scRNA-Seq methods and NGI services

Henrik Gezelius

Single Cell Genomics specialist, NGI Uppsala

Researcher, Molecular Precision Medicine, UU

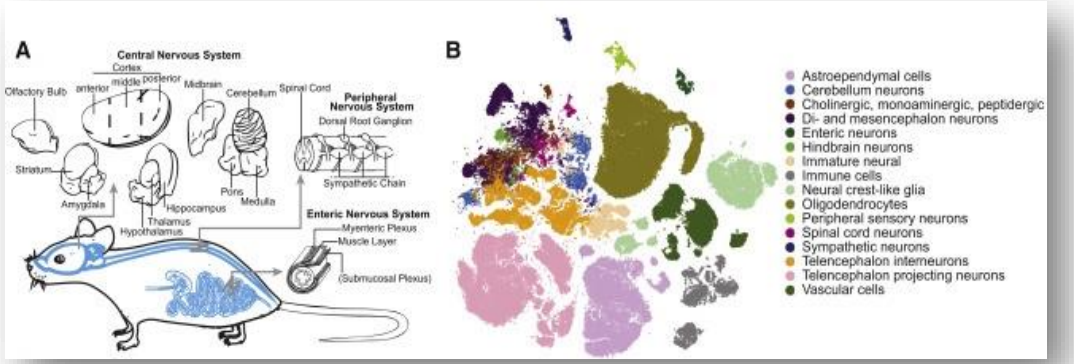
2025-03-31

Outline

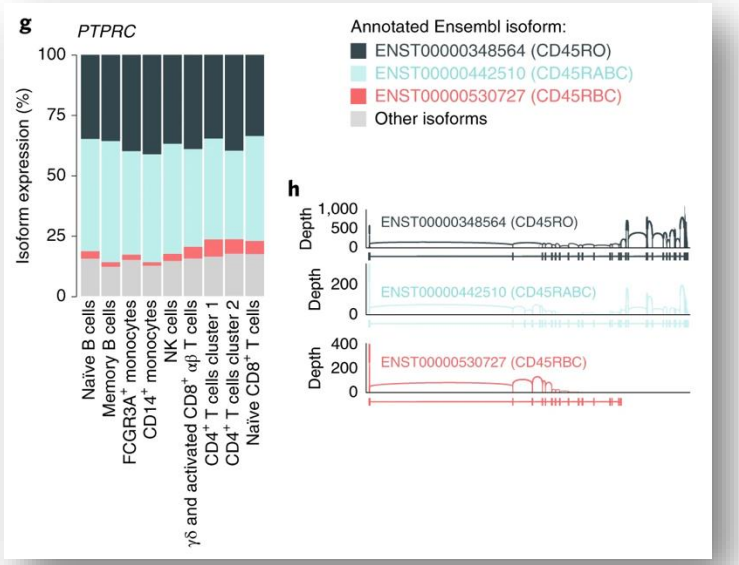
- The workflow
- Various methods
- General principles
- Examples of common methods
- Summary
- NGI services

Applications

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

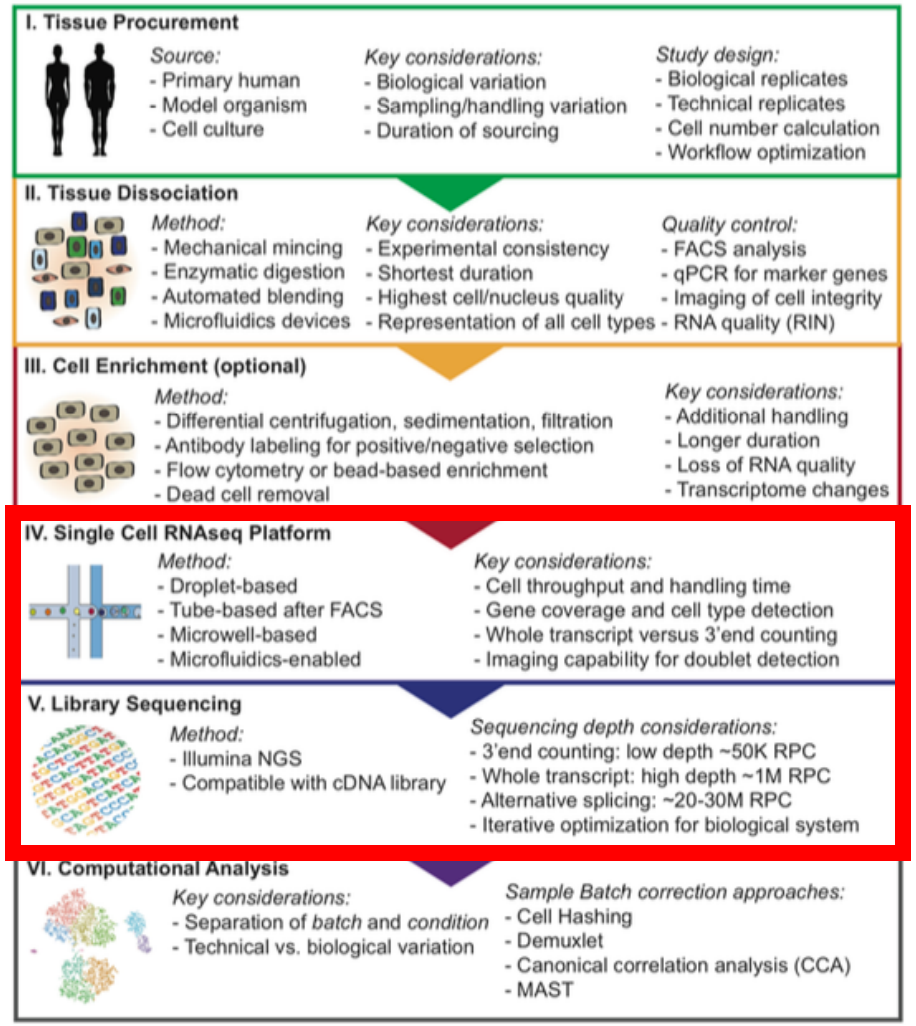


Zeisel et al, Cell 2018



Hagemann-Jensen et al, Nat Biotech 2020

Single cell RNA-seq workflow



Verify your results with orthogonal method!

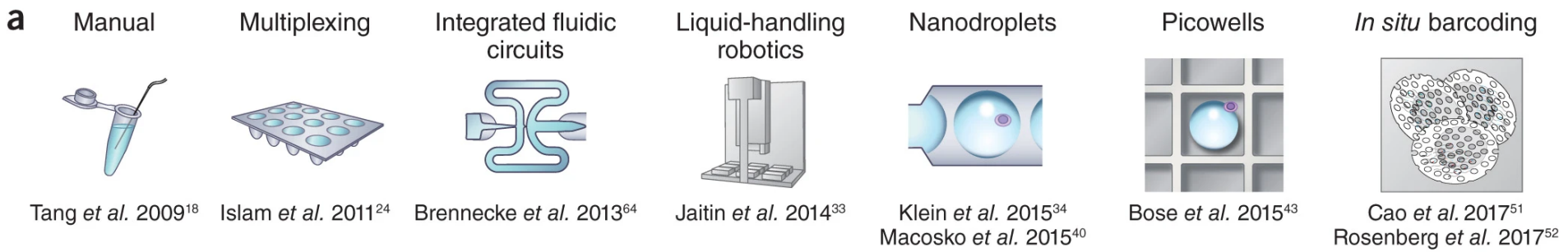
SciLifeLab

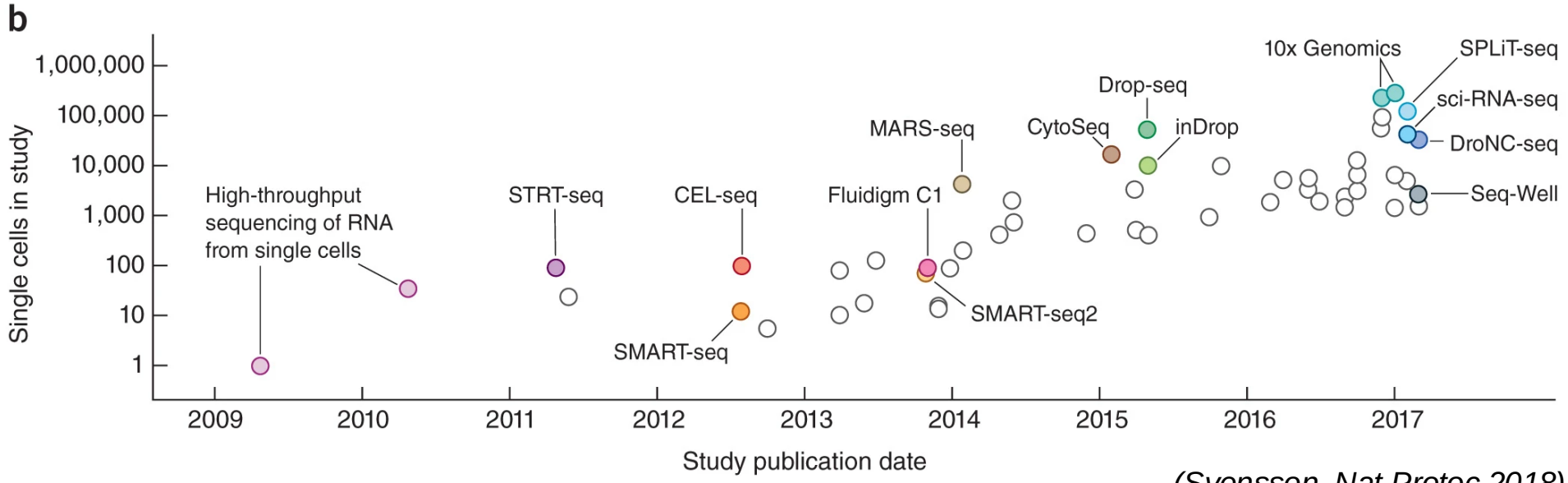
Omics type	Read out		Complexity	Sample requirements			Spatial resolution
	NGS	Imaging	(number of targets)	Fresh-frozen	FFPE	TMAs	
Spatial transcriptimics (10X Visium)	RNA	✓	✓	Unbiased transcriptomome-wide	✓	(✓)	✗	Anatomical features of 55 µm
In situ sequencing	RNA	✗	✓	200-300	✓	✓	✓	Subcellular
Spatial 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

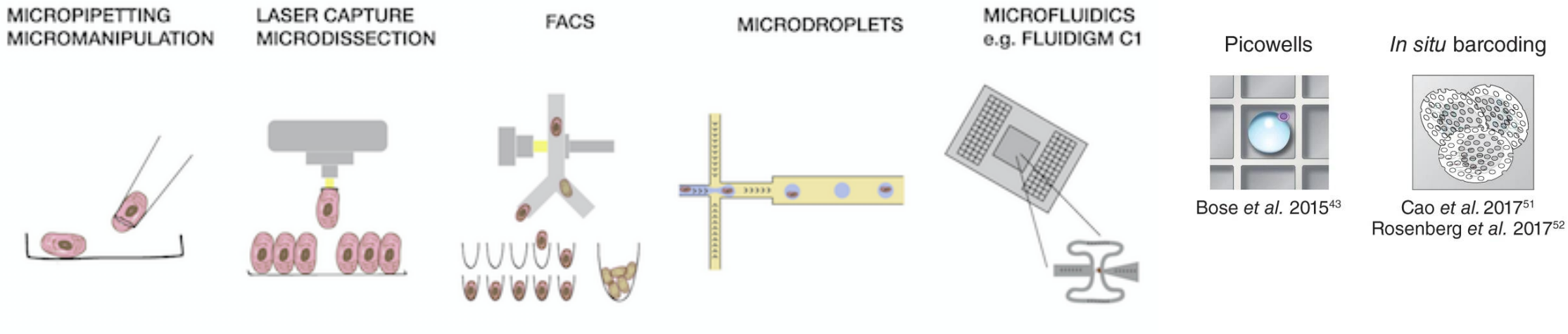
Short history of scRNA-seq methods





(Svensson, Nat Protoc 2018)

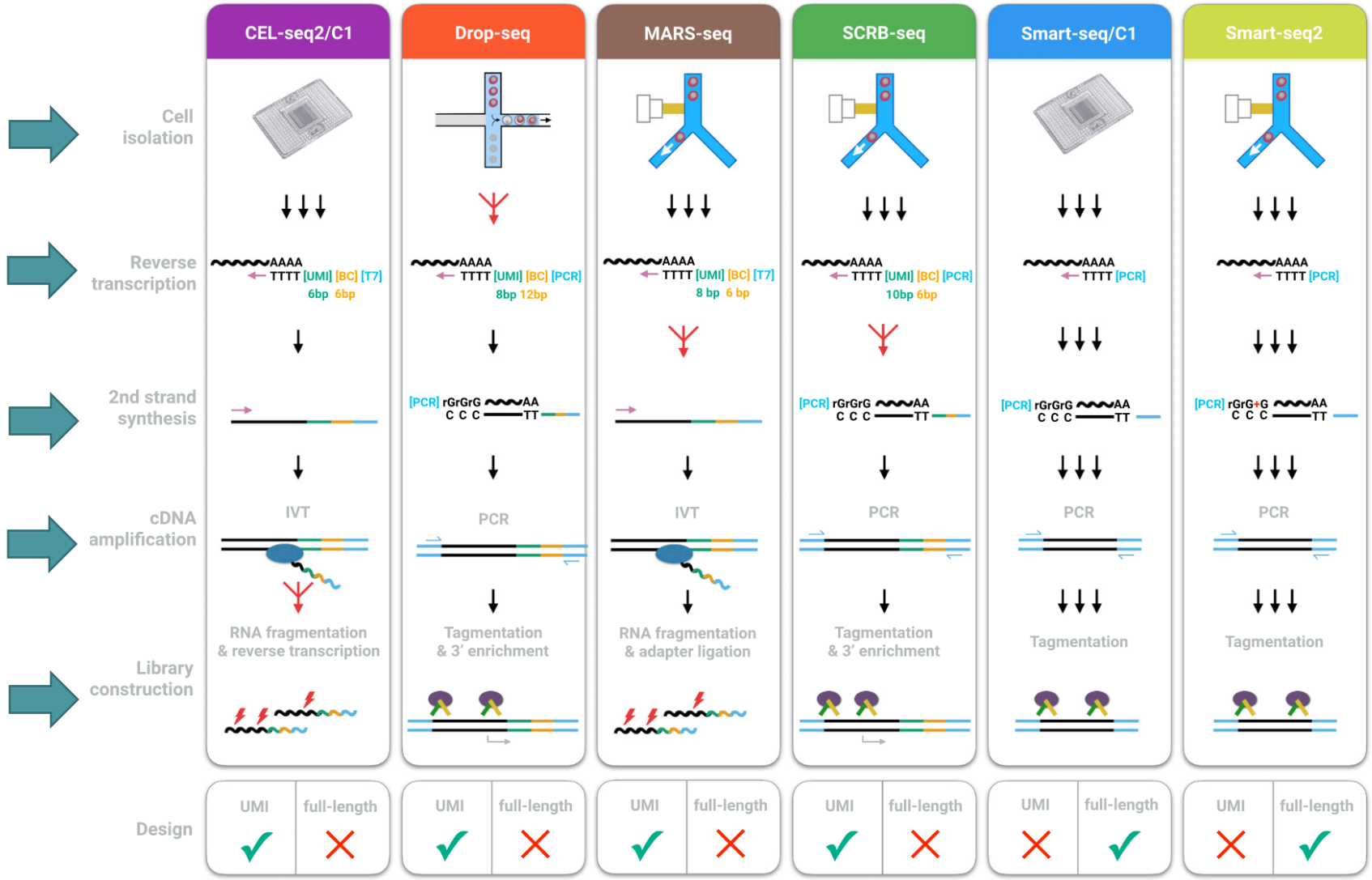
Single-cell isolation or capture



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)

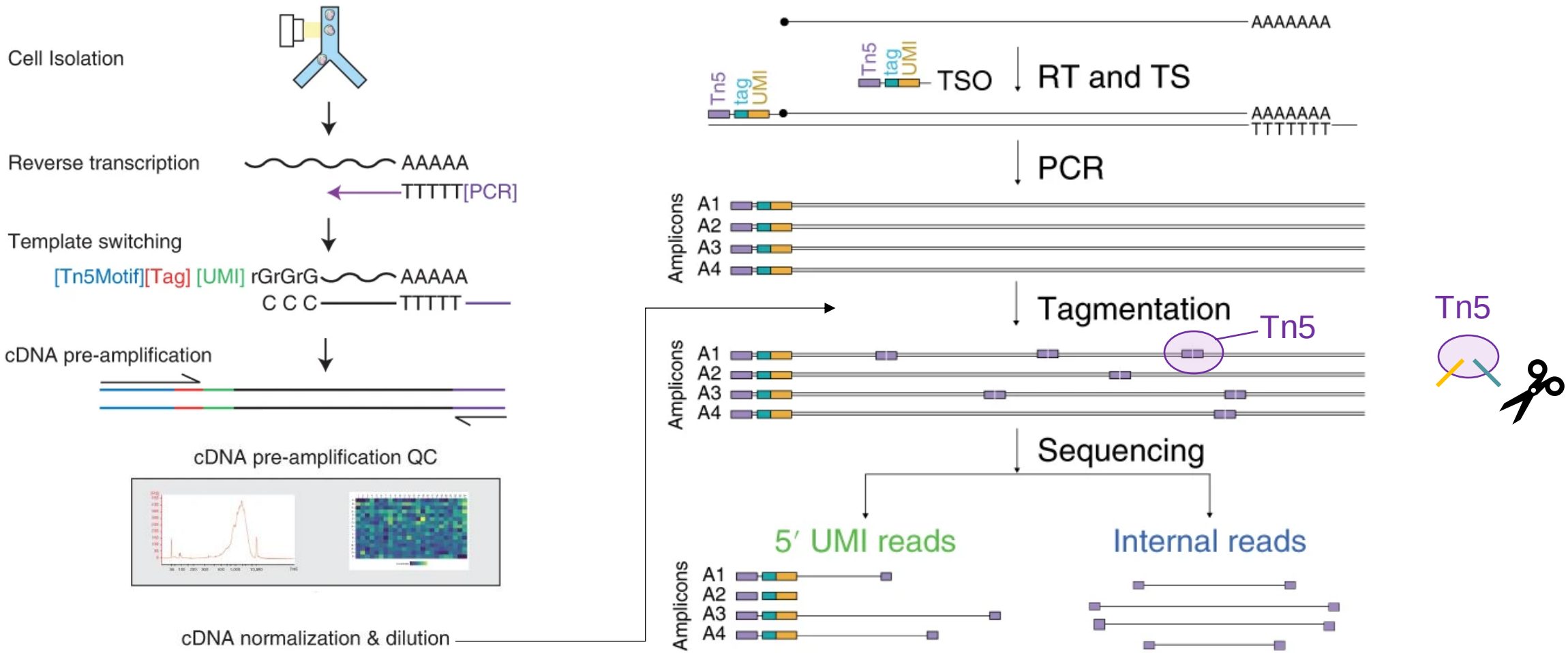
TSO (annealing handle)

PCR (or in vitro transcript)

Fragmentation / Tagment-

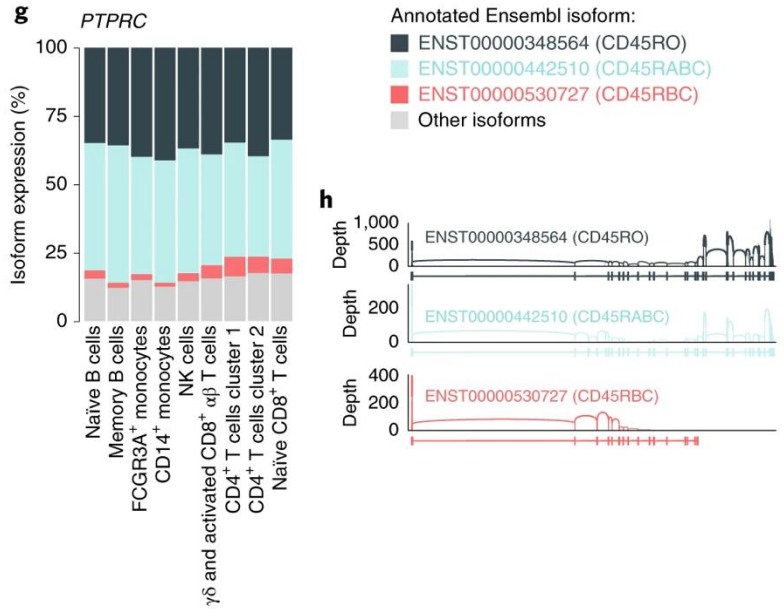
Zieghain et al. Mol Cell 2017

Example scRNA-seq: SMART-seq3



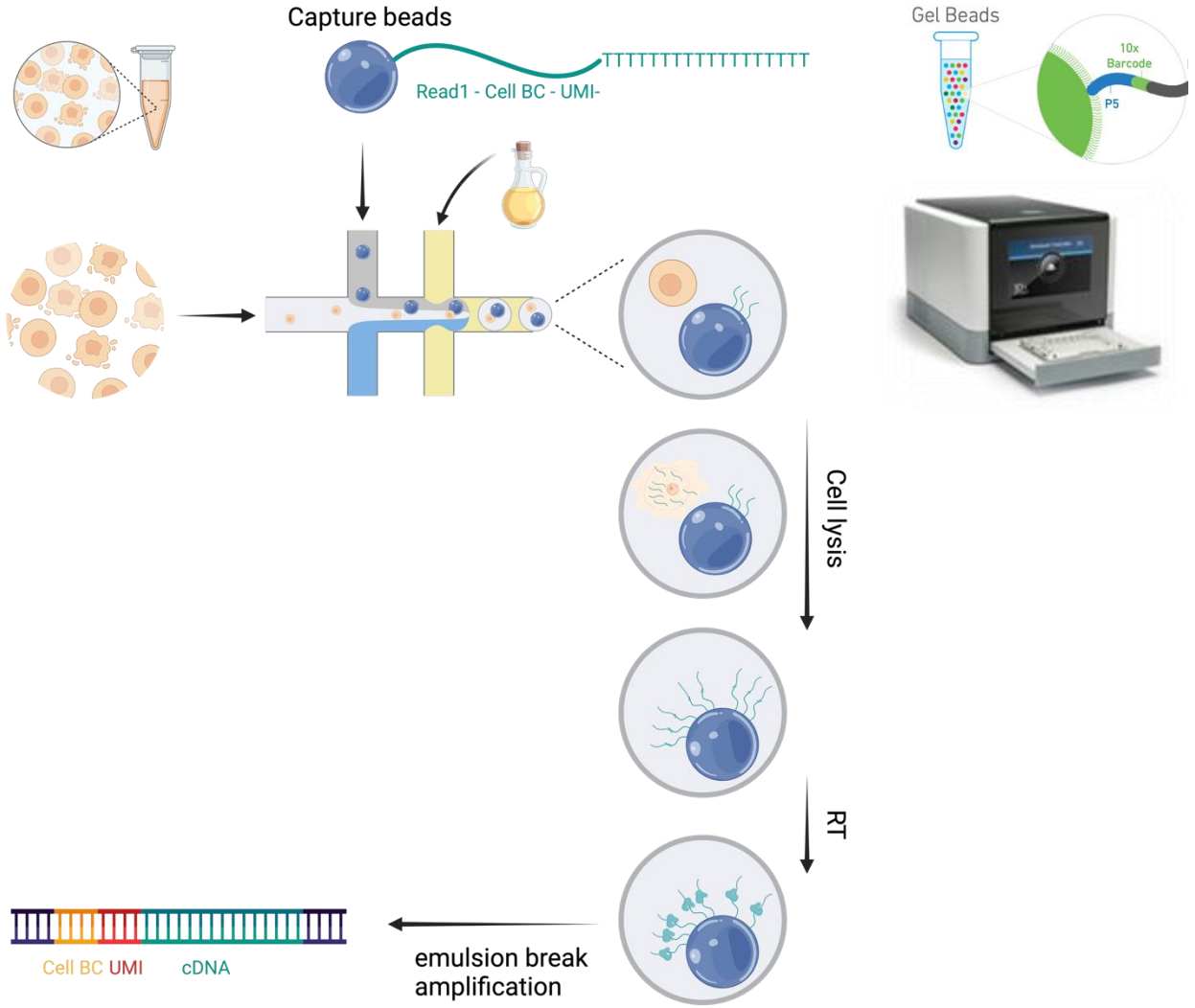
Adapted from Hagemann-Jensen, Nat Biotechnol , 2020 & 2022

SMART-seq3 Sequencing



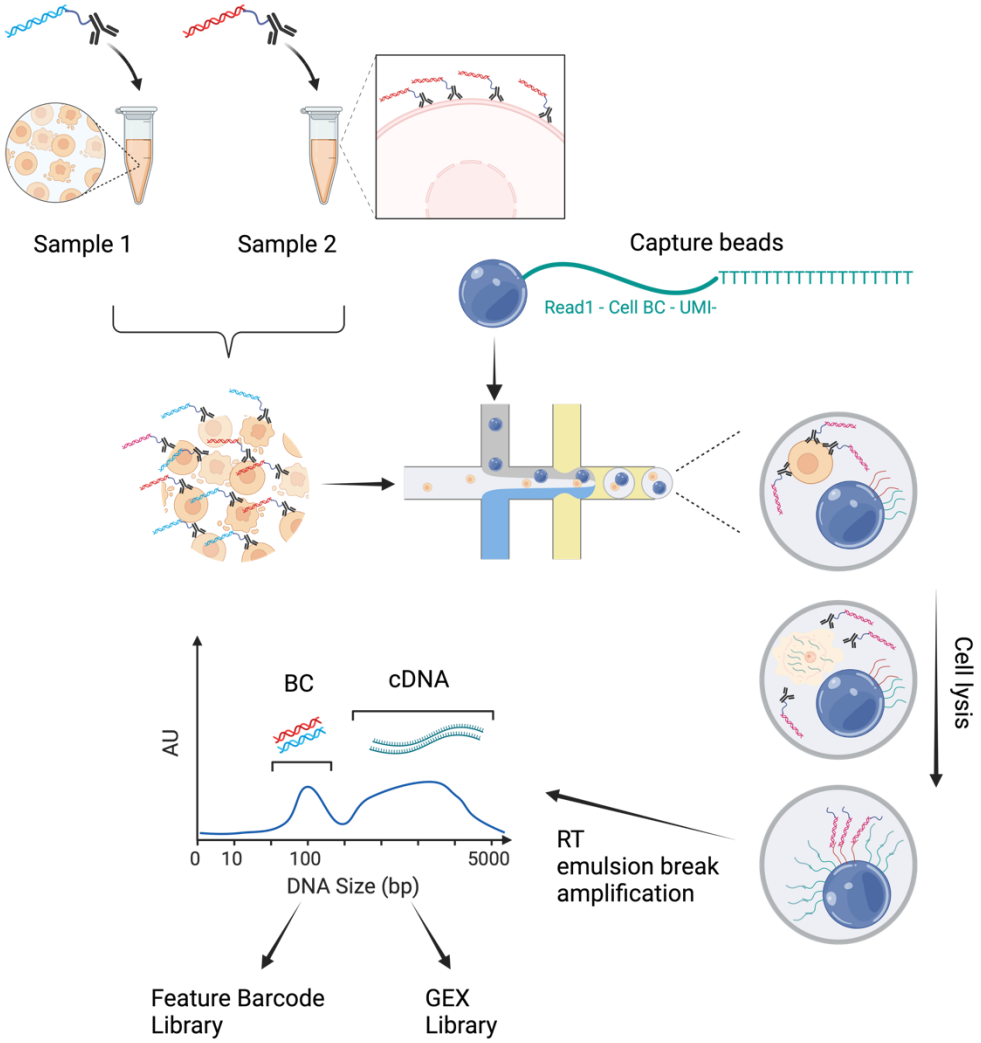
Adapted from Hagemann-Jensen, Nat Biotechnol , 2020 & 2022

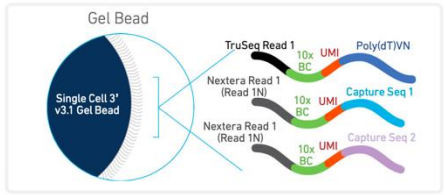
10x Genomics Chromium 3’

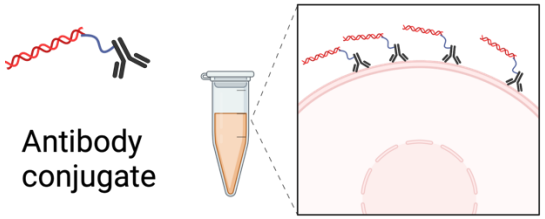


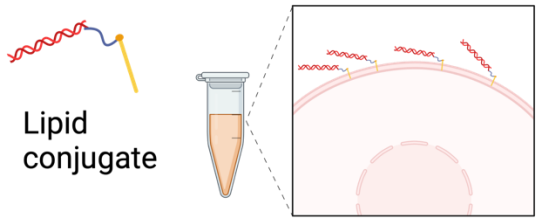
Created with BioRender.com

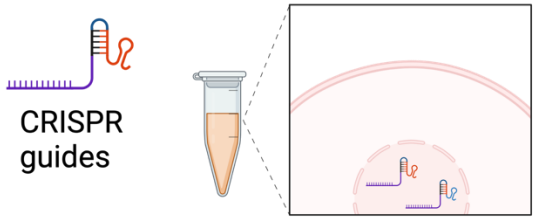
Cell multiplexing in 10x Chromium

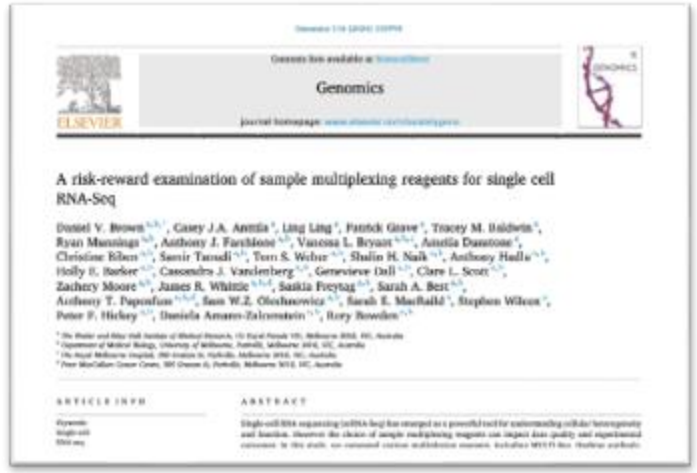










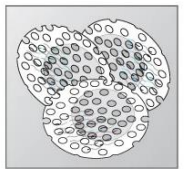


(Brown et al. 2024)

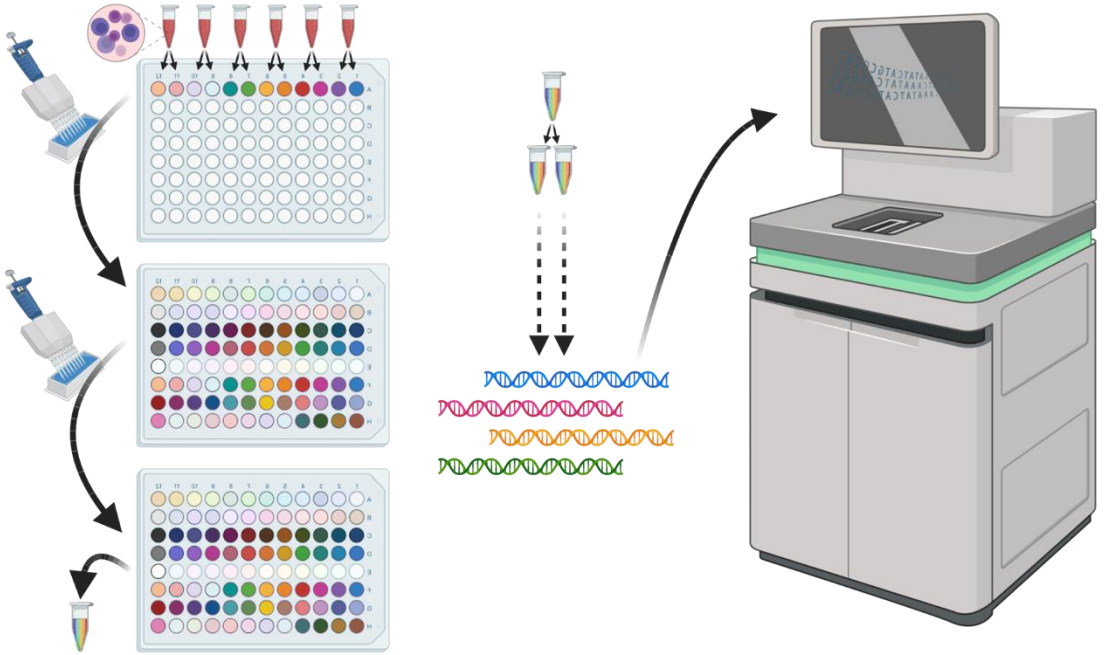
Created with BioRender.com

SPLiT-seq workflow

In situ barcoding

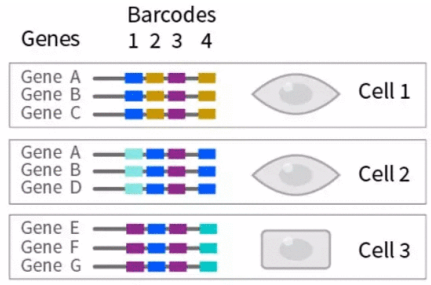


Cao *et al.* 2017[51]
Rosenberg *et al.* 2017[52]



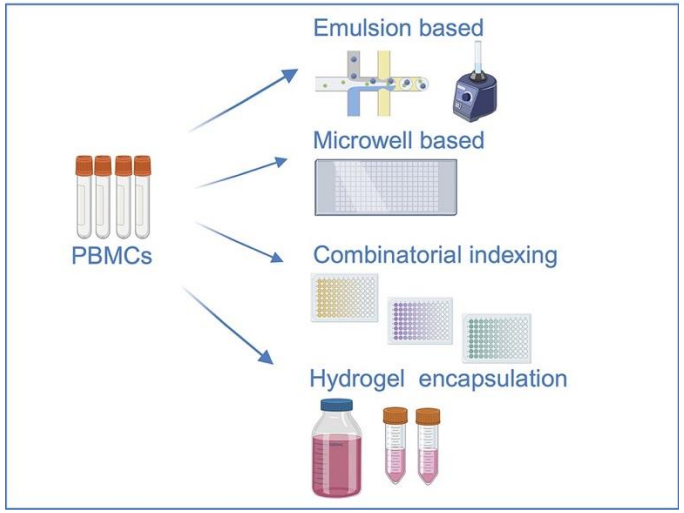
- 12 (BC 1)
- 96 (BC 2)
- 96 (BC 3)
- 2 (BC 4)

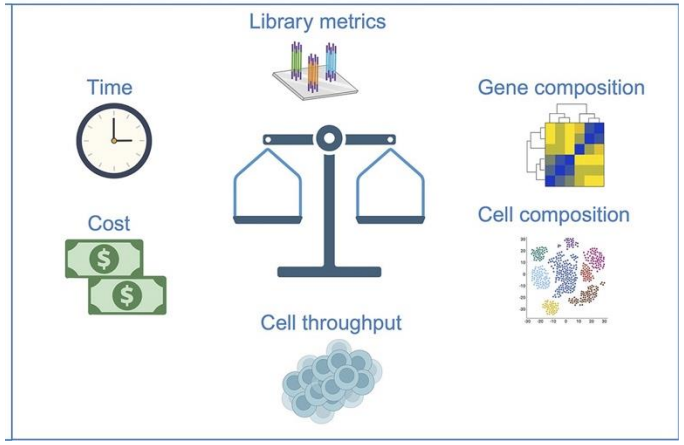
= 221 184
Possible combinations

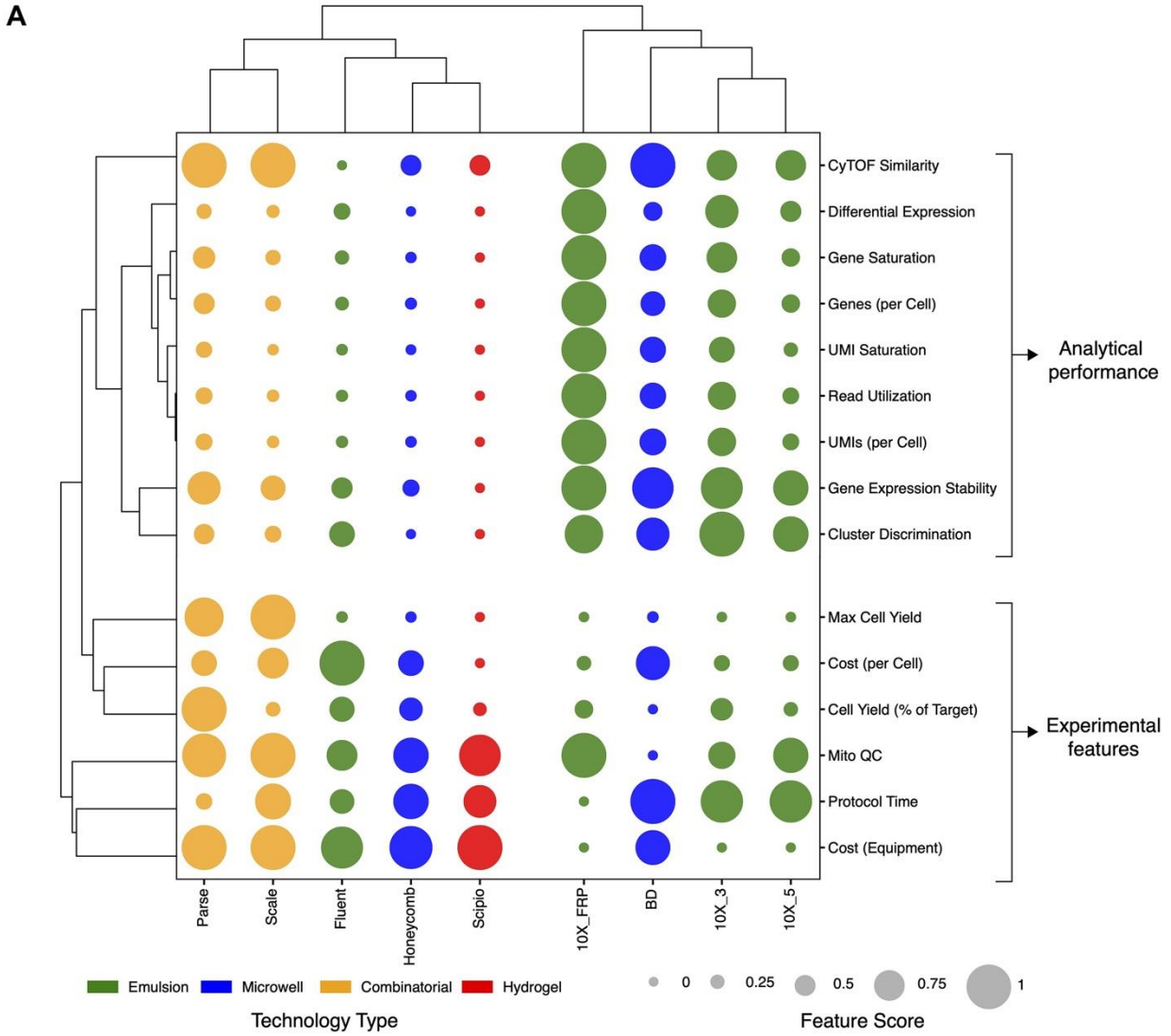


Up to 10 000 cells (with low doublet rate)
100 000 cell with 48 initial barcode
1 000 000 cells with 96 initial barcodes

Comparison of current methods

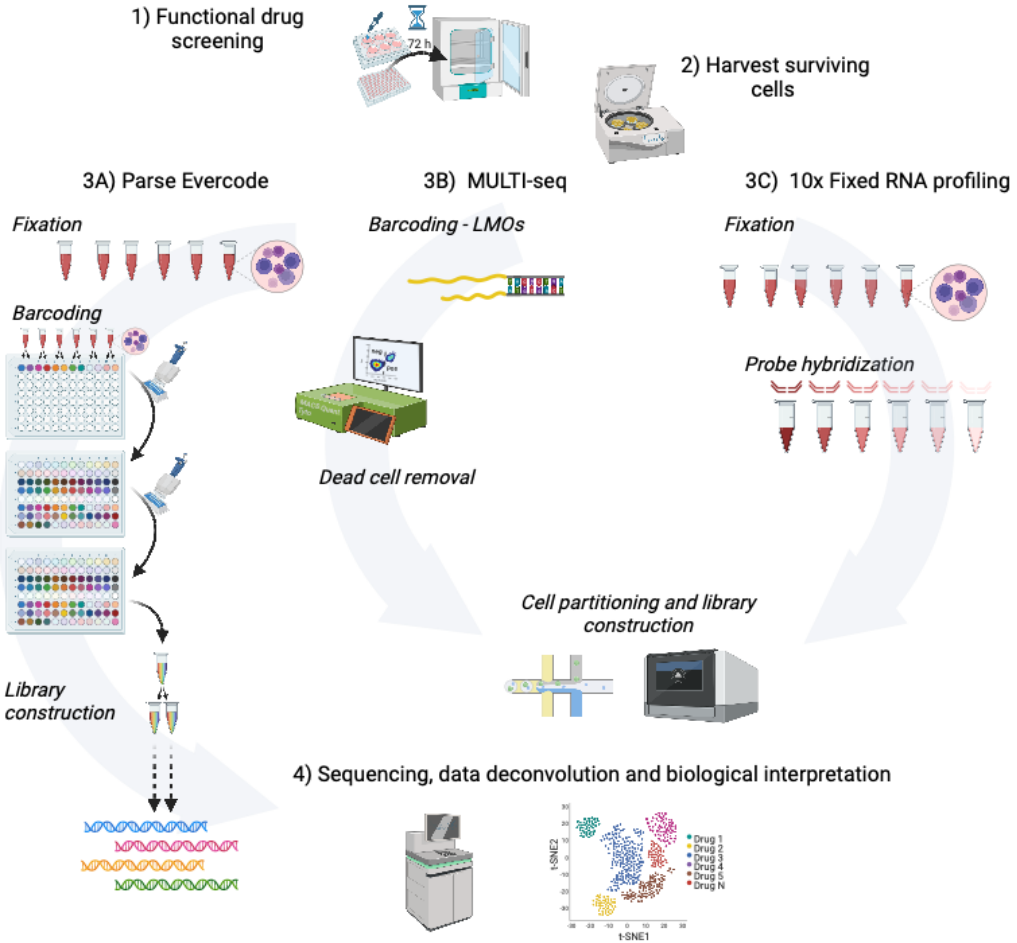


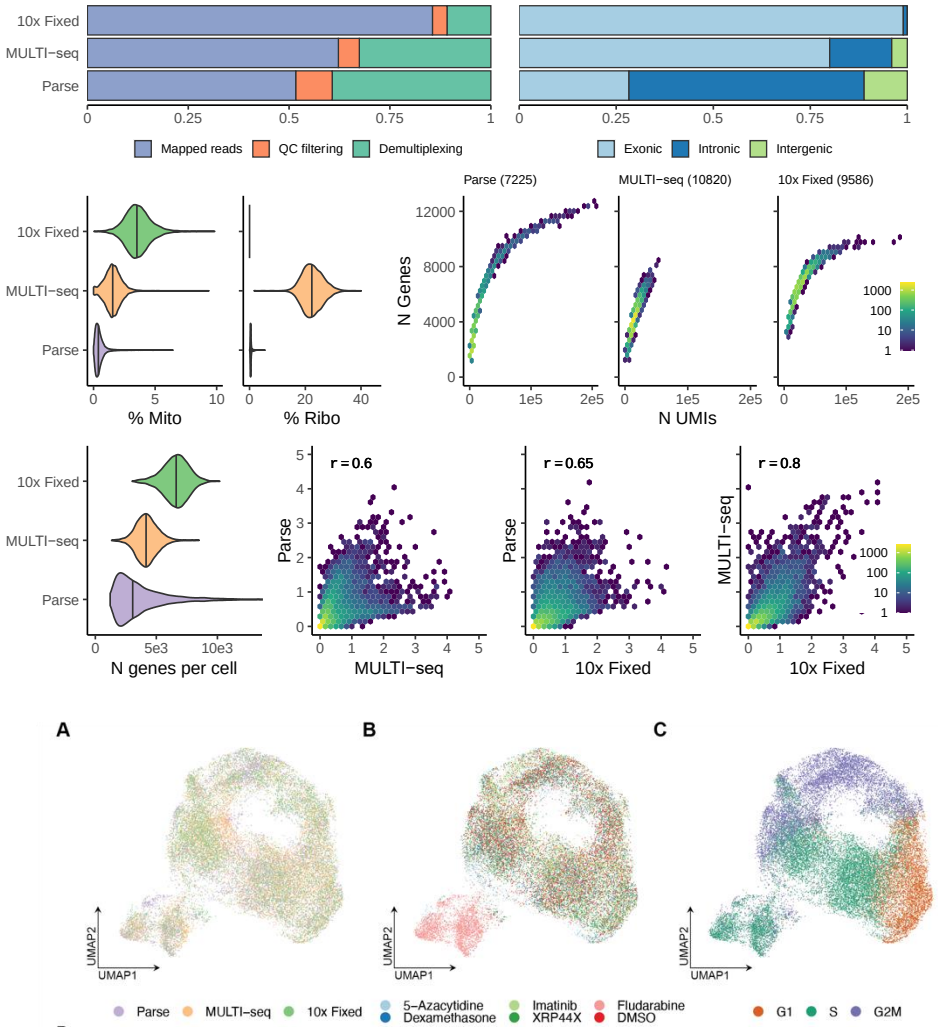




(De Simone et al. NAR 2025)

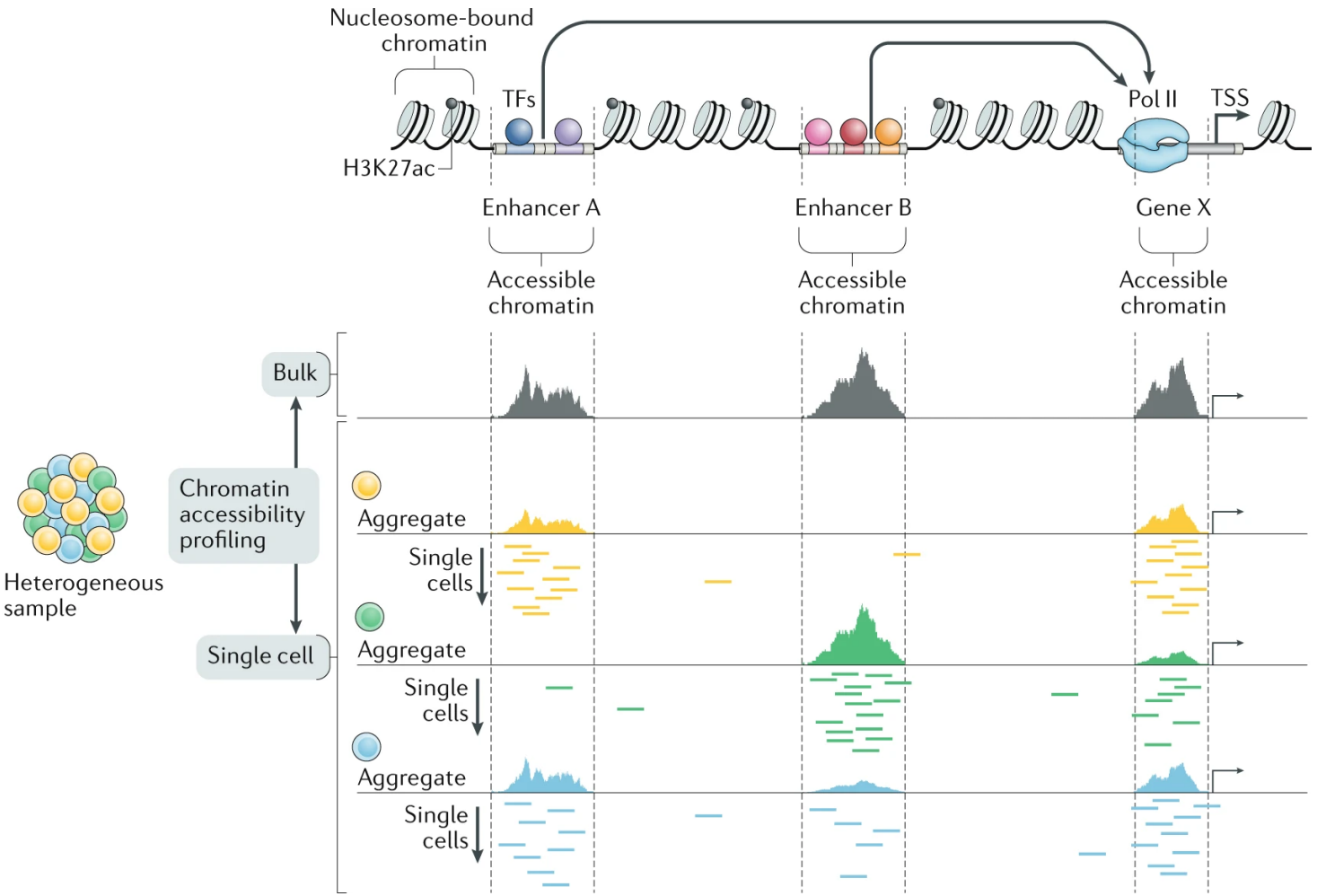
Comparison of multiplexing methods

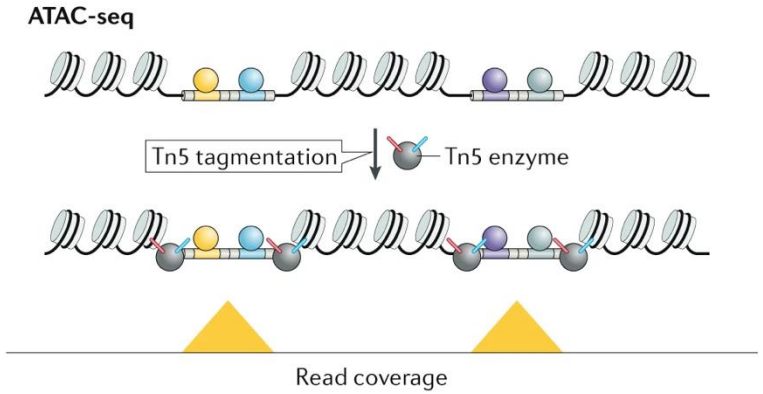




(Gezelius et al. 2024)

Beyond transcriptomics - Chromatin accessibility





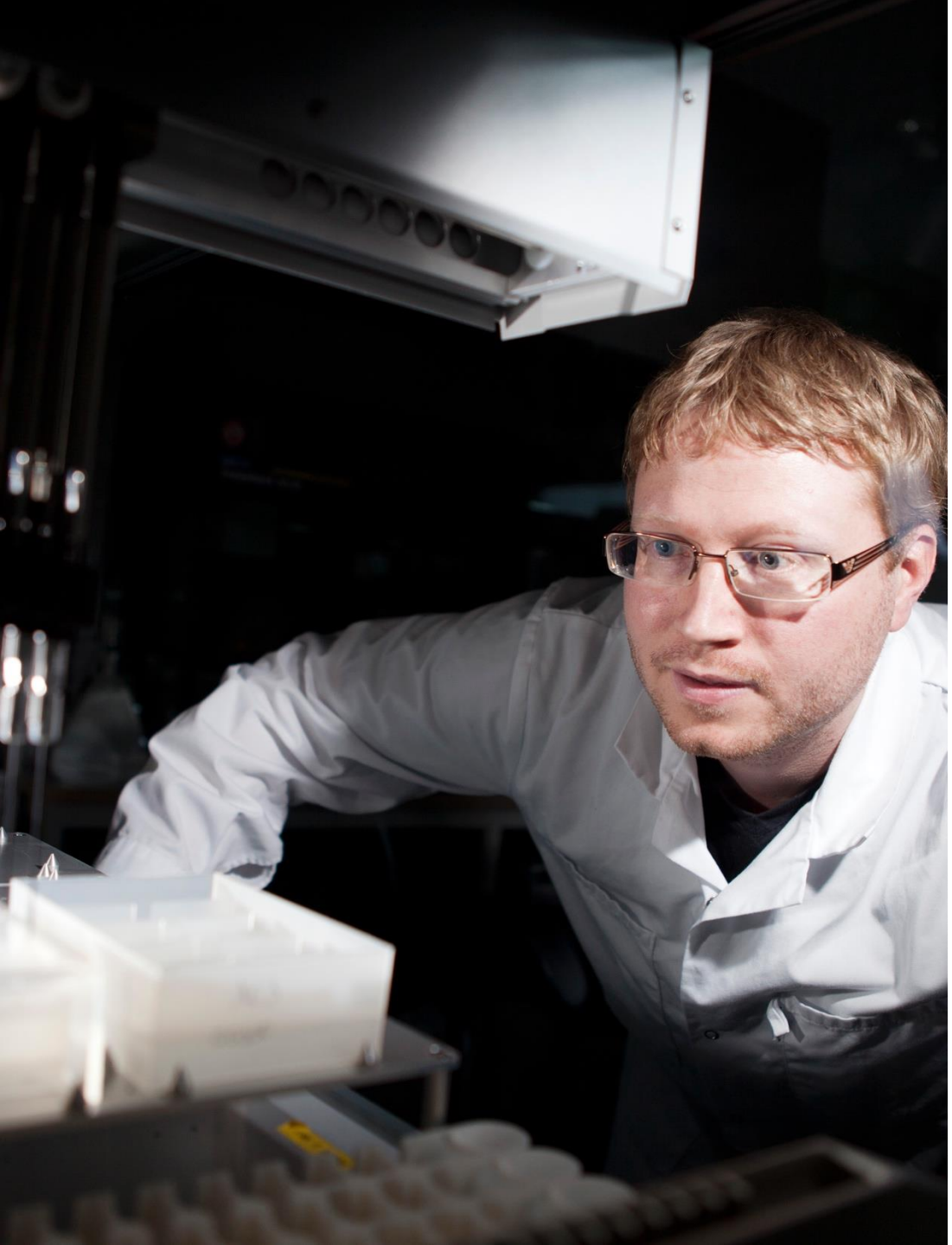
Minnoye, Nat Rev Methods Primers, 2021

Summary single cell sequencing

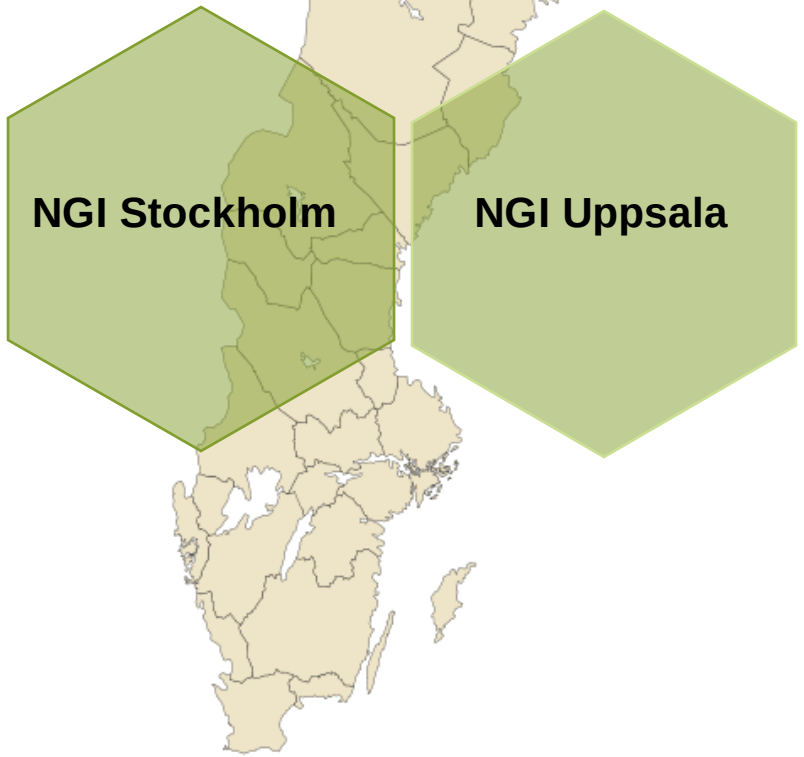
- ✓ Isolate cells in compartments
- ✓ Add UMI & barcode for later pooling
- ✓ Amplify
- ✓ Fragment (e.g Tn5 tagmentation)
- ✓ Sequence pooled libraries
- ✓ Analyze your data!

Questions?

henrik.gezelius@medsci.uu.se



NATIONAL GENOMICS INFRASTRUCTURE



NGI-Stockholm

NGI-Uppsala
Uppsala
Genome Center

NGI-Uppsala
SNP&SEQ
Technology
platform

Infrastructure, services and expertise in genomic technologies and applications

Multi-omics services

Genome Sequencing
De novo, re-seq, targeted...

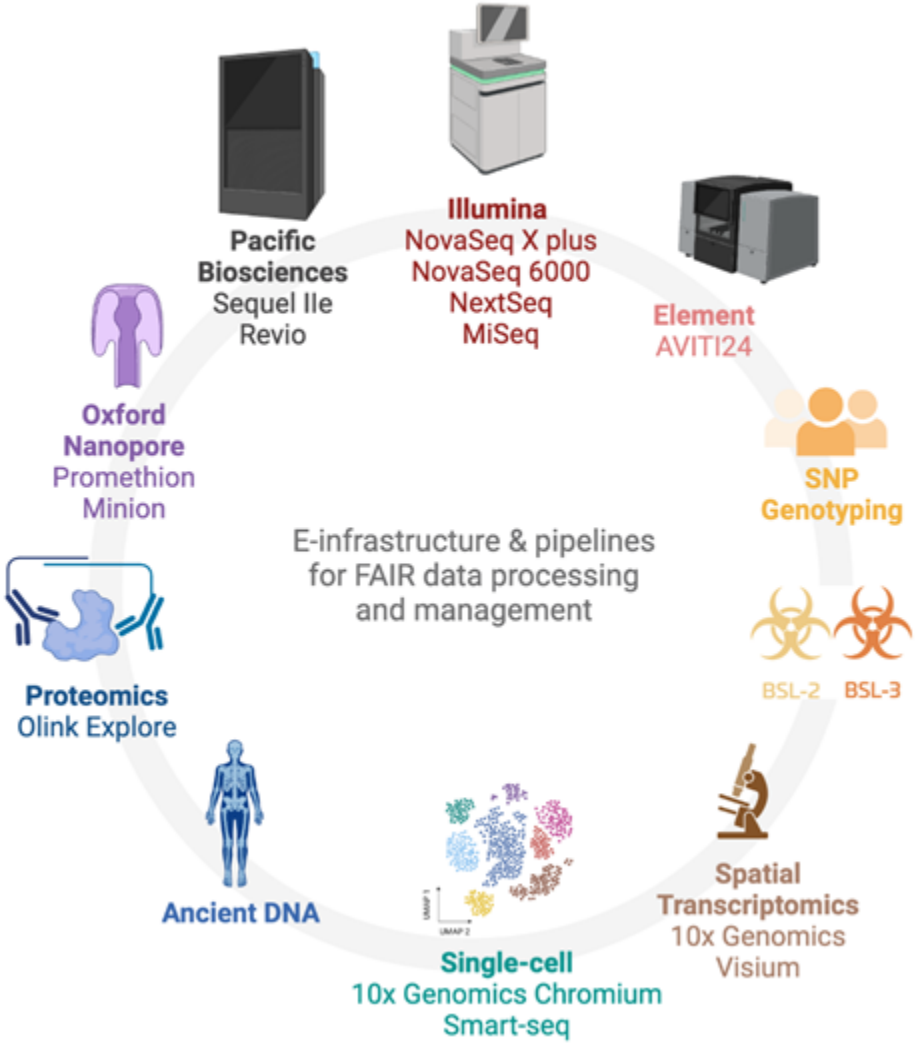
Epigenomics
Methylation, chromatin state, HiC...

Transcriptomics
Short-read, long-read

Proteomics
Olink Explore

Arrays
SNPs, methylation

Source material
Tissues
Cells
Microbes
Plasma
Nucleic acids
Archaeological material
Environmental samples
Read-made libraries

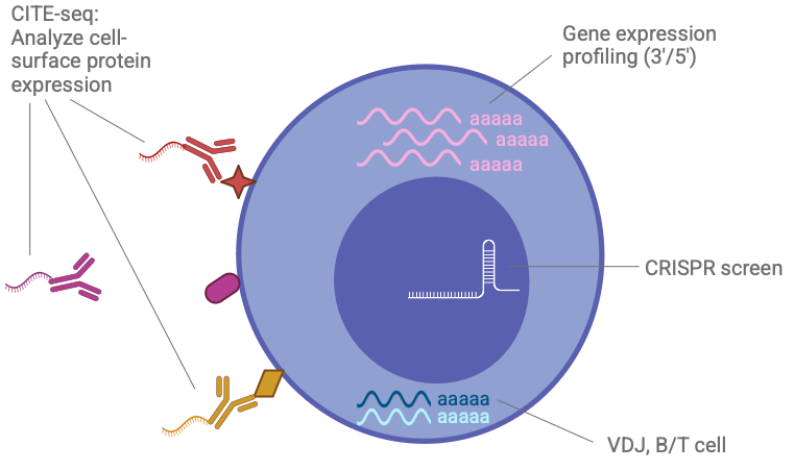


Disease genetics
Cancer research
Molecular biology
Drug development
Infectious diseases
Population genetics
Archaeology
Agriculture
Forestry
Ecology & biodiversity
Evolutionary genetics
Biotechnology
Methods development

All SciLife capabilities

NGI Single Cell services

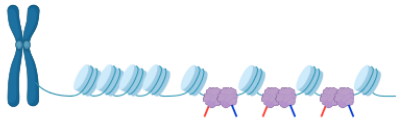
Gene Expression



10x Genomics

- *Flex (fixed)*
- *3'/5' GEX*
  - *optional LR*
- *CITE-seq*
- *Cell hashing*
- *VDJ*
- *CRISPR screen*

Epigenetics



10x Genomics

- *scATAC-seq*
- *scATAC + GEX*

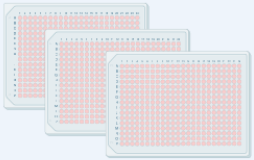
Split-pool

- *GEX*
- *VDJ*
- *CRISPR*

scWBGS (SPLAT)

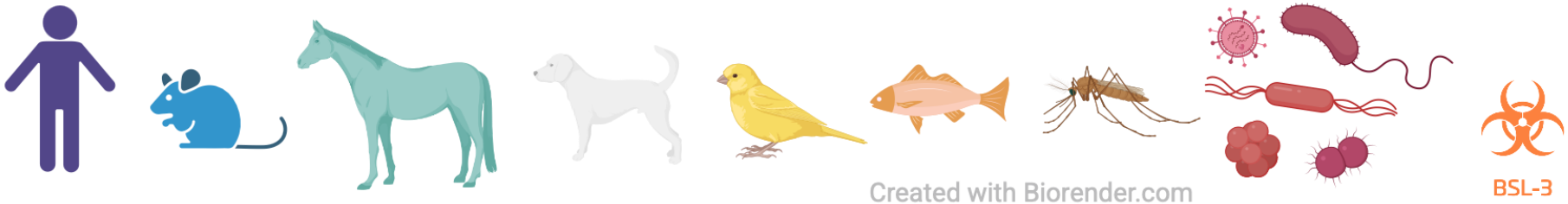
- *In house method: Raine et al, 2022*

DNA-sequencing

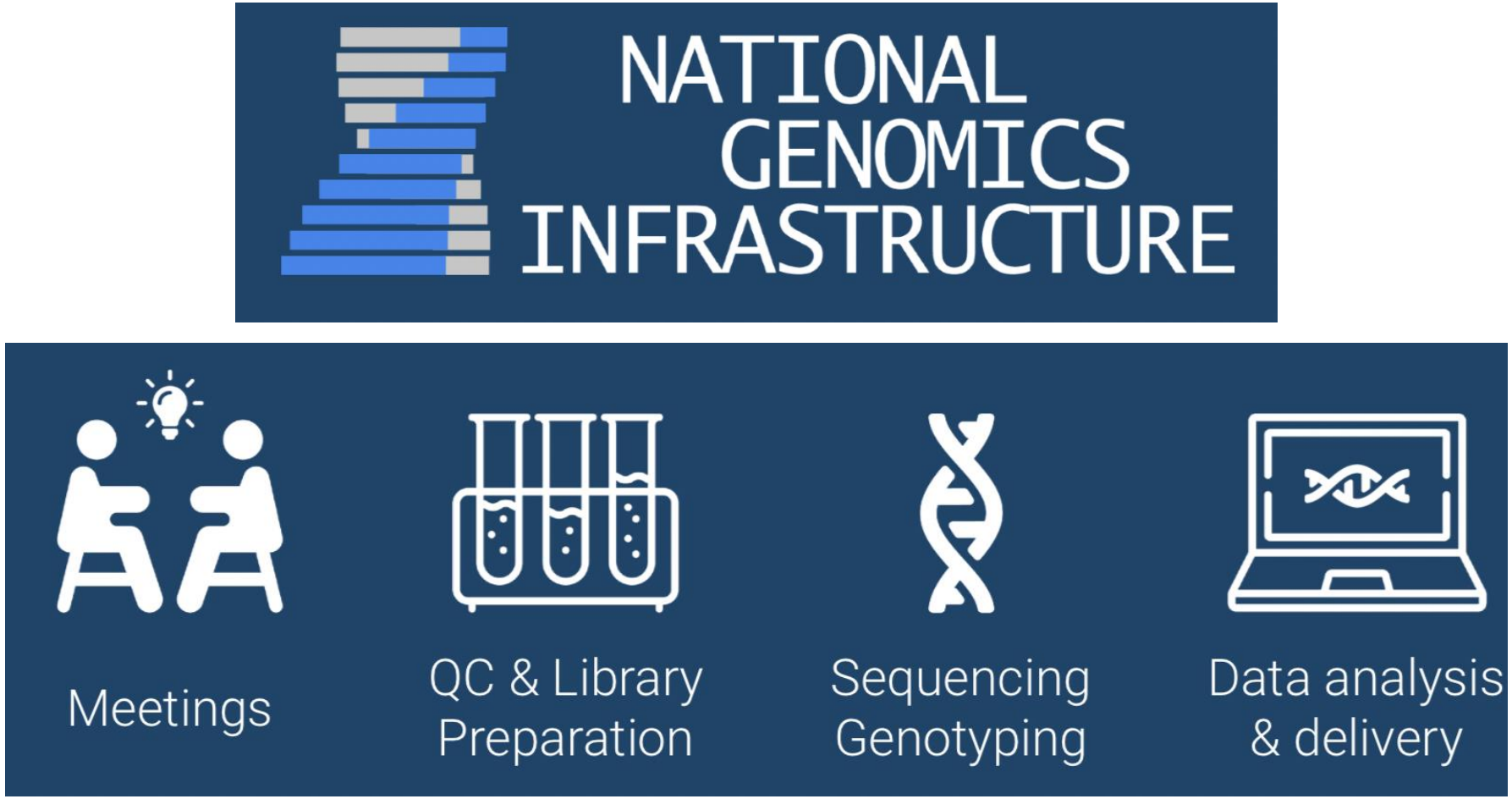


scWGS

- MDA or PTA genome amplification



Project workflow at NGI

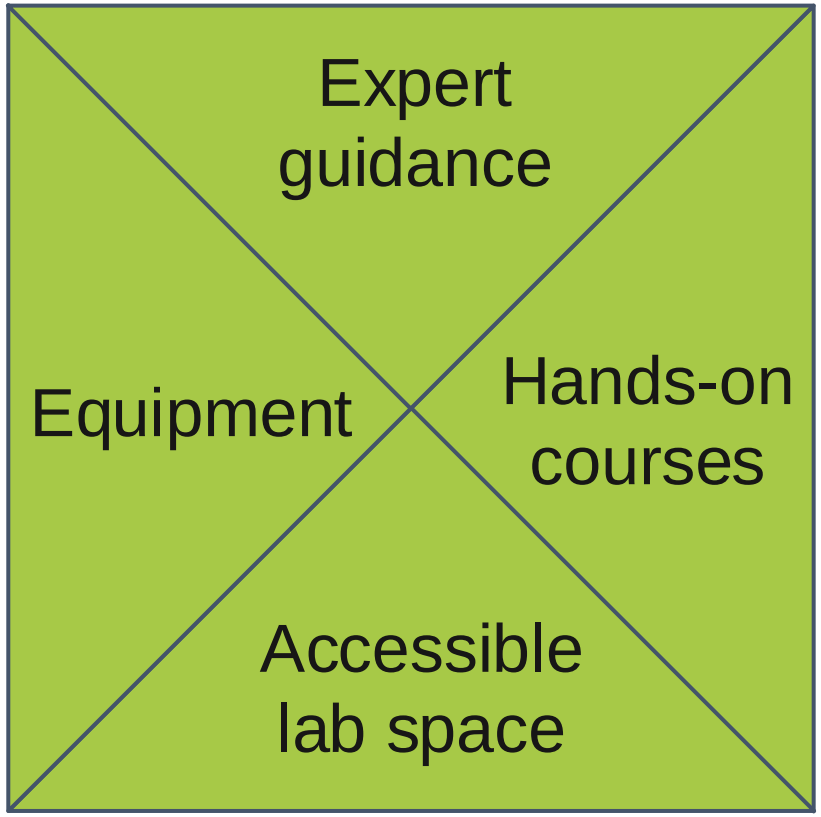


NGI OpenLab

User needs in niche assays, e.g.

- Complex biospecimens
- Customization
- New assays

NGI OpenLab



Platform service

- Assays with robust demand
- Automization
- Large-scale equipment
- Strategic and selective R&D

Users **Platform**

For more details and project requests

Contact NGI at **support@ngisweden.se**

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

https://ngisweden.scilifelab.se/

henrik.gezelius@medsci.uu.se