Sequencing from single cells!

Lia Chappell
Wellcome Sanger Institute, Cambridge, UK
LC5@sanger.ac.uk
Twitter: @LiaVLChappell

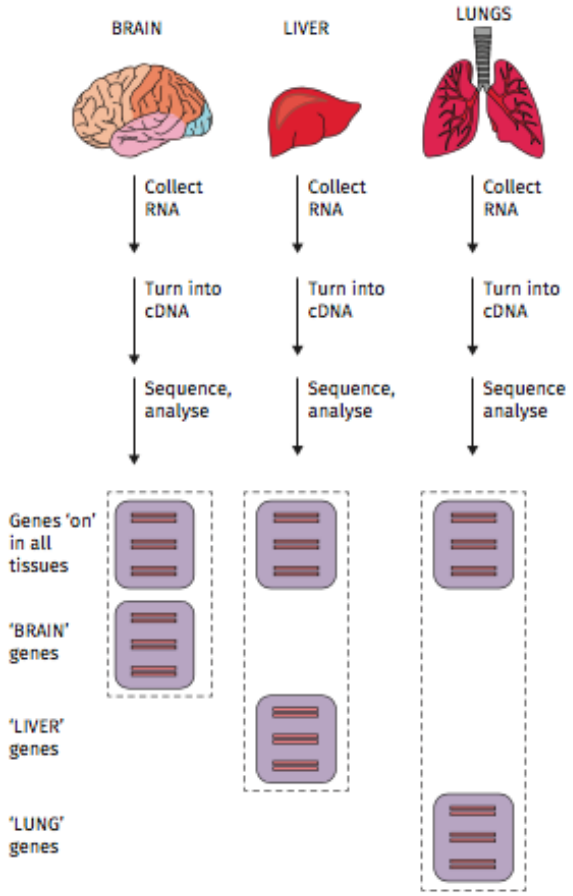
I’m based the Sanger Institute

I grew up in Oxfordshire…

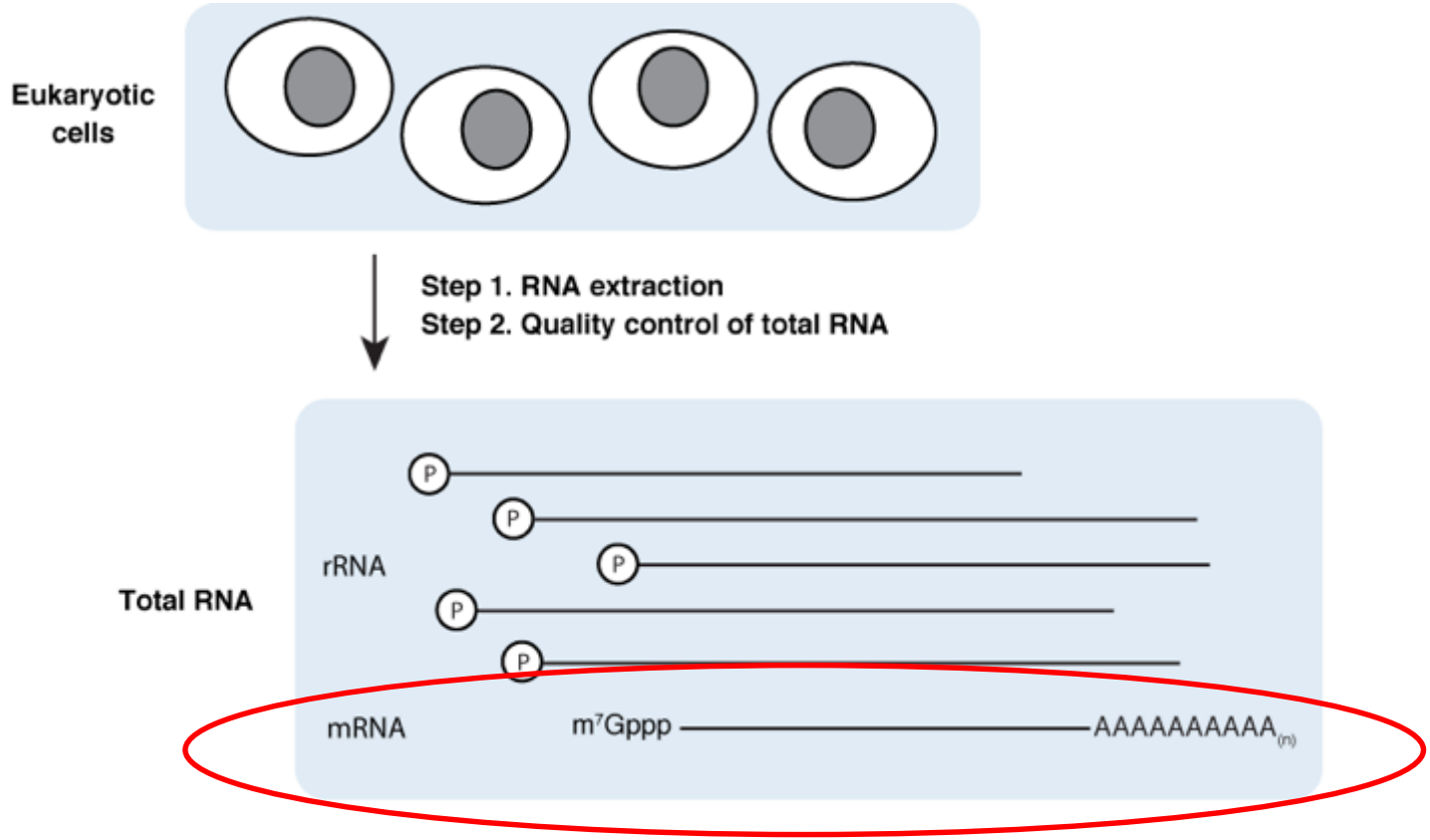
I’m from Oxfordshire

“Bulk” RNA-seq

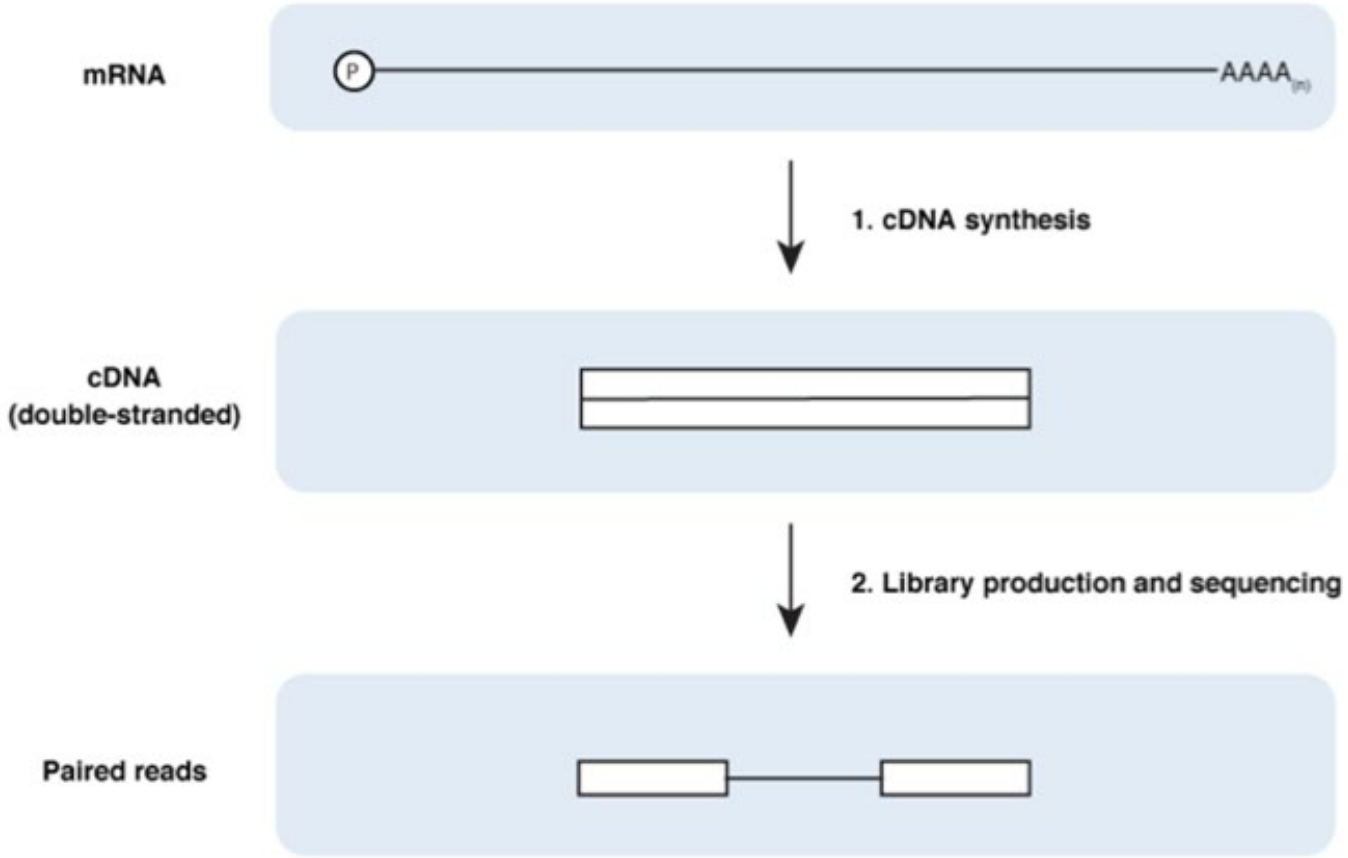
RNA-seq measures “active” genes with sequencing



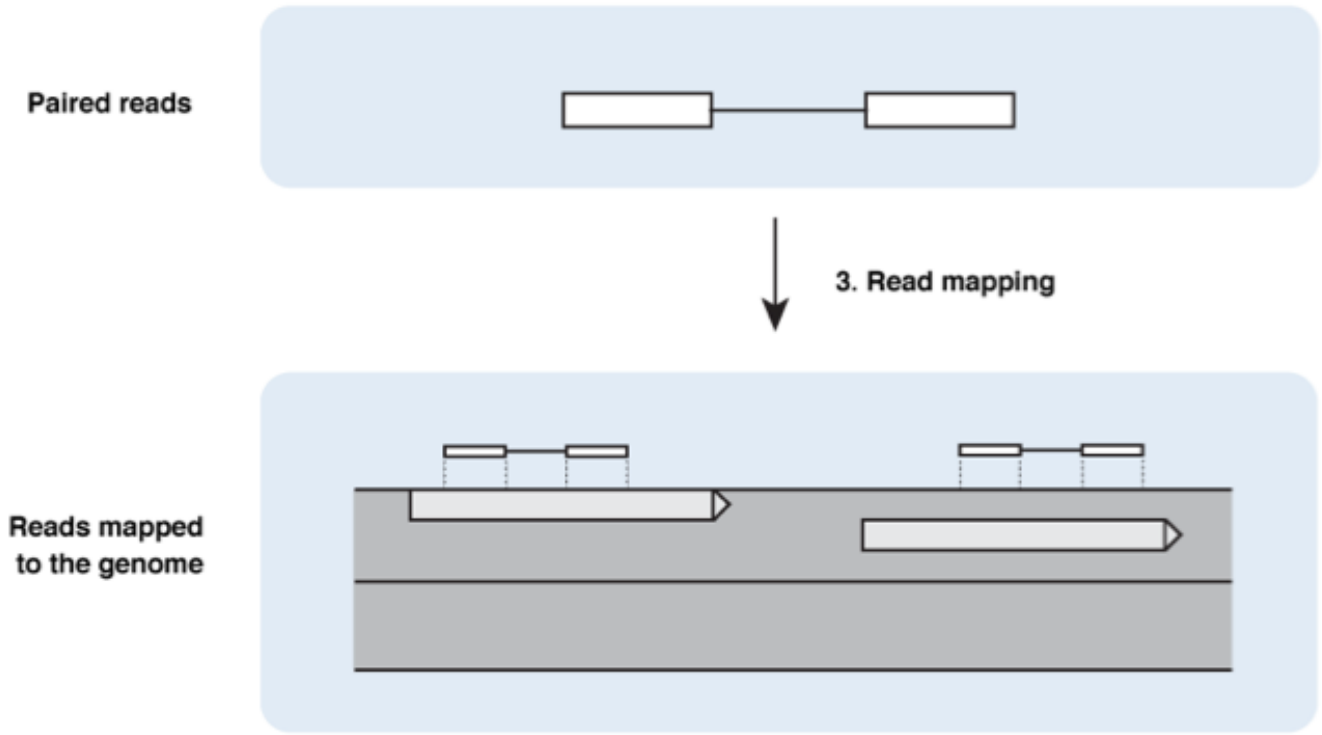
RNA


Eukaryotic cells
Step 1. RNA extraction
Step 2. Quality control of total RNA
Total RNA
rRNA
mRNA
m7Gppp
AAAAAAAAAA(n)

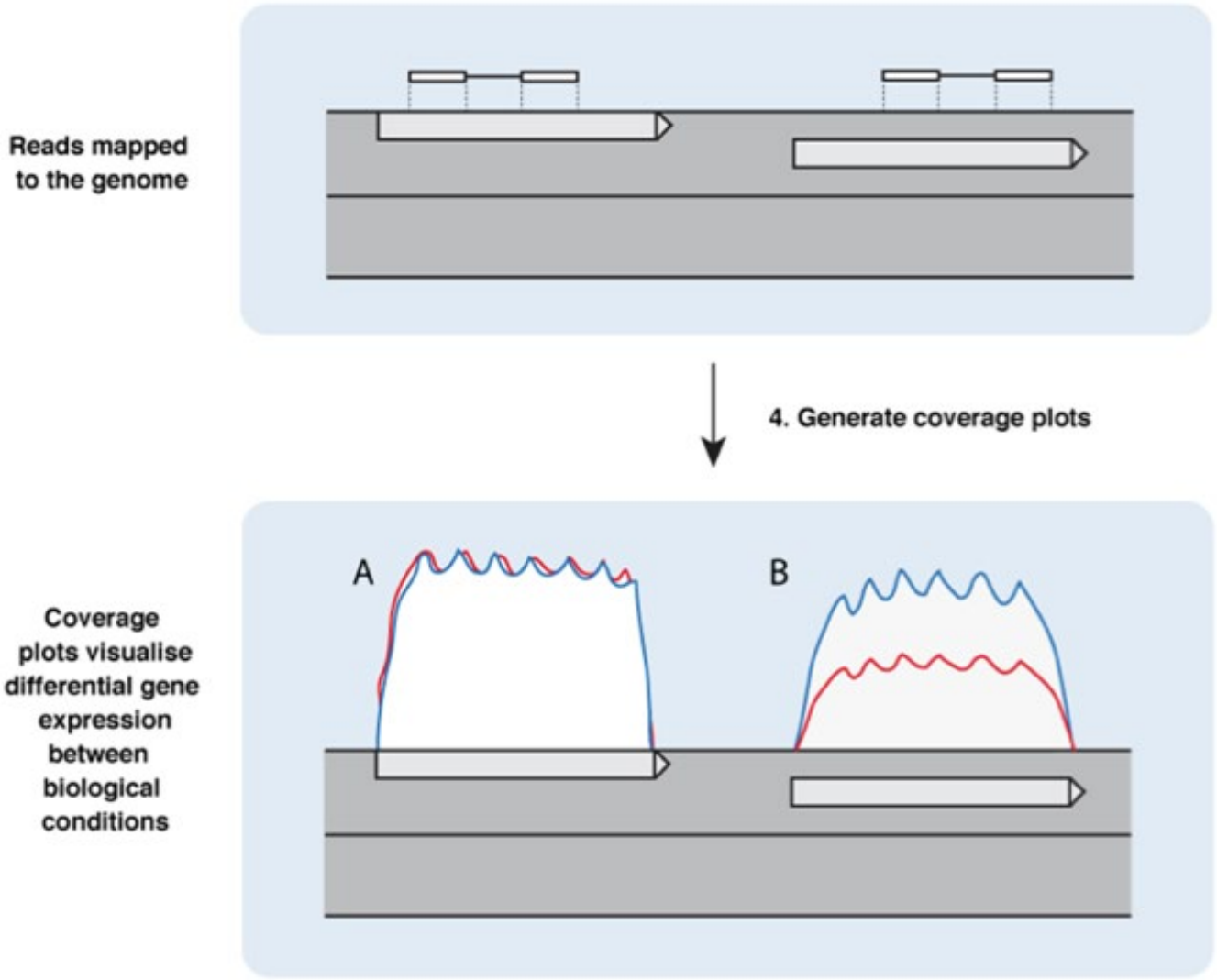
From RNA to RNA-seq



From RNA to RNA-seq

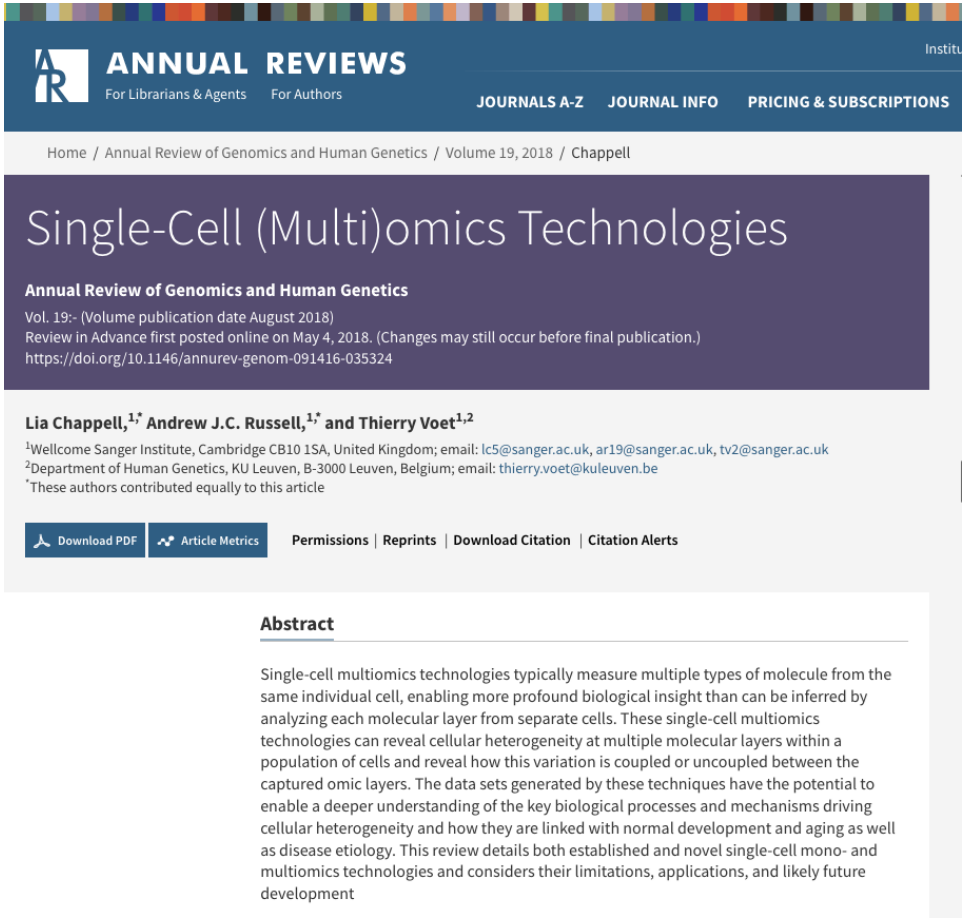


From RNA to RNA-seq



Sequencing from single cells: why and how?

Too long and full of too many technical details…..



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Single-Cell (Multi)omics Technologies

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Lia Chappell,[1,*] Andrew J.C. Russell,[1,*] and Thierry Voet[1,2]
[1]Wellcome Sanger Institute, Cambridge CB10 1SA, United Kingdom; email: lc5@sanger.ac.uk, ar19@sanger.ac.uk, tv2@sanger.ac.uk
[2]Department of Human Genetics, KU Leuven, B-3000 Leuven, Belgium; email: thierry.voet@kuleuven.be
[*]These authors contributed equally to this article

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Abstract

Single-cell multiomics technologies typically measure multiple types of molecule from the same individual cell, enabling more profound biological insight than can be inferred by analyzing each molecular layer from separate cells. These single-cell multiomics technologies can reveal cellular heterogeneity at multiple molecular layers within a population of cells and reveal how this variation is coupled or uncoupled between the captured omic layers. The data sets generated by these techniques have the potential to enable a deeper understanding of the key biological processes and mechanisms driving cellular heterogeneity and how they are linked with normal development and aging as well as disease etiology. This review details both established and novel single-cell mono- and multiomics technologies and considers their limitations, applications, and likely future development

Andy Russell

Thierry Voet

Table 1 Single-cell multiomics technologies

Technology	Genomic layer	Chromatin accessibility epigenetic layer	CpG methylation epigenetic layer	Transcriptomic layer	Protein layer	Strategy	Cell isolation[a]	Cell throughput	Automation	Constituent methods
DR-seq (31)	Genome	—	—	Transcriptome	—	Preamplification and tagging of DNA and RNA followed by splitting	Mouth pipette	Low	No	Modified CEL-seq (59) and modified MALBAC (161)
G&T-seq (92, 94)	Genome	—	—	Transcriptome	—	Separation (DNA and polyadenylated mRNA)	FACS	Medium	Yes	Modified Smart-seq2 (112, 113) and PicoPLEX WGA or MDA WGA (92, 94)
scTrio-seq (62)	CNVs (from scRRBS data)	—	Reduced-representation DNA CpG methylation	Transcriptome	—	Separation (nucleus and cytoplasm) followed by bisulfite conversion	Mouth pipette	Low	No	scRNA-seq method of Tang et al. (143) and scRRBS (54)
scMT-seq (64)	SNPs (from scRRBS and scRNA-seq data)	—	Reduced-representation DNA CpG methylation	Transcriptome	—	Separation (nucleus and cytoplasm)	Microcapillary pipette	Low	Partial	Modified Smart-seq2 protocol (112, 113) and modified scRRBS (54)
scGEM (20)	Targeted genotyping (Sanger and next-generation sequencing)	—	Targeted DNA CpG methylation (qPCR)	Targeted (RT-qPCR)	—	Restriction digestion, preamplification, and splitting	Fluidigm C1	Medium	Yes	Modified SCRAM (21)
scM&T-seq (5)	—	—	DNA CpG methylation	Transcriptome	—	Separation (DNA and polyadenylated mRNA) followed by bisulfite conversion	FACS	Medium	Yes	Modified G&T-seq (92, 94) and modified scBS-seq (24, 131)

(*Continued*)

Why?

Bulk population based methods

Bulk population based methods

Single Cell Methods

The Art of Clean Up, Ursus Wehrli, Kimberly Vardeman

Bulk population based methods

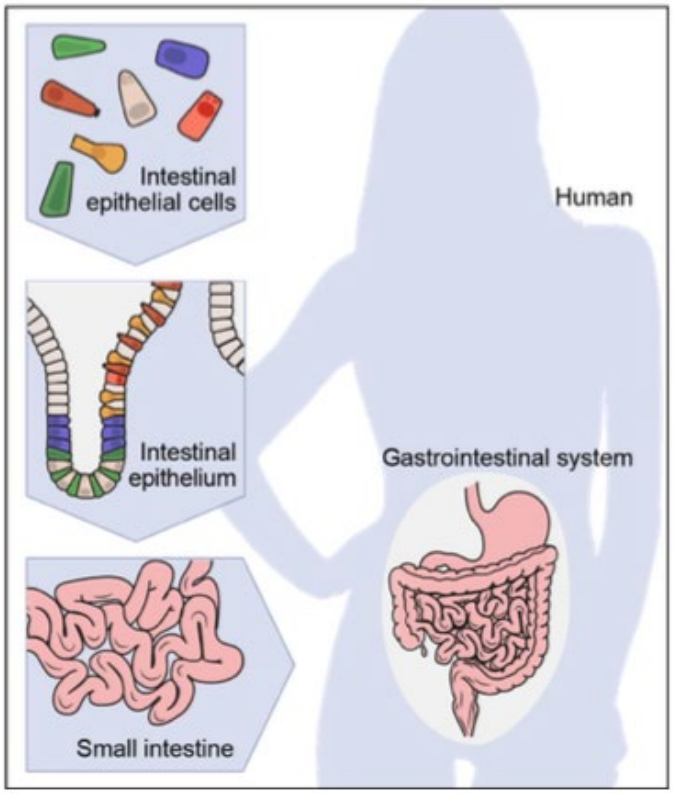
Single Cell Methods

Spatial methods

The Art of Clean Up, Ursus Wehrli, Kimberly Vardeman

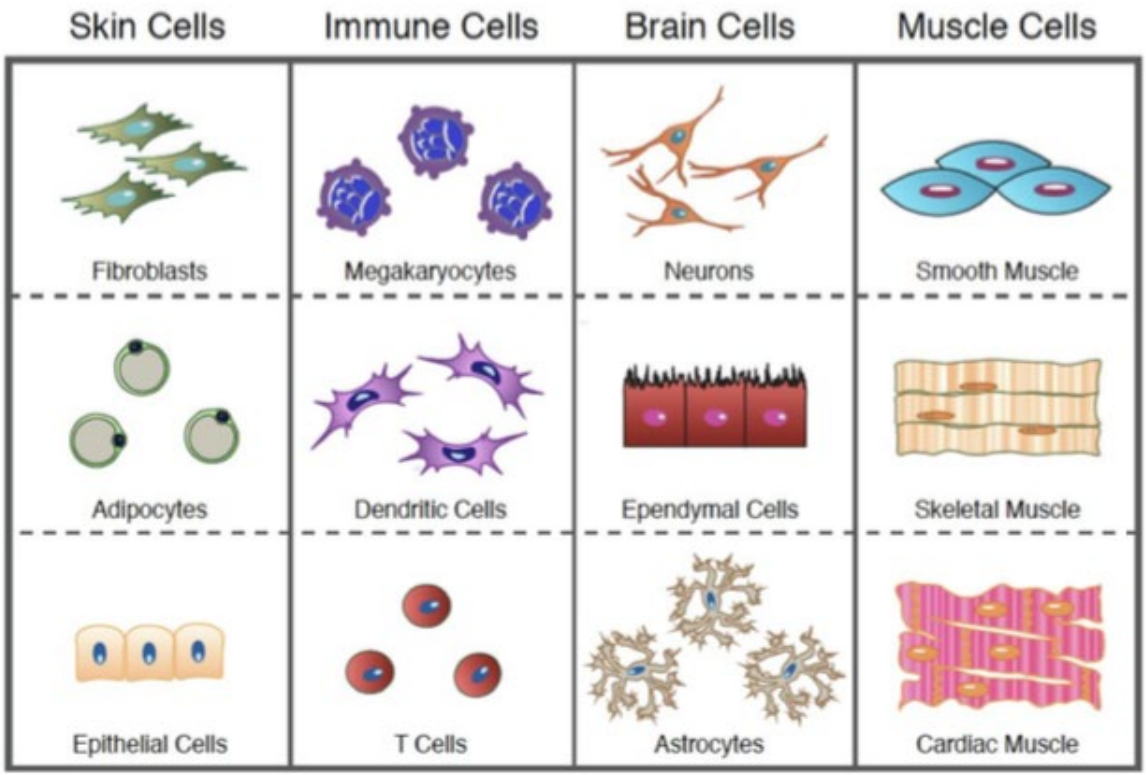
Human Cell Atlas

A “google maps” of human anatomy

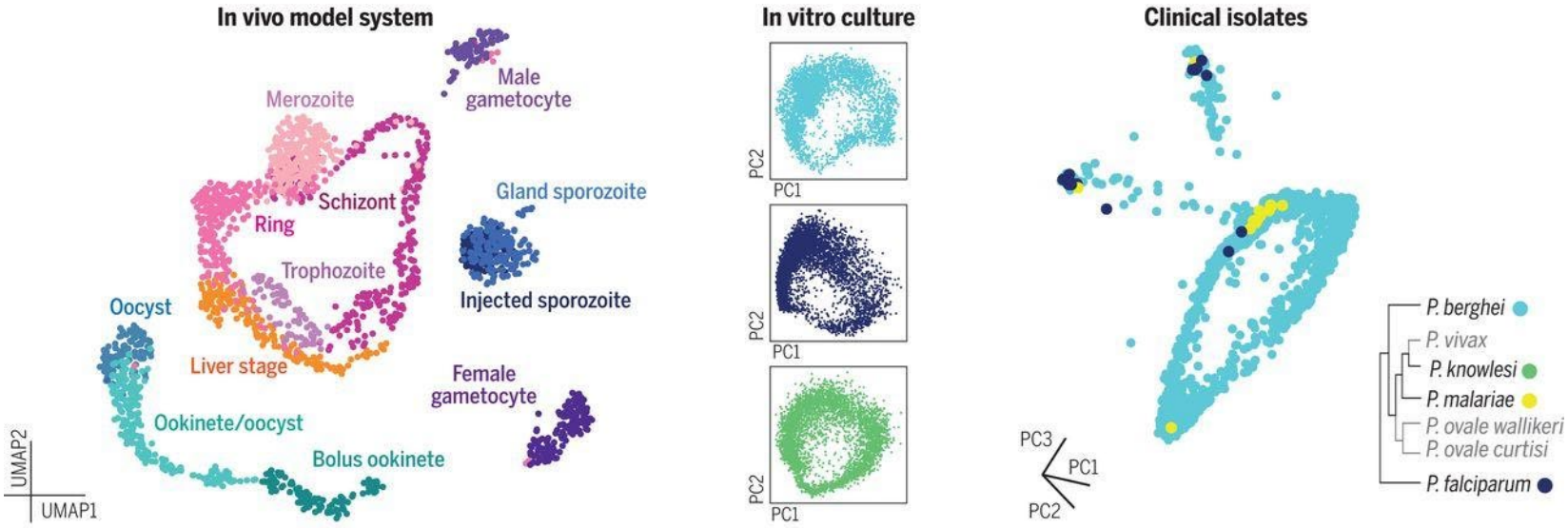


Human Cell Atlas

Goal : A periodic table of our cells



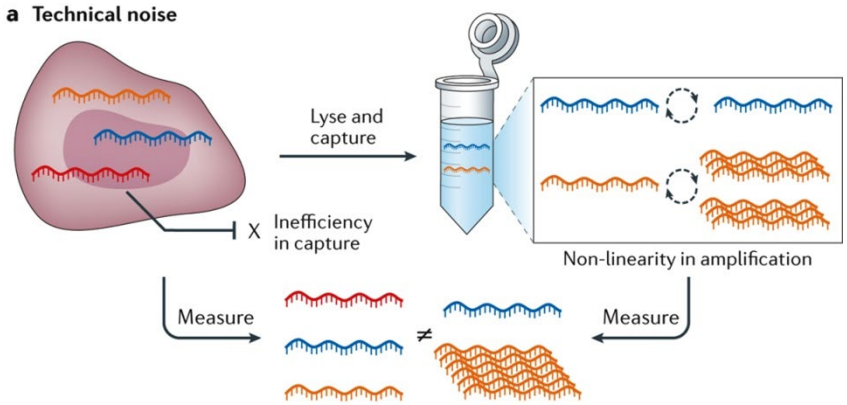
Malaria Cell Atlas



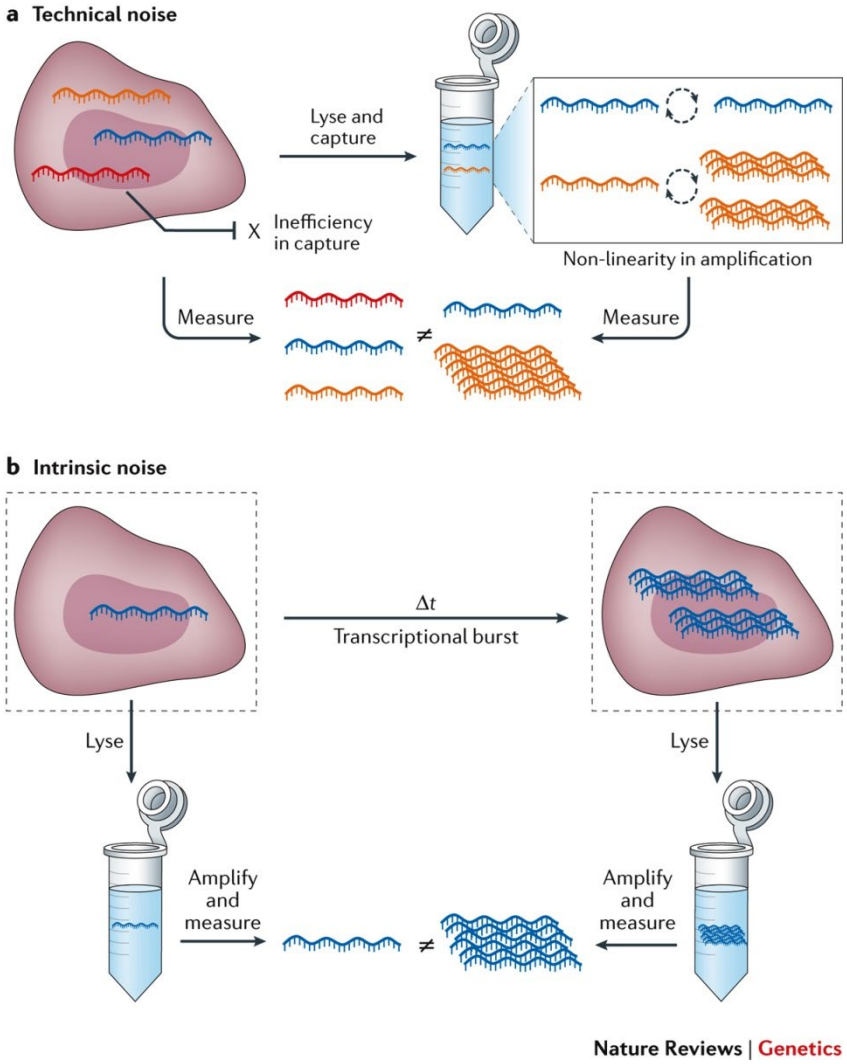
Virginia M. Howick et al. Science 2019;365:eaaw2619

Science
AAAS

Noise in scRNA-seq



Noise in scRNA-seq



How?

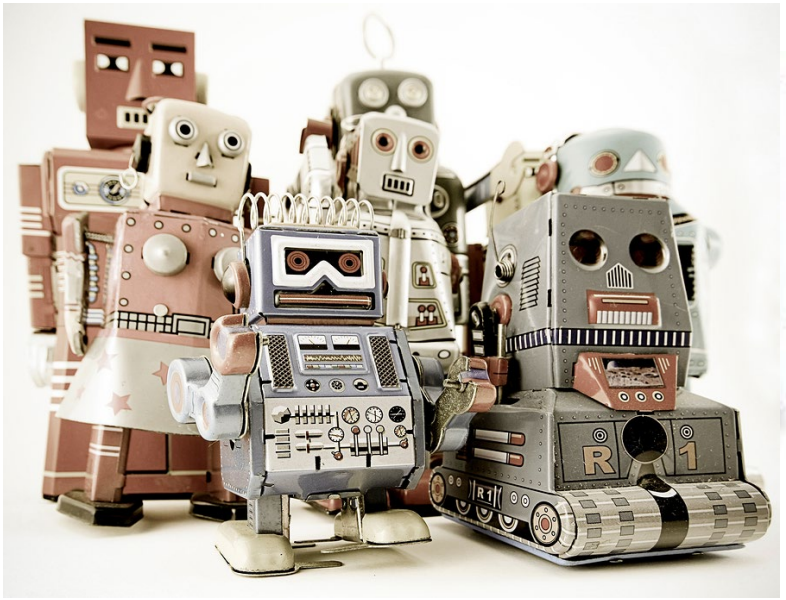
FACS machine

Usually in plates

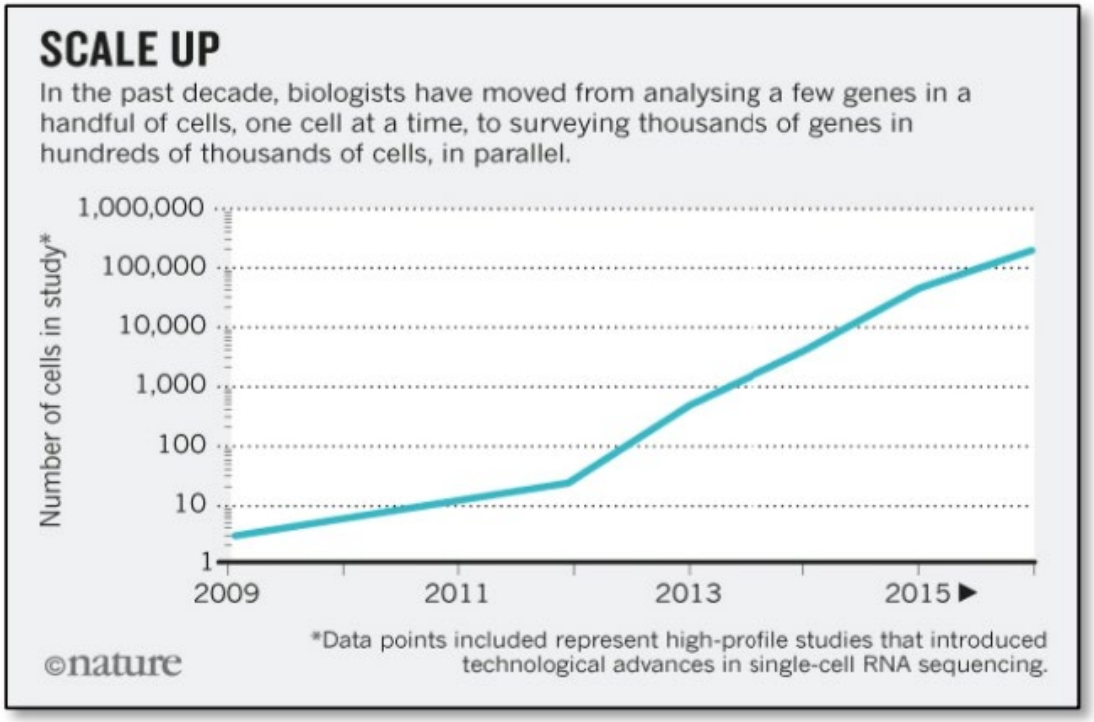
Single cell RNA-seq: plate format

Single cell RNA-seq: plate format

Single cell RNA-seq: plate format

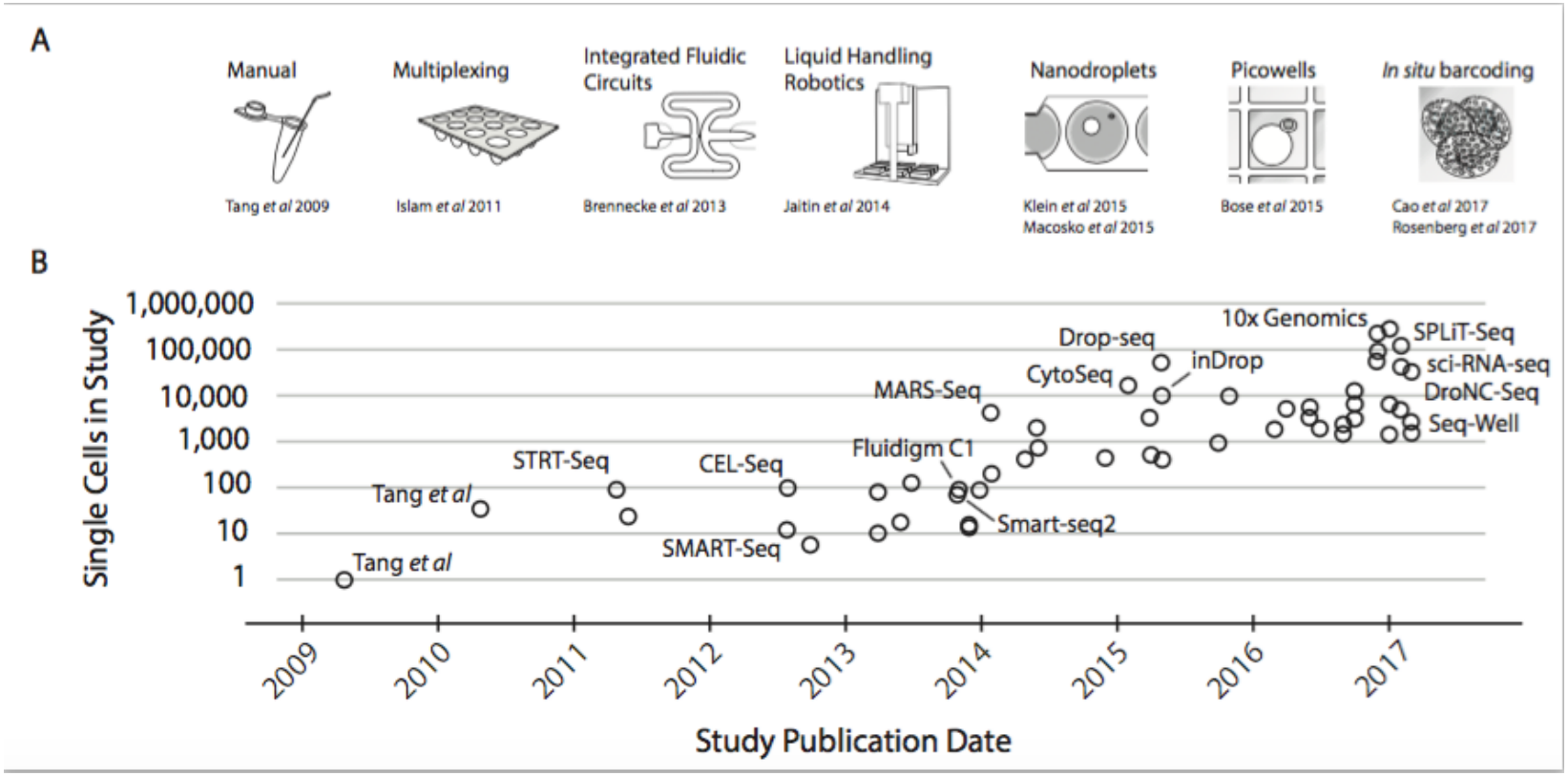


A trend for increasing scale…



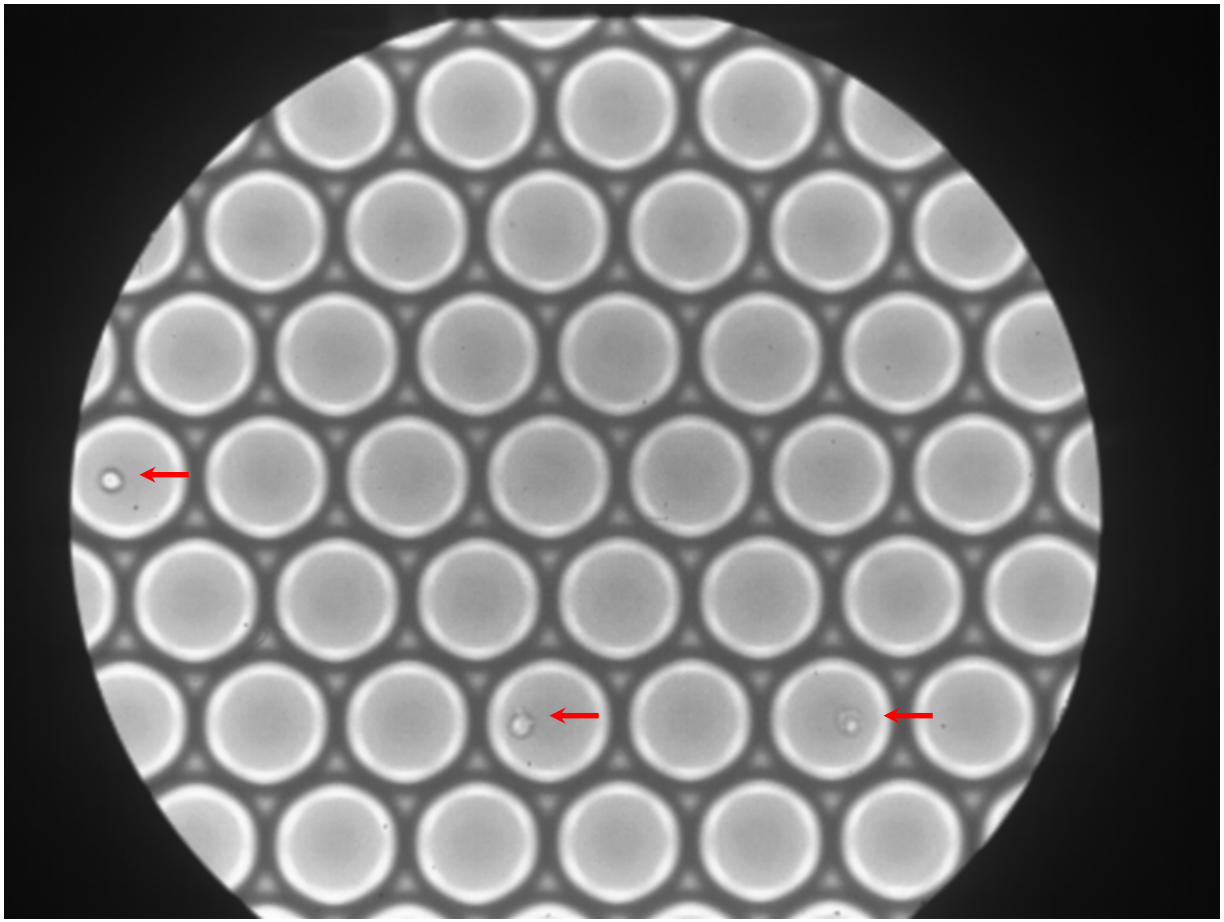
Amir Giladi & Ido Amit, 6 July 2017 | VOL 547 | Nature | 27

A trend for increasing scale…

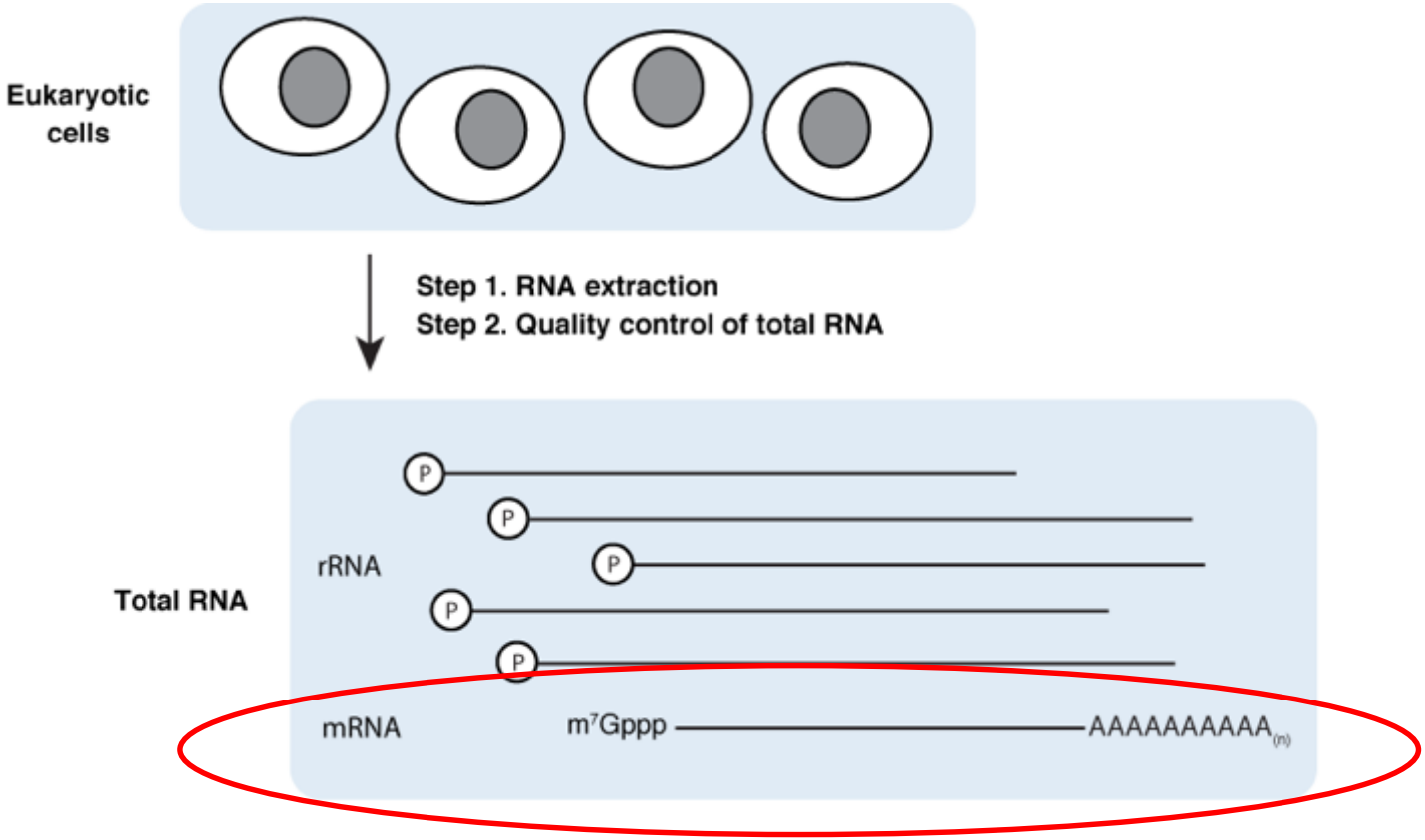


Valentine Svensson, Roser Vento-Tormo, Sarah A Teichmann

Another way: droplets

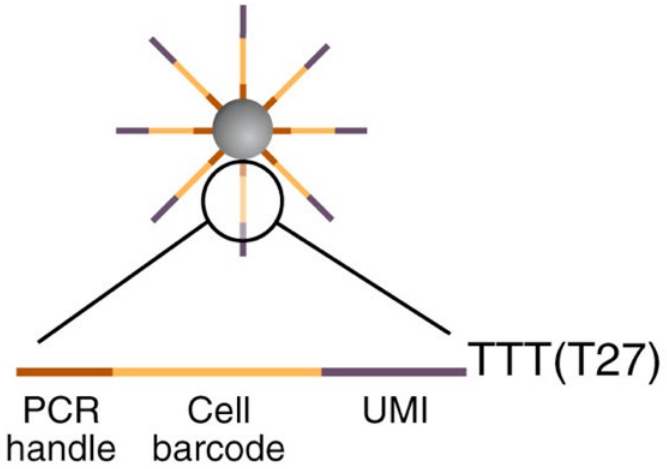


mRNA - a reminder



Barcoded beads…

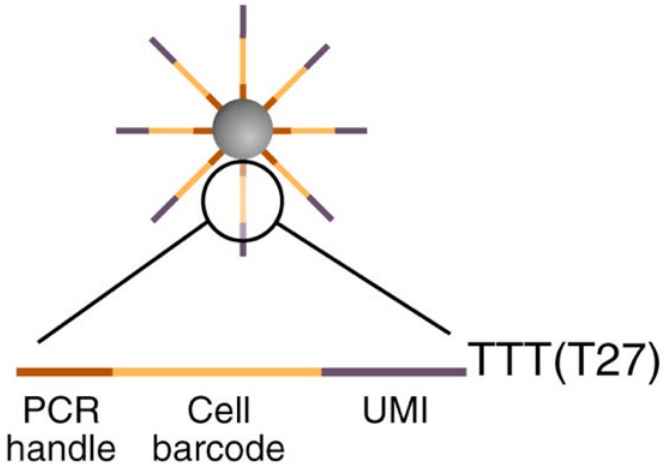
Barcoded beads



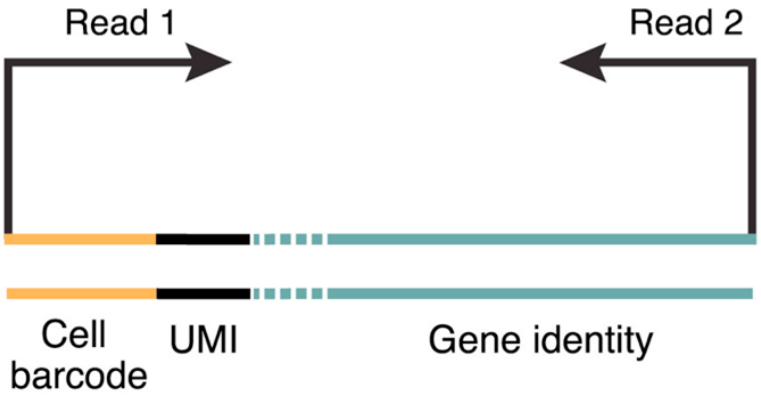
Drop-seq (Macosko et al. 2015)

Barcoded beads…

Barcoded beads



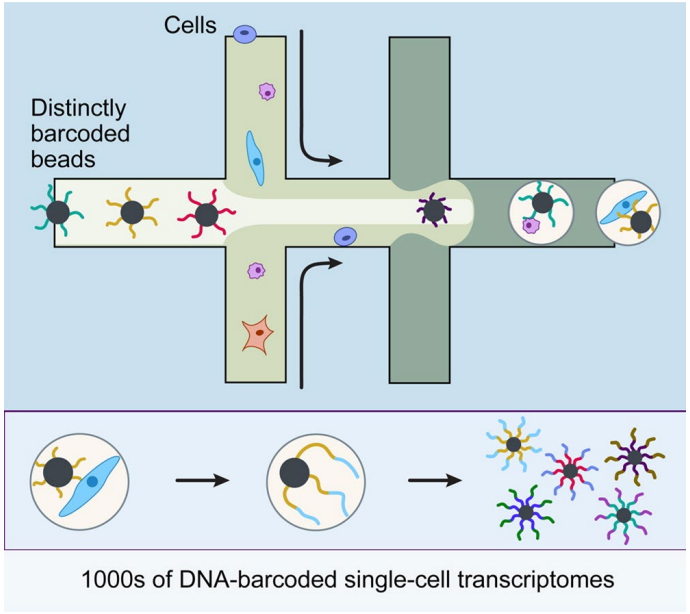
Sequencing reads



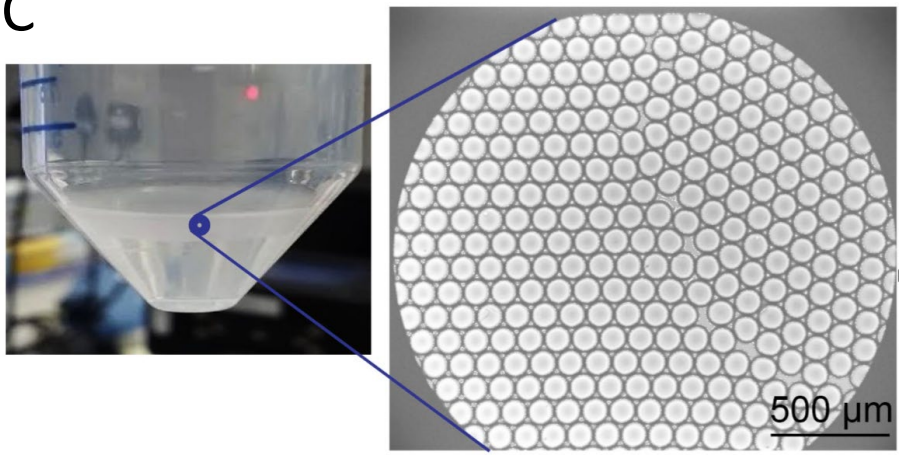
Drop-seq (Macosko et al. 2015)

Drop-seq

A



C



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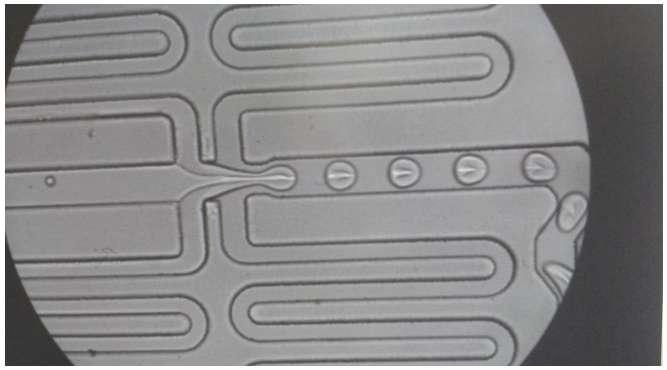

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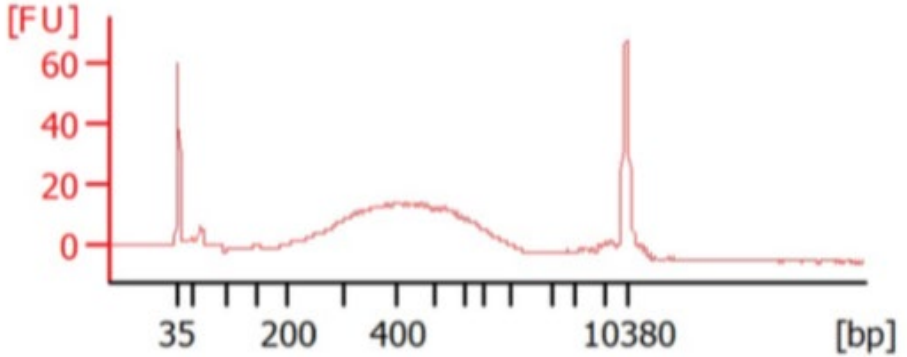
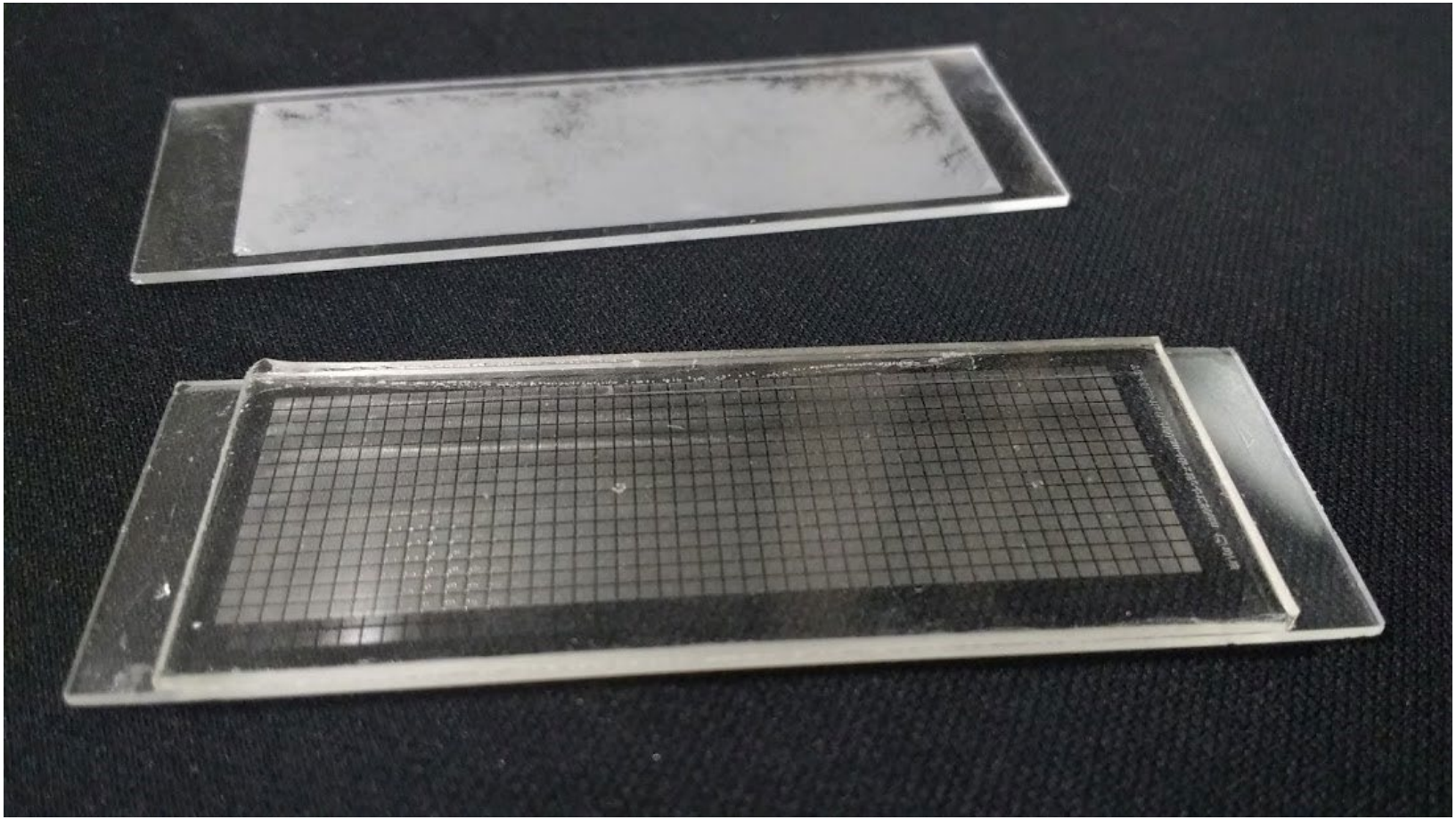
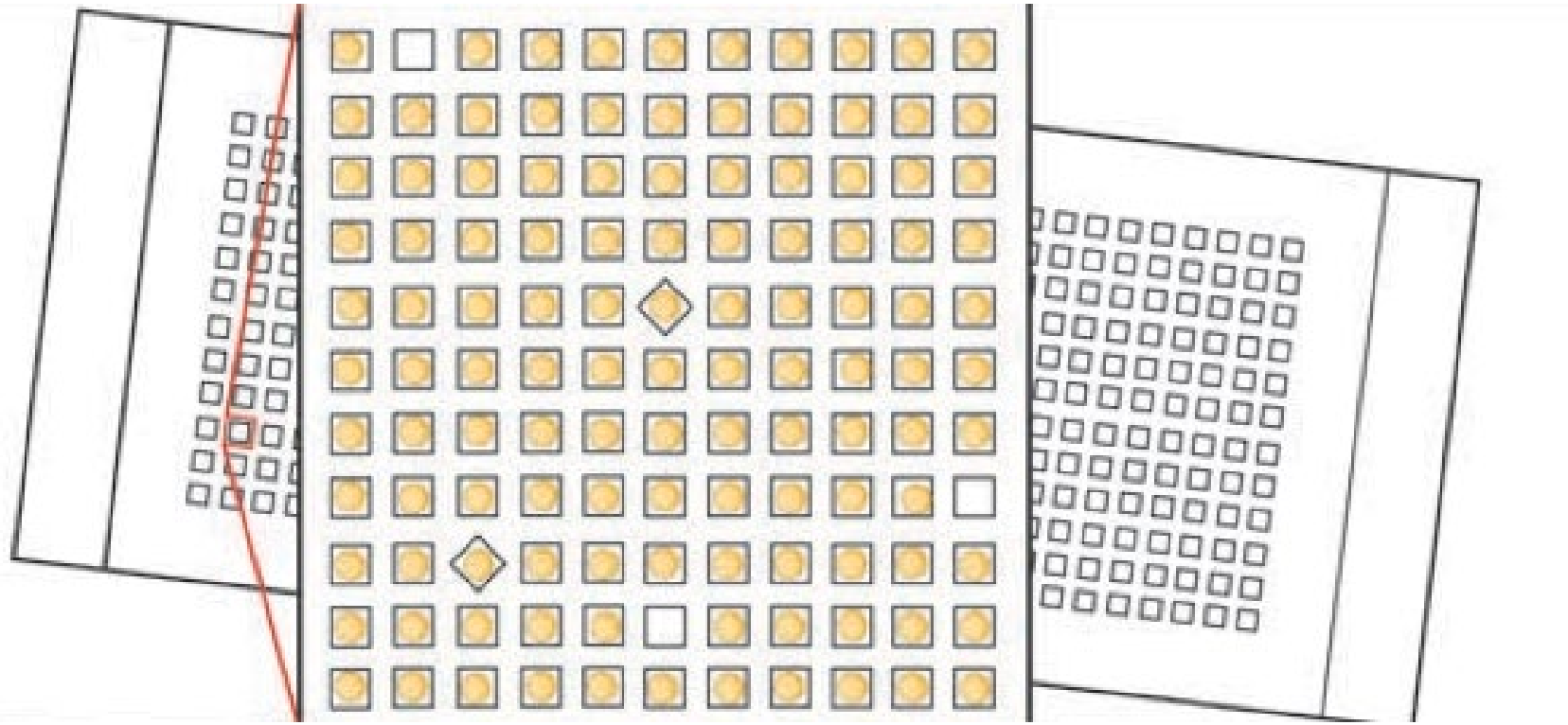


10X “black box”

Nanowells: e.g. Seq-Well



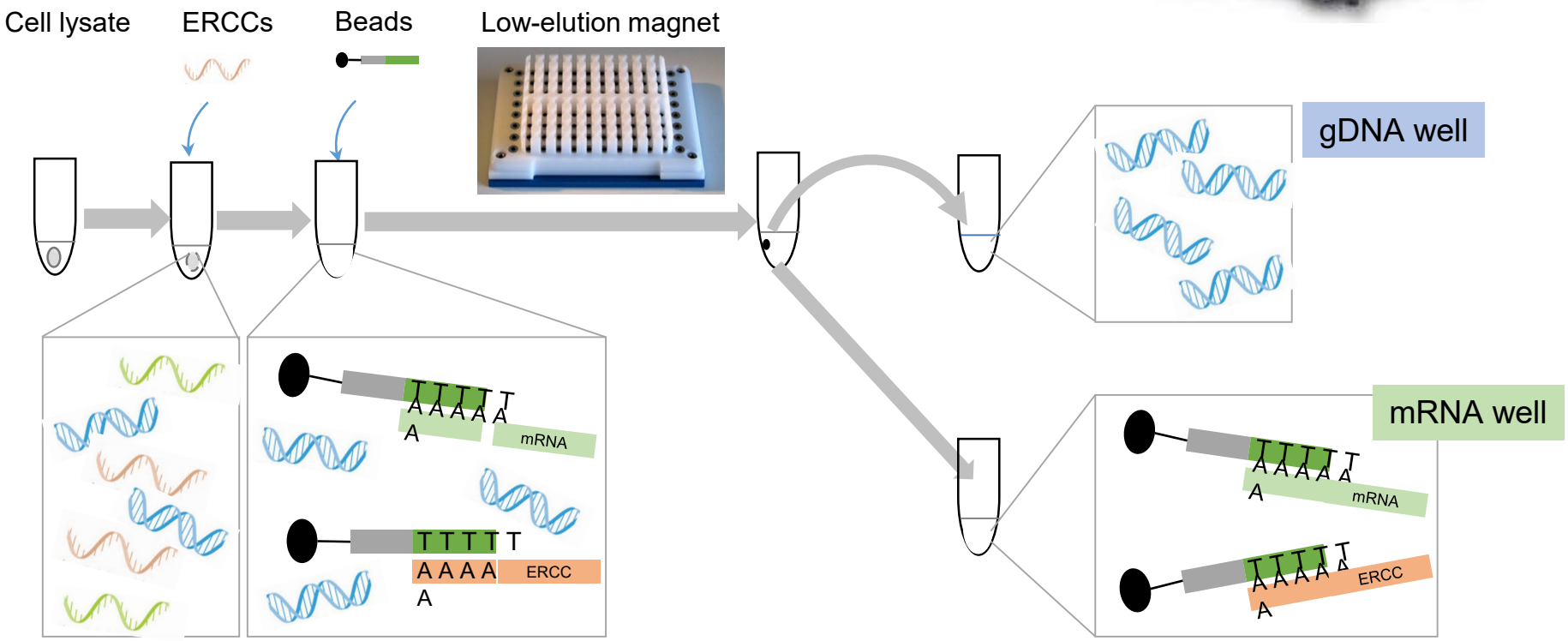
Beads fit into wells…



Multiple layers from the same cell

Single-cell G&T-seq (Voet group)

In plate format, automated on robotic platforms



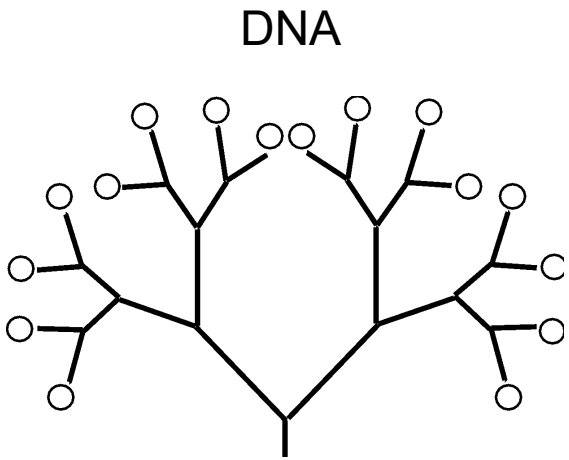
Nature Protocols. 2016 Nov;11(11):2081-103.
Nature Methods. 2015 Jun;12(6):519-22.

Relationship between RNA and DNA maintained by copying plate layout for both layers

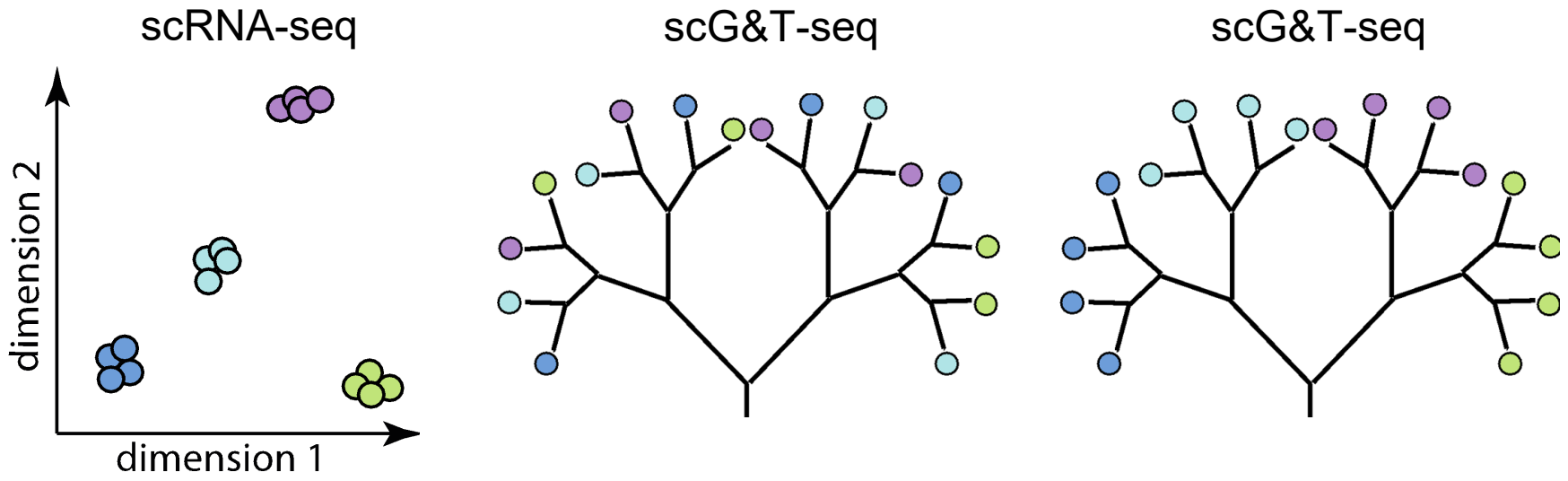
RNA

DNA

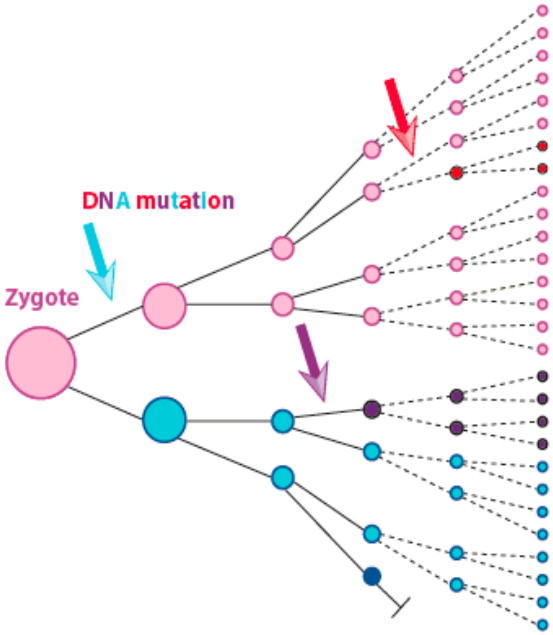
Genotype-phenotype correlation at single cell level

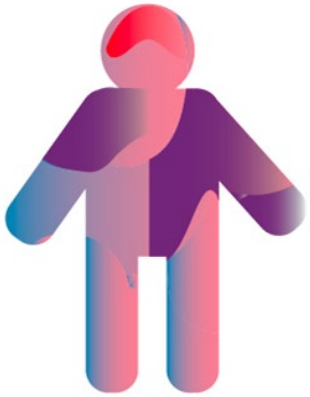


Genotype-phenotype correlation at single cell level



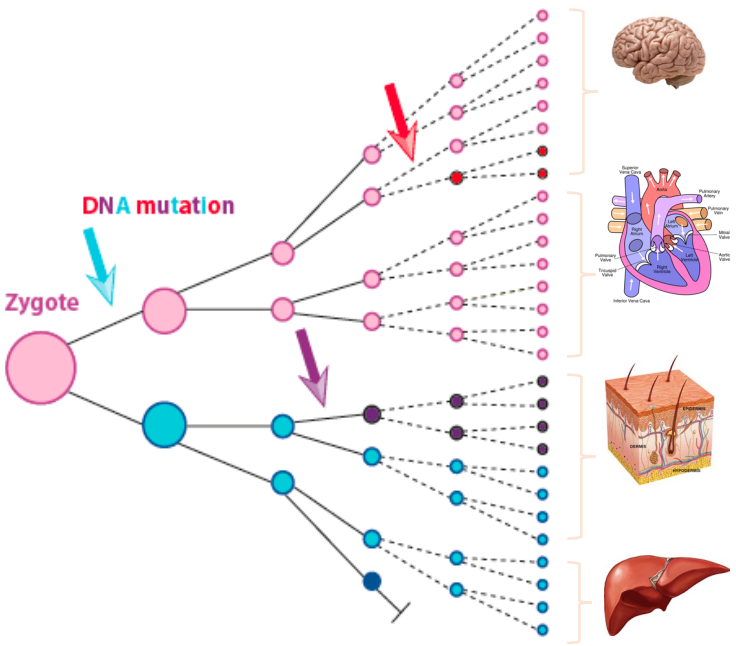
One human, multiple genomes

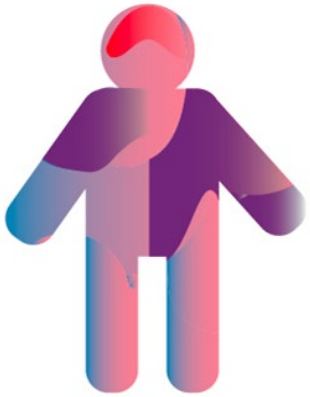




One human, multiple genomes

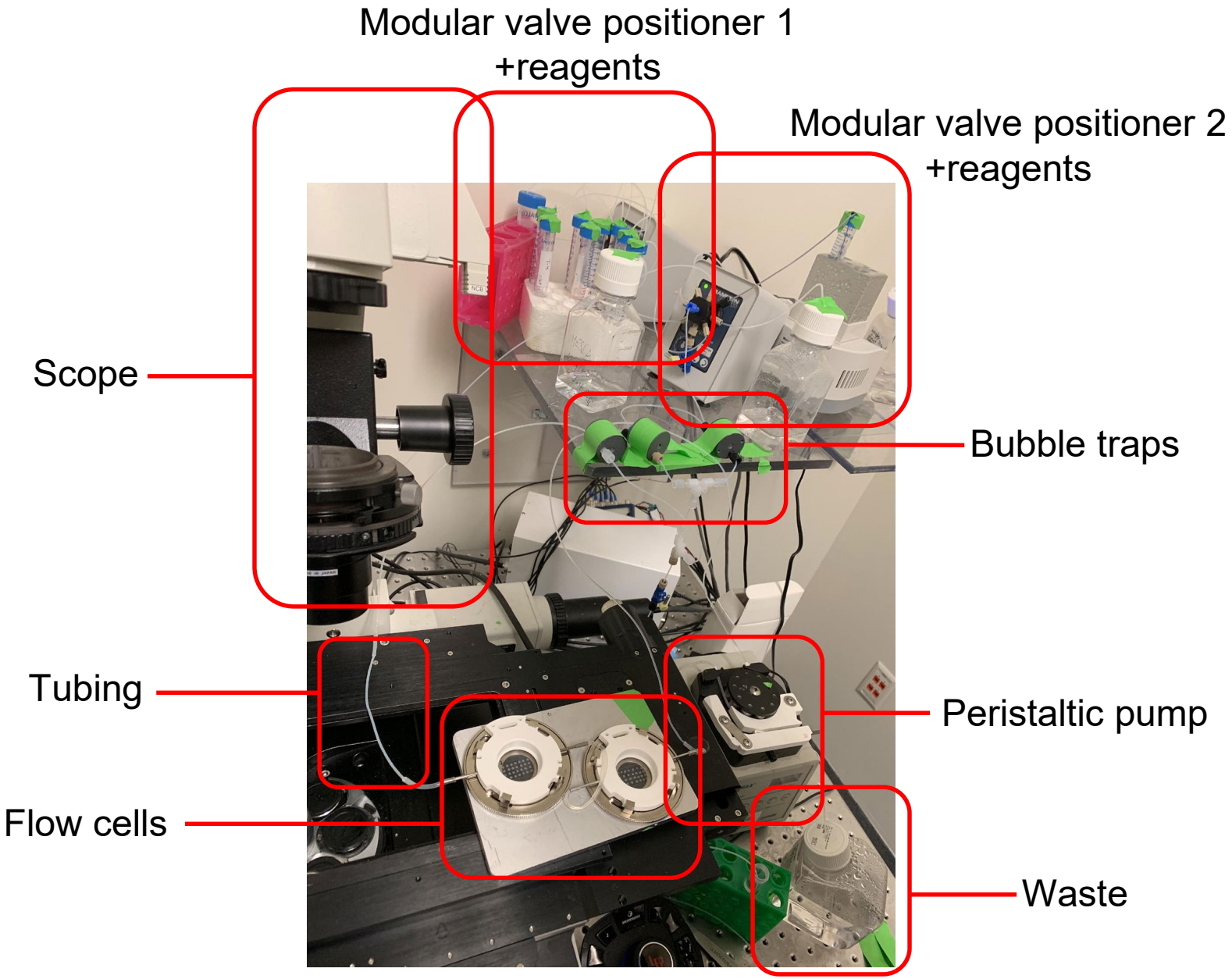
… as a means to study cellular architectures of human organs





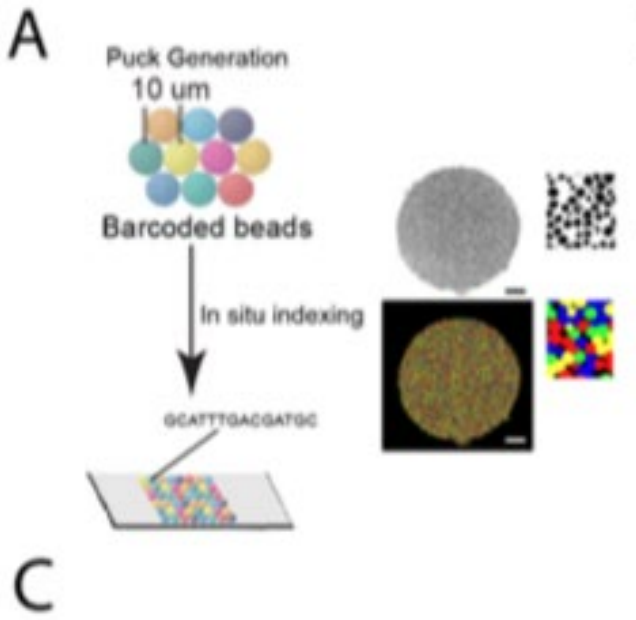
- The relative contribution of embryonic cells to tissues/organs;
- The ‘noise’ in early embryonic development between individuals;
- Developmental and cellular architectures of organs:
  - clonal structures
  - amount of stem cells contributing to functional units
  - differentiation trajectories available to given adult stem cell populations;
- Cell lineage perturbed in diseased tissues/organs
- Nature and role of somatic mutation in phenotypic variation, aging and disease

Another dimension: spatial


Modular valve positioner 1
+reagents
Modular valve positioner 2
+reagents
Scope
Bubble traps
Tubing
Peristaltic pump
Flow cells
Waste

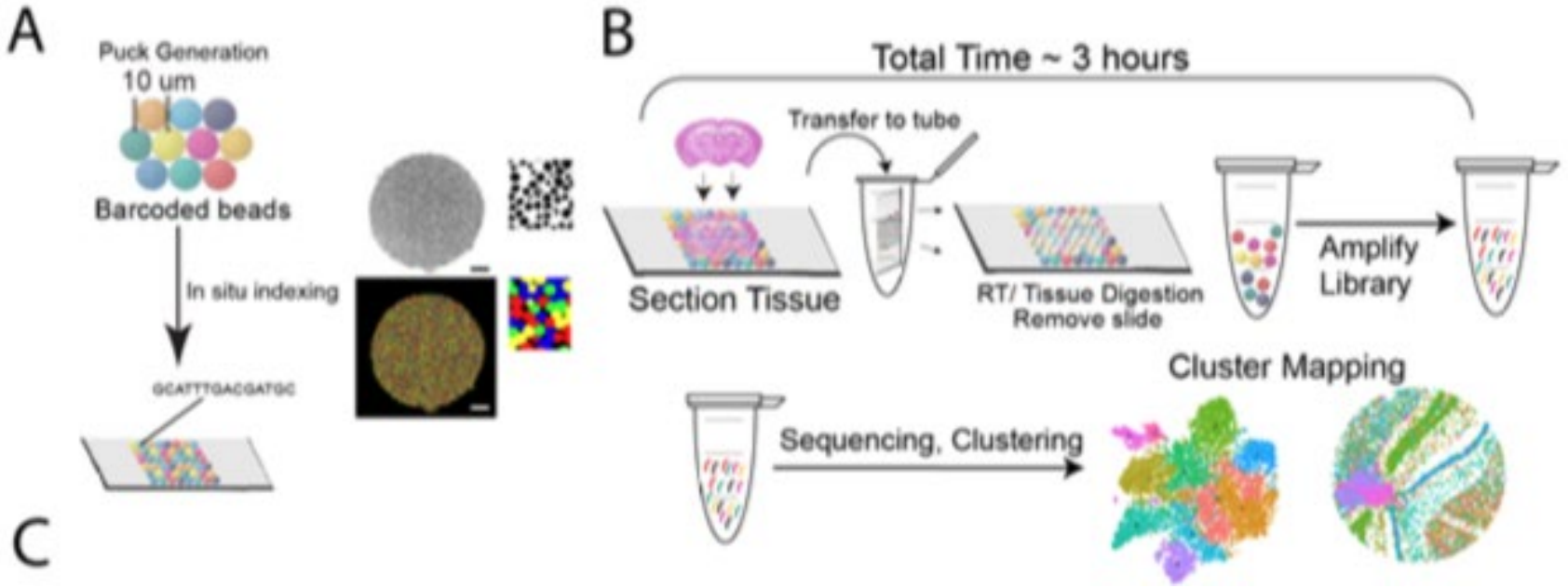
Slide-seq

- each bead barcode has known spatial location



Slide-seq

- each bead barcode has known spatial location



Slide-seq

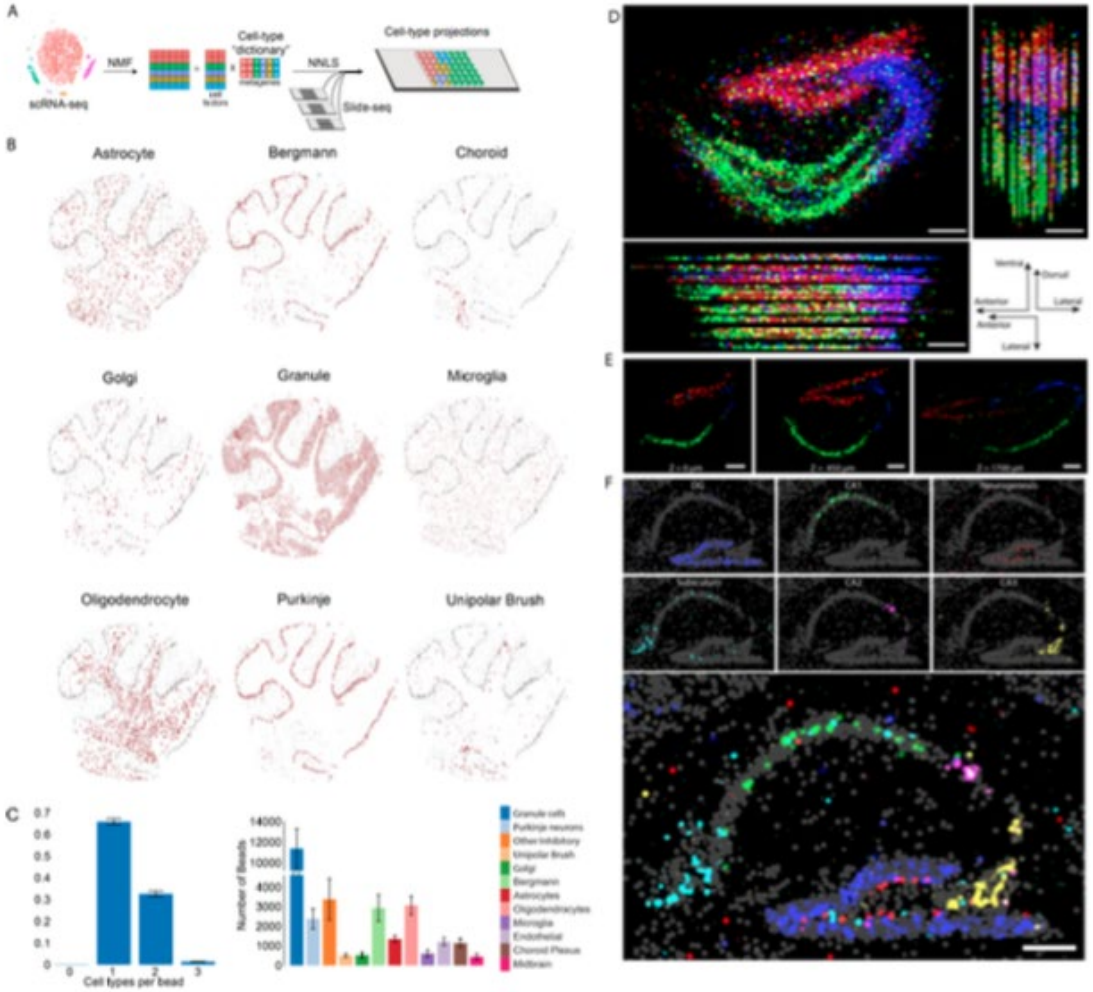
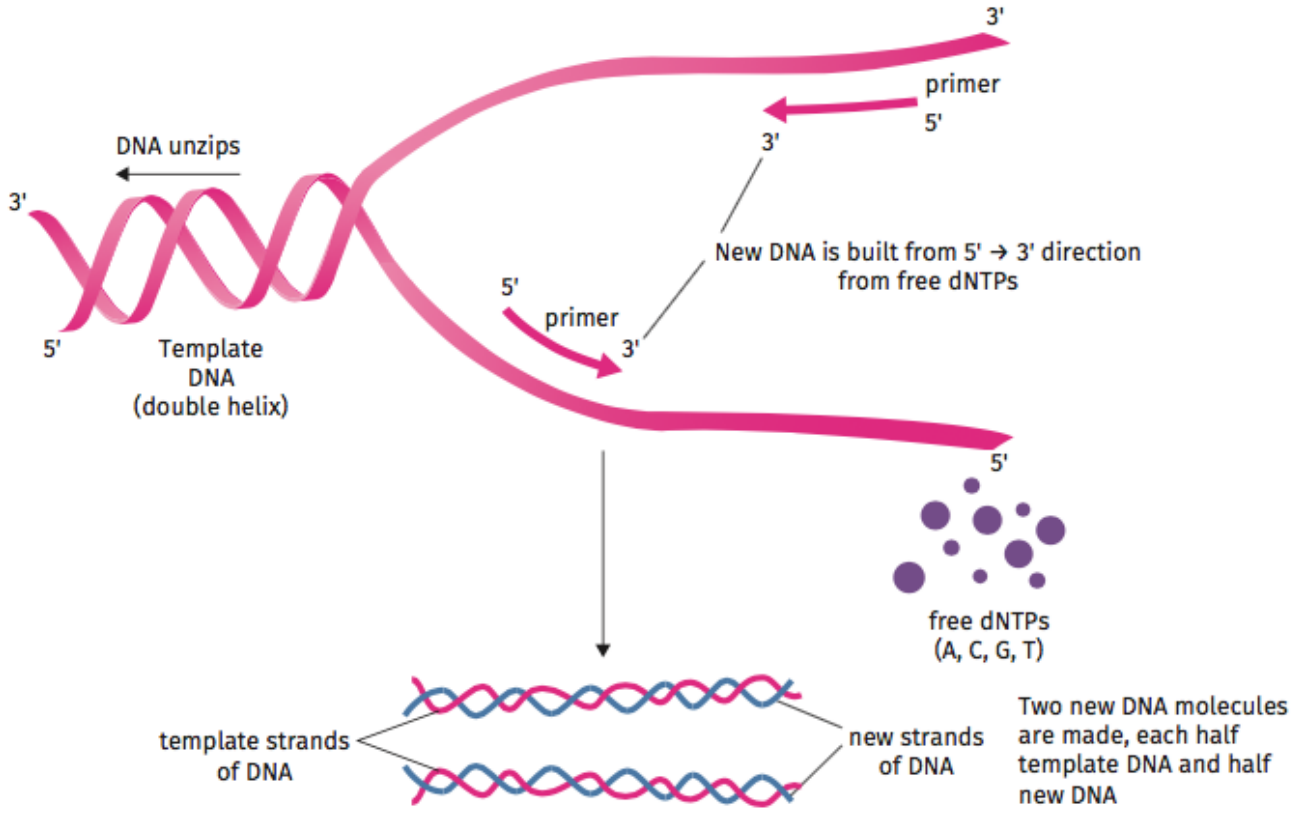


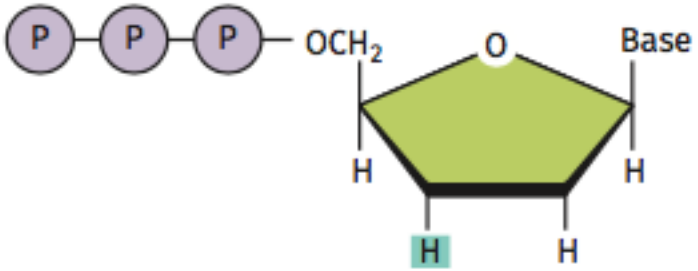
Figure 2: Localization of cell types in cerebellum and hippocampus using Slide-seq. (A)

Thank you for listening!
:)

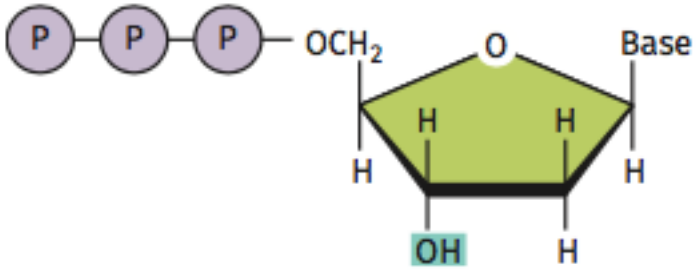
DNA replication: the key principles that underlie the tech



Nucleotide building blocks

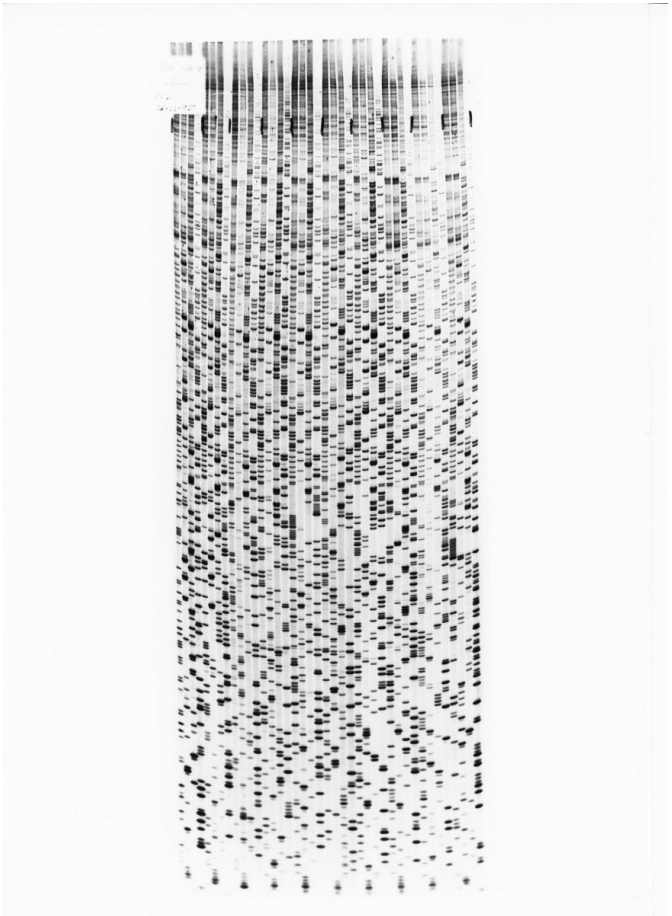


Dideoxynucleotide (ddNTP)

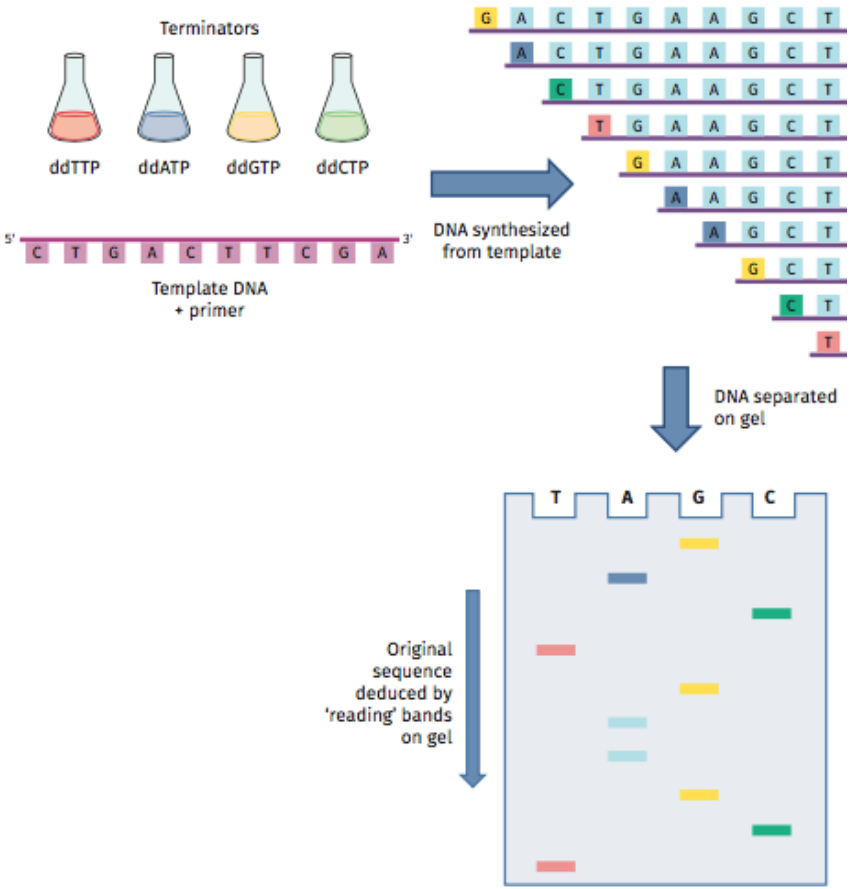


Deoxynucleotide (dNTP)

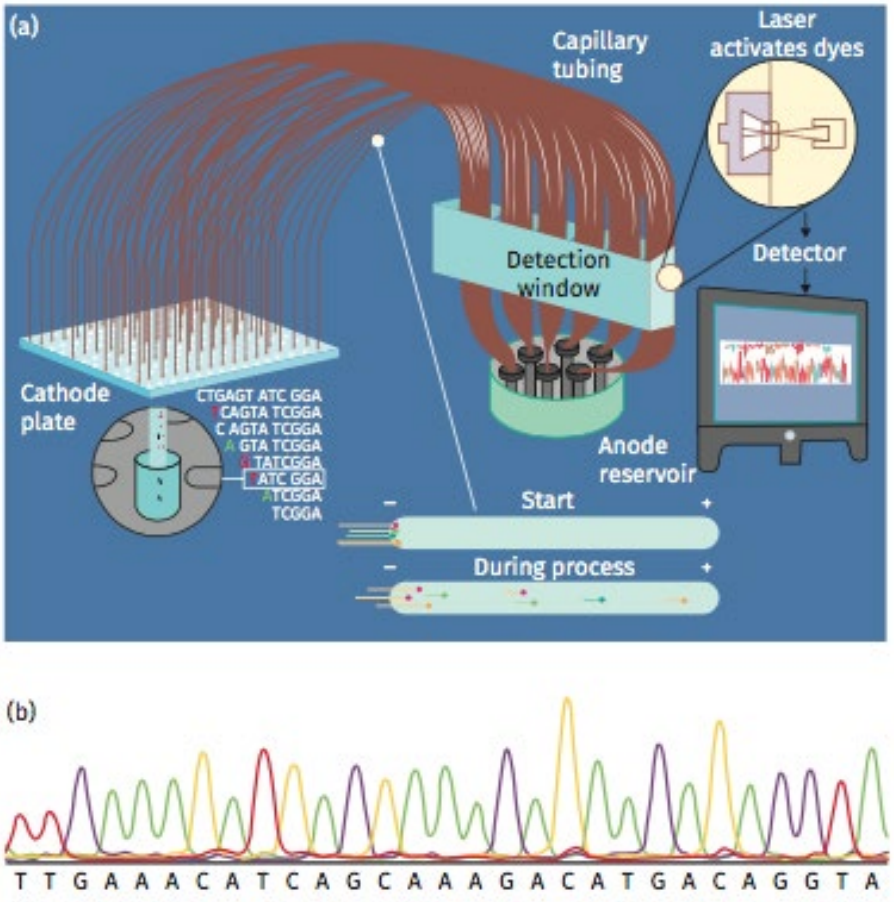
Sanger sequencing



Sanger sequencing

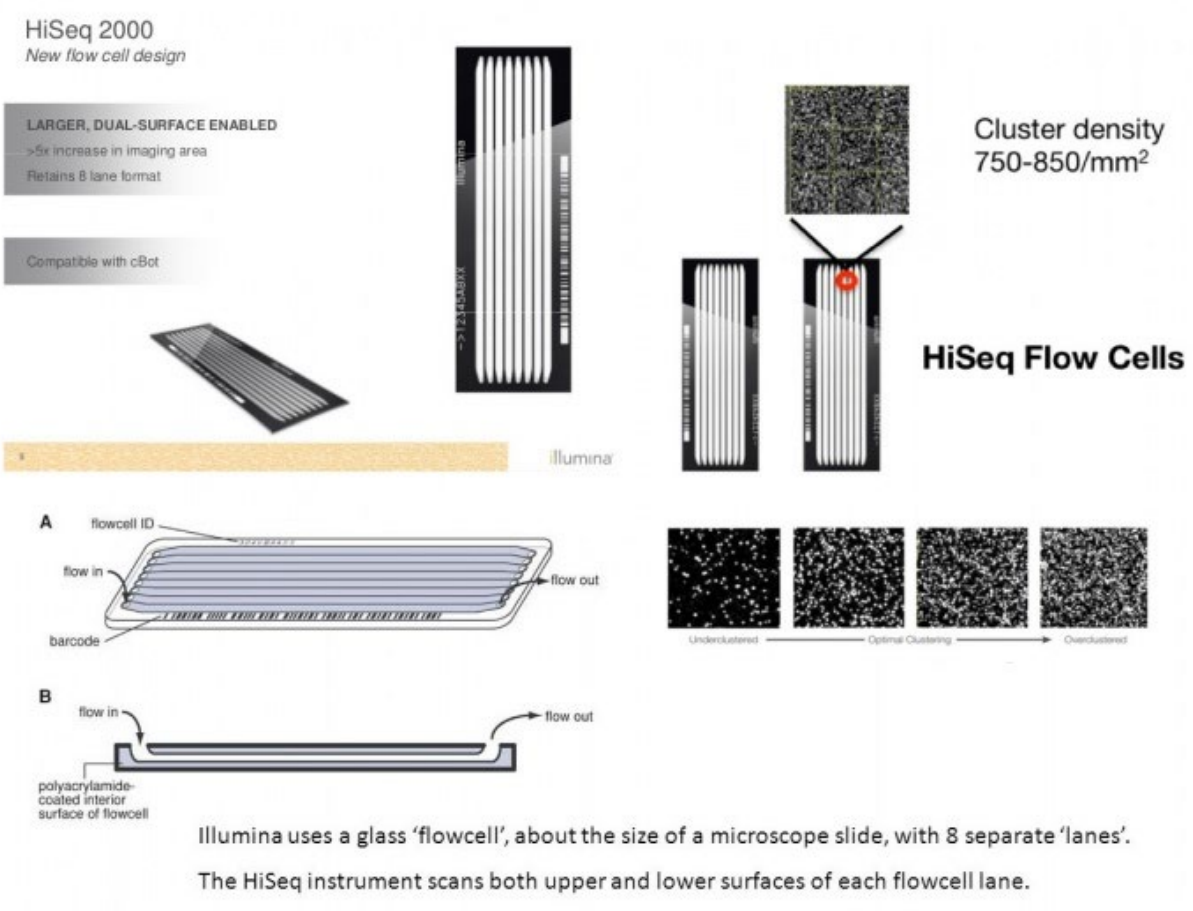


Capillary sequencing



“Next gen” sequencing: Illumina

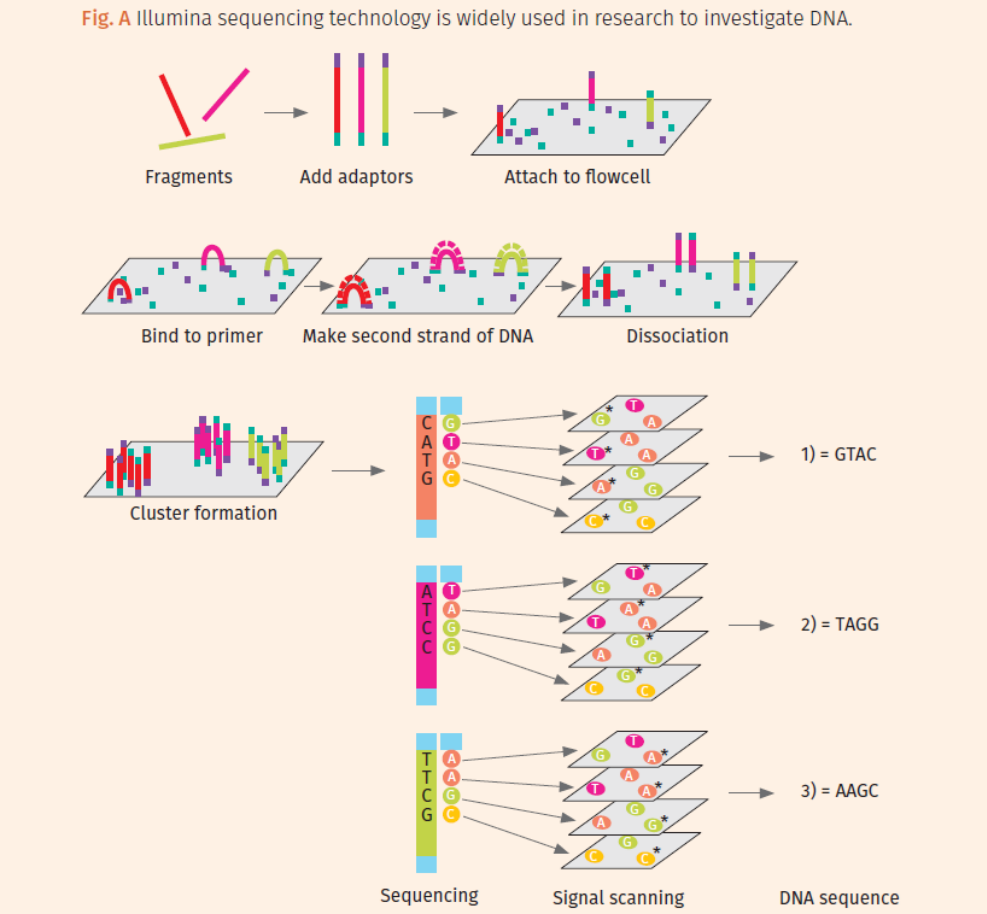
Illumina “flow cells”



Illumina uses a glass ‘flowcell’, about the size of a microscope slide, with 8 separate ‘lanes’.

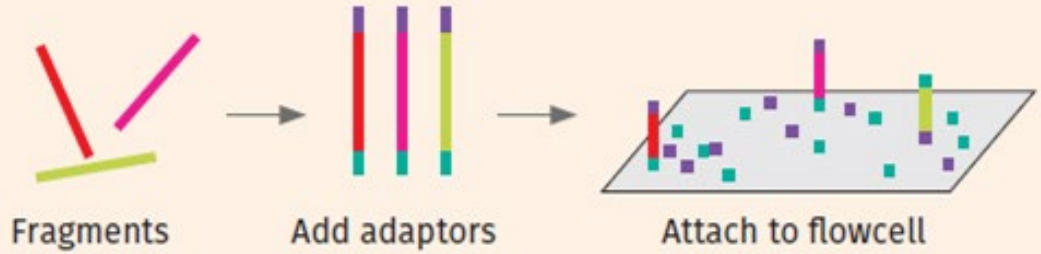
The HiSeq instrument scans both upper and lower surfaces of each flowcell lane.

Illumina sequencing: overview

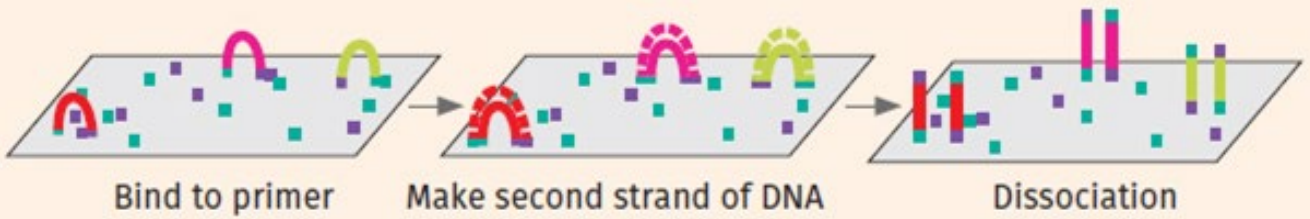


“Seeding” the flow cell

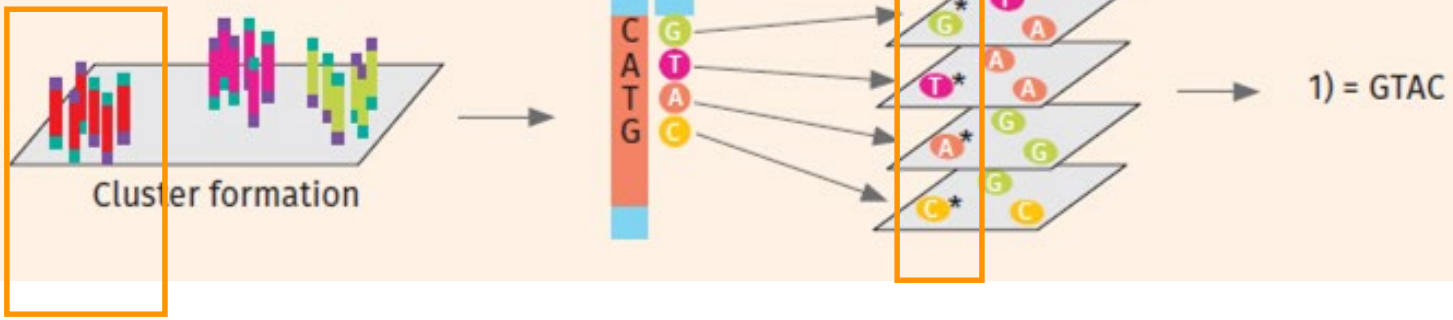
Fig. A Illumina sequencing technology is widely used in research to investigate DNA.



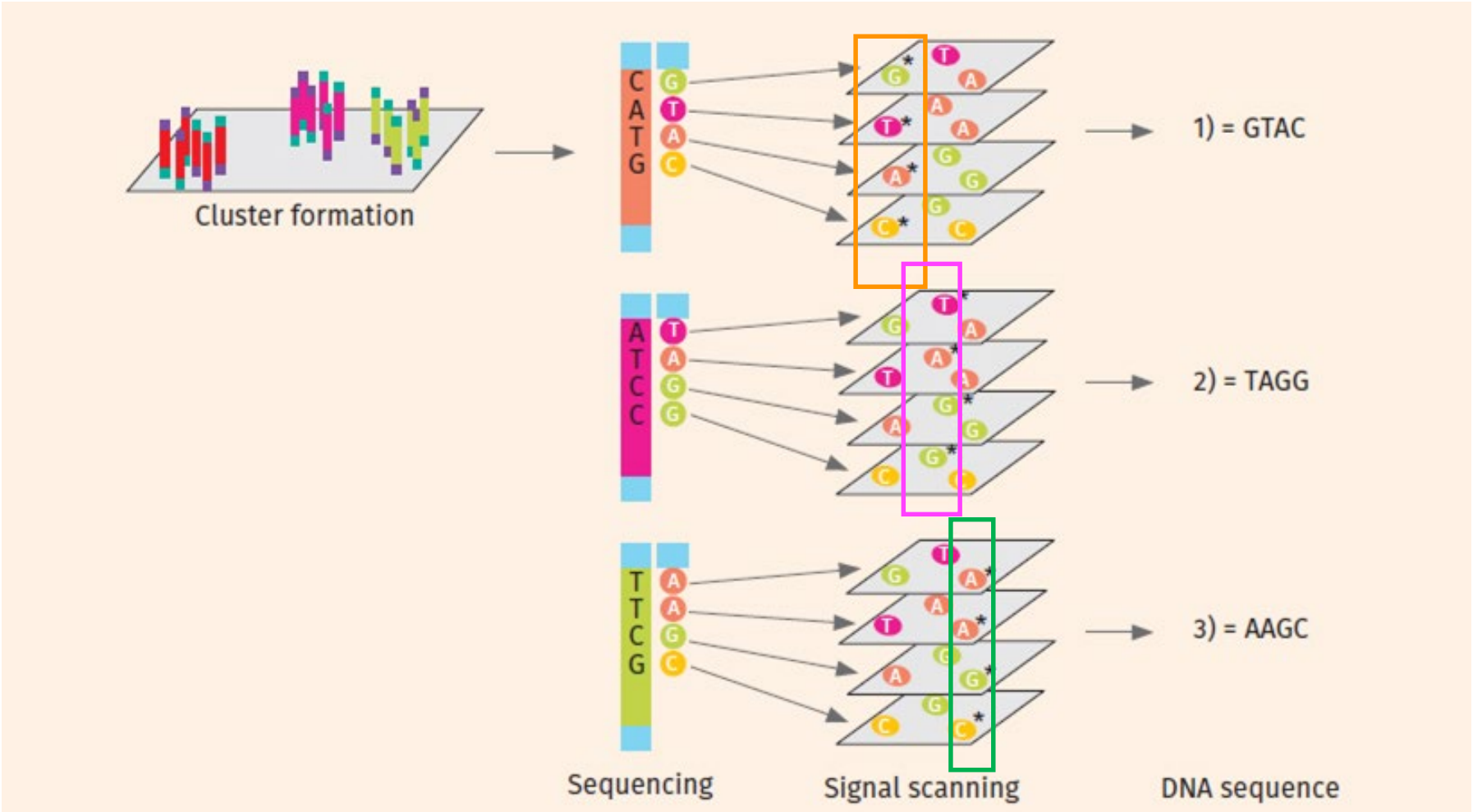
Forming clusters



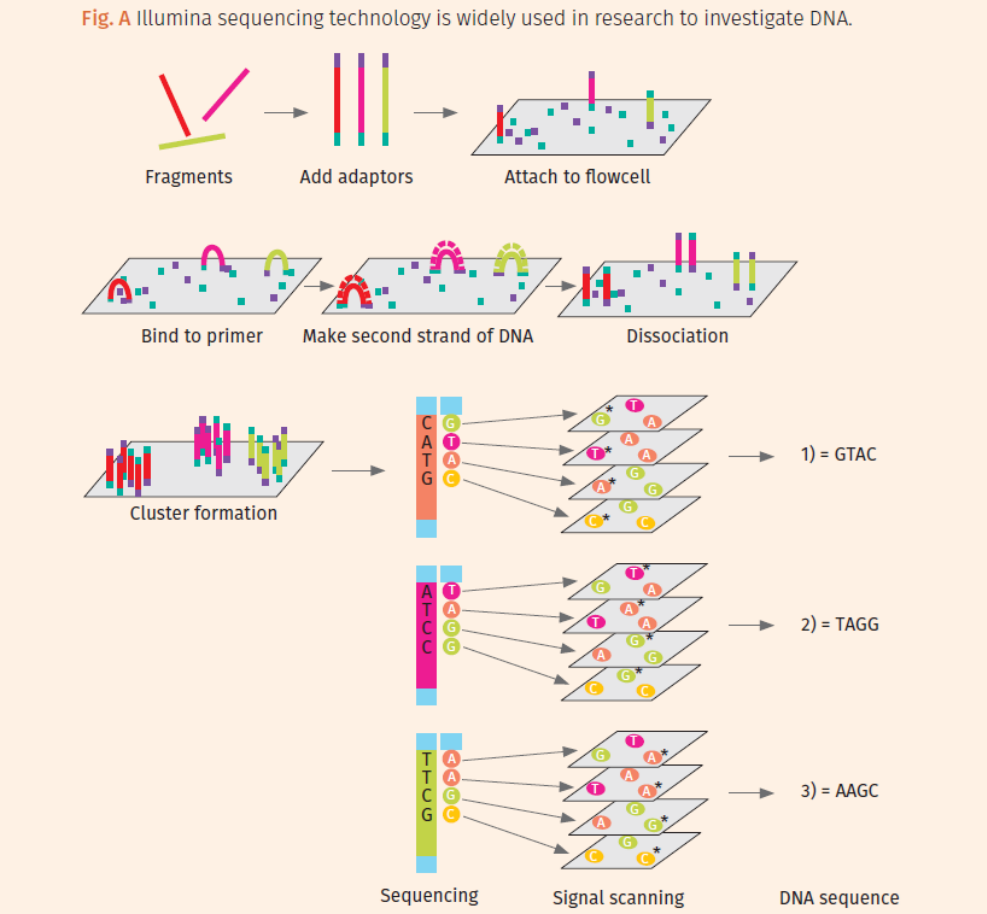
Reading each cluster



Reading each cluster



Illumina sequencing: overview



Data analysis: lots of data!

Sequencing in remote environments: possible?

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