

On the notion of functional regulation in molecular biology — the case of microRNAs

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February 8, 2018

<http://www.igh.cnrs.fr/equip/Seitz/Curie2018.pdf>

<http://www.normalesup.org/~seitz/Curie2018.pdf>

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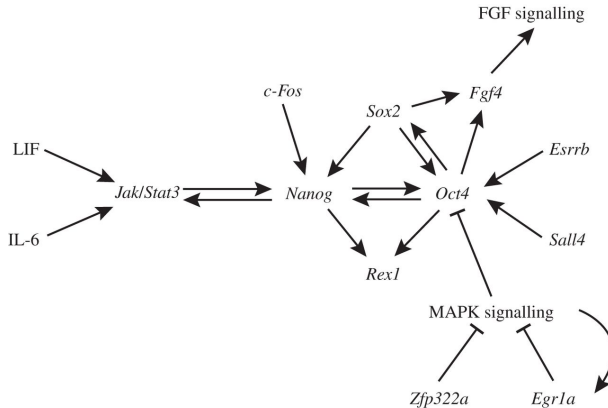
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(Parfitt and Shen, 2014)

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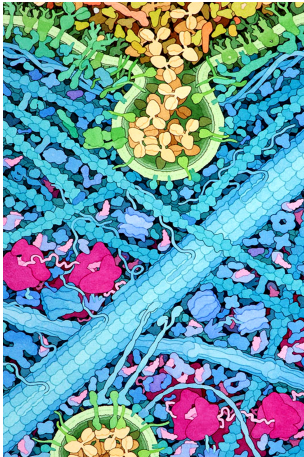
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(illustration by D. Goodsell, Scripps Research Institute and Rutgers University)

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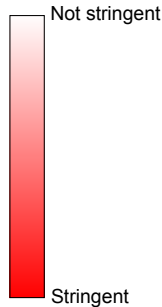
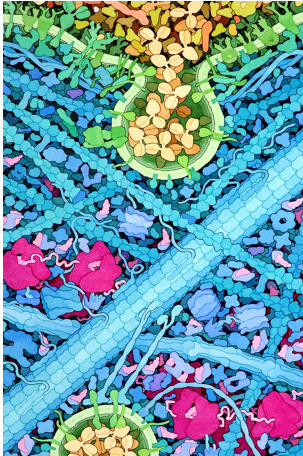
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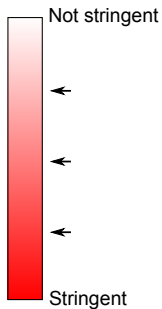
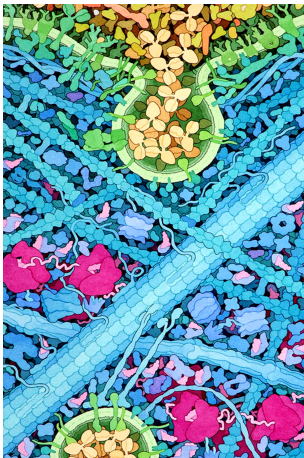
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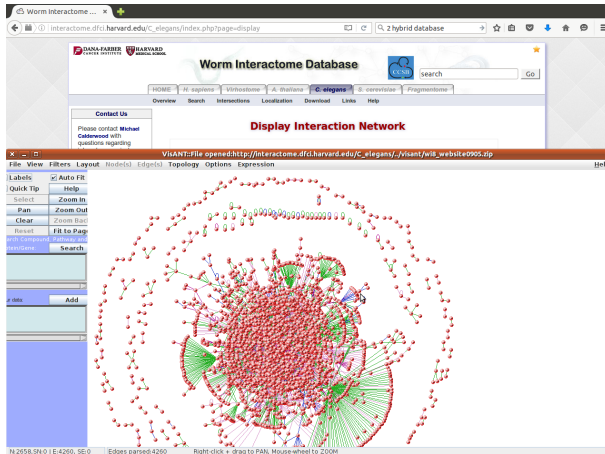
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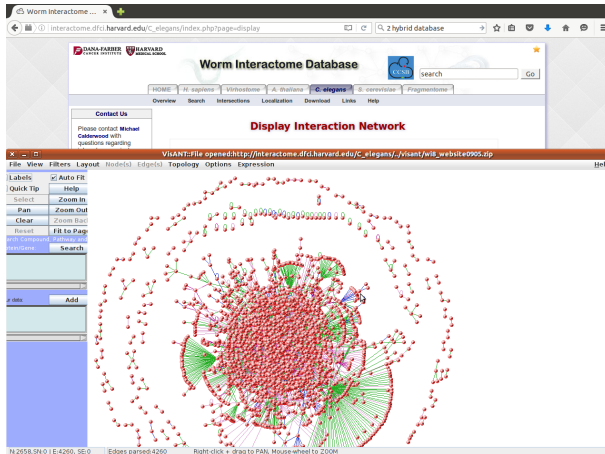
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How can we make sense of *big data* ?

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Comparative genomics dogma:
if it is conserved, it must be functional.

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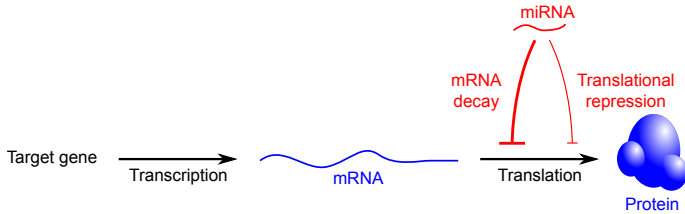
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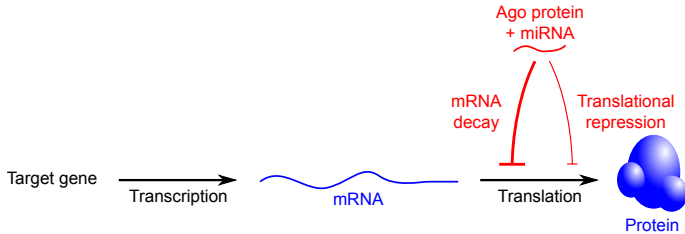
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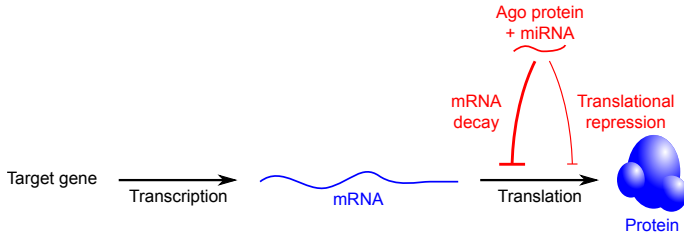
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miRNA sequences are known (for the most part); can we identify their targets ?

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microRNAs

- ▶ Sequence complementarity to the miRNA.
- ▶ Physically bind the miRNA effector complex.
- ▶ mRNA and protein level respond to miRNA.

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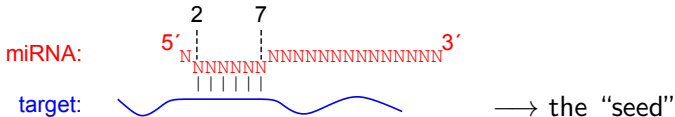
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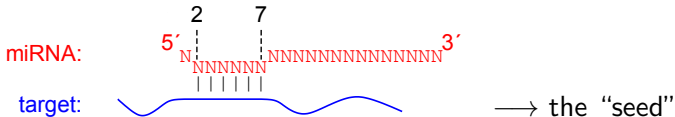
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Comparative genomics (if a seed match is conserved, it must be functional).

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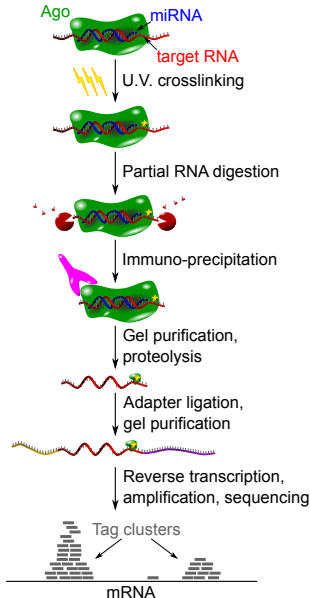
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Functionality of miRNA/target interactions

- ▶ Experimental methods (CLIP and avatars): hundreds of targets per miRNA.
- ▶ Computational methods (seed matches, conserved in evolution): hundreds of targets per miRNA.

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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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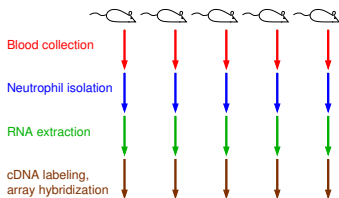
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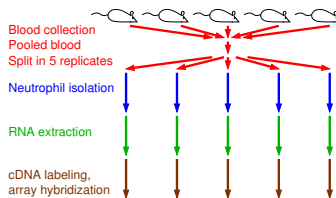
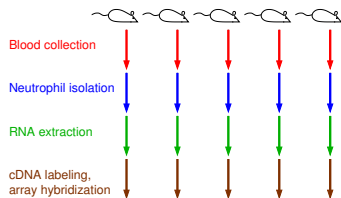
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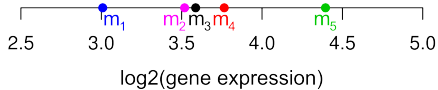
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Gene *Styx*



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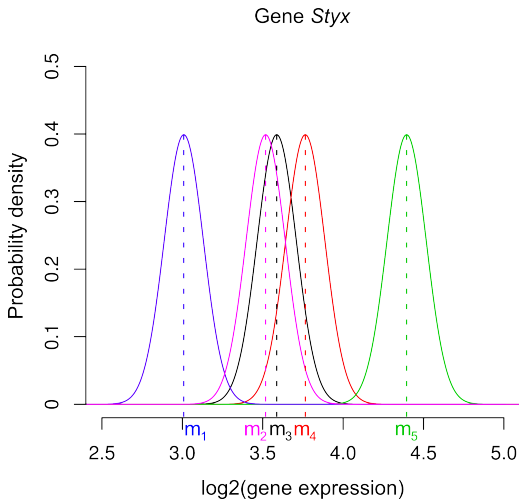
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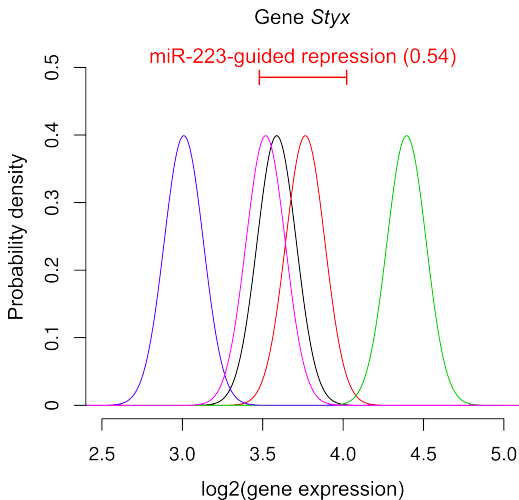
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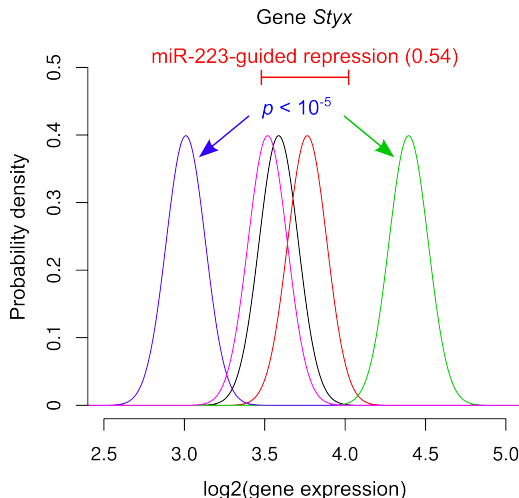
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p : probability that the difference between two individual mice is smaller than miRNA-guided repression

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Robustness of predicted targets

For 150 predicted targets out of 192:
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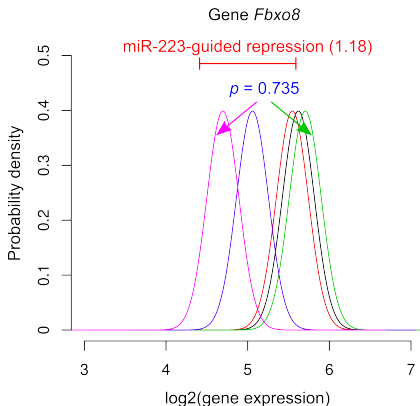
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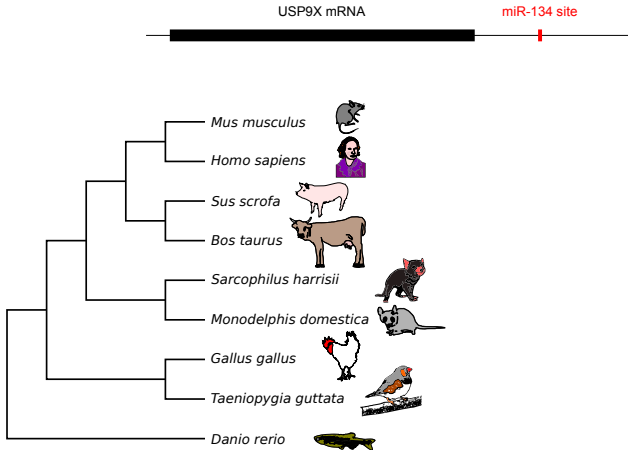
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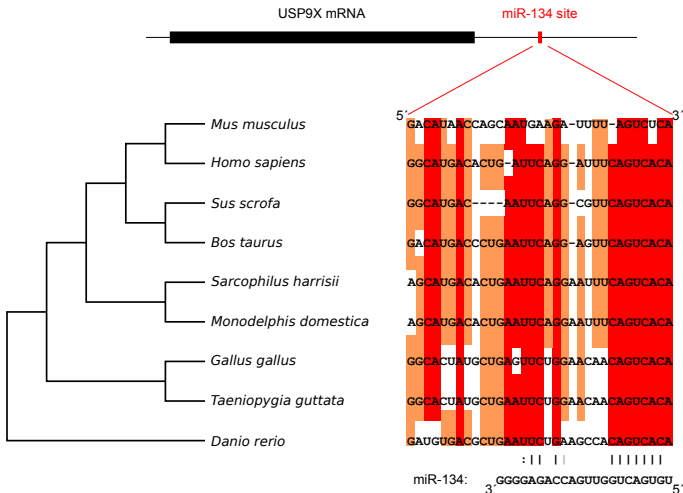
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■ : conserved in 8 or 9 species out of 9 ■ : conserved in 6 or 7 species out of 9 □ : conserved in less than 6 species out of 9

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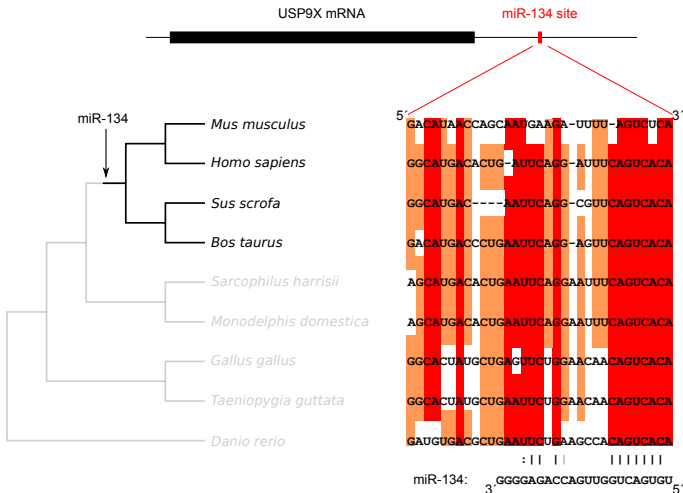
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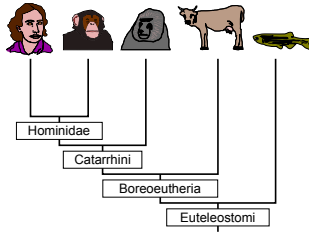
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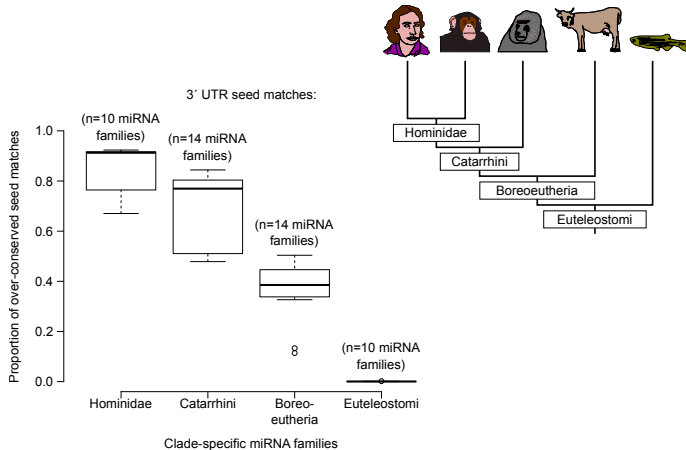
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► Comparison to prediction programs

► Effect of tree architecture

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Most known miRNA “targets” are probably not *functionally* targeted.

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Observing a molecular event does not mean that it has a biological function.

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Observing a molecular **object** does not mean that it has a biological function.

Homeostasy attenuates changes of external origin: it also buffers internal changes.

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S. Mockly
Y. Merlat

Alumni:

A. Sergeeva
L. Martinez
N. Pinzón
B. Li

Collaborators: F. Apparailly, J. Presumey (INM, Montpellier)



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▸ Assessment of miRNA titration by mRNAs

▸ Absolute miRNA quantification

▸ Absolute mRNA quantification

▸ Experimental target identification

▸ Verification of *Tmsb4x* titrating activity

▸ Overconservation statistics

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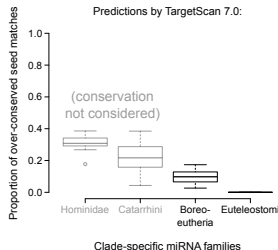
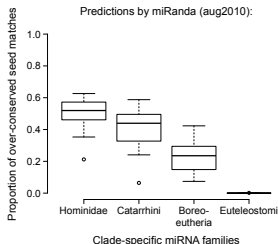
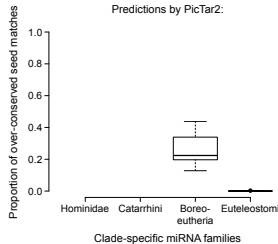
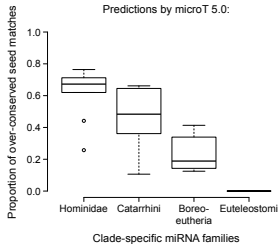
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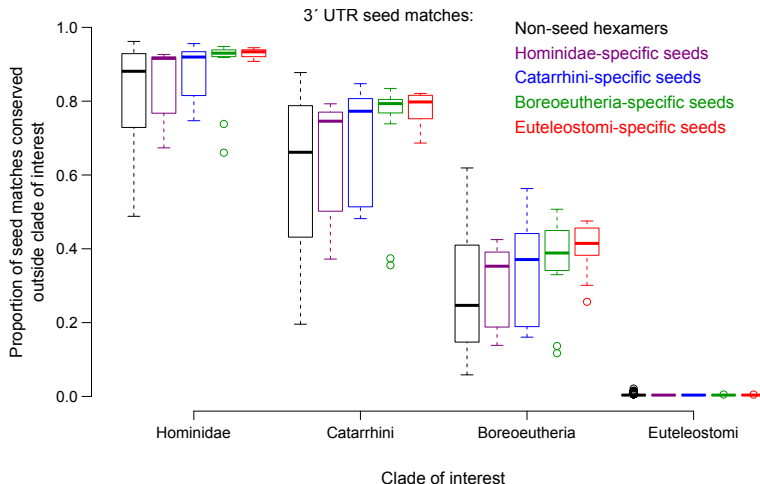
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Effect of tree architecture



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Absolute RNA quantification

For mRNAs to titrate miRNAs, they need to be abundant enough.

► Issues with published examples

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Absolute RNA quantification

For mRNAs to titrate miRNAs, they need to be abundant enough.

► Issues with published examples

Murine cell line “C2C12”: differentiates in myotubes; expresses muscle-specific miR-1, miR-206 and miR-133.

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⇒ absolute quantification of miR-1, miR-206, miR-133 and their predicted targets in differentiating C2C12.

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- miRNAs: Northern blot (calibrated with RNA oligos);

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

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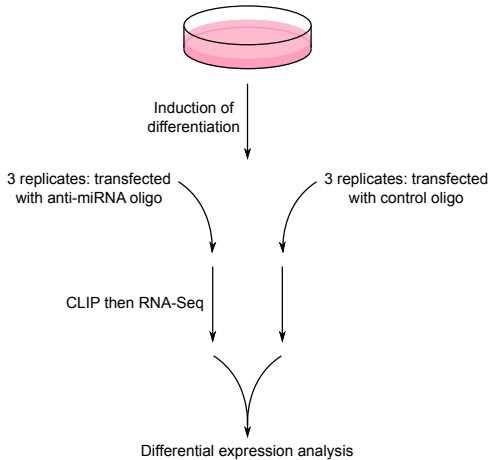
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miR-1a/miR-206 inhibition experiment		
mRNA	log2(fold-change)	Adjusted p-value
L3hydpd	-5.330	4.57e-02
Pla2g4c	-5.180	3.18e-02
Nrk	-3.770	1.86e-02
Park2	-2.870	1.23e-02
Nim1k	-2.690	3.53e-02
Lyplal1	-2.600	4.03e-09
Inca1	-2.540	4.57e-02
Sfrp4	-2.330	8.39e-03
2210408121Rik	-2.140	7.70e-07
Mlph	-2.030	8.12e-03
A430078G23Rik	-2.020	1.28e-02
Tmsb4x	-2.010	1.93e-21
Rpl39	-1.840	1.49e-15
Gbp8	-1.780	1.86e-02
Mab21l3	-1.770	3.60e-02
Sv2b	-1.710	1.23e-02
Dynl1a	-1.590	4.83e-02
Gpr39	-1.550	3.22e-02
Prkag2	-1.480	2.50e-02
Osbpl10	-1.450	2.50e-02
Tesk2	-1.330	8.39e-03
Elof1	-1.330	8.64e-06
Arrdc2	-1.280	4.08e-02
Serpine2	-1.150	2.50e-02
Atp1a1	-1.130	3.78e-06
Yif1b	-1.110	2.75e-04
Ascc1	-1.030	2.36e-02
Mtpap	-1.000	2.50e-02
Tprn	-0.993	1.21e-02
Nprl2	-0.975	4.32e-02
Tmem55b	-0.881	1.21e-02
Pdpn	-0.879	5.72e-03
Cap1	-0.836	8.12e-03
Slc25a17	-0.815	2.50e-02
Tpm4	-0.798	1.23e-02
Cox6a2	-0.777	3.60e-02
Spp1	-0.762	2.00e-02

miR-133 inhibition experiment		
mRNA	log2(fold-change)	Adjusted p-value
Ccdc65	-2.680	4.45e-02
Ctsc	-2.430	8.36e-03
Samd9l	-2.360	9.87e-03
Cnksr1	-2.240	2.49e-04
Npbr	-2.020	3.70e-04
Zfp563	-1.800	3.70e-04
Snap23	-1.410	8.18e-08
Gsg1l	-1.380	2.54e-03
Hsd17b11	-1.180	5.33e-03
Fam132a	-1.180	4.45e-02
Btbd17	-1.150	1.05e-02
Tm6sf1	-1.110	2.62e-02
Eid1	-1.050	4.62e-02
Kcnk5	-1.030	8.18e-03
Pgam2	-0.993	3.70e-04
Nmrk2	-0.881	9.87e-03
Rpl7a	-0.796	9.57e-03

Red: perfect seed match in 3' UTR

Blue: one of the top 3 imperfect seed matches in 3' UTR

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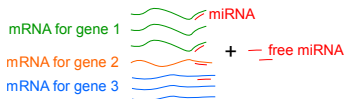
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(K_d value taken from Wee *et al.*, 2012: 26 pM)

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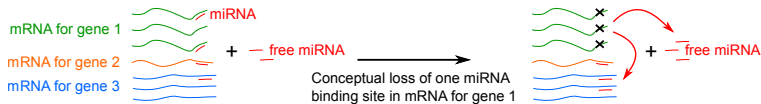
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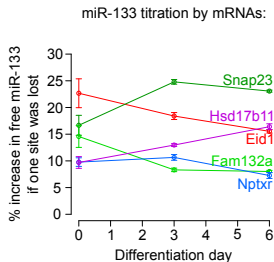
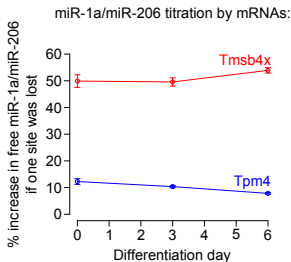
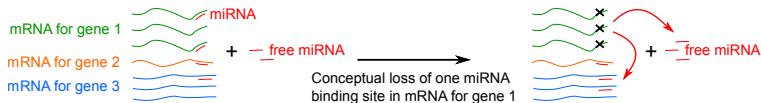
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Absolute miRNA quantification	Experimental target identification	Absolute mRNA quantification
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(in differentiating C2C12 cells)

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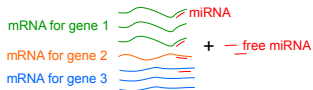
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Absolute miRNA quantification	Experimental target identification	Absolute mRNA quantification
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(K_d value taken from Wee *et al.*, 2012: 26 pM)

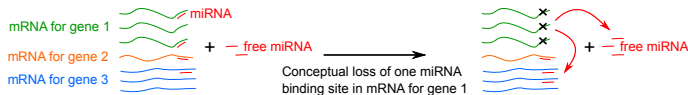
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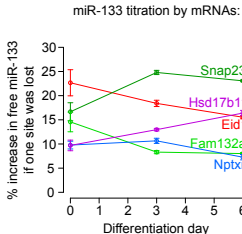
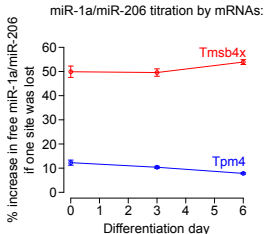


(K_d value taken from Wee *et al.*, 2012: 26 pM)

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Absolute miRNA quantification Experimental target identification Absolute mRNA quantification

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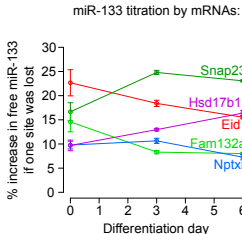
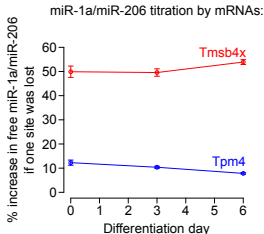


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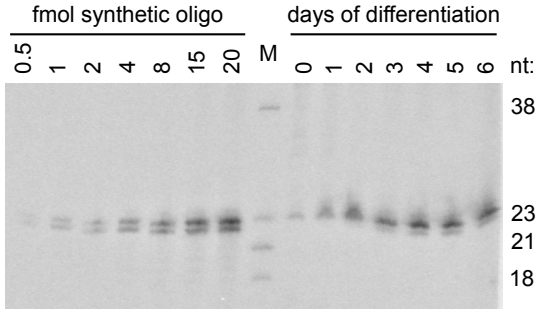
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(in differentiating C2C12 cells)



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miRNA quantification



(quantification of miR-1 and miR-206)

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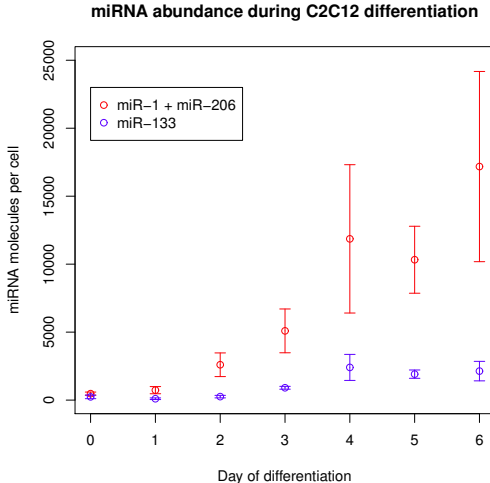
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Very deep sequencing: three time points (day 0, day 3, day 6) in triplicate; each replicate: between 267 and 333 million transcriptome-matching reads.

► Statistics



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27 synthetic spike-ins, for calibration.

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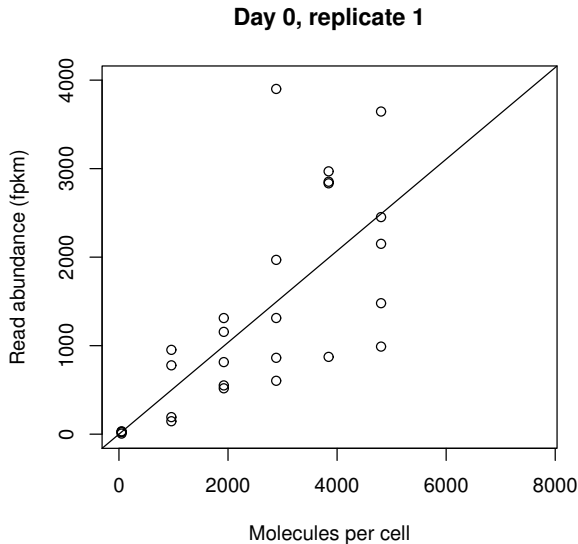
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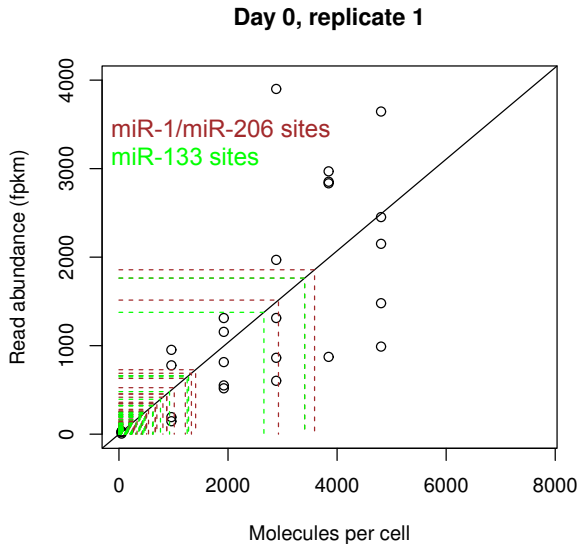
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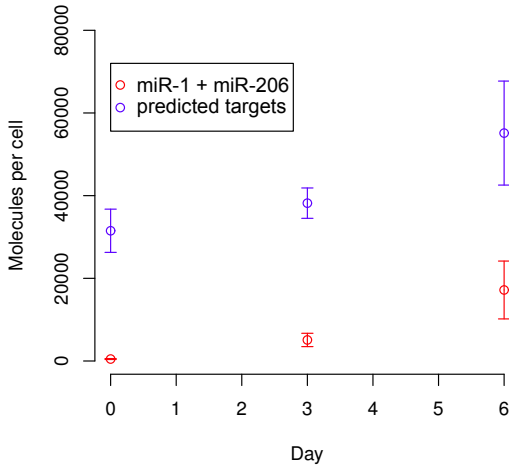
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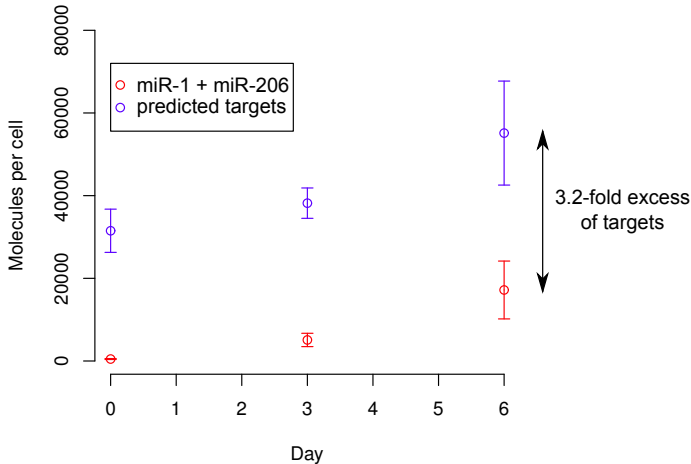
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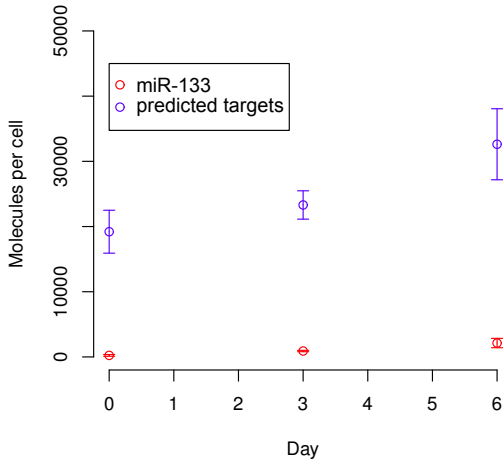
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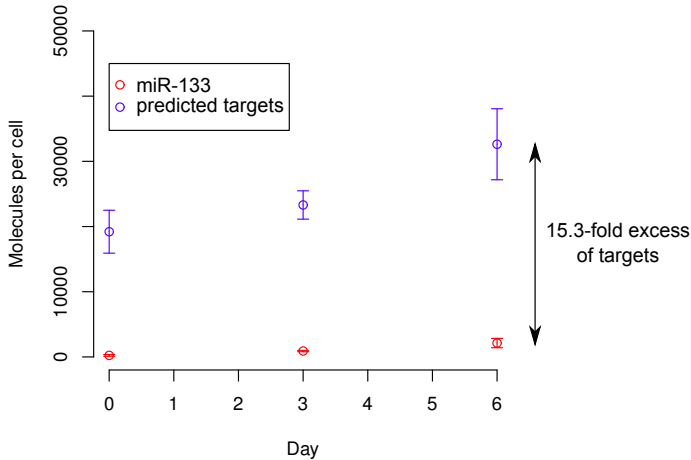
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Deep-sequencing statistics

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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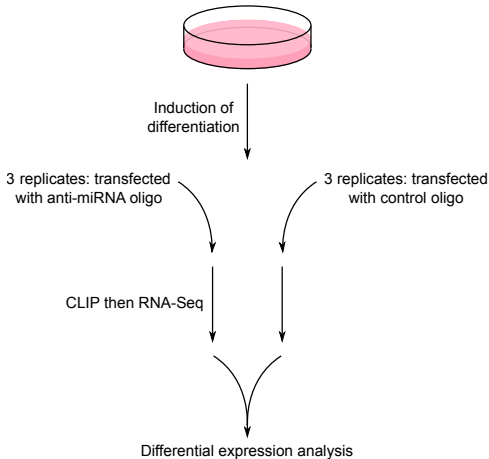
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Pdpn	-0.879	5.72e-03
Cap1	-0.836	8.12e-03
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Tpm4	-0.798	1.23e-02
Cox6a2	-0.777	3.60e-02
Spp1	-0.762	2.00e-02

miR-133 inhibition experiment		
mRNA	log2(fold-change)	Adjusted p-value
Ccdc65	-2.680	4.45e-02
Ctsc	-2.430	8.36e-03
Samd9l	-2.360	9.87e-03
Cnksr1	-2.240	2.49e-04
Nptxr	-2.020	3.70e-04
Zfp563	-1.800	3.70e-04
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Pgam2	-0.993	3.70e-04
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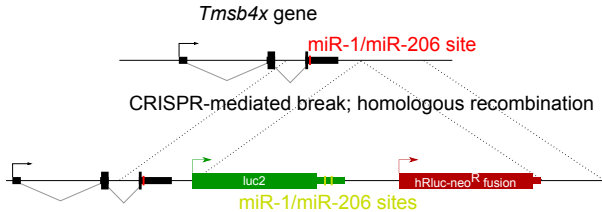
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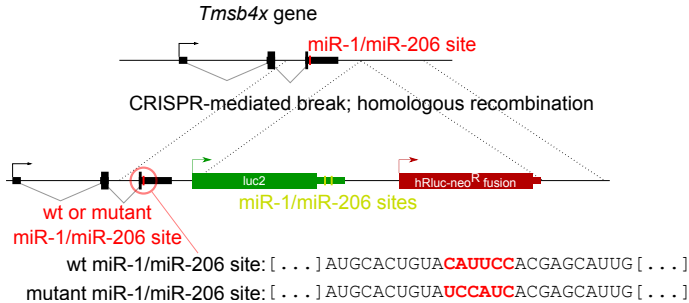
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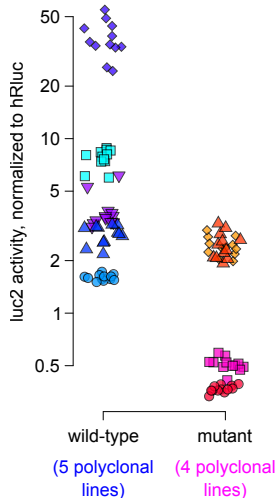
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(effect of genotype: $p = 0.0285$)

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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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Reported examples of pseudo-targets

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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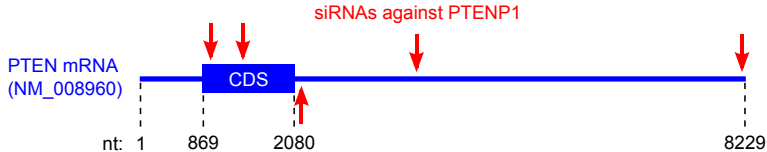
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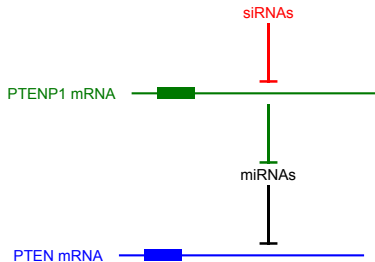
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Proposed by Poliseno *et al* (2010):



More probably:



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

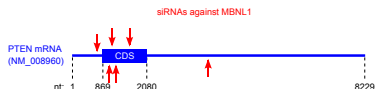
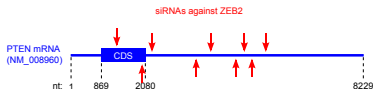
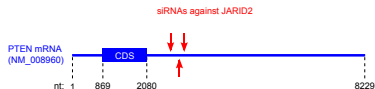
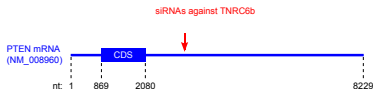
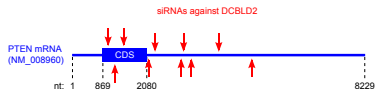
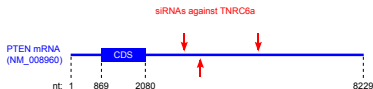
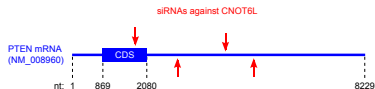
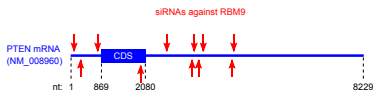
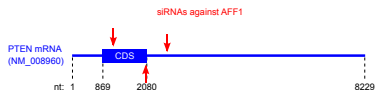
siRNA #2 against PTENP1: 5' UAAUAAUC-AUCAUUCUGGC 3'
||||| |:| |:|
PTEN mRNA (nt 8189-8208): 3 UUUUUUACUUGGAAAAUUA 5'

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 AGGAUUAUUGACGUUAGACUGU 5'

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

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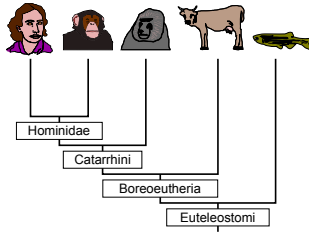
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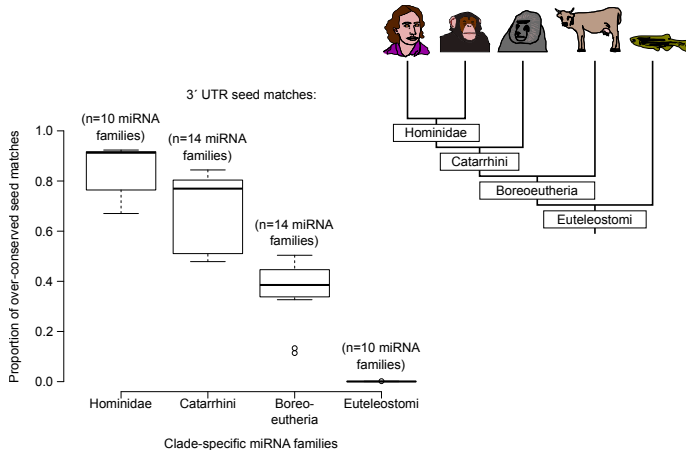
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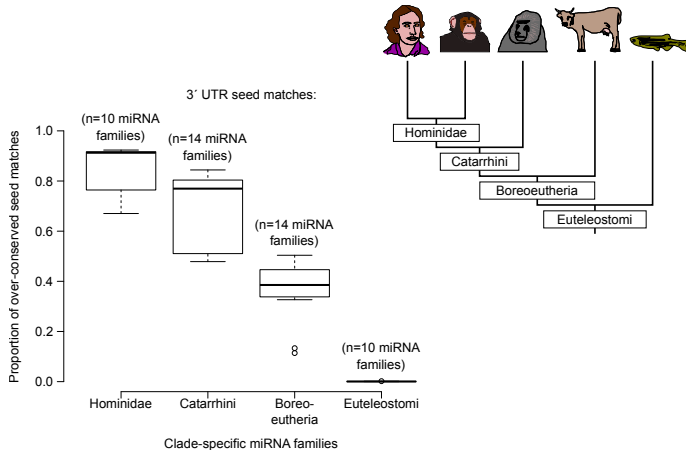
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