

scSeq methodologies and NGI services

Henrik Gezelius

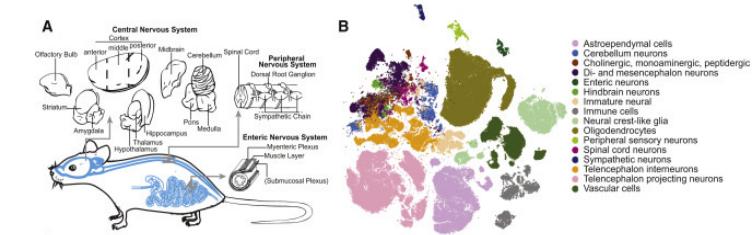
Acting head of Single Cell Genomics team Uppsala

2023-01-30

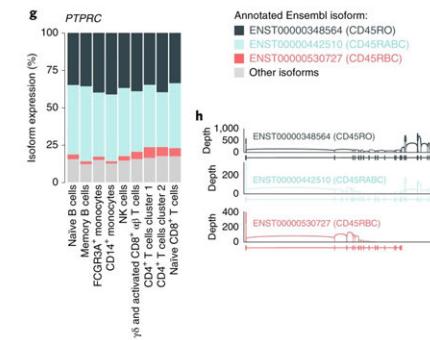
Applications for scRNA-sequencing



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

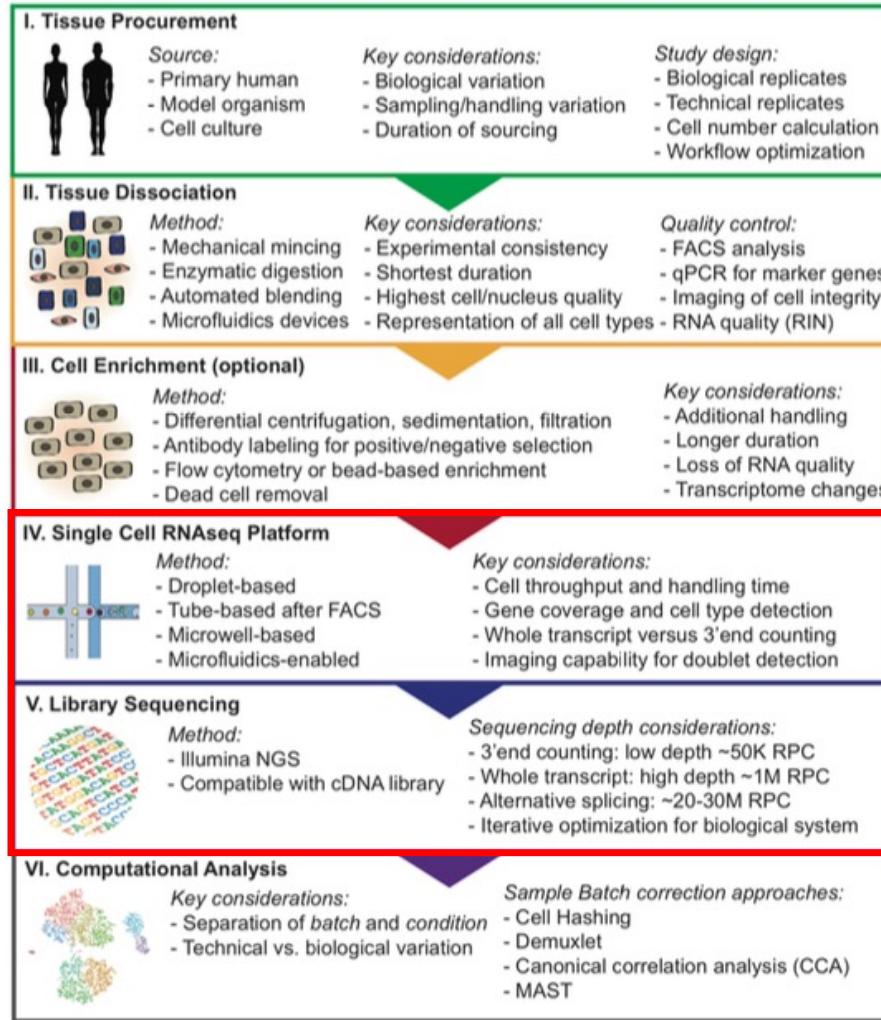


Zeisel et al, Cell 2018



Hagemann-Jensen Nat Biotech 2020

Single cell RNA-seq workflow



Verify your results with orthogonal method!

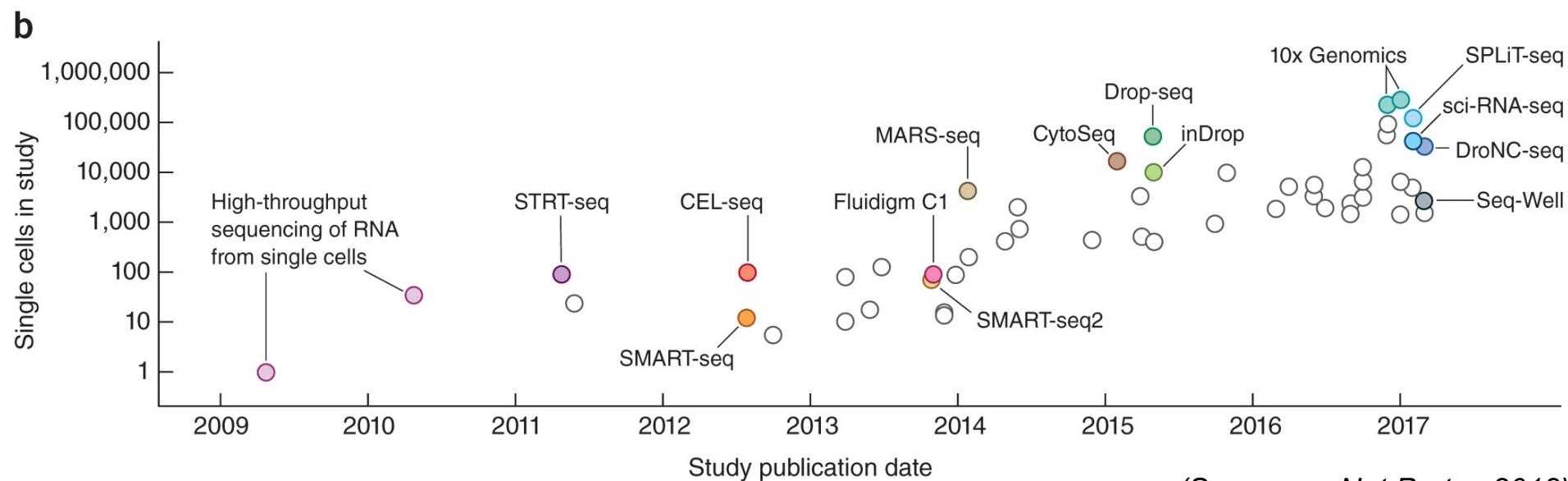
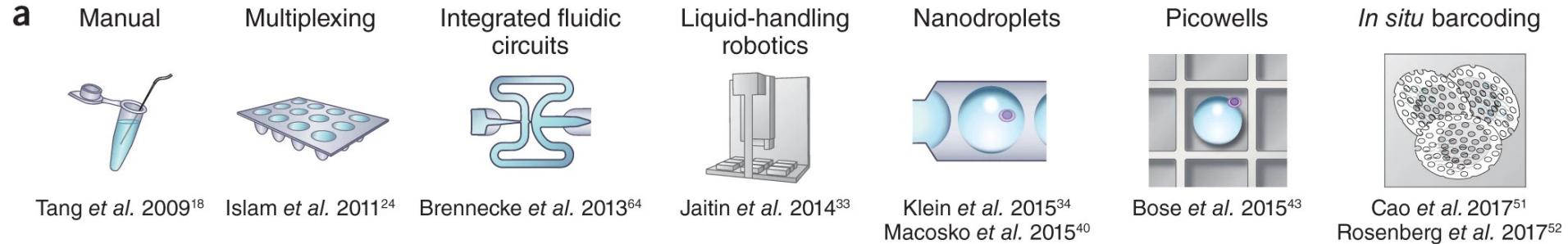


| Omics type | Read out | | Complexity (number of targets) | Sample requirements | | | Spatial resolution |
|--------------------------------------|----------|---------|-------------------------------------|---------------------|------|-----|------------------------------|
| | NGS | Imaging | | Fresh-frozen | FFPE | TMA | |
| Spatial transcriptomics (10X Visium) | ✓ | ✓ | Unbiased transcriptomewide | ✓ | (✓) | ✗ | Anatomical features of 55 µm |
| In situ sequencing | ✗ | ✓ | 200-300 | ✓ | ✓ | ✓ | Subcellular |
| Spatial proteomics (Codex) | ✗ | ✓ | 40 | ✓ | ✓ | ✓ | Subcellular |
| Advanced FISH technologies (smFISH) | ✗ | ✓ | 6 | ✓ | ✓ | ✓ | Subcellular |
| Spatial Mass Spectrometry | ✗ | ✓ | Multiplexed, targeted or untargeted | ✓ | ✗ | ✗ | Anatomical features of 15 µm |



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



| MICROPIPETTING MICROMANIPULATION | LASER CAPTURE MICRODISSECTION | FACS | MICRODROPLETS | MICROFLUIDICS e.g. FLUIDIGM C1 | Picowells | <i>In situ</i> barcoding |
|---|---|---|---|---|-------------------------------|-------------------------------|
| | | | | | | |
| low number of cells | low number of cells | hundreds of cells | large number of cells | hundreds of cells | large number of cells | huge number of cells |
| any tissue | any tissue | dissociated cells | dissociated cells | dissociated cells | dissociated cells | dissociated cells |
| enables selection of cells based on morphology or fluorescent markers | enables selection of cells based on morphology or fluorescent markers | enables selection of cells based on size or fluorescent markers | no selection of cells (can presort with FACS) | no selection of cells (can presort with FACS) | no selection | no selection |
| visualisation of cells | visualisation of cells | fluorescence and light scattering measurements | no visualisation | visualisation of cells | no visualisation | no visualisation |
| time consuming | time consuming | fast | fast | fast | fast | fast |
| reaction in microliter volumes | reaction in microliter volumes | reaction in microliter volumes | reaction in nanoliter volumes | reaction in nanoliter volumes | Multi-plexed Pooled reactions | Multi-plexed Pooled reactions |

- Cytoplasmic aspiration
- Patch-seq

Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015

(Adapted from: Svensson, Nat Protoc 2018)

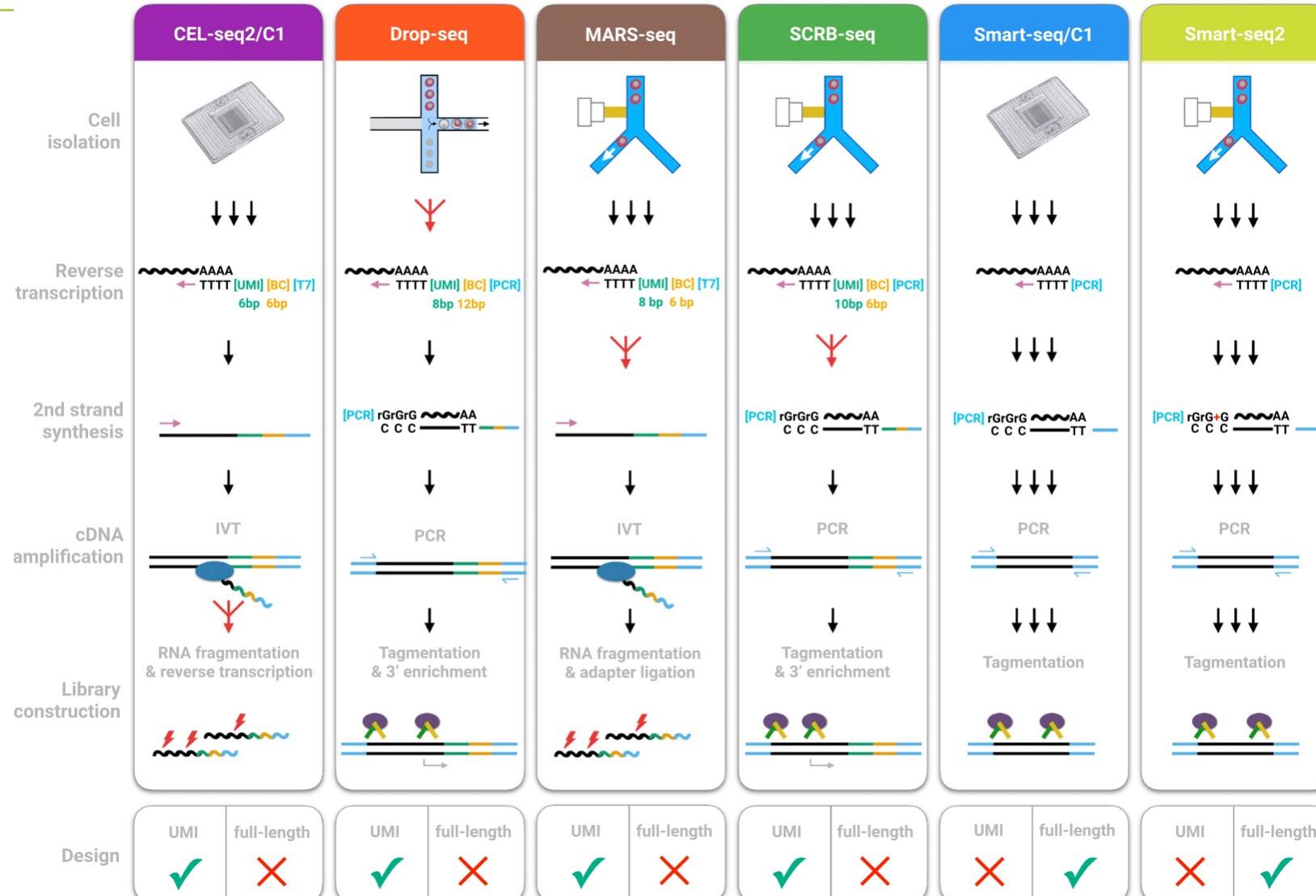
Bose et al. 2015⁴³

Cao et al. 2017⁵¹
Rosenberg et al. 2017⁵²

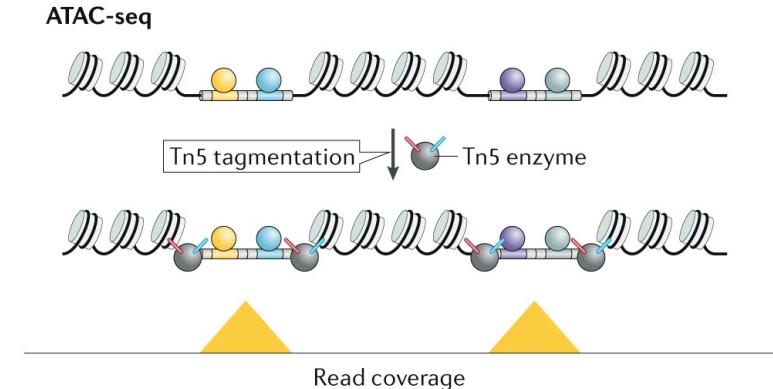
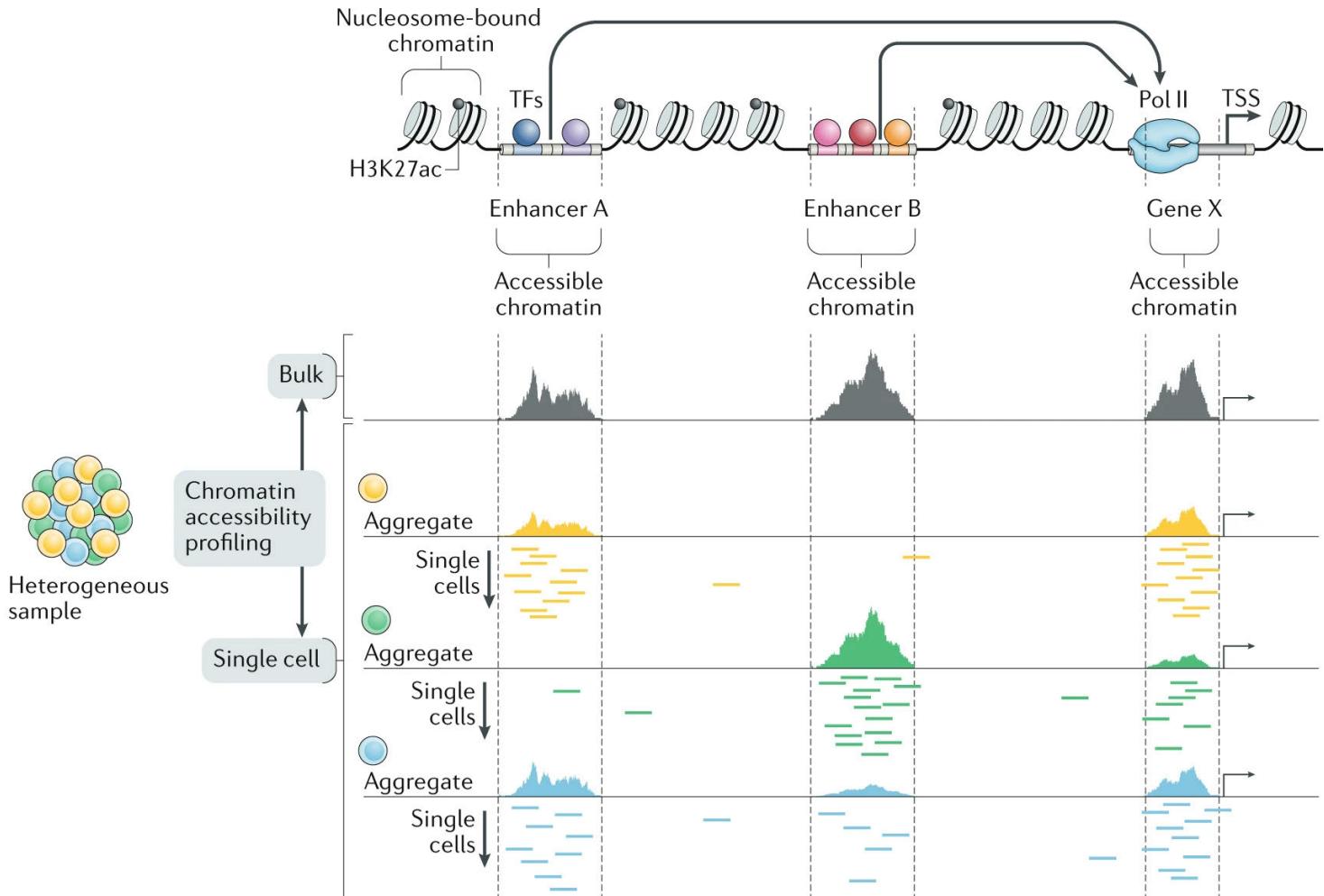
Example scRNA-seq: SMART-seq3



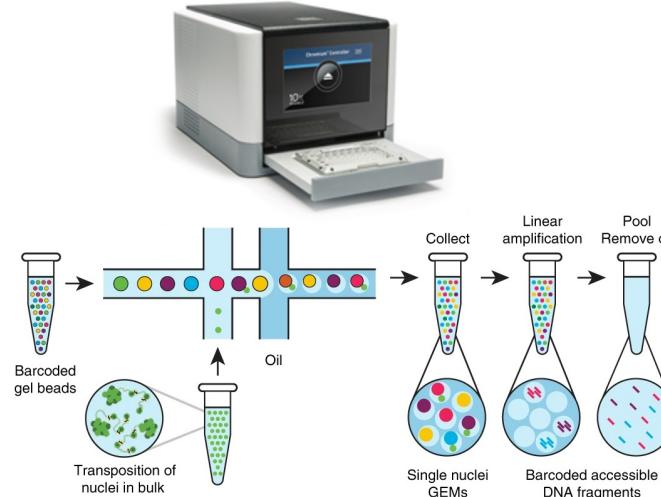
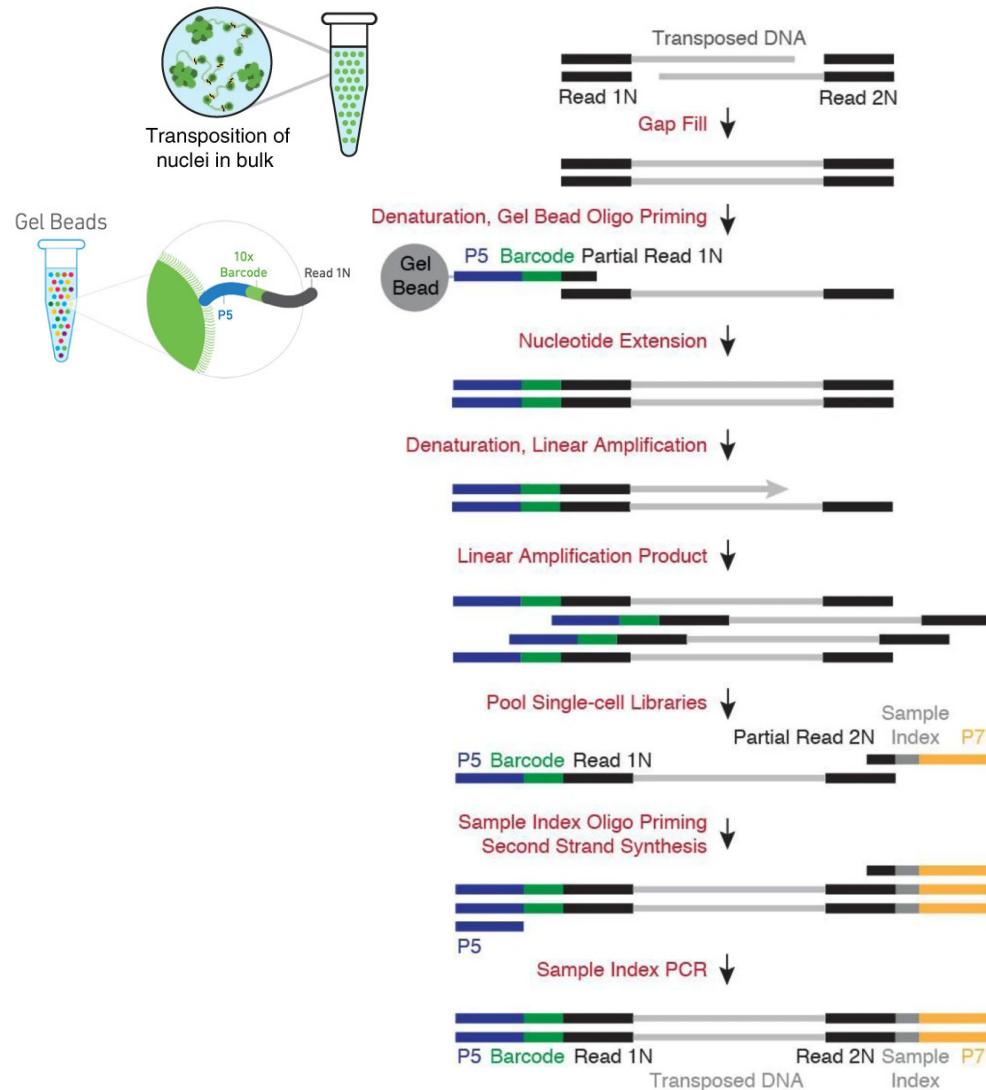
scRNA-sequencing protocol examples



Beyond transcriptomics - Chromatin accessibility



Example: 10x Genomics scATAC-seq



10x Genomics uses equivalent capture principles for all their single cell methods

- For some methods multiple capture sequences on the beads (e.g. multiome)

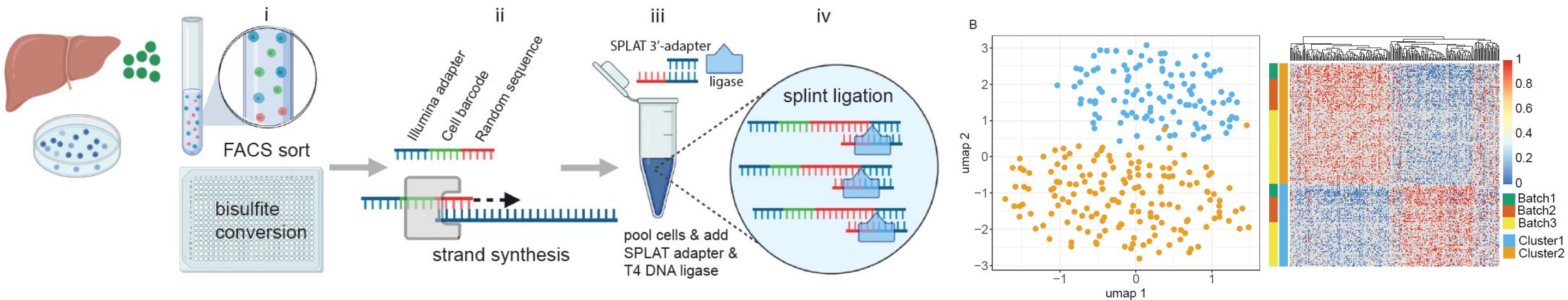
DNA methylation analysis in single cells



Article | Open Access | Published: 06 April 2022

scSPLAT, a scalable plate-based protocol for single cell WGBS library preparation

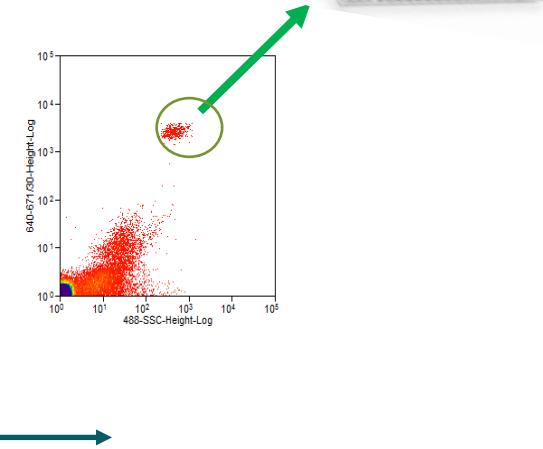
Amanda Raine , Anders Lundmark, Alva Annett, Ann-Christin Wiman, Marco Cavalli, Claes Wadelius,
Claudia Bergin & Jessica Nordlund



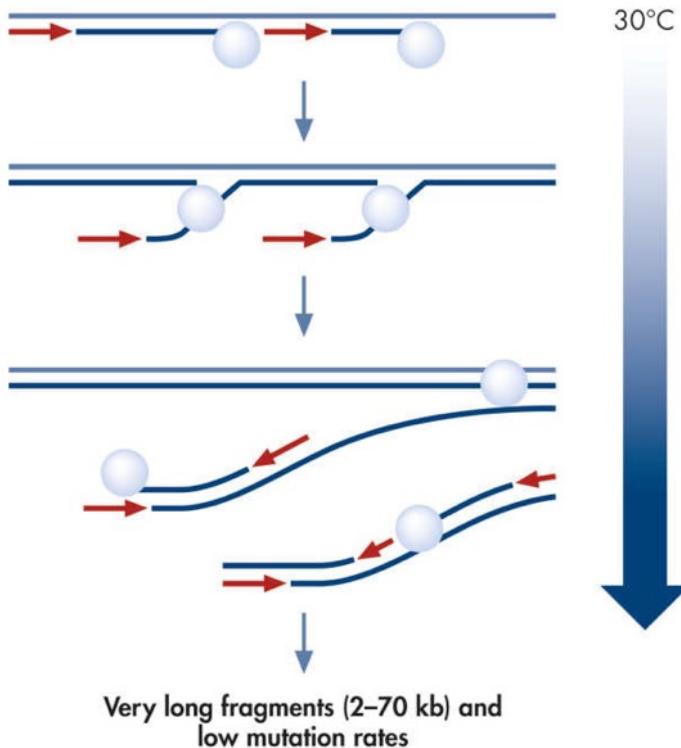
Method validation completed. Open for collaborative projects, contact seq@medsci.uu.se.

MDA – Whole genome DNA seq

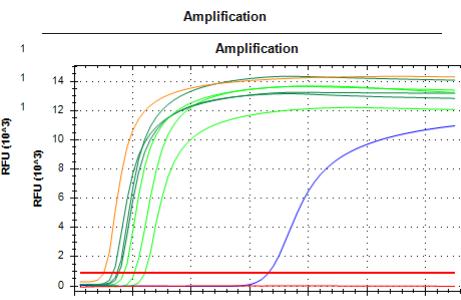
Cell sorting



MDA

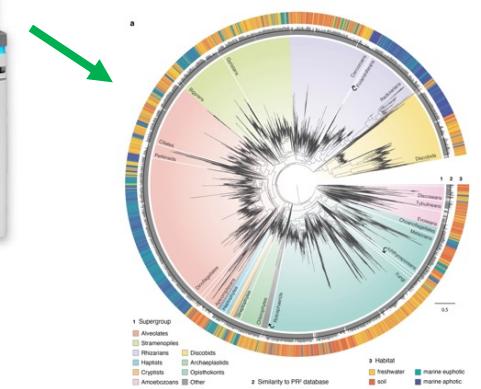


Very long fragments (2-70 kb) and
low mutation rates



1. Sorting, scitur wells
2. Identification
3. Library prep

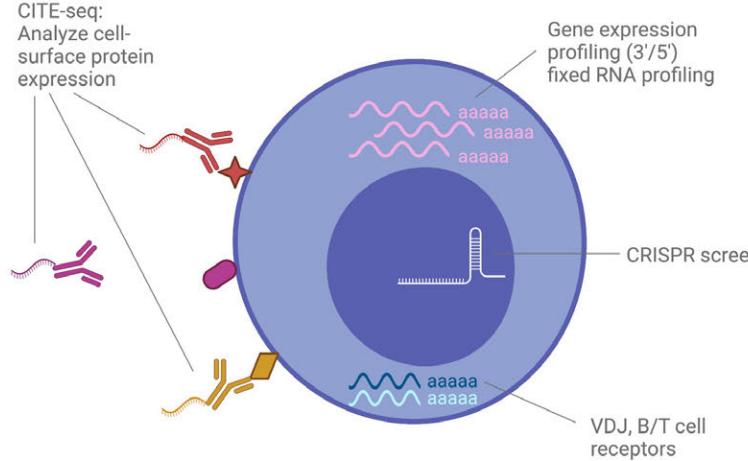
NGS Sequencing



Single Cell Seq at NGI



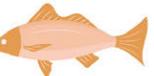
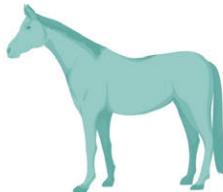
Gene Expression



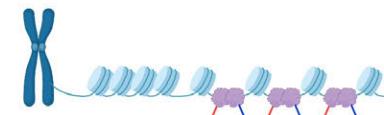
- 10x Genomics
- 3'/5' GEX
 - CITE-seq
 - Cell hashing
 - VDJ
 - CRISPR screen
 - fixed RNA profiling

Smart-seq 2/3

Drop-seq
(Dolomite Nadia)



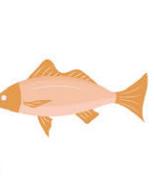
Epigenetics



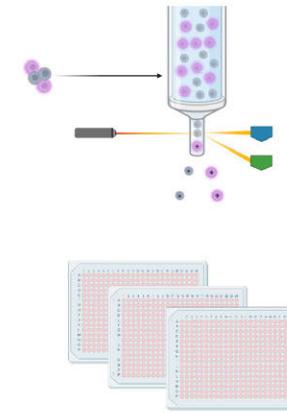
- 10x Genomics
- scATAC-seq
 - scATAC + GEX



- scWBGS (SPLAT)
- Raine et al, 2022



DNA-sequencing



- FACS sorting combined with cell lysis and genome amplification (MDA)



Includes services of ESCG and MSCG, now merged with NGI.

Cellular Immunomonitoring

Enables deep phenotypic characterization and functional profiling of millions of cells with >40 markers tagged/cell that can be readily analyzed (Mass Cytometry through CyTOF™, Fluidigm Inc.).

[Learn More →](#)

Eukaryotic Single Cell Genomics

Provides service for high-throughput single cell genomics analysis

[Learn More →](#)

Microbial Single Cell Genomics

Provides customized single cell genomic services for Swedish and international researchers working with prokaryotic and eukaryotic microbes. The unit also capacitates work with live microbial pathogens up to and including biosafety-level 3 (BSL3).

[Learn More →](#)

National Bioinformatics Infrastructure (NBIS)

Provides custom-tailored support with analysis of proteomics data generated at SciLifeLab or elsewhere, as well as tools and training.

[Learn More →](#)

National Genomics Infrastructure (NGI)

1

[Learn More →](#)

Spatial Proteomics

As part of Human Protein Atlas this unit resource a near proteome wide collection of antibodies used for immunofluorescence to analyze proteins in a broad panel of cell types.

[Learn More →](#)

Project workflow at NGI



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/>