False positives in microRNA target prediction

Hervé Seitz

IGH du CNRS, Montpellier, France

February 12, 2016

Introduction
  Comparative genomics
  microRNA target prediction
Robustness of predicted targets
Comparative genomics yield false positives
Conclusion
Supplementary data

Introduction

The availability of genome sequences profoundly changed the practice of biology:

Introduction

The availability of genome sequences profoundly changed the practice of biology:

- use *grep* instead of Southern blotting;

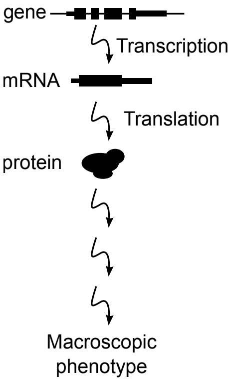
Introduction

The availability of genome sequences profoundly changed the practice of biology:

- use *grep* instead of Southern blotting;
- compare genomic sequences to find phylogenetically conserved elements.

Comparative genomics

Comparative genomics



gene

Transcription

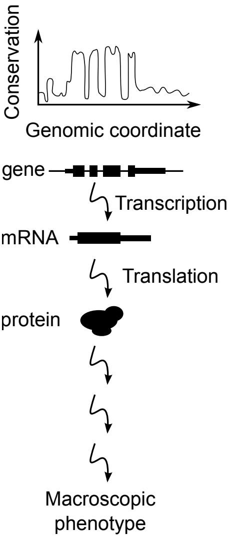
mRNA

Translation

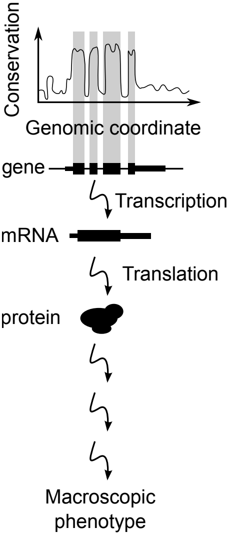
protein

Macroscopic phenotype

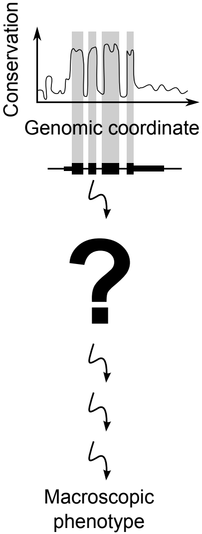
Comparative genomics



Comparative genomics

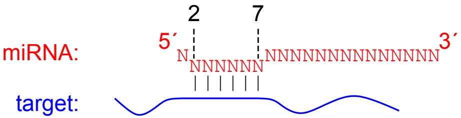


Comparative genomics



microRNA target prediction

microRNA target prediction

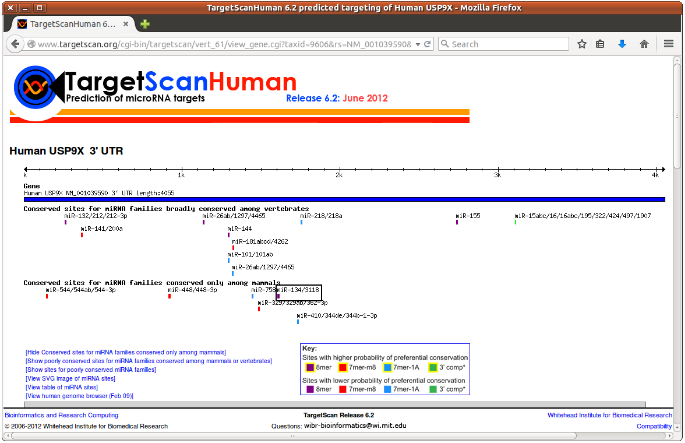


⟶ the “seed”

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

microRNA target prediction



Human USP9X 3' UTR

Gene
Human USP9X NM_001039590 3′ UTR length:4055

Conserved sites for miRNA families broadly conserved among vertebrates

Conserved sites for miRNA families conserved only among mammals

[Hide Conserved sites for miRNA families conserved only among mammals]
[Show poorly conserved sites for miRNA families conserved among mammals or vertebrates]
[Show sites for poorly conserved miRNA families]
[View SVG image of miRNA sites]
[View table of miRNA sites]
[View human genome browser (Feb 09)]

Key:
Sites with higher probability of preferential conservation
🟪 8mer 🟥 7mer-m8 🟦 7mer-1A 🟩 3' comp*
Sites with lower probability of preferential conservation
🟪 8mer 🟥 7mer-m8 🟦 7mer-1A 🟩 3' comp*

Bioinformatics and Research Computing
TargetScan Release 6.2
Whitehead Institute for Biomedical Research
© 2006-2012 Whitehead Institute for Biomedical Research
Questions: wibr-bioinformatics@wi.mit.edu
Compatibility

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. - PubMed - NCBI - Mozilla Firefox

www.ncbi.nlm.nih.gov/pubmed/?term=miR-26a+suppresses+tumour+proliferation+and+metastasi

See 1 citation found by title matching your search:

Cancer Lett. 2015 Feb 1;357(1):384-92. doi: 10.1016/j.canlet.2014.11.050. Epub 2014 Nov 27.

miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer.

Liu P[1], Tang H[1], Chen B[1], He Z[2], Deng M[2], Wu M[3], Liu X[1], Yang L[1], Ye F[1], Xie X[4].

Author information

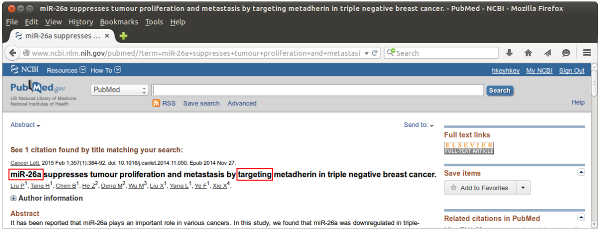
Abstract

It has been reported that miR-26a plays an important role in various cancers. In this study, we found that miR-26a was downregulated in triple-

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).



See 1 citation found by title matching your search:

Cancer Lett. 2015 Feb 1;357(1):384-92. doi: 10.1016/j.canlet.2014.11.050. Epub 2014 Nov 27.

miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer.

Liu P[1], Tang H[1], Chen B[1], He Z[2], Deng M[2], Wu M[3], Liu X[1], Yang L[1], Ye F[1], Xie X[4].

⊕ Author information

Abstract

It has been reported that miR-26a plays an important role in various cancers. In this study, we found that miR-26a was downregulated in triple-

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

$\Longrightarrow$ miRNAs are implicated in every physiological process in animals.

microRNA target prediction

Computational programs for target prediction: look for seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

$\Longrightarrow$ miRNAs are implicated in every physiological process in animals.

miRNA-mediated repression is very modest (usually $<$ 2-fold): lower than well tolerated fluctuations in gene expression (*e.g.*, haplosufficiency). Why have these sites been conserved if they are not functional?

Robustness of predicted targets

Robustness of predicted targets

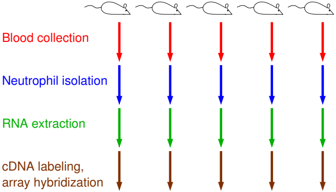
Robustness of predicted targets

Robustness of predicted targets

Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.

Robustness of predicted targets

Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.



Blood collection

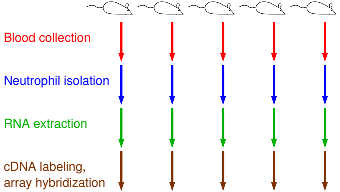
Neutrophil isolation

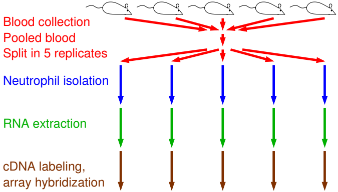
RNA extraction

cDNA labeling,
array hybridization

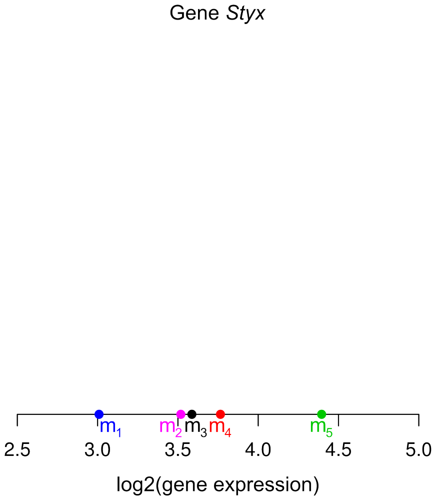
Robustness of predicted targets

Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.

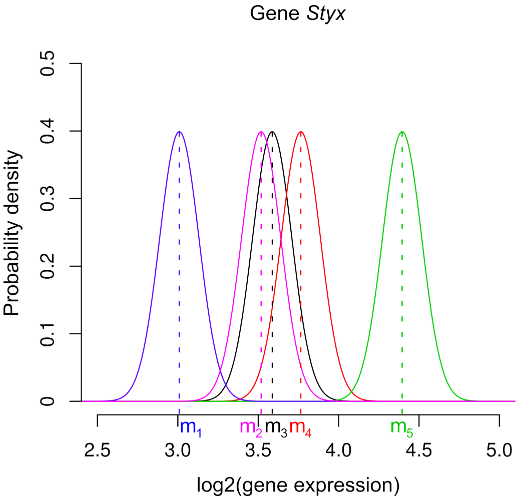




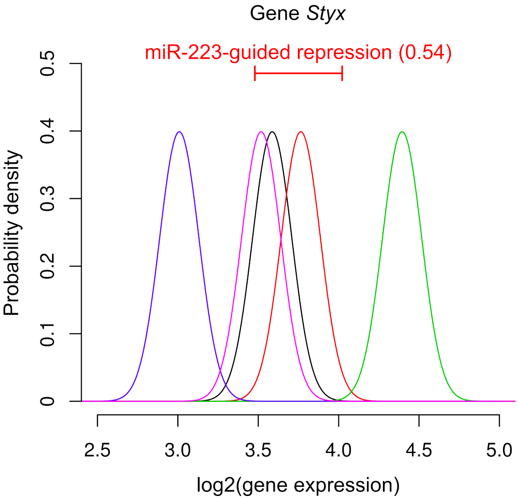
Robustness of predicted targets



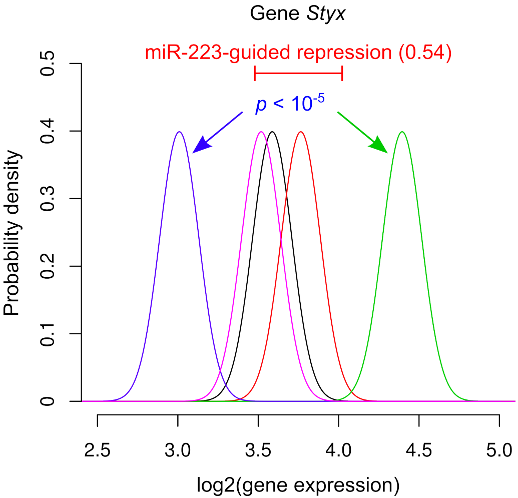
Robustness of predicted targets



Robustness of predicted targets



Robustness of predicted targets



p: probability that the difference between two individual mice is smaller than miRNA-guided repression

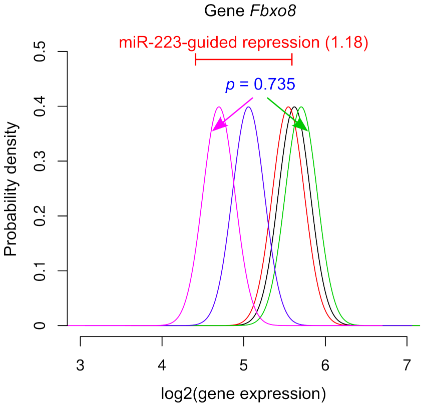
Robustness of predicted targets

Robustness of predicted targets

For 150 predicted targets out of 192:
inter-individual fluctuations across 5 wild-type mice exceeds miRNA-mediated regulation (p-value < 0.05).

Robustness of predicted targets

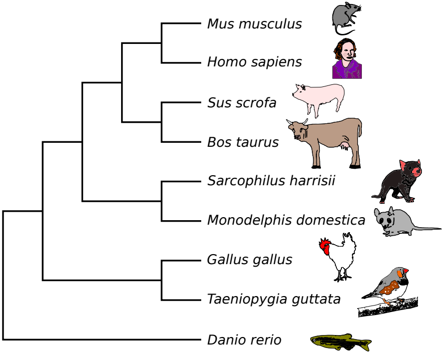
For 150 predicted targets out of 192:
inter-individual fluctuations across 5 wild-type mice exceeds miRNA-mediated regulation (p-value < 0.05).



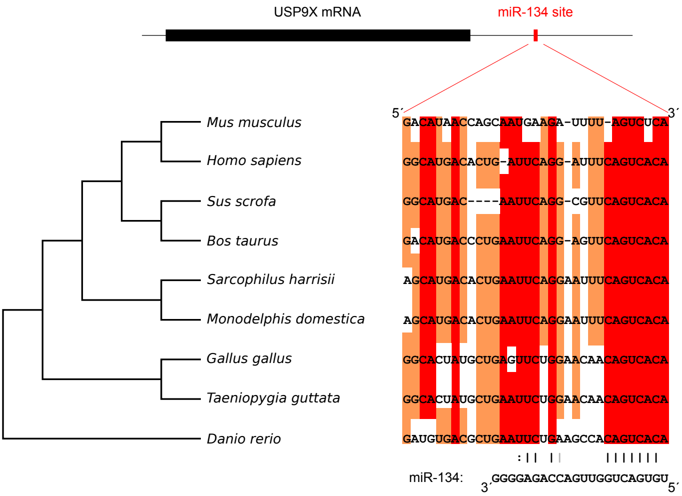
Comparative genomics yield false positives

Comparative genomics yield false positives

Comparative genomics yield false positives

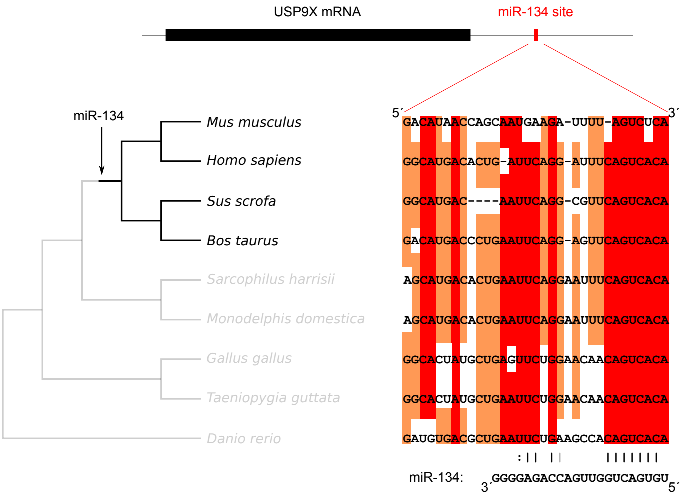


Comparative genomics yield false positives



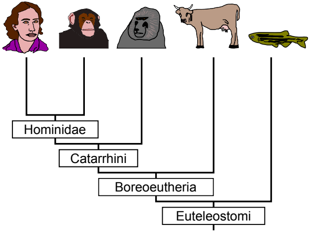
🟥: conserved in 8 or 9 species out of 9 🟧: conserved in 6 or 7 species out of 9 ☐: conserved in less than 6 species out of 9

Comparative genomics yield false positives

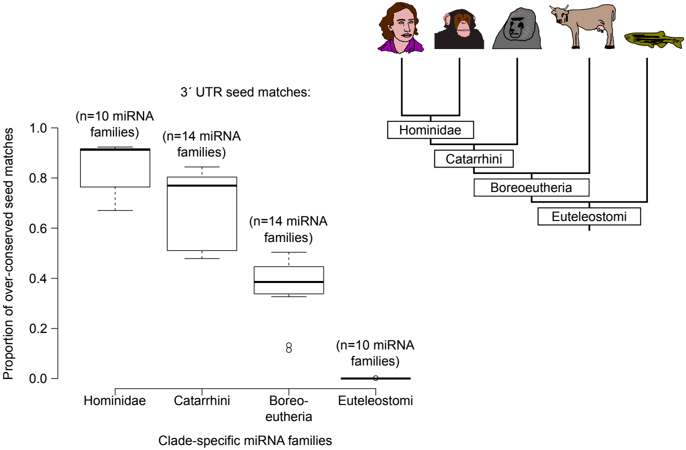


🟥: conserved in 8 or 9 species out of 9 🟧: conserved in 6 or 7 species out of 9 ⬜: conserved in less than 6 species out of 9

Comparative genomics yield false positives



Comparative genomics yield false positives



▸ Comparison to prediction programs

▸ Effect of tree architecture

Conclusion: revisiting miRNA target definition

Conclusion: revisiting miRNA target definition

Every measurable change in gene expression does not translate into a macroscopic, evolutionarily selectable phenotype.

Conclusion: revisiting miRNA target definition

Every measurable change in gene expression does not translate into a macroscopic, evolutionarily selectable phenotype.

Among the (many !) predicted targets: finding those that are responsible for visible phenotypes.


Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites. ▸ Supplementary data

Conclusion: revisiting "gene regulation" definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites. ▸ Supplementary data

Microscopic events which are neutral in evolutionary terms.

Conclusion: revisiting “gene regulation” definition

High-throughput identification of transcription factor or RNA-binding protein targets: thousands of genes are bound, many of them are not under selective pressure to keep these binding sites. ▸ Supplementary data

Microscopic events which are neutral in evolutionary terms.

A central feature of biological systems: their robustness to external insults. Hard to reconcile with the extreme sensitivity required for fine-tuning (the “butterfly effect” has probably been counter-selected).

Acknowledgements

Anna Sergeeva:

Laura Martinez:

Blaise Li:

Natalia Pinzón:

Isabelle Busseau:

Delphine Mazé:

Acknowledgements

Anna Sergeeva:

Laura Martinez:

Blaise Li:

Natalia Pinzón:

Isabelle Busseau:

Delphine Mazé:

Jessy Presumey and Florence Apparailly (INM, Montpellier)

Acknowledgements

Anna Sergeeva:

Laura Martinez:

Blaise Li:

Natalia Pinzón:

Isabelle Busseau:

Delphine Mazé:

Jessy Presumey and Florence Apparailly (INM, Montpellier)

Supplementary data

Introduction:

- ▸ Mechanism of target repression

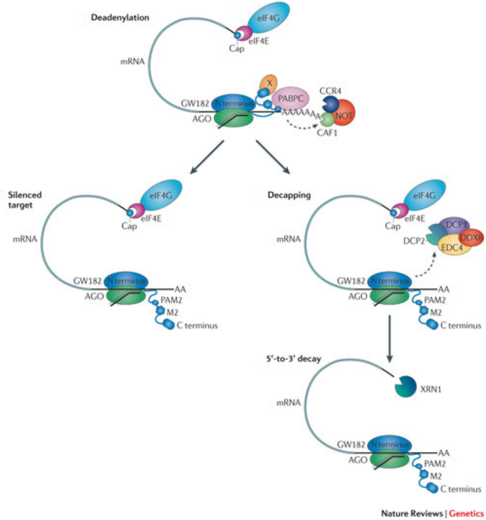
Results:

- ▸ Robustness of biological pathways
- ▸ Over-conserved sites in prediction programs
- ▸ Published evidence for genome-wide targeting
- ▸ Issues with published pseudo-targets
- ▸ Absolute RNA quantification results
- ▸ RNA-Seq statistics

Conclusion:

- ▸ Pseudo-targets for other regulators?
- ▸ Propagation of gene expression perturbation

miRNA target repression

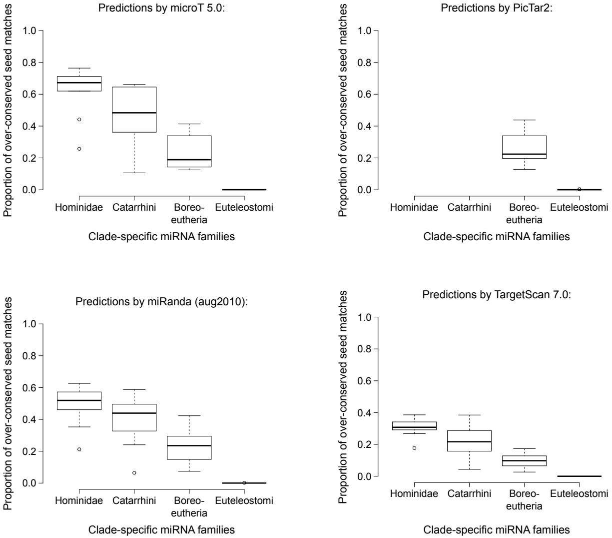


(adapted from Huntzinger and Izaurralde, 2011)

Biological robustness

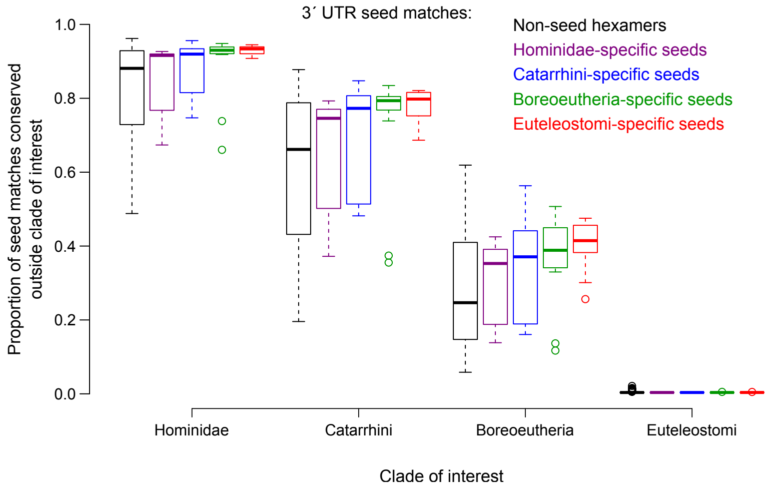
$$\text{Substrate} \xrightarrow{\text{enzyme 1}} \text{Product 1} \xrightarrow{\text{enzyme 2}} \text{Product 2} \xrightarrow{\text{enzyme 3}} \text{Product 3}$$

Over-conservation in prediction programs



Return

Effect of tree architecture

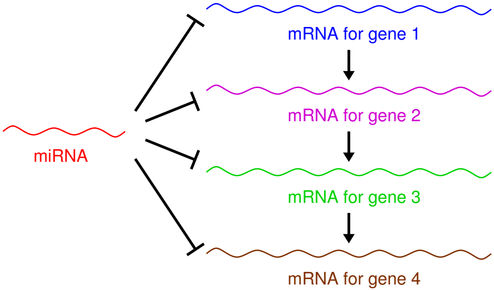


◂ Return

Published evidence

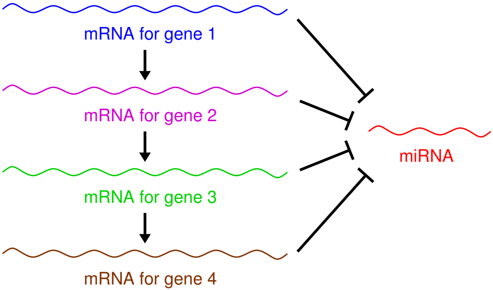
Published evidence

Targets for a given miRNA often belong to the same biological pathways.



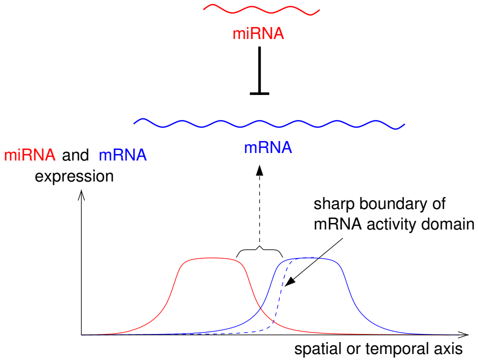
Published evidence

Targets for a given miRNA often belong to the same biological pathways.



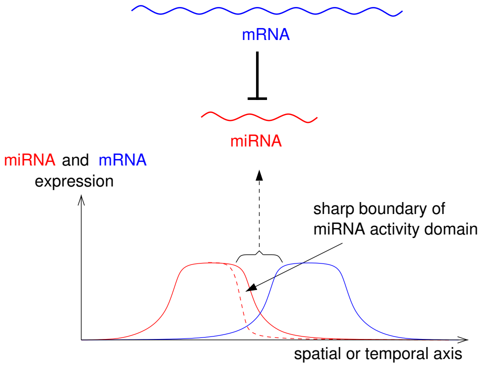
Published evidence

Expression domains for miRNAs and their predicted targets overlap at their boundaries.



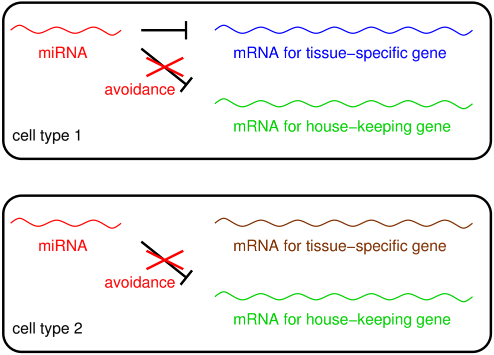
Published evidence

Expression domains for miRNAs and their predicted targets overlap at their boundaries.



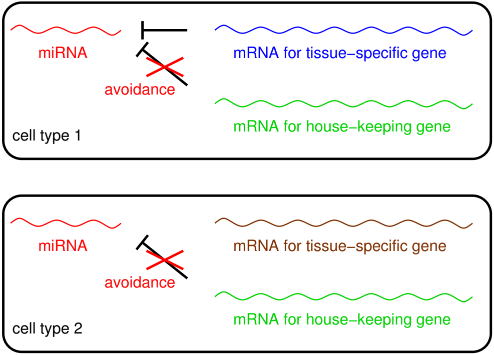
Published evidence

House-keeping genes are rarely predicted to be targeted.



Published evidence

House-keeping genes are rarely predicted to be targeted.



Reported examples of pseudo-targets

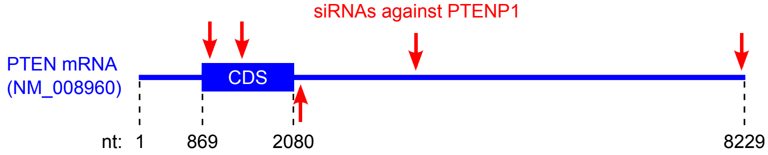
A proposed pseudo-target: PTENP1, that de-repressed PTEN (Poliseno *et al.*, 2010).

Reported examples of pseudo-targets

A proposed pseudo-target: PTENP1, that de-repressed PTEN (Poliseno *et al.*, 2010).

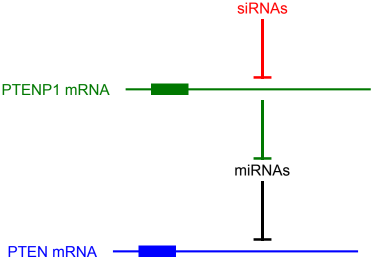
But PTENP1 mRNA is ≈ 100 times less abundant than the PTEN mRNA (Ebert and Sharp, 2010).

Reported examples of pseudo-targets



Reported examples of pseudo-targets

Proposed by Poliseno *et al* (2010):



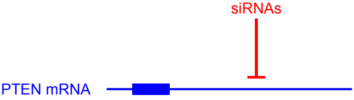
siRNAs

PTENP1 mRNA

miRNAs

PTEN mRNA

More probably:



siRNAs

PTEN mRNA

Reported examples of pseudo-targets

siRNA #2 against PTENP1:
PTEN mRNA (nt 948-961):

```
5´ UAAUAAUCAUCAUUCUGGC 3´
    ||||||    ||| |||
3´ GUUAUUA----UAA-ACCU 5´
```

siRNA #2 against PTENP1:
PTEN mRNA (nt 8189-8208):

```
5´ UAAUAAUC-AUCAUUCUGGC 3´
    ||||||  |:|    |::
3´ UUUAUUACUUGGAAAAAUUA 5´
```

siRNA #2 against PTENP1:
PTEN mRNA (nt 1383-1401):

```
5´ UAAUAAUCAUCAUUCUGGC 3´
    ||||||   |||  :| |
3´ AUUAUUAUAUGUAUCGCGG 5´
```

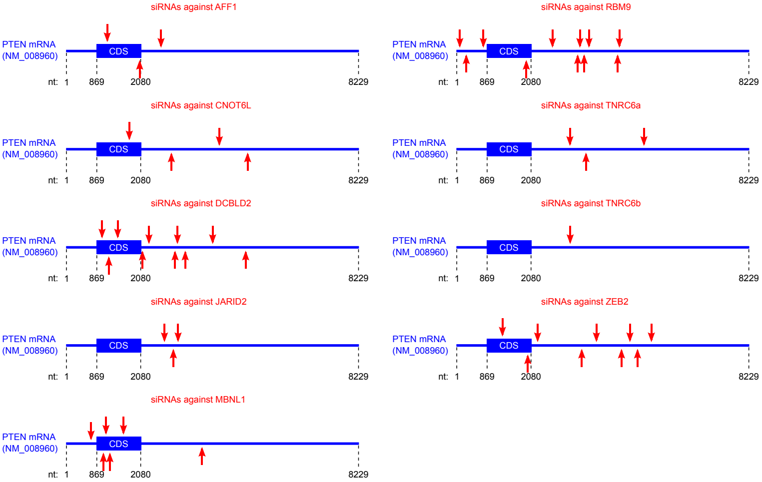
siRNA #3 against PTENP1:
PTEN mRNA (nt 2192-2212):

```
5´ UCCUAUA--UGAUCUCUGAUG 3´
    ||||||  ||   |||||::
3´ AGGAUAUUGACGUUAGACUGU 5´
```

siRNA #2 against PTENP1:
PTEN mRNA (nt 3769-3782):

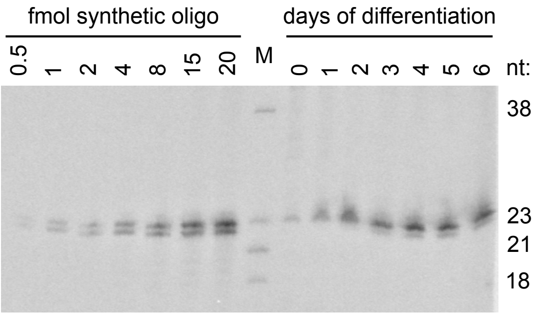
```
5´ UAAUAAUCAUCAUUCUGGC 3´
    ||||||       ||||
3´ AUUAUUACC-----GACCU 5´
```

Reported examples of pseudo-targets



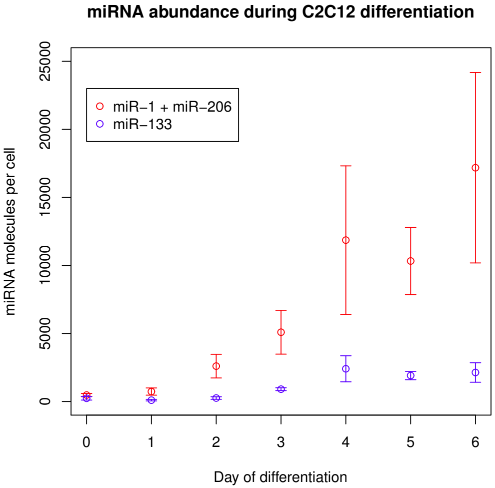
Return

miRNA quantification



(quantification of miR-1 and miR-206)

miRNA quantification



mRNA quantification

mRNA quantification

Very deep sequencing: three time points (day 0, day 3, day 6) in triplicate; each replicate: between 267 and 333 million transcriptome-matching reads. ▸ Statistics

华大基因 BGI

mRNA quantification

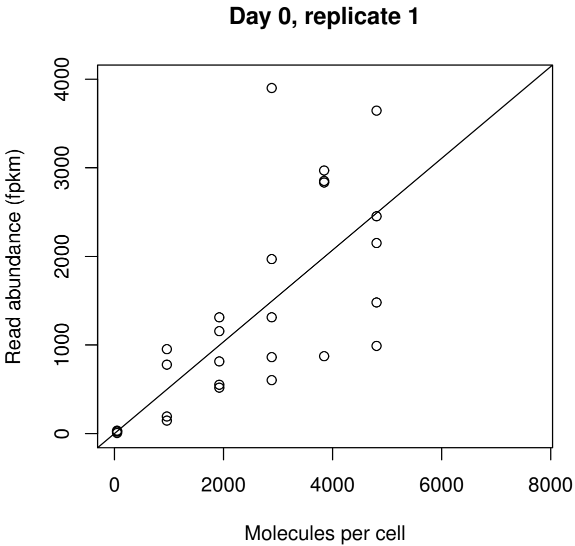
Very deep sequencing: three time points (day 0, day 3, day 6) in triplicate; each replicate: between 267 and 333 million transcriptome-matching reads.

▸ Statistics

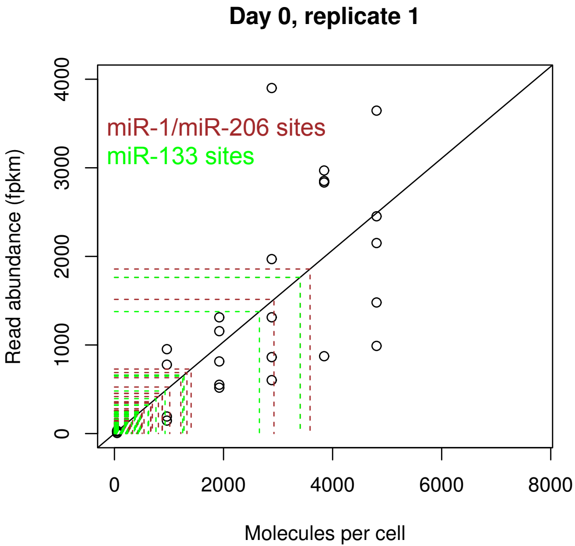
华大基因
BGI

27 synthetic spike-ins, for calibration.

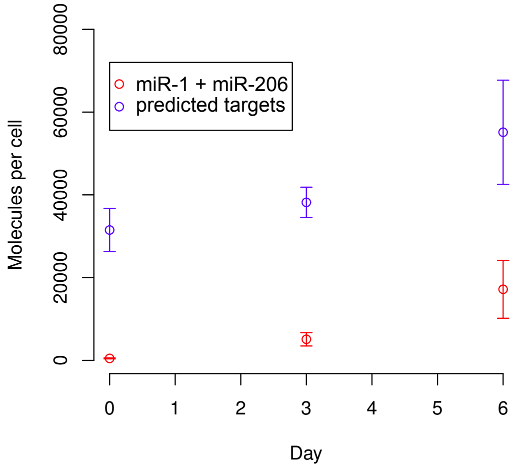
mRNA quantification



mRNA quantification

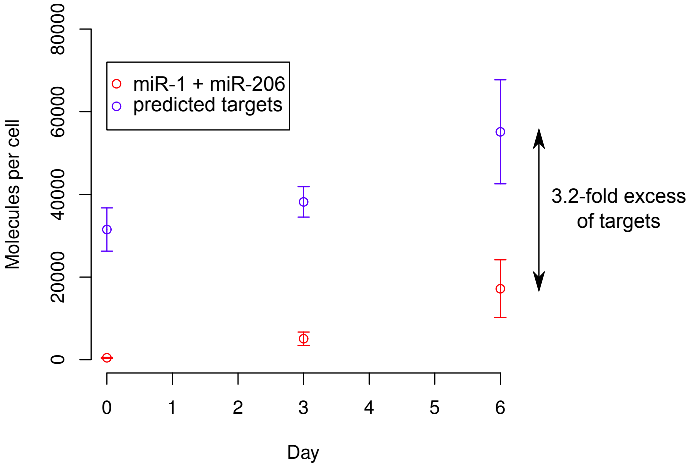


mRNA quantification



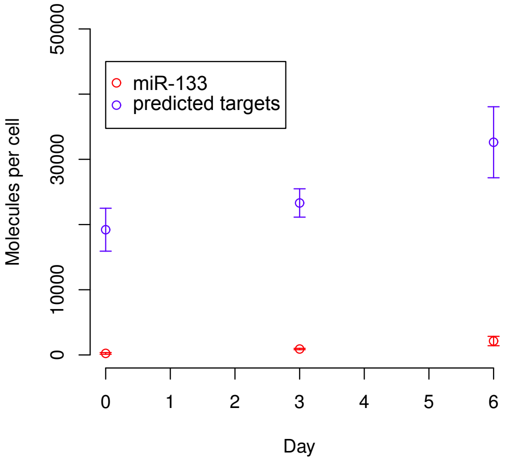
◂ Return

mRNA quantification



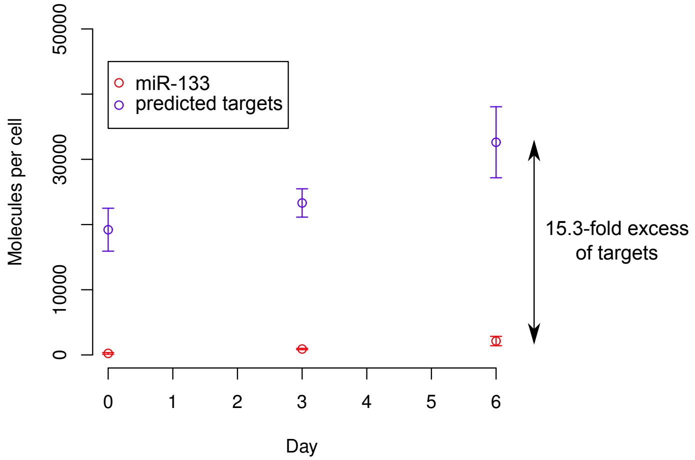
◂ Return

mRNA quantification



Return

mRNA quantification



◂ Return

Deep-sequencing statistics

Number of reads per kb (median transcript, excluding spike-ins):

Day 0	Replicate 1	159.26
	Replicate 2	140.53
	Replicate 3	150.03
Day 3	Replicate 1	213.09
	Replicate 2	167.77
	Replicate 3	198.48
Day 6	Replicate 1	223.57
	Replicate 2	243.86
	Replicate 3	220.23

◂ Return

Pseudo-targets for other regulators?

- Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).

Pseudo-targets for other regulators?

- Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).
- RNA-binding proteins are poorly specific (thousands of experimentally validated targets for each analyzed protein: Hafner *et al.*, 2010; Lebedeva *et al.*, 2011 and Hafner *et al.*, 2013).

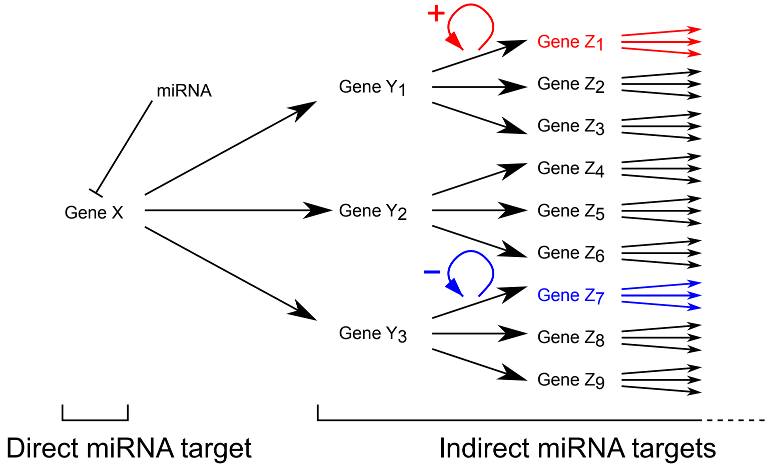
Pseudo-targets for other regulators?

- Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).
- RNA-binding proteins are poorly specific (thousands of experimentally validated targets for each analyzed protein: Hafner *et al.*, 2010; Lebedeva *et al.*, 2011 and Hafner *et al.*, 2013).

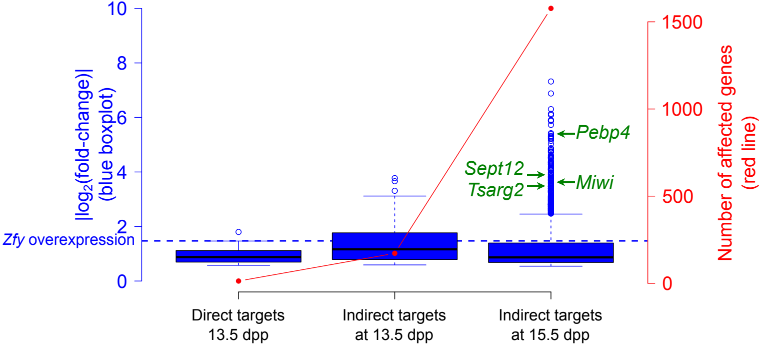
Real molecular events, which are neutral in evolutionary terms?

◂ Return

Propagation of gene expression perturbation



Propagation of gene expression perturbation



(in collaboration with H. Royo and J. Turner, MRC, London)