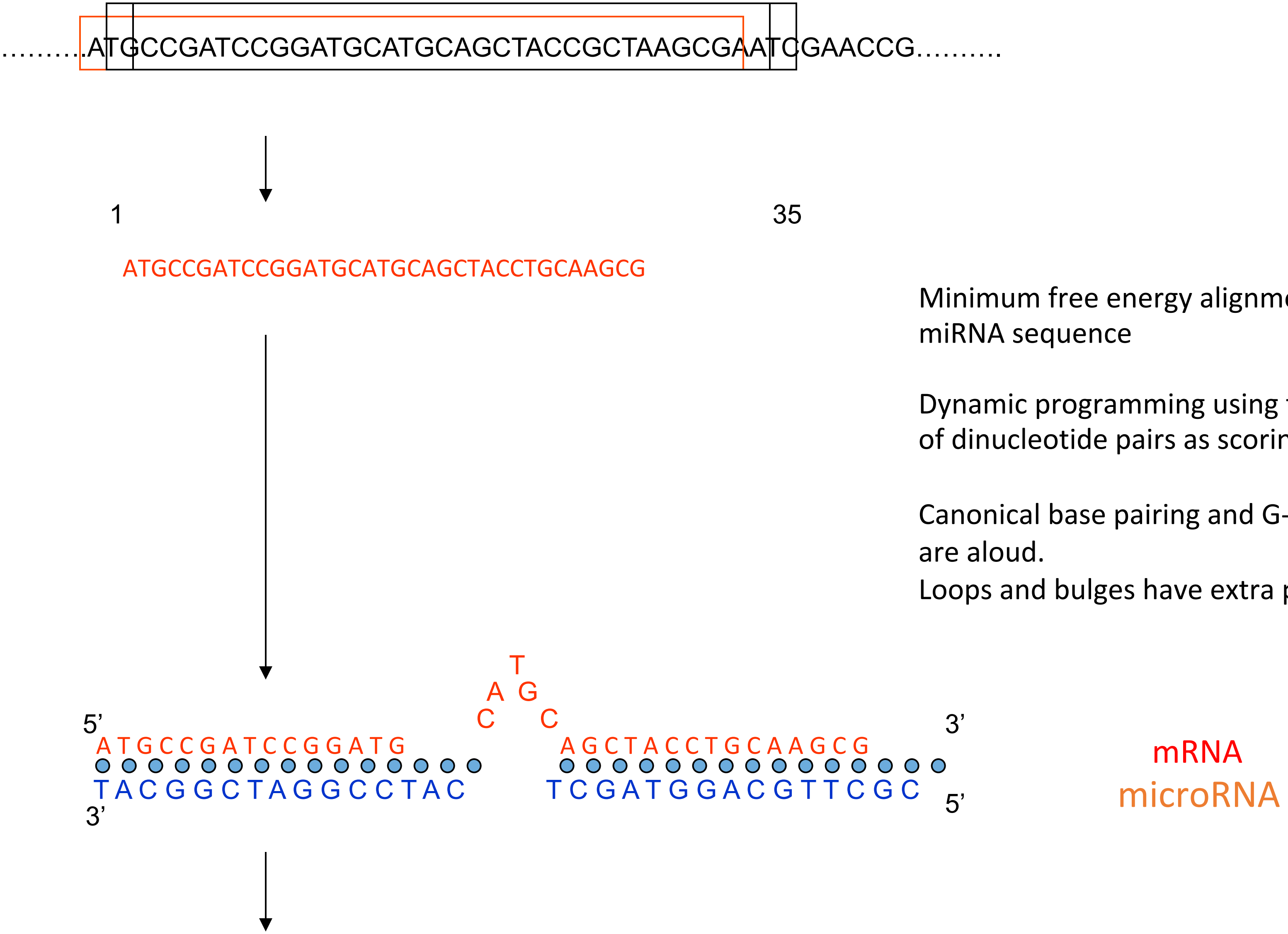
```
              U
             A U
5' UUAUACAACC  CUGCCUC  3' lin-41
   *|||||||||  ||*||||
3' GAUAUGUUGG  GAUGGAG  5' let-7
  U                   U
             AU
```



Reinhart BJ et al. Nature v.403,2000,901-906

```
5' UUGCAC      UCUCAGGA  3' lin-28
   |**|||      ||||||||
3' AGUGUG      AGAGUCCU  5' lin-4
         A     C       U
          A   C
           C U
```

Moss EG et al. Cell v.88,1997,637-646



..........ATGCCGATCCGGATGCATGCAGCTACCGCTAAGCGAATCGAACCG..........

1 35

ATGCCGATCCGGATGCATGCAGCTACCTGCAAGCG

Minimum free energy alignment with a miRNA sequence

Dynamic programming using free energies of dinucleotide pairs as scoring matrix.

Canonical base pairing and G-U wobbles are aloud.
Loops and bulges have extra penalty.

```
                 T
               A   G
5’            C     C              3’
 ATGCCGATCCGGATG     AGCTACCTGCAAGCG
 TACGGCTAGGCCTAC     TCGATGGACGTTCGC
3’                                 5’
```

mRNA
microRNA

Minimum binding free energy score for this mRNA window

Adding conservation

For each miRNA:

- Sort all targets based on the minimum free energy binding score

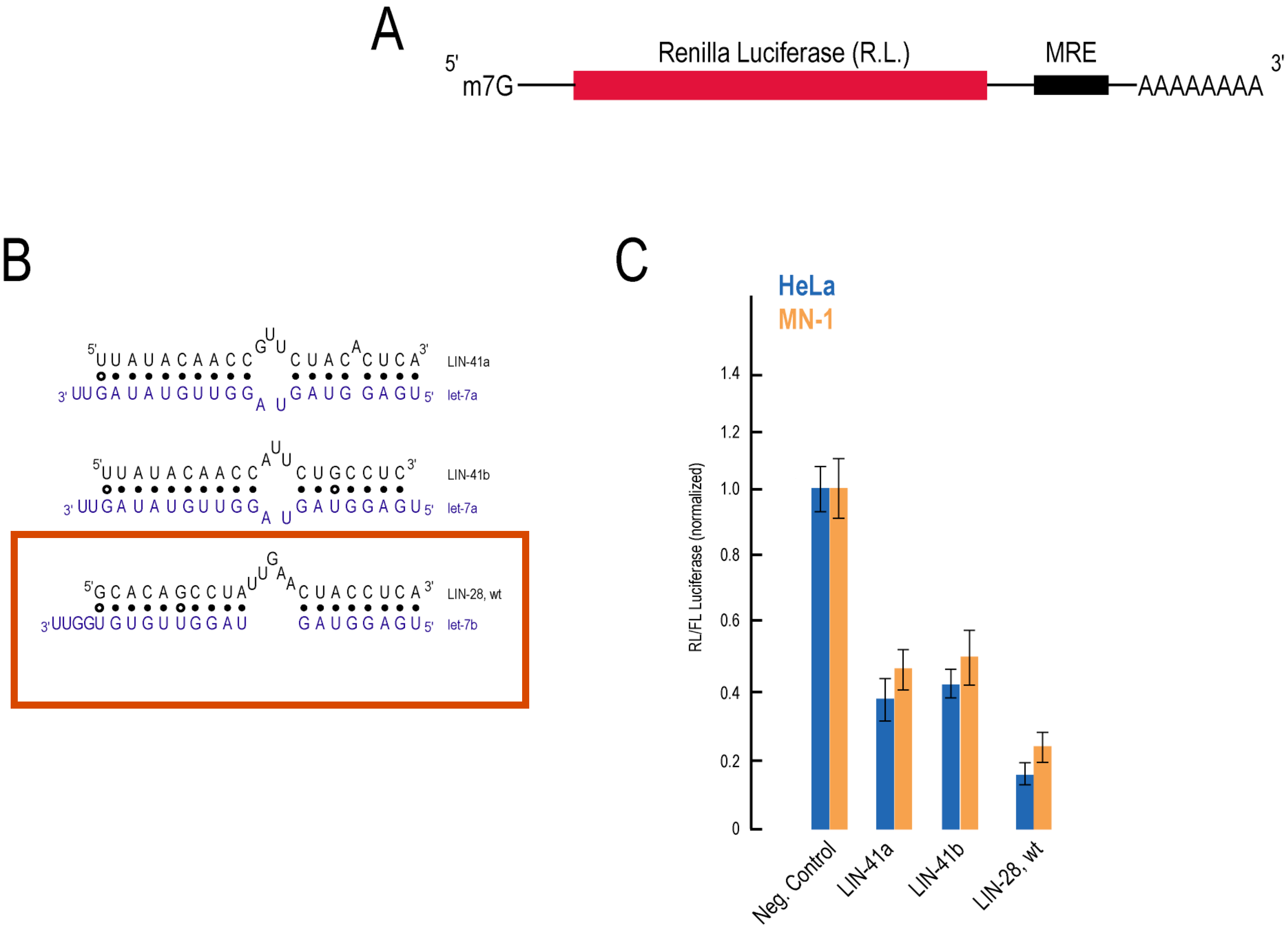
- Keep targets conserved in human / mouse orthologs.

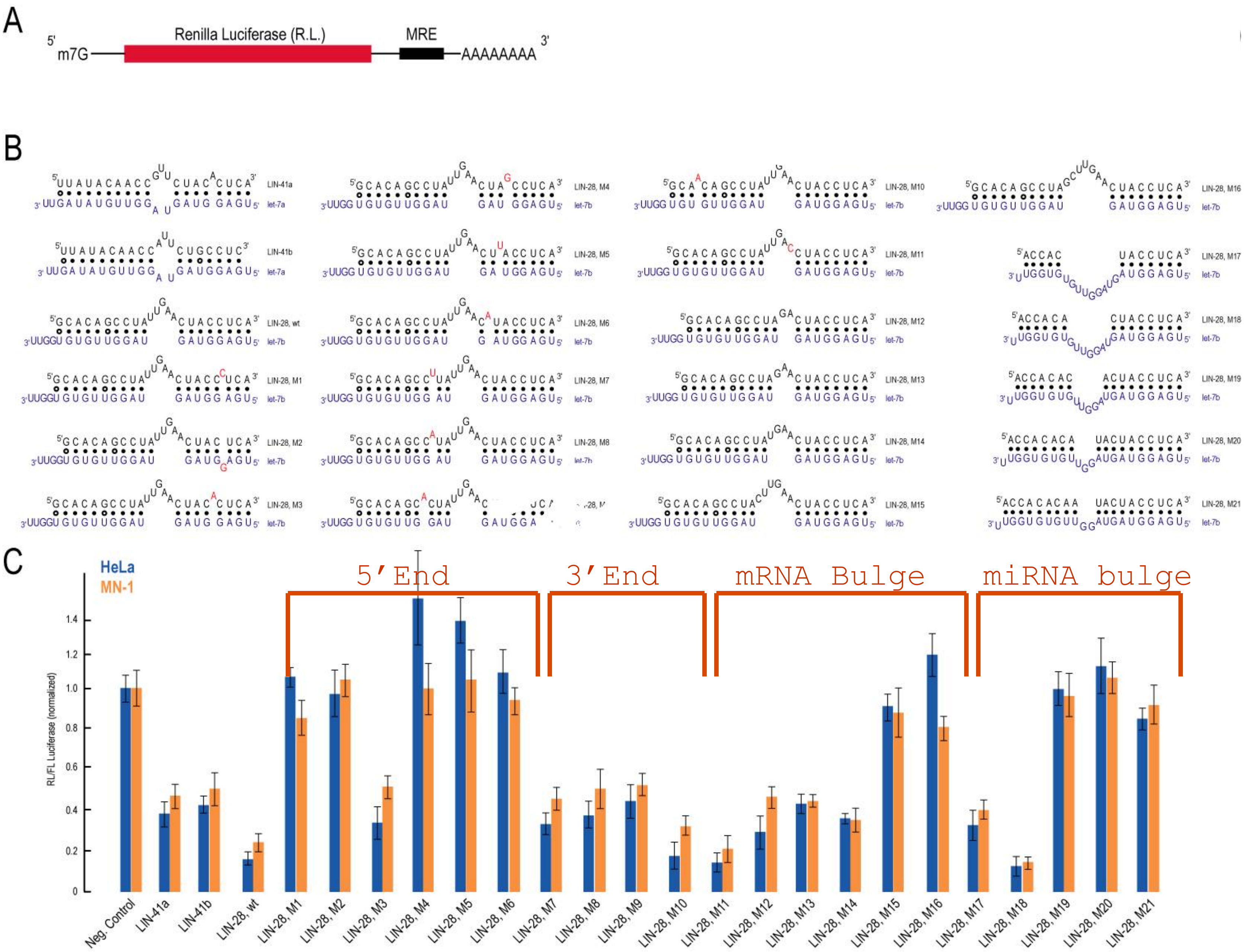
Human 3’ UTR

Mouse 3’ UTR

- -> Top 13 targets selected for experiments.

the wet experiment




A
5'
m7G
Renilla Luciferase (R.L.)
MRE
AAAAAAAA
3'
B
LIN-41a
let-7a
LIN-41b
let-7a
LIN-28, wt
let-7b
LIN-28, M1
LIN-28, M2
LIN-28, M3
LIN-28, M4
LIN-28, M5
LIN-28, M6
LIN-28, M7
LIN-28, M8
LIN-28, M10
LIN-28, M11
LIN-28, M12
LIN-28, M13
LIN-28, M14
LIN-28, M15
LIN-28, M16
LIN-28, M17
LIN-28, M18
LIN-28, M19
LIN-28, M20
LIN-28, M21
C
HeLa
MN-1
5’End
3’End
mRNA Bulge
miRNA bulge
RL/FL Luciferase (normalized)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Neg. Control
LIN-41a
LIN-41b
LIN-28, wt
LIN-28, M1
LIN-28, M2
LIN-28, M3
LIN-28, M4
LIN-28, M5
LIN-28, M6
LIN-28, M7
LIN-28, M8
LIN-28, M9
LIN-28, M10
LIN-28, M11
LIN-28, M12
LIN-28, M13
LIN-28, M14
LIN-28, M15
LIN-28, M16
LIN-28, M17
LIN-28, M18
LIN-28, M19
LIN-28, M20
LIN-28, M21

Computational evaluation

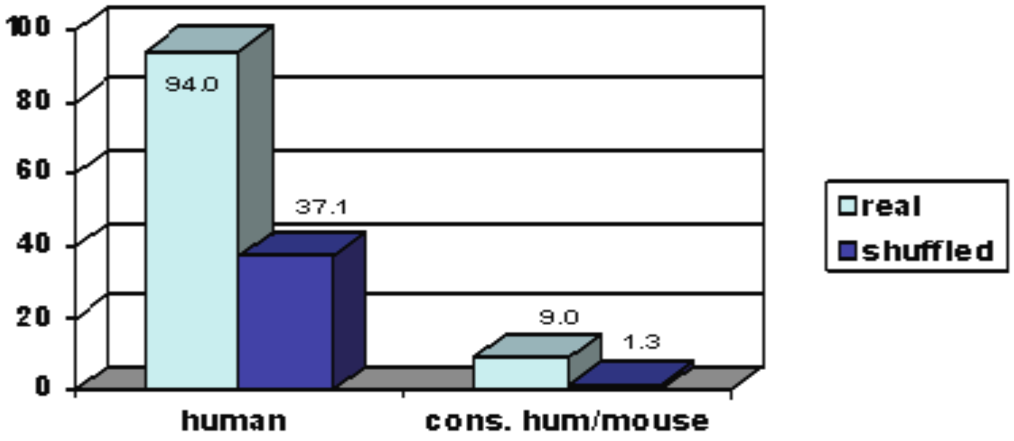
using random sequences (shuffled/artificial miRNA’s)
and conservation through human & mouse.

Signal : noise ratio

1. X = # total target site predictions for a set of real miRNAs
2. Y = # total target site predictions for a set of randomized miRNAs
3. X:Y is the signal:noise ratio which provides a measure of specificity

Example

1. X = 2000
2. Y = 1000
3. Signal:noise ratio is 2:1. For every 2 predicted targets, 1 is likely to be false (50% FPR).



Only energy	real	5,094	shuffled	4,974
Energy & conserv.	real	168	shuffled	158

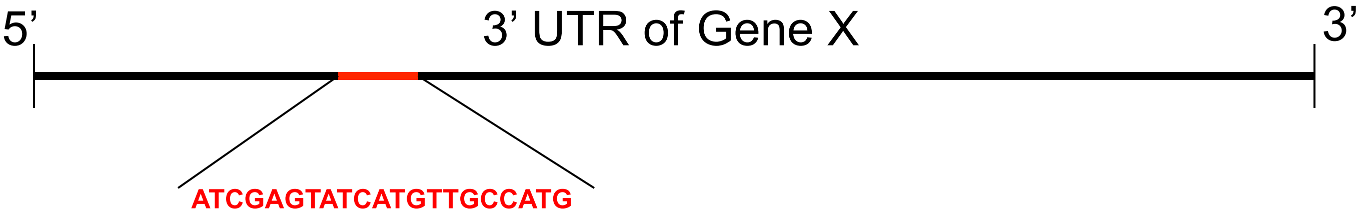
A combined computational-experimental approach predicts human microRNA targets

Marianthi Kiriakidou,[1,2] Peter T. Nelson,[1] Andrei Kouranov,[3] Petko Fitziev,[3,6] Costas Bouyioukos,[3] Zissimos Mourelatos,[1,7] and Artemis Hatzigeorgiou[3,4,5,8]

Departments of [1]Pathology, [2]Medicine, and [3]Genetics, School of Medicine, [4]Center for Bioinformatics, and [5]Computer and Information Science, School of Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Computational miRNA targets:
MiRNA Recognition Elements (MRE) and miRNA:gene intercations

- Predicting Individual binding sites or MREs



5’

3’ UTR of Gene X

3’

ATCGAGTATCATGTTGCCATG

MRE for miRNA y ?

- Predicting miRNA:gene interactions

5’

3’ UTR of Gene X

3’

MRE 1

MRE 2

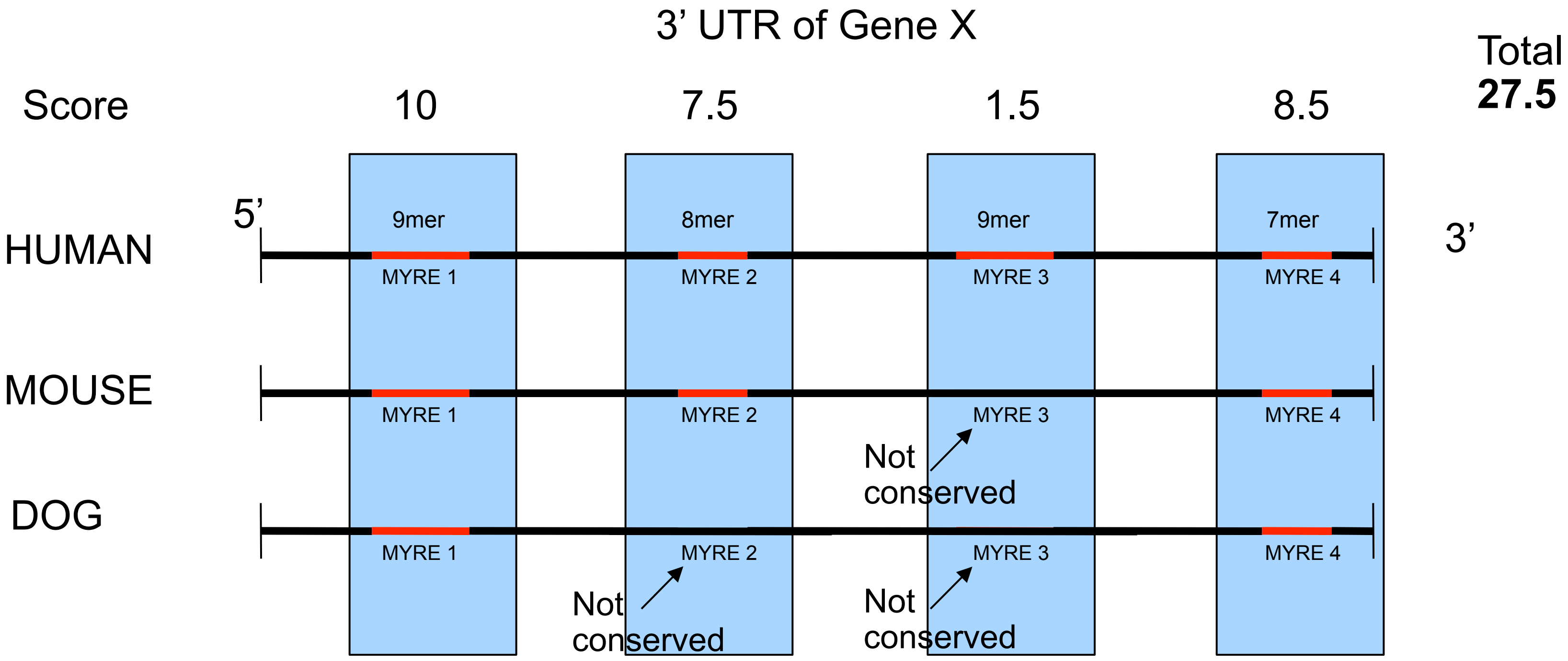
MRE 3

MRE 4

How likely is it that Gene X is a target of miRNA Y?

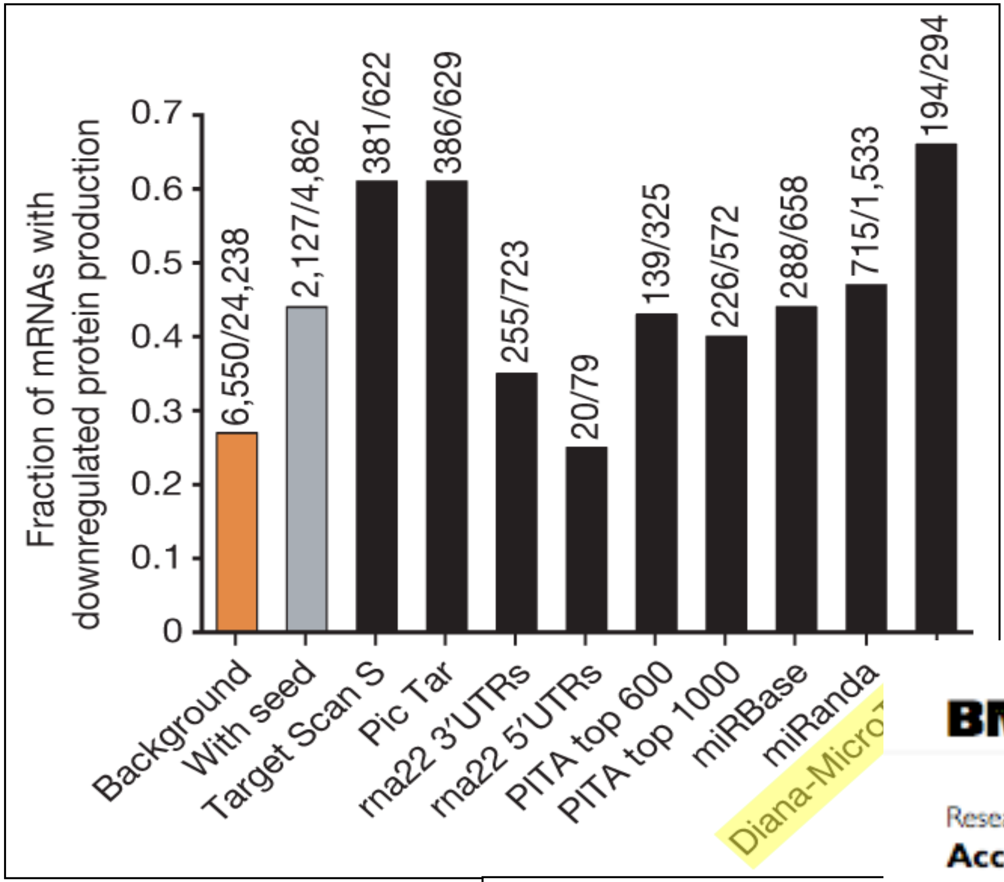
3'UTR score

Weighted sum over of all putative target scores per 3'UTR.



Widespread changes in protein synthesis induced by microRNAs

Matthias Selbach[1], Björn Schwanhäusser[1]*, Nadine Thierfelder[1]*, Zhuo Fang[1], Raya Khanin[2] & Nikolaus Rajewsky[1]



NATURE, 2008

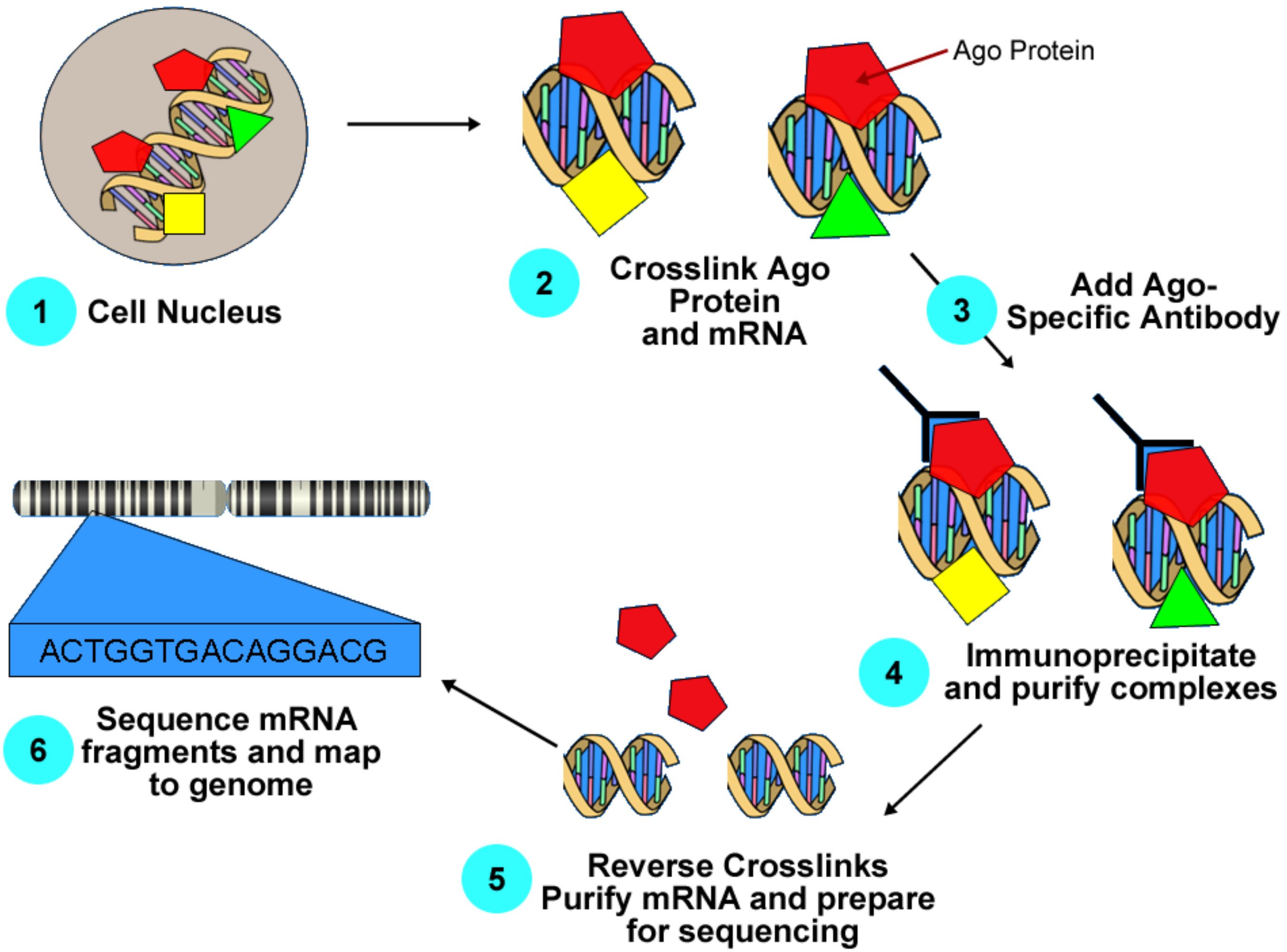
BMC Bioinformatics

Research article

Accurate microRNA target prediction correlates with protein repression levels

Manolis Maragkakis*†[1,2], Panagiotis Alexiou†[1,3], Giorgio L Papado
Martin Reczko[1,4], Theodore Dalamagas[5], George Giannopoulos[5,6],
George Goumas[7], Evangelos Koukis[7], Kornilios Kourtis[7], Victor A S
Praveen Sethupathy[8], Thanasis Vergoulis[5,6], Nectarios Koziris[7],
Timos Sellis[5,6], Panagiotis Tsanakas[7] and Artemis G Hatzigeorgiou

CLIP data (Chi *et al.* 2009, Hafner *et al*. 2010)



Note

PAR-CLIP (Hafner et al. 2010) , HITS-CLIP (Chi et al. 2009).

T->C mutation on the tags specifies binding sites within a region of 5 nts.

Computational miRNA targets: The second (next) generation

2002-2006

Limited number of experimentally identified MRE's.

2006-2008

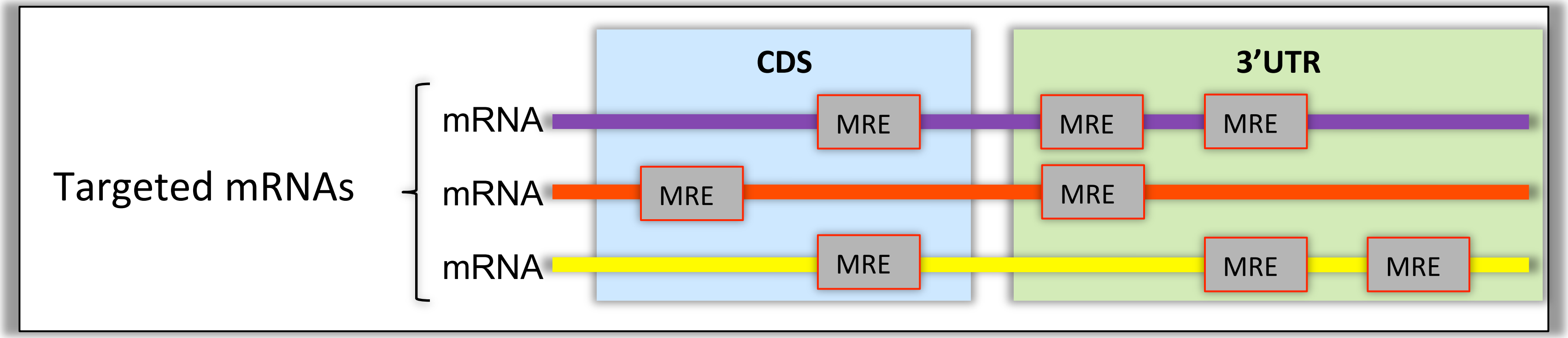
Microarrays (mRNA level) or proteomics (protein level) of miRNA knock down or over expression

Target genes are characterized but MRE's are unknown

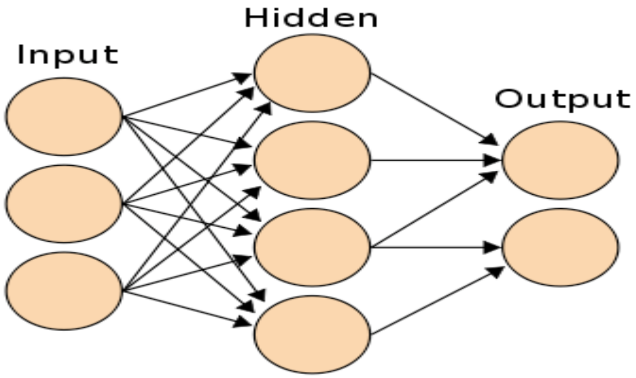
2009/2010

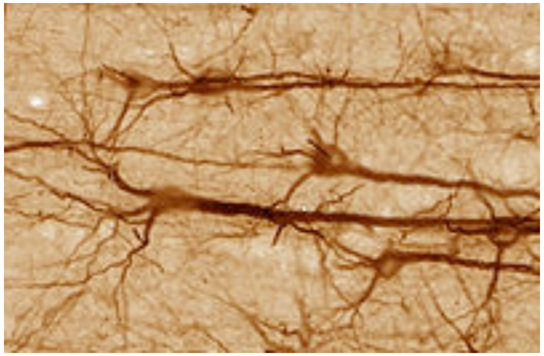
Sequencing data of AGO protein (PAR-CLIP, HITS-CLIP)

Identification of thousands of MRE's



Artificial neural networks are computational models based on biological neural networks.

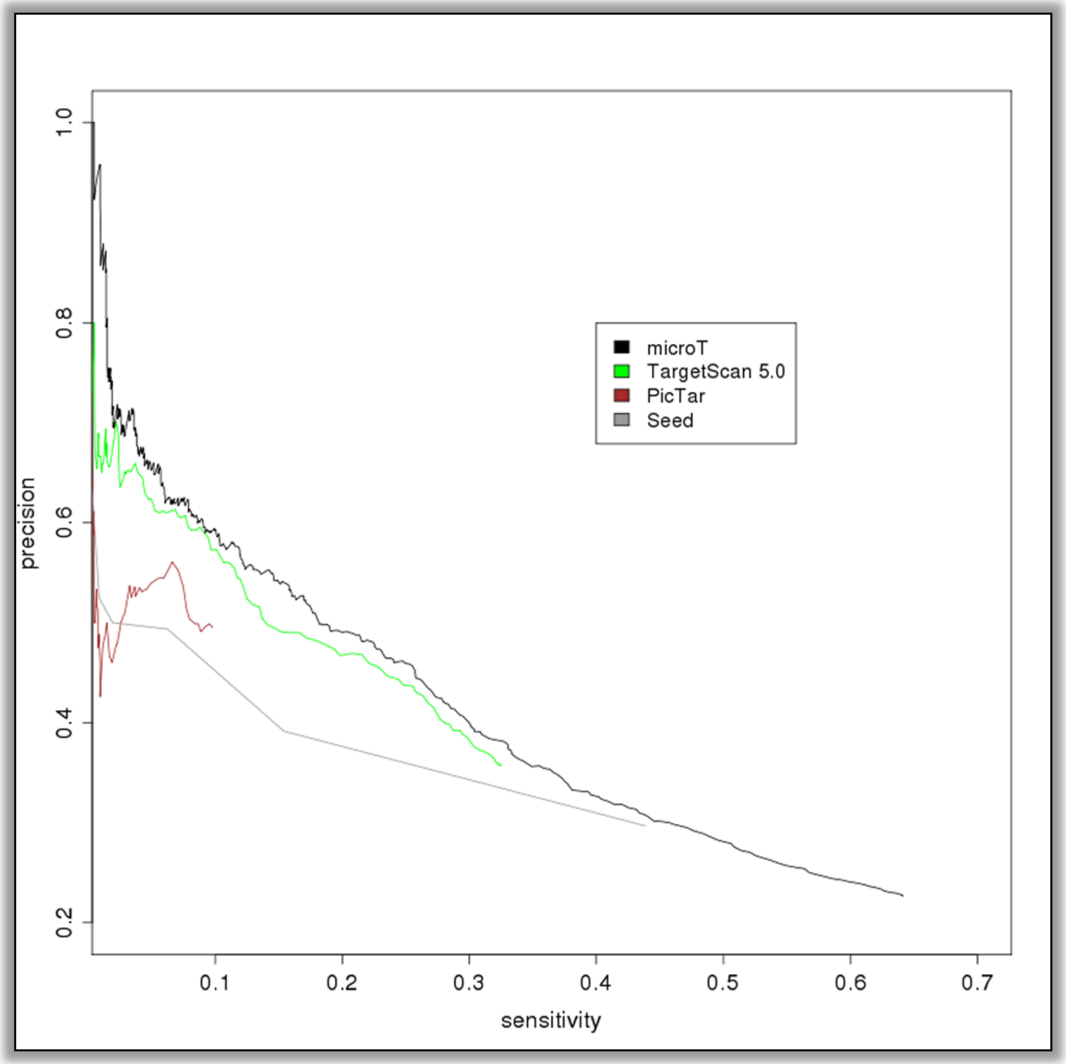




Mostly an adaptive system that changes its structure based on the information that flows through the network during the training.

Learning procedure:
Positive and negative data are represented to the ANN.
In iterative - small - steps the weights are adjusted in order to minimize the "error" in the separation of the two groups of data.

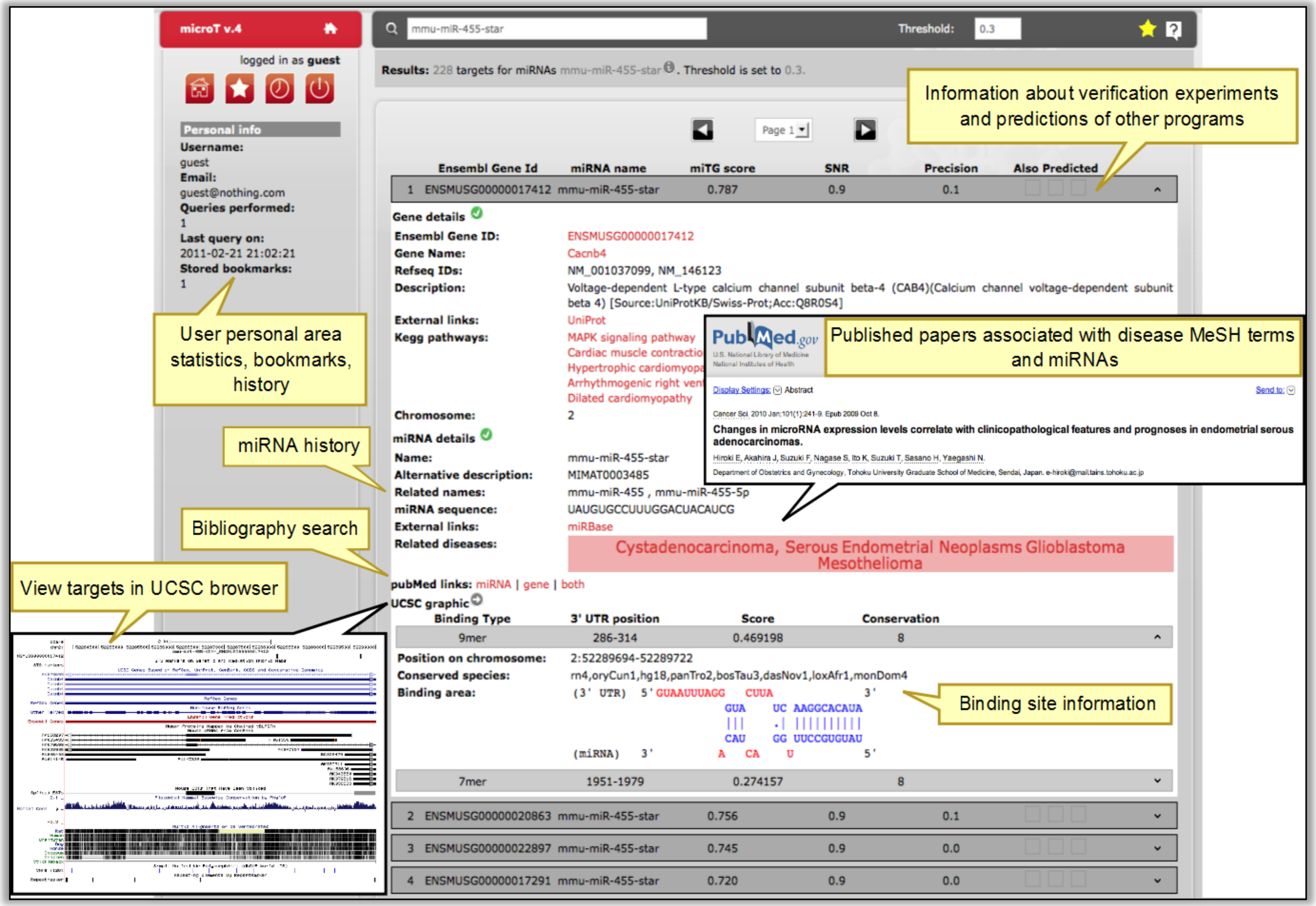
Computational miRNA targets: Performance comparison.



Compared to other widely used programs microT-CDS reaches:
higher precision & higher sensitivity

Reczko, M., et al., Bioinformatics , 2012

Computational miRNA targets: DIANA-microT interface



Username: guest
Email: guest@nothing.com
Queries performed: 1
Last query on: 2011-02-21 21:02:21
Stored bookmarks: 1

Results: 228 targets for miRNAs mmu-miR-455-star. Threshold is set to 0.3.

	Ensembl Gene Id	miRNA name	miTG score	SNR	Precision	Also Predicted
1	ENSMUSG00000017412	mmu-miR-455-star	0.787	0.9	0.1	
2	ENSMUSG00000020863	mmu-miR-455-star	0.756	0.9	0.1	
3	ENSMUSG00000022897	mmu-miR-455-star	0.745	0.9	0.0	
4	ENSMUSG00000017291	mmu-miR-455-star	0.720	0.9	0.0	

Gene details

Ensembl Gene ID: ENSMUSG00000017412
Gene Name: Cacnb4
Refseq IDs: NM_001037099, NM_146123
Description: Voltage-dependent L-type calcium channel subunit beta-4 (CAB4)(Calcium channel voltage-dependent subunit beta 4) [Source:UniProtKB/Swiss-Prot;Acc:Q8R0S4]
External links: UniProt
Kegg pathways: MAPK signaling pathway, Cardiac muscle contractio, Hypertrophic cardiomyopa, Arrhythmogenic right vent, Dilated cardiomyopathy
Chromosome: 2

miRNA details

Name: mmu-miR-455-star
Alternative description: MIMAT0003485
Related names: mmu-miR-455 , mmu-miR-455-5p
miRNA sequence: UAUGUGCCUUUGGACUACAUCG
External links: miRBase
Related diseases: Cystadenocarcinoma, Serous Endometrial Neoplasms Glioblastoma Mesothelioma

pubMed links: miRNA | gene | both

UCSC graphic

Binding Type	3' UTR position	Score	Conservation
9mer	286-314	0.469198	8
7mer	1951-1979	0.274157	8

Position on chromosome: 2:52289694-52289722
Conserved species: rn4,oryCun1,hg18,panTro2,bosTau3,dasNov1,loxAfr1,monDom4
Binding area:

```
(3' UTR)  5'GUAAUUUAGG   CUUA          3'
                     GUA    UC AAGGCACAUA
                     |||    .| ||||||||||
                     CAU    GG UUCCGUGUAU
(miRNA)   3'         A   CA   U         5'
```

PubMed.gov
U.S. National Library of Medicine
National Institutes of Health

Display Settings: Abstract
Send to:

Cancer Sci. 2010 Jan;101(1):241-9. Epub 2009 Oct 8.

Changes in microRNA expression levels correlate with clinicopathological features and prognoses in endometrial serous adenocarcinomas.

Hiroki E, Akahira J, Suzuki F, Nagase S, Ito K, Suzuki T, Sasano H, Yaegashi N.

Department of Obstetrics and Gynecology, Tohoku University Graduate School of Medicine, Sendai, Japan. e-hiroki@mail.tains.tohoku.ac.jp

Information about verification experiments and predictions of other programs

User personal area statistics, bookmarks, history

Published papers associated with disease MeSH terms and miRNAs

miRNA history

Bibliography search

View targets in UCSC browser

Binding site information

Paraskevopoulou, M.D., et al,. Nucleic Acids Res. , 2013

Experimental supported microRNA targets.

DIANA-TarBase

- A reference database devoted to the indexing of experimentally supported miRNA-targets
- More than a decade of continuous support in the non-coding RNA field
- The largest and oldest repository with thousands of high-quality experimentally supported miRNA-gene interactions
- Last update DIANA-TarBase v8 www.microrna.gr/tarbase

Dimitra Karagkouni, Maria D. Paraskevopoulou, Serafeim Chatzopoulos, Ioannis S. Vlachos, Spyros Tastsoglou, Ilias Kanellos, Dimitris Papadimitriou, Ioannis Kavakiotis, Sofia Maniou, Giorgos Skoufos, Thanasis Vergoulis, Theodore Dalamagas, Artemis G. Hatzigeorgiou; ***DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions****,* ***Nucleic Acids Research****, 2017*

Experimental Determination of miRNA – Gene Interactions

✓Gene Specific Techniques

direct

- Reporter genes
- Northern blotting
- qPCR
- Western blotting
- ELISA
- Immunihistochemistry

indirect

✓High Throughput Techniques

direct

- CLIP-Seq (HITS-CLIP, PAR-CLIP, iCLIP)
- CLASH/CLEAR-CLIP
- Microarrays
- RNA-Seq
- Proteomics (such as pSILAC)
- PARE-Seq
- Degradome-Seq
- Biotin tagged miRNA

indirect

Specific techniques:

- Reveal individual miRNA:mRNA interactions
- Complex networks of miRNA regulation can be missed

High-Throughput techniques:

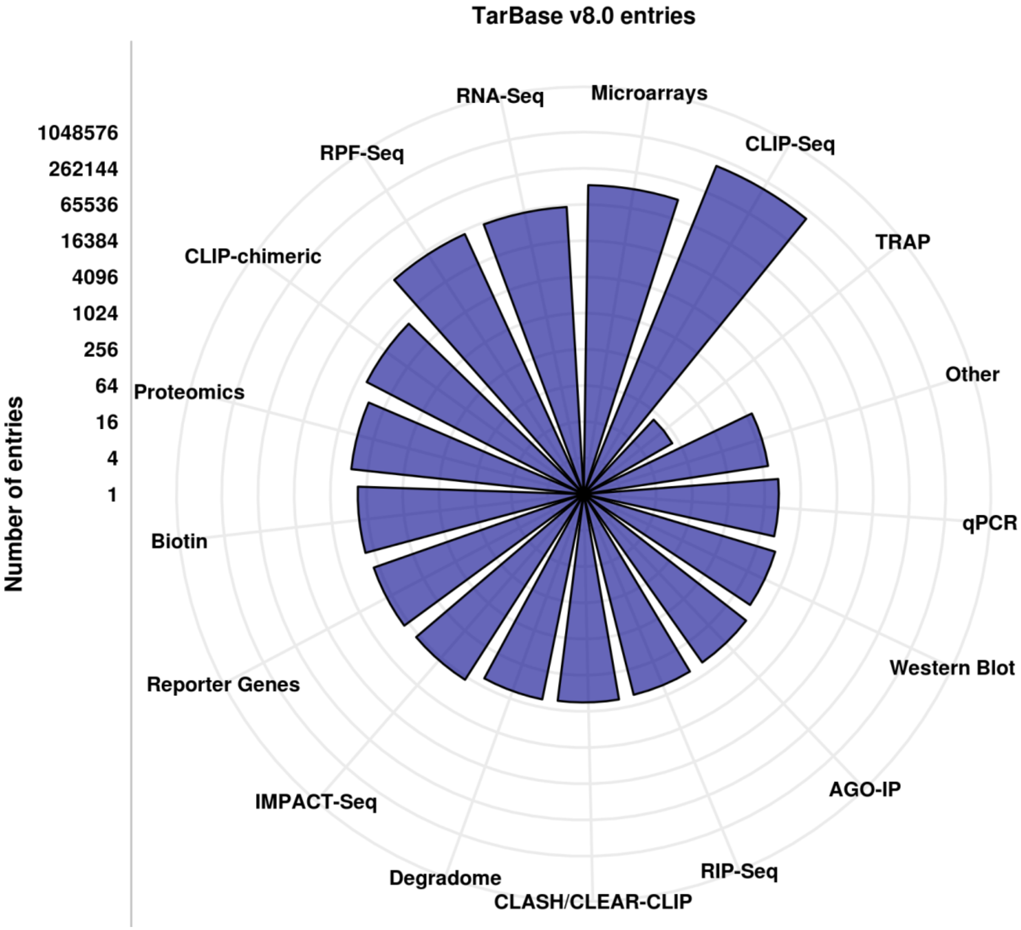
- Characterize numerous miRNA targets
- Necessitate extra computational steps

DIANA-TarBase

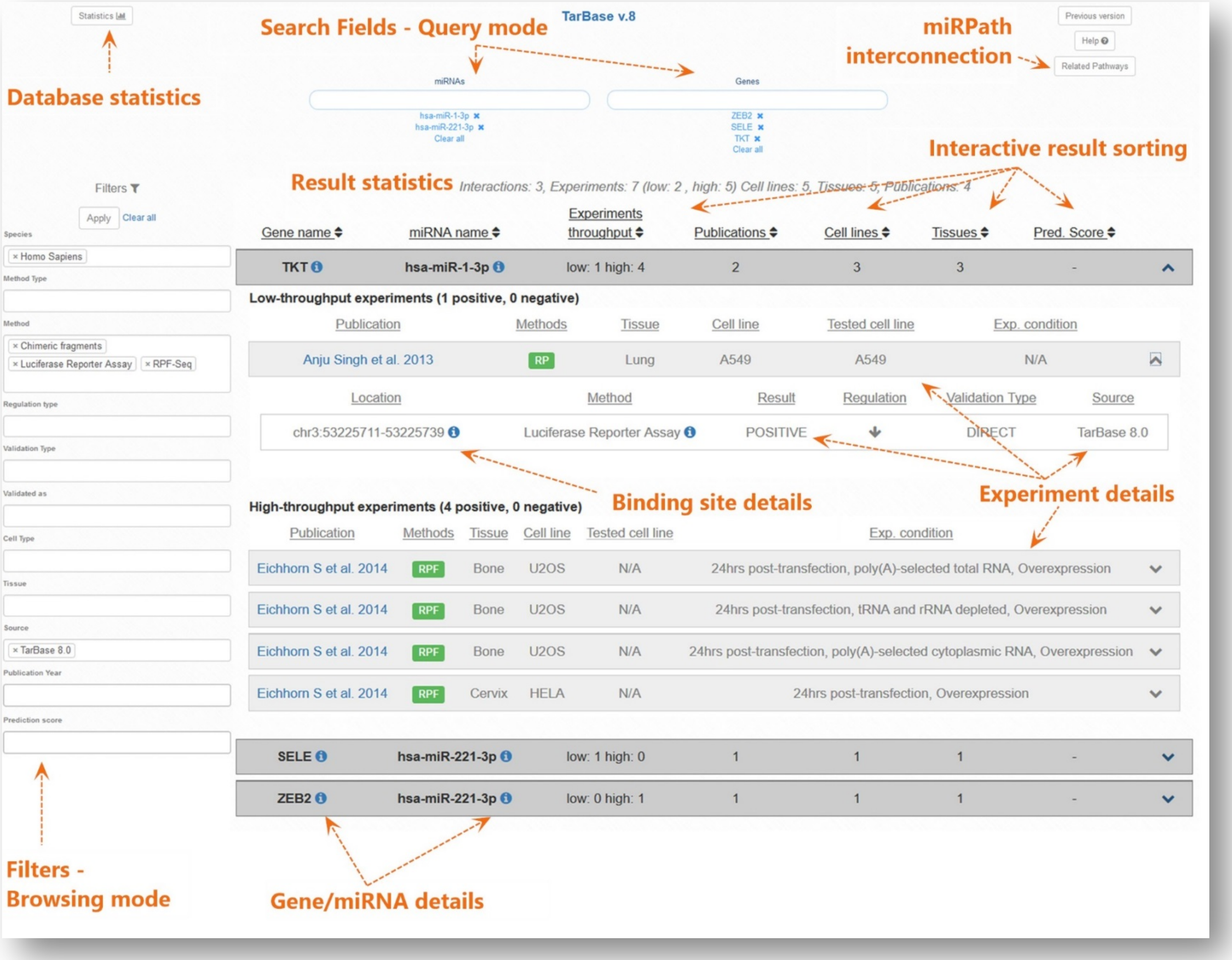
- TarBase v8.0 indexes **more than a million entries**
- This collection has been derived from experiments employing more than **33 distinct methods**, on **592 tissues/cell types** and **~430 experimental conditions** from **18 species**

TarBase hosts

- **> 10,000** interactions from specific techniques (~**5,000 Reporter gene entries**)
- **> 14,000** direct miRNA-mRNA chimeric fragments
- **> 230,000 entries** from miRNA perturbation experiments
- more than **700,000 entries from >150 CLIP-Seq** datasets



TarBase v8 interface



miRNAs: hsa-miR-1-3p, hsa-miR-221-3p
Genes: ZEB2, SELE, TKT

Filters
Apply | Clear all

Species: × Homo Sapiens
Method Type:
Method: × Chimeric fragments, × Luciferase Reporter Assay, × RPF-Seq
Regulation type:
Validation Type:
Validated as:
Cell Type:
Tissue:
Source: × TarBase 8.0
Publication Year:
Prediction score:

Interactions: 3, Experiments: 7 (low: 2 , high: 5) Cell lines: 5, Tissues: 5, Publications: 4

Gene name	miRNA name	Experiments throughput	Publications	Cell lines	Tissues	Pred. Score
TKT	**hsa-miR-1-3p**	low: 1 high: 4	2	3	3	-

Low-throughput experiments (1 positive, 0 negative)

Publication	Methods	Tissue	Cell line	Tested cell line	Exp. condition
Anju Singh et al. 2013	RP	Lung	A549	A549	N/A

Location	Method	Result	Regulation	Validation Type	Source
chr3:53225711-53225739	Luciferase Reporter Assay	POSITIVE	↓	DIRECT	TarBase 8.0

High-throughput experiments (4 positive, 0 negative)

Publication	Methods	Tissue	Cell line	Tested cell line	Exp. condition
Eichhorn S et al. 2014	RPF	Bone	U2OS	N/A	24hrs post-transfection, poly(A)-selected total RNA, Overexpression
Eichhorn S et al. 2014	RPF	Bone	U2OS	N/A	24hrs post-transfection, tRNA and rRNA depleted, Overexpression
Eichhorn S et al. 2014	RPF	Bone	U2OS	N/A	24hrs post-transfection, poly(A)-selected cytoplasmic RNA, Overexpression
Eichhorn S et al. 2014	RPF	Cervix	HELA	N/A	24hrs post-transfection, Overexpression

Gene name	miRNA name	Experiments throughput	Publications	Cell lines	Tissues	Pred. Score
SELE	**hsa-miR-221-3p**	low: 1 high: 0	1	1	1	-
ZEB2	**hsa-miR-221-3p**	low: 0 high: 1	1	1	1	-

miRNA targeting LncRNA

Long non coding RNA (lncRNA)

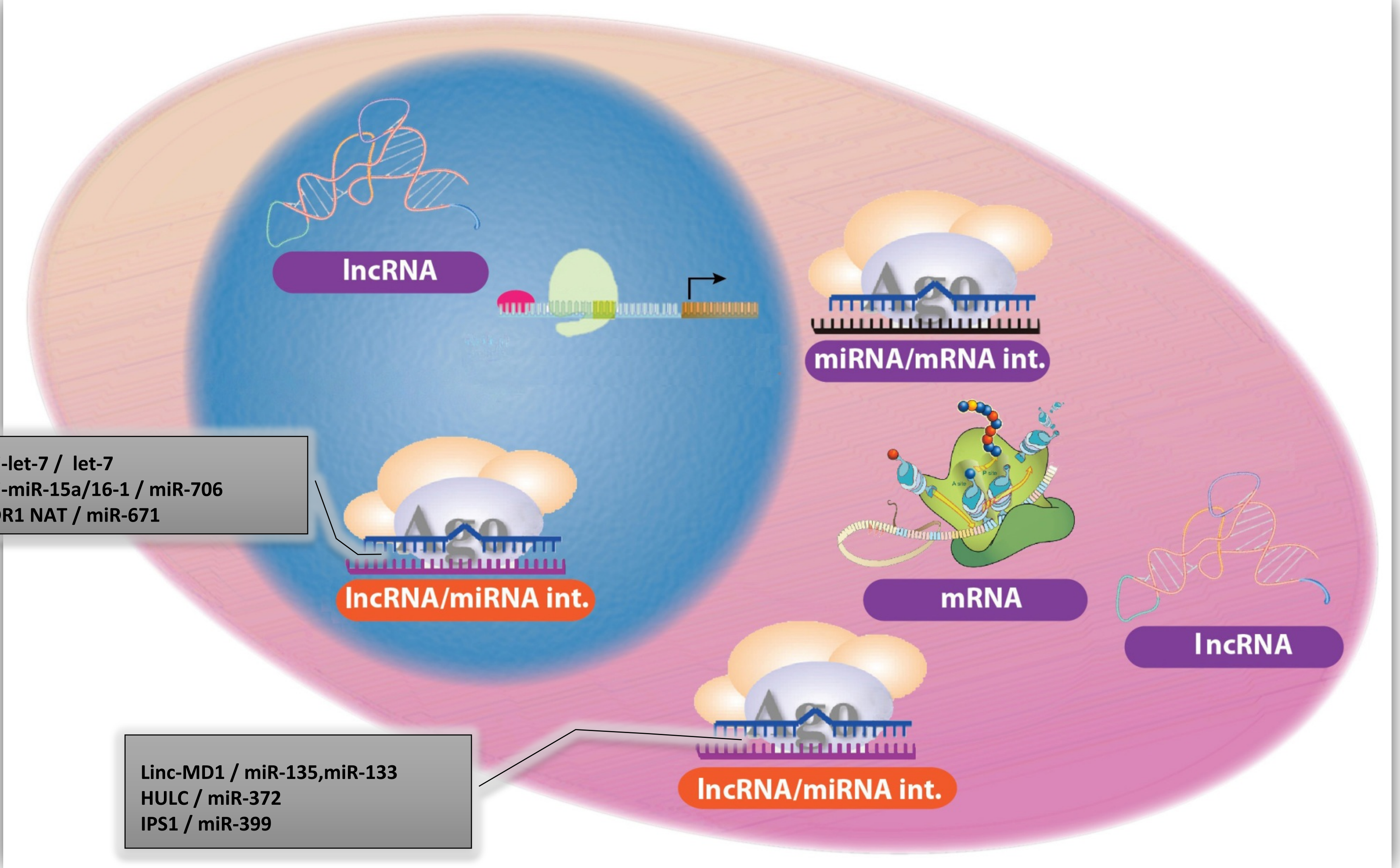
- >200 nts, no clearly defined ORF
- splicing, polyA // non-polyA
- Variable conservation
- Usually low expression
- highly specific disease, tissue, developmental stage expression
- categorized according to their loci of origin

ncRNA Regulation level

“**competing endogenous RNA**” (ceRNA) activity in the transcriptome level. mRNAs, pseudogenes, ncRNAs communicate through a ceRNA language, forming a **large-scale regulatory network**

Salmena L et al. Cell. 2011 Aug 5;146(3)

miRNA:ncRNA regulation



pri-let-7 / let-7
pri-miR-15a/16-1 / miR-706
CDR1 NAT / miR-671

Linc-MD1 / miR-135,miR-133
HULC / miR-372
IPS1 / miR-399

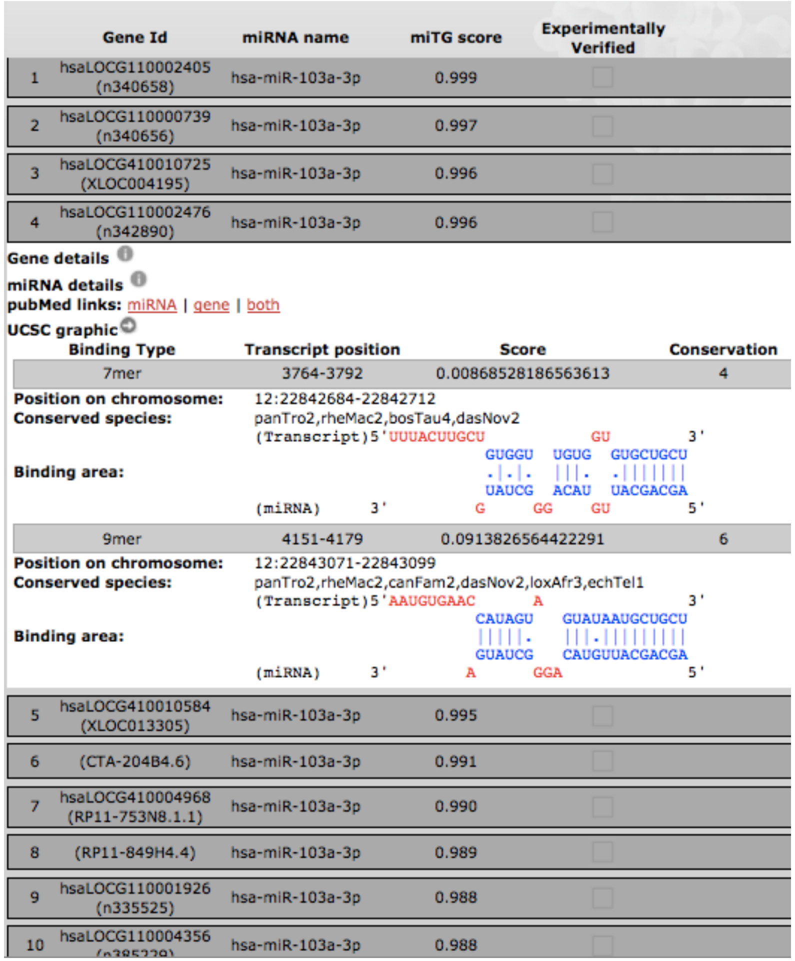
Salmena L et al. Cell. 2011 Aug 5;146(3)
Marcella Cesana et al. Cell. 2011 October 14; 147(2)
Zisoulis DG et al. Nature. 2012 Jun 28;486(7404)

Long Non Coding RNAs

LncBase is the largest available repository of miRNA LNC RNA interactions

- The **Experimental Module** contains more than 5,000 interactions between 2,958 lncRNAs and 120 miRNAs.
- The **Prediction Module** contains detailed information between 56,097 lncRNAs and 3,078 miRNAs.

Integration into RNAcentral (EBI)



	Gene Id	miRNA name	miTG score	Experimentally Verified
1	hsaLOCG110002405 (n340658)	hsa-miR-103a-3p	0.999	☐
2	hsaLOCG110000739 (n340656)	hsa-miR-103a-3p	0.997	☐
3	hsaLOCG410010725 (XLOC004195)	hsa-miR-103a-3p	0.996	☐
4	hsaLOCG110002476 (n342890)	hsa-miR-103a-3p	0.996	☐

Gene details

miRNA details

pubMed links: miRNA | gene | both

UCSC graphic

Binding Type	Transcript position	Score	Conservation
7mer	3764-3792	0.00868528186563613	4

Position on chromosome: 12:22842684-22842712

Conserved species: panTro2,rheMac2,bosTau4,dasNov2

Binding area:

```
(Transcript)5'UUUACUUGCU          GU        3'
                       GUGGU  UGUG  GUGCUGCU
                       .|.|.  |||.  .|||||||
                       UAUCG  ACAU  UACGACGA
(miRNA)     3'        G      GG     GU      5'
```

Binding Type	Transcript position	Score	Conservation
9mer	4151-4179	0.0913826564422291	6

Position on chromosome: 12:22843071-22843099

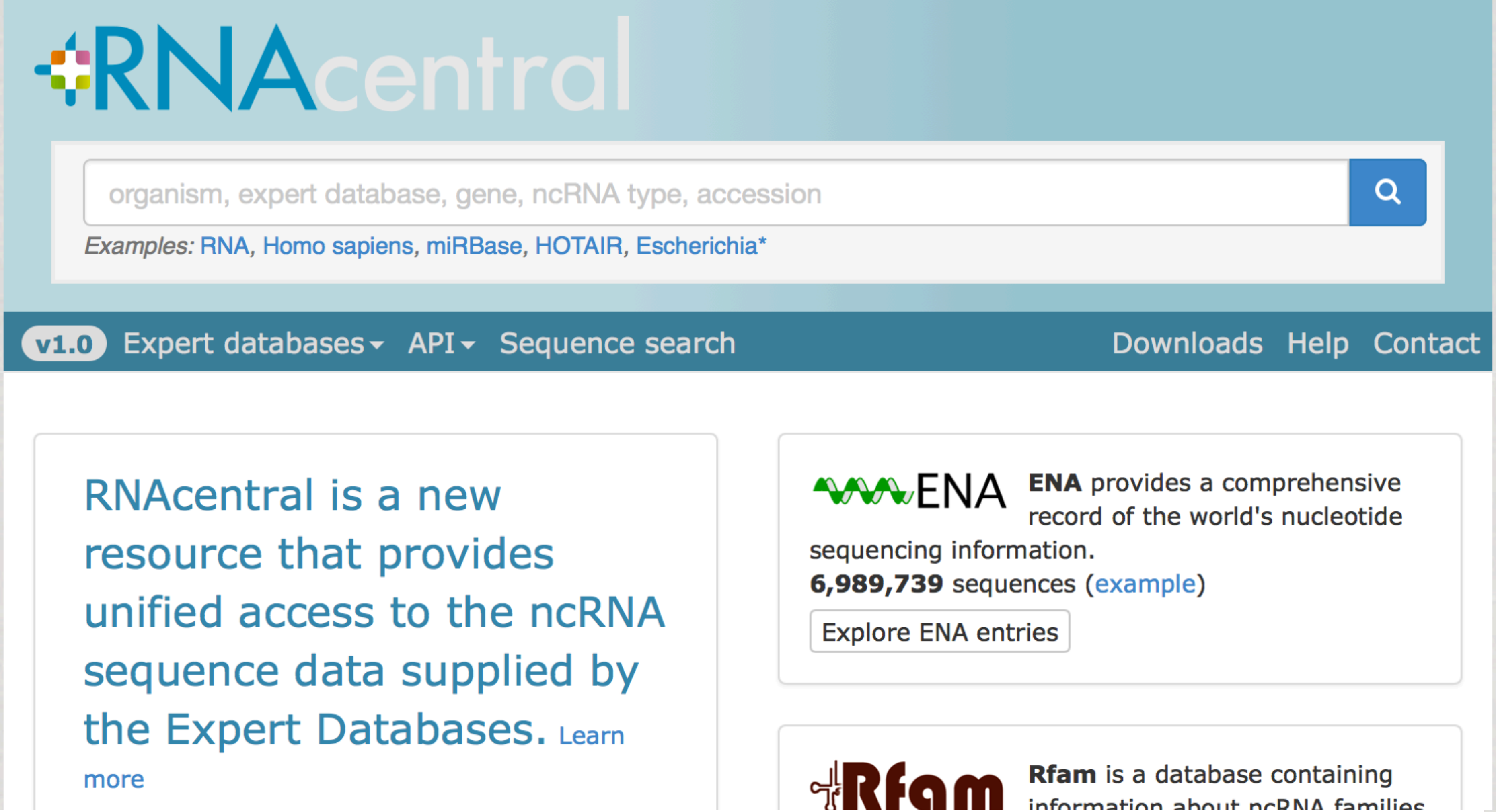
Conserved species: panTro2,rheMac2,canFam2,dasNov2,loxAfr3,echTel1

Binding area:

```
(Transcript)5'AAUGUGAAC      A              3'
                      CAUAGU  GUAUAAUGCUGCU
                      |||||.  |||.|||||||||
                      GUAUCG  CAUGUUACGACGA
(miRNA)     3'       A       GGA            5'
```

	Gene Id	miRNA name	miTG score	Experimentally Verified
5	hsaLOCG410010584 (XLOC013305)	hsa-miR-103a-3p	0.995	☐
6	(CTA-204B4.6)	hsa-miR-103a-3p	0.991	☐
7	hsaLOCG410004968 (RP11-753N8.1.1)	hsa-miR-103a-3p	0.990	☐
8	(RP11-849H4.4)	hsa-miR-103a-3p	0.989	☐
9	hsaLOCG110001926 (n335525)	hsa-miR-103a-3p	0.988	☐
10	hsaLOCG110004356	hsa-miR-103a-3p	0.988	☐

Paraskevopoulou, MD, Vlachos, IS, Karagkouni D, Georgakilas, G, Kanellos I, N., Vergoulis, Tsanakas P, Floros E, Dalamagas T, Hatzigeorgiou AG. DIANA-LncBase v2: Indexing microRNA targets on non-coding transcripts ***Nucleic Acids Res***. 2016 Jan 4;44(D1):D231-8. doi: 10.1093/nar/gkv1270.

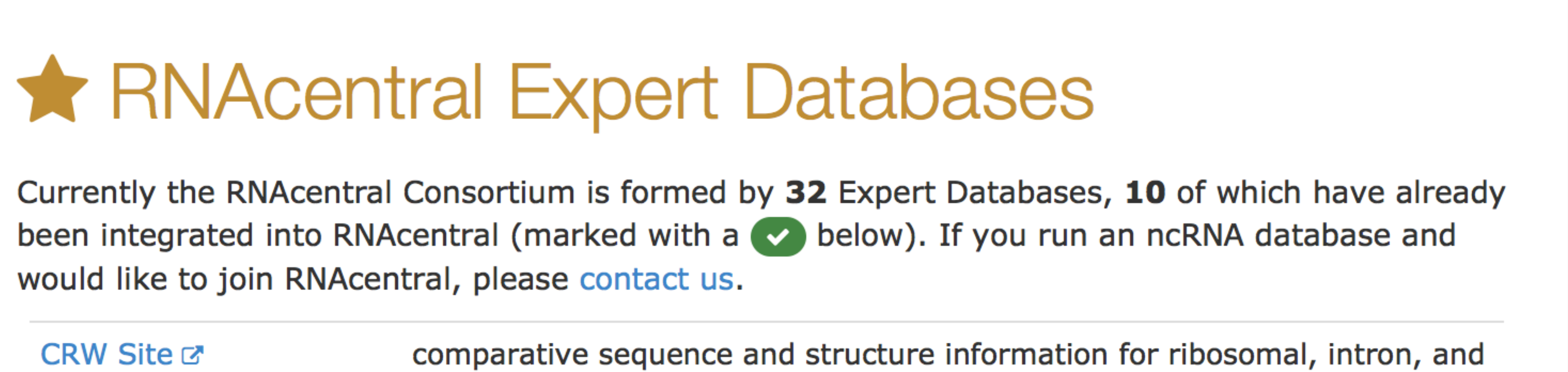


Examples: RNA, Homo sapiens, miRBase, HOTAIR, Escherichia*

RNAcentral is a new resource that provides unified access to the ncRNA sequence data supplied by the Expert Databases. Learn more

ENA **ENA** provides a comprehensive record of the world's nucleotide sequencing information.
6,989,739 sequences (example)

Rfam **Rfam** is a database containing information about ncRNA families



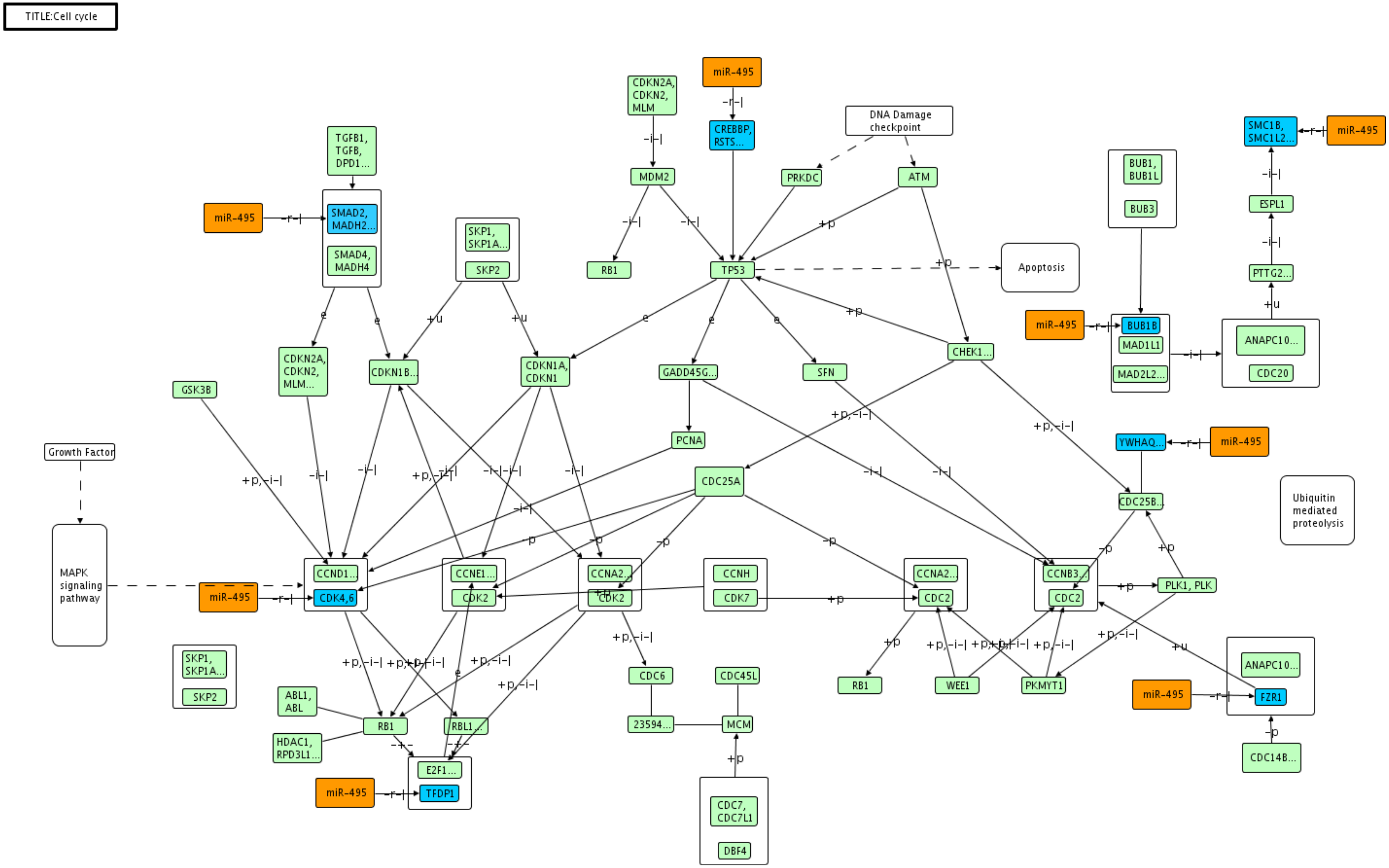
★ RNAcentral Expert Databases

Currently the RNAcentral Consortium is formed by **32** Expert Databases, **10** of which have already been integrated into RNAcentral (marked with a ✓ below). If you run an ncRNA database and would like to join RNAcentral, please contact us.

CRW Site	comparative sequence and structure information for ribosomal, intron, and

Connecting microRNAs to pathways

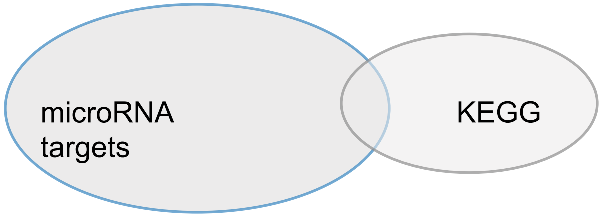
KEGG cell cycle pathways with miRNA targets of miR-495



www.microrna.gr/mirpath/

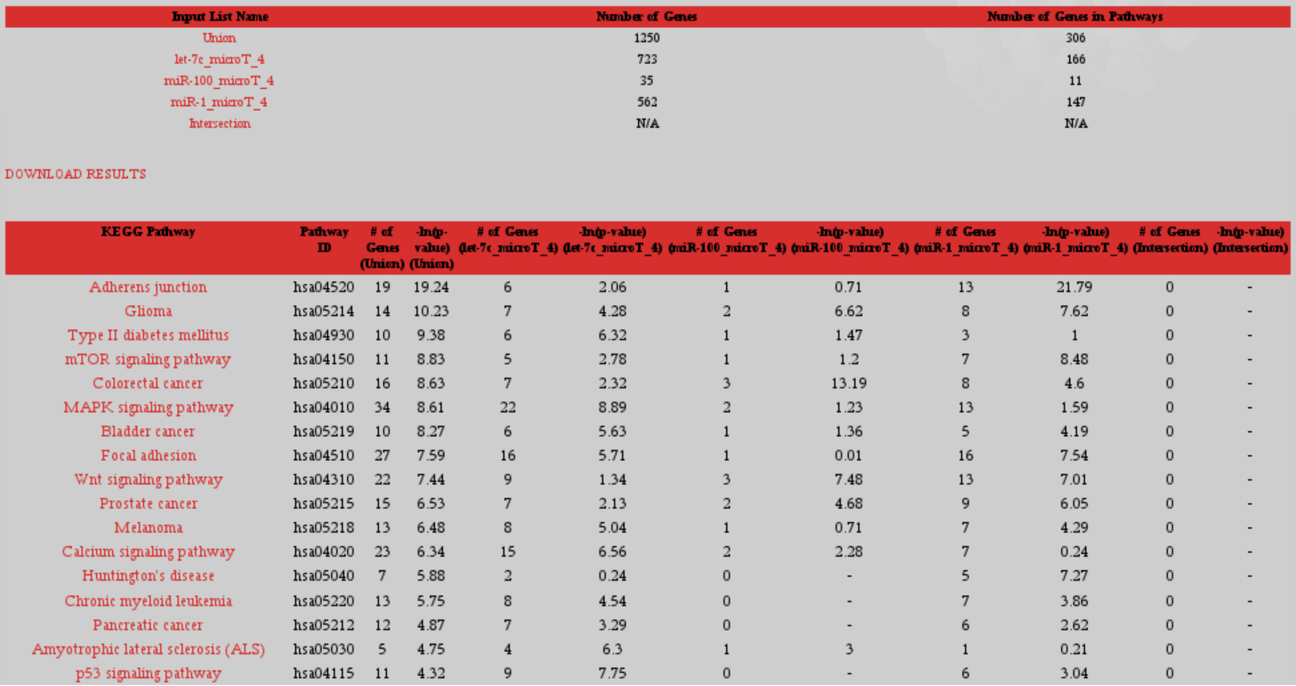
DIANA miRPath

Integrating human and mouse microRNAs in pathways



Small overlap – Not significant

Large overlap – Significant

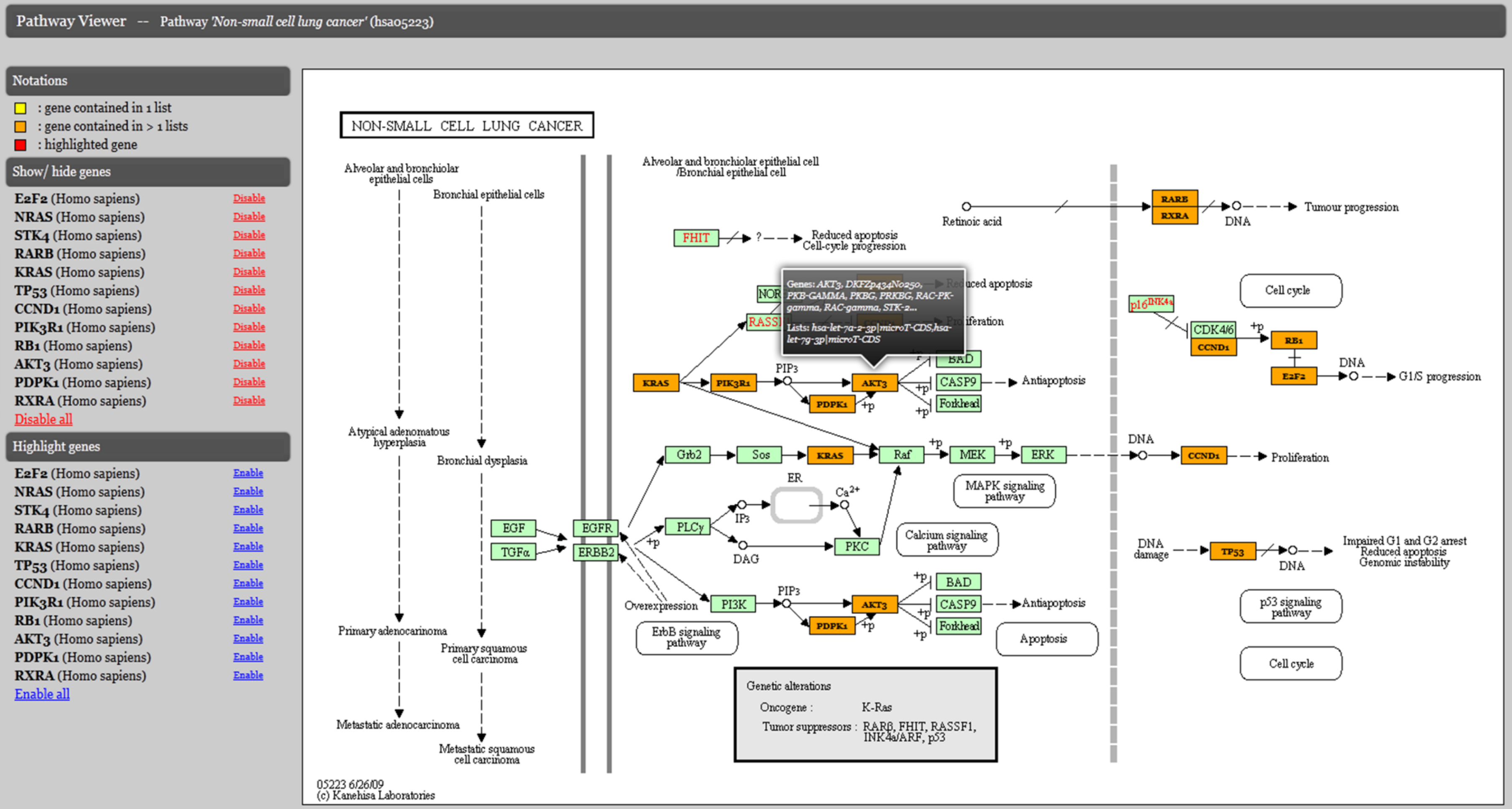


Input List Name	Number of Genes	Number of Genes in Pathways
Union	1250	306
let-7c_microT_4	723	166
miR-100_microT_4	35	11
miR-1_microT_4	562	147
Intersection	N/A	N/A

DOWNLOAD RESULTS

KEGG Pathway	Pathway ID	# of Genes (Union)	-ln(p-value) (Union)	# of Genes (let-7c_microT_4)	-ln(p-value) (let-7c_microT_4)	# of Genes (miR-100_microT_4)	-ln(p-value) (miR-100_microT_4)	# of Genes (miR-1_microT_4)	-ln(p-value) (miR-1_microT_4)	# of Genes (Intersection)	-ln(p-value) (Intersection)
Adherens junction	hsa04520	19	19.24	6	2.06	1	0.71	13	21.79	0	-
Glioma	hsa05214	14	10.23	7	4.28	2	6.62	8	7.62	0	-
Type II diabetes mellitus	hsa04930	10	9.38	6	6.32	1	1.47	3	1	0	-
mTOR signaling pathway	hsa04150	11	8.83	5	2.78	1	1.2	7	8.48	0	-
Colorectal cancer	hsa05210	16	8.63	7	2.32	3	13.19	8	4.6	0	-
MAPK signaling pathway	hsa04010	34	8.61	22	8.89	2	1.23	13	1.59	0	-
Bladder cancer	hsa05219	10	8.27	6	5.63	1	1.36	5	4.19	0	-
Focal adhesion	hsa04510	27	7.59	16	5.71	1	0.01	16	7.54	0	-
Wnt signaling pathway	hsa04310	22	7.44	9	1.34	3	7.48	13	7.01	0	-
Prostate cancer	hsa05215	15	6.53	7	2.13	2	4.68	9	6.05	0	-
Melanoma	hsa05218	13	6.48	8	5.04	1	0.71	7	4.29	0	-
Calcium signaling pathway	hsa04020	23	6.34	15	6.56	2	2.28	7	0.24	0	-
Huntington's disease	hsa05040	7	5.88	2	0.24	0	-	5	7.27	0	-
Chronic myeloid leukemia	hsa05220	13	5.75	8	4.54	0	-	7	3.86	0	-
Pancreatic cancer	hsa05212	12	4.87	7	3.29	0	-	6	2.62	0	-
Amyotrophic lateral sclerosis (ALS)	hsa05030	5	4.75	4	6.3	1	3	1	0.21	0	-
p53 signaling pathway	hsa04115	11	4.32	9	7.75	0	-	6	3.04	0	-

DIANA-miRPath v3.0 www.microrna.gr/miRPathv3



Pathway Viewer -- Pathway *'Non-small cell lung cancer'* (hsa05223)

Notations

🟨 : gene contained in 1 list
🟧 : gene contained in > 1 lists
🟥 : highlighted gene

Show/ hide genes

E2F2 (Homo sapiens)	Disable
NRAS (Homo sapiens)	Disable
STK4 (Homo sapiens)	Disable
RARB (Homo sapiens)	Disable
KRAS (Homo sapiens)	Disable
TP53 (Homo sapiens)	Disable
CCND1 (Homo sapiens)	Disable
PIK3R1 (Homo sapiens)	Disable
RB1 (Homo sapiens)	Disable
AKT3 (Homo sapiens)	Disable
PDPK1 (Homo sapiens)	Disable
RXRA (Homo sapiens)	Disable

Disable all

Highlight genes

E2F2 (Homo sapiens)	Enable
NRAS (Homo sapiens)	Enable
STK4 (Homo sapiens)	Enable
RARB (Homo sapiens)	Enable
KRAS (Homo sapiens)	Enable
TP53 (Homo sapiens)	Enable
CCND1 (Homo sapiens)	Enable
PIK3R1 (Homo sapiens)	Enable
RB1 (Homo sapiens)	Enable
AKT3 (Homo sapiens)	Enable
PDPK1 (Homo sapiens)	Enable
RXRA (Homo sapiens)	Enable

Enable all

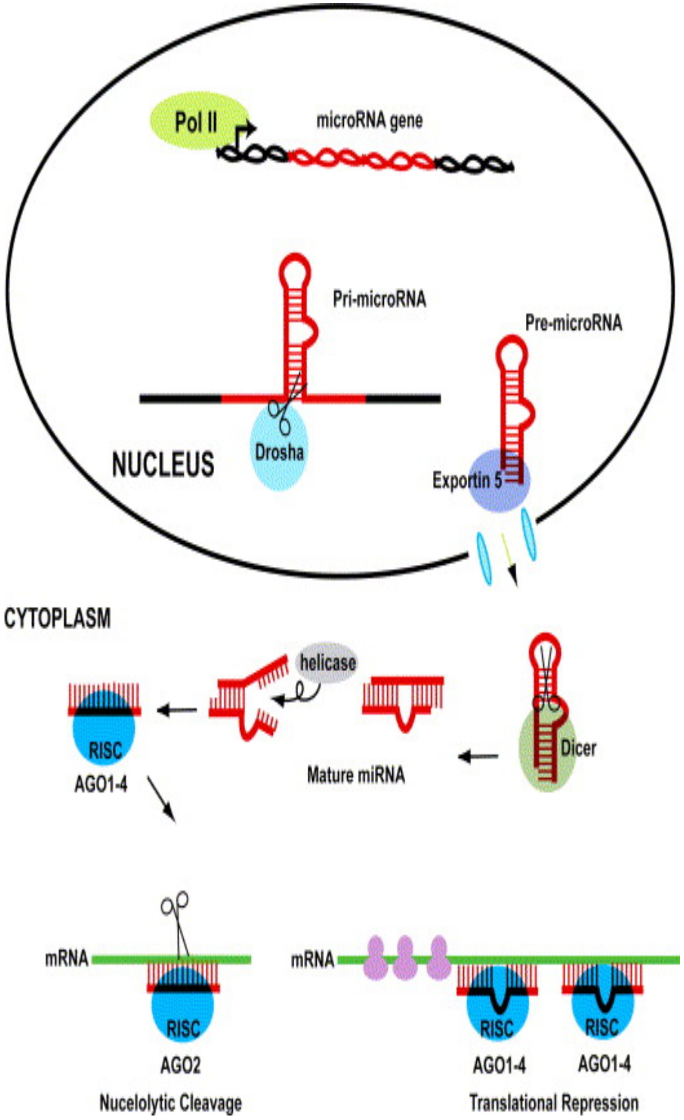
Genes: *AKT3, DKFZp434N0250, PKB-GAMMA, PKBG, PRKBG, RAC-PK-gamma, RAC-gamma, STK-2...*

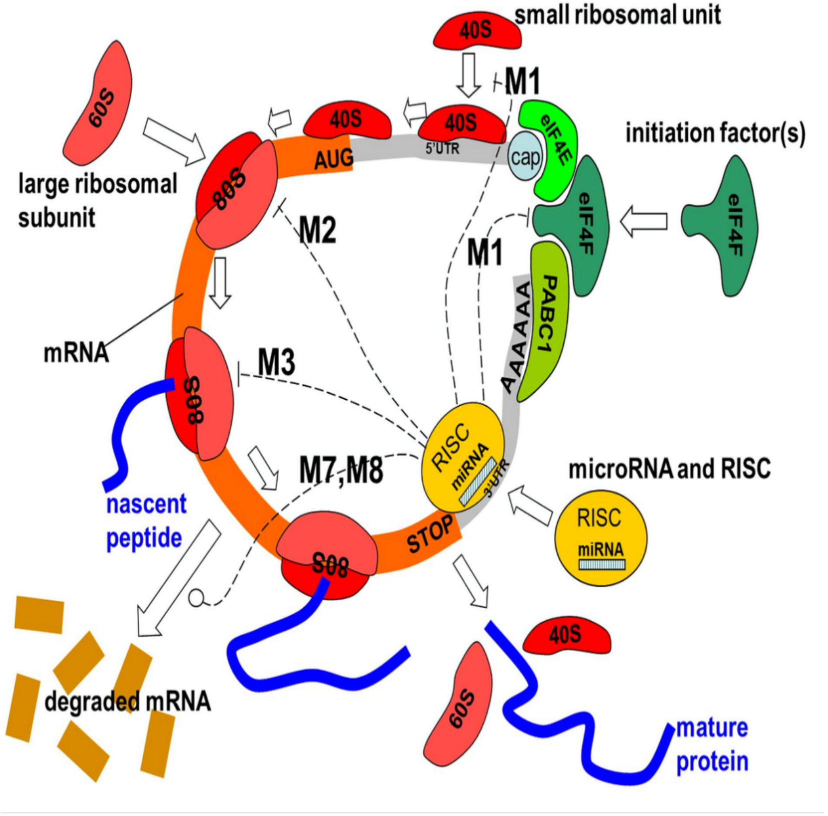
Lists: *hsa-let-7a-2-3p|microT-CDS,hsa-let-7g-3p|microT-CDS*

Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. ***Nucleic Acids Res.*** 2015 Jul 1;43(W1):W460-6.

REGULATION

microRNA Biogenesis & Function

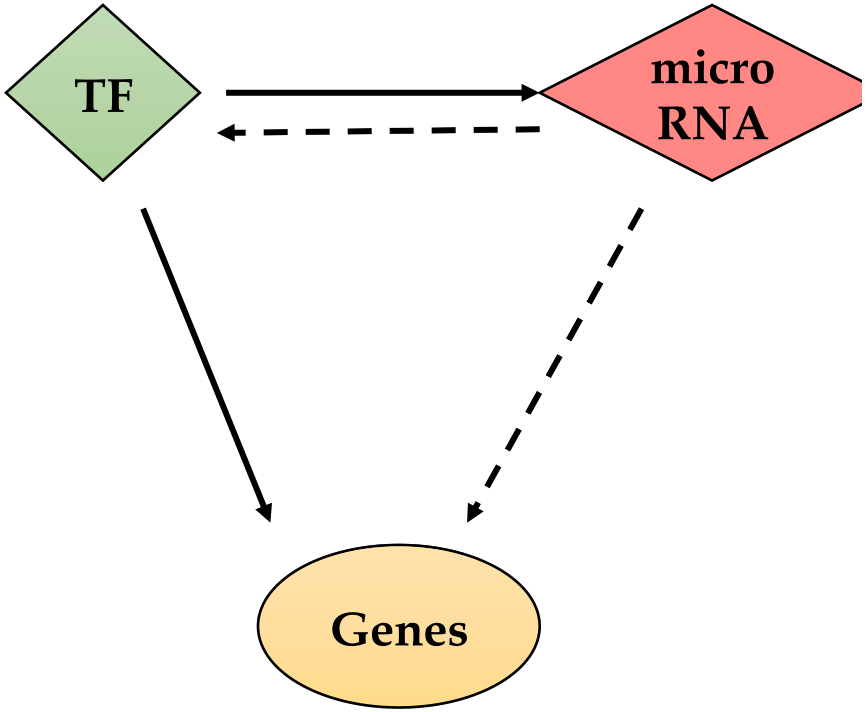




Hammond et al. **Dicing and slicing: The core machinery of the RNA interference pathway. Febs Letters,** Volume 579, Issue 26, 2005, Pages 5822–5829

Refining Gene Regulatory Networks

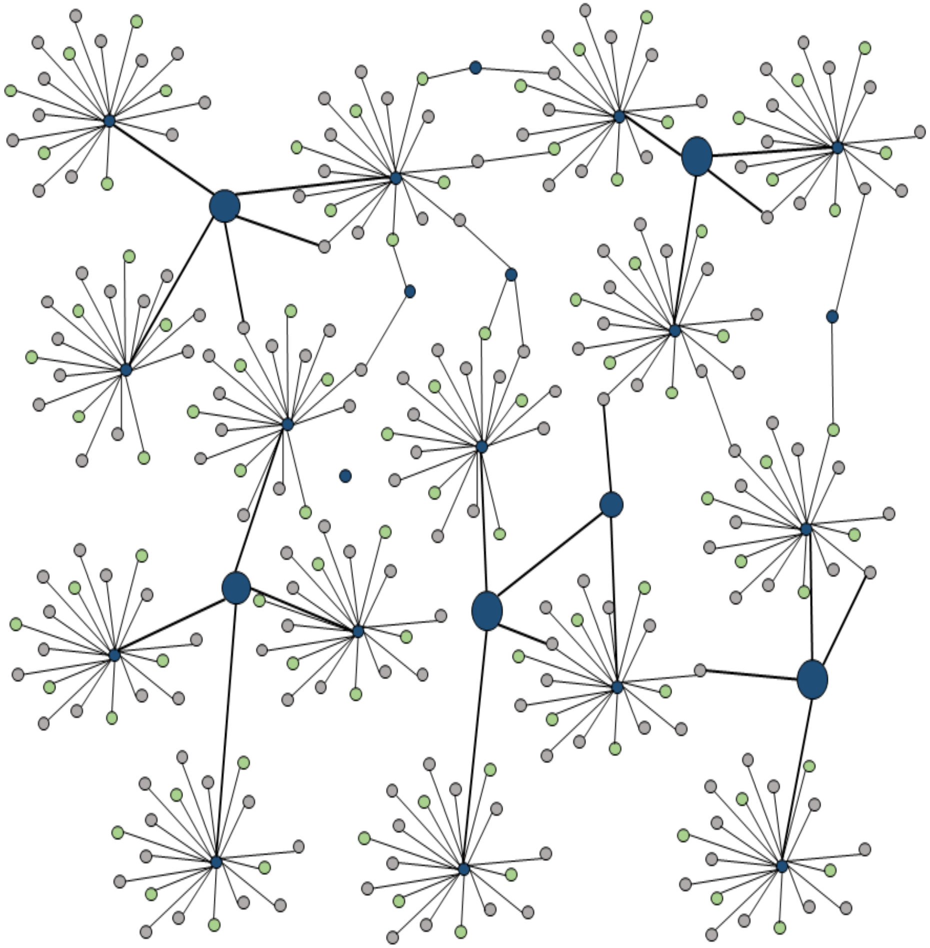
Evolution of Gene Regulatory Networks



TF

micro RNA

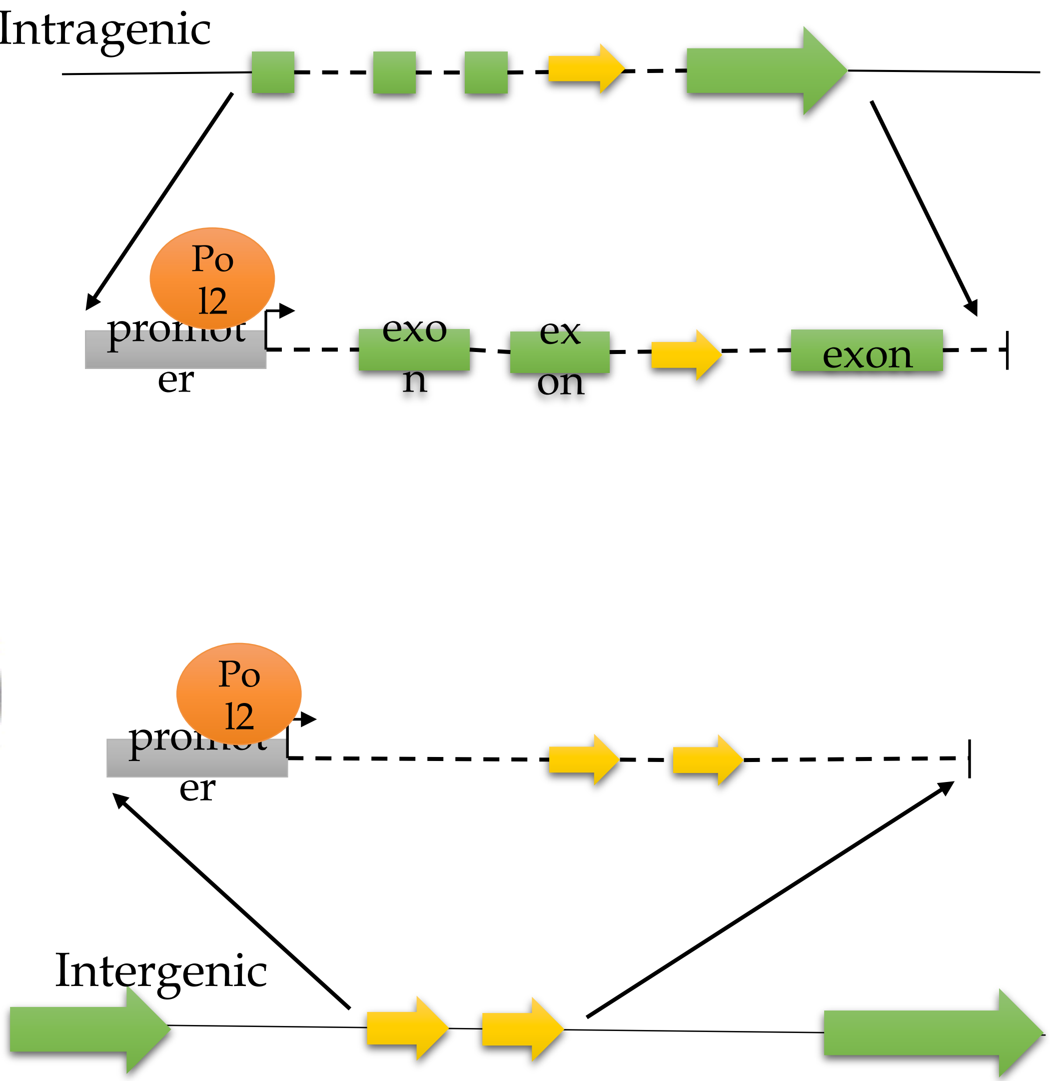
Genes

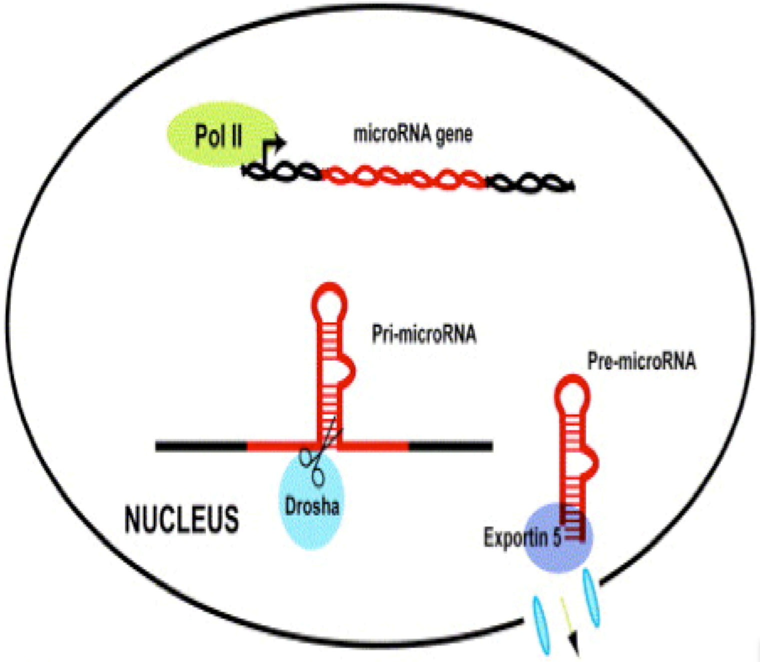


miRNA classification

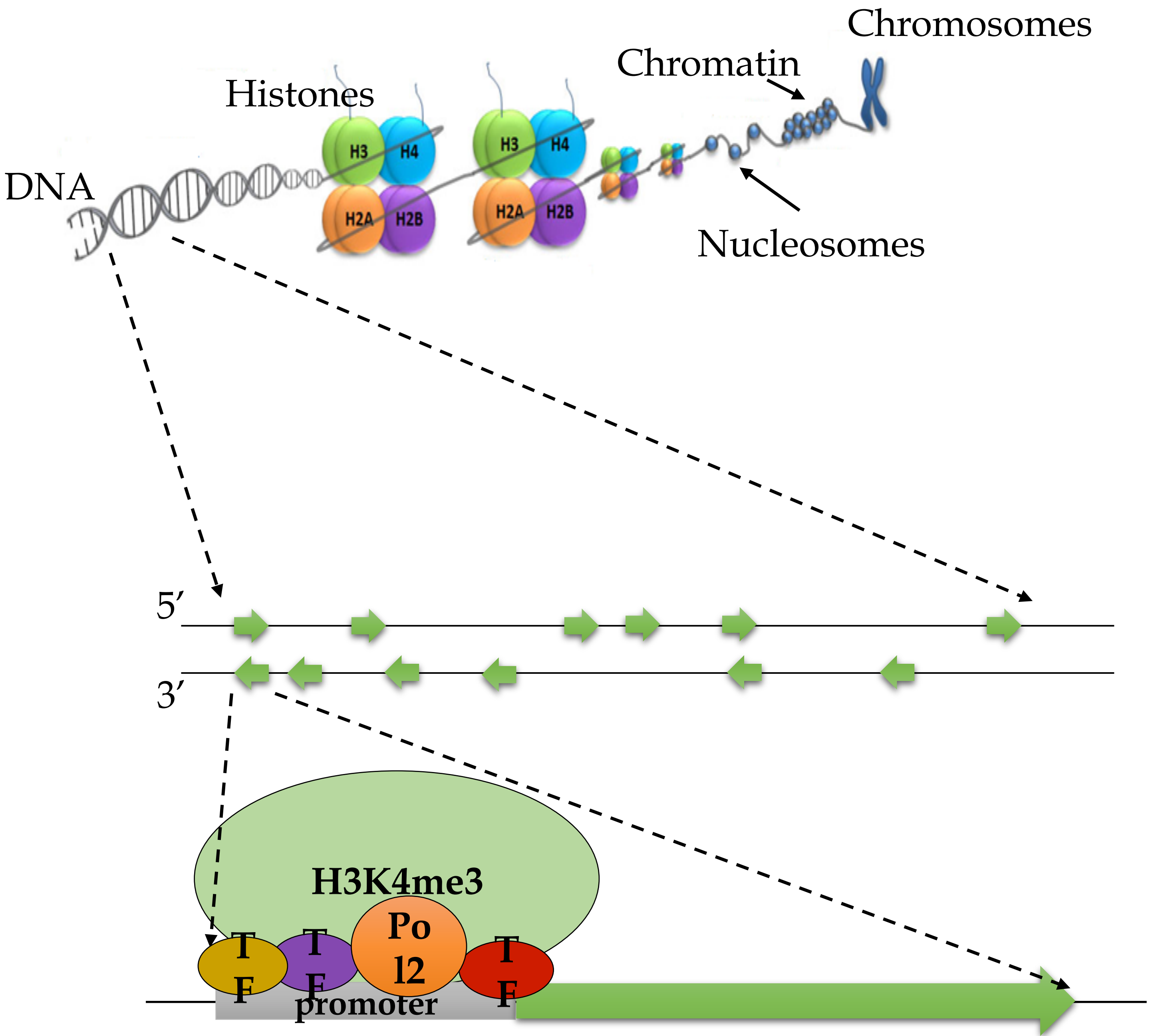
🟩 Coding

🟨 miRNA

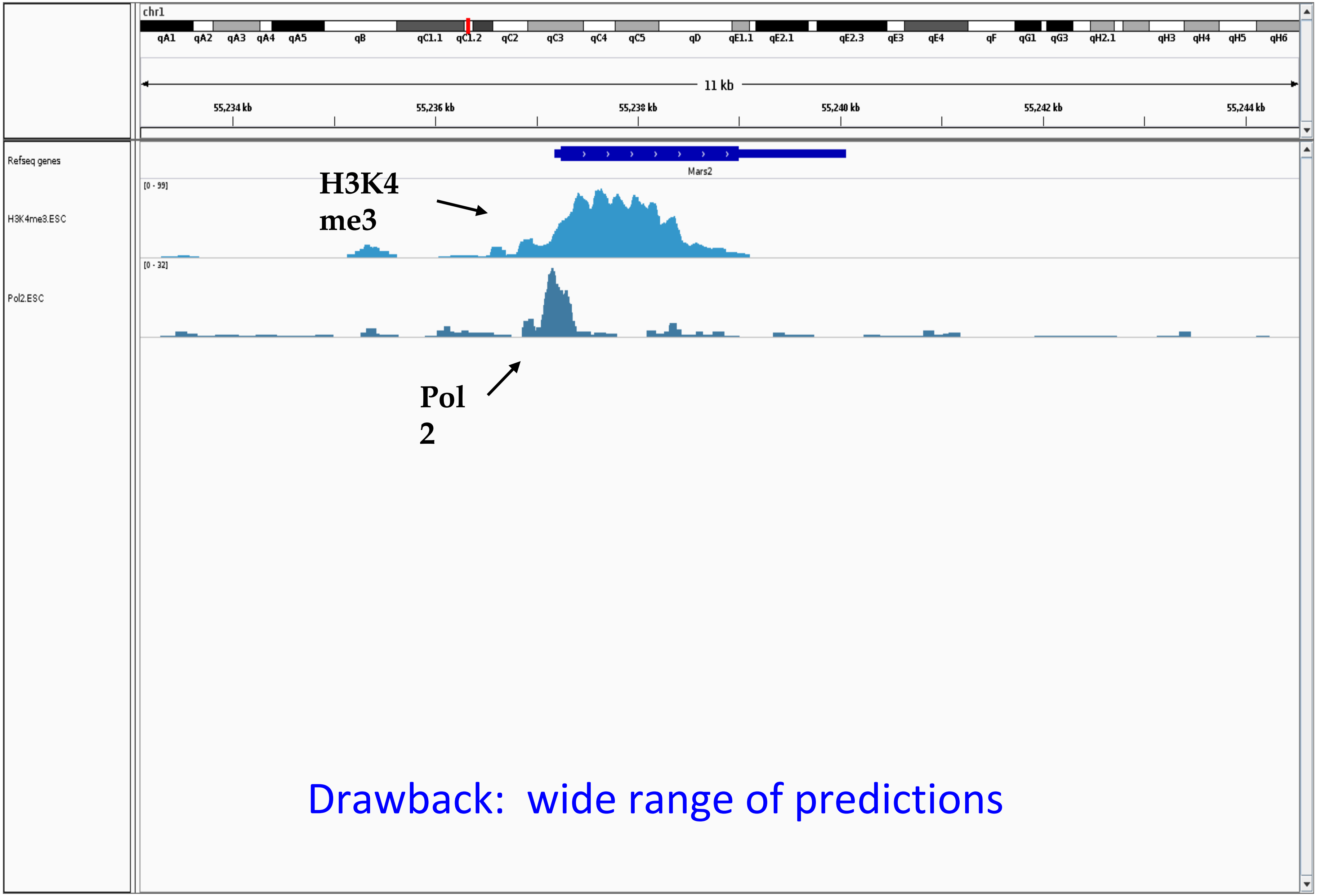




Chromatin structure and transcription



ChIP Sequencing Visualization

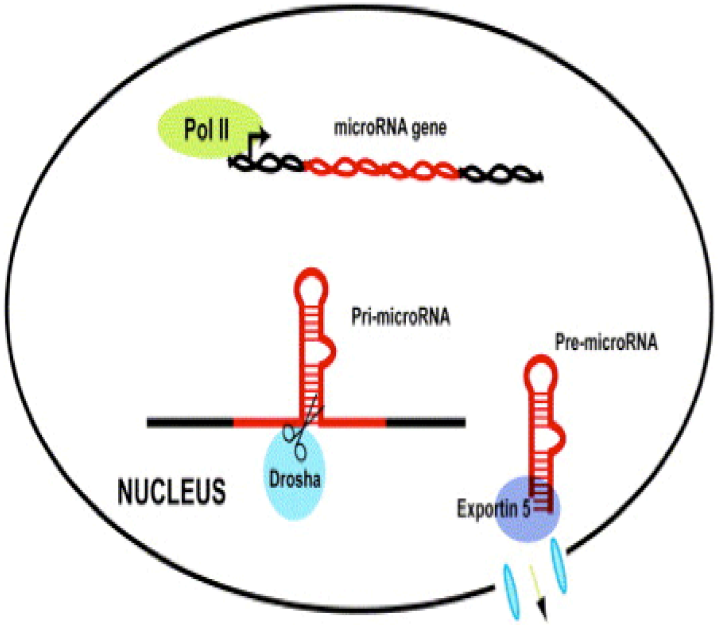


H3K4 me3

Pol 2

Drawback: wide range of predictions

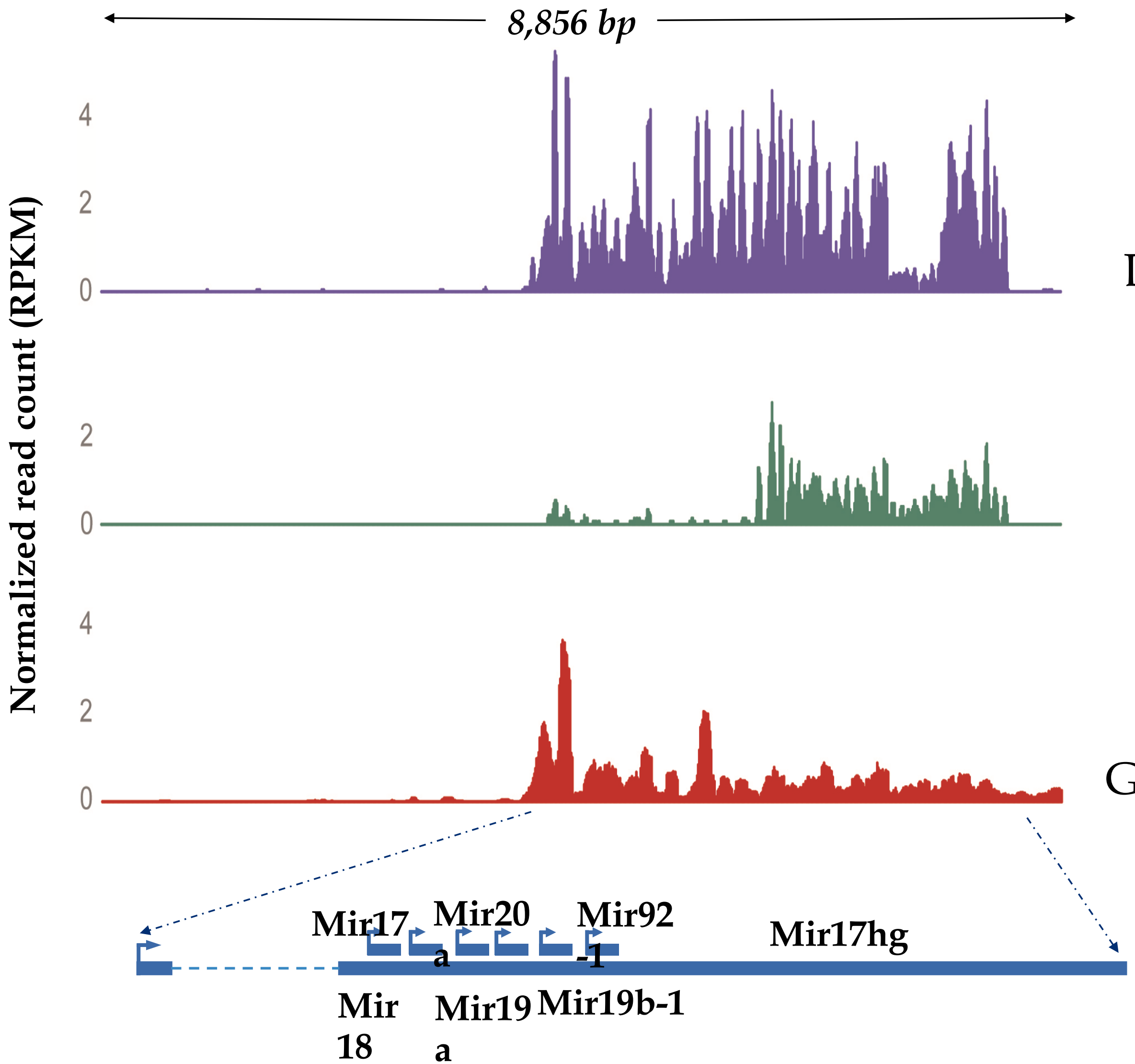
How do we validate a miRNA TSS prediction ?



Drosha null/conditional-null (*Drosha$^{LacZ/e4COIN}$*) mouse model that has been generated using the conditional by inversion (COIN) methodology from Aris Economides @ REGENERON Pharmaceuticals

Comparison other RNA-Seq experiments

RNA-seq coverage over the Mir17hg lncRNA locus



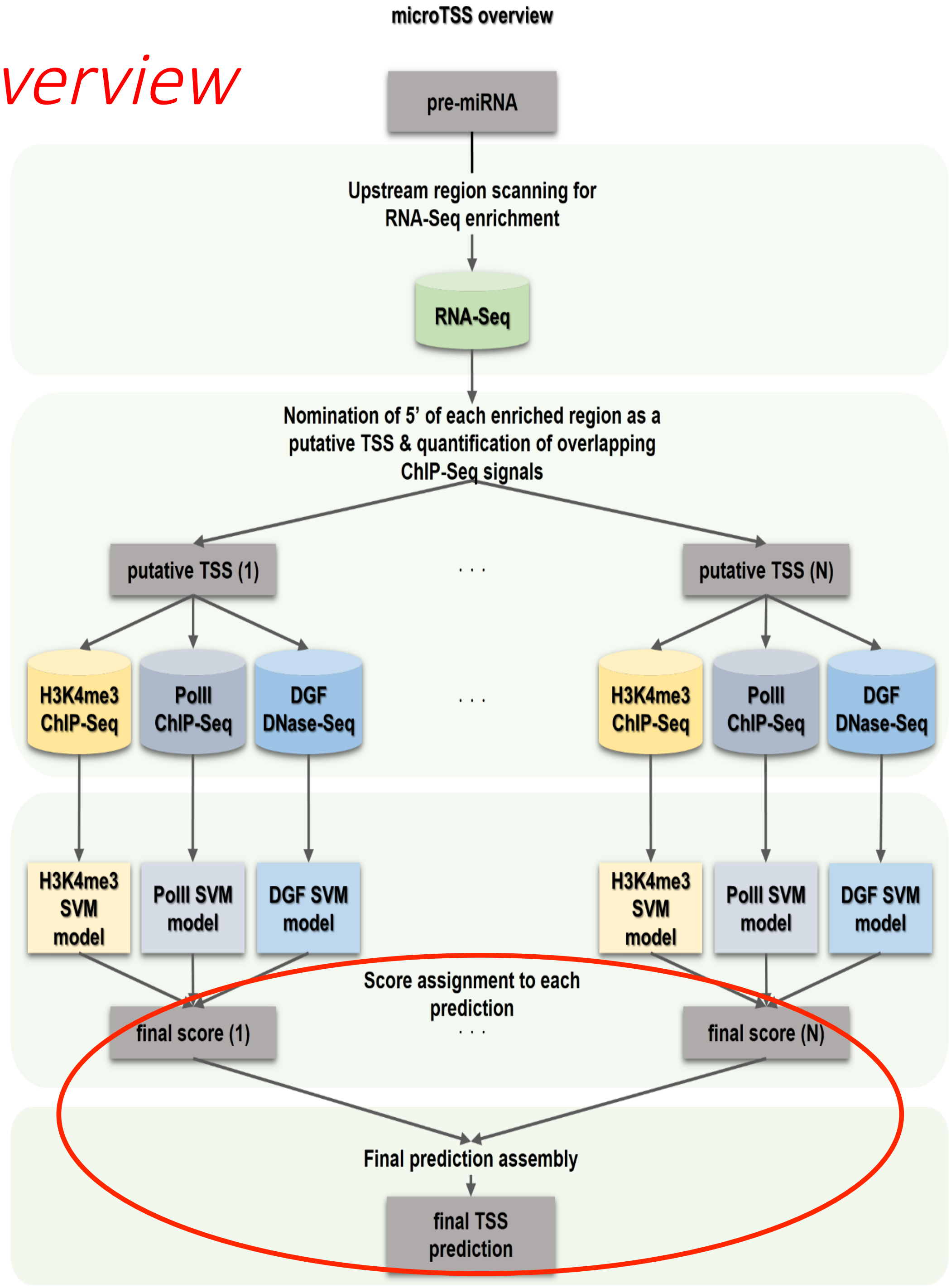
Drosha -/- mESCs with **27M reads**

Drosha +/+ mESCs with **19M reads**

GSM973235 WT mESCs **180M reads**

RNA-seq read depth is essential!

Algorithm overview



microTSS overview

pre-miRNA

First step

Upstream region scanning for RNA-Seq enrichment

RNA-Seq

Second step

Nomination of 5' of each enriched region as a putative TSS & quantification of overlapping ChIP-Seq signals

putative TSS (1) . . . putative TSS (N)

H3K4me3 ChIP-Seq
PolII ChIP-Seq
DGF DNase-Seq
. . .
H3K4me3 ChIP-Seq
PolII ChIP-Seq
DGF DNase-Seq

H3K4me3 SVM model
PolII SVM model
DGF SVM model
H3K4me3 SVM model
PolII SVM model
DGF SVM model

Score assignment to each prediction

final score (1) . . . final score (N)

Final step

Final prediction assembly

final TSS prediction

Comparison with existing methods

Performance on test set:
47 miRNA TSS derived from Drosha depleted mice.

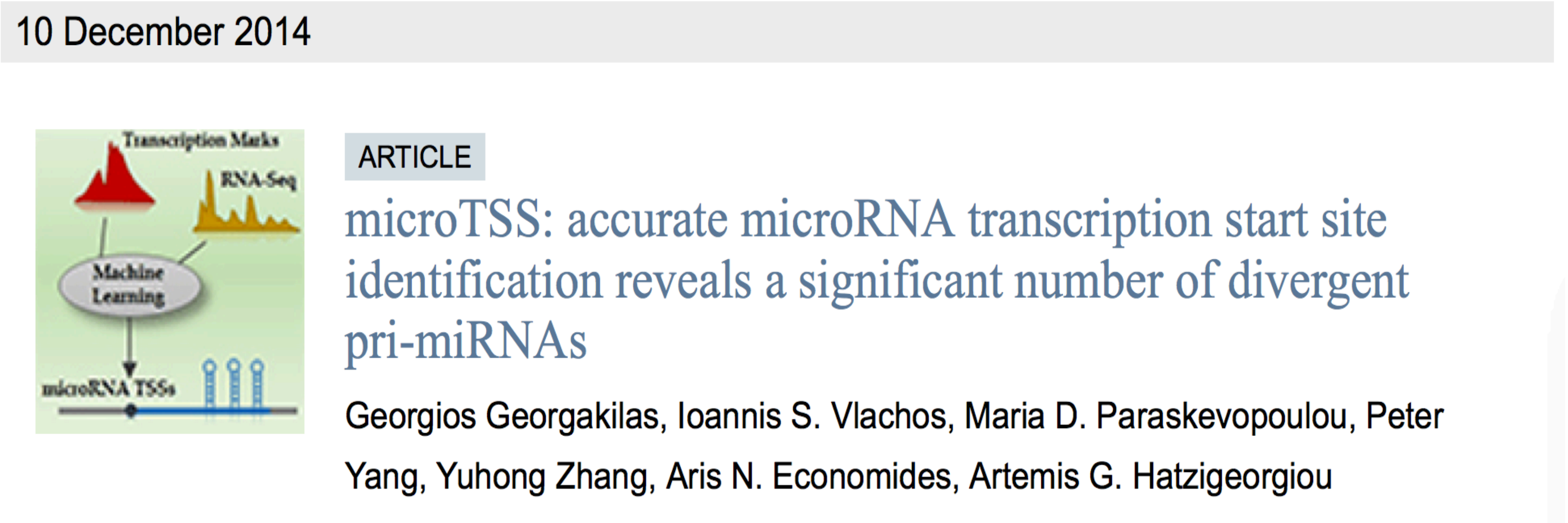
Predictions < 1,000 bp from the validated TSS are considered True

Precision = TP / (TP+FP)

Sensitivity = Correct Predictions / Total Correct

Algorithms Precision and Sensitivity at 1kbp distance threshold from validated TSSs in mESC

	mESCs (N=47)	
	Sensitivity	**Precision**
Marson et al	**54% (20/37)**	**64.5% (20/31)**
PROmiRNA	**78.7% (37/47)**	**25.4% (95/373)**
S-Peaker	**76.5% (36/47)**	**18.8% (77/409)**
microTSS	**93.6% (44/47)**	**100% (44/44)**

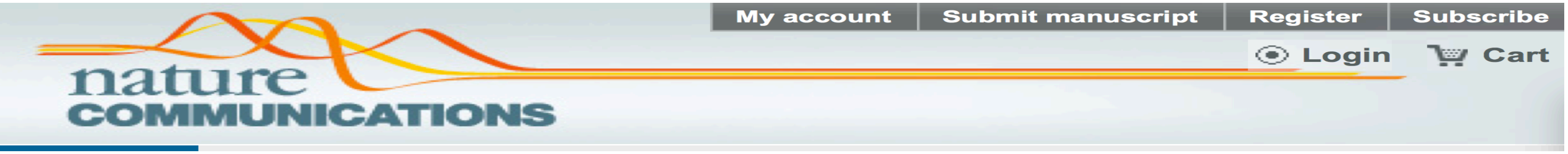


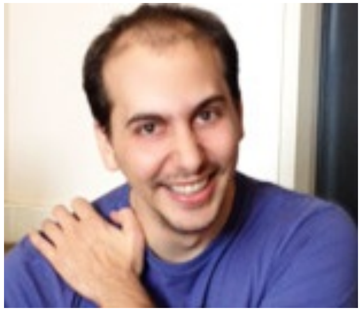
10 December 2014

ARTICLE

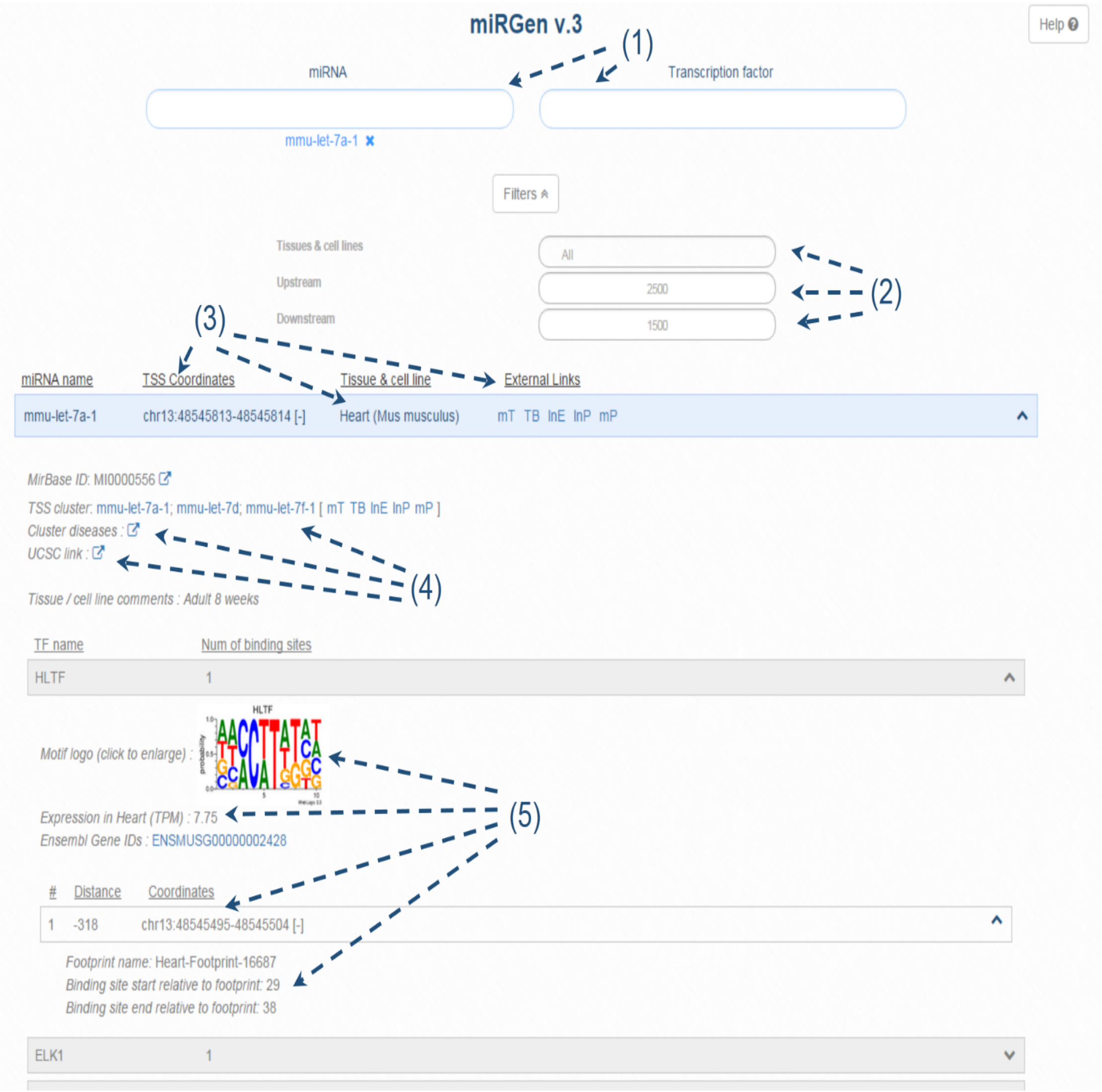
microTSS: accurate microRNA transcription start site identification reveals a significant number of divergent pri-miRNAs

Georgios Georgakilas, Ioannis S. Vlachos, Maria D. Paraskevopoulou, Peter Yang, Yuhong Zhang, Aris N. Economides, Artemis G. Hatzigeorgiou





DIANA-miRGen v3.0



miRNA: mmu-let-7a-1 ✖

Tissues & cell lines: All

Upstream: 2500

Downstream: 1500

miRNA name	TSS Coordinates	Tissue & cell line	External Links
mmu-let-7a-1	chr13:48545813-48545814 [-]	Heart (Mus musculus)	mT TB lnE lnP mP

MirBase ID: MI0000556

TSS cluster: mmu-let-7a-1; mmu-let-7d; mmu-let-7f-1 [mT TB lnE lnP mP]

Cluster diseases :

UCSC link :

Tissue / cell line comments : Adult 8 weeks

TF name	Num of binding sites
HLTF	1

Motif logo (click to enlarge) :

Expression in Heart (TPM) : 7.75

Ensembl Gene IDs : ENSMUSG00000002428

#	Distance	Coordinates
1	-318	chr13:48545495-48545504 [-]

Footprint name: Heart-Footprint-16687

Binding site start relative to footprint: 29

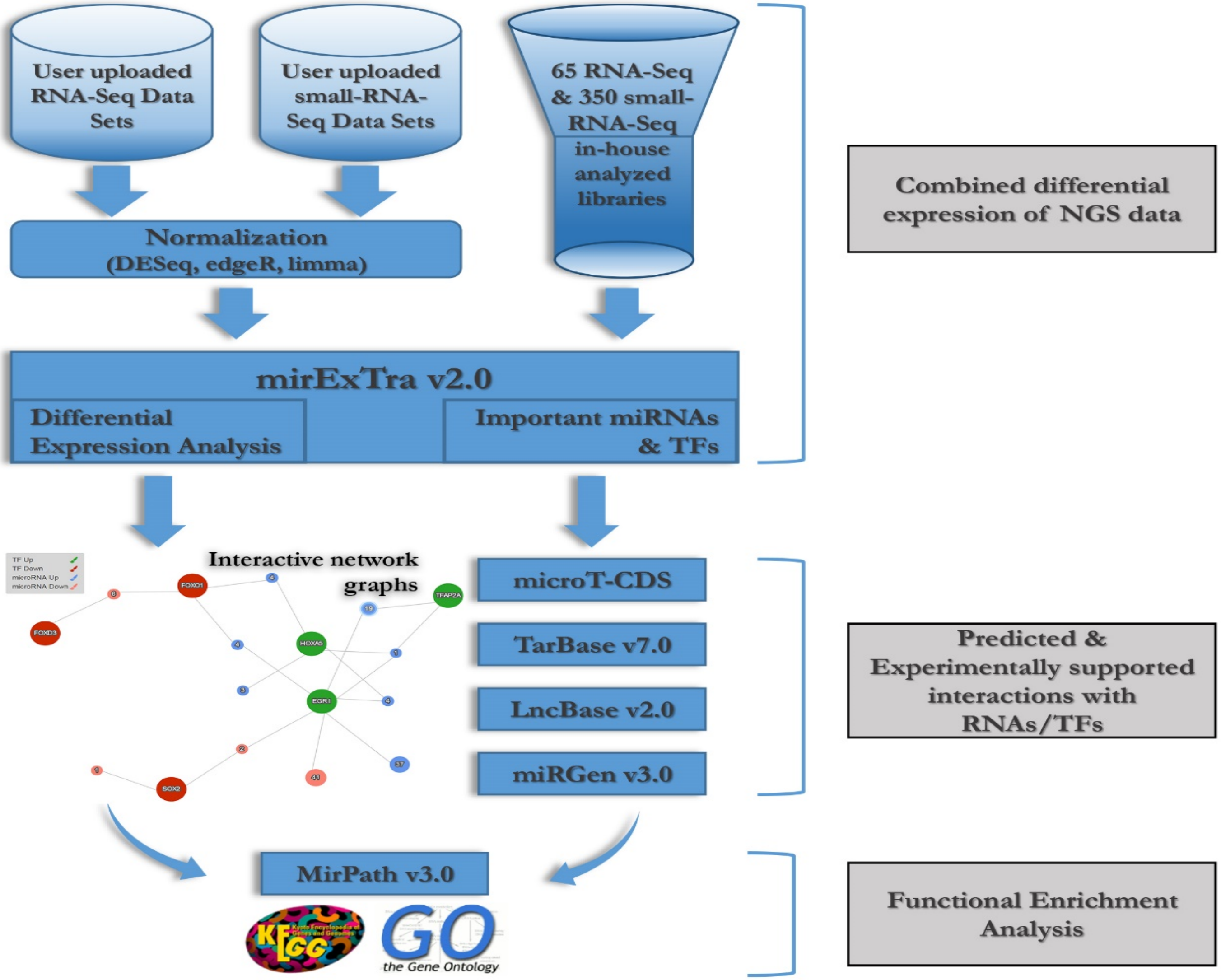
Binding site end relative to footprint: 38

TF name	Num of binding sites
ELK1	1

www.microrna.gr/miRGenv3

Georgakilas, G et al. DIANA-miRGen v3.0: accurate characterization of microRNA promoters and their regulators. Nucleic Acid Research, 2015.

miRNA - biomarkers:
combined analysis of mRNA and miRNA with miRExtra V2.0



User uploaded RNA-Seq Data Sets

User uploaded small-RNA-Seq Data Sets

65 RNA-Seq & 350 small-RNA-Seq in-house analyzed libraries

Normalization
(DESeq, edgeR, limma)

mirExTra v2.0

Differential Expression Analysis

Important miRNAs & TFs

Interactive network graphs

microT-CDS

TarBase v7.0

LncBase v2.0

miRGen v3.0

MirPath v3.0

Combined differential expression of NGS data

Predicted & Experimentally supported interactions with RNAs/TFs

Functional Enrichment Analysis

SNPs & miRNAs

Polymorphic disease associations and microRNAs.

- SNPs that occur in functional miRNA target sites could affect miRNA binding
- Map all annotated SNPs from dbSNP onto all experimentally supported target sites from TarBase
- 2 of the 5 SNPs occur in a region that disrupts the 5’-dominant binding
- 1 of these 2 SNPs is genotyped according to ALFRED (ALlele FREquency Database)
- Does this SNP impair miR-155 binding and silencing of *AGTR1*?

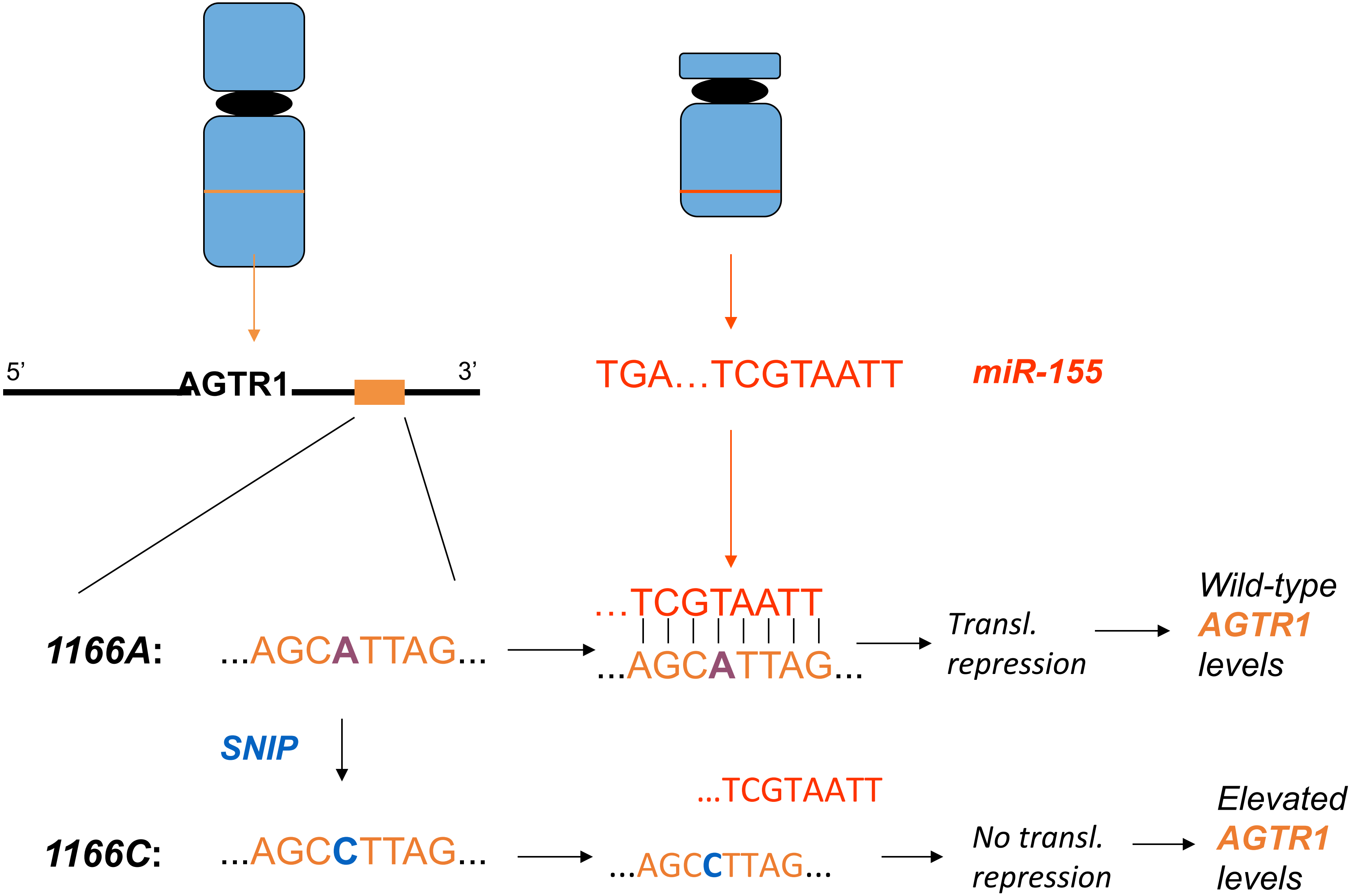
```
5' UUCACUACCAAAUGAGCAUUAG 3'                      Human AGTR1
   ..|    |   || ||||||||.
3' GGGAUAGUGCUAAUCGUAAUU 5'                       Hsa-miR-155
   ..|    |   || ||| |||.
5' UUCACUACCAAAUGAGCCUUAG 3'                      Polymorphic Human AGTR1

5' GCAGUUUGAAAUUCUGAAUUUGCAAAGUACUGUA 3'          Human EZH2
    |||||                || |||||||||
3' AGUCAA--------------UAGUGUCAUGACAU 5'          Hsa-miR-101
    |||||                || ||||||| |
5' GCAGUUUGAAAUUCUGAAUUUGCAAAGUACUGGA 3'          Polymorphic Human EZH2

5' CCCG-CAAGAAAGUGAATCTCACTACUACCUA 3'            Human HOXA7
   |||. |||    .||||       ||||||||
3' GGGUUGUUG---UACUU-------UGAUGGAU 5'            Hsa-miR-196
   | |. |||    .||||       ||||||||
5' CACG-CAAGAAAGUGAATCTCACTACUACCUA 3'            Polymorphic Human HOXA7

5' UGCCU---CUGGAAAACUAAAGAGCCUUGCAUGUACUUGAA 3'   Human SMAD1
    ||||   |||||                    ||||||||
3' UCGGAUAGGACCU-------------------AAUGAACUU 5'   Hsa-miR-26
    |||    |||||                    ||||||||
5' UGCCC---CUGGAAAACUAAAGAGCCUUGCAUGUACUUGAA 3'   Polymorphic Human SMAD1

5' CCGGCCGCCUGCGGCACUGCCU 3'                      Human DLL1
    |..||. ||. |.||||||||
3' UGUUGGUCGAUUCUGUGACGGU 5'                      Hsa-miR-34
    ...||. ||. |.||||||||
5' CUGGCCGCCUGCGGCACUGCCU 3'                      Polymorphic Human DLL1
```



5' **AGTR1** 3'

TGA…TCGTAATT

miR-155

***1166A*:** …AGC**A**TTAG…

…TCGTAATT

…AGC**A**TTAG…

Transl. repression

Wild-type ***AGTR1*** *levels*

SNIP

***1166C*:** …AGC**C**TTAG…

…TCGTAATT

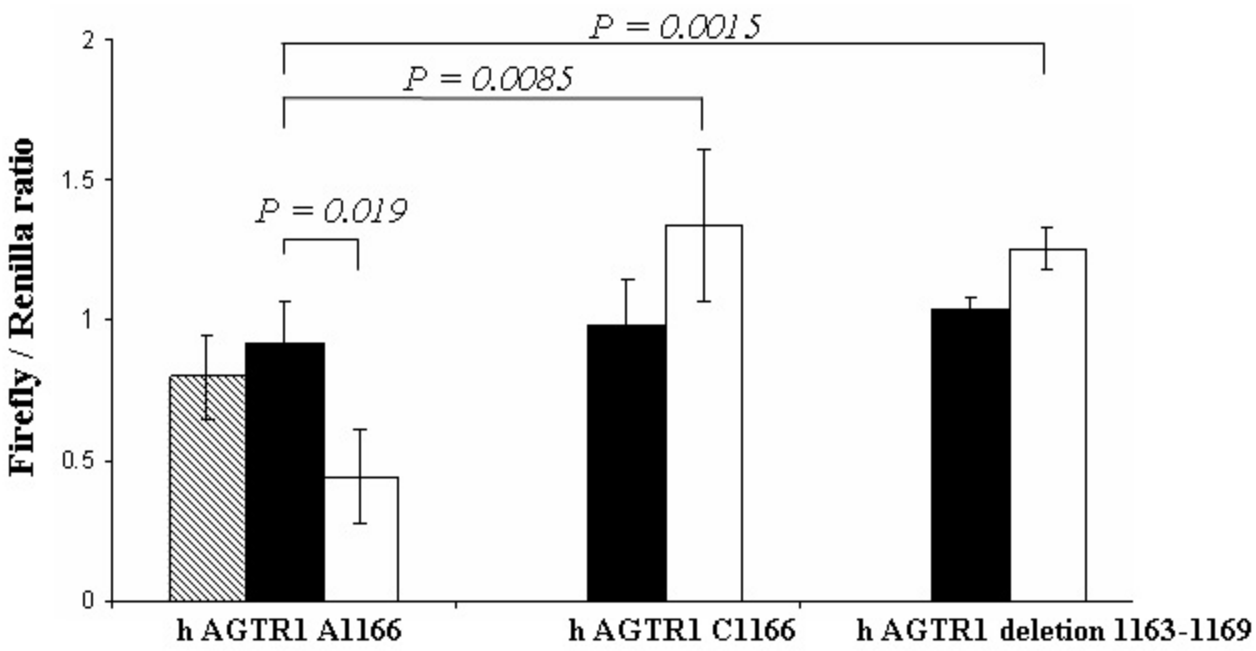
…AGC**C**TTAG…

No transl. repression

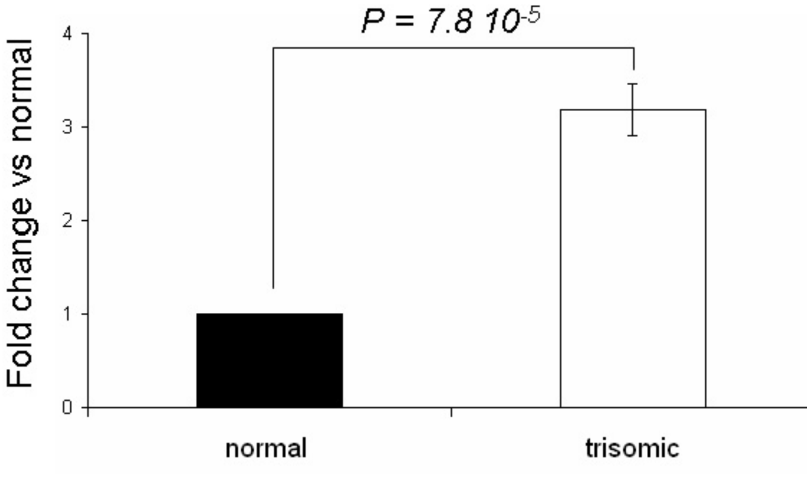
Elevated ***AGTR1*** *levels*

Experimental validation in vitro.

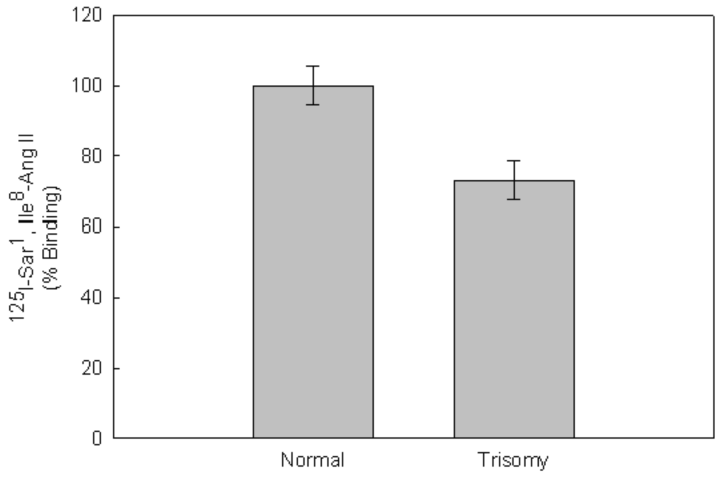
- In vitro luciferase assay to test the prediction



Fibroblast cells from monozygotic twins discordant for trisomy 21. In vivo evidence of mir-155 and ATGR1.



qRT-PCR for mature miR-155 expression



Whole cell AGTR1 binding assays

Sethupathy, P., Borel, C., Gagnebin, M., Grant, G.R, Deutsch S, Eltion TS, Hatzigeorgiou*, A.G, and Antonarakis, S.E. (2006) Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes.***Am J Hum Genet.*** 2007 Aug;81(2):405-13.

DIANA-Lab

DIANA-Lab – a decade-long lab specialized in non-coding RNAs. Provides algorithms, databases and web-servers for the systematic archiving and interpretation of ncRNA-related biological data.

www.microrna.gr

- **DIANA TarBase**

Dimitra Karagkouni, Maria D. Paraskevopoulou, Serafeim Chatzopoulos, Ioannis S. Vlachos, Spyros Tastsoglou, *et al.* **DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions**, **Nucleic Acids Research**, 2017

- **DIANA LncBase**

Maria D. Paraskevopoulou, Ioannis S. Vlachos, Dimitra Karagkouni *et al* "**DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts" Nucleic Acids Research**, 2016

- **DIANA microCLIP**

M.D. Paraskevopoulou***,** D. Karagkouni*, I.S. Vlachos, S. Tastsoglou & A.G. Hatzigeorgiou, **microCLIP: Super learning uncovers functional transcriptome-wide miRNA interactions**, **Nat Commun** , September 2018

- **DIANA – microTSS**

Georgakilas G., Vlachos I.S., Paraskevopoulou M.D., Yang P., Zhang Y., Economides A.N., Hatzigeorgiou A.G. **microTSS: accurate microRNA transcription start site identification reveals a significant number of divergent pri-miRNAs**. Nat. Commun. 2014

- **DIANA-miRGen v3**

Georgios Georgakilas, Ioannis S. Vlachos, Konstantinos Zagganas *et al.* **DIANA-miRGen v3.0: accurate characterization of microRNA promoters and their regulators** Nucl. Acids Res. 2016

DIANA-miRPath v3

Vlachos, Ioannis S., Konstantinos Zagganas, Maria D. Paraskevopoulou *et al.* **DIANA-miRPath v3. 0: deciphering microRNA function with experimental support.** Nucleic Acids Res, 2015

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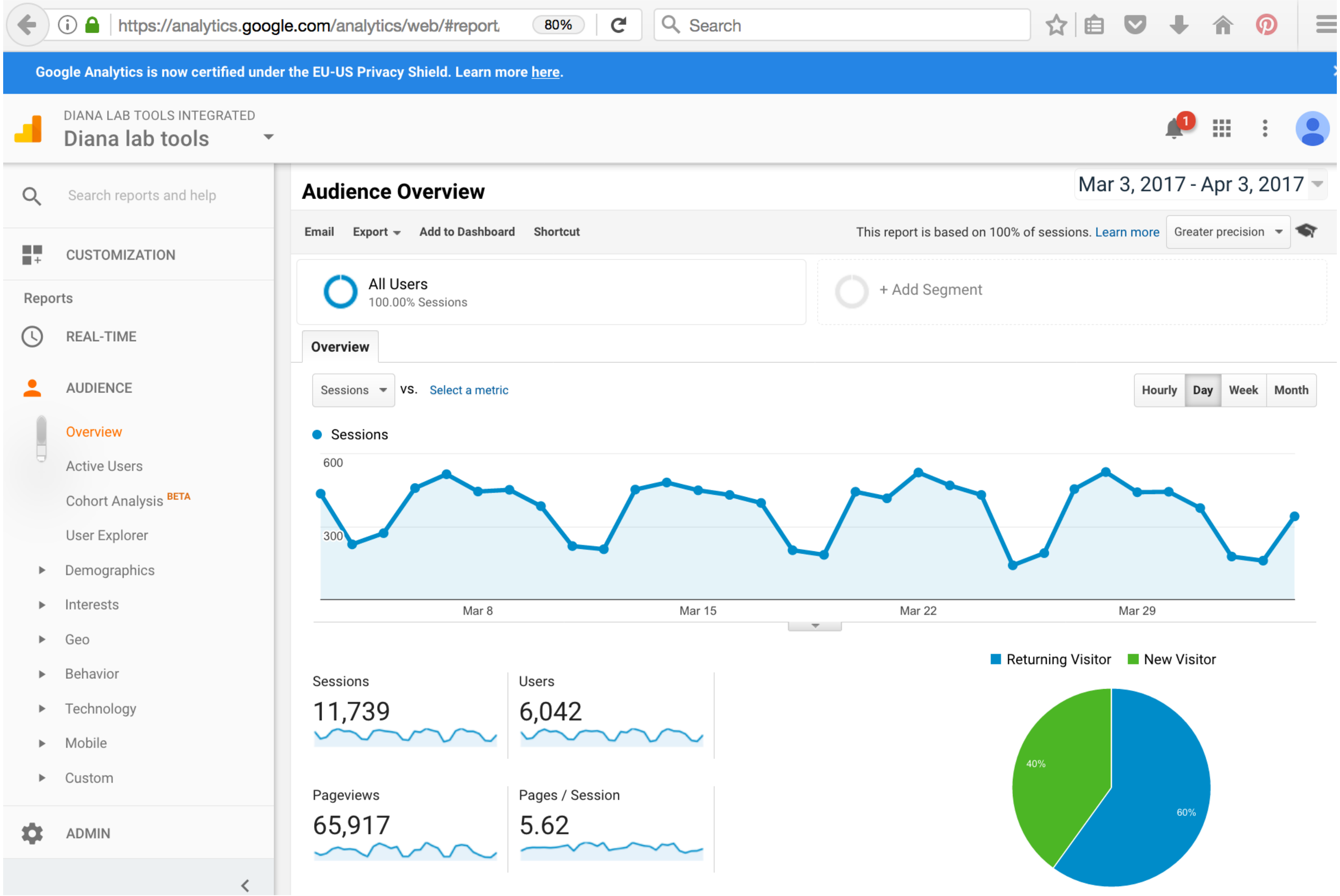

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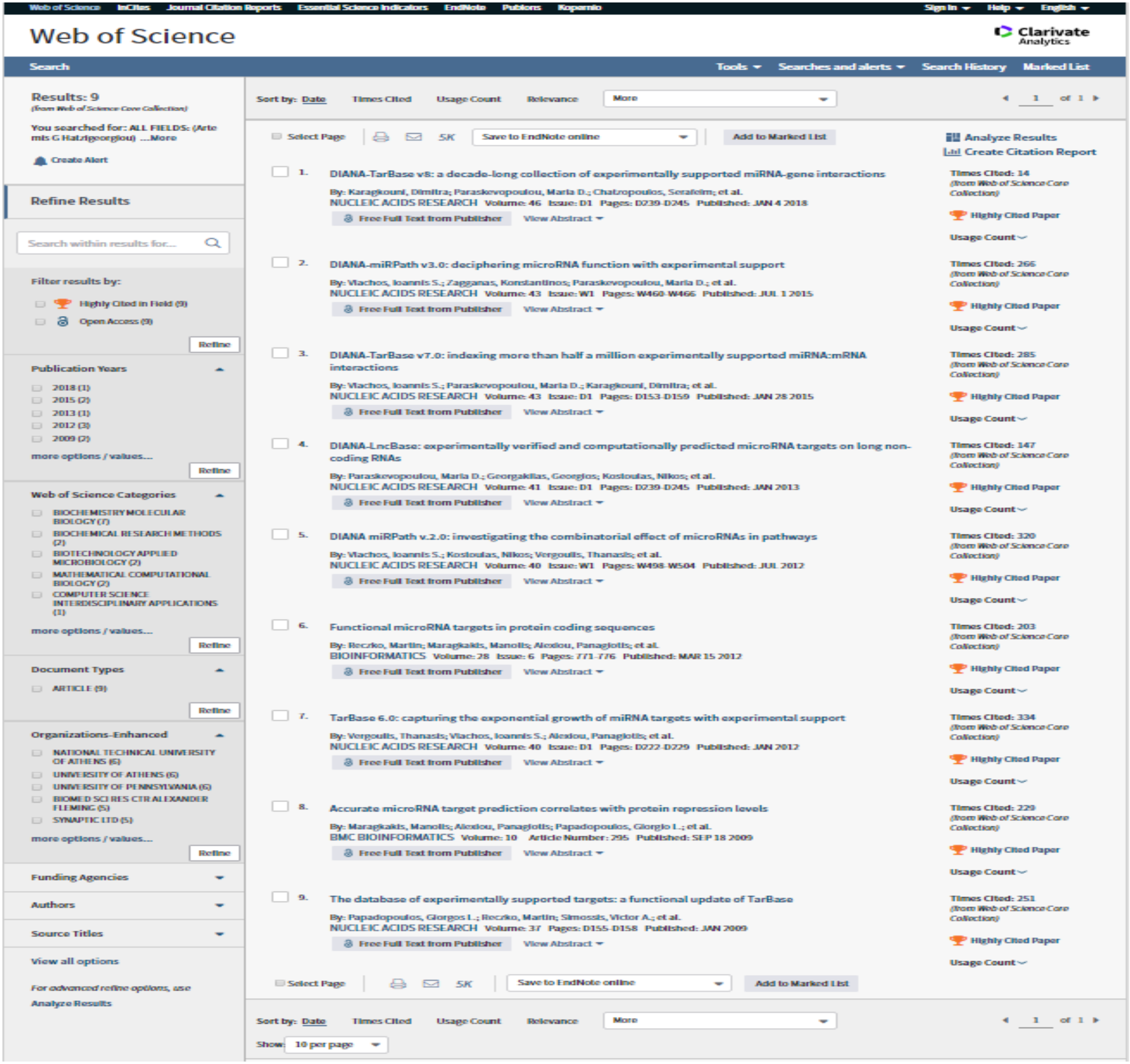

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By: Karagkouni, Dimitra; Paraskevopoulou, Maria D.; Chatzopoulos, Serafeim; et al.
NUCLEIC ACIDS RESEARCH Volume: 46 Issue: D1 Pages: D239-D245 Published: JAN 4 2018
Times Cited: 14 *(from Web of Science Core Collection)*

☐ 2. DIANA-miRPath v3.0: deciphering microRNA function with experimental support
By: Vlachos, Ioannis S.; Zagganas, Konstantinos; Paraskevopoulou, Maria D.; et al.
NUCLEIC ACIDS RESEARCH Volume: 43 Issue: W1 Pages: W460-W466 Published: JUL 1 2015
Times Cited: 266 *(from Web of Science Core Collection)*

☐ 3. DIANA-TarBase v7.0: indexing more than half a million experimentally supported miRNA:mRNA interactions
By: Vlachos, Ioannis S.; Paraskevopoulou, Maria D.; Karagkouni, Dimitra; et al.
NUCLEIC ACIDS RESEARCH Volume: 43 Issue: D1 Pages: D153-D159 Published: JAN 28 2015
Times Cited: 285 *(from Web of Science Core Collection)*

☐ 4. DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs
By: Paraskevopoulou, Maria D.; Georgakilas, Georgios; Kostoulas, Nikos; et al.
NUCLEIC ACIDS RESEARCH Volume: 41 Issue: D1 Pages: D239-D245 Published: JAN 2013
Times Cited: 147 *(from Web of Science Core Collection)*

☐ 5. DIANA miRPath v.2.0: investigating the combinatorial effect of microRNAs in pathways
By: Vlachos, Ioannis S.; Kostoulas, Nikos; Vergoulis, Thanasis; et al.
NUCLEIC ACIDS RESEARCH Volume: 40 Issue: W1 Pages: W498-W504 Published: JUL 2012
Times Cited: 320 *(from Web of Science Core Collection)*

☐ 6. Functional microRNA targets in protein coding sequences
By: Reczko, Martin; Maragkakis, Manolis; Alexiou, Panagiotis; et al.
BIOINFORMATICS Volume: 28 Issue: 6 Pages: 771-776 Published: MAR 15 2012
Times Cited: 203 *(from Web of Science Core Collection)*

☐ 7. TarBase 6.0: capturing the exponential growth of miRNA targets with experimental support
By: Vergoulis, Thanasis; Vlachos, Ioannis S.; Alexiou, Panagiotis; et al.
NUCLEIC ACIDS RESEARCH Volume: 40 Issue: D1 Pages: D222-D229 Published: JAN 2012
Times Cited: 334 *(from Web of Science Core Collection)*

☐ 8. Accurate microRNA target prediction correlates with protein repression levels
By: Maragkakis, Manolis; Alexiou, Panagiotis; Papadopoulos, Giorgio L.; et al.
BMC BIOINFORMATICS Volume: 10 Article Number: 295 Published: SEP 18 2009
Times Cited: 229 *(from Web of Science Core Collection)*

☐ 9. The database of experimentally supported targets: a functional update of TarBase
By: Papadopoulos, Giorgos L.; Reczko, Martin; Simossis, Victor A.; et al.
NUCLEIC ACIDS RESEARCH Volume: 37 Pages: D155-D158 Published: JAN 2009
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2012

- Vergoulis, T., et al., *TarBase 6.0: Capturing the exponential growth of miRNA targets with experimental support.* **Nucleic Acids Res. Highly Cited Paper**
- Reczko, M., et al., *Functional microRNA targets in protein coding sequences.* **Bioinformatics Highly Cited Paper**
- Vlachos, I.S., et al. *DIANA miRPath v.2.0: Investigating the combinatorial effect of microRNAs in pathways.* **Nucleic Acids Res***.* **Highly Cited Paper**

2013

- Paraskevopoulou, M.D., et al., *DIANA-LncBase: Experimentally verified and computationally predicted microRNA targets on long non-coding RNAs*. **Nucleic Acids Res***.* **Highly Cited Paper**
- Paraskevopoulou, M.D., et al. *DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows*. **Nucleic Acids Res. Highly Cited Paper**

2014

- Georgakilas G, et al. *microTSS: accurate microRNA transcription start site identification reveals a significant number of divergent pri-miRNAs.* **Nature Commun.**

2015

- Vlachos IS, et al.. *DIANA-miRPath v3.0: deciphering microRNA function with experimental support.* **Nucleic Acids Res***.* **Highly Cited Paper**
- Vlachos IS, et al. *DIANA-TarBase v7.0: indexing more than half a million experimentally supported miRNA:mRNA interactions.* **Nucleic Acids Res. Highly Cited Paper**

2016

- Georgakilas G, et al*. miRGen v3.0: accurate characterization of microRNA promoters and their regulators.* **NAR.**
- Paraskevopoulou, MD, et al., *DIANA-LncBase v2: Indexing microRNA targets on non-coding transcripts.* **NAR.**
- Vlachos IS, et al., *DIANA-mirExTra v2.0: Uncovering microRNAs and transcription factors with crucial roles in NGS expression data*. **NAR**.

DIANA-LAB

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Lab members

- Karagkouni Dimitra
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- Skoufos Giorgos
- Dimitris Zisis
- Thanos Alexou
- Antonis Kousounadis
- Marios Miliotis
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- Maria D Paraksevopoulou
- George Georgakilas
- Maragkakis Manolis
- Alexiou Panagiotis

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