Spatially resolved transcriptomics

Anna Maria Reuss

JC

18/09/2018

Overview

- Introduction: ISH techniques
- 1st paper: Shah *et al.,* Neuron, 2016
- Introduction: RCA and *in situ* RNAseq
- 2nd paper: Wang *et al.,* Science, 2018

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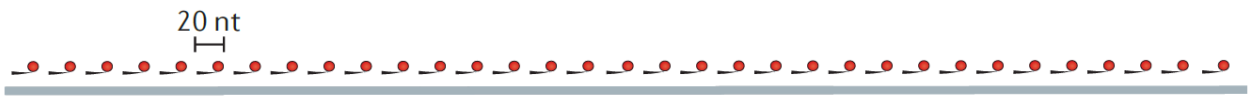
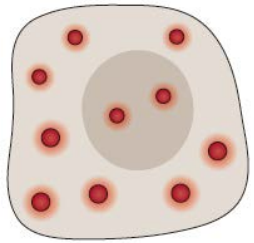
Present challenges

- Next generation sequencing technologies → unbiased genomic, epigenomic and transcriptomic information in cells and tissues

- Multiple spatially resolved omic measurements in the same biological sample could reveal completely new spatiotemporal interdependencies that would advance our understanding of how complex biological systems operate

“Traditional” smFISH

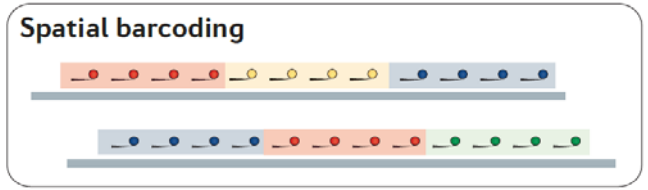


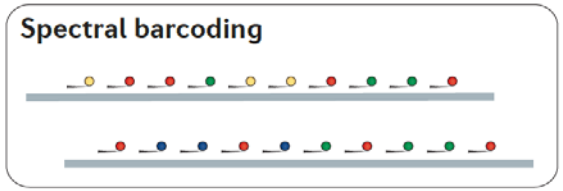


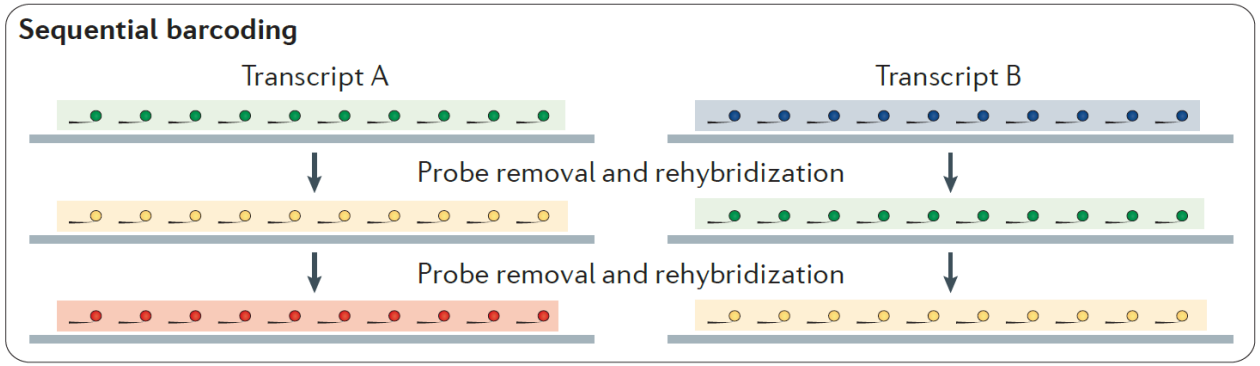
Limited availability of fluorophores with non-overlapping spectra → simultaneous detection of only few transcripts

Itzkovitz *et al.*, Nature Cell Biol., 2012

smFISH - multiplexing approaches







Barcode Type	Hybridization Pattern	Spatial Reconstruction Fidelity	Resolution Requirement	Minimum required Flurophore Emission	Linearization required	Multiplex Scaling
Spectral	distributed	100%	100 nm	~400 photons	No	$p!/(p-n)!/n!$
Spatial	localized	74%	20 nm	~3000 photons	Yes	$p!/(p-n)!/2$

p = number of fluorophores
n = number of positions

F^N transcripts can be detected
F = number of dyes
N= number of hybridization rounds

Lubeck *et al.*, Nature Methods, 2012

Lubeck, *et al.*, Nature Methods, 2014

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Neuron

NeuroResource

In Situ Transcription Profiling of Single Cells Reveals Spatial Organization of Cells in the Mouse Hippocampus

Highlights

- Amplified seqFISH enables in situ detection of hundreds of genes in single cells in tissues
- Combinatorial expression patterns of genes define cell classes in the mouse brain
- Spatial transcriptomics defines regions within the hippocampus
- Heterogeneity in cell class compositions increases along the dorsal to ventral axis

Authors

Sheel Shah, Eric Lubeck, Wen Zhou, Long Cai

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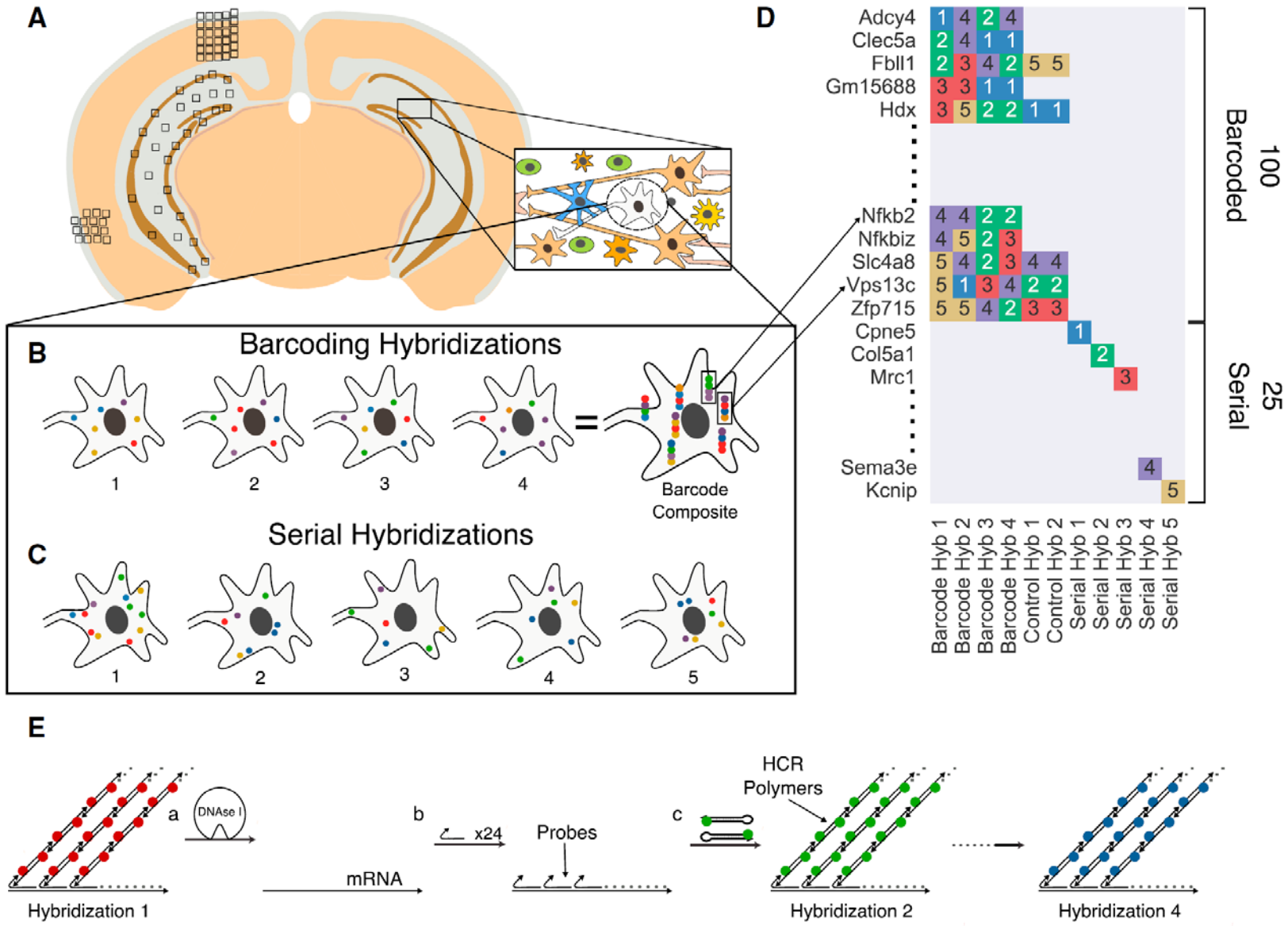
In Brief

Shah et al. demonstrated multiplexing of 250 genes in situ in mouse brain slices using amplified seqFISH. They found that there are distinct subregions of the hippocampus consisting of different combinations of cell classes, defined by the expression patterns in single cells.

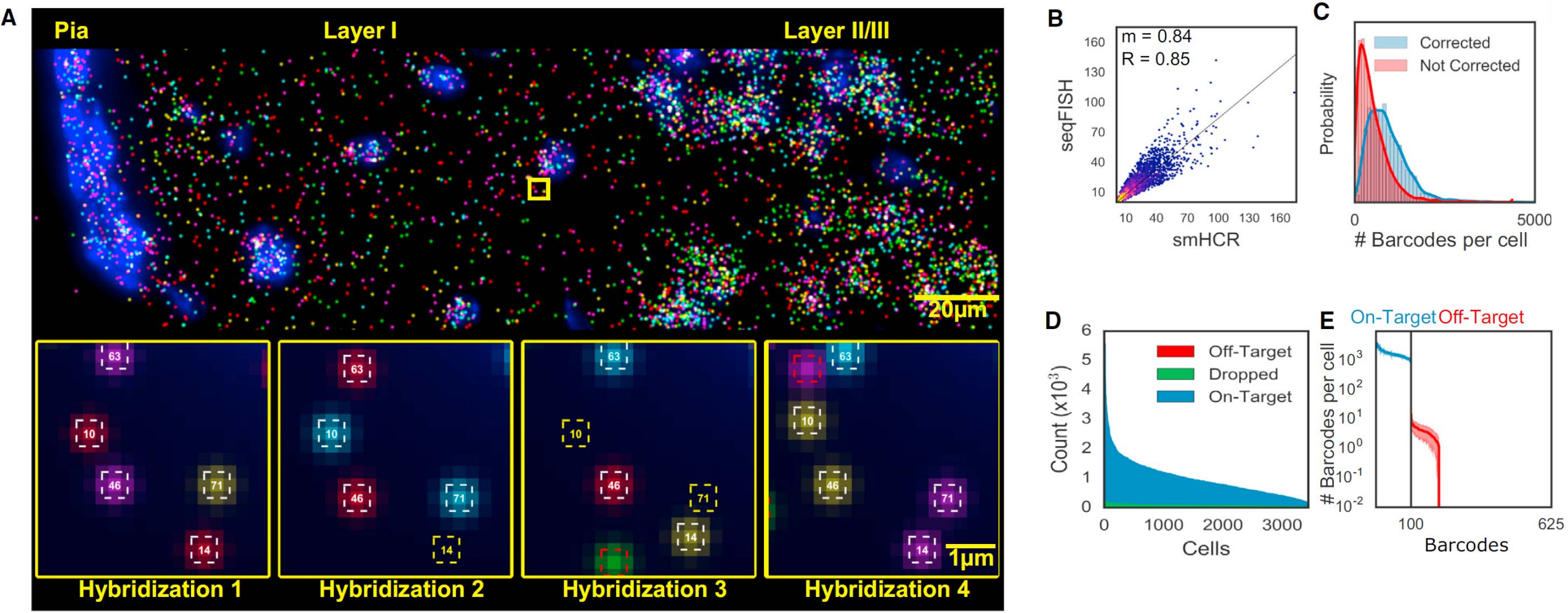
seqFISH in brain slices - principle

F^n → 625 combinations

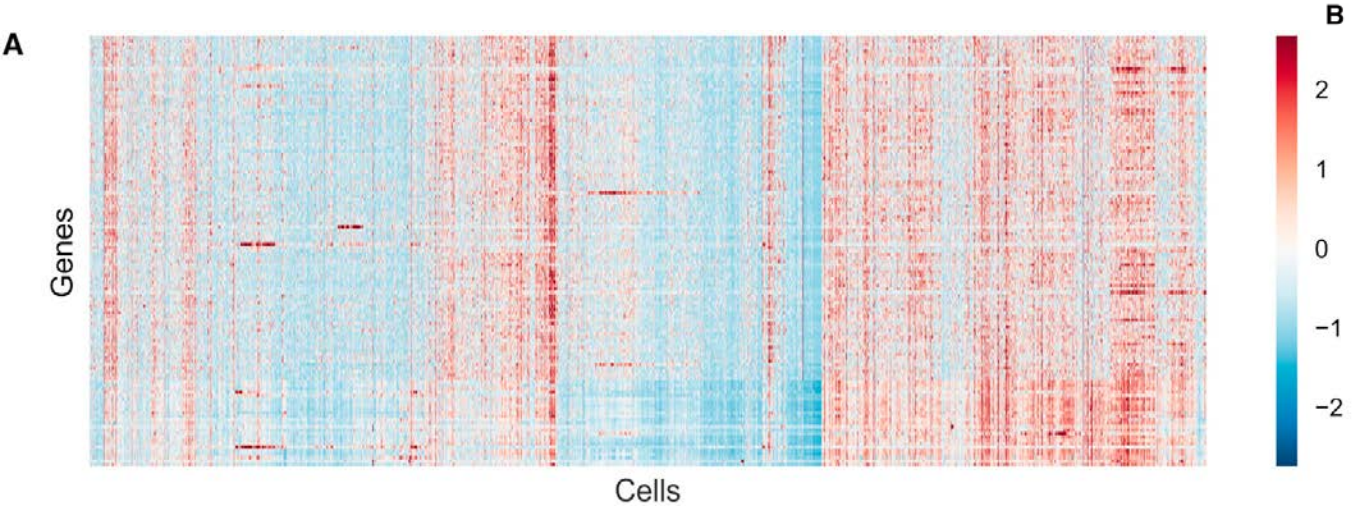
F*n → 25 combinations

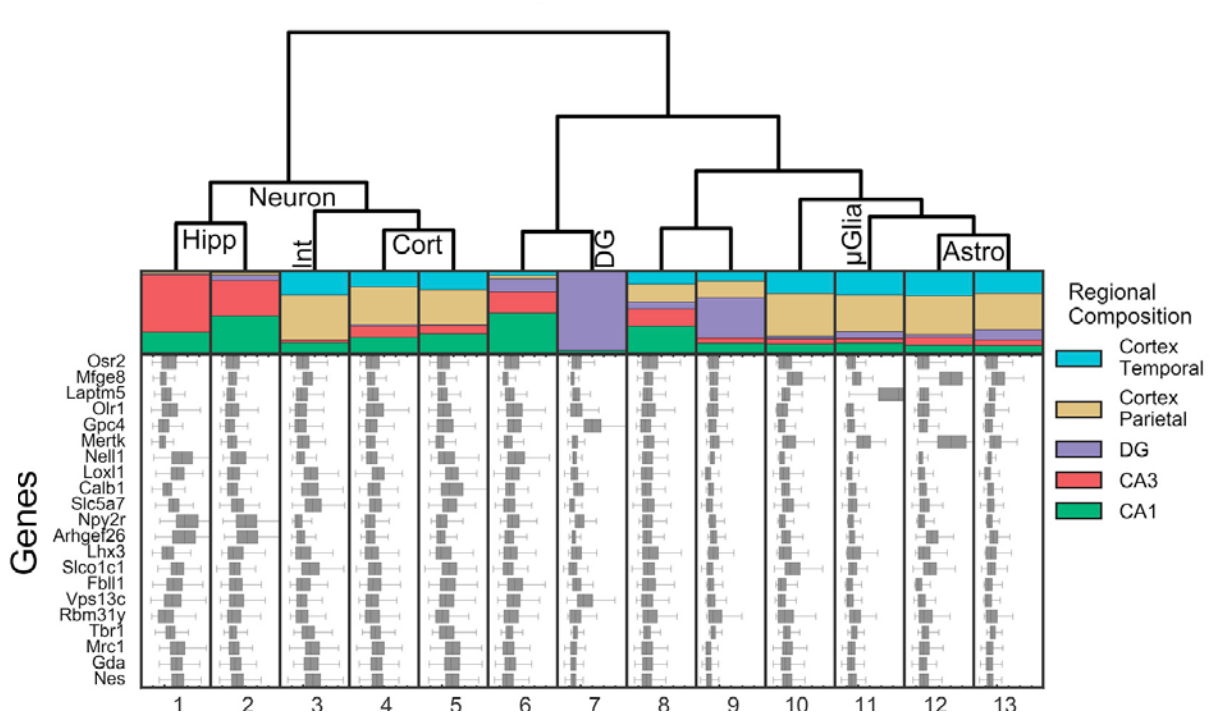


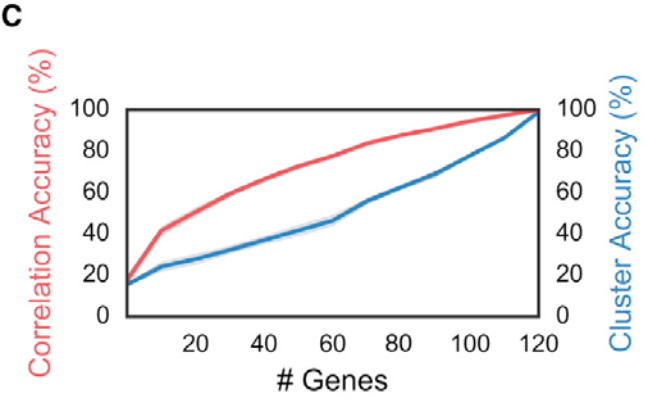
seqFISH in brain slices - validation

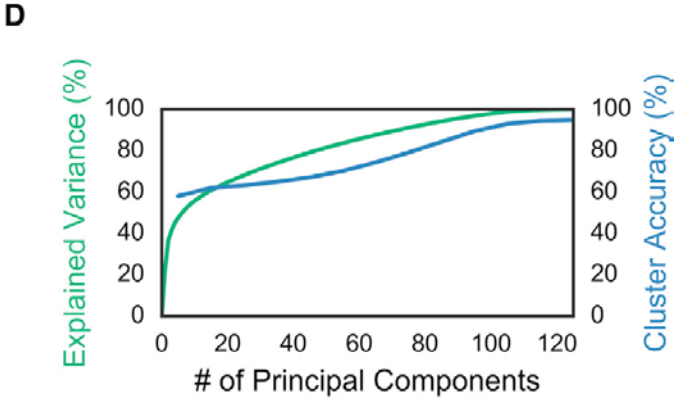


Distinct Clusters of Cells Exhibit Different Regional Localization in the Brain

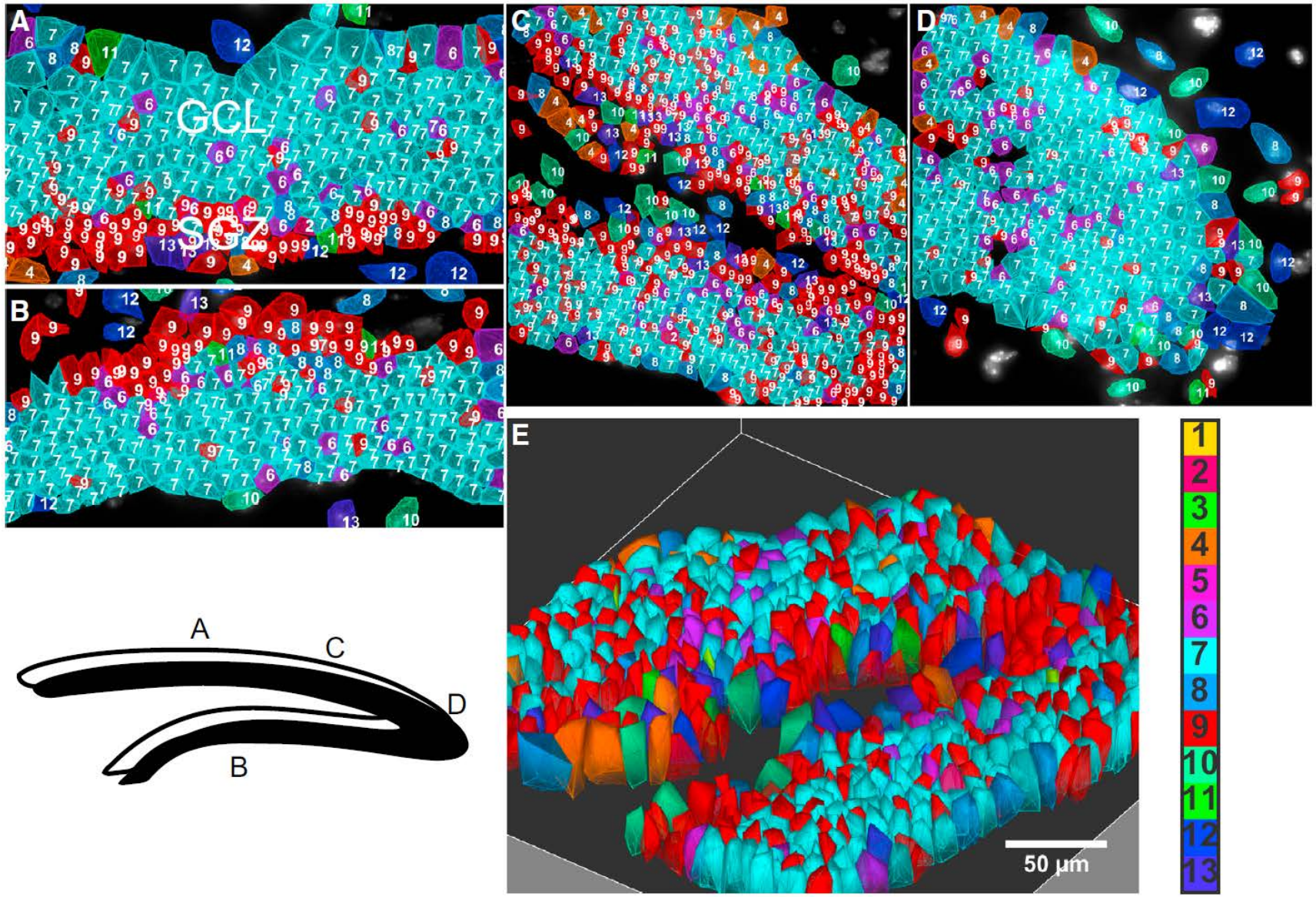




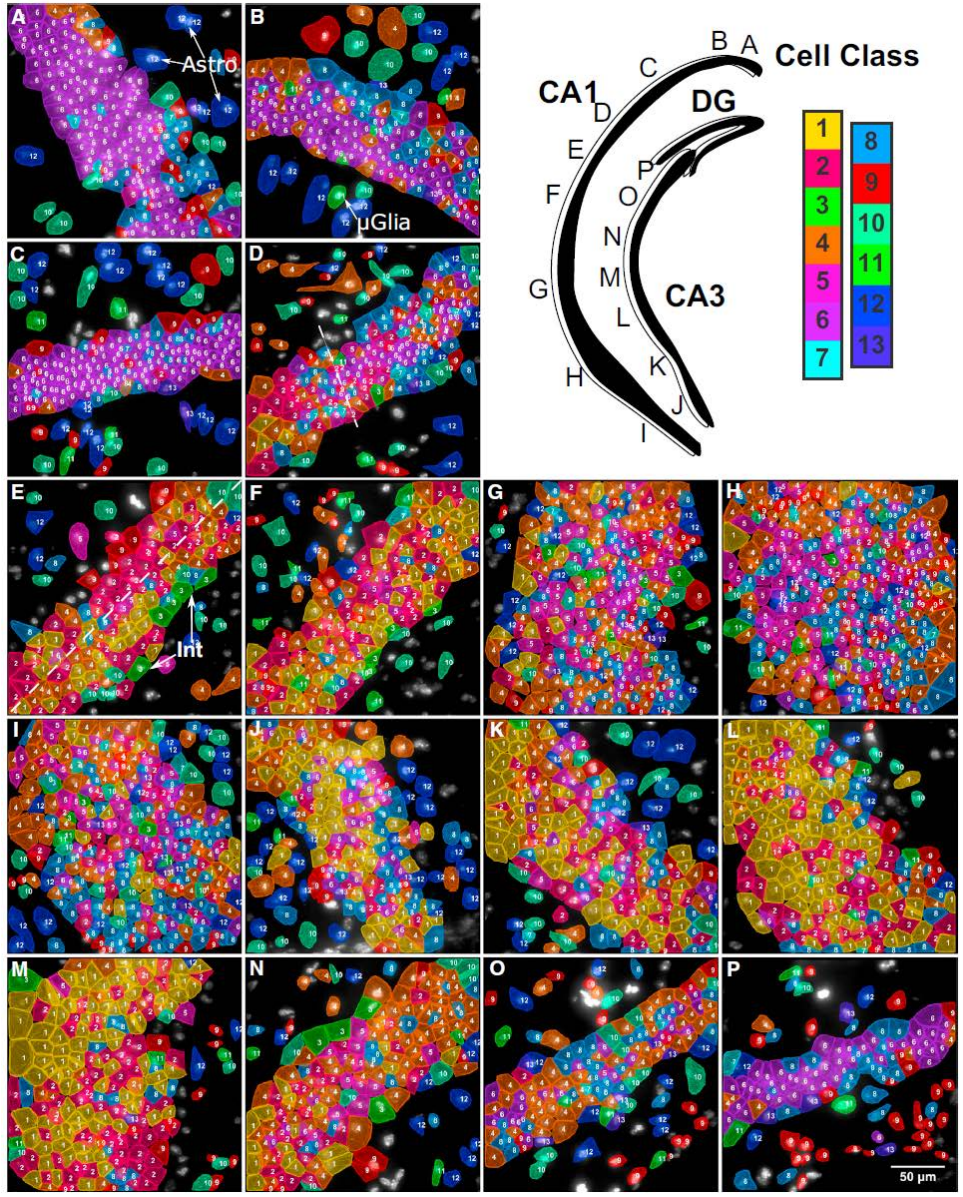




Spatial Layering of Cell Classes in the Dentate Gyrus



Subregions of the Hippocampus Are Composed of Distinct Compositions of Cell Classes



Summary

- Multiplexed spatial assessment of ~100 genes in single cells
- In mammalian brain tissue
- High sensitivity and specifity (almost no off-target effects)
- Identification of cell types in distinct regions and layers

- Target genes have to be known
- Time consuming → only study of one coronal section in the whole paper
- Only 2 mice → biological reproducibility?
- Real 3D ? → no information about slice thickness
- Lack of sufficient raw data

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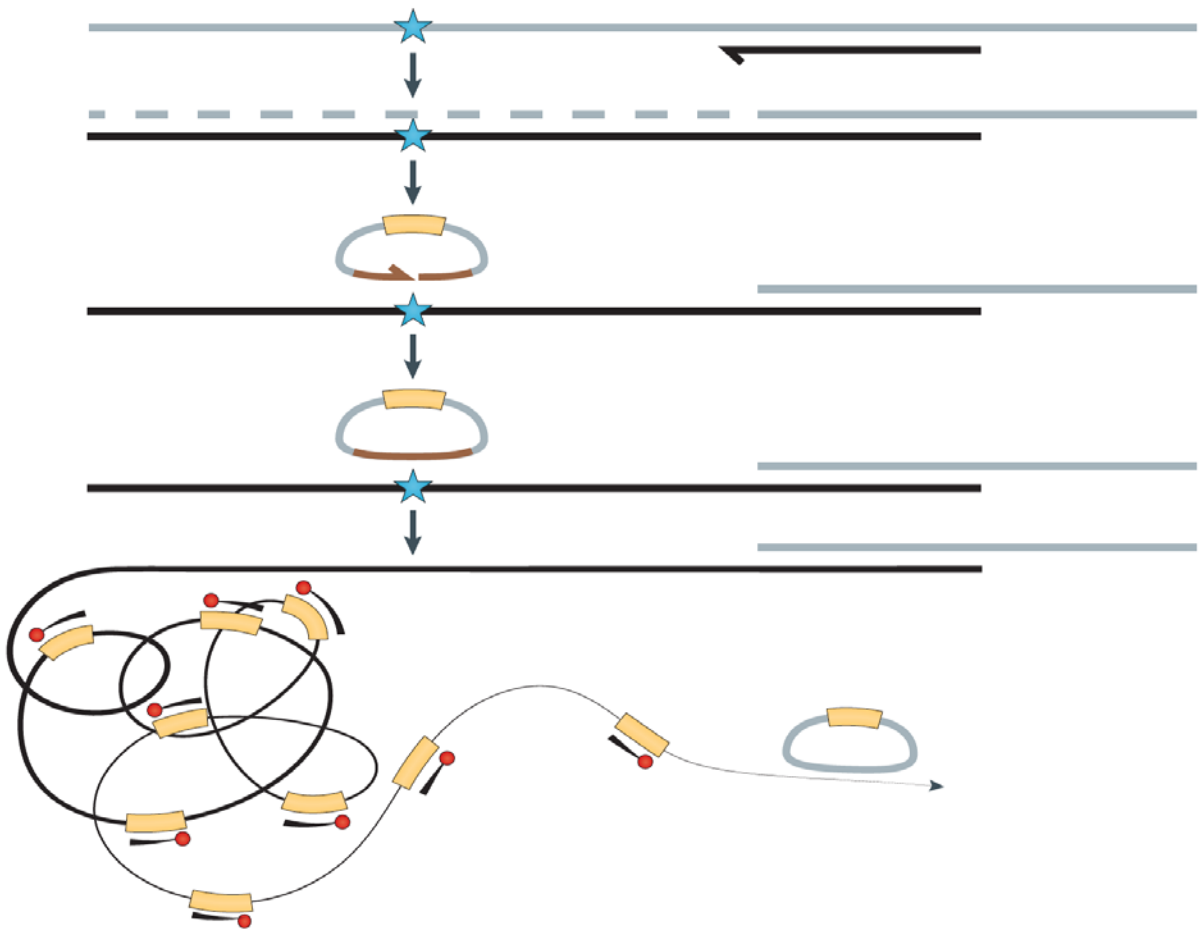
Padlock probes and rolling circle amplification



LNA-modified oligonucleotides prime reverse transcription upstream of the site of a SNV

Padlock probe with 5’- and 3’-homology arms and a linker sequence

Only if both homology arms are complementary to the cDNA target sequence → ligation and circularization

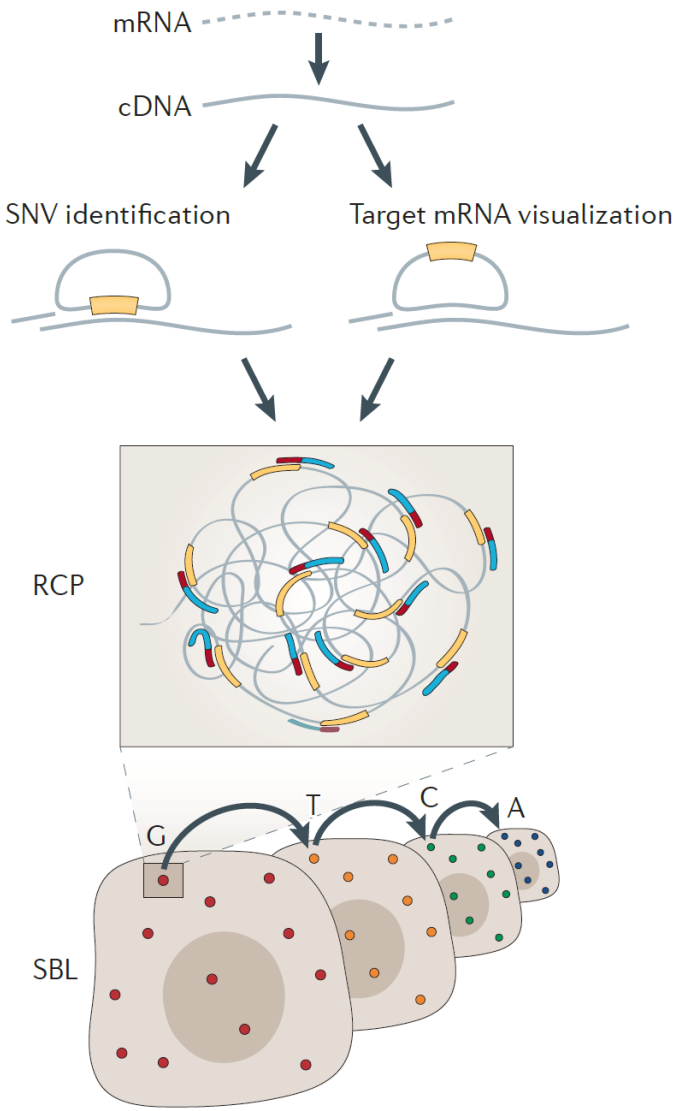
100 – 1 000’s of copies by amplification of the padlock probe via an isothermal DNA polymerase

Linker copies are detected by fluorescently labeled oligonucleotides

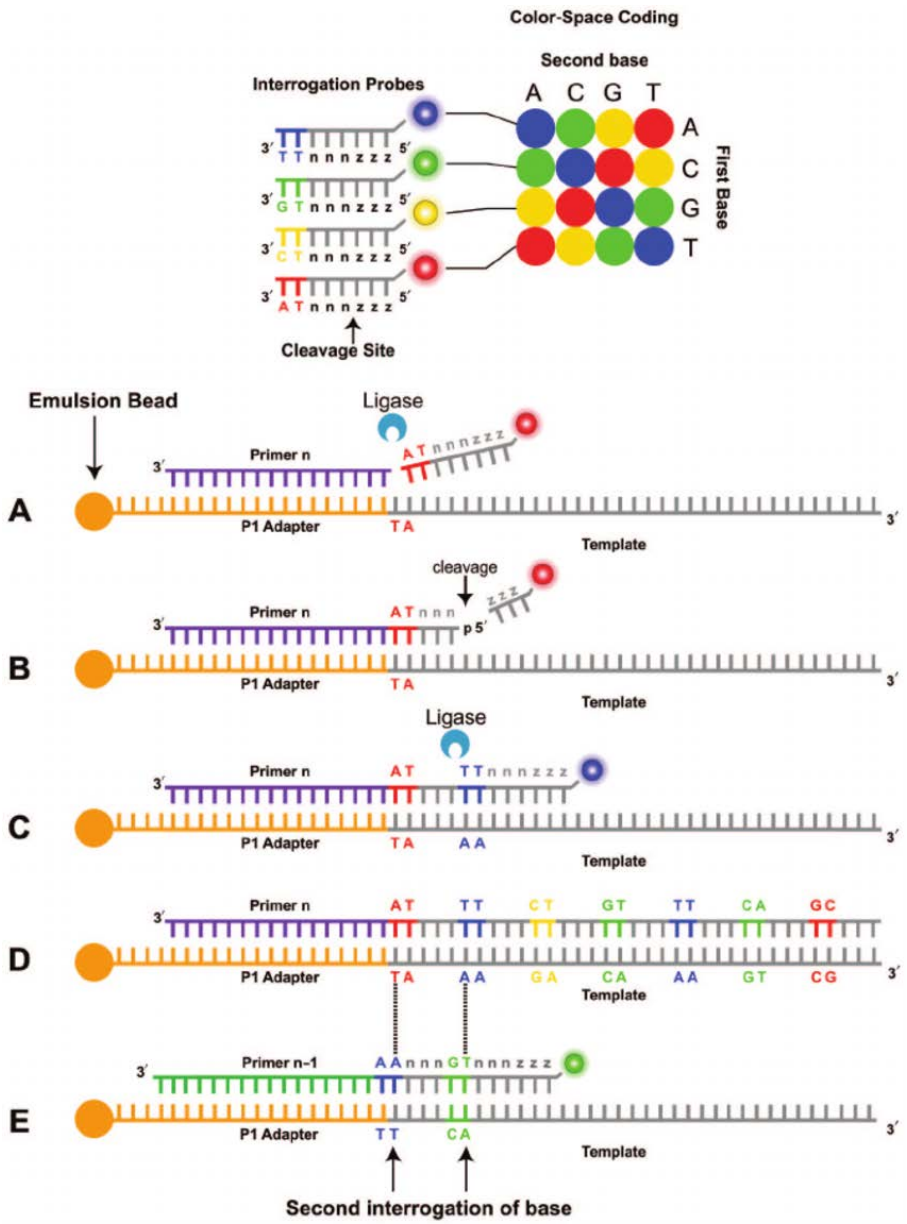
Larsson *et al.*, Nature Methods, 2010

In situ RNAseq

4-base sequences encompassing an expressed SNV



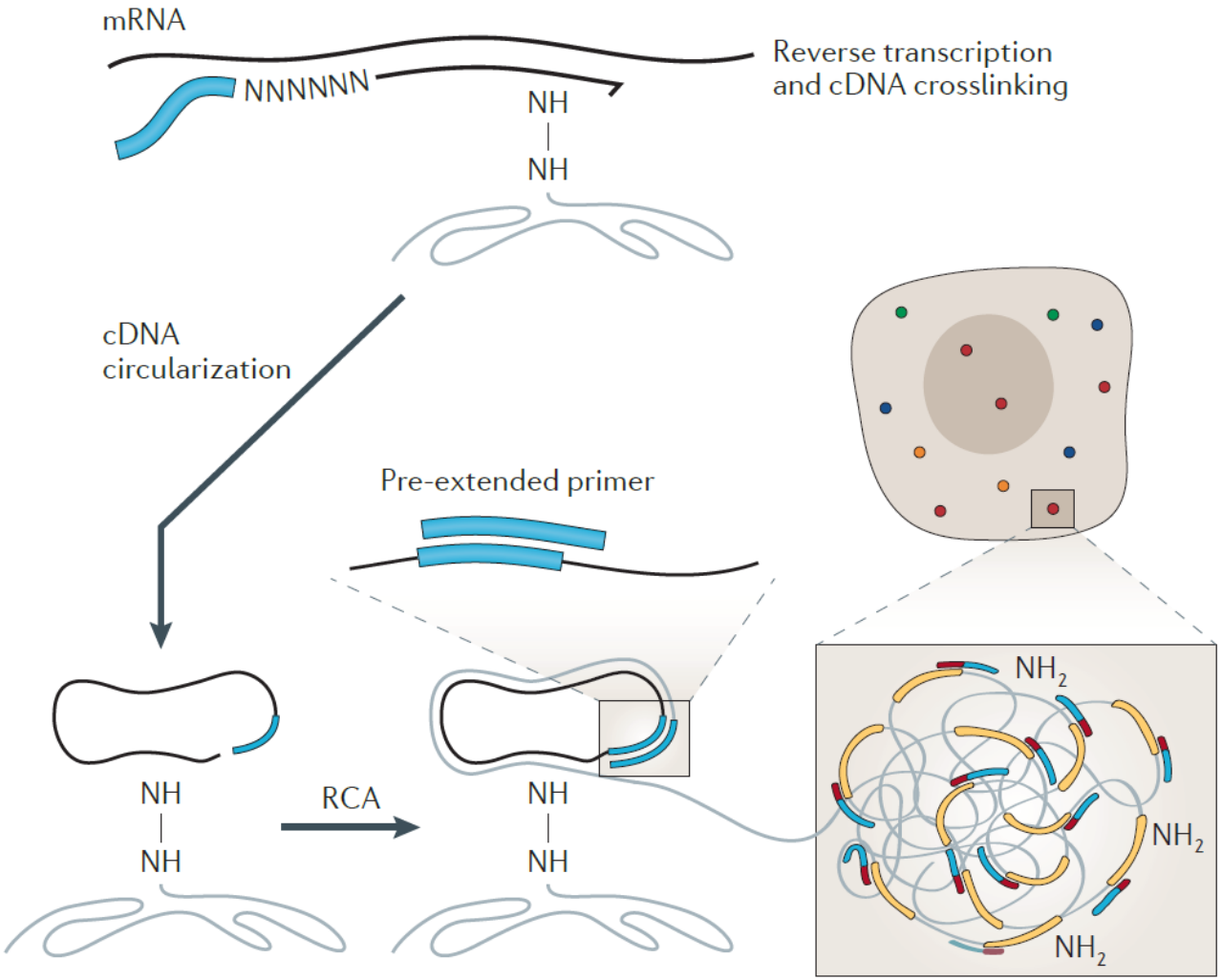
4-base sequence barcodes inserted in padlock probes targeting selected transcripts



Ke *et al.*, Nature Methods, 2013

Voelkerding *et al.*, Clin. Chem., 2009

FISSEQ



Lee *et al.*, Science, 2014

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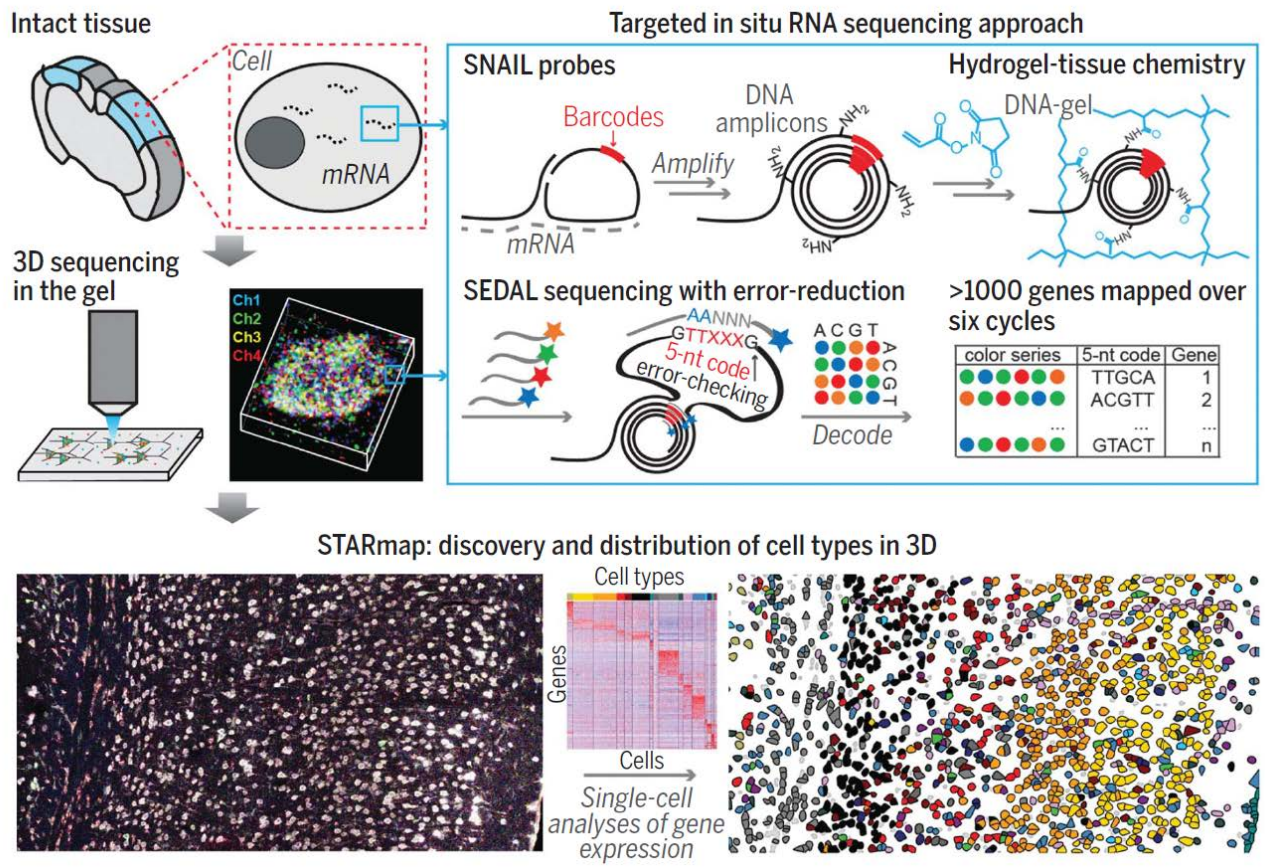
RESEARCH ARTICLE

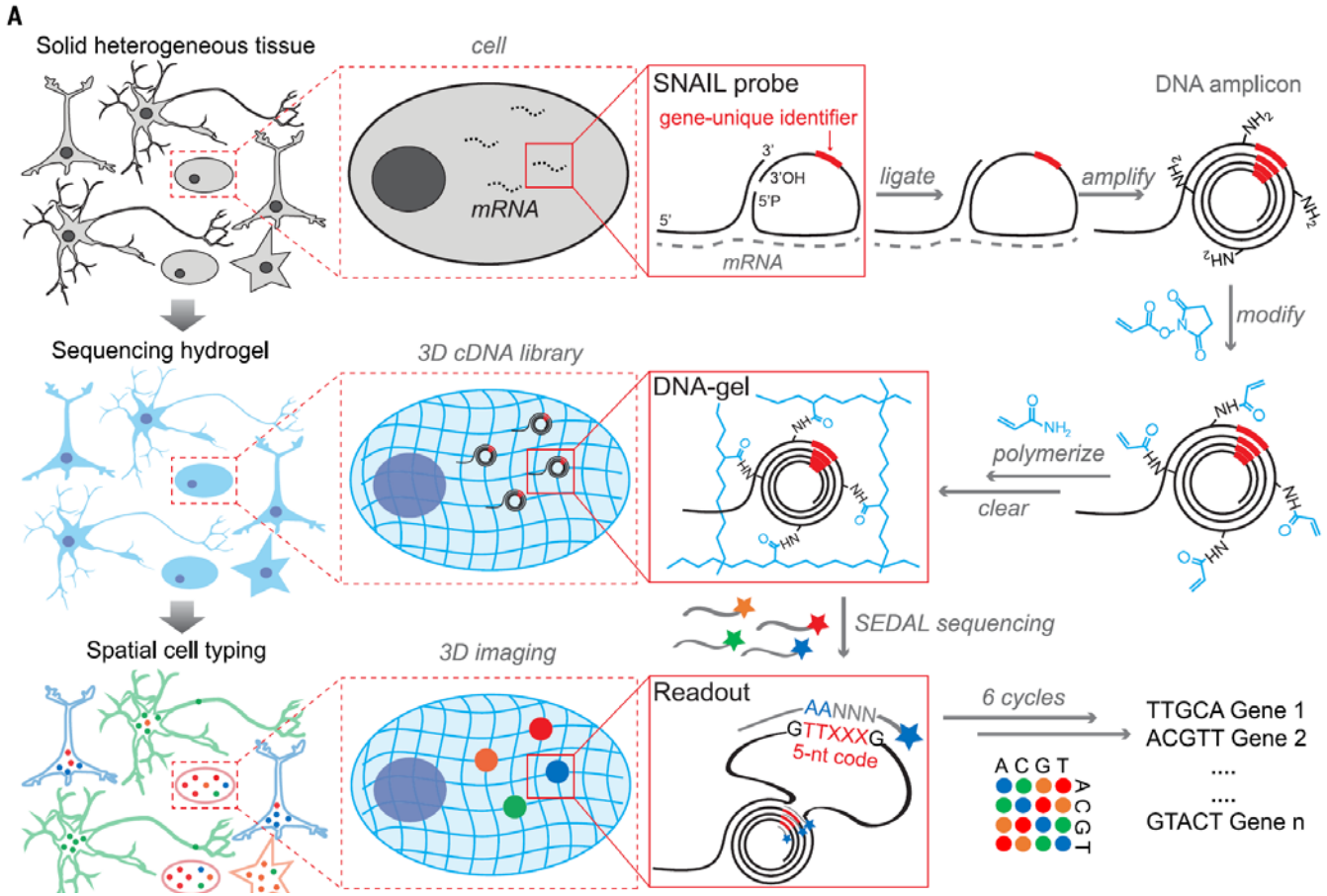
BIOTECHNOLOGY

Three-dimensional intact-tissue sequencing of single-cell transcriptional states

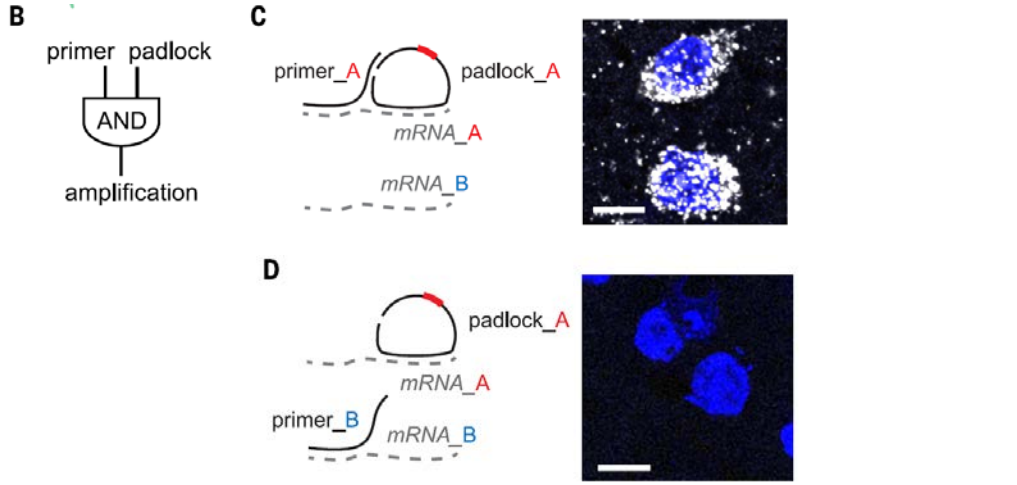
Xiao Wang[1*], William E. Allen[1,2*], Matthew A. Wright[1,3], Emily L. Sylwestrak[1], Nikolay Samusik[4], Sam Vesuna[1], Kathryn Evans[1], Cindy Liu[1], Charu Ramakrishnan[1], Jia Liu[5], Garry P. Nolan[4]†, Felice-Alessio Bava[4]†‡, Karl Deisseroth[1,3,6]†

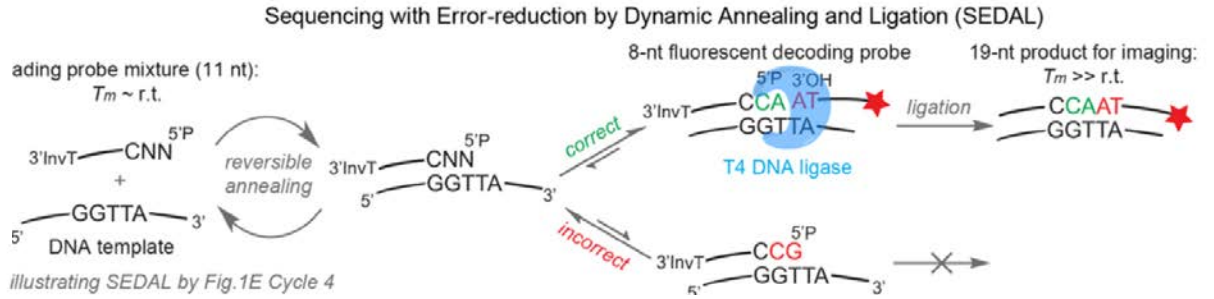


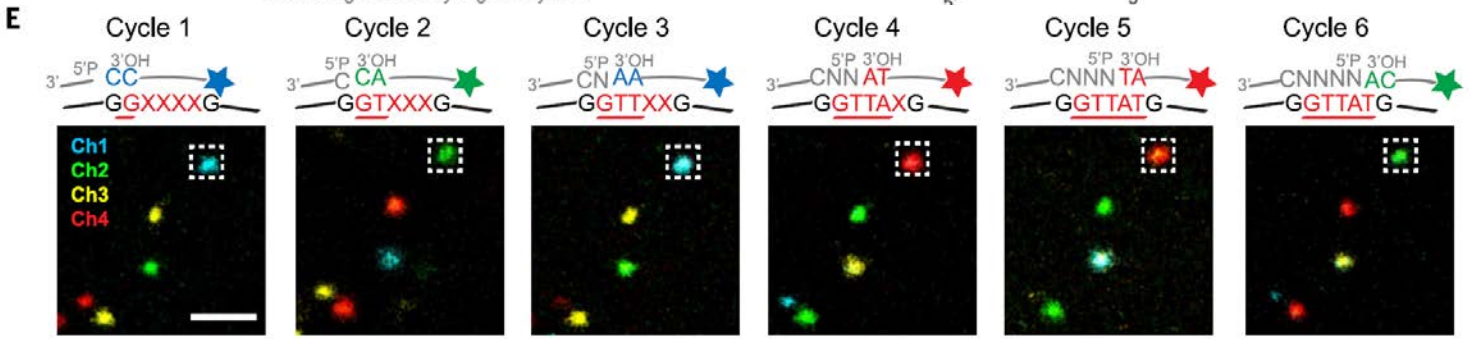
Design and validation of STARmap principles



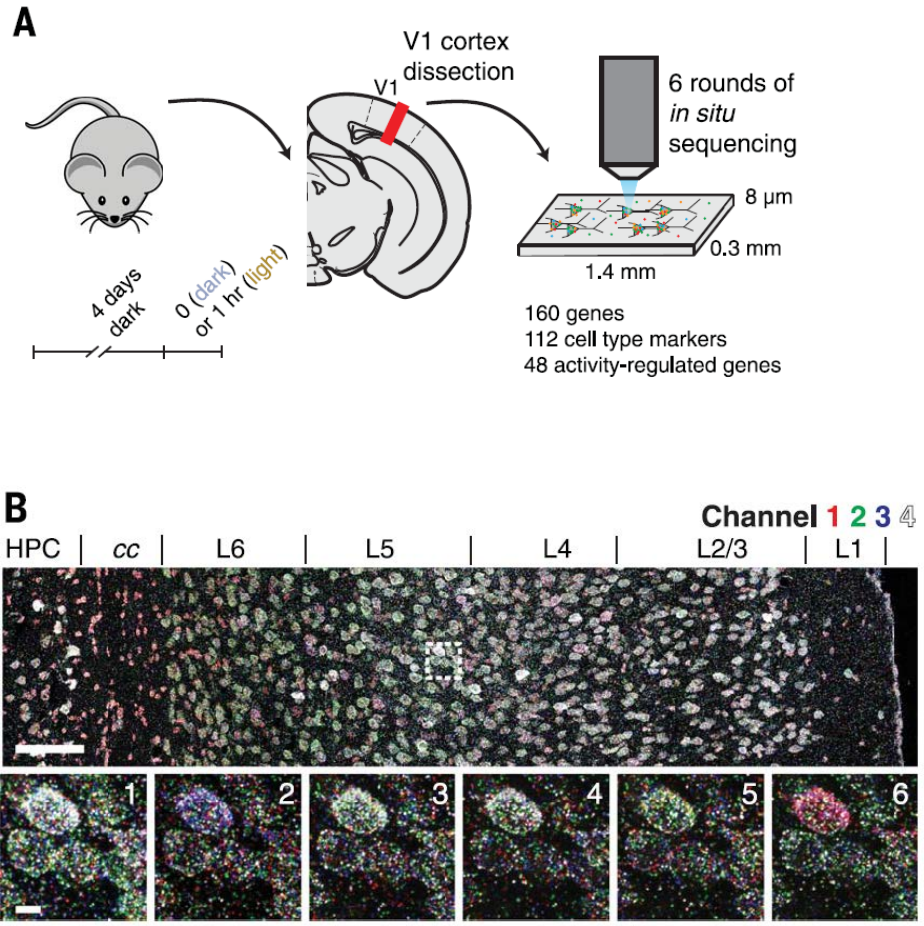
Primer and padlock A for GAPDH

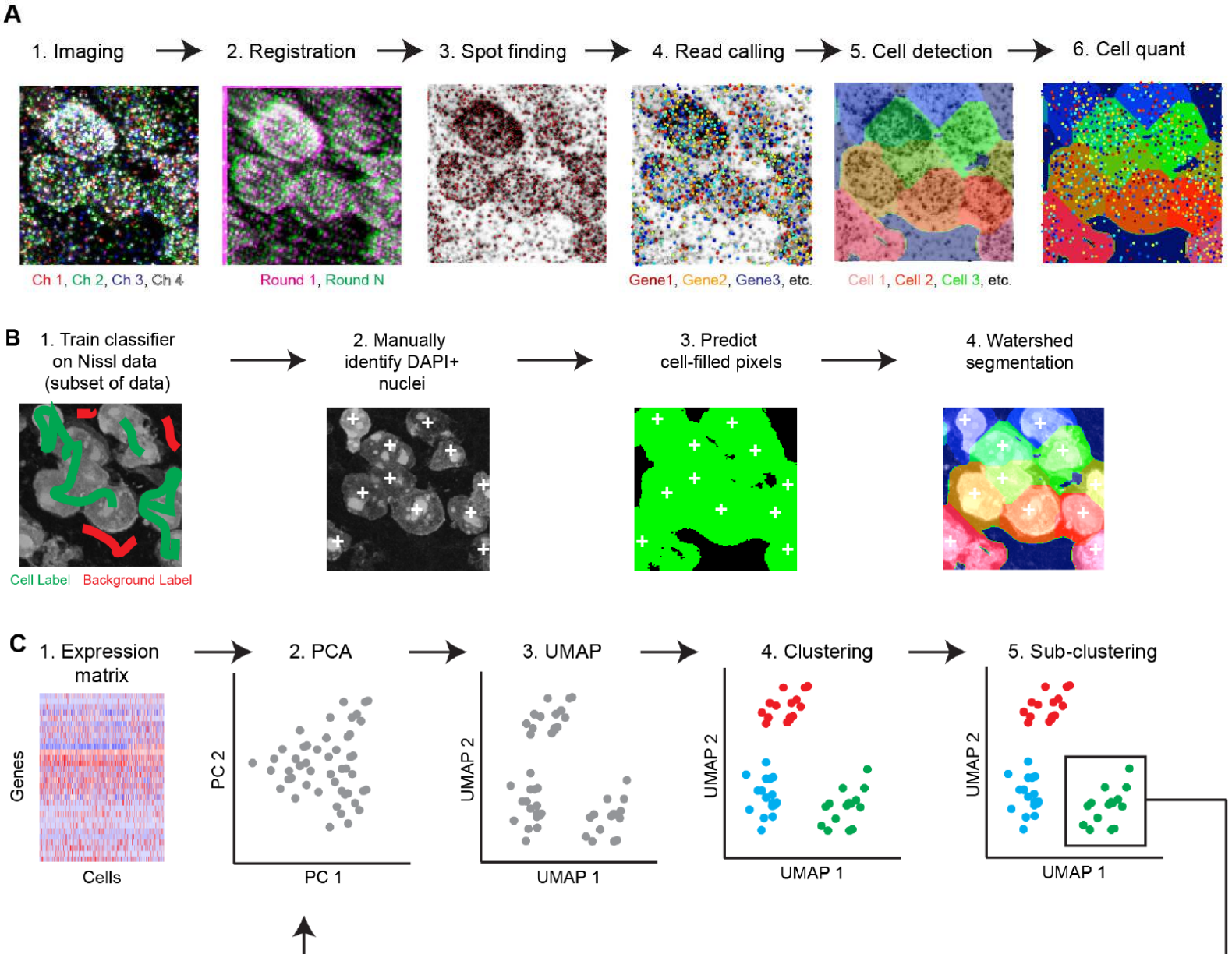






Spatial cell typing in V1 with 160-gene STARmapping





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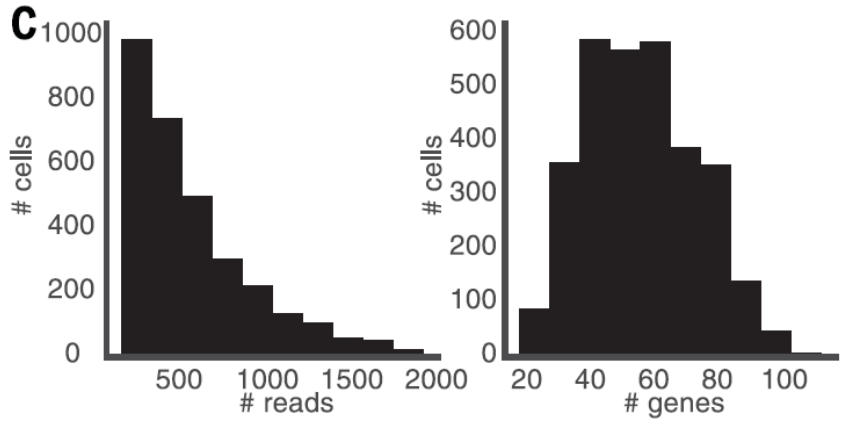

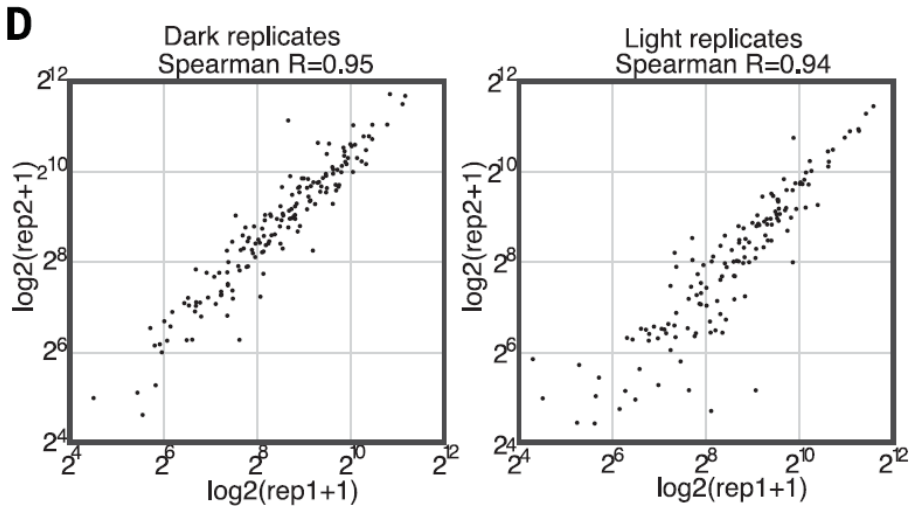

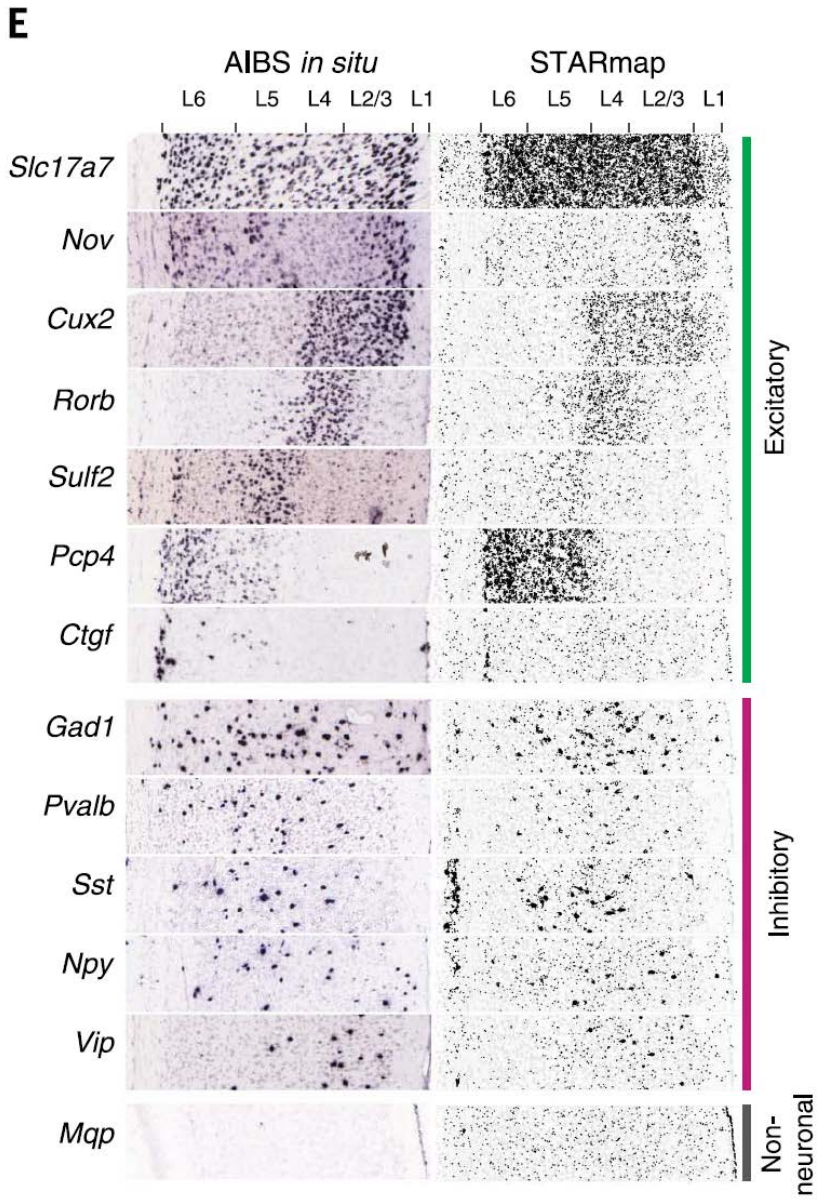

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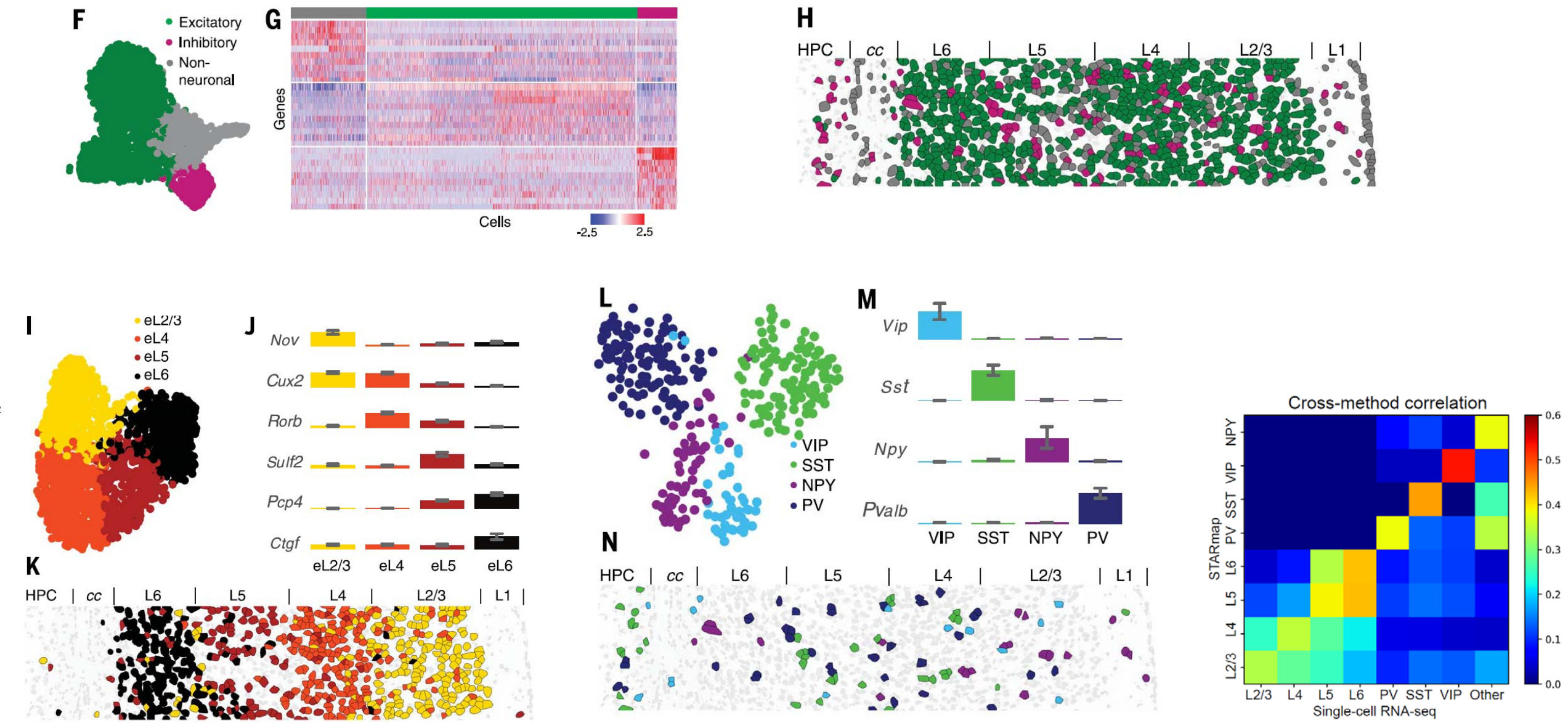

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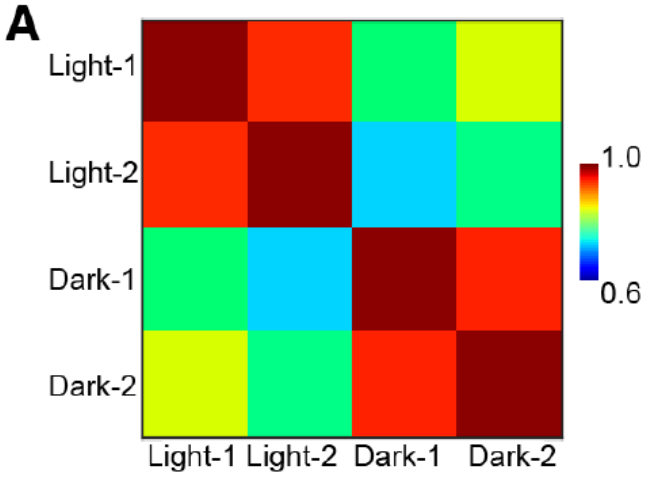
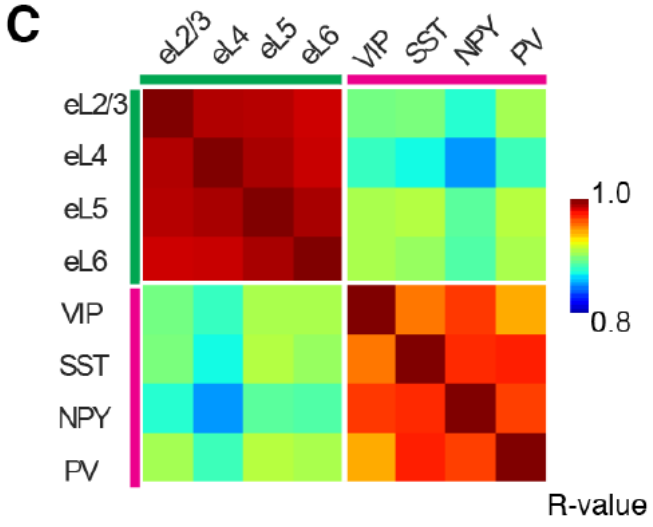
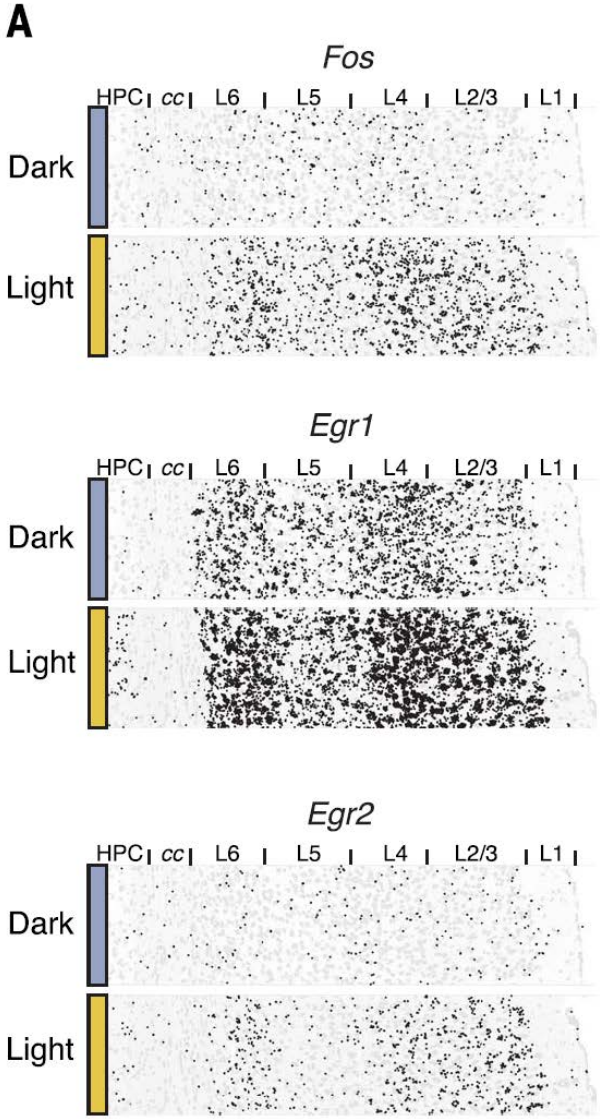
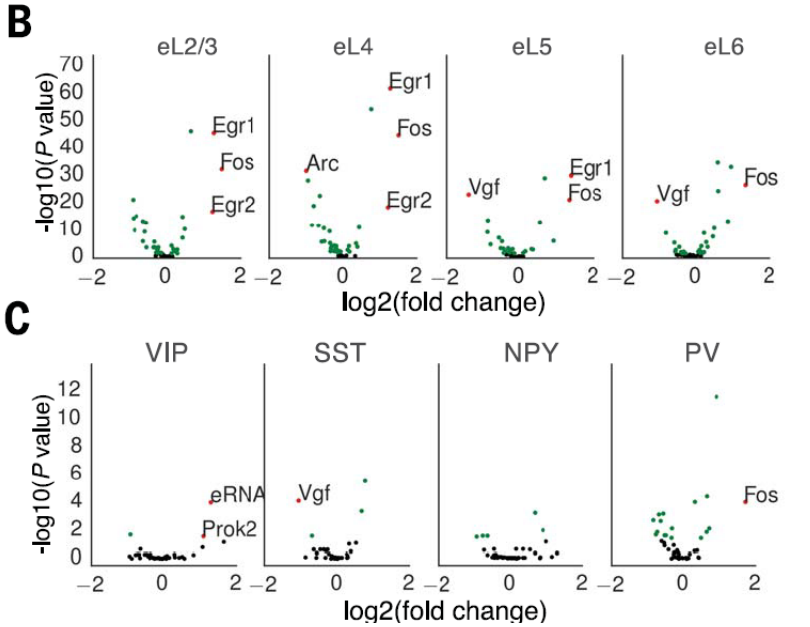
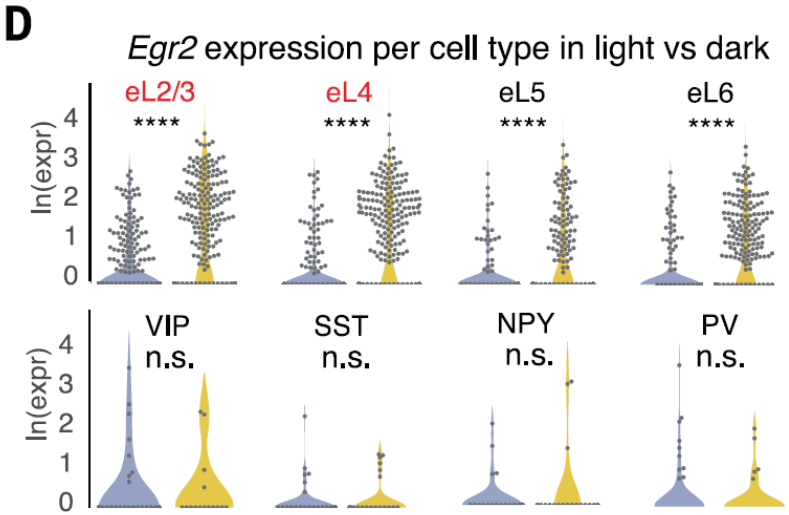


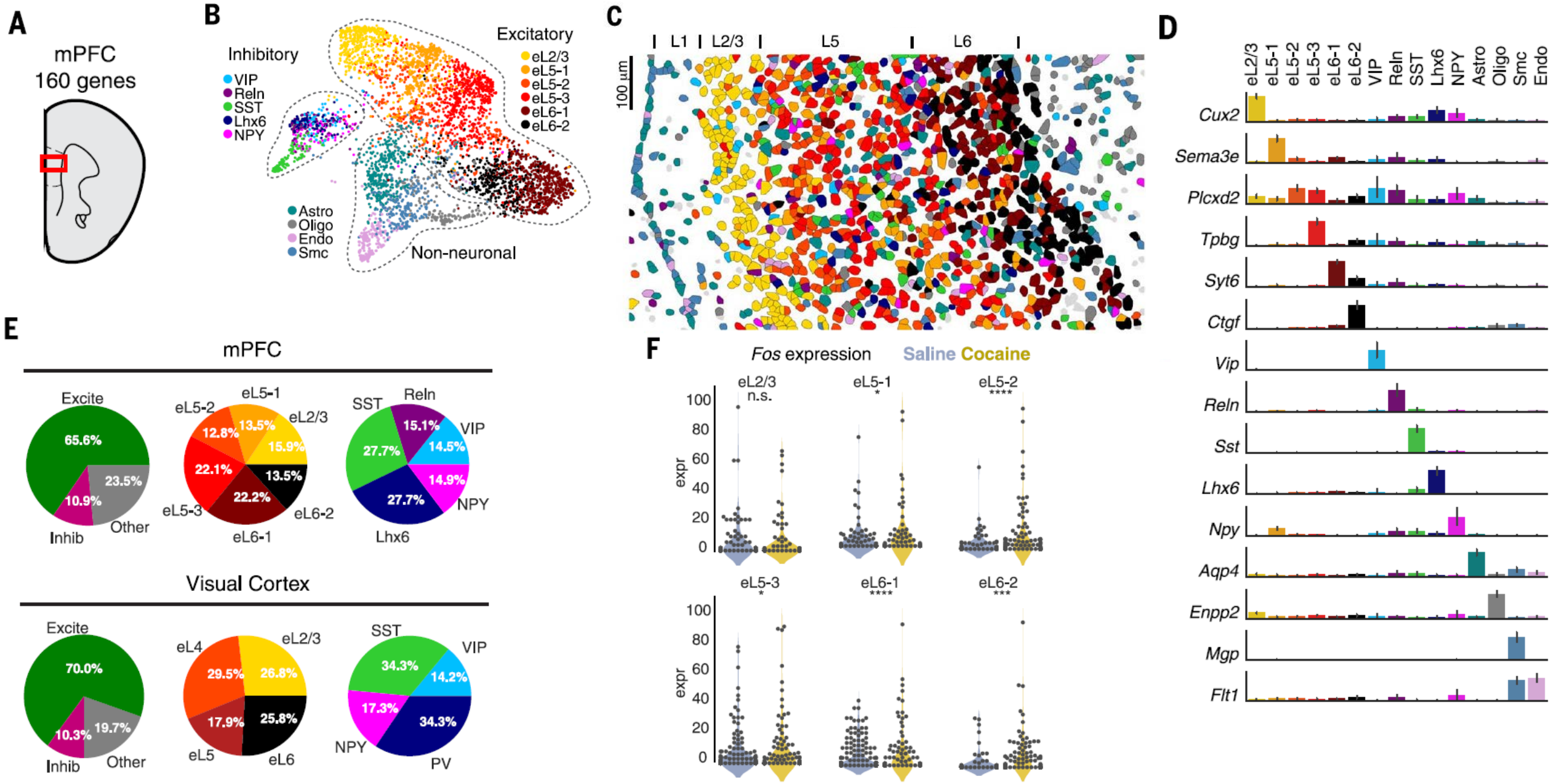




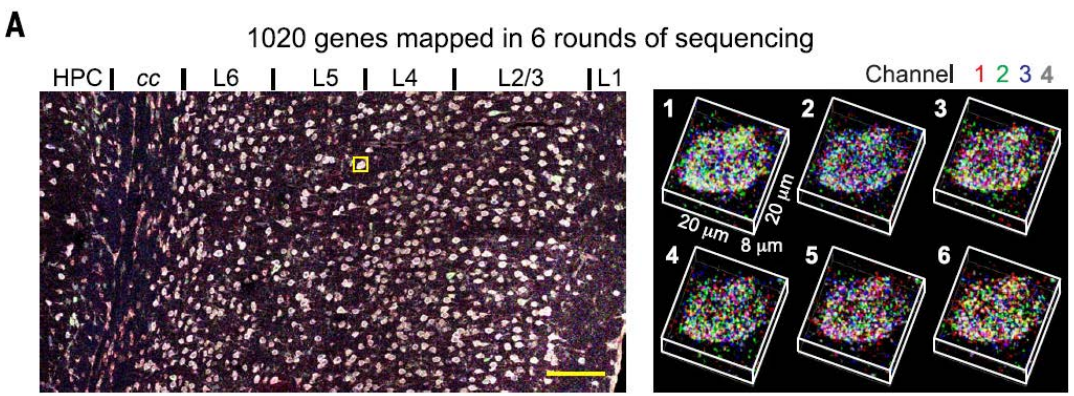


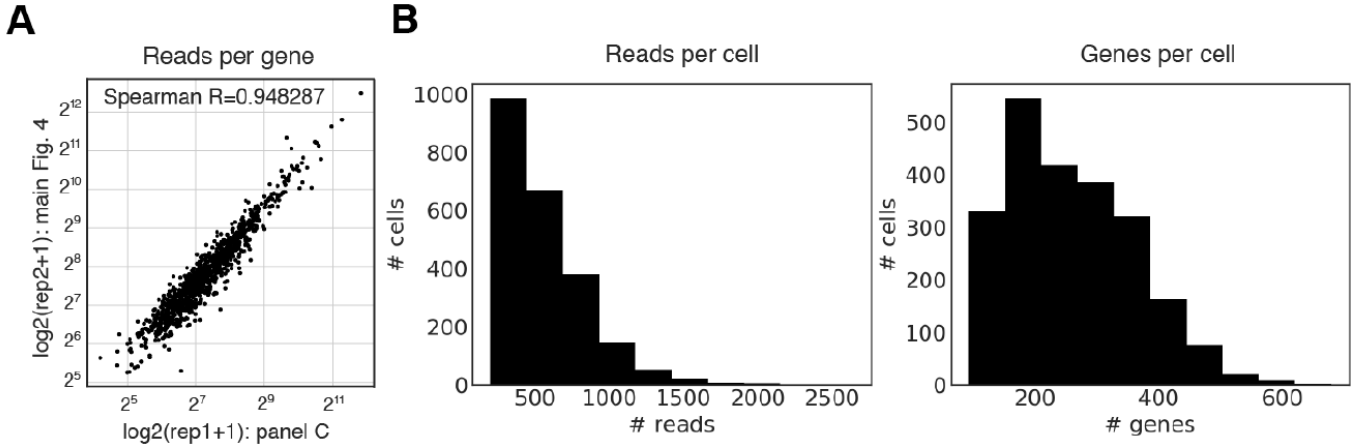


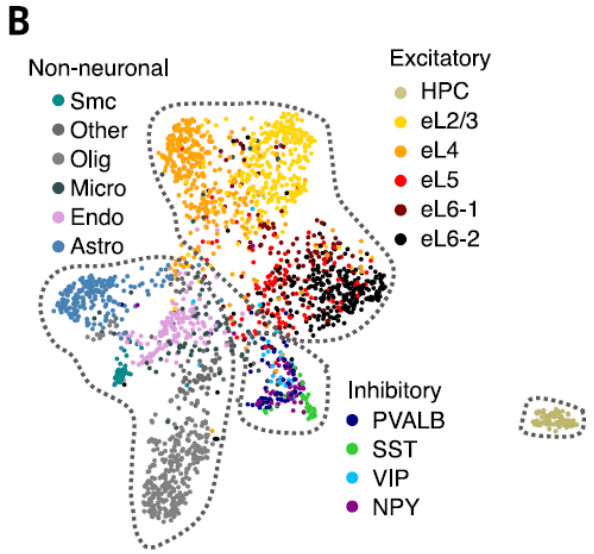
STARmapping cell types and neural activity in mPFC

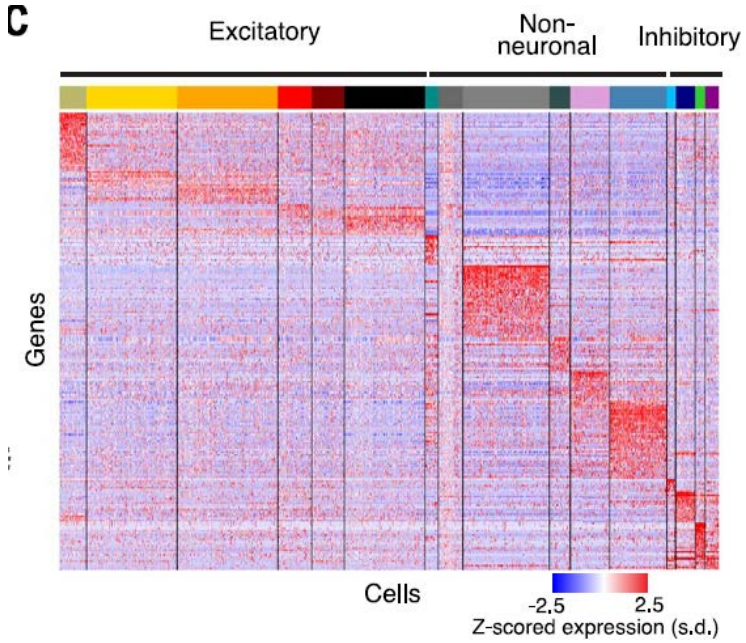


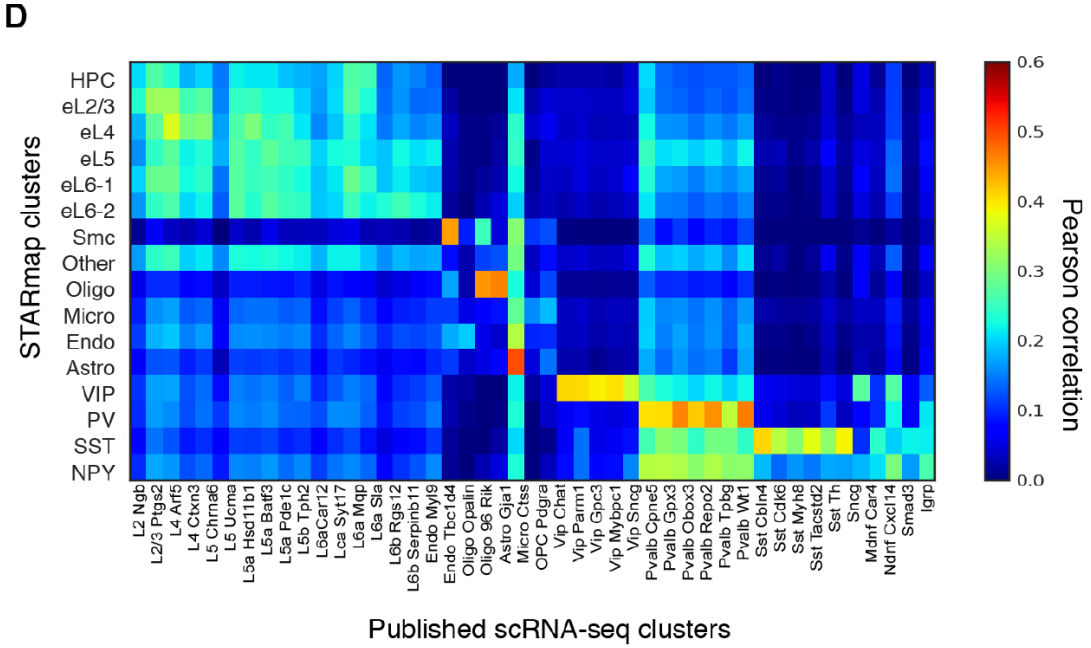
Simultaneous mapping of 1020 genes in V1 by STARmap









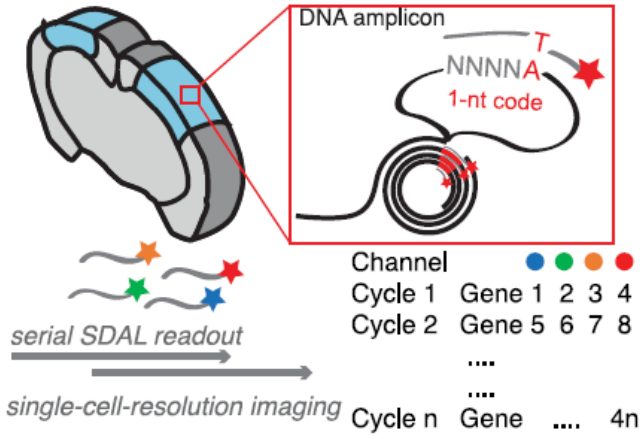


STARmap in thick tissue blocks for 3D analyses

A

	Thin tissue (z < 16 μm, cell monolayer)	Thicker tissue (z >100 μm, multiple cell layers)
Sample dimension		
Library preparation	***Day 1*** Freshly frozen mouse brain → Cryostat slicing → PFA fixation → Permeablization → Hybridization → ***Day 2*** Ligation & amplification → Hydrogel embedding & tissue clearing	***Day 1*** PFA-fixed mouse brain → Vibratome slicing → ***Day 1-2*** Permeablization & hybridization (modified probes) → ***Day 3*** Hydrogel embedding & tissue clearing → ***Day 4-5*** Ligation & amplification
Imaging & sequencing	***Day 3-5*** Single-amplicon resolution; High NA oil-immersed objective; Imaging 200 cells per hour; SEDAL reaction with degenerate probes; Exponential readout from cycles to genes	***Day 6-9*** Single-cell resolution; Low NA water-immersed objective; Imaging 10, 000 cells per hour; SEDAL reaction with orthogonal probes; Linear readout from cycles to genes
Data Output	3D amplicons 2D cell typing (main Figure 1-5)	3D cell typing (main Figure 6)

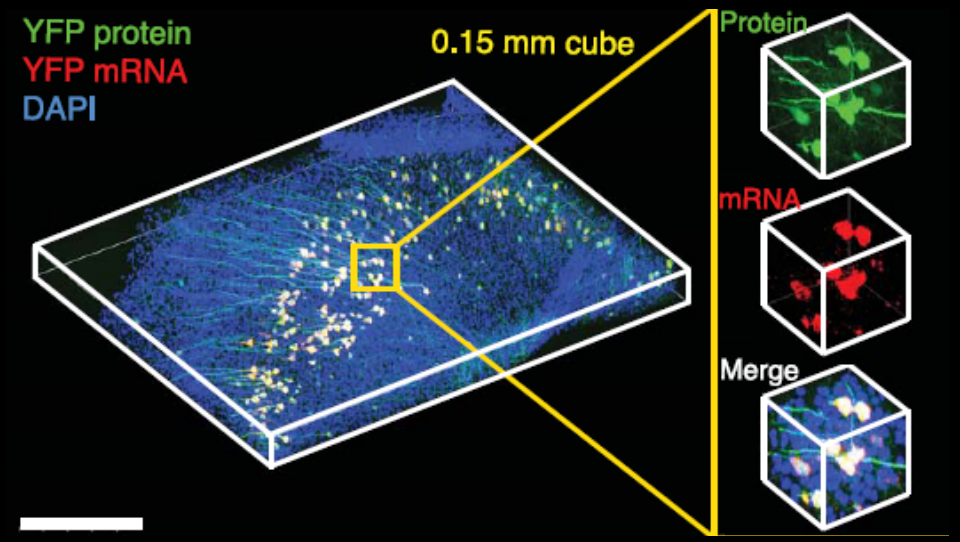
A STARmap optimized for rapid imaging of large tissue volume

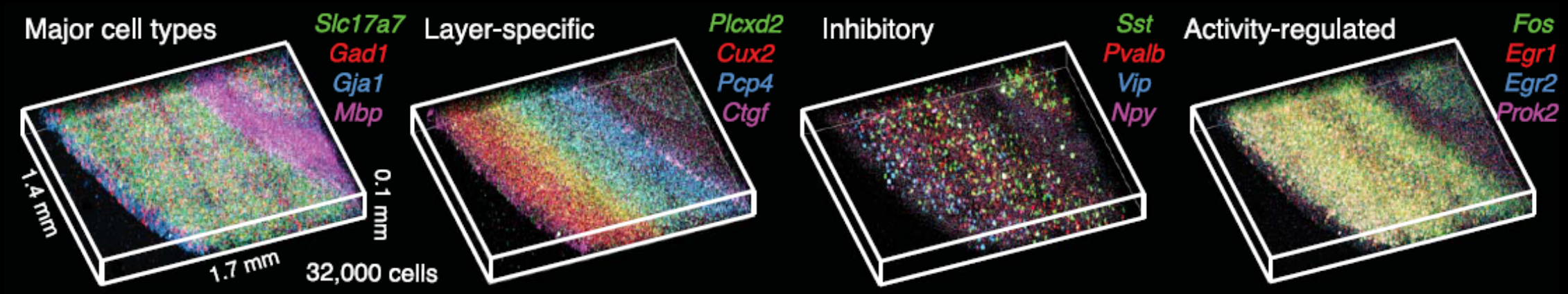


C Time vs. # genes

Tissue Type	Genes	Time
Thin tissue	4	2 days
Thin tissue	160	5 days
Thin tissue	1020	5 days
Thick tissue	1	5 days
Thick tissue	28	9 days

STARmap in thick tissue blocks for 3D analyses





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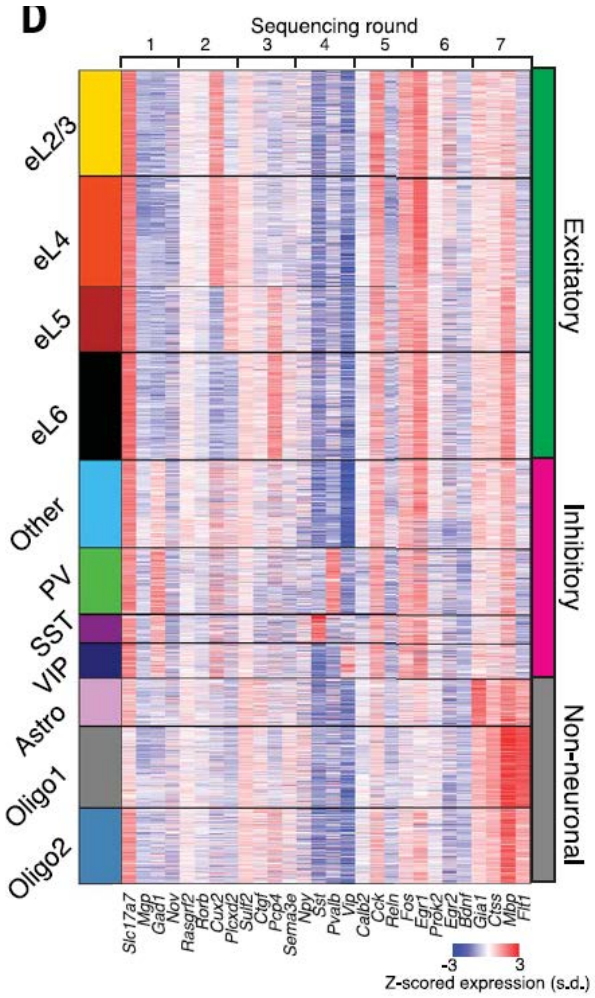

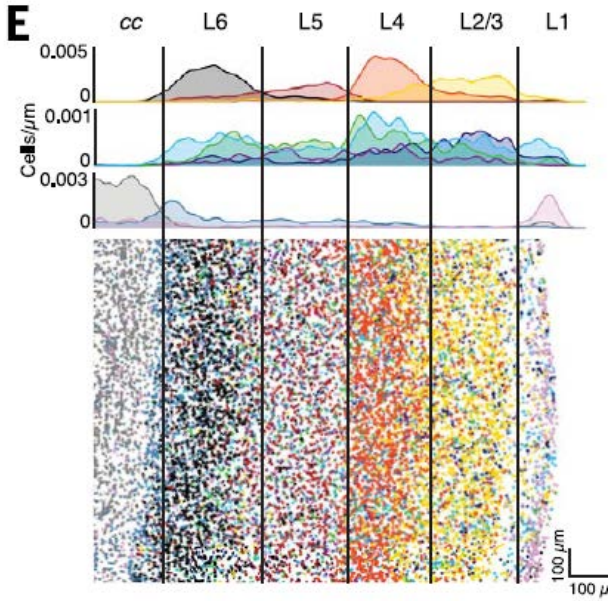

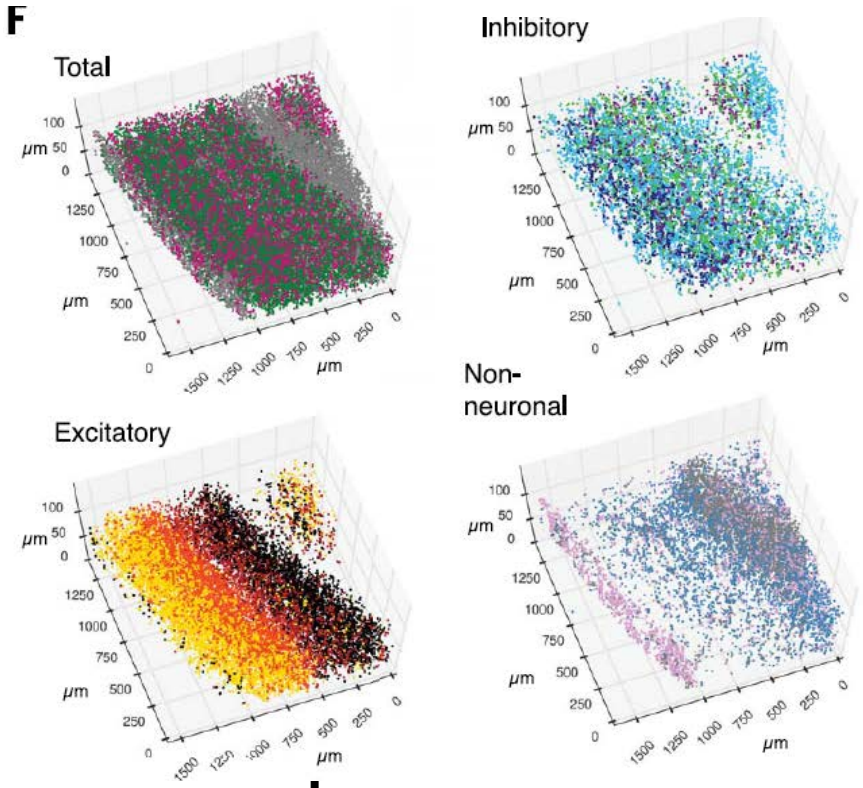

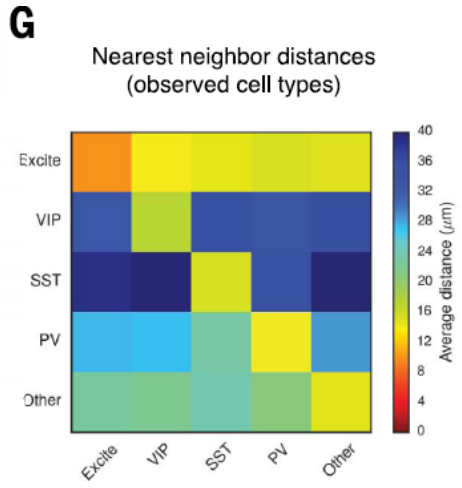

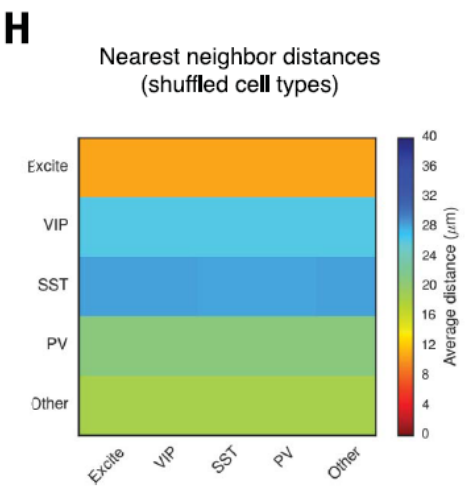

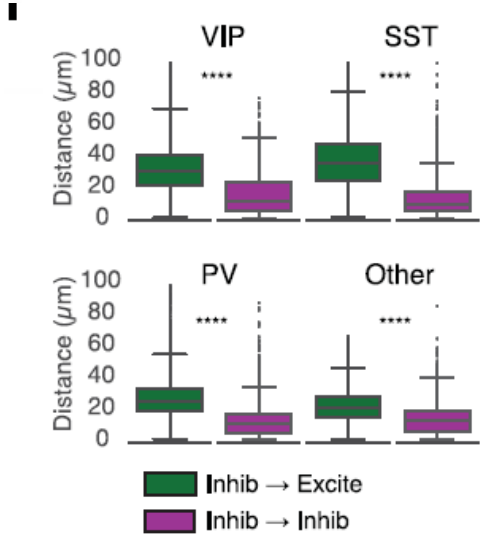

Summary

- STARmap defines a platform for 3D *in situ* transcriptomics
- Study molecularly defined cell types and activity-regulated gene expression in mouse cortex
- Scalable to larger 3D tissue blocks so as to visualize short- and long-range spatial organization of cortical neurons on a volumetric scale
- In theory, ~1 Mio. codes → upper limit of the optical volume of cells at ~1020 genes → serial sequencing rounds, higher super-resolution microscopy

Scalability of STARmap

Capacity	Experimentally verified	Theoretical estimation
Coding limits	≥ 1,000 codes	≤ 10^6 codes (by SEDAL)
Physical limits	≥ 1,000 genes	≤ 10^6 amplicons/cell
Optical limits	≥ 1×10^4 amplicons/cell (cell culture, high-quality through 6 rounds) ≥ 2,600 amplicons/cell (brain tissue, high-quality through 6 rounds)	≤ 2×10^4 amplicons/cell

Comparisons of STARmap and single-cell PCR/RNA sequencing

Method	RNA species	Spatial resolution	Quantification	#Cells
STARmap thin-tissue	≥ 40 nt	single-amplicon (250 nm)	absolute RNA copies	10^2~ 10^3
STARmap thick-tissue	≥ 40 nt	single-cell (1 μm)	relative intensities	10^4~ 10^5
single-cell PCR	≥ 100 nt	No	relative amount or absolute RNA copies	10^1~ 10^2
single-cell RNA sequencing	poly(A)+	No or 100 μm	absolute RNA copies	10^2~ 10^5

Thank you for your attention!