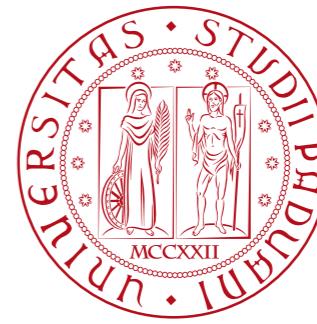




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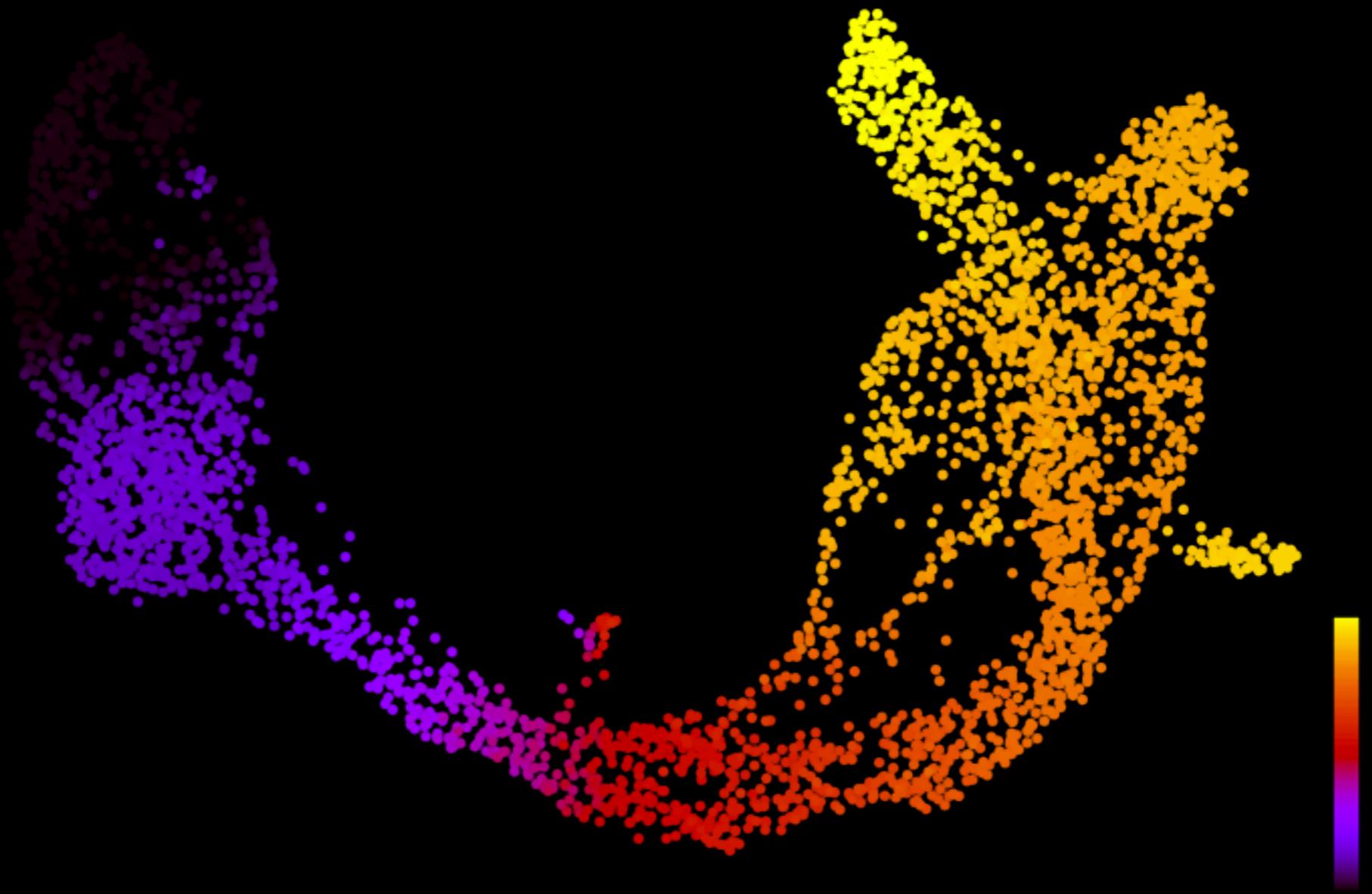
CSAMA 2023 - BRIXEN/BRESSANONE

SINGLE-CELL OMICS II

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@drisso



**Trajectories, pseudo-time,
bifurcations**

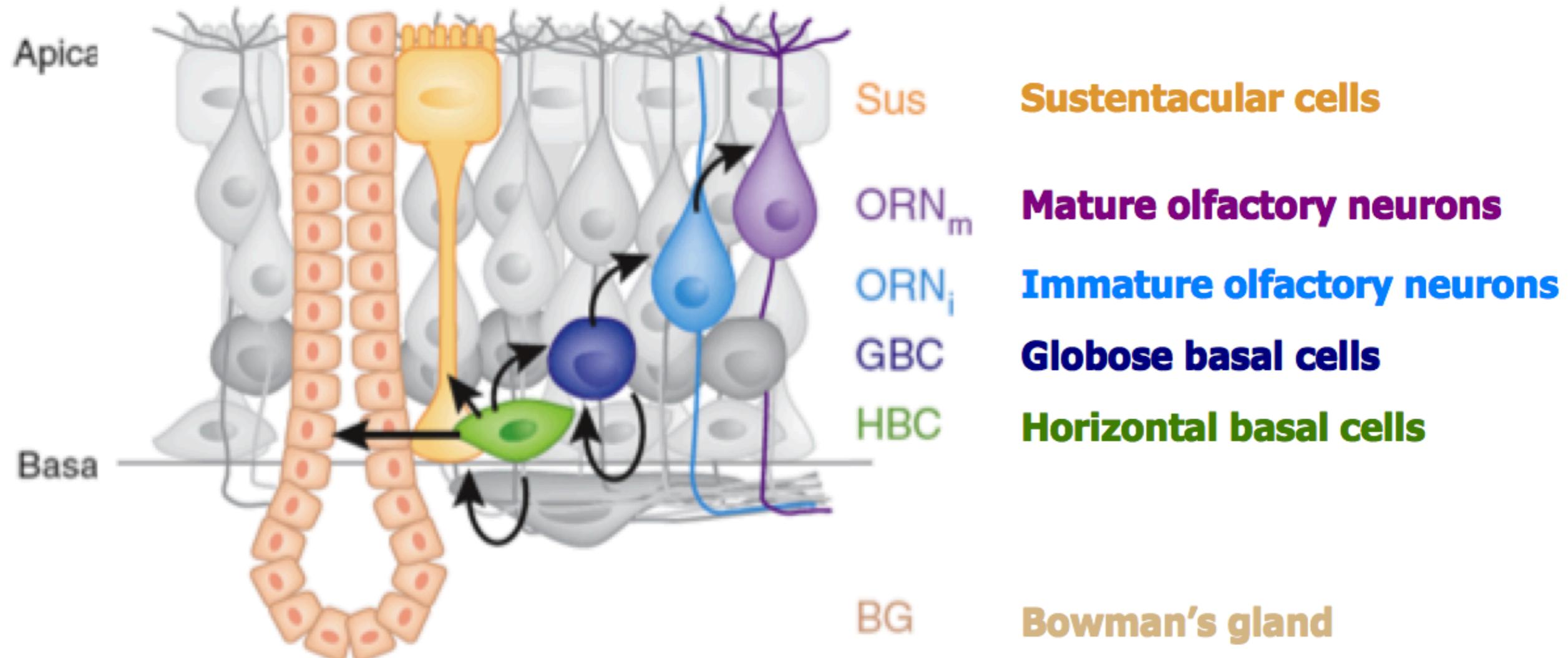
PSEUDOTIME INFERENCE

In development, it is important to know the mechanisms by which stem cells develop into mature cells.

A question of great interest is how and when do cells “decide” their fate.

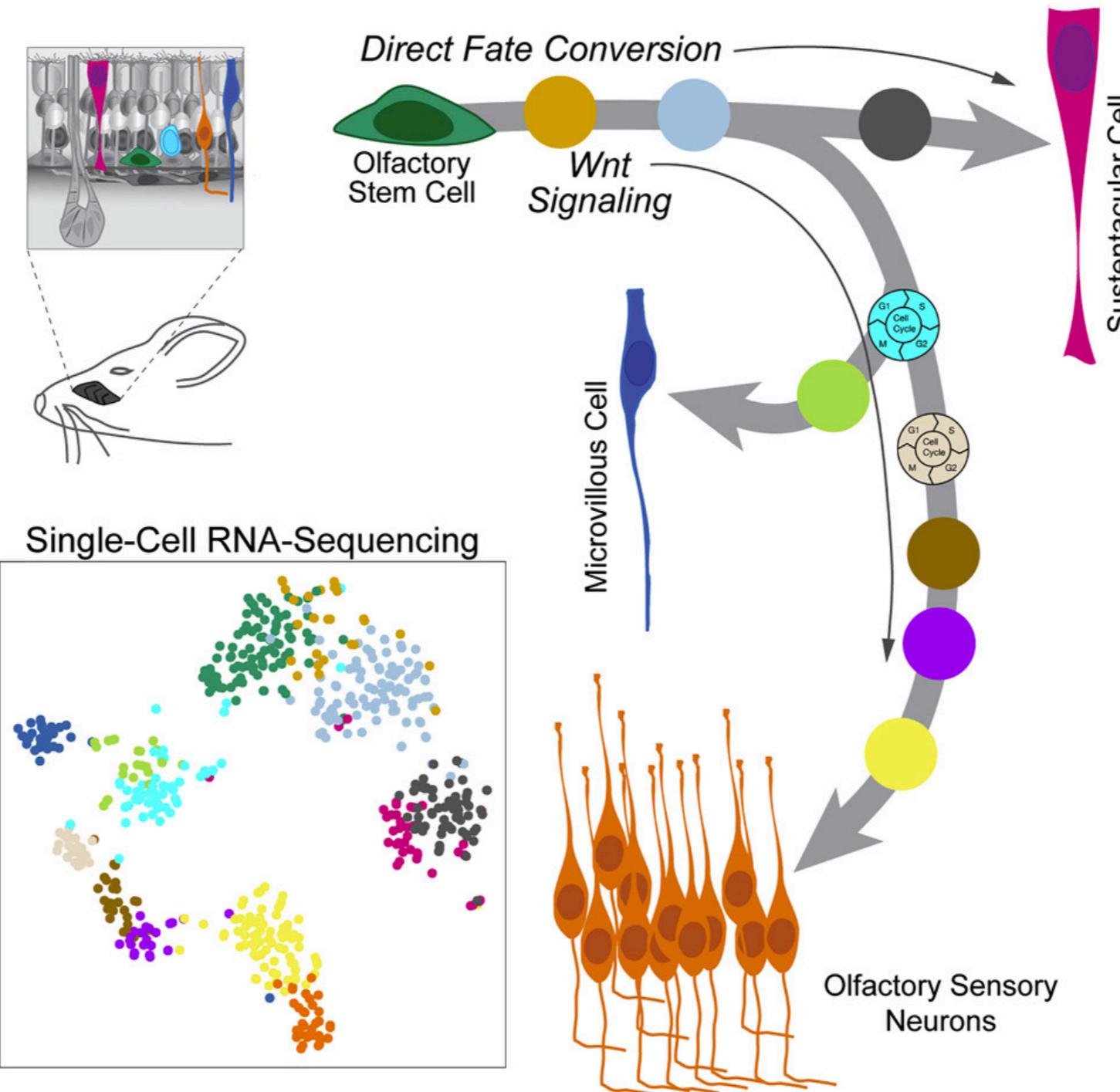
Here we use as an example the mouse olfactory epithelium, which contains a stem cell niche.

OLFACTORY EPITHELIUM



OLFACTORY EPITHELIUM

Olfactory Epithelium Stem Cell Lineage Trajectory



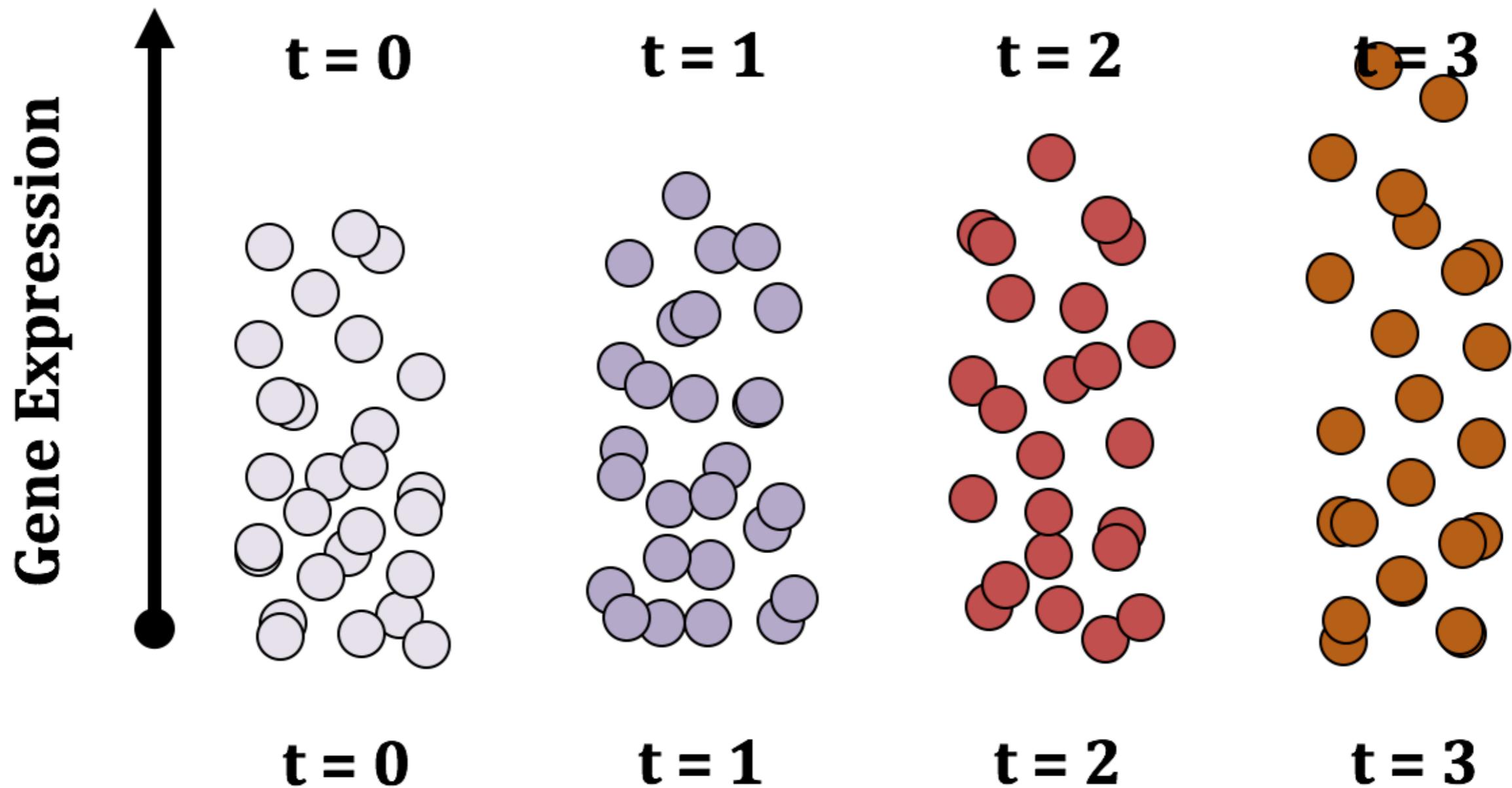
Fletcher et al. (2017). Cell Stem Cell.

PSEUDOTIME INFERENCE

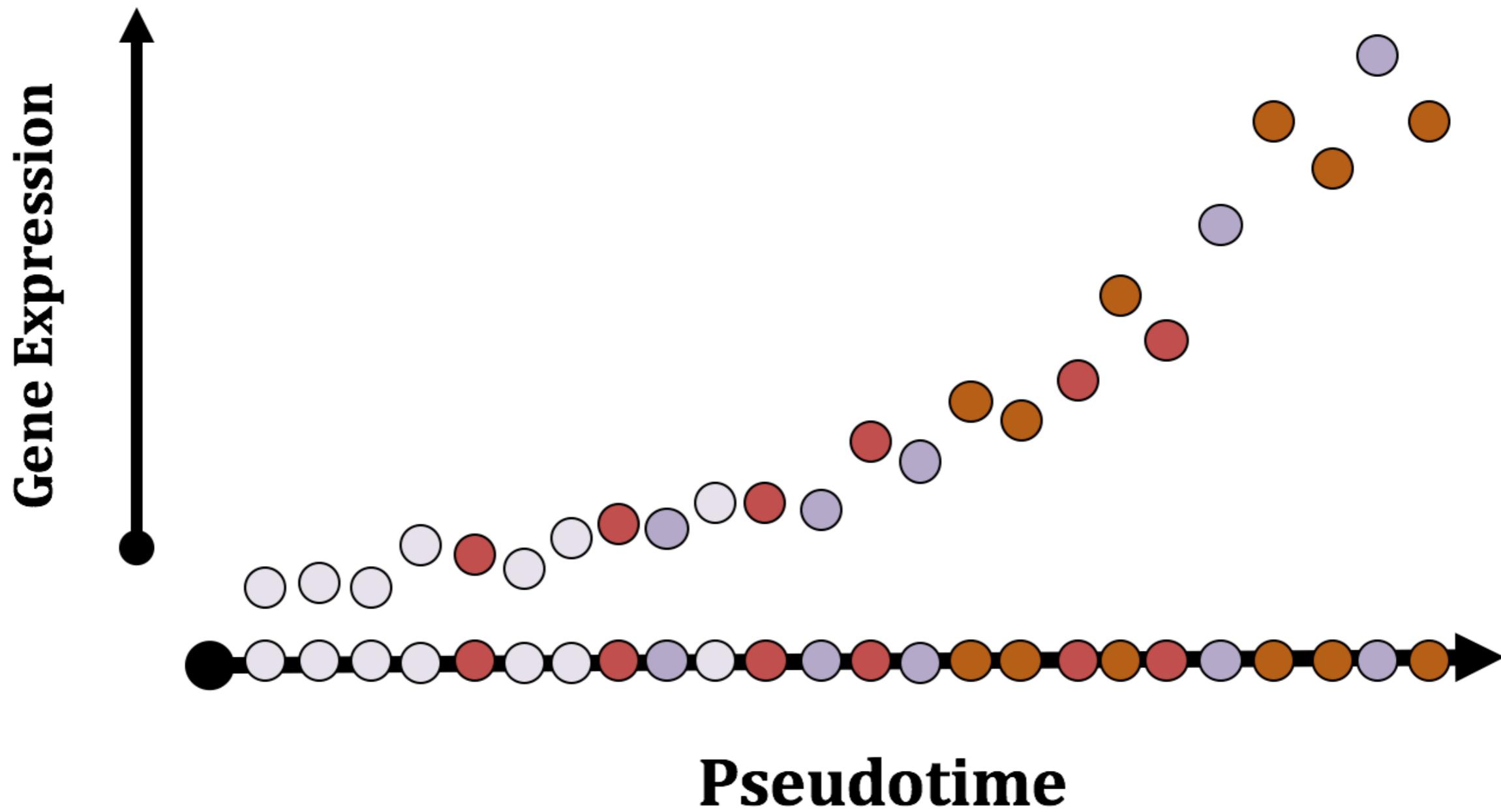
To study cell differentiation, we typically induce an injury in the system and observe how stem cells react.

We can collect the data at different time points, but since cells are not synchronized, we will not have a precise temporal ordering.

PSEUDOTIME INFERENCE

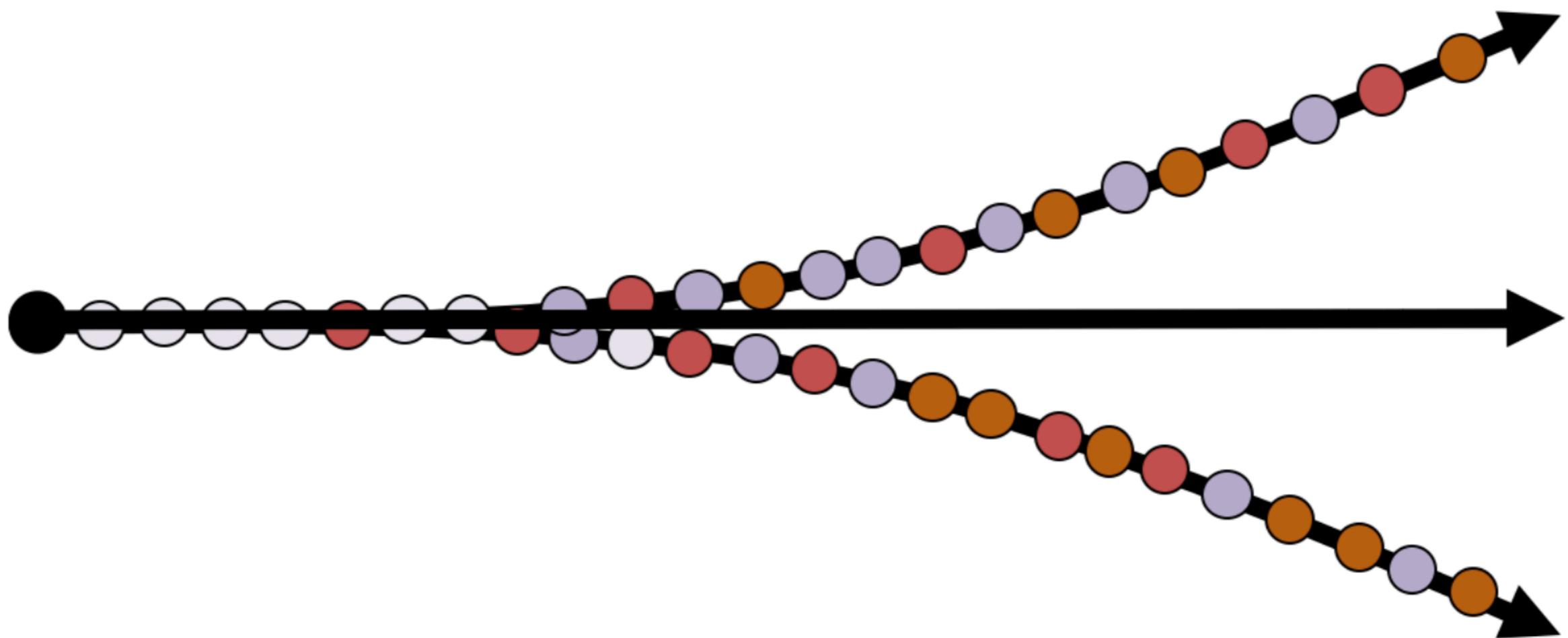


PSEUDOTIME INFERENCE



PSEUDOTIME INFERENCE

Pseudotime



THE SLINGSHOT METHOD

There are several methods for pseudotime analysis, here we see one algorithm, called **Slingshot**.

Slingshot's starting points are:

- ▶ A low dimensional representation of the data (usually PCA with 2-5 components).
- ▶ A clustering.

THE SLINGSHOT METHOD

Suppose to have K clusters. Slingshot consists of two steps:

1. Identify cell lineages.
2. Identify a smooth function that represents the differentiation between a stem cell and a mature cell.

LINEAGE IDENTIFICATION

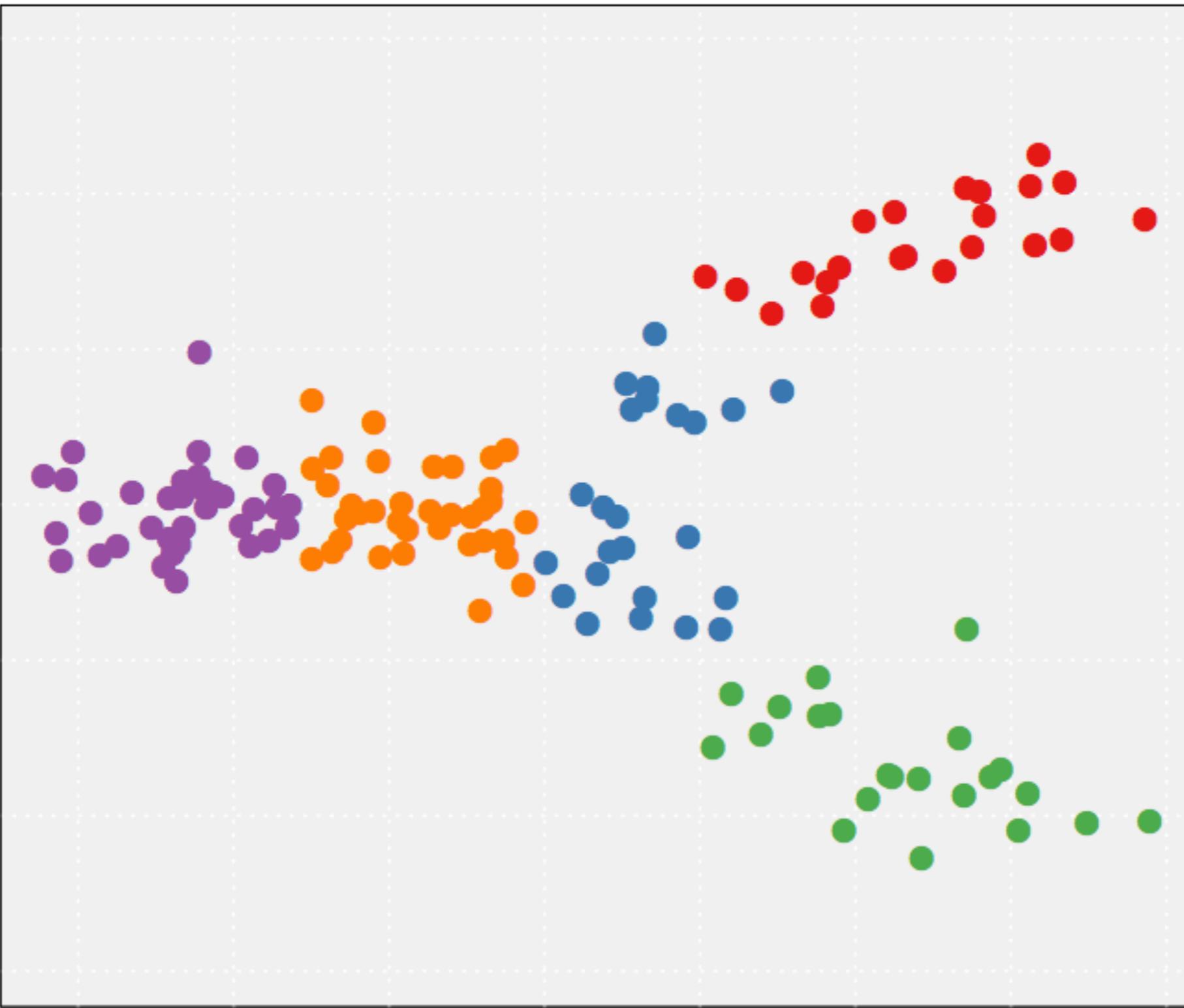
- ▶ We treat clusters as nodes in a network.
- ▶ We create the *minimum spanning tree* (MST) among the nodes.
- ▶ We choose the initial node (the root of the tree).
- ▶ We define the lineages as an ordered set of nodes (clusters).

This is a semi-supervised method.

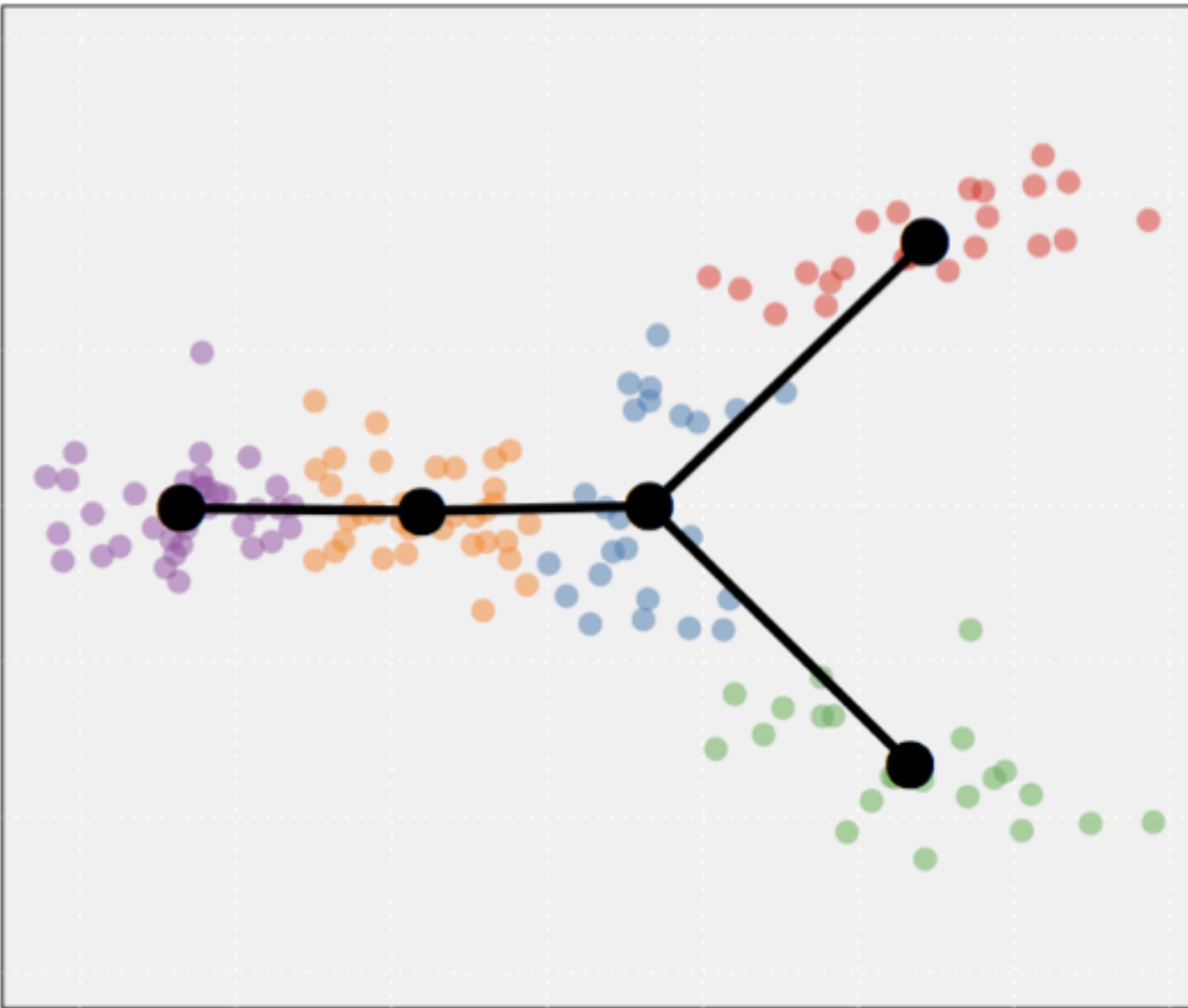
SMOOTHING

- ▶ To smooth the trajectories we use the **principal curves**.
- ▶ We shrink the curves together in the shared part of the lineage.
- ▶ We project the cells onto the principal curves to obtain a measure of **pseudotime**.
- ▶ We define a pseudotemporal ordering of the cells.

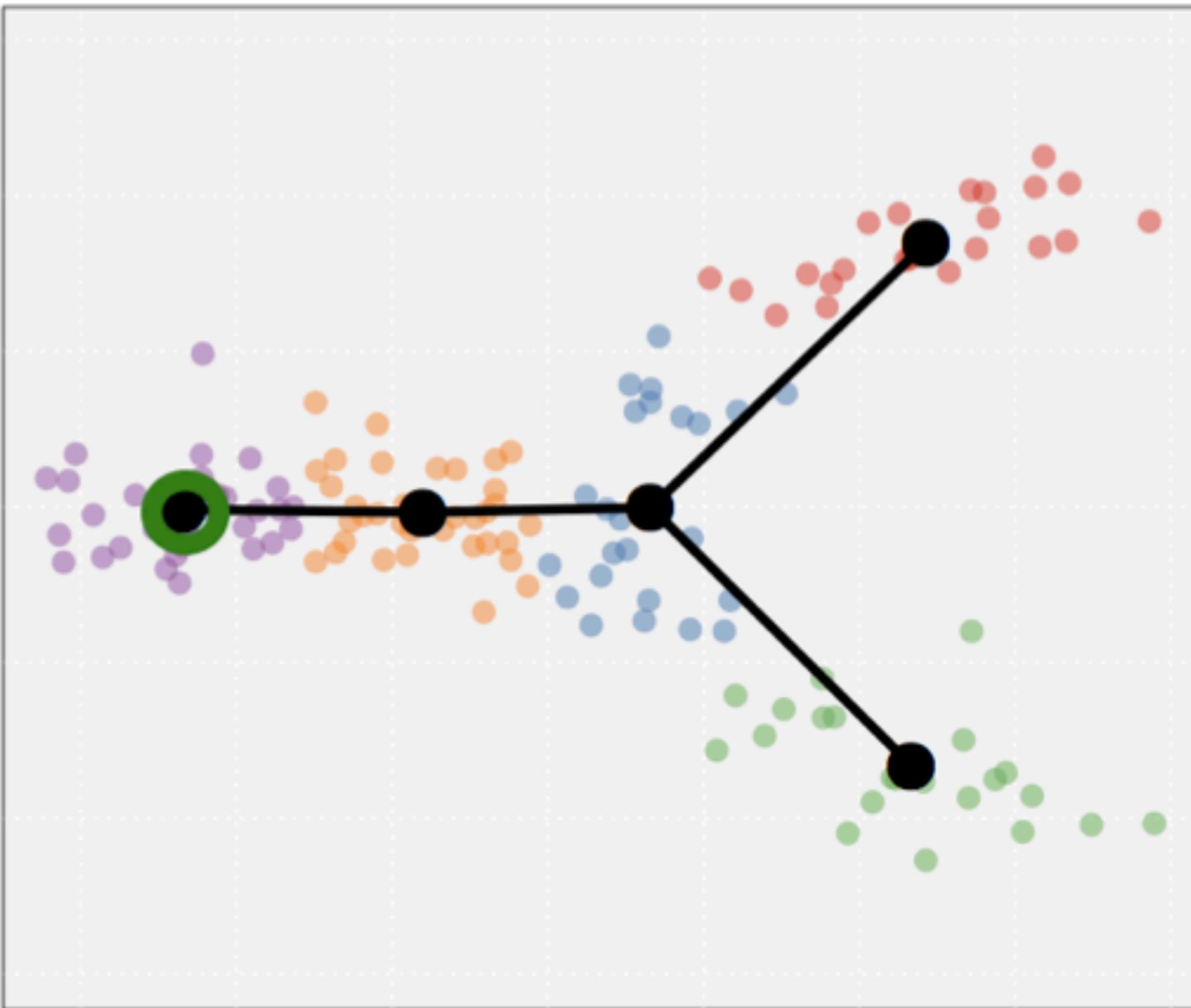
SLINGSHOT



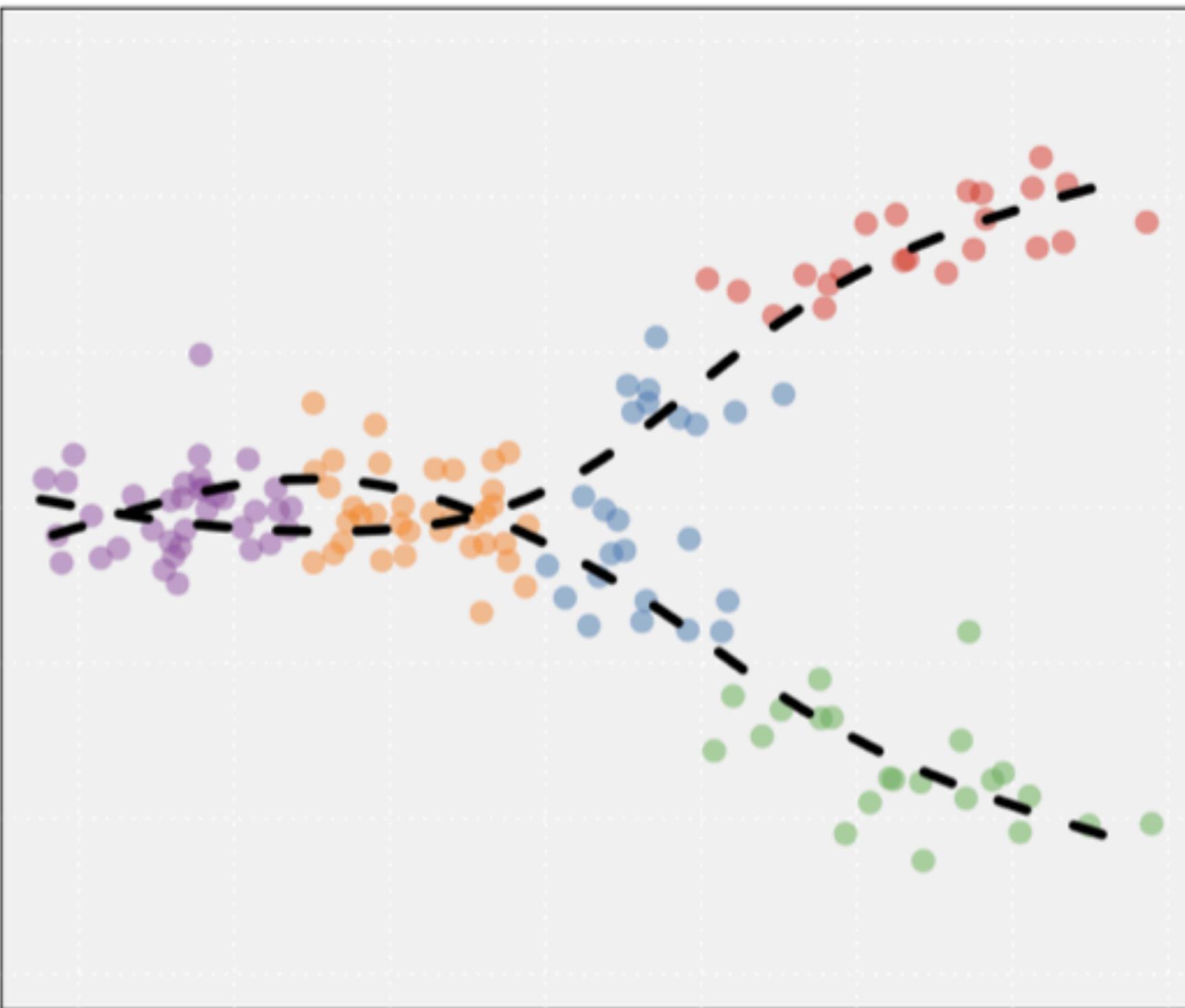
SLINGSHOT



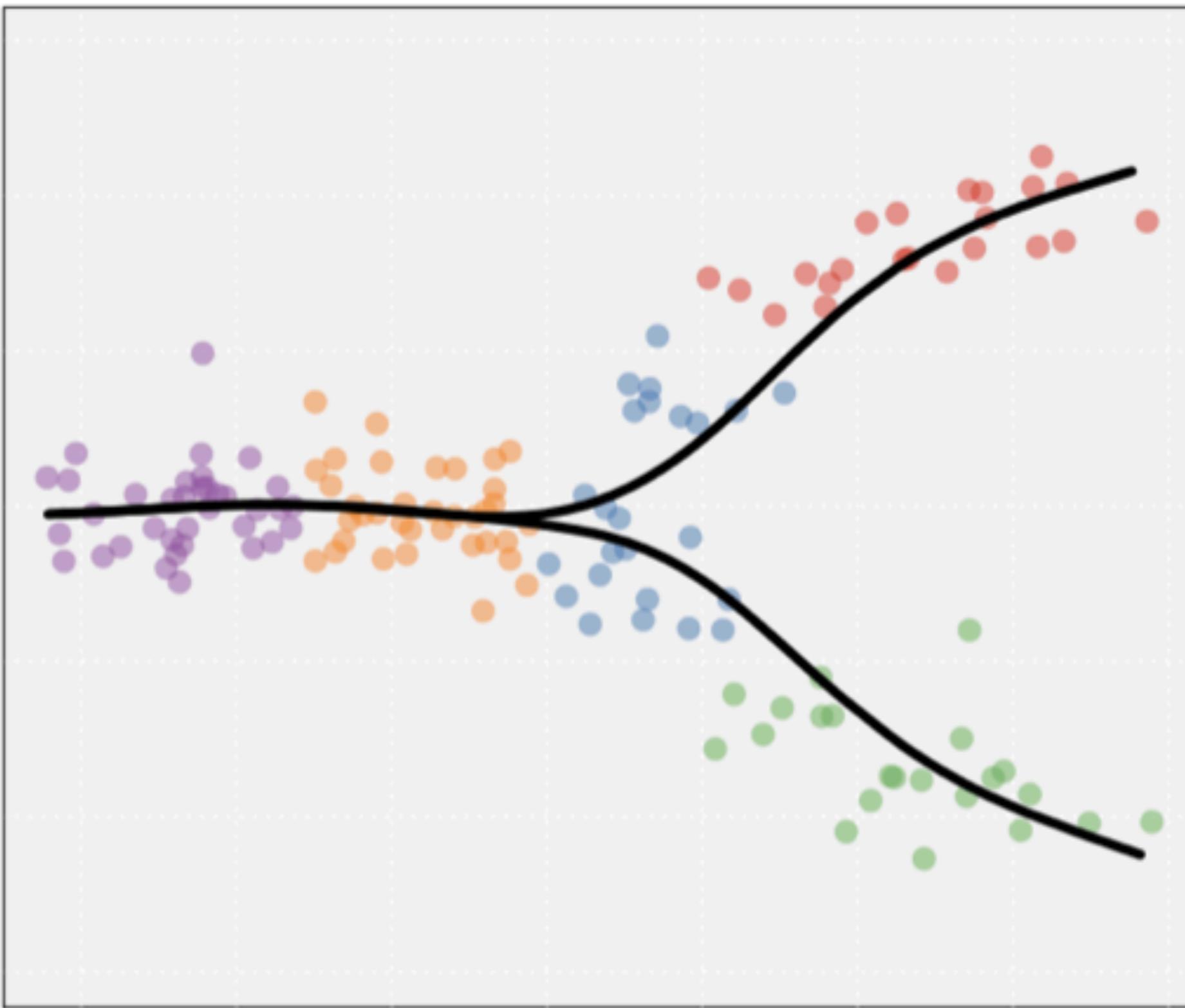
SLINGSHOT



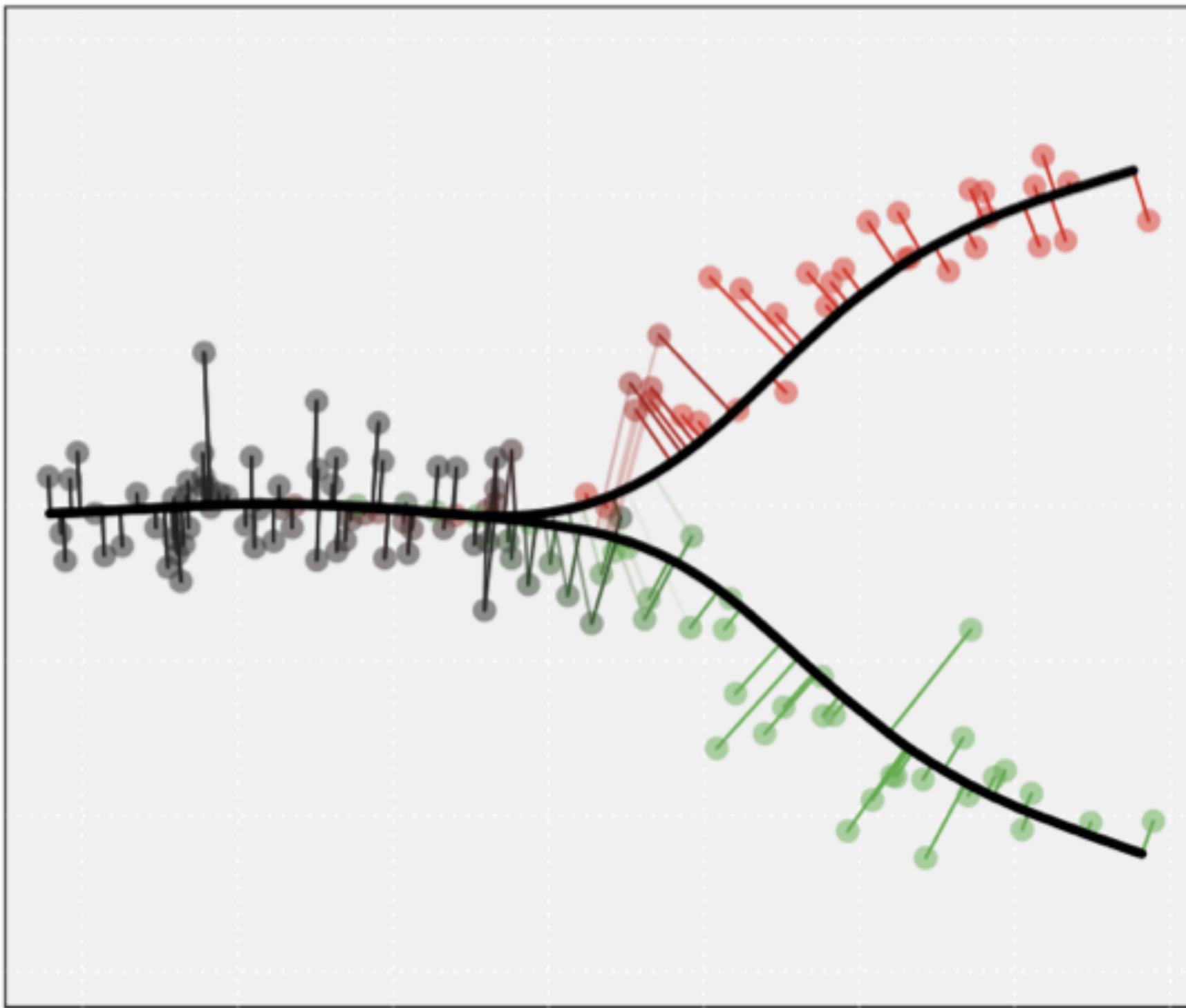
SLINGSHOT



SLINGSHOT



SLINGSHOT

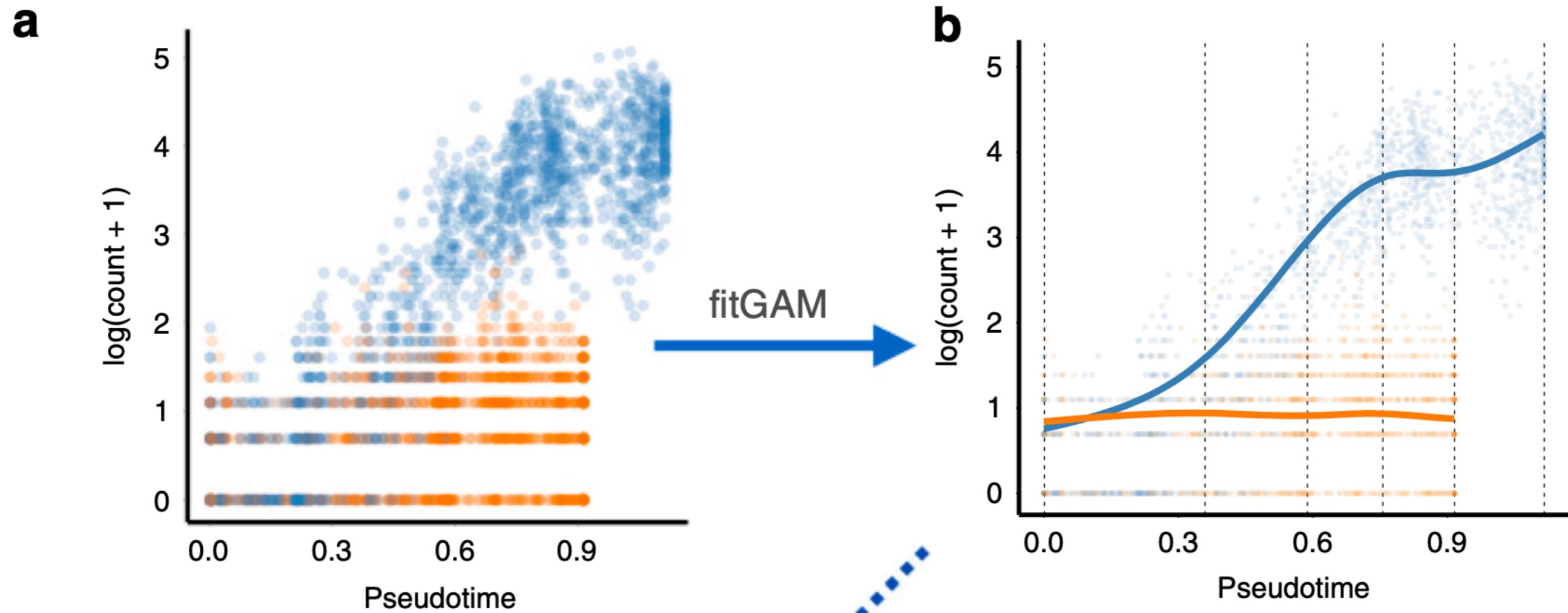


IDENTIFICATION OF PSEUDOTIME-ASSOCIATED GENES

To identify genes associated with pseudotime, we can fit a GAM (generalized additive model) to each gene and determine if the expression of the gene depends on pseudotime. This approach is implemented in the **tradeSeq** Bioconductor package.

The advantage of GAMs is that they allow the identification of nonlinear patterns.

IDENTIFICATION OF PSEUDOTIME-ASSOCIATED GENES

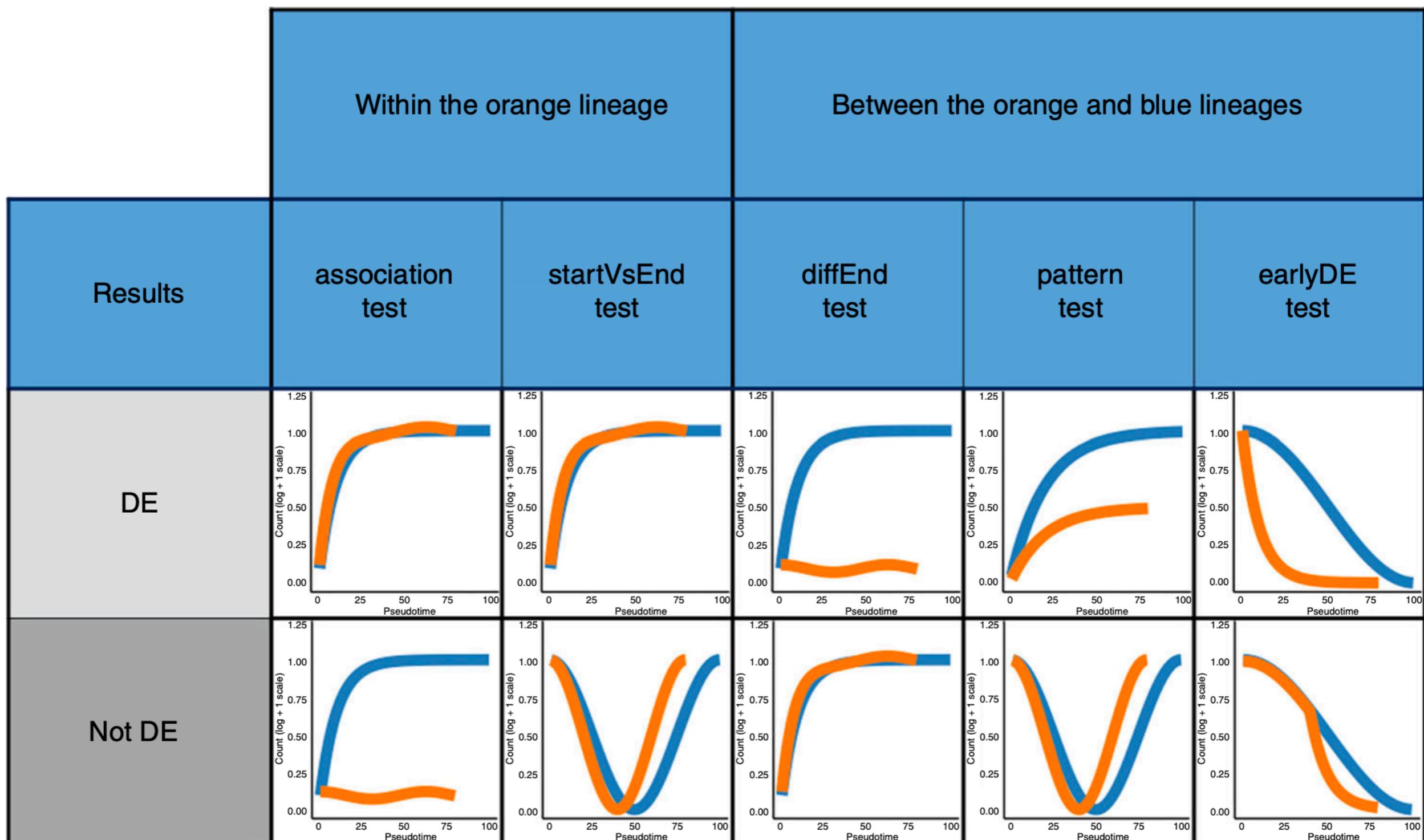


IDENTIFICATION OF PSEUDOTIME-ASSOCIATED GENES

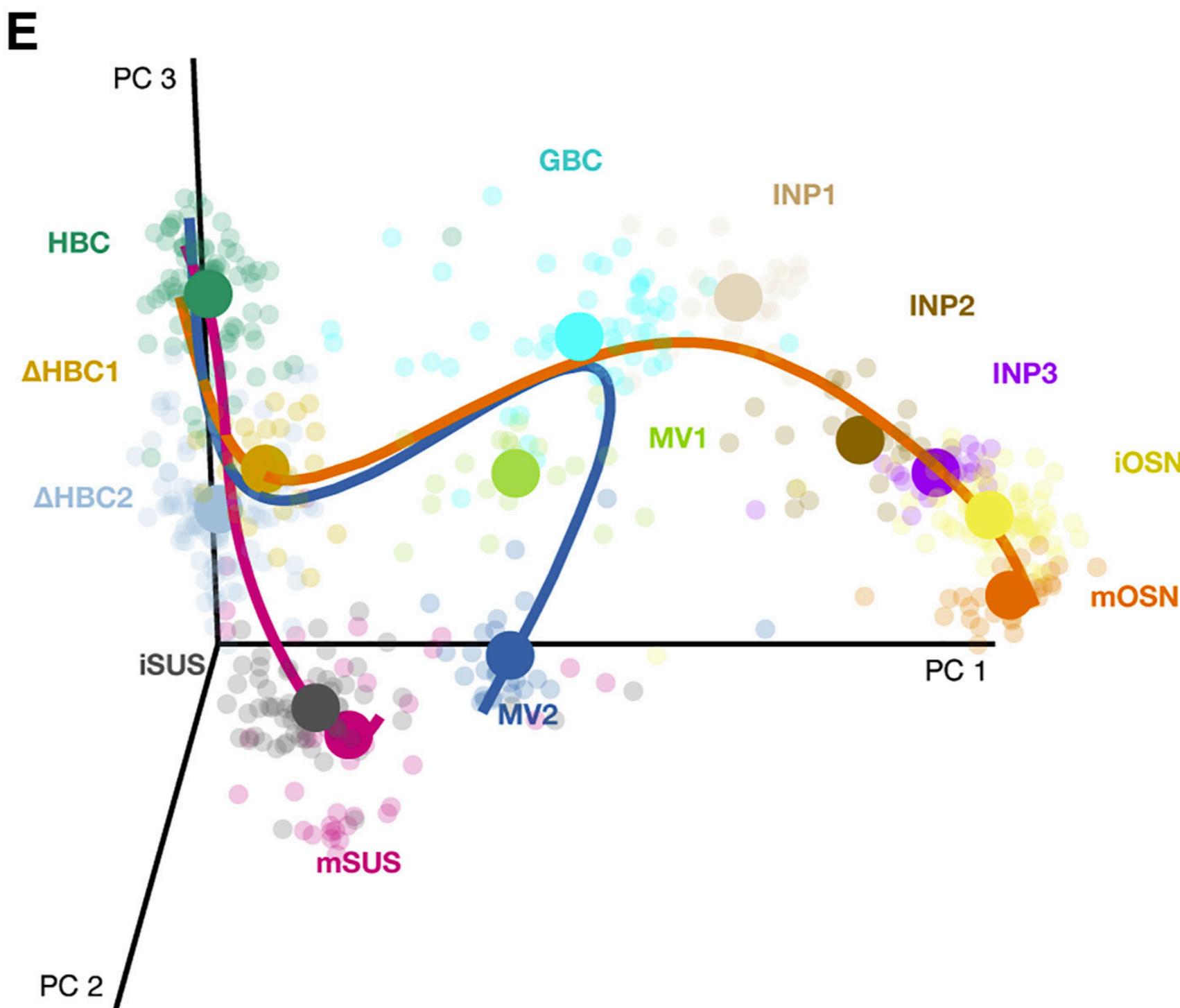
Several genes are of biological interest:

- ▶ Genes associated with pseudotime in a single trajectory.
- ▶ Genes differentially expressed between the start and the end of the trajectory.
- ▶ Genes differentially expressed between two trajectories.

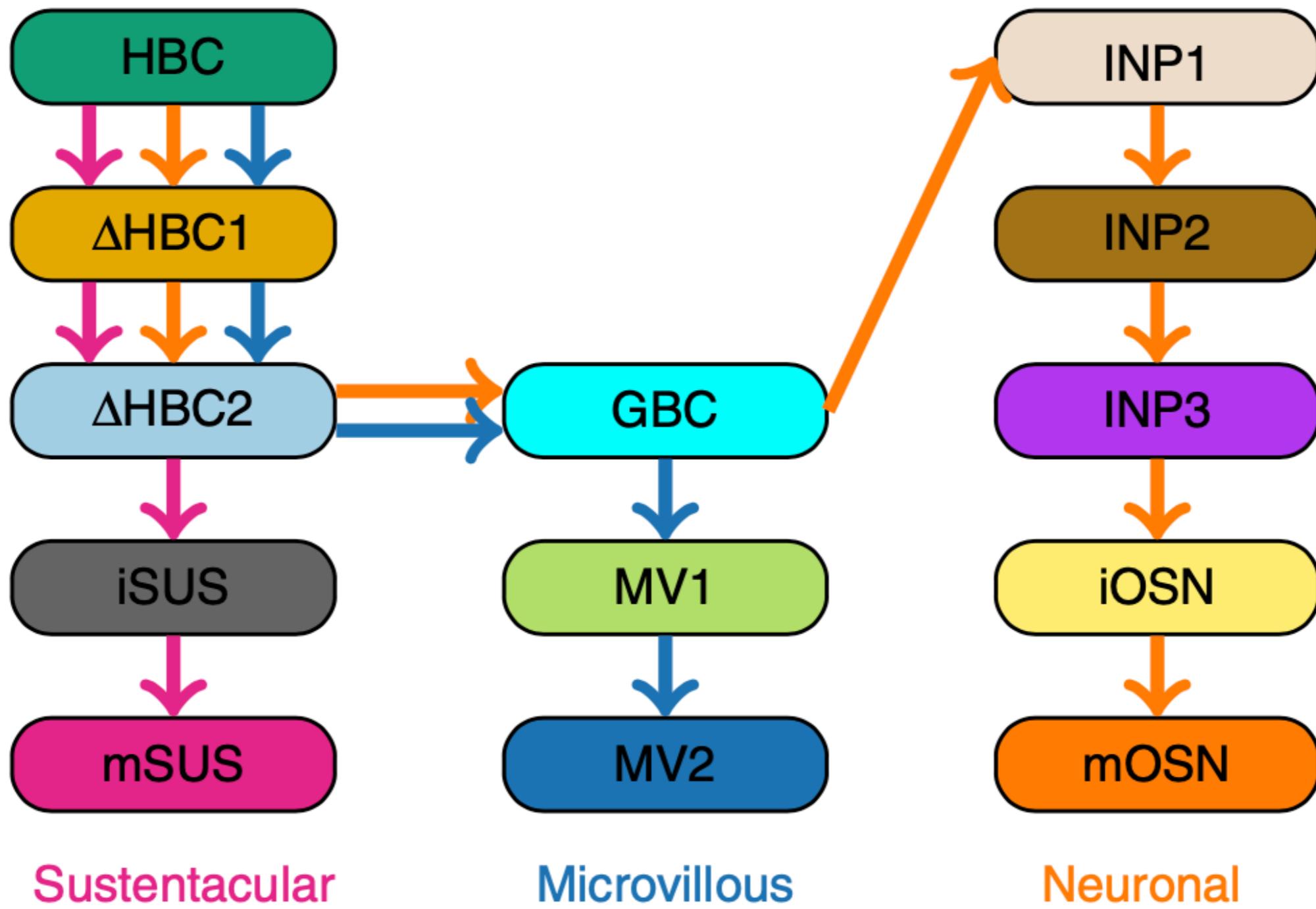
IDENTIFICATION OF PSEUDOTIME-ASSOCIATED GENES



EXAMPLE: OLFACTORY EPITHELIUM

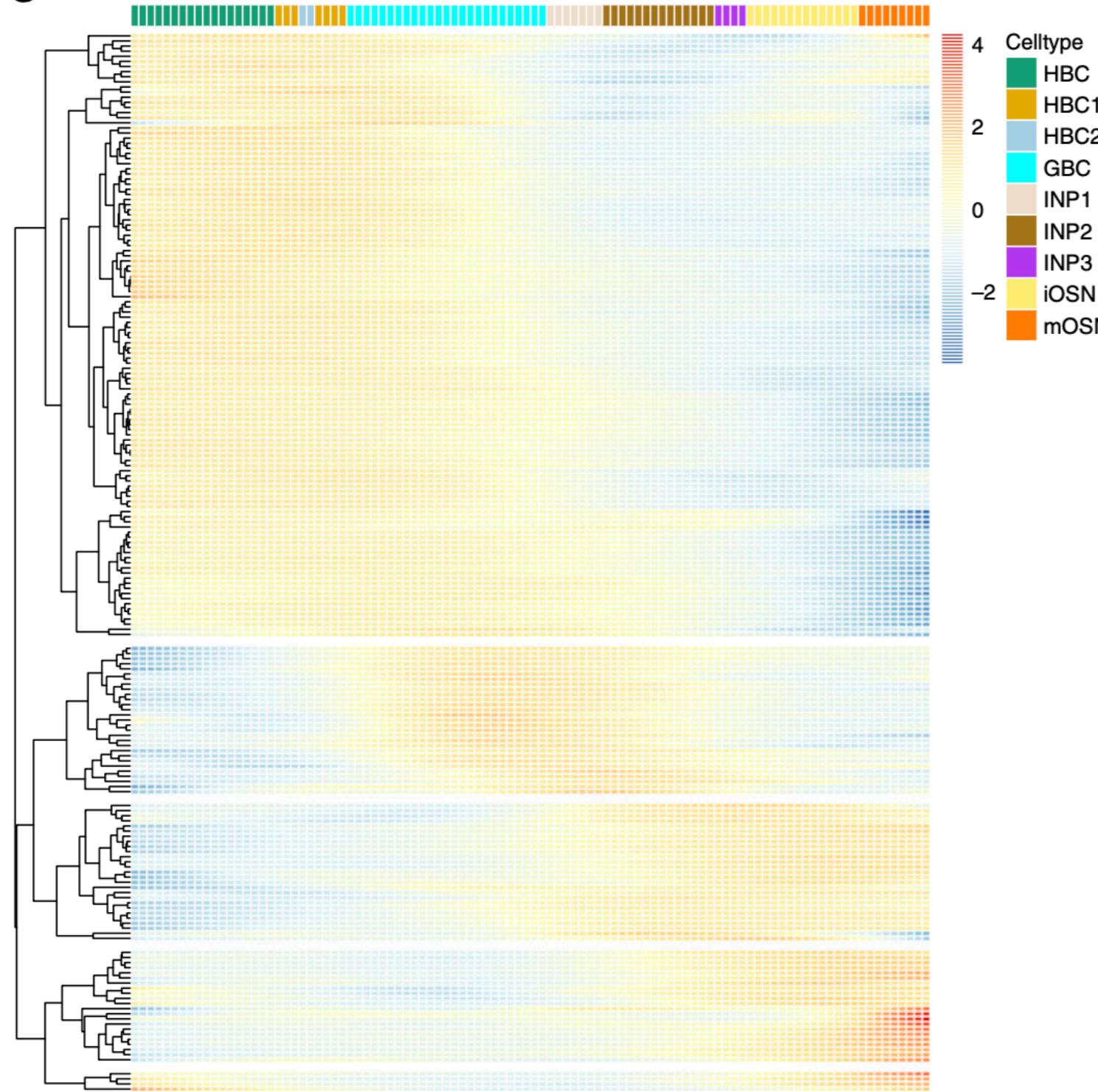


EXAMPLE: OLFACTORY EPITHELIUM

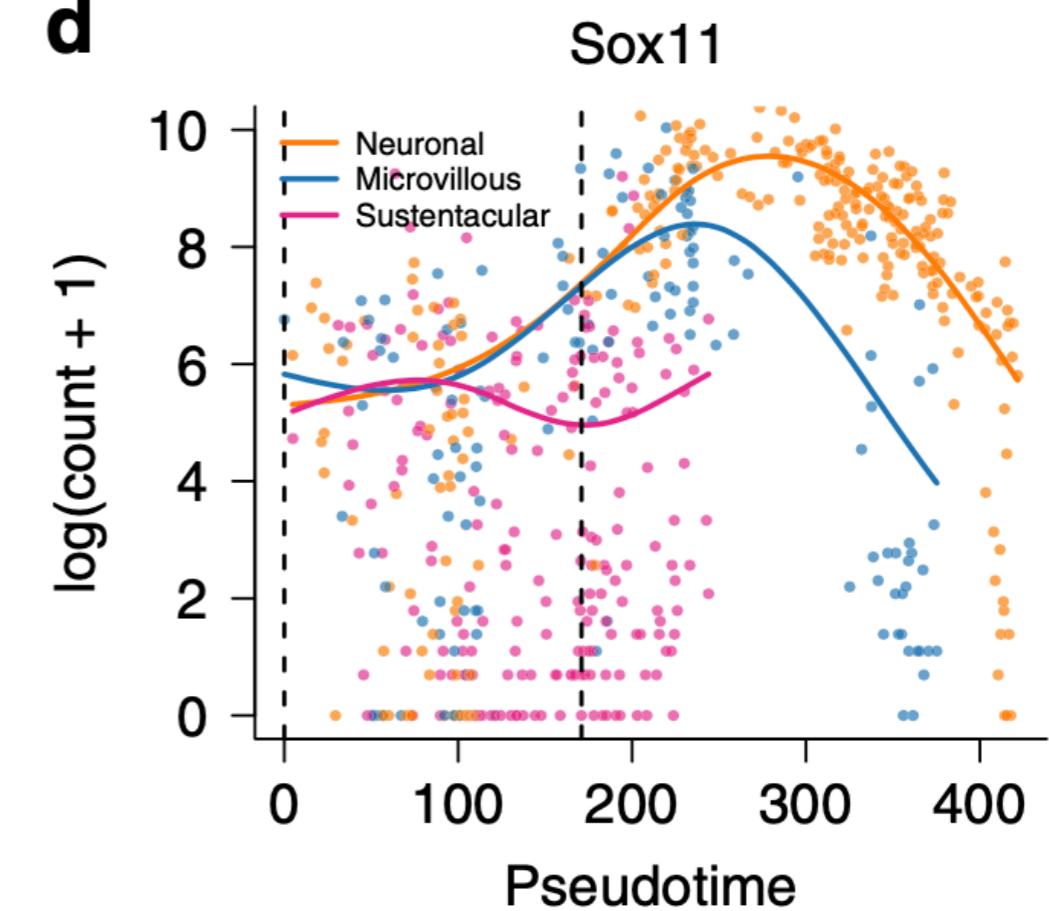


EXAMPLE: OLFACTORY EPITHELIUM

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THE OSCA BOOKS

<http://bioconductor.org/books/release/OSCA/>

Orchestrating Single-Cell Analysis

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Welcome

- What you will learn
- What you won't learn
- Who we wrote this for
- Why we wrote this

Book contents

- Introduction
- Basics
- Advanced
- Multi-sample
- Workflows

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Source: <https://github.com/OSCA-source/OSCA>

Welcome

This is the landing page for the “**Orchestrating Single-Cell Analysis with Bioconductor**” book, which teaches users some common workflows for the analysis of single-cell RNA-seq data (scRNA-seq). This book will show you how to make use of cutting-edge Bioconductor tools to process, analyze, visualize, and explore scRNA-seq data. Additionally, it serves as an online companion for the [paper of the same name](#).



What you will learn

The goal of this book is to provide a solid foundation in the usage of Bioconductor tools for single-cell RNA-seq analysis by walking through various steps of typical workflows using example datasets. We strive to tackle key concepts covered in the manuscript, “**Orchestrating Single-Cell Analysis with Bioconductor**”, with each workflow covering these in varying detail, as well as essential preliminaries that are important for following along with the workflows on your own.

THANKS FOR YOUR ATTENTION!



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