

## **DIANA Tools and Databases: *In silico* investigation of miRNA functions**

Anna Karavangeli  
Eliza Zacharopoulou

Prof. Artemis G Hatzigeorgiou

DIANA-Lab, University of Thessaly, Lamia, Greece

# Sections

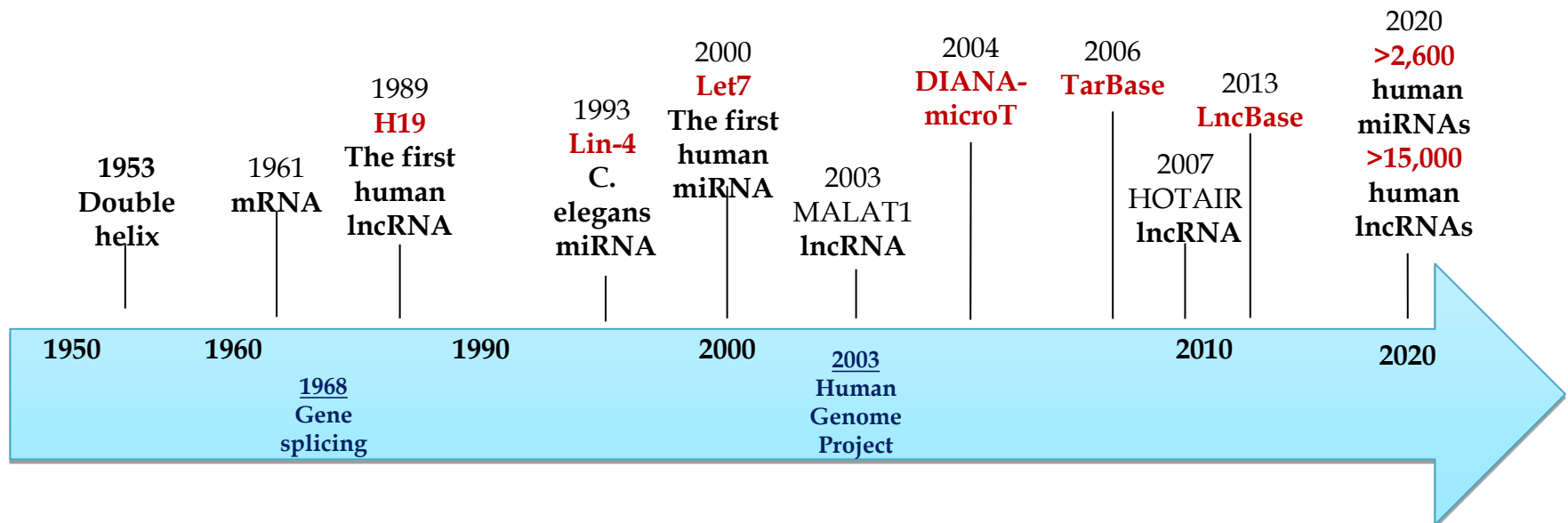
- Introduction to the microRNA world
- **DIANA-TarBase/LncBase:**  
databases with experimentally supported miRNA-gene pairs
- **DIANA-microT-CDS:**  
a web-server of *in silico* predicted miRNA-gene interactions

# Introduction to the microRNA world

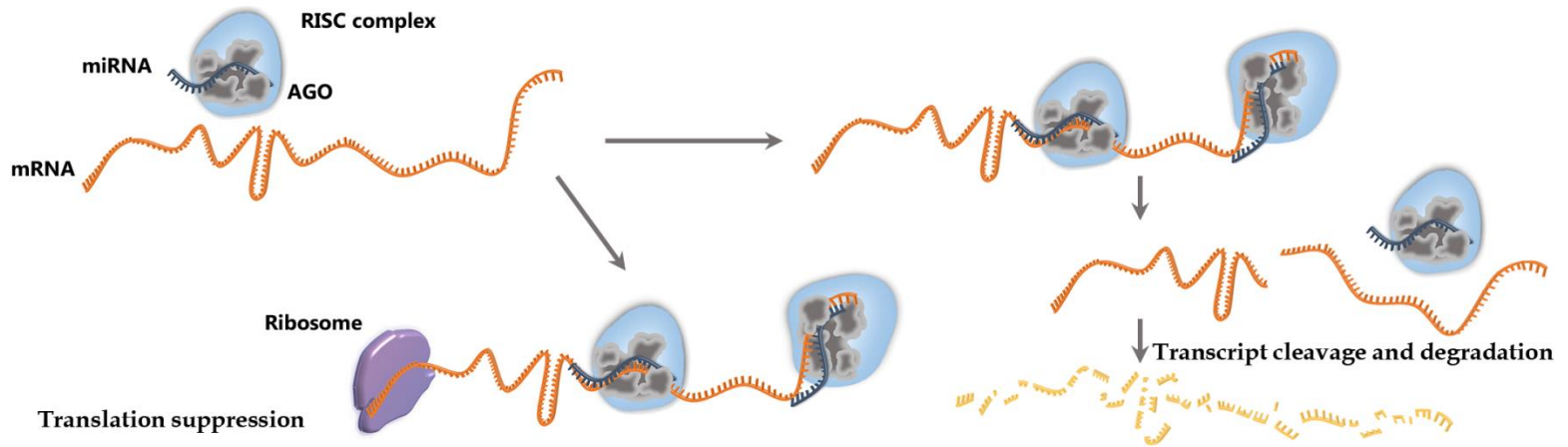
# non-coding RNAs (ncRNAs)



- ~3% of the human genome encodes proteins
- ~62% of the human genome is transcribed
- 10s of thousands of regulatory non-coding RNAs
- Emerging technological developments turned **non-coding RNAs from dark matter into a biological research hotspot**
- Associated with numerous physiological & pathological mechanisms
- Considered as biomarkers or therapeutic targets for numerous diseases



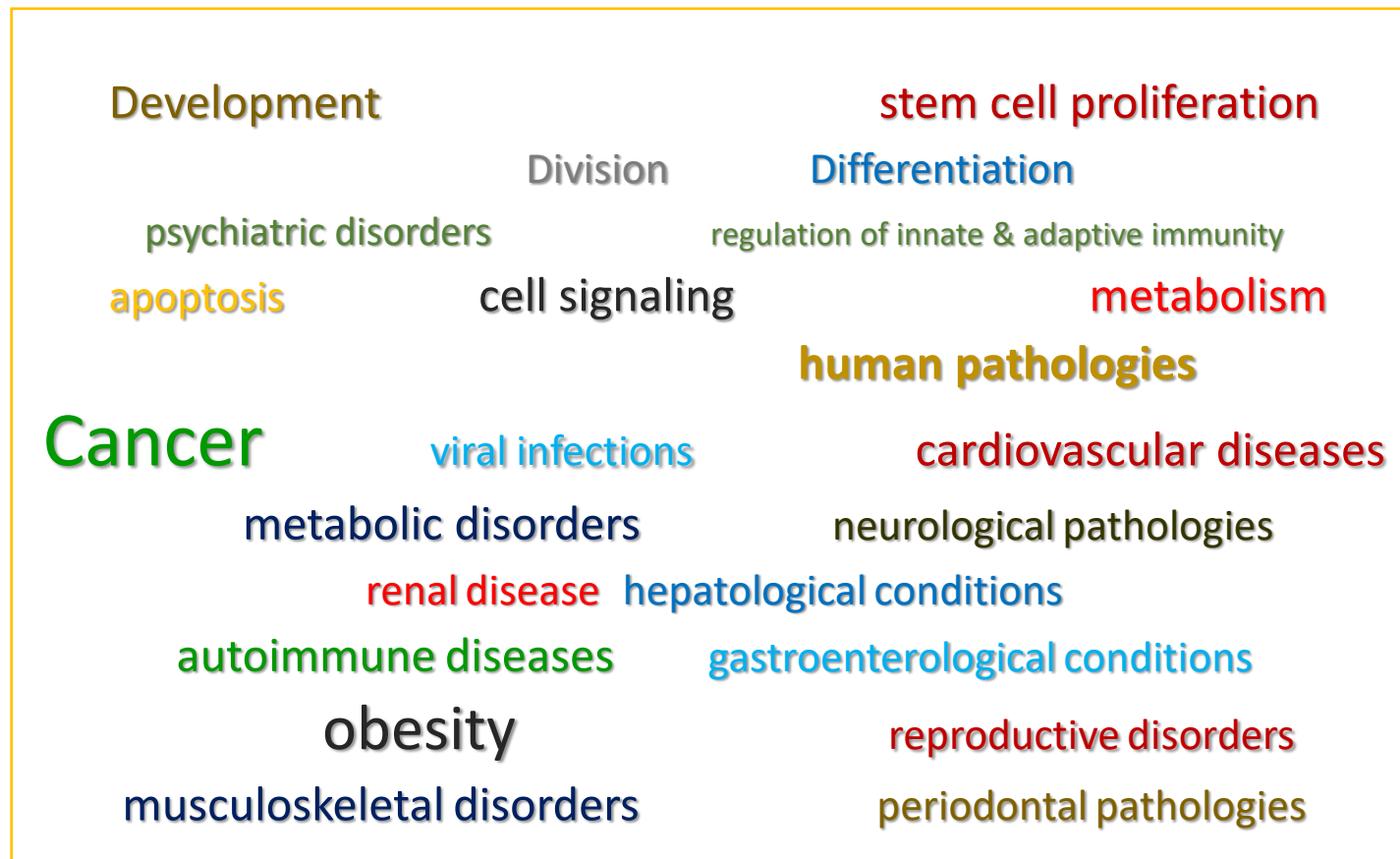
# microRNAs



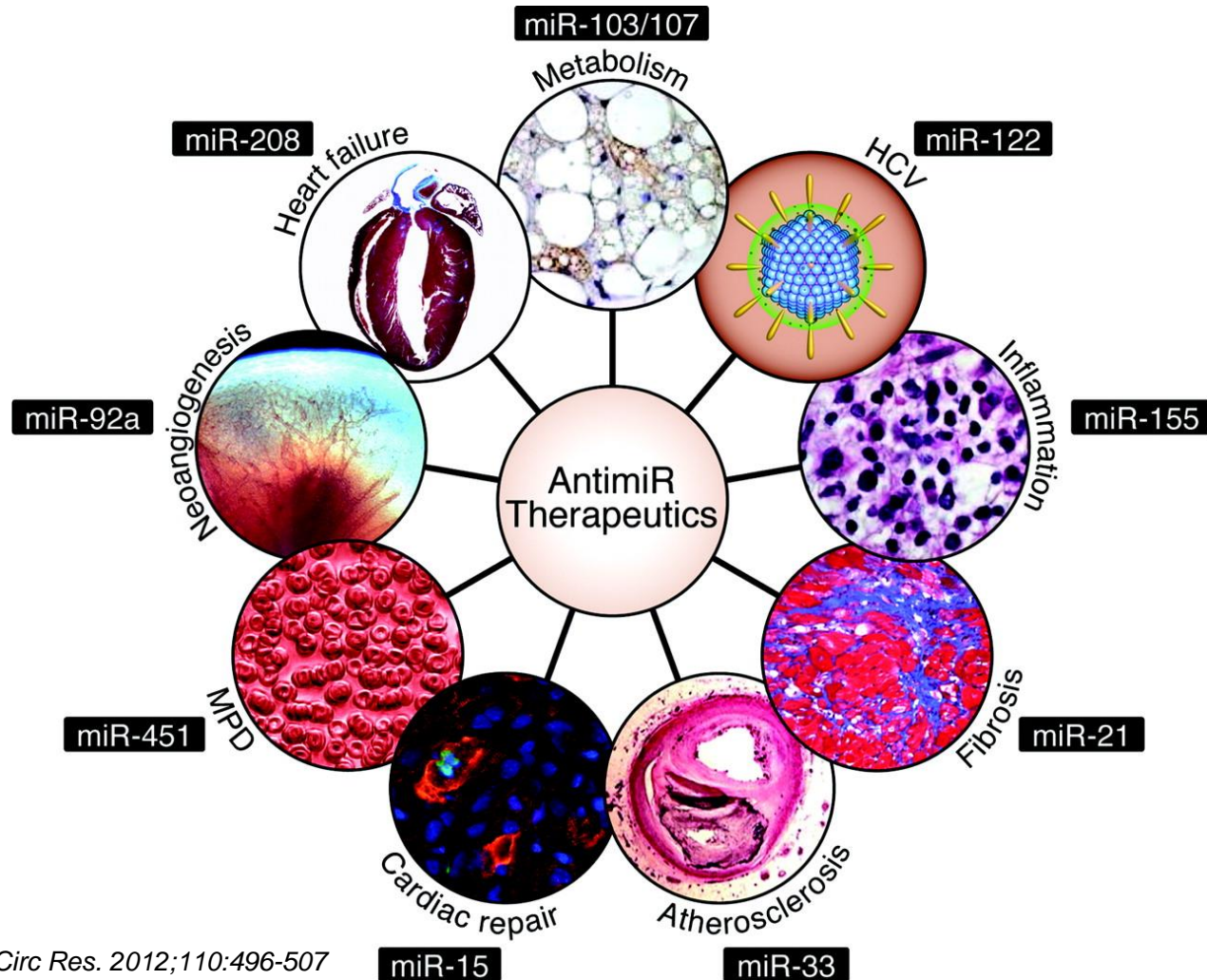
- transcribed in the nucleus
- maturation process in the cytoplasm
- small ncRNAs ~22nt
- central post-transcriptional regulators
- >50% human mRNAs are under miRNA regulation
- conserved in species

# microRNA impact

Many studies have shown that microRNAs (miRNAs) have multiple functions in a wide range of biological processes, such as proliferation, apoptosis, cell cycle arrest, cell migration, and invasion.



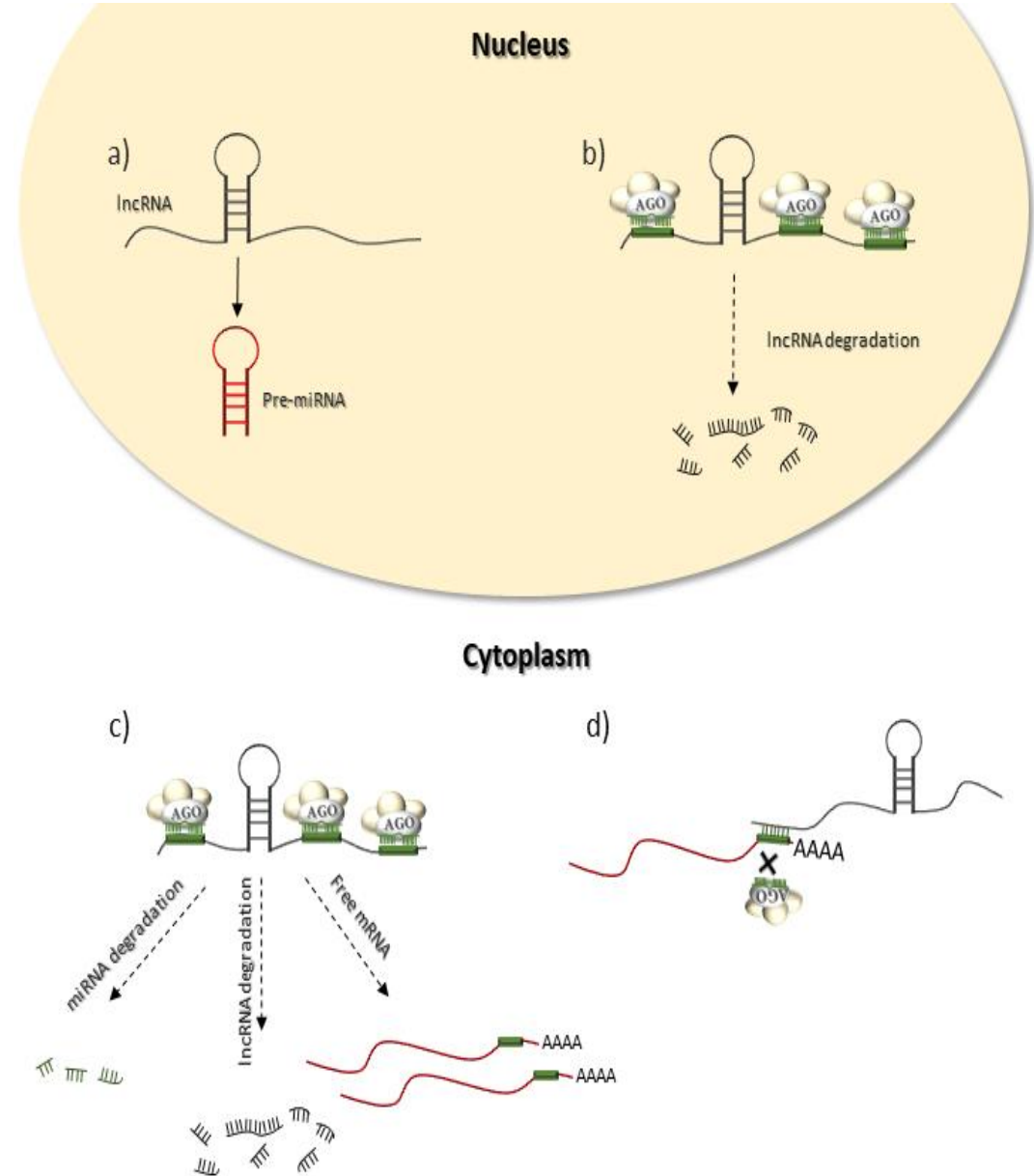
# Specific miRNAs that are currently being pursued as clinical candidates



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

# Long non coding RNA (lncRNA)

- >200 nts, no clearly defined ORF
- splicing, polyA // non-polyA
- Variable conservation
- Usually low expression
- Categorized according to their loci of origin
  - Involved in gene regulation (protein synthesis, RNA maturation, RNA transport and gene silencing)
- Variable subcellular localization





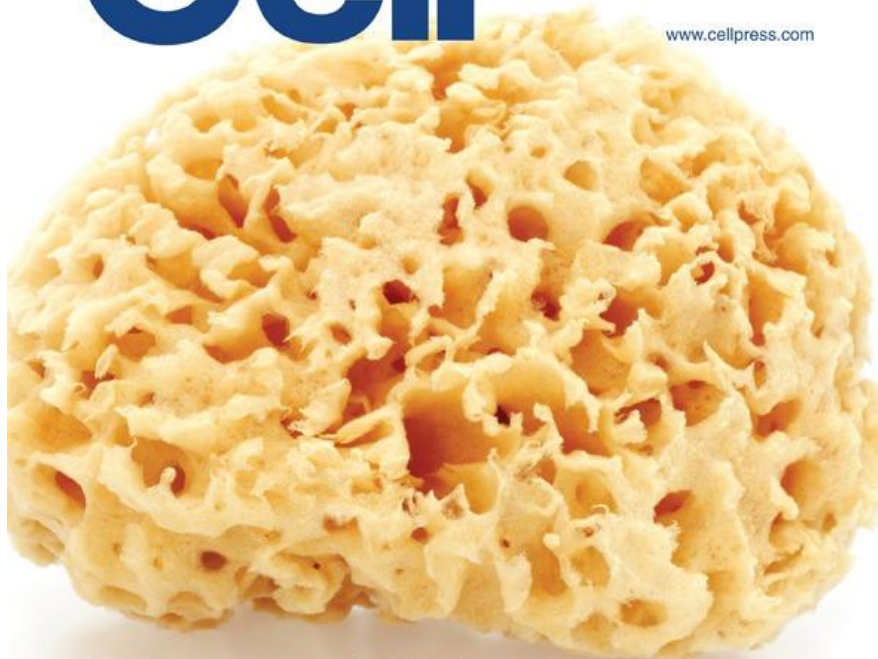
# lncRNAs: miRNA decoys

## Developmental Cell

Volume 25  
Number 1

April 15, 2013

[www.cellpress.com](http://www.cellpress.com)



**Endogenous miRNA Sponge  
Maintains Embryonic Stem Cells**

Ebert MS *et al.* *Nature Meth.* 2007,

Wang *et al.* *NAR* 2010,

Cesana *et al.* *Cell* 2011,

Zisoulis DG *et al.* *Nature.* 2012,

Yoon JH *et al.* *Mol Cell* 2012,

Hansen TB *et al.* *Nature* 2013,

Memczak S *et al.* *Nature* 2013,

Wang *et al.* *J Biol Chem* 2013,

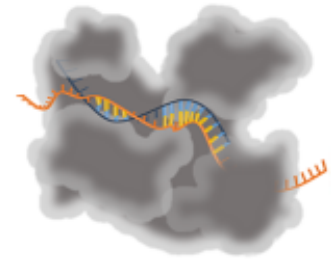
Kallen *et al.* *Molecular Cell* 2013 etc.

# Identification of miRNA targets

## ■ Computational methods

### ✓ *In silico* target prediction programs

- DIANA-microT: [www.microrna.gr](http://www.microrna.gr)
- TargetScan: [www.targetscan.org](http://www.targetscan.org)



## ■ Experimental methods

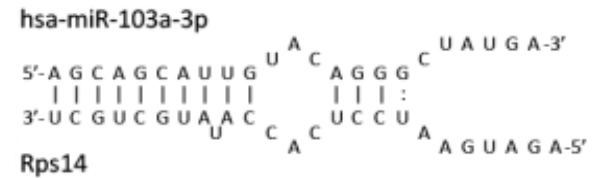
### ✓ Essential to identify genuine miRNA targets

### ✓ Main categories:

- **Low Throughput Techniques:** Reveal individual miRNA:mRNA interactions (e.g. Reporter Luciferase Assays, Northern Blot)
- **High Throughput Techniques:** Characterize numerous miRNA targets. Necessitate extra computational steps (e.g. CLIP-Seq, microarrays, RNA-Seq)

### ✓ Divided into:

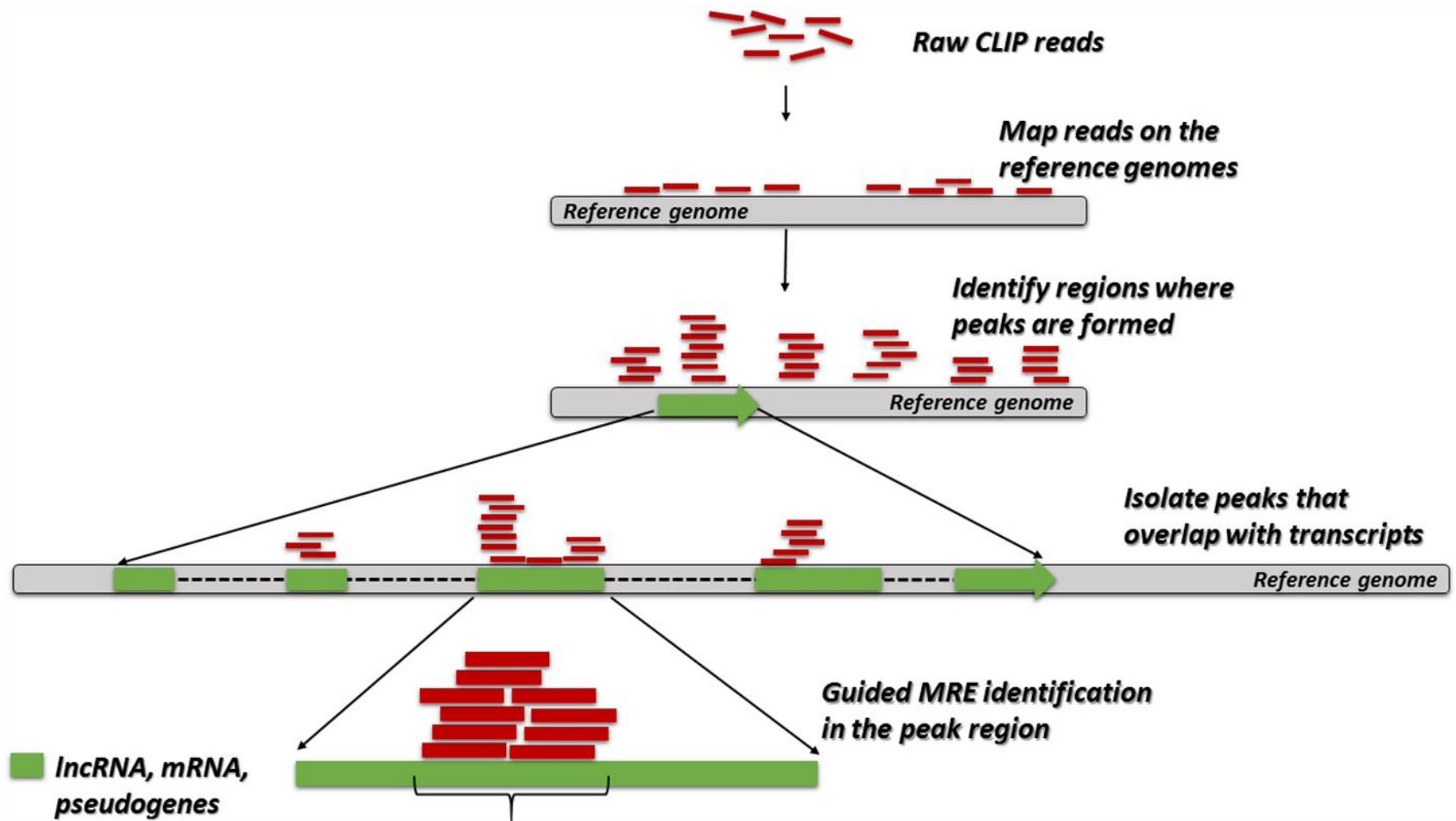
- **Direct Techniques:** Provide the exact miRNA binding location
- **Indirect Techniques:** Reveal the indirect effect of miRNAs on gene expression or on protein levels



# AGO-CLIP-Seq experimental methods

- The most widely used **high-throughput direct** methods
- Provide miRNA binding events on a **transcriptome-wide scale**
- Several CLIP-Seq variants
  - ✓ **HITS-CLIP** (the first AGO-CLIP-Seq methodology)
  - ✓ **PAR-CLIP** (the most widely used methodology)
  - ✓ **CLASH/CLEAR-CLIP** (more accurate/provide miRNA chimeric fragments by incorporating an extra ligation step)

# Analysis of CLIP-Seq data



DIANA-microT-CDS: a web-server of *in silico* predicted miRNA-gene interactions

# *In silico* determination of miRNA-gene interactions

- Ranging from simple to very sophisticated implementations
  - Sequence Features
  - Physics Models
  - Machine Learning
  - Expression Data
- Widely used implementations:
  - TargetScan: [www.targetscan.org](http://www.targetscan.org)
  - DIANA-microT: [www.microrna.gr](http://www.microrna.gr)
  - miRanda: [www.microrna.org](http://www.microrna.org)
  - ....

**Even the most sophisticated implementations achieve 65% sensitivity and 32% specificity**

*M. Reczko, M. Maragkakis, P. Alexiou, I. Grosse, and A. G. Hatzigeorgiou, "Functional microRNA targets in protein coding sequences," Bioinformatics, Jan 27 2012.*

# DIANA-microT-CDS

- One of the first implemented algorithms for miRNA:mRNA target identification with **increased** performance in terms of **sensitivity** and **precision**
- Accurate detection of **canonical** miRNA targets both in CDS and 3' UTR
- Important microT-CDS features are the **target site complementarity**, **A/U upflanking content**, **accessibility**, **pairing stability**, and **conservation** of miRNA targeted CDS and 3' UTR regions

*Reczko, M., Maragkakis, M., Alexiou, P., Grosse, I. & Hatzigeorgiou, A. G. Functional microRNA targets in protein coding sequences. *Bioinformatics* **28**, 771-776 (2012)*

*Paraskevopoulou, M. D. et al. DIANA-microT web server v5. 0: service integration into miRNA functional analysis workflows. *Nucleic acids research* **41**, W169-W173 (2013)*

# RNA features

1D

## RNA sequence

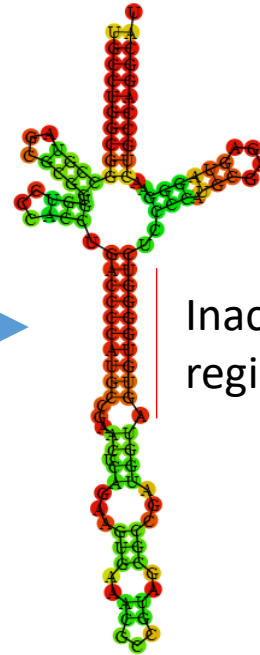
UGCCUGGCGGCCGUAGCGCGGU  
GGUCCCACCUGACCCCAUGCCGA  
ACUCAGAAGUGAAACGCCGUAGC  
GCCGAUGGUAGUGUGGGGUCUC  
CCCAUGCGAGAGUAGGGAACUGC  
CAGGCAU

### Sequence Features

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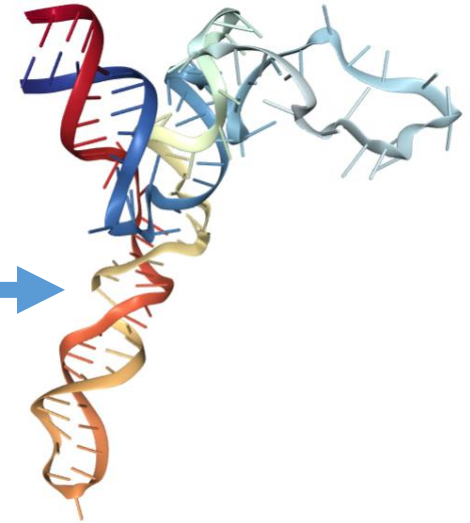
**target site**  
**complementarity**  
**A/U upflanking content**  
**conservation**

2D



Inaccessible  
region

3D



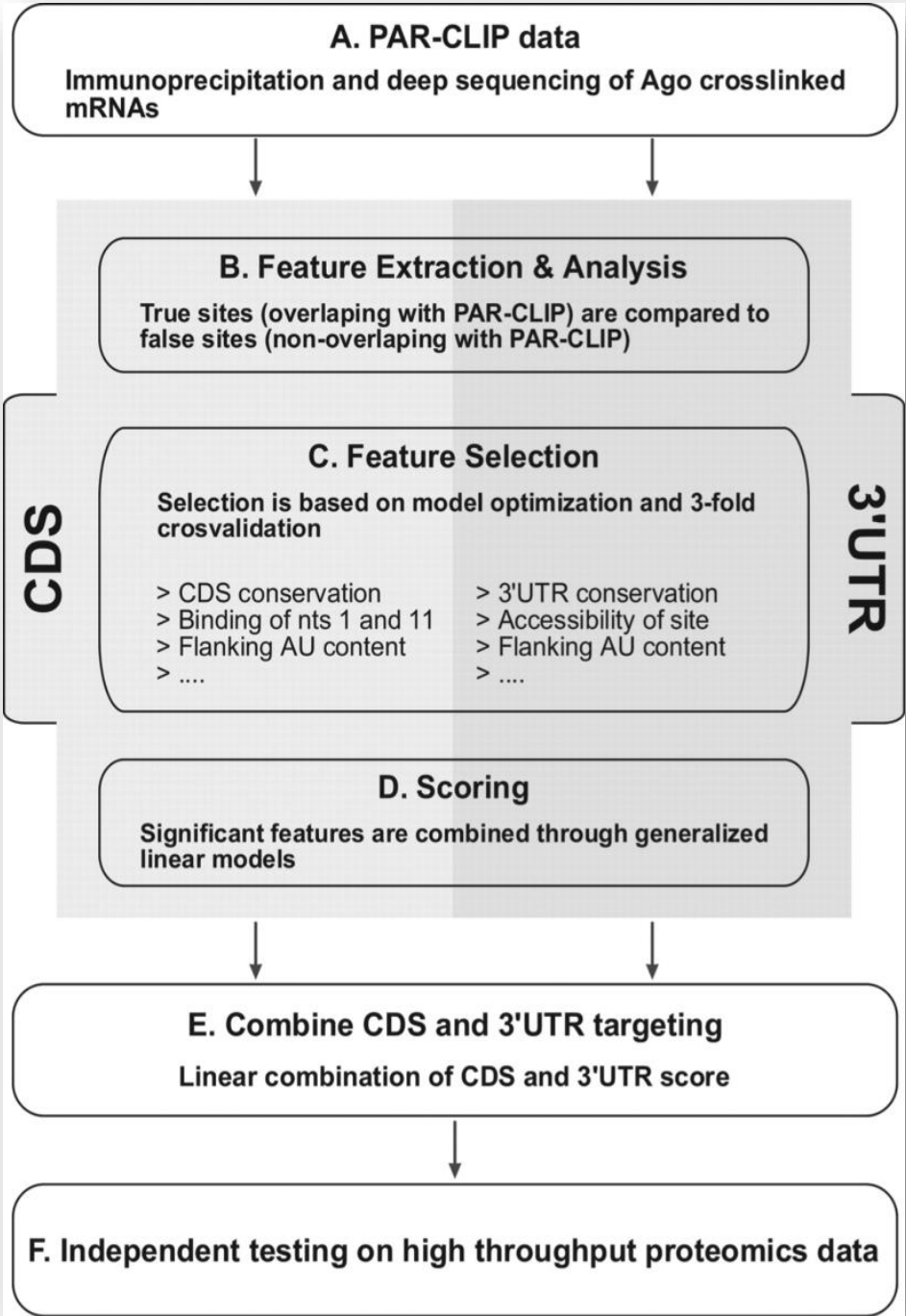
### Physics models

---

**Accessibility**  
**Pairing stability**



# DIANA-microT-CDS



# DIANA-microT-CDS Interface

Software » **MicroT-CDS**

FAQ user: Dimitra

MANAGE HISTORY  
Delete All History

VIEW HISTORY  
Tarbase v6.0  
microT-CDS

hsa-let-7a-5p  
19:34:4...-03-2017  
(2)

Tool-specific history panel

mmu-mir-3-41-3p  
mmu-miR-409-3p  
mmu-miR-369-3p  
mmu-miR-154-3p  
mmu-miR-300-3p  
mmu-miR-4...  
mmu-miR-1...  
mmu-miR-4...  
mmu-miR-3...  
mmu-miR-3...  
mmu-miR-3...  
mmu-miR-460-3p

MRE visualization through the UCSC browser

09:14:37 25-11-2014  
(1)

hsa-miR-27a-3p,  
ENSG00000205189  
17:25:57 04-08-2014  
(1)

mirPath

HOME SO **SEARCH** CATIONS CONTACT

hsa-let-7a-5p Filters

Results: 1051 targets for miRNAs hsa-let-7a-5p. Threshold is set to 0.7.

General information regarding the query

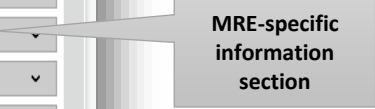
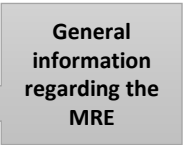
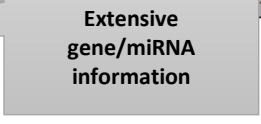
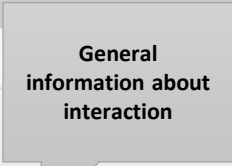
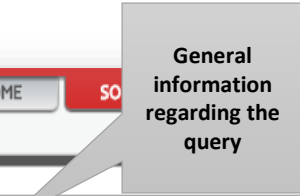
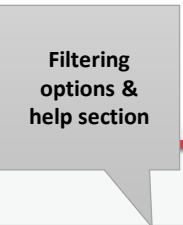
General information about interaction

Ensembl Gene Id	miRNA name	miTG score	Also Predicted										
1 ENSG00000206557 (TRIM71)	hsa-let-7a-5p	0.999999994819048	<input type="checkbox"/> <input type="checkbox"/>										
2 ENSG00000182263 (FIGN)	hsa-let-7a-5p	0.999999994819048	<input type="checkbox"/> <input type="checkbox"/>										
<p>Gene details   miRNA details   PubMed links: <a href="#">miRNA</a>   <a href="#">gene</a>   <a href="#">both</a></p> <p>UCSC graphic</p> <table border="1"> <thead> <tr> <th>Region</th> <th>Binding Type</th> <th>Transcript position</th> <th>Score</th> <th>Conservation</th> </tr> </thead> <tbody> <tr> <td>UTR3</td> <td>7mer</td> <td>6285-6299</td> <td>0.040110047568918</td> <td>16</td> </tr> </tbody> </table> <p>Position on chromosome: 2:164465406-164465420</p> <p>Conserved species: panTro2,rheMac2,rn4,mm9,oryCun2,bosTau4,canFam2,dasNov2,loxAfr3,echTel1,monDom5,galGal3,xenTro2,etNig2,fr2,danRer6</p> <p>(Transcript) 5' AAAGUGAUUGUGAU 3'</p> <p>Binding area:</p> <pre>                     UUGUACAG      UACCUCA                     . .    .                              GAUAUUUU    AUGGAGU                     (miRNA) 3'      U      GGAUG      5'                 </pre>				Region	Binding Type	Transcript position	Score	Conservation	UTR3	7mer	6285-6299	0.040110047568918	16
Region	Binding Type	Transcript position	Score	Conservation									
UTR3	7mer	6285-6299	0.040110047568918	16									
UTR3	7mer	6198-6213	0.0225024140547264	13									
UTR3	8mer	5532-5556	0.0502496831570267	12									
UTR3	6mer	5376-5395	0.00120087302654954	3									
UTR3	8mer	2037-2058	0.0974798729541372	16									
UTR3	8mer	1935-1954	0.0452141077427323	16									
UTR3	7mer	28-41	0.0451015011813675	12									
UTR3	8mer	924-943	0.00714702187331479	0									
3 ENSG00000174498 (IGDCC3)	hsa-let-7a-5p	0.999999163327332	<input type="checkbox"/> <input type="checkbox"/>										

Extensive gene/miRNA information

General information regarding the MRE

MRE-specific information section



# DIANA-microT-CDS Interface

TarBase v6.0

microT-CDS

hsa-let-7a-5p ✓

19:34:47 24-03-2017 (2)

mmu-miR-679-3p  
mmu-miR-134-3p  
mmu-miR-541-3p  
mmu-miR-341-3p  
mmu-miR-409-3p  
mmu-miR-369-3p  
mmu-miR-154-3p  
mmu-miR-300-3p  
mmu-miR-495-3p  
mmu-miR-127-3p  
mmu-miR-412-3p  
mmu-miR-337-3p  
mmu-miR-380-3p  
mmu-miR-377-3p  
mmu-miR-4661-5p

09:14:37 25-11-2014 (1)

hsa-miR-27a-3p,  
ENSG00000205189

17:25:57 04-08-2014 (1)

mirPath

	Ensembl Gene Id	miRNA name	miTG score	Also Predicted
1	ENSG00000206557 (TRIM71)	hsa-let-7a-5p	0.99999994819048	<span style="color: green;">■</span> <span style="color: blue;">■</span>
2	ENSG00000182263 (FIGN)	hsa-let-7a-5p	0.99999967965059	<span style="color: green;">■</span> <span style="color: blue;">■</span>

**Gene details** ✓

**Gene ID:** FIGN

**Expression:**

**External Gene ID:** [ENSG00000182263](#)

**Ensembl version:** 77

**Description:** fidgetin [Source:HGNC Symbol;Acc:13285]

**Chromosome:** 2

**DIANA resources:** [microT-CDS](#) [TarBase v.7](#) [LncBase Experimental](#) [LncBase Predicted](#)

**miRNA details** ✓

**Name:** hsa-let-7a-5p

**Alternative description:** MIMAT0000062

**Related names:** ugagguaguagguuguauaguu

**miRNA sequence:** [miRBase](#)

**External links:** [microT-CDS](#) [TarBase v.7](#) [LncBase Experimental](#) [LncBase Predicted](#) [mirPath v.3](#)

**Related diseases:**

[Acute Disease](#) [Adenocarcinoma](#) [Adenoma](#) [Adenoma, Bile Duct](#) [Alzheimer Disease](#) [Anemia, Sickle Cell](#) [Aortic Valve Stenosis](#) [Ascariasis](#) [Asthma](#) [Autoimmune Diseases](#) [Azoospermia](#) [Bites and Stings](#) [Bone Neoplasms](#) [Brain Neoplasms](#) [Breast Neoplasms](#) [Breast Neoplasms, Male](#) [Burkitt Lymphoma](#) [Carcinoma](#) [Carcinoma, Hepatocellular](#) [Carcinoma, Non-Small-Cell Lung](#) [Carcinoma, Papillary](#) [Carcinoma, Renal Cell](#) [Carcinoma, Small Cell](#) [Carcinoma, Squamous Cell](#) [Cardiomyopathies](#) [Cardiomyopathy, Hypertrophic](#) [Cell Transformation, Neoplastic](#) [Chromosome Aberrations](#) [Chromosome Deletion](#) [Chronic Periodontitis](#) [Colitis, Ulcerative](#) [Colonic Neoplasms](#) [Colorectal Neoplasms](#) [Cystadenocarcinoma, Serous](#) [Dermatitis, Atopic](#) [Diabetes Mellitus](#) [Diabetes Mellitus, Type 2](#) [Disease](#) [Disease Models, Animal](#) [Disease Progression](#) [Disorders of Sex Development](#) [Down Syndrome](#) [Echinococcosis](#) [Esophageal Neoplasms](#) [Facial Neoplasms](#) [Fatty Liver](#) [Fibrosis](#) [Genetic Predisposition to Disease](#) [Glioma](#) [Head and Neck Neoplasms](#) [Hematologic Neoplasms](#) [Hepatitis C, Chronic](#) [HIV Infections](#) [Hodgkin Disease](#) [Huntington Disease](#) [Hypertension](#) [Inflammation](#) [Influenza in Birds](#) [Insulin Resistance](#) [Intellectual Disability](#) [Ischemia](#) [Kidney Failure, Chronic](#) [Kidney Neoplasms](#) [Laryngeal Neoplasms](#) [Leiomyoma](#) [Leprosy](#) [Leukemia](#) [Leukemia, Lymphocytic, Chronic, B-Cell](#) [Leukemia, Myeloid, Acute](#) [Leukemia, Promyelocytic, Acute](#) [Liver Cirrhosis](#) [Liver Neoplasms](#) [Lung Neoplasms](#) [Lupus Erythematosus, Systemic](#) [Lupus Nephritis](#) [Lymphatic Metastasis](#) [Lymphoma, Follicular](#) [Lymphoma, Large B-Cell, Diffuse](#) [Malaria](#) [Falciparum](#) [Marek Disease](#) [Melanoma](#) [Mesothelioma](#) [Mouth Neoplasms](#) [Multiple Myeloma](#) [Multiple Sclerosis](#) [Muscular Dystrophies](#) [Myocardial Infarction](#) [Myocardial Reperfusion Injury](#) [Myotonic Dystrophy](#) [Nasopharyngeal Neoplasms](#) [Neoplasm Invasiveness](#) [Neoplasm Metastasis](#) [Neoplasm Recurrence, Local](#) [Neoplasms](#) [Neoplasms, Experimental](#) [Neoplasms, Glandular and Epithelial](#) [Nerve Degeneration](#) [Nerve Sheath Neoplasms](#) [Neuralgia](#) [Neuroblastoma](#) [Obesity](#) [Ovarian Neoplasms](#) [Pancreatic Neoplasms](#) [Pituitary Neoplasms](#) [Poultry Diseases](#) [Precursor Cell Lymphoblastic Leukemia-Lymphoma](#) [Prostatic Neoplasms](#) [Psoriasis](#) [Pulmonary Embolism](#) [Retinoblastoma](#) [Schistosomiasis japonica](#) [Scrapie](#) [Sepsis](#) [Skin Neoplasms](#) [Small Cell Lung Carcinoma](#) [Stomach Neoplasms](#) [Stroke](#) [Thyroid Neoplasms](#) [Translocation, Genetic](#) [Trichuriasis](#) [Tuberculosis](#) [Urinary Bladder Neoplasms](#) [Uterine Cervical Neoplasms](#) [Uterine Neoplasms](#) [Uveal Neoplasms](#)

**pubMed links:** [miRNA](#) | [gene](#) | [both](#)

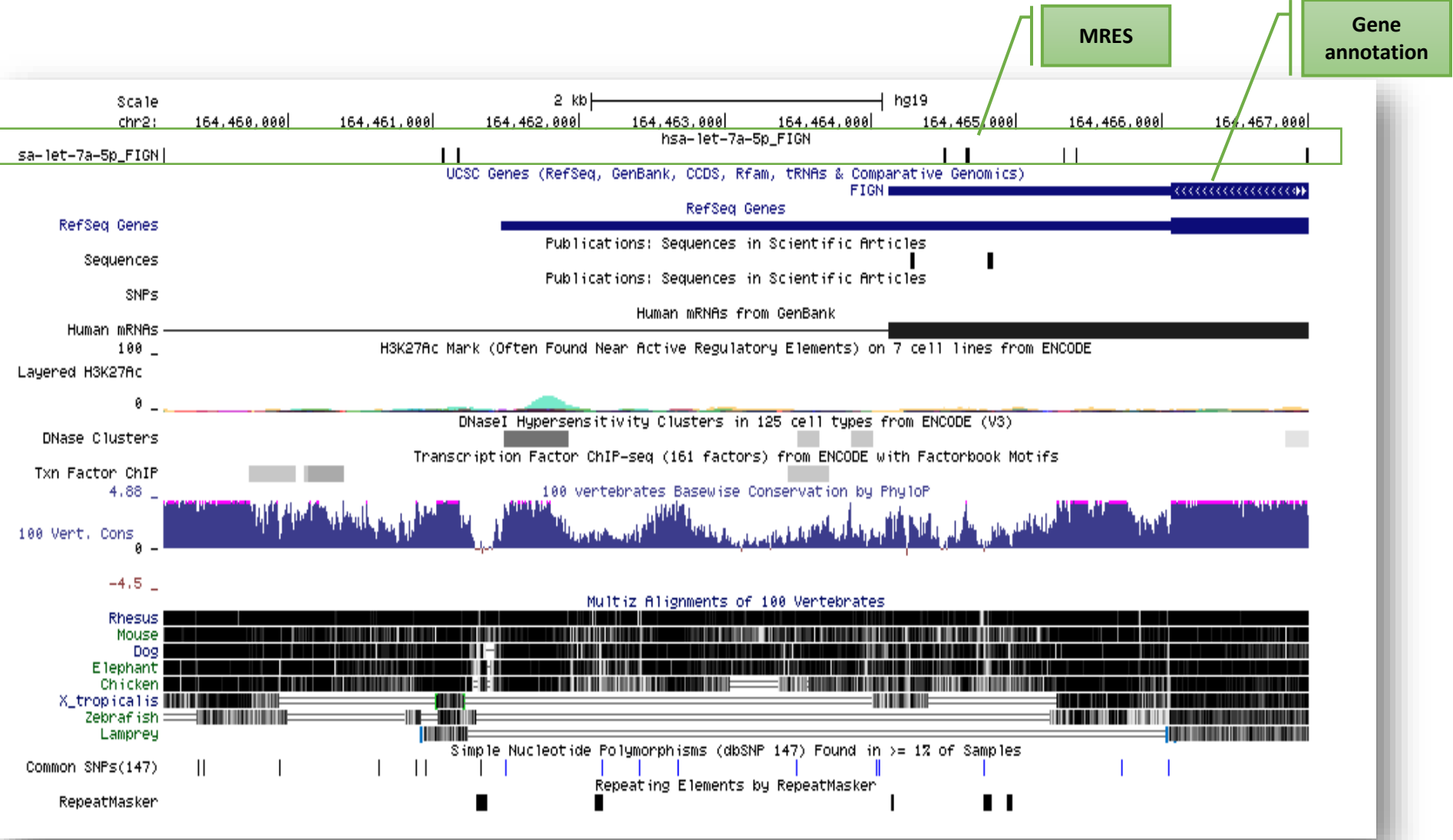
**UCSC graphic**

Region	Binding Type	Transcript position	Score	Conservation
UTR3	7mer	6285-6299	0.040110047568918	16

Extensive gene information

Extensive miRNA information

# Active UCSC graphic of MREs



**DIANA-TarBase/LncBase, databases with experimentally supported  
miRNA-gene pairs**

# 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

*Karagkouni D, Paraskevopoulou MD, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions, Nucl. Acids Res. gkx1141(2018)*

# Data collection

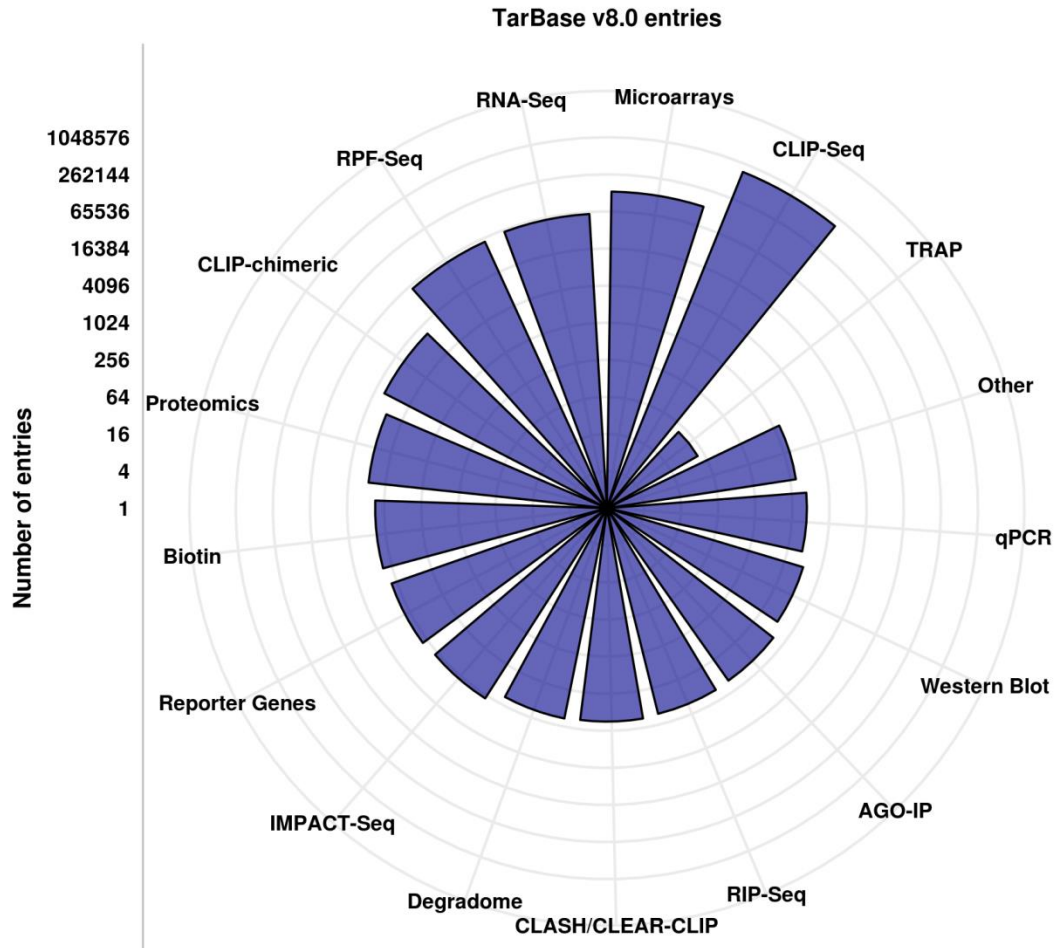
- ✓ Use of text mining-assisted pipeline to:
  - extract publications harboring miRNA-gene interactions, in XML format
  - recognize associated miRNA and Gene Names
  - score the publications and mark them up
  
- ✓ ~370 publications have been manually curated
  
- ✓ >230 collected/analyzed high-throughput datasets

# TarBase v8 Statistics


- TarBase v8.0 indexes **~700,000** unique miRNA-gene interactions and **more than a million entries**
- This collection has been derived from experiments employing more than **33 distinct experiments**, on **592 tissues/cell types** and **~430 experimental conditions** from **18 species**



# TarBase v8 Statistics



# DIANA-TarBase Interface

Statistics 

**Search Fields - Query mode** TarBase v.8

miRNAs: hsa-miR-1-3p, hsa-miR-221-3p, Clear all

Genes: ZEB2, SELE, TKT, Clear all

**miRPath interconnection** [Previous version](#) [Help](#) [Related Pathways](#)

**Database statistics**

Filters

Species:

Method Type:

Method:

Regulation type:

Validation type:

Validated as:

Cell Type:

Tissue:

Source:

Publication Year:

Prediction score:

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

**Interactive result sorting**

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/miRNA details**

SELE	hsa-miR-221-3p	low: 1 high: 0	1	1	1	-
ZEB2	hsa-miR-221-3p	low: 0 high: 1	1	1	1	-

**Binding site details**

**Experiment details**

**Filters - Browsing mode**

← Back

# TarBase statistics

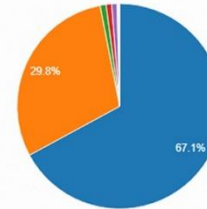
Tarbase v.8 statistics

## Database Summary

Total Entries:	1077279
Interactions:	691188
Cell Types:	510
Tissues:	82
Publications:	1165
Low-yield Methods:	15
High-throughput Methods:	19

Chart of indexed interactions per species

## Interactions per Species



## Species filter

- Homo Sapiens
- Mus musculus
- Caenorhabditis elegans
- Epstein Barr virus
- KSHV
- Rattus norvegicus
- Zea mays
- Physcomitrella patens
- Medicago truncatula
- Oryza sativa japonica
- Arabidopsis thaliana
- Danio rerio
- Vitis vinifera
- Drosophila melanogaster
- Gallus gallus
- Pan troglodytes

Select species to update statistics

× Homo Sapiens × KSHV × Epstein Barr virus

Apply

## Interactive statistics

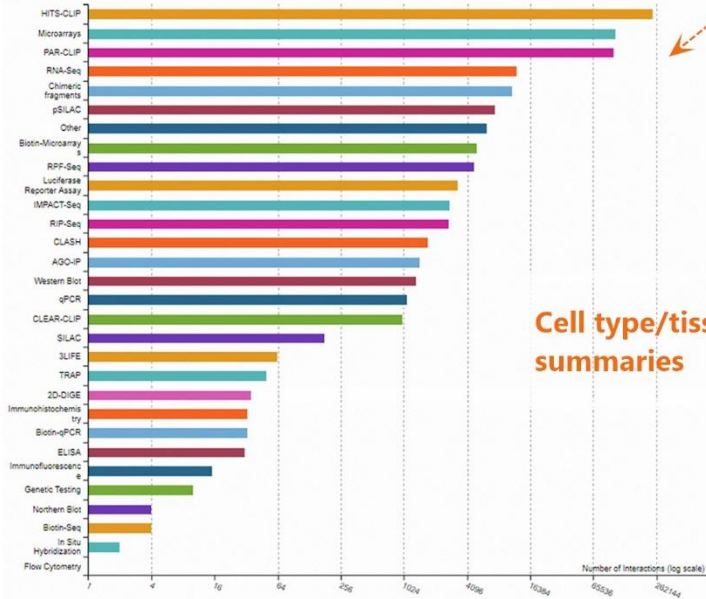
## Dynamic statistics

### Dynamic Statistics Panel

Interactions:	475416
Cell Types:	450
Tissues:	71
Publications:	1003
Low-yield Methods:	13
High-throughput Methods:	17

Interaction counts per method

### Dynamic Methods Barplot



Cell type/tissue summaries

### Cell Types

Cell Types	Interaction Count	Freq
108CA	1	0.0002%
113_6_4L	266	0.0512%
131_4_5B1	218	0.0420%
1386LN	250	0.0481%
16HBE14O	664	0.1278%
16HBET	4	0.0008%
210ZEP	3	0.0006%
22RV1	7	0.0013%

### Tissues

Tissues	Interaction Count	Freq
Adipose	11	0.0023%
Adrenal Gland	10	0.0021%
Aortic Endothelium	3611	0.7474%
Bile duct	3	0.0006%
Bladder	9	0.0019%
Bone	5822	1.2051%
Bone marrow	79	0.0164%
Bone Marrow	50500	10.4529%

# Ensembl/RNAcentral integration

Ensembl release 75 - February 2014 © WTSI / EBI

Permanent link - View in archive site

organism, expert database, gene, ncRNA type, accession

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

v1.0 Expert databases - API - Sequence search Downloads Help Contact

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)  
Explore ENA entries

**Rfam** Rfam is a database containing information about ncRNA families and other structured RNA elements.  
**2,493,782** sequences (example)

# Indexing miRNA targets on non-coding transcripts

*Nucleic Acids Research*, 2019 1–10  
doi: 10.1093/nar/gkz1036

## **DIANA-LncBase v3: indexing experimentally supported miRNA targets on non-coding transcripts**

**Dimitra Karagkouni<sup>1,2,3,\*</sup>, Maria D. Paraskevopoulou<sup>1,2</sup>, Spyros Tastsoglou<sup>1,2,†</sup>,  
Giorgos Skoufos<sup>1,2,†</sup>, Anna Karavangeli<sup>1,3</sup>, Vasilis Pierros<sup>1,2</sup>, Elissavet Zacharopoulou<sup>1,4</sup>  
and Artemis G. Hatzigeorgiou<sup>1,2,3,\*</sup>**

<sup>1</sup>DIANA-Lab, Department of Electrical and Computer Engineering, Univ. of Thessaly, 38221 Volos, Greece, <sup>2</sup>Hellenic Pasteur Institute, 11521 Athens, Greece, <sup>3</sup>Department of Computer Science and Biomedical Informatics, Univ. of Thessaly, 351 31 Lamia, Greece and <sup>4</sup>Department of Informatics and Telecommunications, Postgraduate Program: 'Information Technologies in Medicine and Biology', University of Athens, 15784 Athens, Greece

Received September 22, 2019; Revised October 16, 2019; Editorial Decision October 17, 2019

# DIANA-LncBase v3

- A reference database devoted to the cataloguing of miRNA-lncRNA interactions
- LncBase was initially released in 2012 and since then it is constantly updated.
- LncBase v3.0 indexes **approximately half a million entries**, corresponding to **~240,000** unique tissue and cell type specific miRNA-lncRNA pairs
- This collection has been derived from
  - The incorporation of **236 publications**
  - Text mining and manual curation of publications and the analysis of **>300 high-throughput datasets.**
  - Experiments employing **~15 distinct methods, 243 tissues/cell types** and **~162 experimental conditions** for human and mouse species

# From literature to Database

## EXAMPLE OF SENTENCES EXTRACTION

TFAP2A-AS1 is the target of miR-33.

Dual-Luciferase reporter assay was used to verify the interaction between TFAP2A and miR-33 in MCF-7 cells.

## DATABASE DECODING



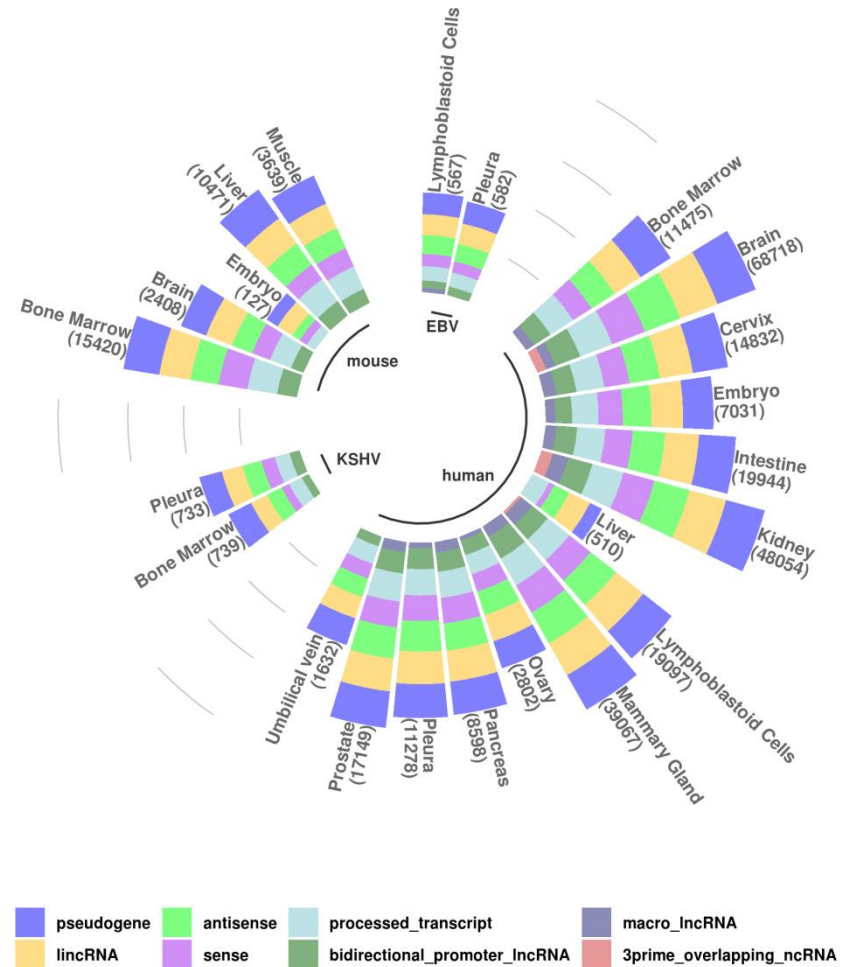
## FINAL RECORD

microRNA	target	method	cell line
miR-33	TFAP2A-AS1	LRE	MCF-7

# DIANA-LncBase v3

## LncBase hosts

- lncRNAs from **GENCODE v30**, **RefSeq** and **Cabili *et al.*** study
- **>730 manually curated** miRNA-lncRNA entries
- **2,094** interactions, extracted from the re-analysis of 86 miRNA-specific transfection/knockdown **microarray** experiments.
- **2,220 viral** miRNA binding events on host lncRNA transcripts
- **>235,000** interactions have been retrieved from the re-analysis of **236 AGO-CLIP-Seq datasets** with **microCLIP** algorithm



85 ± 10% of interactions is spatially classified into sense, antisense, lincRNAs and pseudogenes



# DIANA-LncBase v3

LncBase v3 provides new features and functionalities

- **Known short variant information** on **9,318** miRNA target sites, from the reference databases dbSNP, ClinVar and COSMIC
- **lncRNA transcript expression profiles** in 34 cell types/tissues and in **nucleus/cytoplasm** sub-cellular compartments, by analyzing **103 raw RNA-Seq** libraries, encompassing ~19.3 billion reads
- **Interactive visualization plots** portraying
  - correlations of miRNA-lncRNA pairs, retrieved from CLIP-Seq experiments
  - lncRNA expression profiles within the cell and/or in different subcellular compartments, among distinct cell types

# DIANA-LncBase v3: miRNA-lncRNA Module

(1) Menu bar --> LncBase miRNA-lncRNA LncRNA expression Visualizations Previous Versions - Help

(2) Search field miRNA-lncRNA interactions (3) Search by location

(4) Filters-Browsing results

(5) Results statistics Execute Search Clear All (6) Results sorting (7) Gene/miRNA details (8) Experimental details (9) Short Variant info (10) miRNA Conf. Level (11) Binding site details (12) lncRNA Expression (13) UCSC (14) DIANA-Tools interconnections

miRNA: hsa-miR-185-5p, hsa-miR-106b-5p, Clear All

lncRNA: XIST, PTENP1, Clear All

Filters: Tissue, Cell Type, Method, Validated As, Validation Type, miRNA Conf. Level, Biotypes, Species, lncRNA Source, Variants

Interactions: 4, Experiments: 4 (low: 1, high: 3) Cell lines: 3, Tissues: 3, Publications: 6

Gene name	miRNA name	Experiments	Publications	Cell Lines	Tissues	miRNA Conf.	IncRNA Expr	UCSC	mT	TB	InP	mP	DIANA Links																																			
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# DIANA-LncBase v3: LncRNA Expression Module

**(a)**

(1) Search field - - - ->

(2) Filters

Filters | Clear Filters

Tissue: Select Tissues

Cell Type: LCLBAC x LCLBACD1 x HEK293 x MCF7 x HUVEC x

Species: Homo sapiens x

TPM Level: Medium x

(3) Gene info

IncRNA

MALAT1  
XIST  
Clear All

Expression | Localization | Execute Search

First Previous 1 2 3 Next Last

Gene name	Transcript ID	Cell Types	Tissues	
MALAT1	ENST00000619449	3	1	miRNA-lncRNA
Cell Type Tissue Category TPM				
LCLBACD1	NA	Normal/Primary	243.575003	(5) miRNA-lncRNA
LCLBAC	NA	Normal/Primary	173.677956	
MCF7	Mammary Gland	Cancer/Malignant	29.282278	
XIST	ENST00000421322	1	1	miRNA-lncRNA
Cell Type Tissue Category TPM				
HEK293	Kidney	Embryonic/Fetal	170.8511875	

(4) Experimental details

(6) Change Mode

**(b)**

(1) Search field - - - ->

(2) Filters

Filters | Clear Filters

Tissue: Select Tissues

Cell Type: GM12878 x HELAS3 x

Species: Homo sapiens x

(3) Gene info

IncRNA

H19  
MALAT1  
Clear All

Expression | Localization | Execute Search

First Previous 1 2 Next Last

Gene name	Transcript ID	Cell Types	Tissues			
H19	ENST00000417089	1	1	miRNA-lncRNA		
Cell Type Tissue Category TPM Nucleus TPM Cytoplasm RCI Compartment						
HELAS3	Cervix	Cancer/Malignant	1.7179	2.2355	0.38	Cytoplasm
H19	ENST00000411861	1	1	miRNA-lncRNA		
Cell Type Tissue Category TPM Nucleus TPM Cytoplasm RCI Compartment						
HELAS3	Cervix	Cancer/Malignant	5.4064	3.6427	-0.5696	Nucleus
MALAT1	NR_144567.1	2	2	miRNA-lncRNA		
Cell Type Tissue Category TPM Nucleus TPM Cytoplasm RCI Compartment						
GM12878	Blood	Normal/Primary	100.8411	1.6013	-5.9767	Nucleus
HELAS3	Cervix	Cancer/Malignant	215.4643	3.0875	-6.1249	Nucleus

(4) Experimental details

(5) miRNA-lncRNA

(7) Localization info

# DIANA-LncBase v3: Interactive Visualizations

miRNA-lncRNA plot

Tissue:

Cell Type:

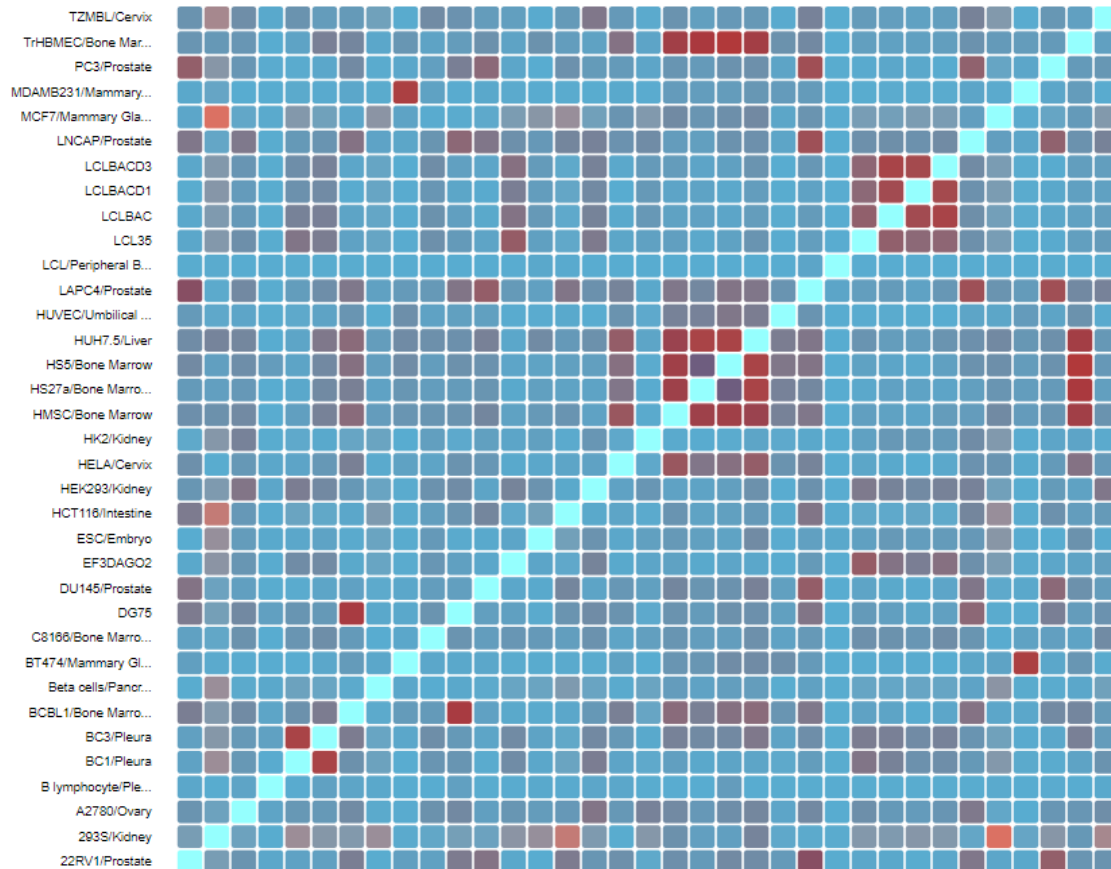
Biotypes:

Species:

Select more than one cell type and/or tissue to explore how they cluster based on absence/presence of miRNA-lncRNA interactions. For each pair-wise combination of cell type/tissue, Pearson's r coefficient values are calculated.

[View](#)

Cell Type / Tissue



Cell Type / Tissue



# DIANA-LncBase v3: Interactive Visualizations

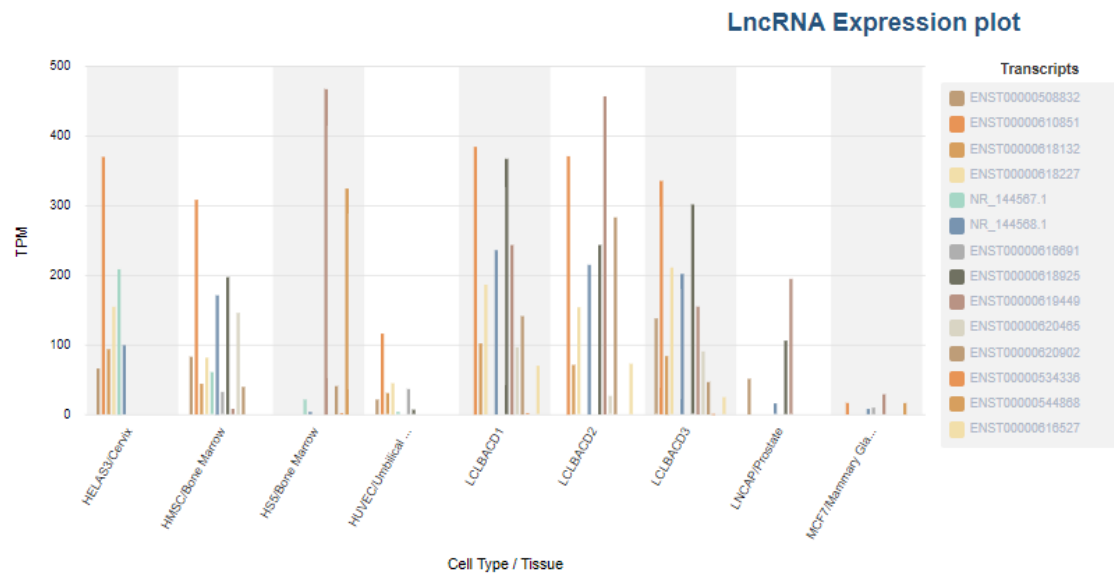
IncRNA:    
 MALAT1   
 Clear All

Tissue:

Cell Type:  HMSC  HS5  HUVEC  LCLBACD1    
 LCLBACD2  LCLBACD3  HELAS3    
 LNCAP  MCF7

Species:

Select an lncRNA, and optionally cell type(s), tissue(s), to view its expression profile. TPM Level Low:(1-10), Medium: (11-600), High: (>600)



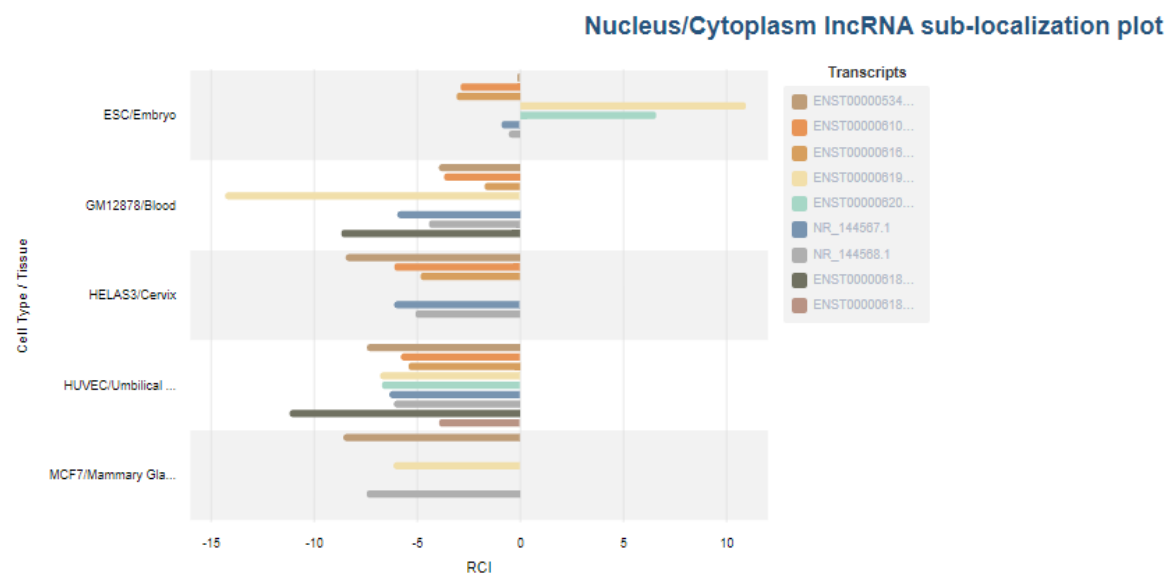
IncRNA:    
 MALAT1   
 Clear All

Tissue:

Cell Type:  ESC  GM12878  HELAS3  HUVEC    
 MCF7

Species:

Select an lncRNA and, optionally cell type(s), tissue(s) to compare its abundance in nuclear and cytoplasmic compartments. Relative Concentration Index (RCI) =  $\log_2(\text{TPM}_{\text{cyt}}/\text{TPM}_{\text{nuc}})$ .   
 Inclination towards nucleus: RCI < 0   
 Inclination towards cytoplasm: RCI > 0



# Querying DIANA-microT-CDS

*Paraskevopoulou, M. D., Vlachos, I. S. & Hatzigeorgiou, A. G. DIANA-TarBase and DIANA Suite Tools: Studying Experimentally Supported microRNA Targets. Current Protocols in Bioinformatics, 12.14. 11-12.14. 18 (2016).*

1. Begin at the microT-CDS home page (<http://www.microrna.gr/microT-CDS>).
2. Continue with the previous query of miRNA “hsa-miR-34a-5p”. In the central search text box, enter the mature miRNA name “hsa-miR-34a-5p” or its MIMAT “MIMAT0000255” (miRBase mature miRNA accession) identifier. Press Enter.
3. Expand the downward-pointing arrows to access further information regarding the predicted miRNA binding location on the CDS and 3’ UTR of the gene, relative positions of miRNA binding sites on the transcript, conservation of each site across different vertebrate species, and predicted graphical representations of every miRNA-mRNA duplex pair.
4. To save the *in silico* predictions results file, select the “Download” button above the results panel.
5. Explore the miRNA-mRNA targeted regions in a UCSC genome browser by pressing the relevant right-pointing arrow near the “UCSC graphic” section.

6. Search for a miRNA related to a specific disease. Open a new tab in order to keep the previous query available. In the search text box, enter “hsa-miR-34a-5p "breast cancer" ” and press enter.

7. Click Filters button and set microT-CDS threshold to 0.9. Press Enter.

### ***Multiple search terms***

8. Open a new tab in order to keep the previous query available.

9. Search microT-CDS for interactions of multiple miRNAs and genes. Include spaces in-between the queried terms. The retrieved results are restricted to the searched microRNAs and Ensembl Gene identifiers. Insert the following terms in the search box “hsa-miR-34a-5p hsa-miR-107 hsa-miR-15a-5p ENSG00000104969 ENSG00000138735 ENSG00000139645” and browse the results.



# Querying DIANA-TarBase

*Paraskevopoulou, M. D., Vlachos, I. S. & Hatzigeorgiou, A. G. DIANA-TarBase and DIANA Suite Tools: Studying Experimentally Supported microRNA Targets. Current Protocols in Bioinformatics, 12.14. 11-12.14. 18 (2016).*

1. Begin at the TarBase home page (<http://www.microna.gr/tarbase>).
2. In the miRNA text box, enter the mature miRNA name “hsa-miR-34a-5p” or its MIMAT “MIMAT0000255” (miRBase mature miRNA accession) identifier. Press Enter.
3. Press the “(i)” information button next to the gene name (CD44) to explore supplementary gene details, including expressed cell types and links to Ensembl entry.
4. Click the link next to Ensembl gene identifier (“*Gene ID*”) to be redirected to Ensembl database.
5. Select a valid microT-CDS score “Pred. Score” to view miRNA-gene interactions predicted from the microT Web-server.
6. Select “Related Pathways” to see the related KEGG pathways in which this miRNA participates.

7. Press the “(i)” information button next to the miRNA name (hsa-miR-34a-5p). miRNA sequence, MIMAT identifier, links to other DIANA-resources and a weighted disease tag cloud will pop-up.
  
8. Click on the downward-pointing arrow to view the details of each miRNA-gene interaction.
  
9. Press the downward-pointing arrow in each publication panel to access further information. The revealed panel shows binding-site coordinates (if available), the name of the supporting experimental methodology, type of exerted regulation (POSITIVE/NEGATIVE, UP/DOWN and DIRECT/INDIRECT), and the source of the indexed interaction.
  
10. Select the “(i)” button next to the “Location” column to invoke further information in a new pop-up window. The window presents the primers used for cloning in case of Reporter Gene Assay–verified interactions, as well as supplementary comments for a specific miRNA recognition target site.
  
11. Follow the provided “Binding info@ensEMBL” link to be redirected to an Ensembl Genome Browser showing this exact binding site location.

12. Select the “Method Type” box in the filters panel in order to reveal the relevant options. Select “Low-Throughput” and “Apply” to reduce results to low-yield methods only. Unselect the “Low-Throughput” filter and hit “Apply”.

13. Click “Method” box. In this menu we can directly select only the methods that we want included in the results. Select “Luciferase Reporter Assay” to see interactions supported by this gold standard technique.

14. Press “Remove all” on the top of the filter panel. Perform a complex query by selecting “Validated as”: POSITIVE, “Prediction score”: 0.6, “Validation Type”: Direct. This query identifies positive interactions from direct experiments that have also been predicted *in silico* (score 0.6).

15. Press “Clear all” in order to restore the query to its original state.

## ***Multiple search terms***

16. Open TarBase (<http://www.microrna.gr/tarbase>) in a new tab in order to keep the previous query available.

17. Search TarBase for experimentally supported interactions of multiple miRNAs and genes. The retrieved results are restricted to the searched microRNAs and Ensembl gene identifiers.

Insert the following terms in the corresponding search boxes one-by-one:

miRNAs: hsa-miR-34a-5p hsa-miR-107 hsa-miR-15a-5p

Genes: ENSG00000104969 ENSG00000138735 ENSG00000139645

## ***Query free search***

18. Remove all queries and select *Mus musculus* “Species” and Luciferase Reporter Assay “Method”. Hit “Apply”.

The top results are displayed (max 3,000)

# Querying DIANA-LncBase

Dimitra Karagkouni, Maria D Paraskevopoulou, Spyros Tastsoglou, Giorgos Skoufos, Anna Karavangeli, Vasilis Pierros, Elissavet Zacharopoulou, Artemis G Hatzigeorgiou, DIANA-LncBase v3: indexing experimentally supported miRNA targets on non-coding transcripts, *Nucleic Acids Research*, Volume 48, Issue D1, 08 January 2020, Pages D101–D110, <https://doi.org/10.1093/nar/gkz1036>

1. Begin at the LncBase home page (<https://diana.e-ce.uth.gr/lncbasev3/interactions>).
2. In the miRNA text box, enter the mature miRNA name “hsa-miR-34a-5p”. From the filters section select **Tissue** “Pancreas”, “Cervix”, **Biotypes** “antisense”, “lincRNA”, “sense”, **Species** “Homo sapiens”. Press Enter.
3. Sort results based on gene names by clicking the arrows in the Gene name column of the results.
4. Press the “(i)” information button next to the gene name (ZBED3-AS1) to explore supplementary gene details and links to Ensembl entry.
5. Click the link next to Ensembl gene identifier (“*Gene ID*”) to be redirected to Ensembl database.
6. Notice the “Var” tag in Z93241.1 entry, indicating a known variant resides in the miRNA binding site. Expand the pane by clicking the downward arrow to view the details for the interaction.

7. Press the downward-pointing arrow in each publication pane to access further information. The revealed pane shows binding-site coordinates (if available), the name of the supporting experimental methodology, type of exerted regulation (POSITIVE/NEGATIVE and DIRECT/INDIRECT), the annotation source of the lncRNA and variant information (if available).

8. Select the “(i)” button in the “Var” column to access further information for known variants residing in the miRNA binding site.

9. Follow the link to the UCSC genome browser in the interaction pane of Z93241.1 – miR-34a-5p.

10. Follow the link to DIANA-TarBase (TB) to obtain the experimentally verified protein-coding targets of miR-34a-5p.

### **Query free search**

11. Remove all queries and select *Homo Sapiens* “Species”, Luciferase Reporter Assay “Method” and Brain “Tissue” to retrieve all miRNA-lncRNA interaction identified in the Brain by Luciferase Reporter Assay.



12. Perform a new search based on genome location. Select “Location” instead of “Keyword” in the search type options. Reset filters and type in the Location field chr21:1-24547942. On the “Based On” field select “Transcripts”. Execute Search.

13. Filter the results by selecting Method: “PAR-CLIP”, miRNA confidence level: “High” and press “Execute Search”.

14. TEKT4P2 gene residing in chr21 is targeted by several miRNAs. Explore the expression of the TEKT4P2 gene by clicking on “lncRNA expr” link and switching to LncBase expression module.

15. How many transcripts have available expression information? Press the downward-pointing arrow in each transcript pane to access further information. The revealed pane shows Transcripts Per Million (TPM) values, describing lncRNA abundance, along with tissue, cell type and replicates information.

16. Expression module can be utilized separately from interaction module to obtain lncRNA abundance information. Click on the “LncRNA Expression” button. Perform multiple lncRNA search by typing GAS5 and XIST in the lncRNA field. Execute Search.

17. Explore the localization of GAS5 by clicking the “Localization” button. Different transcripts of GAS5 are reported.

18. Expand the panel for the first transcript by clicking the downward arrow. Expression information and experimental details are provided. Information, including lncRNA expression TPM values both in nucleus and cytoplasm, RCI value (depicting inclination of lncRNAs to localize either towards the nucleus or cytoplasm respectively) and lncRNA localization tendency.

### **Visualization plots**

19. Explore the similarity of miRNA-gene interactions across different cell types. Select several cell types of interest and click “View” button. See the correlation between the different cell types.

20. Explore lncRNA expression across different cell lines and tissues. Type “GAS5” in the lncRNA field and select cell lines or tissues of interest. Click “View” button.

21. Explore lncRNA localization across different cell lines and tissues. Type “GAS5” in the lncRNA field and select cell lines or tissues of interest. Click “View” button. All GAS5 transcripts localize to the same cellular compartment? Is there a difference between different cell types?

# Thank you

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