

scSeq methodologies and NGI services

Henrik Gezelius

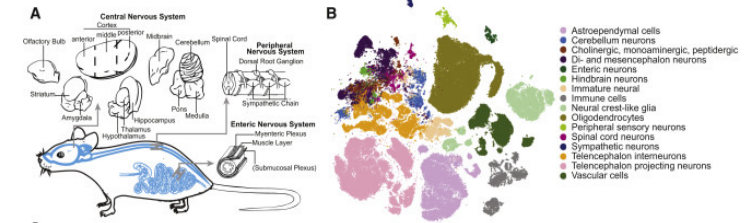
Acting head of Single Cell Genomics team Uppsala

2023-01-30

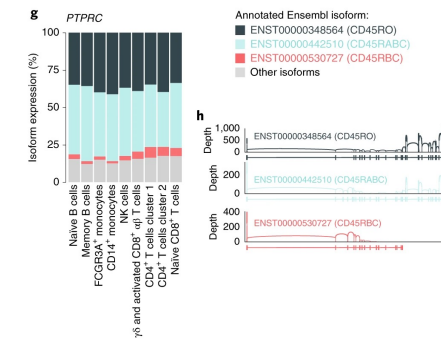
Applications for scRNA-sequencing



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

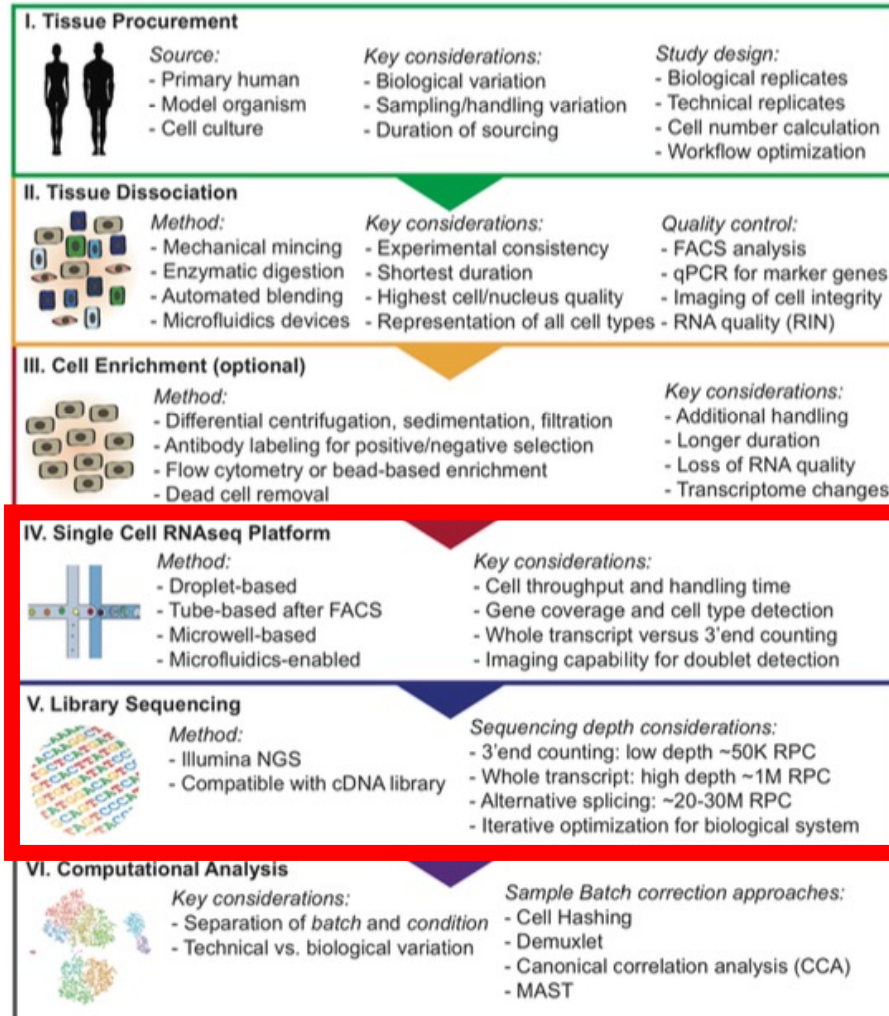


Zeisel et al, Cell 2018



Hagemann-Jensen Nat Biotech 2020

Single cell RNA-seq workflow



Verify your results with orthogonal method!

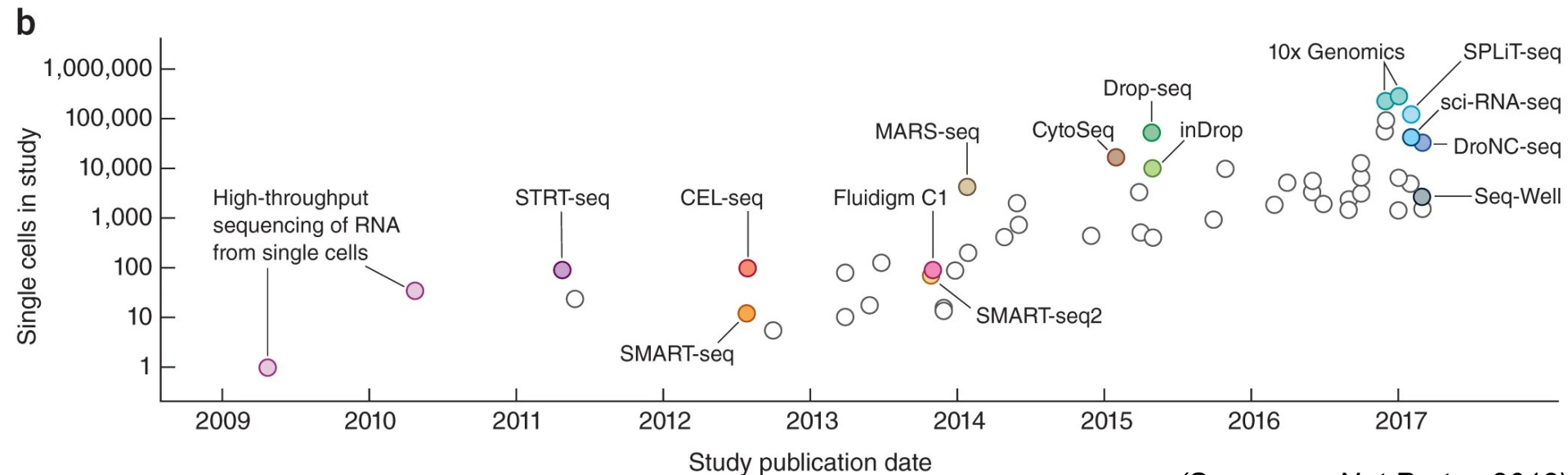
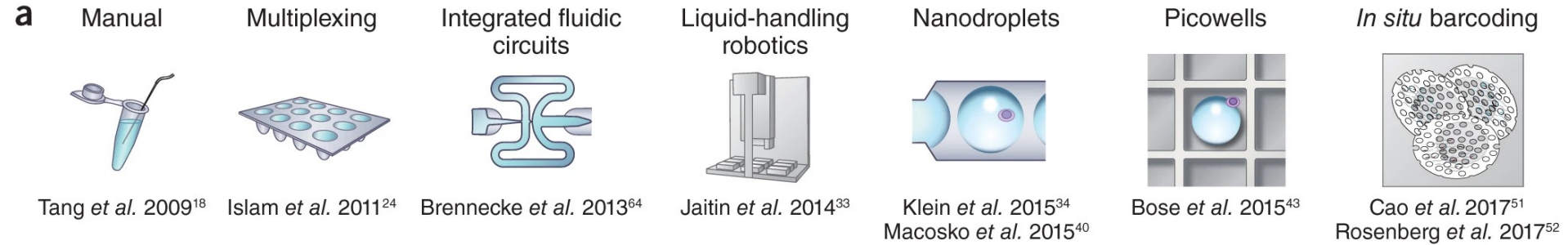


	Omics type	Read out		Complexity (number of targets)	Sample requirements			Spatial resolution
		NGS	Imaging		Fresh-frozen	FFPE	TMA	
Spatial transcriptomics (10X Visium)	RNA	✓	✓	Unbiased transcriptome-wide	✓	(✓)	✗	Anatomical features of 55 µm
In situ sequencing	RNA	✗	✓	200-300	✓	✓	✓	Subcellular
Spatial proteomics (Codex)	Protein	✗	✓	40	✓	✓	✓	Subcellular
Advanced FISH technologies (smFISH)	DNA/RNA	✗	✓	6	✓	✓	✓	Subcellular
Spatial Mass Spectrometry	Small molecules	✗	✓	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**



low number of cells
any tissue

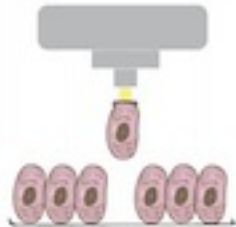
enables selection of cells based on morphology or fluorescent markers

visualisation of cells

time consuming

reaction in microliter volumes

**LASER CAPTURE
MICRODISSECTION**



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

FACS



hundreds of cells
dissociated cells

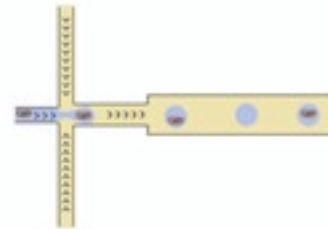
enables selection of cells based on size or fluorescent markers

fluorescence and light scattering measurements

fast

reaction in microliter volumes

MICRODROPLETS



large number of cells
dissociated cells

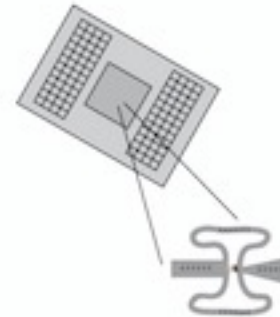
no selection of cells (can presort with FACS)

no visualisation

fast

reaction in nanoliter volumes

**MICROFLUIDICS
e.g. FLUIDIGM C1**



hundreds of cells
dissociated cells

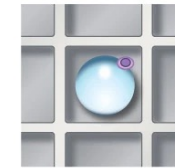
no selection of cells (can presort with FACS)

visualisation of cells

fast

reaction in nanoliter volumes

Picowells



Bose et al. 2015⁴³

large number of cells
dissociated cells

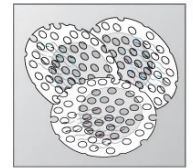
no selection

no visualisation

fast

Multi-plexed
Pooled reactions

In situ barcoding



Cao et al. 2017⁵¹
Rosenberg et al. 2017⁵²

huge number of cells
dissociated cells

no selection

no visualisation

fast

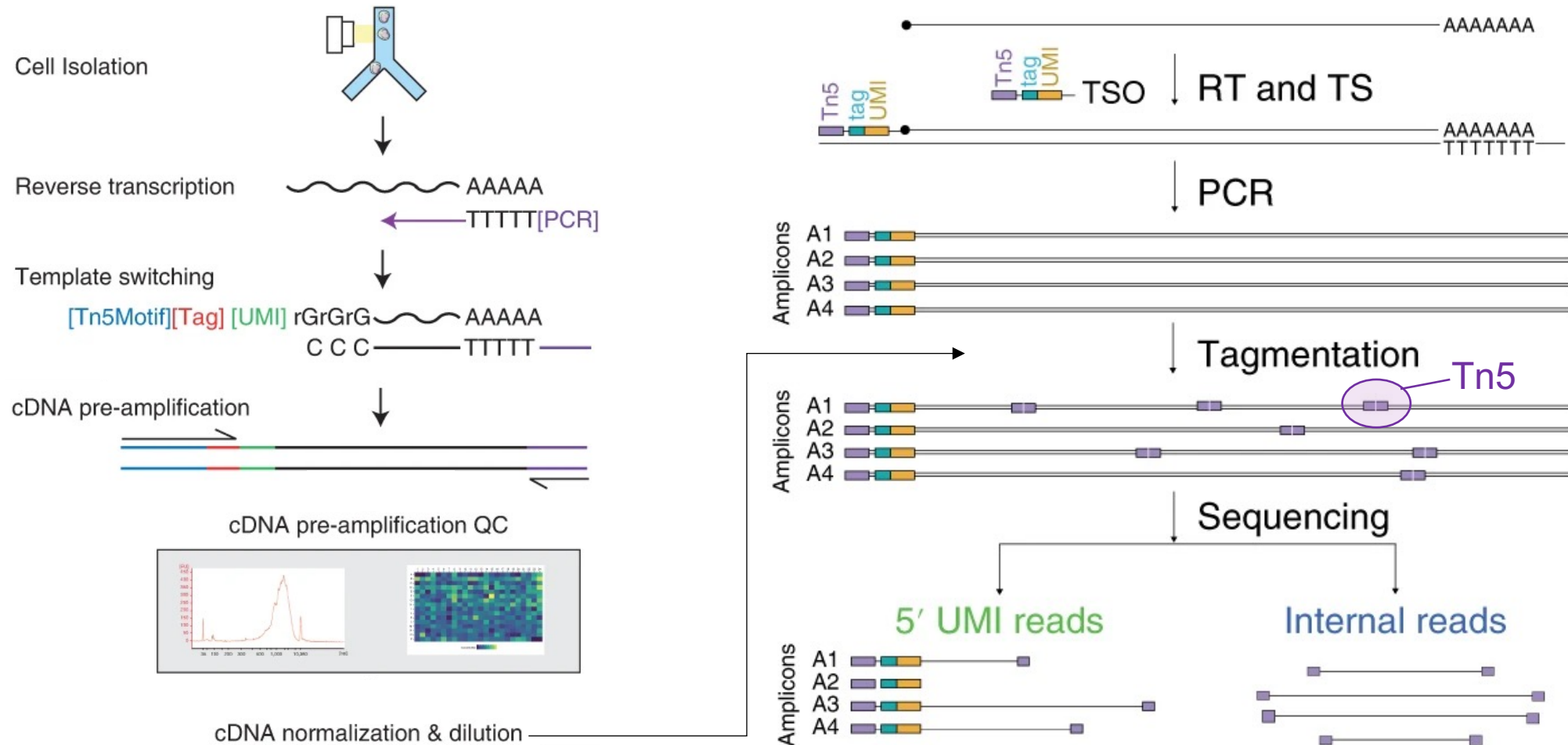
Multi-plexed
Pooled reactions

- Cytoplasmic aspiration
- Patch-seq

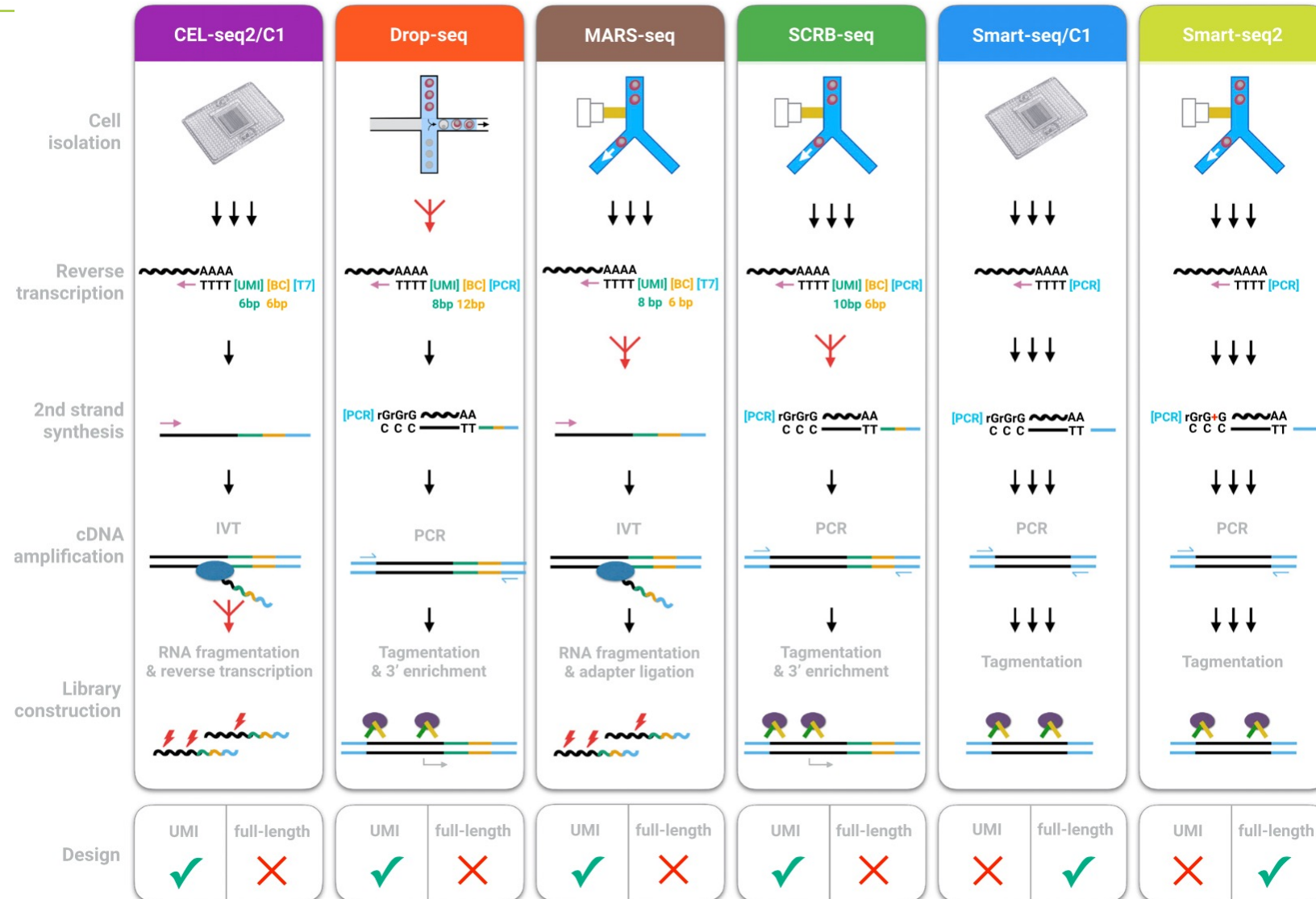
Adapted from: Kolodziejczyk A et al, *Molecular Cell*, 2015

(Adapted from: Svensson, *Nat Protoc* 2018)

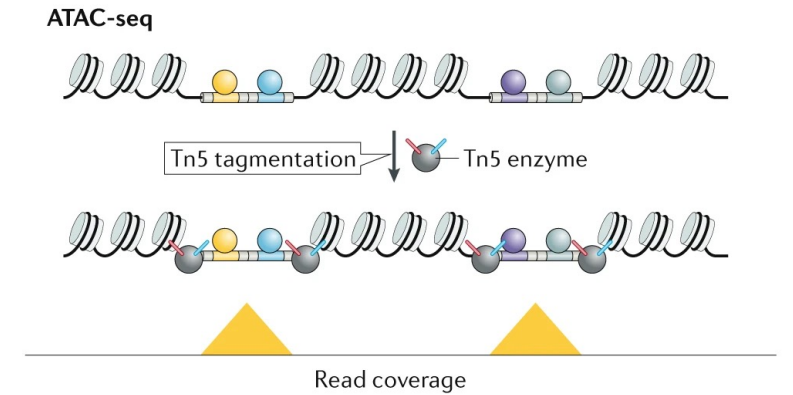
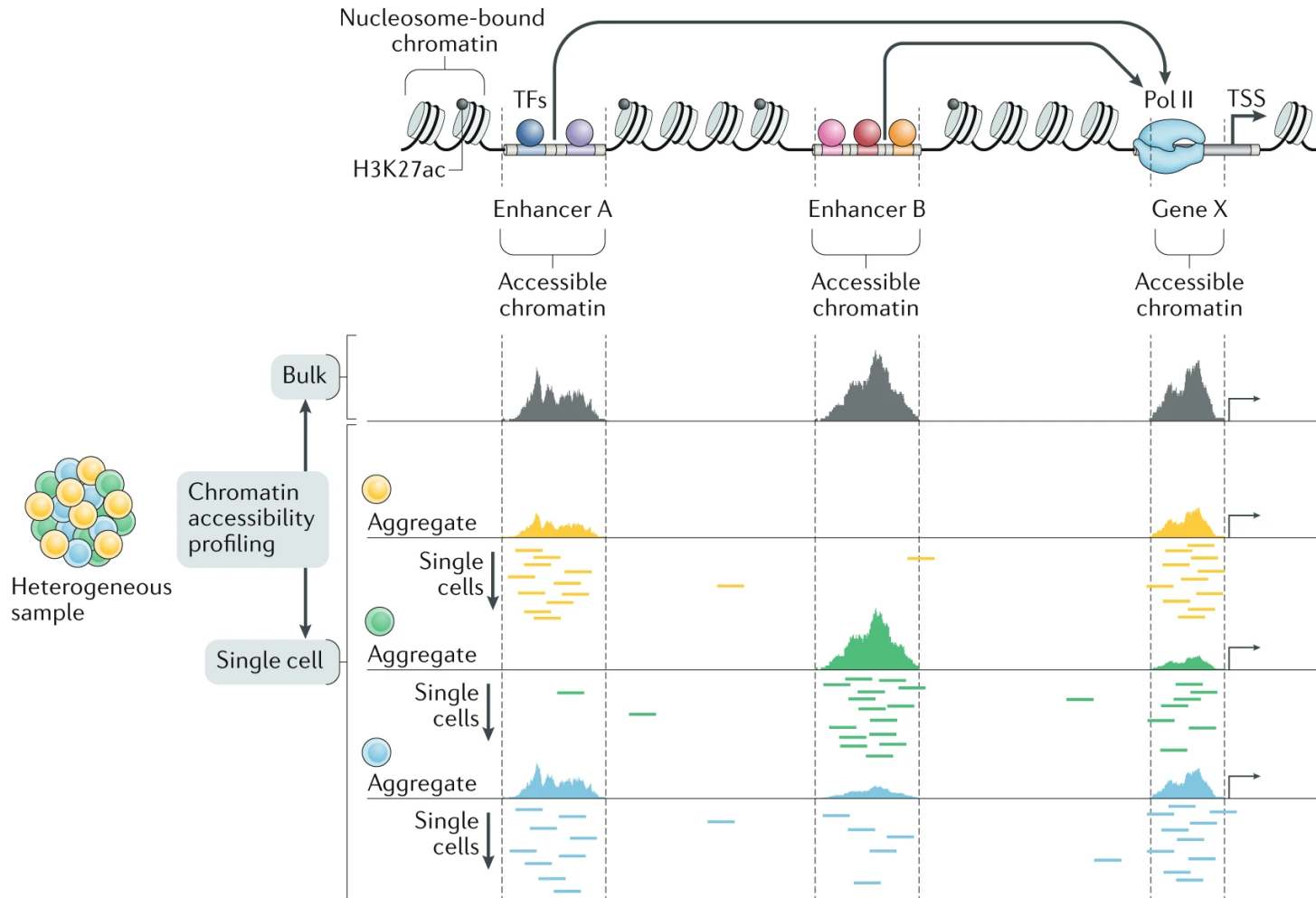
Example scRNA-seq: SMART-seq3



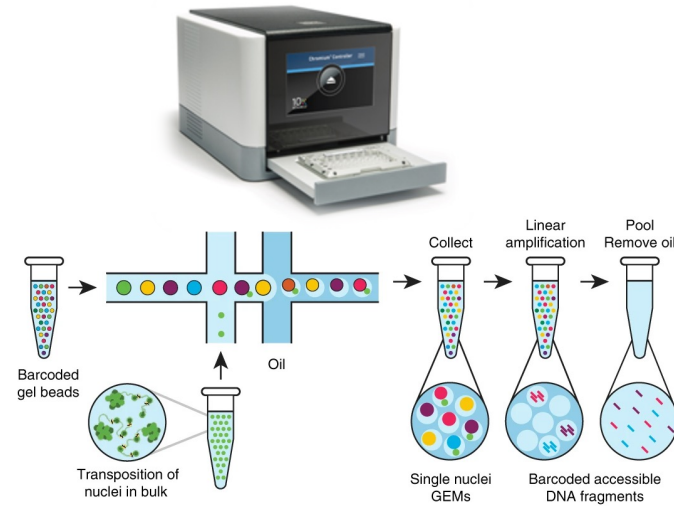
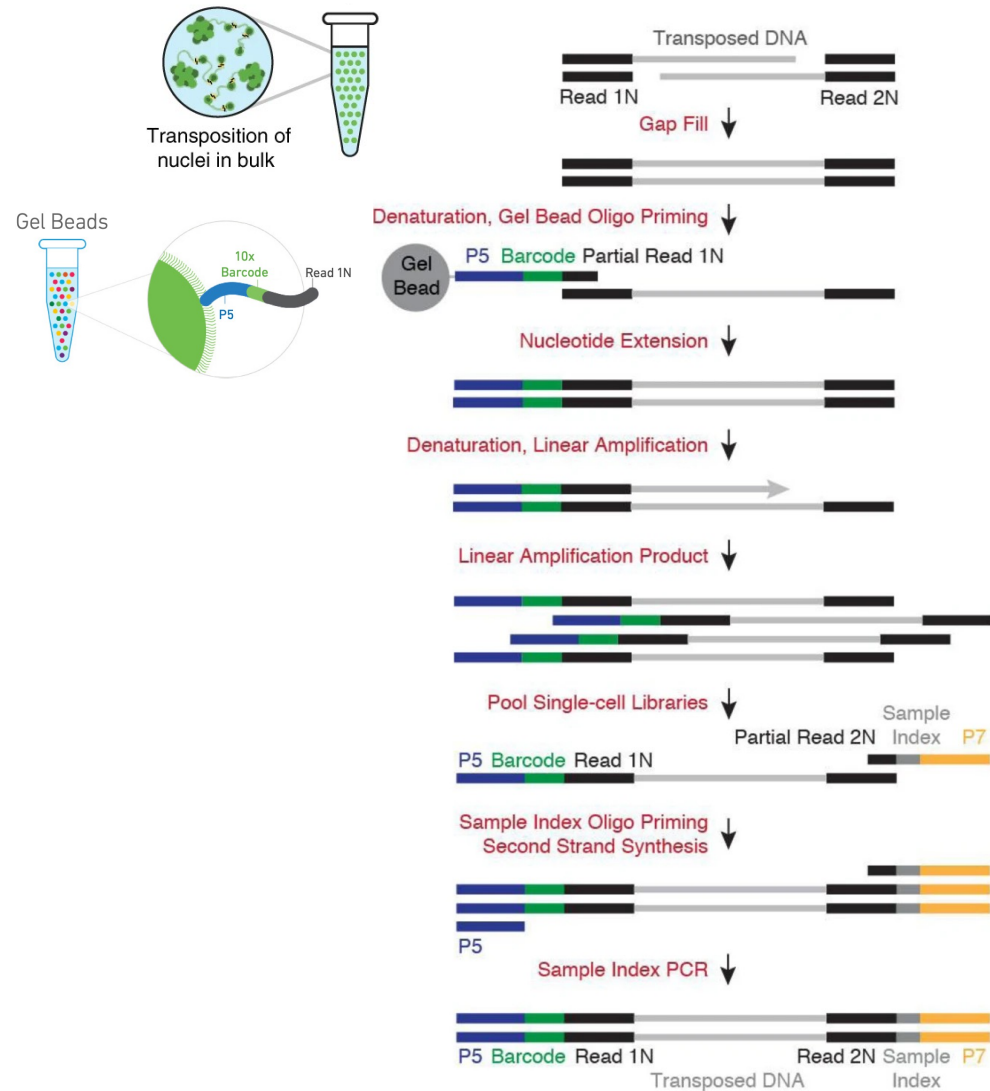
scRNA-sequencing protocol examples



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

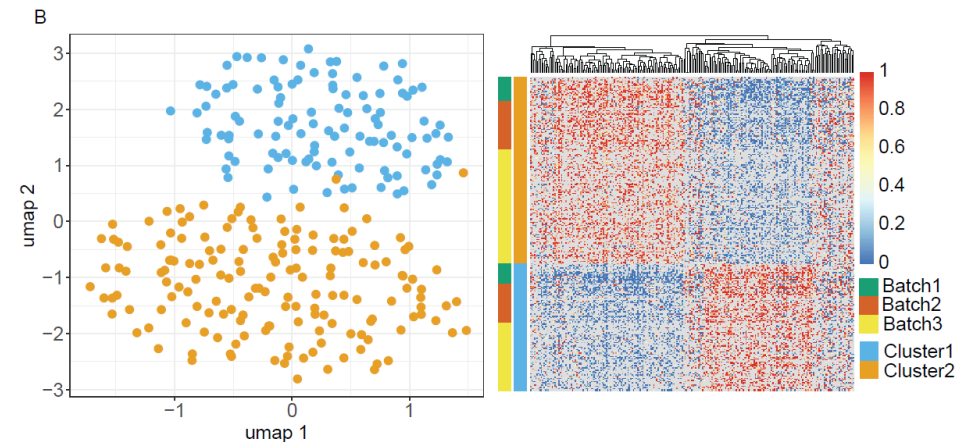
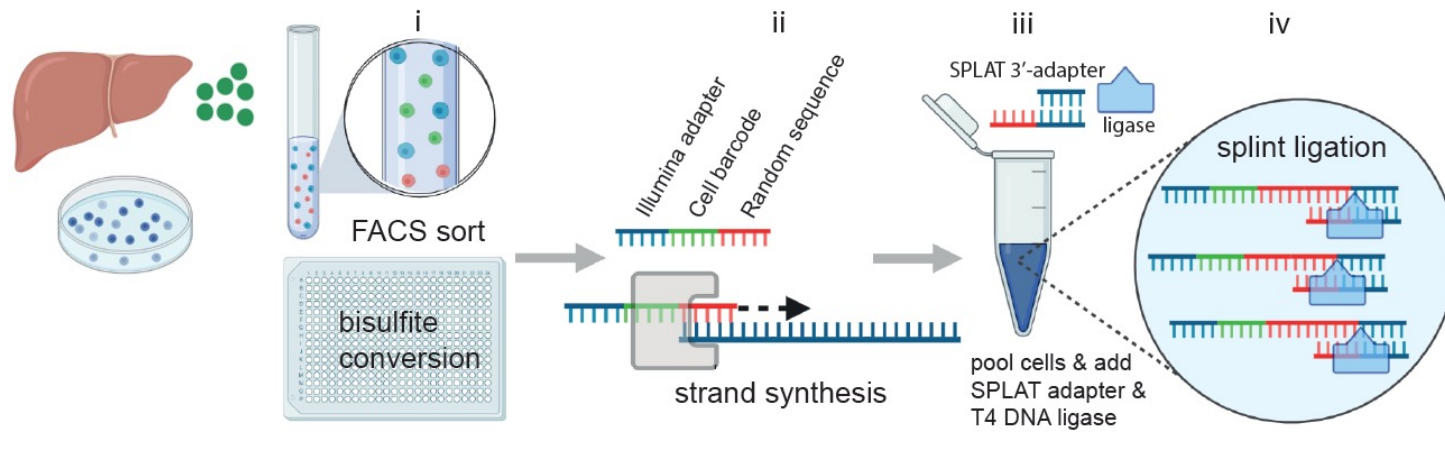


Article | [Open Access](#) | [Published: 06 April 2022](#)

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](#),

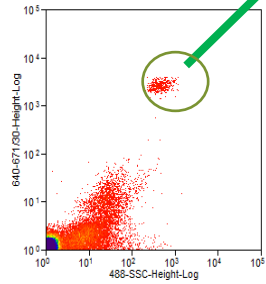
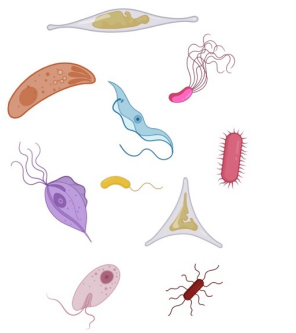
[Claudia Bergin](#) & [Jessica Nordlund](#)



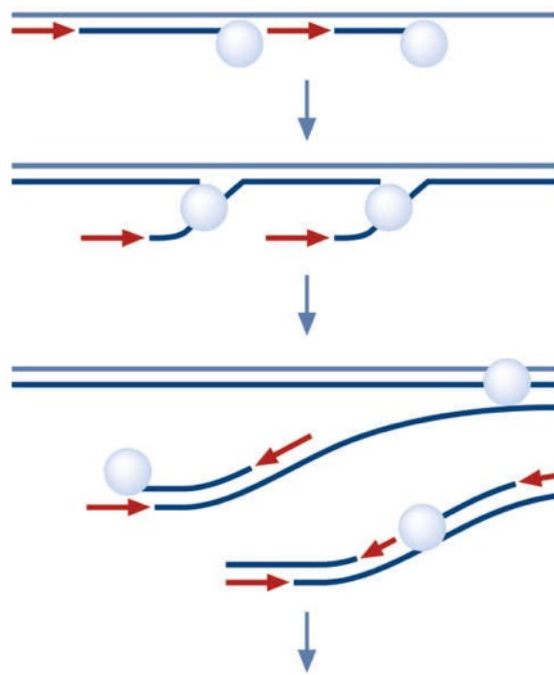
Method validation completed. Open for collaborative projects, contact seq@medsci.uu.se.

MDA – Whole genome DNA seq

Cell sorting

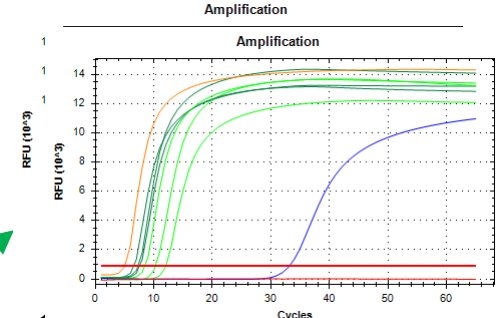


MDA



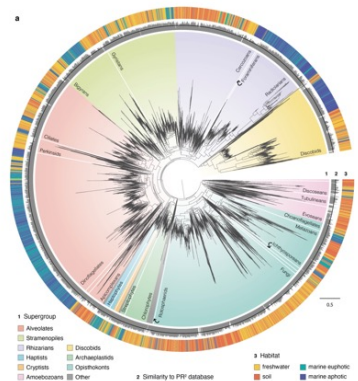
30°C

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



1. SCREENING, select wells
2. Identification
3. Library prep

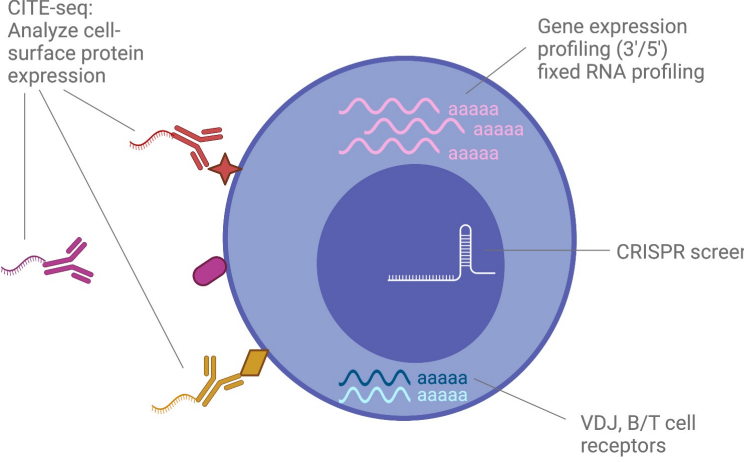
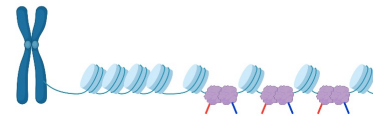

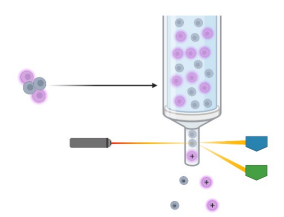
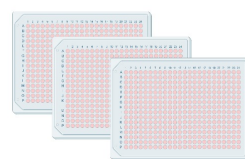
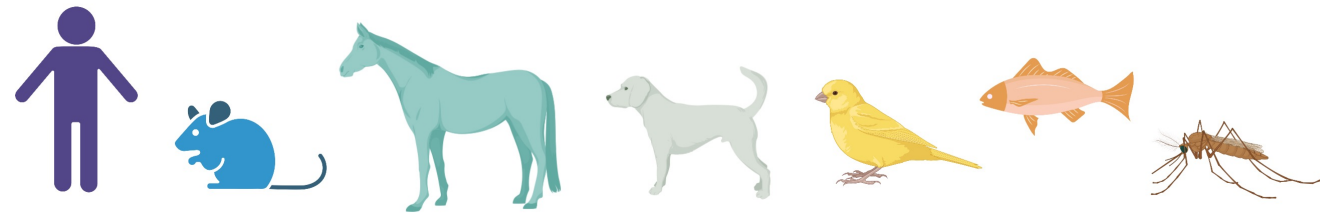

NGS Sequencing



Legend for phylogenetic tree:
1 Supergroup
2 Ancestors
3 Stramenopiles
4 Rhizaria
5 Haptophyta
6 Cryptophytes
7 Amoebozoans
8 Other
9 Chlorobionts
10 Archaeplastids
11 Opisthokonta
12 Other
13 Habitat
14 Freshwater
15 Marine aquatic
16 Soil
17 Marine aphotic
18 Similarity to PP database

Single Cell Seq at NGI



Gene Expression	Epigenetics	DNA-sequencing
<p>CITE-seq: Analyze cell-surface protein expression</p>  <p>Gene expression profiling (3'/5') fixed RNA profiling</p> <p>CRISPR screen</p> <p>VDJ, B/T cell receptors</p>	 <p>10x Genomics</p> <ul style="list-style-type: none"> • scATAC-seq • scATAC + GEX  <p>scWBGS (SPLAT)</p> <ul style="list-style-type: none"> • Raine et al, 2022 	  <p>FACS sorting combined with cell lysis and genome amplification (MDA)</p>
<p>10x Genomics</p> <ul style="list-style-type: none"> • 3'/5' GEX • CITE-seq • Cell hashing • VDJ • CRISPR screen • fixed RNA profiling <p>Smart-seq 2/3</p> <p>Drop-seq (Dolomite Nadia)</p> 		 <p>BSL-2</p> <p>BSL-3</p>

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/>