# microRNAs:
# general overview, recent findings

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June 4, 2015

# Introduction: a brief chronology

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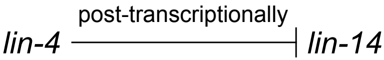
In *Cænorhabditis elegans* development:



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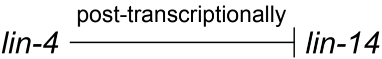

1993: the *lin-4* locus is non-coding.

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$$\textit{lin-4}\ \overset{\text{post-transcriptionally}}{\longrightarrow\!\!\!\!\dashv}\ \textit{lin-14}$$

1993: the *lin-4* locus is non-coding. Mature product is a 22 nt long RNA (+ a stem-loop-folded 61 nt long precursor).

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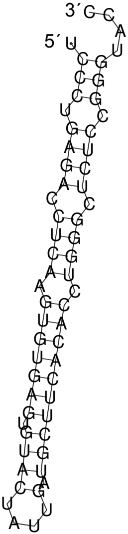

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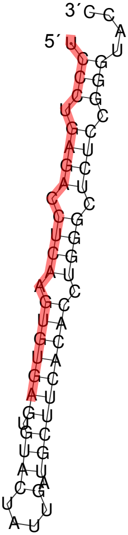

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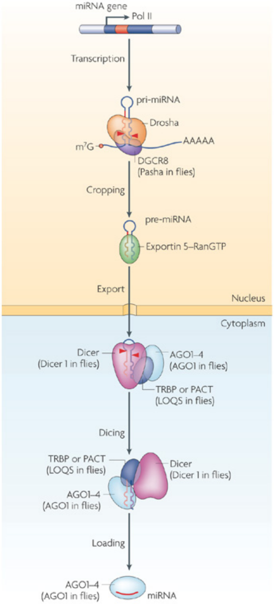

(Kim *et al*, 2009)

# microRNA target prediction

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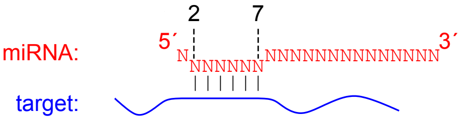

⟶ the “seed”

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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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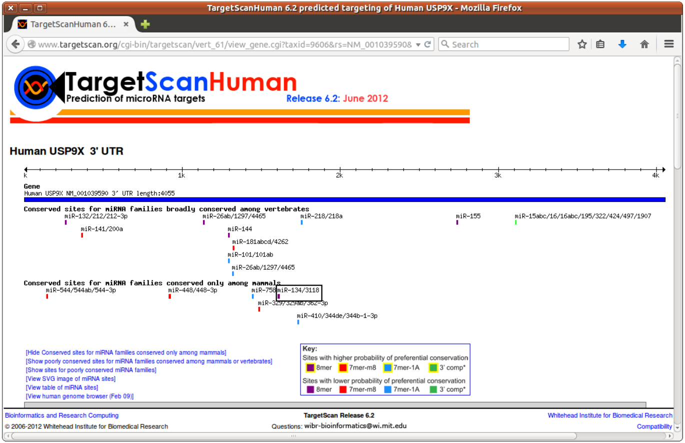

TargetScanHuman 6.2 predicted targeting of Human USP9X - Mozilla Firefox

**Human USP9X 3' UTR**

[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
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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

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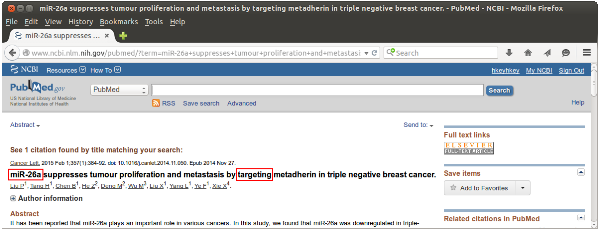

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$\Longrightarrow$ miRNAs are implicated in every physiological process in animals.

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?

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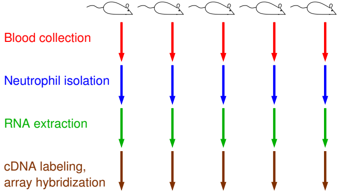

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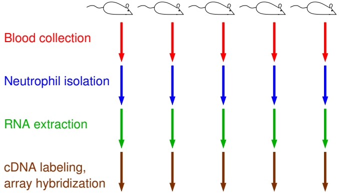
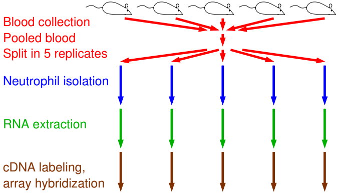


Blood collection
Neutrophil isolation
RNA extraction
cDNA labeling,
array hybridization



Blood collection
Pooled blood
Split in 5 replicates
Neutrophil isolation
RNA extraction
cDNA labeling,
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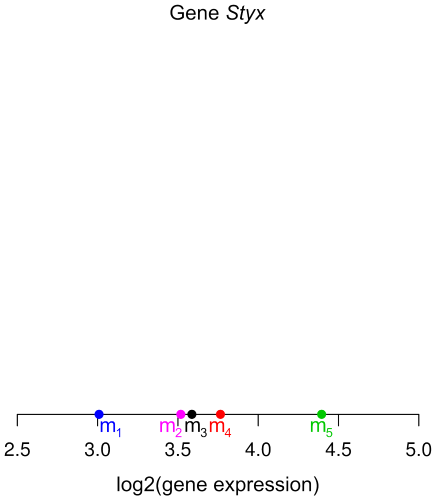

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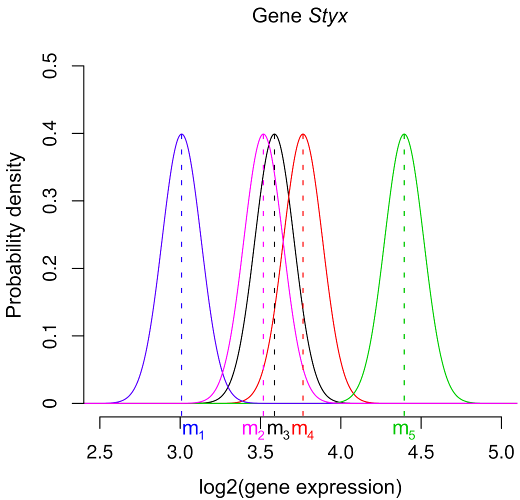

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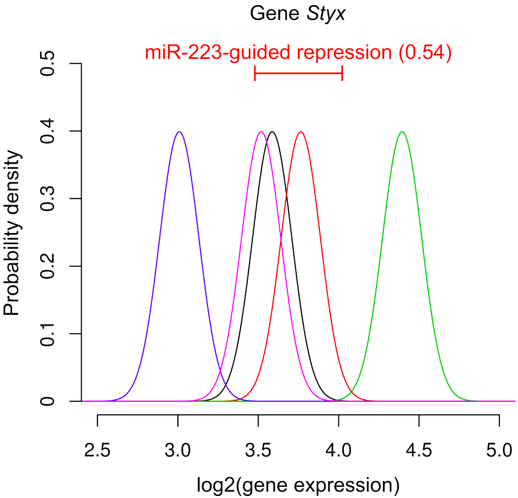

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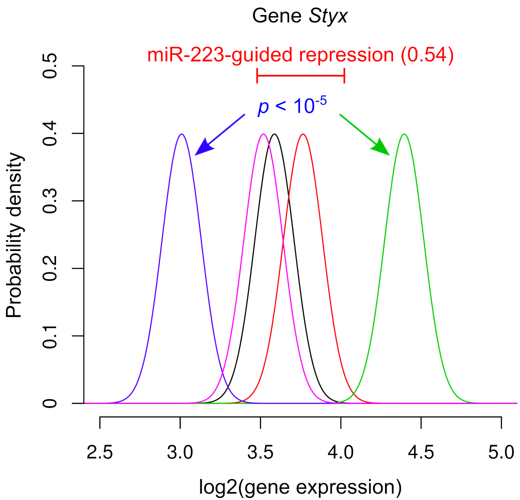

$p$: probability that the difference between two individual mice is smaller than miRNA-guided repression

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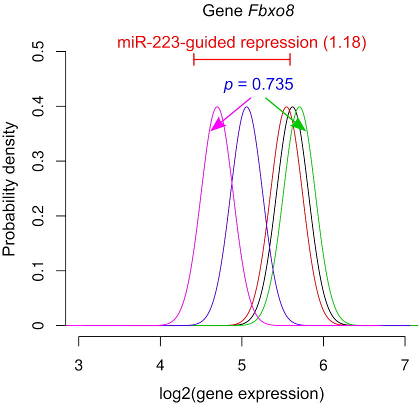

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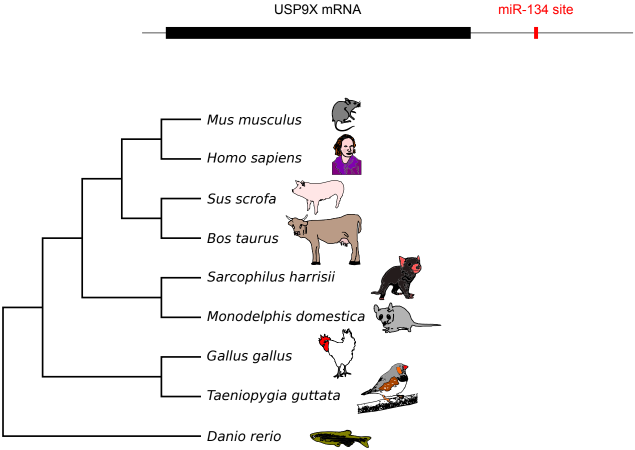

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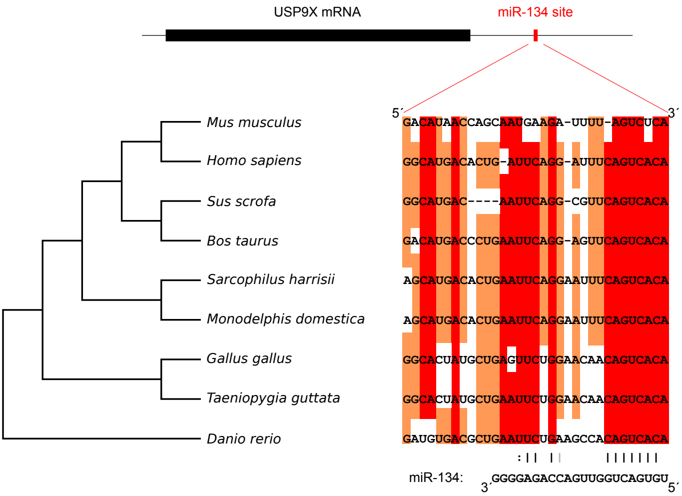

🟥 : conserved in 8 or 9 species out of 9 🟧 : conserved in 6 or 7 species out of 9 ⬜ : conserved in less than 6 species out of 9

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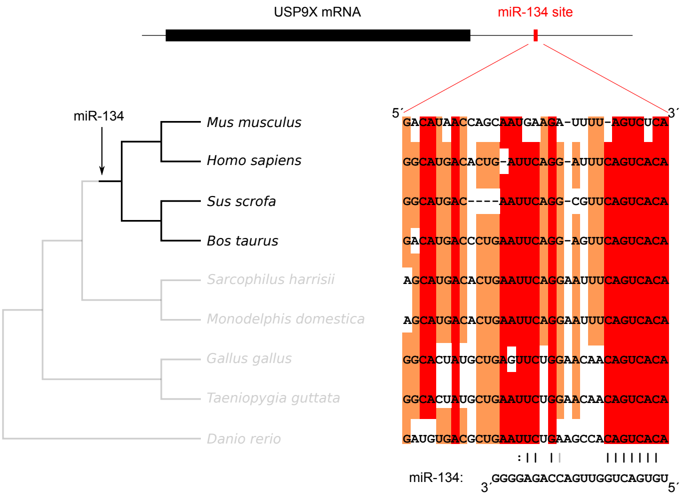

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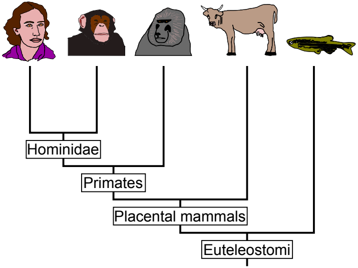

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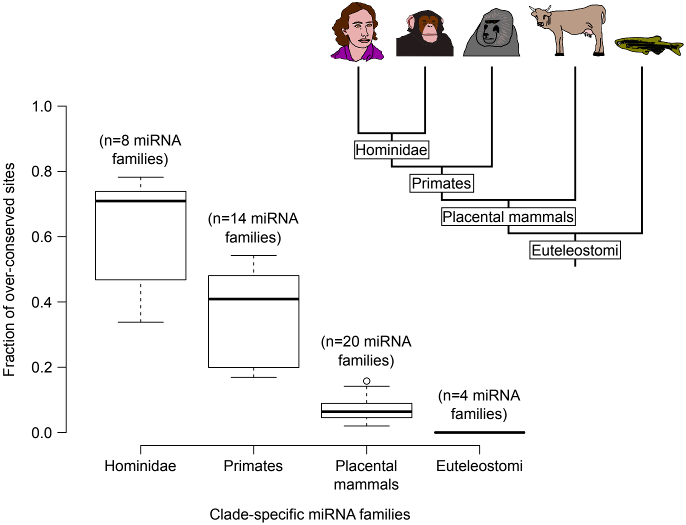

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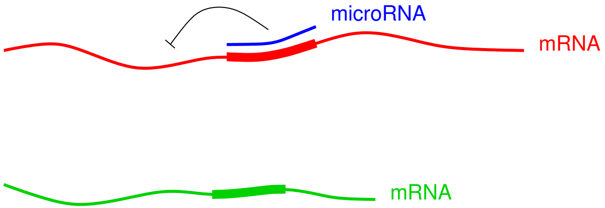

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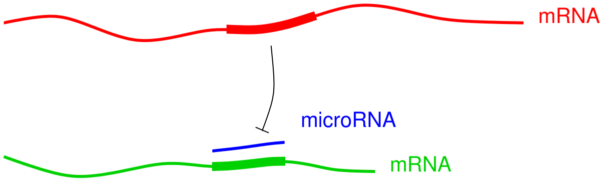

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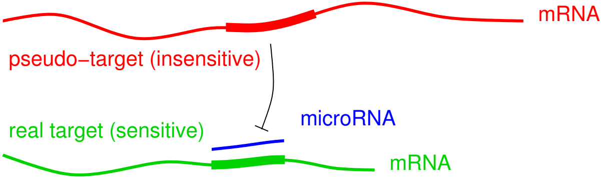

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- miRNAs: Northern blot (calibrated with RNA oligos);
- mRNAs: RNA-Seq (calibrated with *in vitro* transcripts).

▸ Representative results

# Absolute RNA quantification

| miRNA family | Predicted targets | |
|---|---|---|
| | Total | Above $K_d$ |
| miR-1 and miR-206 | 436 | 115 (26 %) |
| miR-133 | 384 | 84 (22 %) |

($K_d$ value taken from Wee *et al.*, 2012: 26 pM)

# Absolute RNA quantification

Titration effect of individual miRNA binding sites:

| mRNA | Sample | Increase in free miR-1/miR-206 if site is lost |
|---|---|---|
| *Anxa2* | Day 0 | 8 % |
| *Ptma* | Day 0 | 9 % |
| *Tmsb4x* | Day 0 | 10 % |
| *Actb* | Day 3 | 15 % |
| *Tmsb4x* | Day 3 | 9 % |
| *Actb* | Day 6 | 13 % |
| *Tmsb4x* | Day 6 | 11 % |

# Absolute RNA quantification

Titration effect of individual miRNA binding sites:

| mRNA | Sample | Increase in free miR-133 if site is lost |
|---|---|---|
| *Tpm4* | Day 0 | 5 % |
| *Ptma* | Day 0 | 16 % |
| *Eif4a1* | Day 0 | 5 % |
| *Tagln2* | Day 0 | 6 % |
| *Ptma* | Day 3 | 7 % |
| *Eef1a1* | Day 3 | 8 % |
| *Ptma* | Day 6 | 6 % |
| *Eef1a1* | Day 6 | 6 % |

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

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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

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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

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# Supplementary data

## Introduction:

- Mechanism of target repression

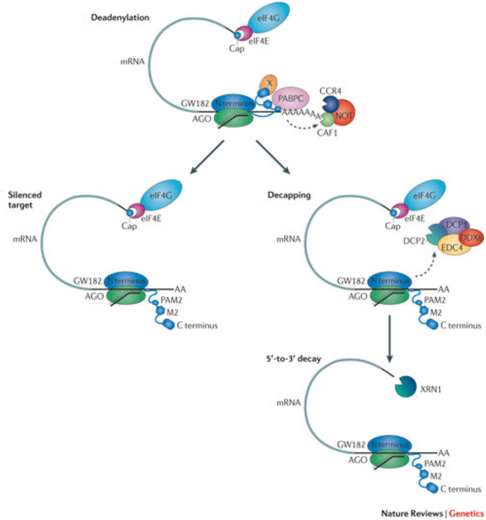
## Results:

- Robustness of biological pathways
- Published evidence for genome-wide targeting
- Issues with published pseudo-targets
- Absolute RNA quantification results
- RNA-Seq statistics
- Candidate titrators

## Conclusion:

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

# miRNA target repression



(adapted from Huntzinger and Izaurralde, 2011)

# Biological robustness

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

# Published evidence

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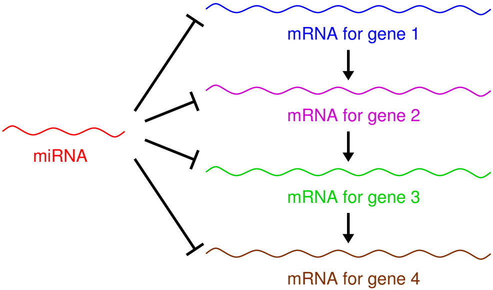
Targets for a given miRNA often belong to the same biological pathways.



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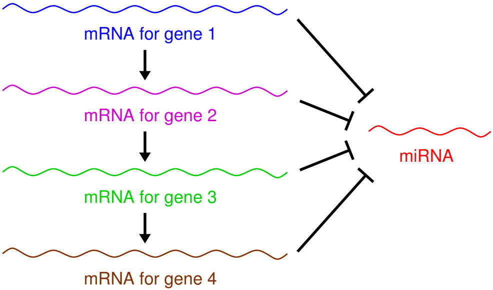

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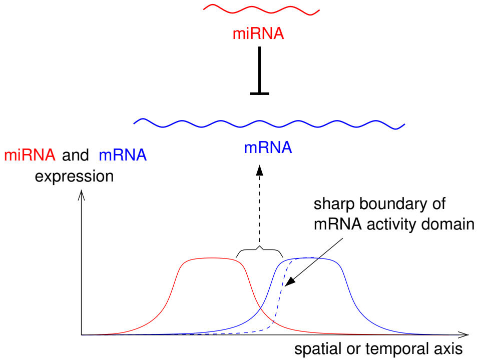

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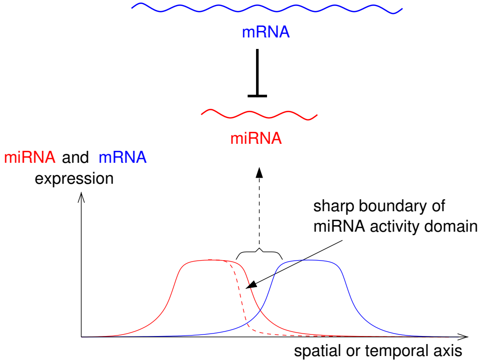

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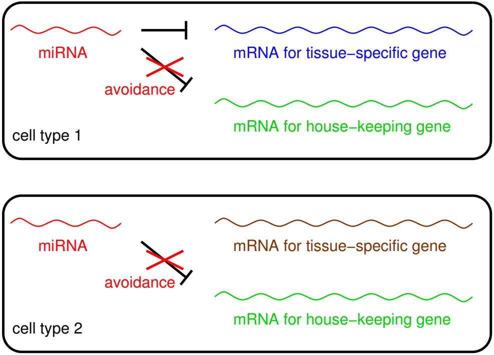
House-keeping genes are rarely predicted to be targeted.



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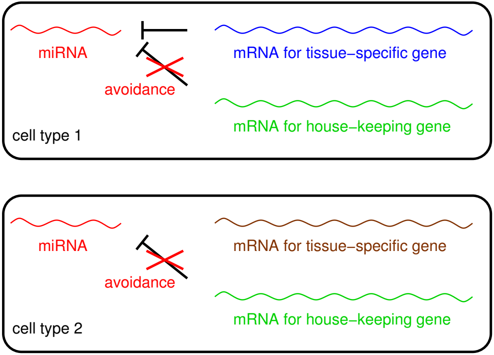

# Reported examples of pseudo-targets

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

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

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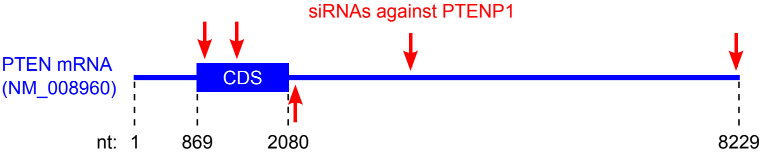

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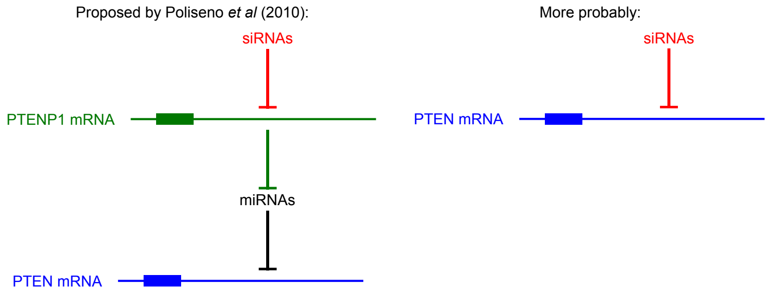

Proposed by Poliseno *et al* (2010):

siRNAs

PTENP1 mRNA

miRNAs

PTEN mRNA

More probably:

siRNAs

PTEN mRNA

# Reported examples of pseudo-targets

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

```
siRNA #2 against PTENP1:      5′ UAAUAAUCAUCAUUCUGGC 3′
                                  ||||||   |||  :| |
PTEN mRNA (nt 1383-1401):     3′ AUUAUUAUAUGUAUCGCGG 5′
```

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

```
siRNA #2 against PTENP1:      5′ UAAUAAUC-AUCAUUCUGGC 3′
                                  ||||||  |:|    |::
PTEN mRNA (nt 8189-8208):     3′ UUUAUUACUUGGAAAAAUUA 5′
```

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

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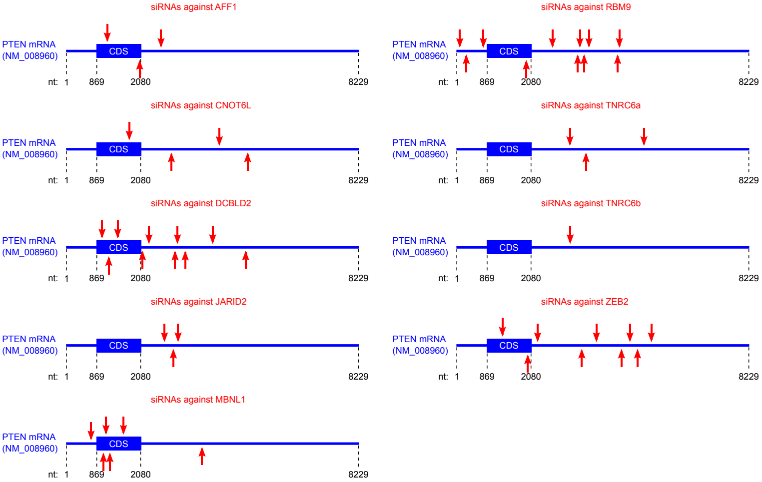
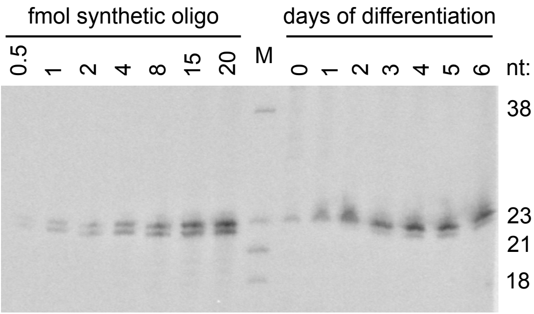


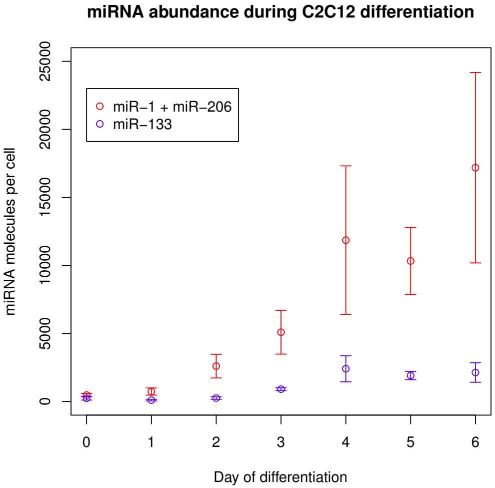
Return

# miRNA quantification



(quantification of miR-1 and miR-206)

# miRNA quantification



# mRNA quantification

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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

华大基因
BGI

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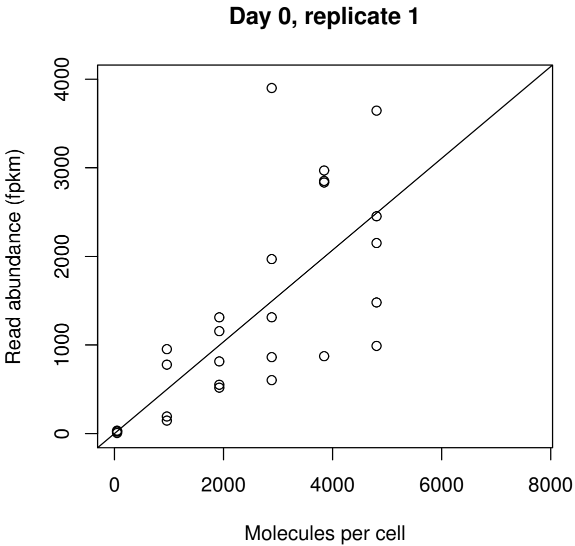
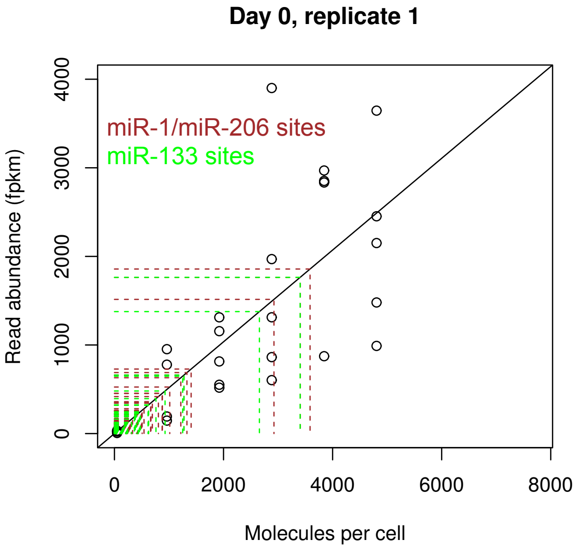
27 synthetic spike-ins, for calibration.

# mRNA quantification



# mRNA quantification



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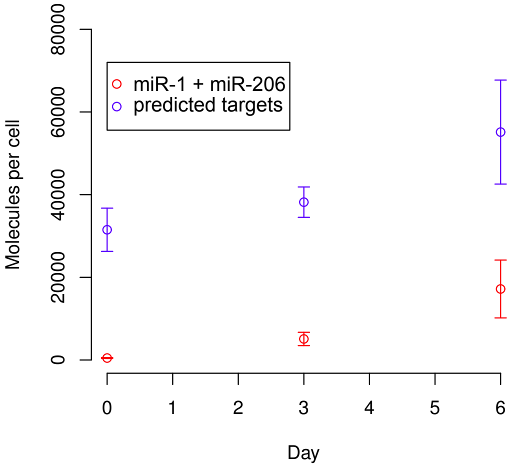

◂ Return

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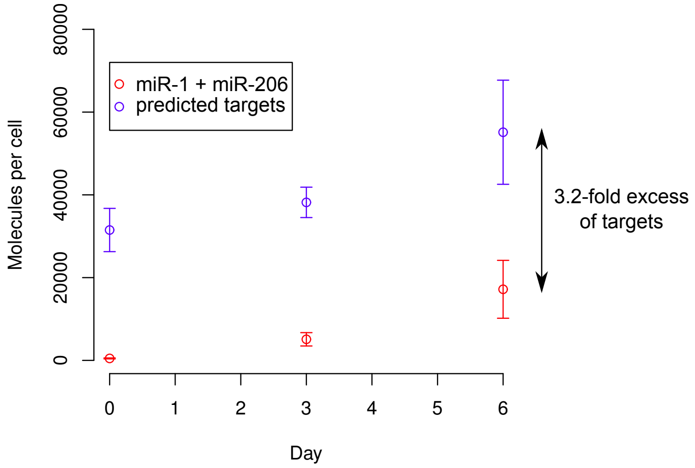

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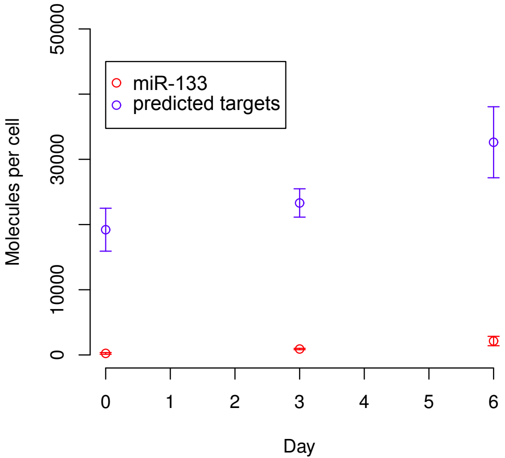

◂ Return

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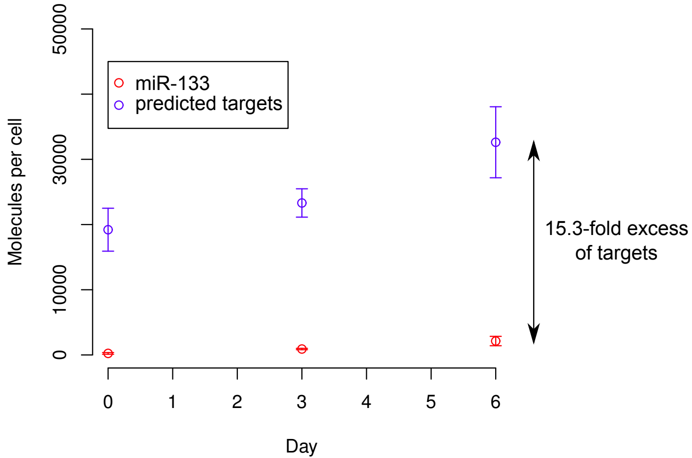


◂ Return

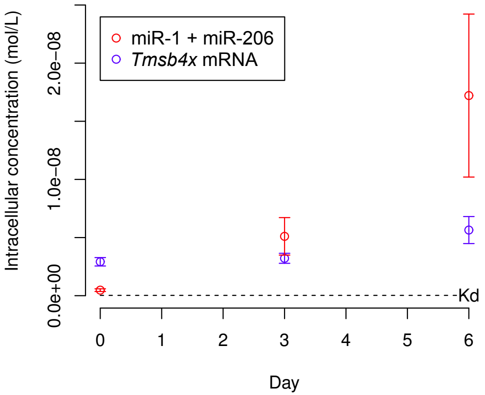
# Deep-sequencing statistics

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

# Concentration effects



◂ Return

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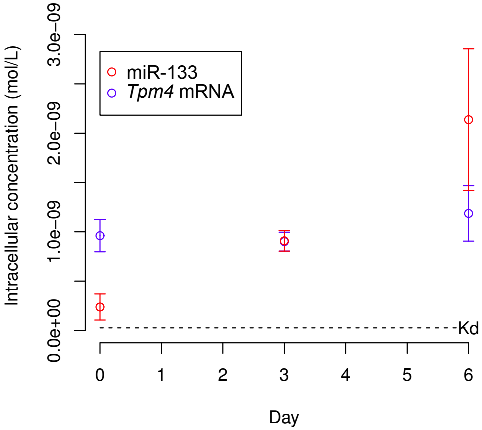

Return

# Pseudo-targets for other regulators?

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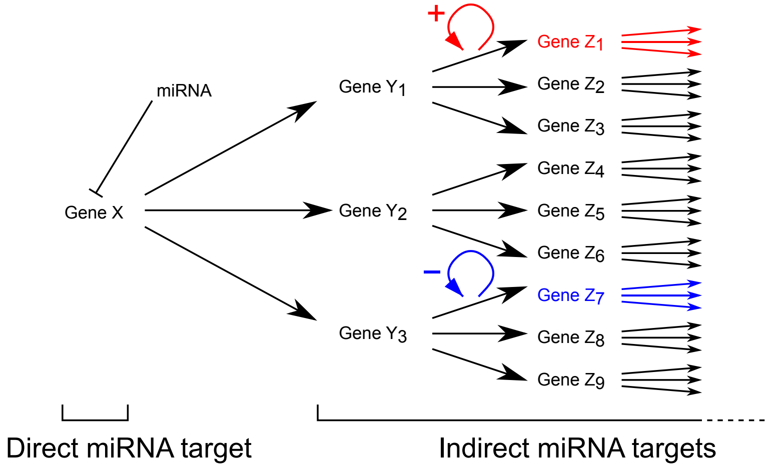
Real molecular events, which are neutral in evolutionary terms?

◂ Return

# Propagation of gene expression perturbation



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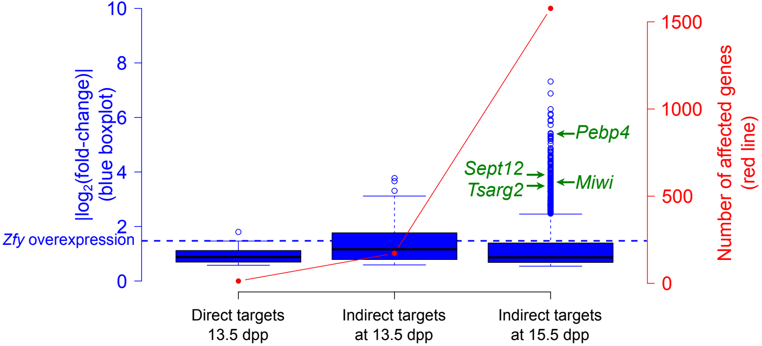

(in collaboration with H. Royo and J. Turner, MRC, London)