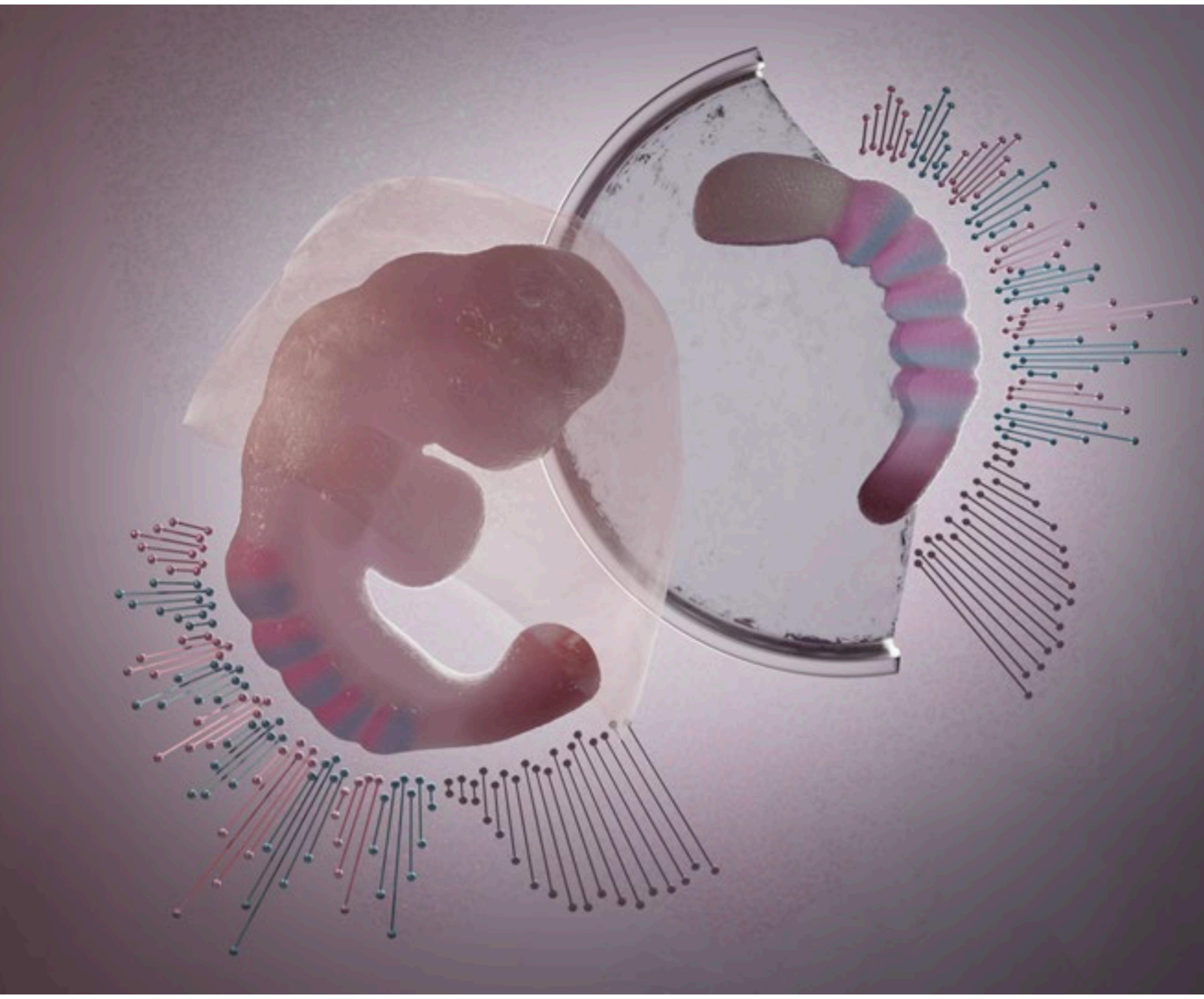


Spatial Transcriptomics

Anna Alemany
Dpt. Anatomy and Embryology
(LUMC)

20/09/2021, MGC/BioSB course

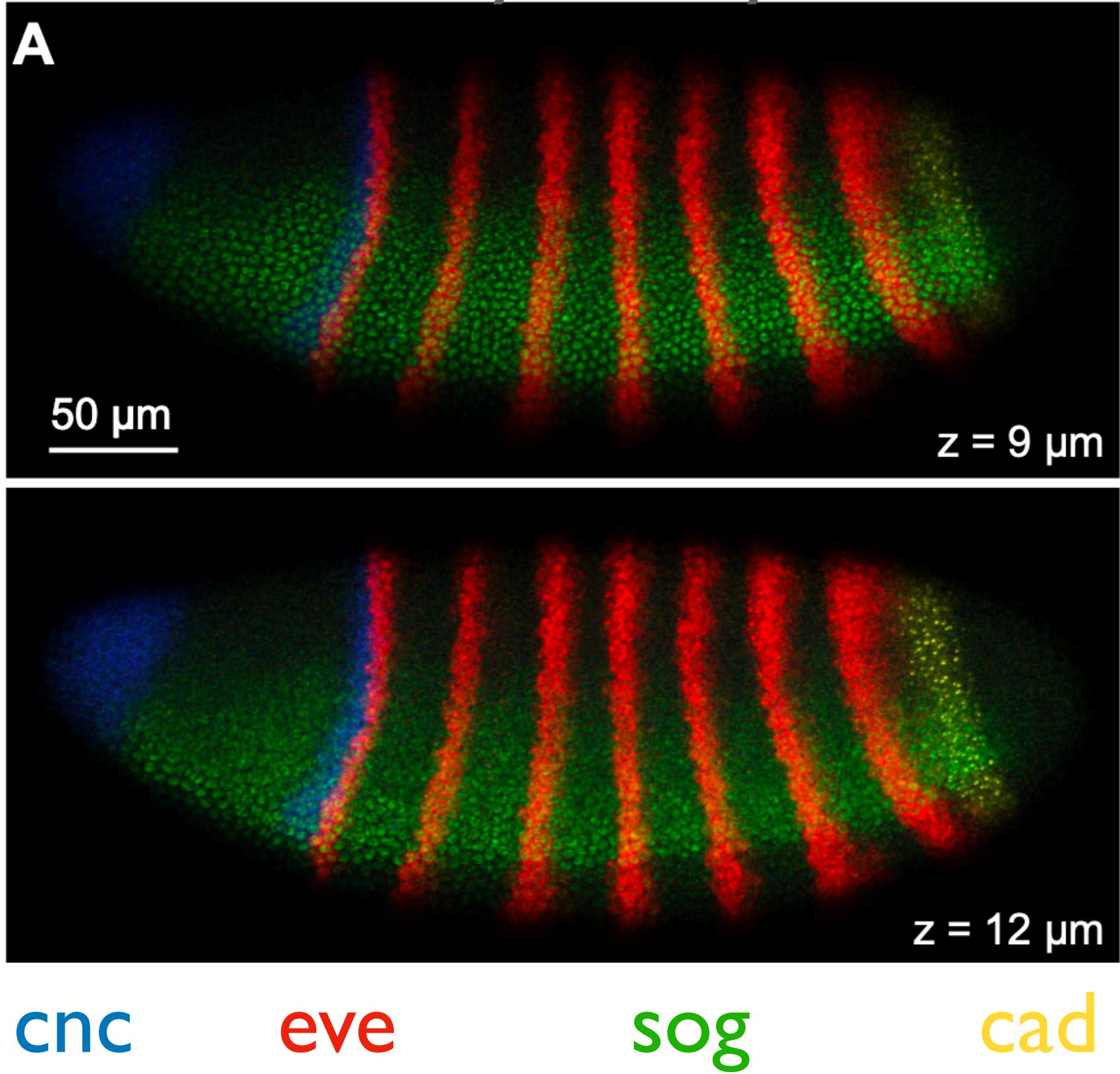


“Spatial transcriptomics is a molecular profiling method that allows to measure all gene activity in a tissue and map where the activity is occurring”

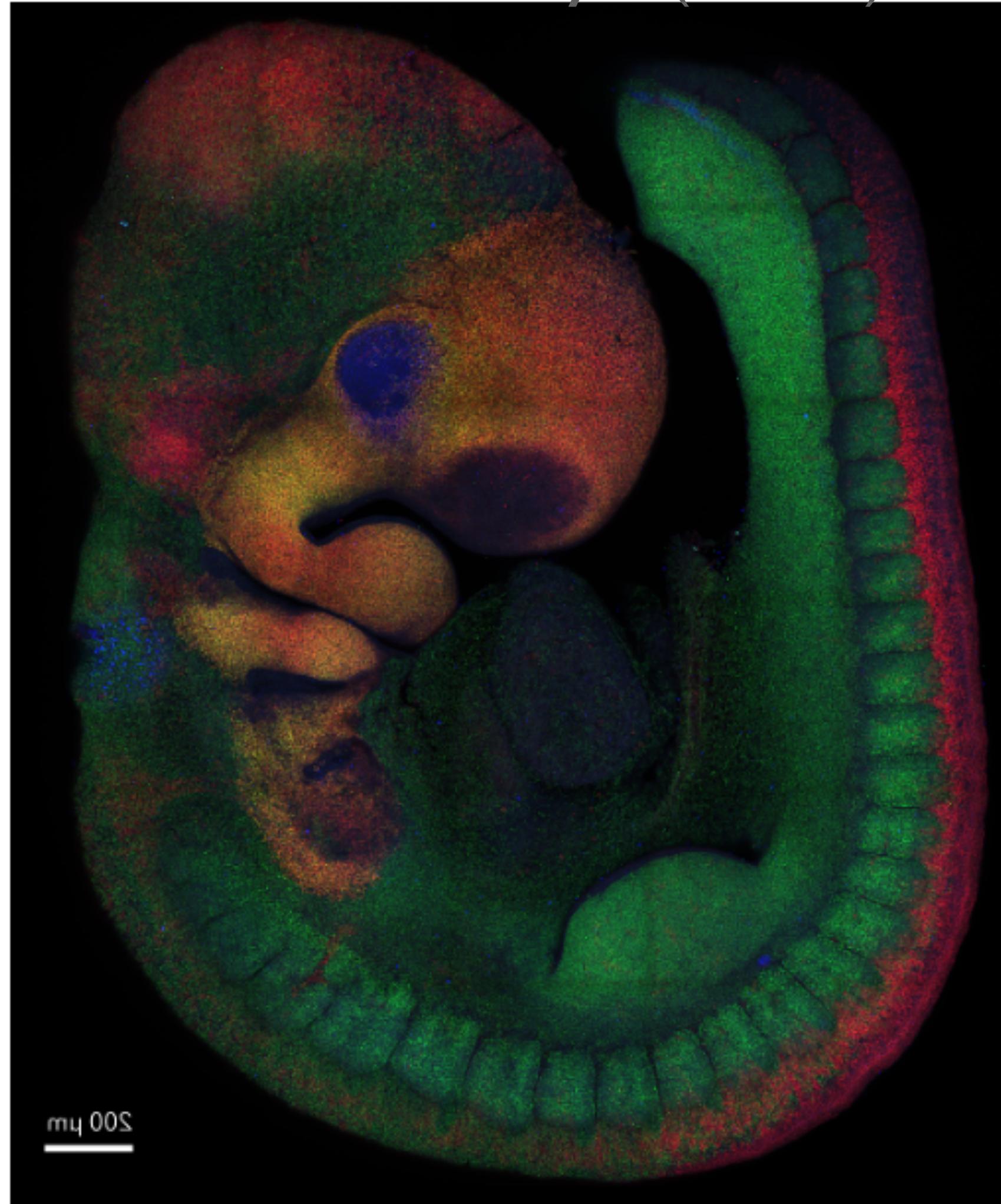
From 10xgenomics website

Tissues have architecture

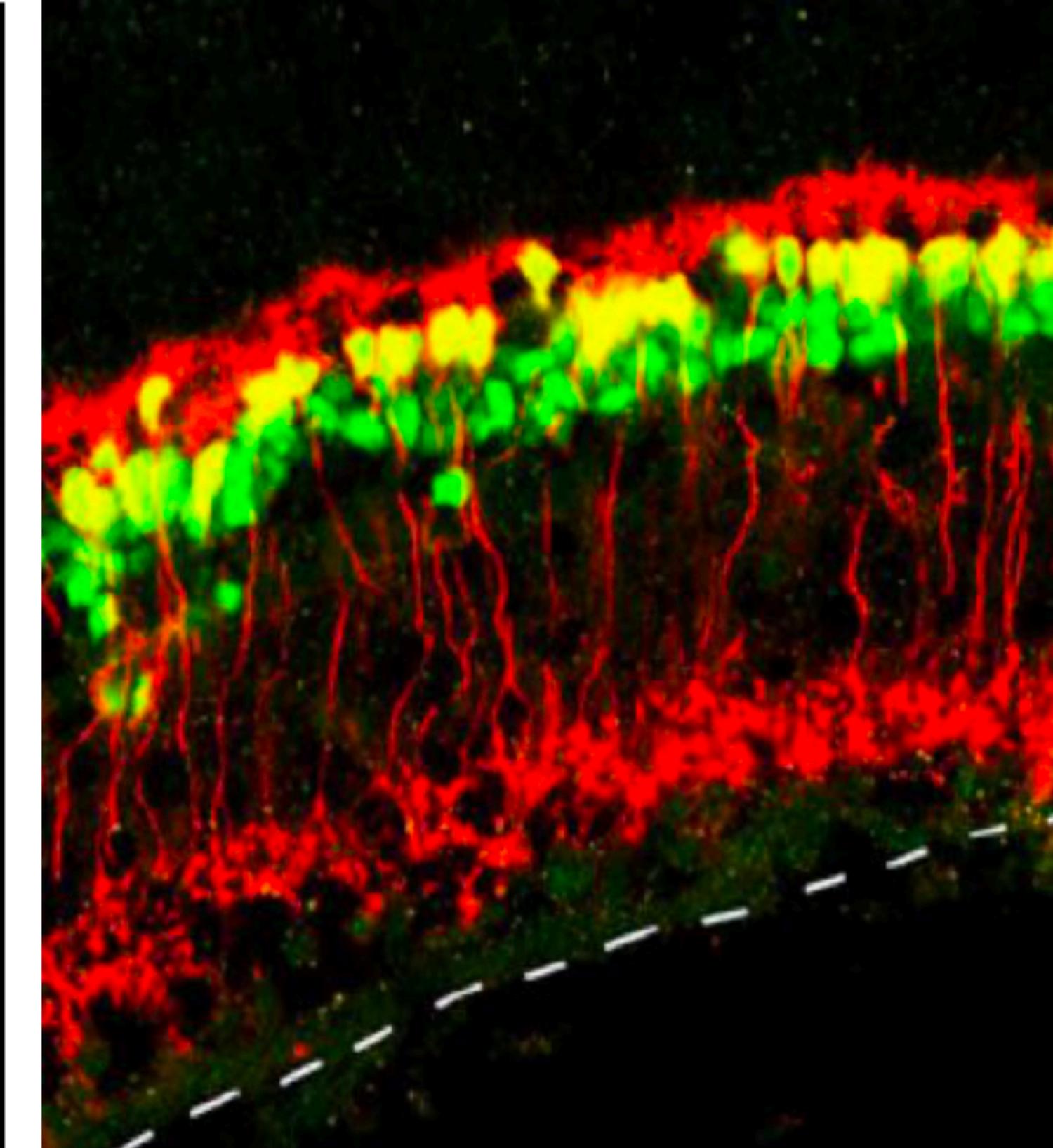
Fruit fly embryo

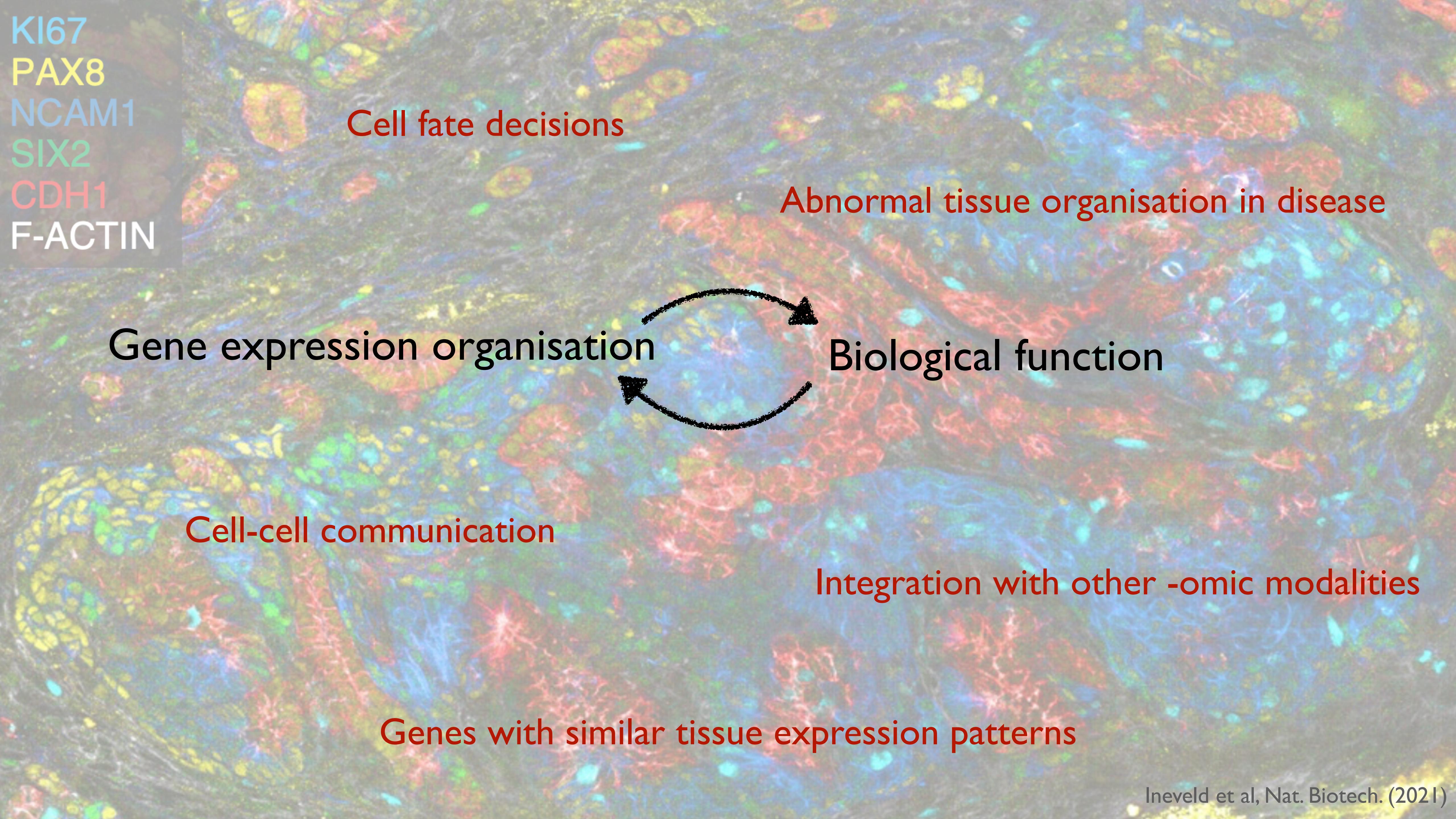


Mouse embryo (E10.5)



Mouse retina





KI67
PAX8
NCAM1
SIX2
CDH1
F-ACTIN

Cell fate decisions

Abnormal tissue organisation in disease

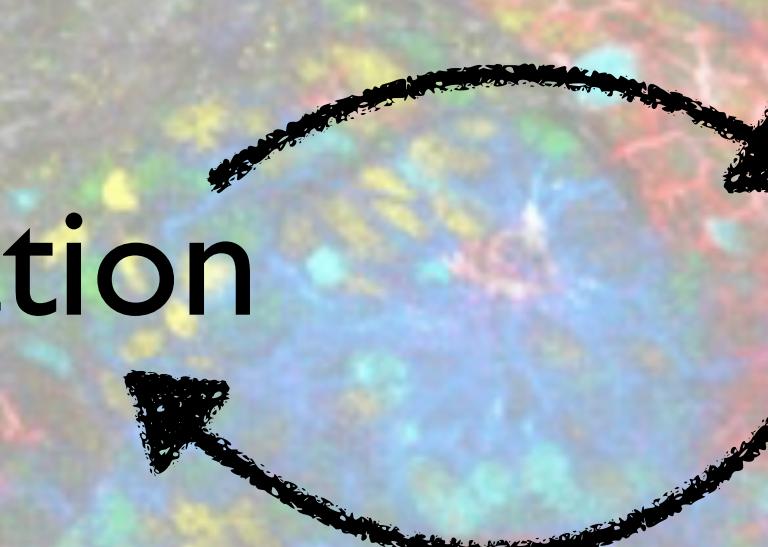
Gene expression organisation

Biological function

Cell-cell communication

Integration with other -omic modalities

Genes with similar tissue expression patterns



Method of the Year: spatially resolved transcriptomics

Nature Methods has crowned spatially resolved transcriptomics Method of the Year 2020.

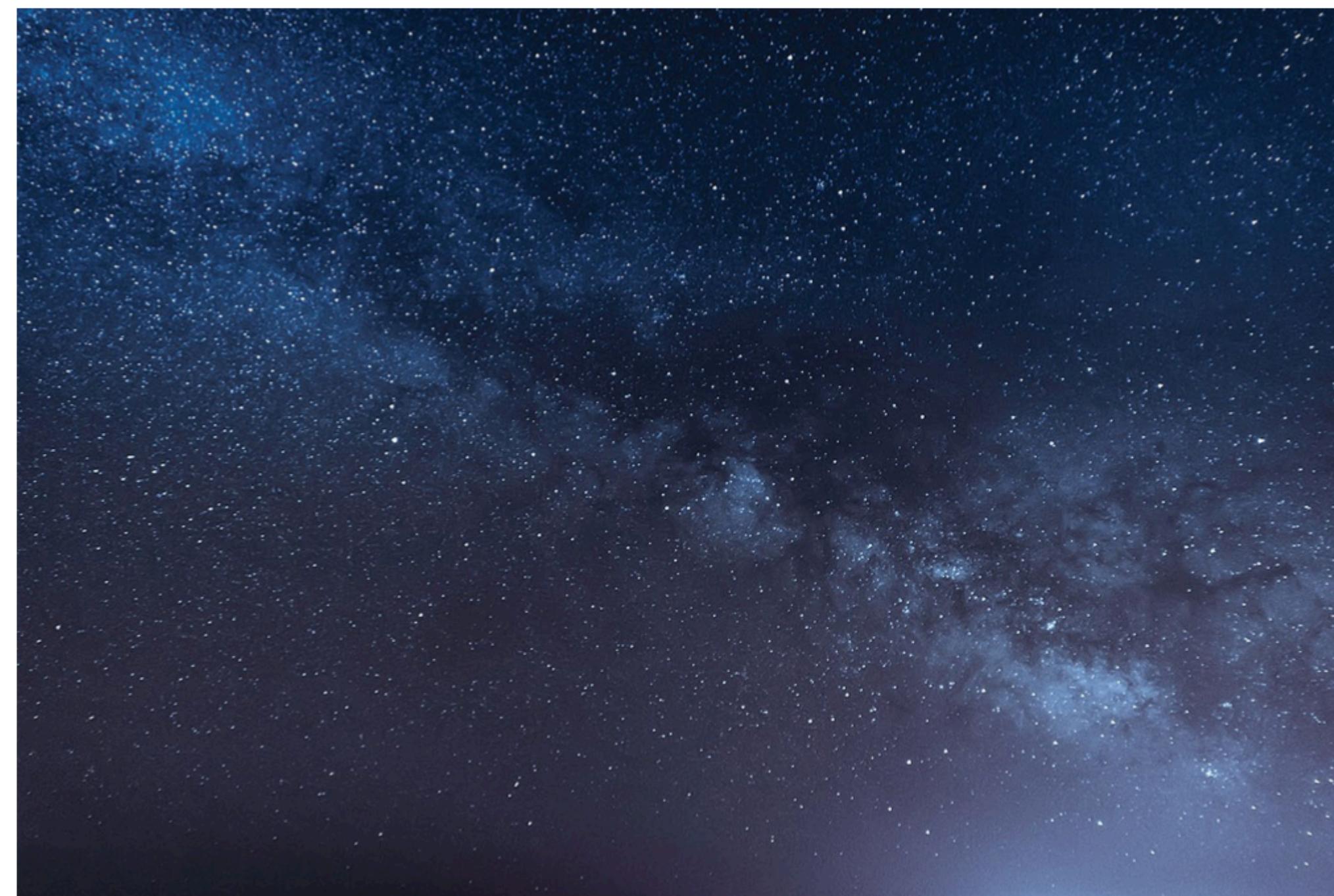
Vivien Marx

If a researcher is making a smoothie, it might be snack time. Or it could be the moment to prepare a sample for bulk RNA sequencing, in which tissue is homogenized and analyzed to yield averaged gene expression from the mRNAs in a tissue's cells — its transcriptome.

When single-cell RNA sequencing (scRNA-seq) was developed, it brought a more fine-grained assessment of each cell's transcriptome. In scRNA-seq, researchers dissociate cells from tissue to, for example, discern cell types on the basis of gene expression. Working with single cells is more like digging into a fruit salad than a smoothie, says Hongkui Zeng, who directs the Allen Institute for Brain Science. Now,

with spatially resolved transcriptomic methods, scientists can get transcriptomic data and know the positional context of those cells in a tissue^{1–3}. “Fruit tart is spatial transcriptomics,” says Bosiljka Tasic, an Allen Institute researcher who was interviewed jointly with Zeng. “You know exactly where each piece of fruit is and what is the relationship of each piece of fruit to the other,” she says. Given how

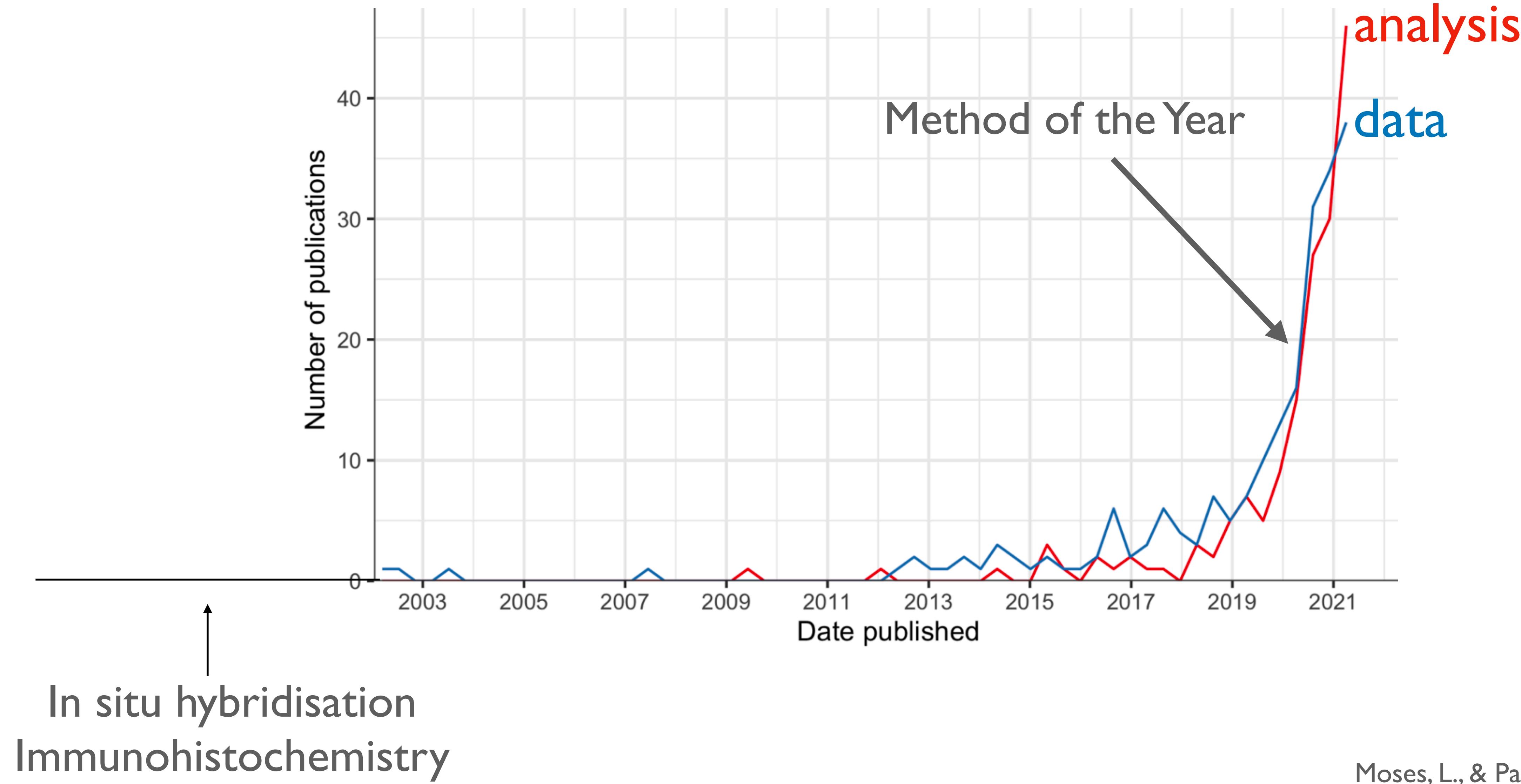
much one can learn from single cells and spatially resolved cells, the ‘smoothie’ approach is fast becoming passé, she says. It’s not yet routine for spatial analysis to deliver transcriptome-wide information of all single cells, but the field is fast moving



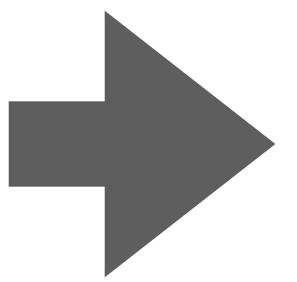
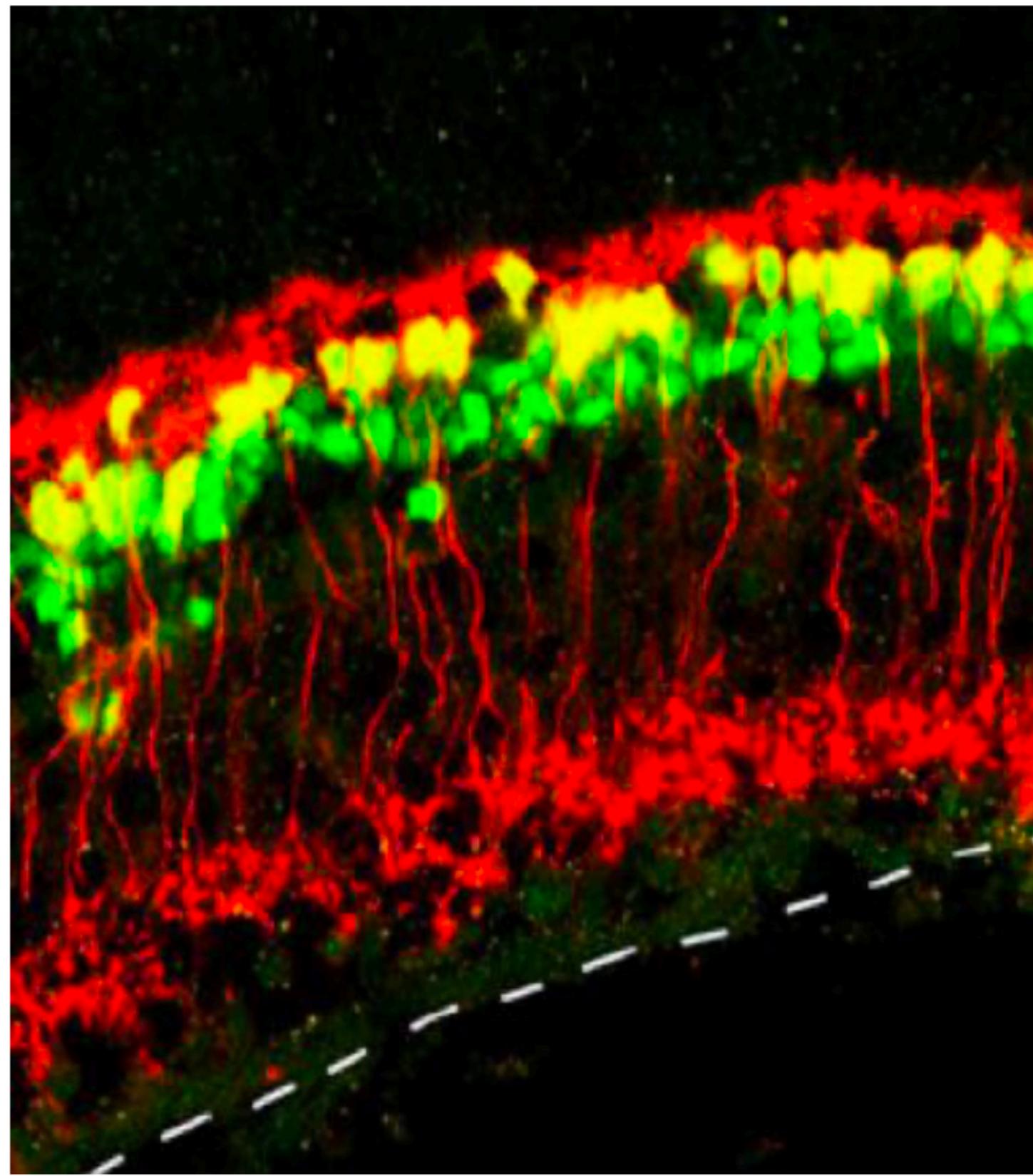
Starry skies invite space exploration. In transcriptomics, spatial resolution opens up new worlds too.
Credit: bjdlzx/Getty Images

says Jay Shendure from the University of Washington. He is most excited about ways to apply these methods to obtain spatially resolved atlases of development that combine cell states, histories and

says Alexander van Oudenaarden, a physicist turned biologist who directs the Hubrecht Institute in Utrecht. He and his lab have combined single-cell and spatial transcriptomics to compare gene expression



From the tissue to a count table



	Gene 1	Gene 2	...
Position 1	3	10	..
Position 2	7	1	..
..

Classification of spatial transcriptomics methods

Imaged-based

An image is processed to generate
a gene-expression matrix

- In situ hybridisation (ISH)
 - MERFISH
 - seqFISH
- In situ sequencing (ISS)
 - Sequencing by ligation
 - STARMap
 - Sequencing by synthesis
(BaristaSeq, Barseq)
 - Sequencing by hybridisation (HybISS)
 - FISSEQ
 - ExSeq (expansion)

Sequencing-based

Encode positional information
onto the transcripts

- Tomo-seq
- Capture and sequencing (Visium)
- Slide-seq
- HDST
- DBiT-seq
- Stereo-seq
- Seq-scope
- PIXEL-seq

Classification of spatial transcriptomics methods

Imaged-based

An image is processed to generate a gene-expression matrix

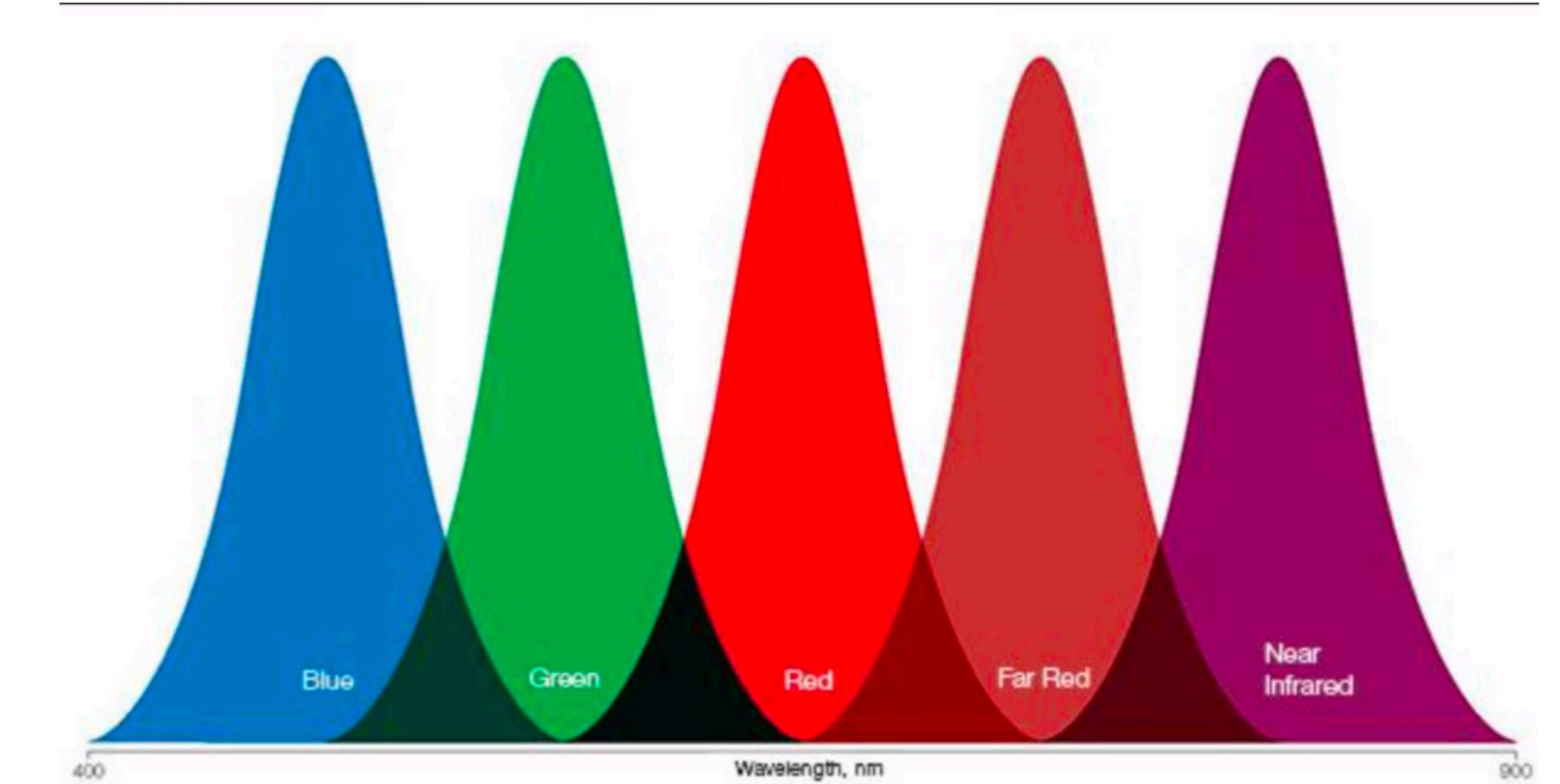
- In situ hybridisation (ISH)
 - MERFISH
 - seqFISH
- In situ sequencing (ISS)
 - Sequencing by ligation
 - STARMap
 - Sequencing by synthesis (BaristaSeq, Barseq)
 - Sequencing by hybridisation (HybISS)
 - FISSEQ
 - ExSeq (expansion)

Sequencing-based

Encode positional information onto the transcripts

- Tomo-seq
- Capture and sequencing (Visium)
- Slide-seq
- HDST
- DBiT-seq
- Stereo-seq
- Seq-scope
- PIXEL-seq

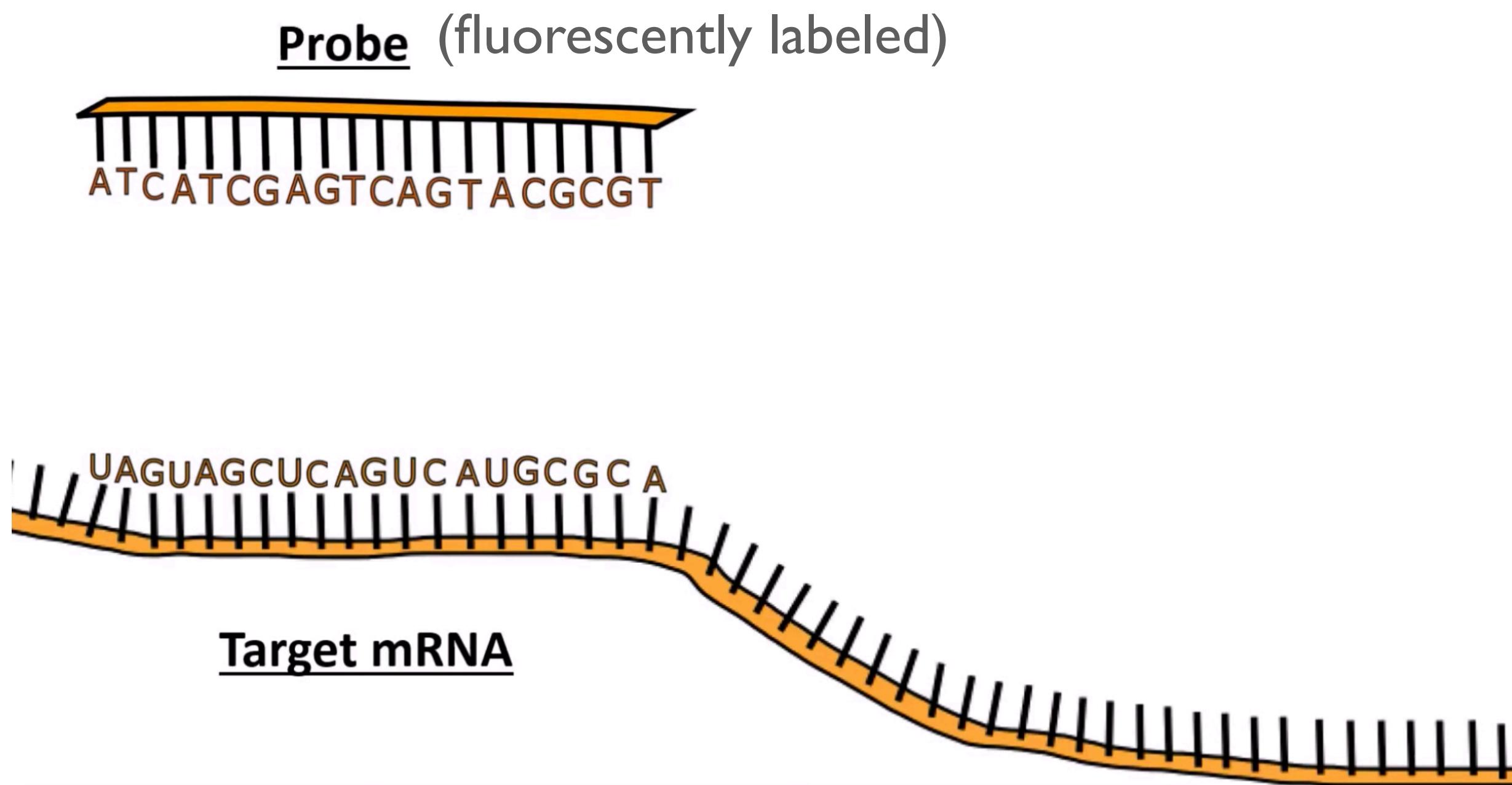
Most imaging-based approaches are limited by the amount of distinguishable fluorophores



Spectral overlap

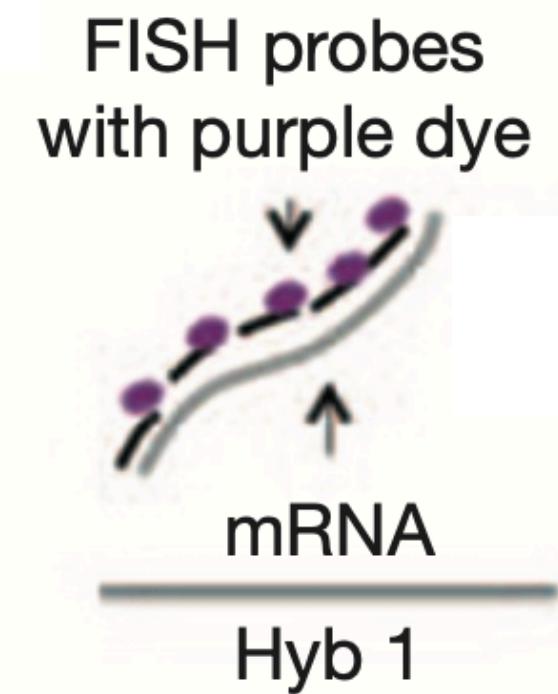
Imaged-based *ISH* methods

Targeted approaches



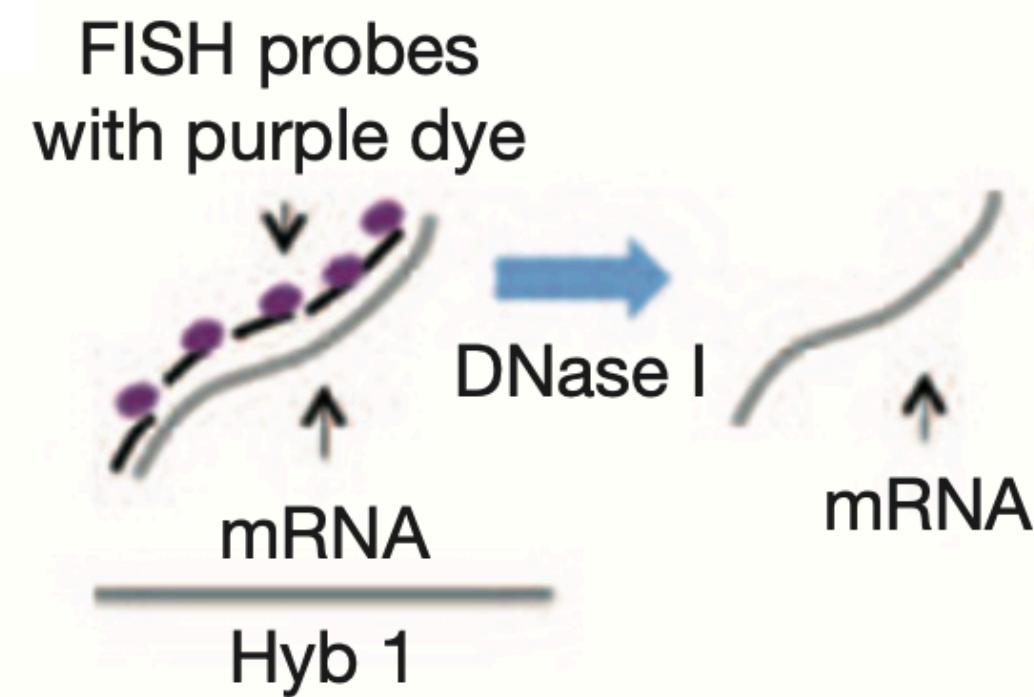
seqFISH - sequential FISH

Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.



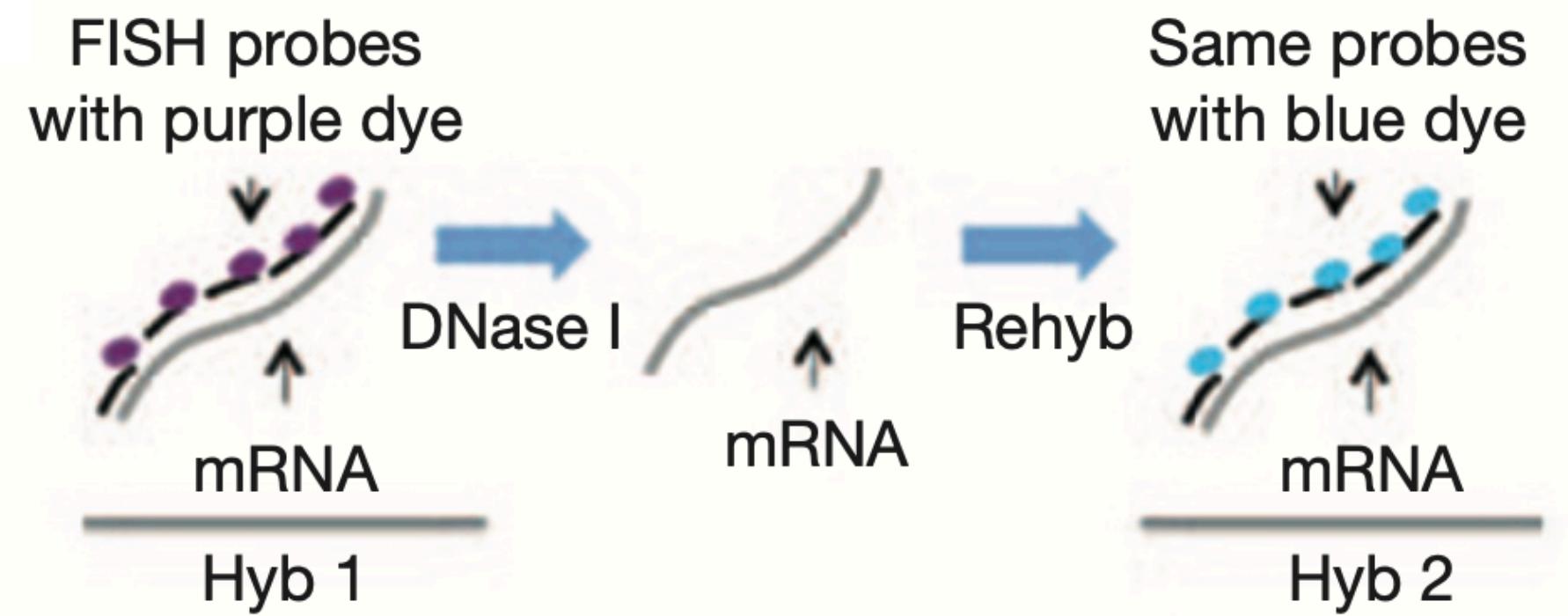
seqFISH - sequential FISH

Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.



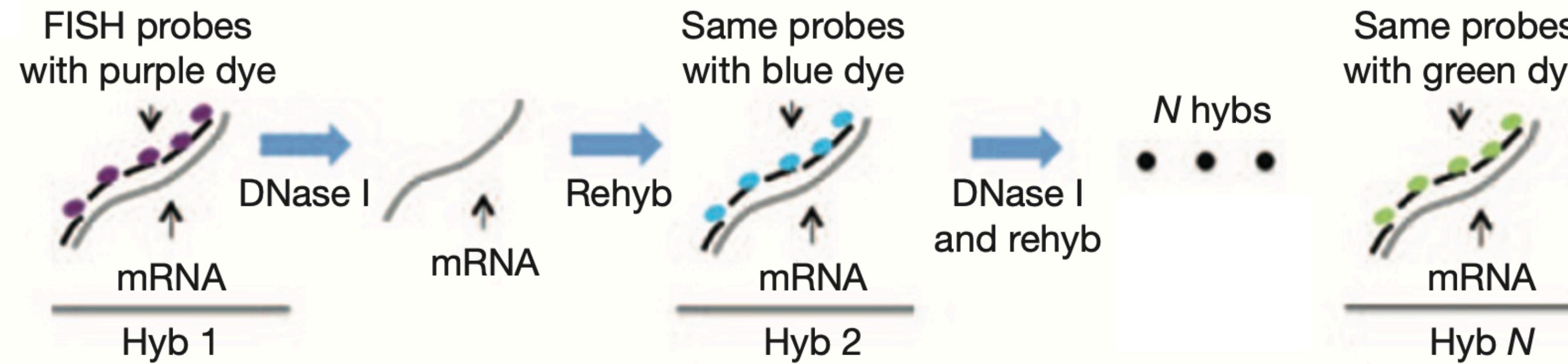
seqFISH - sequential FISH

Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.



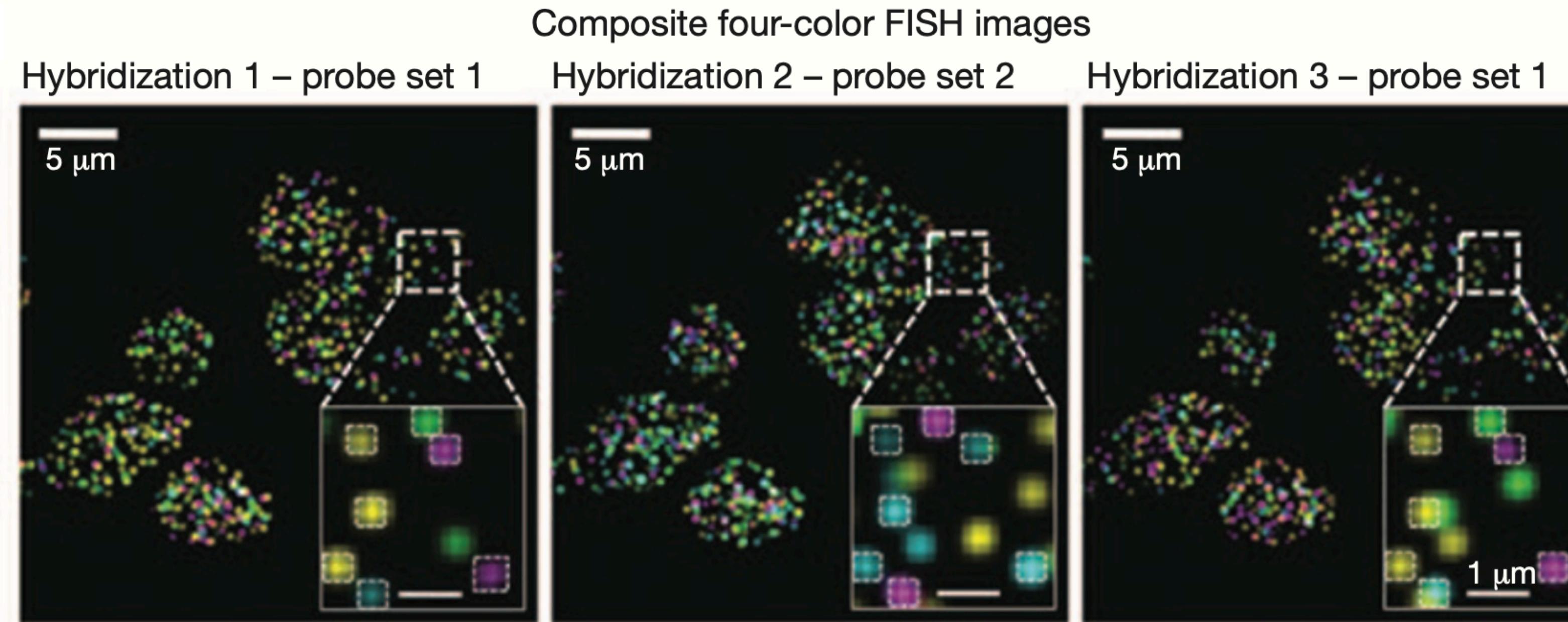
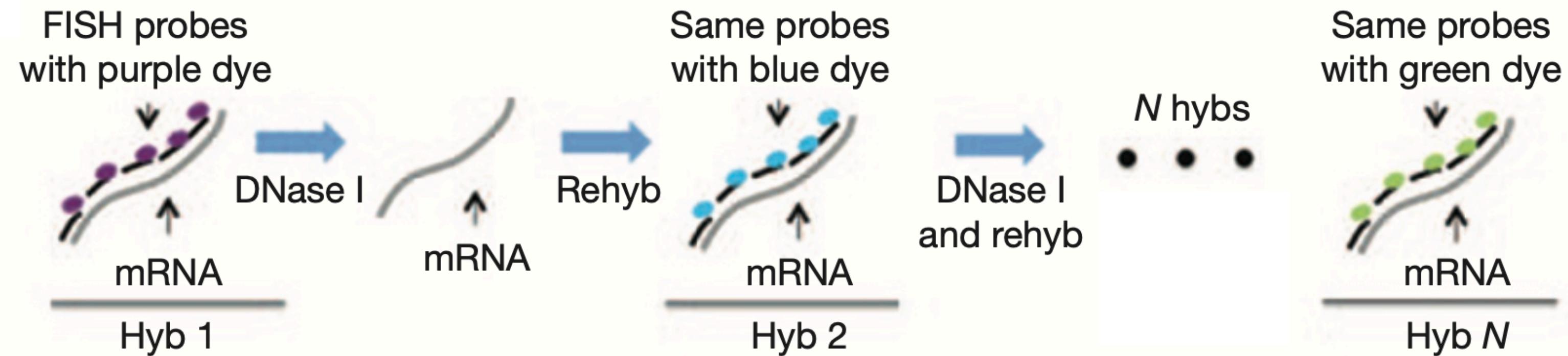
seqFISH - sequential FISH

Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.



seqFISH - sequential FISH

Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.

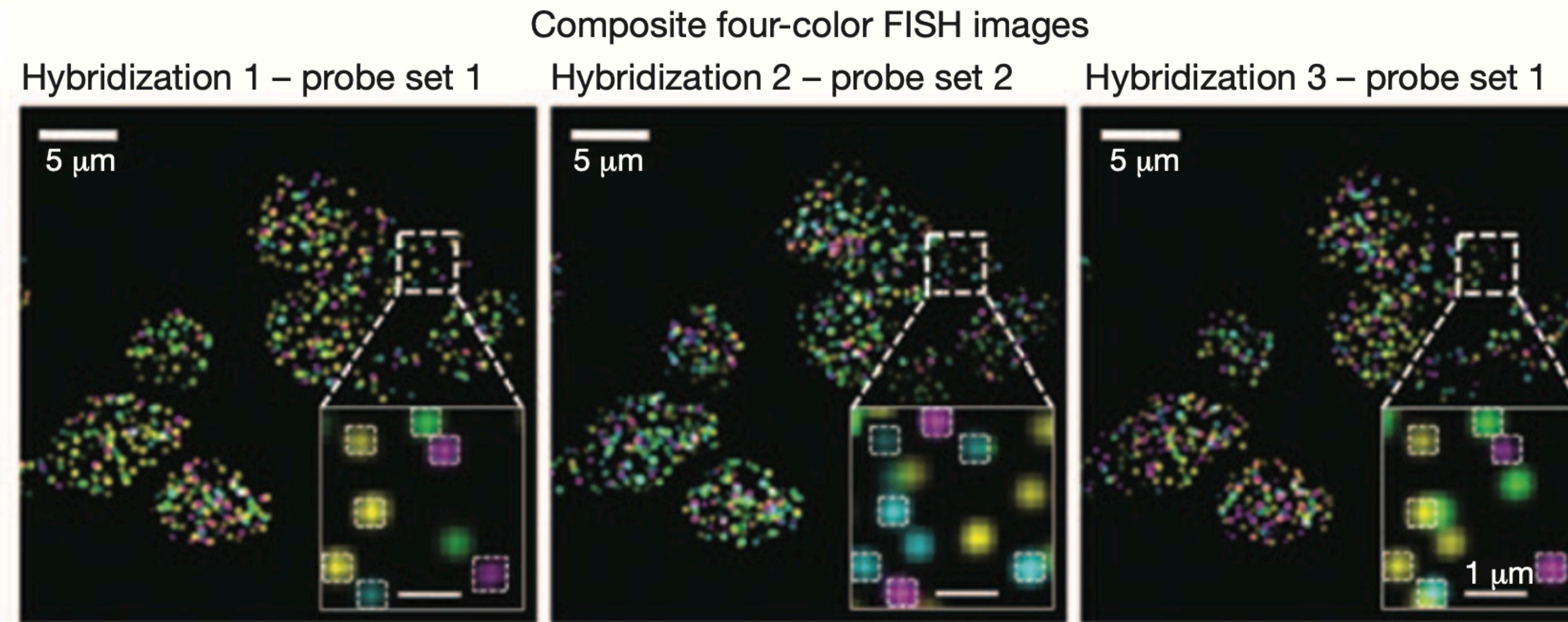
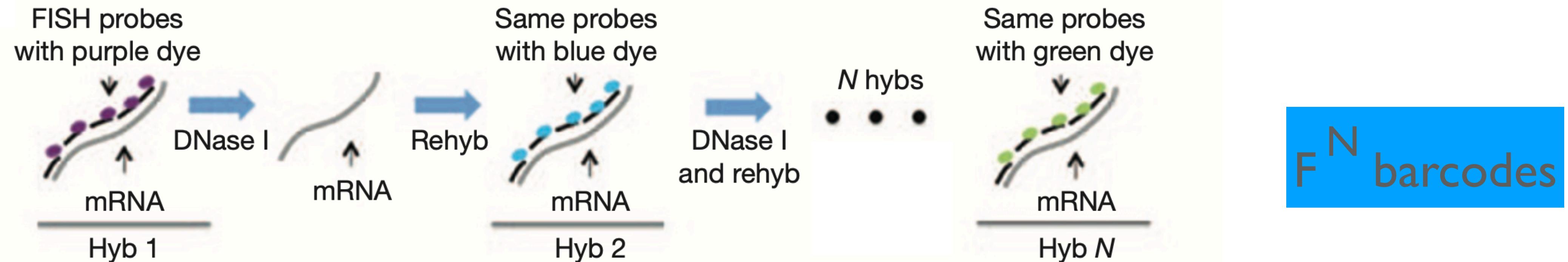


Barcode Key: (for $N=6$)

mRNA1	● ● ○ ○ ○ ○
mRNA2	○ ○ ● ● ○ ○
mRNA3	○ ○ ○ ○ ○ ○

seqFISH - sequential FISH

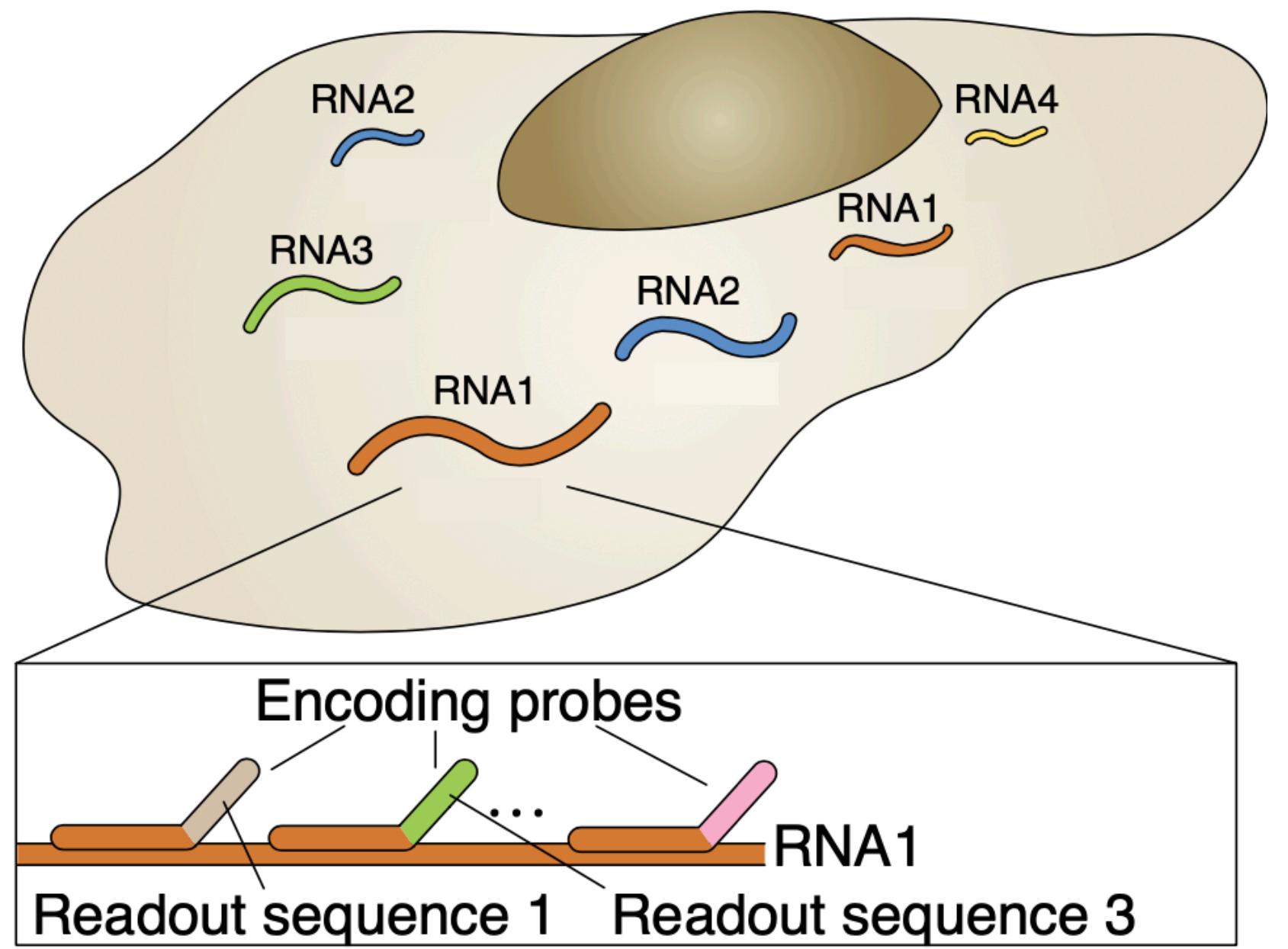
Individual transcripts are detected several times by serial rounds of hybridization, imaging and probe stripping.



Barcode Key: (for $N=6$)

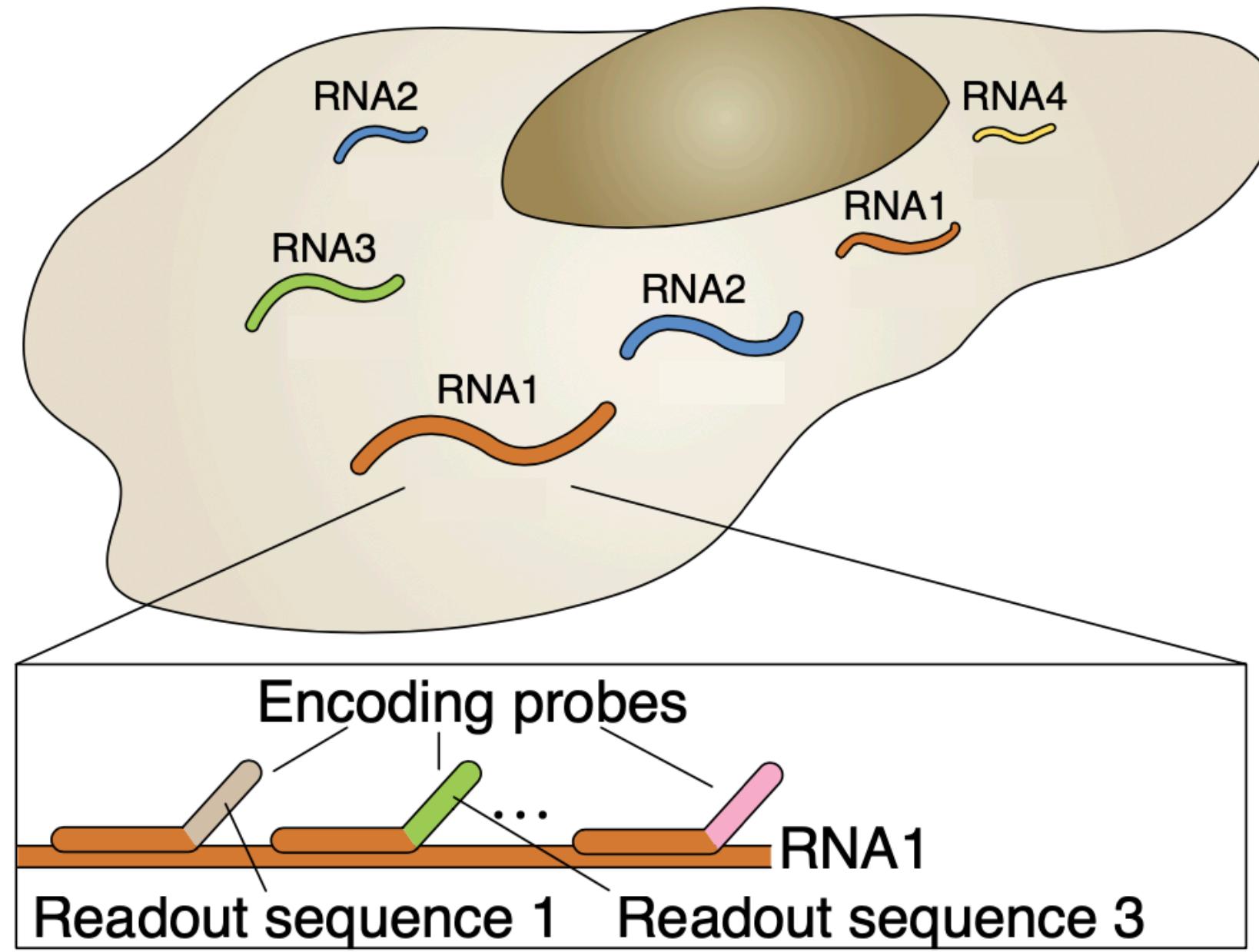
mRNA1	● ● ○ ○ ○ ○
mRNA2	○ ○ ● ● ○ ○
mRNA3	○ ○ ○ ○ ○ ○

MERFISH

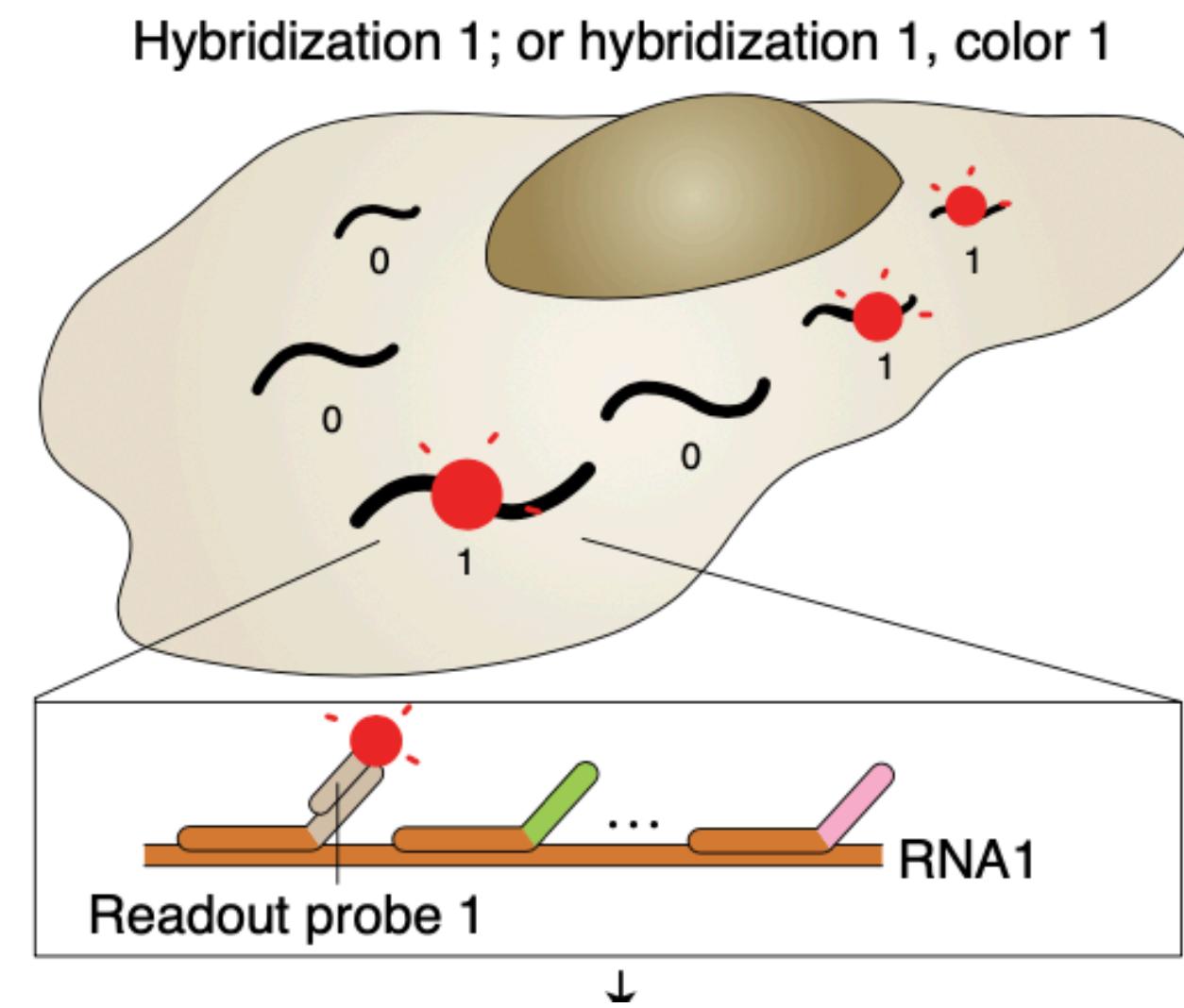


Non-readout probes carrying two flanking regions are first hybridized to the target transcript

MERFISH

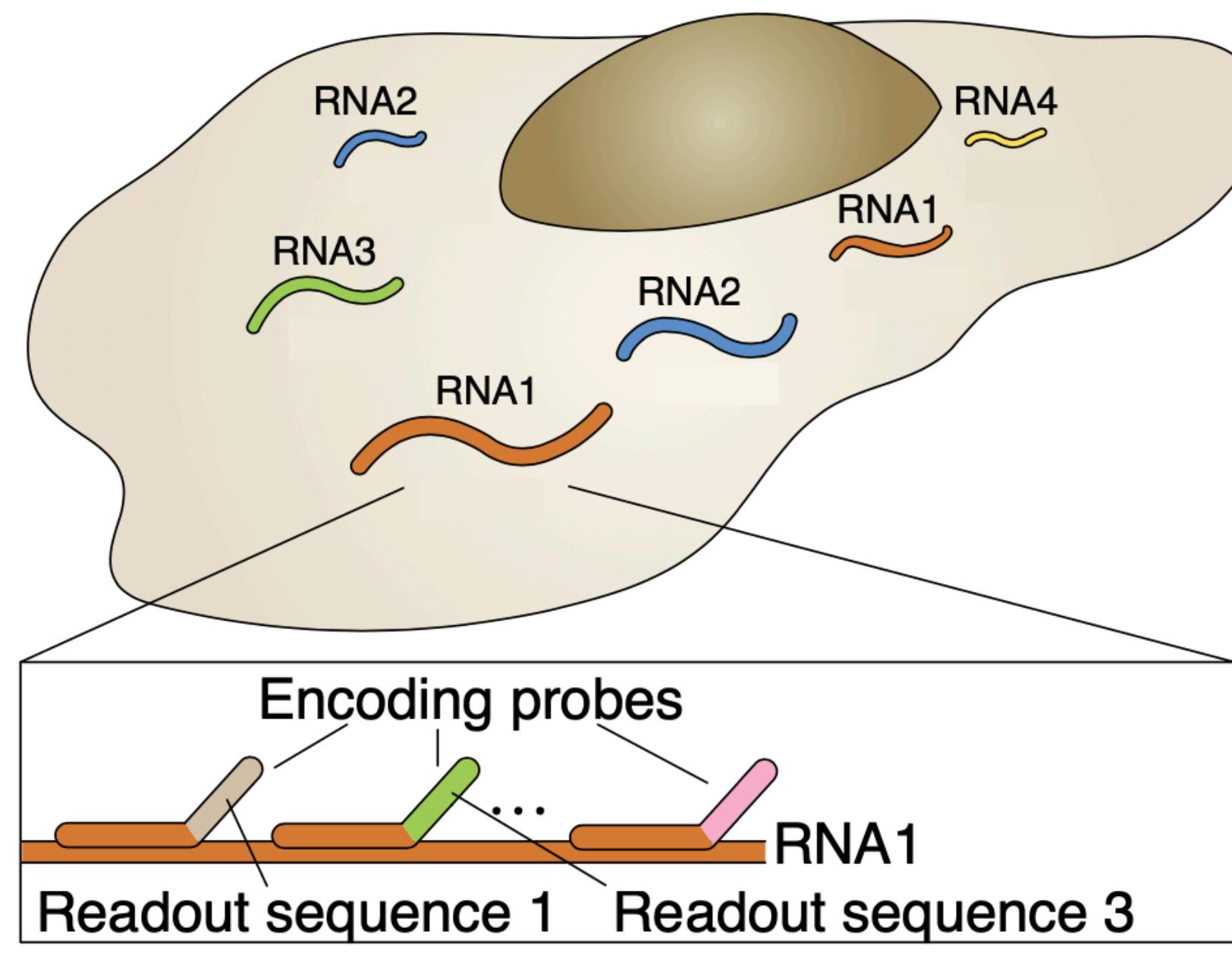


Non-readout probes carrying two flanking regions are first hybridized to the target transcript

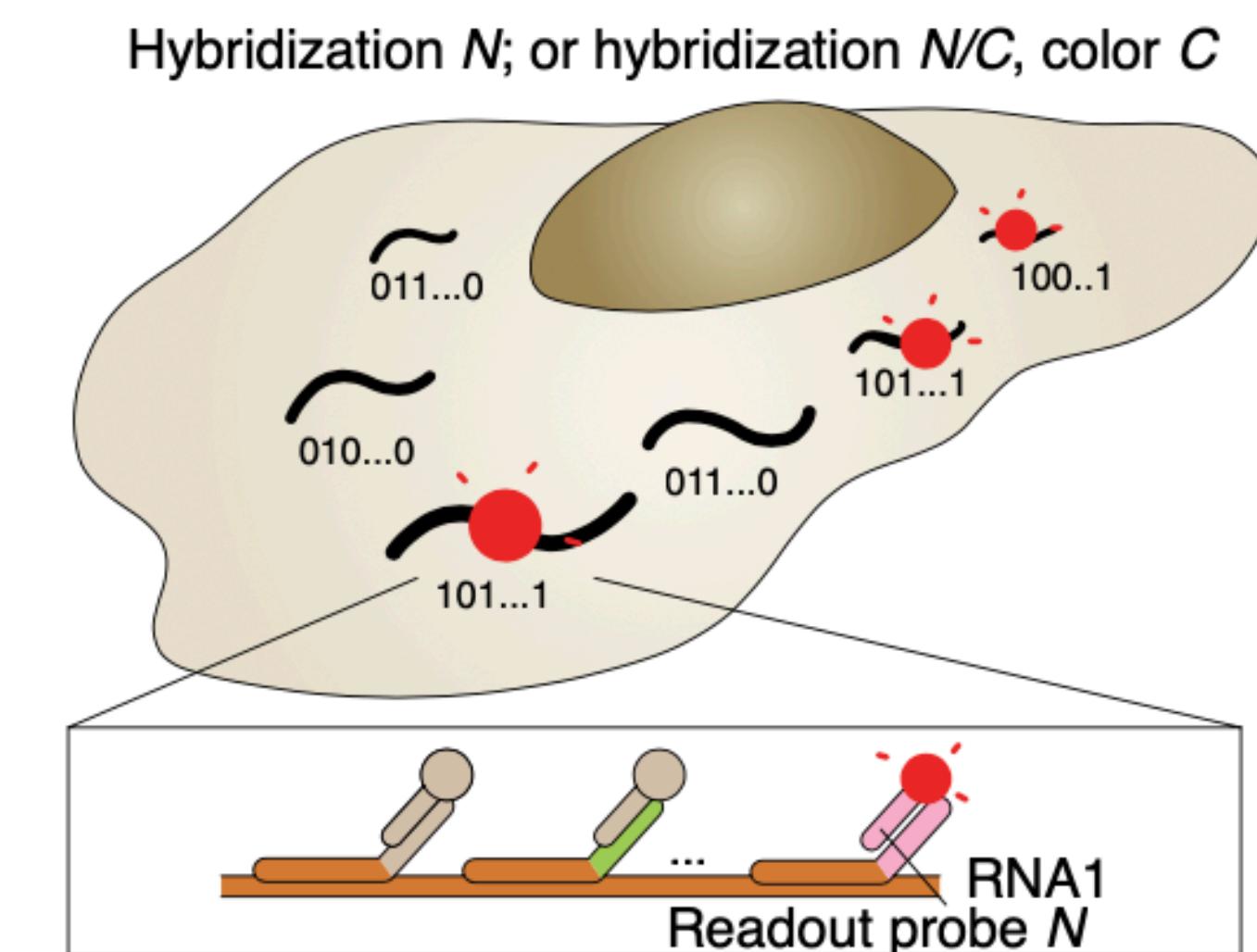
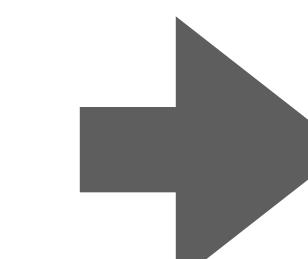
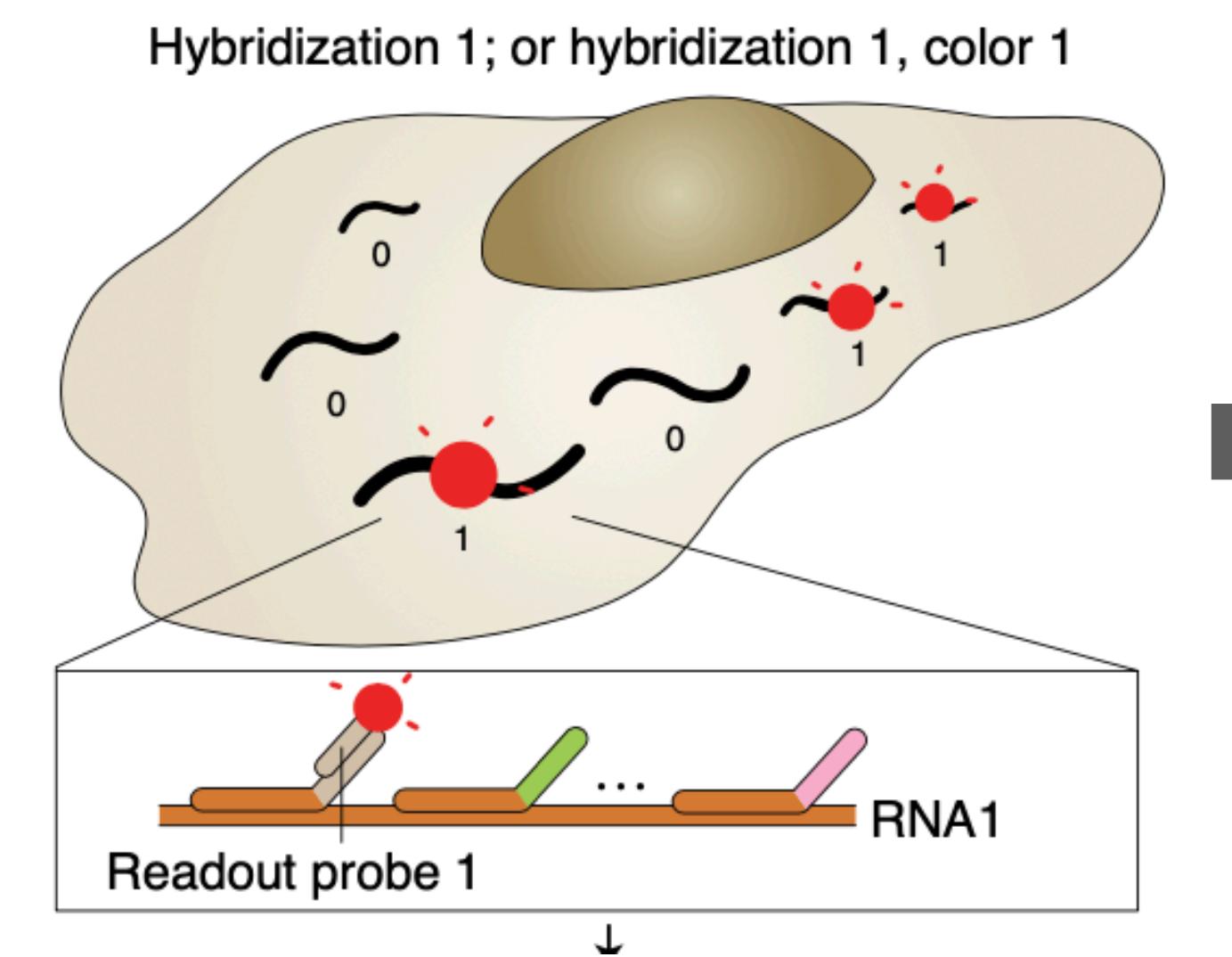


Fluorescent readout-probes are hybridized to non-readout probes in several rounds of hybridization, imaging, and signal extinguishing

MERFISH

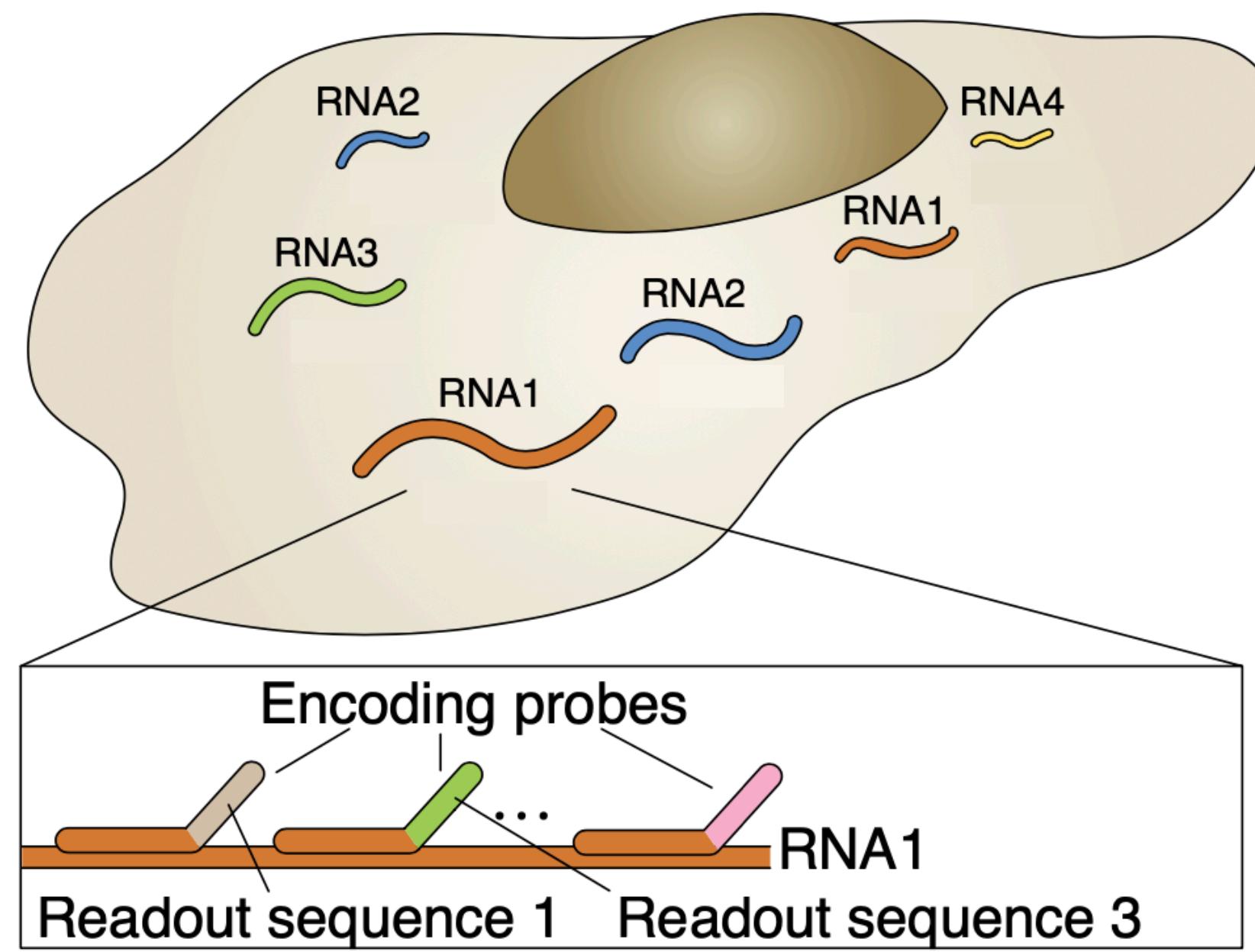


Non-readout probes carrying two flanking regions are first hybridized to the target transcript

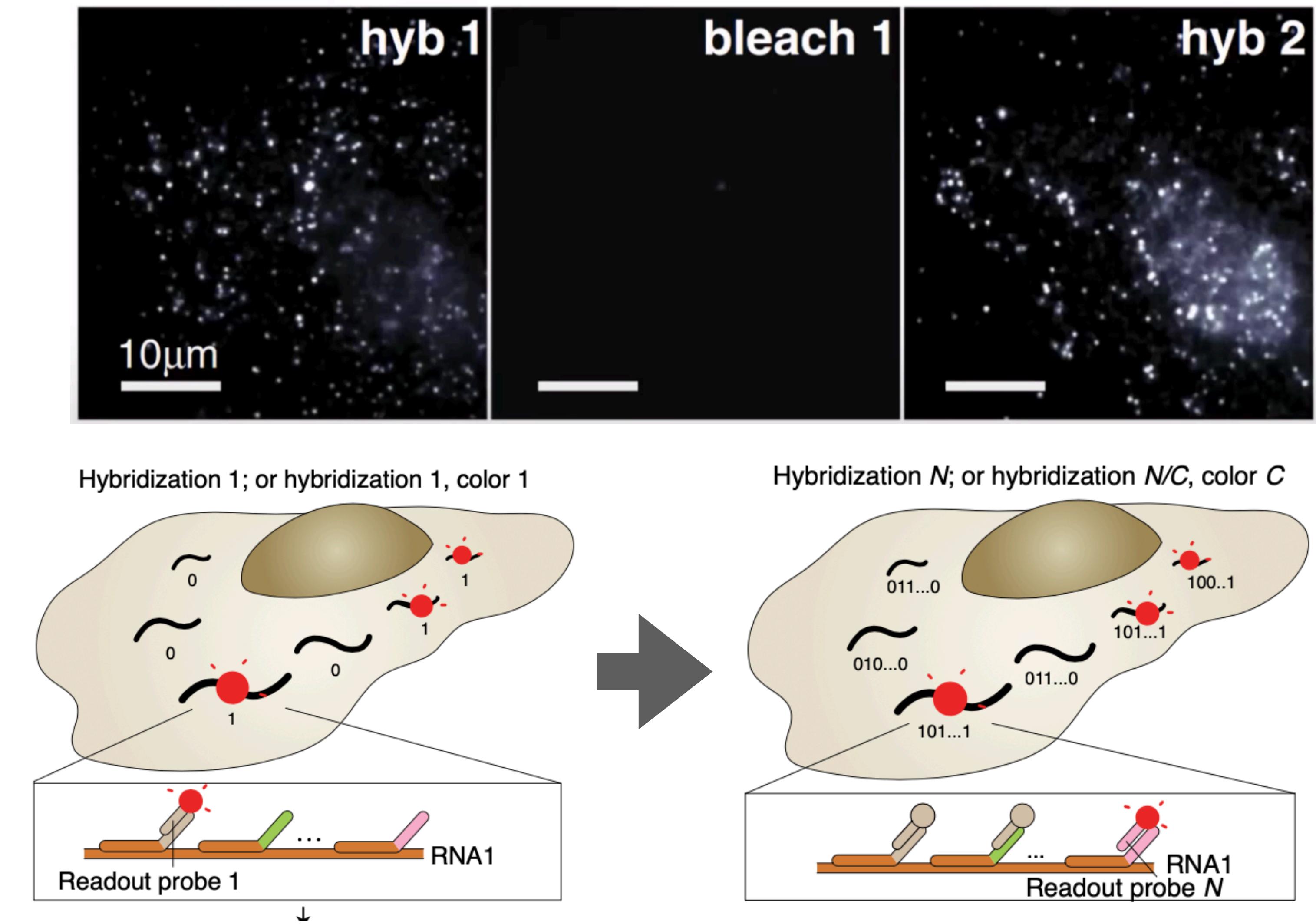


Fluorescent readout-probes are hybridized to non-readout probes in several rounds of hybridization, imaging, and signal extinguishing

MERFISH

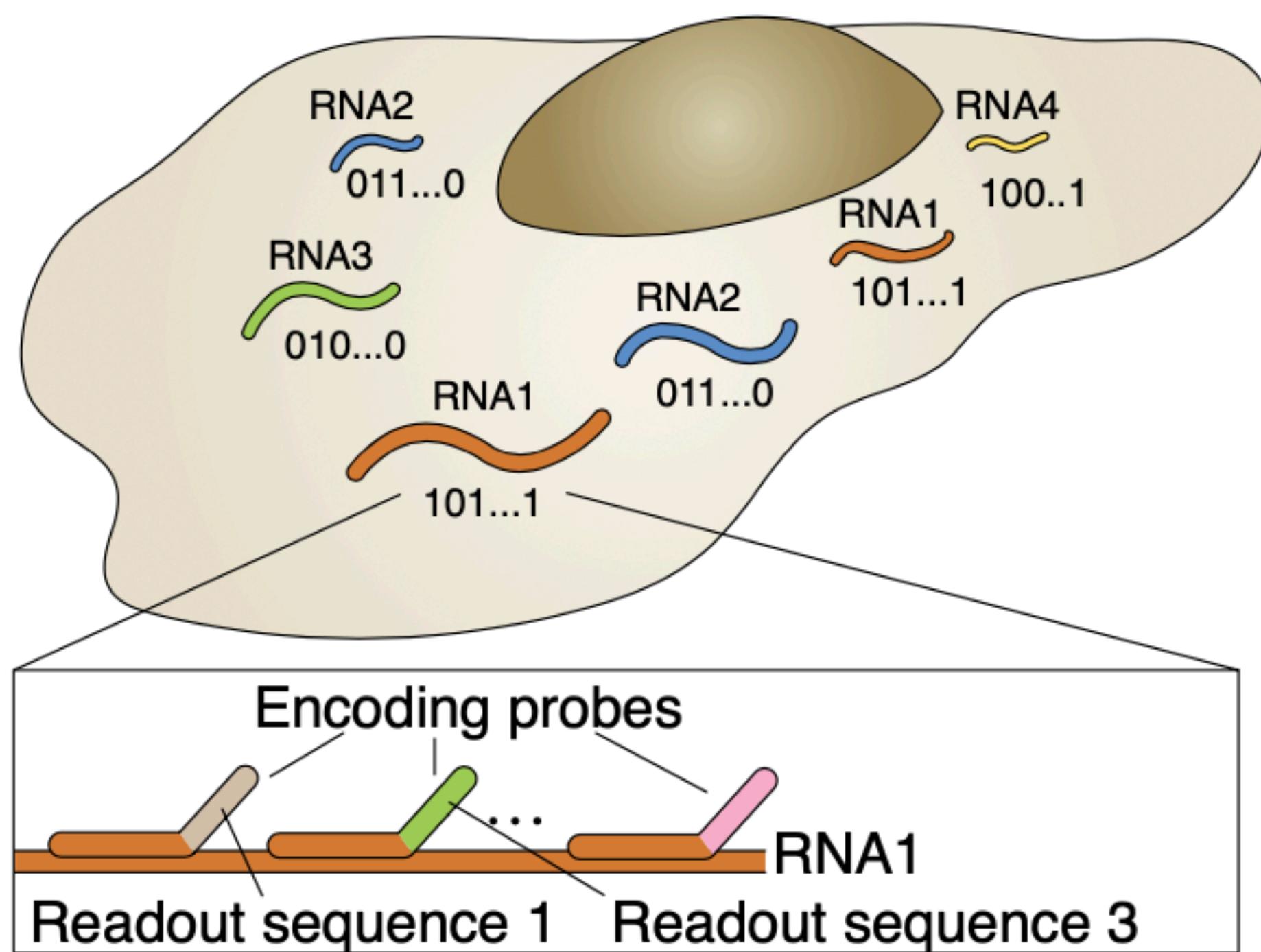


Non-readout probes carrying two flanking regions are first hybridized to the target transcript



Fluorescent readout-probes are hybridized to non-readout probes in several rounds of hybridization, imaging, and signal extinguishing

MERFISH

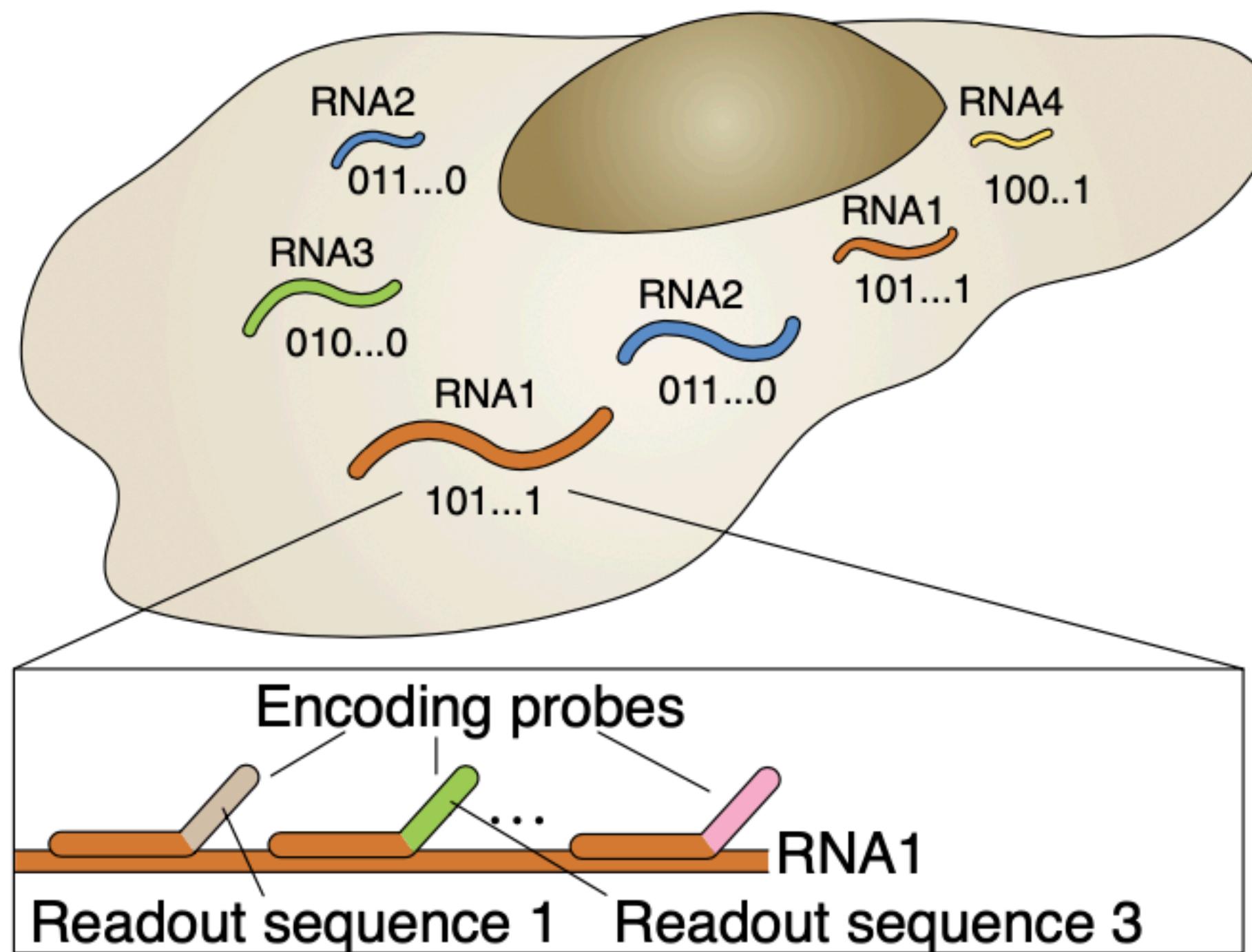


mRNA

- A 111100
- B 110010
- C 101001
- D 100111
- E 011111
- F 010001
- G 001010

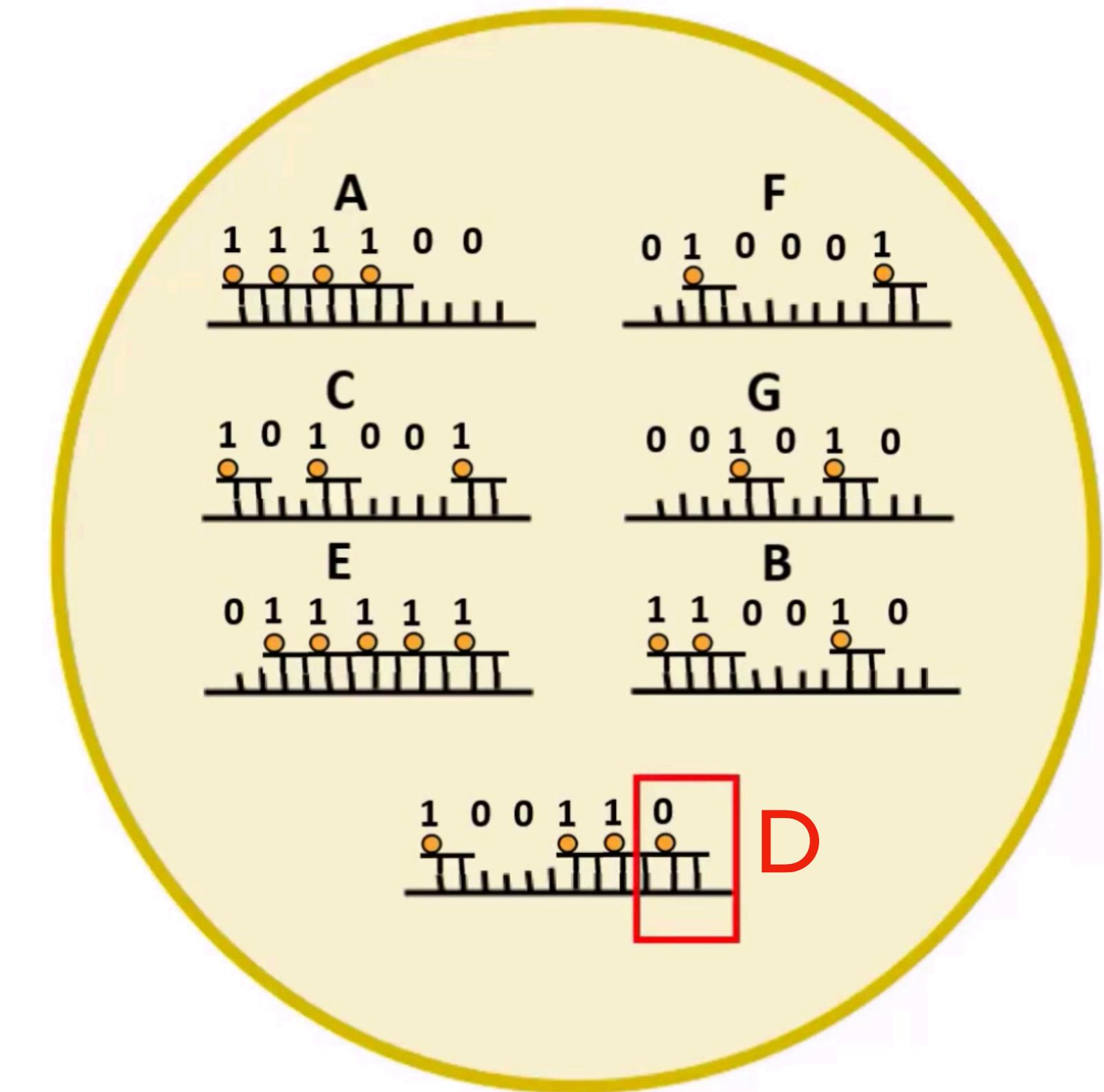
2^{NF}
genes

MERFISH - Multiplex error-robust FISH



mRNA

A	111100
B	110010
C	101001
D	100111
E	011111
F	010001
G	001010



2^{NF} genes

Classification of spatial transcriptomics methods

Imaged-based

An image is processed to generate
a gene-expression matrix

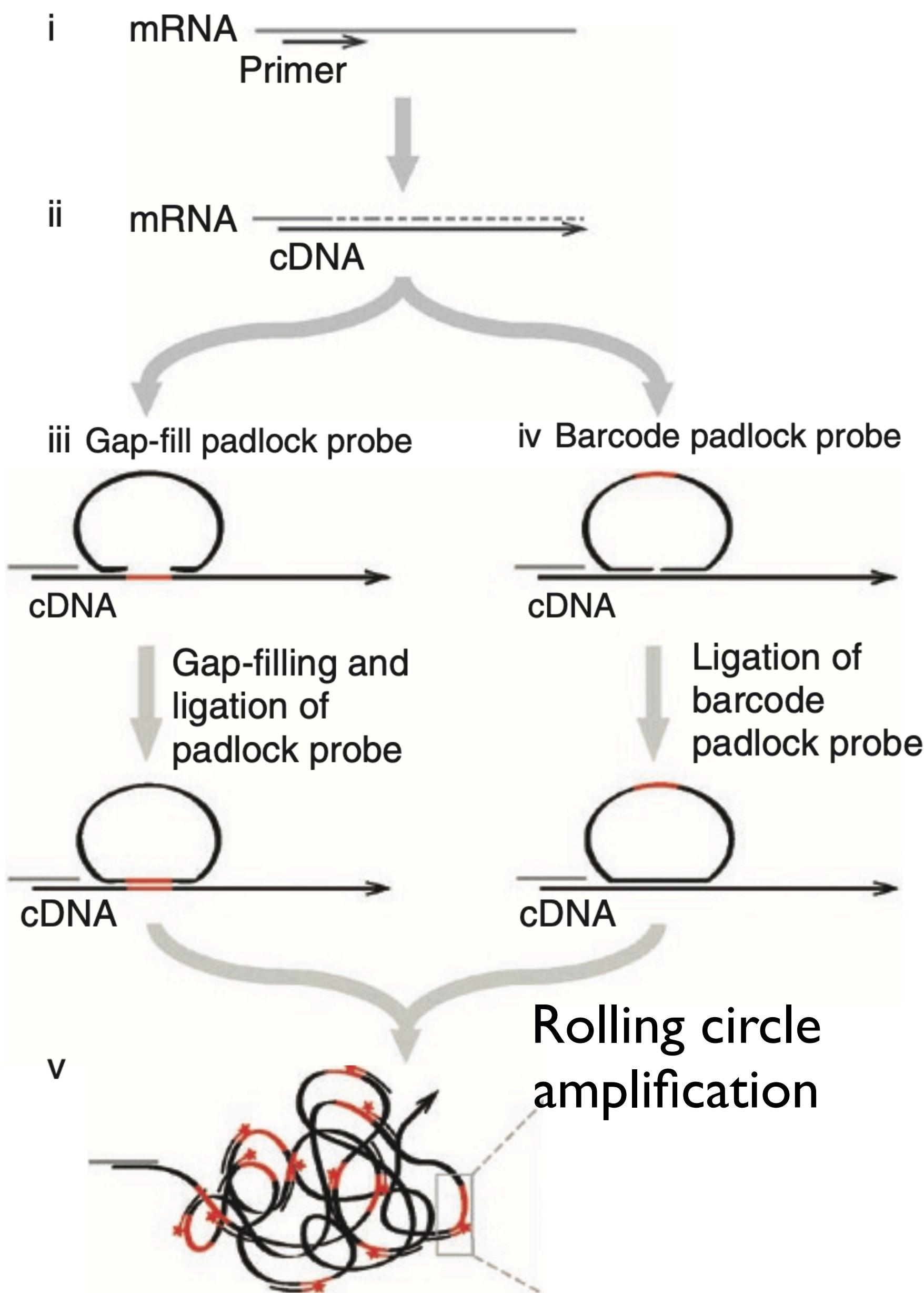
- In situ hybridisation (ISH)
 - MERFISH
 - seqFISH
- In situ sequencing (ISS)
 - Sequencing by ligation
 - STARMap
 - Sequencing by synthesis
(**BaristaSeq**, **Barseq**)
 - Sequencing by hybridisation (HybISS)
 - FISSEQ
 - ExSeq (expansion)

Sequencing-based

Encode positional information
onto the transcripts

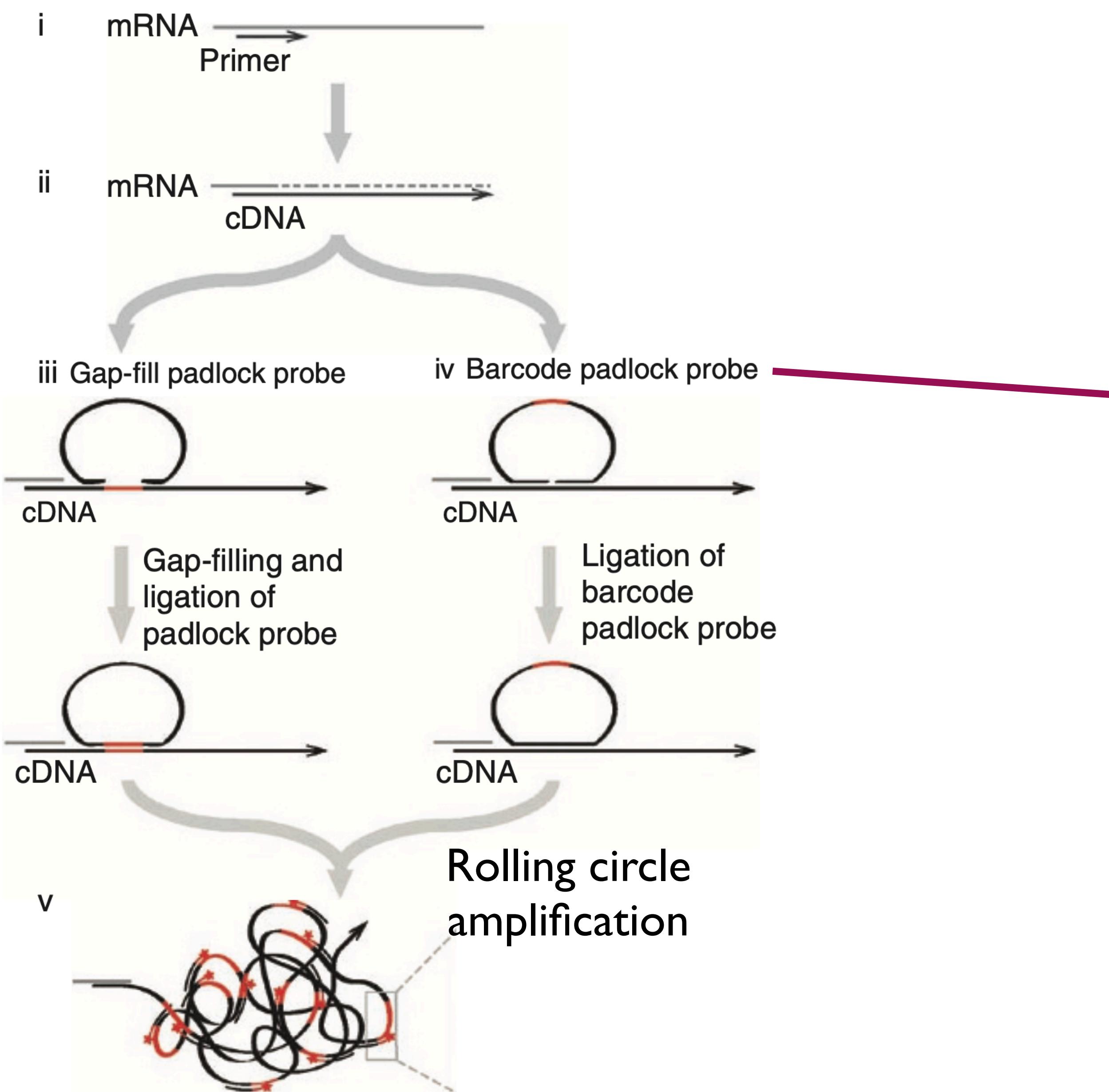
- Tomo-seq
- Capture and sequencing (Visium)
- Slide-seq
- HDST
- DBiT-seq
- Stereo-seq
- Seq-scope
- PIXEL-seq

Imaged-based ISS methods



RNA sequencing is done
directly on the tissue

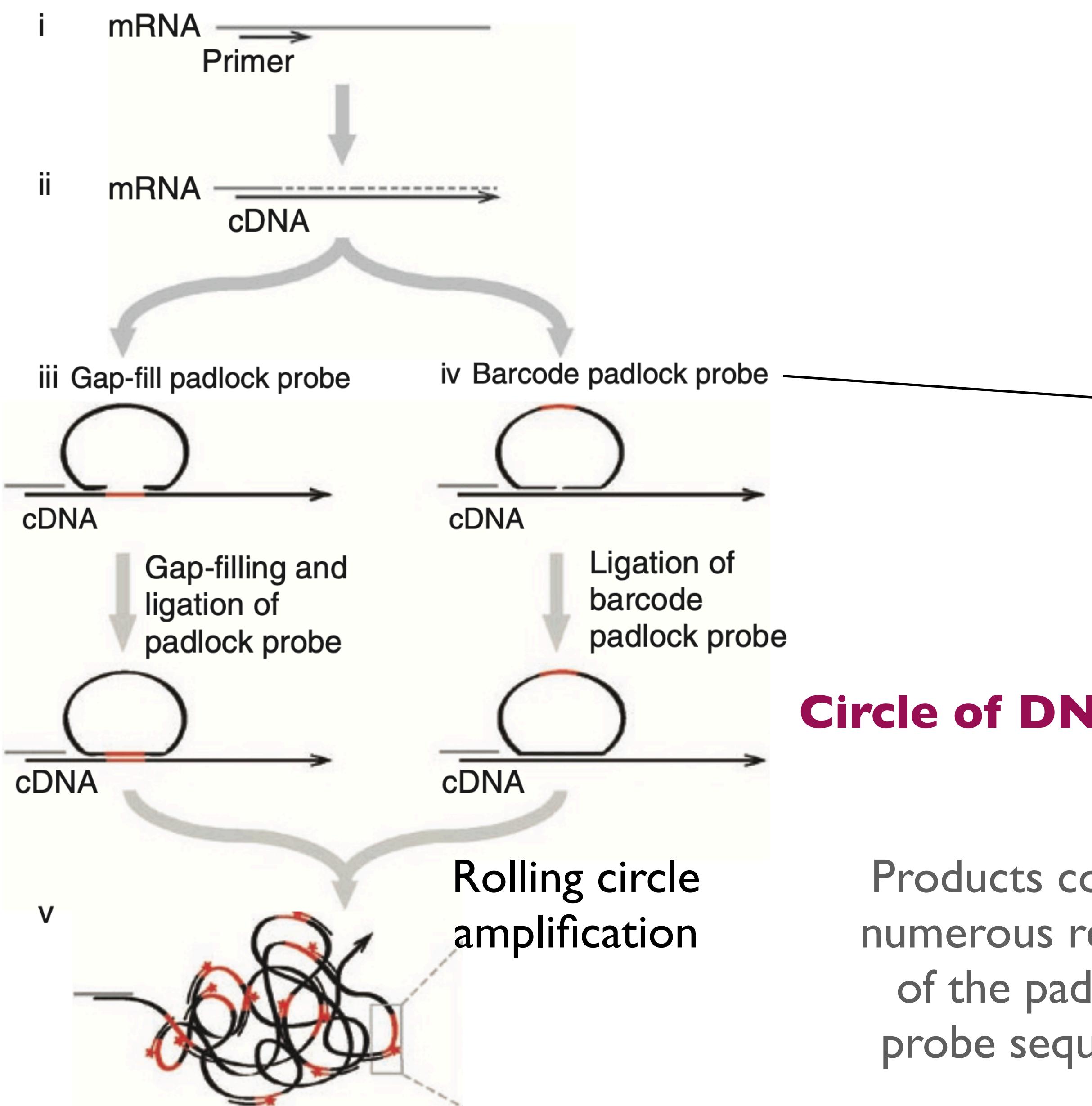
Imaged-based ISS methods



RNA sequencing is done directly on the tissue

Padlock probe:
Single stranded DNA containing regions complementary to a target cDNA

Imaged-based ISS methods



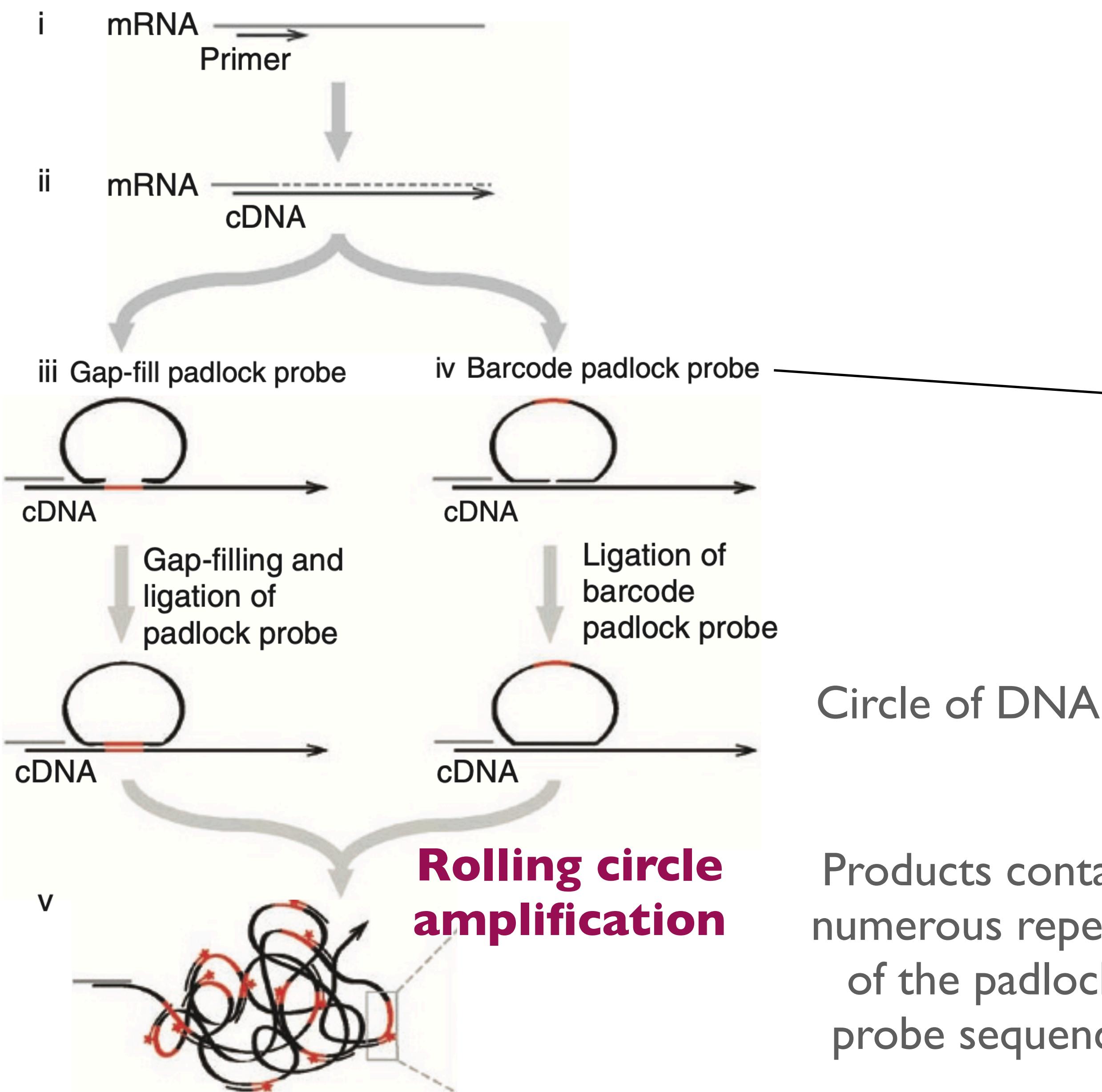
RNA sequencing is done directly on the tissue

Padlock probe:
Single stranded DNA containing regions complementary to a target cDNA

Circle of DNA

Products contain numerous repeats of the padlock probe sequence

Imaged-based ISS methods



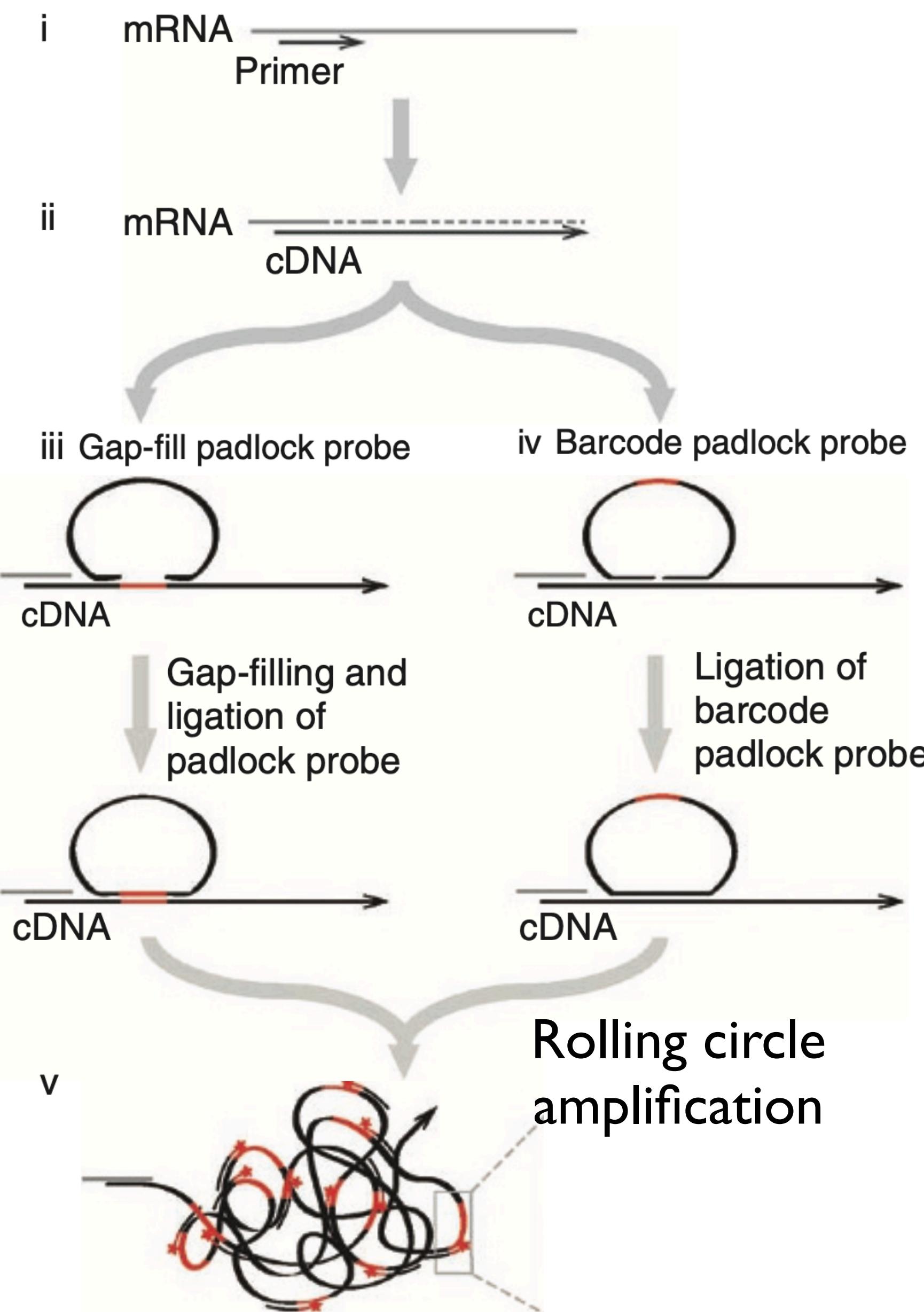
RNA sequencing is done directly on the tissue

Padlock probe:
Single stranded DNA containing regions complementary to a target cDNA

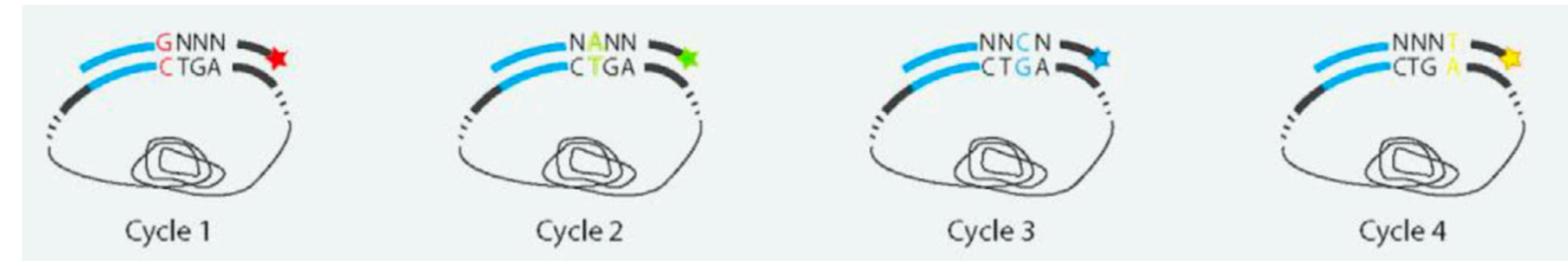
Circle of DNA

Products contain numerous repeats of the padlock probe sequence

Imaged-based ISS methods

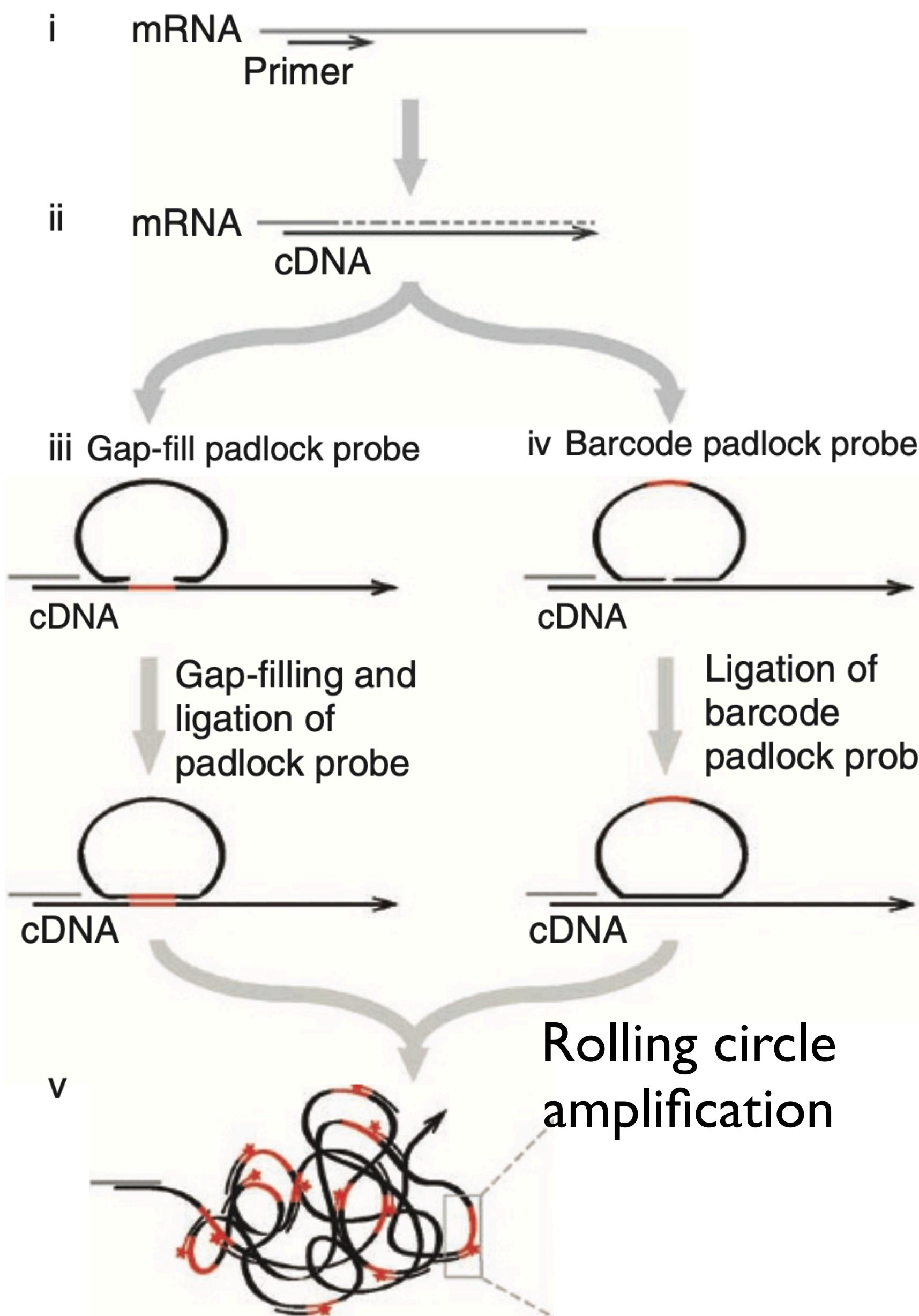


Sequencing by ligation



Ke et al, Nature Methods 2013

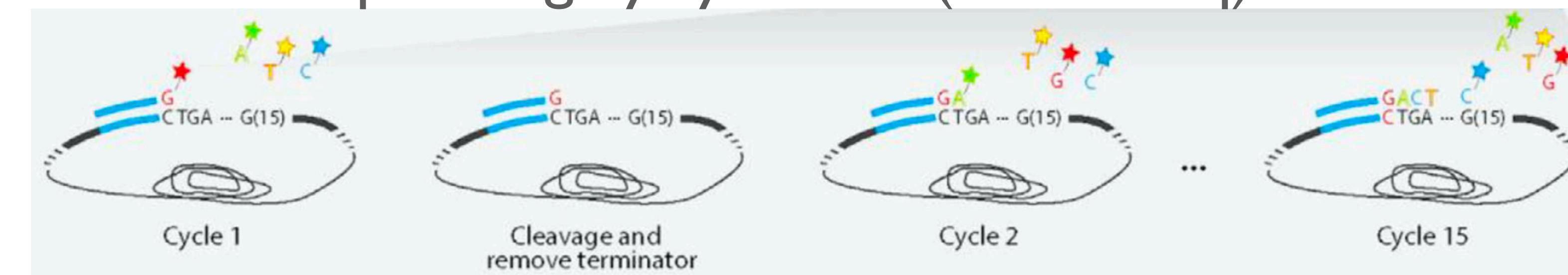
Imaged-based ISS methods



Sequencing by ligation



Sequencing by synthesis (BaristaSeq)



Low detection efficiency

Classification of spatial transcriptomics methods

Imaged-based

An image is processed to generate
a gene-expression matrix

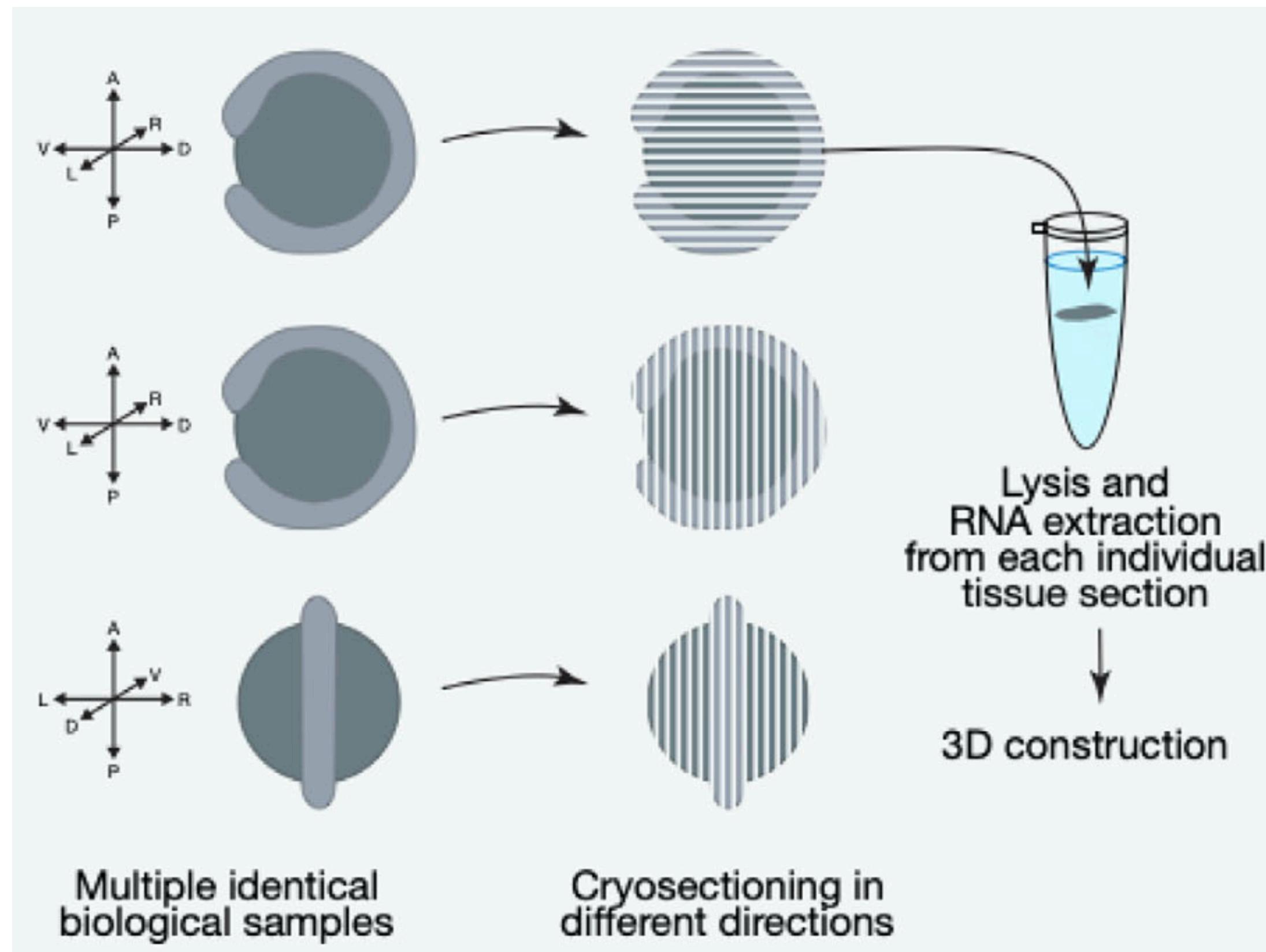
- In situ hybridisation (ISH)
 - MERFISH
 - seqFISH
- In situ sequencing (ISS)
 - Sequencing by ligation
 - STARMap
 - Sequencing by synthesis
(BaristaSeq, Barseq)
 - Sequencing by hybridisation (HybISS)
 - FISSEQ
 - ExSeq (expansion)

Sequencing-based

Encode positional information
onto the transcripts

- Tomo-seq
- Capture and sequencing (Visium)
- Slide-seq
- HDST
- DBiT-seq
- Stereo-seq
- Seq-scope
- PIXEL-seq

Tomo-seq: genome-wide RNA tomography



Junker et al, Cell 2014

Krusse et al, Methods Cell Biology 2016

Brink et al, Nature 2020

ST/Visium & Slide-seq

Tissue section



3D stacks



- No single cell resolution
- Sparse data
- “Easy” to extend to other omics

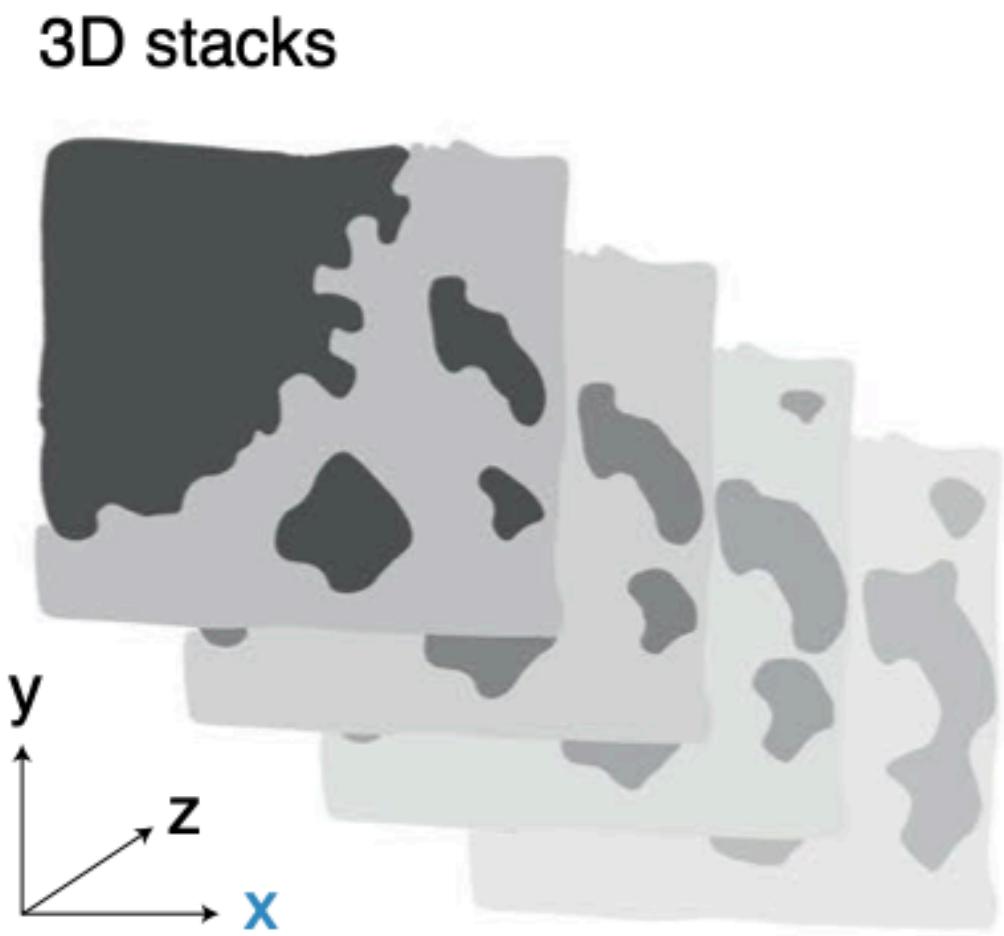
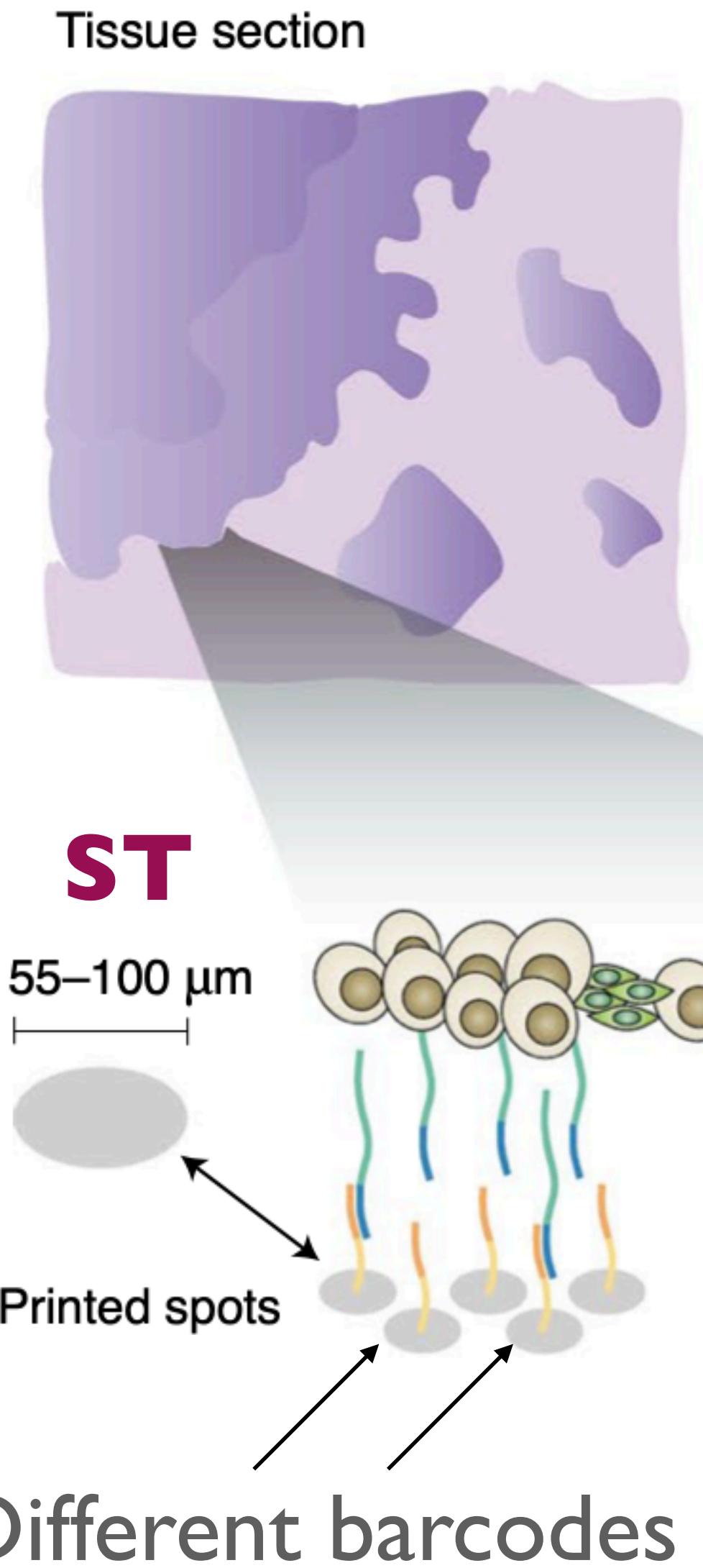
Ståhl et al, Science 2016

Rodrigues et al, Science 2019

Larsson et al, Nat. Methods Comment, 2021

ST/Visium & Slide-seq

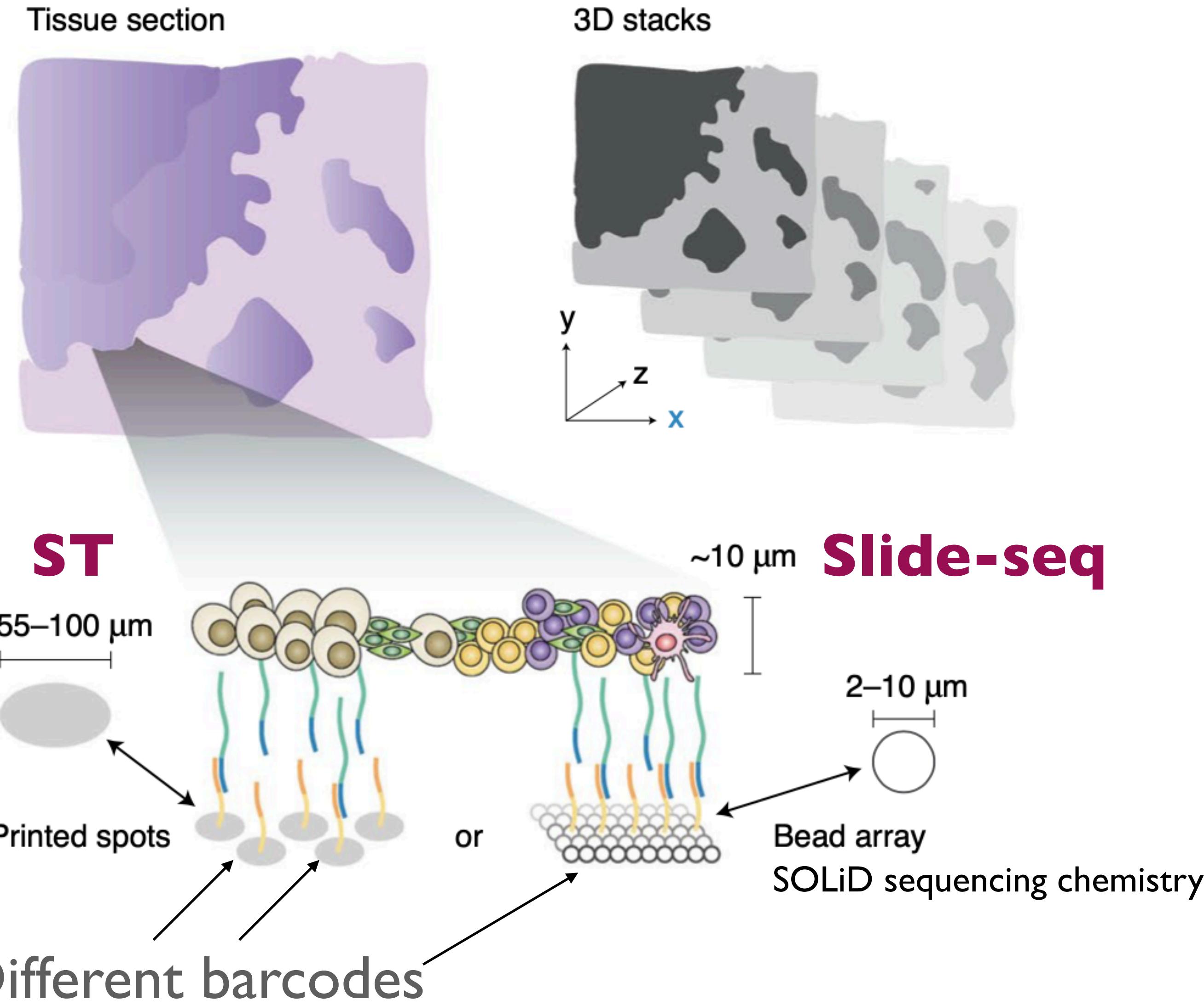
- No single cell resolution
- Sparse data
- “Easy” to extend to other omics



Glass slides:
Barcoded RT
primers specify
(x,y) coordinates

ST/Visium & Slide-seq

- No single cell resolution
- Sparse data
- “Easy” to extend to other omics



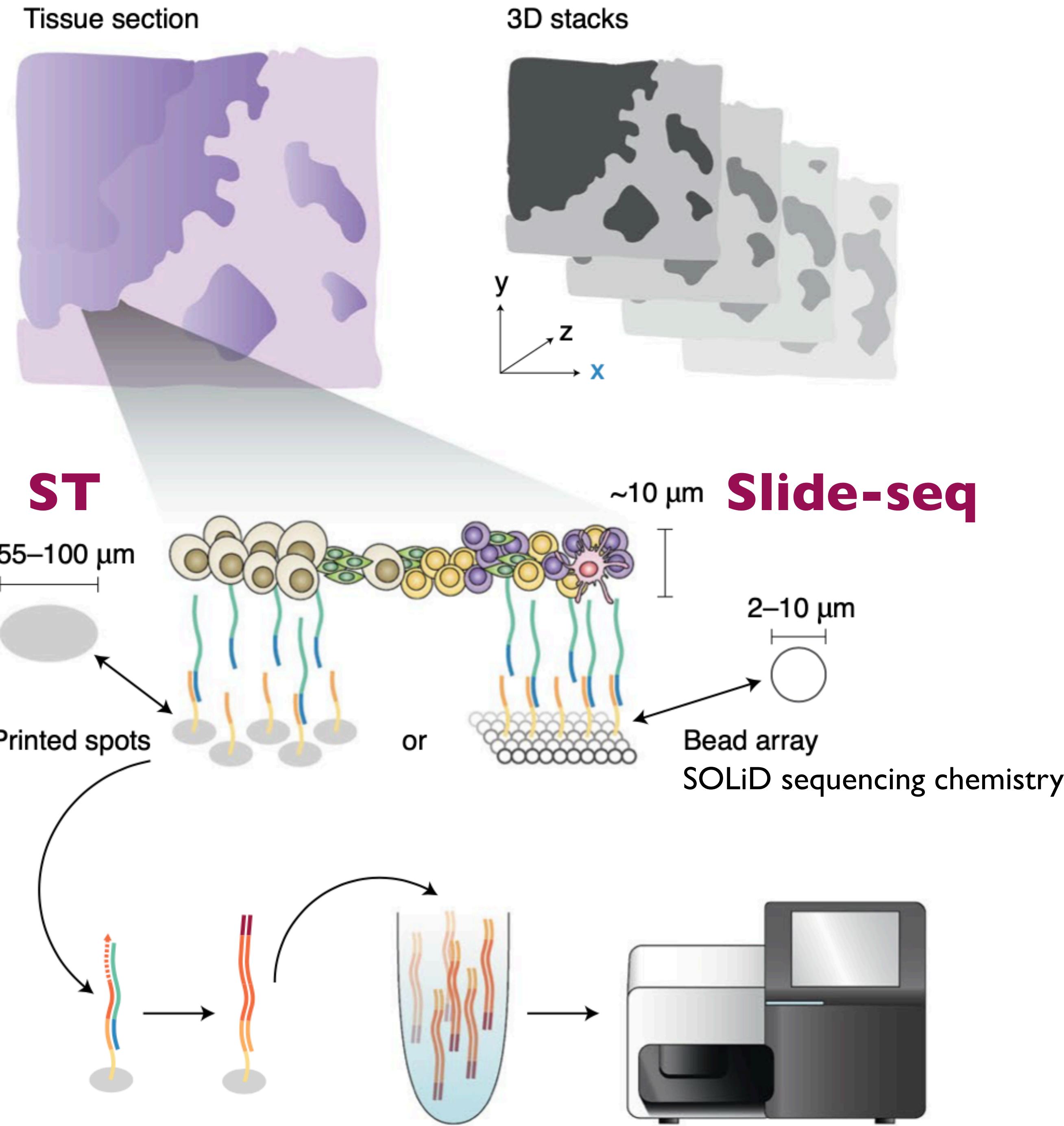
Ståhl et al, Science 2016

Rodrigues et al, Science 2019

Larsson et al, Nat. Methods Comment, 2021

ST/Visium & Slide-seq

- No single cell resolution
- Sparse data
- “Easy” to extend to other omics



Overview of the techniques

	Gene throughput	Sensitivity	Resolution (transcript distances)
Sequenced-based	Unbiased polyA transcripts	Low (100 transcripts/ μm^2)	~Tens of cells Distance between spots (~1 μm)
ISS-based	Prior selection of genes to target (up to 10k now!) - Exc: FISSEQ, ExSeq	0.2%	Sub-cellular Diffraction limit
ISH-based	All types of RNA (polyA and non-polyA)	80%	(Expansion microscopy: 100 nm)

Integration of spatial transcriptomics with scRNA-seq

nature
biotechnology

ARTICLES

<https://doi.org/10.1038/s41587-021-01006-2>



OPEN

Integration of spatial and single-cell transcriptomic data elucidates mouse organogenesis

T. Lohoff^{1,2,3,17}, S. Ghazanfar^{4,17}, A. Missarova^{4,5}, N. Koulena⁶, N. Pierson⁶, J. A. Griffiths^{ID 4,16}, E. S. Bardot⁷, C.-H. L. Eng^{ID 6}, R. C. V. Tyser^{ID 8}, R. Argelaguet^{ID 3,5}, C. Guibentif^{ID 1,9,10}, S. Srinivas^{ID 8}, J. Briscoe^{ID 11}, B. D. Simons^{ID 1,12,13}, A.-K. Hadjantonakis⁷, B. Göttgens^{ID 1,9}, W. Reik^{ID 1,3,14,15}✉, J. Nichols^{1,2}✉, L. Cai^{ID 6}✉ and J. C. Marioni^{ID 4,5,15}✉

Molecular profiling of single cells has advanced our knowledge of the molecular basis of development. However, current approaches mostly rely on dissociating cells from tissues, thereby losing the crucial spatial context of regulatory processes. Here, we apply an image-based single-cell transcriptomics method, sequential fluorescence in situ hybridization (seqFISH), to detect mRNAs for 387 target genes in tissue sections of mouse embryos at the 8–12 somite stage. By integrating spatial context and multiplexed transcriptional measurements with two single-cell transcriptome atlases, we characterize cell types across the embryo and demonstrate that spatially resolved expression of genes not profiled by seqFISH can be imputed. We use this high-resolution spatial map to characterize fundamental steps in the patterning of the midbrain-hindbrain boundary (MHB) and the developing gut tube. We uncover axes of cell differentiation that are not apparent from single-cell RNA-sequencing (scRNA-seq) data, such as early dorsal-ventral separation of esophageal and tracheal progenitor populations in the gut tube. Our method provides an approach for studying cell fate decisions in complex tissues and development.

Integration of spatial transcriptomics with scRNA-seq

nature
biotechnology

ARTICLES

<https://doi.org/10.1038/s41587-021-01006-2>



OPEN

Integration of spatial and single-cell transcriptomic data elucidates mouse organogenesis

T. Lohoff^{1,2,3,17}, S. Ghazanfar^{4,17}, A. Missarova^{4,5}, N. Koulena⁶, N. Pierson⁶, J. A. Griffiths^{ID 4,16}, E. S. Bardot⁷, C.-H. L. Eng^{ID 6}, R. C. V. Tyser^{ID 8}, R. Argelaguet^{ID 3,5}, C. Guibentif^{ID 1,9,10}, S. Srinivas^{ID 8}, J. Briscoe^{ID 11}, B. D. Simons^{ID 1,12,13}, A.-K. Hadjantonakis⁷, B. Göttgens^{ID 1,9}, W. Reik^{ID 1,3,14,15}✉, J. Nichols^{1,2}✉, L. Cai^{ID 6}✉ and J. C. Marioni^{ID 4,5,15}✉

Molecular profiling of single cells has advanced our knowledge of the molecular basis of development. However, current approaches mostly rely on dissociating cells from tissues, thereby losing the crucial spatial context of regulatory processes. Here, we apply an image-based single-cell transcriptomics method, sequential fluorescence in situ hybridization (seqFISH), to detect mRNAs for 387 target genes. By combining seqFISH with spatial transcriptomics and single-cell RNA sequencing (scRNA-seq) data, such as early dorsal-ventral separation of esophageal and tracheal progenitor populations in the gut tube. Our method provides an approach for studying cell fate decisions in complex tissues and development.

Integration of spatial transcriptomics with scRNA-seq

Article

Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids

<https://doi.org/10.1038/s41586-020-2024-3>

Received: 4 December 2018

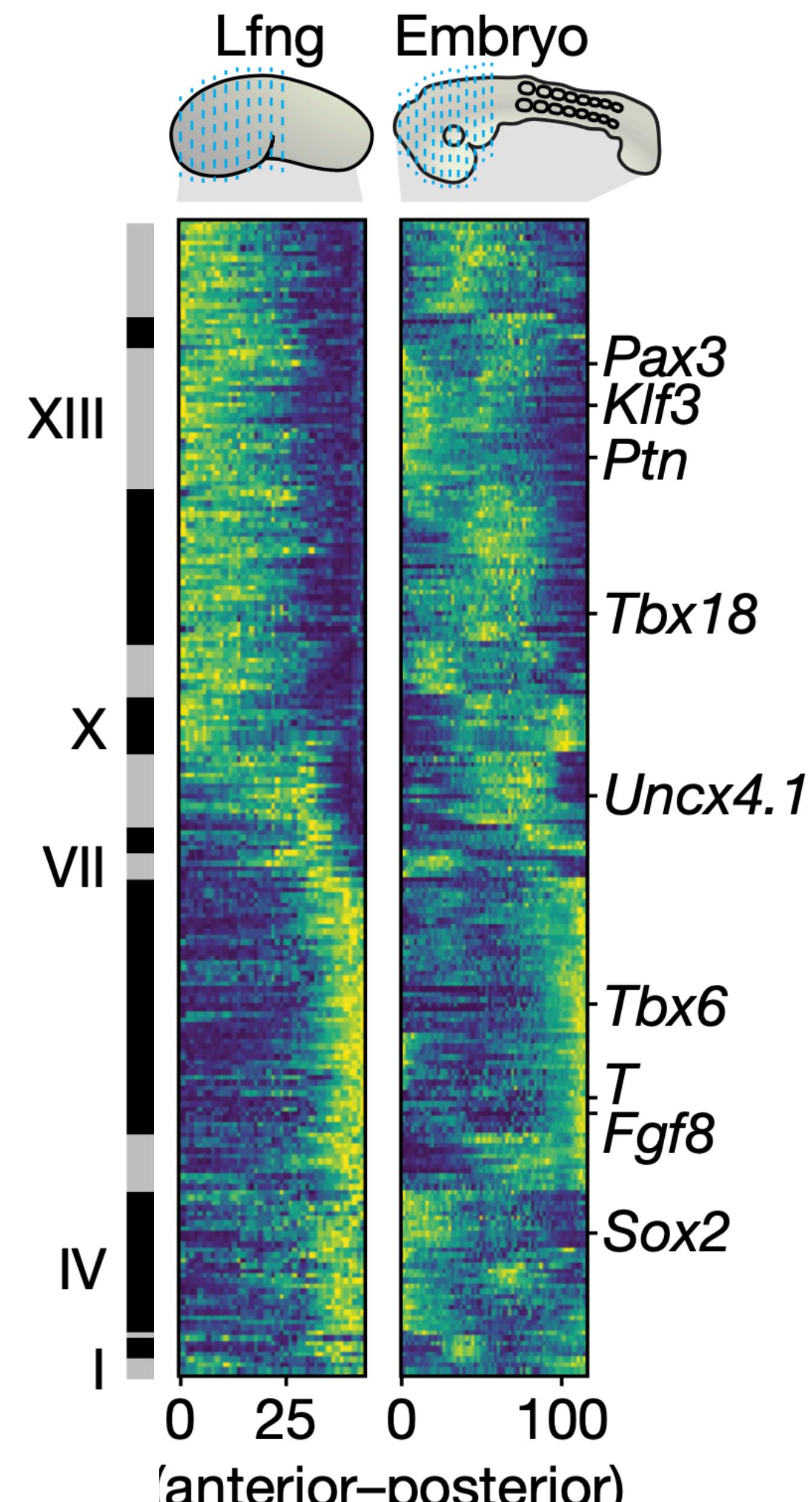
Accepted: 19 December 2019

Published online: 19 February 2020

 Check for updates

Susanne C. van den Brink^{1,6}✉, Anna Alemany^{1,6}, Vincent van Batenburg^{1,6}, Naomi Moris², Marloes Blotenburg¹, Judith Vivié¹, Peter Baillie-Johnson², Jennifer Nichols^{3,4}, Katharina F. Sonnen⁵, Alfonso Martinez Arias² & Alexander van Oudenaarden¹✉

Gastruloids are three-dimensional aggregates of embryonic stem cells that display key features of mammalian development after implantation, including germ-layer specification and axial organization^{1–3}. To date, the expression pattern of only a small



Integration of spatial transcriptomics with scRNA-seq

Article

Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids

<https://doi.org/10.1038/s41586-020-2024-3>

Received: 4 December 2018

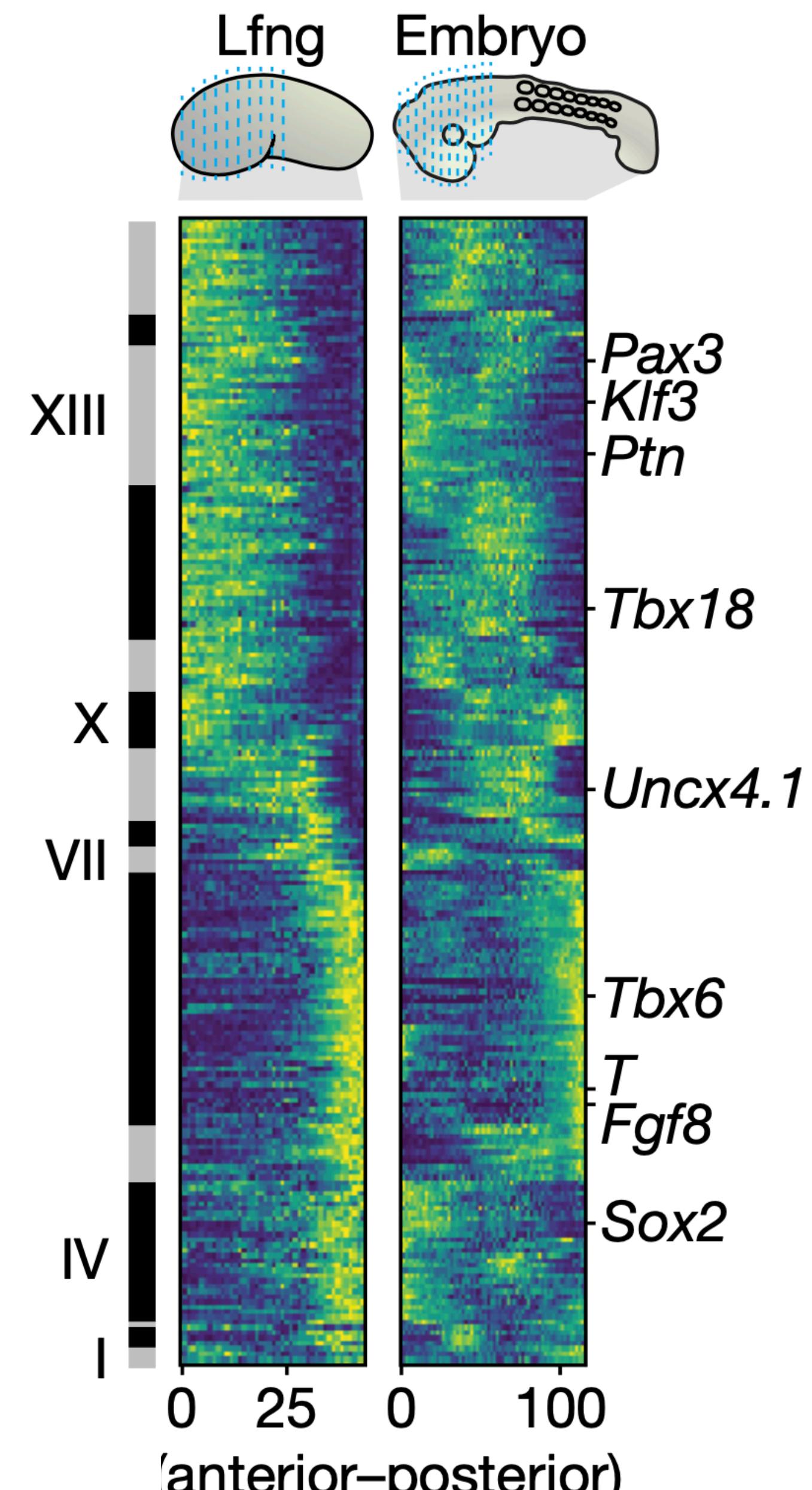
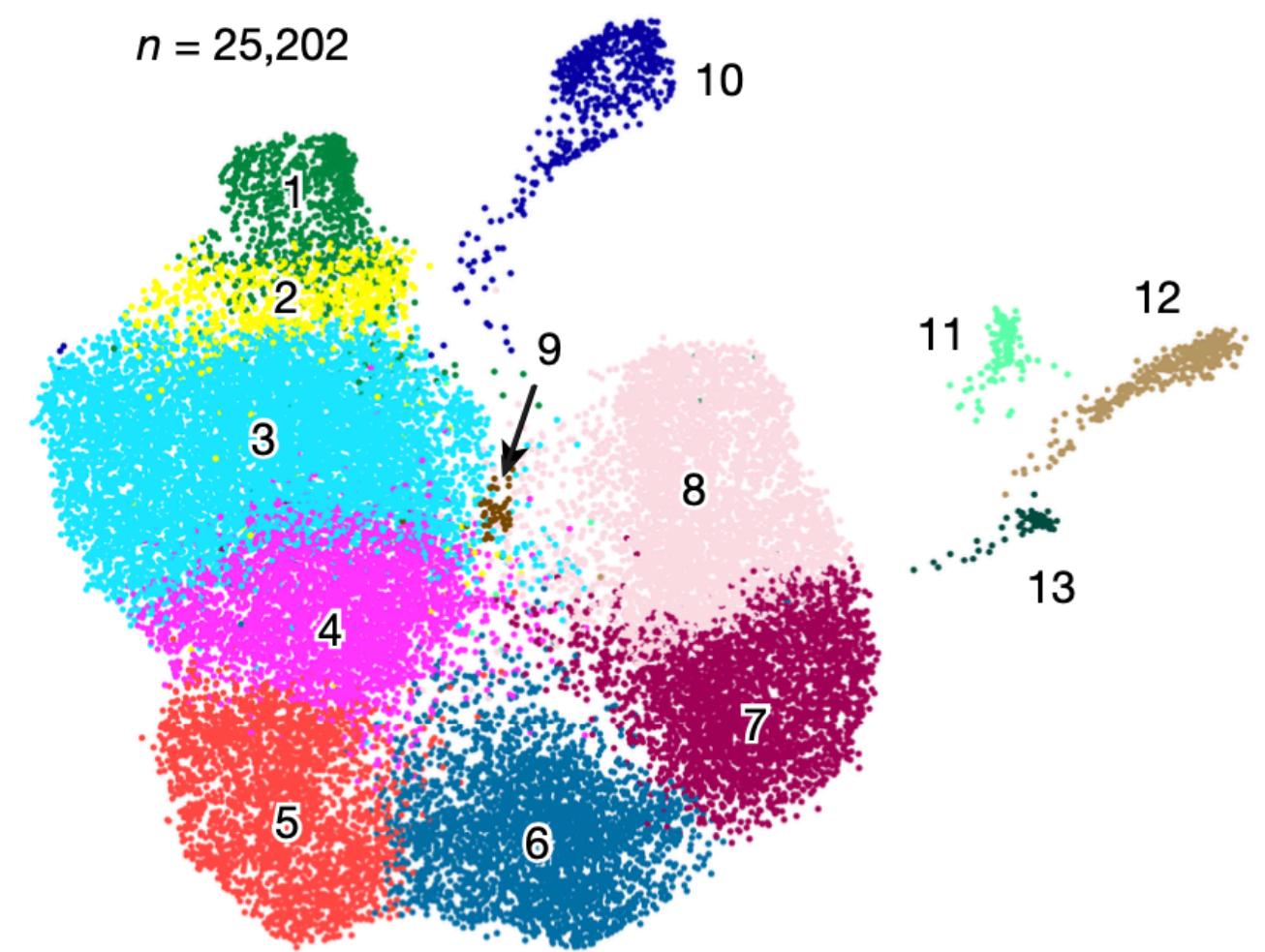
Accepted: 19 December 2019

Published online: 19 February 2020

 Check for updates

Susanne C. van den Brink^{1,6}✉, Anna Alemany^{1,6}, Vincent van Batenburg^{1,6}, Naomi Moris², Marloes Blotenburg¹, Judith Vivié¹, Peter Baillie-Johnson², Jennifer Nichols^{3,4}, Katharina F. Sonnen⁵, Alfonso Martinez Arias² & Alexander van Oudenaarden¹✉

Gastruloids are three-dimensional aggregates of embryonic stem cells that display key features of mammalian development after implantation, including germ-layer specification and axial organization^{1–3}. To date, the expression pattern of only a small



Integration of spatial transcriptomics with scRNA-seq

Article

Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids

<https://doi.org/10.1038/s41586-020-2024-3>

Received: 4 December 2018

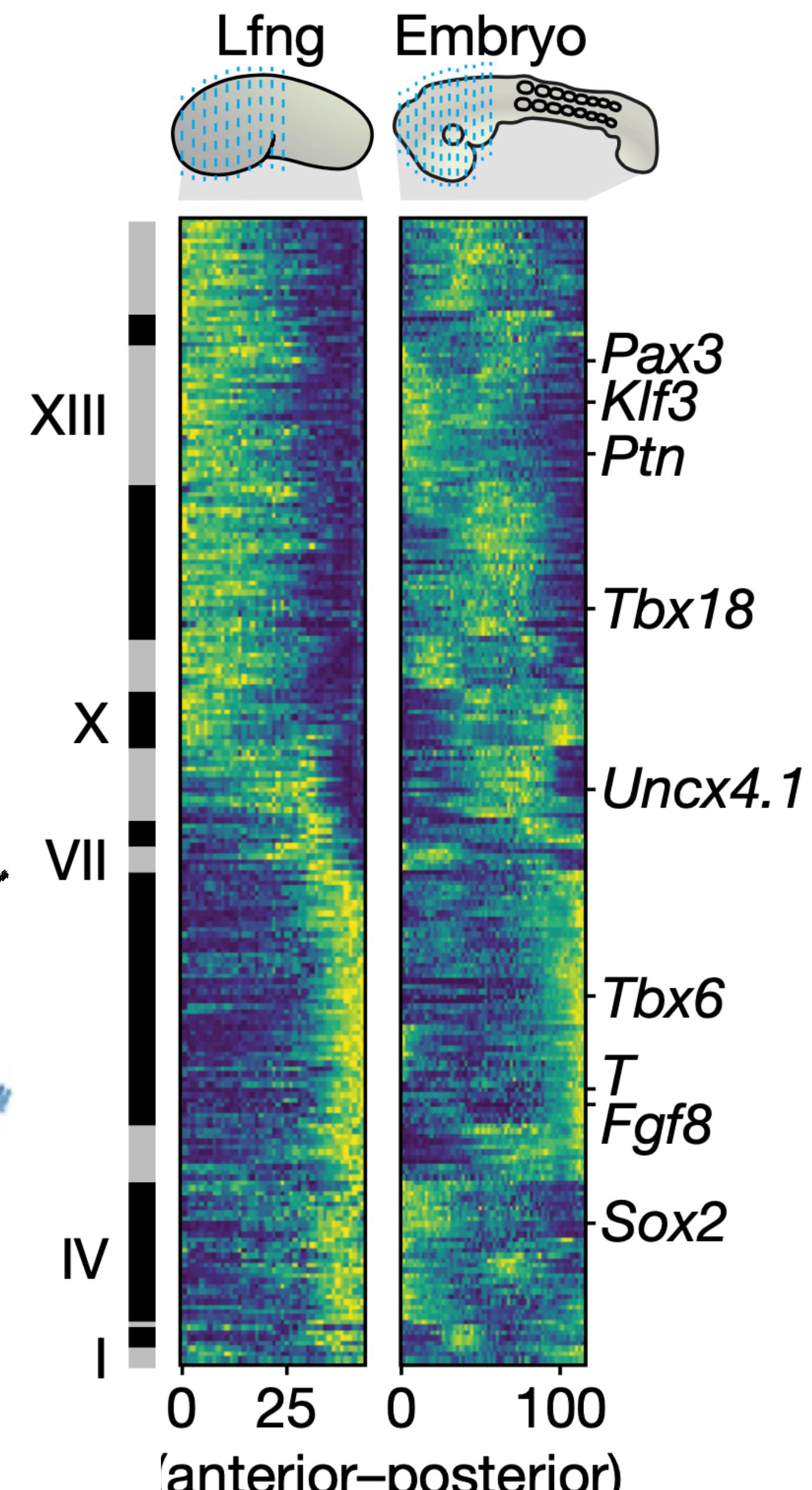
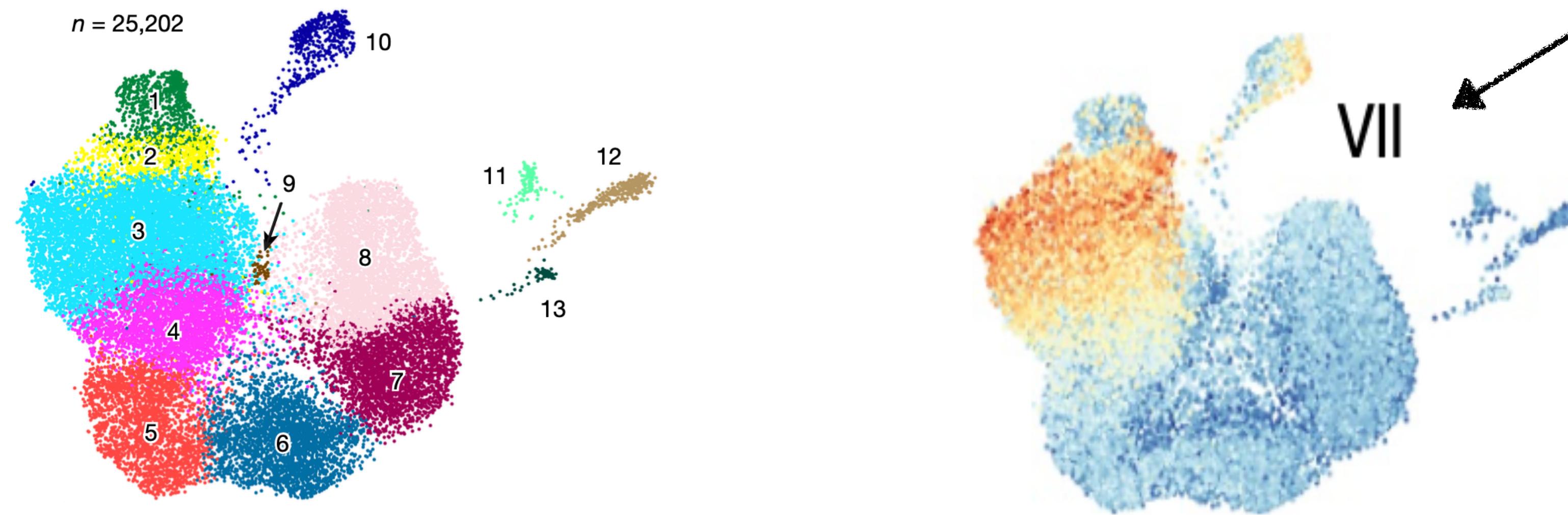
Accepted: 19 December 2019

Published online: 19 February 2020

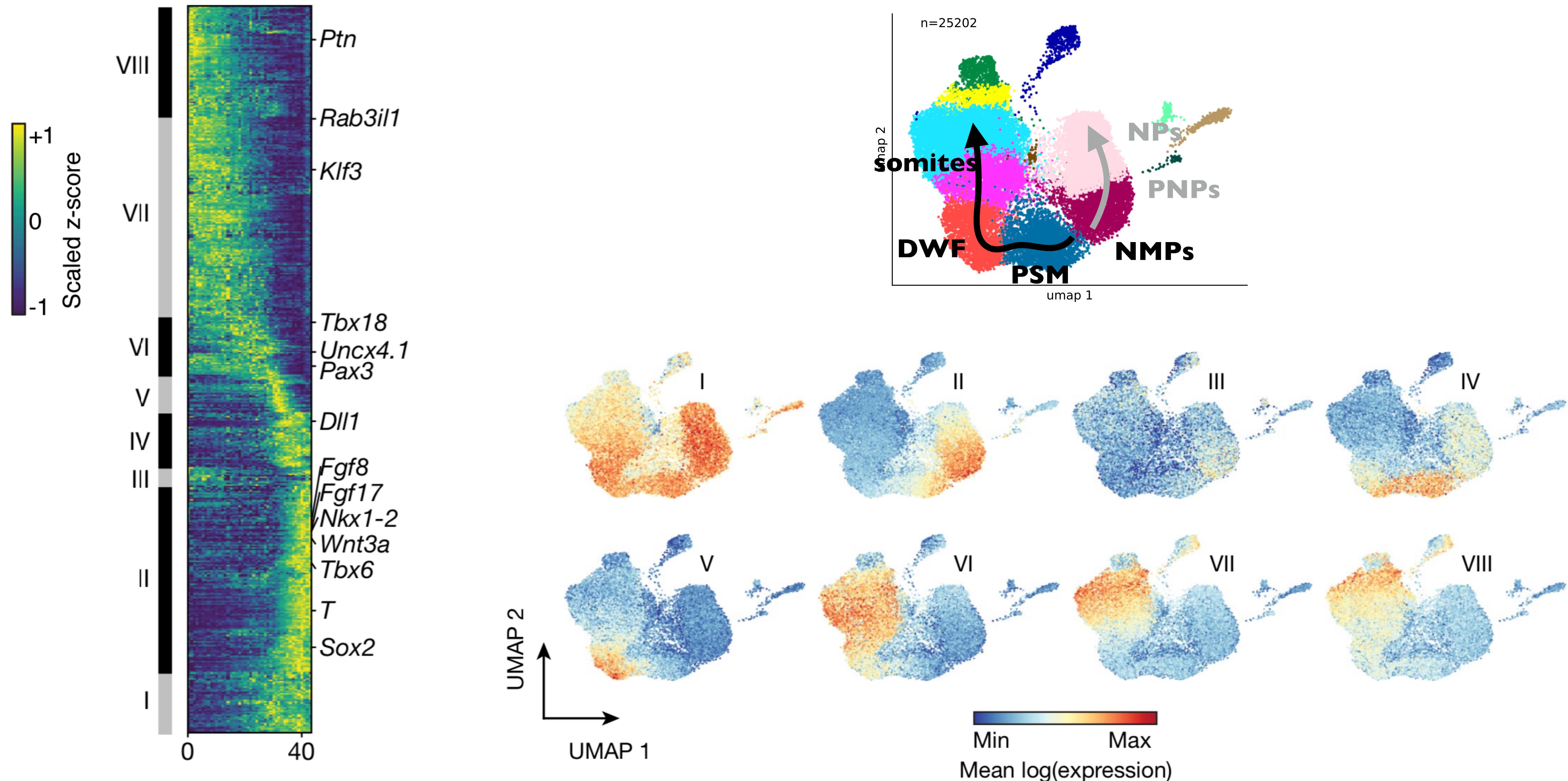
 Check for updates

Susanne C. van den Brink^{1,6}✉, Anna Alemany^{1,6}, Vincent van Batenburg^{1,6}, Naomi Moris², Marloes Blotenburg¹, Judith Vivié¹, Peter Baillie-Johnson², Jennifer Nichols^{3,4}, Katharina F. Sonnen⁵, Alfonso Martinez Arias² & Alexander van Oudenaarden¹✉

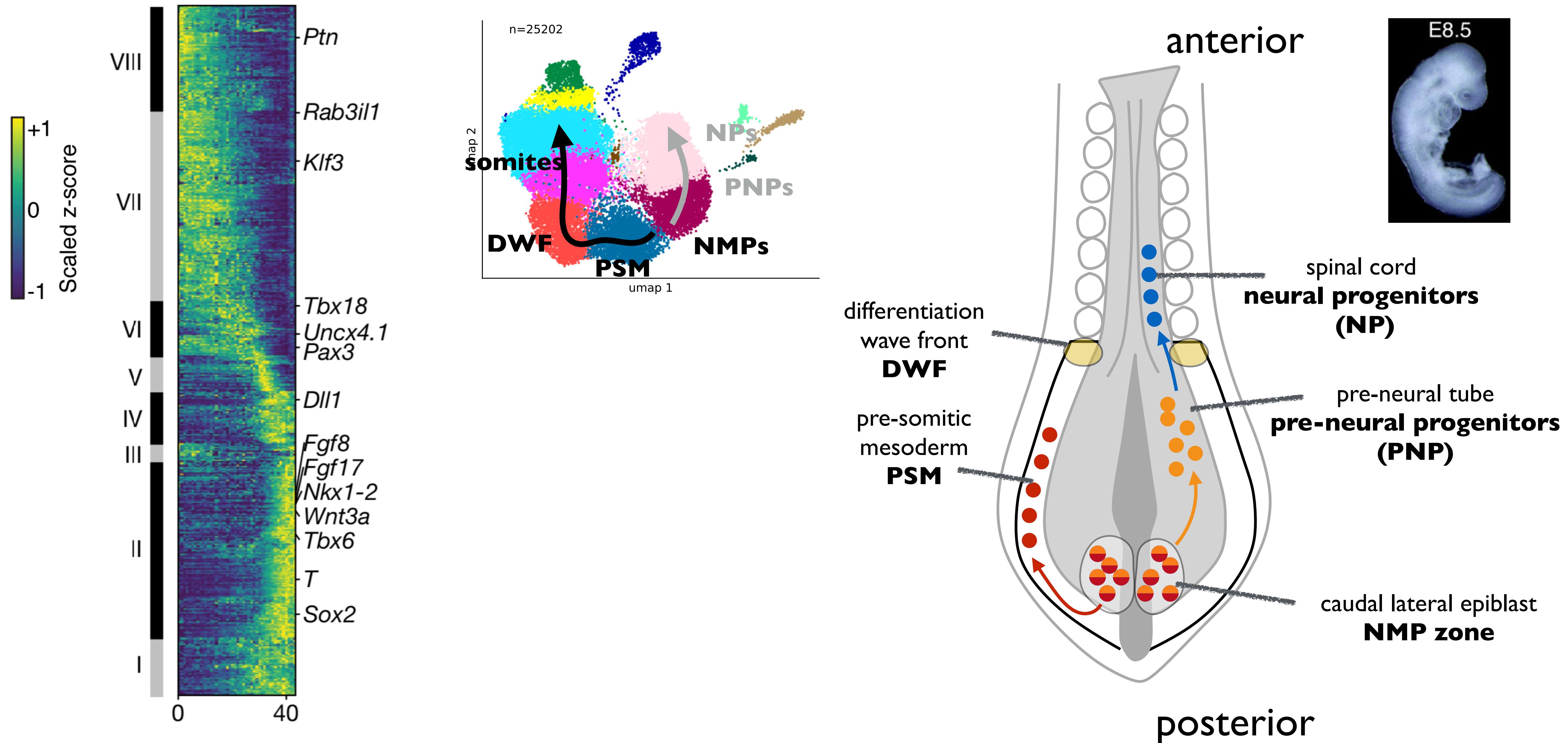
Gastruloids are three-dimensional aggregates of embryonic stem cells that display key features of mammalian development after implantation, including germ-layer specification and axial organization^{1–3}. To date, the expression pattern of only a small



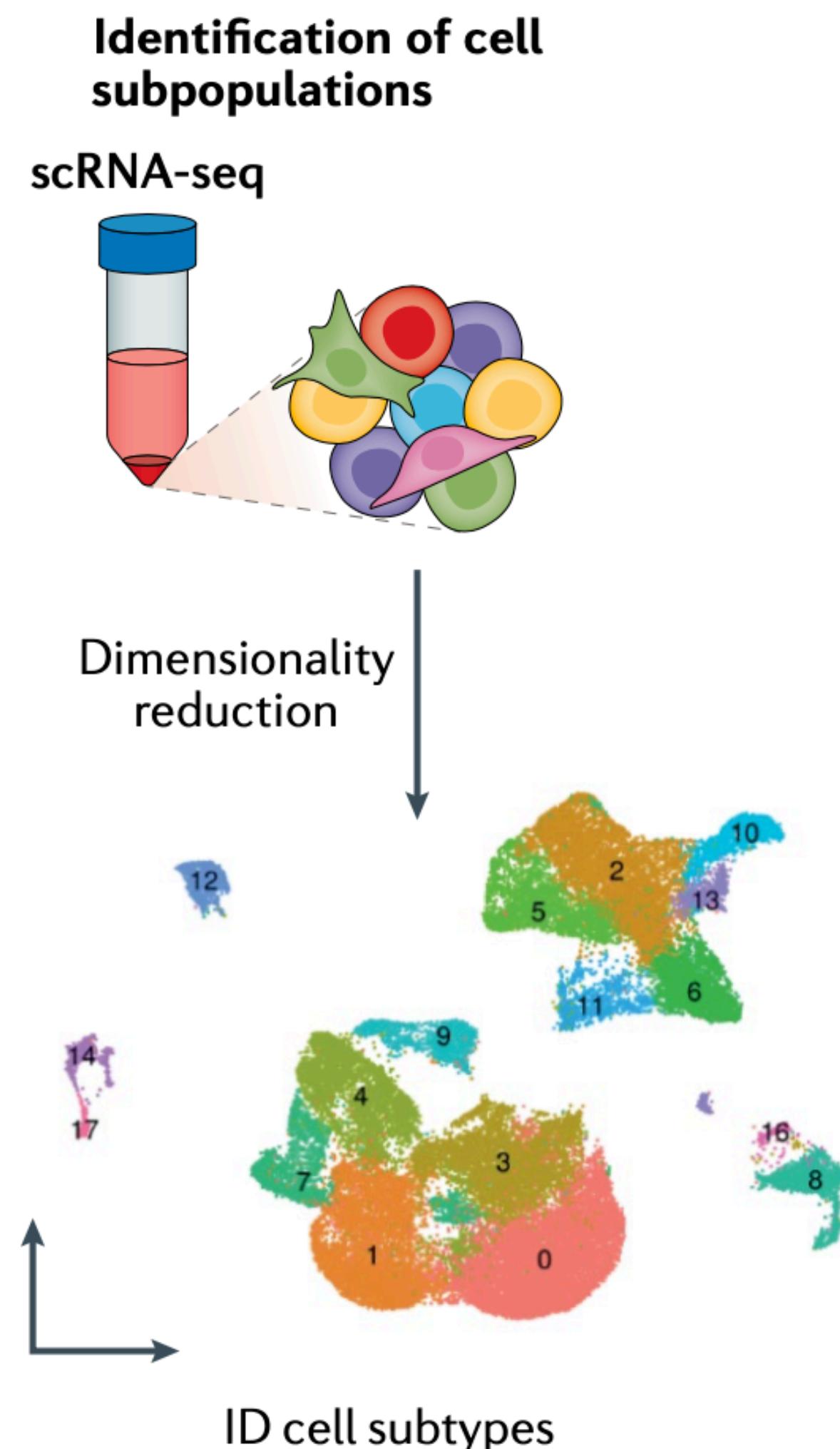
Gastruloids recapitulate the spatial components of somitogenesis



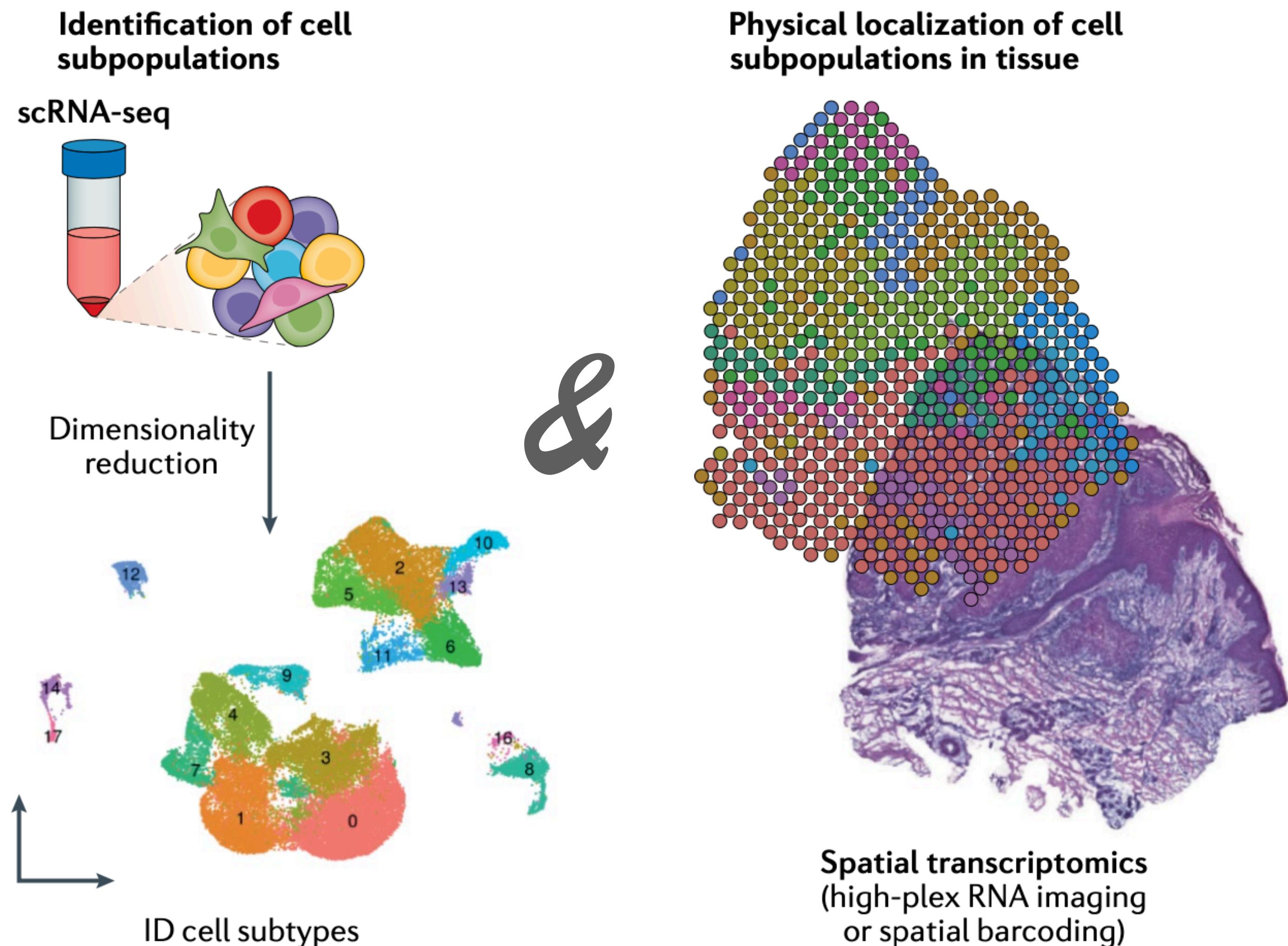
Gastruloids recapitulate the spatial components of somitogenesis



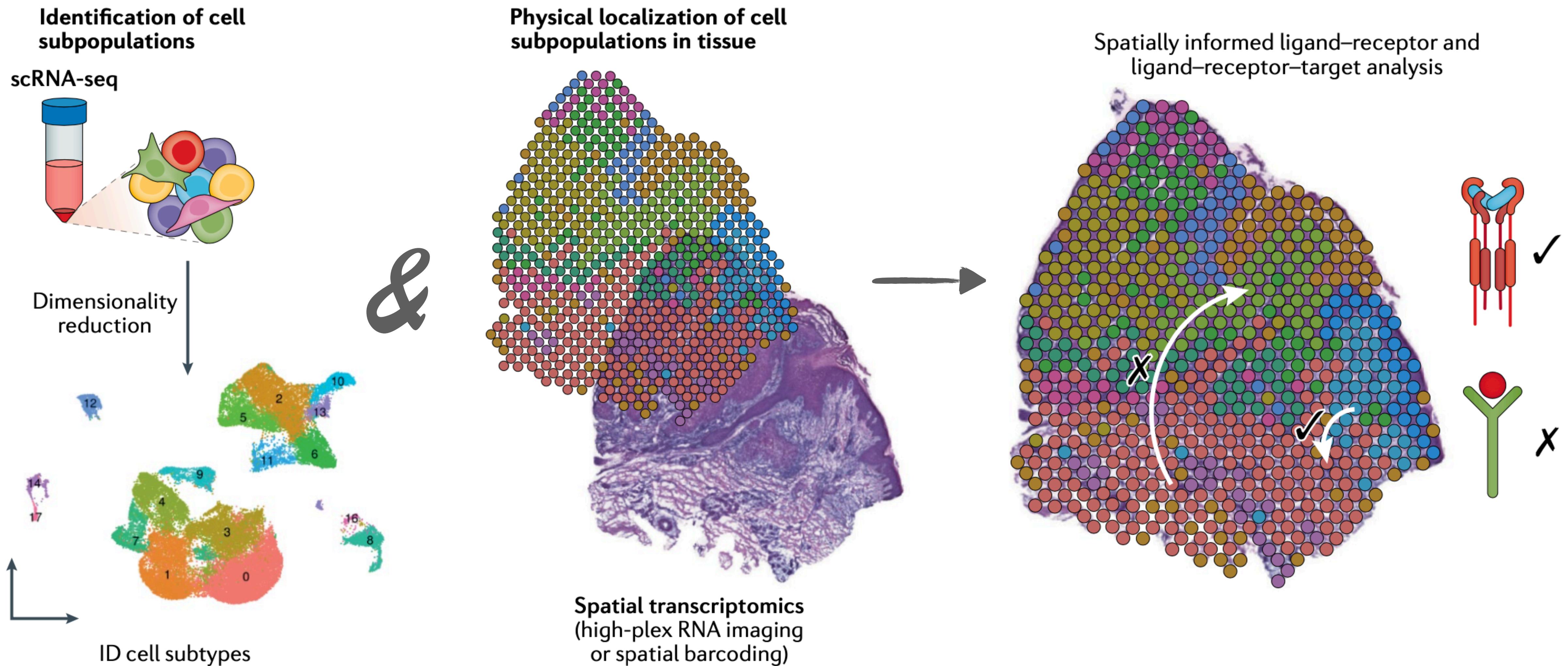
Integration of spatial transcriptomics with scRNA-seq



Integration of spatial transcriptomics with scRNA-seq



Integration of spatial transcriptomics with scRNA-seq



Summary

- Spatial transcriptomics methods allow you to measure mRNA expression while retaining tissue structure
- There are two categories of spatial methods: imaging- and sequencing-based
- Combining spatial transcriptomics will lineage tracing and other *omics* methods will improve our ability to decipher the mechanisms underpinning cell fate choice and tissue patterning.

Thank you for your attention!