Announcement

- ★ Analysis assignment due Feb 28
- ★ Optional midcourse feedback (bonus point) due March 1
- ★ Discussion session for your project on March 6, 12 - 1 PM

Functional interpretation of genomics analysis by enrichment analysis

Yongjin Park, UBC Path&Lab, STAT, BC Cancer

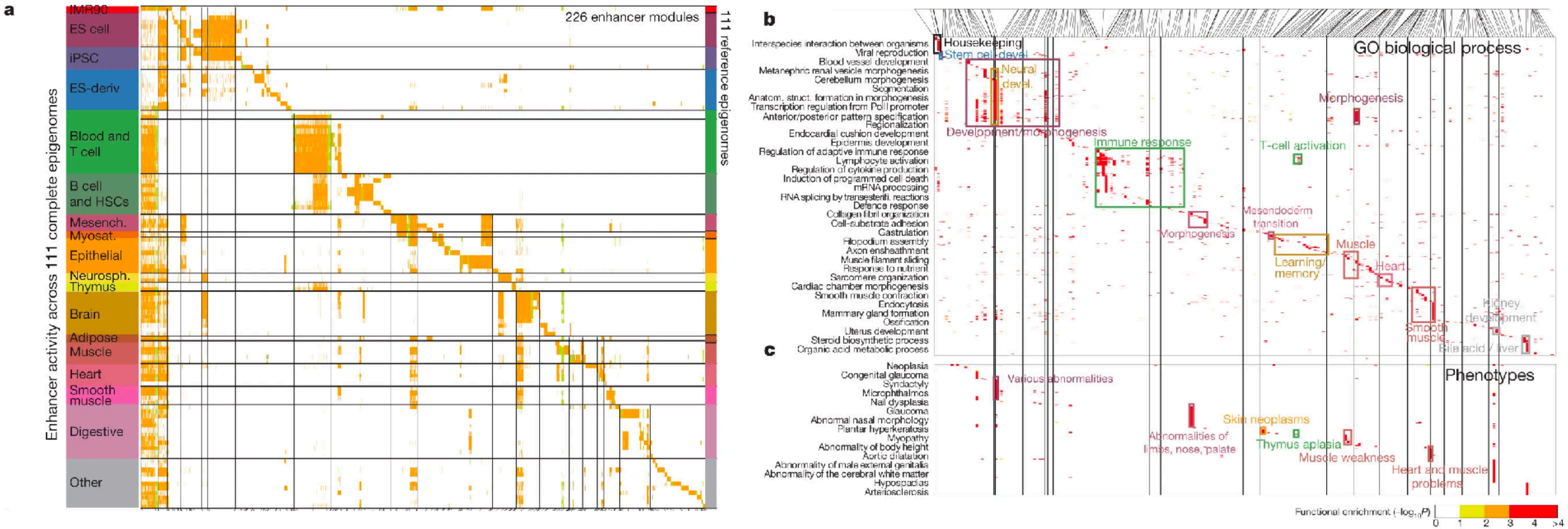
Today’s lecture: Enrichment Analysis

- **Motivations: What’s next after genomics analysis?**
  - What have we learned?
  - How do we know that our discovery is meaningful?
- **Gene set enrichment analysis**
  - Set-based approach: Hypergeometric test
  - Rank-based approach: GSEA by KS statistic
- **Can we engineer new gene sets?**
  - Principal Component Analysis
  - Matrix factorization of count data

Biological mechanisms/processes are modular

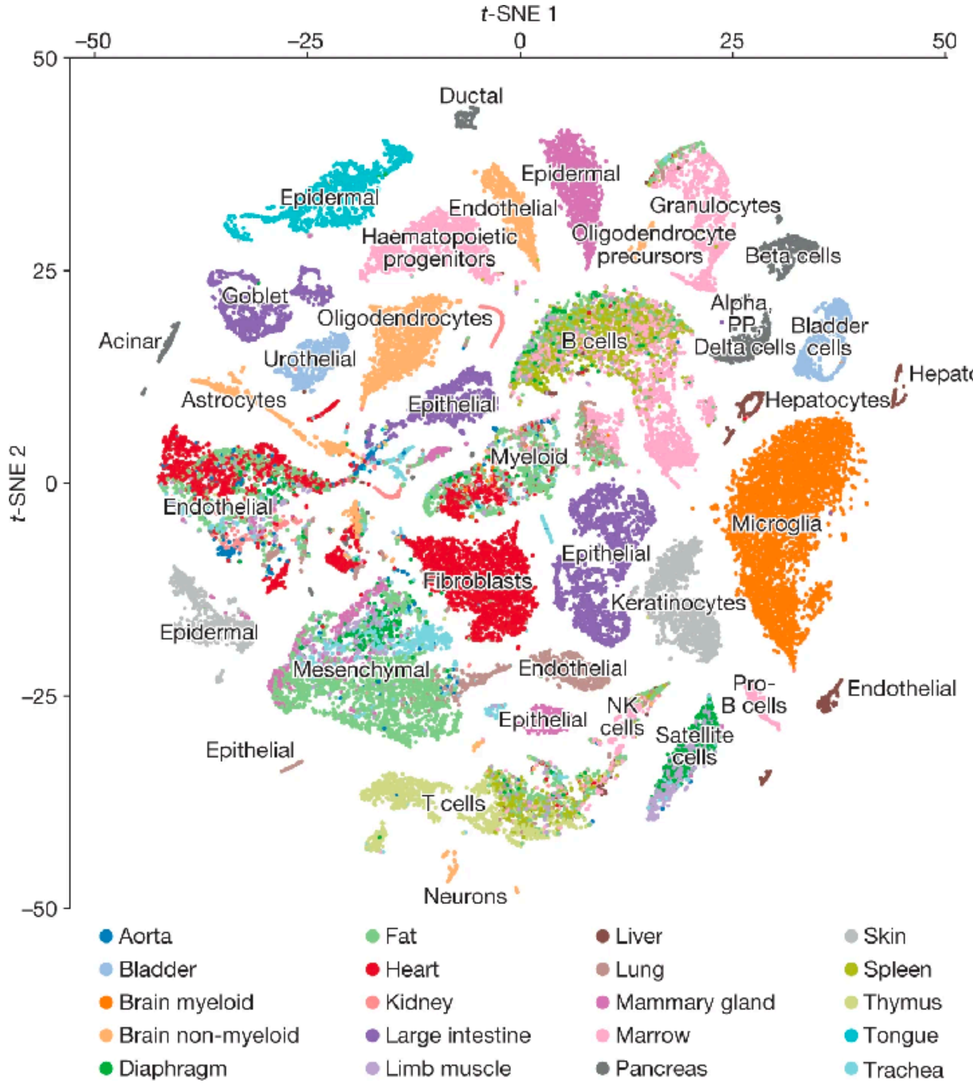
Epigenomic (enhancer element) modules across 111 tissues

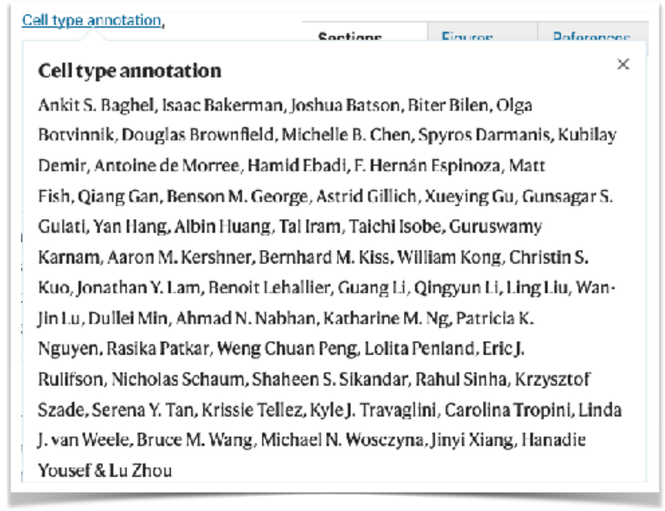
Genie ontology information confirms cell-type- or tissue-specific biological pathways



Epigenomics Roadmap, *Nature* (2015)

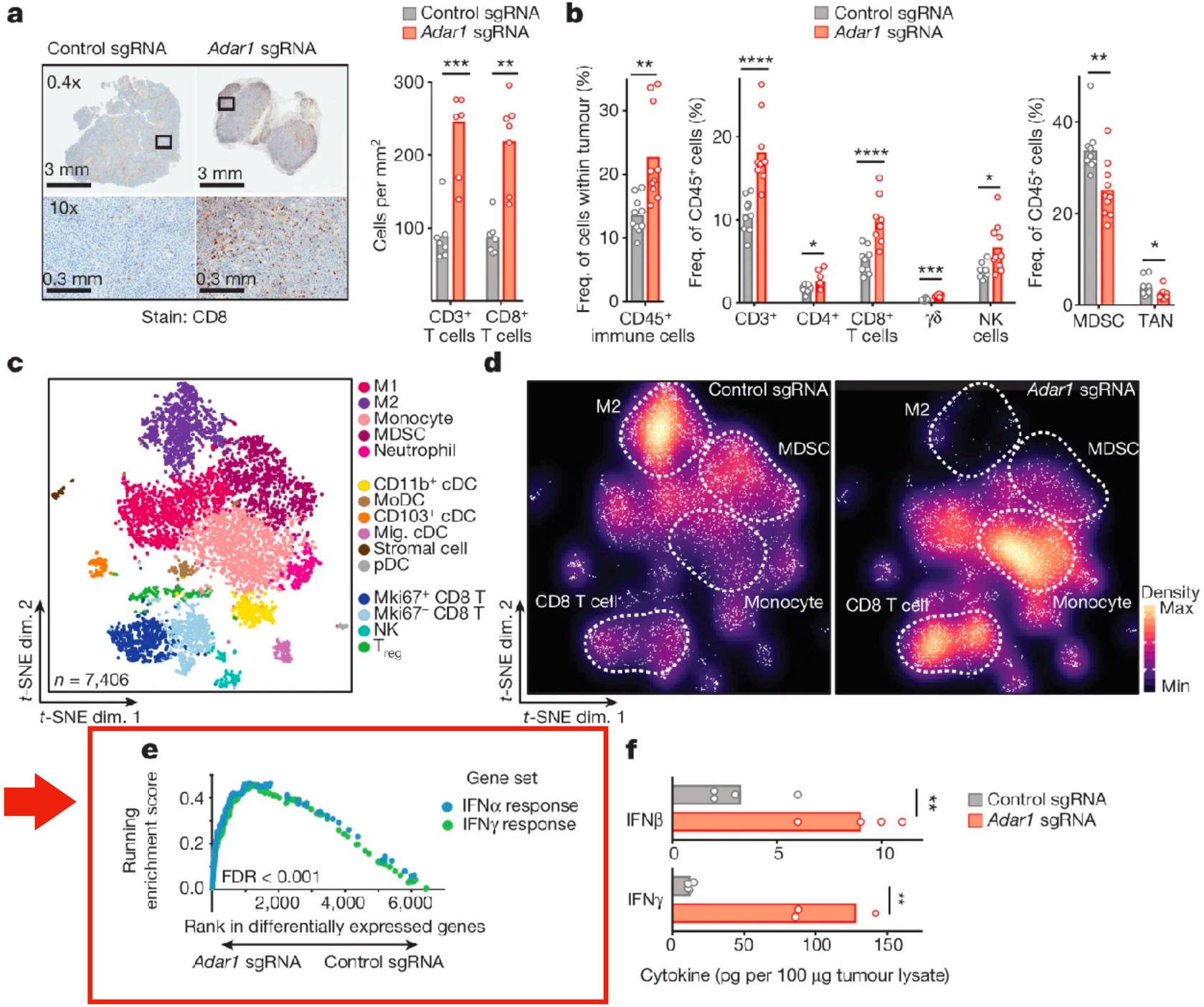
How do we know clusters of cells correspond to cell types?





Tabular Muris, *Nature* (2018)

Enrichment analysis is also embedded a larger analysis



Gene set enrichment analysis checks with previous knowledge

Ishizuka *et al. Nature* (2019)

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What is Gene Set Analysis?

(Discrete) Gene Set Analysis

Input:

1. A dictionary of gene sets that map genes to sets (gene-to-set mapping)
2. A list of **top** genes identified in our own study (after FDR control)

(Rank-based) Gene Set Enrichment

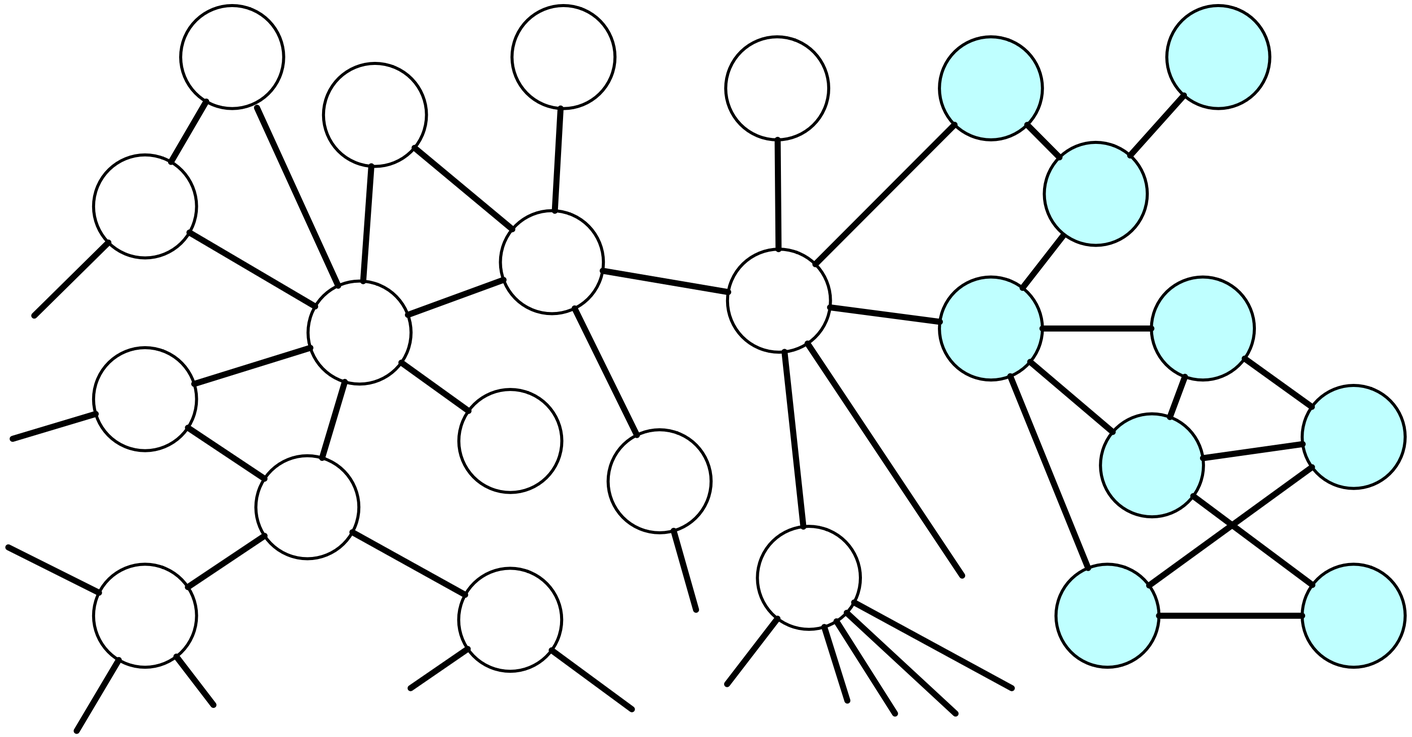
Input:

1. A dictionary of gene sets that map genes to sets
2. A **full** list of gene-level **scores** (e.g., p-values)

Output:

A table of scores for all the gene sets in the dictionary.

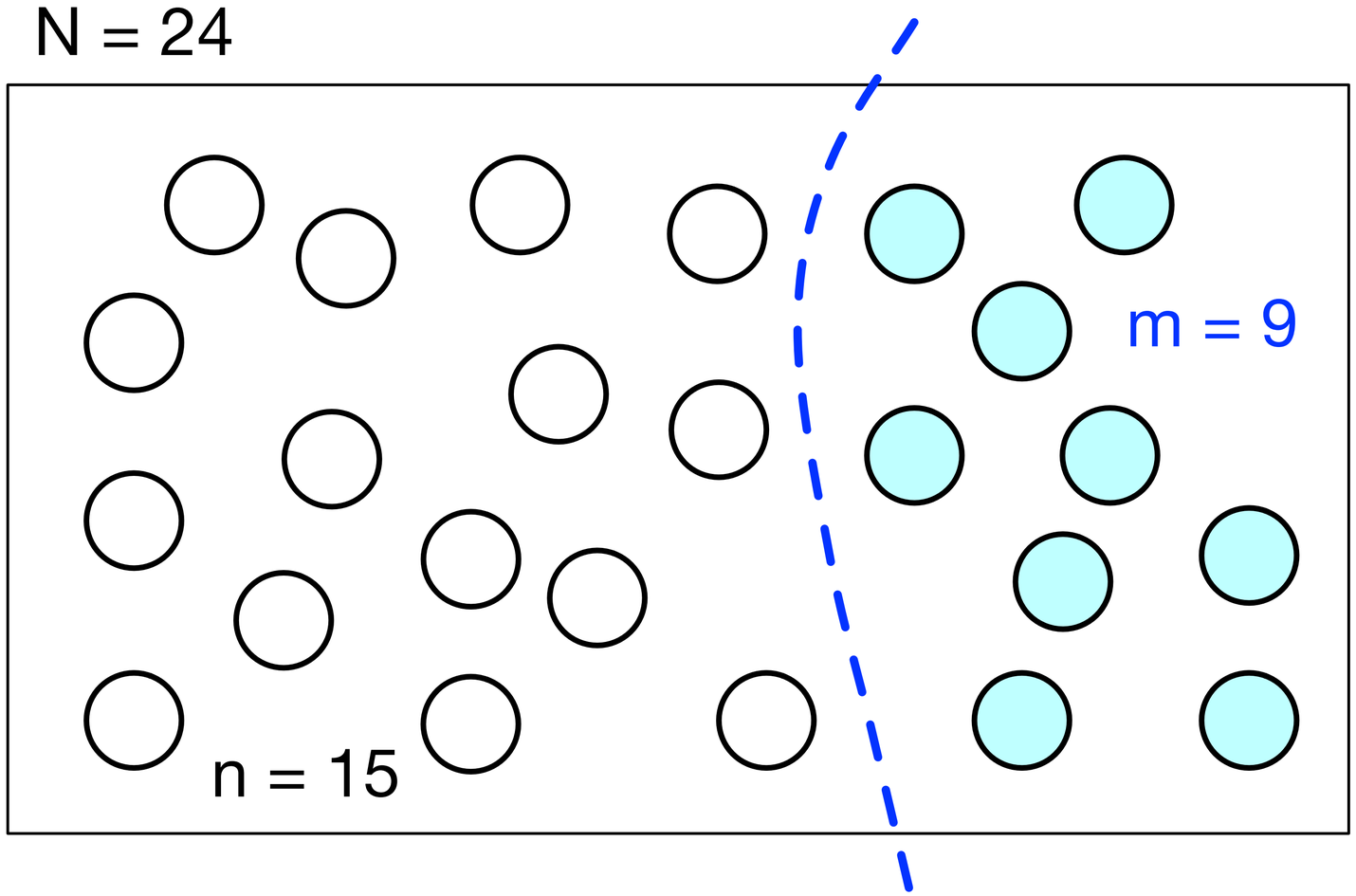
What have we learned from our differential expression analysis?



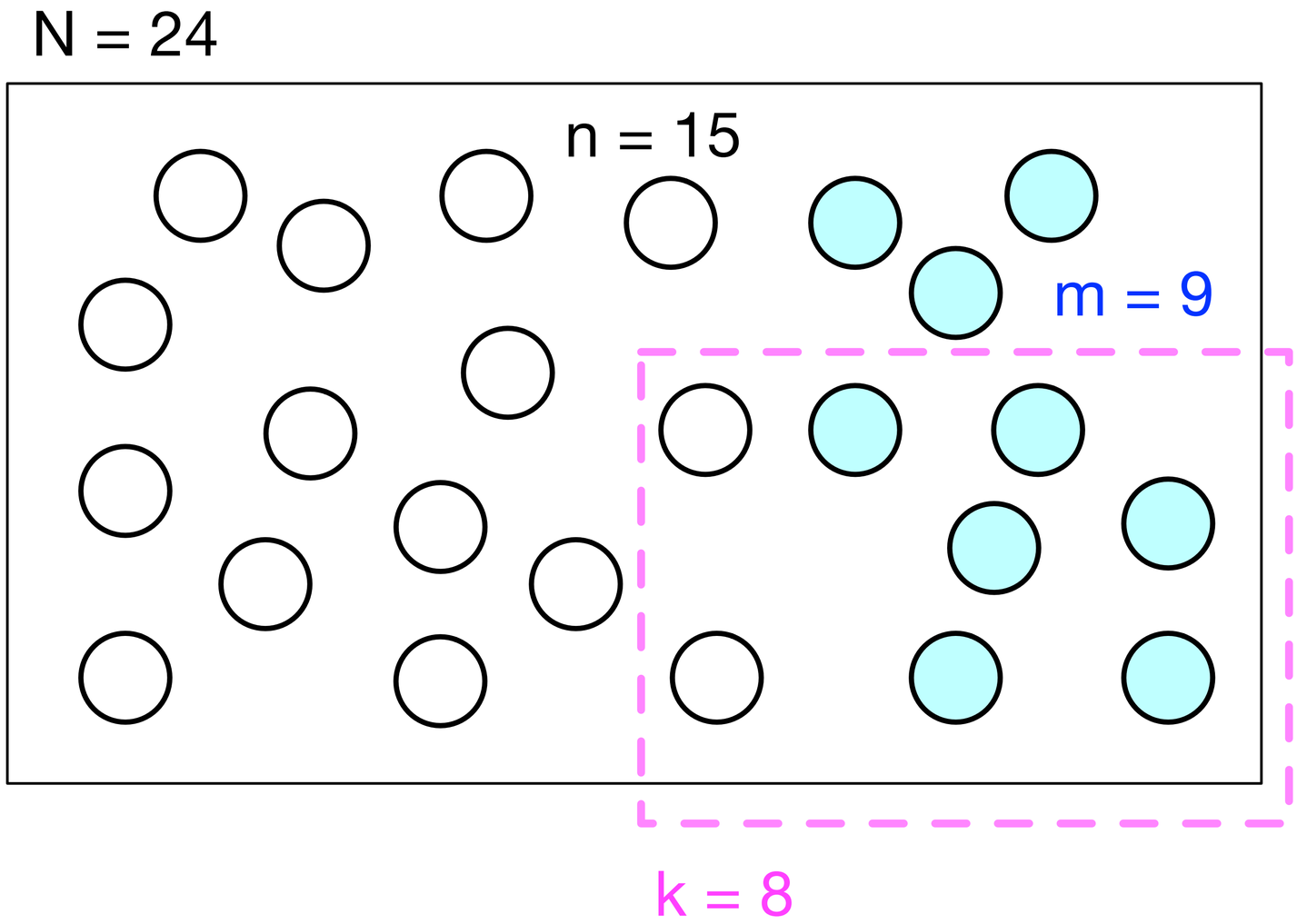
→ **coloured:** some pathway of interest

Let's drop the edges in biological networks

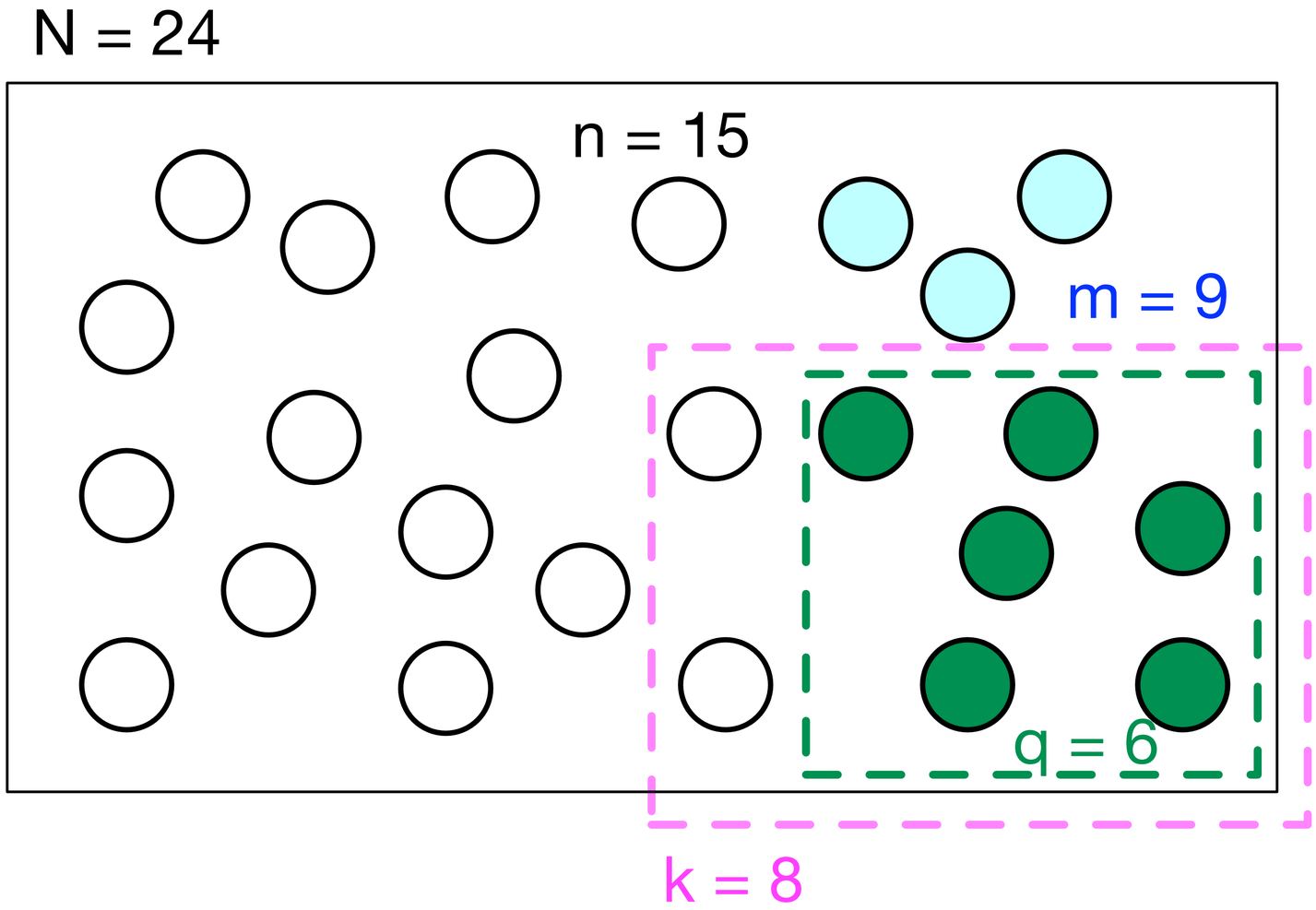
Prior Knowledge of biological pathways define a set of genes (coloured)



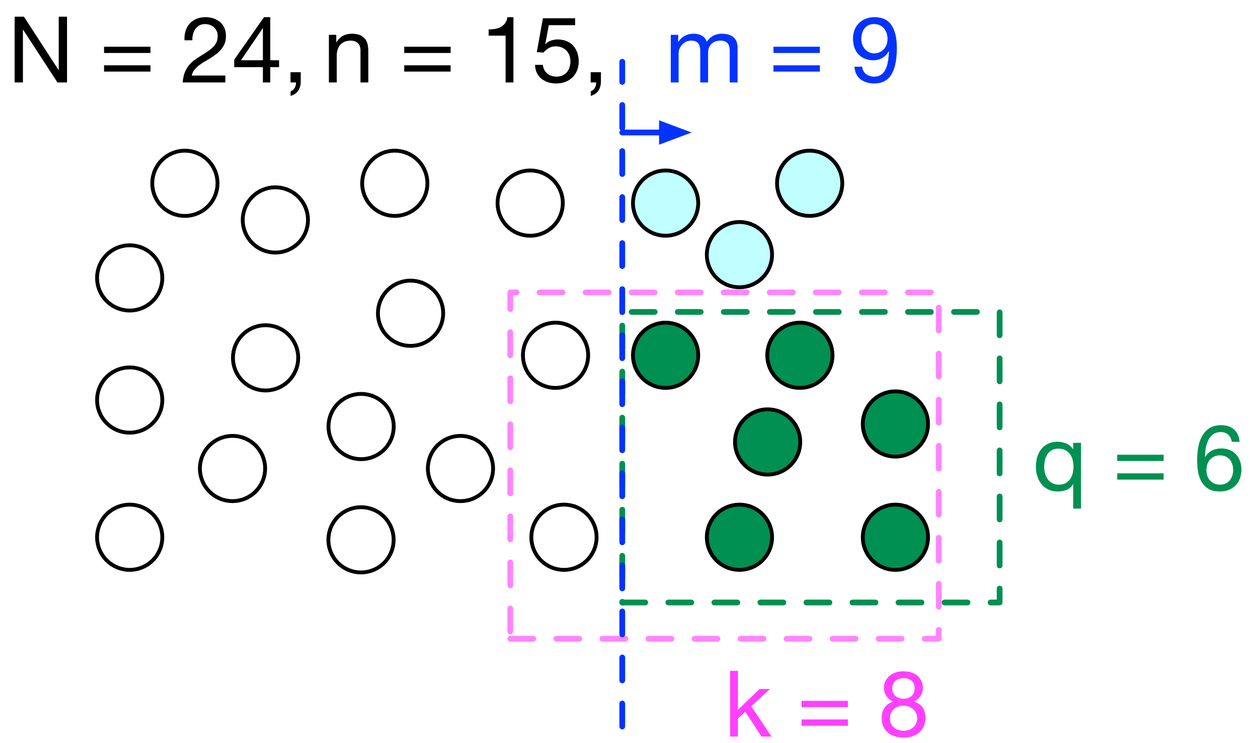
Does this pathway overlap with our DEG list?



Say that we found q genes are overlapping with this pathway



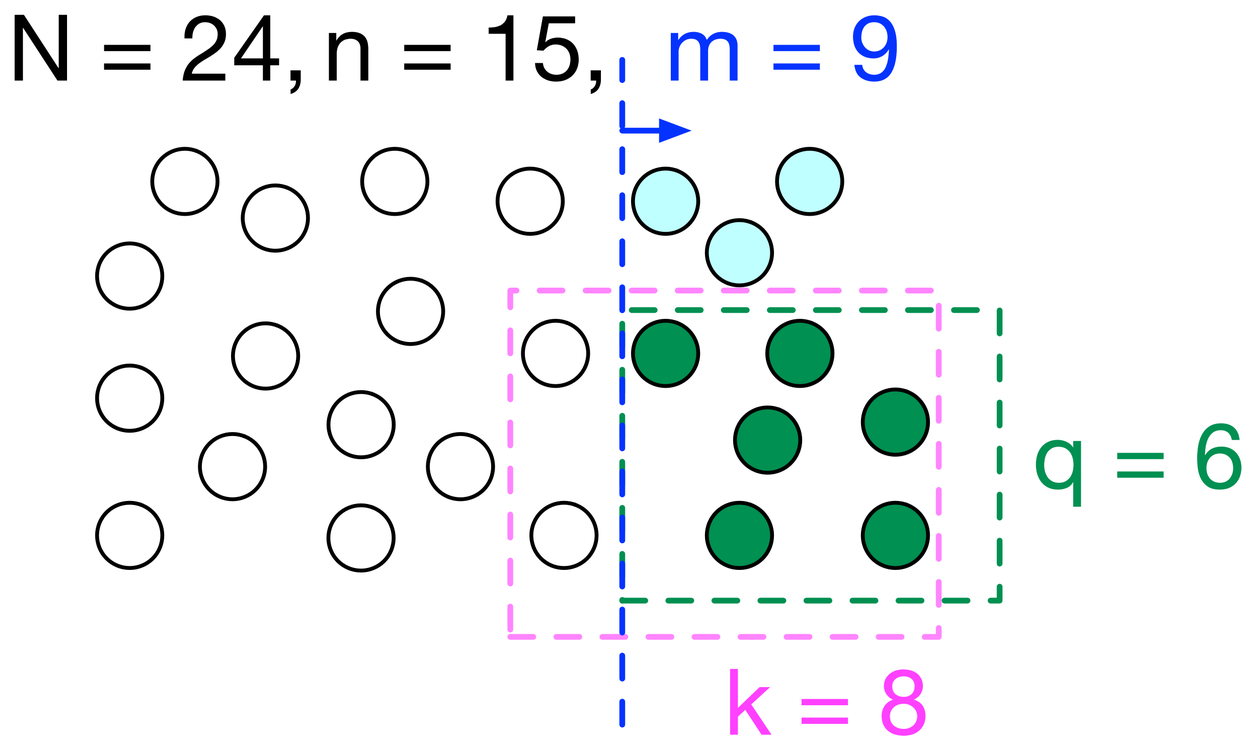
Gene Set Analysis testing over-representation of DEGs



What are the numbers to count?

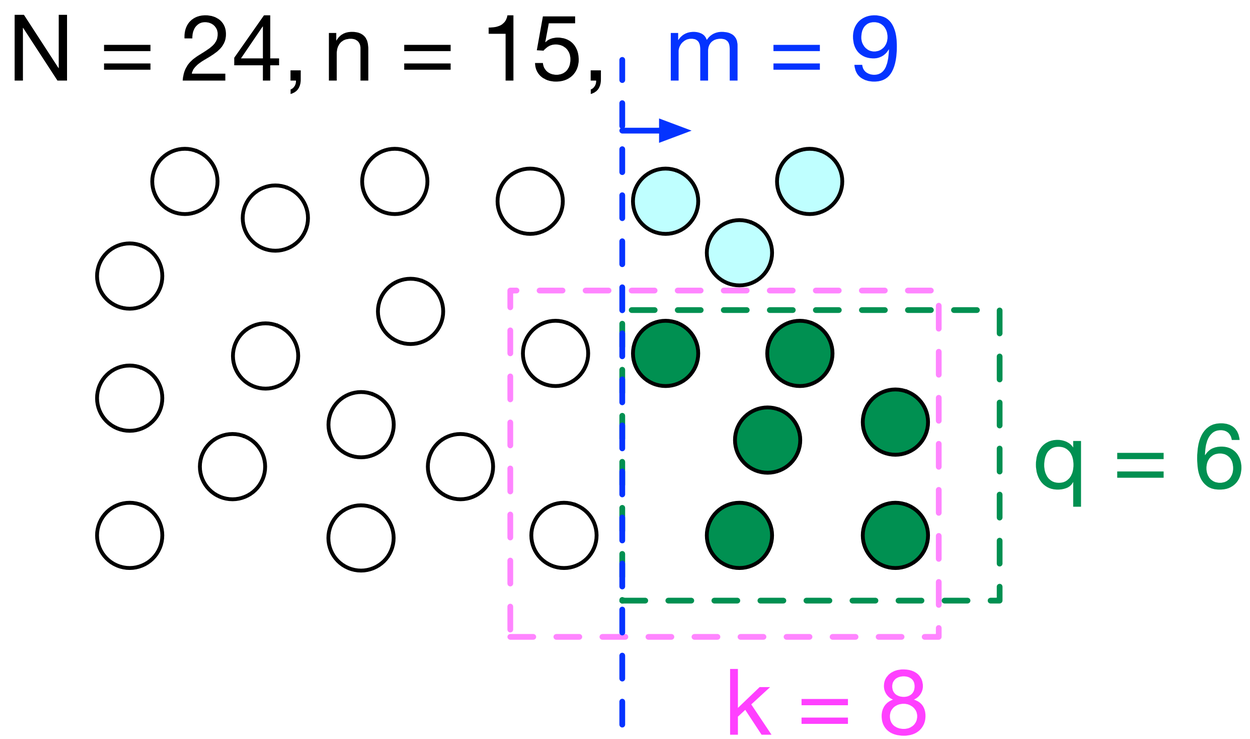
- N: # genes in this universe
- m: # genes in this set
- n: # genes *not* in this gene set
- k: # DEGs in our analysis
- q: # DEGs (of k) overlapping with the set of m genes

Is this overlap of $q = 6$ of $k = 8$ genes significant?



- $N = 24$ # a total of 24 genes
- $m = 9$ genes in this set
- $n = N - m = 15$
- $k = 8$ DEGs
- $q = 6$ out of $k = 8$ overlap

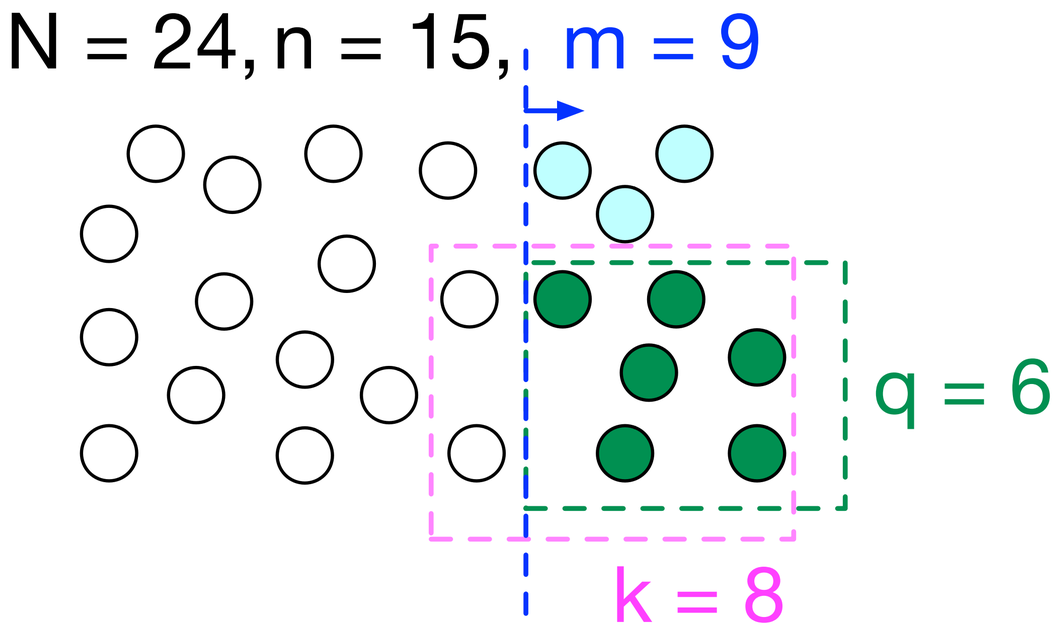
Is this overlap of $q = 6$ of $k = 8$ genes significant?



Questions:

- Is it meaningful enough to report?
- Is it surprising enough that we recapitulated 6/9 (~67 %)?
- What is the null distribution?
- What is the generative/simulation scheme?

How do we find q out of k DEGs overlapping with a gene set of m genes?



Under the null of hypergeometric distribution

1. Sample k DEGs out of N genes
2. Of these k genes, q overlap with a gene set consisting of m genes
3. The rest $k - q$ genes overlap with genes outside of the gene set $N - m$

Binomial coefficient: Let's see if we can estimate the null distribution by counting

- How many all possible ways to select $k = 8$ out of $N = 24$ genes, ignoring the order of k selected genes and $N - k$ *not* selected genes?
- We can think of this as three steps: (1) enumerating N genes, (2) partition them into the first k genes and the rest, (3) ignore the order within each partition.

$$\binom{24}{8} = \frac{\{\text{all possible ways to enumerate 24 genes}\}}{} = \frac{24!}{}$$

Okay, using binomial coefficient, let's count the numbers.

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$$\binom{24}{8} = \frac{\{\text{all possible ways to enumerate 24 genes}\}}{\{\text{enumerating 8 genes}\}\{\text{enumerating 16 genes}\}} = \frac{24!}{8!16!}$$

Okay, using binomial coefficient, let's count the numbers.

Hypergeometric distribution

- What is the probability of uniformly selecting k DEGs out of N genes? $\binom{N}{k}^{-1}$

Hypergeometric distribution

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Hypergeometric distribution

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- How many possible ways of finding q DEGs overlapping with m genes in the gene set? $\binom{m}{q}$
- How many possible ways of finding the rest $k - q$ DEGs overlapping with $(N - m = n)$ genes in the gene set? $\binom{N-m}{k-q}$

Hypergeometric distribution

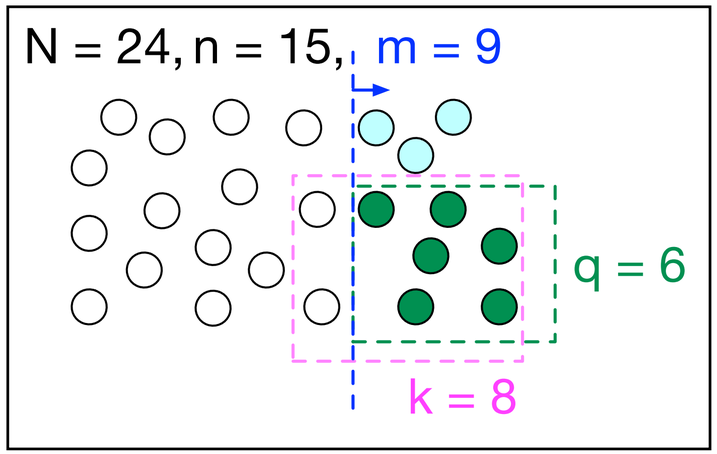
$$P_0(q|N,m,k) = \sum$$

ways to select q out of m

ways to select $(k-q)$ out of $N-m$

the probability
of choosing a set size k
out of total N

$$= $$



Hypergeometric distribution

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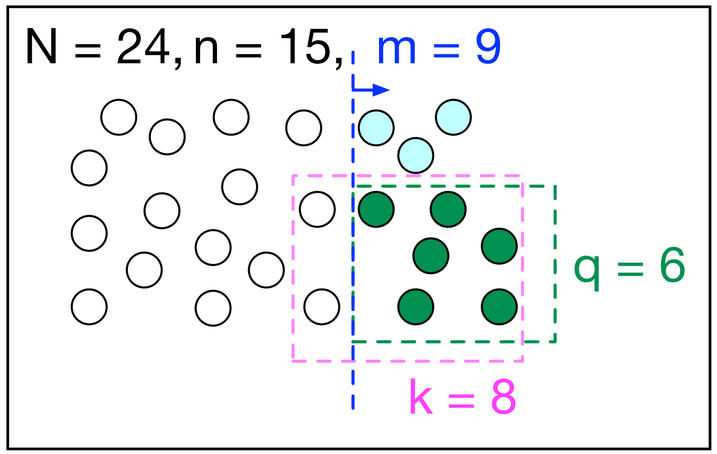
ways to select $(k-q)$ out of $N-m$

the probability
of choosing a set size k
out of total N

$$= \underbrace{\binom{m}{q}}$$

ways to choose
q overlap out of m

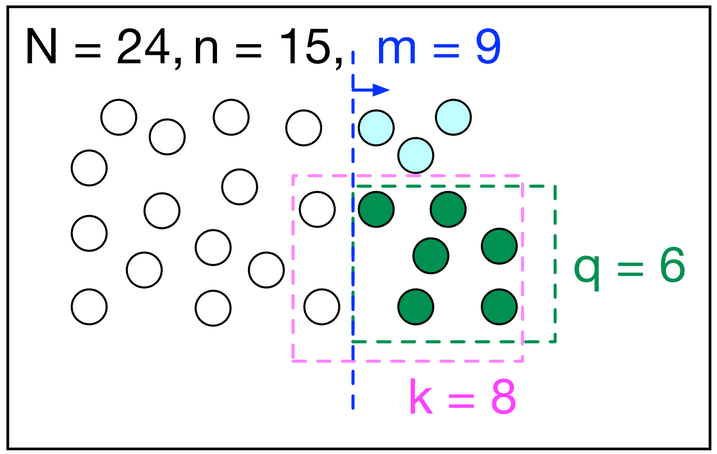


Hypergeometric distribution

$$P_0(q|N,m,k) = \sum_{\substack{\#\text{ ways to select } q \text{ out of } m \\ \#\text{ ways to select } (k-q) \text{ out of } N-m}}$$

the probability
of choosing a set size k
out of total N

$$= \underbrace{\binom{m}{q}}_{\substack{\#\text{ ways to choose} \\ q \text{ overlap out of } m}} \times \underbrace{\binom{N-m}{k-q}}_{\substack{\#\text{ ways to choose} \\ (k-q) \text{ out of } N-m}}$$



Hypergeometric distribution

$$P_0(q|N,m,k) = \sum$$

the probability
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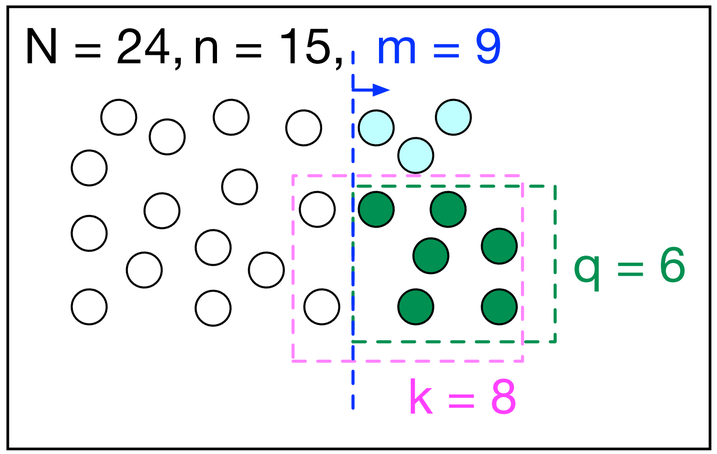
\# ways to select q out of m
\# ways to select $(k-q)$ out of $N-m$

$$= \underbrace{\binom{m}{q}}_{\text{\# ways to choose } q \text{ overlap out of } m} \times \underbrace{\binom{N-m}{k-q}}_{\text{\# ways to choose } (k-q) \text{ out of } N-m} \times \binom{N}{k}^{-1}$$



What is the probability of k overlapping DEGs?

Hypergenometric PMF

$$p(x|N,m,k) = \frac{\binom{m}{x}\binom{N-m}{k-x}}{\binom{N}{k}}$$

Hypergeometric CDF

$$p(q|N,m,k) = \sum_{x=0}^{q} \frac{\binom{m}{x}\binom{N-m}{k-x}}{\binom{N}{k}}$$

Hypergeometric test for testing significant overlap

$$H_0 : x \leq q \quad \text{vs.} \quad H_1 : x > q$$

We may observe overlap q genes by random sampling of k genes **without** replacement.

Therefore, we can calculate the p-value:

What is the definition of p-value?

```
phyper(q=6, m=9, n=15, k=8, lower.tail=FALSE)
```

```
## [1] 0.0007464604
```

Hypergeometric test for testing significant overlap

$$H_0 : x \leq q \quad \text{vs.} \quad H_1 : x > q$$

We may observe overlap q genes by random sampling of k genes **without** replacement.

Therefore, we can calculate the p-value:

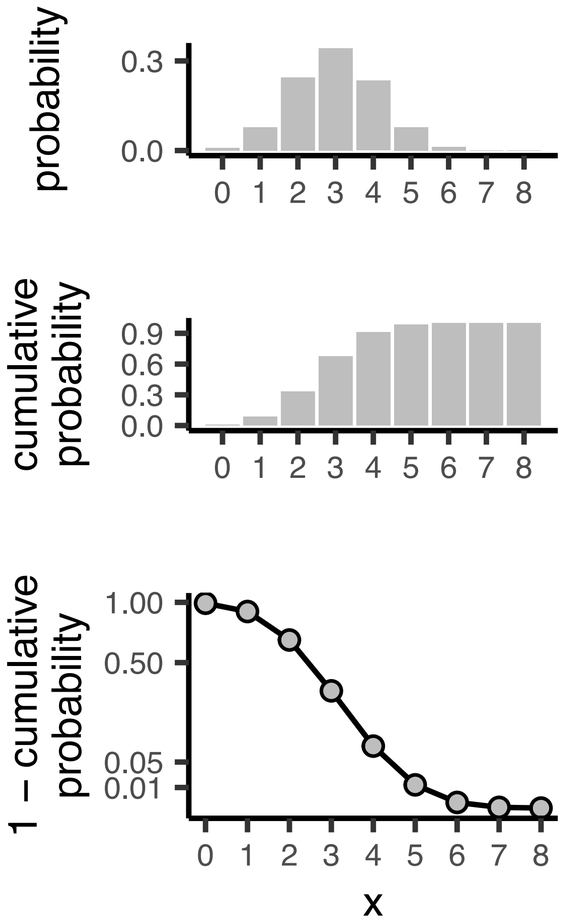
$$P(x > q | n, m, k) = 1 - \sum_{x=0}^{q} \frac{\binom{m}{x}\binom{n}{k-x}}{\binom{n+m}{k}}$$

```
phyper(q=6, m=9, n=15, k=8, lower.tail=FALSE)
```

```
## [1] 0.0007464604
```

How significant is q overlap in our discovery?

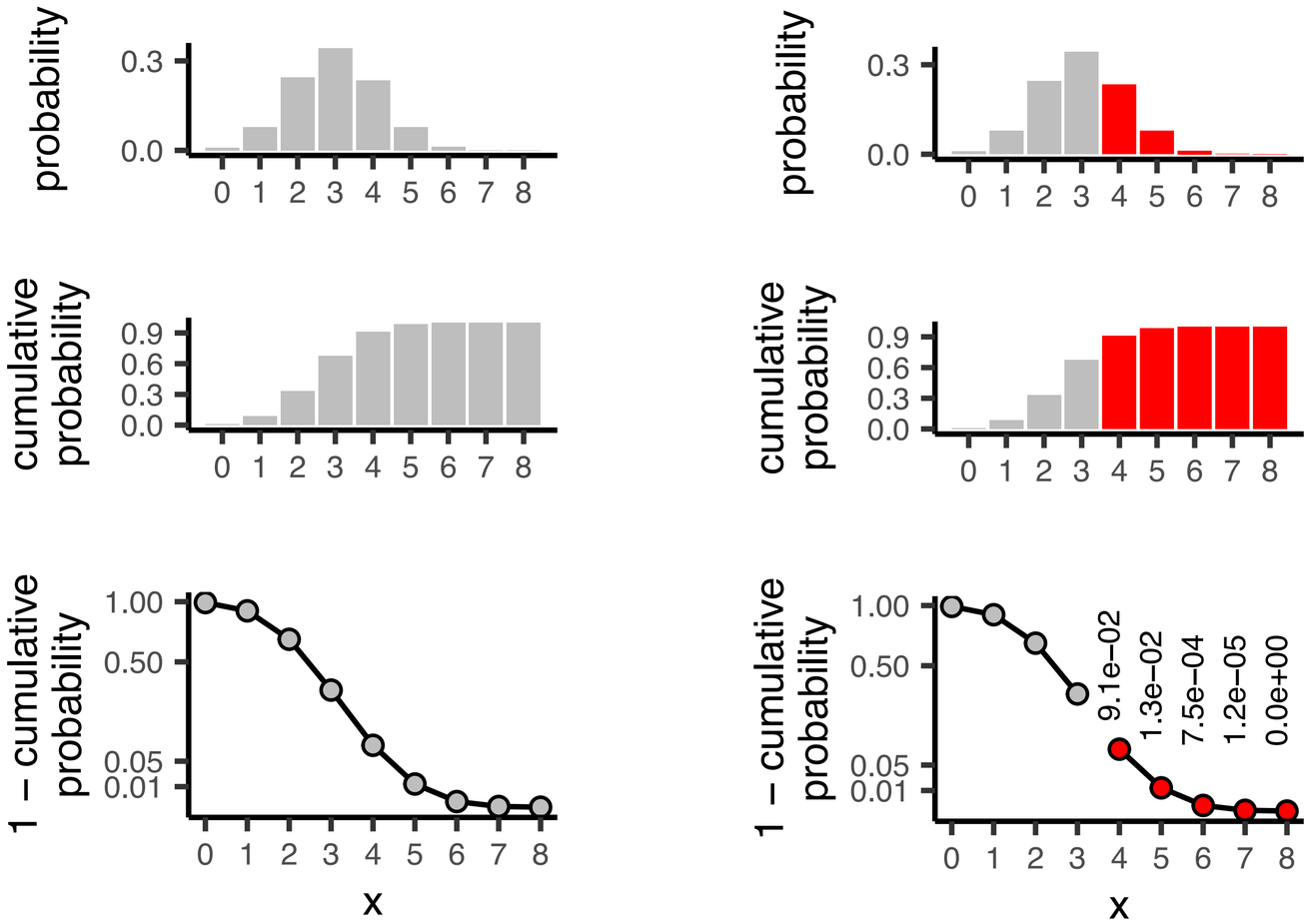
$m = 9$, $n = 15$, and $k = 8$,



How significant is q overlap in our discovery?

$m = 9$, $n = 15$, and $k = 8$,

If $q = 3$:



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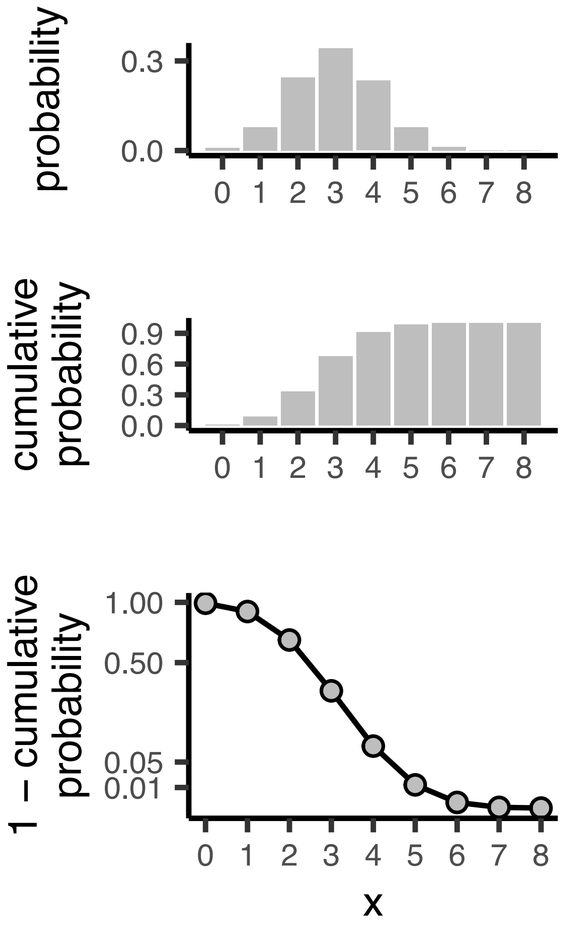

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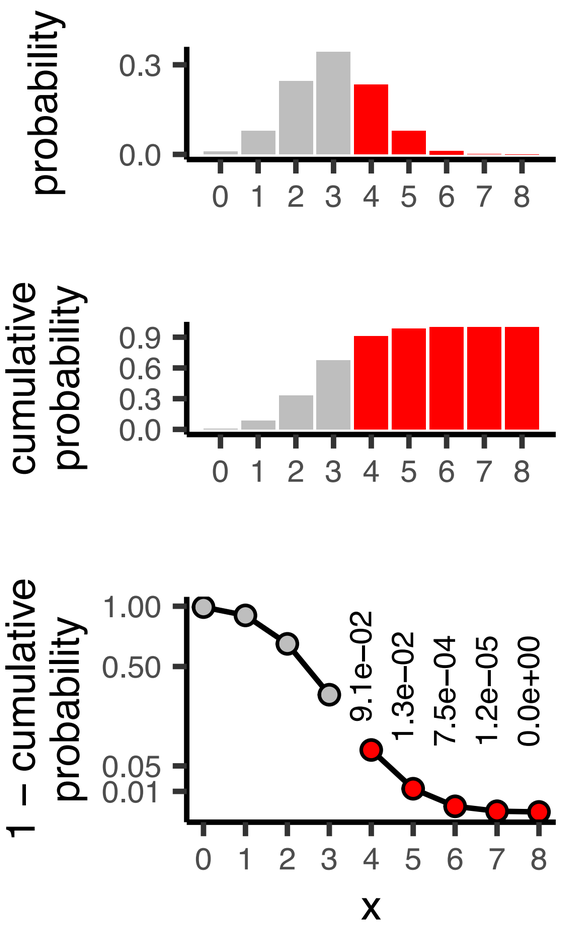
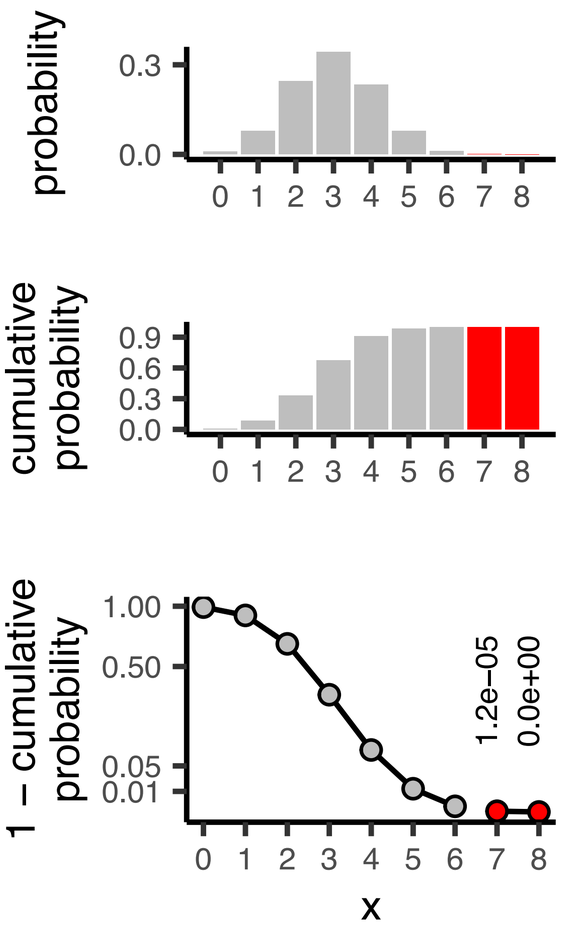


If $q = 6$:



Summary of the gene set analysis by hypergeometric test

When does it work?

- A routine to construct a set of differentially expressed genes by handling multiple hypothesis testing
- Gene sets are of similar sizes and *nearly* disjoint/independent from one another
- Genes are *nearly* independent (there is no overwhelmingly favourite genes)

When does it not work?

- **Don't** have a good way to make a set:
  - Our discovery data may lack statistical power, i.e., no (or a few) significant genes left after multiple hypothesis correction
- There is a hidden factor that can affect two steps: (1) gene set selection (annotations/knowledge) and (2) differential expression calling

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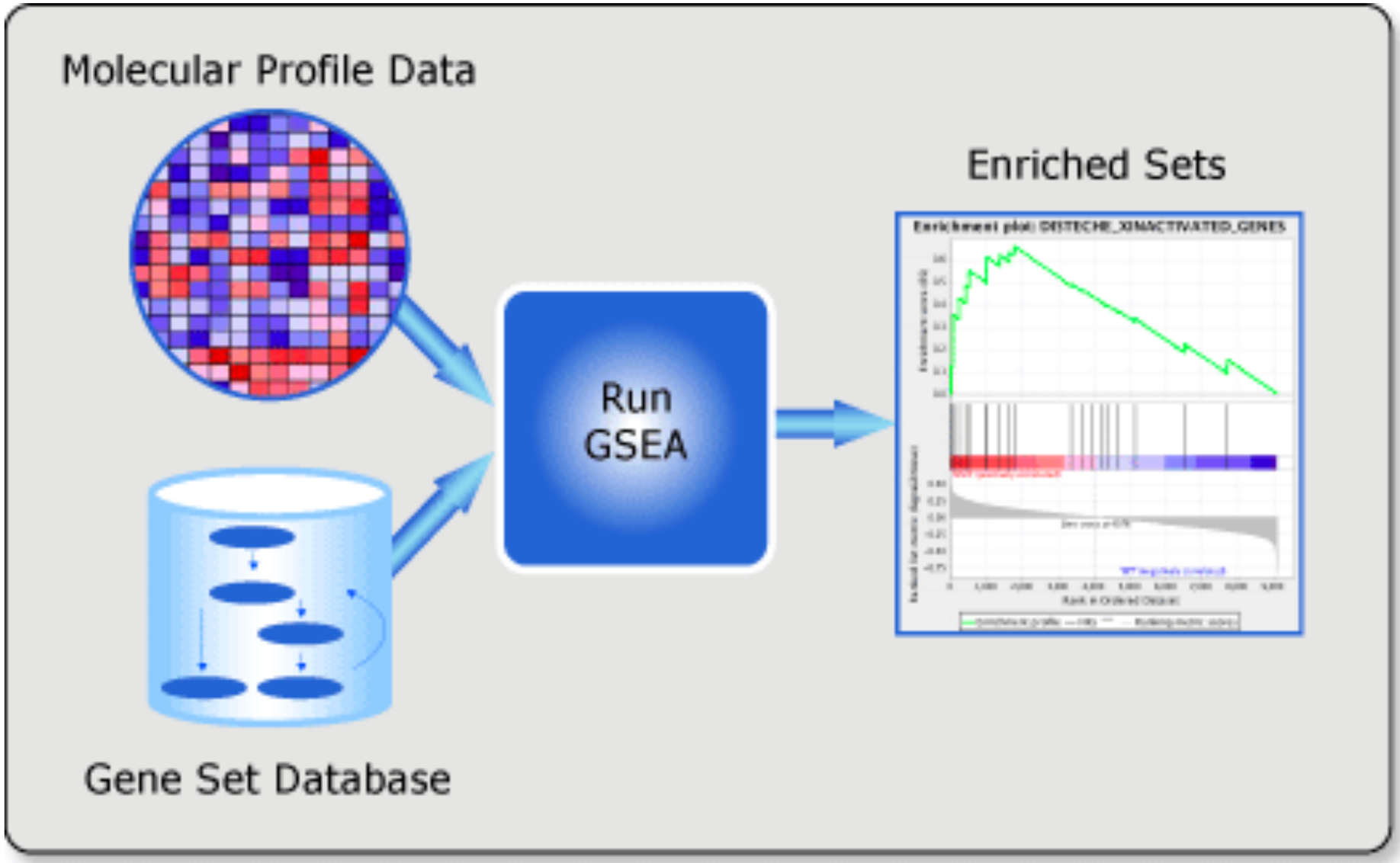


https://www.gsea-msigdb.org/gsea/index.jsp

Rank-based Gene Set Enrichment Analysis method (Subramanian *et al.* 2005)

- A collection of gene-sets: $\mathcal{C}_1, \ldots, \mathcal{C}_K$
- A vector of gene-level scores (G genes): $z_1, \ldots, z_G$
- Each z_g could come from differential expression analysis

GSEA algorithm

- For each k
  - Compute a set-level score $S_k(\mathbf{z}, \mathcal{C}_k)$
  - E.g., Kolmogorov-Smirnov statistic comparing

$$\{z_g : g \in \mathcal{C}_k\} \text{ vs. } \{z_g : g \notin \mathcal{C}_k\}$$

Gene Set Enrichment Analysis method (Subramanian *et al.* 2005)

- Construct null distribution of $S_1, \ldots, S_K$ by sample label (case-control) or gene-to-set membership permutation
- Using null distribution by permutation, estimate p-values and false discovery rates
- If we knew null distribution, we would not need expensive permutations.

Good:

- No cutoff/assumptions needed to estimate null distribution
- Aggregate scores across many genes! (boost the power)

Bad:

- What is an appropriate statistic?
- What should be permuted? For how long?

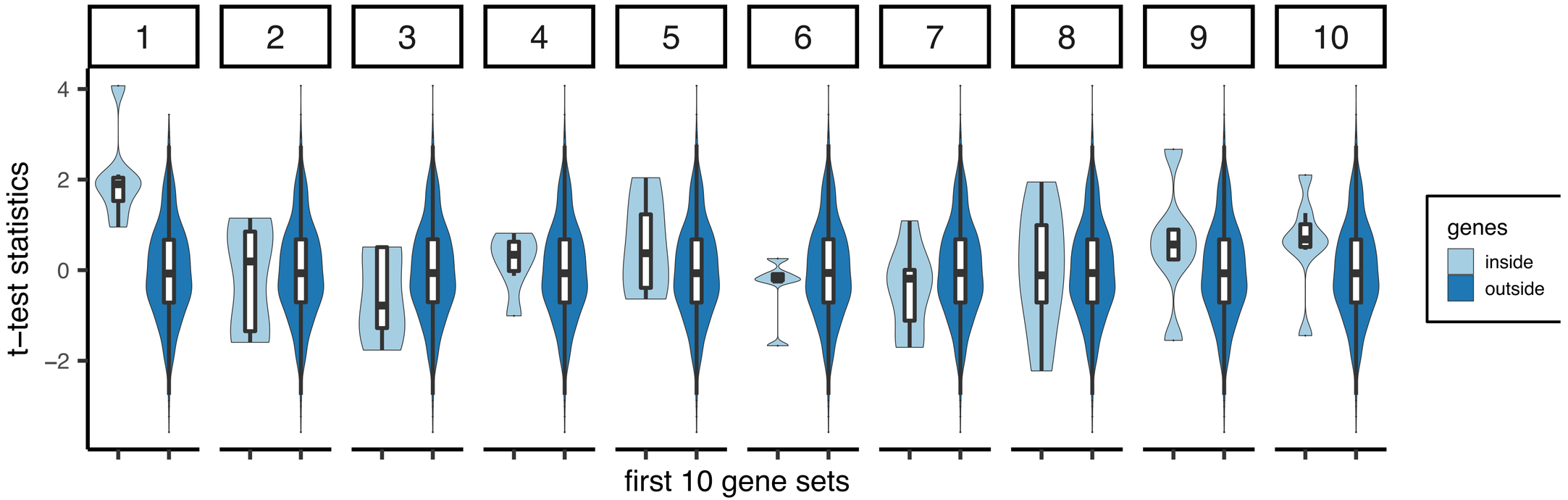
Simulation (Efron and Tibshirani 2007)

1. Generate basal gene expression

$$X_{i,g} \sim \mathcal{N}(0, 1)$$

2. Sample case vs. control membership (the rows of X) uniformly at random
3. Sample membership gene to gene set uniformly at random
4. For the first gene set, select a certain fraction of genes to perturb
5. For the selected genes g^*, add some Δ value to X_{i,g^*} if the sample i belongs to the control group

The goal is to come up with a representative score for all the genes within each set



What will be a proper gene set score?

Can we simply aggregate gene-level z-scores (or t-statistics) within each set?

Irizarry *et al.* (2009), using Stouffer Z-score

$$S_k = \sum_{g \in \mathcal{C}_k} z_g / \sqrt{|\mathcal{C}_k|} \sim \mathcal{N}(0, 1)$$

if $Z_g \sim \mathcal{N}(0, 1)\,, \forall g$

Aggregating z-scores within a set to have one number for the set

```
geneset.score <- function(X, S, Y) {
    z.genes <- run.t.test(X, Y)
    n.sets <- apply(S, 1, sum)
    z.sets <- (S %*% z.genes /
                sqrt(n.sets))
}
```

```
z.sets <- geneset.score(dat$X, dat$S, dat$Y)
```

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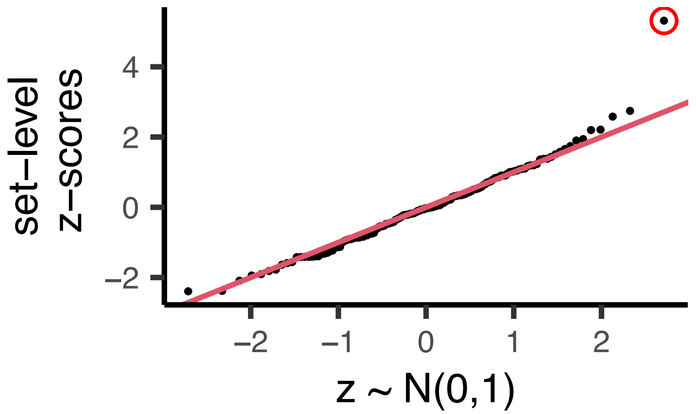

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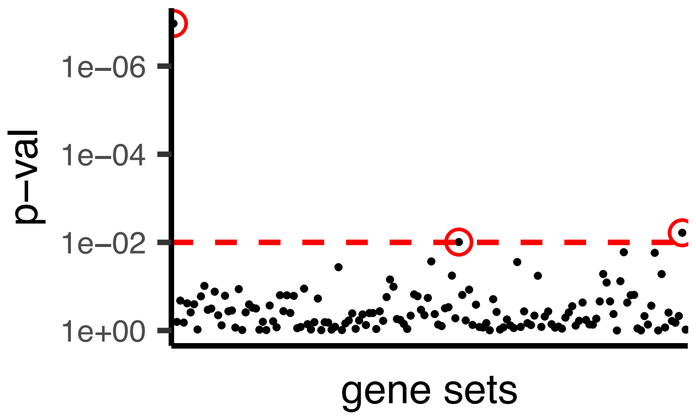
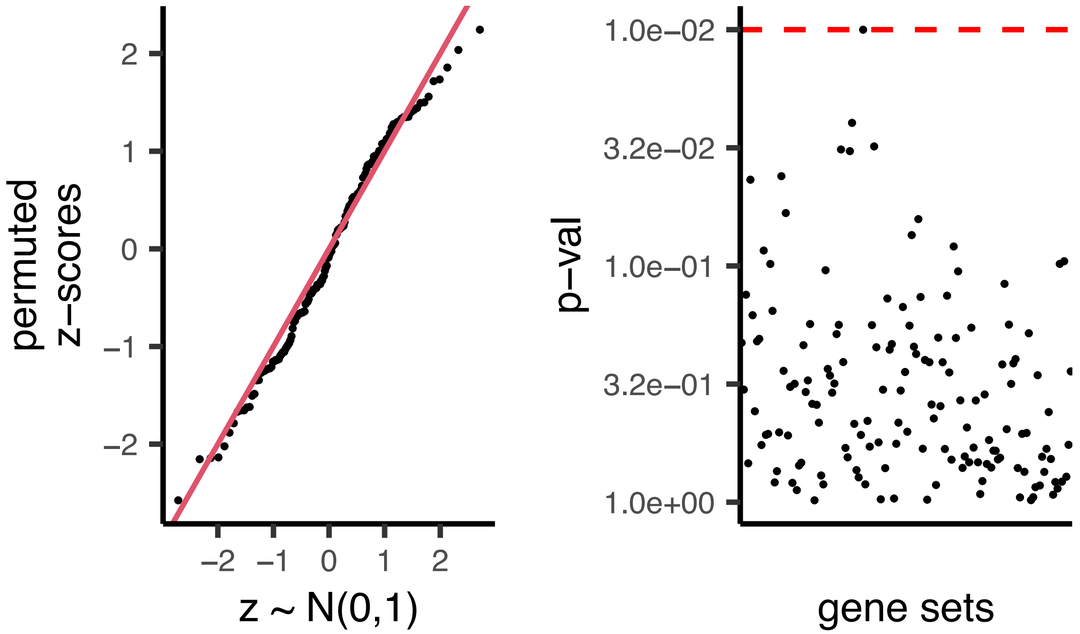


Constructing null distribution by gene permutation

What if we don't know the distribution of set-wise scores?

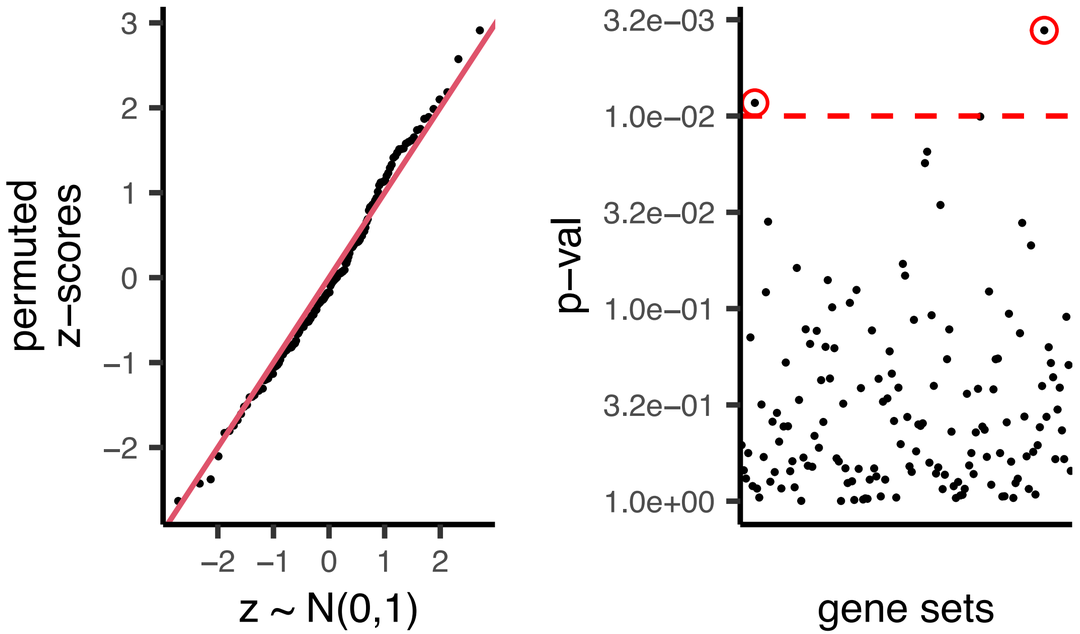
```
S.perm <- t(apply(dat$S, 1, sample))
z.perm <- geneset.score(dat$X, S.perm, dat$Y)
```



- Repeat the permutation of gene set membership matrix while preserving the number of genes within each set
- Compute set-level z-scores (or a similar kind) and construct null distribution
- Calculate p-values by counting the frequency of observed $S_k^* > S_k^{\text{perm}}$

Constructing null distribution by sample permutation

```
Y.perm <- sample(dat$Y)
z.perm <- geneset.score(dat$X, S.perm, Y.perm)
```



- Repeat the permutation of case-control labels while preserving the same number of cases and controls
- Compute set-level z-scores (or a similar kind) and construct null distribution
- Calculate p-values by counting the frequency of observed $S_k^* > S_k^{\text{perm}}$

Young *et al. Genome Biology* 2010, **11**:R14
http://genomebiology.com/2010/11/2/R14

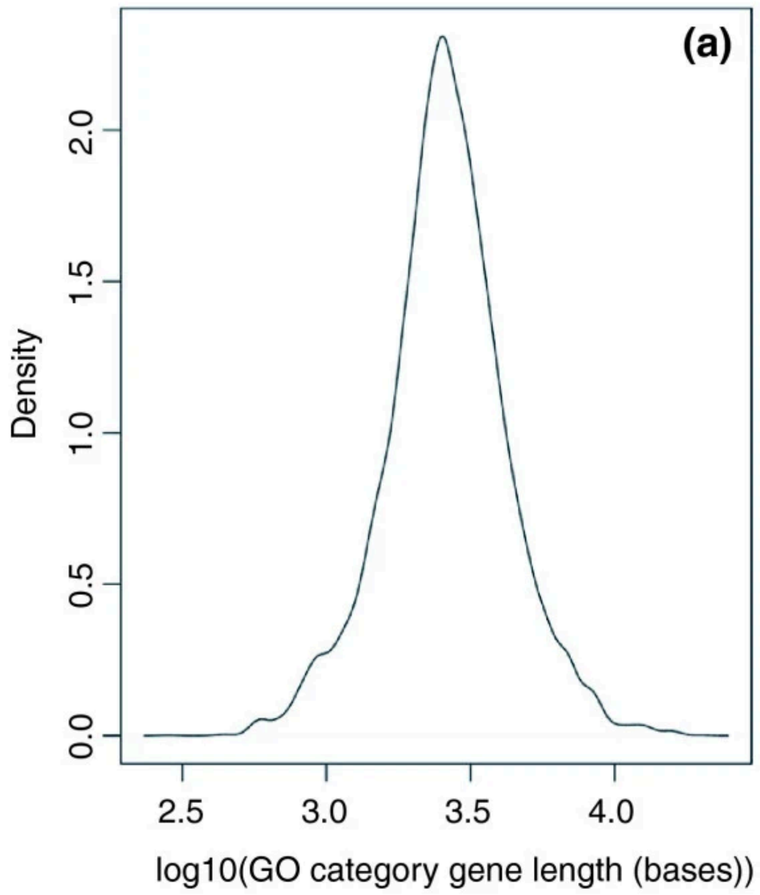
Genome Biology

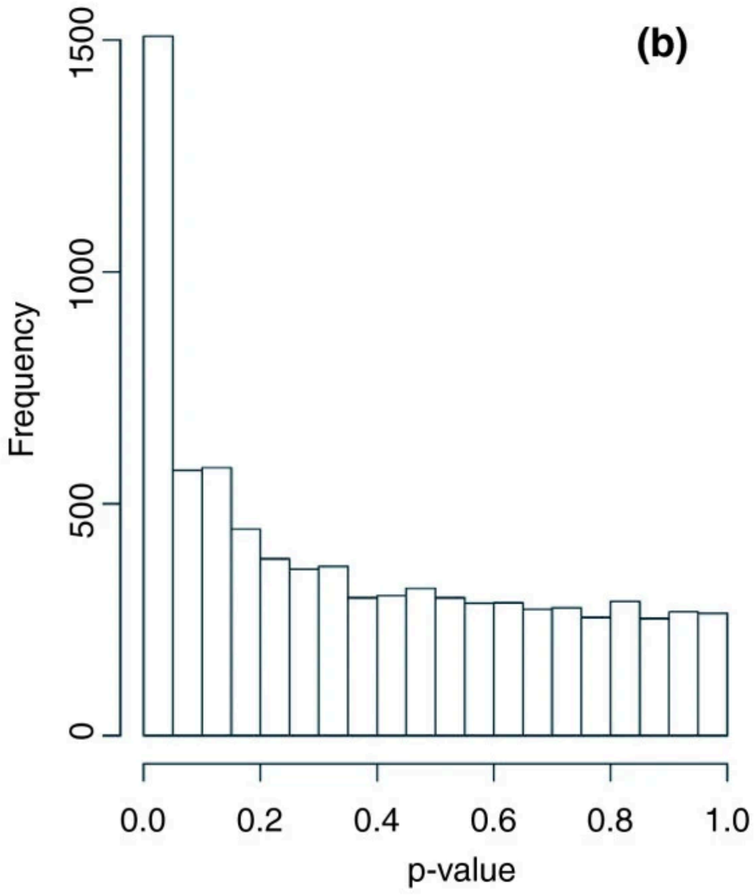
METHOD Open Access

Gene ontology analysis for RNA-seq: accounting for selection bias

Matthew D Young, Matthew J Wakefield, Gordon K Smyth and Alicia Oshlack*

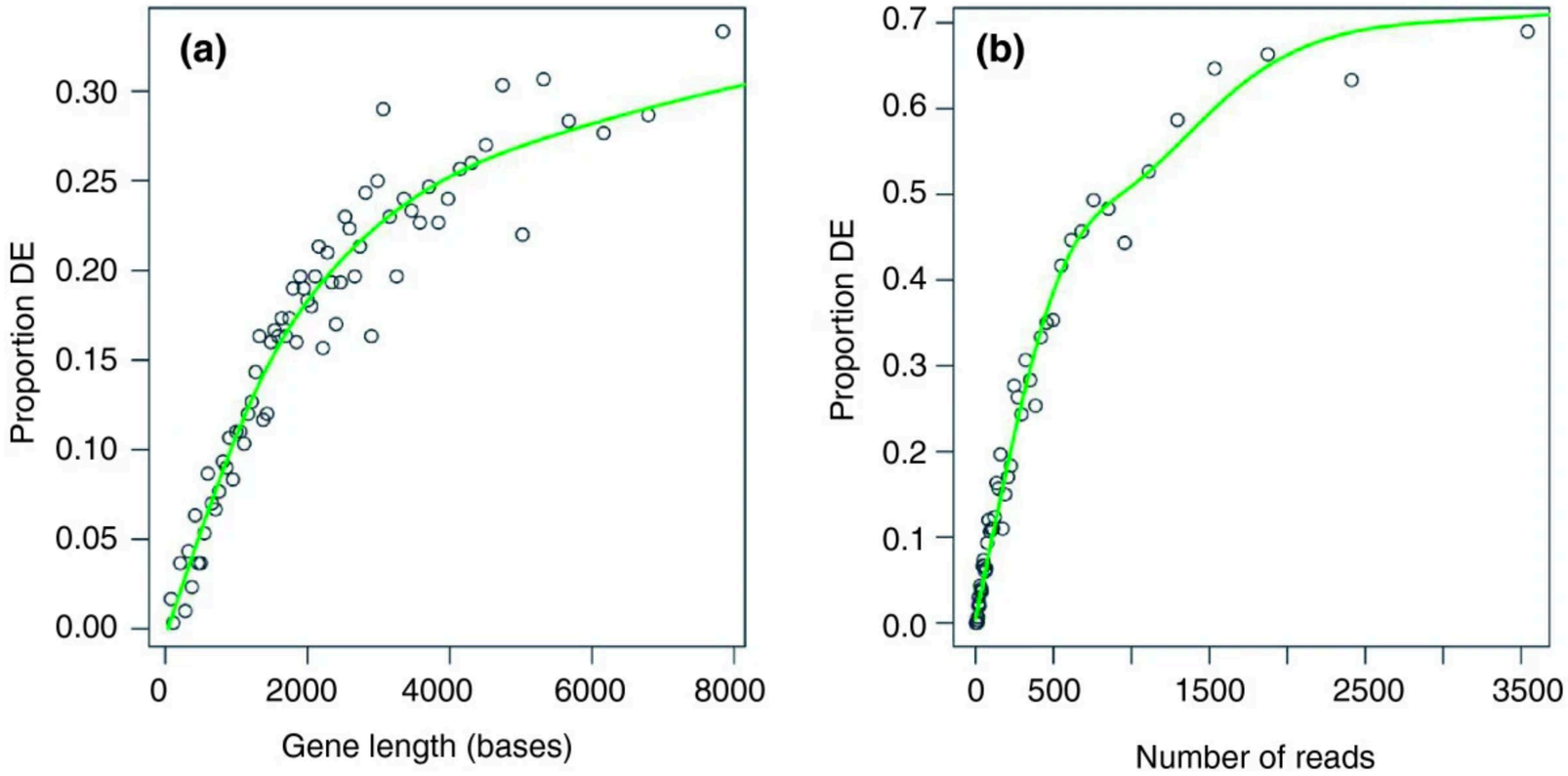
Not all gene sets are the same (gene length bias)





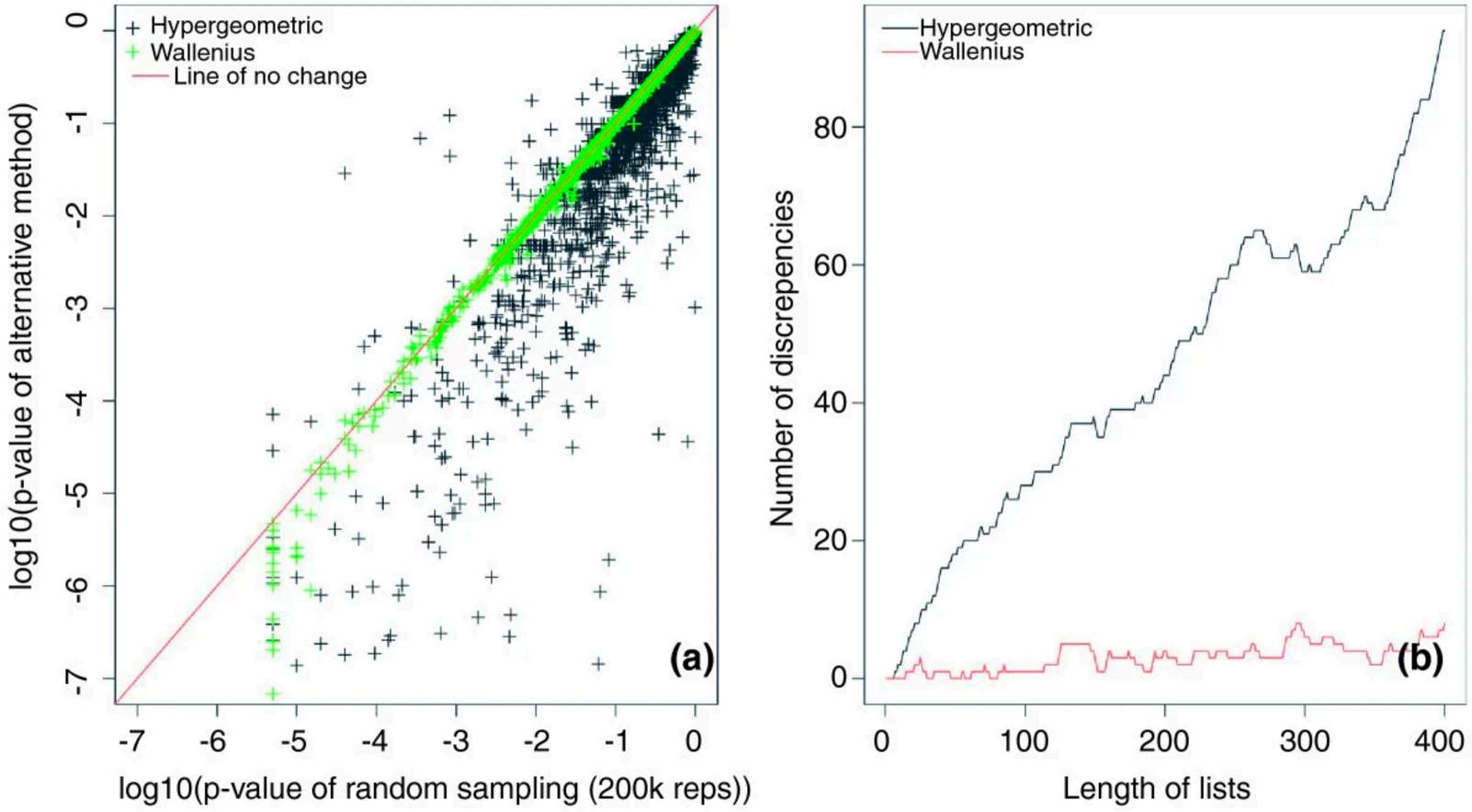
Young .. Oshlack, *Genome Biology* (2010)

Longer genes tend to be more differentially expressed



Young .. Oshlack, *Genome Biology* (2010)

A theoretical distribution of set-level scores may inflate p-values, thus wrong FDR calibration



Young .. Oshlack, *Genome Biology* (2010)

The Annals of Applied Statistics
2007, Vol. 1, No. 1, 107–129
DOI: 10.1214/07-AOAS101

ON TESTING THE SIGNIFICANCE OF SETS OF GENES

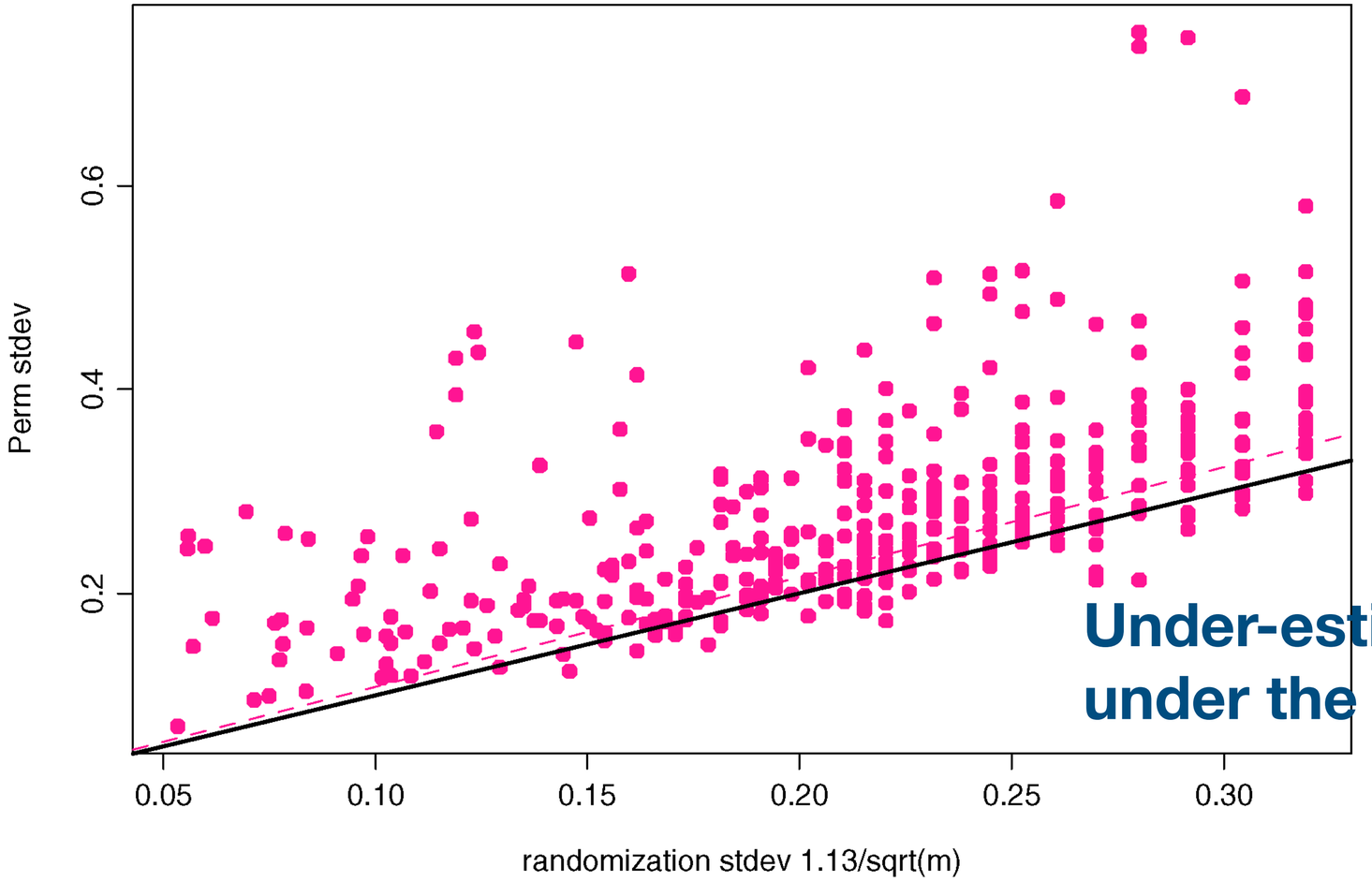
BY BRADLEY EFRON[1] AND ROBERT TIBSHIRANI[2]

Stanford University

This paper discusses the problem of identifying differentially expressed groups of genes from a microarray experiment. The groups of genes are externally defined, for example, sets of gene pathways derived from biological databases. Our starting point is the interesting Gene Set Enrichment Analysis (GSEA) procedure of Subramanian et al. [*Proc*. *Natl*. *Acad*. *Sci*. *USA* **102** (2005) 15545–15550]. We study the problem in some generality and propose two potential improvements to GSEA: the *maxmean* statistic for summarizing gene-sets, and *restandardization* for more accurate inferences. We discuss a variety of examples and extensions, including the use of gene-set scores for class predictions. We also describe a new R language package *GSA* that implements our ideas.

Row vs. column (gene vs. sample) permutation

sample to disease/ condition label permutation

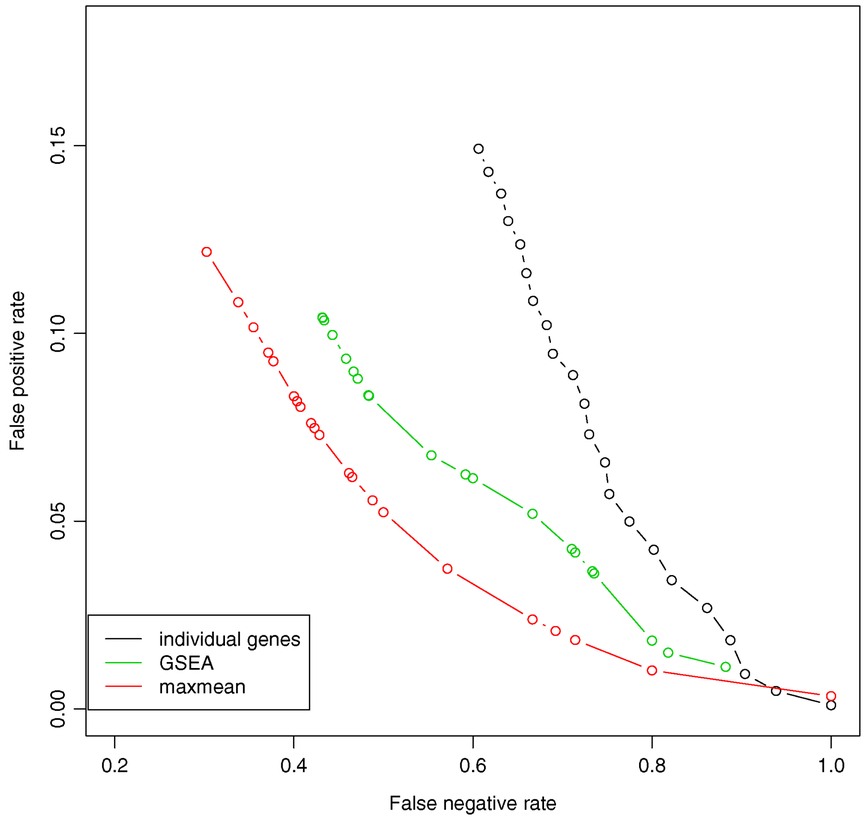


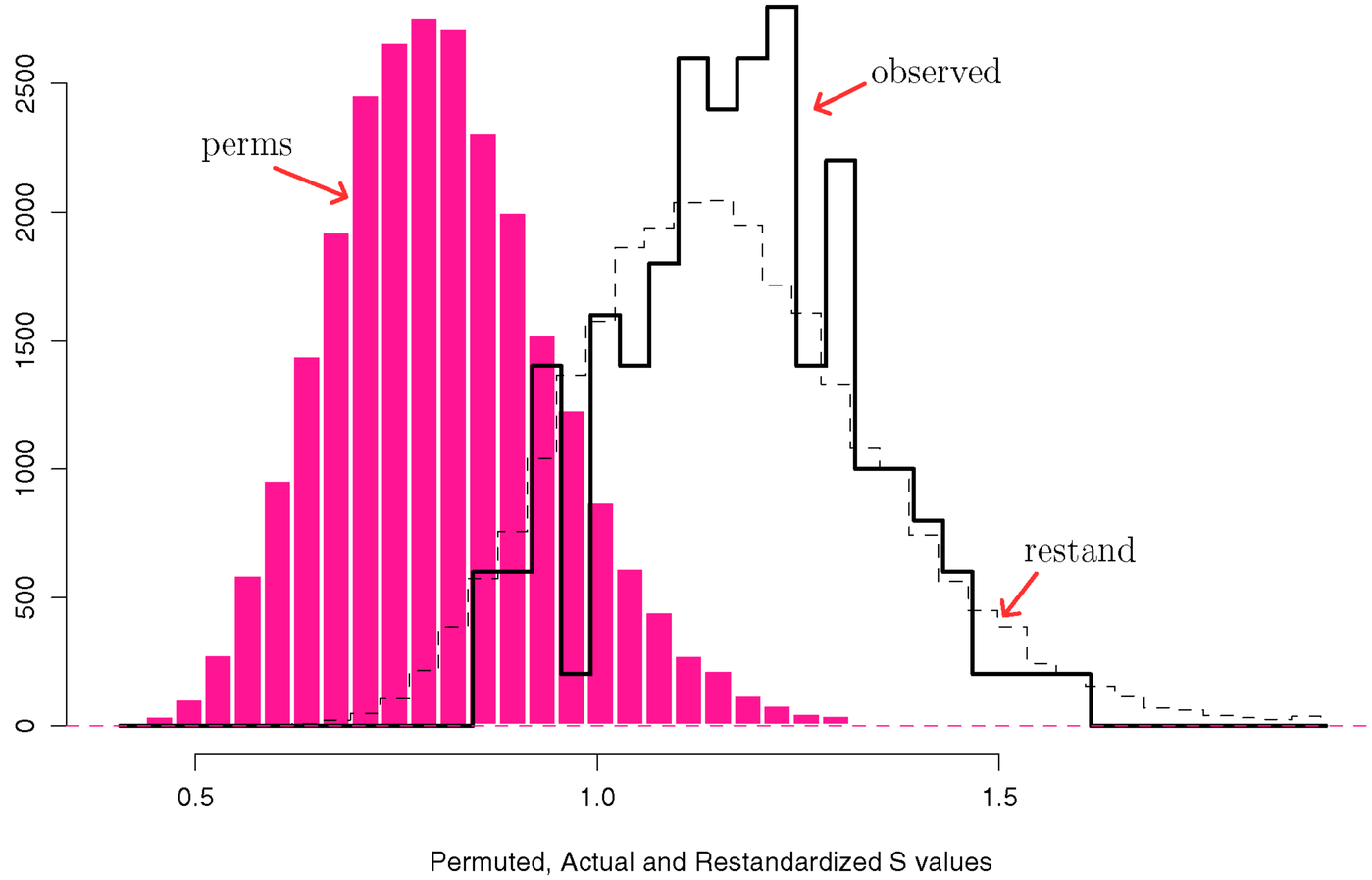
Under-estimating variance under the null

gene-to-set membership permutation

Efron & Tibshirani, *AOAS* (2007)

Mis-calibrating the null distribution will make almost everything very significant





Efron & Tibshirani, *AOAS* (2007)

Today’s lecture: Enrichment Analysis

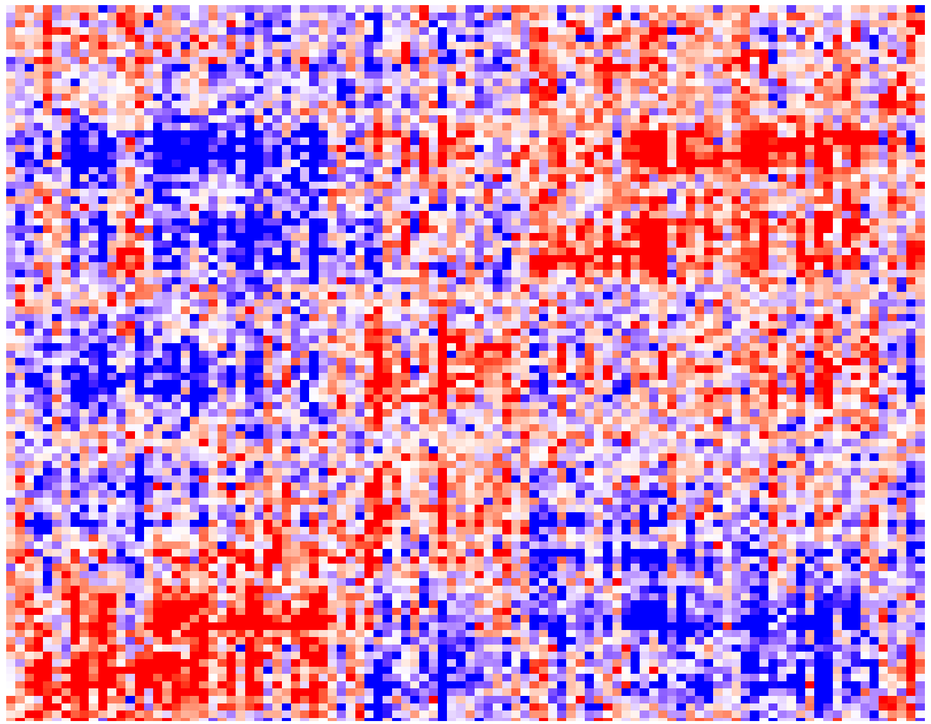
- **Motivations: What’s next after genomics analysis?**
  - What have we learned? How do we know that our discovery is meaningful?
- **Gene set enrichment analysis**
  - Set-based approach: Hypergeometric test
  - Rank-based approach: GSEA by KS statistic
- **Can we engineer new gene sets/scores?**
  - Principal Component Analysis
  - Matrix factorization of count data

A toy example: Can we uncover hidden structures in a gene expression matrix?

Suppose the data matrix (gene expression data) were generated by the following:

$$\mathbf{x}_i = \mathbf{u}_i V^\top + \epsilon_i$$

for all i with $\epsilon_i \sim \mathcal{N}(\mathbf{0}, I)$



Can we reverse engineer the U and V matrices from the data X?

$$X \to UV^\top$$

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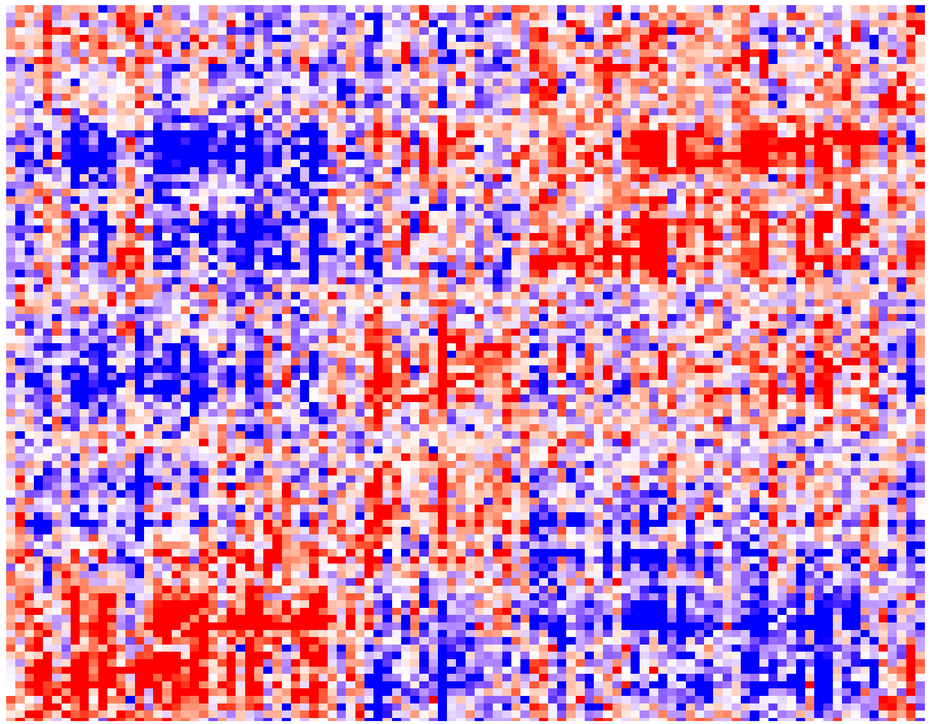

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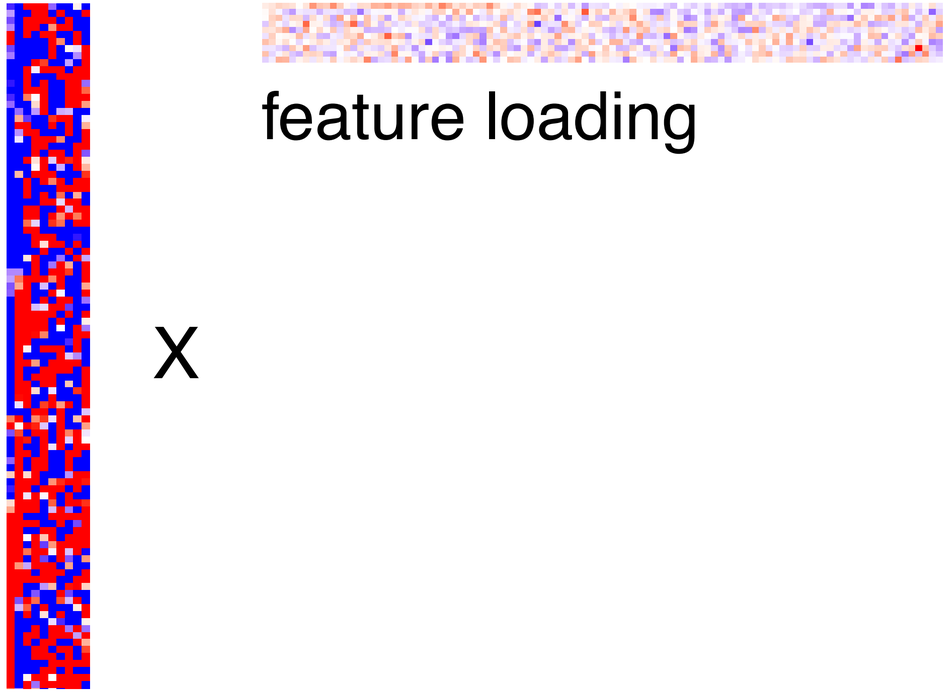

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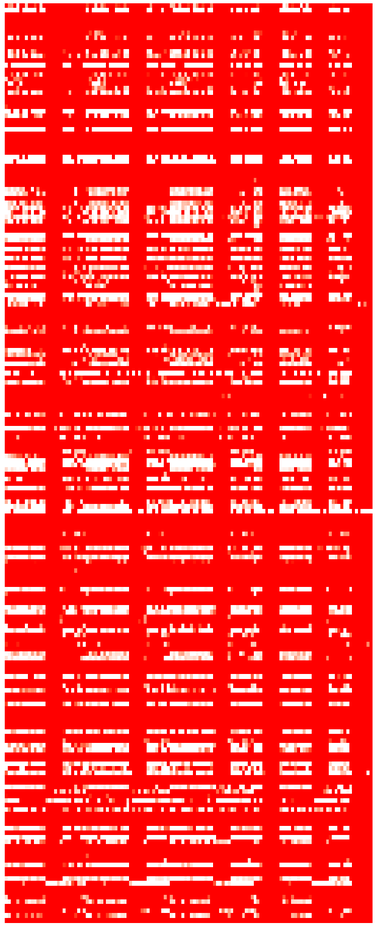
Working example: GSE107011

Top 200 most variable genes in the data matrix:



- We will call such a high-dimensional matrix X $(m \times n)$
- X has m=58,311 rows (transcripts/genes/features)
- X has n=127 columns (samples/#data points)
- The rows were log-transformed and scaled by `scale()` for visualization
- Each sample is a 58,311-dimensional vector!
- Each gene is a 127-dimensional vector...

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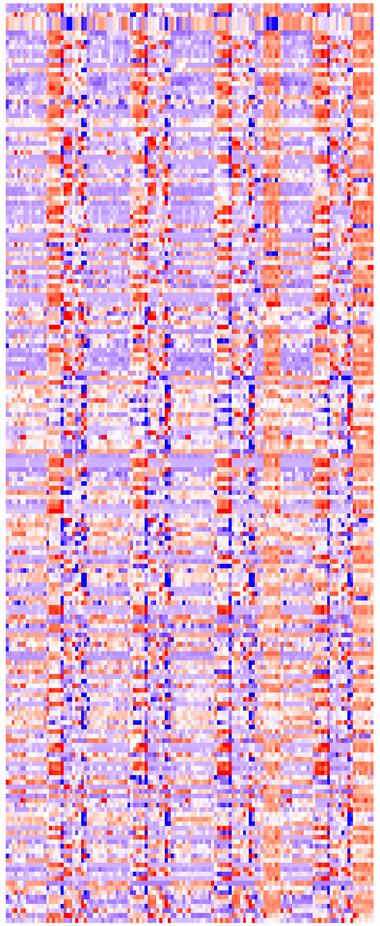

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1-dimensional representations/summary of data matrix

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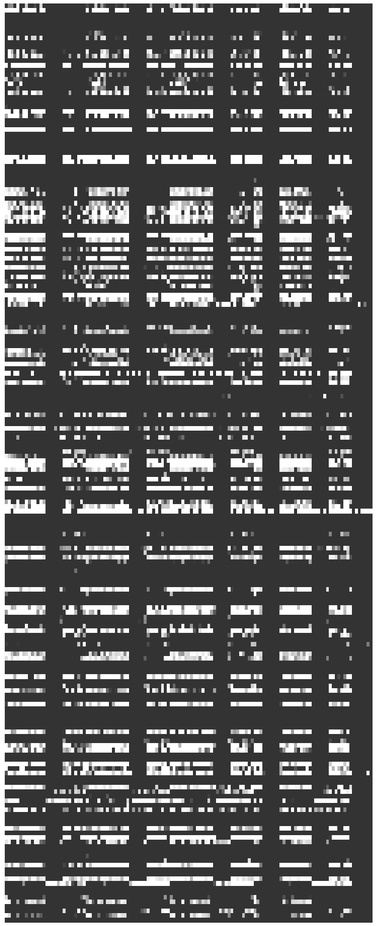
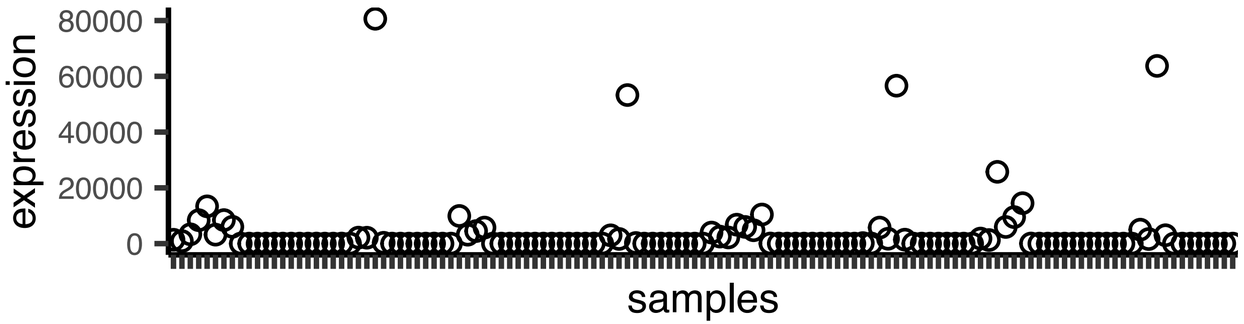


Each sample (column):



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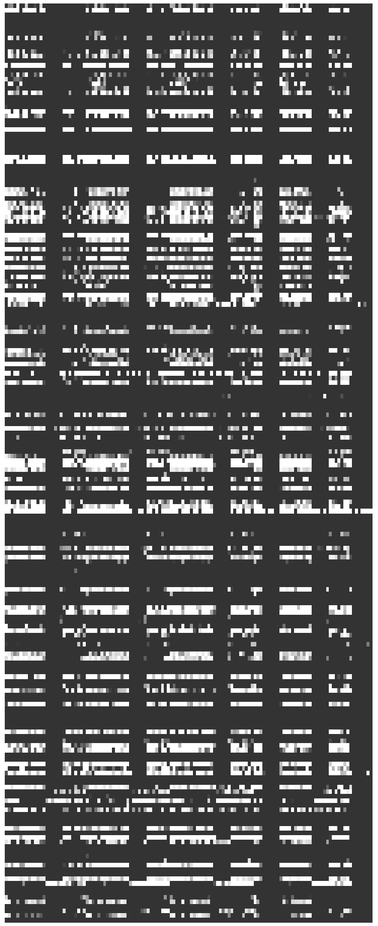

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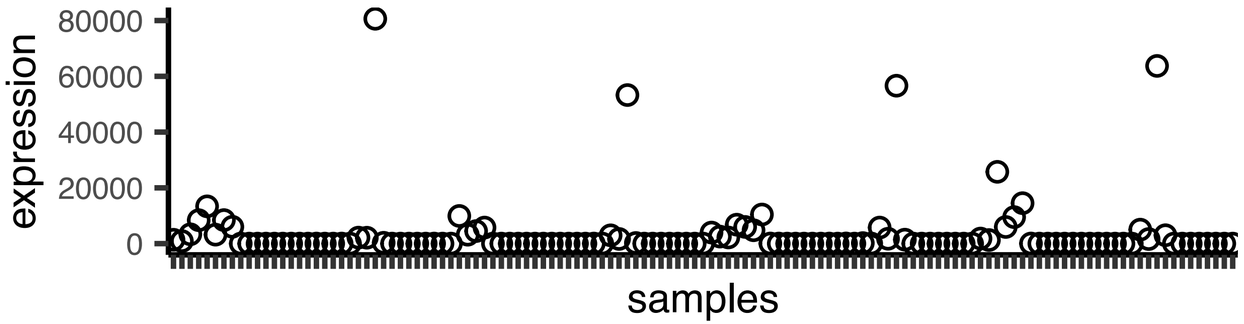
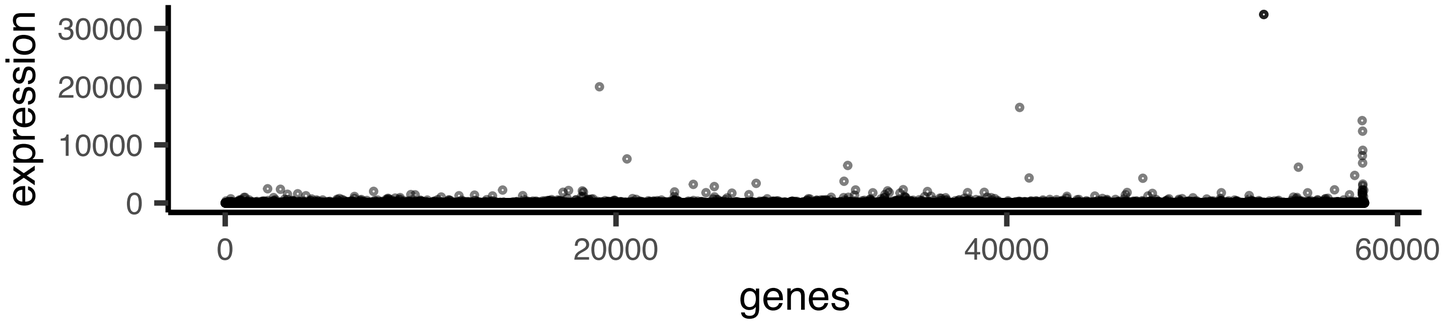
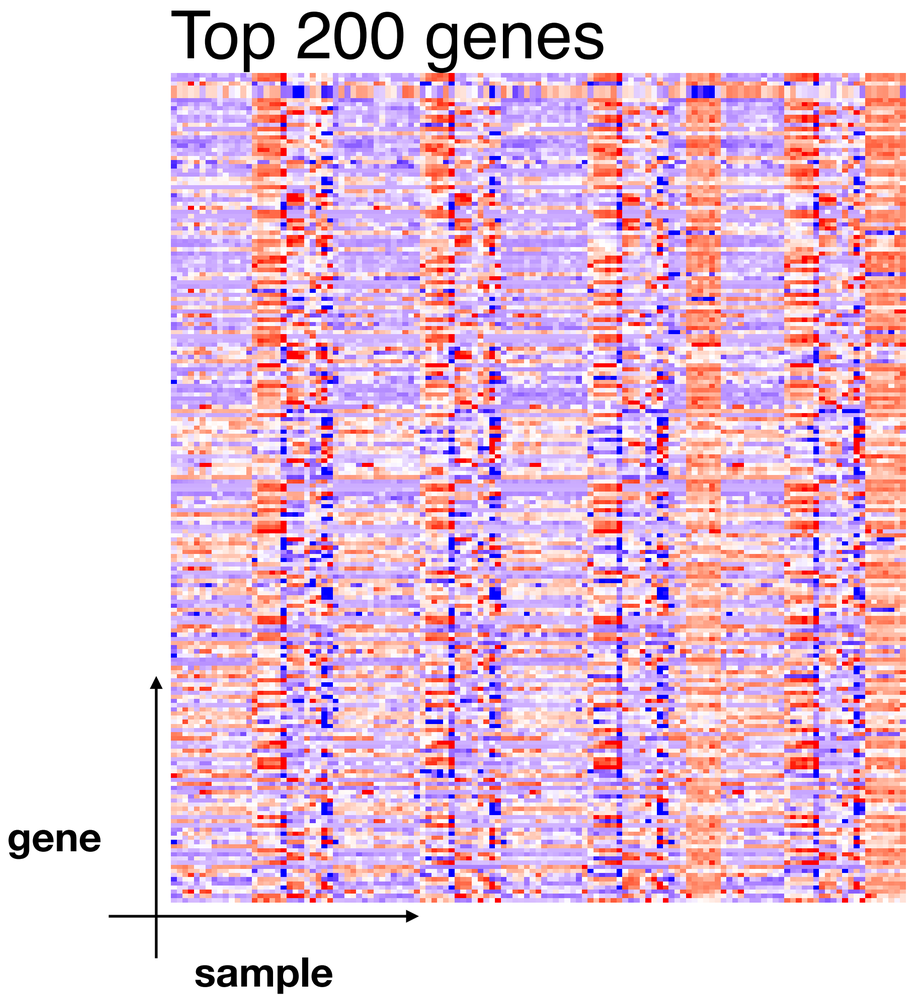


Each gene (row):



Can you see some common patterns?



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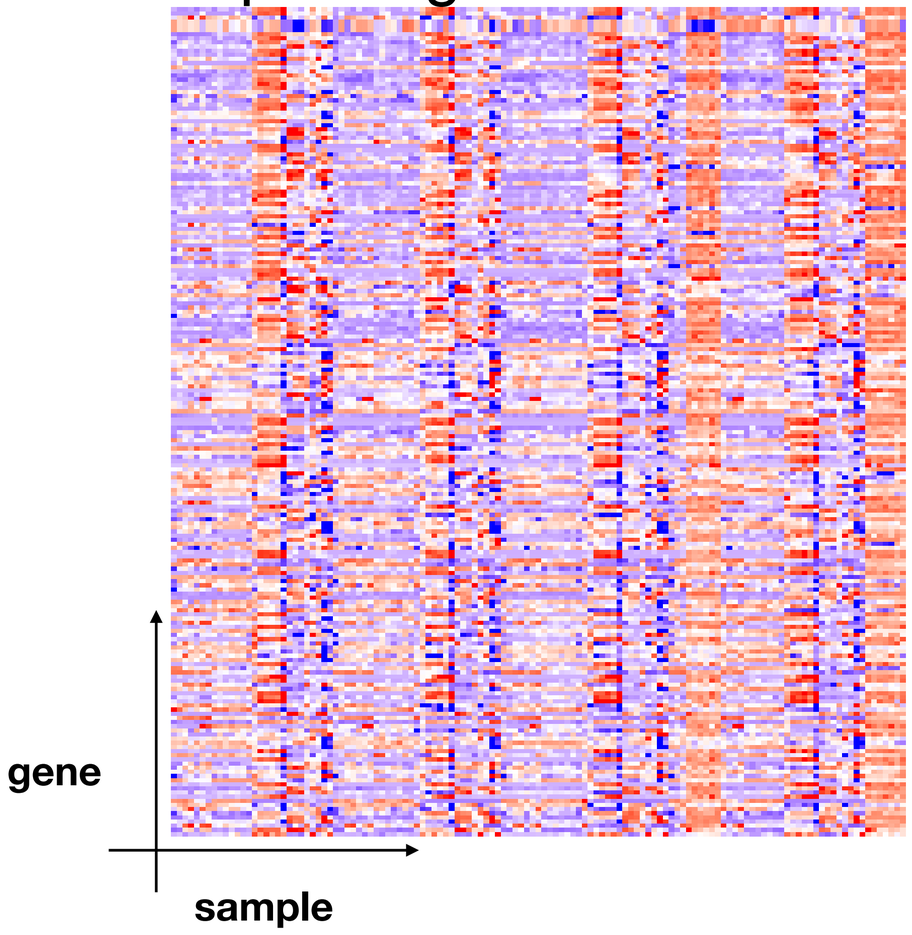
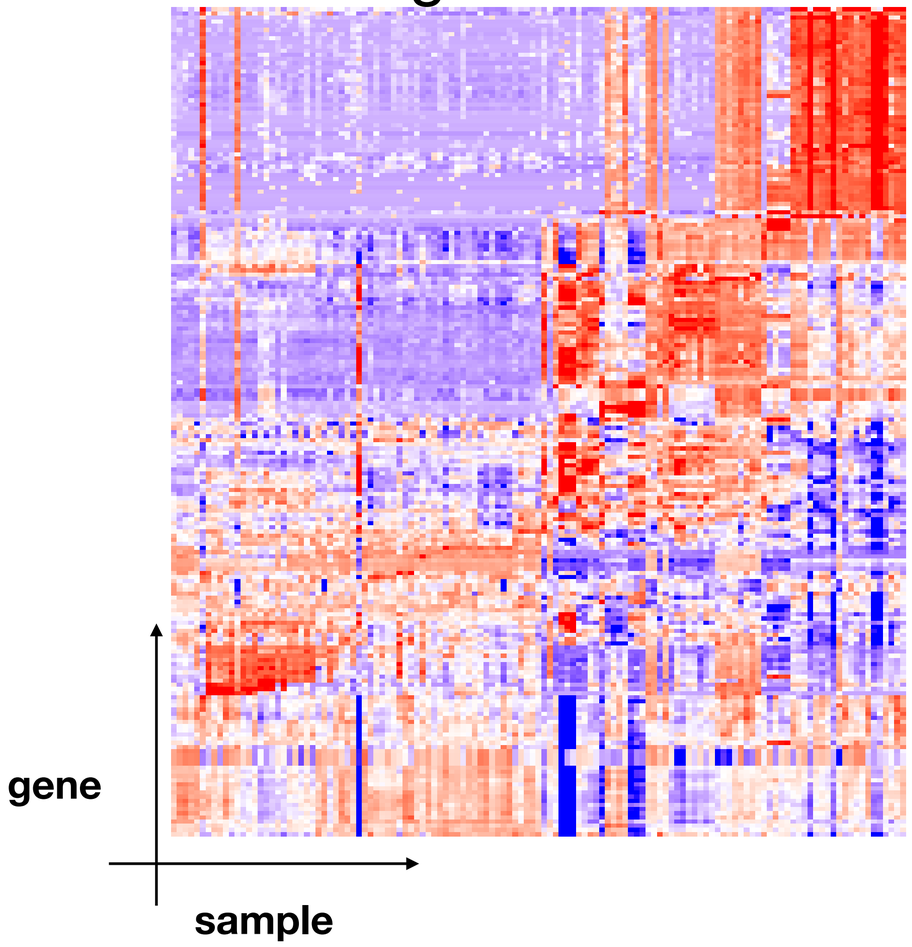


Rearrange them...



PCA: How do we recover “common” patterns from data?

Principal Component Analysis

(Pearson 1901)

- Projection [of the original data] that minimizes the projection cost between the original and projected
- The cost = mean squared distance

(Hotelling 1933)

- Orthogonal projection of data into a lower-dimensional **[principal]** sub-space,
- such that the total **variation of the projected** is maximized

What do we mean by projection to a low-dimensional space?

Recap: We learned that a multivariate linear regression projects a data vector $\mathbf{x}$ onto the column space of the design matrix U:

$$\begin{pmatrix} X_1 \\ X_2 \\ \vdots \\ X_m \end{pmatrix} = \begin{pmatrix} U_{11} & \dots & U_{1k} \\ U_{21} & \dots & U_{2k} \\ \vdots & \vdots & \vdots \\ U_{m1} & \dots & U_{mk} \end{pmatrix} \begin{pmatrix} W_1 \\ \vdots \\ W_k \end{pmatrix} + \begin{pmatrix} \epsilon_1 \\ \epsilon_2 \\ \vdots \\ \epsilon_m \end{pmatrix}$$

or

$\mathbf{x} = U\mathbf{w} + \epsilon$, for many columns, $X = UW + E$.

- If we **knew** U, we would be able to solve weights W. If $k = 2$, it would result in a projection to the 2D space.
- Unlike regression, we need ask: How do we know this unknown U matrix?

What is a projection matrix?

The purpose of a multivariate regression-based prediction:

$$\mathbf{x} \rightarrow \hat{\mathbf{x}} = U\mathbf{w}$$

The least-square solution for the W:

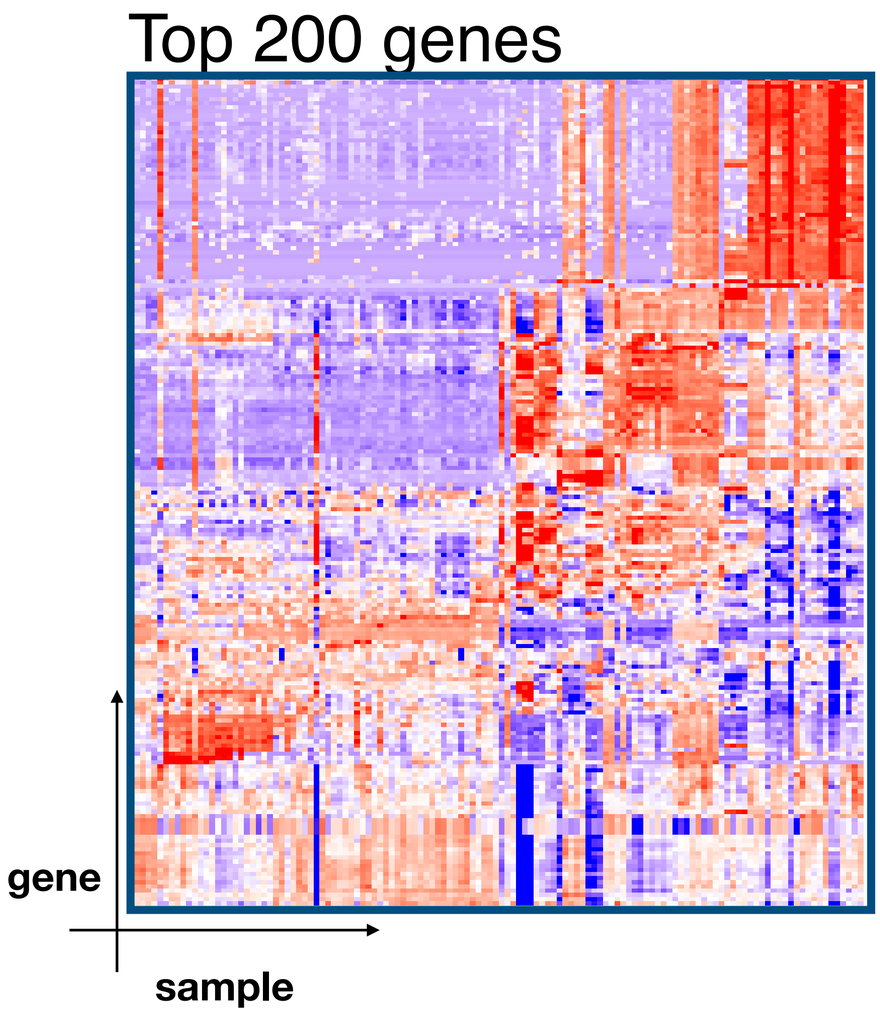
$$\hat{\mathbf{w}} = (U^\top U)^{-1} U^\top \mathbf{x}$$

Then we have

$$\hat{\mathbf{x}} = \underbrace{U(U^\top U)^{-1} U^\top}_{\text{projection matrix}} \mathbf{x}$$

You can think of "projection" as **"prediction" in the linear space** defined by the columns of a design matrix.

What will be “good” features (the U matrix) to regress on?



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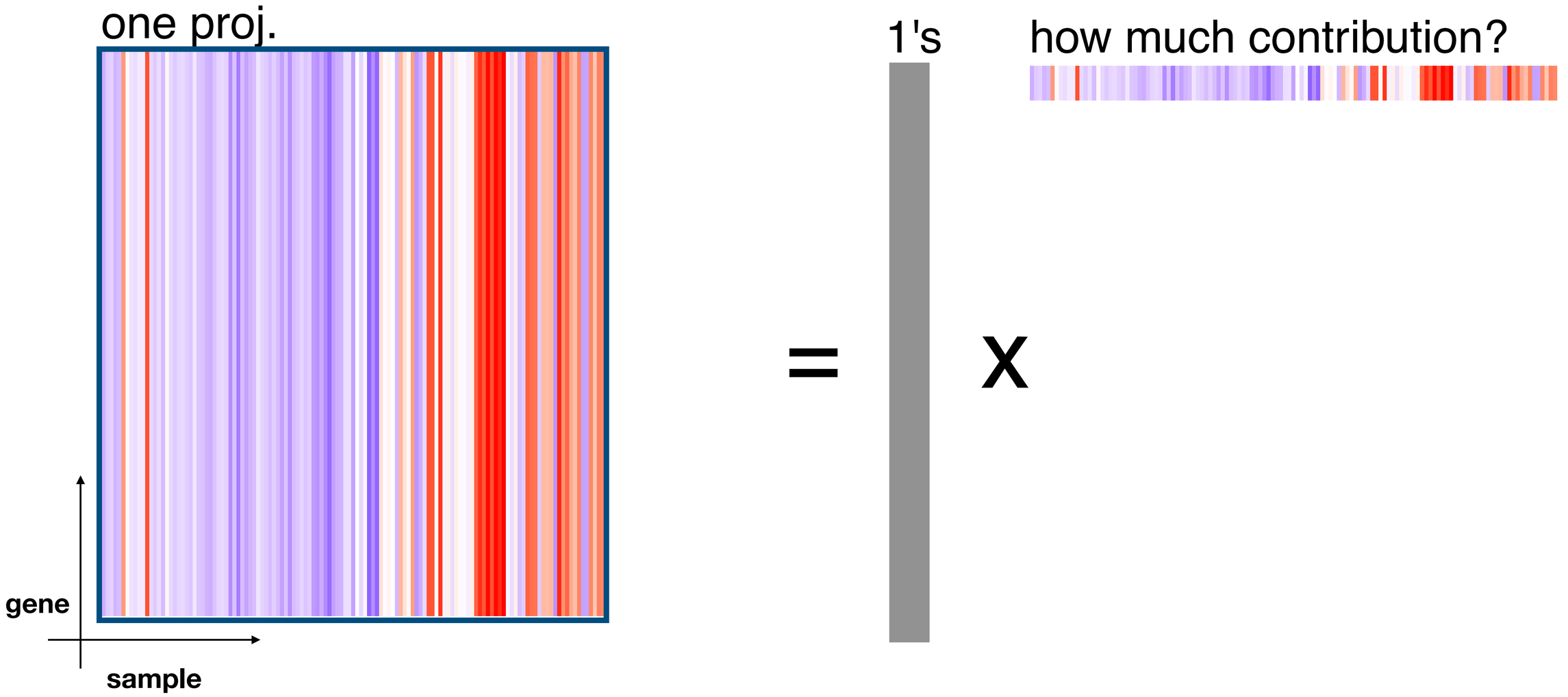

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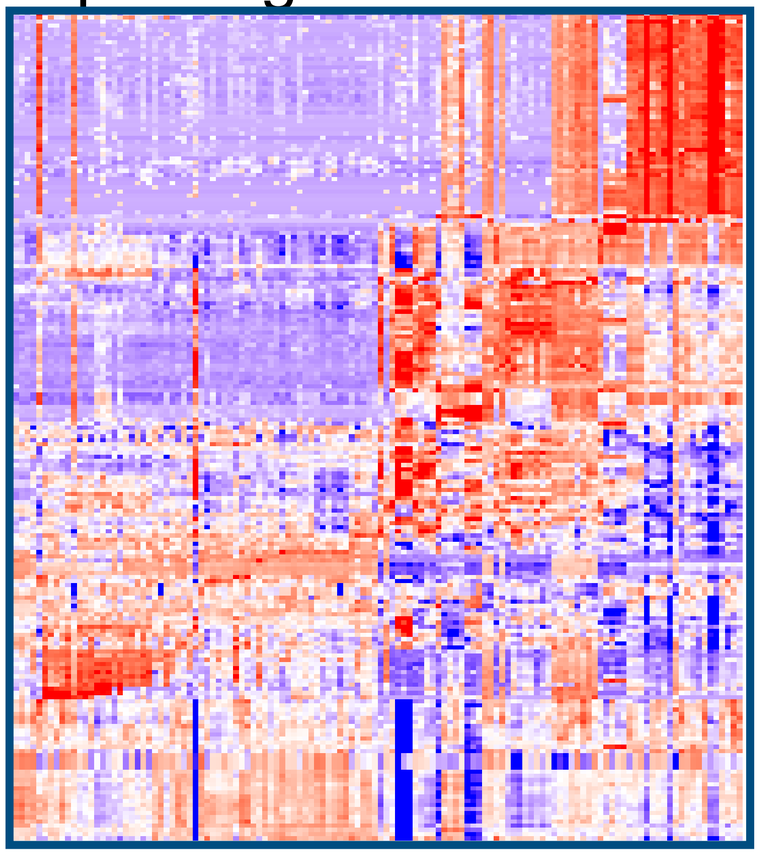
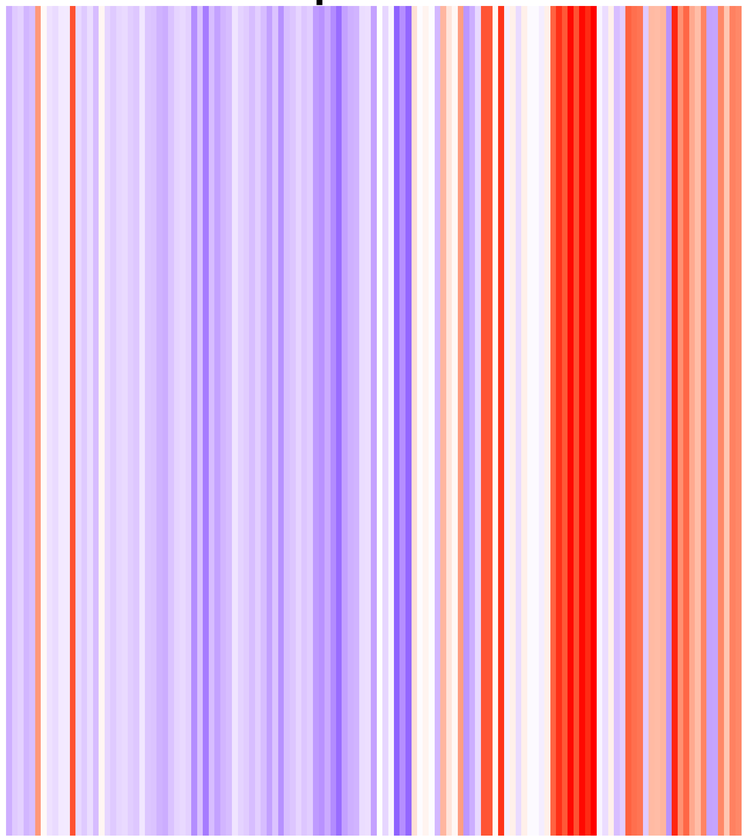


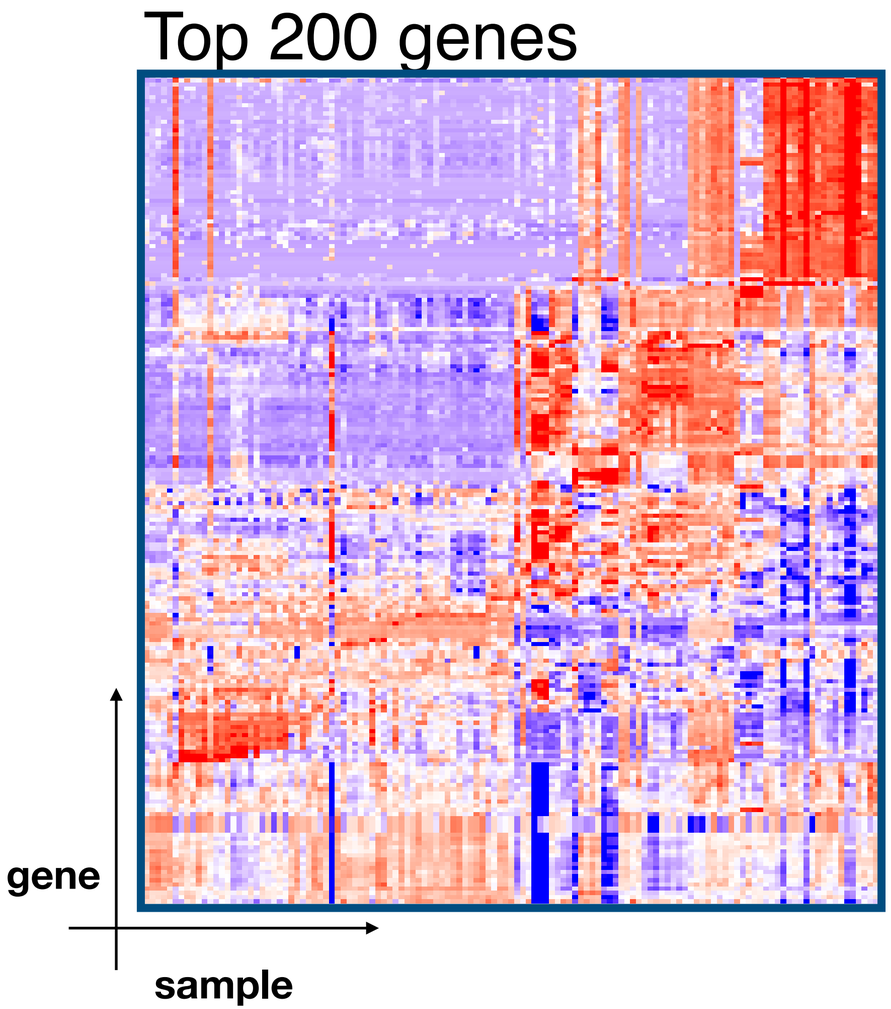
gene

sample

1% var. explained



How about an average vector?



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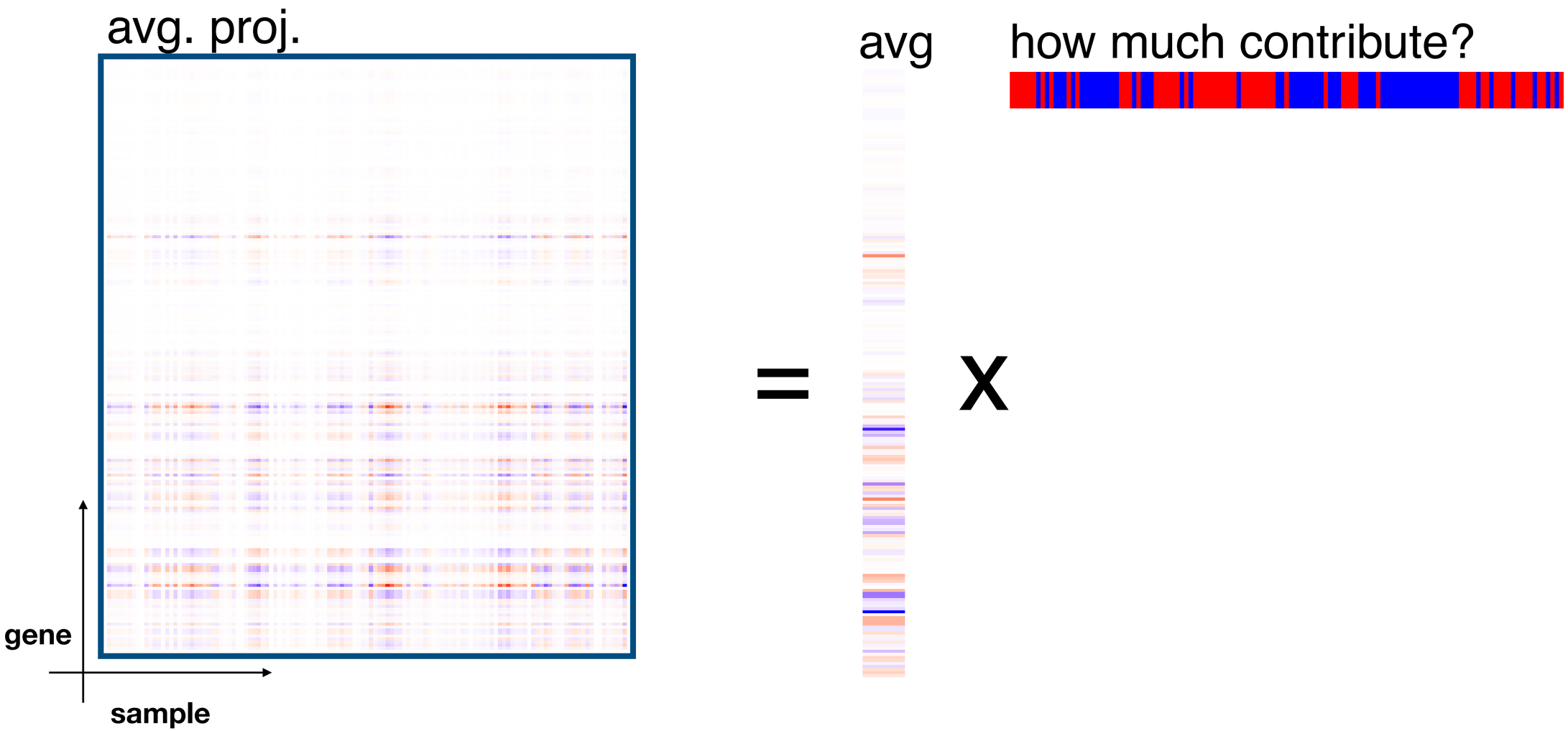

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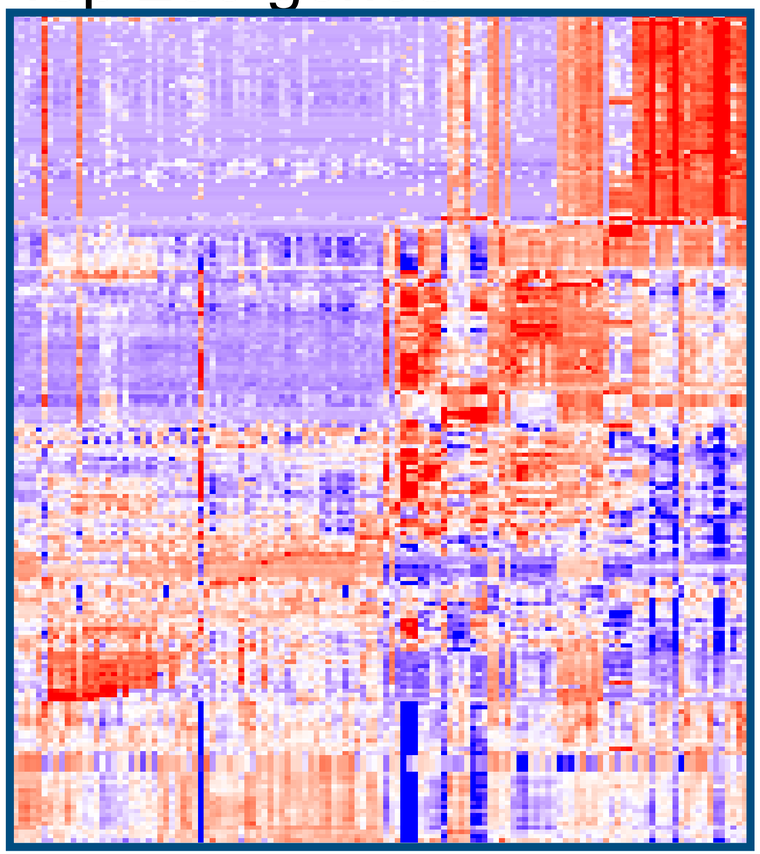

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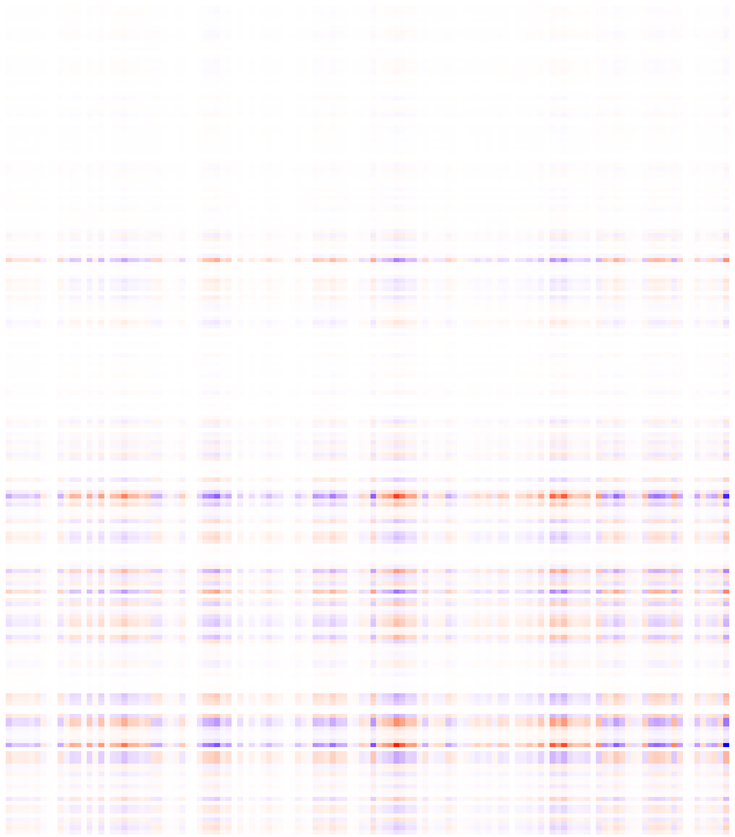
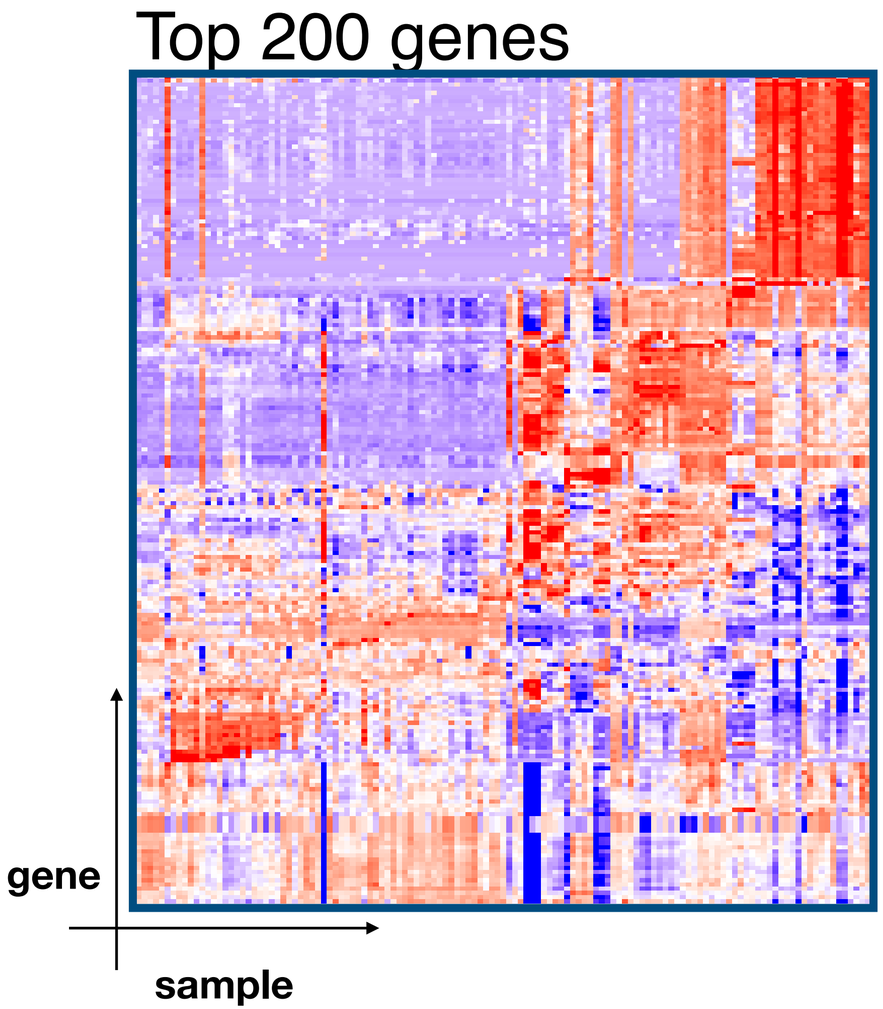


Let's try out random projection



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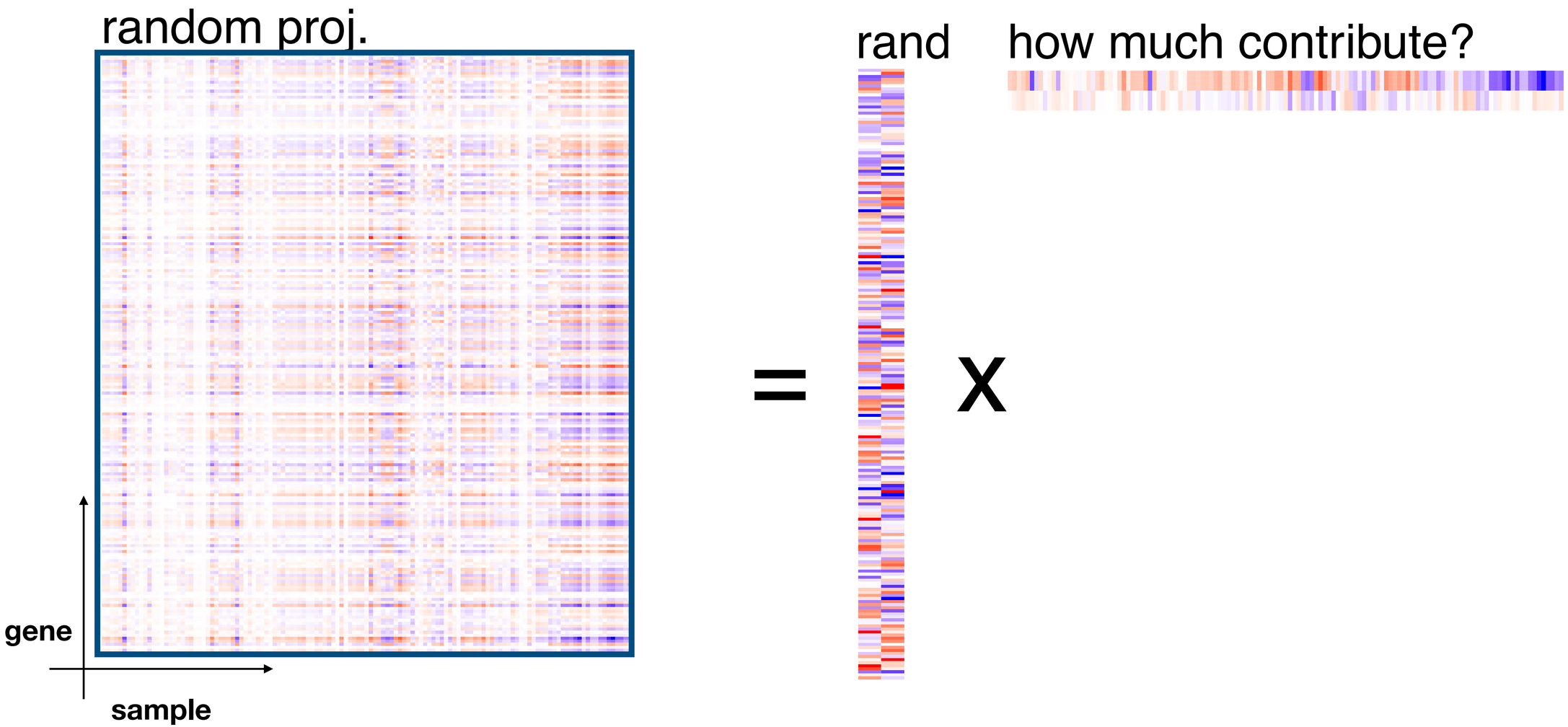

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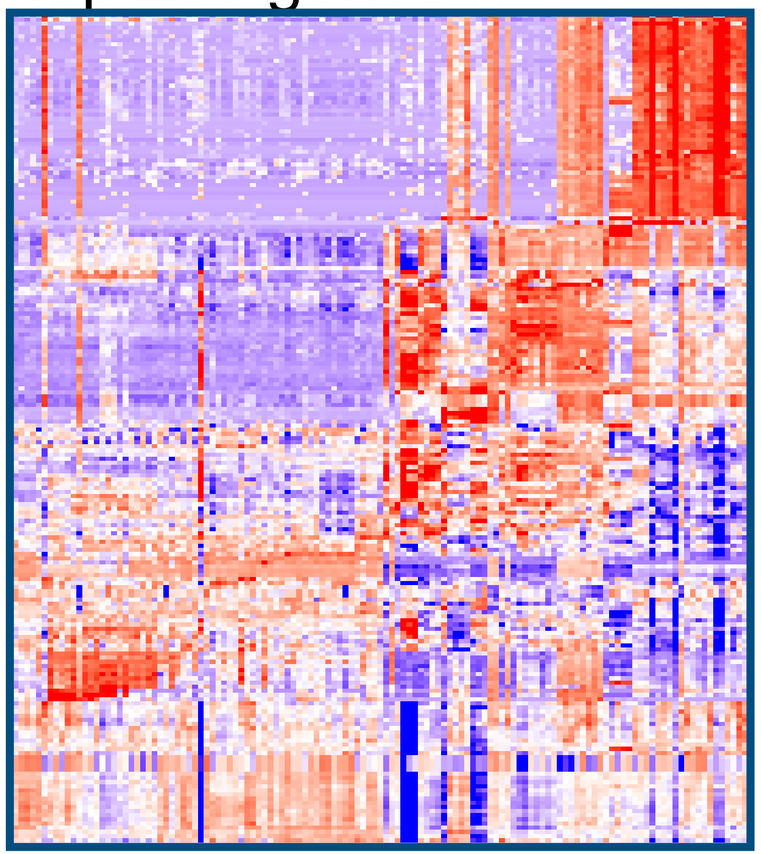

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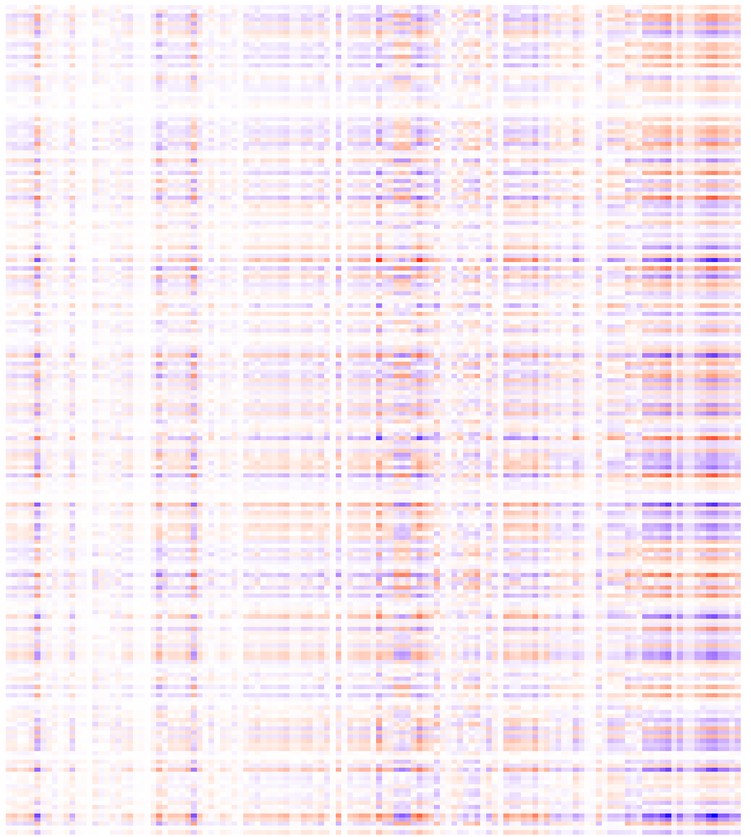
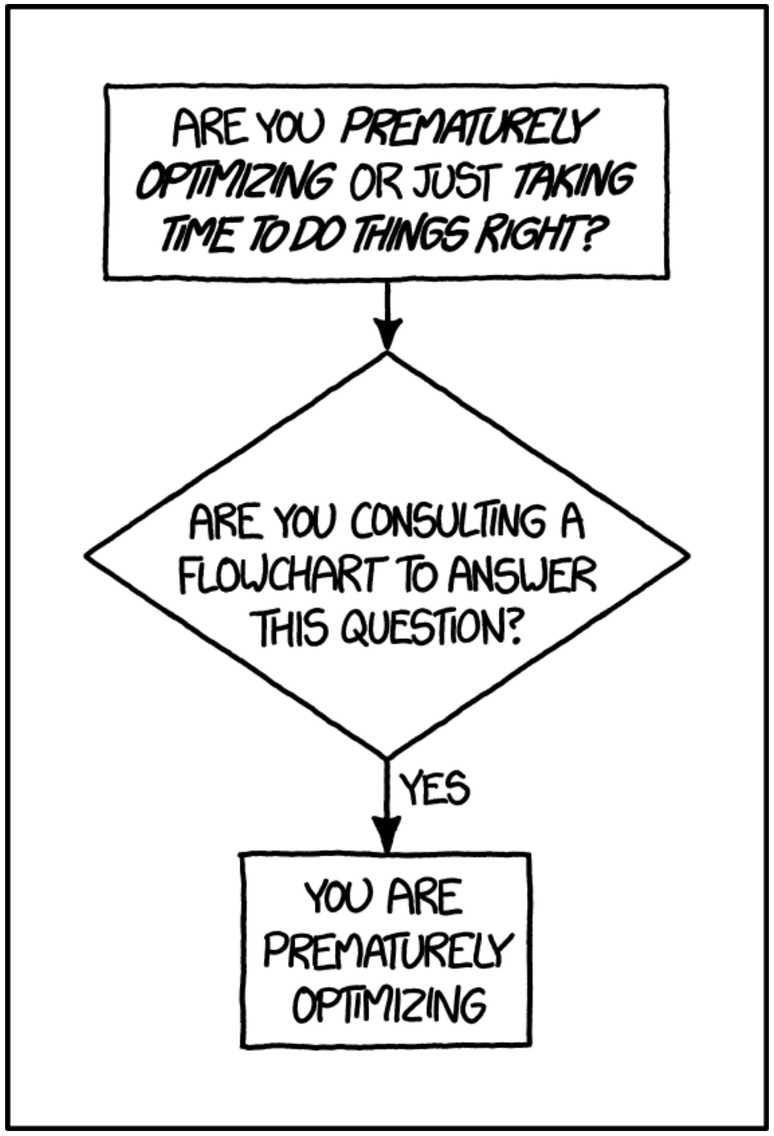



ARE YOU PREMATURELY OPTIMIZING OR JUST TAKING TIME TO DO THINGS RIGHT?
ARE YOU CONSULTING A FLOWCHART TO ANSWER THIS QUESTION?
YES
YOU ARE PREMATURELY OPTIMIZING

Can we find a set of "good" vectors to maximize the explained variability?

Recap: Sample covariance matrix

- Sample mean: $\bar{X}_i = \sum_{j=1}^{m} X_{ji}/m$
- Sample variance: $\sum_{j=1}^{m}(X_{ji} - \bar{X}_i)^2/(m-1)$
- Sample covariance between i and k:

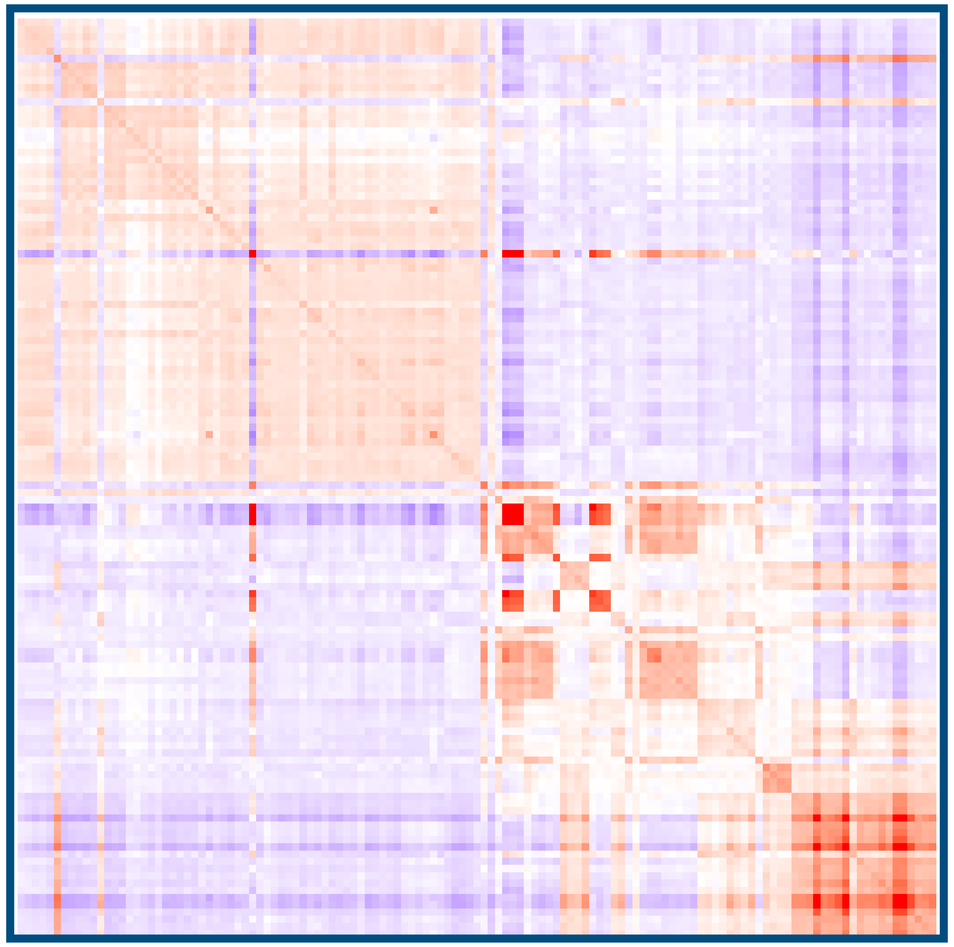
$$\frac{1}{m-1}\sum_{j=1}^{m}(X_{ji} - \bar{X}_i)(X_{jk} - \bar{X}_k)$$

If all the column vectors $\mathbf{x}_i$ are standardized, the column-by-column covariance $X^\top X/(m-1)$.

If all the row vectors $\mathbf{x}_d$ are standardized, the row-by-row covariance $XX^\top/(n-1)$.

Sample covariance matrix

$$\hat{\Sigma} = X^\top X/(m-1)$$



Theory: Total variance of the projected data

Total variance

Given the projected, $\hat{X} = \mathbf{u}_1 \cdot (W_{11}, \ldots, W_{1n})$, our goal is

$$\max \mathbb{V}\left[\hat{X}\right]$$

- We have two unknown variables U and W
- There are an infinite number of solutions.

Theory: Total variance of the projected data

Constrained total variance

Given the projected, $\hat{X} = \mathbf{u}_1\mathbf{w}$, and a unit vector, namely $\|\mathbf{u}_1\| = 1$, our goal is equivalent to

$$\max \mathbf{u}^\top XX^\top \mathbf{u}$$

Because each $\hat{W}_i$ is the solution to the least-square problem:

$$\hat{W}_i = \arg\min \|\mathbf{x}_i - \mathbf{u}W_i\|$$

by solving the least square:

$$\hat{W}_i = \mathbf{x}_i^\top \mathbf{u}/\mathbf{u}^\top \mathbf{u}, \forall i$$

Theory: Why is Principal Component Analysis an eigen value problem?

PCA

Letting the feature-by-feature sample covariance matrix $\hat{\Sigma} = XX^\top/(n-1)$, we want to find a unit vector $\mathbf{u}$ by

$$\max \mathbf{u}^\top \hat{\Sigma} \mathbf{u}$$

subject to $\mathbf{u}^\top \mathbf{u} = 1$.

Theory: Why is Principal Component Analysis an eigen value problem?

Eigen value problem

Given the covariance matrix $\hat{\Sigma}$, we can resolve an eigen-value λ and the corresponding eigen-vector $\mathbf{u}$ such that

$$\hat{\Sigma}\mathbf{u} = \lambda\mathbf{u}$$

("eigen" mean "own" in German).

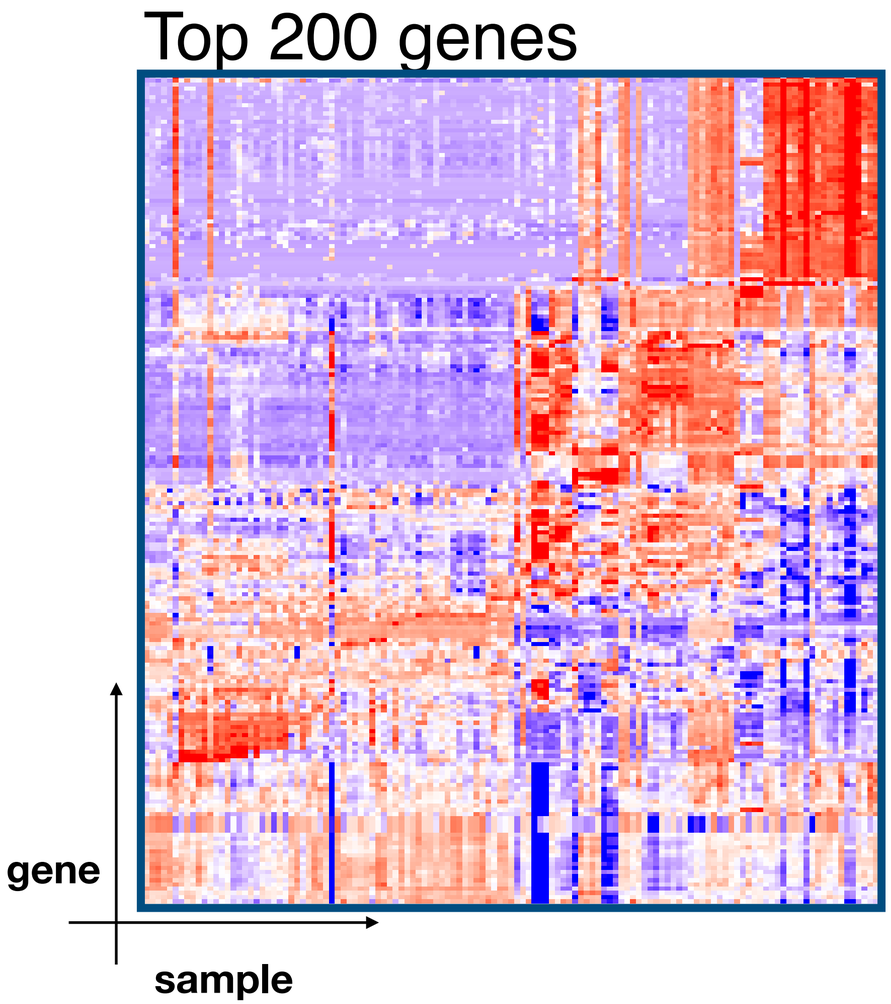
Theory: Why is Principal Component Analysis an eigen value problem?

Why are they equivalent? Solving the PCA problem is

$$\iff \max \quad \underbrace{\mathbf{u}^\top \hat{\Sigma} \mathbf{u}}_{\text{total variation}} + \underbrace{\lambda \left(1 - \mathbf{u}^\top \mathbf{u}\right)}_{\text{constraint}}, \lambda > 0 \quad \text{a.k.a. Lagrangian}$$

Taking the derivative with respect to $\mathbf{u}$ and setting it to zero, we get the eigen-value problem.

Let's see how much the first eigenvector can explain



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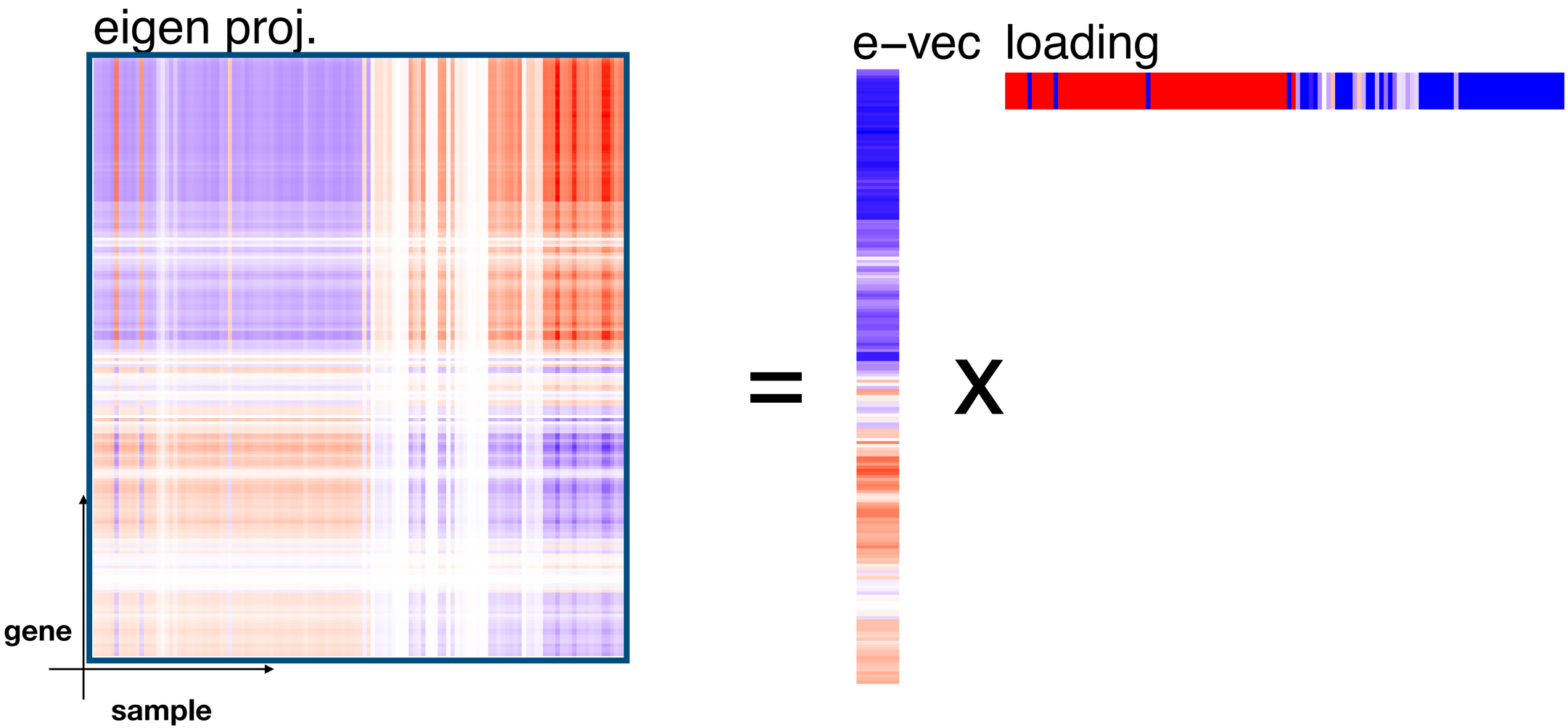
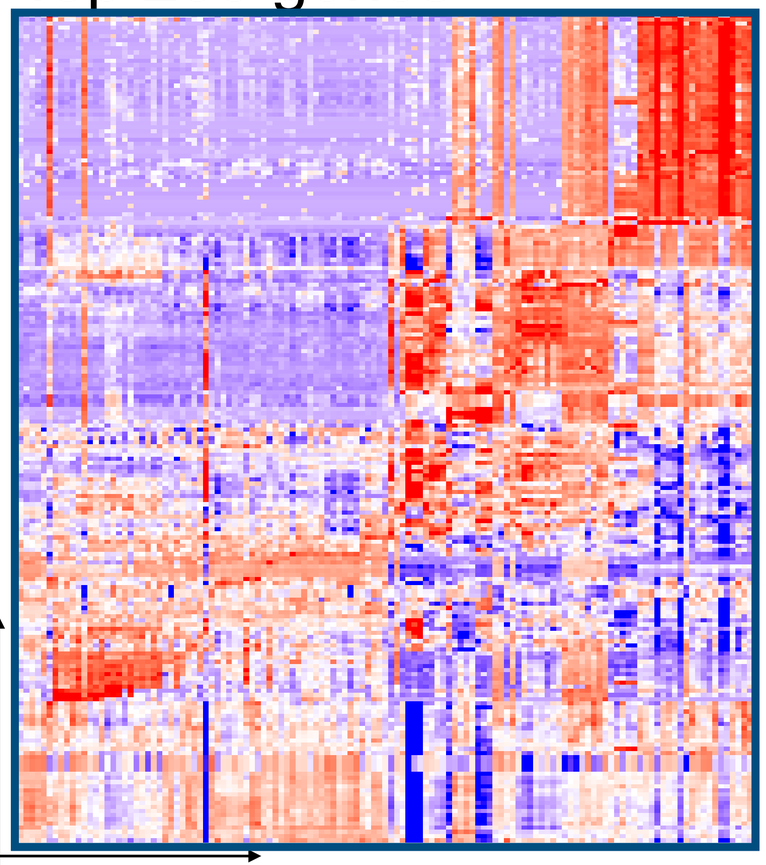
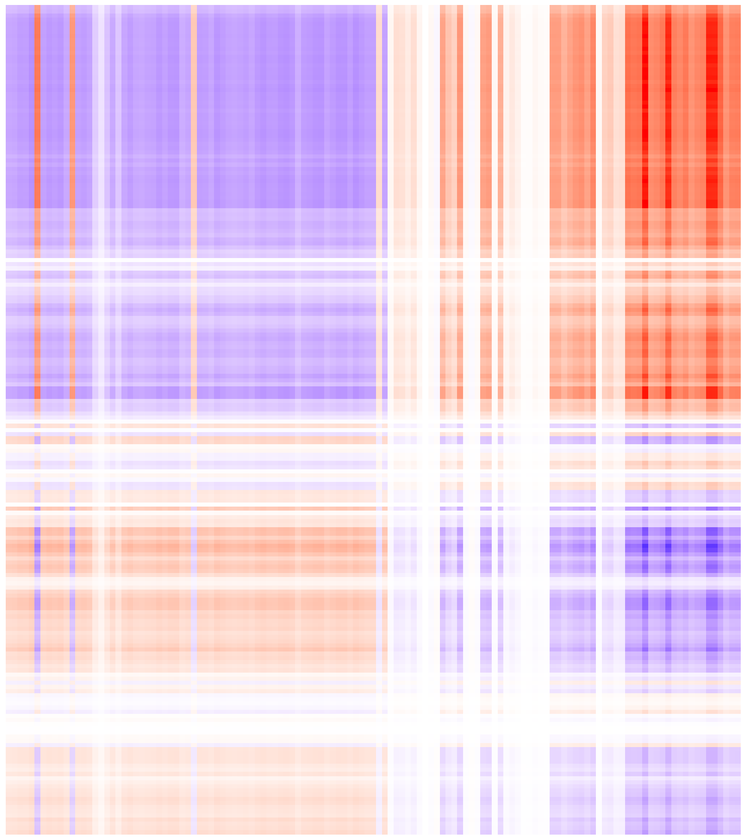


Let's see how much the first eigenvector can explain

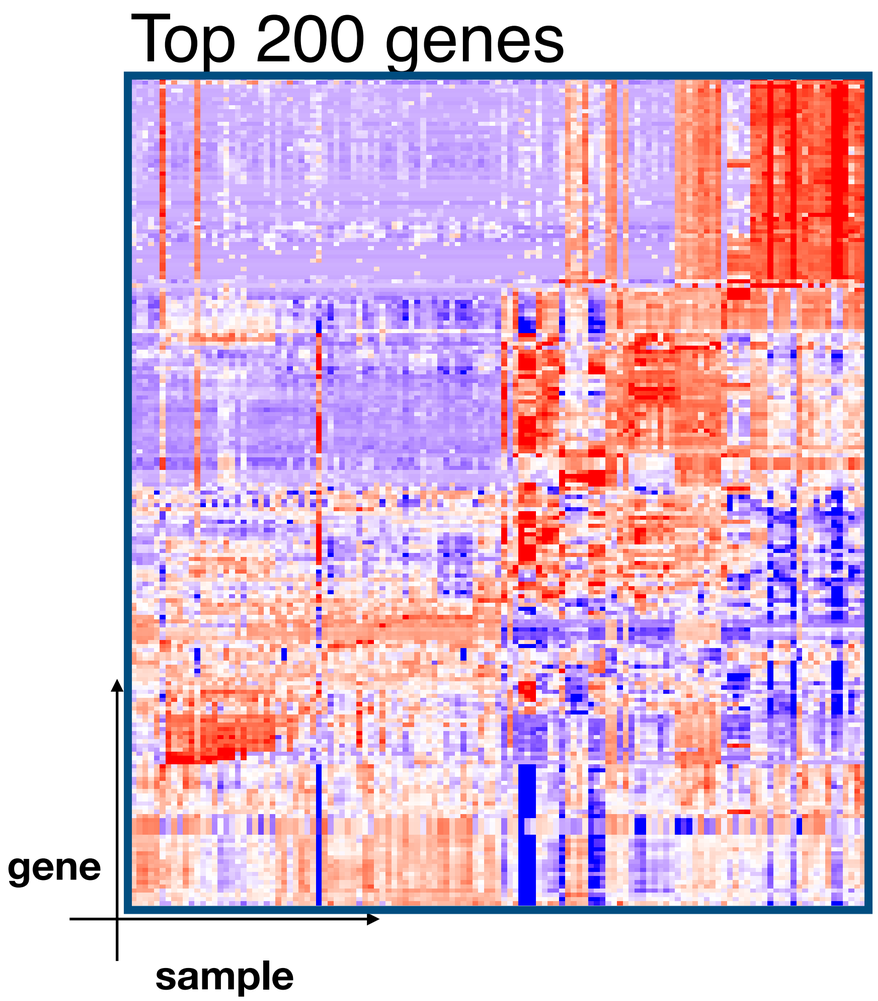
Top 200 genes



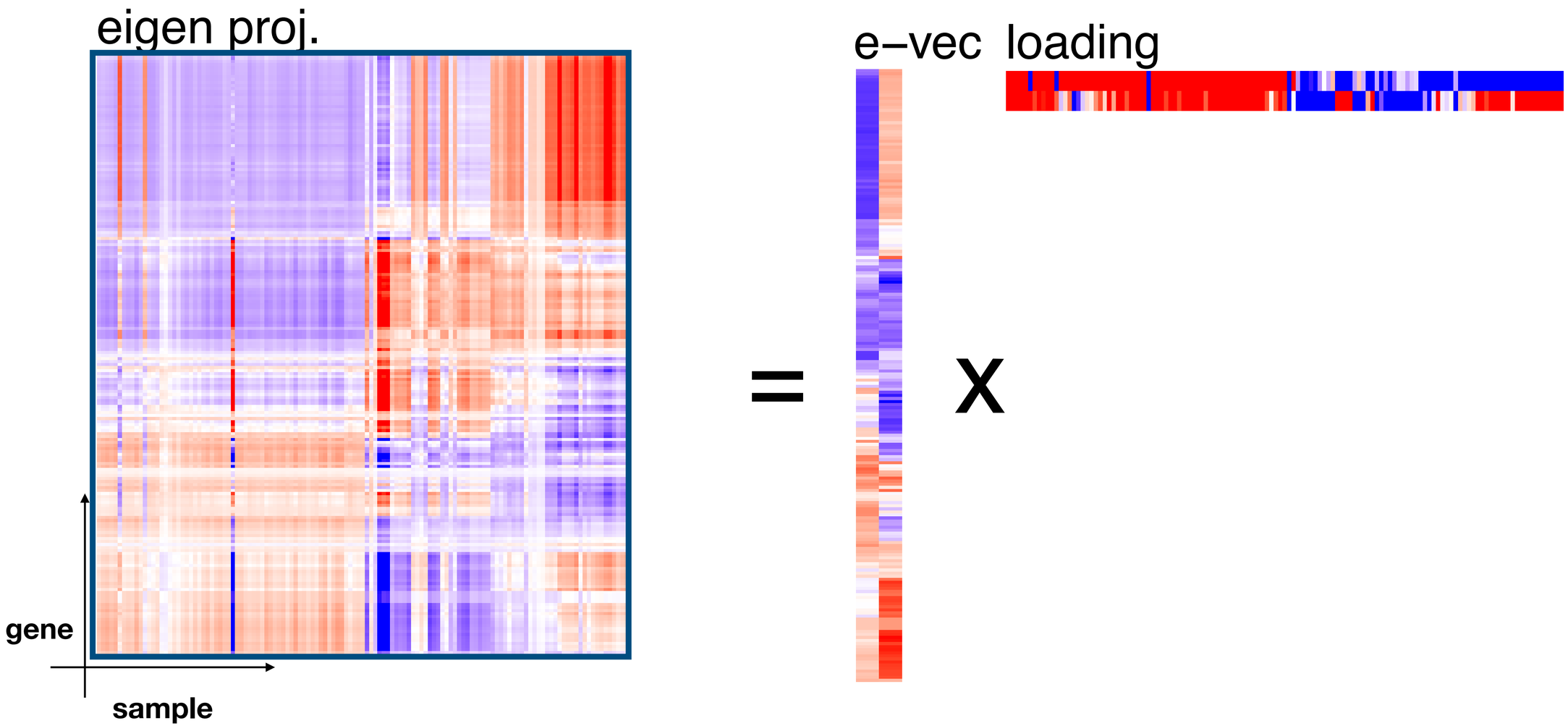
42%



Top two eigen-vectors

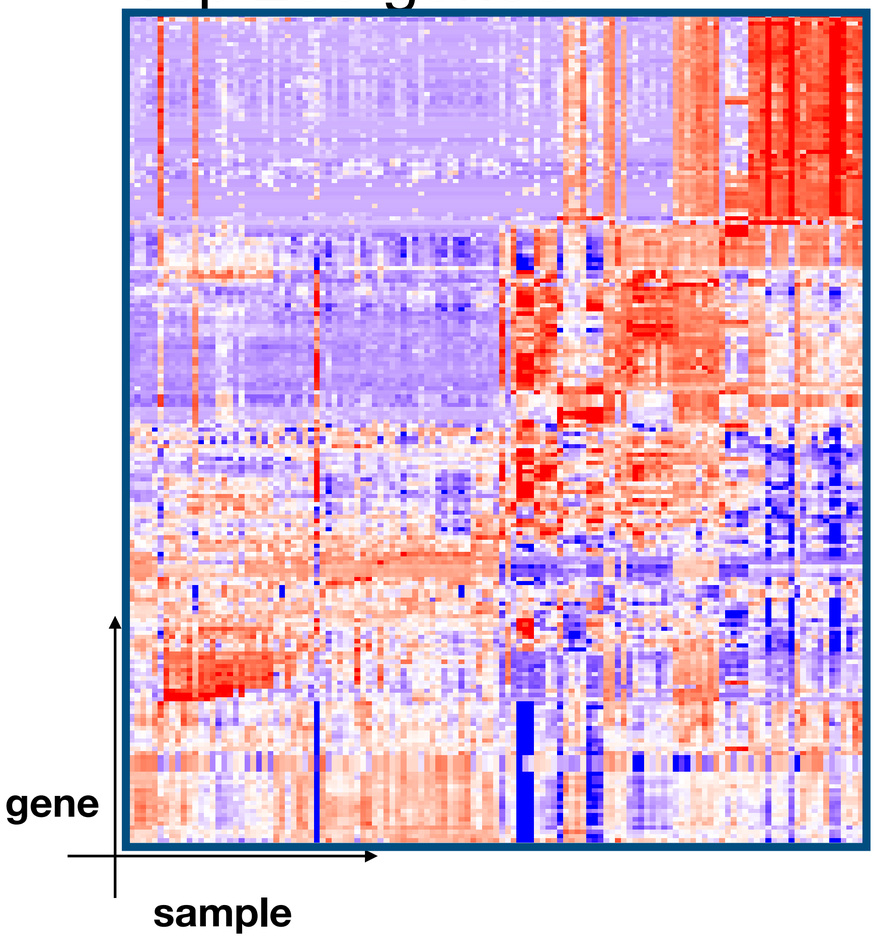


Top two eigen-vectors

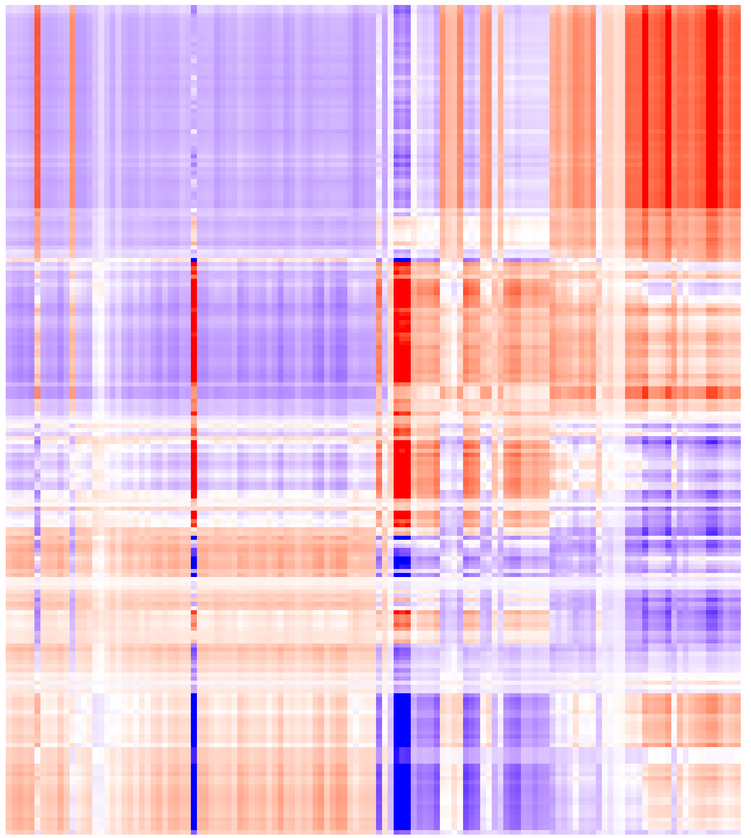


Top two eigen-vectors

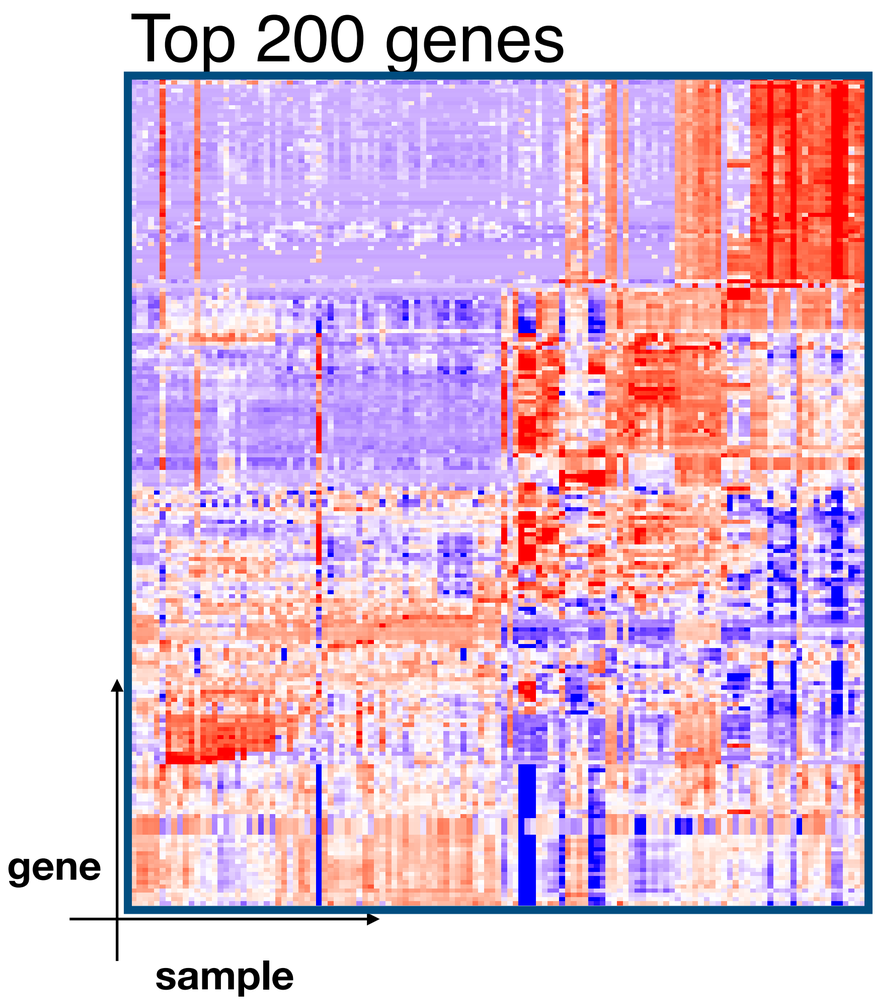
Top 200 genes



58%



Top three eigen-vectors



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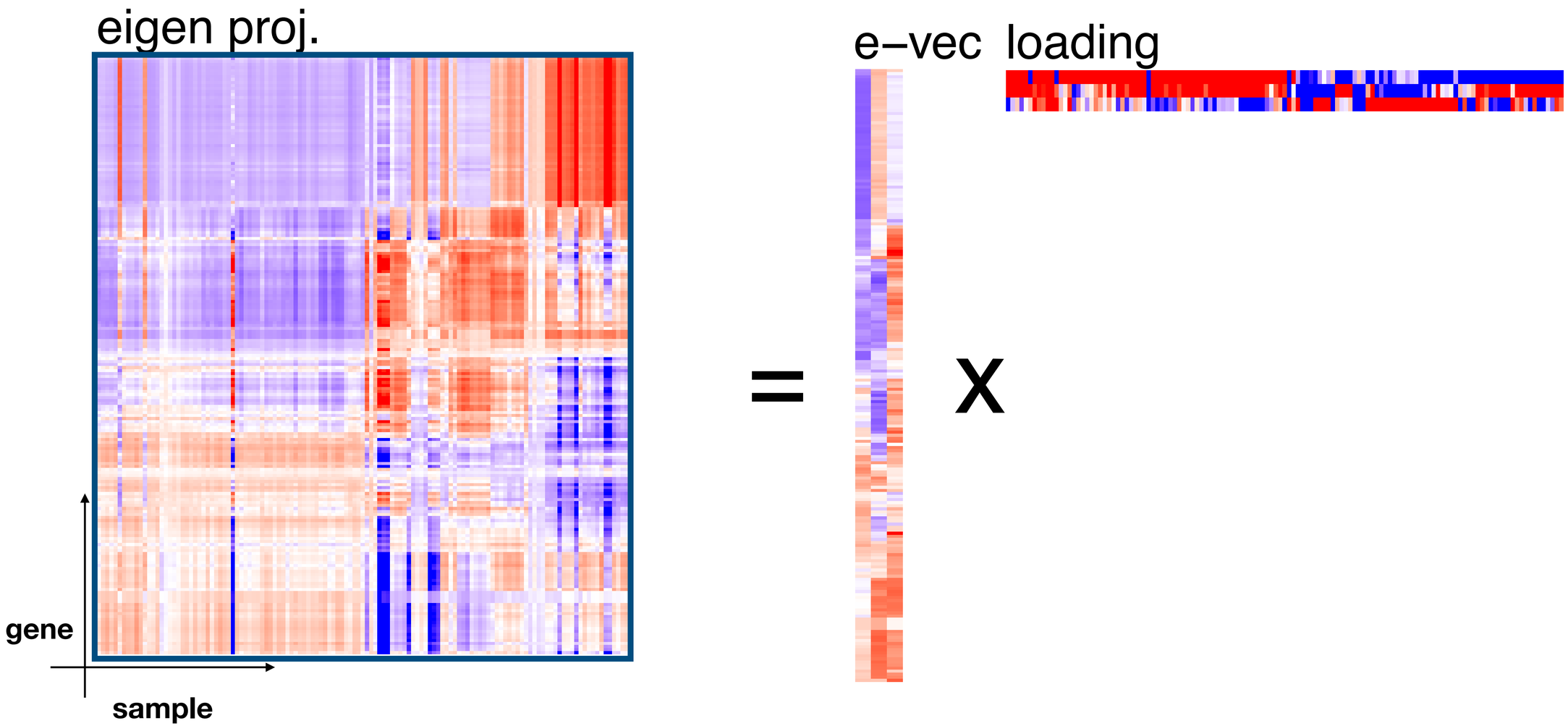
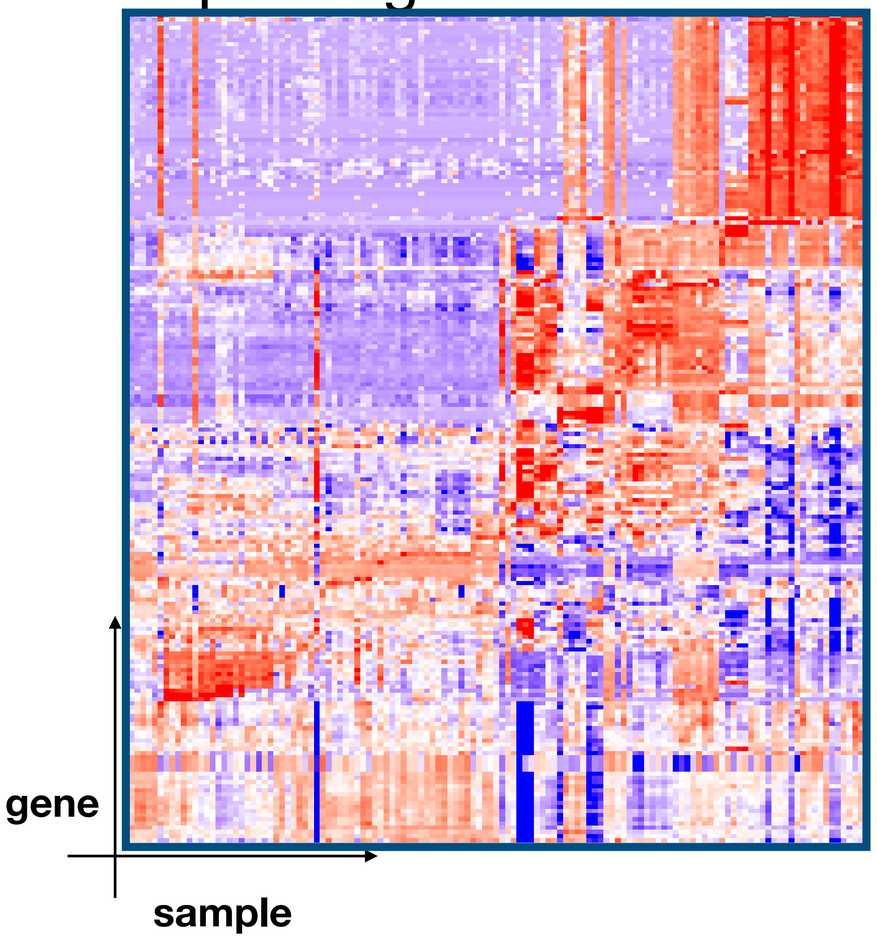
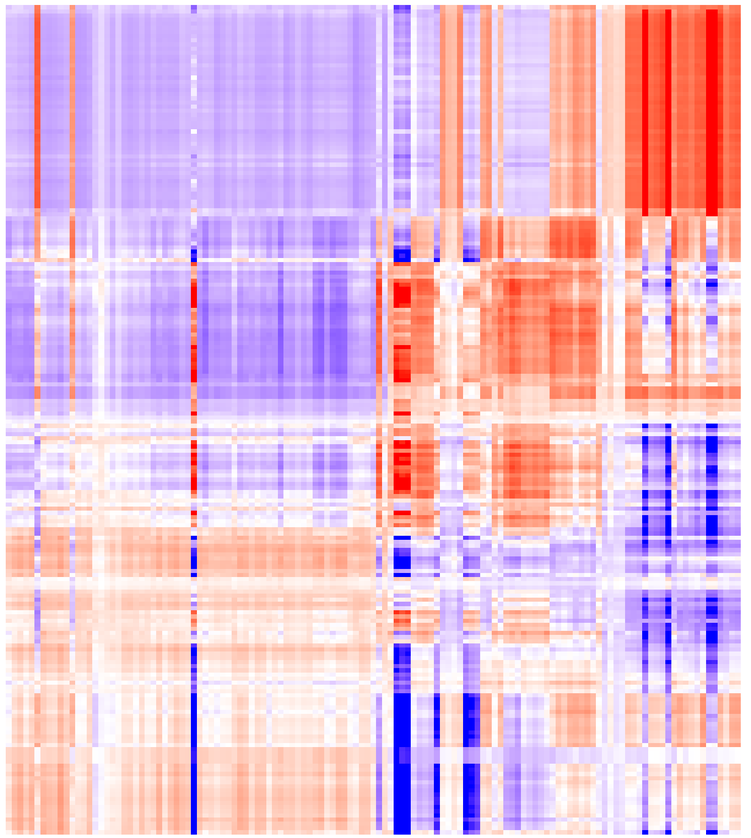


Top three eigen-vectors

Top 200 genes



59%



Singular Value Decomposition can find a PCA solution

Singular Value Decomposition

SVD identifies three matrices of X:

$$X = UDV^\top$$

where both U and V vectors are orthonormal, namely,

- $U^\top U = I$, $\mathbf{u}_k^\top \mathbf{u}_k = 1$ for all k,
- $V^\top V = I$, $\mathbf{v}_k^\top \mathbf{v}_k = 1$ for all k.

SVD: another equivalent method for PCA

Singular Value Decomposition

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Covariance by SVD

Covariance across the columns (samples)

$$X^\top X/(m-1) = VD^2V^\top/(m-1)$$

Covariance across the rows (genes)

$$XX^\top/(n-1) = UD^2U^\top/(n-1)$$

Remark: standardized matrix

How can SVD find an equivalent solution for PCA?

$$\underbrace{\left(\frac{1}{m-1}X^\top X\right)}_{\text{sample covariance}}\mathbf{v}_1 \quad = \quad \frac{1}{m-1}(\mathbf{v}_1, \mathbf{v}_2, \ldots, \mathbf{v}_k)\begin{pmatrix} D_1^2 & 0 & \ldots & \ldots \\ 0 & D_2^2 & 0 & \ldots \\ 0 & \ldots & \ddots & 0 \\ 0 & \ldots & 0 & D_k^2 \end{pmatrix}\begin{pmatrix} \mathbf{v}_1^\top \\ \mathbf{v}_2^\top \\ \vdots \\ \mathbf{v}_k^\top \end{pmatrix}\mathbf{v}_1$$

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$$= \frac{1}{m-1}(\mathbf{v}_1,\mathbf{v}_2,\ldots,\mathbf{v}_k)\begin{pmatrix} D_1^2 & 0 & \ldots & \ldots \\ 0 & D_2^2 & 0 & \ldots \\ 0 & \ldots & \ddots & 0 \\ 0 & \ldots & 0 & D_k^2 \end{pmatrix}\begin{pmatrix} 1 \\ 0 \\ \vdots \\ 0 \end{pmatrix}$$

How can SVD find an equivalent solution for PCA?

$$
\begin{aligned}
\underbrace{\left(\frac{1}{m-1}X^\top X\right)}_{\text{sample covariance}}\mathbf{v}_1 &= \frac{1}{m-1}(\mathbf{v}_1,\mathbf{v}_2,\ldots,\mathbf{v}_k)\begin{pmatrix} D_1^2 & 0 & \ldots & \ldots \\ 0 & D_2^2 & 0 & \ldots \\ 0 & \ldots & \ddots & 0 \\ 0 & \ldots & 0 & D_k^2 \end{pmatrix}\begin{pmatrix} \mathbf{v}_1^\top \\ \mathbf{v}_2^\top \\ \vdots \\ \mathbf{v}_k^\top \end{pmatrix}\mathbf{v}_1 \\
&= \frac{1}{m-1}(\mathbf{v}_1,\mathbf{v}_2,\ldots,\mathbf{v}_k)\begin{pmatrix} D_1^2 & 0 & \ldots & \ldots \\ 0 & D_2^2 & 0 & \ldots \\ 0 & \ldots & \ddots & 0 \\ 0 & \ldots & 0 & D_k^2 \end{pmatrix}\begin{pmatrix} 1 \\ 0 \\ \vdots \\ 0 \end{pmatrix} \\
&= \frac{1}{m-1}(\mathbf{v}_1,\mathbf{v}_2,\ldots,\mathbf{v}_k)\begin{pmatrix} D_1^2 \\ 0 \\ 0 \\ 0 \end{pmatrix}
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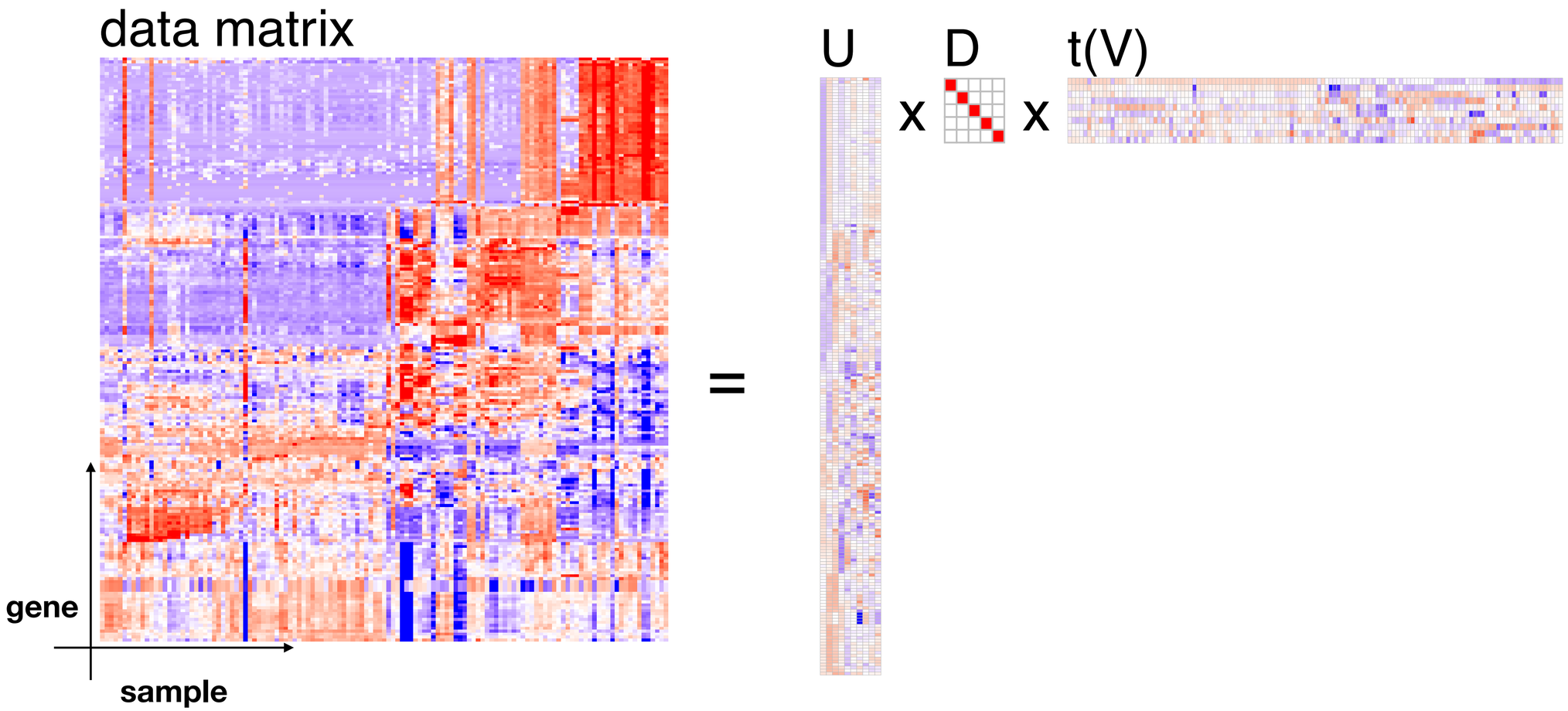
$$\hat{\mathbf{\Sigma}}\,\mathbf{v}_1 = \frac{1}{m-1}(\mathbf{v}_1,\mathbf{v}_2,\ldots,\mathbf{v}_k)\begin{pmatrix} D_1^2 \\ 0 \\ 0 \\ 0 \end{pmatrix} = \underbrace{\frac{D_1^2}{m-1}}_{\text{eigenvalue}}\underbrace{\mathbf{v}_1}_{\text{eigenvector}}$$

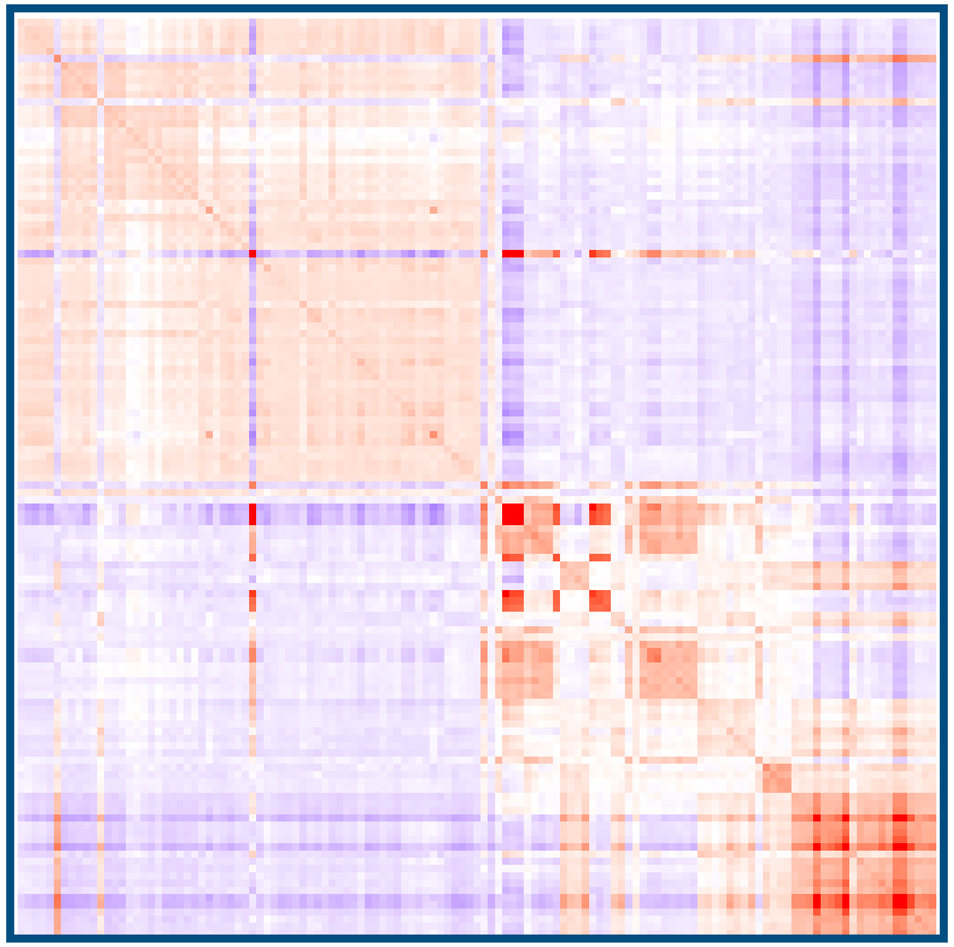
Run SVD to find principal components

```
svd.out <- svd(x.sub, nu = 10, nv = 10)
U <- svd.out$u; D <- diag(svd.out$d[1:5]); V <- svd.out$v
```



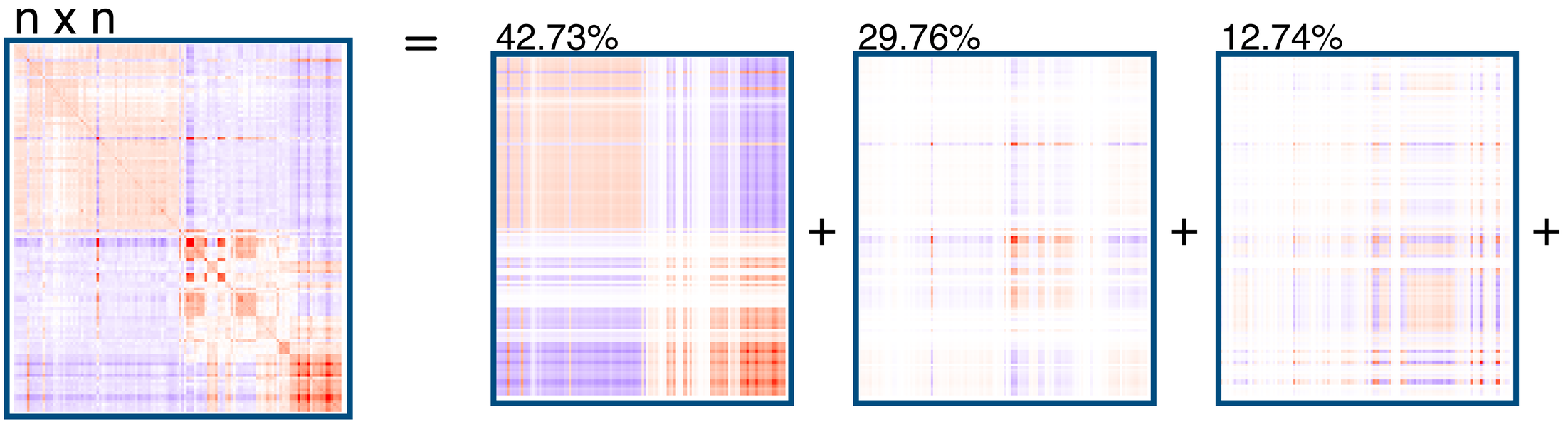
Sample covariance matrix

$$\hat{\Sigma} = X^\top X/(m-1)$$



Principal components decompose the covariance matrix

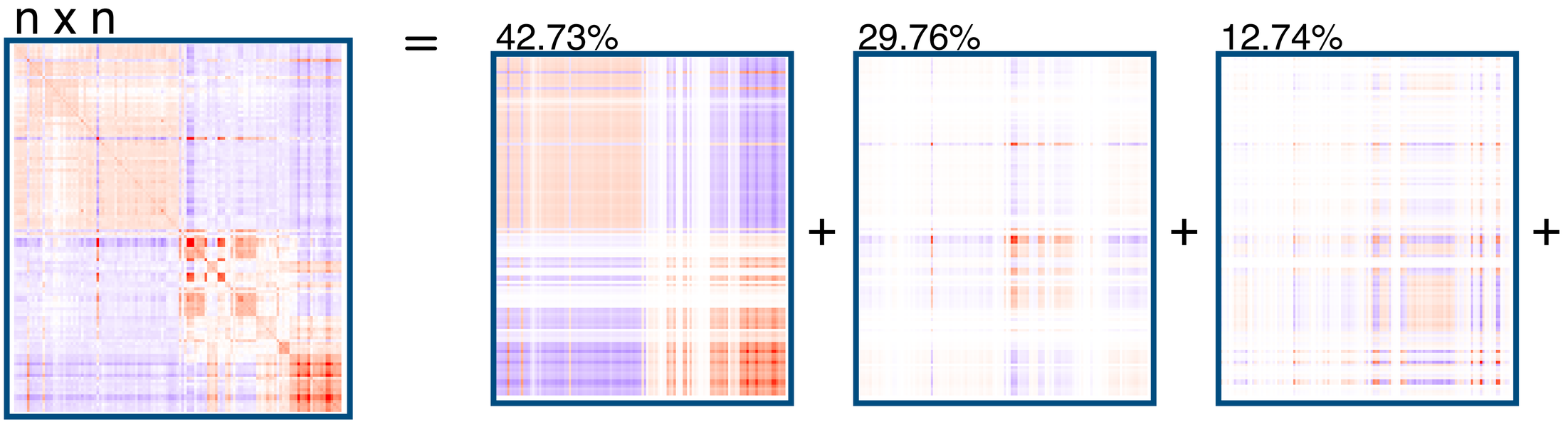
$$\hat{\Sigma} = \frac{X^\top X}{m-1}$$



- How much variance is explained by each component?

Principal components decompose the covariance matrix

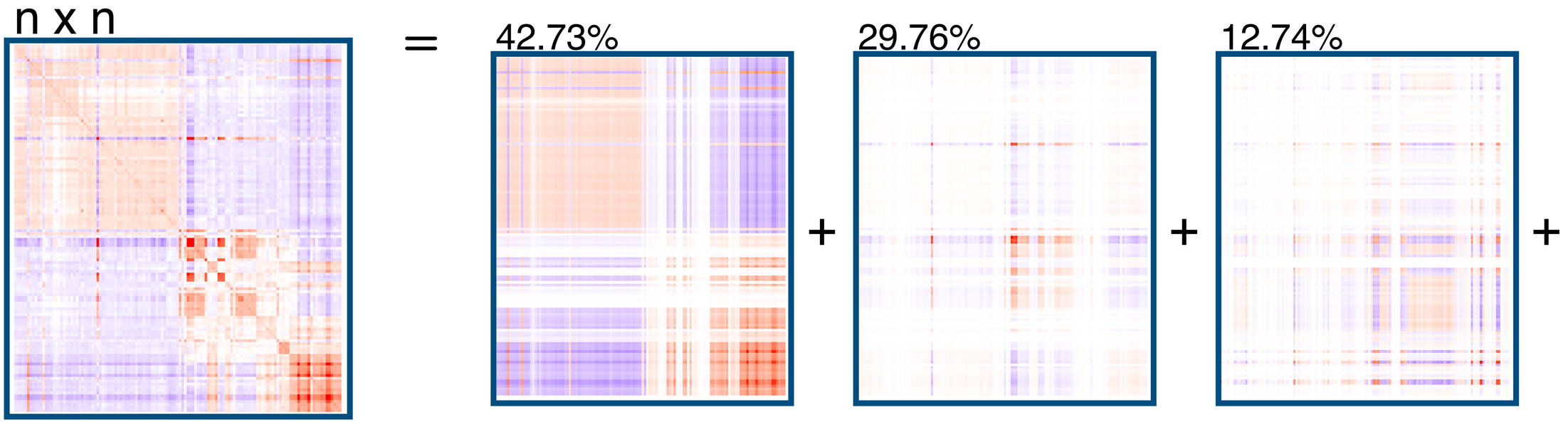
$$\hat{\Sigma} = \frac{X^\top X}{m-1} = V \frac{D^2}{m-1} V^\top$$



- How much variance is explained by each component?

Principal components decompose the covariance matrix

$$\hat{\Sigma} = \frac{X^\top X}{m-1} = V \frac{D^2}{m-1} V^\top = \sum_{k=1} \lambda_k \mathbf{v}_k \mathbf{v}_k^\top, \quad \lambda_k$$



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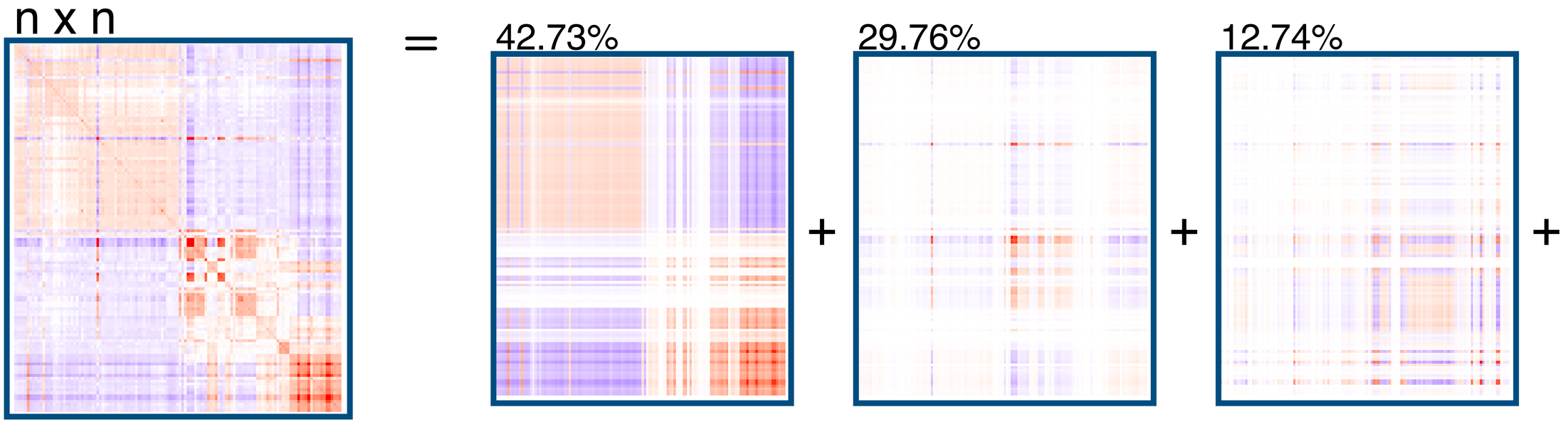
$$\hat{\Sigma} = \frac{X^\top X}{m-1} = V \frac{D^2}{m-1} V^\top = \sum_{k=1} \lambda_k \mathbf{v}_k \mathbf{v}_k^\top, \quad \lambda_k = \frac{D_k^2}{m-1}$$



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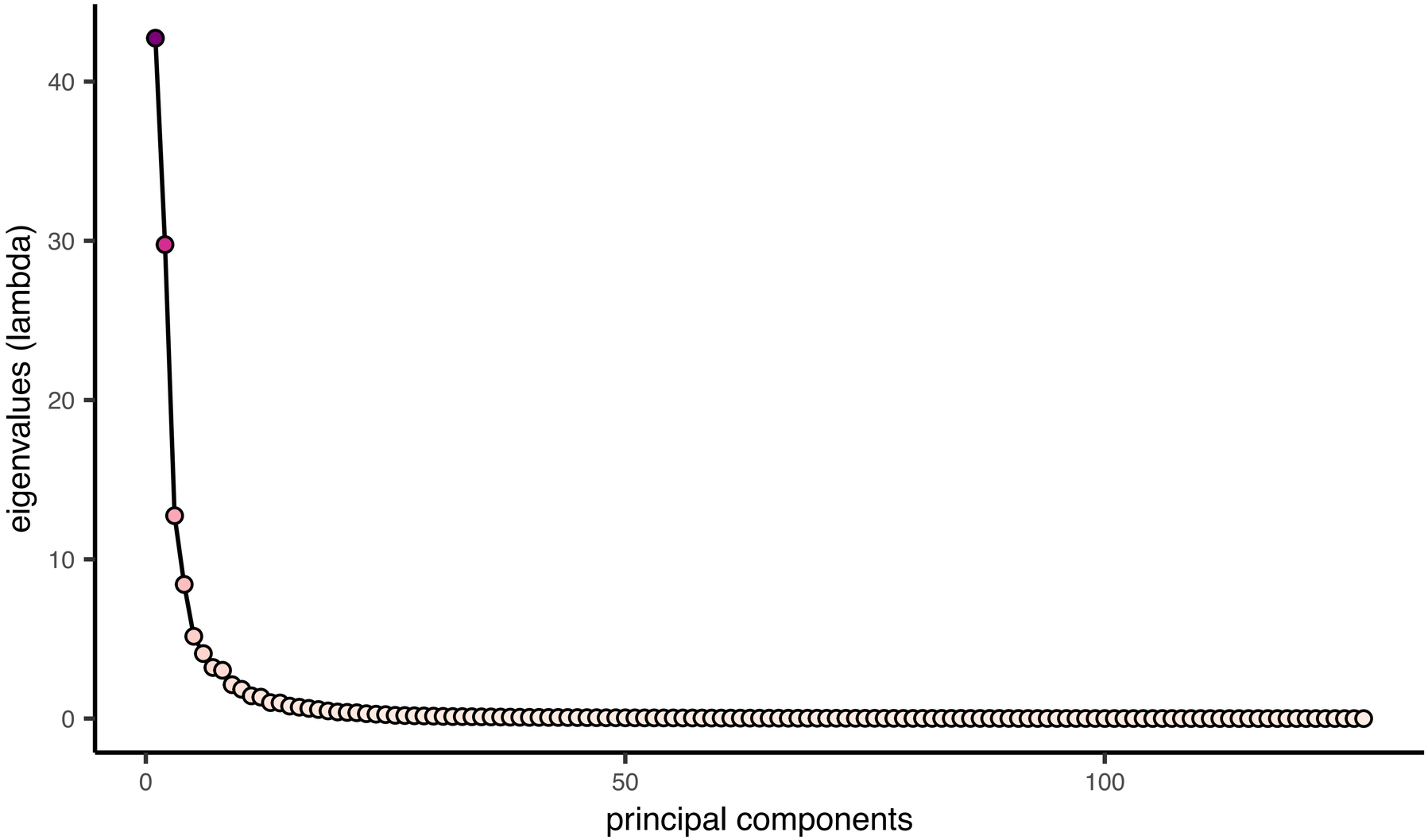
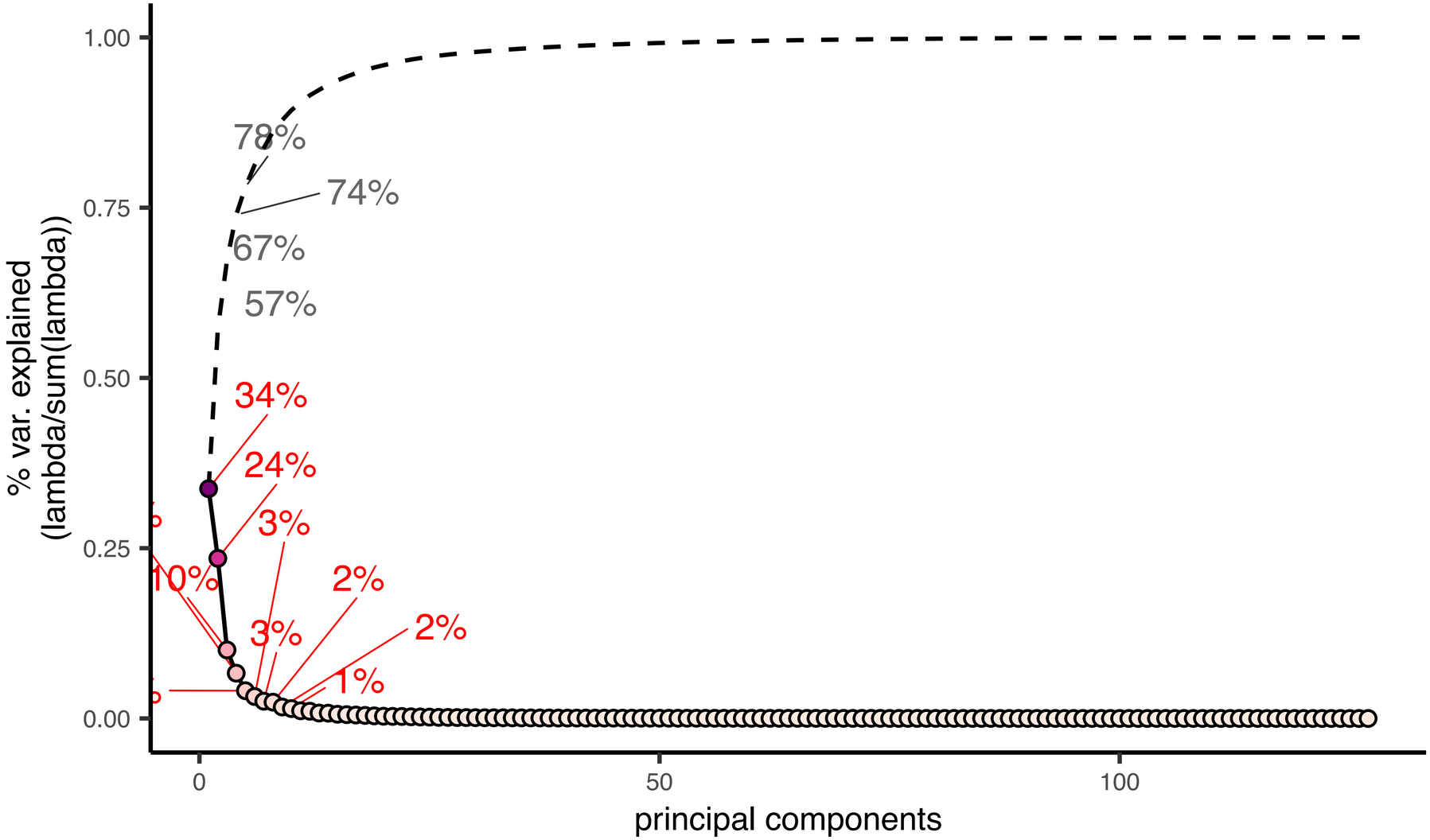


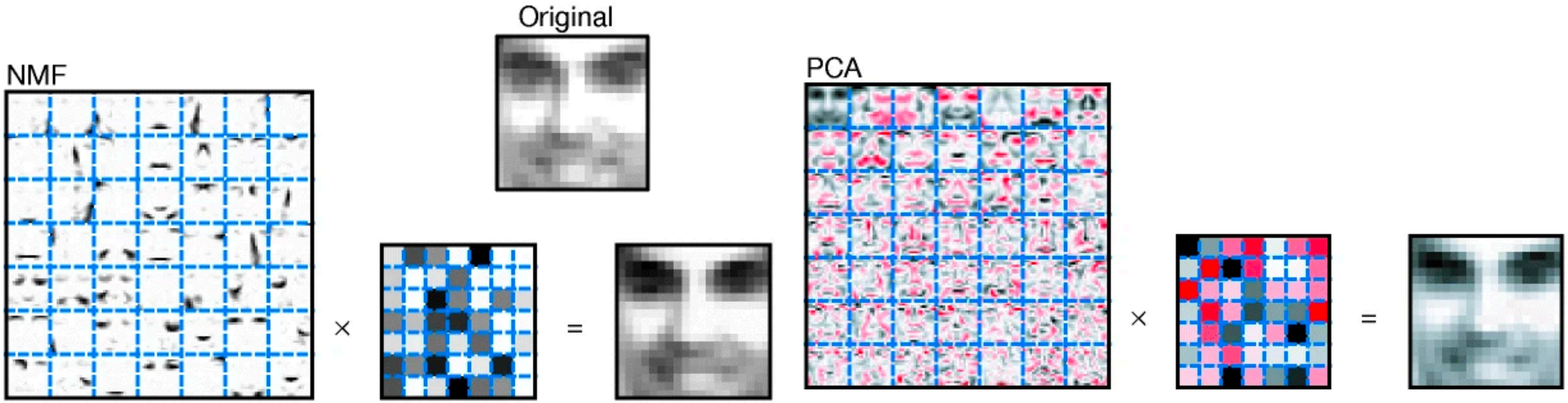
How much variance is explained by each principal component?



Today’s lecture: Enrichment Analysis

- **Motivations: What’s next after genomics analysis?**
  - What have we learned? How do we know that our discovery is meaningful?
- **Gene set enrichment analysis**
  - Set-based approach: Hypergeometric test
  - Rank-based approach: GSEA by KS statistic
- **Can we engineer new gene sets/scores?**
  - Principal Component Analysis
  - Matrix factorization of count data

Why another factorization method?

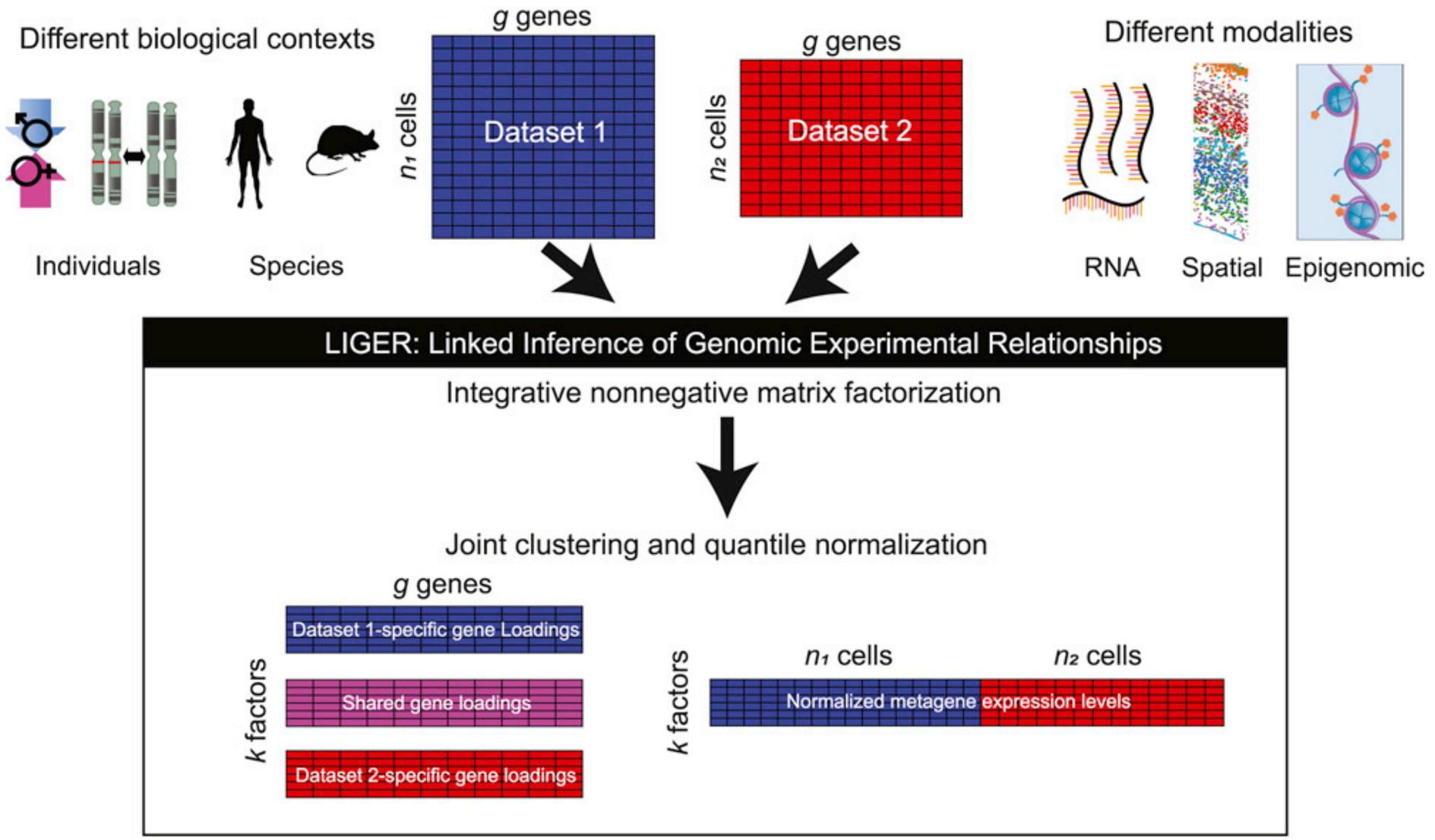


Decomposition by "parts"

Positive + negative can cancel each other

Lee and Seung, *Nature* (1999)

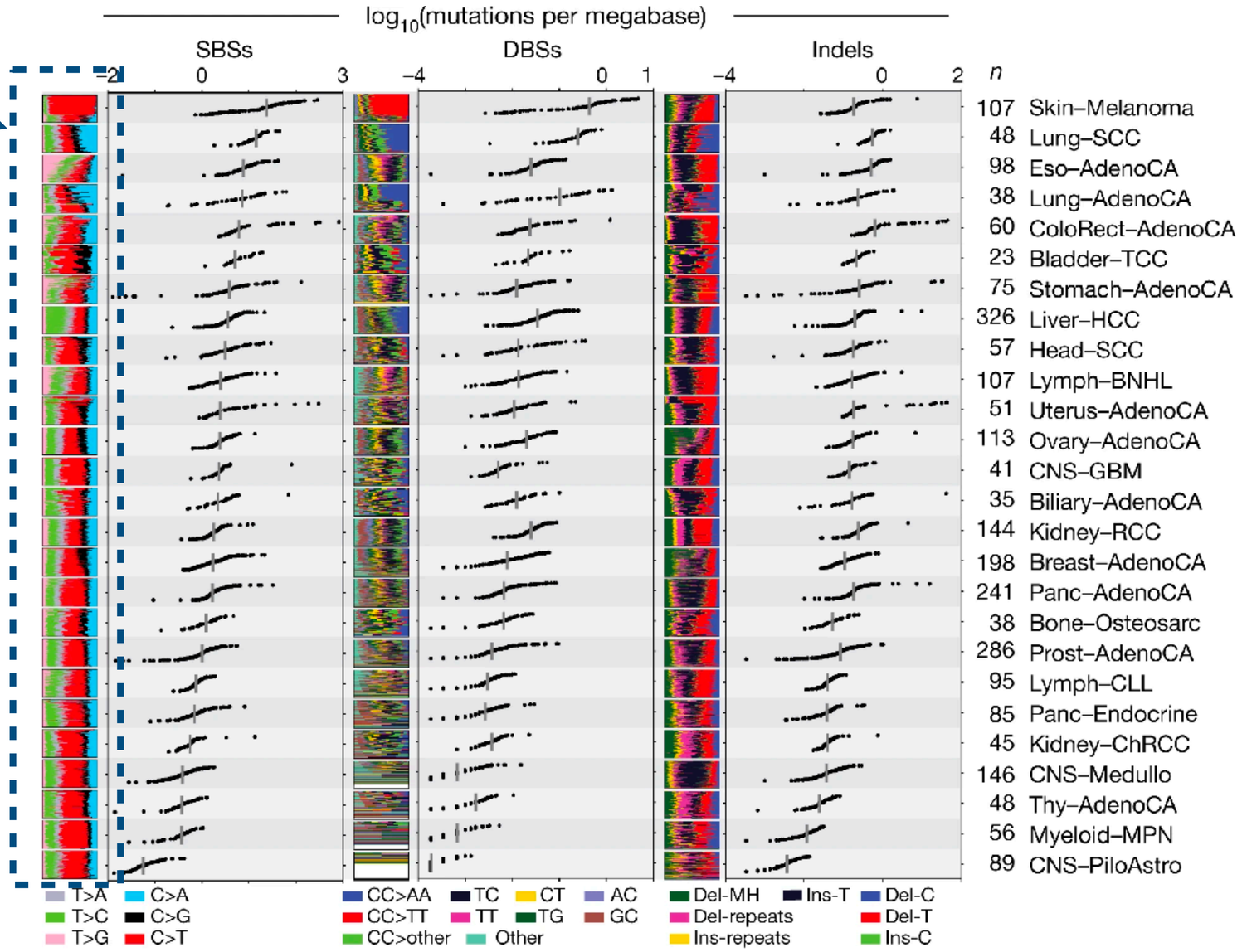
NMF-based method can facilitate data integration



Welch .. Macosko, *Cell* (2019)

NMF can identify hidden patterns in high-dimensional count data

Proportion of mutation "topic" or "signatures" per tumour sample



Alexandrov *et al.*, *Nature* (2020)

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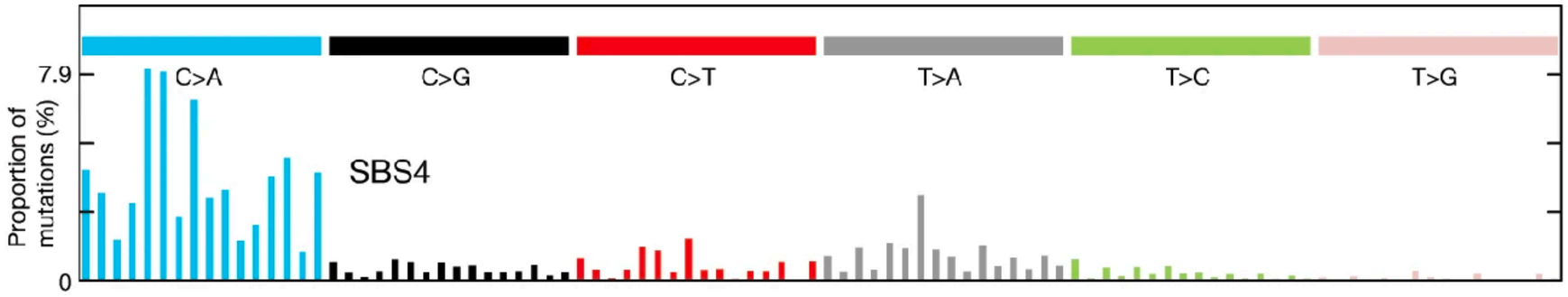
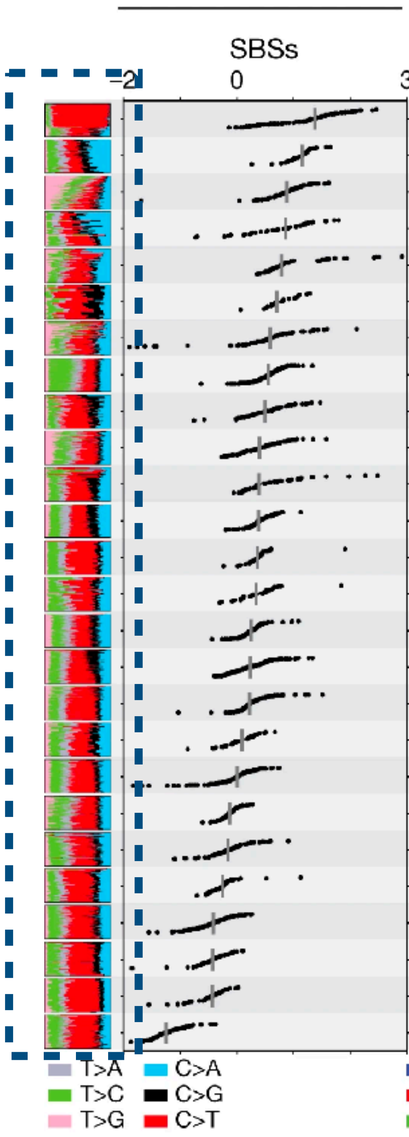


One of the single-base substitution signatures

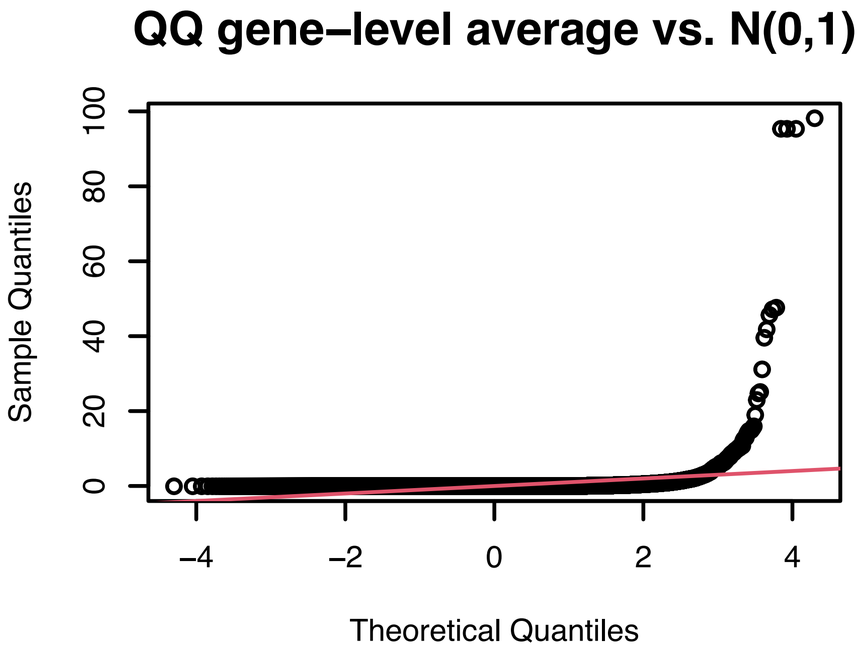
Proportion of mutation "topic" or "signatures" per tumour sample

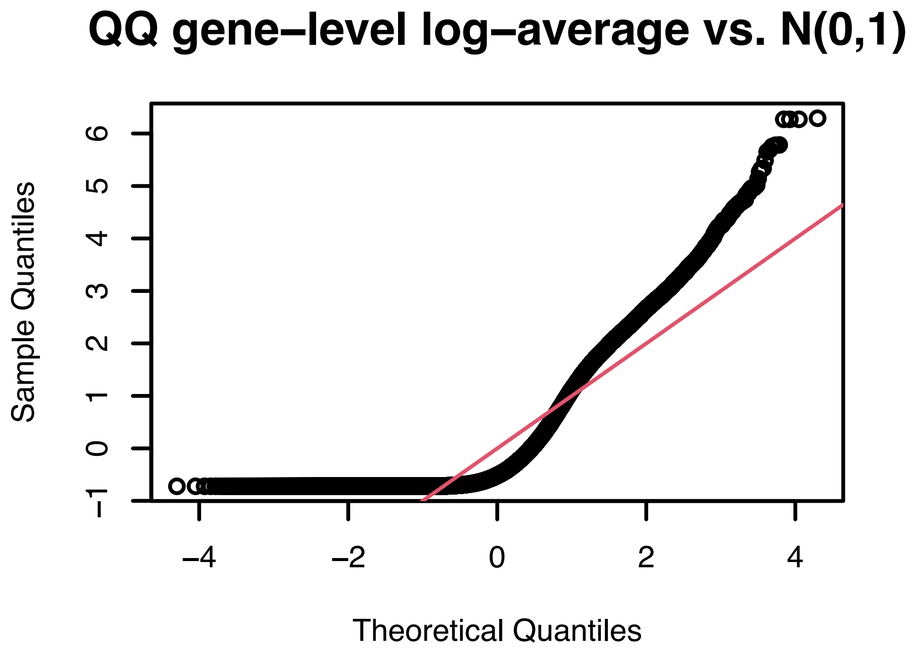


Alexandrov *et al.*, *Nature* (2020)

Why another factorization method for genomics data?

Most genomics data were measured by counting the number of short reads. It is generally hard to make it look "normal," and the normality assumption may introduce unwanted bias.





What is NMF? How can we find NMF solutions?

For each **non-negative** element X_{ij} of a matrix, we want to find non-negative U and V (factors and factor-loading values):

$$X_{ij} \approx \sum_{k=1}^{K} U_{ik} V_{kj}$$

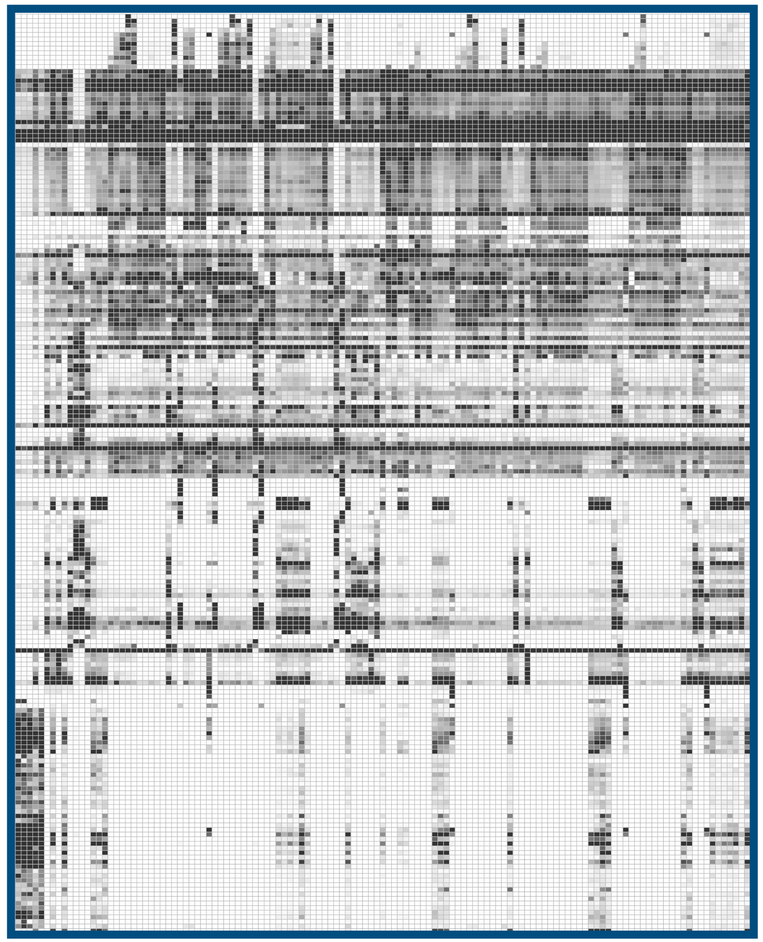
with

$$U_{ik} \geq 0,\ V_{ik} \geq 0.$$

We can use some packages e.g., `NMF` in `R`.

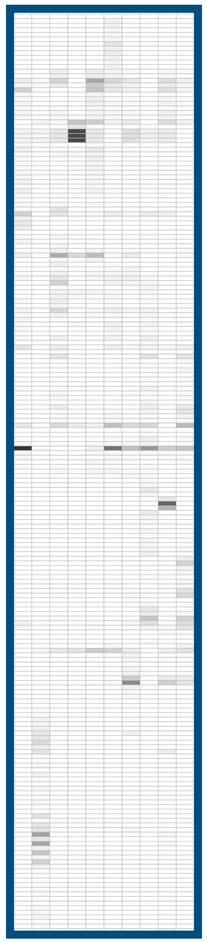
```
library(NMF)
out <- nmf(x.sub, rank=10)
U <- basis(out)
V <- t(coef(out))
```

NMF naturally induces (1) gene sets (2) sample loading



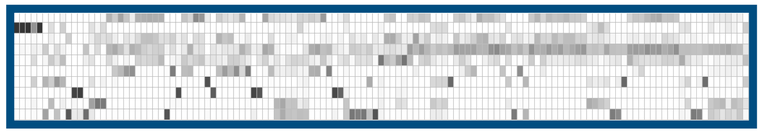
Y

$\sim$



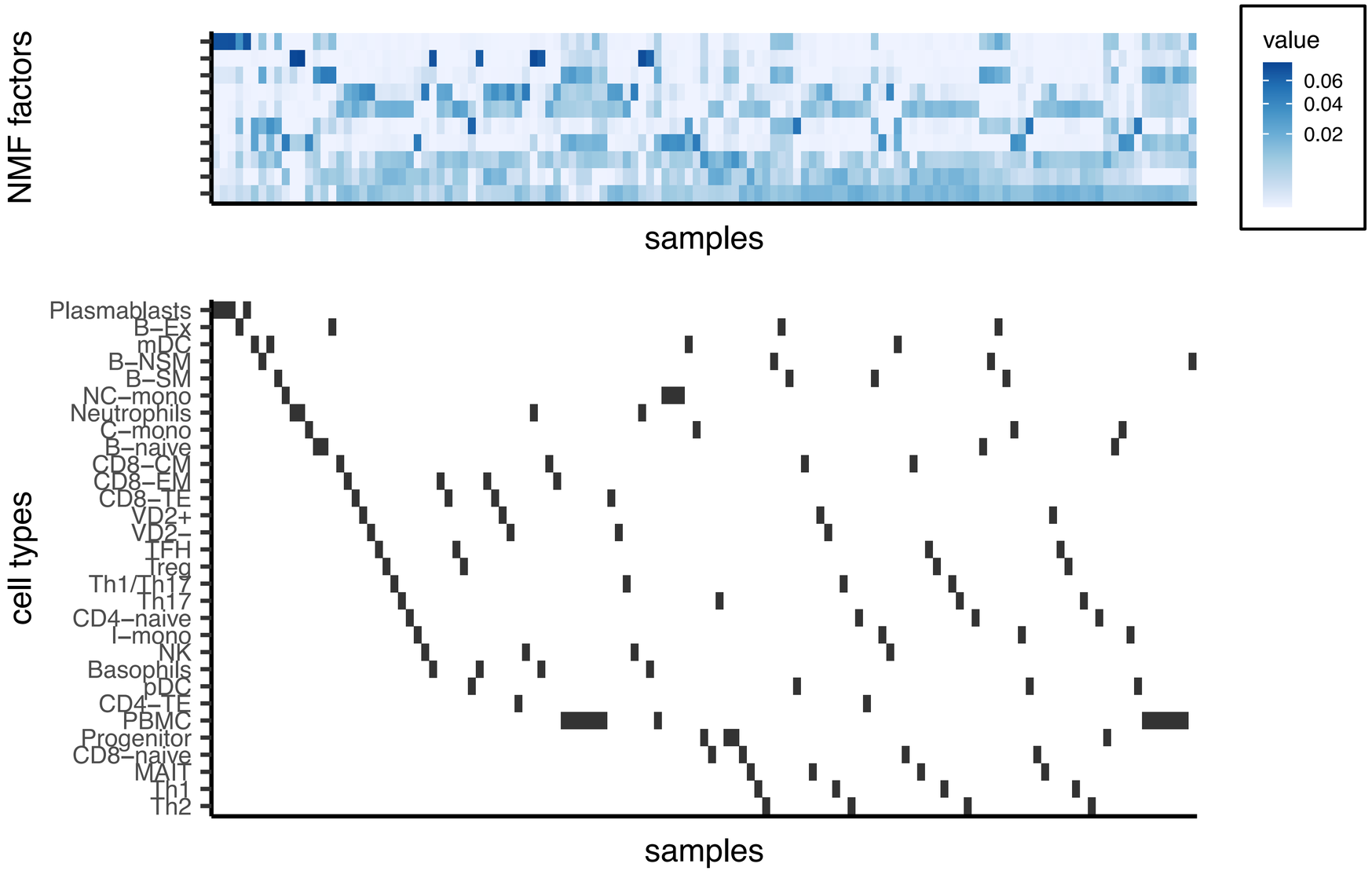
U

$\times$



V

Some NMF factors are highly correlated with cell types



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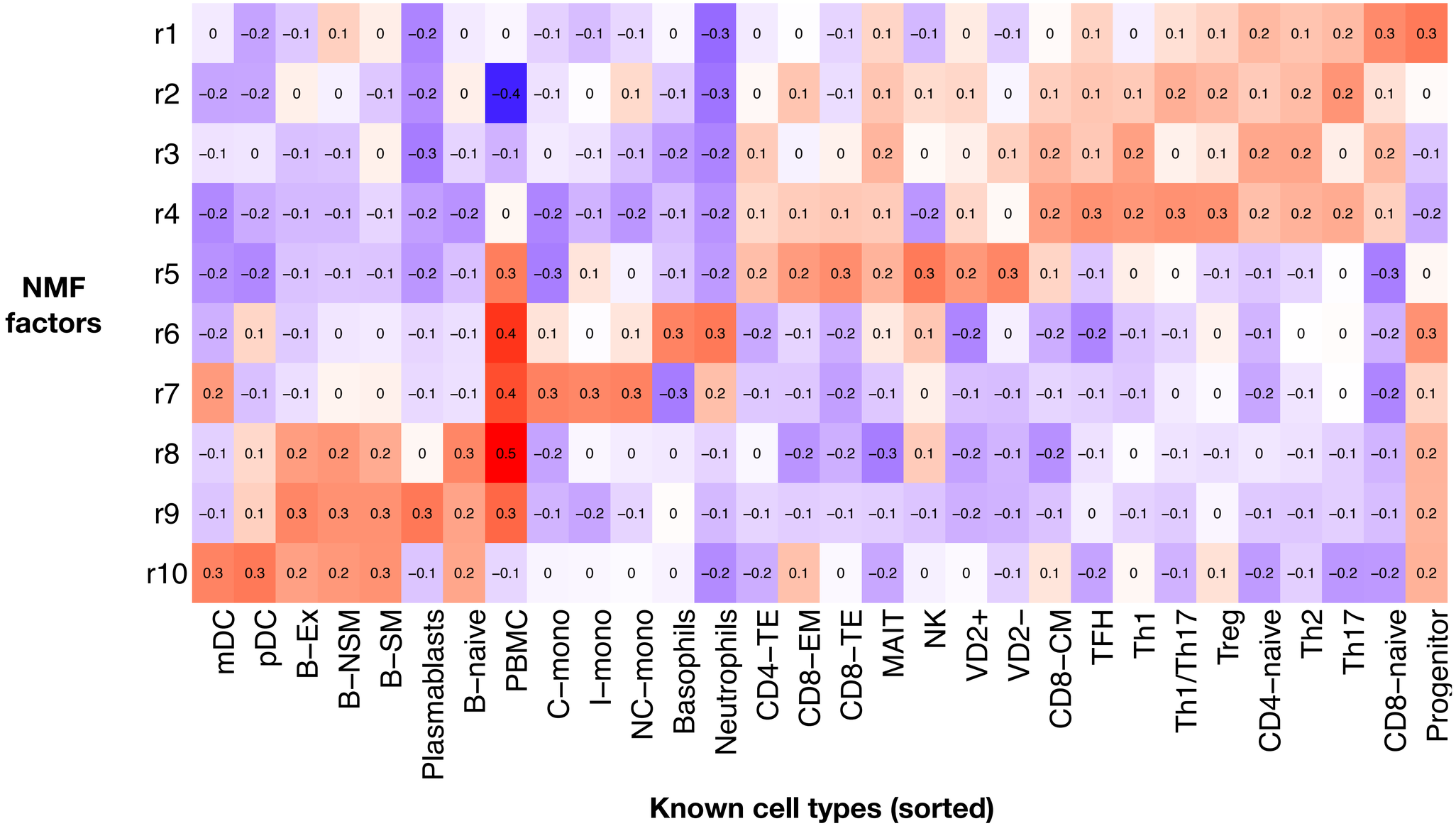
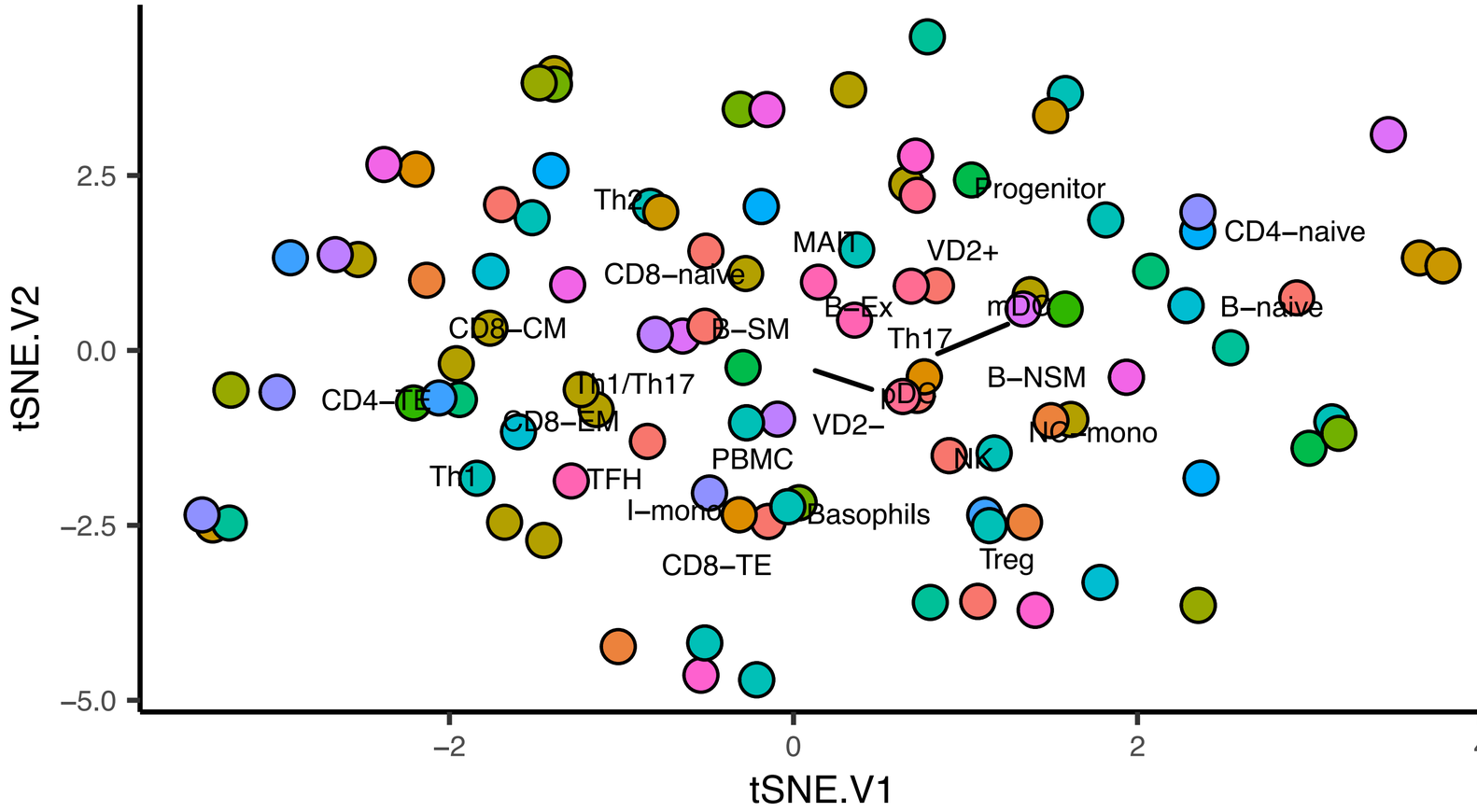


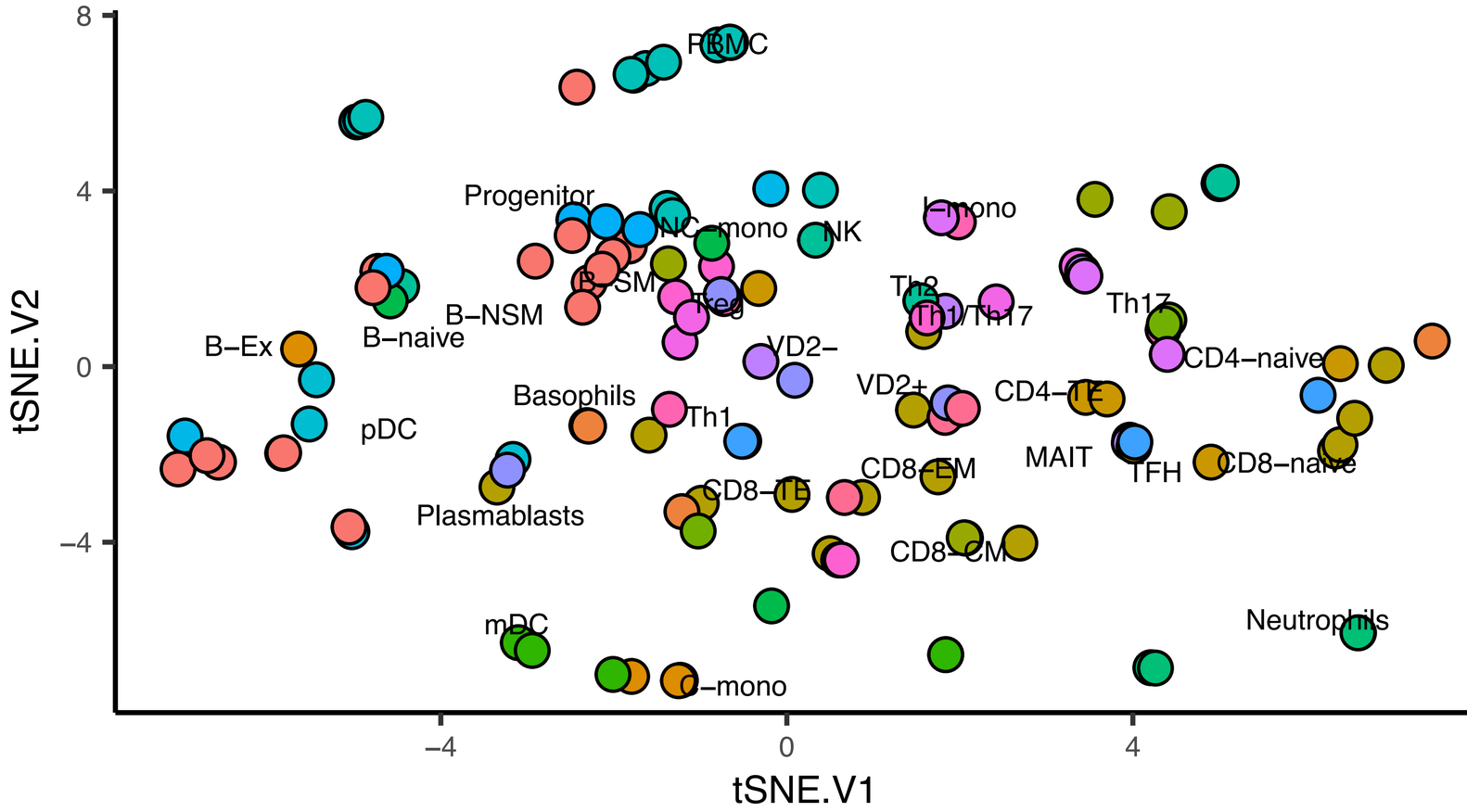
Sample-specific factor loading matrix can be an input to other methods

```
.tsne <- Rtsne::Rtsne(apply(.svd$v, 2, scale))
```

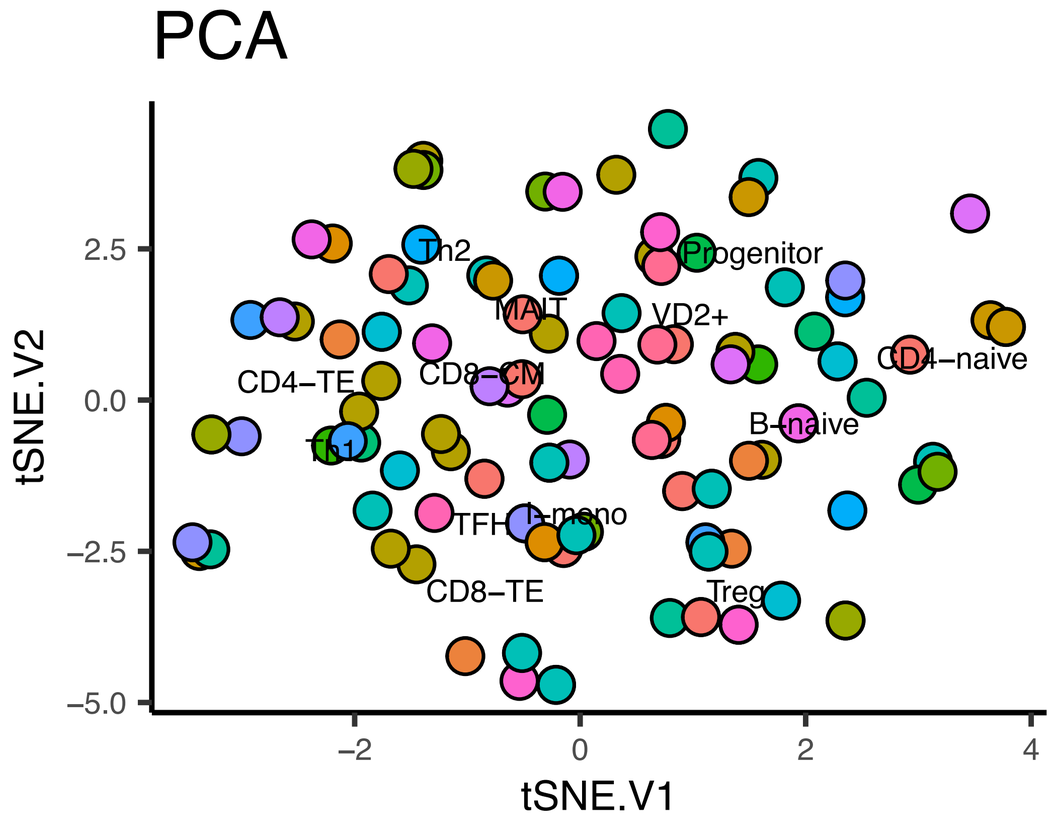


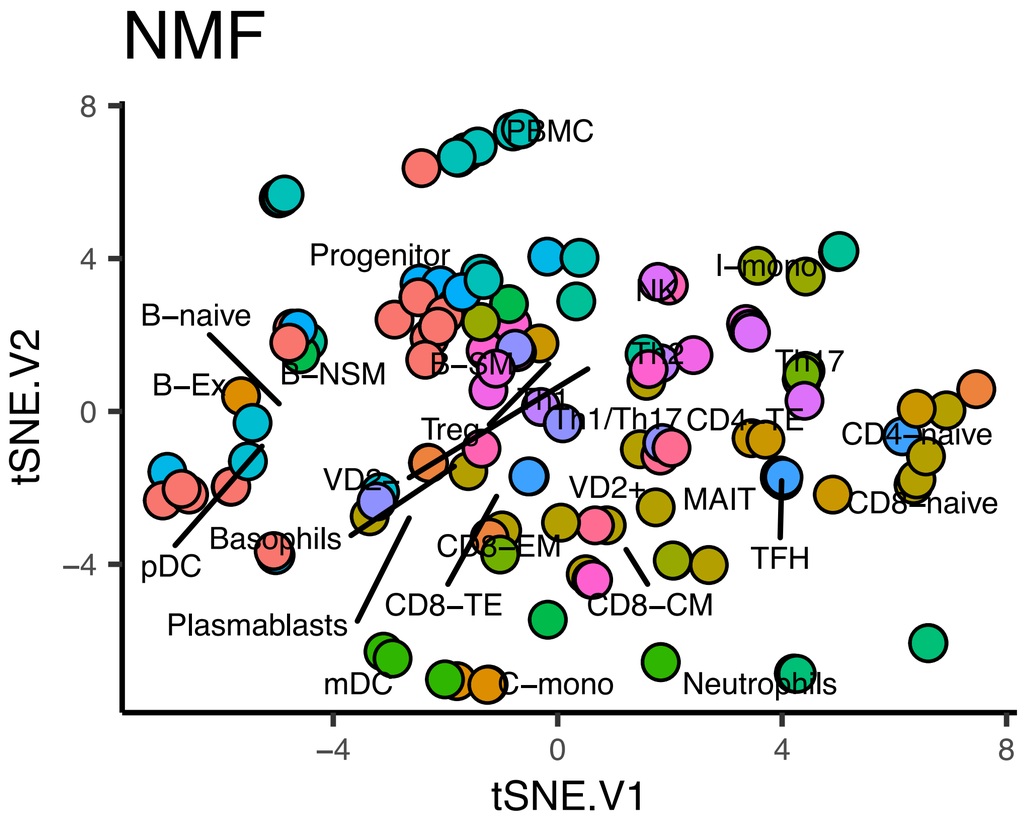
Sample-specific factor loading matrix can be an input to other methods

```
.tsne <- Rtsne::Rtsne(apply(log(V), 2, scale))
```



PCA vs. NMF: Which one looks better?





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