

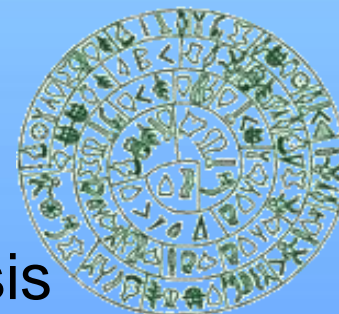


Application (MiRs): A new microRNA target prediction tool identifies a novel interaction of a putative miRNA with CCND2



UOC
Medical
Division

Anastasis Oulas, Nestoras Karathanasis
Annita Louloui, Ioannis Iliopoulos, Kriton
Kalantidis, Panayiota Poirazi



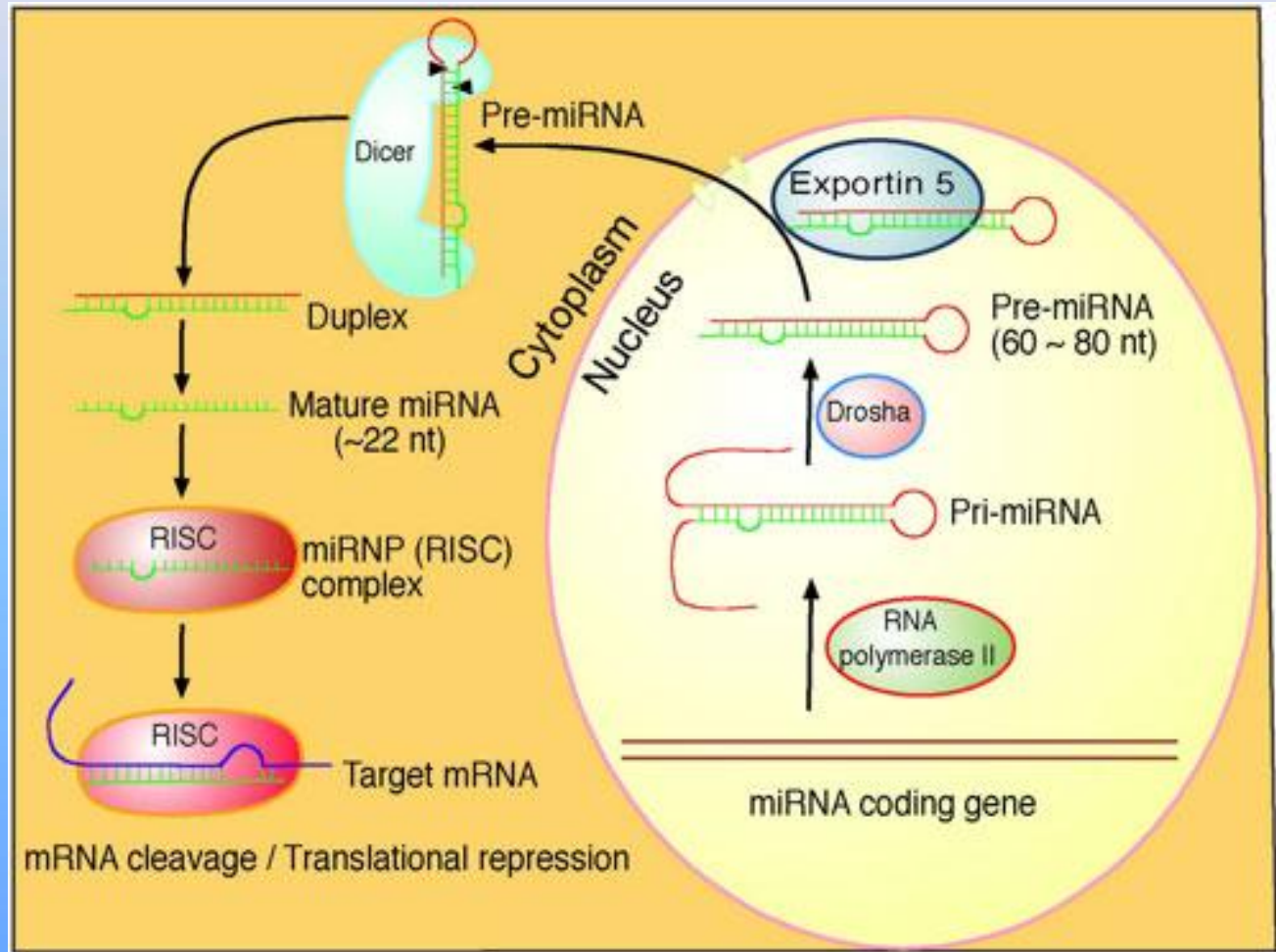
IMBB
FORTH

Talk outline



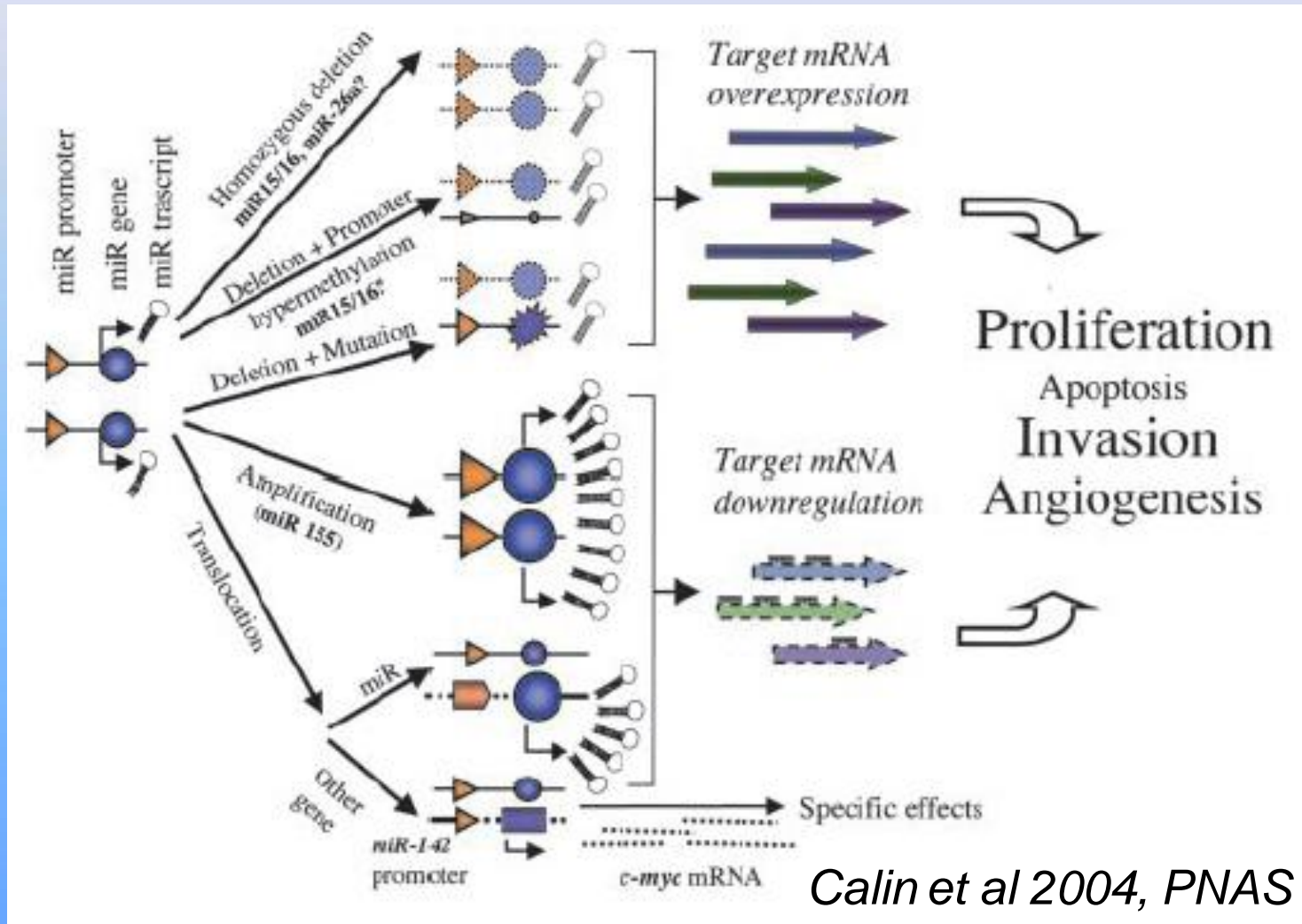
- **A new microRNA target prediction tool identifies a novel interaction of a putative miRNA with CCND2**
 - **Intro on miRNAs & Cancer associated miRs**
 - **Improving miRNA Target Prediction**
 - **Back to the lab**
 - **Conclusion and Future work**

Biogenesis of microRNAs

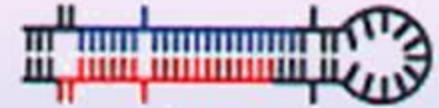


Complementary
base-pair
binding
Highly specific!

MiRNAs and Cancer Associated Genomic Regions (CAGRs)



Known MiRNAs in CAGRs



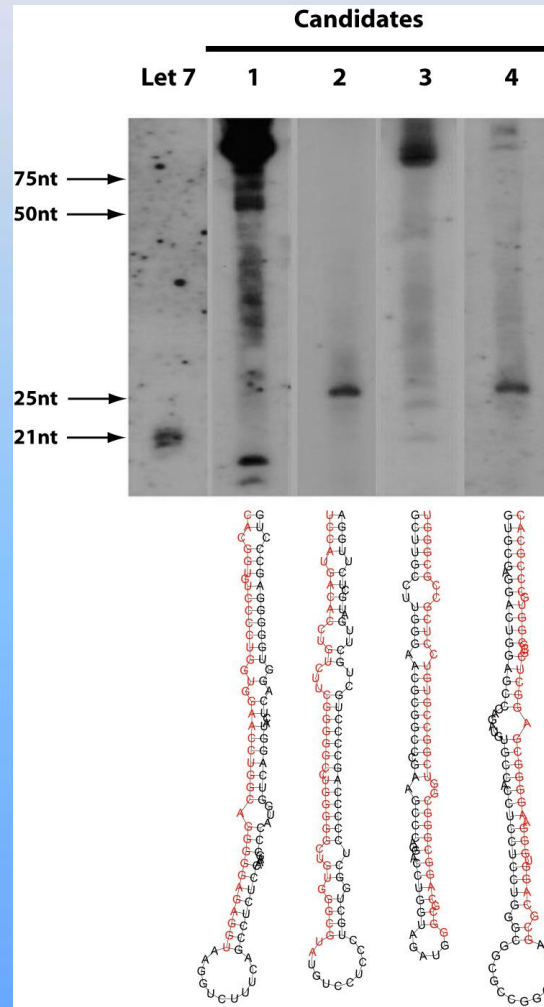
CAGR = minimal deleted regions, minimal amplified regions, and breakpoint regions involved in human cancers.

Chromosome	Location (defining markers)	Size, Mb	miR	Hystotype	Known OG/TS
3p21.1–21.2-D	ARP-DRR1	7	<i>let-7g/miR-135-1</i>	Lung, breast cancer	—
3p21.3(AP20)-D	GOLGA4-VILL	0.75	<i>miR-26a</i>	Epithelial cancer	—
3p23–21.31(MDR2)-D	D3S1768-D3S1767	12.32	<i>miR-26a; miR-138-1</i>	Nasopharyngeal cancer	—
5q32-D	ADRB2-ATX1	2.92	<i>miR-145/miR-143</i>	Myelodysplastic syndrome	—
9q22.3-D	D9S280-D9S1809	1.46	<i>miR-24-1/miR-27b/miR-23b; let-7a-1/let-7f-1/let-7d</i>	Urothelial cancer	PTC, FANCC
9q33-D	D9S1826-D9S158	0.4	<i>miR-123</i>	NSCLC	—
11q23-q24-D	D11S927-D11S1347	1.994	<i>miR-34a-1/miR-34a-2</i>	Breast, lung cancer	PPP2R1B
11q23-q24-D	D11S1345-D11S1328	1.725	<i>miR-125b-1/let-7a-2/miR-100</i>	Breast, lung, ovary, cervix cancer	—
13q14.3-D	D13S272-D13S25	0.54	<i>miR-15a/miR-16a</i>	B-CLL	—
13q32–33-A	stSG15303-stSG31624	7.15	<i>miR-17/miR-18/miR-19a/miR-20/ miR-19b-1/miR-92-1</i>	Follicular lymphoma	—
17p13.3-D	D17S1866-D17S1574	1.899	<i>miR-22; miR-132; miR-212</i>	HCC	—
17p13.3-D	ENO3-TP53	2.275	<i>miR-195</i>	Lung cancer	TP53
17q22-t(8;17)	miR-142s/c-MYC		<i>miR-142s; miR-142as</i>	Prolymphocytic leukemia	c-MYC
17q23-A	CLTC-PPM1D	0.97	<i>miR-21</i>	Neuroblastoma	—
20q13-A	FLJ33887-ZNF217	0.55	<i>miR-297-3</i>	Colon cancer	—
21q11.1-D	D21S1911-ANA	2.84	<i>miR-99a/let-7c/miR-125b</i>	Lung cancer	—

D, deleted region; A, amplified region; NSCLC, non-small-cell lung cancer; HCC, hepatocellular carcinoma; PTC, patched homolog (*Drosophila*); FANCC, Fanconi anemia, complementation group C; PPP2R1B, protein phosphatase 2, regulatory subunit A (PR 65), β isoform, miRs in a cluster are separated by a slash. For references, see Table 6.

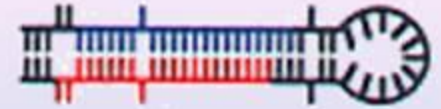
Calin et al 2004, PNAS

Four novel miRNA gene candidates



Oulas, et al, 2009, Nucleic Acid Research.

Tumour Suppressors?



Candidate	Candidate Information ^[1]	CAGR	Type of Cancer	Closest miRNA	Expression in HeLa
1	chr9:123327358-123327460 st-	chr9:121 153509-128793509	bladder ca	miR-181a; miR-199b	1667.5
2	chr5:148958951-148959053 st-	chr5:144 121683-156051683	prostate ca aggressiveness	miR-145/miR-143	363.5
2	chr5:148958951-148959053 st-	chr5:148 181683-151101683	myelodysplastic syndrome	miR-145/miR-143	363.5
3	chr22:40863894-40863996 st+	chr22:31 530000-43583971	colorectal ca,	miR-33a	345.0
3	chr22:40863894-40863996 st+	chr22:31 530000-42193557	astrocytomas	miR-33a	345.0
4	chr5:149984684-149984786 st-	chr5:144 121683-156051683	prostate ca aggressiveness	miR-145/miR-143	264.0
4	chr5:149984684-149984786 st-	chr5:148 181683-151101683	myelodysplastic syndrome	miR-145/miR-143	264.0

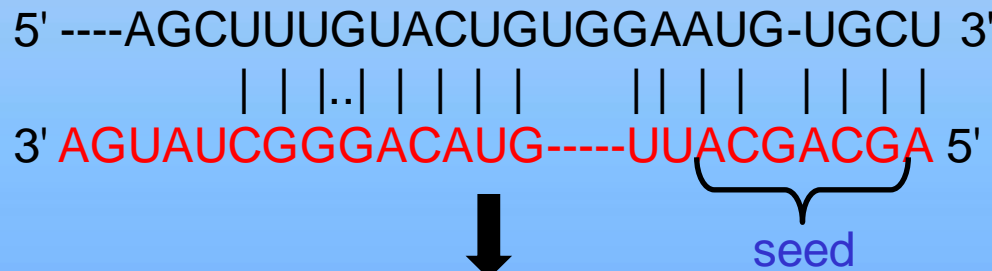
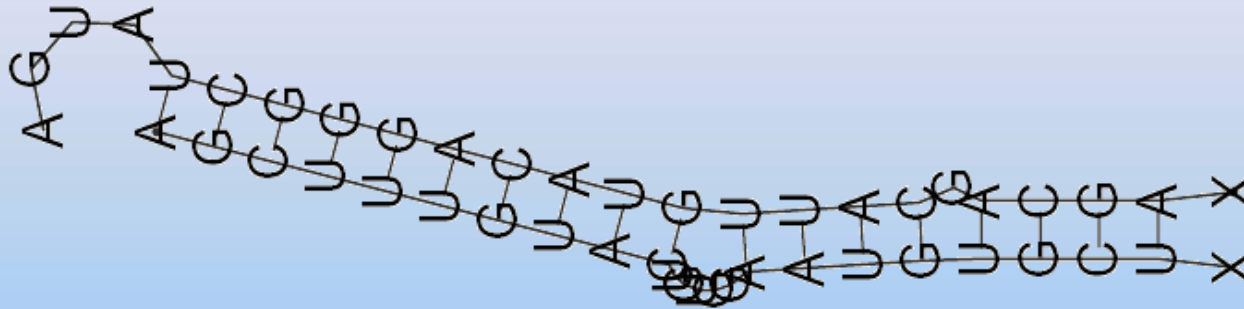
^[1] Chromosomal location and st, strand (+ or -)

Talk outline



- **A new microRNA target prediction tool identifies a novel interaction of a putative miRNA with CCND2**
 - Cancer associated miRs
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Methodology – RNAcofold to HMM



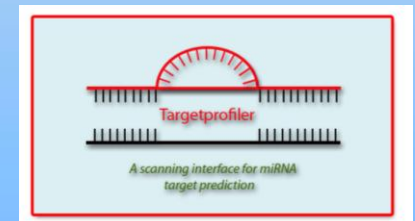
LLLLMMMFFMMMMMLLLLLMMMMLMMMM



TRAIN HMM

Multiple
Sequence
Alignment

Java



Targetprofiler

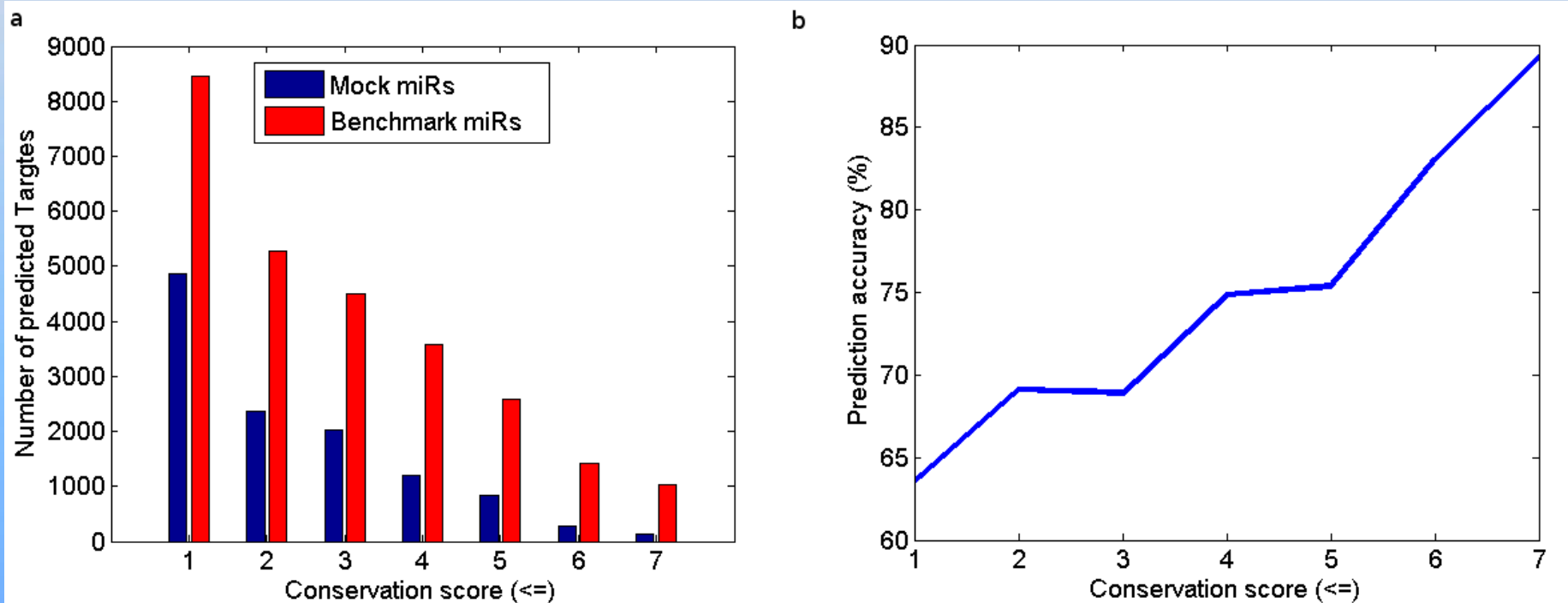
Additional
features used:

Conservation of
target site



Tool Validation

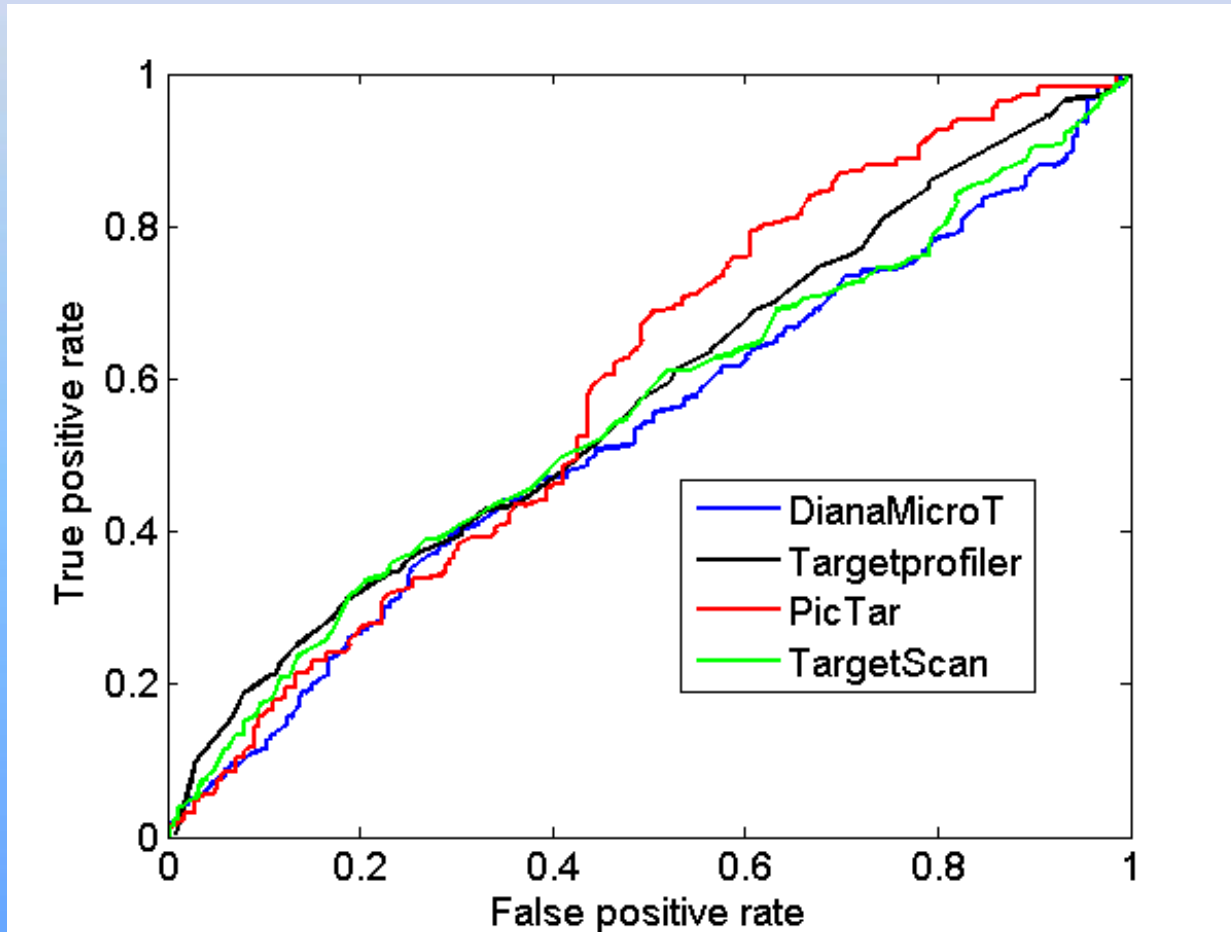
- Mock miRNA vs 5 Benchmark miRNA – conservation score



Tool Validation



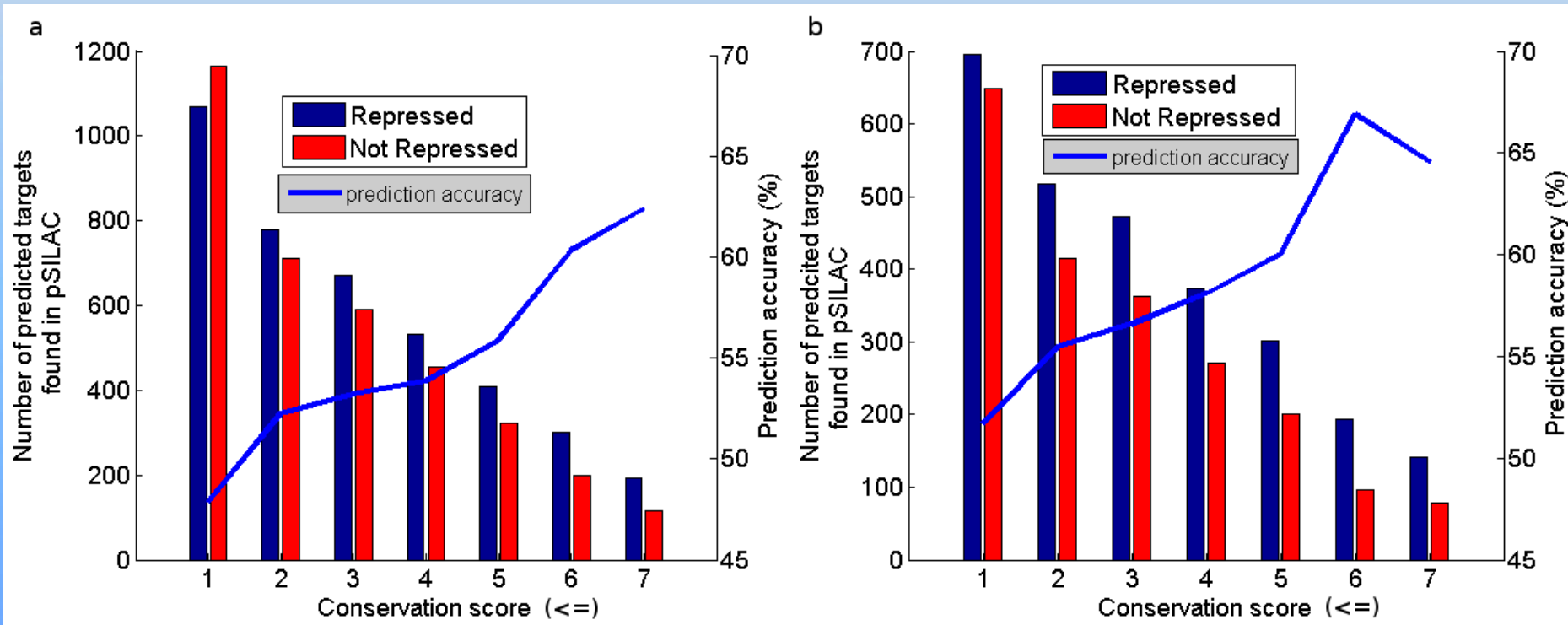
- ROC curve comparing Targetprofiler with 3 other tools



Tool Validation



- Using large scale protein repression (Selbach et,al, Nature 2008) assay to validate predictions



Tool Comparison



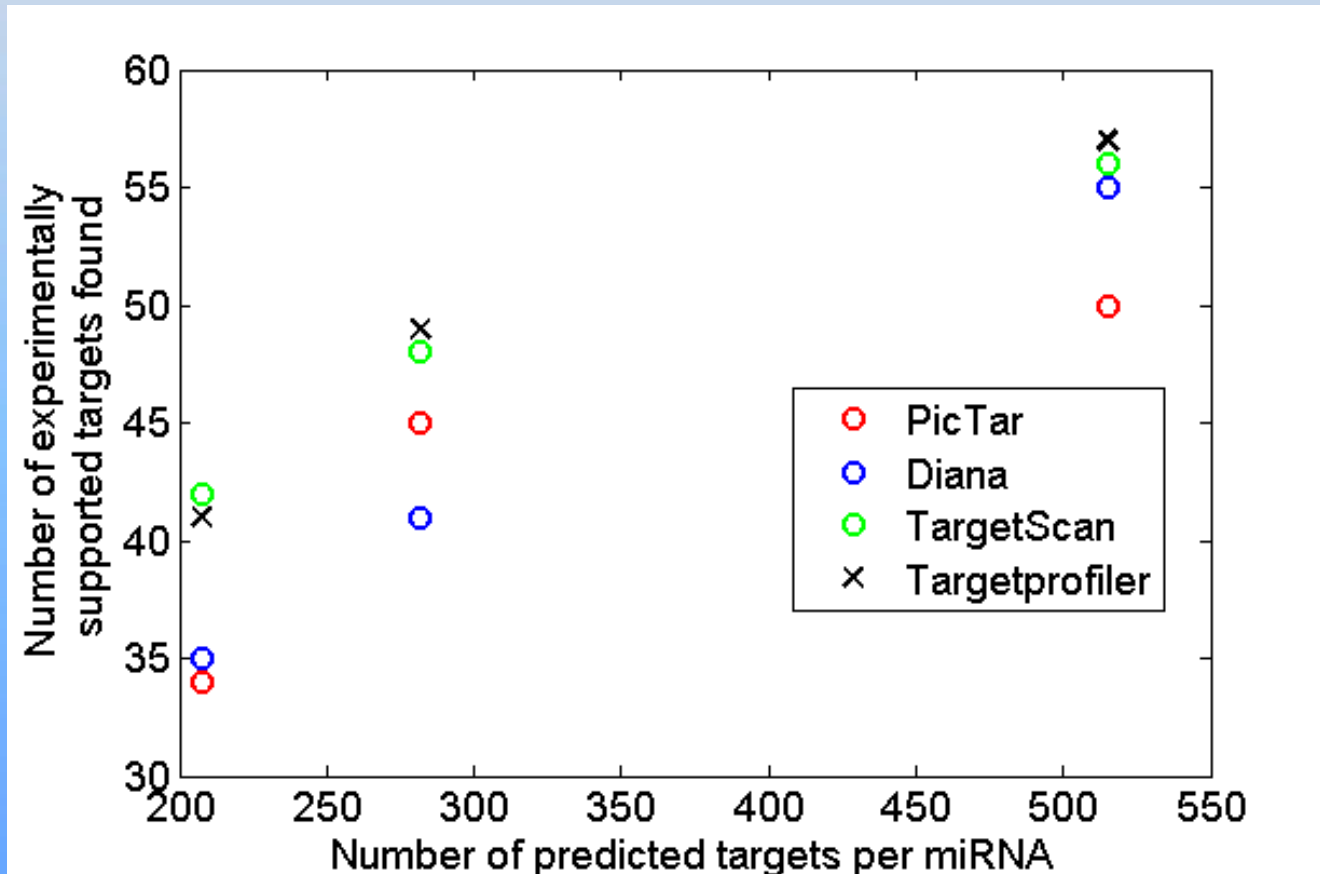
Correlation of target predictions with changes in protein production for five miRNAs (*miR-1*, *miR-16*, *miR-30a*, *miR-155*, *let7b*)

Prediction algorithm	Number of predicted targets mapped to Refseq	Number of targets measured by pSILAC	Number of down-regulated targets (log ₂ FC <-0.1)	Fraction of down-regulated targets (log ₂ FC <-0.1)
TargetScanS	2842	622	381	61.25%
PicTar	3289	629	386	61.37%
rna22 on 3'UTRs	4112	723	255	35.27%
rna22 on 5'UTRs	607	79	20	25.32%
PITA to 600	3000	325	139	42.77%
PITA top 1000	5000	572	226	39.51%
miRbase	3347	658	288	43.77%
miRanda	8605	1533	715	46.64%
Diana-MicroT 3.0	1678	294	194	65.99%
TargetProfiler	1879	290	194	66.90%

Tool Validation



- Comparison of Targetprofiler vs. 3 other tools using experimentally verified miRNAs

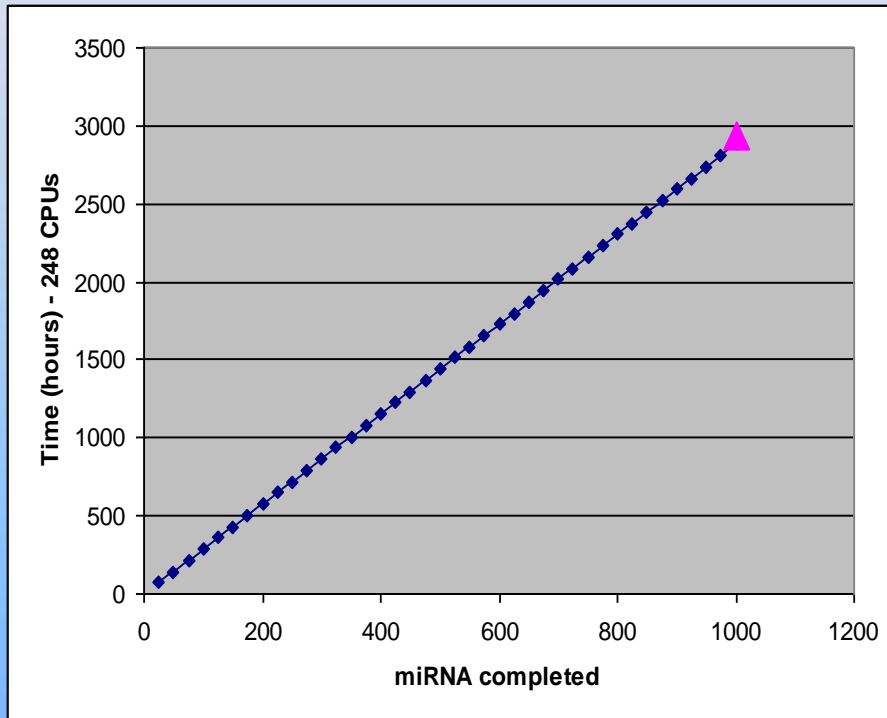


Scanning for human miRNA targets



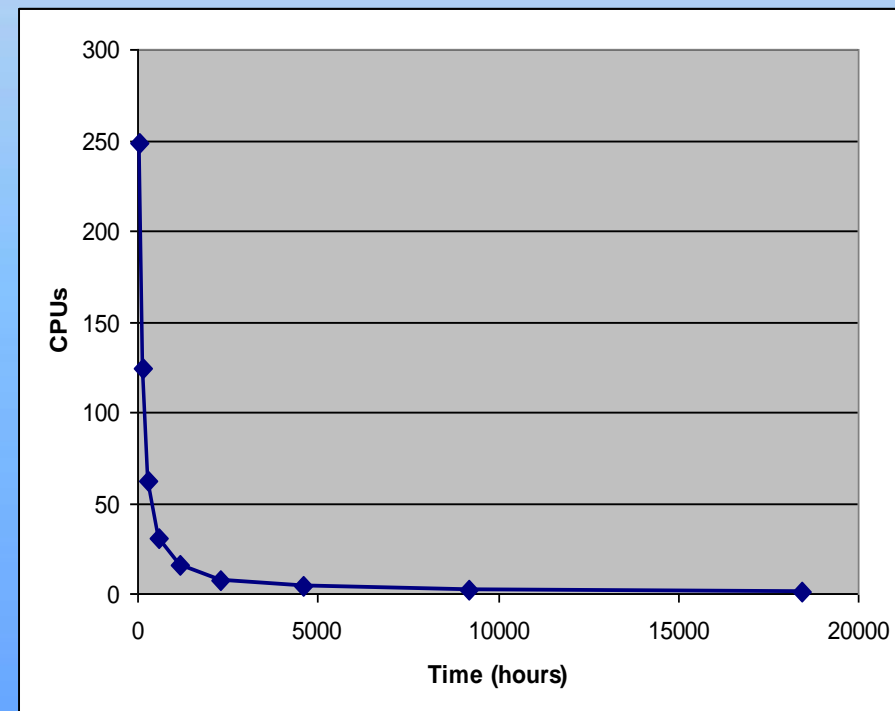
- ~30,000 3'UTRs
- ~1000 miRNA genes
- Home system: IFIN_Bio
- Achieved scalability: 31 nodes – 248 CPUs
- Serial application split in parametric processes using pbs (job manager).
- Completion of the full job is reversely proportional to the number of CPUs utilized.

Progress of application – Production ready

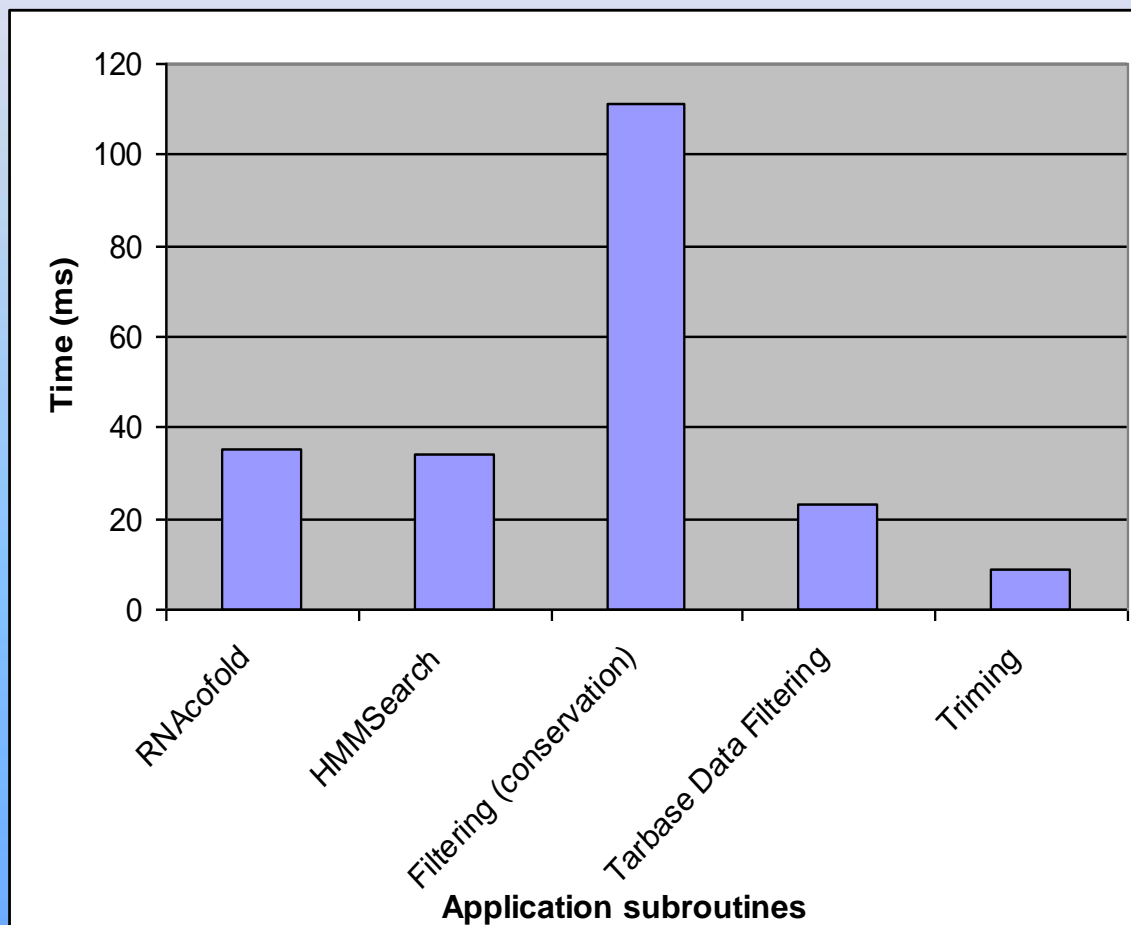


▲ status
of application
so far -
completed

CPU usage



Profiling and performance studies



•Results show the time in ms needed for one miRNA to scan one target site applying all the subroutines of the application

Scanning for human miRNA targets

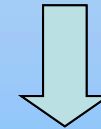


TargetProfiler flowchart

Scanning 3'UTRs
using sliding
window



- Filtering:
 - Conserved in seed region
 - Thermodynamics (ΔG)



- Trained HMM
model
score >3.0



- Candidate
miRNA target
(Experimental Verification)



IFIN_Bio

PC cluster

Targetprofiler on the Web



- MySQL Database store of data from HP-SEE infrastructure runs.
- Search by Human miRNA.
- Search by Human gene.
- User flexibility in specifying filtering parameter.
- Additional feature - search by RNA sequence or miRNA sequence of interest.

<http://mirna.imbb.forth.gr/Targetprofiler.html>



Targetprofiler

A scanning interface for miRNA target prediction

Targetprofiler is running on a server at the Computational Biology Lab at the Institute of Molecular Biology and Biotechnology(IMBB), Heraklion, Greece.

toggle between search by ID or Sequence

Enter an Entrez gene ID (i.e. CCND2):

Enter a microRNA ID (i.e. hsa-miR-182):

*You can search for a specific gene targeted by a specific miRNA by providing both gene and miRNA ID.

Conservation Threshold:

HMM Threshold:

Energy Threshold:

Oulas et, al, RNA Biology 9:9, 1–12; September 2012

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HP-SEE Forum - Belgrade,
17-19, Oct, 2012



High scoring target predictions



Target Type	HMM Score	Location (chr:start-end)	Sequence (targetX&XmiRNA)	Bracket notation	Conservation score	Strand
7mer-m8	6.2	10:6019369-6019401	GAAGAGGACACCAGCCCAA GCUGGACCUGCCAUX &XCUGGCAGGGGGAG AGGUA	.(((....((...((((((..&..))))))..)).)))	7	1
8mer	6.2	11:26973747-26973779	GAGGUUCAAGGUGCUGCUU UGCAUGCCUGCCAAX & XCUGGCAGGGGGAGA GGUA((((....(.((((((..&..))))).-.))))	7	1
7mer-m8	6.2	1:156619755-156619787	AACCUUGUCAGCUUGCACCA UCCCACCUGCCACX & XCUGGCAGGGGGAGA GGUA(((....((((((..&..)))))))..))))	7	1
8mer	6.2	11:72794632-72794664	CUCAAAAGGUGAUUUUGUC CUUAGACCUGCCAAX & XCUGGCAGGGGGAGA GGUA(((....(((((..&..)))))))..))	7	-1
7mer-m8	6.2	12:3771033-3771065	CCCGCUGUUAACUGCAUA GGGCAGCCUGCCACX &XCUGGCAGGGGGAG AGGUA((((((..&..))))).....	7	-1
8mer	6.2	12:4282963-4282995	AGUGGGGGCCGAGUUGUUC CCCCAGCCUGCCAAX & XCUGGCAGGGGGAGA GGUA	(((....((((((..&..))))..))..))	7	1

Target Selection



- **miRNA**

Candidate miRNA	Candidate Information	CAGR	Type of Cancer	Closest miRNA	Expression in HeLa
miR-ch9	chr9:123327358-123327460 st-	chr9:121153509-128793509	bladder ca	miR-181a; miR-199b	1667.5

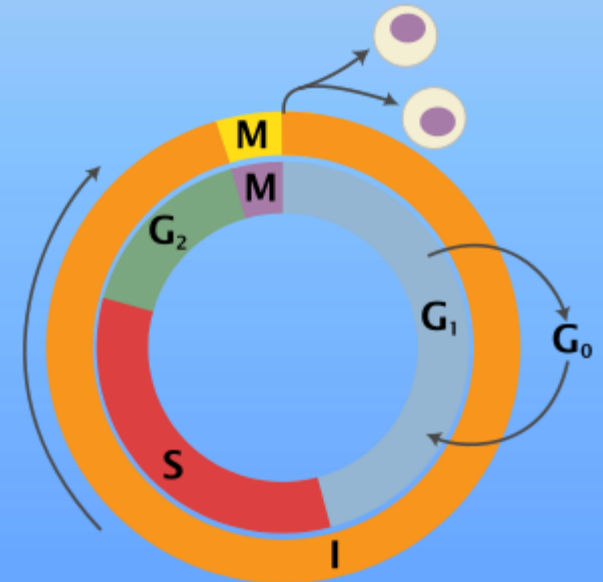
- **Target Gene**

- ***CCND2***

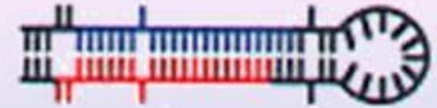
- **Aliases & Descriptions**

- cyclin D2

- G1/S-specific cyclin D2

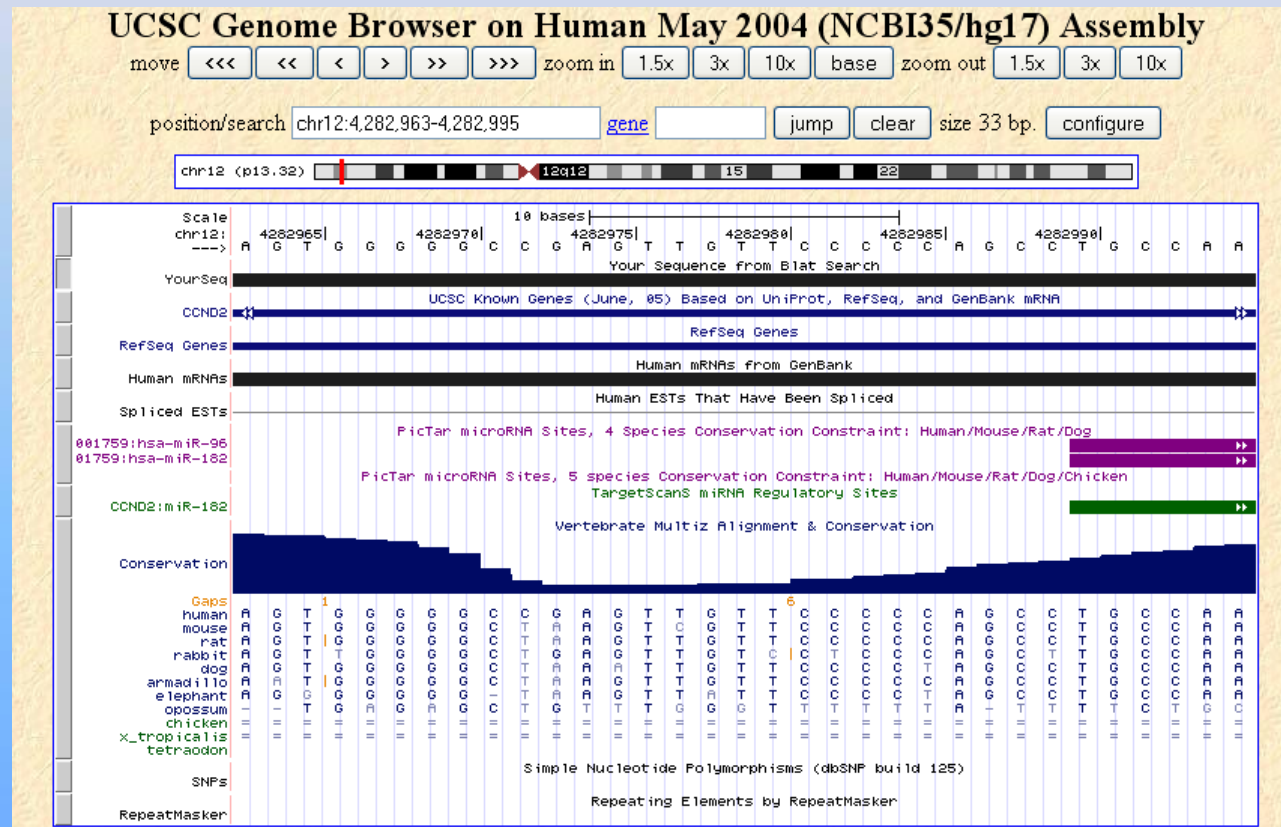


Site for known miRNAs

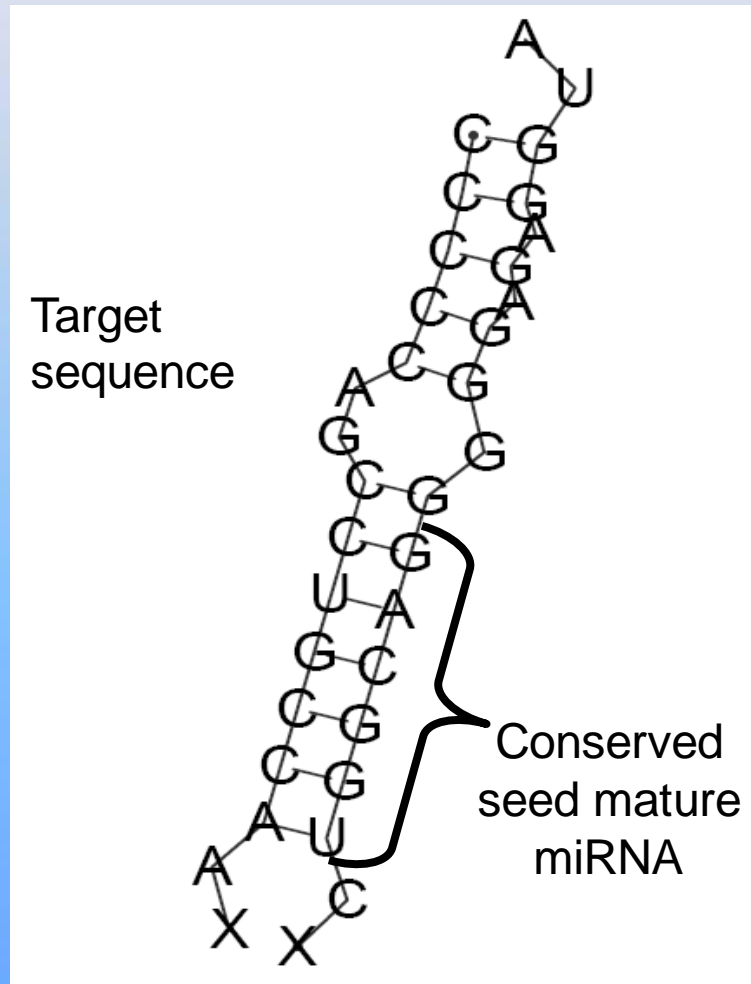


- PicTar
 - miR-96
 - miR-182

- TargetScan
 - miR-182
 - miR-96
 - miR-1271



Structure of miRNA:target-site



Constructs



- Triplet cassette preparation for cloning

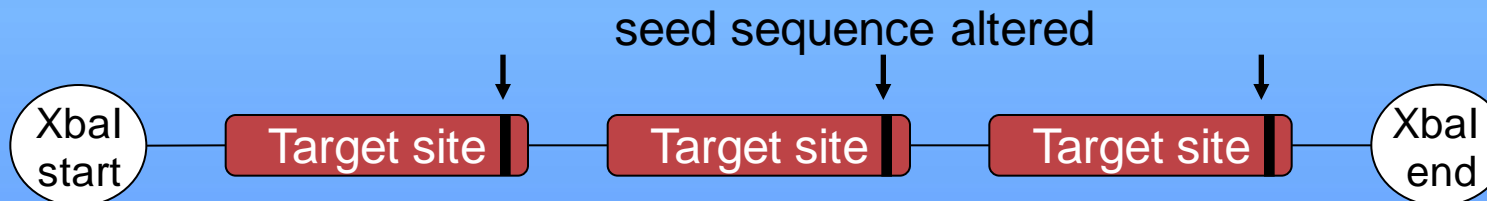
Restriction Site Sequence : *Xba*I

...TCTAGA...
...AGATCT...

- Wild-type triplet cassette



- Mutated triplet cassette



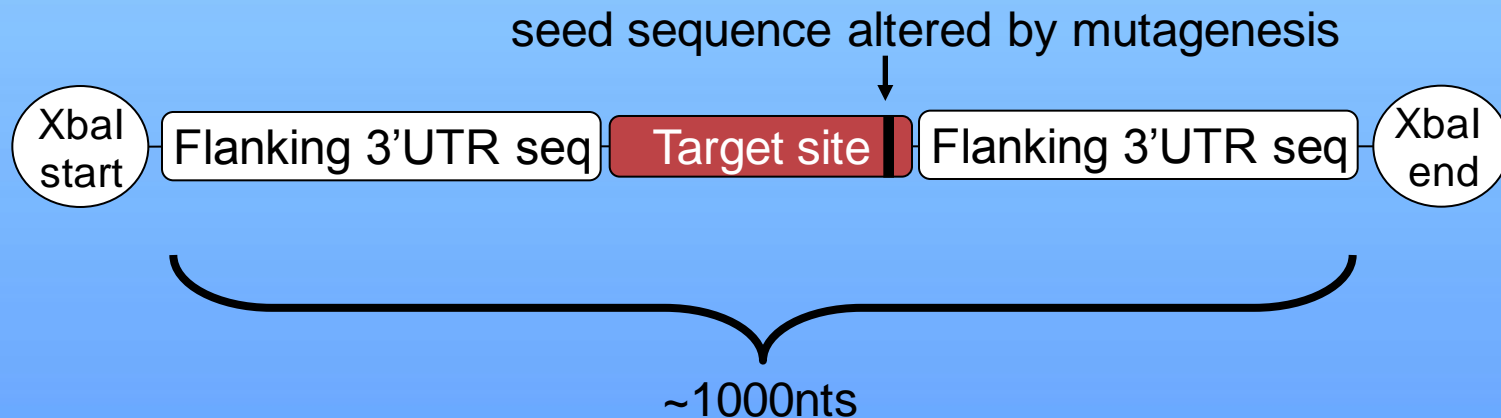
Constructs



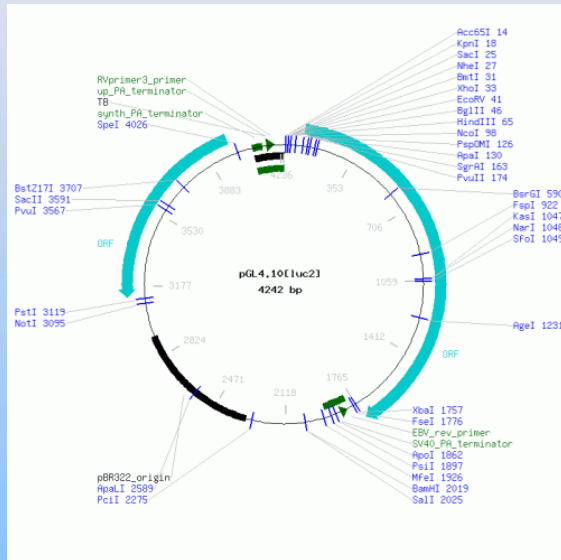
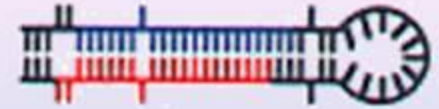
- Wild-type 3'UTR



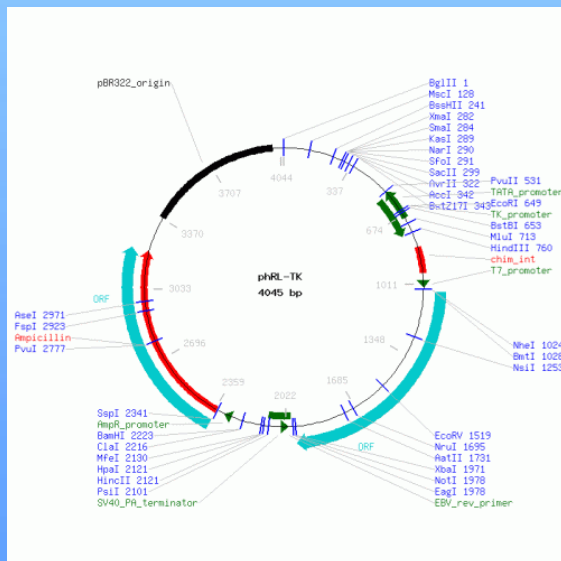
- Mutated 3'UTR



Luciferase Assays



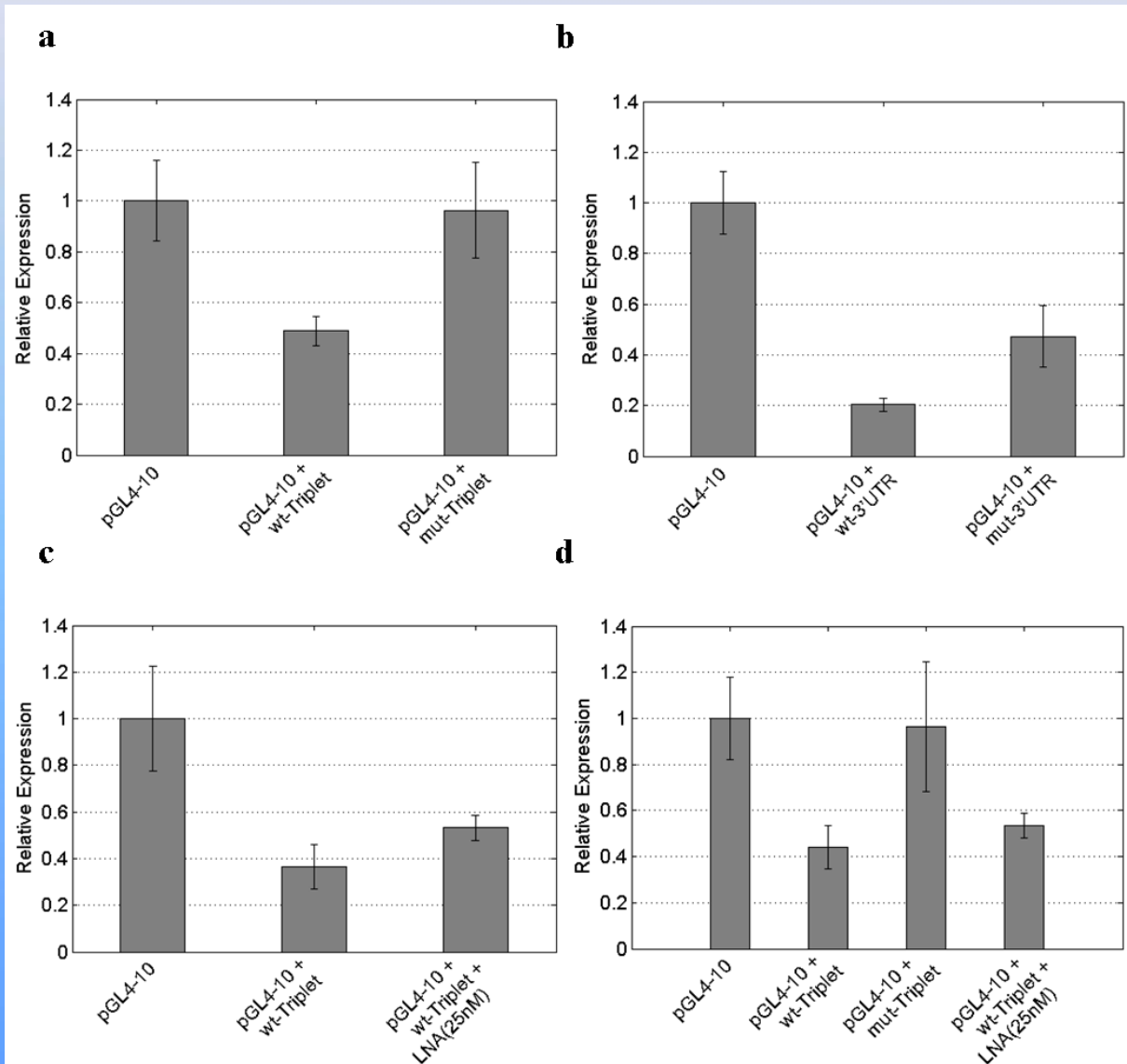
pGL4



phRL-TK

- Cut with restriction enzymes add CCND2 triplet cassette.
- Transfect into HeLa – measure repression of Luc
- Same for triplet cassette containing mutated target sequence at seed region.
- Same for empty vector as control
- Same with no expression vector as blank.

Relative Luciferase repression standardized to a transfection control



Conclusions and future work



- Novel miRNA target prediction tool - Targetprofiler
- Utilization to predict a biologically significant target – CCND2 validated by reporter assays
- Tumour suppressor miRNA?
- Other known miRNA target same site?
- Transfection of bladder cancer cell lines with expression vector for specific miRNA.
- Use assays for cancer processes, i.e. proliferation, invasion to assess the phenotypic effect of specific miRNA on bladder cancer cells.

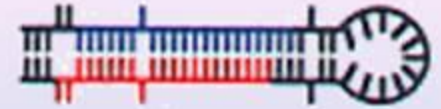
Expanding miRs application



- Batch jobs submission is being initiated for marine species.
- Give the production ready stage of the miRs application and the completion of the human miRNA target prediction runs we are now expanding the capabilities of the application to include marine species such as:
 - **Zebrafish (*Danio rerio*)**
 - **Stickleback (*Gasterosteus aculeatus*)**
 - **Tetraodon (*Tetraodon nigroviridis*)**
 - **Fugu (*Takifugu rubripes*)**
 - **Medaka (*Oryzias latipes*)**



PUBLICATIONS



Generating Scientific Results!

- A new microRNA target prediction tool identifies a novel interaction of a putative miRNA with CCND2 - Anastasis Oulas, Nestoras Karathanasis, Annita Louloui, Ioannis Iliopoulos, Kriton Kalantidis and Panayiota Poirazi. [RNA Biology 9:9, 1–12; September 2012]
- MicroRNA target prediction identifies a novel interaction of a putative miRNA with CCND2 - Anastasis Oulas, Nestoras Karathanasis, Annita Louloui, Ioannis Iliopoulos, Kriton Kalantidis and Panayiota Poirazi. Poster presentation, 7th Microsymposium on Small RNAs, May 21 - 23, 2012 in Basel, Switzerland
- Prediction of miRNA gene targets in cancer associated genomic regions – Oral presentation in the 62nd Conference of the Hellenic Society of Biochemistry and Molecular Biology, 9-11, December, 2011.
- Prediction of miRNA gene targets - a combined computational and experimental approach - Poster - ISMB/ECCB 2011, Vienna Austria
- Oulas, A, Karathanassis N., Louloui, A. and Poirazi, P. “Finding cancer-associated miRNAs: methods and tools” Molecular Biotechnology, 1-11, May 2011.



MiRs Acknowledgments



• IFIN_Bio Cluster

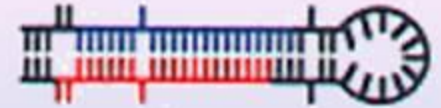
- Dr. Kriton Kalantidis (IMBB/UOC)
- Nestoras Karathanasis (IMBB)
 - Annita Louloui (UOC)



Dr. Ioannis Iliopoulos
(UOC)

HP-SEE Forum - Belgrade,
17-19, Oct, 2012

Dr. Panayiota Poirazi
(IMBB)



THANK YOU!

FOR YOUR ATTENTION!

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