

# scSeq methodologies and NGI services

Henrik Gezelius

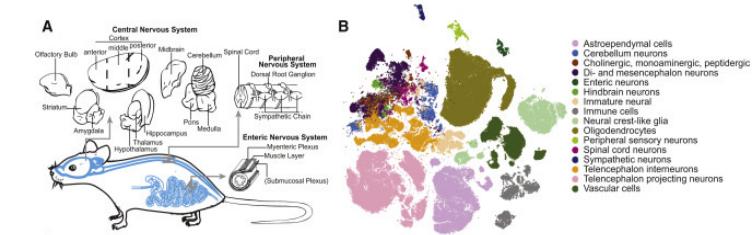
Acting head of Single Cell Genomics team Uppsala

2023-01-30

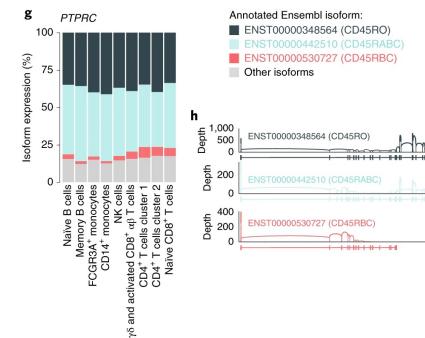
# Applications for scRNA-sequencing



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

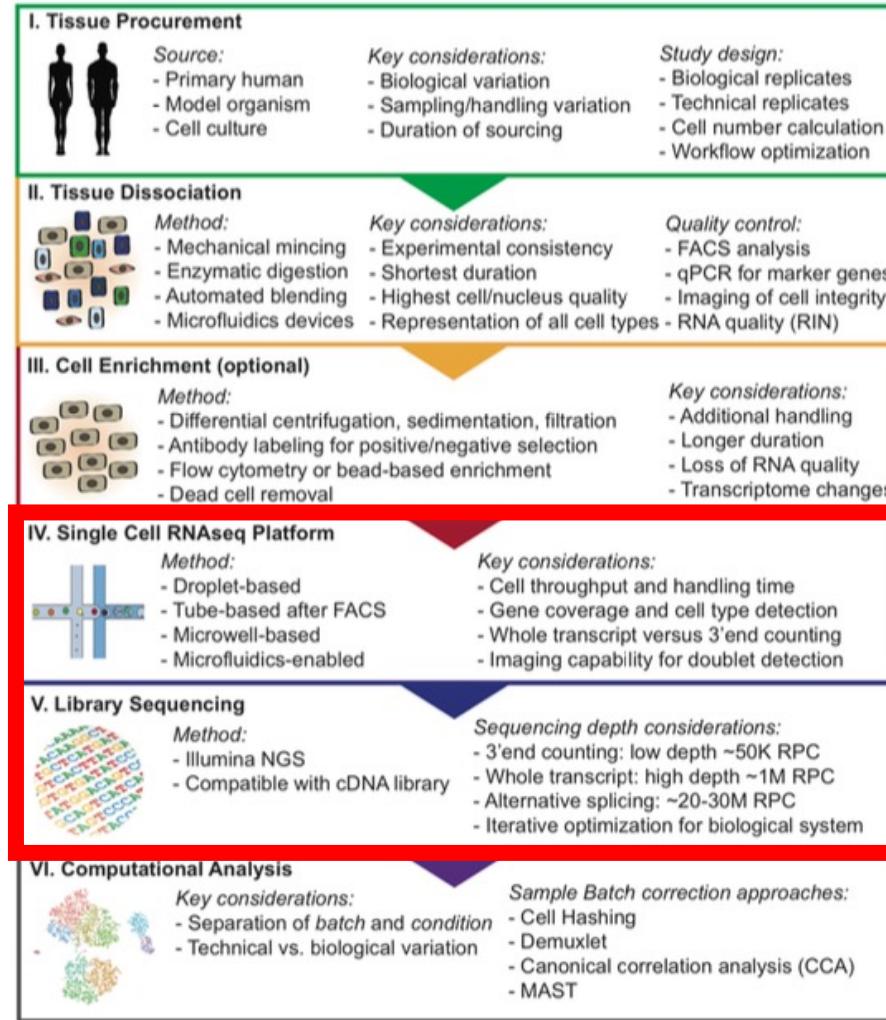


Zeisel et al, Cell 2018



Hagemann-Jensen Nat Biotech 2020

# Single cell RNA-seq workflow



Verify your results with orthogonal method!

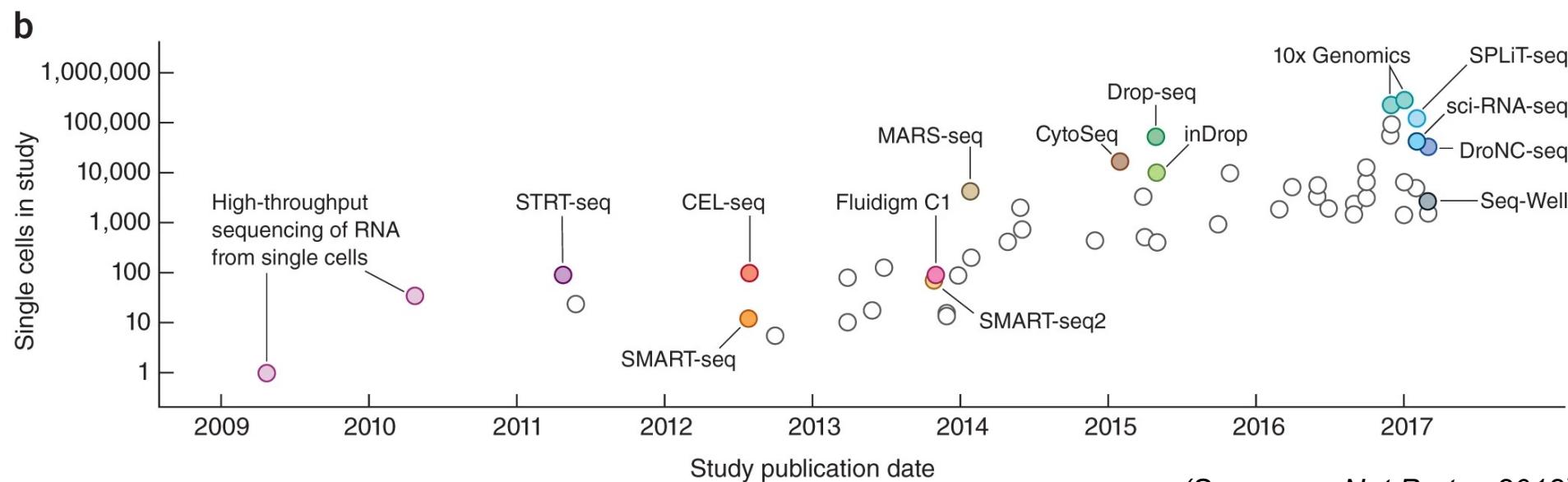
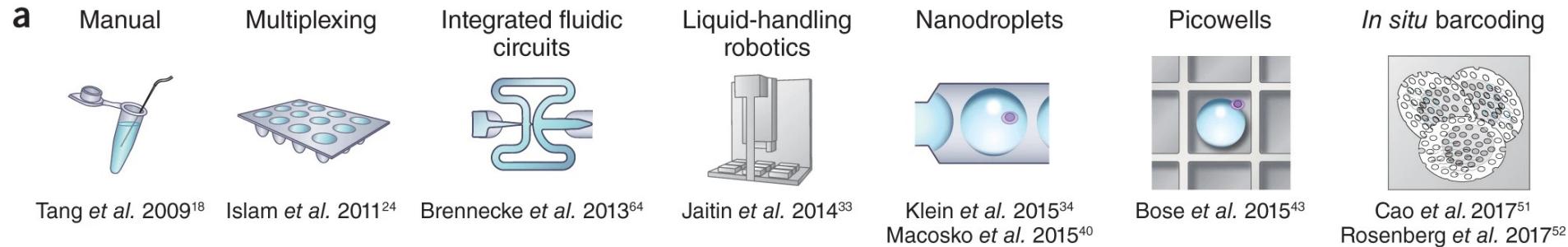


Omics type	Read out		Complexity (number of targets)	Sample requirements			Spatial resolution
	NGS	Imaging		Fresh-frozen	FFPE	TMA	
Spatial transcriptomics (10X Visium)	✓	✓	Unbiased transcriptome-wide	✓	(✓)	✗	Anatomical features of 55 µm
In situ sequencing	✗	✓	200-300	✓	✓	✓	Subcellular
Spatial proteomics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



# Single-cell isolation or capture



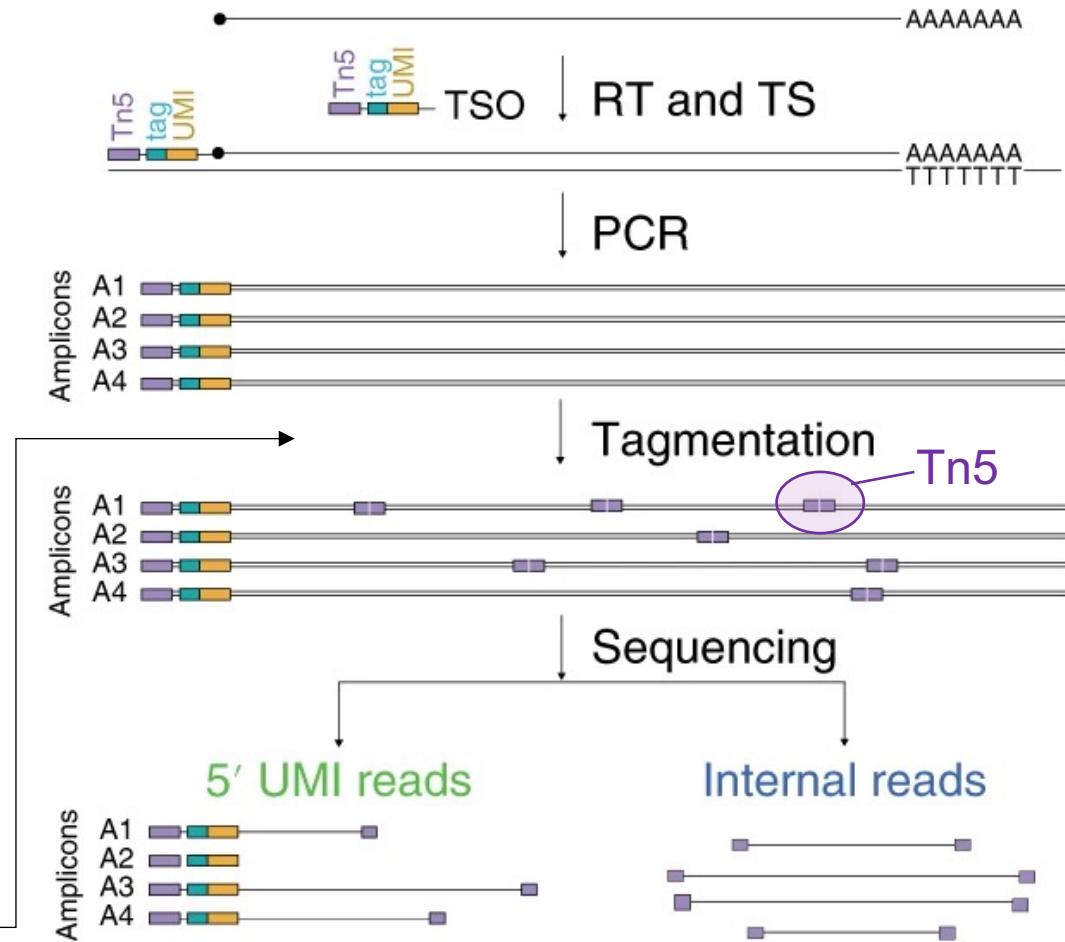
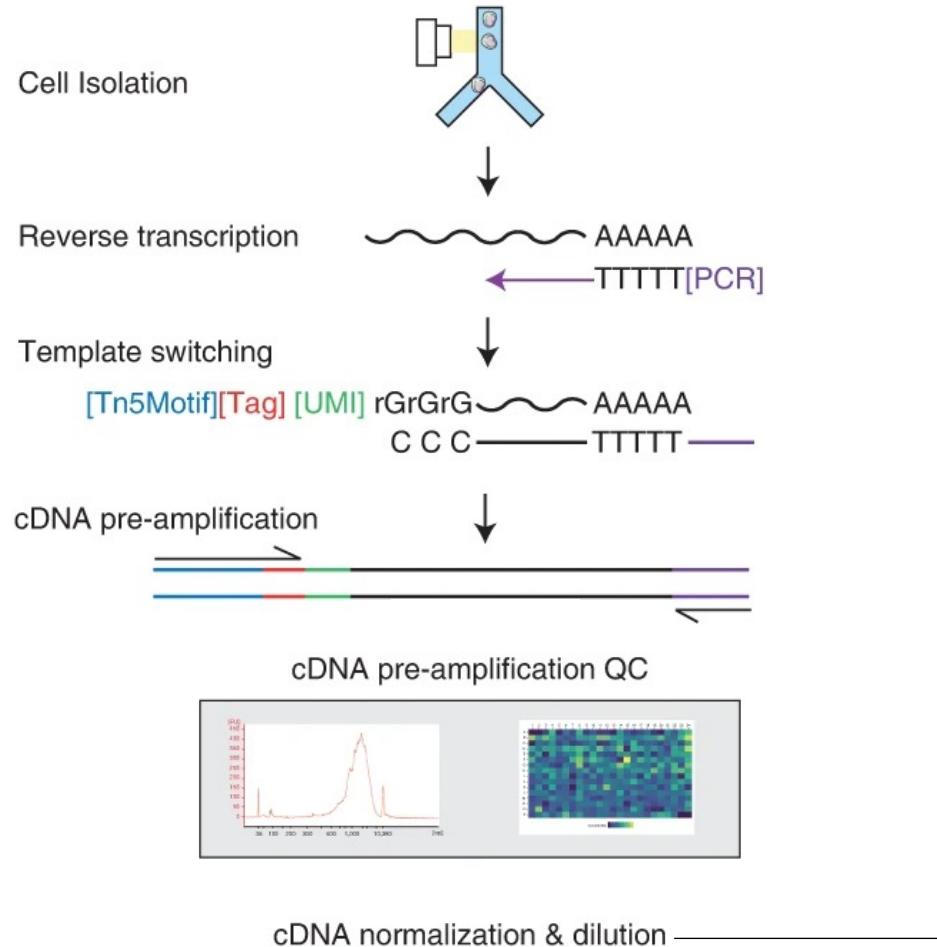
MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1	Picowells	<i>In situ</i> barcoding
low number of cells any tissue enables selection of cells based on morphology or fluorescent markers visualisation of cells time consuming reaction in microliter volumes	low number of cells any tissue enables selection of cells based on morphology or fluorescent markers visualisation of cells time consuming reaction in microliter volumes	hundreds of cells dissociated cells enables selection of cells based on size or fluorescent markers fluorescence and light scattering measurements fast reaction in microliter volumes	large number of cells dissociated cells no selection of cells (can presort with FACS) no visualisation fast reaction in nanoliter volumes	hundreds of cells dissociated cells no selection of cells (can presort with FACS) visualisation of cells fast reaction in nanoliter volumes	large number of cells dissociated cells no selection no visualisation fast Multi-plexed Pooled reactions	huge number of cells dissociated cells no selection no visualisation fast Multi-plexed Pooled reactions
Bose et al. 2015 <sup>43</sup>						Cao et al. 2017 <sup>51</sup> Rosenberg et al. 2017 <sup>52</sup>

- Cytoplasmic aspiration
- Patch-seq

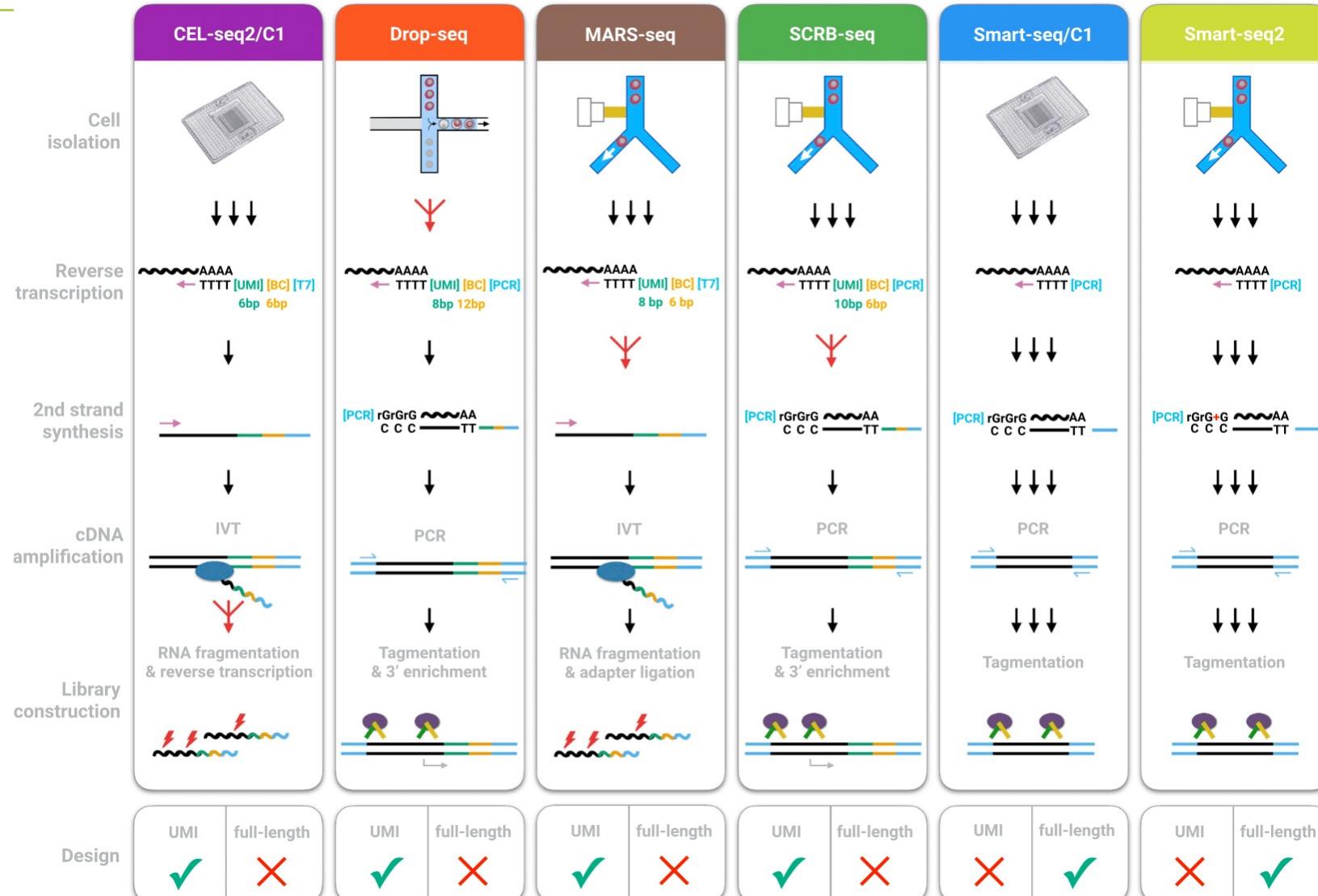
Adapted from: Kolodziejczyk A et al, Molecular Cell, 2015

(Adapted from: Svensson, Nat Protoc 2018)

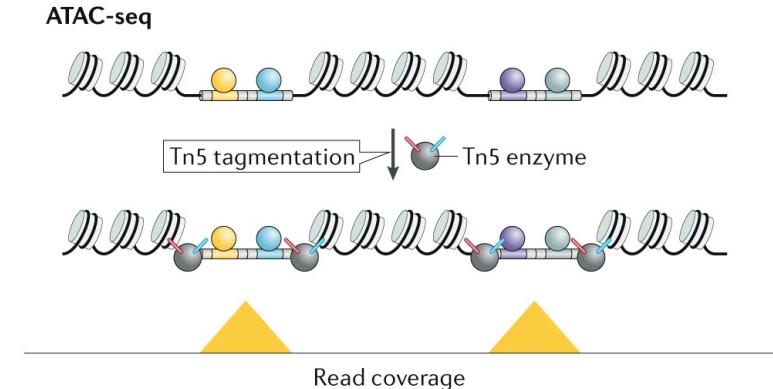
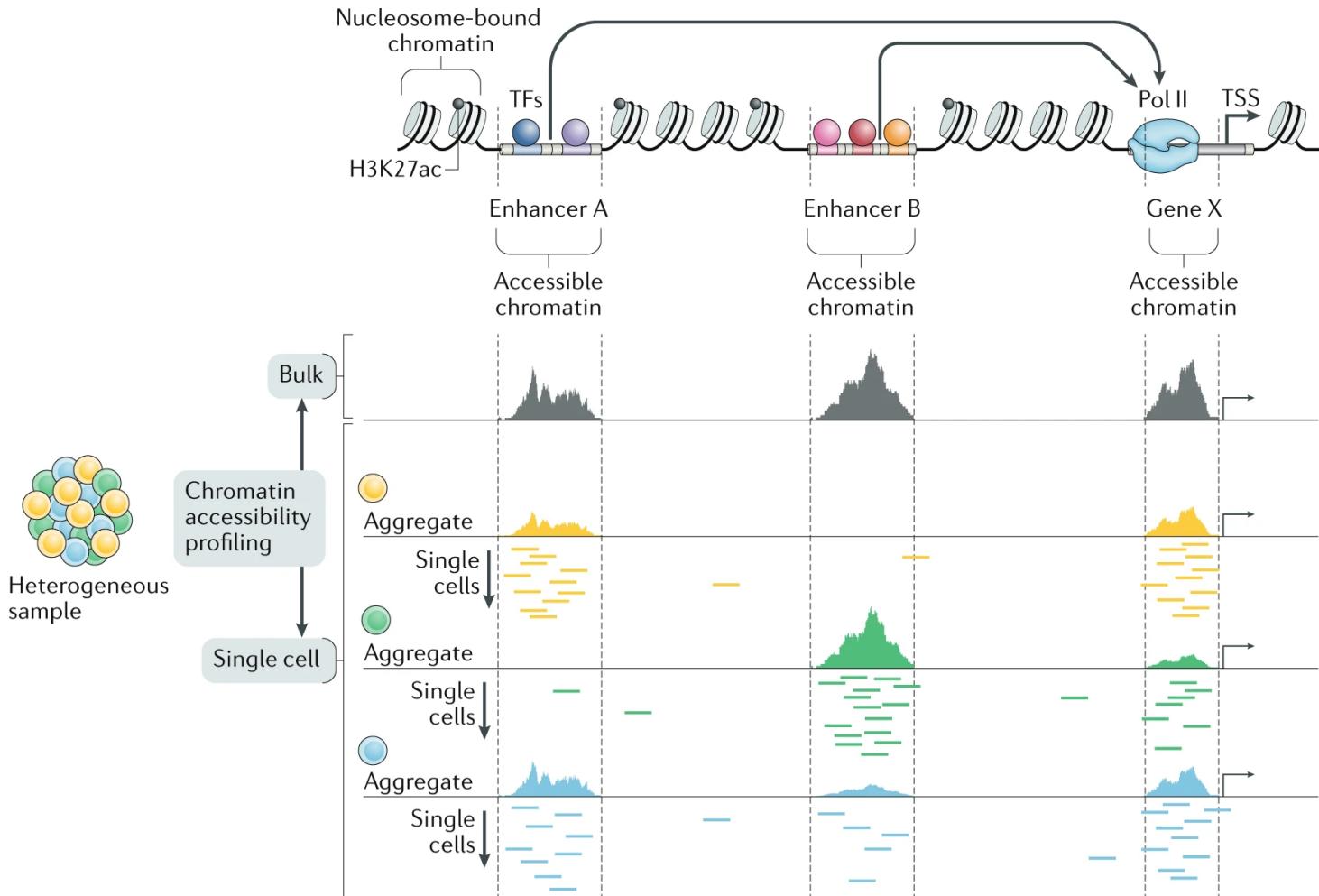
# Example scRNA-seq: SMART-seq3



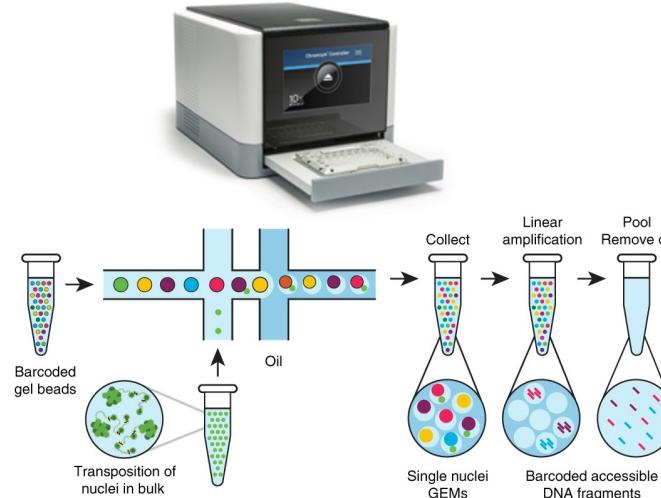
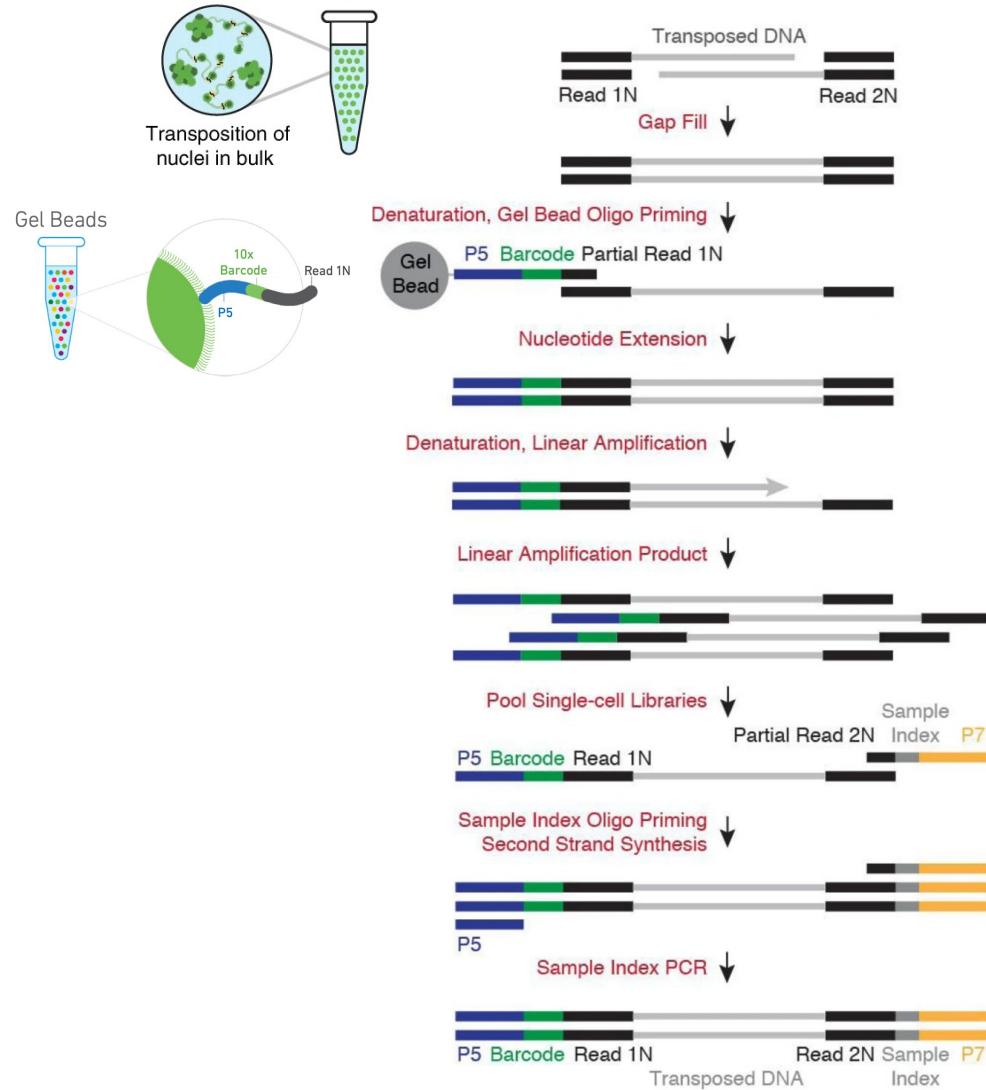
# scRNA-sequencing protocol examples



# 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)

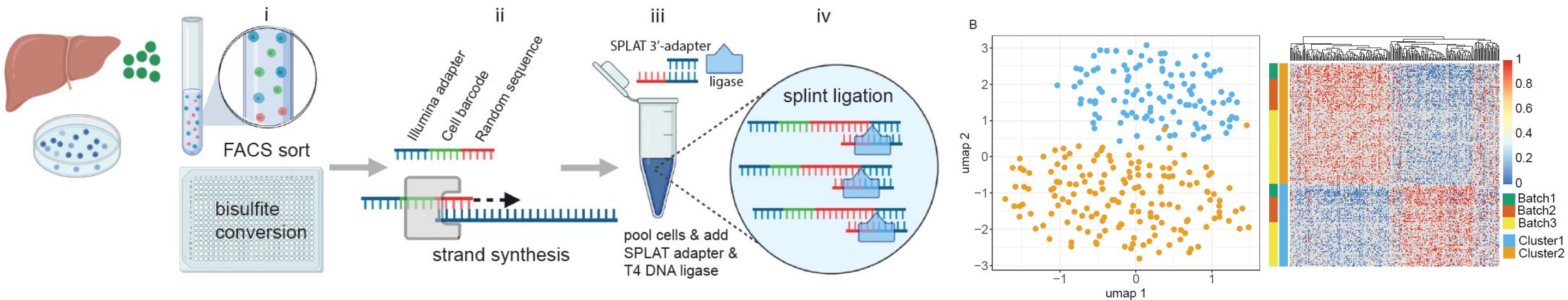
# DNA methylation analysis in single cells



Article | Open Access | Published: 06 April 2022

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

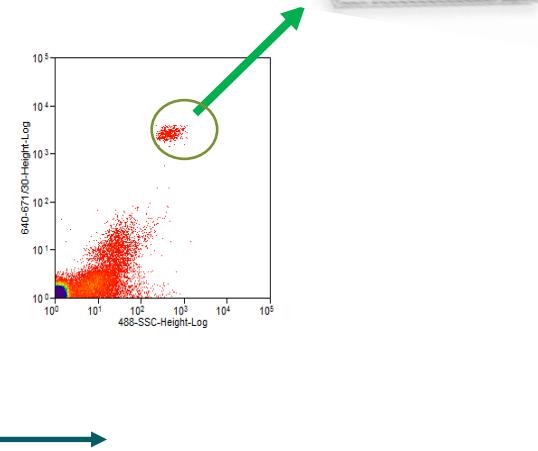
Amanda Raine , Anders Lundmark, Alva Annett, Ann-Christin Wiman, Marco Cavalli, Claes Wadelius,  
Claudia Bergin & Jessica Nordlund



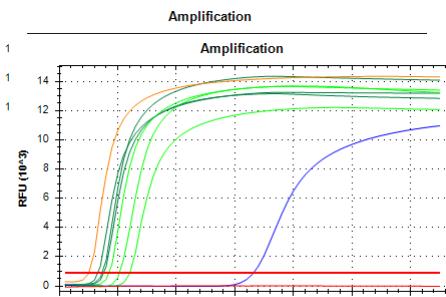
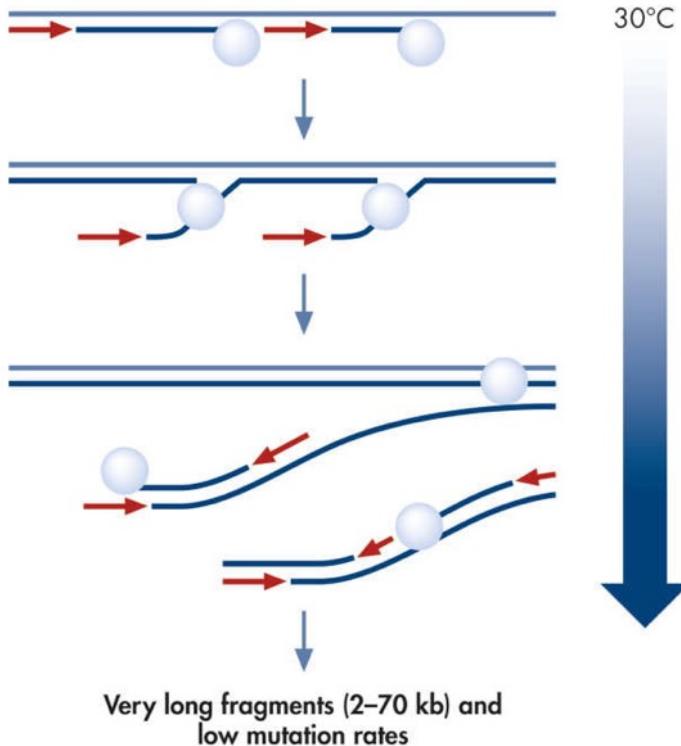
Method validation completed. Open for collaborative projects, contact [seq@medsci.uu.se](mailto:seq@medsci.uu.se).

# MDA – Whole genome DNA seq

## Cell sorting

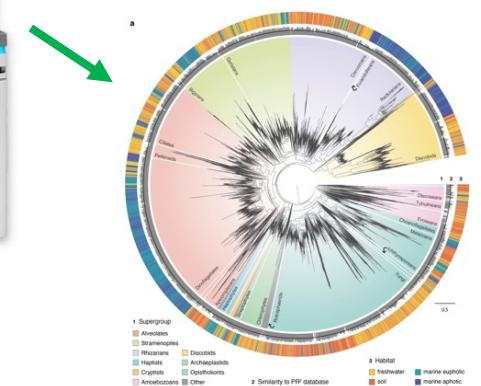


## MDA



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

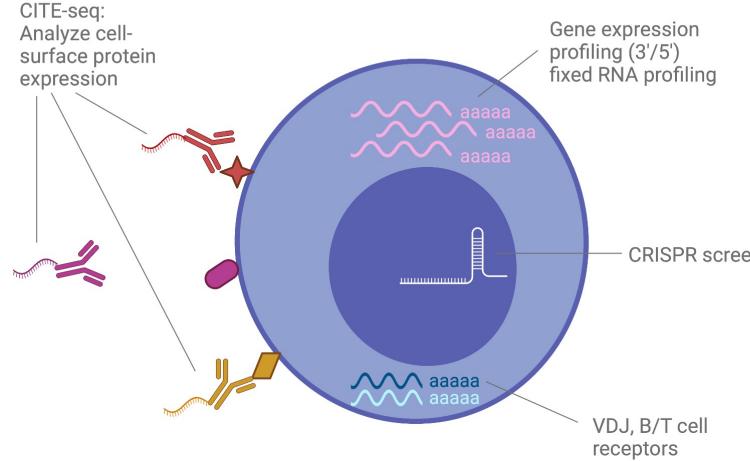
NGS Sequencing



# Single Cell Seq at NGI



## Gene Expression



## Epigenetics

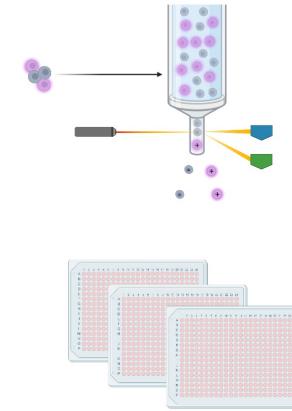


- 10x Genomics
- scATAC-seq
  - scATAC + GEX



- scWBGS (SPLAT)
- Raine et al, 2022

## DNA-sequencing



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



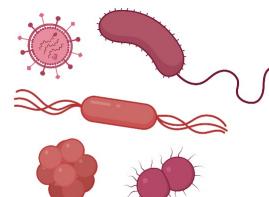
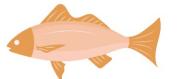
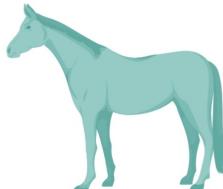
Includes services of  
ESCG and MSCG,  
now merged with  
NGI.

10x Genomics

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

Smart-seq 2/3

Drop-seq  
(Dolomite Nadia)



### 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)

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[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](mailto:support@ngisweden.se)

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

<https://ngisweden.scilifelab.se/>