Single cell RNA sequencing data analysis, 2023

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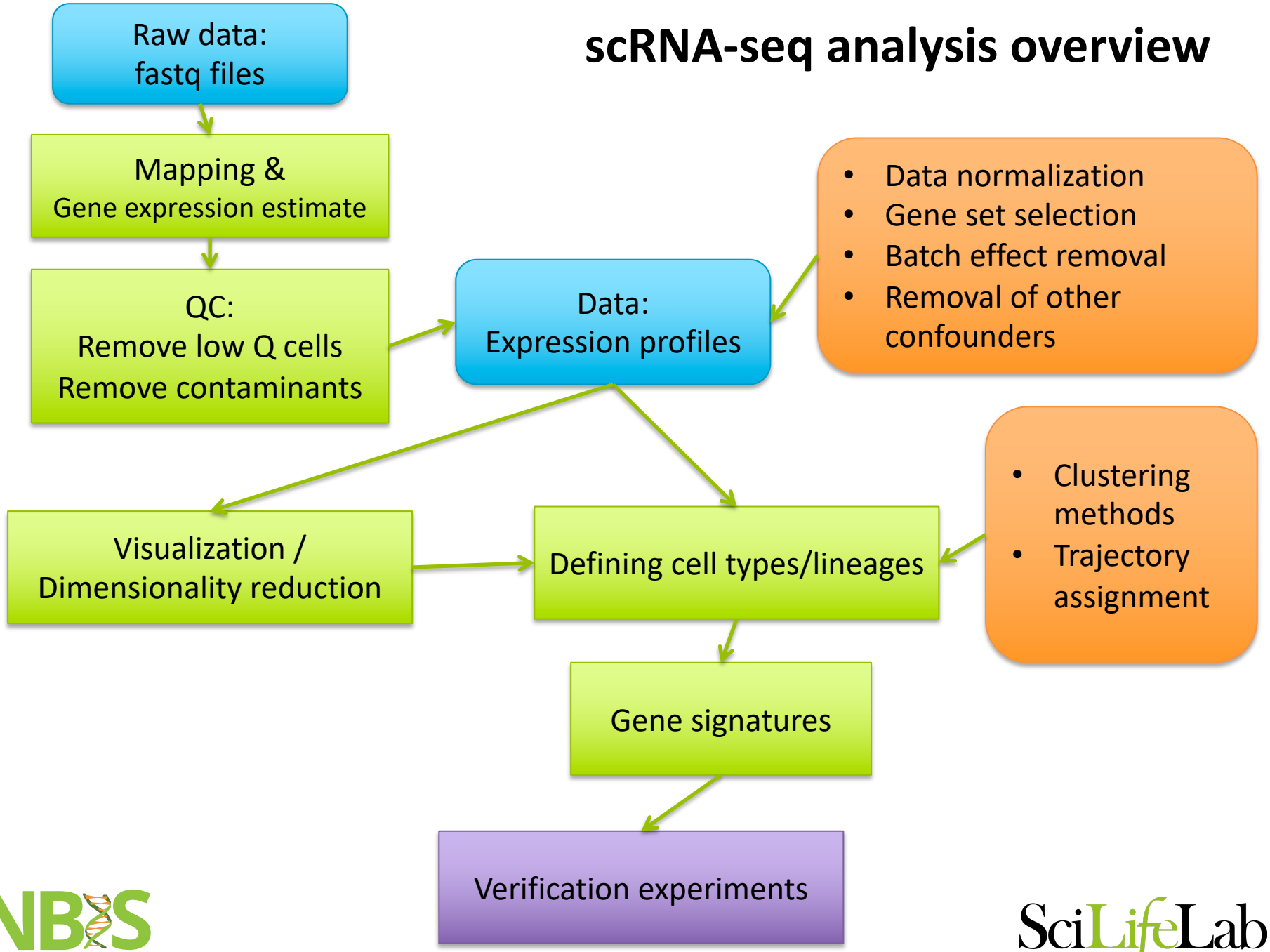
scRNA-seq analysis overview



Some take-home messages

- Data analysis is very seldom a straight line – one pipeline fits all.
  - Often requires several iterations of filtering data, exploring data, refiltering, exploring again, discovering technical artifacts, normalization, exploring again, etc. etc.

- Get to know your data – what types of variation do you have?
  - PCA is a good tool for exploring data
- Apply appropriate methods to control for problems that you see.

- Always check for:
  - Batch effects – think of all possible batches.
  - Cell cycle effects if appropriate
  - Separation due to nUMI / nGene / percent mito
- Both at the start of a project and at the end for your final clustering.

- Variable gene selection is a very critical step
  - Filter to much and you may lose populations
  - Keep to much and you may have too much noise

- Similar for choice of PCs

- Clustering – try out a few different approaches
  - Consensus of different methods gives confidence
  - If they do not agree – figure out why!

- Use your biological knowledge to evaluate the results
- Warning! Do not overfit your data to fit your initial hypotheses. Keep an open mind ;-)

- Remember that bioinformatics tools are giving predictions not the truth – always keep a critical mind!
  - Clustering
  - Differential expression
  - GSEA
  - Celltype prediction
  - Deconvolution

- In this course we point out many of the problems that can occur..
- Do not worry too much, in most cases, a standard workflow works well!

- scRNAseq analysis is a fast evolving field with new methods being published all the time.
  - Try to keep up with development
  - **BUT!** You cannot test every new method out there!

Reproducible research in R

- R / Rstudio in Docker containers
  - https://www.andrewheiss.com/blog/2017/04/27/super-basic-practical-guide-to-docker-and-rstudio/
  - https://github.com/rocker-org/rocker
- OBS! On Uppmax – only Singularity containers are allowed. Most Docker images can be converted.
- Learn more on containers etc:
  - http://nbis-reproducible-research.readthedocs.io/en/latest/
- Rstudio package management – Packrat
  - https://rstudio.github.io/packrat/
- Conda installations of packages – can use conda on both bianca and rackham – `module load conda`

NBIS course in reproducible research:
https://uppsala.instructure.com/courses/73110

Compute resources

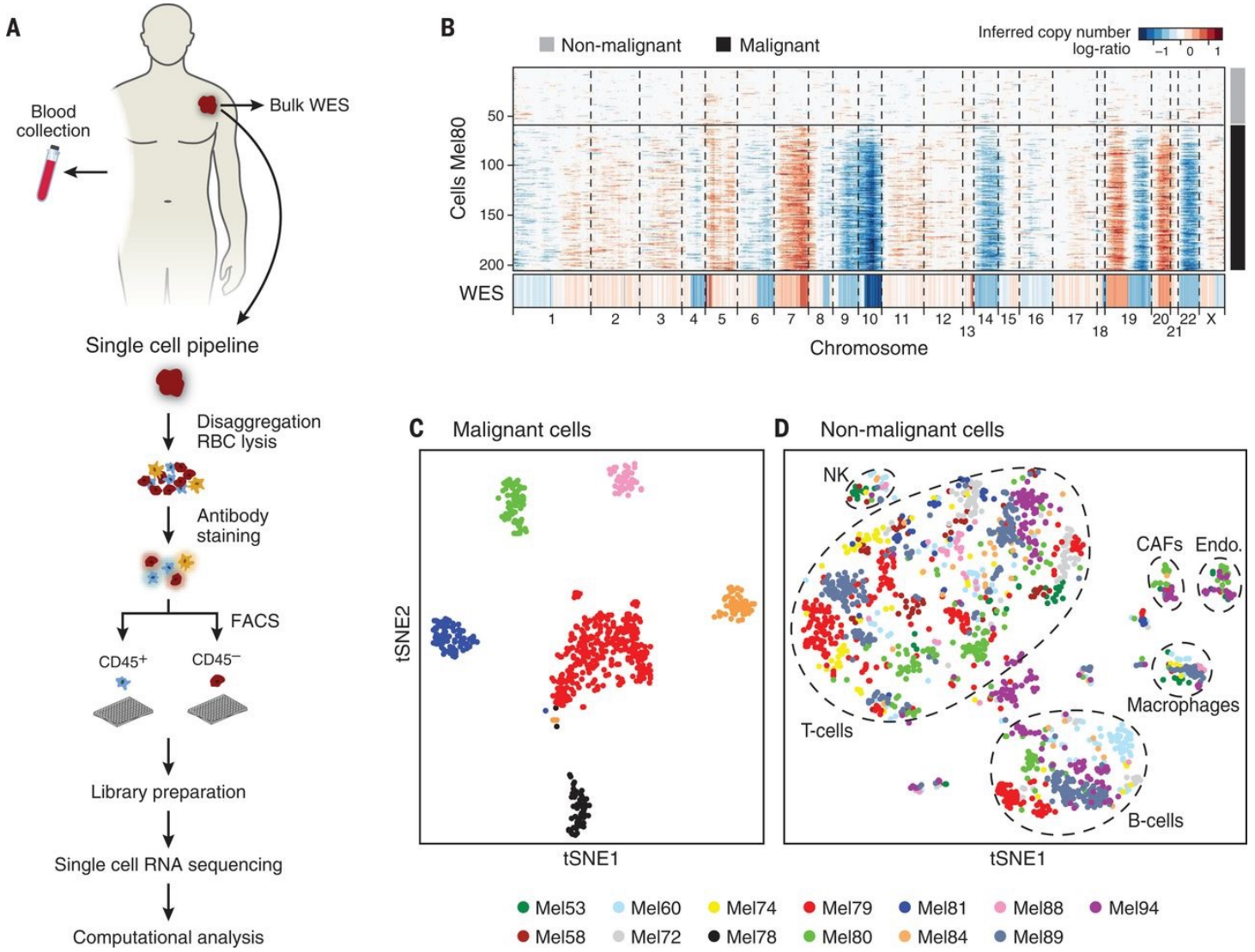
- In these exercises the datasets were small, but you may have many more cells/samples.
- Structure your code to avoid duplication of matrices and expansion of sparse matrices
  - `rm() & gc()`
- Plan ahead for compute resourses, local computer, uppmax or other HPC clusters.
- Human data – raw reads only on encrypted servers like Bianca. Count matrices is fine to use in other places.

Some conda comments

- Conda disc space usage
- `conda env remove –n myenv`
  - Will remove an environment
- `conda clean –all`
  - Will remove all tarballs and packages that are not used.

- We have covered the basic processing, but there is much more you can do…

Copy-number variation (CNV) profiling with RNAseq



(Tirosh et al. *Science* 2016)

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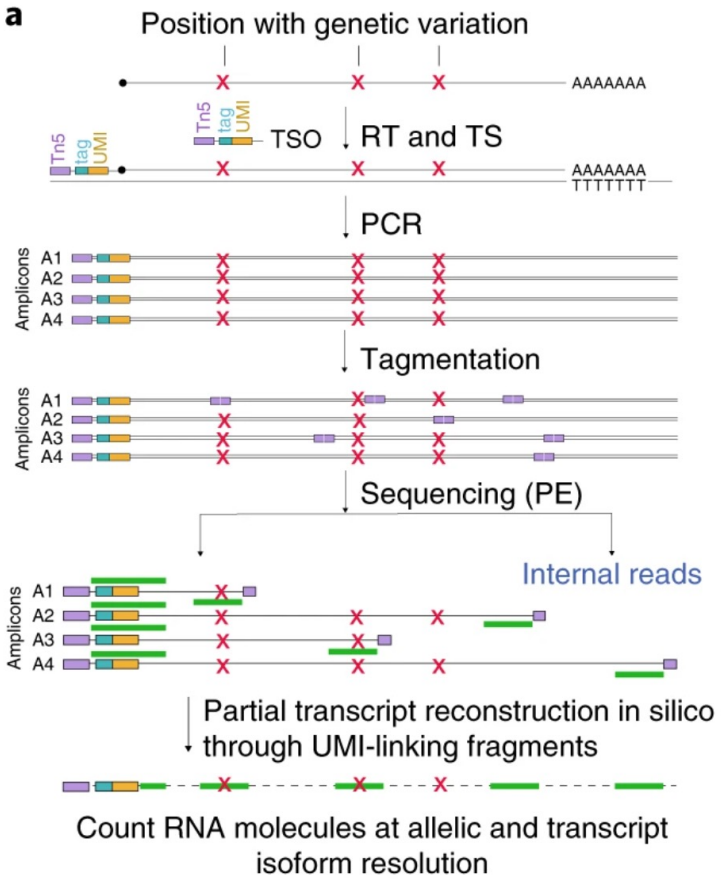
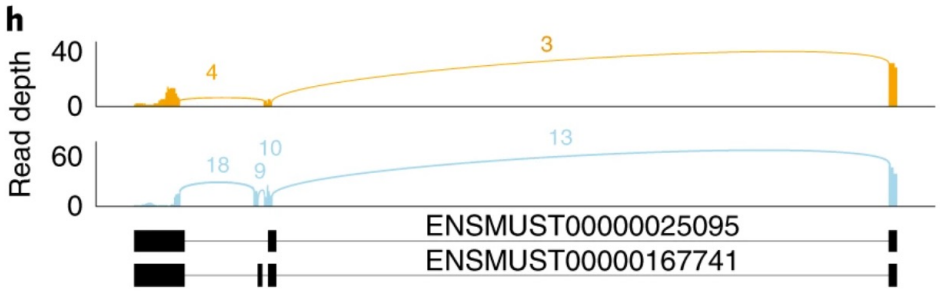
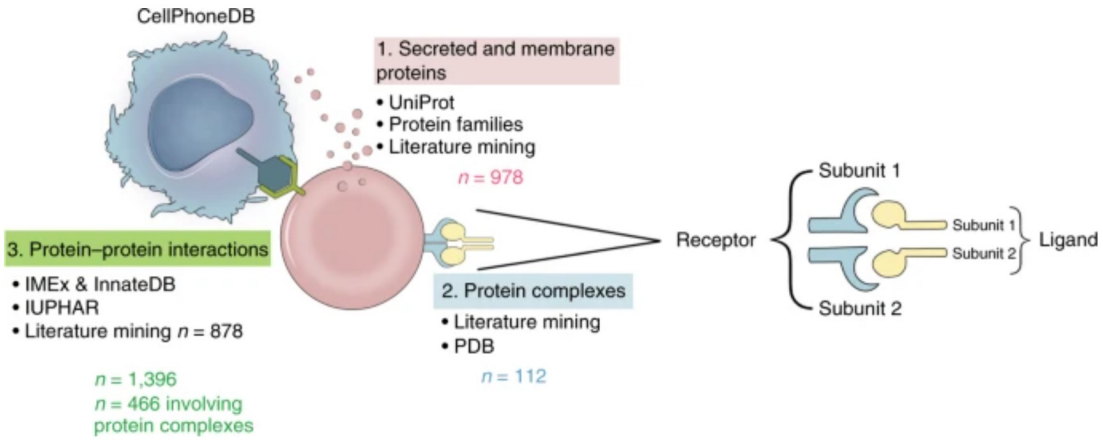
Allele and isoform information with SmartSeq3

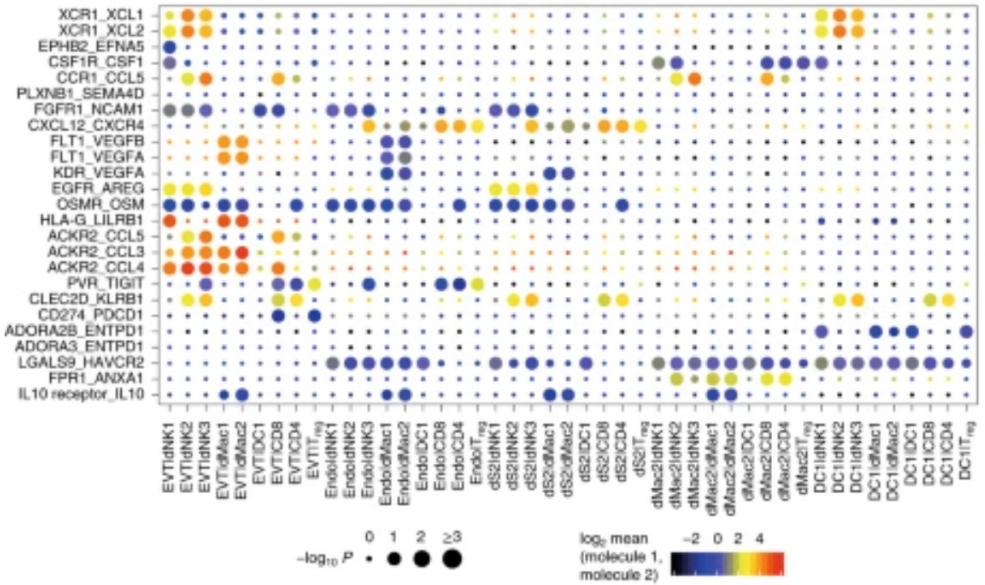




(Hagemann-Jensen et al. Nat. Biotech 2020)

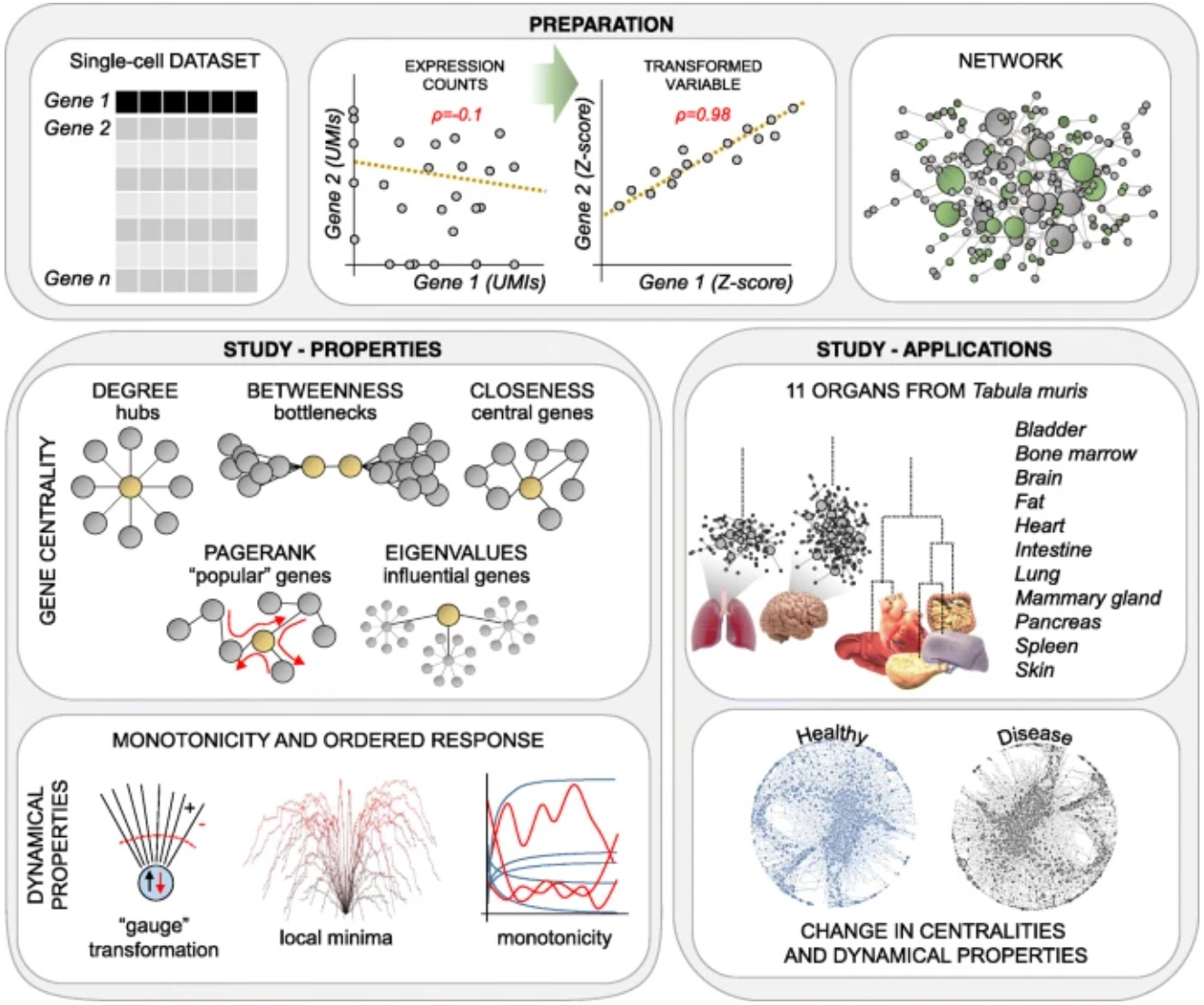
Receptor ligand interaction





(Efremova et. Al. Nat. Protocols 2020)

Gene regulatory networks



(Iacono et al. Genome Biology 2019)

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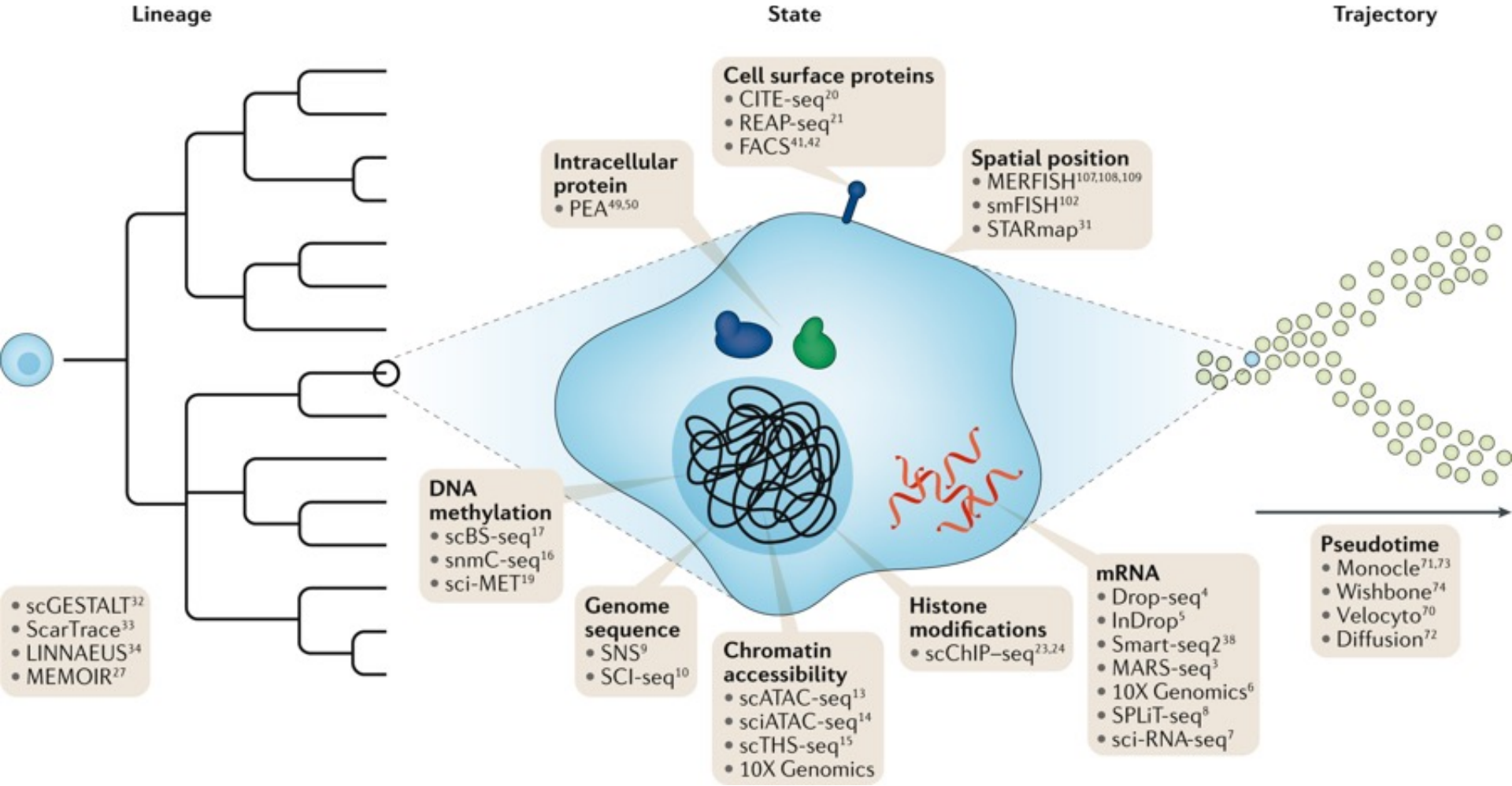
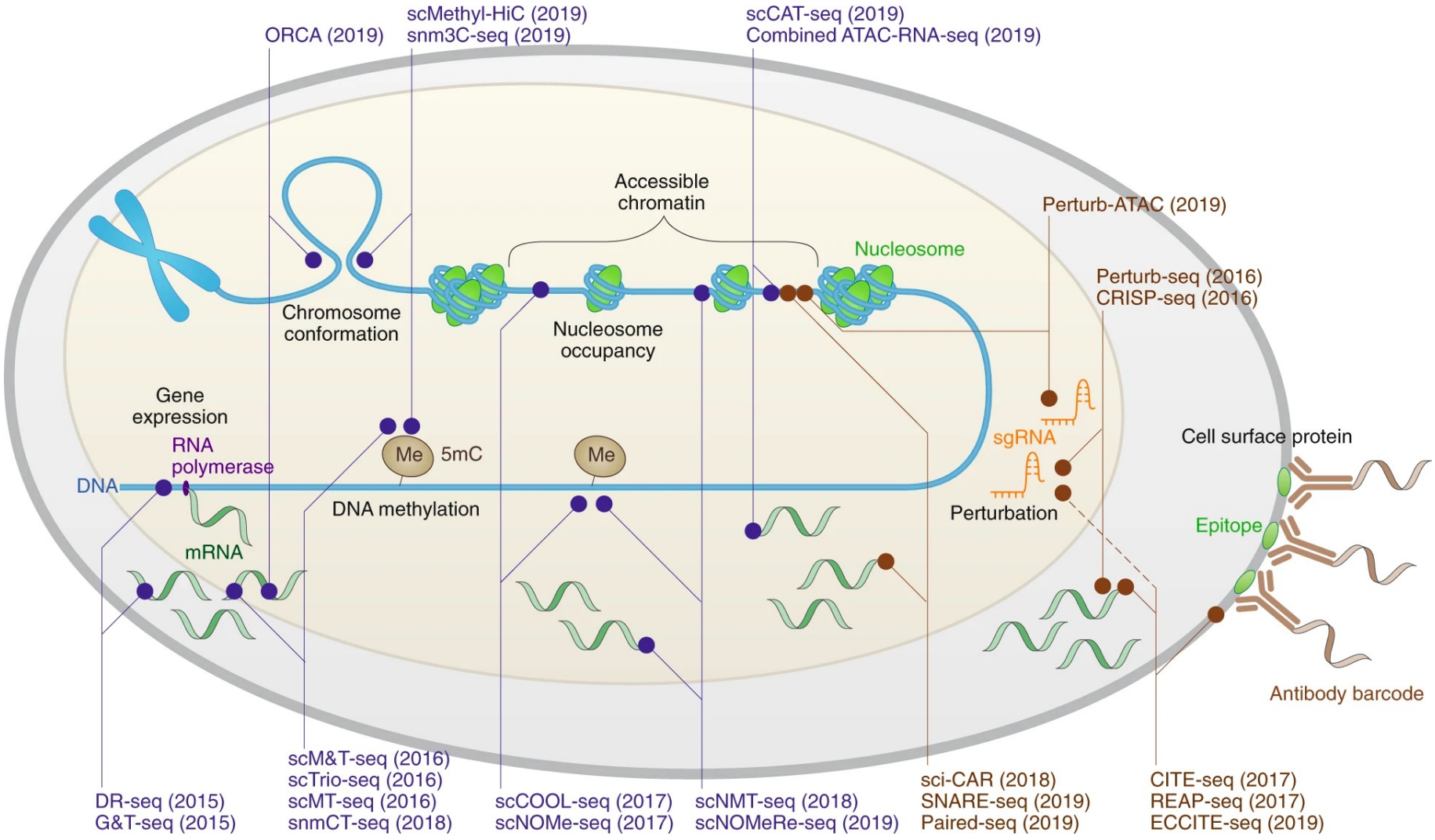
Single cell omics



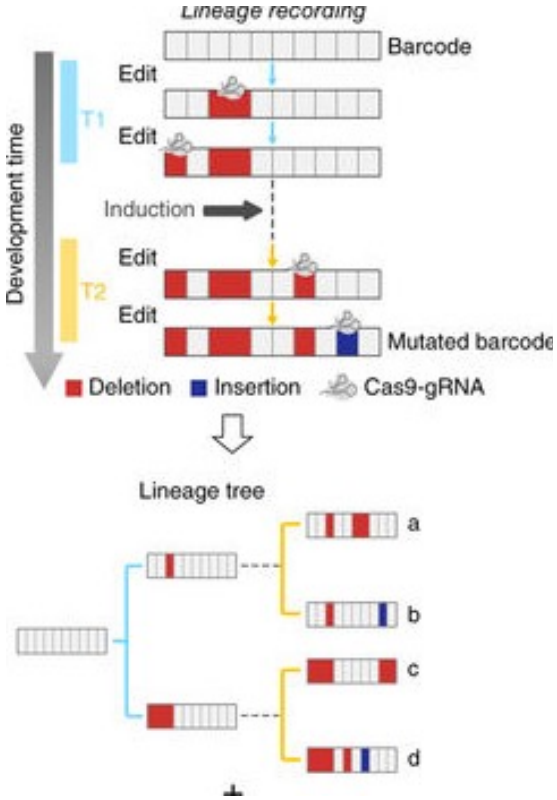
(Stuart & Satija, Nature Rev. Genetics 2019)

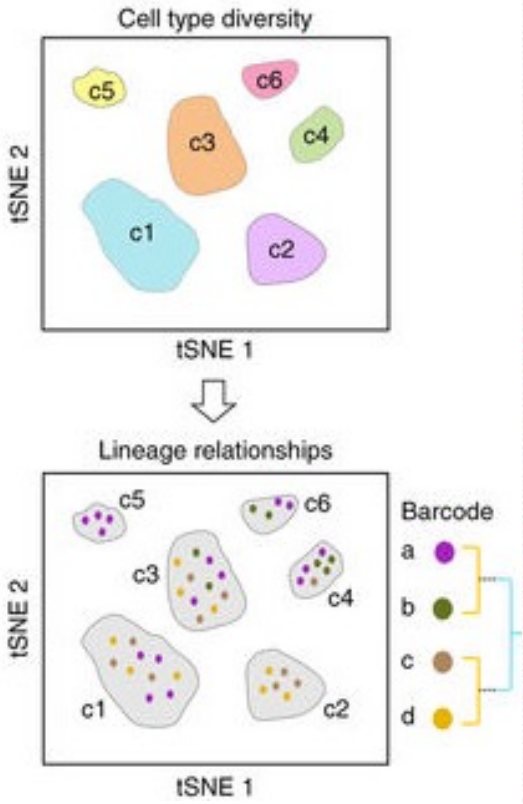
SC Multimodal omics

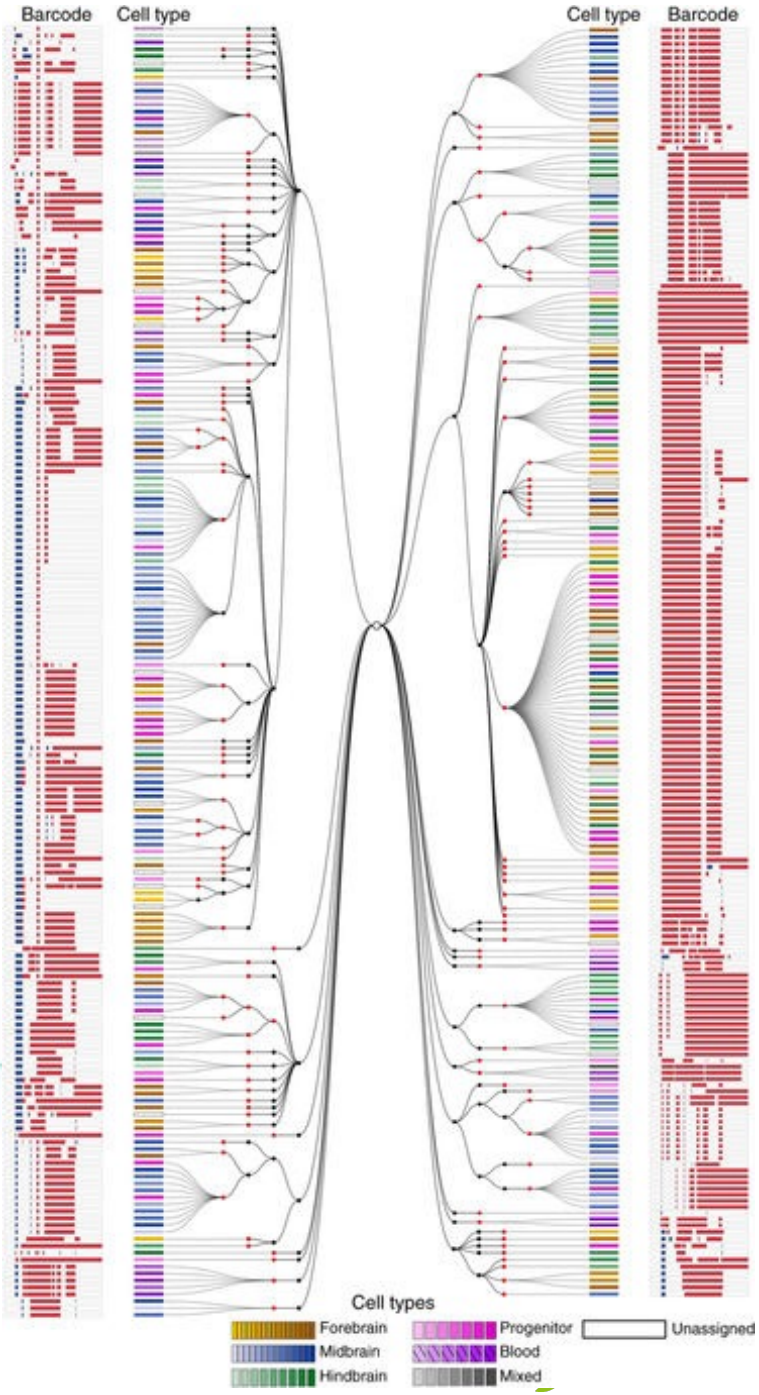


(Zhu et al, Comment in Nature Methods, 2020)

scGESTALT – lineage tracing and cell profiling with CRISPR-Cas9 editing of barcodes







(Raj et al. Nature Biotech 2018)

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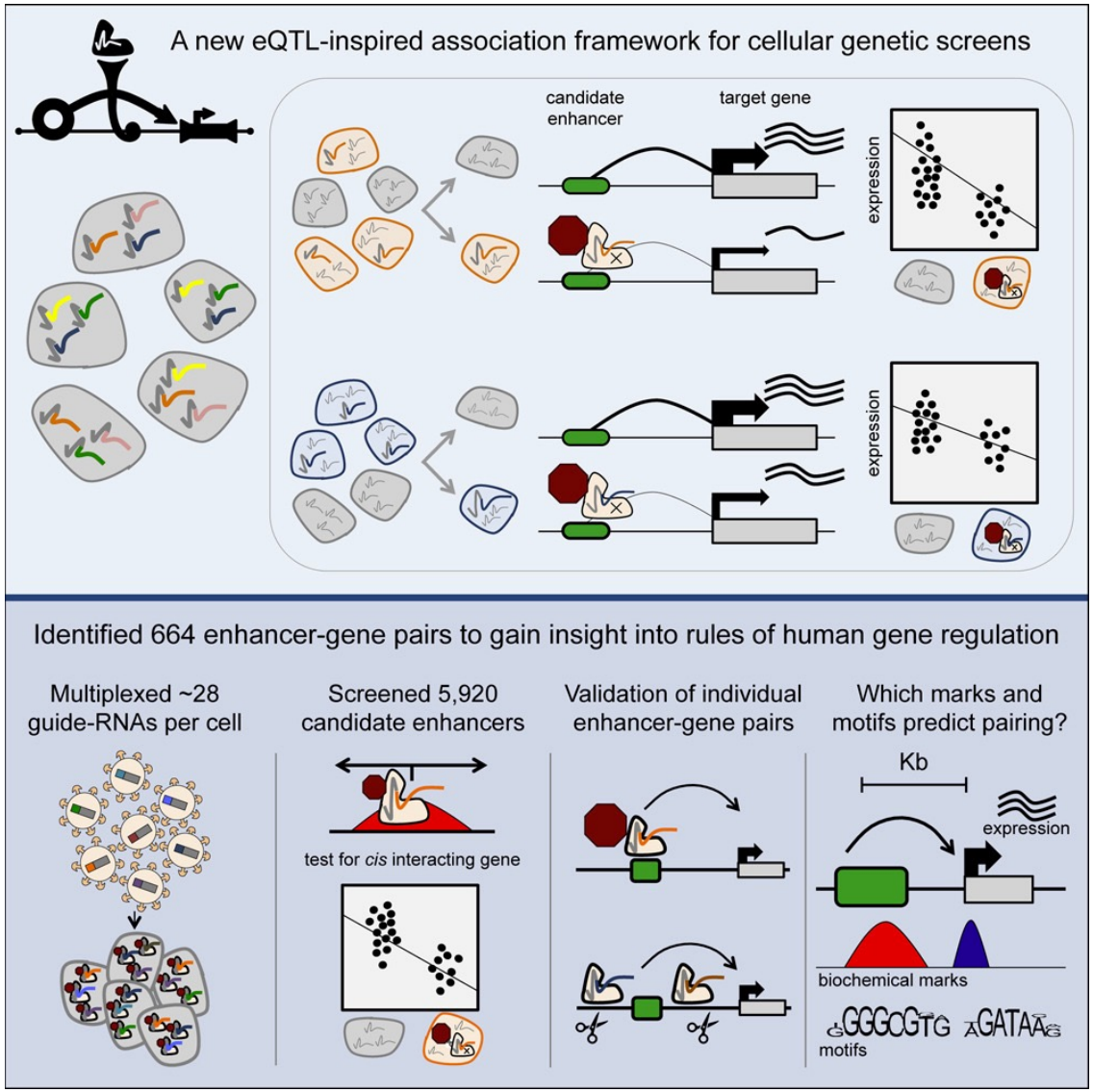
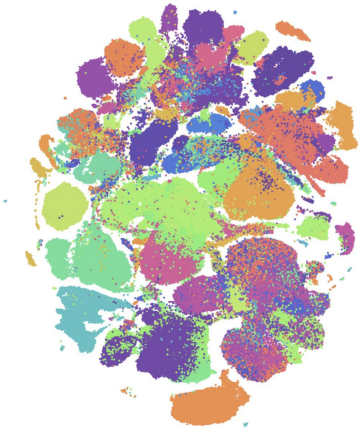
crisprQTL mapping for enhancer-gene pairs



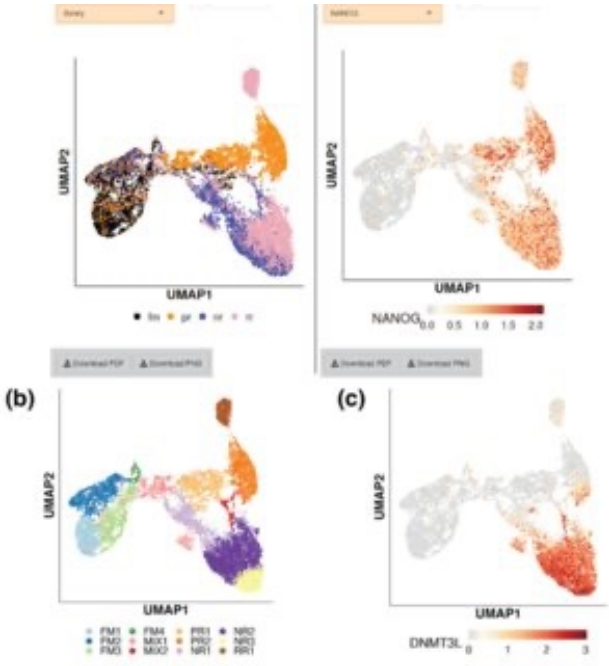
(Gasperini et. al. Cell 2019)

Interactive visualization

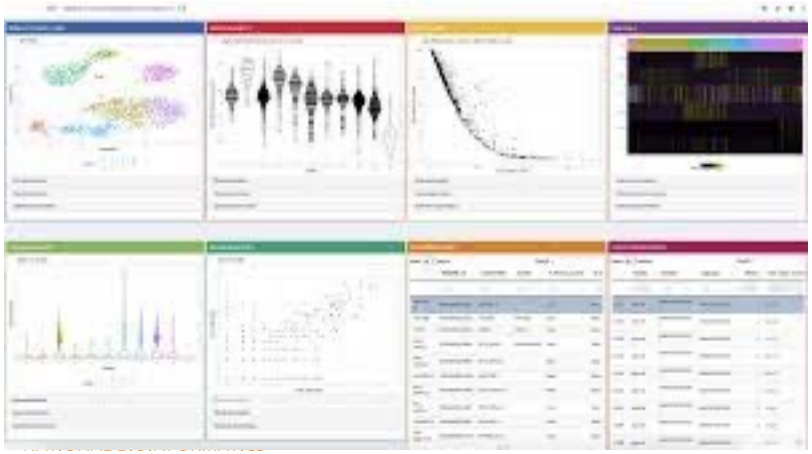
Cellxgene



Shinycell



iSEE



TissUUmaps

Resources

- Course at: https://hemberg-lab.github.io/scRNA.seq.course/
- Scanpy course: https://www.sc-best-practices.org/
- Orchestrating Single-Cell Analysis with Bioconductor http://bioconductor.org/books/3.13/OSCA/
- Many of the packages have good tutorials on their websites
- Repo with scRNA-seq tools: https://github.com/seandavi/awesome-single-cell
- Single cell assay objects for many datasets: https://hemberg-lab.github.io/scRNA.seq.datasets/
- EBI Single cell expression atlas: https://www.ebi.ac.uk/gxa/sc

Need help?

- NBIS project support
- Courses in programming and other types of analyses.
- Drop-in sessions every Tuesday 14.00
- More info at: http://nbis.se/

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Please fill in the Evaluation Form

Your feedback is important so that we can help improve the course.

Good luck with your analyses!

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