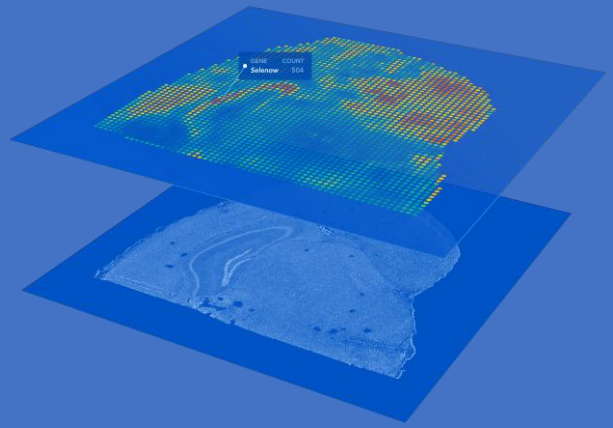
Single cell approaches



Emmanouil I. Athanasiadis, PhD

Staff Research Scientist B' – Associate Professor level

Biomedical Research Foundation of the Academy of Athens – Greek Genome Center

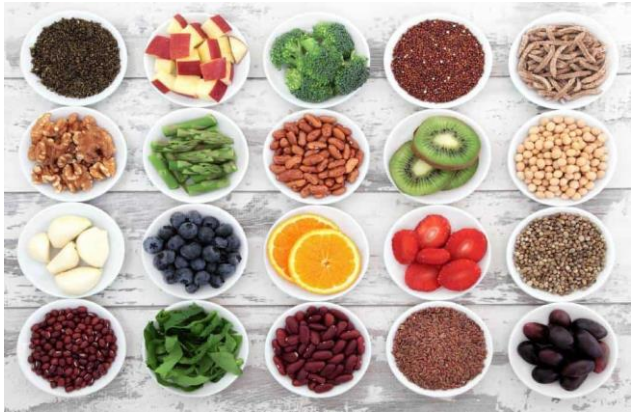
3rd Immunology Workshop for Clinicians
08 – 10 October 2021

Why single cell?

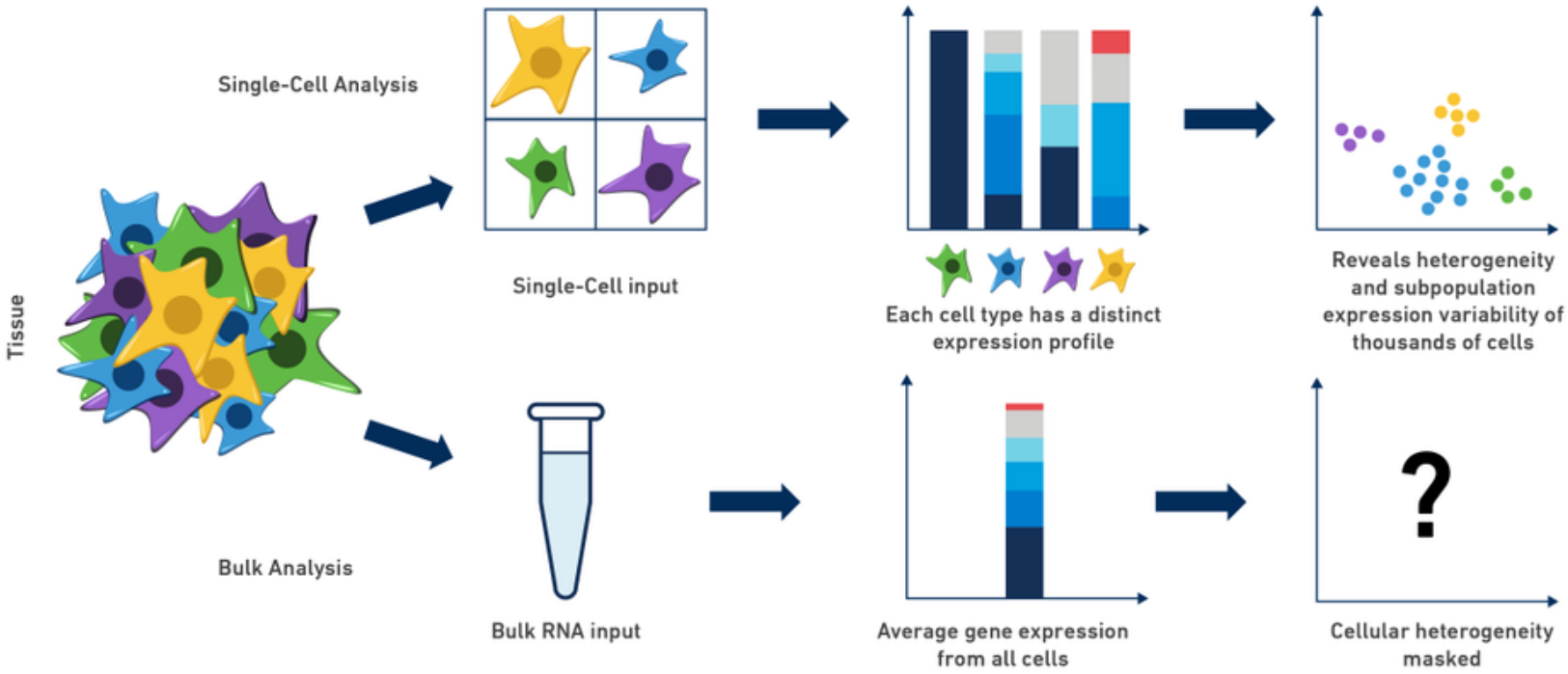
Initial Sample (fruit salad)

Bulk RNAseq analysis is like putting a fruit salad into a blender, the **taste will be an average** of all ingredients

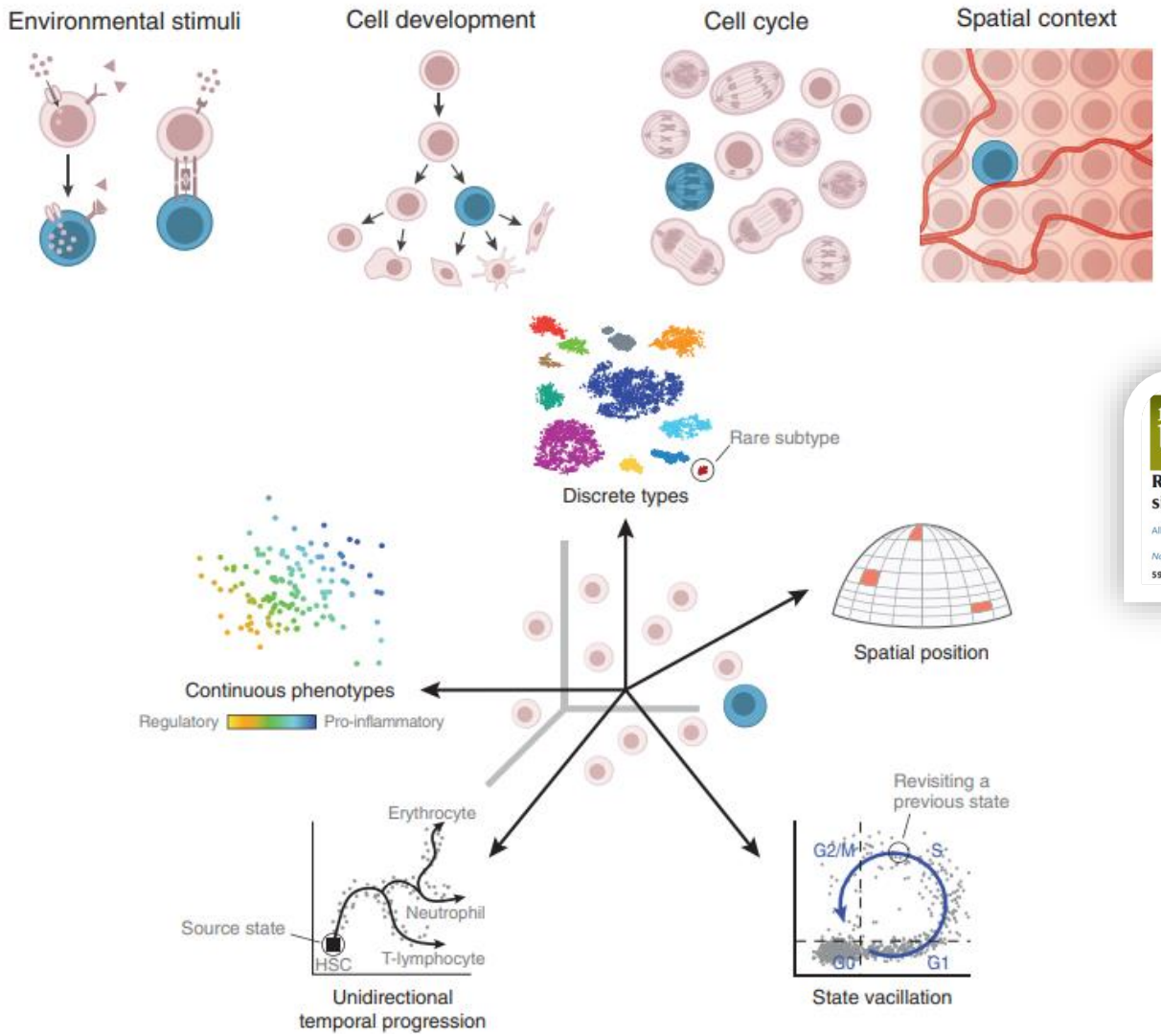
Single Cell RNAseq analysis is like tasting **each individual** piece of fruit to understand of the composition of the fruit salad

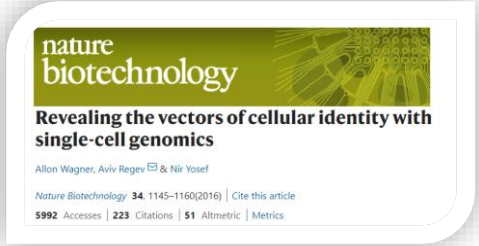


Why single cell RNA sequencing?



What type of biological questions can we address?





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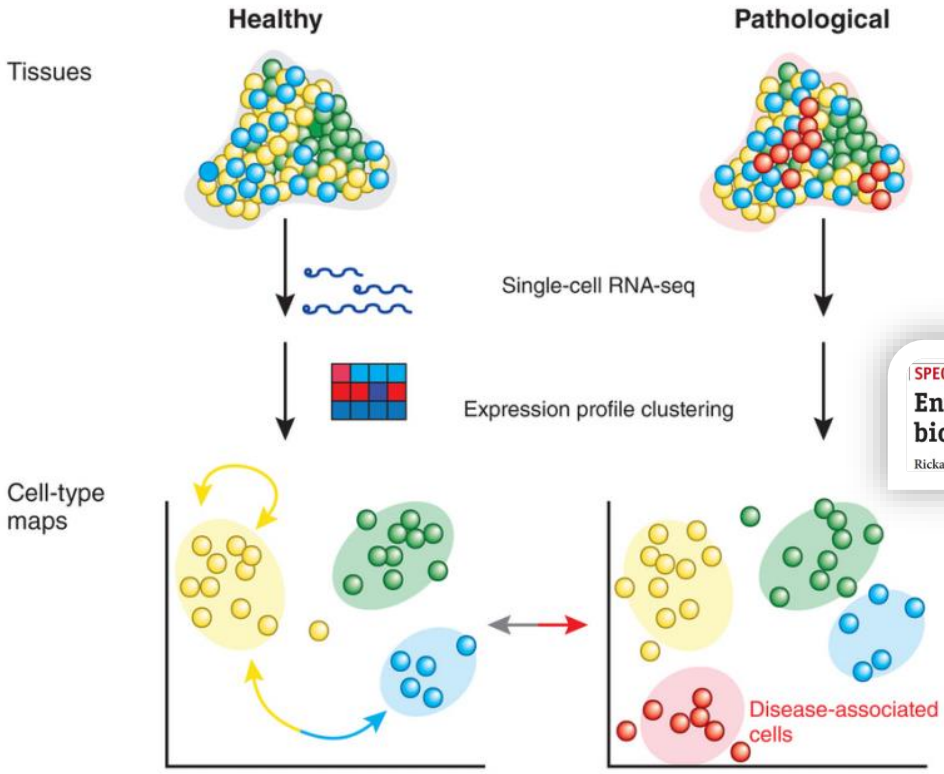



SPECIAL FEATURE | COMMENTARY METHOD OF THE YEAR

Entering the era of single-cell transcriptomics in biology and medicine

Rickard Sandberg

22 | VOL.11 NO.1 | JANUARY 2014 | NATURE METHODS

Types of analyses

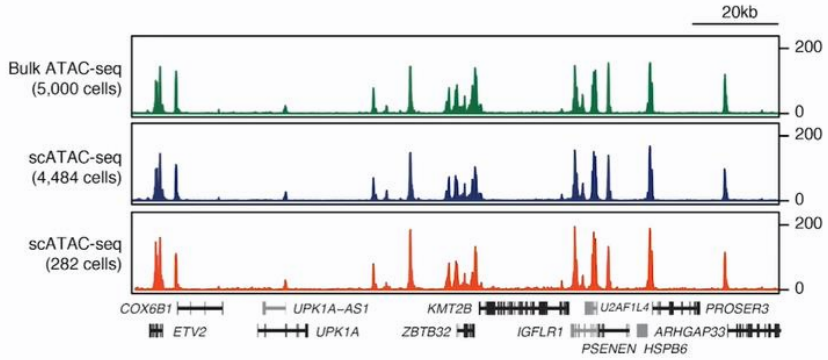
Within cell type
- Stochasticity, variability of transcription
- Regulatory network inference
- Allelic expression patterns
- Scaling laws of transcription

Between cell types
- Identify biomarkers
- (Post)-transcriptional differences

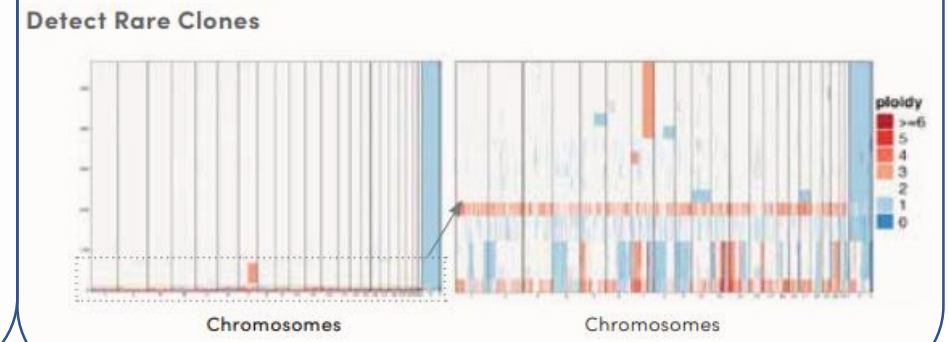
Between tissues
- Cell-type compositions
- Altered transcription in matched cell types

What else we can sequence at single cell resolution?

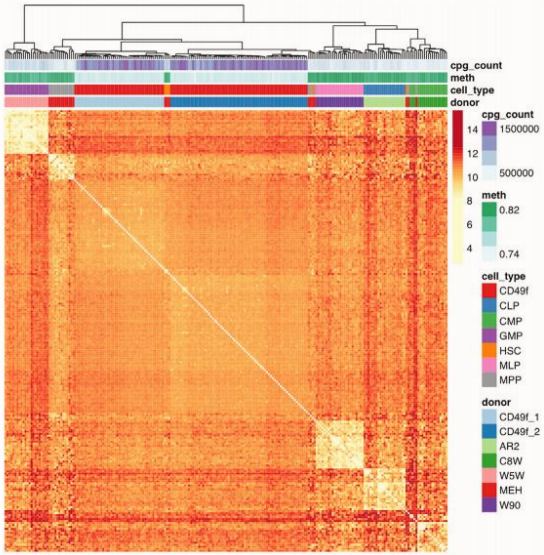
Chromatin accessibility (*scATACseq*)



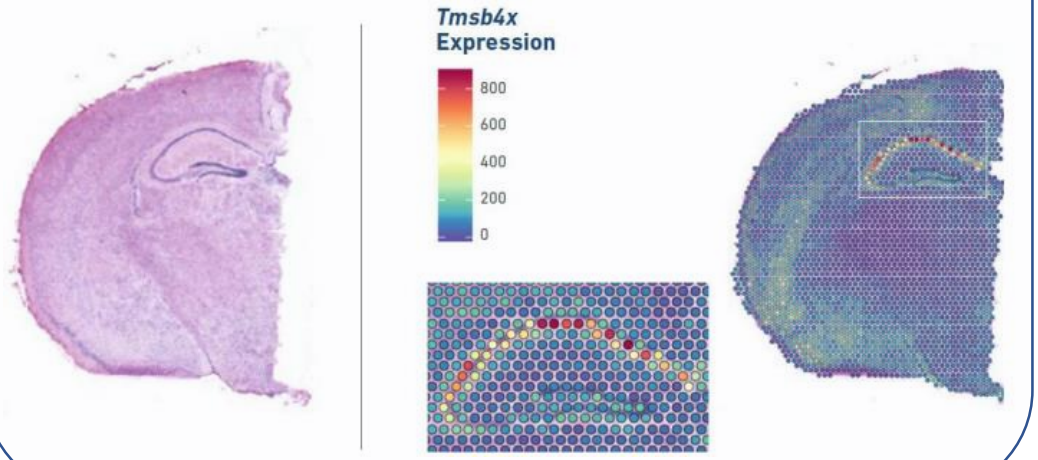
Mutation & CNV (*scDNAseq*)



Single cell Methylation

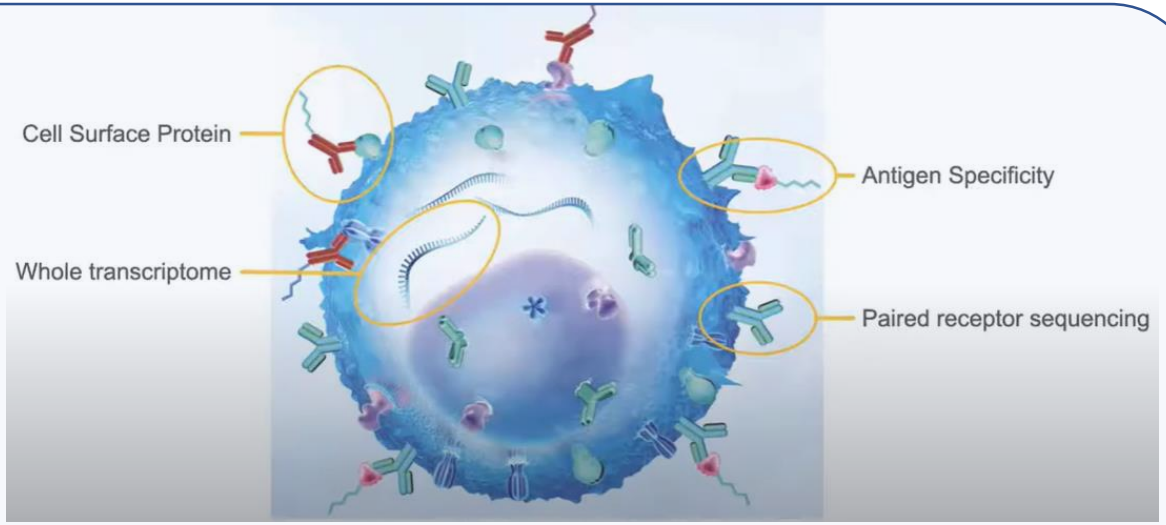


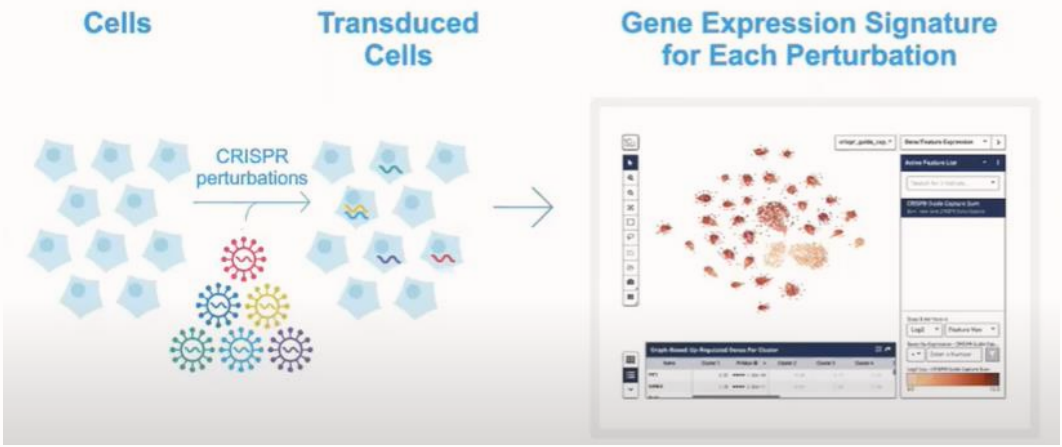
Single cell Spatial Transcriptomics (*STx*) Protein Co-detection



What else we can sequence at single cell resolution?

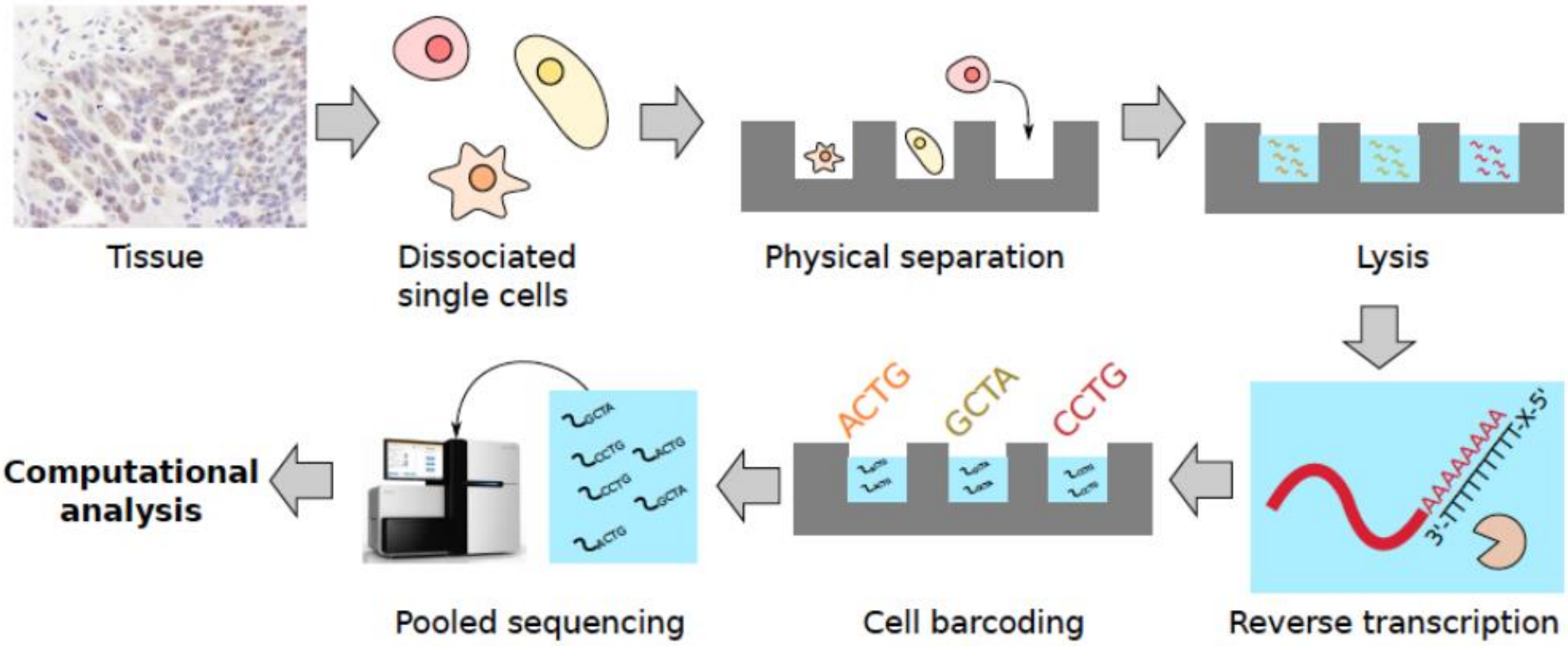
Single Cell Surface Protein
Single Cell Antigen Profiling
Single Cell Immune Repertoire Profiling





Single cell CRISPR Screening
Perturb-Seq (Adamson et al., 2016, and Dixit et al., 2016),
CRISP-seq (Jaitin et al., 2016),
CROP-seq (Datlinger et al., 2017), or
CRISPR-QTL (Gasperini et al., 2018)

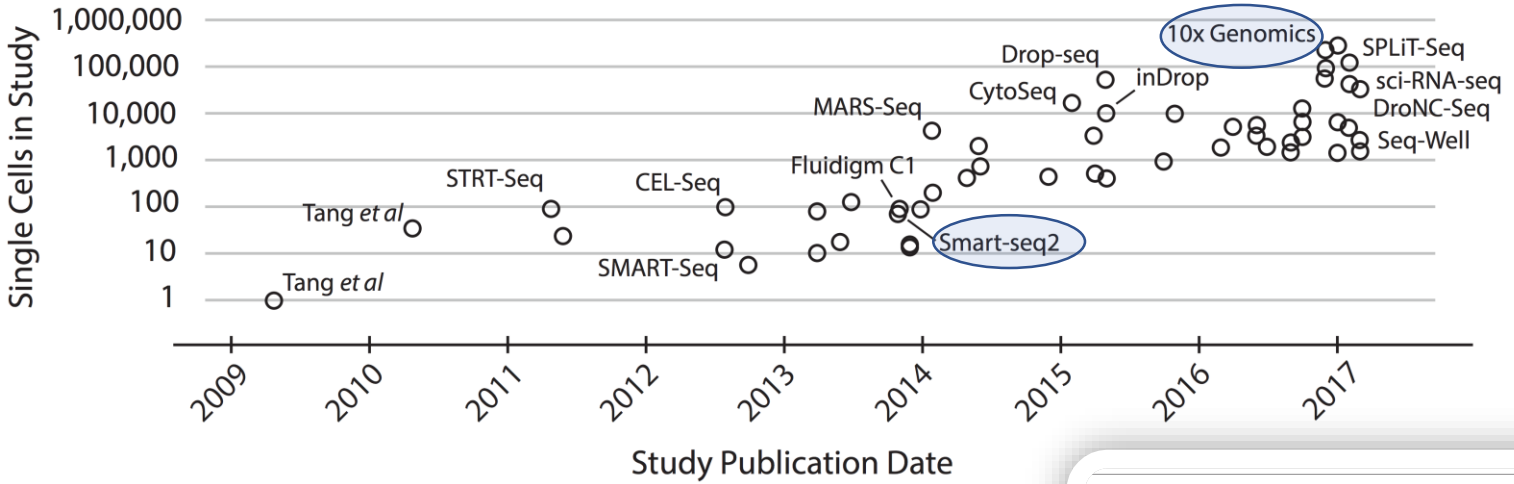
Single Cell RNA Sequencing Workflow (*scRNAseq*)

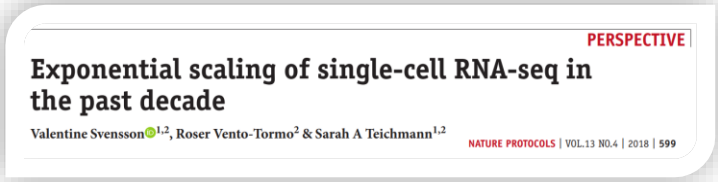


- Dissociation can be easy (blood) or hard (collagenous tissue)
- Separation and RT differ by protocol

Image courtesy of Aaron Lun

Experimental methods




PERSPECTIVE

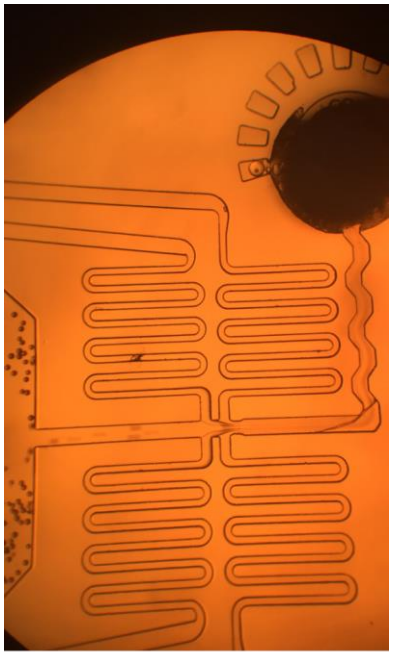
Exponential scaling of single-cell RNA-seq in the past decade

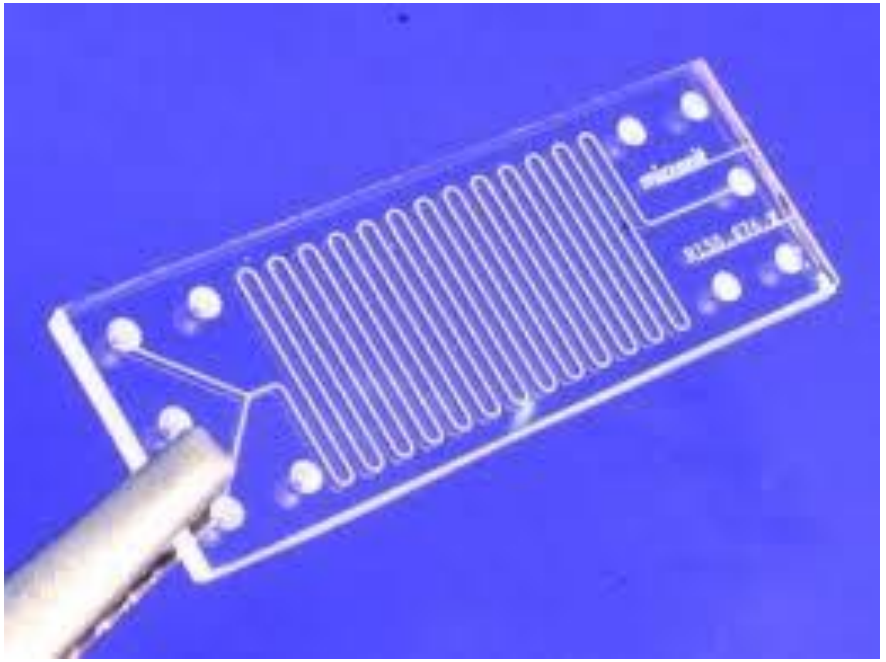
Valentine Svensson[1,2], Roser Vento-Tormo[2] & Sarah A Teichmann[1,2]

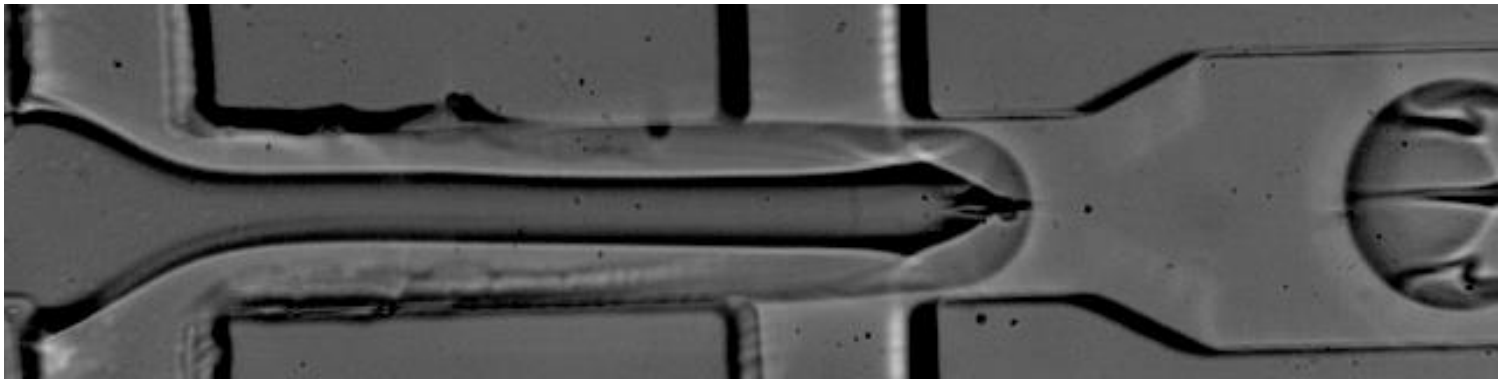
NATURE PROTOCOLS | VOL.13 NO.4 | 2018 | 599

Development of new methods and protocols for scRNA-seq is currently a very active area of research, and several protocols have been published over the last few years. A non-comprehensive list includes: CEL-seq (Hashimshony et al. 2012), CEL-seq2 (Hashimshony et al. 2016), Drop-seq (Macosko et al. 2015), InDrop-seq (Klein et al. 2015), MARS-seq (Jaitin et al. 2014), SCRB-seq (Soumillon et al. 2014), Seq-well (Gierahn et al. 2017), Smart-seq (Picelli et al. 2014), **Smart-seq2** (Picelli et al. 2014), SMARTer, STRT-seq (Islam et al. 2013)

Drop-seq: Assay Overview





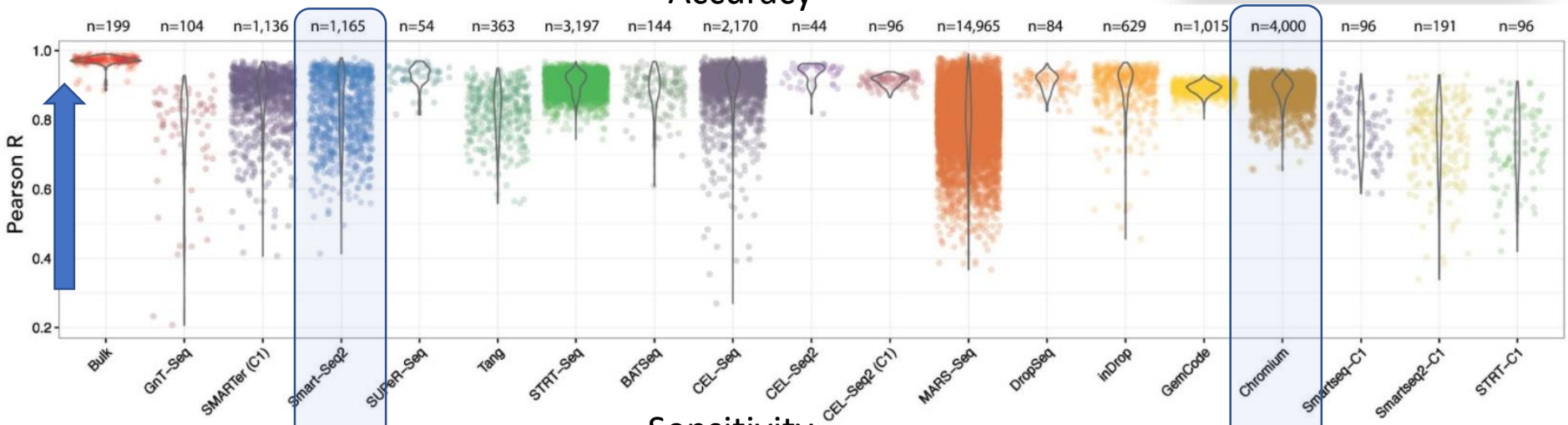


http://mccarrolllab.org/dropseq/

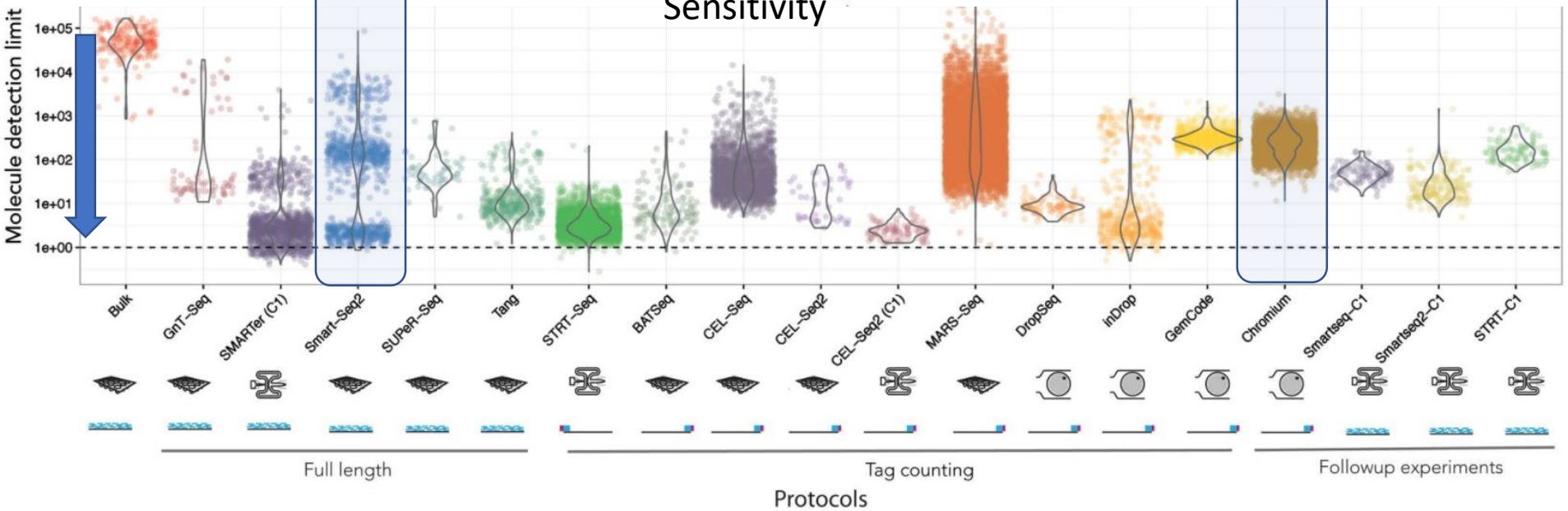
What Assay to use?

nature methods
Published: 06 March 2017
Power analysis of single-cell RNA-sequencing experiments
Valentine Svensson, Kedar Nath Natarajan, Lam-Ha Ly, Ricardo J Miragaia, Charlotte Labalette, Iain C Macaulay, Ana Cvejic & Sarah A Teichmann
Nature Methods 14, 381–387(2017) | Cite this article

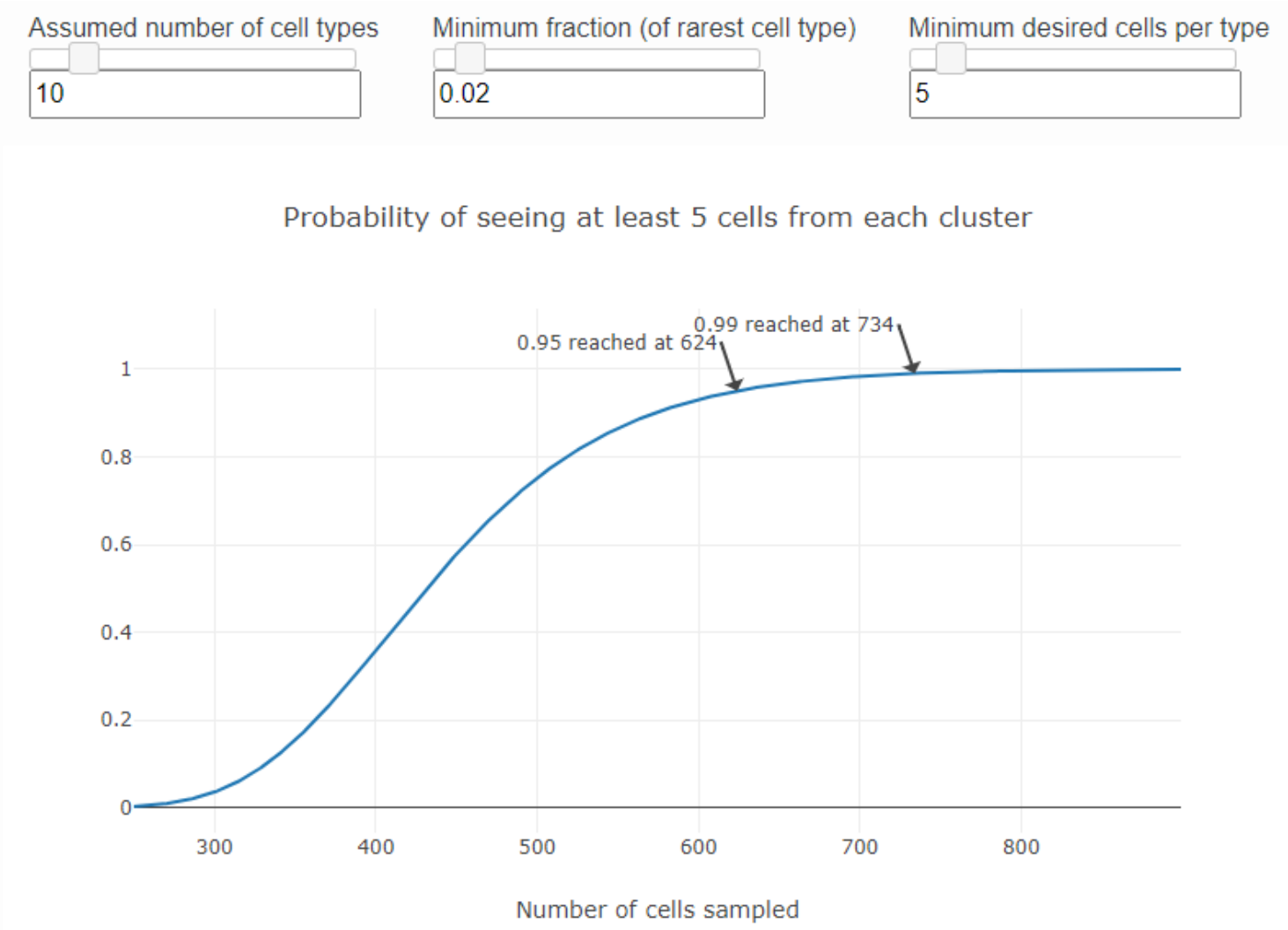
Accuracy



Sensitivity

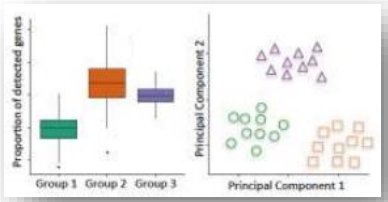


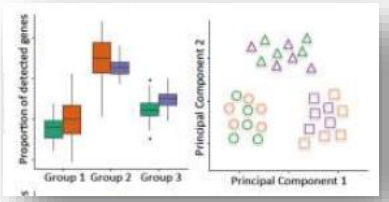
How many cells to sequence?



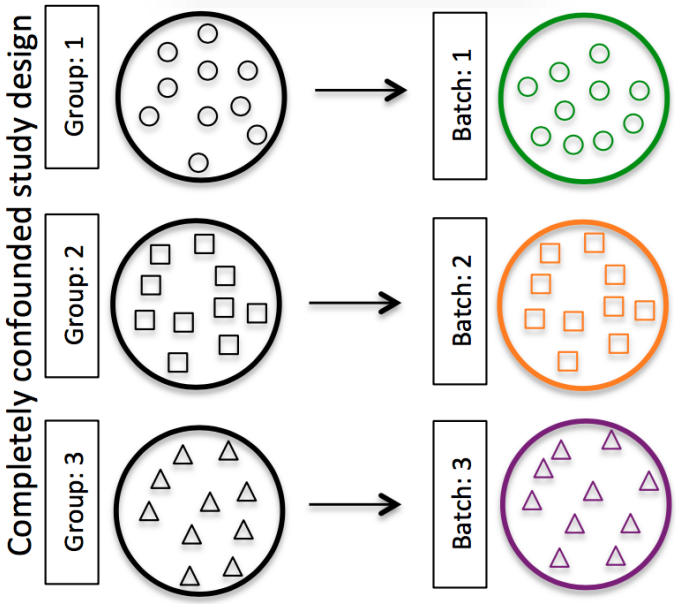
https://satijalab.org/howmanycells

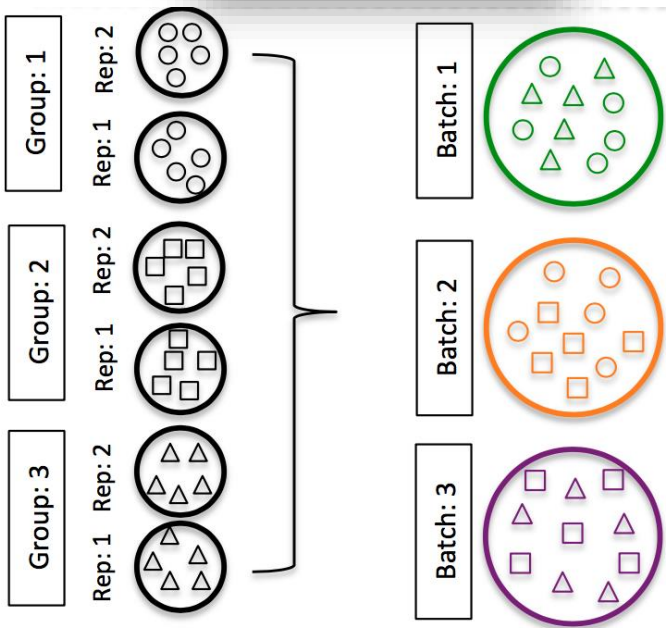
Confounding by Design

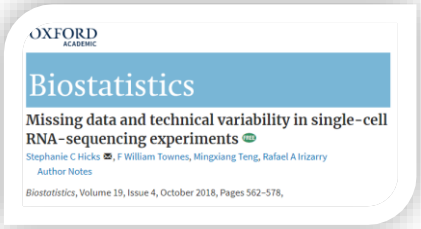




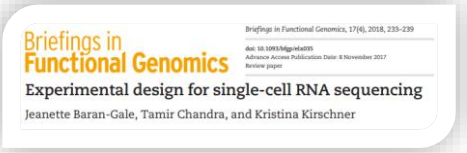
Completely confounded study design

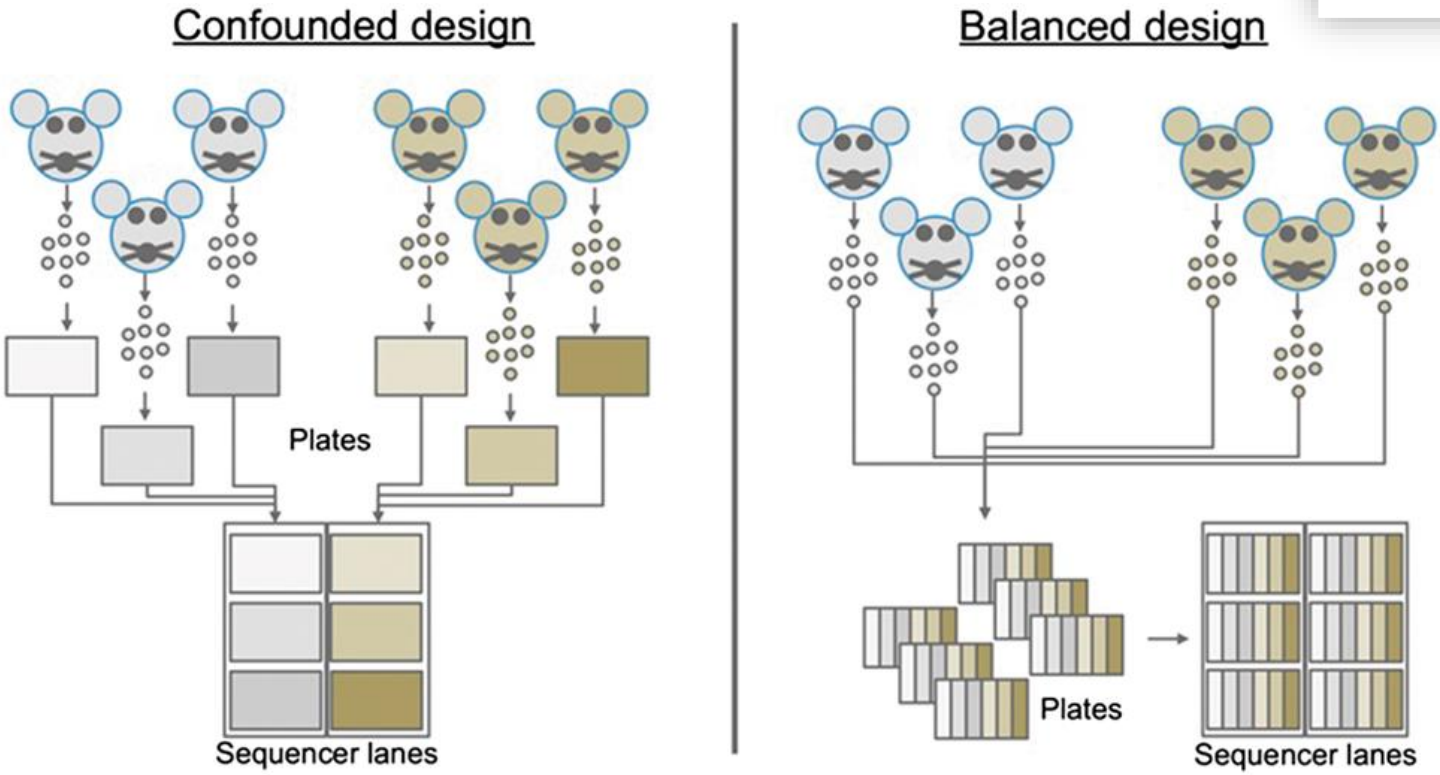






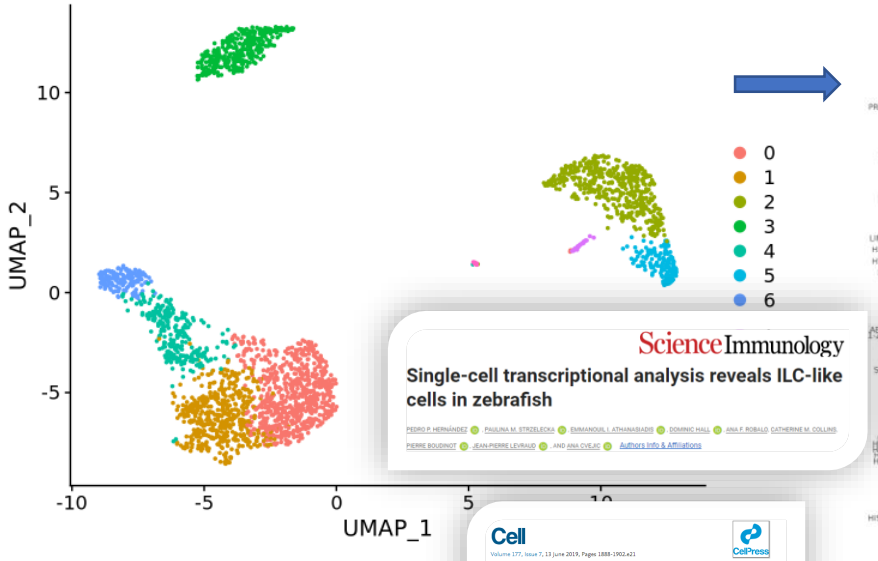
Confounding by Design

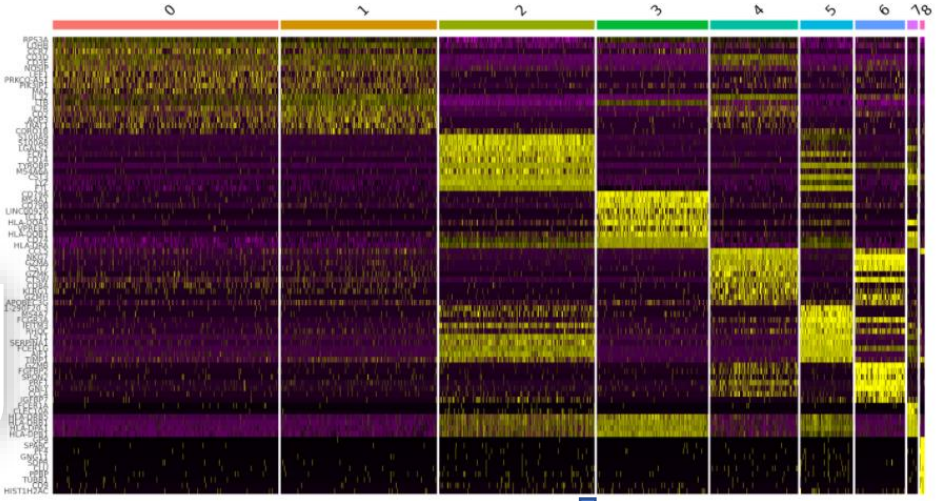


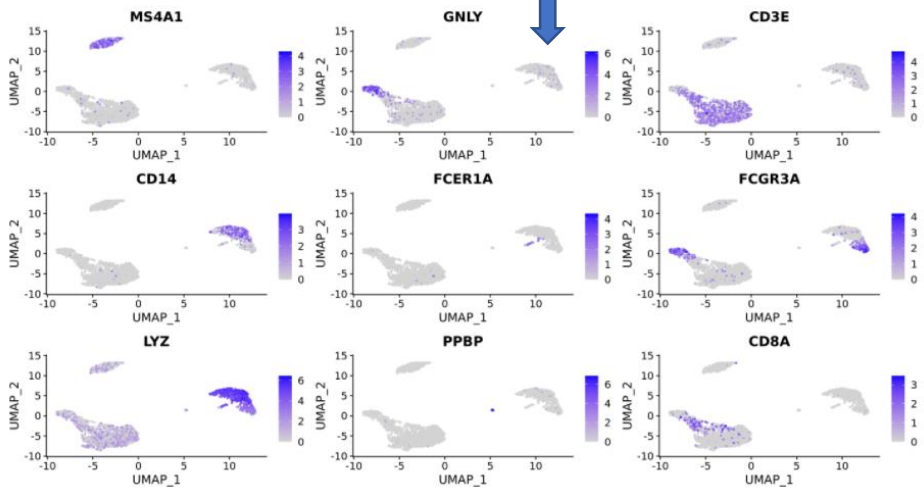


Computational analysis

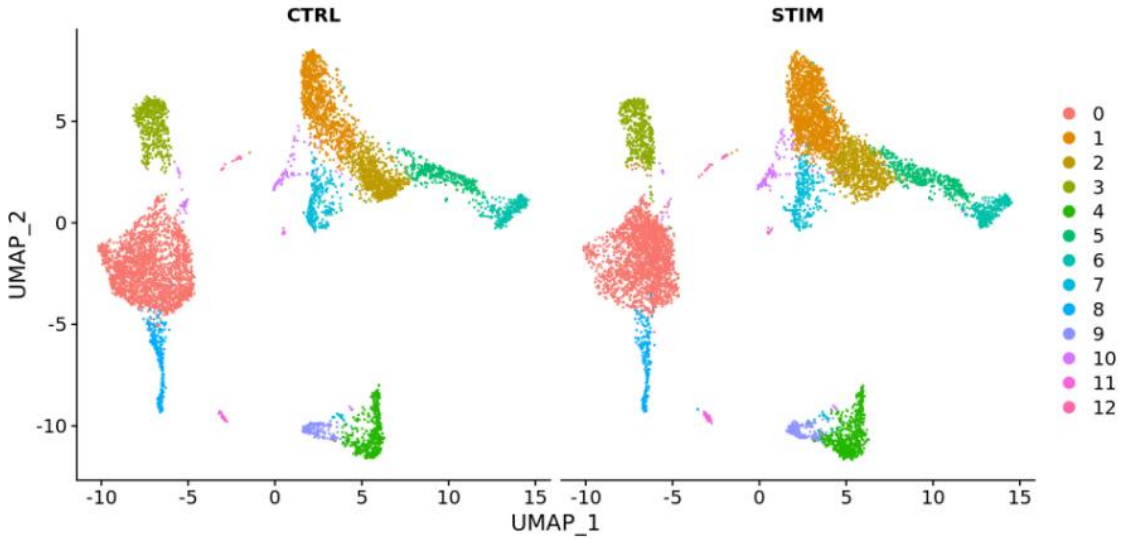
scRNAseq Analysis

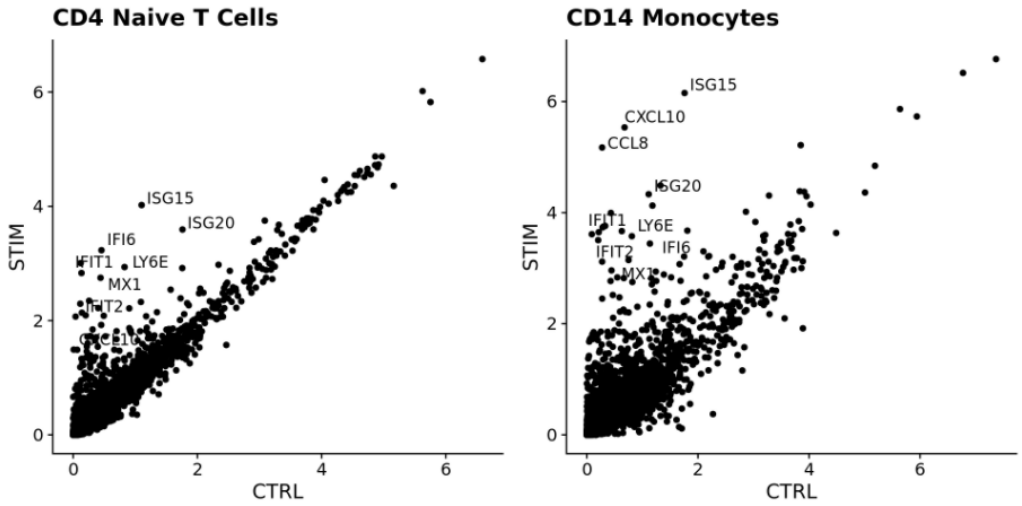


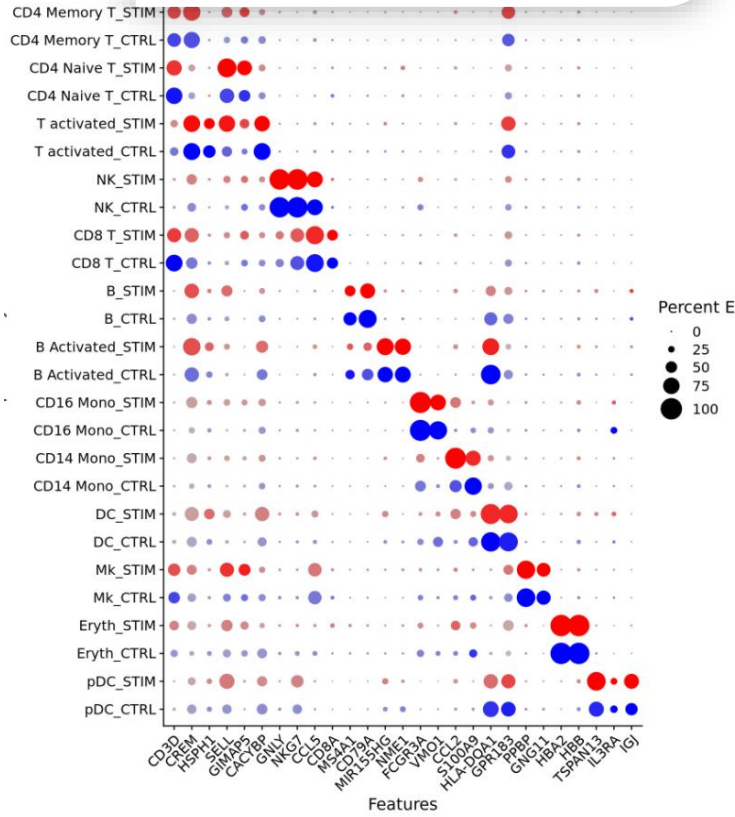




Comparative analysis (i.e. CTRL vs STIM)







Cell

Volume 177, Issue 7, 13 June 2019, Pages 1888-1902.e21

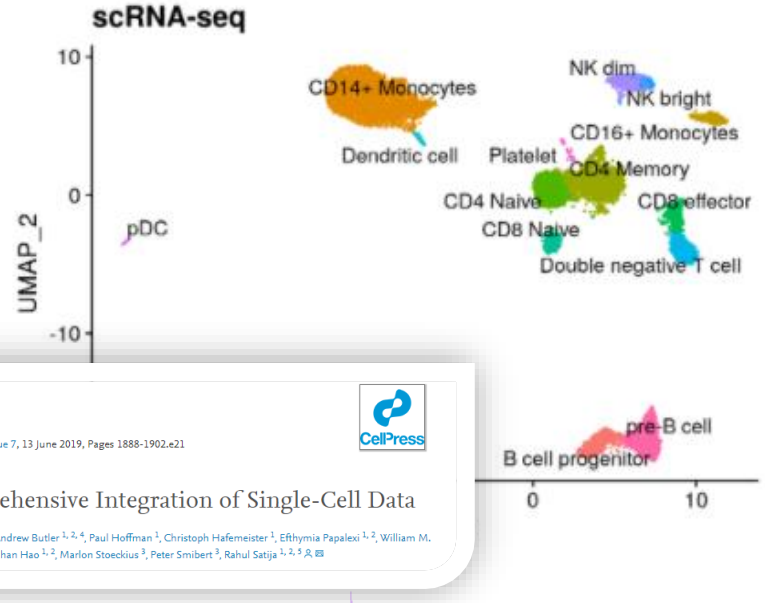
CellPress

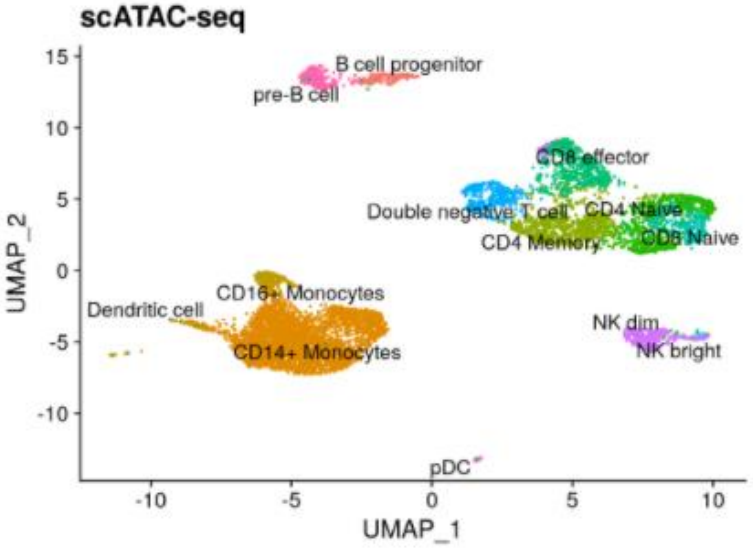
Resource

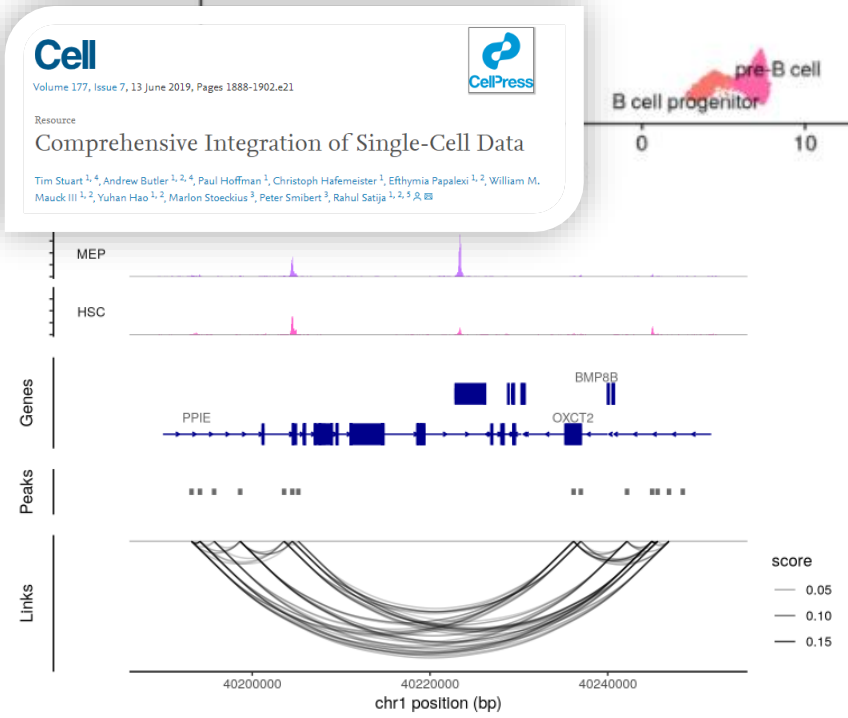
Comprehensive Integration of Single-Cell Data

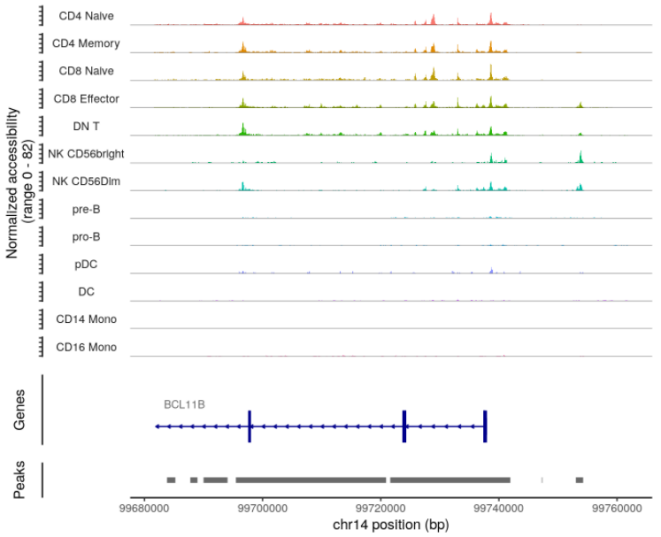
Tim Stuart [1, 4], Andrew Butler [1, 2, 4], Paul Hoffman [1], Christoph Hafemeister [1], Efthymia Papalexi [1, 2], William M. Mauck III [1, 2], Yuhan Hao [1, 2], Marlon Stoeckius [3], Peter Smibert [3], Rahul Satija [1, 2, 5]

scRNAseq / scATACseq integration









Cell
Volume 177, Issue 7, 13 June 2019, Pages 1888-1902.e21
CellPress
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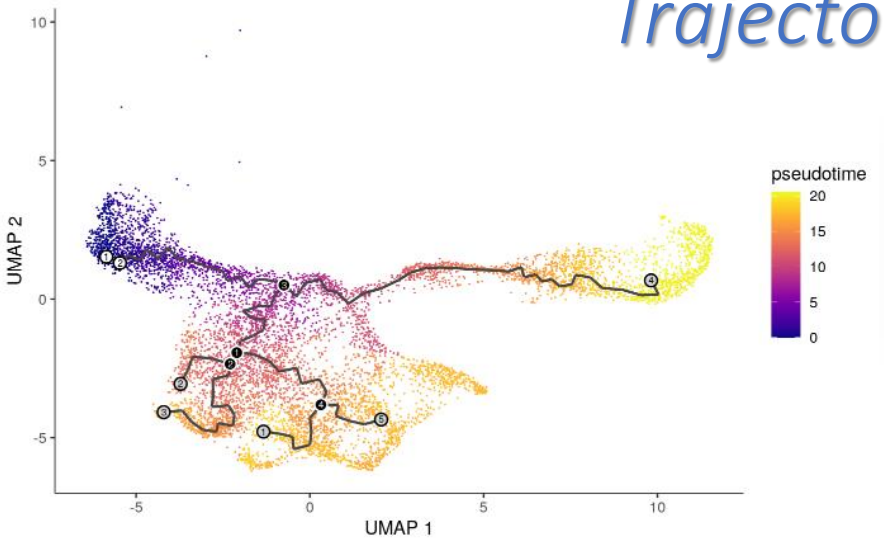
Comprehensive Integration of Single-Cell Data
Tim Stuart [1, 4], Andrew Butler [1, 2, 4], Paul Hoffman [1], Christoph Hafemeister [1], Efthymia Papalexi [1, 2], William M. Mauck III [1, 2], Yuhan Hao [1, 2], Marlon Stoeckius [3], Peter Smibert [3], Rahul Satija [1, 2, 5]

Trajectory analysis



nature methods

Reversed graph embedding resolves complex single-cell trajectories

Xiaojie Qiu, Qi Mao, Ying Tang, Li Wang, Raghav Chawla, Hannah A Pliner & Cole Trapnell

Nature Methods **14**, 979–982(2017) | Cite this article

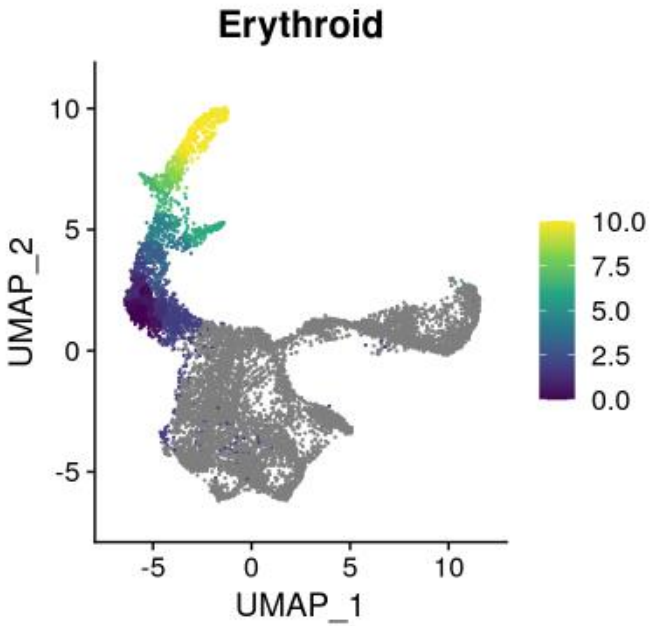
nature COMMUNICATIONS

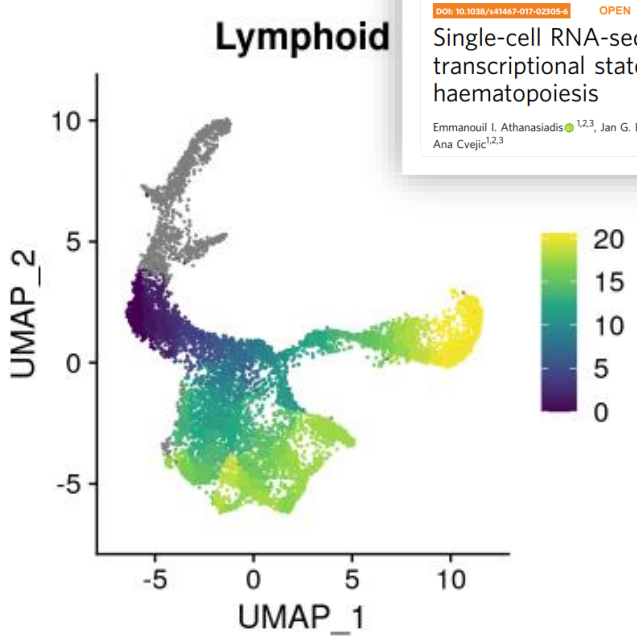
ARTICLE

DOI: 10.1038/s41467-017-02305-6 OPEN

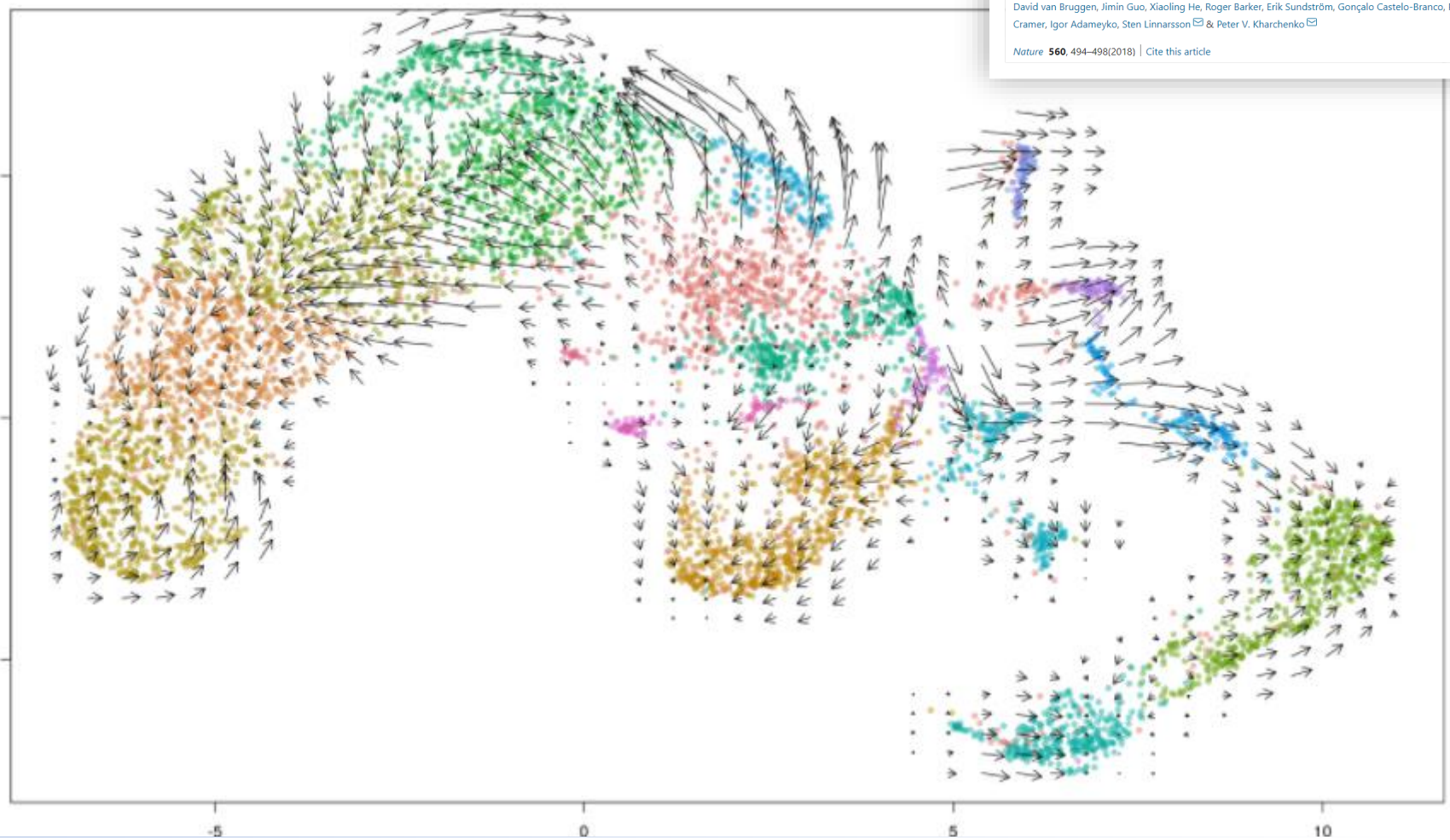
Single-cell RNA-sequencing uncovers transcriptional states and fate decisions in haematopoiesis

Emmanouil I. Athanasiadis[1,2,3], Jan G. Botthof[1,2,3], Helena Andres[4], Lauren Ferreira[1,2,3,5], Pietro Lio[4] & Ana Cvejic[1,2,3]





Estimating RNA Velocity



nature

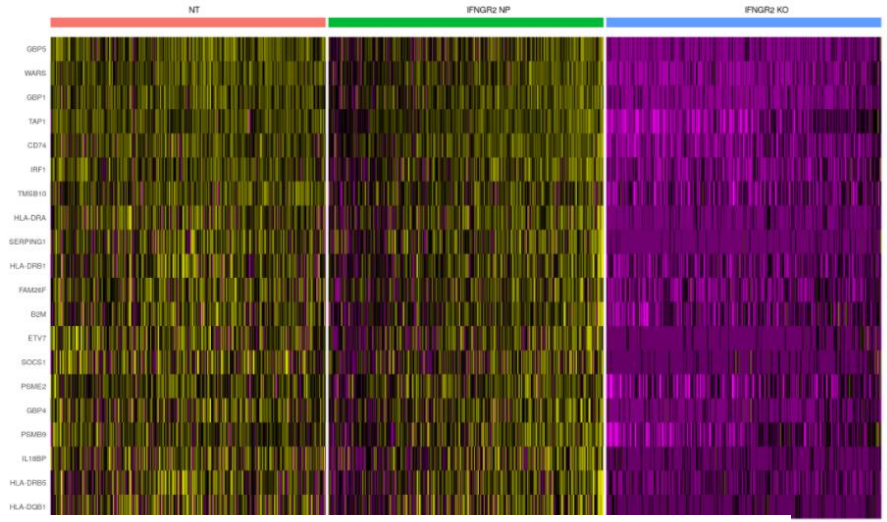
Letter | Published: 08 August 2018

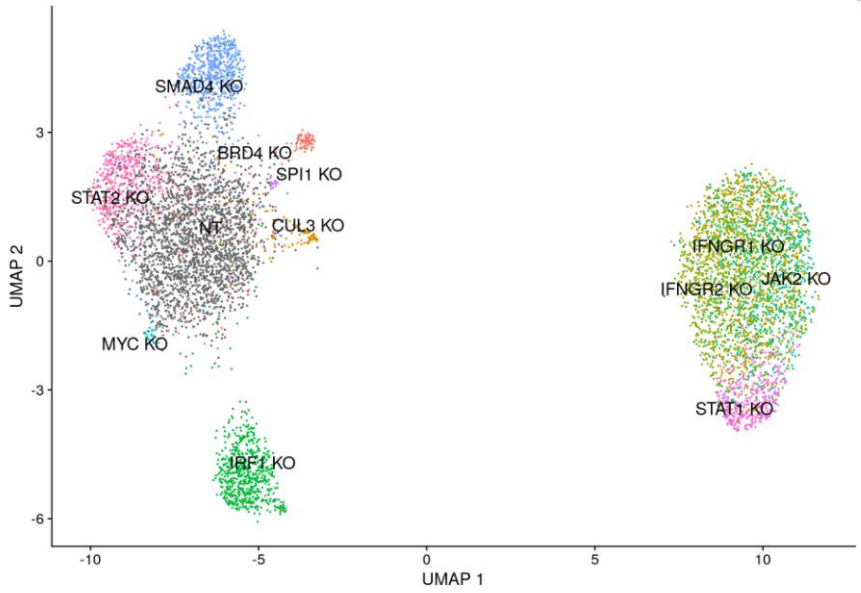
RNA velocity of single cells

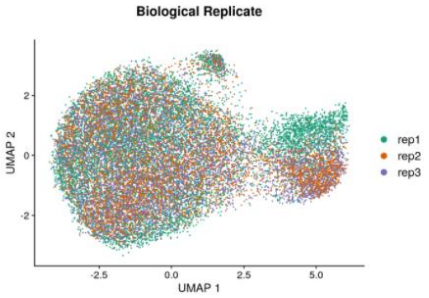
Gioele La Manno, Ruslan Soldatov, Amit Zeisel, Emelie Braun, Hannah Hochgerner, Viktor Petukhov, Katja Lidschreiber, Maria E. Kastriti, Peter Lönnerberg, Alessandro Furlan, Jean Fan, Lars E. Borm, Zehua Liu, David van Bruggen, Jimin Guo, Xiaoling He, Roger Barker, Erik Sundström, Gonçalo Castelo-Branco, Patrick Cramer, Igor Adameyko, Sten Linnarsson & Peter V. Kharchenko

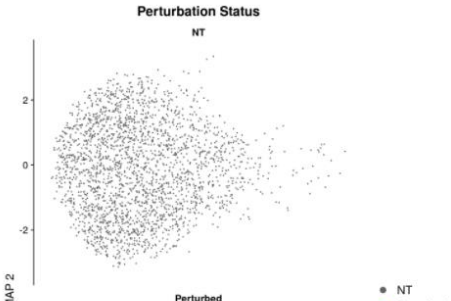
Nature **560**, 494–498(2018) | Cite this article

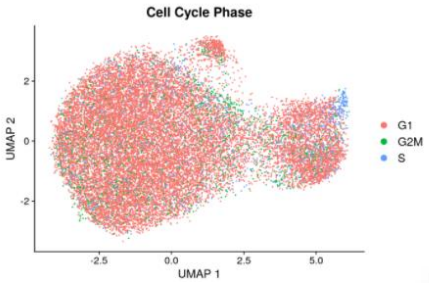
Detect Perturbations (pooled CRSIPR screens)

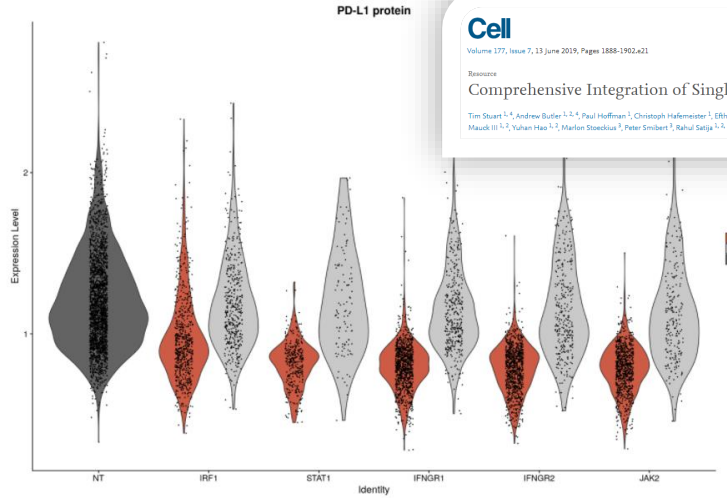




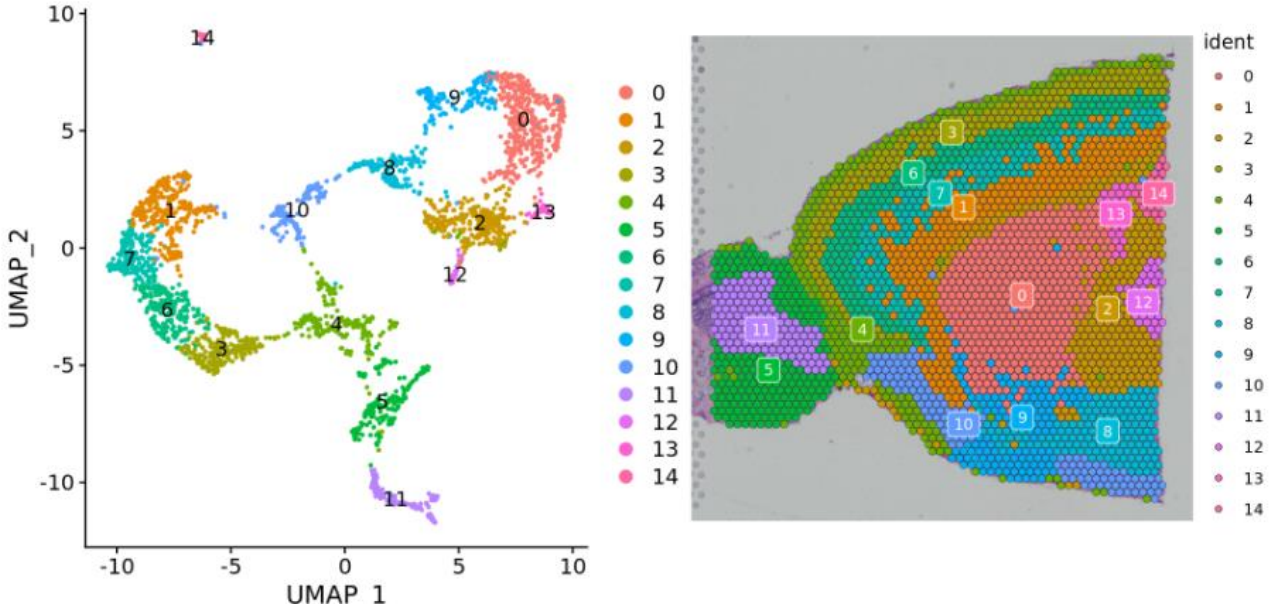


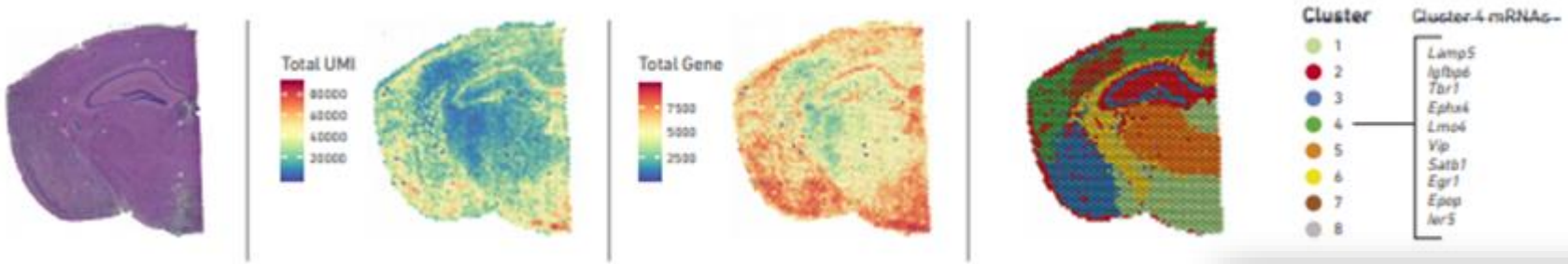






Spatial Transcriptomics





Cell
Volume 177, Issue 7, 13 June 2019, Pages 1888-1902.e21
Resource
Comprehensive Integration of Single-Cell Data

Overall recommendations

• **Do not perform** single-cell RNA-seq unless it is necessary for the experimental question of interest. Could you answer the question using bulk sequencing, which is simpler and less costly? Perhaps FACS sorting the samples could allow for bulk analysis?

• **Understand the details of the experimental question you wish to address**. The recommended library preparation method and analysis workflow can vary based on the specific experiment.

• **Avoid technical sources of variability**, if possible:
  - Discuss experimental design with experts prior to the initiation of the experiment
  - Isolate RNA from samples at same time
  - Prepare libraries at same time or alternate sample groups to avoid batch confounding
  - Do not confound sample groups by sex, age, or batch

Additional links to study

- https://satijalab.org/
- https://scanpy.readthedocs.io/en/stable/tutorials.html
- https://github.com/hbctraining/scRNA-seq/tree/master/lessons
- https://scrnaseq-course.cog.sanger.ac.uk/website/index.html
- http://www2.stat.duke.edu/~sayan/Sta613/2018/singlecellrnaseq-170131050320.pdf
- http://bioinformatics.org.au/ws17/wp-content/uploads/sites/13/2016/02/Joseph-Powell_1_2017-Winter-School.pdf
- https://bioinformatics-core-shared-training.github.io/cruk-summer-school-2018/SingleCell/slides/2018-07-25_CRUK_CI_summer_school-scRNAseq.pdf

Thank you for your attention