SCIENCE MEETS LIFE

Single cell and spatial transcriptomics

Life Science Technology Specialist
VIB Tech Watch team
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VIB Tech Watch

Scout

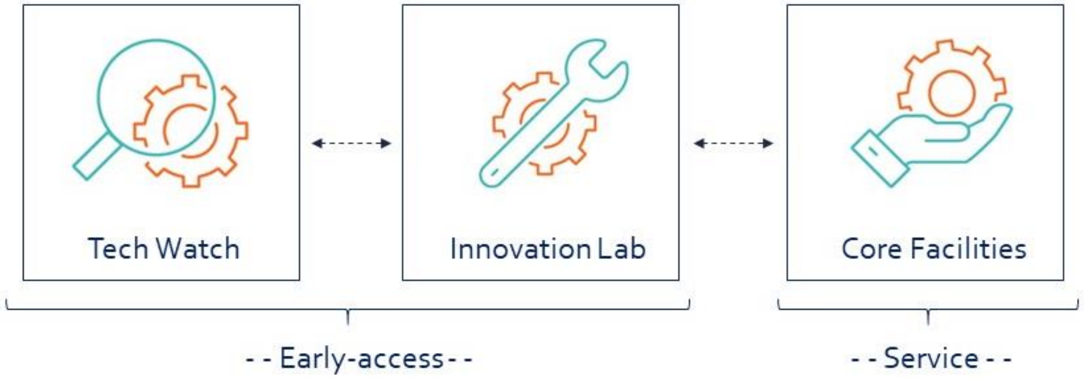
- Scout for disruptive technologies.
- Partner with companies

De-risk

- Funding.
- Hands-on support.

Facilitate

- Increase adoption of novel technologies.
- Staying at the forefront of life science research



Tech Watch

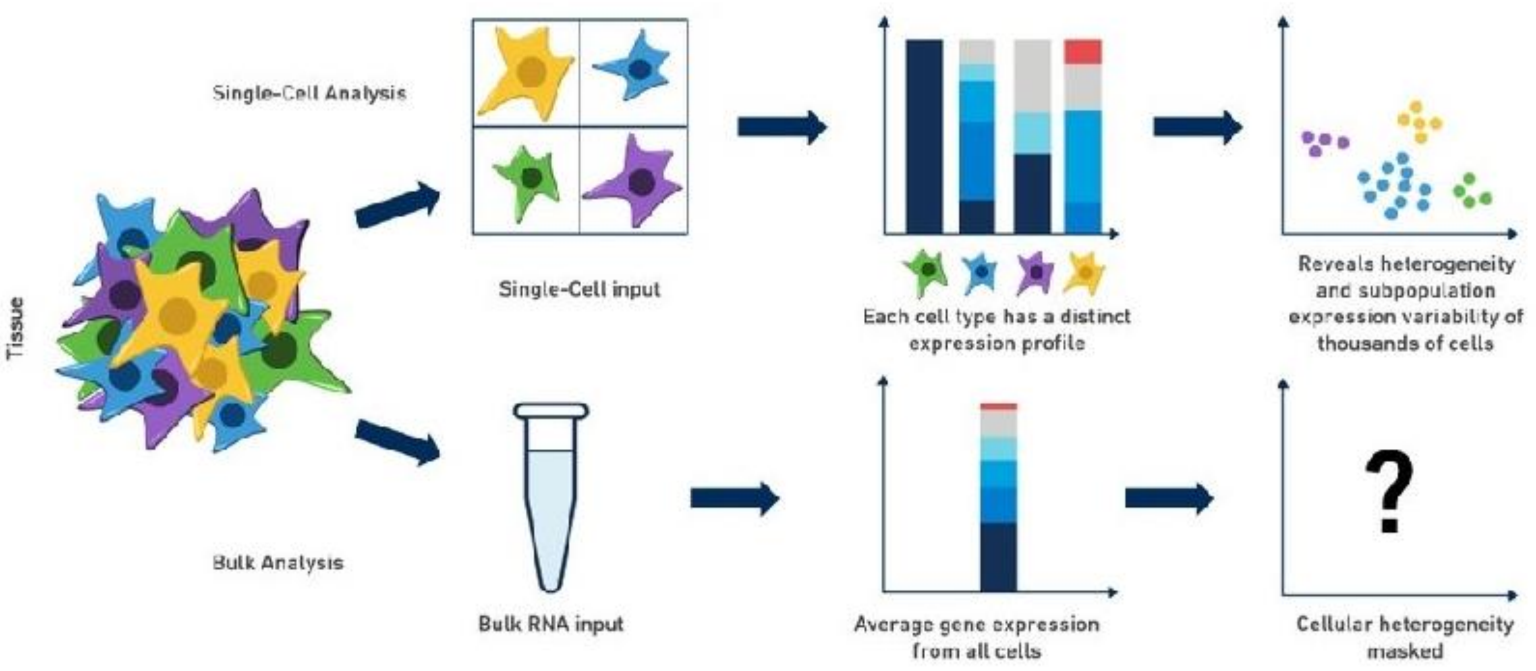
Innovation Lab

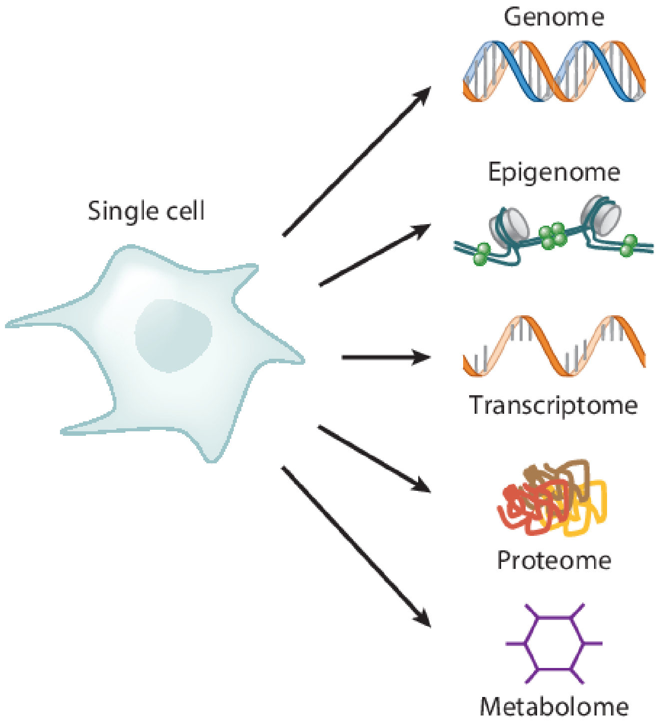
Core Facilities

- - Early-access - -

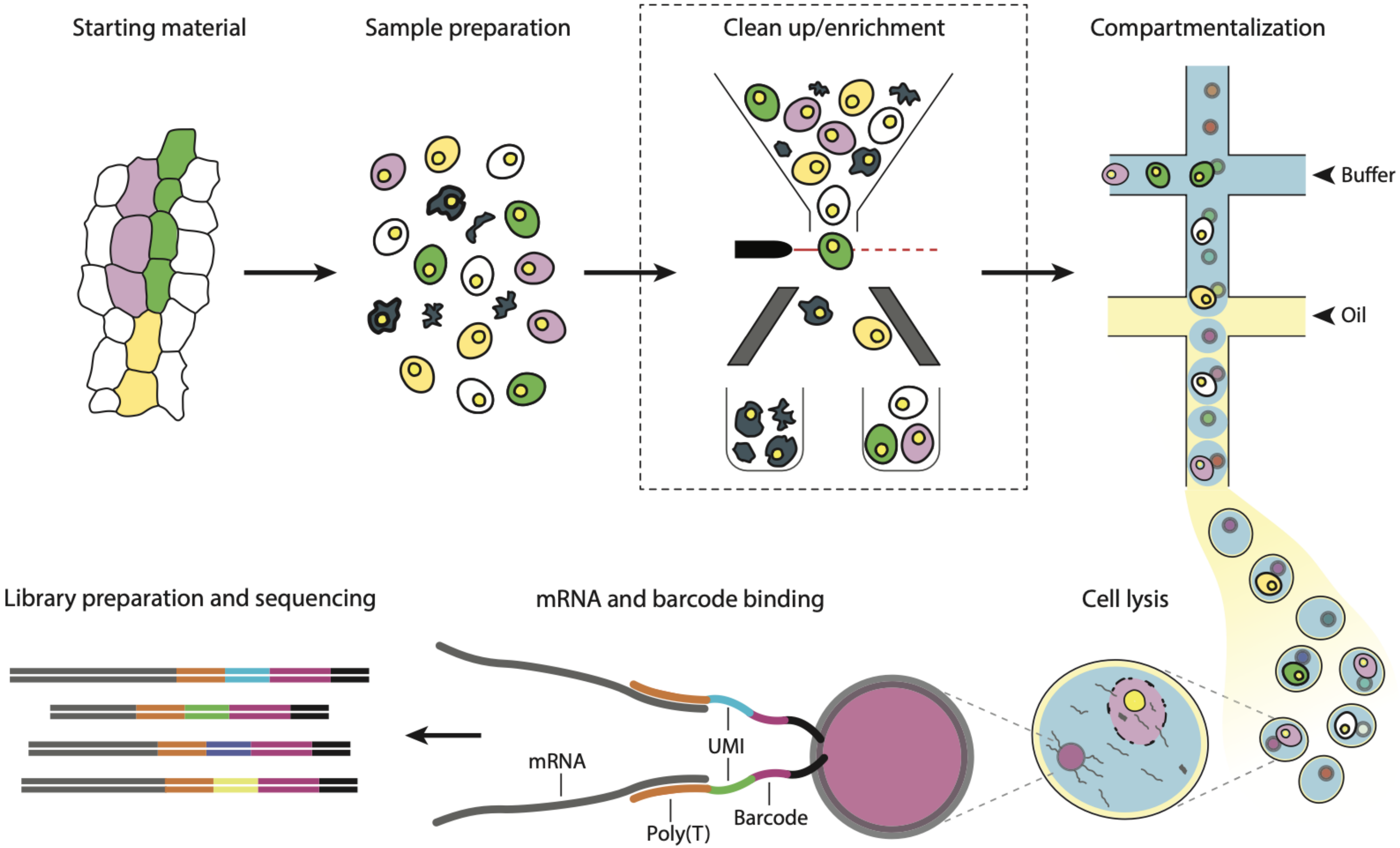
- - Service - -

The premise of single cell analysis



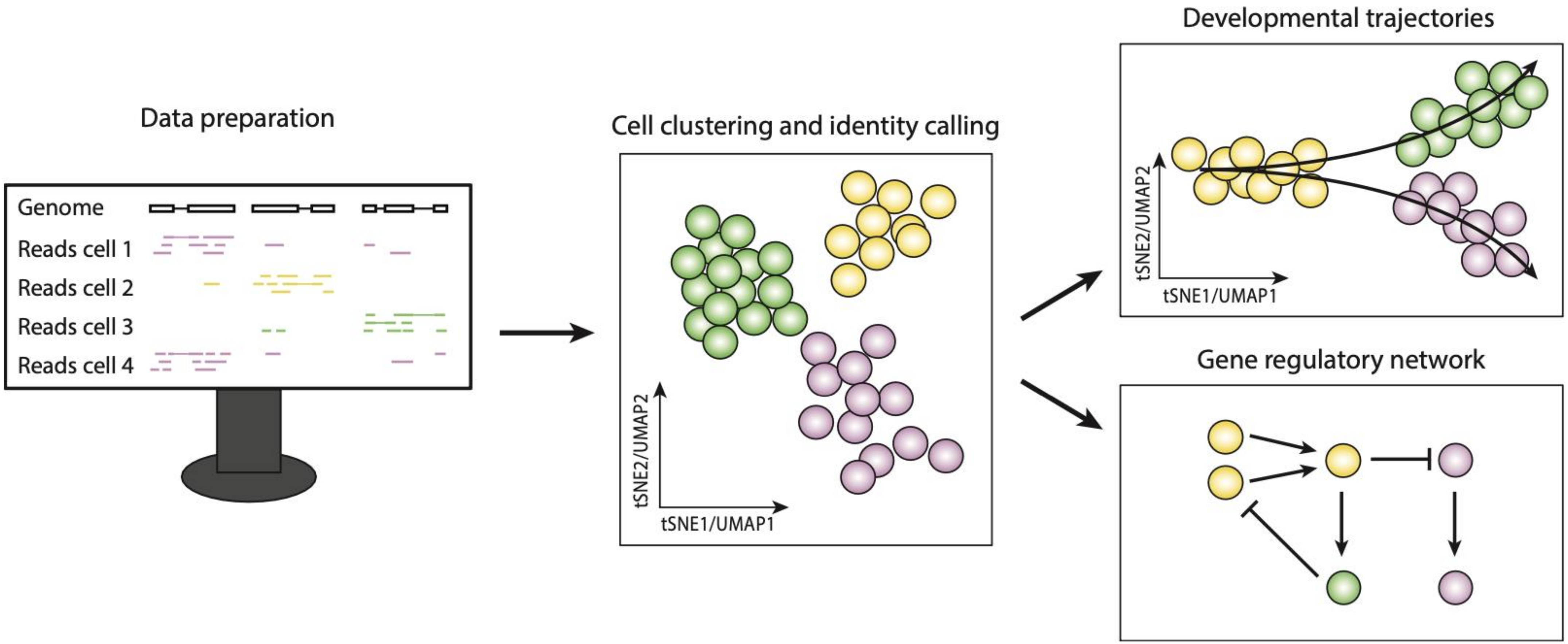


Single cell transcriptomics workflow



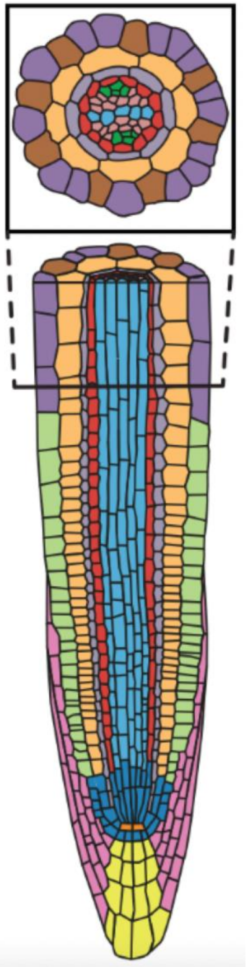
Seyfferth C, et al. (in preparation)

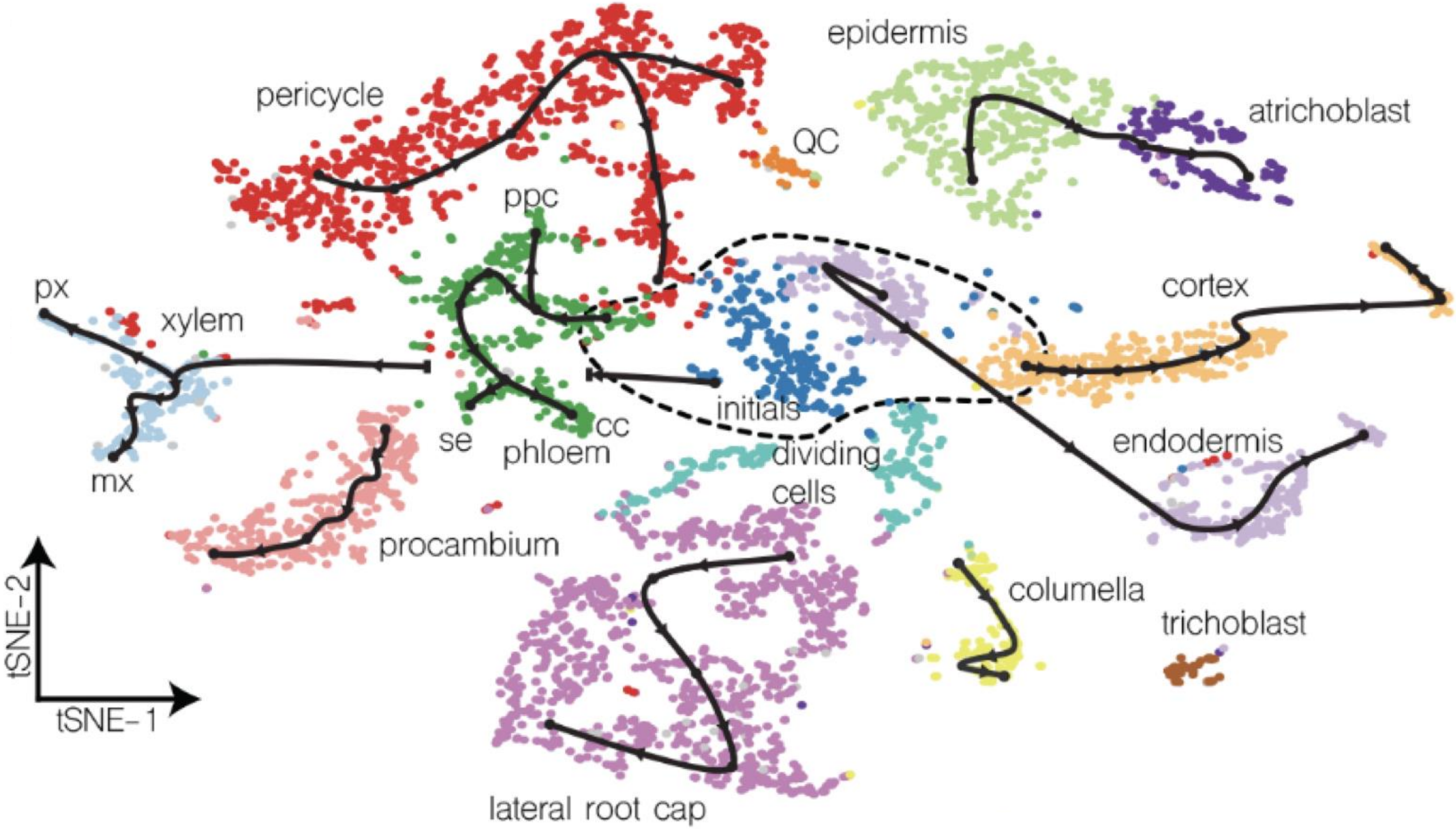
Single cell transcriptomics workflow



Seyfferth C, et al. (in preparation)

Arabidopsis root single cell atlas

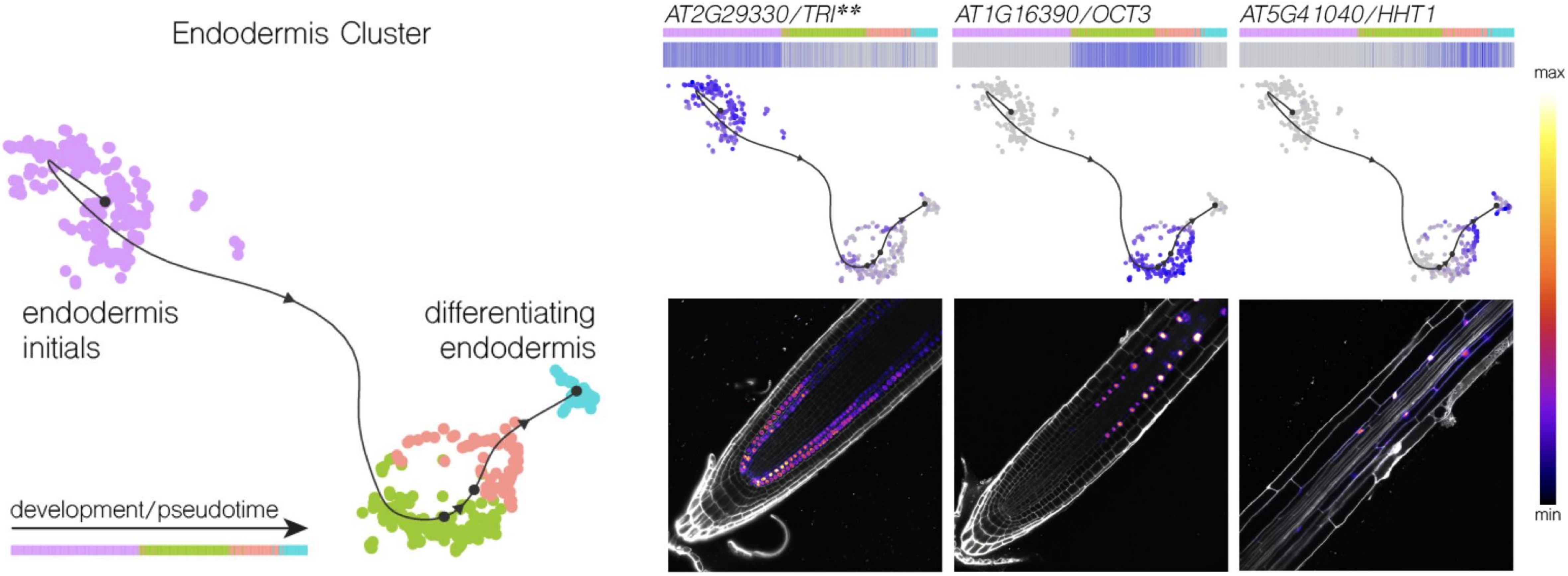




De Rybel lab

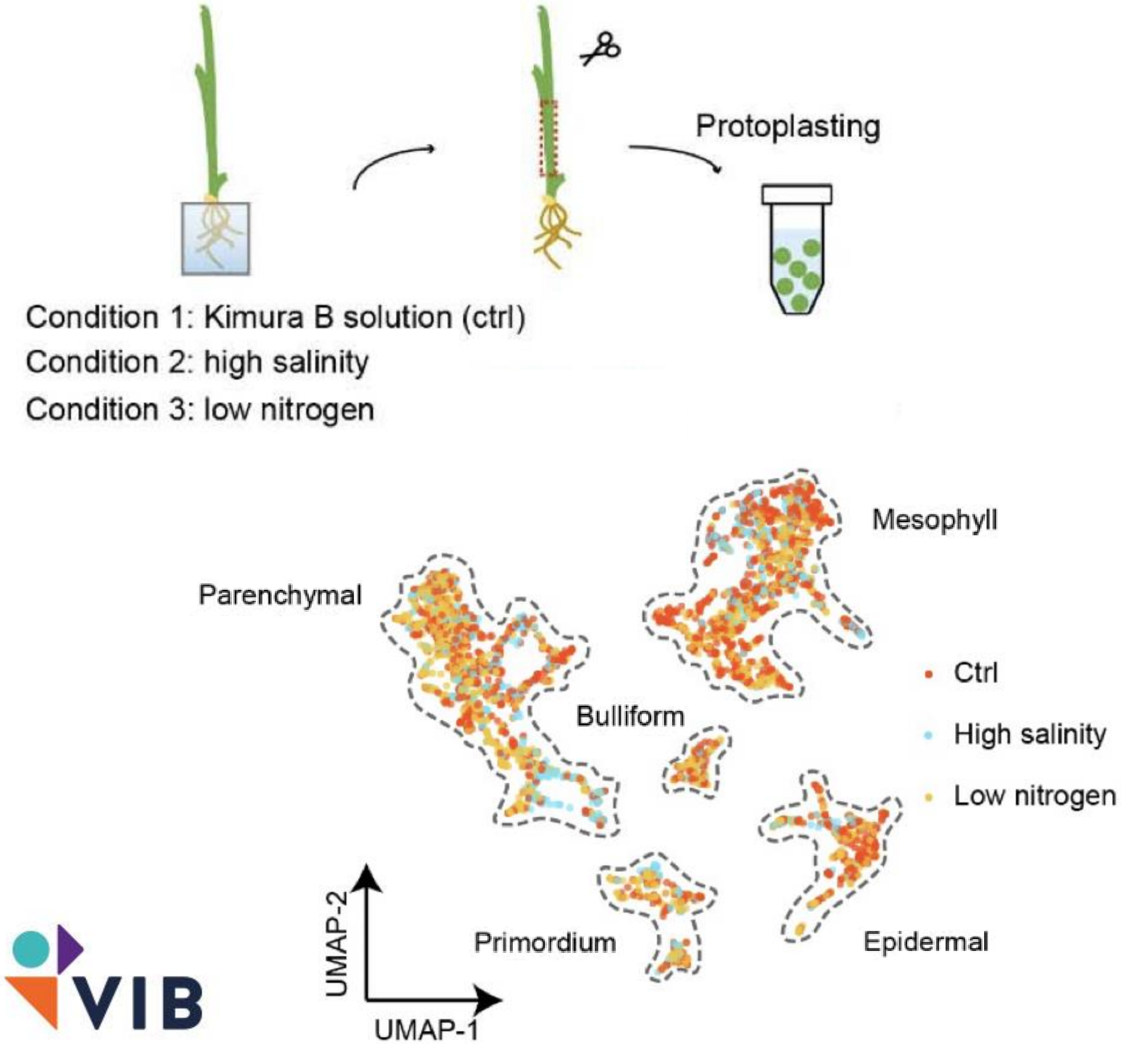
Wendrich J, et al. (2020) Science

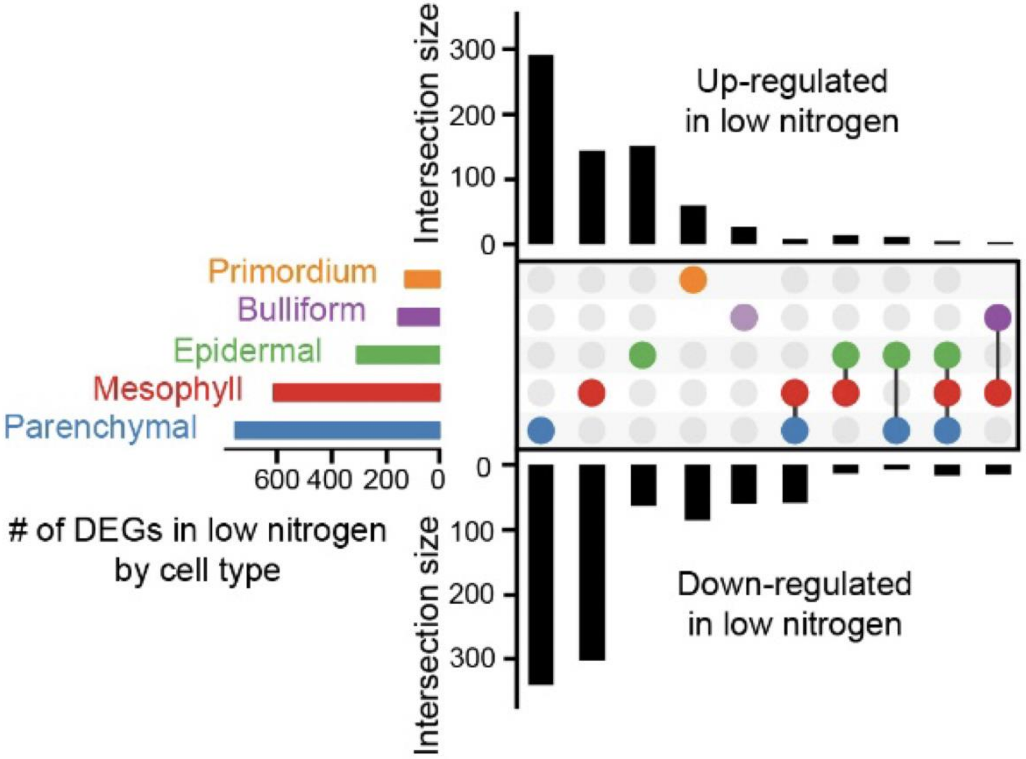
Atlassing reveals new marker genes



Wendrich J, et al. (2020) Science

Cell-type specific responses to stress (rice)

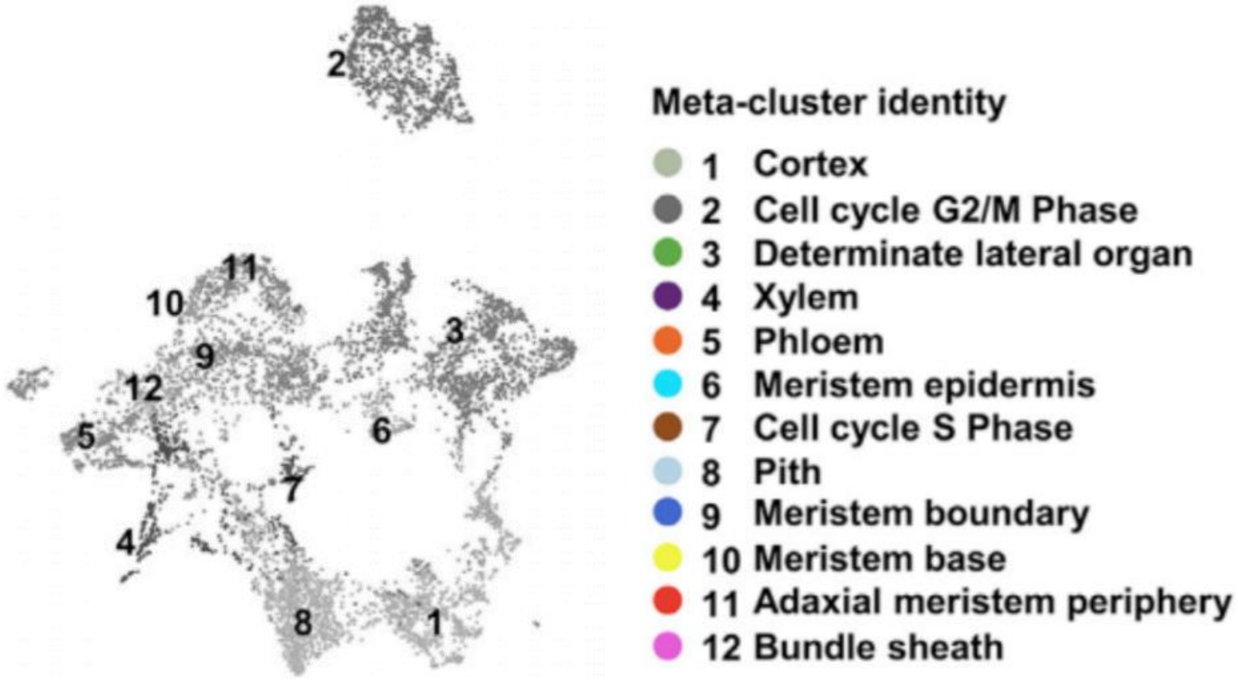


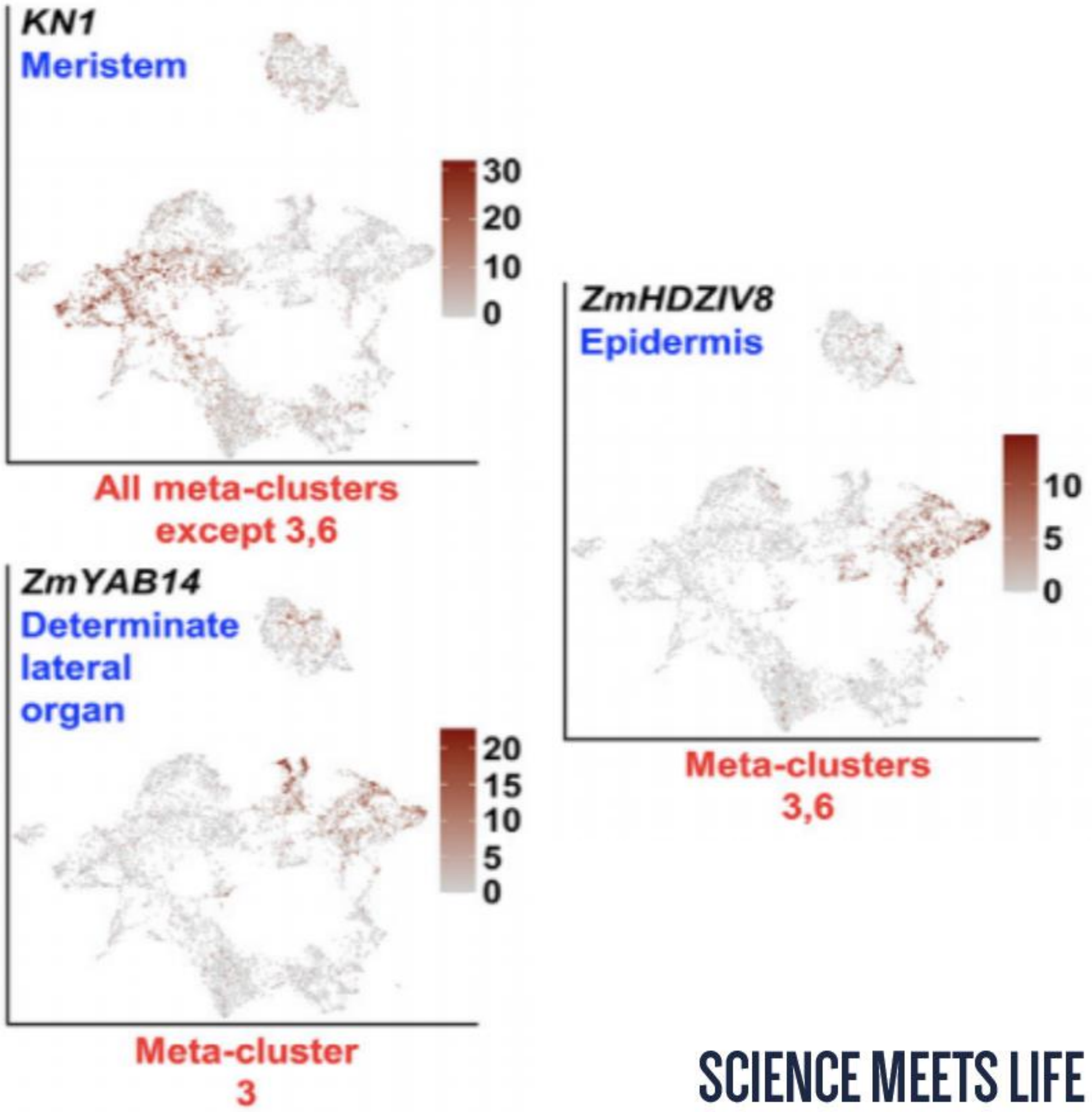


Wang Yu, et al. (2020) BioRxiv

Cluster annotation

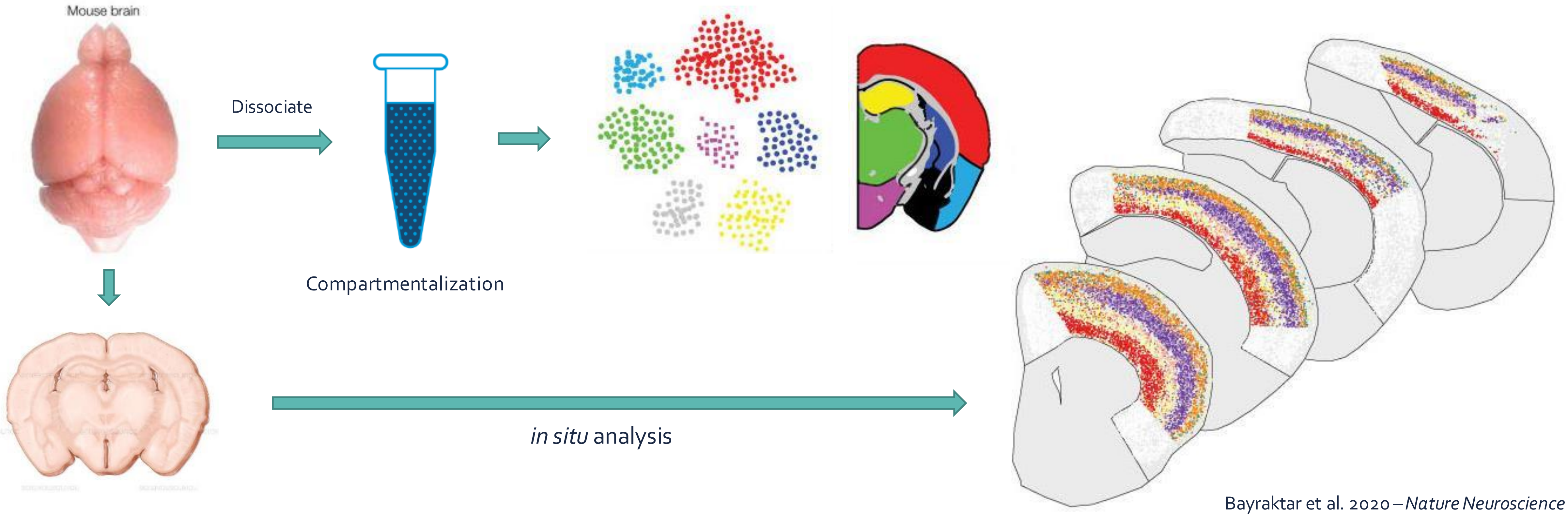
Maize ear atlas





Xu X, et al. (2021) Developmental Cell

Spatial transcriptomics



Dissociate

Compartmentalization

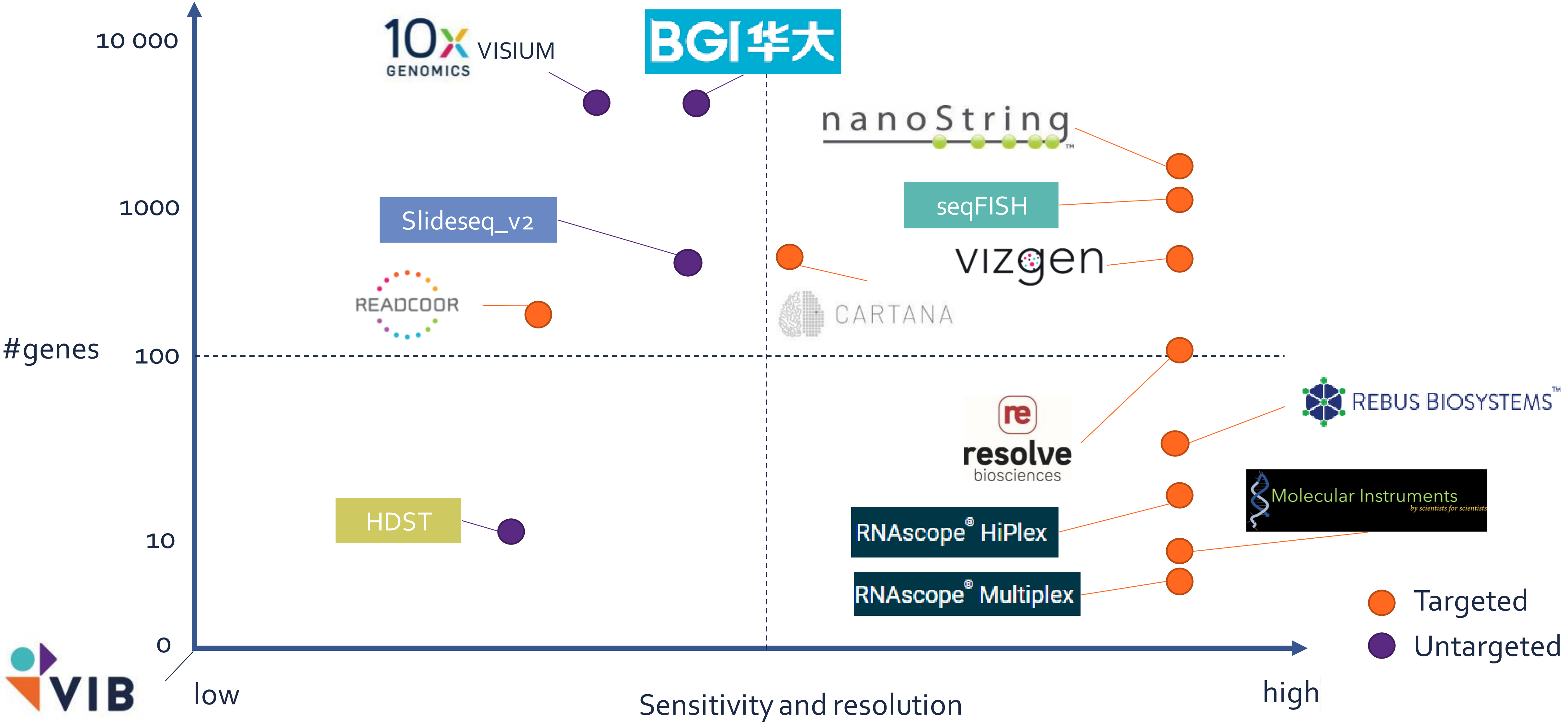
in situ analysis

Bayraktar et al. 2020 – *Nature Neuroscience*

Method of the Year 2020: spatially resolved transcriptomics

Spatially resolved transcriptomics methods are changing the way we understand complex tissues.

Technological landscape



Targeted spatial transcriptomics

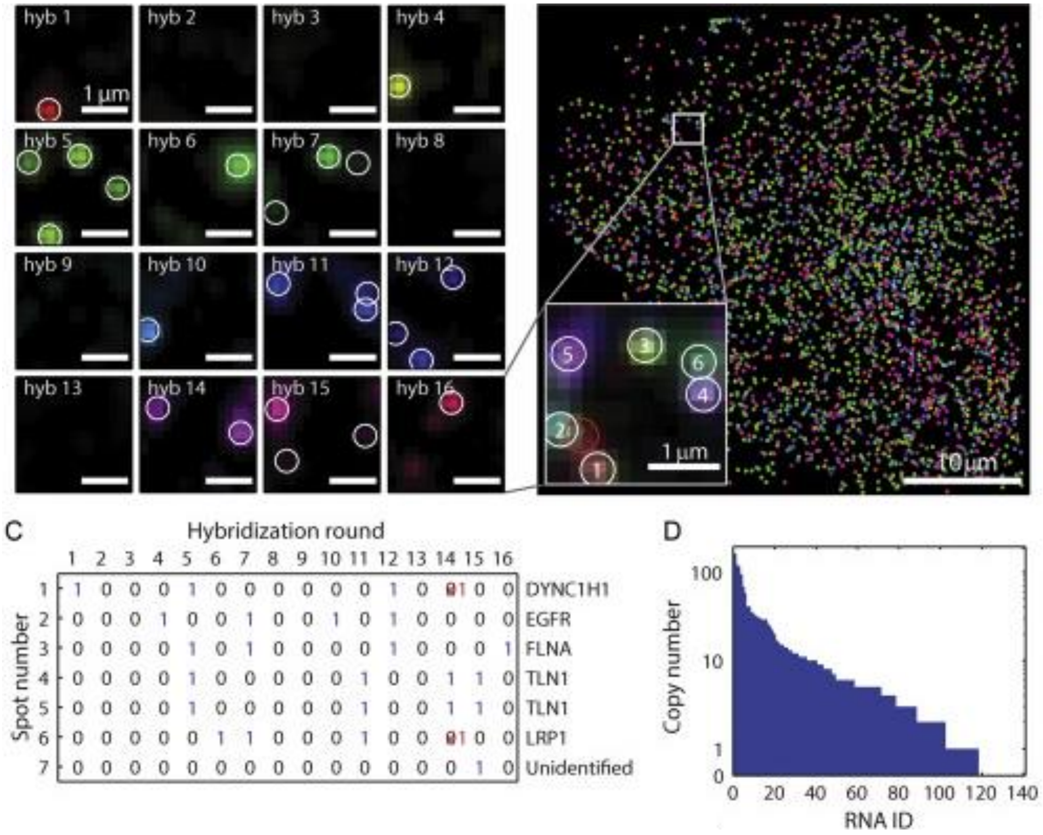
Fixed tissue section

Stain with target probes

Hybridize fluorescent readout probes

Image and erase signal

Decode RNA identity



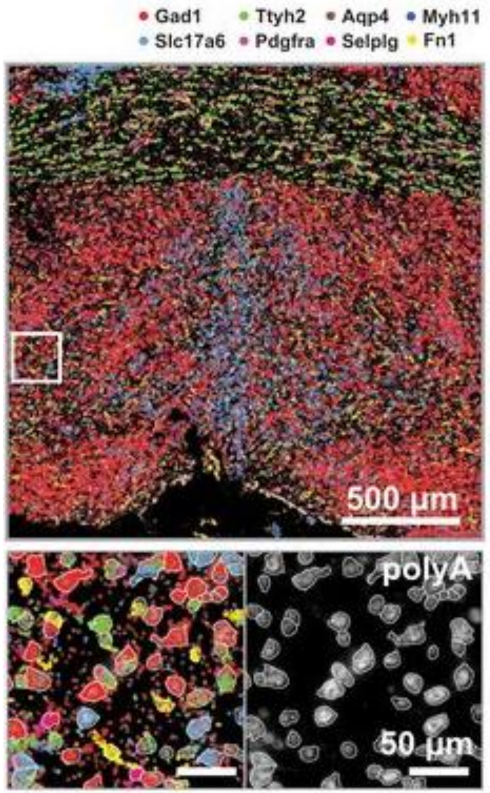
Hybridization round

Spot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1	1	0	0	0	1	0	0	0	0	0	0	1	0	01	0	0	DYNC1H1
2	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	EGFR
3	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	FLNA
4	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	TLN1
5	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	TLN1
6	0	0	0	0	0	1	1	0	0	0	1	0	0	01	0	0	LRP1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unidentified

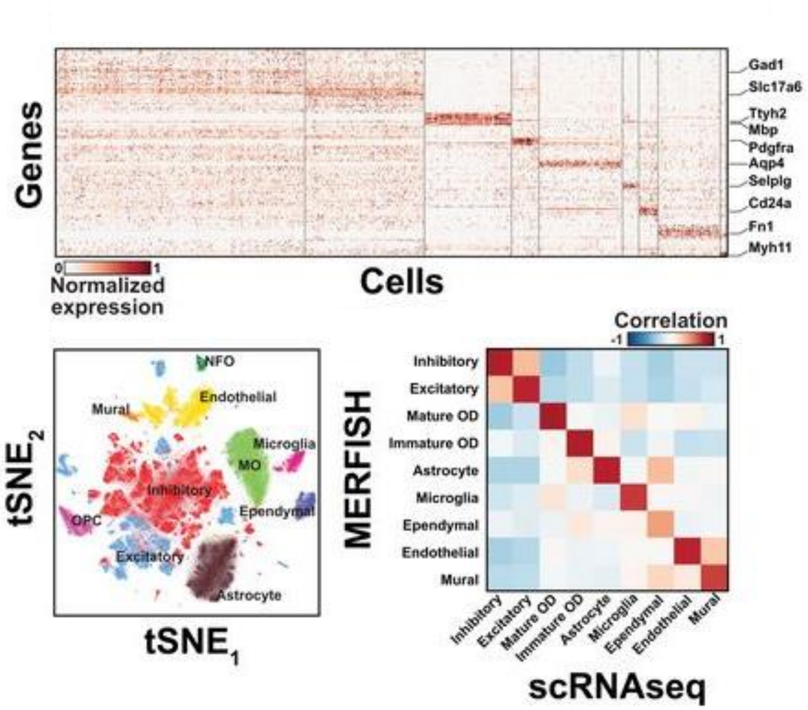
Moffitt JR, Zhuang X,. (2016) Methods Enzymol

Reconstructing spatial transcriptomics data to cell-type maps

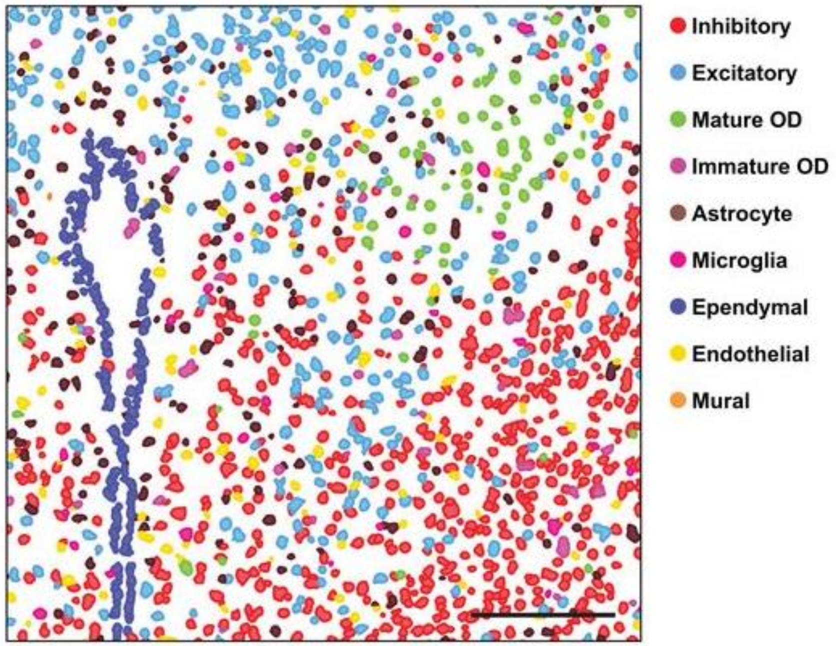
Raw data and segmentation



Clustering on marker expression



Construct map



Moffitt JR, et al. (2018) Science

Targeted spatial transcriptomics

Fixed tissue section

↓

Stain with target probes

↓

Hybridize fluorescent readout probes

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Image and erase signal

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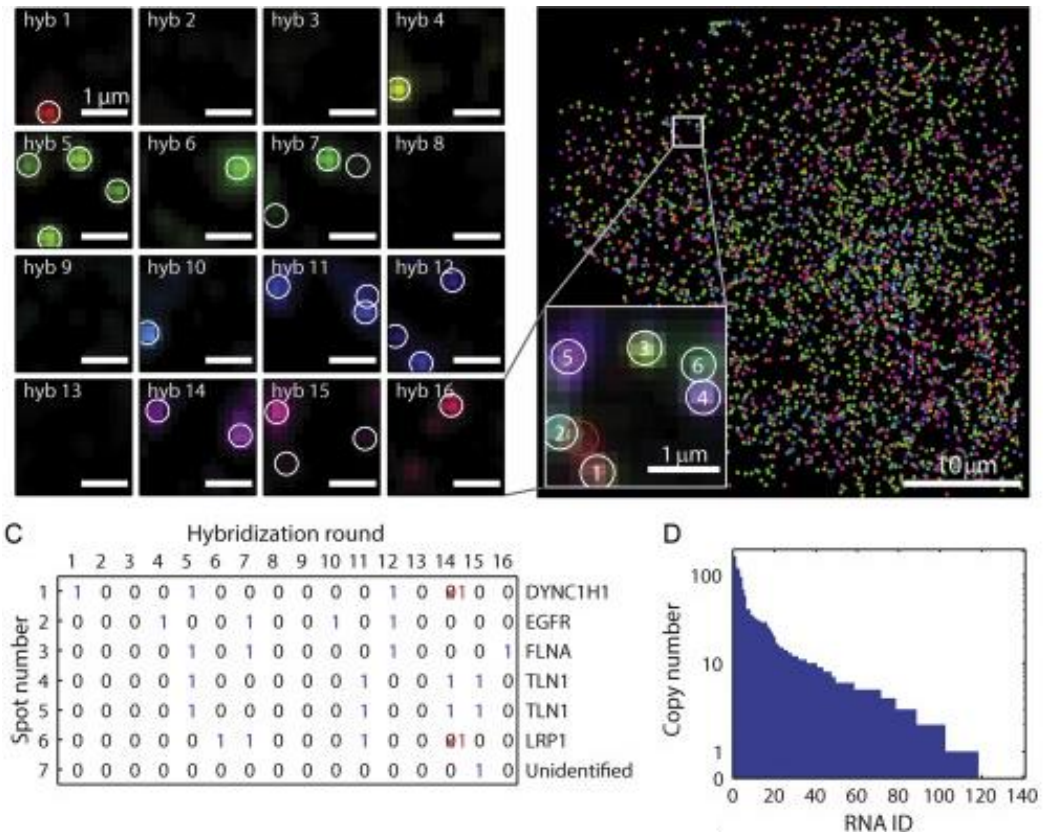
Decode RNA identity



Hybridization round

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3	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	FLNA
4	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	TLN1
5	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	TLN1
6	0	0	0	0	0	1	1	0	0	0	1	0	0	01	0	0	LRP1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	Unidentified

Moffitt JR, Zhuang X,. (2016) Methods Enzymol

- ✓ Superior resolution and sensitivity
- ✓ Method of choice for validating scRNA-seq markers
- ✗ Limited panel “budget”
- ✗ Specialized equipment

VIB

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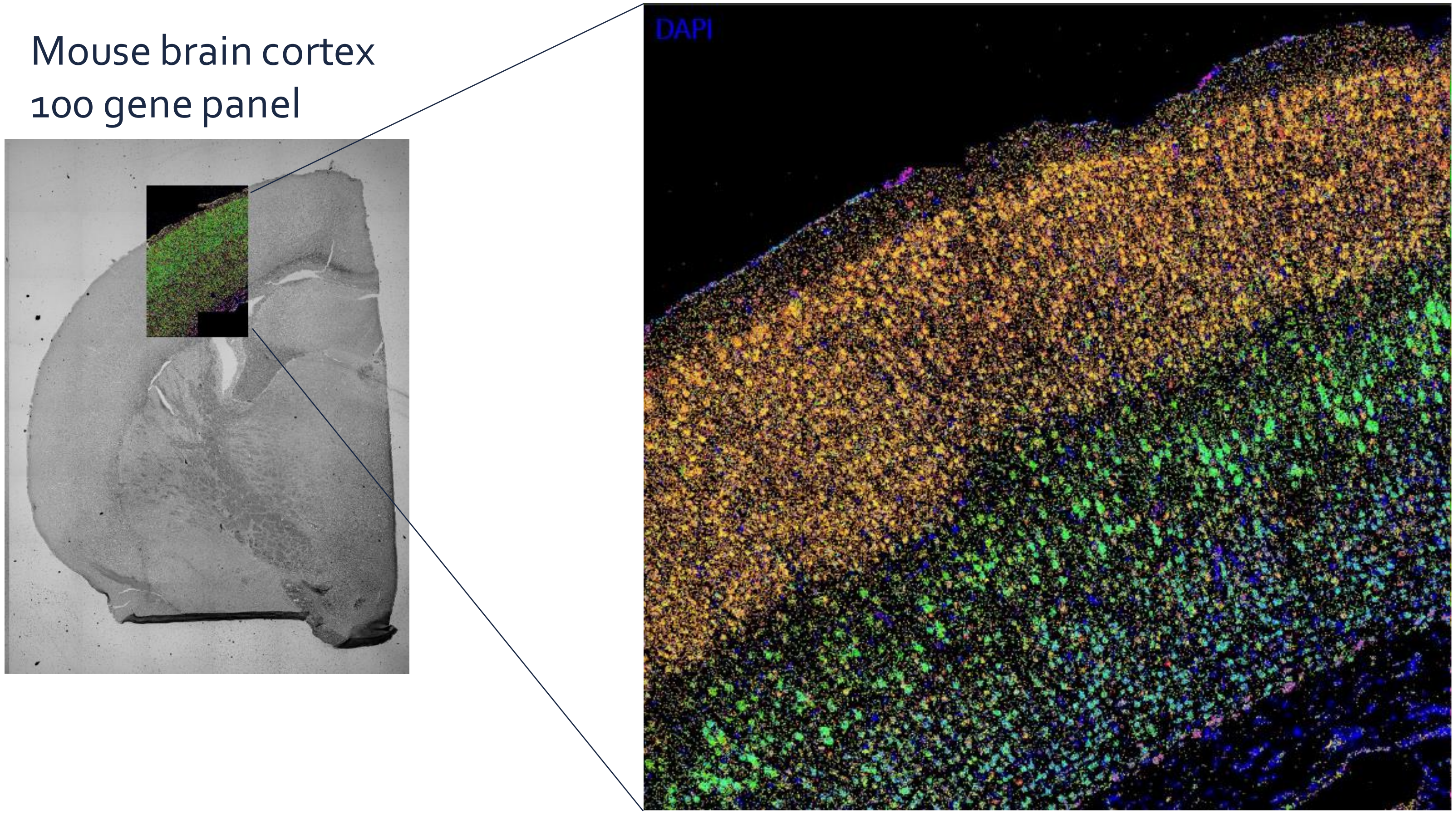
Targeted spatial transcriptomics at VIB

Mouse brain cortex
100 gene panel



Unpublished data

$1mm^2$
2million transcripts detected

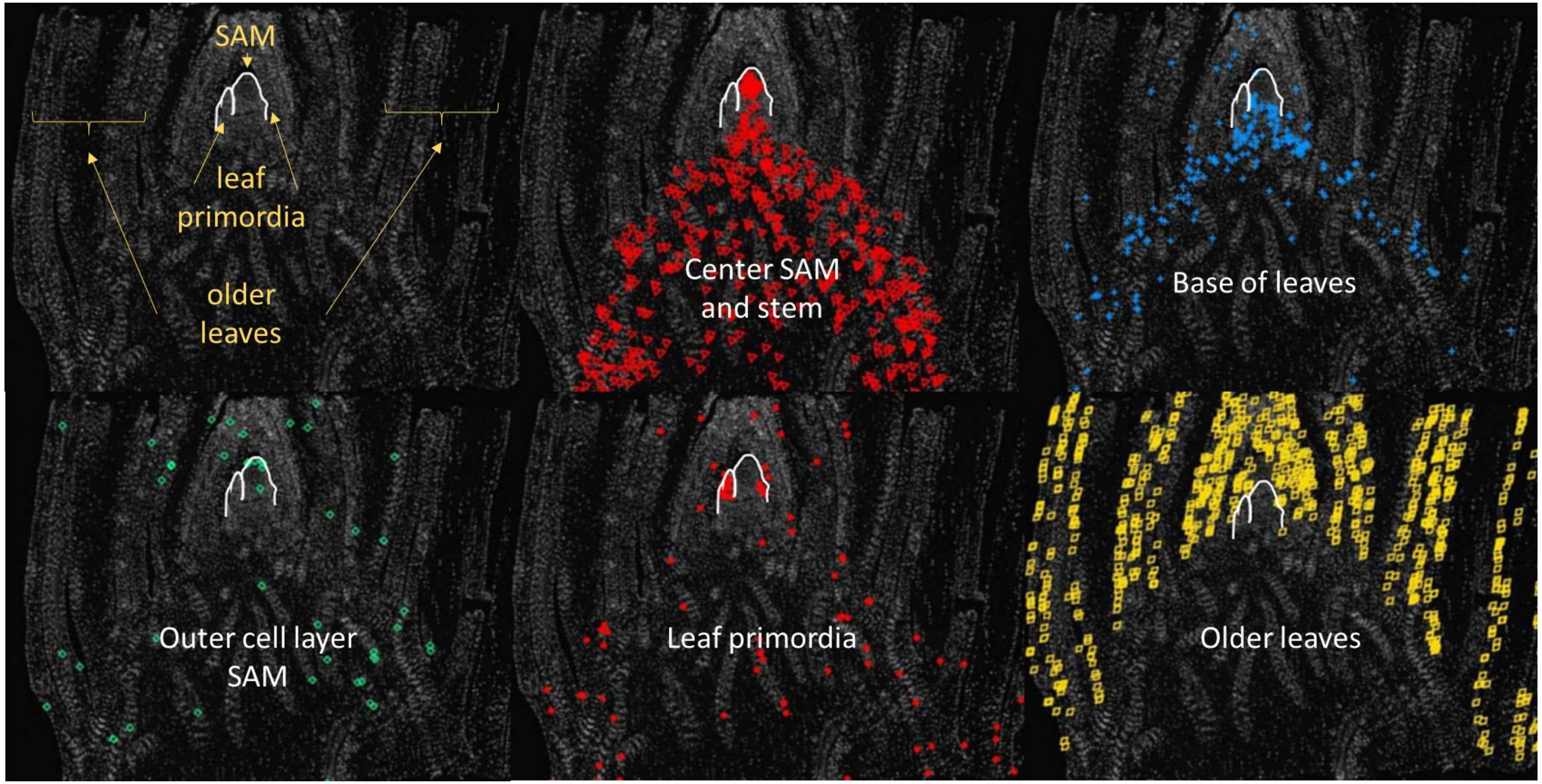
🔴	Reln	layer 1
🔴	Rasgrf2	layer 2/3
🟠	Cux1	layer 2-4
🟡	Cux2	layer 2-4
🟡	Pou3f2	layer 2-5
🟢	Pcp4	layer 5
🟢	Fezf2	layer 5
🔵	Bcl11b	layer 5-6
🔵	Foxp2	layer 6
🔵	Tle4	layer 6
🟣	Tbr1	layer 6
🟣	Ccn2	layer 6b

Targeted spatial transcriptomics at PSB

CARTANA

10x GENOMICS®

Maize shoot apical meristem, 90 gene panel



Laureyns R, et al. (in preparation)

Nelissen lab

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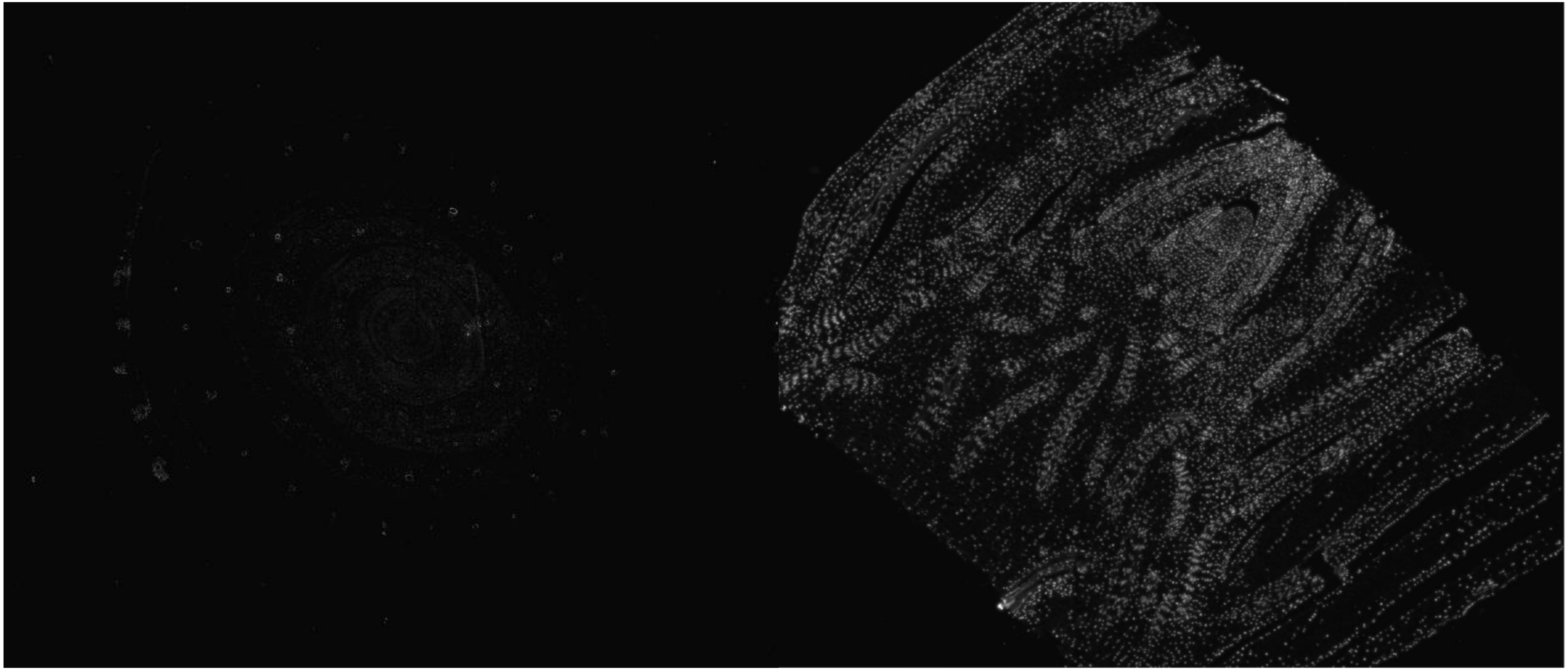
Targeted spatial transcriptomics at PSB



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Untargeted spatial transcriptomics

Fixed tissue section

↓

Application of 2D barcode matrix

↓

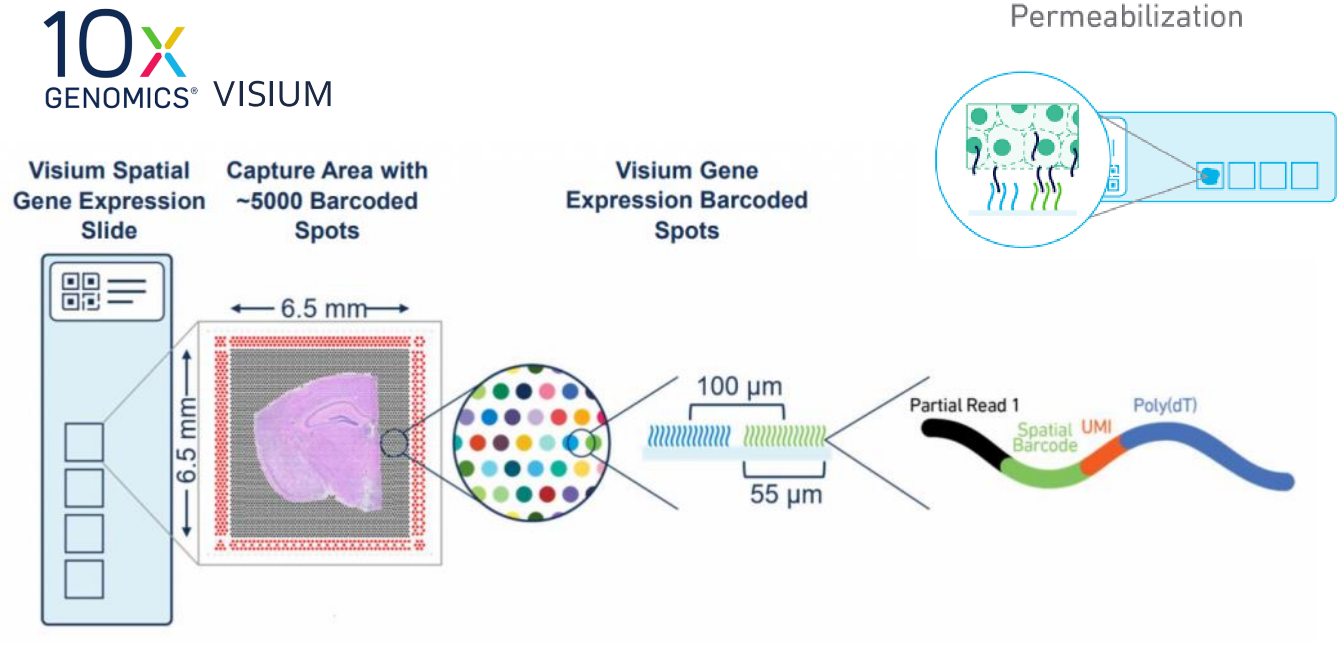
Permeabilisation and ligation of 2D barcodes

↓

Bulk library prep and sequencing

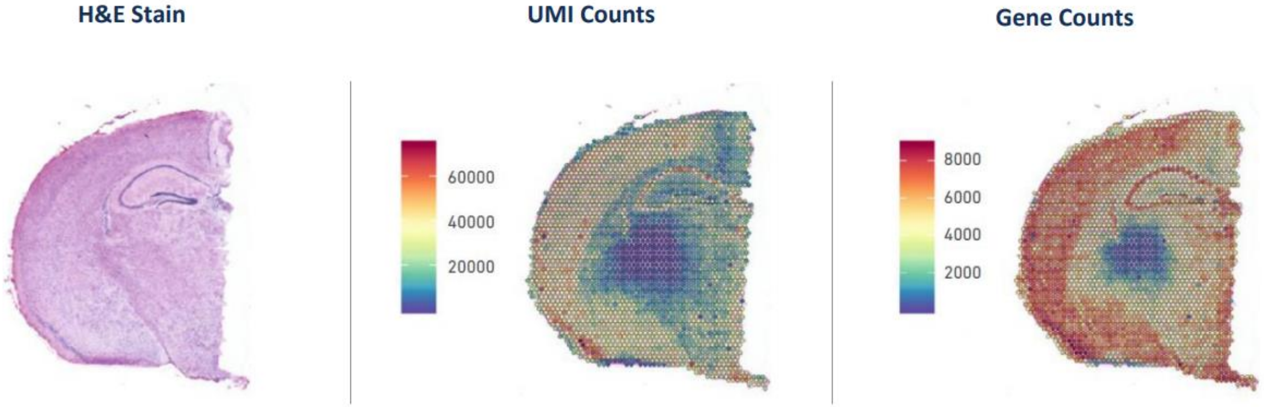
↓

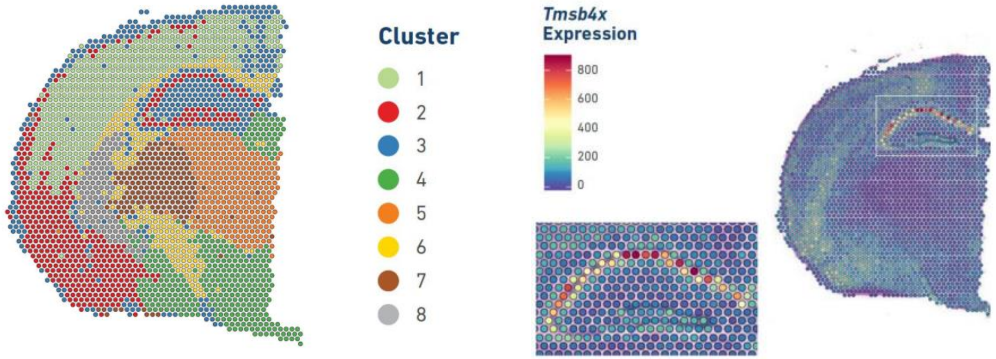
Clustering and spatial reconstruction

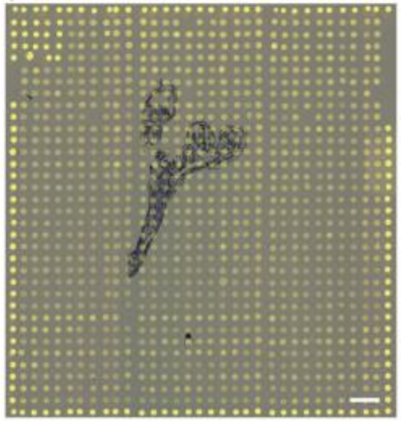


Untargeted spatial transcriptomics

10x GENOMICS



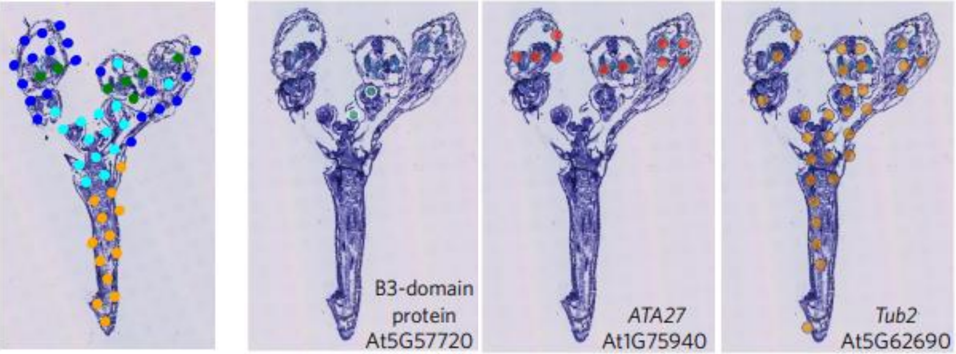




SPATiAL TRANSCRIPTOMICS → 10x GENOMICS

100µm spots

55µm spots



Giacomello S, et al. (2017) Nature plants
Giacomello S, et al. (2018) Nature protocols

Untargeted spatial transcriptomics

Fixed tissue section

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Application of 2D barcode matrix

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Permeabilisation and ligation of 2D barcodes

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Bulk library prep and sequencing

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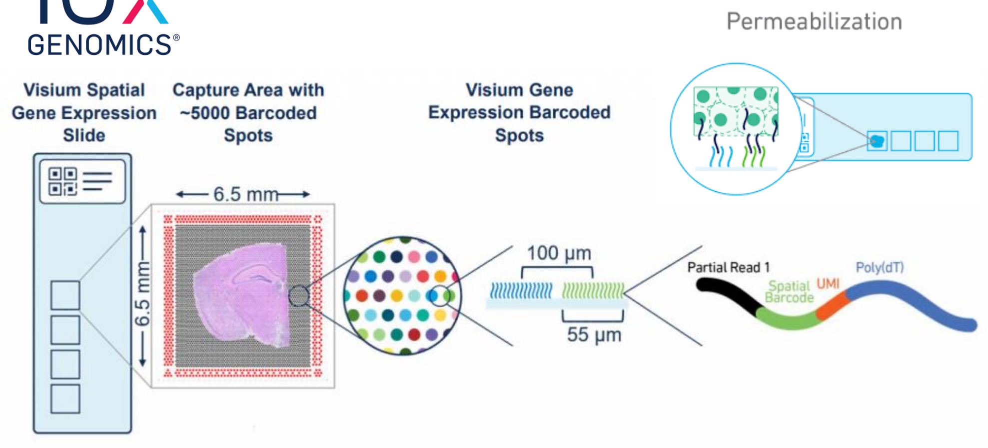
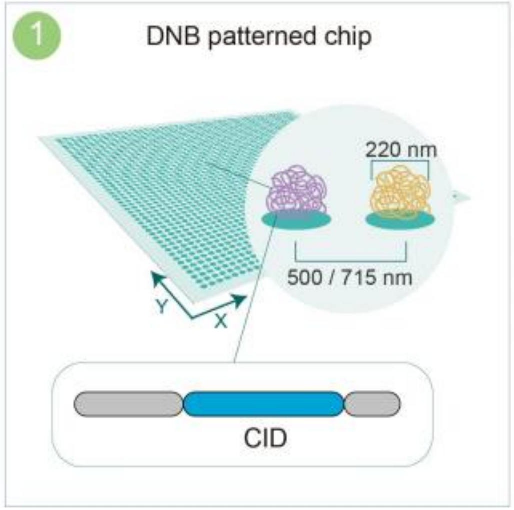
Clustering and spatial reconstruction

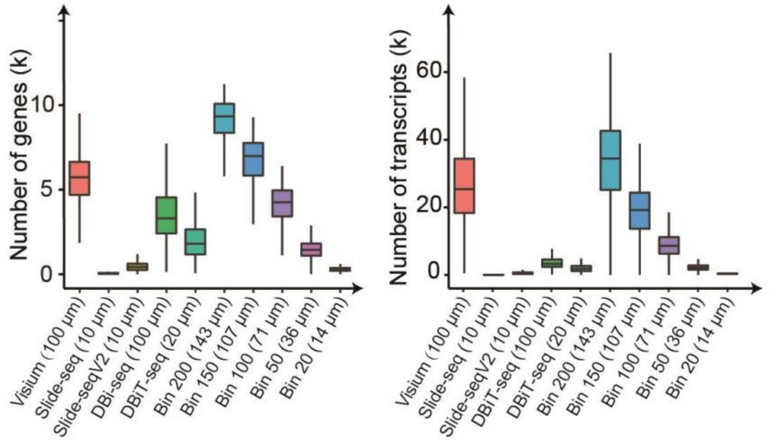
10x GENOMICS®





Chen Ao, et al. 2021 (BioRxiv)

- ✓ Transcriptome-wide = clustering + spatial info
- ✓ Readout using illumina sequencing
- ✗ Resolution/capture efficiency



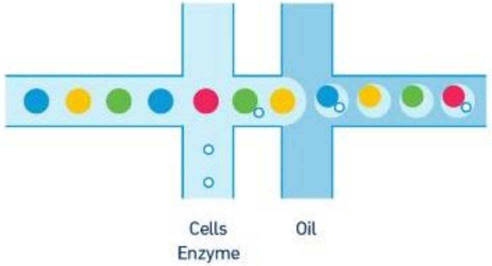
Chen Ao, et al. 2021 (BioRxiv)

Additional benefits of spatial transcriptomics

- No protoplasting required
  - No protoplasting/stress- induced genes
  - No loss of cell types due to inefficient tissue dissociation or sample prep
- Throughput
  - Max output of a single 10X lane = 10k cells
  - Depending on sample, ST can interogate 100k of cells in a single run
- Beyond marker validation
  - Spatial co-expression analsysis
  - Cell-cell interactions
  - Subcellular localisation of transcripts

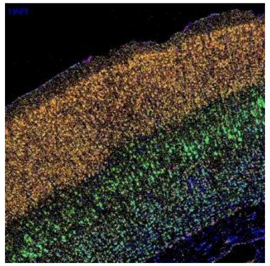
Take home

Single cell transcriptomics



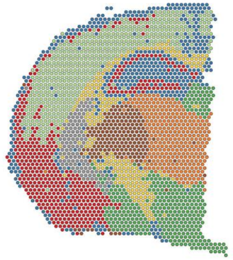
- ✓ Discover novel markers for cell types and developmental states
- ✓ Study response to environment or genetic perturbation at single cell level
- ✗ Lacks spatial component

Targeted spatial



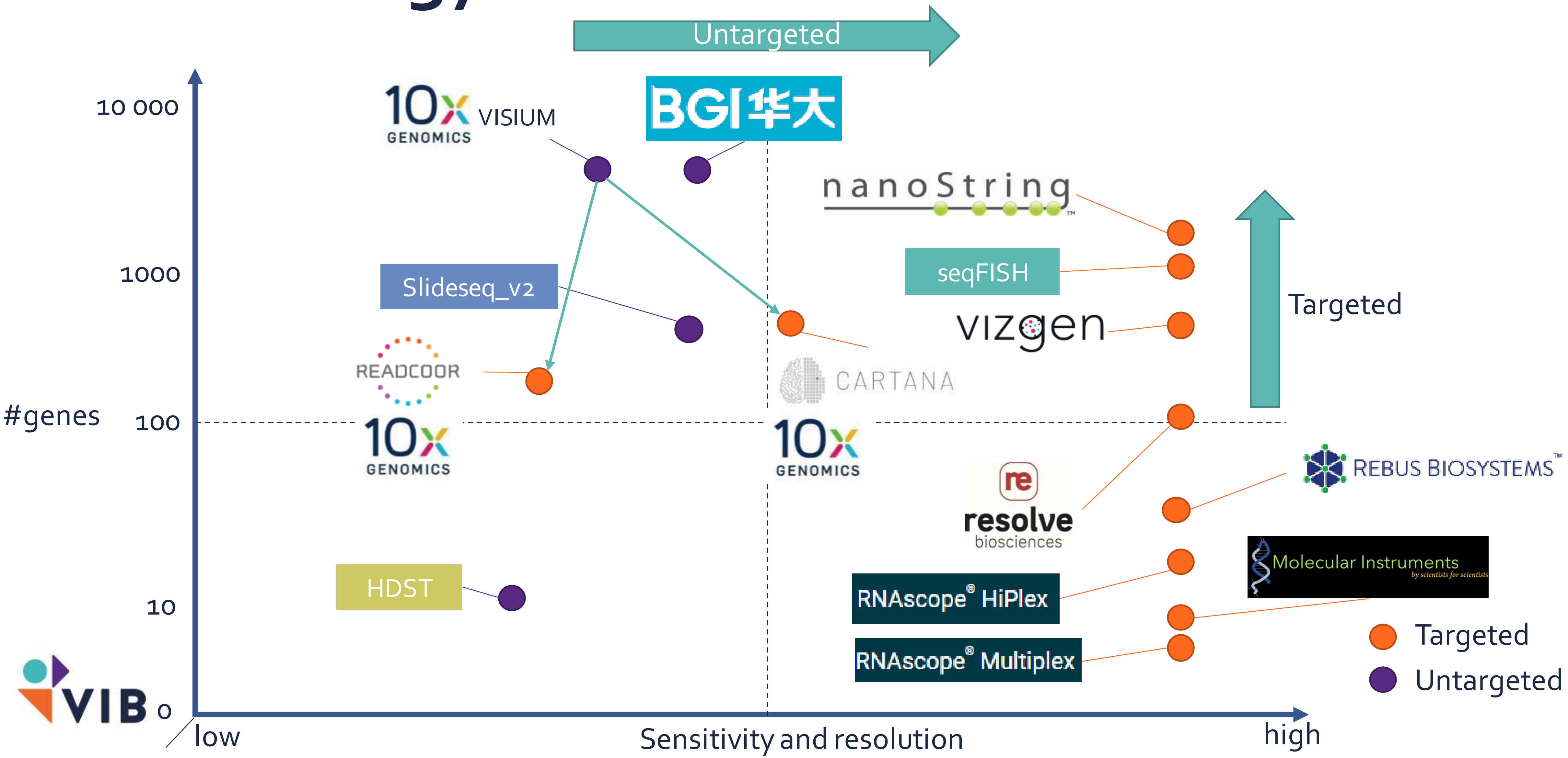
- ✓ Validate novel single cell markers or locate new cell-types
- ✓ Study spatial transcriptomes at (sub)cellular resolution
- ✗ Requires rational target panel design

Untargeted spatial



- ✓ Transcriptome-wide spatial mapping
- ✗ Limited resolution

Technology evolution forecast



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