

Searching for biologically consequential and inconsequential miRNA/target interactions using the evolutionary history of vertebrate miRNA genes

Hervé Seitz

May 7, 2025

Slideshow accessible for download at:

<http://www.igh.cnrs.fr/equip/Seitz/ISHG25.pdf>

A shift in the
definition of
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microRNAs

Consequential vs.
inconsequential
targets

False positives in
target prediction

Binding site
evolution after
miRNA loss

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Observed losses

Evolution of seed match
count after loss

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Supplementary
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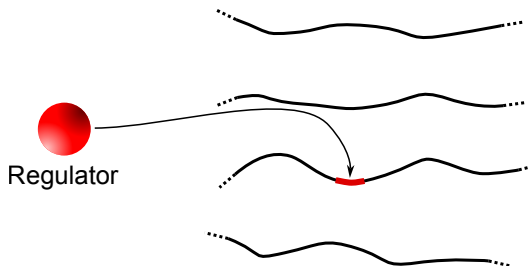
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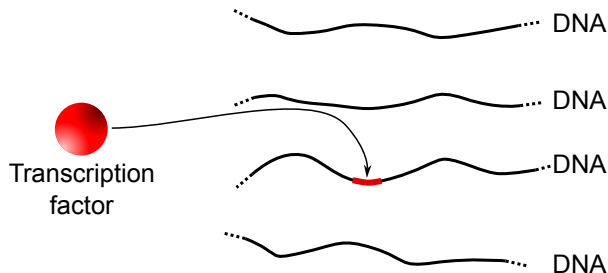
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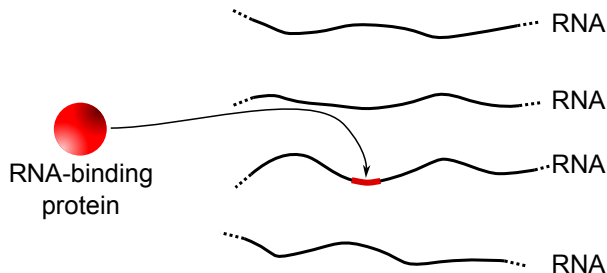
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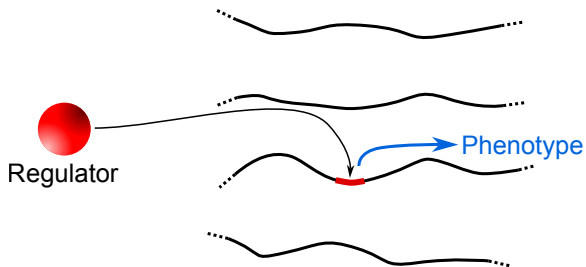
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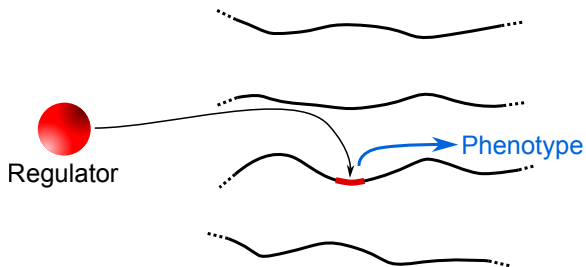
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The “lock and key” model

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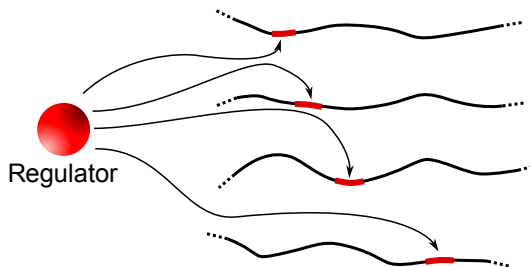
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Since the advent of high-throughput molecular biology (years 2000's):



ChIP-on-chip, ChIP-Seq, CLIP, ...

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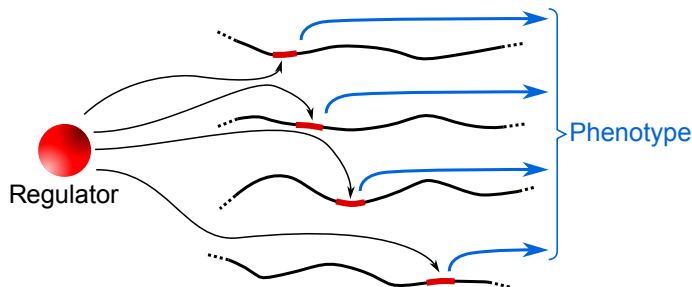
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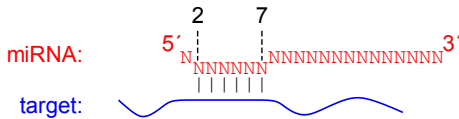
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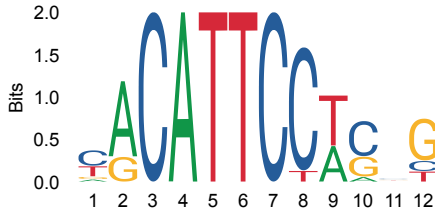
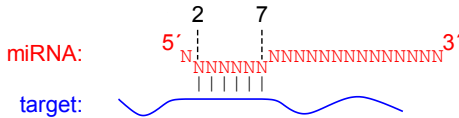
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(Human TEAD1 PWM from JASPAR)

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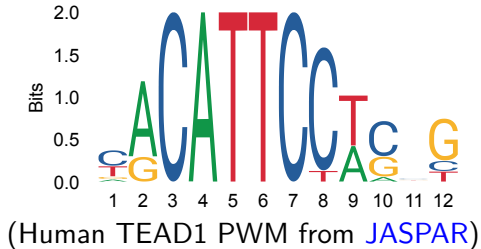
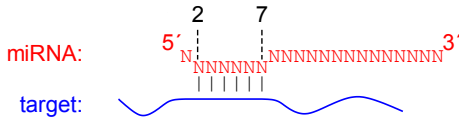
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Among regulators: miRNAs have a “digital” target recognition rule.

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CLIP experiments: miRNAs also bind hundreds to thousands of distinct targets.

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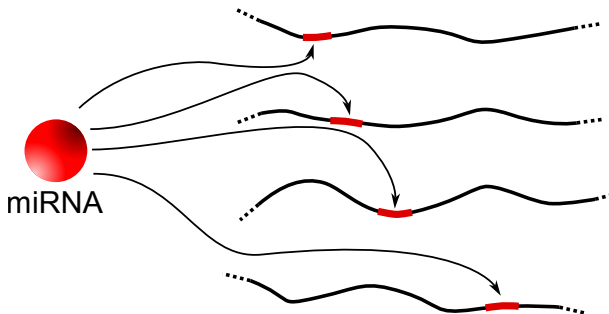
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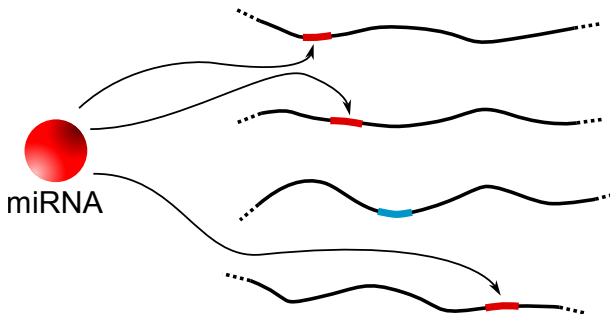
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Regulator	Functional target(s)
<i>Mus musculus</i> miR-155	<i>AID</i> and <i>SOCS1</i>
<i>Cænorhabditis elegans</i> <i>let-7</i>	<i>lin-41</i>
<i>C. elegans</i> miR-791	<i>akap-1</i> and <i>cah-3</i>
<i>M. musculus</i> miR-200	<i>Zeb1</i>
<i>C. elegans</i> miR-35	<i>egl-1</i>
<i>Drosophila melanogaster</i> miR-iab-4/8	<i>hth</i>
<i>M. musculus</i> miR-140-5p	<i>Wnt11</i>
<i>Oryzias latipes</i> miR-202	<i>Tead3b</i>

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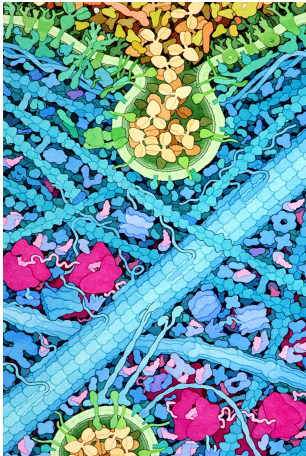
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→ *In vivo* genetics: just a few important targets.

Consequential vs. inconsequential targets

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(D. Goodsell, Rutgers university)

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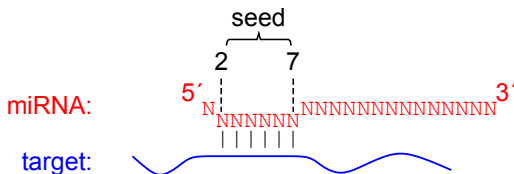
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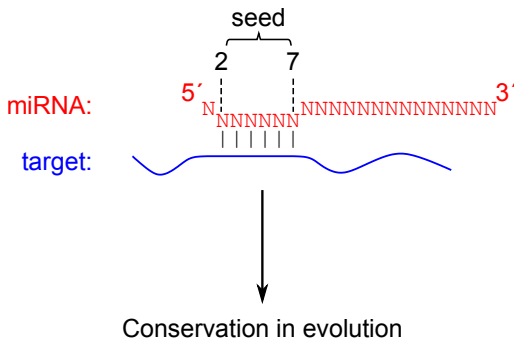
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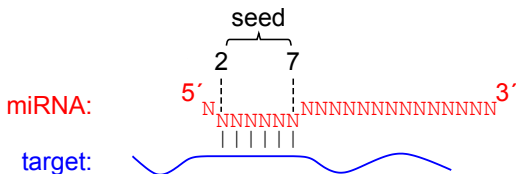
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Conservation in evolution

List of 3' UTRs with conserved complementarity
to miRNA seeds (→ "predicted targets").

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Paradox:

- ▶ *In vivo* genetics: just a few targets appear to be biologically important.
- ▶ Comparative genomics: hundreds of distinct mRNAs have conserved seed matches in their 3' UTR.

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Paradox:

- ▶ *In vivo* genetics: just a few targets appear to be biologically important.
- ▶ Comparative genomics: hundreds of distinct mRNAs have conserved seed matches in their 3' UTR.

Why have all these sites been conserved in evolution if they are inconsequential ?

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Some 3' UTR seed matches are conserved for miRNA-independent reasons.

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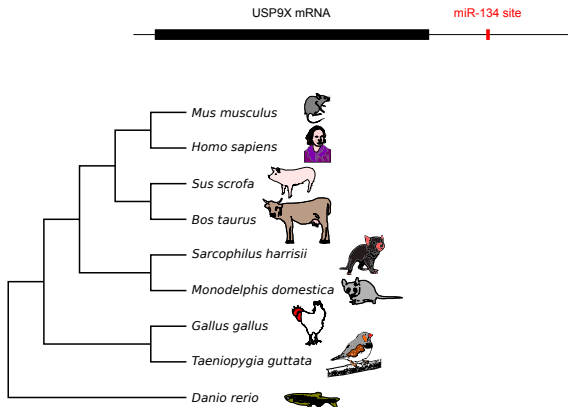
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(Pinzón *et al.*, 2017)

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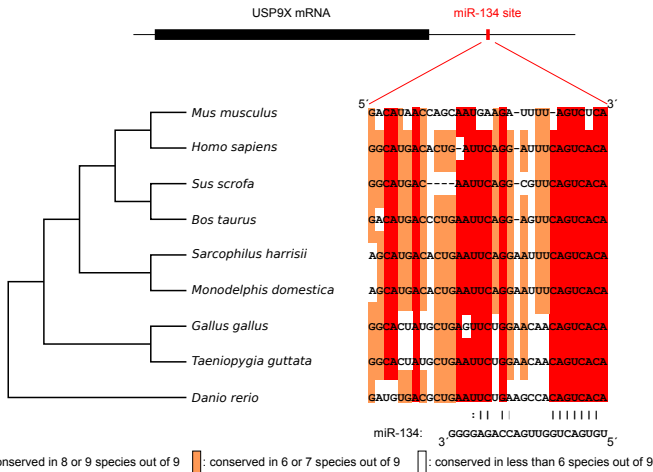
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(Pinzón et al., 2017)

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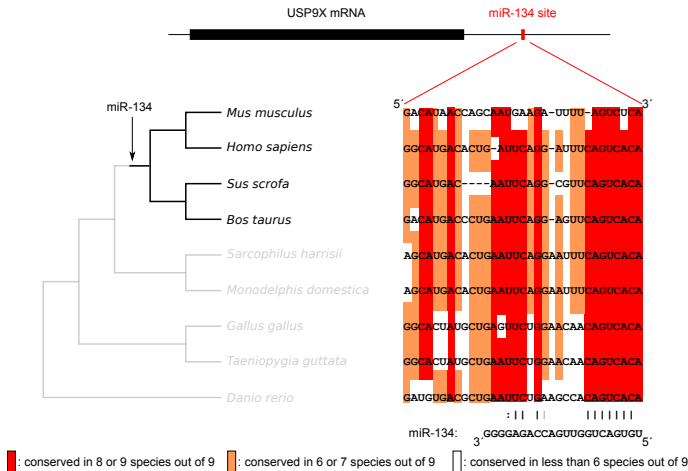
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(Pinzón et al., 2017)

False positives in target prediction

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

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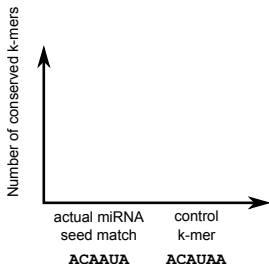
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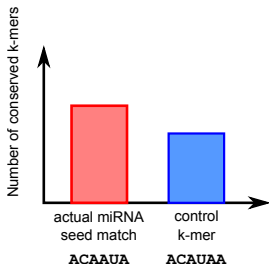
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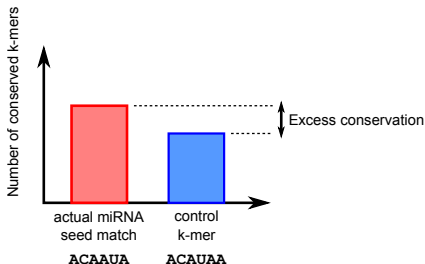
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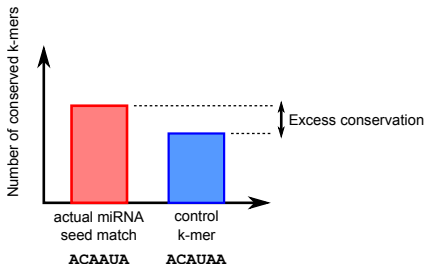


False positives in target prediction

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

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



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Binding site evolution after miRNA loss

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After loss of a *complete* miRNA family (*), how long do its seed matches persist ?

(*): family: all the miRNAs sharing the same seed sequence

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After loss of a *complete* miRNA family (*), how long do its seed matches persist ?

(*): family: all the miRNAs sharing the same seed sequence

We need to be lucky: loss of complete miRNA families!

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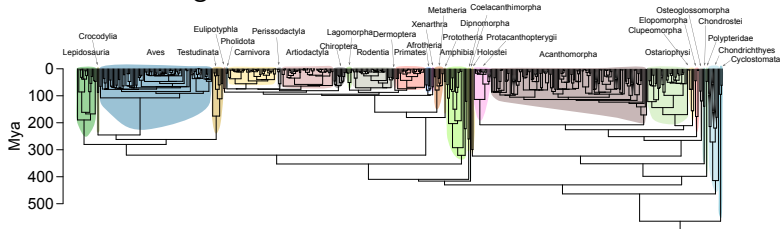
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522 vertebrate genomes:



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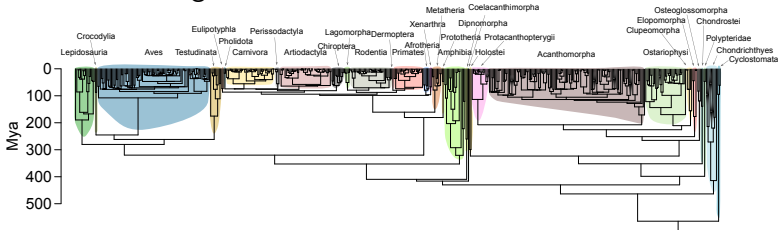
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522 vertebrate genomes:



848 best annotated vertebrate miRNA families.

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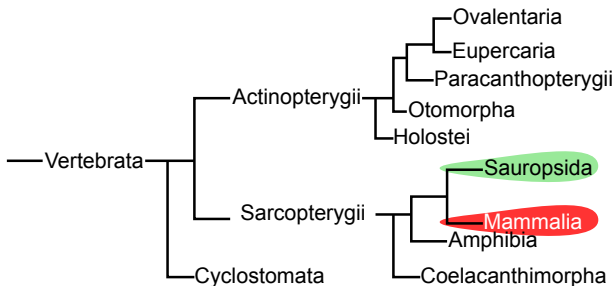
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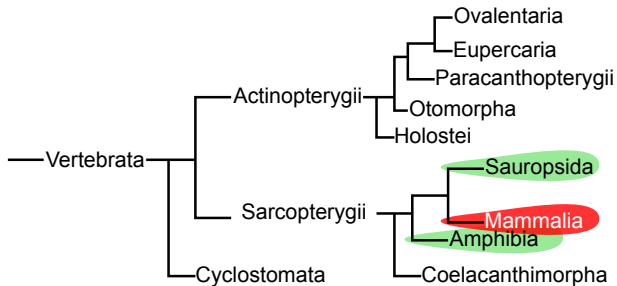
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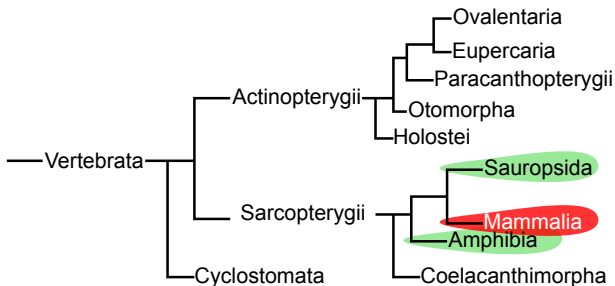
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More convincing if many species in clade of loss.

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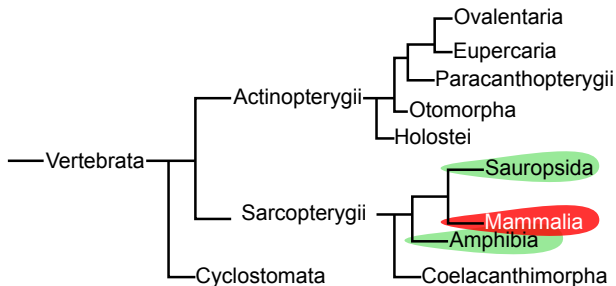
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More convincing if many species in clade of loss.
 ≥ 5 species in clade of loss; presence in sister clade + 3 closest cousin clades.

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Family	Clade of loss
miR-509	Cervidae+Bovidae
miR-541	Feliformia
miR-224	Mustelidae
miR-338	Lagomorpha
miR-338	Syngnathidae
miR-460	Serpentes
miR-660	Murinae
miR-880	Deomyinae+Gerbilinae
miR-452	Mustelidae
miR-456	Balistidae+Tetraodontidae
miR-296-3p	Lagomorpha
miR-2188	Eutheria
miR-2114	Phocidae
miR-656	Myomorpha
miR-654	Delphinoida
miR-460b	Mammalia
miR-460b	Serpentes
miR-676	Cervidae+Bovidae
miR-676	Feloidea
miR-187	Serpentes
miR-769	Yangochiroptera
miR-769	Whippomorpha
miR-1185	Yangochiroptera

Family	Clade of loss
miR-451a	Paralichthyidae+Pleuronectidae
miR-509	Yangochiroptera
miR-31	Protacanthopterygii
miR-31	Lophiiformes+Tetraodontiformes
miR-885	Myomorpha
miR-296-5p	Lagomorpha
miR-296-5p	Canidae
miR-412	Lagomorpha
miR-889	Muridae+Cricetidae
miR-889	Bovinae
miR-458	Theria
miR-374	Yangochiroptera
miR-513b	Bos
miR-653	Yangochiroptera
miR-1662	Endoglyptodonta
miR-297p	Pinnipedia+Musteloidea
miR-2131	Psittaciformes
miR-122b	Paralichthyidae+Pleuronectidae
miR-325-3p	Bovidae
miR-599	Batrachia
miR-599	Corvidae+Passerides
miR-599	Galloanserae

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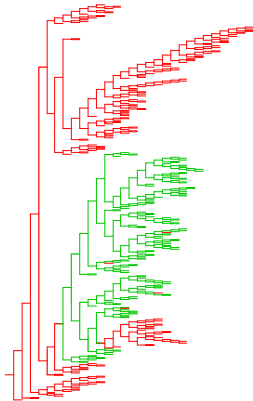
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miR-656 family:



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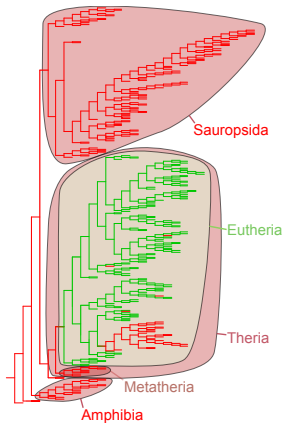
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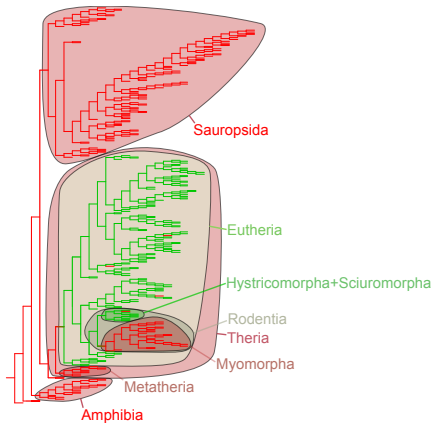
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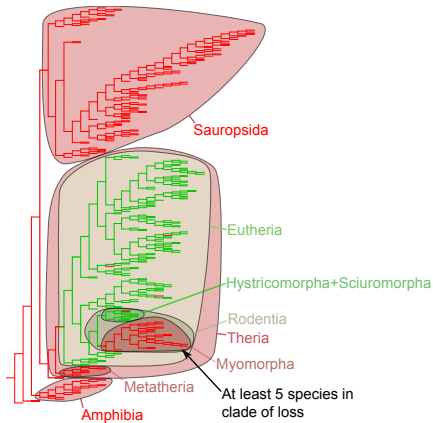
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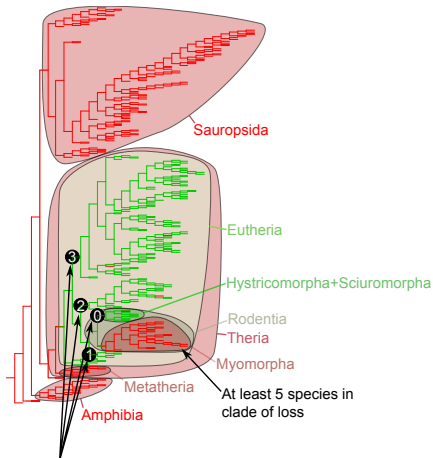
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miR-656 family:



In sister clade + at least 3 degrees of cousinhood: miRNA family with certified presence in >50% of species at each degree

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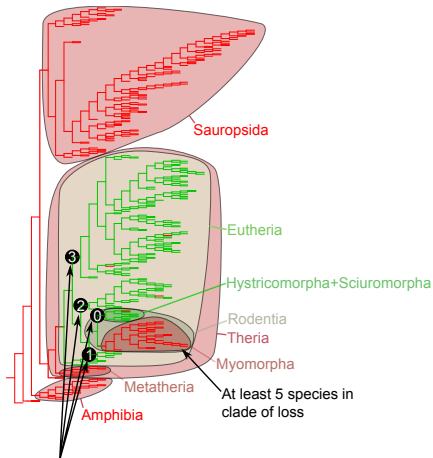
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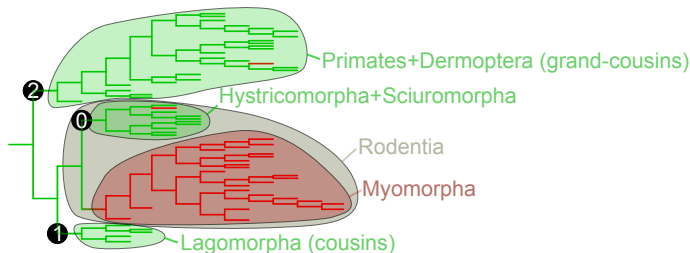
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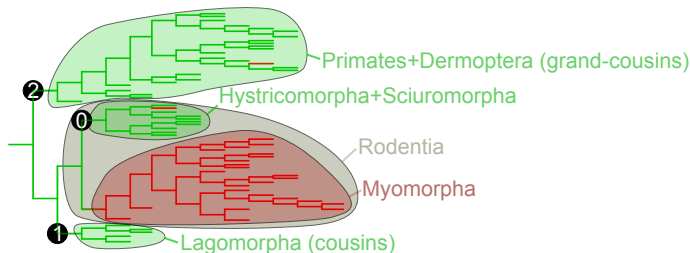
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PhyloP score: measures probability of selective conservation of seed match vs. rest of UTR.

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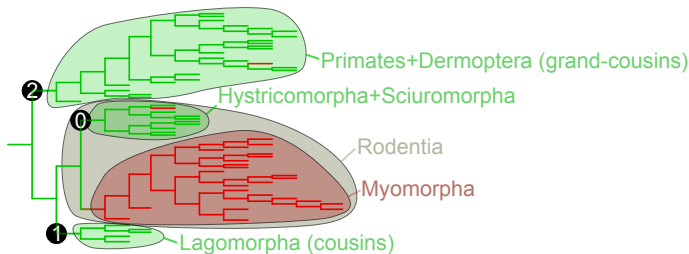
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PhyloP score: measures probability of selective conservation of seed match vs. rest of UTR in a clade vs. rest of tree.

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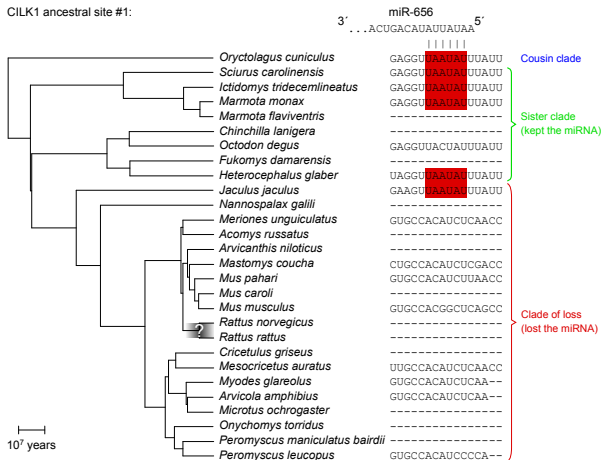
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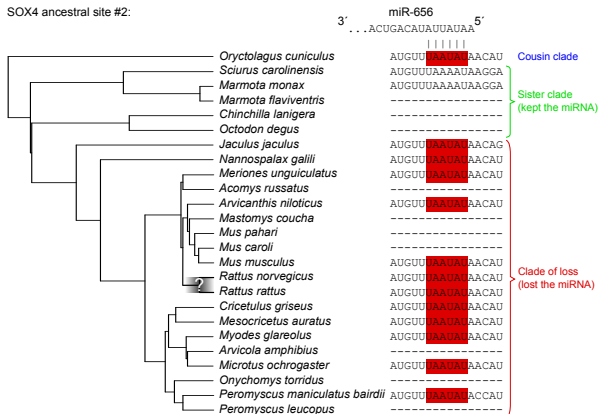
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SOX4 ancestral site #2:



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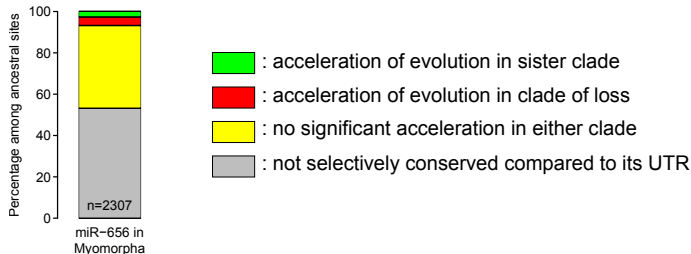
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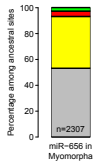
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- acceleration of evolution in sister clade
- acceleration of evolution in clade of loss
- no significant acceleration in either clade
- not selectively conserved compared to its UTR

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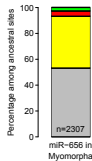
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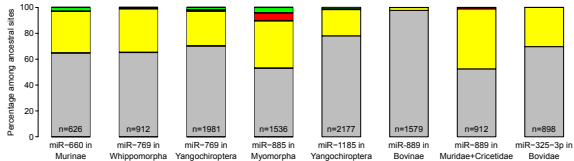
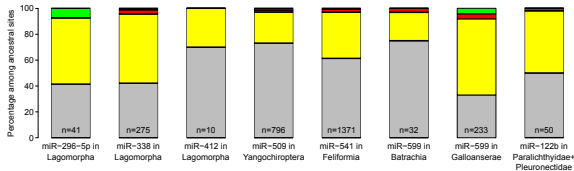
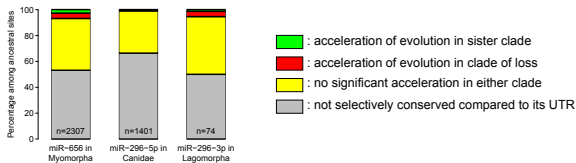
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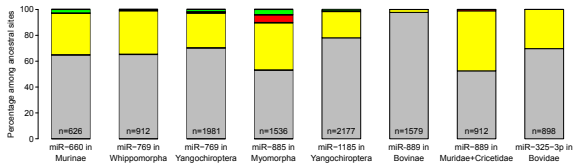
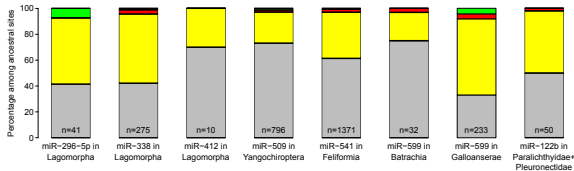
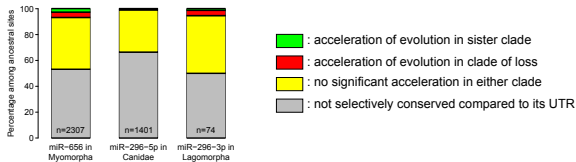
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Most of these sites: conserved independently of the miRNA.

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High-throughput experimental target identification captures many false positives...

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High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

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Improvement: identification of miRNA-independent conservation of binding sites.

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On the analyzed examples: most conserved seed matches are not conserved because of the miRNA!

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On the analyzed examples: most conserved seed matches are not conserved because of the miRNA!

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→ A large part of the miRNA literature probably needs a cleanup.

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May be generalized to other regulators (transcription factors, RNA-binding proteins; [Seitz, 2024](#)).

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In vivo veritas: genome editing of regulator binding site provides a rigorous assessment of functionality.

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May be generalized to other regulators (transcription factors, RNA-binding proteins; [Seitz, 2024](#)).

In vivo veritas: genome editing of regulator binding site provides a rigorous assessment of functionality.

Trying to infer biological functionality from GO term enrichment analysis is a bad idea: enriched GO terms do not reflect biological phenotypes !

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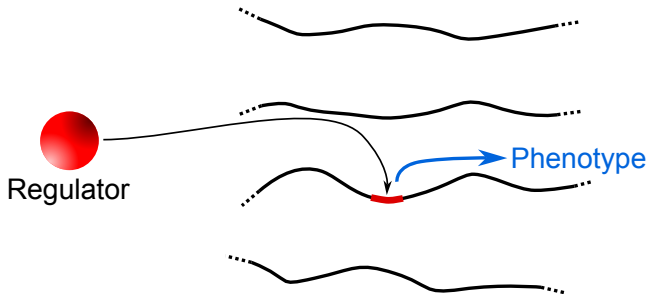
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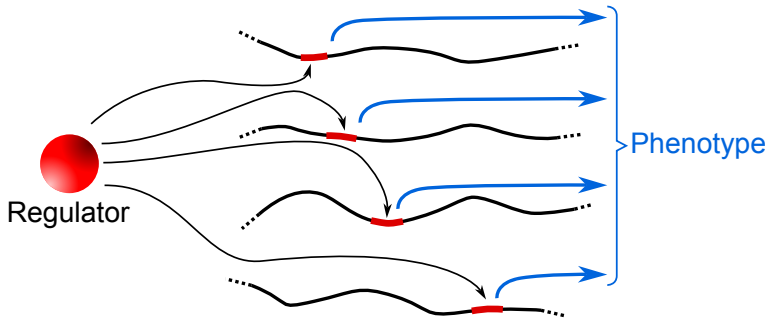
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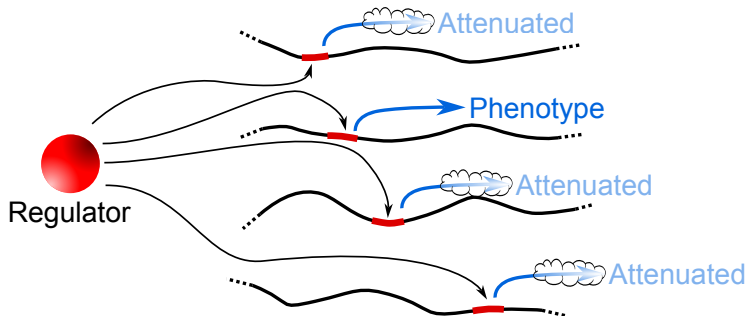
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Acknowledgments



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Laura Martinez Anna Sergeeva

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<https://internat25.sciencesconf.org/>

Current members:

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Estelle Rebecq	Pauline De Sousa	

Former members:

Sophie Mockly	Germain Busto	Natalia Pinzón
Blaise Li	Laura Martinez	Anna Sergeeva
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Fondation
pour la recherche
sur le cancer



Supplementary data

► *mir-656* orthologous loci in mammals

► Missing statistics

► *In vivo veritas*: pitfalls of GO term enrichment analysis.

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miR-656 family:

Percentage identity among 470 mammals: 60 80 100



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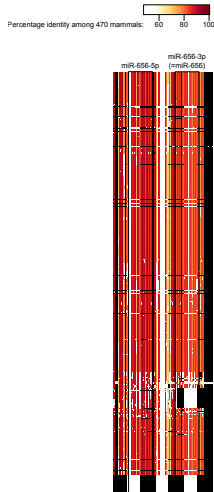
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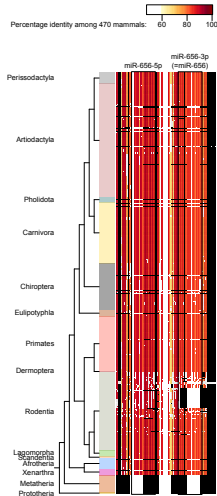
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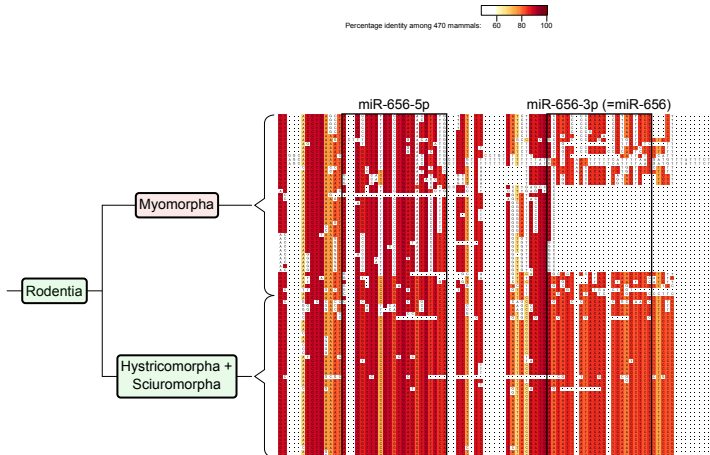
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Family	Seed	Clade of loss	Reason
miR-451a	AACCGU	Paralichthyidae+Pleuronectidae	<10 ancestral sites
miR-224	AAGUCA	Mustelidae	Under-studied sister clade
miR-338	ACAAUA	Syngnathidae	<10 ancestral sites
miR-460	ACAGCG	Serpentes	<10 ancestral sites
miR-880	ACUCCA	Deomyiinae+Gerbillinae	Under-studied sister clade
miR-452	ACUGUU	Mustelidae	Under-studied sister clade
miR-456	AGGCUG	Balistidae+Tetraodontidae	Under-studied sister clade
miR-2188	AGGUCC	Eutheria	<10 ancestral sites
miR-2114	AGUCCC	Phocidae	Under-studied sister clade
miR-654	AUGUCU	Delphinoida	Under-studied sister clade
miR-460b	CCUCAU	Mammalia	<10 ancestral sites
miR-460b	CCUCAU	Serpentes	<10 ancestral sites
miR-676	CGUCCU	Cervidae+Bovidae	Under-studied sister clade
miR-676	CGUCCU	Feloidea	<10 ancestral sites
miR-187	CGUGUC	Serpentes	<10 ancestral sites
miR-509	GAUUGA	Cervidae+Bovidae	Under-studied sister clade
miR-31	GGCAAG	Protacanthopterygii	<10 ancestral sites
miR-31	GGCAAG	Lophiiformes+Tetraodontiformes	<10 ancestral sites
miR-458	UAGCUC	Theria	<10 ancestral sites
miR-374	UAUAAU	Yangochiroptera	In progress
miR-513b	UCACAA	Bos	<10 ancestral sites
miR-653	UGAAAC	Yangochiroptera	In progress
miR-1662	UGACAU	Endoglyptodonta	Under-studied sister clade
miR-297p	UGUAUG	Pinnipedia+Musteloidea	Under-studied sister clade
miR-2131	UGUUAC	Psittaciformes	Under-studied sister clade
miR-599	UUGAUA	Corvidae+Passerides	Under-studied sister clade

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Genome editing *in vivo* can assess biological phenotypes.

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- ▶ For miRNAs: ▶ Target site mutation

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Genome editing *in vivo* can assess biological phenotypes.

- ▶ For miRNAs: ▶ Target site mutation
- ▶ For transcription factors: [Wu et al., 2020](#).

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Genome editing *in vivo* can assess biological phenotypes.

- ▶ For miRNAs: ▶ Target site mutation
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Long, labor-intensive, expensive, low-throughput, ...

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Trying to infer biological function from enriched annotation terms (GO, KEGG, ...) in (predicted) targets.

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Trying to infer biological function from enriched annotation terms (GO, KEGG, ...) in (predicted) targets.

- ▶ Agonists and antagonists often counted together.

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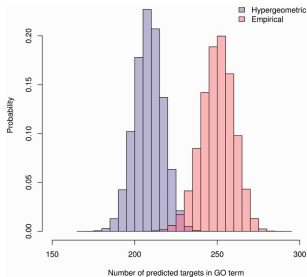
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Trying to infer biological function from enriched annotation terms (GO, KEGG, ...) in (predicted) targets.

- ▶ Agonists and antagonists often counted together.
- ▶ Systematic biases due to inadequate control ([Bleazard et al., 2015](#)).



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Trying to infer biological function from enriched annotation terms (GO, KEGG, ...) in (predicted) targets.

- ▶ Agonists and antagonists often counted together.
- ▶ Systematic biases due to inadequate control ([Bleazard et al., 2015](#)).
- ▶ Usually fails at predicting the most obvious phenotypes.

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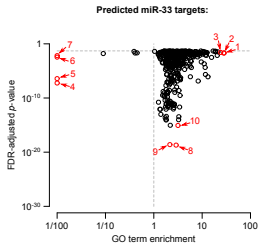
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M. musculus miR-33 KO:
changes in cholesterol
and lipid metabolism
([Horie et al., 2010](#); [Kim
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liver steatosis ([Horie et
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([Nishiga et al., 2017](#)).

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Point on plot	GO term
1	Postsynaptic density protein 95 clustering
2	Pre-B cell differentiation
3	Cell quiescence
4	Sensory perception of chemical stimulus
5	Sensory perception of smell
6	Detection of chemical stimulus involved in sensory perception
7	Detection of chemical stimulus involved in sensory perception of smell
8	Nervous system development
9	Multicellular organism development
10	Neurogenesis

M. musculus miR-33 KO:
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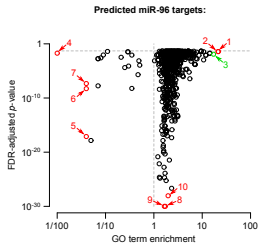
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M. musculus miR-96 KO:
hearing loss (Lewis *et al.*, 2009; Mencía *et al.*, 2009), probably due to deficient differentiation of ciliated cells in the sensory epithelium (Kuhn *et al.*, 2011).

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Point on plot	GO term
1	Forebrain astrocyte differentiation
2	Forebrain astrocyte development
3	Establishment of planar polarity of embryonic epithelium
4	tRNA metabolic process
5	Sensory perception of smell
6	Detection of chemical stimulus involved in sensory perception
7	Detection of chemical stimulus involved in sensory perception of smell
8	Positive regulation of cellular process
9	Positive regulation of biological process
10	System development

M. musculus miR-96 KO:
hearing loss (Lewis *et al.*, 2009; Mencía *et al.*, 2009), probably due to deficient differentiation of ciliated cells in the sensory epithelium (Kuhn *et al.*, 2011).

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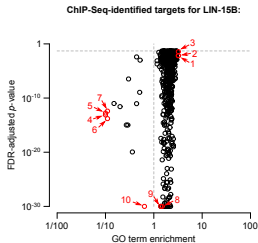
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C. elegans lin-15 KO:
perturbation of vulval de-
velopment ([Herman et
al., 1990](#)).

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Point on plot	GO term
1	DNA replication initiation
2	Regulation of mitotic sister chromatid segregation
3	Axonal transport
4	Detection of chemical stimulus
5	Detection of chemical stimulus involved in sensory perception
6	Detection of stimulus involved in sensory perception
7	Detection of chemical stimulus involved in sensory perception of smell
8	Cellular process
9	Biological process
10	Unclassified

C. elegans lin-15 KO:
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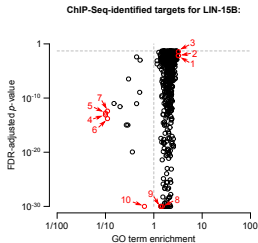
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C. elegans lin-15 KO:
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→ GO term enrichment is a poor predictor of a regulator's biological activity.

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