scSeq methodologies and NGI services

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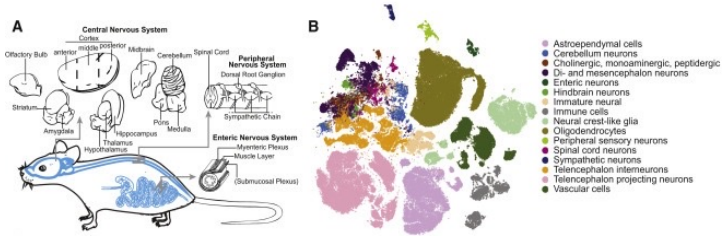
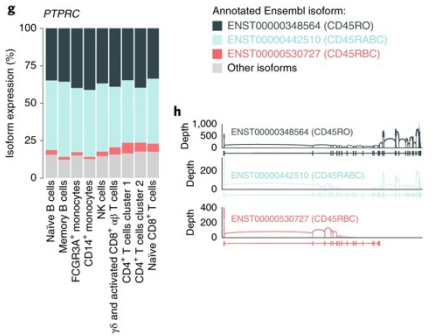
Applications for scRNA-sequencing

- Heterogeneity analysis
- Cell type identification
- Lineage tracing, cellular states in differentiation and development
- Monoallelic gene expression, splicing patterns
- Immune profiling
- More…

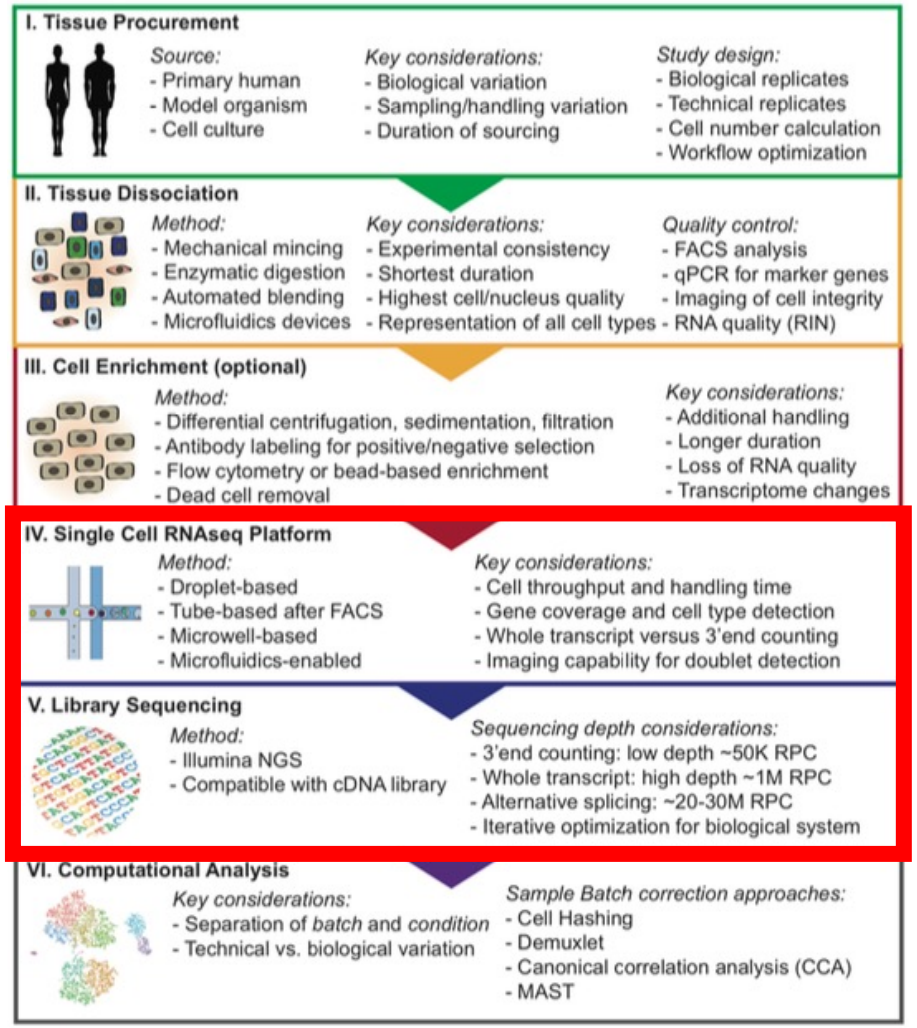


Zeisel et al, Cell 2018



Hagemann-Jensen Nat Biotech 2020

Single cell RNA-seq workflow



Verify your results with orthogonal method!

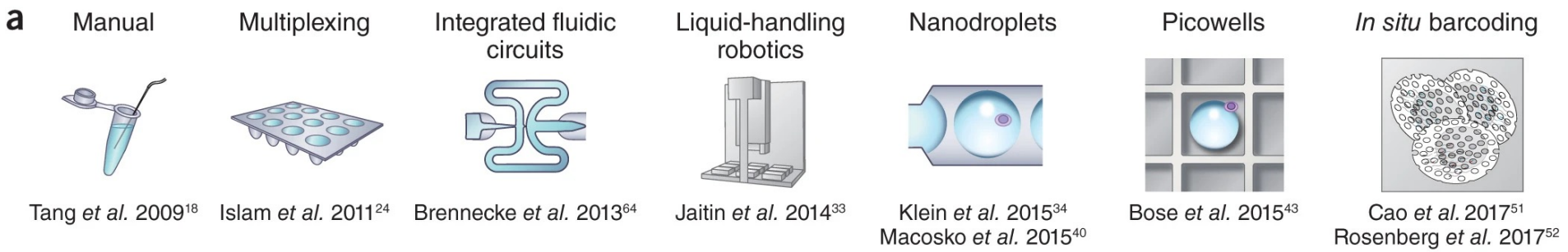
SciLifeLab

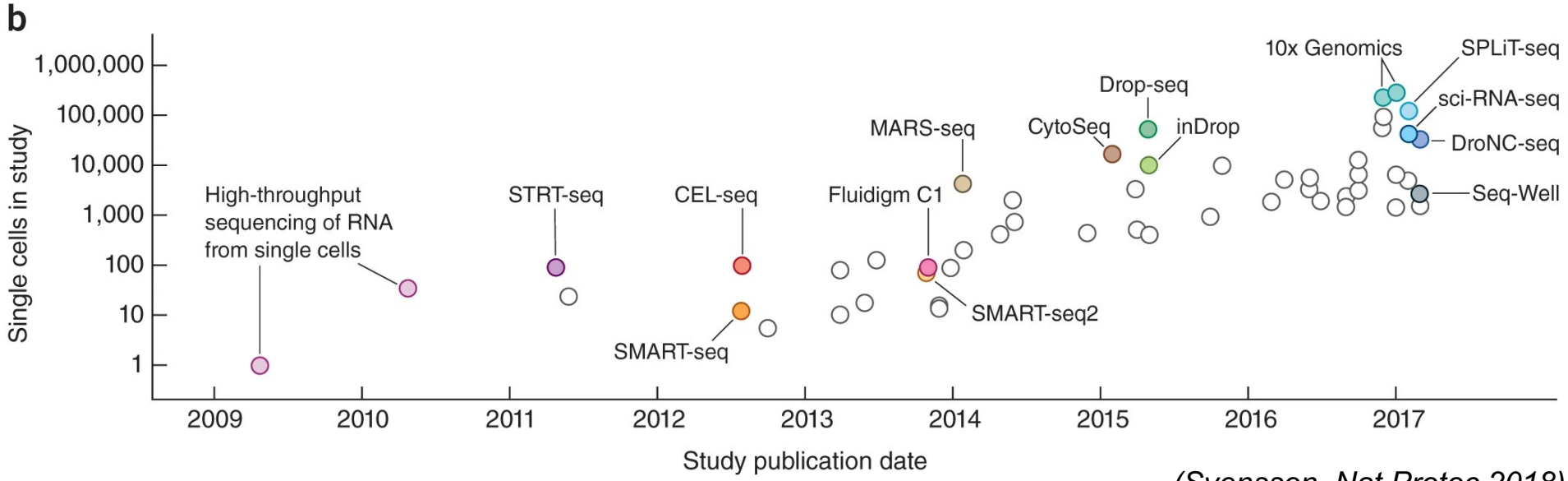
Omics type	Read out		Complexity	Sample requirements			Spatial resolution
	NGS	Imaging	(number of targets)	Fresh-frozen	FFPE	TMAs	
Spatial transcriptimics (10X Visium)	RNA	✓	✓	Unbiased transcriptomome-wide	✓	(✓)	✗	Anatomical features of 55 µm
In situ sequencing	RNA	✗	✓	200-300	✓	✓	✓	Subcellular
Spatial proteomics (Codex)	Protein	✗	✓	40	✓	✓	✓	Subcellular
Advanced FISH technologies (smFISH)	DNA/RNA	✗	✓	6	✓	✓	✓	Subcellular
Spatial Mass Spectometry	Small molecules	✗	✓	Mutliplexed, targeted or untargeted	✓	✗	✗	Anatomical features of 15 µm

NATIONAL GENOMICS INFRASTRUCTURE

Nguyen et al., "Experimental Considerations for Single-Cell RNA Sequencing Approaches." *Frontiers in Cell and Developmental Biology* 2018

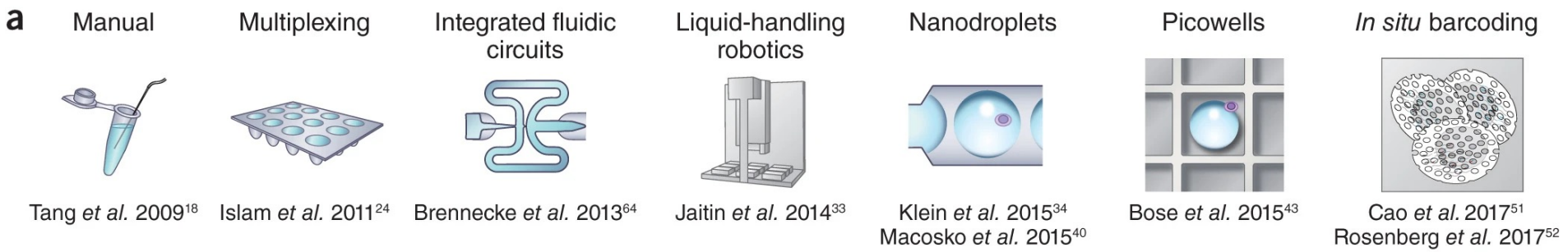
Short history of scRNA-seq methods

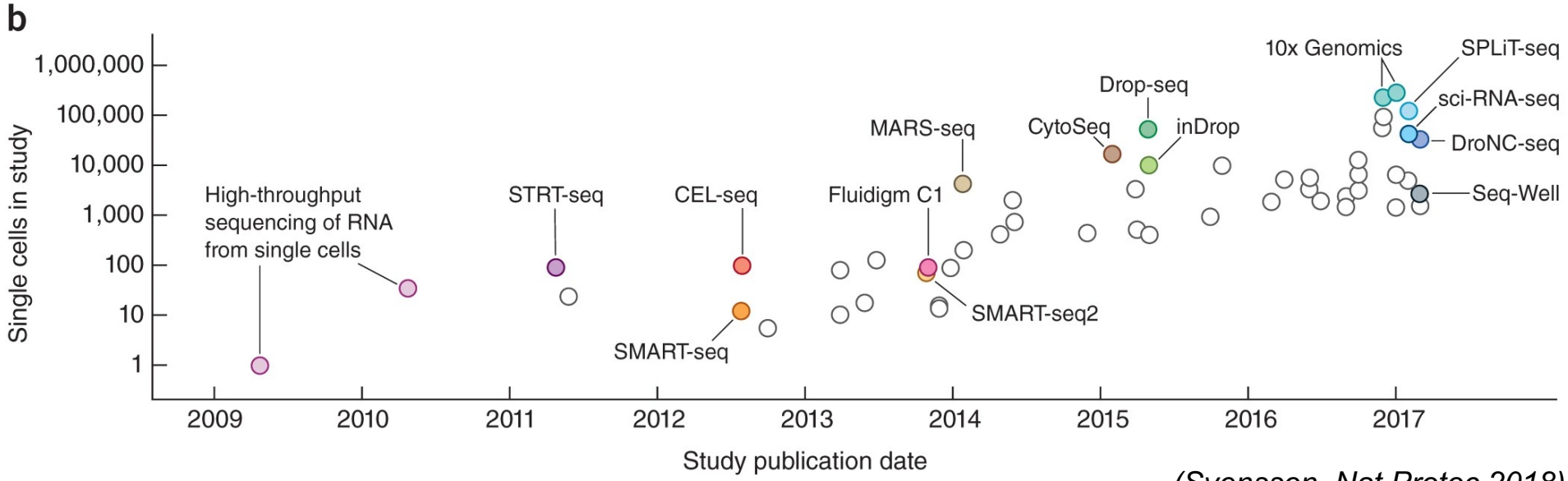




(Svensson, Nat Protoc 2018)

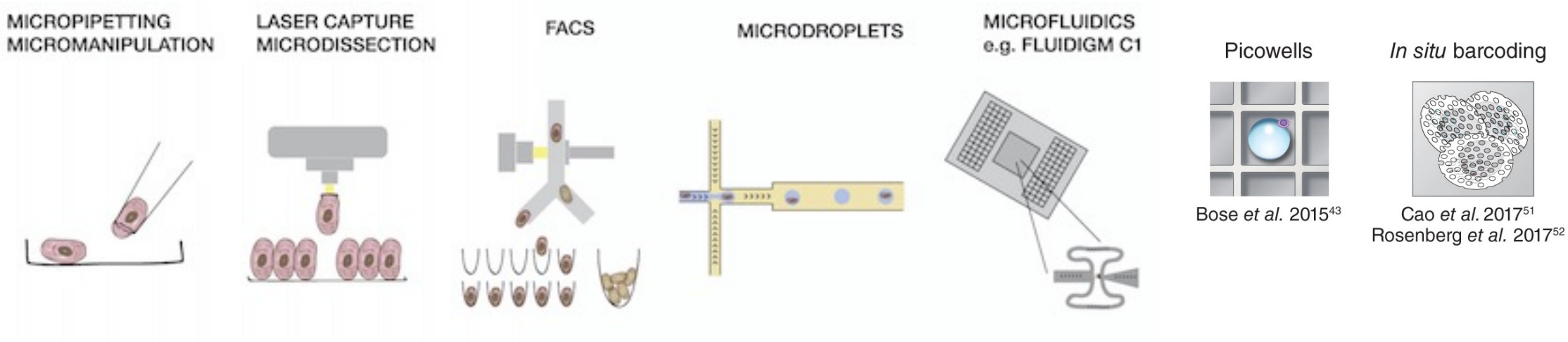
Short history of scRNA-seq methods





(Svensson, Nat Protoc 2018)

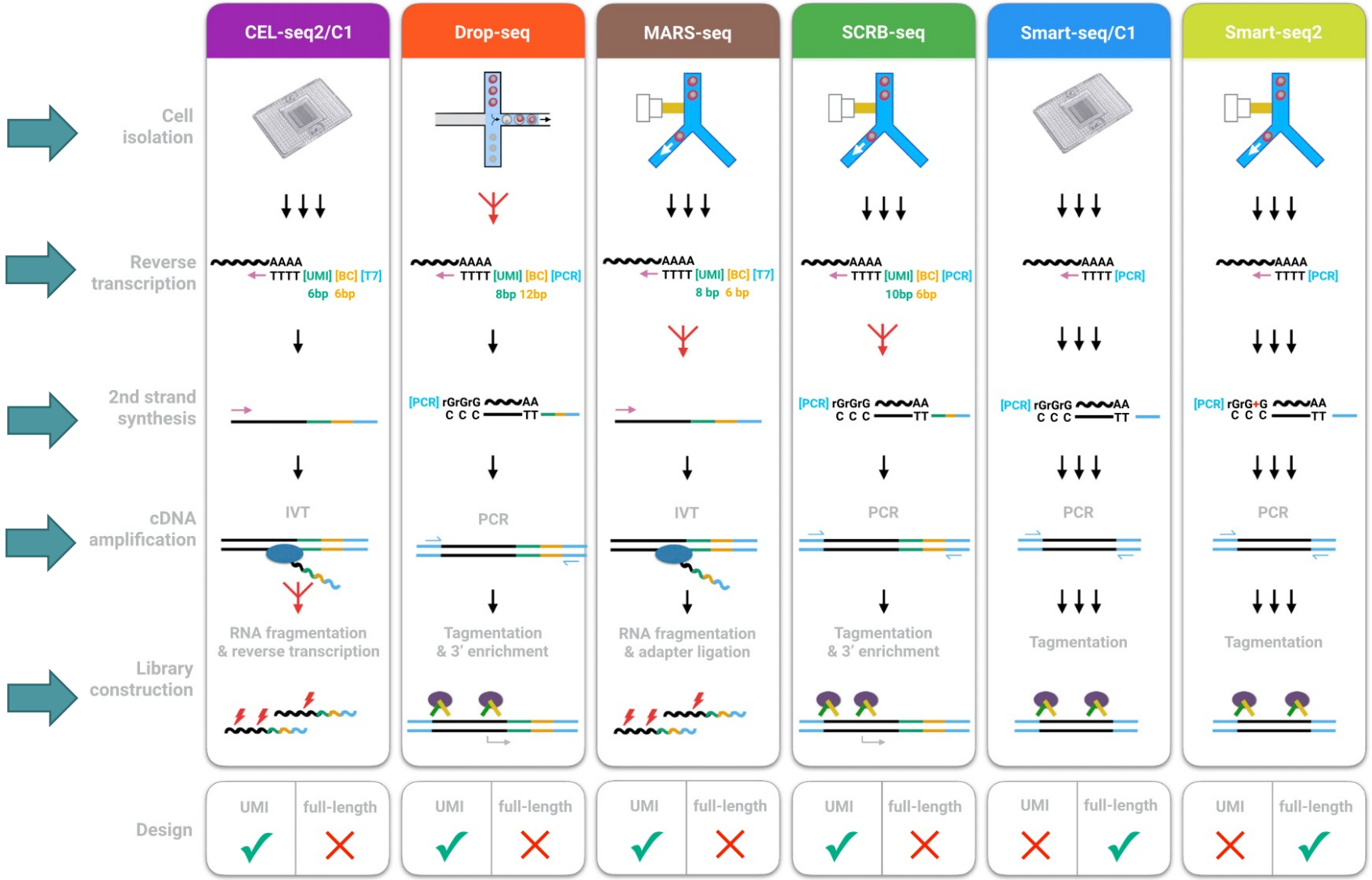
Single-cell isolation or capture



Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015 *(Adapted from: Svensson, Nat Protoc 2018)*

Add a barcode to the cell in the compartment

scRNA-sequencing protocol principles



BC (cell unique sequence)

UMI (random sequence)

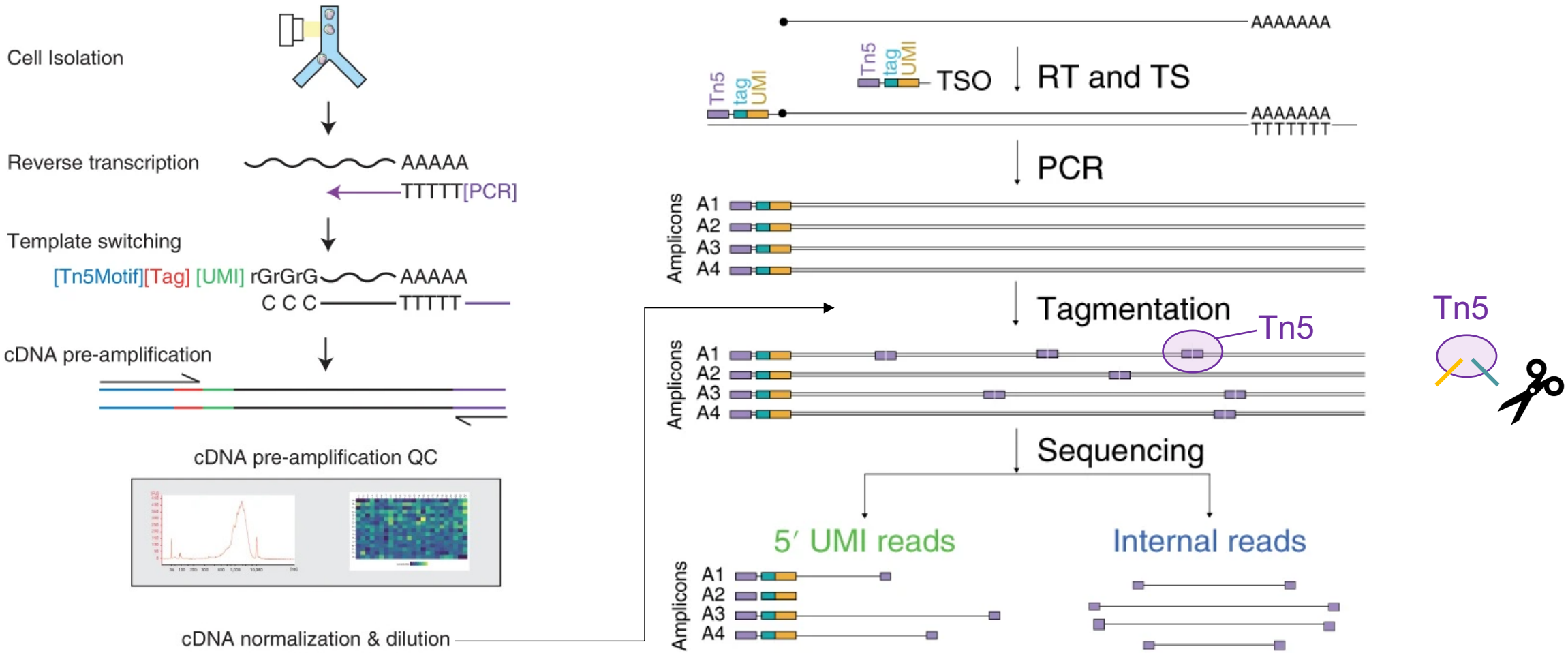
TSO (annealing handle)

In Vitro Transcript or PCR

Fragmentation / Tagment-

Zieghain et al. Mol Cell 2017

Example scRNA-seq: SMART-seq3

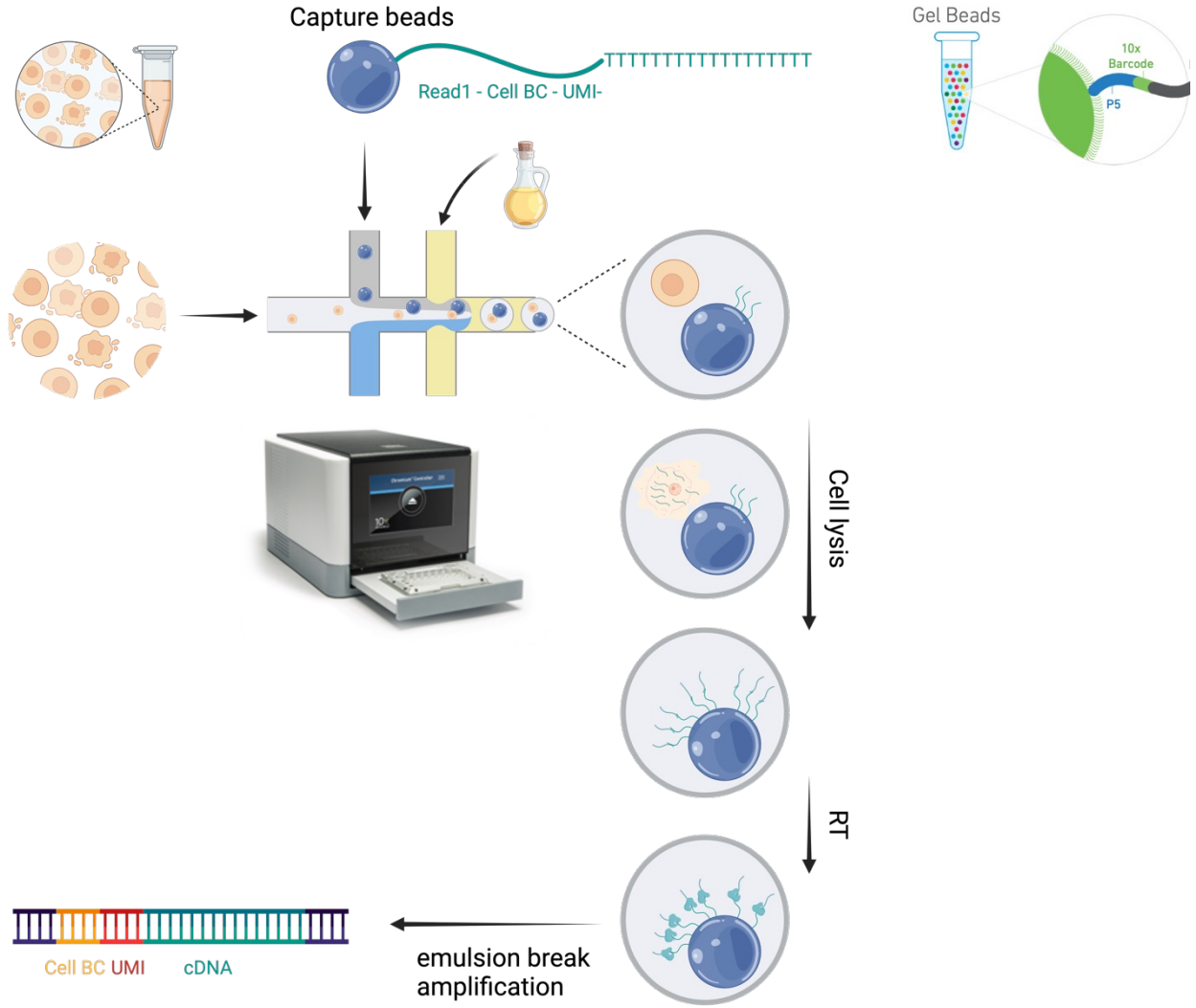


Adapted from Hagemann-Jensen, Nat Biotechnol , 2020 & 2022

SMART-seq3 Sequencing

Adapted from Hagemann-Jensen, Nat Biotechnol , 2020 & 2022

10x Genomics Chromium 3’



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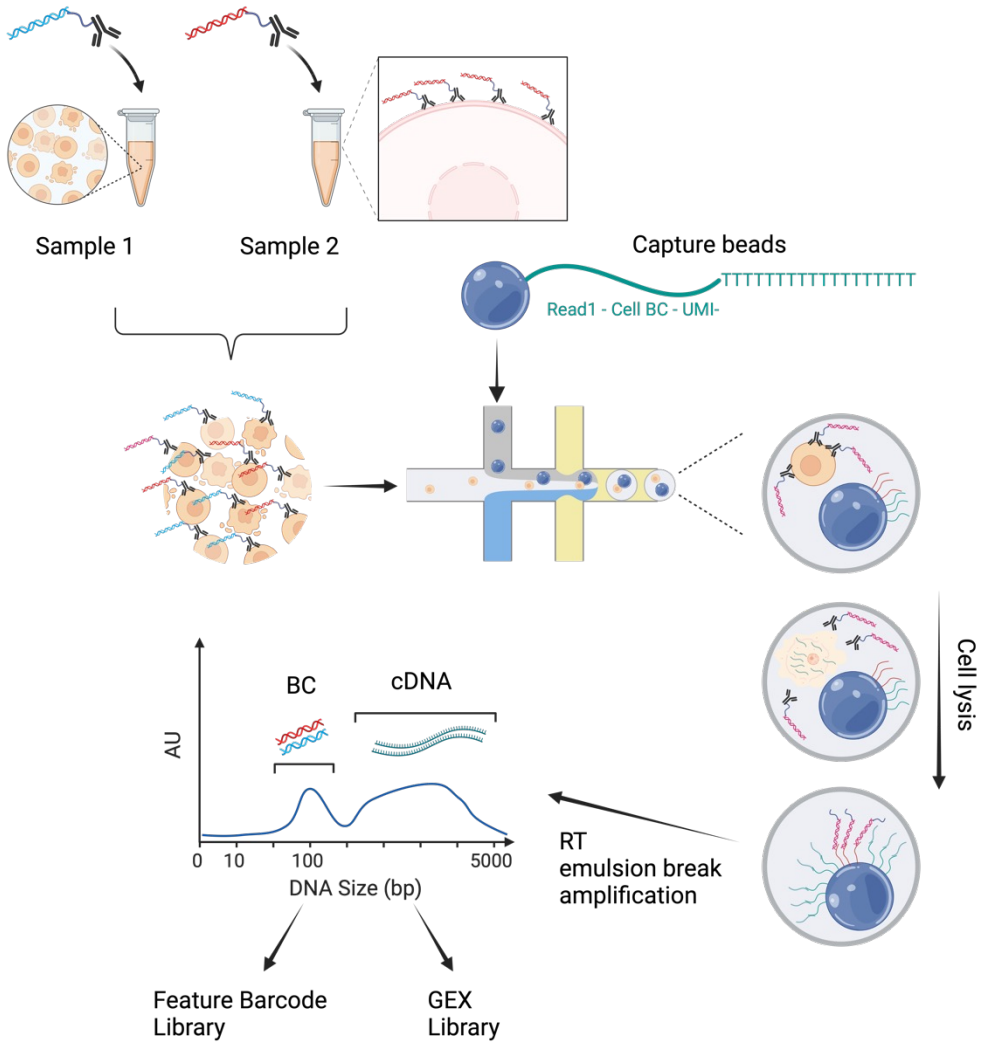
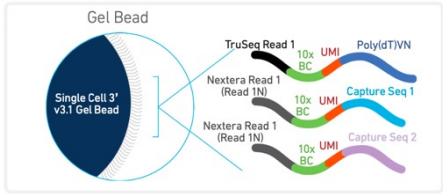
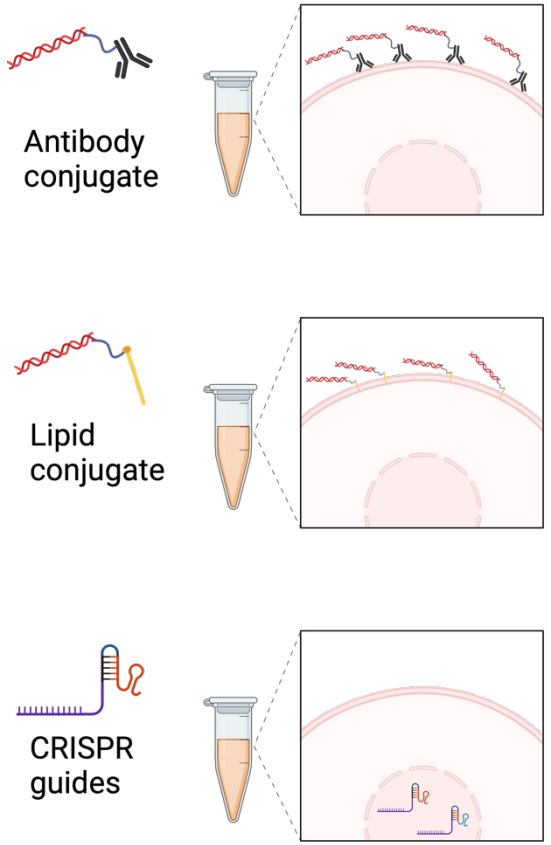
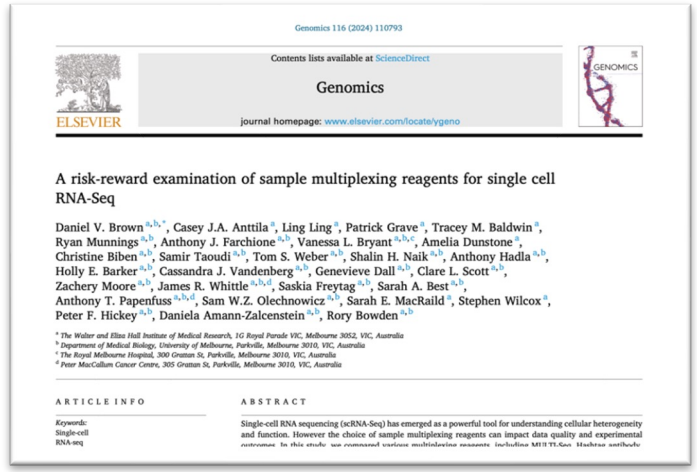
Cell multiplexing in 10x Chromium









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Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

A risk-reward examination of sample multiplexing reagents for single cell RNA-Seq

Daniel V. Brown, Casey J.A. Anttila, Ling Ling, Patrick Grave, Tracey M. Baldwin, Ryan Munnings, Anthony J. Farchione, Vanessa L. Bryant, Amelia Dunstone, Christine Biben, Samir Taoudi, Tom S. Weber, Shalin H. Naik, Anthony Hadla, Holly E. Barker, Cassandra J. Vandenberg, Genevieve Dall, Clare L. Scott, Zachery Moore, James R. Whittle, Saskia Freytag, Sarah A. Best, Anthony T. Papenfuss, Sam W.Z. Olechnowicz, Sarah E. MacRaild, Stephen Wilcox, Peter F. Hickey, Daniela Amann-Zalcenstein, Rory Bowden

ARTICLE INFO

ABSTRACT

(Brown et al. 2024)

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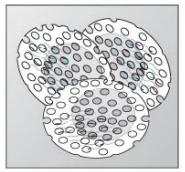
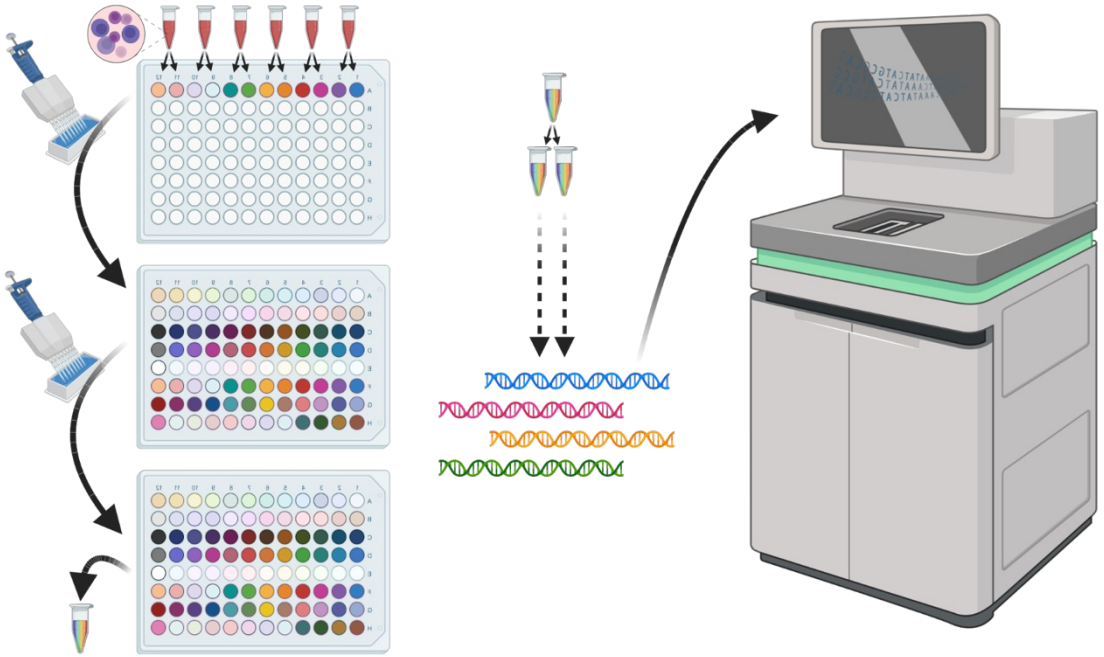
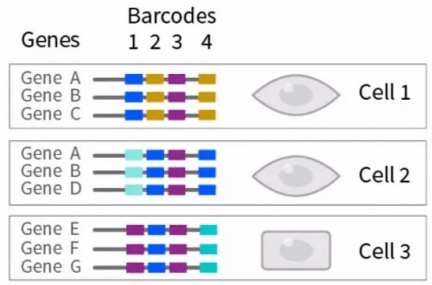
SPLiT-seq workflow

In situ barcoding



Cao *et al.* 2017[51]
Rosenberg *et al.* 2017[52]





Up to 10 000 cells (with low doublet rate)
100 000 cell with 48 initial barcode
1 000 000 cells with 96 initial barcodes

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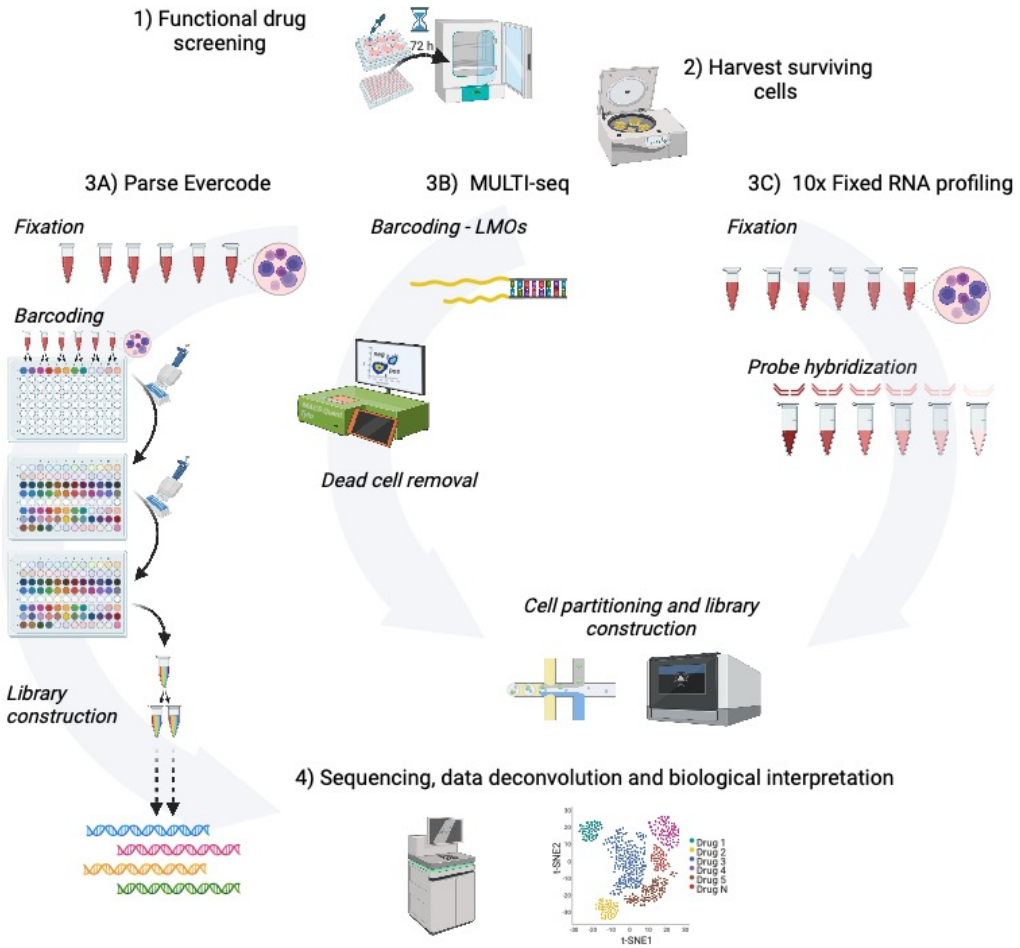
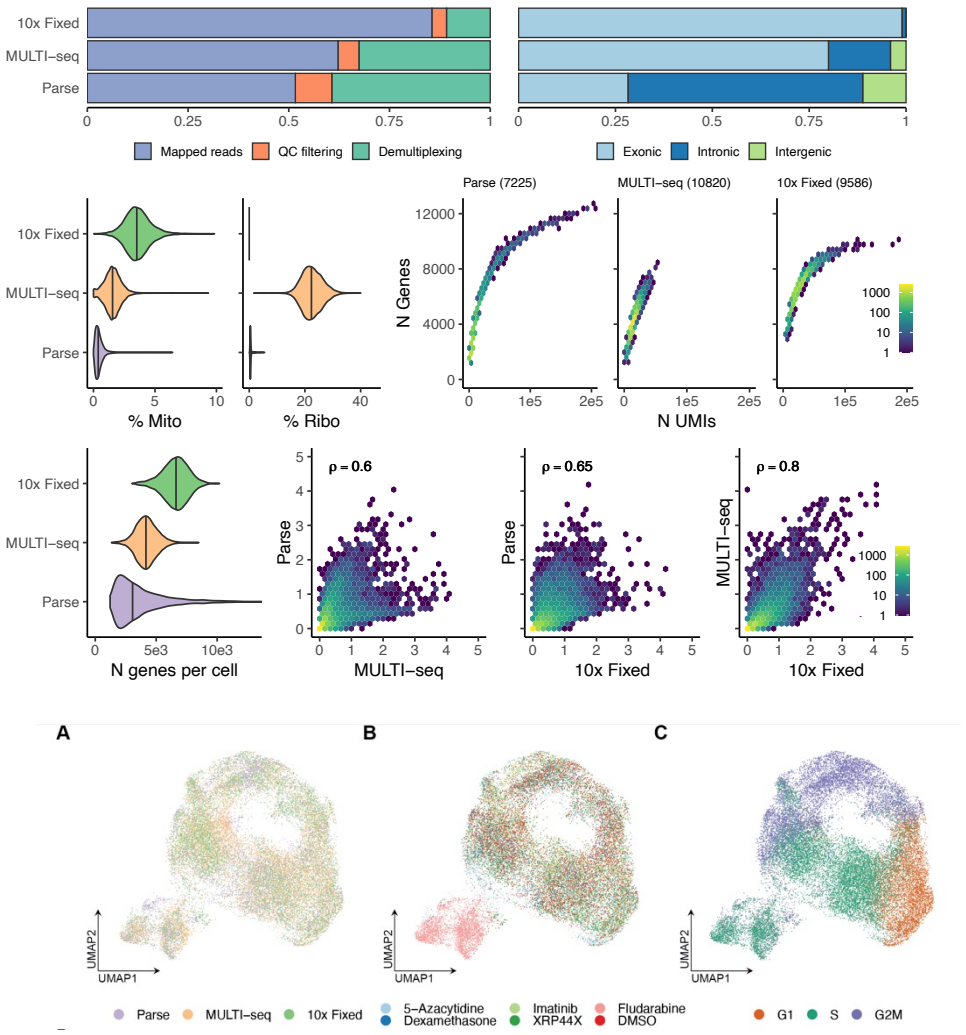
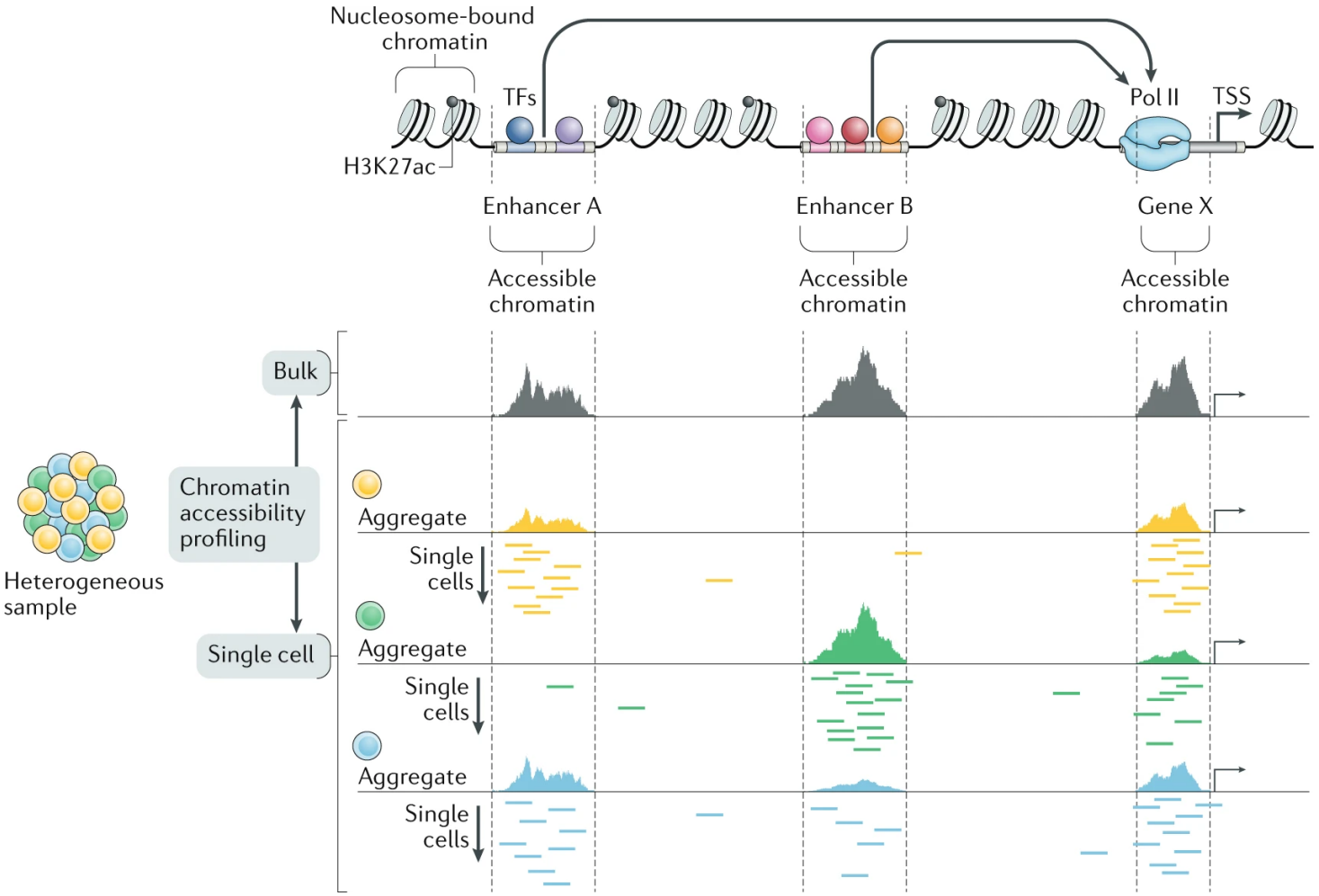
Comparison of current methods

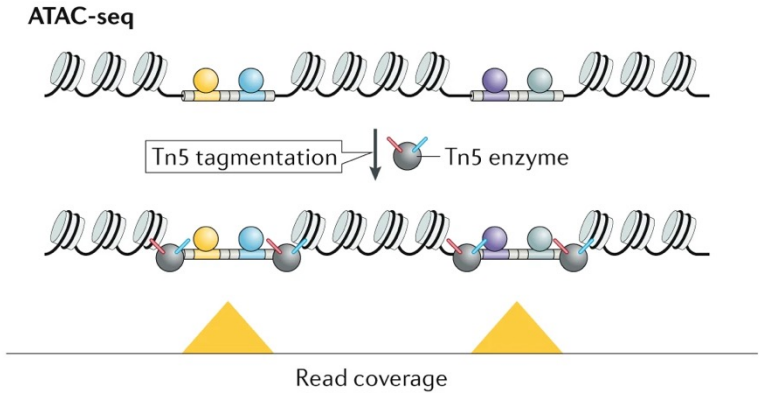




(Gezelius et al. 2024)

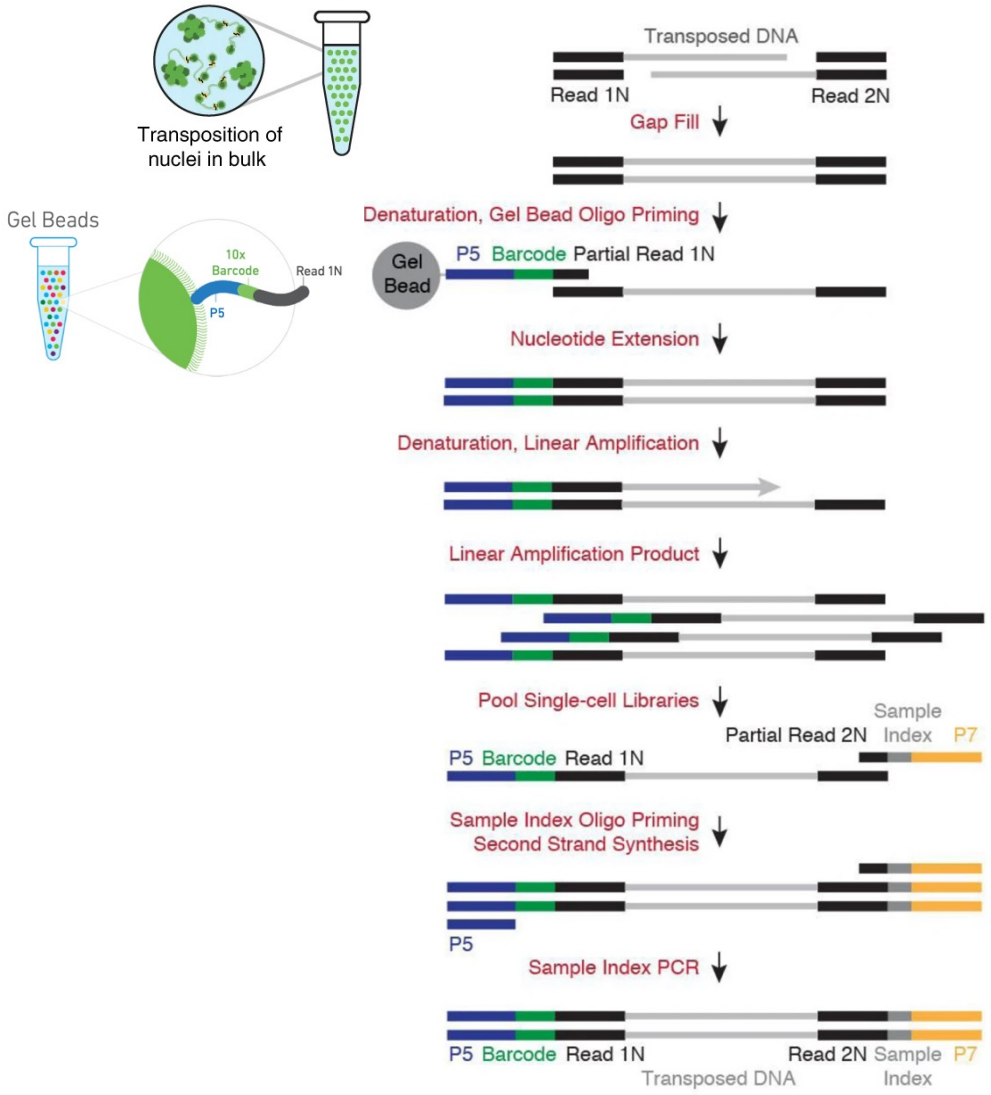
Beyond transcriptomics - Chromatin accessibility

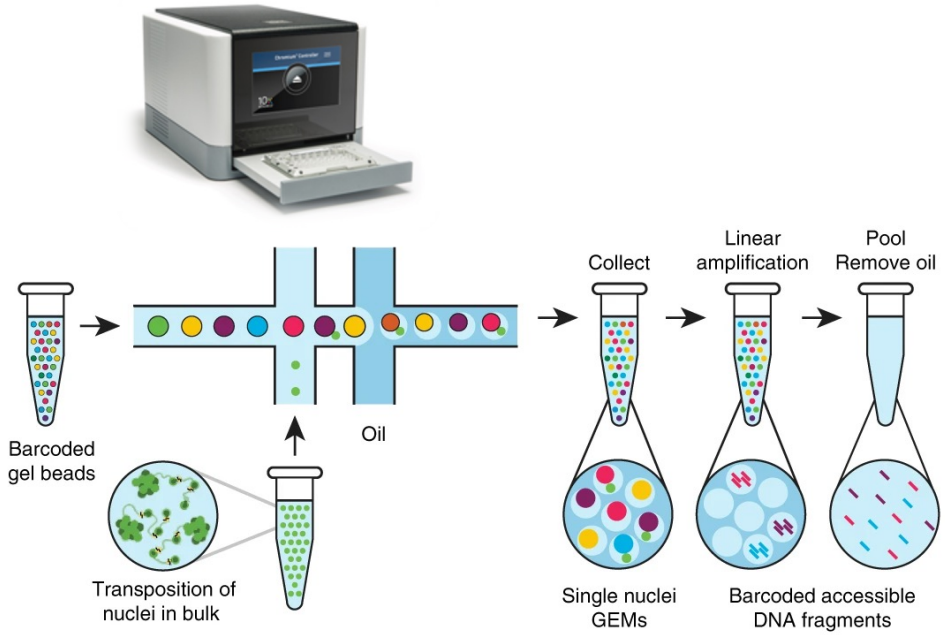




Minnoye, Nat Rev Methods Primers, 2021

Example: 10x Genomics scATAC-seq





Satpathy Nat Biotechnol 2019

Summary single cell sequencing

- ✓ Isolate cells in compartments
- ✓ Add UMI & barcode for later pooling
- ✓ Amplify
- ✓ Fragment (e.g Tn5 tagmentation)
- ✓ Sequence pooled libraries
- ✓ Analyze your data!

Questions?

henrik.gezelius@medsci.uu.se

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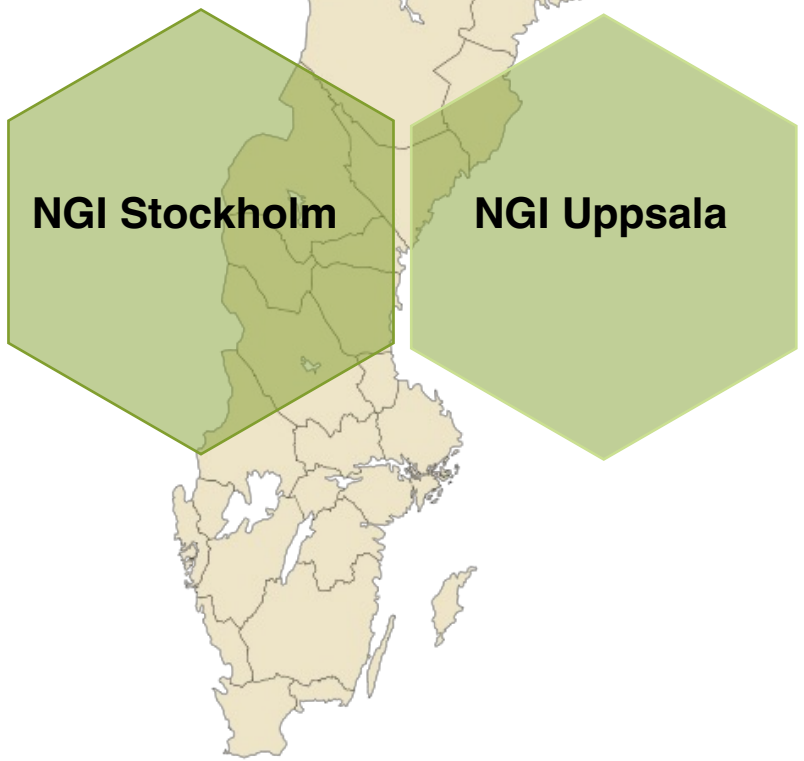
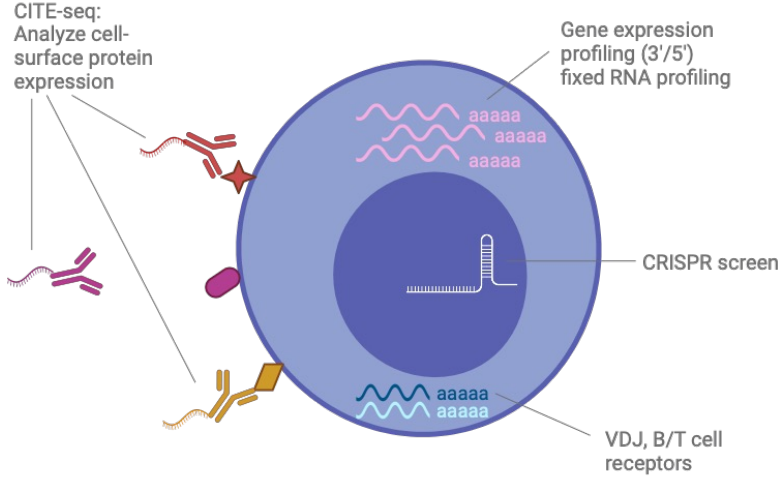


NGI-Stockholm

NGI-Uppsala
Uppsala
Genome Center

NGI-Uppsala
SNP&SEQ
Technology
platform

Gene Expression

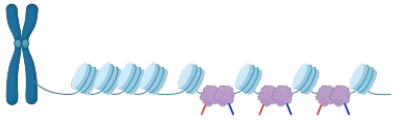


10x Genomics
- *3'/5' GEX*
- *CITE-seq*
- *Cell hashing*
- *VDJ*
- *CRISPR screen*
- *fixed RNA profiling*

Smart-seq3
- *FL GEX*

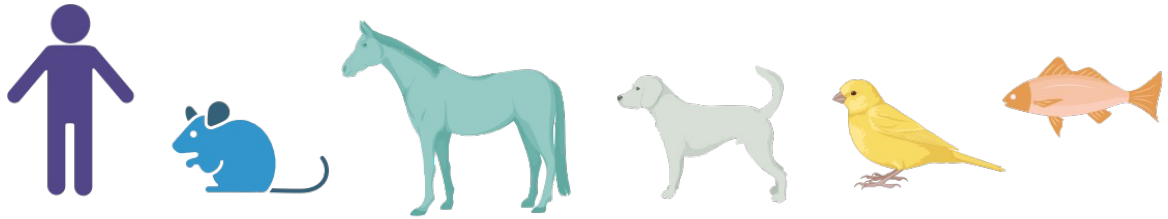
Drop-seq
(Dolomite Nadia)

Epigenetics



10x Genomics
- scATAC-seq
- scATAC + GEX

scWBGS (SPLAT)
- Raine et al, 2022



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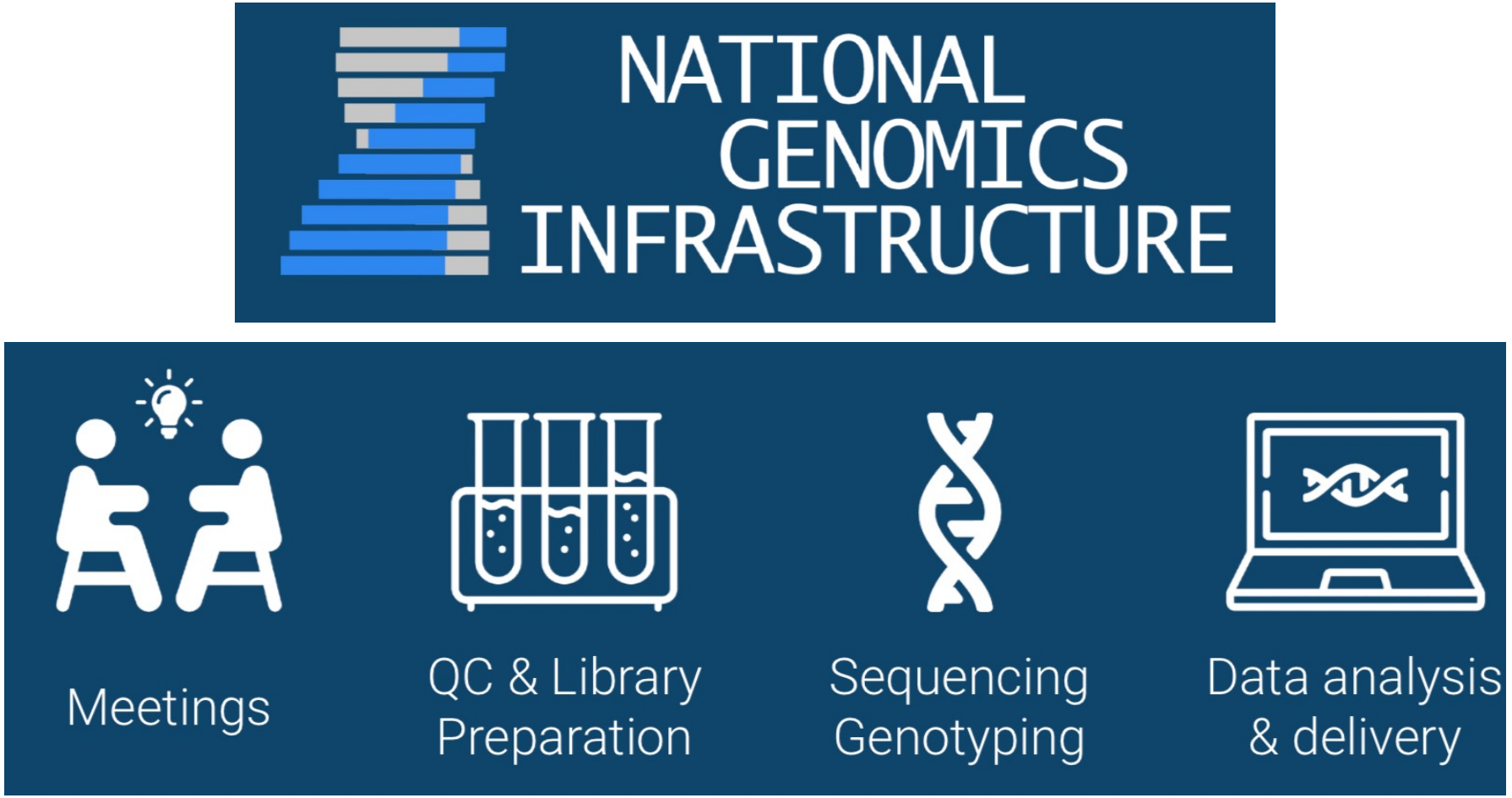
Project workflow at NGI



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NATIONAL GENOMICS INFRASTRUCTURE

For more details and project requests

Contact us at support@ngisweden.se

or place your order or meeting request in our order portal at

https://ngisweden.scilifelab.se/

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