Single-Cell Sequencing in Molecular Recording and Natural Time Tracking

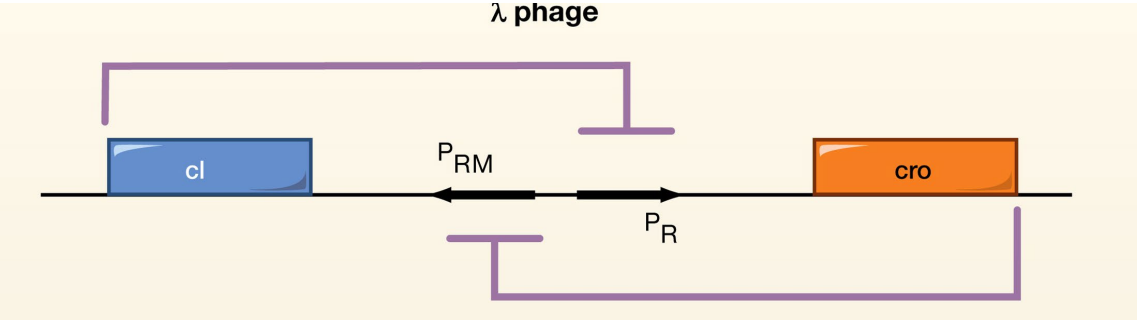
2025

Group1: Lewei He

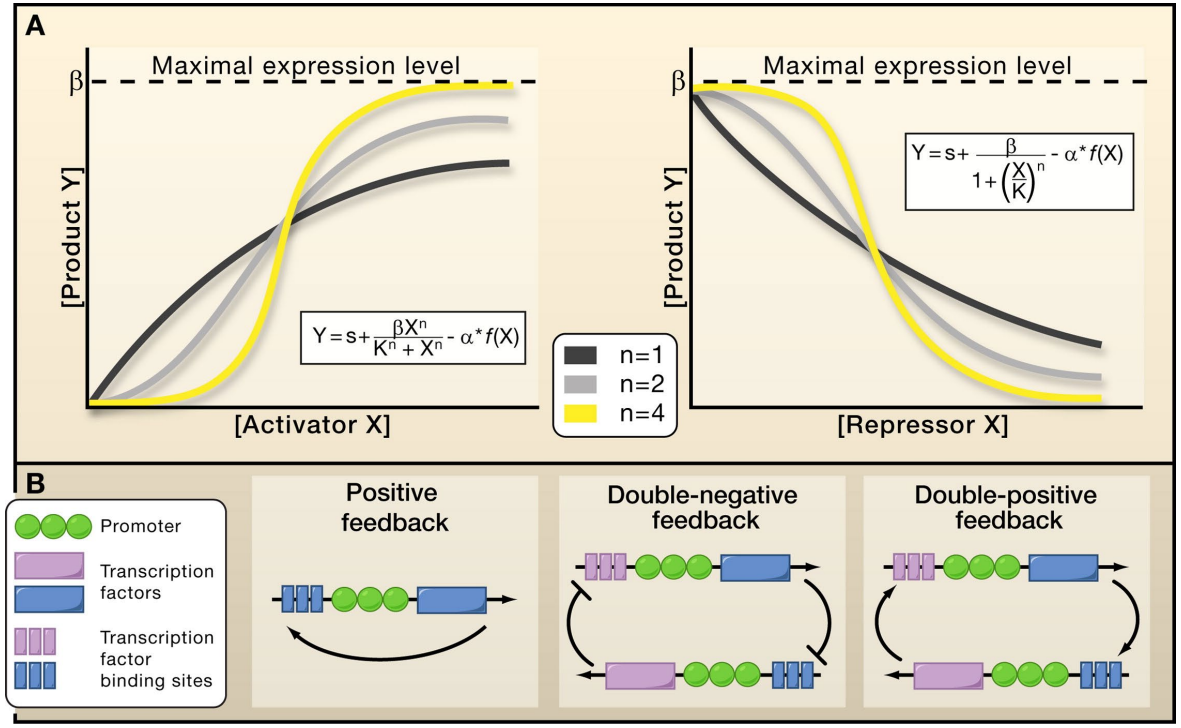
Making Cellular Memories

Biological memory can be defined as a sustained cellular response to a transient stimulus

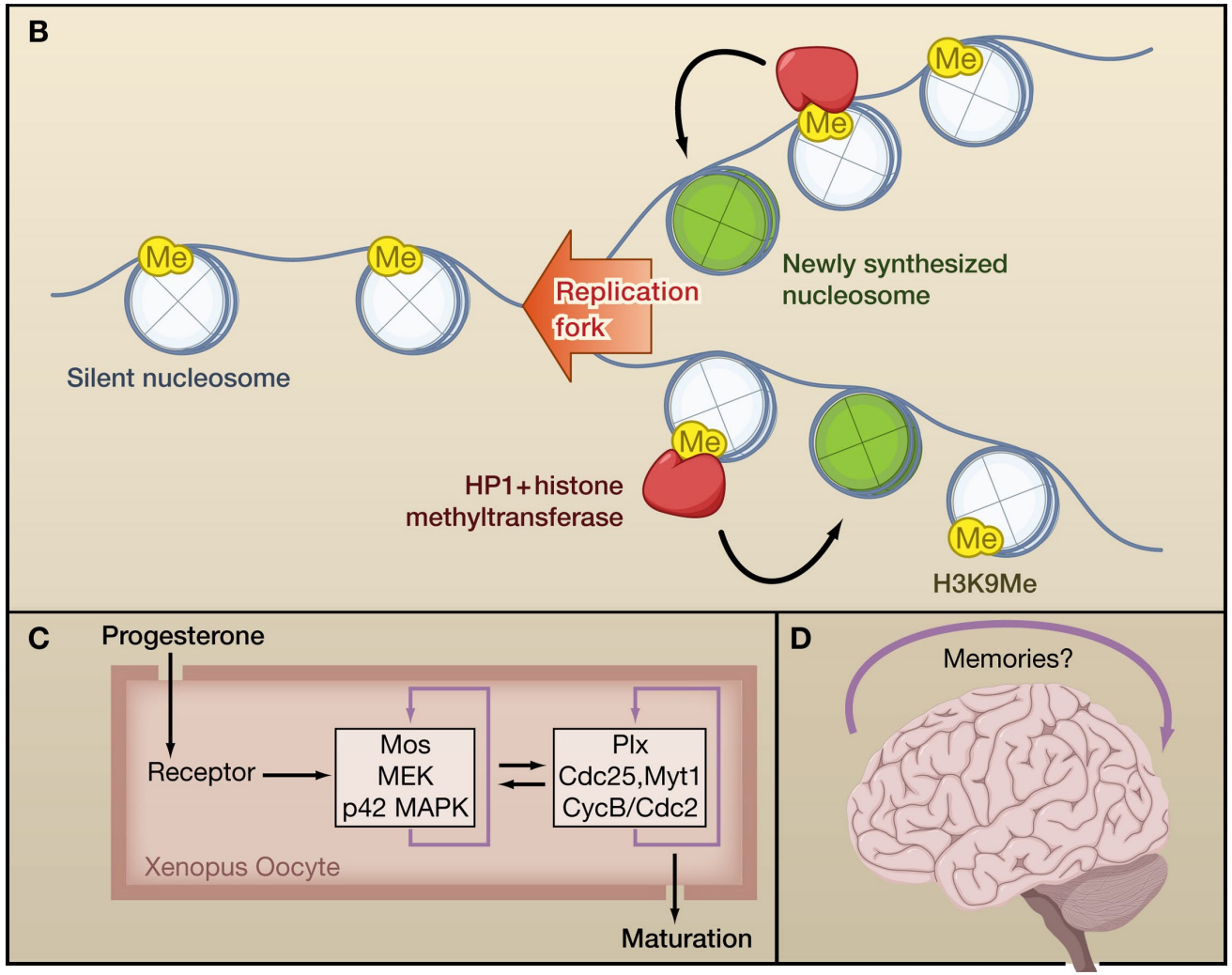
The phage lambda system



Gene Circuits for Cellular Memory

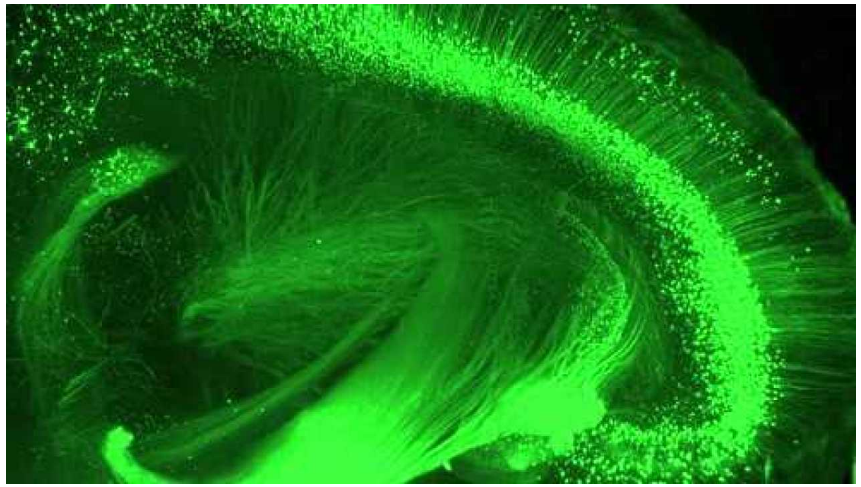


Replication-coupled nucleosome



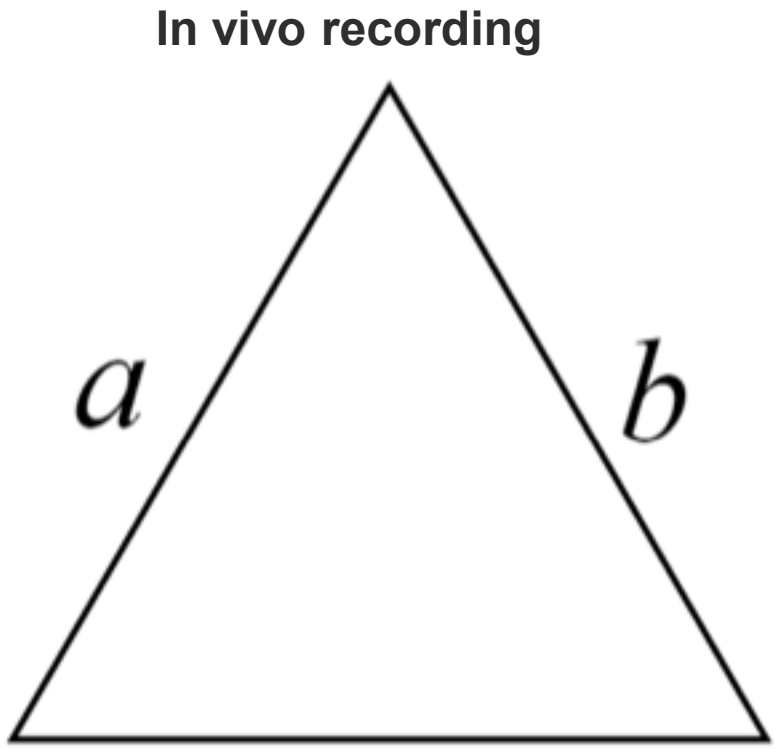
Burrill, Devin R. Cell (2010)

Questions in Recording Cellular Memory

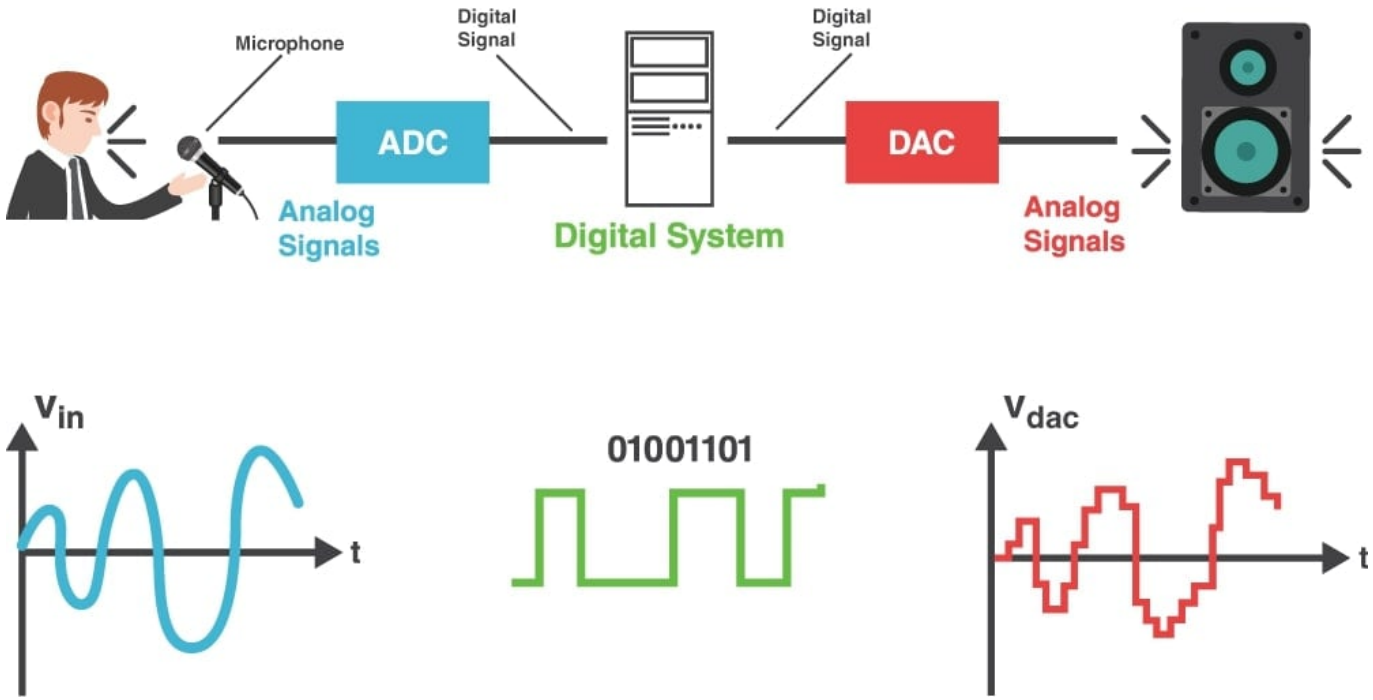


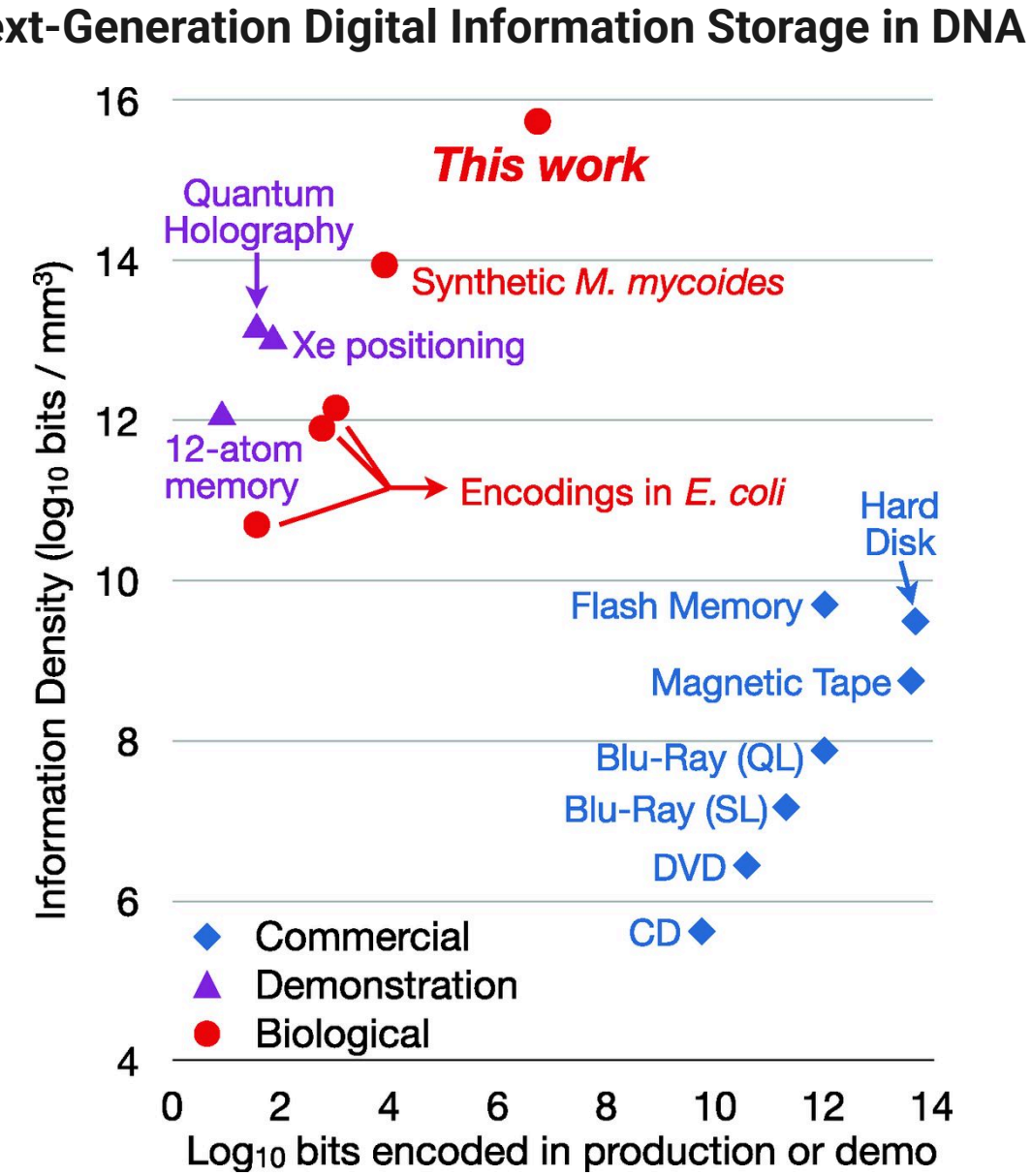
reporter signal

Impossible trinity in molecular recording:



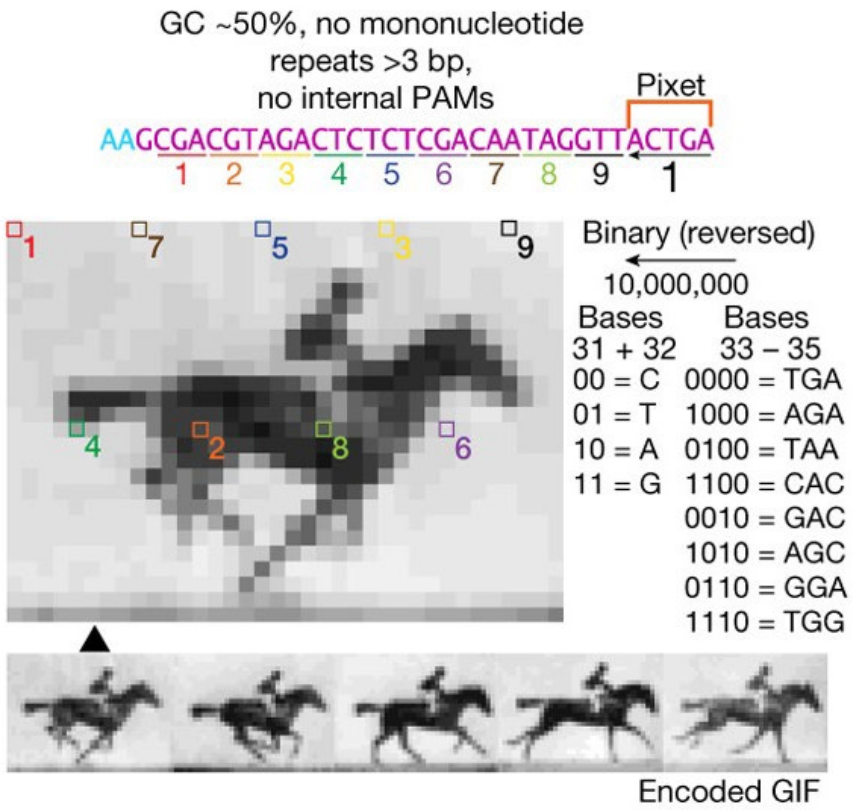
Digital recording in model life





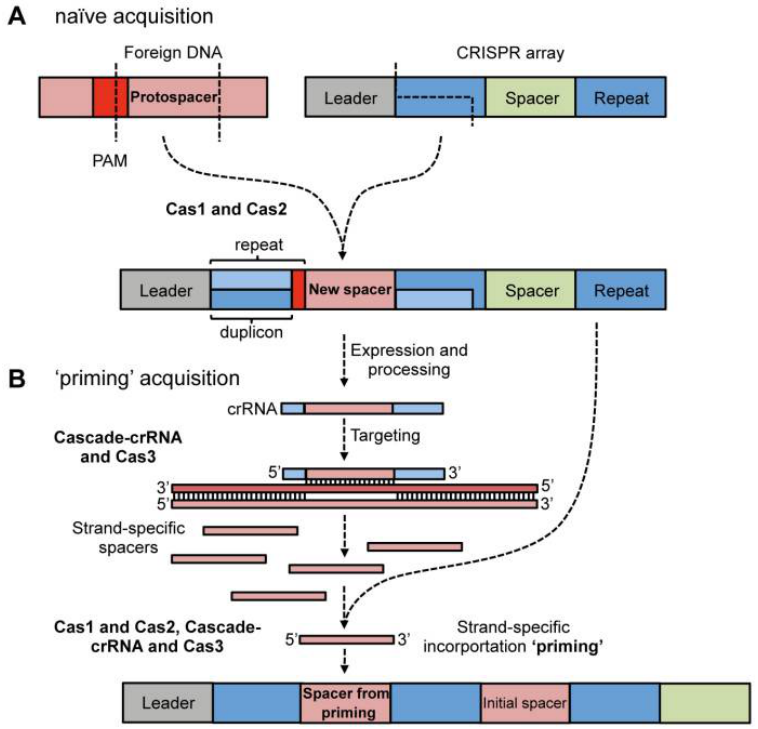
George Church. Science (2012)

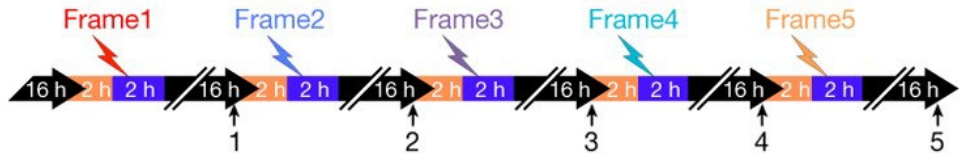
Encoding a digital movie into the genomes of bacteria

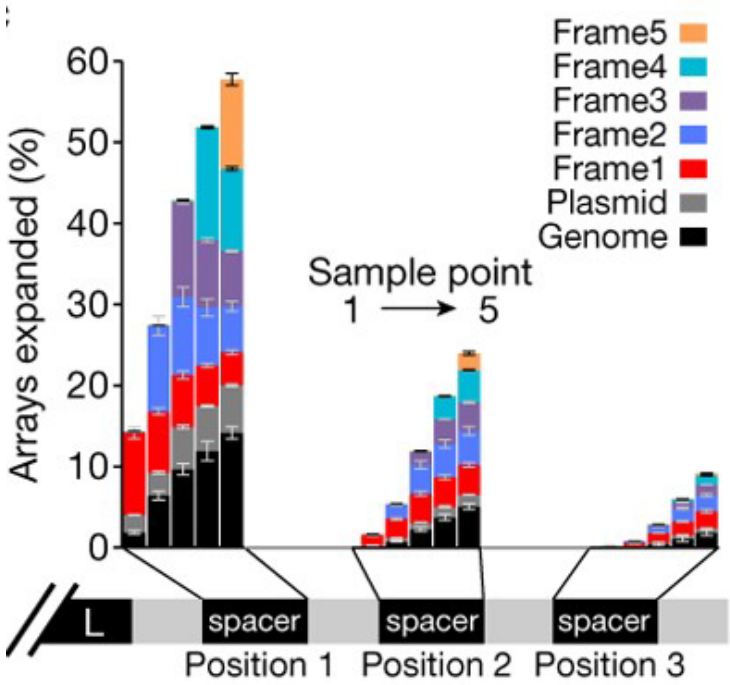


Human and Animal Locomotion

Cas1-Cas2 system

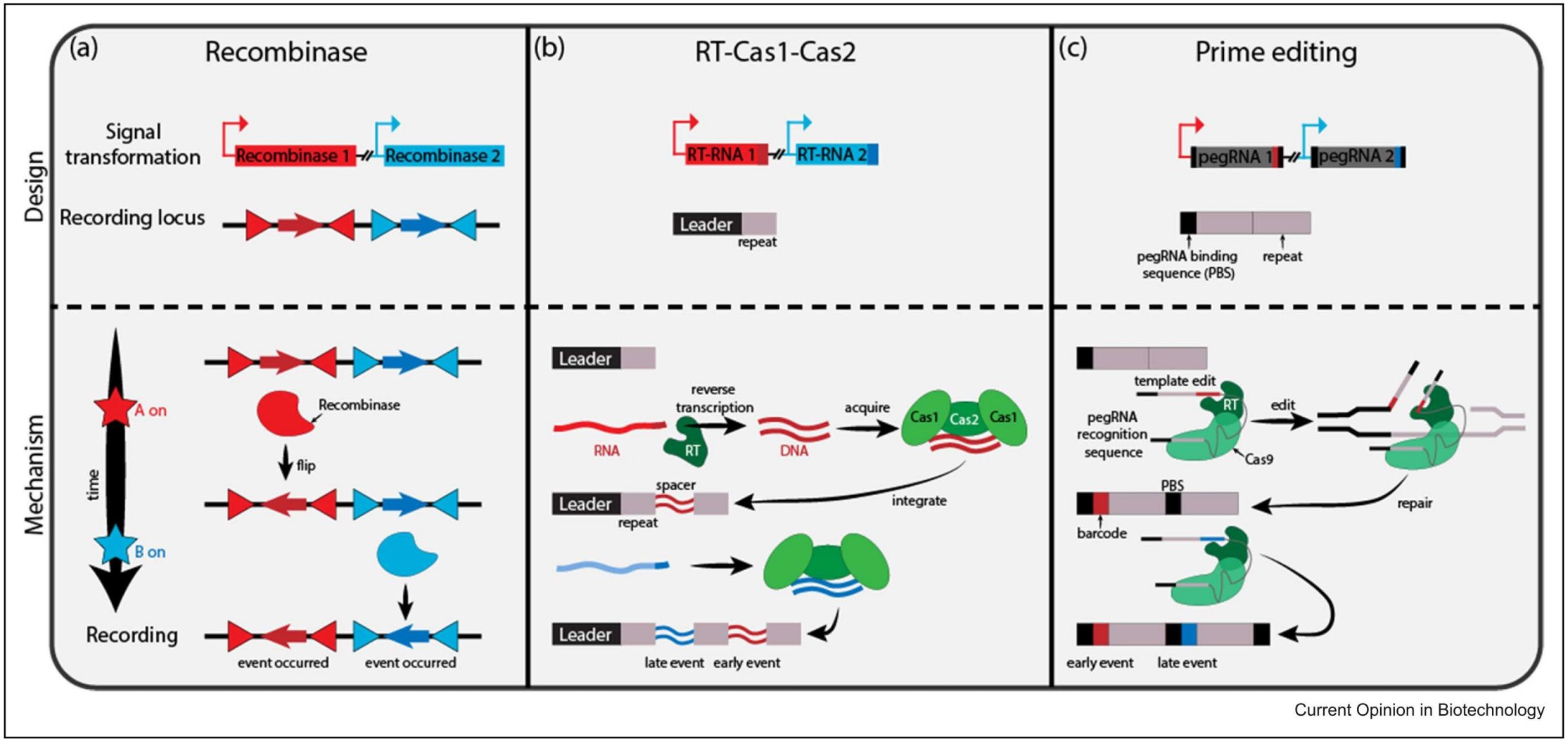






Shipman, S. *Nature* (2017)

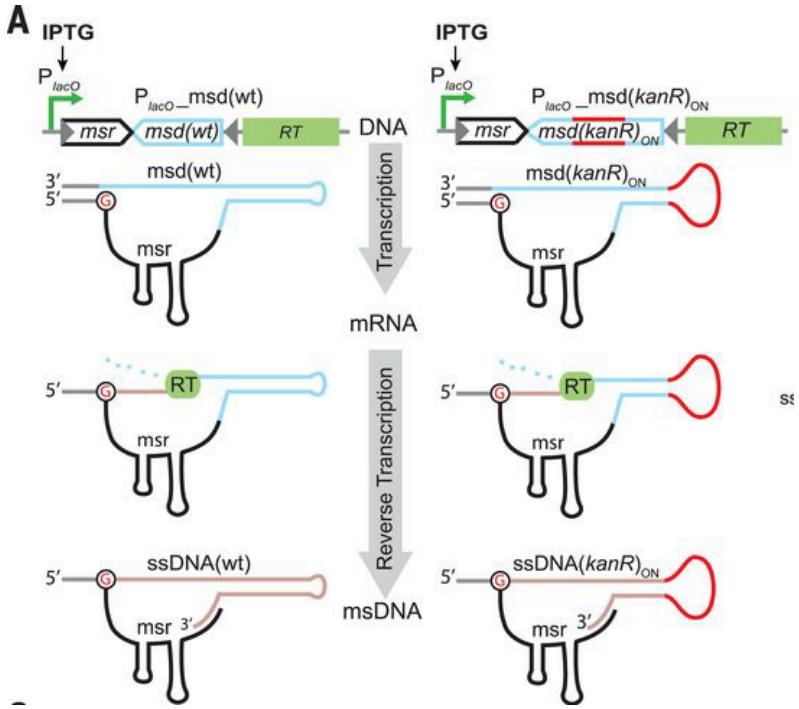
Strategy in Molecular Recording



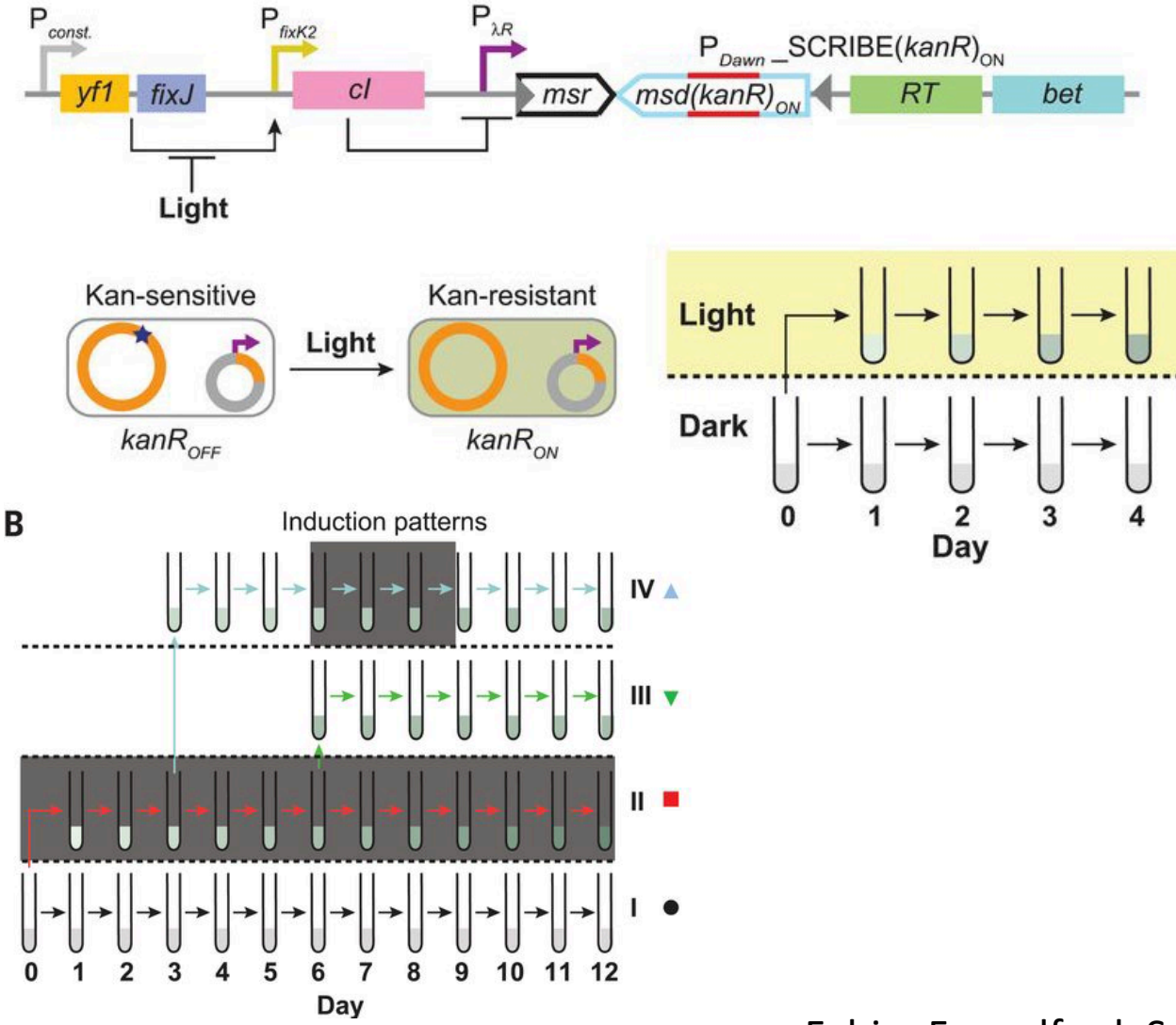
Lear SK. Curr Opin Biotechnol (2023)

Recombinase + Recording in living populations

Synthetic ssDNA

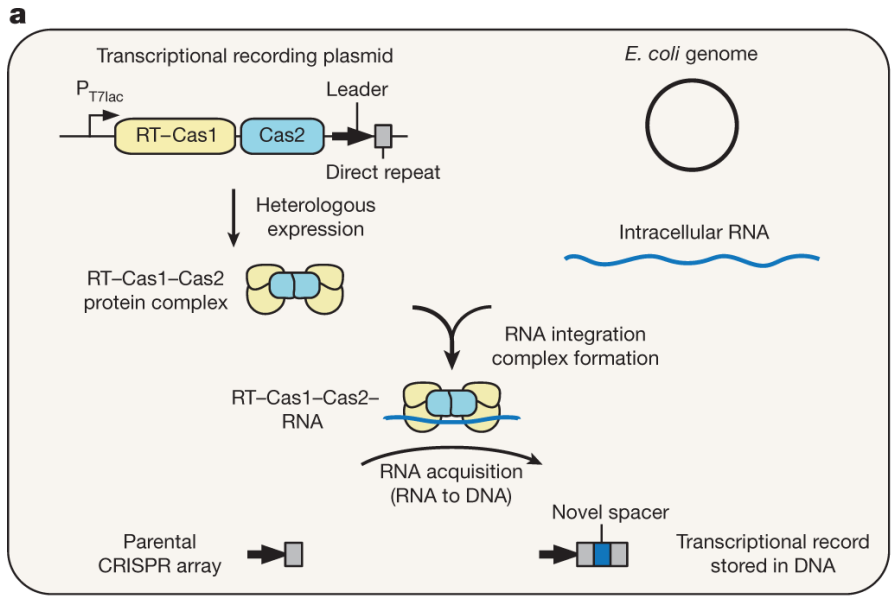


Optogenetic genome editing

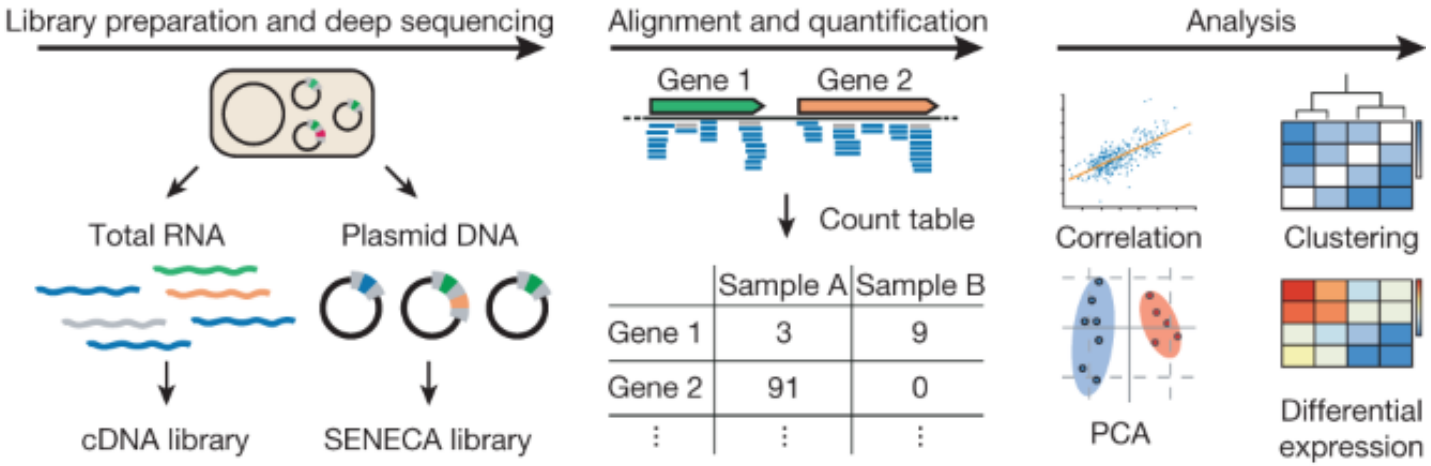


Fahim Farzadfard. Science

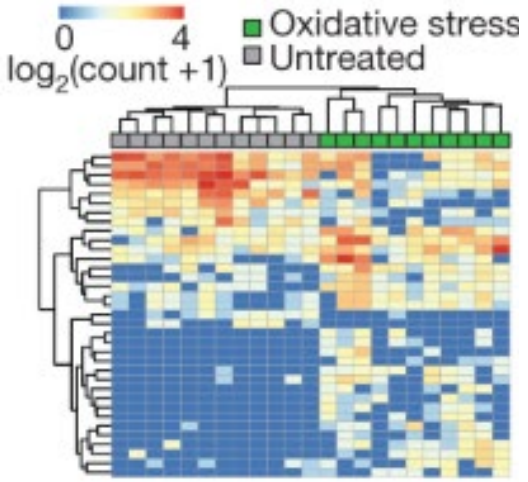
FsRT-Cas1-Cas2+ Recording in living populations



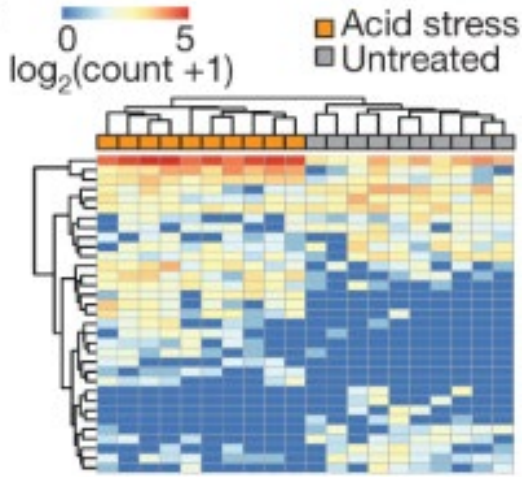
Record-seq



under oxidative stress



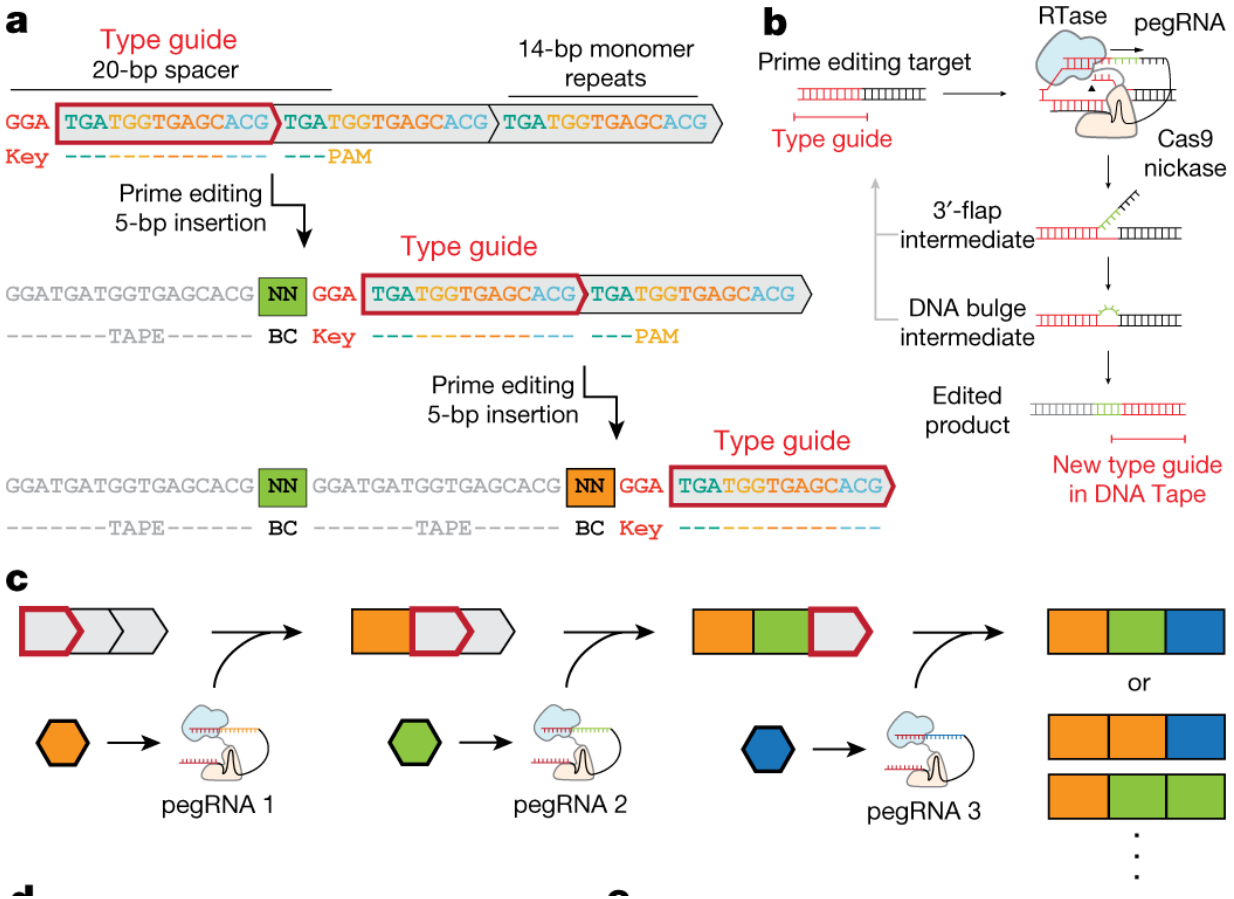
under acid stress



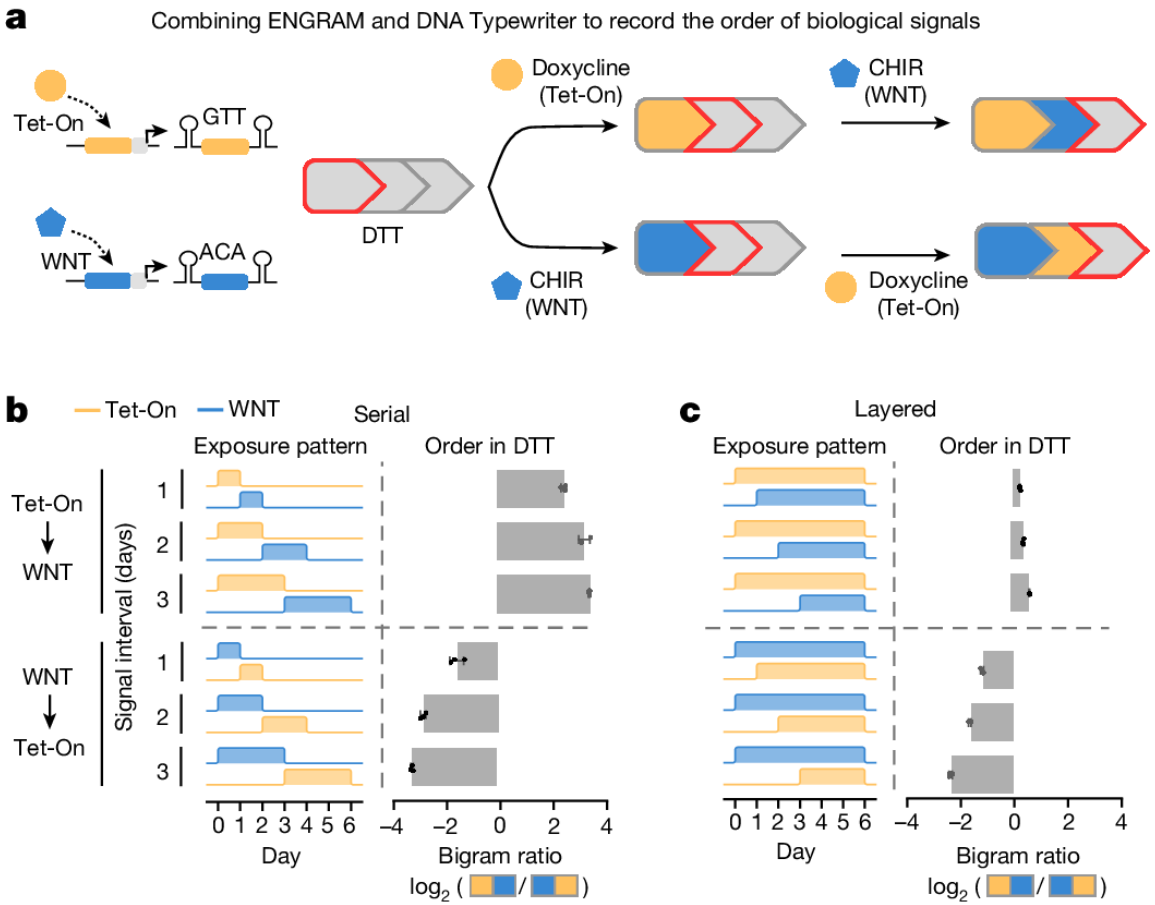
Schmidt, F. *Nature* (2018)

DNA Typewriter: Prime editing + recording in single cell

Sequential genome editing with DNA Typewriter



Multiplex recording of intensity and duration of activity



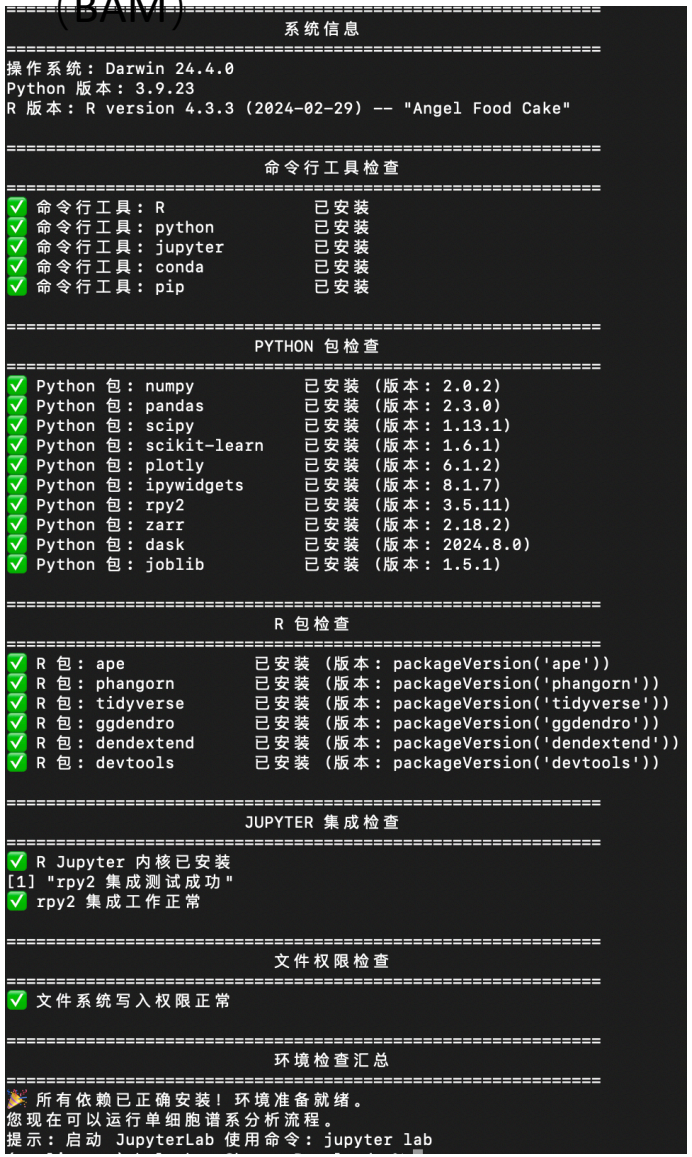
Choi, J. *Nature* (2022)

Chen, W. *Nature* (2024)

Liao, H. *Nat Protoc* (2024)

DNA Typewriter

SRR15584112(175) ➡️ SRA Toolkit(fasta) ➡️ samtools (BAM)



Step 1: TAPE_10X_read2fromBAM

Objective: Extract raw data from CellRanger-generated BAM files to generate a table linking cell barcodes, TAPE barcodes, edit sites (Sites 1–5), and molecule counts.

Input: Position-sorted BAM file (10X Genomics data).

Output: A **cell-TAPE edit association tableStep 2: TAPE_text_sorting**

Objective: Process the output from Step 1 to generate structured statistics for downstream analysis.

Input: The cell-TAPE edit association table from TAPE_10X_read2fromBAM.

Output: Bigram matrix; Unigram frequencies; Transfected barcode order.

	Cell	TargetBC	Site1	Site2	Site3	Site4
1	AGCCATAGCGCCTAC-1	ATTTGGTT	CCGGGA	CCGGGA		
2	AGCCATAGCGCCTAC-1	TTCACGTA	CCGGGA	TATGGA		
3	AGCCATAGCGCCTAC-1	TAGATTTT	CTAGGA	GCCGGA		
4	AGCCATAGCGCCTAC-1	TTGTTTAC	CATGGA	CCGGGA		
5	AGCCATAGCGCCTAC-1	ATTTATAT	ATAGGA	CATGGA	CATGGA	CATGGA
6	AGCCATAGCGCCTAC-1	TTAGATTG	GATGGA	CTAGGA	ACAGGA	
7	AGCCATAGCGCCTAC-1	TGGACGAC	GCCGGA			
8	AGCCATAGCGCCTAC-1	TGGTTTTG	GATGGA	TATGGA	GCCGGA	ACGGGA
9	AGCCATAGCGCCTAC-1	TGCGATTT	CCGGGA	GCCGGA		
10	AGCCATAGCGCCTAC-1	ACCTCGTG	GCTGGA	GCTGGA	TGTGGA	CATGGA
11	AGCCATAGCGCCTAC-1	TTTCGTGA	CCGGGA	TATGGA		
12	AGCCATAGCGCCTAC-1	GTAAAGAT	GCCGGA	GCCGGA	CCGGGA	
13	AGCCATAGCGCCTAC-1	TTGAGGTG	CATGGA	GCCGGA	TATGGA	CCGGGA
14	AGCCATAGCGCCTAC-1	ATGGTAAG	CATGGA	CCGGGA		
15	AGCCATAGCGCCTAC-1	GCAGGGTG	GATGGA	TATGGA	CATGGA	ATAGGA
16	AGCCATAGCGCCTAC-1	ATTAGTCA	CCGGGA	GCCGGA	GCCGGA	
17	AGCCATAGCGCCTAC-1	ATCACGCT	TAAGGA	TATGGA	CATGGA	GCCGGA

Liao, H. *Nat Protoc* (2024)

DNA Typewriter

Step 3 (Parallel Execution): Visualization Tools

3a. PEbigram_plotting

Objective: Visualize bigram frequencies as a heatmap.

Input: Bigram matrix and unigram frequencies from TAPE_text_sorting.

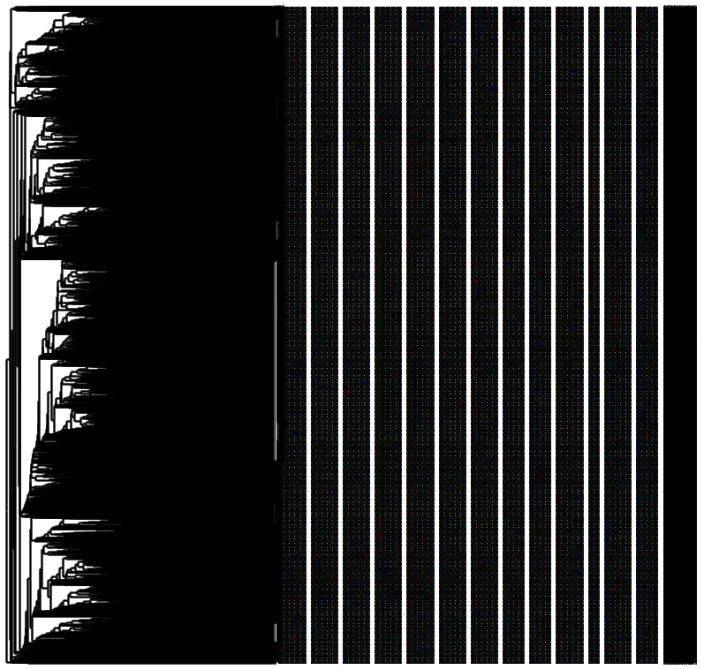
Output: Heatmap plot of bigram frequencies

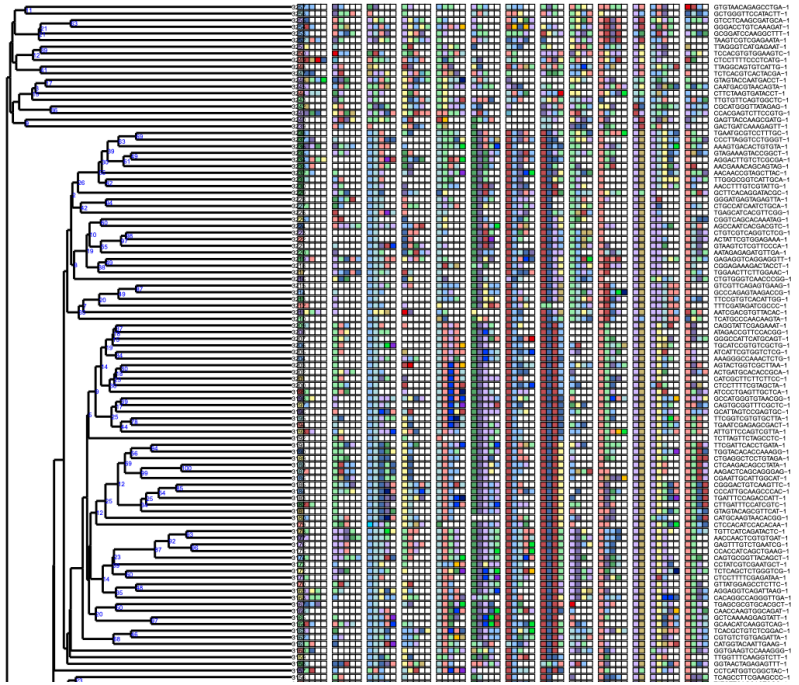
3b. DNATypewriter_SingleCellLineage_Rscript

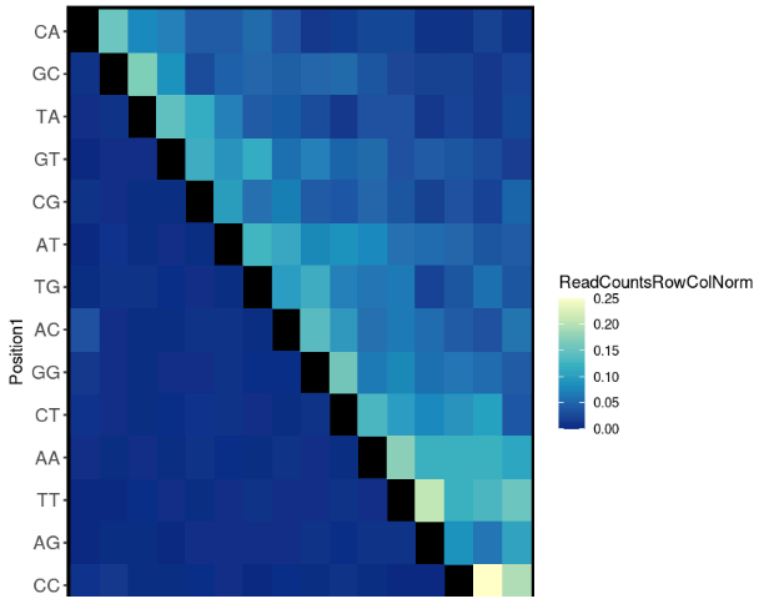
Objective: Generate lineage-specific plots for single-cell editing patterns.

Input: Bigram matrix and unigram frequencies from TAPE_text_sorting.

Output: Lineage-specific visualization

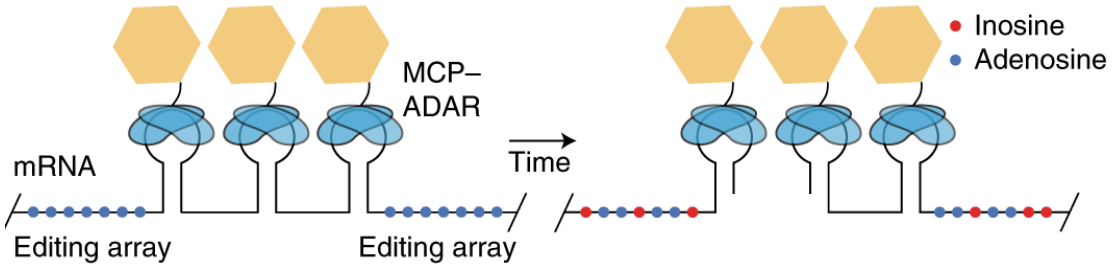


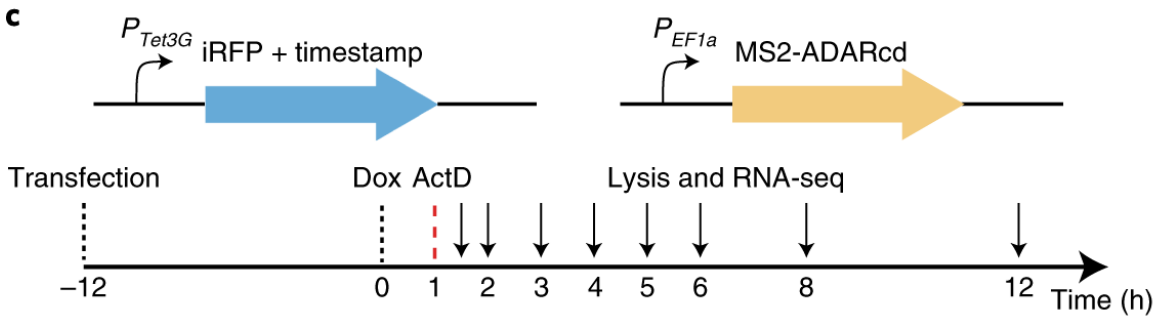


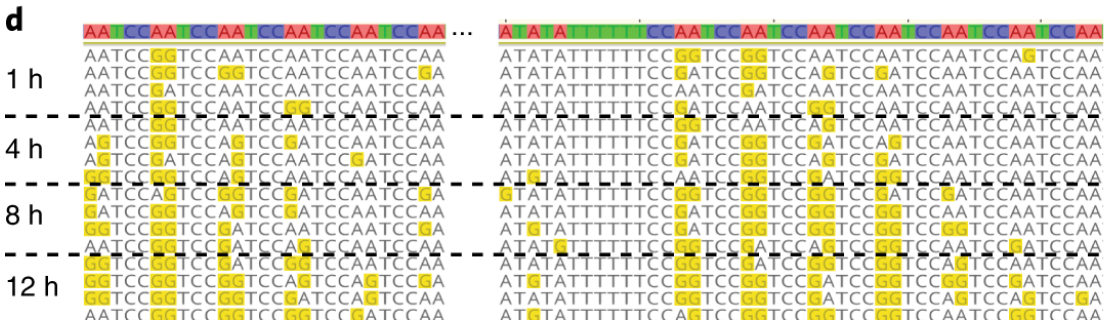


RNA seq + Pseudotime Inference

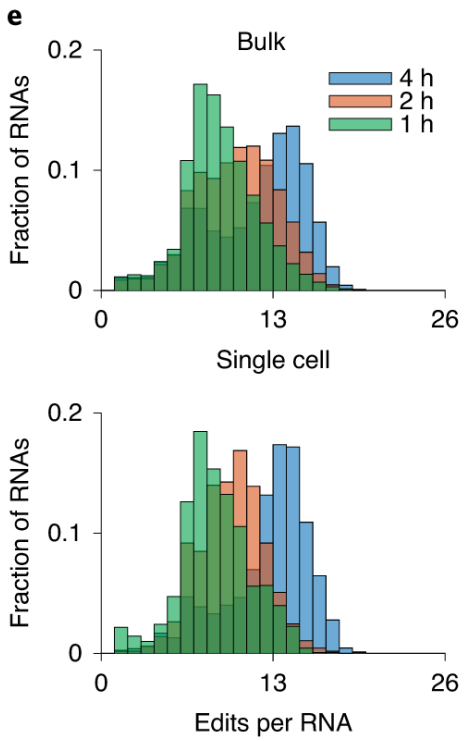
timestamps approach

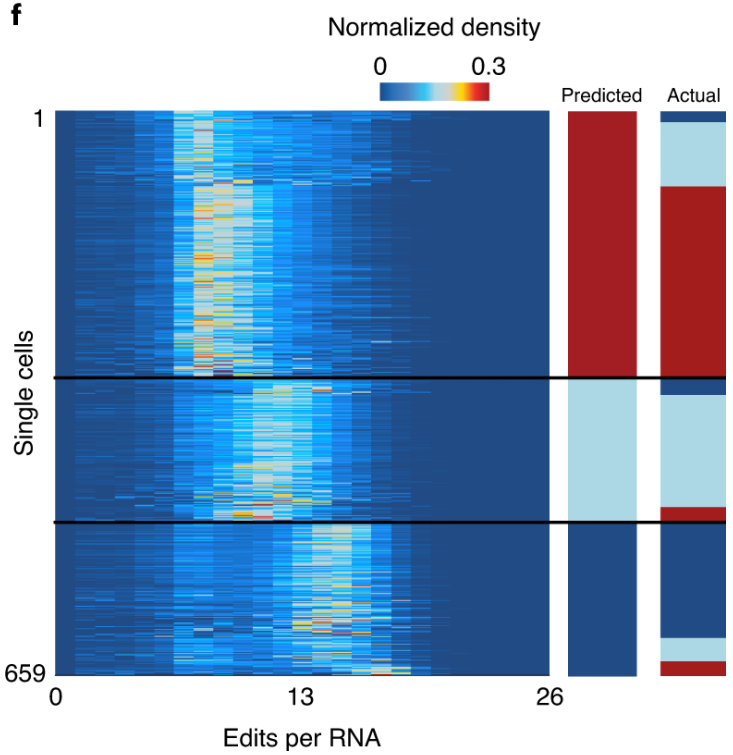






Pseudotime Inference

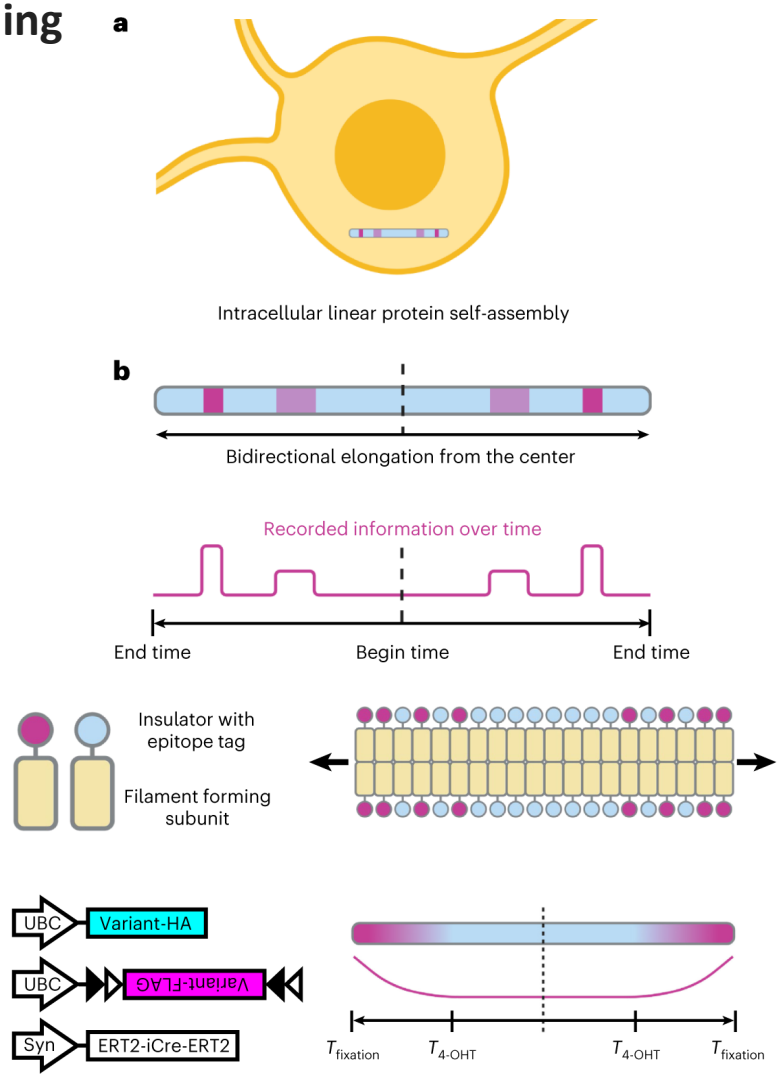




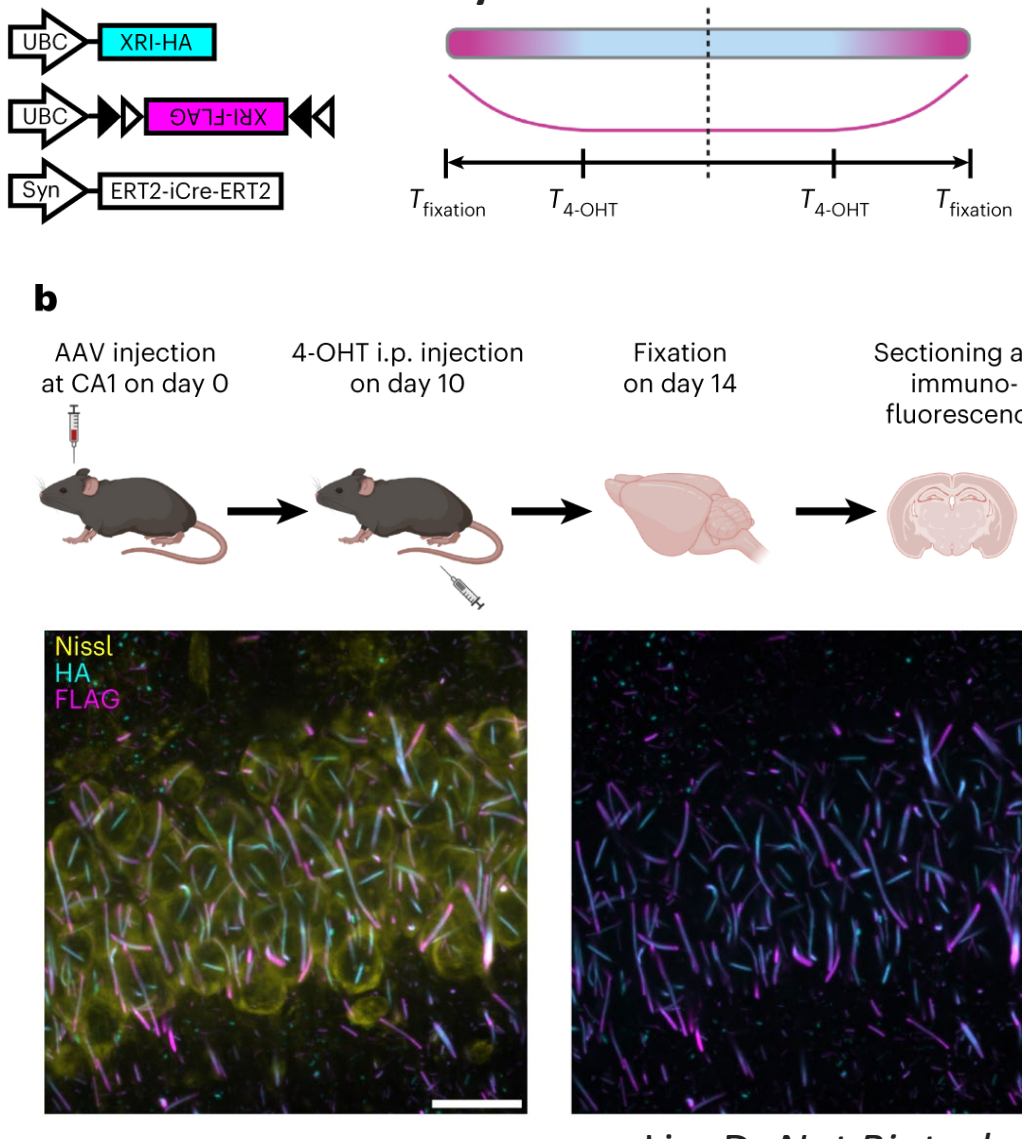
Rodriques, S.G. Nat Biotechnol (2021)

protein chain + absolute timing

linear protein self-assembly-based cellular physiology recording



In vivo XRI self-assembly in mouse brain

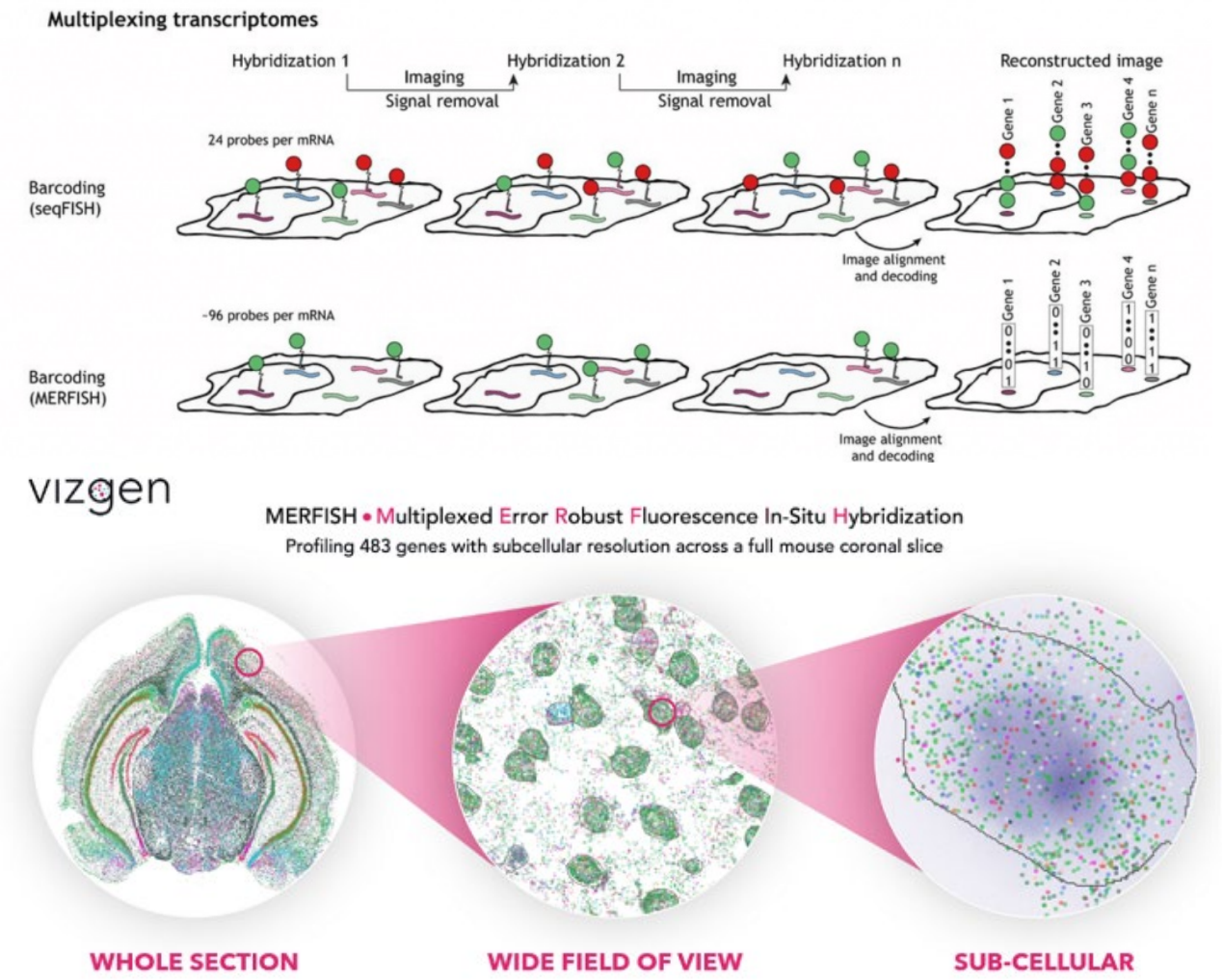


Lin, D. *Nat Biotechnol* (2023).

Linghu, C. *Nat Biotechnol* (2023)

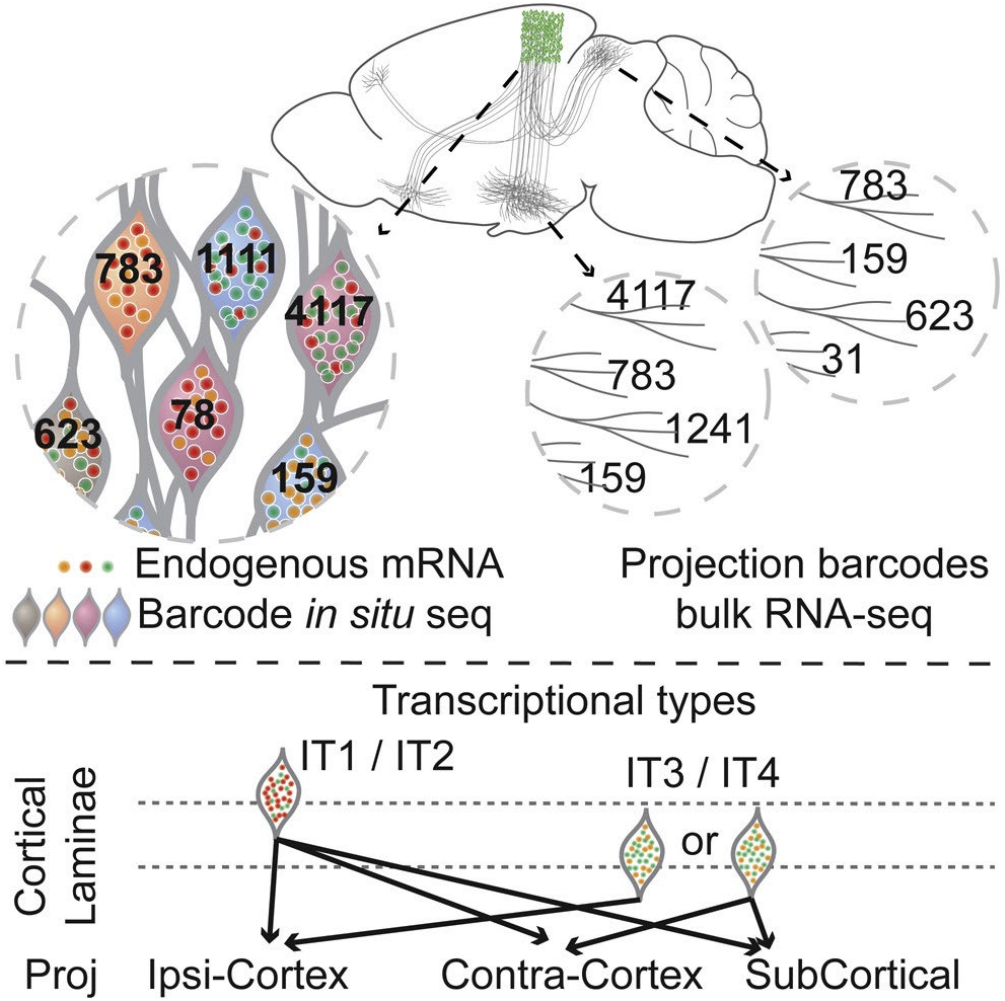
Orchestrate spatiotemporal signals in single-cell sequencing

MERFISH (Multiplexed Error-Robust Fluorescence In Situ Hybridization)



Chen, K. H. Science (2015)

MAPseq for Brain Connectome



Chen X. Cell. (2019)