bio-techne
spatial™

Expand your multiomic capabilities with RNAscope

Any RNA, Any proteins, Any Tissue

Ludovic ARNOLD, PhD
RNAscope Specialist
Ludovic.arnold@bio-techne.com
06 47 44 47 91

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bio-techne®

R&D systems™ by bio-techne



Antibodies



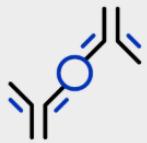
Proteins



Small Molecules



Instruments



ELISAs

bio-techne spatial™



RNAscope



COMET Platform



Spatial Bio



SPYRE Panels

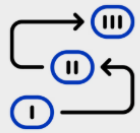
bio-techne diagnostics™



Molecular Kits



Controls & Reagents



Development & Manufacturing

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Overview

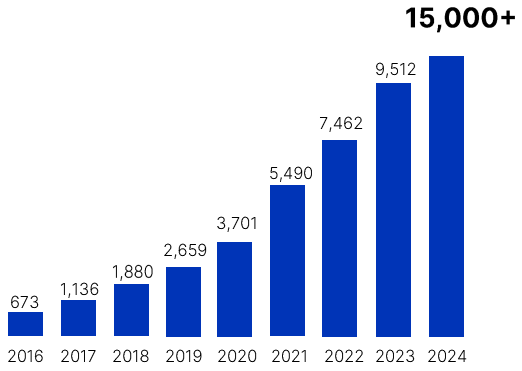
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RNAscope™ - smFISH gold standard

15,000+ Publications

The Gold Standard

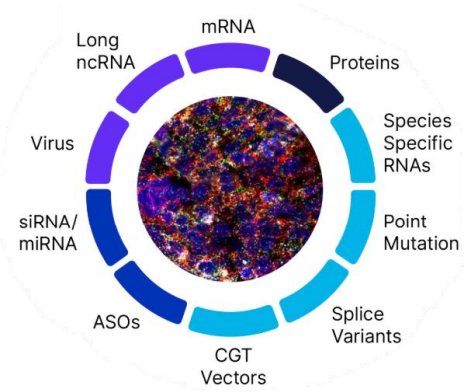
- 33% pubs in 2024 for RNA + Protein co-detection



80,000+ Catalog probes

Catalog and Custom Probes

- Widest menu of Omics- mRNA, lncRNA, Splice Variants, GT Vectors, ASOs +/- Proteins
- Probe guarantee for specificity



300+ Spatial Biology Team

RNA + Protein experts

- 60+ user-facing PhDs: FAS, Tech support and Probe Design scientists
- Part of Bio-Techne family, $10 Billion public company



Manual and automated chromogenic and fluo

Built on Innovation

- High throughput spatial analysis with
  - Leica
  - Ventana
  - COMET

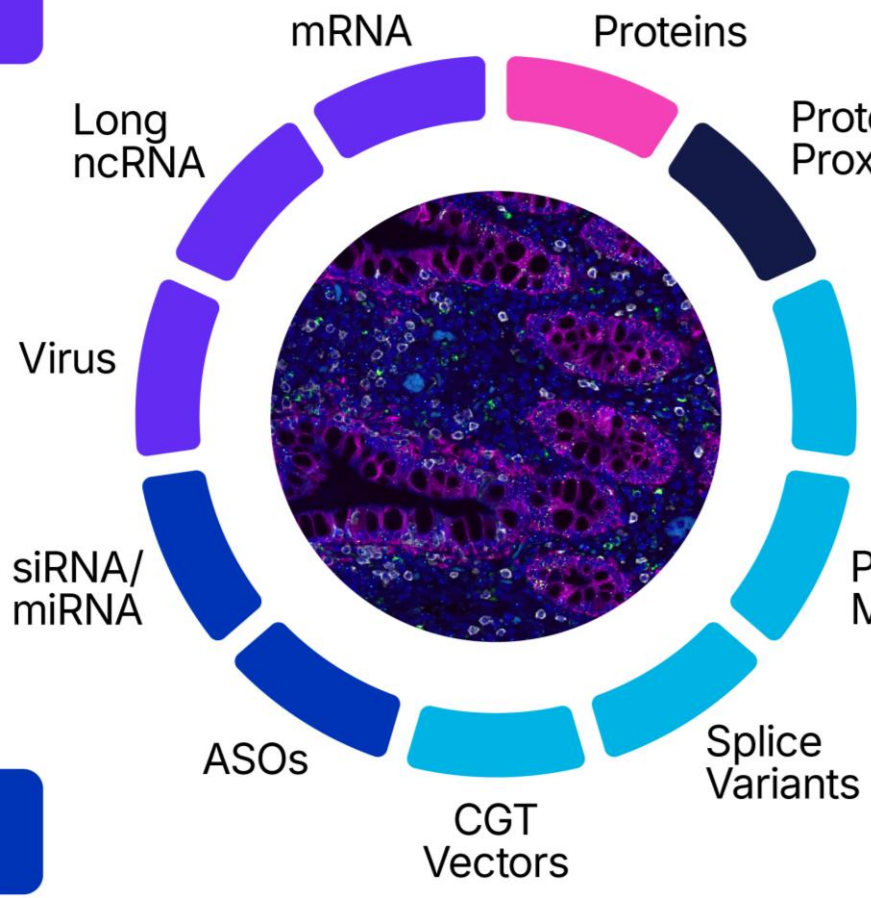


Our shared vision to decode cell function, disease progression, and accelerate therapeutic discoveries.

RNAscope

>300 nt

ProximityScope



miRNAscope

17 to 50 nt

BaseScope

50 to 300 nt

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Multiomics capabilities

We aim to develop an universal assay to combine any omics on the same section

RNAscope (>300 nt) + proteins = Manual assay (> 4 plex) / Leica Bond RX (> 6 plex) / Roche Ventana (2-3 plex) / COMET (>12 plex RNA; protein illimited)

> 4 plex RNAscope (>300 nt) + 1 plex proteins + ProximityScope = Leica Bond RX

> 3 plex > 5 plex 1 plex RNAscope (>300 nt) + 1 plex miRNAscope (17 to 50 nt) + proteins = Manual assay / Leica Bond RX / Roche Ventana

---

> 4 plex RNAscope (>300 nt) + > 2 plex BaseScope (50 to 300 nt) = Leica Bond RX

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Principle

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RNAscope™ principle

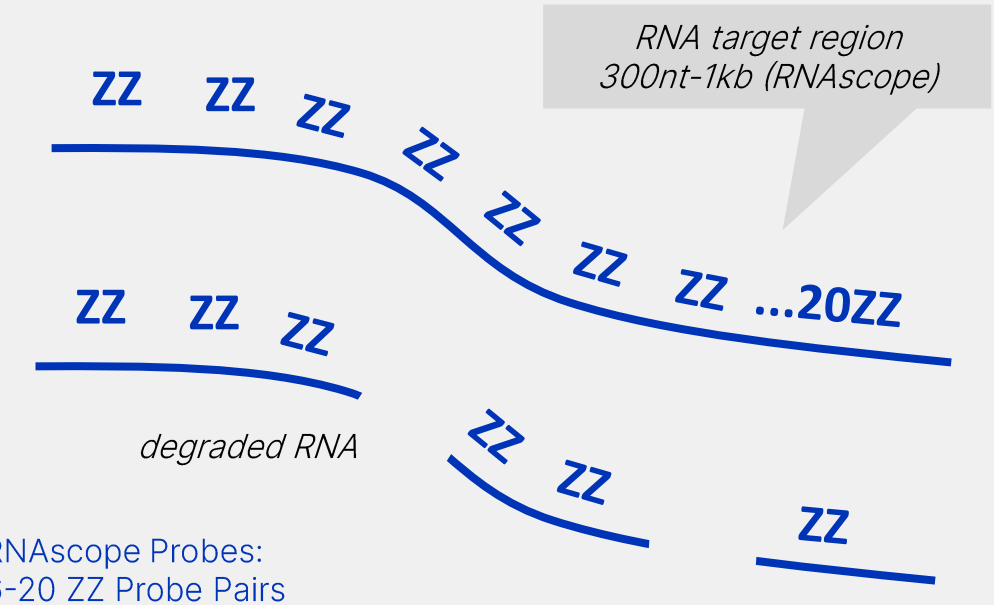
Preamplifier binding site / tail:
~28 nt

ZZ

Target specific binding site:
~50 nt

Double ZZ Design of RNAsope Probe Pairs

Specificity

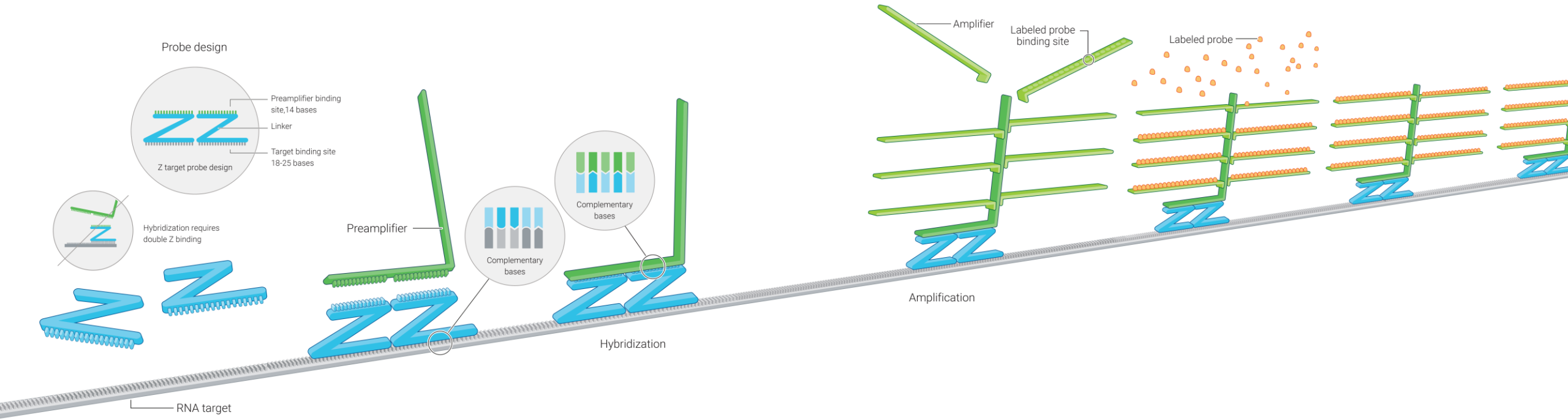


RNAscope Probes:
6-20 ZZ Probe Pairs

Assay Robustness

- Single, Z' probes do not generate a signal
- No need for RNase-free conditions
- Compatible with human archival tissue

RNAscope™ principle

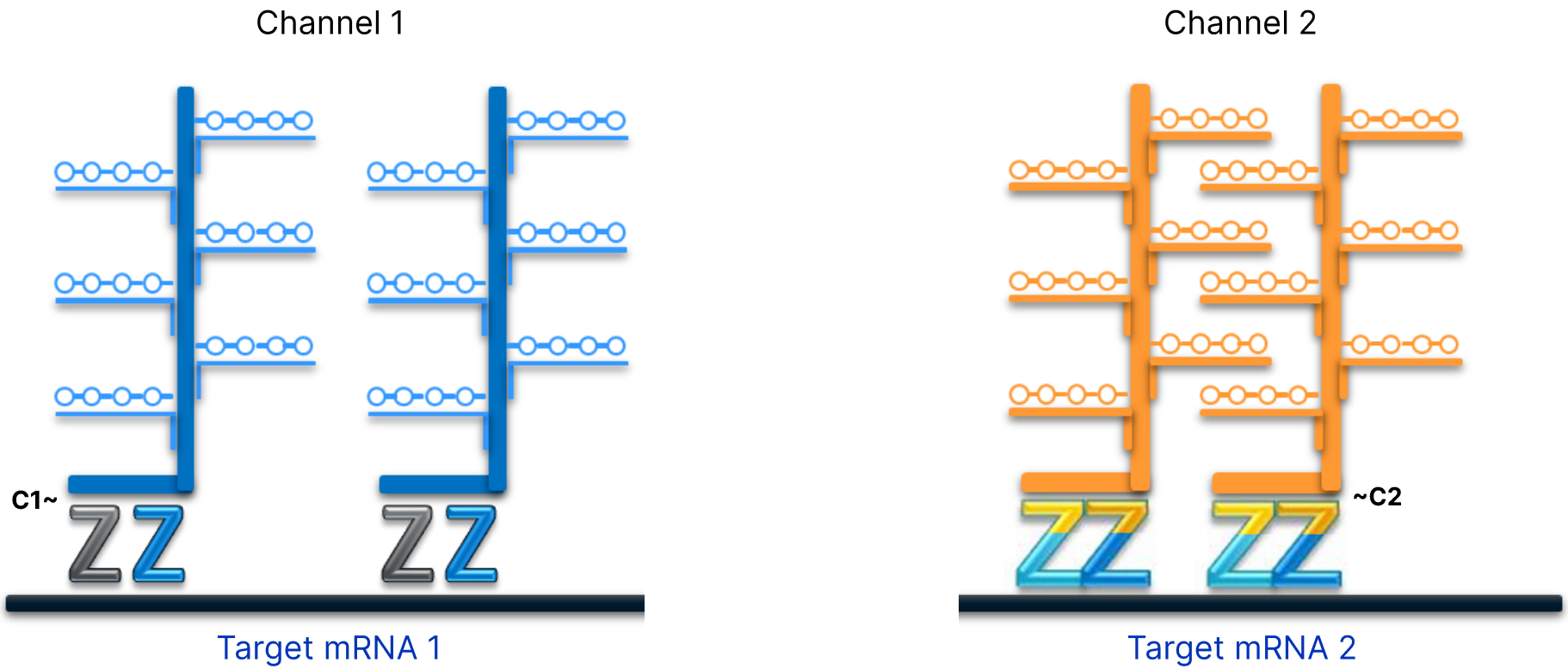


Easy to implement . Easy to perform . Sensitive . Specific . Flexible . Cellular resolution

Multiplex . Multiomic . Economical . Time-Saving . Semi-quantitative . Trust

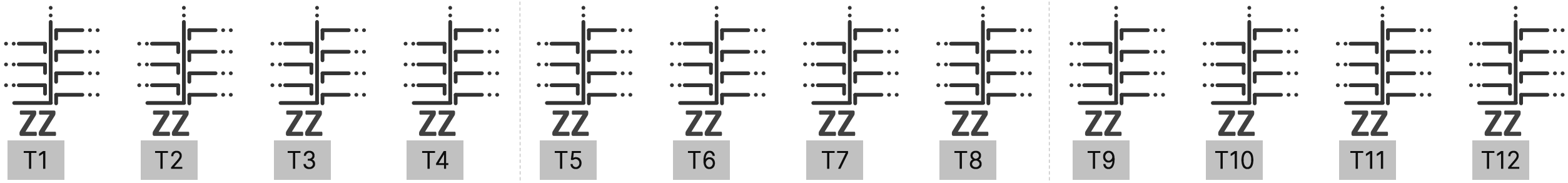
Multiplexing – probes channels

The double ZZ probe design has an upper region sequence that is indicative of a channel/tail designator



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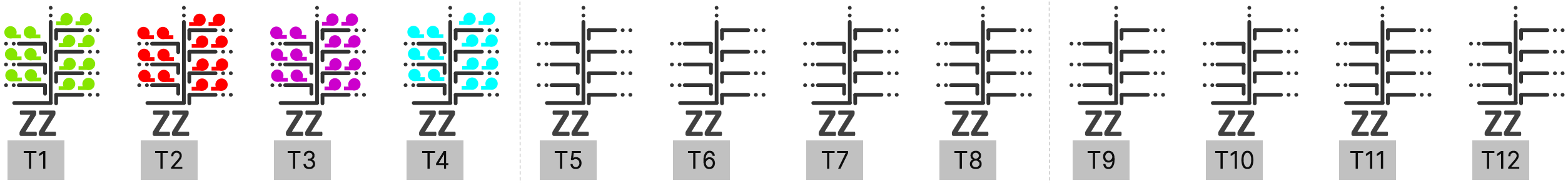
RNAscope **cyclic HiPlex workflow** allows detection of 12mRNA targets



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RNAscope **cyclic HiPlex workflow** allows detection of 12mRNA targets

1st round of imaging

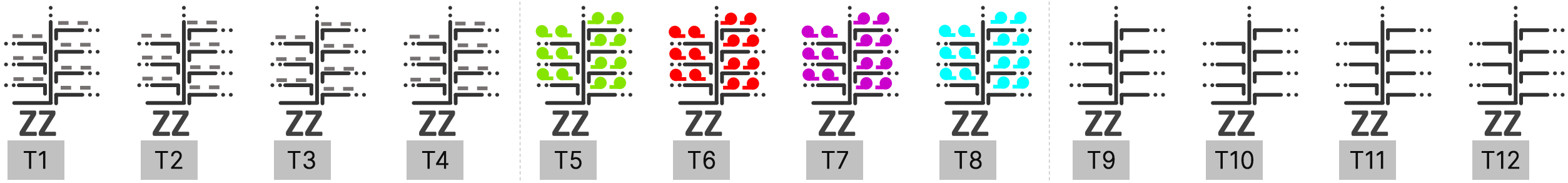


Alexa488 DyLight650
DyLight550 Alexa750

RNAscope **cyclic HiPlex workflow** allows detection of 12mRNA targets

2nd round of imaging

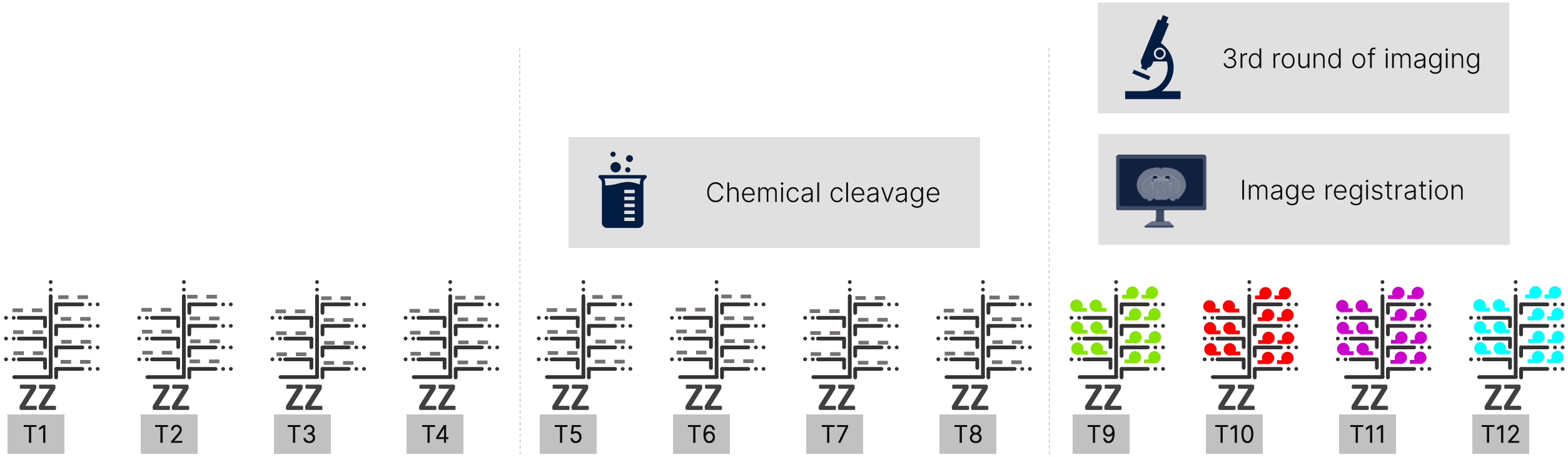
Chemical cleavage



Alexa488 DyLight650
DyLight550 Alexa750

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RNAscope **cyclic HiPlex workflow** allows detection of 12mRNA targets

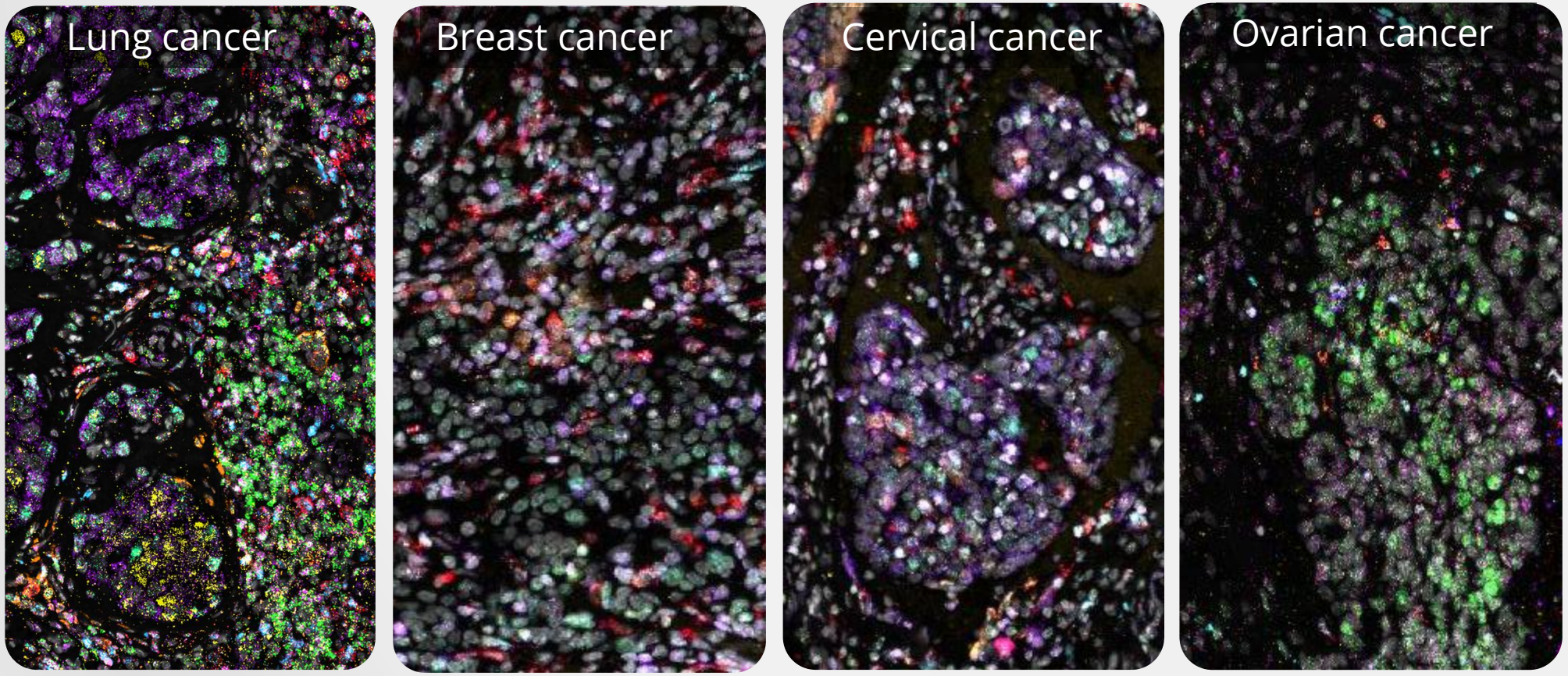


Cell identification: Decipher TME

RNAscope Hiplex Assay (12 plex)

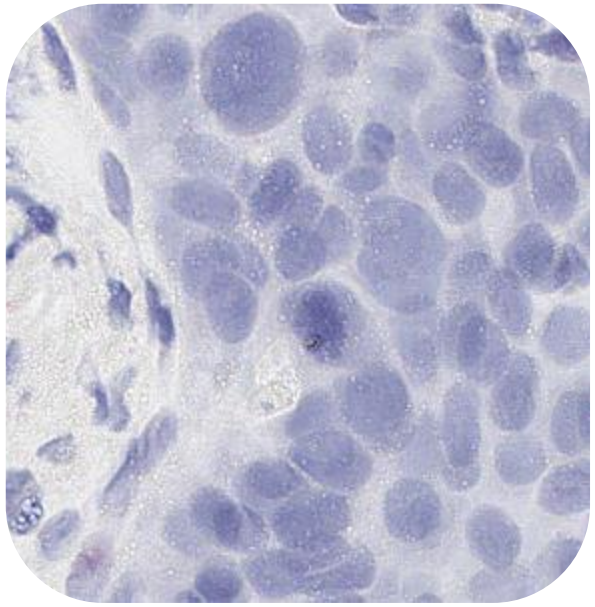
12 Plex TME immune marker panel in FFPE tumor samples

CD3 CD8 IFNG PD1 CD68 CD163 ARG1 CCL22 CXCL10 VEGF HIF1A PDL1



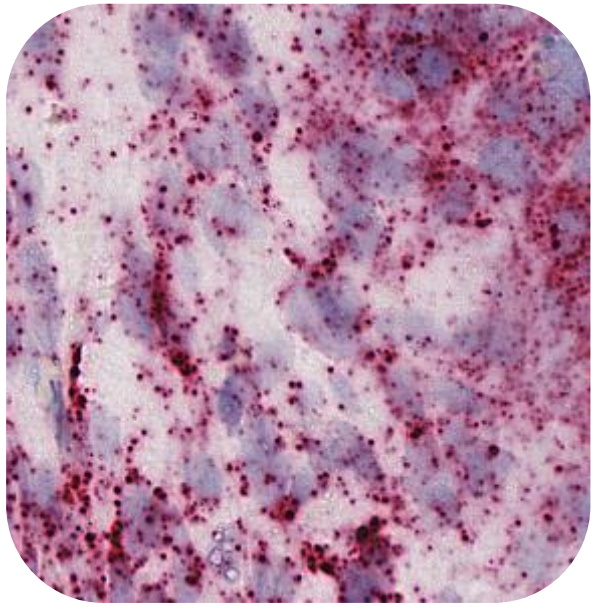
We provide you positive & negative control probes for tissue quality control

Human Lung Cancer



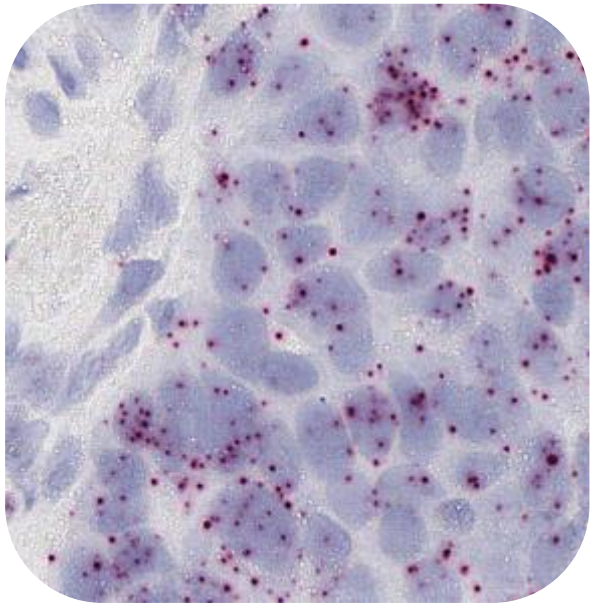
Negative control DapB

NO BACKGROUND



Positive control PPIB

UNIFORM SIGNAL

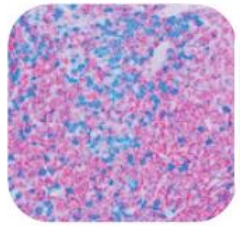
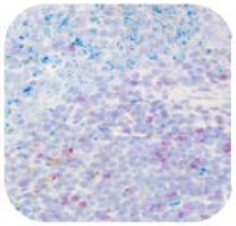


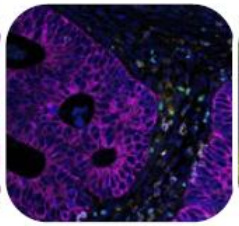
Test probe PD L-1

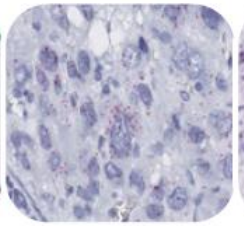
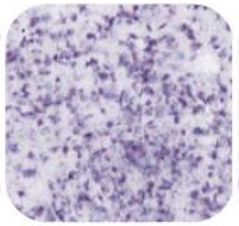
Serial FFPE sections with RED assay

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RNAscope™ Product Portfolio





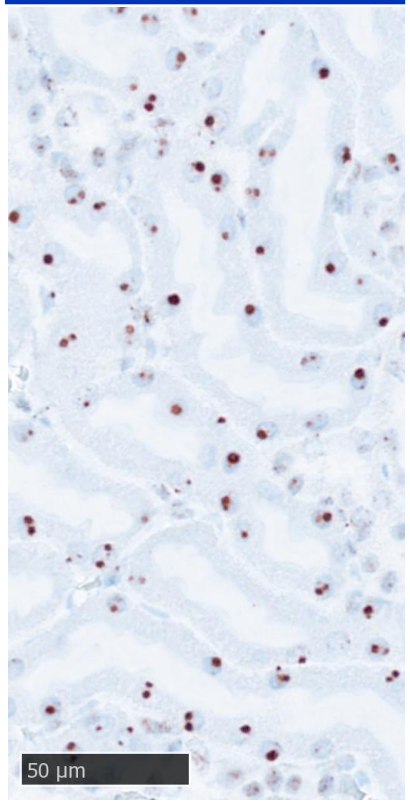
	RNAscope™	RNAscope™ Duplex	RNAscope™ Multiplex v2	RNAscope™ Multiomic LS	RNAscope™ HiPlex	BaseScope™	BaseScope™ Duplex	miRNAscope™	RNAscope™ Plus smRNA-RNA
Transcript Length									
Maximum Targets	Single or	Duplex	4-plex	6-plex	12-plex* x4 * up to 48 w/ HiPlex Up	Single	Duplex	Single	4-plex or
Channels	C1	C1, C2	C1, C2, C3, C4	C1, C2, C3, C4, C5, C6	T1, T2, T3... ...T11, T12	C1	C1, C2	C1, C2, C3, C4	C1, C2, C3, C4, C5, C6
Available Platforms	Manual Roche Leica	Manual Roche Leica	Manual Leica	Leica	Manual COMET	Manual Roche Leica	Manual	Manual Leica	Manual Leica
Add Antibody	Co-Detection Compatible	N	Co-Detection Compatible				N	Co-Detection Compatible	N

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smRNA Detection on DISCOVERY ULTRA

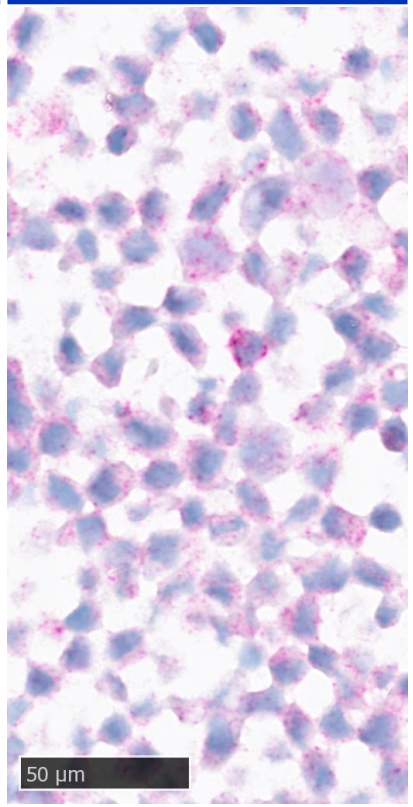
miRNAscope + RNAscope: Chromogenic and Fluorescent

miRNA
VS Universal HRP



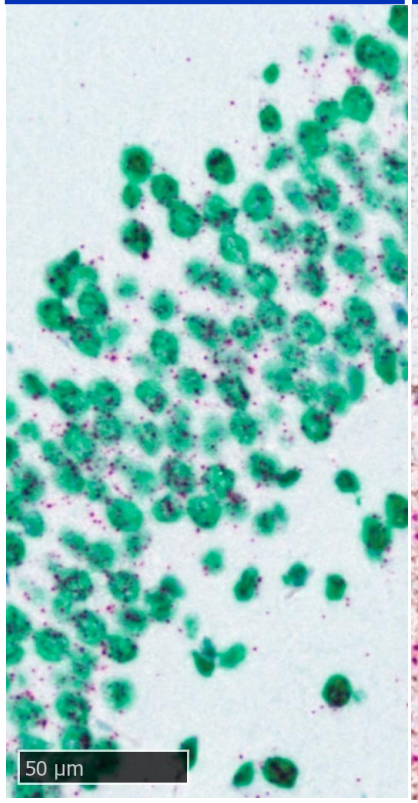
Snord85-S1
Mouse Kidney

miRNA
VS Universal AP



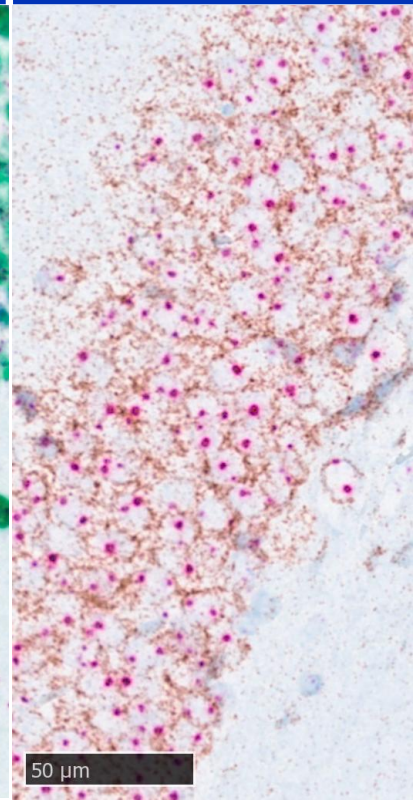
miR21-S1
HeLa

RNA + miRNA
VS Duplex



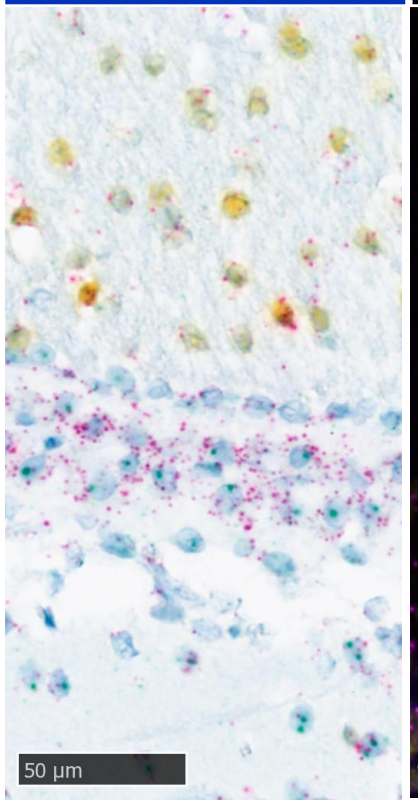
RNU6-S1
PPIB-C2
Mouse Brain

miRNA + miRNA
VS Duplex



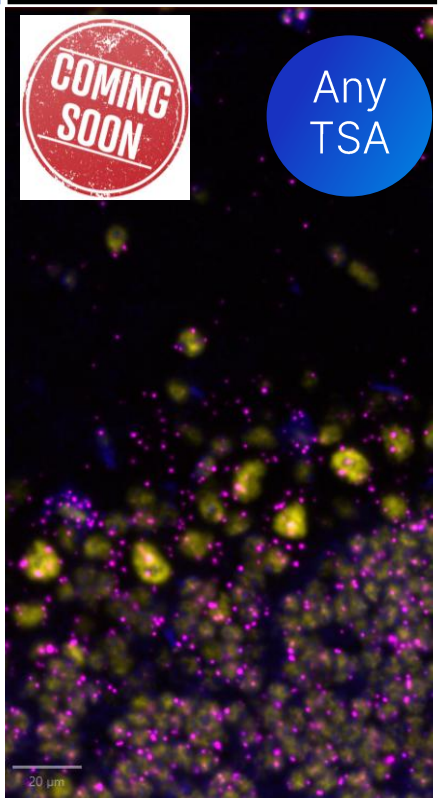
miR124-S1
Snord85-S2
Mouse Brain

RNA + miRNA + protein
VS Duplex + IHC



Snord85-S1
PPIB-C2
Anti-Olig2
Mouse Brain

RNA + miRNA
New VS Multiplex Fluo



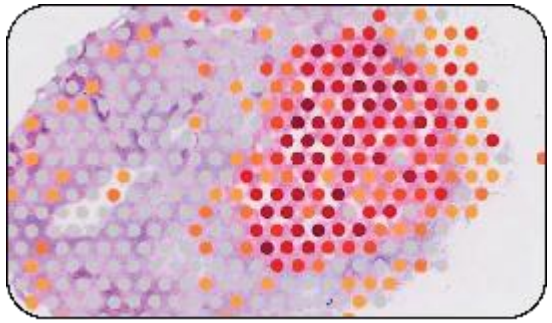
RNU6-S1
POLR2A-C2
Mouse Brain

Spatial transriptomics

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RNAscope and Spatial transcriptomics answer different biological questions

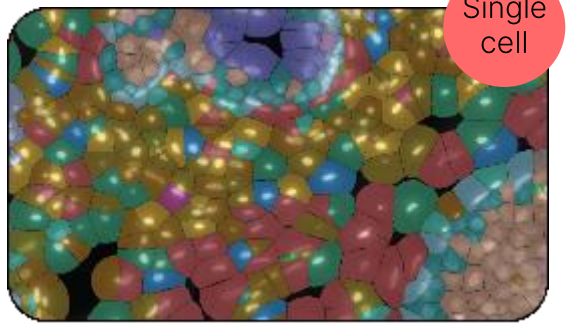
Discovery Tools
(spatial transcriptomic)



Sequencing-based Spatial Transcriptomics

GeoMx / Visium-HD

Up to 18 000 genes

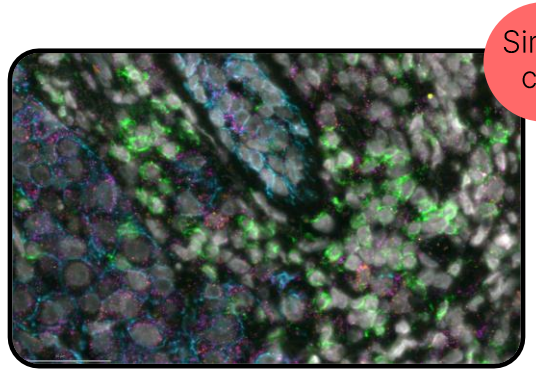


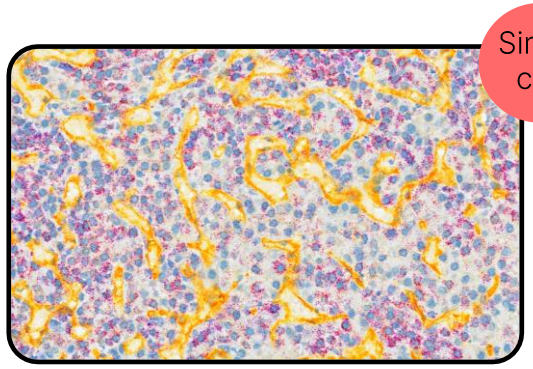
Imaging-based Spatial Transcriptomics

Xenium / CosMx / *Merscope*

50 to 5000 genes

Hypothesis-driven tools
(lo/mid plexing spatial multiomic)





Spatial Proteomics and Multiomics :

COMET

Virtually unlimited proteins plexity or up to 12 RNA/88+ proteins (fluo)

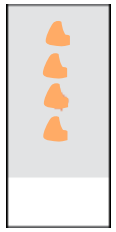
RNAscope and multiplex IF

Bond Rx

Up to 6 RNA/protein (fluo)

Discovery Ultra

up to 4 / 5 proteins up to 2 RNA / 2 proteins (chromo)

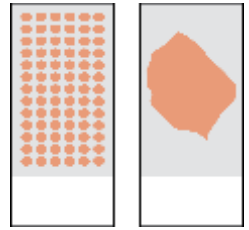


Plexing

Throughput and Imaging area

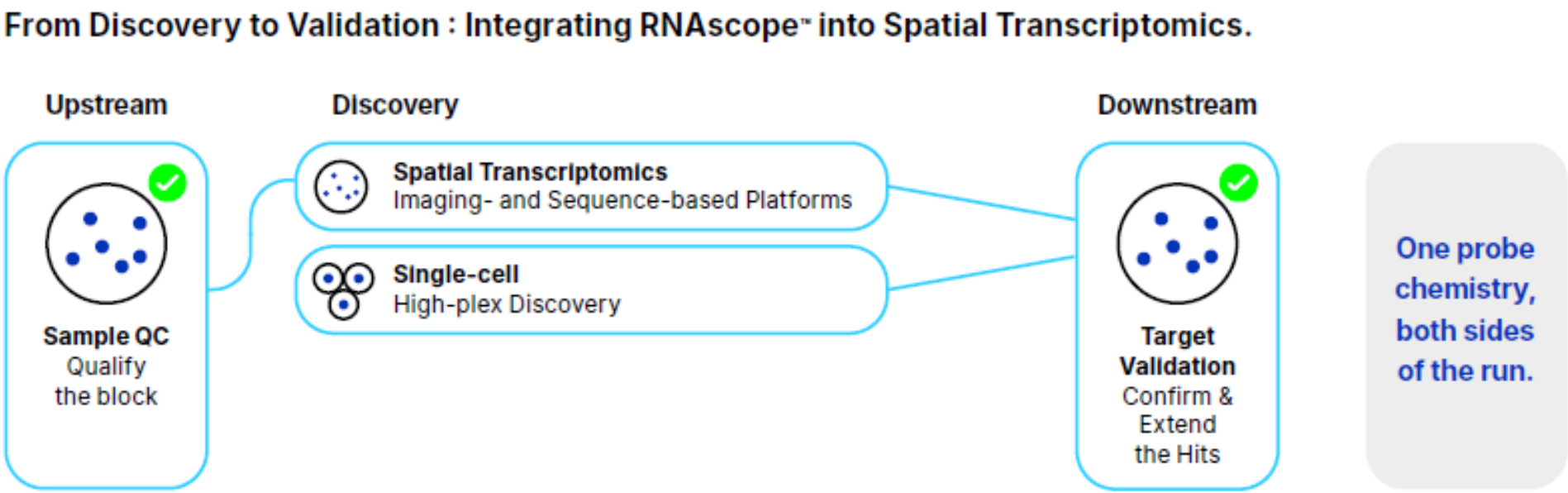
Cost

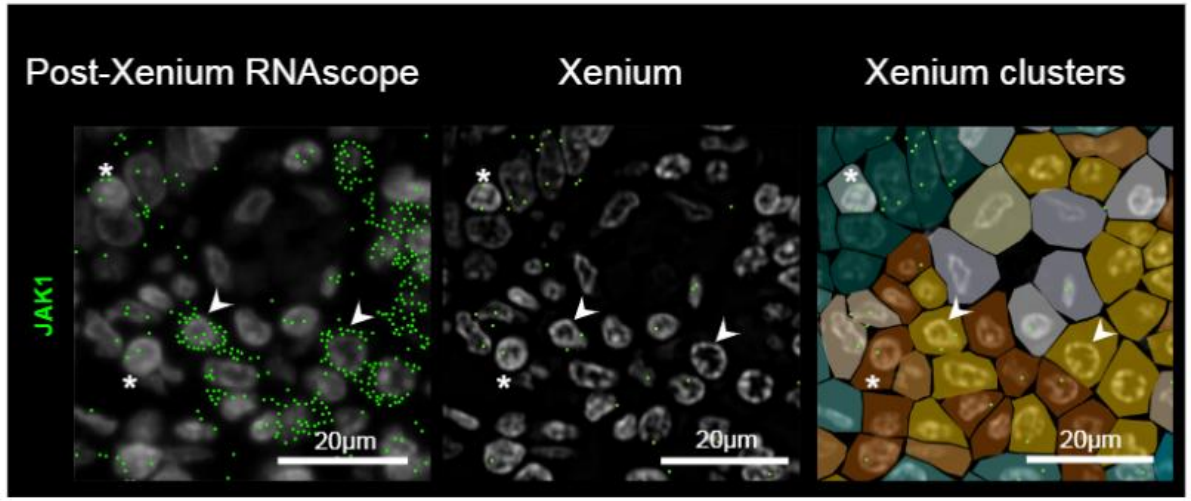
Bioinformatic analysis



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RNAscope for spatial transcriptomics





Cells in the light brown clusters exhibit high JAK1 expression by RNAscope, which is not detected in the Xenium data (arrows), potentially due to signal crowding. In contrast, cells with intermediate JAK1 expression show strong concordance between the two technologies (asterisks).

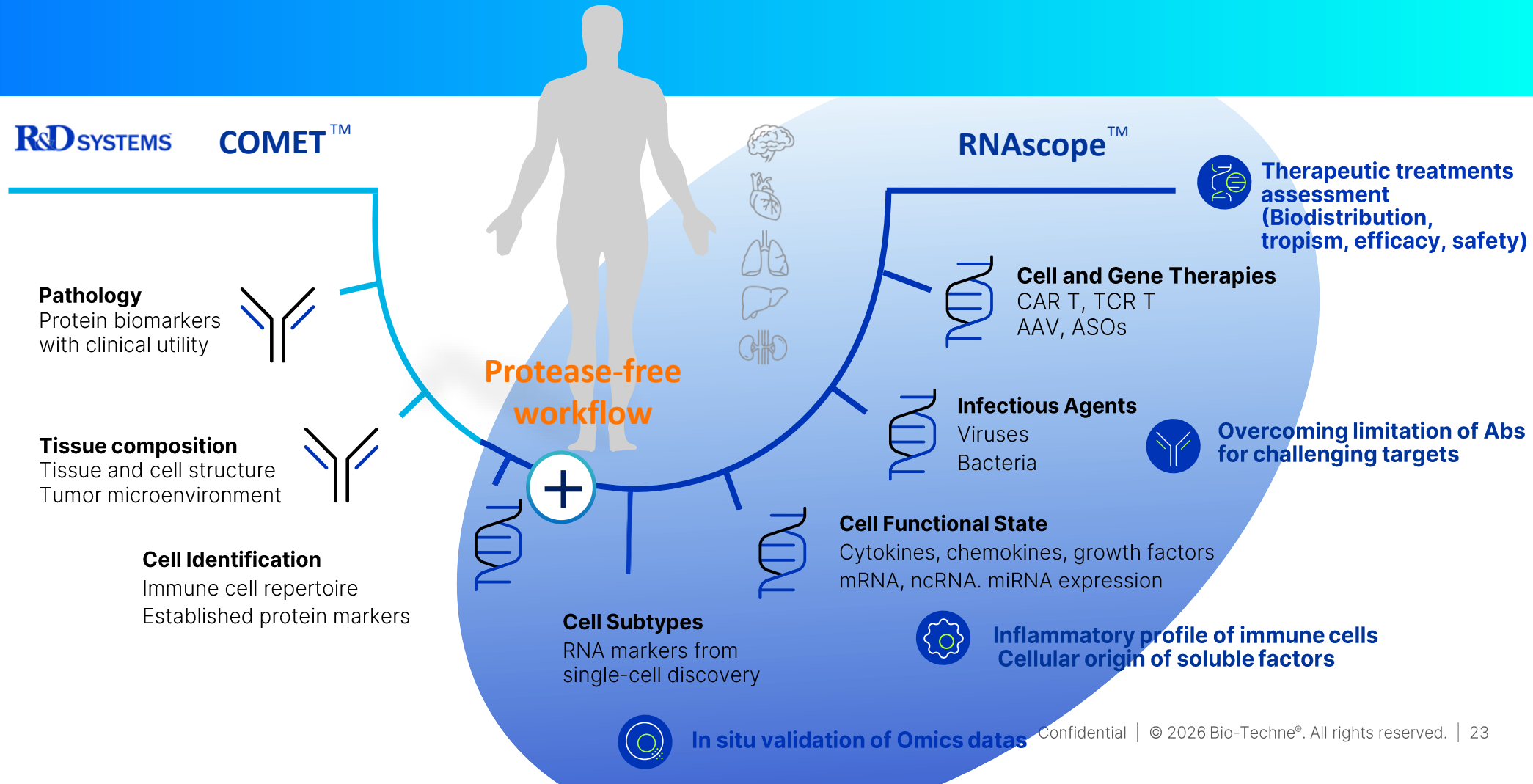
reserved.

Applications

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Multiomic assay : RNA + protein co-detection

Applications

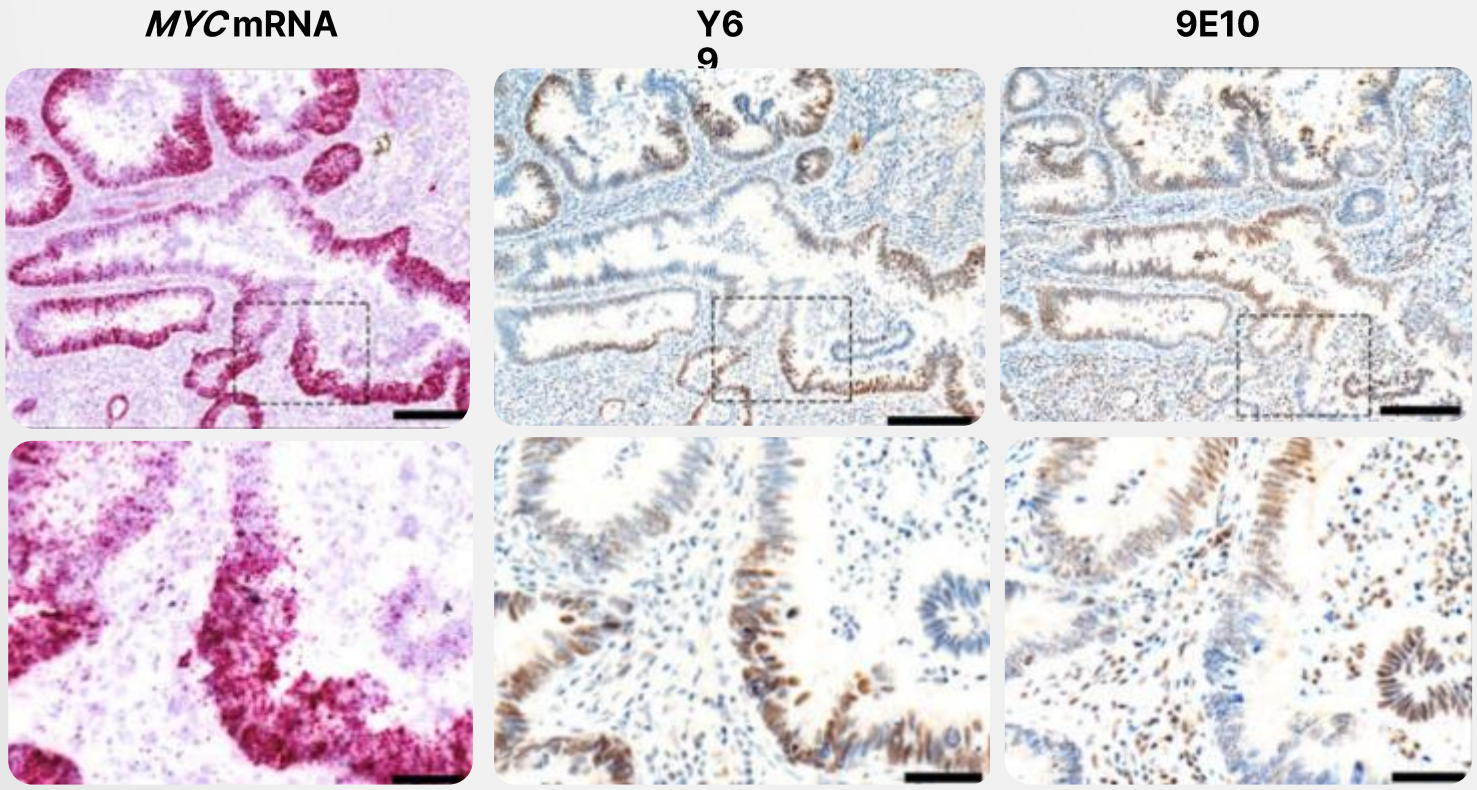


Tissue/Organoid sections
FFPE
Fixed frozen
Fresh frozen

Limitation of Antibodies

RNAscope for *in situ* Abs validation

RNAscope Red Chromogenic Assay



Correlation of RNA and protein

"gold standard" MYC antibody

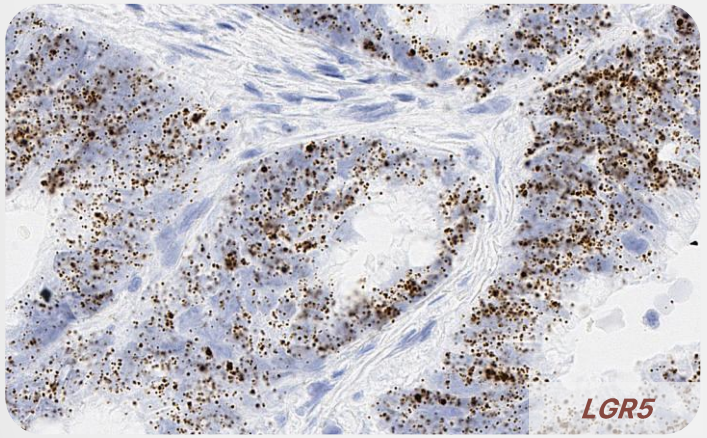
"Localization of MYC mRNA by RNAscope ISH correlated well with IHC antibody Y69, but not 9E10, questioning the reliability of the "gold standard" antibody 9E10"

Source: Baker et al. Histopathology, 2016

Challenging Targets

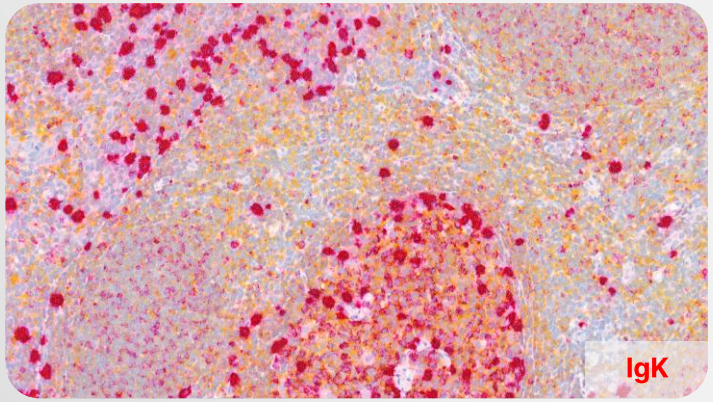
Limitation of Antibodies

Stem cell marker



Colorectal cancer

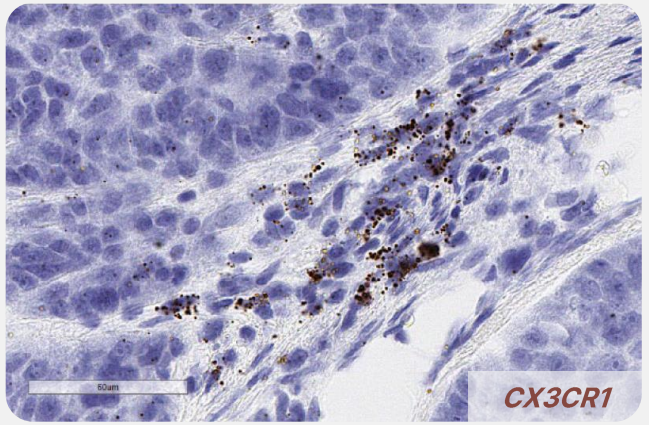
Immunoglobulin



In the absence of good Abs

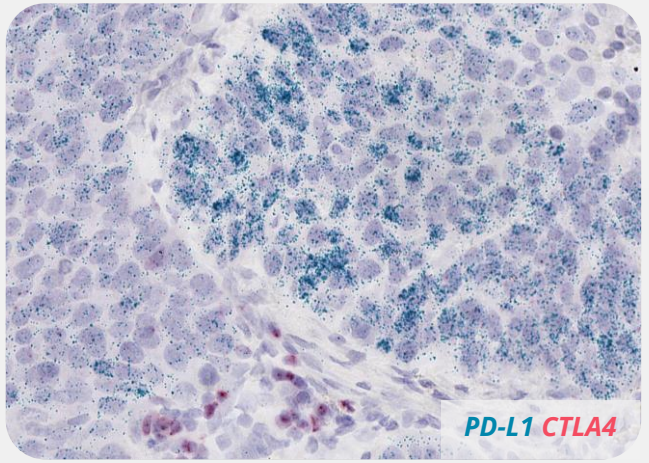
- Rare animal species
- Cytokines
- Immunoglobulin
- ECM
- GPCR
- Virus
- Bacteria
- Transcription factors
- Stem cell markers
- Splice variants
- Fusion transcript
- miRNA
- Circular RNA
- Point mutation
- RNA Tx (siRNA, ASO)
- CAR-T
- Ion channels
- lncRNA

GPCR



Liver cancer

Immune checkpoints



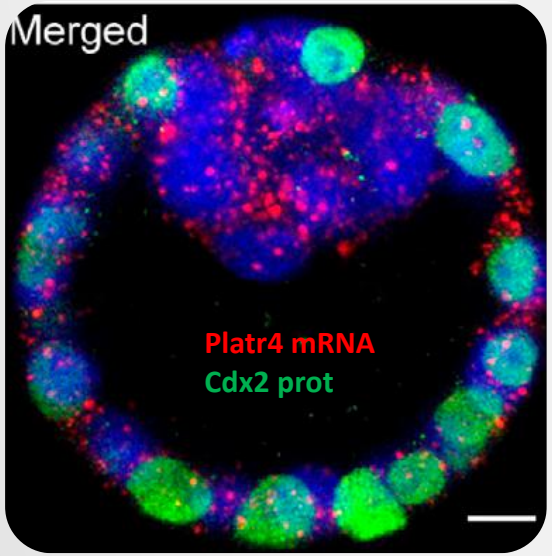
Lung cancer

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Challenging Targets in challenging tissues

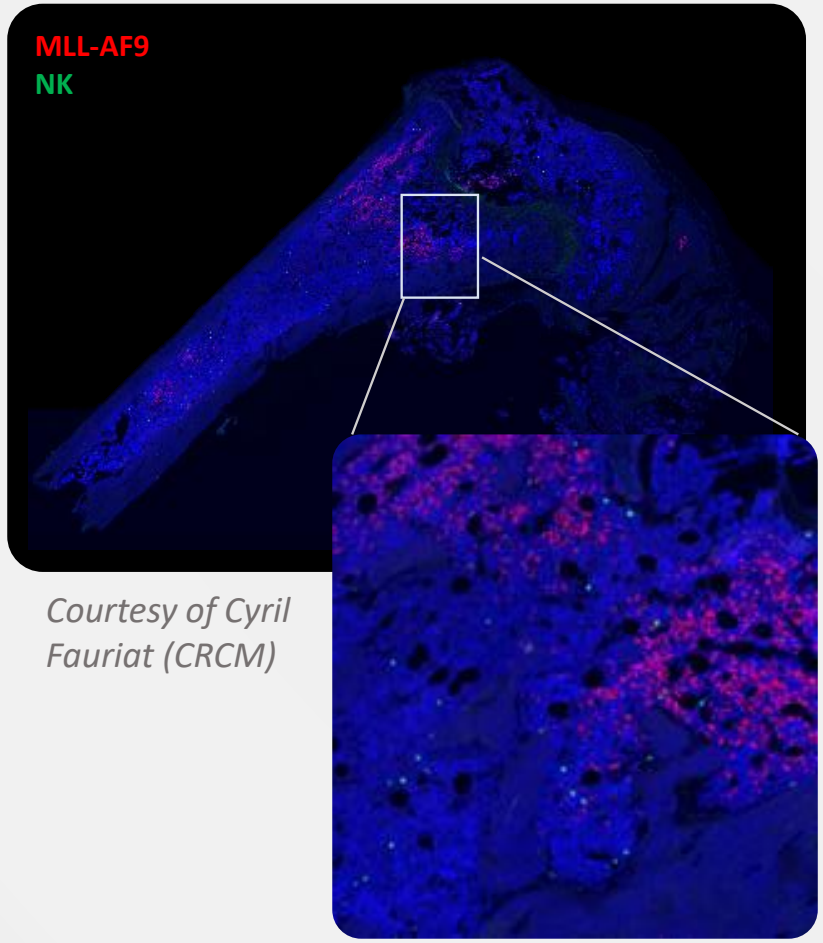
Limitation of Antibodies

lncRNA in mouse embryo



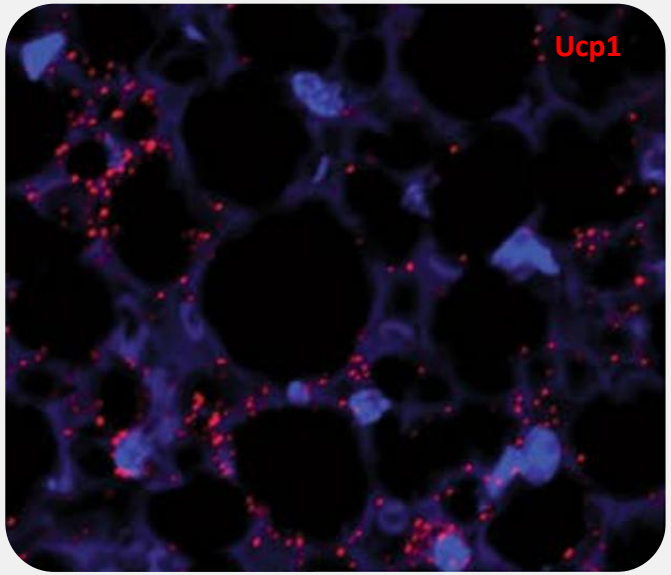
Hazra & Spector et al., 2022

***Fusion transcript** in mouse bone*



Courtesy of Cyril Fauriat (CRCM)

Mouse adipose tissue

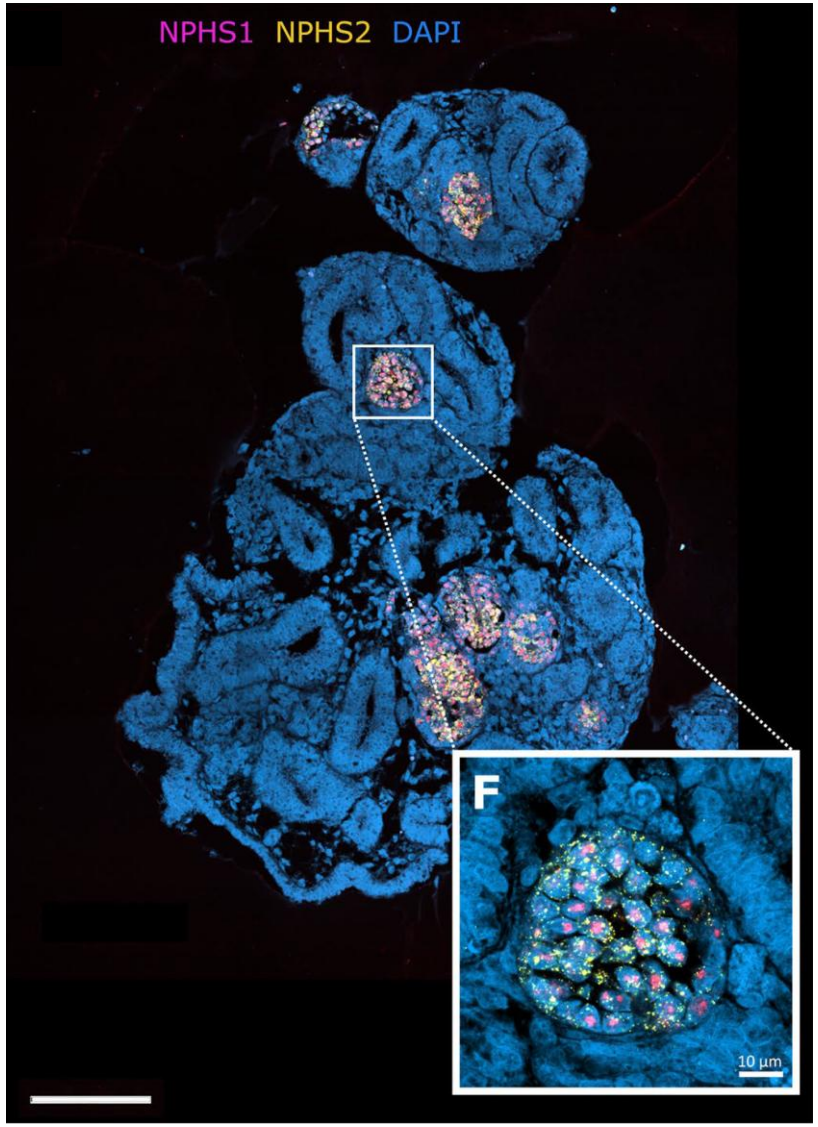


Rajaii et al., 2021

RNAscope on organoids sections

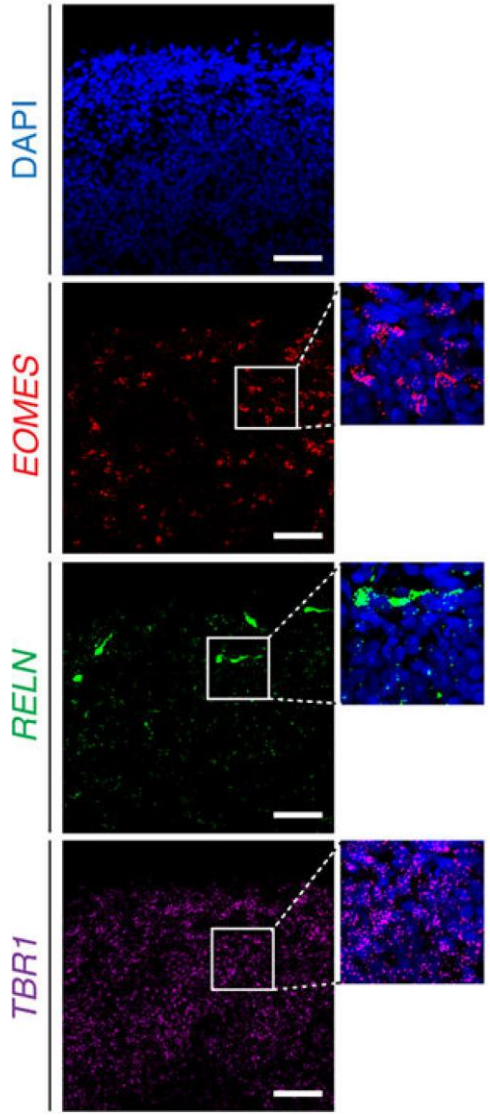
Renal organoids

Dilz et al., 2023



Brain organoids

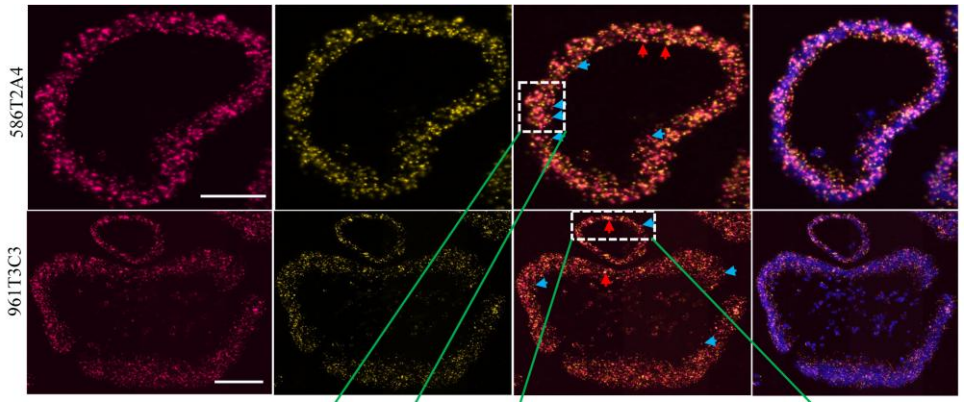
Velasco et al., 2019



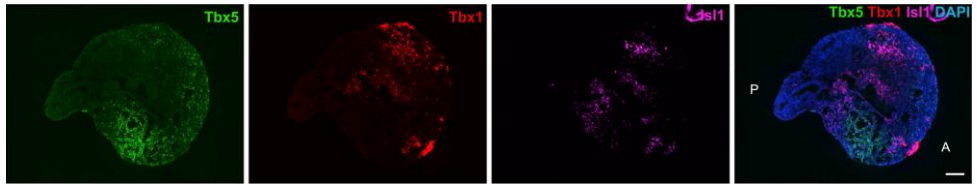
Intestinal tumor organoids

Song et al., 2023

Asxl1 mRNA mutant / Asxl1 mRNA WT

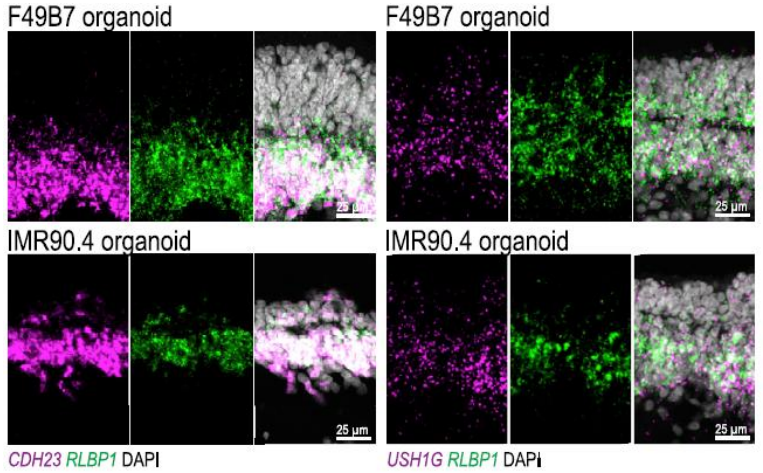


Gastruloids



Retinal organoïds

Cowen et al. *Cell*, 2020

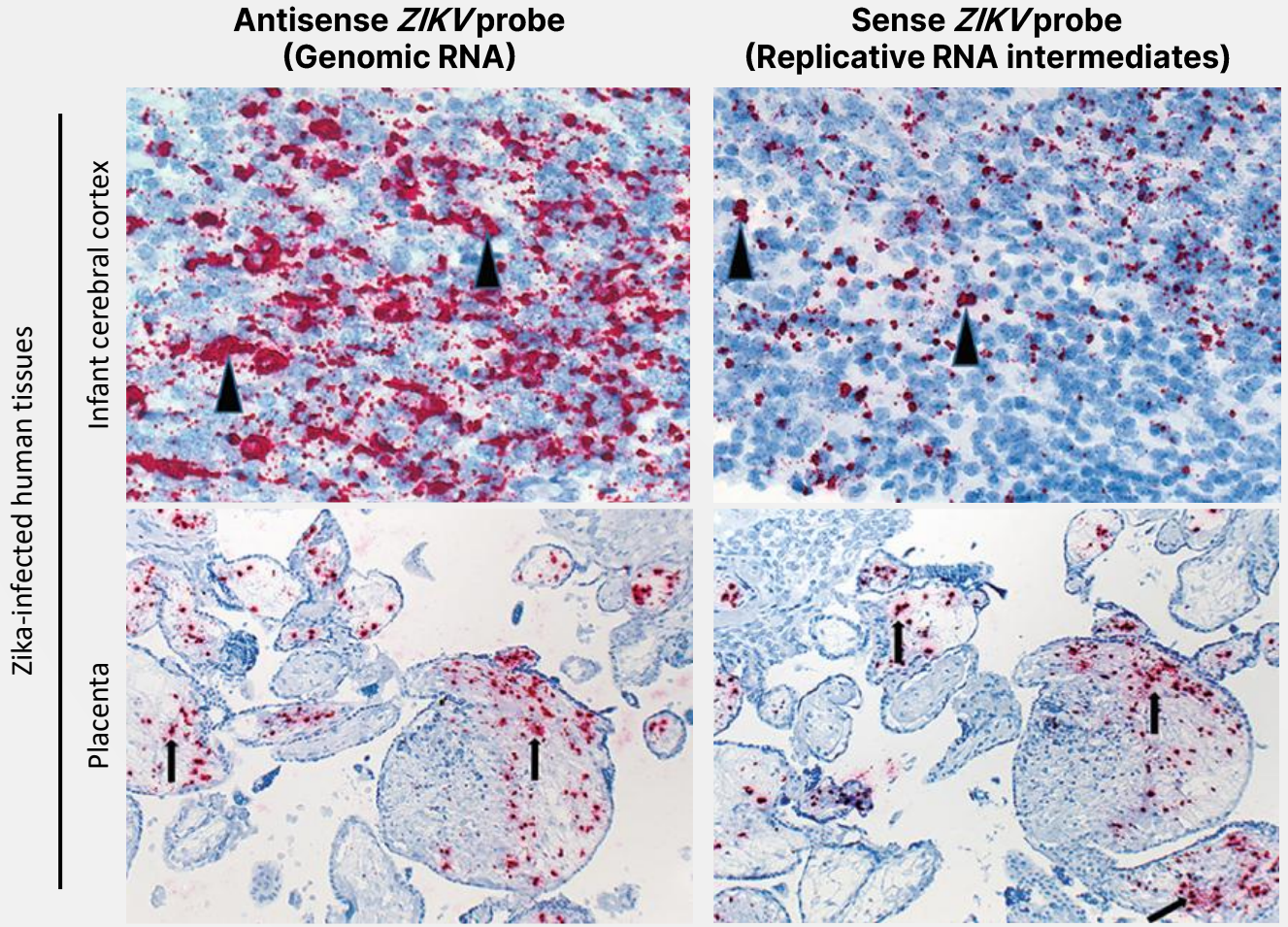


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Virus biodistribution, replicative intermediate and persistence

Limitation of Antibodies

- Zika virus is causally linked with congenital microcephaly and may be associated with pregnancy loss
- Mechanisms of Zika virus intrauterine transmission, replication, tropism, and persistence poorly understood
- RNAscope ISH localized replicative Zika virus RNA in brains of infants and placentas of women with pregnancy loss
- Demonstrate that Zika virus replicates and persists in fetal brains and placentas
    - Direct evidence of ZIKV association with microcephaly



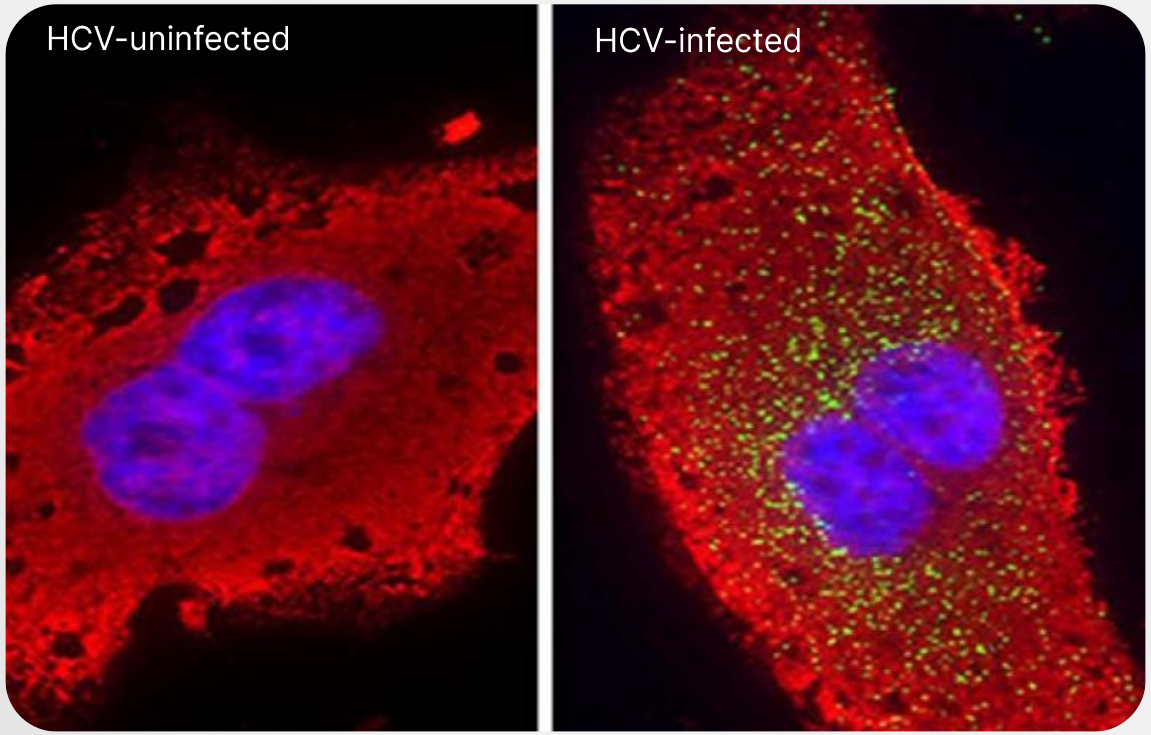
ZIKV RNA replication and persistence in human brain and placenta

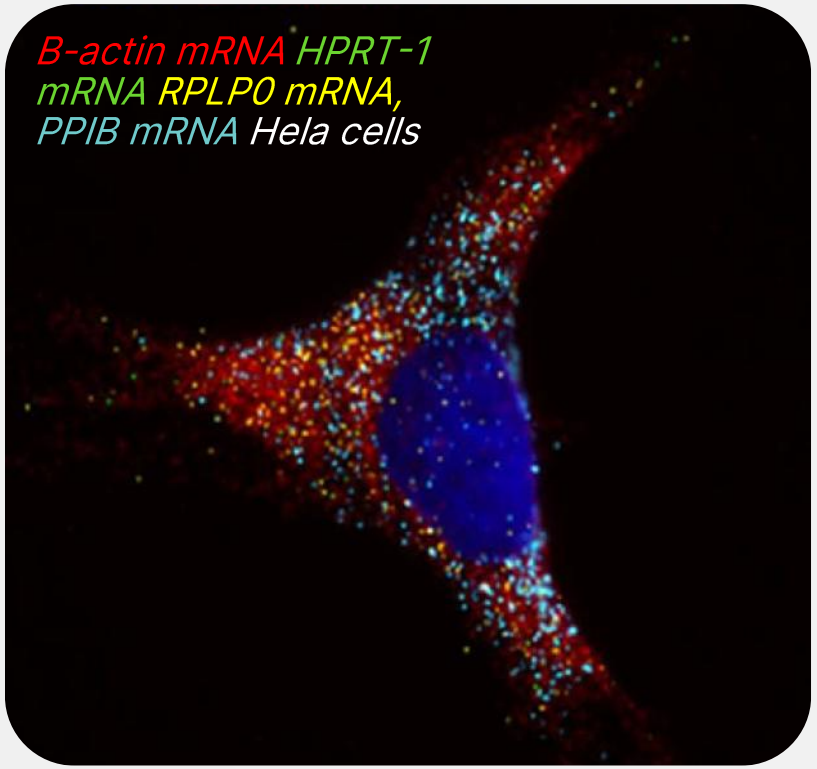
Bhatnagar J, *et al.* Emerg Infect Dis, 2017

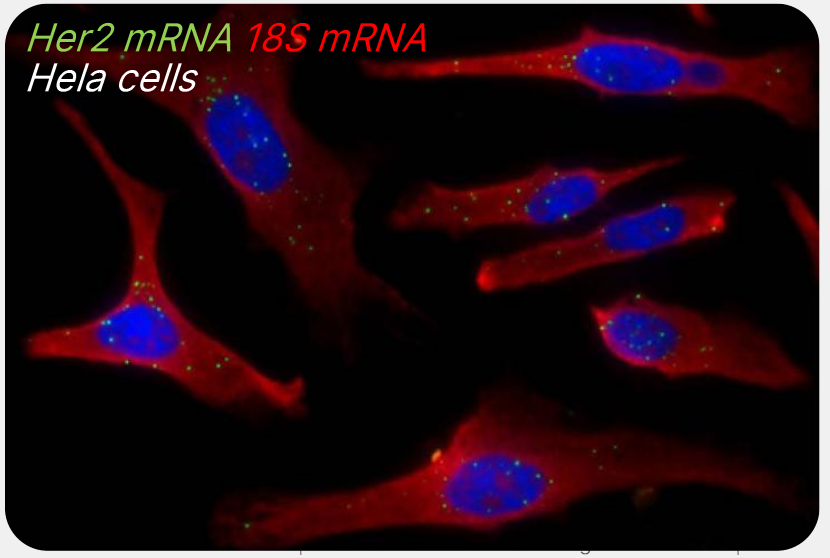
Limitation of Antibodies

Subcellular resolution

Multiplex Fluorescent V2 Assay







Limitation of Antibodies

Splice Variants Detection

BaseScope Single Plex Chromogenic Red Assay

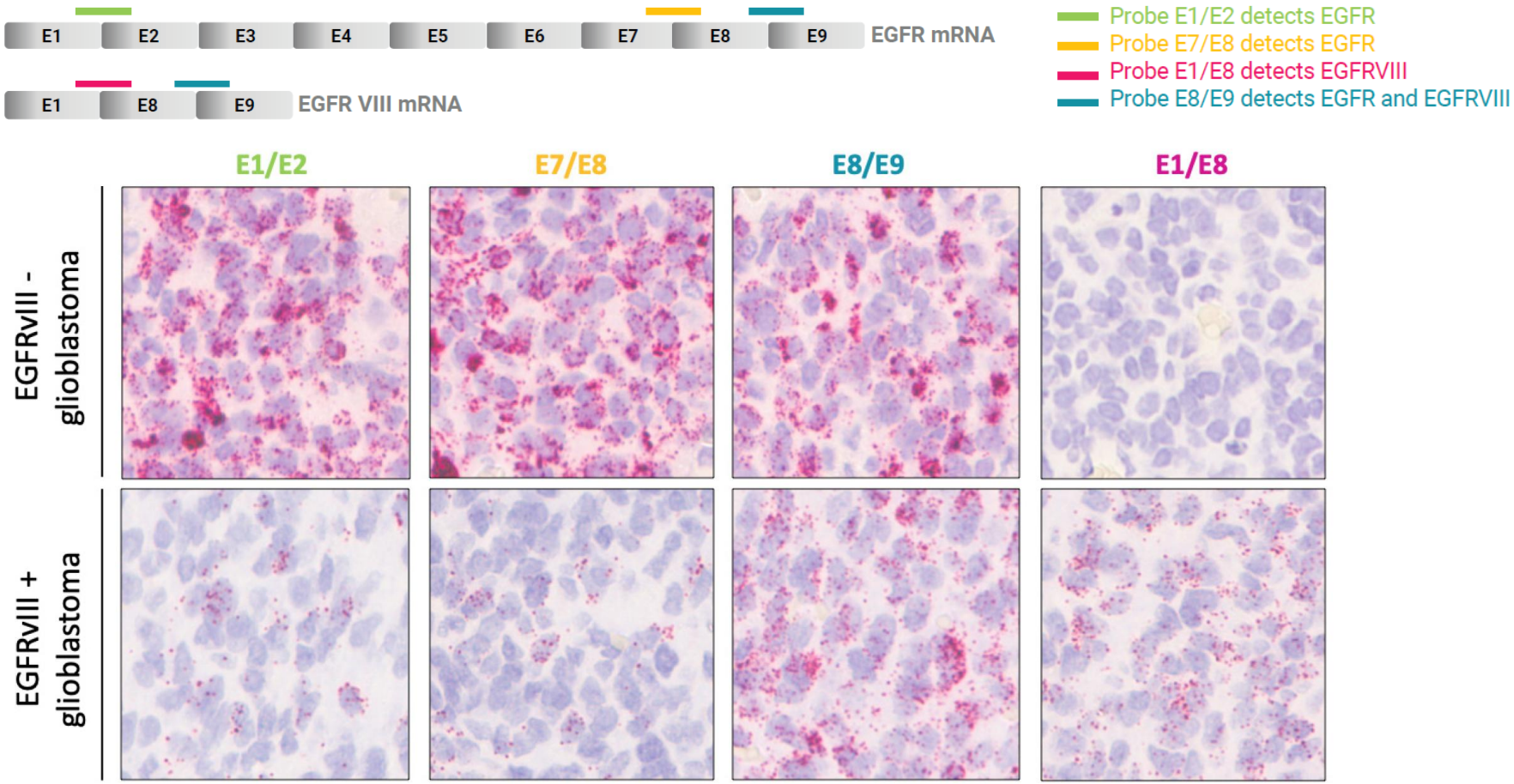
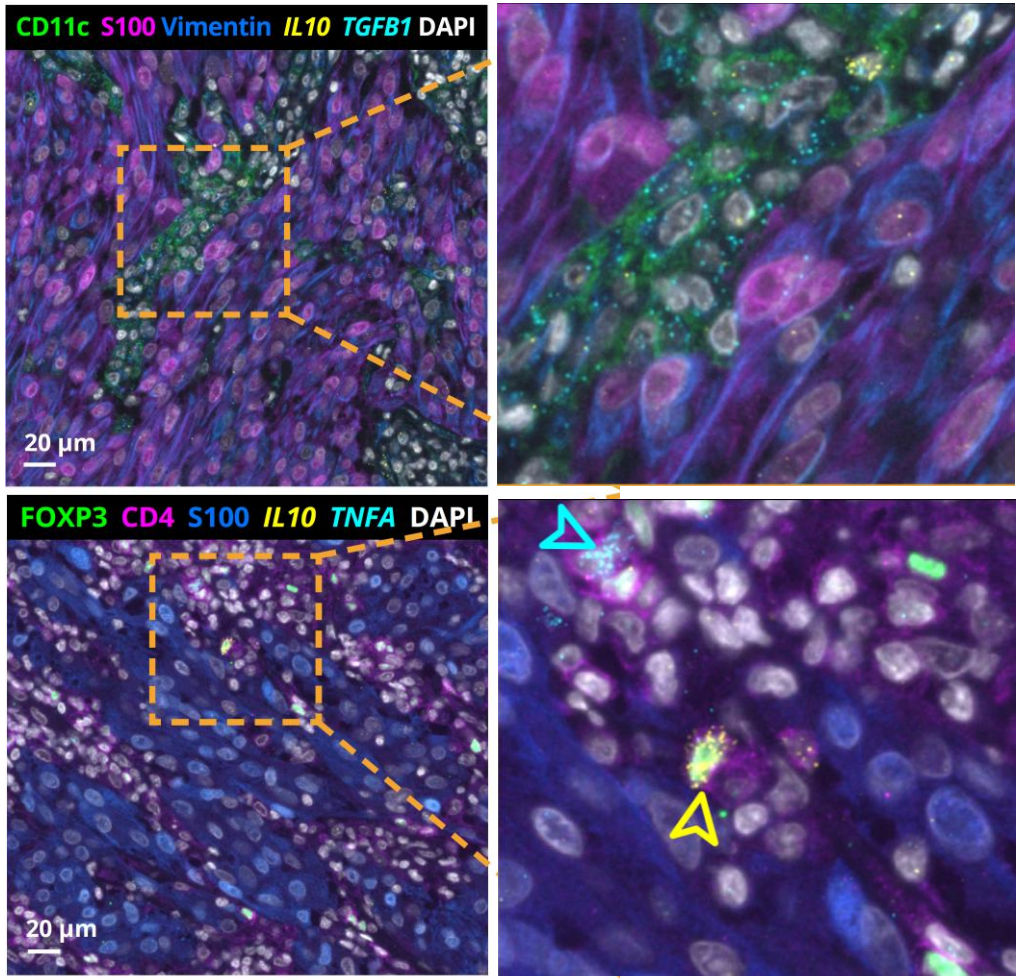


FIGURE 1 – Detection of *EGFR* and *EGFTvIII* mRNA using the BaseScope assay on a WHO grade IV glioblastoma case shows expression of *EGFR* and of the mutant variant *EGFRvIII.*

Evaluating inflammation by both measuring cell infiltration and cytokines expression within the tissue context

Cell phenotype/ function

Melanoma – Tumor micro environment profiling



RNAscope™ + Immunofluorescence enable to :

- **Identify the cellular origin of soluble factors at single cell level within the tissue context**
- **Characterize the inflammatory profile of any cell type** (pro/anti-inflammatory cytokines probes)

It bridges the gap with other existing methods (qPCR, RNA-seq, ELISA, Flow cytometry…) by providing results at single cell level with the tissue context

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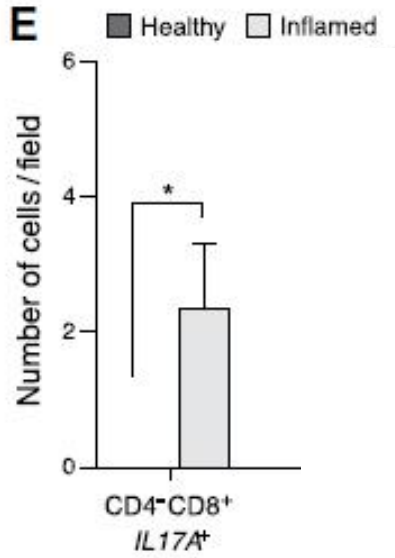
In situ validation of OMICS data

scRNA-seq validation

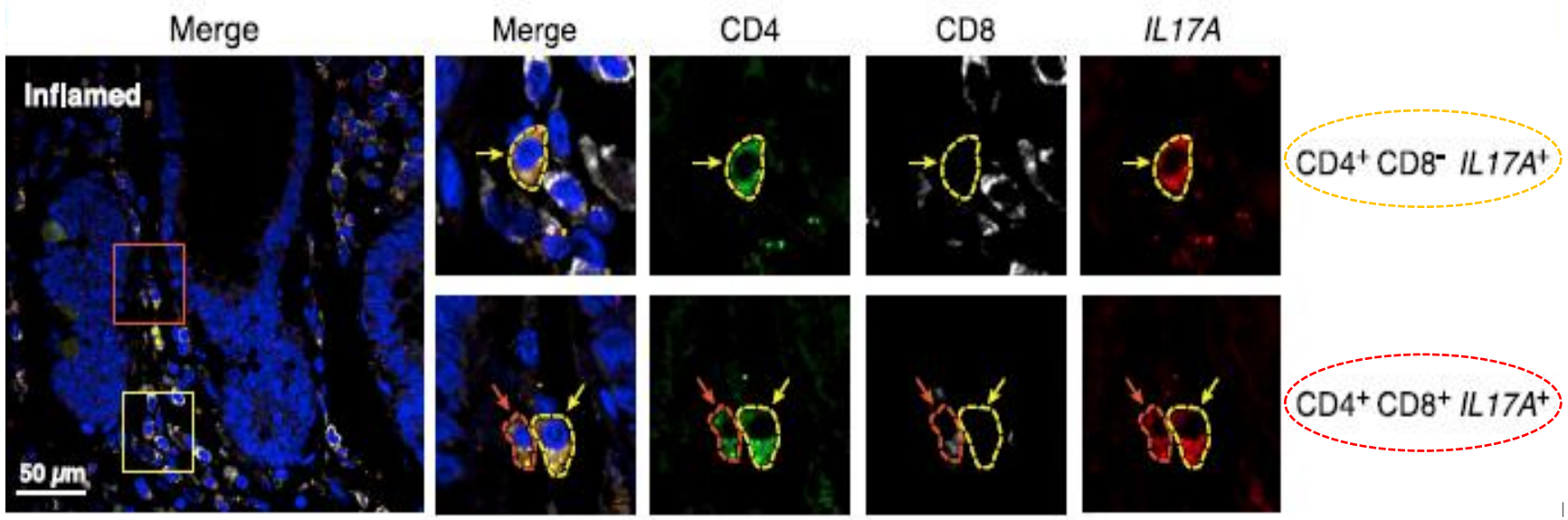
In situ transcriptomic data validation

Single cell analysis with 10X Genomics Chromium → RNAscope

- scRNA-seq performed on 366,650 cells from the colon mucosa of 18 UC patients and 12 healthy individuals
- Revealed 51 epithelial, stromal, and immune cell subsets
- **RNAscope ISH-IF co-detection** was used to map these epithelial, stromal and immune cells subsets.



Previously uncharacterized CD4+/CD8+/IL17A+ T cells were increased in inflamed tissues



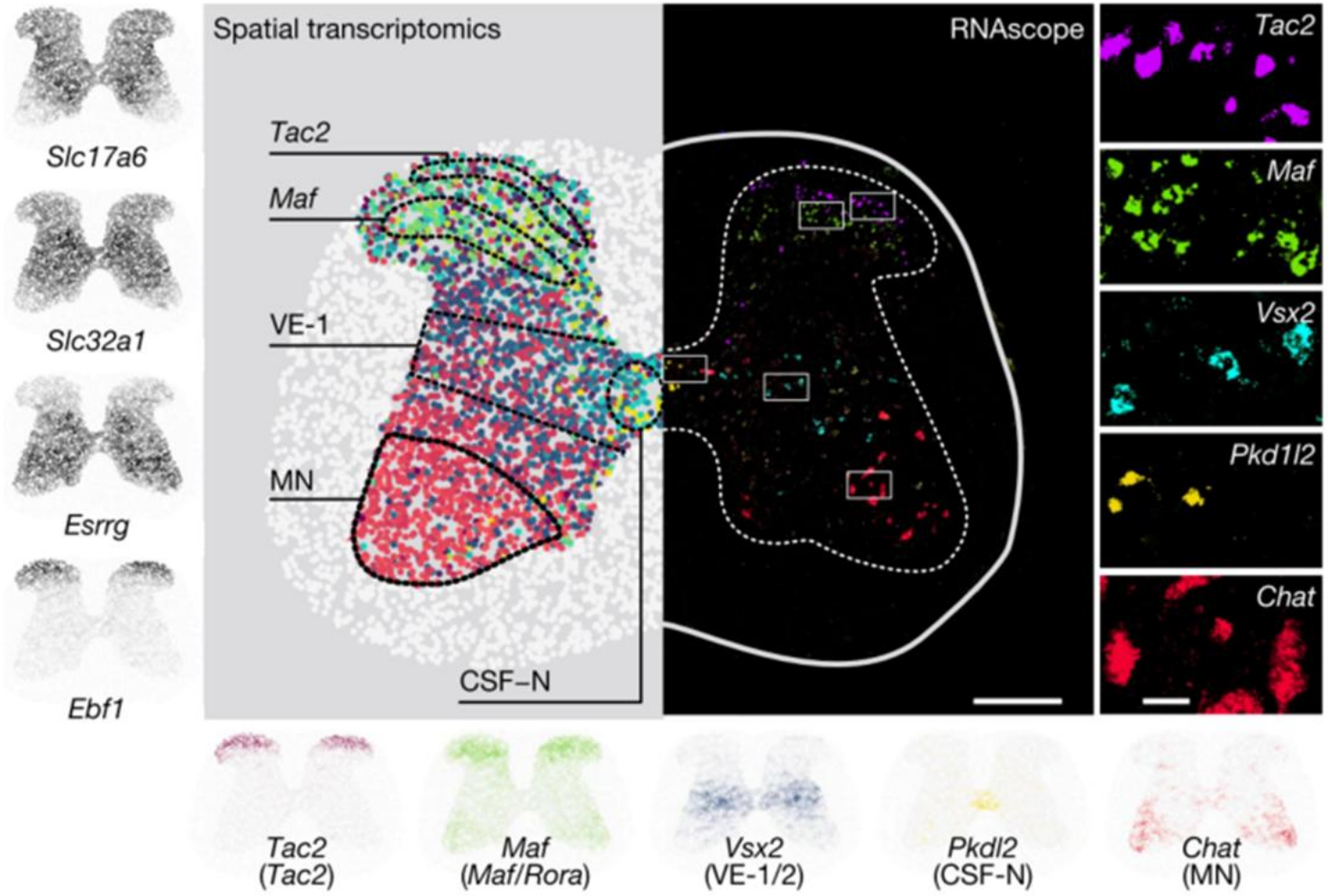
Similie et al., Cell, 2019

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In situ validation of OMICS data

Spatial transcriptomic validation

In situ **transcriptomic data validation**



EPFL

Gregoire Courtine

Sources: Kathe et al. 2022 Nature

RNAscope confirms the spatial location of five neuronal subpopulation

Evaluate therapeutic biodistribution, safety and efficacy

CAR-T Therapy in PDX Preclinical Model

Therapeutic treatments

ACD a bio-techne brand

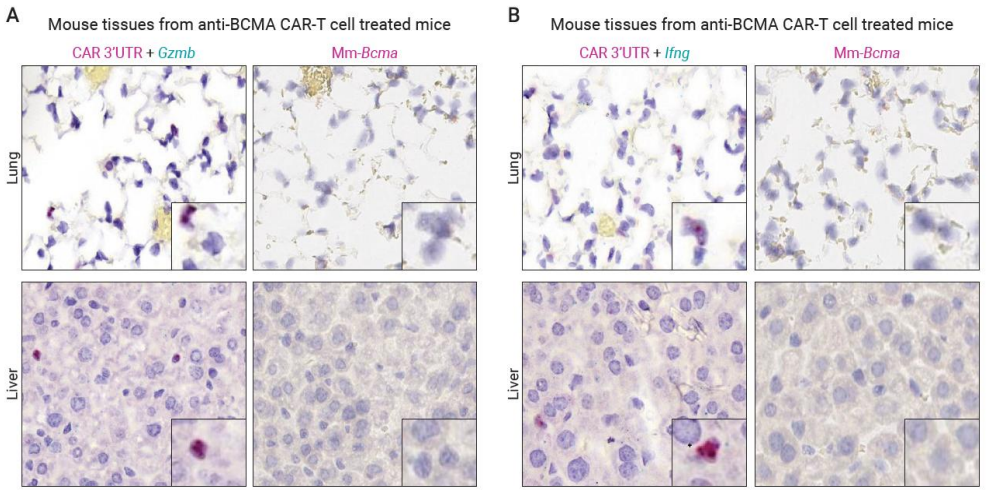
PRECLINICAL CAR-T CELL TARGET SAFETY, BIODISTRIBUTION, AND TUMOR INFILTRATION ANALYSIS USING THE RNASCOPE® ISH ASSAY

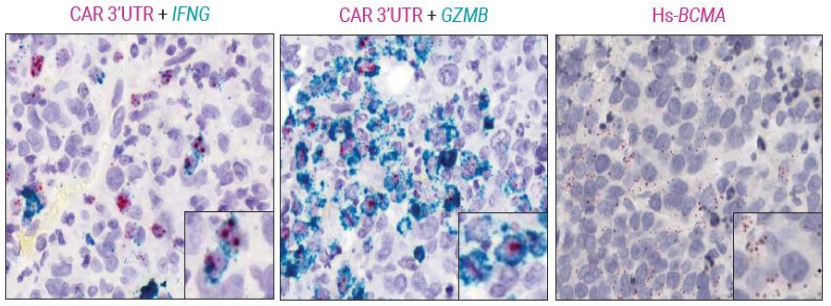
APPLICATION NOTE

Helly Pimentel, Helen Jarnagin, Hailing Zong, Courtney Todorov, Courtney Anderson, Bingqing Zhang, Christopher Bunker, Xiao-Jun Ma
Advanced Cell Diagnostics Inc., 7707 Gateway Blvd, Newark, CA, USA 94560

RNAscope duplex chromogenic assay







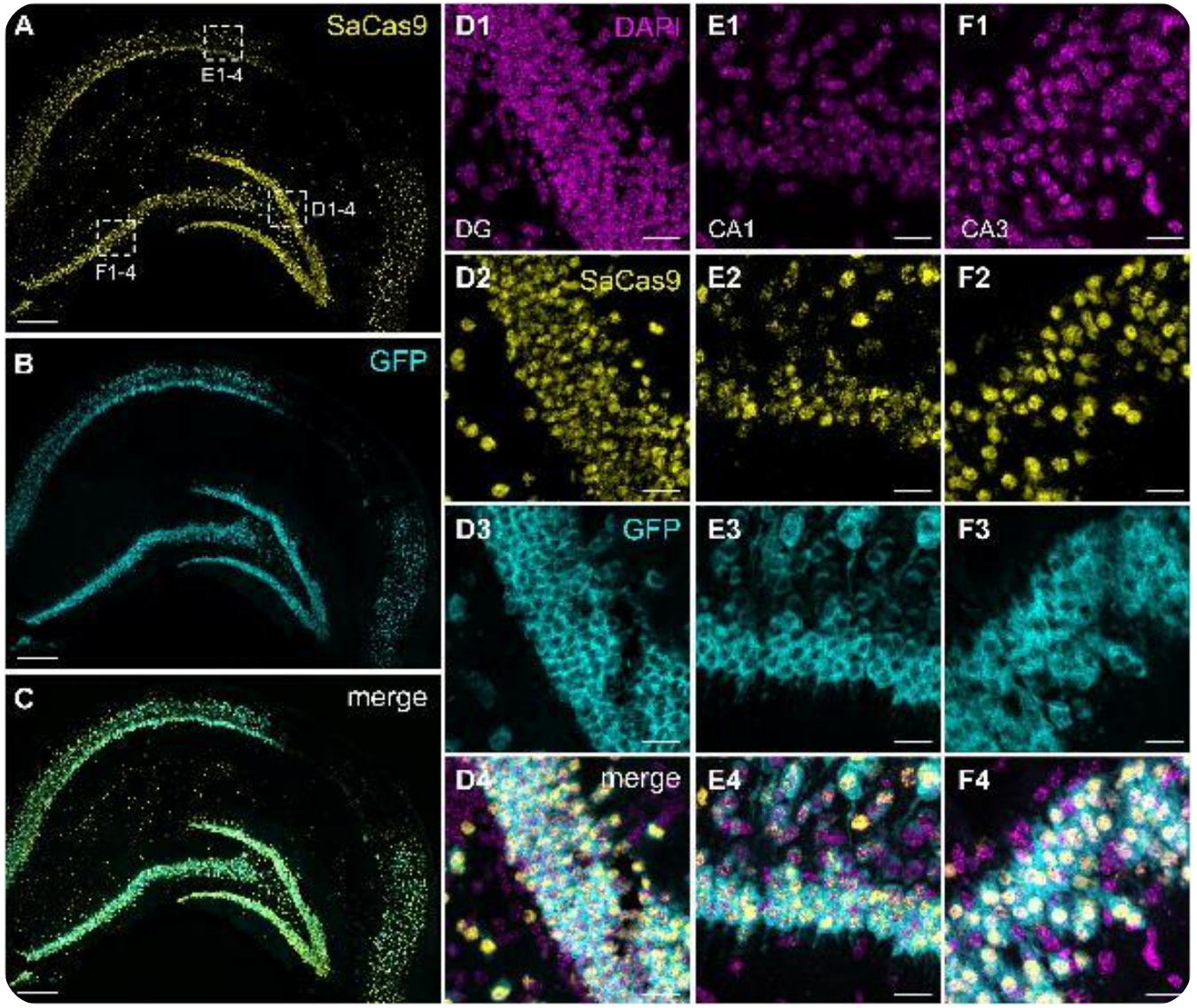
BCMA was only expressed in the BCMA+ xenografted tumor
Anti-BCMA CAR-T cells were active only in the tumor

RNAscope to visualize transgen expression following AAV gene therapy in hippocampus neurons

Therapeutic treatments

Efficacy of the dual AAV viral vector system

RNAscope was used to detect SaCas9 and GFP expression suggesting the AAV viral vectors successfully transduced target neurons in the hippocampus



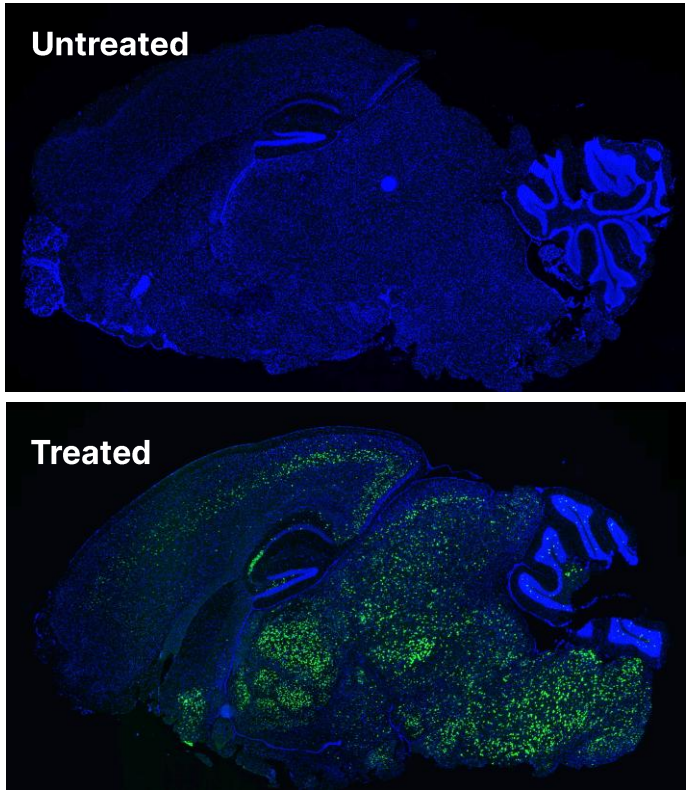
Maria et al., *Front. Mol. Neurosci. , 2020*

Biodistribution, tropism & efficacy

AAV-delivered miRNA in mouse brain

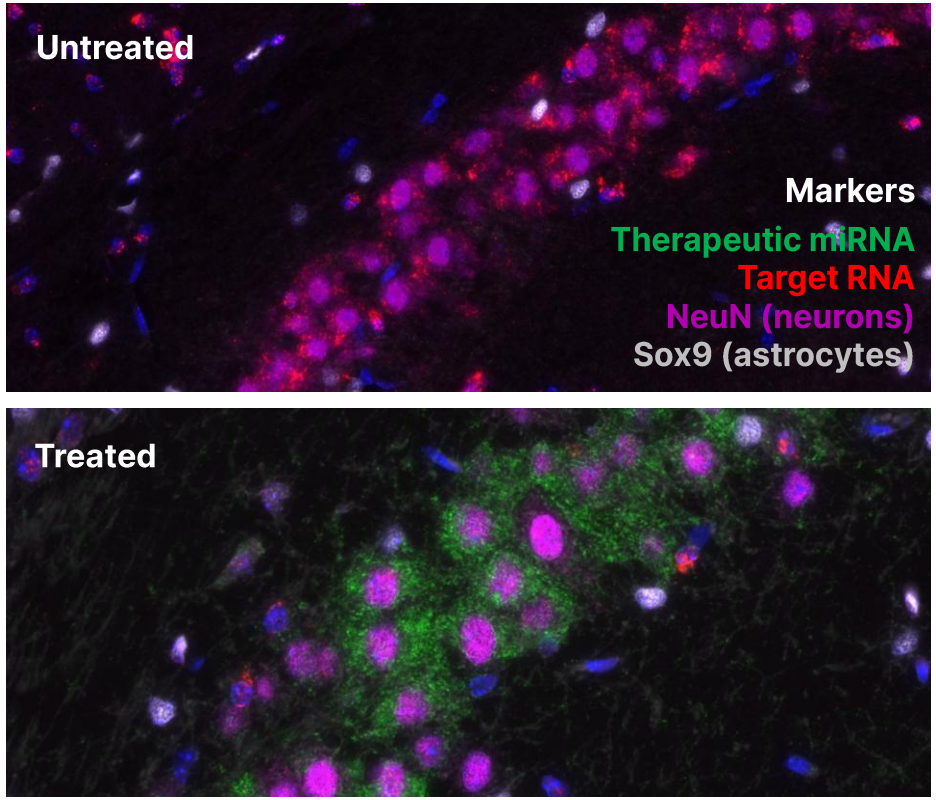
Therapeutic treatments

Whole brain section



Therapeutic miRNA delivered IV by AAV in mouse brain - Biodistribution study

Hippocampus

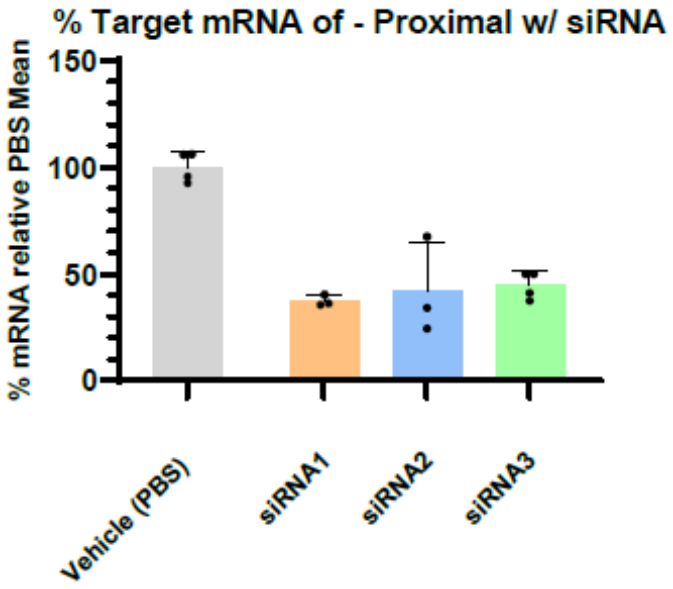


Delivery, tropism & knockdown measured on one slide with RNAscope + IF

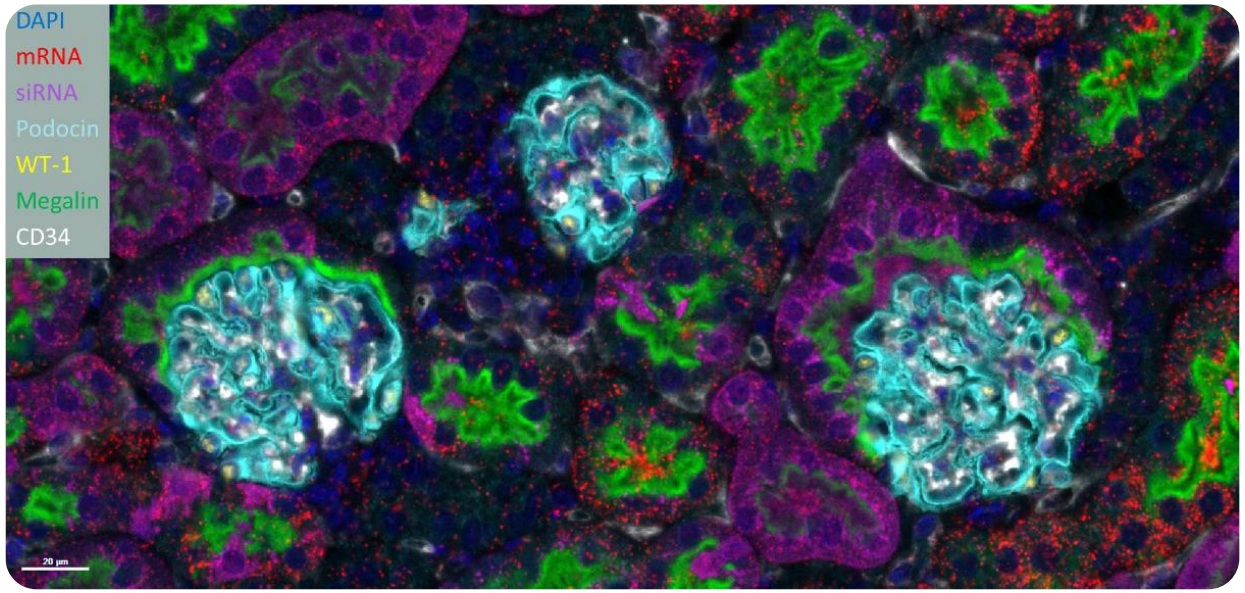
siRNA biodistribution and efficacy on mouse kidney sections

RNAscope™ Plus smRNA-RNA Assay + seqIF

Therapeutic treatments



1 siRNA +1 mRNA +4 Proteins



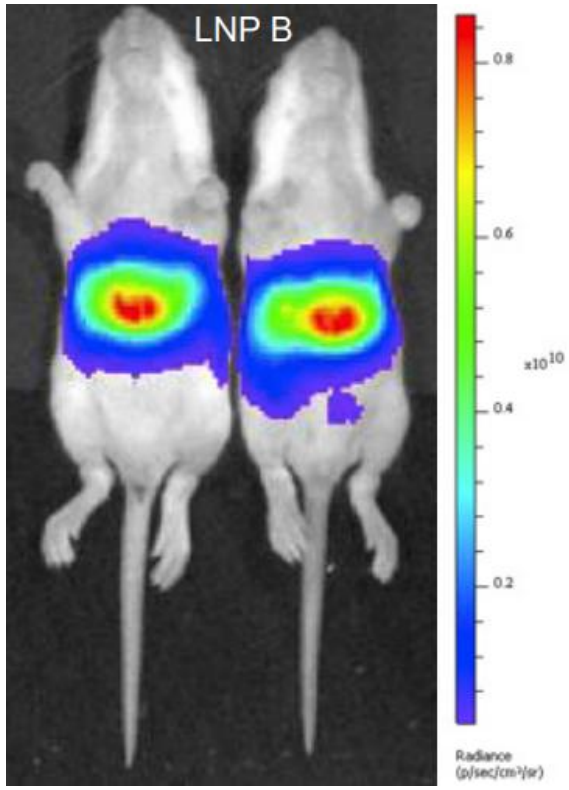
Characterize siRNA biodistribution and efficacy | Mouse Kidney

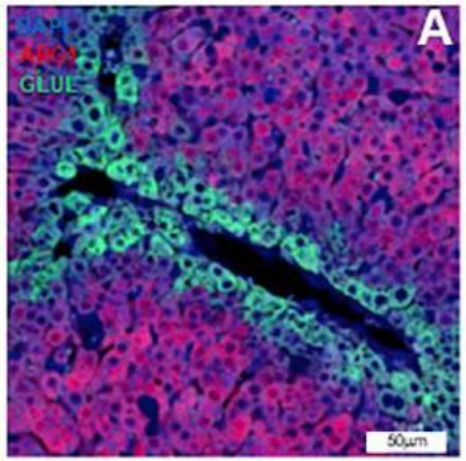
siRNA (purple) and targeted mRNA (red) Podocytes (Podocin, cyan; WT-1, yellow) Proximal tubules (Megalin, green) Endothelial cells (CD34, white)

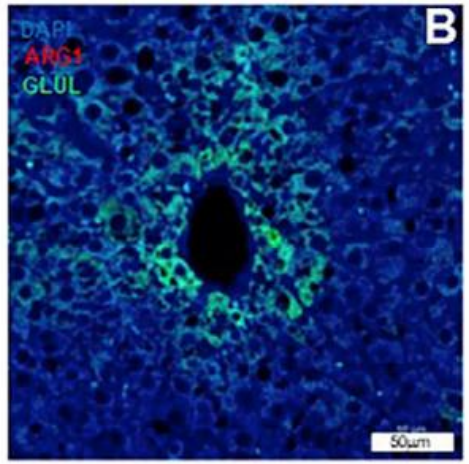
LNP-RNA vaccine – in situ biodistribution at single cell level and knock-out rescue

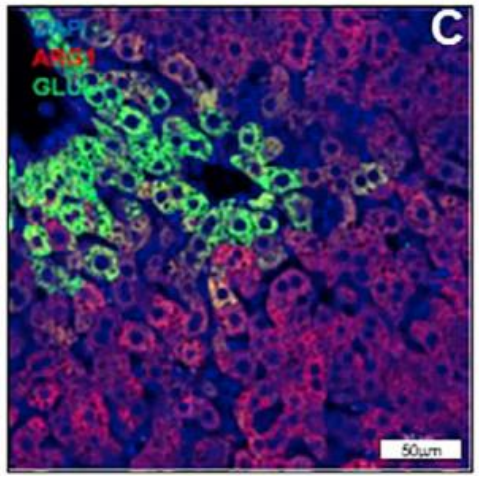
Therapeutic treatments

Arg1-/- mice injected i.p. with Arg1 mRNA loaded LNPs mRNA

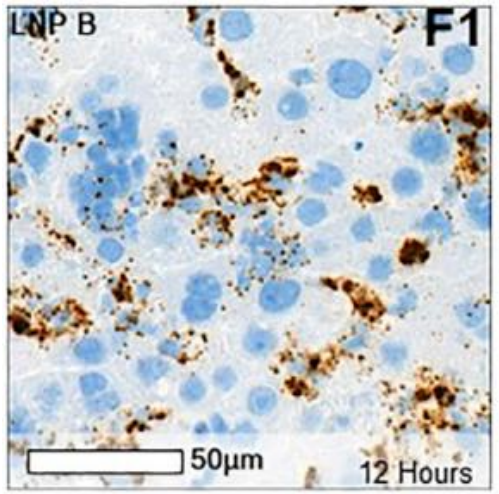


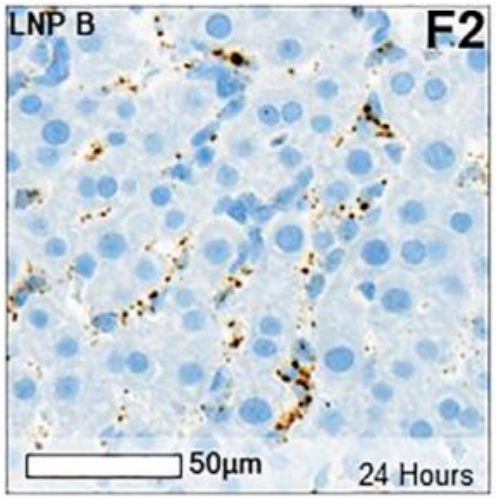






RNAscope



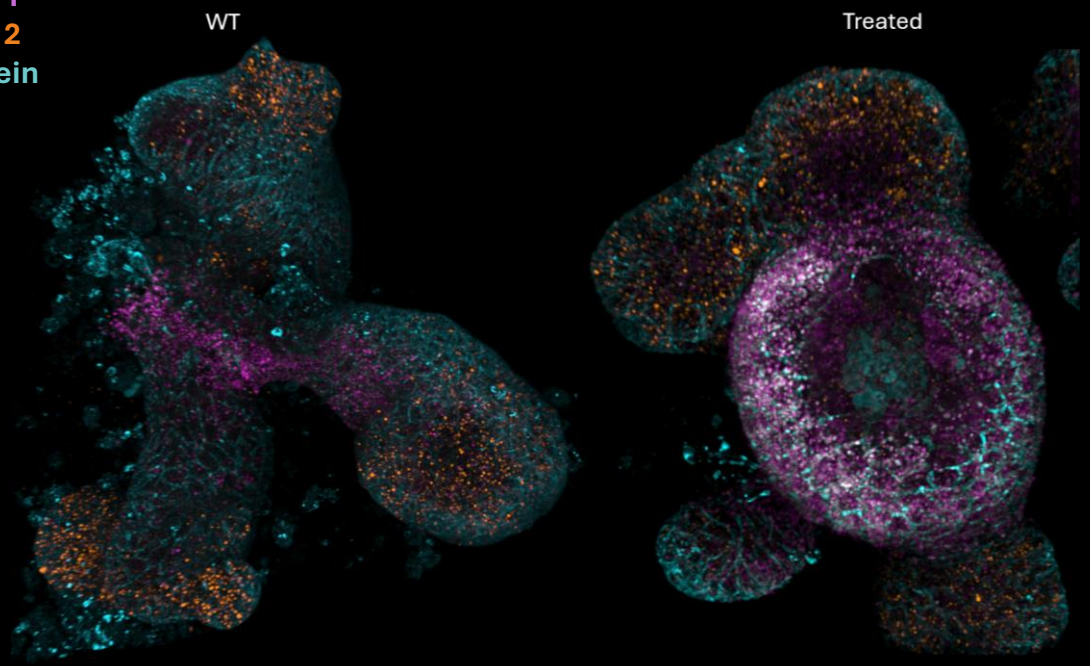


Khoja et al., 2022

3D multiomics – RNAscope + IF

intestinal organoïds

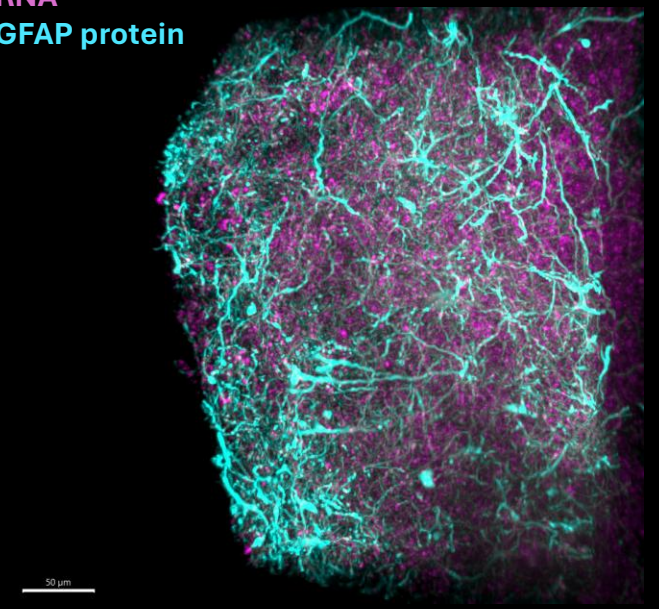
RNA 1
RNA 2
Protein



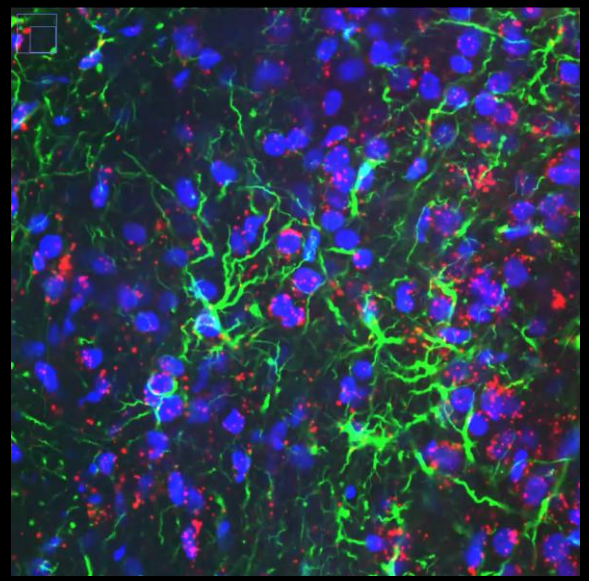
© Giulia Nigro – Institut Pasteur
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midbrains organoïds

RNA
GFAP protein



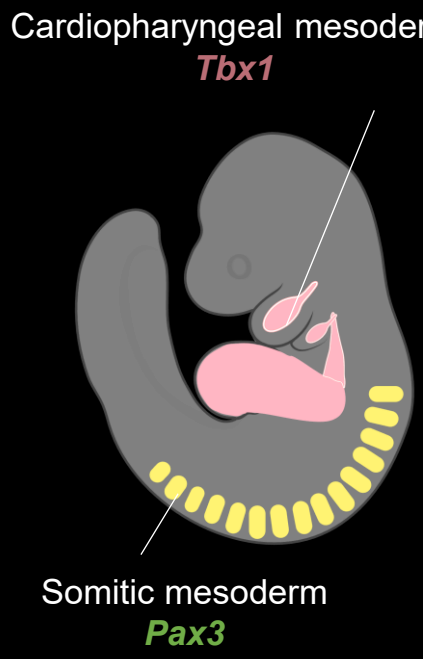
RNA
GFAP protein

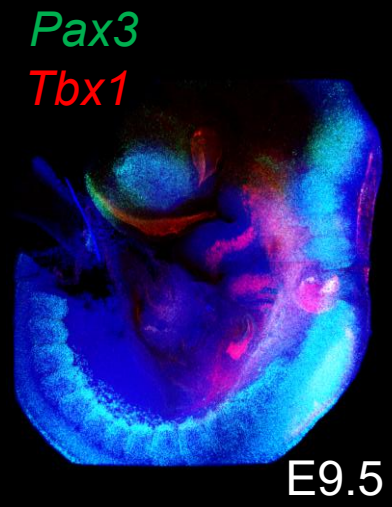


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© Annick Prigent – Paris Brain Institute

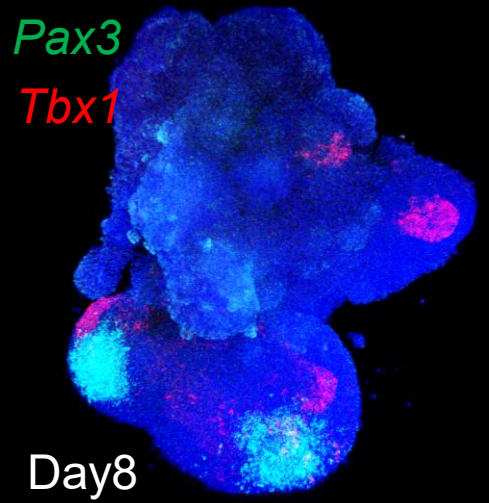
HISTOMICS histocore lab

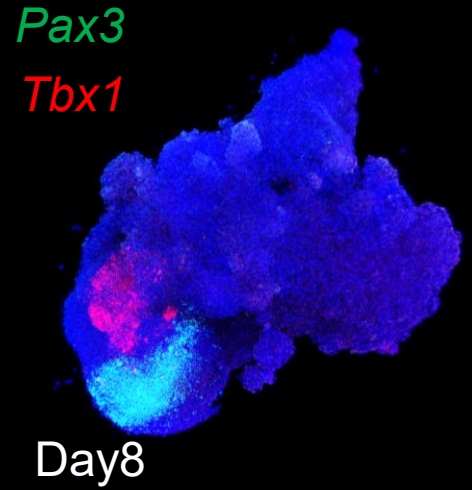
mouse embryo





mouse gastruloids





© Caroline Choquet, Fabienne Lescroart, U1251 MMG
Argiro et al. Nature Com 2024; De Bono Methods Mol Biol. 2025

ProximityScope

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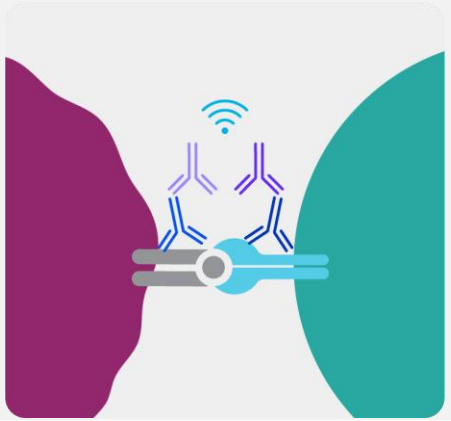
bio-techne

ProximityScope provides key biological insights by enabling visualization of diverse protein interactions

Interactions can be classified as:

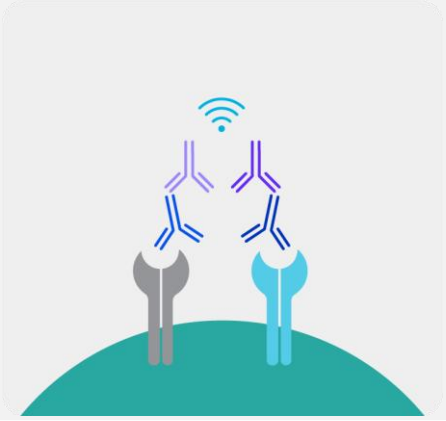
Cell-cell interactions

Interactions between surface proteins from different cells.



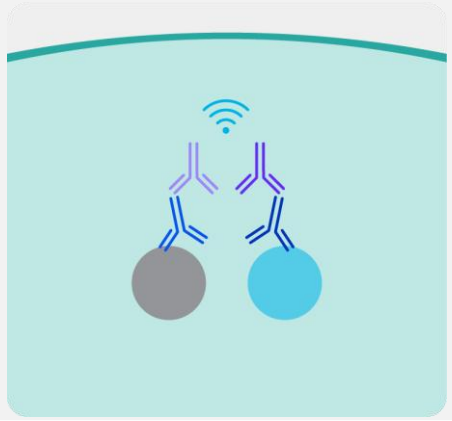
Cell surface interactions

Interactions/proximity between surface proteins on the same cell.



Intracellular interactions

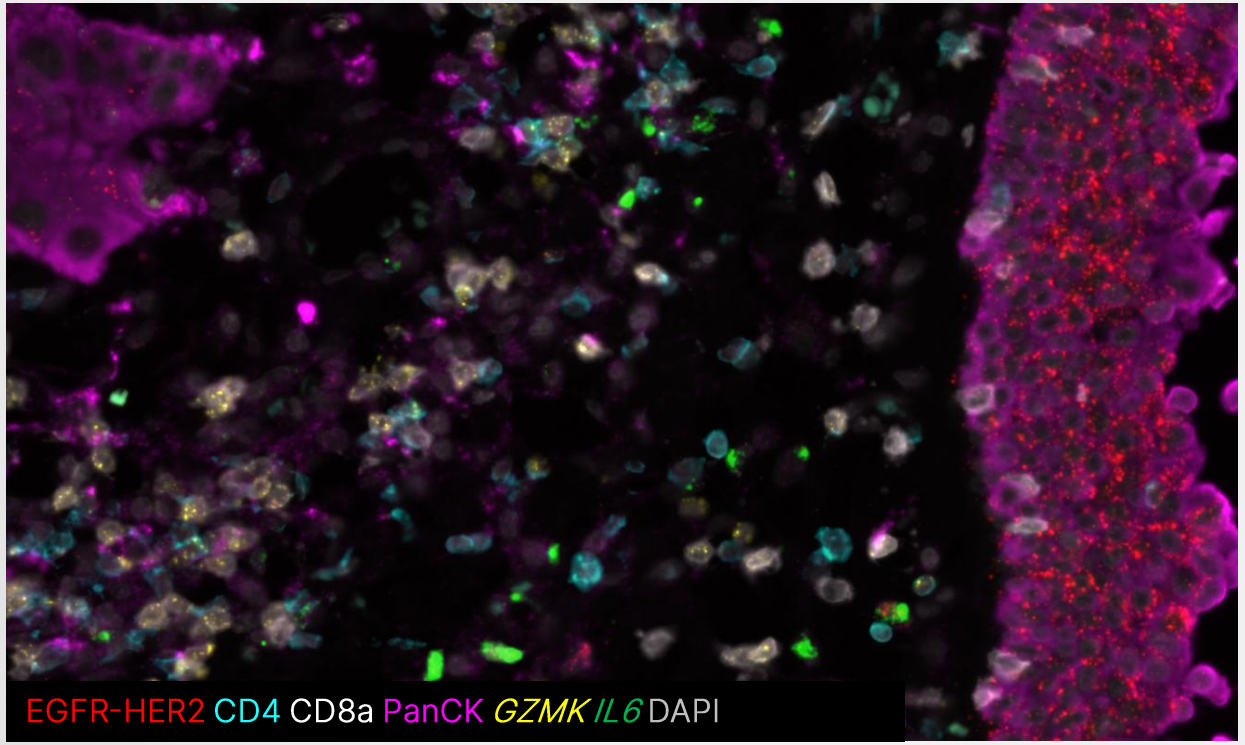
Interactions between protein within cellular complexes or signaling cascades.



ProximityScope provides key biological insights

Cell Surface Interactions

EGFR-HER2 heterodimerization on tumor cells indicated by PanCK



Use Case	**Detect signaling pathway activation through receptor dimerization.**
Examples	EGFR-HER2
Applications	Patient Stratification; Biological Insights

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Conclusions

Immunofluorescence is a technique that sometimes has limitations : RNAscope provides complentary data

- Not always a specific Ab available for the target
- Identify cells subsets in situ based on their gene expression
- Caracterize immune cell inflammatory profile at single cell level
- Therapeutic treatment assessment (biodistribution, uptake/tropism, efficacy, safety)

RNA and proteins of interest can be simultanesoulsy visualized on tissue / organoids cross-sections with the protease free workflow :

- With RNAscope manual assays followed by IF (experiment done by your own) – up to 4 plex
- On the Bond RX automate from Leica (available at ICM, CRC and more) - up to 6 plex
- On the Ventana Discovery Ultra from Roche (available at Micropicell) – up to 3 plex
- On the COMET from Bio-Techne Spatial (available at micropicell) - up to 12 RNA + X proteins

RNA and proteins of interest can be visualized on 3D samples (manual RNAscope/IF)

Learn from your peers' experiences

- Annick Prigent, Sonita Ing (ICM, Paris) : Service project on HISTOMICS platform
- Martine Cohen-Salmon : 200µm brain cross-sections (CIRB, Collège de France)

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spatial™

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