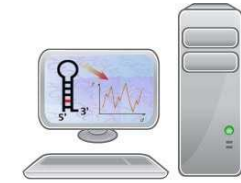


In silico analysis of miRNA promoters



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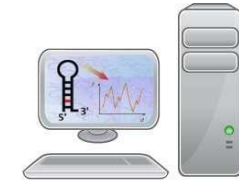


OVERVIEW

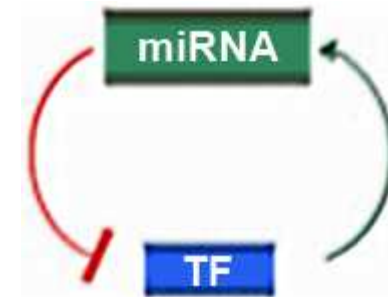


- Introduction
- Problem description
 - MicroRNAs and Transcription Factors
 - Regulation loops
- Predictions
 - Predicting transcription factor binding sites
 - Predicting microRNA targets
 - Predicting regulation loops
- Results
- Conclusions
- Future work

INTRODUCTION – OBJECTIVES OVERVIEW

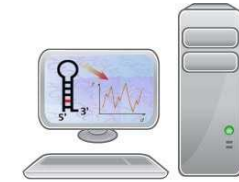


- Several authors have postulated the existence of regulatory feedback loops between transcription factors controlling miRNA expression and the regulatory control exerted by miRNA over the transcription factor expression.

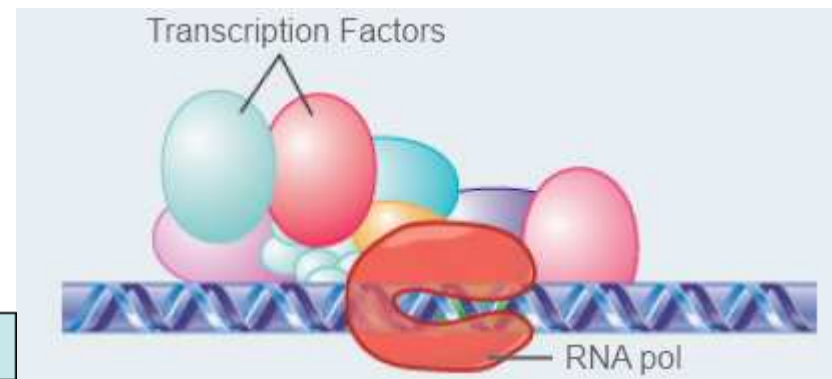


- We were looking for transcription factors regulating miRNA expression and being simultaneously the target protein-coding gene of that same miRNA.
- TF/miRNA regulation loops in humans
- Publish all data

MicroRNAs and Transcription Factors

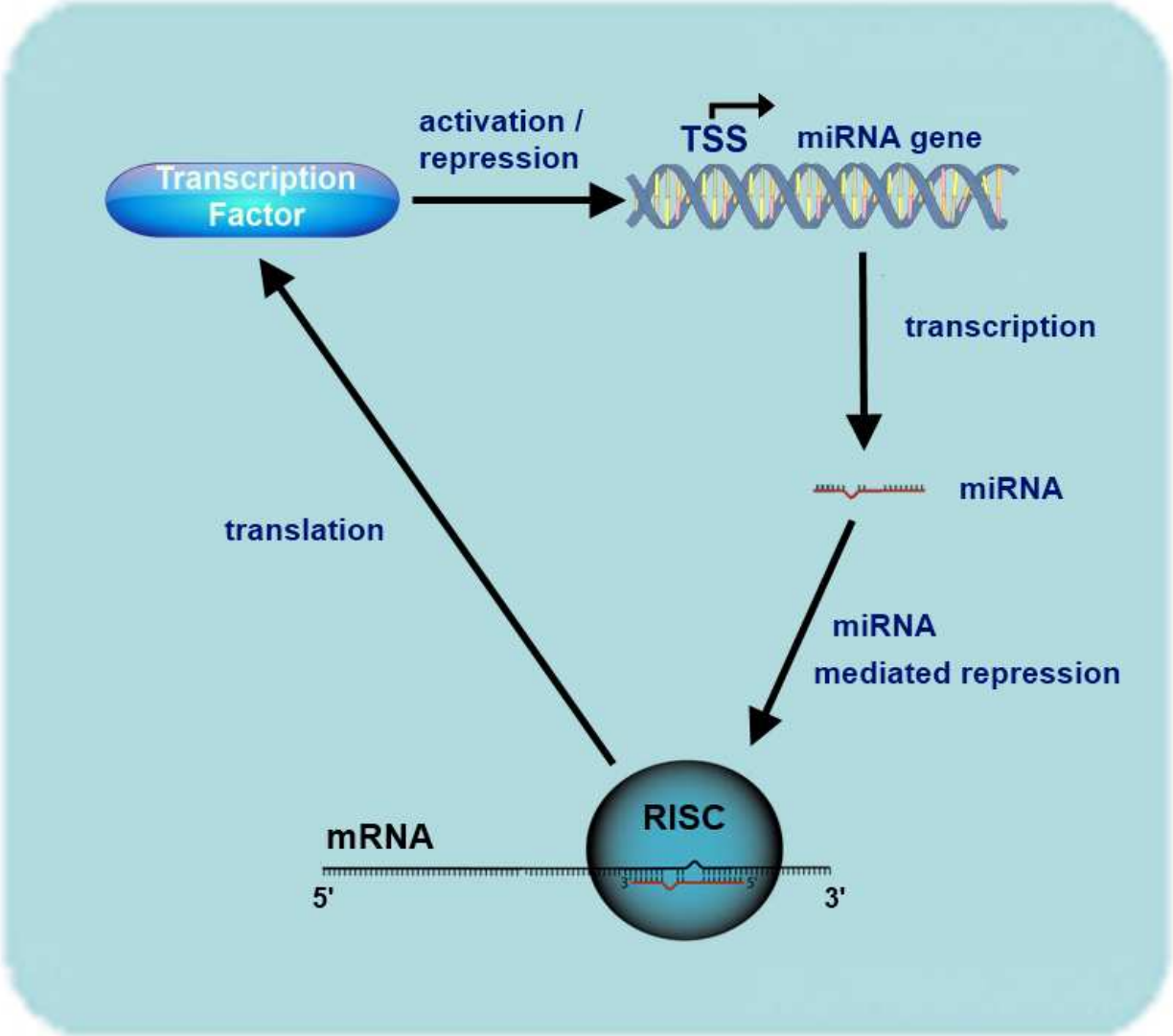
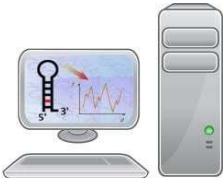


- MicroRNAs (miRNAs) are small (≈ 22 nucleotides), non-protein coding RNA molecules known to regulate the expression of genes by binding to the 3'-untranslated regions (3'-UTR) of mRNAs.
- Transcription factors (TFs) are **proteins** that either activate or repress genes transcription by binding to short cis-regulatory elements called transcription factor binding sites.



Lewin B, Genes IX

REGULATION LOOPS

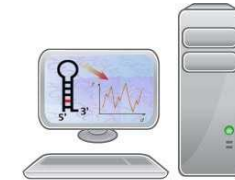


PREDICTING TFBS – PROMOTERS



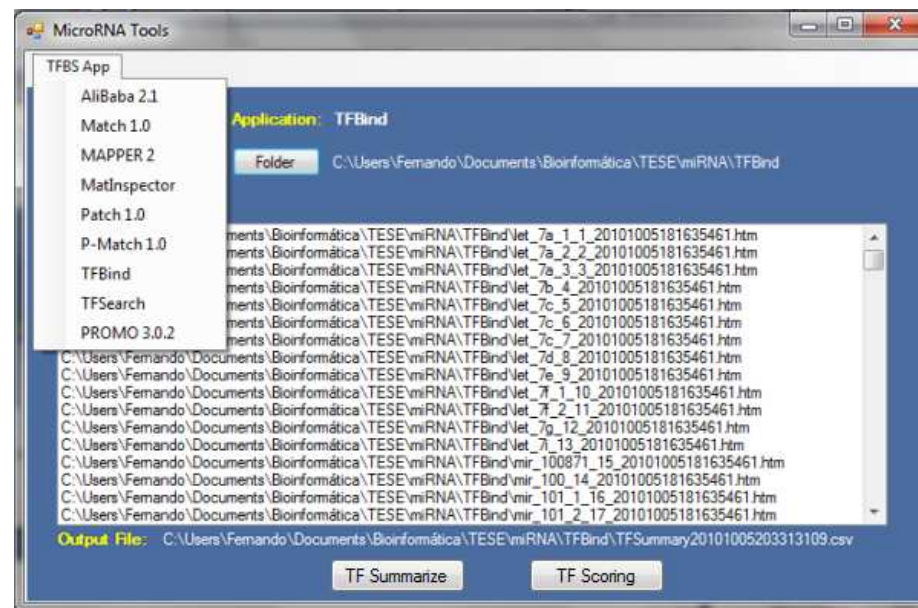
- The first step in the analysis of the transcription factor/microRNA regulation loops was to predict the TFBS for all sequences of miRNA promoters published by Marson *et al.*
 - All chromosomes were downloaded from UCSC Genome Browser (hg17, NCBI build 35)
 - TSS positions were used to identify the sequences of all 550 promoters
 - Results were saved in a FASTA file

PREDICTING TFBS – ONLINE TOOLS



FASTA files were submitted to the following tools:

- Mapper 2
- Match 1.0
- Patch 1.0
- P-Match 1.0
- TFBind
- TFSEARCH 1.3



PREDICTING TFBS – DB OVERLAPPING

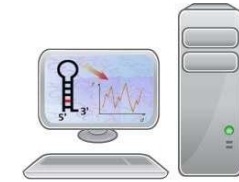


- Pairs of TF/miRNA promoters simultaneously predicted by TFBS prediction tools

#tools	TF/miRNA pairs	Pct.
1	38288	56.87
2	12865	19.11
3	7835	11.64
4	4012	5.96
5	2951	4.38
6	978	1.45
7	395	0.59

- The degree of overlap between databases is often poor

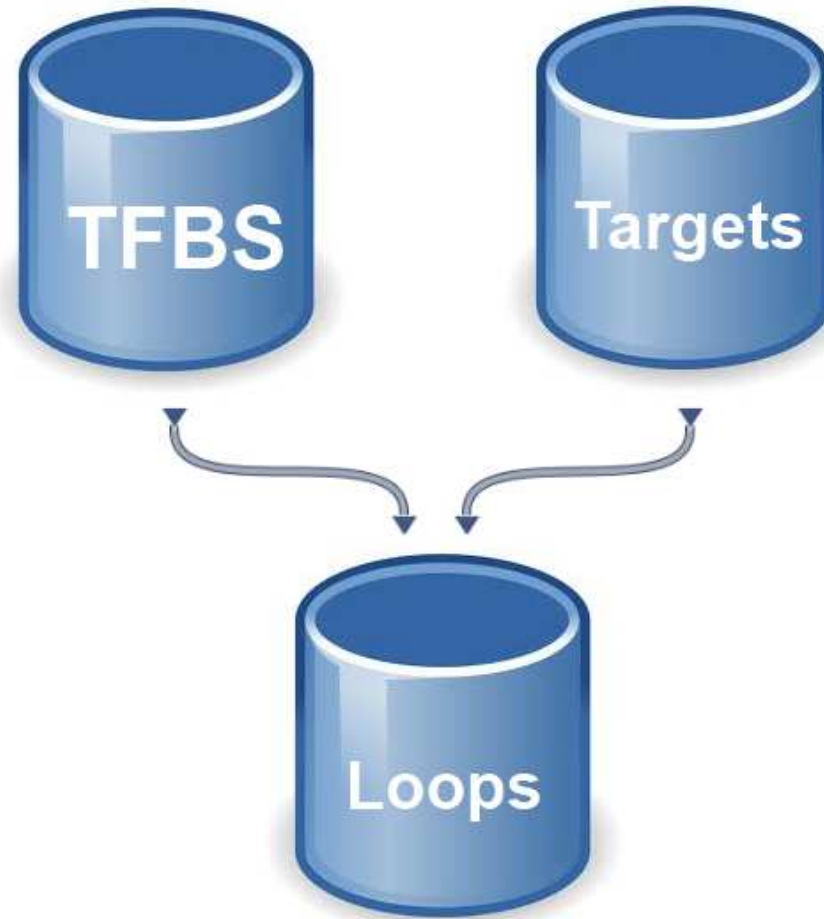
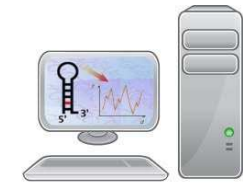
PREDICTING MIRNA TARGETS



- Targets databases

- miRTarBase
- miRWalk
- Diana micro-T v3.0
- MicroCosm
- TargetScan (conserved and non-conserved miRNAs)
- miRanda (conserved and non-conserved miRNAs)

PREDICTING REGULATION LOOPS



RESULTS



- 16450 loops, covering 311 distinct transcription factors and 344 distinct miRNAs
- Rankings of loops

Ranking	Top value
Loops by databases agreement	hsa-mir-9/NFKB1
Genes by databases agreement	NFKB1
MicroRNAs by average of binding sites per loop	hsa-mir-124
Genes by total number of TFBS in all loops	SP1
Genes by target sites average	MYB

...

RESULTS – WEB PLATFORM



MicroRNAs & TFs Regulation Loops

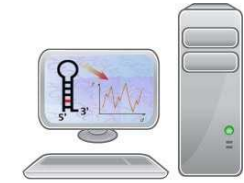
Home Promoters Search for Loops Predicted Loops Predicted Targets Predicted TFBS Downloads About

PREDICTED LOOPS

	miRNA	Gene	Target DB	Tot Targets	MAPPER	Match	Patch	P-Match	PROMO	TFBind	TFSEARCH
Select	hsa-miR-21	ARNT	miRanda_c	2	Y	Y	N	N	N	Y	N
Select	hsa-miR-21	RUNX2	MicroCosm	1	Y	N	N	N	N	N	N
Select	hsa-miR-21	RUNX1	mirWalk	1	Y	Y	Y	Y	N	Y	Y
Select	hsa-miR-21	CBFβ	DIANA	1	N	N	Y	N	N	N	N
Select	hsa-miR-21	CBFβ	miRanda_c	2	N	N	Y	N	N	N	N
Select	hsa-miR-21	CBFβ	TargetScan_nc	2	N	N	Y	N	N	N	N
Select	hsa-miR-21	CDC5L	DIANA	1	Y	N	N	N	N	N	N
Select	hsa-miR-21	CDKN2A	mirWalk	5	Y	N	N	N	N	N	N
Select	hsa-miR-21	CEBPA	mirWalk	1	N	N	N	N	N	Y	N
Select	hsa-miR-21	CEBPB	mirWalk	1	N	N	N	N	N	Y	N
Select	hsa-miR-21	CREB1	mirWalk	1	Y	Y	Y	Y	Y	Y	Y
Select	hsa-miR-21	ATF2	mirWalk	1	N	Y	Y	N	Y	Y	N
Select	hsa-miR-21	ATF2	DIANA	1	N	Y	Y	N	Y	Y	N
Select	hsa-miR-21	CUX1	TargetScan_c	4	Y	Y	Y	Y	N	Y	N
Select	hsa-miR-21	E2F1	mirTarBase	1	N	N	Y	N	Y	N	N
Select	hsa-miR-21	E2F1	mirWalk	3	N	N	Y	N	Y	N	N

<http://mirnatools.eu/TFmiRNA/loops.html>

CONCLUSIONS



- Despite the fact that we cannot yet understand the biological significance of these regulatory loops, their existence seems to be evident and this must be an important mechanism of genes regulation.
- All these data demand for further investigations and experimental validations.

FUTURE WORK



- Analysis of the most reliable loops
- Experimental validations
- Adding other databases or new versions of the already used ones
- To combine all new information in order to get better reliability criteria
- To investigate the biological significance of these loops
- ...

THE END



- Thank you for your attention!
- Questions?

