

Expression of macroscopic phenotypes for miRNA-guided regulation at the molecular level

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March 3, 2016

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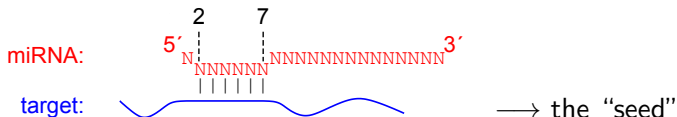
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Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

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

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⇒ miRNAs are implicated in every physiological process in animals.

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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?

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

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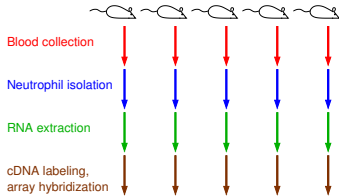
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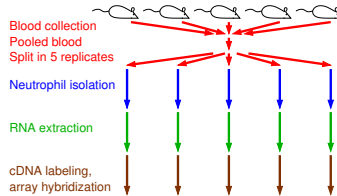
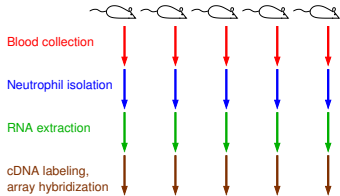
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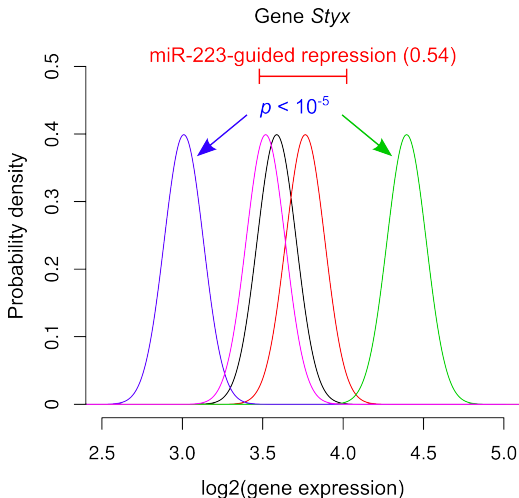
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For 150 predicted targets out of 192:
inter-individual fluctuations across 5 wild-type mice exceeds
miRNA-mediated regulation (p -value < 0.05).

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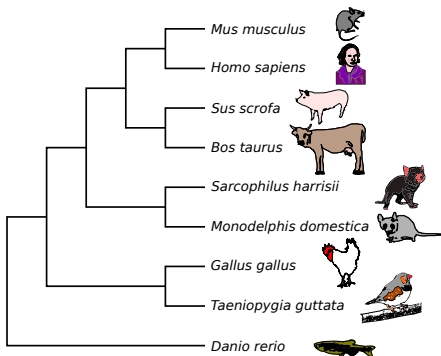
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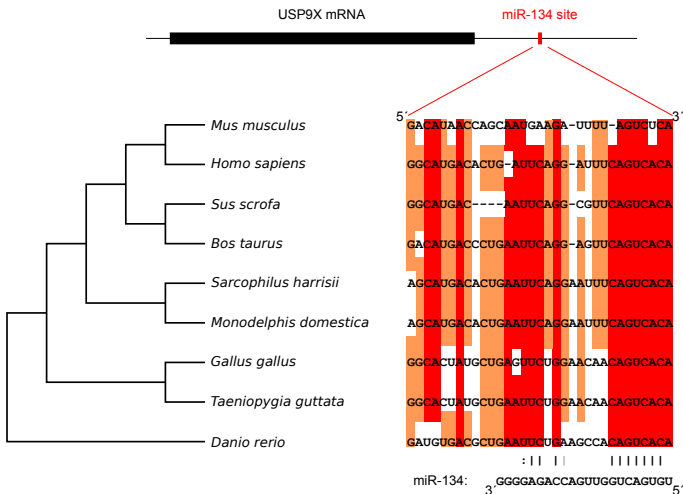
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■ : conserved in 8 or 9 species out of 9 ■ : conserved in 6 or 7 species out of 9 □ : conserved in less than 6 species out of 9

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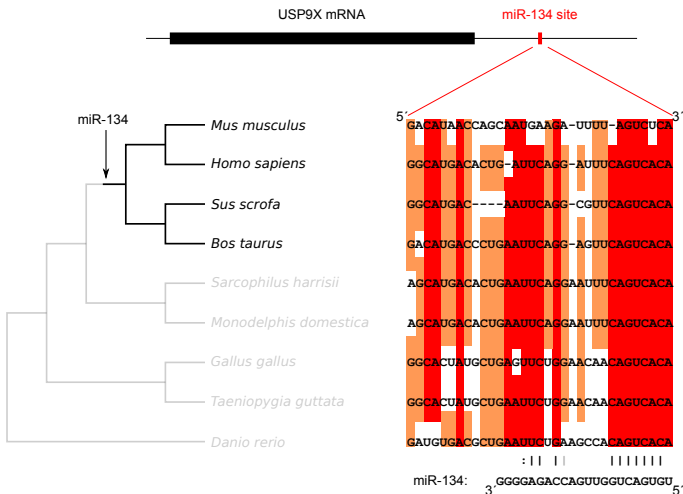
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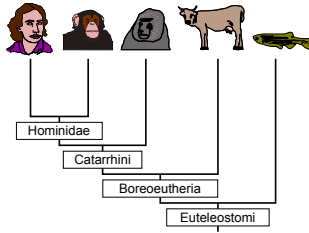
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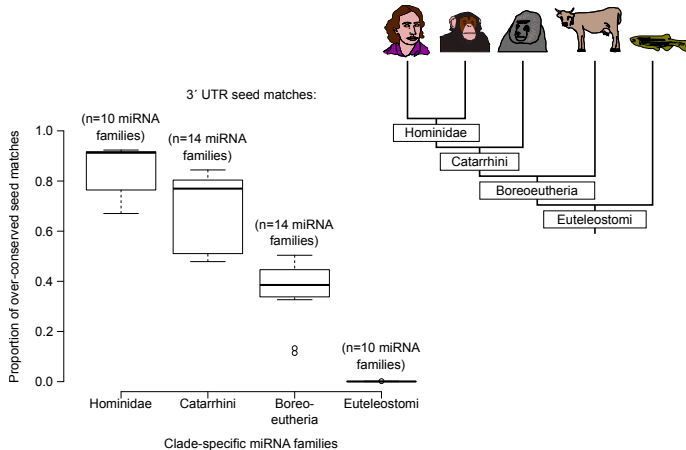
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► Comparison to prediction programs

► Effect of tree architecture

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Conclusion: revisiting miRNA target definition

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

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Conclusion: revisiting miRNA target definition

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

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).

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Acknowledgements

Anna
Sergeeva:



Laura
Martinez:



Blaise
Li:



Natalia
Pinzón:



Isabelle
Busseau:



Delphine
Mazé:



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Jessy Presumey and Florence Apparailly (INM, Montpellier)

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Introduction:

- ▶ Mechanism of target repression

Results:

- ▶ Robustness of biological pathways
- ▶ miR-223 experiment: experimental details
- ▶ 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

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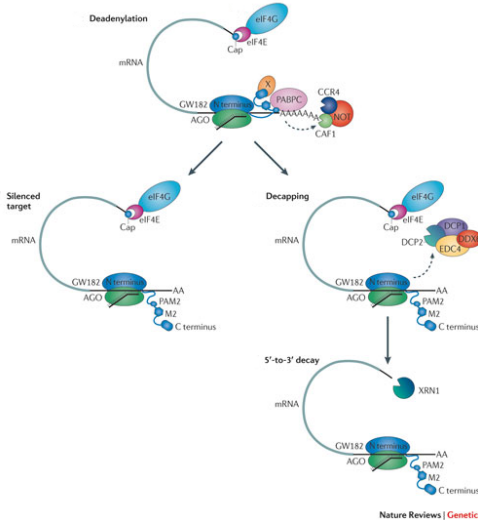
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miRNA target repression

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(adapted from Huntzinger and Izaurralde, 2011)

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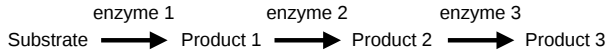
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Biological robustness



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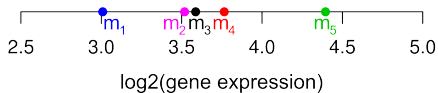
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Experimental details

Gene *Styx*



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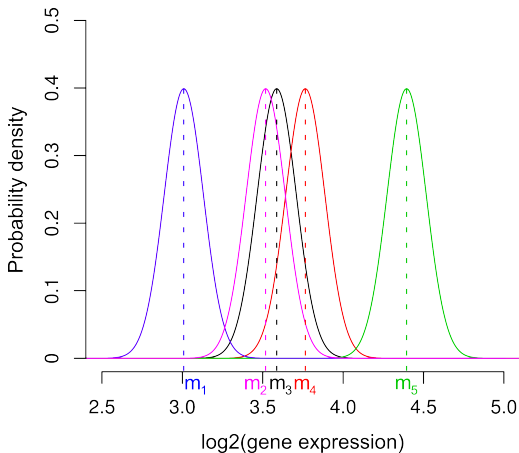
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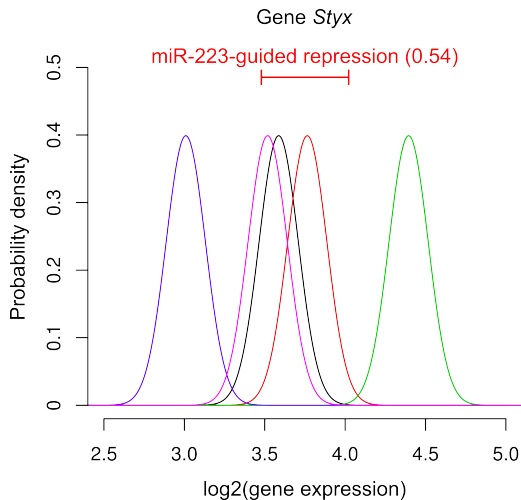
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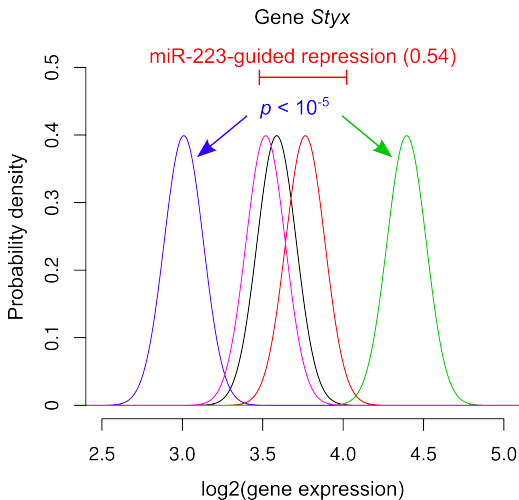
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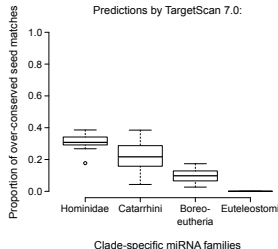
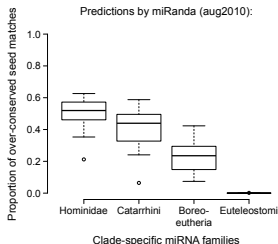
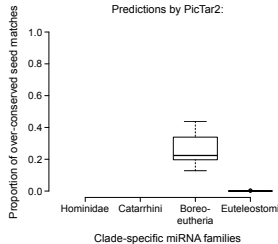
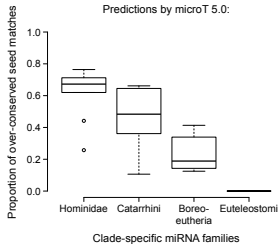
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p : probability that the difference between two individual mice is smaller than miRNA-guided repression

Over-conservation in prediction programs

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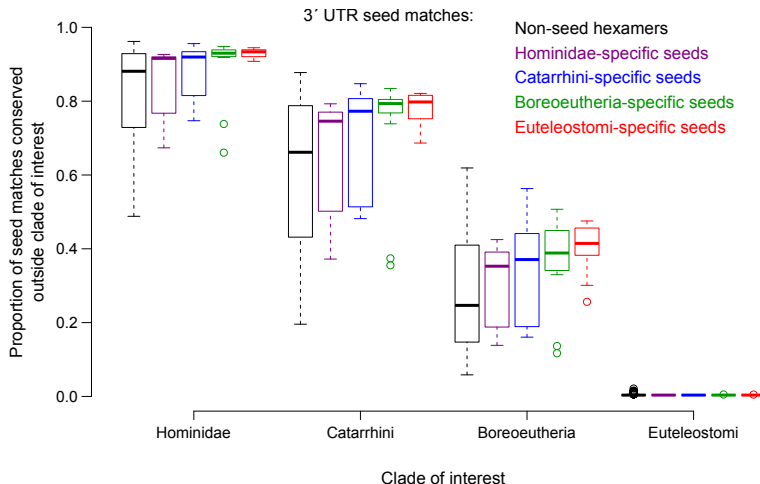
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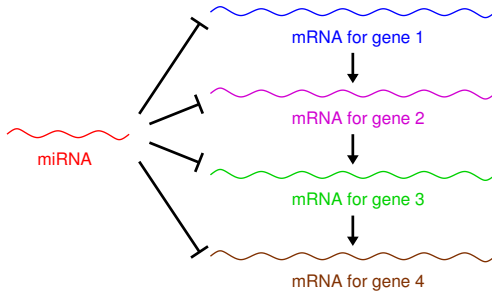
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Targets for a given miRNA often belong to the same biological pathways.



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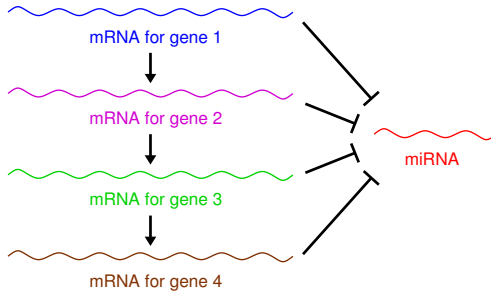
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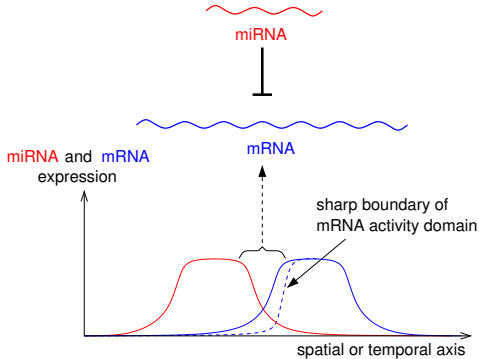
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Expression domains for miRNAs and their predicted targets overlap at their boundaries.



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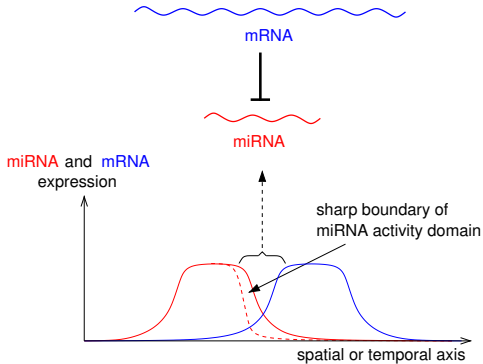
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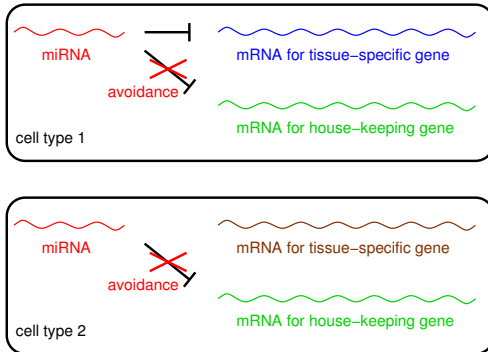
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House-keeping genes are rarely predicted to be targeted.



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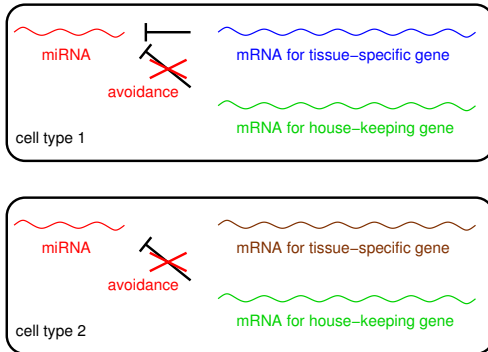
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Reported examples of pseudo-targets

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

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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).

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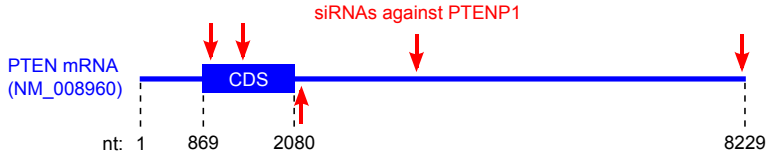
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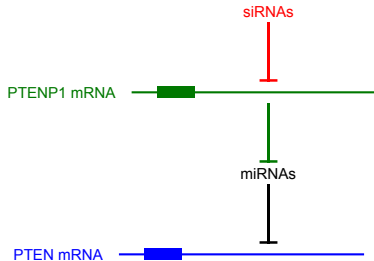
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Proposed by Poliseno *et al* (2010):



More probably:



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siRNA #2 against PTENP1: 5' UAAUAAUCAUCAUUCUGGC 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' UUUUUUACUUGGAAAAUUA 5'

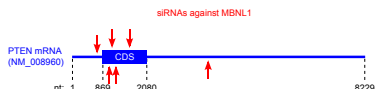
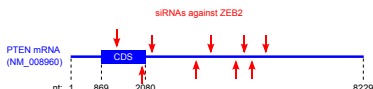
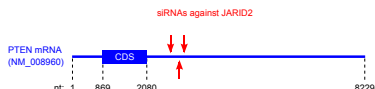
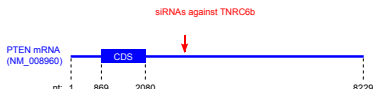
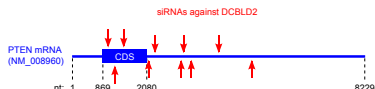
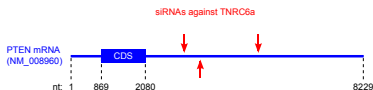
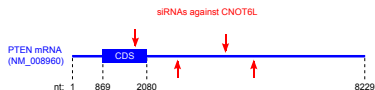
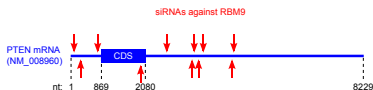
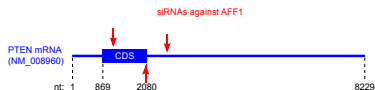
siRNA #2 against PTENP1: 5' UAAUAAUCAUCAUUCUGGC 3'
PTEN mRNA (nt 1383-1401): 3' AUUAUUUAUGUAUCGCGG 5'

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

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

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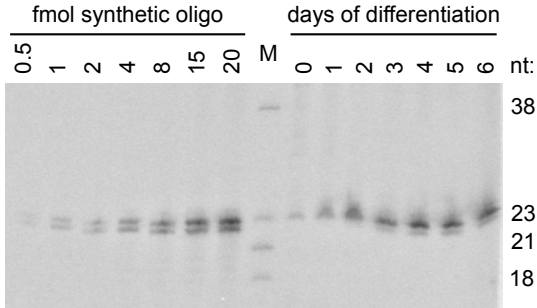
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miRNA quantification

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(quantification of miR-1 and miR-206)

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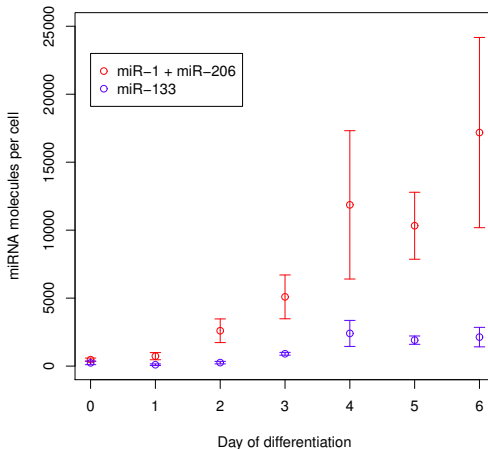
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miRNA abundance during C2C12 differentiation



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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



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27 synthetic spike-ins, for calibration.

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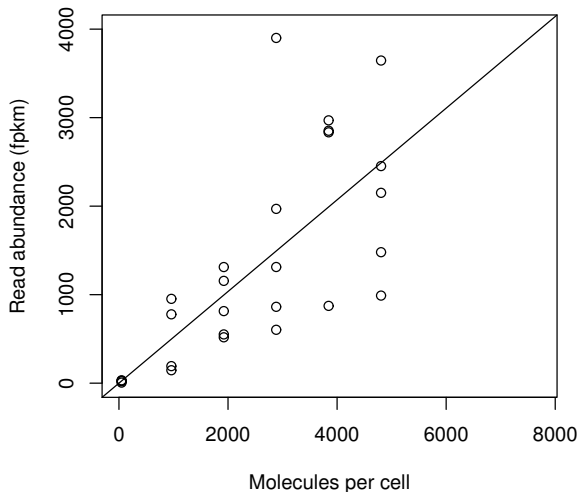
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Day 0, replicate 1



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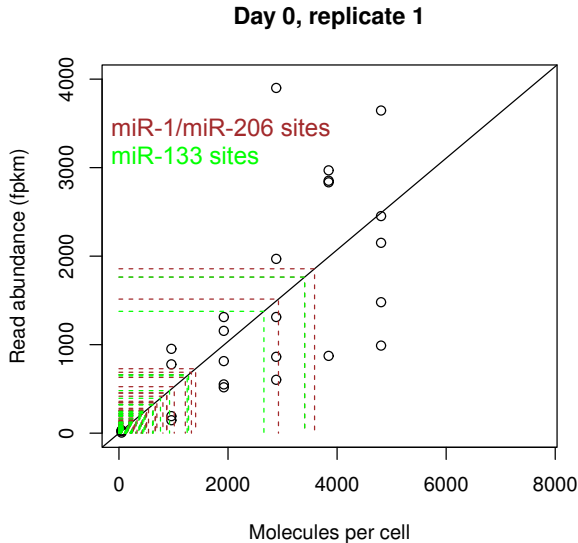
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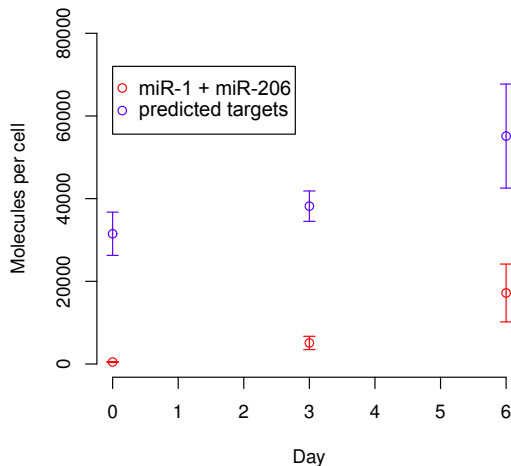
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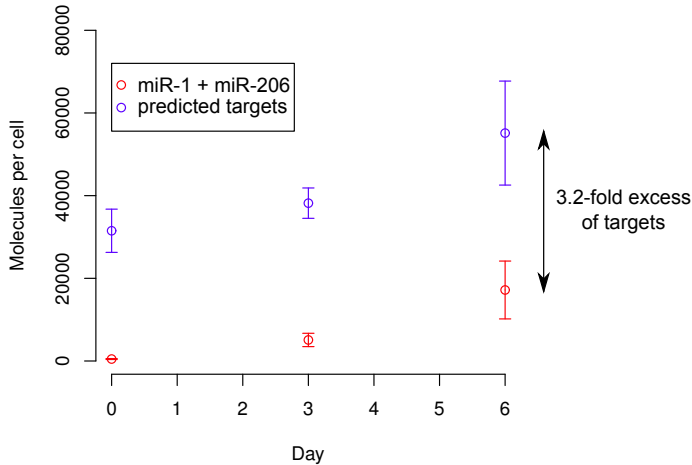
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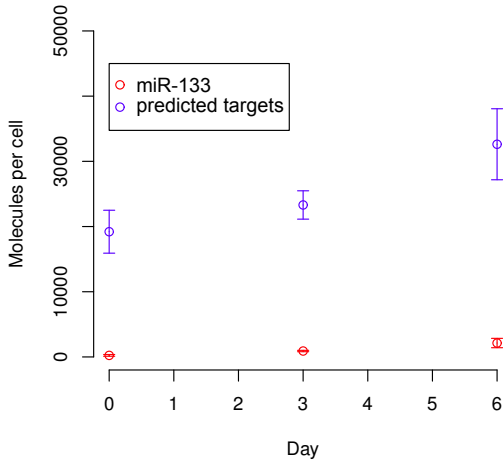
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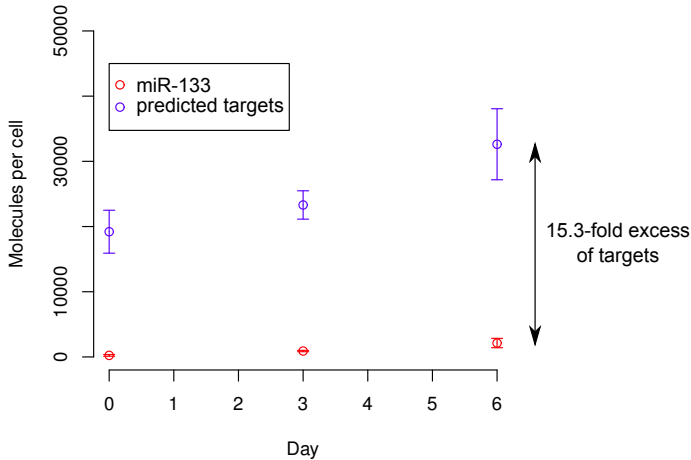
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Deep-sequencing statistics

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

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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).

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Real molecular events, which are neutral in evolutionary terms?

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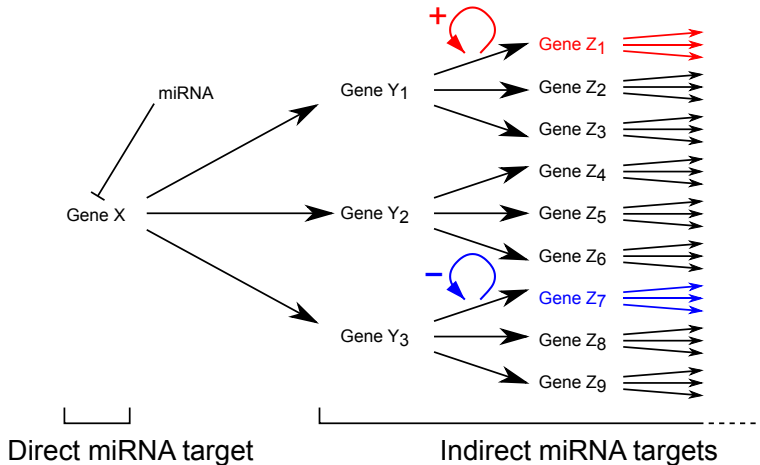
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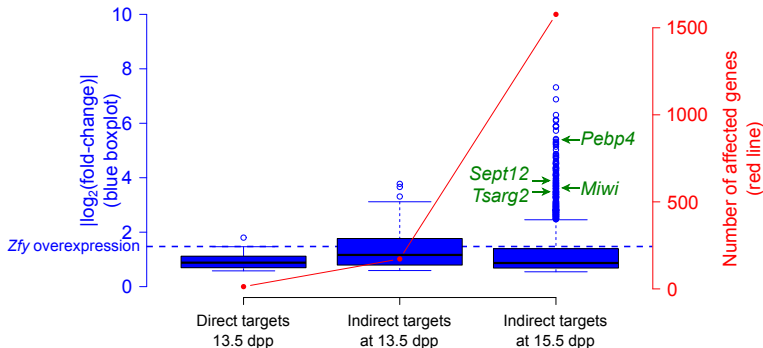
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(in collaboration with H. Royo and J. Turner, MRC, London)

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