Deep generative modelling of bulk transcriptomics

Iñigo Prada Luengo

Agenda

- Why are generative models useful?
  - The weather for my grandma & a scientist
  - Why are generative models useful in biology
- A generative model of the bulk human transcriptome
  - Why even model bulk RNA-seq data?
  - A generative model for bulk RNA-seq
  - Detecting tumor origins
  - A new way of finding differentially expressed genes

Discriminative vs Generative models

- A discriminative model: $P(y|x)$ "Lets learn to predict an outcome given the observations"
- A generative model: $P(y, x)$ "Lets learn a joint distribution of all variables"
- Why are generative models attractive?
- We learn a model of how our data is *generated*.

Modeling the weather

- A discriminative model for the weather: $P(y|x)$

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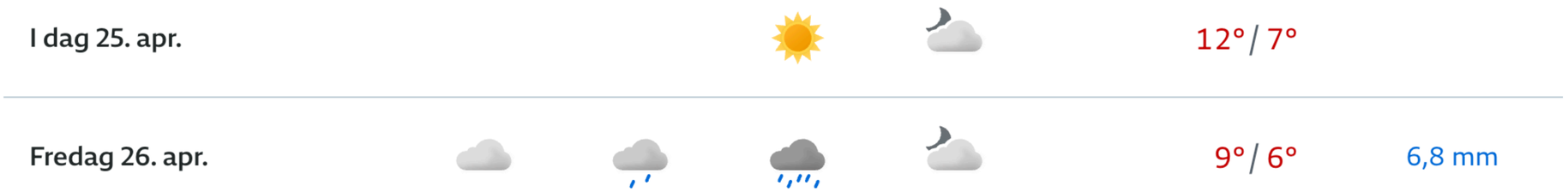

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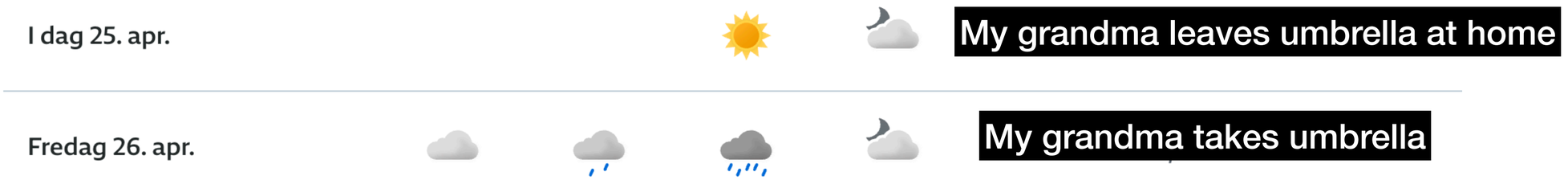

- A generative model for the weather: $P(y, x)$

Temperature

Humidity

Wind

⋮

Precipitation

Sunshine

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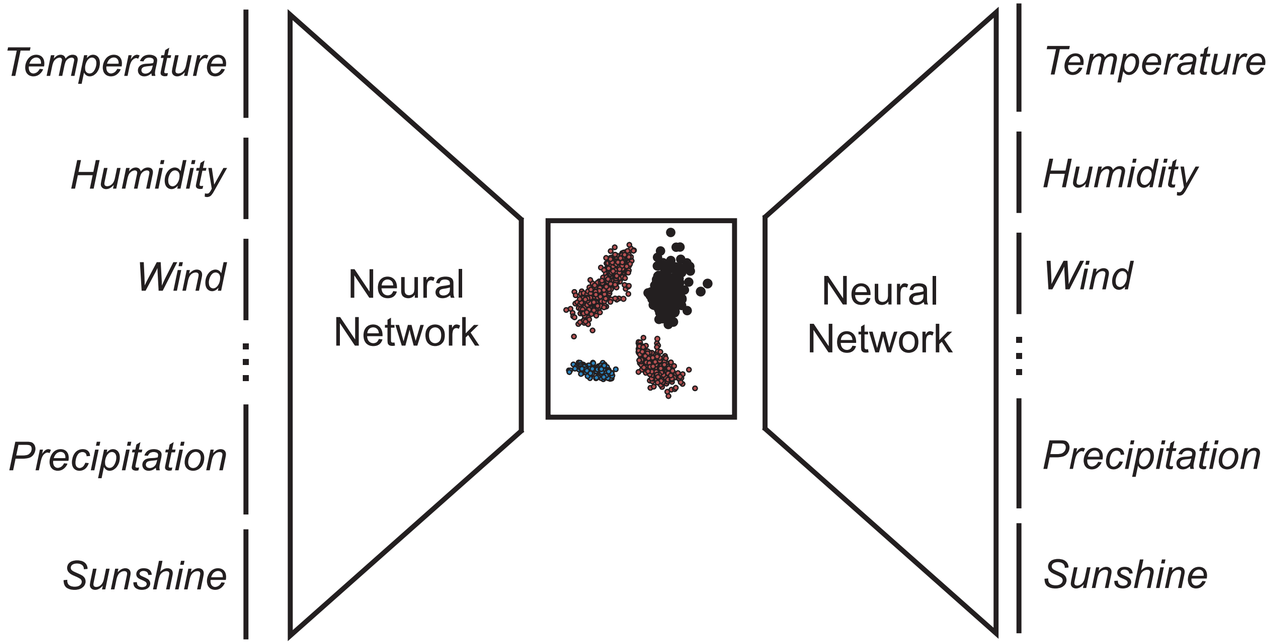

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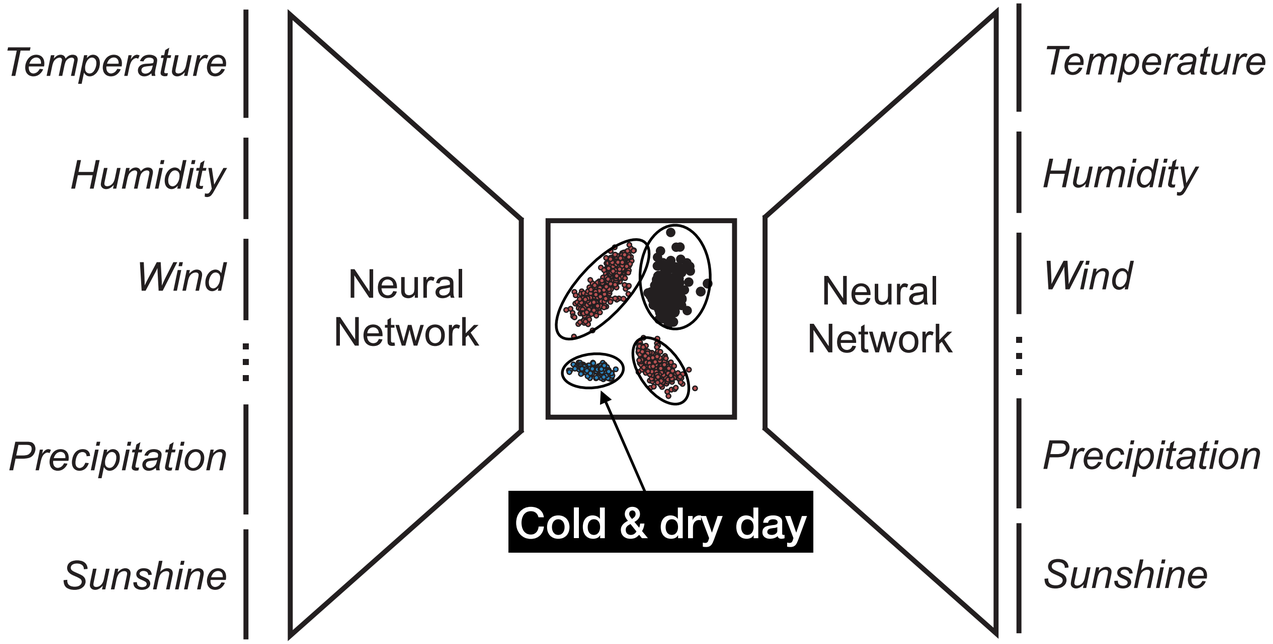

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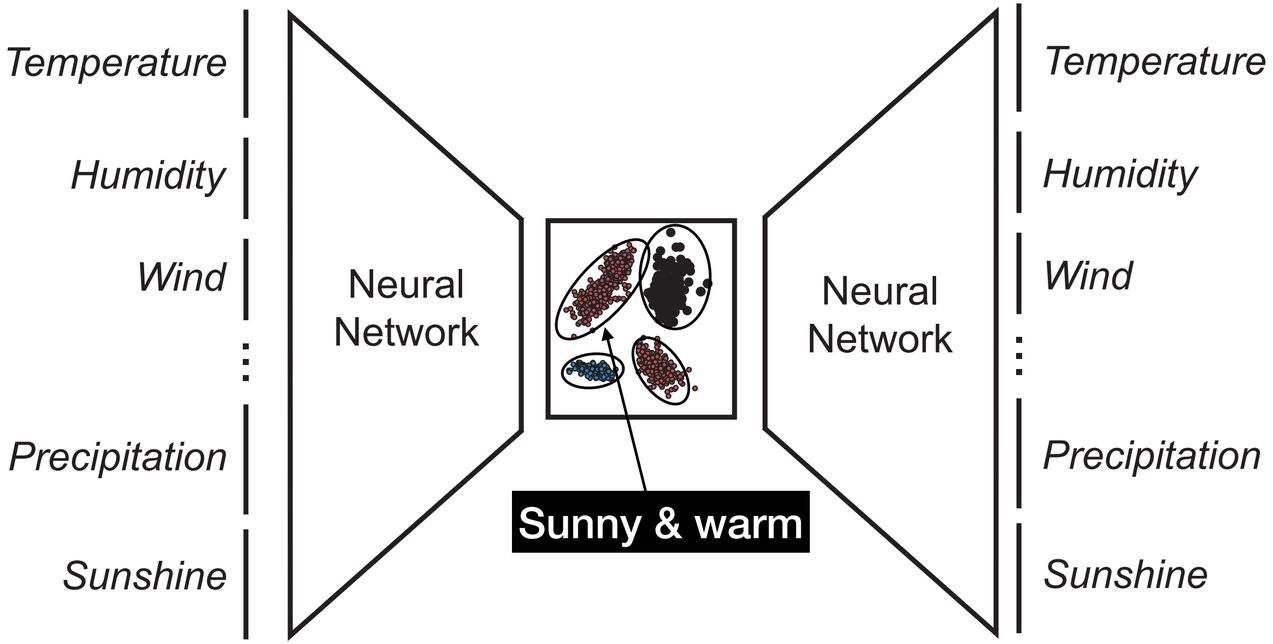

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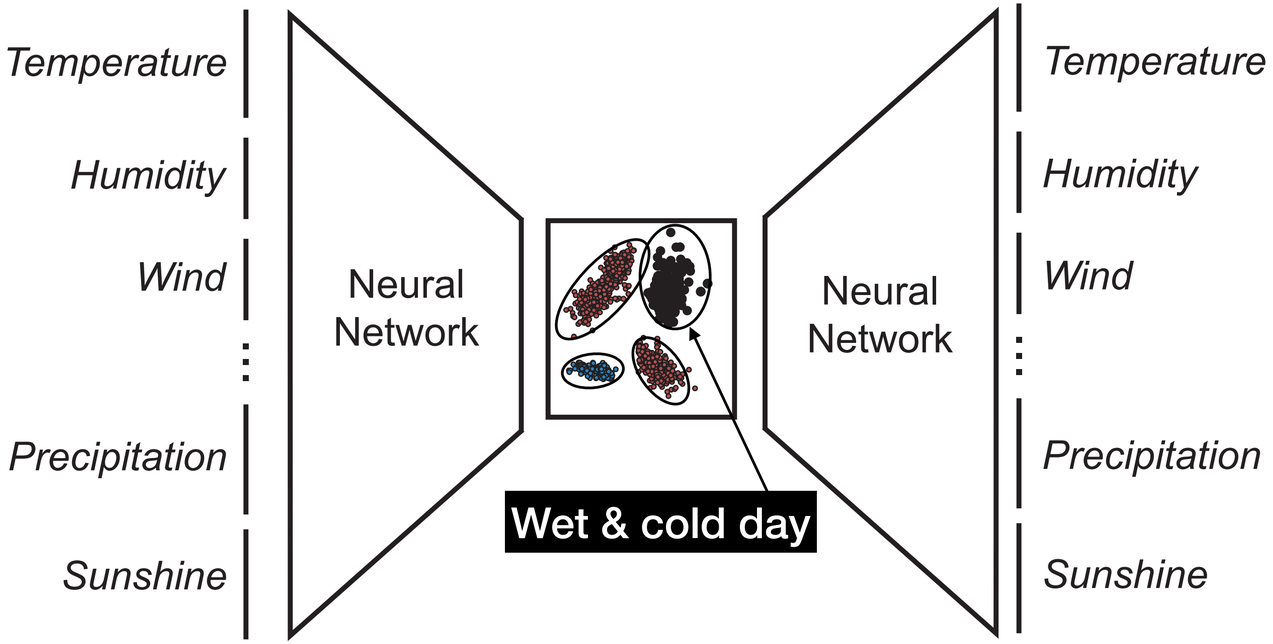

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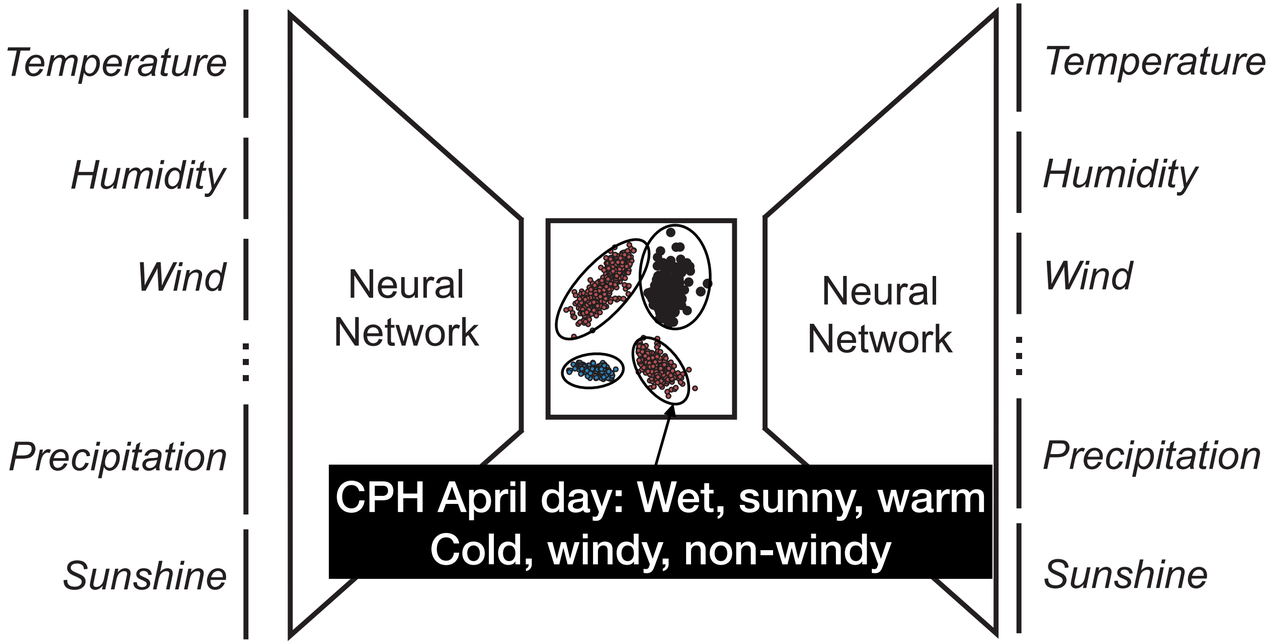
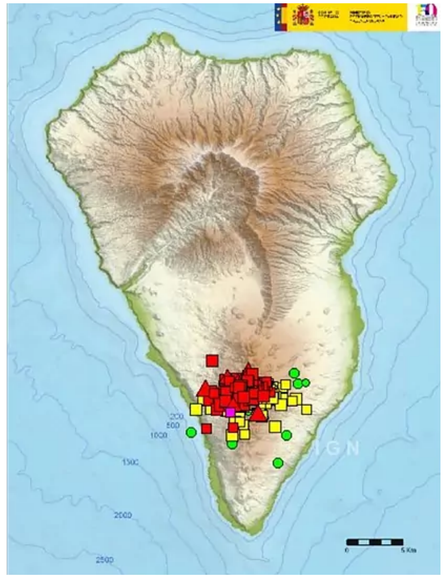


Generative models for science

- We can use generative models to understand the world

2021 La Palma eruption, Canary Islands. Spain



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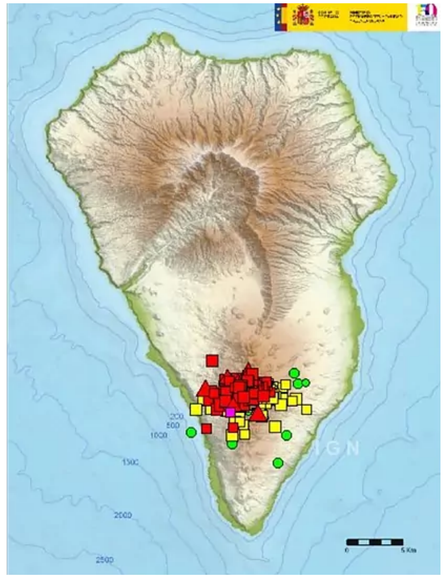

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Generative Model
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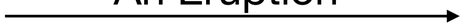

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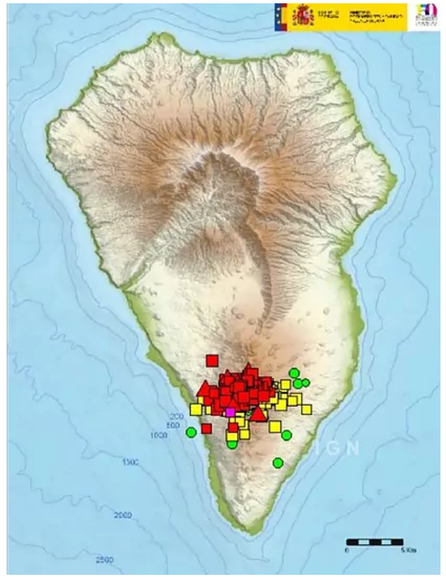

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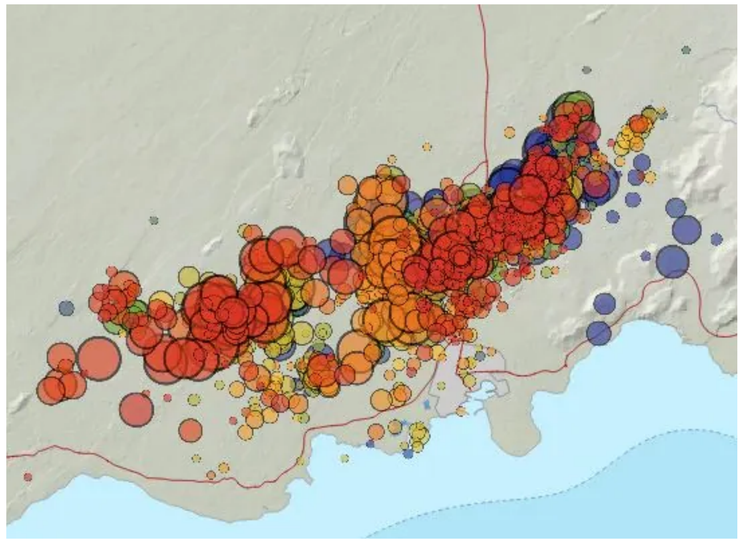
2024 Grindavik eruption, Iceland



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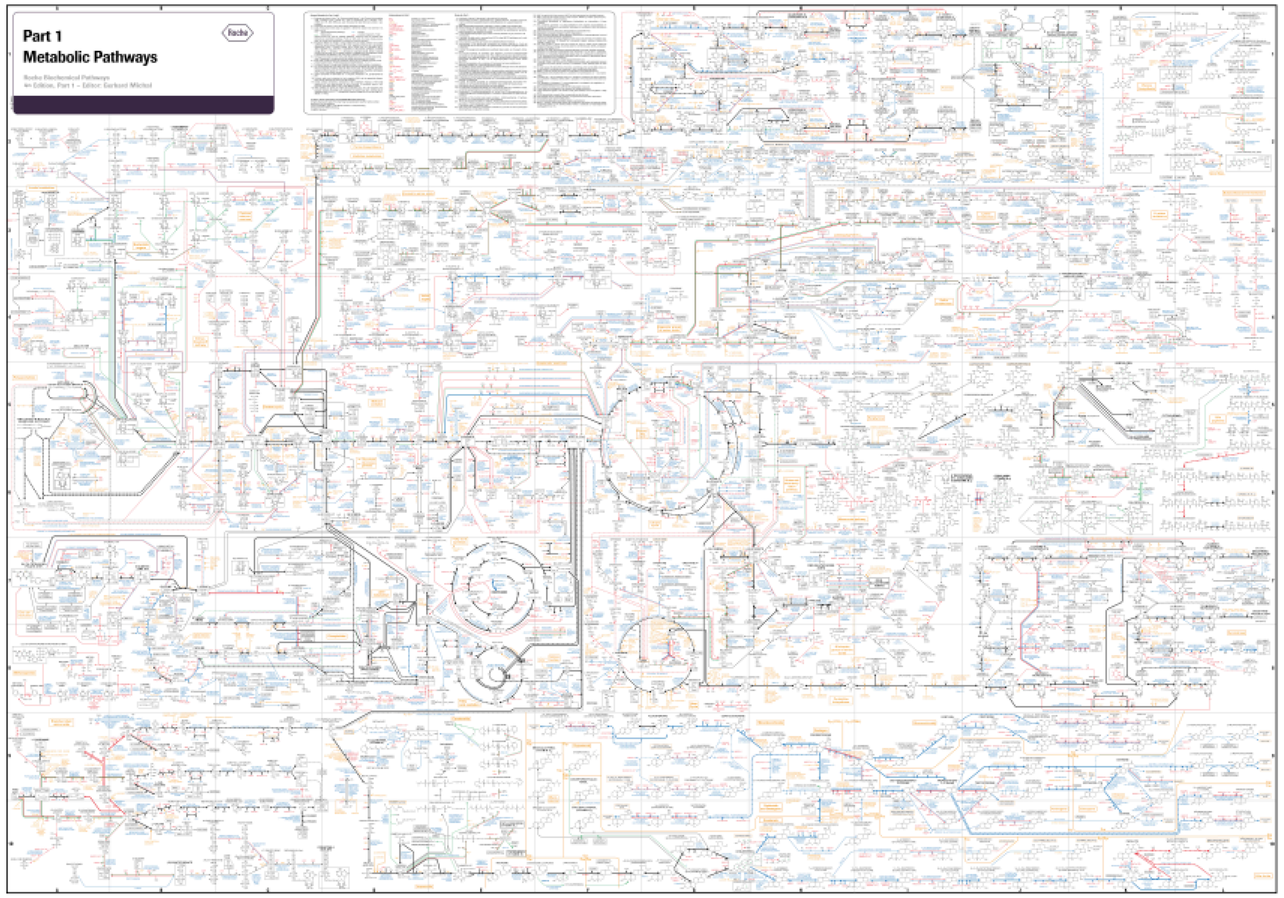

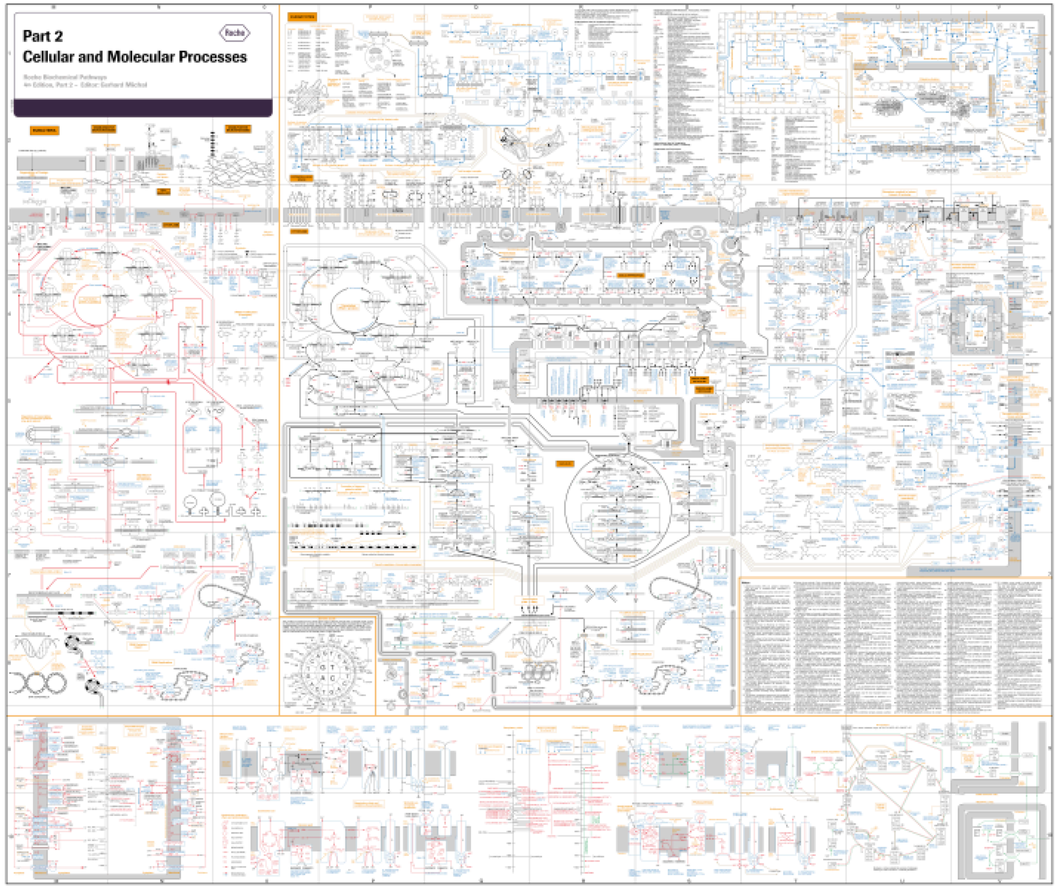

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Deep generative models for biology

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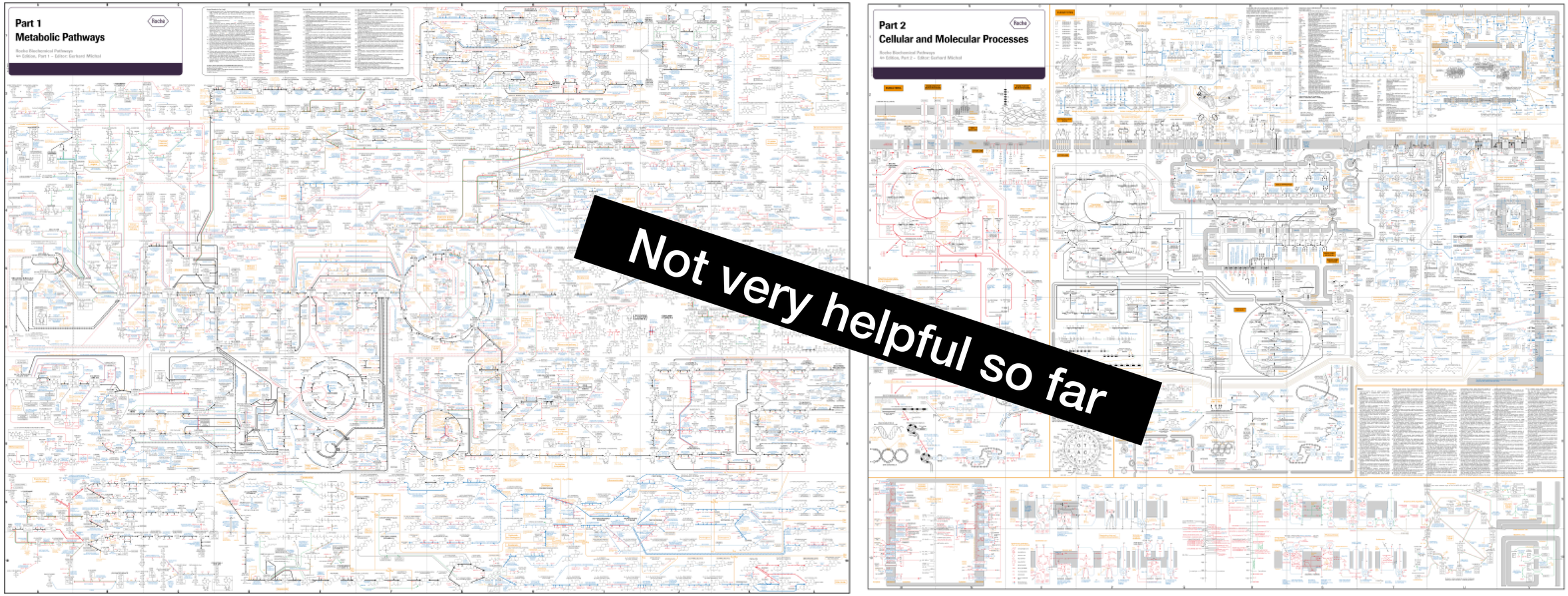

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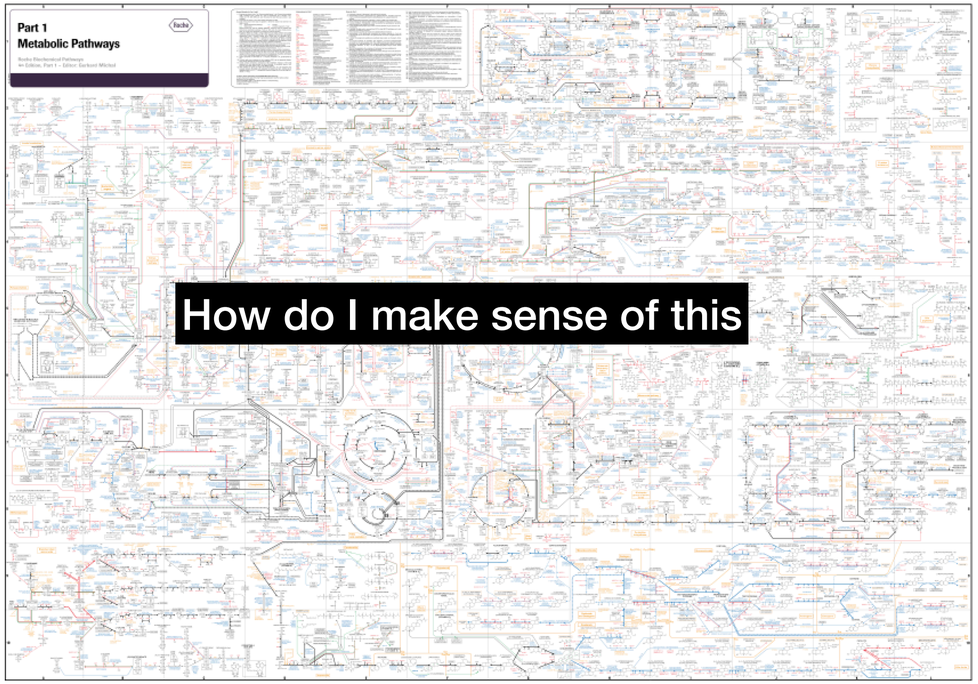
Why don’t we let a computer figure this out?



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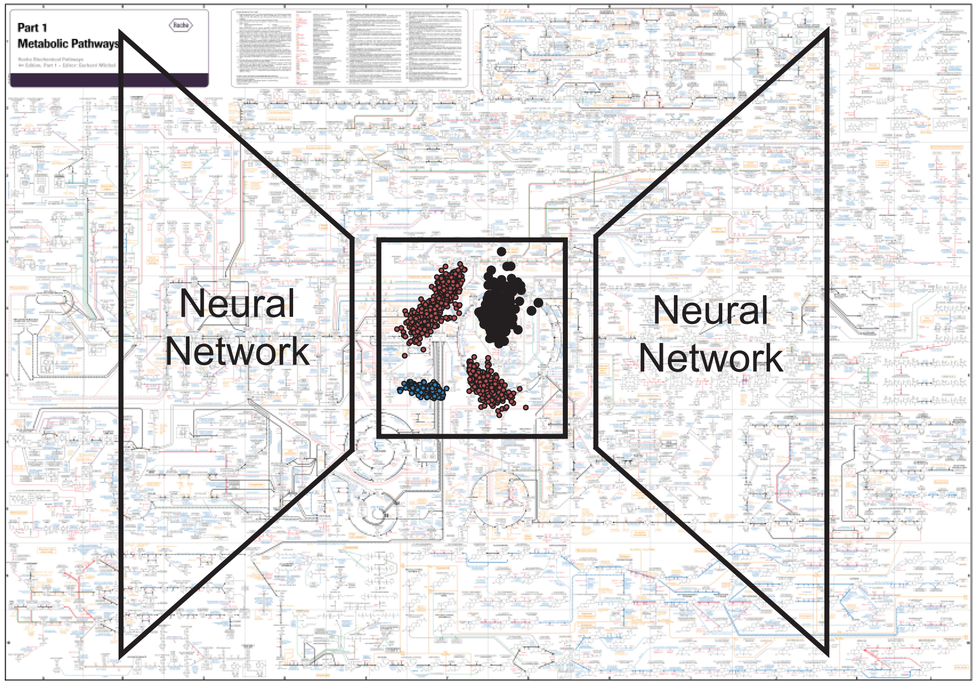
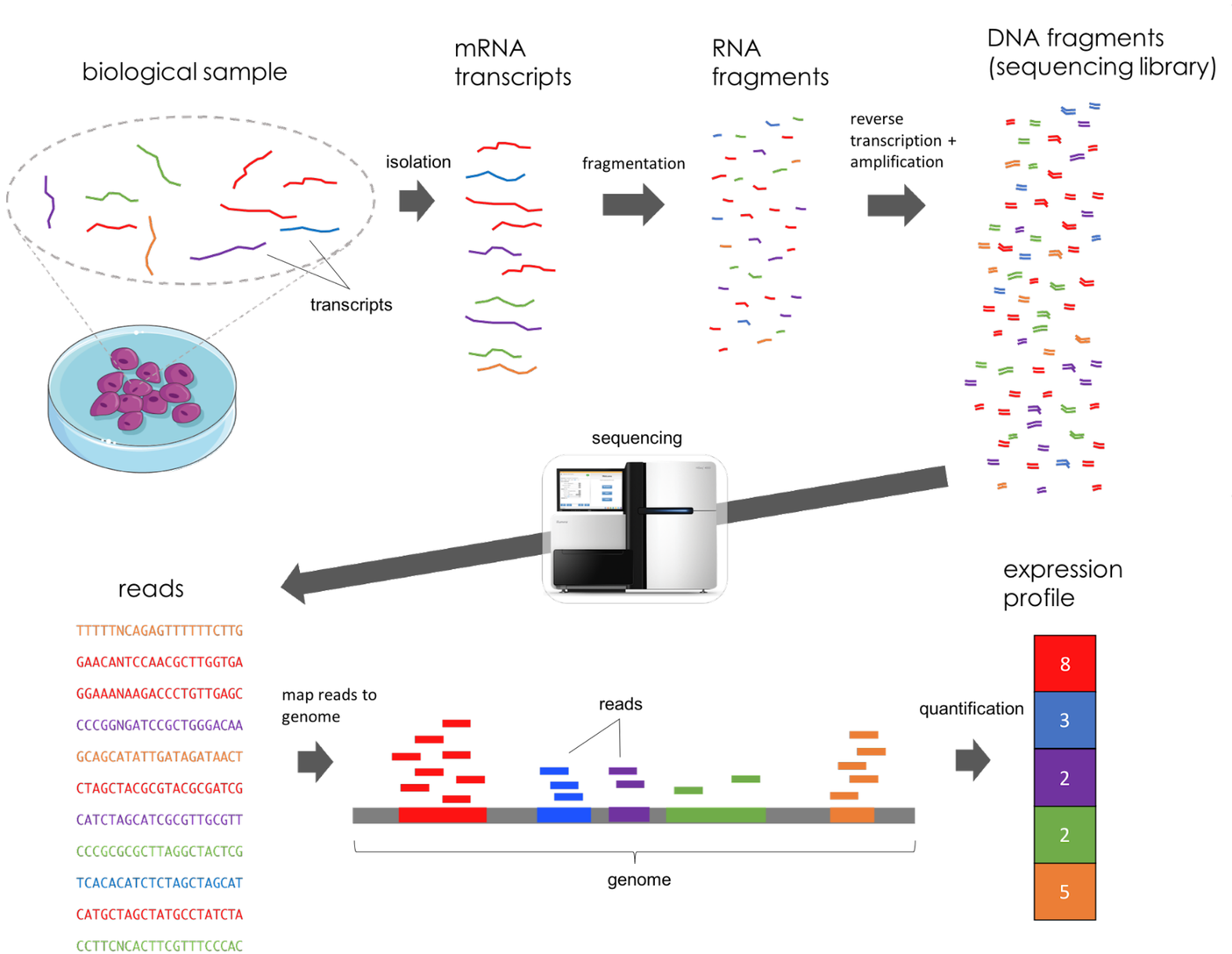


- We nowadays can collect vast amounts of data using omic techniques
- We can leave the task of figuring out all this associations to a neural network
- Highly successful in recent years
  - E.g. AlphaFold, scRNA-seq

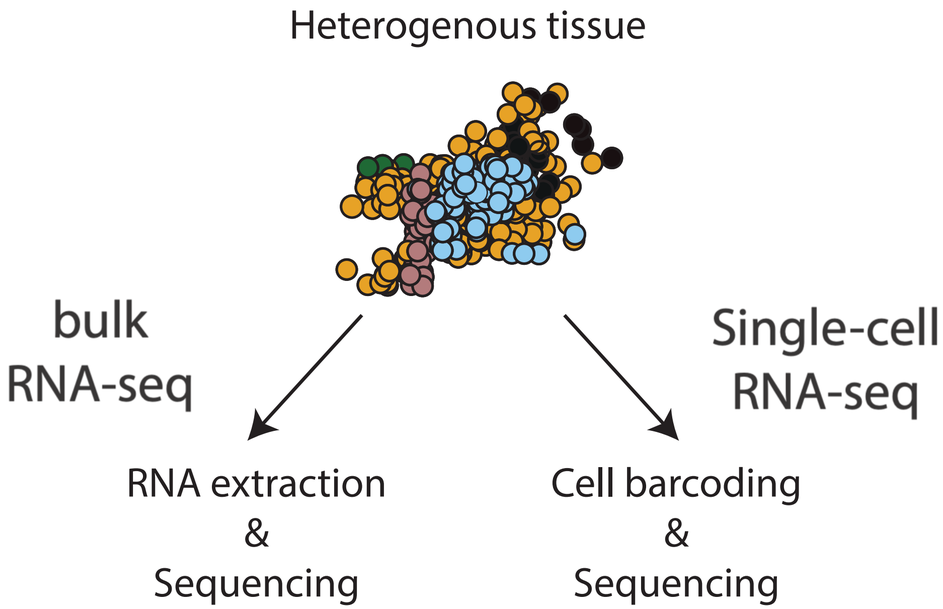
Deep generative modelling of bulk RNA-seq

The RNA-seq workflow



- Highly versatile technique:
  - Isoform, fusion gene detection…
- Main use is the detection of differentially expressed genes
  - E.g. Limma, DEseq2, EdgeR

Why bulk RNA-seq?



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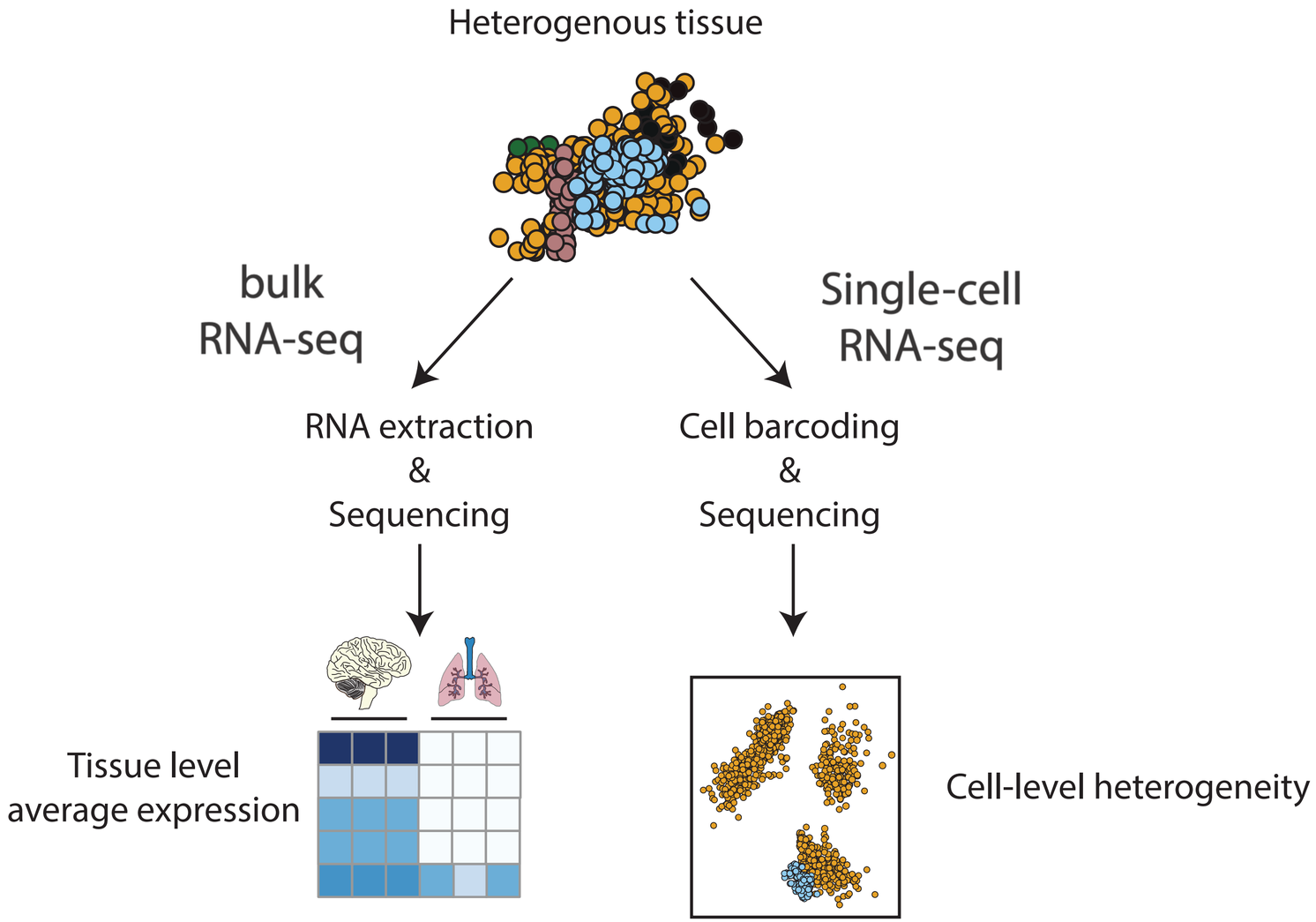

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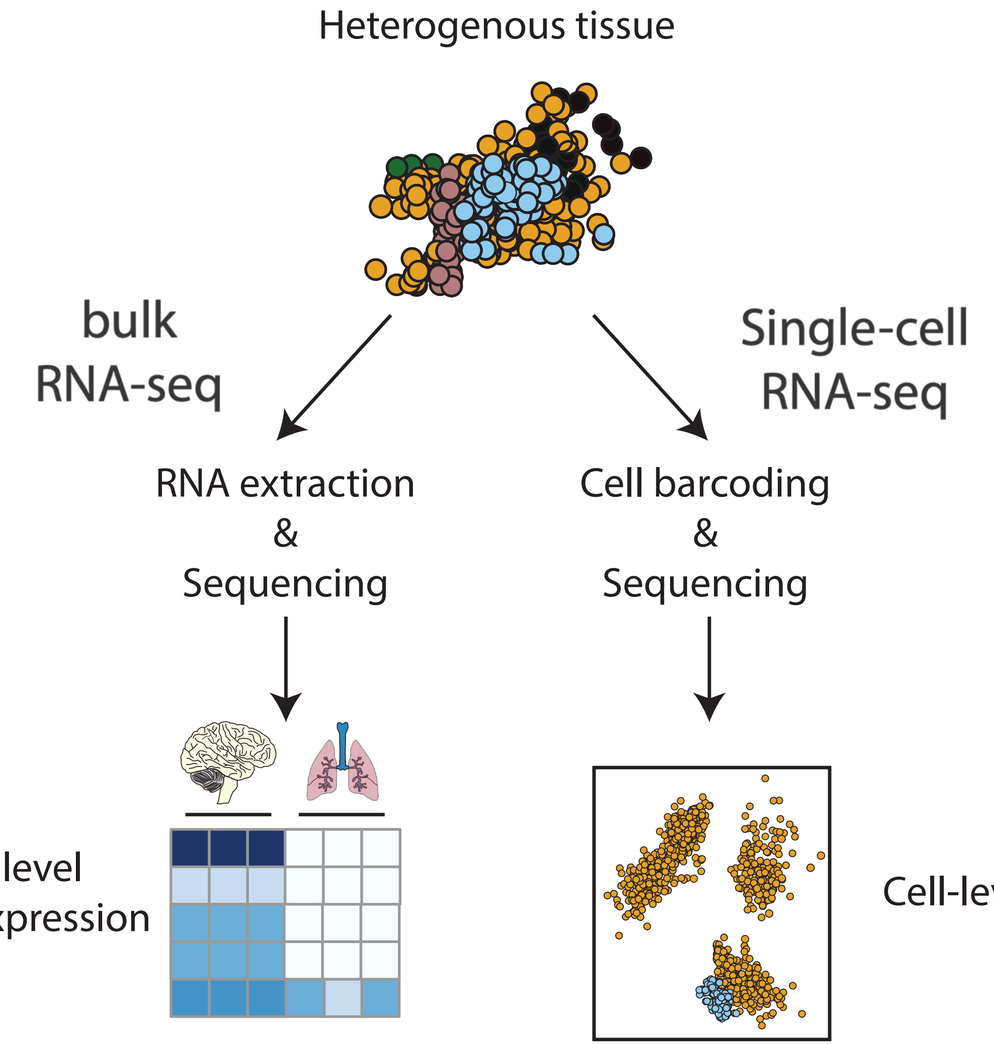
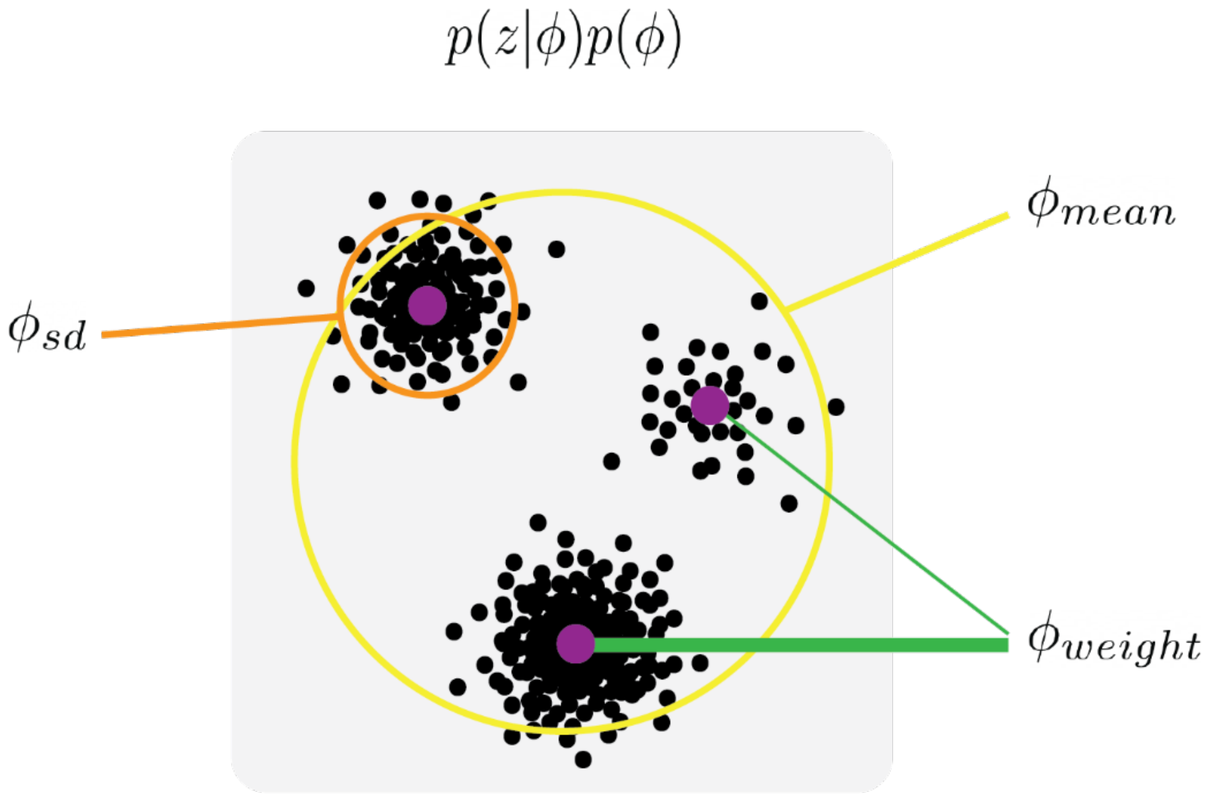
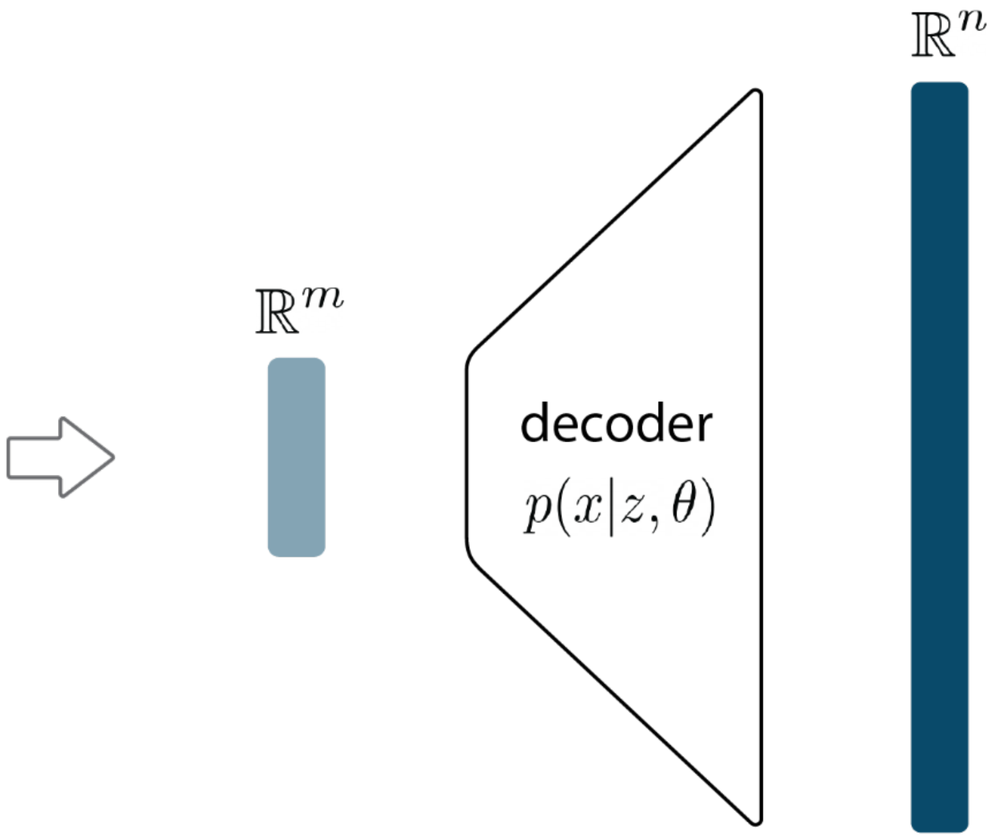


- Bulk RNA-seq
  - Mature technique
  - Less noisy
  - Main technique used in “production”

The Deep Generative Decoder



GMM

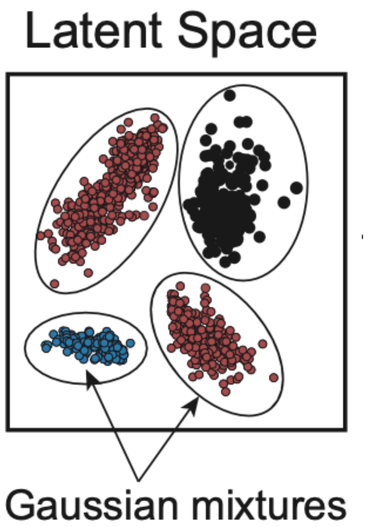


latent Z

data X

Schuster & Krogh. Bioinformatics, 2023

Model set-up



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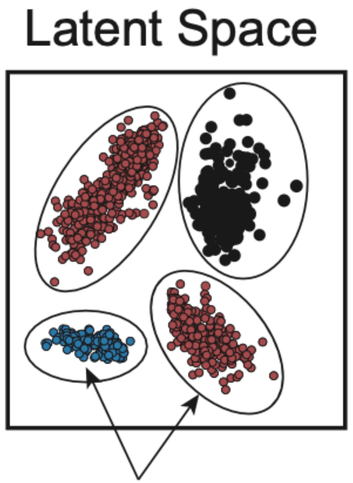
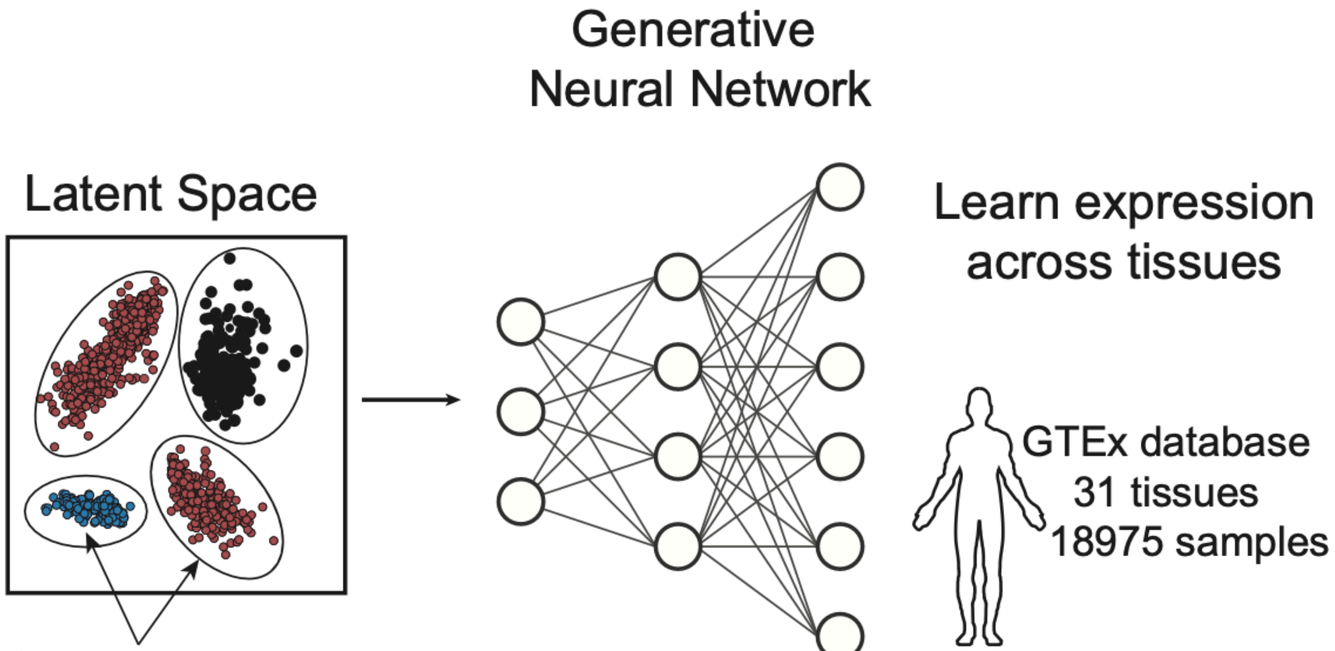


Latent space 50 dimensions
GMM with 45 components
Diagonal covariance

Model set-up


Generative
Neural Network
Latent Space
Learn expression
across tissues
GTEx database
31 tissues
18975 samples
Gaussian mixtures
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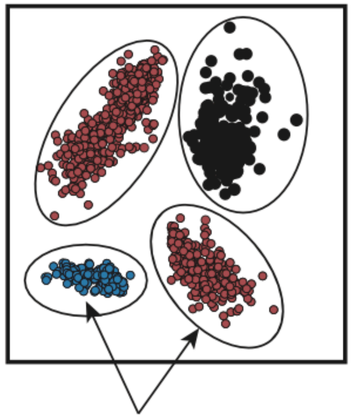

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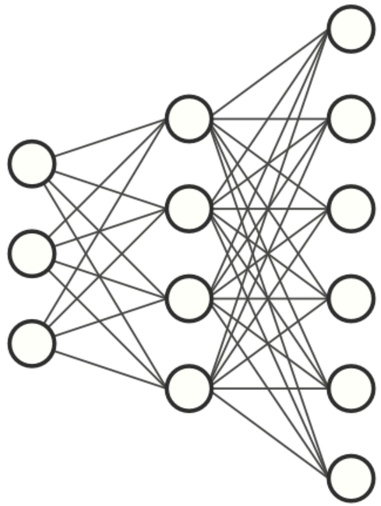

2 Fully connected layers
Negative-binomial output
16883 genes

Learn expression
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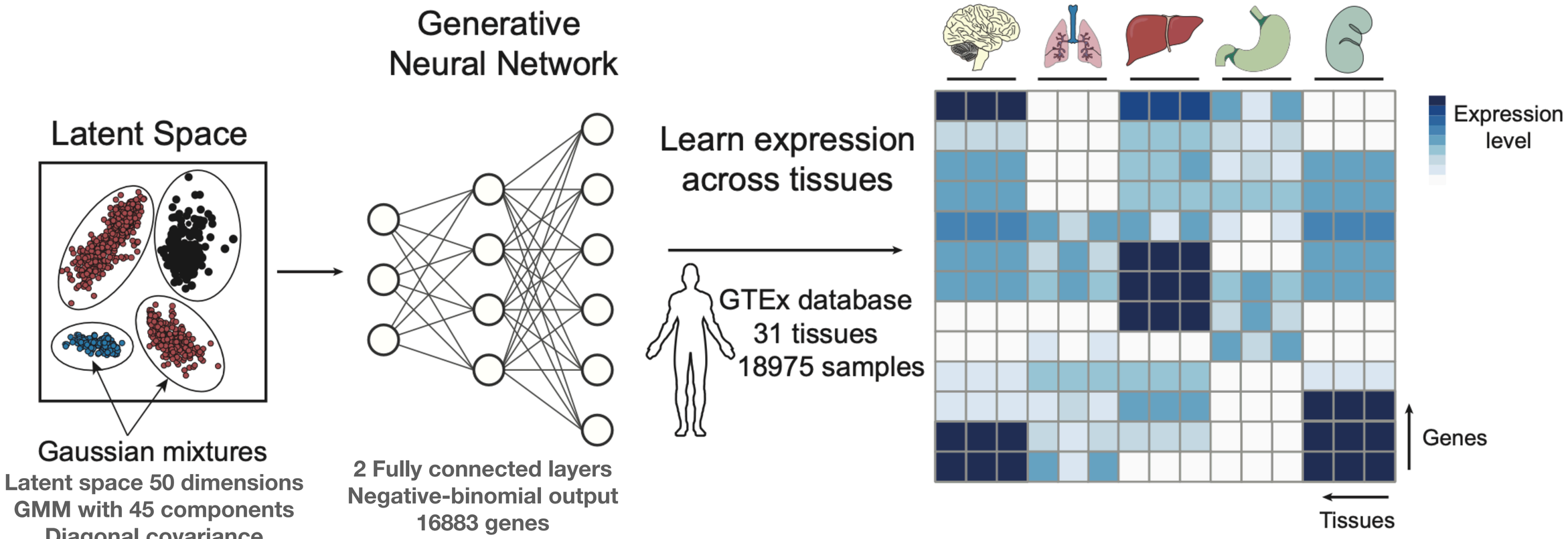
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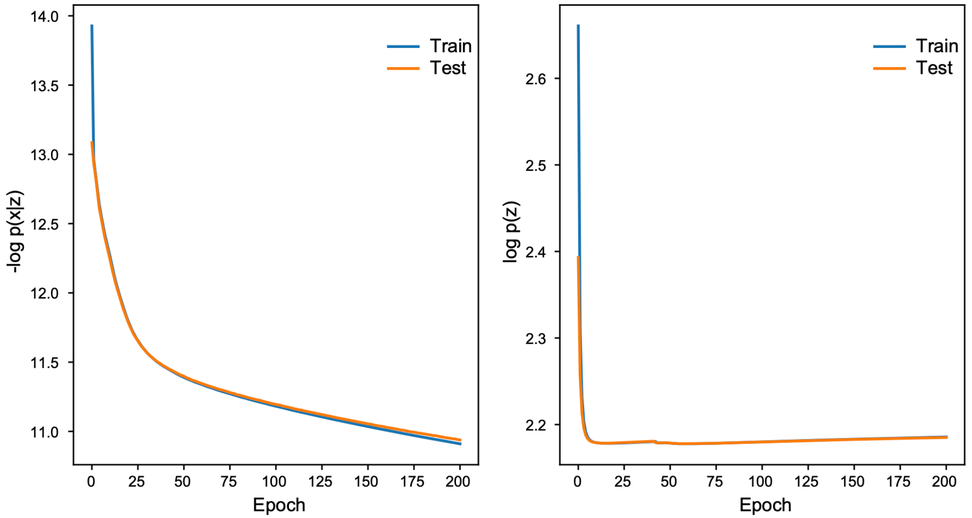

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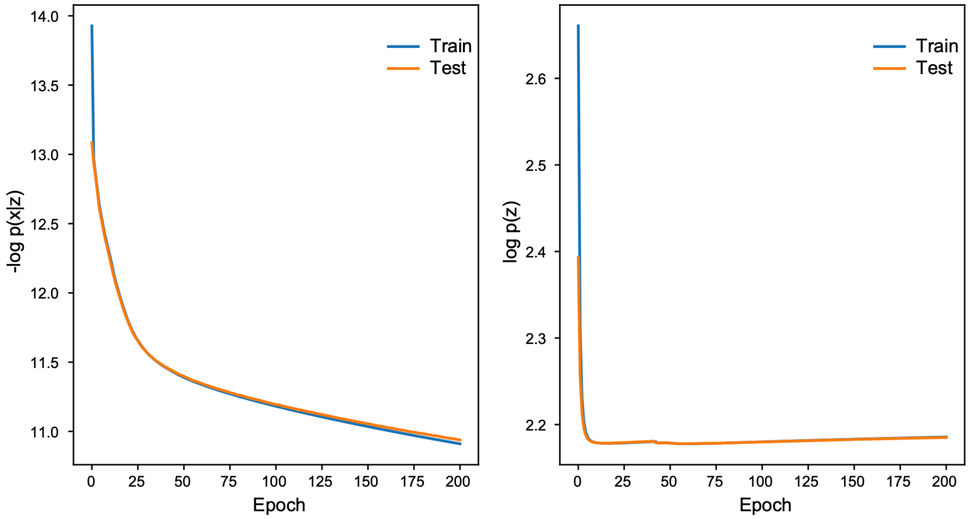

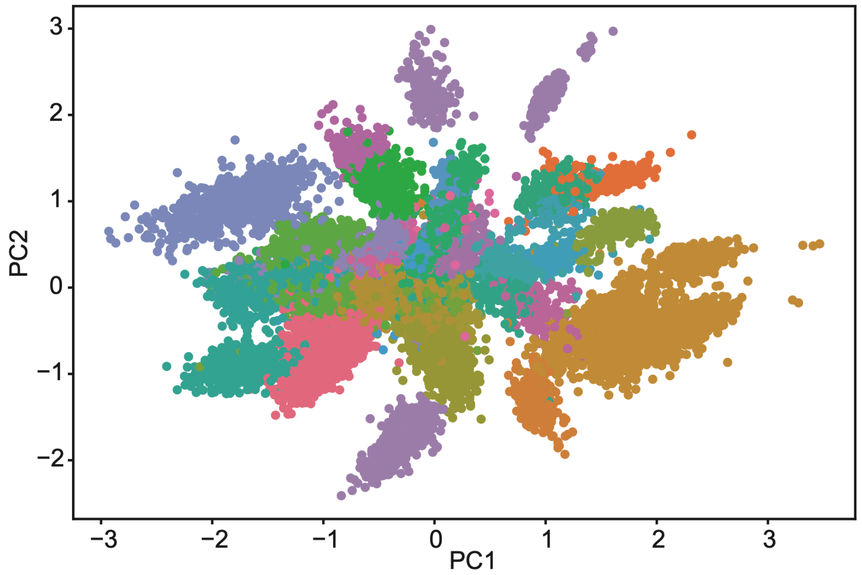

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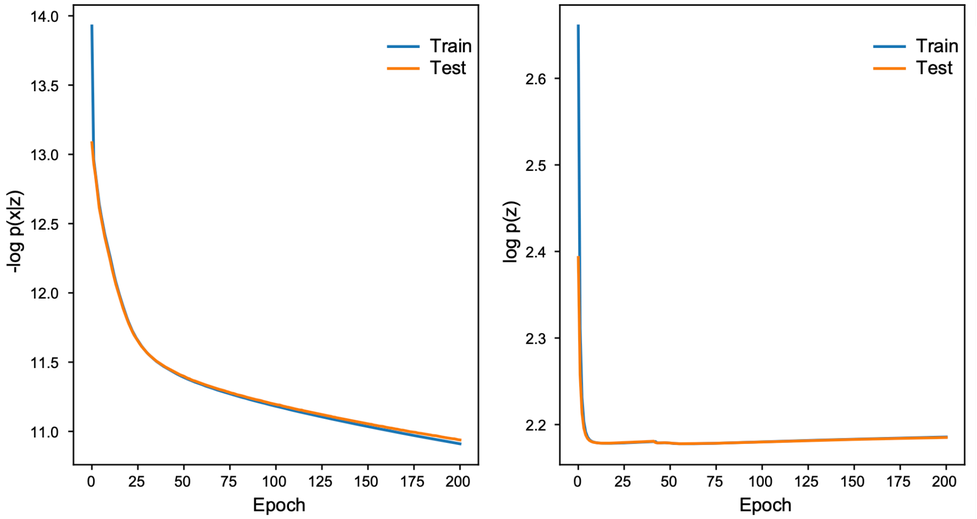

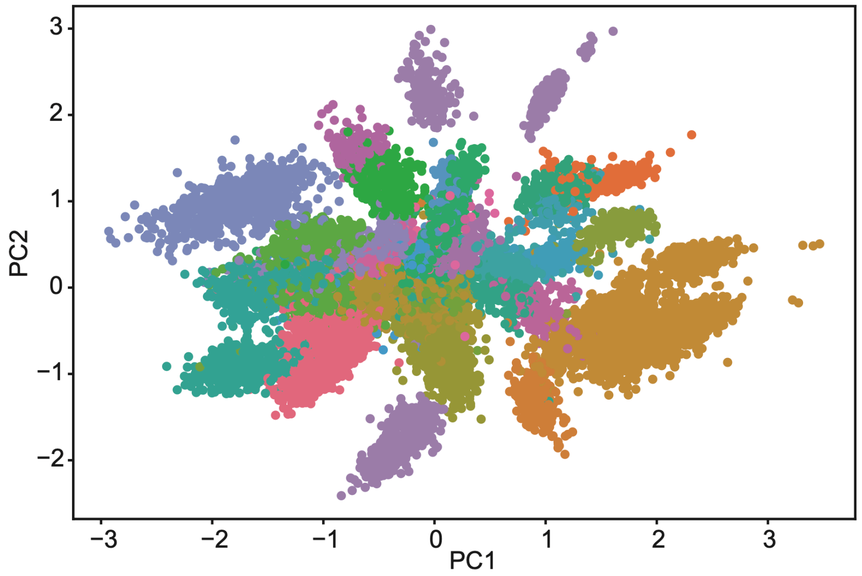

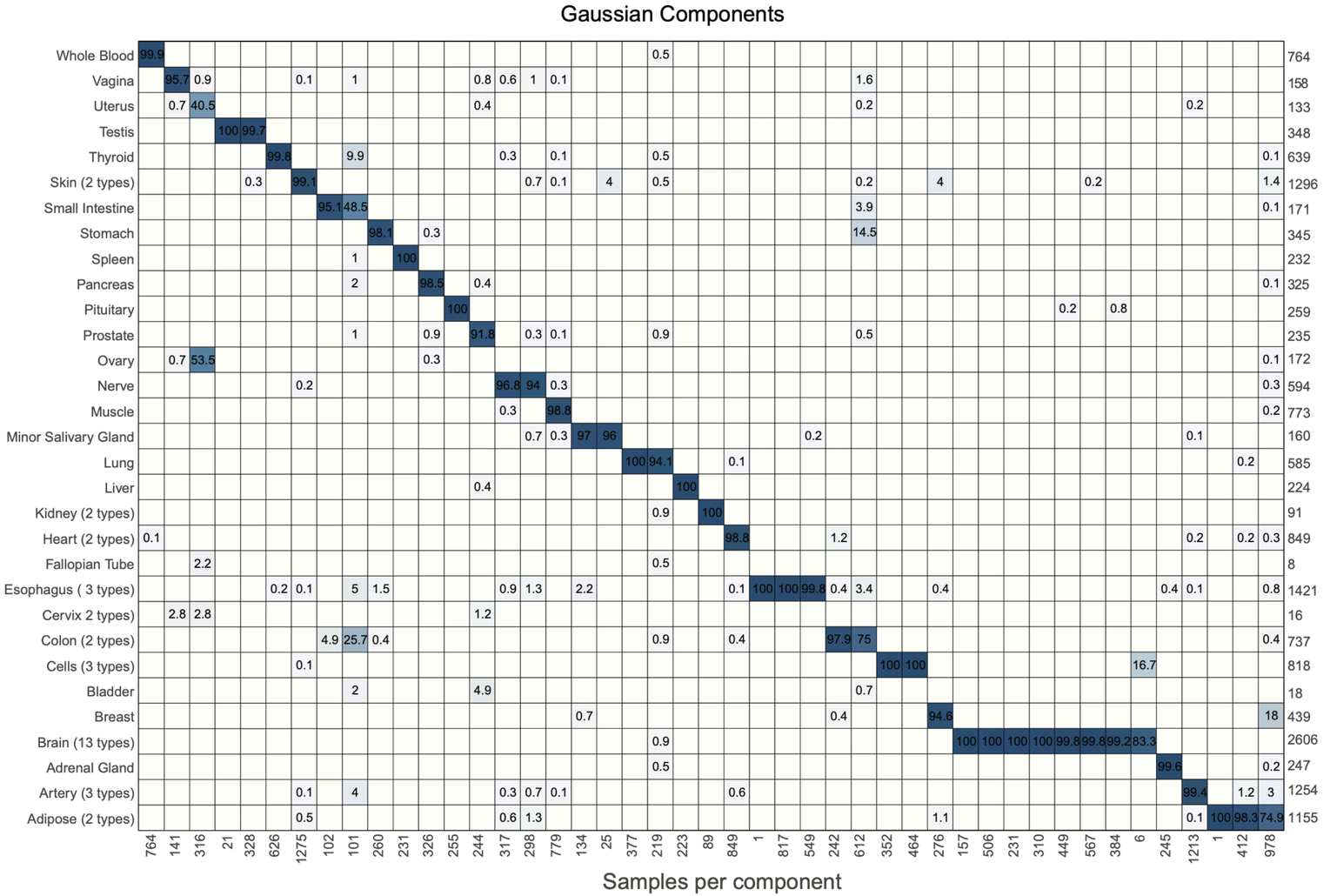

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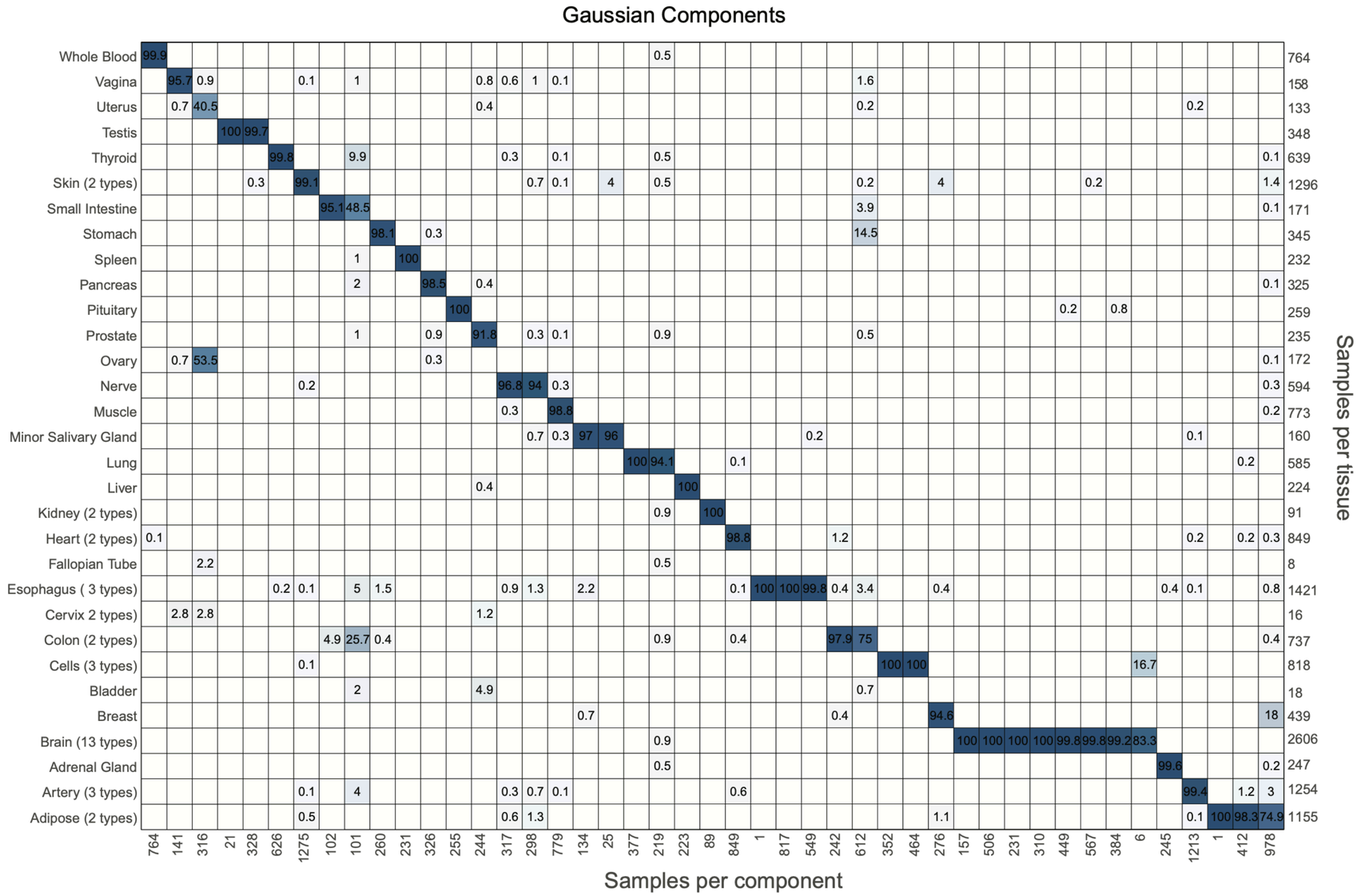

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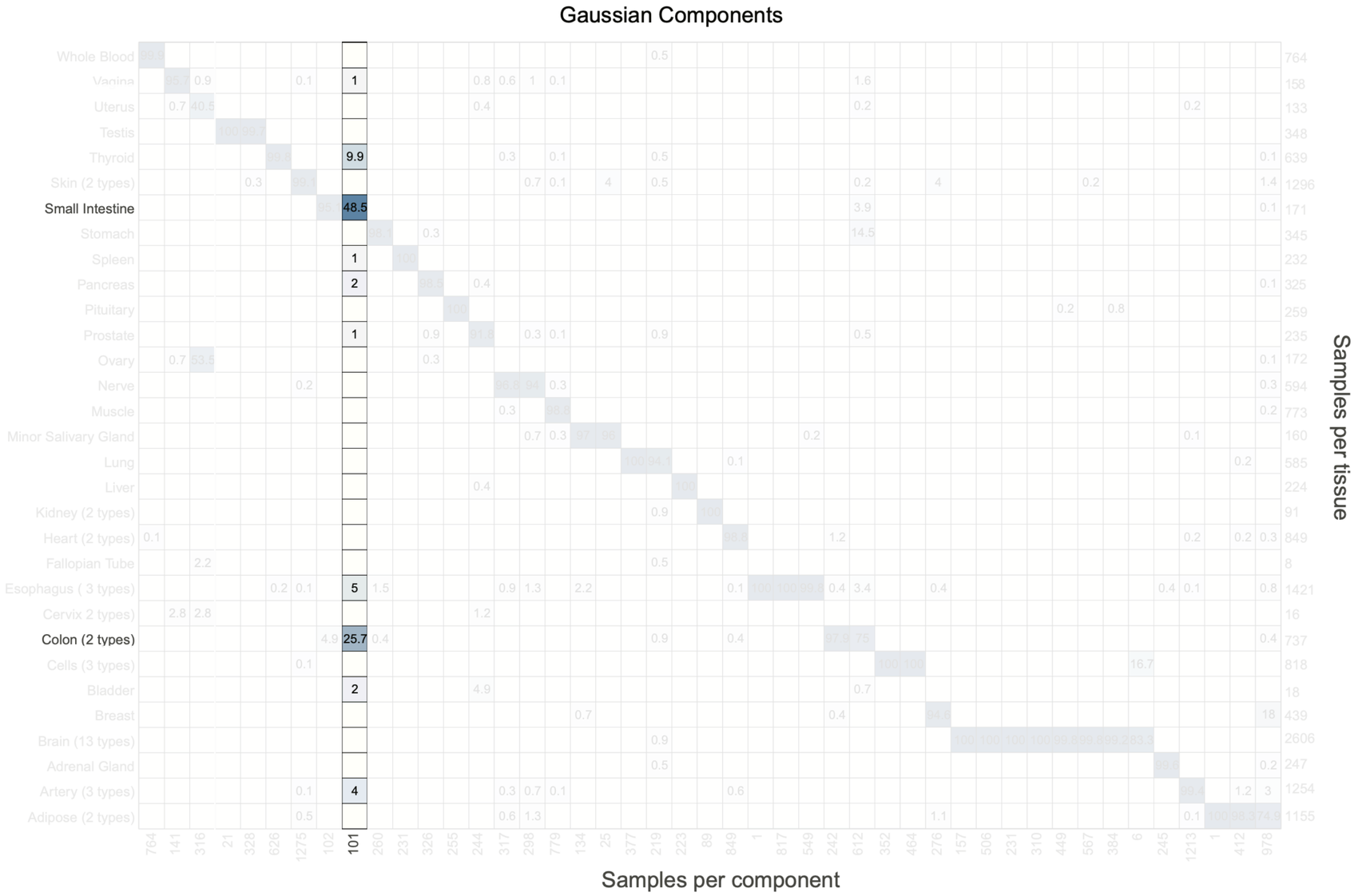

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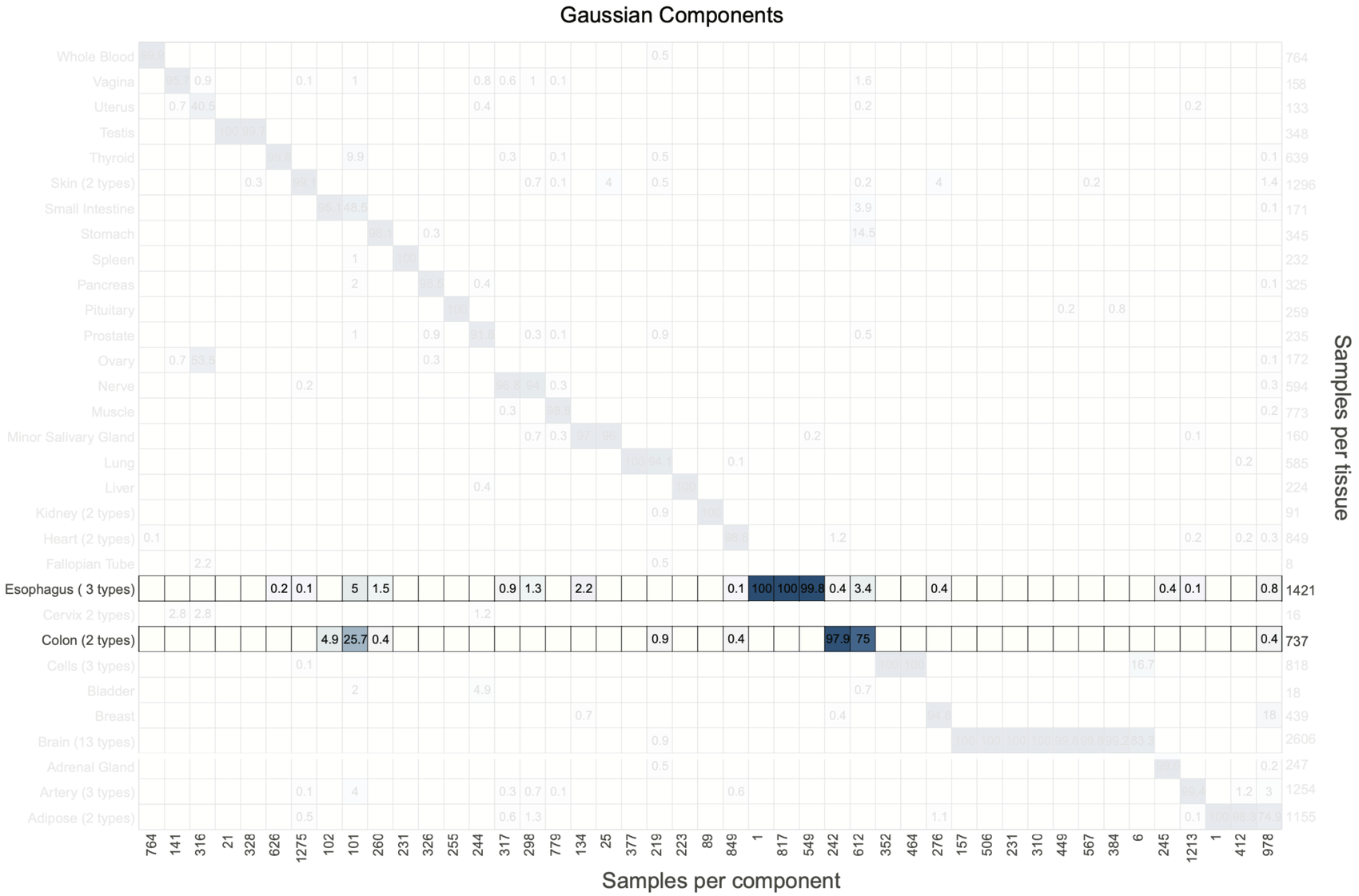

- Single tissue dominates most of components
- Tissues are divided according to biological functions

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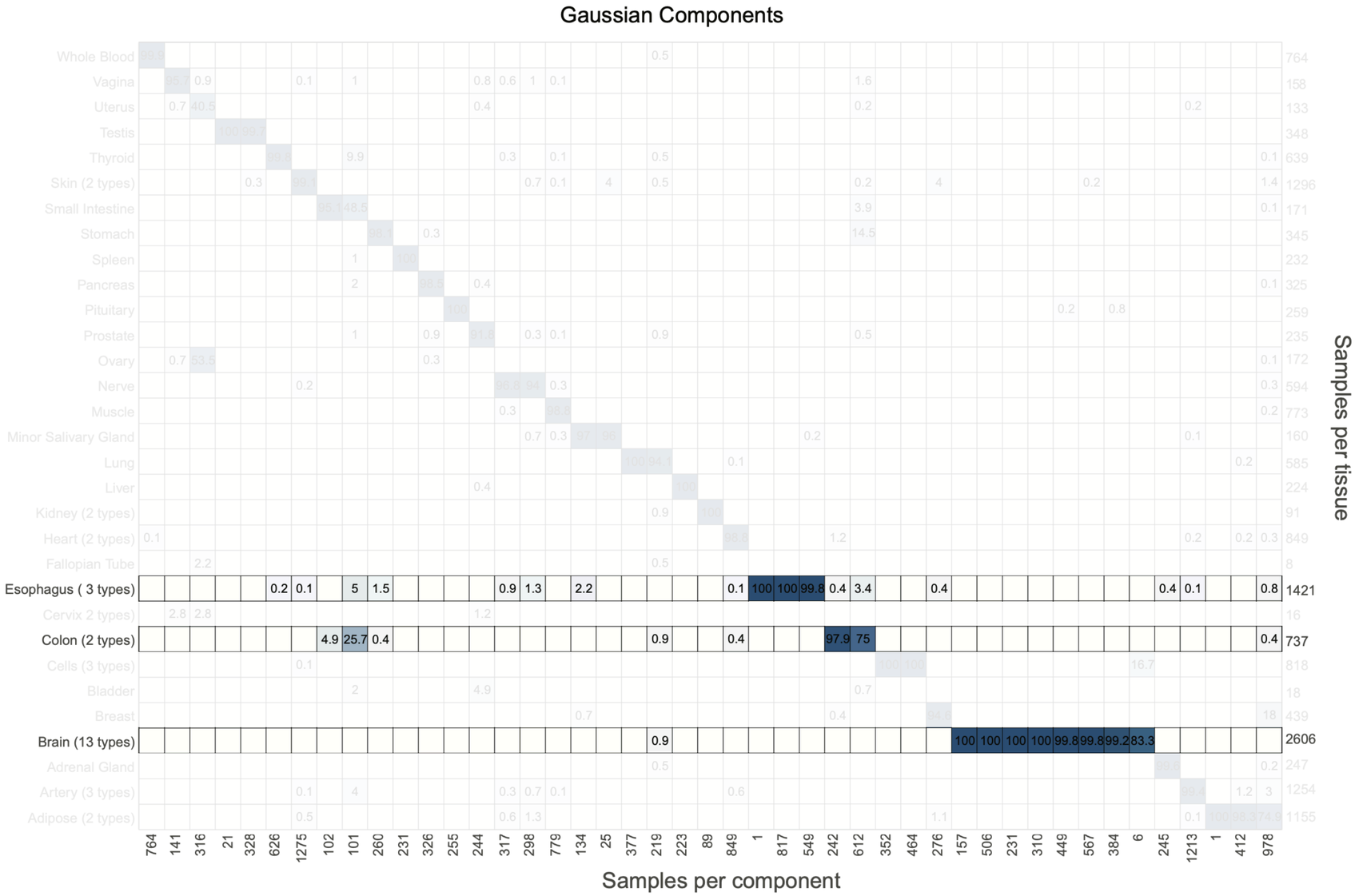

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this is very nice but what can you do with it?

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  - Create *in silico samples* **Often we have no good controls!**

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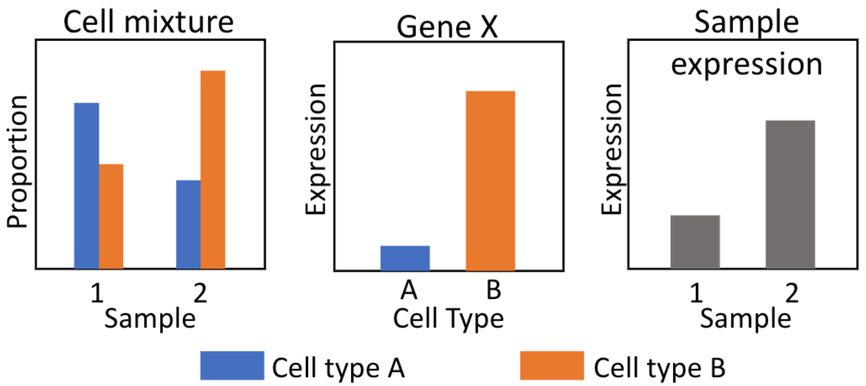
      - Samples from other individuals are genetically different
      - In cancer, adjacent normal samples may resemble disease
    - Even if you have a good control
      - Likely to have cell-to-cell differences



      - This leads to biased differential expression estimates

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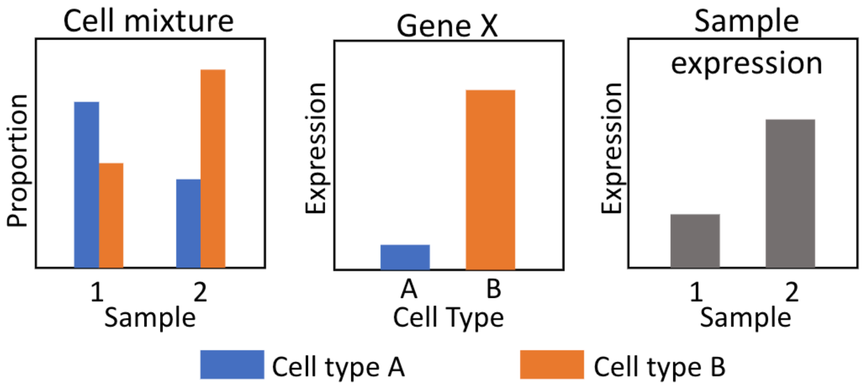
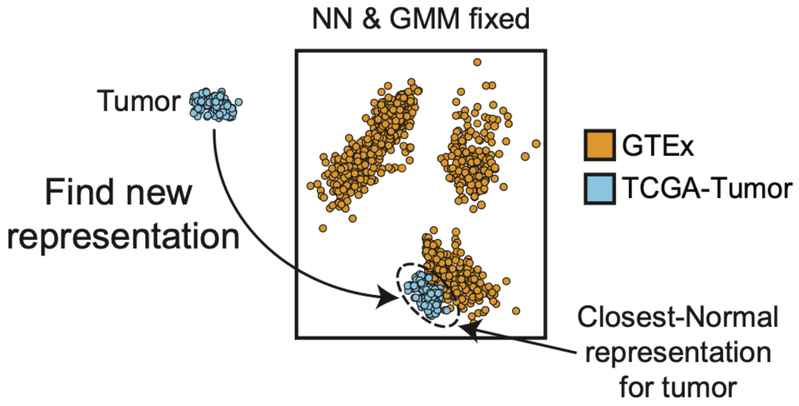
      - Samples from other individuals are genetically different
      - In cancer, adjacent normal samples may resemble disease
    - Even if you have a good control
      - Likely to have cell-to-cell differences



      - This leads to biased differential expression estimates
  - Find comparison tissues and tissues of origin for cancer samples

Finding the ideal comparison sample

What is a good comparison sample?



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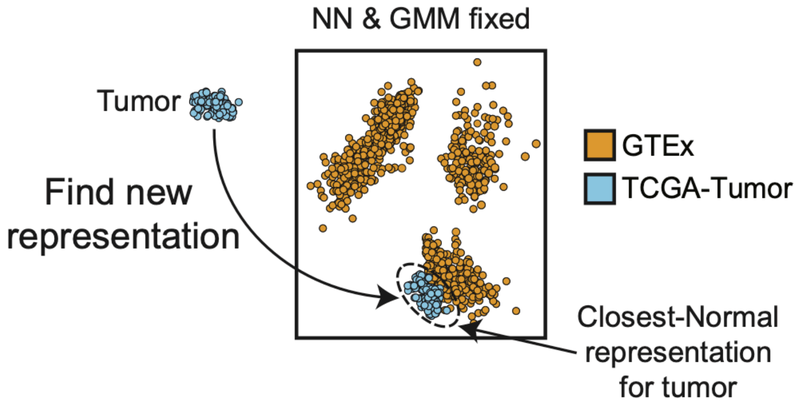

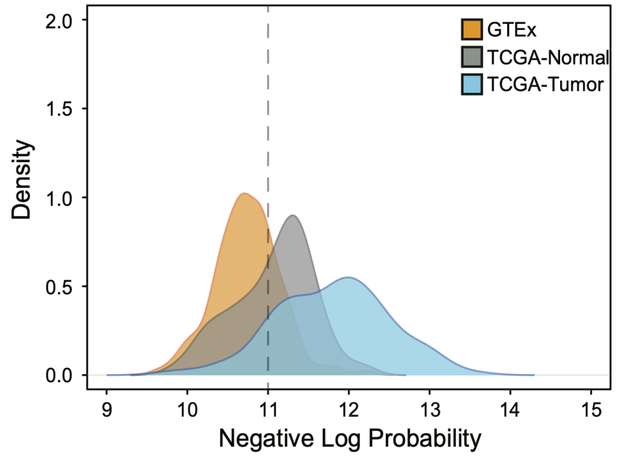

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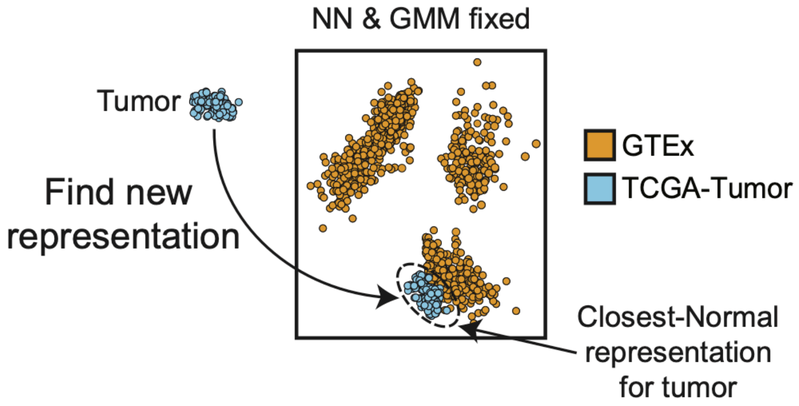

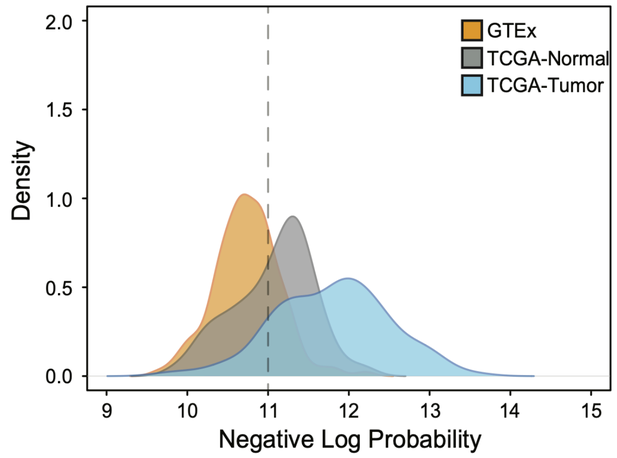

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Thyroid Carcinoma
Stomach Adenocarcinoma
Prostate Adenocarcinoma
Lung Adenocarcinoma
Liver Hepatocellular Carcinoma
Kidney Renal Papillar Cell Carcinoma
Kidney Renal Clear Cell Carcinoma
Kidney Chromophobe
Esophageal Carcinoma
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Breast Invasive Carcinoma
Brain Lower Grade Glioma
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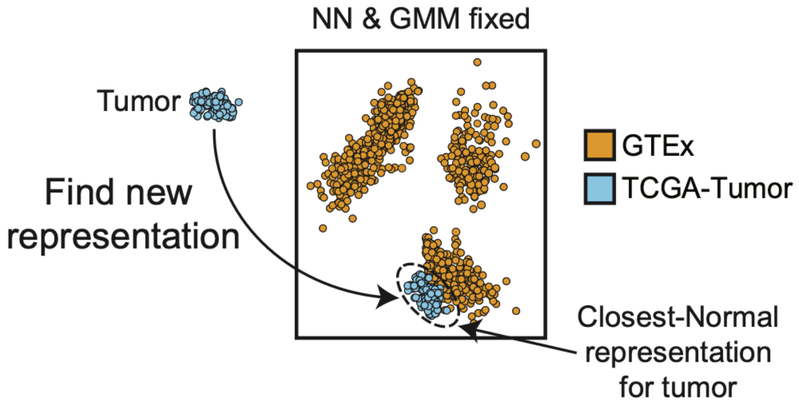

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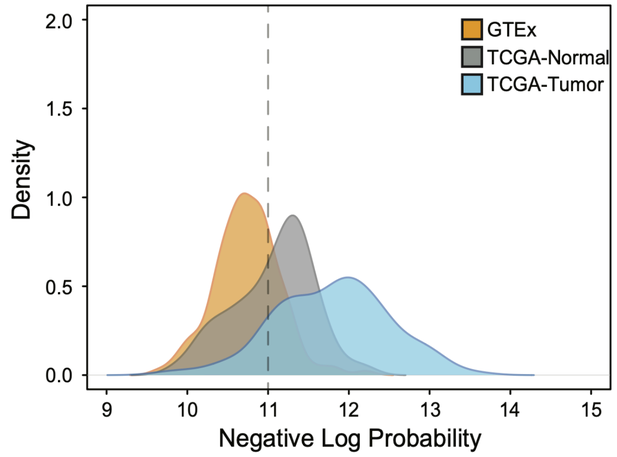

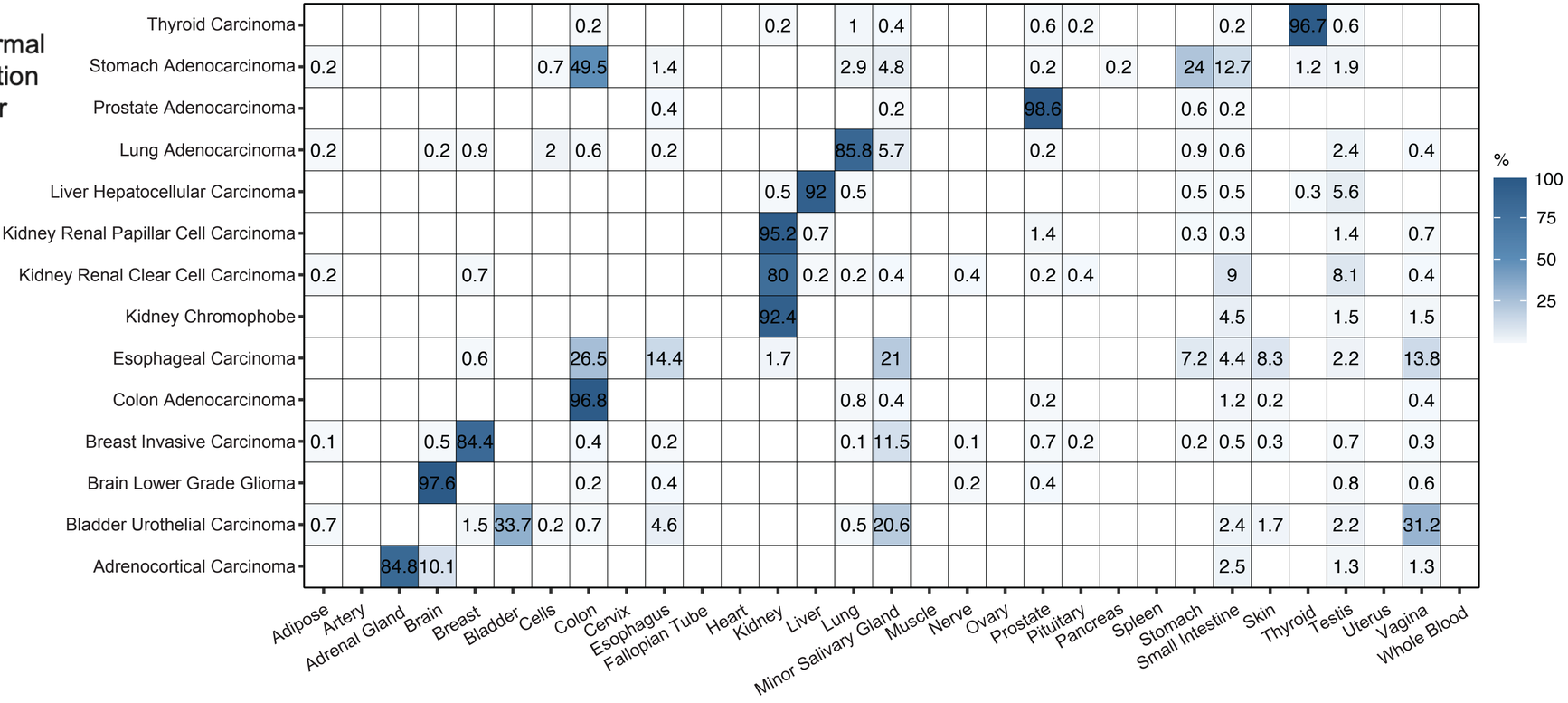

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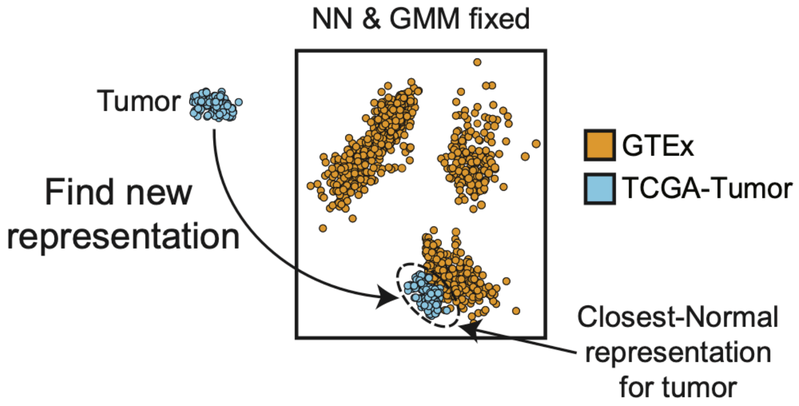

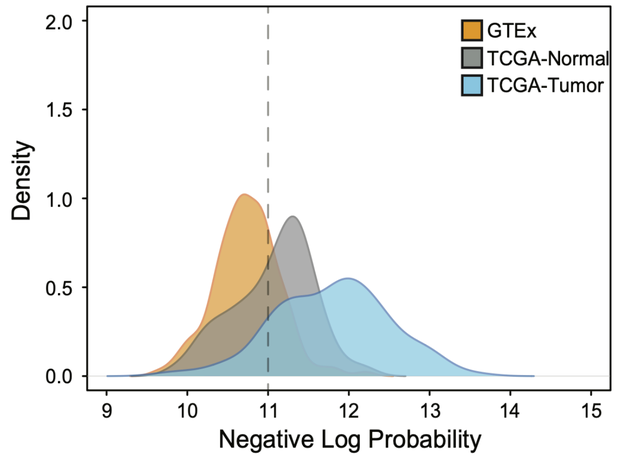

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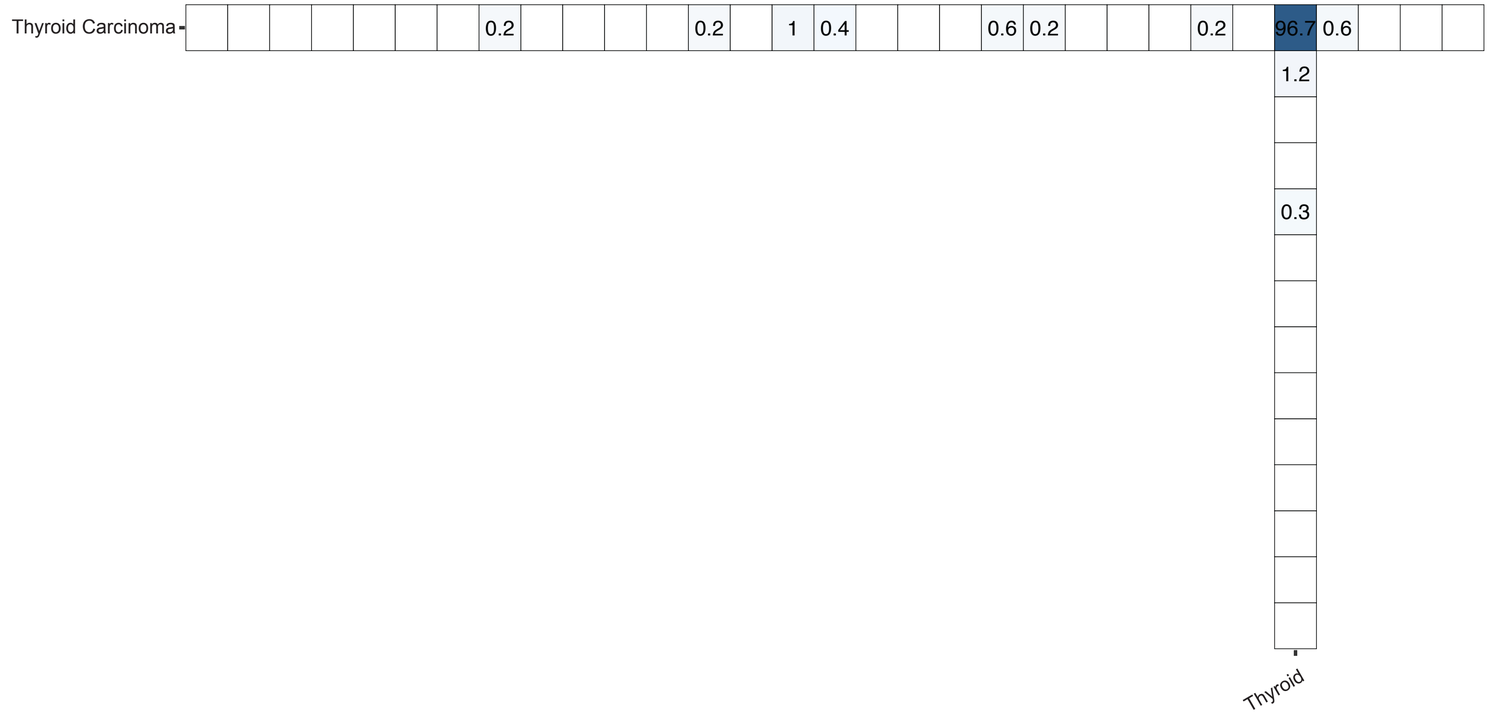

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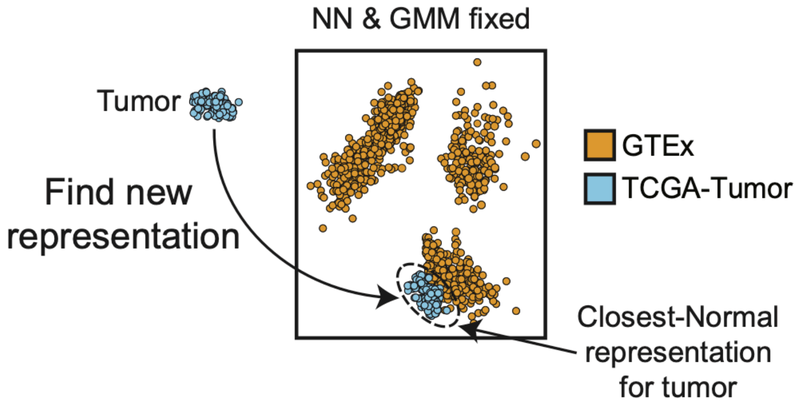

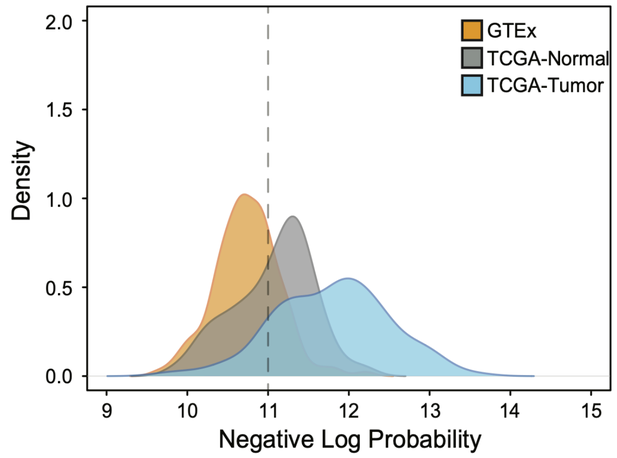

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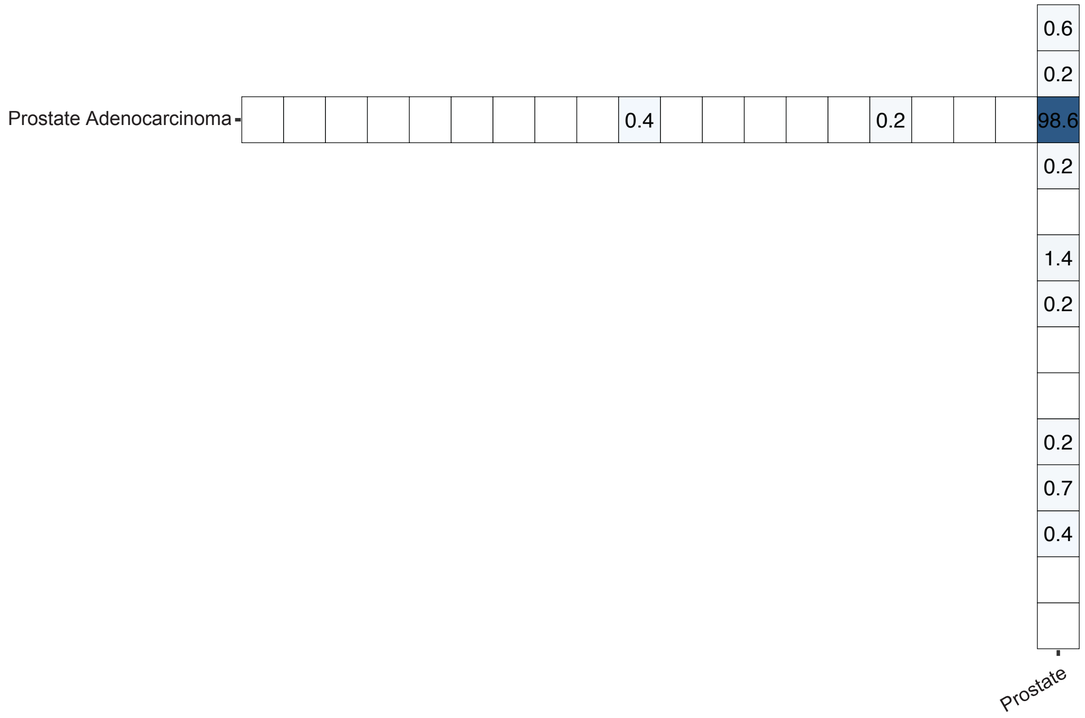

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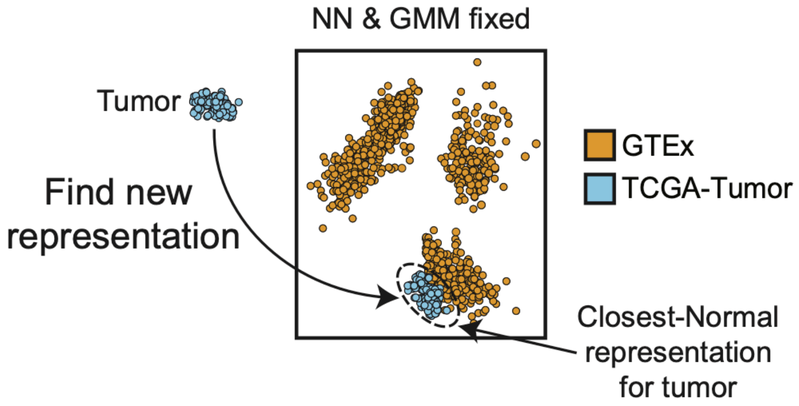

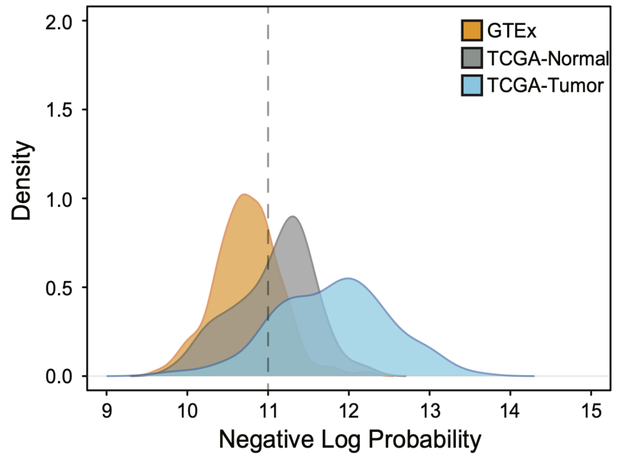

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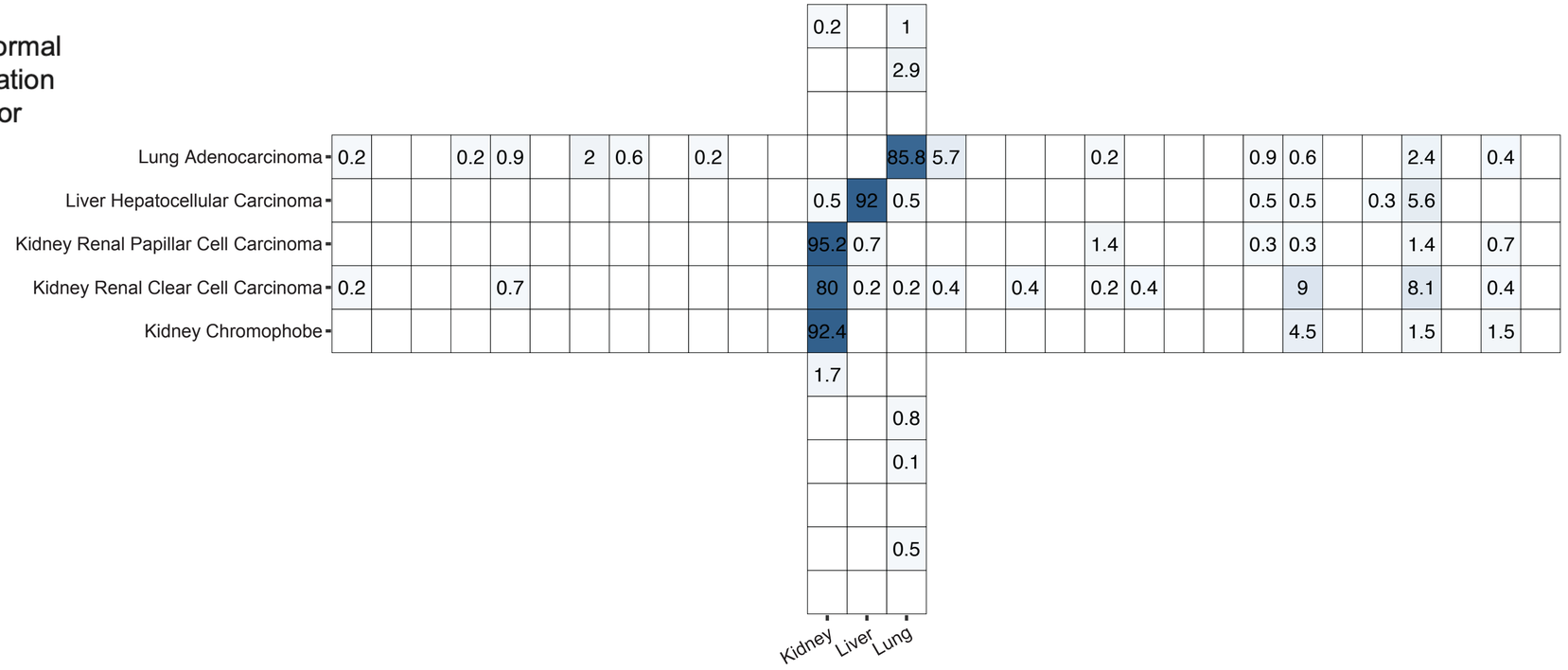

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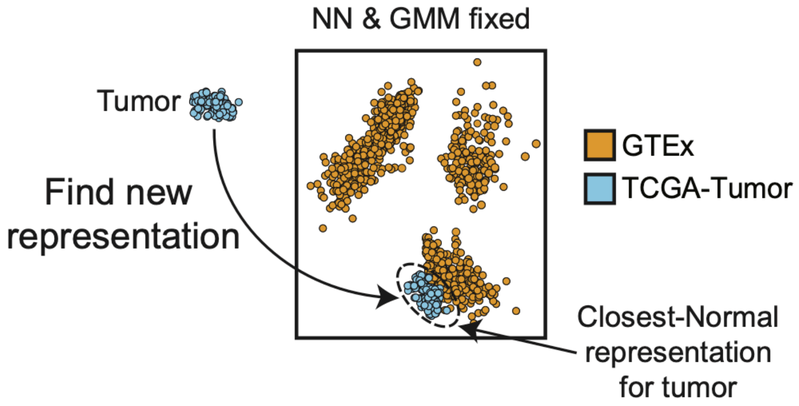

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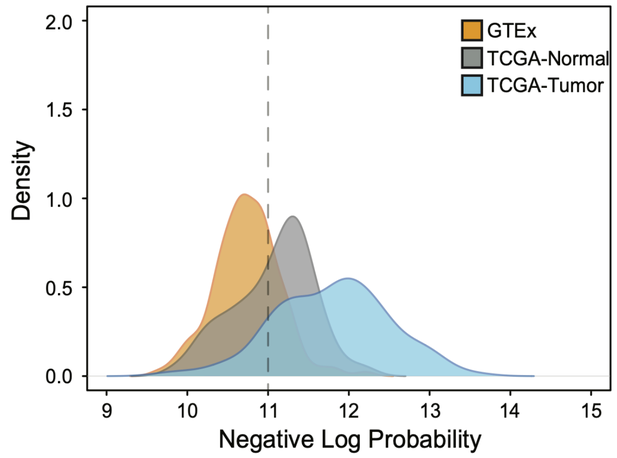
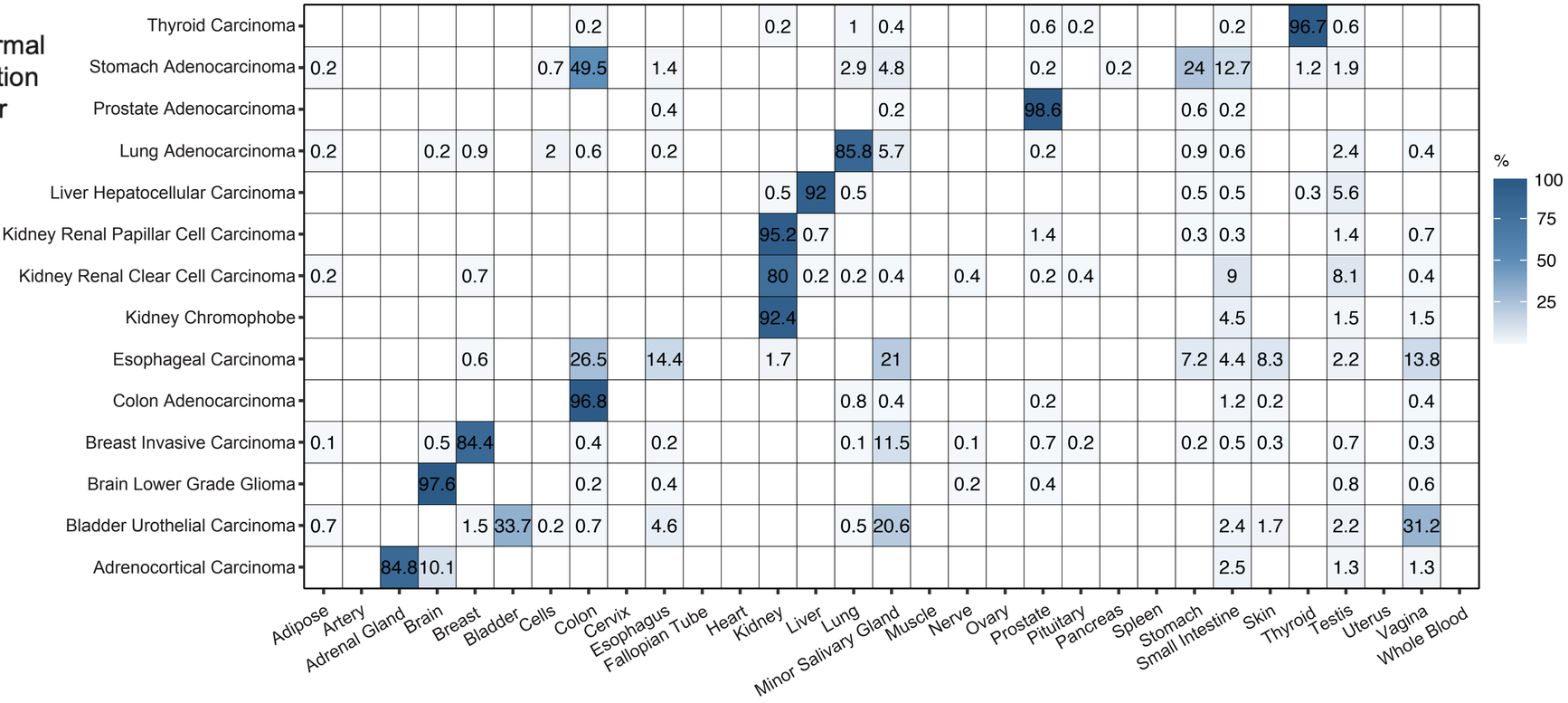


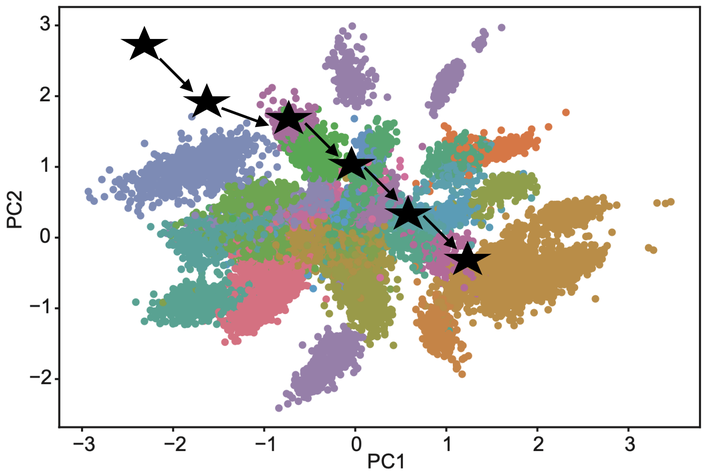


The model finds adequate *in silico* comparison samples

A new way of finding differentially expressed genes?

- Standard set-up: *N* cases vs *N* controls
  - Samples and cases are hard to find.
  - Analysis suffers from low sample sizes
  - Selection of good controls is critical
- We propose: Deep generative model vs single patient
  - Train a generative model on large-scale bulk RNA-seq
  - *Generate* the control you need for you disease sample
  - Allows for personalised analysis in an *N-of-one* manner

A new way of finding differentially expressed genes?



★ Most likely representation

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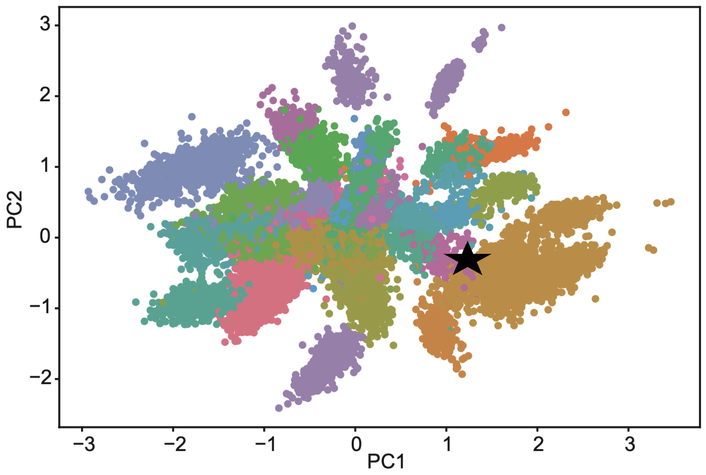

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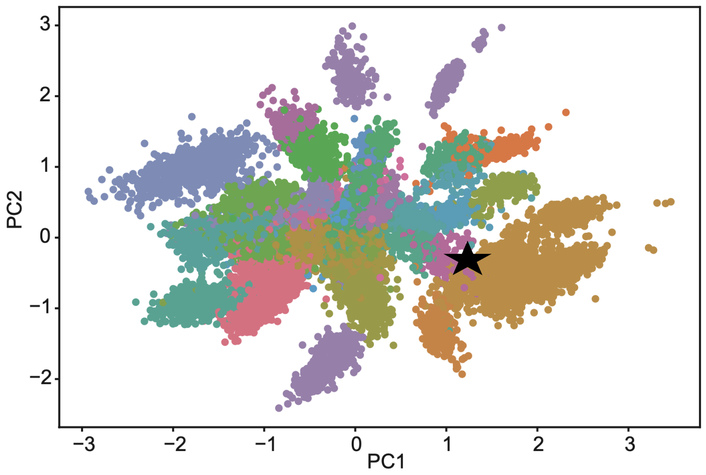

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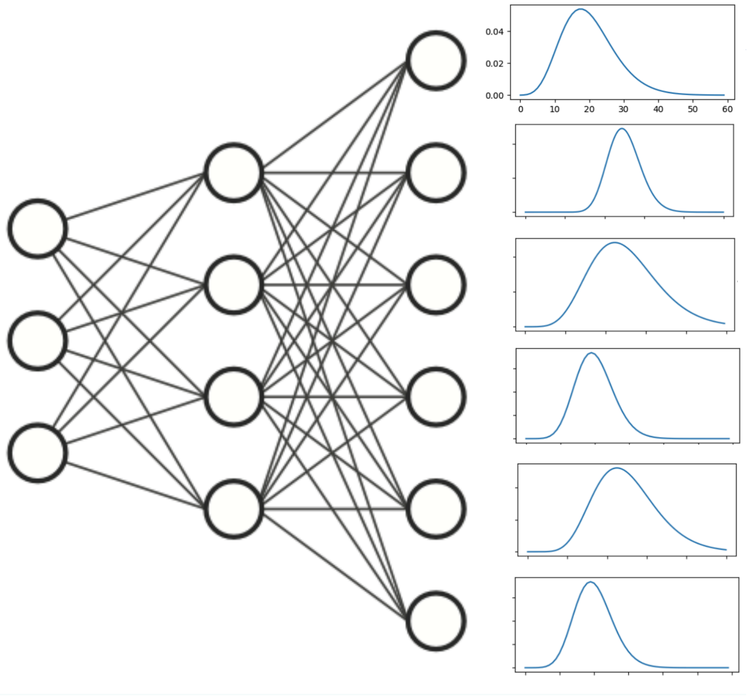

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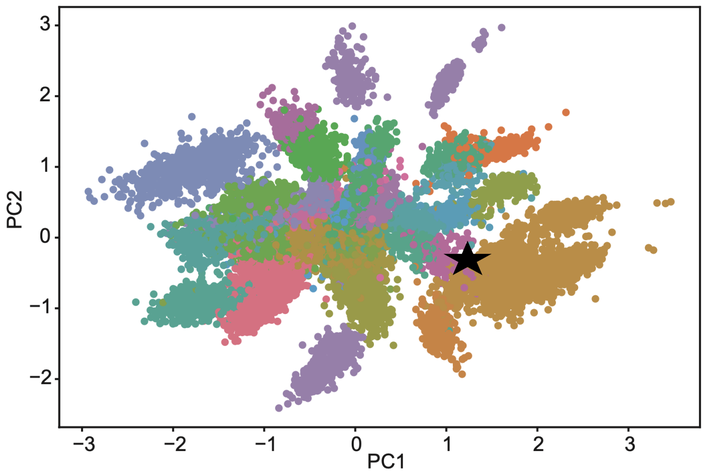

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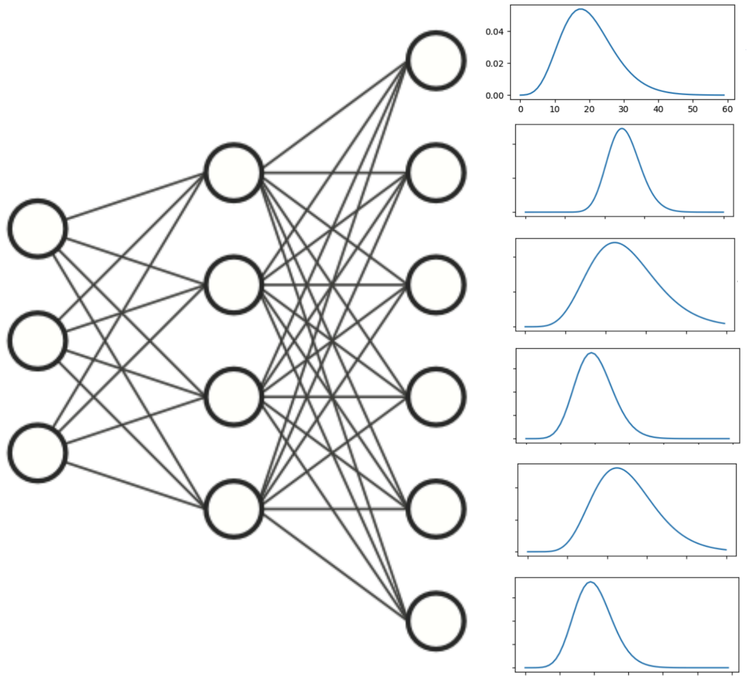
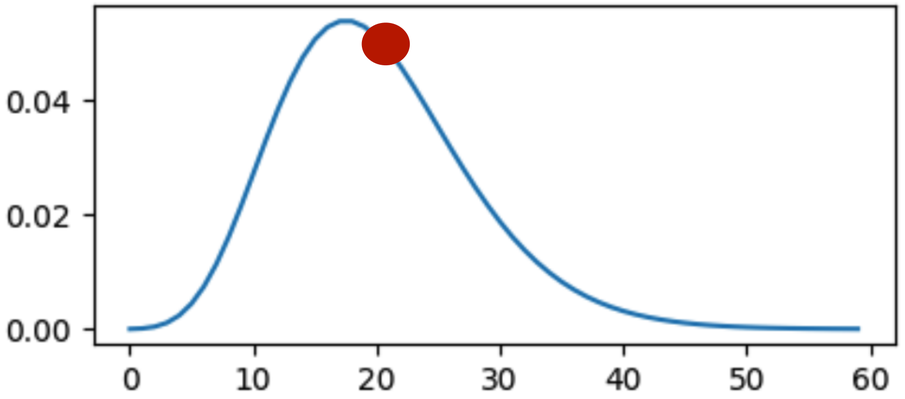


Gene of interest



Observed count

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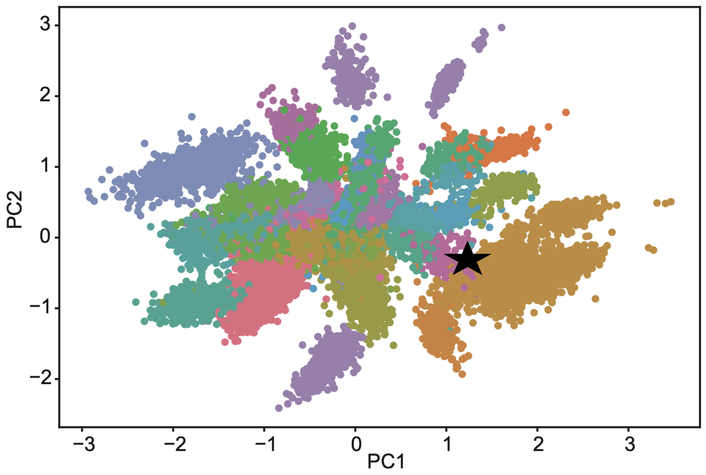

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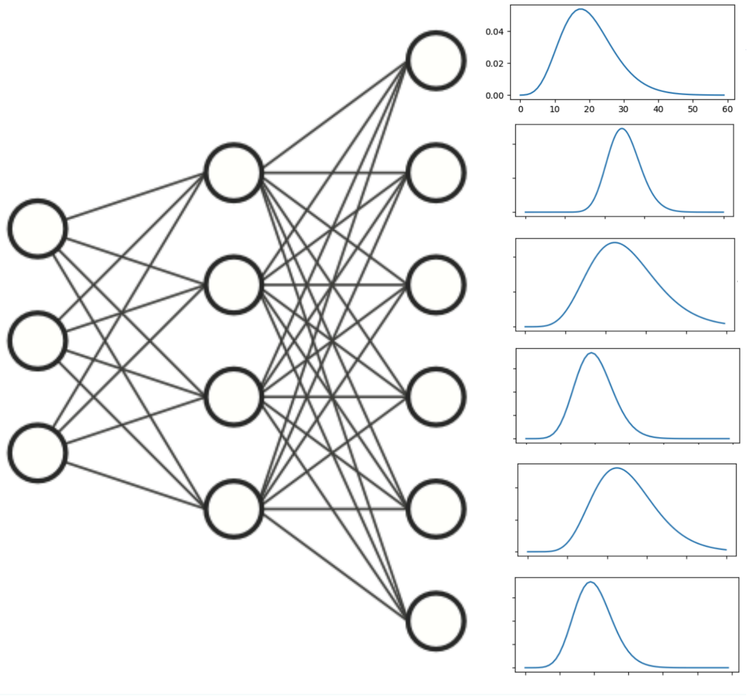

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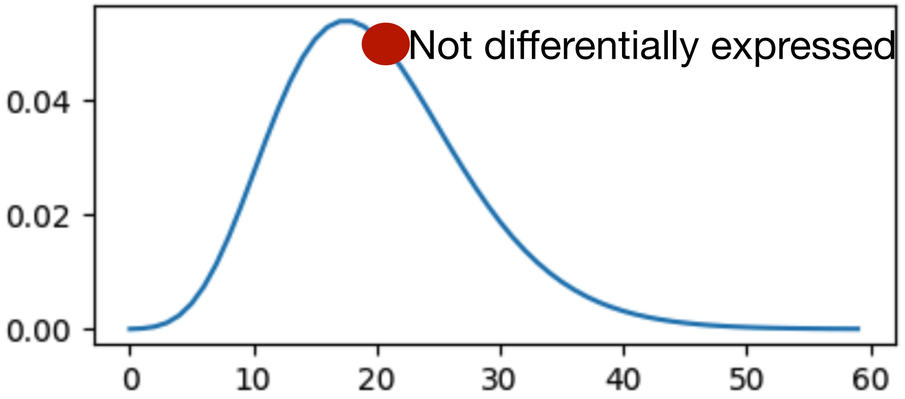

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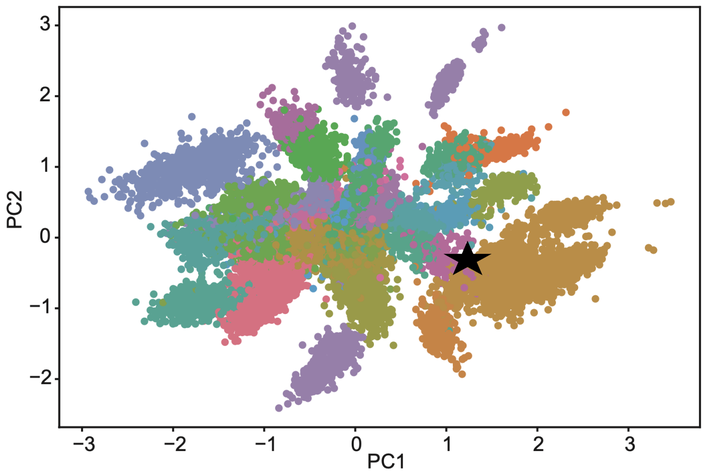

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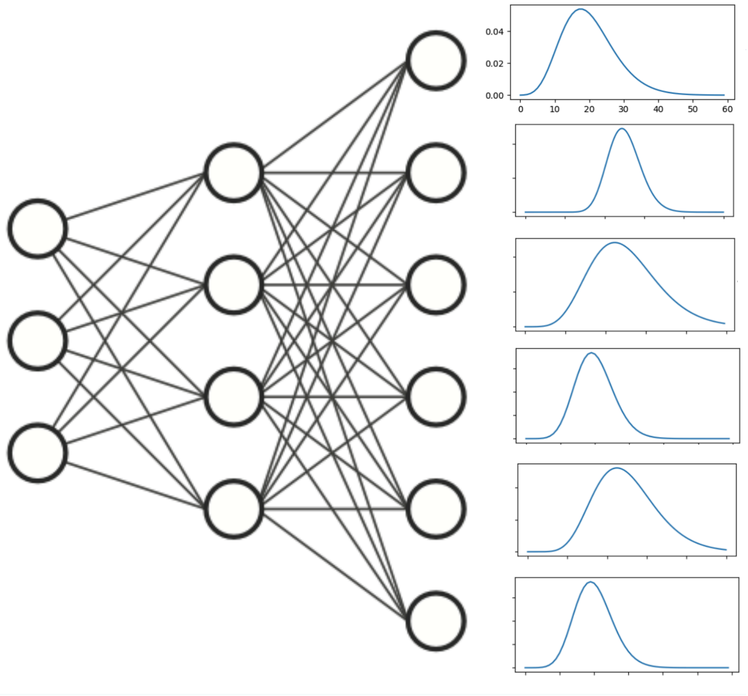

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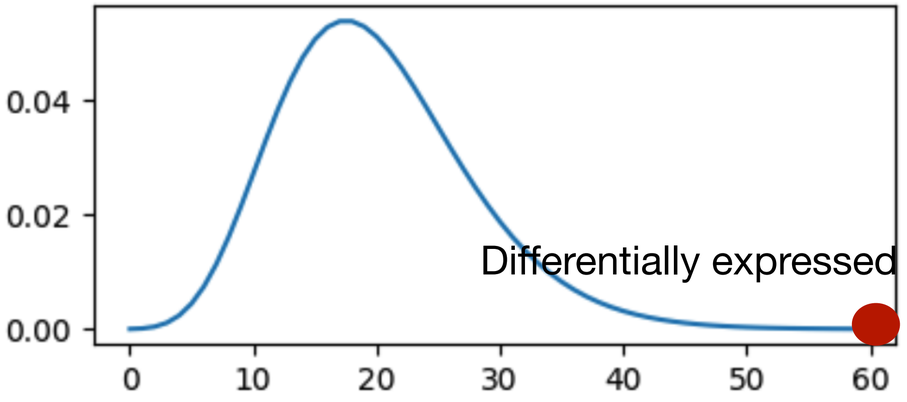

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Can we revisit gene expression?

Experiment:
1-Select a **single** cancer sample
2-Compare to 5 controls or DGD
3-Repeat 30 times

Calling differential expression:
Negative binomial test between closest-normal sample and tumor sample

Methods

- DEseq2
- DGD

Data

Healthy vs healthy:
GTEx breast

Healthy vs cancer:
GTEx breast
TCGA-Tumor

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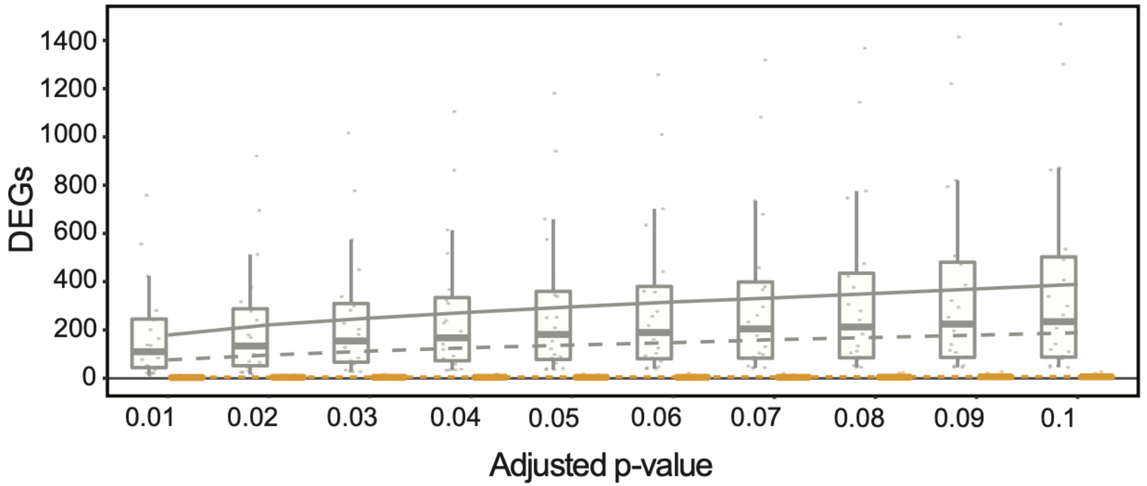

Healthy vs healthy:
Proxy to specificity

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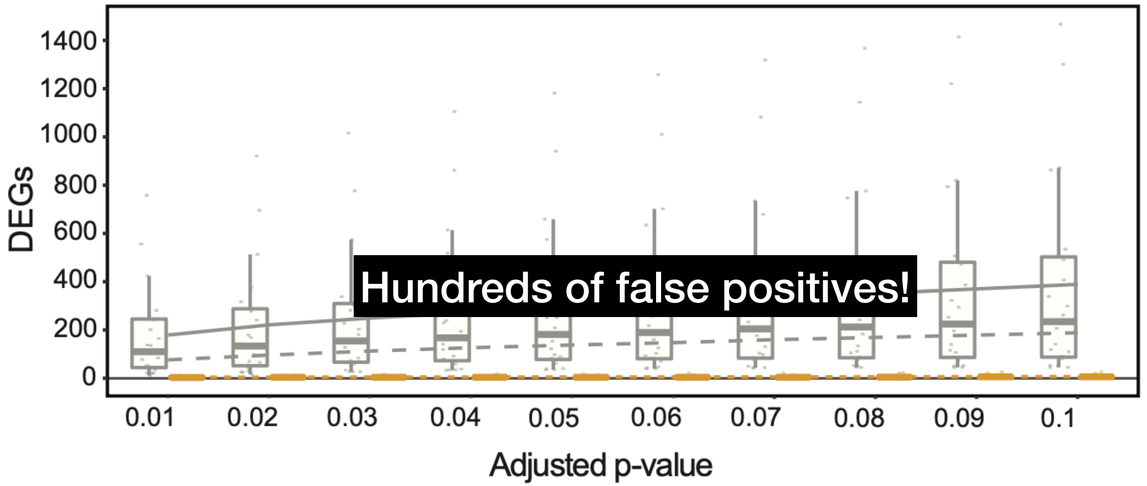

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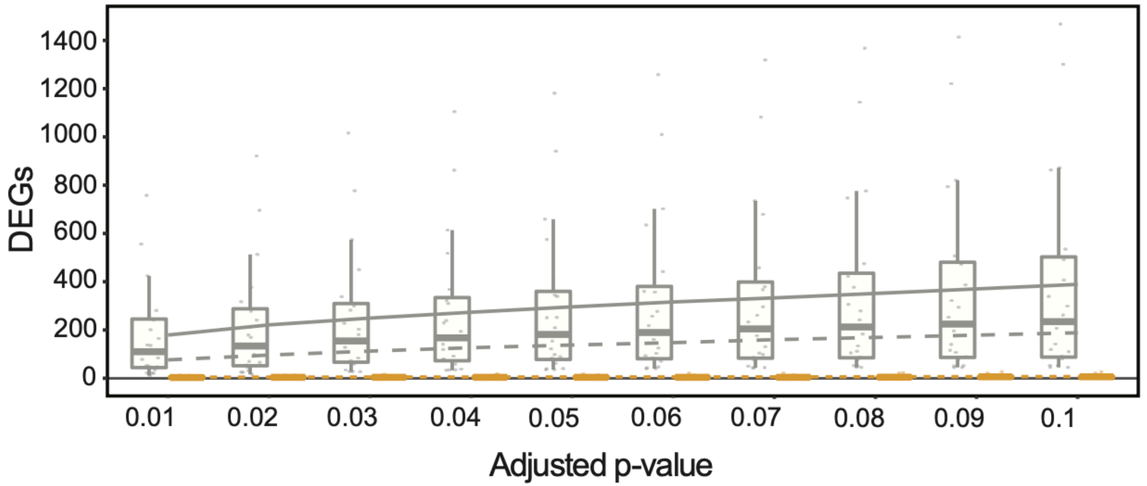

Healthy vs healthy:
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Healthy vs cancer on marker genes
Proxy to sensitivity

Marker gene sets
DriverDB
PAM50

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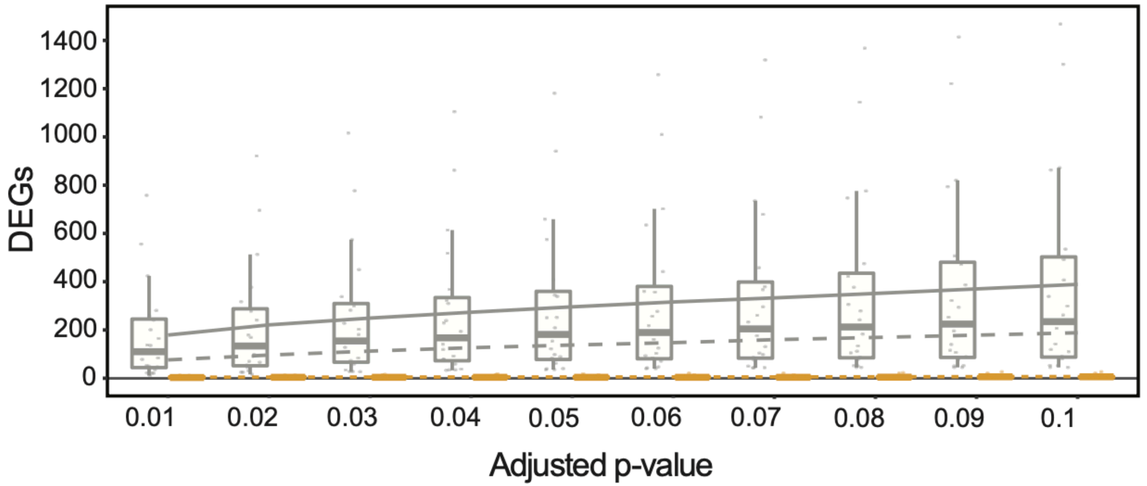

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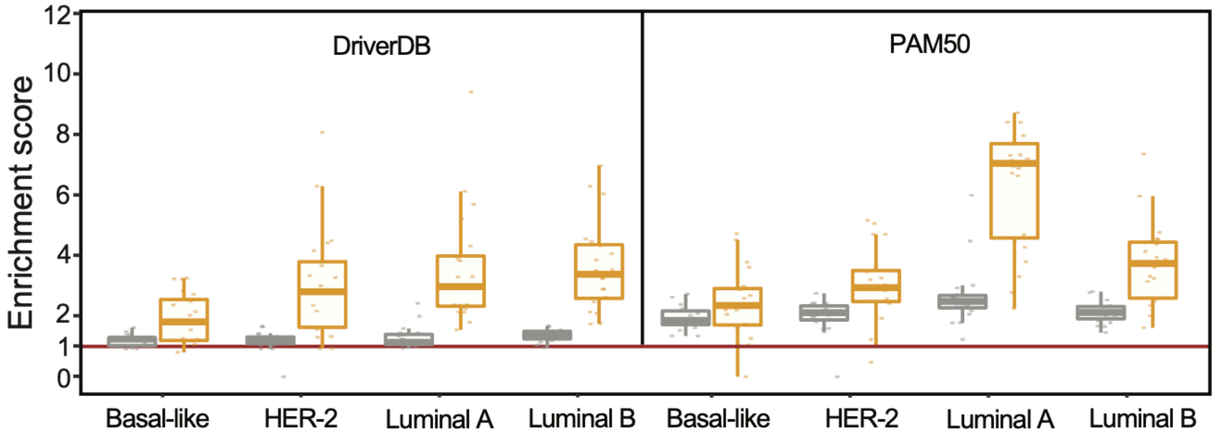

Score: $\frac{\text{Fraction of marker significant genes}}{\text{Fraction of all significant genes}}$

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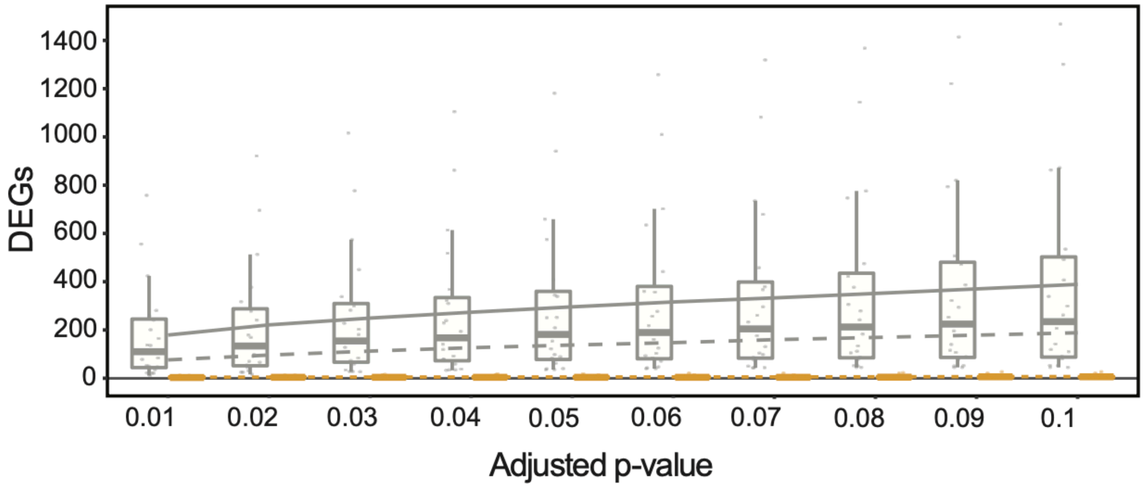

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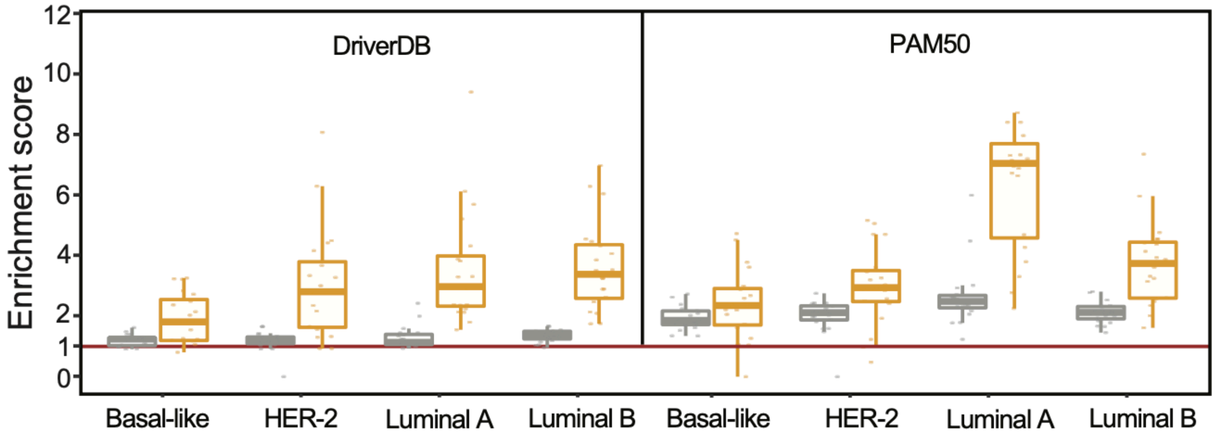

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DGD has a higher sensitivity, while keeping false positives low

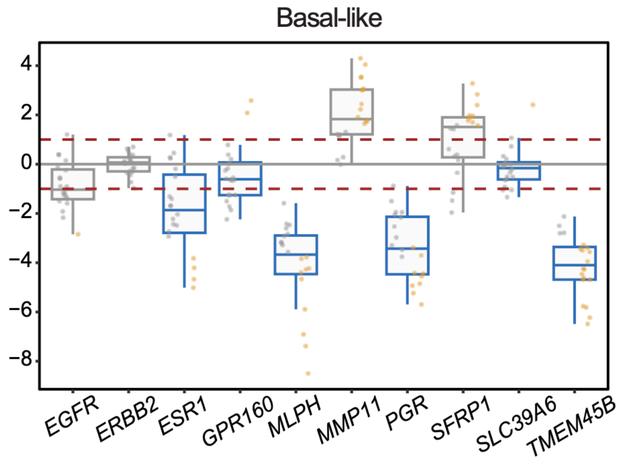
Zooming in breast cancer

Can we find the well known breast cancer genes?

Do we correctly guess expression trends? (Up/down)

P-value < 0.05
P-value > 0.05

Upregulated
Normal expression
Downregulated



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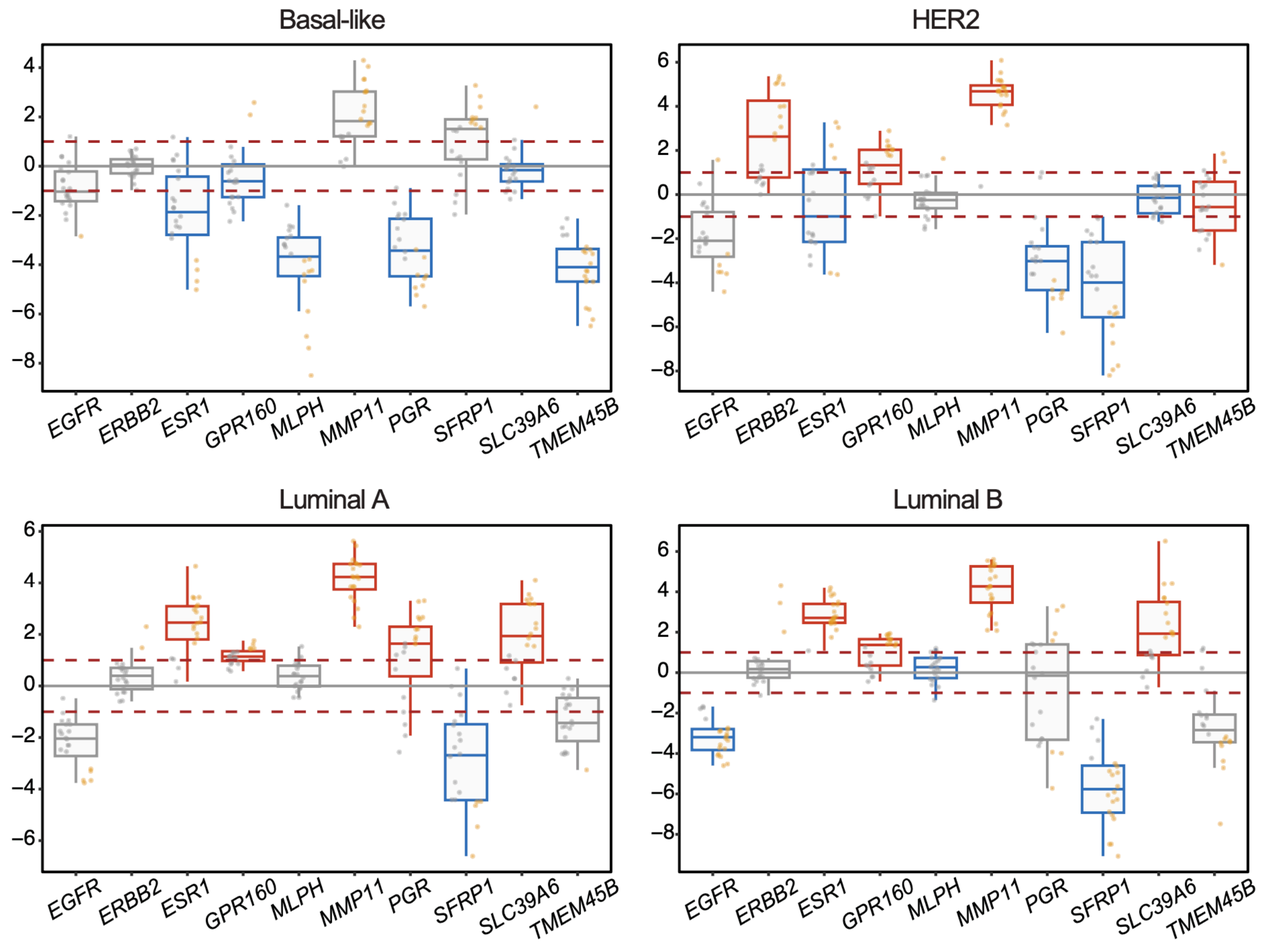
Normal expression
Downregulated



DGD finds the main patterns of gene expression across the subtypes

Do we find enrichment across cancers?

- Good performance in breast cancer
  - Better sensitivity than DEseq2
  - Dramatically low false positives
  - Very few called genes, facilitating interpretation
- How is the performance in other cancers?
  - Extend our analysis across the cancer genome atlas

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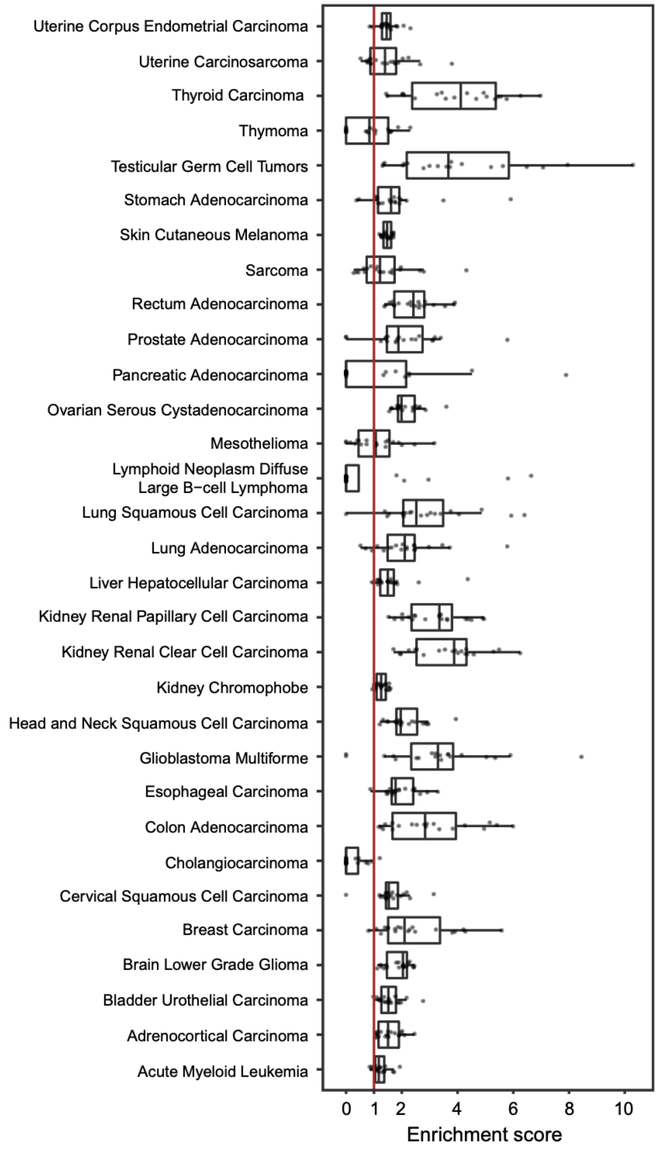


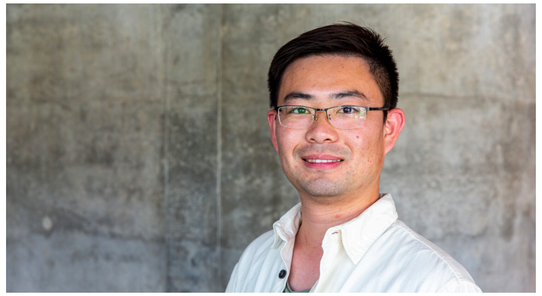
Summary and conclusions

- DGD beats the standards.
  - Note: with only 2 fully-connected layers
- Keeps the gene “haystack” small
- Stop using controls!
  - Are healthy samples useful controls?
  - Generate *in silico* samples instead

What is next?

- We wanna go to the real world and use our model
- Use cases:
  - Lets kill less mice
  - Rare diseases
  - And not also not so rare diseases

Acknowledgements



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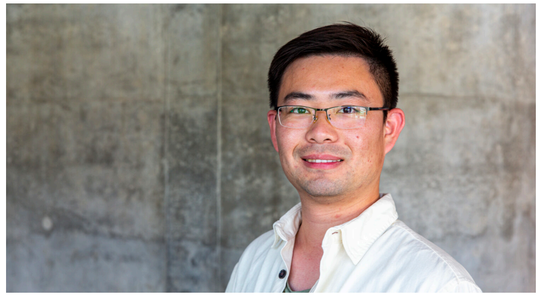

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