Biological function of microRNAs in *Drosophila*

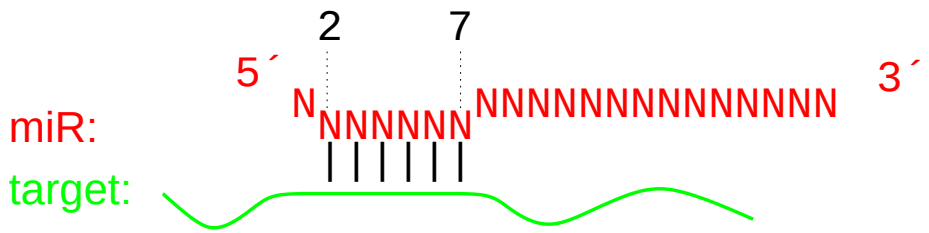
Hervé Seitz

IGH (CNRS), Montpellier, France

September 12, 2012

microRNA target identification

microRNA target identification



$\longrightarrow$ the "seed"

Identification of miRNA targets

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

Identification of miRNA targets

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).

Identification of miRNA targets

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.

Identification of miRNA targets

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?

An alternative hypothesis

An alternative hypothesis

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

An alternative hypothesis

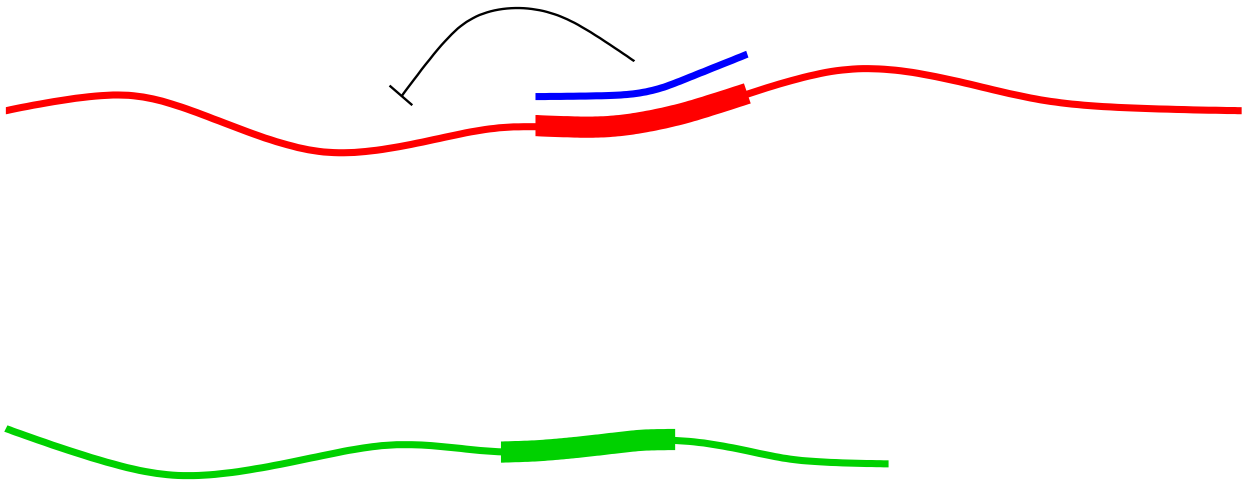
- Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- Their phylogenetic conservation means that these binding sites have a function.

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.

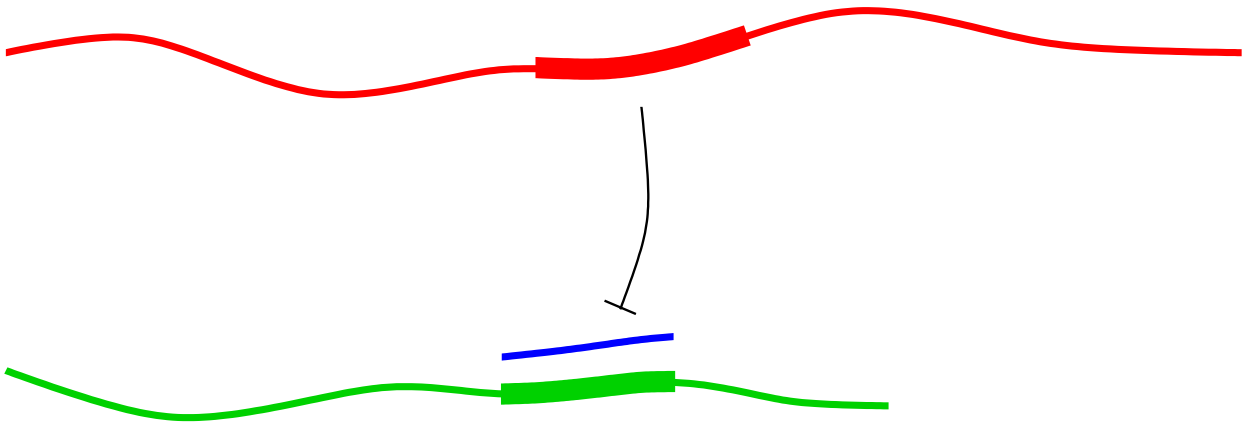
An alternative hypothesis

- 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.



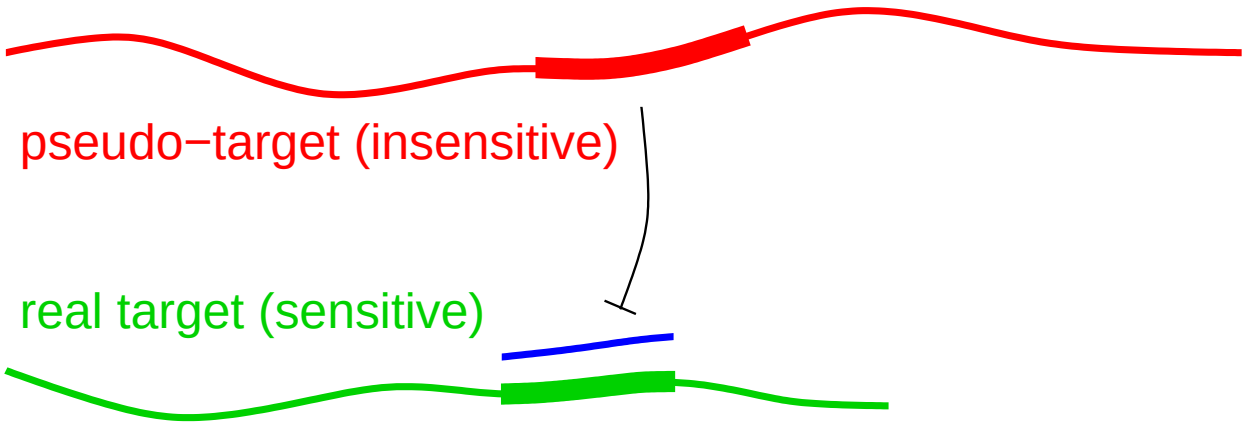
An alternative hypothesis

- 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.



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.



A new interpretation

The molecular event is the same (interaction between an mRNA and a miRNA), but the interpretation of that interaction is different:

- Current theory: the miRNA represses the mRNA.
- New hypothesis: the mRNA represses the miRNA (except for a few real targets).

Confronting predictions

Predicted by the current theory:

Confronting predictions

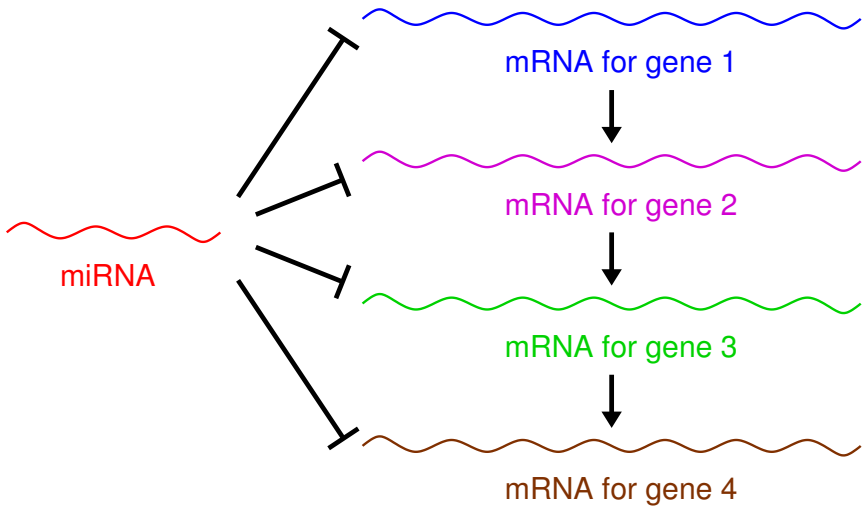
Predicted by the current theory:

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

Confronting predictions

Predicted by the current theory:

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



Confronting predictions

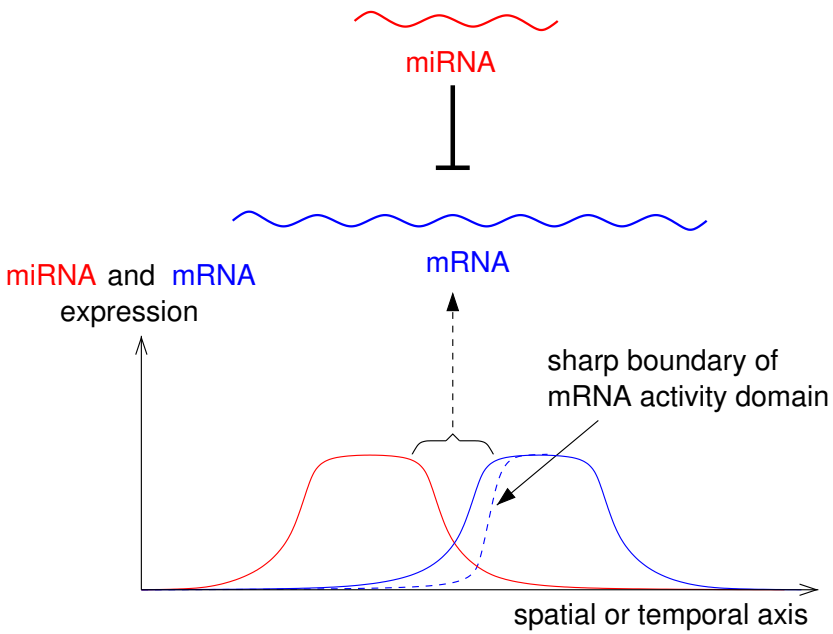
Predicted by the current theory:

1. Targets for a given miRNA often belong to the same biological pathways.
2. miRNA and predicted target expression patterns overlap at their boundaries.

Confronting predictions

Predicted by the current theory:

1. Targets for a given miRNA often belong to the same biological pathways.
2. miRNA and predicted target expression patterns overlap at their boundaries.



Confronting predictions

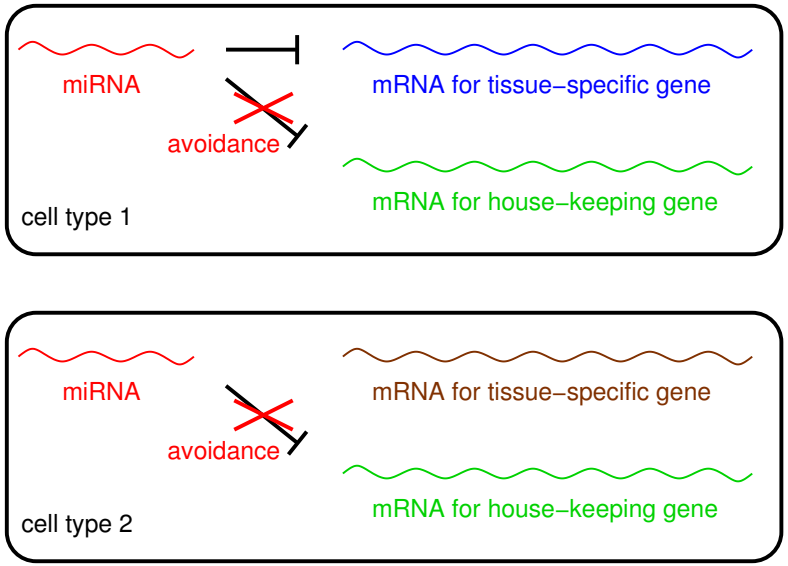
Predicted by the current theory:

1. Targets for a given miRNA often belong to the same biological pathways.
2. miRNA and predicted target expression patterns overlap at their boundaries.
3. Predicted targets are depleted in ubiquitously-expressed genes.

Confronting predictions

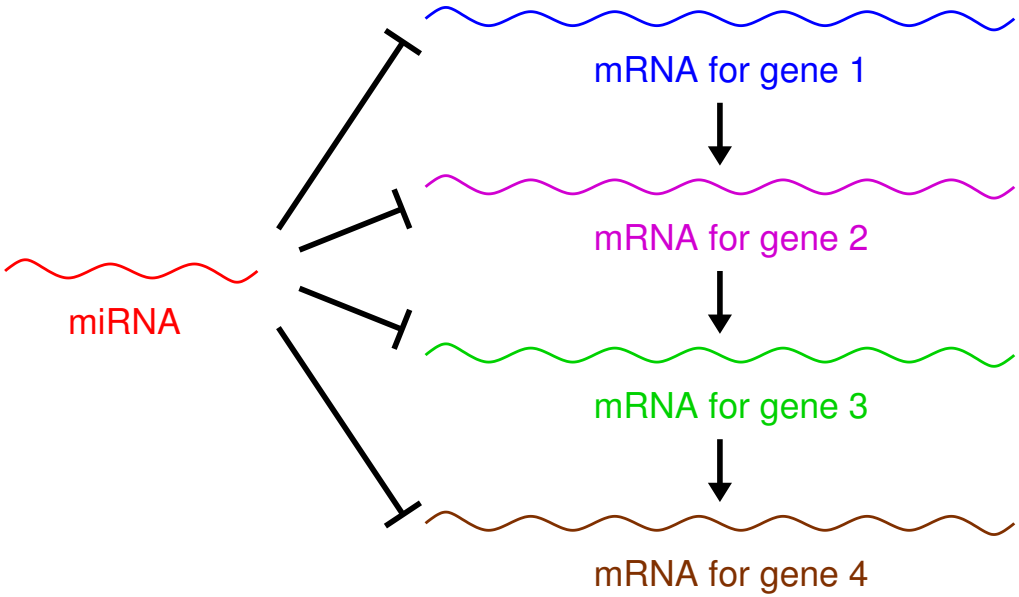
Predicted by the current theory:

1. Targets for a given miRNA often belong to the same biological pathways.
2. miRNA and predicted target expression patterns overlap at their boundaries.
3. Predicted targets are depleted in ubiquitously-expressed genes.

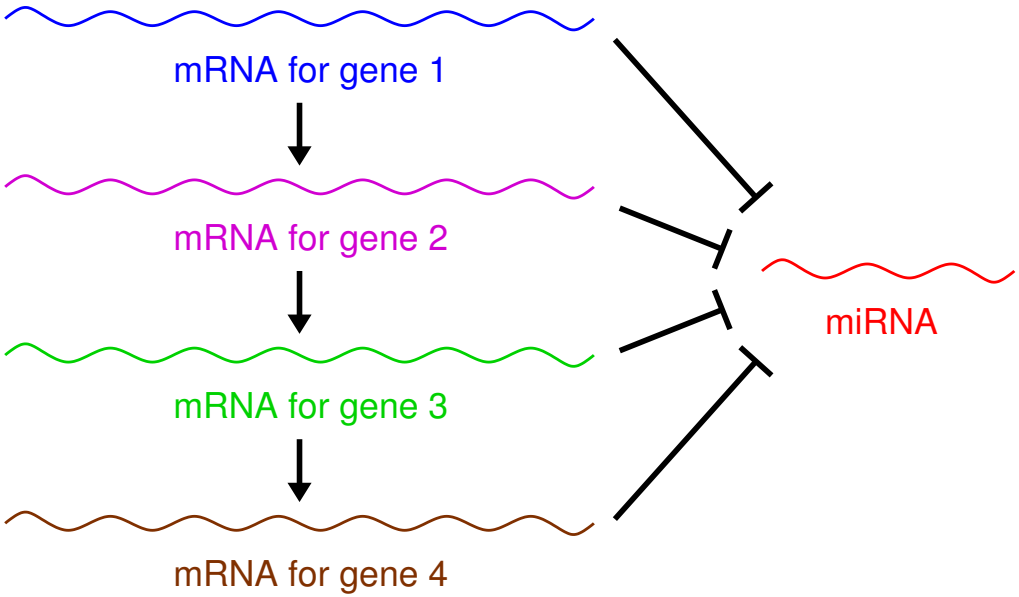


Confronting predictions

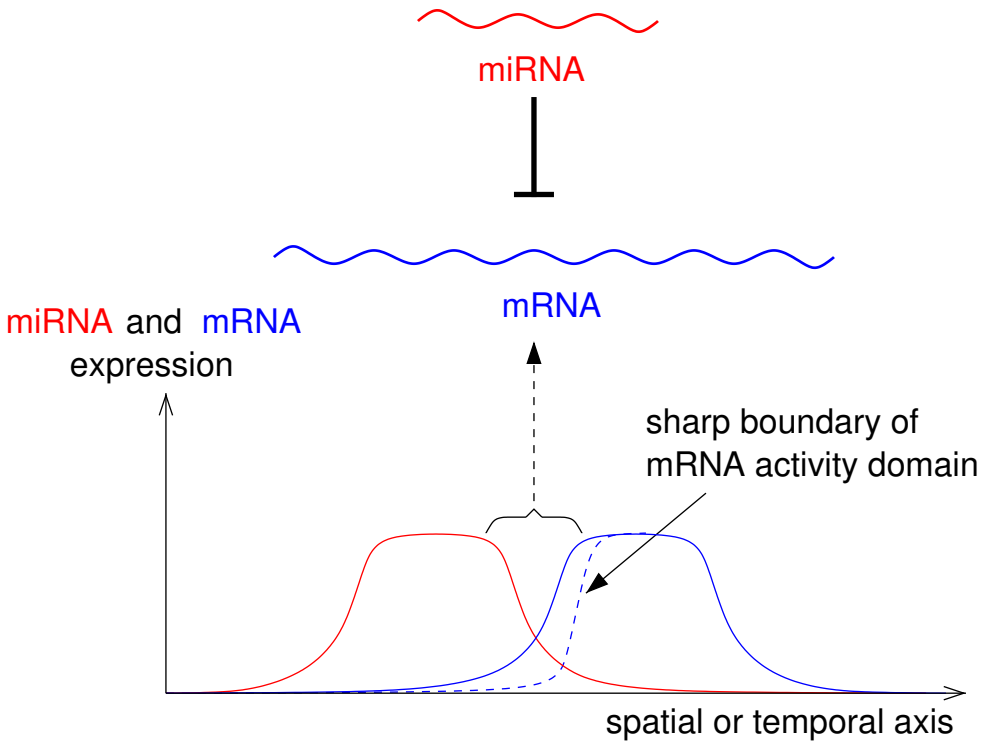
Confronting predictions



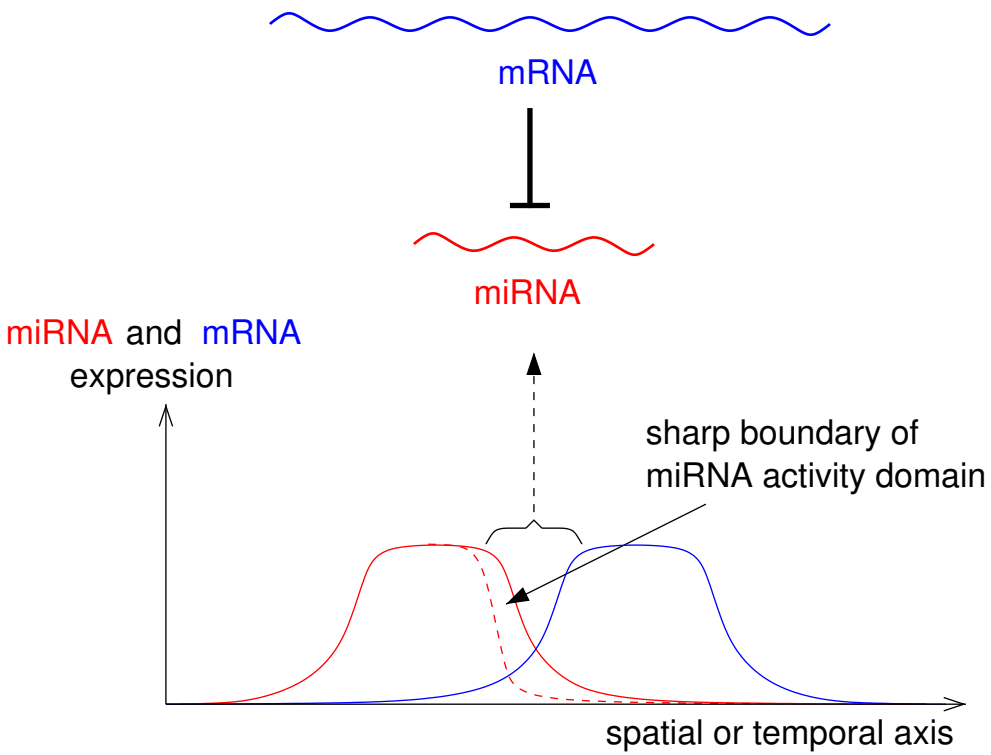
Confronting predictions



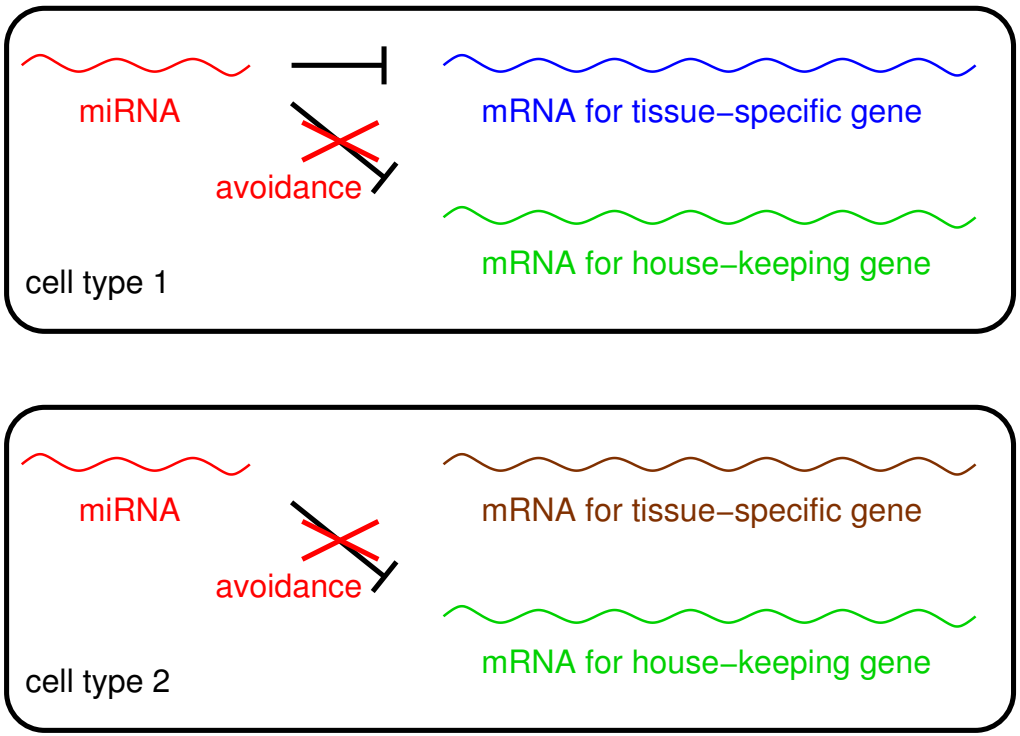
Confronting predictions



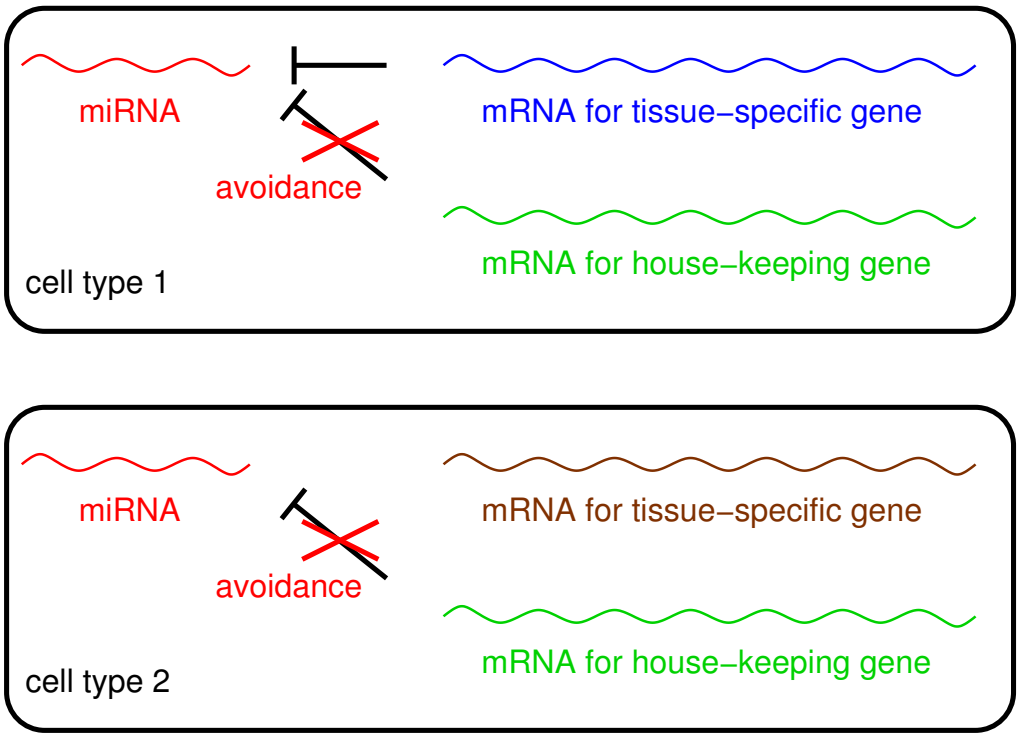
Confronting predictions



Confronting predictions



Confronting predictions



Discriminative predictions

According to the new hypothesis:

According to the current theory:

Discriminative predictions

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:

Discriminative predictions

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 $\Longrightarrow$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

Discriminative predictions

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 $\Longrightarrow$ mRNA abundance should be positively correlated with miRNA binding site conservation.

According to the current theory:

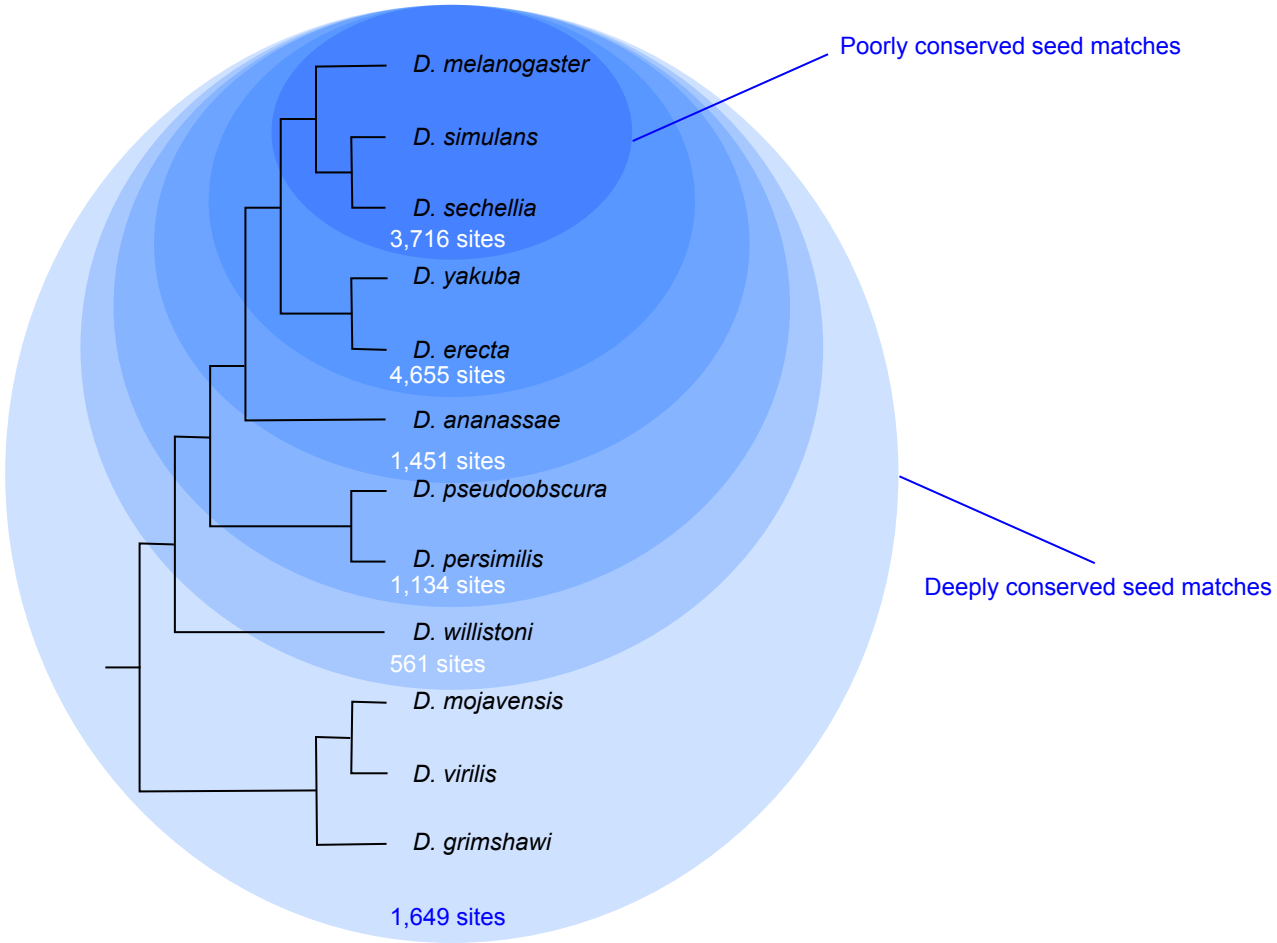
- Each target is a real target, its interaction is more or less conserved depending on the functional importance of the regulation (it is not expected to correlate with gene expression).

mRNA abundance and seed match conservation

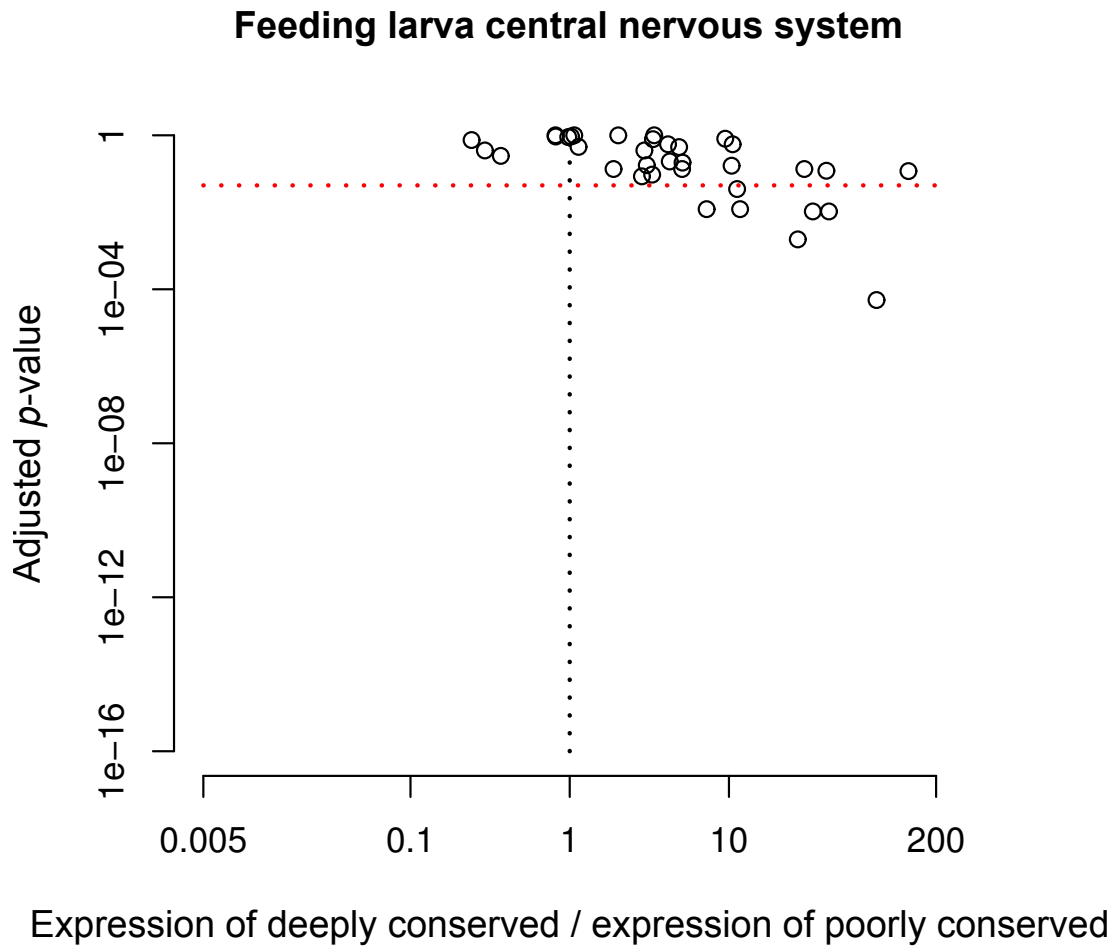
Does mRNA abundance correlate positively with miRNA binding site conservation?

mRNA abundance and seed match conservation

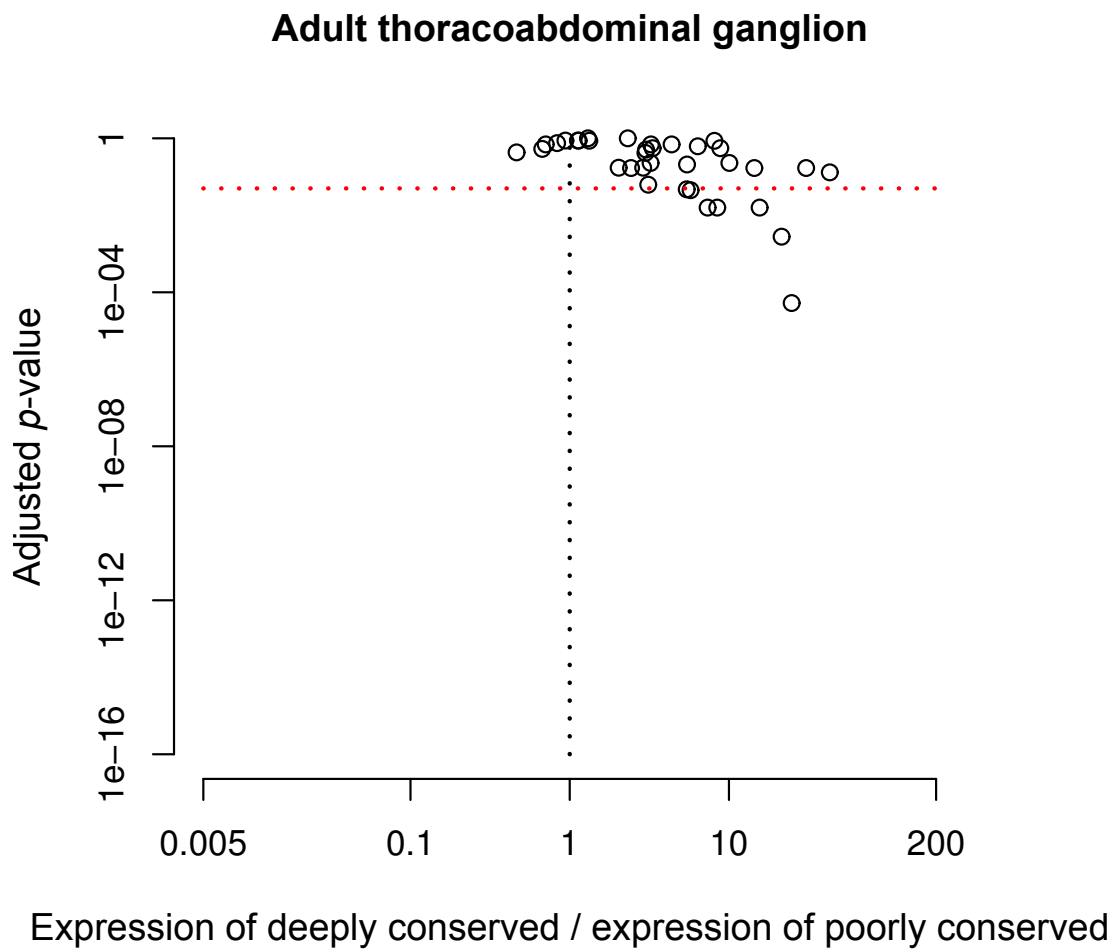
Does mRNA abundance correlate positively with miRNA binding site conservation?



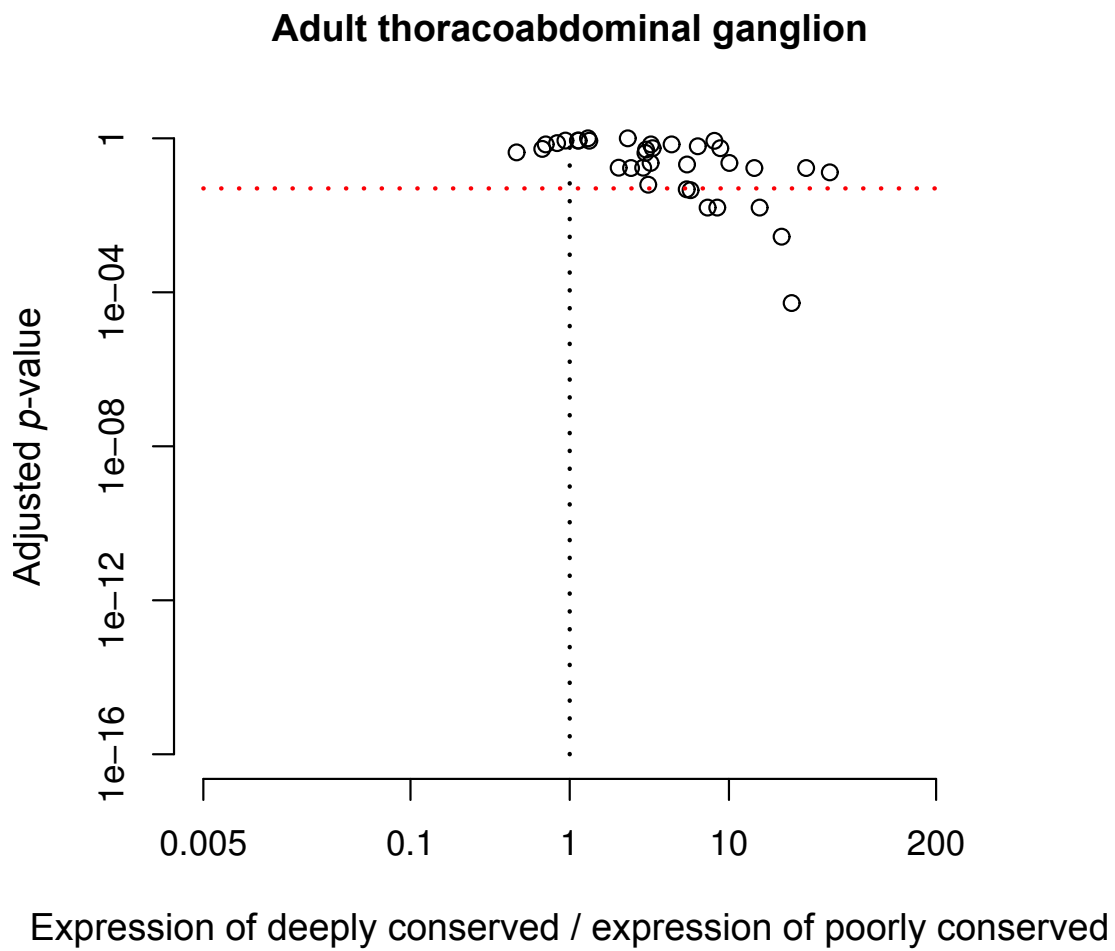
mRNA abundance and seed match conservation



mRNA abundance and seed match conservation



mRNA abundance and seed match conservation



+31 other fly tissues, similar results: ▸ Supplementary data

+36 mouse tissues, same results: ▸ Supplementary data

Discriminative predictions

According to the new hypothesis:

According to the current theory:

Discriminative predictions

According to the new hypothesis:

- Pseudo-targets do not need to be coding to exhibit conserved miRNA binding sites.

According to the current theory:

Discriminative predictions

According to the new hypothesis:

- Pseudo-targets do not need to be coding to exhibit conserved miRNA binding sites $\Longrightarrow$ there could be non-coding RNAs whose only role is to sequester miRNAs.

According to the current theory:

Discriminative predictions

According to the new hypothesis:

- Pseudo-targets do not need to be coding to exhibit conserved miRNA binding sites $\Longrightarrow$ there could be non-coding RNAs whose only role is to sequester miRNAs.

According to the current theory:

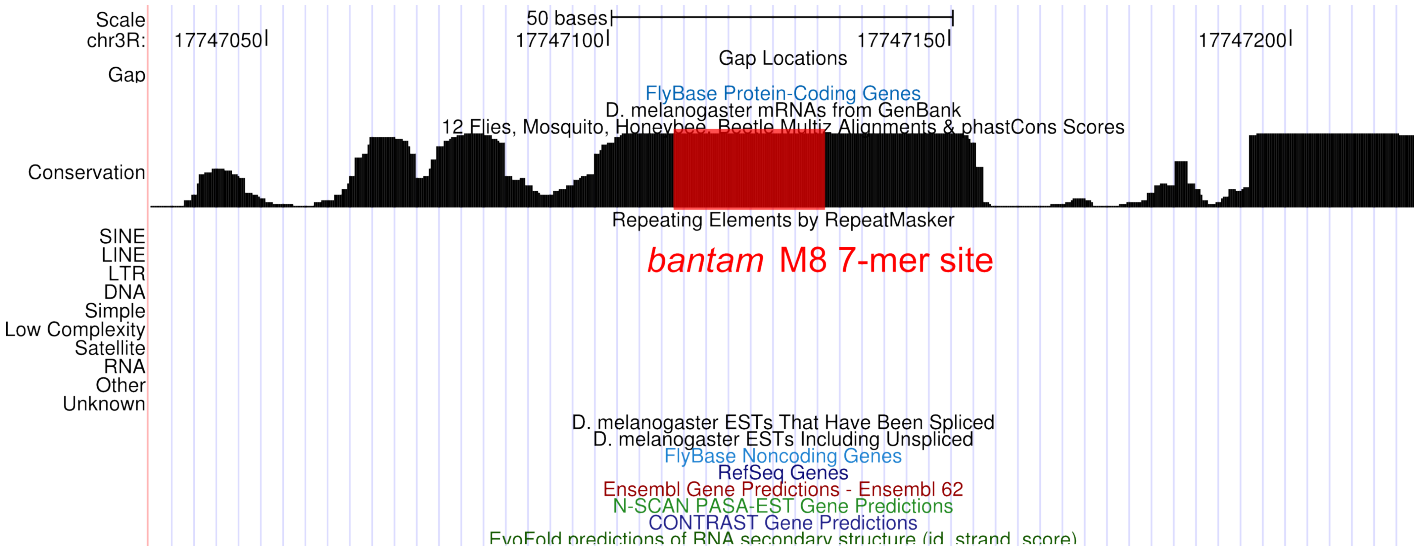
- Non-coding RNAs with conserved miRNA binding sites must have a function distinct from miRNA titration.

Non-coding pseudo-targets

Are there non-coding pseudo-targets?

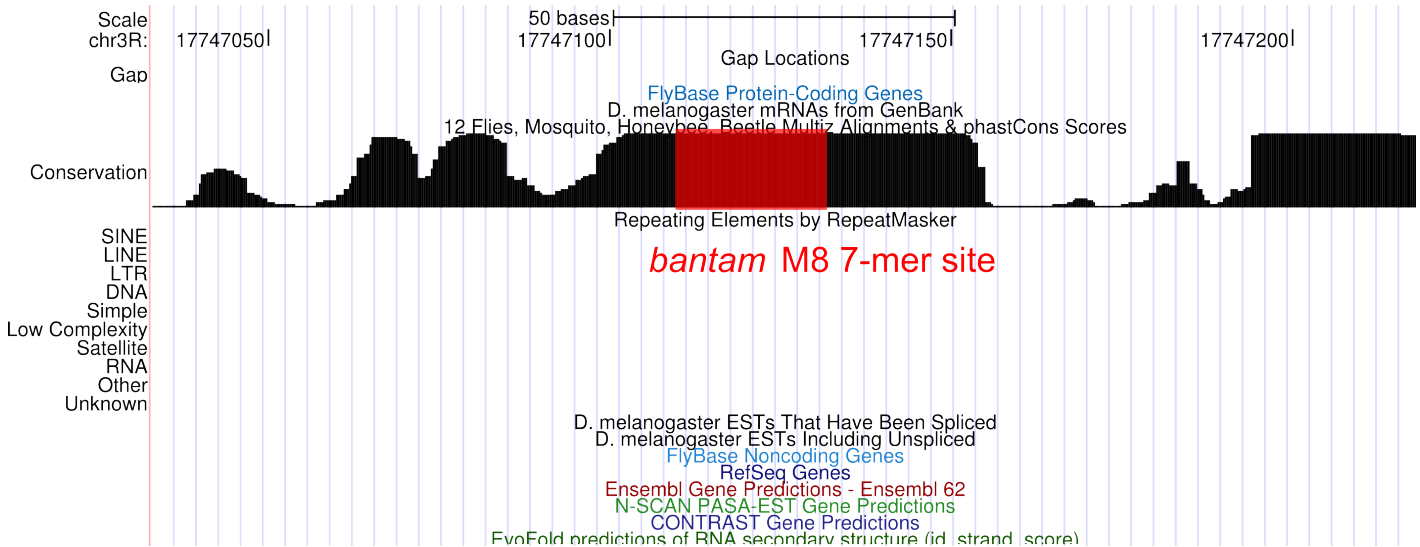
Non-coding pseudo-targets

Are there non-coding pseudo-targets?



Non-coding pseudo-targets

Are there non-coding pseudo-targets?



13 candidates identified, molecular characterization in progress (Natalia Pinzón Restrepo, Anna Sergeeva).

Discriminative predictions

According to the new hypothesis:

According to the current theory:

Discriminative predictions

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:

Discriminative predictions

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).

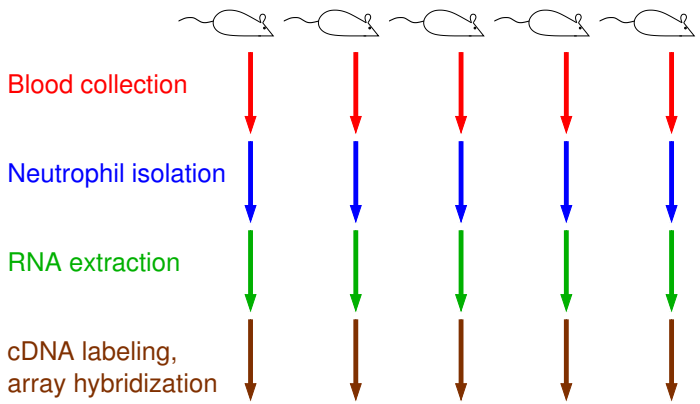
Natural variability vs. miRNA-guided repression

Natural variability vs. miRNA-guided repression

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

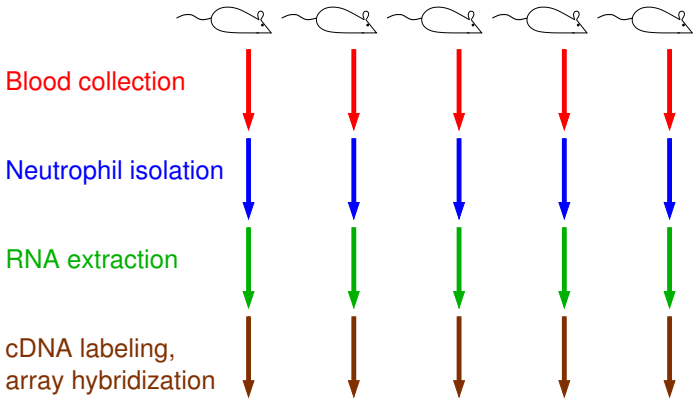
Natural variability vs. miRNA-guided repression

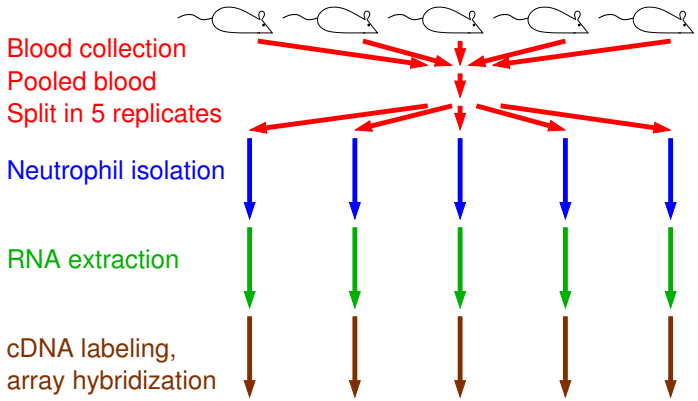
Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.



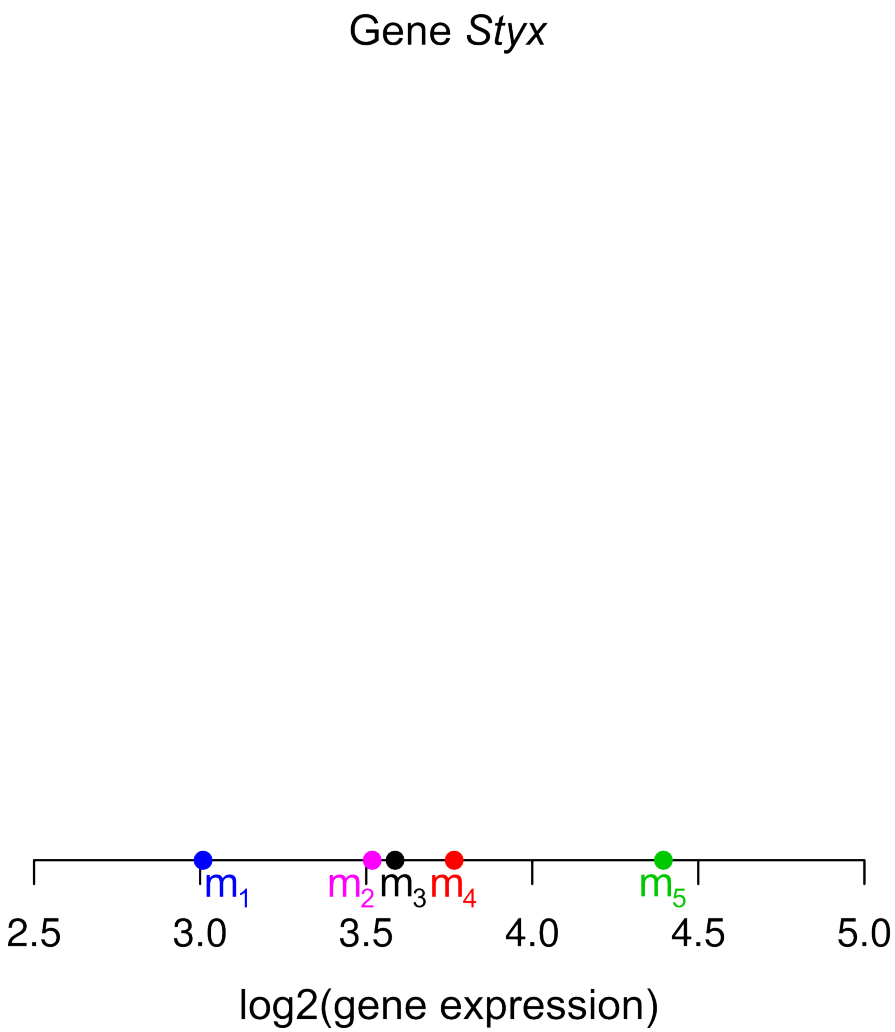
Natural variability vs. miRNA-guided repression

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

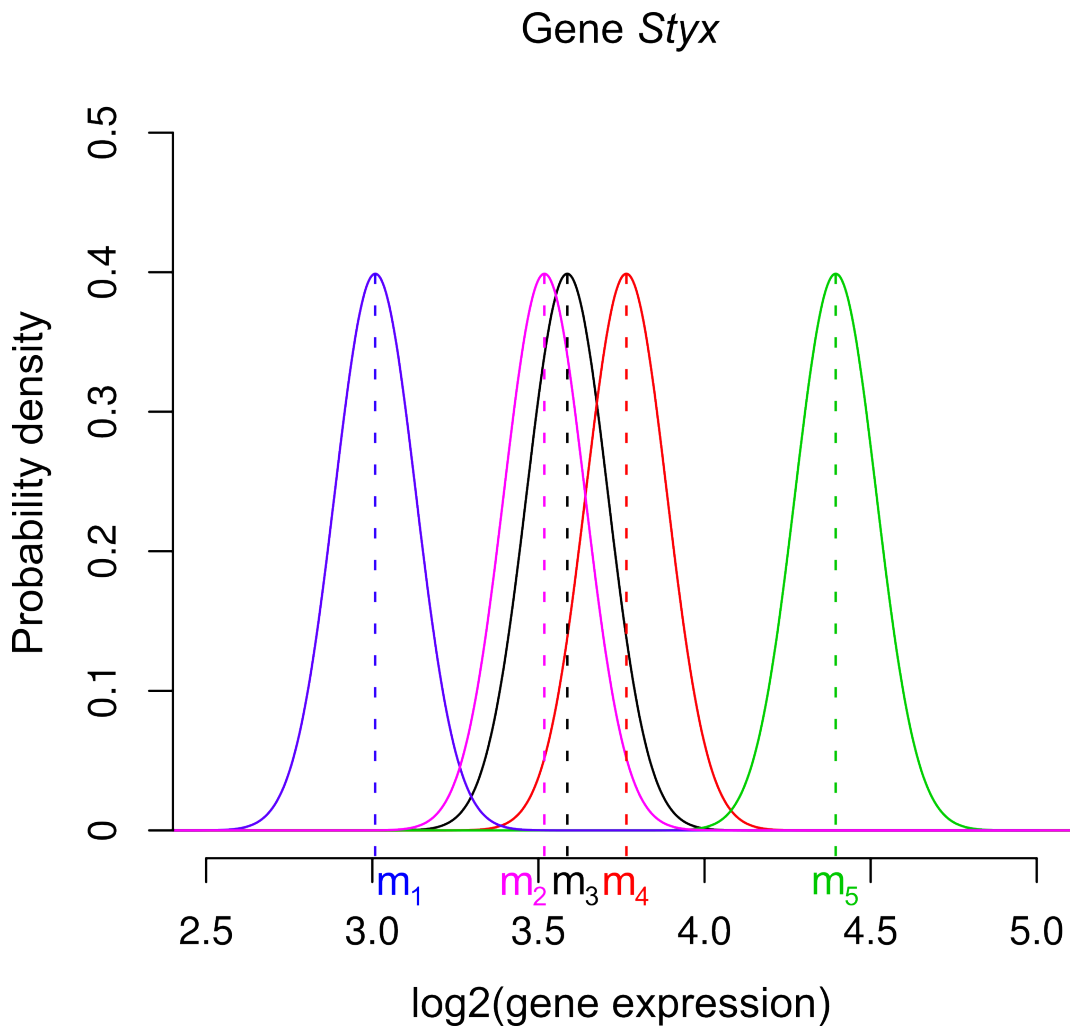




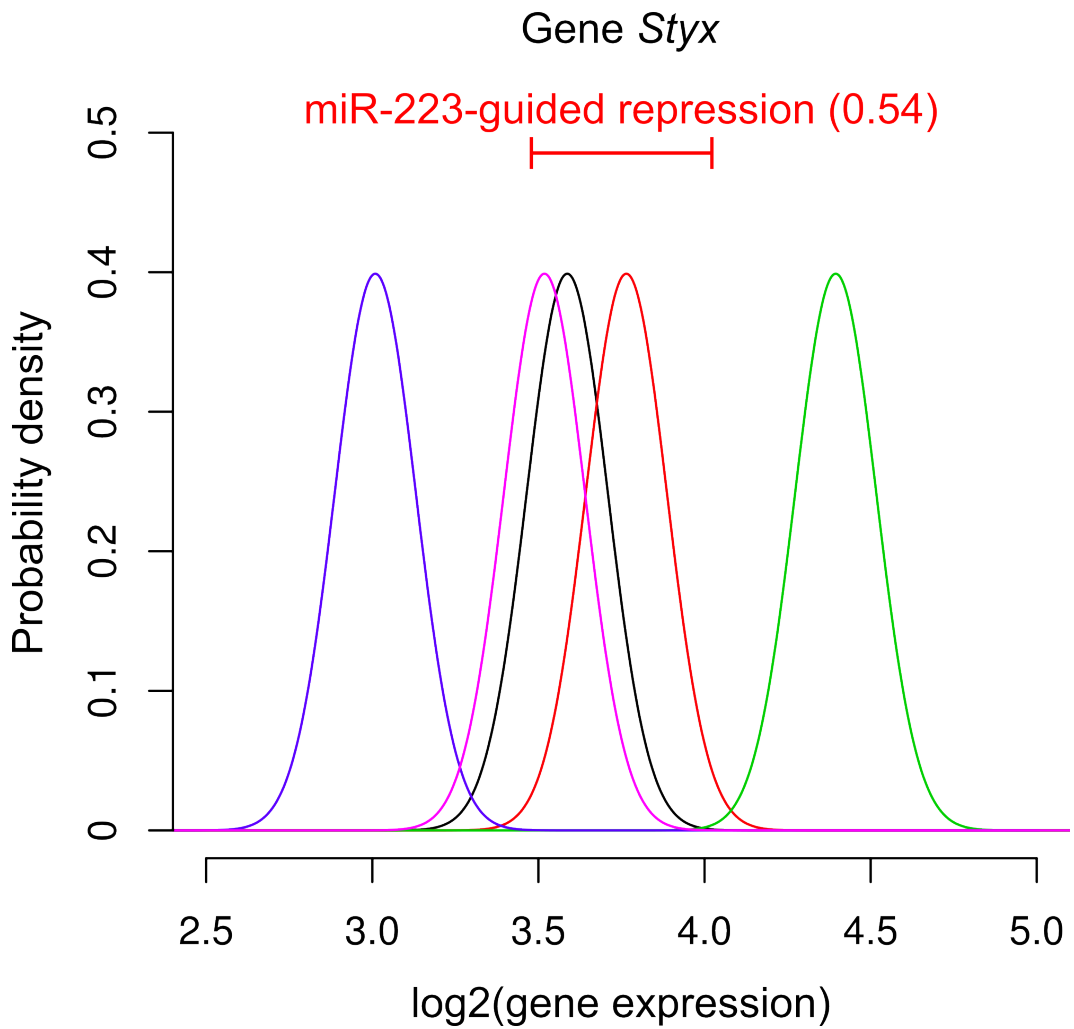
Natural variability vs. miRNA-guided repression



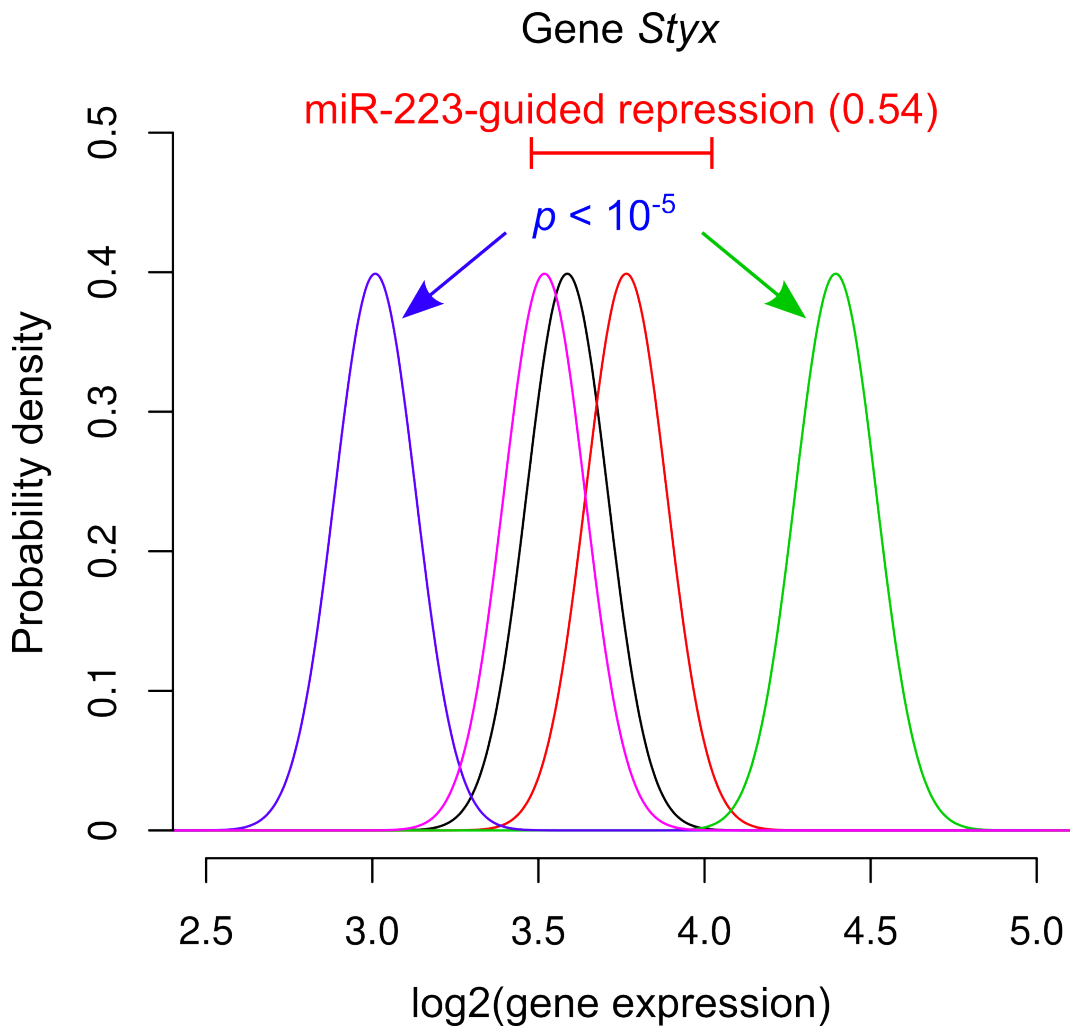
Natural variability vs. miRNA-guided repression



Natural variability vs. miRNA-guided repression



Natural variability vs. miRNA-guided repression



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

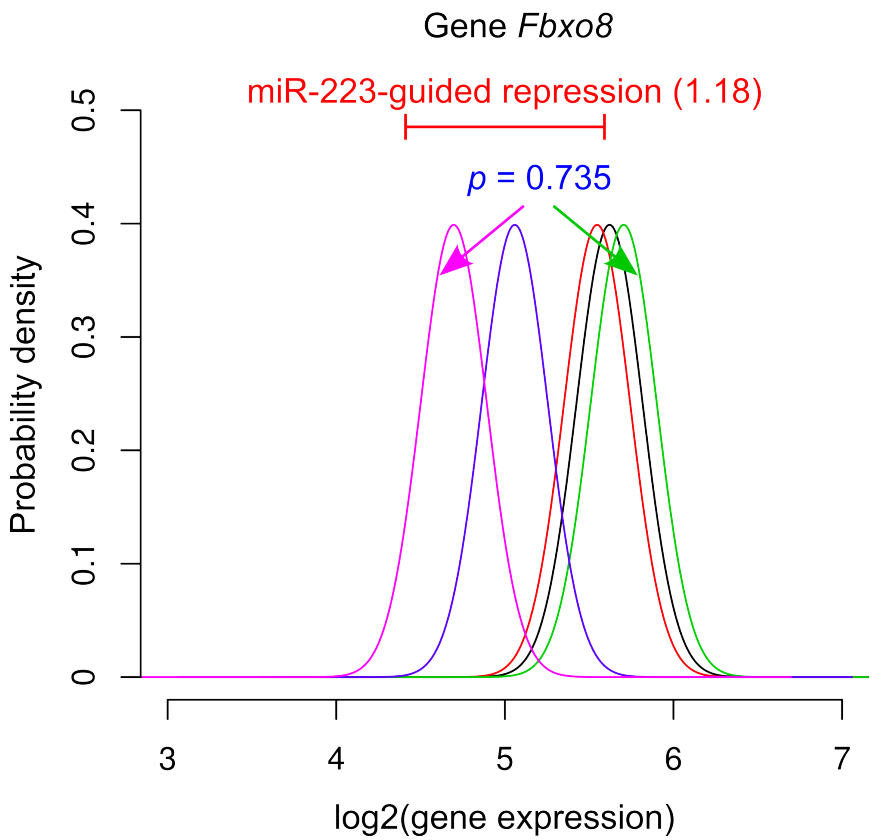
Natural variability vs. miRNA-guided repression

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).

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).



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.

Cells can modulate miRNA activity in a cell type-specific manner (integrating expression information from hundreds of genes on a single regulator)

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.

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.

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.

Microscopic events which are neutral in evolutionary terms.

Are there pseudo-targets for these other regulators?

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.

Microscopic events which are neutral in evolutionary terms.

Are there pseudo-targets for these other regulators?

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

Natalia Pinzón Restrepo

Acknowledgements

Anna Sergeeva

Laura Martinez

Natalia Pinzón Restrepo

Hélène Royo and James Turner (MRC, London, UK)
Jessy Presumey and Florence Apparailly (INM, Montpellier, France)

Acknowledgements

Anna Sergeeva

Laura Martinez

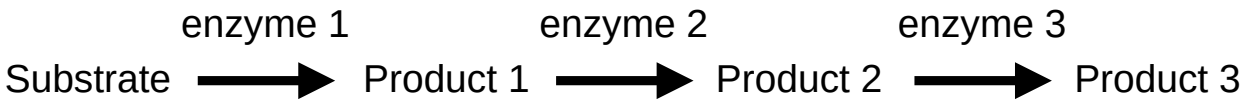
Natalia Pinzón Restrepo

Hélène Royo and James Turner (MRC, London, UK)
Jessy Presumey and Florence Apparailly (INM, Montpellier, France)

Supplementary data

- Robustness of biological pathways
- Conservative approximations in the assessment of abundance/conservation correlation
- Positive correlation between gene expression and conservation of miRNA binding sites

Second paradox

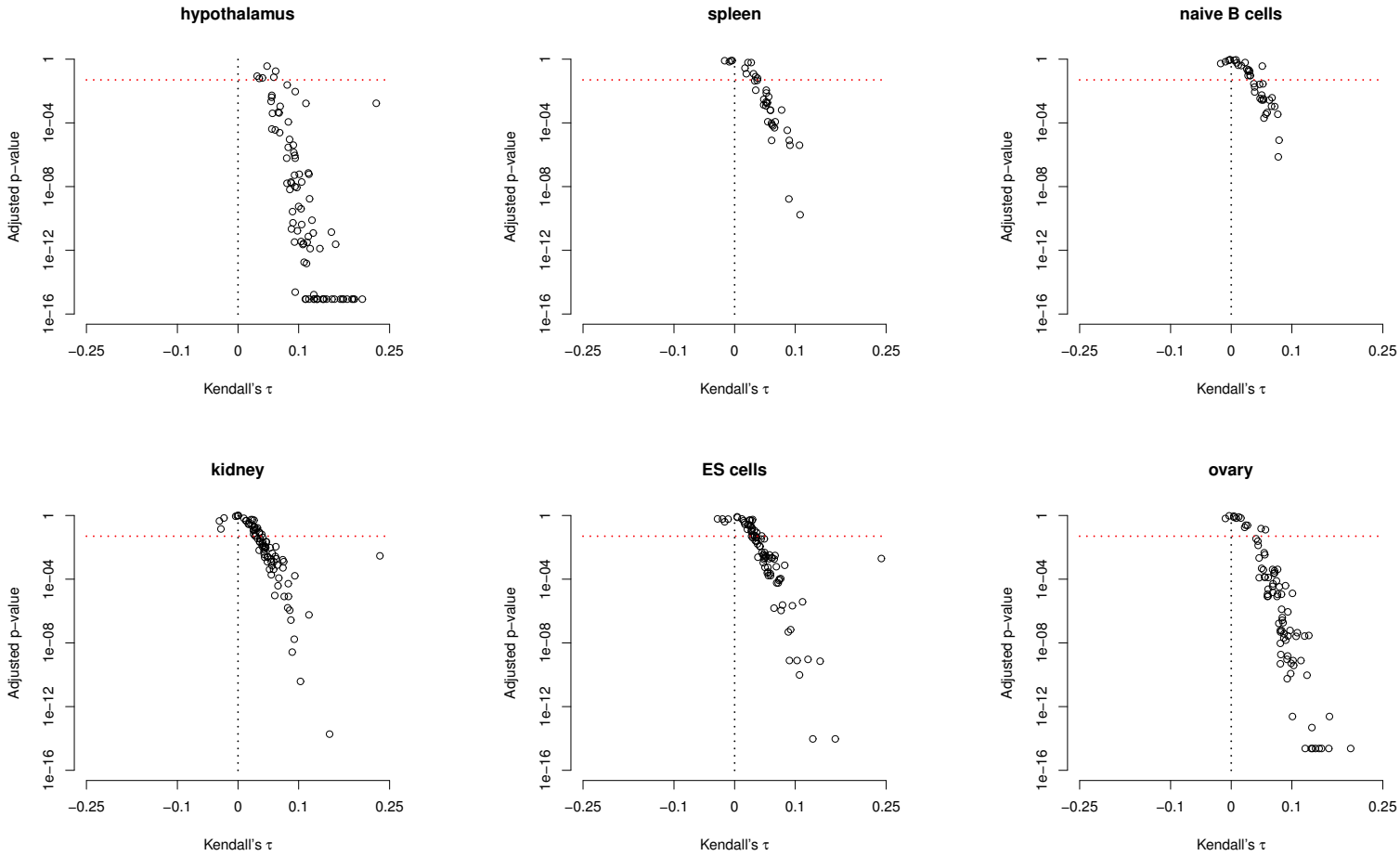


mRNA abundance and seed match conservation

- 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).

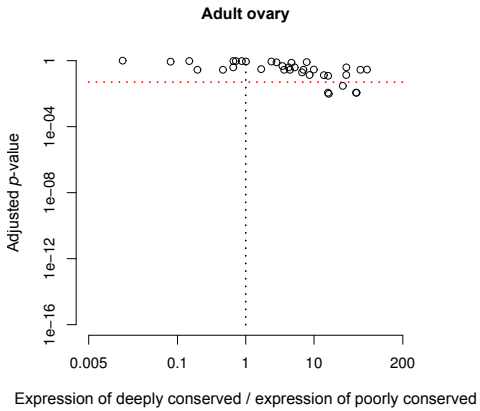
◂ Return

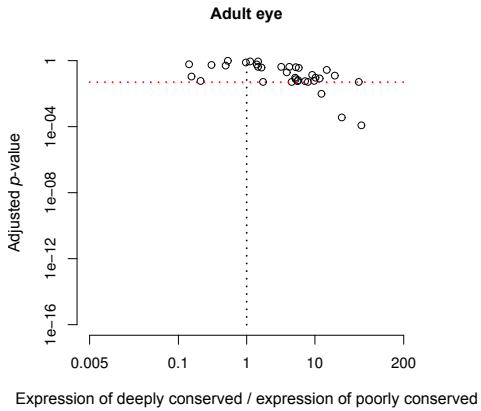
mRNA abundance and seed match conservation

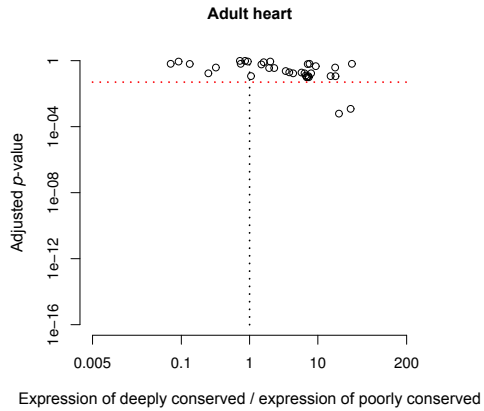


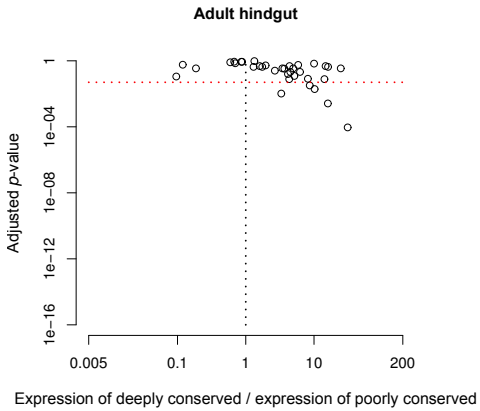
Return

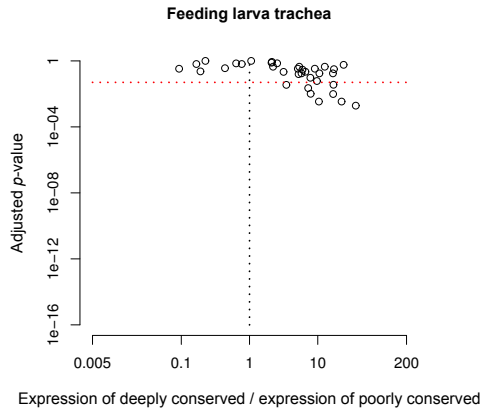
mRNA abundance and seed match conservation











◂ Return