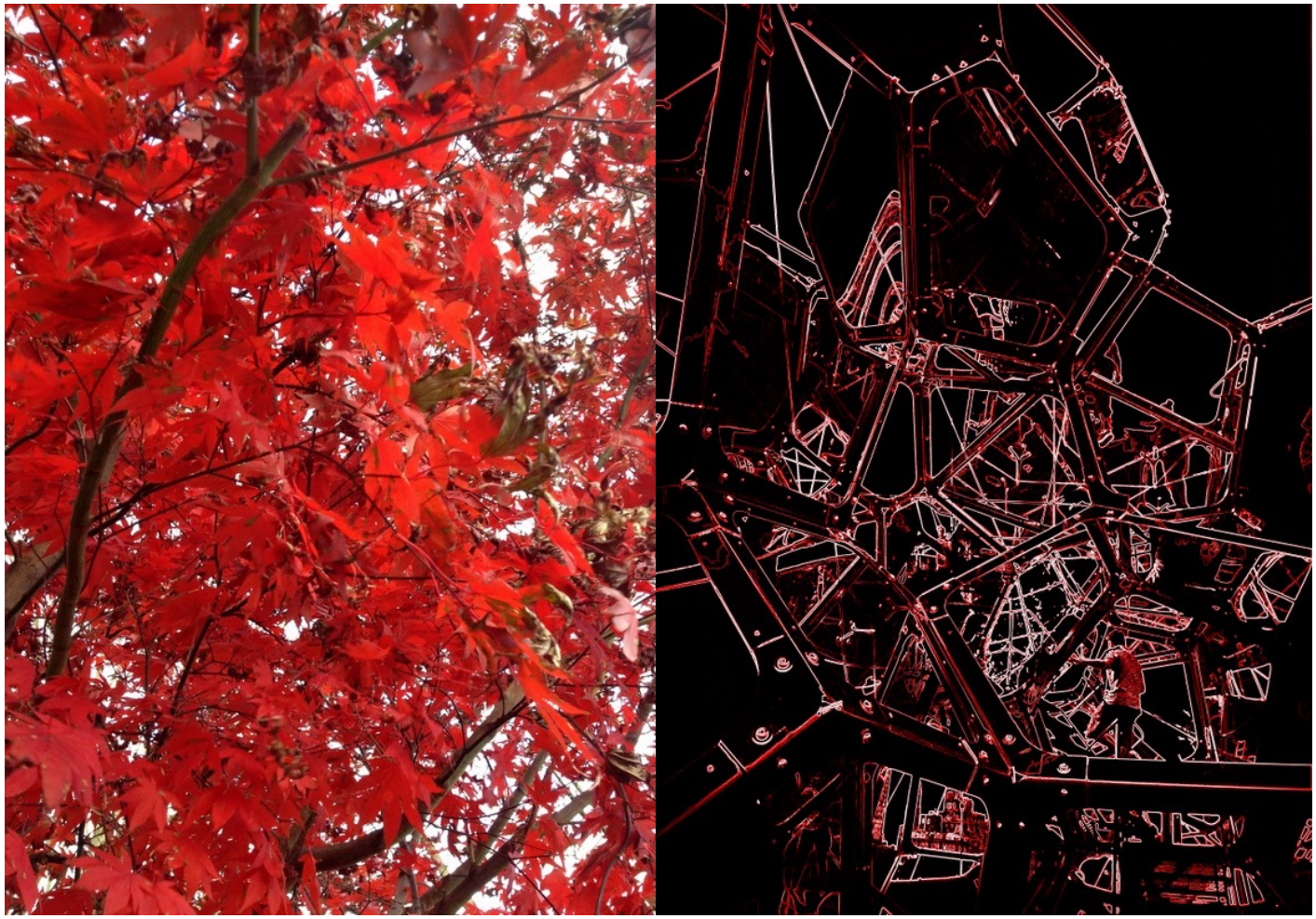
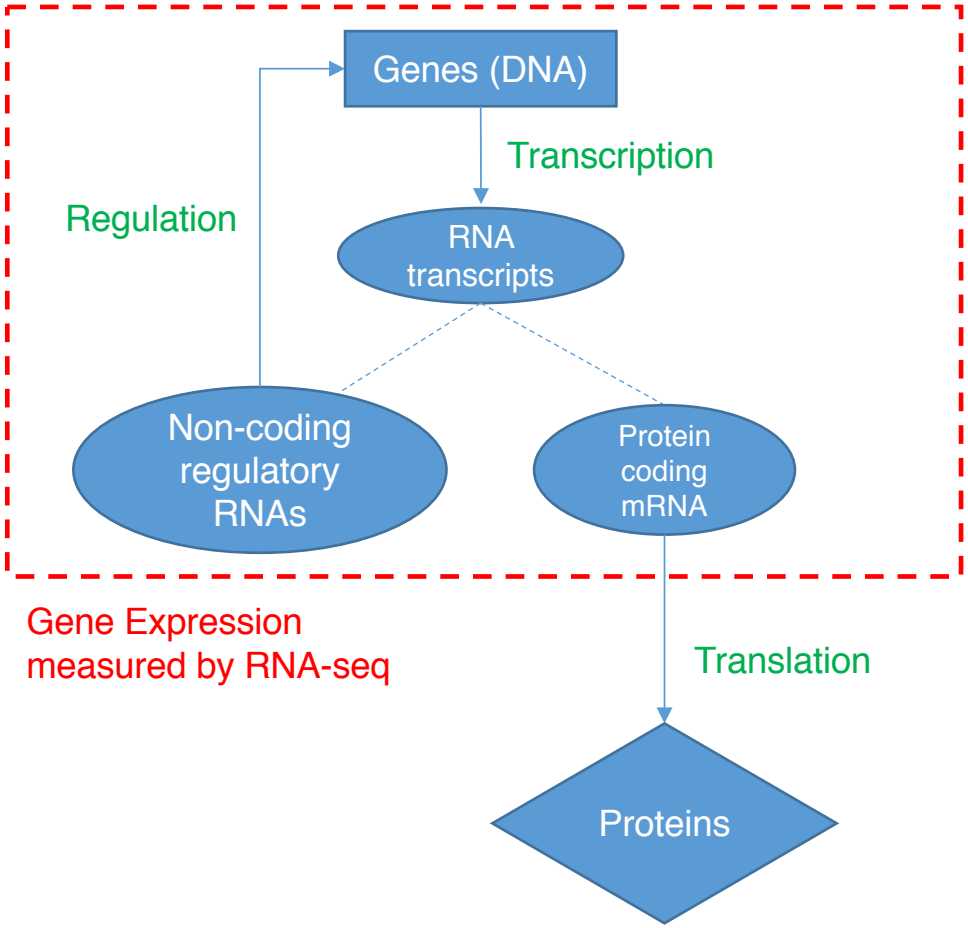
Transcriptome Analysis: Tackling core issues related to gene regulation & also mining the "data exhaust" produced by this activity

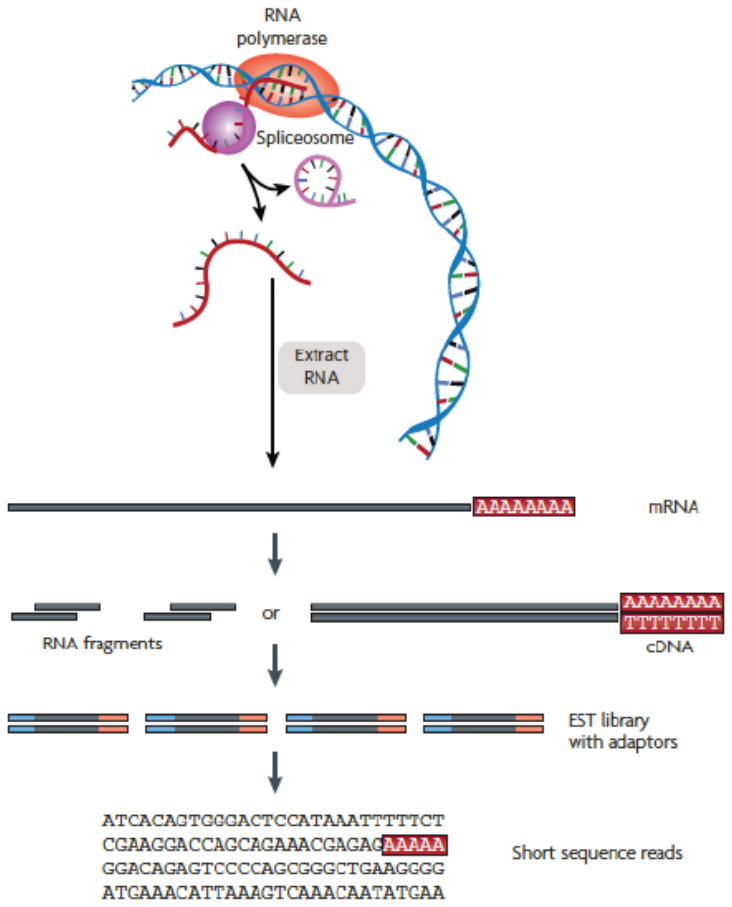


Mark Gerstein, Yale. Slides freely downloadable from **Lectures.GersteinLab.org & "tweetable"** (via @markgerstein). See last slide for more info.

Expression of genes is quantified by transcription: RNA-Seq measures mRNA transcript amounts



Gene Expression measured by RNA-seq



[*NATURE 459: 927; NAT. REV. GEN. 10: 57*]

Activity Patterns

- RNA Seq. gives rise to activity patterns of genes & regions in the genome

Some Core Science Qs Addressed by RNA-seq

- Gene activity as a function of:
  - Developmental stage: basic patterns and clusters of co-active genes across an organisms development
  - Evolutionary relationships: behavior preserved across a wide range of organisms; patterns and clusters in model organisms in relation to those in humans
  - Tissue- and cell-type: relationship of expression and specialized function
  - Disease phenotypes: disruption of patterns in disease

RNA-Seq Overview

Successive steps of Data Reduction

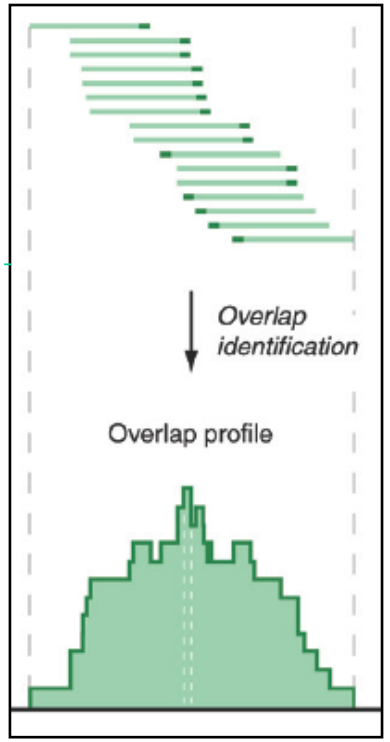
Fastq sequence files
~5-10 GB

```
ATACAAGCAAGTATAAGTTCGTATGCCGTCTT
GGAGGCTGGAGTTGGGGACGTATGCGGCATAG
TACCGATCGAGTCGACTGTAAACGTAGGCATA
ATTCTGACTGGTGTCATGCTGATGTACTTAAA
```

Index-building + Alignment to reference genome

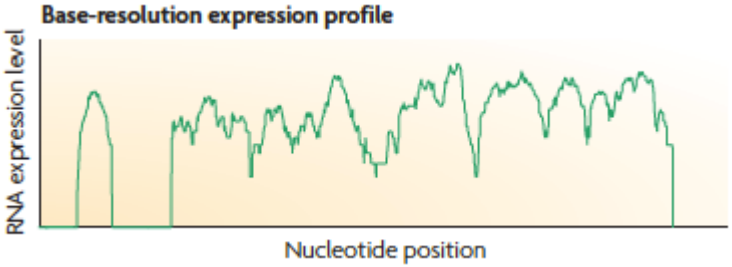
BAM files
~1-2-fold reduction

Conversion to signal track by overlapping reads



Reads => Signal

BigWig files
~25-fold reduction



Quantitative information from RNA-seq signal:
average signals at exon level (RPKMs)

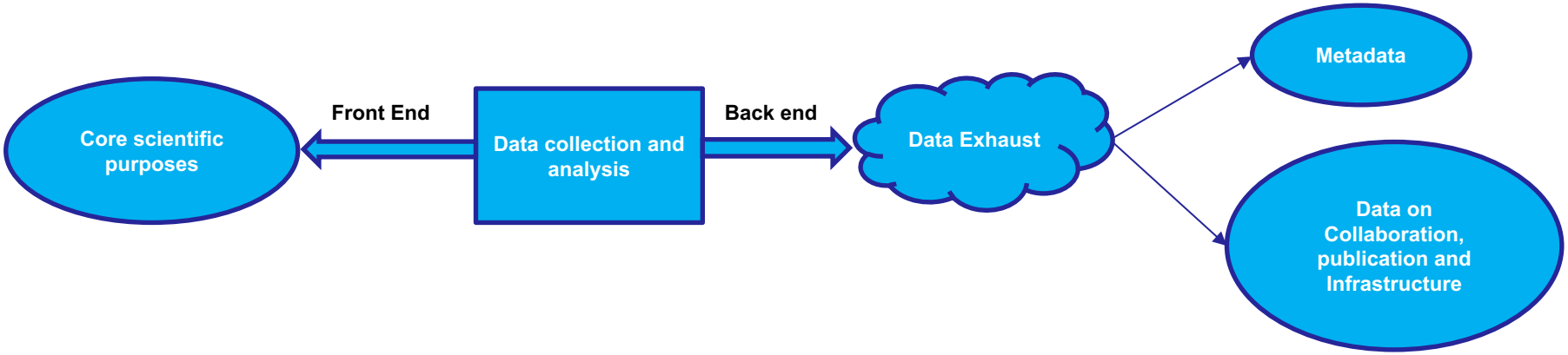
Mapping to genes

Gene/Transcript expression matrix
~20-fold reduction

[*NAT. REV.* 10: 57; *PLOS CB* 4:e1000158; *PNAS* 4:107: 5254]

Studying large-scale functional genomics data also produces

Data Exhaust



- Data Exhaust = Exploitable byproducts of big data collection and analysis

[PHOTO: RELAXNEWS; from http://www.lapresse.ca]

Transcriptome Analysis: Tackling core issues related to gene regulation & also mining the "data exhaust" produced by this activity

- *[Core-1]* **Expression Clustering**, Cross-species
  - Comparative ENCODE – Lots of worm-fly-human matched data & developmental timecourses
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  - Key role of brokers in data dissemination

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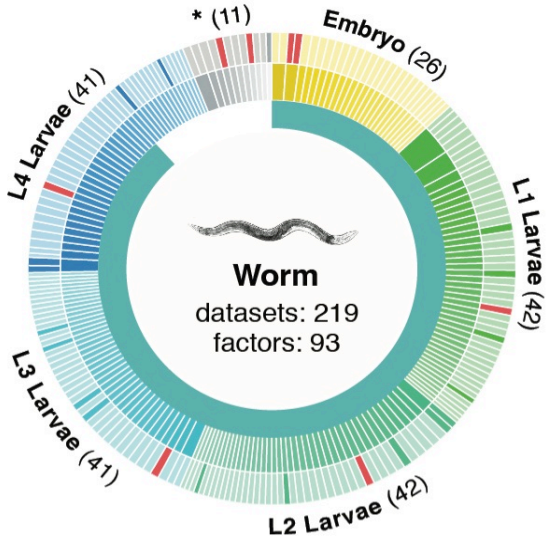
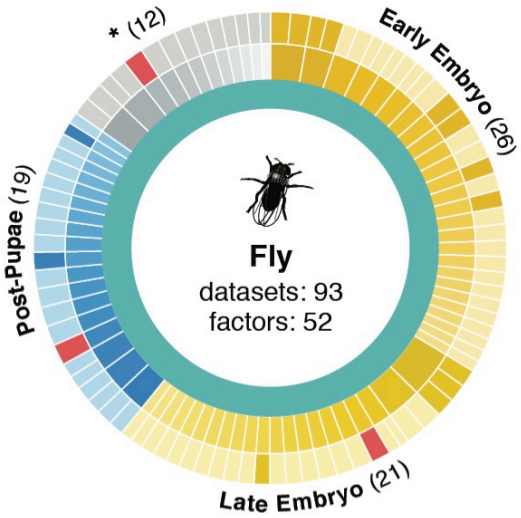
Time-course gene expression data of worm & fly development





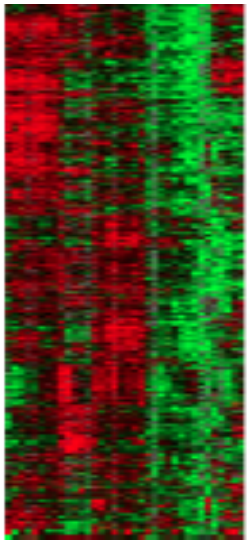
Comparative ENCODE Functional Genomics Resource (EncodeProject.org/comparative)

Organism	Major developmental stages
worm (*C. elegans*)	33 stages: 0, 0.5, 1, …, 12 hours, L1, L2, L3, L4, …, Young Adults, Adults
fly (*D. mel.*)	30 stages: 0, 2, 4, 6, 8,…, 20, 22 hours, L1-L4, Pupaes, Adults

- Broad sampling of conditions across transcriptomes & regulomes for human, worm & fly
  - embryo & ES cells
  - developmental time course (worm-fly)
- In total: ~3000 datasets (~130B reads)

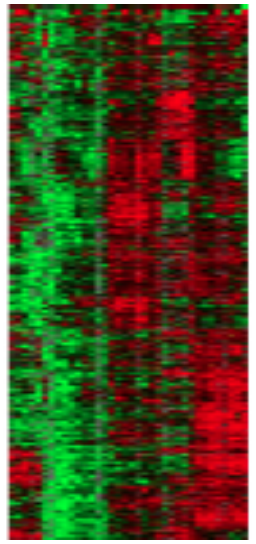
[*Nature* 512:445 ('14); doi: 10.1038/nature13424]

Expression clustering: revisiting an ancient problem



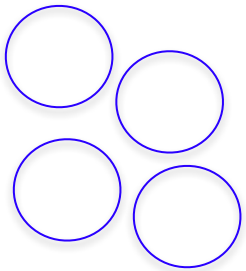
Species A

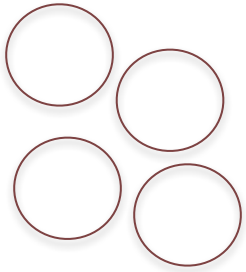
Species B



Clustering algorithm

Clustering algorithm



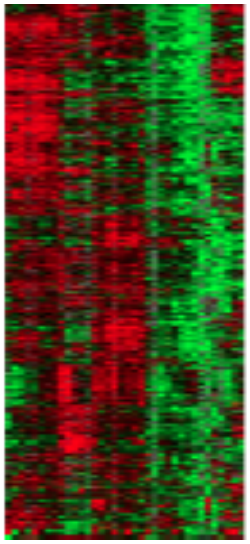


co-expressed genes responsible for the same function in a species

two independent sets of modules

Eisen MB et al. PNAS 1998
Langfelder P et al. BMC Bioinfo. 2008
Tamayo P et al. PNAS 1999
Kluger Y et al. Genome Res. 2003

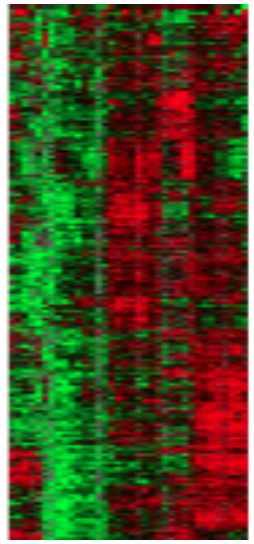
Expression clustering: revisiting an ancient problem



Species A

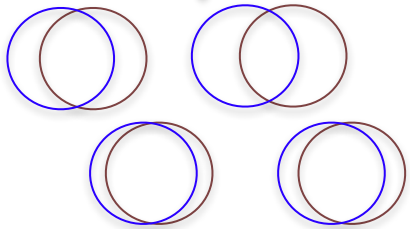
Orthologous pairs between species

Species B



A novel unified framework to integrate co-expression data across species

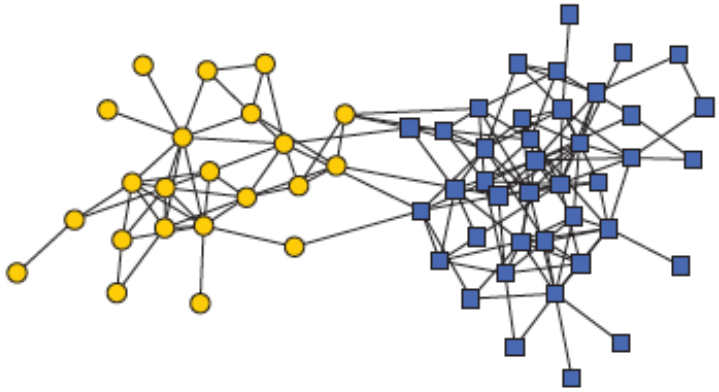
OrthoClust



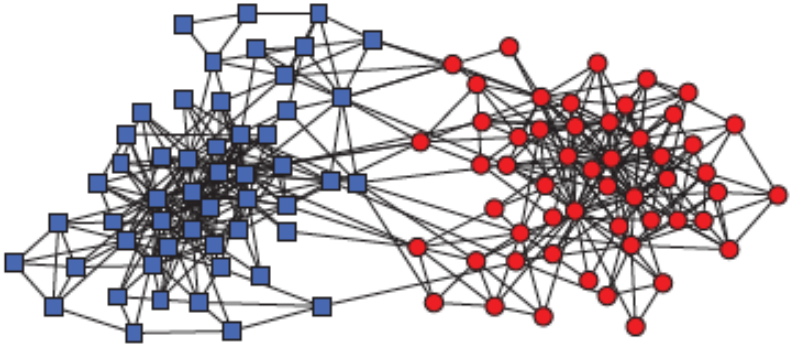
cross species modules

Yan et al. Genome Biol. 2014

Network modularity



Dolphin social network



Political books

Newman Phy. Rev. E 2013

$$Q = \frac{1}{2m} \sum_{i,j} \left(W_{ij} - \frac{k_i k_j}{2m} \right) \delta_{\sigma_i \sigma_j}$$

adjacency matrix

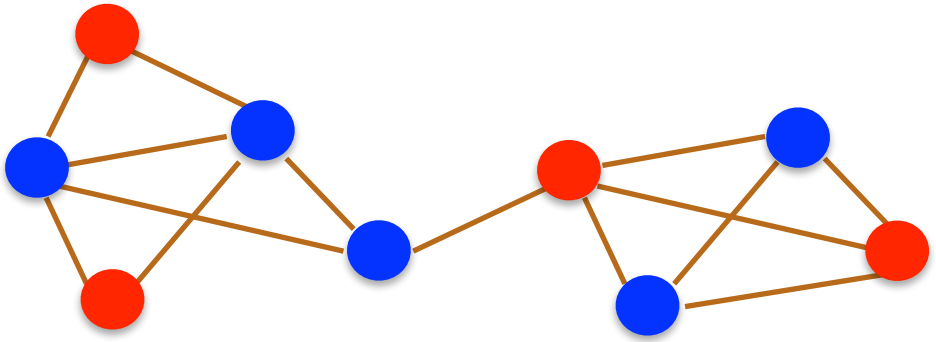
degree of node i

number of edges

expected number of edges between i and j

whether or not i, j are in the same module

Network modularity



$$Q \approx 0$$

$$Q = \frac{1}{2m} \sum_{i,j} \left(W_{ij} - \frac{k_i k_j}{2m} \right) \delta_{\sigma_i \sigma_j}$$

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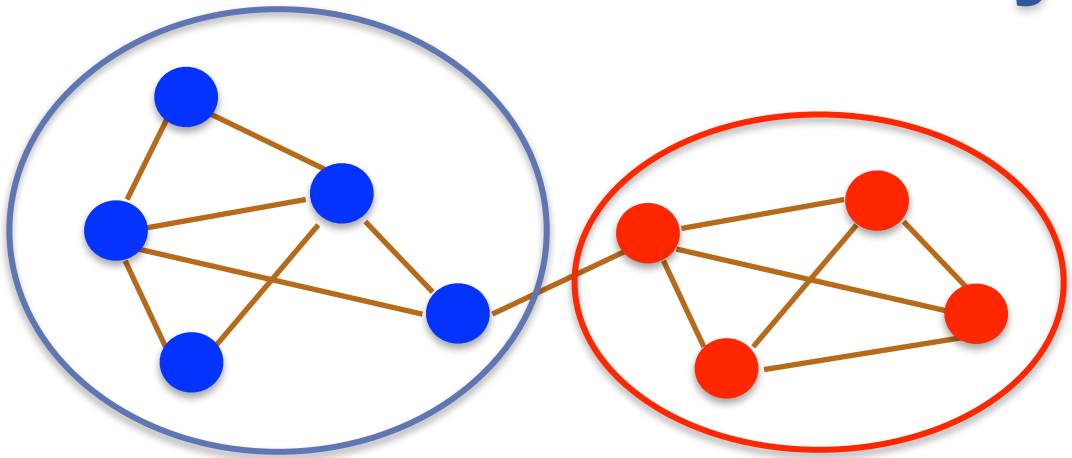

$$Q = Q_{max}$$

Optimization problem for sim. annealing

$$Q = \frac{1}{2m} \sum_{i,j} \left(W_{ij} - \frac{k_i k_j}{2m} \right) \delta_{\sigma_i \sigma_j}$$

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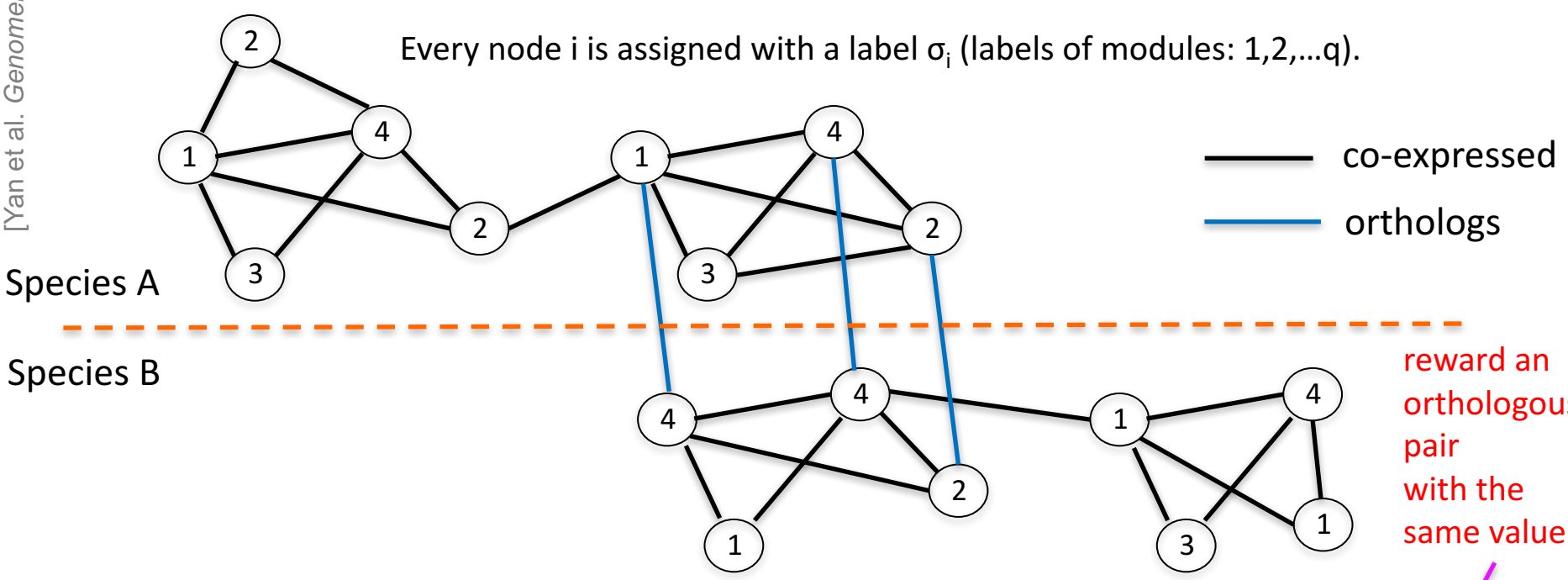
A toy example [orthoclust]

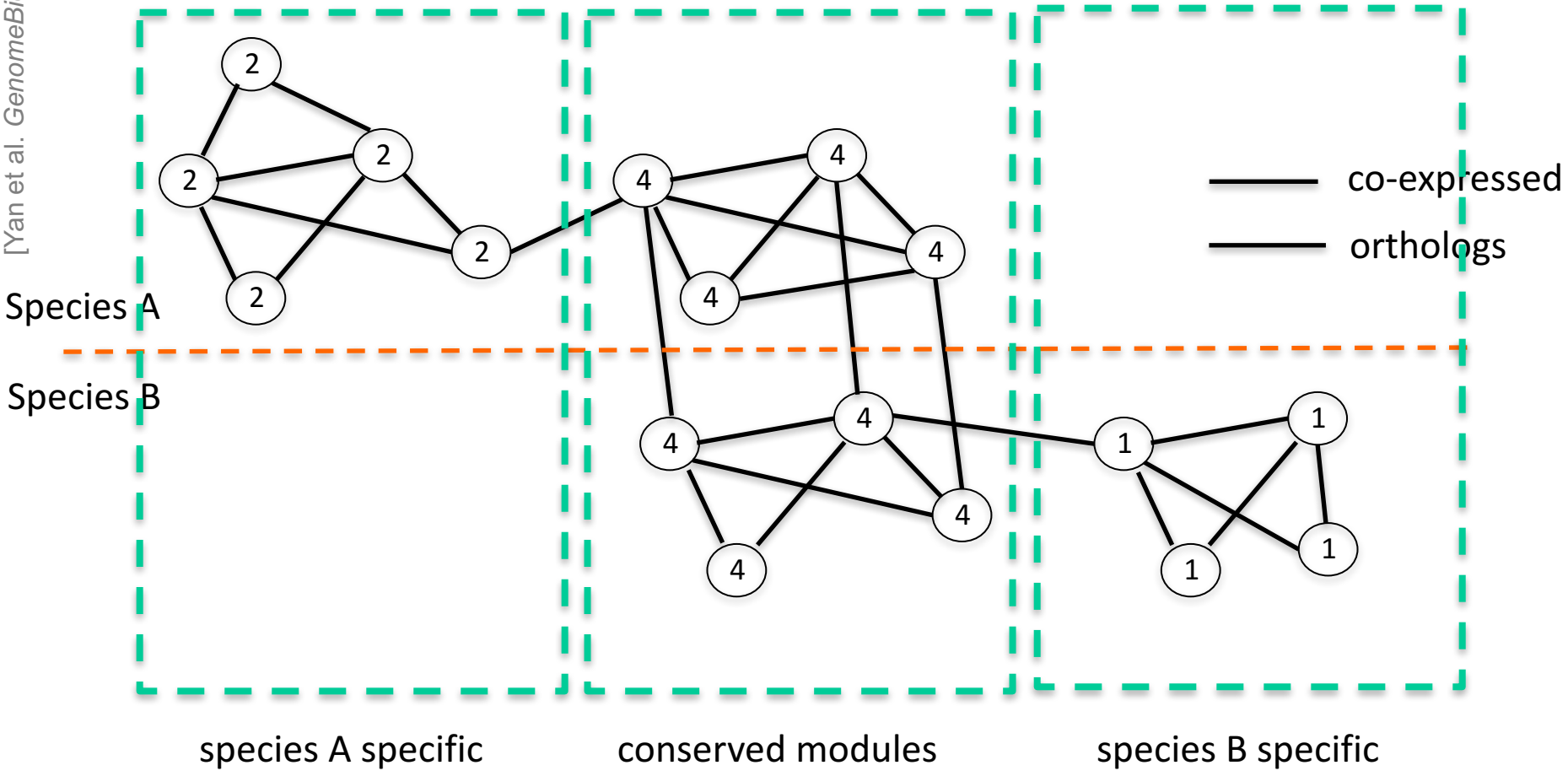
Every node i is assigned with a label σ_i (labels of modules: 1,2,…q).



$$H = \boxed{Q(\text{for all } \sigma_i \text{ in A}) \quad + \quad Q(\text{for all } \sigma_i \text{ in B})} + \kappa \boxed{\sum_{(i,j')\in Ortho} \delta_{\sigma_i \sigma_{j'}}}$$

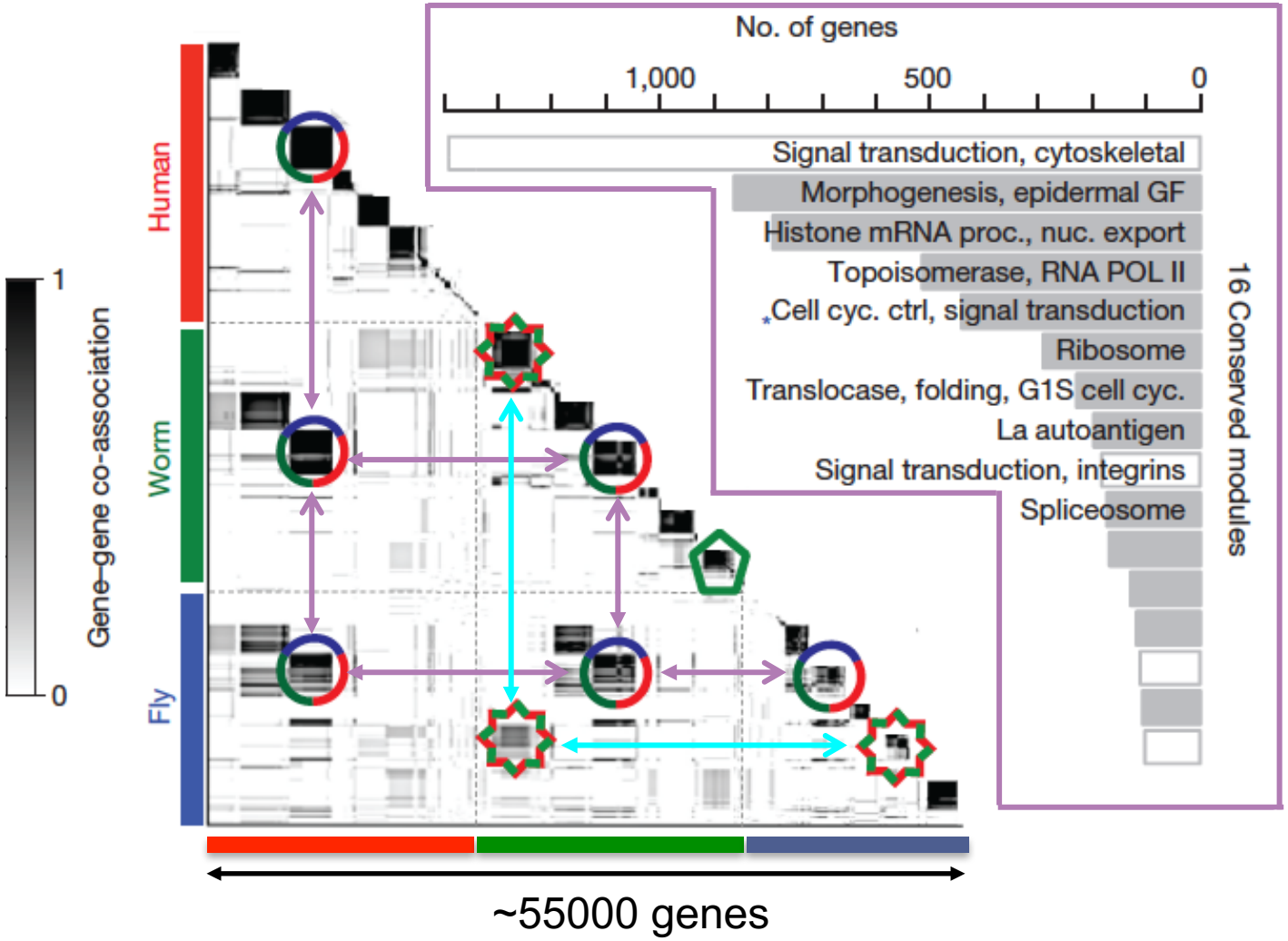
Favorableness = "Modularity" in species A + "Modularity" in species B + consistency betw. A & B

A toy example [orthoclust]



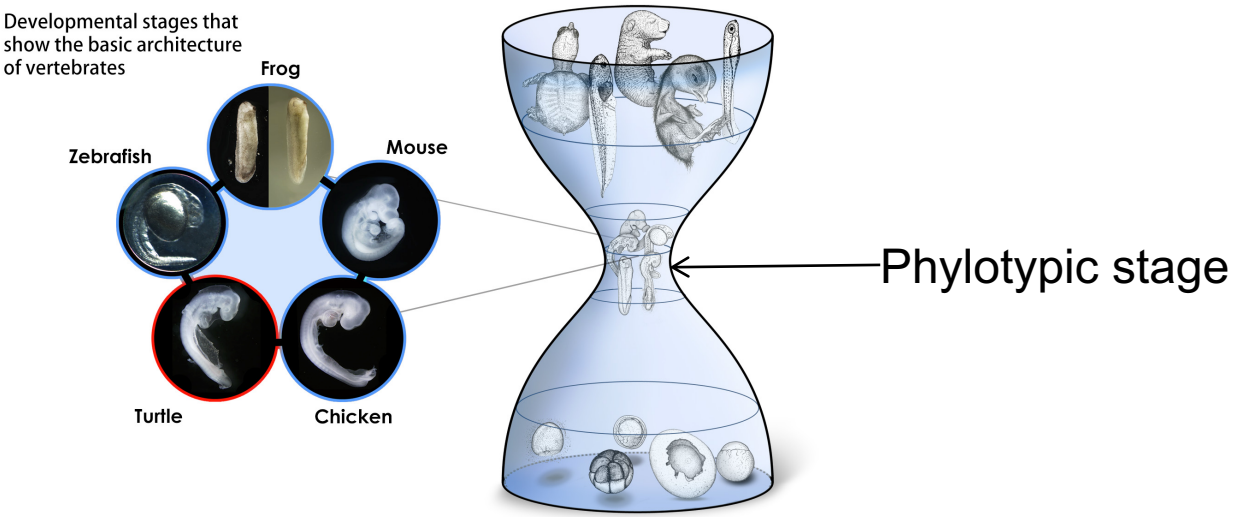
Use Potts model (generalized Ising model) to simultaneously cluster co-expressed genes within an organism as well as orthologs shared between organisms. Here, the ground state configuration correspond to three modules: 1, 2, 4.

Application for more than 2



[*Nature* 512:445 ('14); doi: 10.1038/nature13424]

Conserved modules exhibit canonical hourglass behavior



Illustrations courtesy Naoki Irie

Canonical Inter-organism Behavior

- "Hourglass hypothesis": all organisms go through a particular stage in embryonic development ("phylotypic" stage) where inter-organism expression differences of orthologous genes are smallest.
- **We identify modules (12 out of 16) which have this behavior at the phylotypic stage.**

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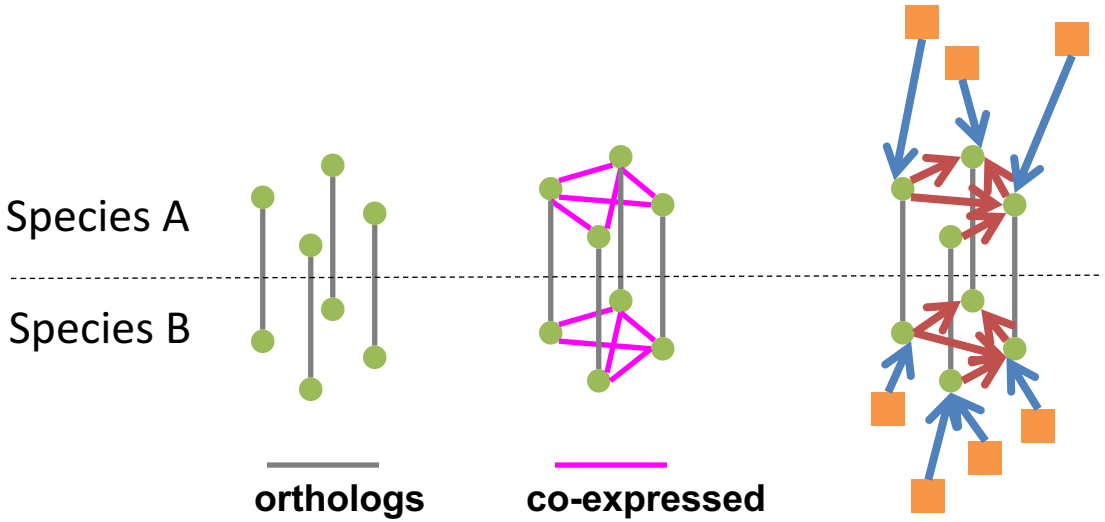
Are gene regulations among orthologs conserved across species?



Are gene regulatory networks among orthologs conserved across species?

Regulation among orthologs (**internal**)

Regulation from species-specific factors (**external**)

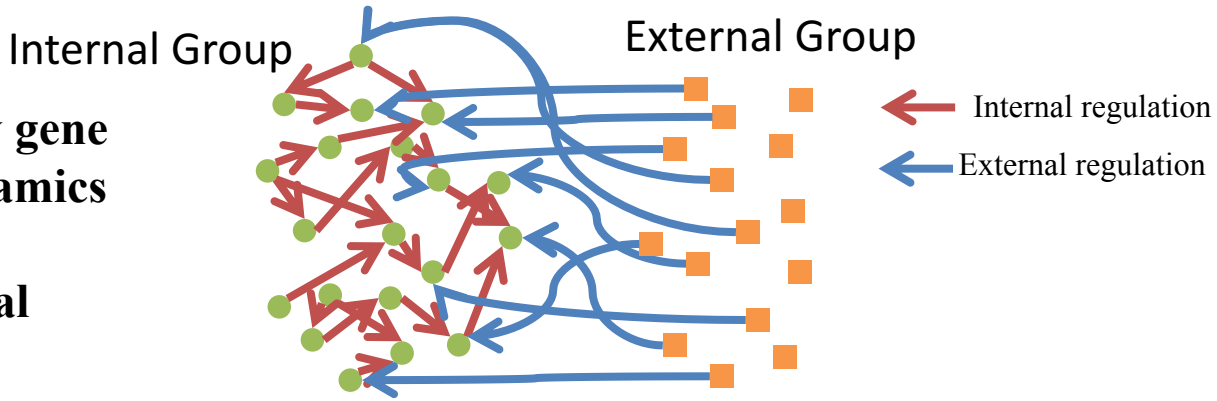
Orthologous genes (orthologs)

Species-specific transcription factors

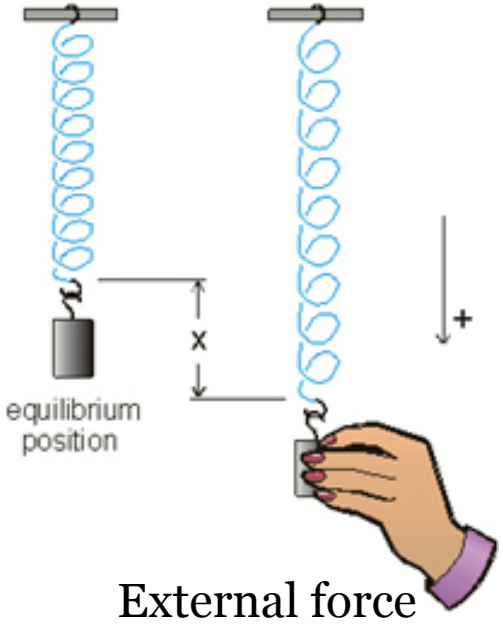
To what degree can't ortholog expression levels be predicted due to species-specific regulation

[Wang et al. *PLOS CB*, '16]

Internal & external gene regulatory networks



How to identify gene expression dynamics driven by internal/external regulation?

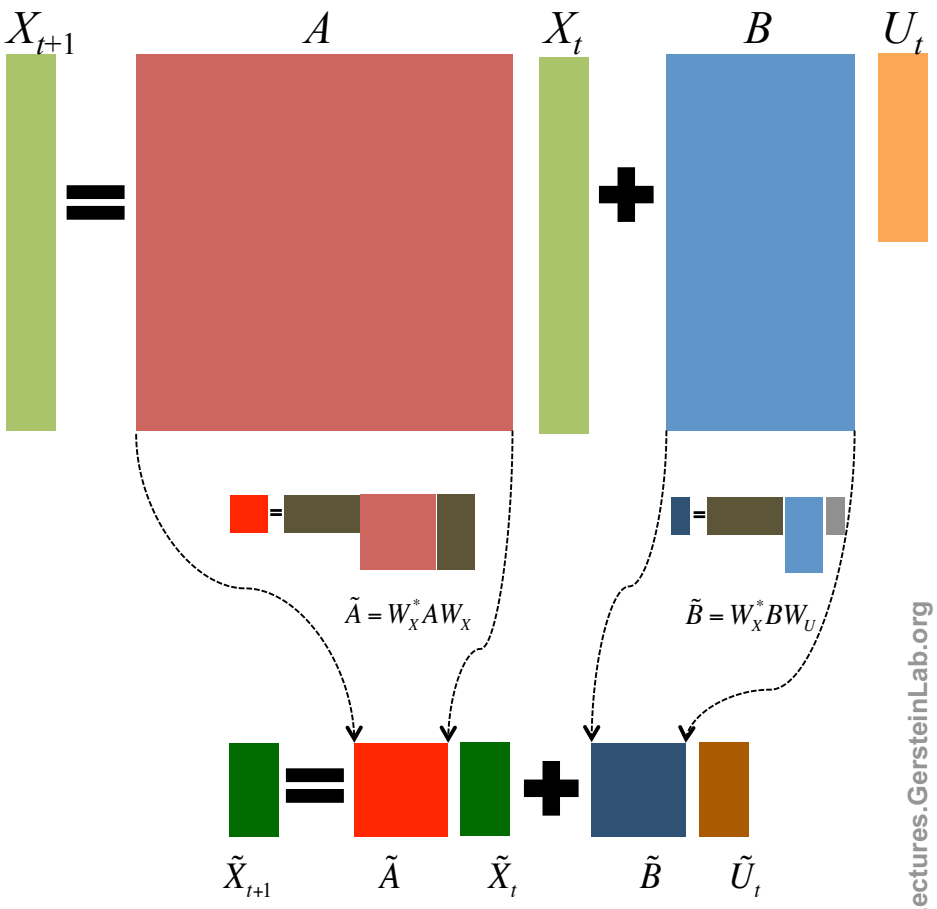


Interested system	Internal regulatory network	External regulatory network
Cross-species conserved genes	Conserved transcriptional factors (TFs)	Non-conserved TFs
Protein-coding genes	TFs	micro-RNAs
Individual's protein coding genes	Wild-type TFs	Somatic mutated TFs
Protein-coding genes in brain	Commonly expressed TFs	Brain-specific expressed TFs
Protein-coding genes in development	House-keeping TFs	Developmental TFs

[Wang et al. *PLOS CB* (in revision, '15)]

State-space model for internal and external gene regulatory networks

- **State X_{t+1}:** Gene expression vector of Group X at time t+1
- A_{ij} captures temporal casual influence from Gene i to Gene j in internal group
- **State X_t:** Gene expression vector of internal group at time t
- B_{kl} captures temporal casual influence from external factor k to Gene l in internal group
- **Control U_t:** Gene expression vector of external factors at time t



[Wang et al. *PLOS CB*, '16]

State-space model for internal and external gene regulatory networks

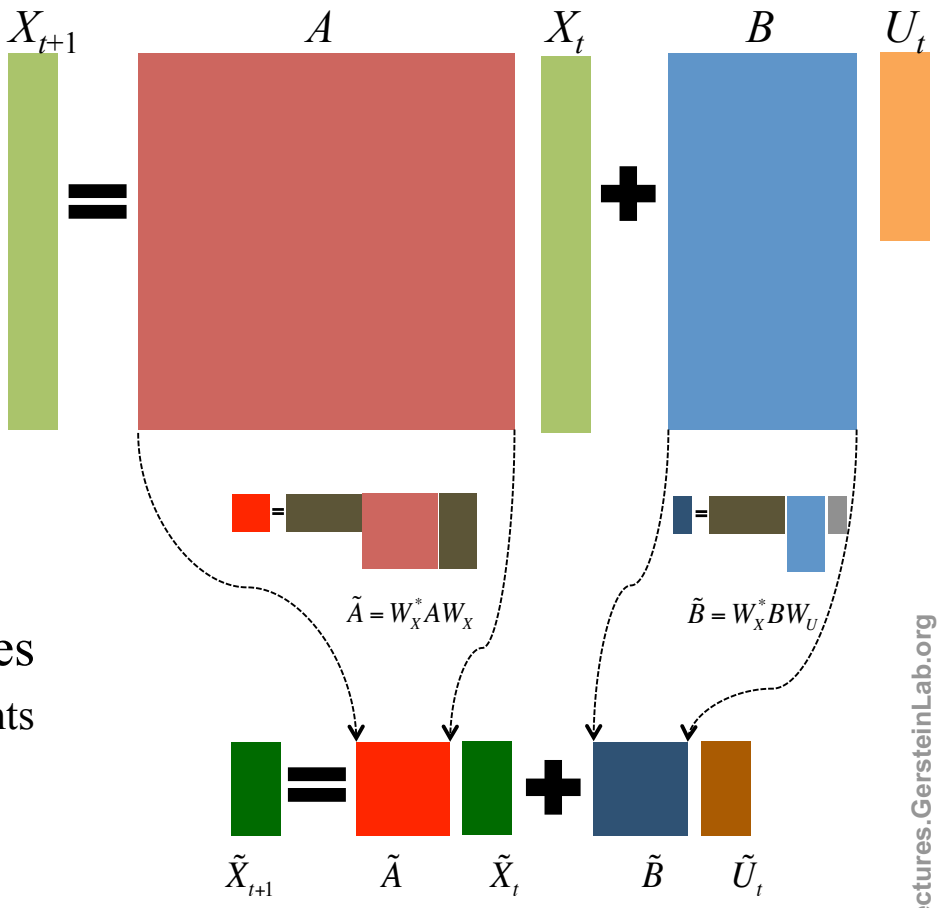
Not enough data to estimate state space model for genes
(e.g., 25 time points per gene to estimate 4 million elements of A or B for 2000 genes)

$$X_{t+1} = AX_t + BU_t$$

Dimensionality reduction from genes to meta-genes (e.g., SVD)

Effective state space model for meta-genes
(e.g., 250 time points to estimate 50 matrix elements if 5 meta-genes)

$$\tilde{X}_{t+1} = \tilde{A}\tilde{X}_t + \tilde{B}\tilde{U}_t$$



[Wang et al. *PLOS CB*, '16]

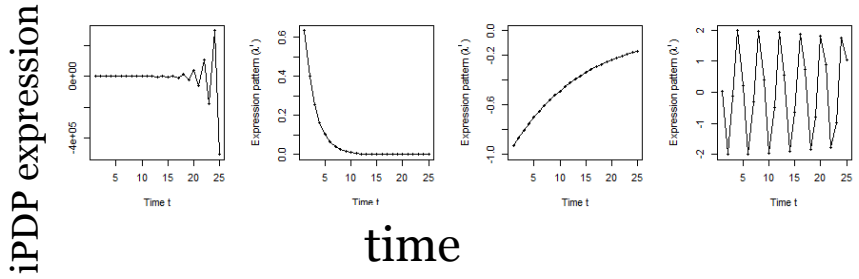
Canonical temporal expression trajectories from effective state space model

Is a std. 1st order homogeneous matrix difference equation. It can solved by diagonalizing A giving….

$$\tilde{X}_{t+1} = \tilde{A}\,\tilde{X}_t + \tilde{B}\,\tilde{U}_t$$

p^{th} internal principal dynamic pattern (iPDP): $[\lambda_p{}^1, \lambda_p{}^2, \ldots, \lambda_p{}^T]$,
where λ_p is p^{th} eigenvalue of $\tilde{A}$.

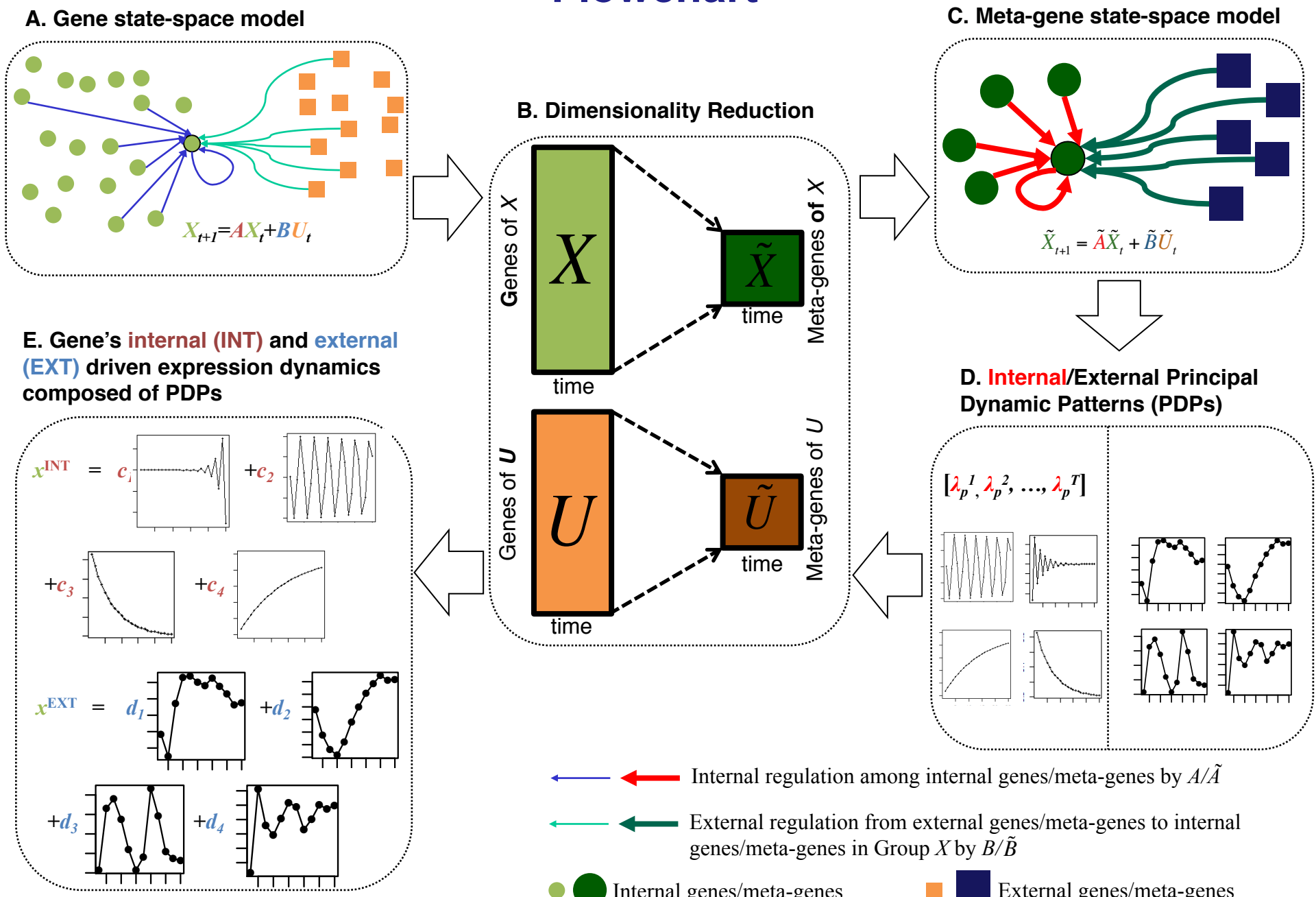
Canonical temporal expression trajectories
(e.g., degradation, growth, damped oscillation, etc.)



[Wang et al. *PLOS CB*, '16]

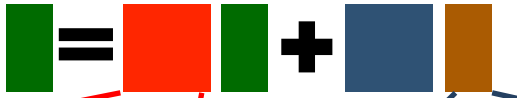
[Wang et al. *PLOS CB*, '16]

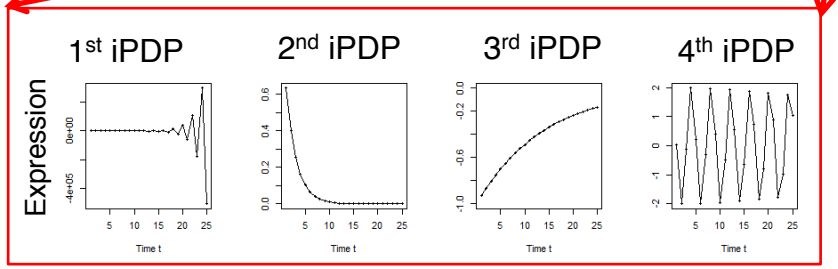
Flowchart

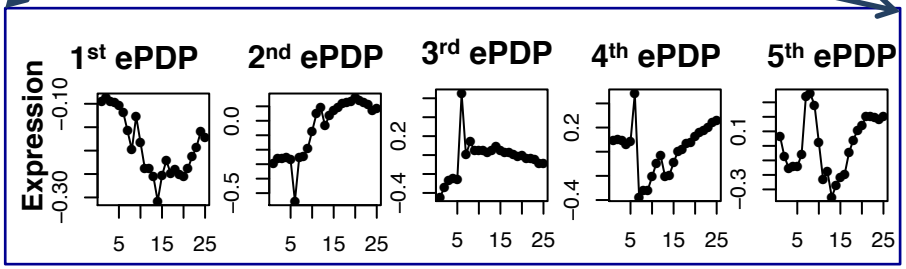


Orthologs have similar internal but different external dynamic patterns during embryonic development

Worm's effective state space model

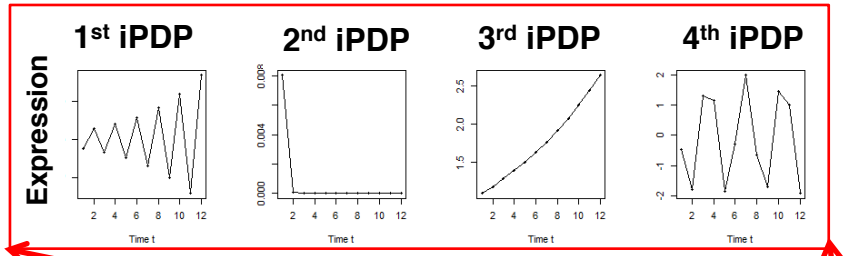


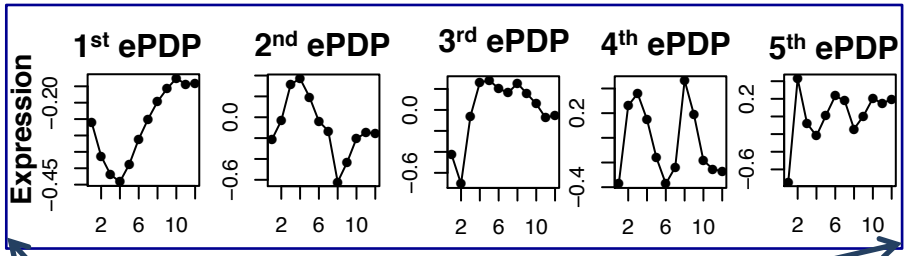




Similar

Different



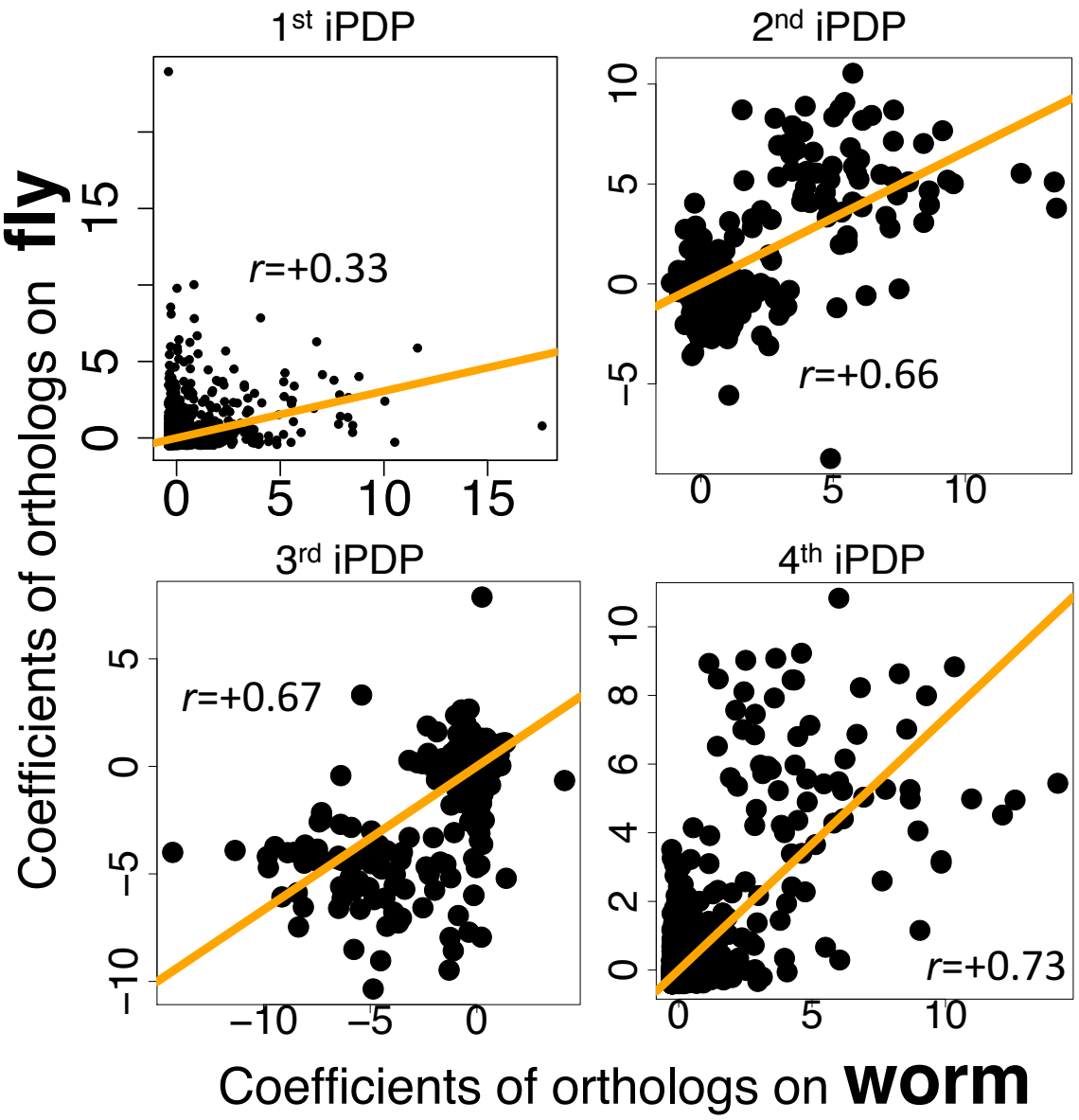


Fly's effective state space model

$$\tilde{X}_{t+1} = \tilde{A}\tilde{X}_t + \tilde{B}\tilde{U}_t$$

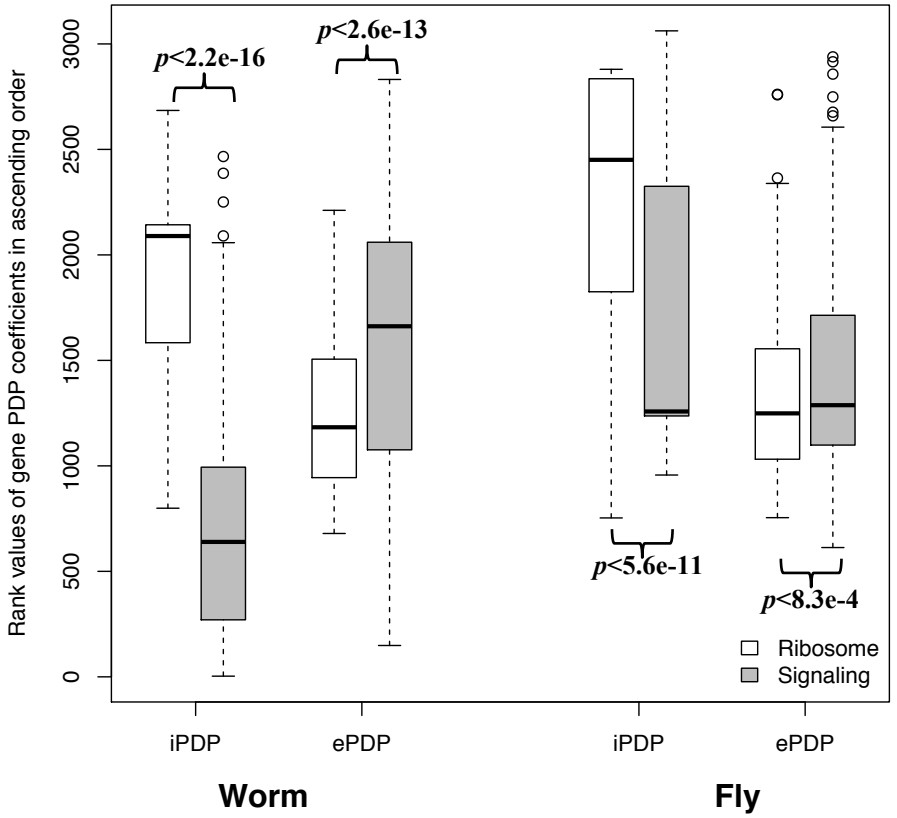
[Wang et al. *PLOS CB*, '16]

Orthologs have correlated iPDP coefficients



[Wang et al. *PLOS CB*, '16]

Evolutionarily conserved & younger genes exhibit the opposite internal & external PDP coefficients



Ribosomal genes have significantly larger coefficients for the internal than external PDPs, but signaling genes exhibit the opposite trend

[Wang et al. *PLOS CB*, '16]

Transcriptome Analysis: Tackling core issues related to gene regulation & also mining the "data exhaust" produced by this activity

- *[Core-1]* **Expression Clustering**, Cross-species
  - Comparative ENCODE – Lots of worm-fly-human matched data & developmental timecourses
  - Optimization gives 16 conserved co-expression modules
- *[Core-2]* **State Space Models** of Gene Expression
  - Using dimensionality reduction to help determine internal & external drivers
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  - Also, conserved genes have similar canonical patterns (iPDPs) in contrast to species specific ones (Ex of ribosomal v signaling genes)
- *[Exhaust-1]* **Genomic Privacy & RNA-seq**
  - The dilemma: The genome as fundamental, inherited info that's very private v need for large-scale mining for med. research
  - Issues w/ current social & tech approaches: inconsistencies & burdensome security
  - RNA-seq presents a particularly tricky privacy issue
  - Quantifying & removing variant info from expression levels + eQTLs using ICI & predictability
  - Instantiating a practical linking attack using extreme expression levels
- *[Exhaust-2]* **Value of Publication Patterns** generated by data producing consortia
  - Co-authorship network statistics relate to publication rollouts & show gradual adoption by a diverse community
  - Key role of brokers in data dissemination

2-sided nature of functional genomics data: Analysis can be very General/Public or Individual/Private

- General quantifications related to overall aspects of a condition & are not tied to an individual's genotype - ie what genes go up in cancer
  - However, data is derived from an individual & tagged with an individual's genotype
- Other calculations aim to use genotype & specific aspects of the quantification to derive general relations related to sequence variation & gene expression
- Some calculations and data derive finding very specific to the variants in a particular individual

Tricky Privacy Considerations in Personal Genomics

- **Genetic Exceptionalism :** The Genome is very fundamental data, potentially very revealing about one's identity & characteristics
- **Personal Genomic info. essentially meaningless currently but will it be in 20 yrs? 50 yrs?**
  - Genomic sequence very revealing about one's children. Is true consent possible?
  - Once put on the web it can't be taken back
- **Culture Clash:** Genomics historically has been a proponent of "open data" but not clear personal genomics fits this.
  - Clinical Medline has a very different culture.
- **Ethically challenged** history of genetics
  - Ownership of the data & what consent means (Hela)
    - Could your genetic data give rise to a product line?

[D Greenbaum & M Gerstein ('08). Am J. Bioethics; D Greenbaum & M Gerstein, Hartford Courant, 10 Jul. '08 ; SF Chronicle, 2 Nov. '08; Greenbaum et al. *PLOS CB* ('11) ; Greenbaum & Gerstein ('13), The Scientist; Photo from NY Times]

Genomics has similar "Big Data" Dilemma in the Rest of Society

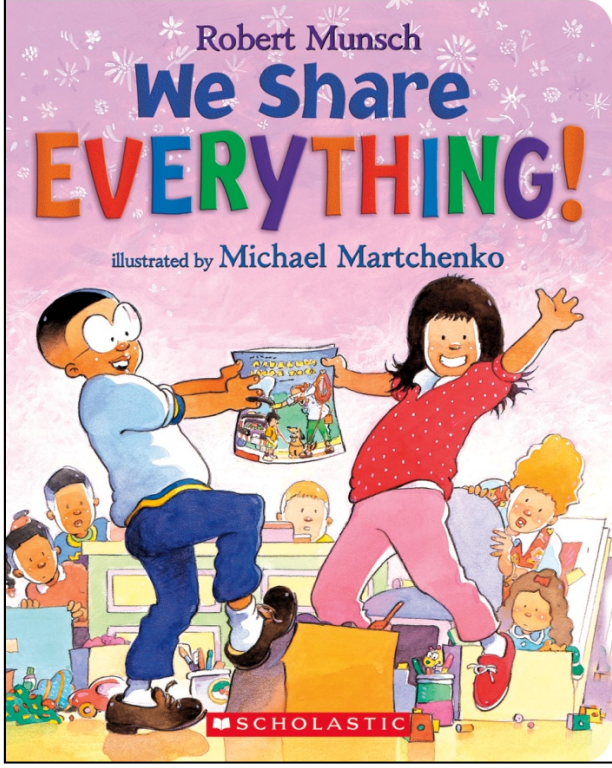
- Sharing & "peer-production" is central to success of many new ventures, with the same risks as in genomics
  - **EG web search**: Large-scale mining essential

- We confront privacy risks every day we access the internet
- (...or is the genome more exceptional & fundamental?)

[Seringhaus & Gerstein ('09), *Hart. Courant* (Jun 5); Greenbaum & Gerstein ('11), *NY Times* (6 Oct)]

The Other Side of the Coin: Why we should share

- Sharing helps **speed research**
  - Large-scale mining of this information is important for medical research
  - Privacy is cumbersome, particularly for big data
- Sharing is important for **reproducible research**
- Sharing is useful for **education**
  - More fun to study a known person's genome
    - Eg Zimmer's Game of Genomes in STAT



[Yale Law Roundtable ('10). Comp. in Sci. & Eng. 12:8; D Greenbaum & M Gerstein ('09). Am. J. Bioethics; D Greenbaum & M Gerstein ('10). SF Chronicle, May 2, Page E-4; Greenbaum et al. *PLOS CB* ('11)]

[Economist, 15 Aug '15]

The Dilemma

- The individual (harmed?) v the collective (benefits)
  - But do sick patients care about their privacy?
- How to balance risks v rewards - Quantification
  - What is acceptable risk? What is acceptable data leakage? Can we quantify leakage?
    - Ex: photos of eye color
  - Cost Benefit Analysis: how helpful is identifiable data in genomic research v. potential harm from a breach?

Current Social & Technical Solutions

- **Closed Data** Approach
  - Consents
  - "Protected" distribution via dbGAP
  - Local computes on secure computer
- Issues with Closed Data
  - Non-uniformity of consents & paperwork
    - Different international norms, leading to confusion
  - Encryption & computer security creates burdensome requirements on data sharing & large scale analysis
  - Many schemes get "hacked"

- **Open Data**
  - Genomic "test pilots" (ala PGP)?
    - Sports stars & celebrities?
  - Some public data & data donation is helpful but is this a realistic solution for an unbiased sample of ~1M

[Greenbuam et al ('04), Nat. Biotech; Greenbaum & Gerstein ('13), The Scientist]

Strawman Hybrid Social & Tech Proposed Solution?

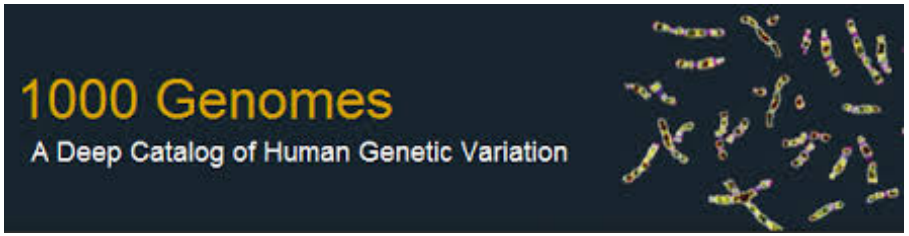
- Fundamentally, researchers have to keep genetic secrets.
  - **Need for an (international) legal framework**
  - Genetic Licensure & training for individuals (similar to medical license, drivers license)
- Technology to make things easier
  - Cloud computing & enclaves (eg solution of Genomics England)
- Technological barriers shouldn't create a social incentive for “hacking”

- **Quantifying Leakage & allowing a small amounts of it**
- **Careful separation & coupling of private & public data**
  - **Lightweight, freely accessible secondary datasets coupled to underlying variants**
  - Selection of stub & "test pilot" datasets for benchmarking
  - Develop programs on public stubs on your laptop, then move the program to the cloud for private production run

[D Greenbaum, M Gerstein (‘11). Am J Bioeth 11:39. Greenbaum & Gerstein, The Scientist ('13)]

Representative Expression, Genotype, eQTL Datasets

- Genotypes are available from the 1000 Genomes Project
- mRNA sequencing for 462 individuals from gEUVADIS and ENCODE
  - Publicly available quantification for protein coding genes
- Approximately 3,000 cis-eQTL (FDR<0.05)



Variants directly in the reads

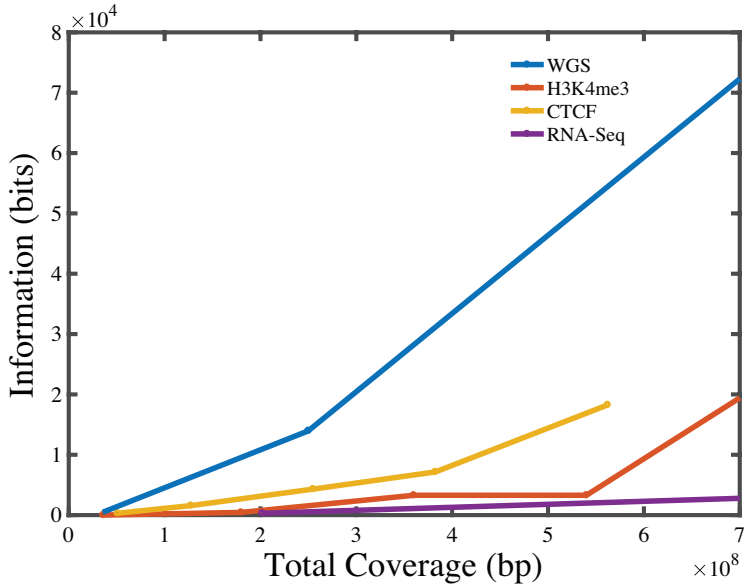
- Functional genomics data comes with a great deal of sequencing
  - NA12878 as case study - 1000 genomes variants are used as gold standard
- How much information, for example, do RNA-Seq reads (or ChIP-Seq) reads contain? Does that information enough to identify individuals?

Variants from RNA-Seq reads

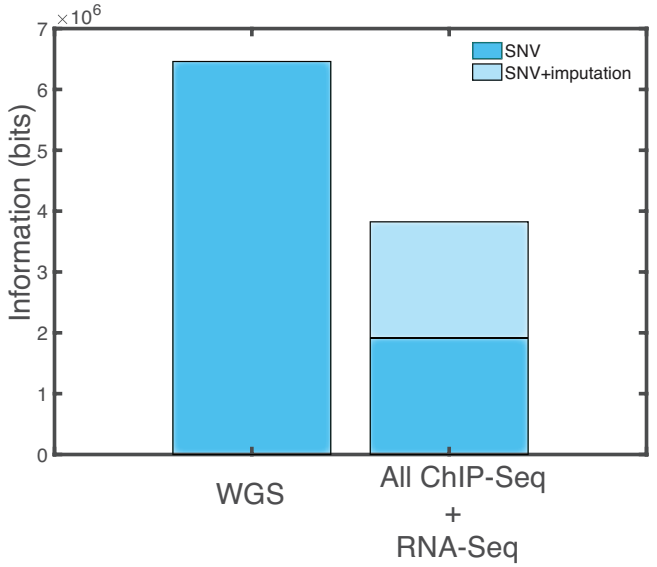
```
R1   start1   end1    ATAAATGAGGATTTAGAGGTGGTGACC
reference genome      ATAAATGAGAATTTTGAGGTGGTGACC

R2   start2   end2    T-- ATTTTCTCTCATACCACCTCAACG
reference genome      TTTATTTTCT ---  ATACCACCTCAACG

R3   start3   end3    TTTATTTTCTATACCACCTCAA
```

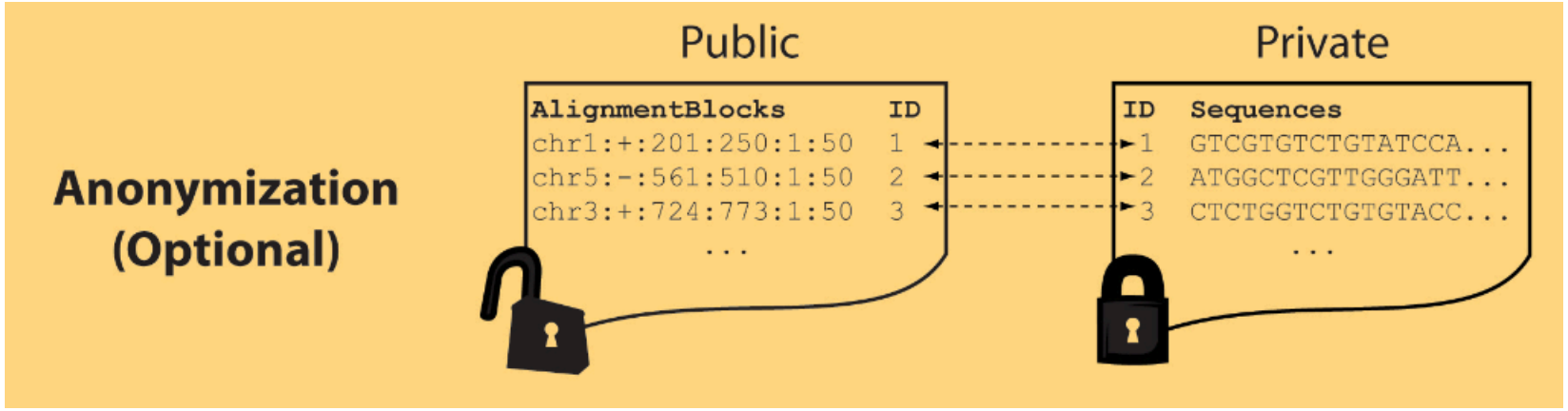


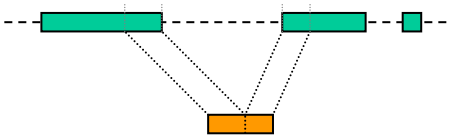
- It might seem like we don't infer much information from single ChIP-Seq and RNA-Seq experiments compared to WGS
  - However putting 10 different ChIP-Seq experiments and RNA-Seq together with imputation provides a great deal of information about the individual



Light-weight formats to Hide Most of the Read Data (Signal Tracks)

- Some lightweight format clearly separate public & private info., aiding exchange
- Files become much smaller
- Distinction between formats to compute on and those to archive with – become sharper with big data

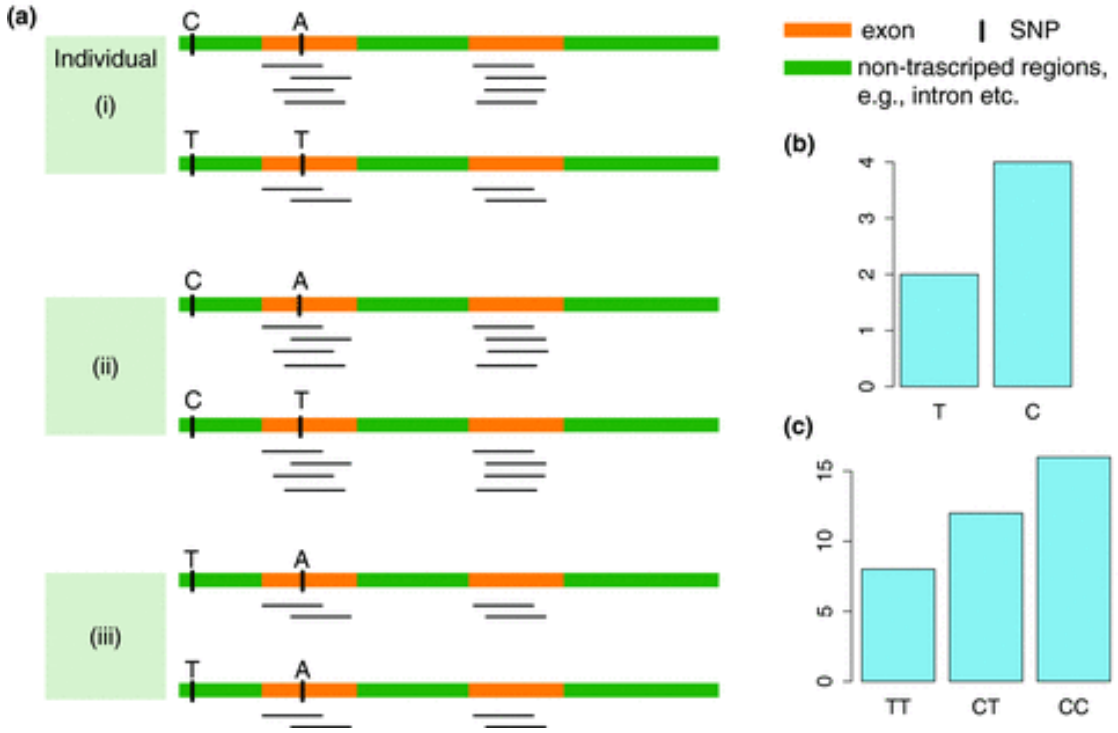




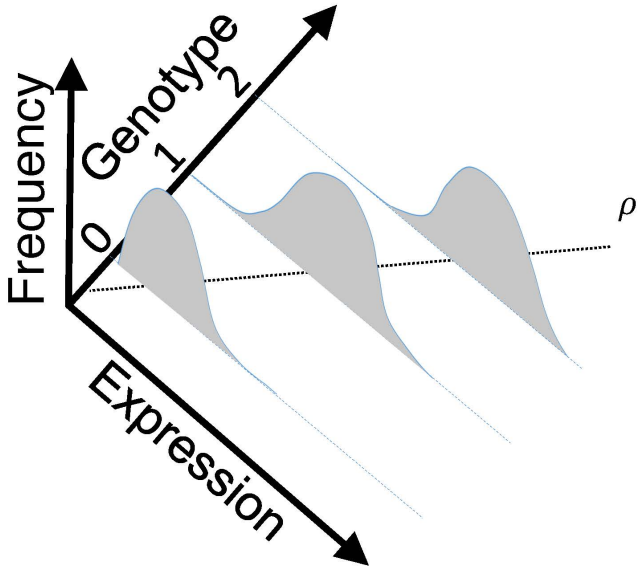
Mapping coordinates without variants (MRF)

Reads (linked via ID, 10X larger than mapping coord.)

[*Bioinformatics* 27: 281]



[*Biometrics 68(1) 1–11*]

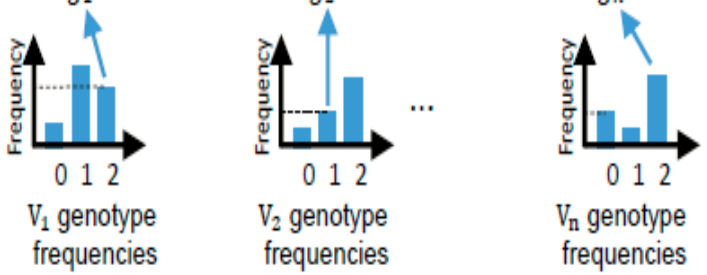


eQTL Mapping Using RNA-Seq Data

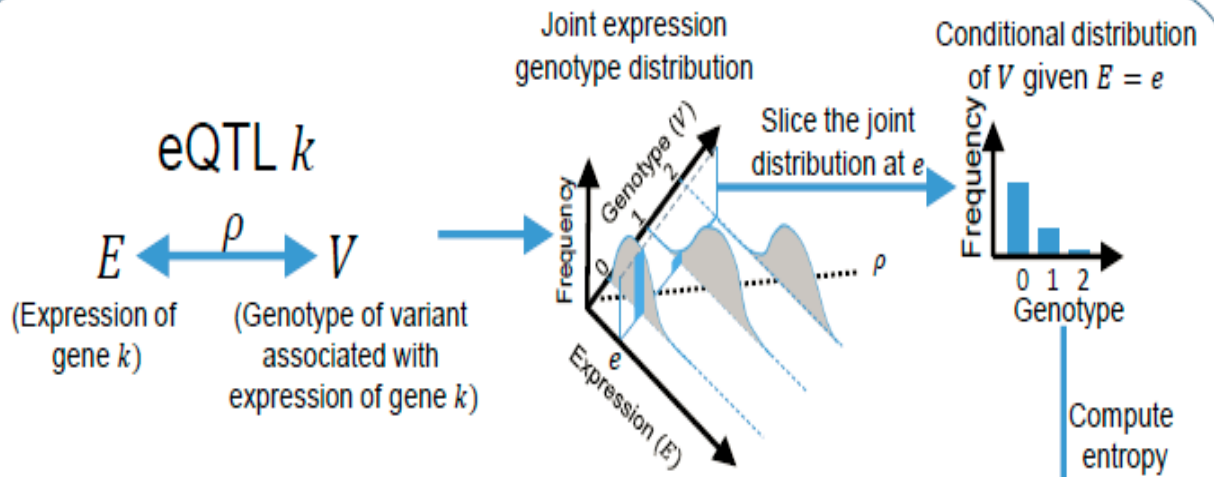
- eQTLs are genomic loci that contribute to variation in mRNA expression levels
- eQTLs provide insights on transcription regulation, and the molecular basis of phenotypic outcomes
- eQTL mapping can be done with RNA-Seq data

Information Content and Predictability

$$ICI\left(\begin{array}{c}\text{Individual has variant}\\ \text{genotypes } g_1, g_2, \ldots, g_n\\ \text{for variants } V_1, V_1, \ldots, V_n\end{array}\right) = \log\left(\frac{1}{\text{Frequency of } V_1 \text{ genotype } g_1 = 2}\right) + \log\left(\frac{1}{\text{Frequency of } V_2 \text{ genotype } g_2 = 1}\right) + \ldots + \log\left(\frac{1}{\text{Frequency of } V_n \text{ genotype } g_n = 2}\right)$$



- **Higher frequency: Lower ICI**
- **Lower frequency: Higher ICI**
- **Additive for multiple variants**



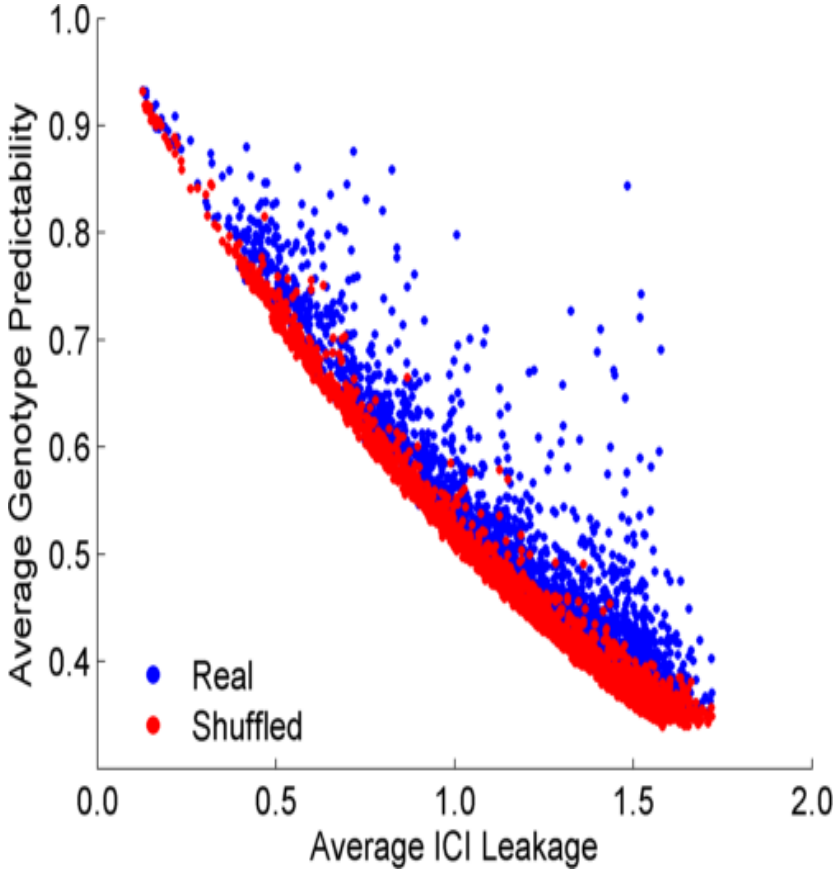
$$\pi\left(V \middle| E = e\right) = e^{-H\left(V \middle| E = e\right)}$$

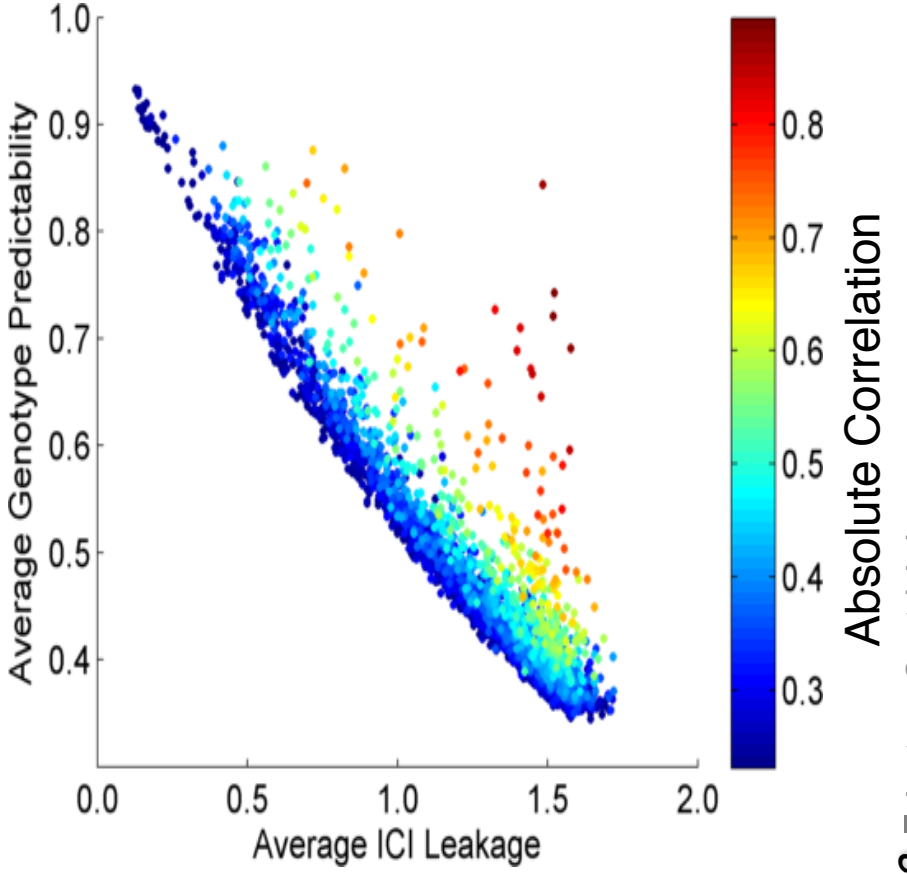
- **Condition specific entropy**
- **Higher cond. entropy: Lower predictability**
- **Lower cond. entropy: Higher predictability**
- **Additive for multiple eQTLs**

[Harmanci et al. Nat. Meth. 2016]

Per eQTL and ICI Cumulative Leakage versus Genotype Predictability

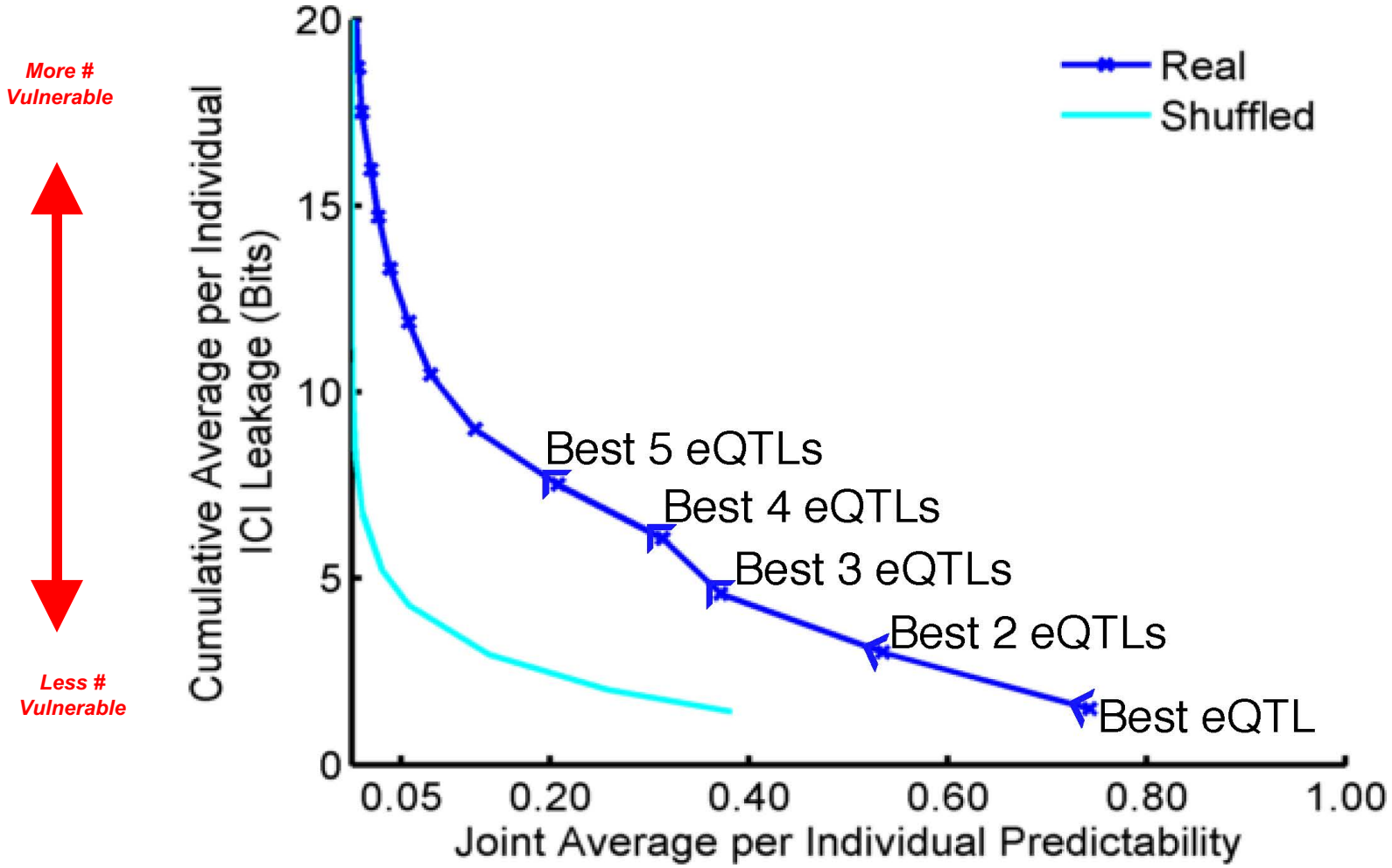
Colors by absolute correlation





[Harmanciet al. Nat. Meth. (in revision)]

Cumulative Leakage versus Joint Predictability



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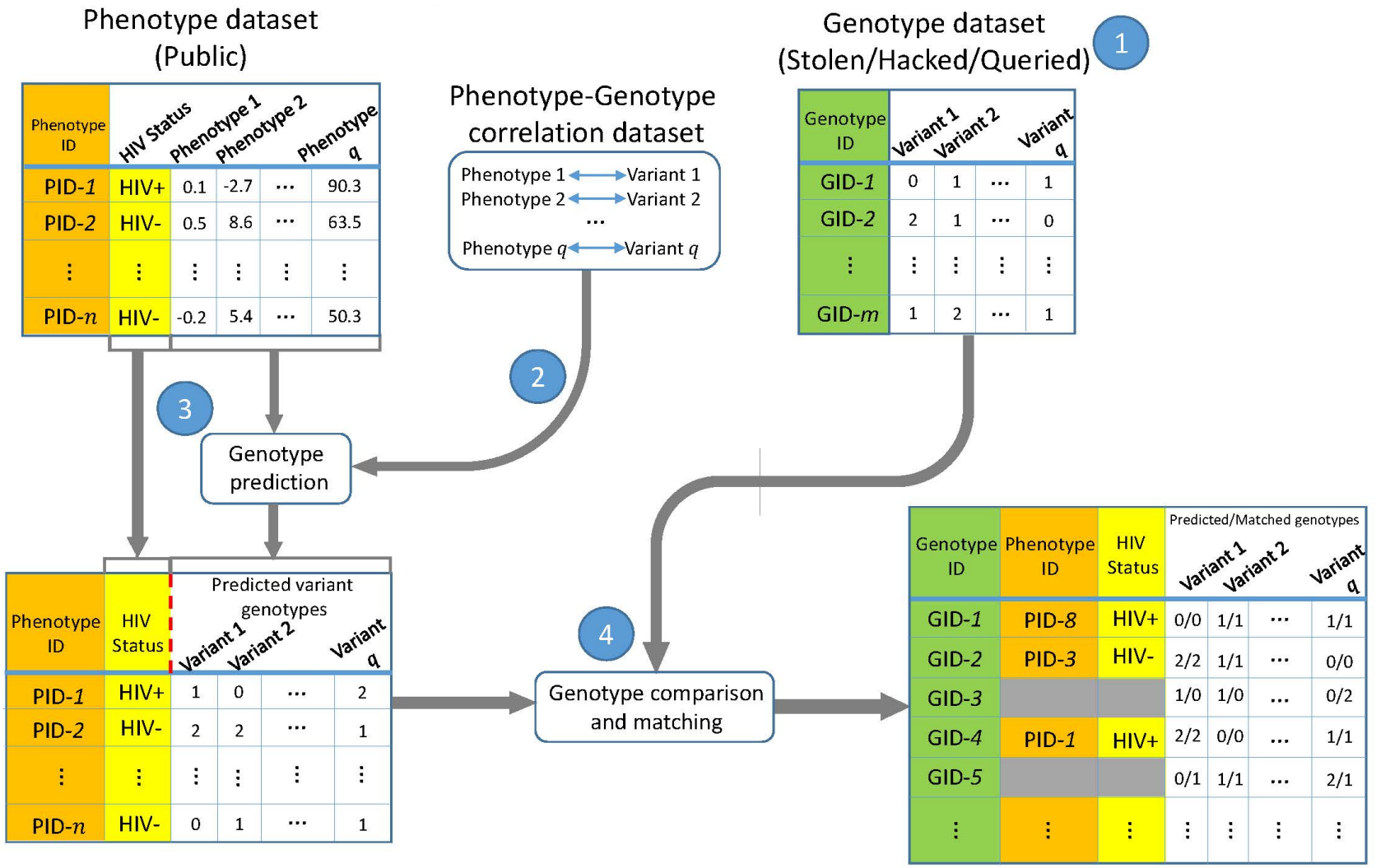
Linking Attack Scenario



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Linking Attacks: Case of Netflix Prize

User (ID)	Movie (ID)	Date of Grade	Grade [1,2,3,4,5]
NTFLX-0	NTFLX-19	10/12/2008	1
NTFLX-1	NTFLX-116	4/23/2009	3
NTFLX-2	NTFLX-92	5/27/2010	2
NTFLX-1	NTFLX-666	6/6/2016	5
…	…	…	…
…	…	…	…

Names available for many users!

User (ID)	Movie (ID)	Date of Grade	Grade [0-10]
IMDB-0	IMDB-173	4/20/2009	5
IMDB-1	IMDB-18	10/18/2008	0
IMDB-2	IMDB-341	5/27/2010	-
…	…	…	…
…	…	…	…
…	…	…	…

- **Many users are shared**
- **The grades of same users are correlated**
- **A user grades one movie around the same date in two databases**

Anonymized Netflix Prize Training Dataset made available to contestants

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- **IMDB users are public**

- **NetFLIX and IMdB moves are public**

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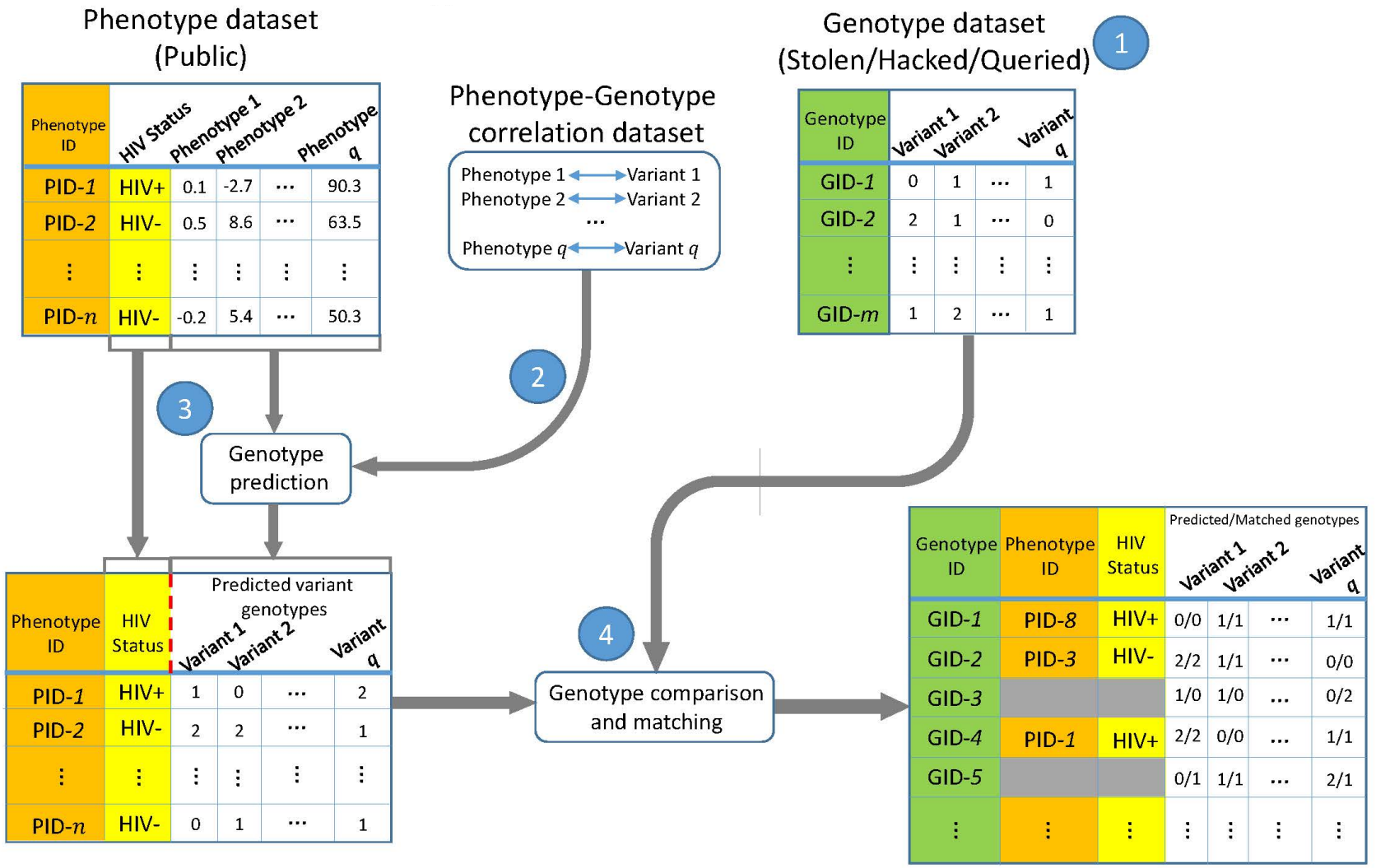

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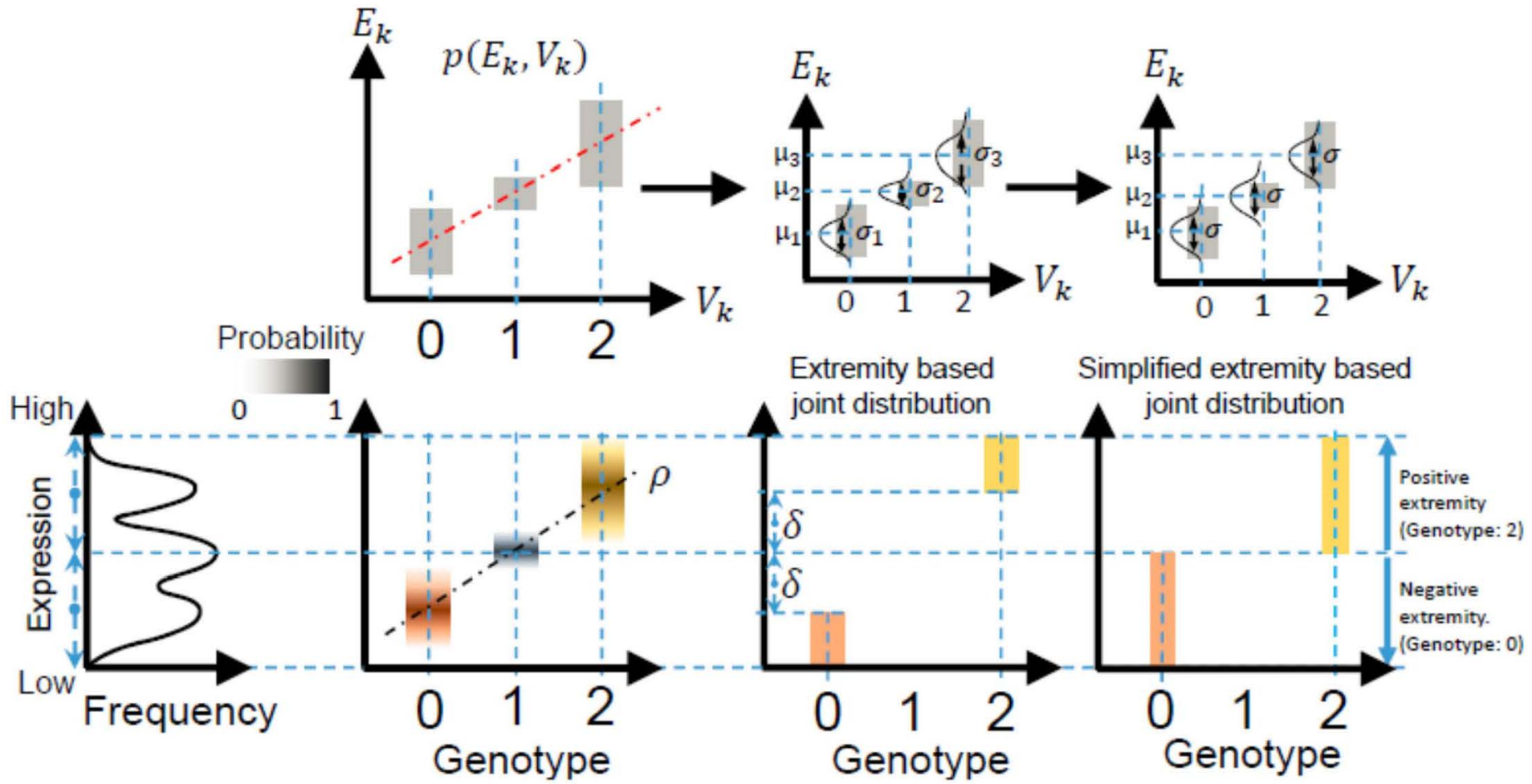
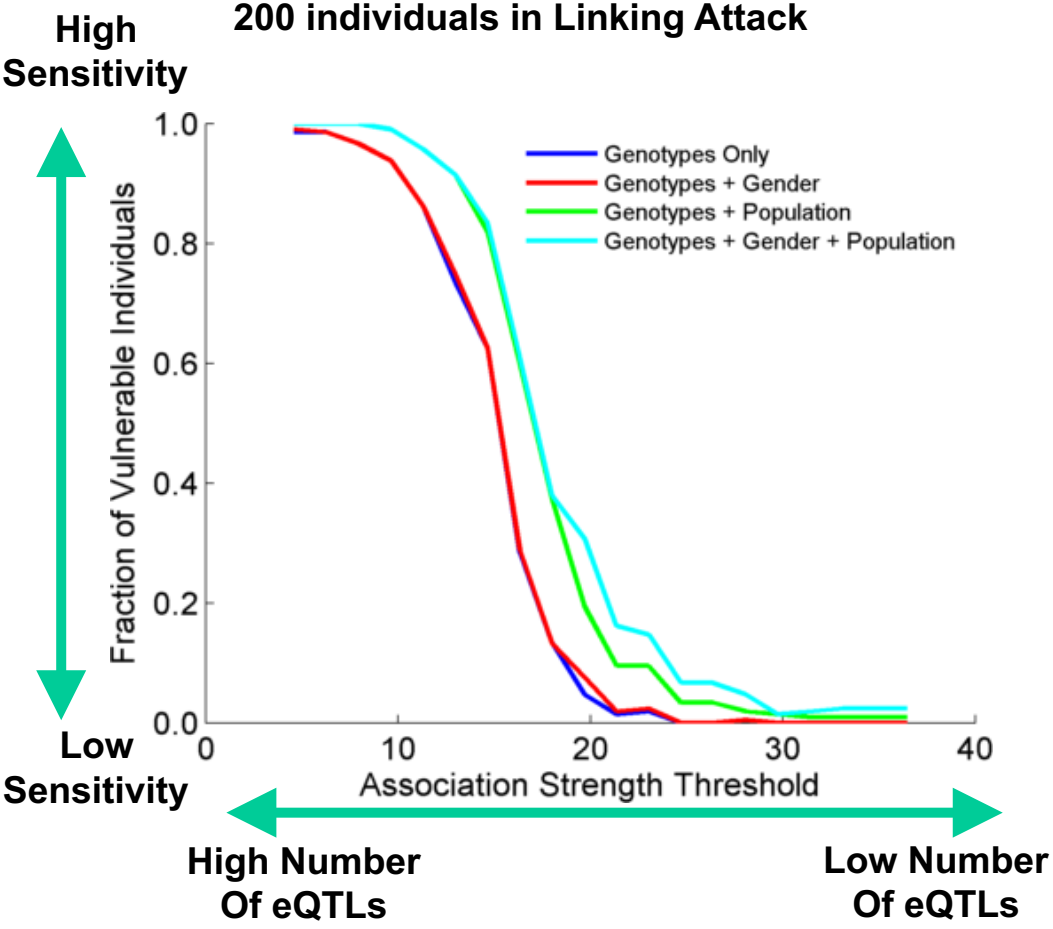
Levels of Expression-Genotype Model Simplifications for Genotype Prediction



[Harmanci et al. *Nat. Meth.* (16)]

Success in Linking Attack with Extremity based Genotype Prediction

200 individuals eQTL Discovery
200 individuals in Linking Attack



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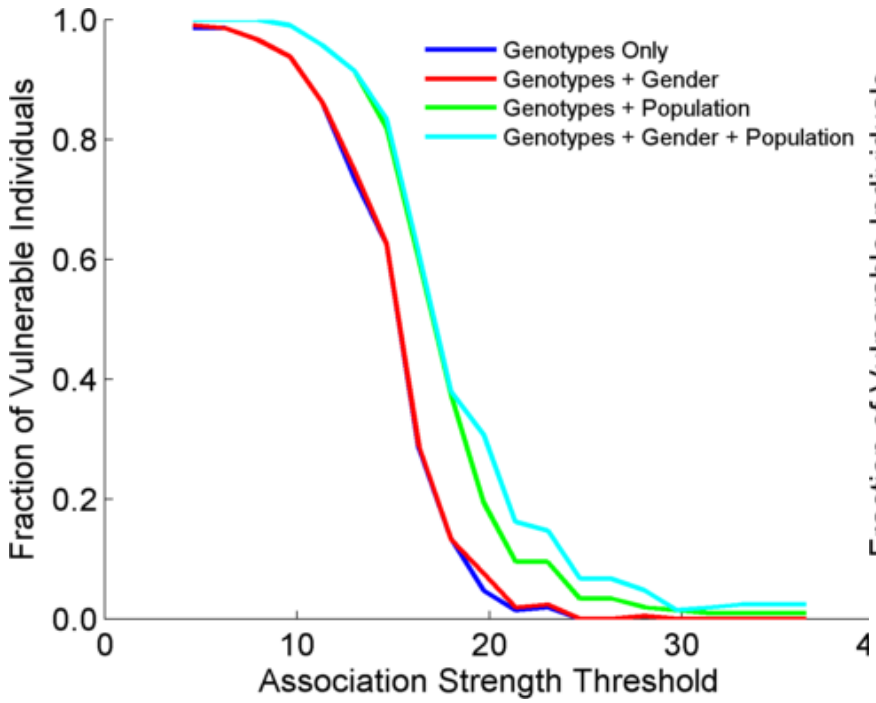
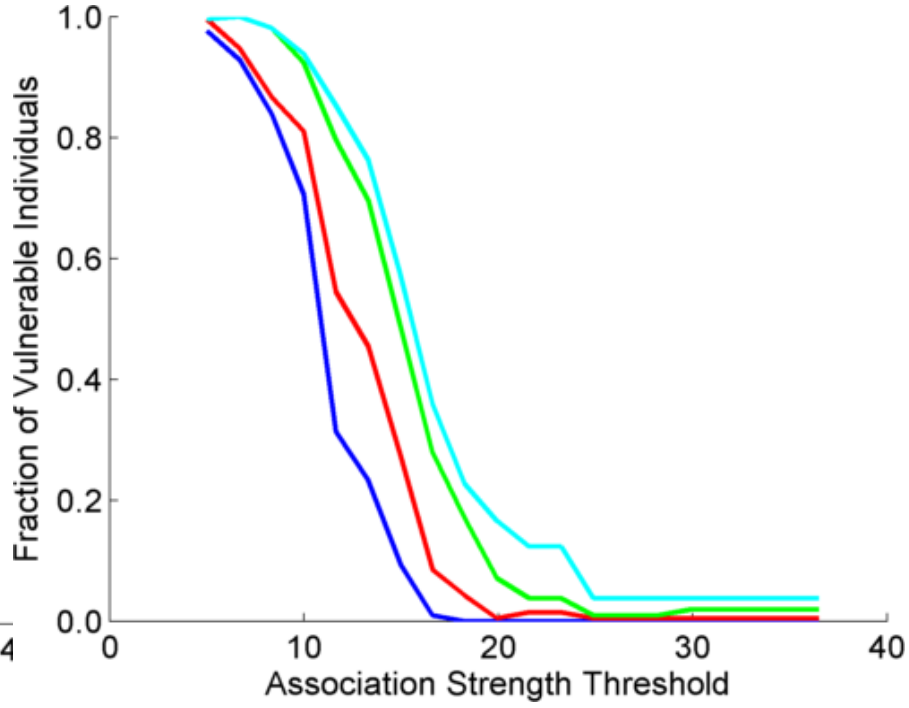


200 individuals eQTL Discovery
100,200 individuals in Linking Attack



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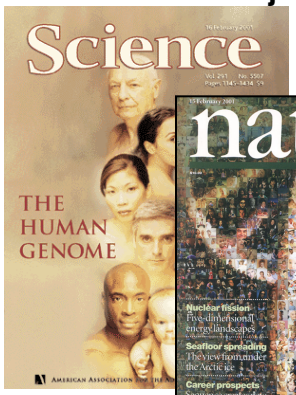
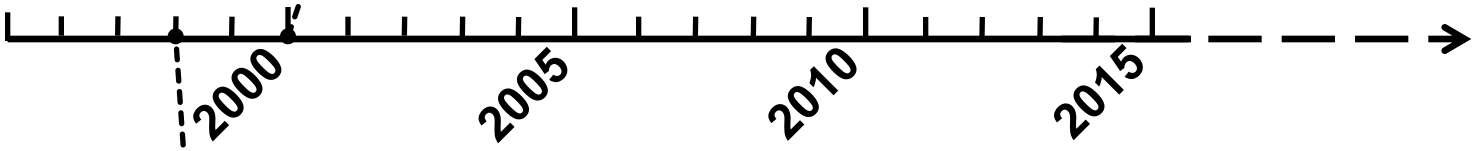
The Human Genome Project



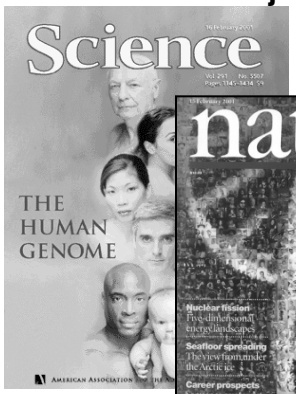


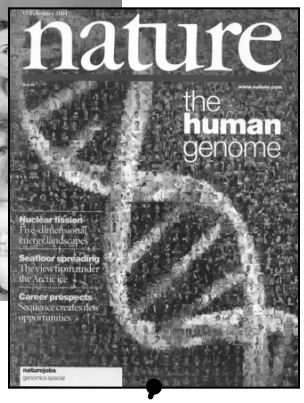
Worm Genome

The Human Genome Project

ENCODE Pilot

ENCODE Production

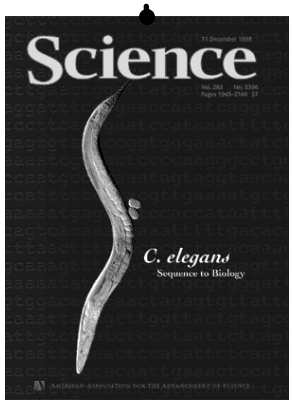




2000

2005

2010



Worm Genome

modENCODE

The Human Genome Project

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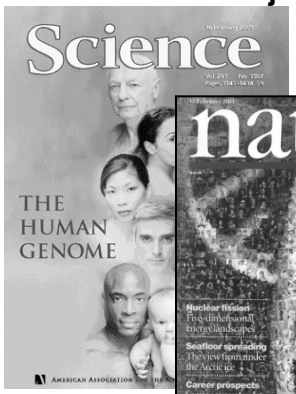
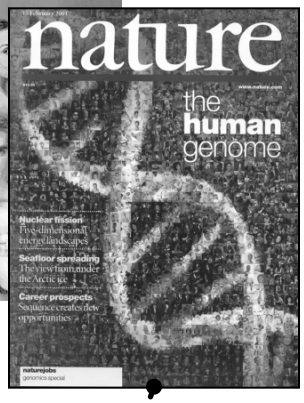
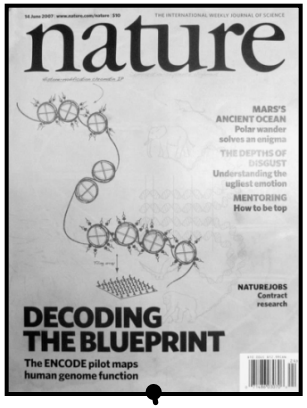
Comparative ENCODE







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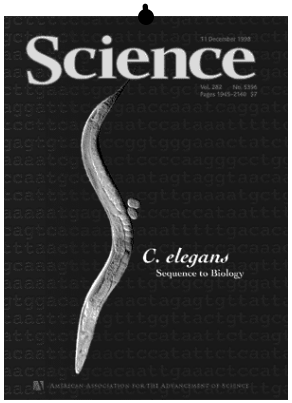

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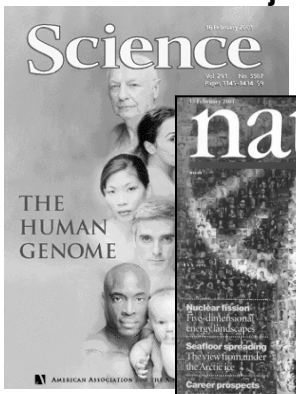

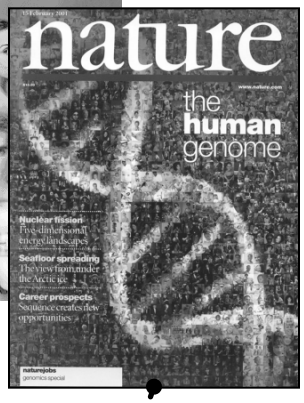

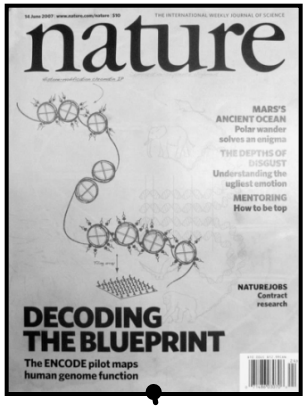

2000

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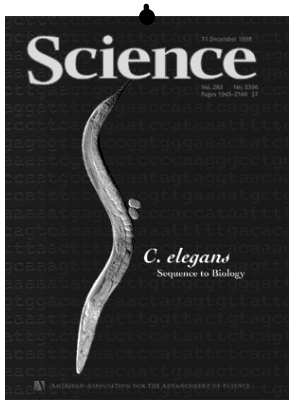
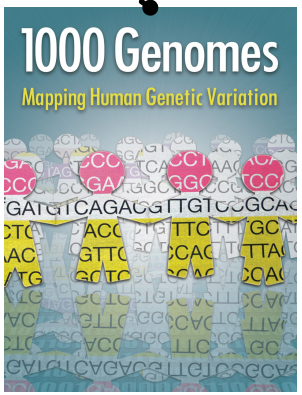




Worm Genome

modENCODE

1000 Genomes Pilot

1000 Genomes Production

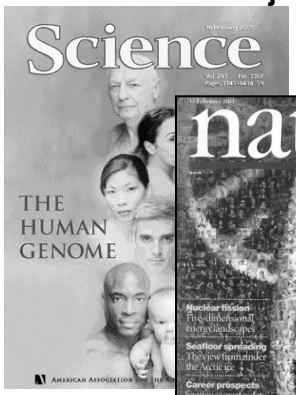
The Human Genome Project

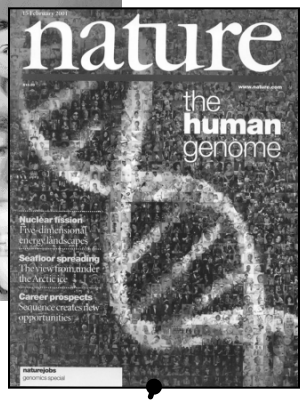
ENCODE Pilot

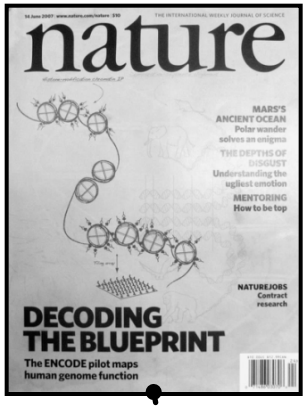
ENCODE Production

Comparative ENCODE

Epigenome Roadmap







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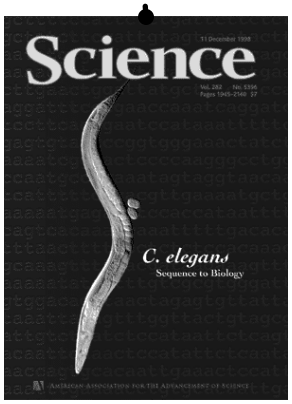

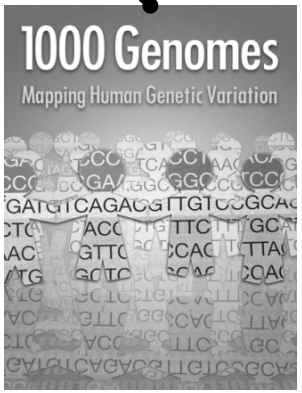

Worm Genome

modENCODE

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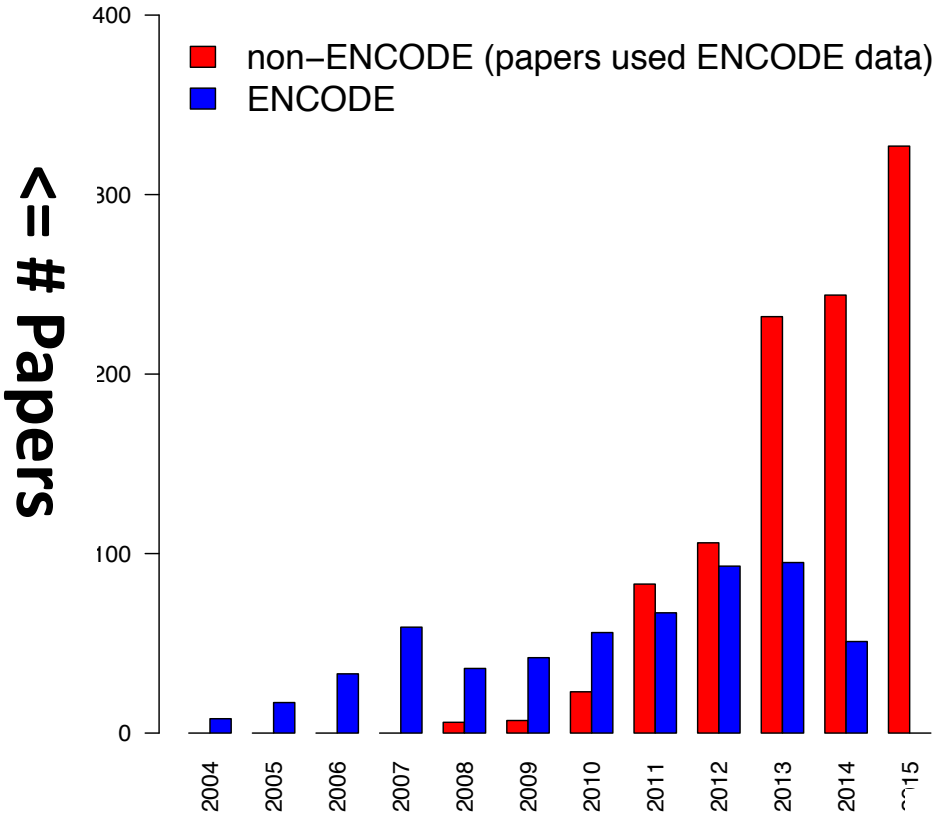
1000 Genomes Production

GTEx

With help of M Pazin at NHGRI, identified: 702 community papers that used ENCODE data but were not supported by ENCODE funding &
558 consortium papers supported by ENCODE funding
(https://www.encodeproject.org/search/?type=Publication for up-to-date query)
Then identified 1,786 ENCODE members & 8,263 non-members .

non–ENCODE (papers used ENCODE data) ENCODE



 # Authors

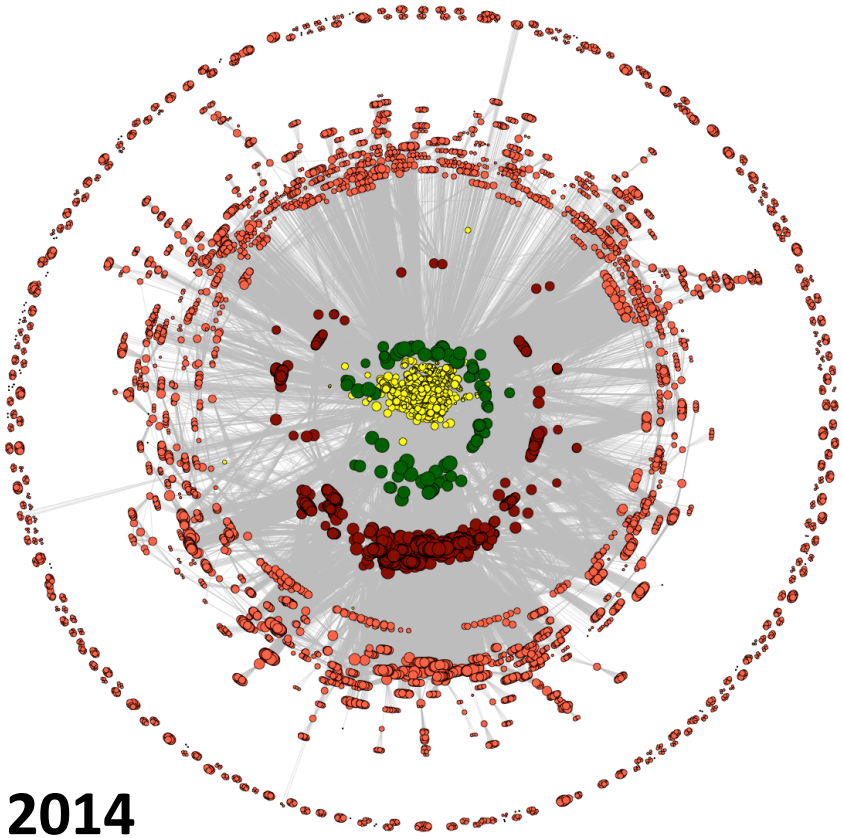
[Wang et al., TIG ('16)]

Yr. ('04 to '15)

Co-authorship Network of ENCODE members & Data Users

- ENCODE member
- non-member
- ENCODE member broker
- non-member broker

co-authorship



2014

[Wang et al., TIG ('16)]

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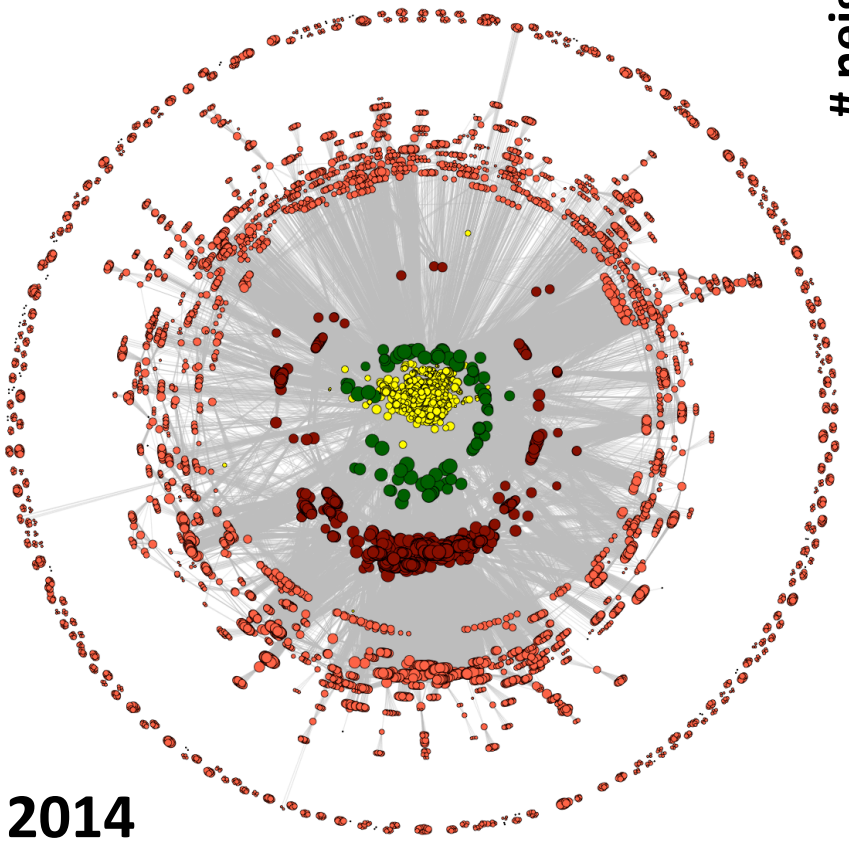

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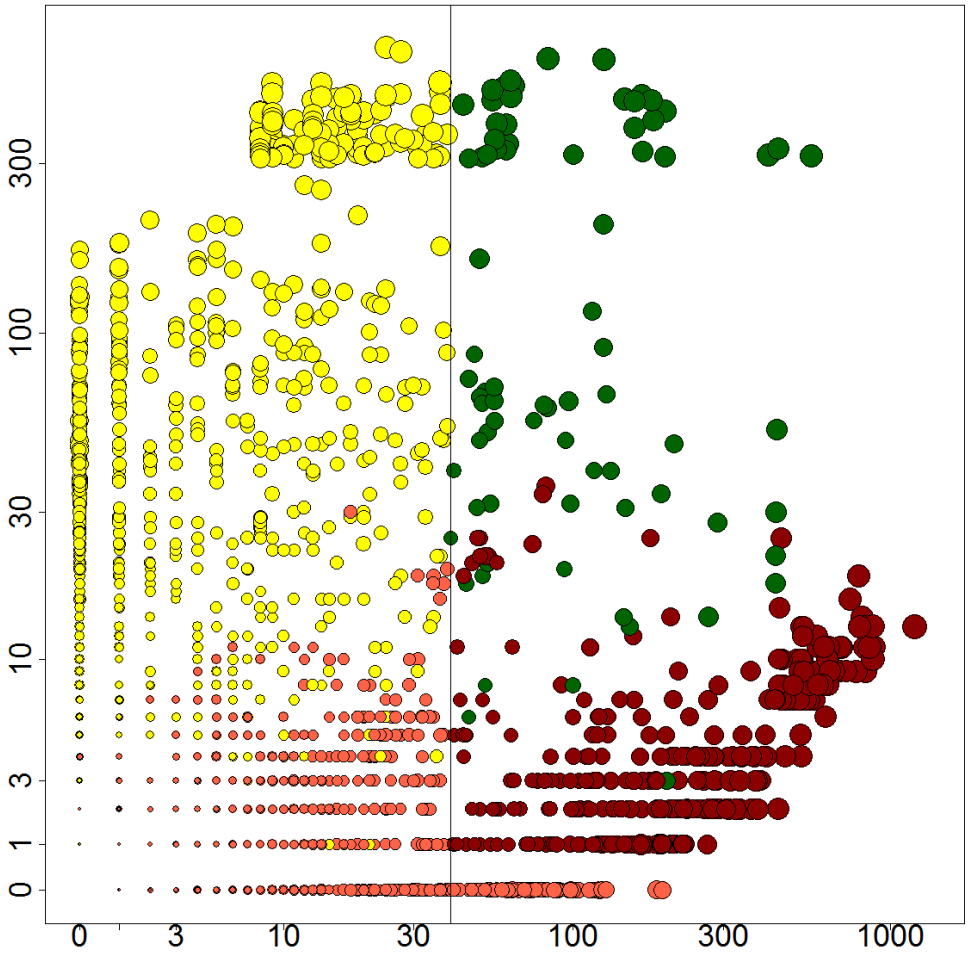

[Wang et al., TIG ('16)]

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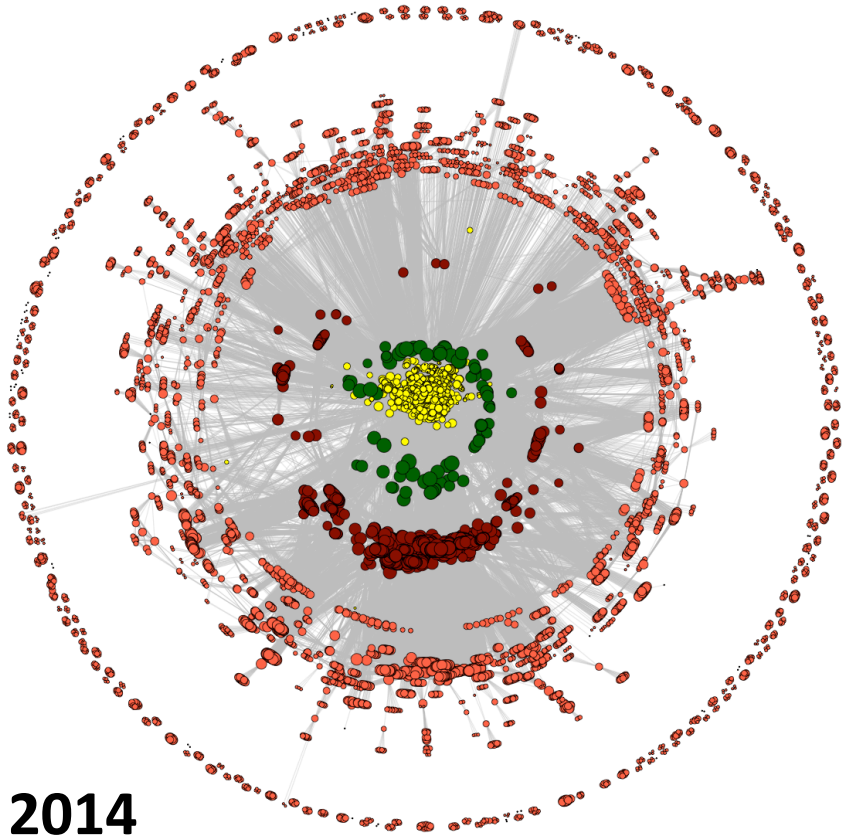
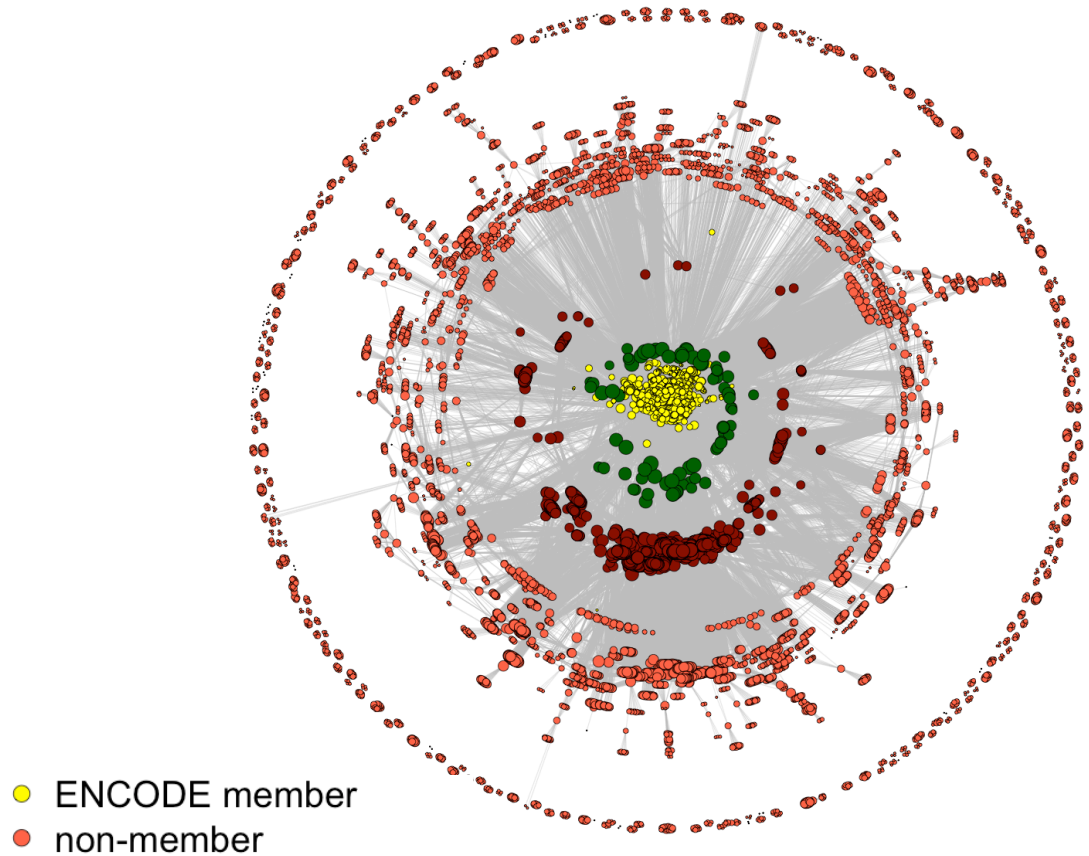
co-authorship



2014

[Wang et al., TIG ('16)]

Dynamics of co-authorship network



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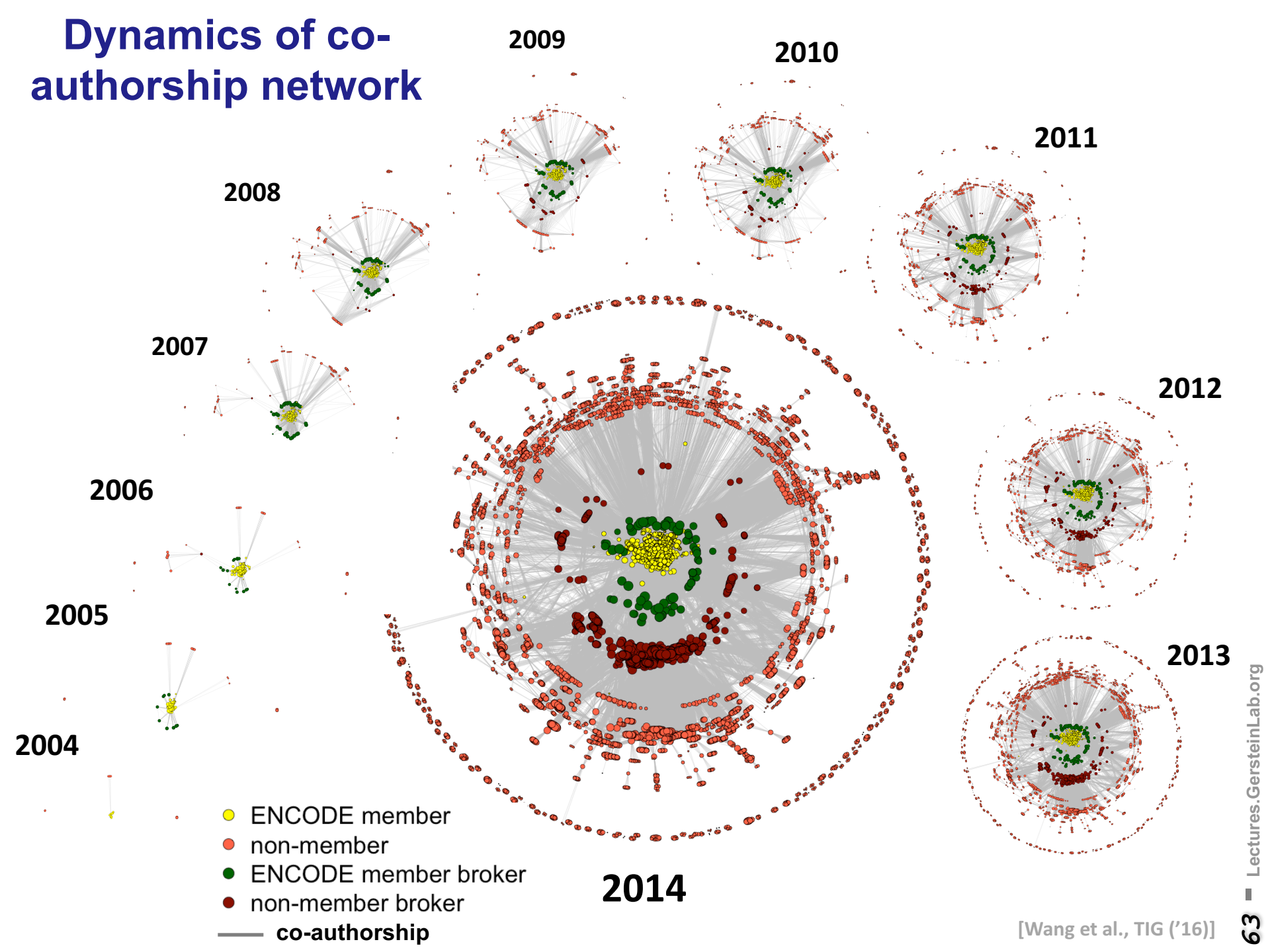
Dynamics of co-authorship network



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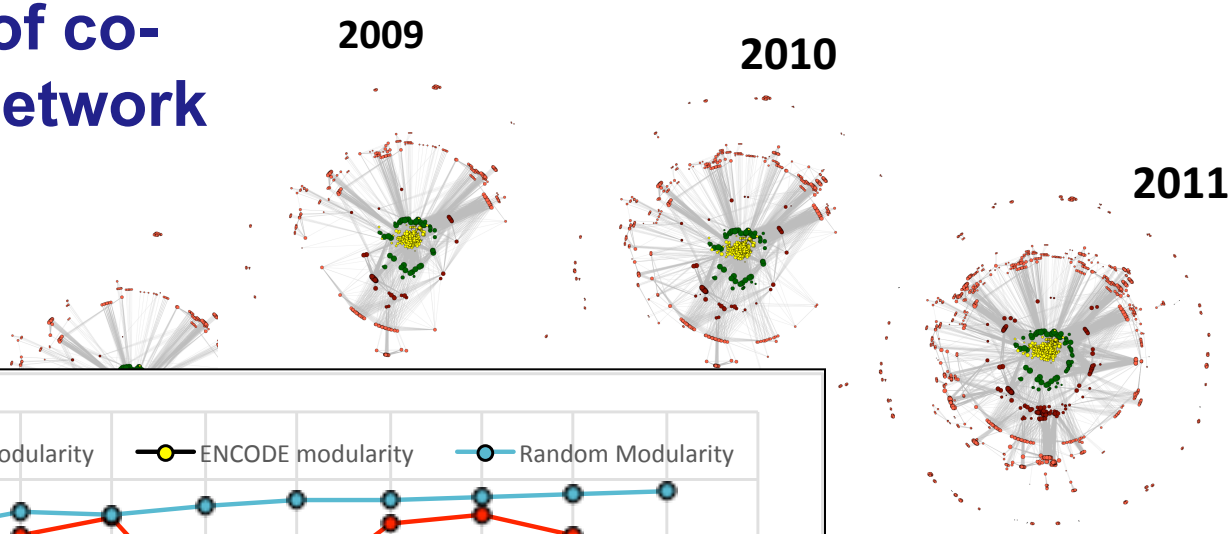

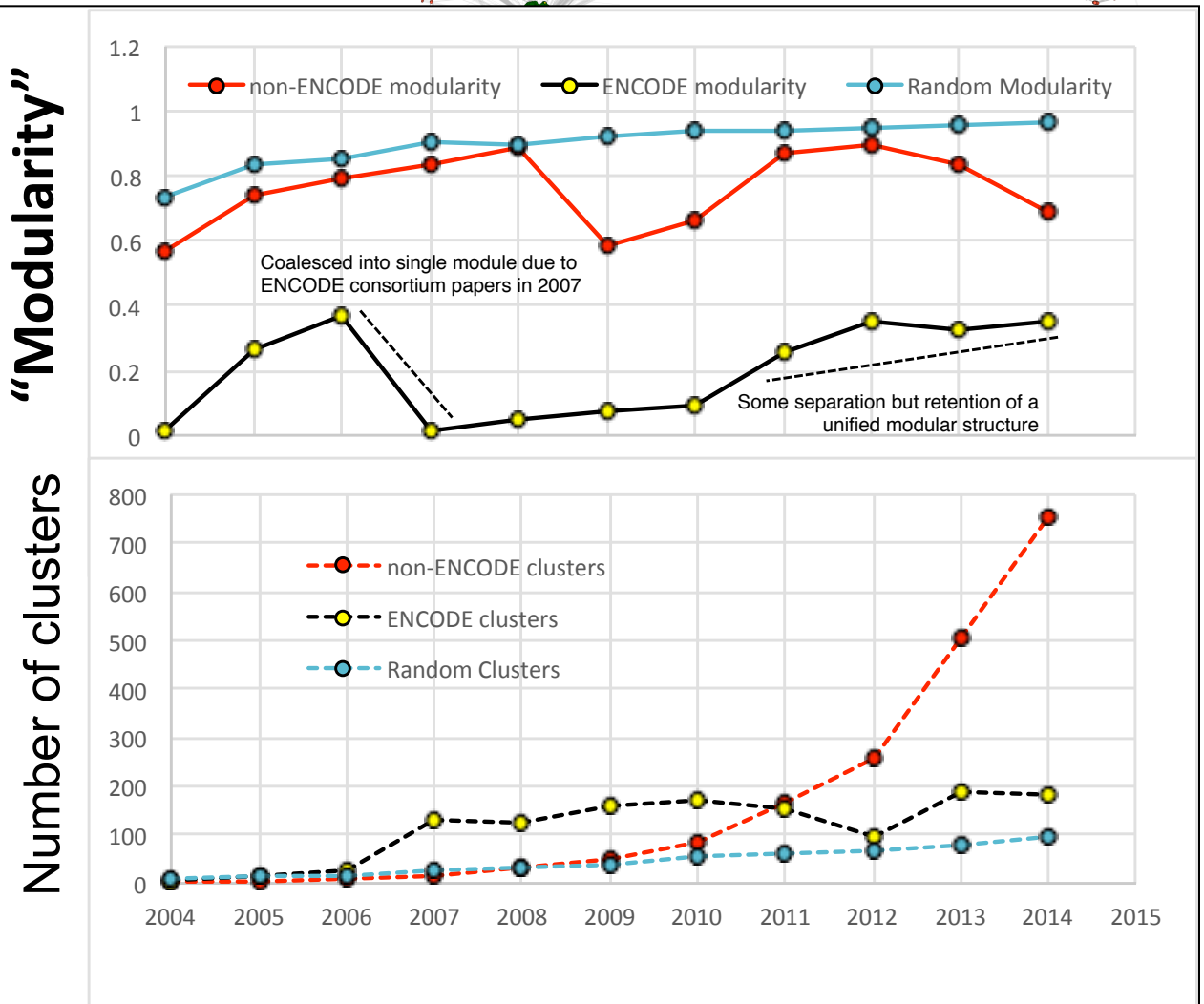

[Wang et al., TIG ('16)]

Transcriptome Analysis: Tackling core issues related to gene regulation & also mining the "data exhaust" produced by this activity

- *[Core-1]* **Expression Clustering**, Cross-species
  - Comparative ENCODE – Lots of worm-fly-human matched data & developmental timecourses
  - Optimization gives 16 conserved co-expression modules
- *[Core-2]* **State Space Models** of Gene Expression
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  - Decoupling expression changes into those from conserved vs species-specific genes
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Acknowledgements

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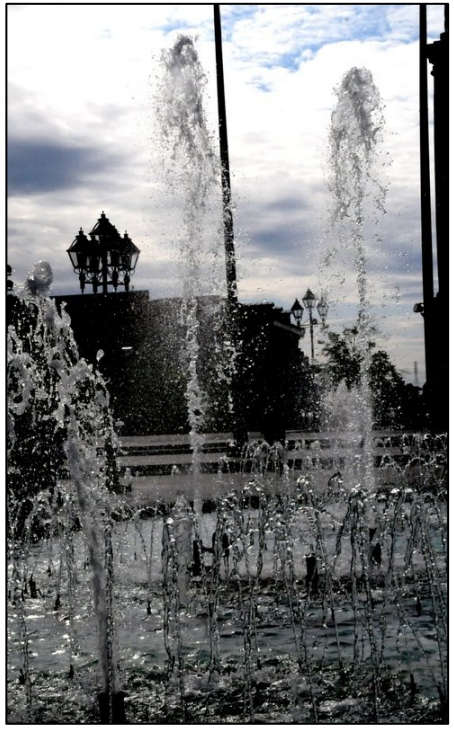

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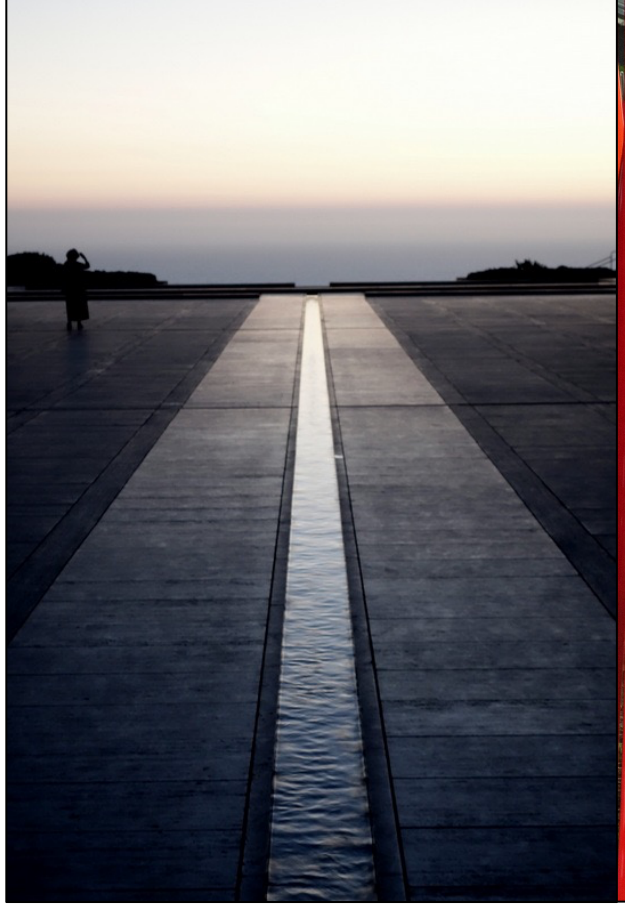

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