Scientific Literature Analysis In Neuroscience

Methods in Neurobiology

BIOENG-451

Academic year: 2025-2026

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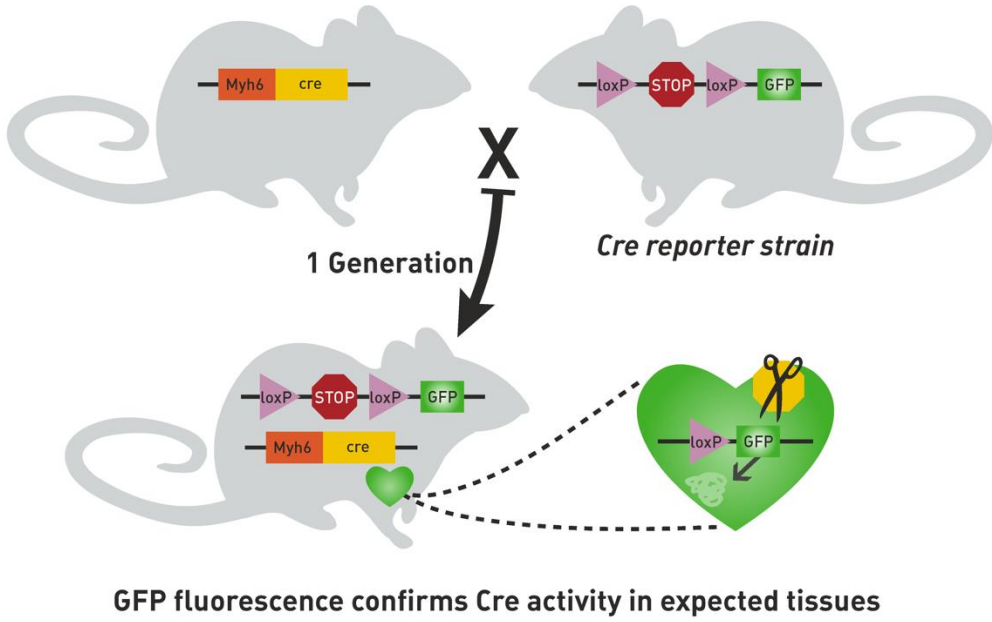
Genetic Mouse Systems

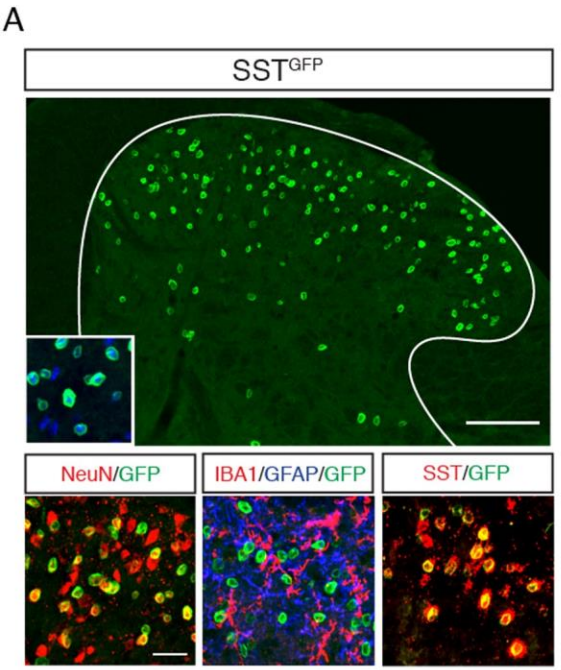
Reporter Mice

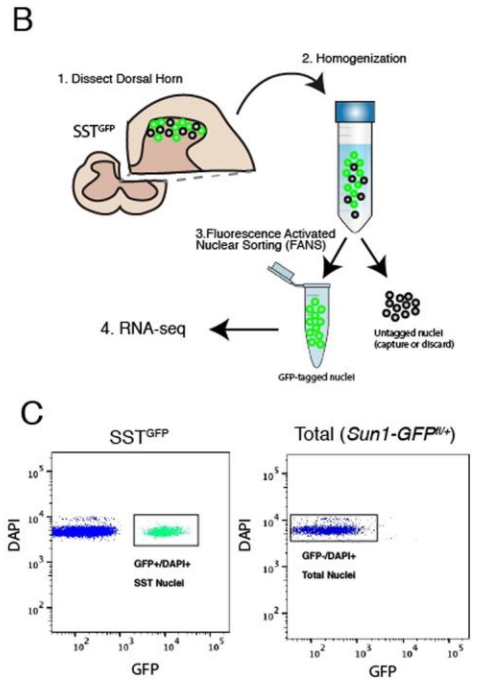
Aim: Visualize gene expression patterns or cellular identities in vivo & live

- Express fluorescent proteins or enzymes under specific promoters
- Allows for labeling of specific cell types or lineages
- Enables live imaging and cell trackin



Reporter Mice





Controls:

- Comparison with known expression patterns
- Validation with other techniques (e.g., immunostaining)

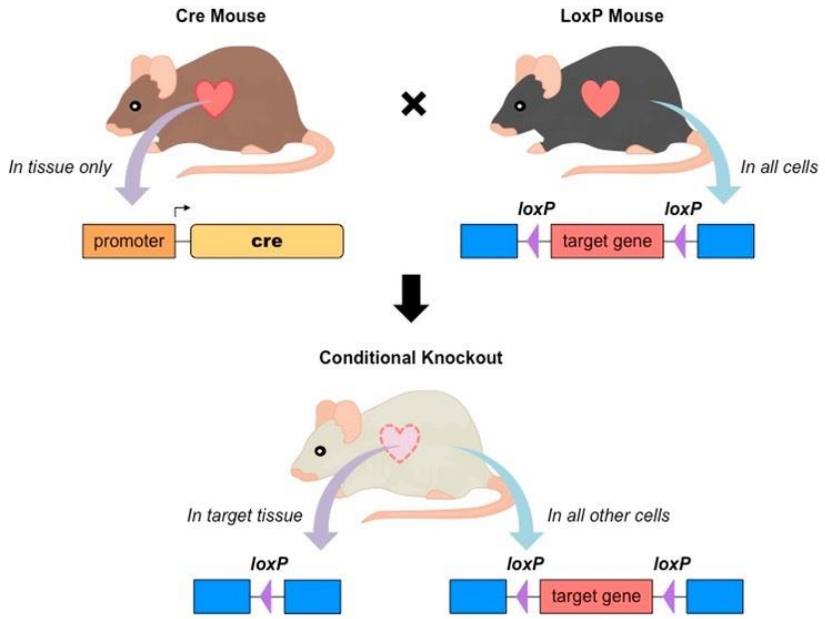
Critical points:

- Fidelity reporter expression / endogenous gene
- Potential interference with normal gene function
- Sensitivity and specificity of the reporter

Conditional Knockout Mice (Cre-lox System)

Aim: Generate tissue-specific or time-specific gene knockouts in mice

- Cre recombinase recognizes loxP sites flanking target gene
- Excises DNA between loxP sites, inactivating the gene
- Cre expression controlled by tissue-specific or inducible promoters
- Results in targeted gene deletion in specific cells or at specific times



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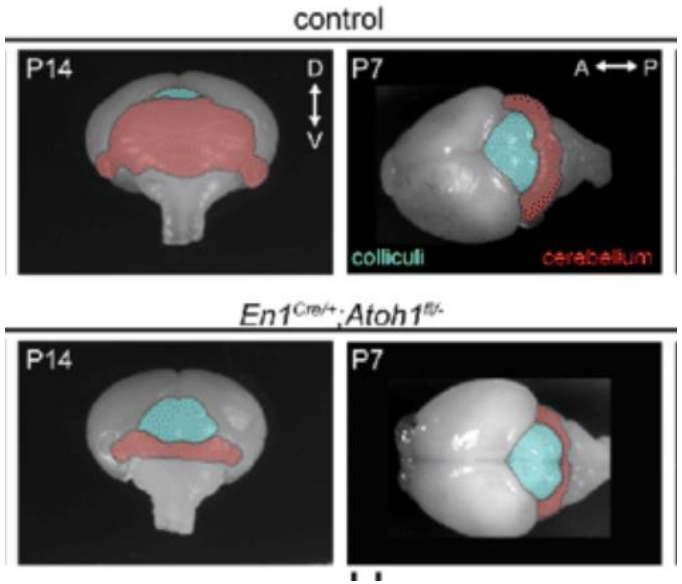


Controls:

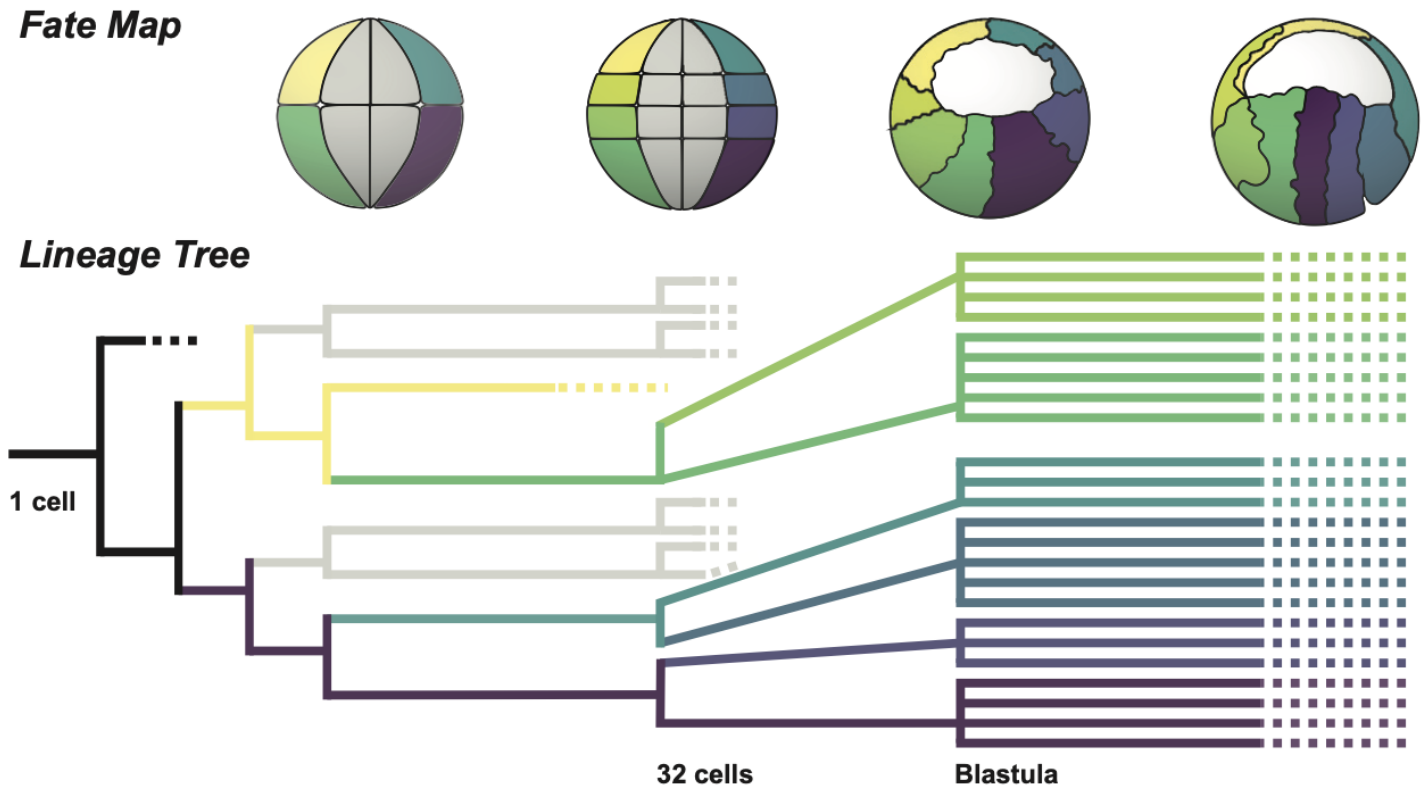
- Cre-only and floxed-only controls
- Comparison with wild-type littermates

Critical points:

- Specificity and efficiency of Cre expression
- Potential for unexpected Cre activity
- Importance of confirming gene deletion

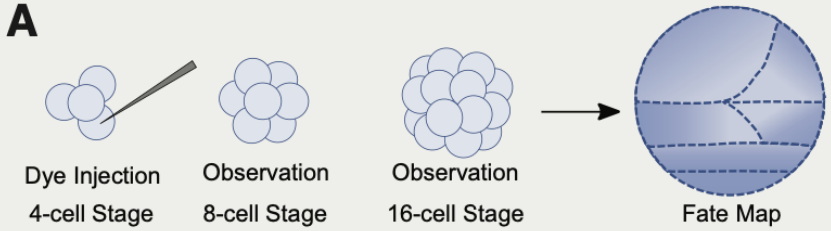
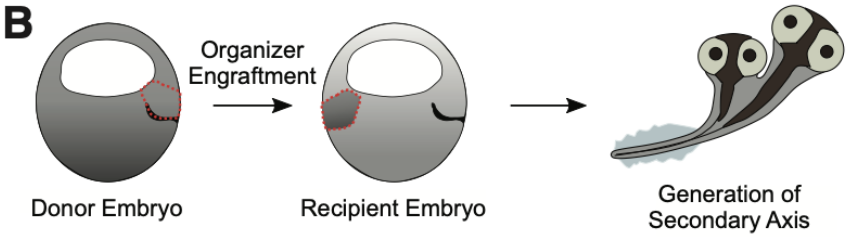
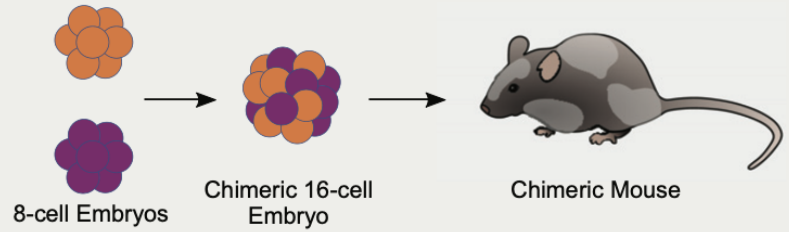
Developmental Tracing

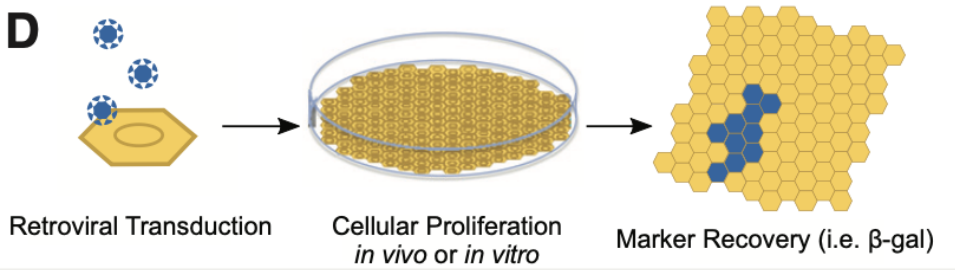
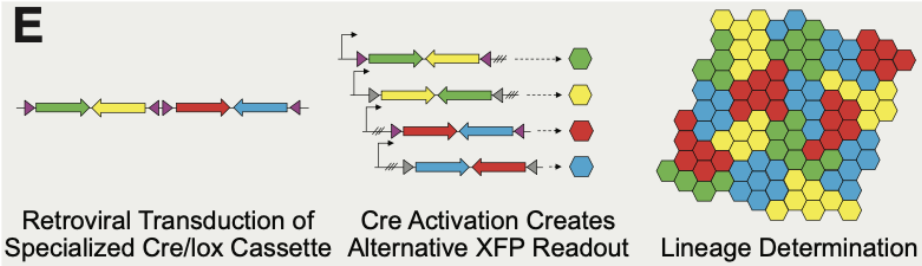
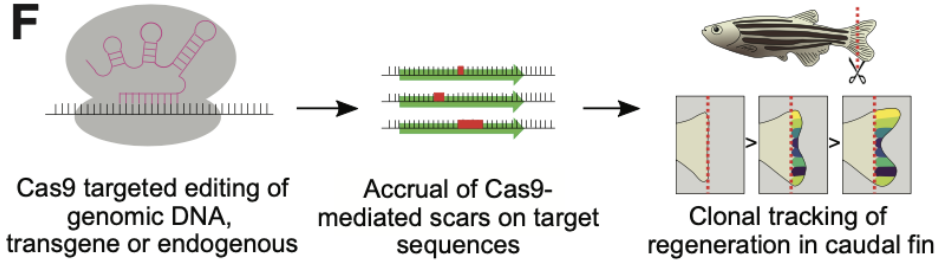
Fate Map vs Lineage tree

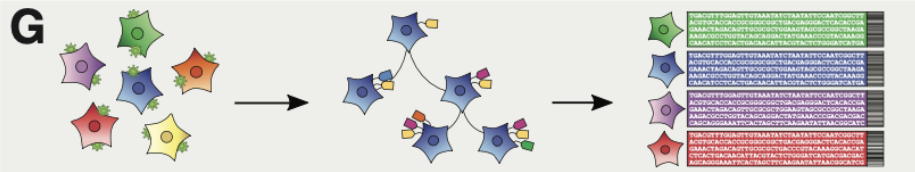
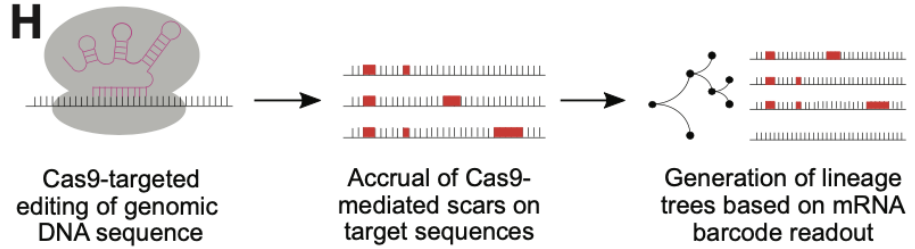
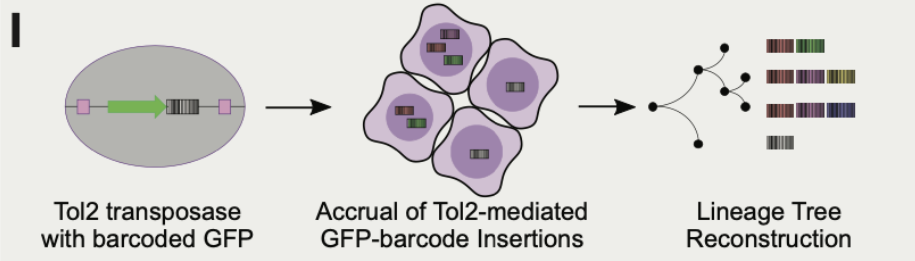


Fate maps are schematics representing the developmental potential of specific cells or regions of cells within an embryo at a defined stage. Upper panels: early *Xenopus* development fate map, retaining cell-cell relationships and position. Lower panels: lineage tracing, the identification of all progeny arising from an individual cell. Each color in the branching tree relates to a region of the corresponding fate map. Dashed lines depict lineages not shown for simplicity.

Evolution of lineage tracing tech

Era	Year	Lineage Tracing Technique	Resolution	Scalability	Limitation	Technique & Citation
Observational Biology	1890s	A 	Single-cell limited by injection	10s of cells limited by observation	Observational data	**Dye Injection and Time Lapse** Conklin, 1905 Vogt, 1924
	1920-30s	B 	N/A	Tissues	Observational data	**Organizer Grafts** Spemann and Mangold, 1924 Wetzel, 1929
	1960s	C 	N/A	Tissues	Only specific to embryo of origin	**Chimera Generation** Tarkowski, 1965 Mintz, 1965

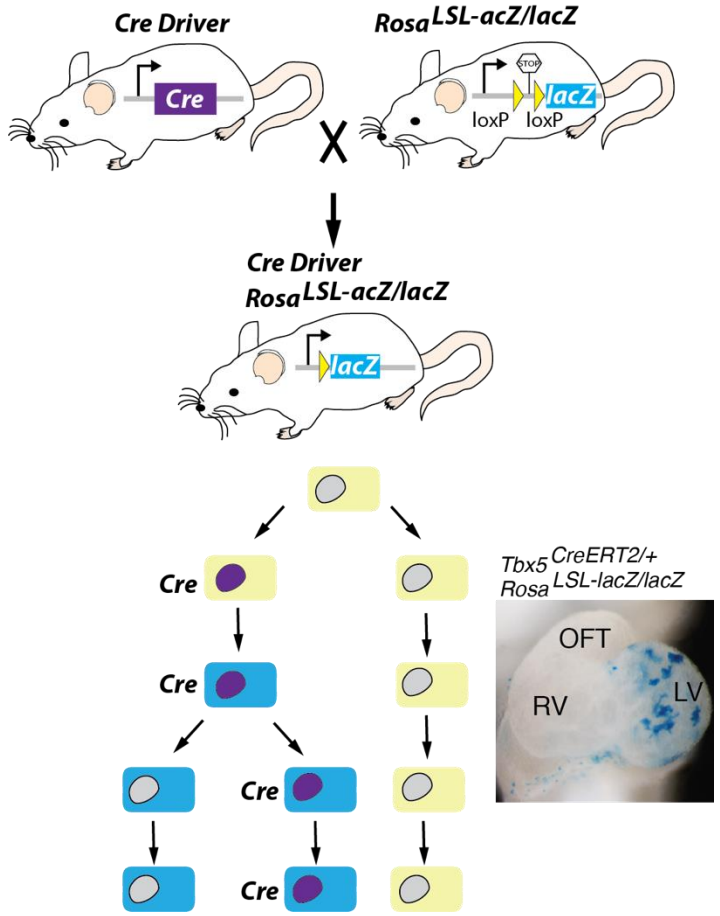
Molecular Biology	1980s		Theoretically single clones	10s of cells limited by observation	Observational data	**Retroviral Labelling** Cepko *et al*, 1987
	2000s		Theoretically single clones	100s of cells limited by observation	Observational data	**Randomized Recombination Cassettes** Livet *et al*, 2007 Snippert *et al,* 2010
	2010s		Theoretically single clones	100s of cells limited by observation	Dataset limitation based on collection method	**Cas9 Targeted Scar Accrual** McKenna *et al,* 2016 Junker *et al*, 2017

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

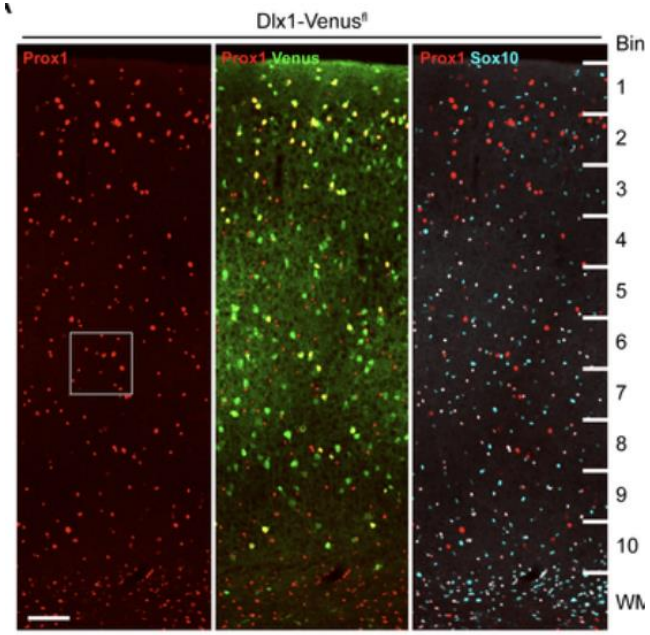
Inducible Lineage Tracing (CreERT2-loxP System)

Aim: Achieve highly efficient, temporally controlled labeling and tracking of specific cell populations and their progeny

- Uses CreERT2 (Cre recombinase fused to mutated estrogen receptor) and loxP-flanked reporter
- Tamoxifen administration activates CreERT2
- Induces permanent genetic labeling of target cells and their progeny
- Allows for precise temporal and spatial control of labeling



Lineage Tracing (Inducible Dox-based Reporters)



Controls:

- Vehicle-only (no tamoxifen) controls
- Cre-negative; reporter-positive controls

Critical points:

•Timing, dose, and route of tamoxifen administration

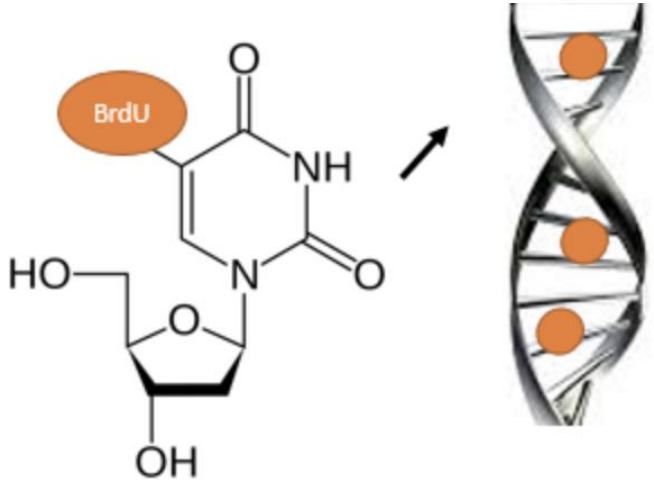
•Background recombination in absence of tamoxifen

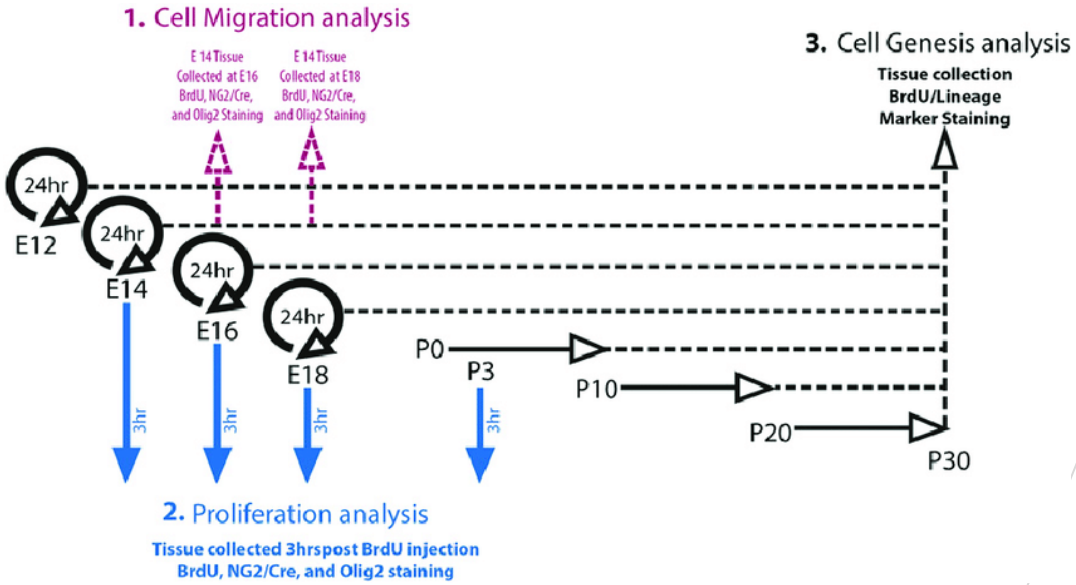
•Recombination efficiency and labeling sensitivity

Birth Dating with EdU/BrdU

Aim: Label and track newly born cells in the developing or adult nervous system

- EdU/BrdU are thymidine analogs incorporated into DNA during S-phase
- Pulse-labeling marks cells undergoing division at specific time points
- Detection via immunohistochemistry (BrdU) or click chemistry (EdU)
- Allows determination of cell birth timing and fate





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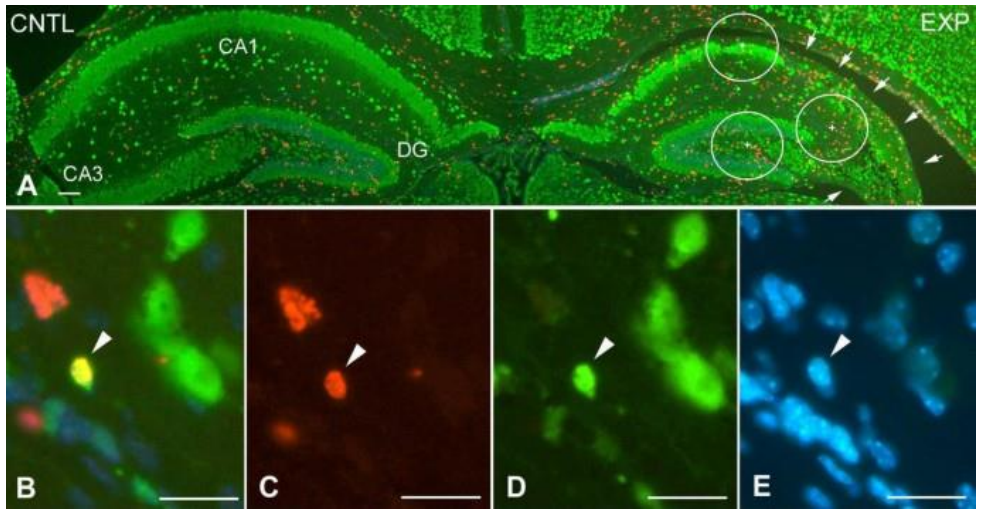


Controls:

- Untreated samples for background
- Known proliferative regions as positive controls

Critical points:

•Toxicity and potential effects on cell cycle

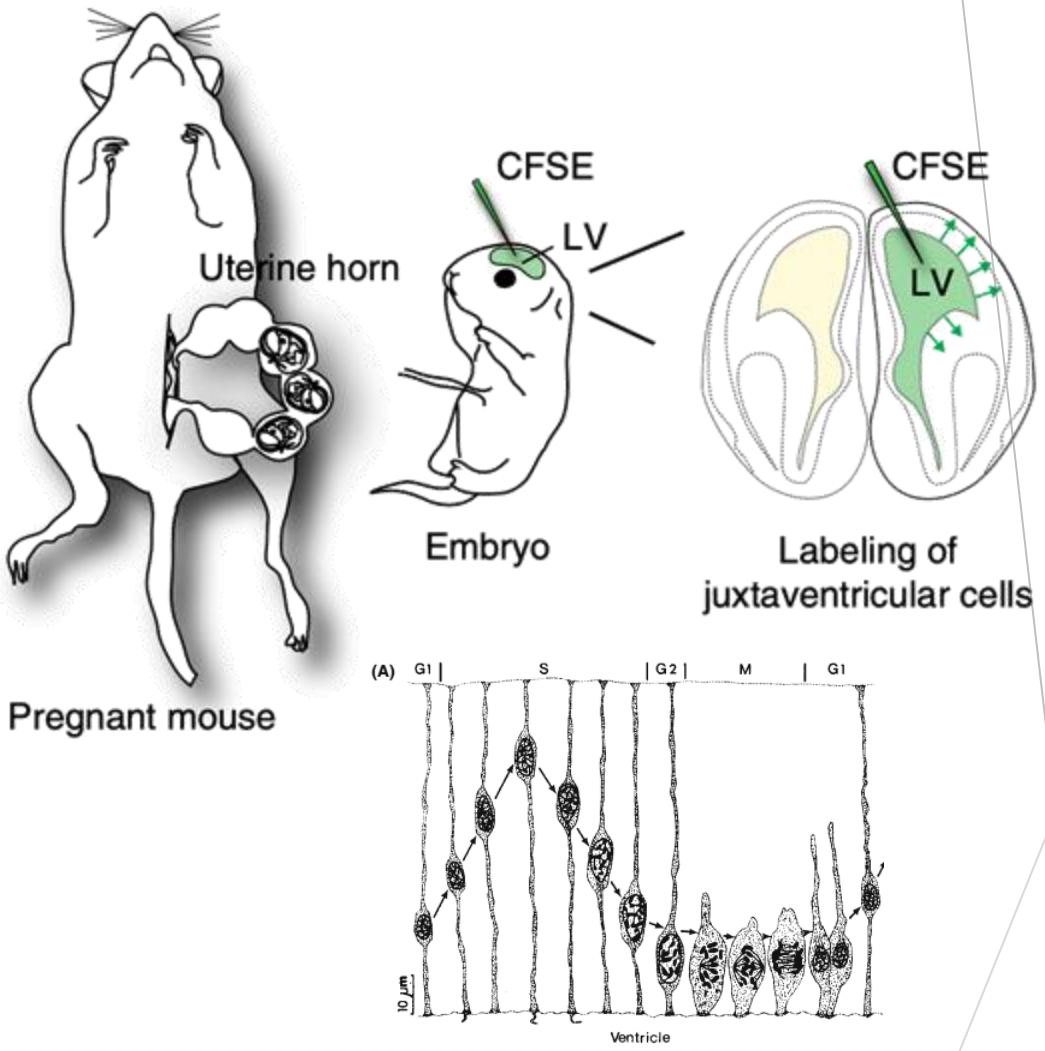
•Dosage and timing of administration

•Sensitivity and specificity of detection methods

Flash Tag Technique

Aim: Precisely label and track newborn neurons from the ventricular zone

- Intraventricular injection of fluorescent molecule (e.g., CFSE)
- Rapid uptake by ventricular zone progenitors
- Fluorescence retained in daughter cells
- Allows for high temporal resolution birth dating



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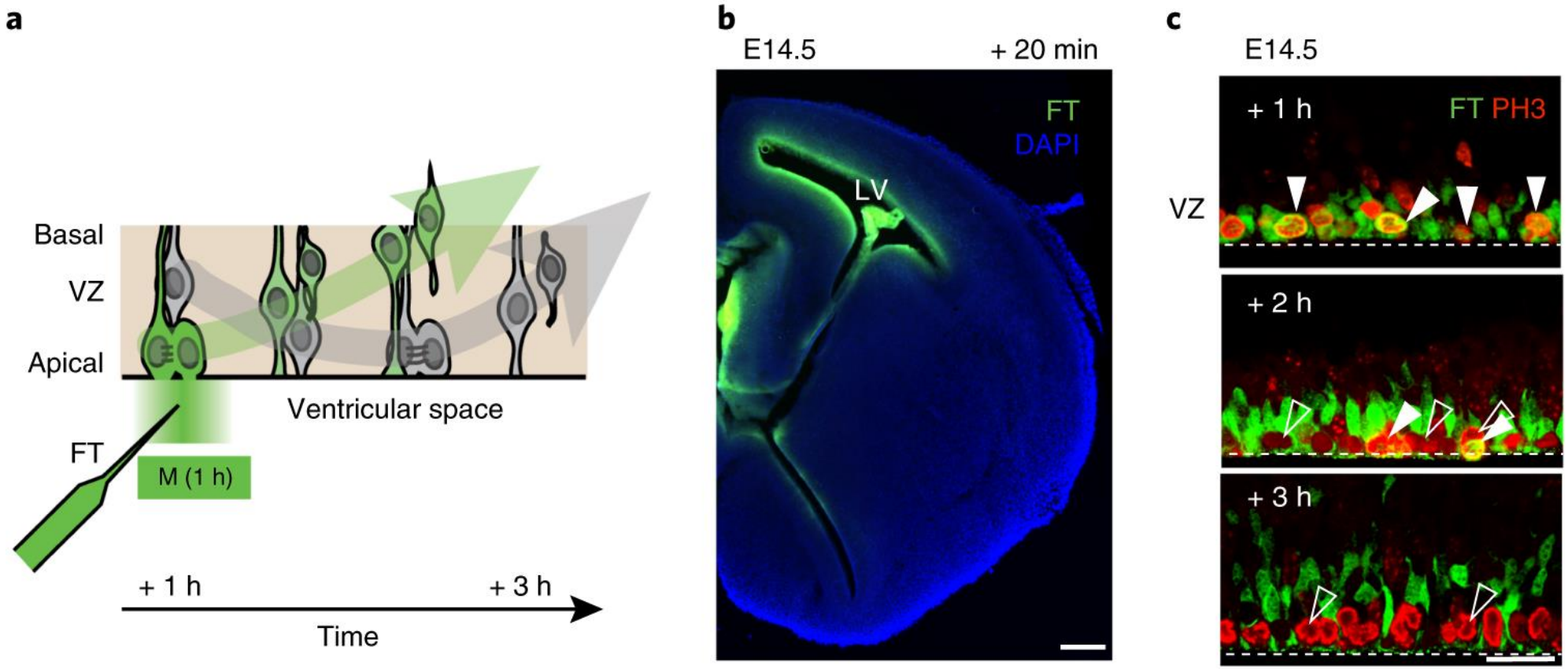


Flash Tag Technique

Controls:

- Vehicle-only injections
- Comparison with traditional birth dating methods (EdU/BrdU)

Critical points:

•Injection technique and timing

•Potential for labeling non-dividing cells

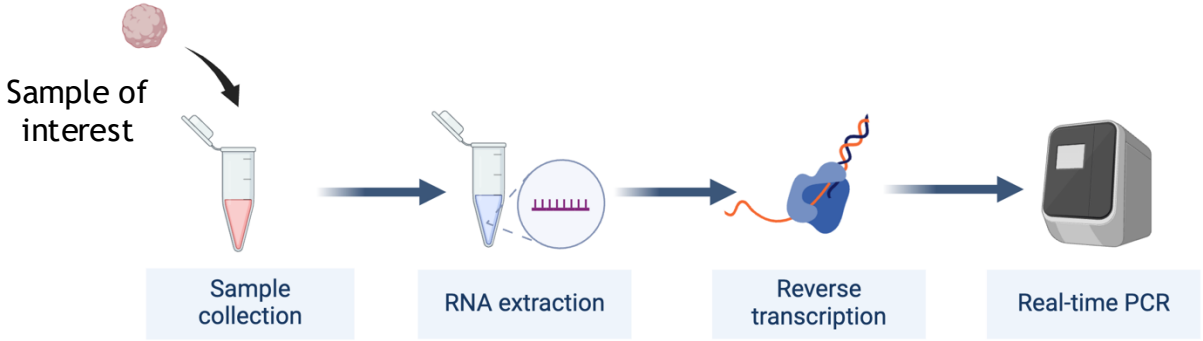
•Signal dilution with successive divisions

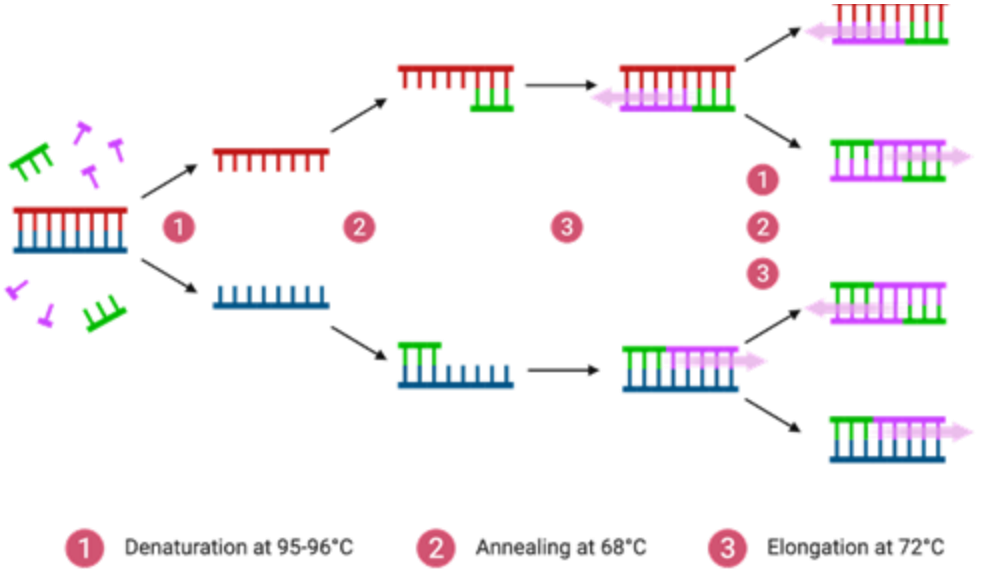
Molecular Techniques

Quantitative PCR (qPCR)

Aim: *Measure relative or absolute gene expression levels*

- Amplifies and quantifies targeted DNA sequences,
- Uses fluorescent reporters to detect amplification in real-time.
- Cycle threshold (Ct) values indicate relative abundance.





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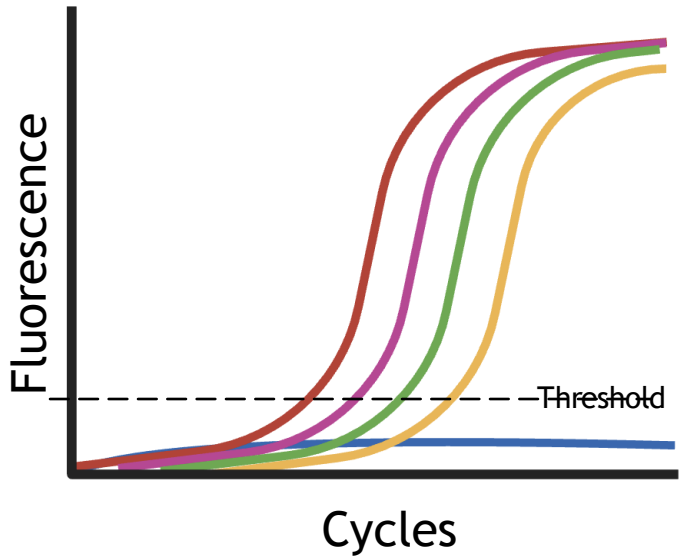


Controls:

- Housekeeping genes for normalization
- No template control (NTC)

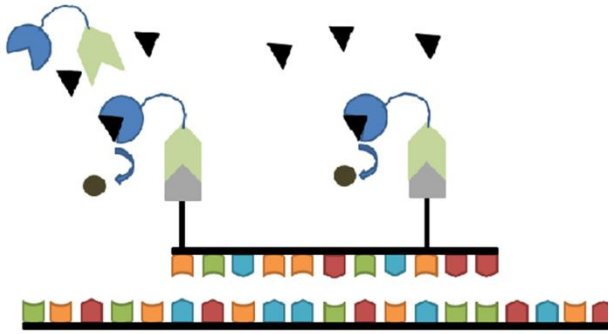
Critical points:

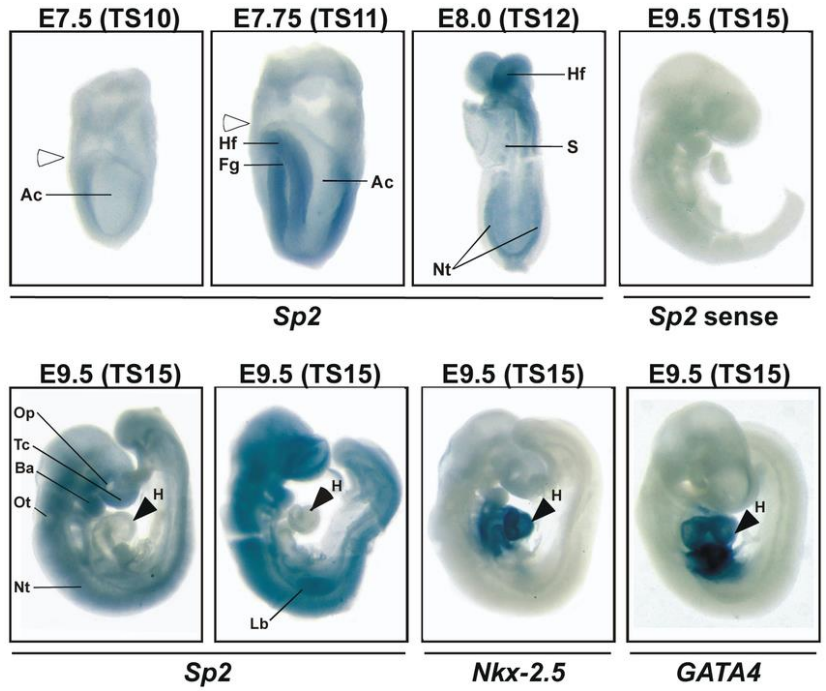
- Primer design and efficiency
- Sample quality and consistency
- Proper data analysis and interpretation

In Situ Hybridization (ISH)

Aim: Visualize and localize specific RNA sequences within tissues or cells

- Uses labeled complementary DNA or RNA probes
- Hybridizes to target mRNA in fixed tissue sections
- Colorimetric detection reveals spatial gene expression patterns





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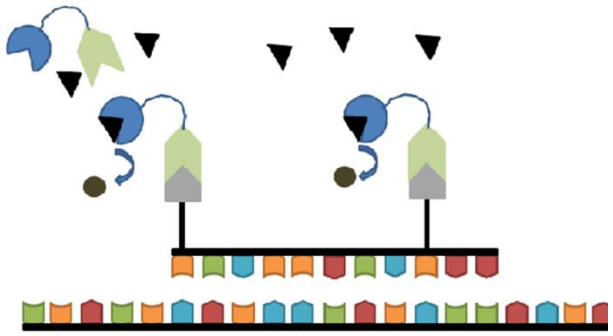


Controls:

- Sense probe as negative control
- Known expression patterns as positive control

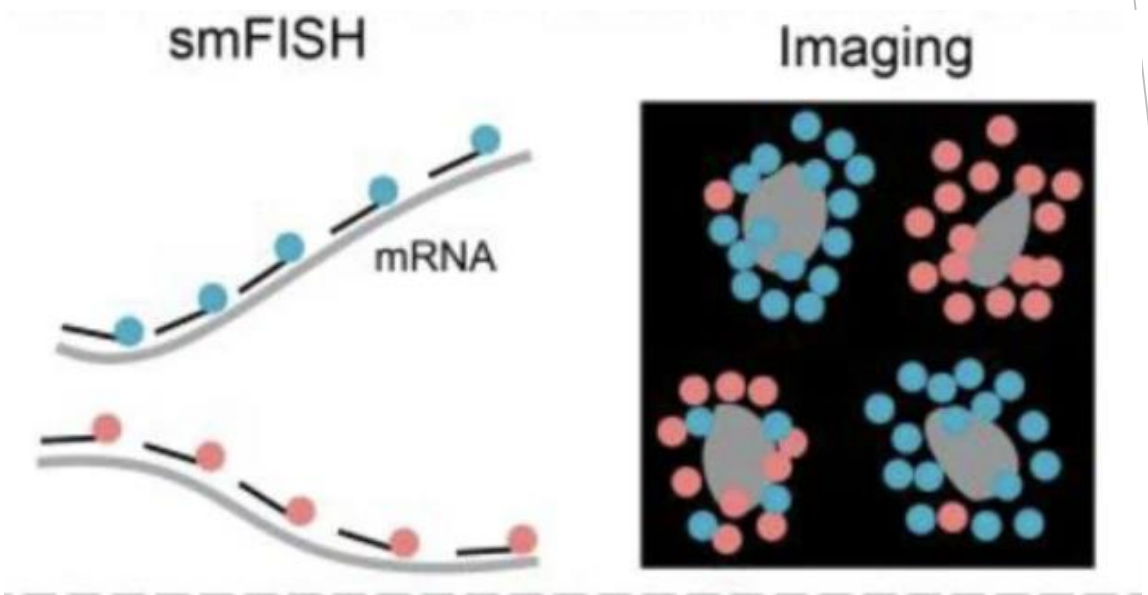
Critical points:

- Probe specificity and sensitivity
- Tissue fixation and permeabilization
- Signal-to-noise ratio and background

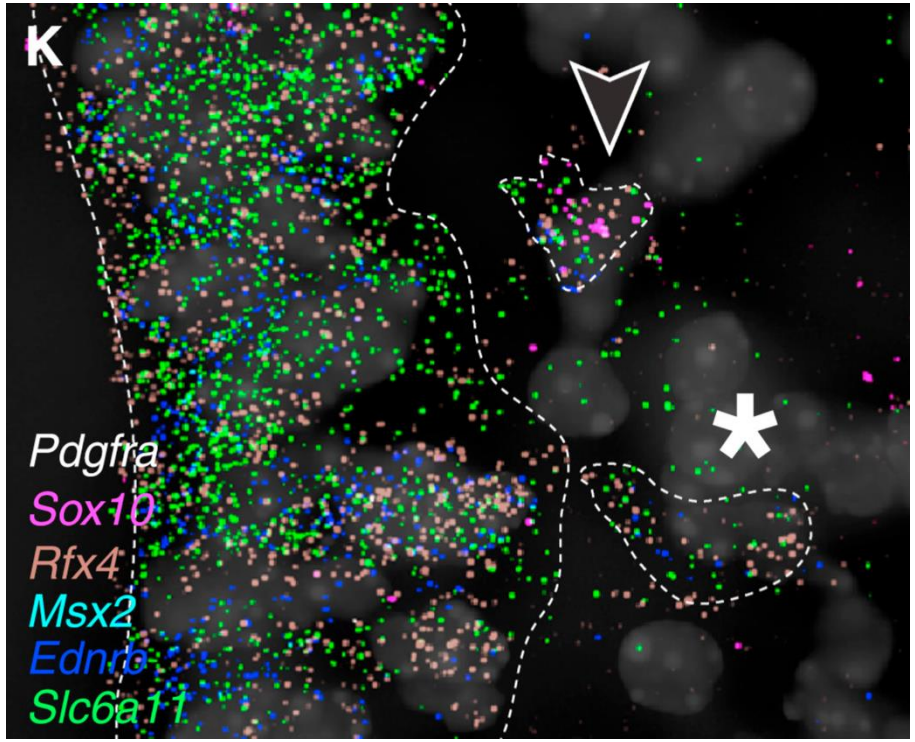
Quantitative smFISH (single-moleucle Fluorescent ISH)

Aim: Detect and quantify specific RNA sequences at single-molecule resolution

- Uses fluorescently labeled oligonucleotide probes
- Hybridizes to target mRNA in fixed cells or tissues
- Visualizes individual mRNA molecules as fluorescent dots



Quantitative FISH (Fluorescent In Situ Hybridization)



Controls:

- Negative control probes
- Positive control for known abundant transcripts

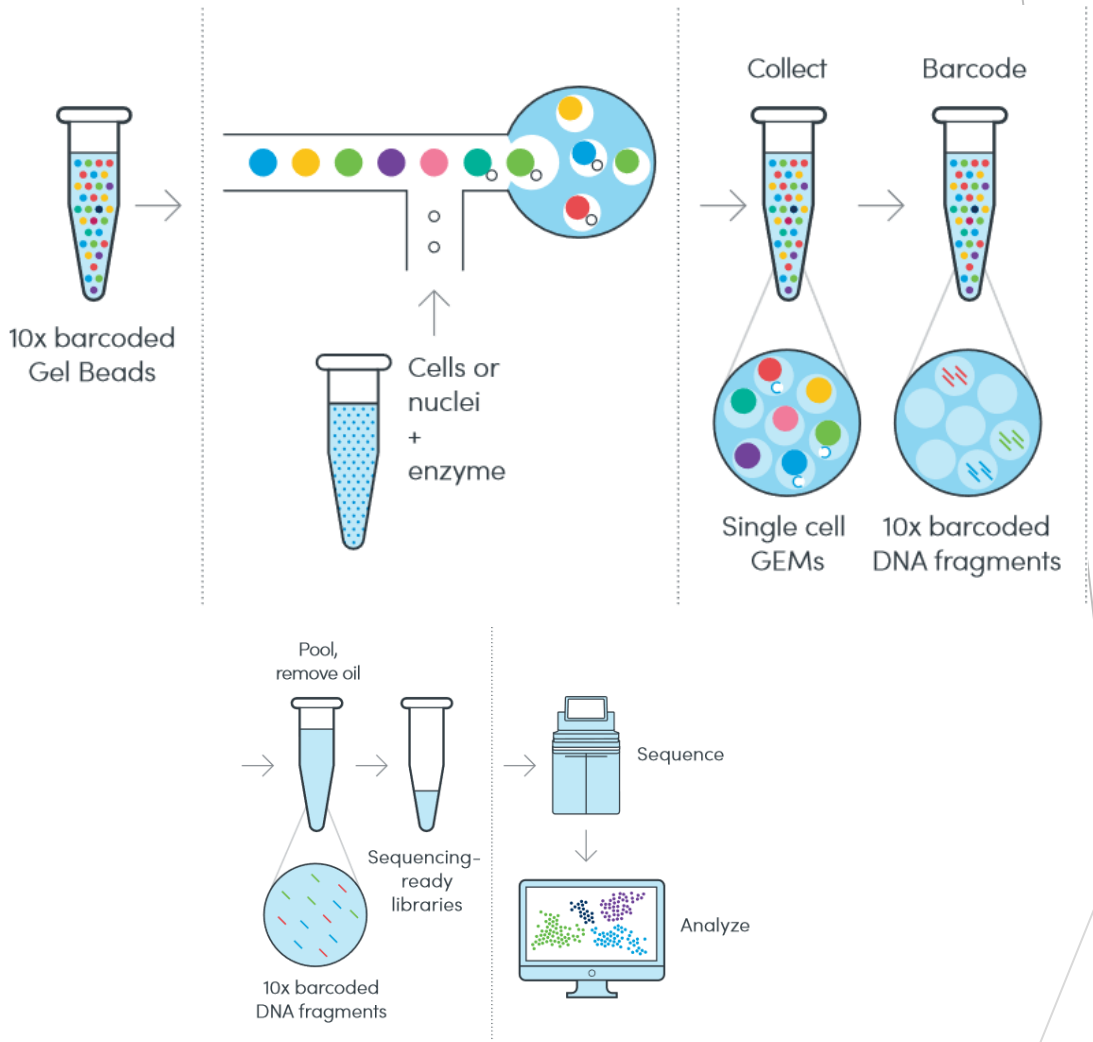
Critical points:

- Probe design and specificity
- Image acquisition and analysis parameters
- Thresholding and spot counting algorithms

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



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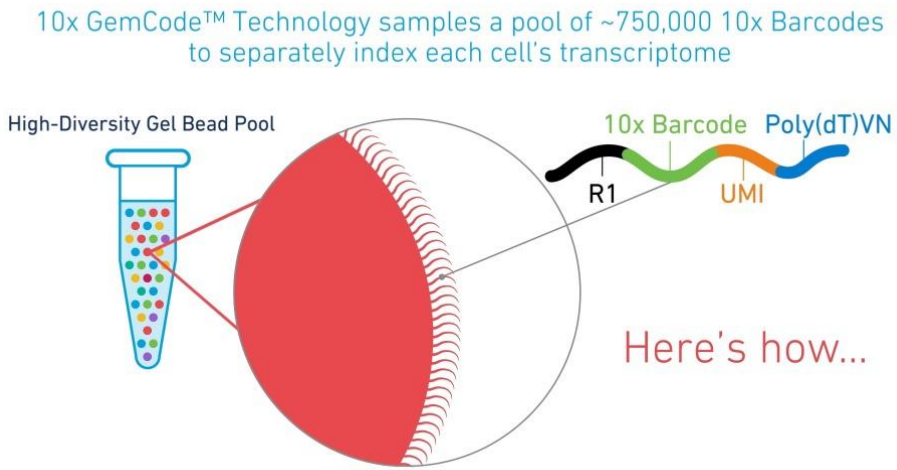


Controls:

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

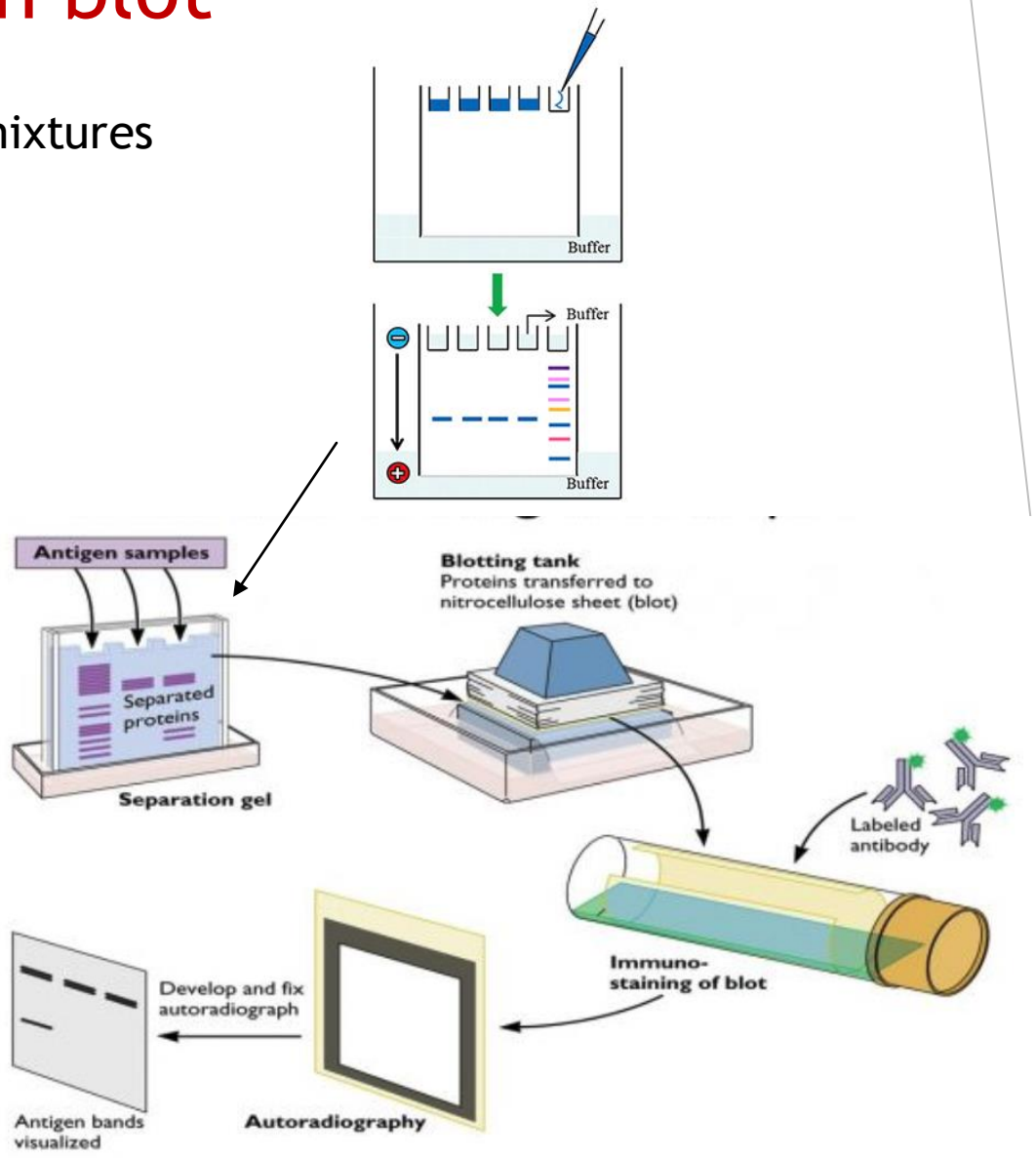
Critical points:

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

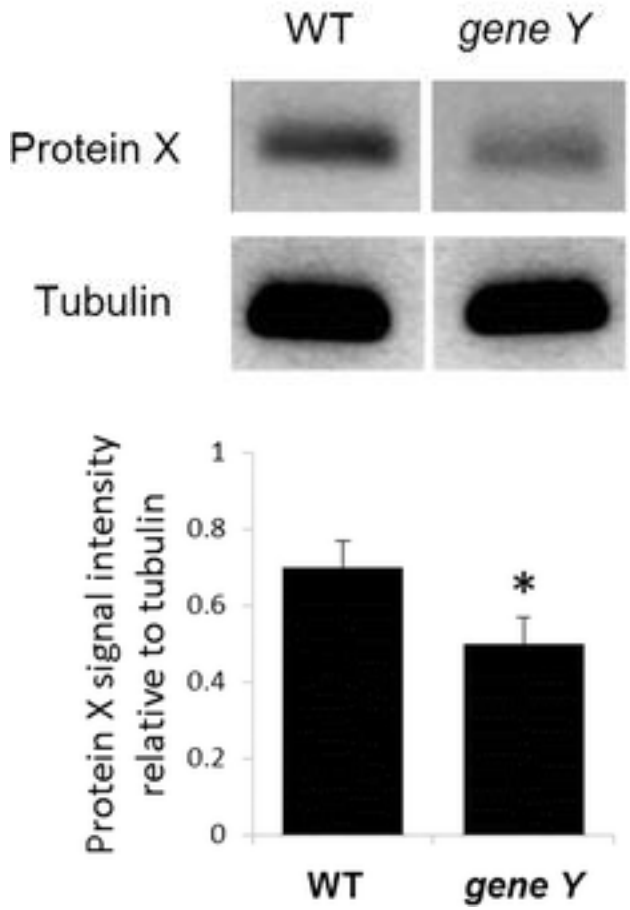
Western blot

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

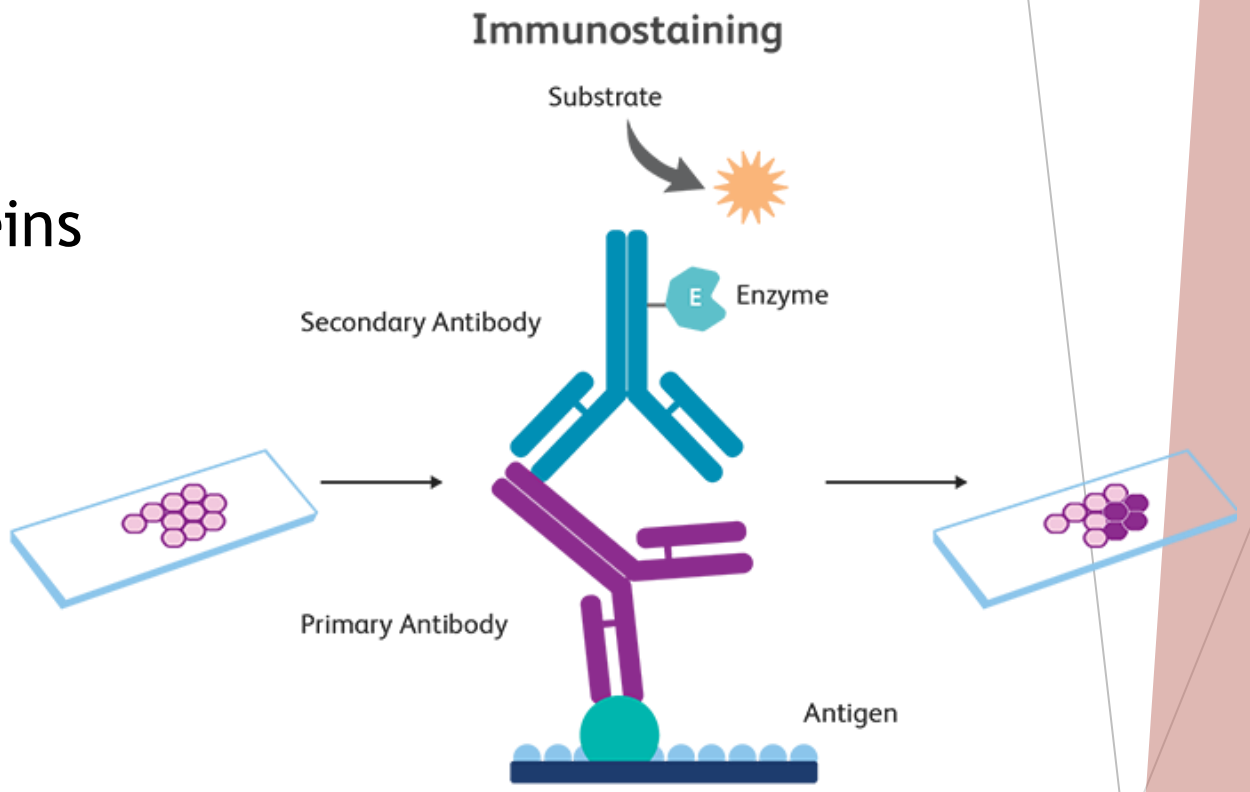
Critical points:

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

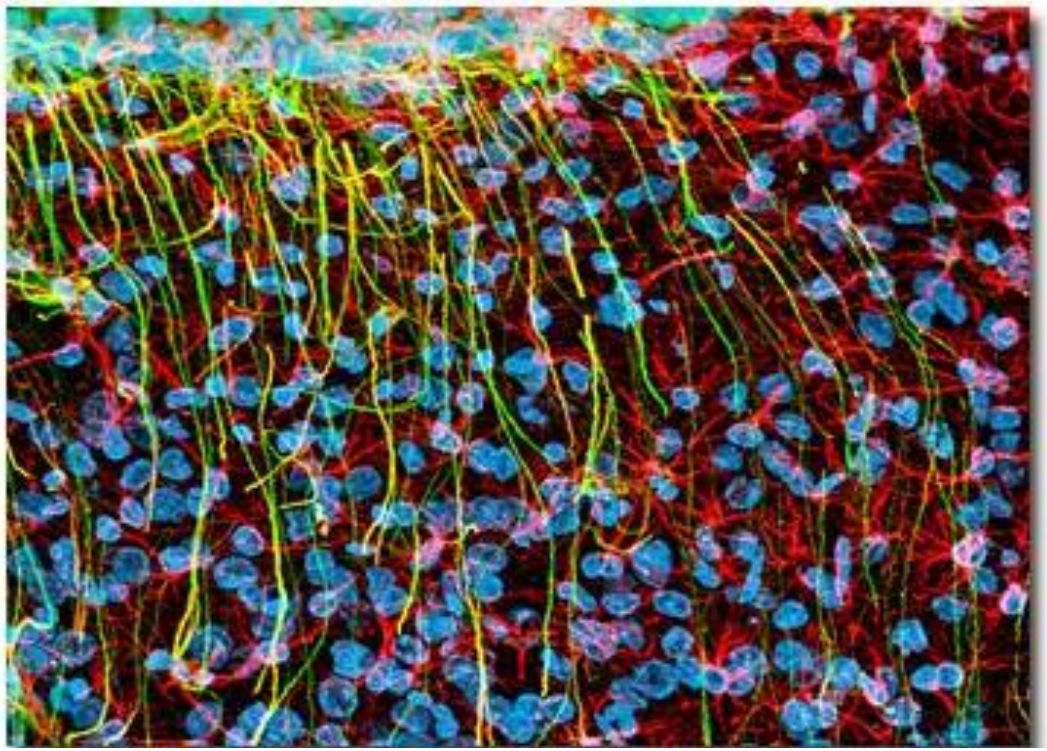
Immunostaining

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

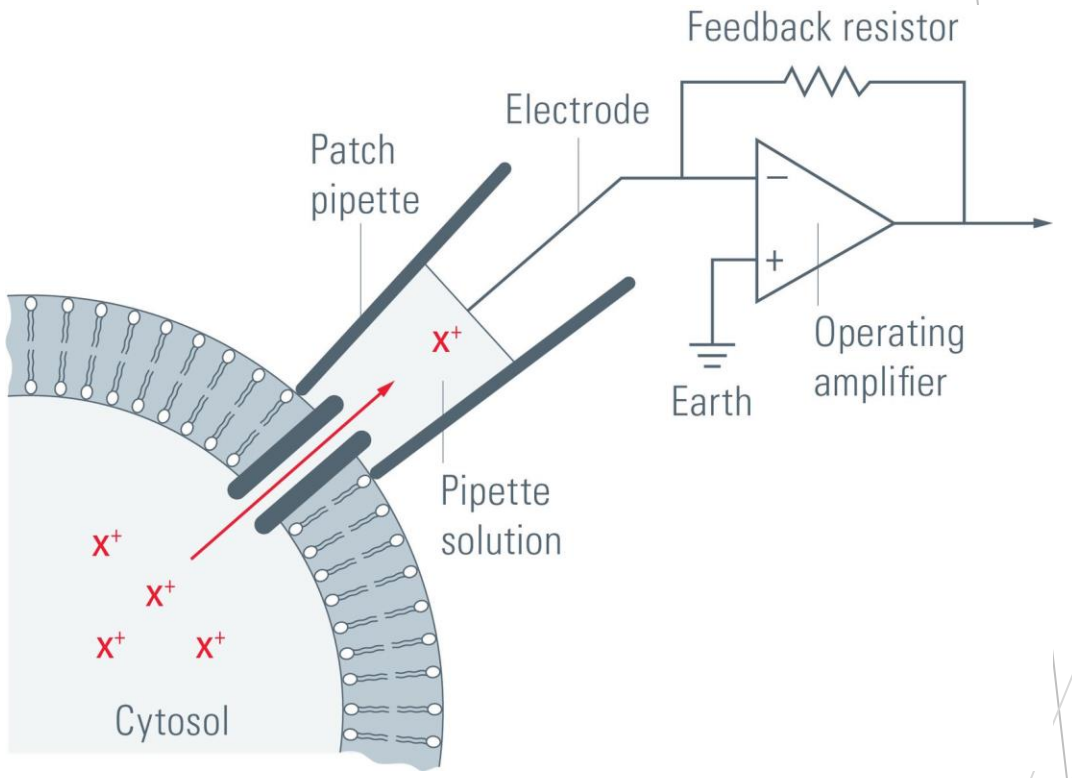
•Autofluorescence and background signal

Neuronal activity

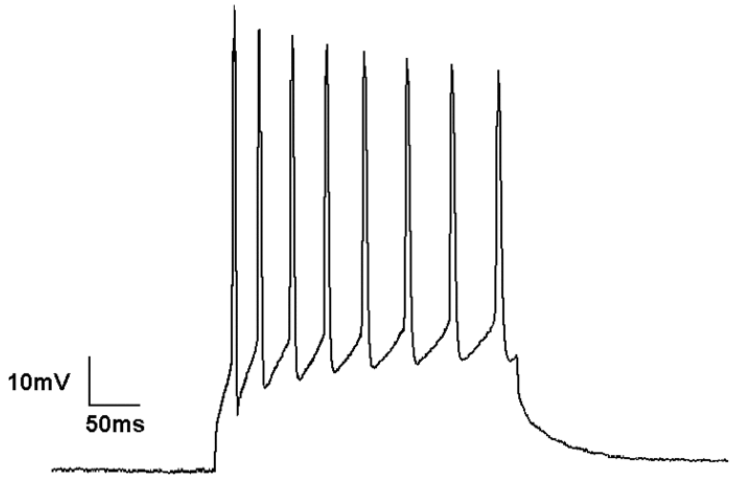
Patch Clamp Electrophysiology

Aim: Measure electrical activity and membrane properties of individual neurons

- Forms tight seal between pipette and cell membrane
- Records current flow through ion channels
- Allows manipulation of intracellular environment
- Provides high-resolution temporal data on neuronal activity



Patch Clamp Electrophysiology



Controls:

- Known ion channel blockers or activators
- Comparison with model cell parameters

Critical points:

•Cell health and viability during recording

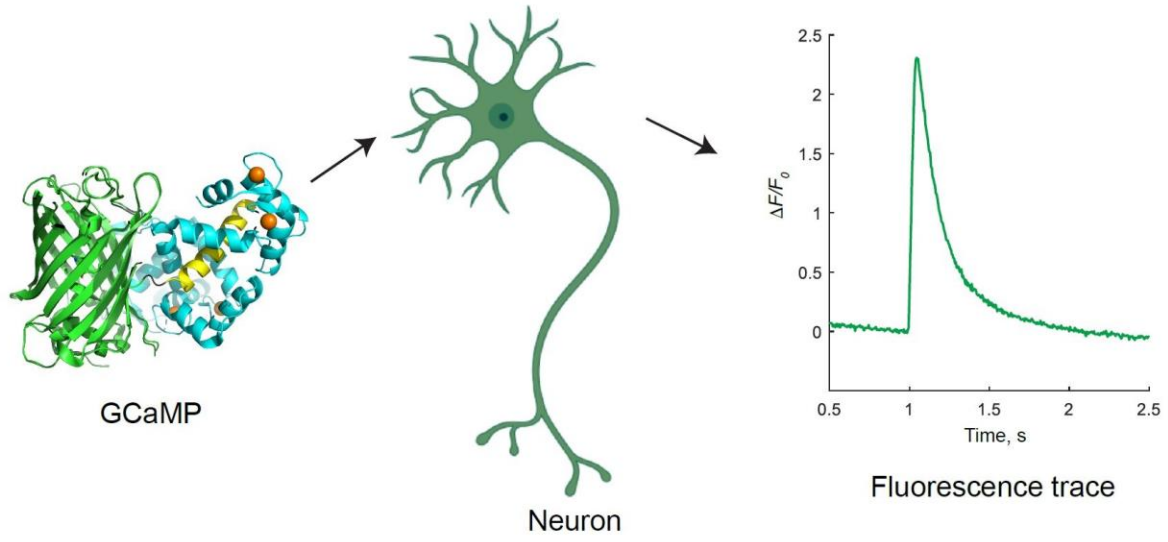
•Series resistance and capacitance compensation

•Liquid junction potential correction

Calcium Imaging with GCaMP

Aim: Visualize neuronal activity through calcium-dependent fluorescence changes

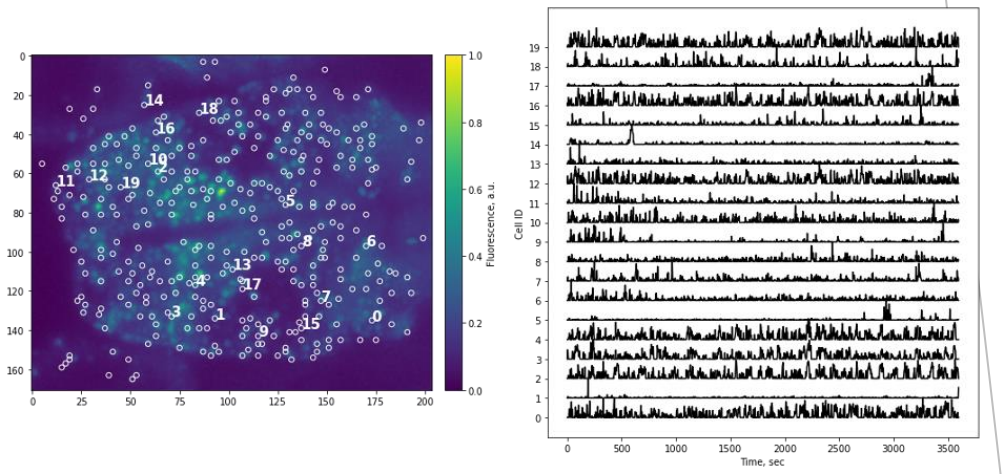
- GCaMP: Genetically encoded calcium indicator
- Binds to Ca2+ upon neuronal firing
- Increases fluorescence intensity
- Optical imaging captures activity patterns



Calcium Imaging with GCaMP

Controls:

- Calibration with known stimuli
- Comparison with electrophysiological recordings



Critical points:

- Temporal resolution and calcium dynamics
- Potential interference with cellular calcium homeostasis
- Relationship between fluorescence and spike rate