Multiomics at Illumina: Sequencing Beyond the Genome

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May 15, 2025

Illumina install base generates 8 human genome equivalents of data per minute, every minute, of every day[1]

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[1] Illumina Internal Data on file, 2025

The power of genomics across clinical applications

REPRODUCTIVE HEALTH

GENETIC DISEASE

ONCOLOGY

RESEARCH / APPLIED GENOMICS

INFECTIOUS DISEASE

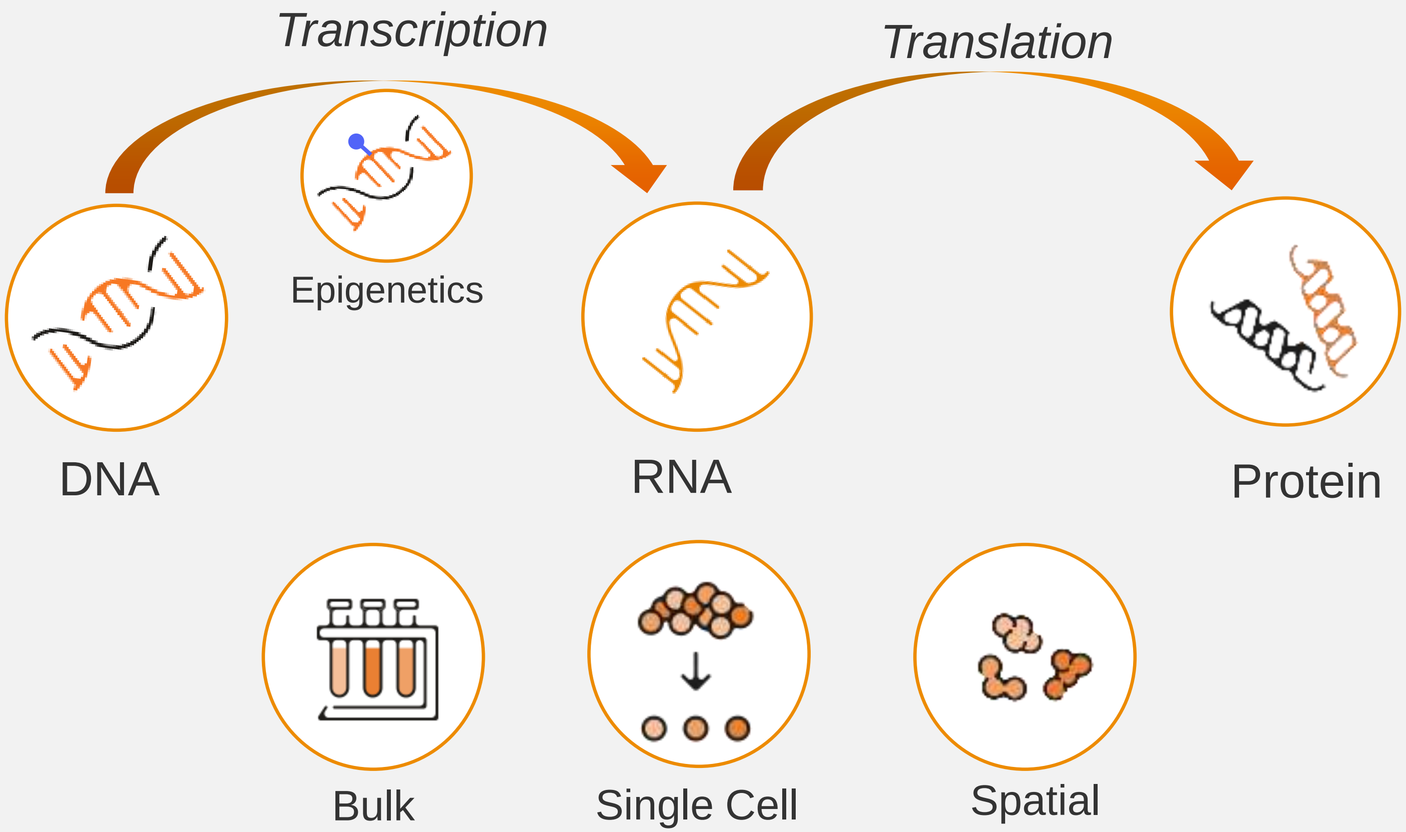
DRUG DISCOVERY

Multiomics is shaping the next era of precision medicine

Requires highly accurate, scalable, and flexible analysis

- Genomics
- Transcriptomics
- Epigenomics
- Proteomics
- Spatial

From genomics to multiomics - studying the entire central dogma



Transcription

Translation

Epigenetics

DNA

RNA

Protein

Bulk

Single Cell

Spatial

Preparing non-genomic modalities for sequencing requires innovation

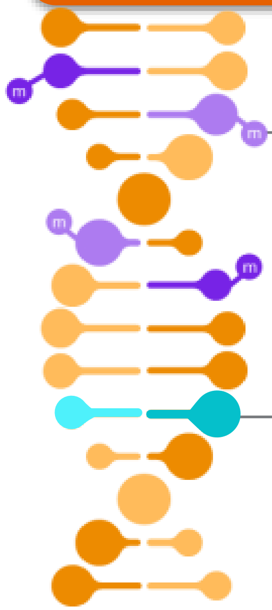
DNA is inherently multiomic: *Introducing the 5-base genome*

5-Base Technology

One sample

Dual-omic insights

Empowering discovery



DNA **methylation** regulates gene expression

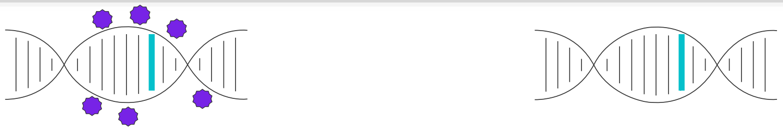
DNA **variants** inform static predisposition

Increase discovery power

Single-ome

Dual-ome

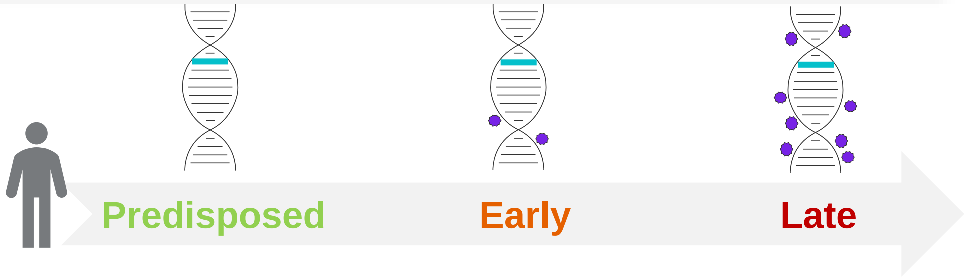
Intersectional changes inform interpretations



Benign

Malignant

Profile longitudinal disease biology



Predisposed

Early

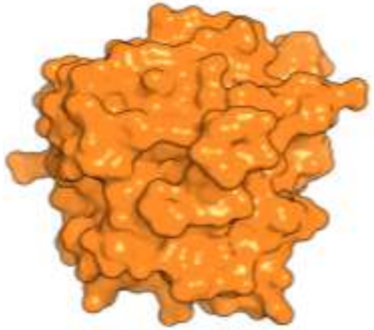
Late

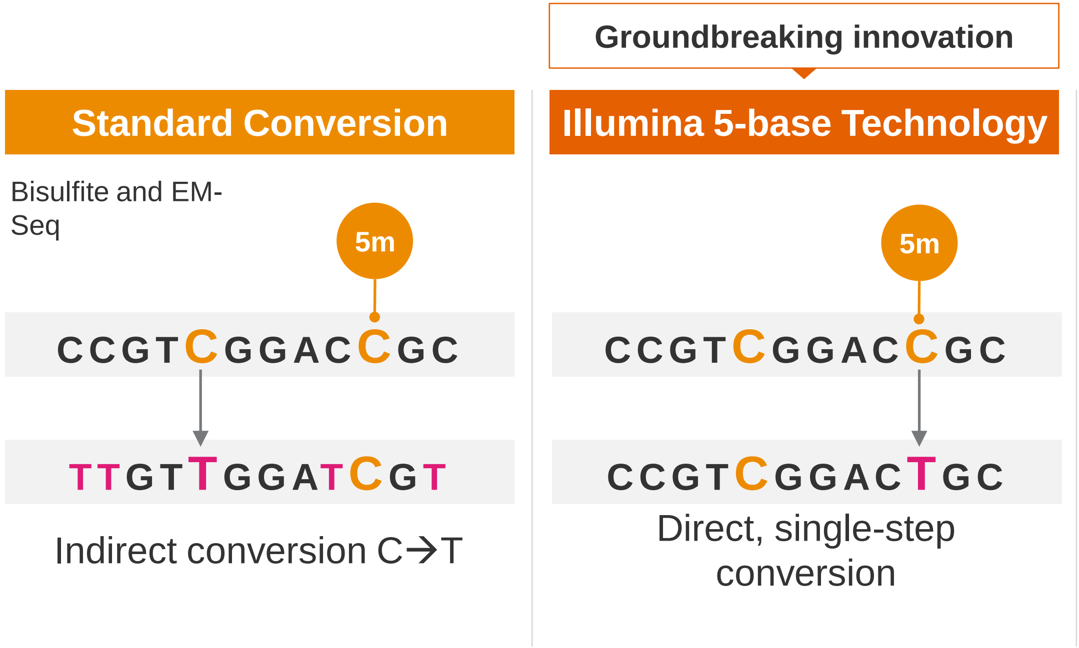
Illumina methylation enzyme

An engineered enzyme to selectively convert 5mC into T

Millions of mutants screened over multiple rounds. Final variant with multiple mutations achieving:

- High selectivity
- High efficiency
- High stability





Groundbreaking innovation

Standard Conversion

Bisulfite and EM-Seq

5m

CCGTCGGACCGC

TTGTTGGATCGT

Indirect conversion C→T

Illumina 5-base Technology

5m

CCGTCGGACCGC

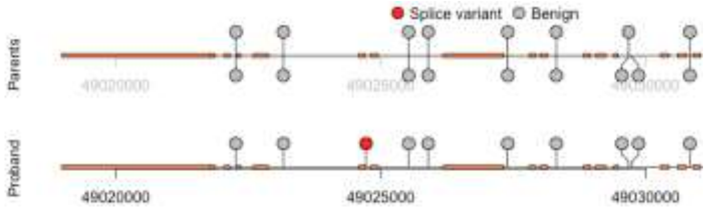
CCGTCGGACTGC

Direct, single-step conversion

Dualomic 5-base readout guides interpretations

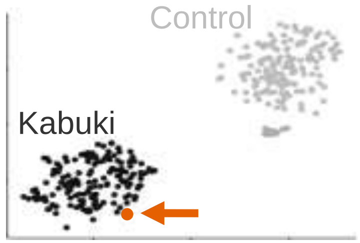
DNA Variant | **Disease Episignature** | **Resolution**

Case 1



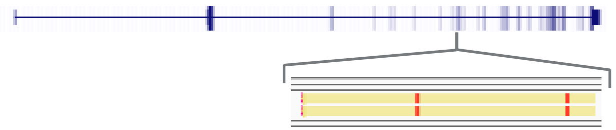
KMT2D c.15922-12C>G: splicing variant

&



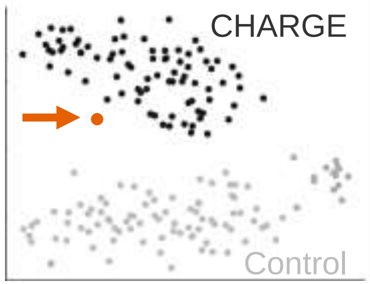
Kabuki Syndrome

Case 2



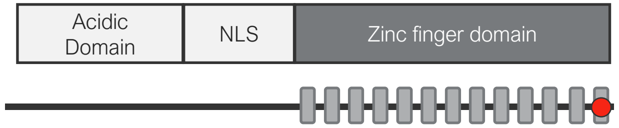
CHD7 278 bp ALU insertion

&



CHARGE Syndrome

Case 3



ZFX c.2357G>A p.(Arg786Gln)

&

X-linked Intellectual Disorder

3 RUGD trios

No diagnosis obtained from standard genetic testing

- DNA variant in disease-associated gene
- Methylome pattern matches the same disease
- **Convergent findings from orthogonal omes confirm classification of novel disruption**

Transcriptomics has evolved dramatically

Information content increases from Bulk to Single-Cell to Spatial

Bulk RNA expression

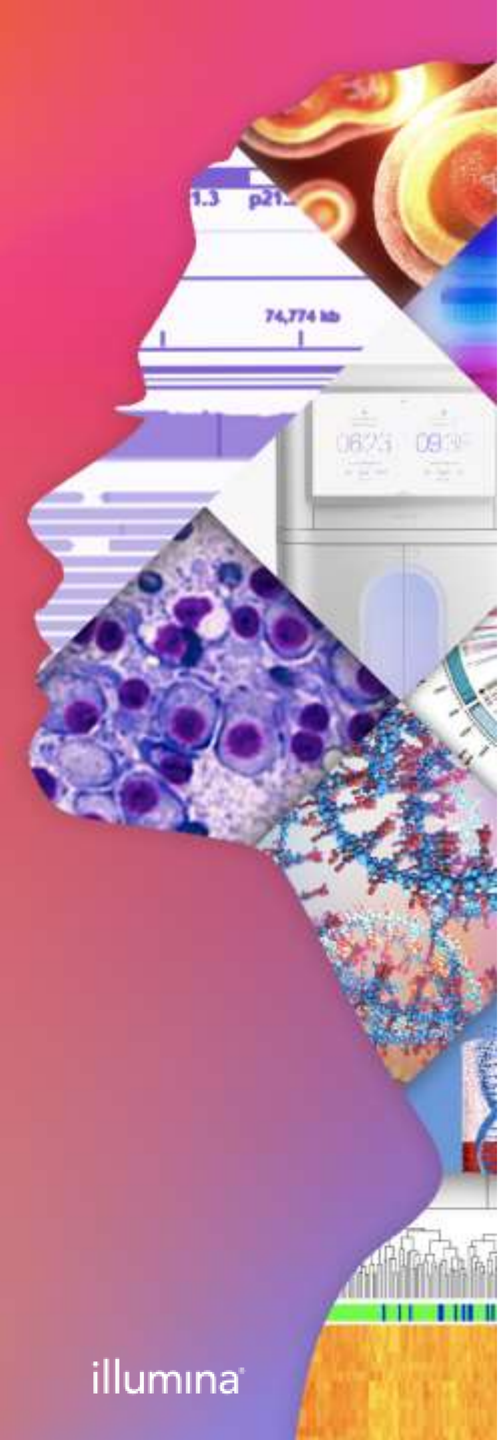
- Homogenize tissue and analyze changes in gene signatures.
- Expression changes are averaged across a tissue

Single-cell RNA expression

- Reveal the expression profile of all cell types present in a tissue sample
- Determine the specific cells or cell types where expression changes occur

Spatial RNA expression

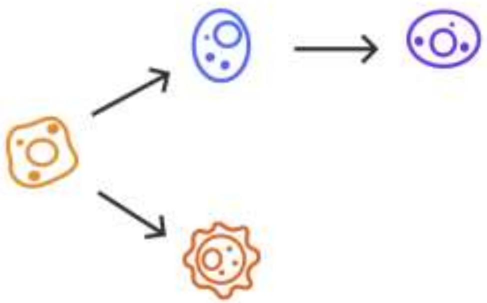
- Examine how gene expression within a cell relates to gene expression in nearby cells
- Do this with a richness of information similar to single-cell



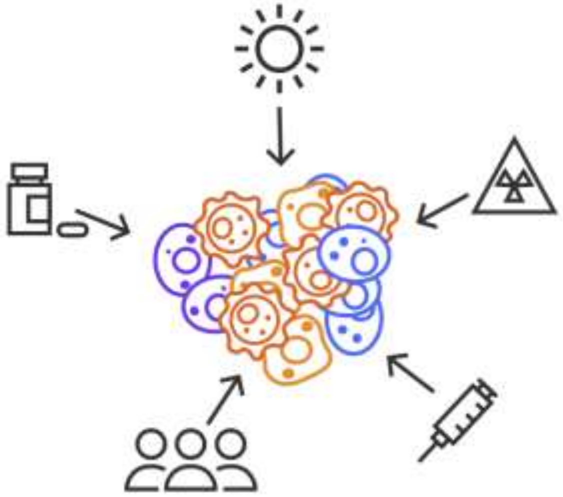
Single-cell sequencing is one of the fastest-growing NGS methods, with 62% of NGS researchers currently using single cell methods

Understand the development of complex organs, and their diseases

Model and predict behavior of cells during development



Detect how different cell populations react to external factors



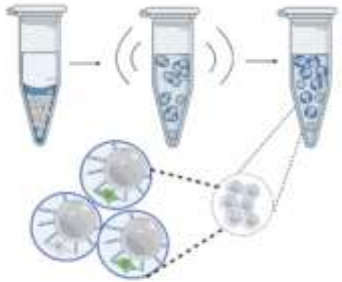
DNA, RNA, Epigenomics, and Protein can all be studied at the single cell level, alongside popular screening methods like CRISPR/Cas9

2023 Study, Percepta Associates, Report on file. Illumina Single Cell Methods Guide, 2024

How is RNA from individual cells captured and sequenced?

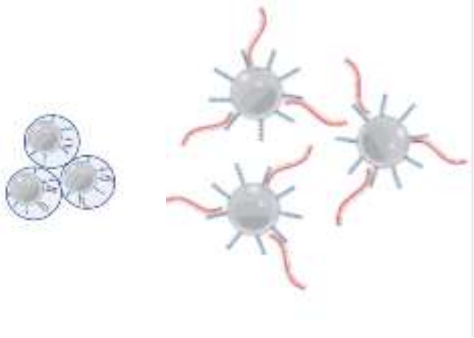
Isolate cells

Separate cells into droplets



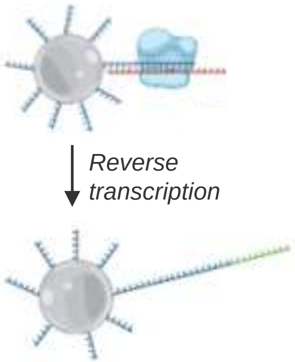
Digital vortexer creates emulsion of tiny droplets each containing a single cell

Lyse cells and capture RNA



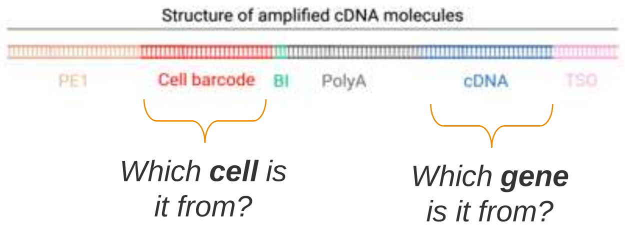
mRNA has a poly-A tail. This sequence can be used to capture RNA on beads

Copy RNA onto bead



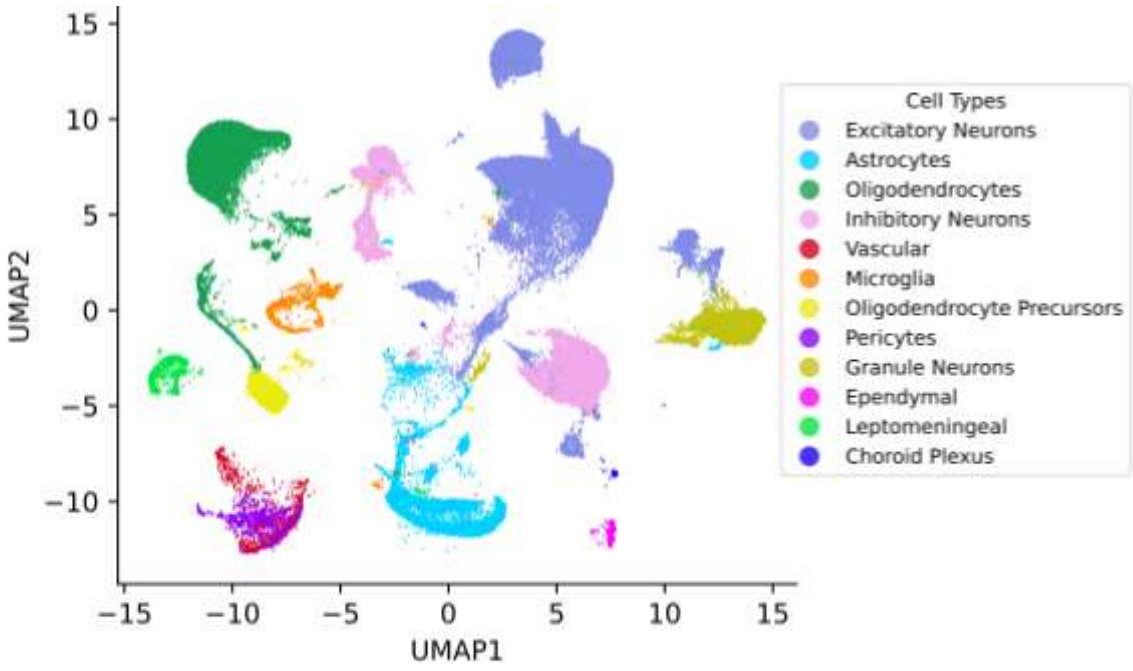
Copy DNA & Sequence

Sequencing reads contain a unique sequence from the bead and the RNA sequence (cDNA)

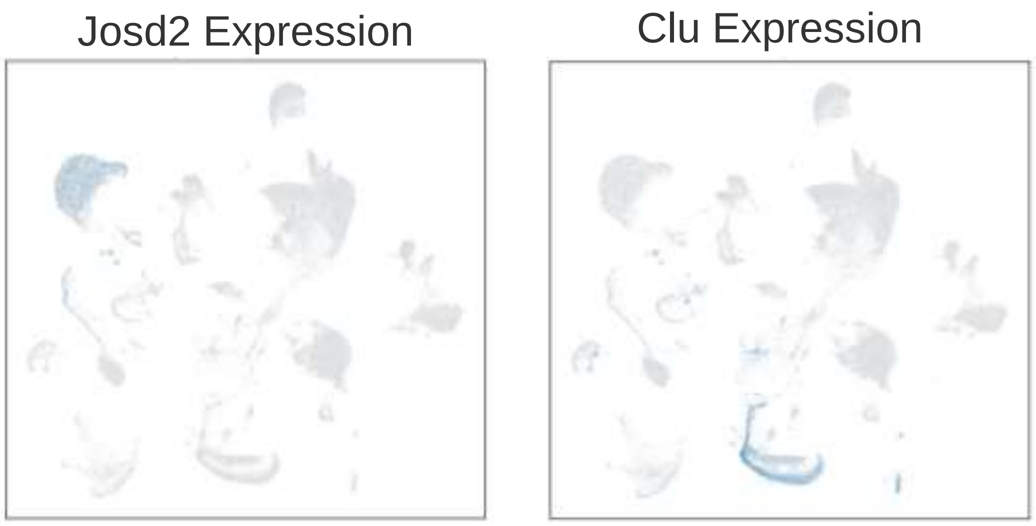


Single cell analysis reveals unique characteristics of cell types

Cell types separate into distinct clusters based on gene expression profile



Different cell types have elevated expression of specific marker genes



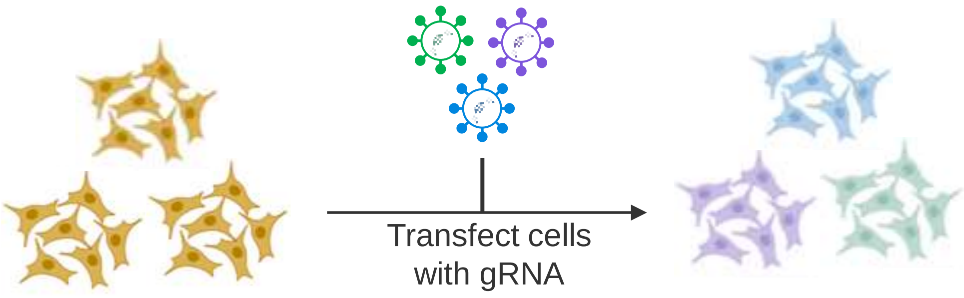
Neurons

Astrocytes

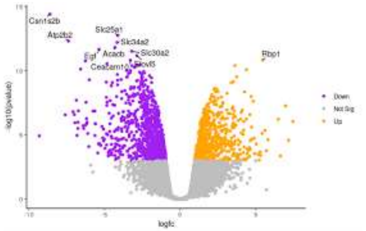
scRNA with CRISPR provides deeper insights

Gene regulation and cell differentiation can be studied at the cellular level

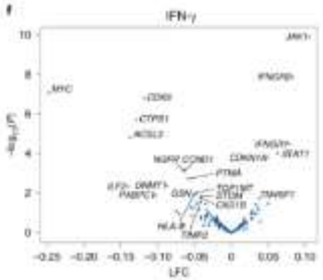
Deactivated CRISPR-Cas9 can be used to interfere with gene expression, providing an incredibly rich dataset of regulatory information



Transfect cells with gRNA

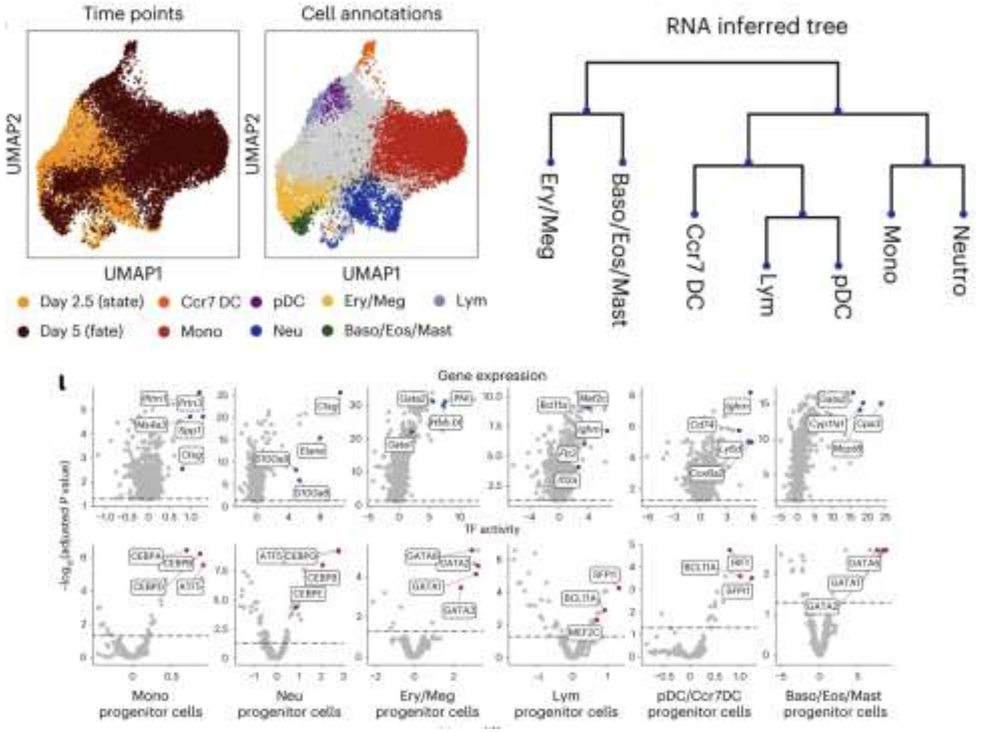


Differential expression analysis between perturbations



Analysis of perturbation impact on pathway activation & suppression

CRISPR can be used to tag cells & study cell lineage during cell differentiation



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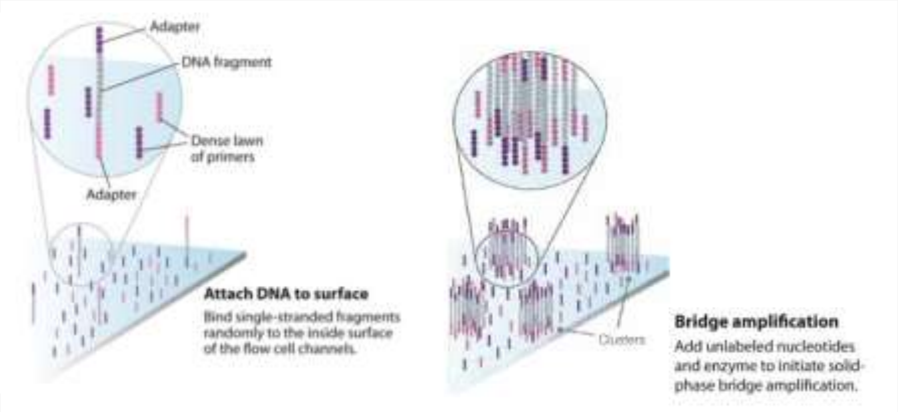
1. Bock, Christoph et al. "High-content CRISPR screening." *Nature reviews. Methods primers* vol. 2,1 (2022): 9. doi:10.1038/s43586-022-00098-7
2. Frangieh, Chris J., et al. "Multimodal pooled Perturb-CITE-seq screens in patient models define mechanisms of cancer immune evasion." Nature genetics 53.3 (2021): 332-341
3. Jindal, K., Adil, M.T., Yamaguchi, N. *et al.* Single-cell lineage capture across genomic modalities with CellTag-multi reveals fate-specific gene regulatory changes. *Nat Biotechnol* **42**, 946–959 (2024). https://doi.org/10.1038/s41587-023-01931-4

An innovative use of Illumina technology

Traditional genomic sequencing involves creating clusters of DNA on a flow cell surface

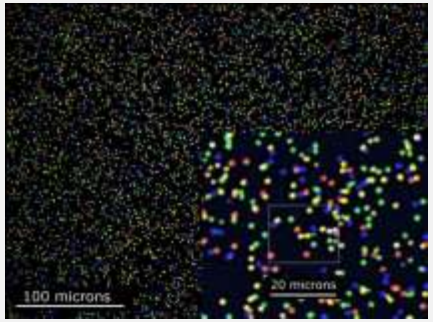


Attach DNA to surface

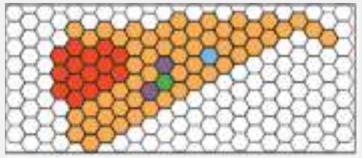
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

Bridge amplification

Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

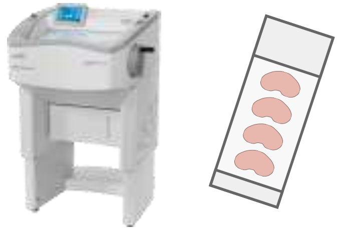


Adding a poly-A capture sequence to each cluster creates a surface of “pixels” that can be used to image spatial transcriptomics data

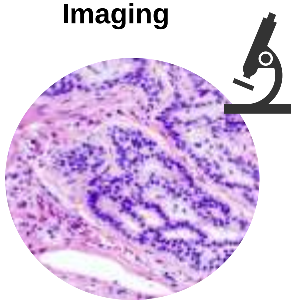


Placing tissue directly on the flow cell surface enables spatial transcriptomics

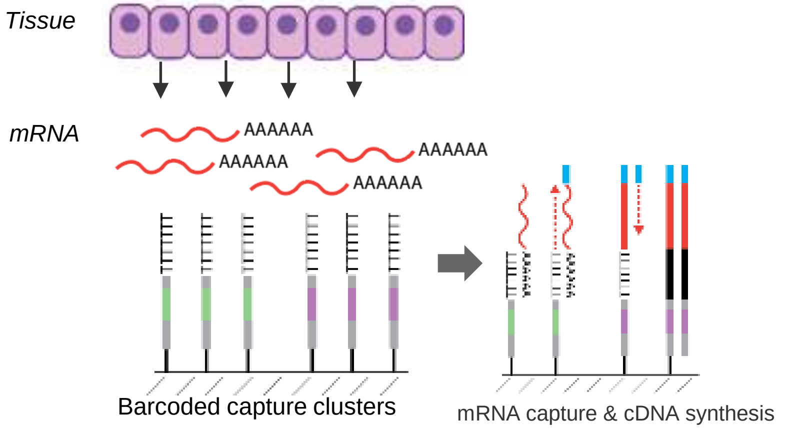
Sectioning and mounting on barcoded surface



Staining & Imaging



Release of mRNA from tissue



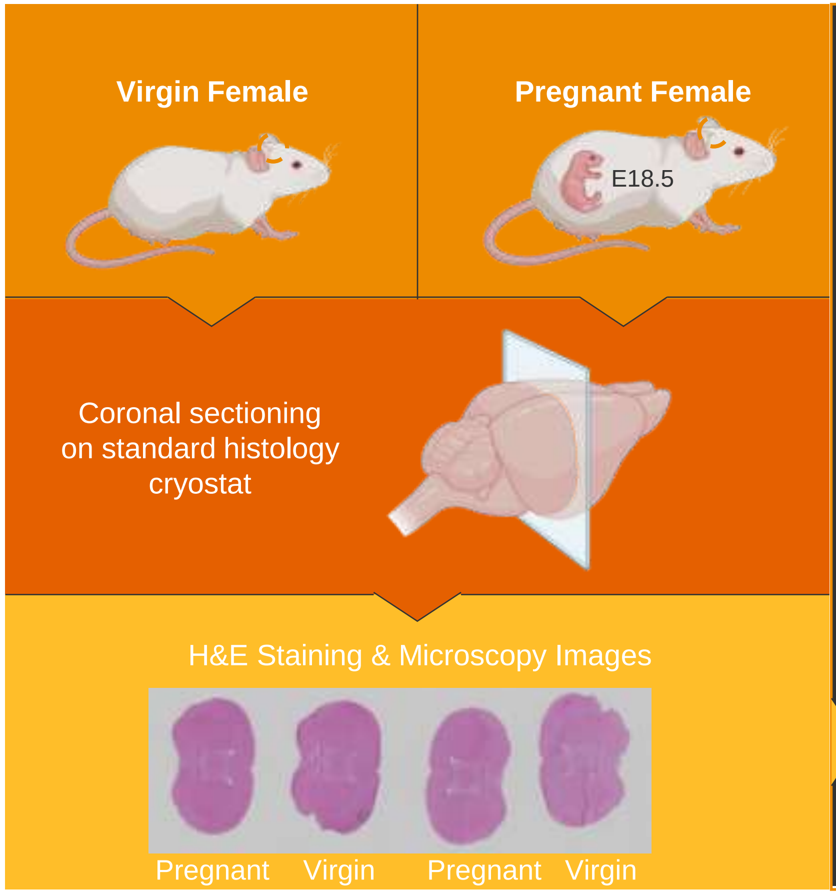
Barcoded capture clusters

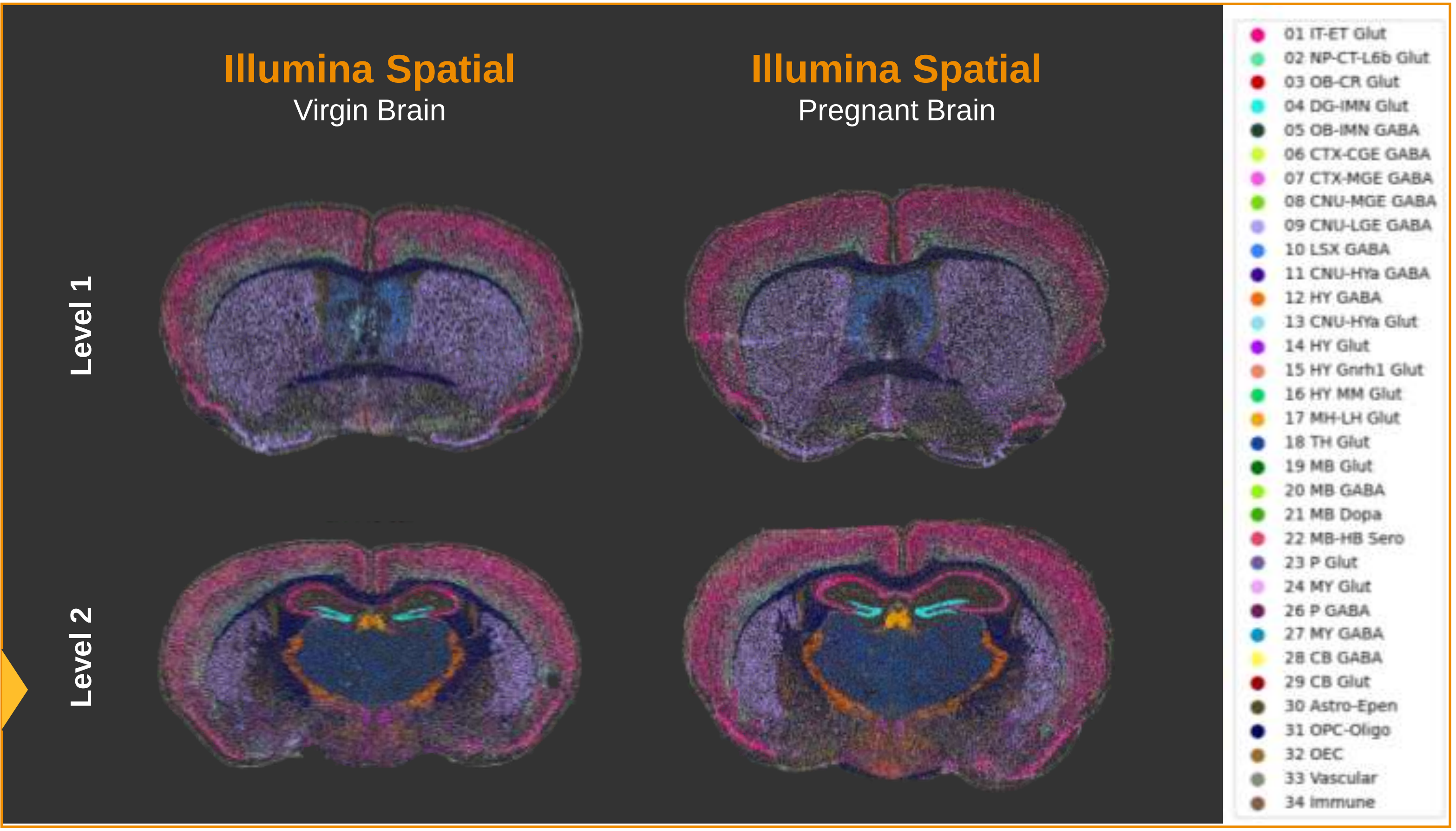
mRNA capture & cDNA synthesis

Sequencing

Sequencing on a standard flow cell

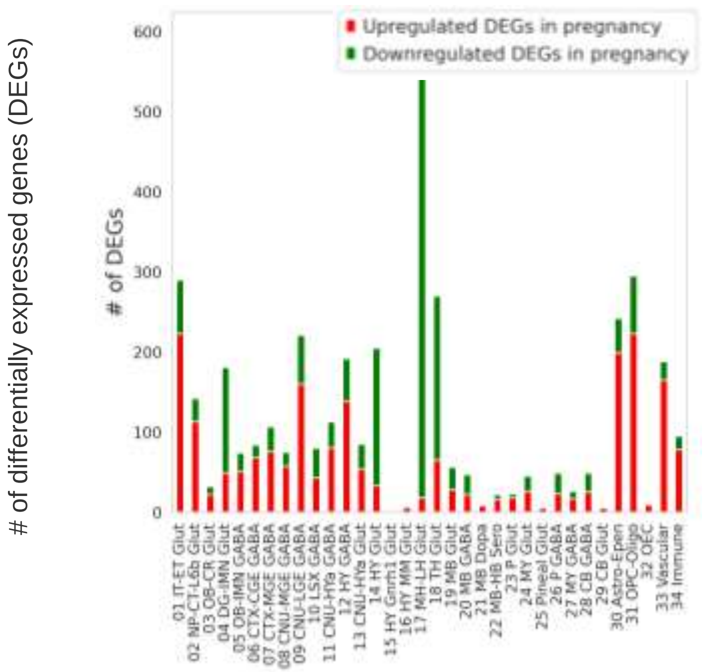
Annotated cell typing using scRNA-seq reference data produced detailed spatial maps across all brain regions



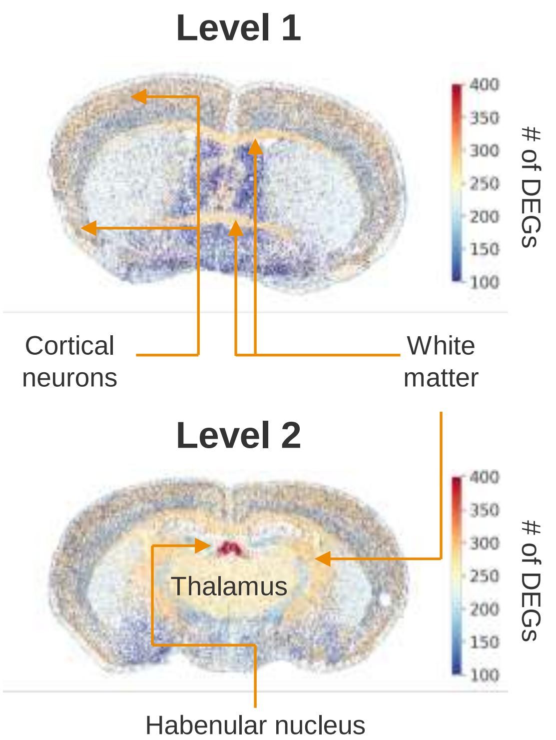


Pregnancy changes expression in >2000 genes

Significant changes in the habenular nucleus, known to be is involved in mood, stress and anxiety regulation



Significantly changed gene cutoff: $p < 0.05$ & log fold change > 0.5 or < -0.5

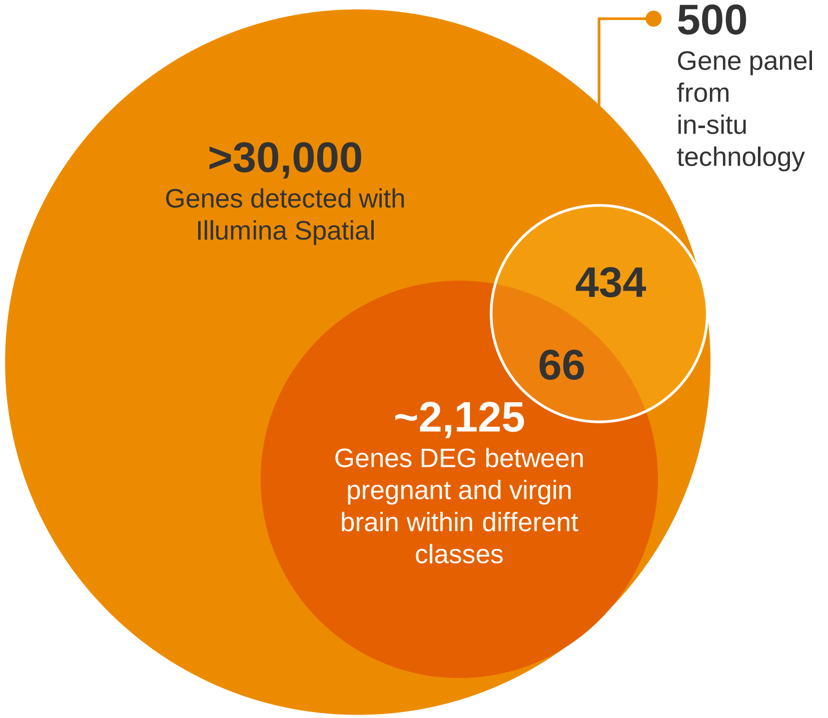


Cortical neurons

White matter

Thalamus

Habenular nucleus



500
Gene panel from in-situ technology

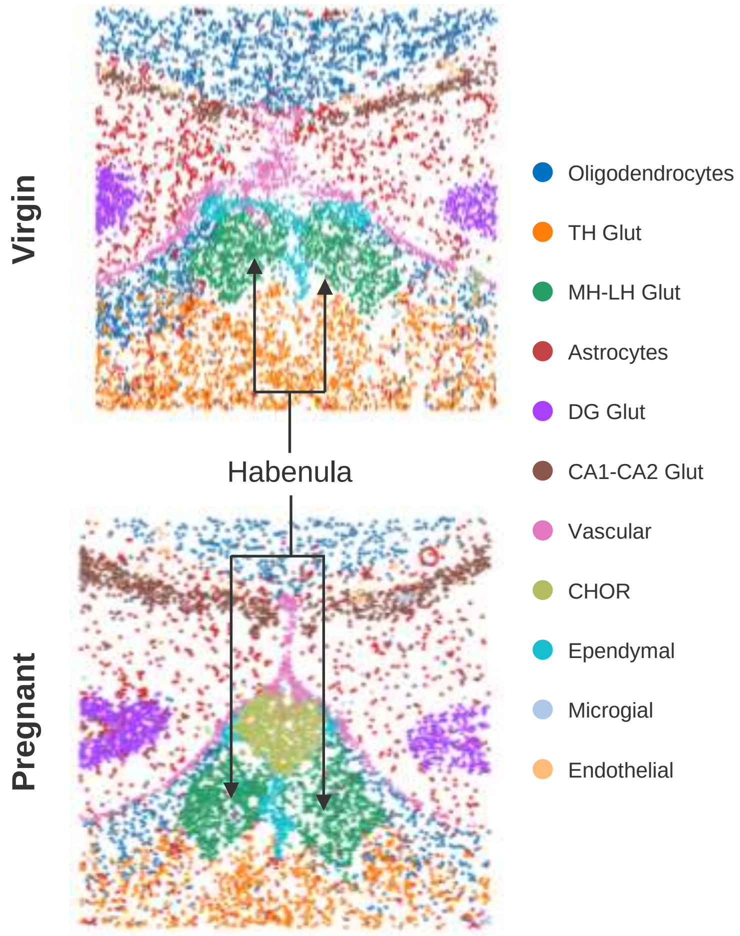
>30,000
Genes detected with Illumina Spatial

434

66

~2,125
Genes DEG between pregnant and virgin brain within different classes

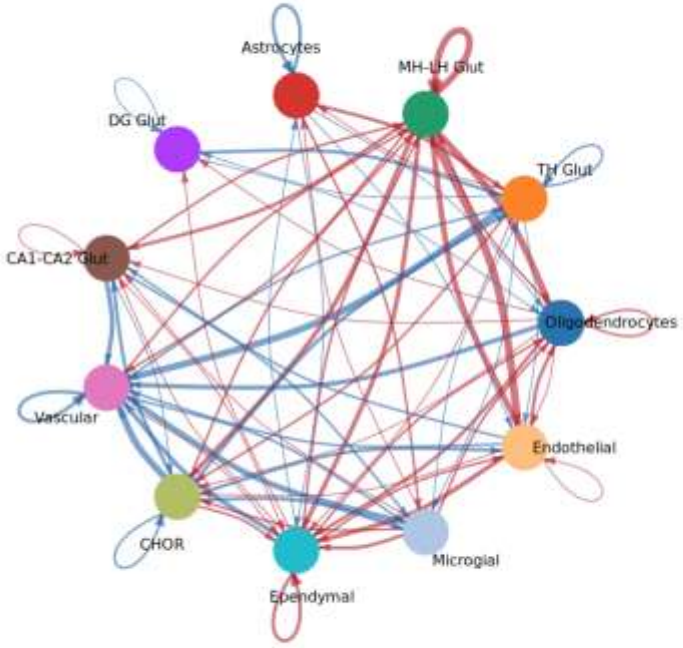
Cell-to-cell communication is altered in the habenular region



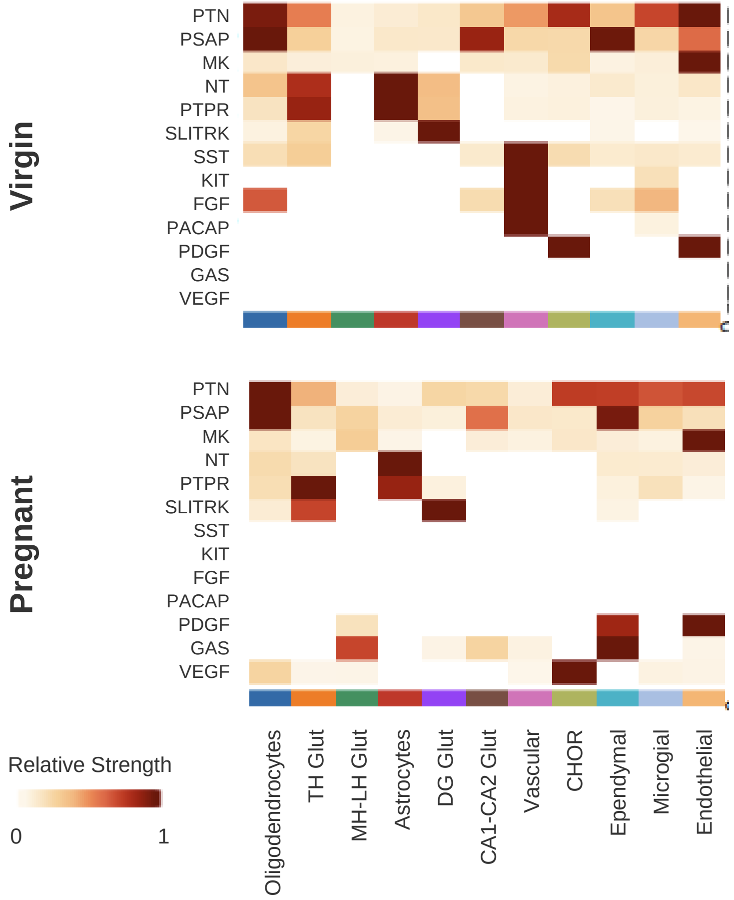
Cell type interactions (cellchat2)

● Red connection increased in pregnant

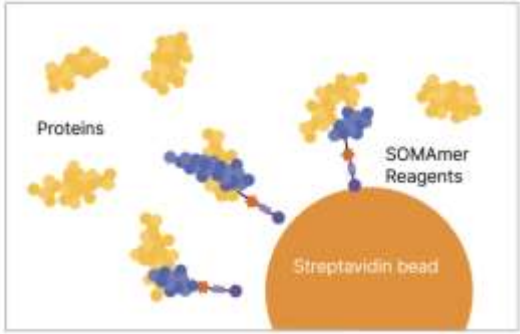
● Blue connection decreased in pregnant



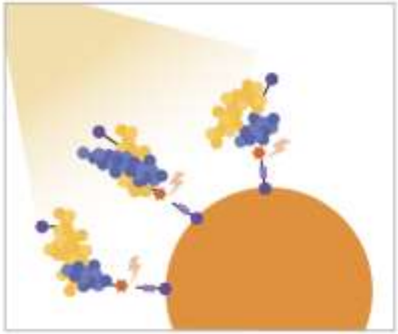
Signaling pathway Strength



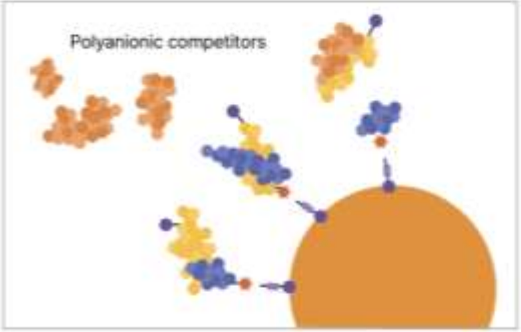
Illumina Protein Prep delivers highly sensitive protein capture using SOMAmer Reagents and Illumina library preparation



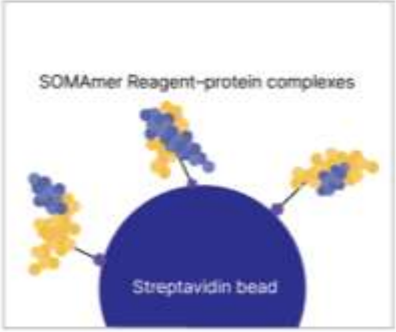
Protein capture using SOMAmer Reagents



UV light releases complexes back into solution



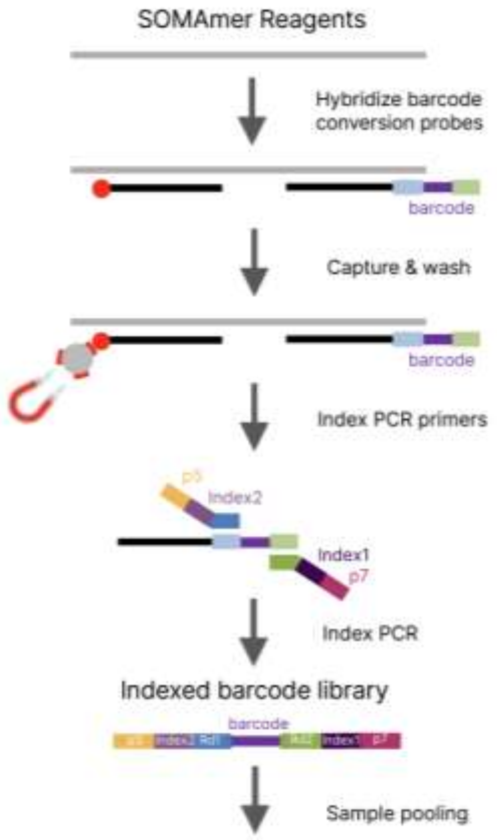
Specific complexes remain bound. Polyanionic competitor prevents rebinding of nonspecific complexes



Biotinylated SOMAmer Reagent–protein complexes bind to streptavidin beads

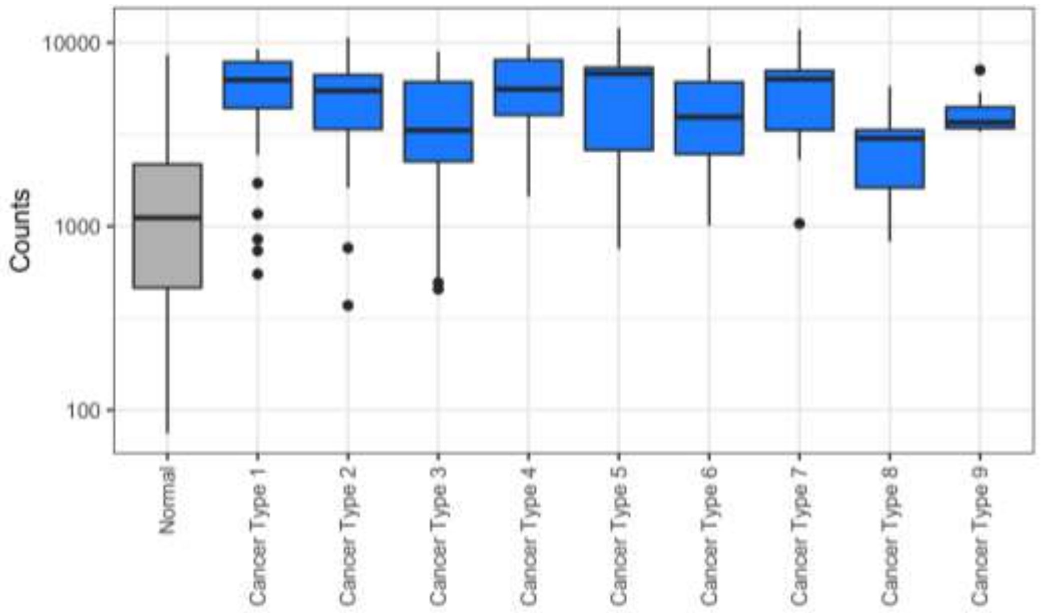
The SomaScan assay quantitatively **transforms the protein epitope availability** in a biological sample into a specific signal. Relative protein concentrations are then converted into SOMAmer Reagent abundances.

SOMAmer Reagent abundances are converted to barcoded sequencing-ready libraries using a hybridization approach.



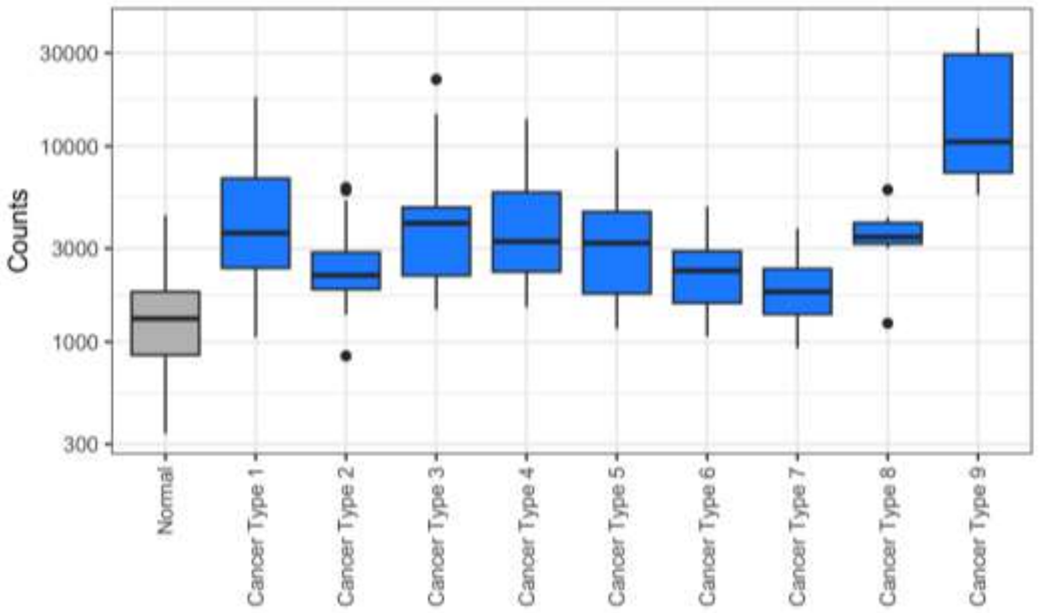
Illumina Protein Prep used to study differentially expressed proteins in different types of cancer

Inflammatory Protein



Inflammatory proteins may reflect general host response to tumor or treatment

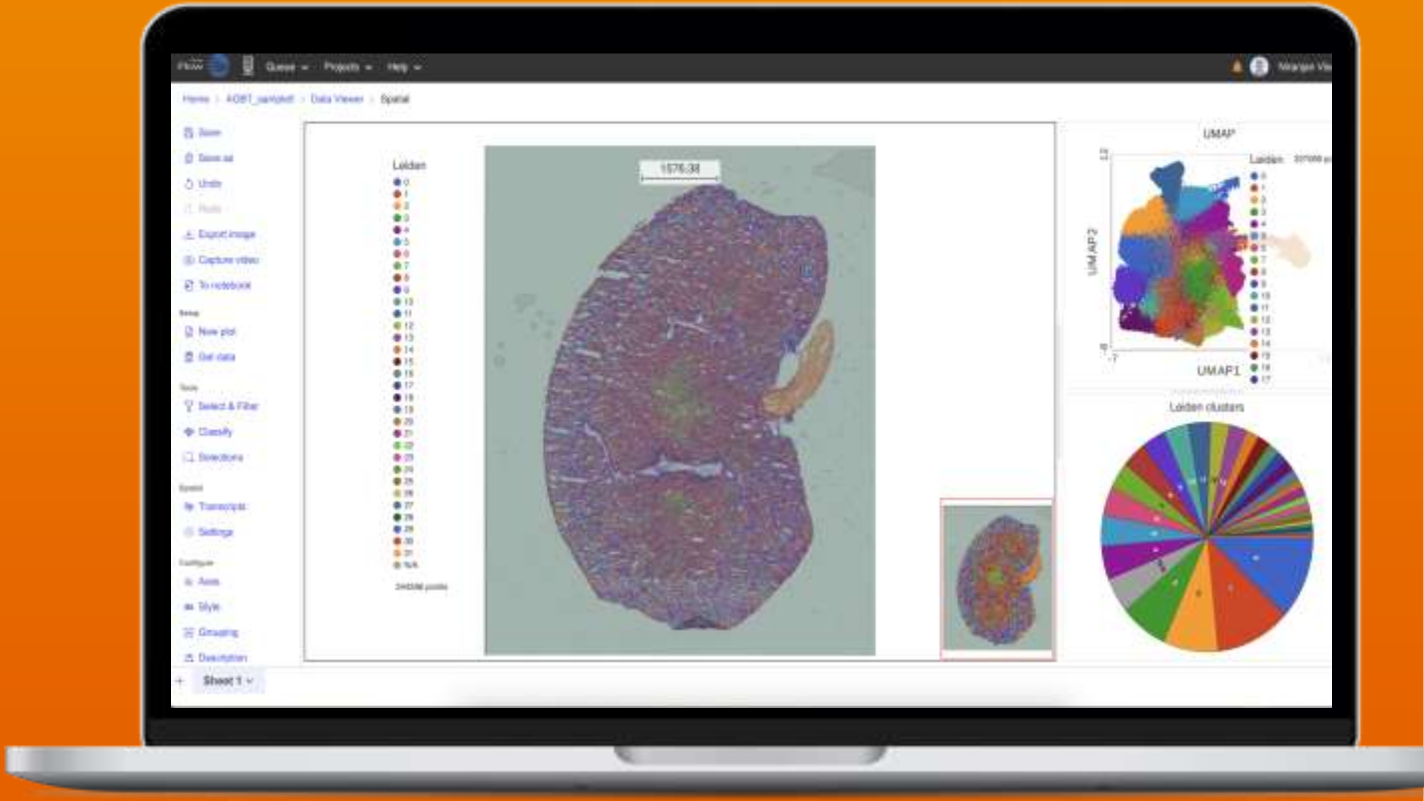
Fatty Acid Binding Protein



Other proteins may be more over-expressed in particular cancer types

Illumina Connected Multiomics: One platform for multiomic and multimodal analysis and discovery

- Combines multiple omic data types and modalities
- Pre-configured and fully customizable workflows reduce time to result
- Produces publication-ready graphics



Integrated multiomics analysis on ICM

Combining proteomics with other modalities provides deeper insights

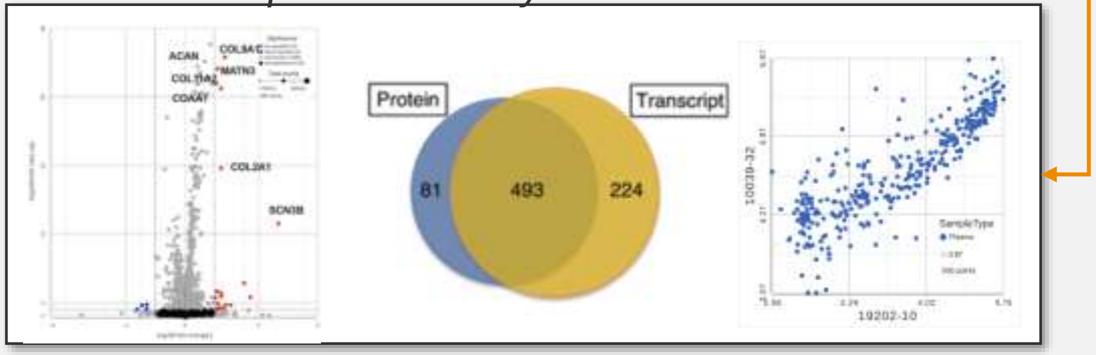
Proteomics and Bulk RNA data on paired samples can be analyzed together

Create paired ICM study

Transcriptome

Proteome

Differential expression analysis on ICM



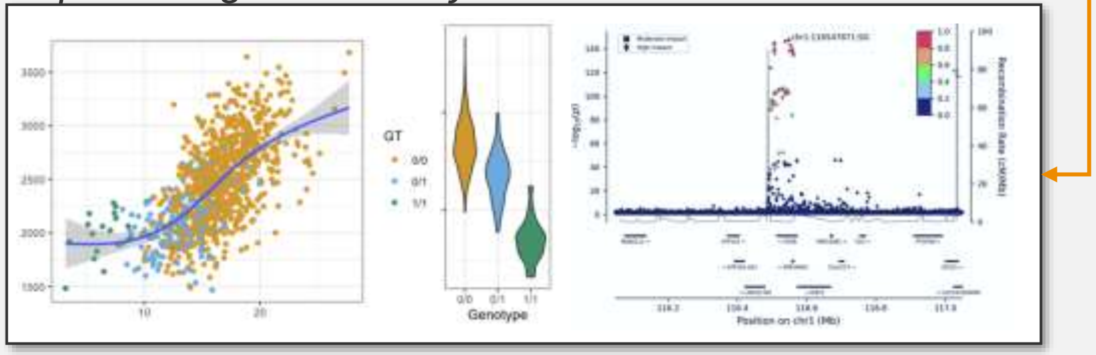
Proteomics and genomics or epigenomics data enables identification of cis- & trans-pQTLs

Create paired ICM study

5-base Genome

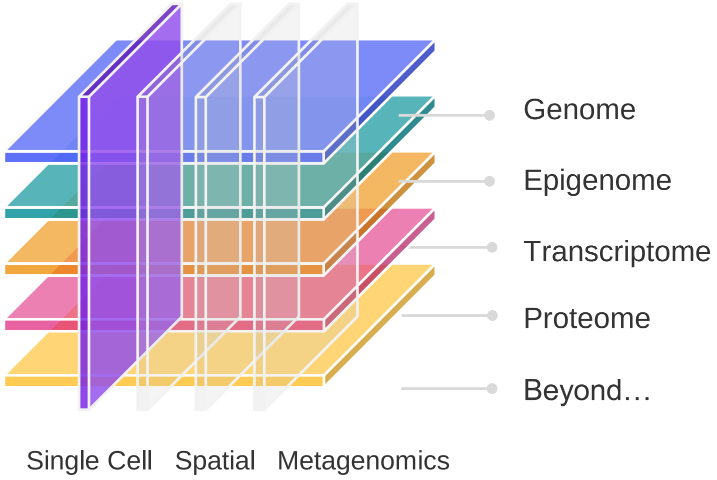
Proteome

Population genetic analysis on ICM



Unlock an unprecedented depth of biological insights

Expanded Biological Content



New library prep kits

Accessible and scalable library kits

Powerful Platforms

Illumina sequencers

Trusted technology, proven performance

State of art insights

Connected Multiomics

Powerful, intuitive, scalable multiomic analysis

One platform for all your ”omes”

Thank You