MicroRNA titration: a novel function for conserved microRNA binding sites.

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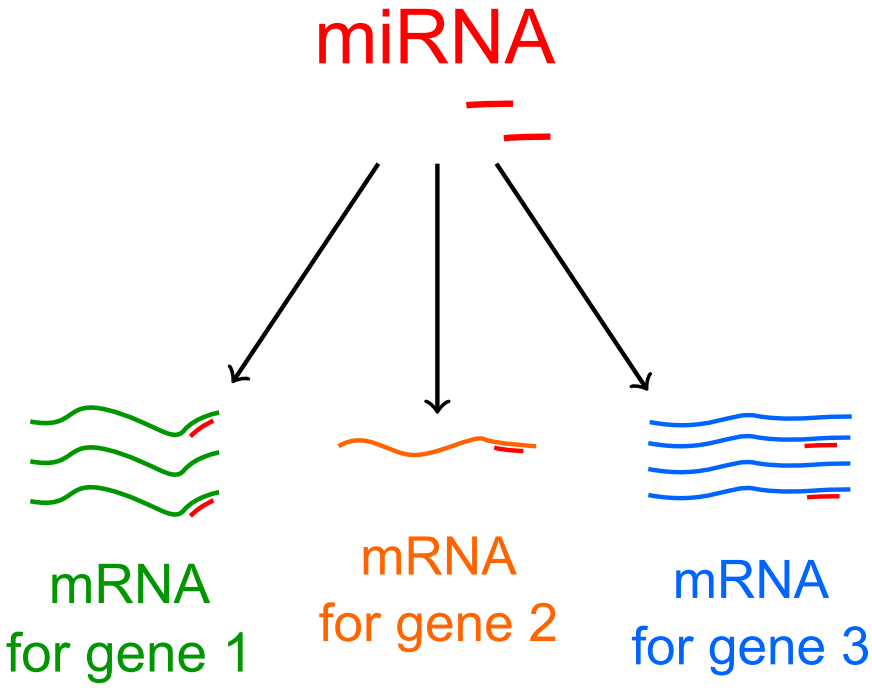
Monday, January 25 2016

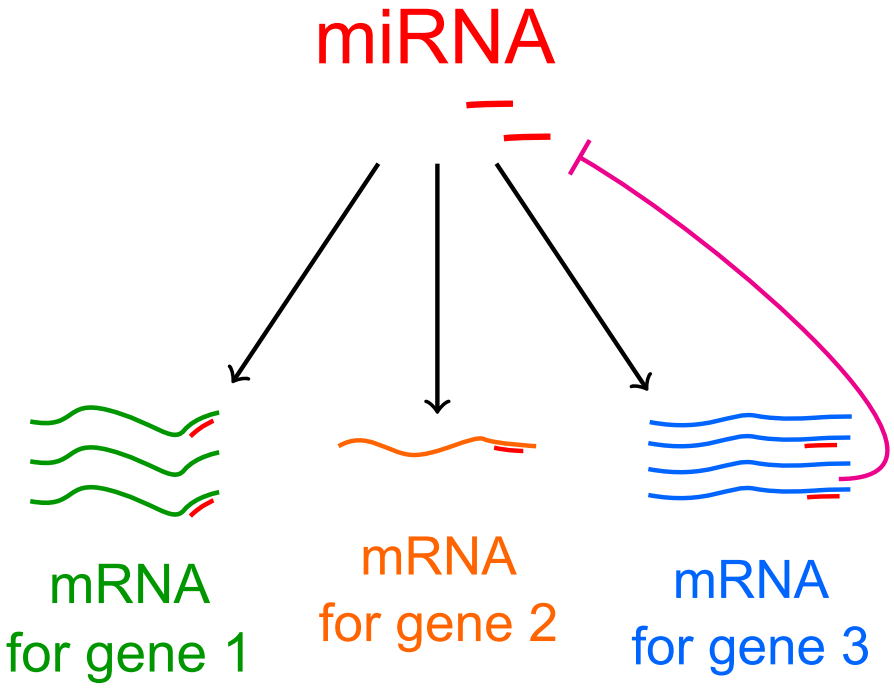
microRNA-mediated regulation

- Each microRNA regulates many mRNAs.
- Usually several hundreds of predicted targets bearing conserved binding sites.



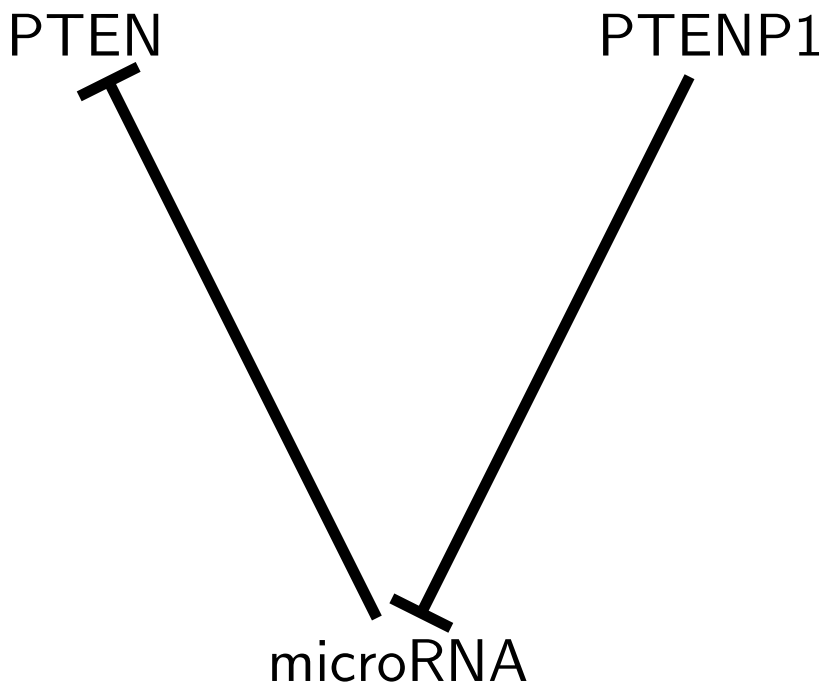
microRNA-mediated regulation

- Each microRNA regulates many mRNAs.
- Usually several hundreds of predicted targets bearing conserved binding sites.
- Some of the targets could function as titrators.



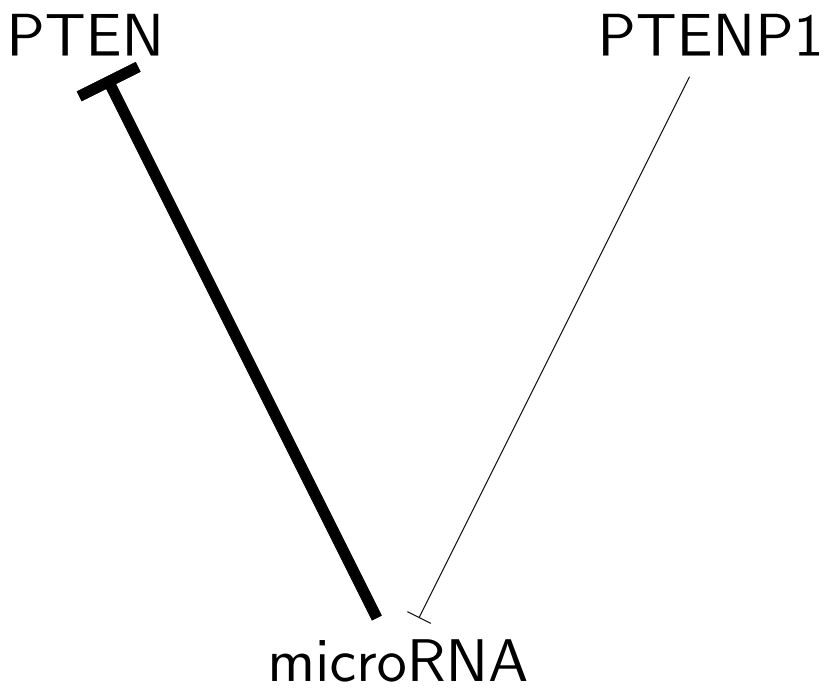
Controversy

ceRNAs:



Controversy

ceRNAs: not abundant enough.



Controversy

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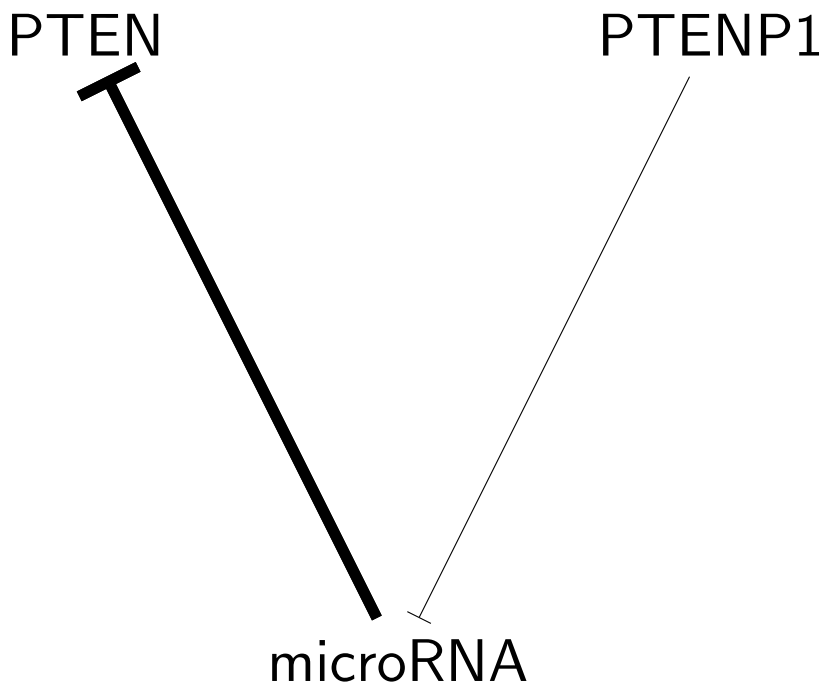

Susceptibility of microRNAs to competition is low in endogenous concentrations

Controversy

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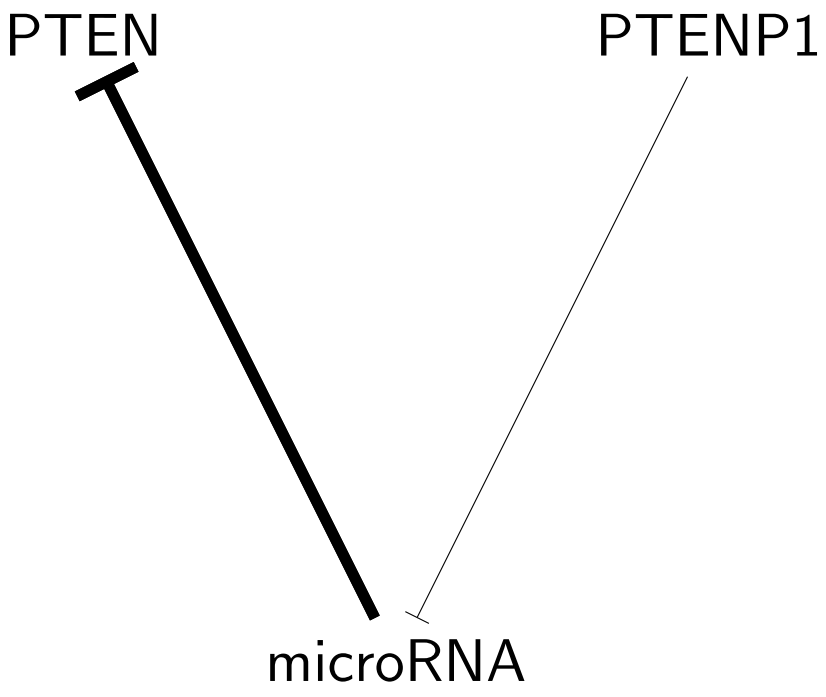
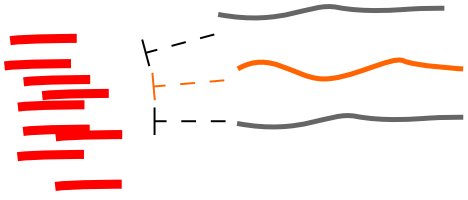


Susceptibility of microRNAs to competition is low in endogenous concentrations

microRNAs should not be in large excess:



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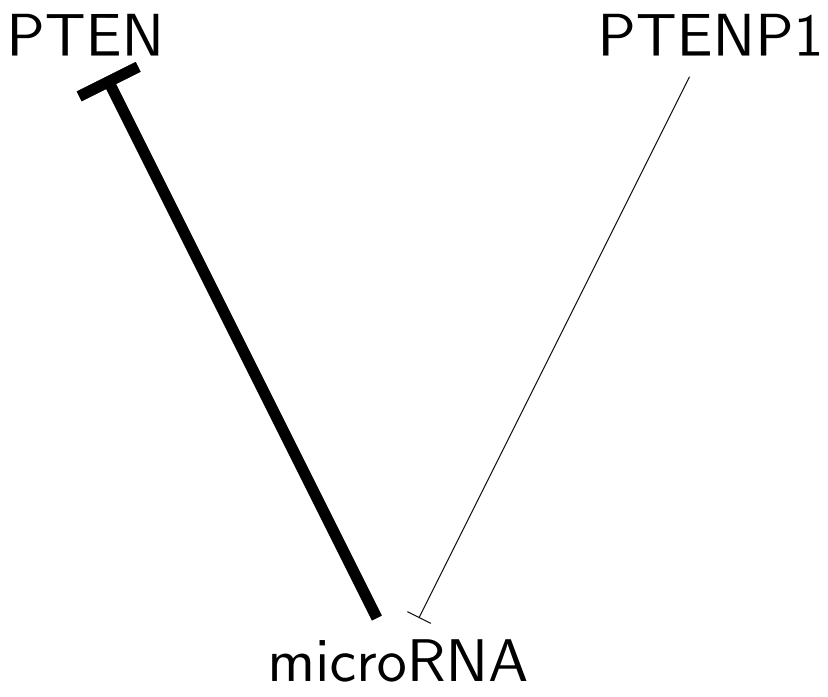
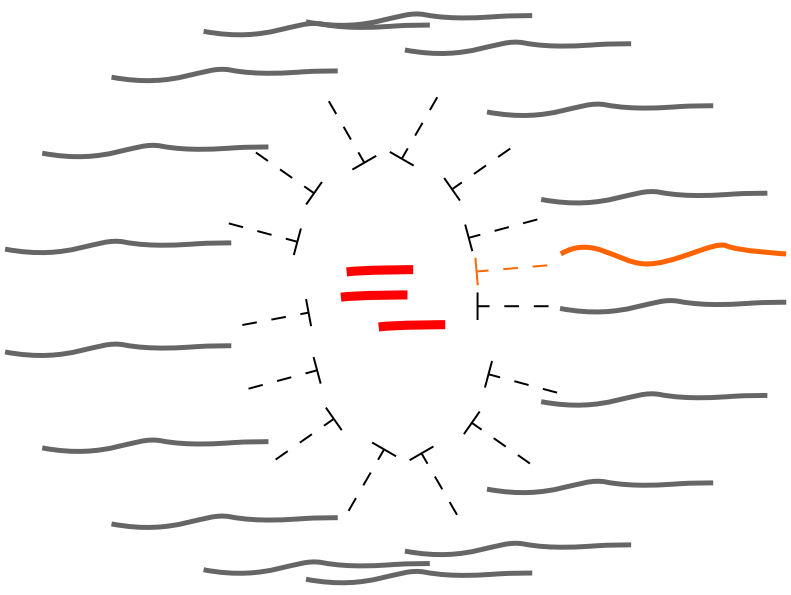


Susceptibility of microRNAs to competition is low in endogenous concentrations

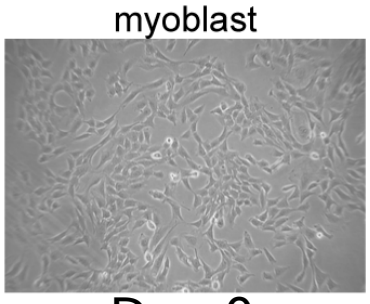
mRNAs should not be in large excess:



Endogenous mRNAs can titrate microRNAs?

"C2C12" cells:

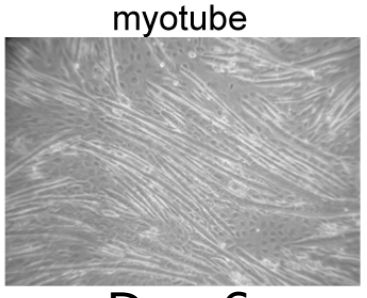
induction
of
differentiation



Day 0

miR-1/206

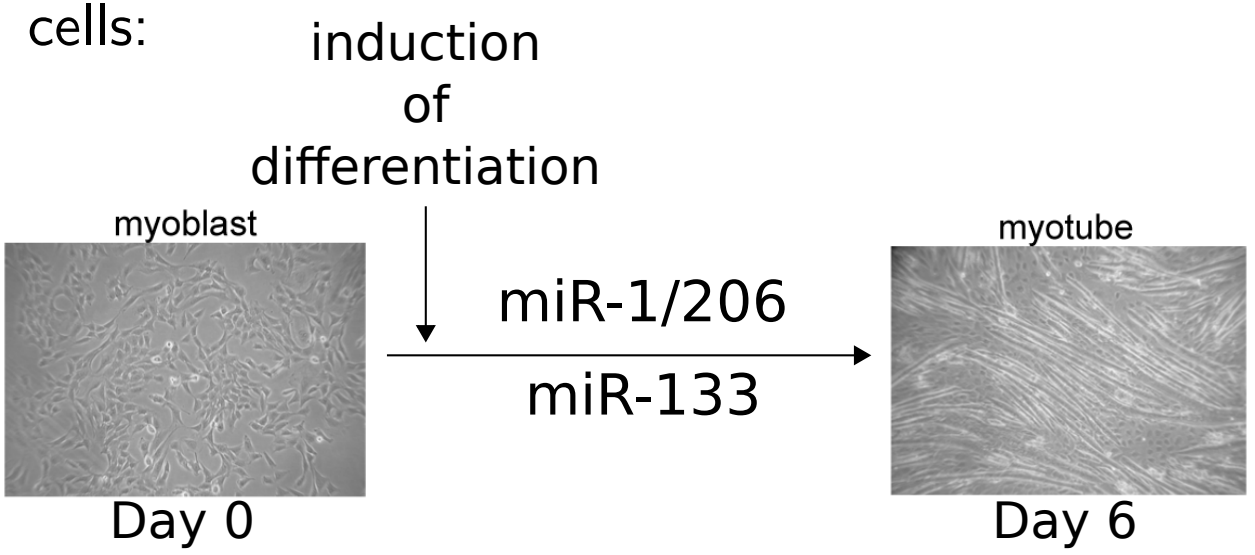
miR-133



Day 6

Endogenous mRNAs can titrate microRNAs?

"C2C12" cells:

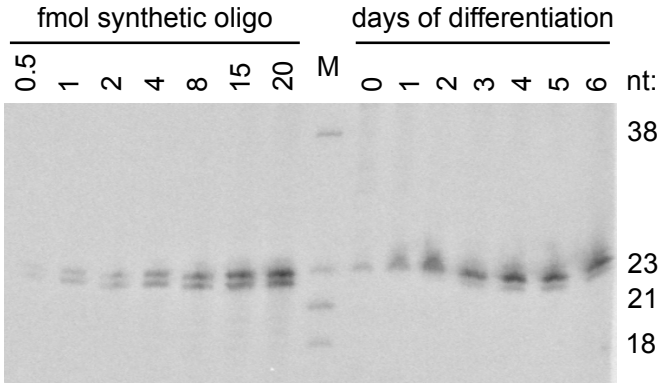


For individual titration to occur, concentrations must be in specific ranges.

- Goal: absolute quantification of intracellular concentration of miR-133, miR-1/miR-206 and their targets.

Absolute mRNA and microRNA quantification

microRNAs:



3 replicates

Absolute mRNA and microRNA quantification

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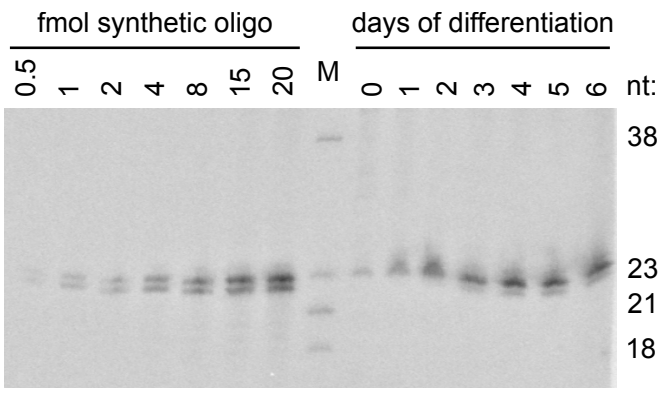

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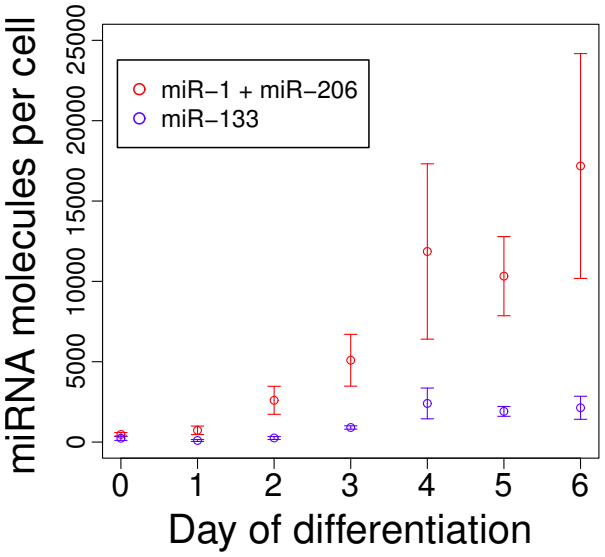

Absolute mRNA and microRNA quantification

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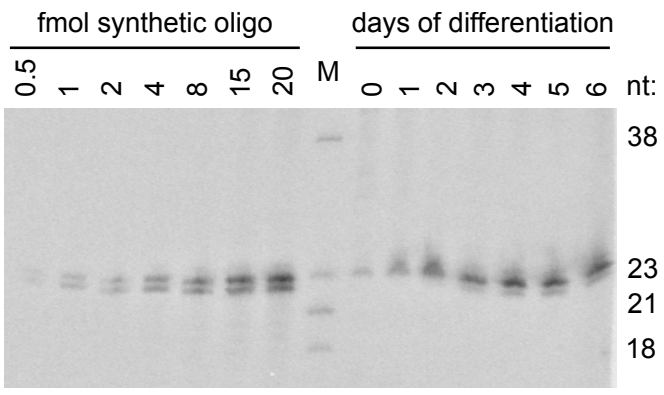

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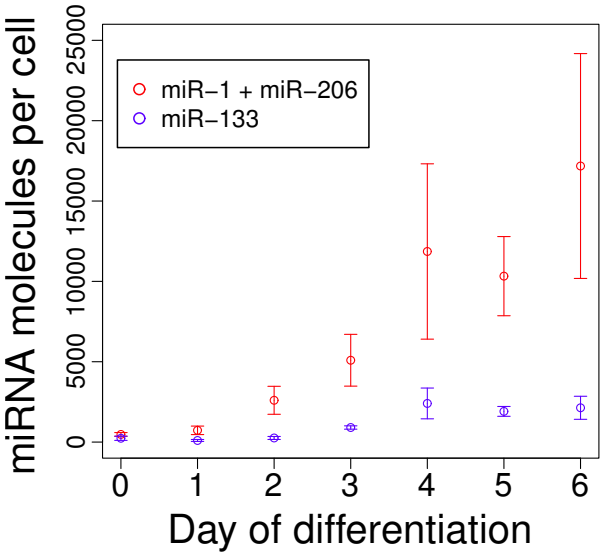
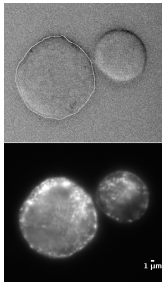


Cell volume



Absolute mRNA and microRNA quantification

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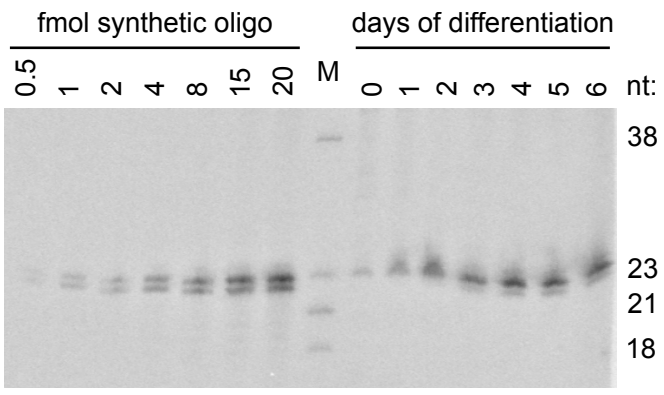

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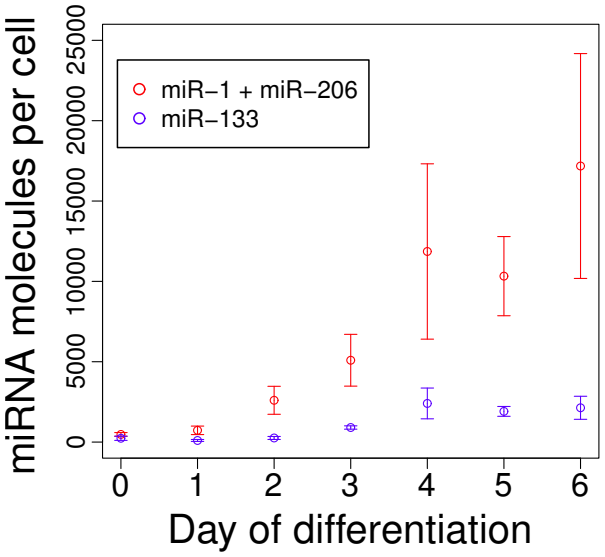

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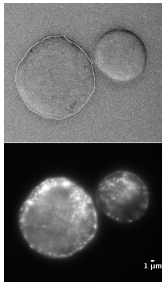
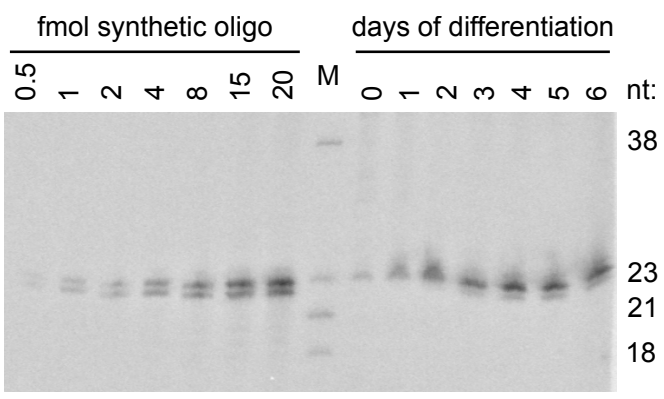


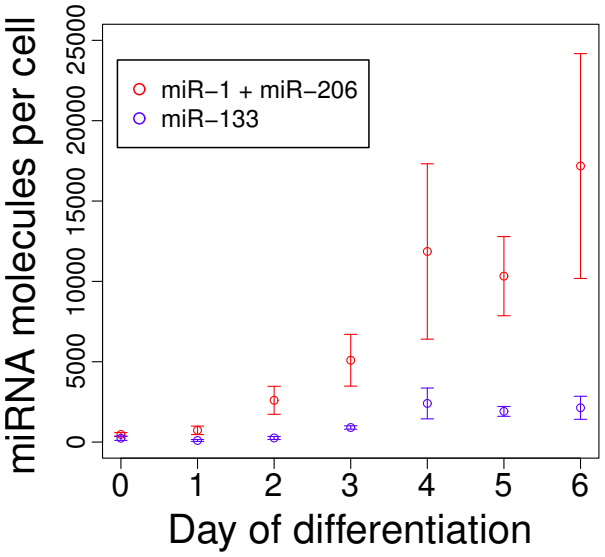
intracellular concentration

Absolute mRNA and microRNA quantification

microRNAs:



3 replicates



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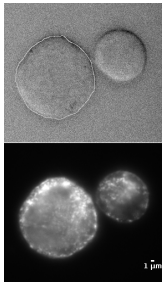


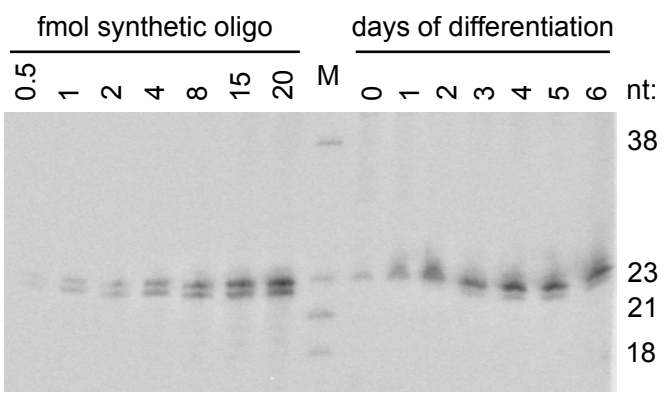
intracellular concentration

spiked-in RNAseq

at day 0,
day 3,
and day 6
(3 replicates)

Absolute mRNA and microRNA quantification

microRNAs:



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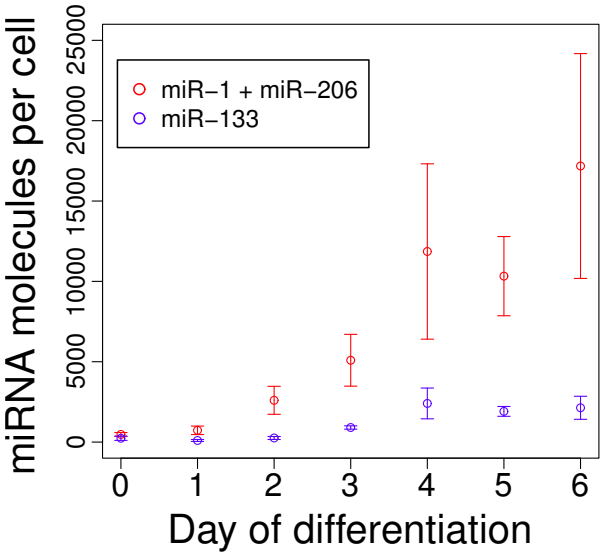

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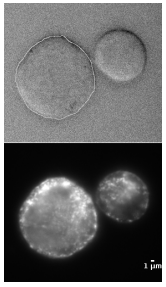

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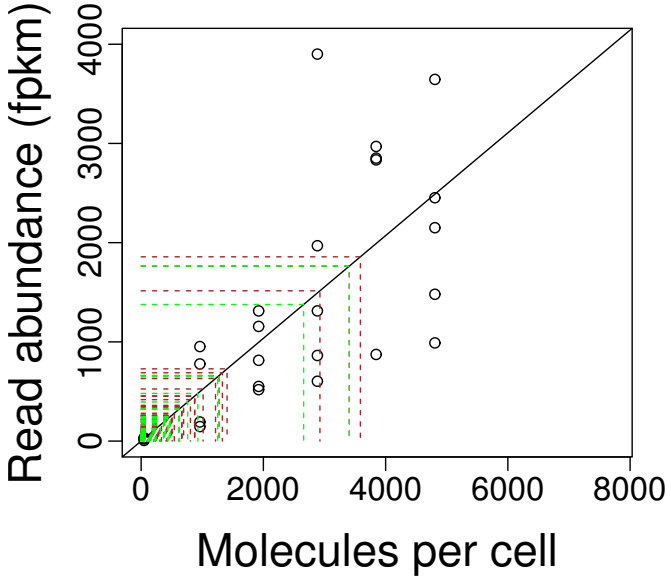
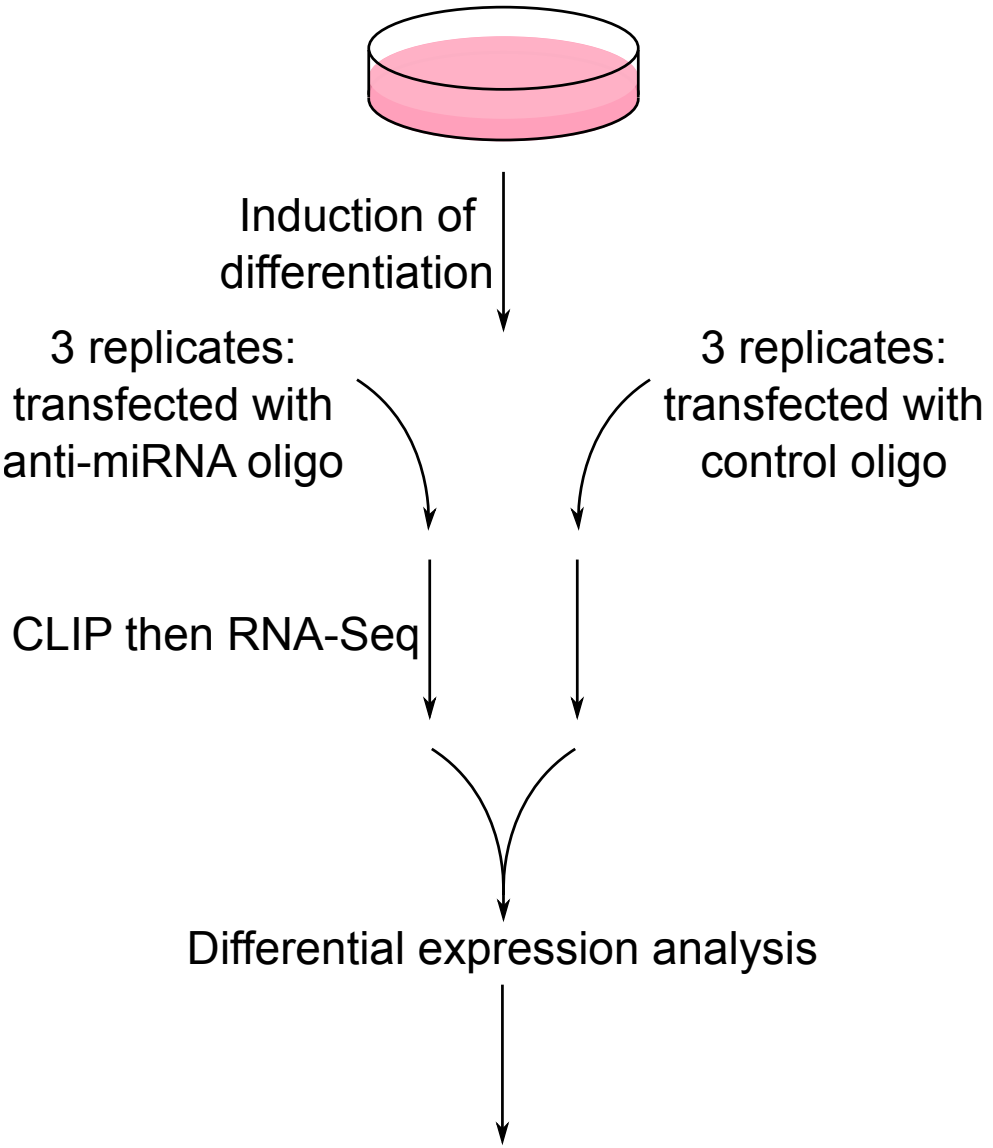


Experimental identification of targets: differential CLIP

- Predictions exclude non canonical binding that may contribute to general titration.
- Predictions may bear false positives.
- Some sites may not be really bound (sites not accessible).

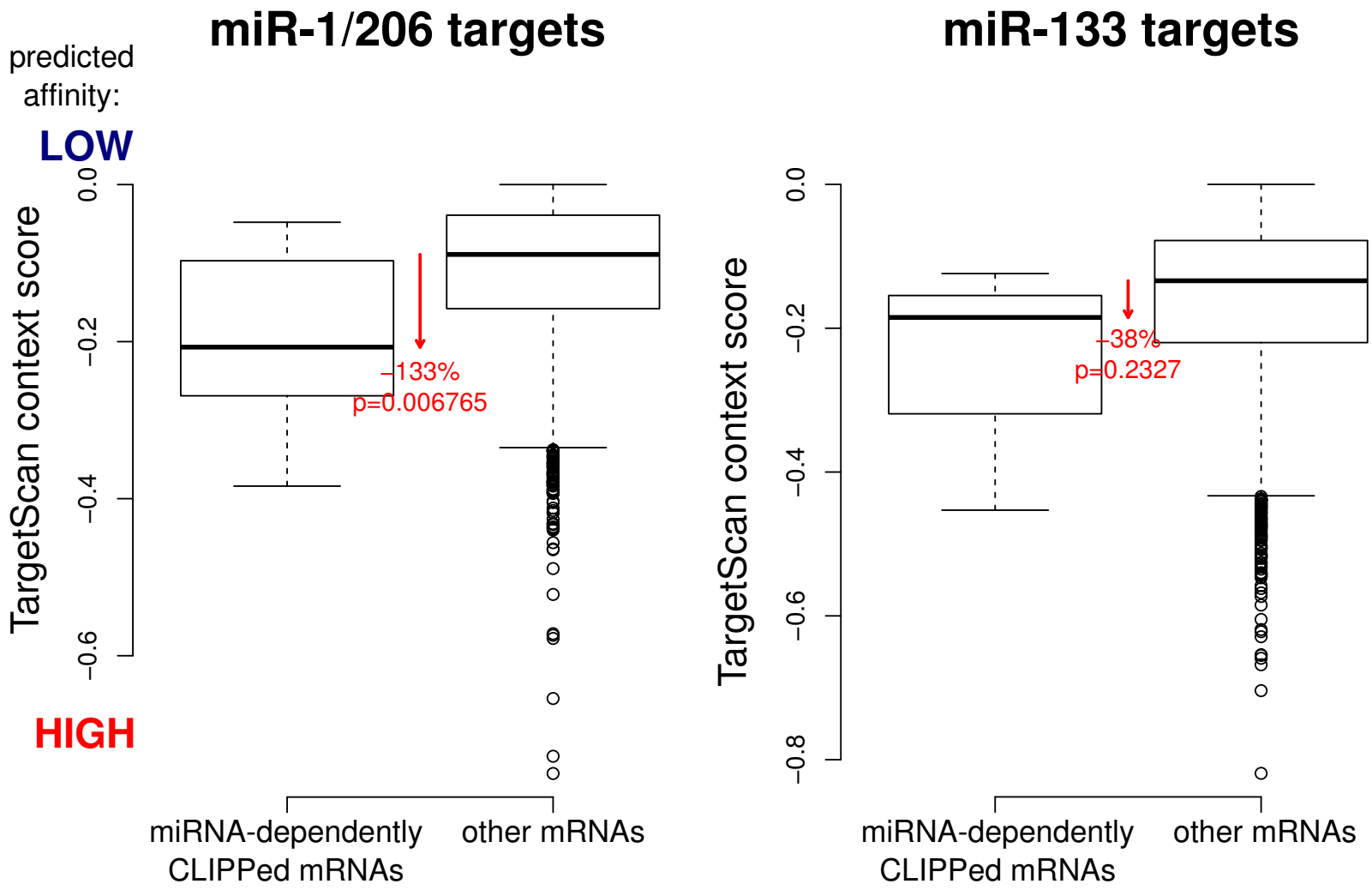
Experimental identification of targets: differential CLIP



mRNAs depleted from the CLIP after transfection with anti-miRNAs:

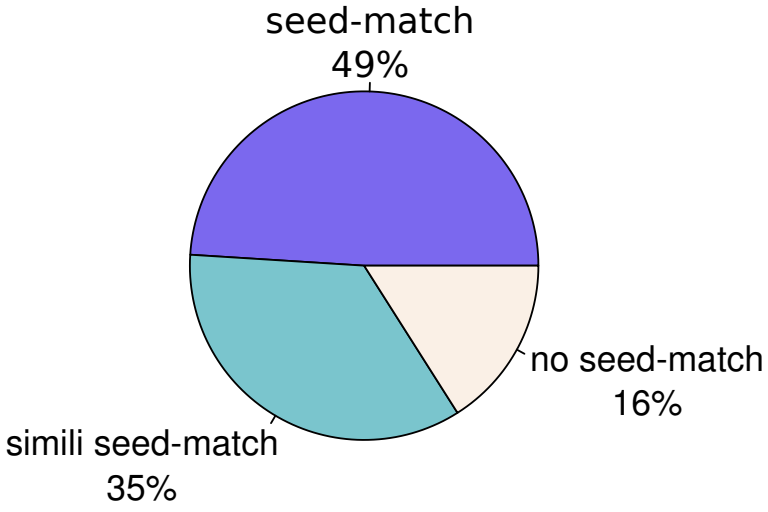
- Excludes background binding
- Excludes microRNA-independent binding
- Identification of specific microRNA targets

Clipped targets have high predicted affinity



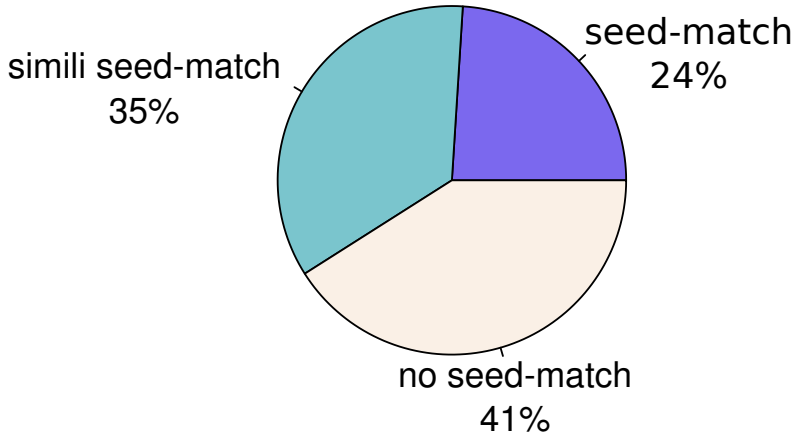
Clipped targets are enriched in seed and simili-seed matches

miR-1/206 targets



n=37

miR-133 targets

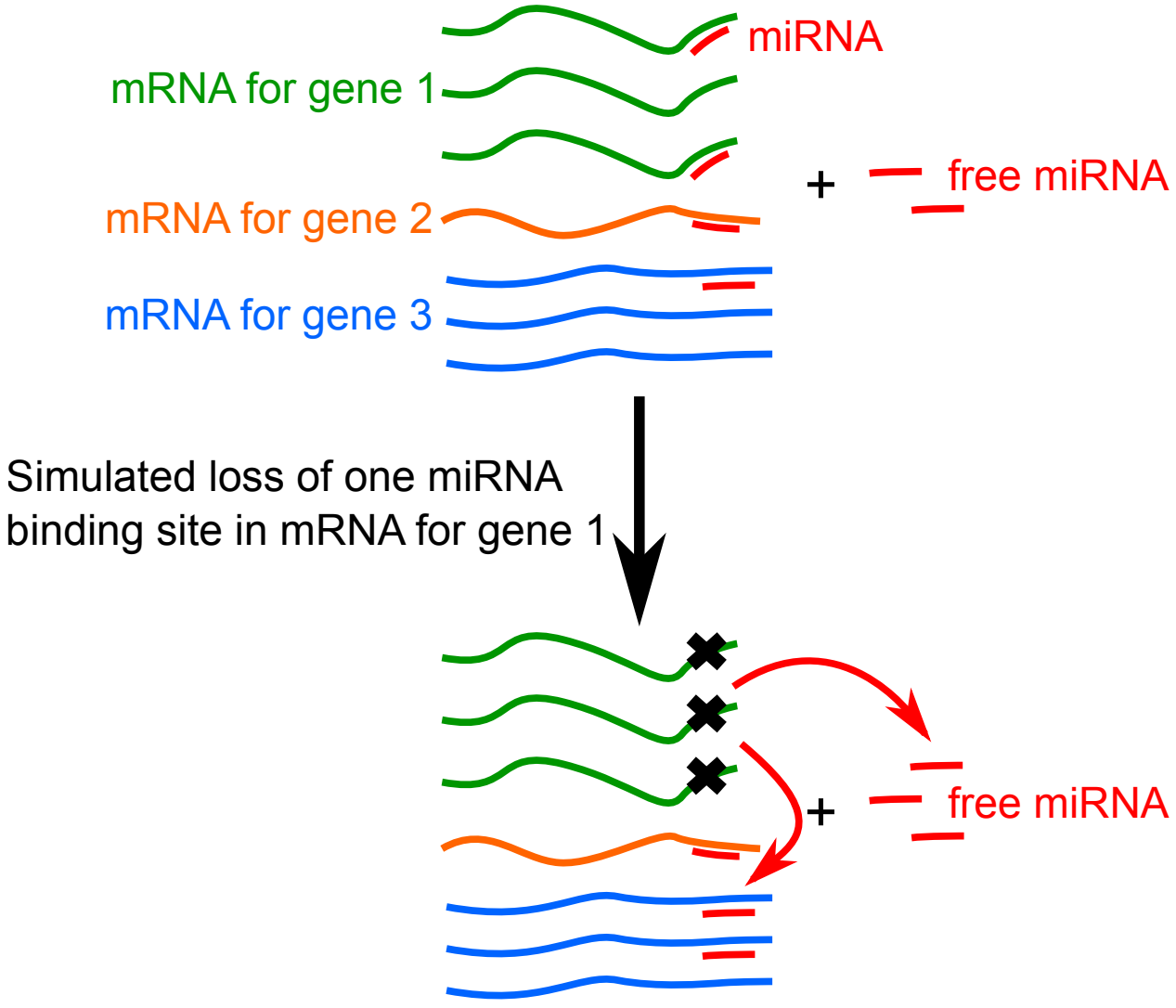


n=17

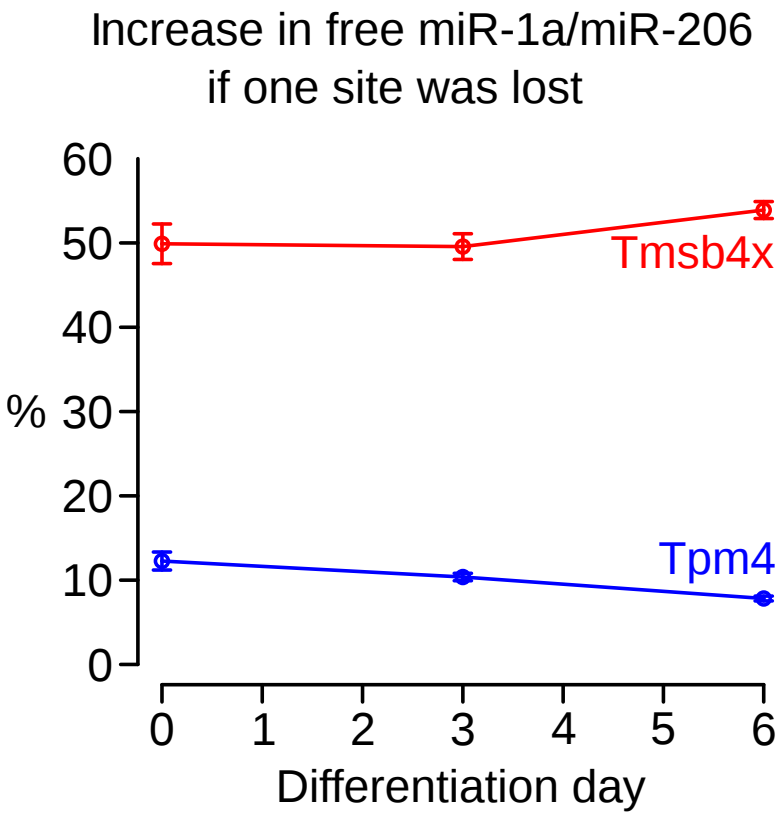
simili seed-match: top 3 most abundant single mismatch seed-matches.

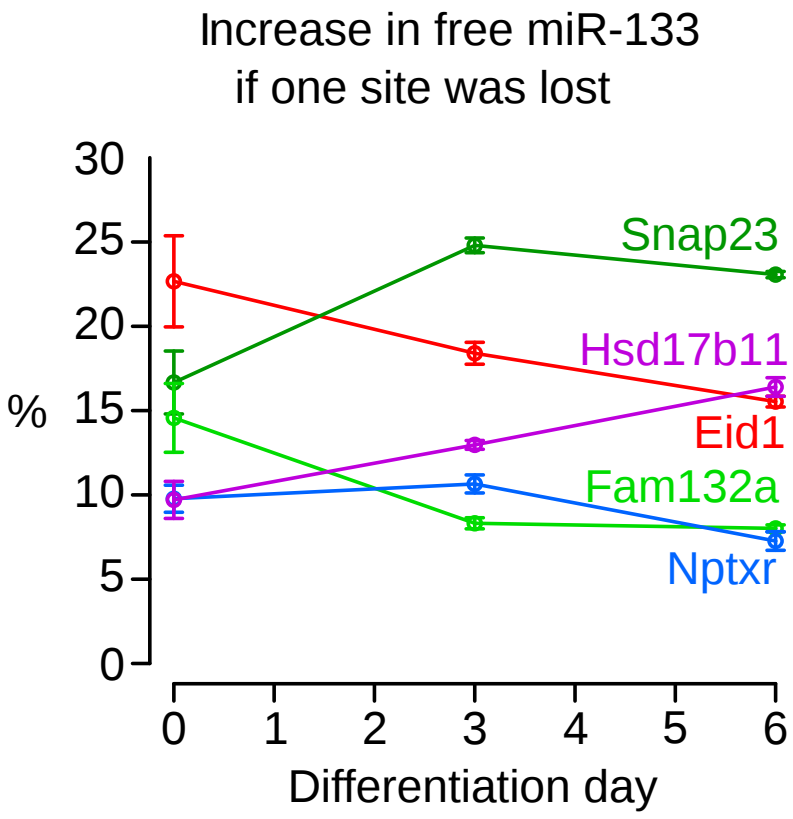
Titration capacity of each individual binding site:

Incorporation of concentration data:



Strongest titrators





Titration occurs

- Some mRNAs can efficiently titrate miRNAs in endogenous concentrations.
- This could be another function for conserved miRNA binding sites.
- Each cell type may express a variable set of titrators, resulting in a set of conserved binding sites with miRNA titrating activity.

Acknowledgements

Hervé Seitz
Blaise Ii
Laura Martinez