Butterfly effect in molecular biology: the case of microRNAs

Hervé Seitz

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April 24, 2012

microRNA target identification

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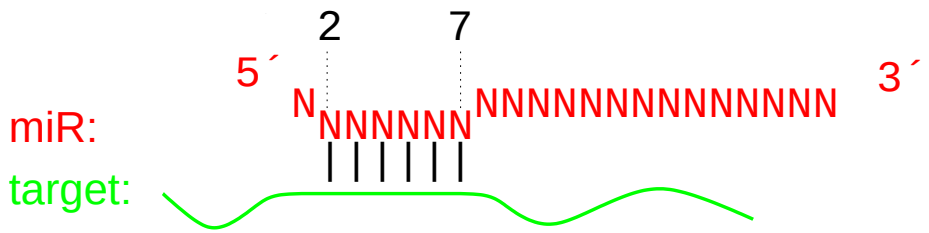

$\longrightarrow$ the "seed"

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miRNA-mediated repression is very modest (usually $<$ 2-fold): have these binding sites been conserved because they trigger a butterfly effect (large consequences for minor causes)?

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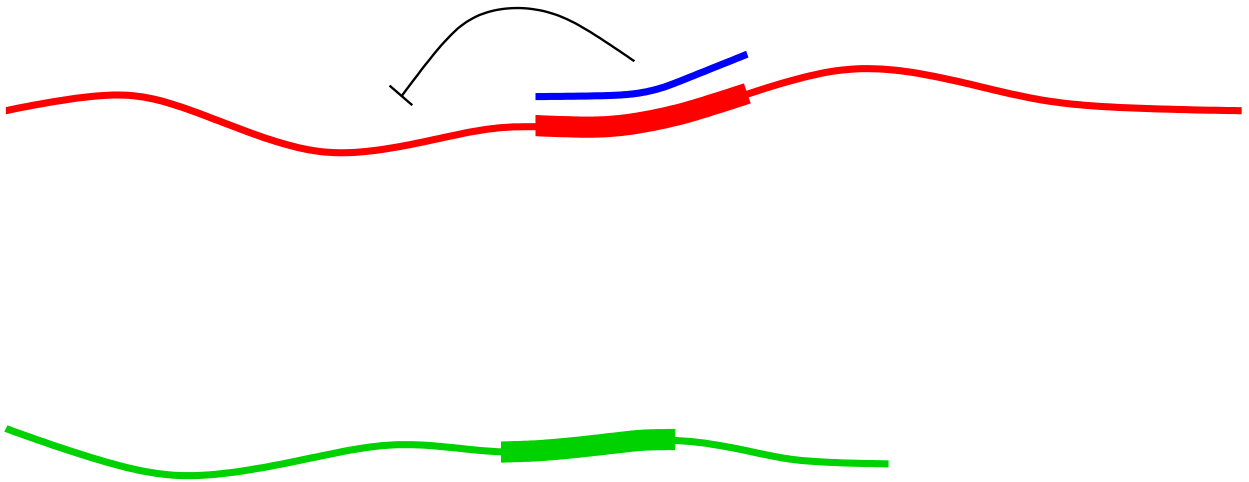

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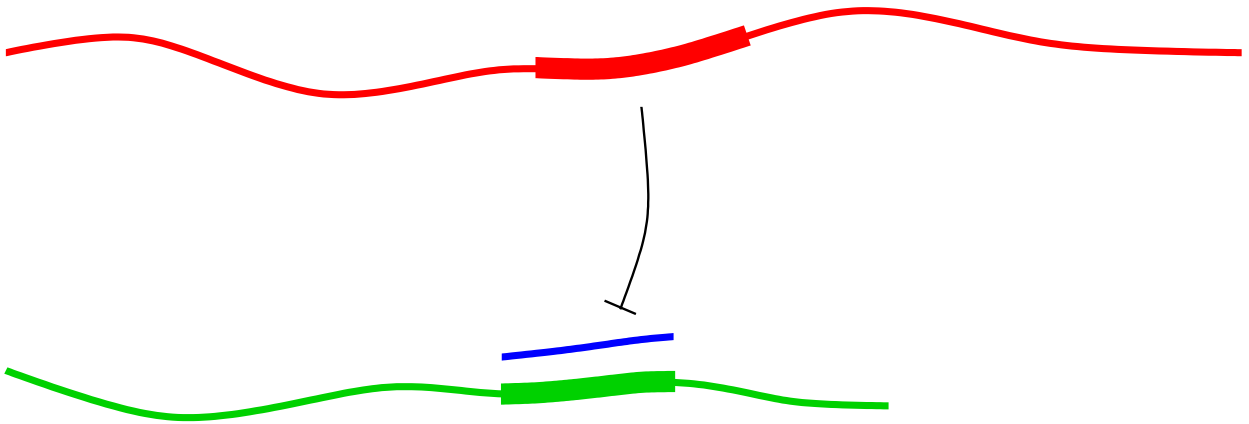

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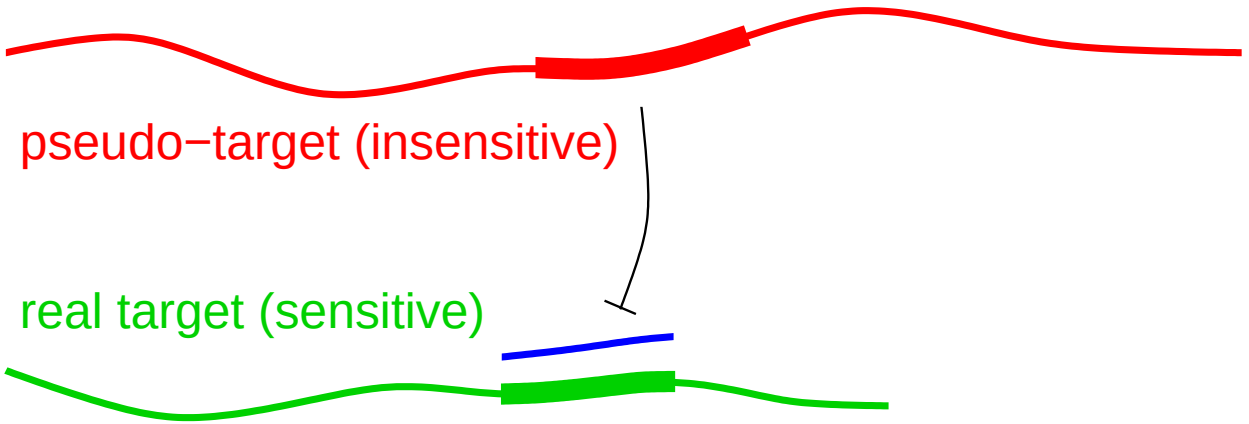

A new interpretation

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).

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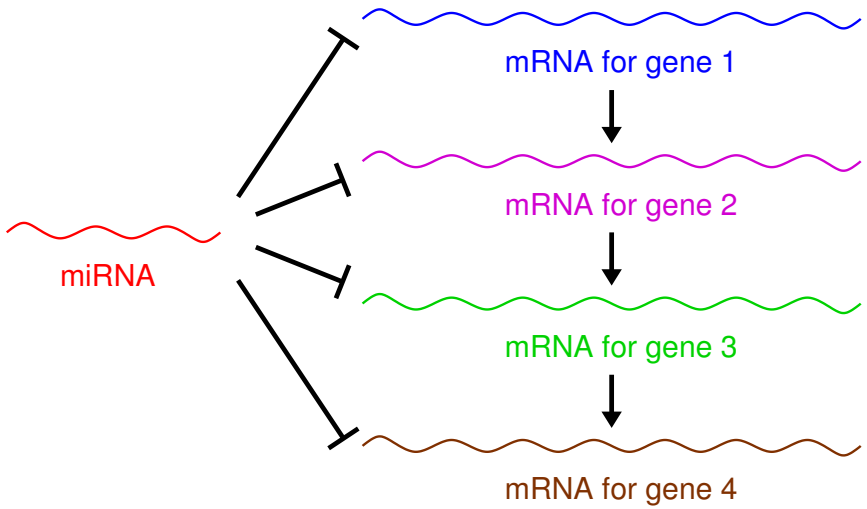

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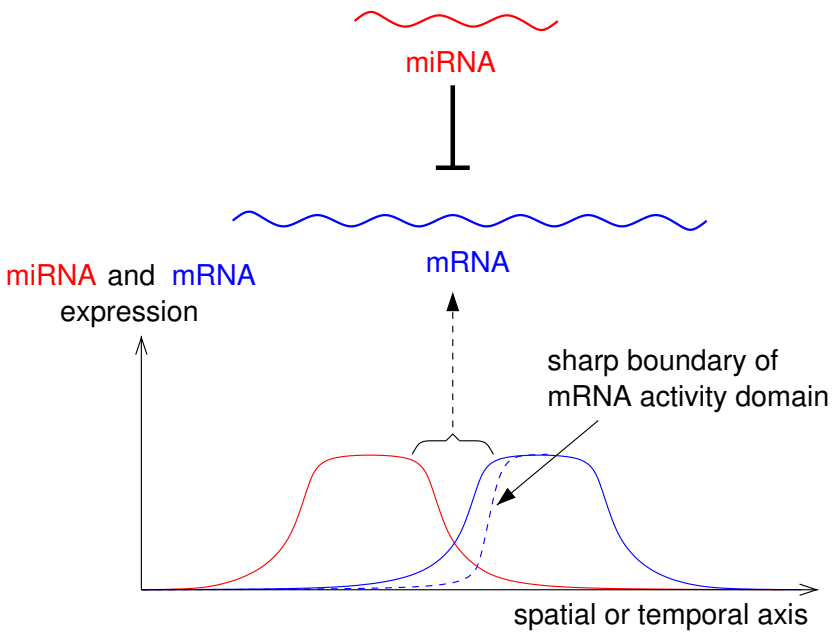

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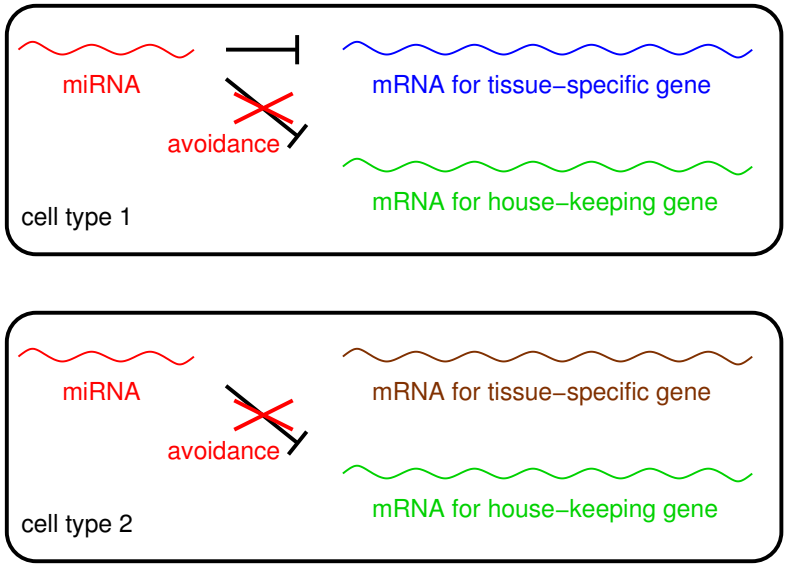

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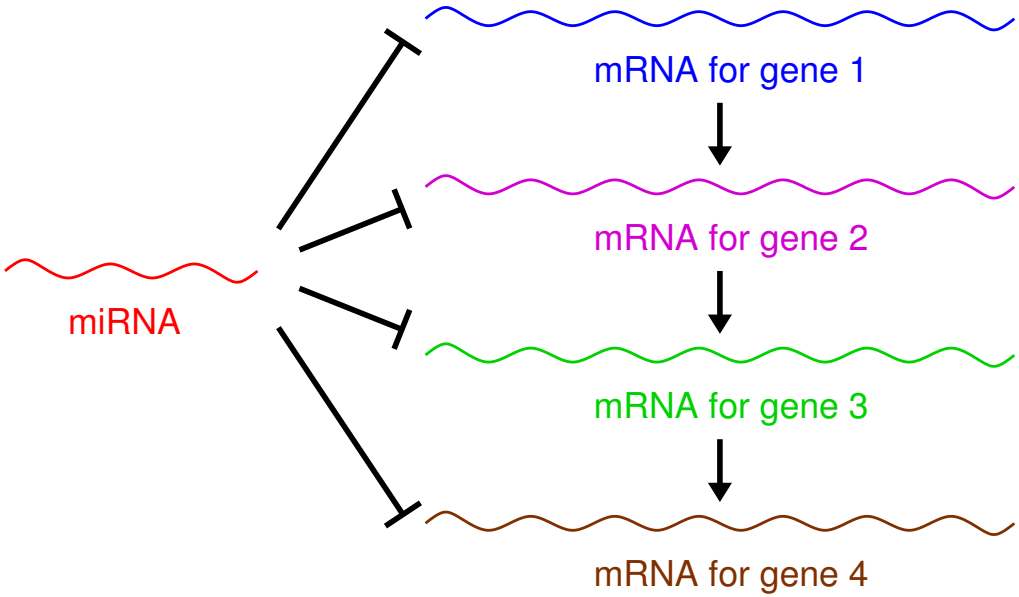

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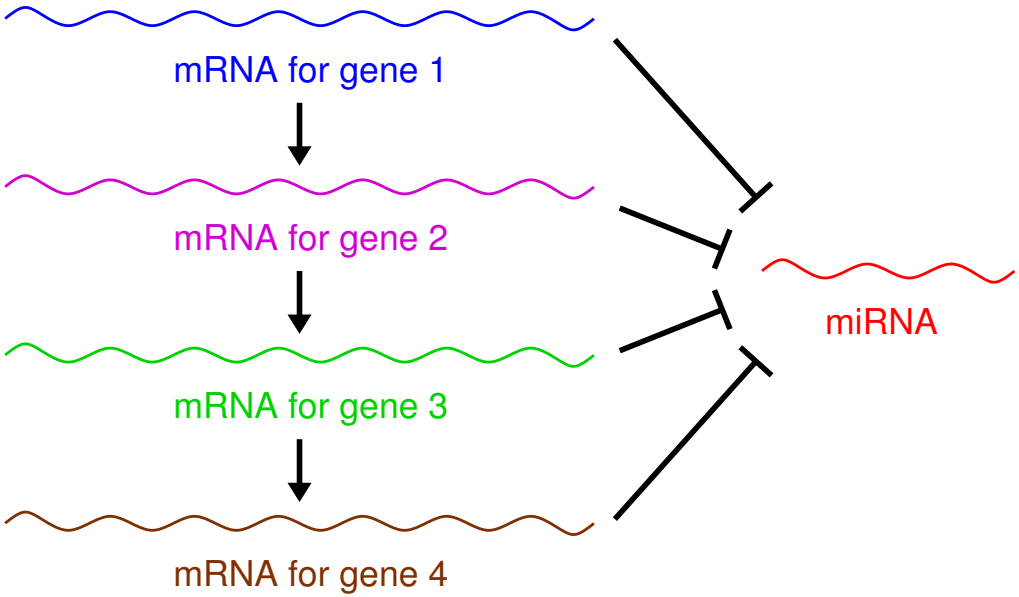

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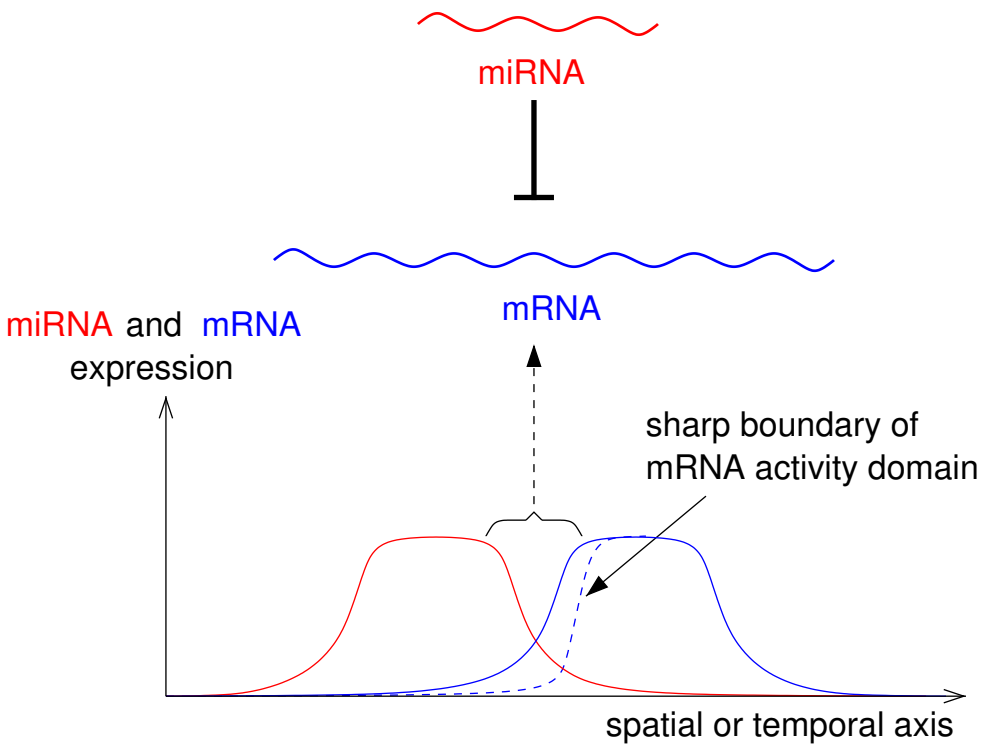

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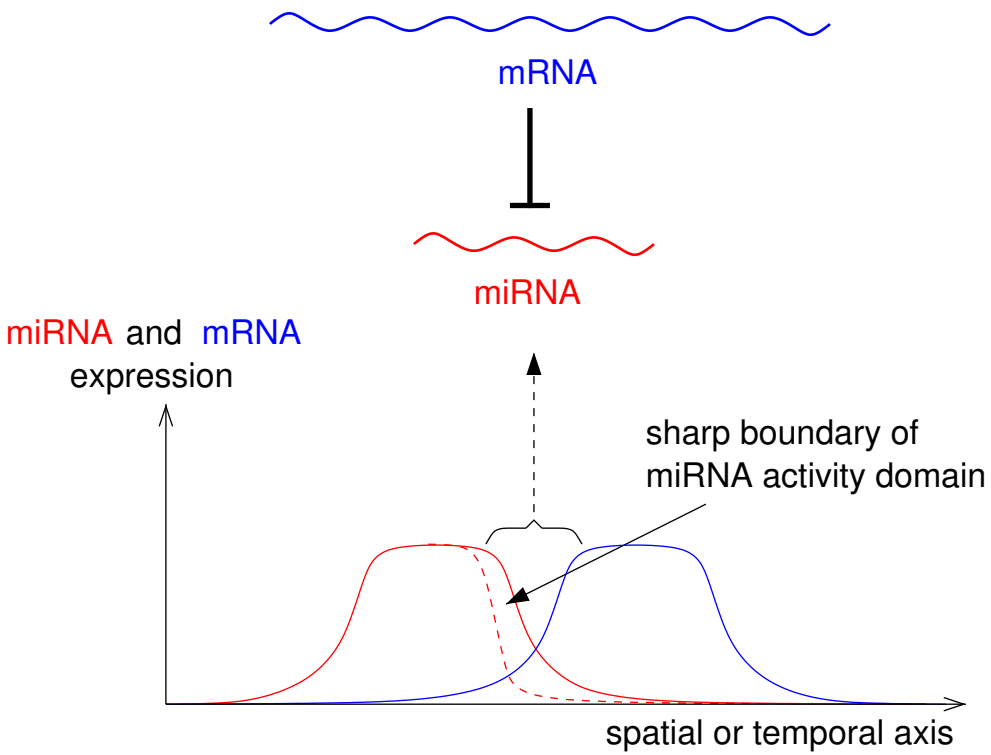

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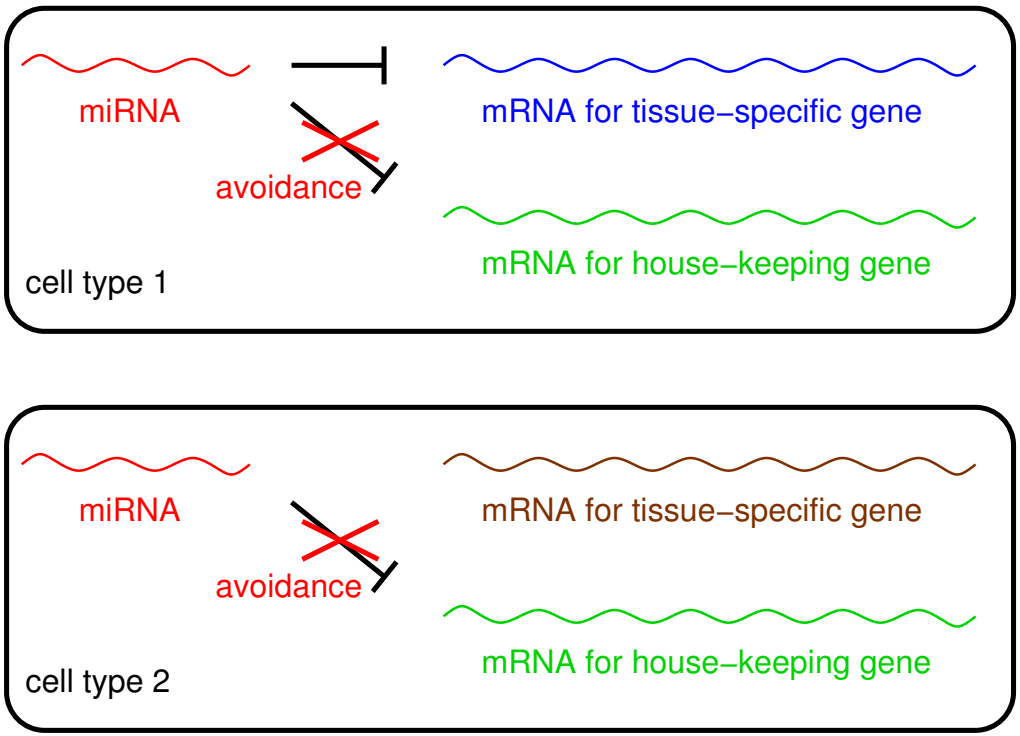

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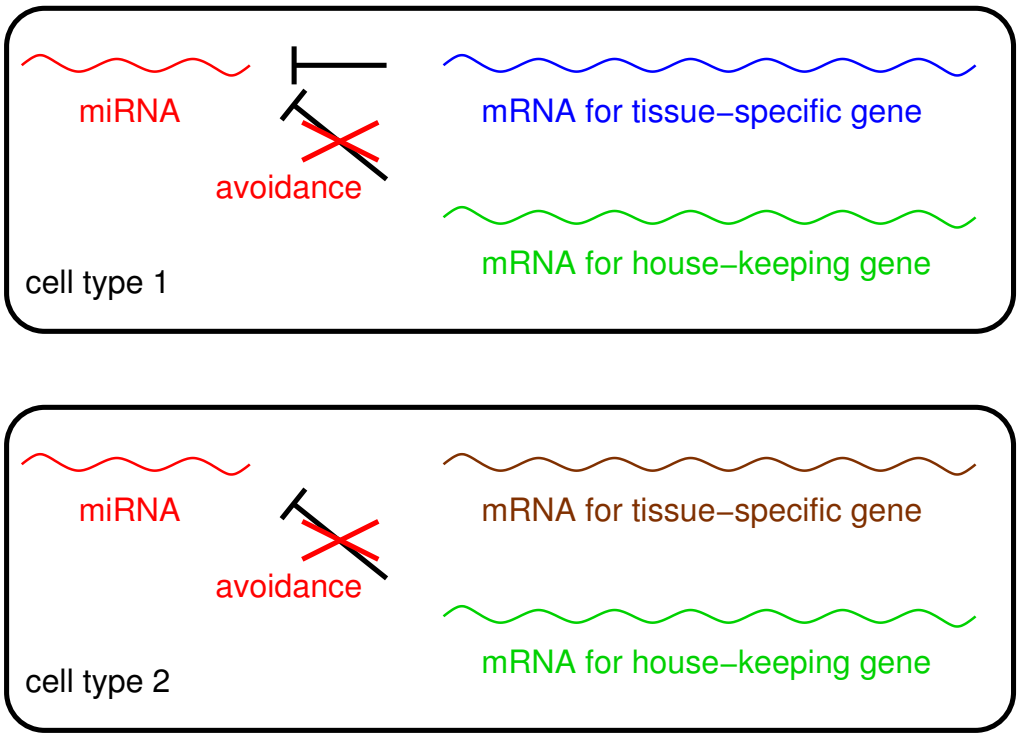

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According to the current theory:

- 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

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

▸ Supplementary data

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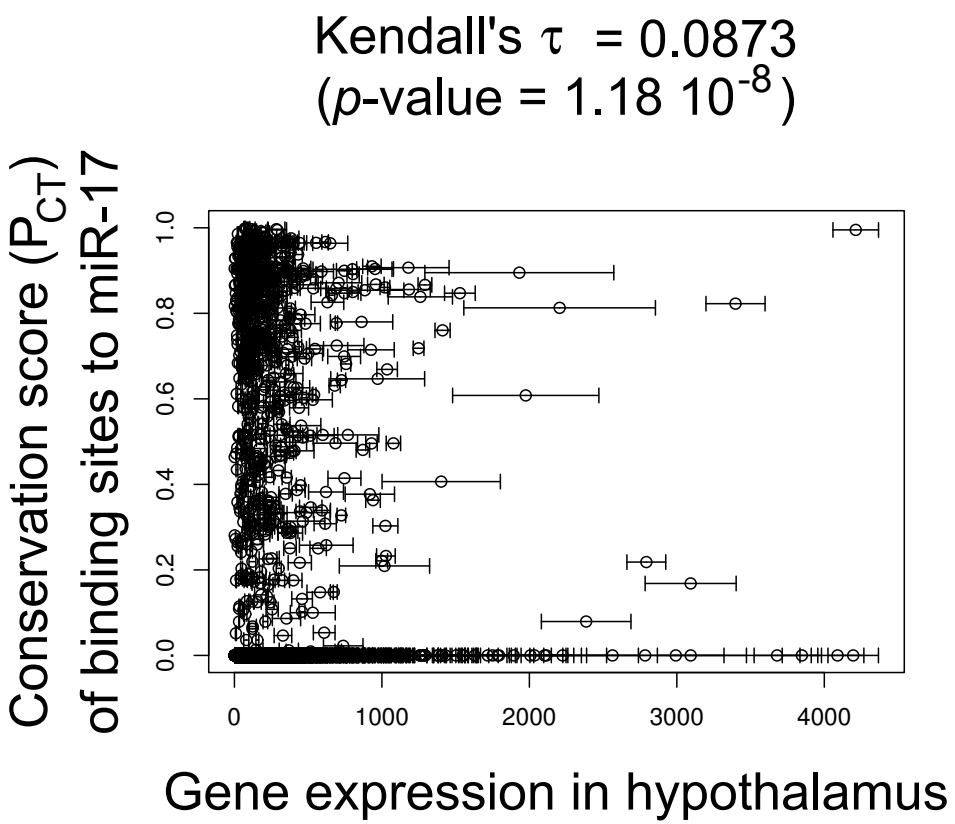

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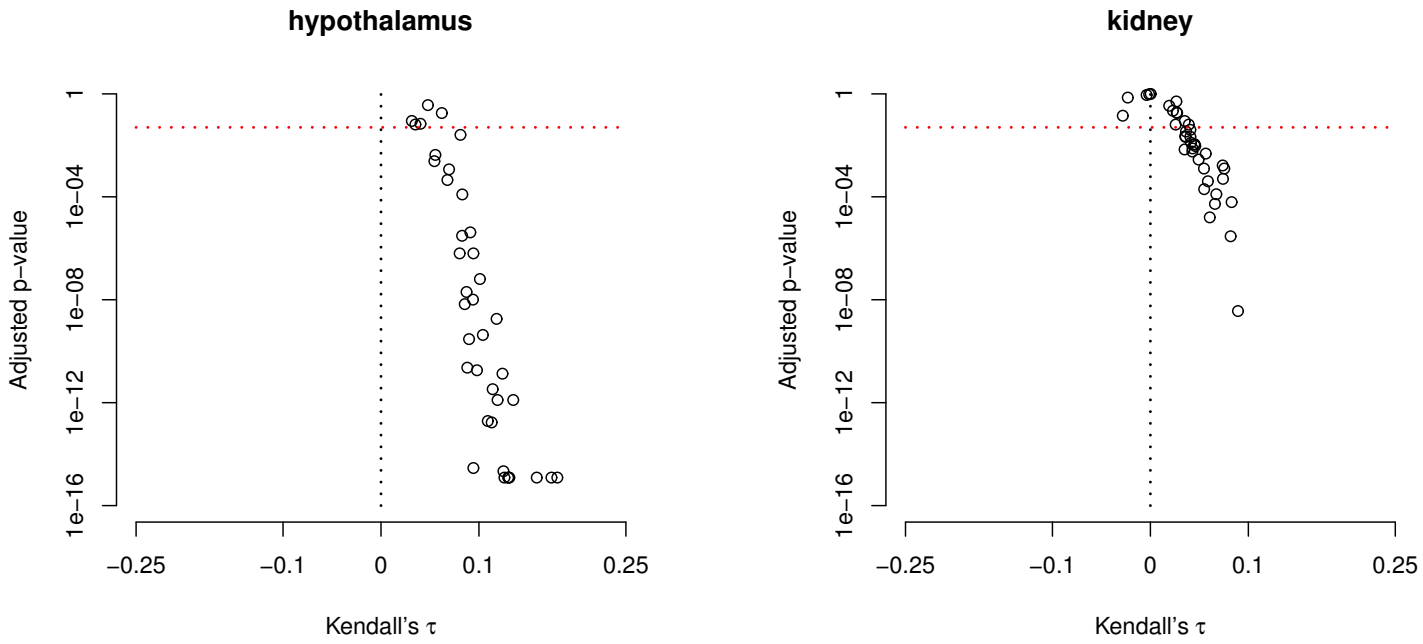

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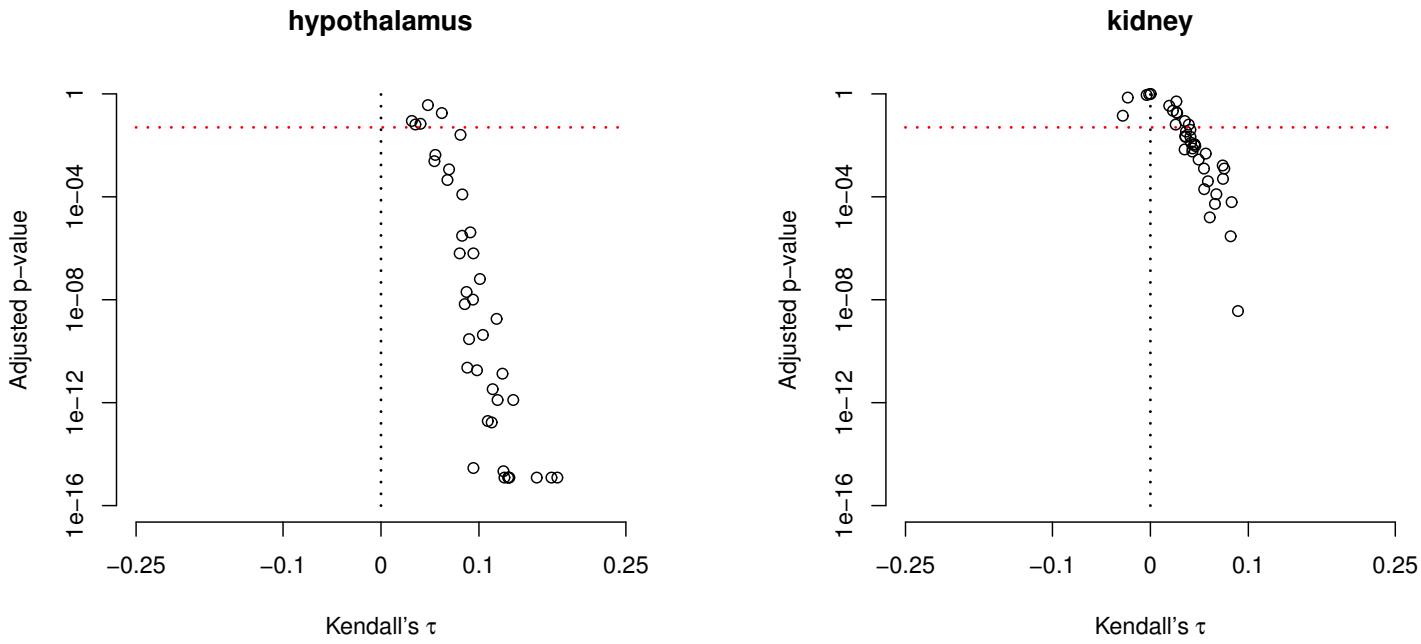

+34 other mouse tissues, same results: ▸ Supplementary data

+33 fly tissues, same results: ▸ Supplementary data

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According to the current theory:

- miRNA binding site conservation is not expected to correlate with dose-sensitivity.

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- Counting the number of gene members in each gene family: measures the duplicability of ancestral genes.
- Liang *et al.*, 2008: estimating the structural robustness of the components of multi-protein complexes (protein "under-wrapping" leads to dose-sensitivity in invertebrates).

Dose-sensitivity and seed match conservation

Expected	Correlation coefficient
Positive correlation between haplo-insufficiency probability and site conservation	human: $+0.190$ ($p < 2.2\ \ 10^{-16}$)

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Positive correlation between protein under-wrapping and site conservation	worm: $+0.070$ ($p = 0.64$) fly: $+46$ % ($p = 1.57\ \ 10^{-5}$)

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

Non-coding pseudo-targets

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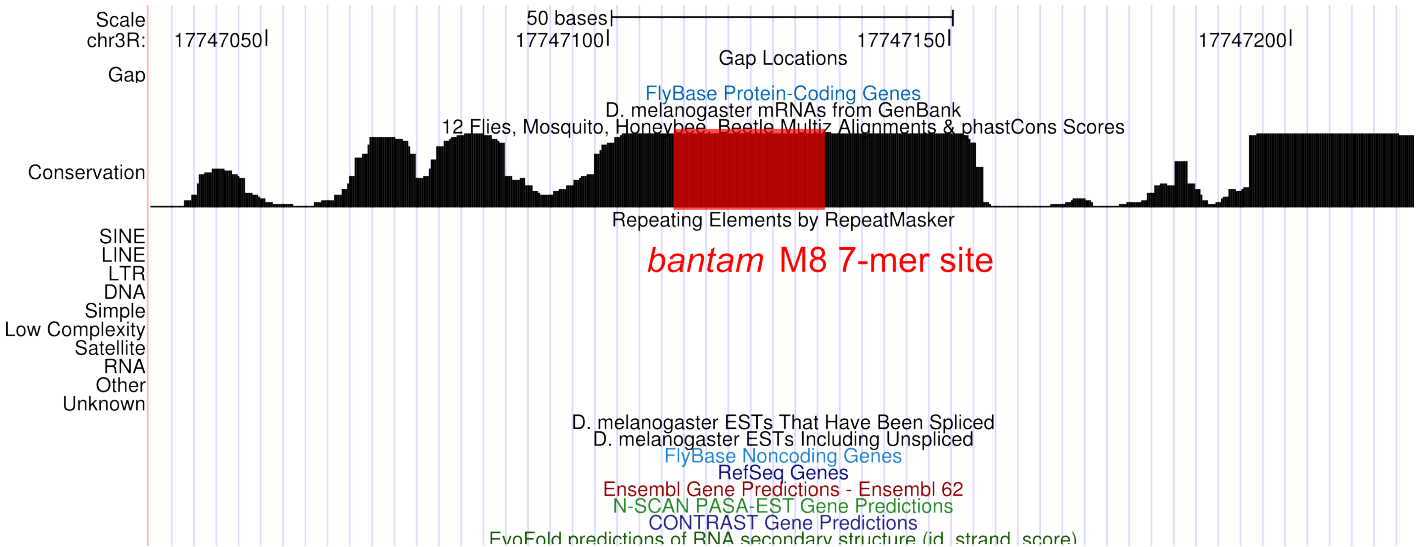

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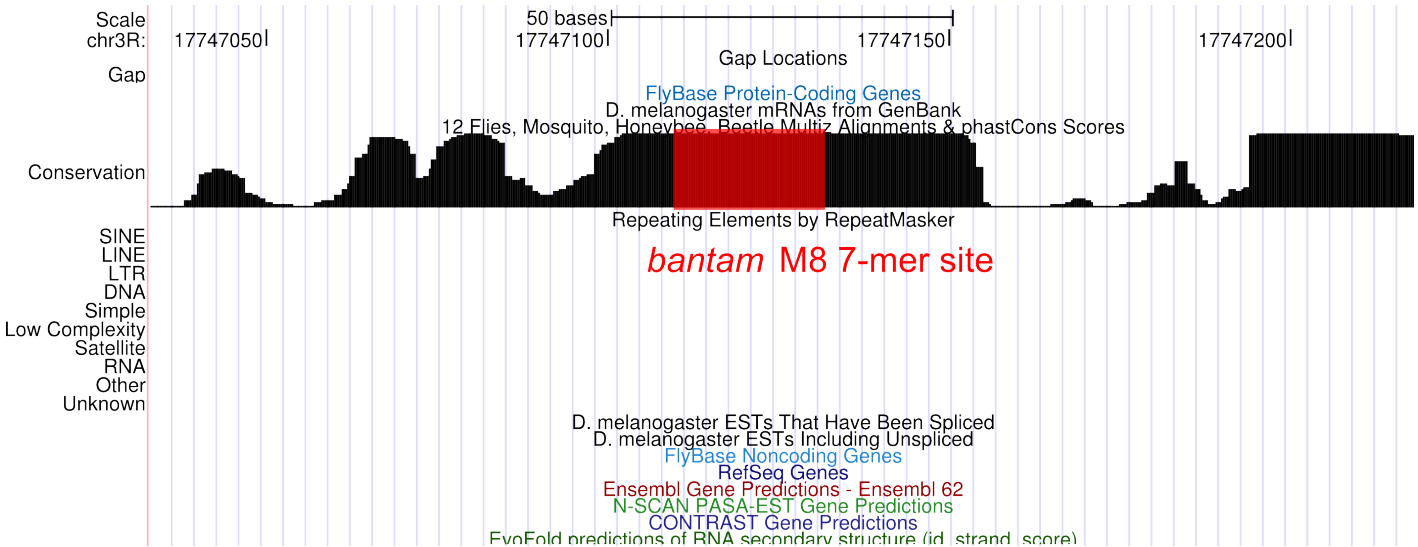

18 candidates identified, molecular characterization in progress (Natalia Pinzon Restrepo, Anna Sergeeva).

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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

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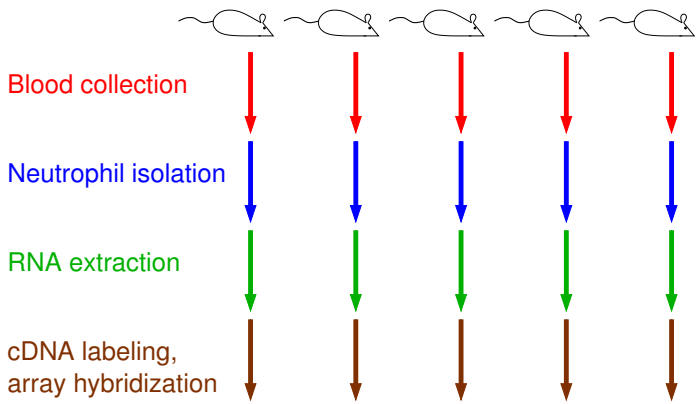

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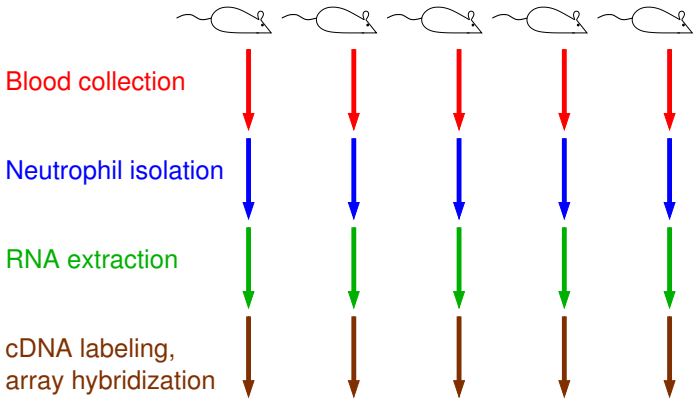

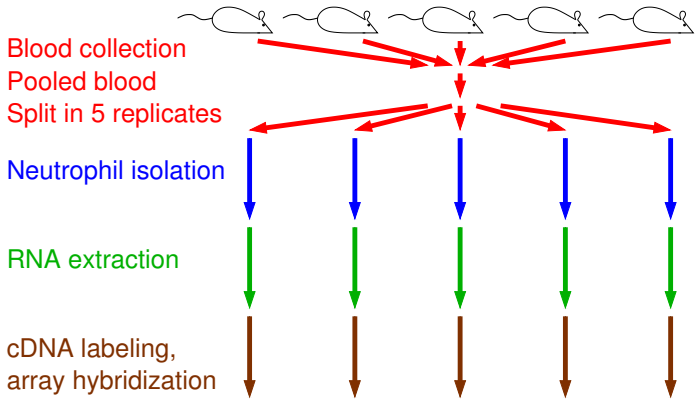

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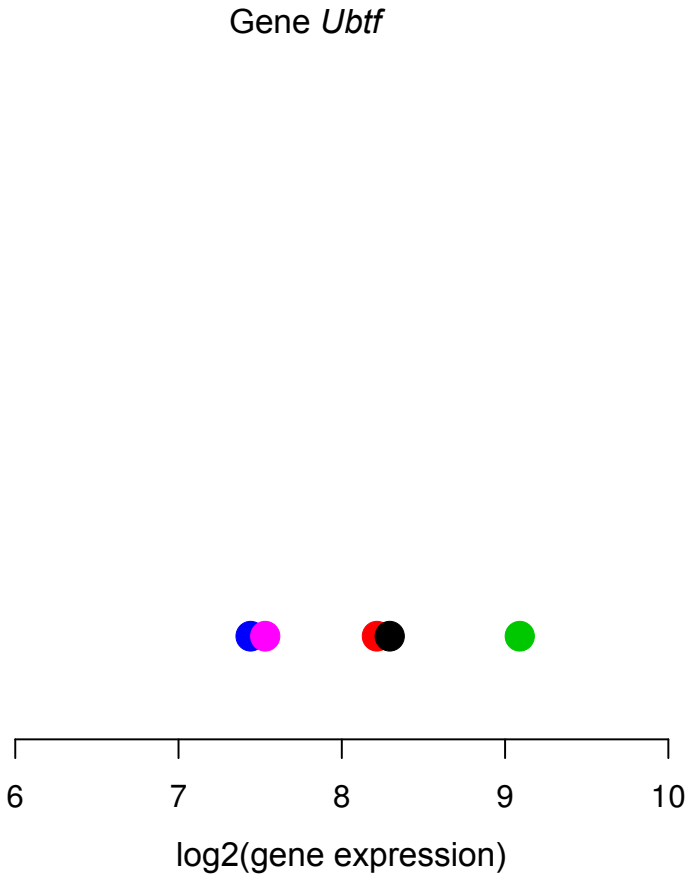

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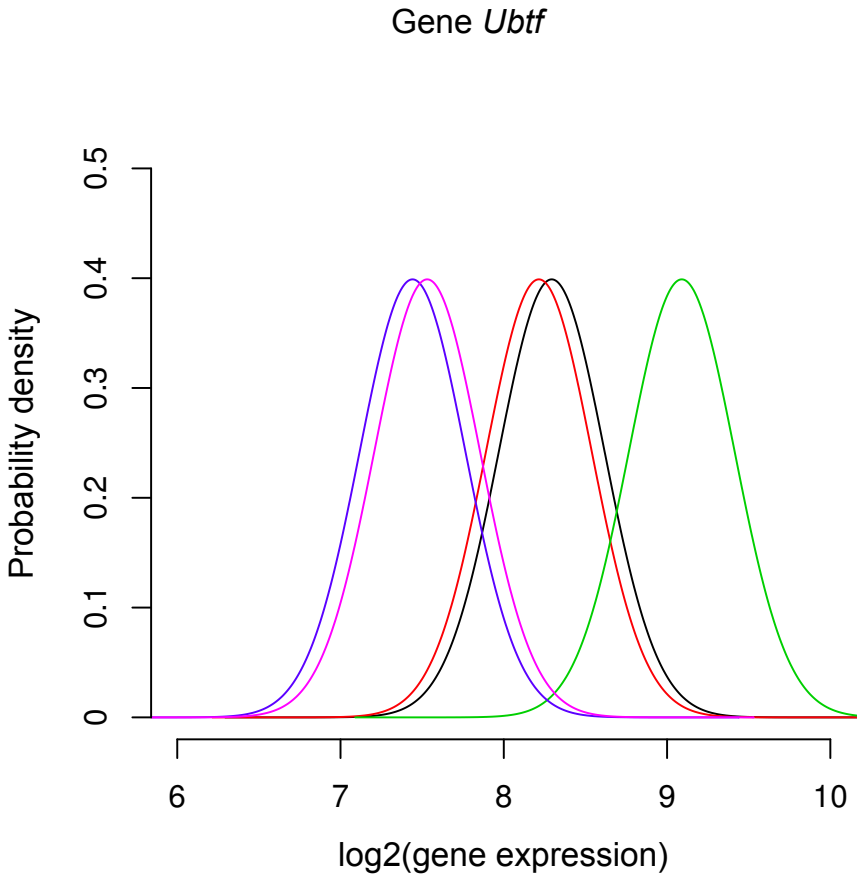

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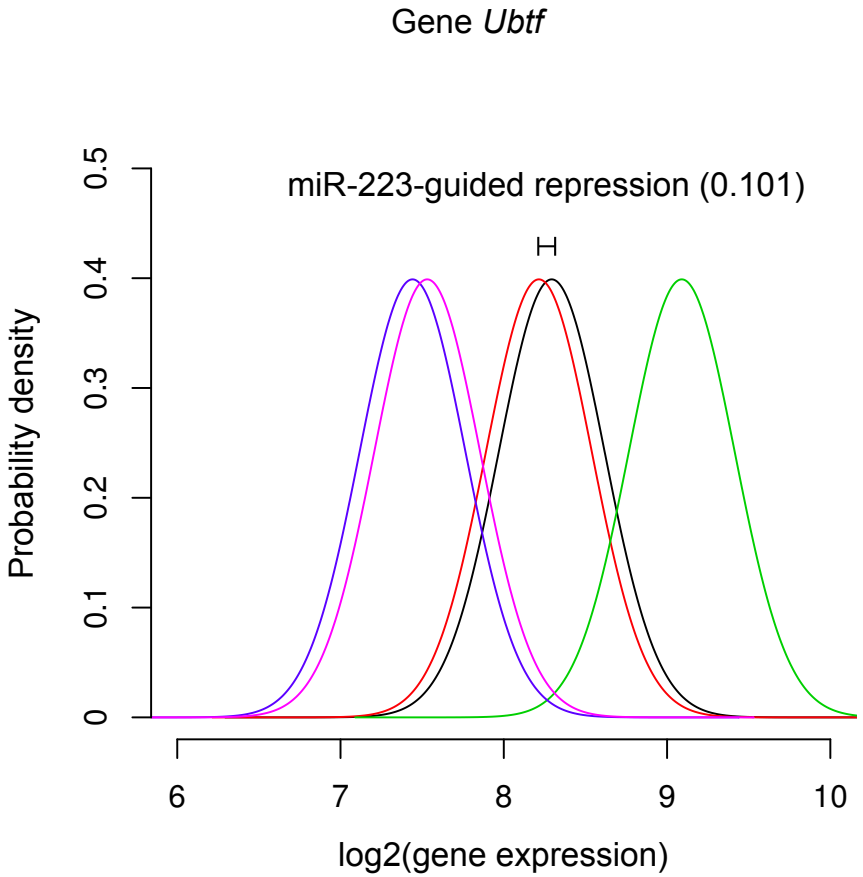

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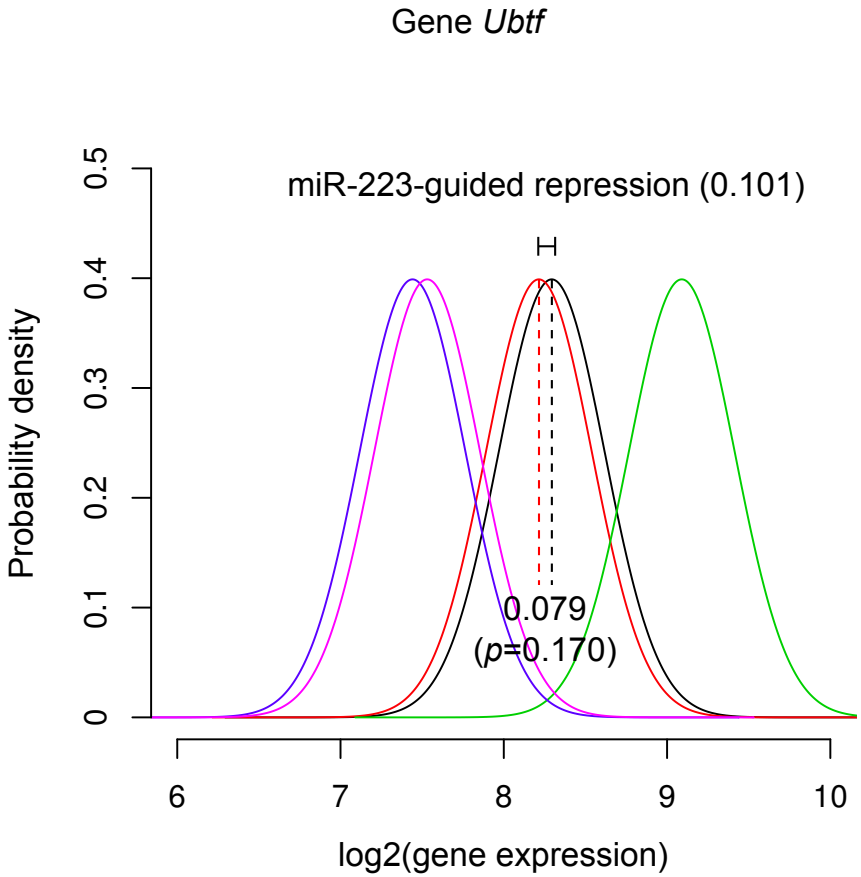

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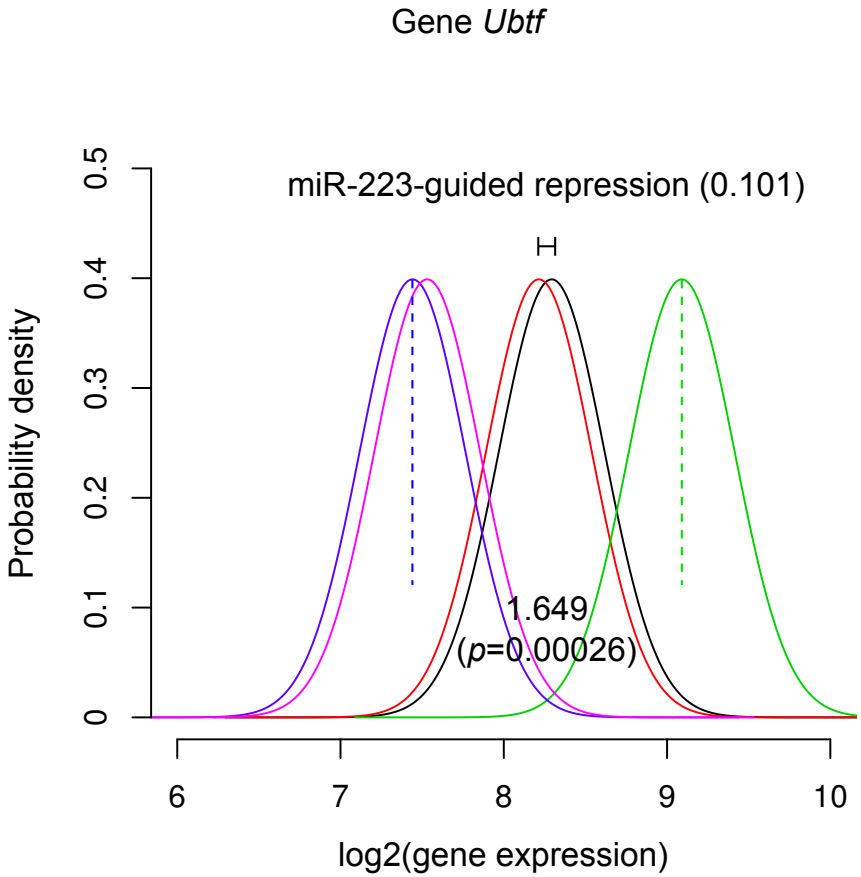

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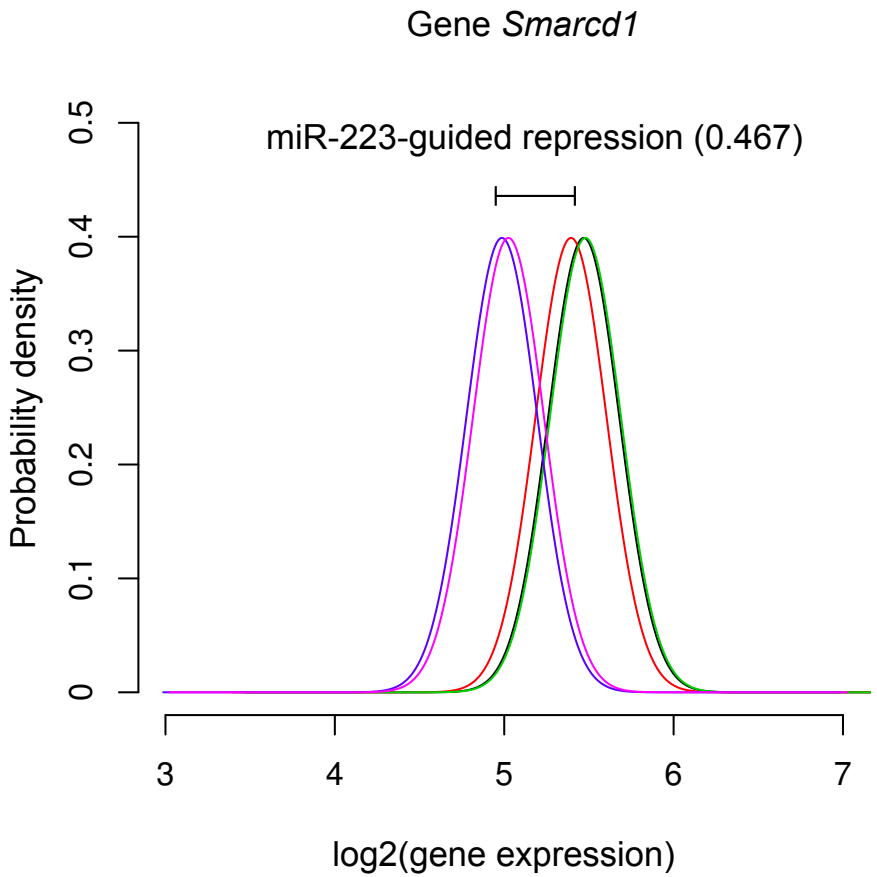

Natural variability vs. miRNA-guided repression

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Stim1	1.54 (0.246)	
Rasa1	1.44 (0.170)	
Carm1	0.824 (0.167)	
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Transcription factor Zfy-overexpressing mice (Hélène Royo, Turner lab, MRC, London). Transgene is overexpressed in the testis from day 13.5, cells enter apoptosis at day 15.5. mRNA quantification by microarray at these two stages.

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Zfy binds the [A/T]GGCC pentamer.

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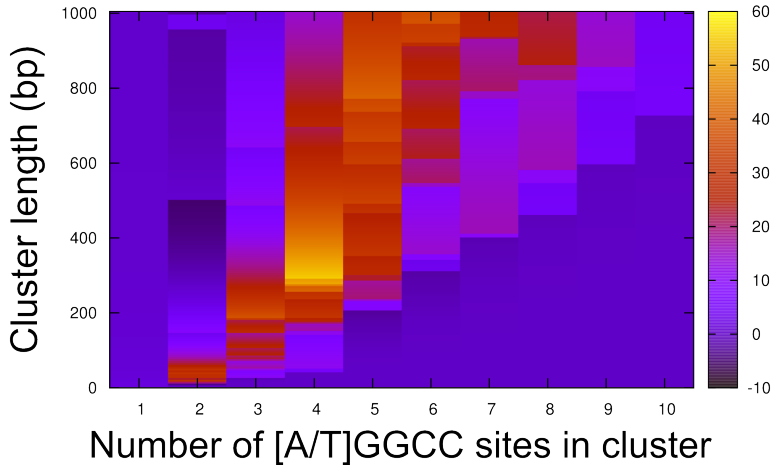
(% of up-regulated genes with cluster) - (% of unaffected genes with cluster)



▸ Supplementary data

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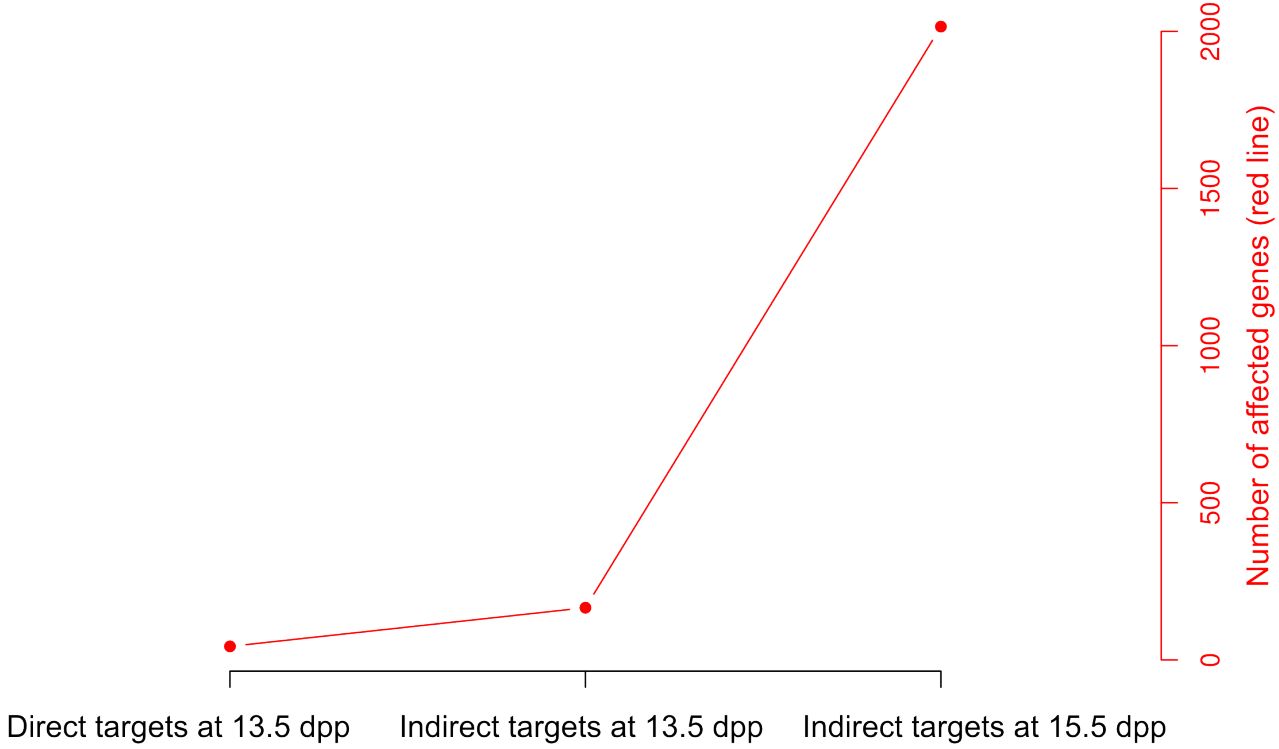

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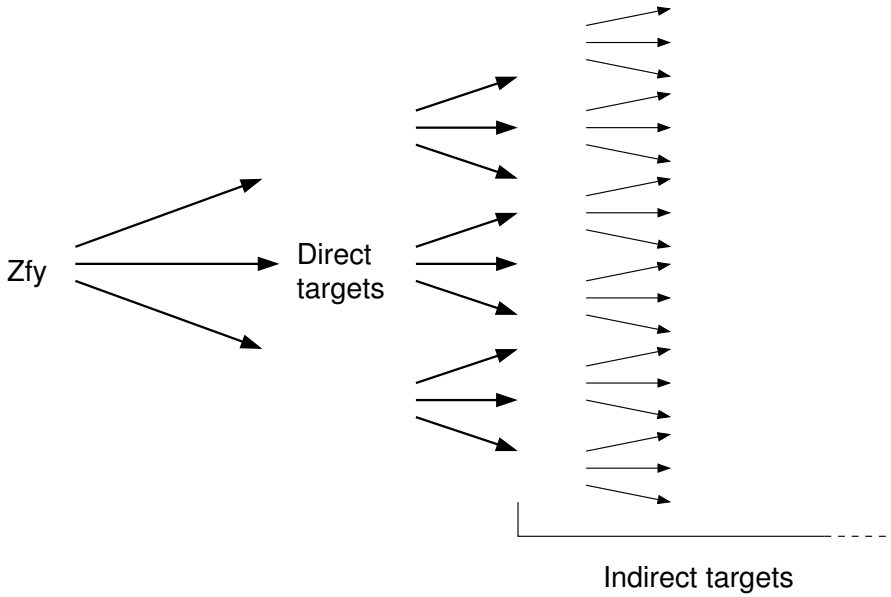

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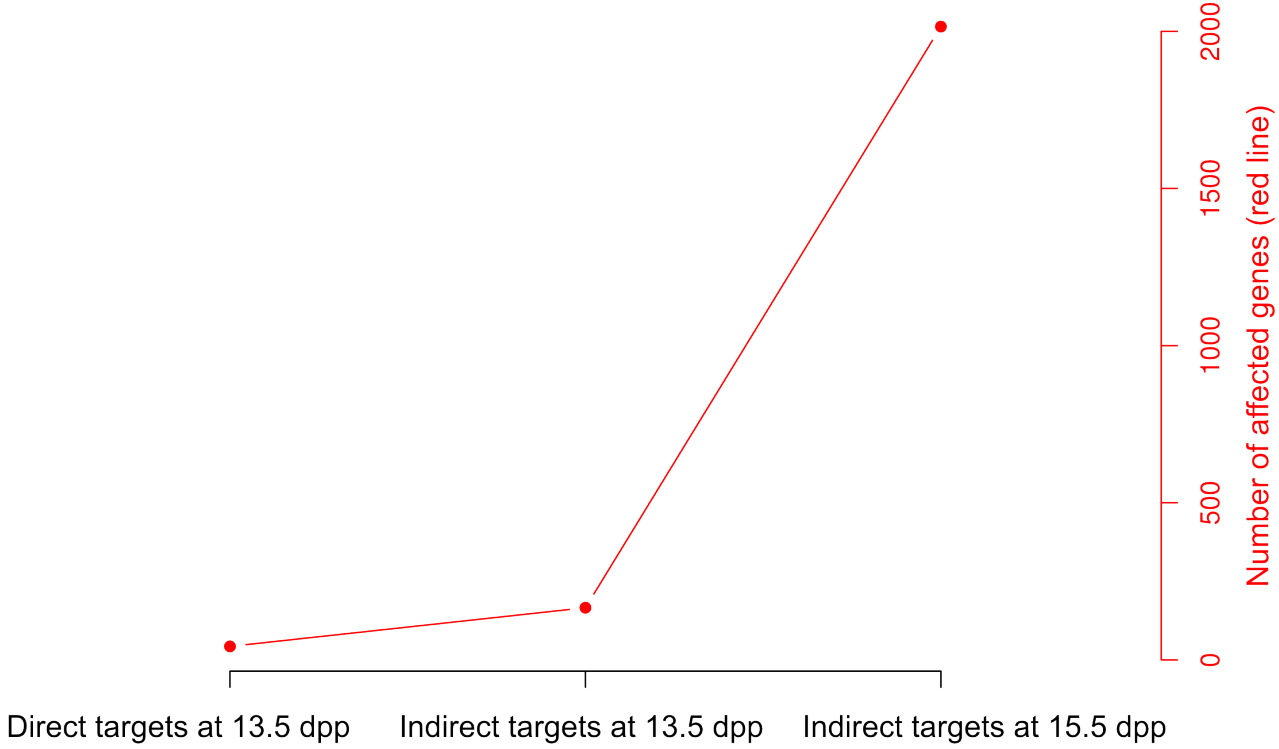

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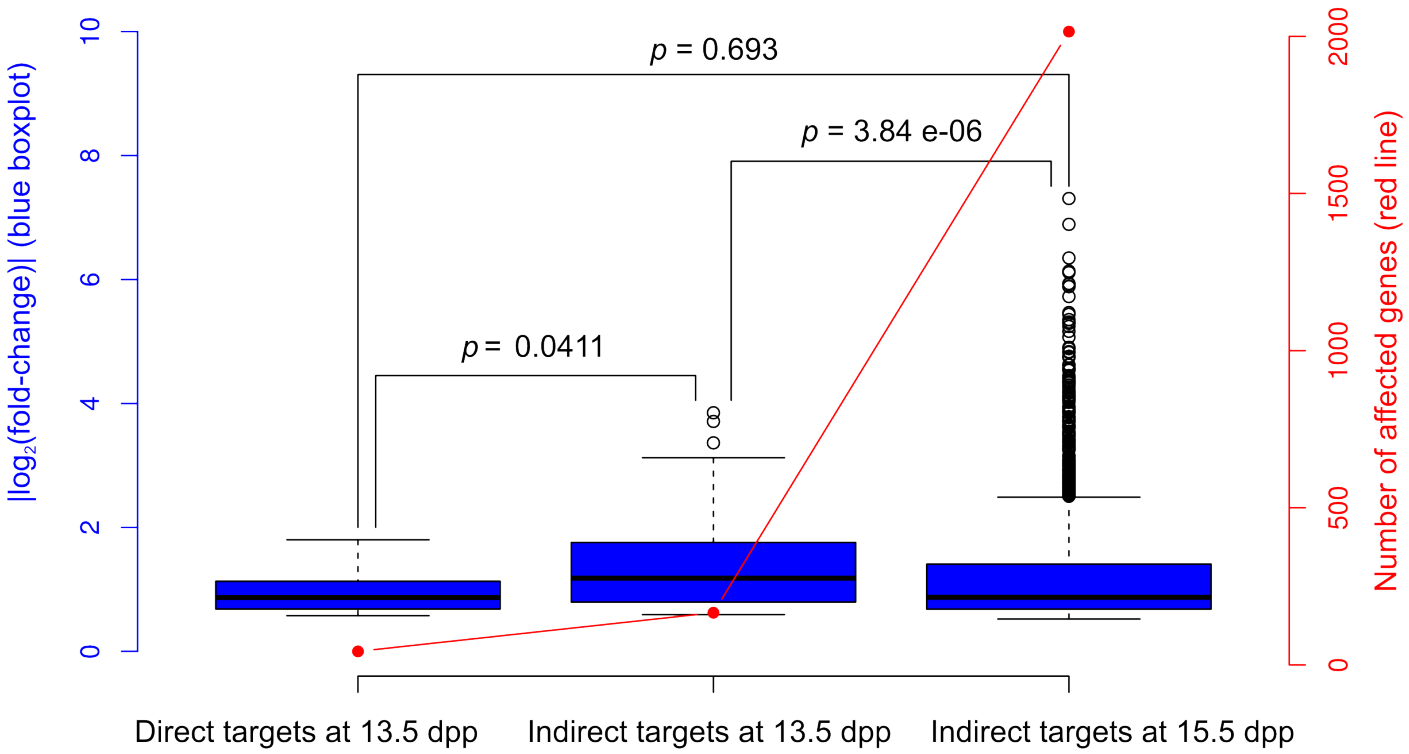

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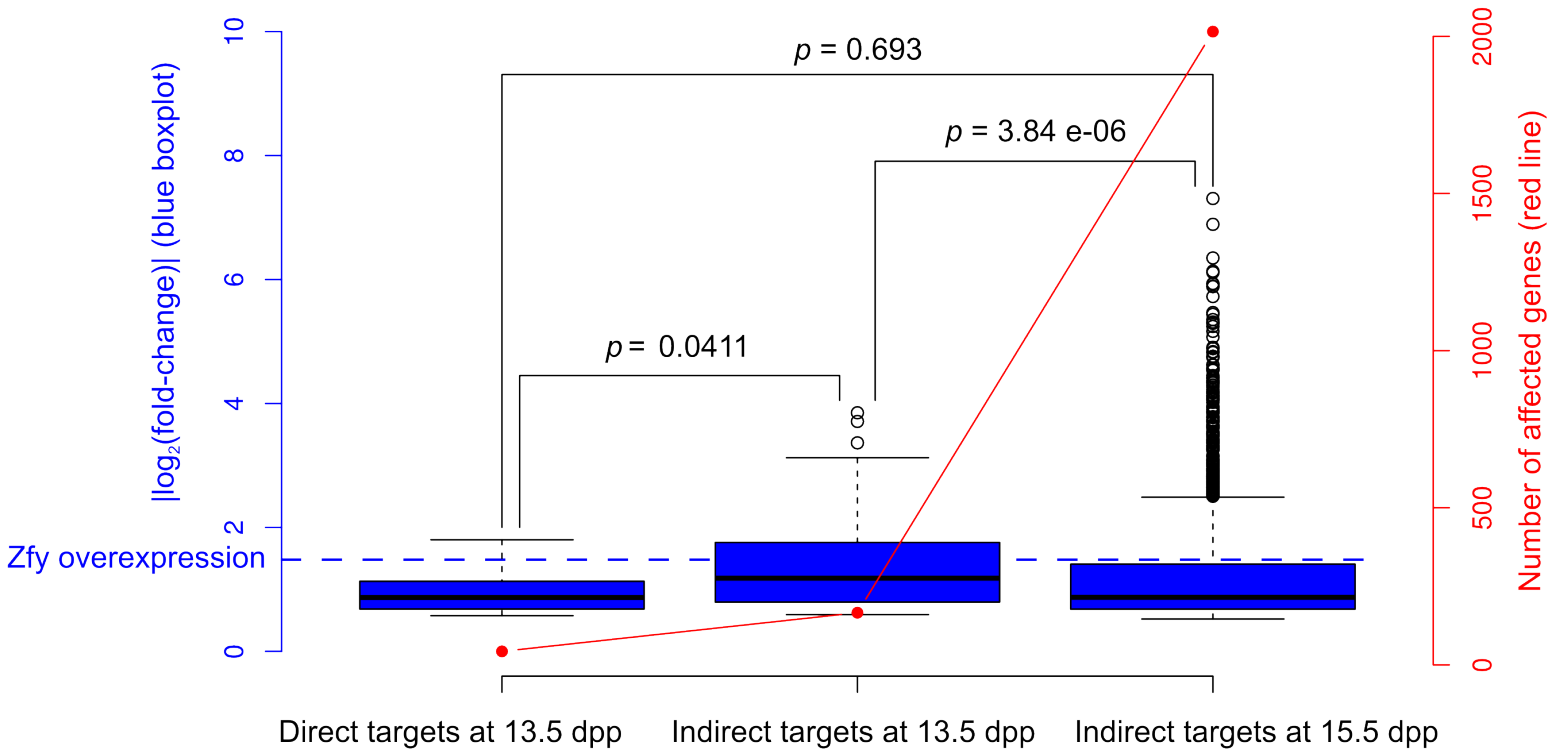

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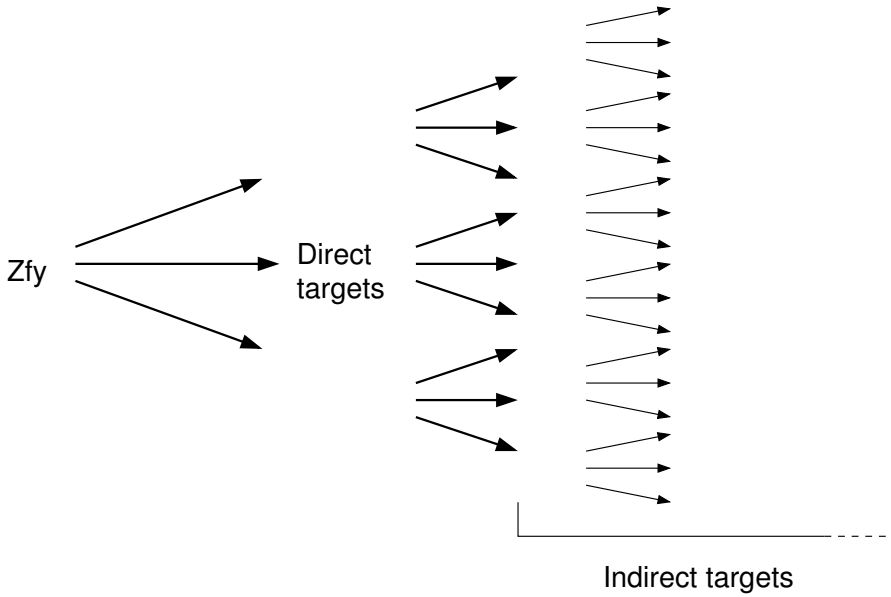

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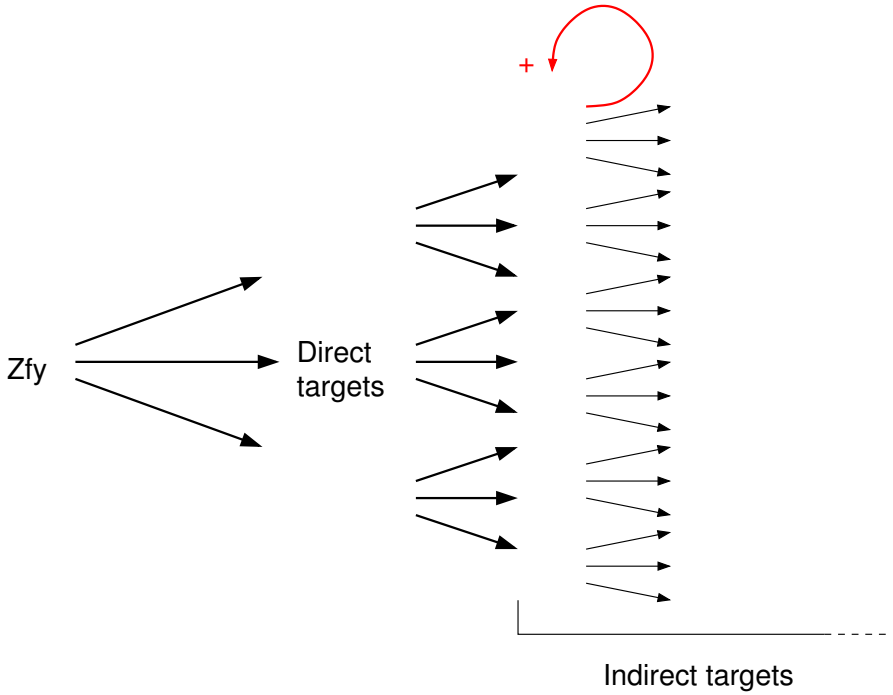

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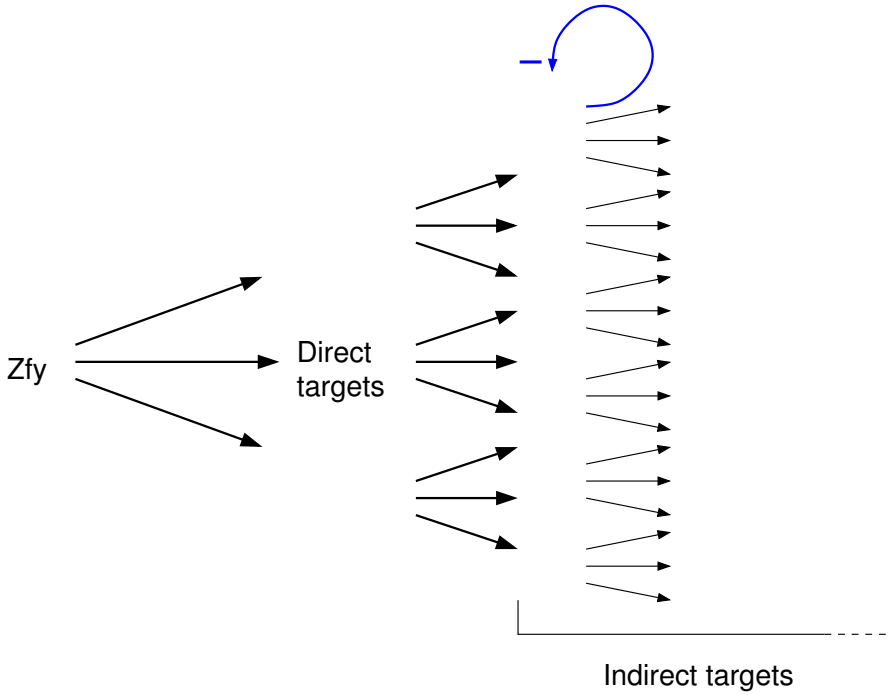

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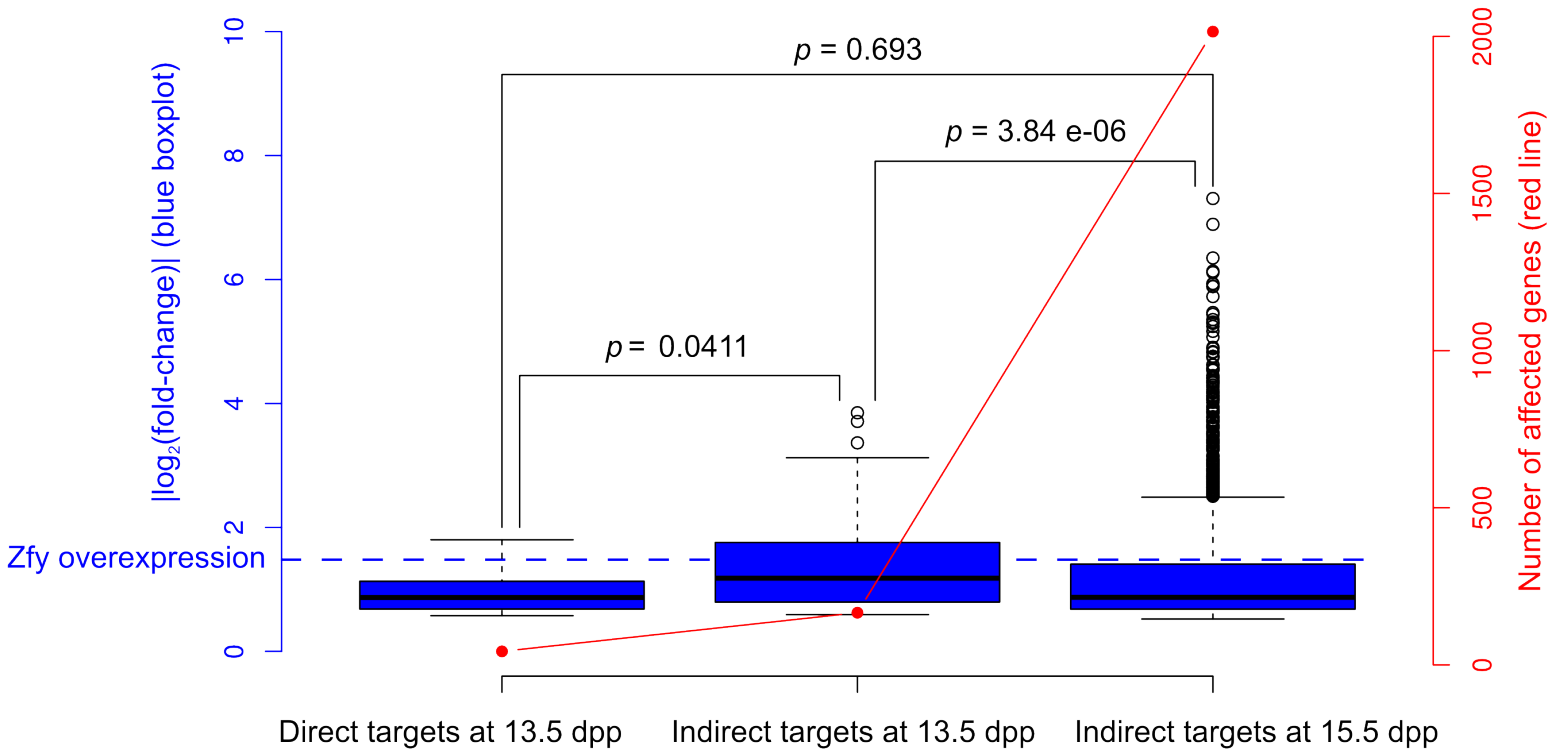

Conclusion: revisiting miRNA target definition

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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.

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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

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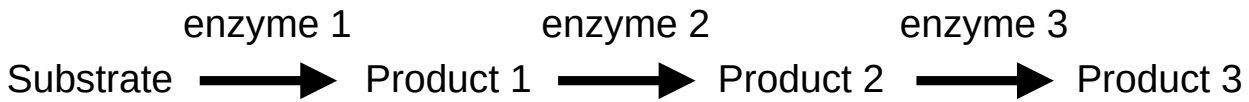
Supplementary data

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

Second paradox

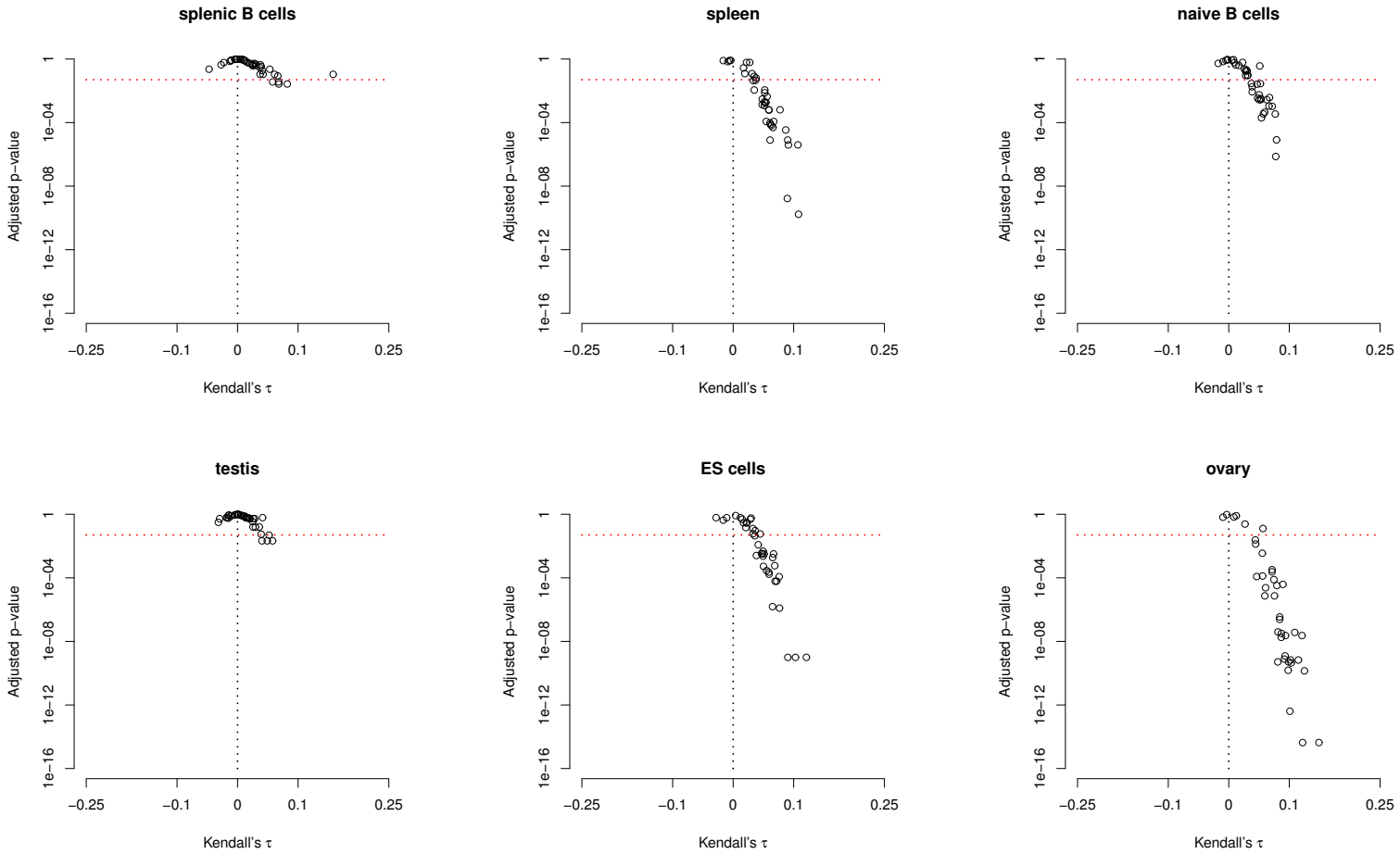


mRNA abundance and seed match conservation

- 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



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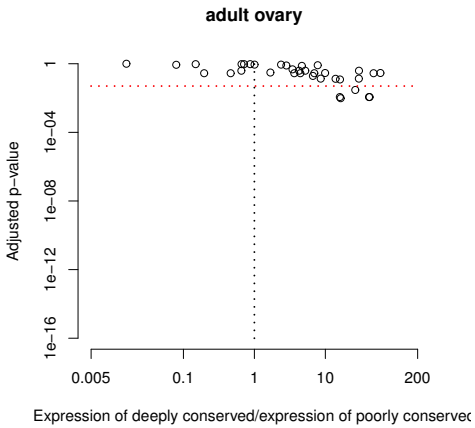
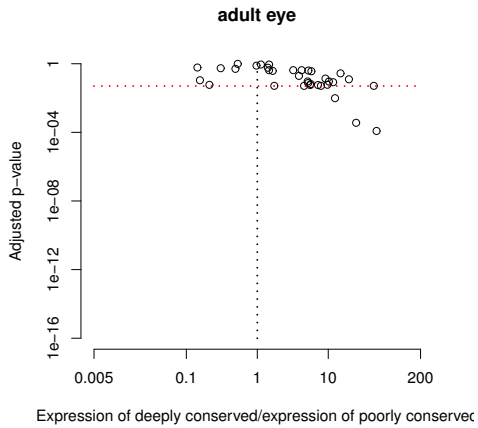
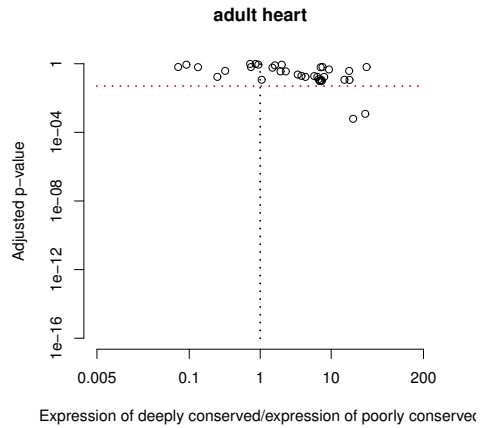
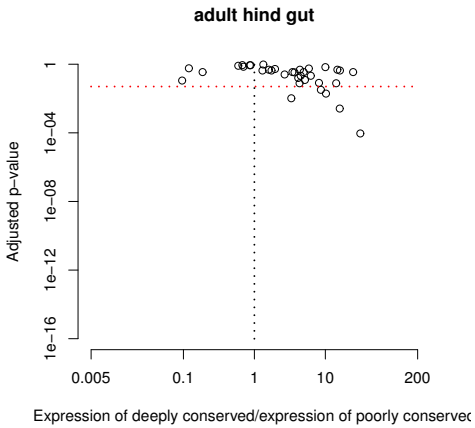
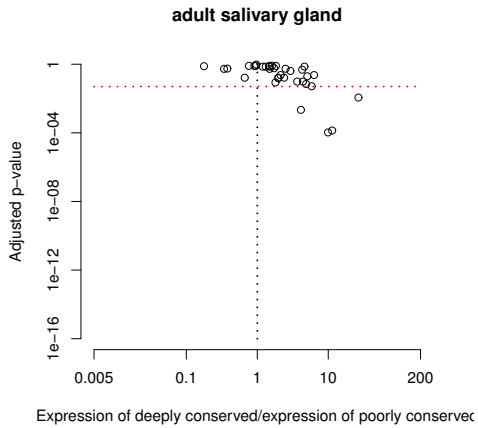
mRNA abundance and seed match conservation

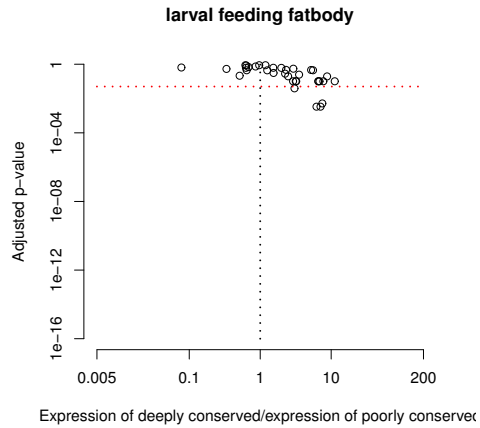












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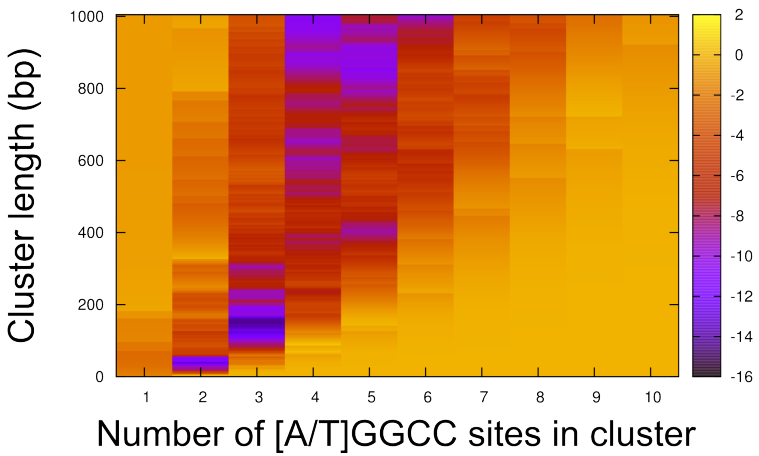
Generalization

(% of down-regulated genes with cluster) - (% of unaffected genes with cluster)



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