

Experimental Strategies

- Perturb a microRNA in system of interest
- Measure transcript expression as a readout
- Transcripts whose expression changes may be direct targets of miRNA
- Computational analysis of genelist for confirmation



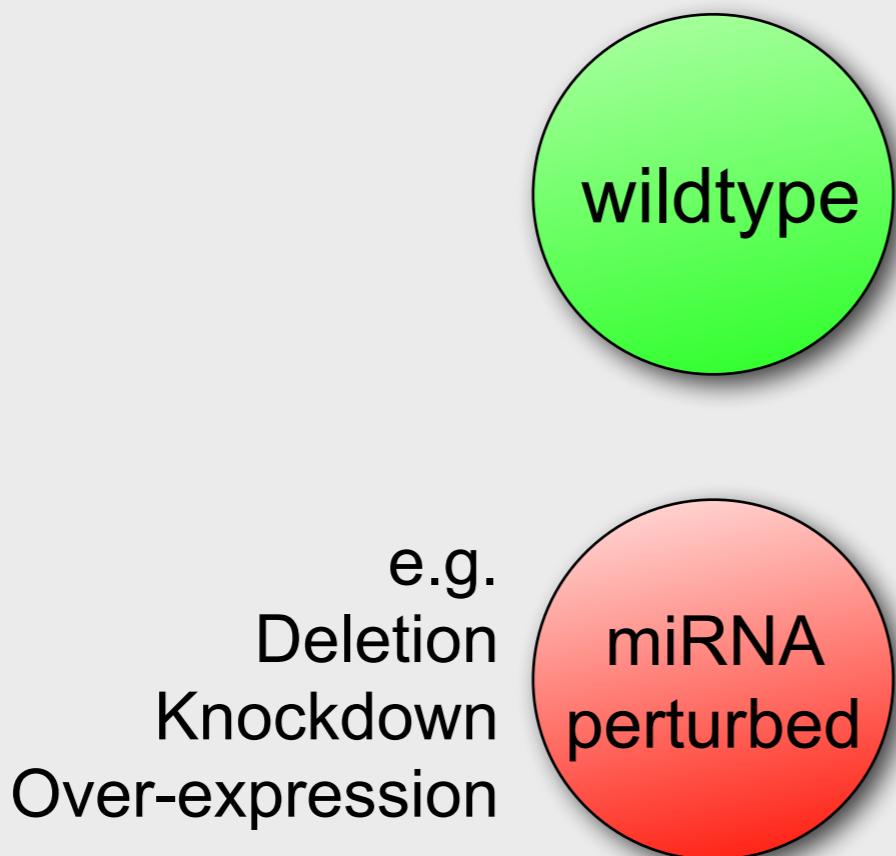
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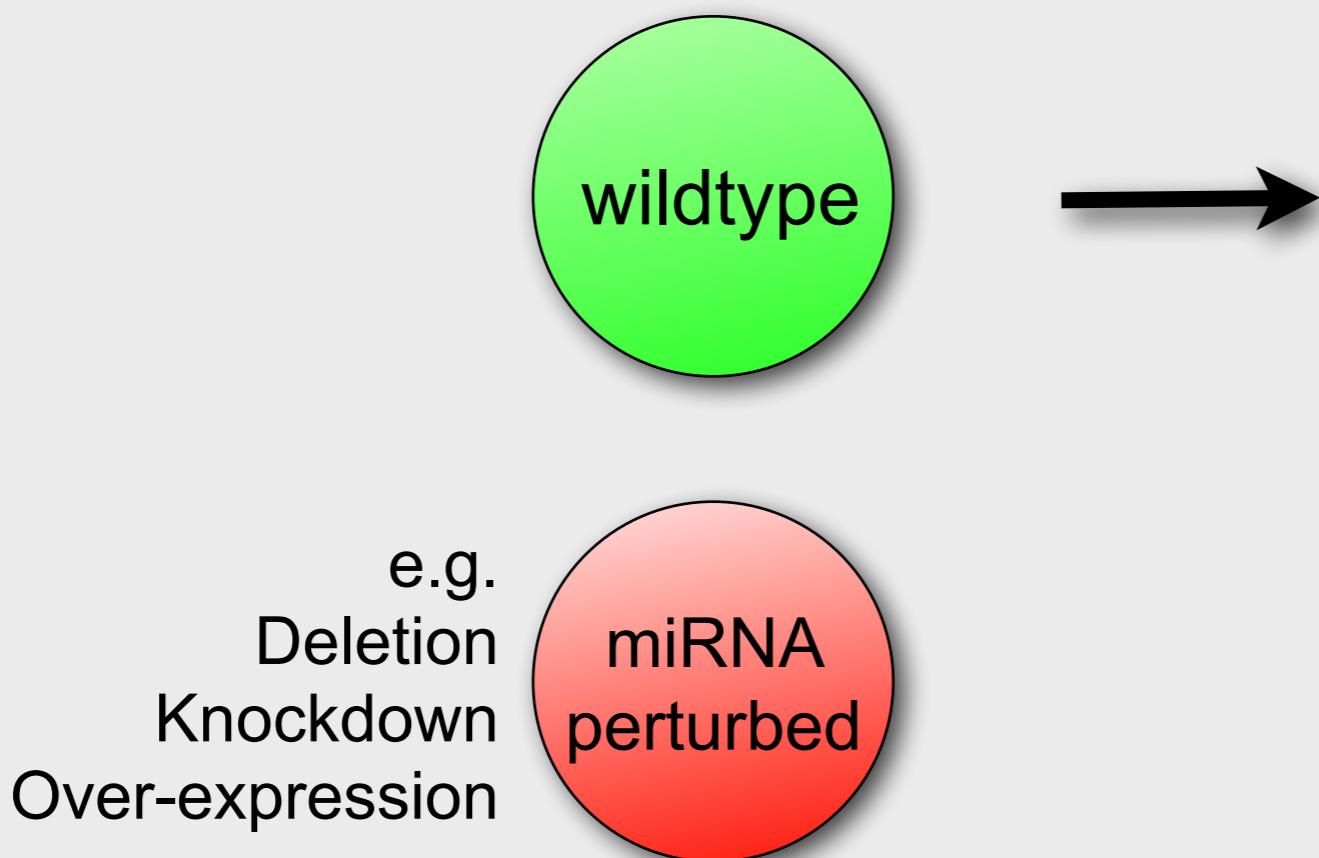
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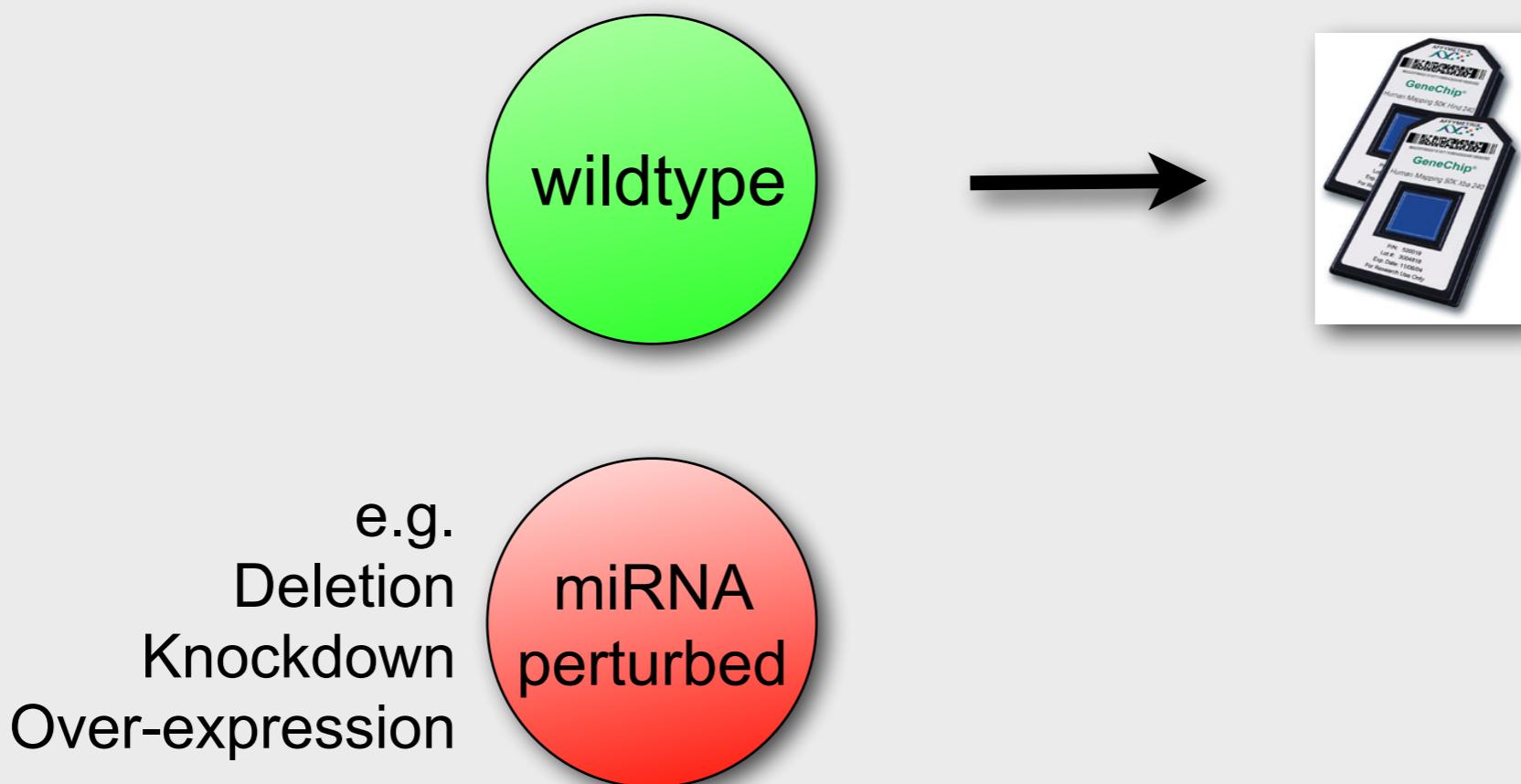
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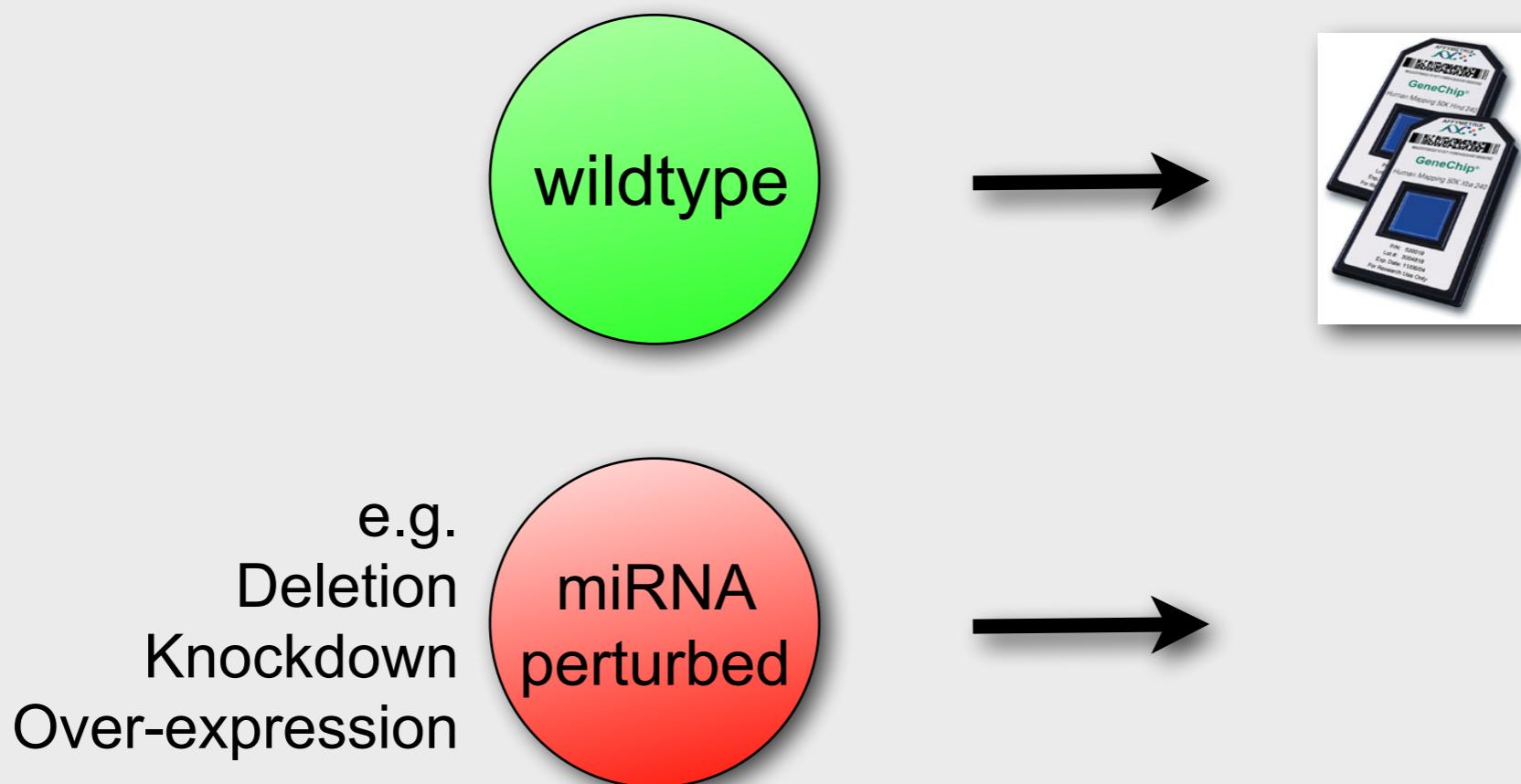
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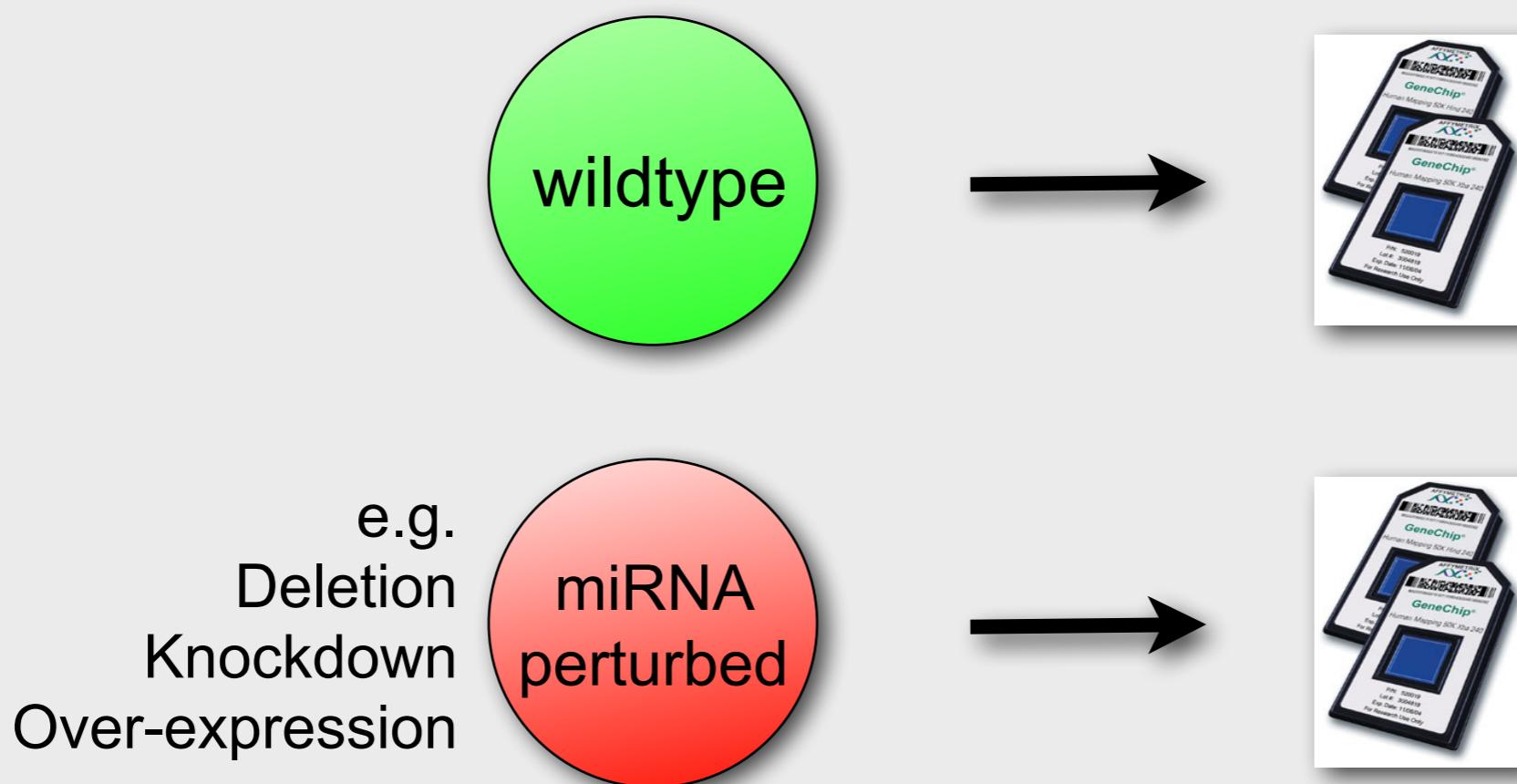
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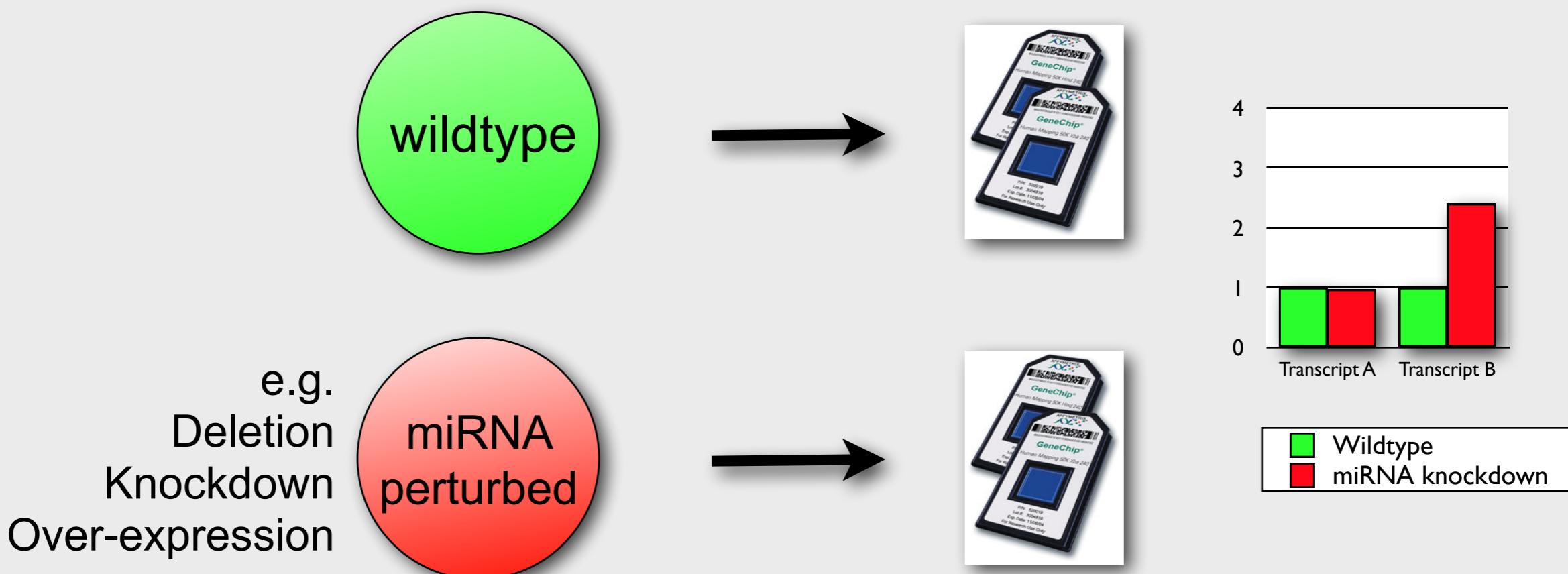
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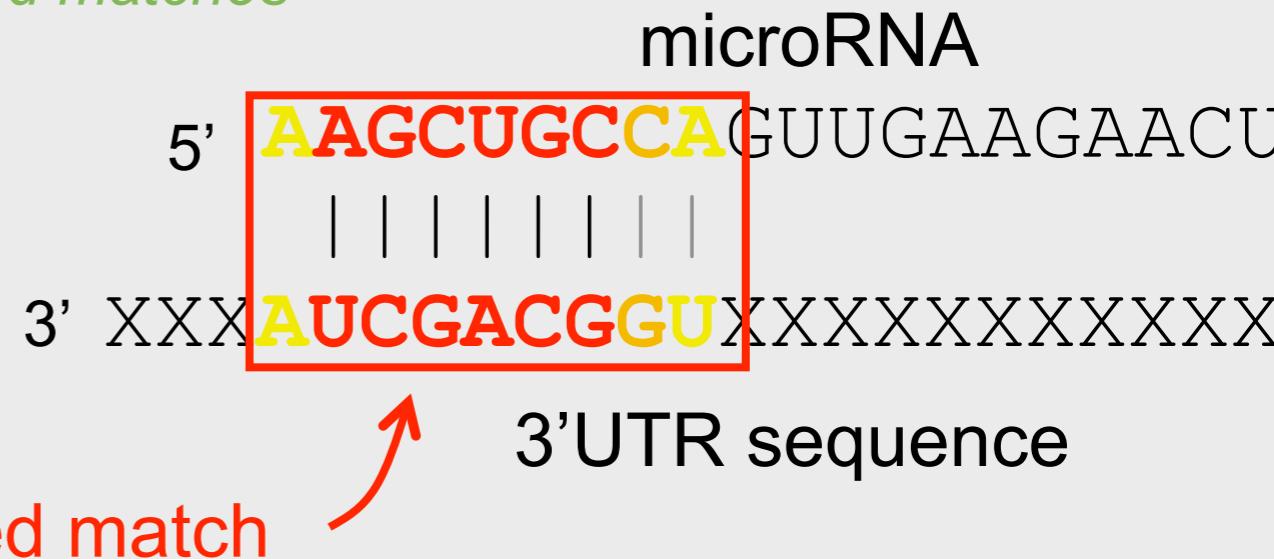
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Are expression changes direct miRNA target regulation ?

If so, then they should be enriched in miRNA seed matches

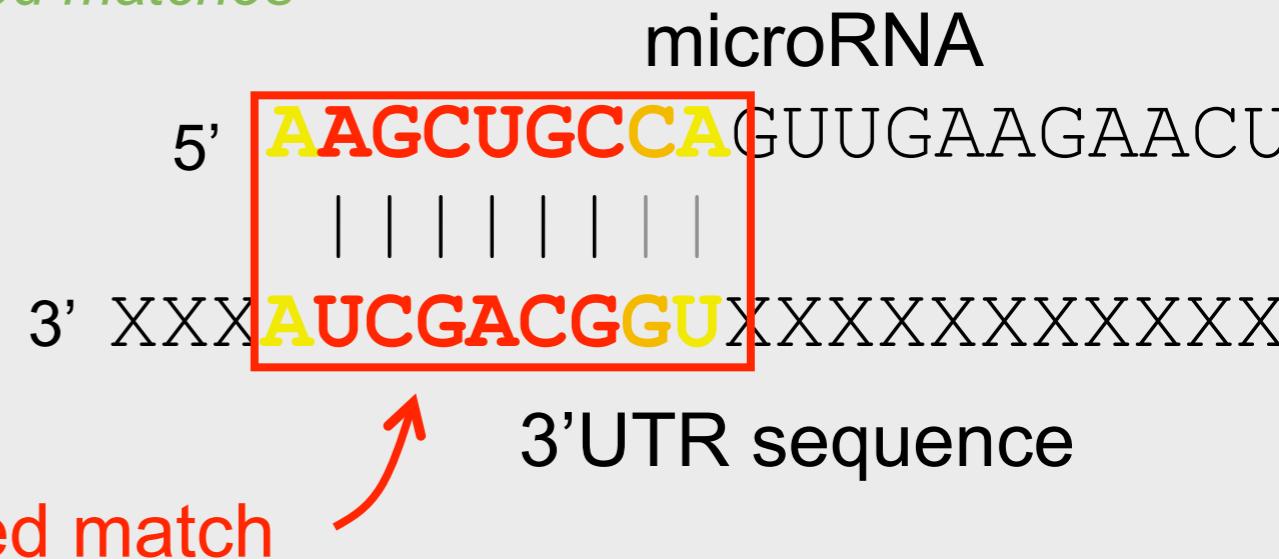
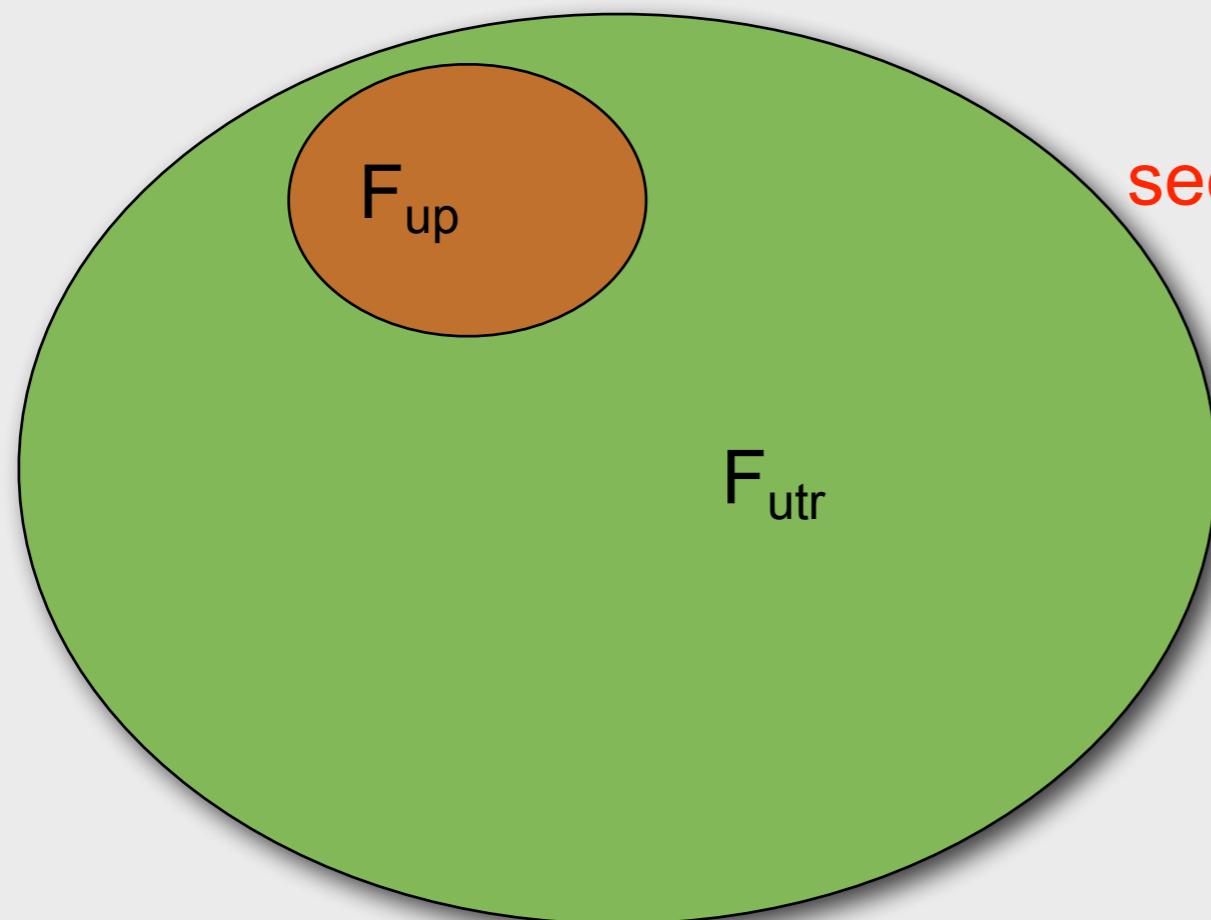


F = Frequency of 6mer in 3' UTRs
eg: 'UCGACG'



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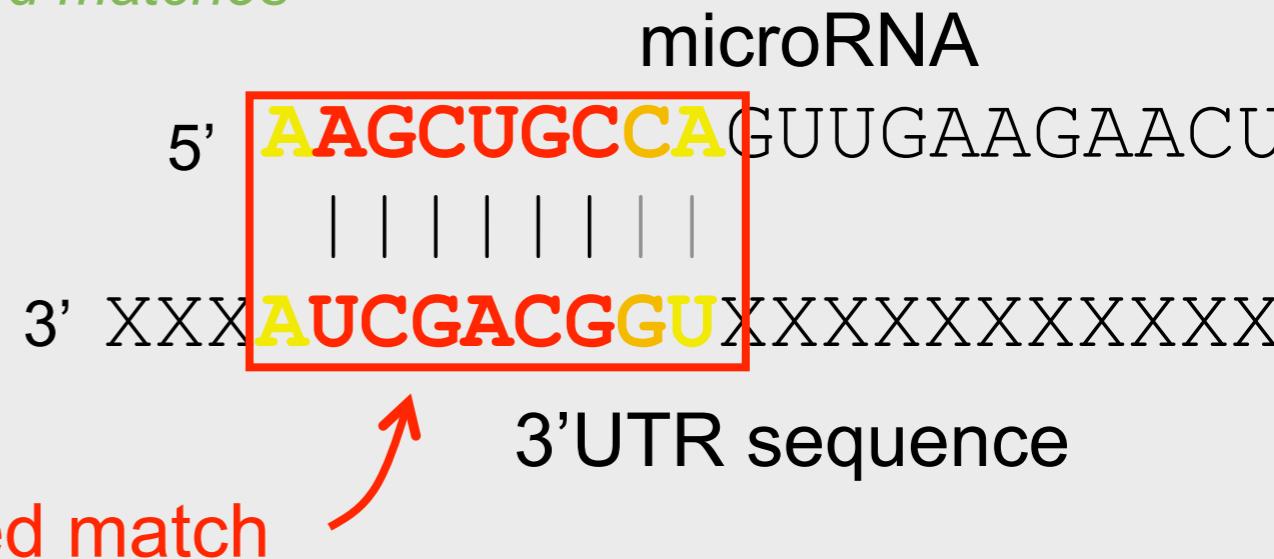
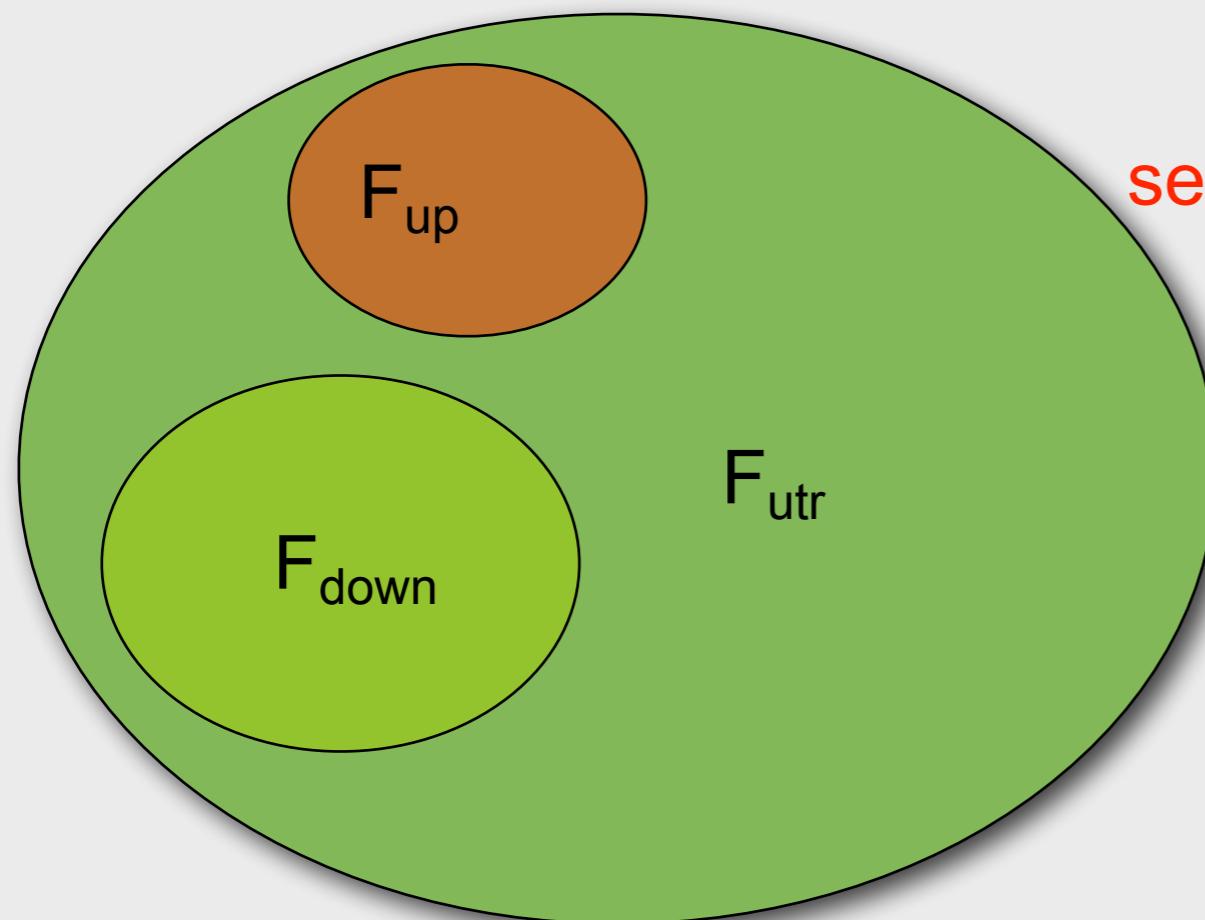


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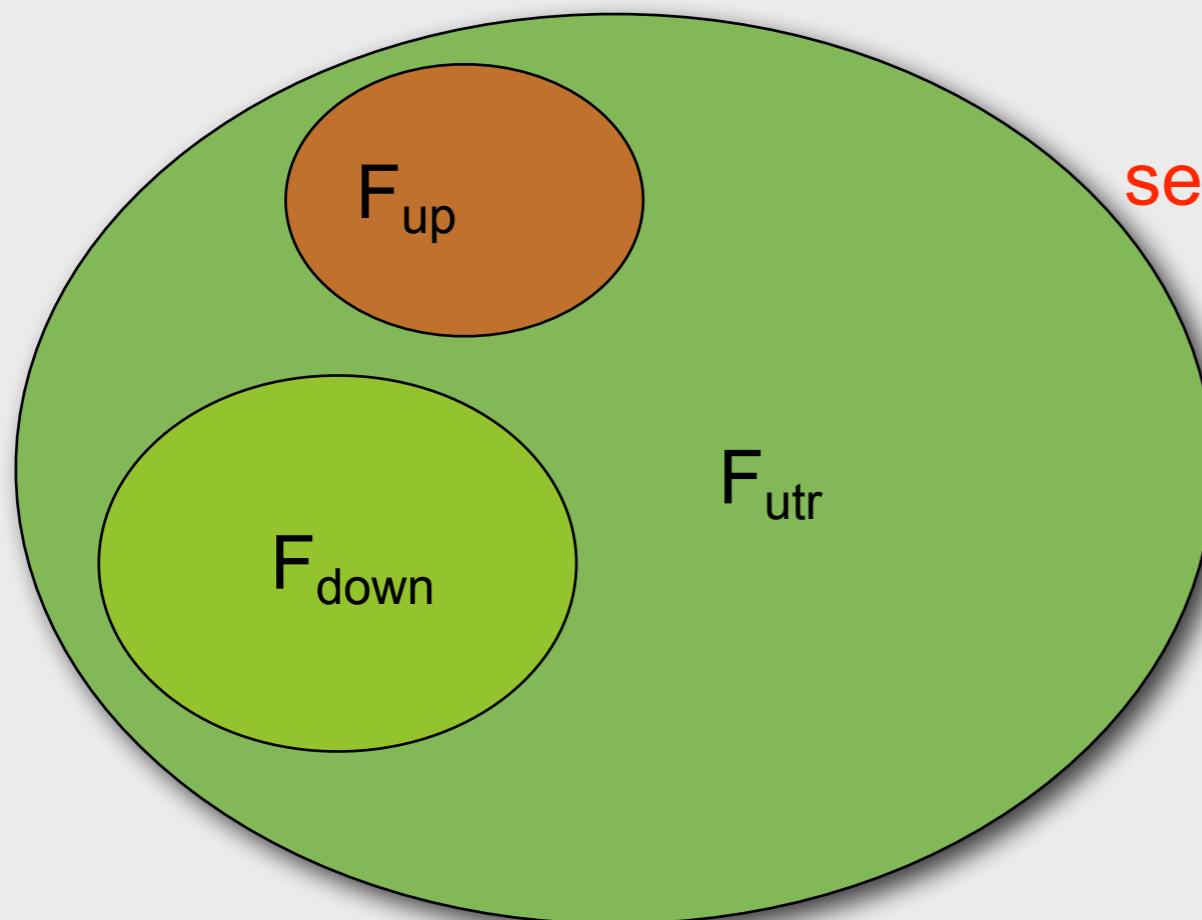


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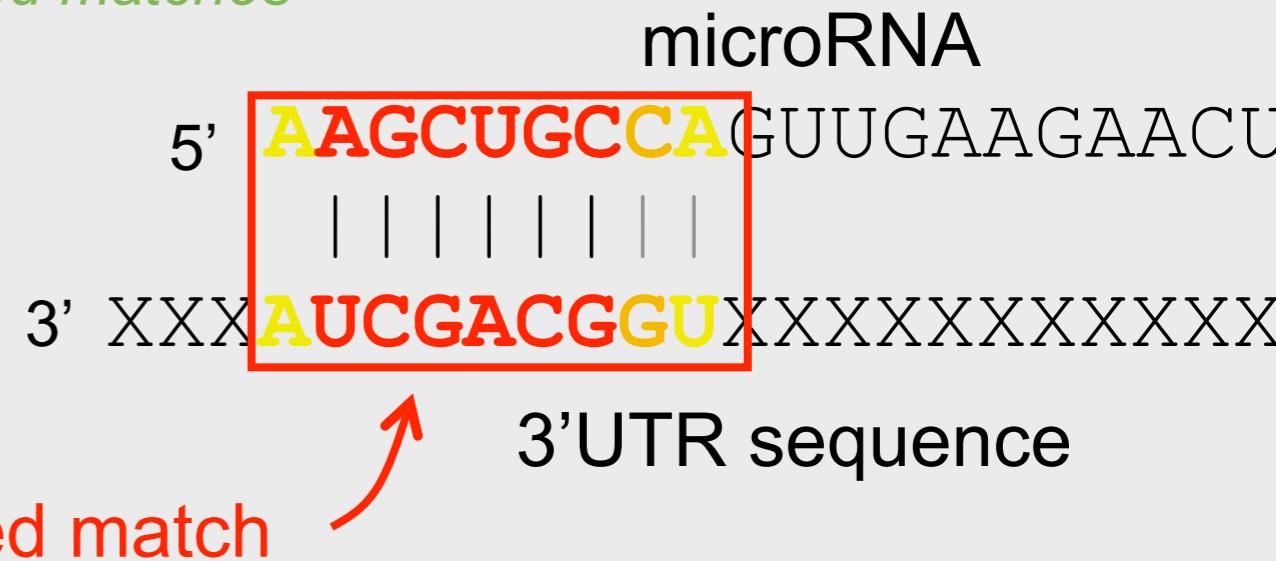


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miRNA Addition

Enrichment = F_{down} / F_{utr}

Controls

Control 6mers
Random Trials

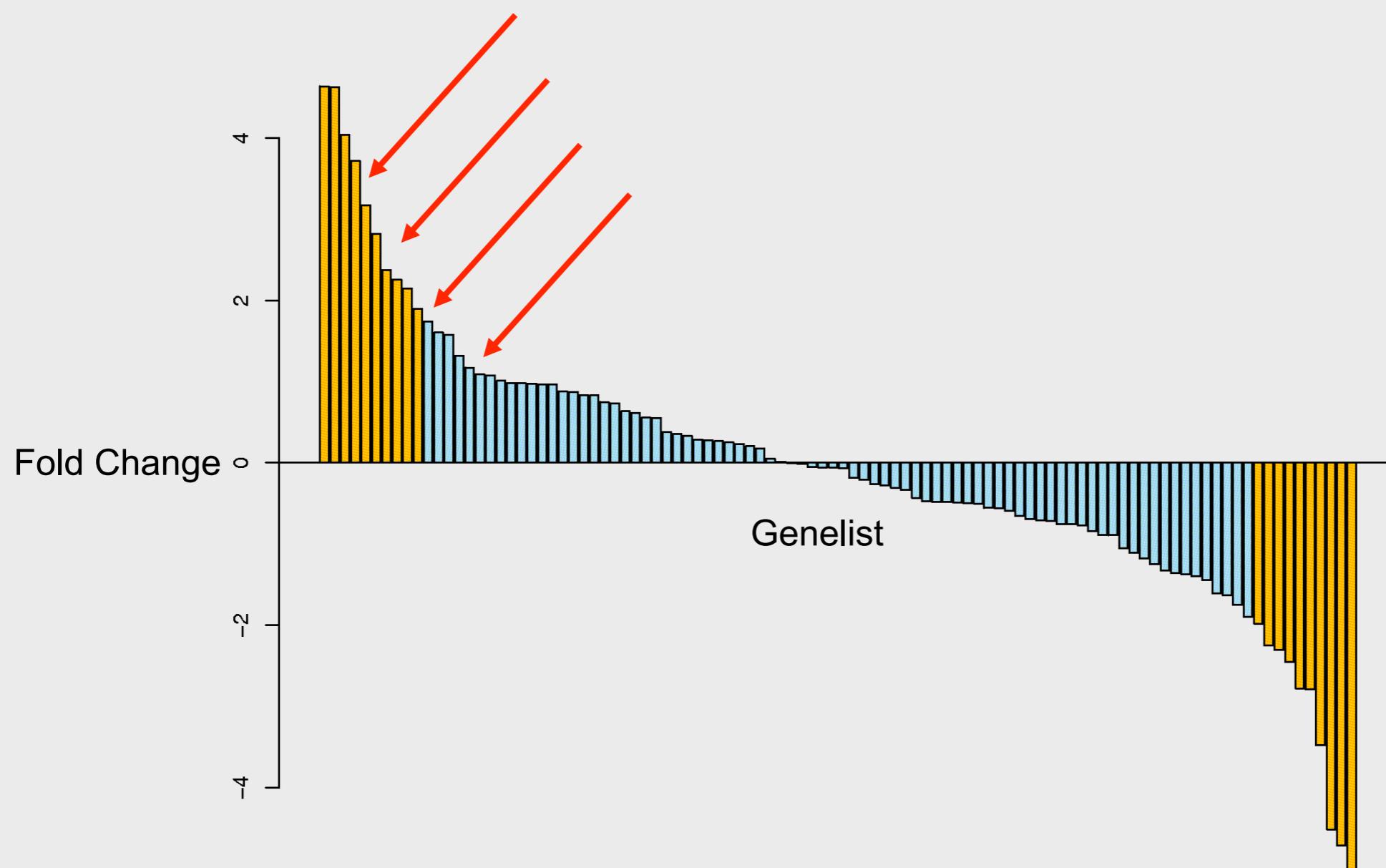
Significance Testing

Hypergeometric P-Value

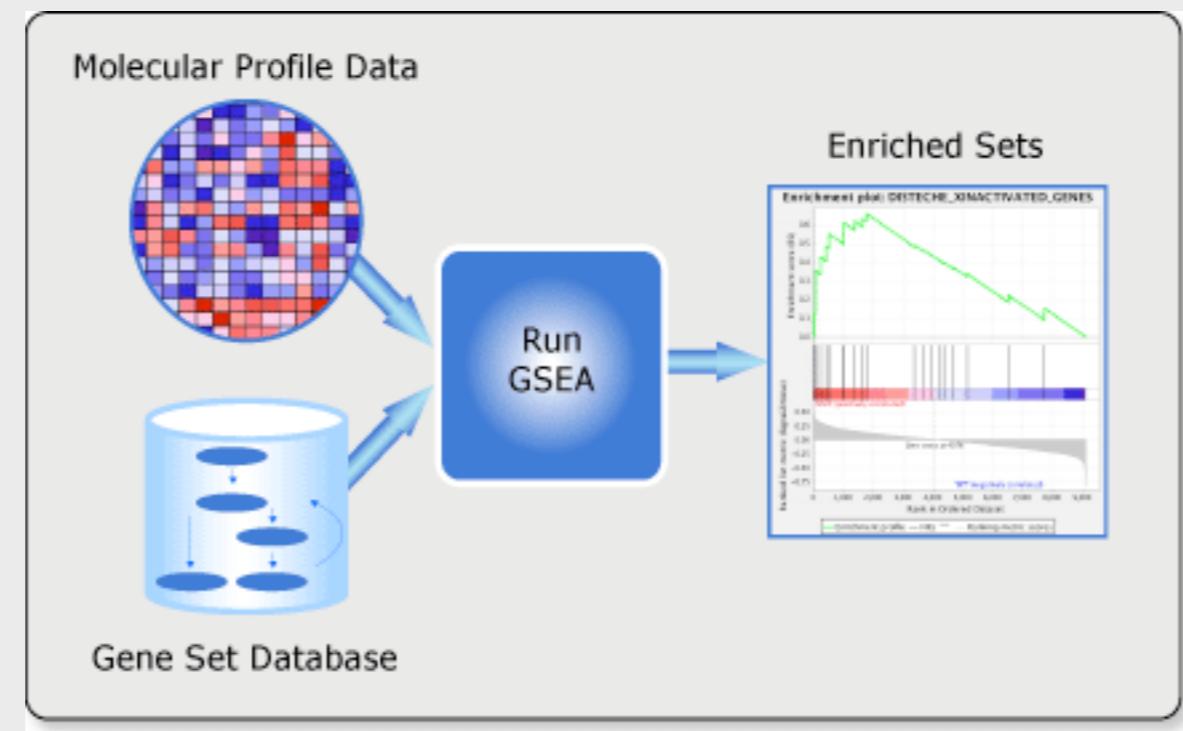


What “cut-off” should one choose?

how do we know where to look for enrichment ?



Enrichment of annotations from genelists of expression experiments

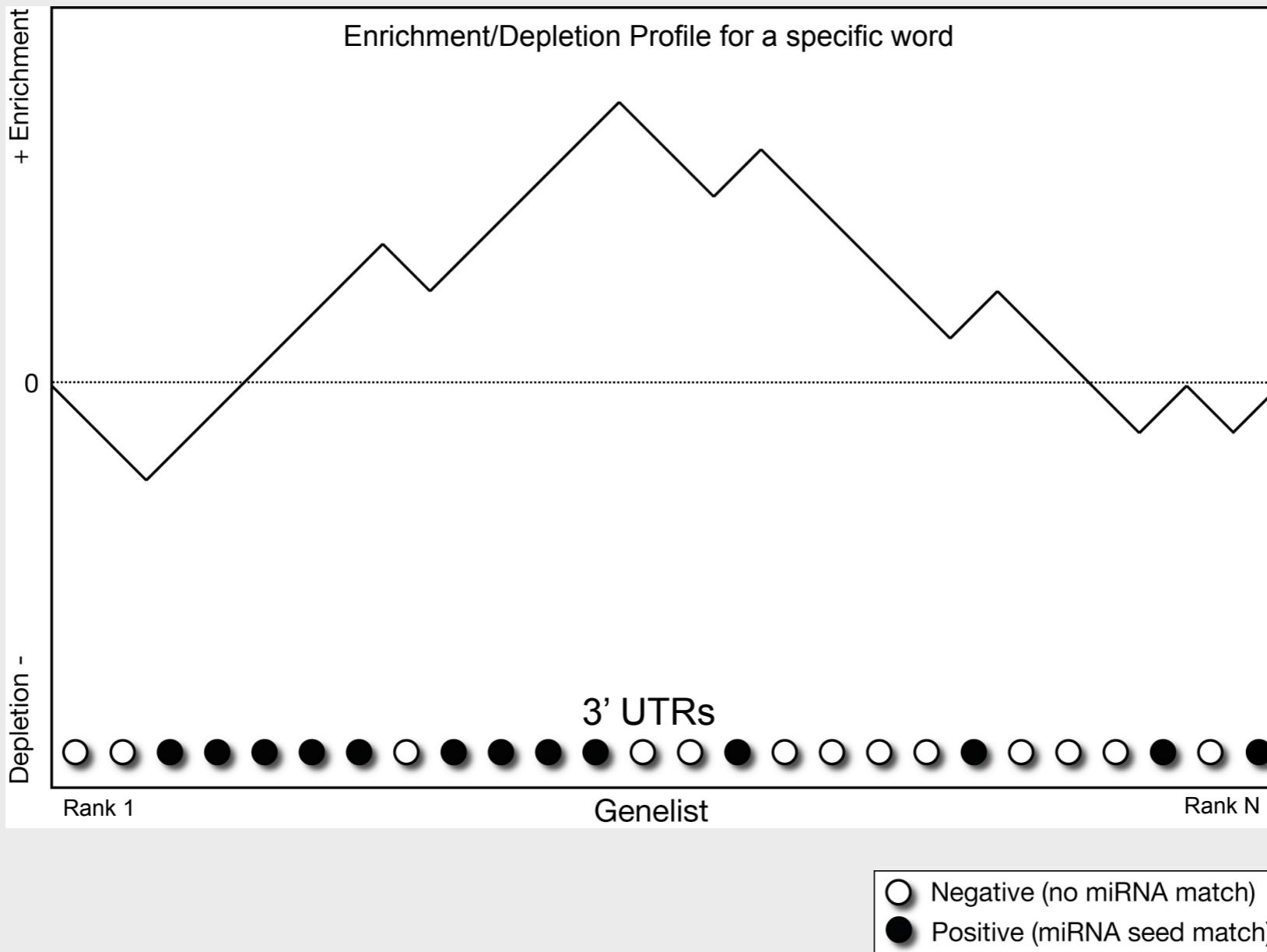


Can we do this with sequence motifs instead of annotations ?

Mootha et al (2003) Nat Genet 34, 267-273
<http://www.broad.mit.edu/gsea/>



microRNA Gene Set Enrichment Analysis



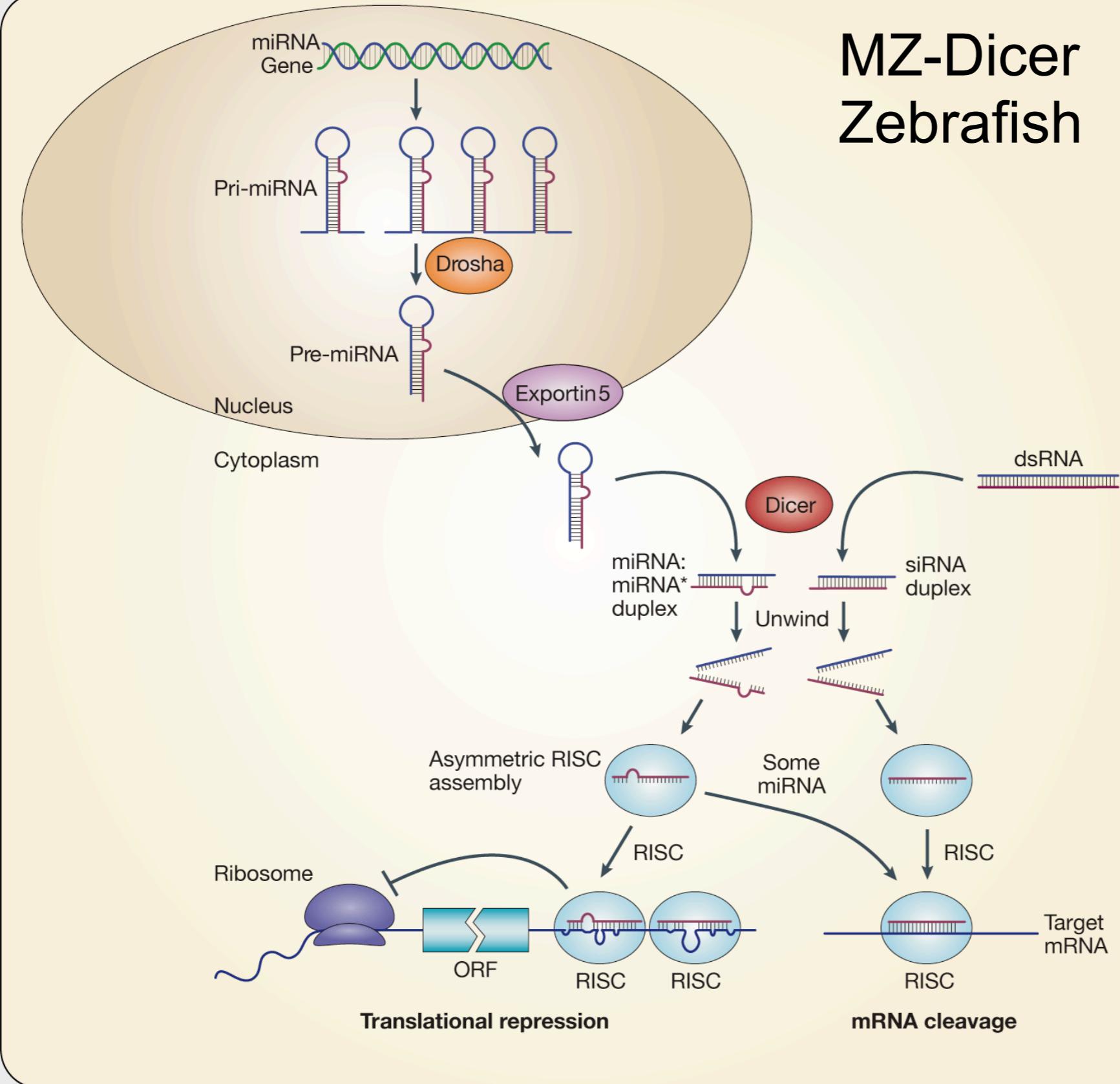
Sylamer: a tool for motif enrichment in genelists

- Requires:
 - Ordered Genelist
 - Associated 3'UTR sequences
- Fast C Implementation
 - Sequence hashing
 - GSL library for hypergeometric tests
 - 16000 motifs across 30000 genes in less than 60 seconds
- Java GUI Available & Web-based Service
- Open Source (GPL)
 - <http://www.ebi.ac.uk/enright/sylamer>

Fast assessment of miRNA binding and siRNA off-targets from Expression Data
van Dongen S., Abreu-Goodger C., Enright AJ. *Nature Methods* 2008



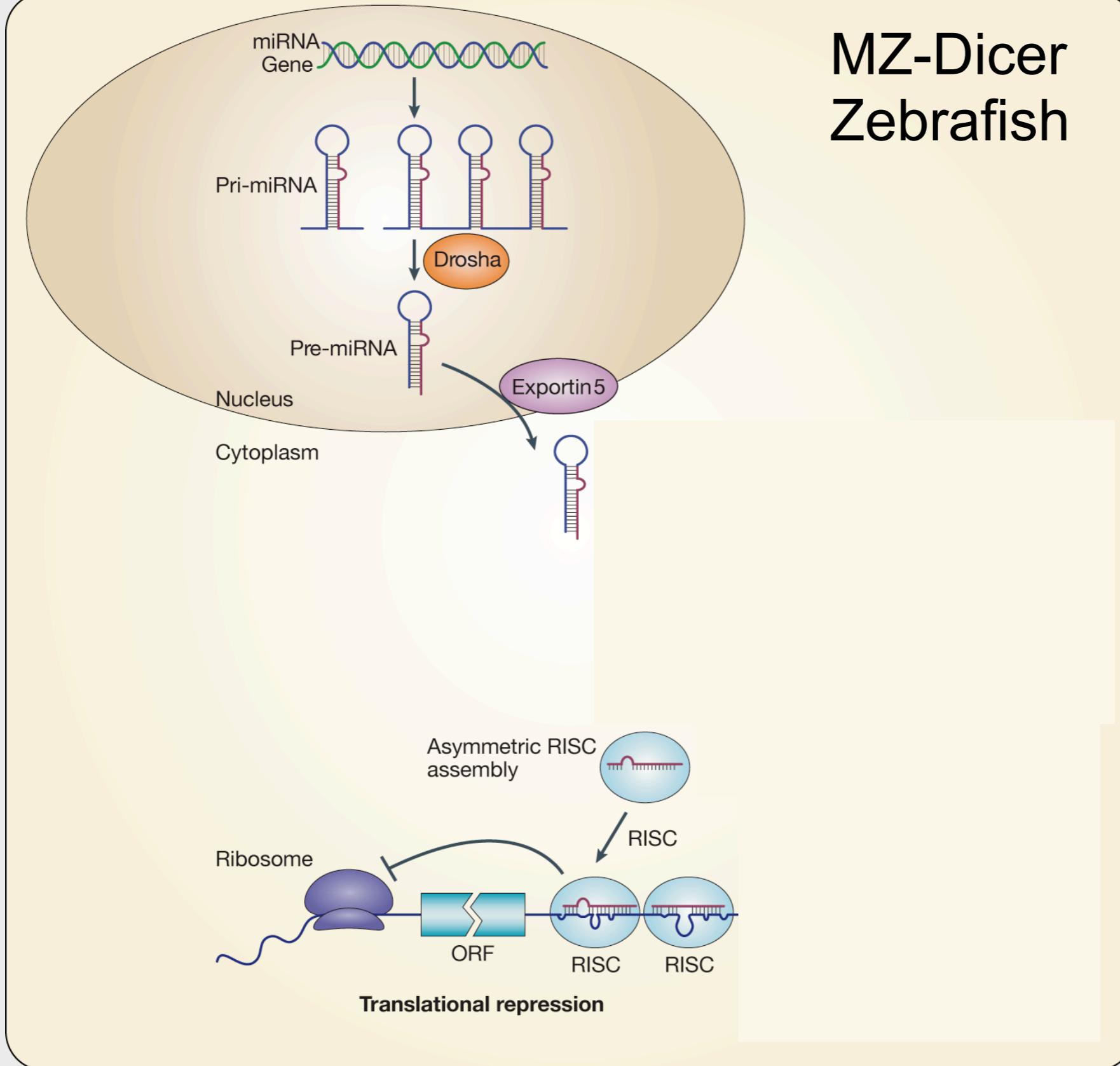
MZ-Dicer Zebrafish



MicroRNAs Regulate Brain Morphogenesis in Zebrafish.
Giraldez AJ, et al Science. (2005)

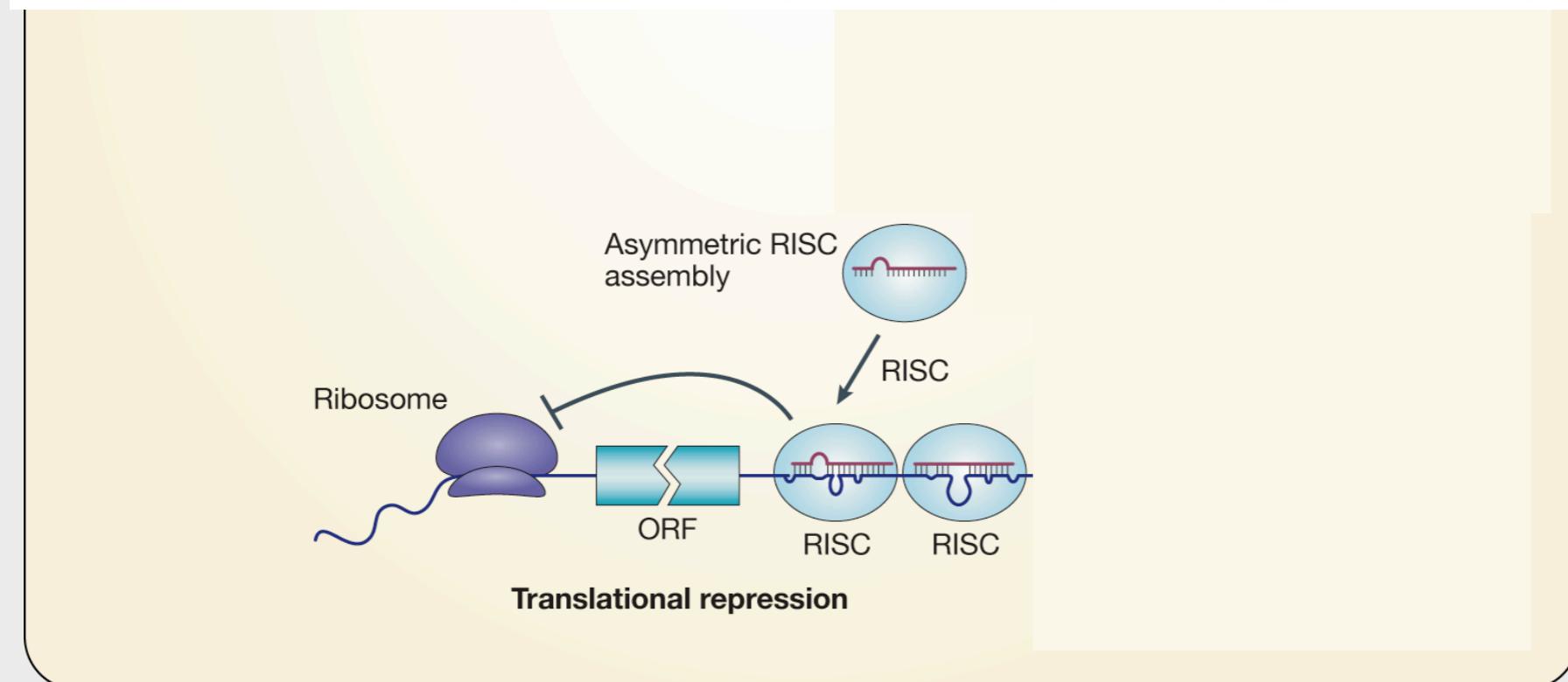
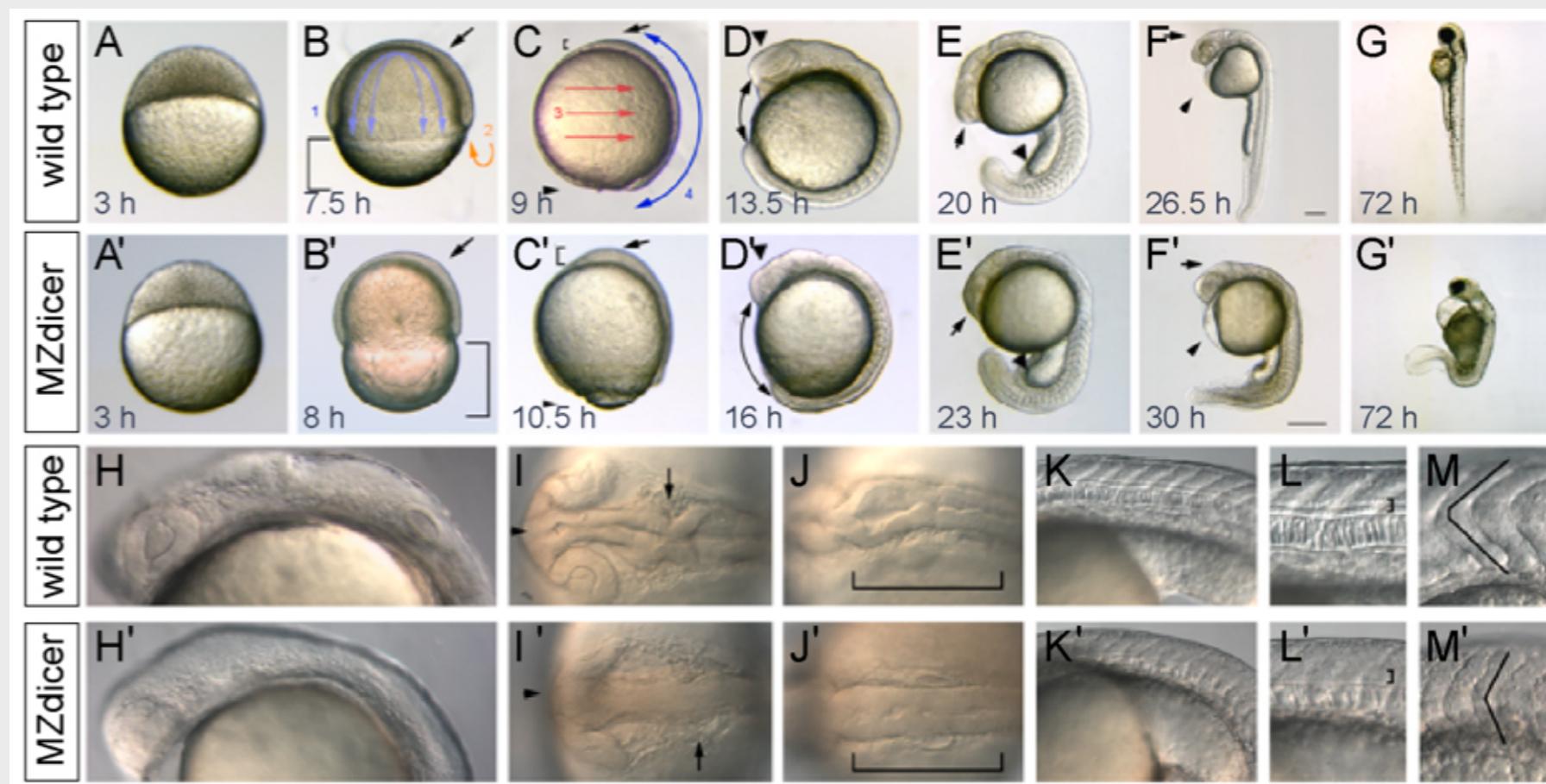


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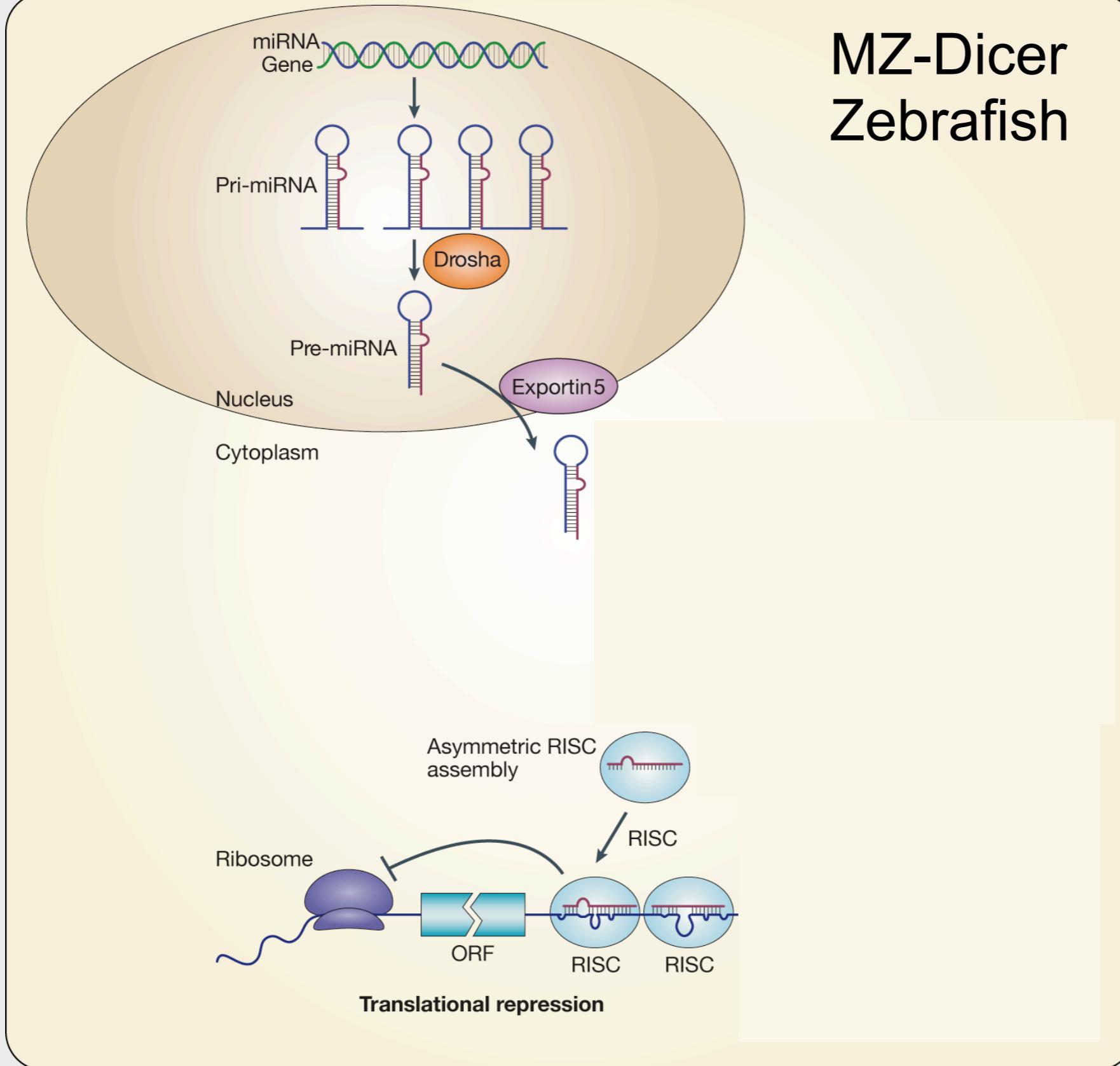




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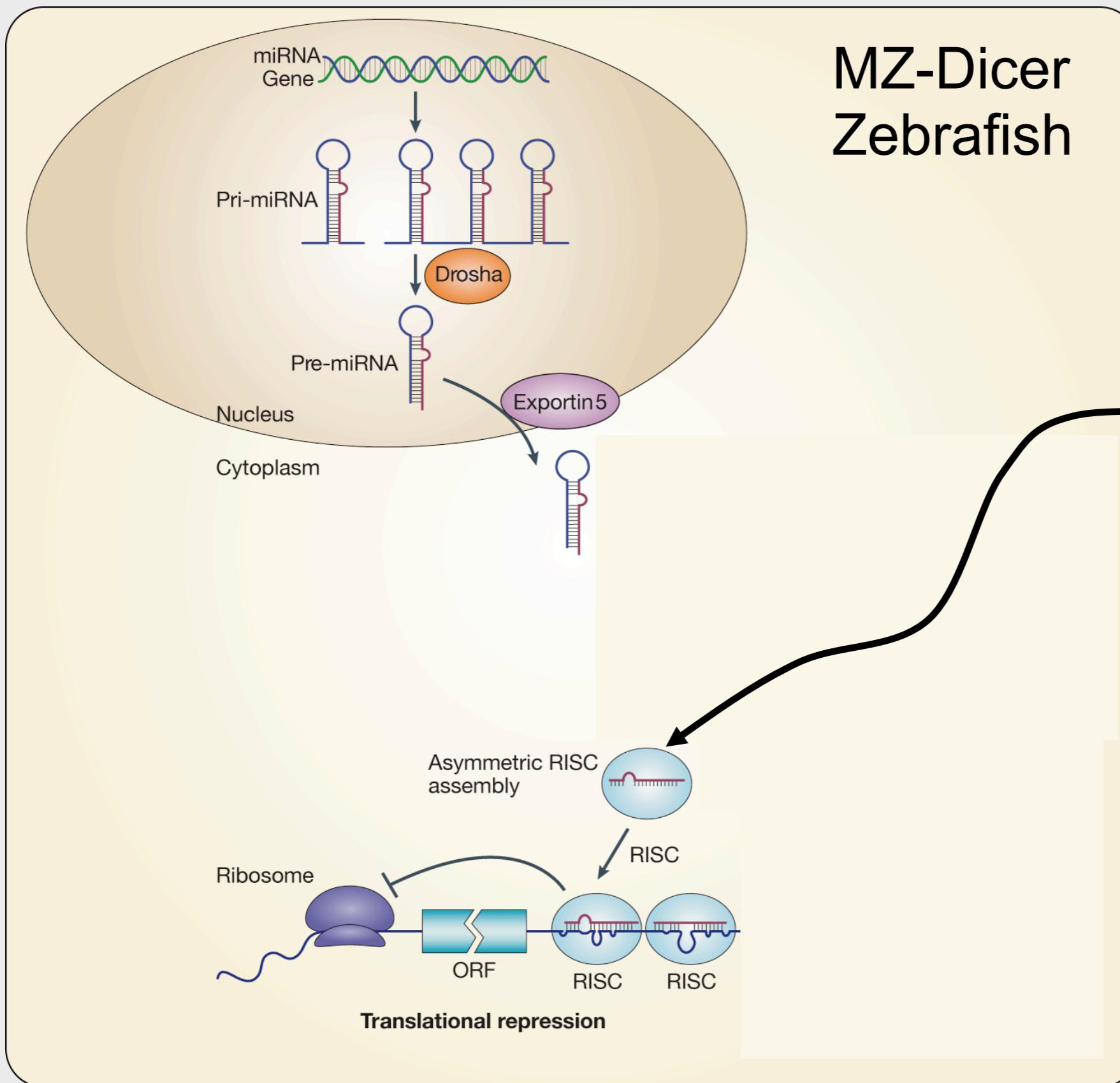


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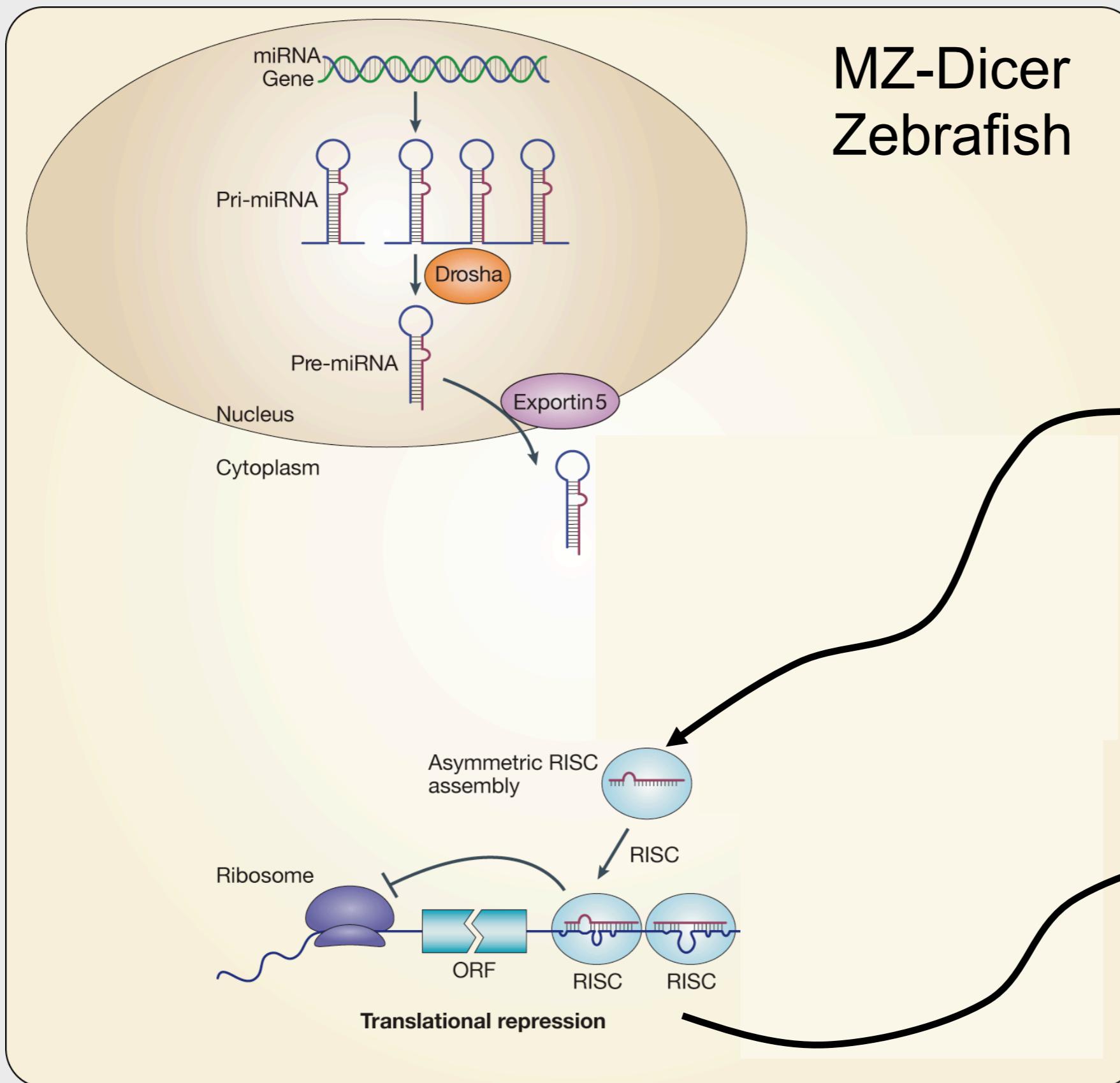
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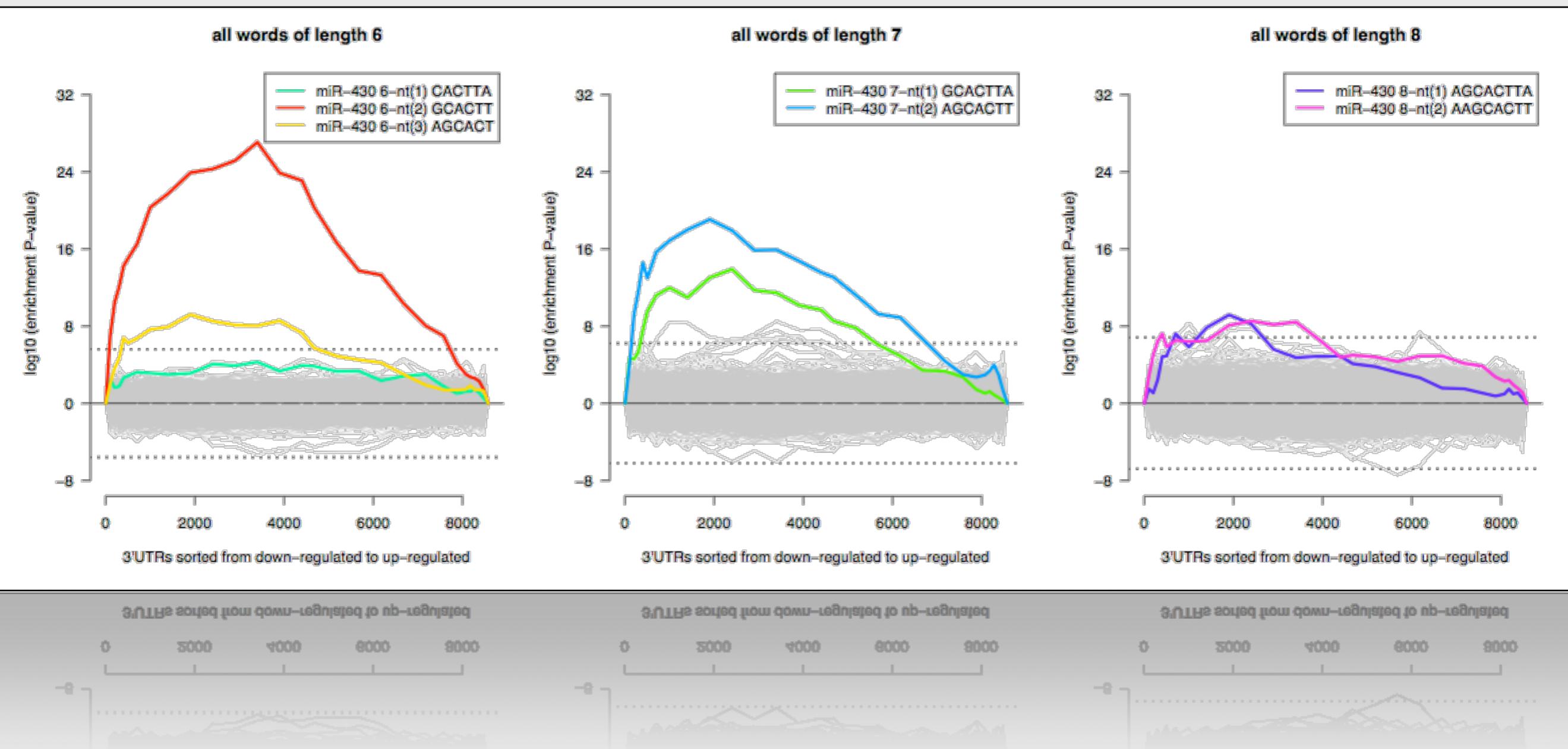


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Zebrafish MZ-Dicer embryos, miR-430



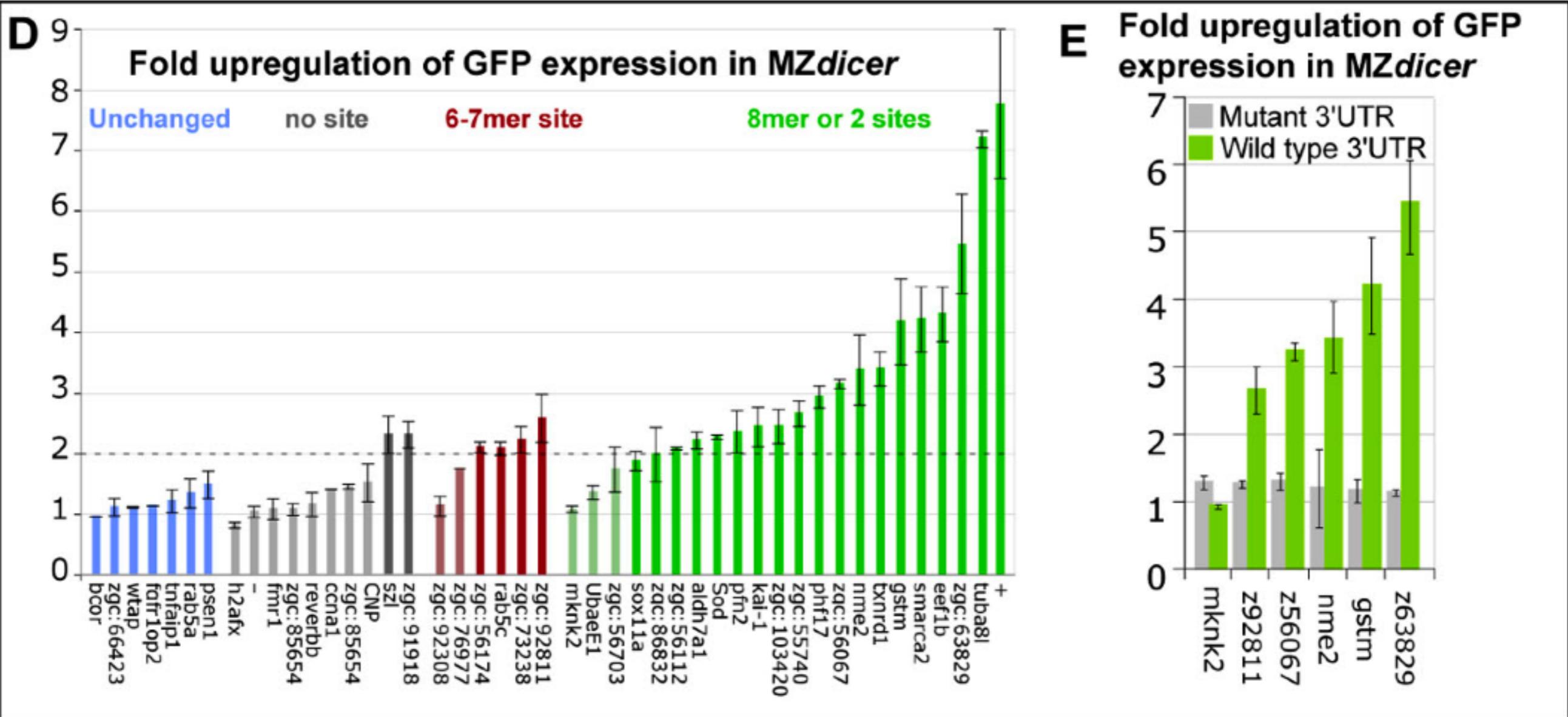
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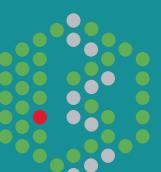


Targets Validate Experimentally

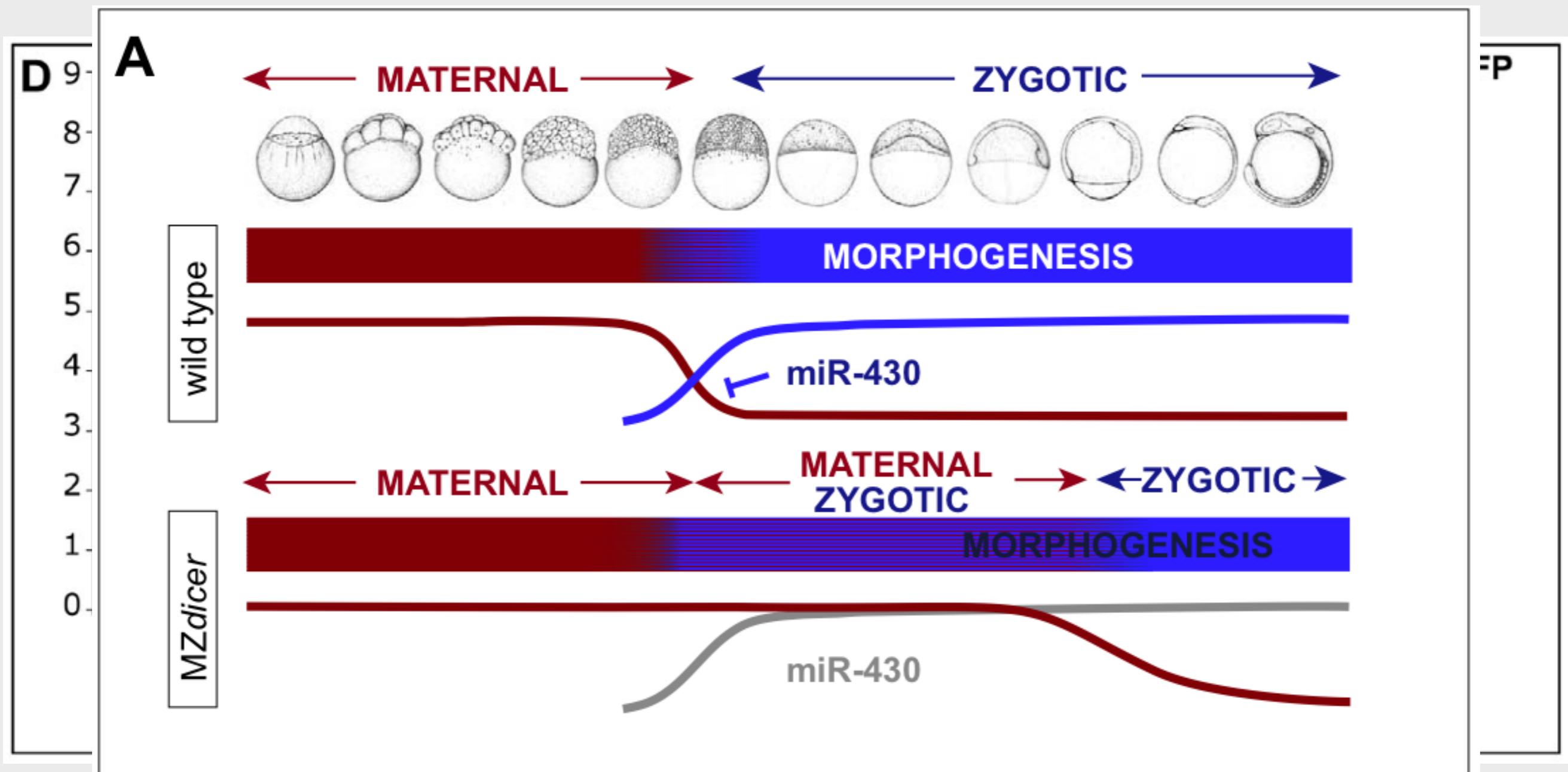


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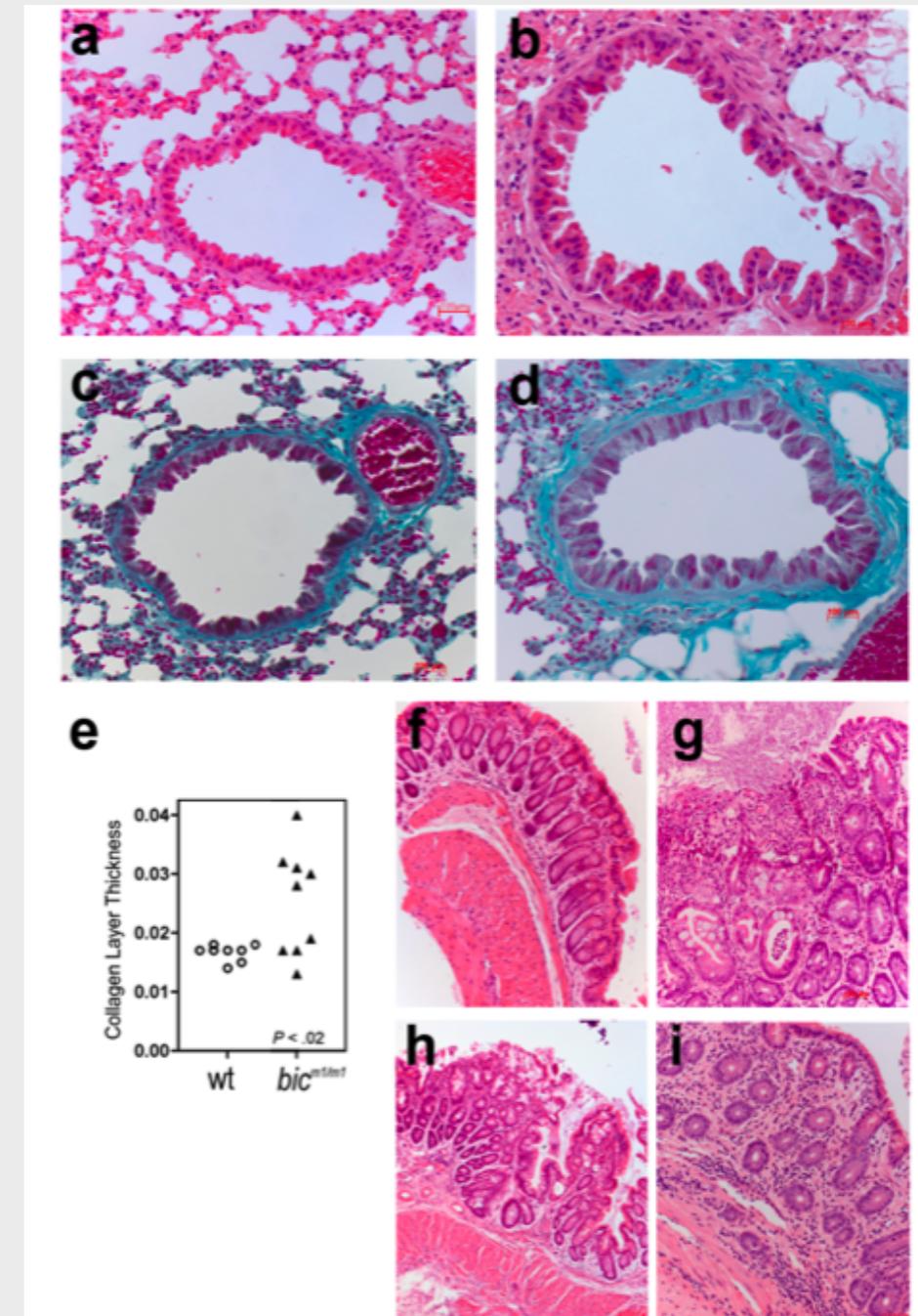
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Testing Sylamer Bic (Δ miR-155) Mouse

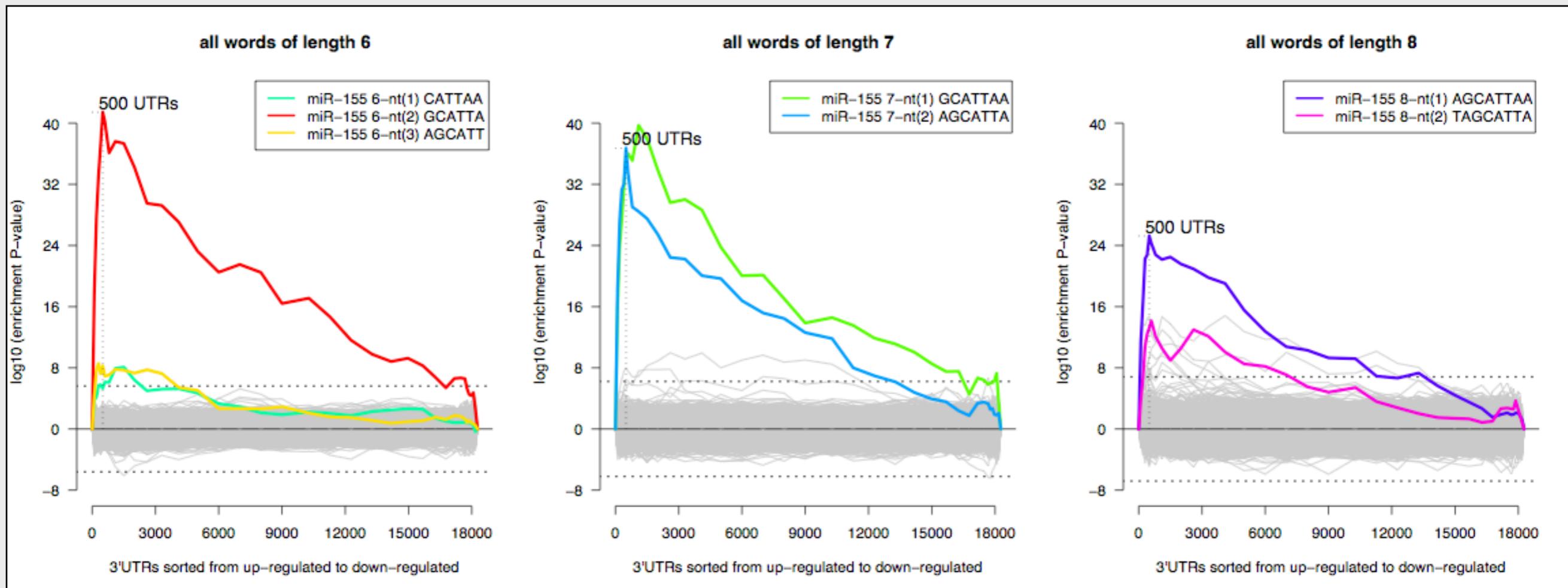
- **Mouse miR-155 Knockout**
- Lung GI tract inflammation
- Immuno-compromised
- B/T & dendritic cell function impaired
- Affymetrix analysis of Th1 & Th2 cells wt vs bic



Requirement of bic/microRNA-155 for normal immune function.
Rodriguez A, et al. Science. (2007);316:608-11.



Mouse miR-155 knock-out, Th1 cells



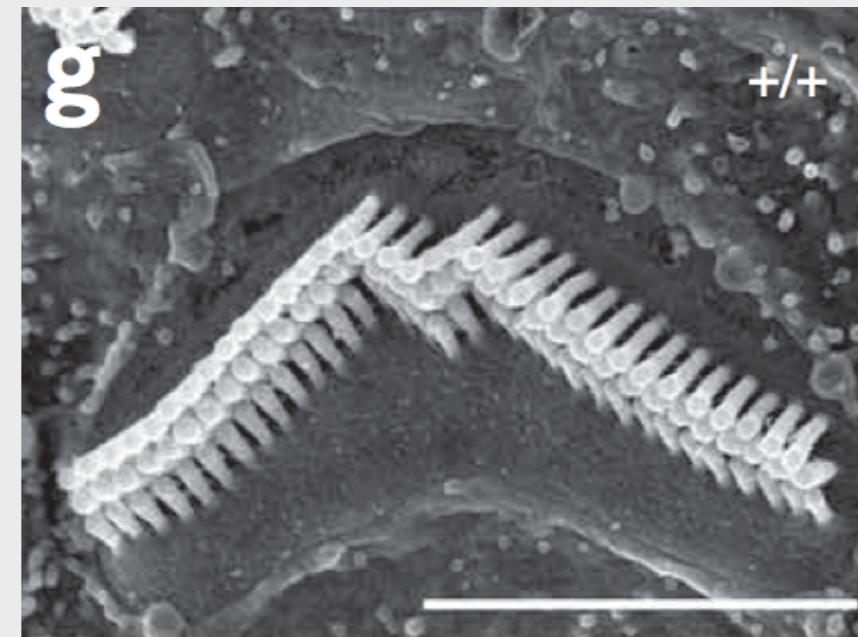
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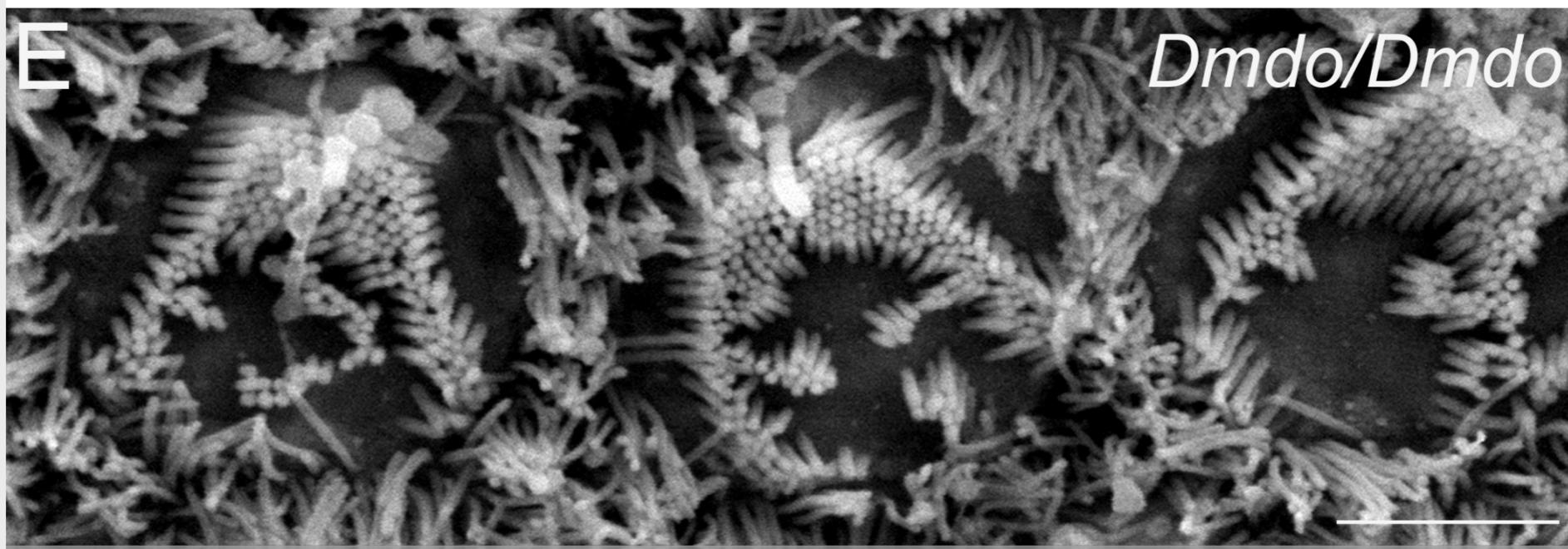
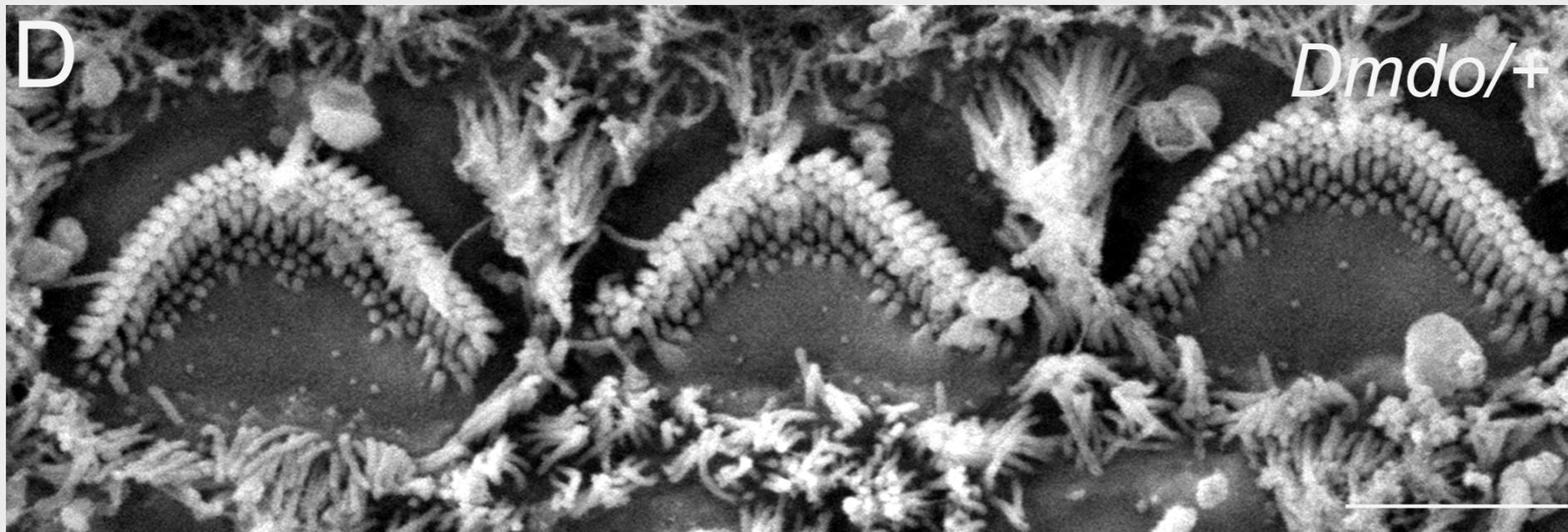
MicroRNAs & Deafness



An ENU-induced mutation of a miRNA associated with progressive hearing loss
Lewis M., et al (Nature Genetics) 2009



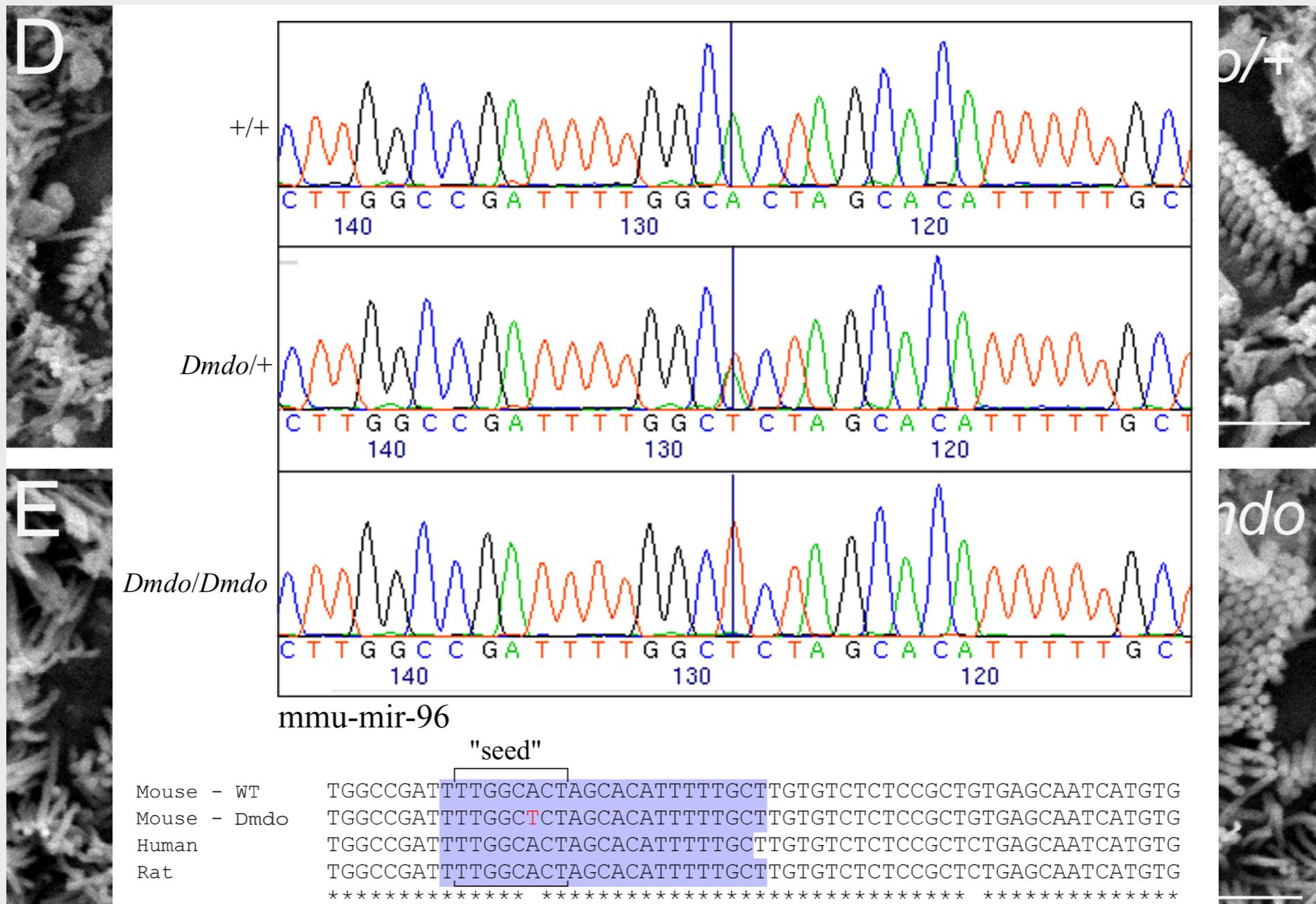
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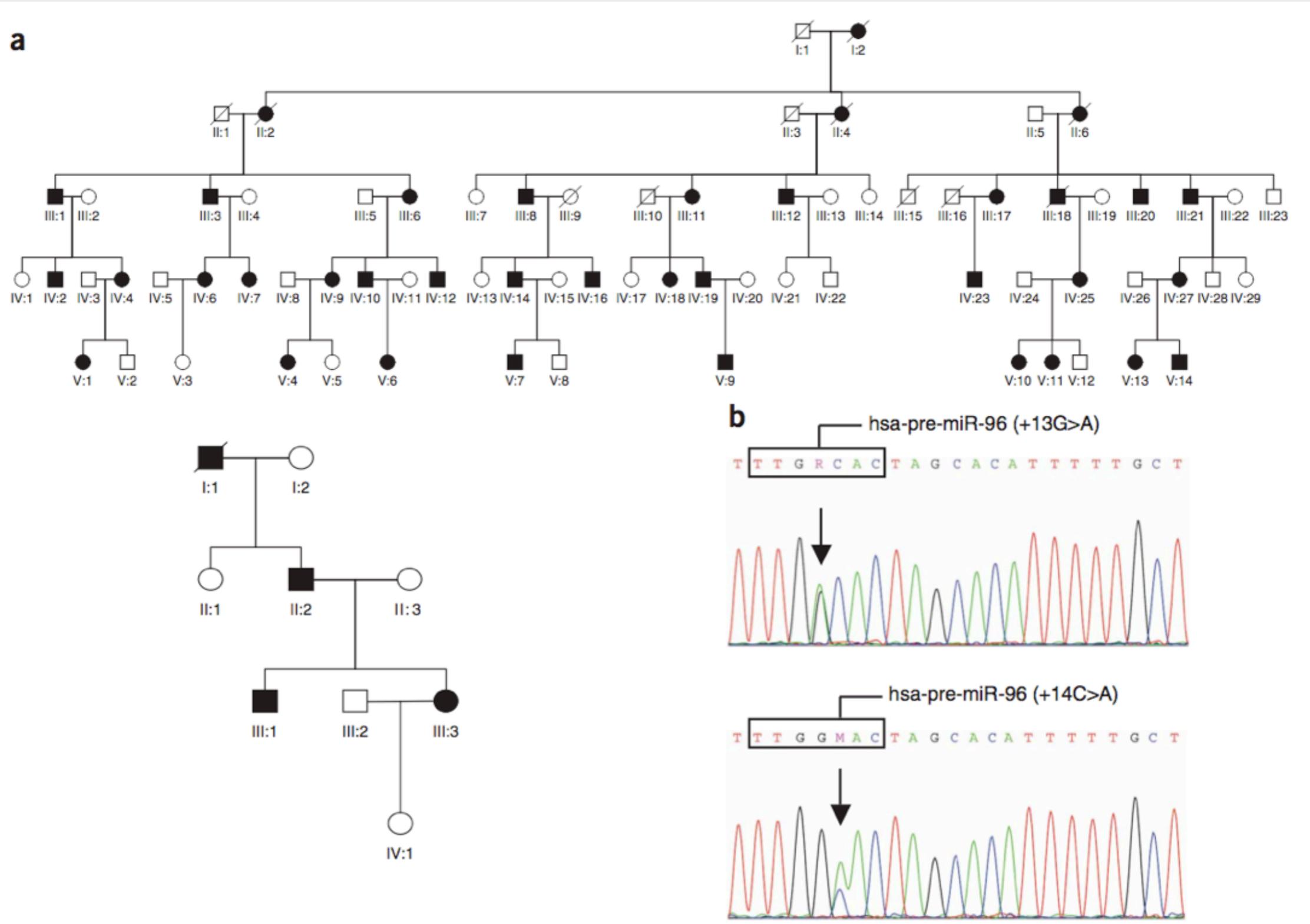
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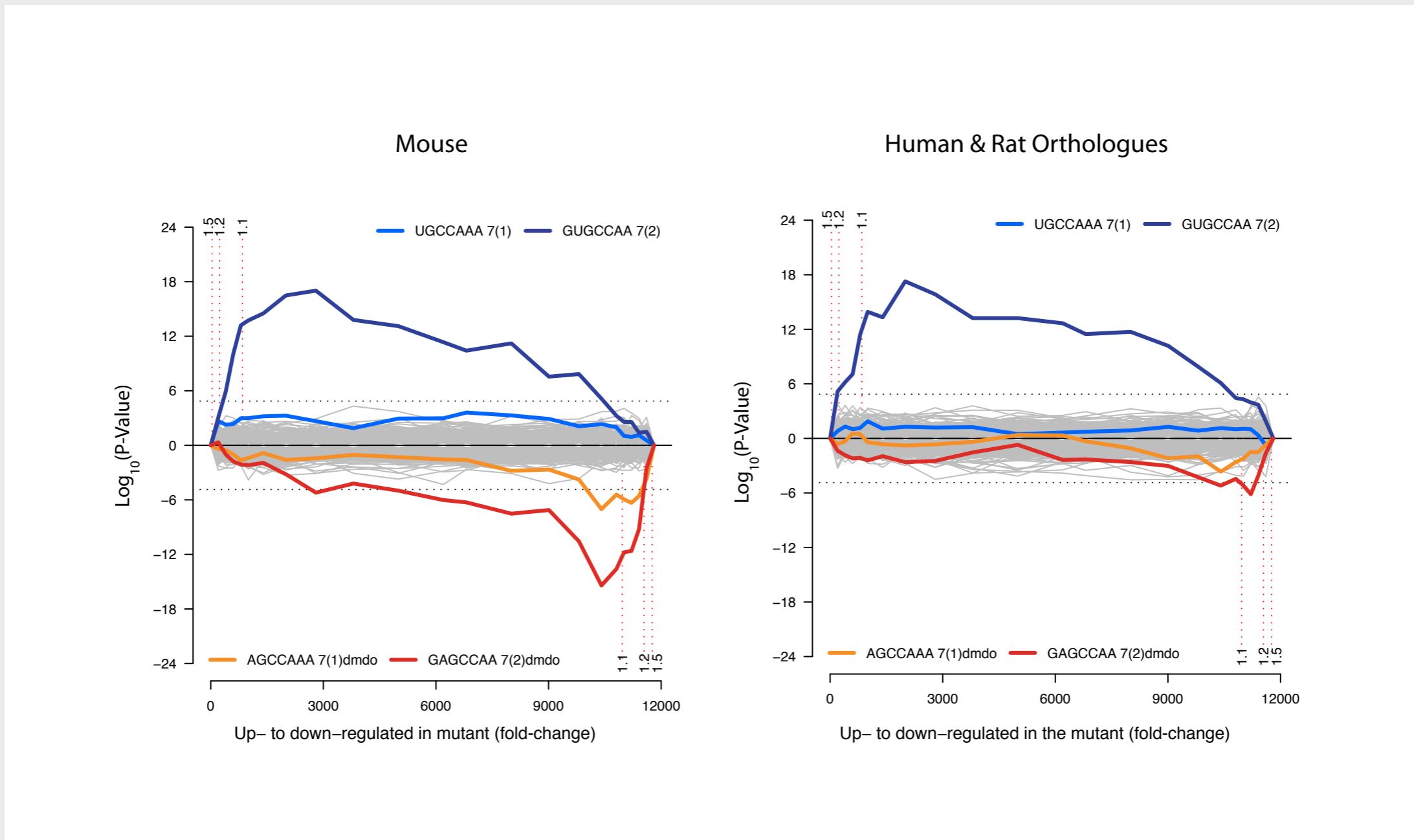
Just in Mouse ?



Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss
Mencia A., et al (Nature Genetics) 2009



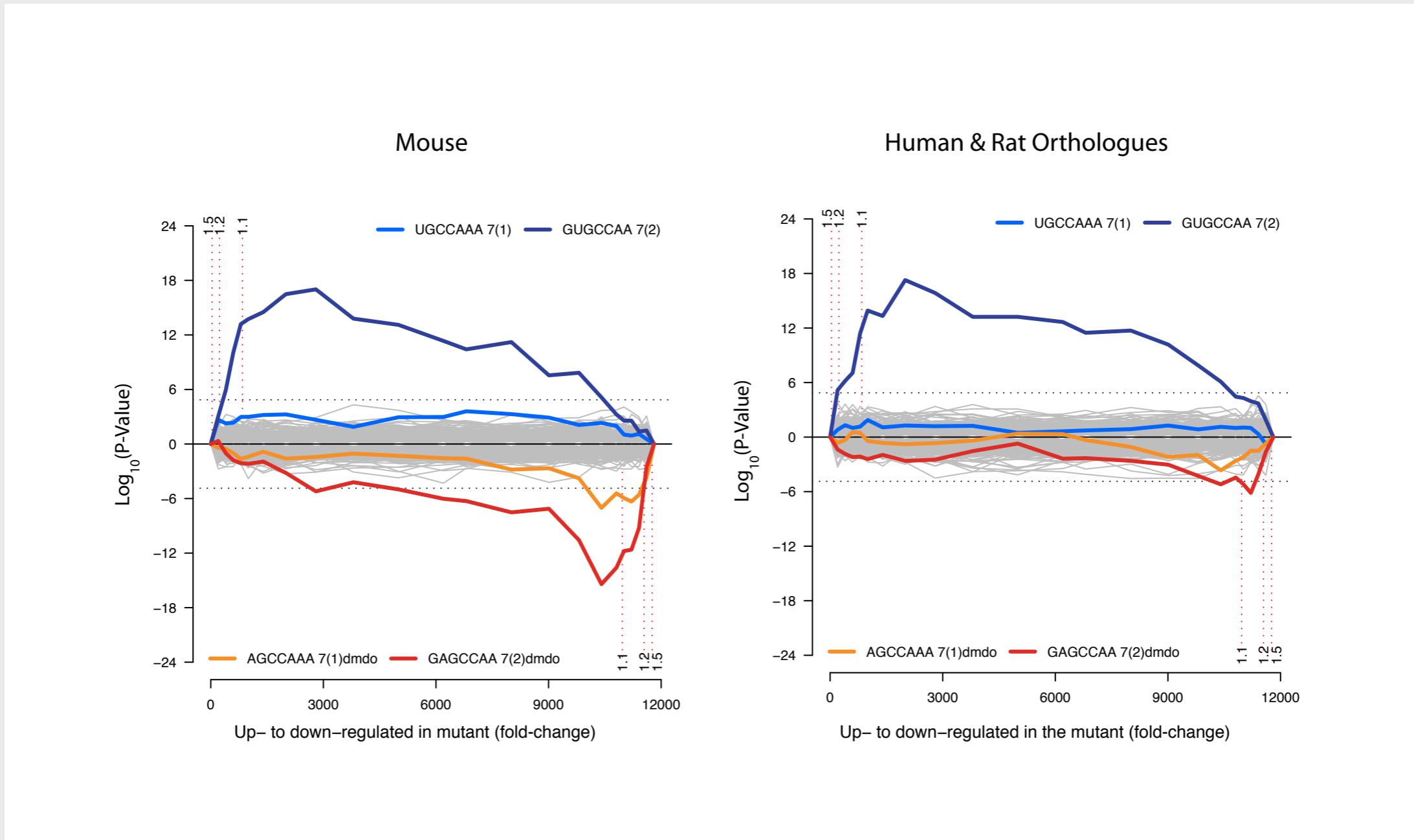
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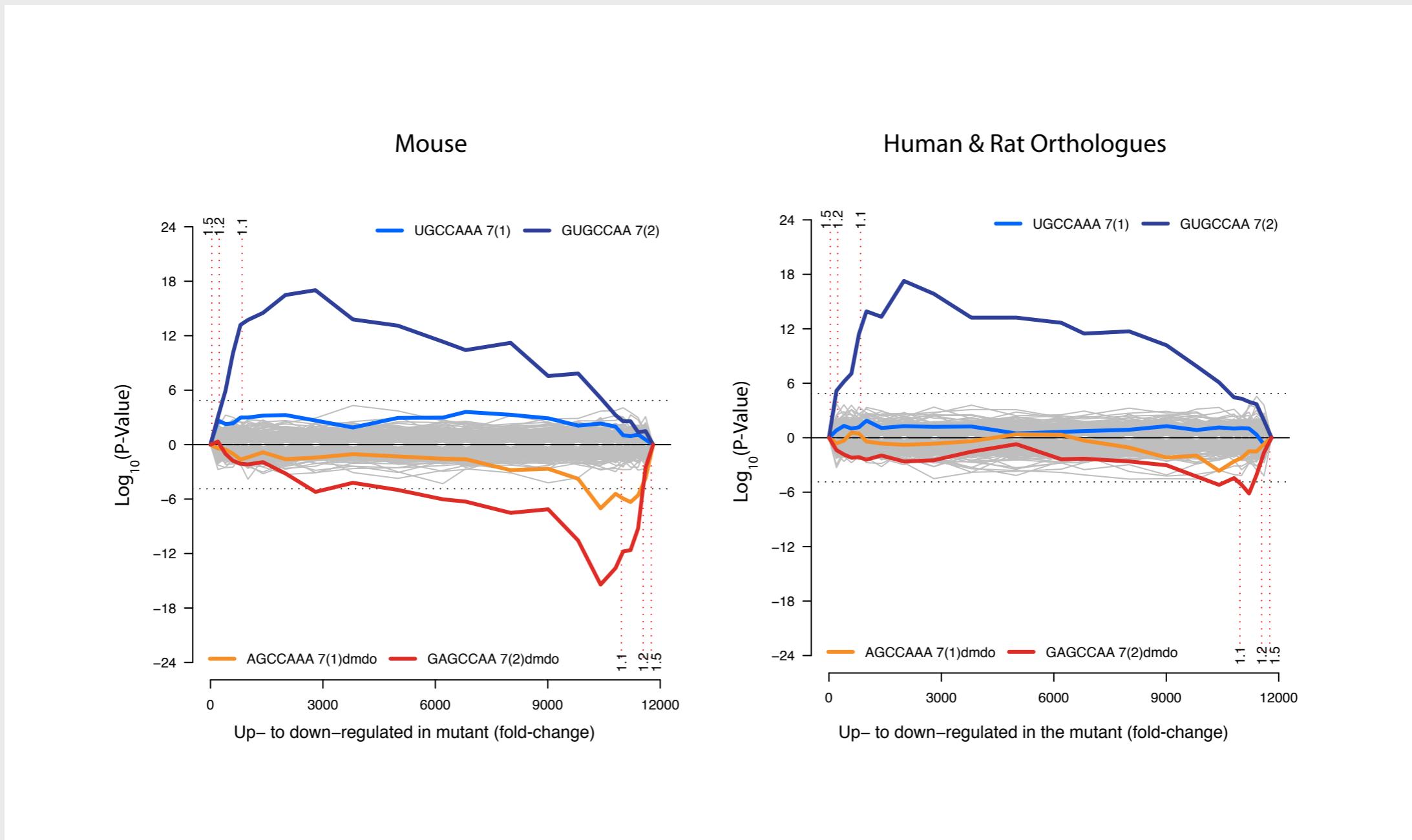
5' uuuggcacuagcacauuuuugcu 3'

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Mutant miR-96

