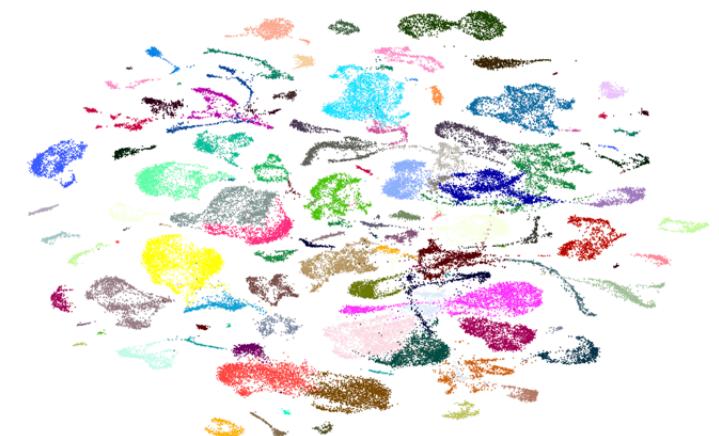
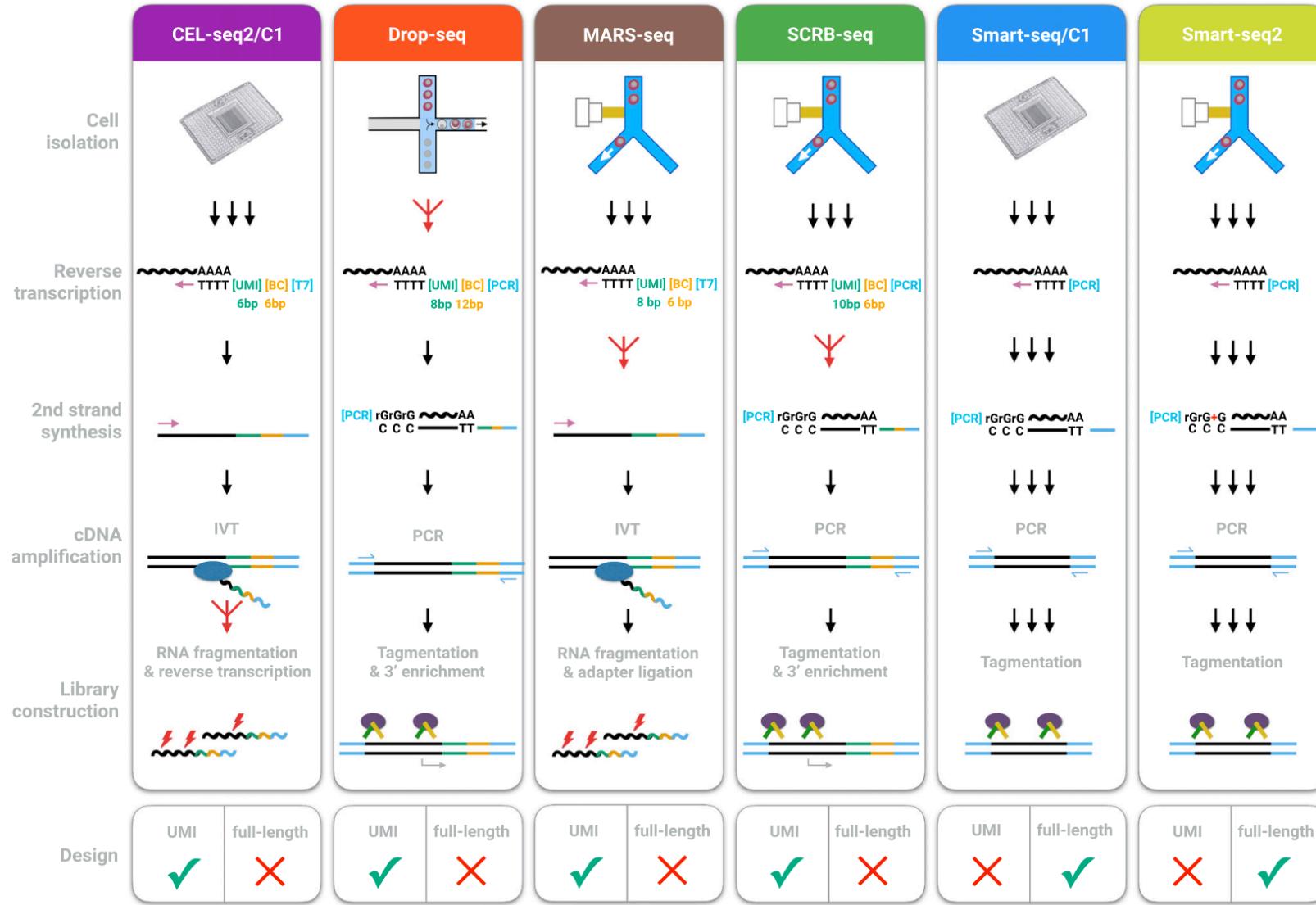


# Single-cell methodologies and the ESCG facility

**SciLifeLab**

Karolina Wallenborg  
January 27, 2020





RNA molecule capture and reverse transcription

Amplification

Library preparation

3' -tag

5'- tag

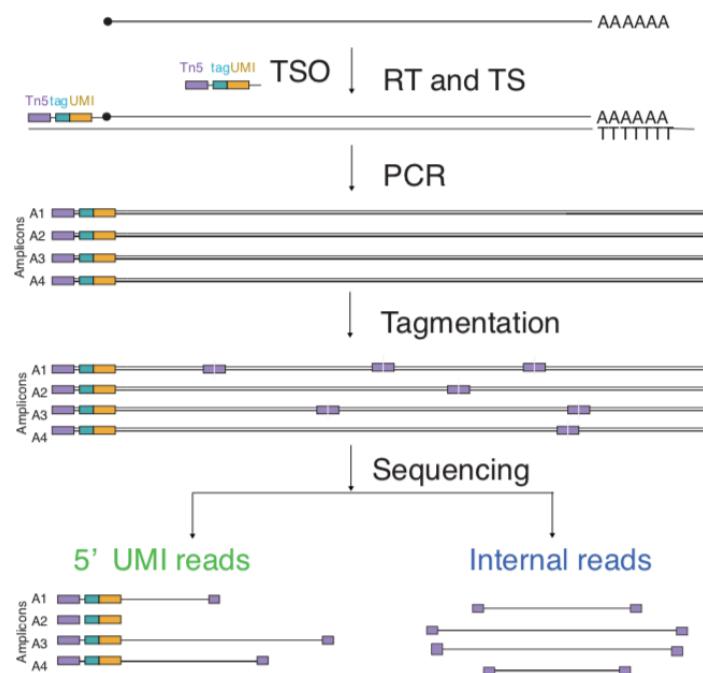
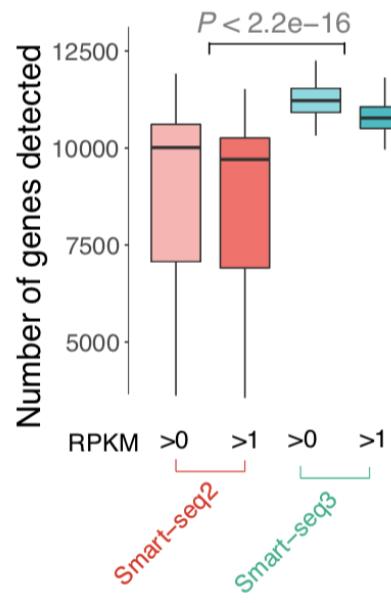
Full-length

Adapted from  
Kolodziejczyk A et al, Molecular Cell, 2015

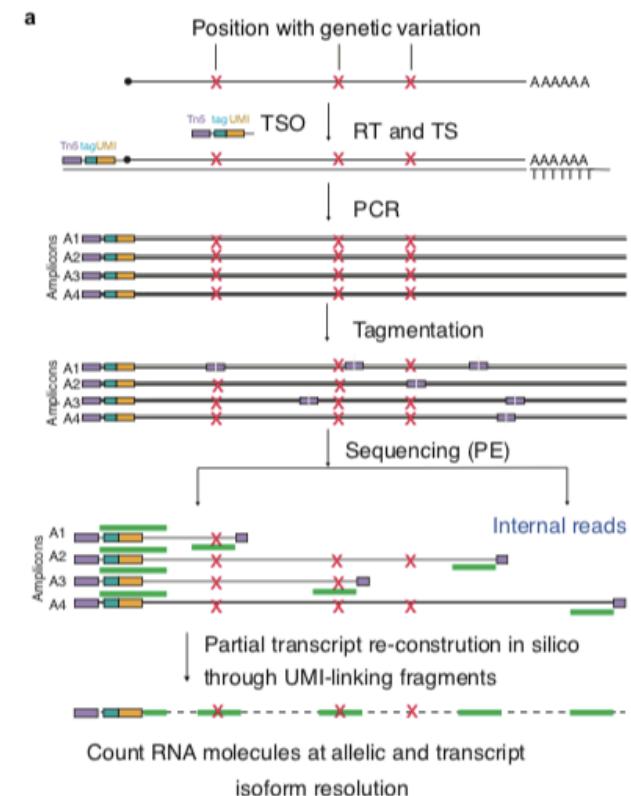
# Full-length vs 3' or 5'-end transcript sequencing

- 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

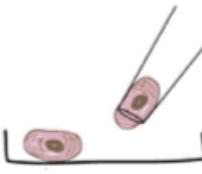
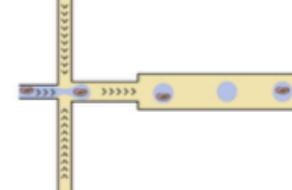
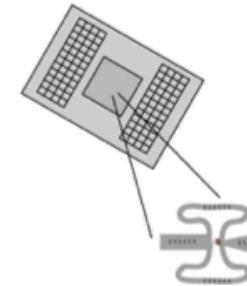
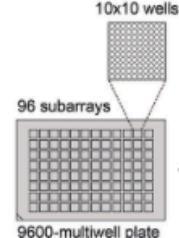


- Highly sensitive (80% of molecules detected by smRNA-FISH per cell)



Hagemann-Jensen et al BioRxiv 2019

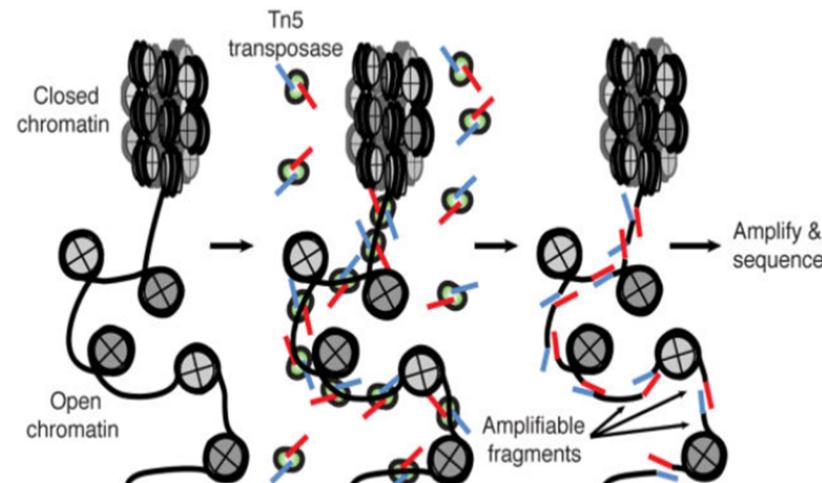
# Single-cell isolation and capture

MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1	Multi sample nanodispenser
					
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) fluorescence detection 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 visualisation of cells fast reaction in nanoliter volumes

- Cytoplasmic aspiration
- Patch-seq

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

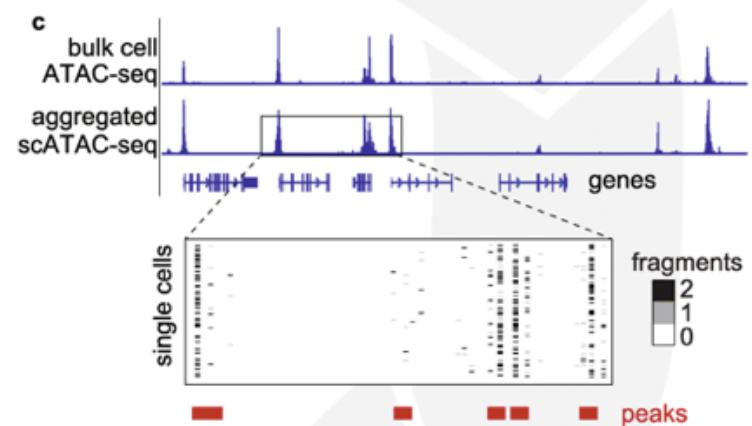
# Single-cell ATAC-sequencing (Assay for Transposase Accessible Chromatin)



(Buenrostro et al, Nature Methods, 2013)

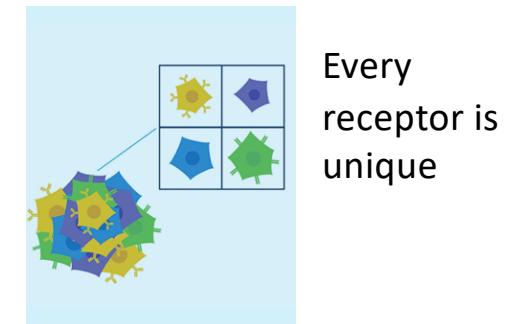
Chromatin and DNA-binding proteins regulate gene expression

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

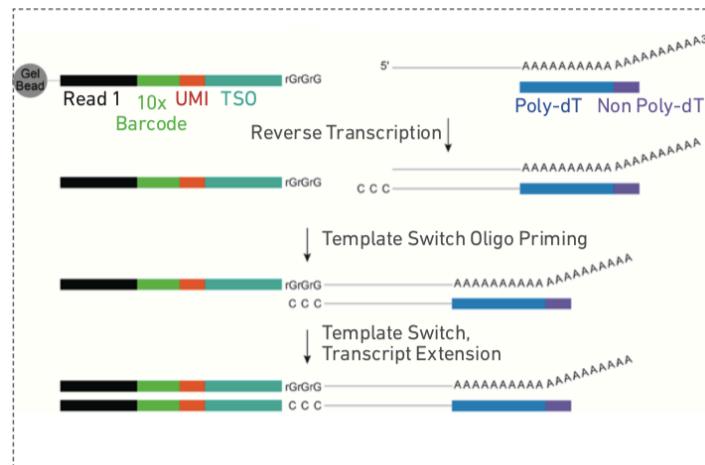


Adapted from Chen et al, Genome Biology, 2019

# Single-cell immune profiling, V(D)J



Example from 10XGenomics protocol



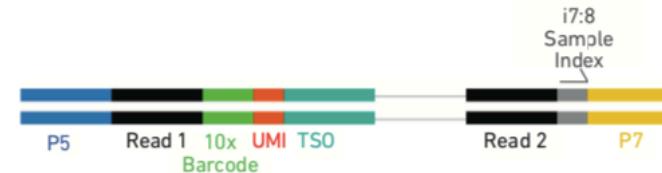
cDNA amplification

Target enrichment with specific primers  
for TCR or Ig constant regions

Chromium Single Cell V(D)J Enriched Library



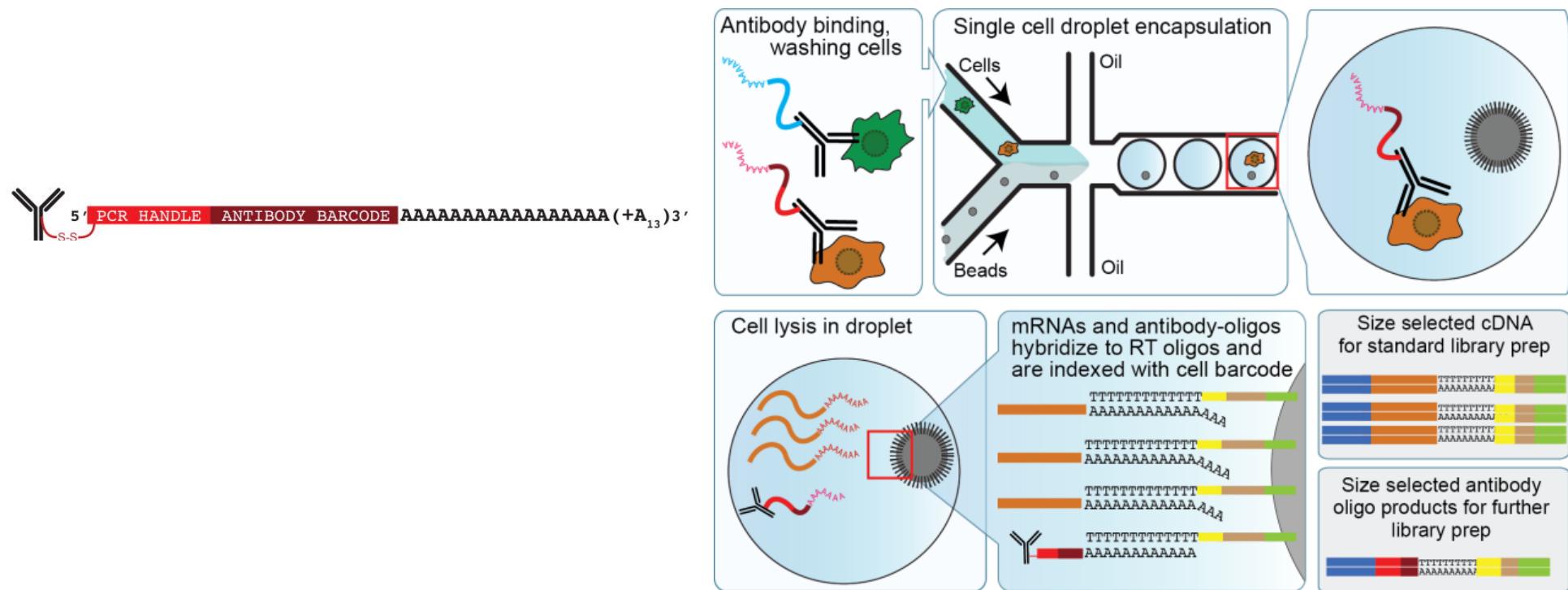
Chromium Single Cell 5' Gene Expression Library



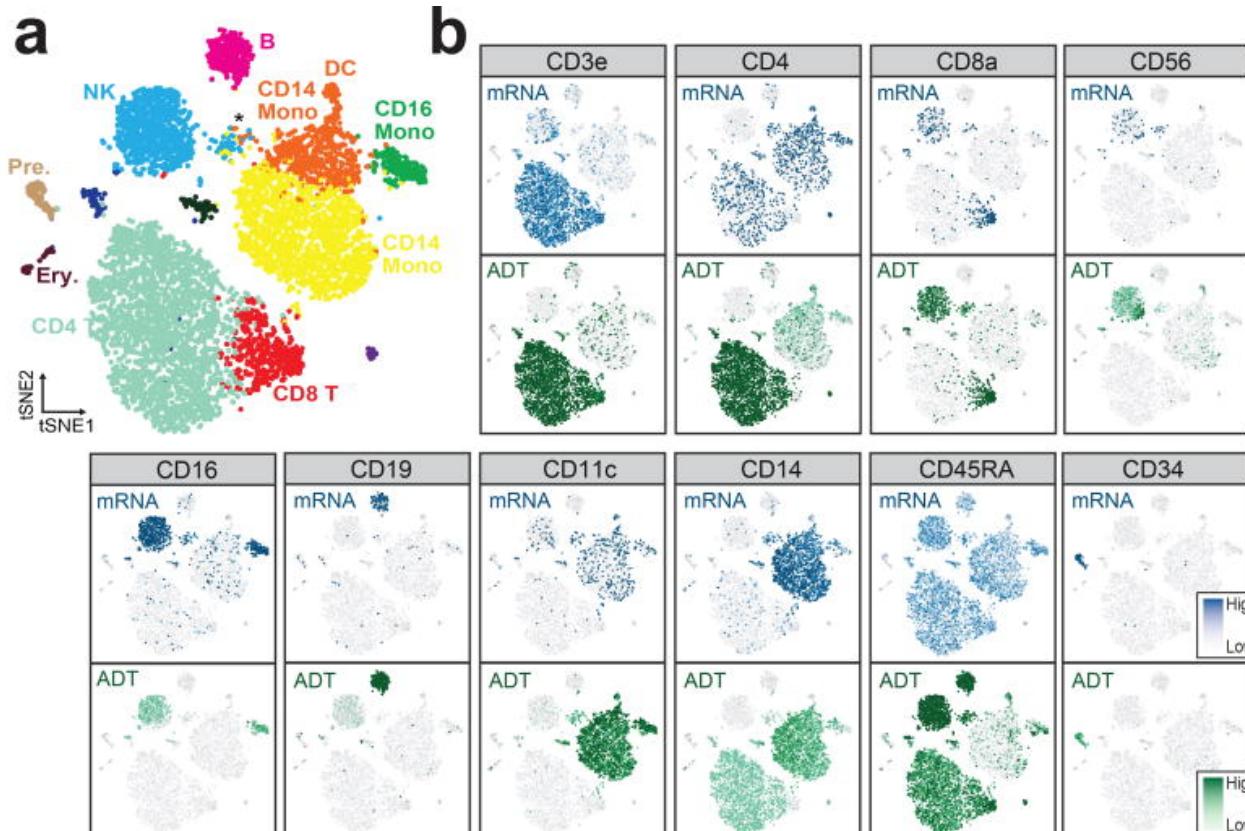
# CITE-seq

## simultaneously measure gene expression and cell surface protein abundance

a



Adapted from Stoeckius et al., 2017

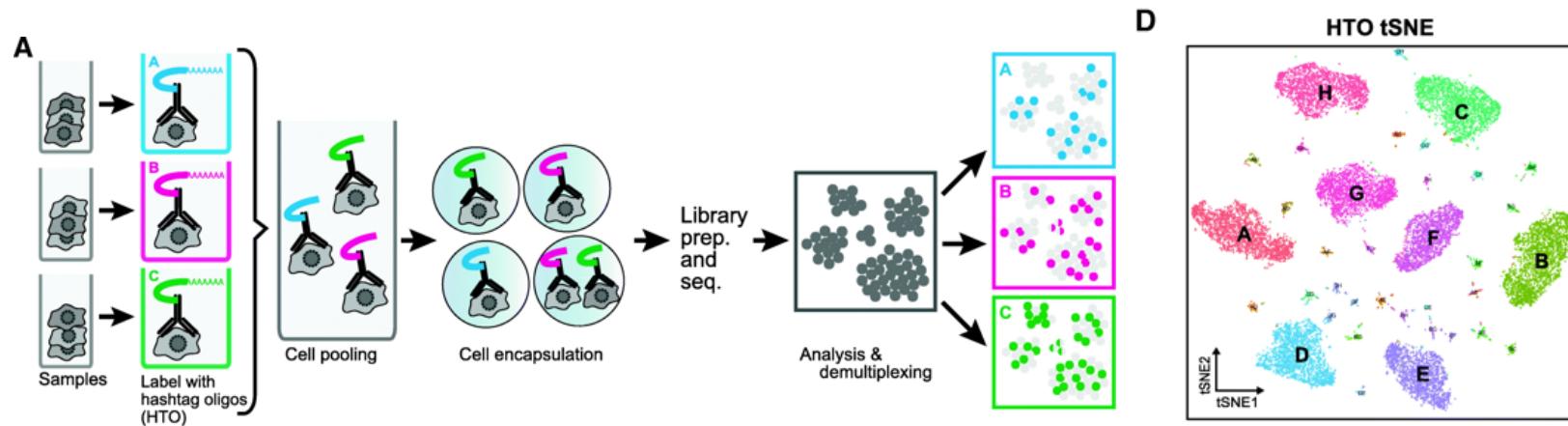


Antibody panels can  
be purchased from  
Biolegend

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

Volume 17 Issue 1, January 2020

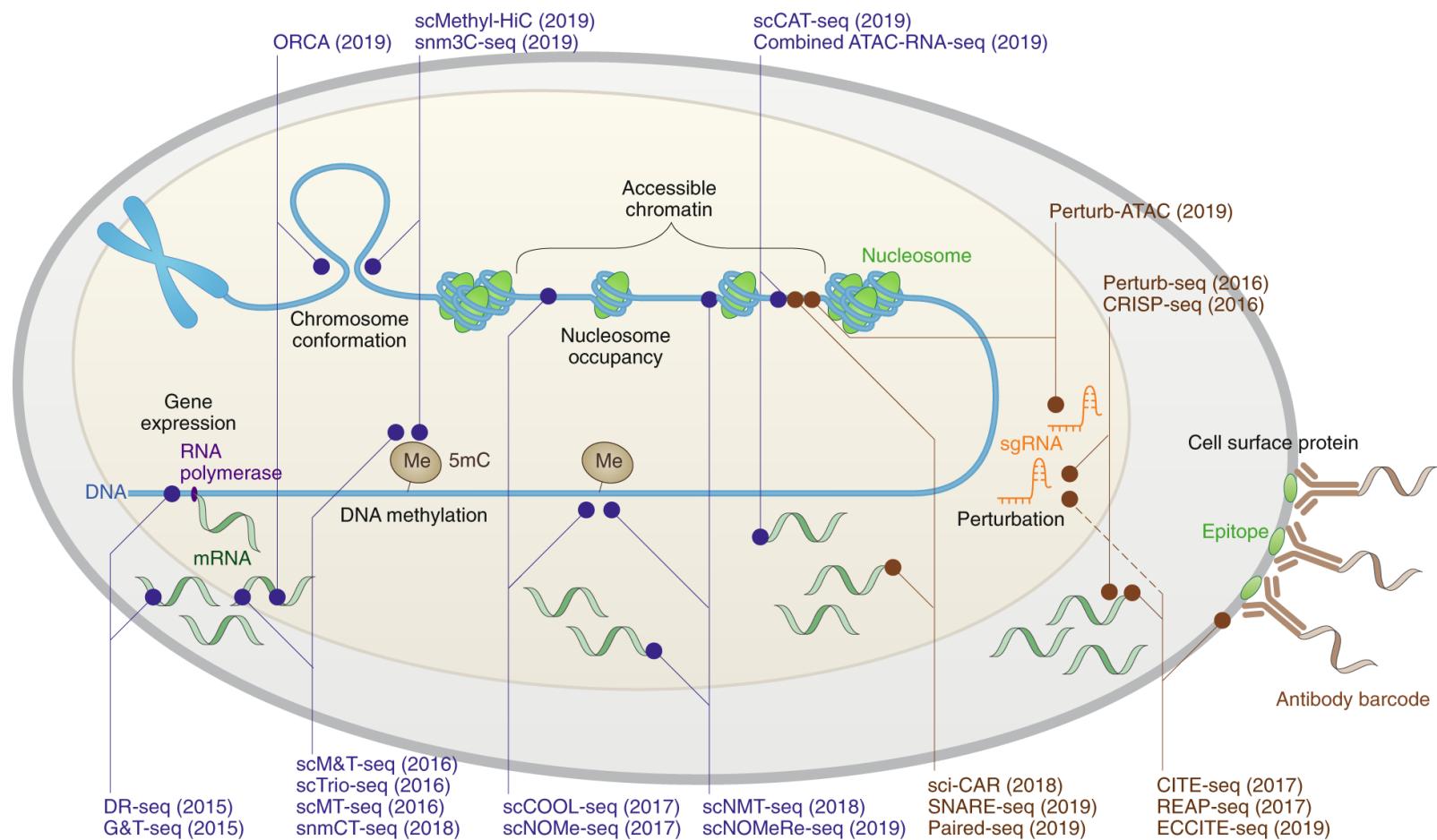


## METHOD OF THE YEAR 2019

Our choice for Method of the Year 2019 is single-cell multimodal omics analysis.

Cover design: Erin DeWalt

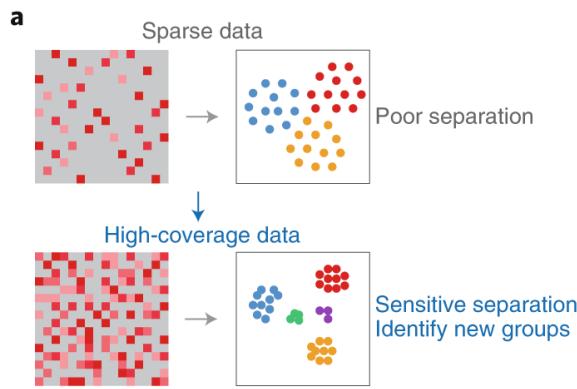
# Single-cell multimodal omics



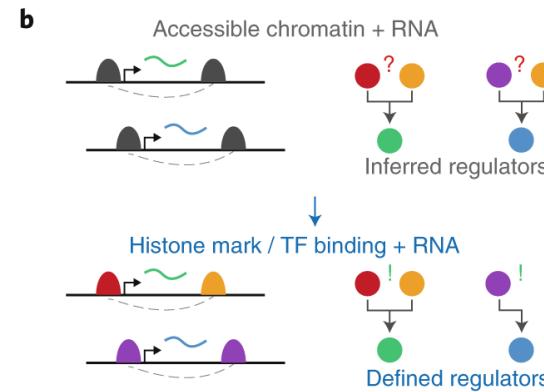
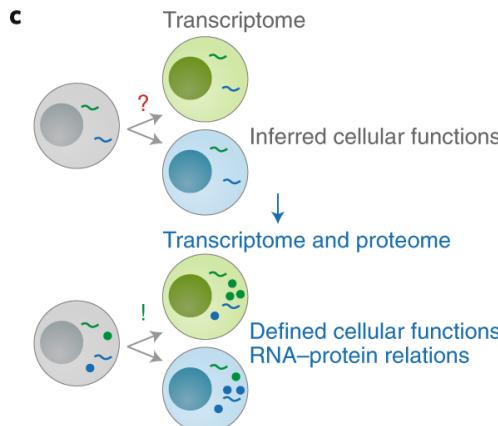
Zhu et al, Comment in Nature Methods, 2020

# Improvements for single-cell multimodal omics

Improve sensitivity and coverage

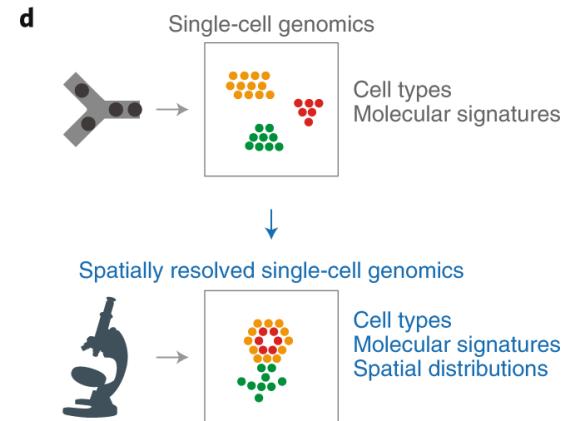


Transcriptome + intracellular proteome



Co-assays of histone marks /TFs binding sites & gene expression

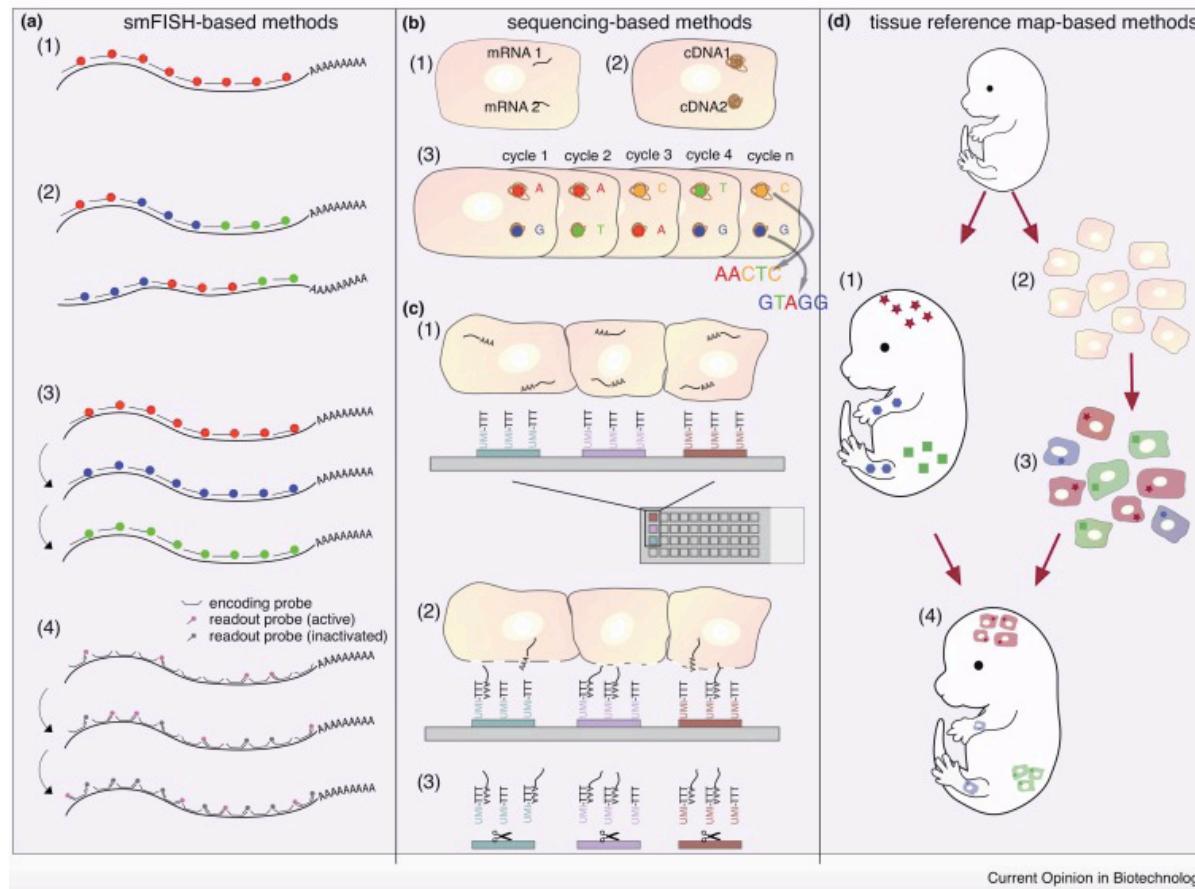
Single-cell CUT&TAG  
From Henikoff lab



Spatial transcriptomics + epigenome analysis

Zhu et al, Comment in Nature Methods, 2020

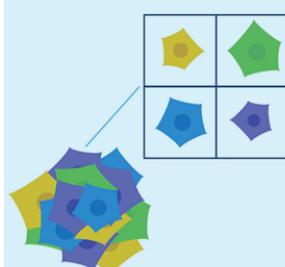
# Spatial transcriptomics



Moor & Itzkovitz, 2017

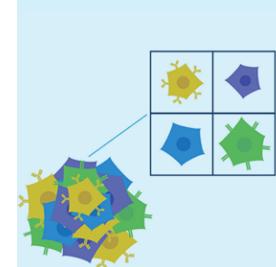
# Services at ESCG

## Gene expression



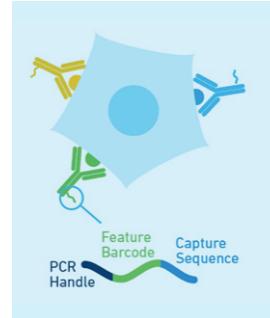
- 10XGenomics
  - 3'GE
  - 5'GE
- Smart-seq2
- Smart-seq3

## Gene expression + Immune profiling



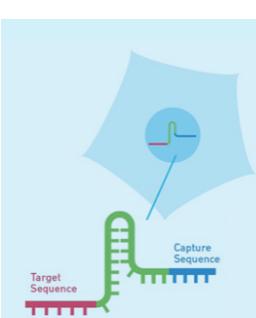
- 10X + VDJ
  - 3'GE
  - 5'GE
  - CITE-seq
  - Cell hashing

## Gene expression + Cell surface protein expression



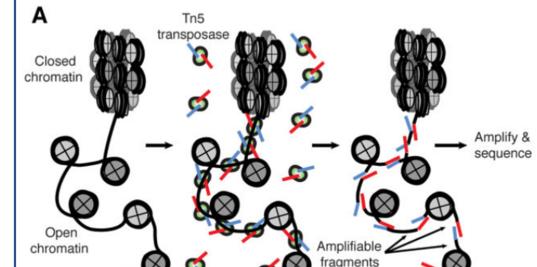
- 10X + CITE-seq
  - 3' GE
  - 5' GE
- Cell hashing
  - 3' GE
  - 5' GE

## Gene expression + CRISPR screen



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

## Chromatin accessibility



- 10XGenomics
  - scATAC-seq

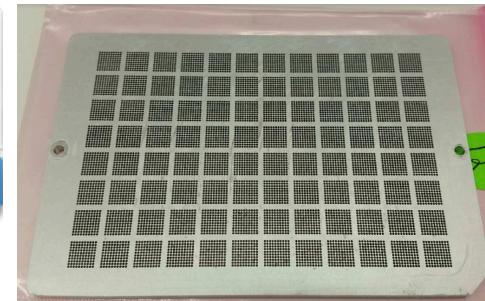
ESCG should enable scale in single-cell genomics hard to achieve in individual labs



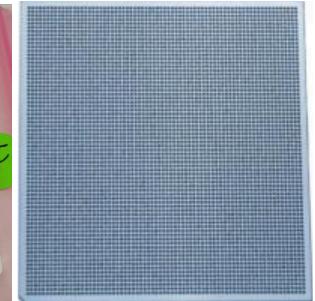
TECAN EVO, liquid handling robot



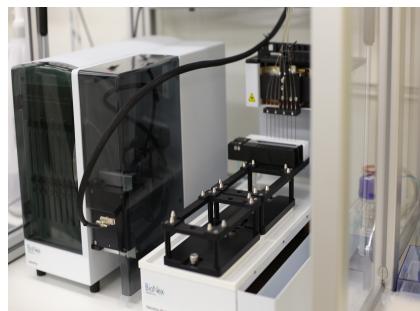
ICELL8cx, TAKARA



9600 wells



5184 wells



NanoDrop dispenser, GC Biotech



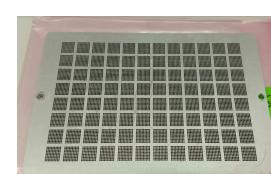
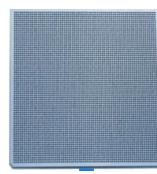
Chromium Controller, 10XGenomics



8 x 10,000 cells

# Single-cell RNA-seq technologies offered through ESCG

Method	Smart-seq2 Smart-seq3	Smart-Seq ICELL8	STRT-seq-2i	10xGenomics
Format	384-wells	Microwell chip 5184 wells	Microwell chip 9600 wells	Chromium microfluidics chip 8 x 10,000 cells
Input	FACS-sorted cells	Suspension	Suspension / FACS	Suspension
Transcript coverage	Full-length	Full-length	5'	3'



# 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)

# Selected publications in 2019 based on data generated at ESCG

- ESCG has sequenced ~1.5M cells (2015-2019)
- Contributed to ~21 publications.

## Altered human oligodendrocyte heterogeneity in multiple sclerosis

Sarah Jäkel<sup>1,5</sup>, Eneritz Agirre<sup>2,5</sup>, Ana Mendanha Falcão<sup>2</sup>, David van Bruggen<sup>2</sup>, Ka Wai Lee<sup>2</sup>, Irene Knuesel<sup>3</sup>, Dheeraj Malhotra<sup>3,6</sup>, Charles ffrench-Constant<sup>1,6\*</sup>, Anna Williams<sup>1,6\*</sup> & Gonçalo Castelo-Branco<sup>2,4,6\*</sup>

Nature, 2019

## Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution

Takahiro Masuda<sup>1,17</sup>, Roman Sankowski<sup>1,2,17</sup>, Ori Staszewski<sup>1,17</sup>, Chotima Böttcher<sup>3</sup>, Lukas Amann<sup>1,4</sup>, Sagar<sup>6</sup>, Christian Scheiwe<sup>5</sup>, Stefan Nessler<sup>7</sup>, Patrik Kunz<sup>7</sup>, Geert van Loo<sup>8,9</sup>, Volker Arnd Coenen<sup>10</sup>, Peter Christoph Reinacher<sup>10</sup>, Anna Michel<sup>11</sup>, Ulrich Sure<sup>11</sup>, Ralf Gold<sup>12</sup>, Dominic Grün<sup>6</sup>, Josef Priller<sup>3,13,14</sup>, Christine Stadelmann<sup>7</sup> & Marco Prinz<sup>1,15,16\*</sup>

Nature, 2019

### RESEARCH ARTICLE SUMMARY

#### NEURODEVELOPMENT

## Spatiotemporal structure of cell fate decisions in murine neural crest

Ruslan Soldatov<sup>\*</sup>, Marketa Kaucka<sup>\*</sup>, Maria Eleni Kastriti<sup>9</sup>, Julian Petersen, Tatiana Chontorotzea, Lukas Englmayer, Natalia Akkuratova, Yunshi Yang, Martin Häring, Viacheslav Dyachuk, Christoph Bock, Matthias Farlik, Michael L. Piacentino, Franck Boismoreau, Markus M. Hilscher, Chika Yokota, Xiaoyan Qian, Mats Nilsson, Marianne E. Brommer, Laura Croci, Wen-Yu Hsiao, David A. Guertin, Jean-François Brunet, Gian Giacomo Consalez, Patrik Ernfors, Kaj Fried, Peter V. Kharchenko<sup>†</sup>, Igor Adameyko<sup>†</sup>

Science, 2019

# Outlook – for ESCG

- Multi-omics -- combine scRNA-sequencing with:
  - Surface protein expression (CITE-seq, Cell hashing)
  - CRISPR perturbations
  - Accessible chromatin (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

# Contacts

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

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

## Funding

- Science for Life Laboratory
- Karolinska Institutet (KI Core)
- SFO/Stratregen (KI)

**SciLifeLab**

