

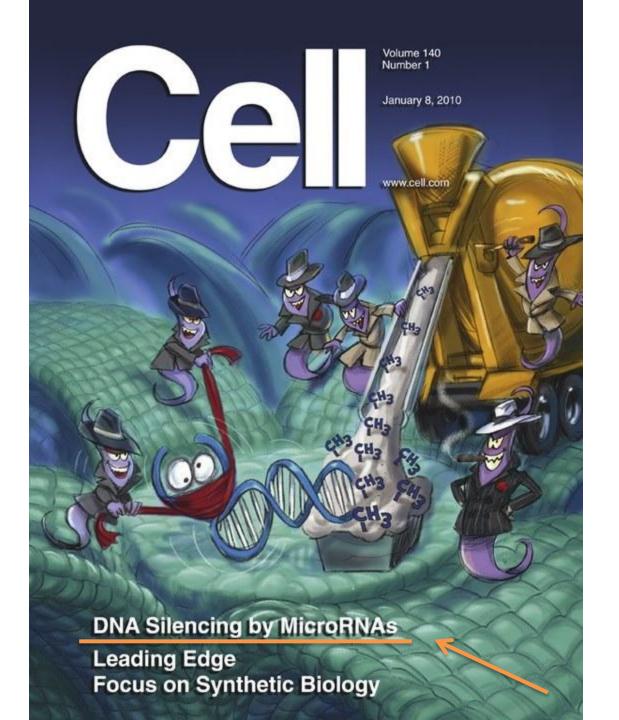






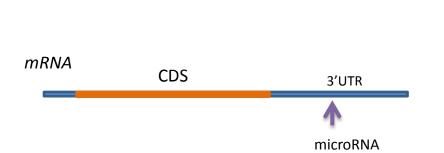
Group 11 Ma Ming

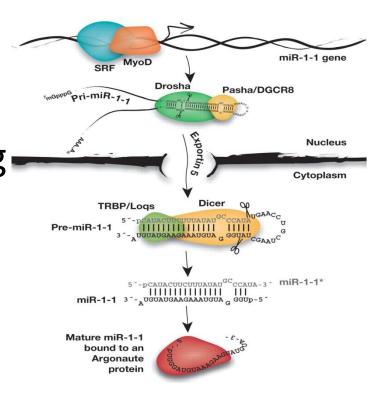




Background

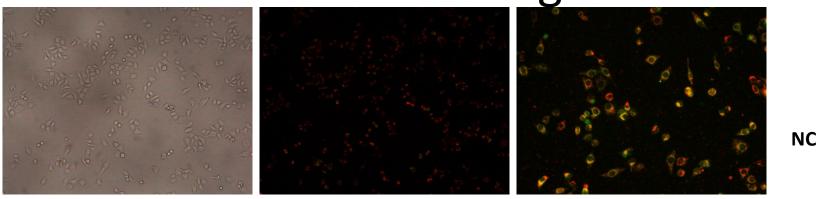
- What is microRNA
- Apoptosis
- High Throughput Screening



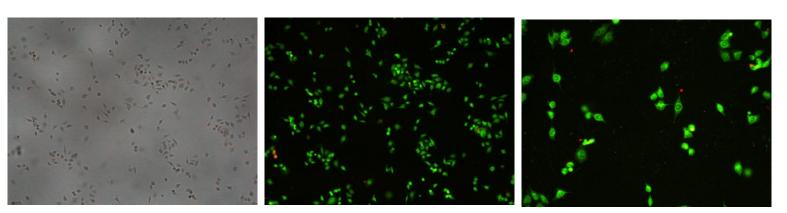


Apoptosis Screen Method:

JC-1 Staining



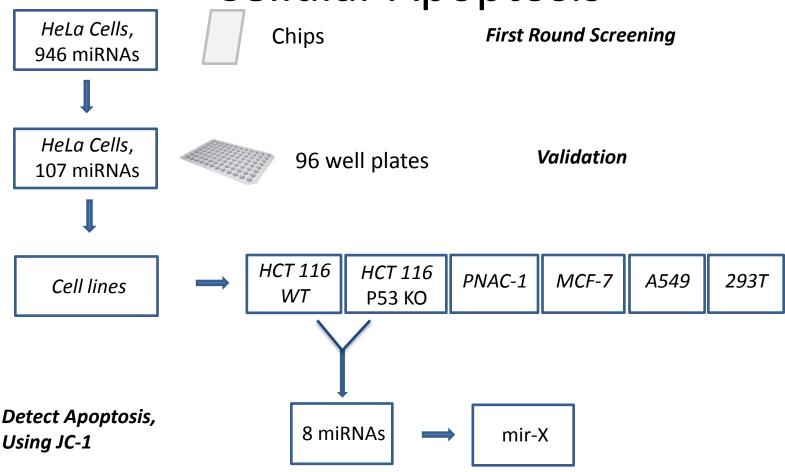
Control



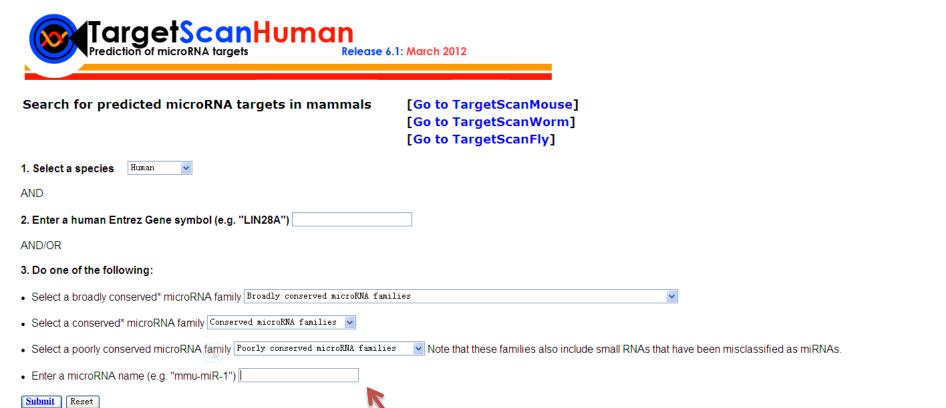
Apoptosis

Alcohol Induced

Screening of miRNAs that Promotes Cellular Apoptosis



How to find its Targets?



TargetScan predicts biological targets of miRNAs by searching for the presence of conserved 8mer and 7mer sites that match the seed region of each miRNA (ref. 1). As an option, nonconserved sites are also predicted. Also identified are sites with mismatches in the seed region that are compensated by conserved 3' pairing (ref. 2). In mammals, predictions are ranked based on the predicted efficacy of targeting as calculated using the context+ scores of the sites (ref. 3, 4). As an option, predictions are also ranked by their probability of conserved targeting (P_{CT}, ref. 2).

* broadly conserved = conserved across most vertebrates, usually to zebrafish

conserved = conserved across most mammals, but usually not beyond placental mammals



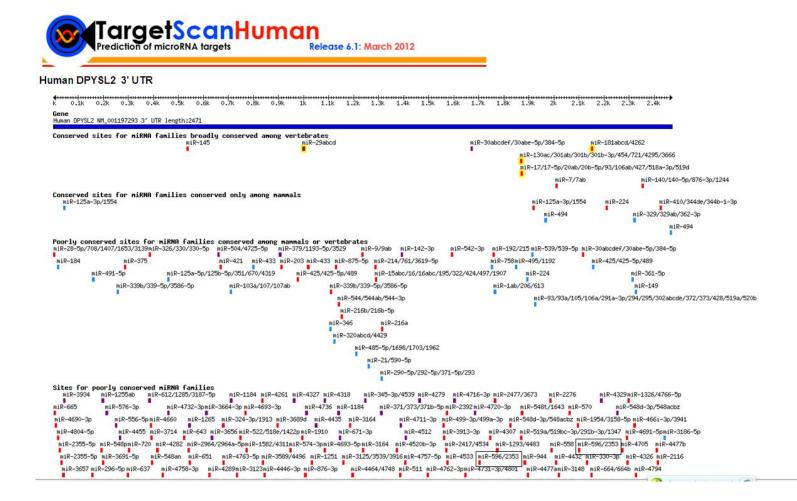
129 conserved targets, with a total of 130 conserved sites and 51 poorly conserved sites.

Table sorted by total context score

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

The table shows at most one transcript (with the longest 3' UTR) per gene. [Show all transcripts]

Target	Representative	Gene name		Conserved sites					onserv ites	/ed	Repre- sentative	Total context+	Aggregate	Links to	
gene	transcript	Gene name			7mer- m8	7mer- 1A	total	8mer	7mer- m8	7mer- 1A	miRNA	score	Рст	UTRs	
DPYSL2	NM_001197293	dihydropyrimidinase-like 2	1	0	1	0	2	0	2	0	hsa-miR-596	-0.61	N/A	Sites in UTR	١,
SMAD3	NM_001145102	SMAD family member 3	1	0	1	0	4	0	3	1	hsa-miR-596	-0.60	N/A	Sites in UTR	П
C18orf19	NM_001098801	chromosome 18 open reading frame 19	1	1	0	0	2	0	0	2	hsa-miR-596	-0.56	N/A	Sites in UTR	į.
IGF2BP2	NM_001007225	insulin-like growth factor 2 mRNA binding protein 2	2	0	2	0	0	0	0	0	hsa-miR-596	-0.53	N/A	Sites in UTR	
RPP14	NM_001098783	ribonuclease P/MRP 14kDa subunit	1	1	0	0	0	0	0	0	hsa-miR-596	-0.53	N/A	Sites in UTR	
ST8SIA2	NM_006011	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2	1	1	0	0	0	0	0	0	hsa-miR-596	-0.51	N/A	Sites in UTR	
USP30	NM_032663	ubiquitin specific peptidase 30	1	0	1	0	2	0	1	1	hsa-miR-596	-0.50	N/A	Sites in UTR	
ATP1A2	NM_000702	ATPase, Na+/K+ transporting, alpha 2 polypeptide	1	1	0	0	0	0	0	0	hsa-miR-596	-0.49	N/A	Sites in UTR	
RELA	NM_001145138	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	1	0	1	0	1	0	1	0	hsa-miR-596	-0.46	N/A	Sites in UTR	
PPP1R3A	NM_002711	protein phosphatase 1, regulatory (inhibitor) subunit 3A	1	1	0	0	0	0	0	0	hsa-miR-596	-0.45	N/A	Sites in UTR	
CNRIP1	NM_015463	cannabinoid receptor interacting protein 1	1	0	1	0	1	0	1	0	hsa-miR-596	-0.45	N/A	Sites in UTR	
NEGR1	NM_173808	neuronal growth regulator 1	1	0	1	0	1	0	0	1	hsa-miR-596	-0.44	N/A	Sites in UTR	
CBX2	NM_005189	chromobox homolog 2	1	0	1	0	1	1	0	0	hsa-miR-596	-0.42	N/A	Sites in UTR	
RBM19	NM_001146699	RNA binding motif protein 19	1	0	1	0	2	0	1	1	hsa-miR-596	-0.41	N/A	Sites in UTR	
SRSF7	NM_001031684	serine/arginine-rich splicing factor 7	1	0	1	0	1	0	0	1	hsa-miR-596	-0.39	N/A	Sites in UTR	Ĺ
NFYC	NM_001142587	nuclear transcription factor Y, gamma	1	1	0	0	0	0	0	0	hsa-miR-596	-0.39	N/A	Sites in UTR	
ZNF516	NM_014643	zinc finger protein 516	1	0	1	0	1	0	1	0	hsa-miR-596	-0.38	N/A	Sites in UTR	
HAO1	NM_017545	hydroxyacid oxidase (glycolate oxidase) 1	1	1	0	0	0	0	0	0	hsa-miR-596	-0.38	N/A	Sites in UTR	
PFKFB2	NM_001018053	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	1	1	0	0	0	0	0	0	hsa-miR-596	-0.36	N/A	Sites in UTR	
C19orf26	NM_152769	chromosome 19 open reading frame 26	1	1	0	0	0	0	0	0	hsa-miR-596	-0.36	N/A	Sites in UTR	Ĺ
C5orf13	NM_001142474	chromosome 5 open reading frame 13	1	0	1	0	0	0	0	0	hsa-miR-596	-0.34	N/A	Sites in UTR	
BL CAP	NM_001167820	hladder cancer associated protein	1	1	0	0	0	0	0	r_	h	0.01	N/A	Sites in UTR	



Conserved

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site- type contri- bution	3' pairing contri- bution	local AU contri- bution	position contri- bution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	P _{CT}
Position 2096-2102 of DPYSL2 3' UTR	5'AACCUGACGAUUUCU <mark>GCAGGCU</mark> G	7mer-	-0.120	0.003	-0.037	-0.009	-0.006	-0.083	-0.25	89	2.016	N/A
hsa-miR-596	3' GGGCUCCUCGGCCCGUCCGAA	m8										

Context+ score and features that contribute to the context+ score are evaluated as in Garcia et al., 2011.

Conserved branch lengths and P_{CT} are evaluated as in Friedman et al., 2008.

Poorly conserved

	P	redicted consequential pairing of target region (top) and miRNA (bottom)		contri-	3' pairing contri- bution	contri-	contri-	TA contribution		context+ score	context+ score percentile	conserved branch length	P _{CT}
Position 679-685 of DPYSL2 3' UTR hsa-miR-596	5' 3'	GCUGUUGAUAAAGGGGCAGGCUU GGGCUCCUCGGCCCGUCCGAA	7mer- m8	-0.120	0.003	0.045	0.034	-0.006	-0.083	-0.13	45	0.193	N/A
Position 1700-1706 of DPYSL2 3' UTR hsa-miR-596	5' 3'	AGCAAUGUUGUAUUUGCAGGCUU GGGCUCCUCGGCCCGUCCGAA	7mer- m8	-0.120	0.040	-0.105	0.047	-0.006	-0.083	-0.23	84	1.194	N/A

Context+ score and features that contribute to the context+ score are evaluated as in Garcia et al., 2011.

Mutant Design



Human TP53 3' UTR Human TP53 NM_001126114 3' UTR length:1496 Conserved sites for miRNA families broadly conserved among vertebrates let-7/98/4458/4500 miR-22/22-3p [Show conserved sites for miRNA families conserved only among mammals] Sites with higher probability of preferential conservation

Show poorly conserved sites for miRNA families conserved among mammals or 8mer 7mer-m8 7mer-1A 3' comp* [Show sites for poorly conserved miRNA families] Sites with lower probability of preferential conservation

[View SVG image of miRNA sites] [View table of miRNA sites]

View human genome browser (Feb 09)]										
510	520	530	540		550	560.	570.		58	0	.590
Hsa AGAUUUU-AAG-	-GUUUUU-A	CUGUGAGGGAUGU	UUGGGAG	-A	-UGUAAG-AI	AAUGUUCUUG	CAGUUAAGGGU	UA	GUUUA	CAAUCAGO	CACA
Ptr AGAUUUU-AAG-	-GUUUUU-A	CUGUGAGGGAUGU	UUGGGAG	A	UGUAAG-A	AAUGUUCUUG	CAGGUAAGGGU	UA	GUUUA	CAAUCAGO	CCACA
Mml AGAUUUU-AAG-	-GUUUUU-A	CUGUGAGGGAUGU	UUGGGAG	A	UGUAAG-A	AAUGUUCUUG	CAGGUAAGGGU	UA	GUUUA	CAAUCAGO	CCACA
OgaAG-	-GUUUUU-A	CUAUGAGGAAUAU	UUGGAAG	-A	GGUAAGGA	AAUGUUCUUGA	AGUUUAAGGGU	UA	GAUUUUA	AGUCAGO	CCAUA
Tbe GGCUUUU-AAU-							JGUGGAAGCGU	UA	GGUUUGA	AACUGGO	CAUA
Mmu											
Rno											
Cpo AGUUUUU-AAG-	-GUUUUUUZ	ACCAUGAGCAGAGU	UAGGAC	A	GAGAAGAGI	AAUGUUCUUG	CAUAUAAAGAU	CA	GAGUUUA	CAAUCAGO	CCAUA
Ocu AGCUUUU-AAA-	-AUUUUG-A	CUGUGAGGAAUGU	UUGGGGAAAUU	LAA	GAAAAAAA	AAAAGGCUC	UG		A0	AAGCAG	CCCUA
Sar CGCUUUU-AAU-	-GUUUUU-A	CUGUGAGGAA-AU	UUGGGAG	A	GGUAGAAA	AAGGUUCUUGU	JAUGUAAGAAC	AG	UCUAG	AAU	A
Eeu AACUUGAAGGUUU-UUU-	-UUUUUUU-A	ACCCUGAGAAACAU	UUGGGAG	-AACUAGGA	AGAUAAGAA	AAGAUUUCUA	CACACAAGAAA	UGAUUUAGG:	AAGGAUCUAG	AAACAGO	CAAA
Cfa GGUUUUU-AAG-	-0000000-2	CUGUGUAGA-ACC	AUGGGAG	A	GGUAGGAAI	AAUGUUCCUGA	AAUGUUGGGAG	CA	JAUUUU	JAGCCCCU	JAAGA
Fca AGUUUUU-UAGG	AUUUUUU-A	CUAUGAGGA-ACC	GUGAGGG	A	GGUAGGAGI	AAUGUUCCUGO	CCUAUAAAGAG	CA	UUCUAG	AACCAAU	JCAUA
Eca GU											
Bta AGCUUUC-AAG-							CAUGUGAGGAA	CA	UCUUAU	JAACCAGO	CCAUA
Dno AGCUUUC-ACG-											
Laf CUAUUUUUAAG-											
Ete UCAGCUC		-UUACAAAGAACCC	UUGGGAG	C	GAUAGGAAI	UAUGGUCGUGO	CUUGUGAGAGU	UA	GCUUUUC	CAACCAGO	CUAUA
Mdo											

8mer 7mer-m8 7mer-1A 3' comp*



miRBase provides the following services:

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

Highlights, Web watch - Nature Reviews

Genetics 5:244 (2004)

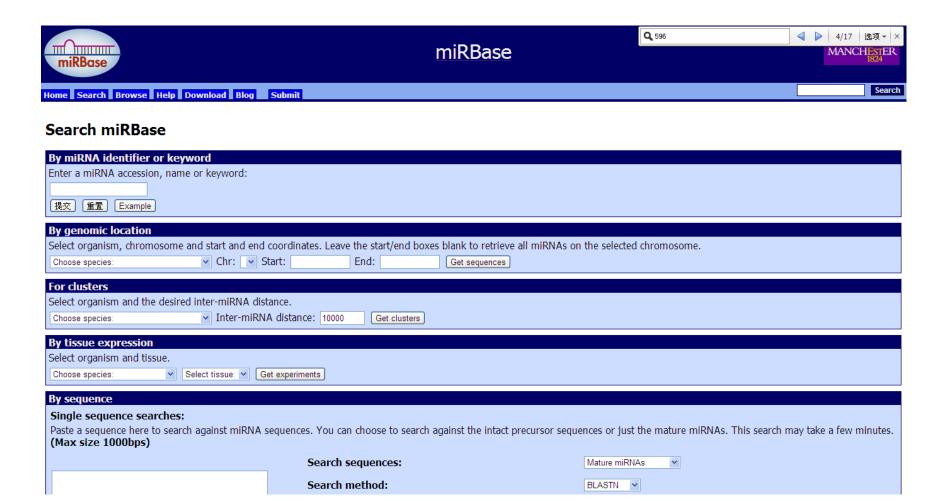
To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

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

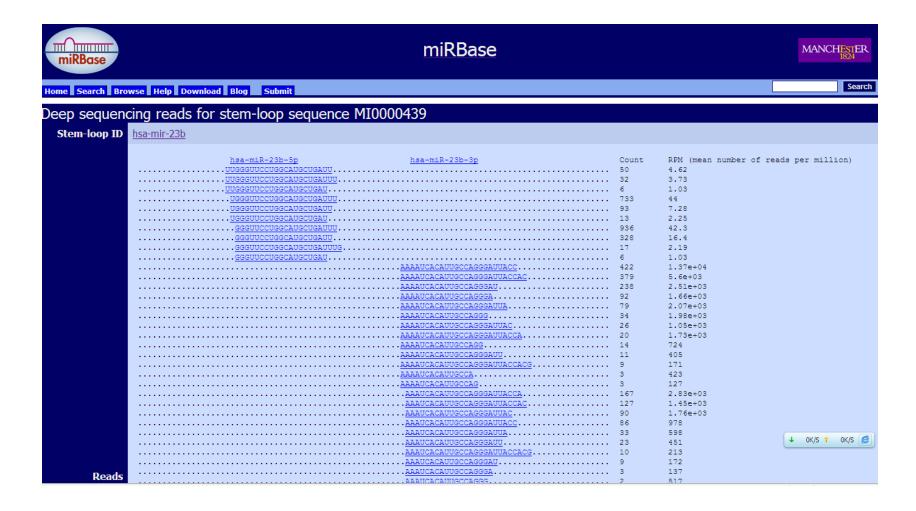
References

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

miDRaco: integrating microPNA apportation and doop-coguencing data









miRBase Sequence Download

- ► Go to the FTP site
- ► Previous releases

► README Release notes - read these first!

► <u>miRNA.dat</u> all published miRNA data in EMBL format

► <u>hairpin.fa</u> Fasta format sequences of all miRNA hairpins

► mature.fa Fasta format sequences of all mature miRNA sequences

► miRNA.diff Changes between the last release and this

► miRNA.dead List of entries that have been removed from the database

► <u>miFam.dat</u> Family classification of miRNA hairpin sequences

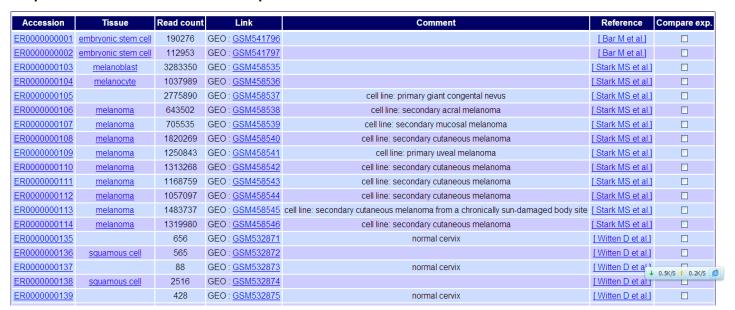
Genome coordinates

aae.gff3	ame.gff3	ath.gff3	bmo.gff3	bta.gff3	cbr.gff3	cel.gff3	cfa.gff3
cre.gff3	<u>dme.gff3</u>	dps.gff3	<u>dre.gff3</u>	ebv.gff3	<u>fru.gff3</u>	gga.gff3	<u>hcmv.gff3</u>
hsa.gff3	kshv.gff3	mdo.gff3	mghv.gff3	mml.gff3	mmu.gff3	osa.gff3	ptc.gff3
ptr.gff3	rno.gff3	sme.gff3	tae.gff3	tni.gff3	<u>vvi.gff3</u>	xtr.gff3	zma.gff3

Comments, questions? Email mirbase@manchester.ac.uk



9 experiments for all tissues in Homo sapiens





and gene-related to information to their on, the ental validation blogies and their

Gene name miRNA name Methods score

1 PRODH (Homo sapiens) hsa-miR-23b* R N W Q P M A D O - ^

Gene ^ details

Ensembl Gene ID: ENSG00000100033

Gene Name: PRODH
Refseq IDs: NM_016335

Description: Proline dehydrogenase, mitochondrial Precursor (EC 1.5.99.8)(Proline oxidase)(Proline

oxidase 2)(p53-induced gene 6 protein) [Source:UniProtKB/Swiss-Prot;Acc:O43272]

Pred.

Validated Targets

External links: UniProt

Kegg pathways: Arginine and proline metabolism

Metabolic pathways

Chromosome: 22

Transcirpts: ENST00000399694, ENST00000334029, ENST00000313755, ENST00000357068

miRNA details

Name: hsa-miR-23b*
Alternative description: MIMAT0004587

Related names: There are no related names for this entry.

miRNA sequence: UGGGUUCCUGGCAUGCUGAUUU

External links: miRBase
Related MeSH terms:

Adenocarcinoma Breast Neoplasms Carcinoma,

Hepatocellular Cardiomegaly Cell Transformation, Viral Cholangiocarcinoma Choline Deficiency Colonic Neoplasms Cytomegalovirus Infections

Disease Models, Animal Disease Progression

Endometriosis Folic Acid Deficiency Hypertrophy, Left Ventricular

Kidney Neoplasms Leiomyoma Leukemia, Myeloid, Acute Liver Neoplasms Melanoma Neoplasm Metastasis

Neoplasms Nevus, Pigmented Precursor Cell Lymphoblastic

Leukemia-Lymphoma Prostatic Neoplasms Reperfusion Injury Urinary Bladder

Neoplasms Uterine Neoplasms Ventricular Remodeling

Authors Year Methods Regulation Valid. type Region

Liu W et al. 2010 R N W O P M A D O not specified UNKNOWN UNKNOWN \

DNA Intelligent Analysis

'ALEXANDER: HOME SOFTWARE DATABASES MEMBERS PUBLICATIONS HELP CSLab Q ? Threshold: 0.3 Please cite: M. Maragkakis, T. Vergoulis, P. Alexiou, M. Reczko, K. Plomaritou, M. Gousis, K. Kourtis, N. Koziris, T. Dalamagas, AG. Hatzigeorgiou DIANA-microT Web server upgrade supports Fly and Worm miRNA target prediction and bibliographic miRNA to disease association. Nucleic Acids Res. 2011 Jul;39(Web Server issue):W145-8 Results: 1668 targets for miRNAs hsa-miR-23b . Threshold is set to 0.3. ires are aistered **Predicted Targets** Page 1 Ensembl Gene Id miRNA name miTG score SNR Precision Also Predicted ENSG00000095015 hsa-miR-23b 0.881 5.8 0.9 (MAP3K1) ENSG00000108256 hsa-miR-23b 0.871 5.8 0.9 count? (NUFIP2) ere!, or ENSG00000113615 ng the 5.8 hsa-miR-23b 0.851 0.9 (SEC24A) ENSG00000196323 hsa-miR-23b 0.829 5.8 0.9 (ZBTB44) ENSG00000090905 hsa-miR-23b 0.826 5.8 0.9 (TNRC6A) ENSG00000173698 hsa-miR-23b 5.8 0.9 v 6 0.825 (GPR64) ENSG00000148200 hsa-miR-23b 0.813 5.8 0.9 v (NR6A1) ENSG00000137872 hsa-miR-23b 0.7985.8 0.9 v (SEMA6D) ENSG00000113742 v hsa-miR-23b 0.797 5.8 0.9 (CPEB4) ↓ 43 ENSG00000110497

0.795

5.8

0.9

hsa-miR-23b

(AMBRA1)

Guidance for Further Research

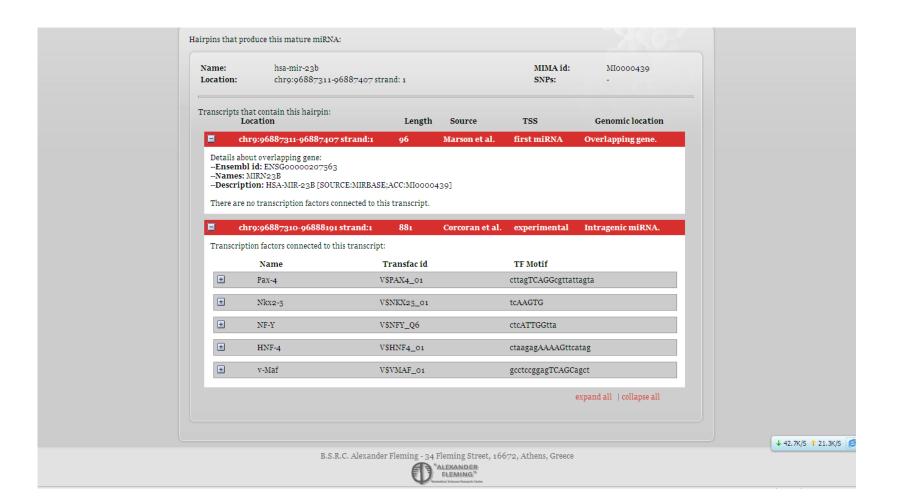
DIANA mirPath



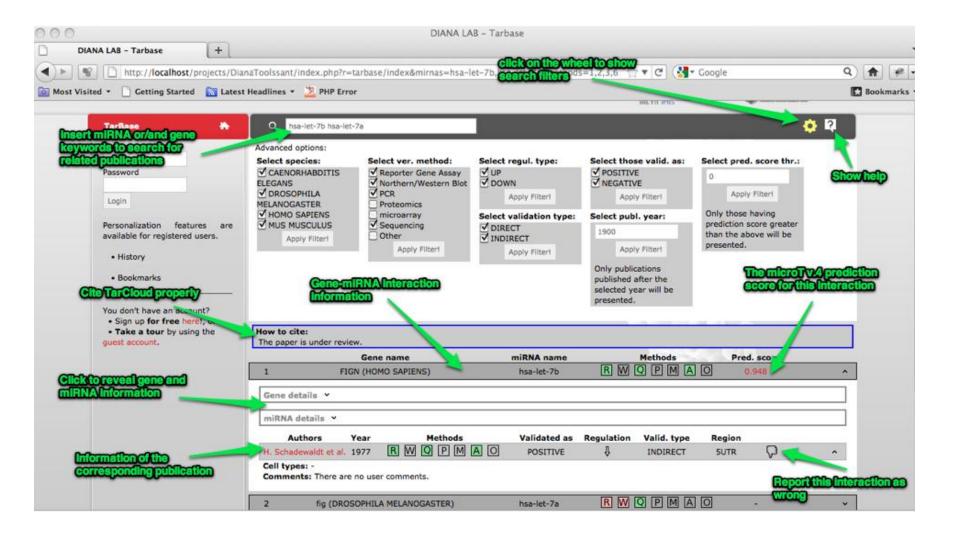
DIANA mirPath



DIANA mirGen



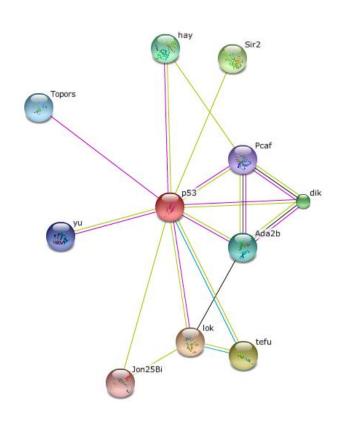
DIANA Tarbase



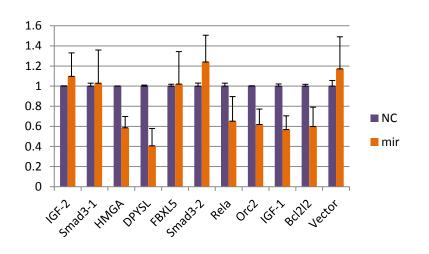
Selection of Candidates

- Genes related with p53
- Tools:
 - String
 - Google scholar

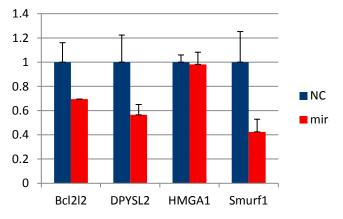
Ten Genes are selected



Further Experiments Validation



Luciferase Experiment



Realtime Detection

