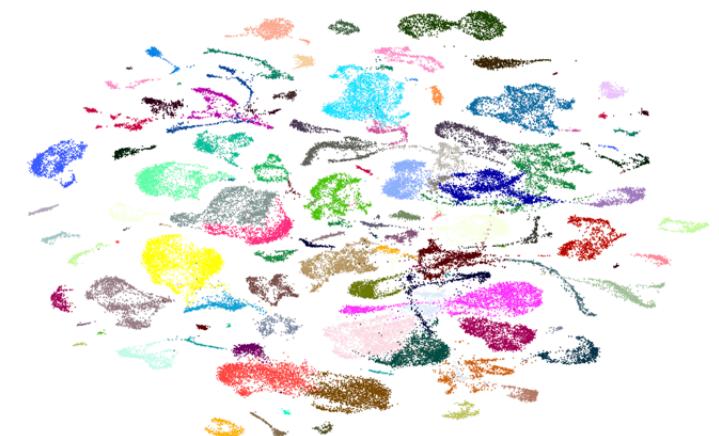


# Single-cell methodologies and the ESCG facility

**SciLifeLab**

Karolina Wallenborg  
January 25, 2021

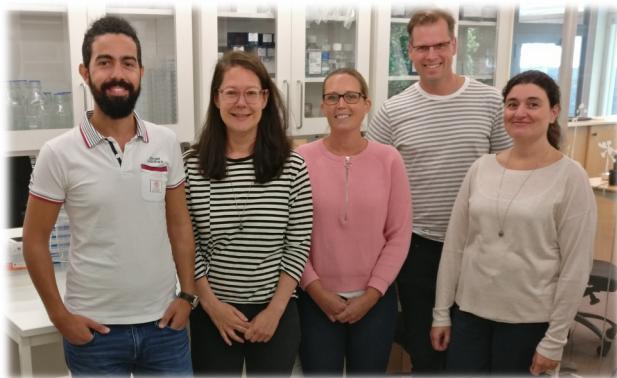


# Eukaryotic Single-cell Genomics (ESCG)

- Established in 2015 with funding from SciLifeLab
- 2021
  - 6 personnel



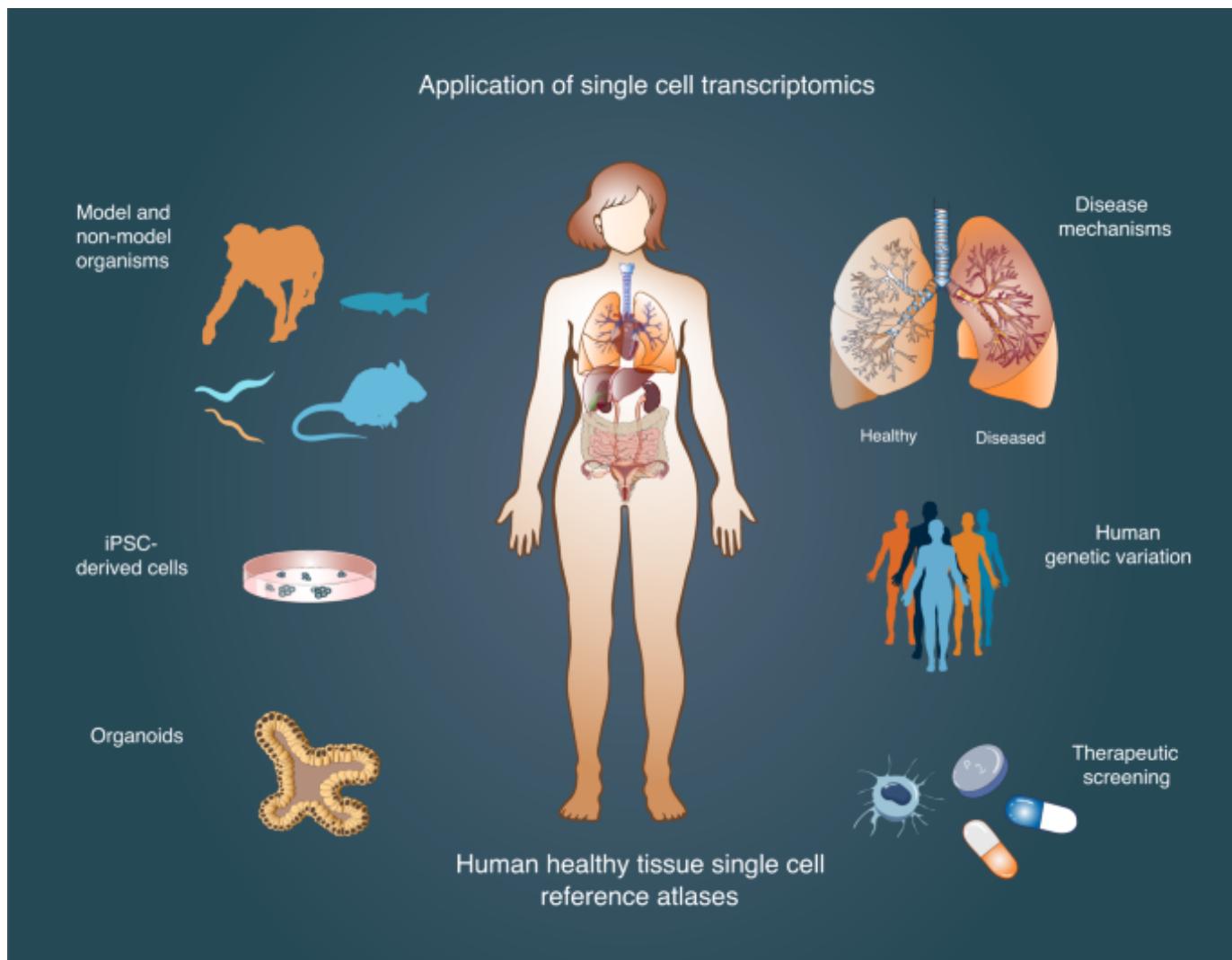
Rickard Sandberg Sten Linnarsson



Anastasios, Karolina, Matilda, Henrik, Marcela, Samaneh & Helena



Fluidigm C1 Auto-prep single cell system



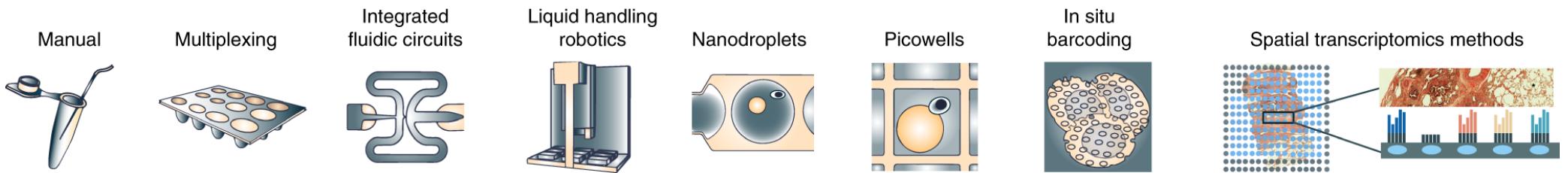
Aldridge & Teichmann, Nature Communication, 2020

# Single cell RNA-sequencing experiment

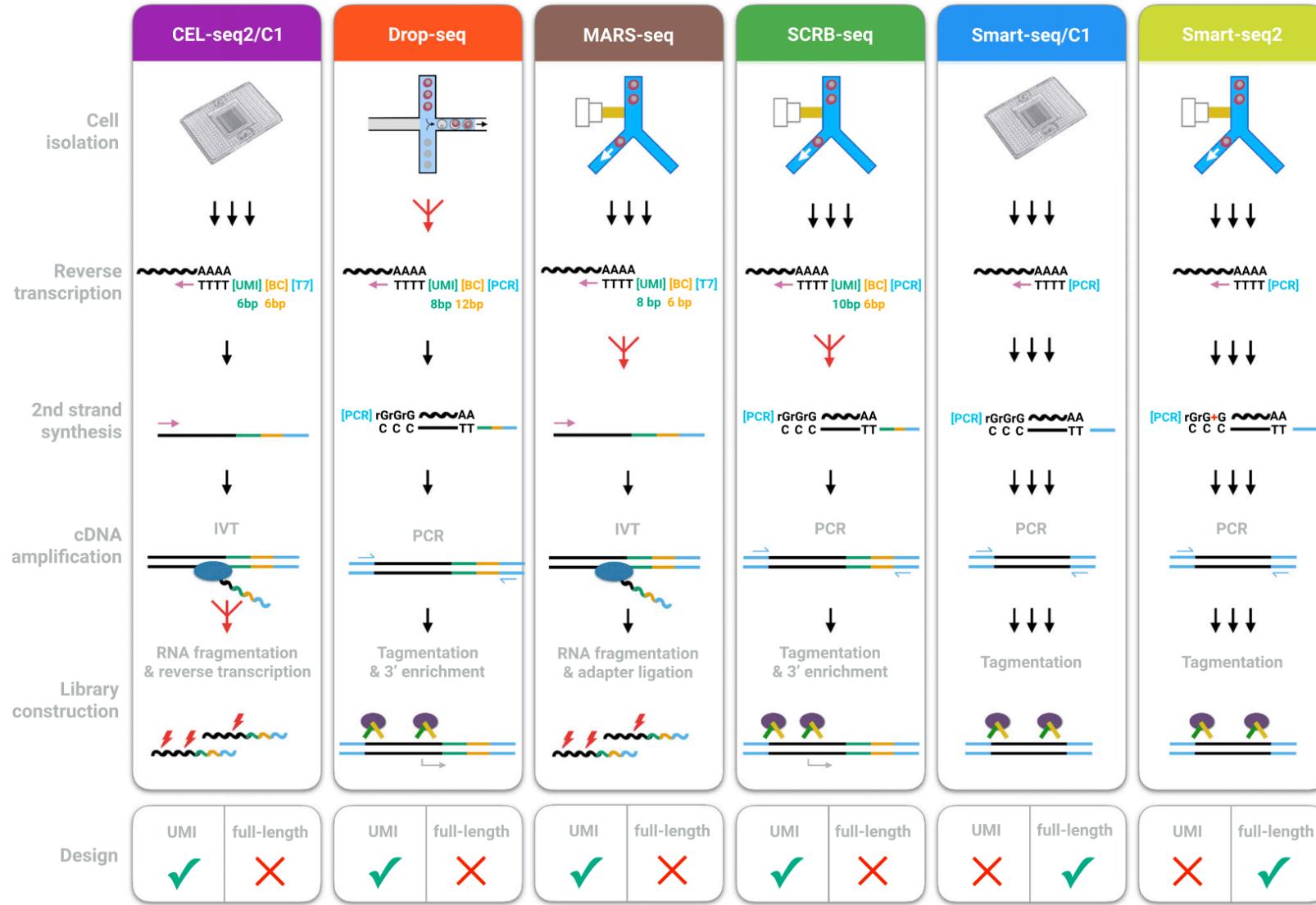


- User Consultations
  - Experimental design
- Quality control of single-cell preparations prior to the experiment
  - Sample submission guidelines for cells/nuclei for different applications
- Initial bioinformatics

# Single-cell isolation and library preparation



Aldridge & Teichmann, Nature Communications, 2020



RNA molecule capture and reverse transcription

Amplification

Library preparation

3' -tag

5'- tag

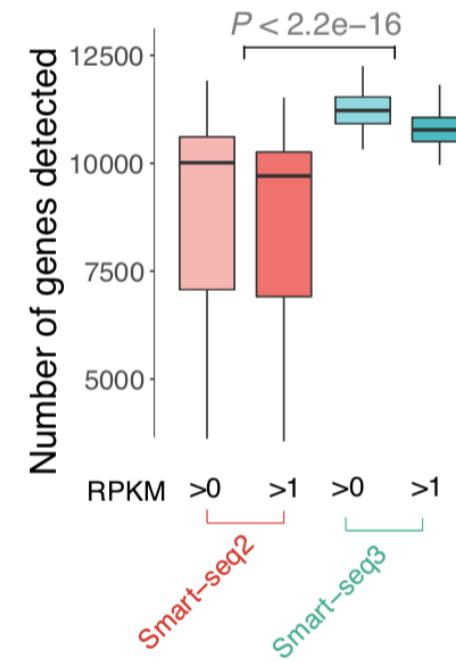
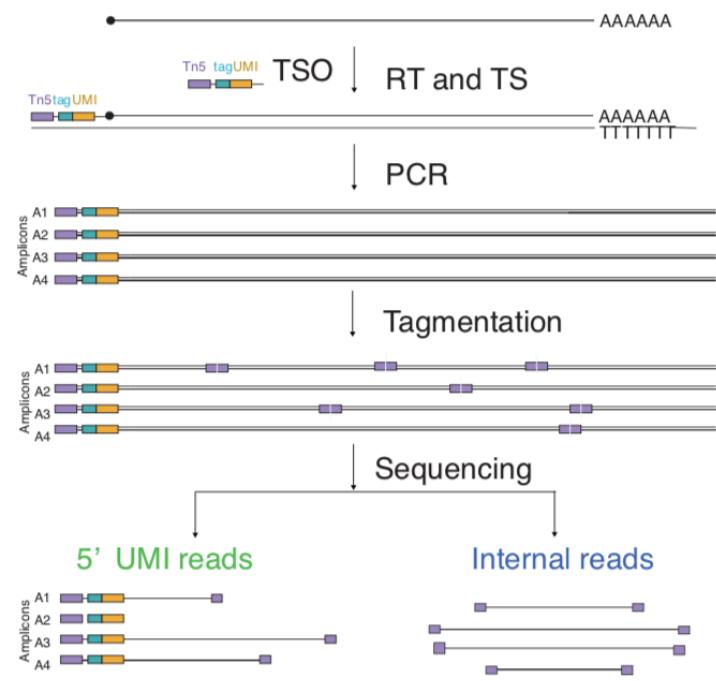
Full-length

Adapted from  
Kolodziejczyk A et al, Molecular Cell, 2015

# Full-length vs 3' or 5'-end transcript sequencing

- Full-length
  - Whole transcript information
  - Gene expression quantification
  - Isoform, SNPs, and mutations
  - Higher sensitivity
- Tag-based methods
  - Estimate of transcript abundance
  - Early multiplexing (lower cost)
  - Combined with molecular counting
  - Retain DNA strand information
  - Higher throughput

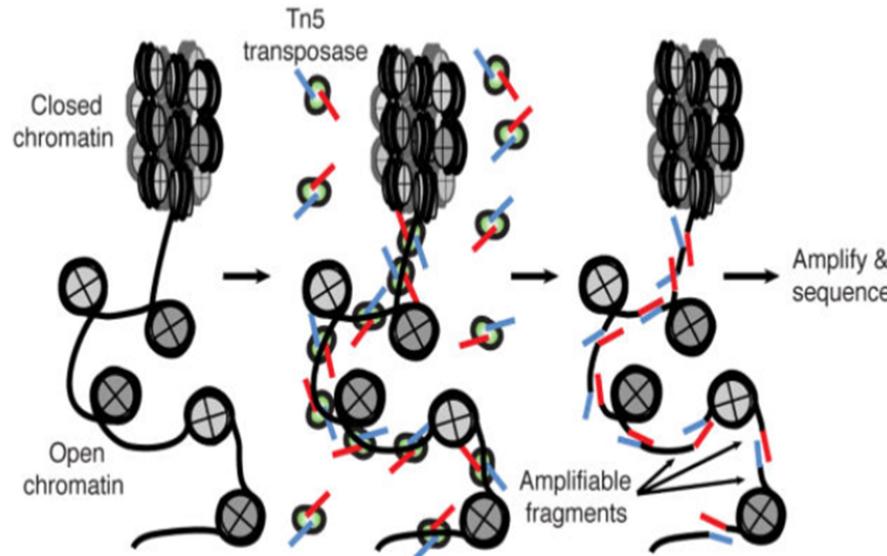
# Full-length transcriptome coverage + 5' UMI RNA counting with Smart-seq3



- Highly sensitive (80% of molecules detected by smRNA-FISH per cell)

Hagemann-Jensen et al , Nature Biotechnology, 2020

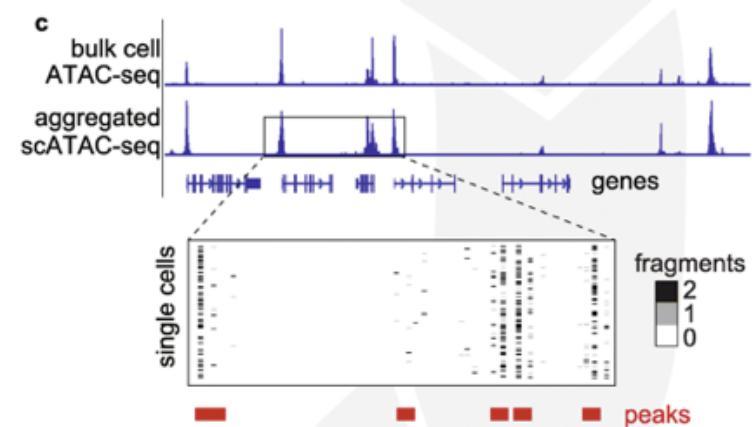
# Single-cell ATAC-sequencing (Assay for Transposase Accessible Chromatin)



(Buenrostro et al, Nature Methods, 2013)

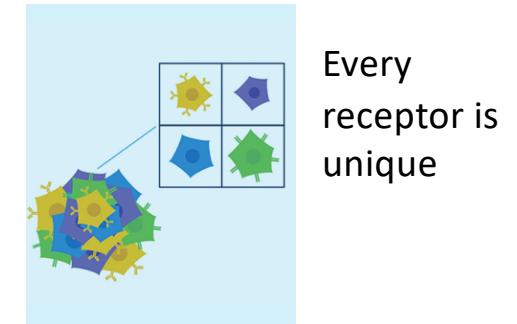
Chromatin and DNA-binding proteins regulate gene expression

- Subpopulations with different chromatin accessibility profiles  
→ Increased understanding of gene regulatory networks upstream on gene expression

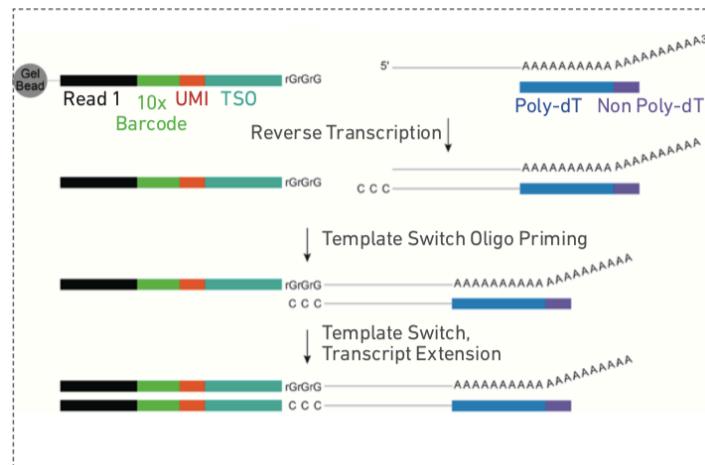


Adapted from Chen et al, Genome Biology, 2019

# Single-cell Immune Profiling, V(D)J



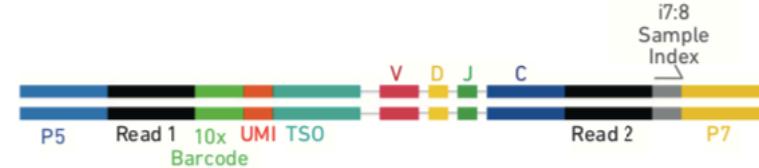
Example from 10XGenomics protocol



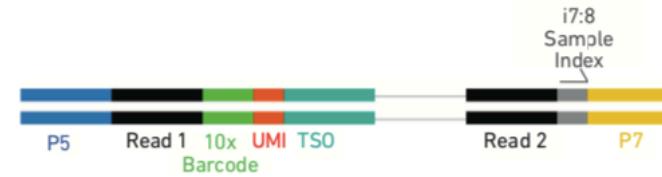
cDNA amplification

Target enrichment with specific primers  
for TCR or Ig constant regions

Chromium Single Cell V(D)J Enriched Library



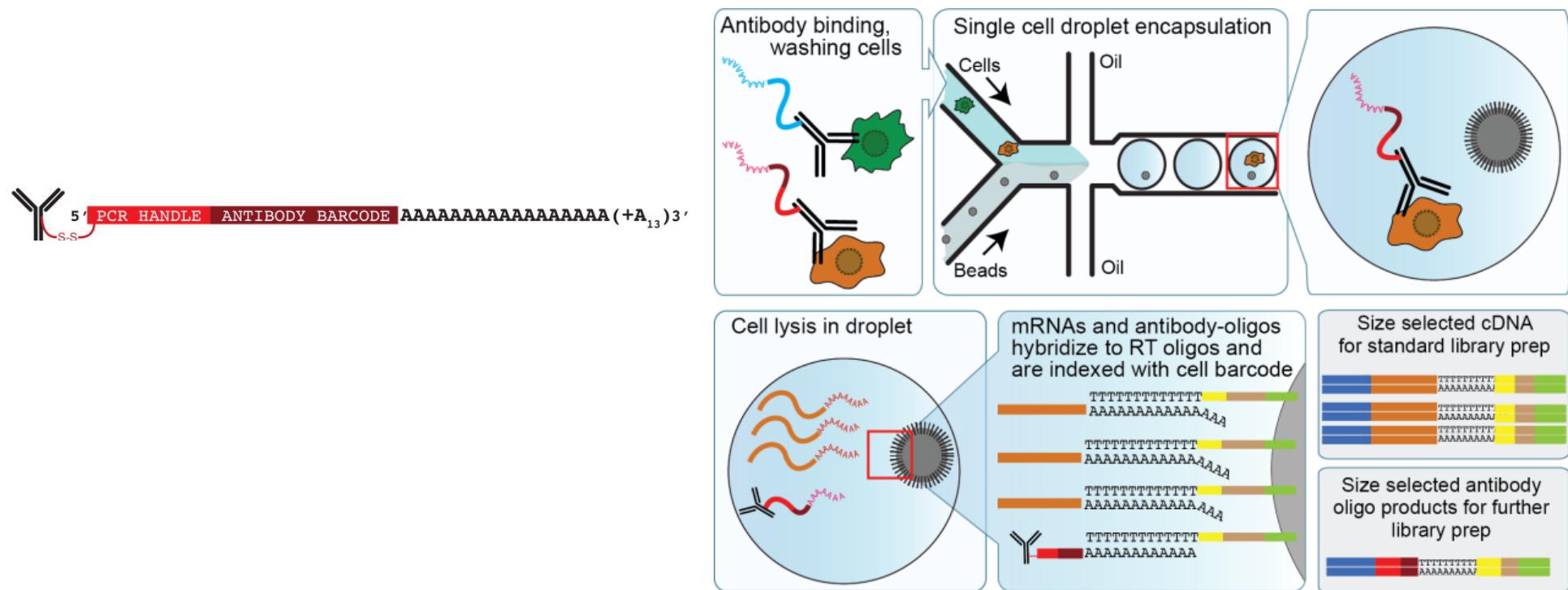
Chromium Single Cell 5' Gene Expression Library



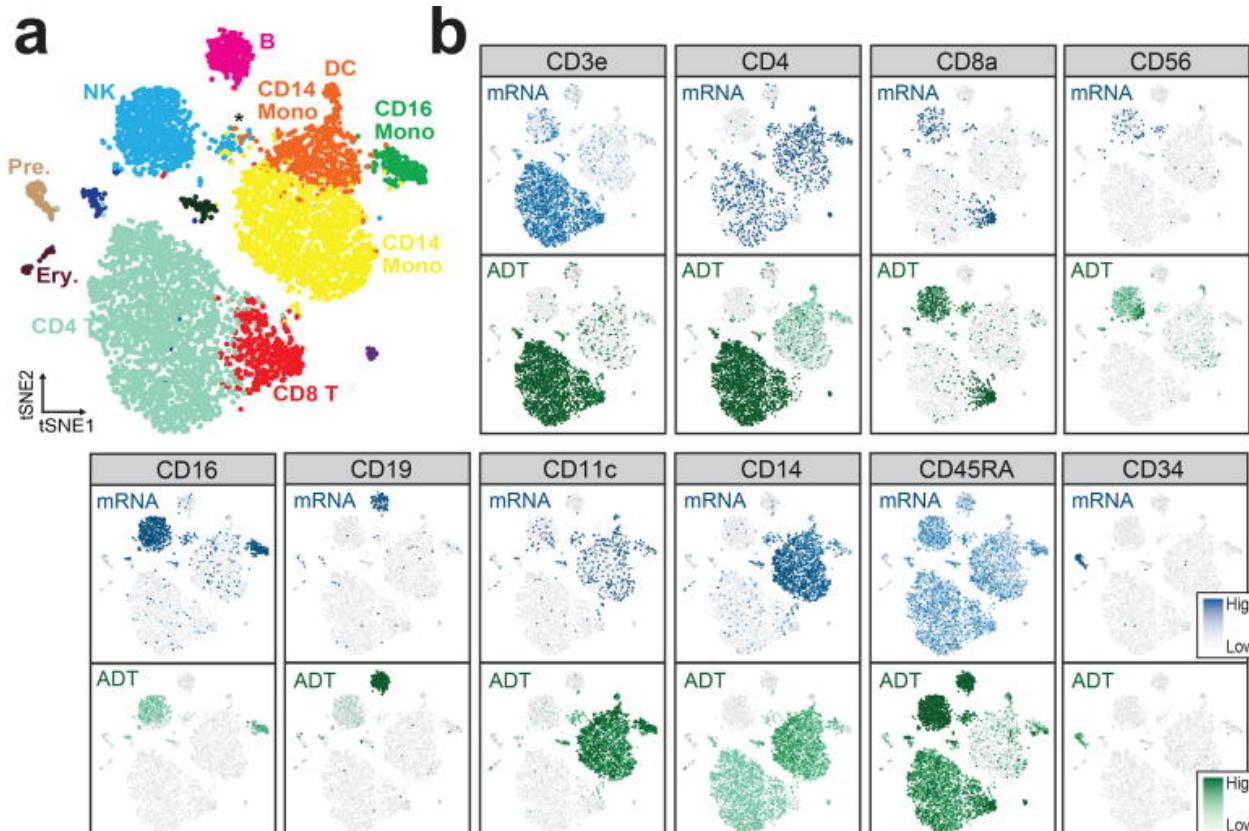
# CITE-seq

## simultaneously measure gene expression and cell surface protein abundance

a



Adapted from Stoeckius et al., 2017

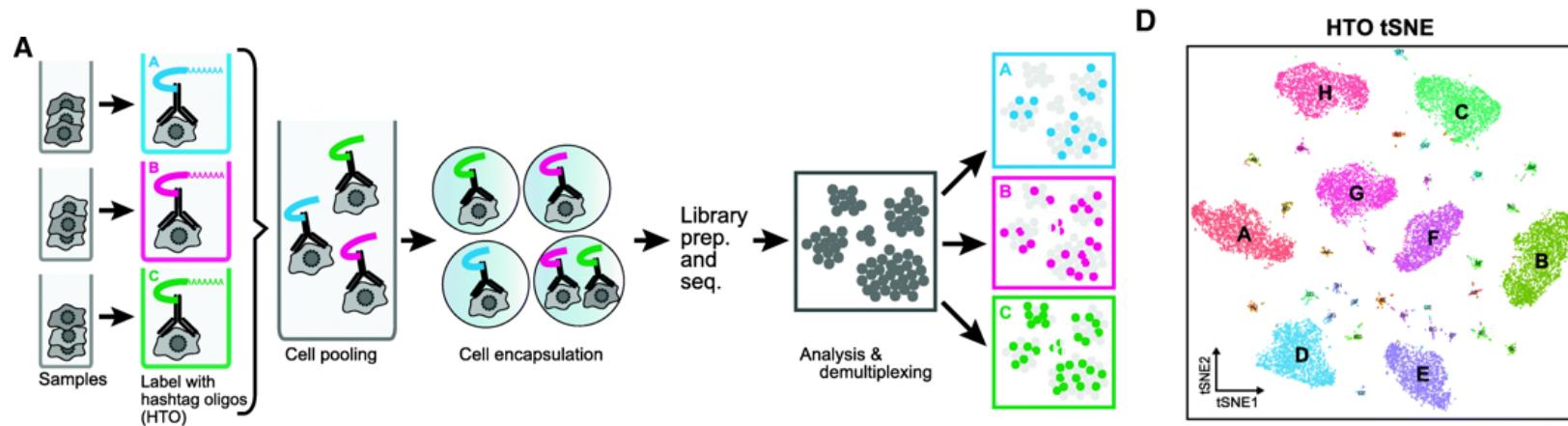


Antibody panels can  
be purchased from  
Biolegend

Stoeckius et al., 2017

→ Integrated protein and RNA measurements enhance cellular phenotyping

# Cell hashing – multiplexing with ubiquitous surface protein expression

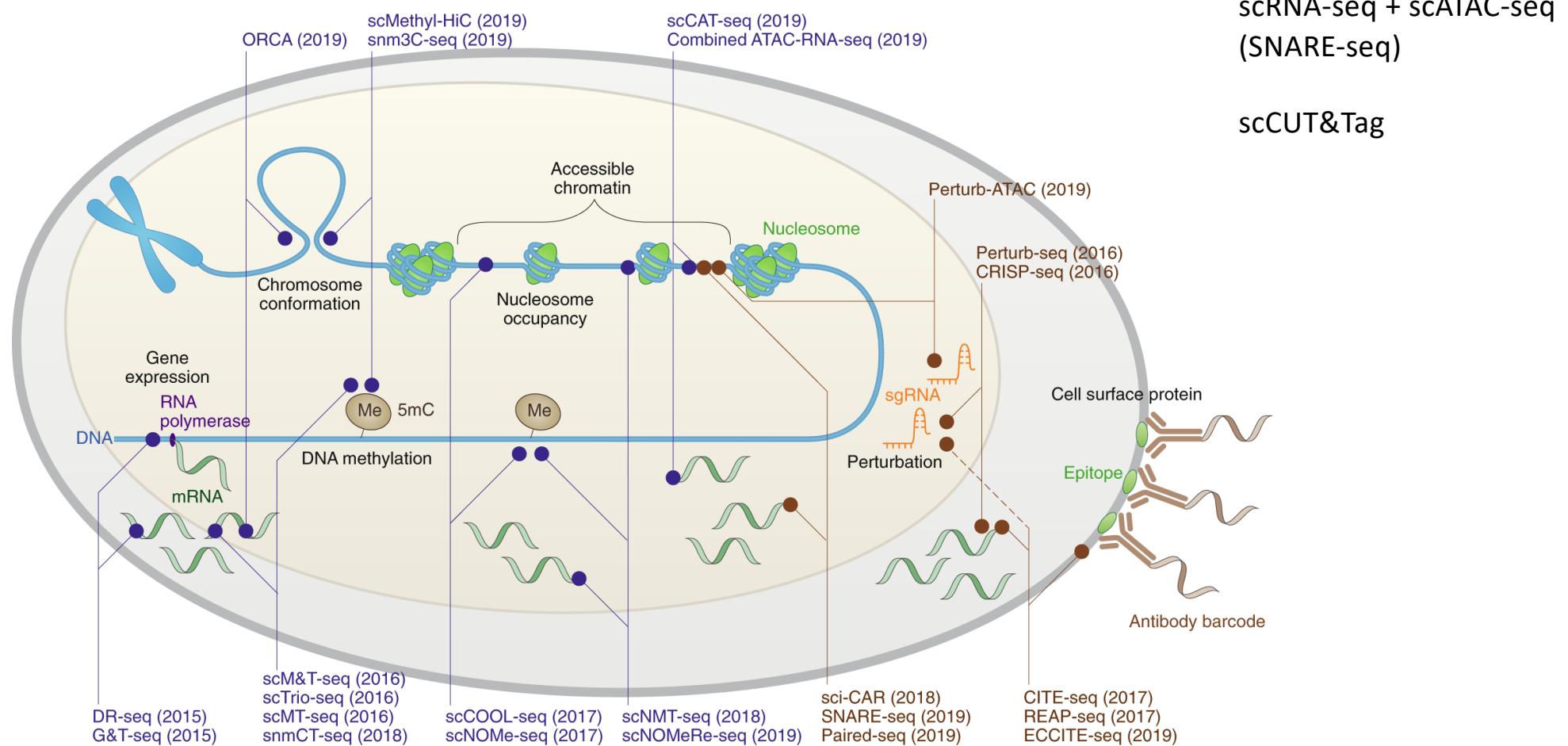


- Cell hashing, Stoeckius, Zheng et al., 2017
- Sample multiplexing
- Identify doublets, superload
- Batch effects
- Save on costs

# Sample multiplexing

- Demuxlet (genetic variation), Kang et al, Nature Biotech, 2018
- MULTI-seq, McGinnis et al Nature Methods, 2019
  - Lipid-tagged indices
- Cell hashing, Stoeckius et al Genome Biology, 2018
- Nuclei multiplexing with barcoded antibodies
- CellTag indexing, Guo et al, Genome Biology, 2019
- Multiplexing by transient barcoding, Shin et al Science Advances, 2019

# Single-cell multimodal omics



Zhu et al, Comment in Nature Methods, 2020

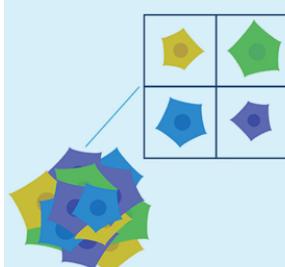
# Services at ESCG

- Single-cell RNAseq
- Single-nucleus RNAseq
- Single-cell ATACseq
- Single-cell CUT&Tag
- Single-cell Immune profiling
- CITE-seq and cell/nuclei hashing
- Single-cell CRISPR screens
- Sequencing is done with NGI and Clinical Genomics in Stockholm

NEW: 10XGenomics Chromium platform in BSL3 for infections biology samples.

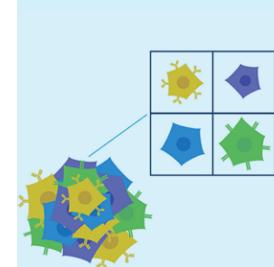
# Services and applications at ESCG

## Gene expression



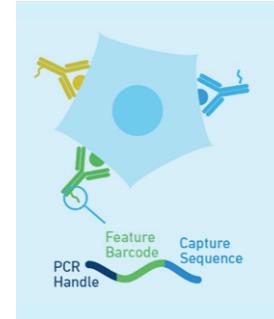
- 10XGenomics
  - 3'GE
  - 5'GE
- Smart-seq2
- Smart-seq3

## Gene expression + Immune profiling



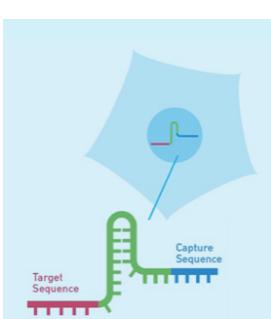
- 10X + VDJ
  - 5'GE
  - CITE-seq
  - Cell hashing

## Gene expression + Cell surface protein expression



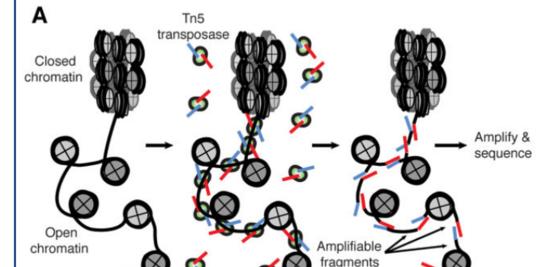
- 10X + CITE-seq
  - 3' GE
  - 5' GE
- Cell hashing
  - 3' GE
  - 5' GE

## Gene expression + CRISPR screen



- 10X + CRISPR
    - 10X 3' GE
- High-throughput  
Genome engineering  
facility

## Chromatin accessibility



- 10XGenomics
  - scATAC-seq

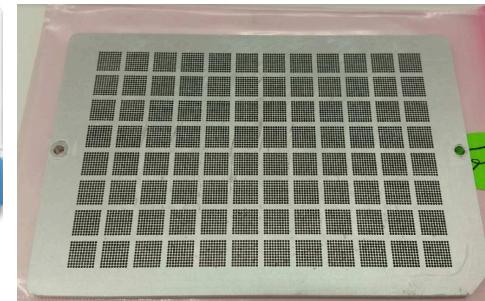
ESCG should enable scale in single-cell genomics hard to achieve in individual labs



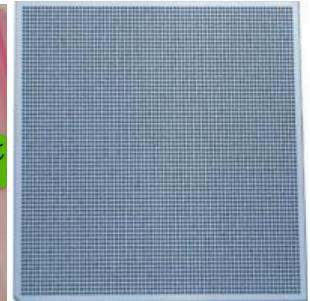
TECAN EVO, liquid handling robot



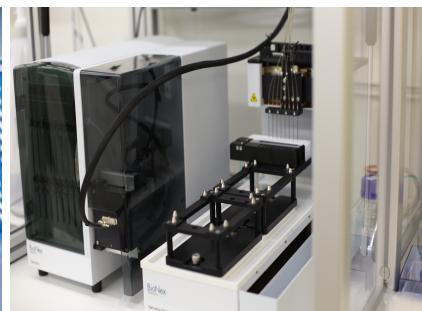
ICELL8cx, TAKARA



9600 wells



5184 wells



NanoDrop dispenser, GC Biotech



Chromium Controller, 10XGenomics



8 x 10,000 cells

# Outlook for single-cell genomics

- Multi-omics -- combine scRNA-sequencing with:
  - Surface protein expression (CITE-seq, Cell hashing)
  - CRISPR perturbations
  - scRNaseq + scATAC-seq
  - Histone modifications (scChIC-seq, CUT&Tag)
  - Low-coverage DNA (DLP+, Vancouver)
  - Methylated DNA (Joe Ecker lab, SALK)
- Spatial techniques
- Improve quality and reduce costs

# Sample preparation

- Limit time of cell isolation
- Be gentle
- Viability >80%
- Careful cell handling
- No debris in the suspension
- No cell aggregations



Contact us for tips & tricks!

- Nuclei
- Cryo-preserved cells
- Fixed cells (methanol)

# Contacts



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Institutet

SciLifeLab

[escg@scilifelab.se](mailto:escg@scilifelab.se)

<https://www.scilifelab.se/facilities/eukaryotic-single-cell-genomics>

<https://ki.se/en/research/core-facilities-for-research>

→iLAB login

Our publications can be found here:

[https://publications.scilifelab.se/label/Eukaryotic%20Single%20Cell%20Genomics%20\(ESCG\)](https://publications.scilifelab.se/label/Eukaryotic%20Single%20Cell%20Genomics%20(ESCG))