

Widespread microRNA-independent conservation of presumptive microRNA binding sites in vertebrates

Hervé Seitz

May 6, 2024

miRNA target
identification

False positives in
target prediction

Binding site
evolution after
miRNA loss

Strategy

Observed losses

Evolution of seed match
count after loss

Generalization:
defining regulatory
targets

Conclusion

Slideshow accessible for download at:

<http://www.igh.cnrs.fr/equip/Seitz/Sympo24.pdf>

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- Low-throughput genetics (measures *in vivo* phenotypes).

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Example: [Ecsedi et al., 2015](#)

miRNA target identification

- ▶ Low-throughput genetics (measures *in vivo* phenotypes).
- ▶ High-throughput molecular biology (finding mRNAs bound, or repressed, by a miRNA).

Example: [Chi et al., 2009](#)

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- ▶ Low-throughput genetics (measures *in vivo* phenotypes).
- ▶ High-throughput molecular biology (finding mRNAs bound, or repressed, by a miRNA).
- ▶ Computational predictions.

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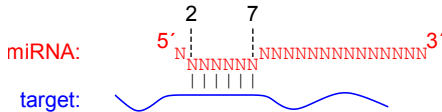
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Example: [Agarwal et al., 2015](#)

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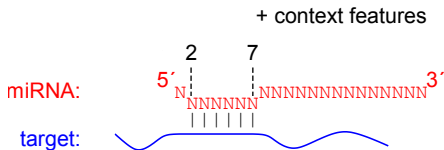
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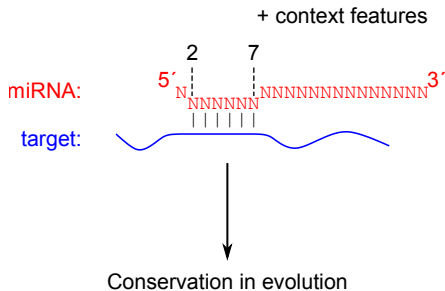
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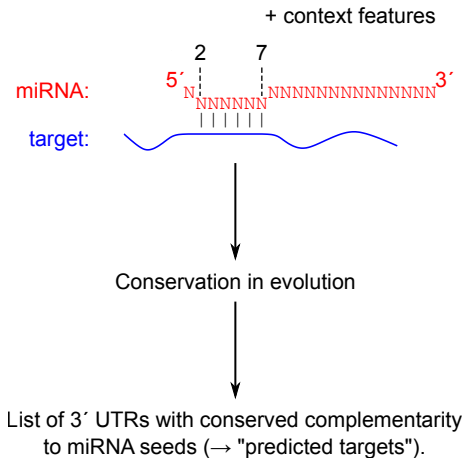
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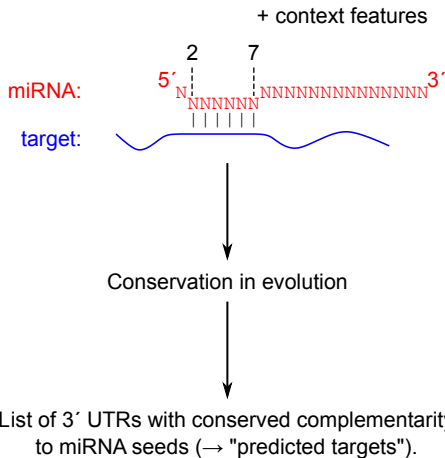
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Even high-throughput identification needs to assess conservation.

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

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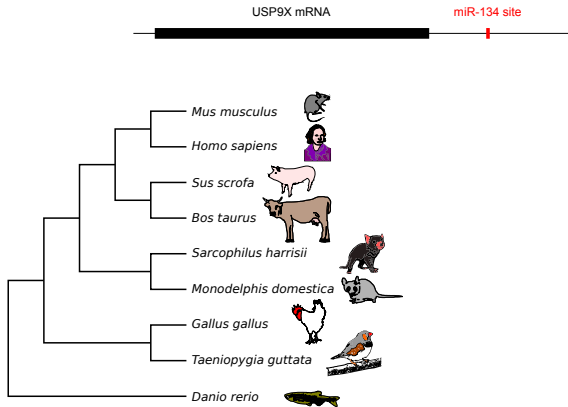
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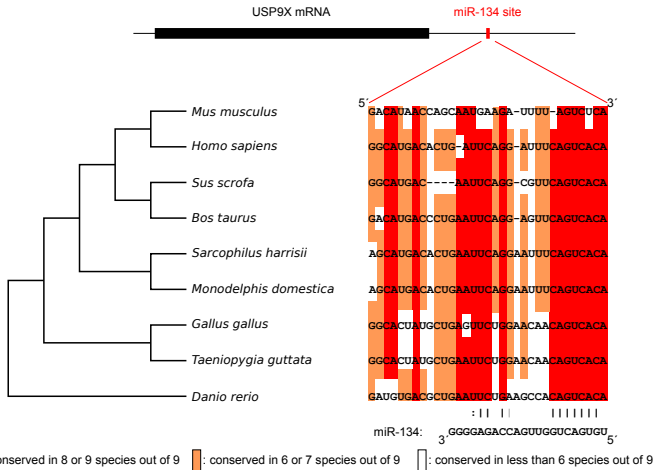
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(Pinzón et al., 2017)

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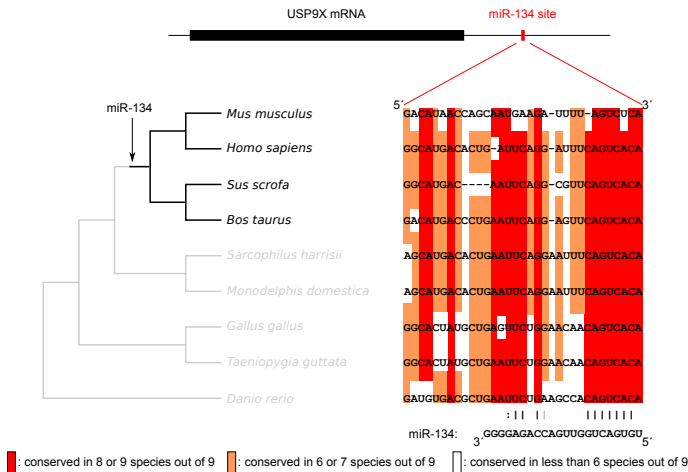
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(Pinzón *et al.*, 2017)

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Seed match “overconservation” could only be precisely measured for the most recently emerged miRNAs.

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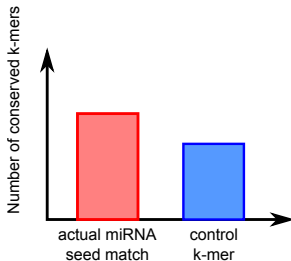
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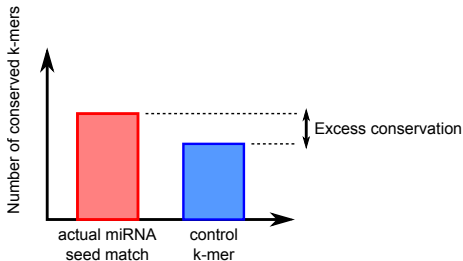
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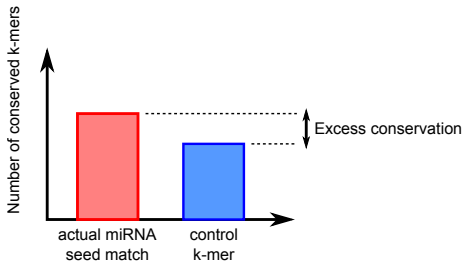
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—→ not submitted to the same selective pressure than actual miRNA seed matches (e.g., protein binding sites).



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Binding site evolution after miRNA loss

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 ?

We need to be lucky: loss of complete miRNA families a long time ago (many species with miRNA loss).

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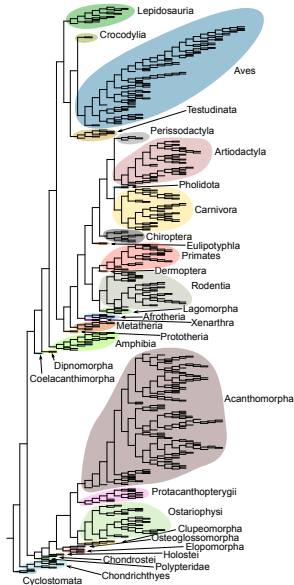
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522 vertebrate genomes:



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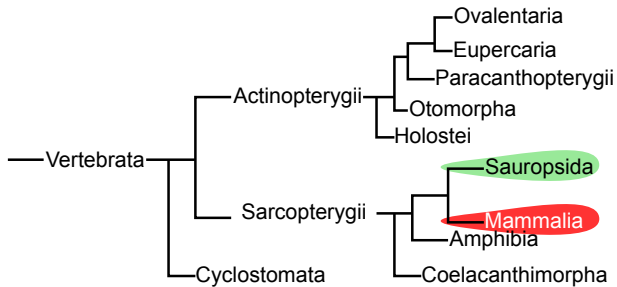
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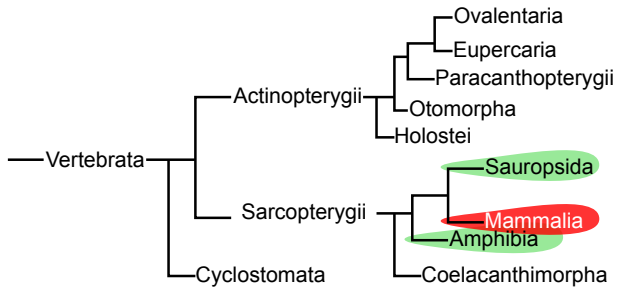
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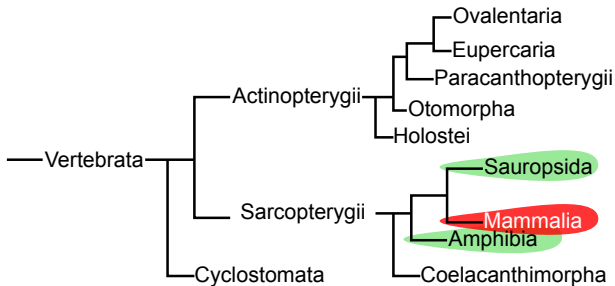
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More convincing if many species in clade of loss.

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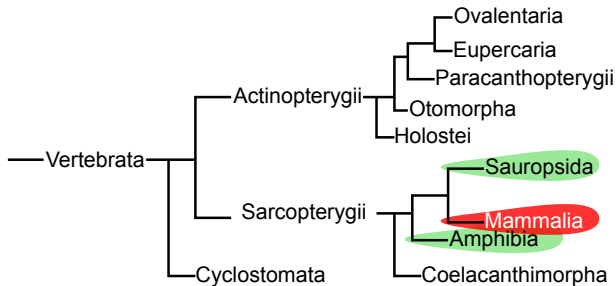
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More convincing if many species in clade of loss.

≥ 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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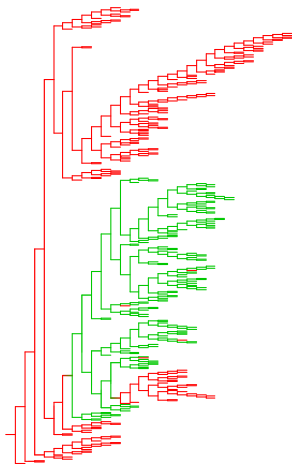
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miR-656 family:



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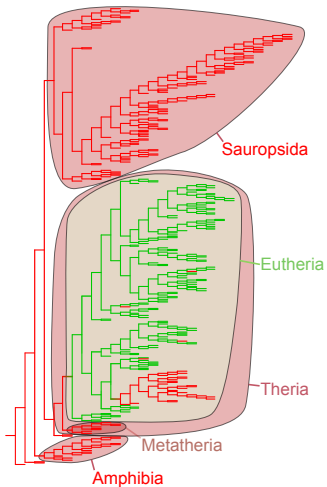
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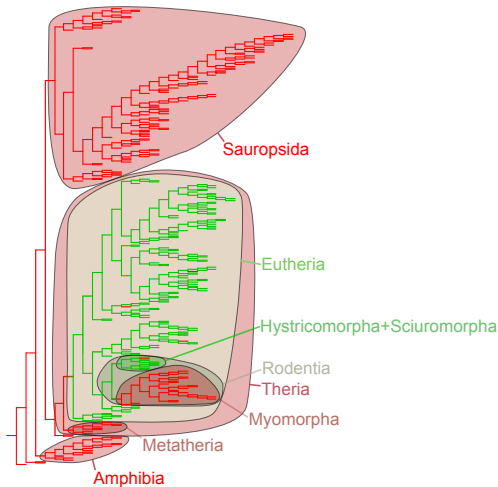
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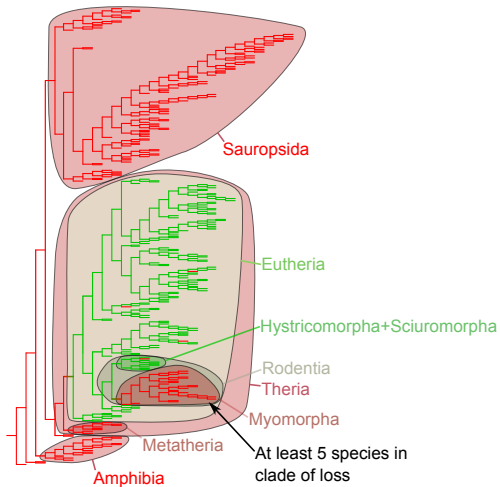
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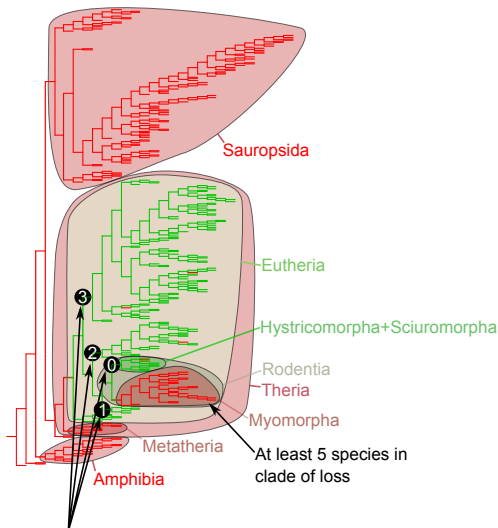
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miR-656 family:



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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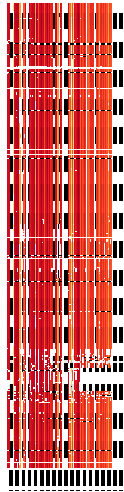
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miR-656 family:

Percentage identity among 470 mammals: 60 80 100



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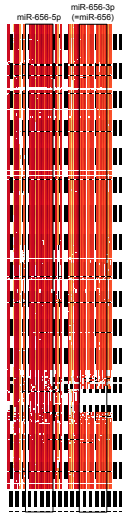
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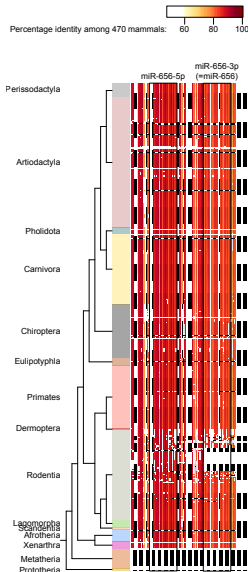
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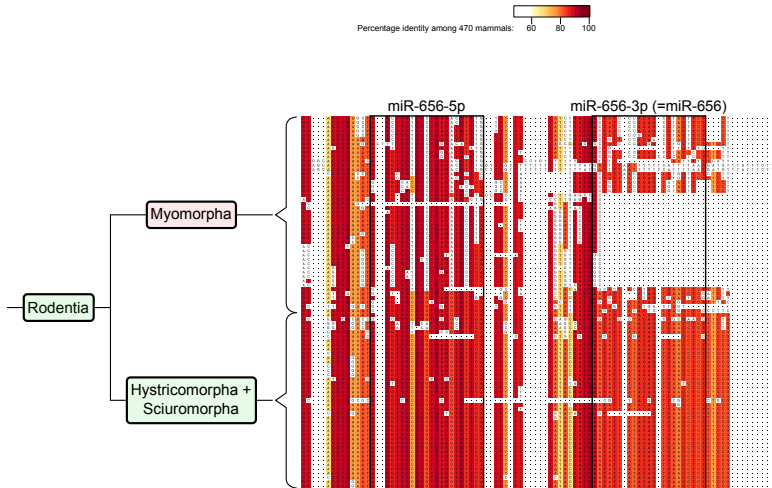
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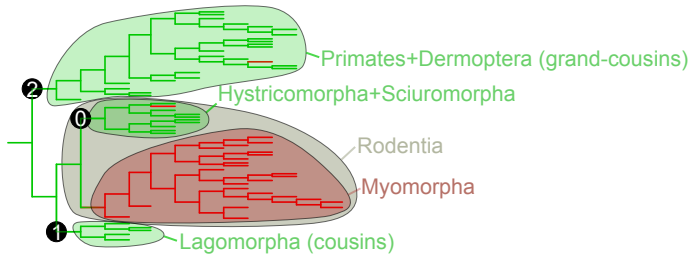
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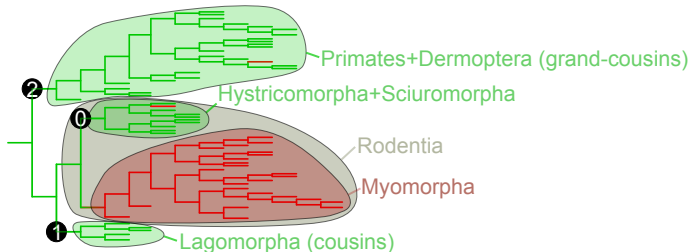
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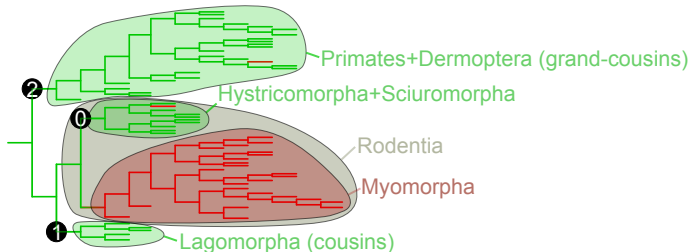
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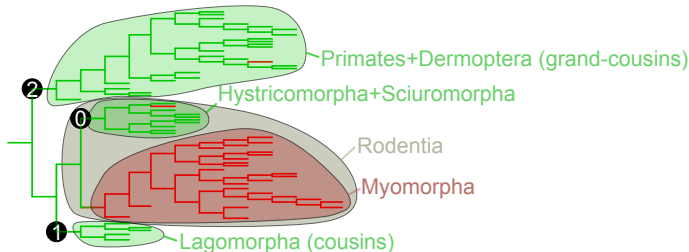
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Align 3' UTR sequences in clade of loss and sister clade.

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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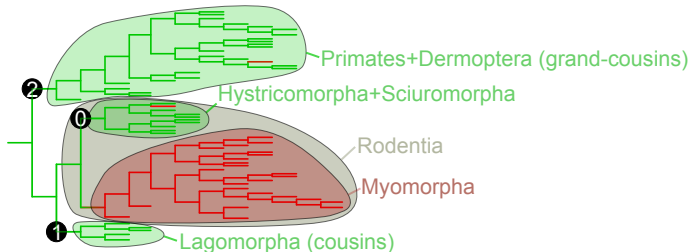
Observed losses

Evolution of seed match count after loss

Generalization: defining regulatory targets

Conclusion

Evolution of seed match count after loss



Align 3' UTR sequences in clade of loss and sister clade.

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

→ Selects seed matches with selective conservation in rodents, then compare their conservation in rodent subclades (clade of loss vs. sister clade).

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Strategy

Observed losses

Evolution of seed match count after loss

Generalization: defining regulatory targets

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Evolution of seed match count after loss

After miR-656 family loss:

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

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Generalization: defining regulatory targets

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After miR-656 family loss:



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miRNA target
identification

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miRNA loss

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defining regulatory
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Evolution of seed match count after loss

Conservation of
presumptive
microRNA binding
sites

After miR-656 family loss:

miRNA target
identification

False positives in
target prediction

Binding site
evolution after
miRNA loss

Cricetulus griseus CDON 3' UTR:



Strategy

Observed losses

Evolution of seed match
count after loss

In Rodentia:

Generalization:
defining regulatory
targets

Conclusion

Site	Conservation vs. rest of UTR
	<i>p</i> -value
nt 3320–3325	0.02815

Evolution of seed match count after loss

Conservation of presumptive microRNA binding sites

After miR-656 family loss:

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Cricetulus griseus CDON 3' UTR:



Strategy

Observed losses

Evolution of seed match count after loss

Acceleration of evolution in subclades:

Generalization: defining regulatory targets

Conclusion

Site	Clade of loss <i>p</i> -value	
nt 3320–3325	0.03585	

Evolution of seed match count after loss

Conservation of presumptive microRNA binding sites

After miR-656 family loss:

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Cricetulus griseus CDON 3' UTR:



Strategy

Observed losses

Evolution of seed match count after loss

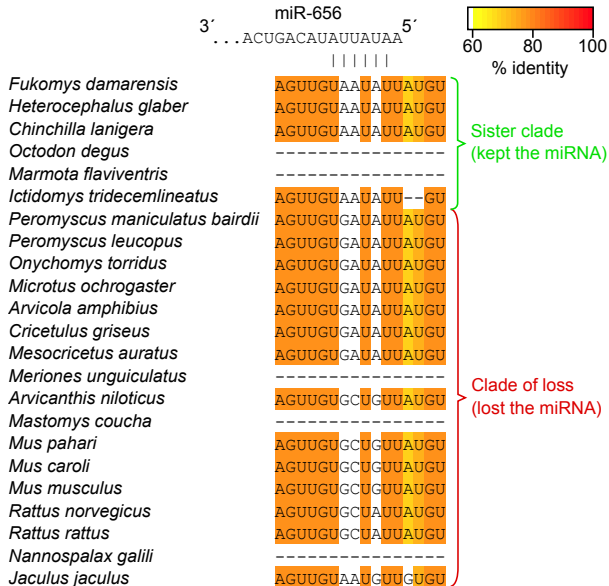
Acceleration of evolution in subclades:

Generalization: defining regulatory targets

Conclusion

Site	Clade of loss <i>p</i> -value	Sister clade <i>p</i> -value
nt 3320–3325	0.03585	0.50000

Evolution of seed match count after loss



Conservation of presumptive microRNA binding sites

miRNA target identification

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Binding site evolution after miRNA loss

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Conservation of presumptive microRNA binding sites

After miR-656 family loss:

miRNA target identification

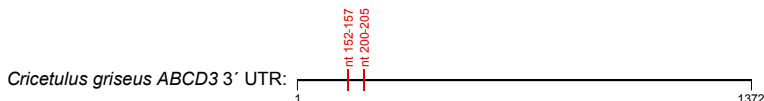
False positives in target prediction

Binding site evolution after miRNA loss

Strategy

Observed losses

Evolution of seed match count after loss



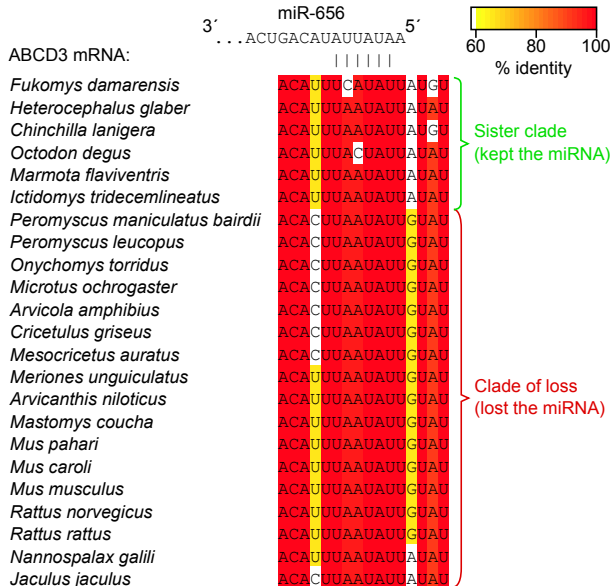
Acceleration of evolution in subclades:

Generalization: defining regulatory targets

Conclusion

Site	Clade of loss <i>p</i> -value	Sister clade <i>p</i> -value
nt 152–157	0.50000	0.00864
nt 200–205	0.23280	0.50000

Evolution of seed match count after loss



Conservation of
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miRNA target
identification

False positives in
target prediction

Binding site
evolution after
miRNA loss

Strategy

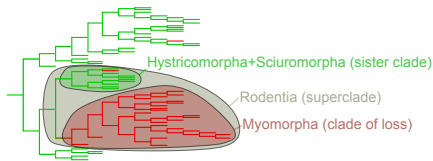
Observed losses

Evolution of seed match
count after loss

Generalization:
defining regulatory
targets

Conclusion

Evolution of seed match count after loss



Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Strategy

Observed losses

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Generalization: defining regulatory targets

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Evolution of seed match count after loss

Conservation of
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sites

miRNA target
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False positives in
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Binding site
evolution after
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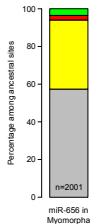
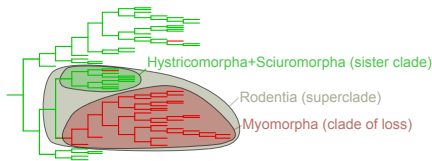
Strategy

Observed losses

Evolution of seed match
count after loss

Generalization:
defining regulatory
targets

Conclusion



- : Accelerated evolution in sister clade
- : Accelerated evolution in clade of loss
- : Equally conserved in clade of loss and sister clade
- : Not significantly conserved in superclade

Evolution of seed match count after loss

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

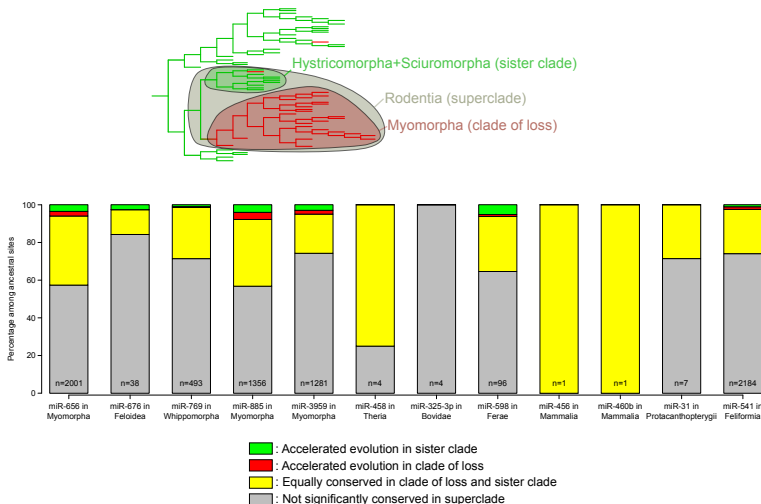
Strategy

Observed losses

Evolution of seed match count after loss

Generalization: defining regulatory targets

Conclusion



Evolution of seed match count after loss

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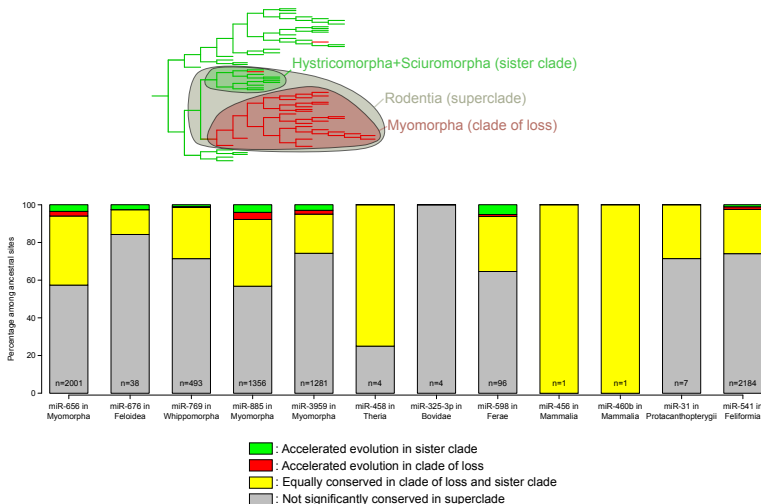
Strategy

Observed losses

Evolution of seed match count after loss

Generalization: defining regulatory targets

Conclusion



→ Most of these seed matches are conserved in a miRNA-independent manner.

Generalization: defining regulatory targets

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Strategy

Observed losses

Evolution of seed match count after loss

Generalization: defining regulatory targets

Conclusion

Generalization: defining regulatory targets

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

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sites

miRNA target
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target prediction

Binding site
evolution after
miRNA loss

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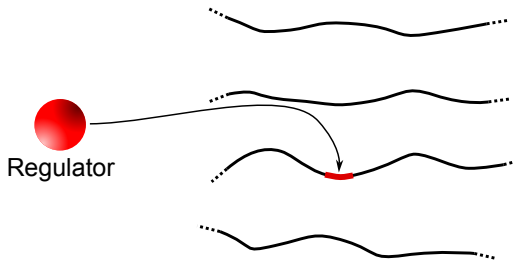
Evolution of seed match
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**Generalization:
defining regulatory
targets**

Conclusion

Generalization: defining regulatory targets

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



The “key and lock” model

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

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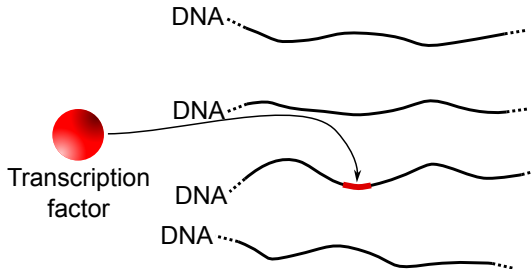
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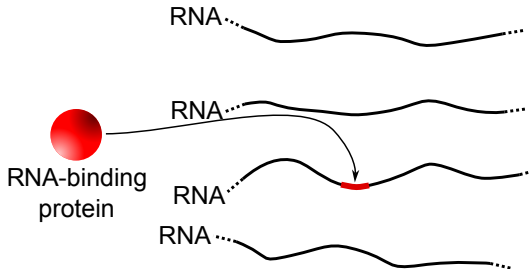
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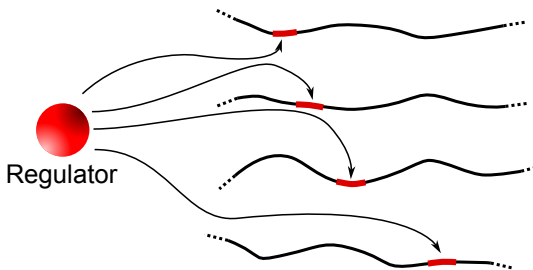
Evolution of seed match count after loss

Generalization: defining regulatory targets

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Generalization: defining regulatory targets

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



Poor biochemical specificity

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miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

Strategy

Observed losses

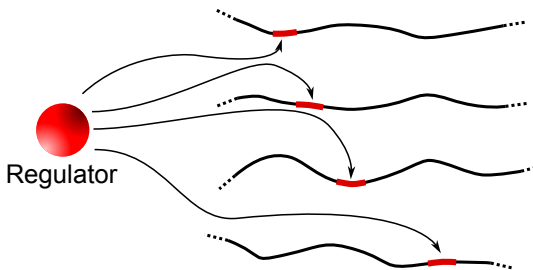
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Generalization: defining regulatory targets

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



Poor biochemical specificity

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

Conservation of presumptive microRNA binding sites

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Generalization: defining regulatory targets

Each regulator tends to have specific biological roles (→ specific phenotypes when mutated).

Conservation of presumptive microRNA binding sites

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Evolution of seed match count after loss

Generalization: defining regulatory targets

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Generalization: defining regulatory targets

Each regulator tends to have specific biological roles (→ specific phenotypes when mutated):

- ▶ “Key and lock model”: specificity in biological response is due to a high biochemical specificity.

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Generalization: defining regulatory targets

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Generalization: defining regulatory targets

Each regulator tends to have specific biological roles (→ specific phenotypes when mutated):

- ▶ “Key and lock model”: specificity in biological response is due to a high biochemical specificity.
- ▶ Newer model: regulators have a poor biochemical specificity, they control phenotypes through the coordinated regulation of many targets.

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Experimental identification of transcription factor binding sites.

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Experimental identification of transcription factor binding sites → short motifs (≈ 6 to 8 bp), very frequent in genomes.

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The “futility theorem” ([Wasserman and Sandelin, 2004](#)): most occurrences of these motifs are non-functional *in vivo*.

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Experimental identification of transcription factor binding sites → short motifs (≈ 6 to 8 bp), very frequent in genomes.

The “futility theorem” ([Wasserman and Sandelin, 2004](#)): most occurrences of these motifs are non-functional *in vivo*.
→ Newer model: in fact many of them bind the regulator *in vivo*.

Conservation of presumptive microRNA binding sites

miRNA target identification

False positives in target prediction

Binding site evolution after miRNA loss

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Generalization: defining regulatory targets

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Consequential vs. inconsequential targets

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

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Generalization: defining regulatory targets

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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>

Consequential vs. inconsequential targets

Some evidence suggests that other regulators also have consequential and inconsequential targets:

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Many experimentally-identified binding sites are not under a selective pressure:

- ▶ For transcription factors: [Odom *et al.*, 2007](#), [Schmidt *et al.*, 2010](#);
- ▶ For RNA-binding proteins: [Litterman *et al.*, 2019](#).

—→ An even-newer model: regulators are specific, but not just through biochemical recognition.

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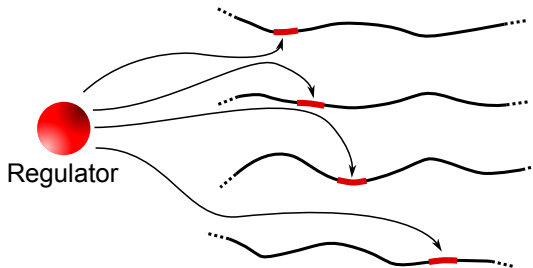
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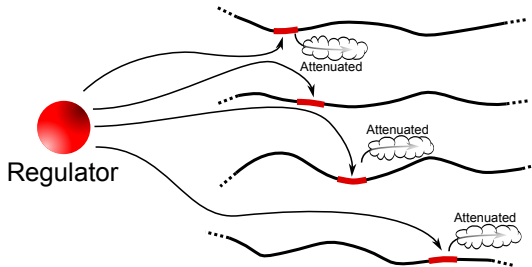
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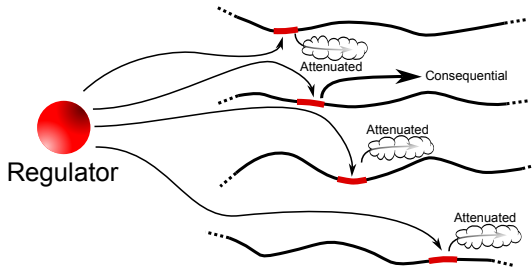
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High-throughput experimental target identification captures many false positives...

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... but comparative genomics too!

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

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

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

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... but comparative genomics too!

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

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

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

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Conclusion

May be generalized to other regulators (transcription factors, RNA-binding proteins).

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In vivo veritas: genome editing may correct a lot of false beliefs.

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A natural antagonism between developmental transitions and homeostasis.

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Conclusion

May be generalized to other regulators (transcription factors, RNA-binding proteins).

In vivo veritas: genome editing may correct a lot of false beliefs.

A natural antagonism between developmental transitions and homeostasis. Gene regulation should not only be described at the molecular scale.

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Acknowledgments



Current members:

Isabelle Busseau
Estelle Rebecq

Élisabeth Houbron
Pauline De Sousa

Rémy Rodriguez

Former members:

Sophie Mockly
Natalia Pinzón
Laura Martinez

Germain Busto
Blaise Li
Anna Sergeeva

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