Scientific Literature Analysis In Neuroscience

Preparation to the analysis of Part 2 “Cell type determination”

BIOENG-451

Academic year: 2025-2026

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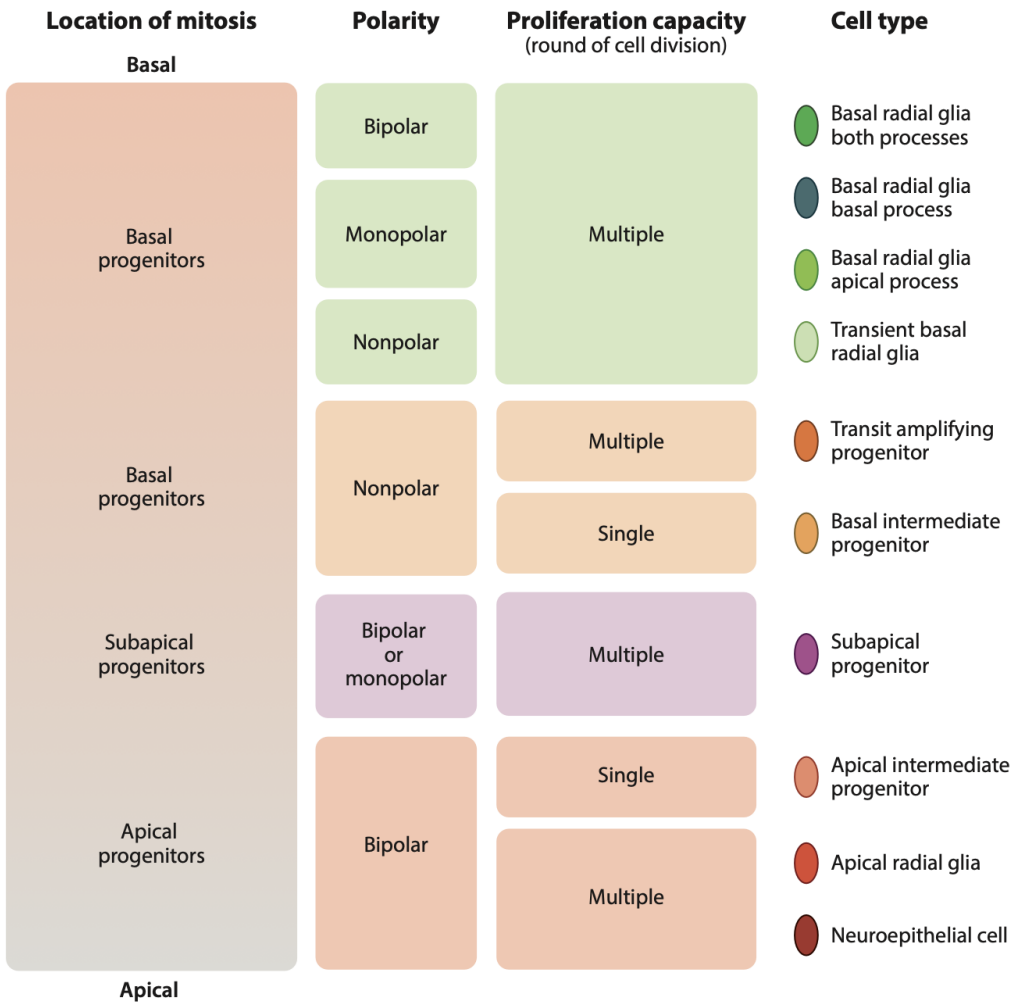
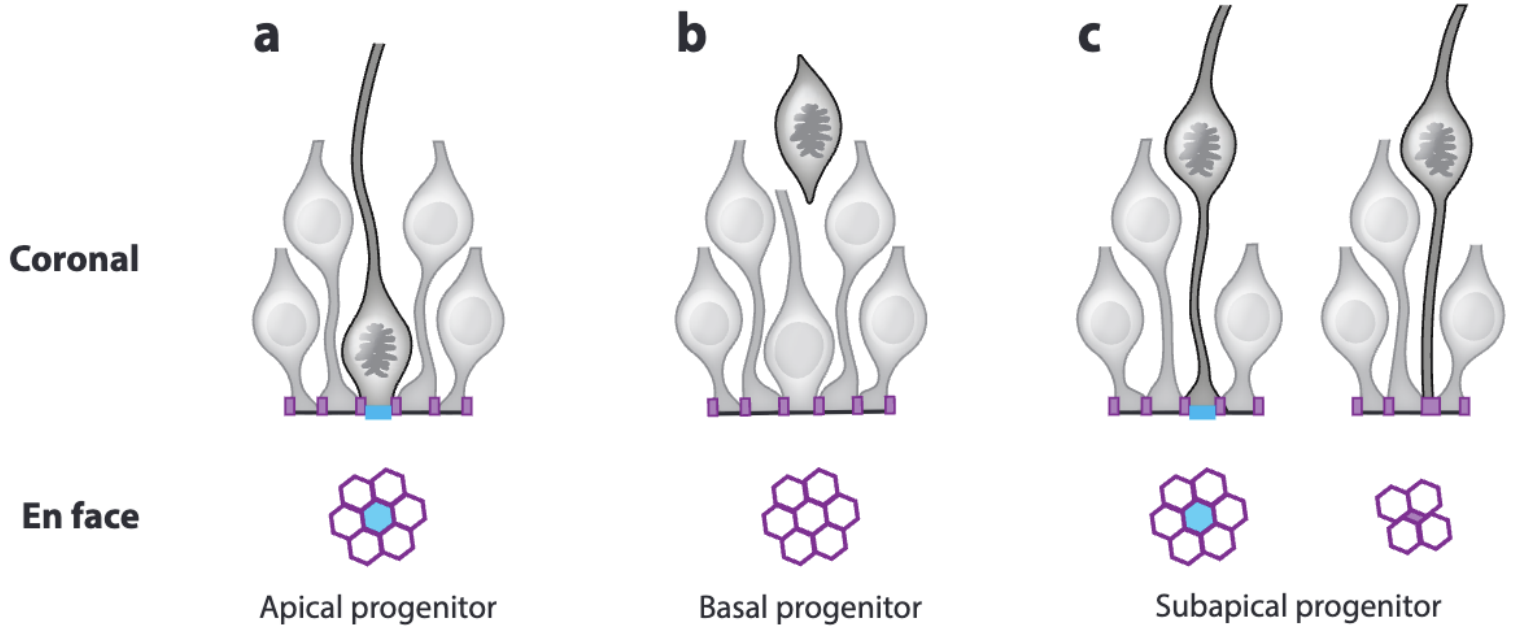
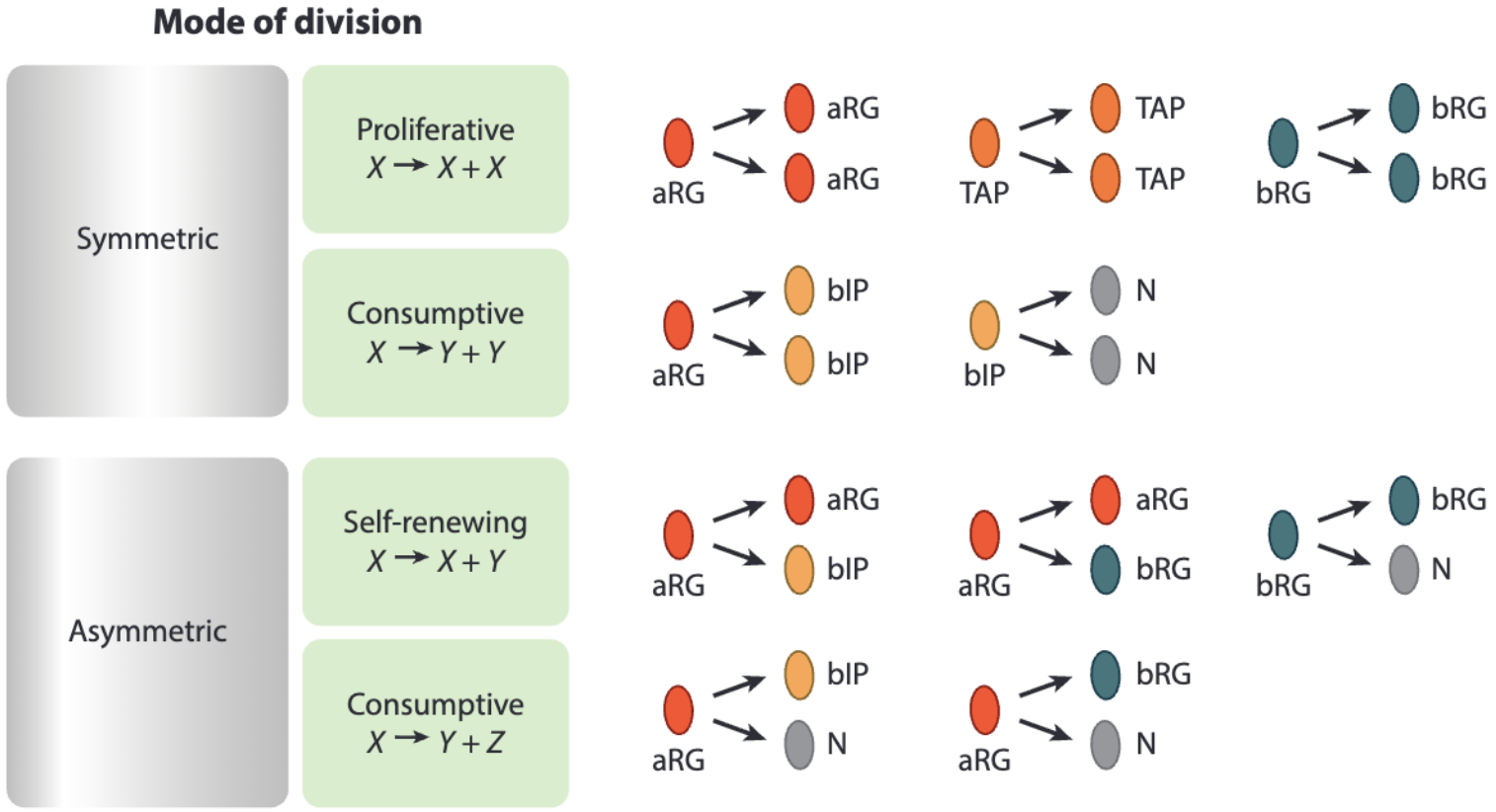
Neural stem and progenitor cell classification



Apical progenitors

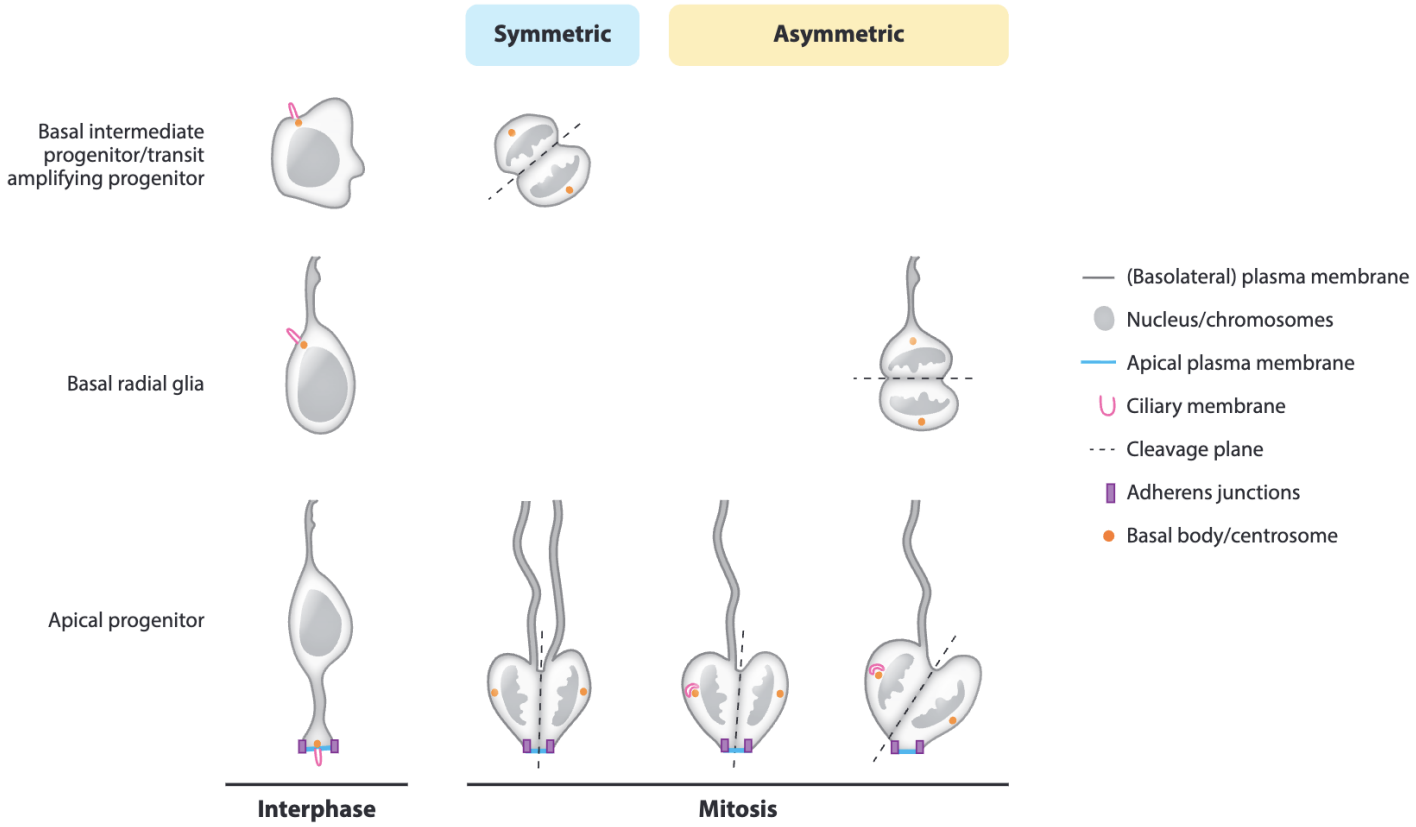


The various modes of cell division of neural stem and progenitor cells



Polarity cues are partitioned during mitosis

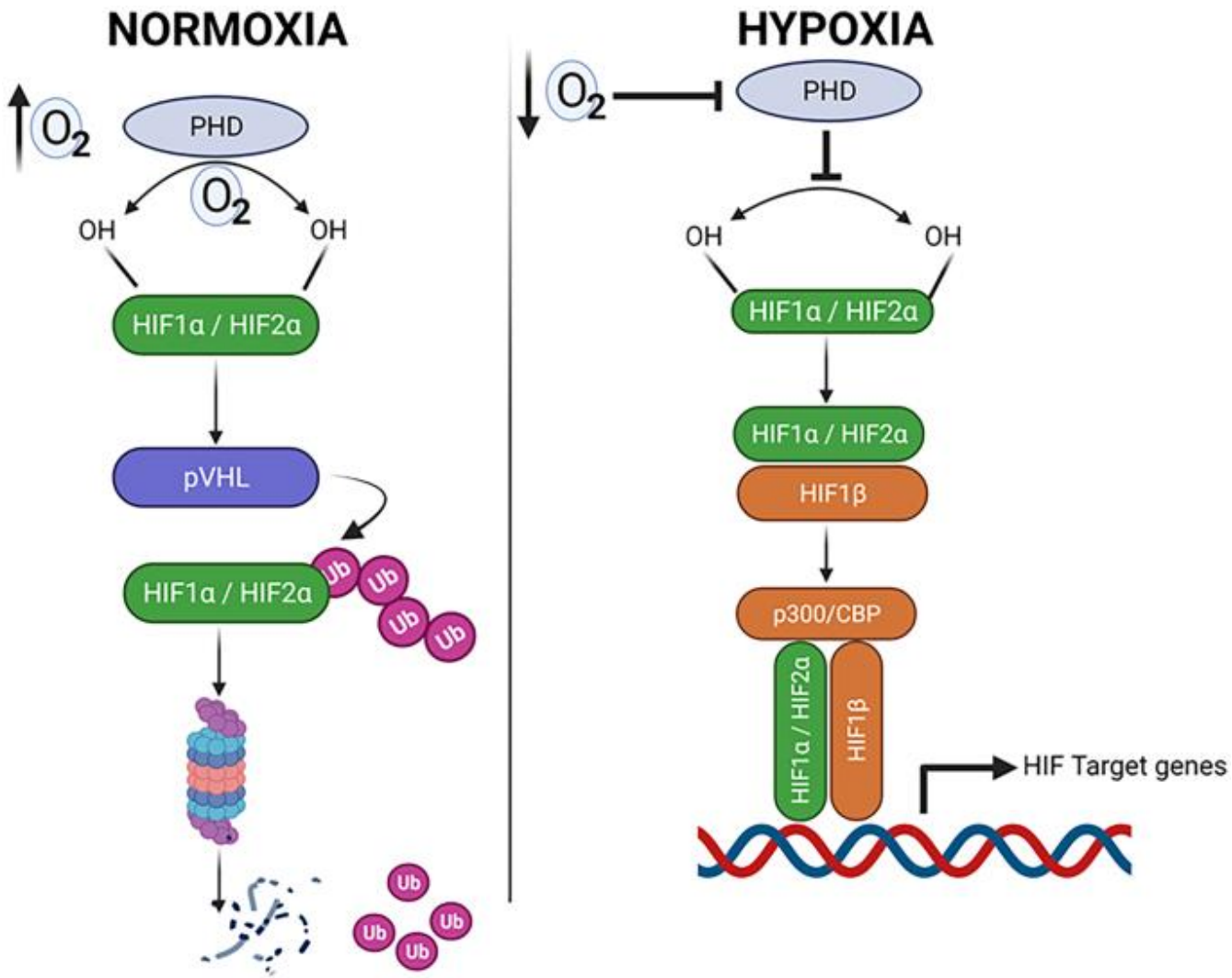
In stem and progenitor cells, polarity cues are represented by the apical plasma membrane (APs) the junctional complexes (APs)the primary cilium (APs and BPs), the centrosomes (APs and BPs), and the basal process (Aps and basal radial glial cells).
These cues are also present in interphase and can be asymmetrically or symmetrically partitioned during mitosis..



HIF1alpha pathway

HIF-1α is a **transcription factor** that mediates the **cellular response to oxygen availability**.

It allows cells to adapt to hypoxia by reprogramming metabolism, angiogenesis, and differentiation.



HIF1alpha pathway

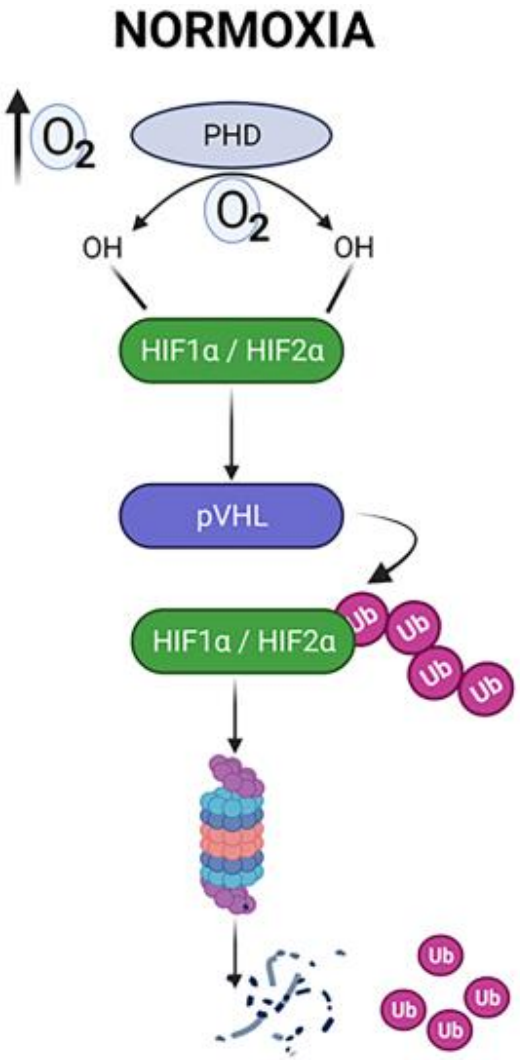
Normoxia (normal O_2):

HIF-1α protein is continuously synthesized but **rapidly degraded**.

Prolyl hydroxylase domain enzymes (**PHD1-3**) hydroxylate specific proline residues on HIF-1α using O_2 and α-ketoglutarate.

Hydroxylated HIF-1α is recognized by **VHL (von Hippel-Lindau)** E3 ubiquitin ligase complex → **ubiquitination** → **proteasomal degradation**.

Thus, under normoxia, HIF-1α is absent from the nucleus.



HIF1alpha pathway

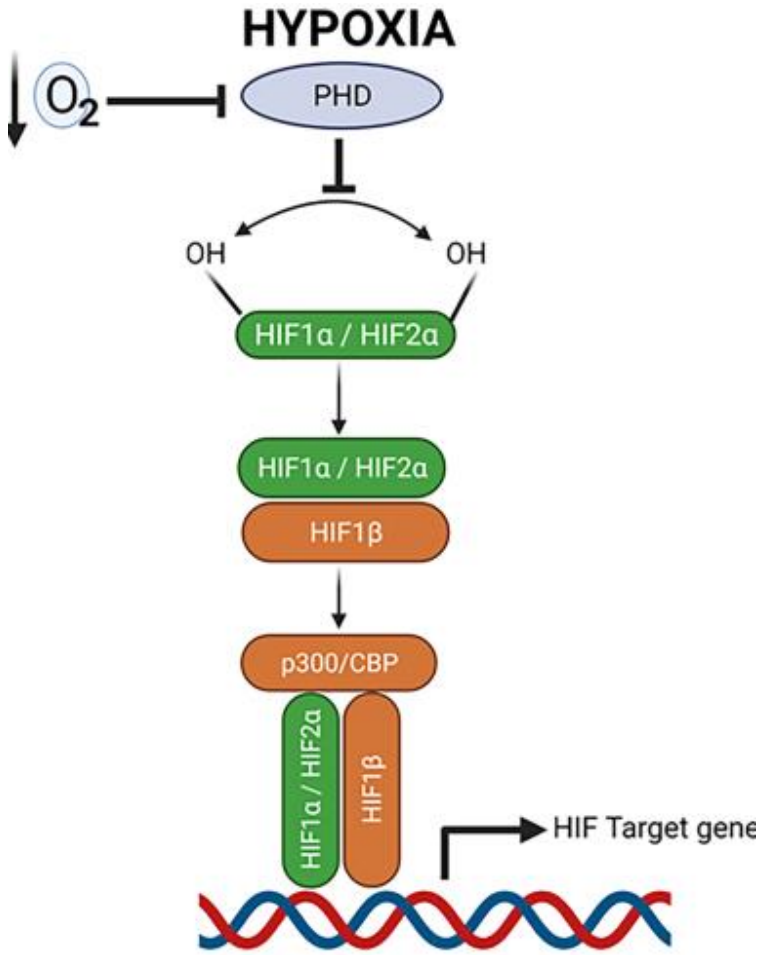
Hypoxia (low O_2):

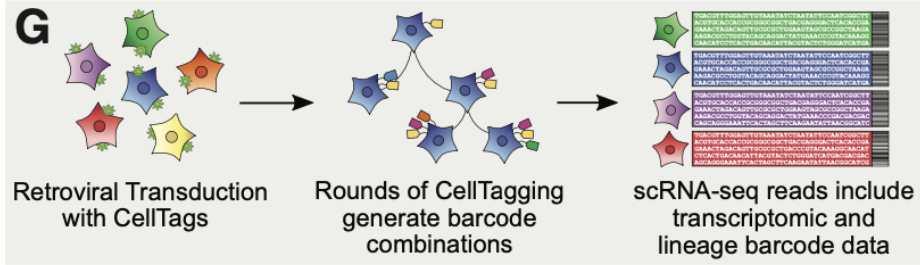
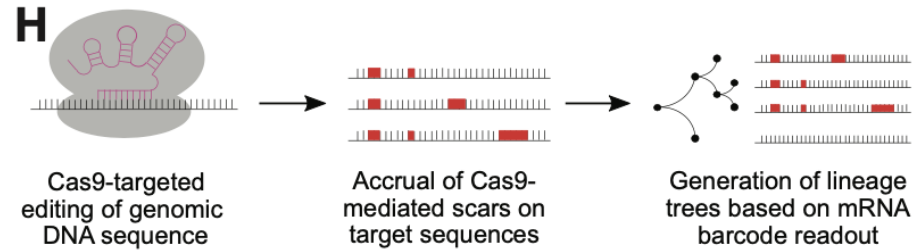
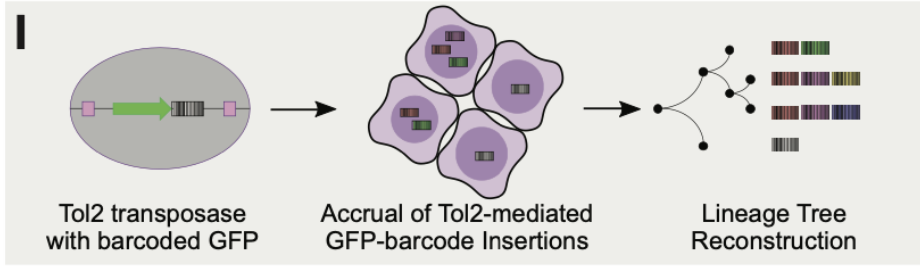
PHD enzymes are inactive (need O_2).

HIF-1α is **stabilized**, accumulates, and translocates to the **nucleus**.

Dimerizes with **HIF-1β (ARNT)** → forms the active HIF-1 complex.

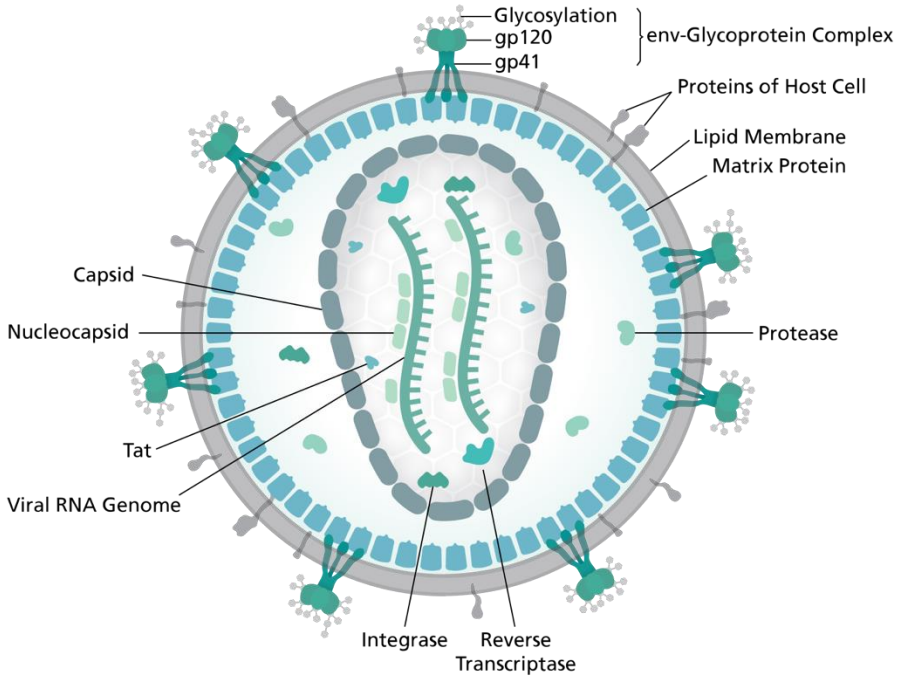
Binds **hypoxia response elements (HREs)** in target gene promoters → transcriptional activation.

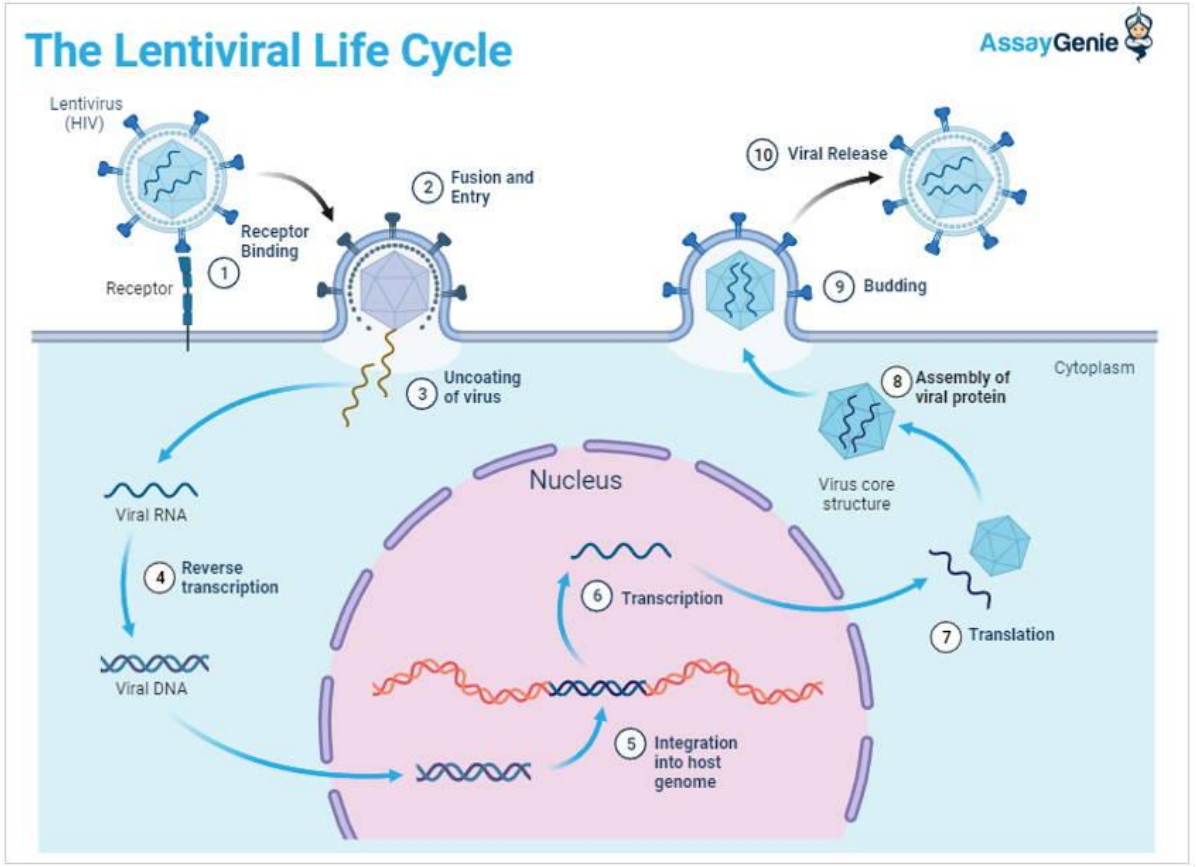


Single-cell Biology	2010s-		Single-cell	1000s - 10,000s of cells	Resolved to clonal and sub-clonal populations	**Retroviral mRNA Barcode Accrual** Yao *et al*, 2017 Biddy *et al*, 2018 Weinreb *et al,* 2020
	2010s-		Single-cell	1000s - 10,000s of cells	Information dropout due to Cas9 induced deletion of previous scars	**Cas9 mRNA Scars** Spanjaard *et al,* 2018 Raj *et al*, 2018 Chan *et al*, 2019 Bowling *et al*, 2020
	2010s-		Single-cell	1000s - 10,000s of cells	None beyond usual scRNA-seq transgene dropout	**Transposon mRNA Barcode Accrual** Wagner *et al*, 2019

Lentiviral Libraries: Principle and Purpose

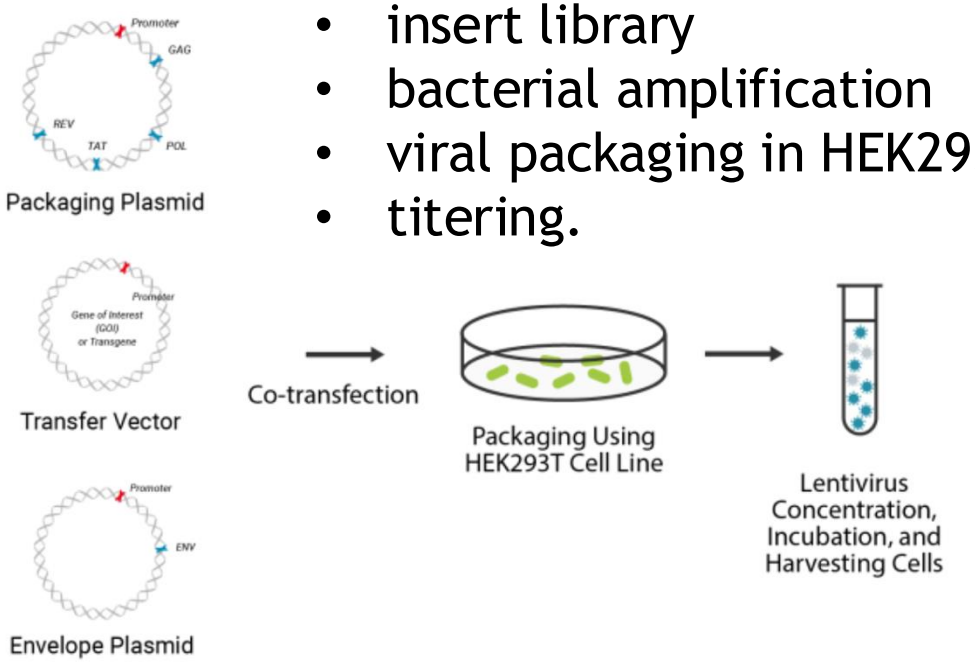
- Lentiviruses are modified retroviruses integrating into host genomes.
- Used for stable delivery of genes, reporters, or barcodes.
- Applications: molecular tagging, perturbation screens, lineage marking.

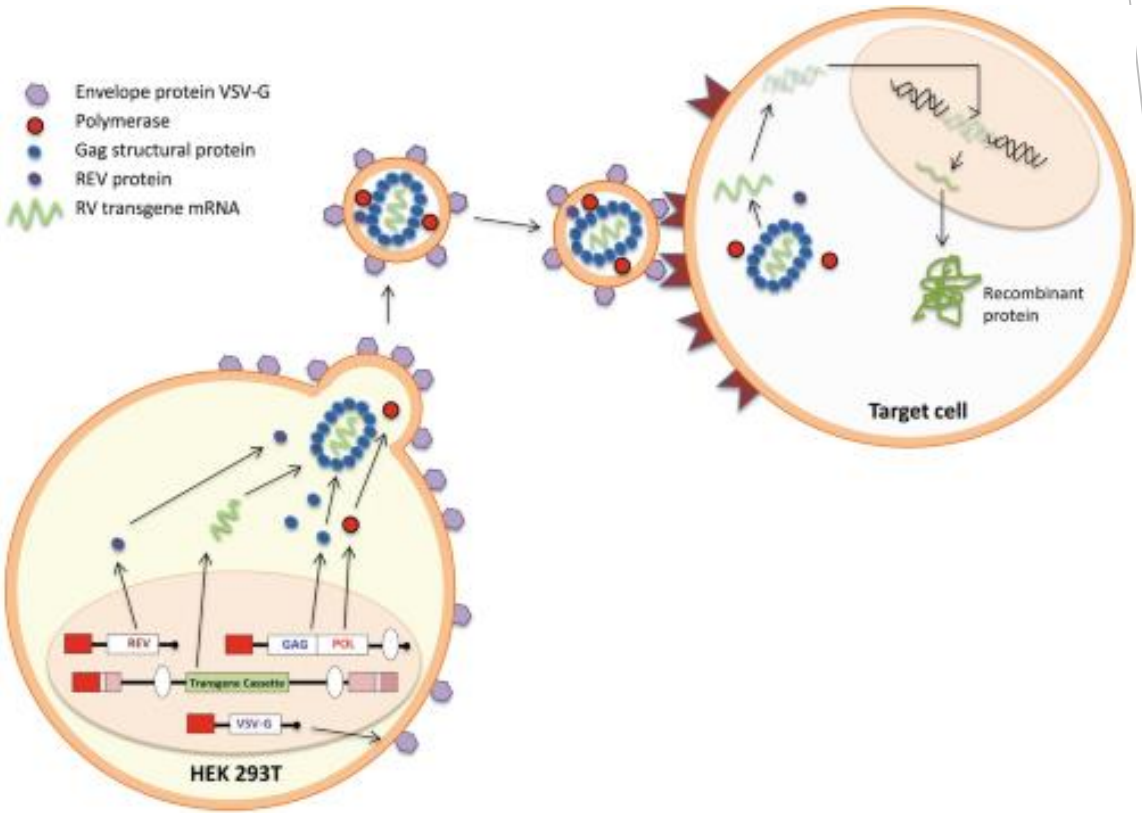




Lentiviral Libraries: How They Are Made

- Steps:
- plasmid backbone
- insert library
- bacterial amplification
- viral packaging in HEK293T
- titering.





Lentiviral Libraries: Experimental Logic

- Library = pool of constructs with randomized sequence (barcode or guide RNA).
- Infect target tissue at low multiplicity of infection (MOI).
- Each cell integrates a unique barcode → stable expression.
- Expression under chosen promoter (ubiquitous or specific).

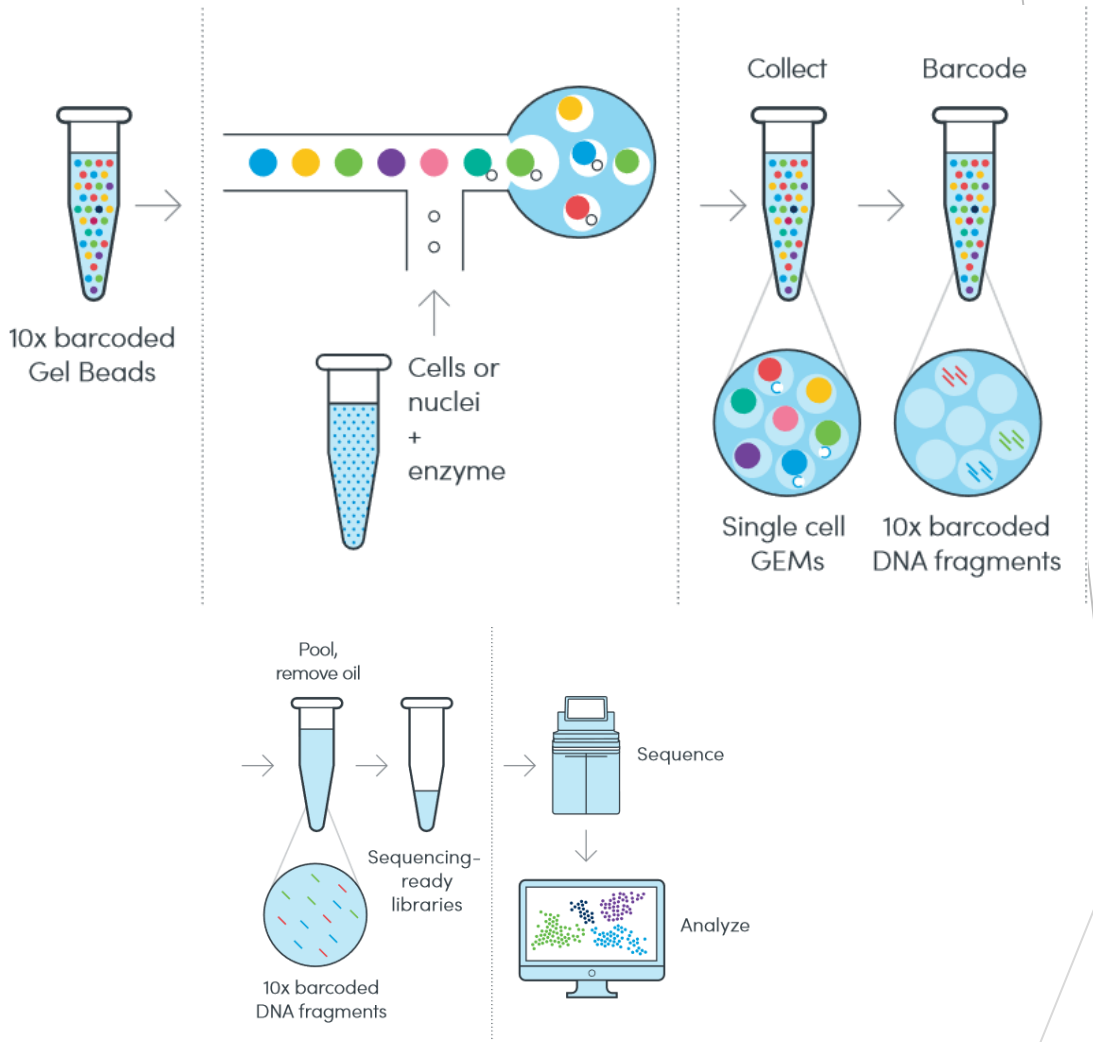
Critical points:

- MOI control: too high → multiple integrations, too low → unlabeled cells
- Integration site variability can affect gene expression
- Titering accuracy essential for reproducible transduction
- Potential for insertional mutagenesis in host genome

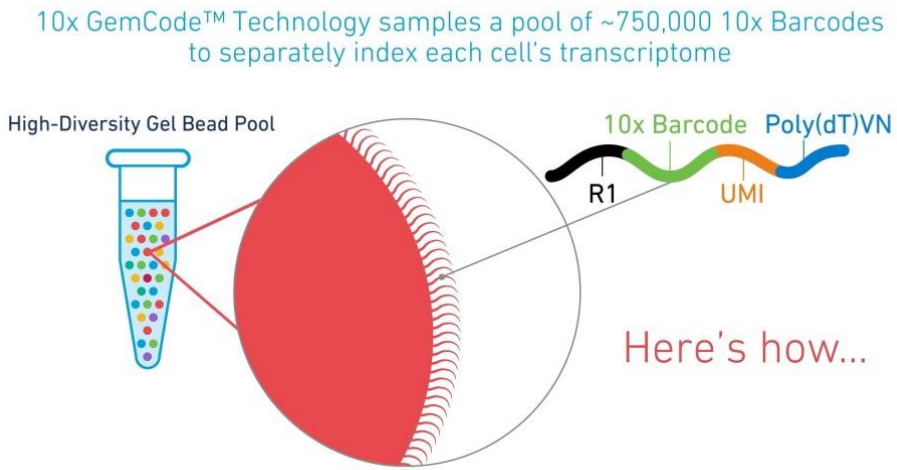
Single-cell RNA Sequencing (scRNA-seq)

Aim: Profile gene expression at individual cell resolution

- Isolates individual cells
- Captures and amplifies mRNA from each cell
- Sequences and quantifies transcripts
- Computational analysis for cell clustering and gene expression patterns



Single-cell RNA Sequencing (scRNA-seq)



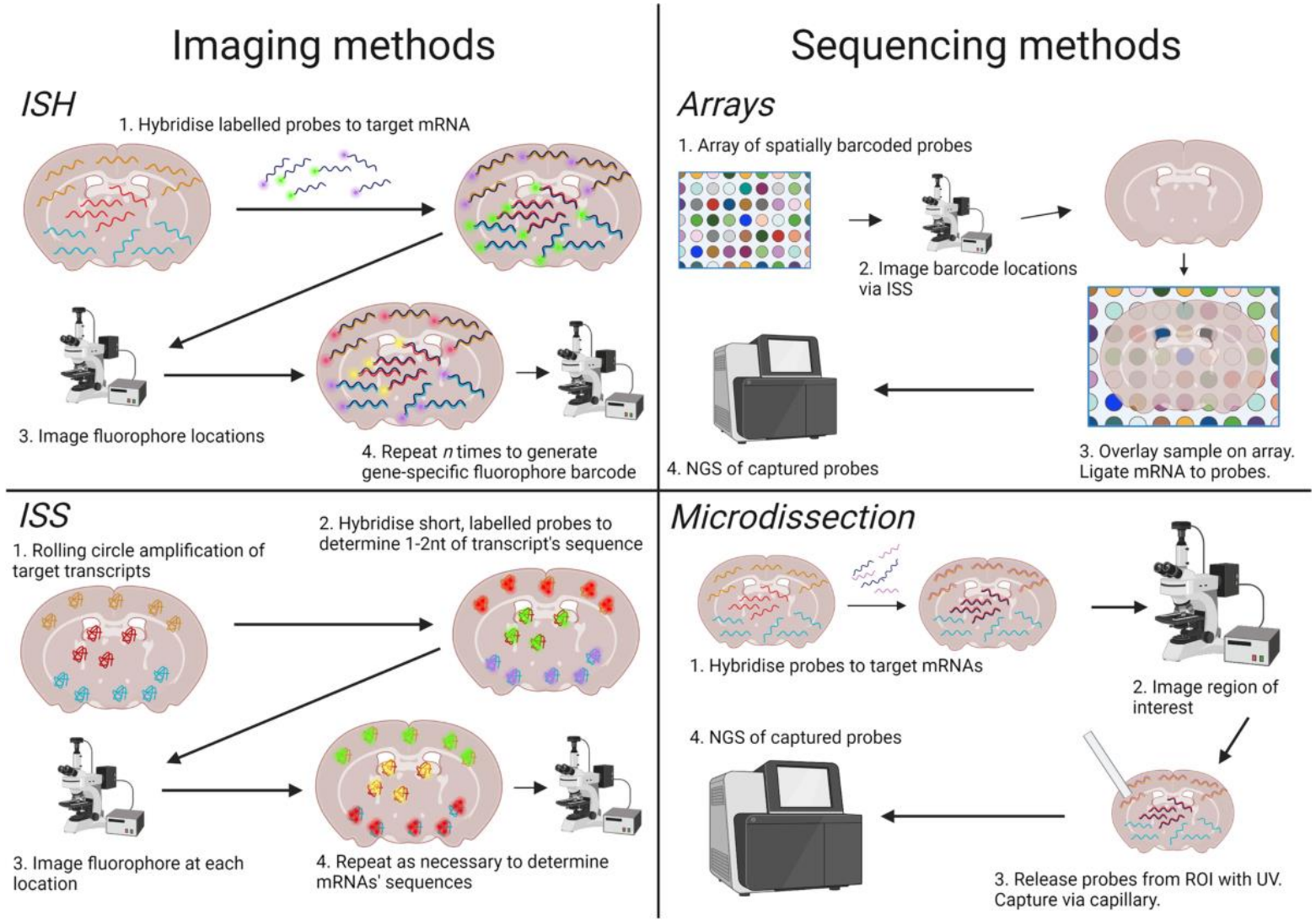
Controls:

- Spike-in controls for technical variability
- Cell type-specific markers for validation

Critical points:

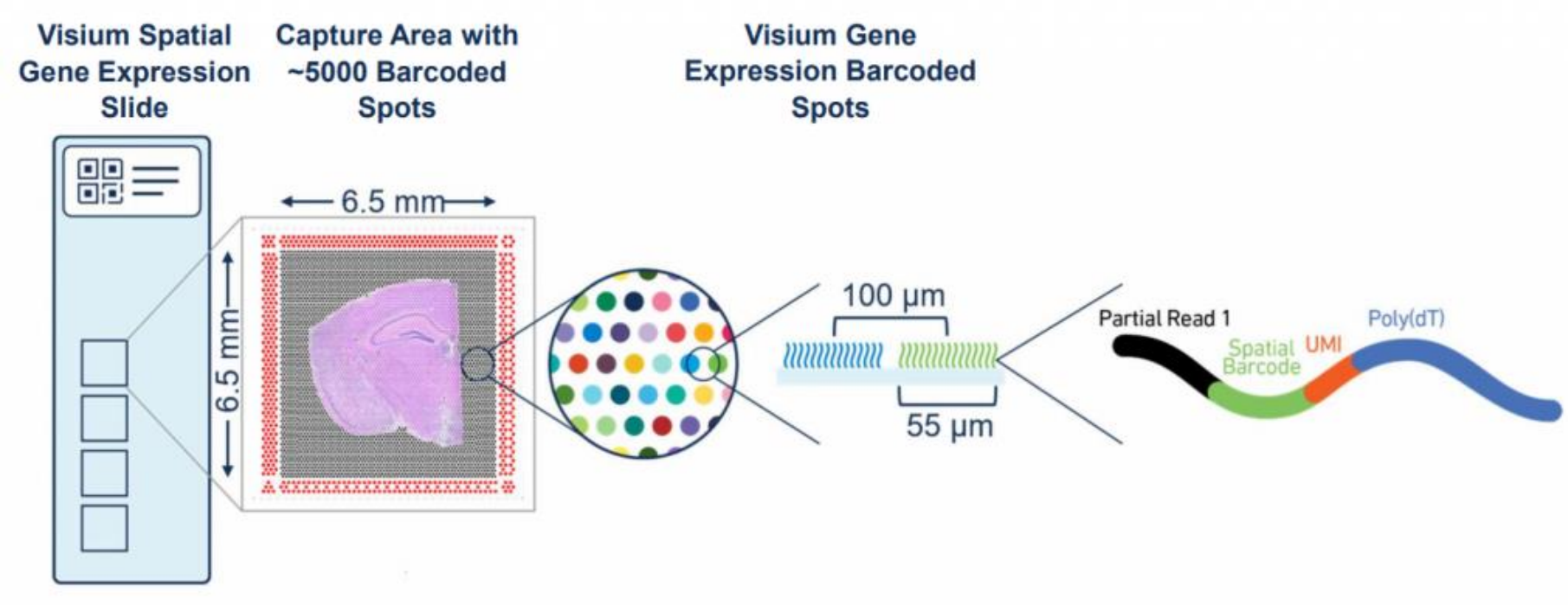
- Cell dissociation and viability
- Dropout events and technical noise
- Batch effects and data normalization

Spatial Transcriptomics – many methods, not one



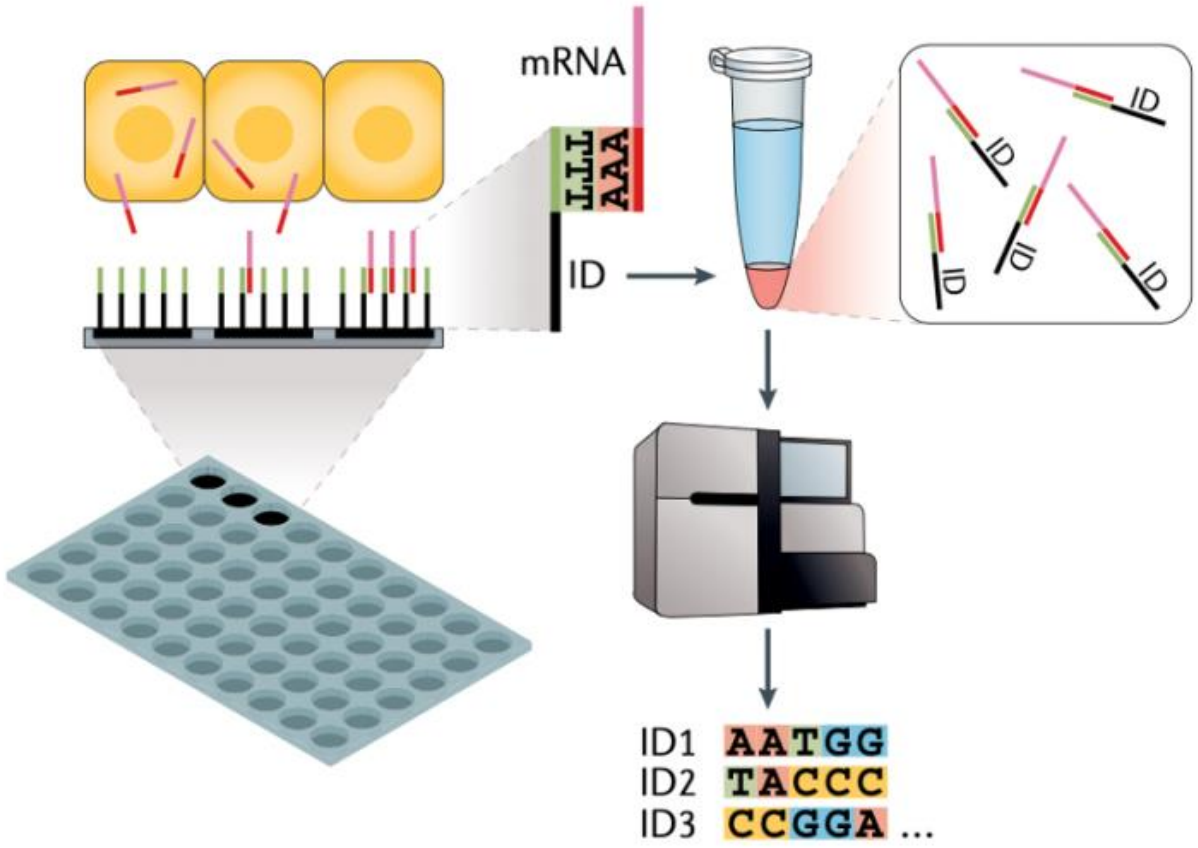
Spatial Transcriptomics: Principle

- Fresh-frozen tissue section placed on array of spatially barcoded oligos.
- mRNA hybridizes → reverse transcription → spatial barcodes captured in cDNA.
- Sequencing yields transcriptomes with coordinates.



Spatial Transcriptomics: Data and Interpretation

- Each spot (~55 µm) covers several cells.
- Generates gene–position matrix.
- Integration with scRNA-seq refines cell-type assignment.



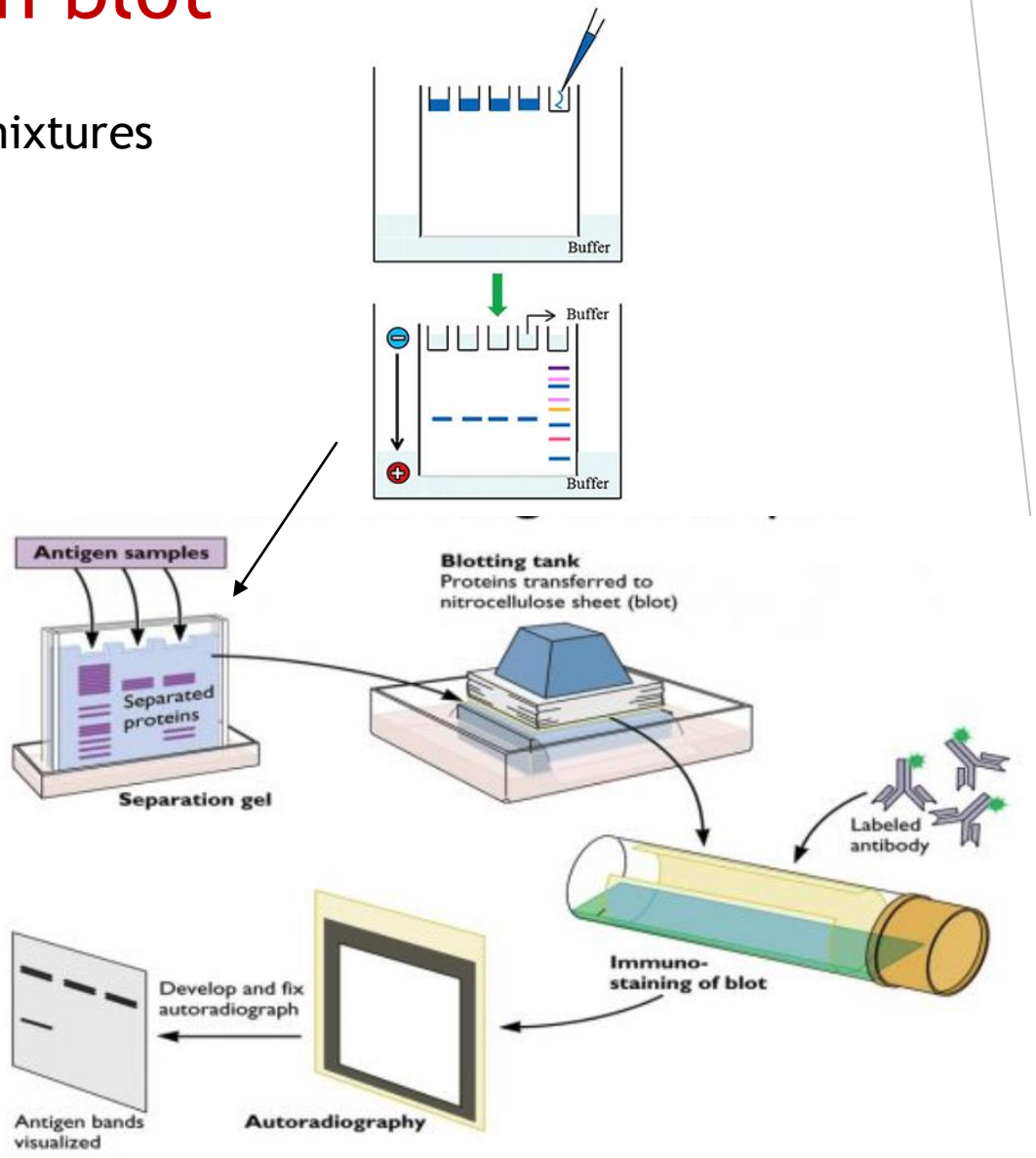
Critical points:

- Resolution limit: spots contain multiple cells (cannot resolve single cells)
- Capture efficiency varies across transcripts
- Spatial resolution vs transcriptome depth trade-off
- Tissue dissociation artifacts during preparation
- Computational deconvolution needed for cell type assignment

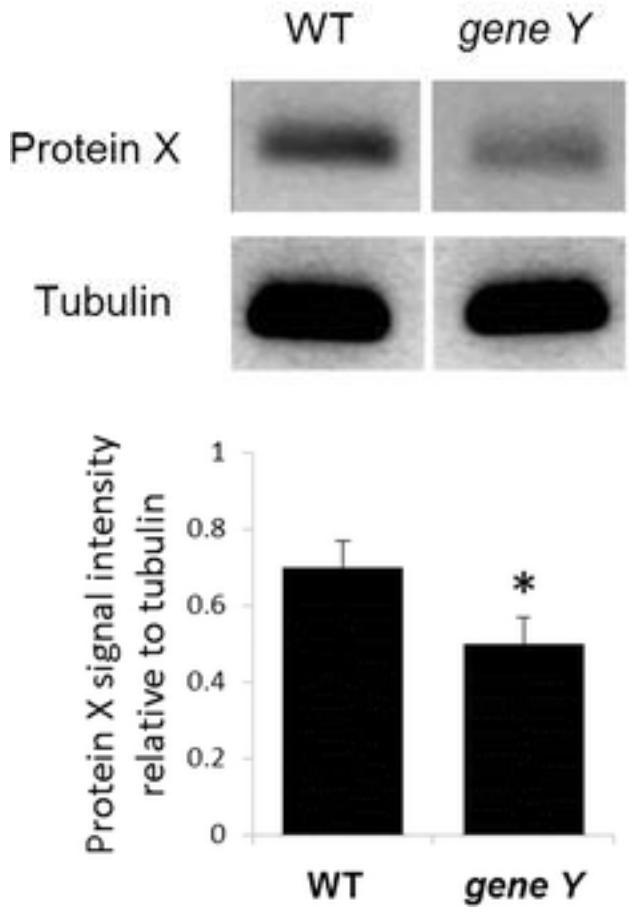
Western blot

Aim: Detect and quantify specific proteins in complex mixtures

- Separates proteins by size using gel electrophoresis.
- Transfers proteins to a membrane.
- Detects target proteins using specific antibodies.
- Visualizes using chemiluminescence or fluorescence.



Western blot



Controls:

- Loading control (e.g., housekeeping proteins)
- Positive and negative controls for antibody specificity

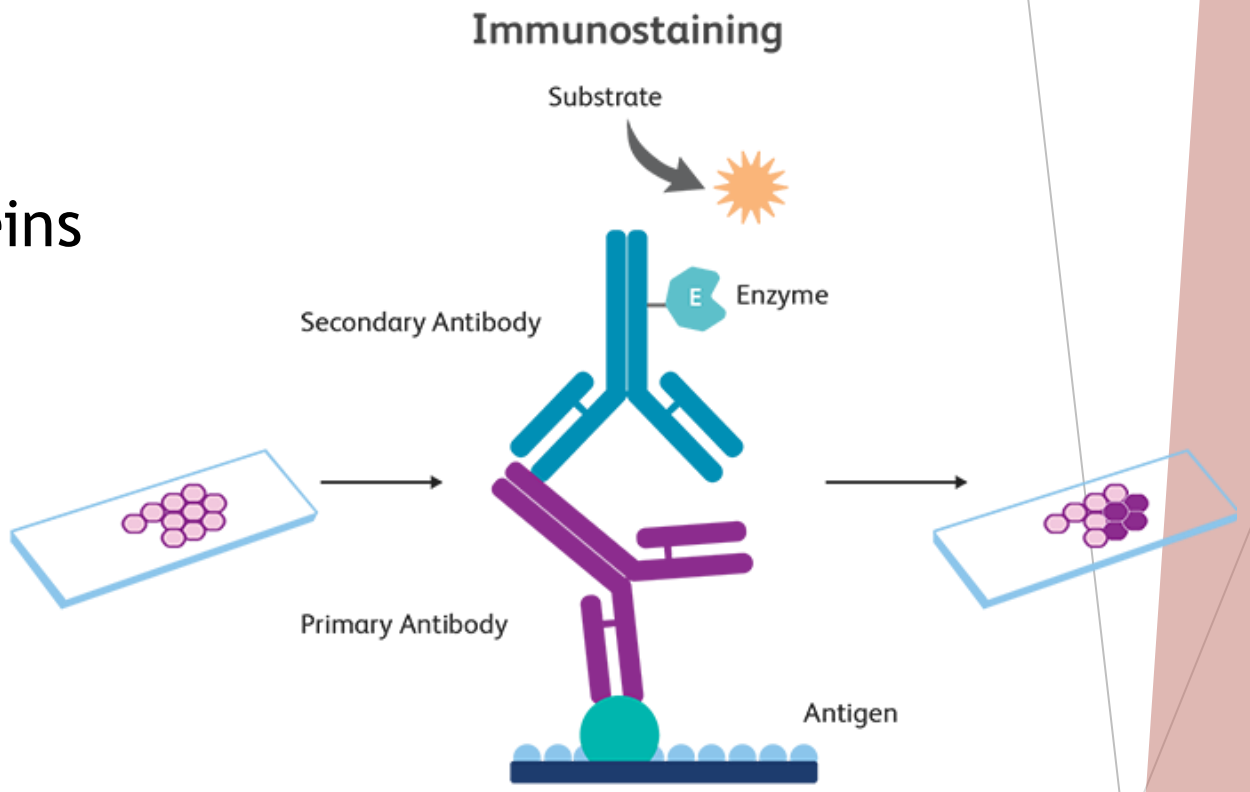
Critical points:

- Antibody specificity and validation
- Proper normalization for quantification
- Linear range of detection

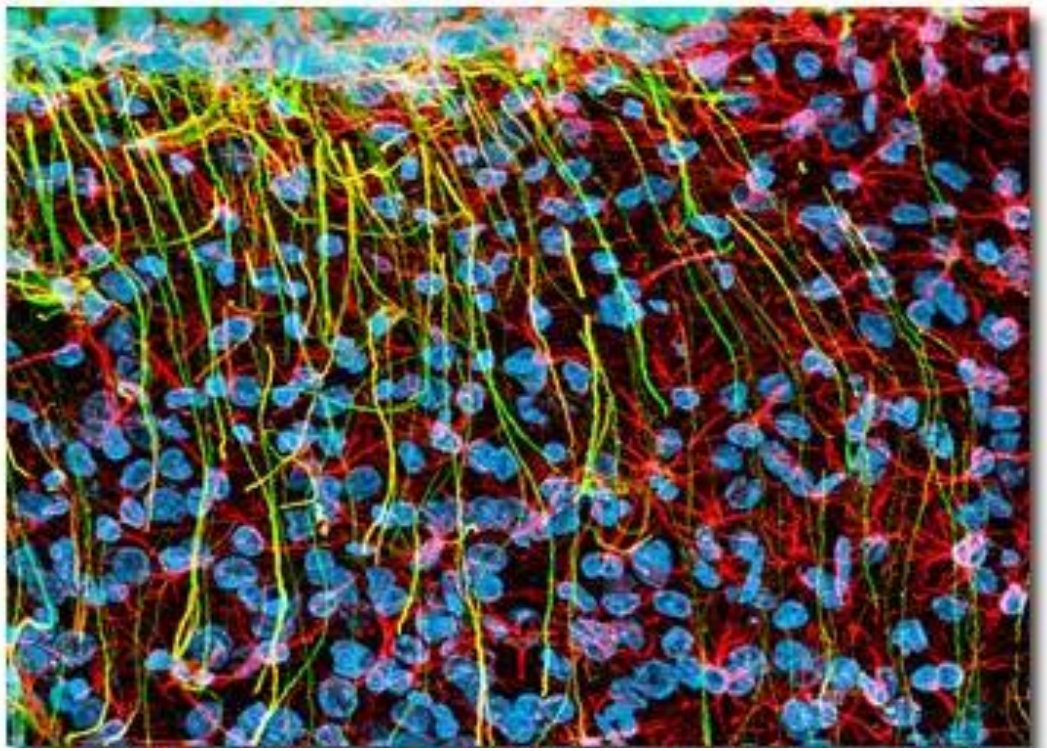
Immunostaining

Aim: Visualize and localize specific proteins within cells or tissues

- Uses antibodies to detect target proteins
- Primary antibody binds to protein of interest
- Secondary antibody (labeled) binds to primary antibody
- Fluorescent (or chromogenic) detection



Immunostaining



Controls:

- Negative control (no primary antibody)
- Positive control (known expression pattern)

Critical points:

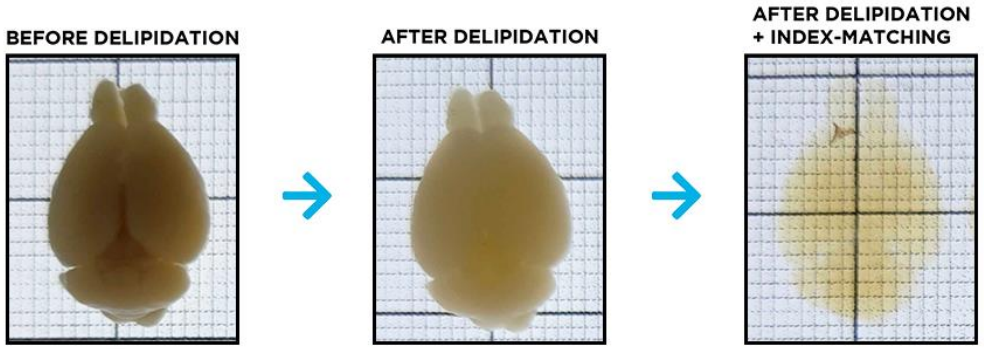
•Antibody specificity and validation

•Fixation and permeabilization methods

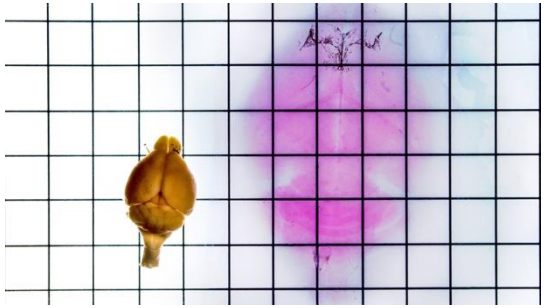
•Autofluorescence and background signal

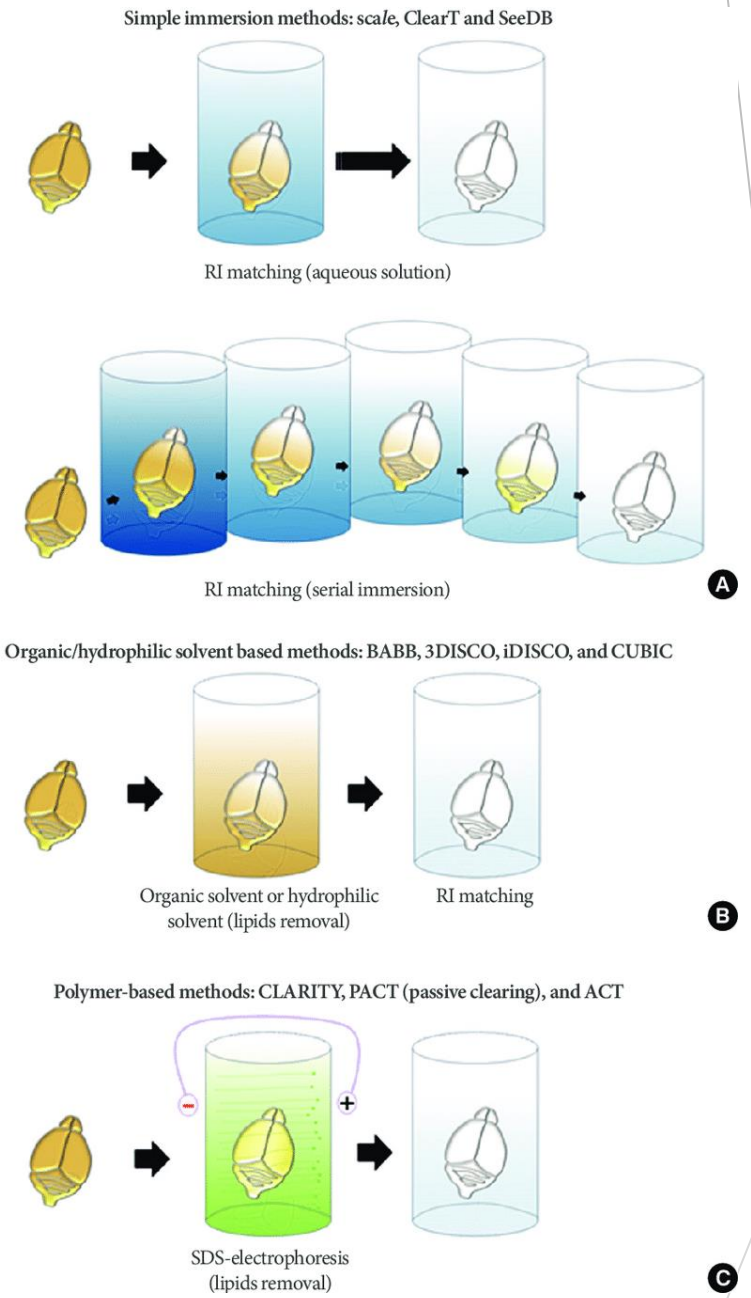
Tissue clearing methods and light sheet imaging

Aim: Visualize intact organs and large tissue volumes in three dimensions at cellular or subcellular resolution



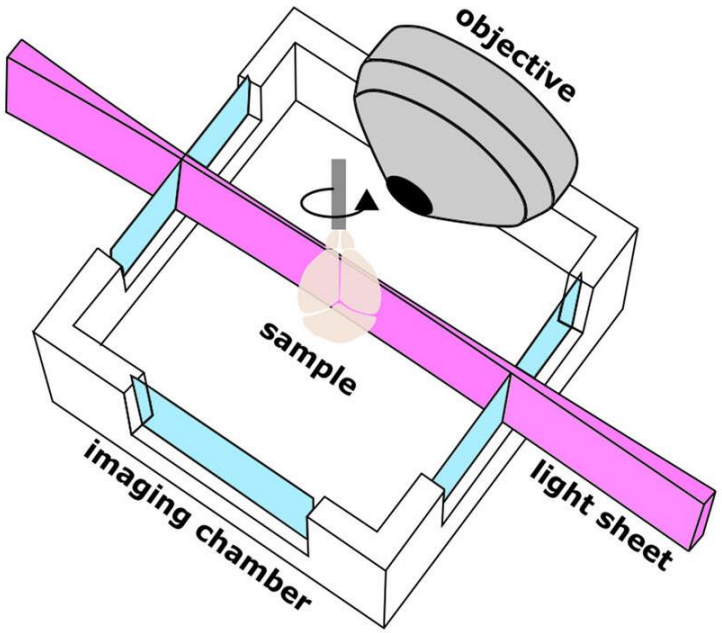
- Tissue clearing renders samples optically transparent by removing lipids and matching refractive index

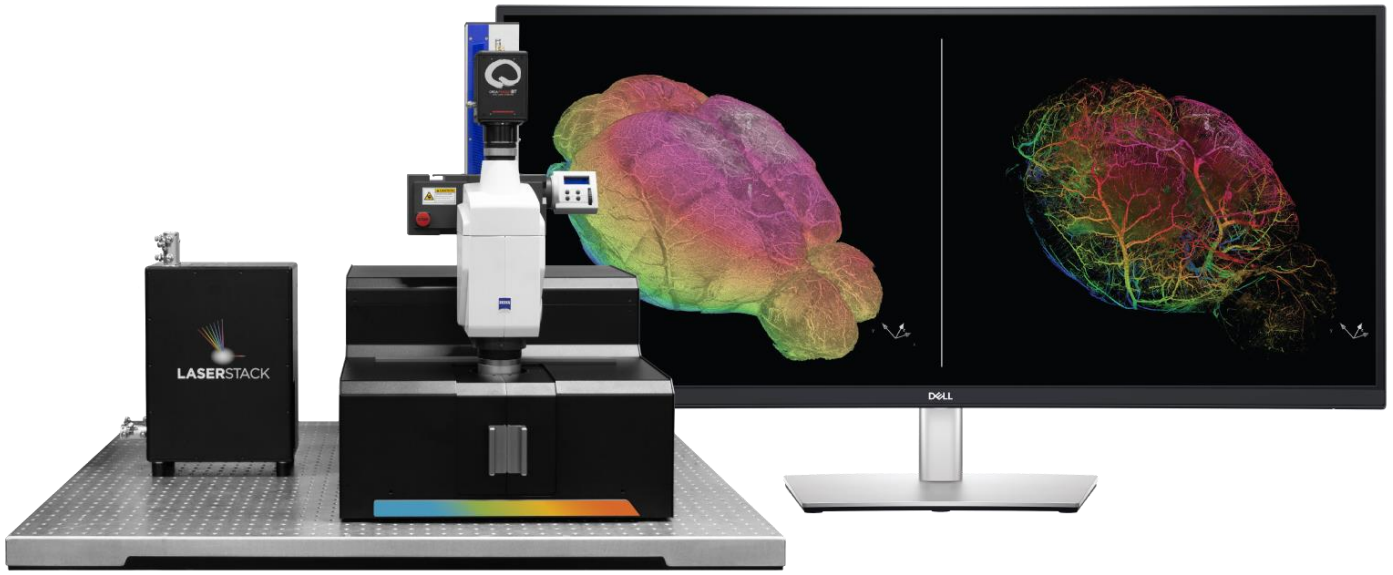




Tissue clearing methods and light sheet imaging

- Light sheet microscopy illuminates only the focal plane with a thin sheet of light perpendicular to the detection axis
- Enables rapid volumetric imaging of millimeter to centimeter-scale specimens





Tissue clearing methods and light sheet imaging – critical aspects

Clearing method compatibility: Does the method preserve the fluorophores or antibody epitopes used? Solvent-based methods quench many fluorescent proteins, while aqueous methods better preserve endogenous fluorescence.

Tissue distortion and shrinkage: Some protocols cause significant tissue expansion or shrinkage that affects spatial measurements and anatomical interpretation.
Imaging depth and resolution: Resolution degrades with depth due to light scattering and aberrations. Validate that quantitative analyses are performed within the reliable imaging depth range.

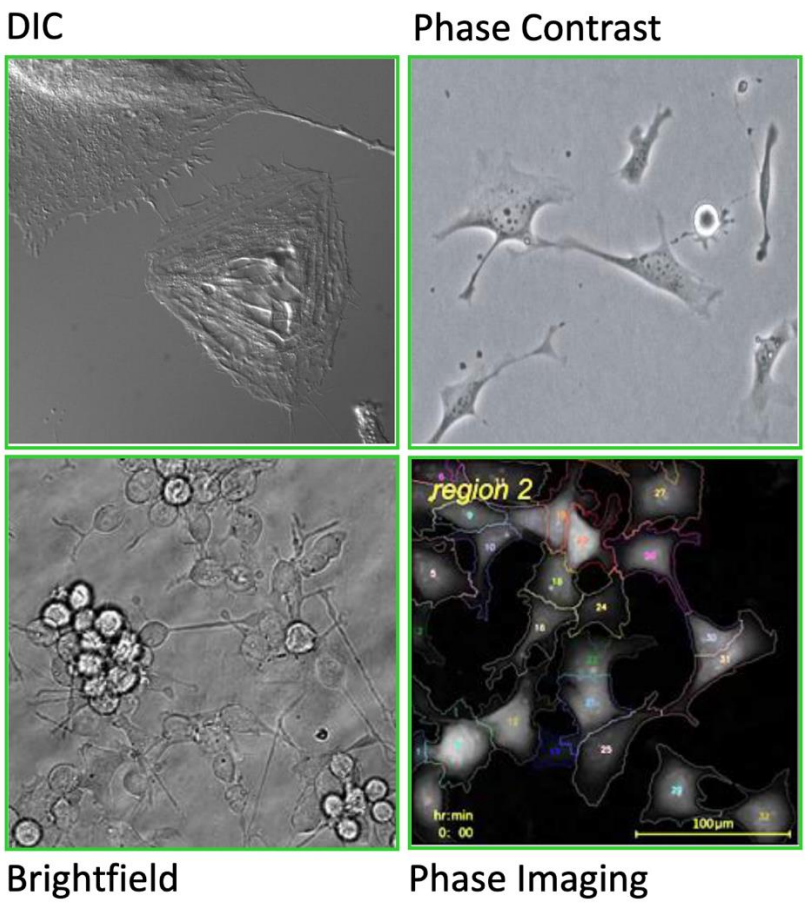
Controls and validation: Proper controls include uncleared tissue comparisons, antibody specificity checks, and validation of cell counts or morphology against established methods.

Computational processing: Image stitching, deconvolution, and cell segmentation algorithms can introduce artifacts. Check whether methods are described and validated, especially for automated quantification.

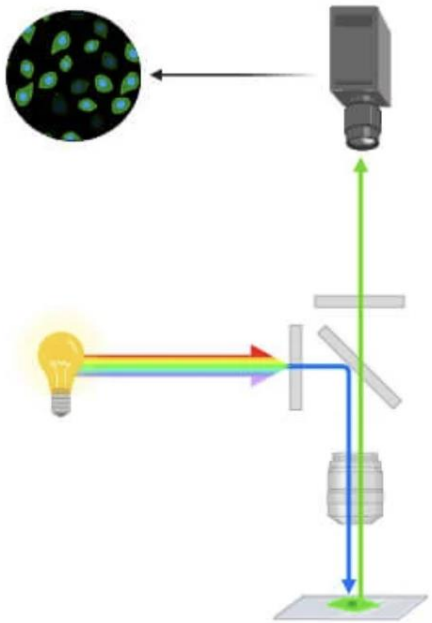
Live Imaging: Principle and Setup

- Observes dynamic processes in real time.

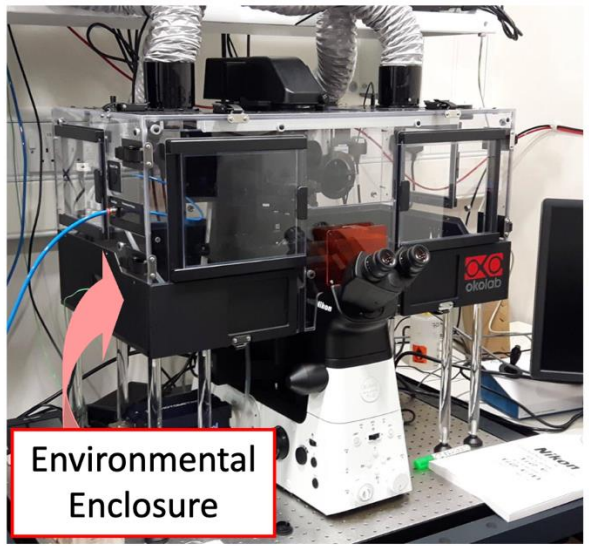
Non-epifluorescence techniques



Epifluorescence

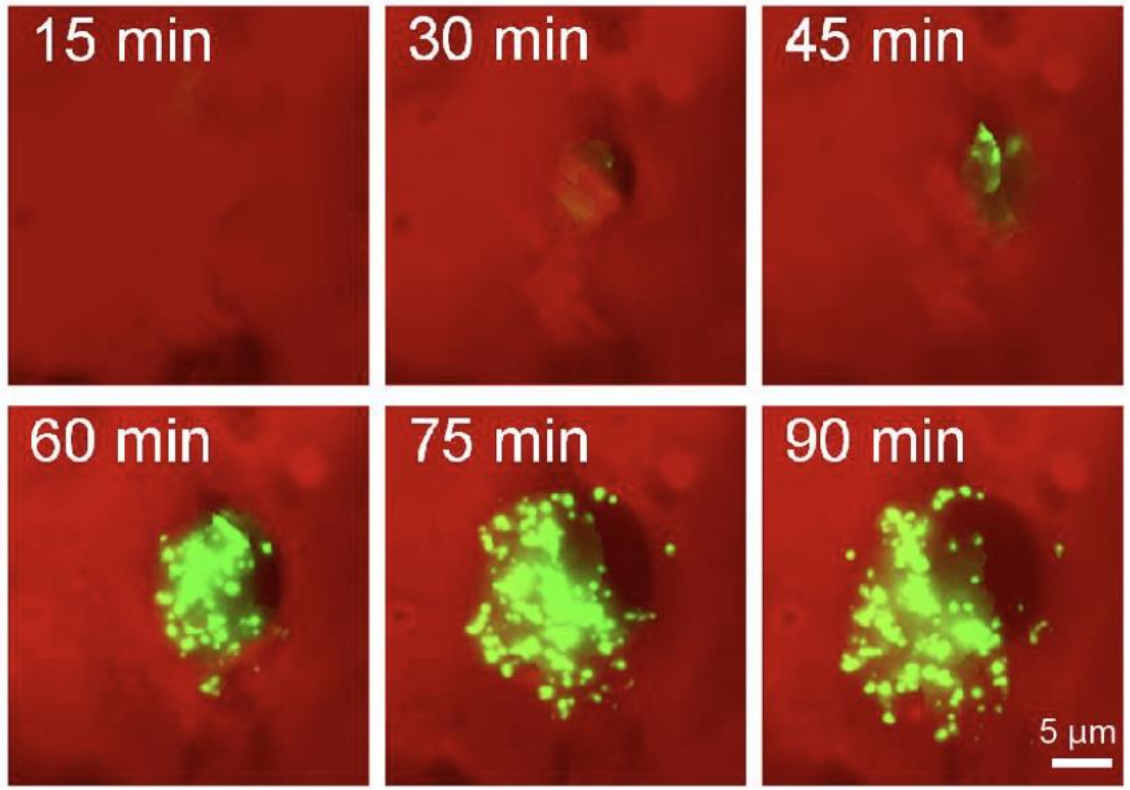


Enclosure

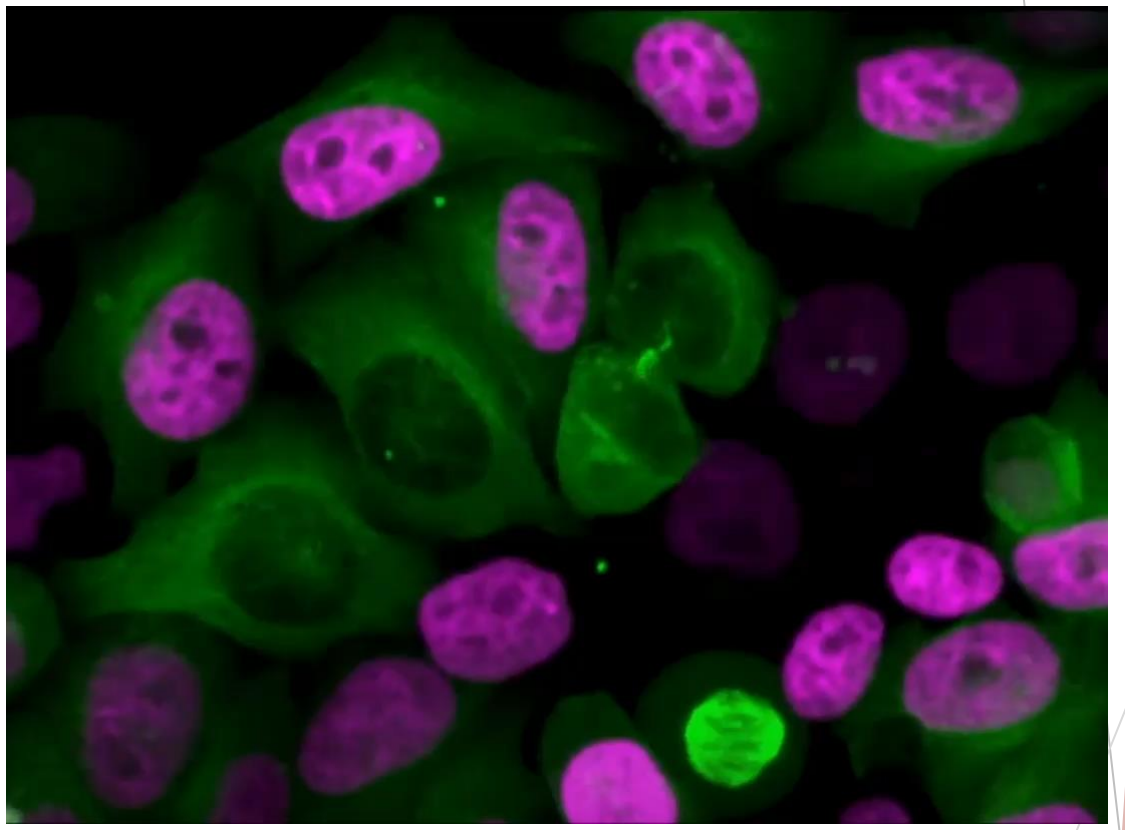


Live Imaging

Fluorescent reporters



Example



Live Imaging: Interpretation and Critical aspects

- Phototoxicity: excessive light exposure damages cells and alters behavior
- Photobleaching: fluorophore degradation limits observation time
- Environmental control: maintain temperature, CO_2, pH, and humidity
- Z-drift during long acquisitions affects tracking accuracy
- Out-of-plane movement can cause cell loss during tracking
- Reporter expression level can affect cell physiology