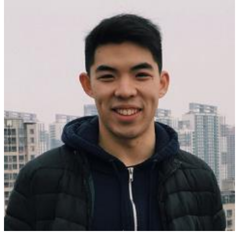
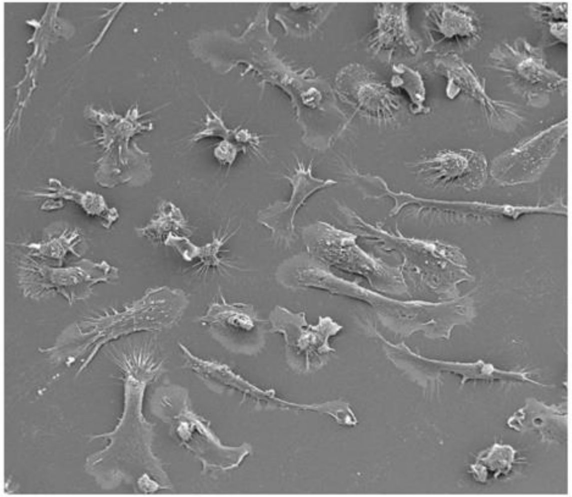
# ACE: Explaining cluster from an adversarial perspective

Yang Lu, Timothy Yu, Giancarlo Bonora, William Stafford Noble

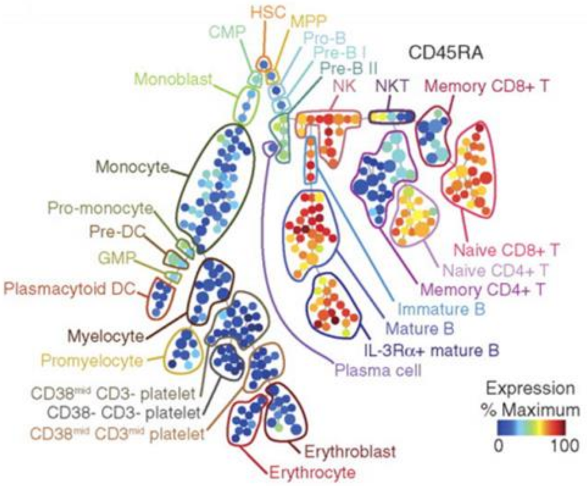


# Single-cell transcriptome profiling (scRNA-seq) is an important technique to study cellular heterogeneity

## Cellular heterogeneity

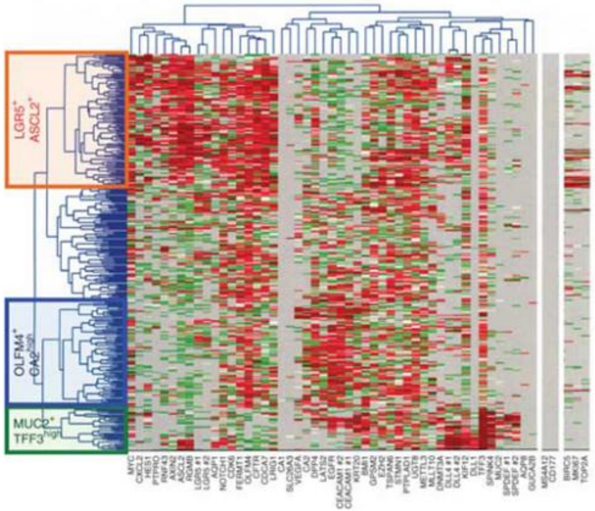


## Differentiation trajectories



Bendall et al. (2011), Science

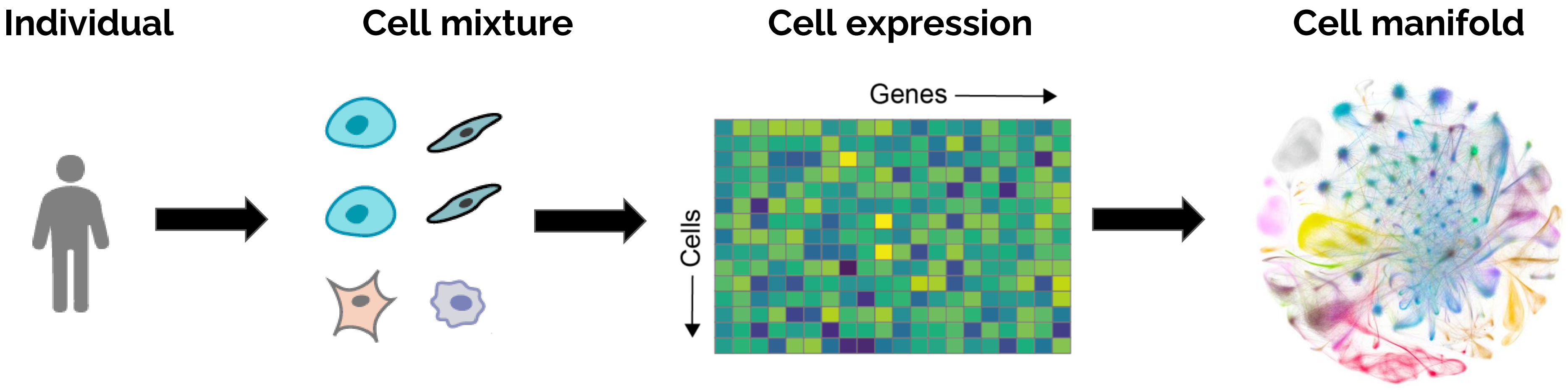
## Within-cell-type differences



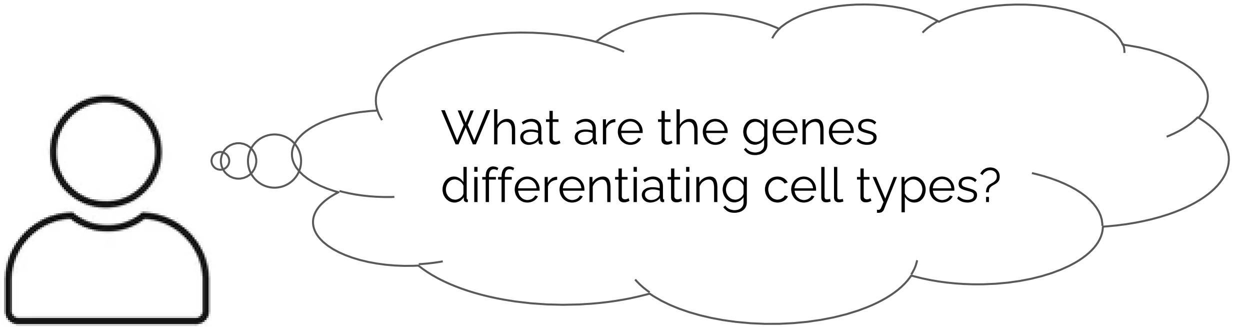
Dalerba et al. (2011), Nature Biotech

**Credit:** *Manolis Kellis, MIT*

# Analysis of scRNA-seq data often involves manifold embedding

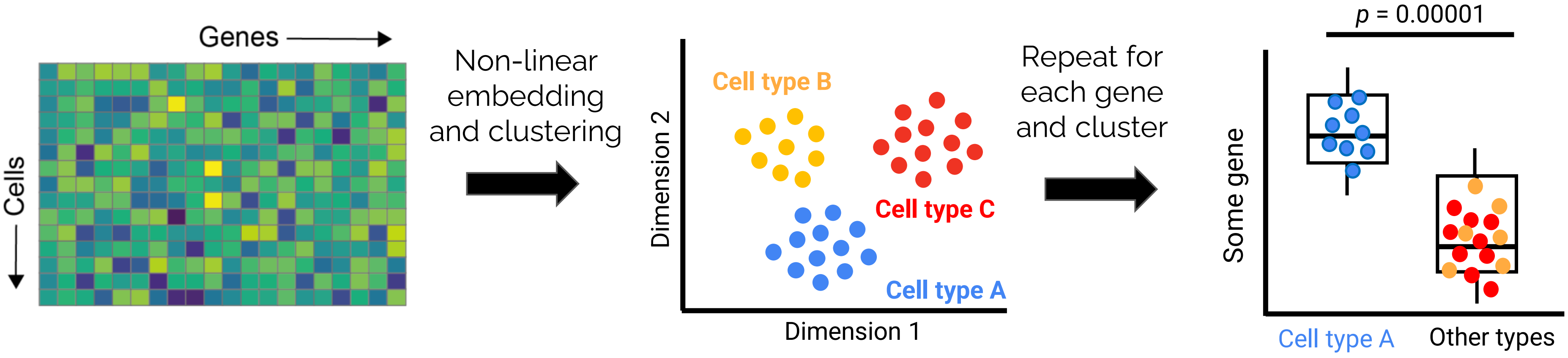


**Individual** **Cell mixture** **Cell expression** **Cell manifold**



What are the genes differentiating cell types?

# Caveats of conventional scRNA-seq differentiation analysis workflow



Non-linear embedding and clustering

Repeat for each gene and cluster

**Caveats:**

❑ The **uncertainty induced by the nonlinear embedding** is ignored

❑ The **stochasticity in cluster assignments** is ignored

❑ The **dependency among genes** is ignored

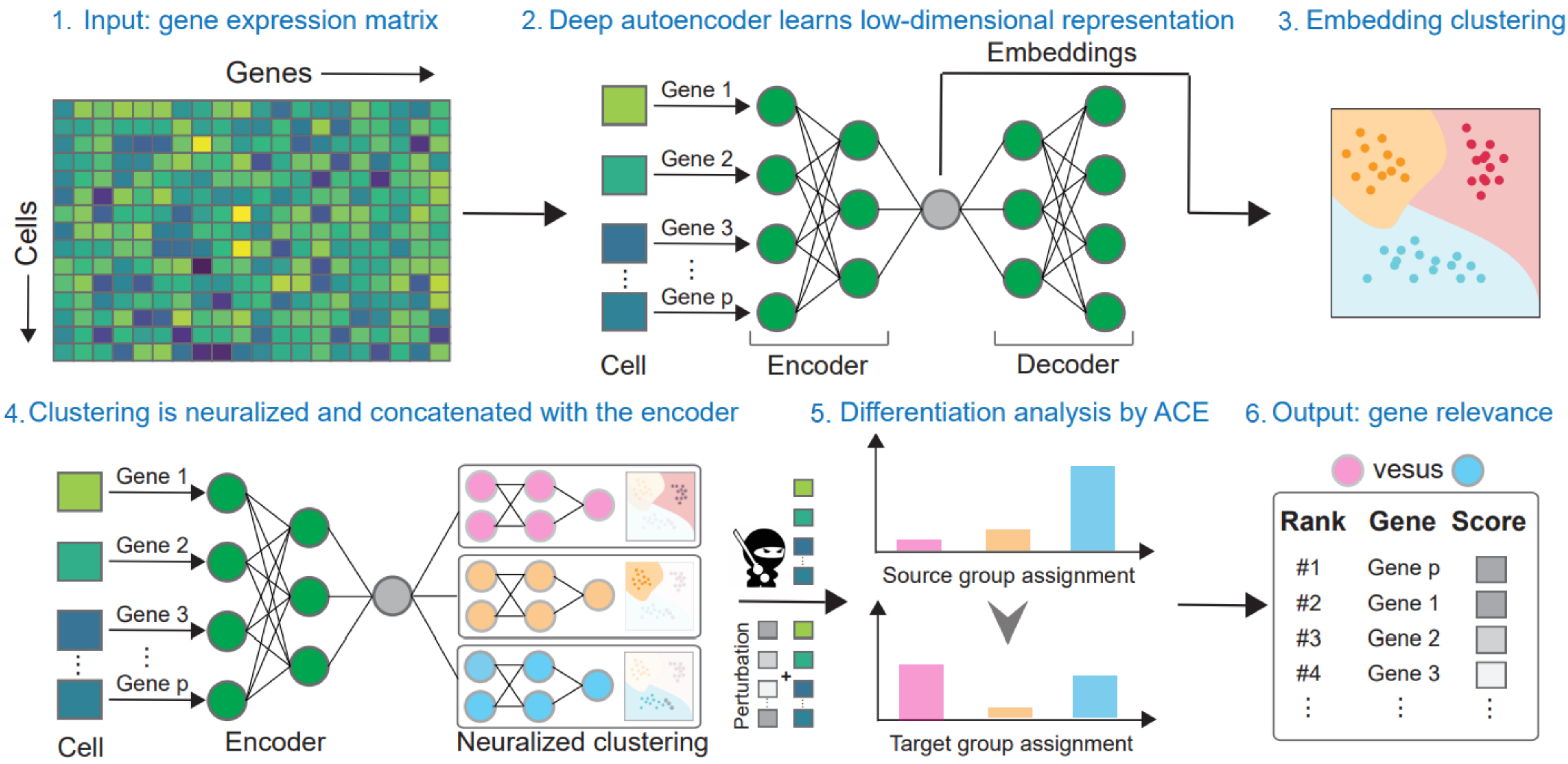
❑ **Only genes enriched in single clusters are highlighted** as the signature

# **A**dversarial **C**lustering **E**xplanation (**ACE**) overcomes limitations of existing methods

| | ACE | DESeq2 | Jensen-Shannon Distance (Monocle 3) | Global Counterfactual Explanation | Gene Relevance Score |
|---|---|---|---|---|---|
| Dependency among genes | ✓ | ✗ | ✗ | ✓ | ✓ |
| Uncertainty by the nonlinear embedding | ✓ | ✗ | ✗ | ✗ | ✓ |
| Stochasticity in cluster assignments | ✓ | ✗ | ✗ | ✗ | ✗ |
| Does not limit to enriched genes | ✓ | ✗ | ✗ | ✓ | ✓ |
| Additional limitations | NA | NA | NA | Limited to linear transformation | Limited to diffusion maps |

*Love et al, Genome Biology (2014)*
*Cao et al, Nature (2019)*
*Plumb et al, ICML (2020)*
*Angerer et al, Bioinformatics (2020)*

# ACE aims to jointly explain the embedding and clustering



1. Input: gene expression matrix

2. Deep autoencoder learns low-dimensional representation

3. Embedding clustering

4. Clustering is neuralized and concatenated with the encoder

5. Differentiation analysis by ACE

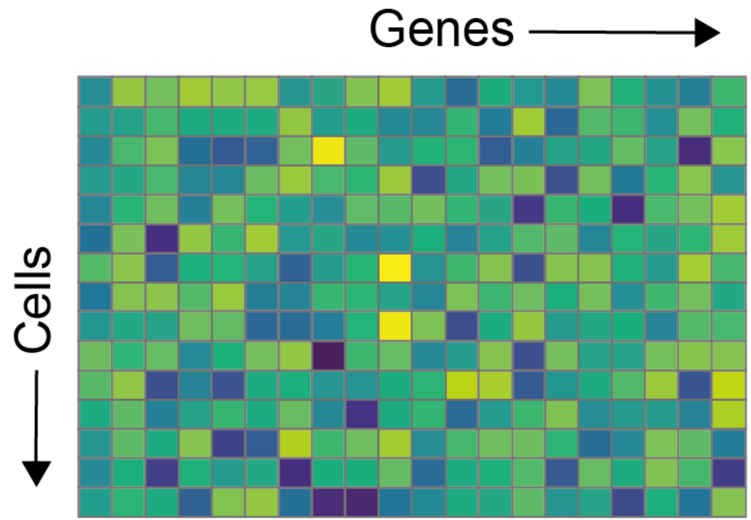
6. Output: gene relevance

| Rank | Gene | Score |
|---|---|---|
| #1 | Gene p | ■ |
| #2 | Gene 1 | ■ |
| #3 | Gene 2 | ▨ |
| #4 | Gene 3 | □ |
| ⋮ | ⋮ | ⋮ |

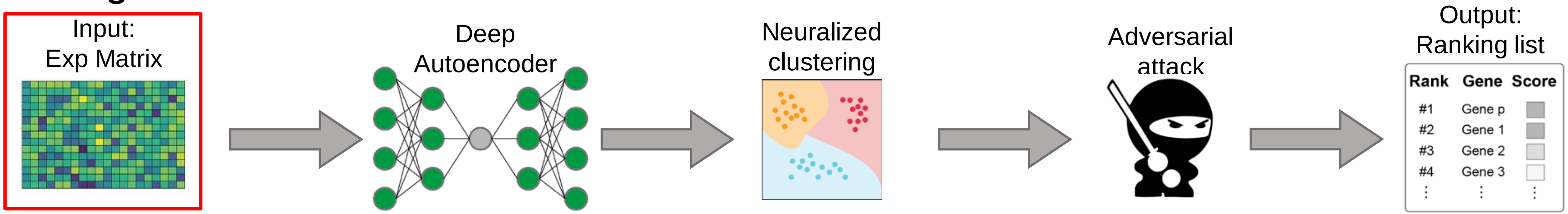
# ACE takes as input the expression matrix and a pre-specified number of clusters

**Input:**

- ❑ The scRNA-seq expression matrix
- ❑ The specified cluster number **k**



**At a high level:**



Input:
Exp Matrix

Deep
Autoencoder

Neuralized
clustering

Adversarial
attack

Output:
Ranking list

| Rank | Gene | Score |
|---|---|---|
| #1 | Gene p | ▨ |
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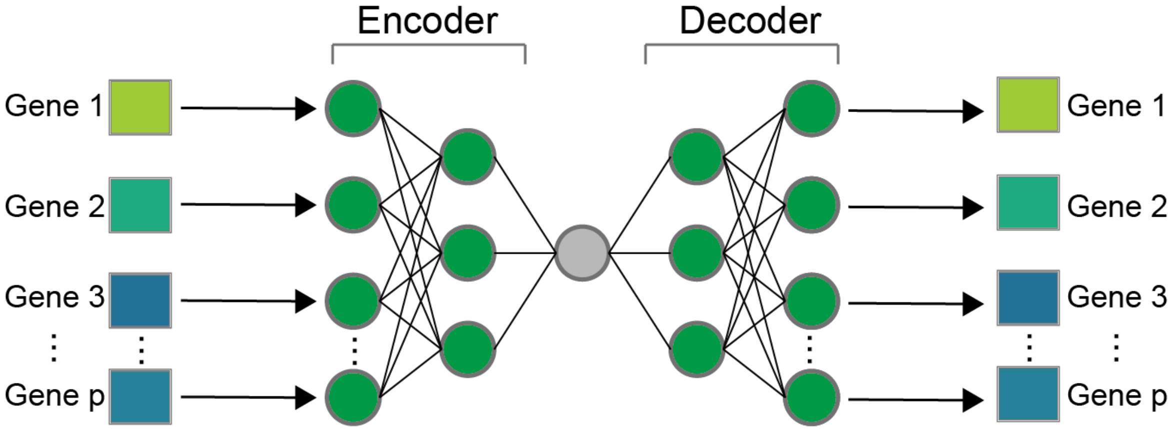
# ACE projects the expression data into a low-dimensional embedding using a deep autoencoder

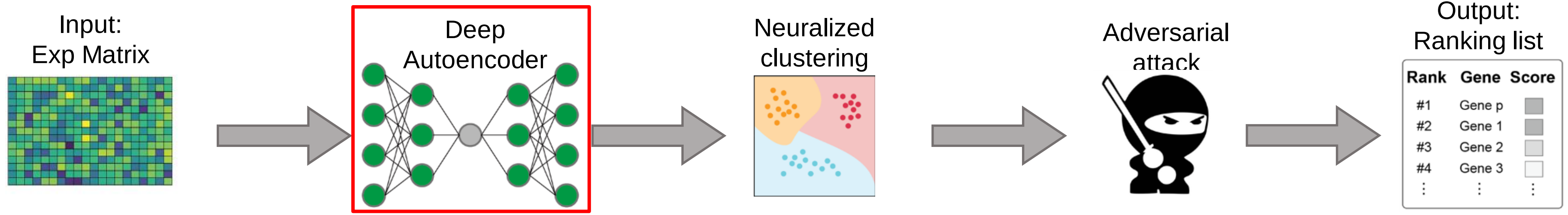
**Embedding by Autoencoder:**

- ❑ Dimension reduction similar to UMAP, t-SNE, or PCA
- ❑ Batch correction
- ❑ Applicable to any scRNA-seq embedding method



*Amodio et al, Nature Methods (2019)*

**At a high level:**



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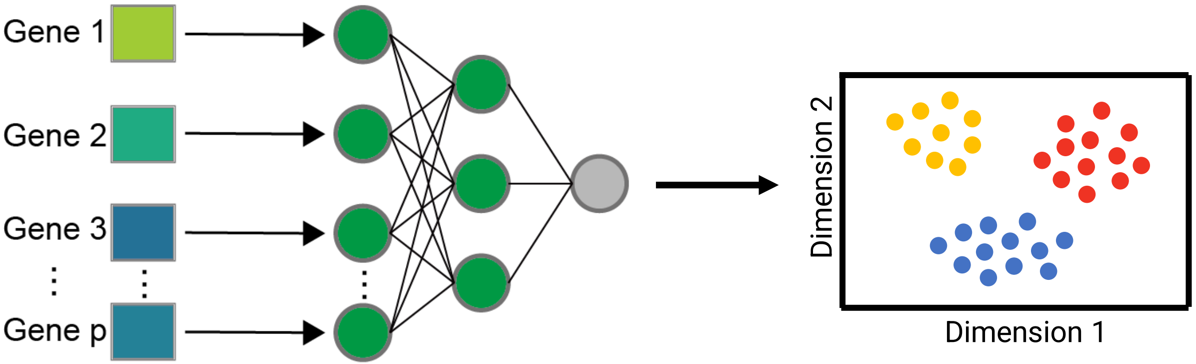
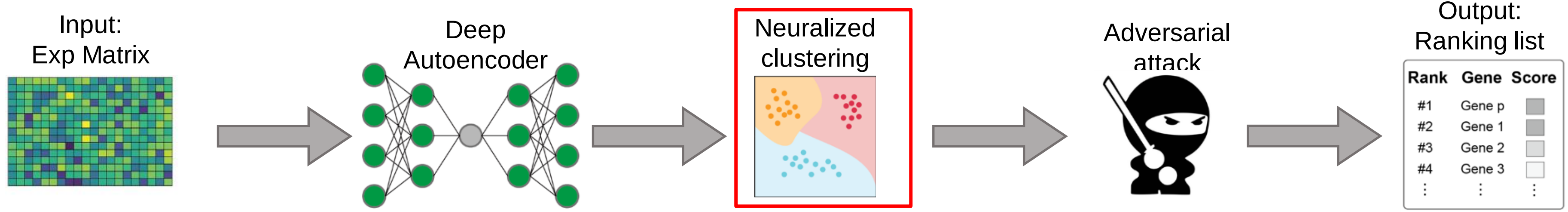
# ACE performs k-means clustering in the learned embedding space

### Clustering:

❑ K-means clustering in the embedding space



### At a high level: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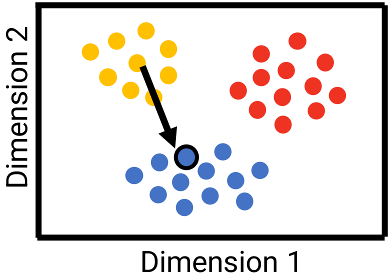
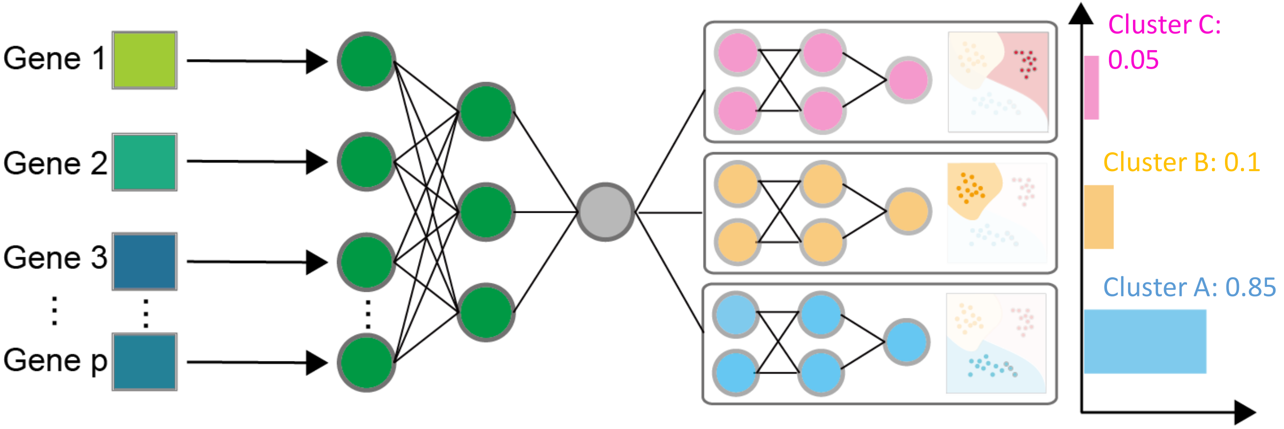
# ACE reformulates the k-means clustering as a functionally equivalent multi-layer neural network

**Cluster neuralization:**

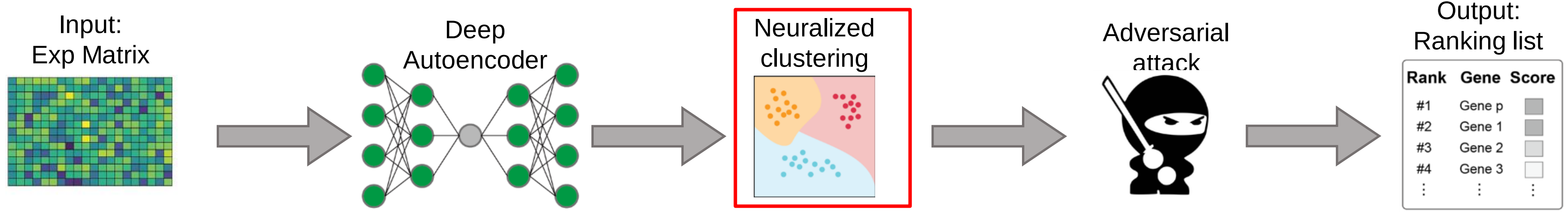
❑ The cluster assignment is preserved for each cell





*Kauffmann et al, arXiv:1906.07633 (2019)*

**At a high level:**



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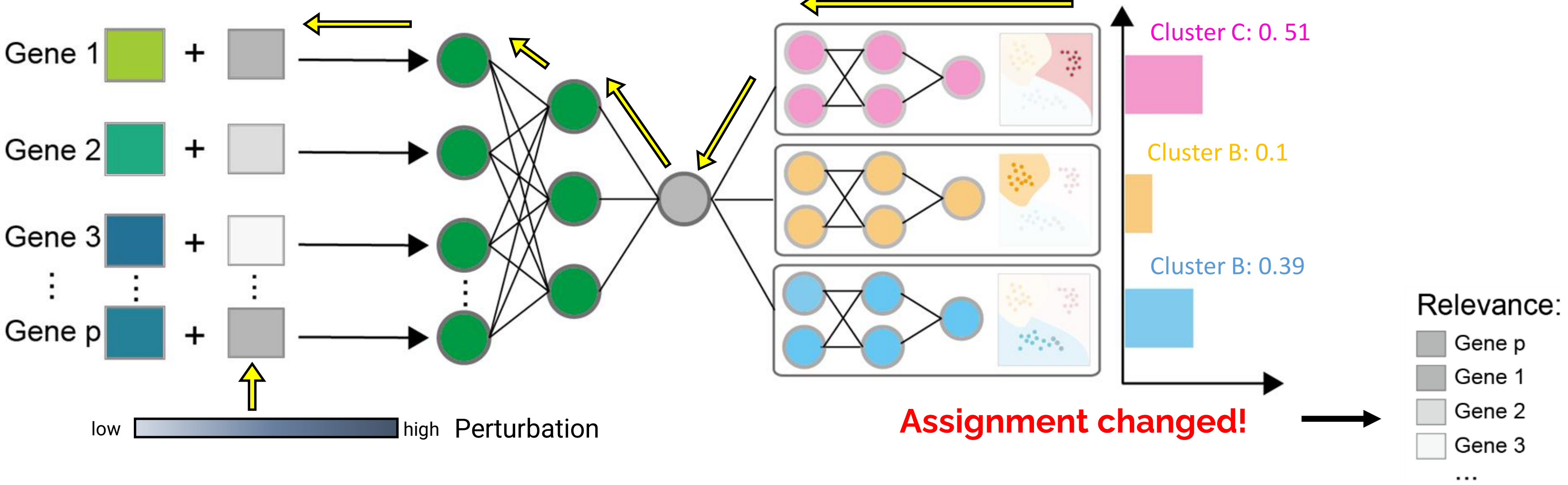
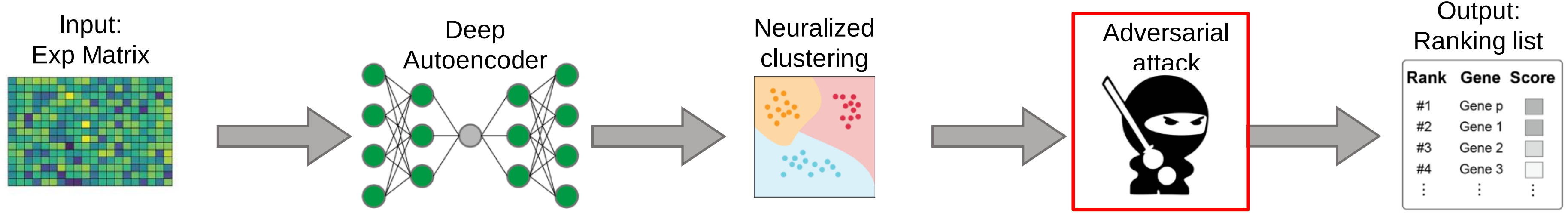
# ACE finds the **minimal perturbation** to a cell that causes the clustering assignment to change

**The assignment changes are propagated back to the perturbation**

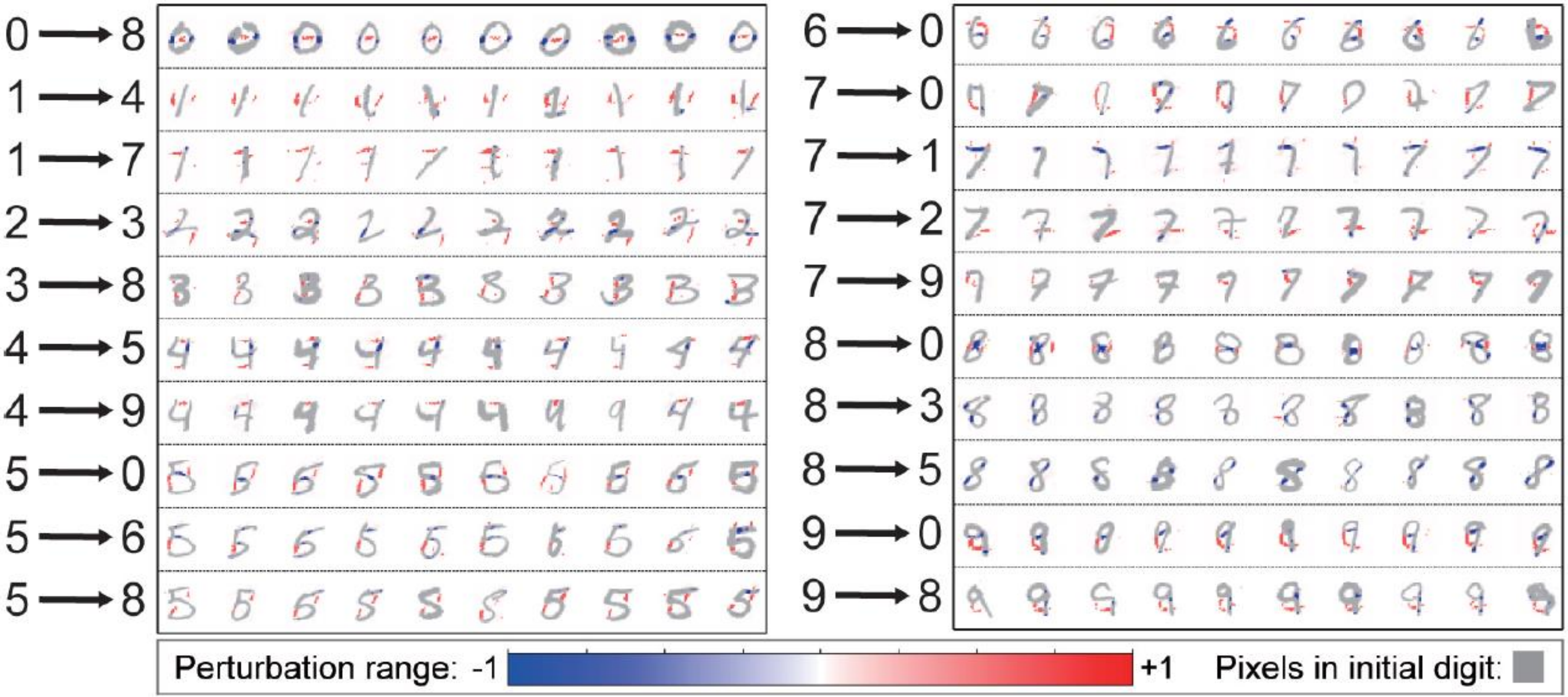


**Assignment changed!**

## At a high level:

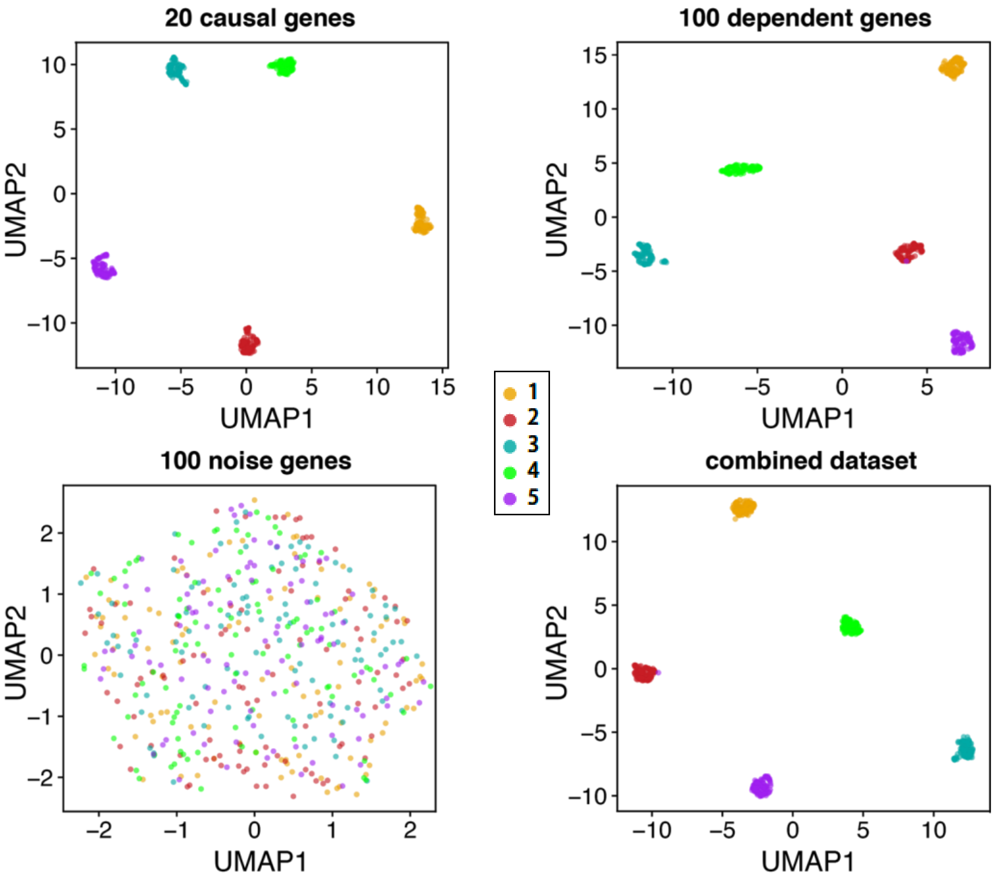


# Sanity check: we applied ACE to identify digit transitions in a pixel-wise manner



*Deng L., IEEE Signal Processing Magazine (2012)*

# ACE is applied to a simulated dataset with many **redundant** genes



Both causal and noise genes are simulated for 500 cells by using **SymSim** toolkit.

**Dependent genes are weighted sums of random causal genes with noises:**

$\text{Dep}_1 = w_1\text{causal}_1 + w_2\text{causal}_3$
$\text{Dep}_2 = w_1\text{causal}_2 + w_2\text{causal}_4 + w_3\text{causal}_6$
$\text{Dep}_3 = w_1\text{causal}_{10}$

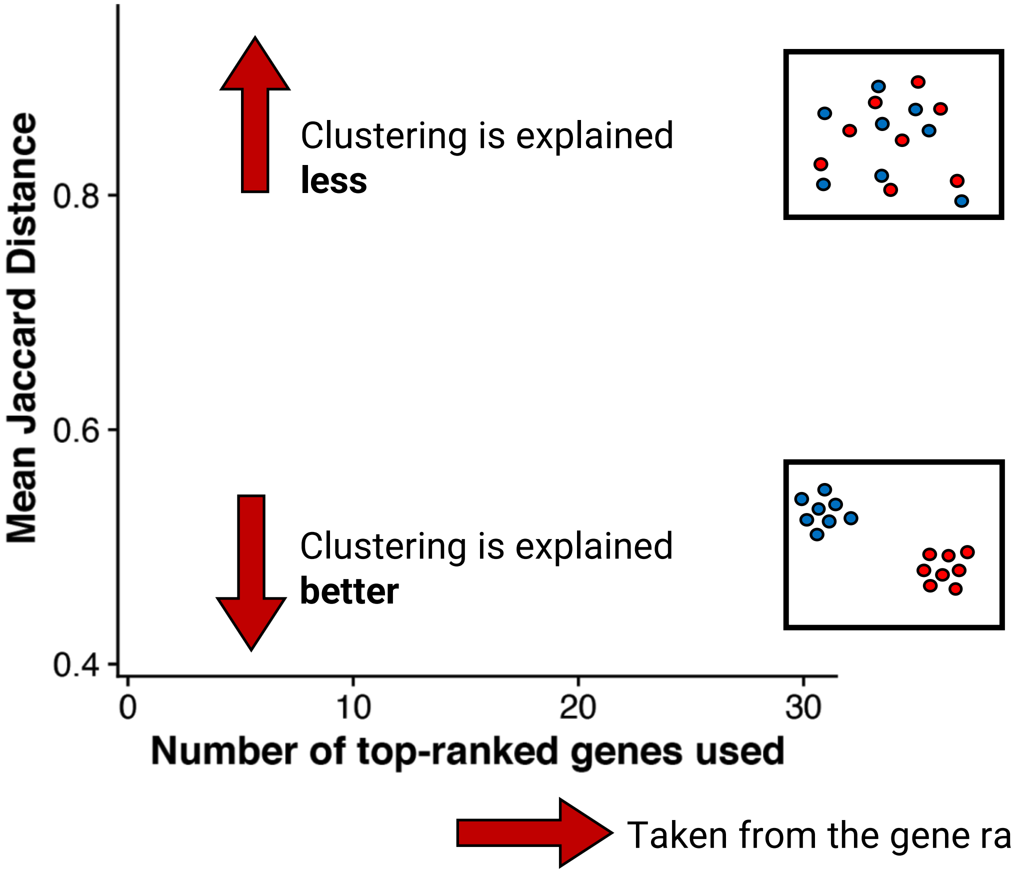
…

**Task:** Rank the genes by relevance

**Metric:** Quantify how well the top *k* genes in the ranking capture the clustering

*Zhang et al, Nature Communications (2019)*

# The **Mean Jaccard Distance** is a metric for how well a subset of features capture the cluster structure

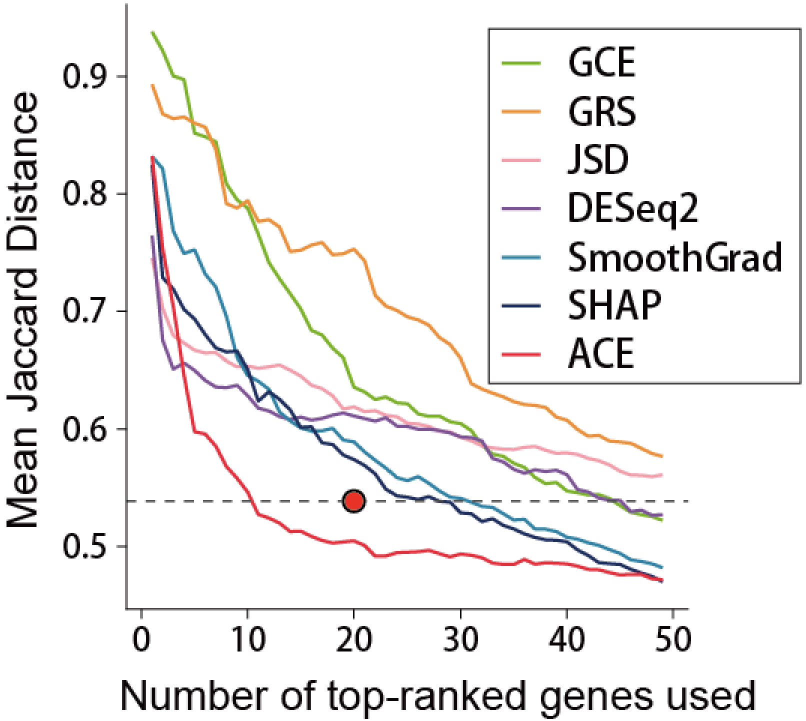


Clustering is explained **less**

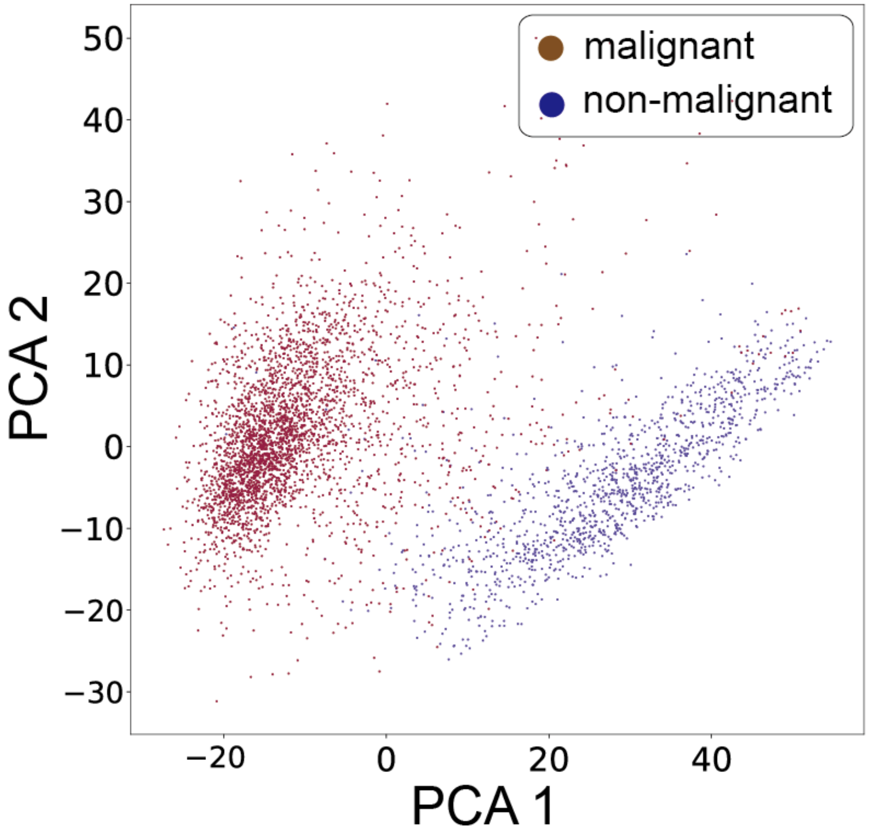
Clustering is explained **better**

Taken from the gene ranking

# ACE is **competitive** against existing methods



# ACE is applied to a real melanoma dataset



2 cell types (malignant vs. non-malignant)
4513 cells (1257 malignant and 3256 non-malignant)
23686 genes

**Task:** Rank the genes by relevance

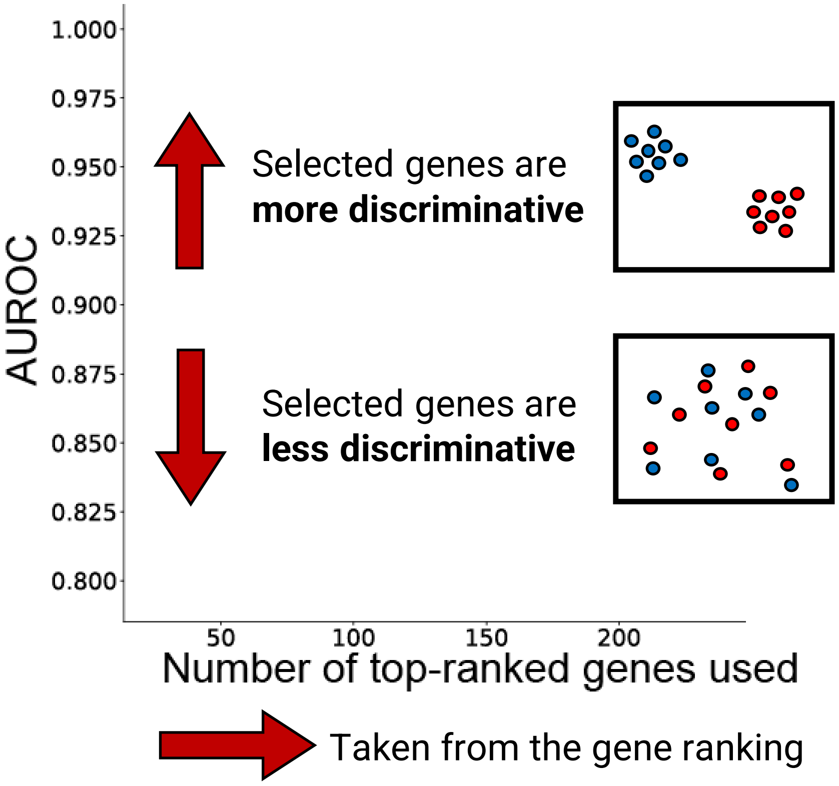
**Metric:**

❑ Quantify how well the top *k* genes in ranking discriminate malignant cells

❑ Quantify how non-redundant/diverse are the top *k* genes in the ranking

*Tiroshi et al, Science (2016)*

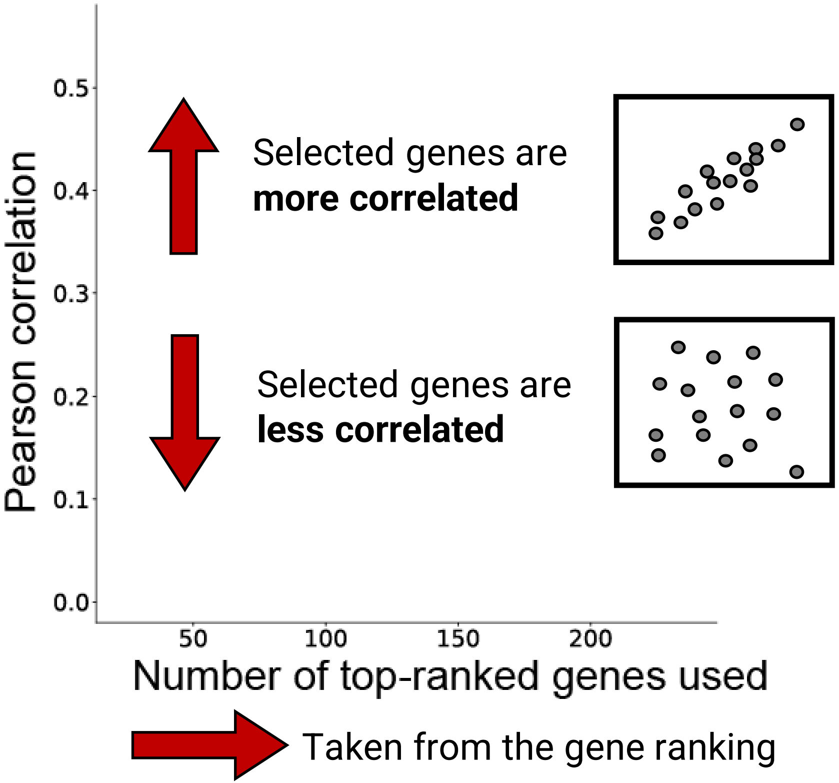
# Ideally we want the selected top-ranked genes to be both **highly discriminative** and **non-redundant**



Selected genes are **more discriminative**

Selected genes are **less discriminative**

Taken from the gene ranking

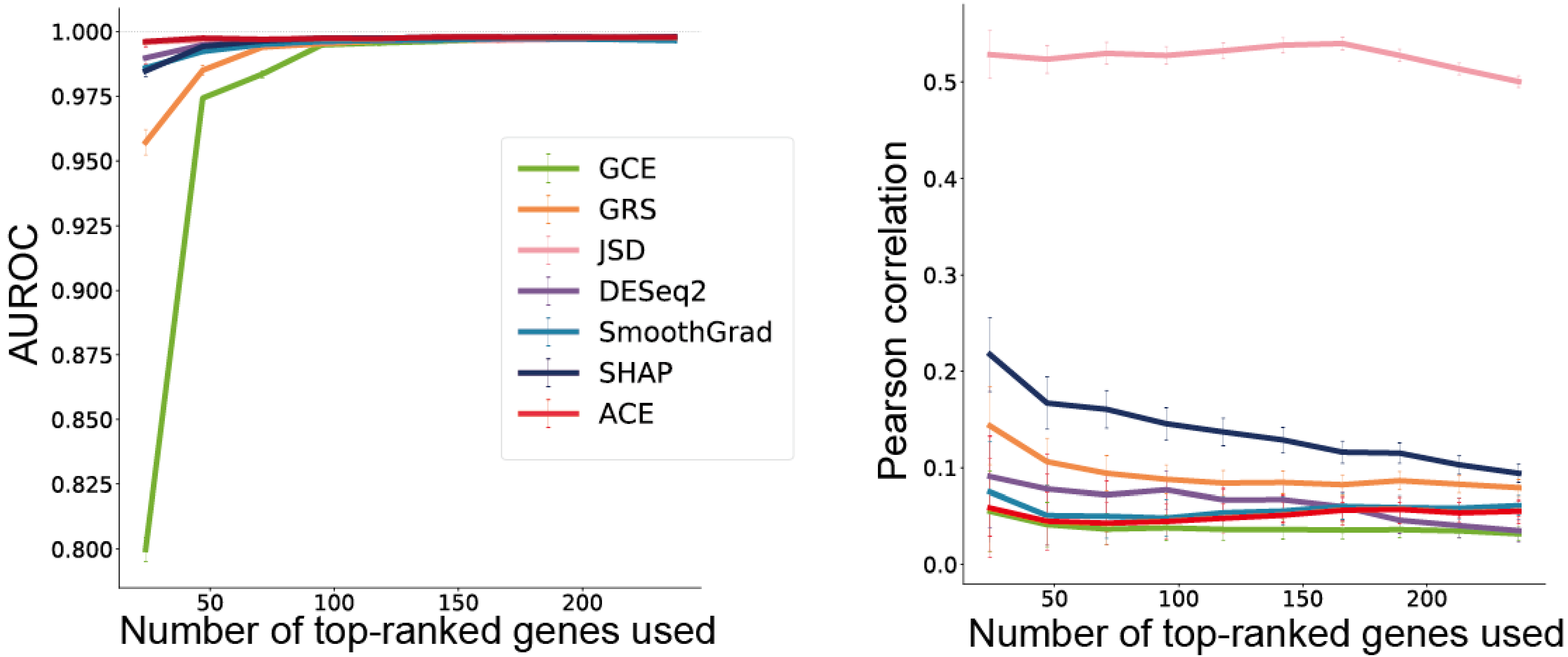


Selected genes are **more correlated**

Selected genes are **less correlated**

Taken from the gene ranking

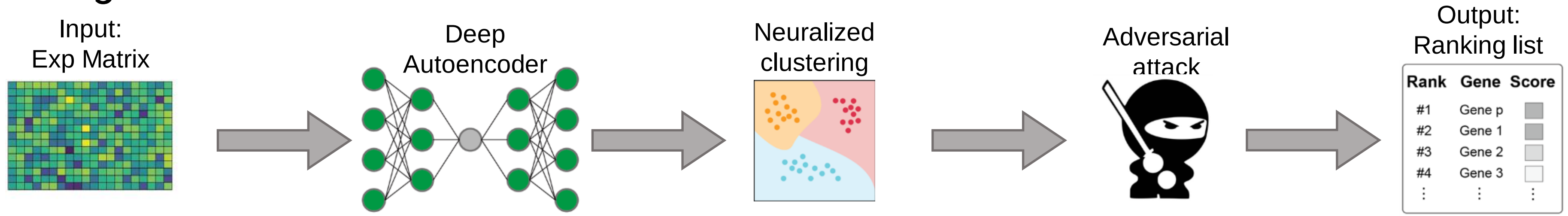
# ACE is **competitive** against existing methods in both discriminative power and minimum redundancy



# Conclusions

- ACE finds the minimal set of genes that best explain clustering and is competitive against existing methods.
- The selected highly-discriminative genes can be both enriched and depleted.
- ACE is potentially useful in domains beyond biology.
- Open-source code availability: [https://bitbucket.org/noblelab/ace](https://bitbucket.org/noblelab/ace)

**At a high level:**



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# Acknowledgements

- Noble lab members:

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UNIVERSITY OF WASHINGTON

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UNIVERSITY *of* WASHINGTON