microRNAs: historical aspects, biogenesis, mode of action, biological functions

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This slideshow is available at:
https://www.igh.cnrs.fr/equip/Seitz/UM2024.pdf

Initial discoveries

(taken from van der Krol *et al.*, 1990)

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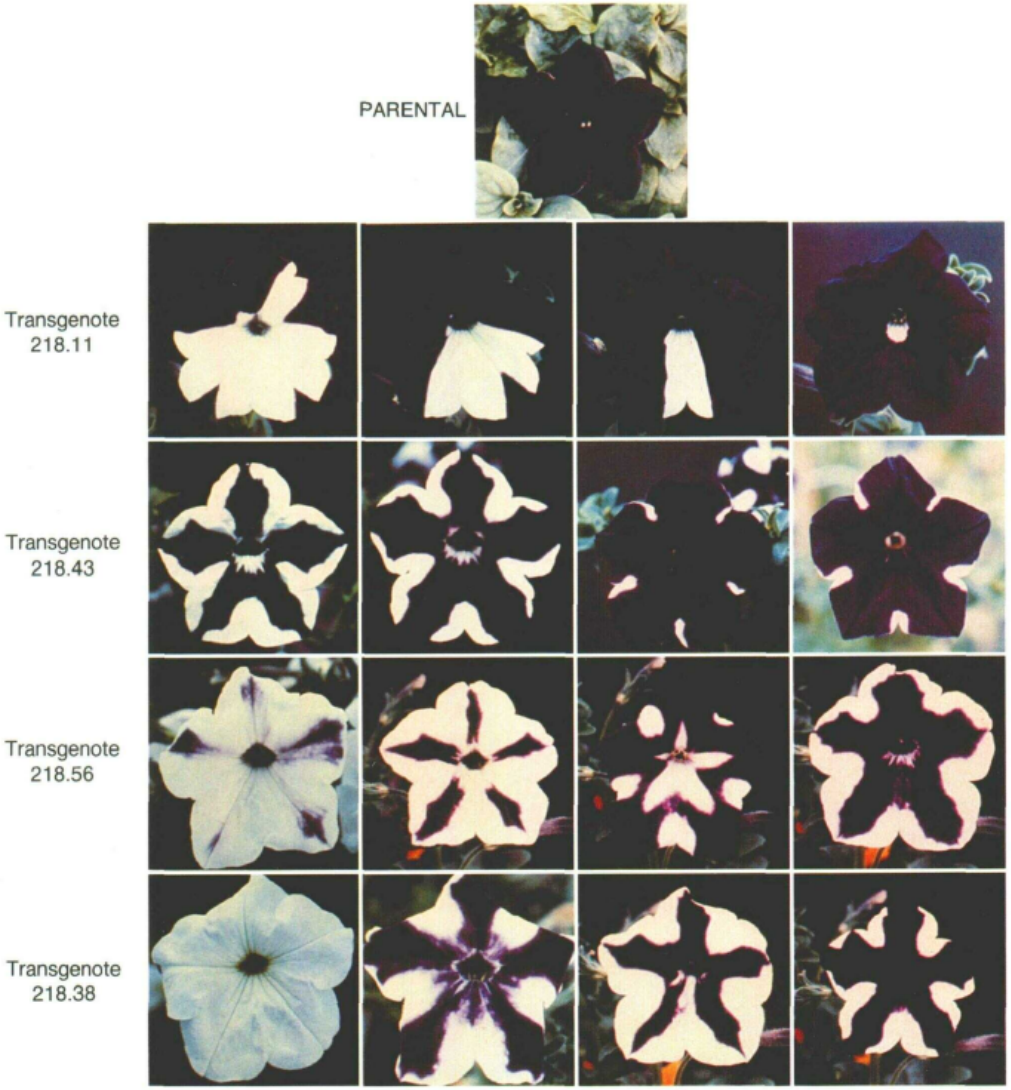

(taken from Napoli *et al.*, 1990)

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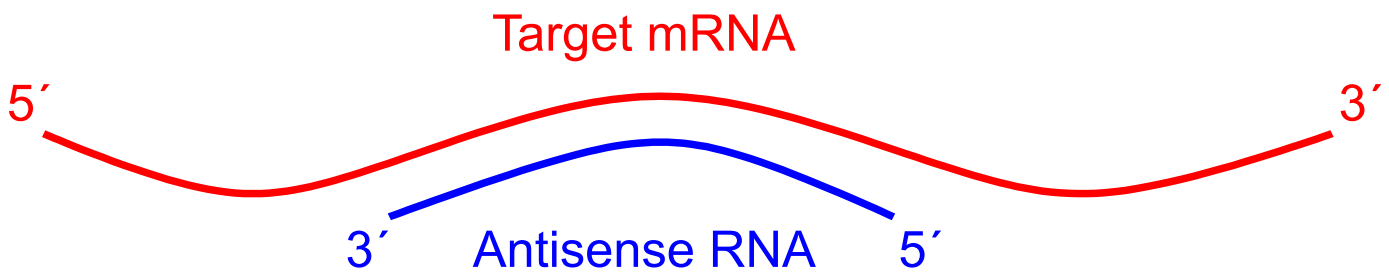
Homology sensing: nucleic acids are probably involved (proteins would not have a universal homology-sensing property) (Baulcombe, 1996).

Identification of the trigger



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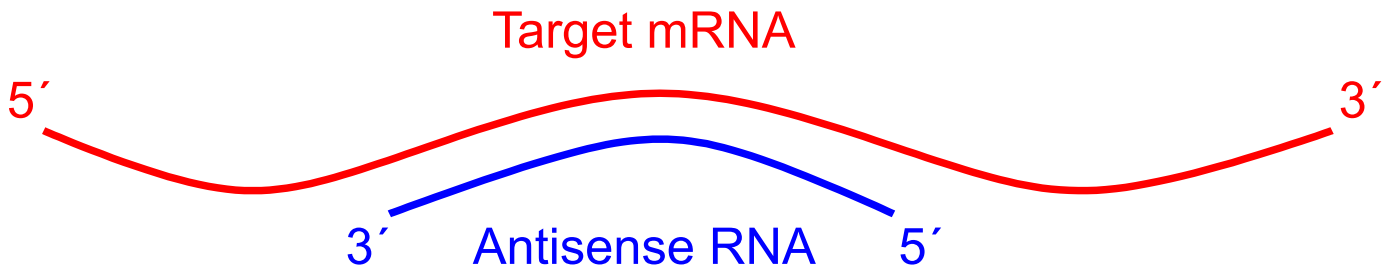

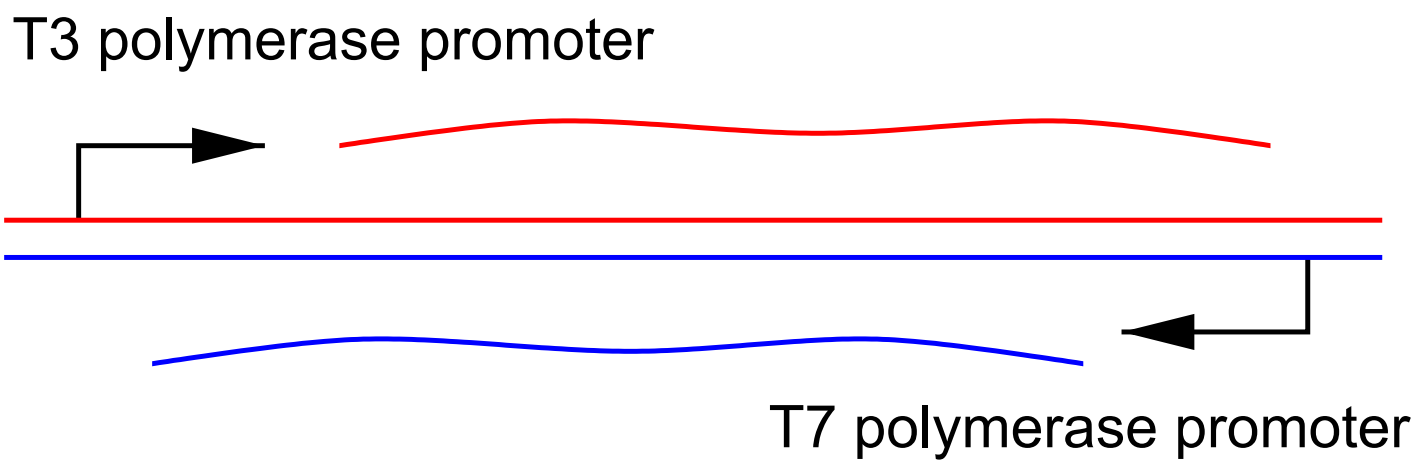

Untreated worms

(video by Darryl Conte Jr., Worcester, MA, USA)

After injection of *unc-22* double-stranded RNA

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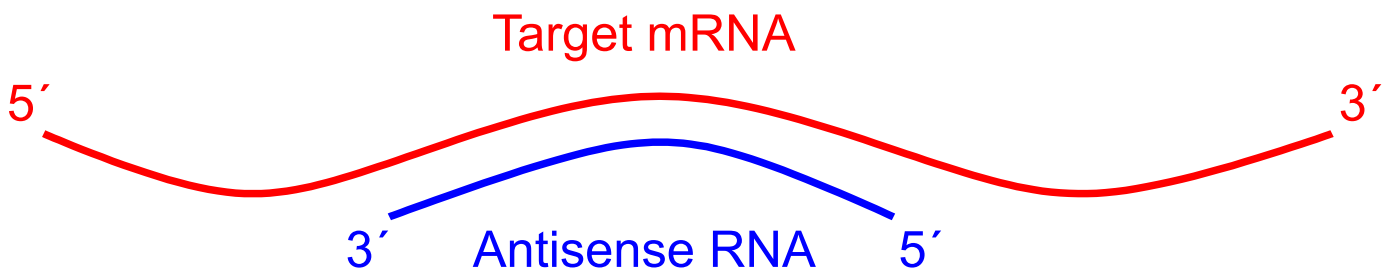

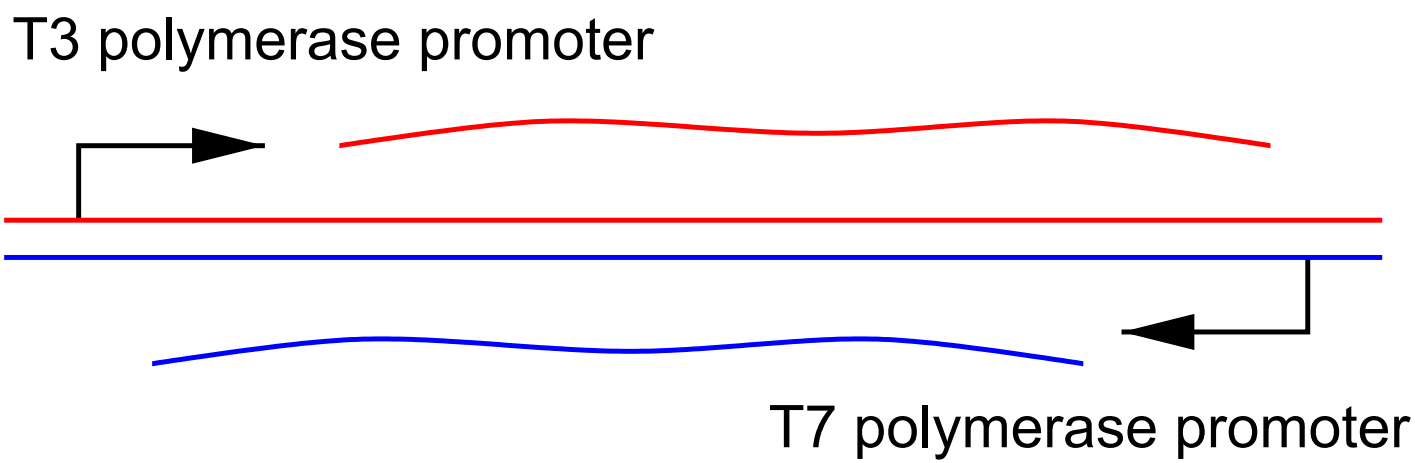

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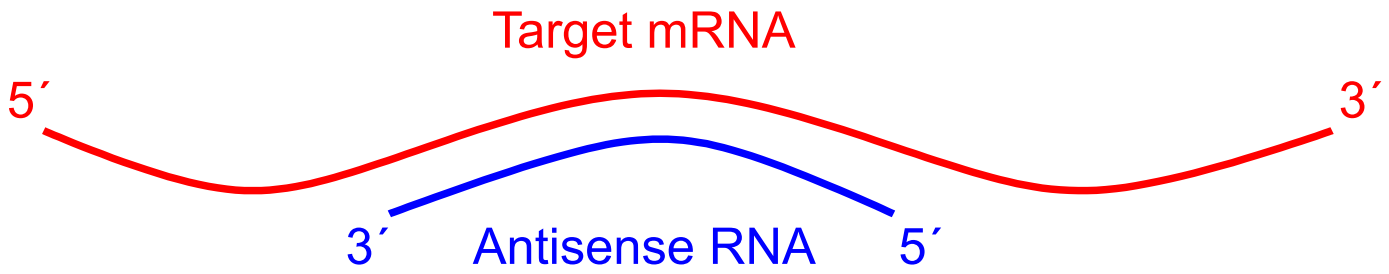

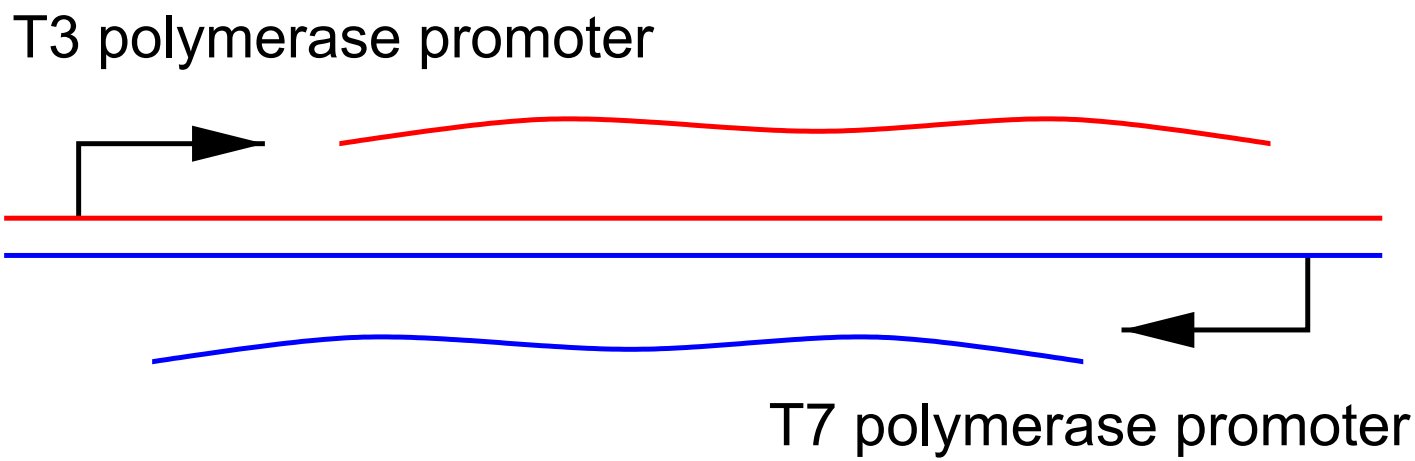

$\longrightarrow$ The trigger: double-stranded RNA (Fire *et al.*, 1998).

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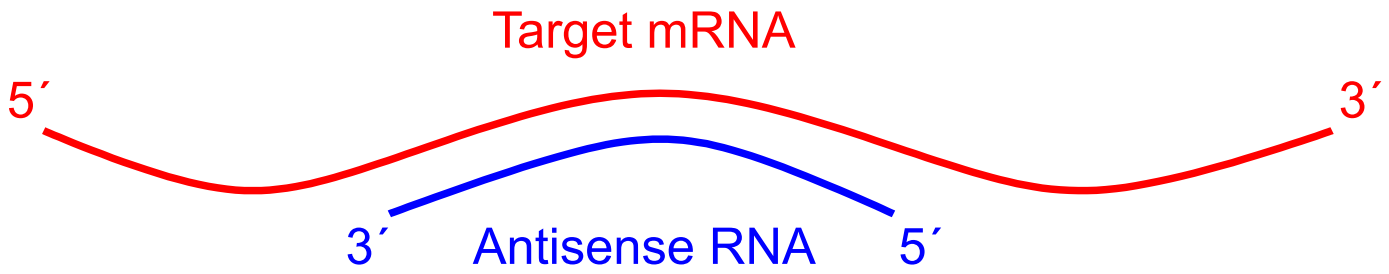

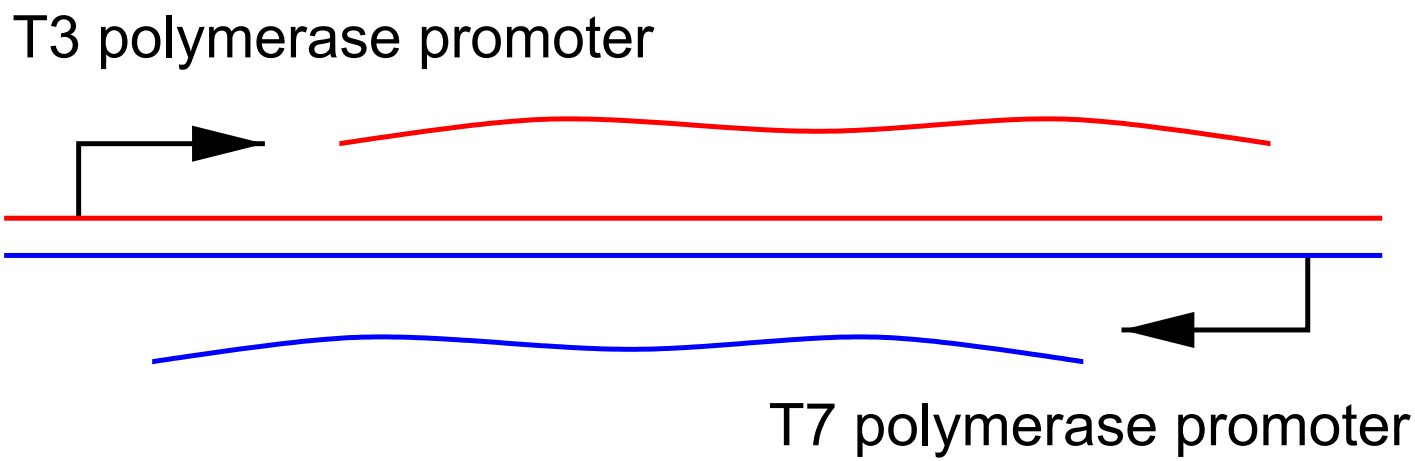

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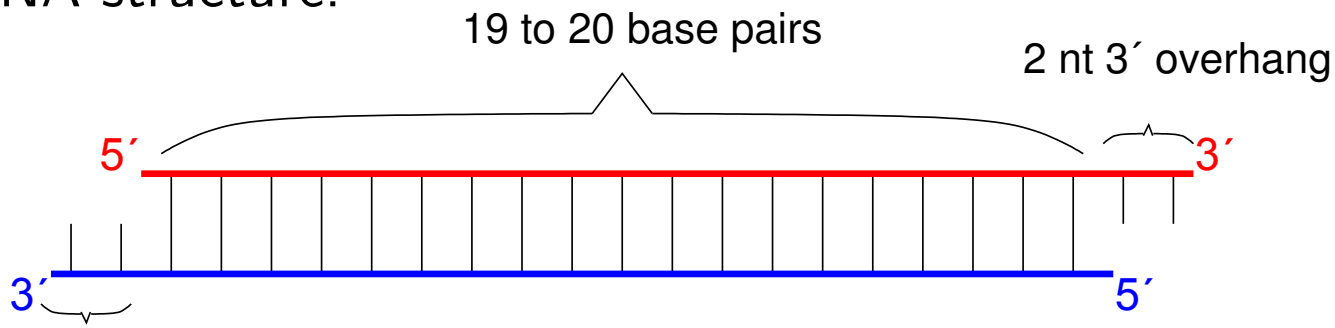
In plants (Hamilton and Baulcombe, 1999), then in the *in vitro Drosophila* system (Zamore *et al.*, 2000) and in cultured *Drosophila* cells (Hammond *et al.*, 2000): observation of small double-stranded RNAs ($\approx$ 20–25 bp) homologous to both the trigger and the target: “siRNAs” (small interfering RNAs).

RNAi-associated small RNAs: “siRNAs”

siRNA structure:



2 nt 3´ overhang

5´ monophosphate, 3´ hydroxyl

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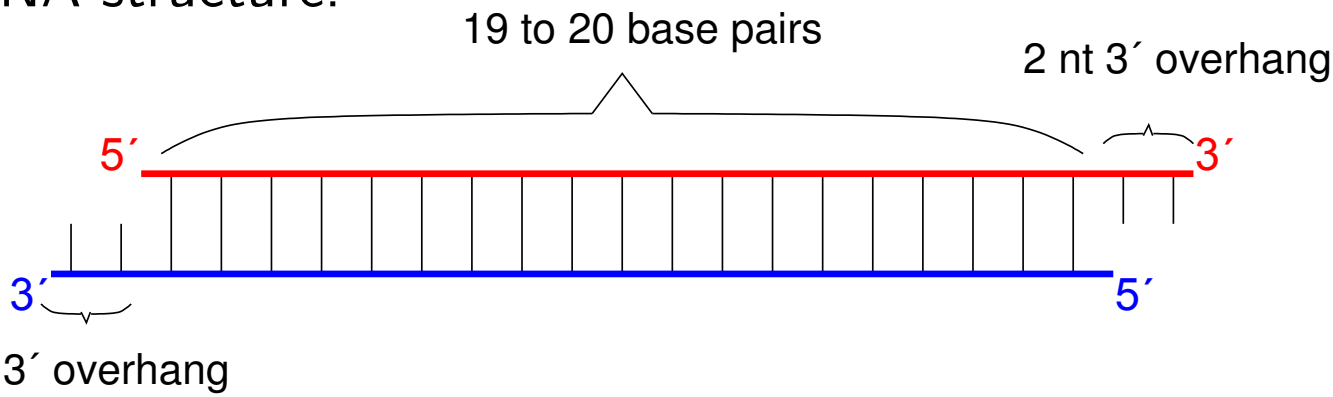

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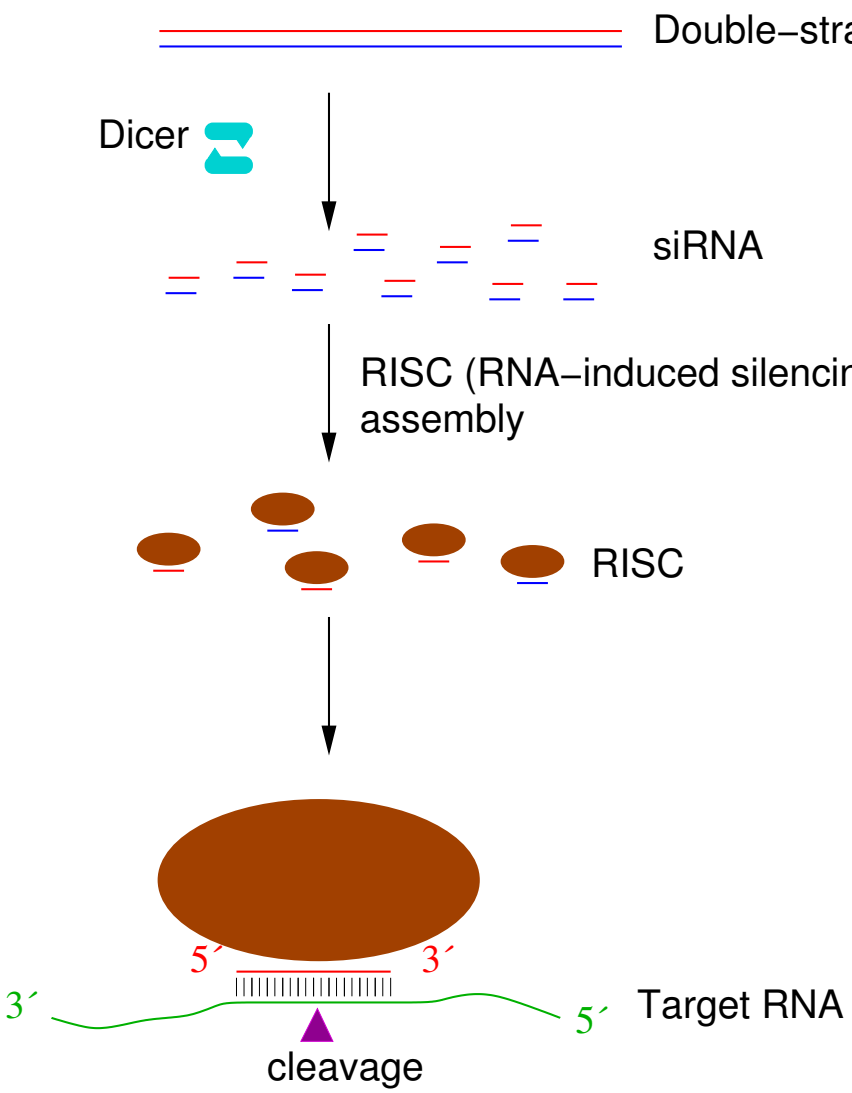
siRNAs come from the digestion of the double-stranded RNA trigger by the “Dicer” enzyme (member of the RNase III family) (Bernstein *et al.*, 2001).

Mechanism of the RNAi response



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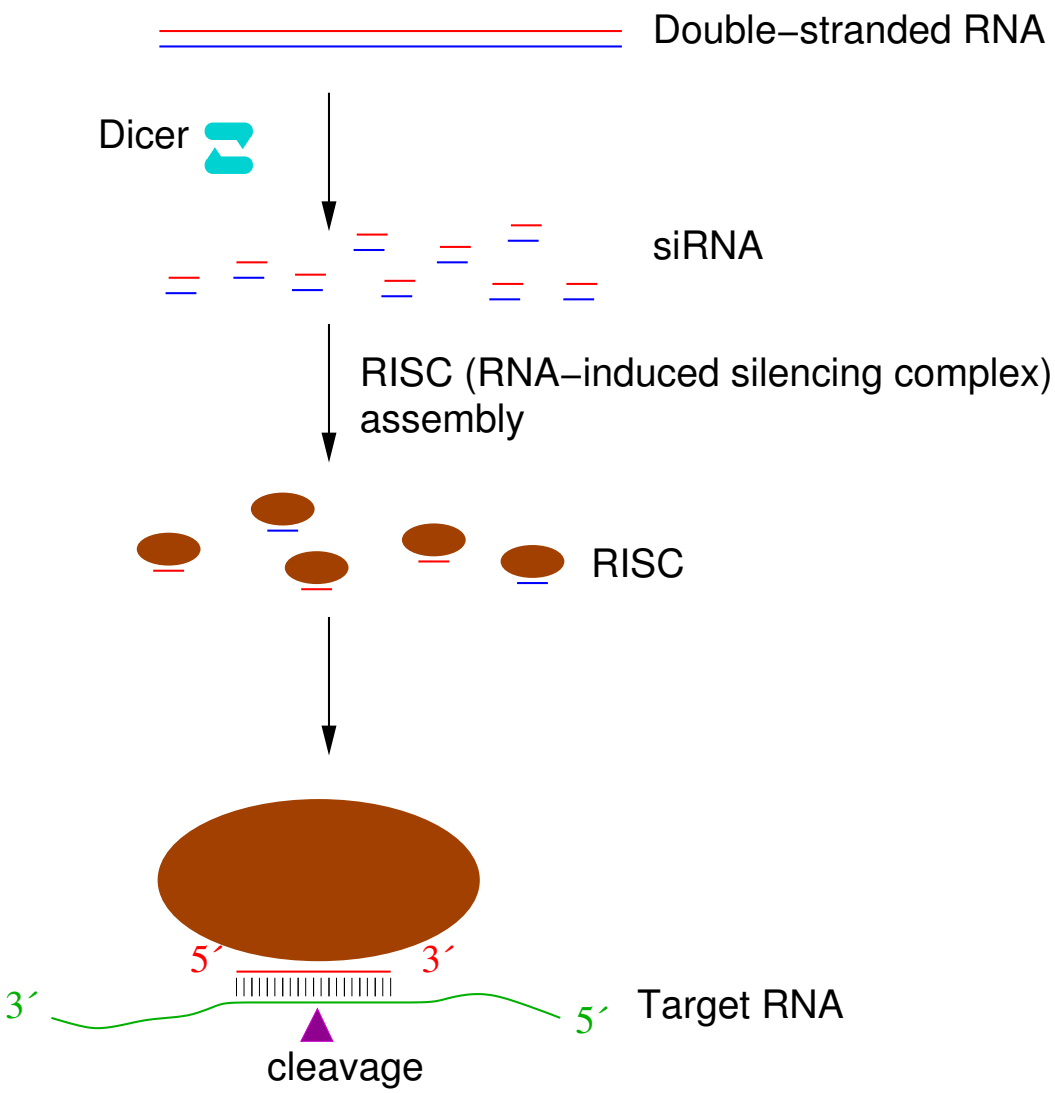

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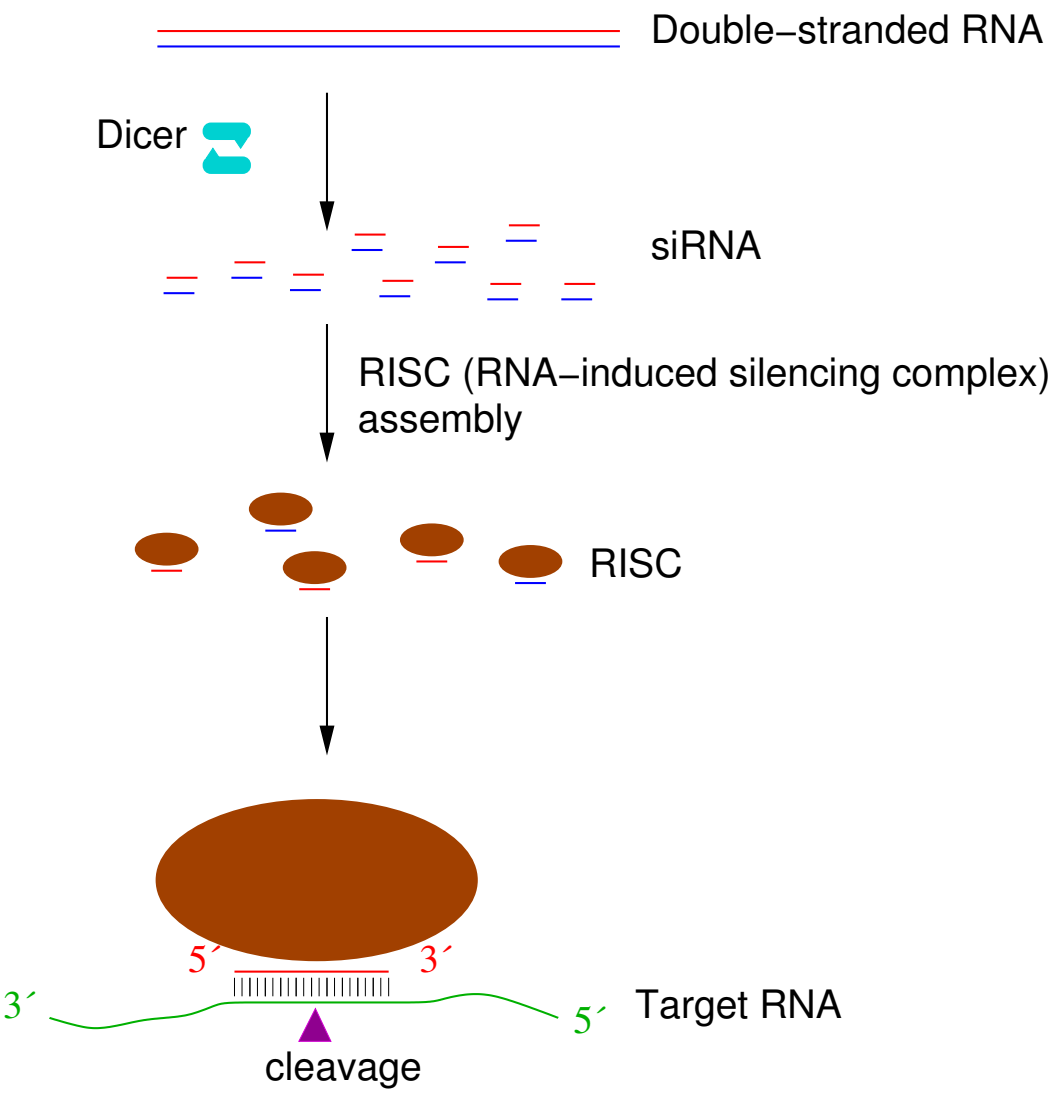

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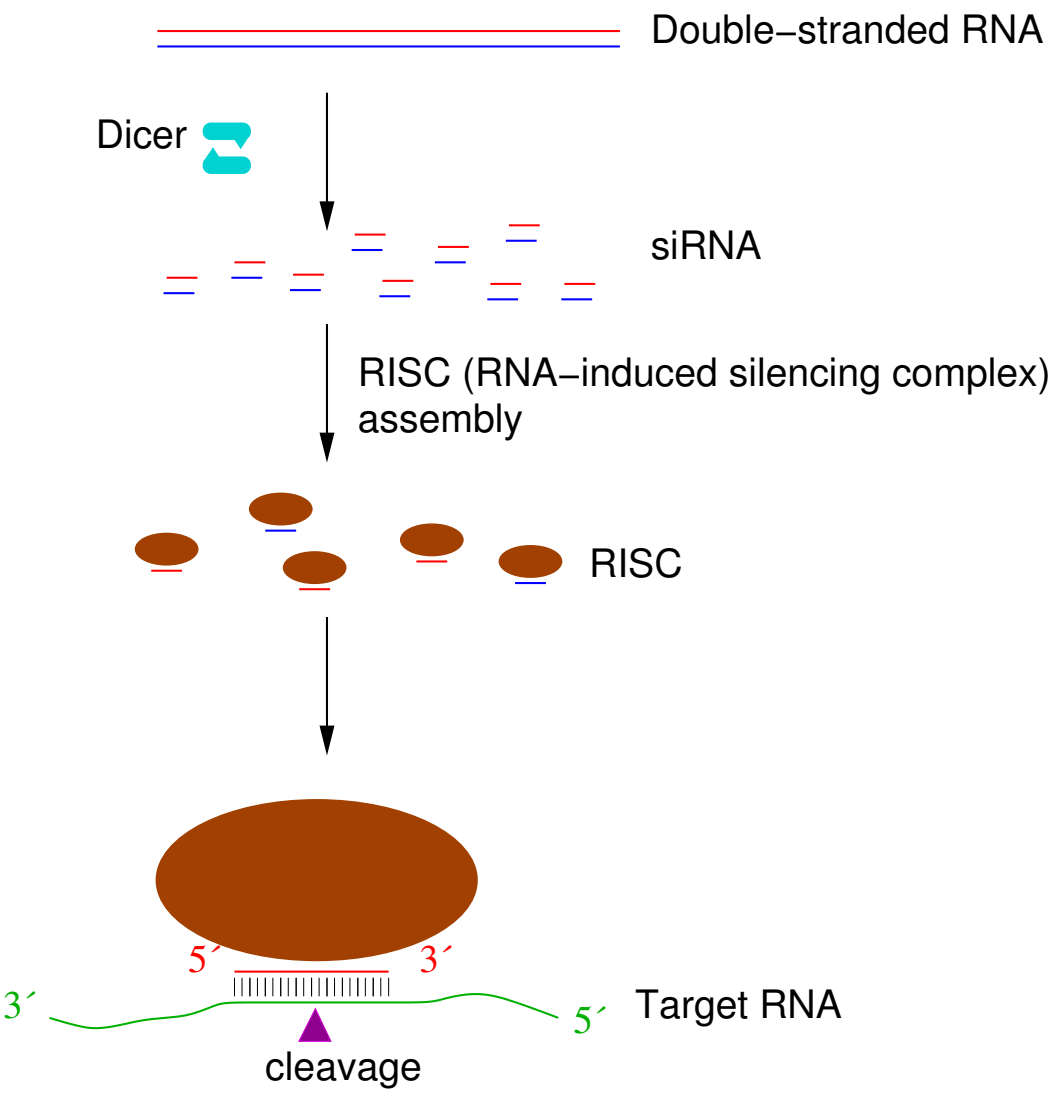

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In the lab: mammalian systems did not have any simple method for specific gene silencing.

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Nomenclature: "rasiRNAs" are not siRNAs (they are "piRNAs").

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A precursor for *lin-4*: stem-loop-folded RNA.

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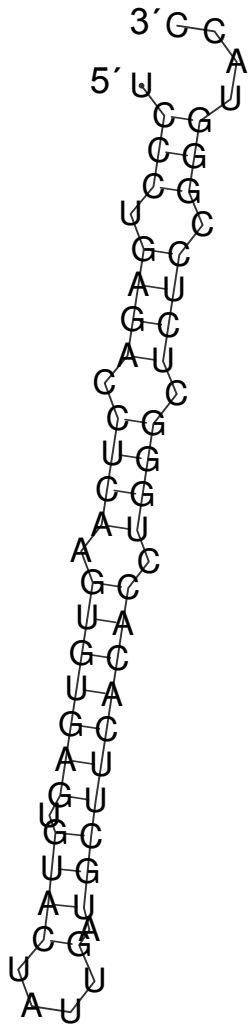

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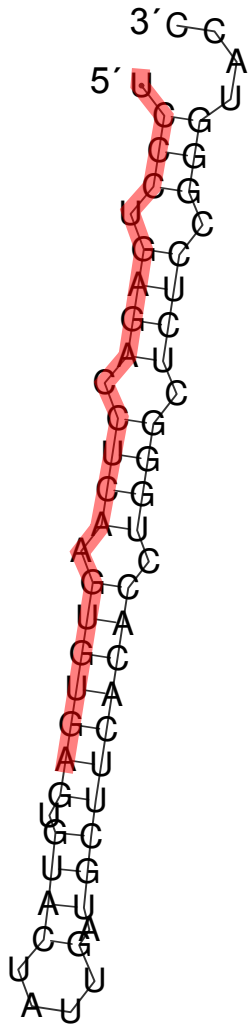

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Since then: in metazoans, metaphytes, *Chlamydomonas*, viruses.

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Location of imperfect binding sites: 3´ UTR, but $\exists$ exceptions !

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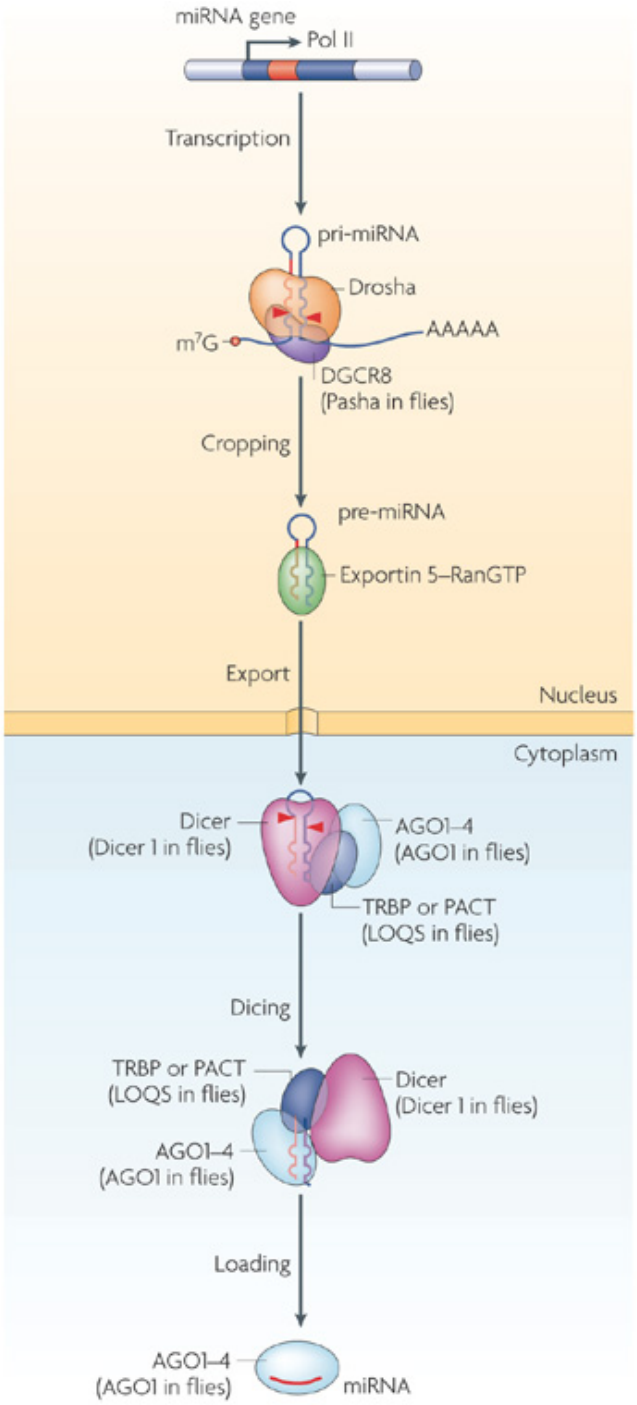
There are a few exceptions.



(taken from Kim *et al.*, 2009)

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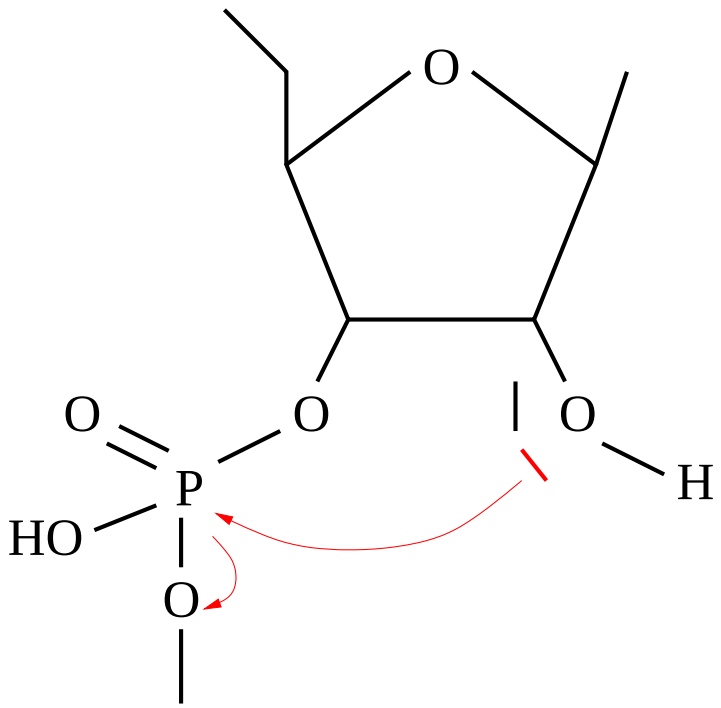
Too short: RNAs are very sensitive to degradation (nucleophilic attack by 2´ OH), and an RNA sample is always contaminated with degradation fragments (the bottom of the gel is traditionally ignored).



microRNA identification

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Large diversity (48,885 known miRNAs in 271 eukaryotic species and viruses: miRBase 22.1)

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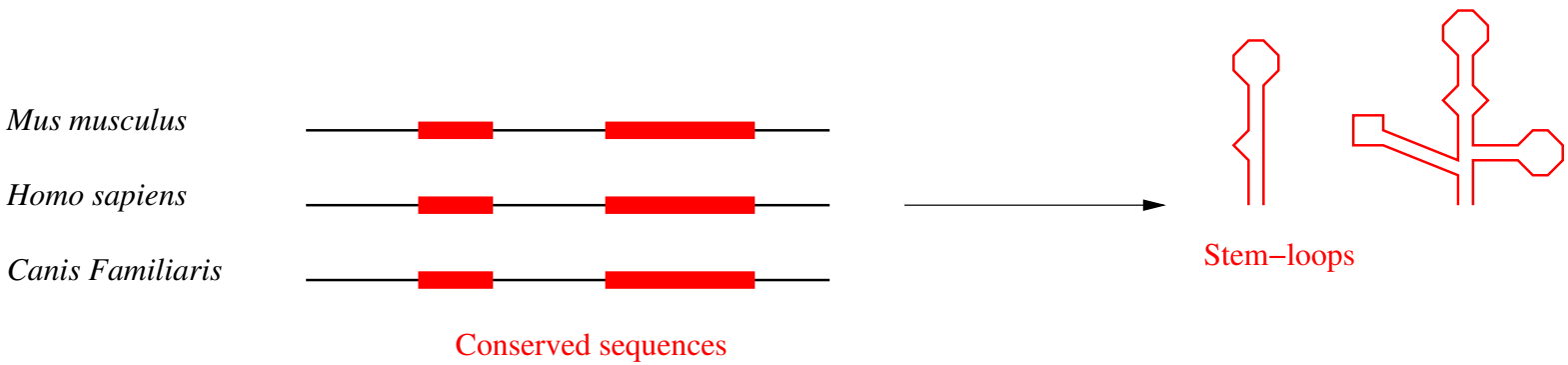

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Each approach is limited:

- Genetics: random, very rare.
- Cloning: abundance (weak expression, spatially or temporally restricted).
- Bio-informatics: choice of selection criteria is often arbitrary; does not identify miRNA extremities.

Differential transcriptomics or proteomics

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Identifies both direct and indirect targets.

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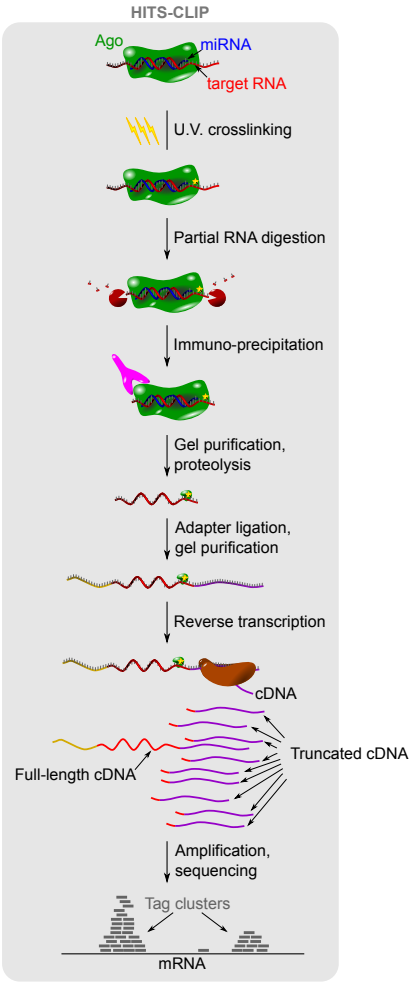

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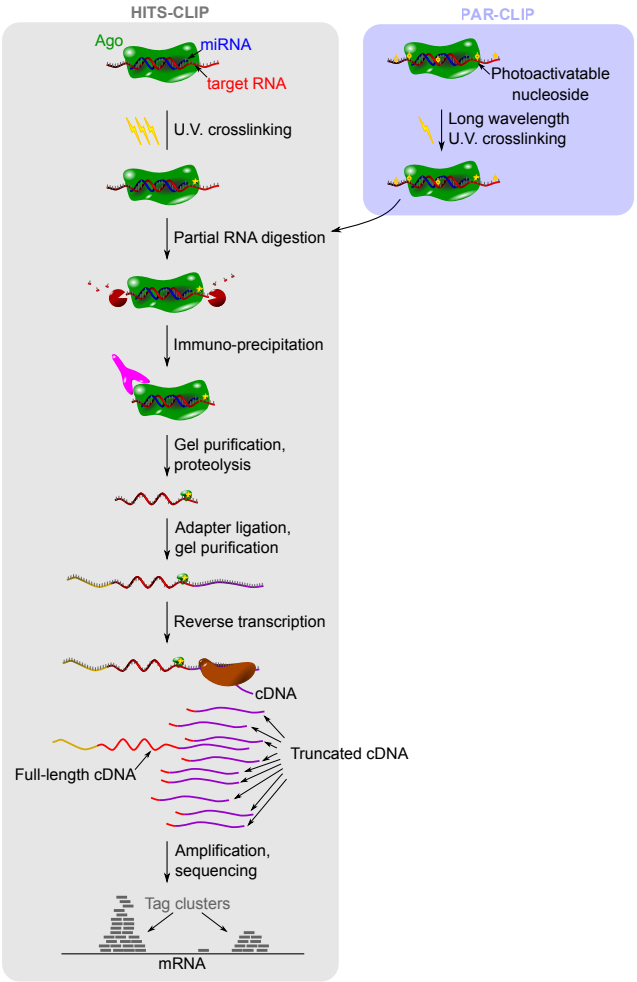

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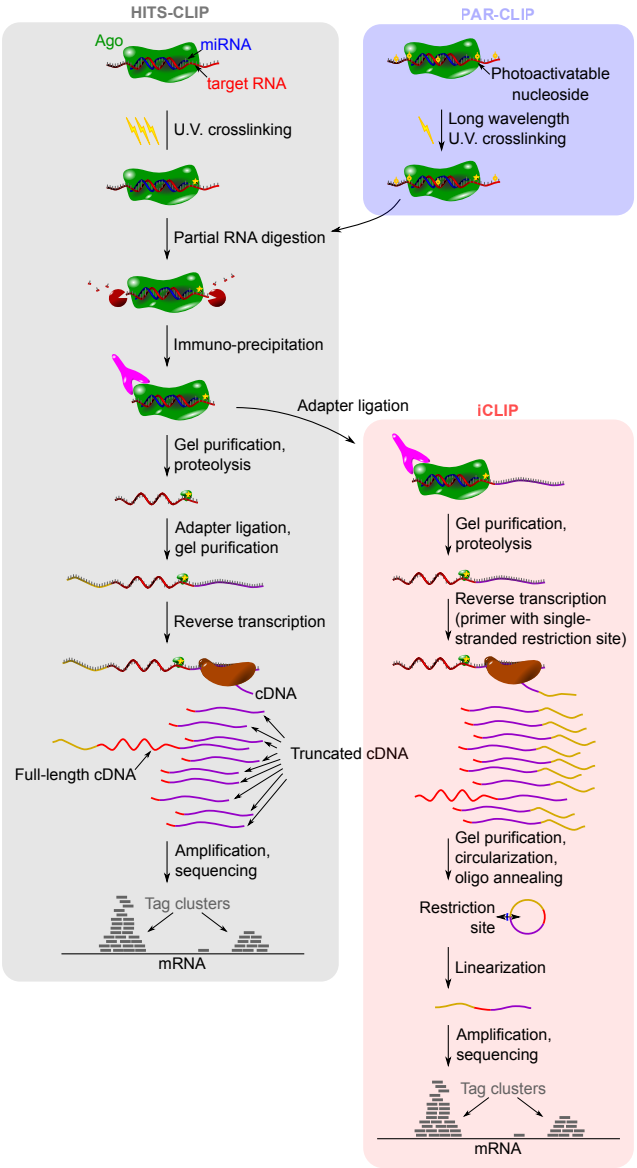

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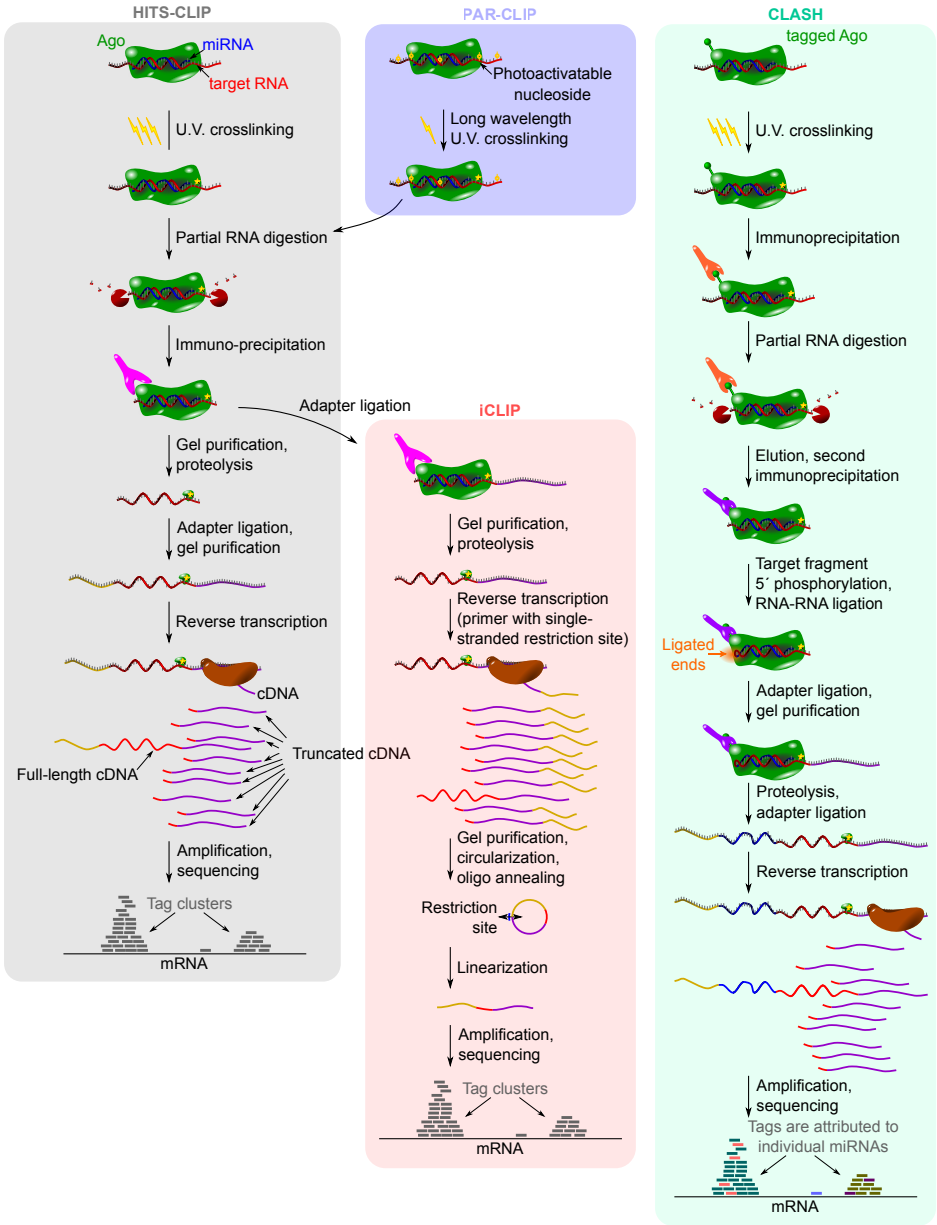

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- look for phylogenetically conserved sites (should capture known and unknown biochemical features) (Friedman and Burge, 2014)

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Perhaps the best: TargetScan (and: frequently updated). But: in their benchmark papers, the authors of TargetScan find that TargetScan is the best, the authors of PicTar find that PicTar is the best (Baek *et al.*, 2008; Selbach *et al.*, 2008) ...

A problem with high-throughput methods

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Showing that the miRNA binds (or: represses) the mRNA $\neq$ showing that this interaction plays a biological role

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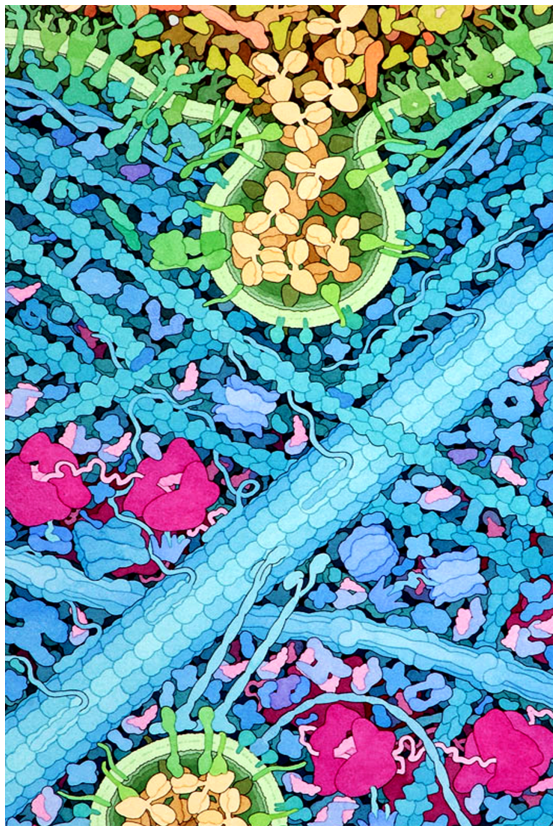

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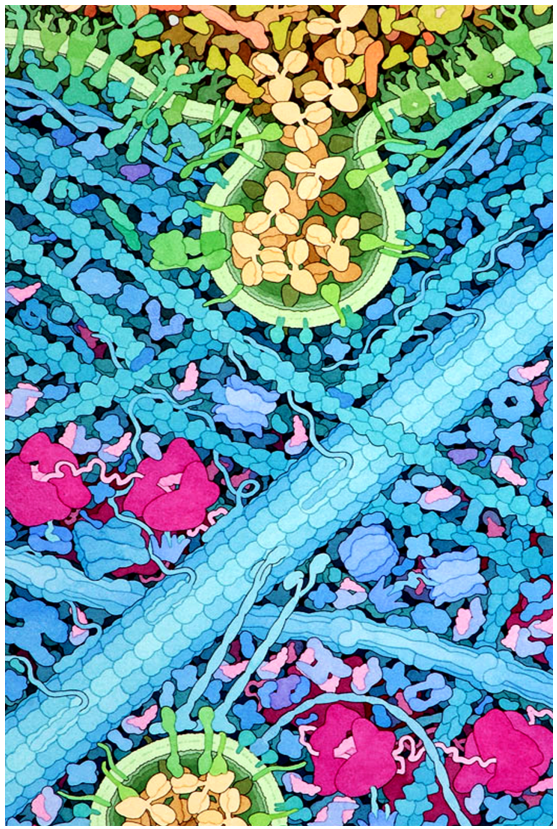

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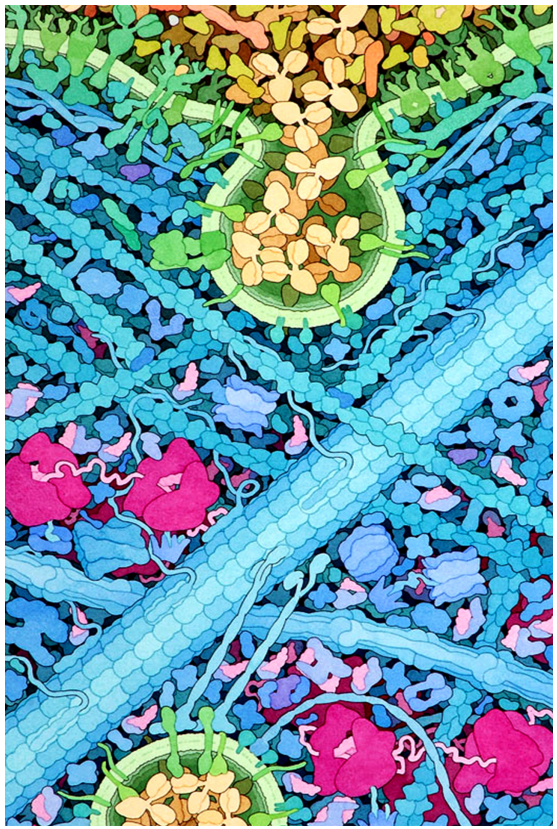

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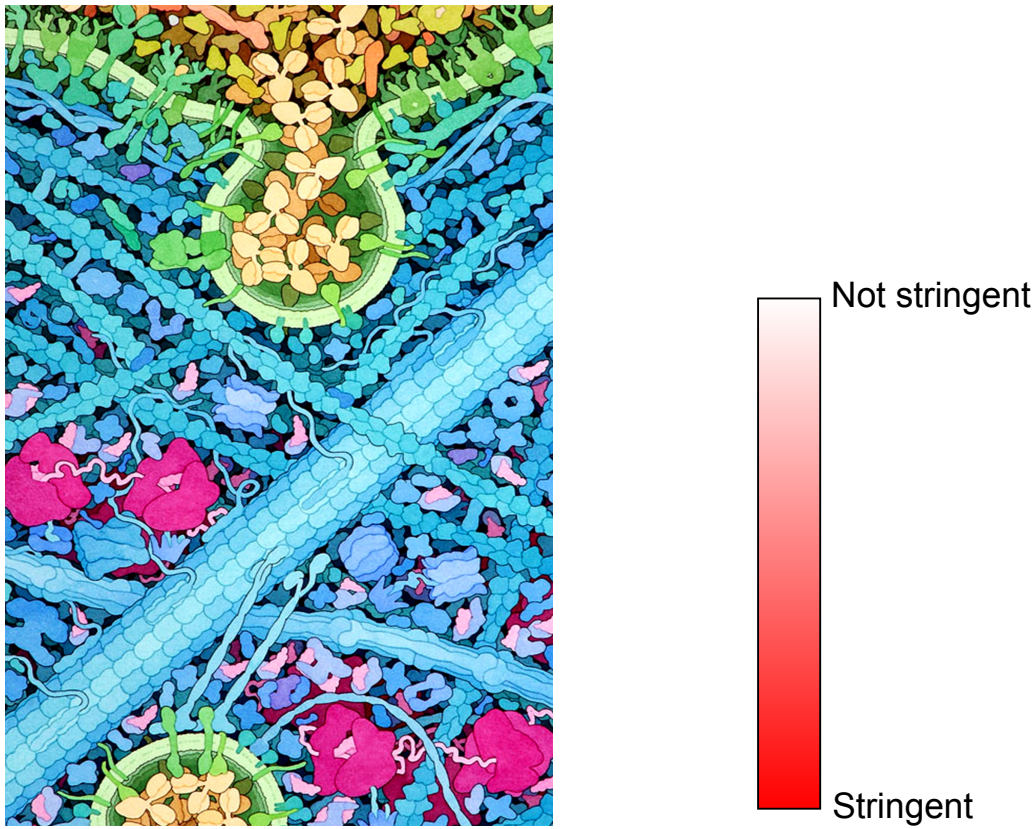

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If it is conserved, it must be functional ($\longrightarrow$ prediction programs are useful even after a CLIP experiment).

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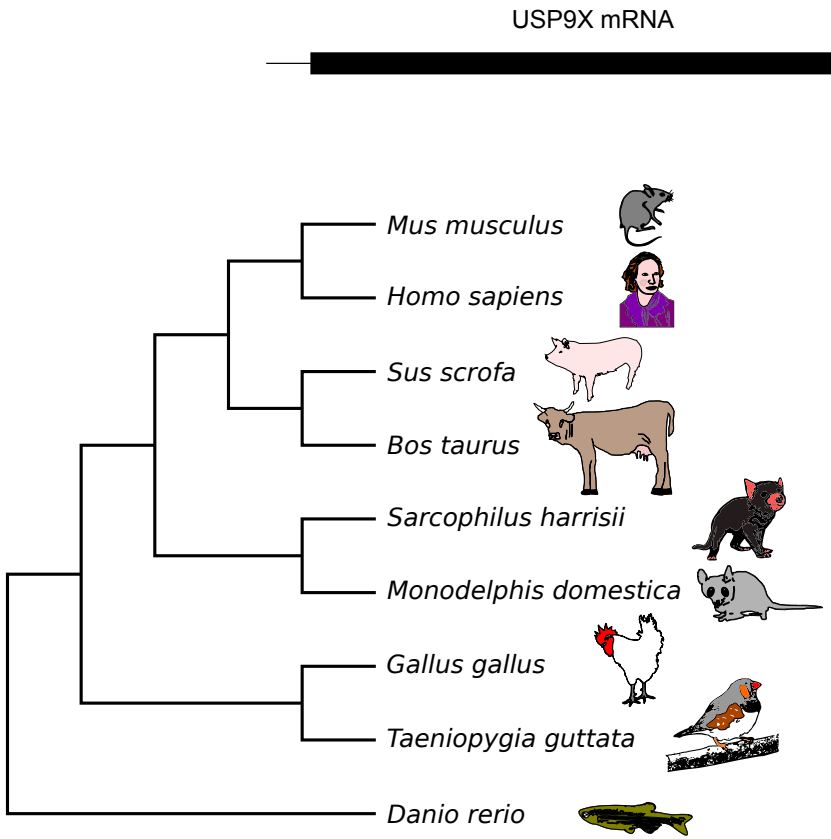

(taken from Pinzón *et al.*, 2017)

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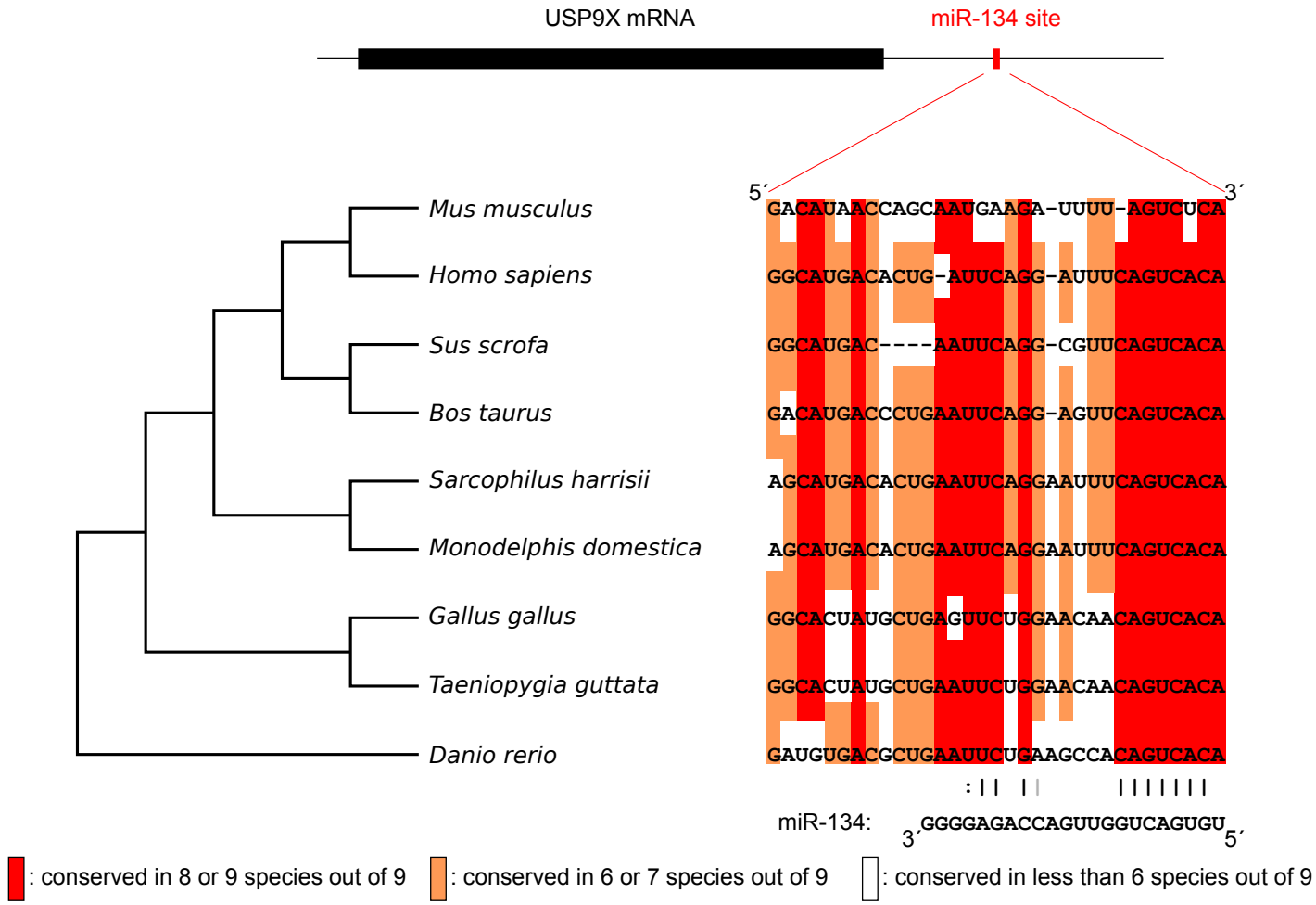

(taken from Pinzón *et al.*, 2017)

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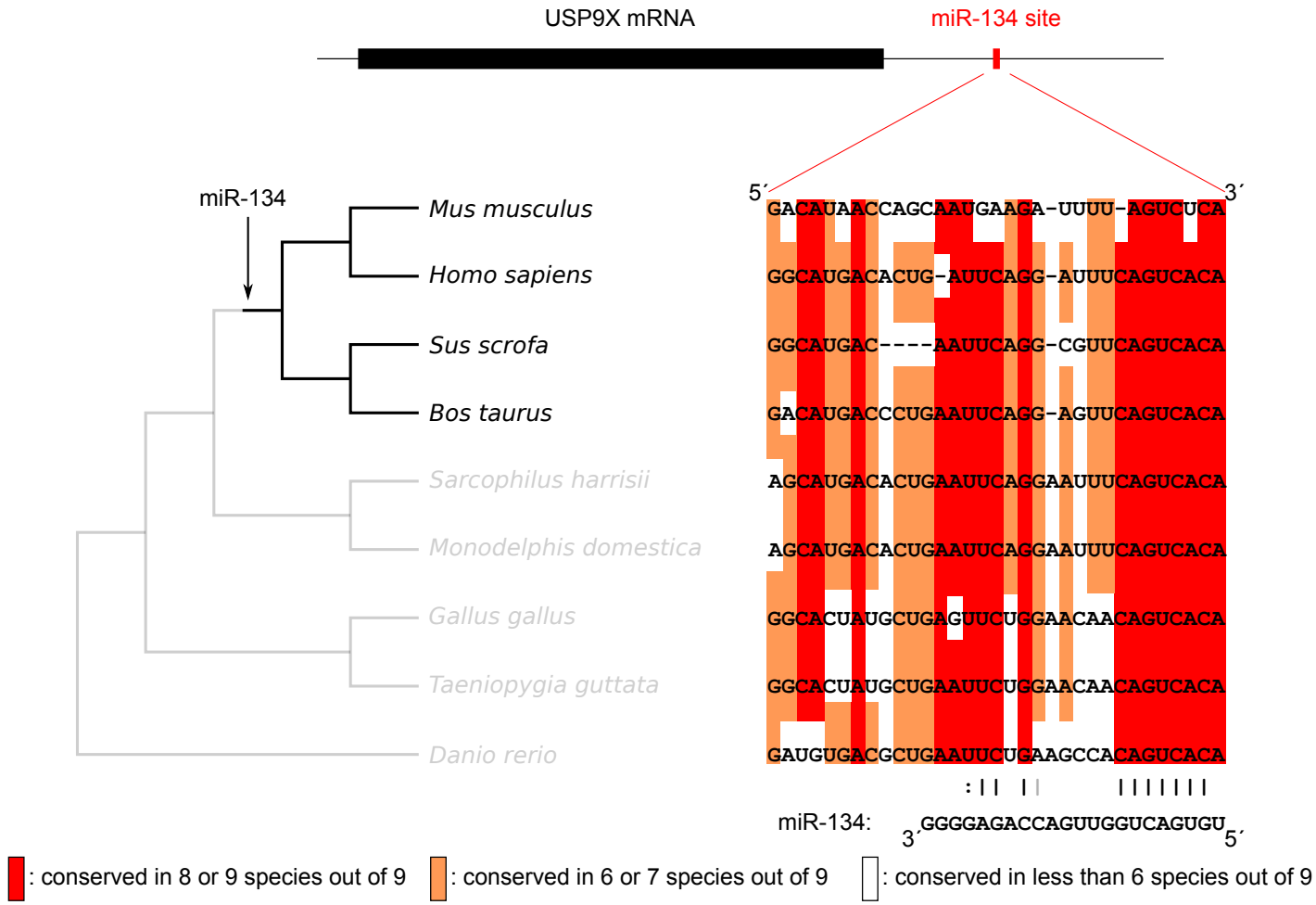

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Conclusion

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None of these methods can probe unambiguously for biological relevance. Low-throughput genetics is required for a reliable assignment.

In vivo veritas: a large part of the miRNA literature certainly needs a cleanup (Seitz, 2024).