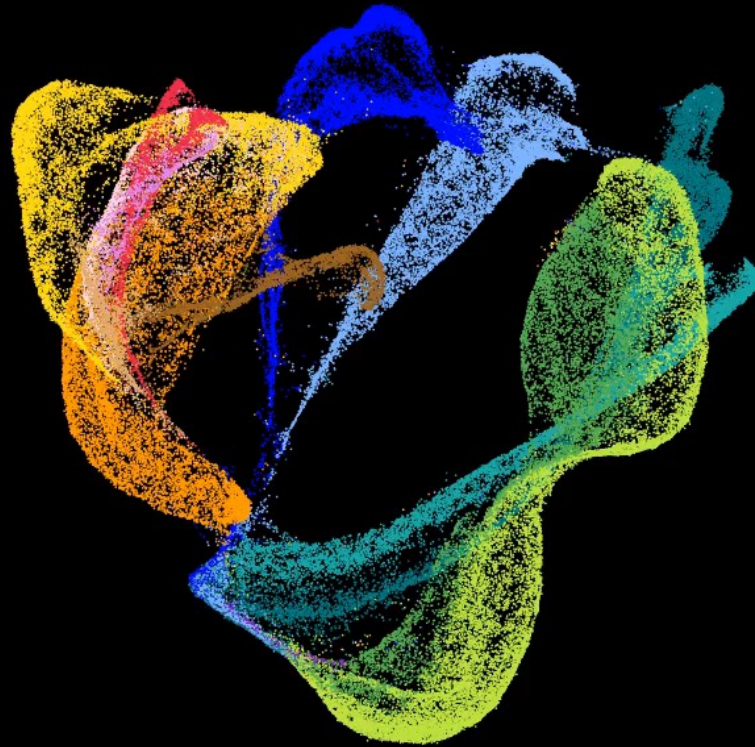


Phenotyping at Single Cell Resolution



Philip Benfey
Duke University, HHMI & Raleigh Biosciences

Six years around the world



Sometimes a Great Notion



Ski bum



Iron mines



Gardening in Japan



Across the USSR



Maybe a day job



Paris in the winter



Graduate school and Post-doc



NYU



Duke

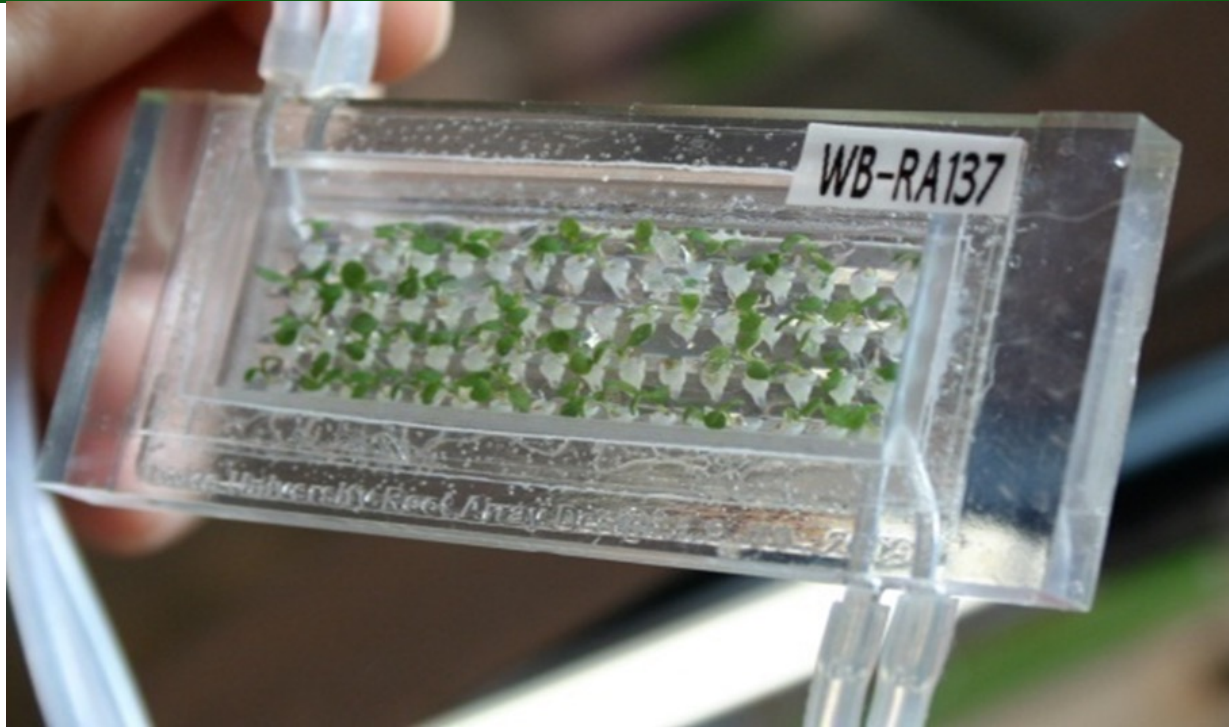
- Arrived 2002
- Chair of Biology 2002-2007
- Director NIH Center for Systems Biology 2007-2013
- HHMI Investigator 2012-present



Grassroots Biotechnology

Superior research. Superior crops.

GrassRoots' RootArray platform



- Study promoter function in real time in living plants
- High-throughput, semi-automated image acquisition and analysis
- Allowed rapid iteration



THE PROBLEM

Farmers need to increase food production in the presence of climate change and a growing population

Novel innovation will be needed:

1. Shift how we design crop varieties
2. Design climate resilient crops



THE CURRENT INDUSTRY SOLUTION

Utilize genomic data from whole plants or tissues

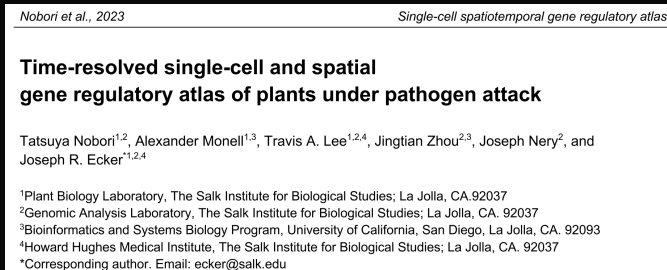
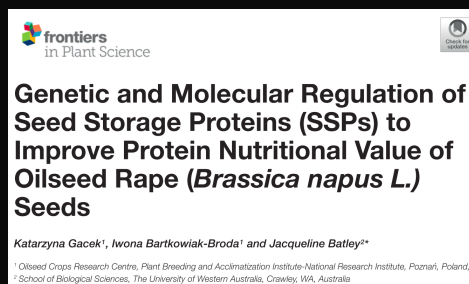
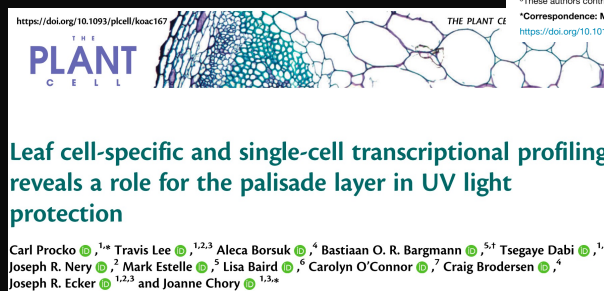
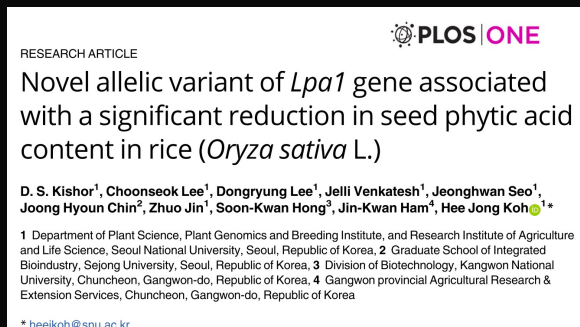
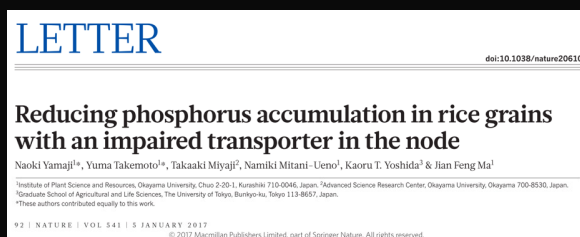
- Gene expression patterns can be obscured
- Lack of spatial specificity

THE RALEIGH BIOSCIENCES SOLUTION

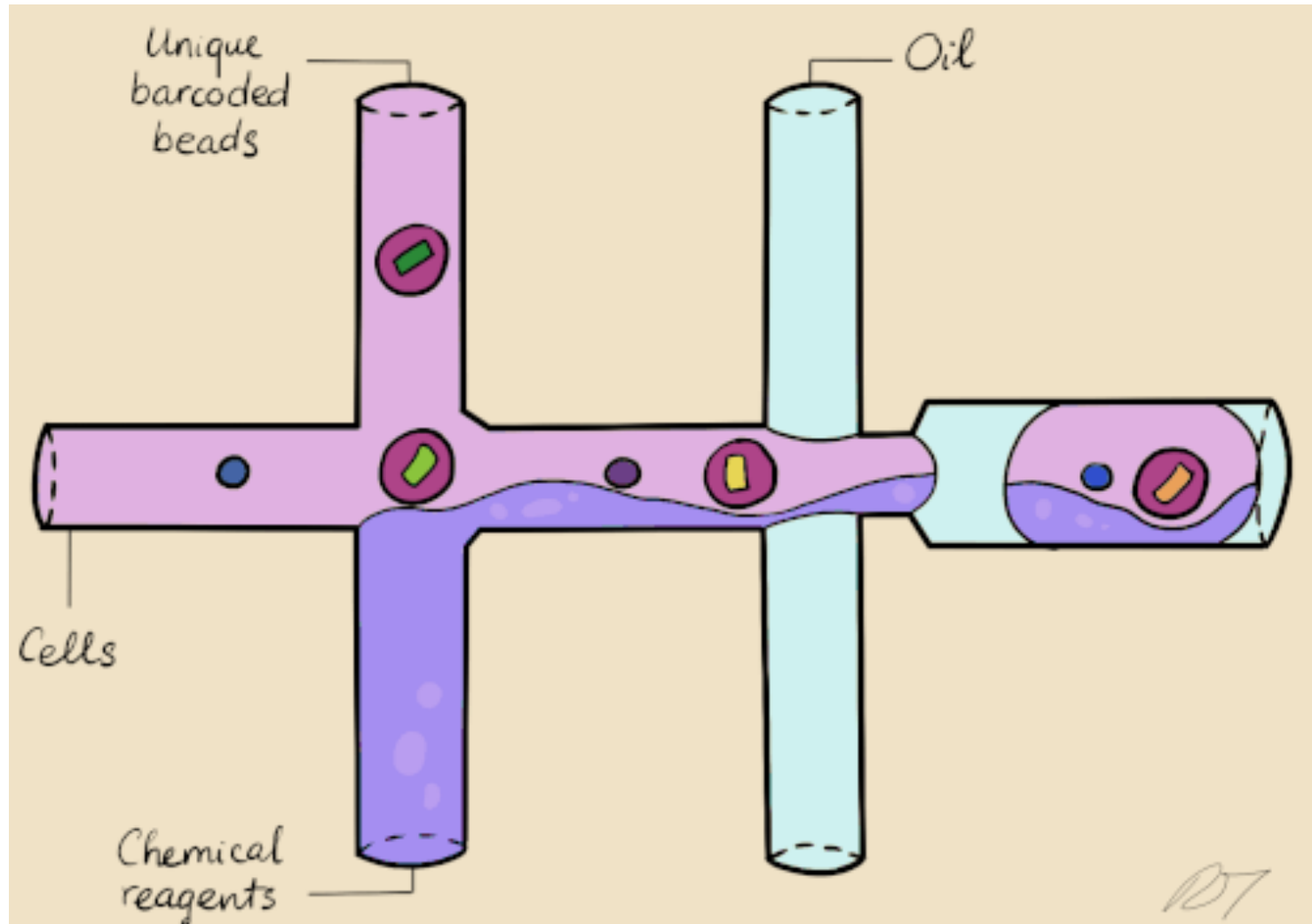
Utilize single cell and spatial genomics for crop design

- Expertise in utilizing single cell genomics in plants
 - Focused solely on plants
- Identify traits in cellular space and developmental time
 - Precise trait expression

Single cell genomics can identify genes with agronomic value

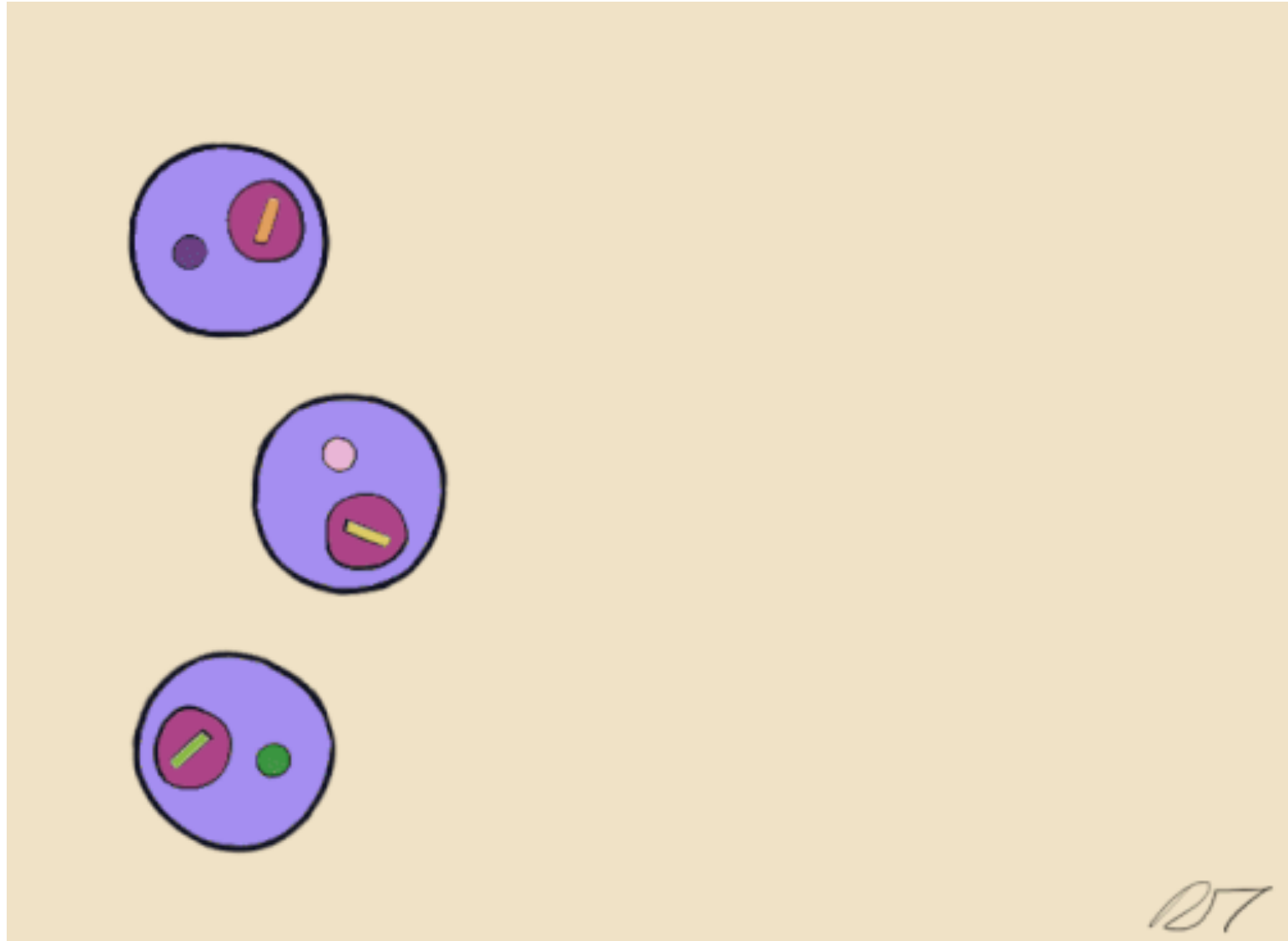


Single cell RNA-seq

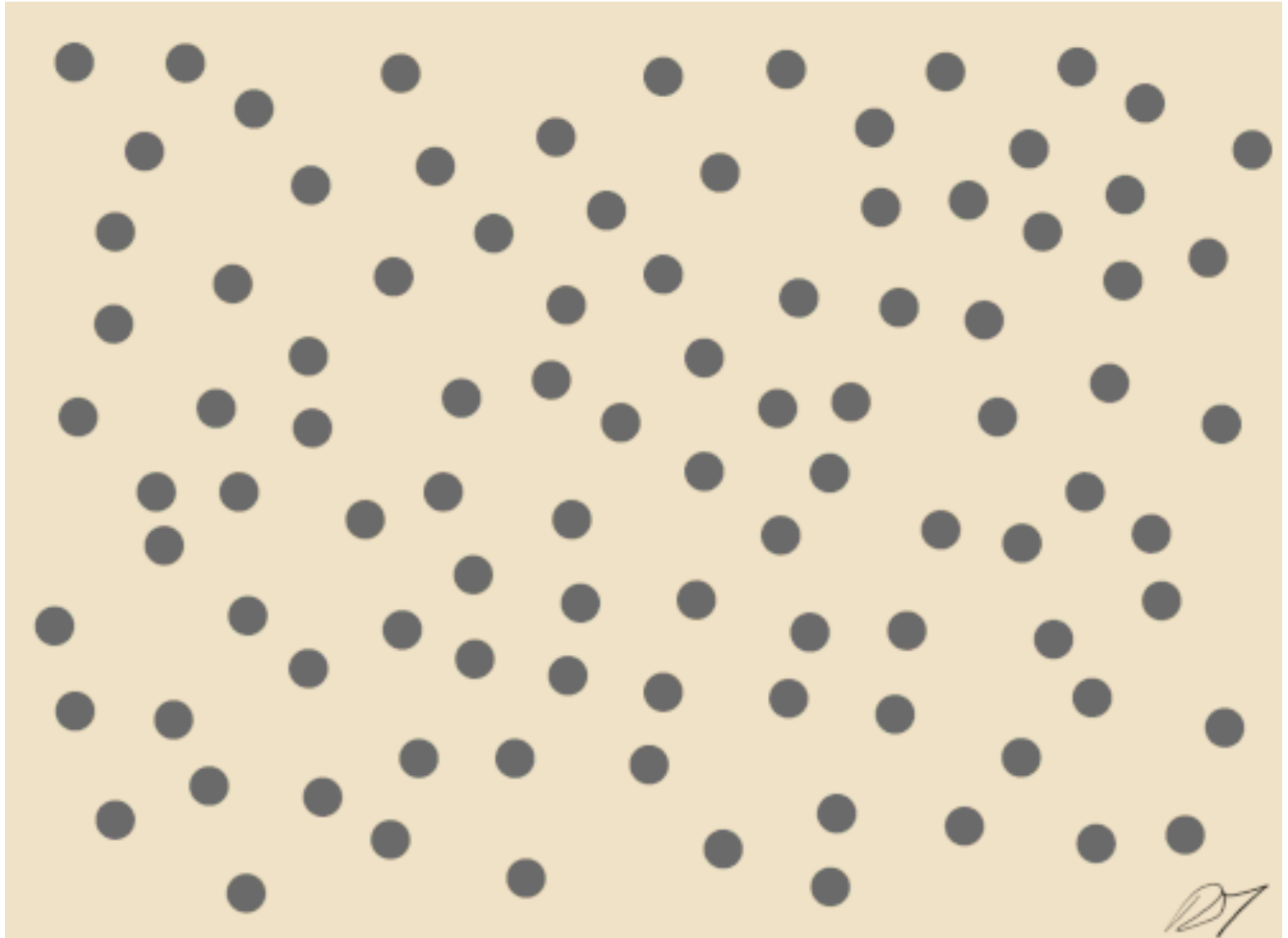


Thai Dao (post-doc Oregon State)

Library construction and sequencing

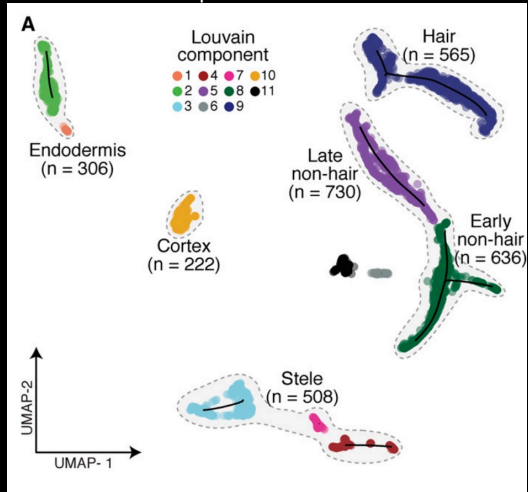


Cell lineage reconstruction

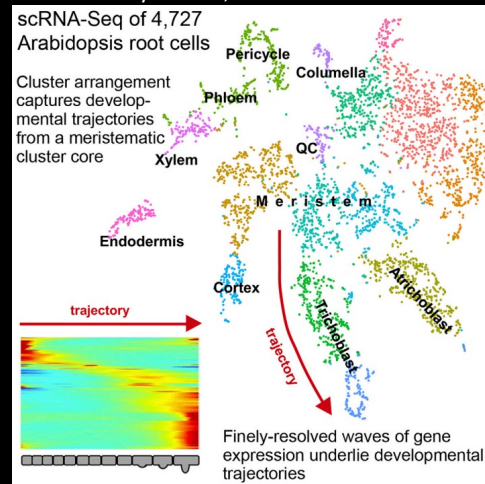


scRNA-seq in Arabidopsis identifies major root cell types

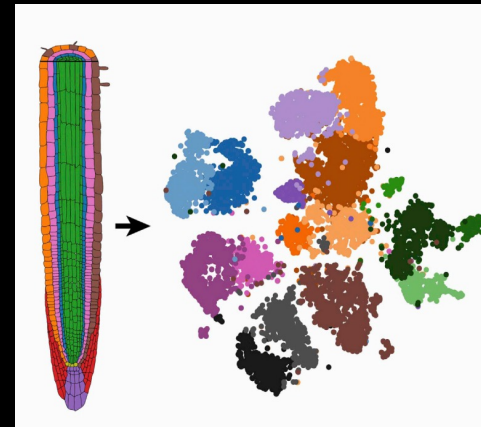
Jean-Baptiste et al 2019 Plant Cell



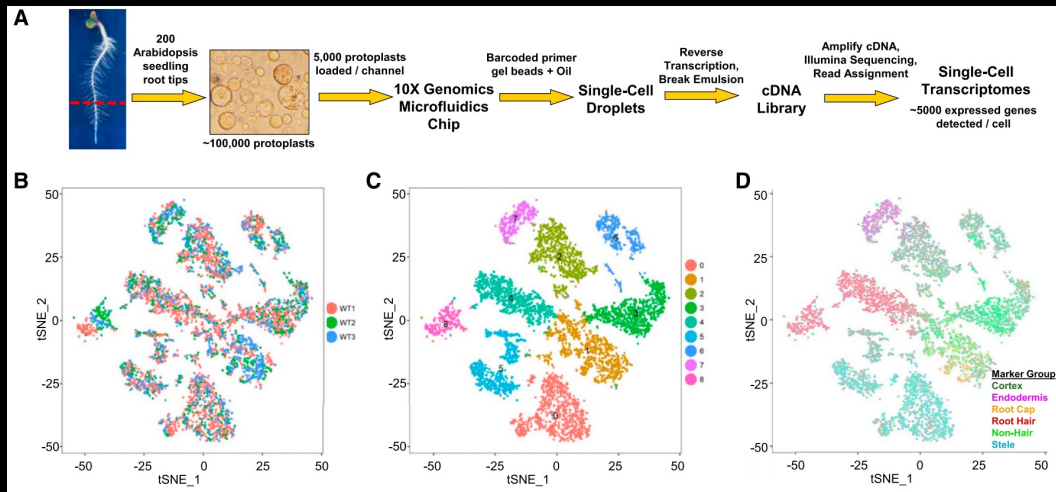
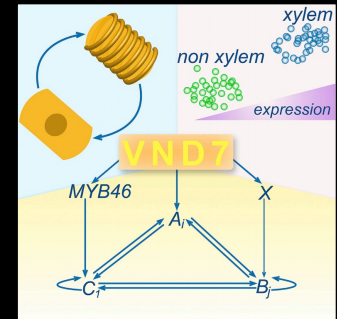
Denyer et al., 2019 Dev. Cell



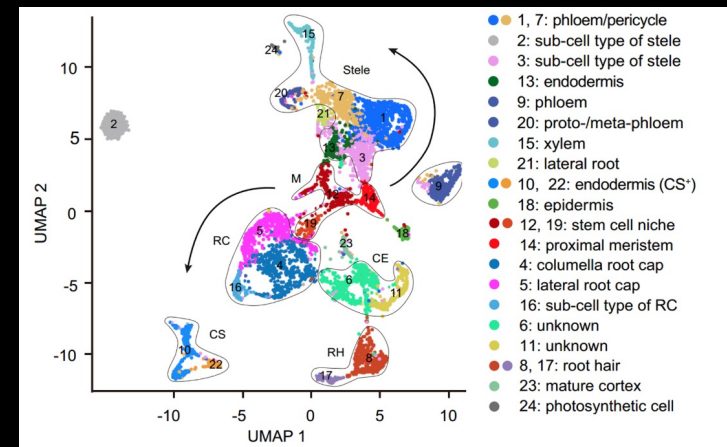
Shulze et al 2019 Cell Reports



Turco et al 2019 Cell Reports

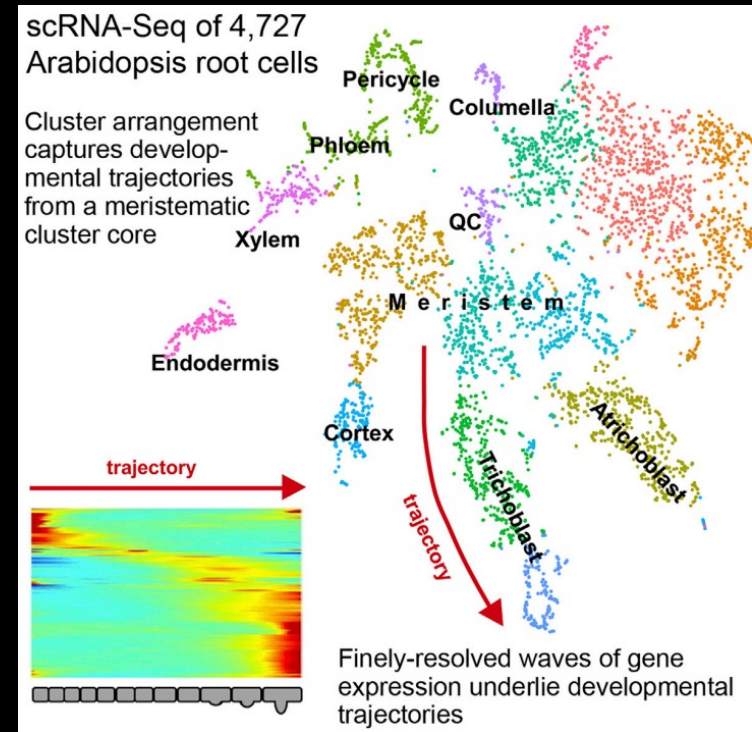
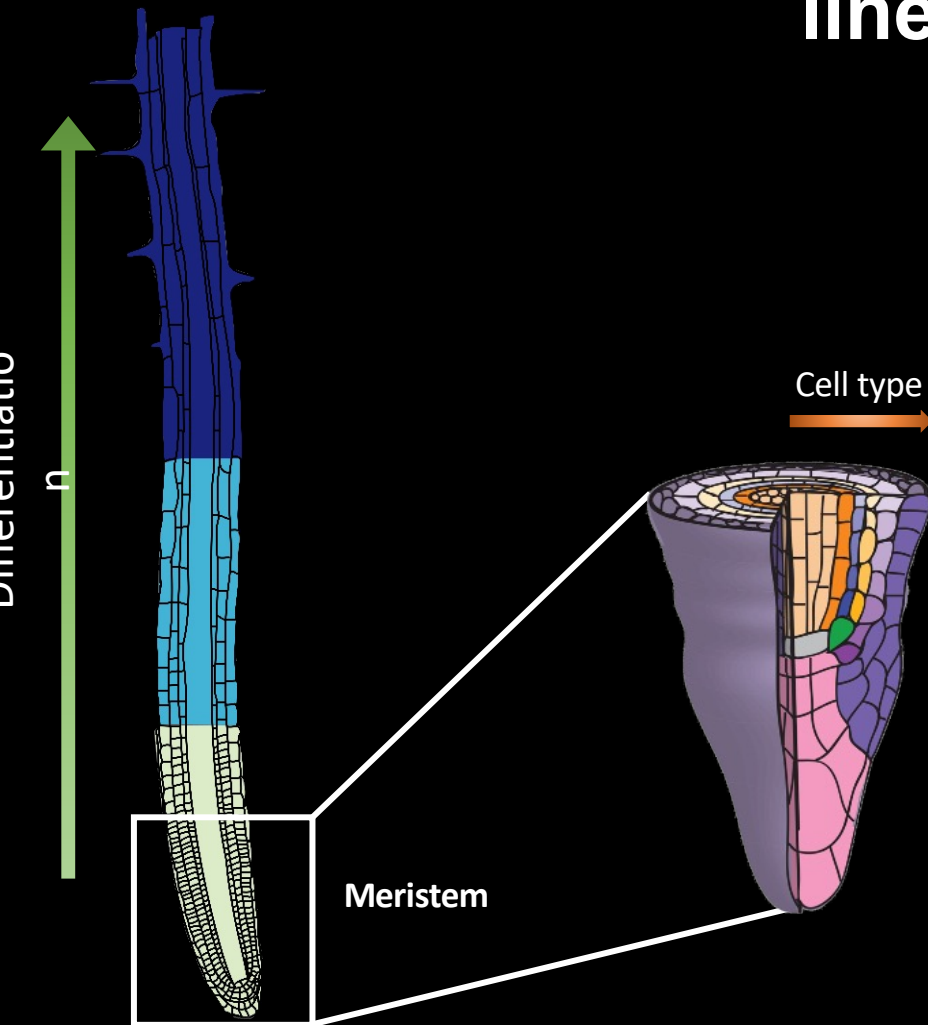


Ryu et al 2019 Plant Phys.



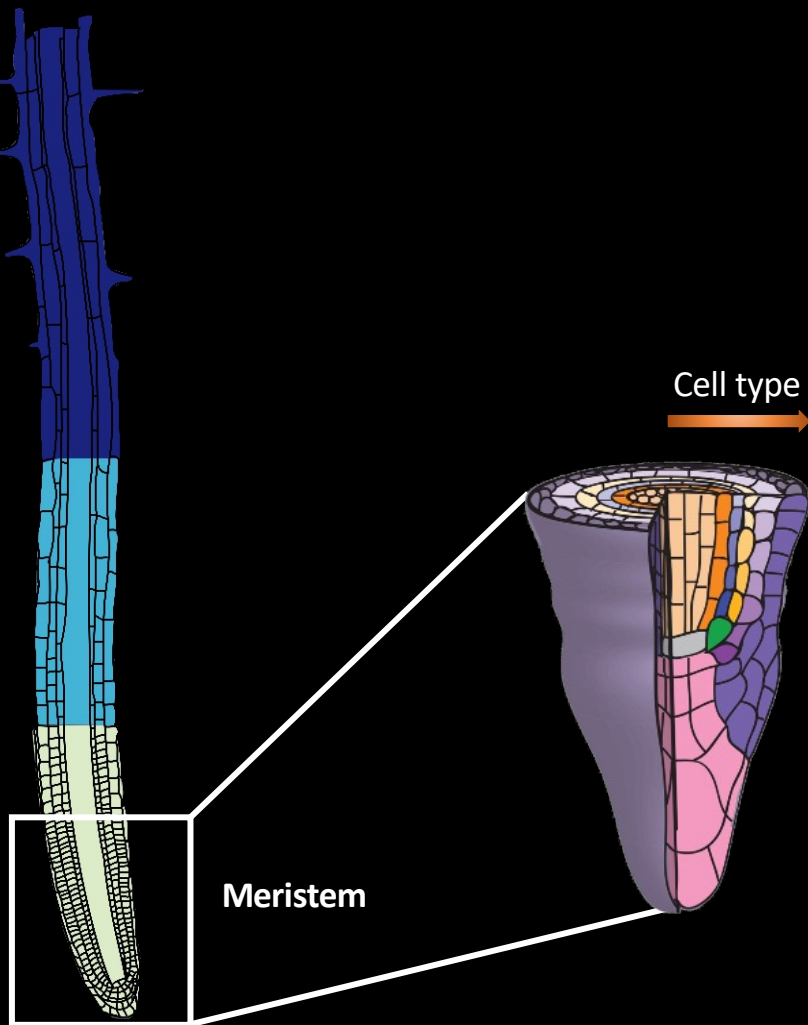
Zhang et al 2019 Mol. Plant

And the developmental progression for some lineages



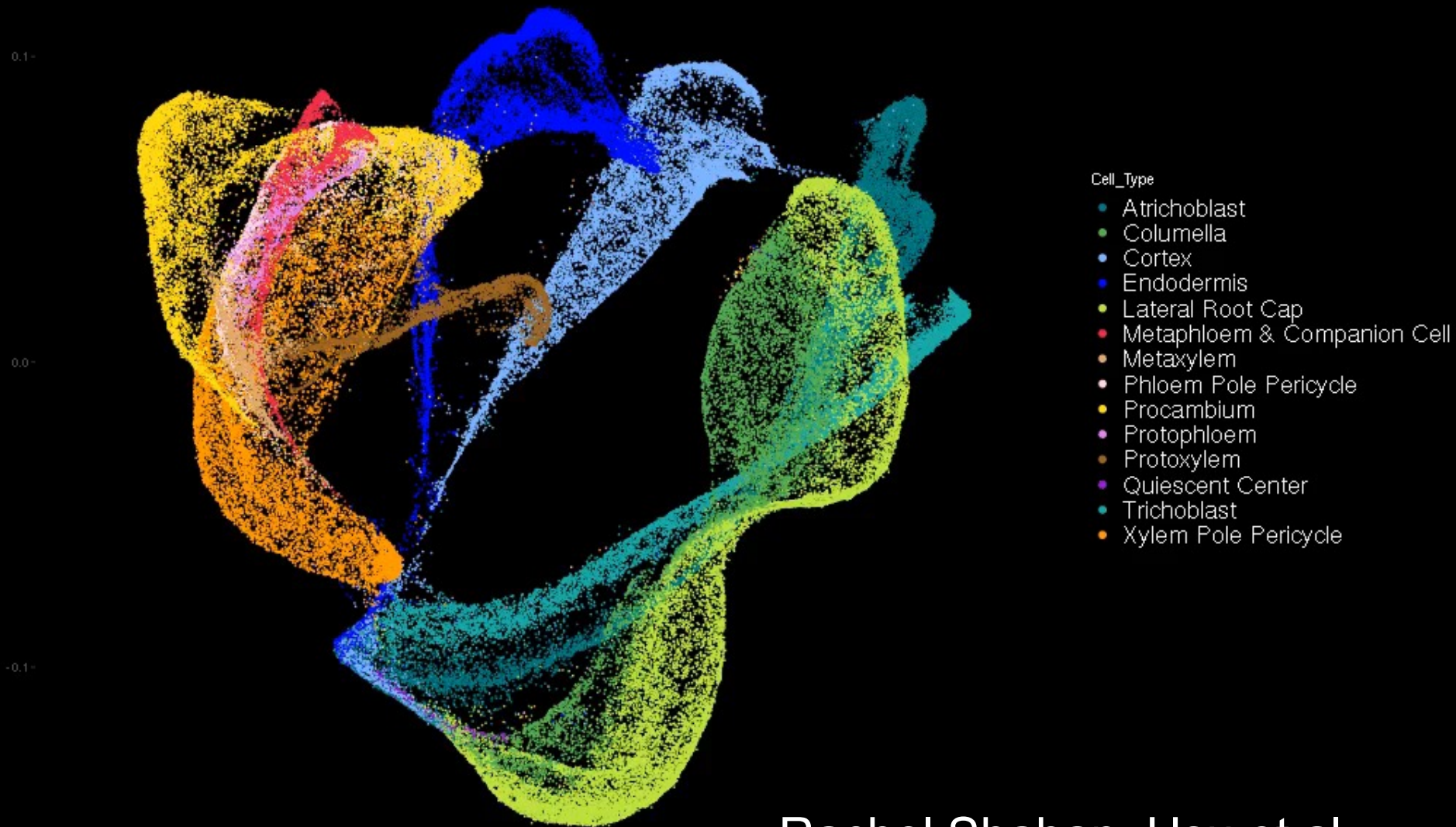
Denyer et al 2019 Dev. Cell

Differentiation



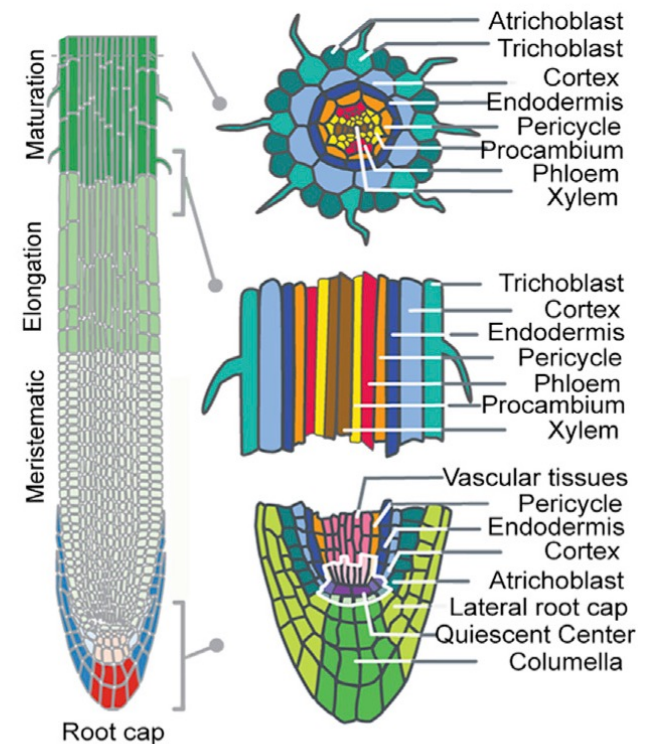
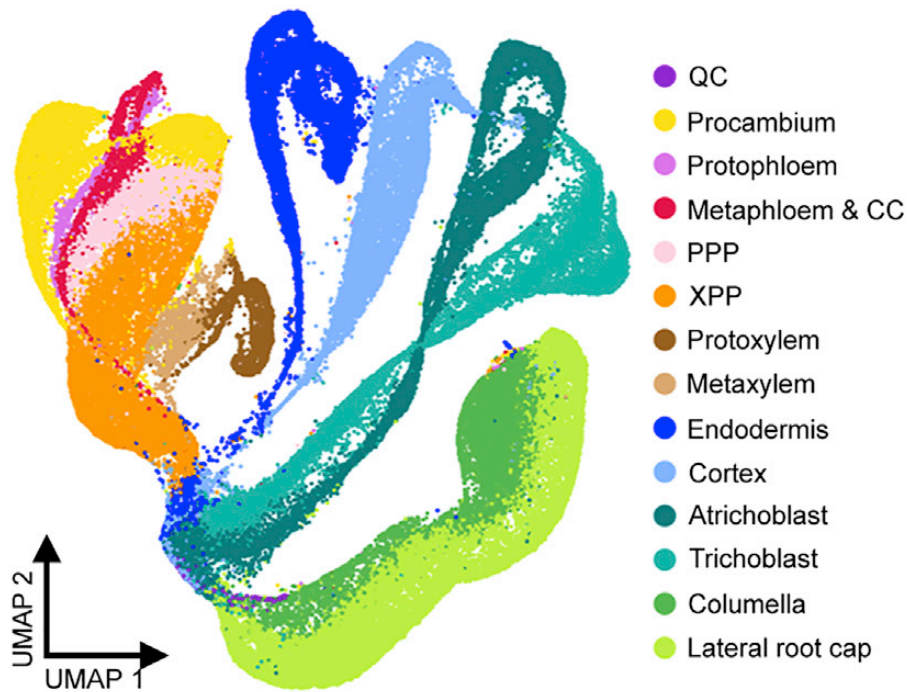
**More cells needed
to map
developmental
trajectories**

A Root Atlas of 110,000 cells

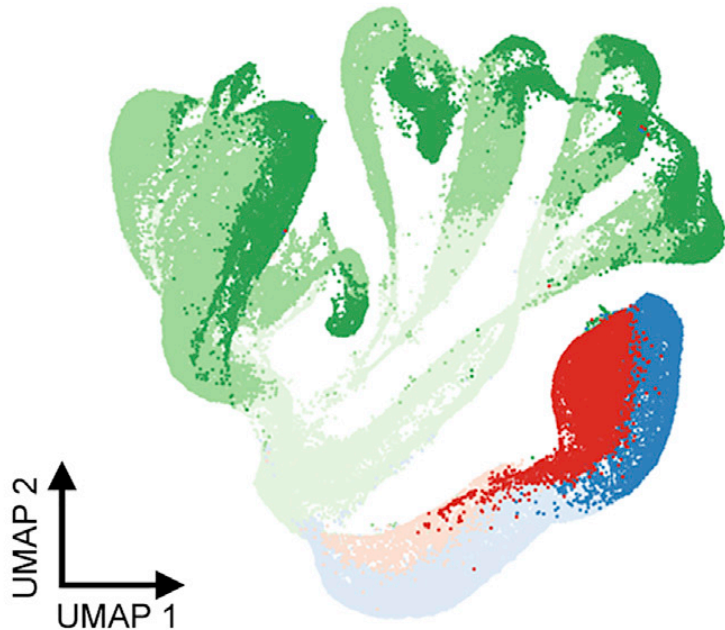


Rachel Shahan, Hsu et al.

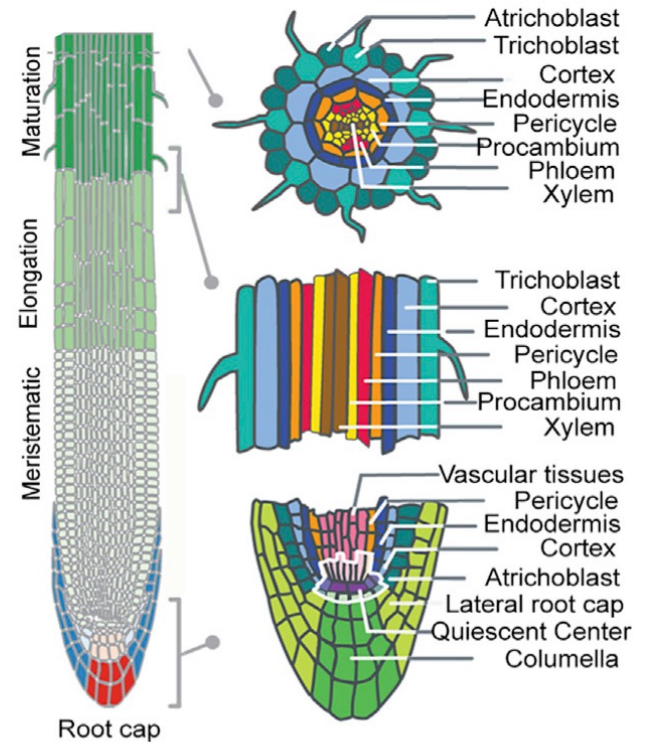
Single-cell atlas of 110K cells captures major cell types



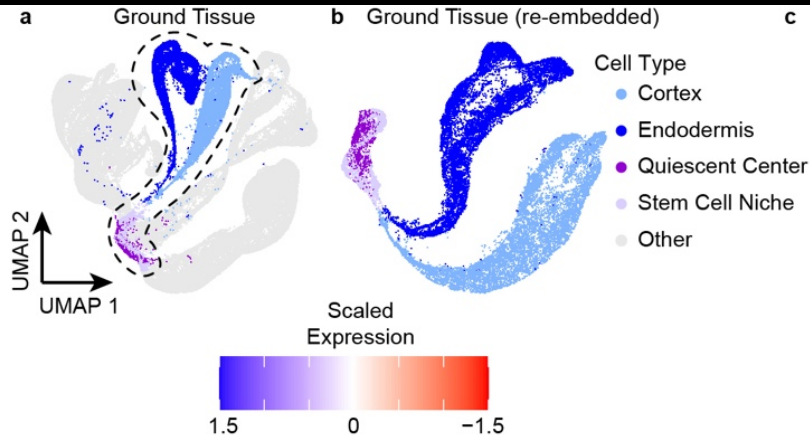
And developmental stages



- Maturation
- Elongation
- Meristem
- Proximal columella
- Distal columella
- Proximal LRC
- Distal LRC



Continuous developmental trajectories are revealed by the atlas



Analysis of scRNA-seq data

Step 1



BIOCONDA

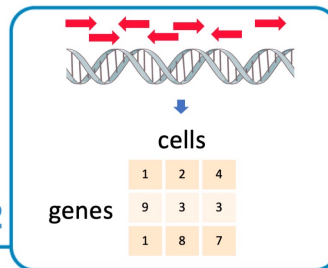


20-50m

Before you begin

Download and install R and packages

Step 2

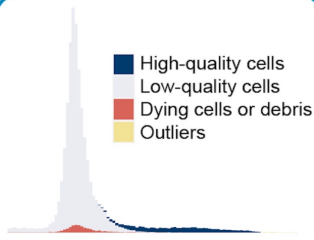


20m-2h

Align reads with scKB (kallisto, bustools)

Fast read alignment and transcript count matrix generation

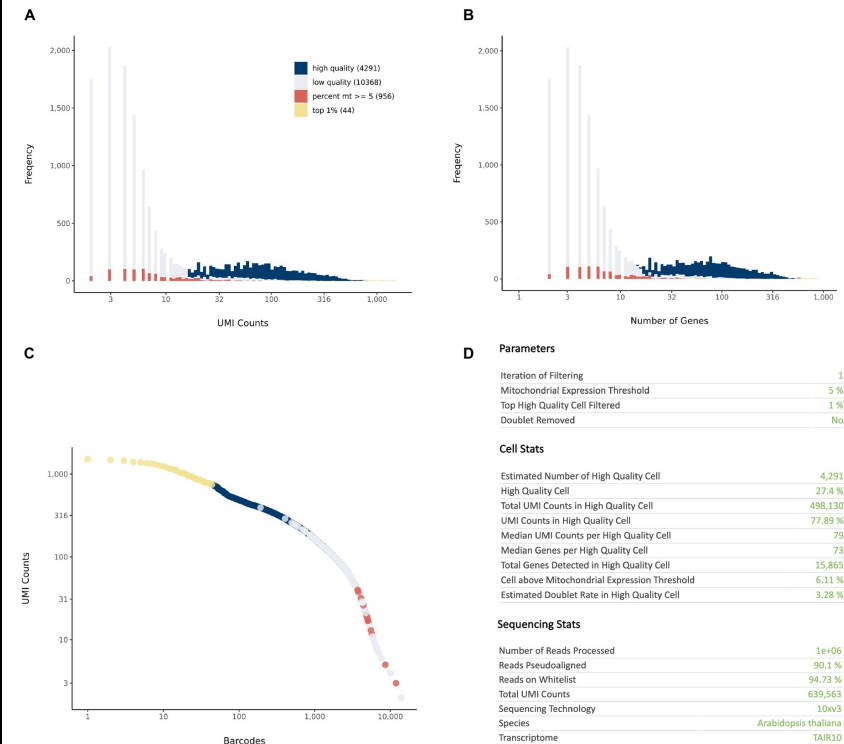
Step 3



30s-50m

Quality filtering with COPILOT

Non-arbitrary, distribution-based background noise detection



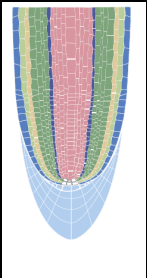
Assign identity to each cell

Used four different approaches to determine likely identity

Majority of approaches used to assign identity

Uses of the Root atlas

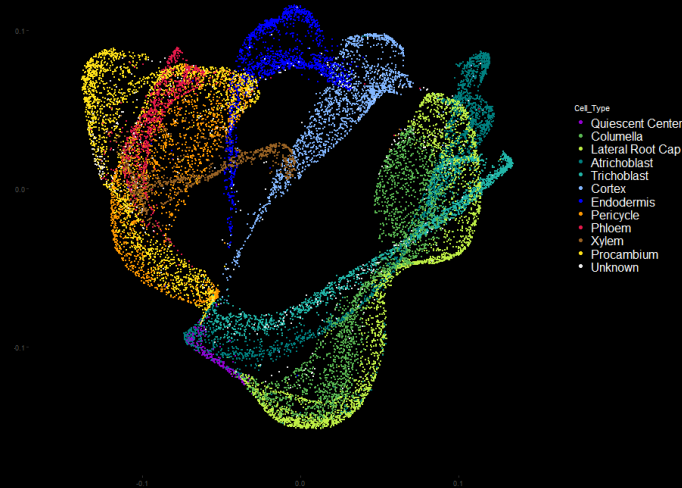
Other species



Mutants



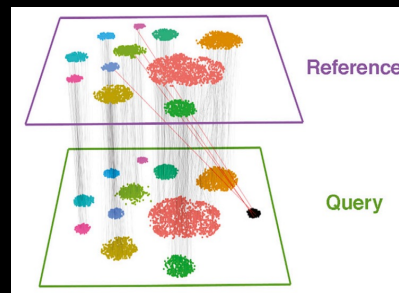
Shahan, Hsu et al.
Dev Cell 2022



Cell_Type

- Quiescent Center
- Columella
- Lateral Root Cap
- Attrichoblast
- Trichoblast
- Cortex
- Endodermis
- Pericycle
- Phloem
- Xylem
- Procambium
- Unknown

Annotation label transfer



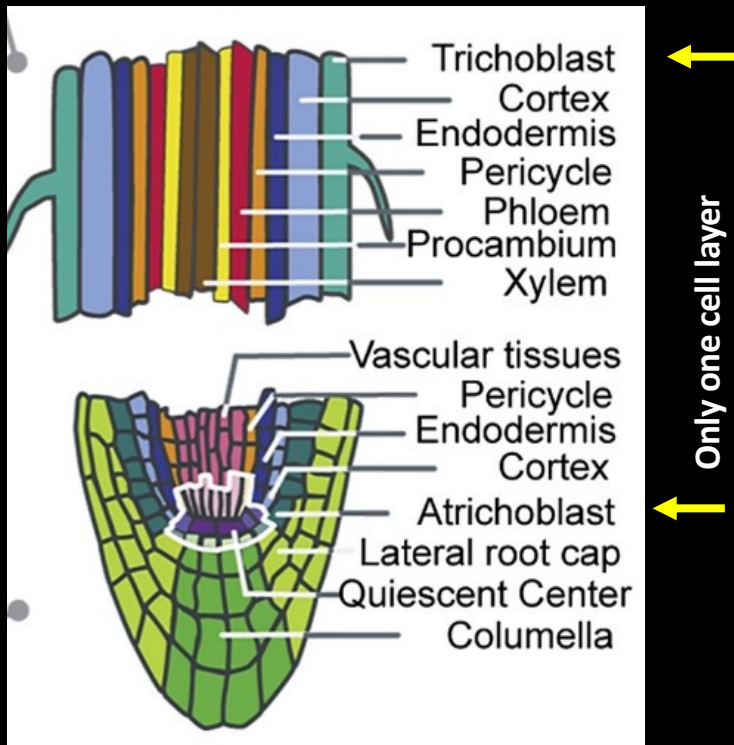
Time courses



Nolan et al.
Science 2023

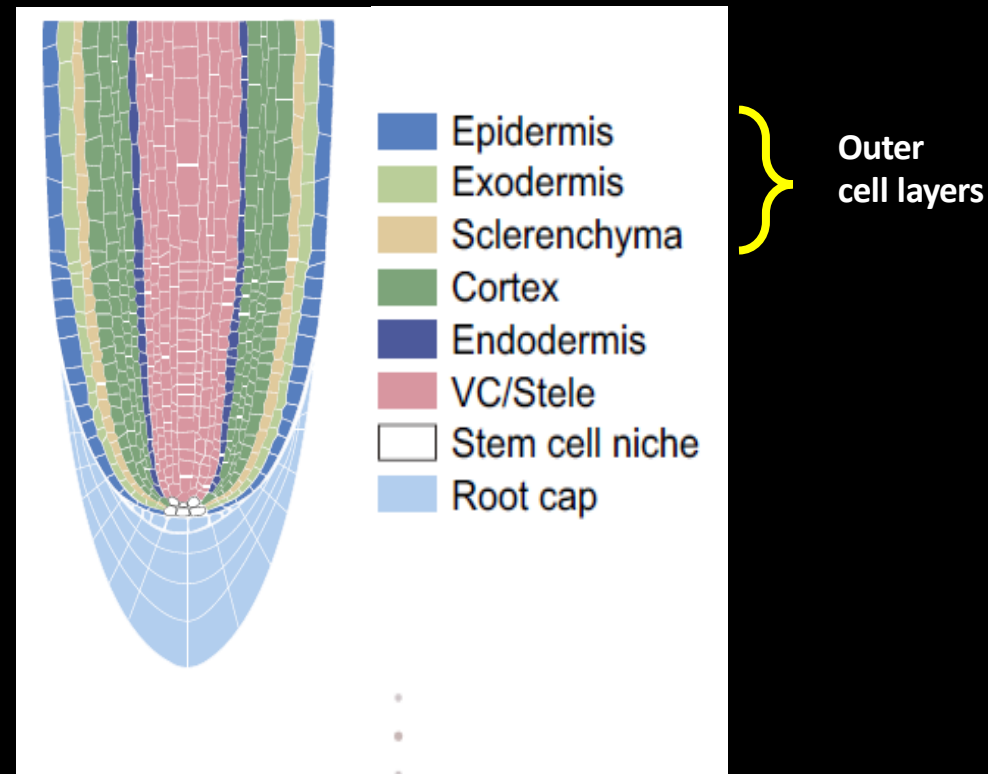
Rice and Arabidopsis have similar root tissue organization

Arabidopsis root



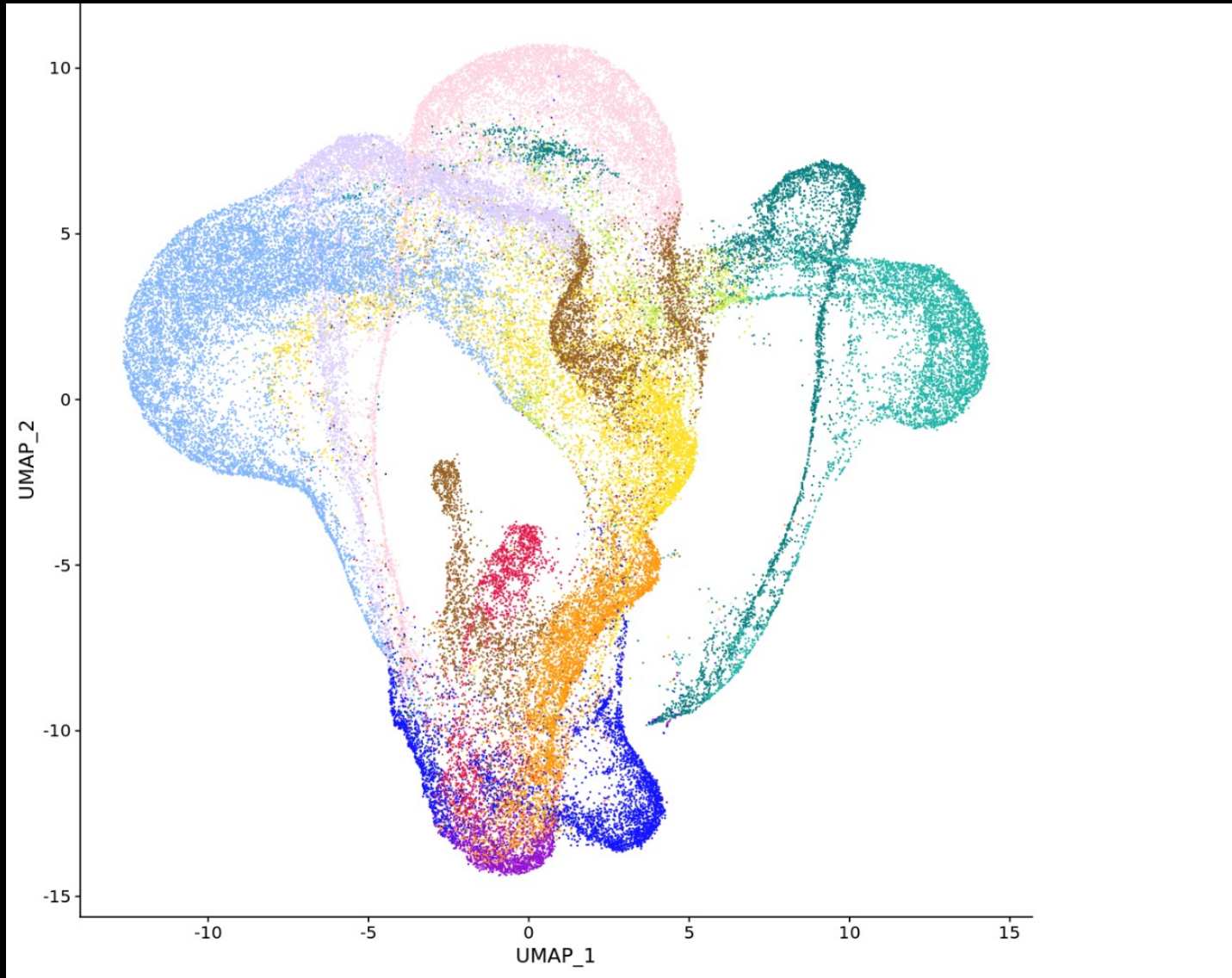
Shahan, Hsu et al.,
2022

Rice root



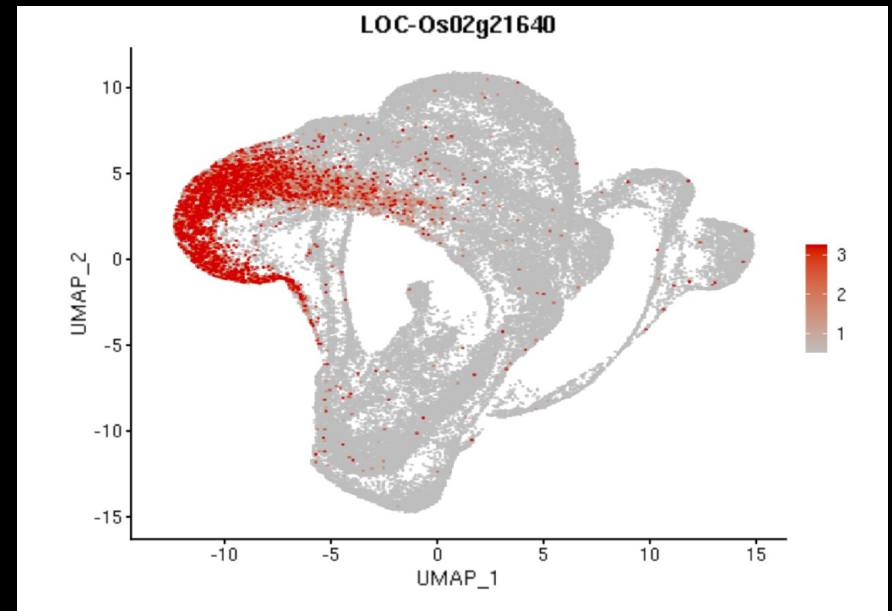
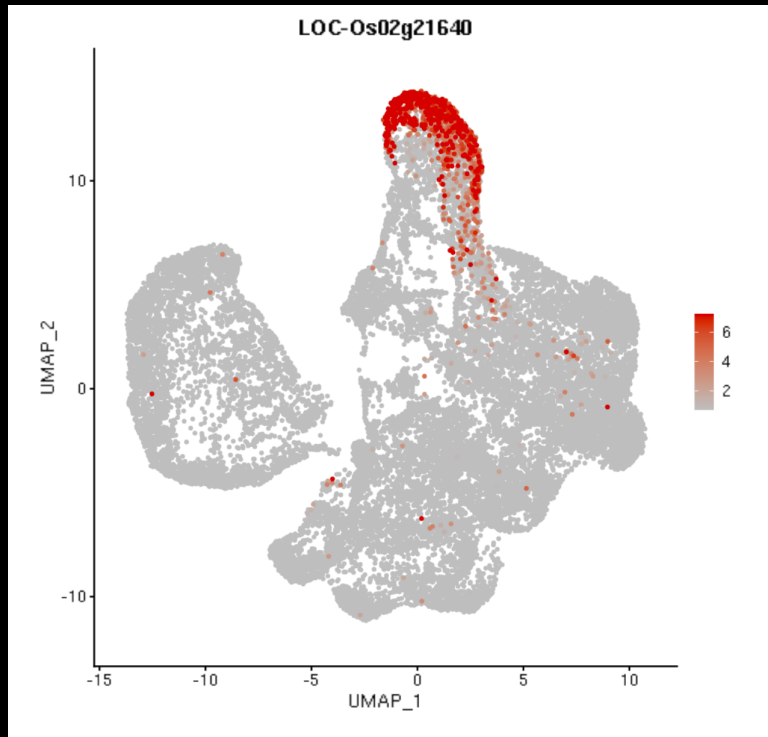
Zhang et al., 2021

What are clusters in rice root scRNAseq map?

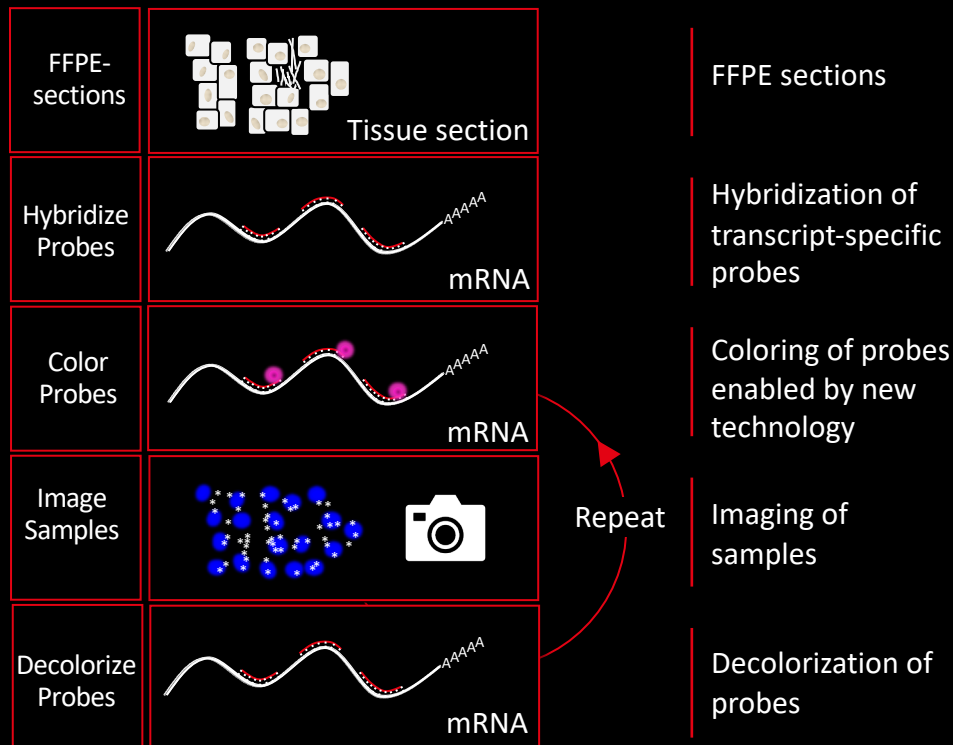


Mingyuan
Zhu &
Isaiah
Taylor

Identify cluster-specific markers



Molecular Cartography™ is a multiplexed fluorescence in situ hybridization (FISH) technique



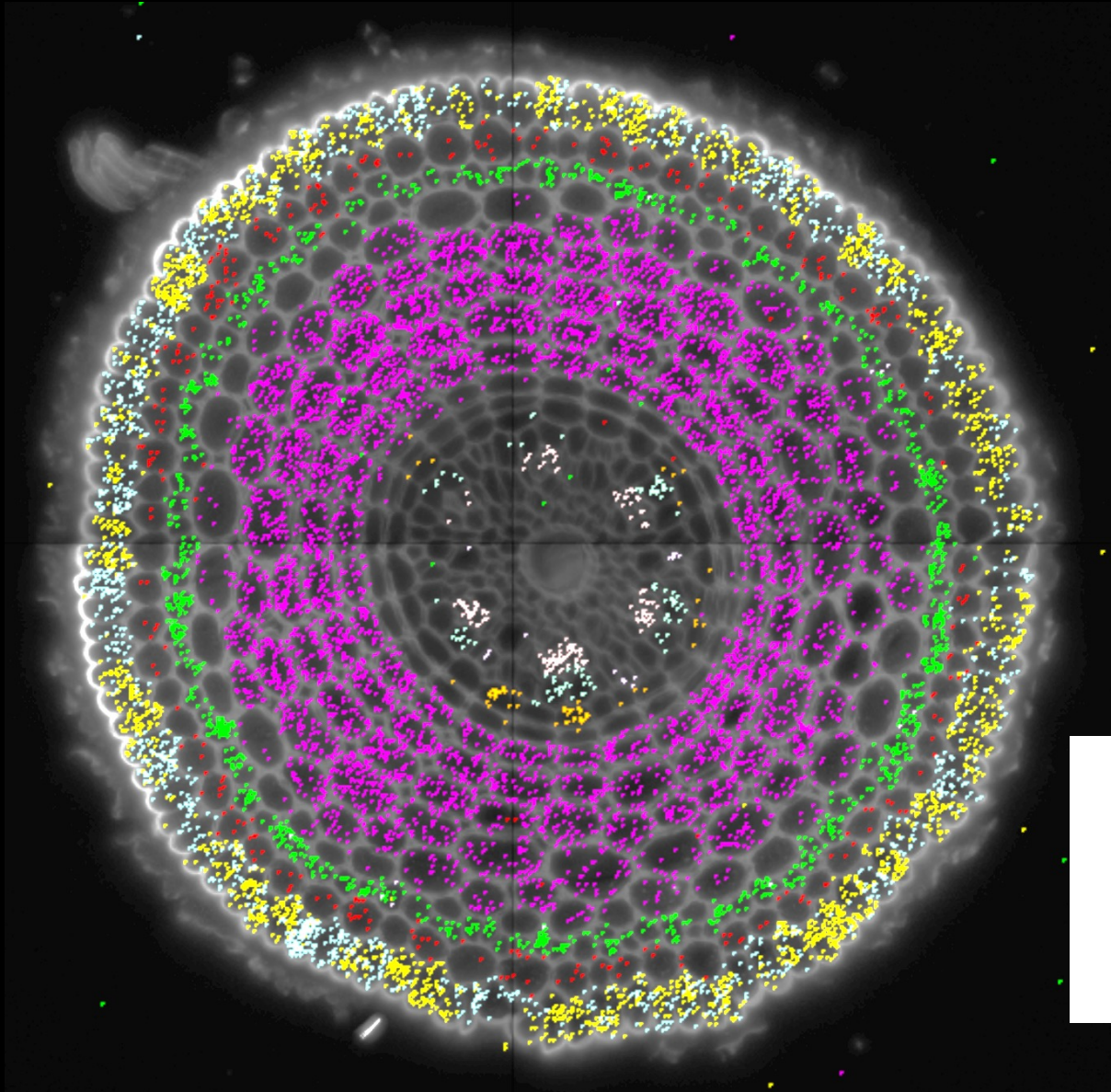
Each gene has its own unique color code

Code:	round 1	round 2	round 3	round 4	round 5	round 6
Transcript A	green	green	red	red	green	red
Transcript B	red	red	green	red	green	red
Transcript C	red	green	red	green	red	green

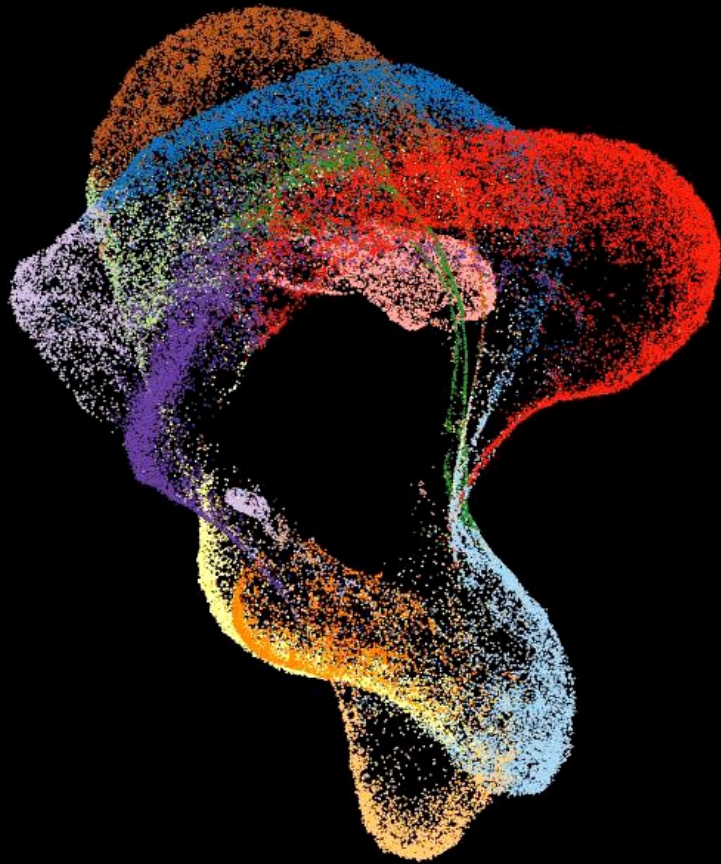


Dr. Samik Bhattacharya

Markers identified for all major cell types



Rice root atlas of 70k cells



Markers:

- Atrichoblast
- Trichoblast
- Exodermis
- Sclerenchyma
- Cortex
- Endodermis
- Pericycle
- Phloem
- Xylem
- Stem cell niche

Other multiplex in situ hybridization technologies

- Vizgen – Merfish

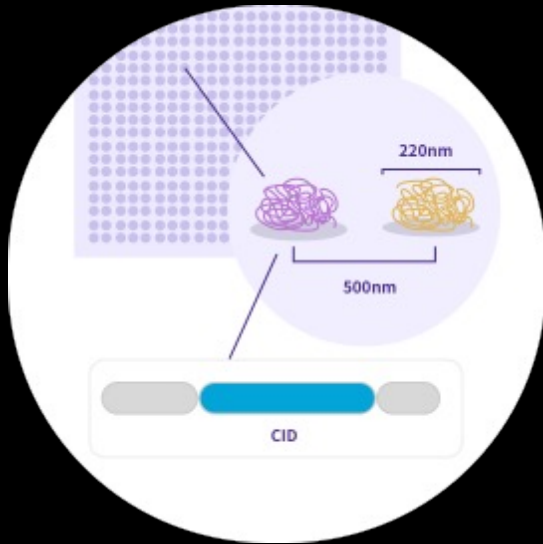


https://vizgen.com/?gclid=CjwKCAjwkLCkBhA9EiwAka9QRrAzcuGj71NpCcbgN_LfQ5xw5iy8iR2VQgXeFg8UlqrVxsekfGY5xRoCTlwQAvD_BwE

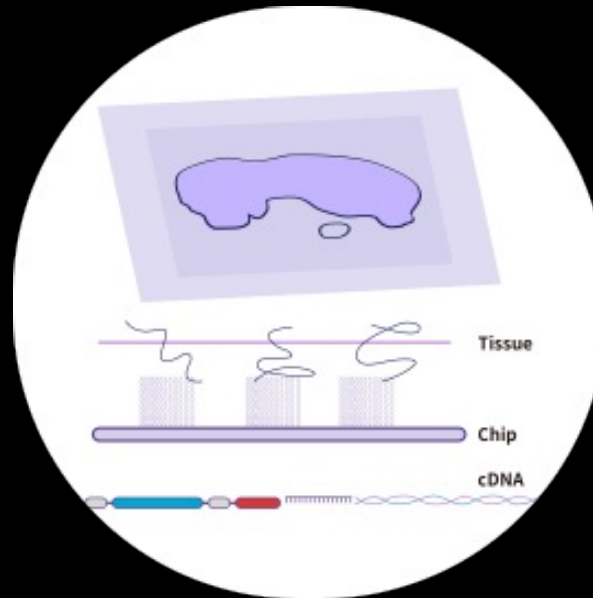
- 10X – Xenium

- Nanostring – GeoMx, CosMx

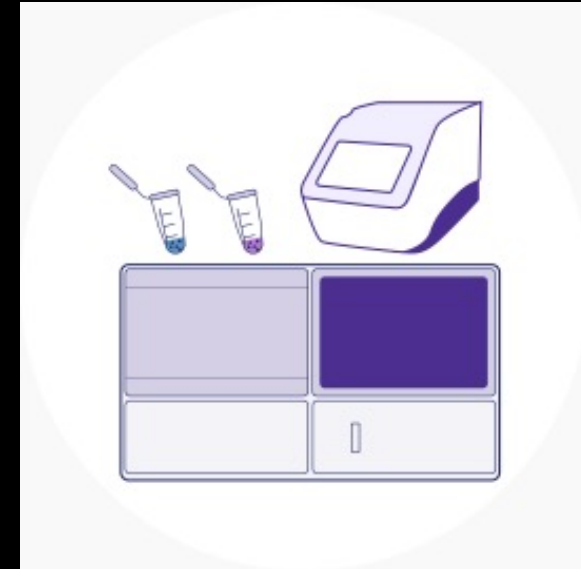
Sequencing based spatial transcriptomics: StereoSeq



DNA nanoballs
arrayed on slide

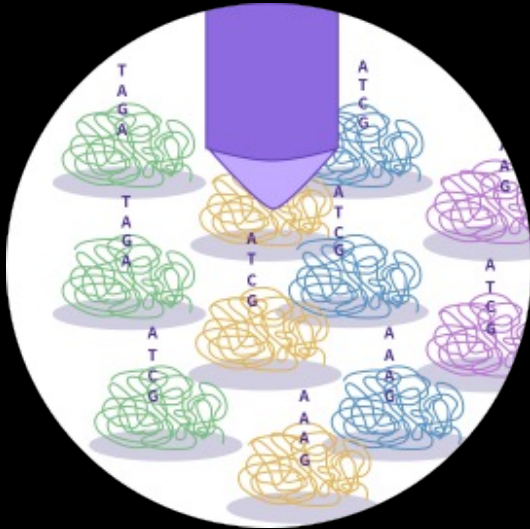


RNA capture

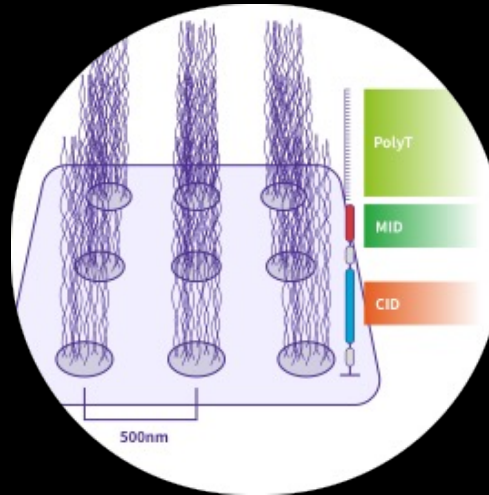


Library construction &
sequencing

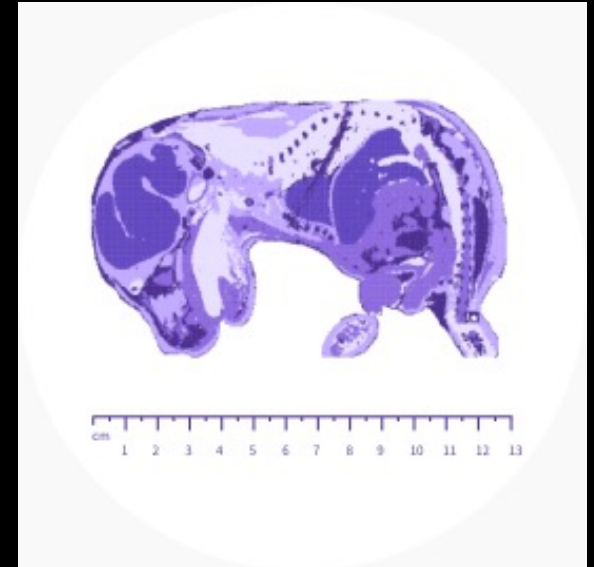
StereoSeq



Library sequencing



Use of coordinate
information to align
sequences with tissue



Spatial resolution:
subcellular

single cell chromatin accessibility: scATAC seq

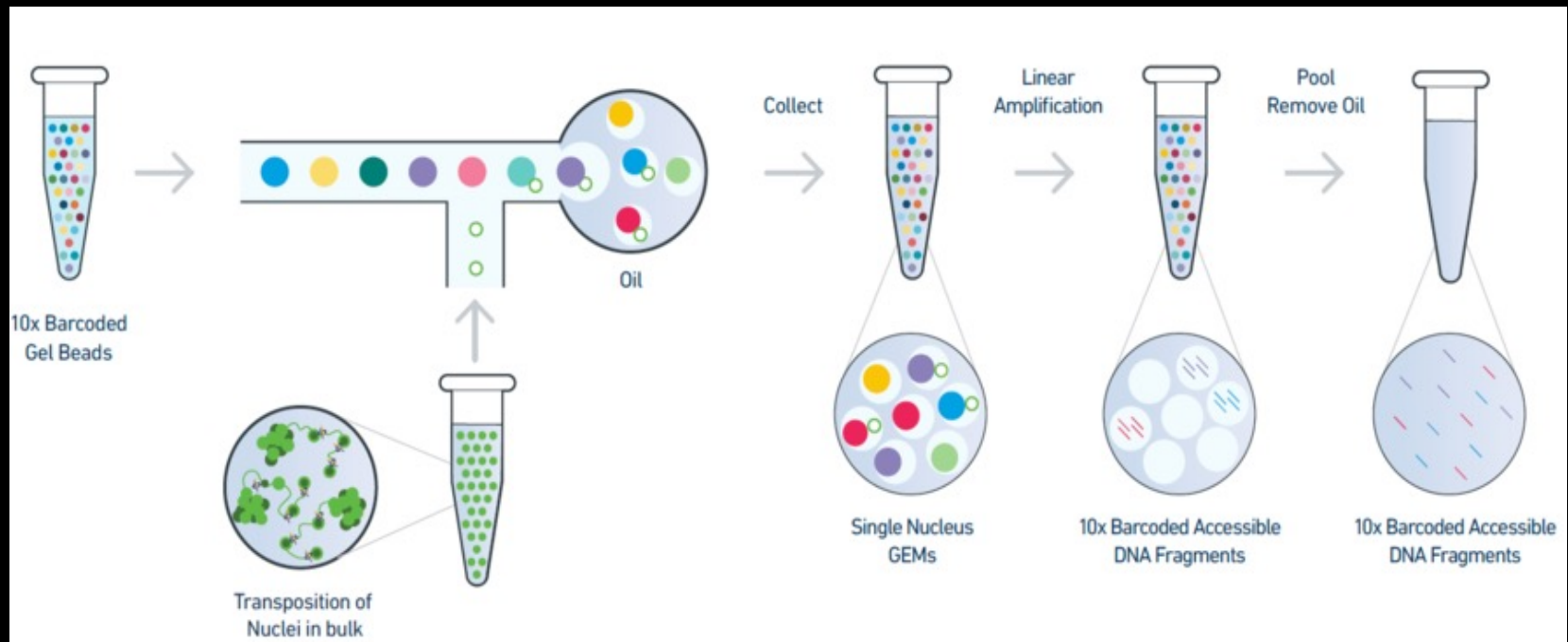


Image : 10X Genomics

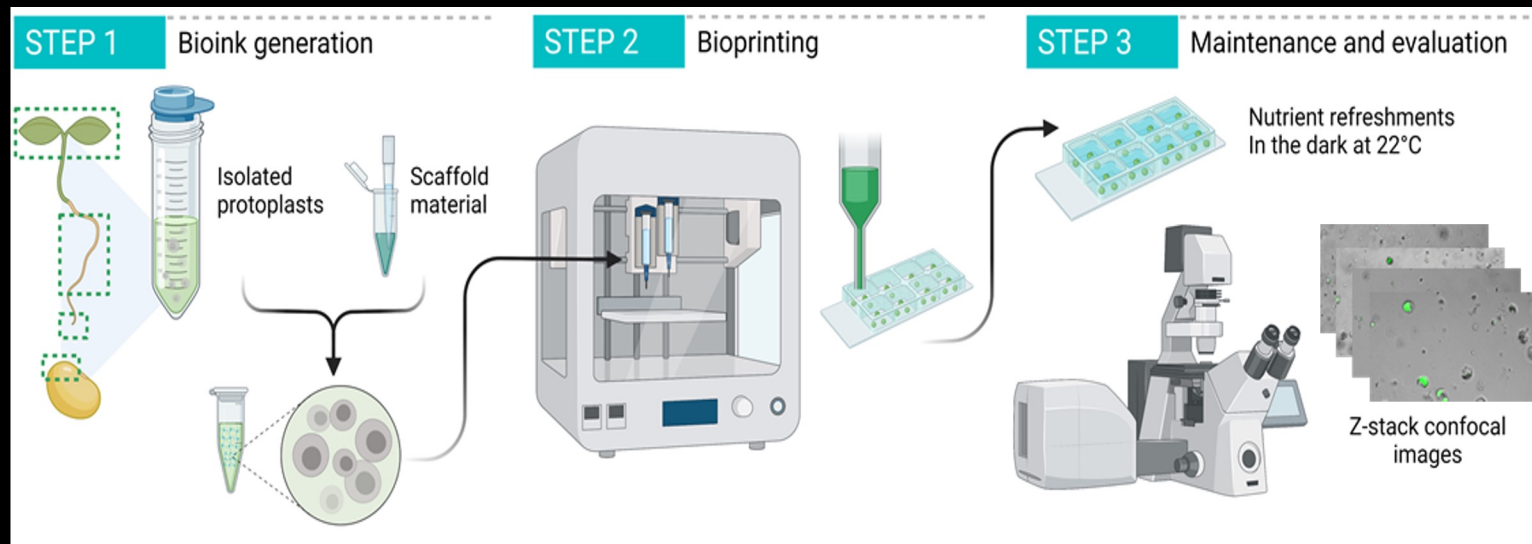
Identifying genomic features driving expression

- Use AI/ML to identify genomic elements responsible for specific expression patterns
- Genome edit native promoters to change expression patterns
- Generate synthetic promoters that confer specific expression

Genome editing in diverse species

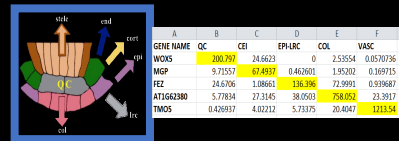
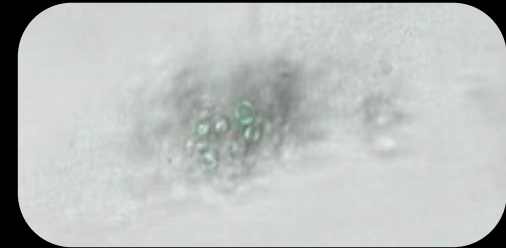
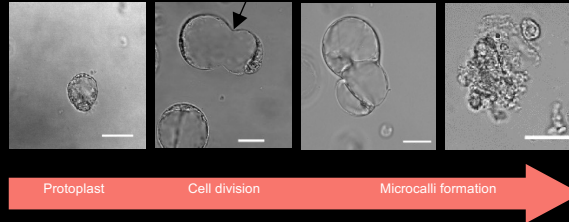
- Transformation bottle neck
- Use 3D Bioprinting to overcome

3D bioprinting

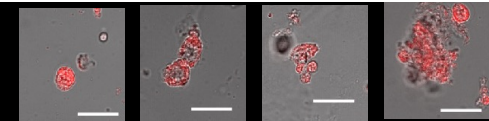
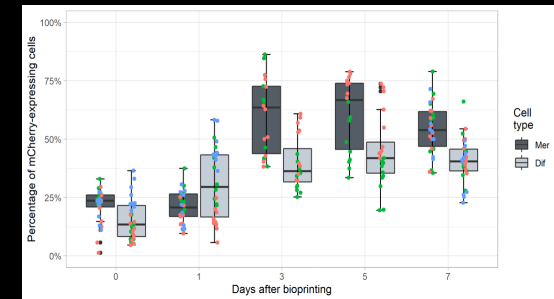
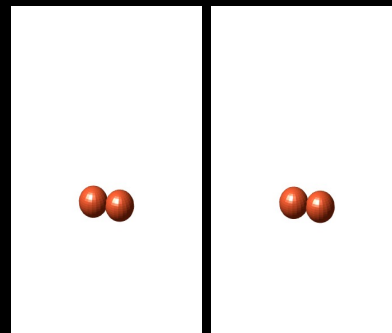
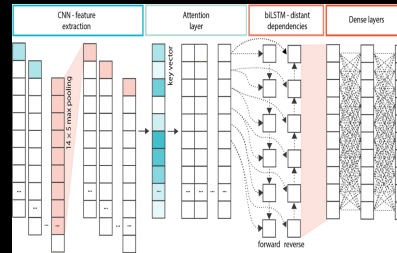


Bioprinting to transform any genotype

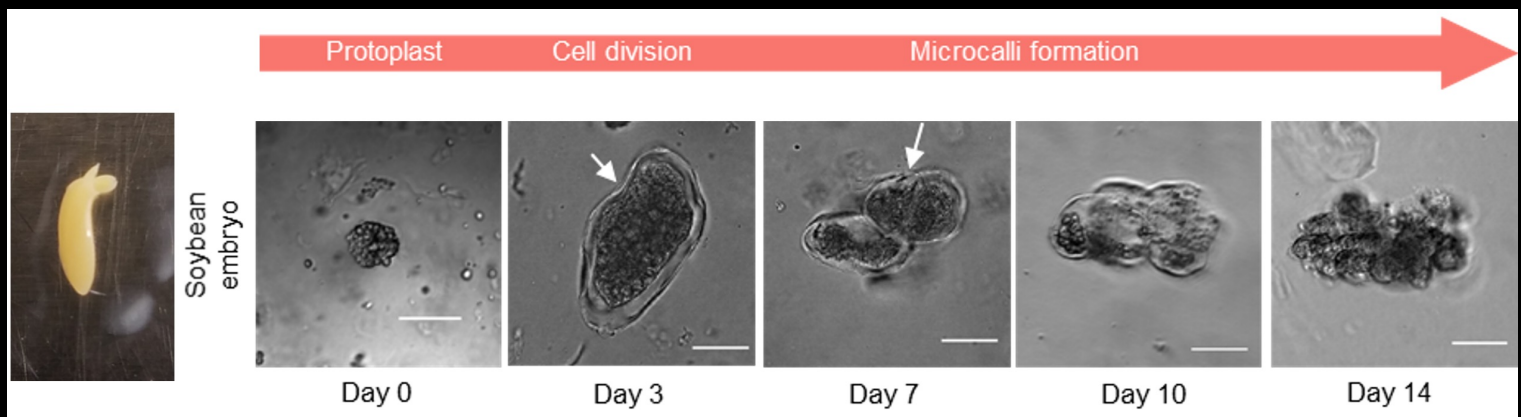
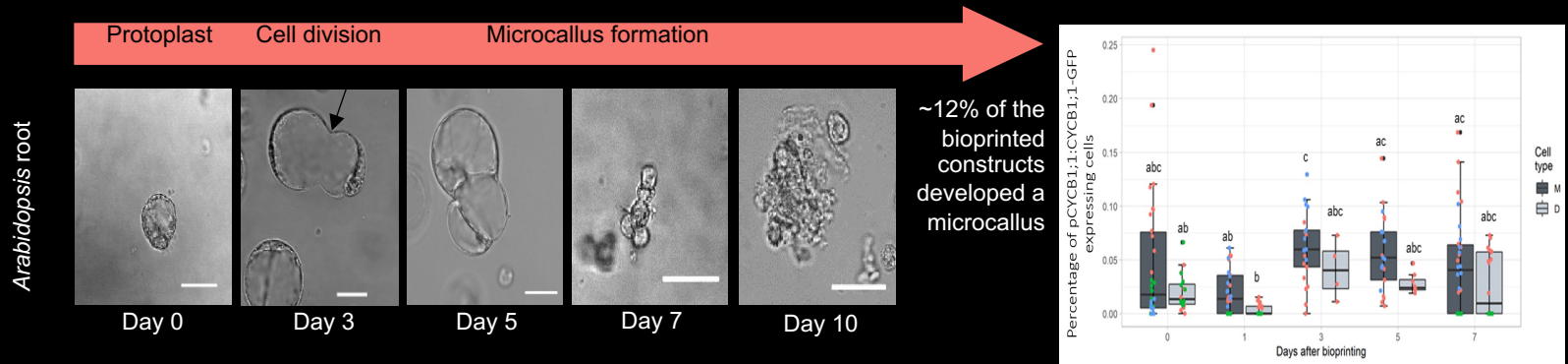
3D bioprinted cells maintain division capacities and form microcalli



Monitoring of cell-type specific markers over time



Cell cycle re-entry of protoplasts derived from *Arabidopsis* roots and soybean embryos



Summary

- Single cell transcriptomics
- Spatial transcriptomics
- Data analysis and interpretation
- Genome features driving gene expression
- Genome editing to change expression patterns
- Repeatable plant regeneration
- 3D bioprinting

Rachel Shahan
Che-Wei Hsu
Trevor Nolan
Mingyuan Zhu
Isaiah Taylor

Uwe Ohler (Humboldt, Berlin)
Ben Cole (LBL Joint Genome Institute)
Geoff Schiebinger (UBC)
Scott Fraser, Thai Truong (USC)



Ross Sozzani
Eric Rogers
Natale Clark

