Transcriptomes as phenotypes

Bringing Genetics to Genomics

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Online Slides Available at dangeles.github.io

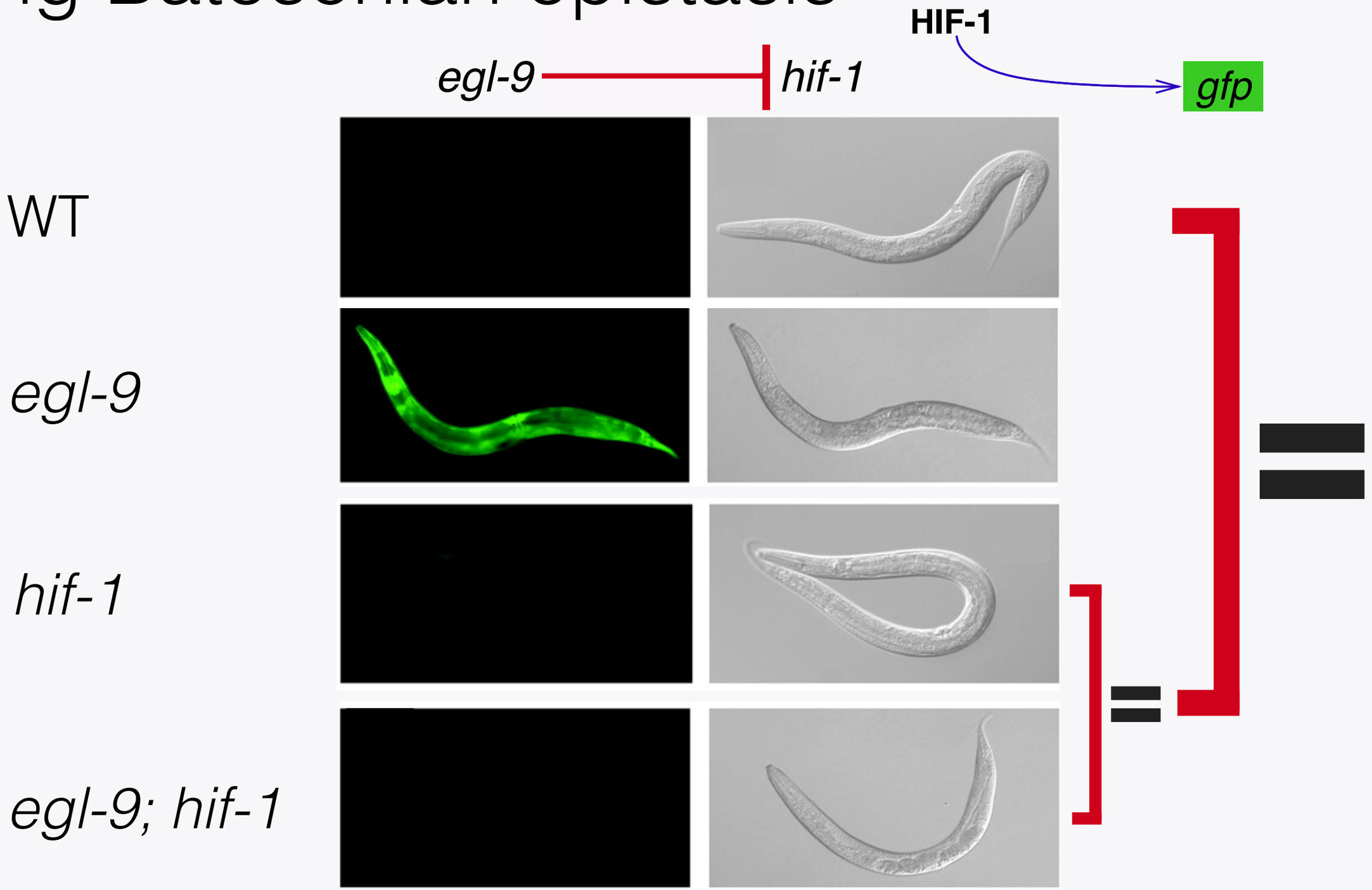
The geneticist's arsenal

Null mutants (**Epistasis**)

Allelic series (**dominance**)

Crosses (**maternal effects**)

Genetics orders genes along pathways using Batesonian epistasis



Shen *et al*, 2006

Epistasis analysis in a nutshell:

(A) Choose phenotype (based on expertise)
(B) Phenotype single, double NULL mutants
(C) Check if double mutant = a single mutant

Yes?

Infer pathway

No?

Genetic interaction is ‘complex’, need more information

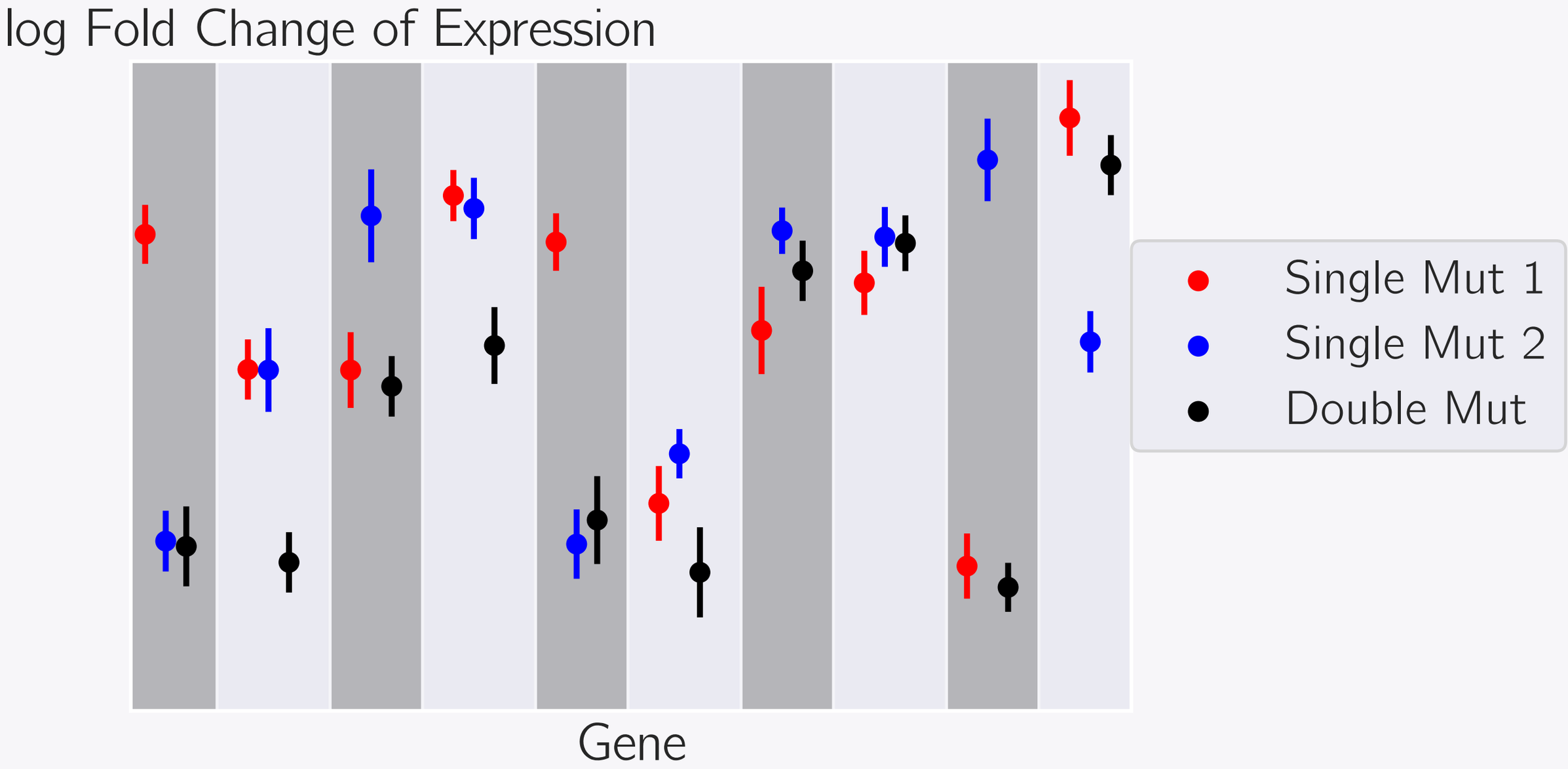
RNA-seq offers the possibility of a new kind of phenotypes

Genome-wide

Quantitative

Unbiased

Transcriptomes are powerful, but complicated



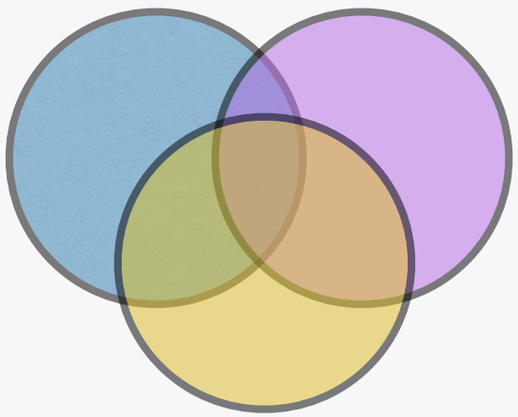
To use genetic methods in a genomic context, we need **specialized statistical machinery**

For details, see:

- **Epistasis**: Angeles-Albores *et al*, *PNAS*, 2018; Angeles-Albores *et al*, *G3*, 2017
- **Dominance**: Angeles-Albores, *Genetics*, 2018

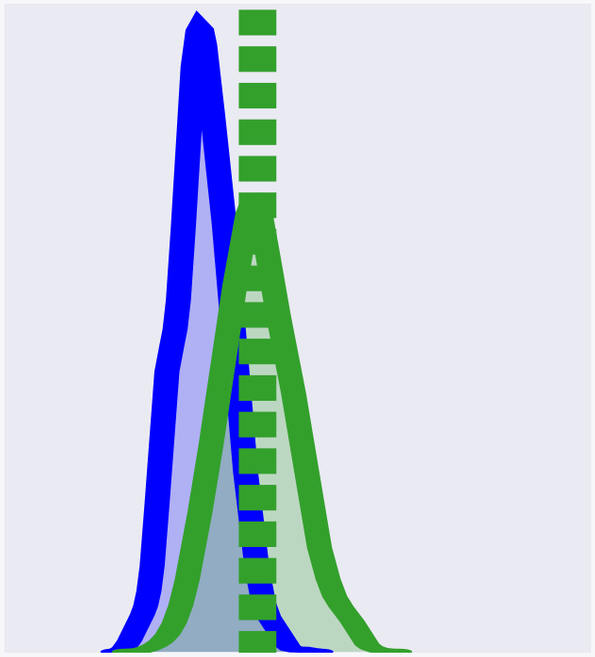
Transcriptome-wide epistasis analysis in a nutshell:

Choose phenotype

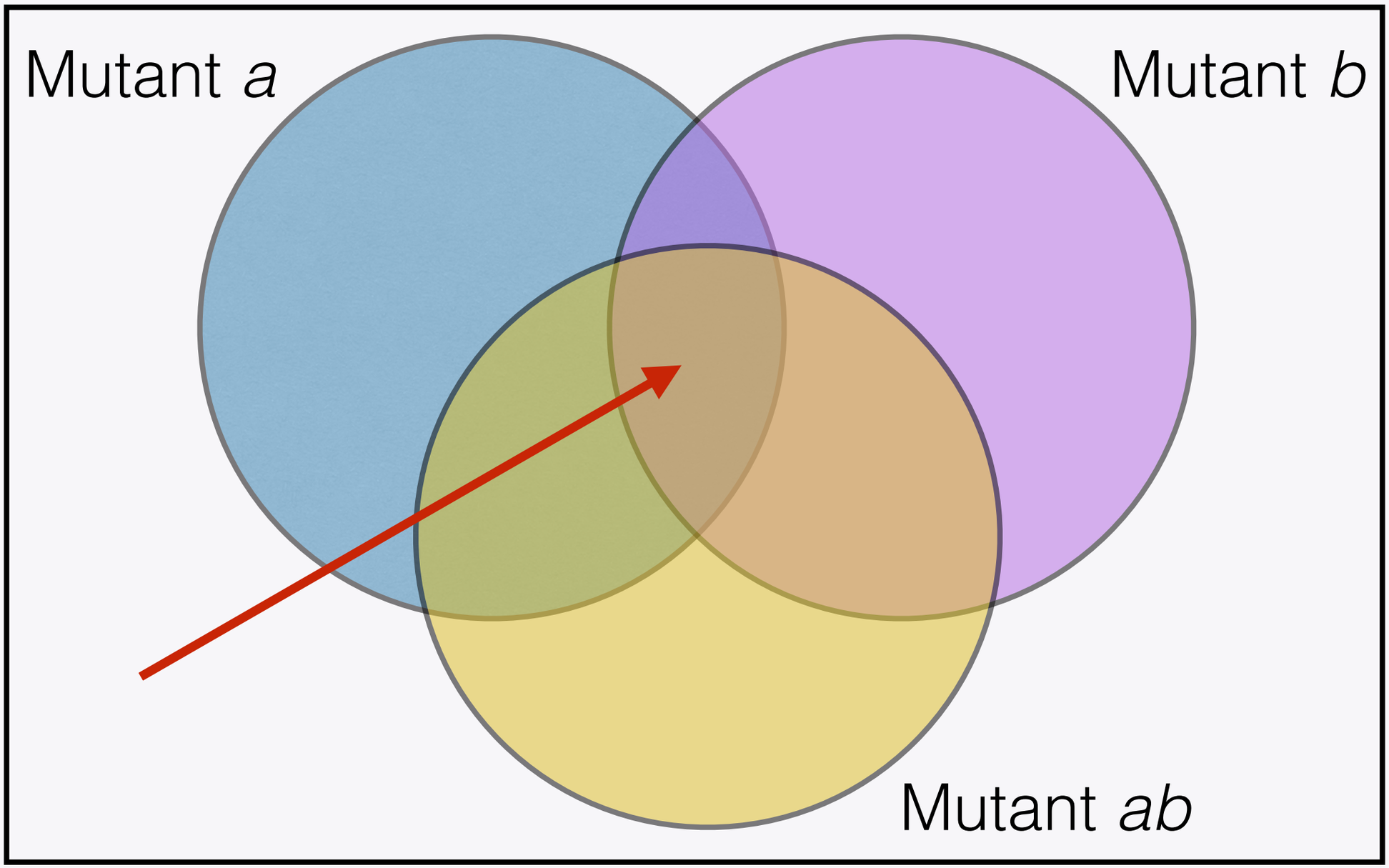


Compute a statistic for all genes in phenotype

Check if statistic is Batesonian



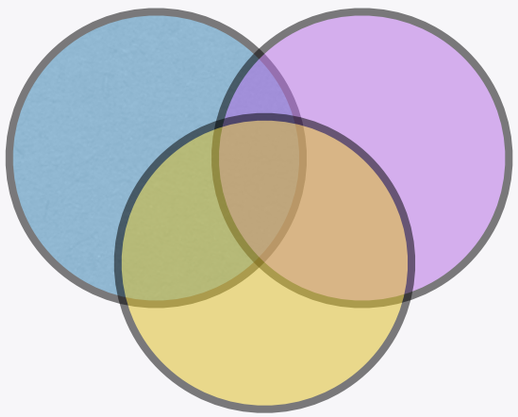
Transcriptome-wide epistasis: Defining a phenotype



Diff. Exp. Genes relative to WT

Transcriptome-wide epistasis analysis in a nutshell:

Choose phenotype



Compute a statistic for all genes in phenotype

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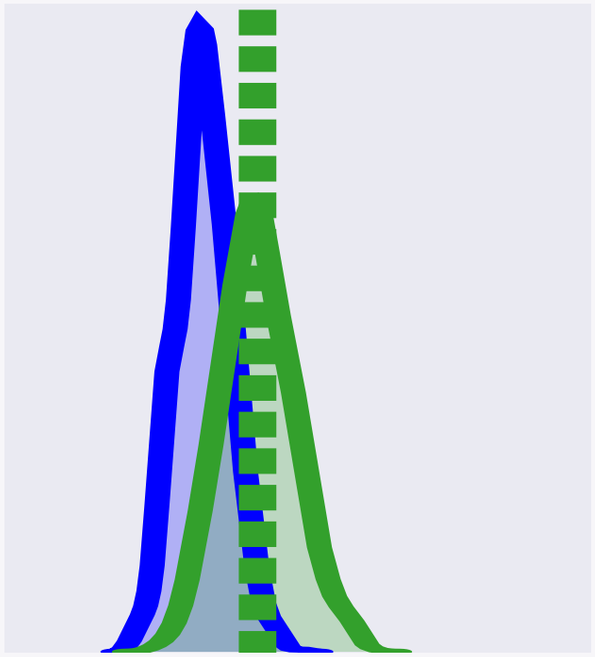


What does our black box do?

(1) Calculate: **expected** double mutant value
(Add the single mutant log Fold Changes)

(2) Compute: **difference = observed - expected**

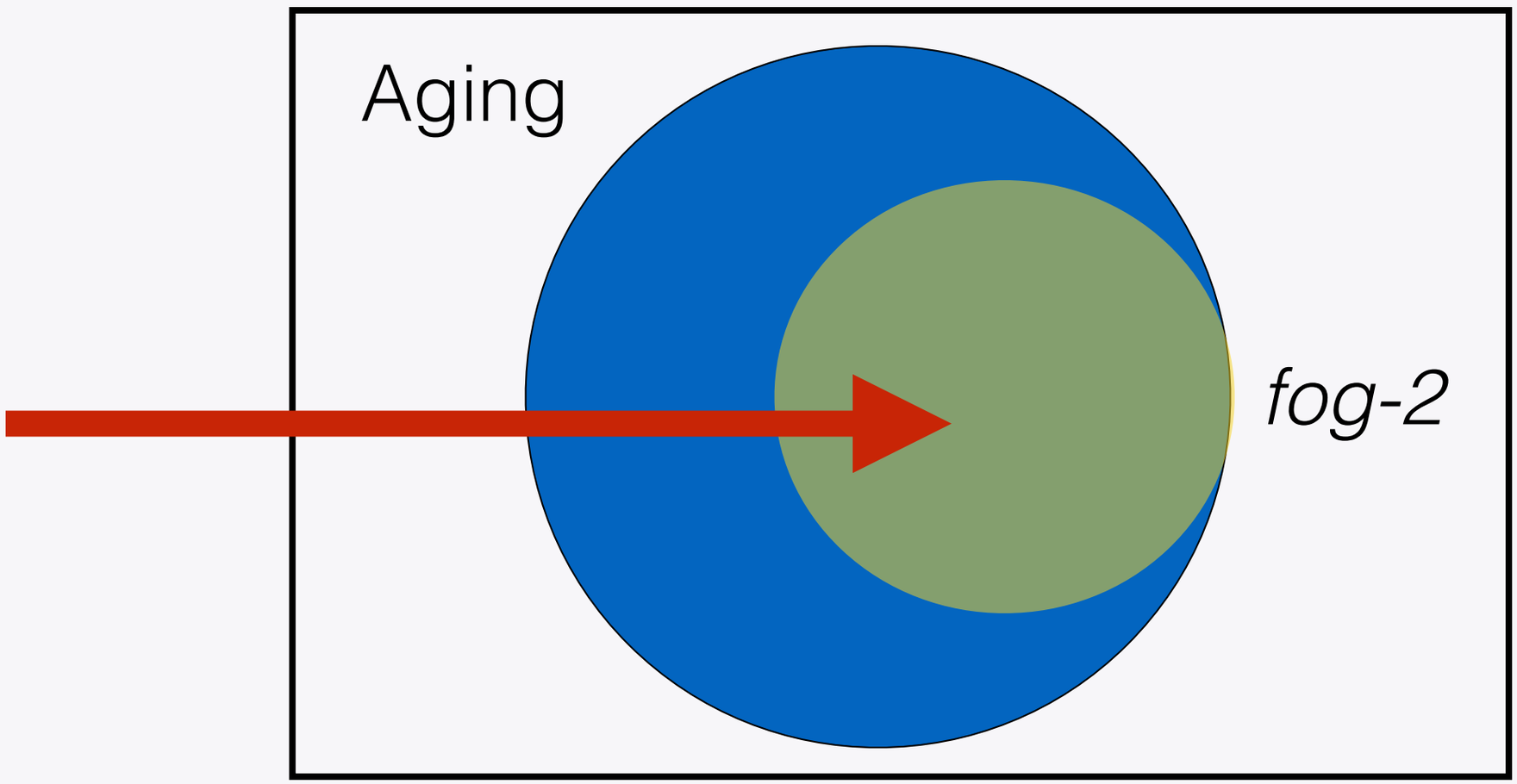
(3) Plot difference vs. expected for all transcripts
and **determine line of best fit**

An example: Does sperm status have effects independent of aging?

	WT	***fog-2***
Young adult	Young, Sperm	Young, NO Sperm,
‘Middle-aged’ adult	Aged, NO Sperm,	Aged, NO Sperm,

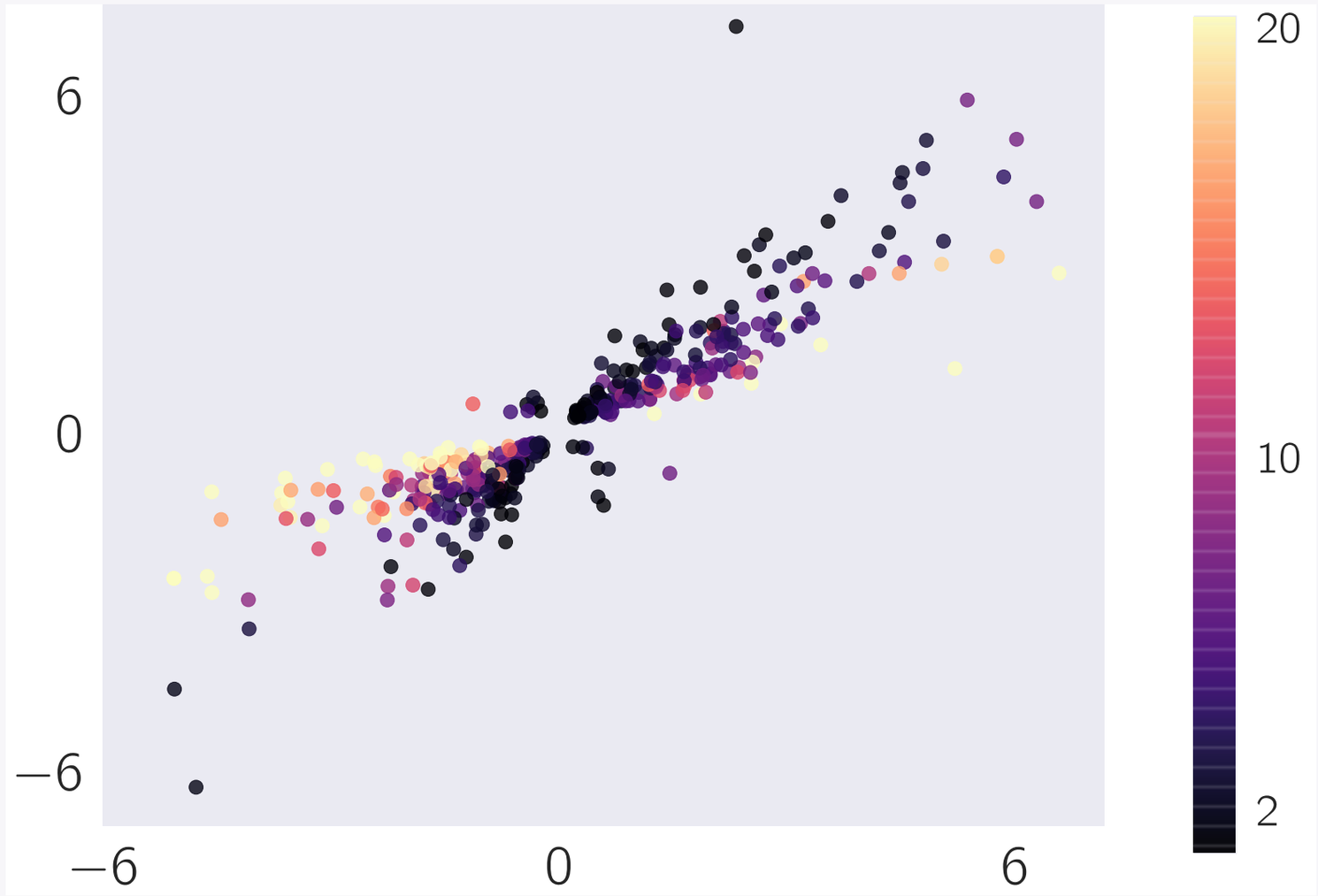
Angeles-Albores, Leighton and Sternberg, *G3,* 2017

Age affects more genes than *fog-2*, so we find the commonly affected subset



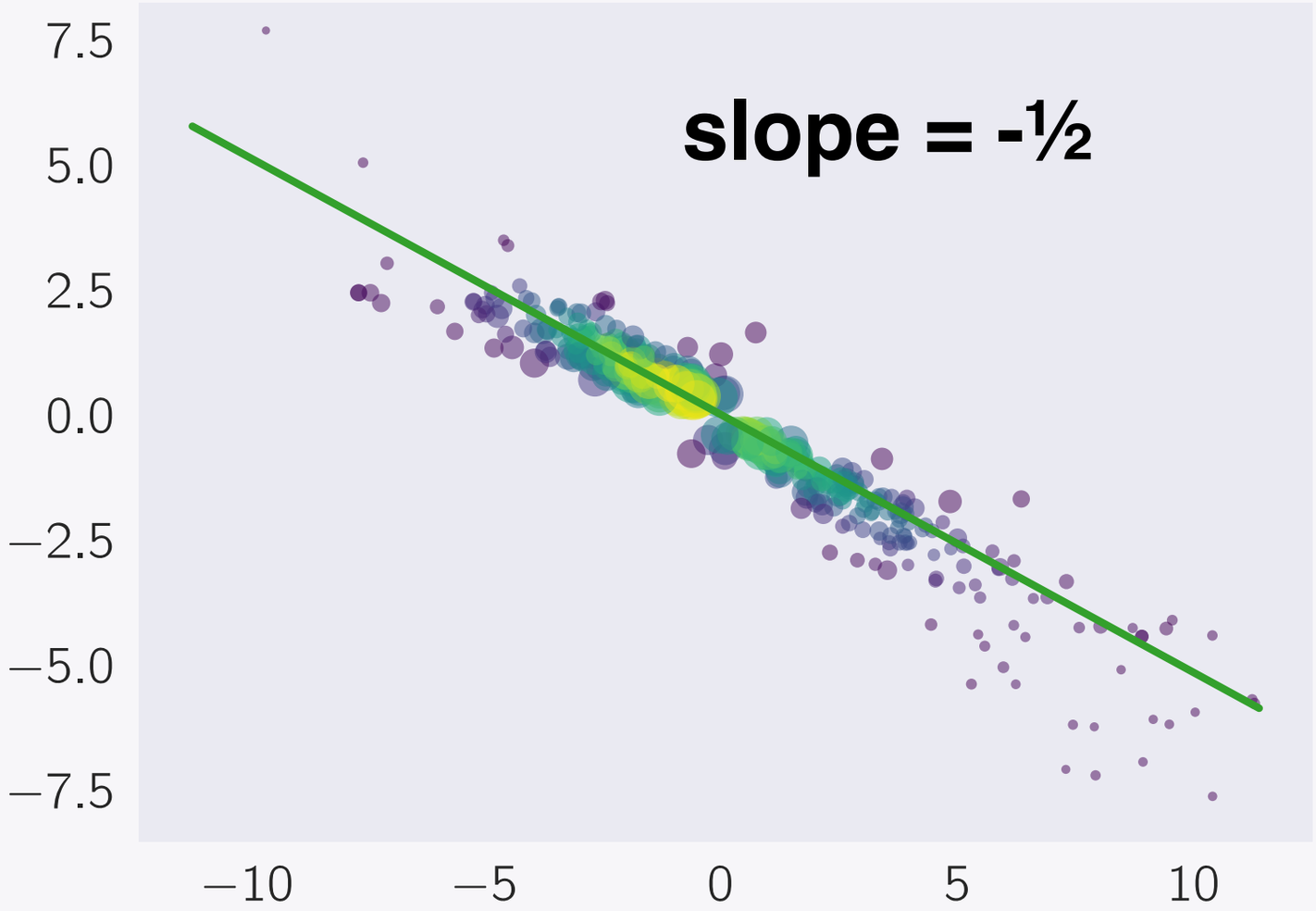
Diff. Exp. Genes relative to WT

First, show that both perturbations have equivalent effects



A slope of -½ indicates that sperm loss through aging is the same as never having sperm

Observed - Expected



Behind the math:

Observed

=

½ Expected

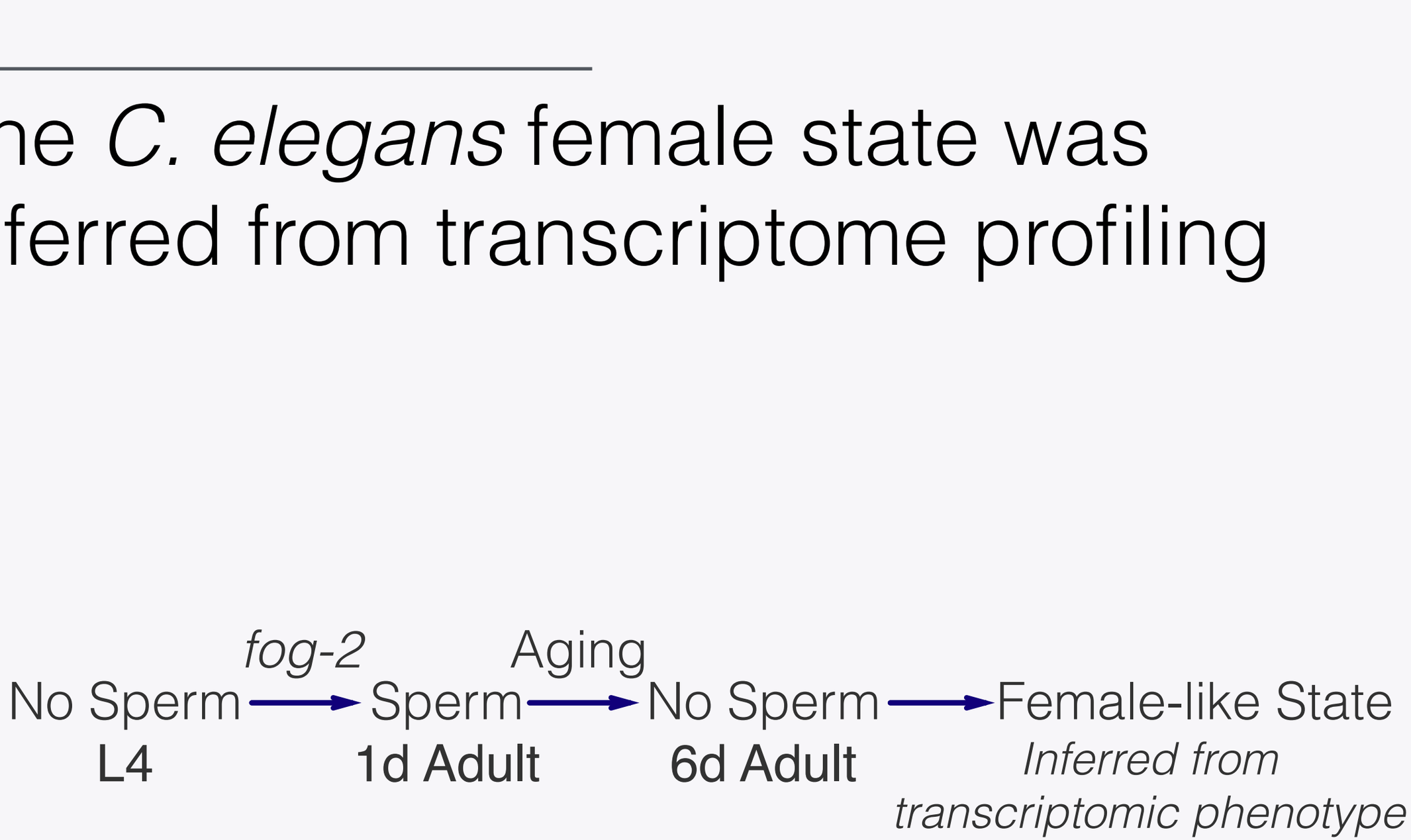
=

ln(FC) time

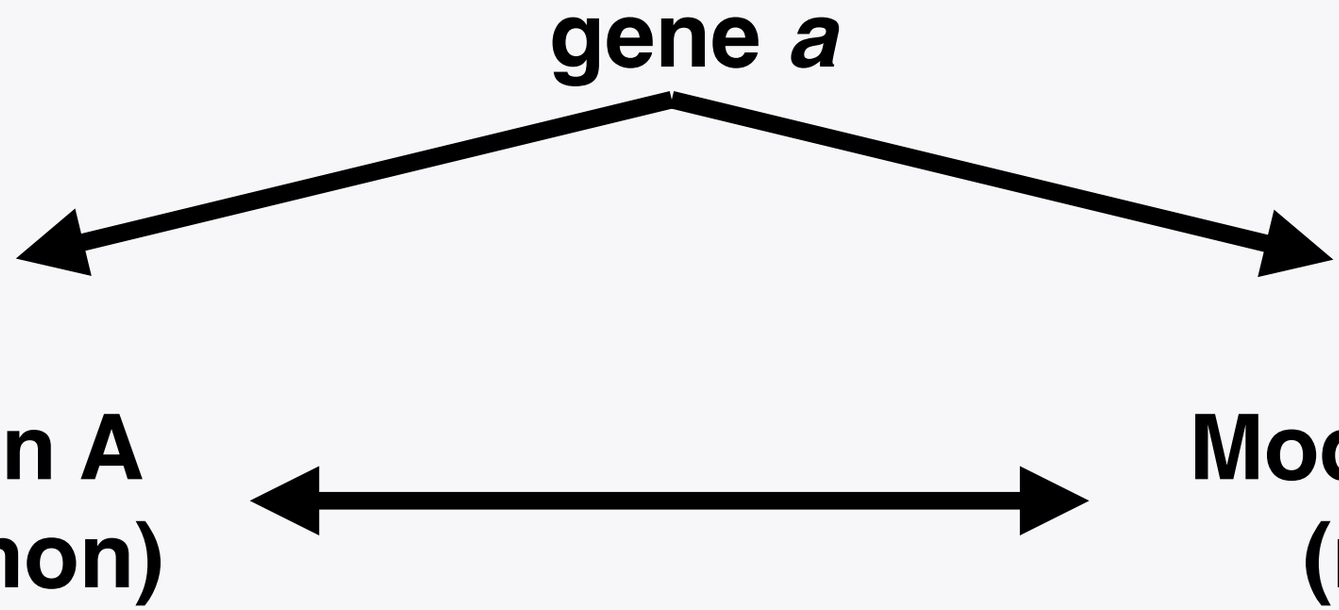
=

ln(FC) fog-2

The *C. elegans* female state was inferred from transcriptome profiling



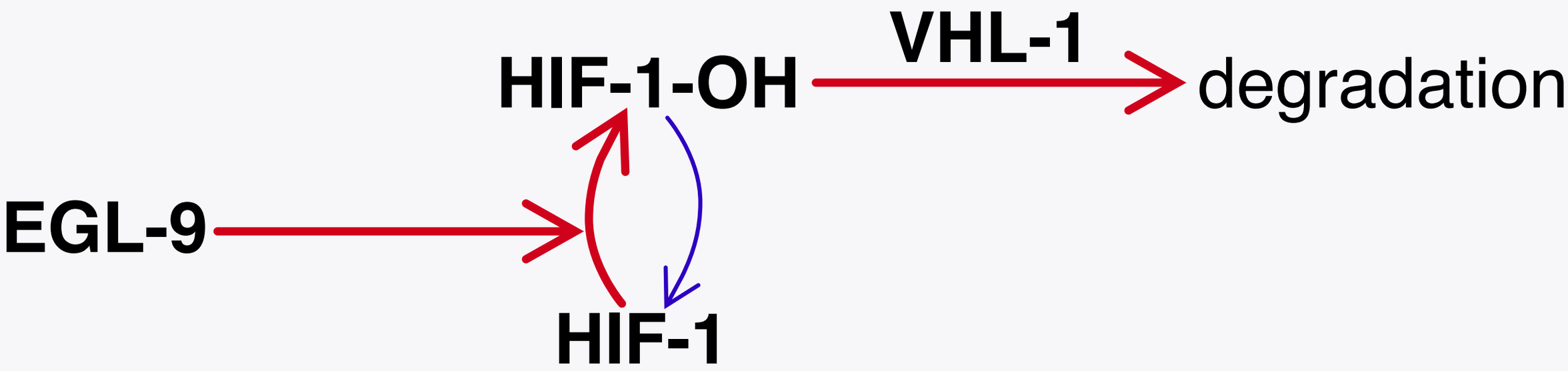
Transcriptomes can be used to think about biochemistry



Accounts for most effects of knocking out ***a***

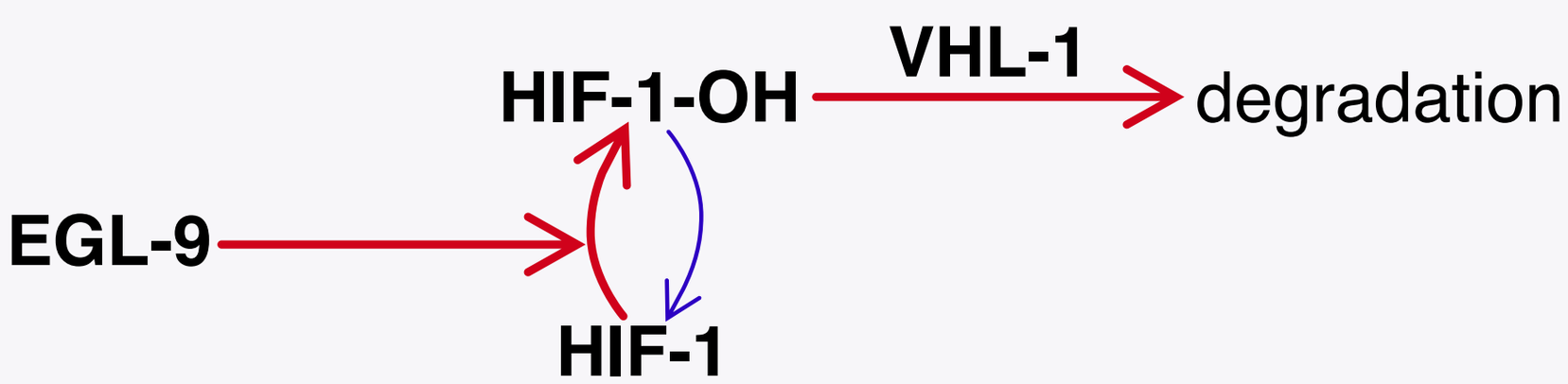
Accounts for a few effects of knocking out ***a***

Hypoxia factor 1 (*hif-1*) is degraded by VHL-1 in an EGL-9 dependent manner

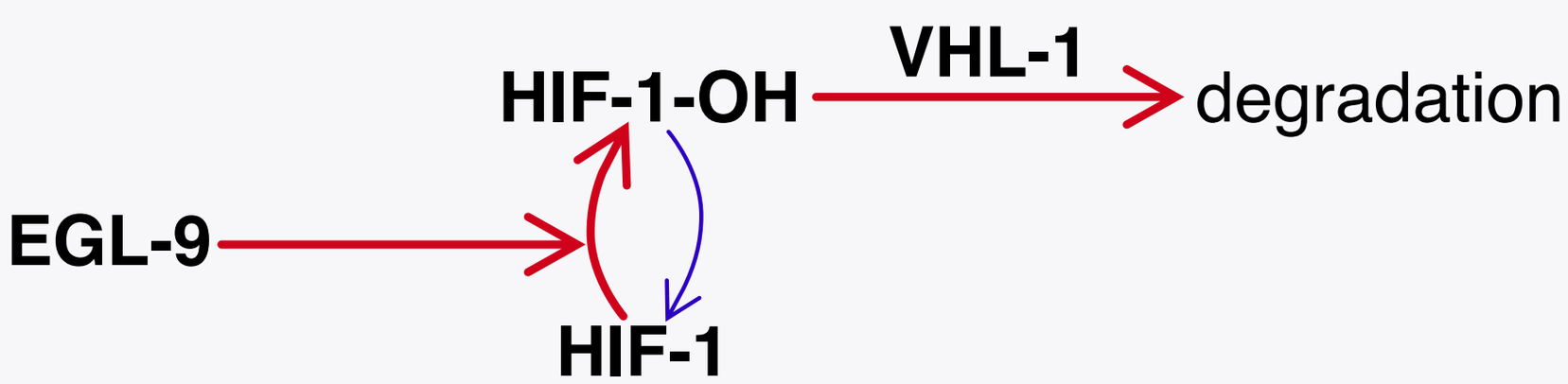


Angeles Albores, Puckett Robins, *et al.*, *PNAS*, 2018

Using HIF-1 abundance as phenotype leads to the canonical genetic diagram:



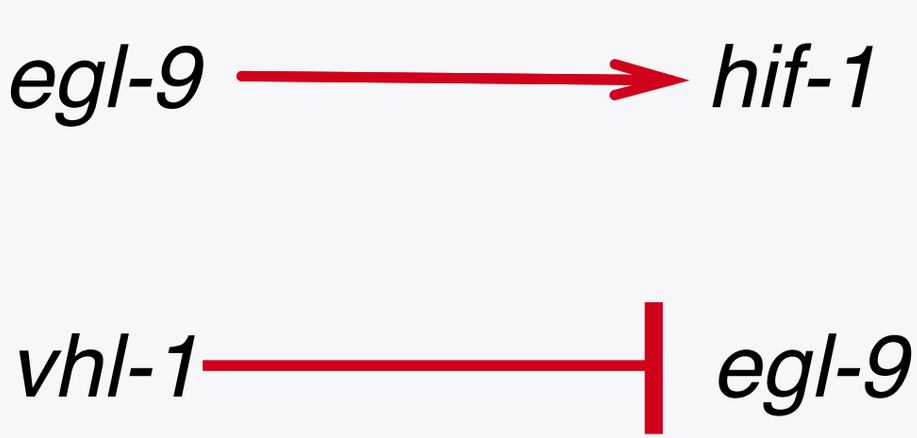
If we could measure HIF-1-OH abundance, we would write the genetic pathway as:



Choosing a phenotype affects the outcome of the genetic reconstruction:

HIF-1 abundance as phenotype

HIF-1-OH abundance as phenotype

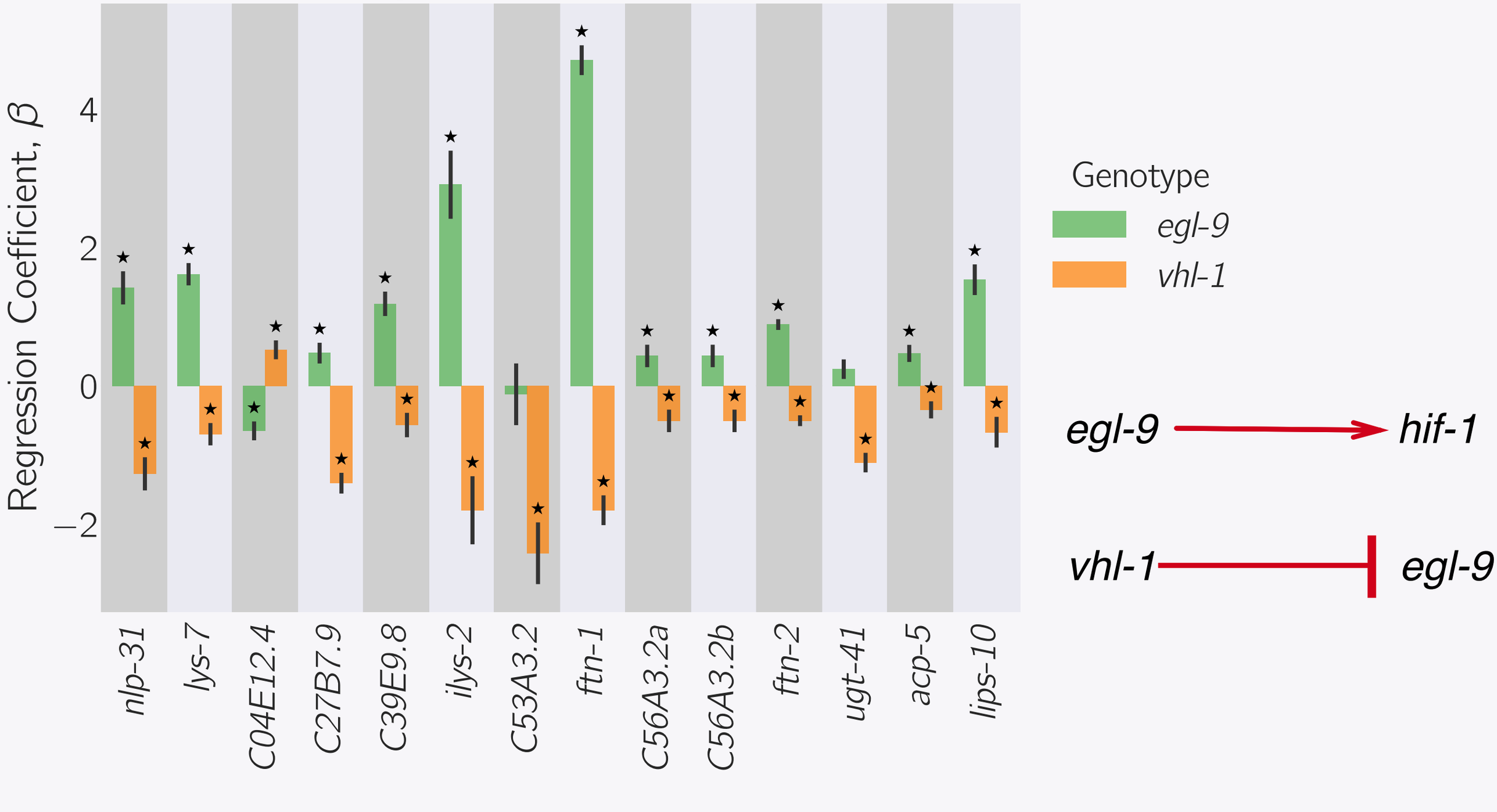


However, both pathways obey the same set of epistatic rules!

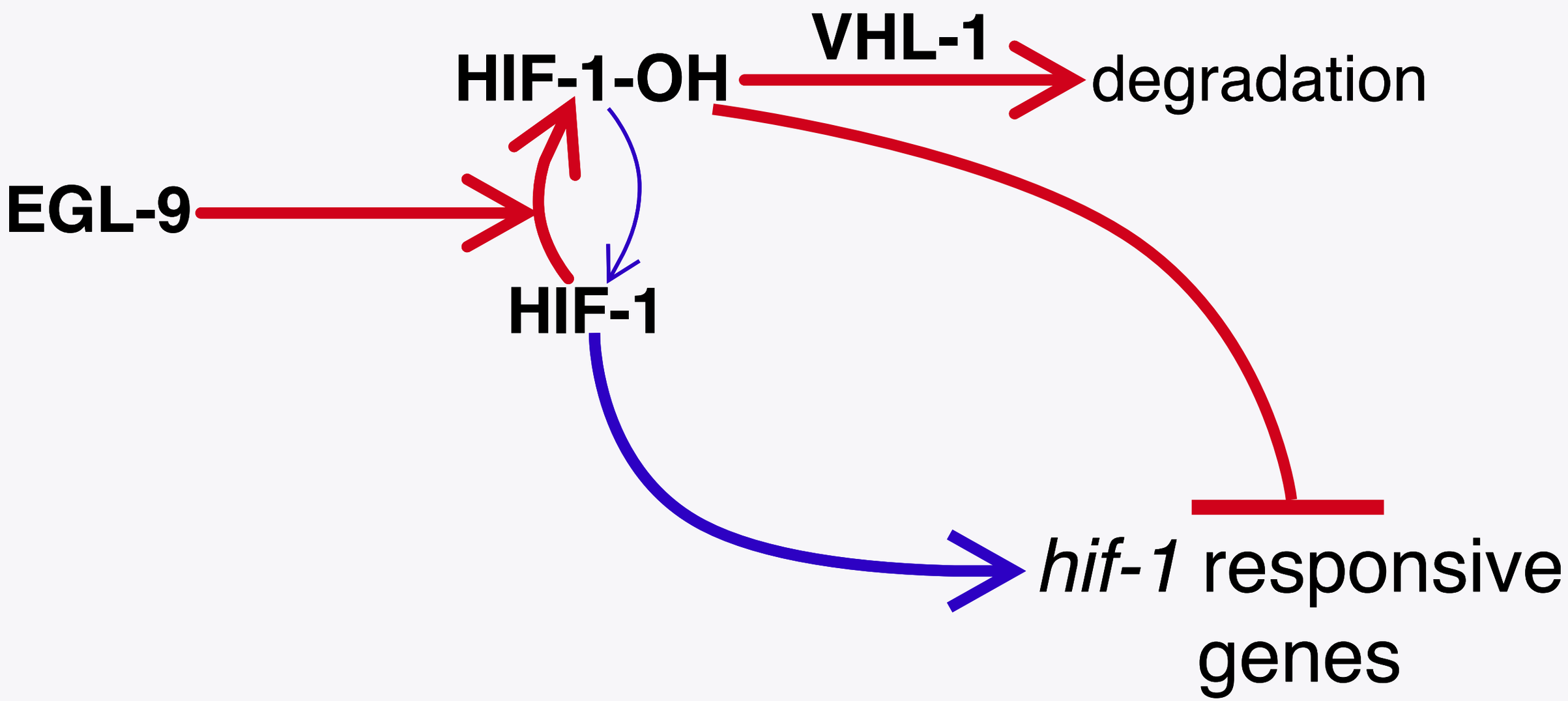
egl-9 = egl-9;vhl-1

hif-1 = egl-9;hif-1

Sequencing hypoxia pathway mutants reveals ~50 genes that behave as if controlled by HIF-1-OH



Hypothesis: A subset of genes is strongly responsive to HIF-1-OH levels



Transcriptomes + Biochemical Models can lead to testable hypotheses about molecular functions.

Transcriptomes are phenotypes in other organisms, such as bacteria!

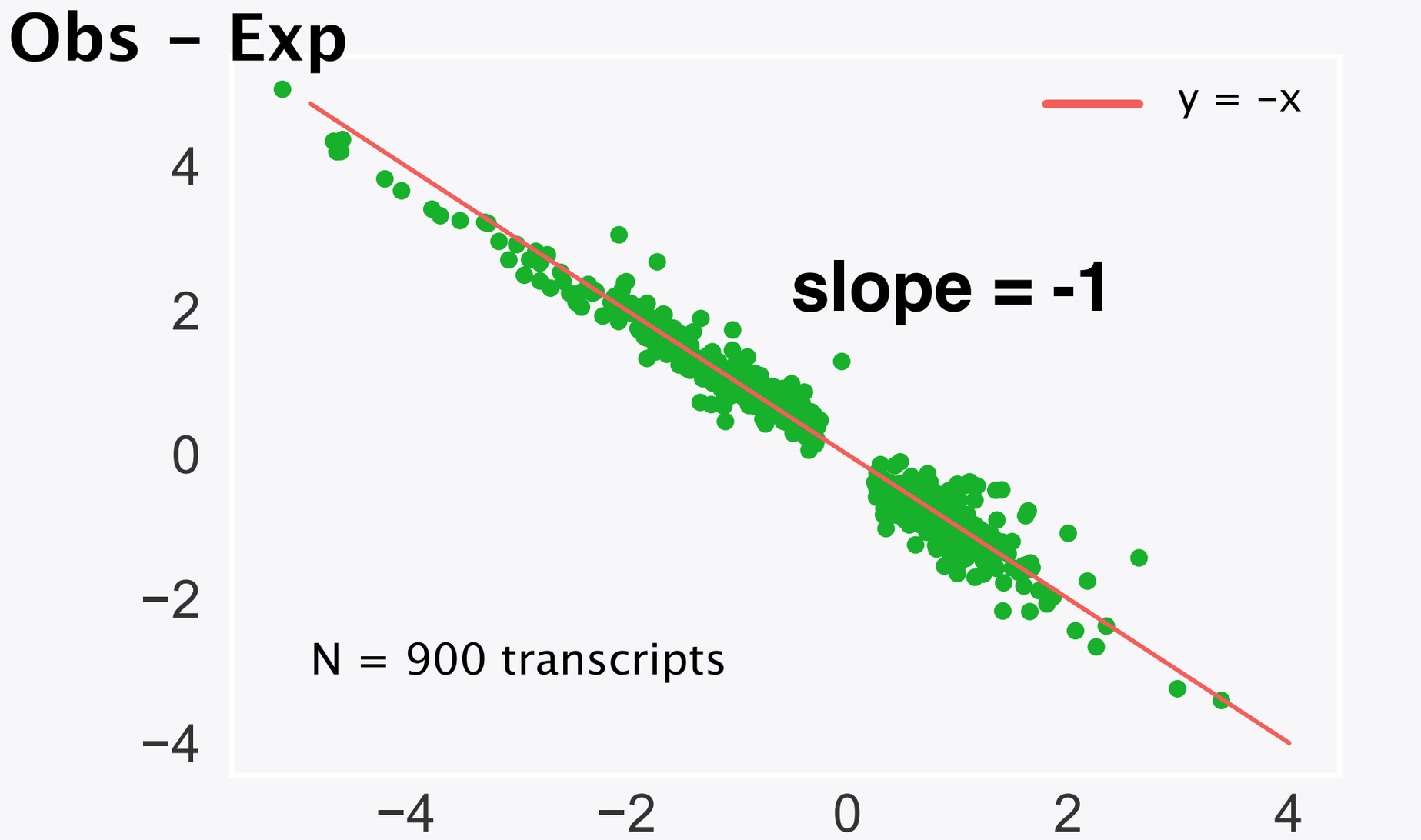
Fuqing's Question:

Do probiotics affect antibiotic response in a gut bacterium?

+/- Probiotic
+/- Antibiotic

Work by Fuqing Wu

A slope of -1 indicates complete inhibition of the effect of antibiotics by probiotics for a subset of genes



Work by Fuqing Wu

Transcriptomes are phenotypes in other organisms, such as bacteria!

Probiotic — **Antibiotic**

Work by Fuqing Wu

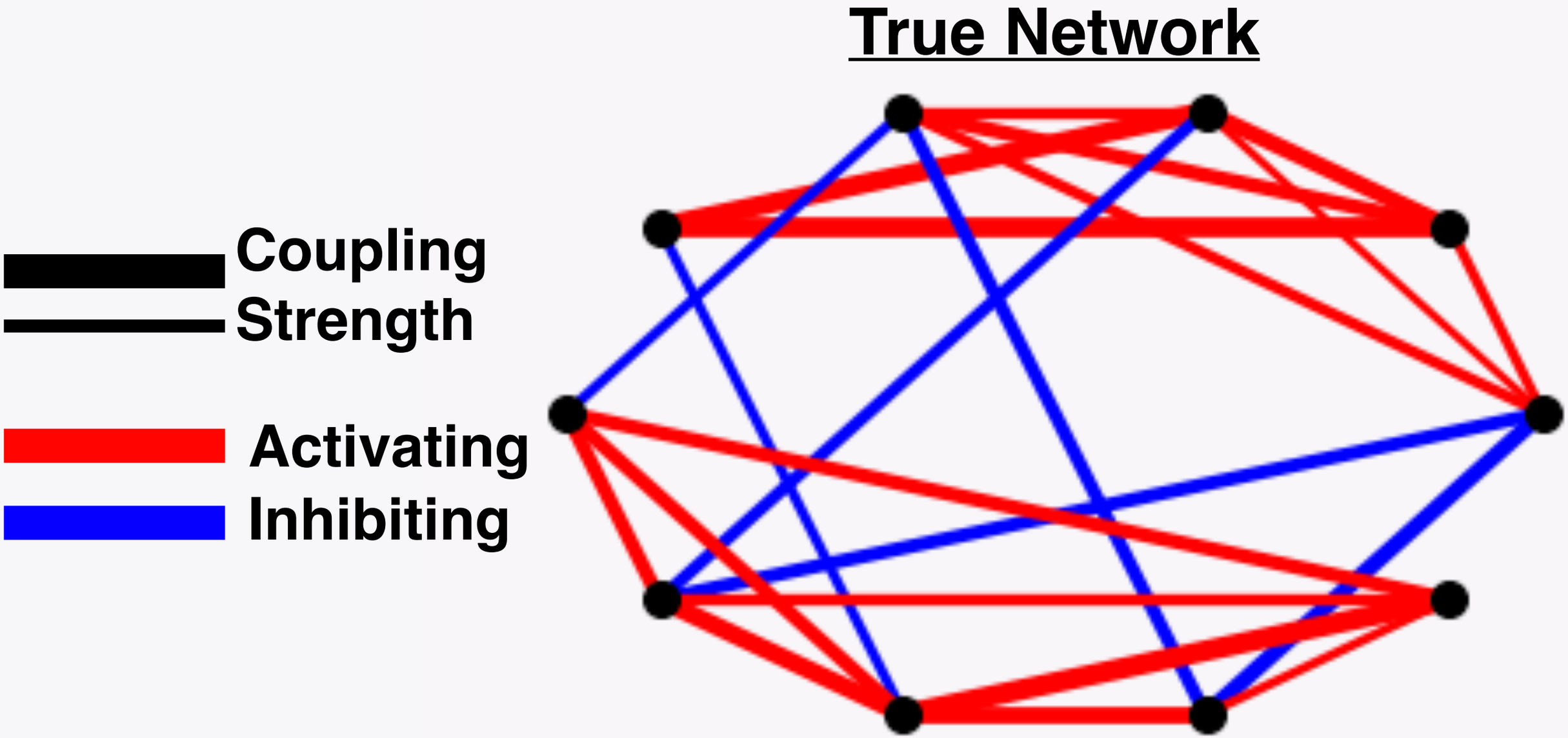
Transcriptomes as phenotypes: The geneticist's new arsenal

Null mutants (**Transcriptome-wide Epistasis**)

Allelic series (**Transcriptome-wide dominance**)

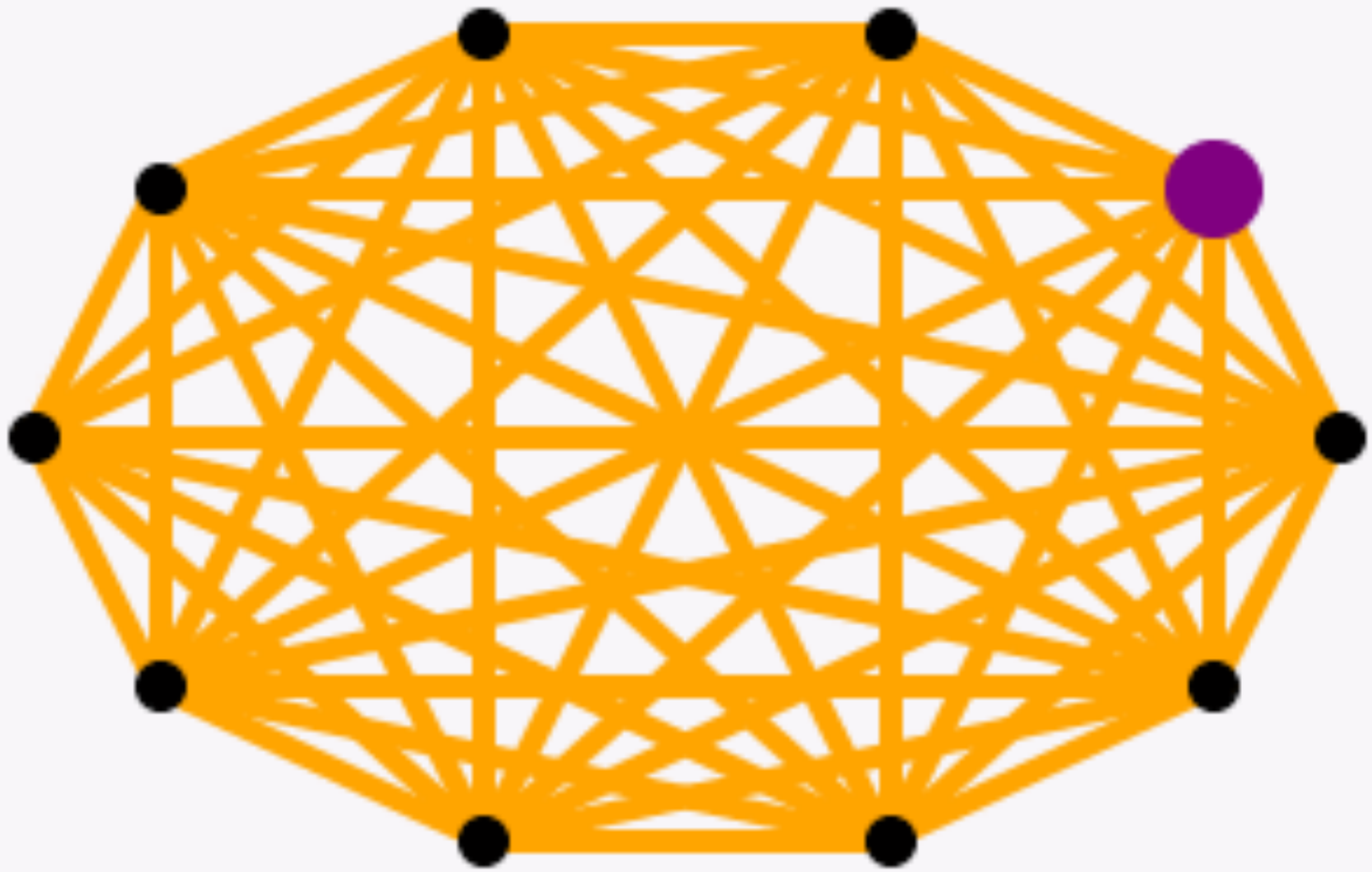
Crosses (**Transcriptome-wide maternal effects**)

Epistasis analyses can be automated



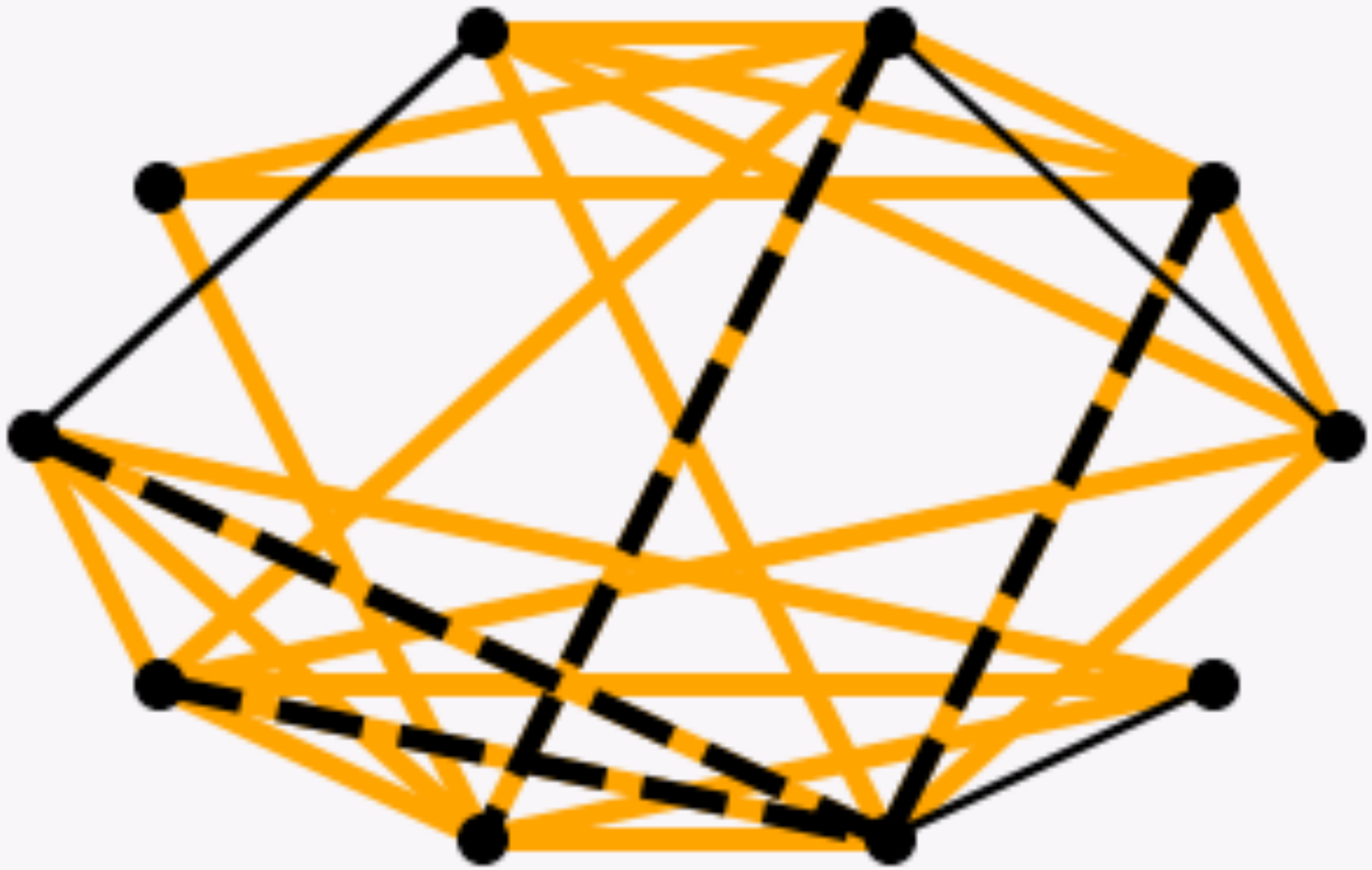
Collaboration with Matt Thomson

An example of automated reconstruction



Mutated Gene (Null)

Reconstructed network structure (no valences!)



Real edges

Missing edges (smaller = weaker)

Extra edges (should not be there)

Transcriptomes are phenotypes

Deploying transcriptomes in a **rich experimental context**
makes them powerful

We developed **statistical and conceptual machinery**
to use transcriptomes productively

Transcriptomes are Phenotypes!

Paul Sternberg

hhmi | Howard Hughes Medical Institute

Sternberg Lab
Carmie Puckett Robinson
Daniel Leighton
Tiffany Khaw
Tiffany Tsou
Hillel Schwartz

Barbara Wold
Brian Williams

Matt Thomson

NATIONAL INSTITUTES OF HEALTH

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