Single-cell methodologies and the ESCG facility
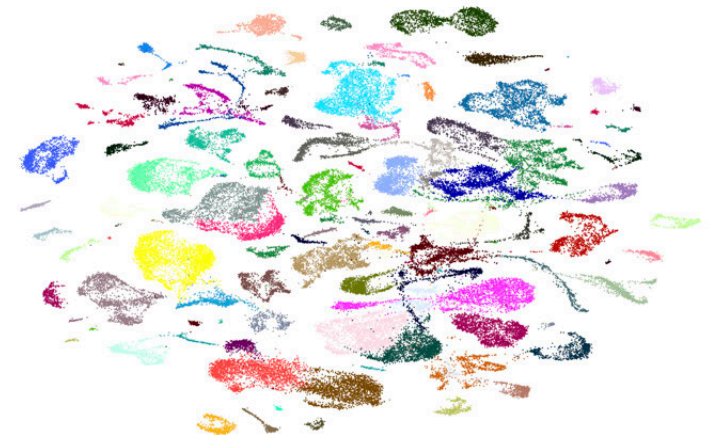
Karolina Wallenborg

January 25, 2021

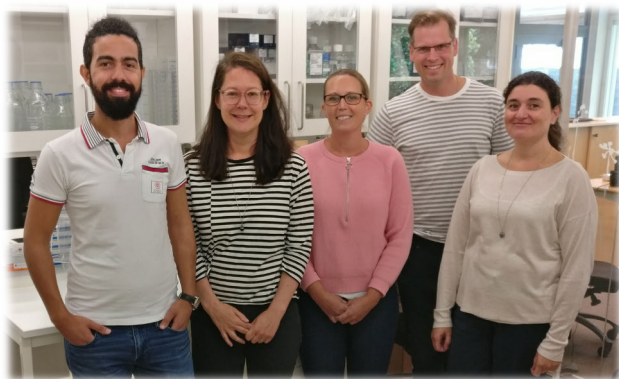# Eukaryotic Single-cell Genomics (ESCG)

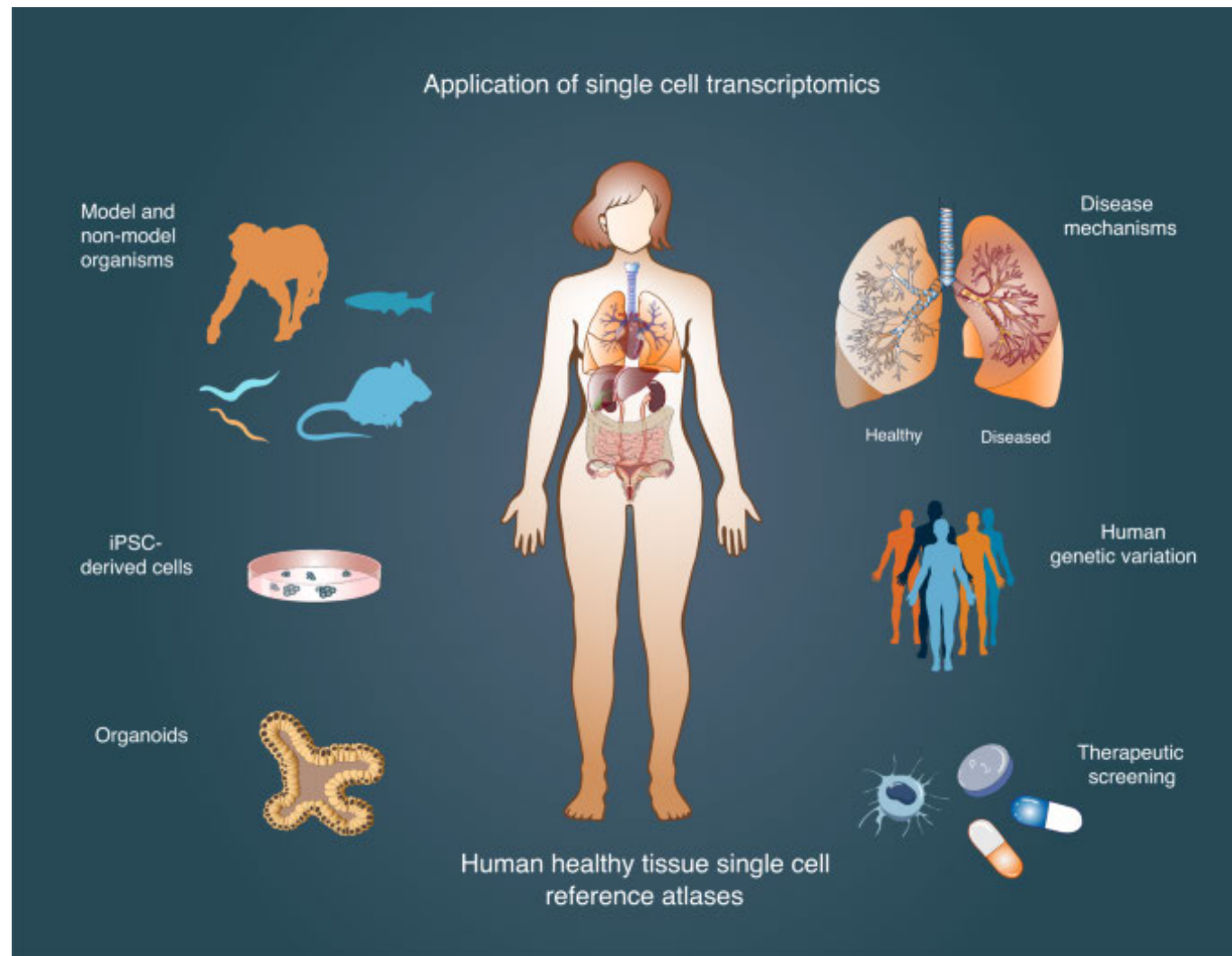- Established in 2015 with funding from SciLifeLab

- 2021
  - 6 personnel

Rickard Sandberg   Sten Linnarsson

Fluidigm C1 Auto-prep single cell system

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

Application of single cell transcriptomics

Model and non-model organisms

iPSC-derived cells

Organoids

Disease mechanisms

Healthy   Diseased

Human genetic variation

Therapeutic screening

Human healthy tissue single cell reference atlases

Aldridge & Teichmann, Nature Communication, 2020

Single cell RNA-sequencing experiment

Isolate tissue of interest → Dissociate cells/nuclei → Single Cell isolation → Single Cell DNA synthesis → Library preparation & Sequencing → Data processing and analysis
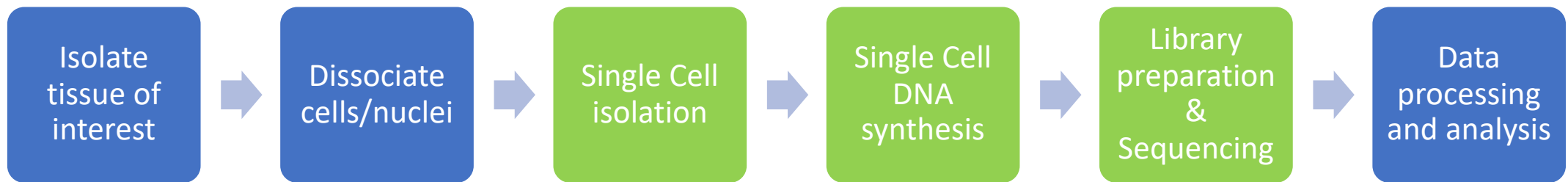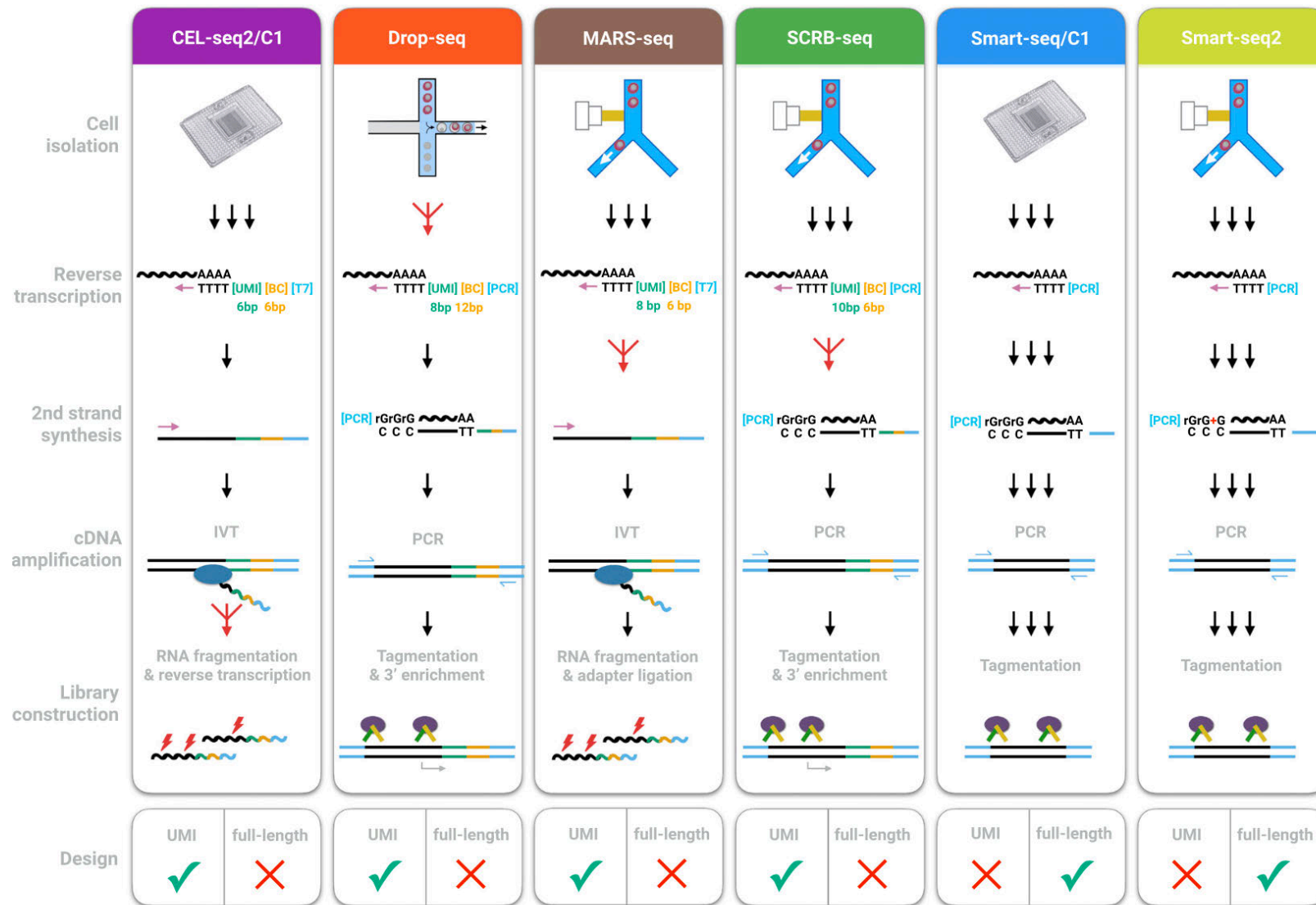
- User Consultations
  - Experimental design
- Quality control of single-cell preparations prior to the experiment
  - Sample submission guidelines for cells/nuclei for different applications
- Initial bioinformatics

Single-cell isolation and library preparation
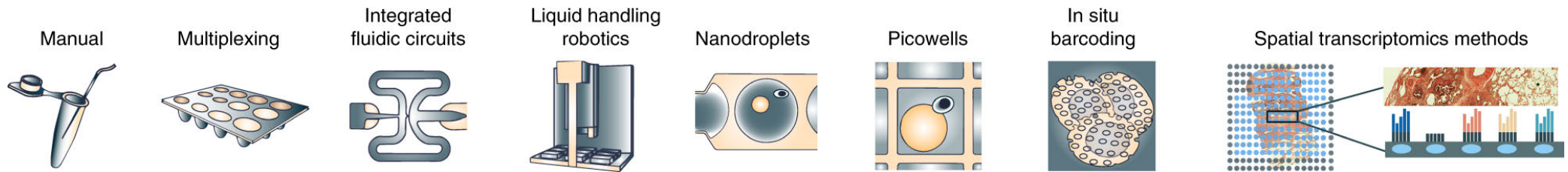
Manual · Multiplexing · Integrated fluidic circuits · Liquid handling robotics · Nanodroplets · Picowells · In situ barcoding · Spatial transcriptomics methods

Aldridge & Teichmann, Nature Communications, 2020

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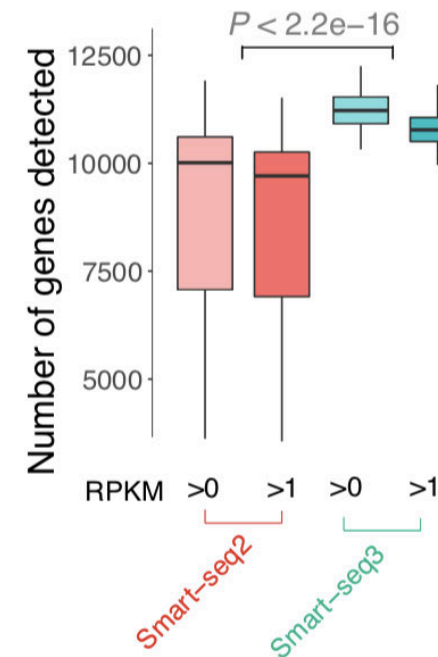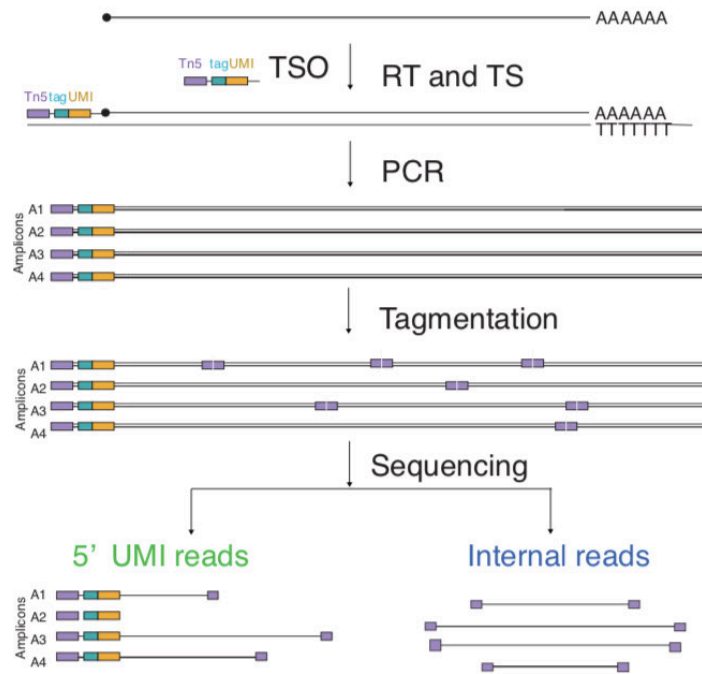
- Highly sensitive (80% of molecules detected by smRNA-FISH per cell)

Hagemann-Jensen et al , Nature Biotechnology, 2020

Single-cell ATAC-sequencing
(Assay for Transposase Accessible Chromatin)
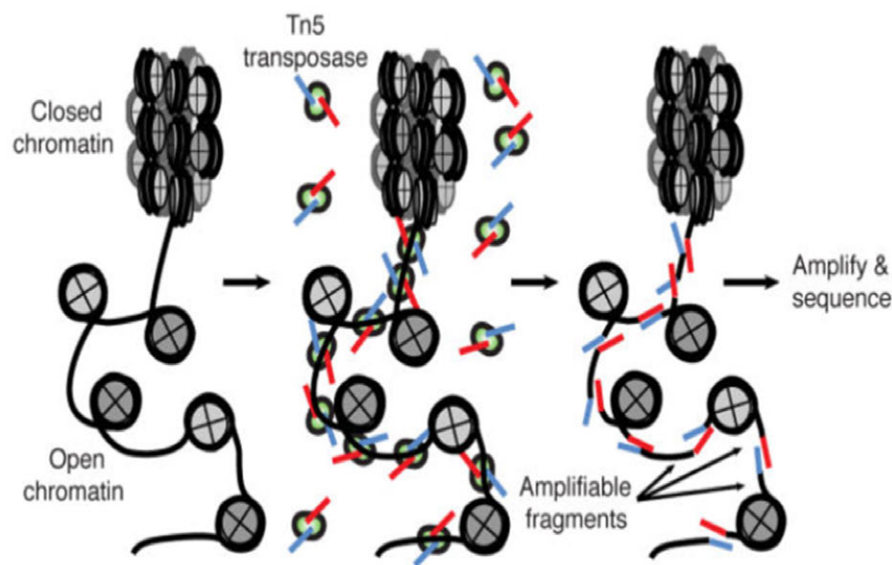
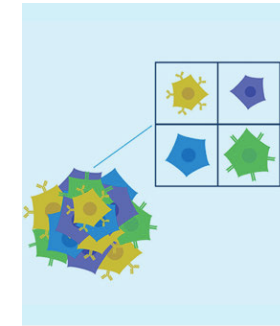(Buenrostro et al, Nature Methods, 2013

Chromatin and DNA-binding proteins regulate gene expression

- Subpopulations with different chromatin accessibility profiles

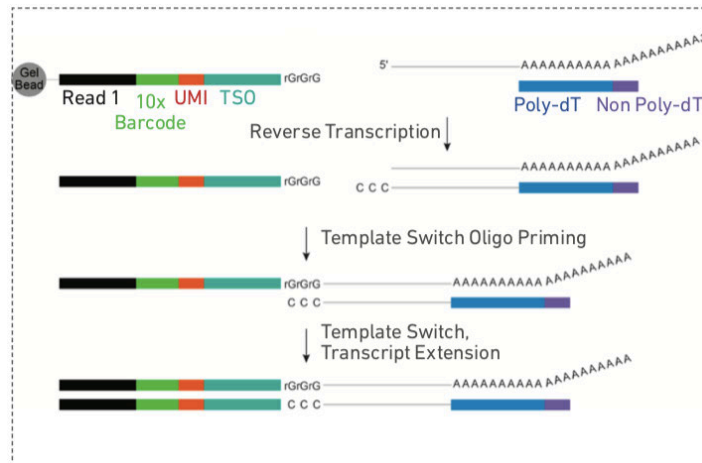→ Increased understanding of gene regulatory networks upstream on gene expression
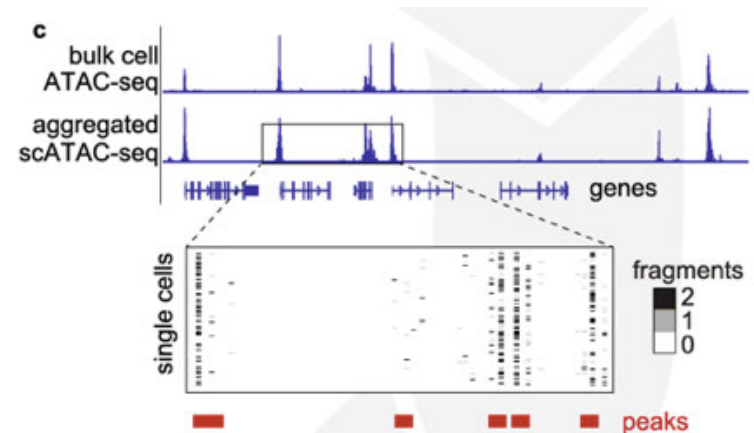
Adapted from Chen etal, Genome Biology,2019

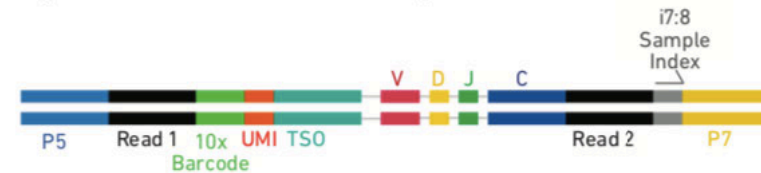# Single-cell Immune Profiling, V(D)J

Every receptor is unique

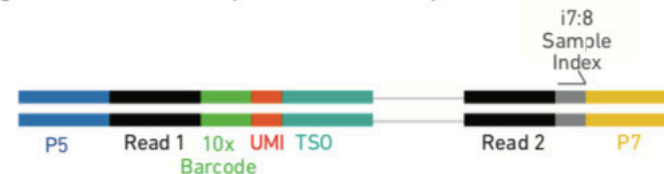Example from 10XGenomics protocol

cDNA amplification

Target enrichment with specific primers for TCR or Ig constant regions

CITE-seq
simultaneously measure gene expression and cell surface protein abundance
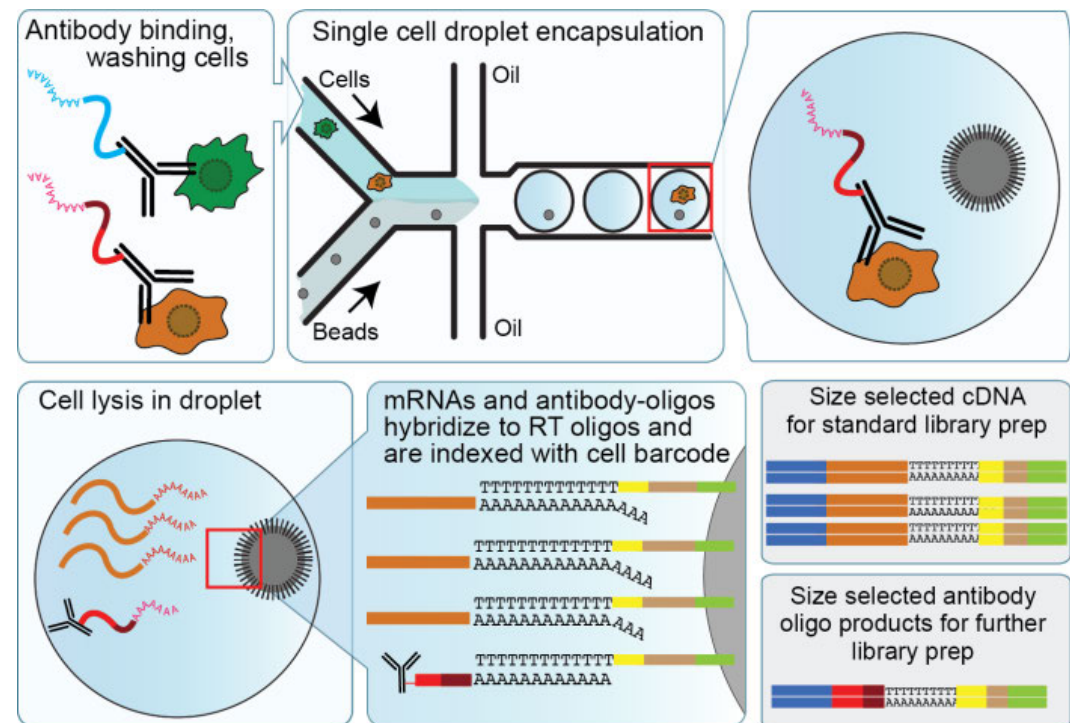
Adapted from Stoeckius et al., 2017
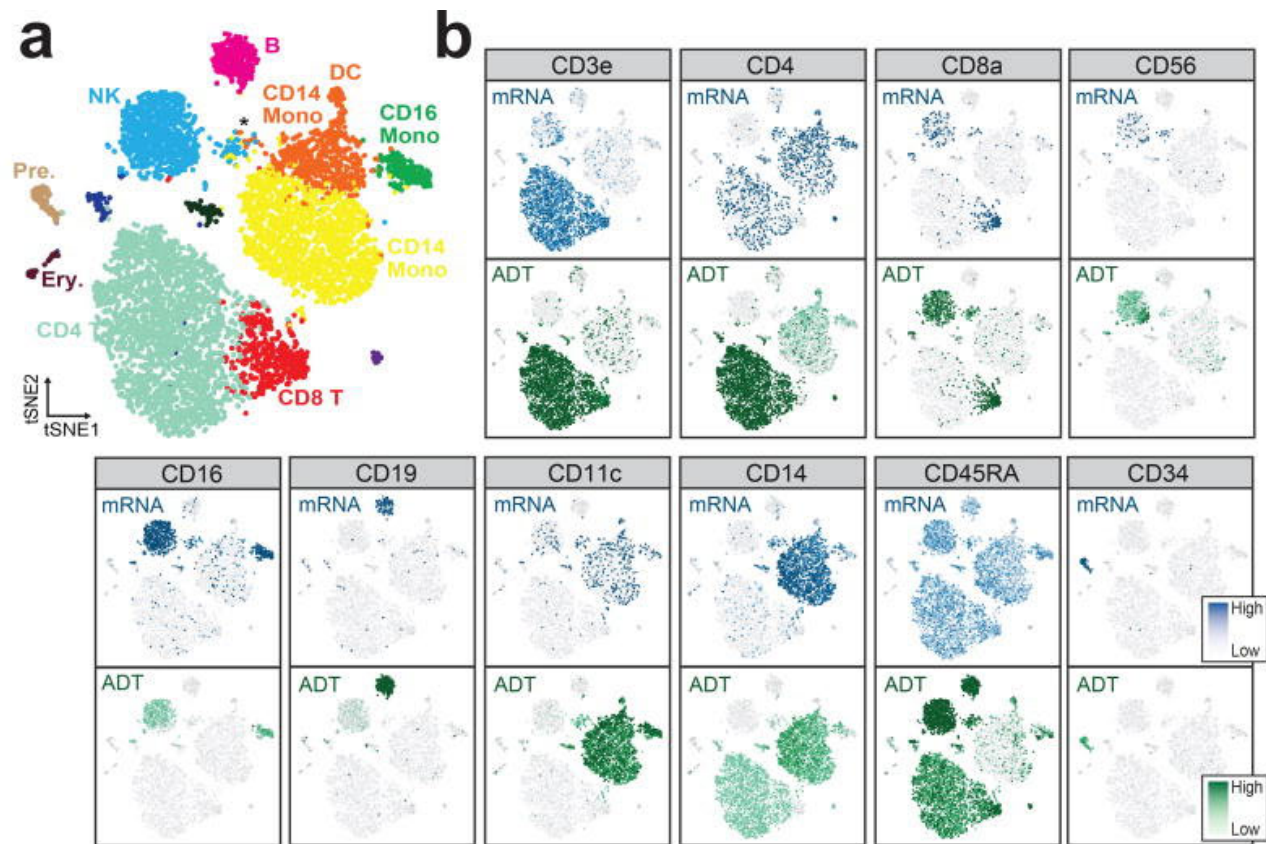
Antibody panels can be purchased from Biolegend

Stoeckius et al., 2017

→ Integrated protein and RNA measurements enhance cellular phenotyping

Cell hashing – multiplexing with ubiquitous surface protein expression
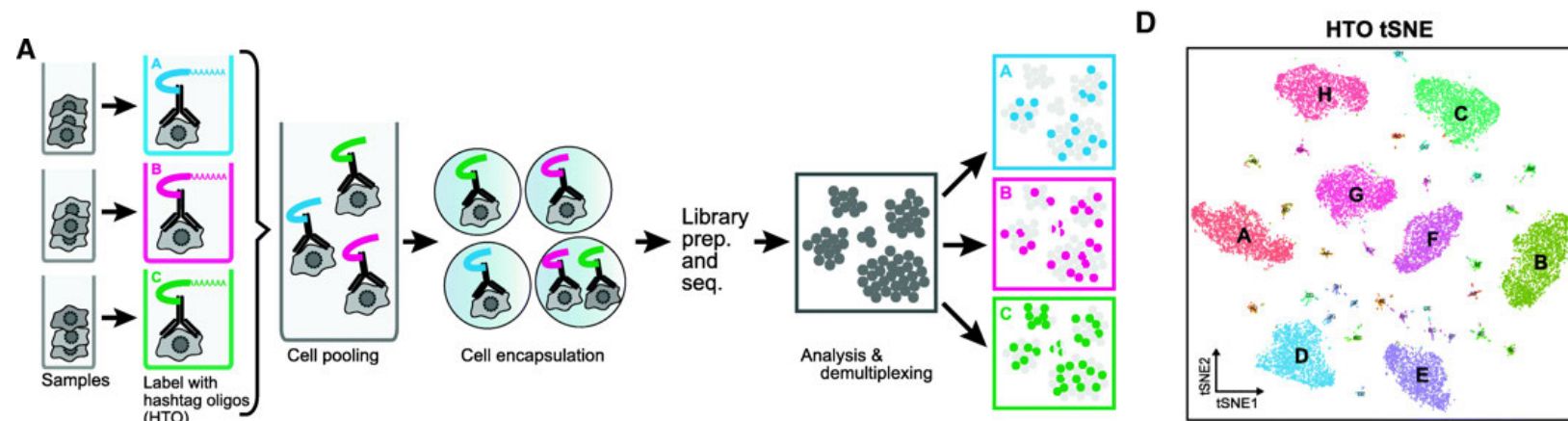
- Cell hashing, Stoeckius, Zheng et al., 2017
- Sample multiplexing
- Identify doublets, superload
- Batch effects
- Save on costs

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
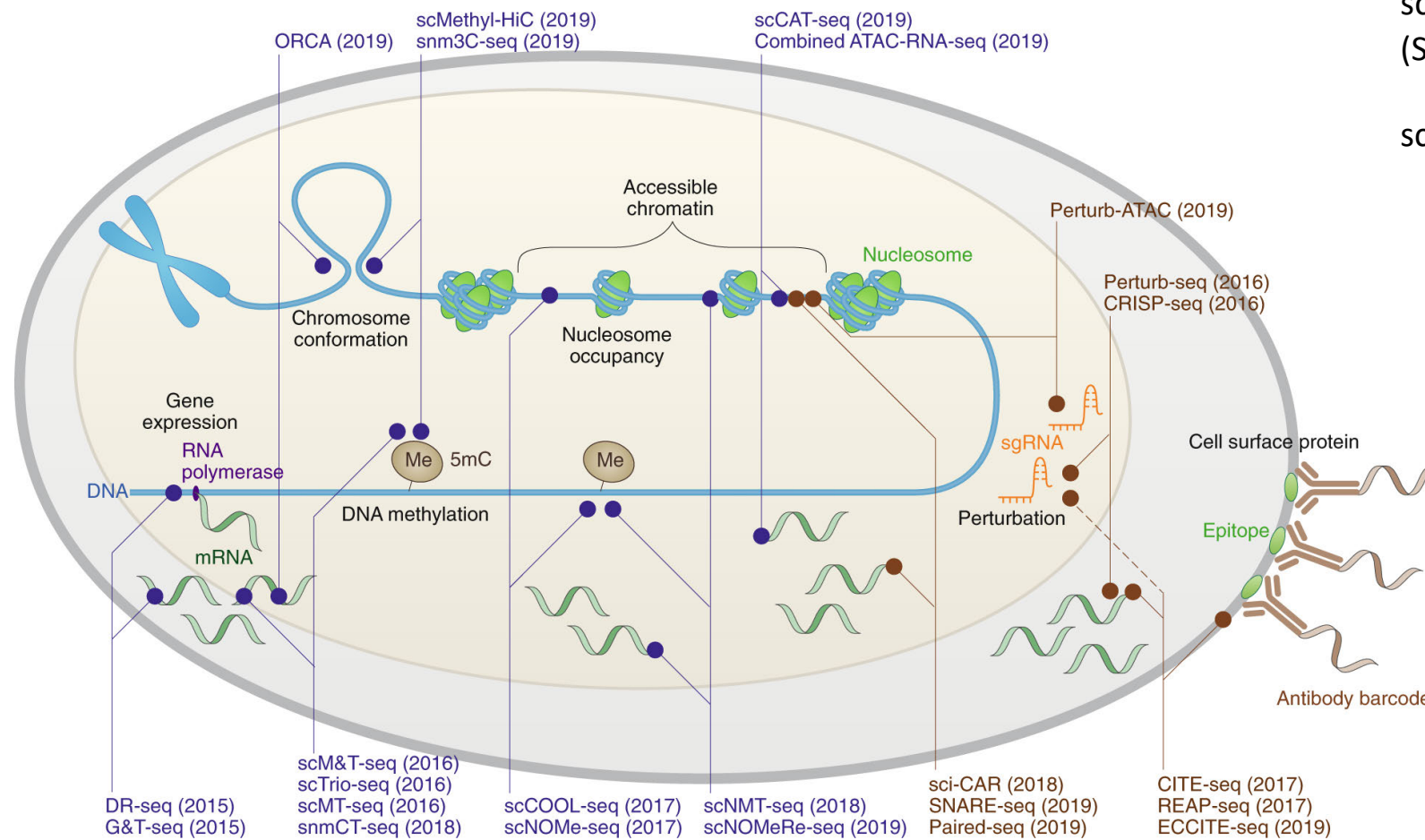
scRNA-seq + scATAC-seq
(SNARE-seq)

scCUT&Tag

ORCA (2019)

scMethyl-HiC (2019)
snm3C-seq (2019)

scCAT-seq (2019)
Combined ATAC-RNA-seq (2019)

Accessible chromatin

Nucleosome

Perturb-ATAC (2019)

Perturb-seq (2016)
CRISP-seq (2016)

Chromosome conformation

Nucleosome occupancy

Gene expression

RNA polymerase

sgRNA

Cell surface protein

DNA

Me 5mC

Me

Epitope

mRNA

DNA methylation

Perturbation

Antibody barcode

scM&T-seq (2016)
scTrio-seq (2016)
scMT-seq (2016)
snmCT-seq (2018)

DR-seq (2015)
G&T-seq (2015)

scCOOL-seq (2017)
scNOMe-seq (2017)

scNMT-seq (2018)
scNOMeRe-seq (2019)

sci-CAR (2018)
SNARE-seq (2019)
Paired-seq (2019)

CITE-seq (2017)
REAP-seq (2017)
ECCITE-seq (2019)

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

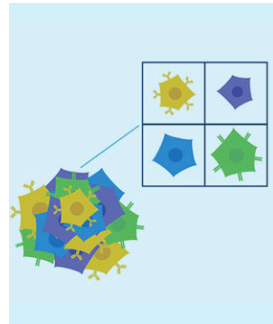NEW: 10XGenomics Chromium platform in BSL3 for infections biology samples.

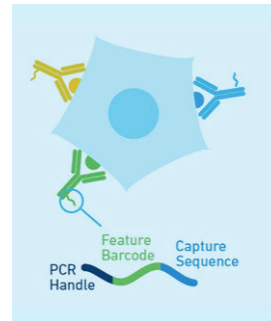# Services and applications at ESCG

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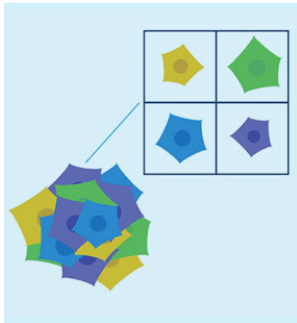
- 10XGenomics
  - 3'GE
  - 5'GE
- Smart-seq2
- Smart-seq3

Gene expression + Immune profiling
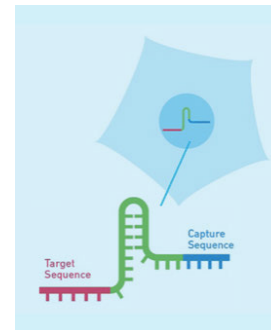
- 10X + VDJ
  - 5'GE
  - CITE-seq
  - Cell hashing

Gene expression + Cell surface protein expression

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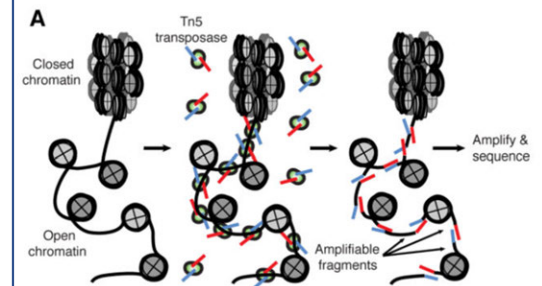 - 5' GE

- Cell hashing
  - 3' GE
  - 5' GE

Gene expression + CRISPR screen

- 10X + CRISPR
  - 10X 3' GE

High-throughput Genome engineering facility

Chromatin accessibility
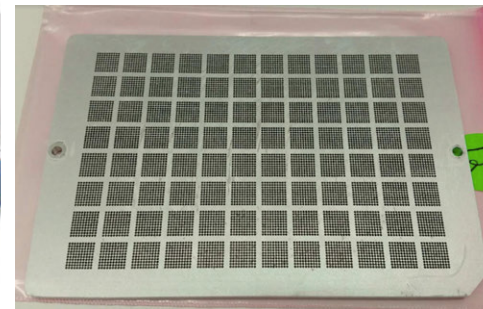
- 10XGenomics
  - scATAC-seq

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

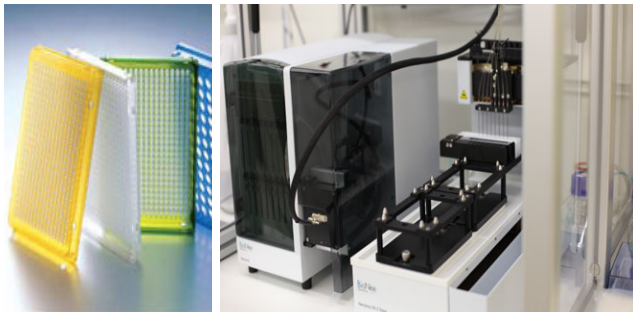TECAN EVO, liquid handling robot

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

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

Contact us for tips & tricks!

Contents

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)