

Every predicted miRNA target is not functionally targeted: revisiting the nature of miRNA/mRNA interaction

Hervé Seitz

IGH (CNRS), Montpellier, France

March 22, 2013

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

microRNA target identification

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

microRNA target identification

Revisiting the
nature of
miRNA/mRNA
interaction

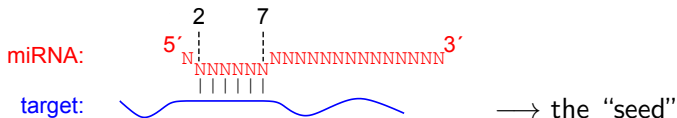
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



microRNA target identification

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

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

microRNA target identification

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).

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

microRNA target identification

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).

⇒ miRNAs are implicated in every physiological process in animals.

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

microRNA target identification

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).

⇒ 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?

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

An alternative hypothesis

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.
- ▶ That function could be to repress the miRNA by titrating it.

Introduction

An alternative hypothesis

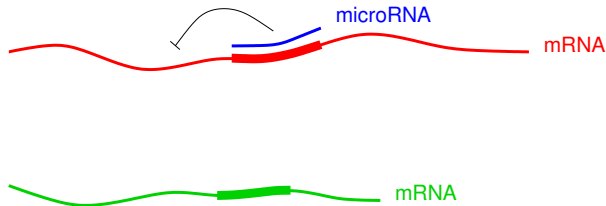
Testing the hypothesis

Conclusion

Supplementary data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.
- ▶ That function could be to repress the miRNA by titrating it.



Introduction

An alternative
hypothesis

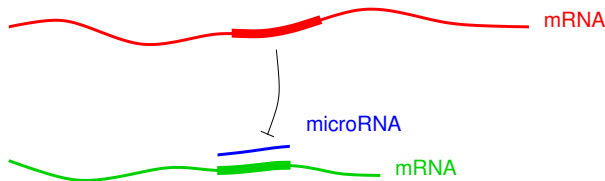
Testing the
hypothesis

Conclusion

Supplementary
data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.
- ▶ That function could be to repress the miRNA by titrating it.



Introduction

An alternative
hypothesis

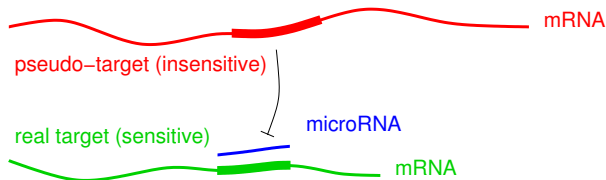
Testing the
hypothesis

Conclusion

Supplementary
data

An alternative hypothesis

- ▶ Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- ▶ Their phylogenetic conservation means that these binding sites have a function.
- ▶ That function could be to repress the miRNA by titrating it.



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Discriminative prediction

According to the new hypothesis:

According to the current theory:

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Discriminative prediction

According to the new hypothesis:

- ▶ Pseudo-target expression levels can fluctuate between individuals in a natural population (phenotype is robust).

According to the current theory:

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

Discriminative prediction

According to the new hypothesis:

- ▶ Pseudo-target expression levels can fluctuate between individuals in a natural population (phenotype is robust).

According to the current theory:

- ▶ miRNA targets are tightly regulated (inter-individual fluctuation should not exceed miRNA-guided repression).

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

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

Introduction

An alternative
hypothesis

Testing the
hypothesis

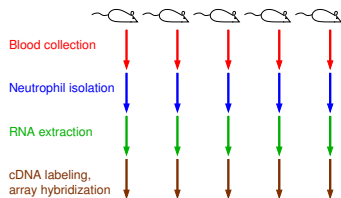
Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction

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



Introduction

An alternative
hypothesis

Testing the
hypothesis

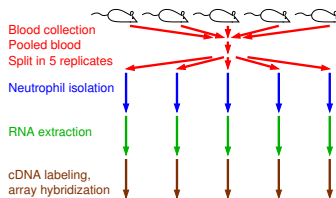
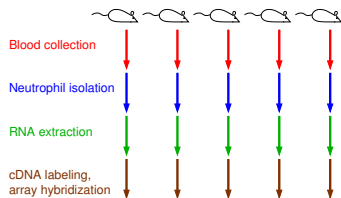
Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction

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



Introduction

An alternative
hypothesis

Testing the
hypothesis

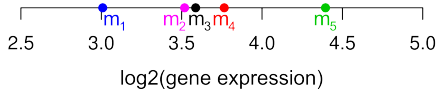
Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction

Gene *Styx*



Introduction

An alternative
hypothesis

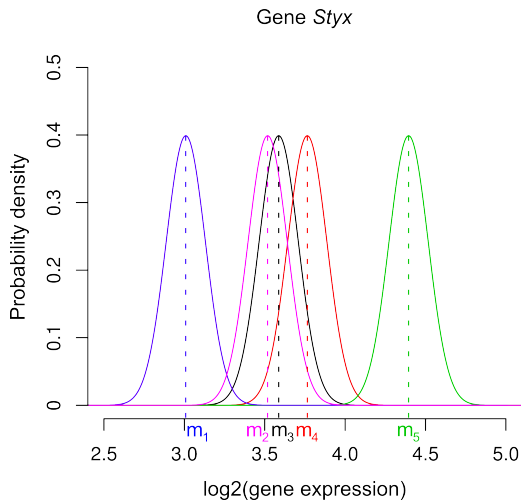
Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction



Introduction

An alternative
hypothesis

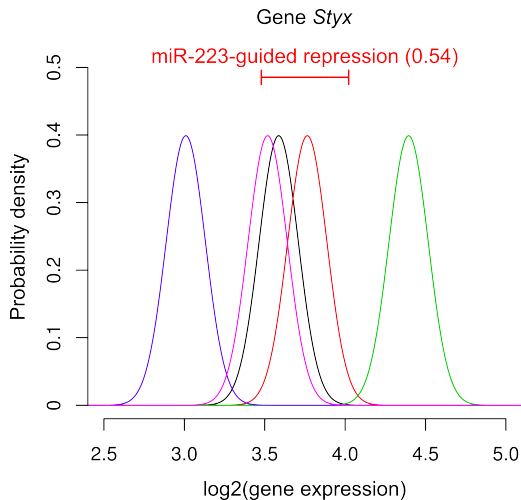
Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction



Introduction

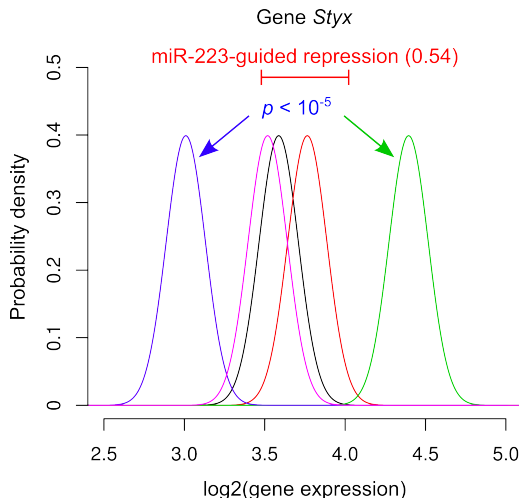
An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression



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

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

Natural variability vs. miRNA-guided repression

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

For 168 predicted targets out of 189:
inter-individual fluctuations across 5 wild-type mice exceeds
miRNA-mediated regulation (p -value < 0.05).

Introduction

An alternative
hypothesis

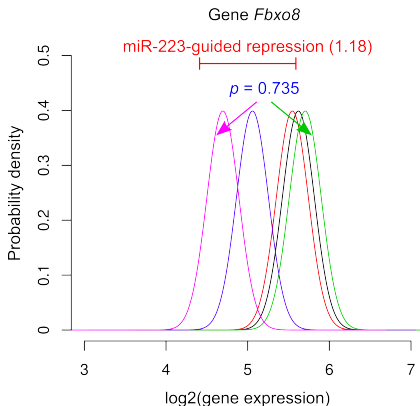
Testing the
hypothesis

Conclusion

Supplementary
data

Natural variability vs. miRNA-guided repression

For 168 predicted targets out of 189:
inter-individual fluctuations across 5 wild-type mice exceeds
miRNA-mediated regulation (p -value < 0.05).



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Conclusion: revisiting miRNA target definition

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Conclusion: revisiting miRNA target definition

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

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

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](#)

[Introduction](#)

[An alternative hypothesis](#)

[Testing the hypothesis](#)

[Conclusion](#)

[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.

[Introduction](#)

[An alternative hypothesis](#)

[Testing the hypothesis](#)

[Conclusion](#)

[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.

Are there pseudo-targets for these other regulators?

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Acknowledgements

Revisiting the
nature of
miRNA/mRNA
interaction

Anna Sergeeva



Laura Martinez



Natalia
Pinzón Restrepo



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Acknowledgements

Revisiting the
nature of
miRNA/mRNA
interaction

Anna Sergeeva



Laura Martinez



Natalia
Pinzón Restrepo



Jessy Presumey and Florence Apparailly (INM, Montpellier,
France)

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Acknowledgements

Revisiting the
nature of
miRNA/mRNA
interaction

Anna Sergeeva



Laura Martinez



Natalia
Pinzón Restrepo



Jessy Presumey and Florence Apparailly (INM, Montpellier,
France)



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Supplementary data

An alternative hypothesis:

- ▶ Robustness of biological pathways
- ▶ Non-discriminative predictions (coordinated targeting)
- ▶ Non-discriminative predictions (expression domains)
- ▶ Non-discriminative predictions (house-keeping genes)

Testing the hypothesis:

- ▶ The most abundant mRNAs bear the most conserved miRNA binding sites
- ▶ miRNA tissue-specificity and abundance/conservation correlation
- ▶ Non-coding pseudo-targets?
- ▶ Correlation between dose-sensitivity and targeting conservation

Conclusion:

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

Introduction

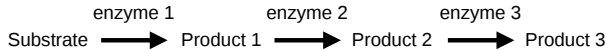
An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

Biological robustness



Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

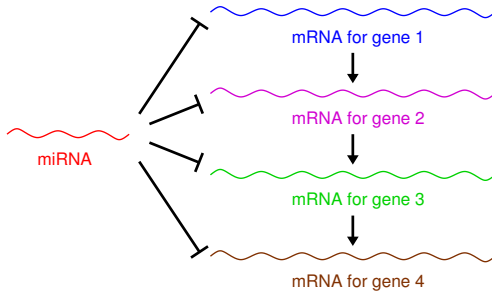
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

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



Introduction

An alternative
hypothesis

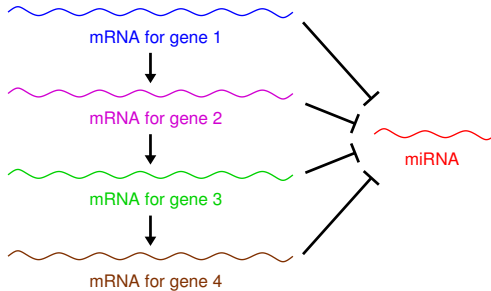
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

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



Introduction

An alternative
hypothesis

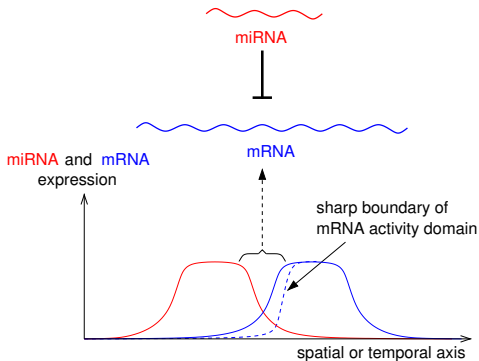
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

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



Introduction

An alternative
hypothesis

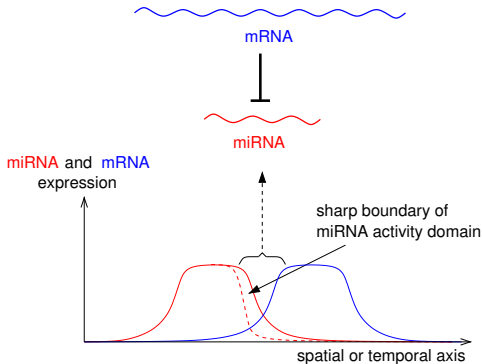
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

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



Introduction

An alternative
hypothesis

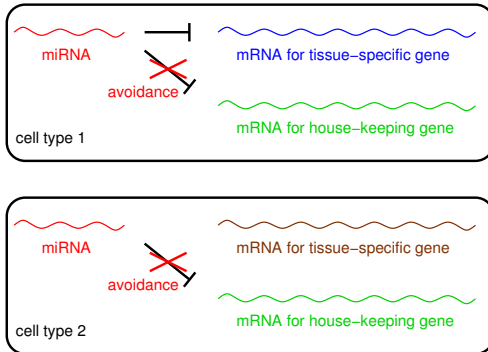
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

House-keeping genes are rarely predicted to be targeted.



Introduction

An alternative
hypothesis

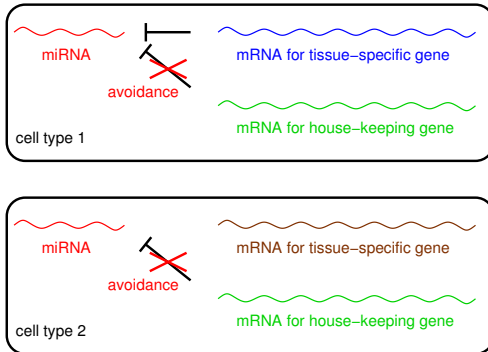
Testing the
hypothesis

Conclusion

Supplementary
data

Non-discriminative predictions

House-keeping genes are rarely predicted to be targeted.



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

mRNA abundance and seed match conservation

- ▶ Most predicted targets are expected to be pseudo-targets (conservative approximation: we will consider every predicted target).
- ▶ miRNA binding site should correlate with that mRNA's abundance in the cells where miRNA titration is beneficial (conservative approximation: we will consider whole tissues and organs).
- ▶ Poorly abundant mRNAs (without a real titration effect on the miRNA) should not exhibit such correlation (conservative approximation: we will consider every mRNA).

Introduction

An alternative
hypothesis

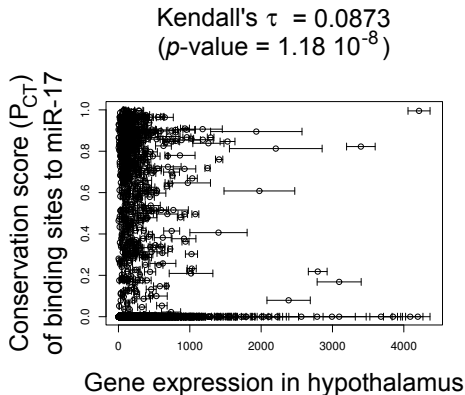
Testing the
hypothesis

Conclusion

Supplementary
data

mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

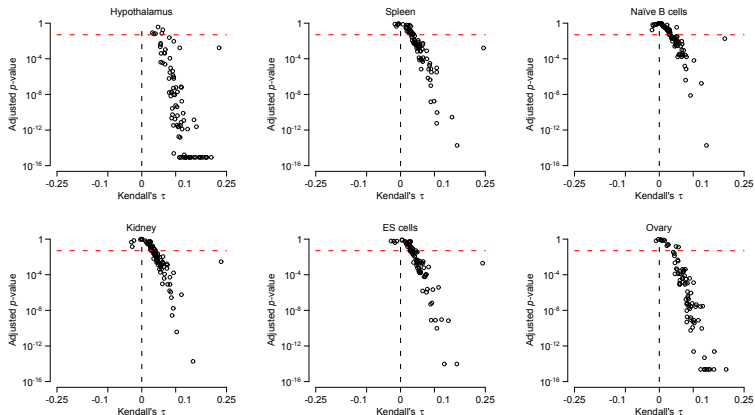
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

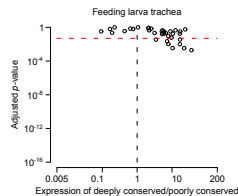
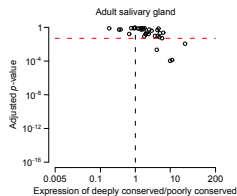
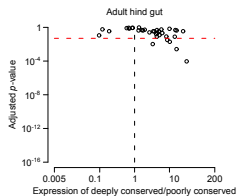
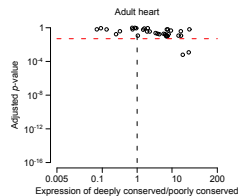
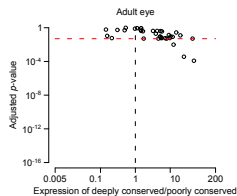
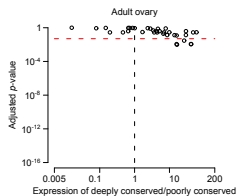
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

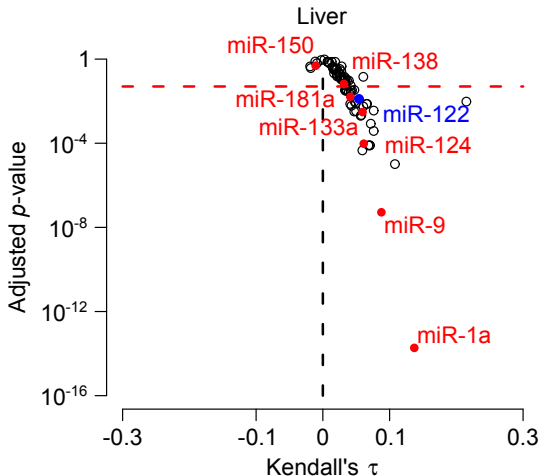
Introduction

An alternative
hypothesis

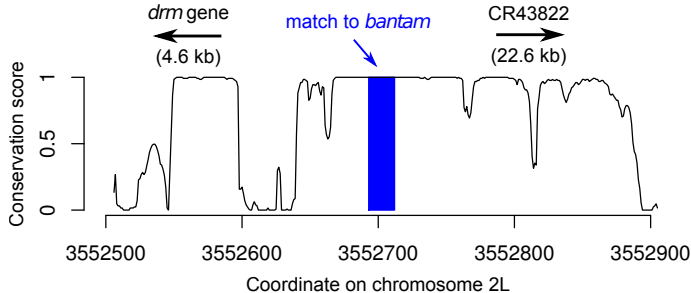
Testing the
hypothesis

Conclusion

Supplementary
data



Non-coding pseudo-targets?



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Dose-sensitivity and targeting conservation

Expected:	Observed:
Positive correlation between haplo-insufficiency probability and miRNA binding site conservation	$\tau = +0.190$ ($p < 2.2 \times 10^{-16}$)
Positive correlation between protein under-wrapping and miRNA binding site conservation	$\tau = +0.070$ ($p = 0.64$) (worm) Deeply conserved: 46% higher than poorly conserved ($p = 1.57 \times 10^{-5}$) (fly)

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

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).

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

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 and Lebedeva *et al.*, 2011).

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

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 and Lebedeva *et al.*, 2011).

Real molecular events, which are neutral in evolutionary terms?

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Propagation of gene expression perturbation

Revisiting the
nature of
miRNA/mRNA
interaction

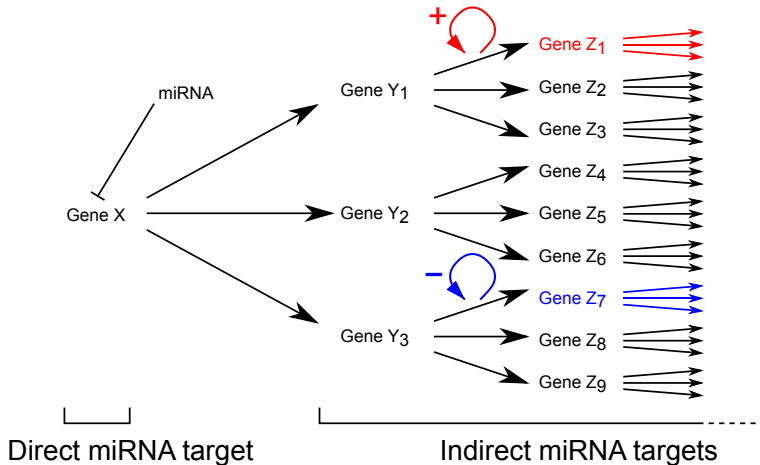
Introduction

An alternative
hypothesis

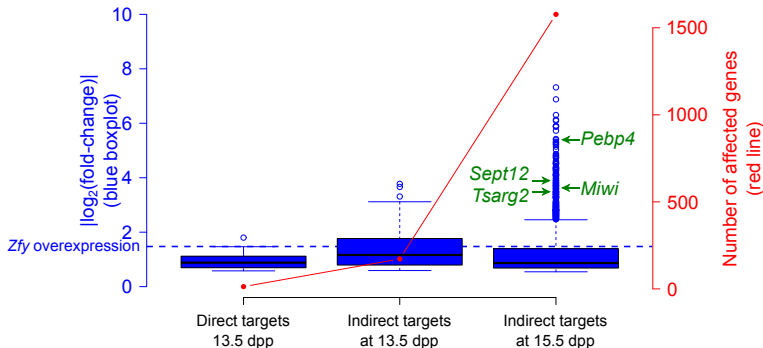
Testing the
hypothesis

Conclusion

Supplementary
data



Propagation of gene expression perturbation



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

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Discriminative prediction

According to the new hypothesis:

According to the current theory:

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Discriminative prediction

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved

According to the current theory:

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Discriminative prediction

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved $\implies$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

Discriminative prediction

According to the new hypothesis:

- ▶ Abundantly expressed pseudo-targets have a stronger miRNA-modulating effect, so their interaction with the miRNA should be better conserved $\implies$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

- ▶ Each target is a real target, its interaction is more or less conserved depending on the functional importance of the regulation (it is not expected to correlate with gene expression).

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

Does mRNA abundance correlate positively with miRNA binding site conservation?

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

mRNA abundance and seed match conservation

Does mRNA abundance correlate positively with miRNA binding site conservation?

miRNA binding site conservation: measured by TargetScan's " P_{CT} " score. mRNA abundance in published microarray experiments.

► [Supplementary data](#)

Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data

mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

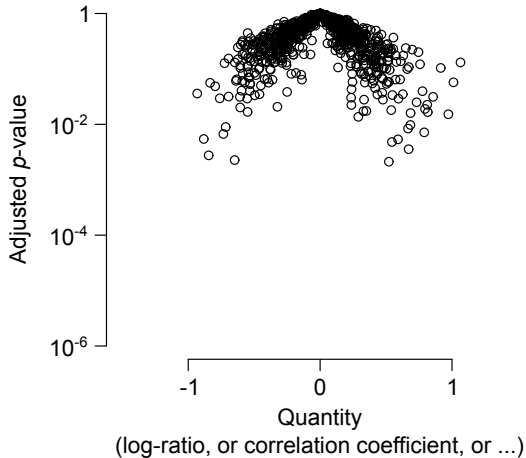
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

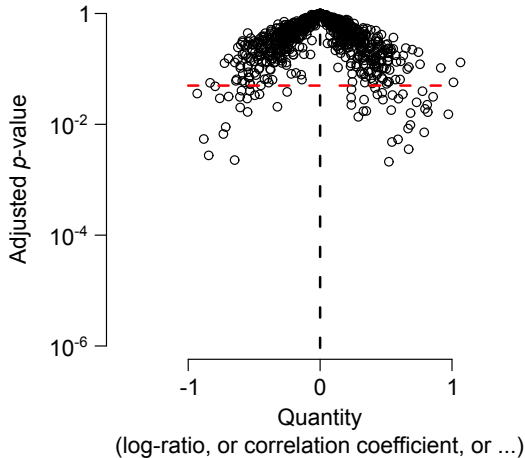
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

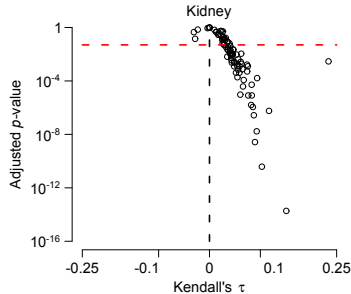
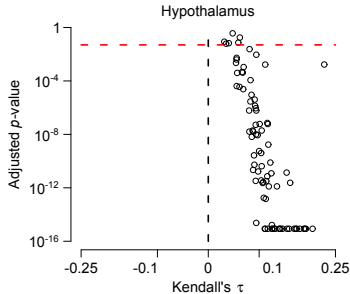
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



mRNA abundance and seed match conservation

Revisiting the
nature of
miRNA/mRNA
interaction

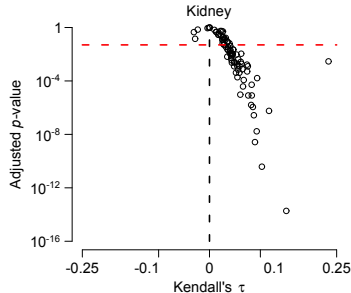
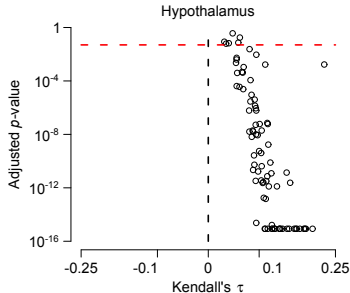
Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data



+34 other mouse tissues, same results:

► [Supplementary data](#)

+33 fly tissues, same results:

► [Supplementary data](#)

Reported examples of pseudo-targets

Revisiting the
nature of
miRNA/mRNA
interaction

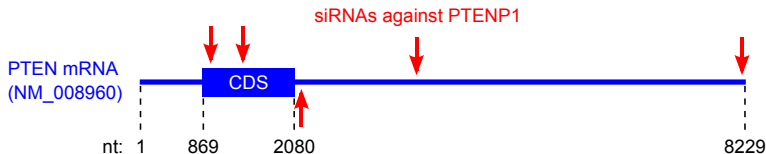
Introduction

An alternative
hypothesis

Testing the
hypothesis

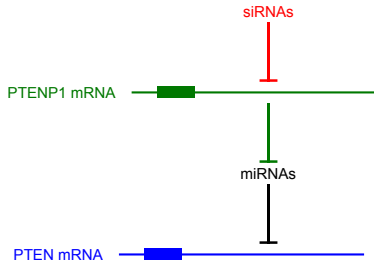
Conclusion

Supplementary
data



Reported examples of pseudo-targets

Proposed by Poliseno *et al* (2010):



More probably:



Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

Reported examples of pseudo-targets

Revisiting the
nature of
miRNA/mRNA
interaction

Introduction

An alternative
hypothesis

Testing the
hypothesis

Conclusion

Supplementary
data

siRNA #2 against PTENP1: 5' UAAUAAUCAUUCUGGC 3'
 ||||| ||| |||
PTEN mRNA (nt 948-961): 3' GUUAUUA-----UAA-ACCU 5'

siRNA #2 against PTENP1: 5' UAAUAAUC-AUCAUUCUGGC 3'
 ||||| |:| |::
PTEN mRNA (nt 8189-8208): 3' UUUUUUACUUGGAAAAAUUA 5'

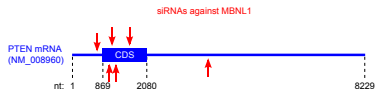
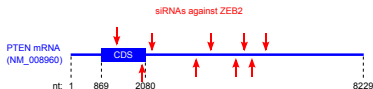
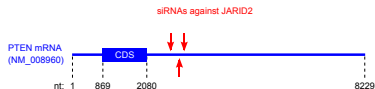
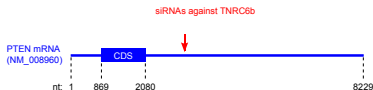
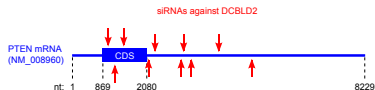
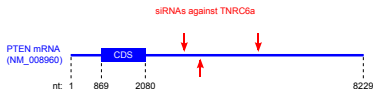
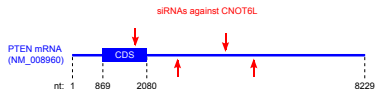
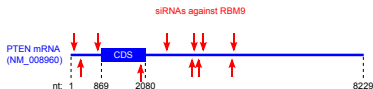
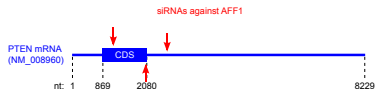
siRNA #2 against PTENP1: 5' UAAUAAUCAUUCUGGC 3'
 ||||| ||| :| |
PTEN mRNA (nt 1383-1401): 3' AUUAUUUAUGUAUCGCGG 5'

siRNA #3 against PTENP1: 5' UCCUAUA--UGAUCUCUGAUG 3'
 ||||| || |||||::
PTEN mRNA (nt 2192-2212): 3' AGGAUUAUUGACGUUAGACUGU 5'

siRNA #2 against PTENP1: 5' UAAUAAUCAUUCUGGC 3'
 ||||| ||| |
PTEN mRNA (nt 3769-3782): 3' AUUAUUACC-----GACCU 5'

Reported examples of pseudo-targets

Revisiting the nature of miRNA/mRNA interaction



Introduction

An alternative hypothesis

Testing the hypothesis

Conclusion

Supplementary data