A functional definition of "microRNA targets": current issues, biological implications, perspectives for improvement

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This slideshow is available at:
http://www.igh.cnrs.fr/equip/Seitz/SRBC2021.pdf

The principles of microRNA target prediction

Inconsequential microRNA/target interactions

Comparative genomics yield false positives

Practical implications: an example

Conclusion

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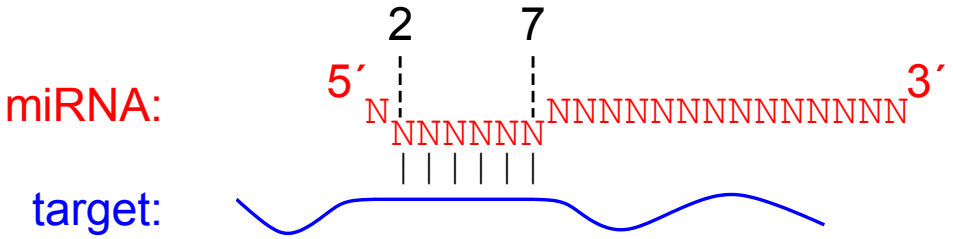

$\longrightarrow$ the "seed"

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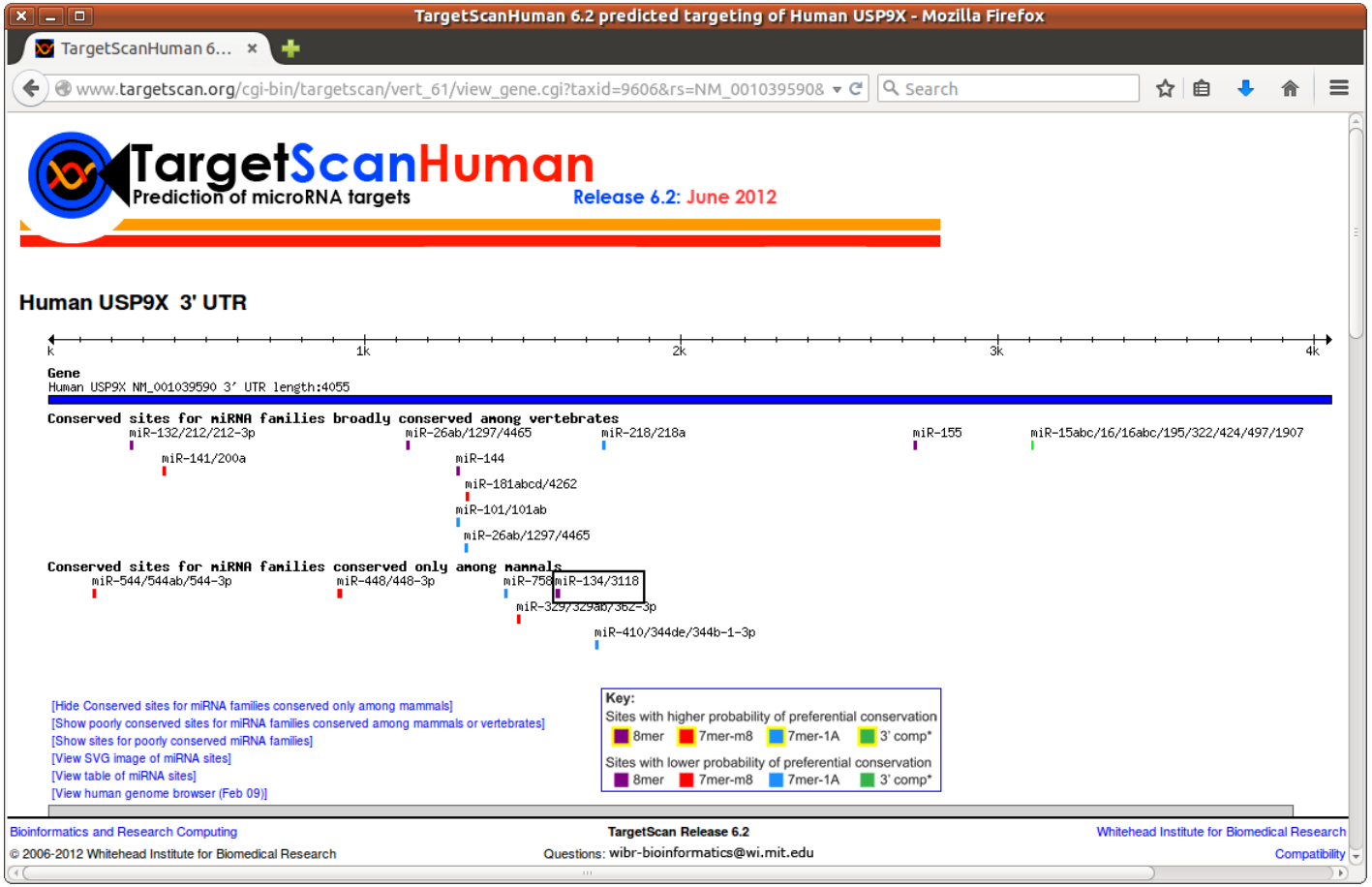

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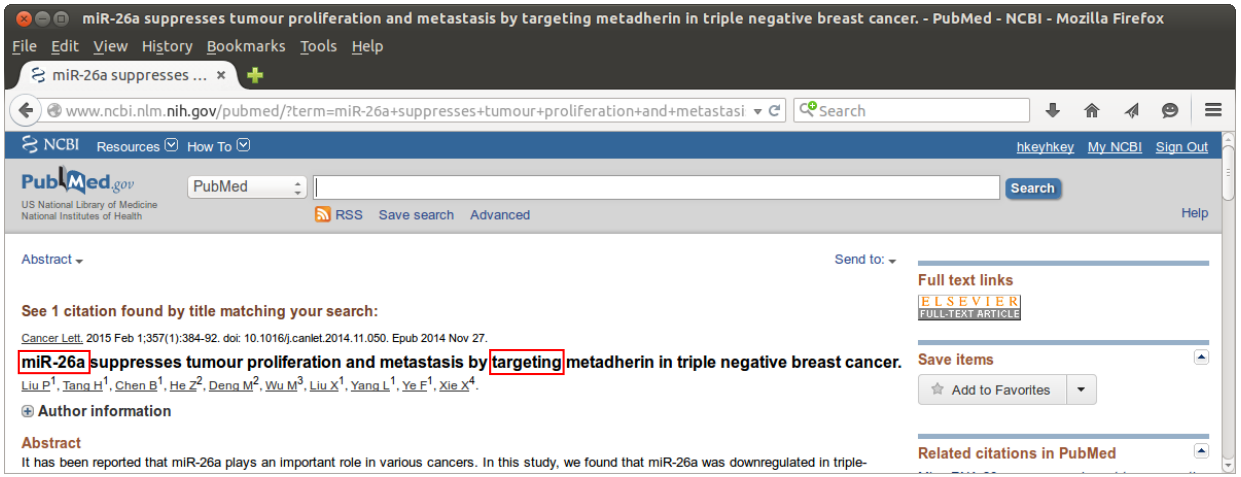

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$\Longrightarrow$ miRNAs are implicated in every physiological process in animals.

Inconsequential microRNA/target interactions

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- In the best studied cases, miRNA-controlled phenotypes appear to be due to a single target each (Ecsedi *et al.*, 2015; Title *et al.*, 2018; Aeschimann *et al.*, 2019; Garaulet *et al.*, 2020; Yang *et al.*, 2020).

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$\longrightarrow$ Why have all predicted sites been conserved if they are not functional ?

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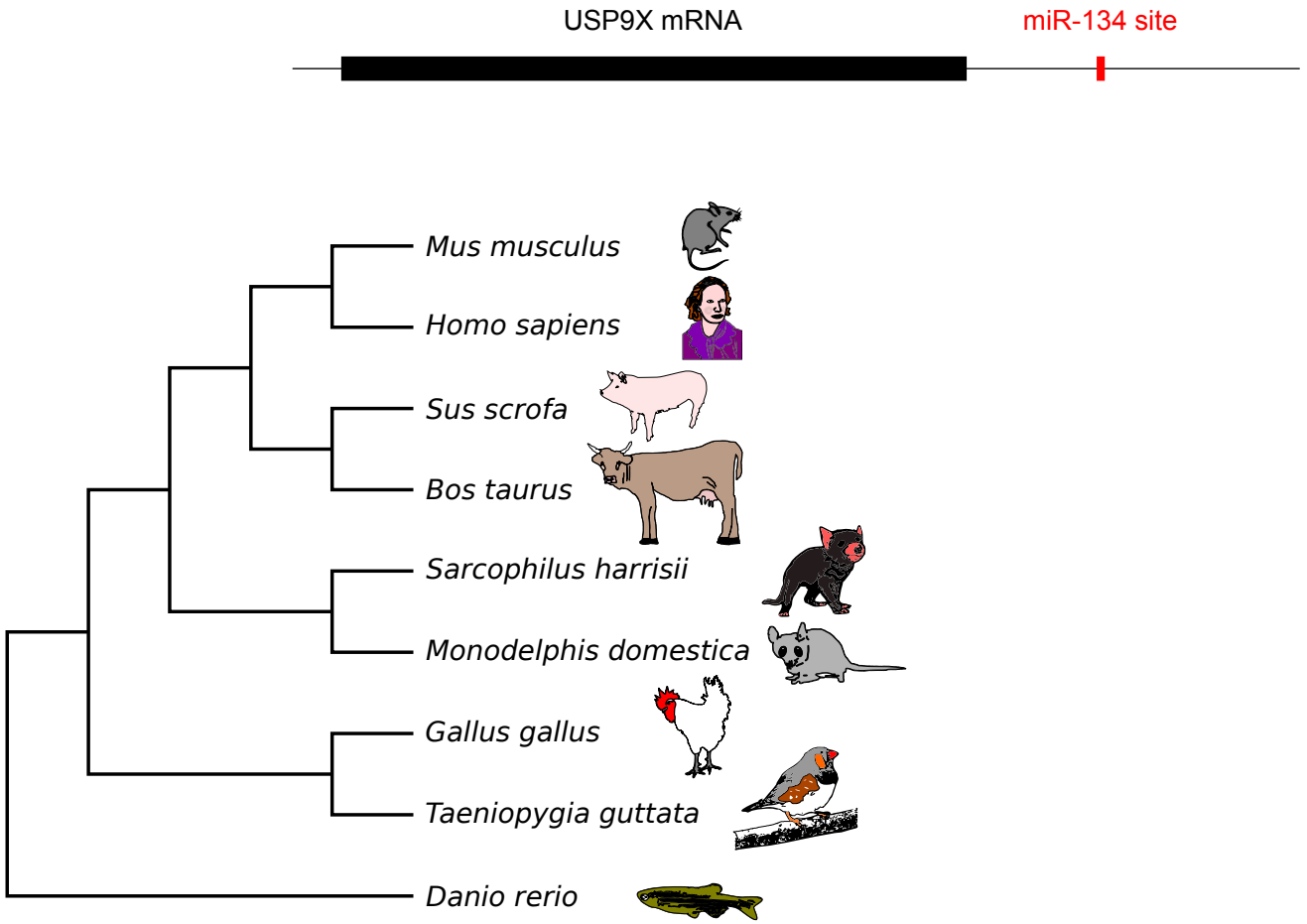

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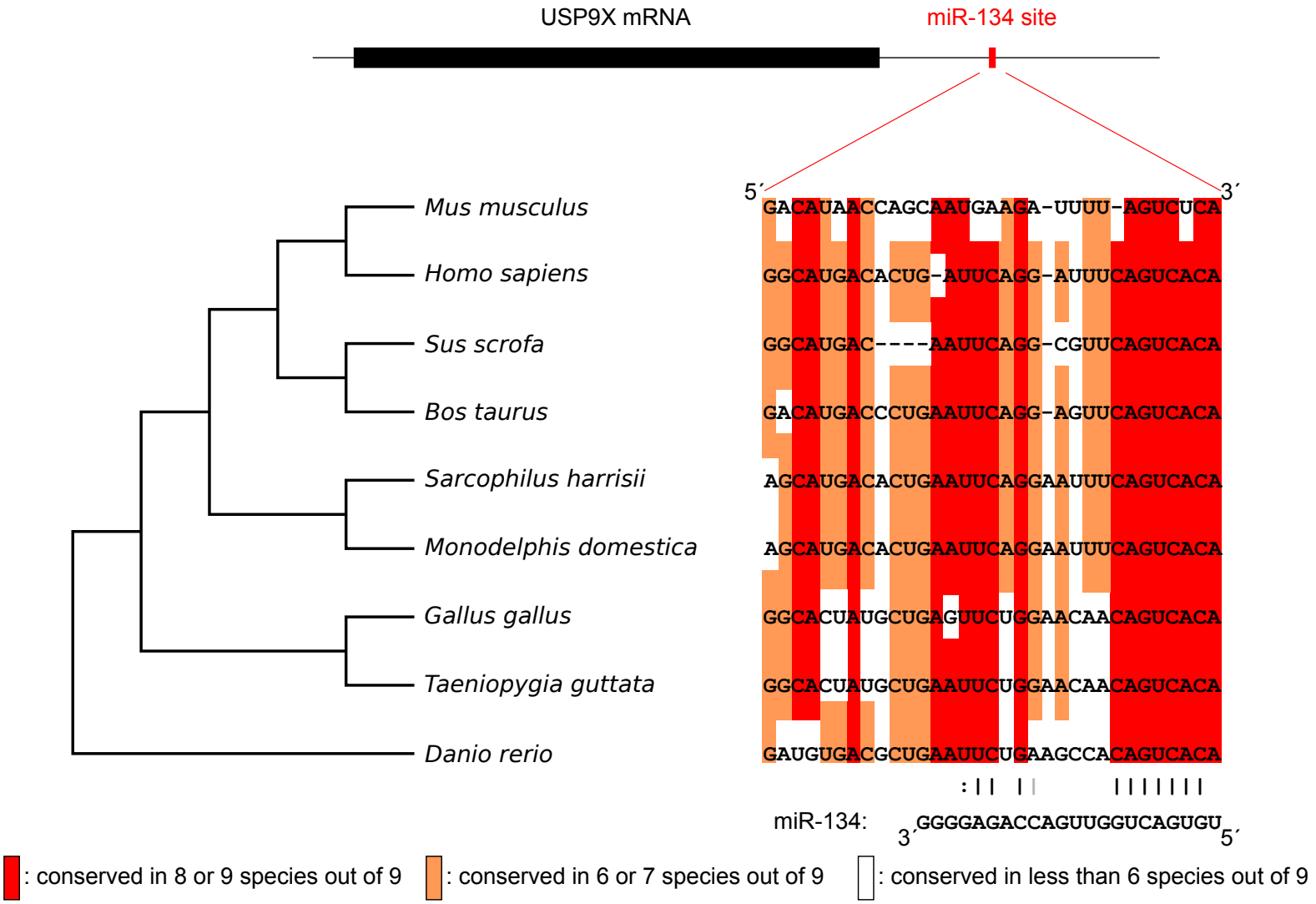

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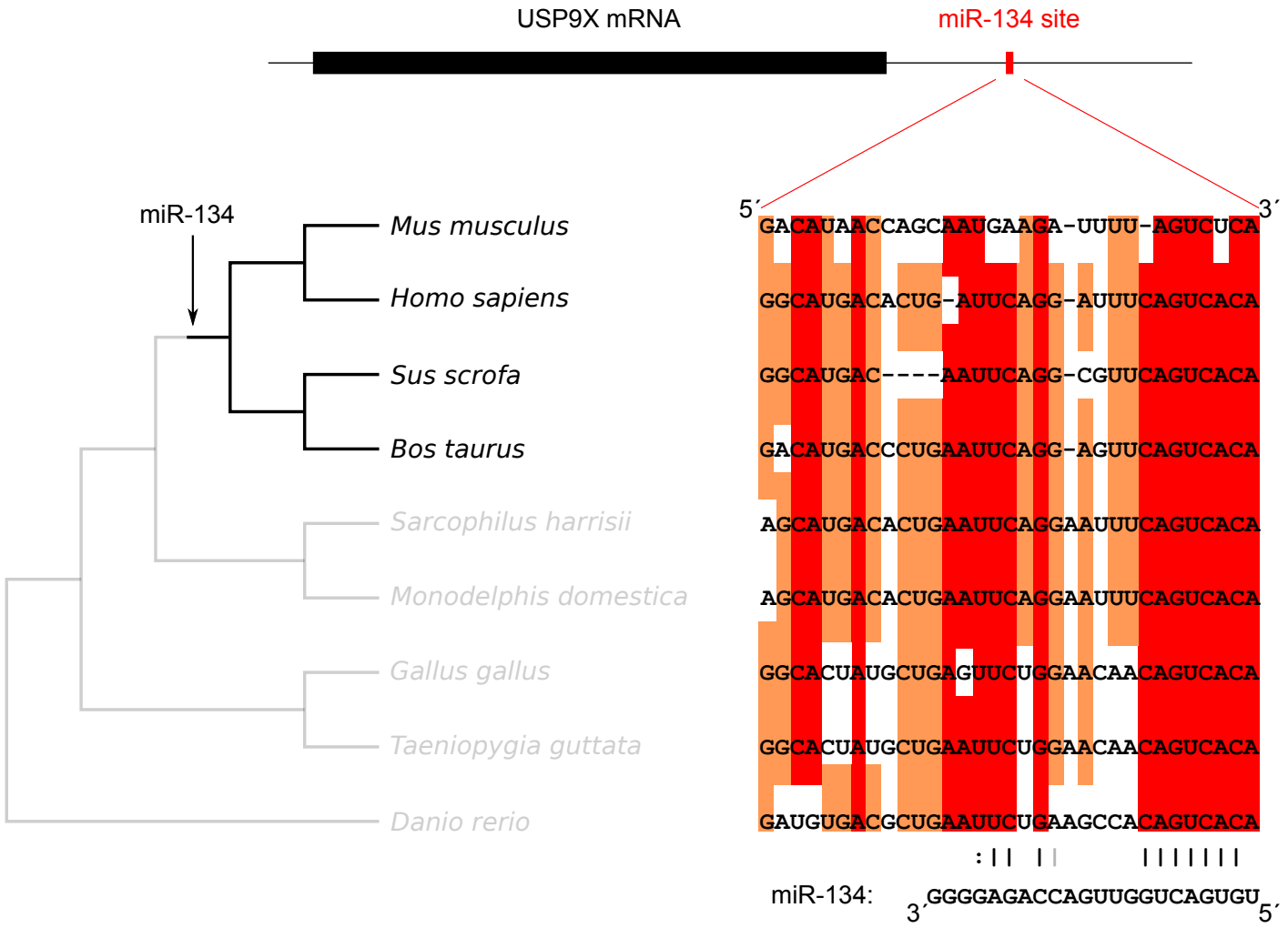

: conserved in 8 or 9 species out of 9 : conserved in 6 or 7 species out of 9 : conserved in less than 6 species out of 9

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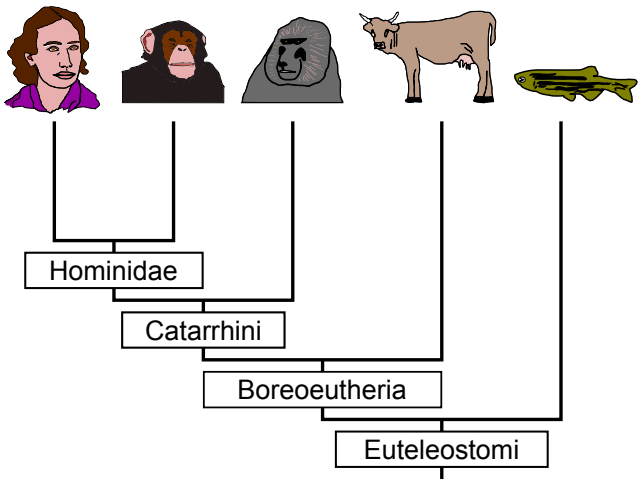

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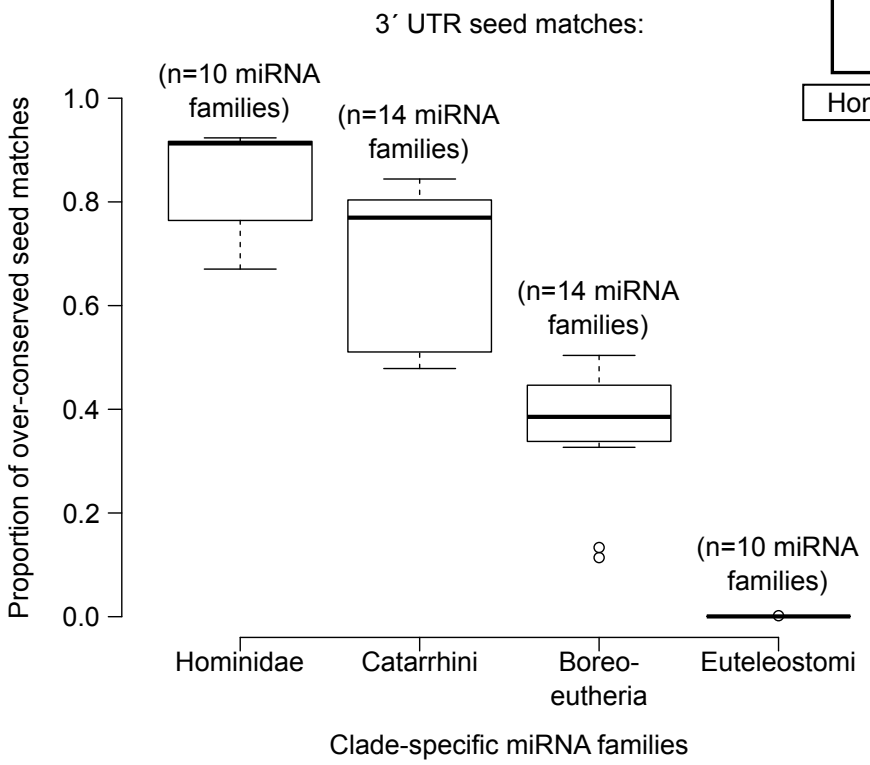

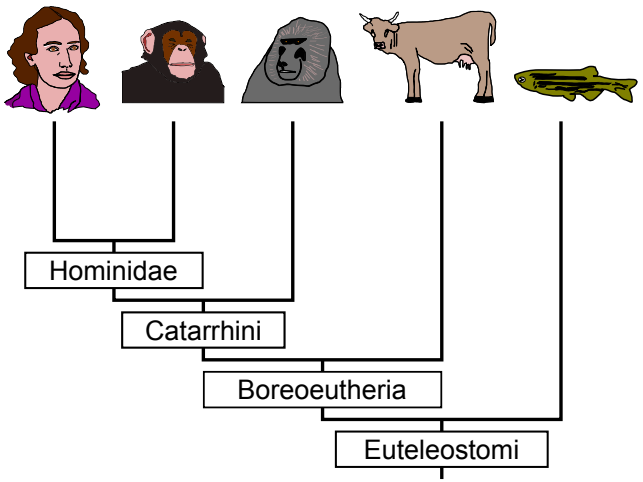

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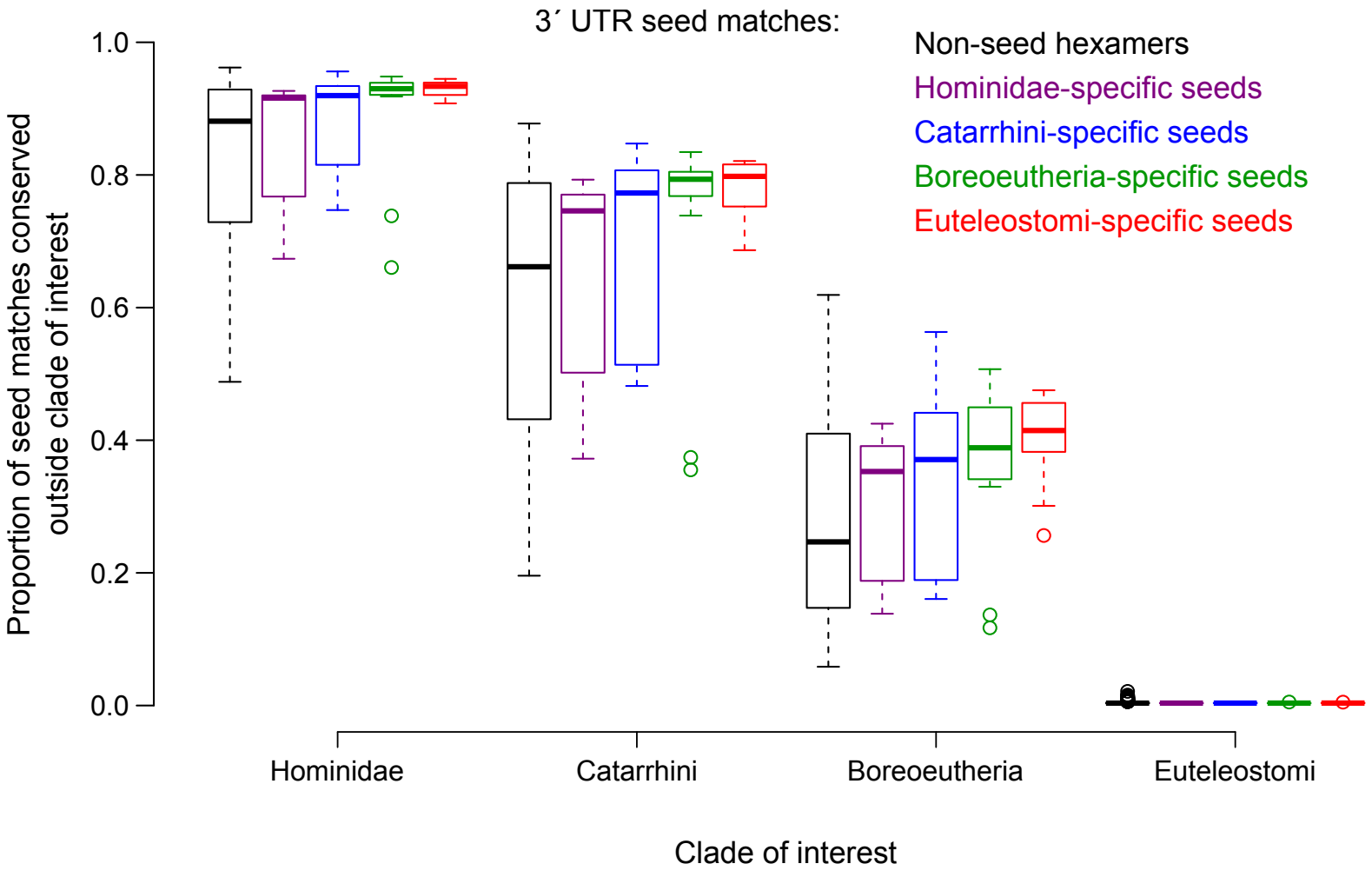

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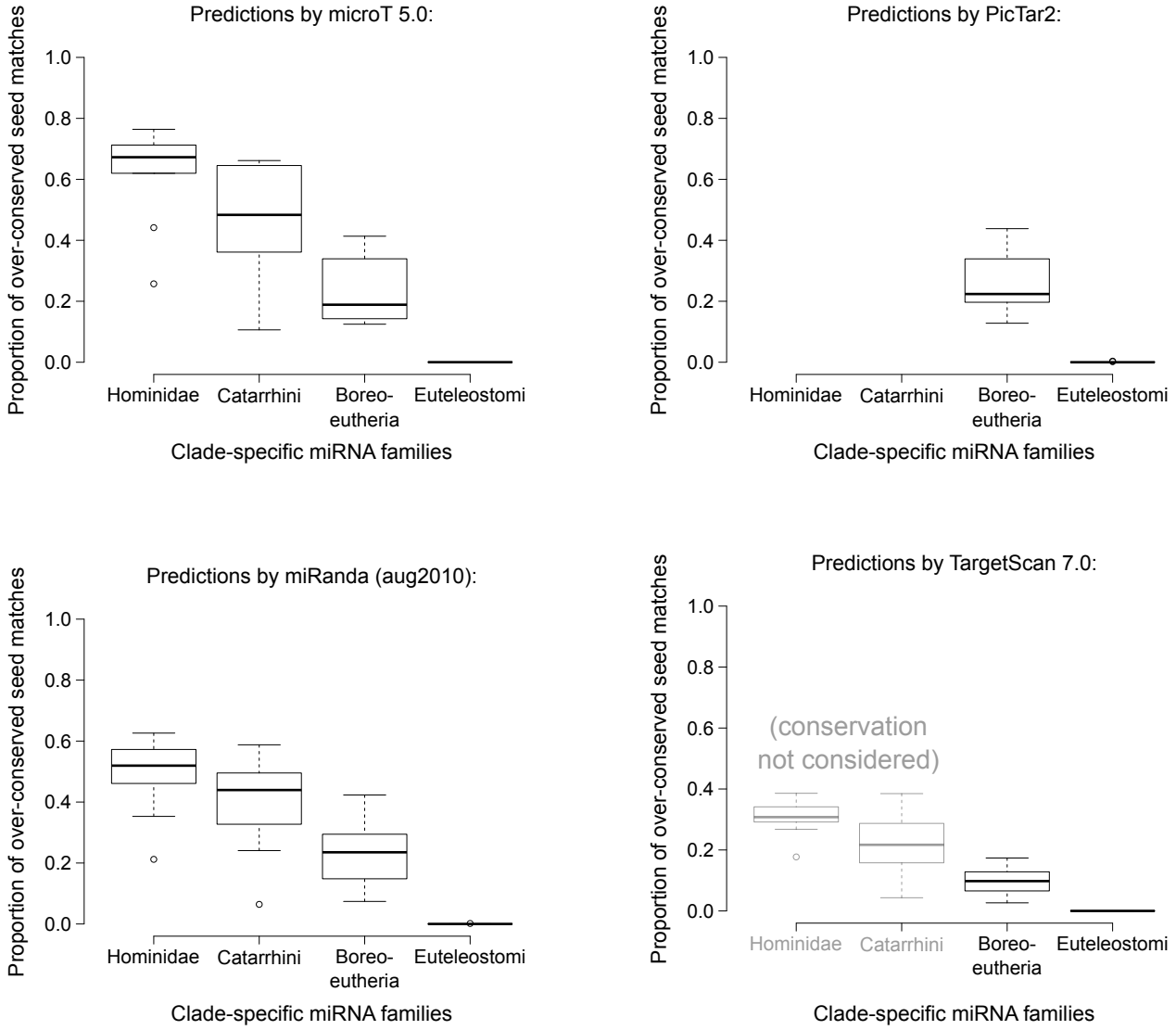

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Measuring miRNA-independent conservation with control *k*-mers (with same G/C content, dinucleotide composition, ...; Friedman *et al.*, 2009).

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Measuring miRNA-independent conservation with control k-mers (with same G/C content, dinucleotide composition, ...; Friedman *et al.*, 2009).

$\longrightarrow$ not submitted to the same selective pressure than actual miRNA seed matches (*e.g.*, protein binding sites).

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The miR-34 family is widely perceived as a tumor suppressor (*e.g.*, Slack and Chinnaiyan, 2019) ...

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TargetscanHuman 8.0: predicted miRNA targets of miR-34-5p/449-5p — Mozilla Firefox

www.targetscan.org/cgi-bin/targetscan/vert_80/targetscan.cgi?species=Human&gid=&mir_sc=miR-34-5p%2F449-5p&mir_c=&mir_nc=&mir_vnc=

Human | miR-34-5p/449-5p

754 transcripts with conserved sites, containing a total of **838** conserved sites and **389** poorly conserved sites.

Please note that these predicted targets include some false positives. [Read more]

Genes with only poorly conserved sites are not shown. [View top predicted targets, irrespective of site conservation]

Table sorted by cumulative weighted context++ score [Sort table by predicted occupancy] [Sort table by aggregate P_{CT}]

The table shows at most one transcript per gene, selected for being the most prevalent, based on 3P-seq tags. [Download table]

Target gene	Representative transcript ENST0000...	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Conserved sites				Poorly conserved sites				6mer sites	Representative miRNA	Predicted occupancy			Cumulative weighted context++ score	Total context++ score	Aggregate P_{CT}	Previous TargetScan publication(s)
					total	8mer	7mer-m8	7mer-A1	total	8mer	7mer-m8	7mer-A1			mod miRNA	high miRNA	trans-fected miRNA				
MDM4	0391947.2	Mdm4 p53 binding protein homolog (mouse)	443	Sites in UTR	**4**	3	0	1	**3**	2	0	1	1	hsa-miR-34c-5p	0.1129	0.7383	3.1800	-2.38	-2.56	0.94	2011, '15
HCN3	0368358.3	hyperpolarization activated cyclic nucleotide-gated potassium channel 3	41	Sites in UTR	**4**	4	0	0	**2**	1	0	1	0	hsa-miR-449b-5p	0.1128	0.6855	2.4728	-1.57	-1.57	> 0.99	2011, '15
FAM76A	0373954.6	family with sequence similarity 76, member A	173	Sites in UTR	**3**	2	0	1	**0**	0	0	0	0	hsa-miR-449b-5p	0.0862	0.5203	1.7444	-1.47	-1.48	> 0.99	2007, '09, '11, '15
SCN2B	0278947.5	sodium channel, voltage-gated, type II, beta subunit	5	Sites in UTR	**3**	2	0	1	**1**	0	0	1	1	hsa-miR-34c-5p	0.1257	0.7301	2.3678	-1.29	-1.29	> 0.99	2005, '07, '09, '11, '15
SYT1	0457153.2	synaptotagmin I	13	Sites in UTR	**4**	1	2	1	**0**	0	0	0	2	hsa-miR-449b-5p	0.1040	0.6559	2.5358	-1.24	-1.24	> 0.99	2005, '07, '09, '11, '15
FAM167A	0284486.4	family with sequence similarity 167, member A	85	Sites in UTR	**1**	1	0	0	**3**	3	0	0	1	hsa-miR-449b-5p	0.0910	0.5730	2.2638	-1.23	-1.23	0.67	2005, '07, '09, '11, '15
RRAS	0246792.3	related RAS viral (r-ras) oncogene homolog	267	Sites in UTR	**1**	1	0	0	**1**	0	0	1	0	hsa-miR-34c-5p	0.0461	0.2752	0.9159	-1.16	-1.16	0.88	2005, '07, '09, '11, '15
VAMP2	0404970.3	vesicle-associated membrane protein 2 (synaptobrevin 2)	1840	Sites in UTR	**3**	1	2	0	**0**	0	0	0	1	hsa-miR-34a-5p	0.0822	0.4989	1.8318	-1.08	-1.11	0.98	2003, '05, '07, '09, '11,

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TargetScan Release 8.0
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Compatibility

Conclusion: revisiting miRNA target definition

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- $\approx$ 1,500 annotated human miRNA families
- out of $4^6 = 4096$ possible hexamers.

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Potential improvements:

- excluding sites more conserved than the cognate miRNA family;
- evaluating phenotypic robustness to expression fluctuations;
- "experimental validation" is a genetic knockout, not a reporter assay.

Conclusion: revisiting miRNA target definition

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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Laura Martinez
Natalia Pinzón
Germain Busto
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Sophie Mockly
Anna Sergeeva

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Supplementary data

- Robustness of biological pathways
- miR-223-guided repression vs. inter-individual variability
- miR-34 and cell proliferation

Biological robustness

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

miR-223-guided repression vs. inter-individual variability

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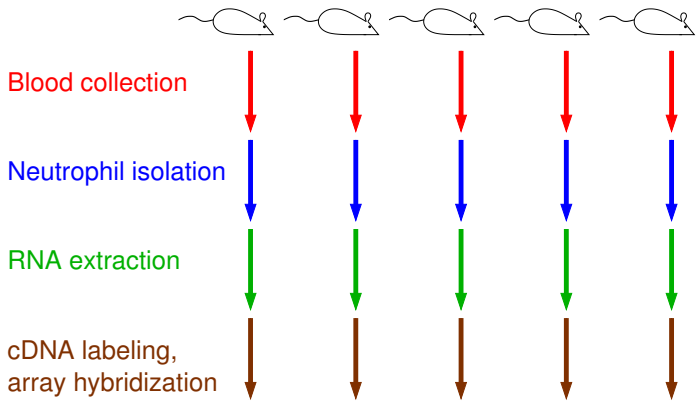

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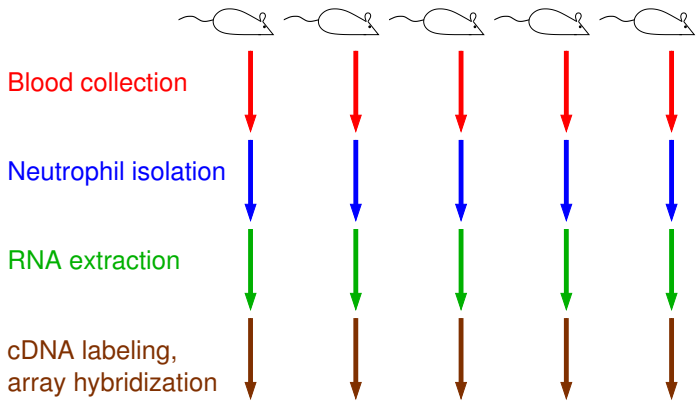
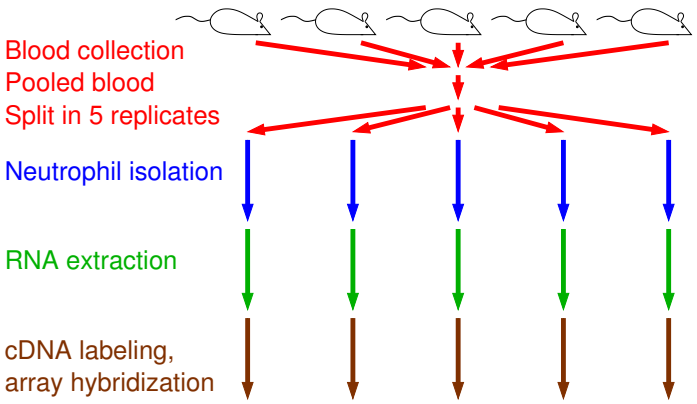
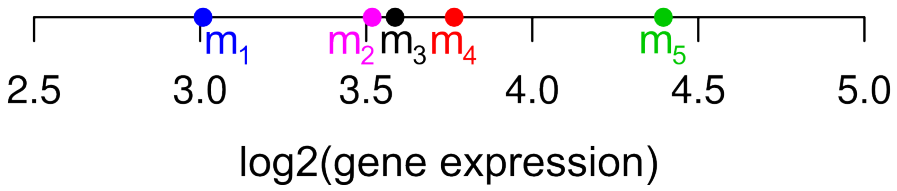




miR-223-guided repression vs. inter-individual variability

Gene *Styx*



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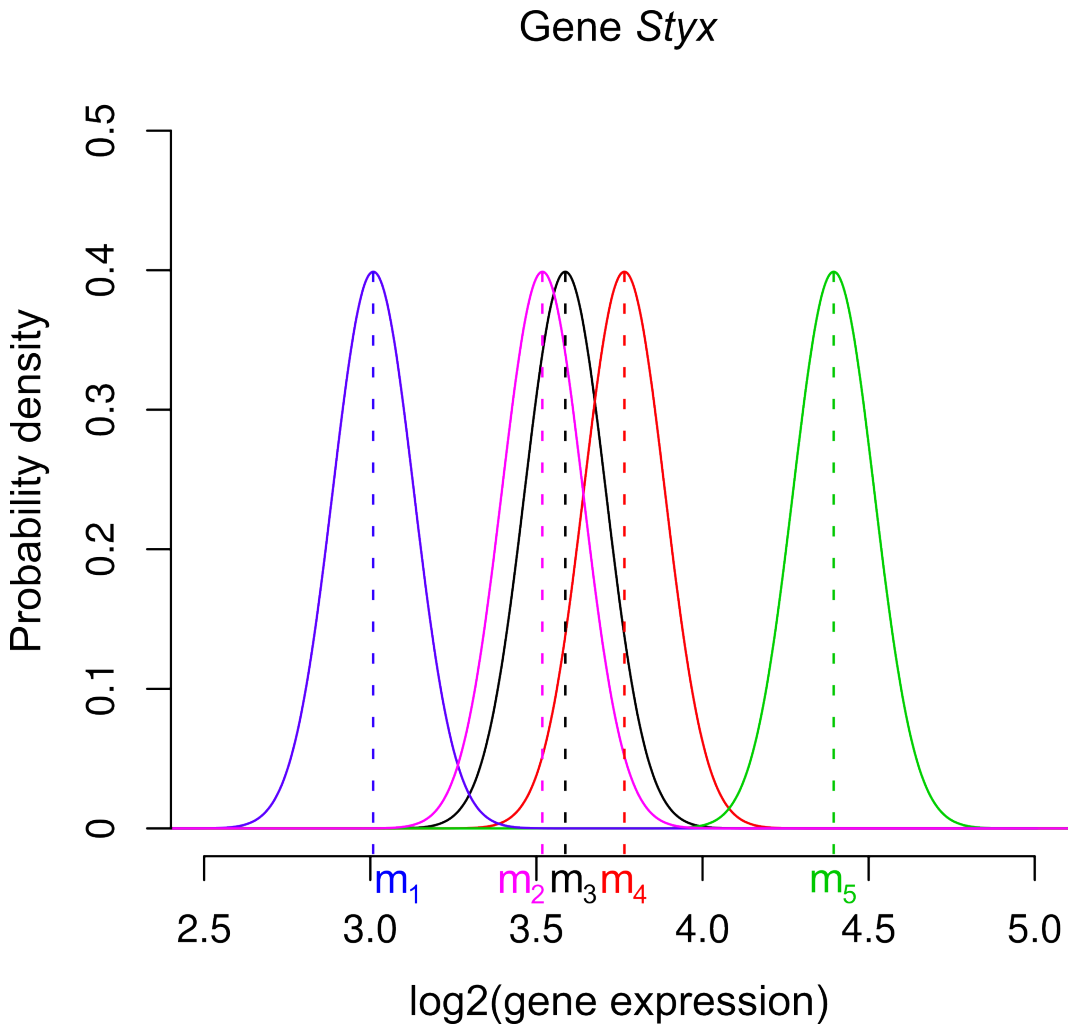

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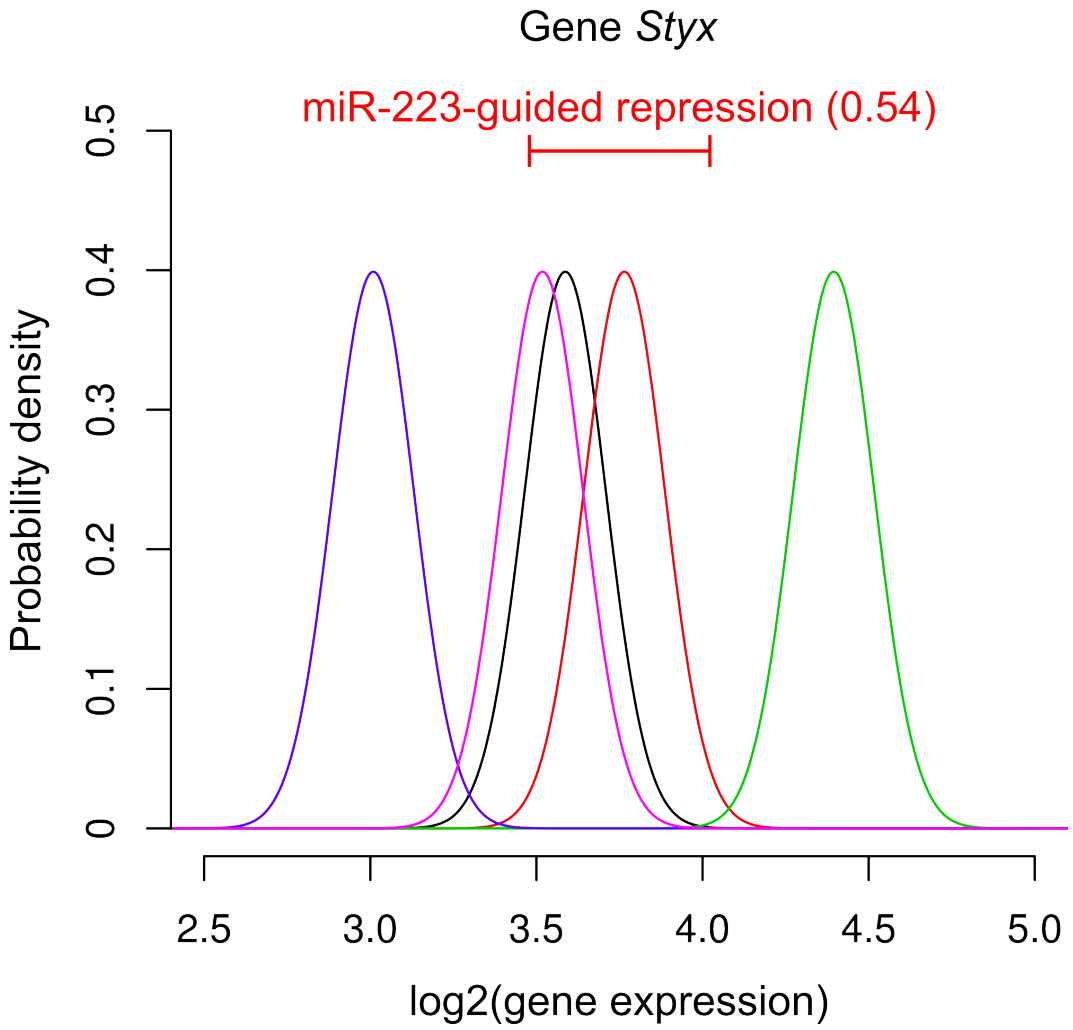

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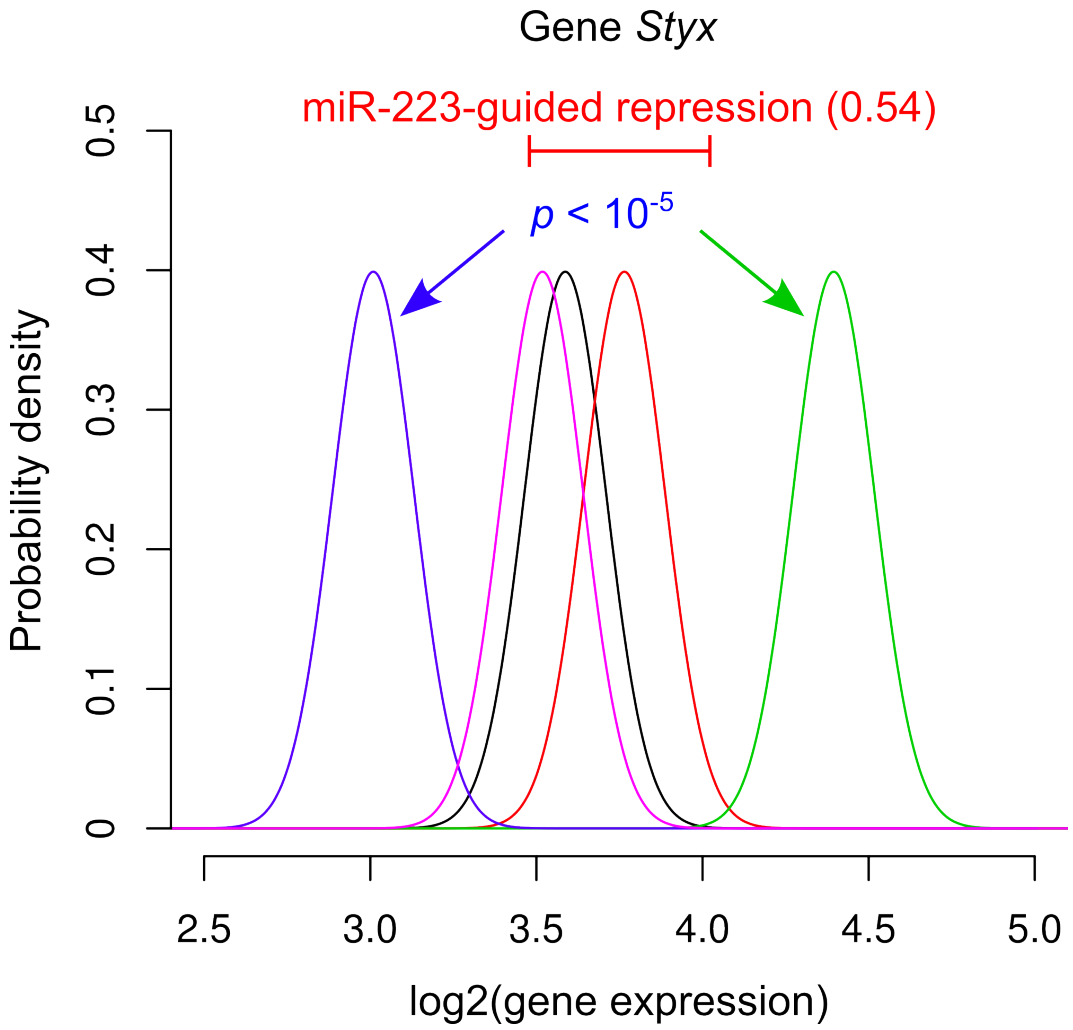

p: probability that the difference between two individual mice is smaller than miRNA-guided repression

miR-223-guided repression vs. inter-individual variability

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inter-individual fluctuations across 5 wild-type mice exceeds miRNA-mediated regulation (p-value < 0.05).

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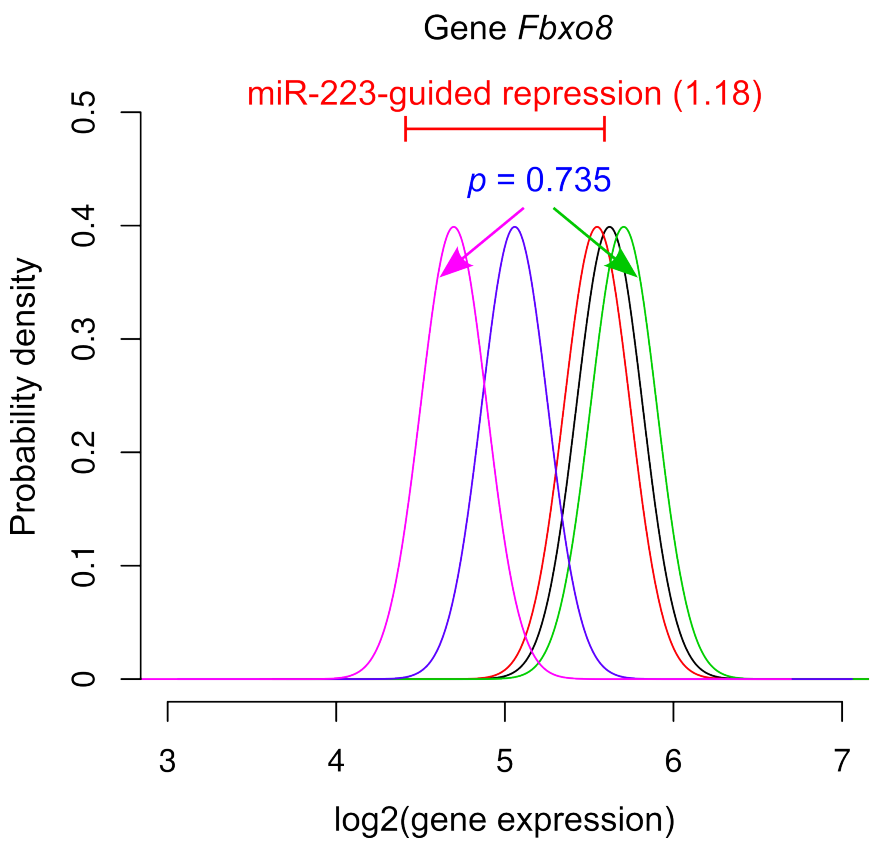
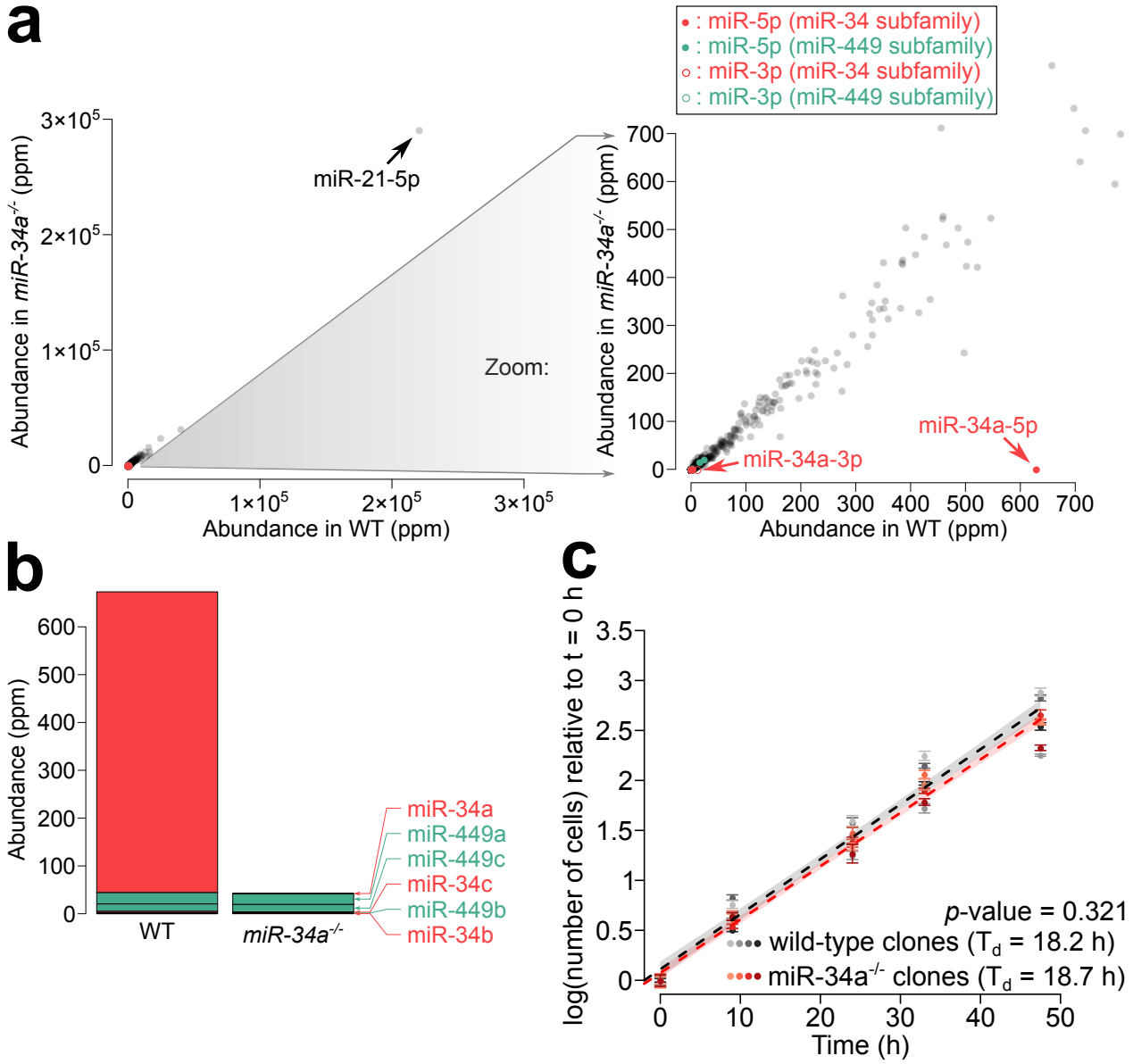


miR-34 and cell proliferation

In HCT-116 cells: