

Functional assessment of individual miRNA binding sites: time to go *in vivo*

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microRNA target prediction

In vivo function of
miRNA binding
sites

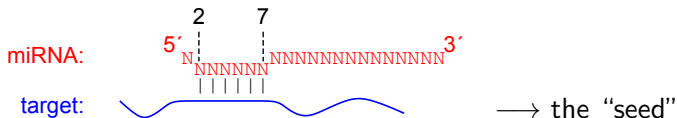
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Comparative
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microRNA target prediction



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Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

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Computational programs for target prediction: look for seed matches in 3' UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

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The screenshot shows a web browser window with the title "miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. - PubMed - NCBI - Mozilla Firefox". The address bar shows the URL "www.ncbi.nlm.nih.gov/pubmed/?term=miR-26a+suppresses+tumour+proliferation+and+metastasi". The search results show "See 1 citation found by title matching your search:" and the citation details: "Cancer Lett. 2015 Feb 1;354(2):354-62. doi: 10.1016/j.canlet.2014.11.050. Epub 2014 Nov 27. miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. Liu P¹, Tang H¹, Chen B¹, He Z², Deng M², Wu M³, Liu X¹, Zhang L¹, Ye F¹, Xia X⁴. Author information Abstract It has been reported that miR-26a plays an important role in various cancers. In this study, we found that miR-26a was downregulated in triple-".

microRNA target prediction

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⇒ miRNAs are implicated in every physiological process in animals.

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⇒ miRNAs are implicated in every physiological process in animals.

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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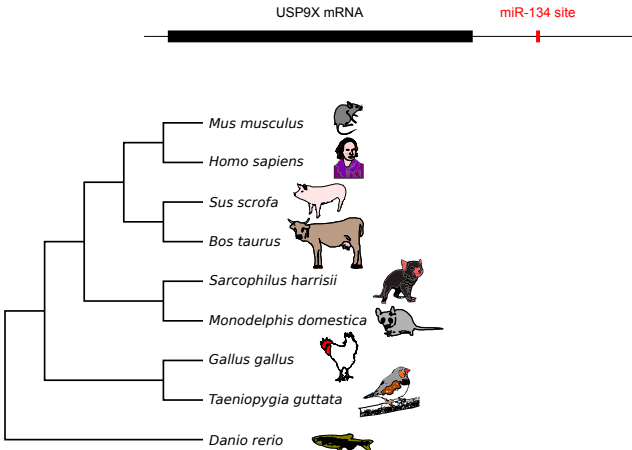
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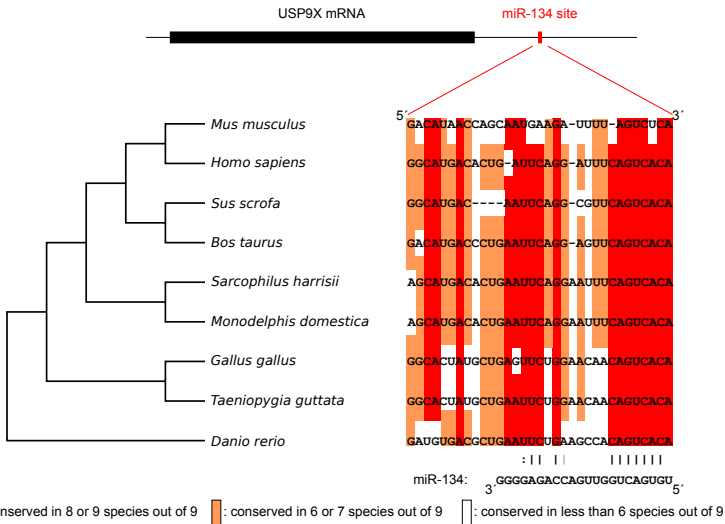
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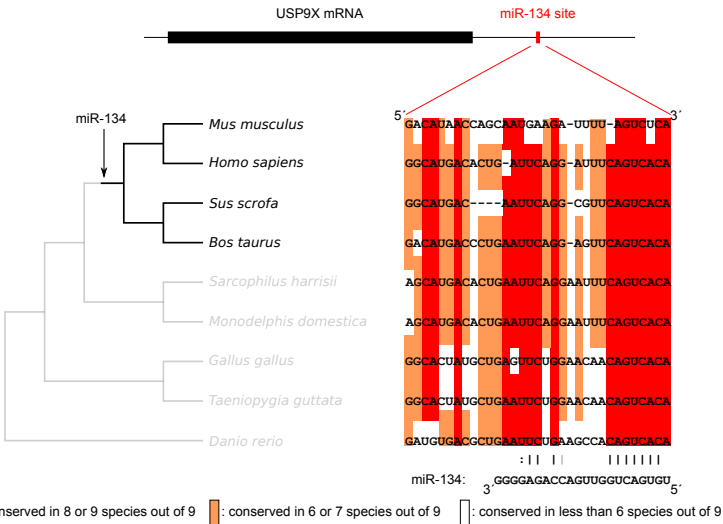
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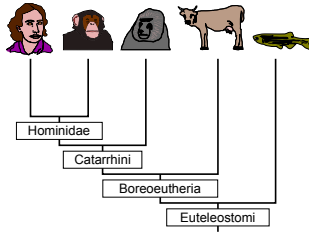
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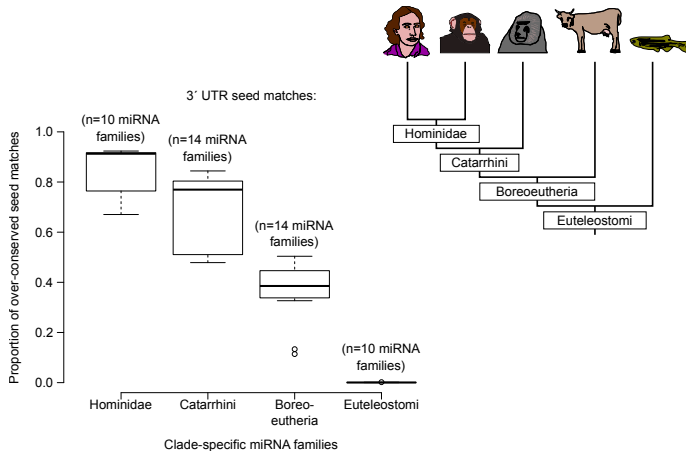
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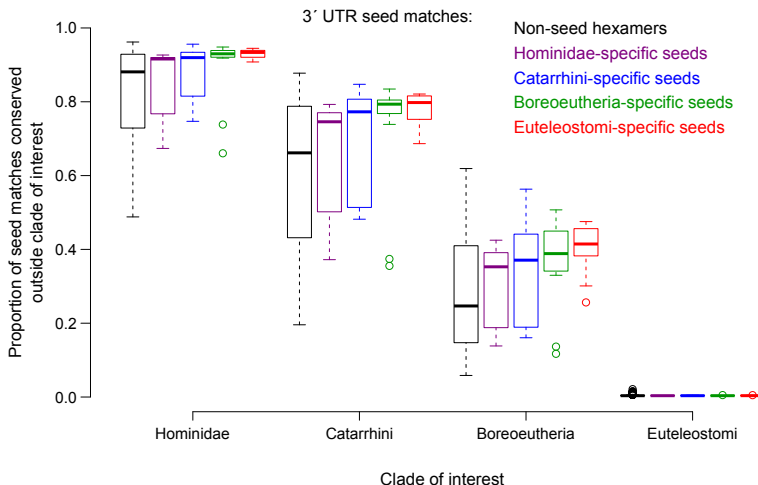
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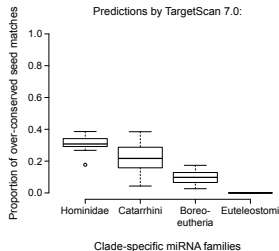
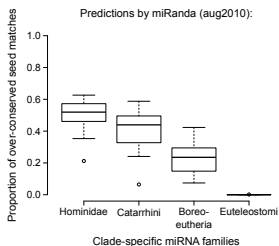
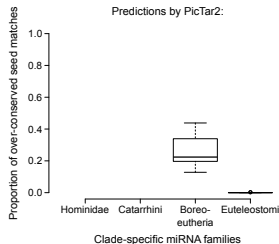
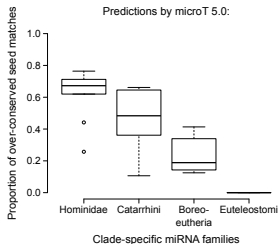
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Conclusion

Many predicted miRNA binding sites are conserved in a miRNA-independent fashion.

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Many predicted miRNA binding sites are conserved in a miRNA-independent fashion.

Eliminate over-conserved sites from predictions.

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Conclusion

Many predicted miRNA binding sites are conserved in a miRNA-independent fashion.

Eliminate over-conserved sites from predictions.

Assess macroscopic *in vivo* phenotypes rather than changes in protein abundance.

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Acknowledgements

Anna
Sergeeva:



Laura
Martinez:



Blaise
Li:



Natalia
Pinzón:



Isabelle
Busseau:



Delphine
Mazé:



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Supplementary data

Introduction:

- ▶ Robustness of biological pathways
- ▶ Issues with published pseudo-targets

Conclusion:

- ▶ Pseudo-targets for other regulators?
- ▶ Propagation of gene expression perturbation

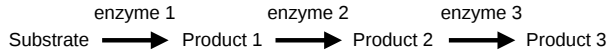
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Biological robustness



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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).

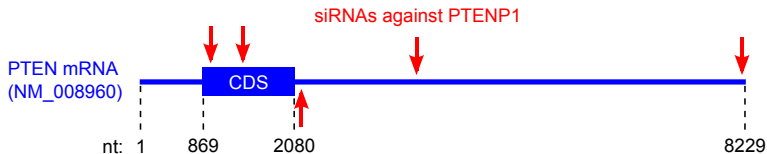
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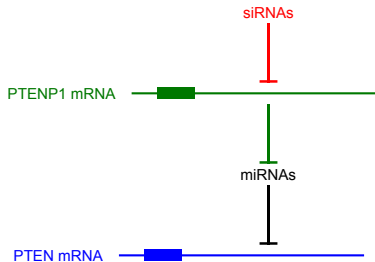
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Proposed by Poliseno *et al* (2010):



More probably:



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

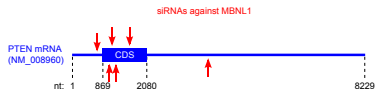
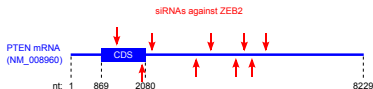
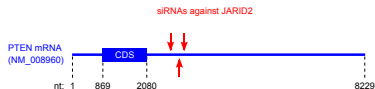
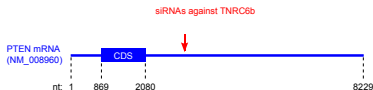
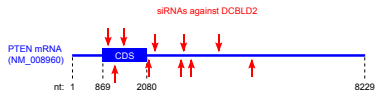
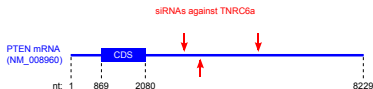
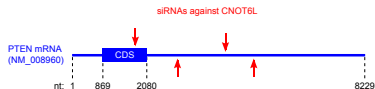
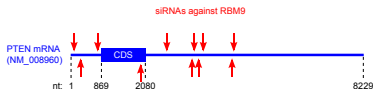
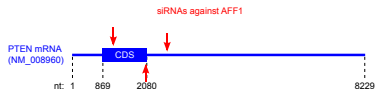
siRNA #2 against PTENP1: 5' UAAUAAUC-AUCAUUCUGGC 3'
 PTEN mRNA (nt 8189-8208): 3' UUUUUUACUUGGAAAAUUU 5'

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' AGGAUAUUGACGUUAGACUGU 5'

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

Reported examples of pseudo-targets



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Pseudo-targets for other regulators?

- ▶ Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).

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Pseudo-targets for other regulators?

- ▶ Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).
- ▶ RNA-binding proteins are poorly specific (thousands of experimentally validated targets for each analyzed protein: Hafner *et al.*, 2010; Lebedeva *et al.*, 2011 and Hafner *et al.*, 2013).

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Real molecular events, which are neutral in evolutionary terms?

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Propagation of gene expression perturbation

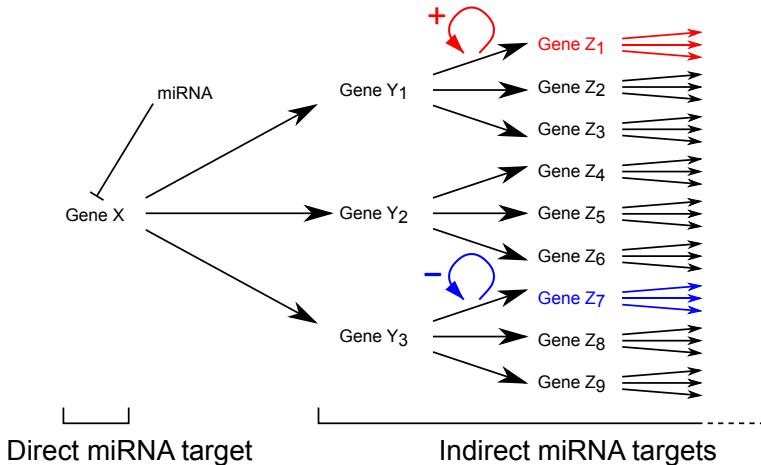
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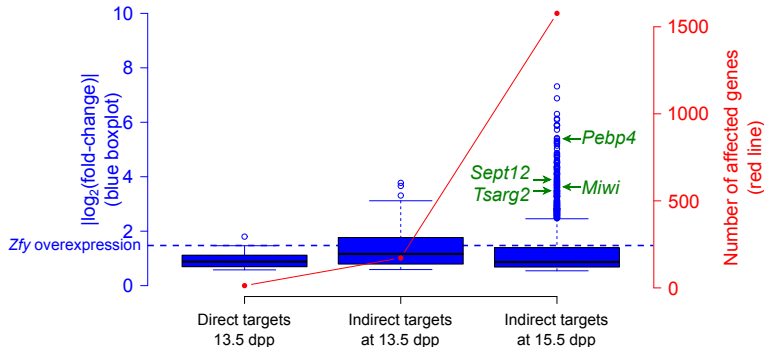
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Propagation of gene expression perturbation



(in collaboration with H. Royo and J. Turner, MRC, London)

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