▼Precisium AI

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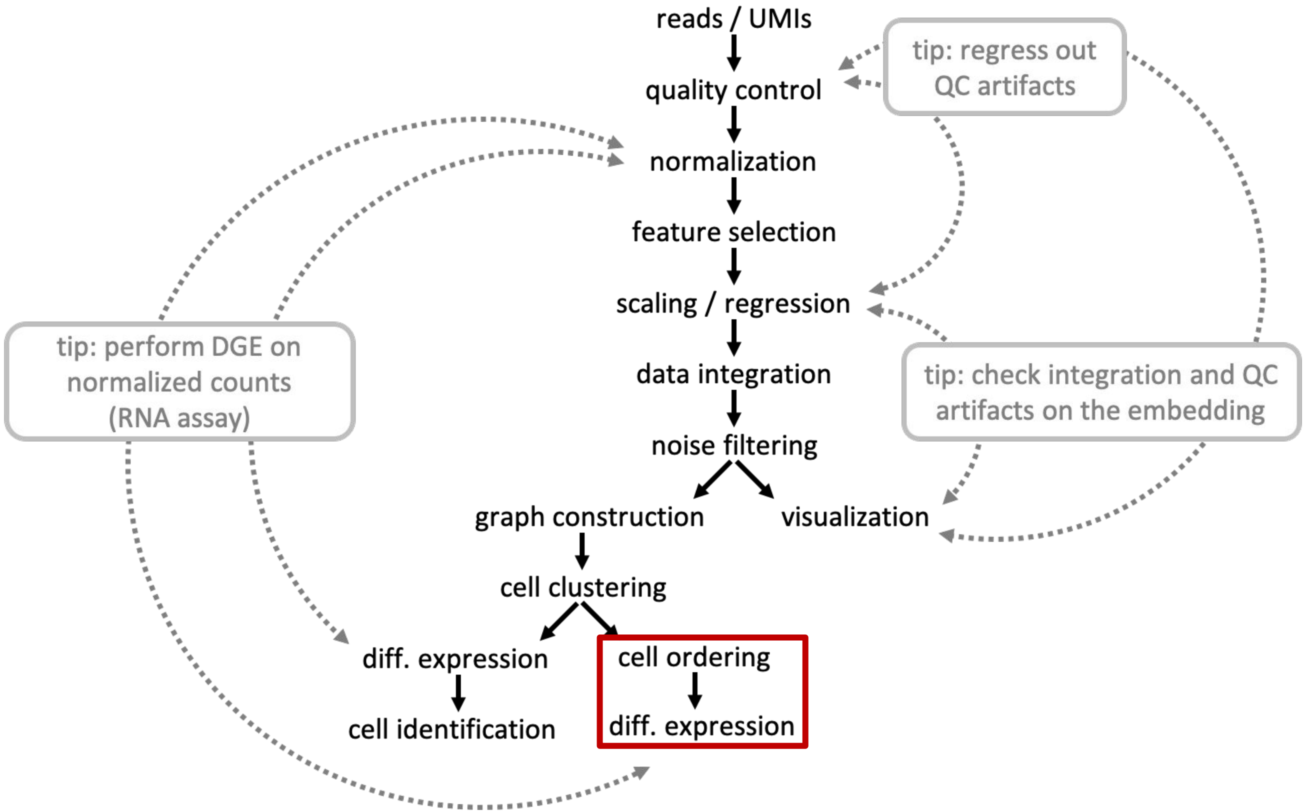
Trajectory Inference Analysis

Introduction

Data analysis workflow



reads / UMIs

quality control

normalization

feature selection

scaling / regression

data integration

noise filtering

graph construction

visualization

cell clustering

diff. expression

cell identification

cell ordering

diff. expression

tip: regress out QC artifacts

tip: perform DGE on normalized counts (RNA assay)

tip: check integration and QC artifacts on the embedding

Single Cell Glossary
https://nbisweden.github.io/single-cell-pbl/glossary_of_terms_single_cell.html

What is pseudotime?

Cells that differentiate display a continuous spectrum of states

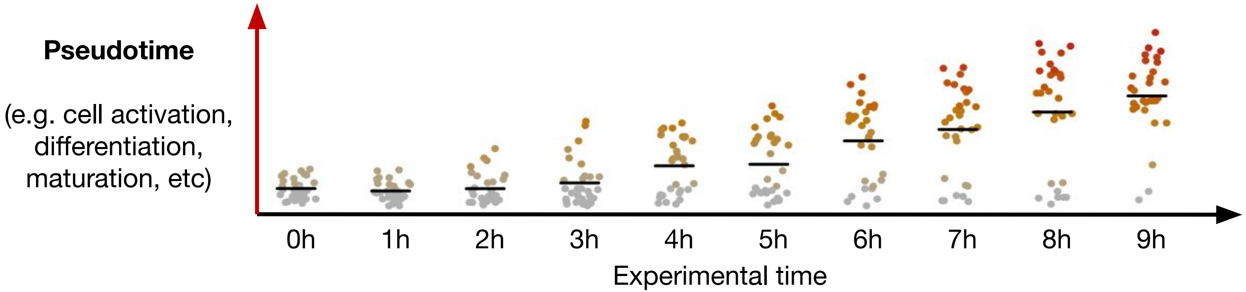
Transcriptional program for activation and differentiation

Individual cells will differentiate in an unsynchronized manner

Each cell is a snapshot of differentiation time

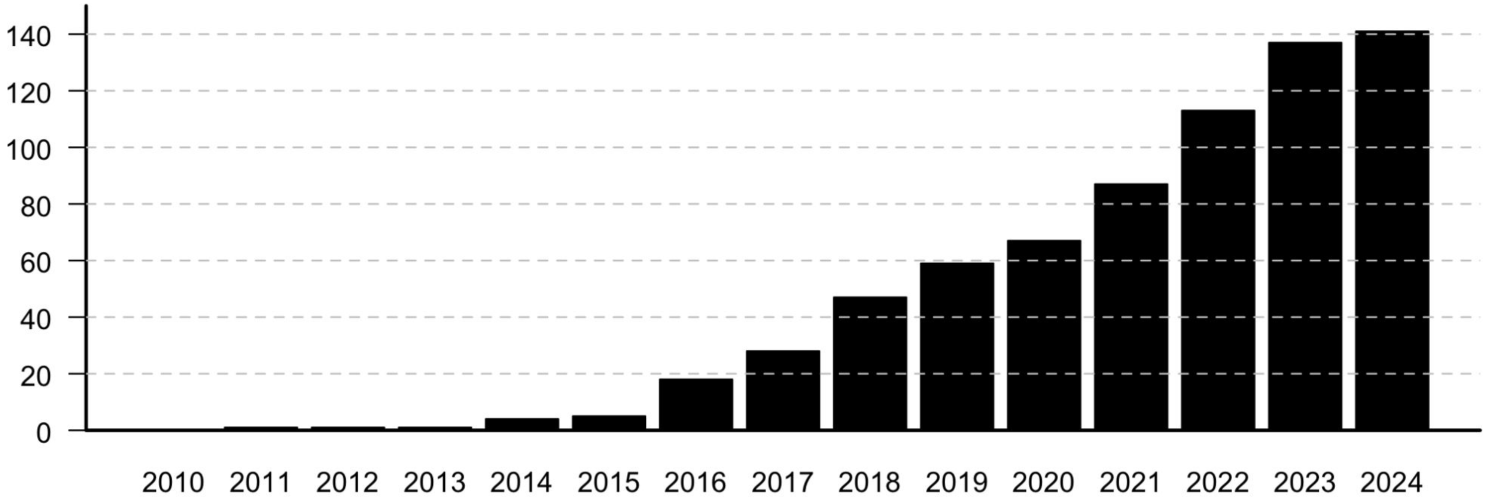
Pseudotime – abstract unit of progress

Distance between a cell and the start of the trajectory



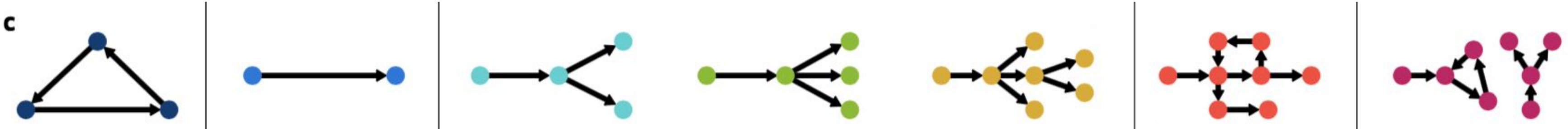
Trajectory inference tools

Number of cell ordering tools by year



Zappia et al. 2021. **Genome Biology**. PMID: 34715899.
https://github.com/scRNA-tools/scRNA-tools (extracted 2025.03.31, by Precisium AI)

Main trajectory types



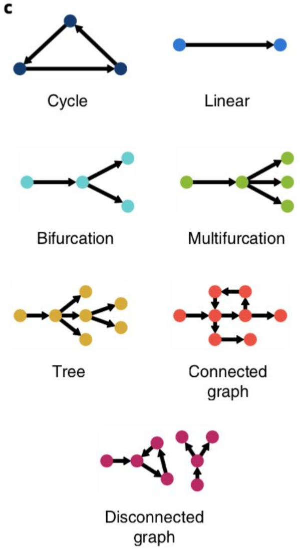
	Cycle	Linear	Bifurcation	Multifurcation	Tree	Connected graph	Disconnected graph
Examples	Cell cycle, circadian rhythm, activation/ deactivation cycles	Transition events during embryogenesis	Most cell differentiation processes. Immune cell maturation in bone marrow. Differentiation of epithelial cells from stem cells.			Stem cell niches	Multiple cell type differentiation

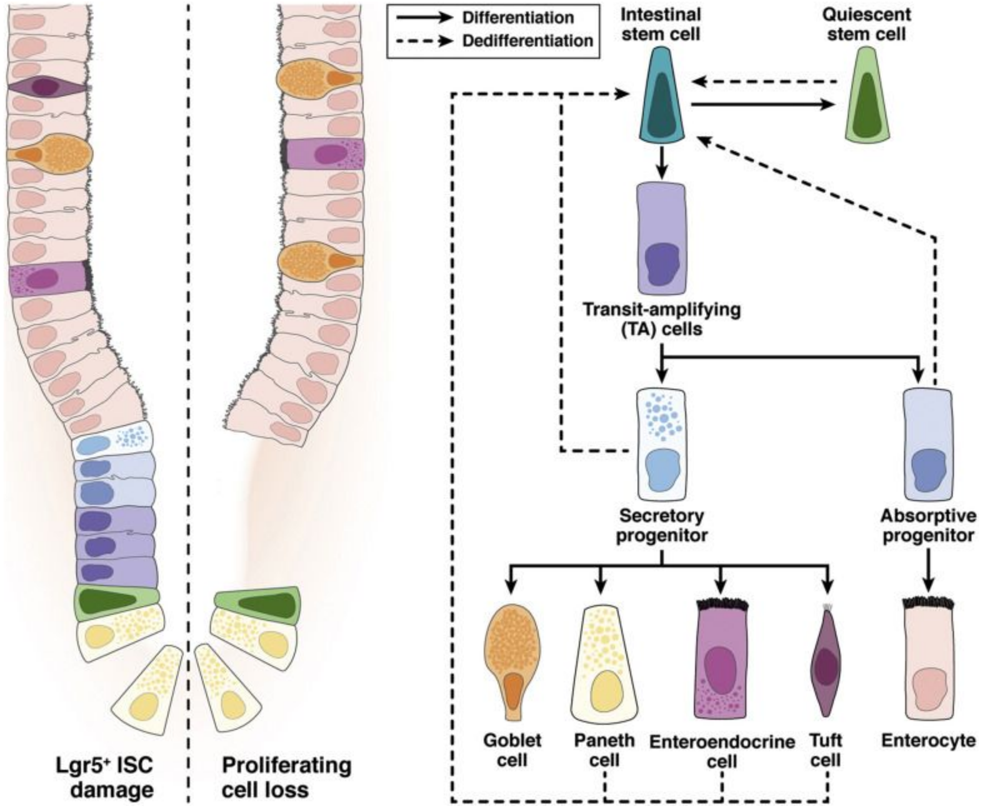
Saelens et al. 2019. **Nat Biotechnol**. PMID: 30936559.

Examples of biological trajectories

Examples: Intestinal epithelial differentiation

Can you split your trajectory into smaller / simpler trajectories?

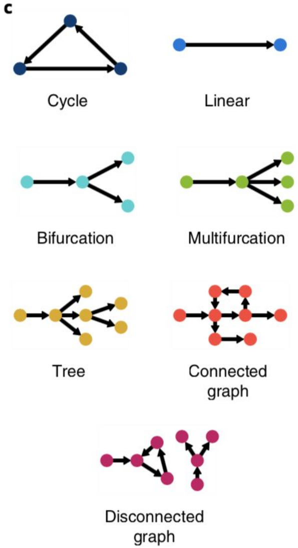


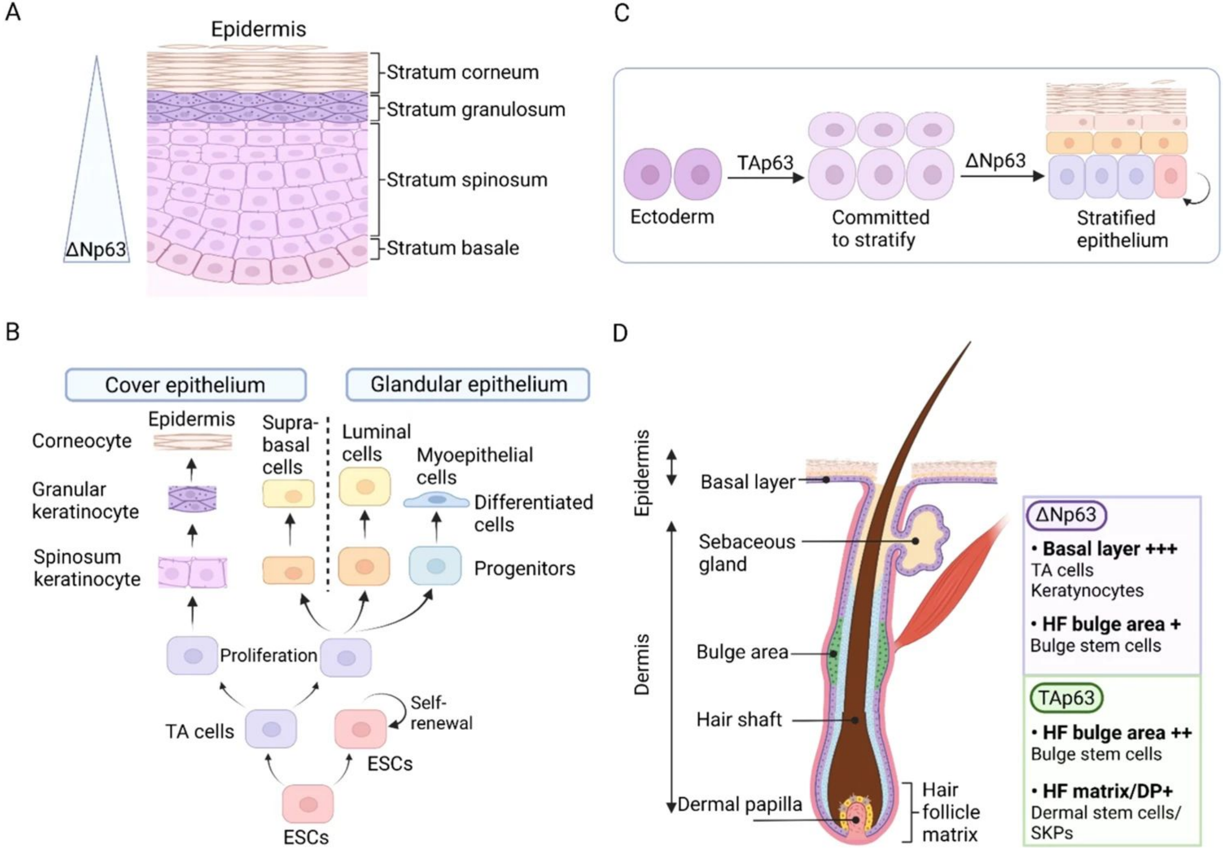


Meyer et al. 2022. **Mol Gastroenterol Hepatol.** PMID: 34915204.

Examples: Skin epithelial differentiation

Can you split your trajectory into smaller / simpler trajectories?

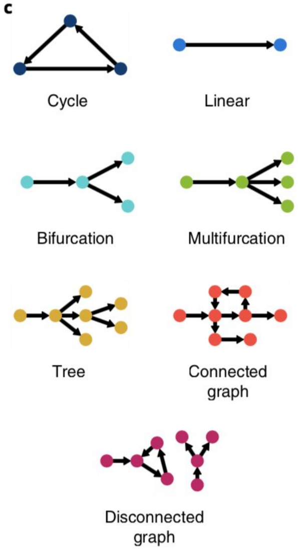


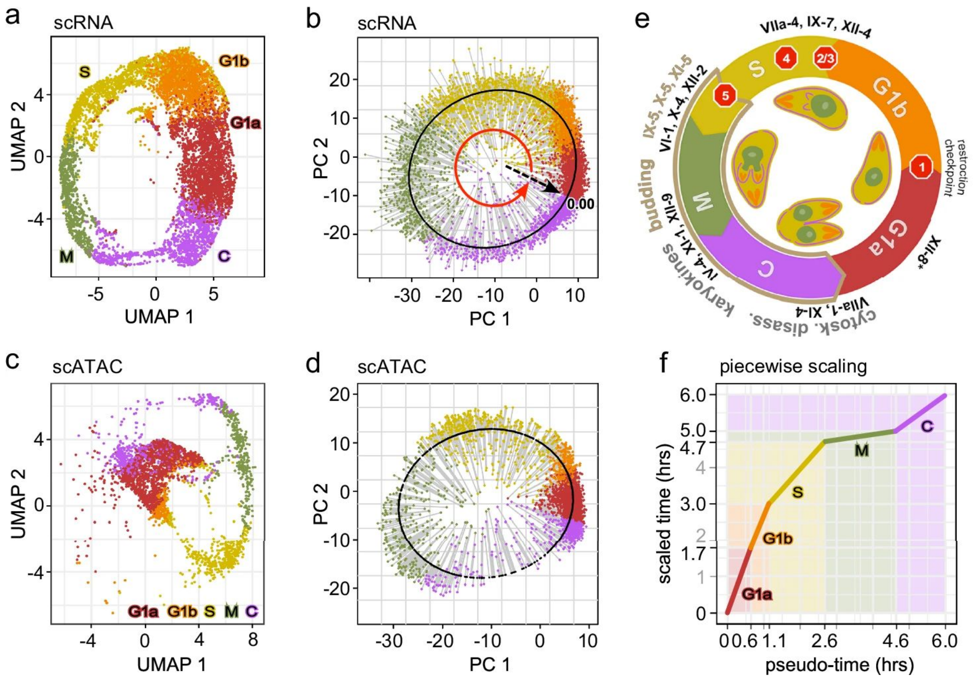


Li et al. 2023. **Oncogene.** PMID: 37848625.

Examples: *T. gondii* cell cycle

Can you split your trajectory into smaller / simpler trajectories?

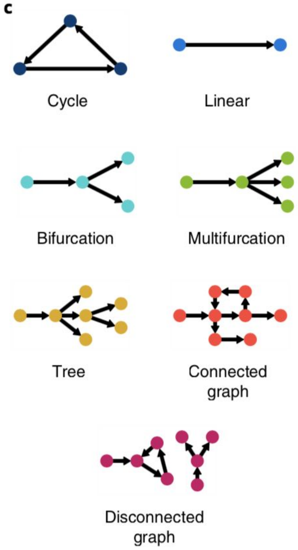


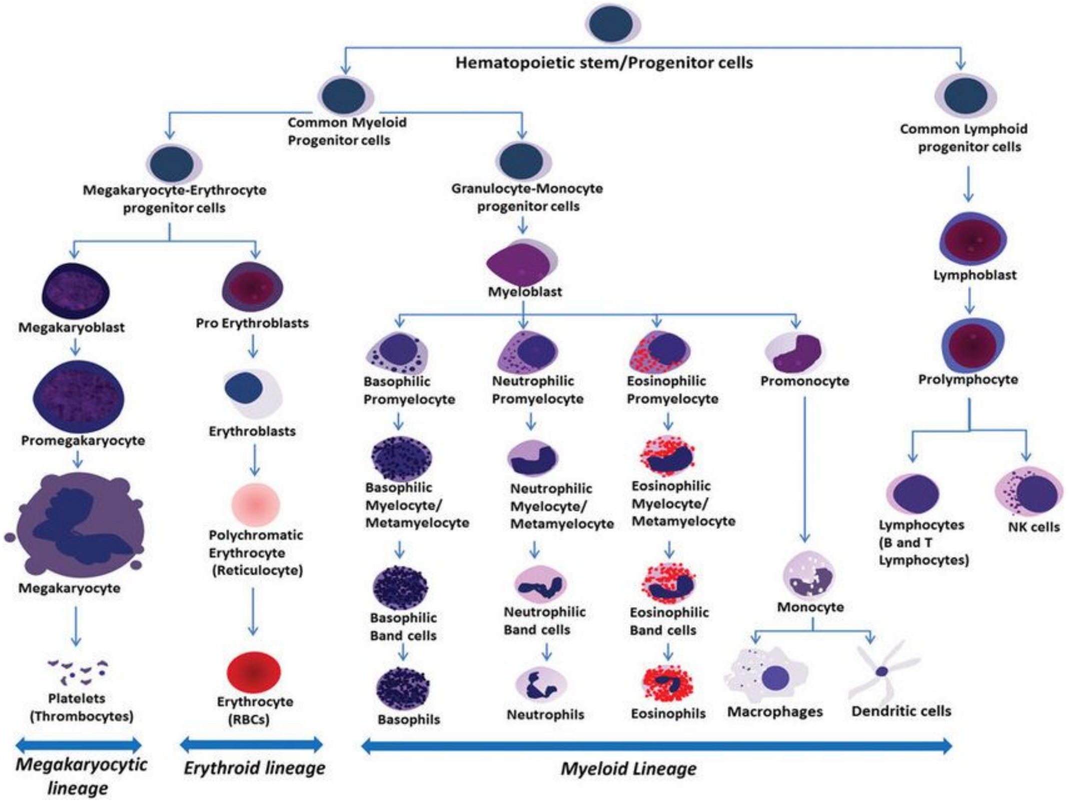


Lou et al. 2024. **Nat Commun.** PMID: 39198388.

Examples: Bone marrow immune differentiation

Can you split your trajectory into smaller / simpler trajectories?

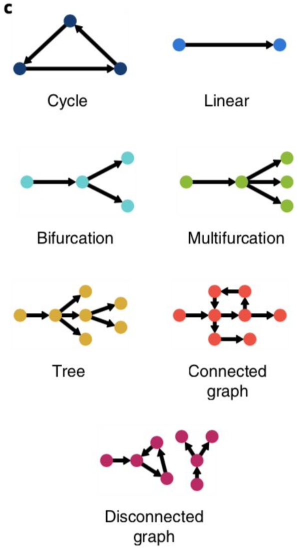


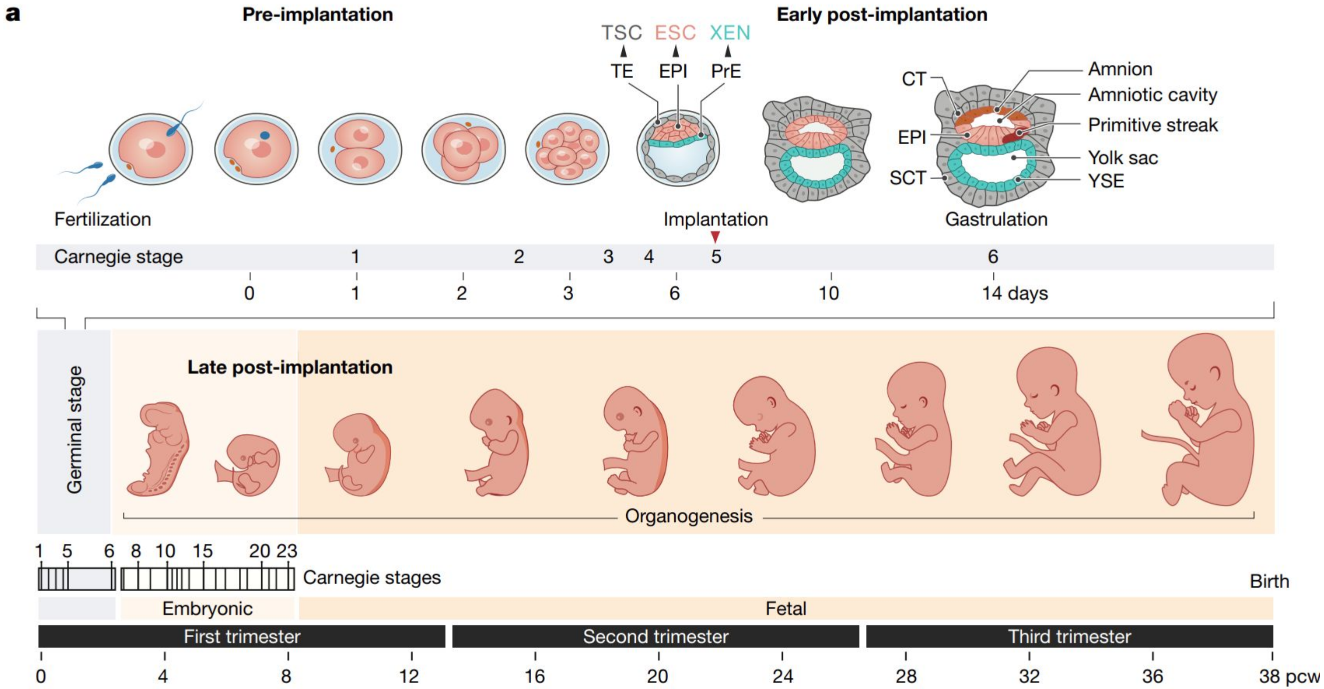


Mahalingaiah et al. 2018. **Curr Protoc Toxicol.** PMID: 30040226.

Examples: Embryonic development

Can you split your trajectory into smaller / simpler trajectories?

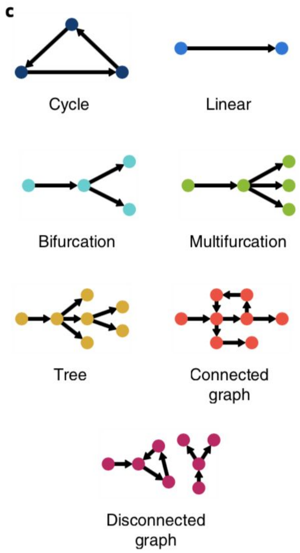


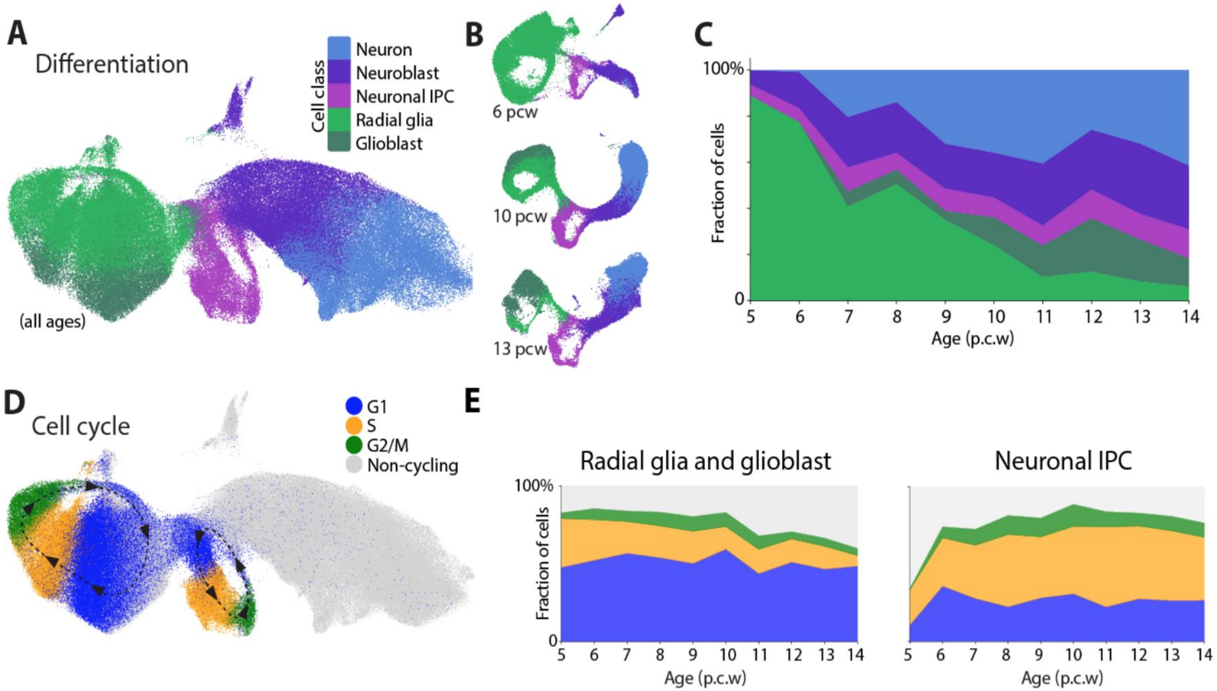


Haniffa et al. 2021. **Nature.** PMID: 34497388.

Examples: Embryonic neuron progenitors

Can you split your trajectory into smaller / simpler trajectories?

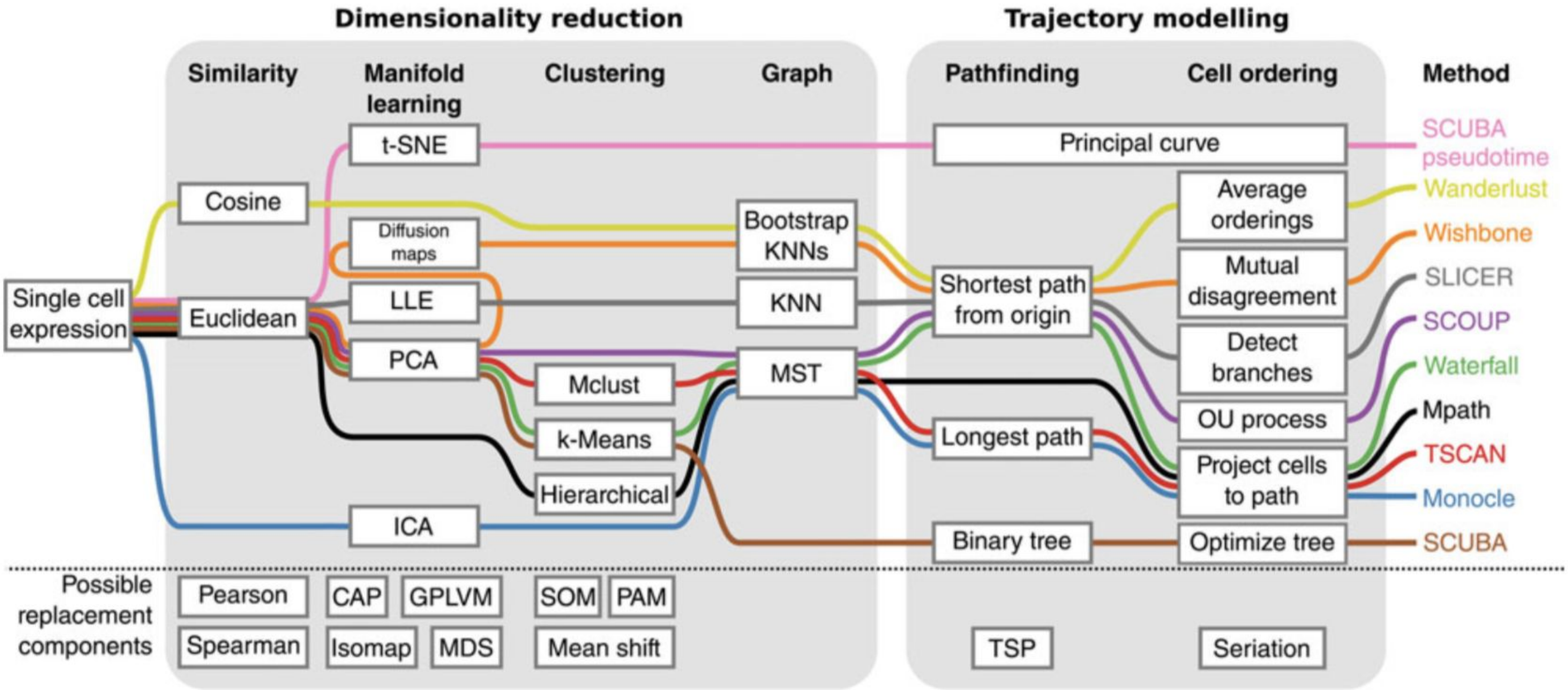




Braun et al. 2023. **Science.** PMID: 37824650.

How do trajectory inference methods work?

Trajectory method overview



Cannoodt et al. 2016. **Eur J Immunol**. PMID: 27682842

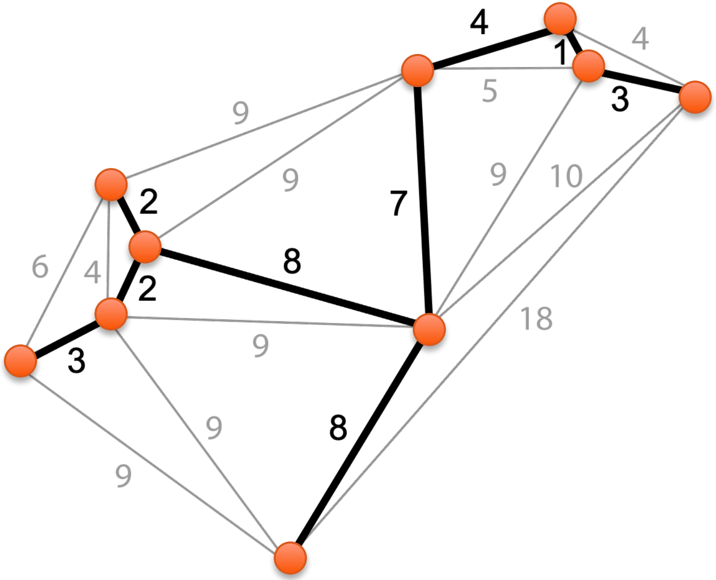
Minimum spanning tree (MST)

Given a set of points, how do we connect them so that the total sum of all distances is minimized?

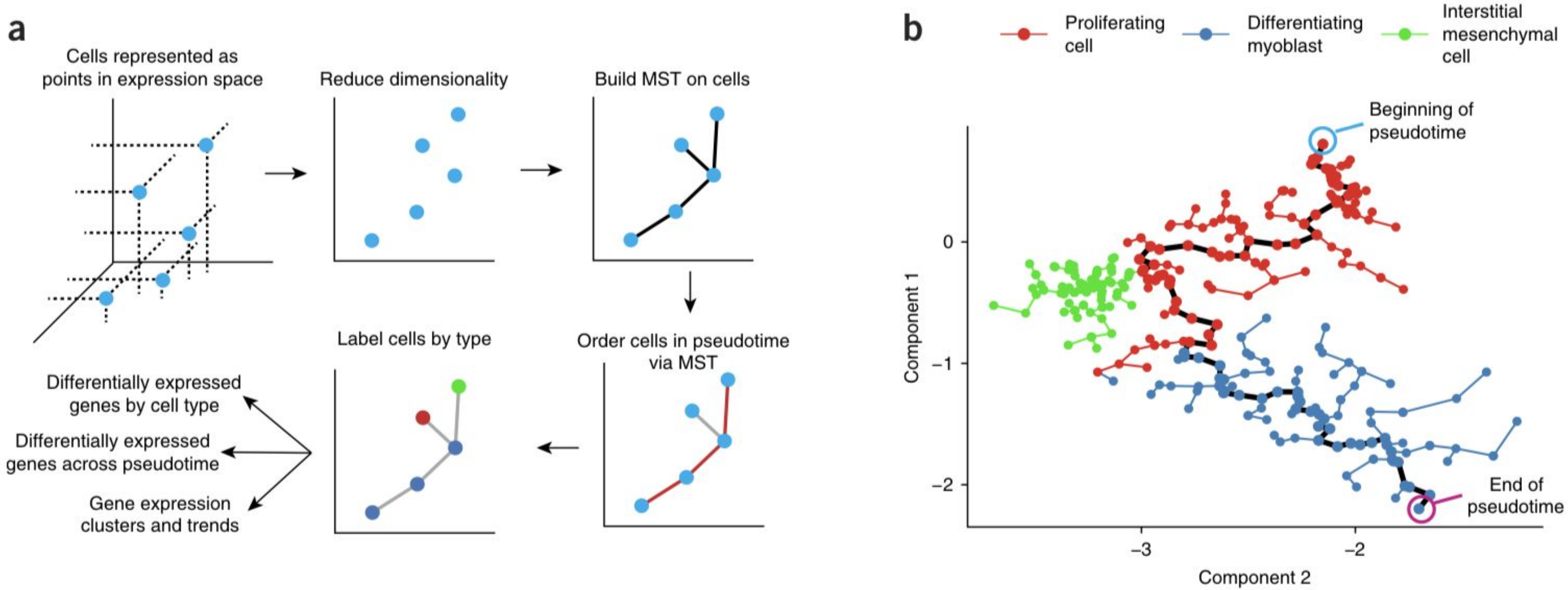
Having more transitional cells improves the definition of the tree

The weights can be the distance is the ICA space or a correlation between cells, etc.

By definition, a MST has no cycles
So you cannot use MST to define cyclic trajectories (i.e. cell cycle)

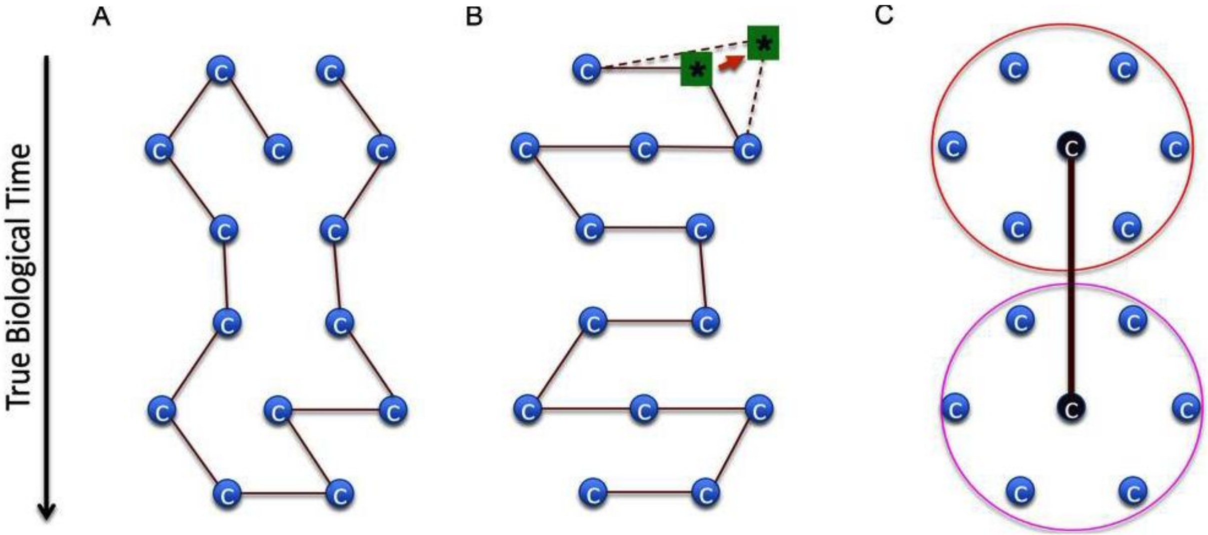


Monocle v1



Trapnell et al. 2023. **Nat Biotech.** PMID: 24658644.

The limitation of MST

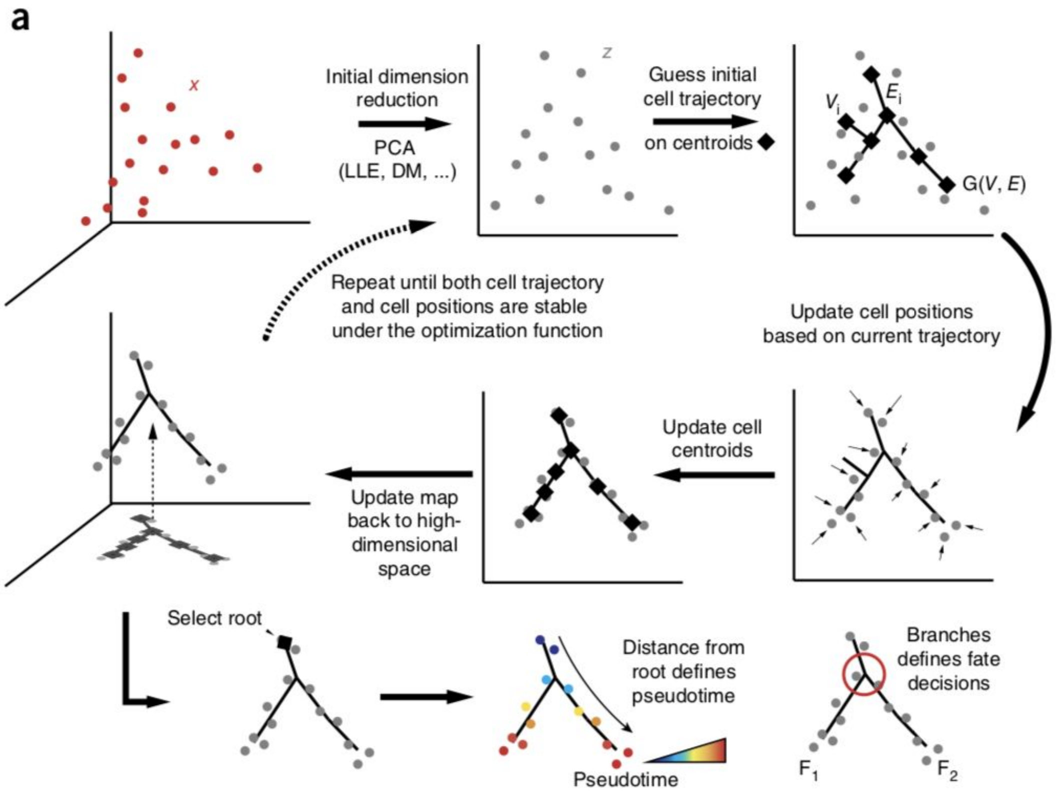


🔗 Zhicheng et al (2016) *Nuc Acid Res*

Trajectory construction using MST is highly dependent on single data points

Zhicheng et al (2016) **Nuc Acid Res**. PMID: 27179027

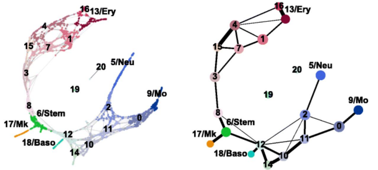
Monocle v2



Qiu et al. 2017. **Nat Methods.** PMID: 28825705.

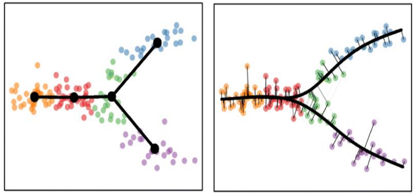
Similar trajectory methods

PAGA



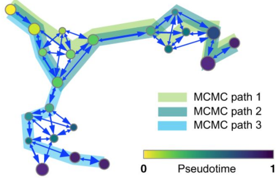
Wolf et al (2019) **Genome Biology.**
PMID: 30890159

Slingshot



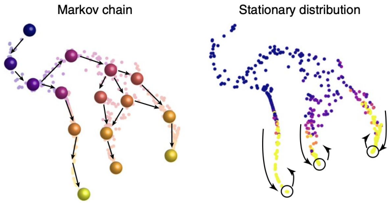
Street et al (2018) **BMC Genomics.**
PMID: 29914354

VIA



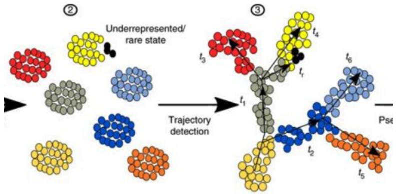
Stassen et al (2021) **Nat Commun.**
PMID: 34545085

Palantir



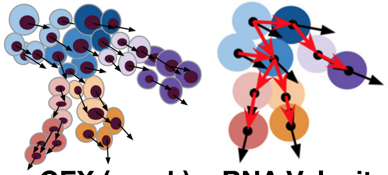
Setty et al (2019) **Nat Biotech.**
PMID: 30899105

Cell Router



Da Rocha et al (2018) **Nat Commun.**
PMID: 29497036

CellPath



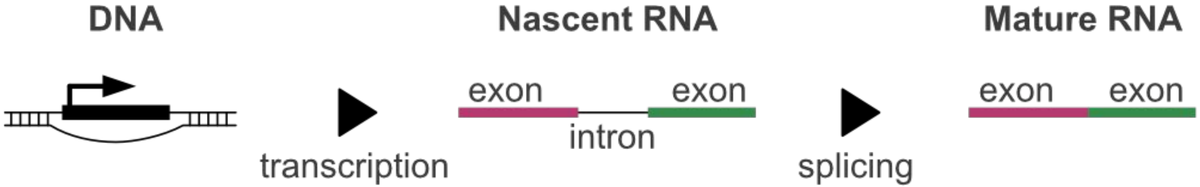
GEX (graph) + RNA Velocity

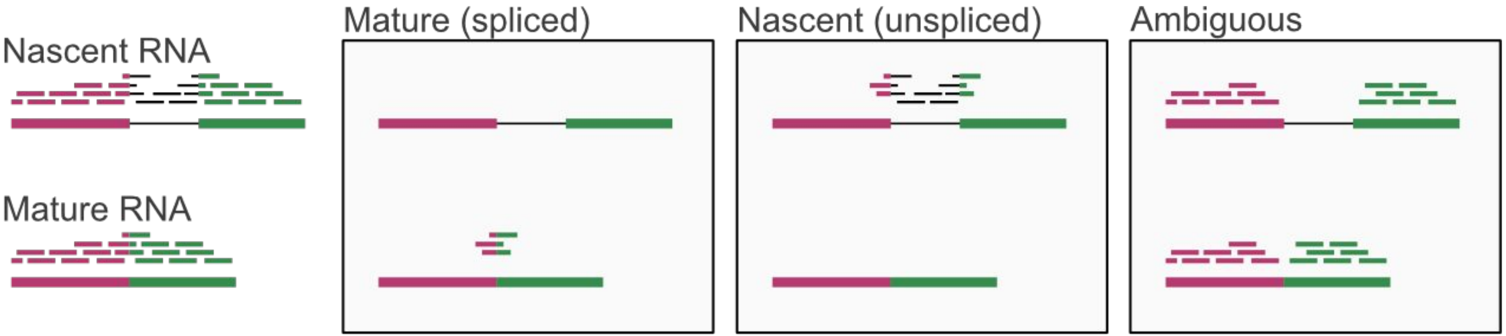
Zhang et al (2021) **Cell Rep. Methods.**
PMID: 35474895

RNA velocity

RNA maturation and quantification

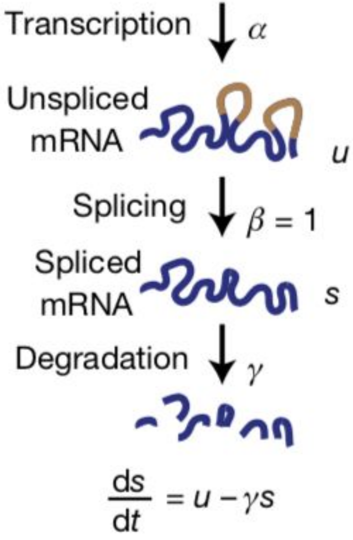
Variations in nascent RNA (unspliced) **are followed** by variations in mature RNA (spliced).



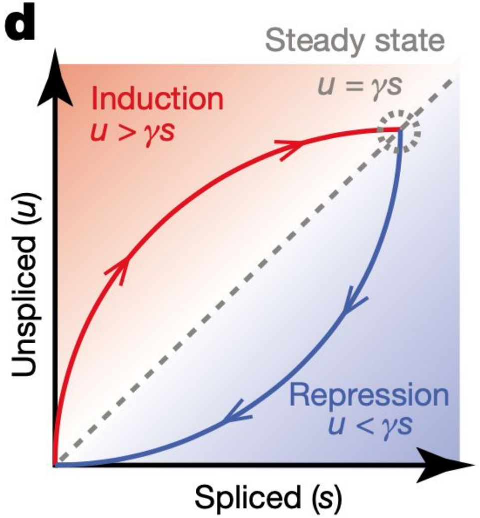


RNA velocity principle

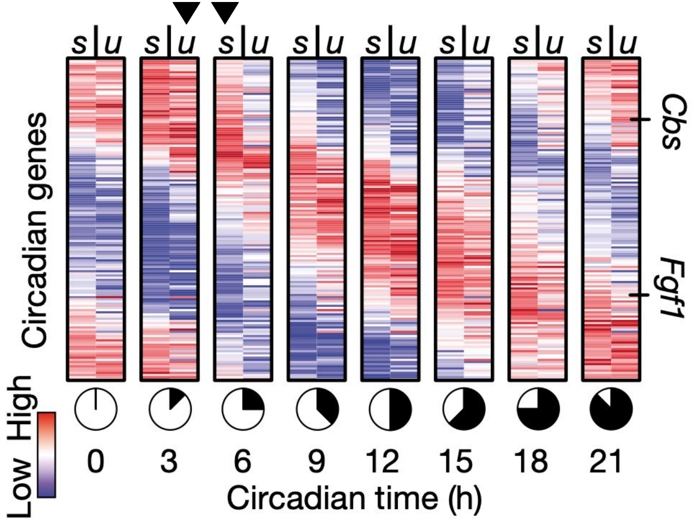
Variations in nascent RNA (unspliced) **are followed** by variations in mature RNA (spliced).



$$\frac{ds}{dt} = u - \gamma s$$



similar!



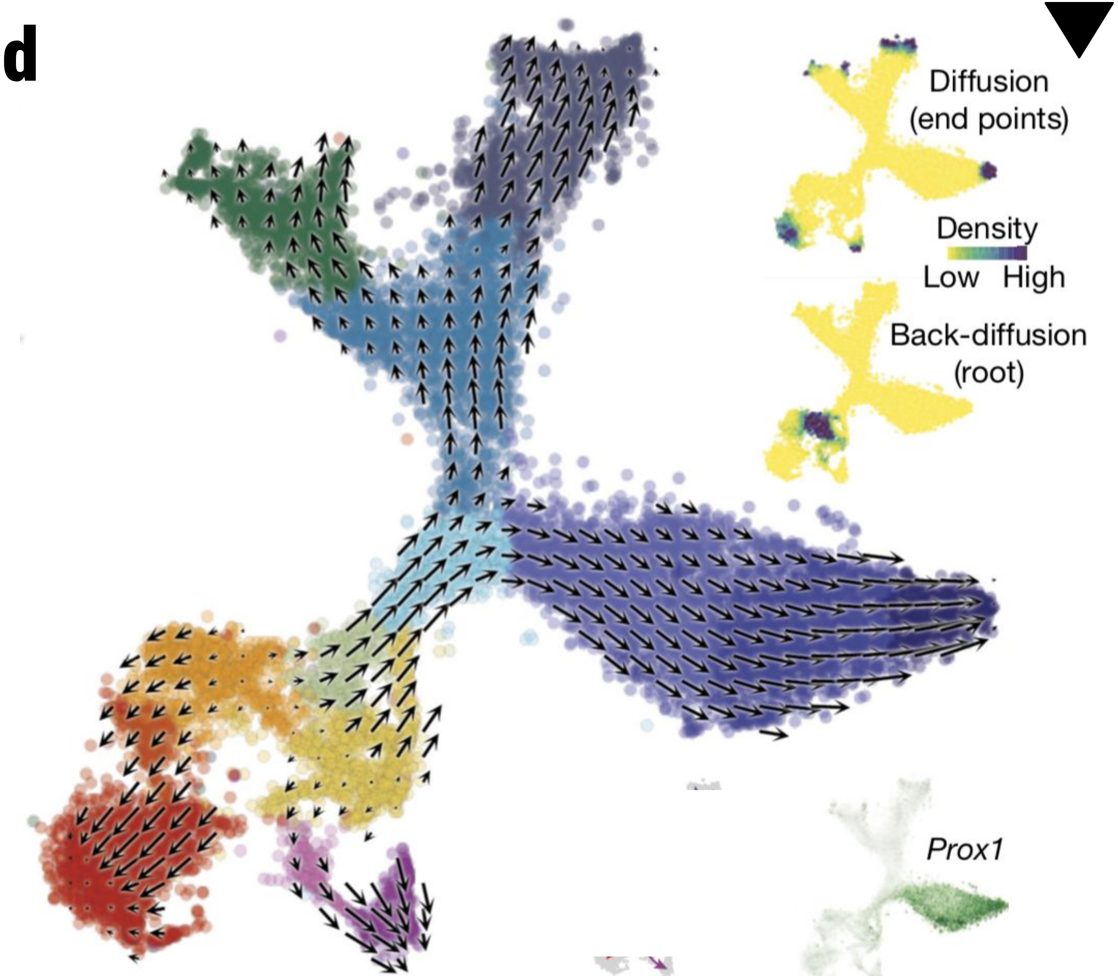
La Manno et al. 2018. **Nature**. PMID: 30089906

RNA velocity vector field

RNA velocity allows a cell-driven identification of cell transcriptional trajectories.

It can help define the start, ends and bifurcations of the trajectory path.

The arrow points to the position of the future state, extrapolated from velocity estimates.

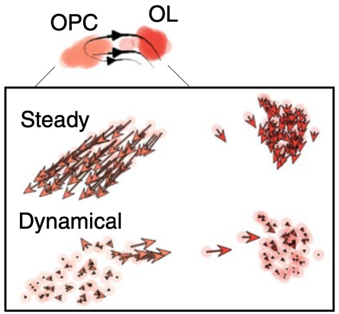


La Manno et al. 2018. **Nature**. PMID: 30089906

New flavors of RNA velocity

scVelo

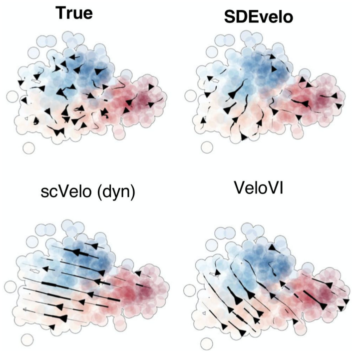
Introduces dynamic modeling



Bergen et al (2020) **Nat Biotech.**
PMID: 32747759

SDEvelo

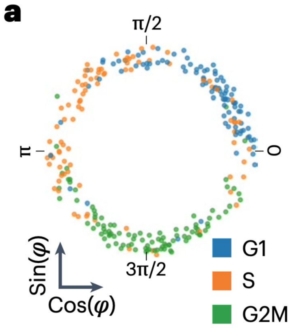
Do not force velocity in control steady state



Liao et al (2024) **Nat Commun.**
PMID: 39738101

VeloCycle

Improved velocity in cell cycles

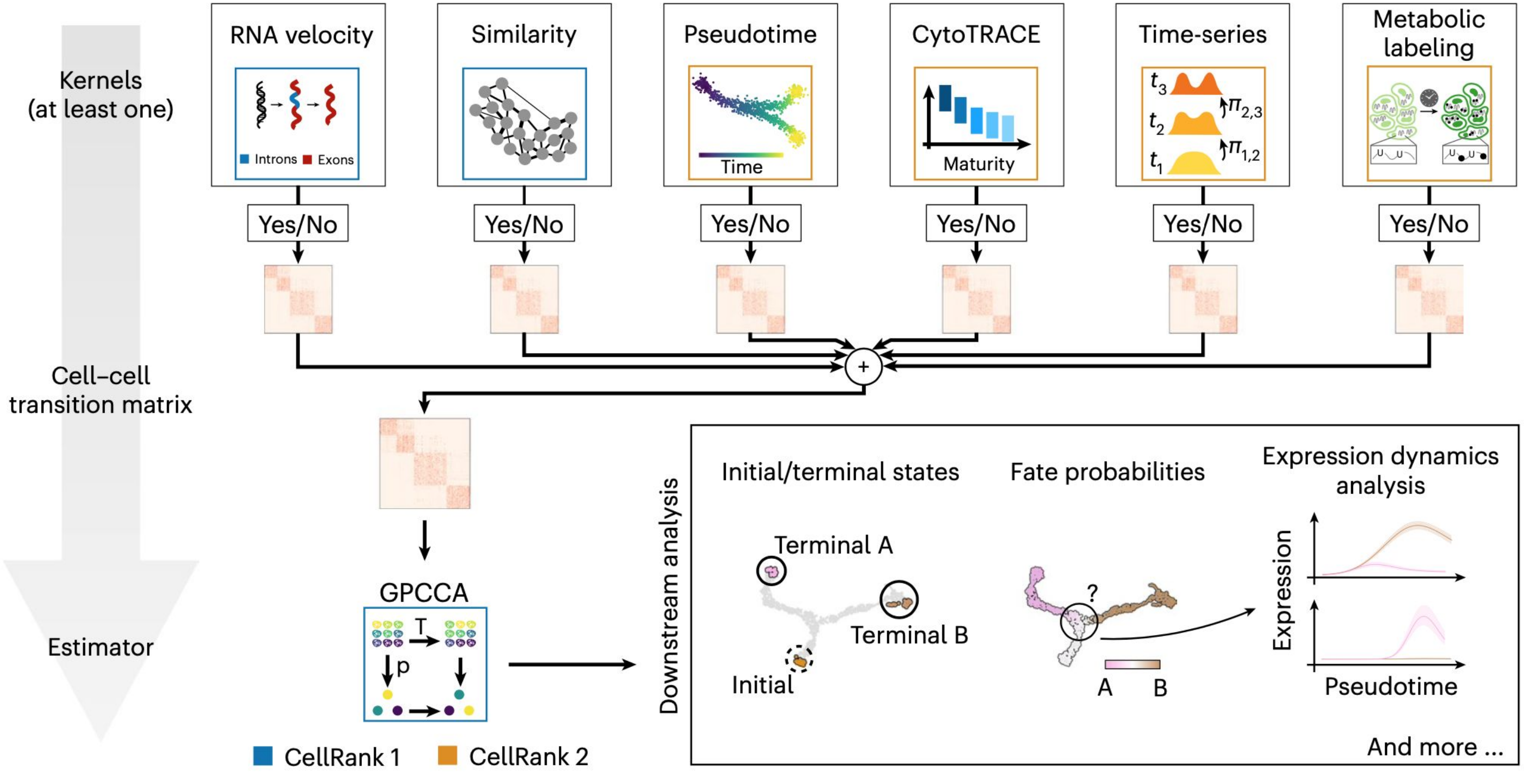


Lederer et al (2024) **Nat Methods.**
PMID: 39482463

Other methods: GeneTrajectory UniTVelo TFvelo VeloVI DeepCycle DeepVelo VeloAE

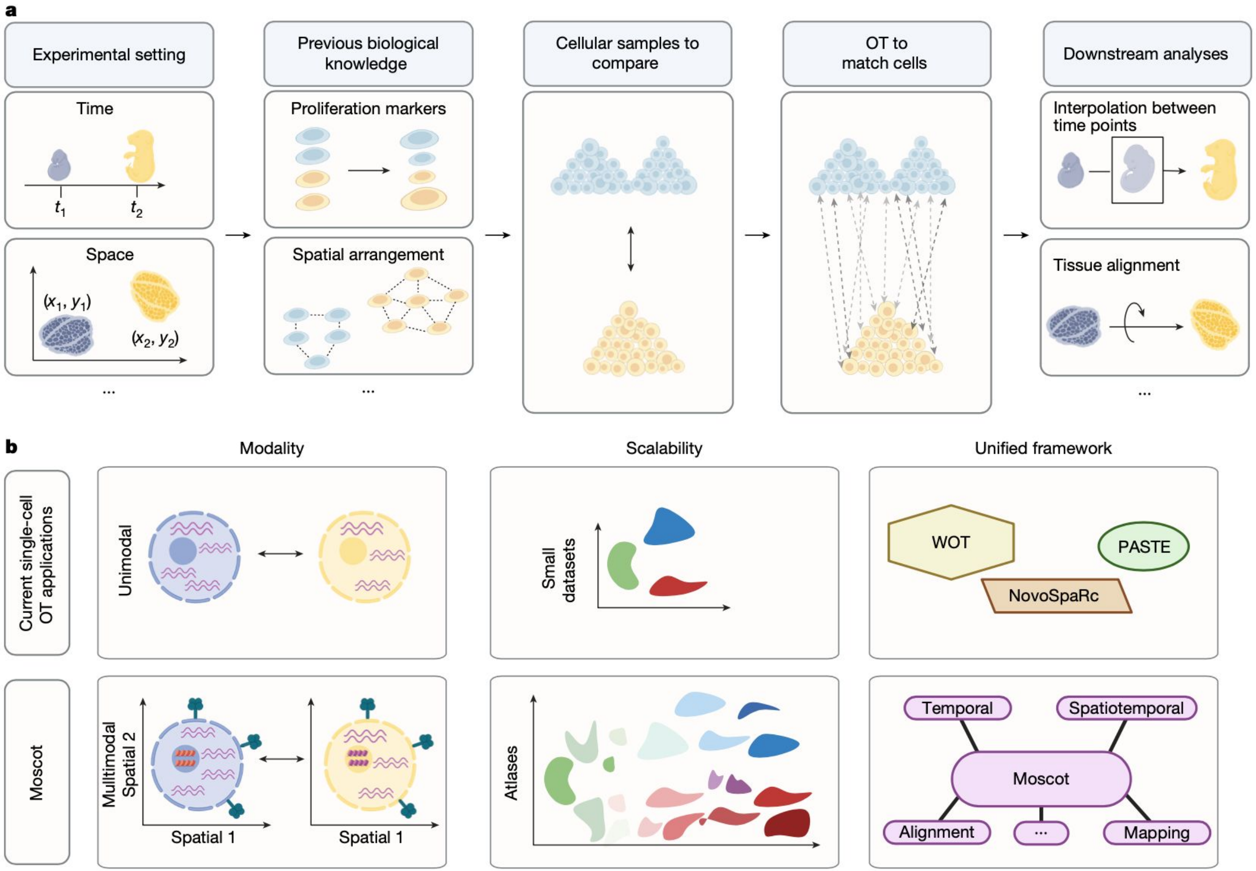
Multimodal trajectory inference tools

CellRank 2



Weiler et al. 2024. **Nature Methods**. PMID: 38871986

Moskot

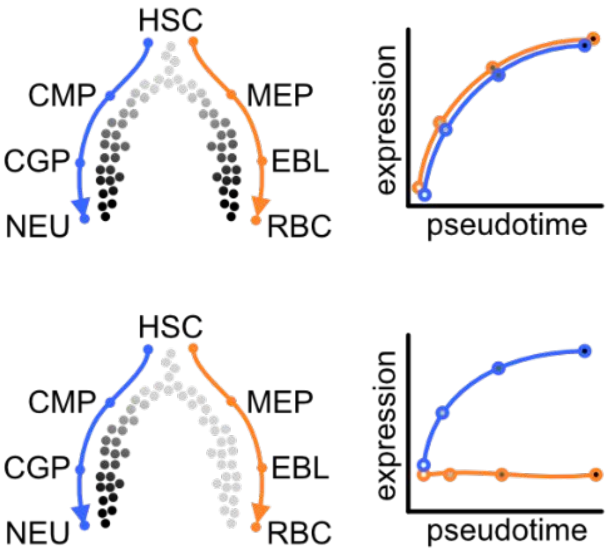


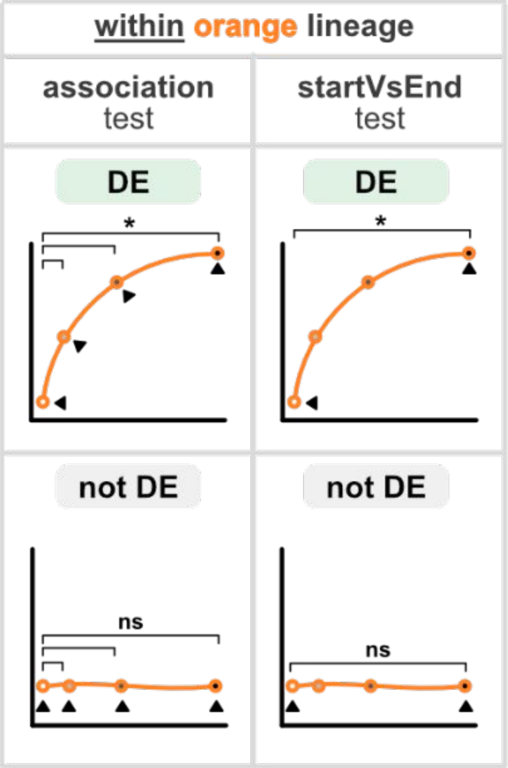
Klein et al. 2025. **Nature**. PMID: 39843746

Paulo Czarnewski, Precisium AI

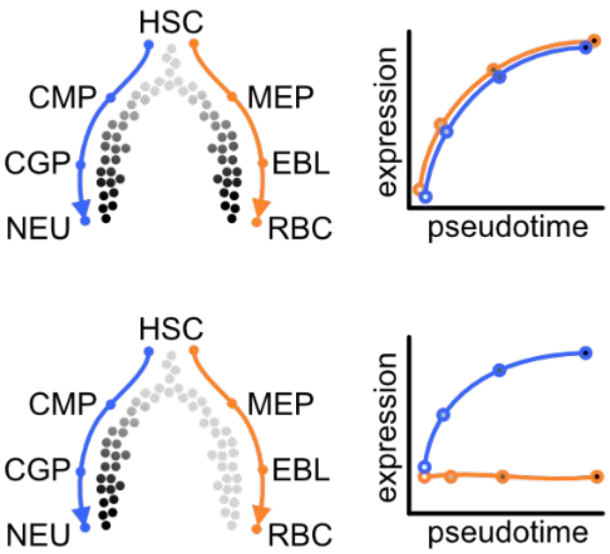
Differential Expression along trajectories

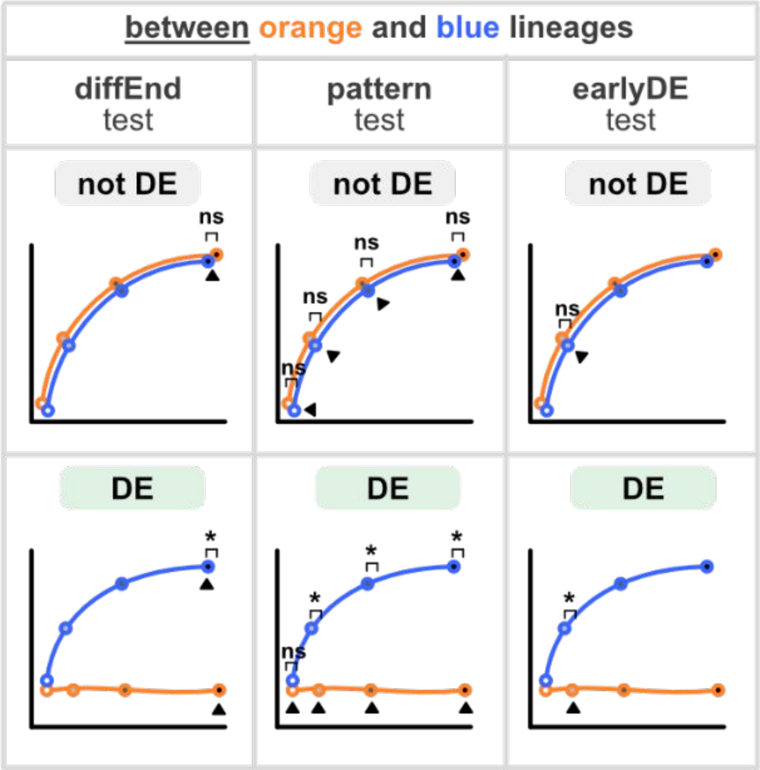
Comparison within lineage



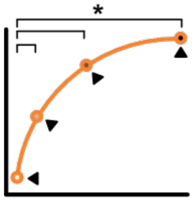


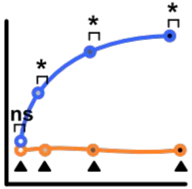
Comparison **between** lineages





Differential expression overview



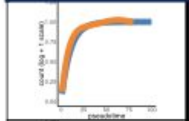
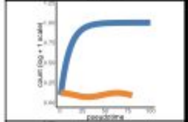
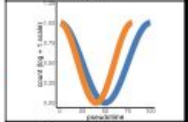
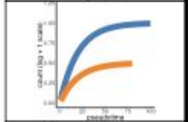
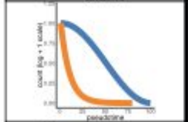
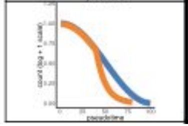


Type	Test name	What is being tested	Analogy
within lineage	**association** test	Are there any differences at any time point along lineage X?	Think as an **ANOVA test** across pseudotime in 1 lineage
	startVsEnd test	What are the differences between the start and end within lineage X?	Think as an **Student t-test** between start and end in 1 lineage
between lineages	**diffEnd** test	What are the differences at the ends between lineage X and Y?	Think as an **Student t-test** between the ends of 2 lineages
	pattern test	Are there any differences at any time point between lineage X and Y?	Think as an **ANOVA test** between pseudotime of 2 lineages
	earlyDE test	What are the differences at the early phase between lineage X and Y?	Think as an **Student t-test** between the early phase of 2 lineages

TradeSeq

There aren’t many dedicated tools for DGE along trajectories. Many toolkits (i.e. PAGA, Monocle, CellRank) include DGE inside their package, but these are somewhat limited.

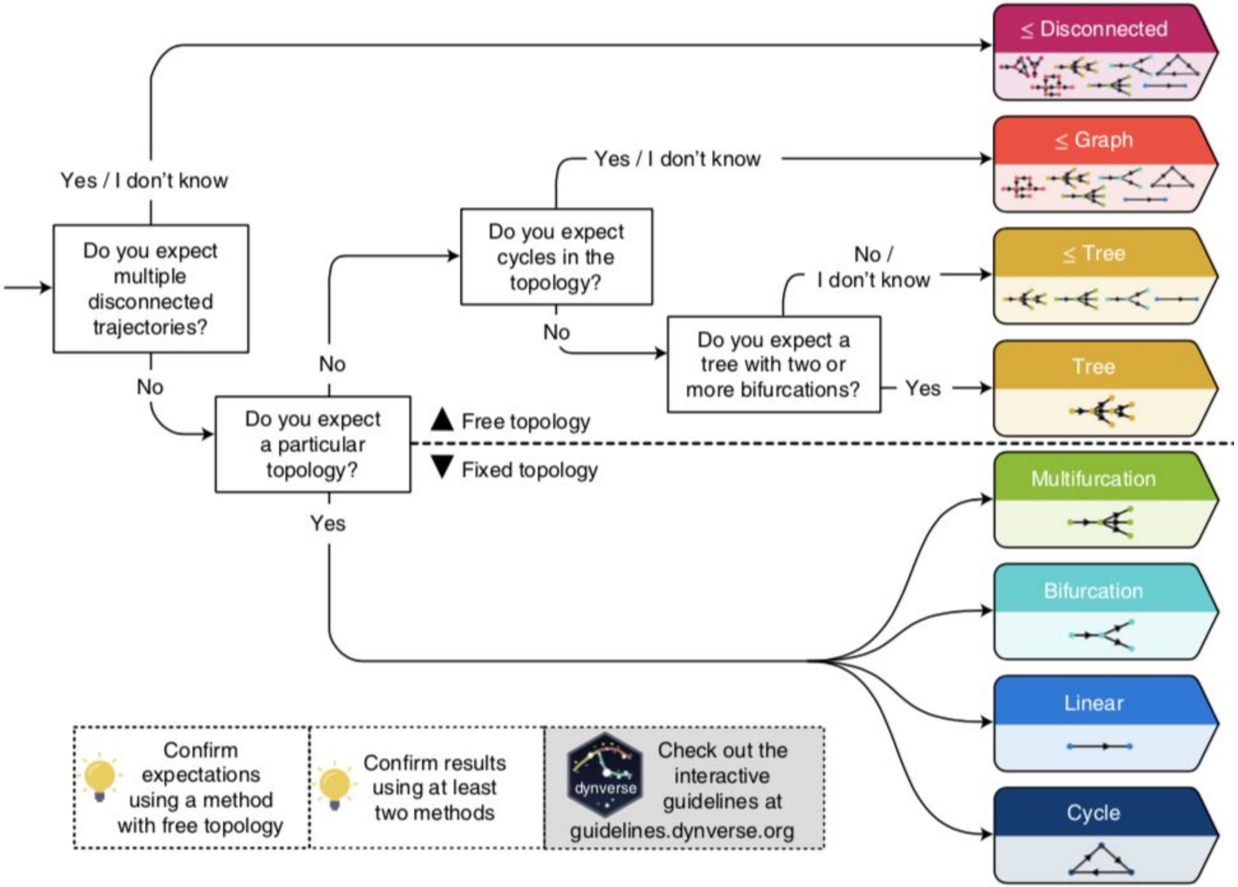
Recommended:
- TradeSeq (R)
- TradeSeq-py (Python, new!)

	Within the orange lineage		Between the orange and blue lineages		
Lineages	**association Test**	**startVsEnd Test**	**diffEnd Test**	**pattern Test**	**earlyDE Test**
	DE	DE	Not DE	Not DE	Not DE
	Not DE	Not DE	DE	DE	DE
	DE	Not DE	Not DE	Not DE	Not DE
	DE	DE	DE	DE	Not DE
	DE	DE	Not DE	DE	DE
	DE	DE	Not DE	DE	Not DE

Berge et al. 2020. **Nat Commun**. PMID: 32139671

Wrap up

Choosing your trajectory method



Do you expect multiple disconnected trajectories?

Yes / I don't know → ≤ Disconnected

No → Do you expect a particular topology?

No → Do you expect cycles in the topology?

Yes / I don't know → ≤ Graph

No → Do you expect a tree with two or more bifurcations?

No / I don't know → ≤ Tree

Yes → Tree

▲ Free topology

▼ Fixed topology

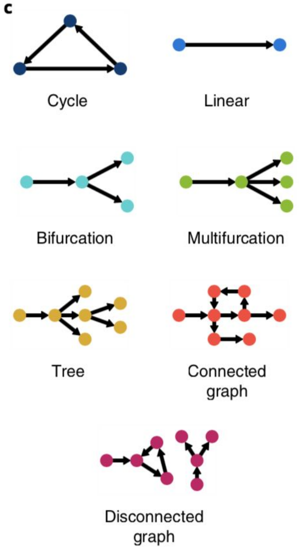
Yes → Multifurcation, Bifurcation, Linear, Cycle

Confirm expectations using a method with free topology

Confirm results using at least two methods

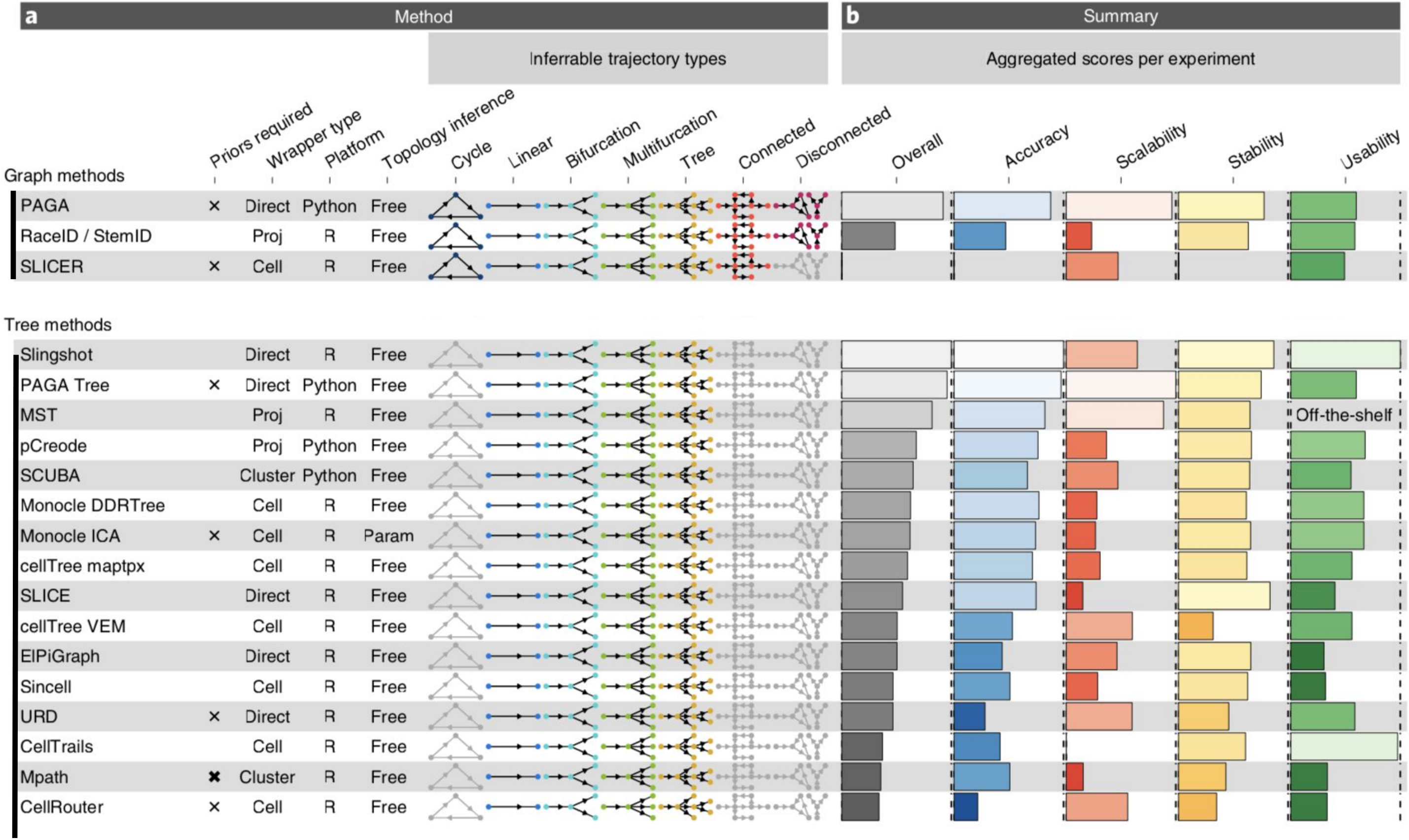
dynverse Check out the interactive guidelines at guidelines.dynverse.org

Can you split your trajectory into smaller / simpler trajectories?



Saelens et al. 2019. **Nat Biotechnol**. PMID: 30936559.

Which method should I use?



Graph methods

	Priors required	Wrapper type	Platform	Topology inference	Usability
PAGA	×	Direct	Python	Free	
RaceID / StemID		Proj	R	Free	
SLICER	×	Cell	R	Free	

Tree methods

	Priors required	Wrapper type	Platform	Topology inference	Usability
Slingshot		Direct	R	Free	
PAGA Tree	×	Direct	Python	Free	
MST		Proj	R	Free	Off-the-shelf
pCreode		Proj	Python	Free	
SCUBA		Cluster	Python	Free	
Monocle DDRTree		Cell	R	Free	
Monocle ICA	×	Cell	R	Param	
cellTree maptpx		Cell	R	Free	
SLICE		Direct	R	Free	
cellTree VEM		Cell	R	Free	
ElPiGraph		Direct	R	Free	
Sincell		Cell	R	Free	
URD	×	Direct	R	Free	
CellTrails		Cell	R	Free	
Mpath	✖	Cluster	R	Free	
CellRouter	×	Cell	R	Free	

Saelens et al. 2019. **Nat Biotechnol**. PMID: 30936559.
http://guidelines.dynverse.org

Key considerations

- Are you sure that you have a trajectory?
- Do you have intermediate states?
- Do you believe that you have branching in your trajectory?
- Can you split your trajectory into smaller trajectories?
- Be aware, any dataset can be forced into a trajectory without any biological meaning!
- First make sure that gene set and dimensionality reduction captures what you expect.

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Precision Medicine at the Atlas Level

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