Spatial isoform Transcriptomics (SiT)

Kévin Lebrigand
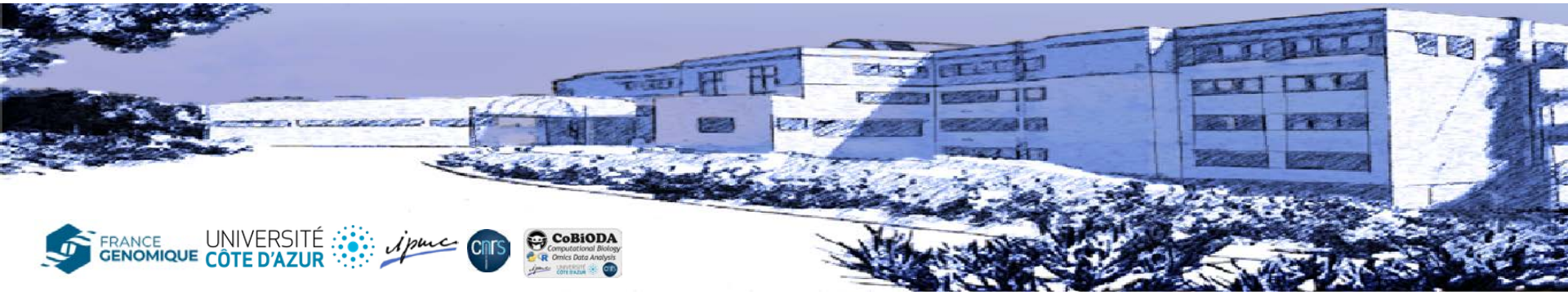Computational Biology and Omics Data Analysis
https://cobioda.github.io
IPMC, CNRS, Côte d'Azur University, France
lebrigand@ipmc.cnrs.fr
@kevinlebrigand

Institute of Molecular and Cellular Pharmacology

Sophia-Antipolis

20 research teams composed of > 220 members

- Ion channels (pain, perception, epilepsy)
- Molecular signaling (molecular trafficking, lipidomics)
- Neurodegenerative disorders (Alzheimer, Parkinson)
- Neuropsychiatric disorders (nervous breakdown, mental retardation)
- Functional genomics and bioinformatics

15 Engineers running 5 technological platforms

- MICA, Imaging and Flow Cytometry
- CAPABIO, Proteomics and Metabolomic
- ANIPRO, animal care and behavior facility
- CoBiODA, BioinformaticsHub
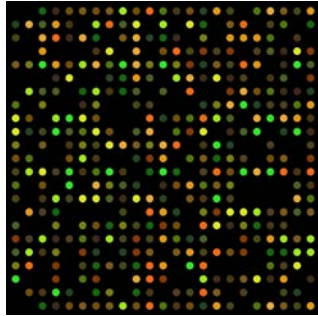- UniCA GenomiX, Functional Genomics platform

Core member of the "France Génomique" network (2008, 60M€) > 500 academics

| 2015 | 2016 | 2018 | 2022 | 2024 |

20 years of transcriptomics

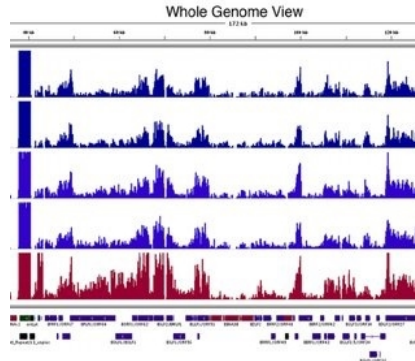Driven by microfluidics technological developments

Early 2000's: DNA microarray

- Large-scale transcriptome
- Oligonucleotide probe tilling
- Fluorochrome signal analysis
- Bulk resolution

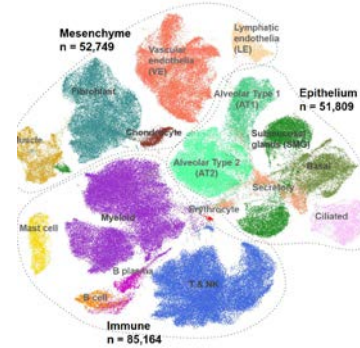Cost : 4k€
20 samples
25k genes
0,5M matrix

Late 2000's : RNA sequencing

- Whole transcriptome
- Next Generation Sequencing
- Full-transcript coverage
- Bulk resolution

Cost : 4k€
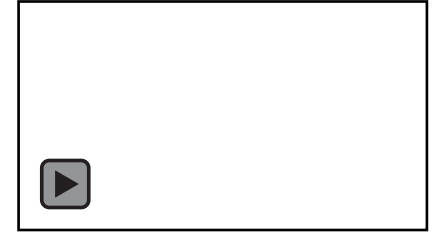20 samples
50k genes
1M matrix

Mid 2010's: Single-cell

- Whole transcriptome
- Microfluidics + NGS
- 3p-end gene signal (UMI)
- Sensitivity (6%)
- Single-cell / state resolution
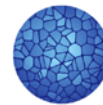
Cost : 4k€
5k cells
50k genes
250M matrix

2020's : Spatial

- 300-1000 gene targets
- Imaging analysis
- Multiplexing FiSH (single molecule)
- Sensitivity (30-80%)
- Sub-cellular resolution

Cost : 4k€
250k cells
1k genes
250M matrix
+ Spatial dimension

Human Cell Atlas

Our contribution

HUMAN CELL ATLAS

2019

TECHNIQUES AND RESOURCES | 23 OCTOBER 2019

Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures 🔓

In collection: Human development

Sandra Ruiz García, Marie Deprez, Kevin Lebrigand, Amélie Cavard, Agnès Paquet, Marie-Jeanne Arguel, Virginie Magnone, Marin Truchi, Ignacio Caballero, Sylvie Leroy, Charles-Hugo Marquette, Brice Marcet, Pascal Barbry ✉ , Laure-Emmanuelle Zaragosi ✉

+ Author and article information

500k
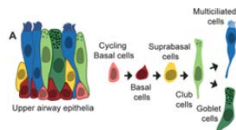
2019

Home > American Journal of Respiratory and Critical Care Medicine > List of Issues > Volume 202, Issue 12

A Single-Cell Atlas of the Human Healthy Airways

Marie Deprez [1*], Laure-Emmanuelle Zaragosi [1*], Marin Truchi [1], Christophe Becavin [1], Sandra Ruiz García [1], Marie-Jeanne Arguel [1], Magali Plaisant [1], Virginie Magnone [1], Kevin Lebrigand [1], Sophie Abelanet [1], Frédéric Brau [1], Agnès Paquet [1], Dana Pe'er [2], Charles-Hugo Marquette [3], Sylvie Leroy [1,3†], and Pascal Barbry [1] ...Show less

+ Author Affiliations

📈 ⬇ 21 125 💬 215

80k

2021

Analysis | Published: 02 March 2021

Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics

Christoph Muus ✉, Malte D. Luecken ✉, Gökcen Eraslan, Lisa Sikkema, Avinash Waghray, Graham Heimberg, Yoshihiko Kobayashi, Eeshit Dhaval Vaishnav, Ayshwarya Subramanian, Christopher Smillie, Karthik A. Jagadeesh, Elizabeth Thu Duong, Evgenij Fiskin, Elena Torlai Triglia, Meshal Ansari, Peiwen Cai, Brian Lin, Justin Buchanan, Sijia Chen, Jian Shu, Adam L. Haber, Hattie Chung, Daniel T. Montoro, Taylor Adams, The NHLBI LungMap Consortium & The Human Cell Atlas Lung Biological Network

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2021

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nature > perspectives > article

Perspective | Published: 08 September 2021

A roadmap for the Human Developmental Cell Atlas

Muzlifah Haniffa ✉, Deanne Taylor, Sten Linnarsson, Bruce J. Aronow, Gary D. Bader, Roger A. Barker, Pablo G. Camara, J. Gray Camp, Alain Chédotal, Andrew Copp, Heather C. Etchevers, Paolo Giacobini, Berthold Göttgens, Guoji Guo, Ania Hupalowska, Kylie R. James, Emily Kirby, Arnold Kriegstein, Joakim Lundenberg, John C. Marioni, Kerstin B. Meyer, Kathy K. Niakan, Mats Nilsson, Bayanne Olabi, Human Cell Atlas Developmental Biological Network + Show authors

65k Accesses 87 Citations 324 Altmetric | Metrics

2022

The discovAIR project: a roadmap towards the Human Lung Cell Atlas

Malte D. Luecken [1,26], Laure-Emmanuelle Zaragosi [2,26], Elo Madissoon [3,4,26], Lisa Sikkema [1,26], Alexandra B. Firsova [5,26], Elena De Domenico [6,26], Louis Kümmerle [1,26], Adem Saglam [6,26], Marijn Berg [7,8,26], Aurore C.A. Gay [7,8,26], Janine Schniering [9,26], Christoph H. Mayr [9,26], Xesús M. Abalo [10,26], Ludvig Larsson [10,26], Alexandros Sountoulidis [5,26], Sarah A. Teichmann [3,11], Karen van Eunen [12,13], Gerard H. Koppelman [8,12], Kourosh Saeb-Parsy [14], Sylvie Leroy [15], Pippa Powell [16], Ugis Sarkans [3], Wim Timens [7,8], Joakim Lundeberg [17], Maarten van den Berge [8,18], Mats Nilsson [10], Peter Horváth [19], Jessica Denning [16], Irene Papatheodorou [4], Joachim L. Schultze [6,20,21], Herbert B. Schiller [9], Pascal Barbry [2], Ilya Petoukhov [22], Alexander V. Misharin [23], Ian M. Adcock [24], Michael von Papen [25], Fabian J. Theis [1], Christos Samakovlis [5], Kerstin B. Meyer [3] and Martijn C. Nawijn [7,8]

2023

nature medicine

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nature > nature medicine > resources > article

Resource | Open access | Published: 08 June 2023

An integrated cell atlas of the lung in health and disease

Lisa Sikkema, Ciro Ramírez-Suástegui, Daniel C. Strobl, Tessa E. Gillett, Luke Zappia, Elo Madissoon, Nikolay S. Markov, Laure-Emmanuelle Zaragosi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Apperloo, Martin Banchero, Christophe Bécavin, Marijn Berg, Evgeny Chichelnitskiy, Mei-i Chung, Antoine Collin, Aurore C. A. Gay, Janine Gote-Schniering, Baharak Hooshiar Kashani, Kemal Inecik, Manu Jain, Theodore S. Kapellos, Lung Biological Network Consortium, ... Fabian J. Theis ✉ + Show authors

72k Accesses 59 Citations 379 Altmetric | Metrics

2,4M

2020

High throughput error corrected Nanopore single cell transcriptome sequencing

Kevin Lebrigand ✉, Virginie Magnone, Pascal Barbry ✉ & Rainer Waldmann ✉

36k Accesses 83 Citations 67 Altmetric | Metrics

10X GENOMICS

NANOPORE technologies

2023

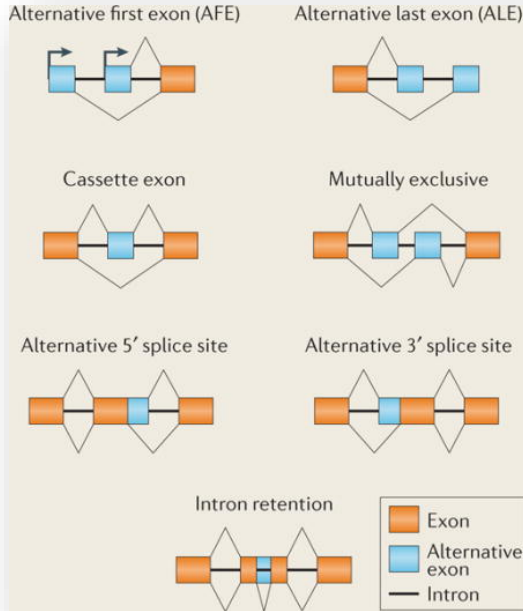## The spatial landscape of gene expression isoforms in tissue sections 🔓

Kevin Lebrigand, Joseph Bergenstråhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbry ✉, Rainer Waldmann, Joakim Lundeberg
Author Notes

10X GENOMICS

Transcriptomics

Complex outcomes of alternative splicing

Scotti and Swanson, Nat Rev Genet., 2016

- **90%** of the genes are subjected to alternative splicing,
- Gencode v42 : **252,416** distinct isoforms for **62,696** genes,
- On average, a human gene contains **8.8 exons**, mean size of **145 nt**,
- Average encodes mRNA **2,410 nt long** :

5' UTR	Coding sequence	3' UTR
770nt	1,340nt	300nt

Alternative splicing and disease
Tazi et al, 2008
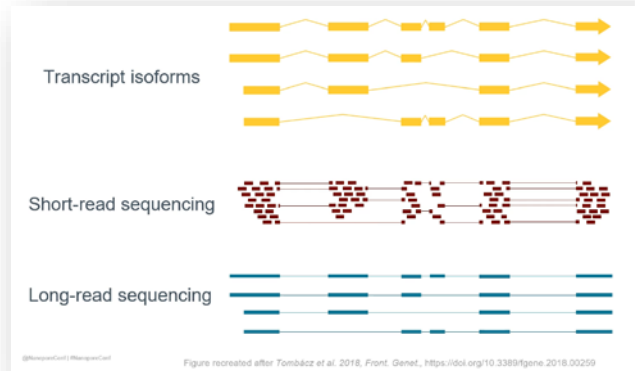
Nature Method of the Year 2022

Inference required

Direct full exonic layout
One read is one molecule

Single-cell long-read transcriptomics
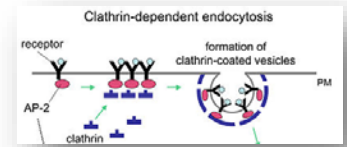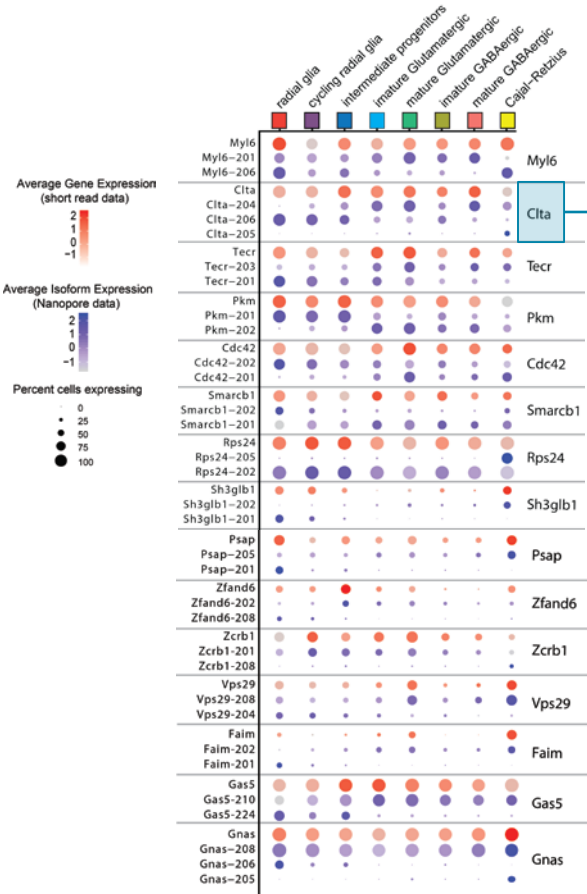
SiCeLoRe, Nature Communication, 2020
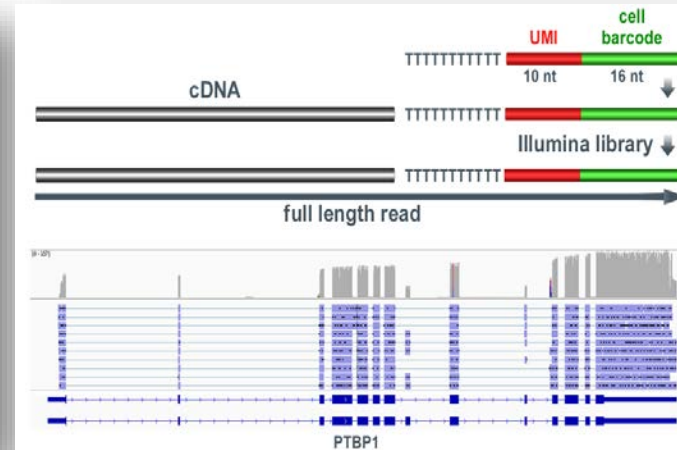
Single-cell long-read transcriptomics reveals diversity

76 isoform-switching genes along neuronal maturation

E18 C57BL/6 mouse hippocampus, cortex, and ventricular zone
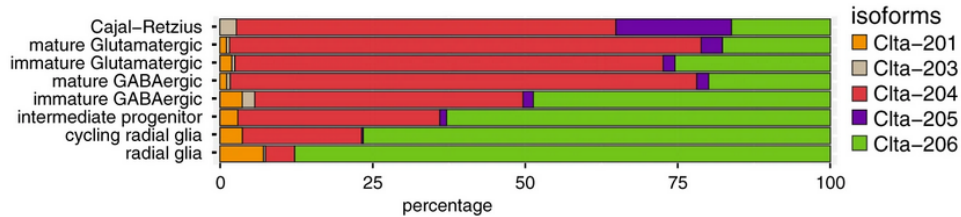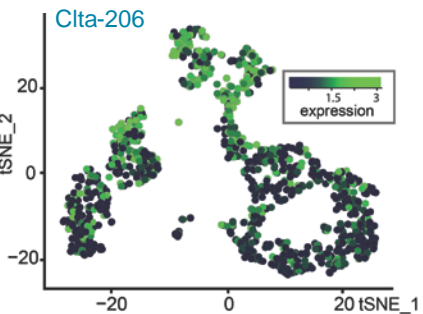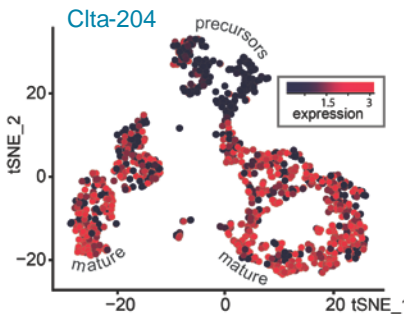
Access to the full length sequence heterogeneity (RNA-A-to-I editing)

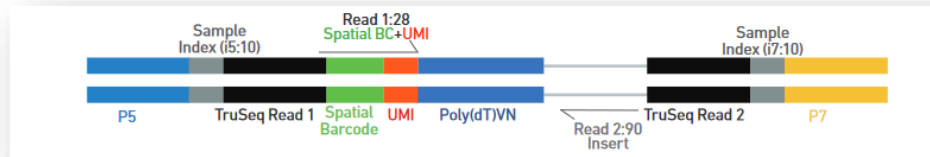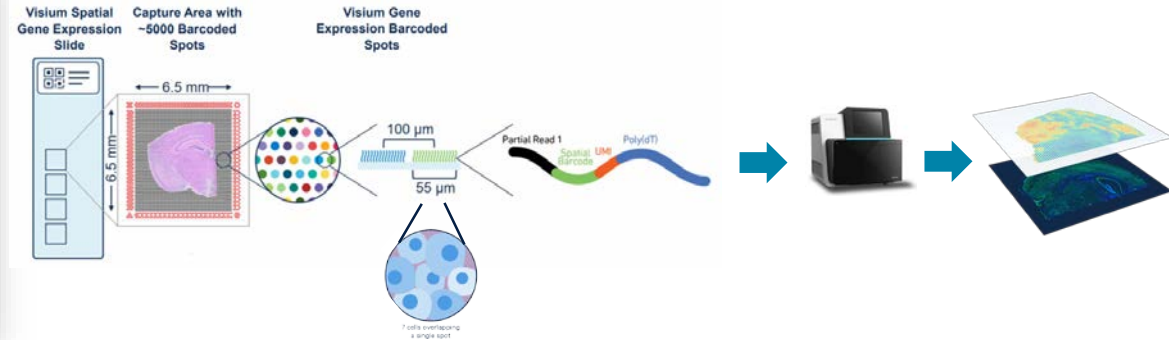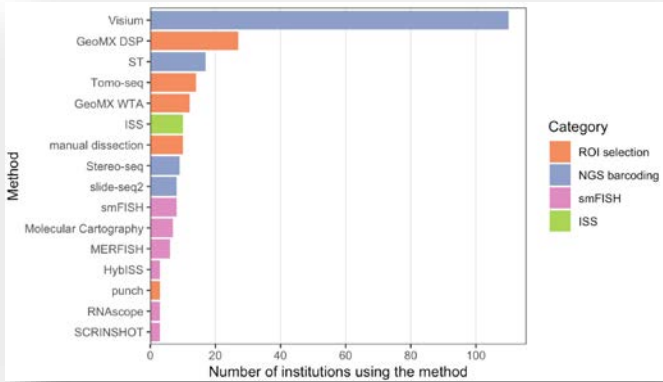# Spatial Transcriptomics approaches

Historical timeline

- Spatial transcriptomics aims at directly visualize gene expression in their original environment

- Tackle the main limitation of single cell experiment missing the spatial organization

- A lot of developments in the last years thanks to recent advances in different fields
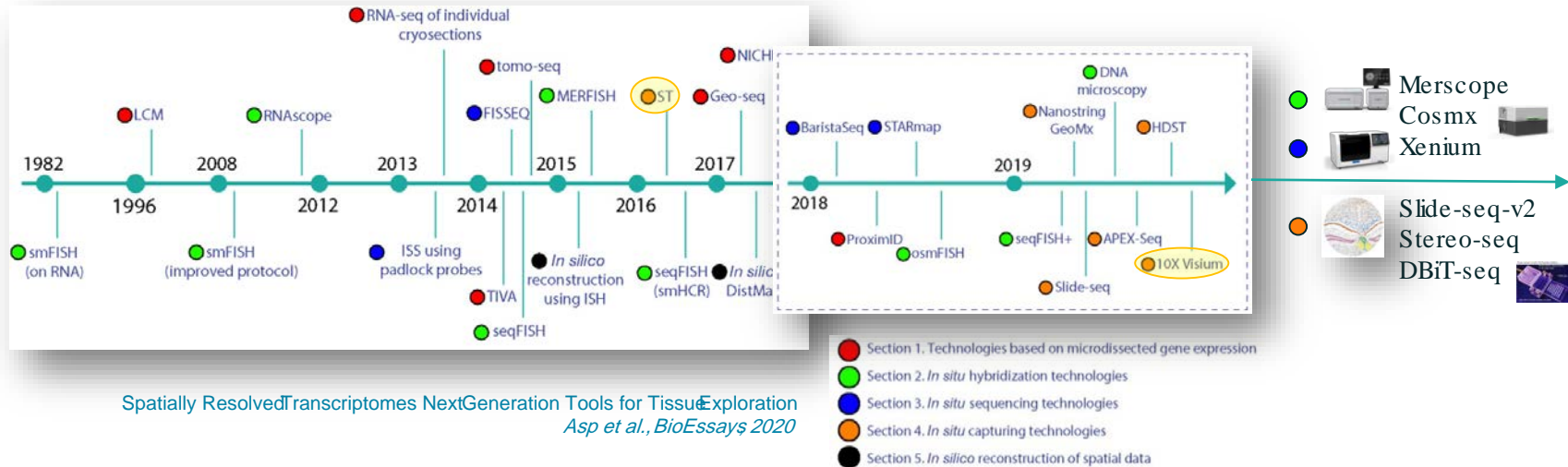
Spatially Resolved Transcriptomes Next Generation Tools for Tissue Exploration
Asp et al., BioEssays 2020

In-situ capture Spatial Transcriptomics (2017 -2022)

Visium is widely adopted by academics

→ Spatial barcode / UMI assignment strategy identical as the droplet-based single-cell approach
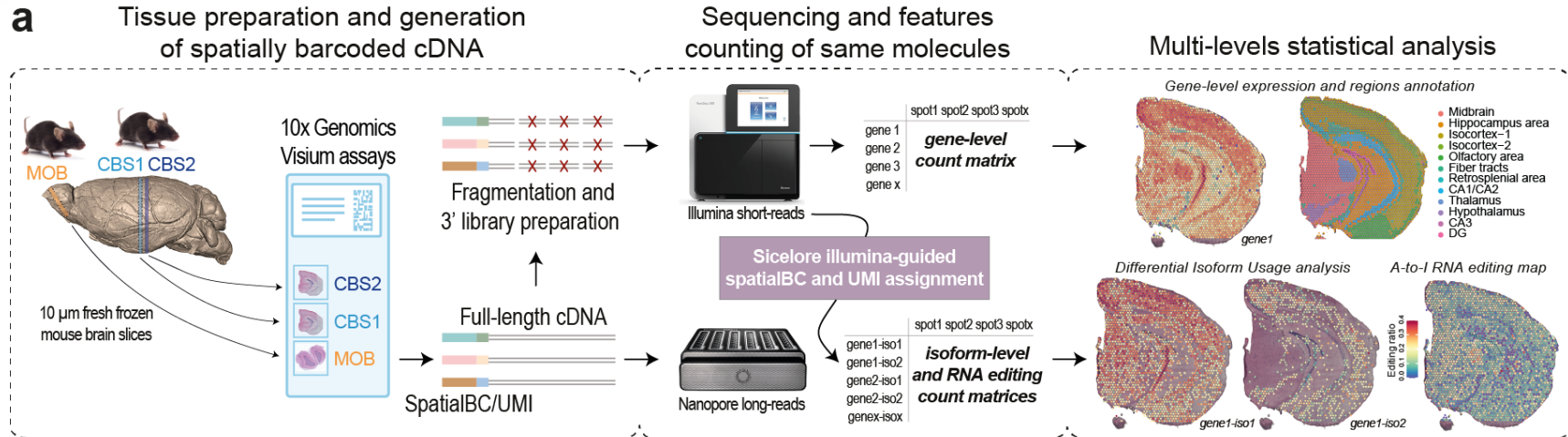
Spatial isoform Transcriptomics (SiT)

Nucleic Acids Research, 2023

a Tissue preparation and generation of spatially barcoded cDNA — Sequencing and features counting of same molecules — Multi-levels statistical analysis
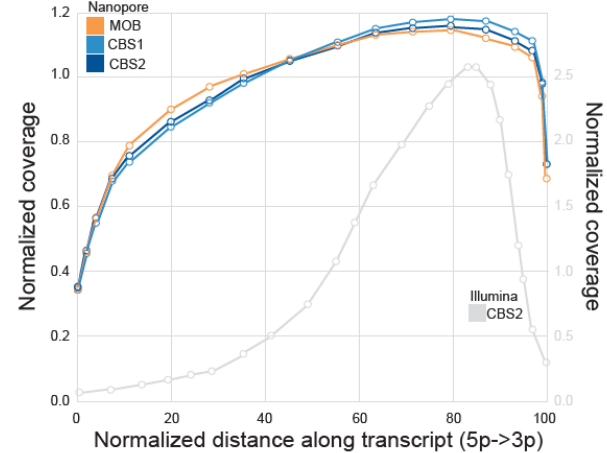
Nanopore promethION long -read sequencing
Sample UMI complexity

*Sequencing saturation curves per sample*
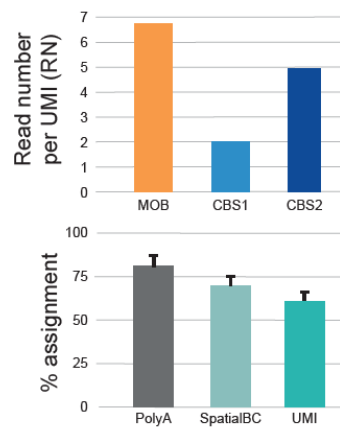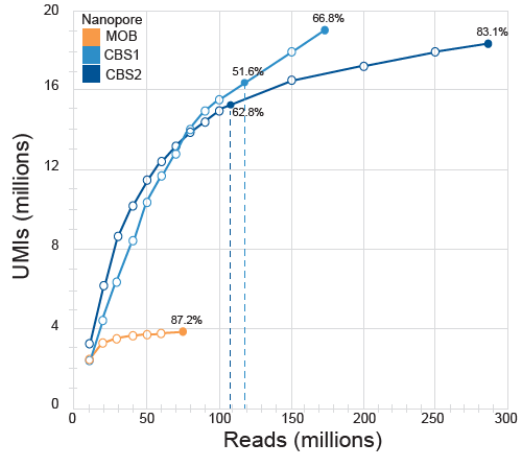
Transcripts full-length coverage

Reads	MOB		CBS1			CBS2								Total		
Date	18 feb. 20	20 mar. 20	18 feb. 20	20 mar. 20	24 feb. 20	12 may 20	13 may 20	19 may 20	25 may 20	25 may 20	26 may 20	27 may 20	09 feb. 21			
Flow cells	PAE06474	PAE59649	PAE01745	PAE59645	PAG52067	PAE59606	PAE59231	PAE32756	PAE32753	PAE31188	PAE21339	PAD99555	PAG56368	13		
Total reads (fastq_pass)	27628000	47272000	24980000	31736000	117280000	22897702	30405384	27492770	18534938	31506774	19108718	25596387	110916000	535354673	%age	
PolyA and Adapter found reads	21318117	47970311	17980183	27286678	80516212	18536047	25199992	22871198	16088962	26777546	15983663	21682530	85837208	428048647	79,96	of Total passed reads
SpatialBC found reads	14506264	29316718	12554655	19051597	54323311	14613934	19867830	14666481	11403706	19099469	11266930	14090779	60154119	294915793	68,90	of PolyA found reads
UMIs found reads	10445006	19328468	7323748	10517081	27584331	8616415	11714126	9347072	7557944	12657620	7448718	9031708	34225619	175797856	59,61	of SpatialBC found reads

CBS1: Oneflow cell, 117 M reads→ 51.6% sequencing saturation
CBS2: One flow cell, 111 M reads → 62.2% sequencing saturation
→ 1 or 2 Promethion flow cells per Visium slice

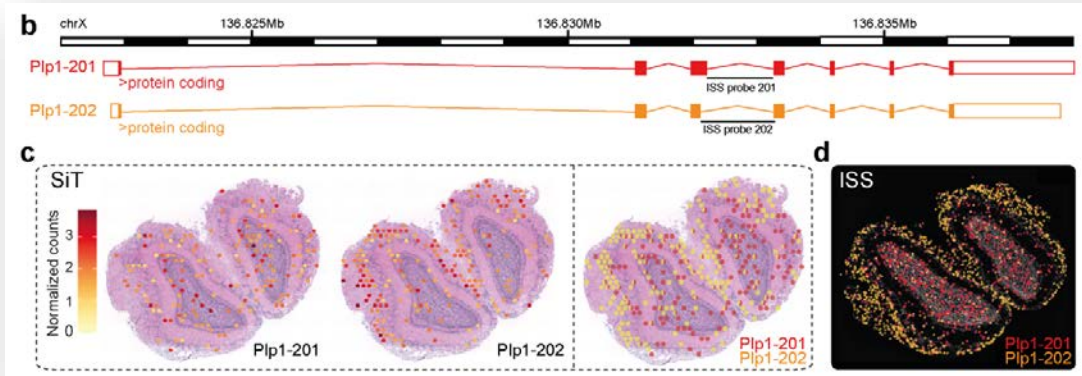# SiT reveals specific splicing pattern across MOB regions

Plp1 Differential Transcript Usage (DTU)
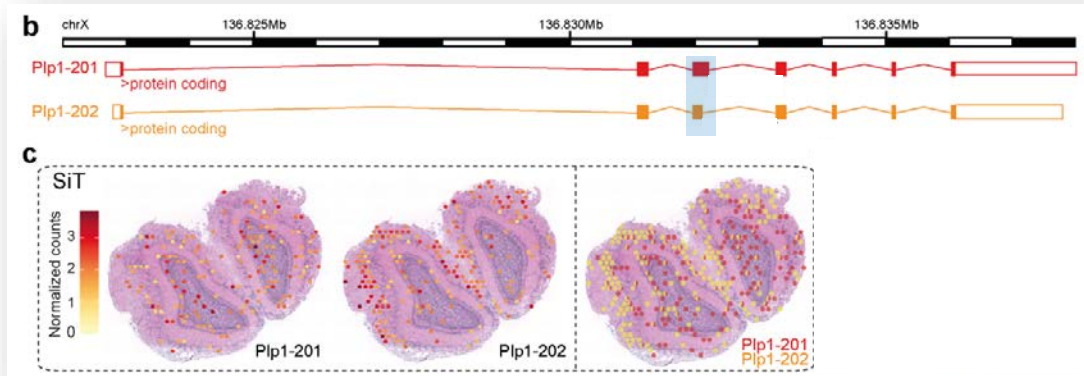
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination

SiT reveals specific splicing pattern across MOB regions
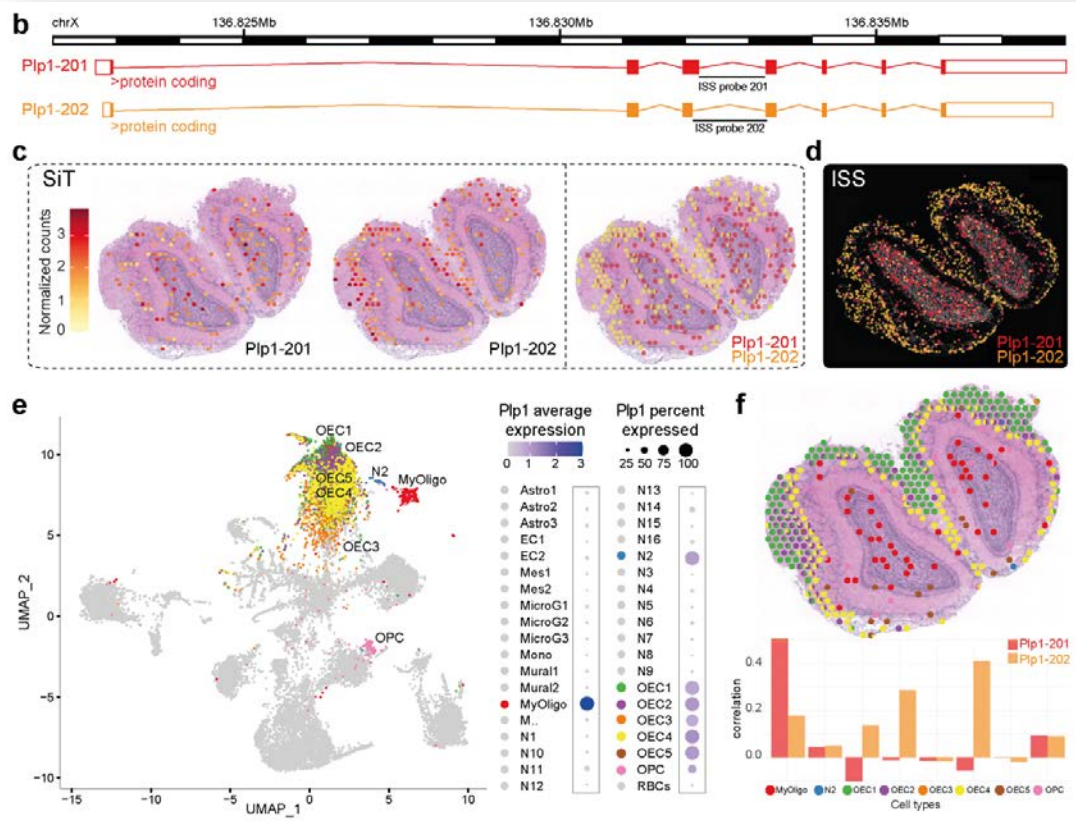
Plp1 Differential Transcript Usage (DTU)

Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination

In Situ Sequencing Data

SiT reveals specific splicing pattern across MOB regions

Cell type deconvolution using single cell external dataset (Tepe et al., 2018)

Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination


In Situ Sequencing Data

Spatial spot deconvolution of *Plp1* expresser cell types
→ *Plp1* is expressed as Plp1-201 by myelinating-oligodendrocytes (MyOligo) in the Granule Cell Layer
→ *Plp1* is expressed as Plp1-202 by olfactory ensheathing cells (OEC) in the Olfactory Nerve Layer

SiT reveals full -length sequence heterogeneity (CBS)

Global A-to-I RNA editing spatial map

- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR), Licht et al., 2019)
- Long read high confidence call thresholding, looking at agreement between long and short read base calls for 88,175 shared UMIs
  — number of reads per UMI >= 3
 — consensus Phred score QV >= 6
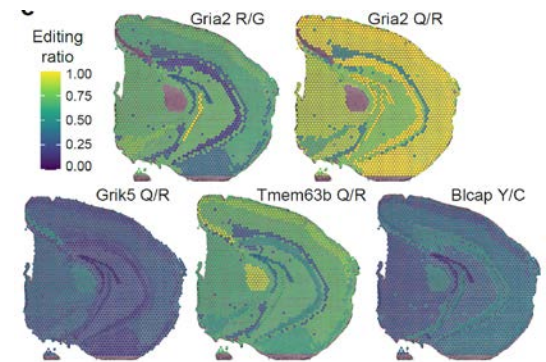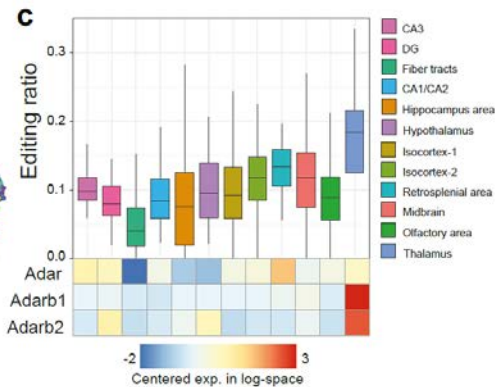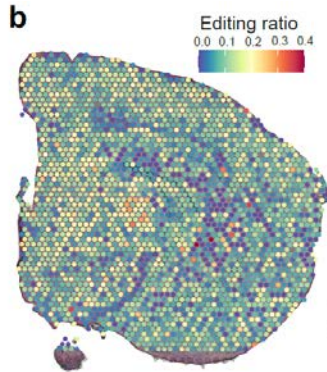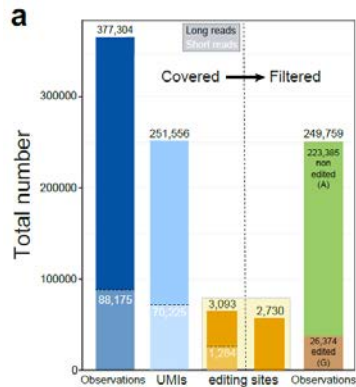
Individual A-to-I editing site editing ratio per region

Imaging -based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution

- Lower gene panel targets (from WT to ~1,000 genes)
- Higher sensitivity (from ~6% to 30-80%)
- Larger imaging area (42 to 236 mm2)
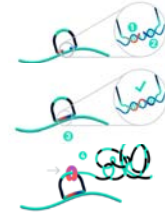- Higher resolution (from 55 µm to subcellular)

WHOLE SECTION   WIDE FIELD OF VIEW   SUB-CELLULAR
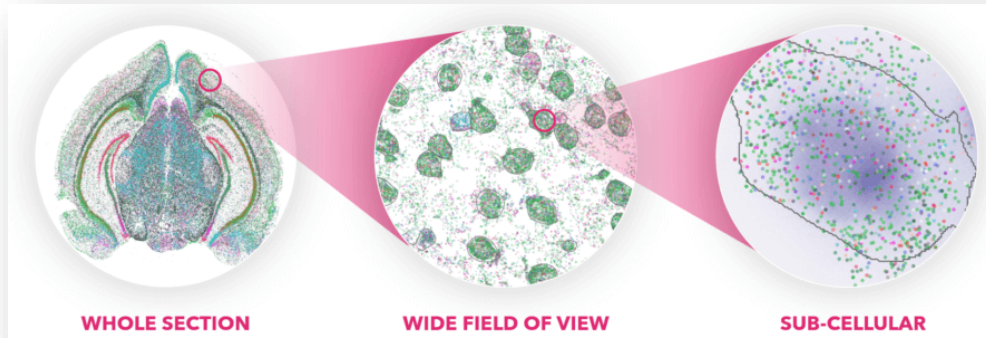
Nanostring CosMx
ISH-based

Vizgen Merscope
Multiplex Error-Robust FISH
Available (oct.2022)

10x Genomics Xenium
Padlock probes / RCA
Available (jan.2024)

Single cell and Spatial isoform transcriptomics

Summary

- Accurate single-cell and spatial transcriptomics using Nanopore long-read sequencing is feasible

- Long reads sequencing reveals transcript diversity that is missed with standard short reads workflows

- Single Nucleotide Variation calls (SNV, editing) in single-cell and in a spatial context can be achieve

- Sicelore-2.1 : we don't need short reads anymore

Raw read accuracy over two years
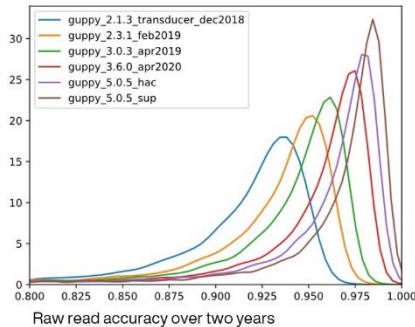
Nanopore PromethION sequencing

2018: 20M reads/FC, 92% raw read accuracy

2022: 150M reads/FC, 98% raw read accuracy

https://github.com/ucagenomix/sicelore2.1
- Visium and single cell 3' and 5' libraries
- Illumina-free profiling available

Acknowledgments

Institut de Pharmacologie Moléculaire et Cellulaire