

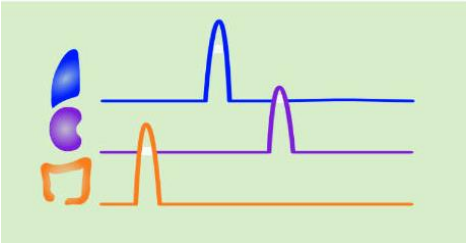
Single cell ATACseq detecting epigenetic heterogeneity

Ulrike Litzenburger

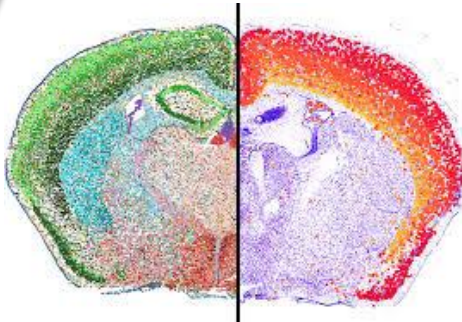
ulrike.litzenburger@nuvisan.com

from bulk to single cell sequencing

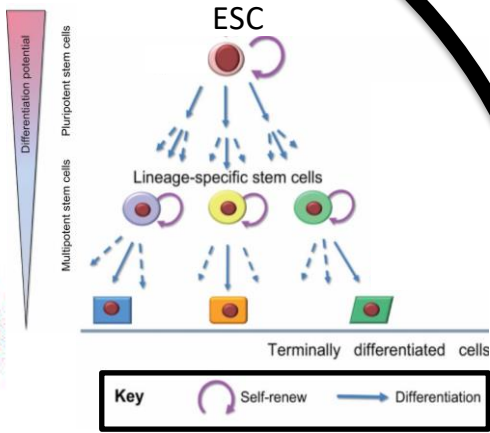
bulk sequencing



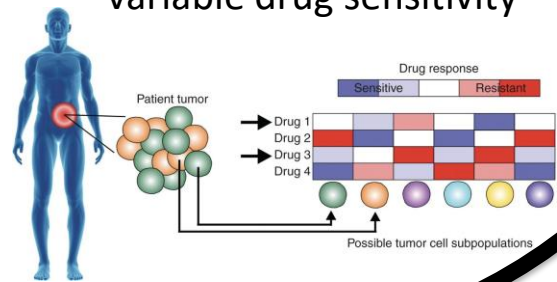
Rare cell populations



Cell type, cell state and non synchronous differentiation



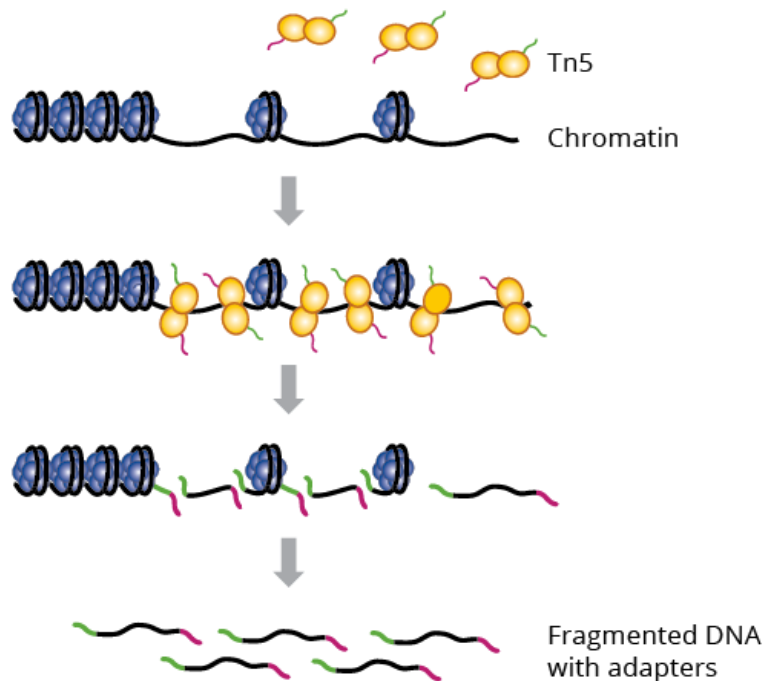
variable drug sensitivity



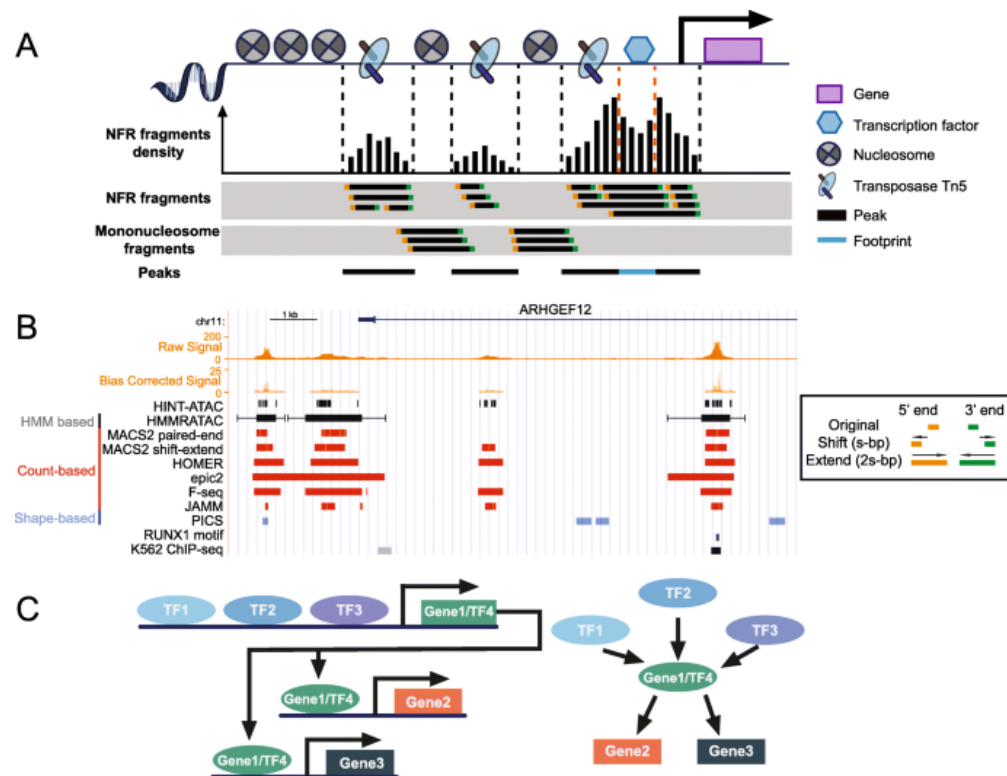
In need of a technique able to analyze epigenetic heterogeneity on a single cell level

Measuring chromatin accessibility using Tn5

key steps in ATACseq



from reads to insights



Scaling of bulk ATAC-seq protocol to single cell level

- Volume and concentrations: maintaining the Tn5 number per cell ratio consistent
- Small volumes makes FACS based assay difficult
- Limited inlet/outlets of the microfluidic device

bulk ATACseq

10,000 cells, in 50 μ l reaction Volume
66 ng DNA with ~2% open chromatin = ~1.32 ng
2 centrifugation steps
1 cleanup step

single cell ATACseq

96 single cells in 4.5 nL/cell
6.6 pg DNA per cell, 0.132 pg open chromatin
no centrifugation
no cleanup

What to expect: SPARSE DATA!

- 2% accessible genome of 3.0×10^9 bp = 60×10^6 bp
 - Average insert size distribution: 350 bp
 - Loss of ~50% due to method limitation
- => ~ 85 k unique fragments per cell
=> Diploid genome so only 2 reads max per location

Microfluidics based scATAC-seq



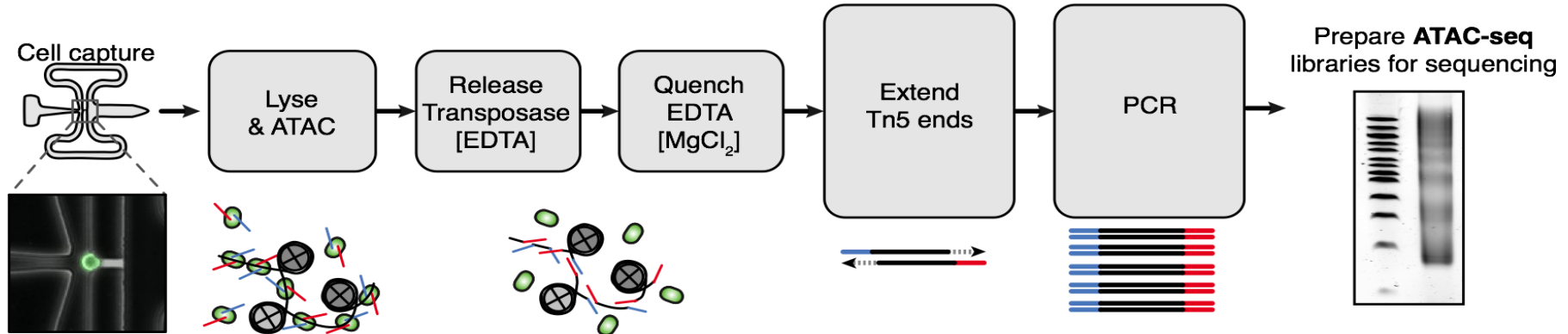
Maximum of 96 cells per C1 chip

Closed system – all reactions on one chip

Short hands-on time

Imaging on chip with wide field

Cell size restricted



since 2015 a lot of Tech dev happened

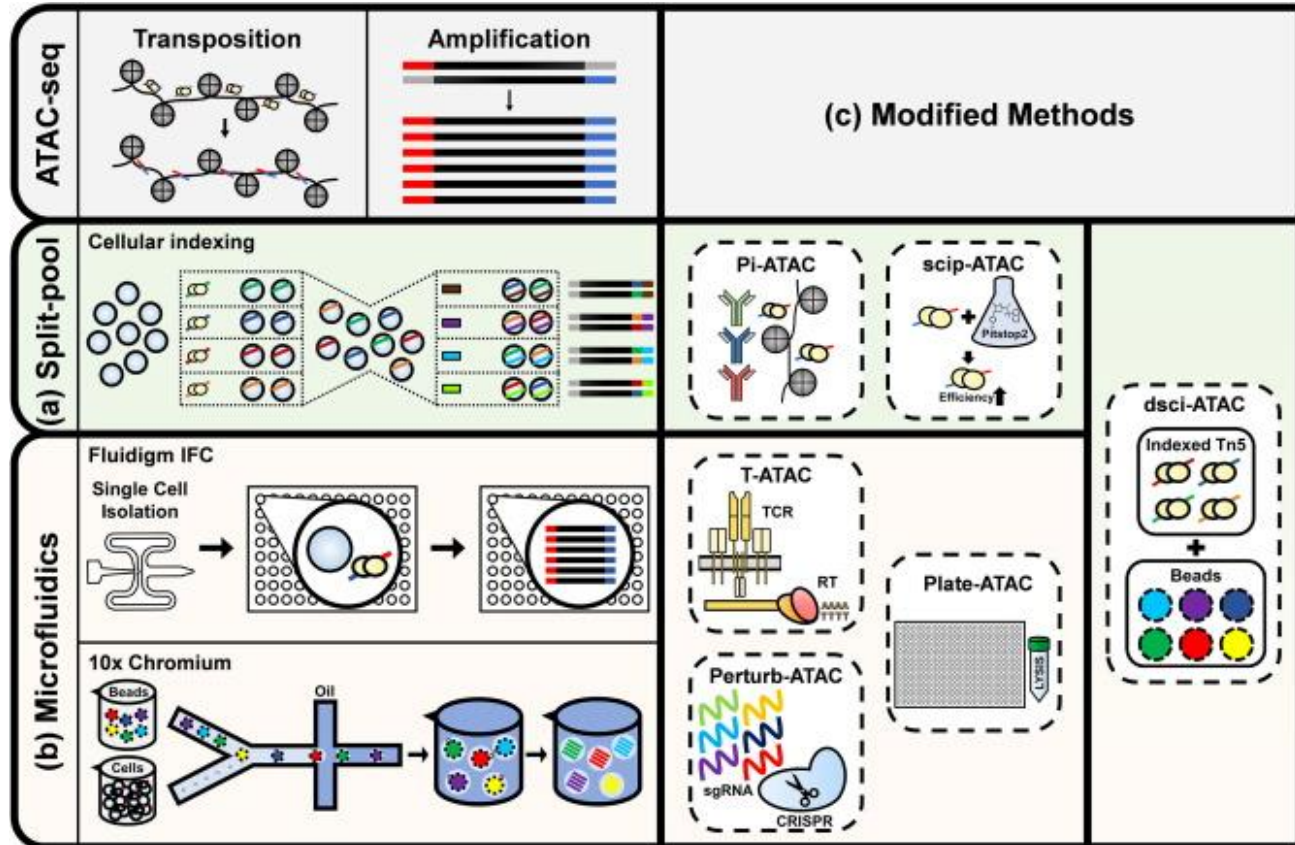
PubMed search

859 results

31 Arabidopsis

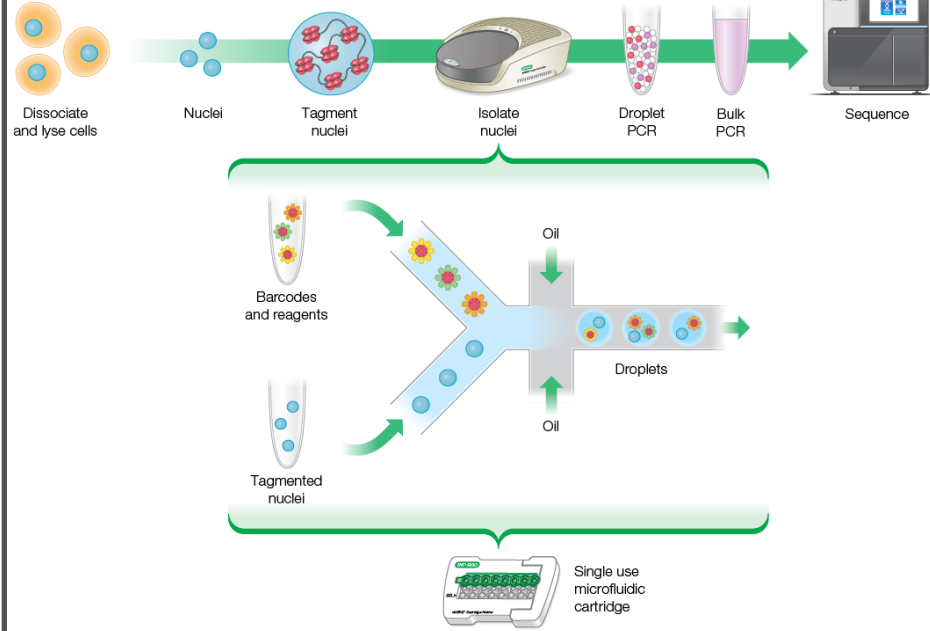
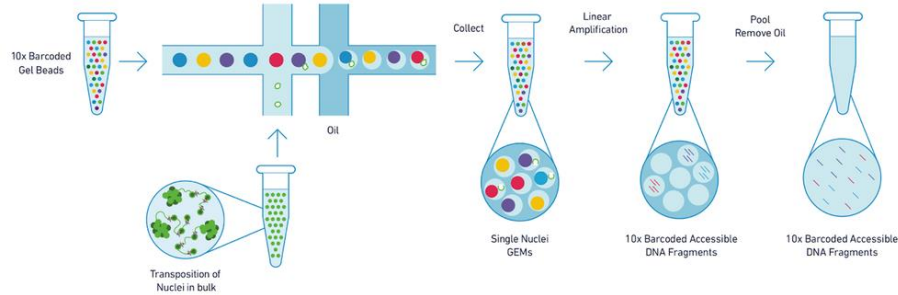
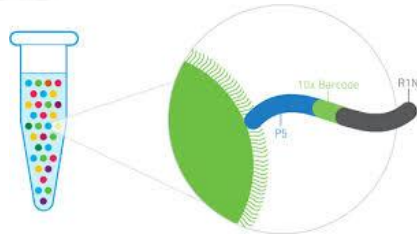
155 drosophila

56 c.elegans



Droplet based scATAC-seq

10 x Genomics, BioRad

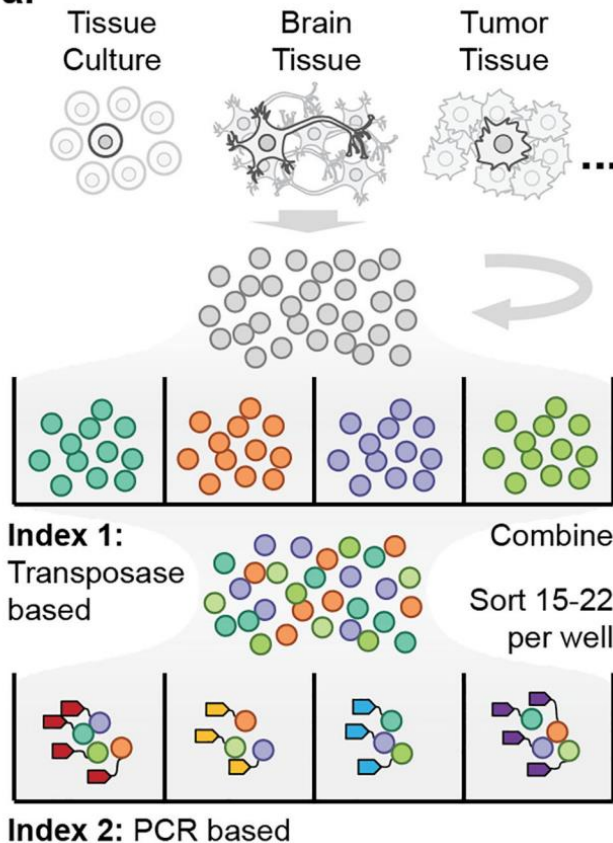


<https://support.10xgenomics.com/single-cell-atac>

<https://www.bio-rad.com/de-de/product/surecell-atac-seq-library-prep-kit?ID=PEXSR1MC1ORV>

Split and Pool based scATAC-seq

a.

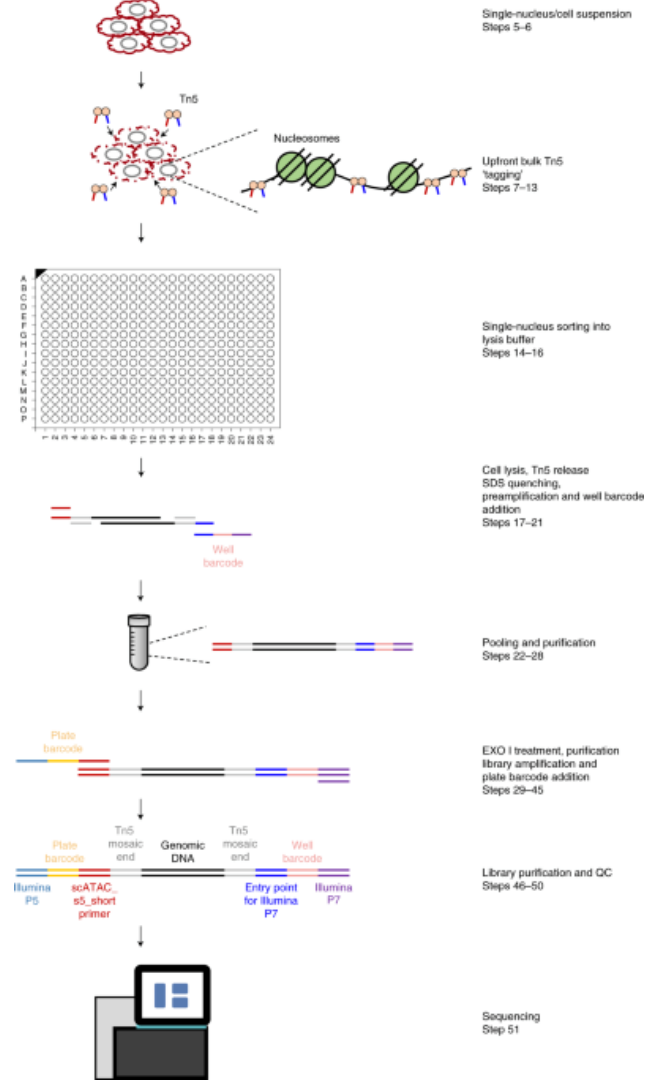


2,000 single nuclei into each well of the 96 well tagmentation plate

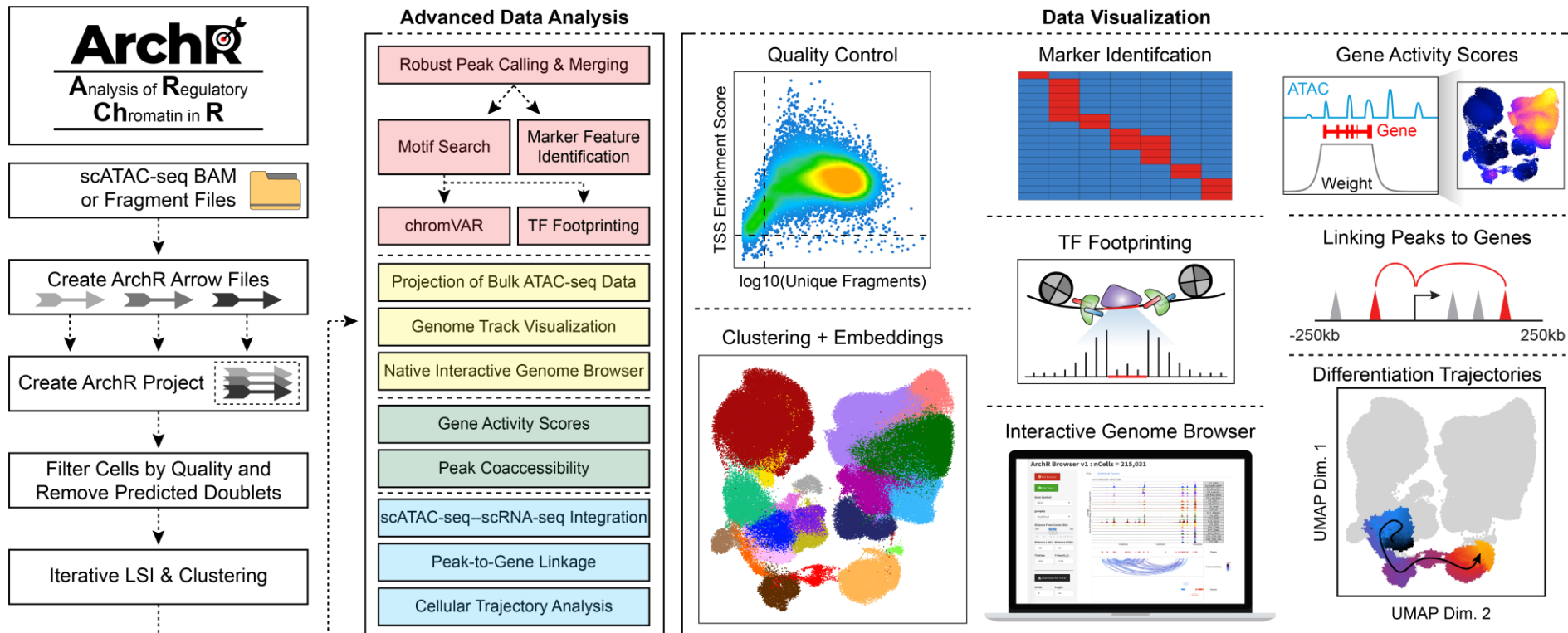
22 post-tagmentation nuclei from the pool of 96 reactions were then flow sorted

Plate based scATAC-seq

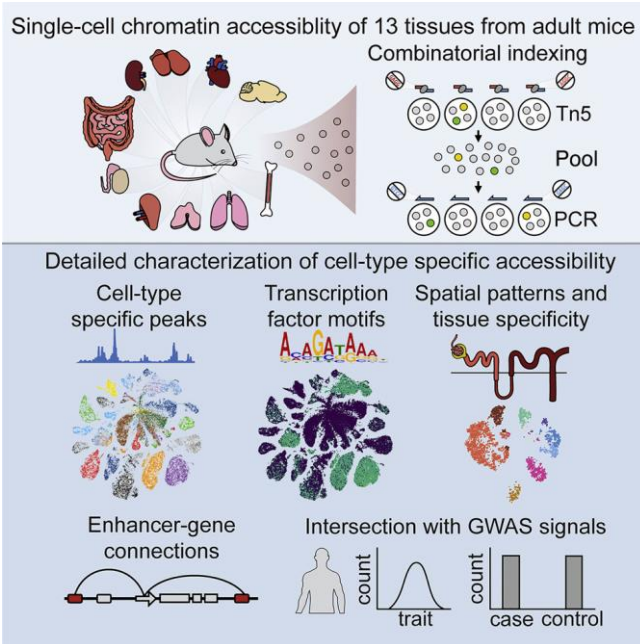
- up-front bulk Tn5 tagging of chromatin with flow cytometry to isolate single nuclei or cells.
- Reagents required to generate sequencing libraries are added to **the same well** in the plate where cells are sorted.
- can be combined with index sorting for in-depth characterization of cell types.
- The whole experimental procedure can be finished within 1 or 2 d with a throughput of hundreds to thousands of nuclei



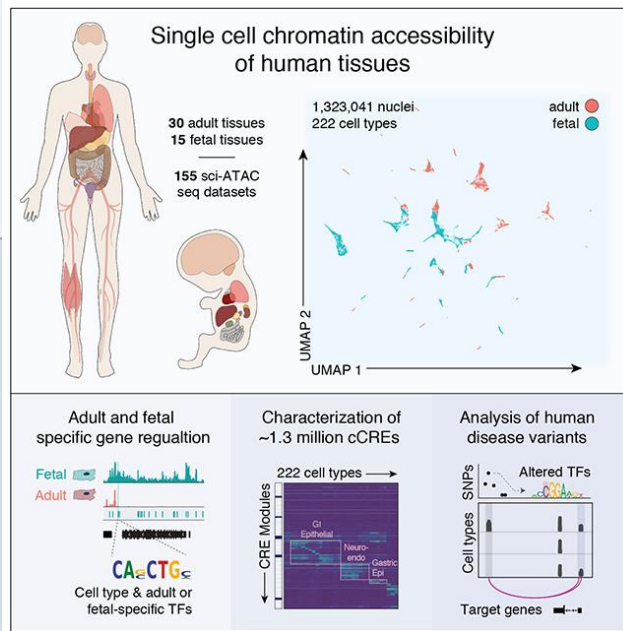
scATACseq data and possible analyses



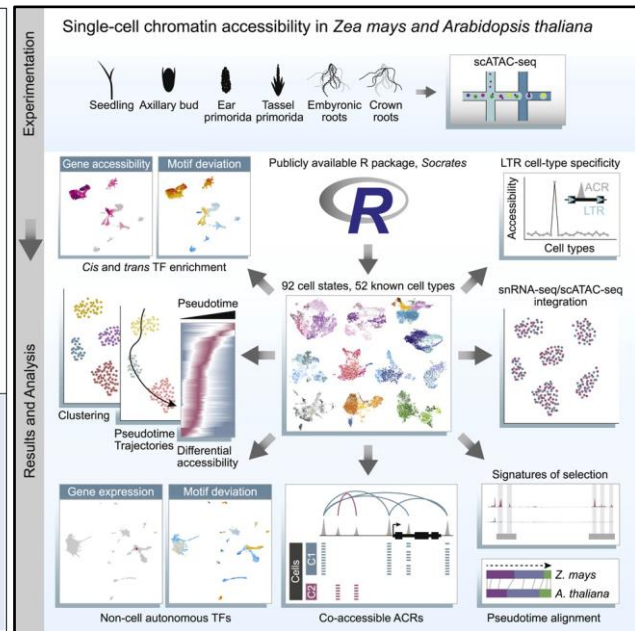
Application Examples



Cell. 2018 Aug 23;174(5):1309-1324.e18. doi: 10.1016/j.cell.2018.06.052. Epub 2018 Aug 2



[Cell. 2021 Nov 24; 184\(24\): 5985–6001.e19.](#)



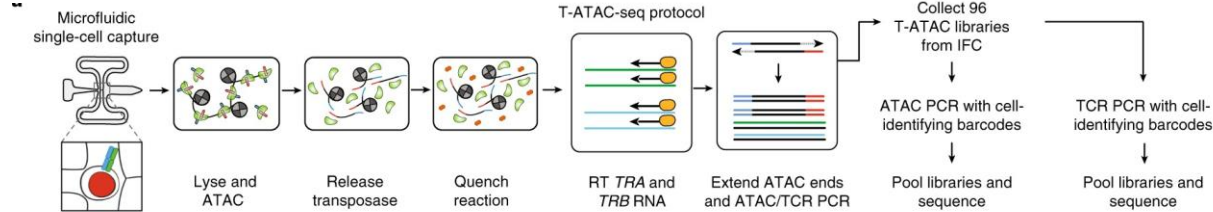
Cell. 2021 May 27;184(11):3041-3055.e21. doi: 10.1016/j.cell.2021.04.014. Epub 2021 May 7.

Comprehensive characterization of tissue-specific chromatin accessibility in L2 *Caenorhabditis elegans* nematodes

Genome Res. 2021 Oct;31(10):1952-1969. doi: 10.1101/gr.271791.120. Epub 2021 Apr 22.

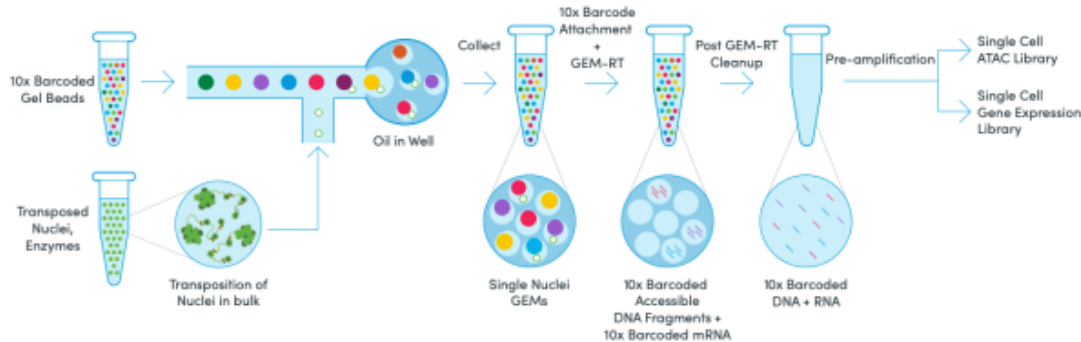
RNA and Chromatin signatures from the same cell

T-ATAC

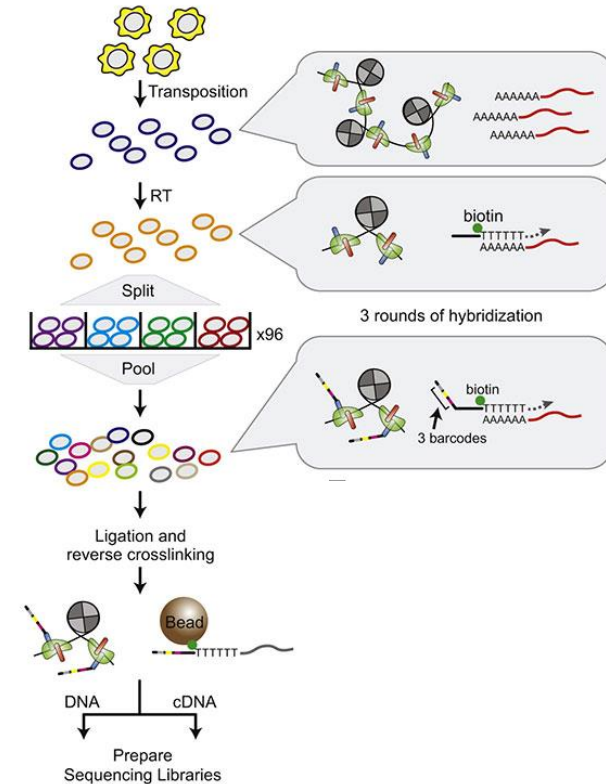


[Nature Medicine volume 24, pages 580–590 \(2018\)](#)

10x Multiomics kit

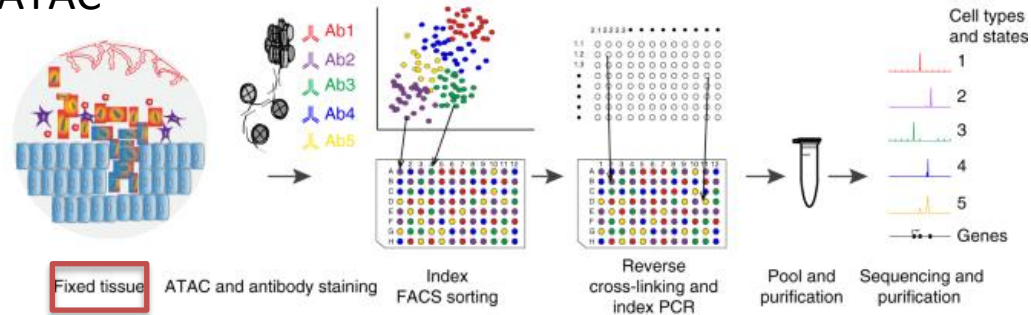


SHARE-seq: RNA-ATAC



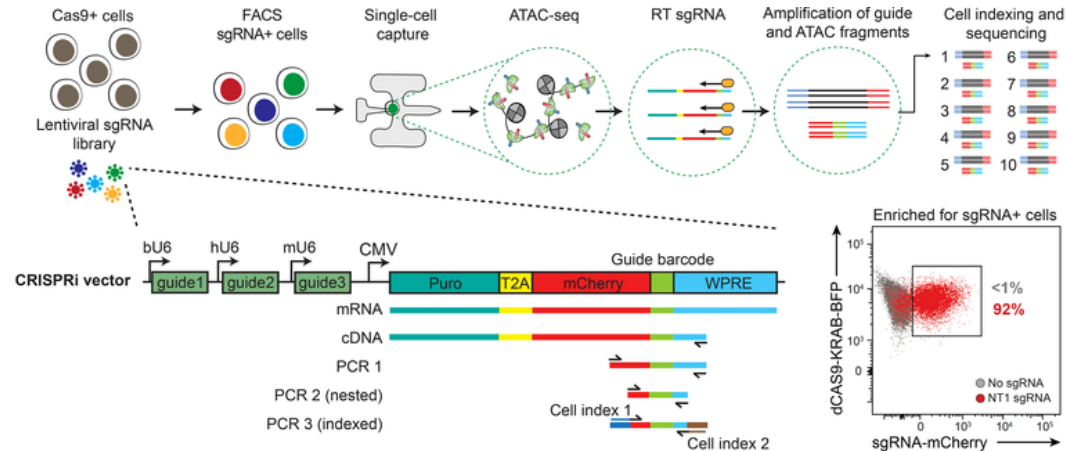
Single Cell Multiomics

PI-ATAC



[Nature Communications](#) volume 9,
Article number: 4590 (2018)

Perturb-ATAC



Cell. 2019 Jan 10;176(1-2):361-376.e17. doi:
10.1016/j.cell.2018.11.022

summary

- from 96 (or less) cells on a Fluidigm C1 chip and immense costs of ~8 \$ per cell (without sequencing!) to tens of thousands of cells for 0,2 \$ per cell within ~5 years
- high throughput technologies are droplet based microfluidics and FACS based split and pool
- with lower sequencing costs, e.g via Novaseq and discussed tech dev scATAC-seq finds more applications
- applications for scATACseq and multiomics in drug discovery
- computational tools more and more available, python and R based
- cell atlas and annotations of cell states made tremendous steps forward
- Increasing data in species other than mouse and human

Looking forward to the interesting single cell chromatin research to come soon from you, your projects and your research teams!

THANK YOU

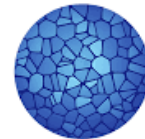


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**HUMAN
CELL
ATLAS**

Microfluidics vs Flow cytometry

Precision of cell capture:
Microscopic observation

Cell type dependent capture efficiency

Single cell transposition

Compatible with targeted RNA measurement

~\$700 per 96 cell chip

1 chip per day per machine

No larger chip available

Precision of cell sorting:
'barnyard experiment'

Cell type independent efficiency

Bulk transposition

Compatible with Protein measurement

Barcoding PCRs compatible
with liquid handling robot

~\$96 per 96 well plate

20-40 384-well plates per day

can be scaled to 384 well plates