

# Sequencing from single cells!

Lia Chappell

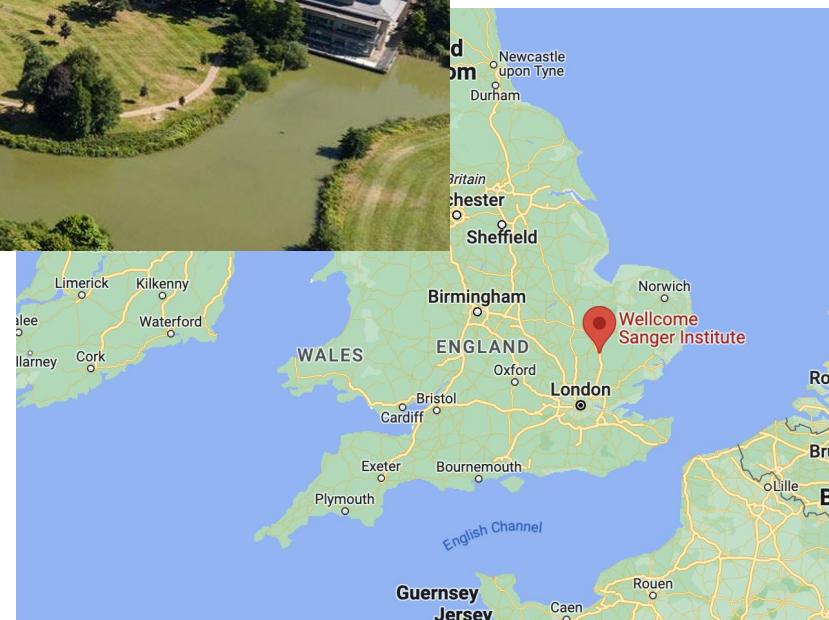
Wellcome Sanger Institute, Cambridge, UK

[LC5@sanger.ac.uk](mailto:LC5@sanger.ac.uk)

Twitter: [@LiaVLChappell](https://twitter.com/@LiaVLChappell)

Extra links are on the  
slides in green :)

# I'm based the Sanger Institute



# I grew up in Oxfordshire...



# I'm from Oxfordshire



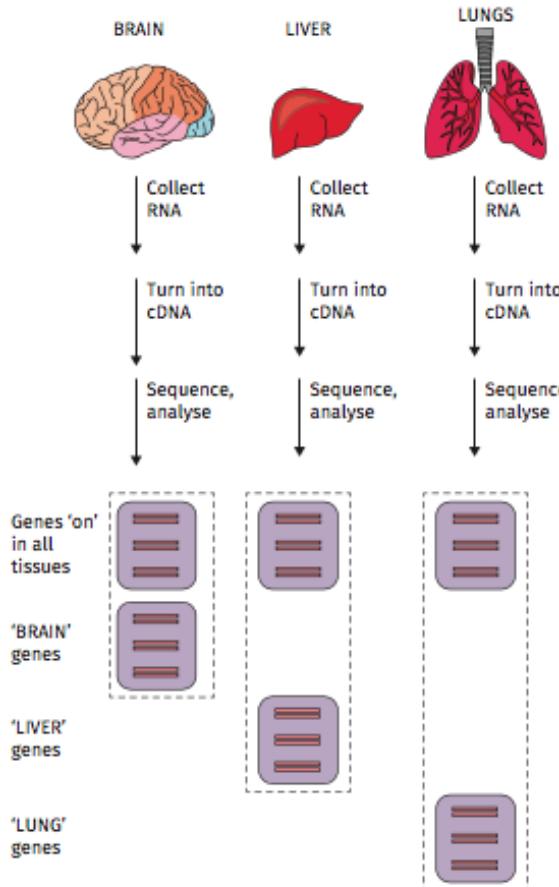
# All about RNA-seq

(In particular, about  
“Bulk” RNA-seq)

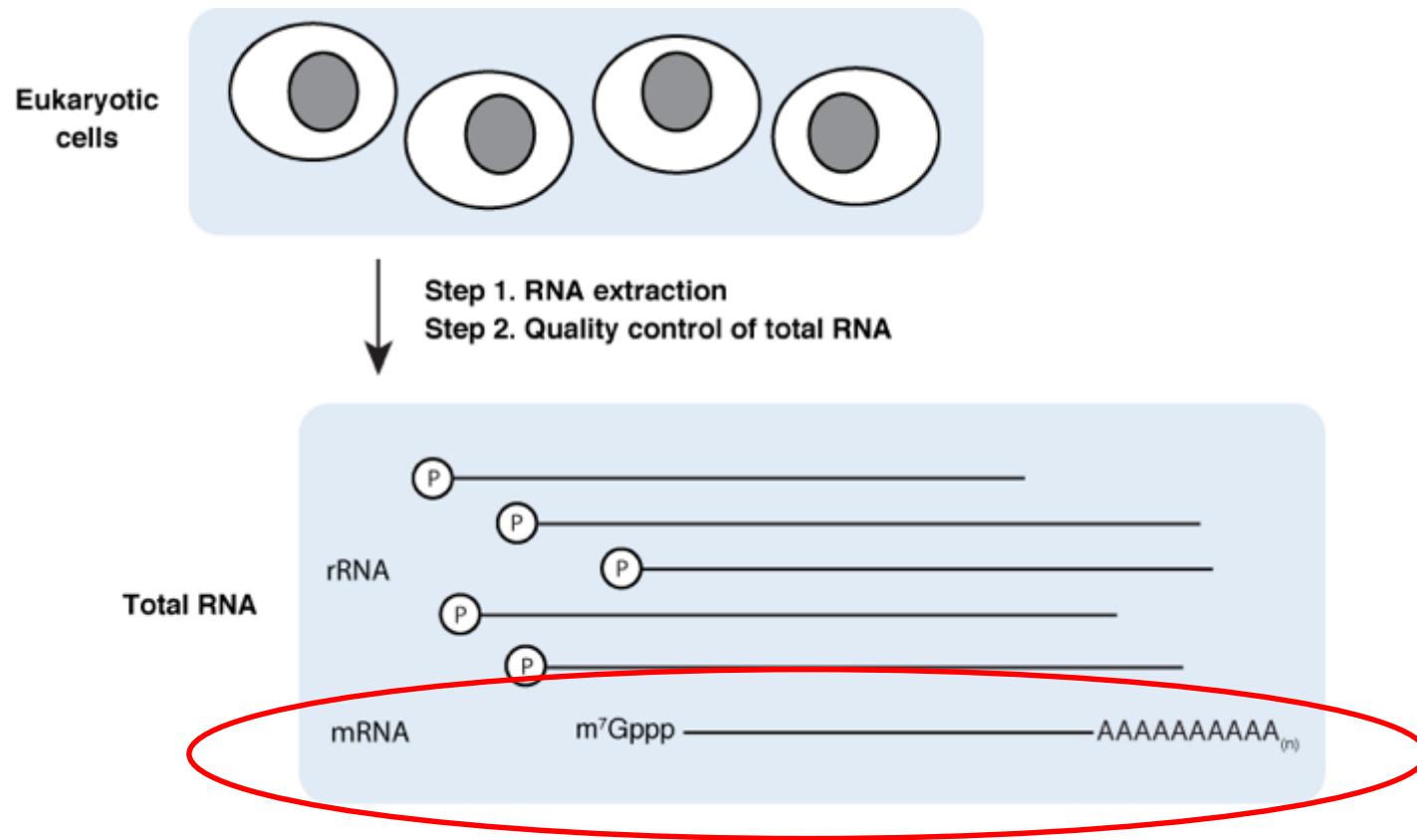
# More about RNA:

<https://www.yourgenome.org/theme/what-is-rna/>

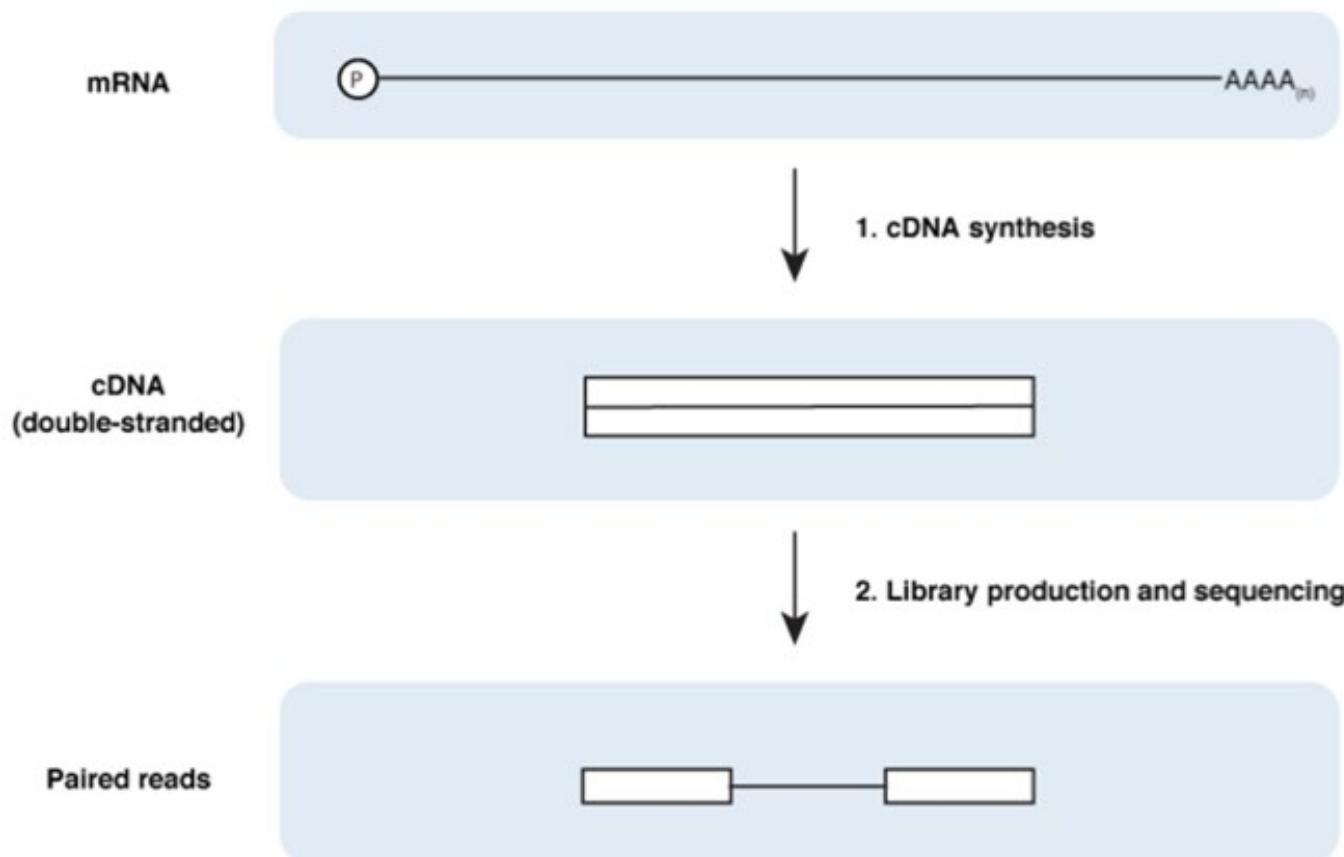
# RNA-seq measures “active” genes with sequencing



# RNA



# From RNA to RNA-seq



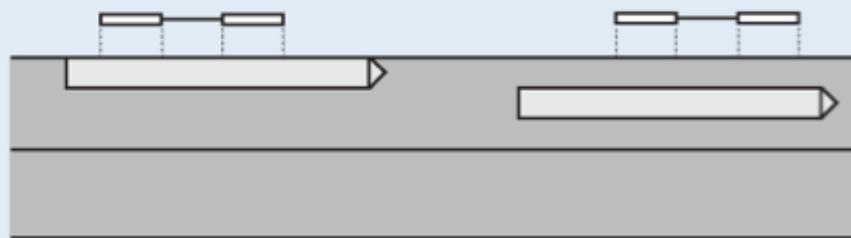
# From RNA to RNA-seq

Paired reads



3. Read mapping

Reads mapped  
to the genome



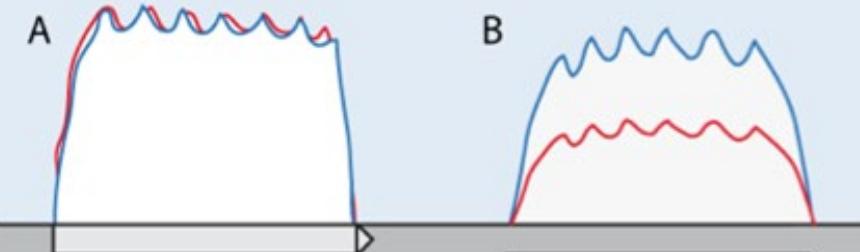
# From RNA to RNA-seq

Reads mapped  
to the genome



4. Generate coverage plots

Coverage  
plots visualise  
differential gene  
expression  
between  
biological  
conditions



# Sequencing from single cells: why and how?

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

The screenshot shows the Annual Reviews website interface. At the top, there's a horizontal bar with the AR logo, followed by the text 'ANNUAL REVIEWS' and 'For Librarians & Agents' and 'For Authors'. Below this is a navigation menu with 'JOURNALS A-Z', 'JOURNAL INFO', and 'PRICING & SUBSCRIPTIONS'. The main content area has a dark blue header with the title 'Single-Cell (Multi)omics Technologies'. Below the header, it says 'Annual Review of Genomics and Human Genetics' and 'Vol. 19:- (Volume publication date August 2018)'. It also mentions 'Review in Advance first posted online on May 4, 2018. (Changes may still occur before final publication.)' and provides the DOI: <https://doi.org/10.1146/annrev-genom-091416-035324>. The author list includes 'Lia Chappell,<sup>1,\*</sup> Andrew J.C. Russell,<sup>1,\*</sup> and Thierry Voet<sup>1,2</sup>'. Footnotes explain the asterisks: <sup>1</sup>Wellcome Sanger Institute, Cambridge CB10 1SA, United Kingdom; email: [lc5@sanger.ac.uk](mailto:lc5@sanger.ac.uk), [ar19@sanger.ac.uk](mailto:ar19@sanger.ac.uk), [tv2@sanger.ac.uk](mailto:tv2@sanger.ac.uk) and <sup>2</sup>Department of Human Genetics, KU Leuven, B-3000 Leuven, Belgium; email: [thierry.voet@kuleuven.be](mailto:thierry.voet@kuleuven.be). A note states, "'These authors contributed equally to this article'". Below the authors, there are links for 'Download PDF' and 'Article Metrics', along with 'Permissions | Reprints | Download Citation | Citation Alerts'. The abstract section starts with the heading 'Abstract'.

**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



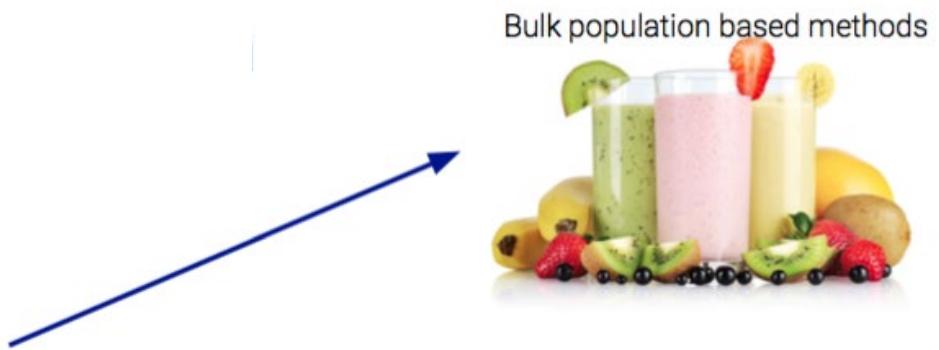
Review in Advance first posted on May 4, 2018. (Changes may still occur before final publication.)

**Table 1** Single-cell multiomics technologies

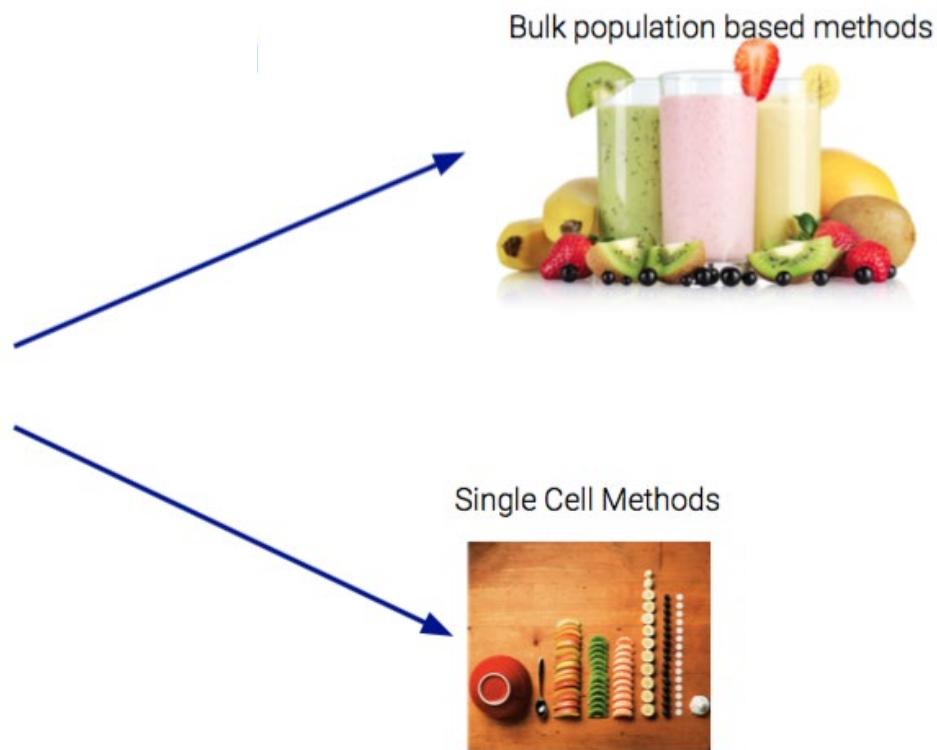
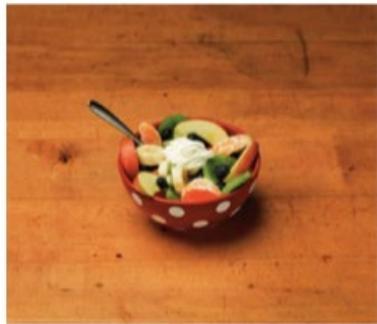
Technology	Genomic layer	Chromatin accessibility epigenetic layer	CpG methylation epigenetic layer	Transcriptomic layer	Protein layer	Strategy	Cell isolation <sup>a</sup>	Cell throughput	Automation	Constituent methods
DR-seq (31)	Genome	—	—	Transcriptome	—	Preamplification and tagging of DNA and RNA 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)

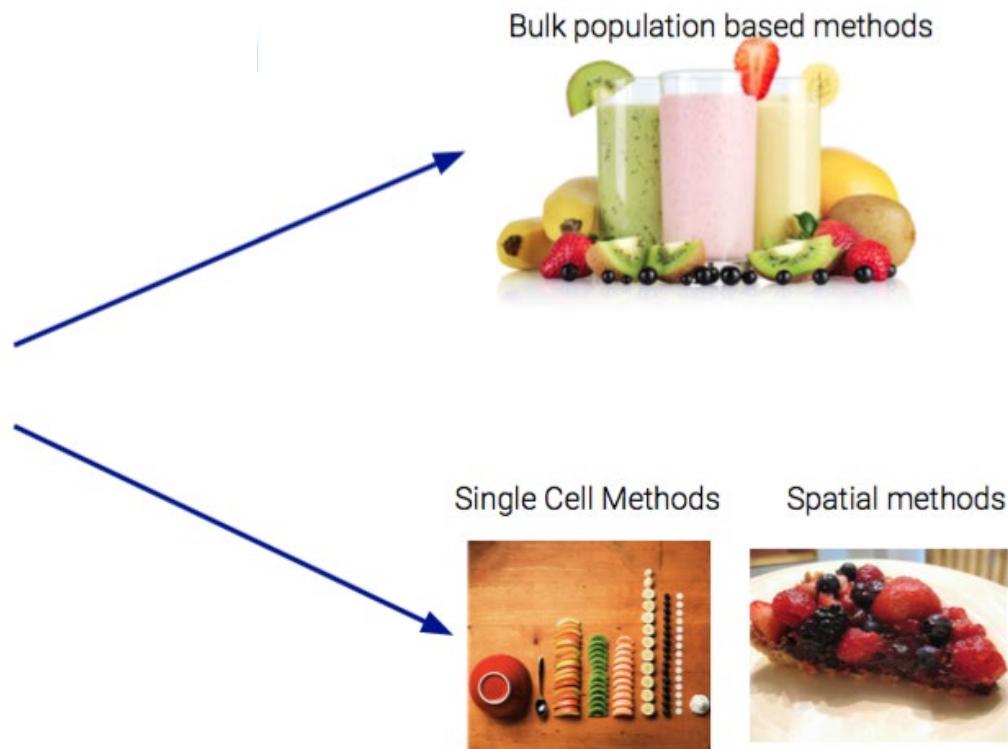
Why?



Bulk population based methods



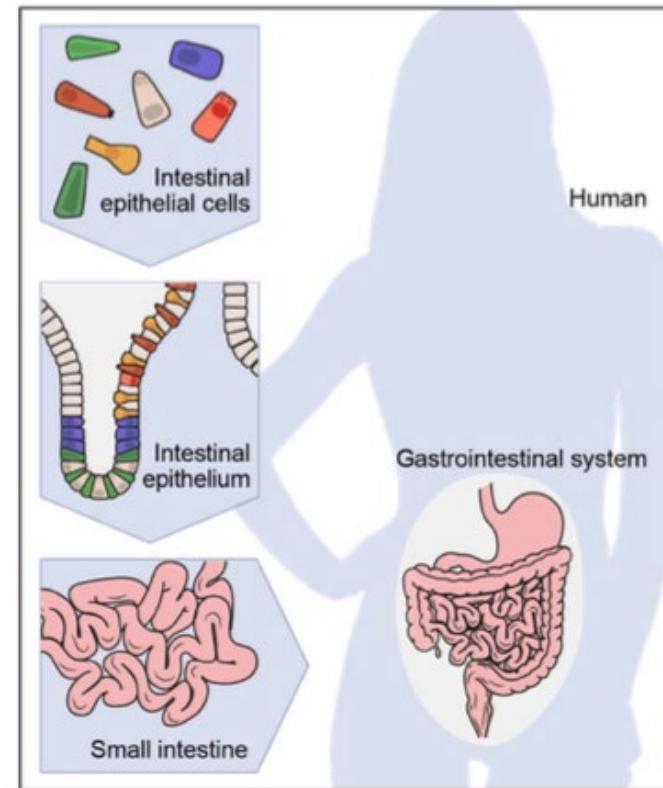
The Art of Clean Up, Ursus Wehrli, Kimberly Vardeman



The Art of Clean Up, Ursus Wehrli, Kimberly Vardeman

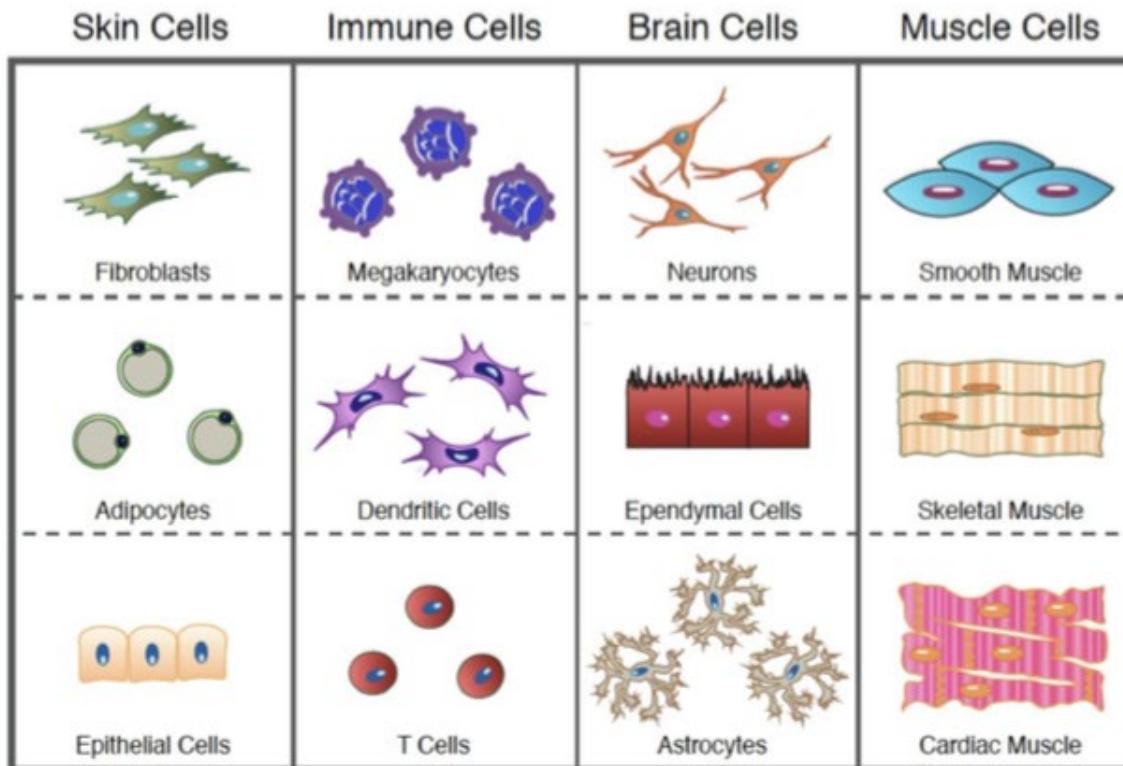
# Human Cell Atlas

A “google maps” of human anatomy



# Human Cell Atlas

**Goal : A periodic table of our cells**

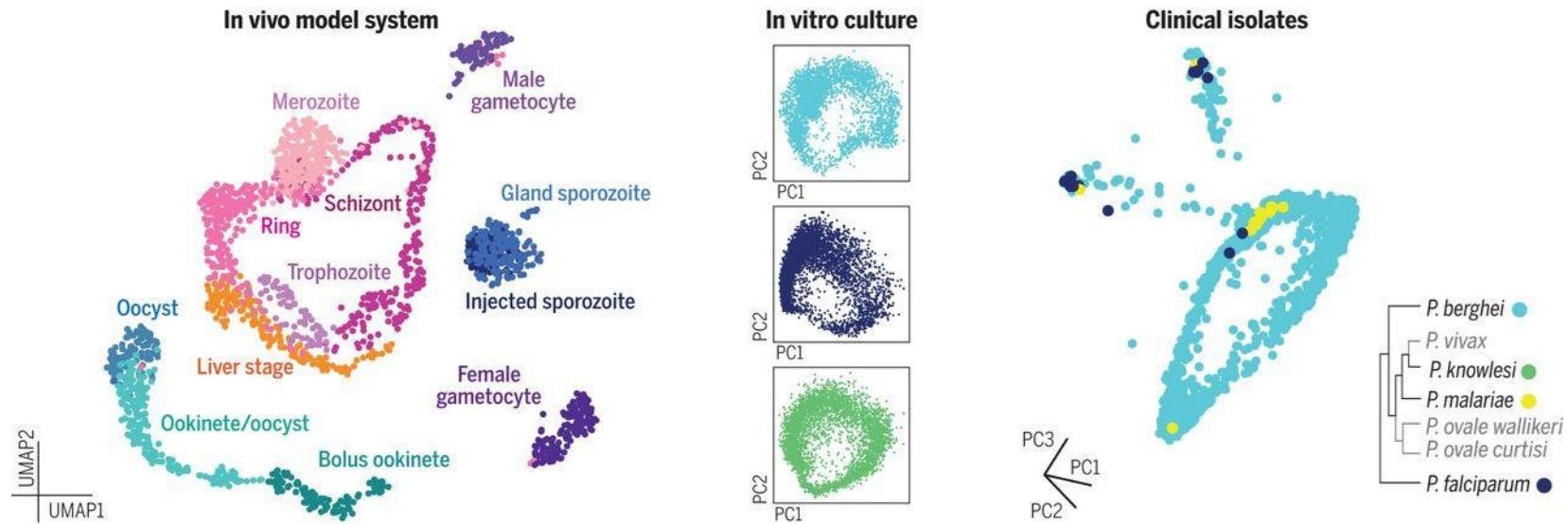


# Find the Human Cell Atlas online...

This page has lots of links for the general public like videos and interviews with scientists:

<https://www.humancellatlas.org/engagement/>

# Malaria Cell Atlas



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

Copyright © 2019 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works

Science  
AAAS

# More about malaria:

Two one page articles:

<https://www.yourgenome.org/theme/using-genomics-to-understand-malaria/>

<https://www.yourgenome.org/theme/malaria-an-ongoing-battle-against-drug-resistance/>

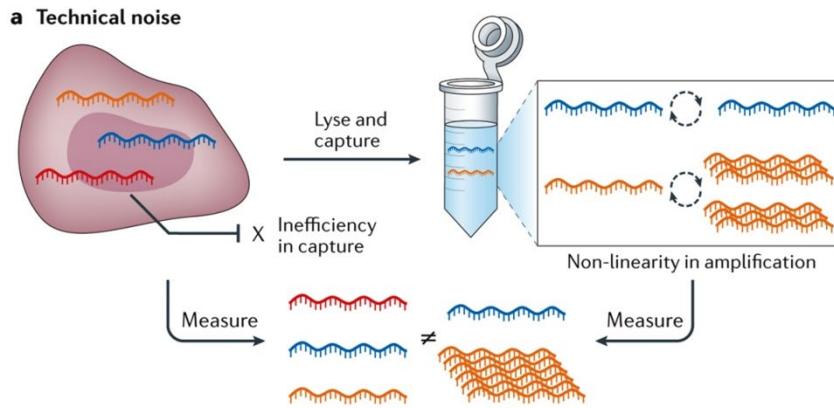
# More about cancer:

Two one page articles:

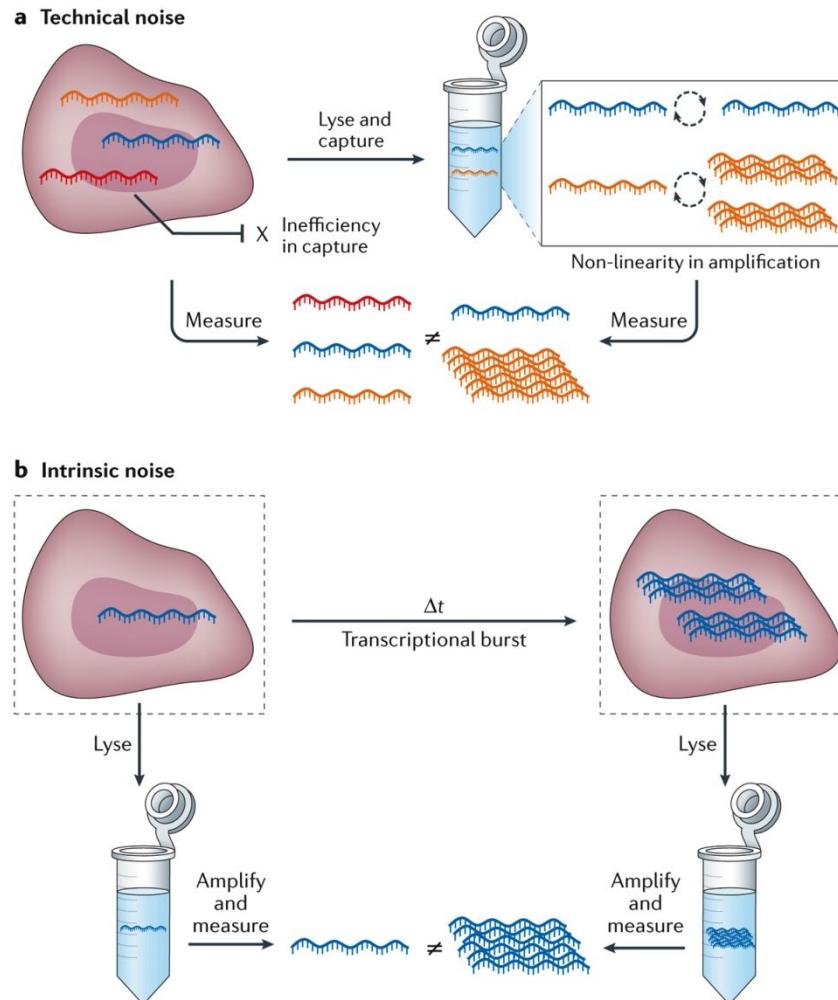
<https://www.yourgenome.org/theme/is-cancer-a-genetic-disease/>

<https://www.yourgenome.org/theme/different-types-of-mutations/>

# Noise in scRNA-seq



# Noise in scRNA-seq



How?

# FACS machine



# Usually in plates



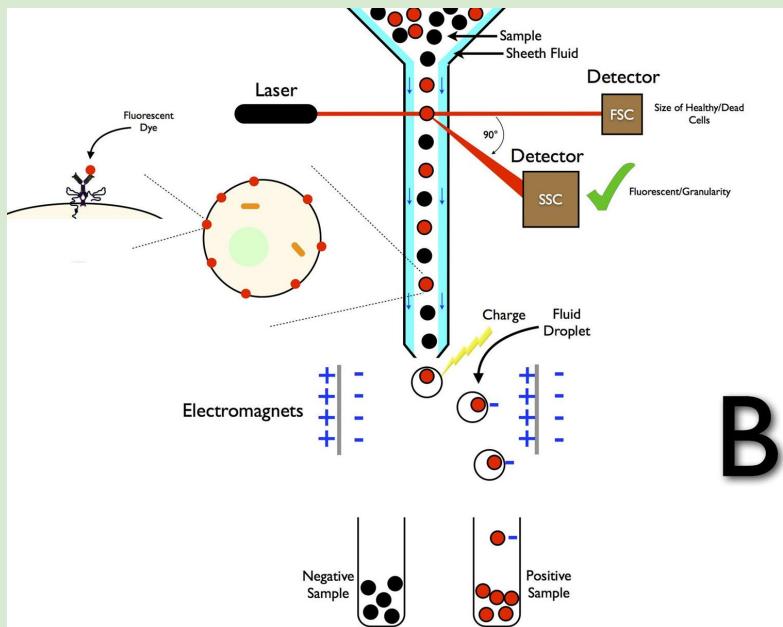
# More about cell sorting

Wikipedia:

[https://en.wikipedia.org/wiki/Cell\\_sorting](https://en.wikipedia.org/wiki/Cell_sorting)

# Cell sorting (from Wikipedia)

- Use a plate instead of the tube shown here
- Put one cell in each of the 0.2 mL wells
- So 96 cells per plastic plate



B



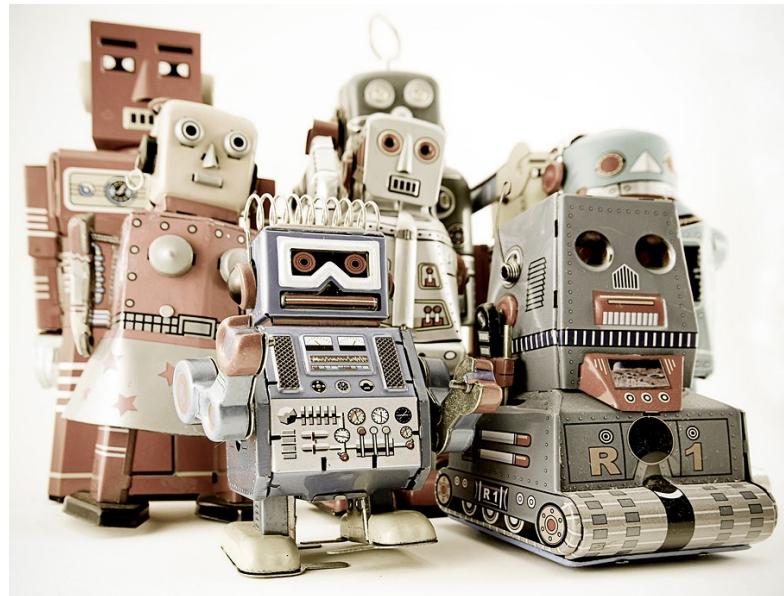
# Single cell RNA-seq: plate format



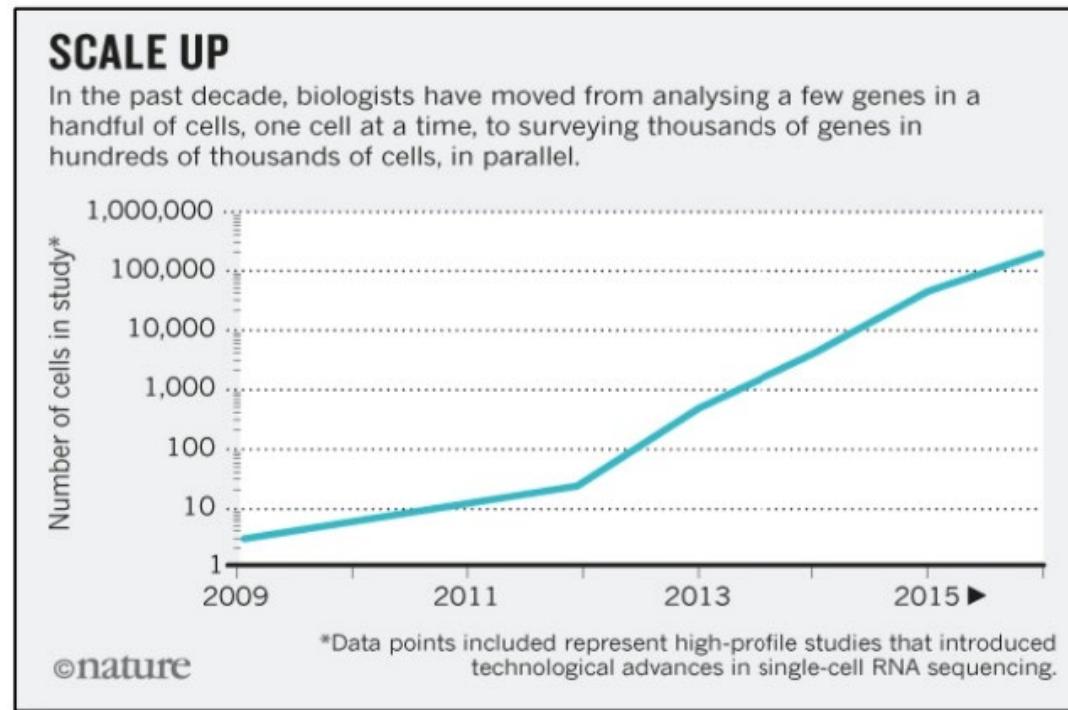
# Single cell RNA-seq: plate format



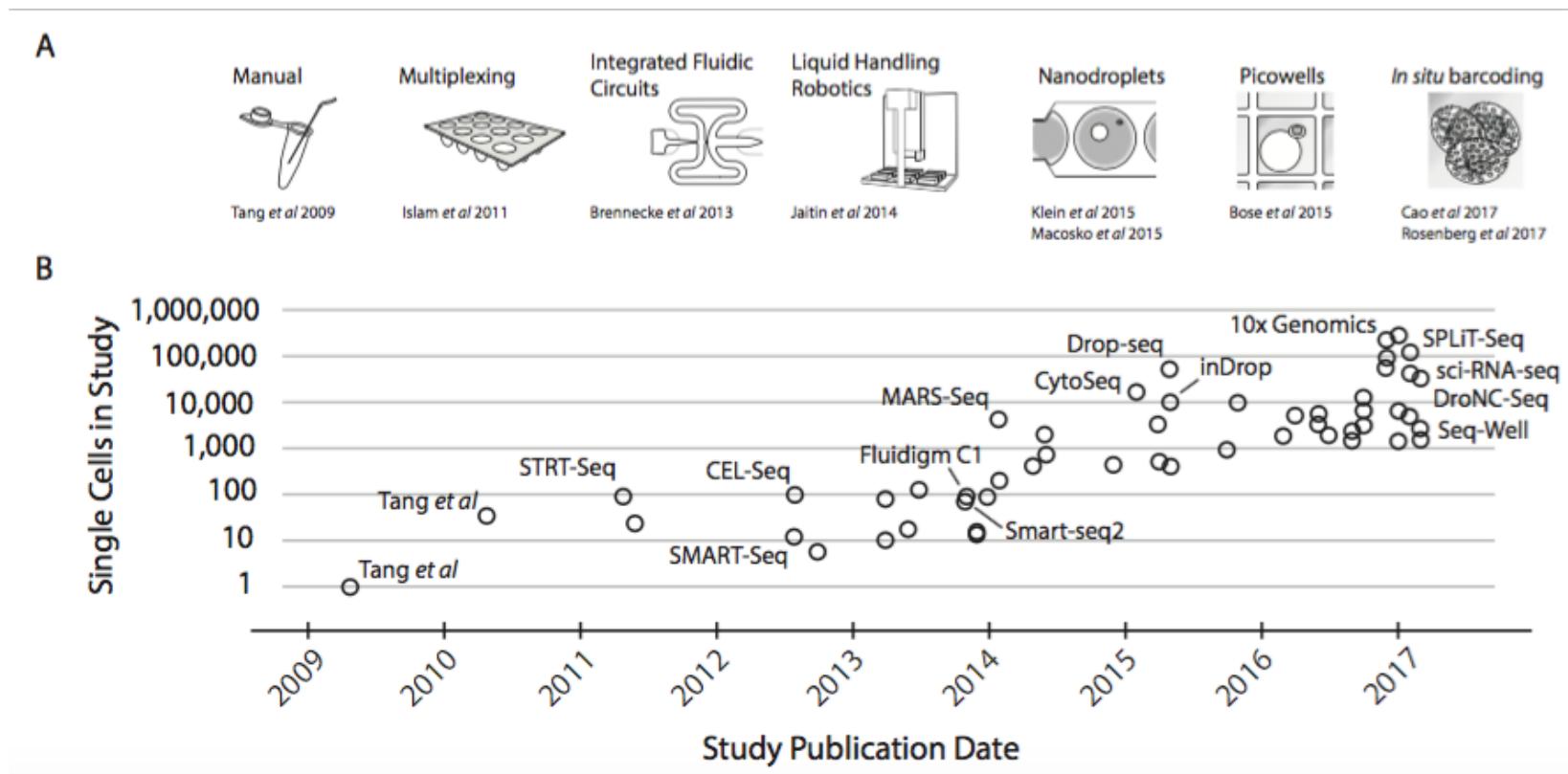
# Single cell RNA-seq: plate format



# A trend for increasing scale...

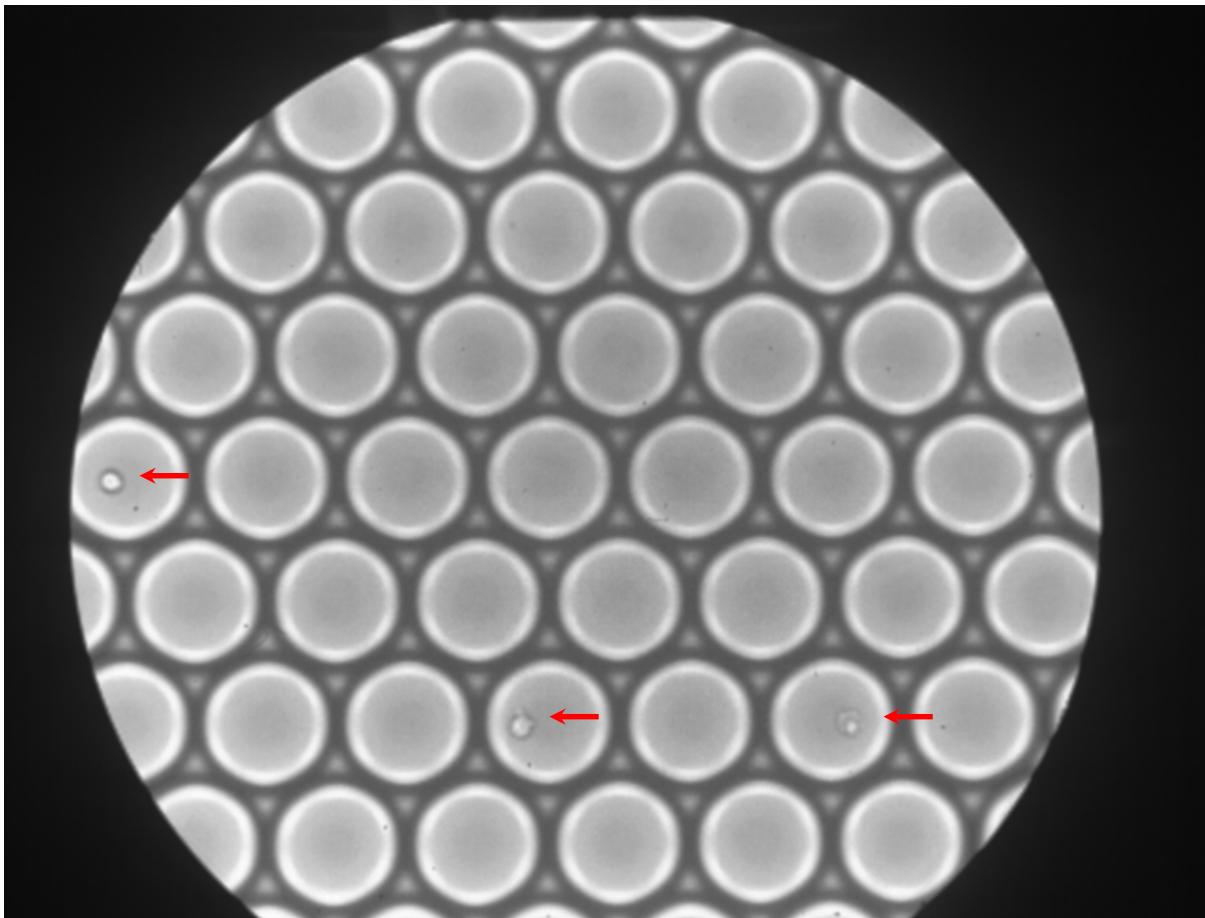


# A trend for increasing scale...

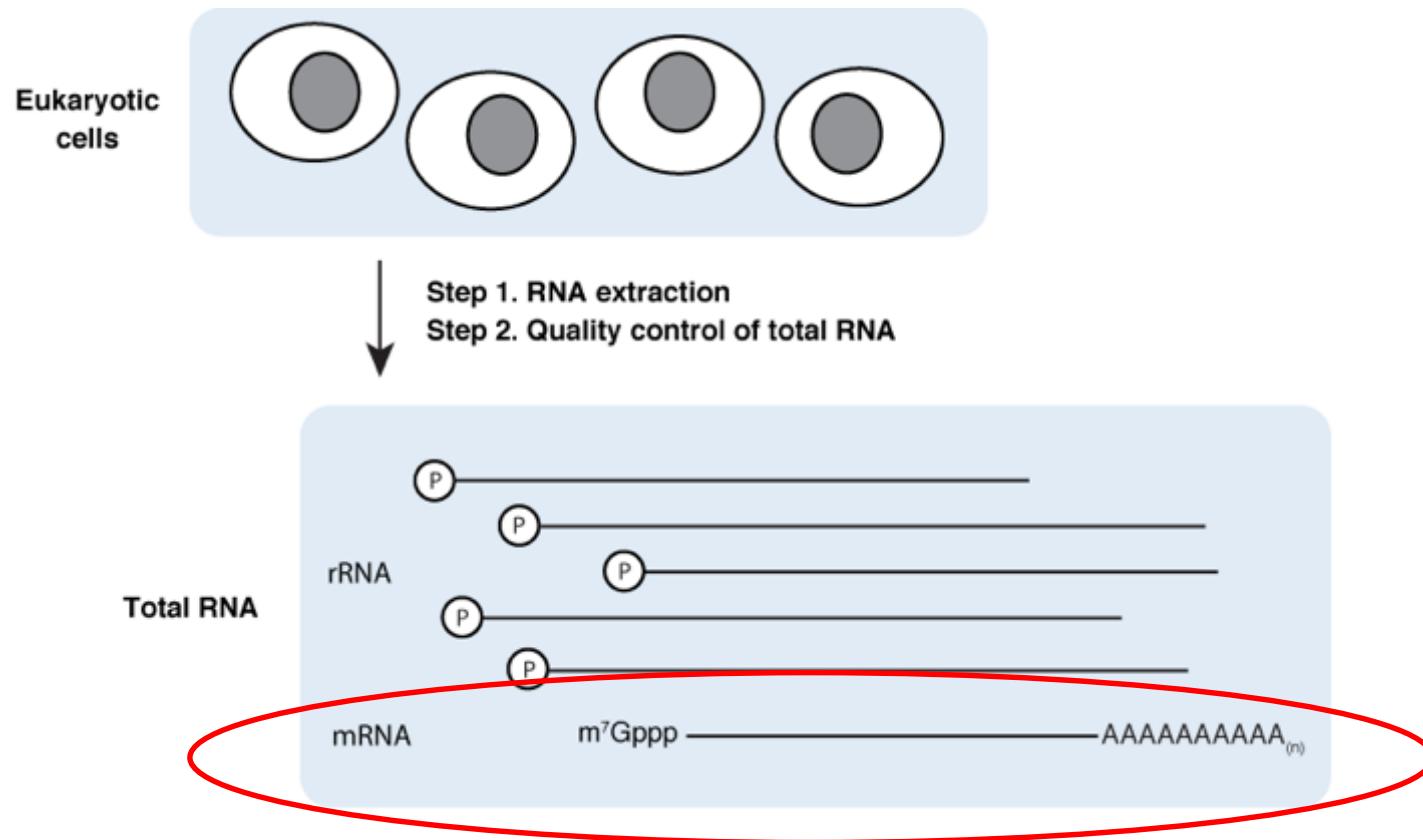


[Valentine Svensson](#), [Roser Vento-Tormo](#), [Sarah A Teichmann](#)

# Another way: droplets

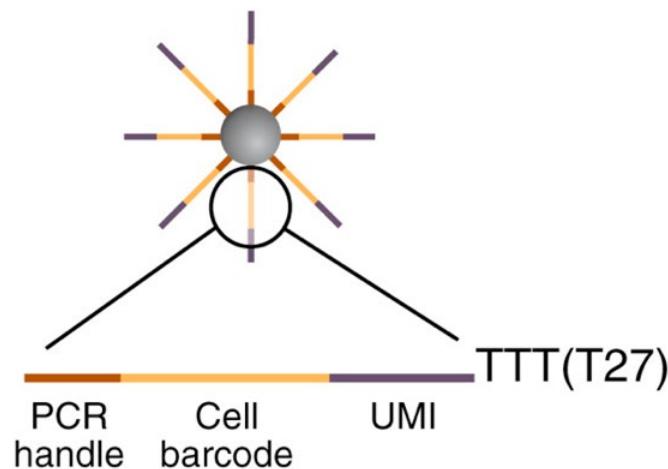


# mRNA - a reminder



# Barcoded beads...

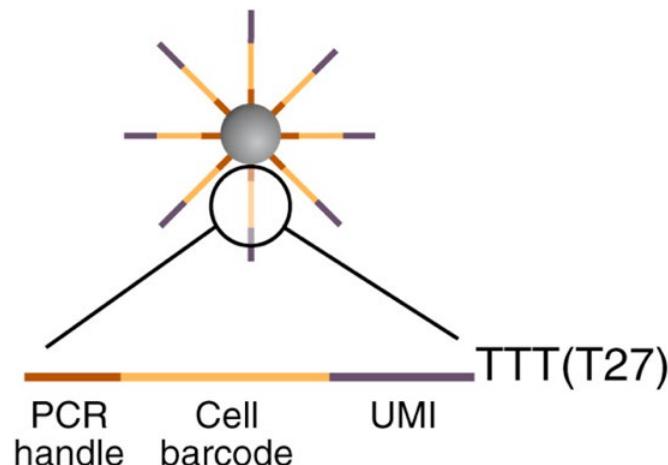
## Barcoded beads



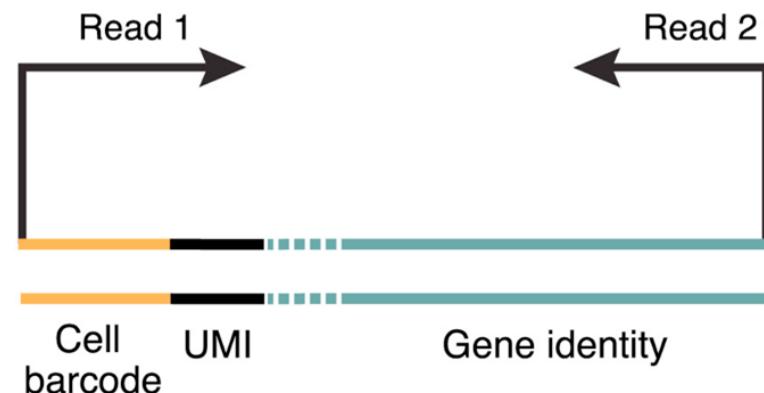
Drop-seq (Macosko et al. 2015)

# Barcoded beads...

## Barcoded beads



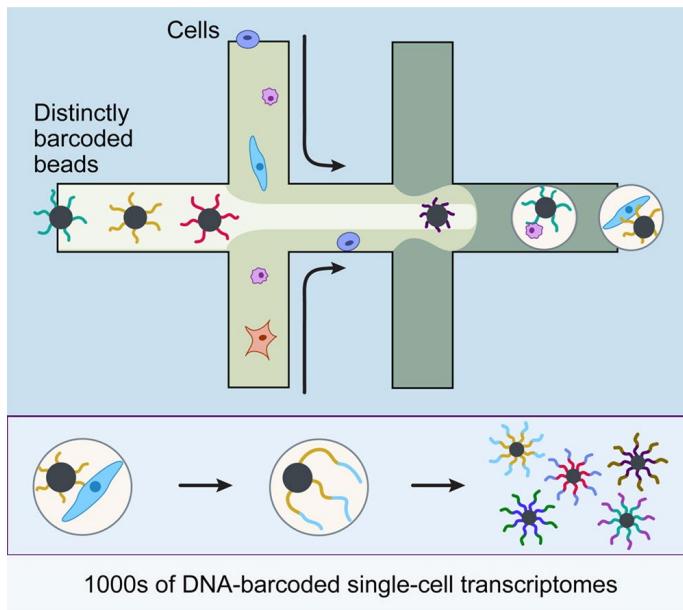
## Sequencing reads



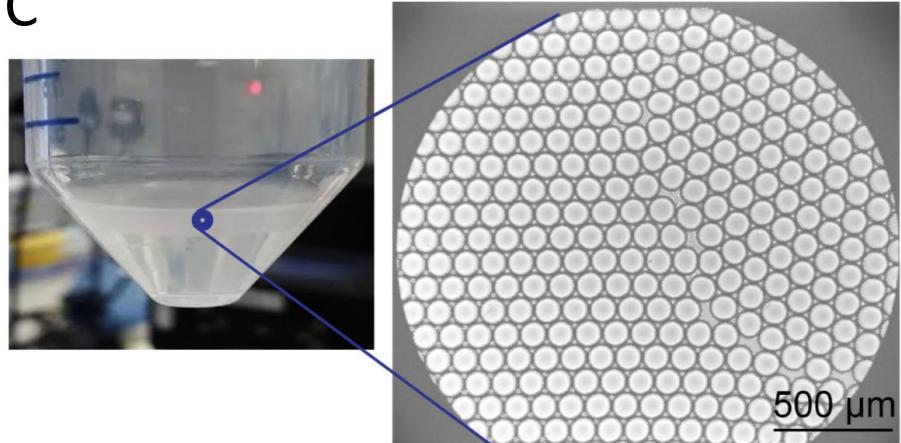
Drop-seq (Macosko et al. 2015)

# Drop-seq

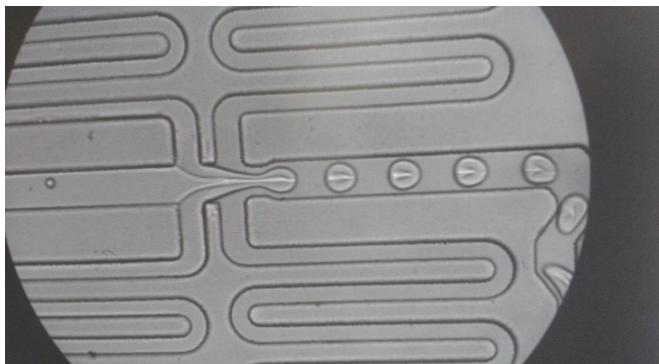
A



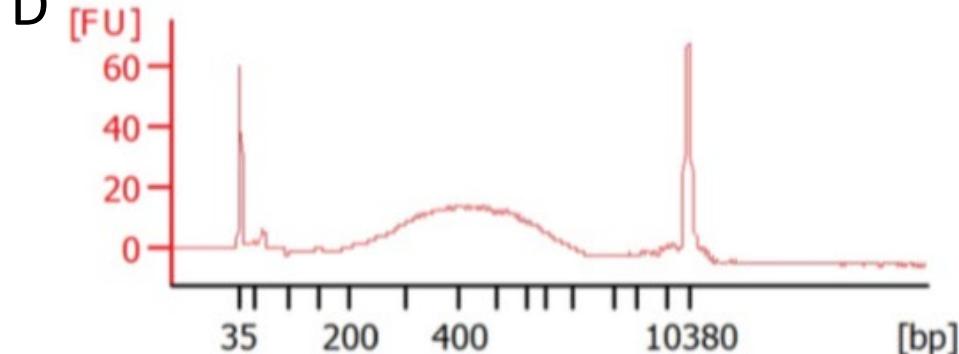
C



B



D



# Drop-seq website

You might enjoy the photos and videos on the Drop-seq website:

<https://mccarrolllab.org/dropseq/>

The lab team did a good job of making it easy for people to make their own in their own labs

# Videos on YouTube:

Short talk (5 min):

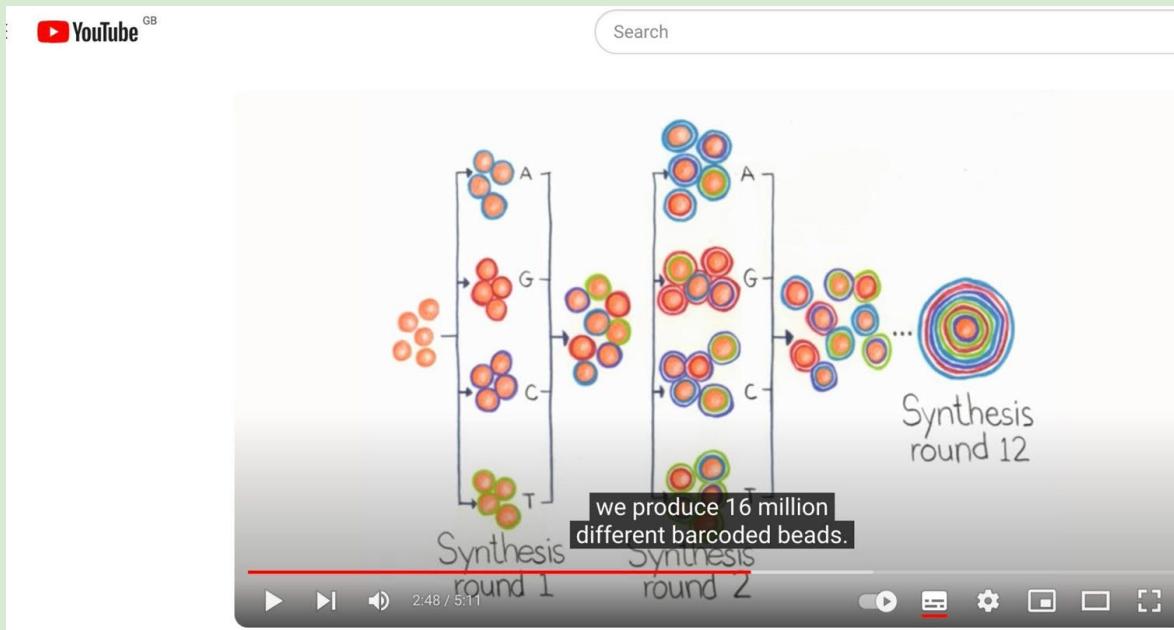
<https://www.youtube.com/watch?v=vL7ptq2Dcf0>

From their lab:

<https://www.youtube.com/@thedrop-seqteammccarrollla2284/videos>

# If you want to know how to make the beads...

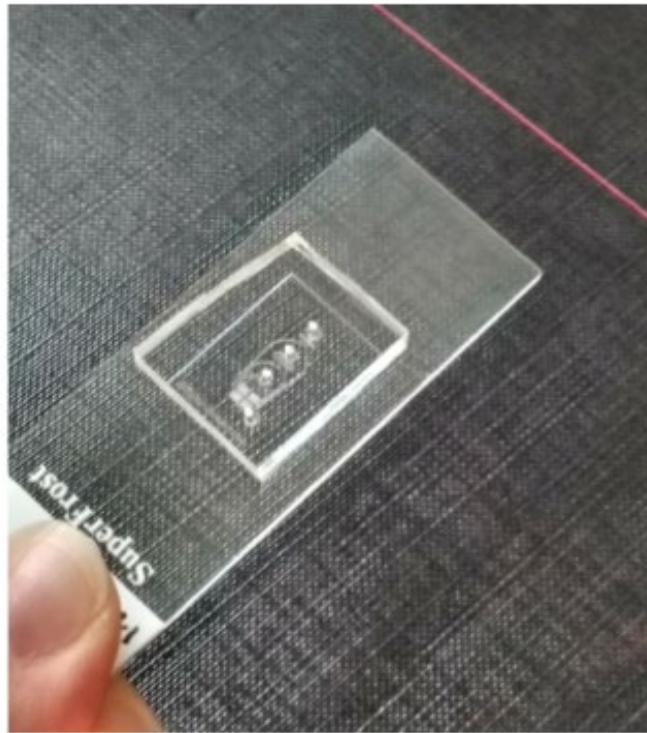
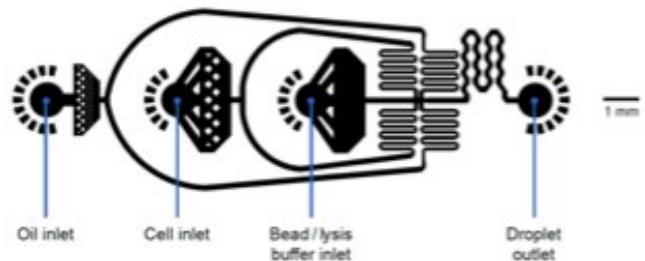
... in the 5 min YouTube video it's explained at about 2.30. Basically the oligos are made on the beads, and are extended one letter at a time.



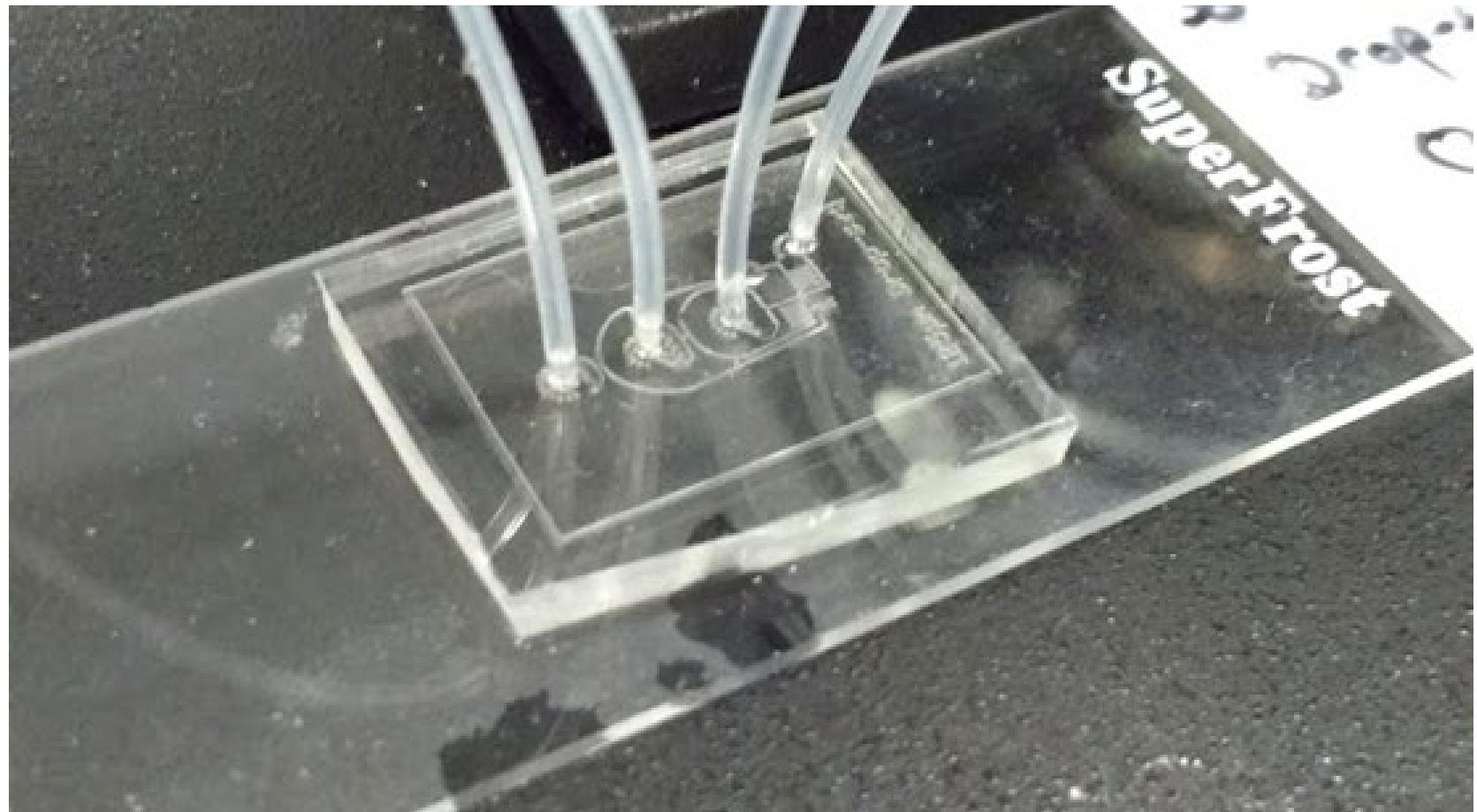
# Droplet rig- check!



# Drop-seq: chip, check!



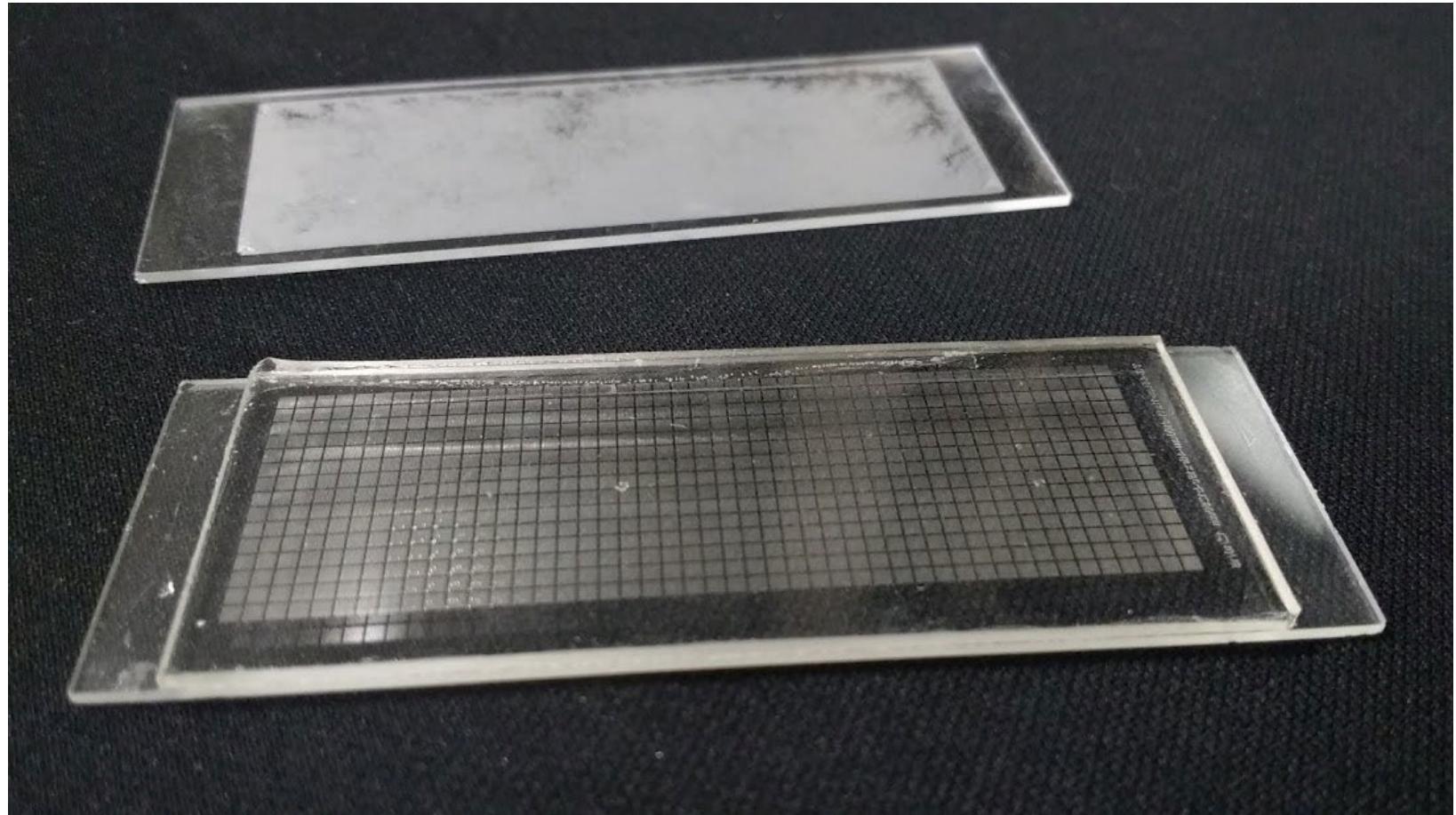
# With tubing...



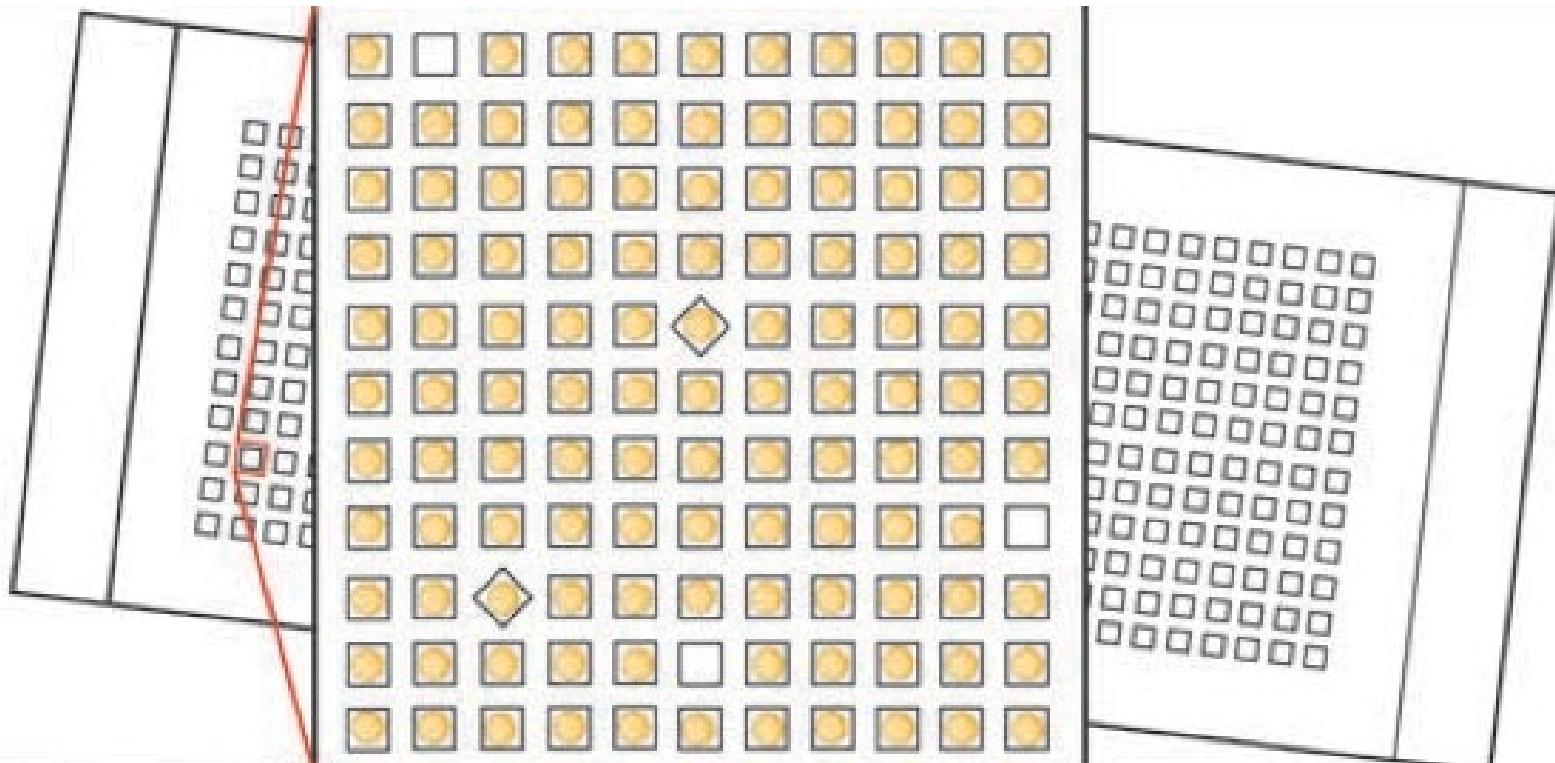
# 10X “black box”



# Nanowells: e.g. Seq-Well



# Beads fit into wells...

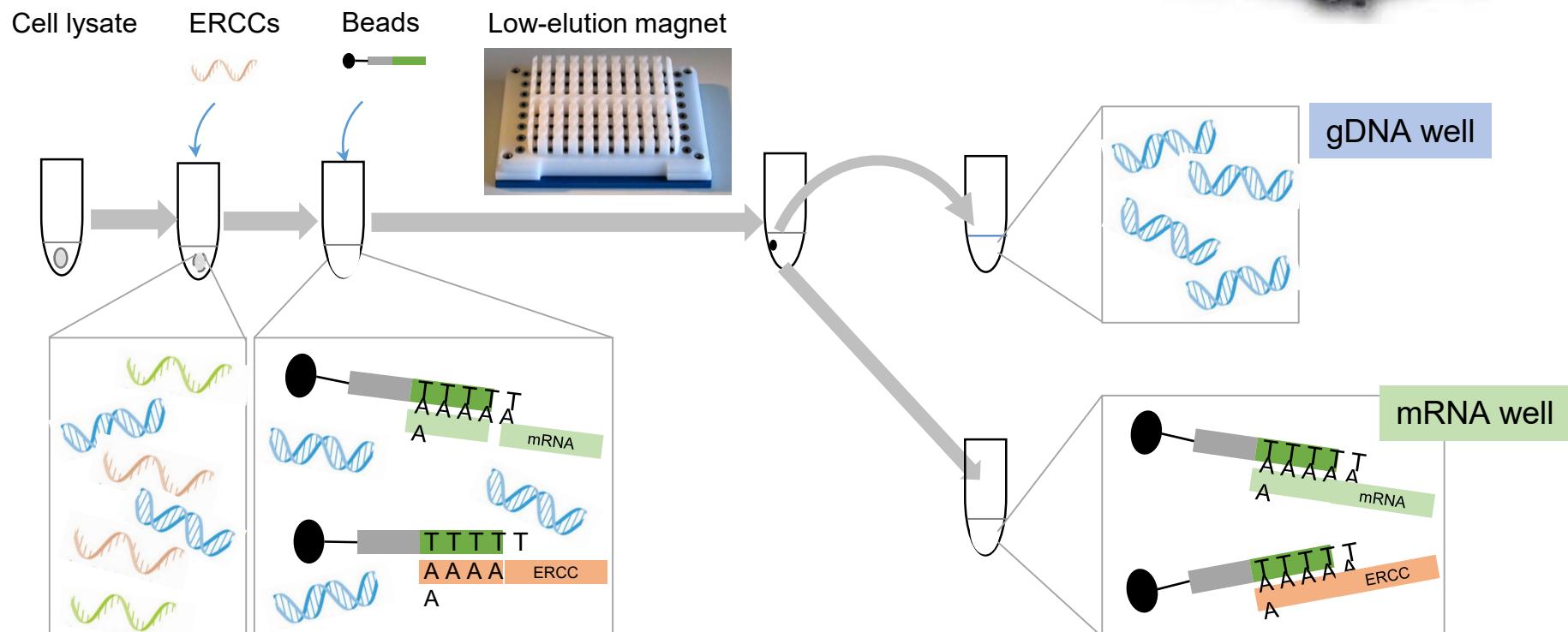


Multiple layers from  
the same cell

# 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

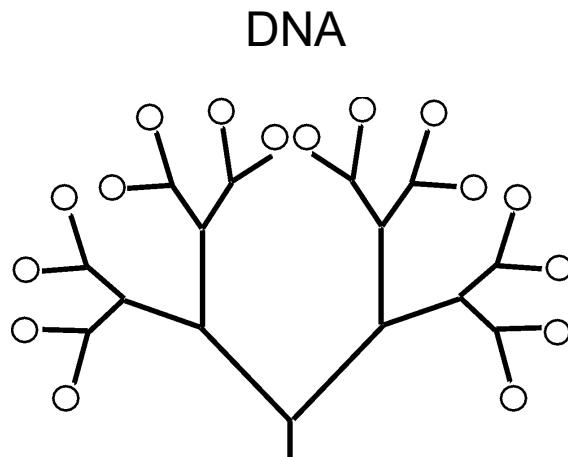
**RNA**



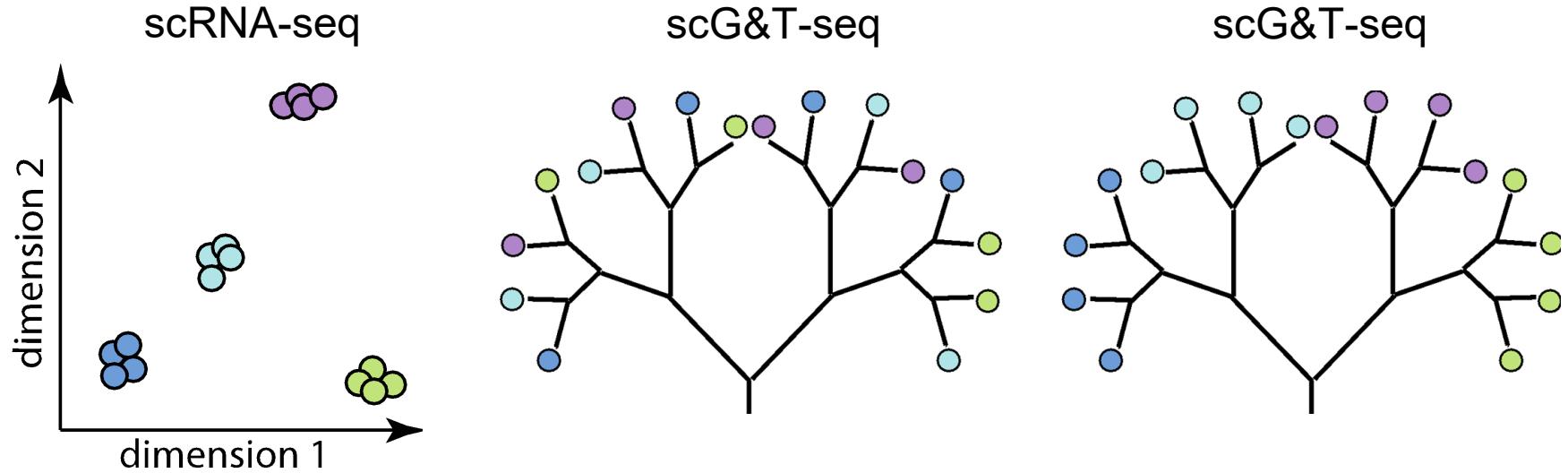
**DNA**



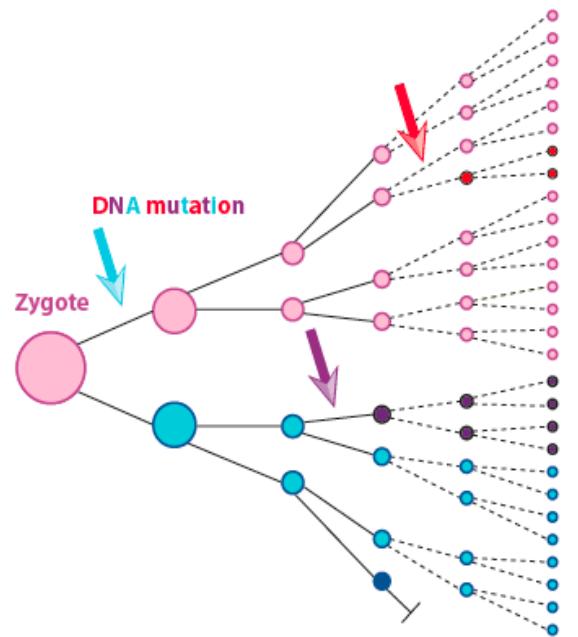
# Genotype-phenotype correlation at single cell level



# Genotype-phenotype correlation at single cell level

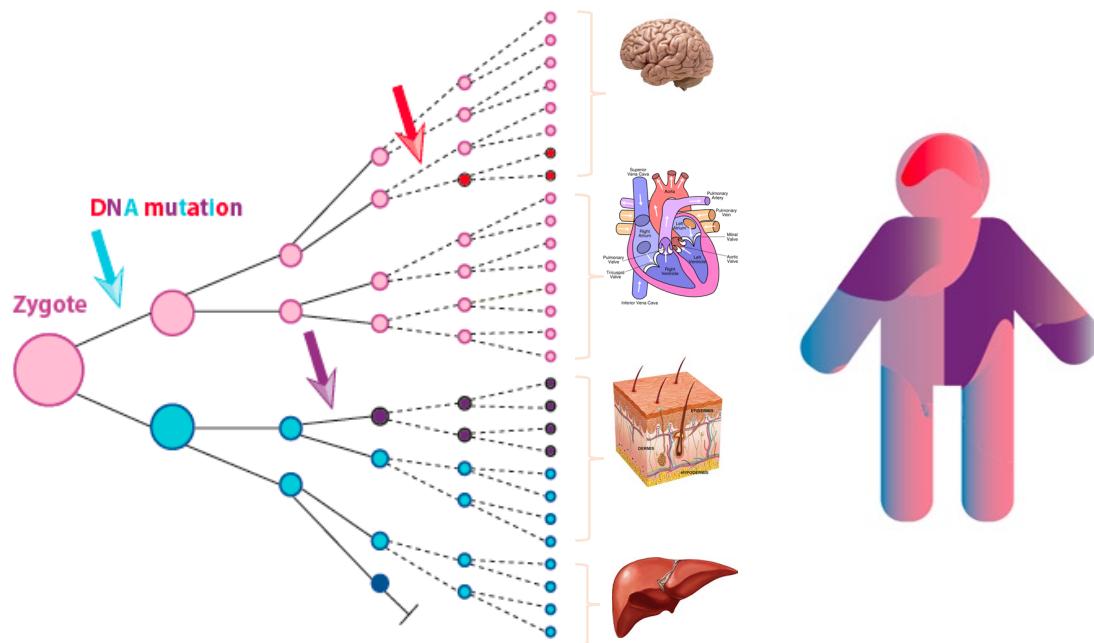


# One human, multiple genomes



# 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

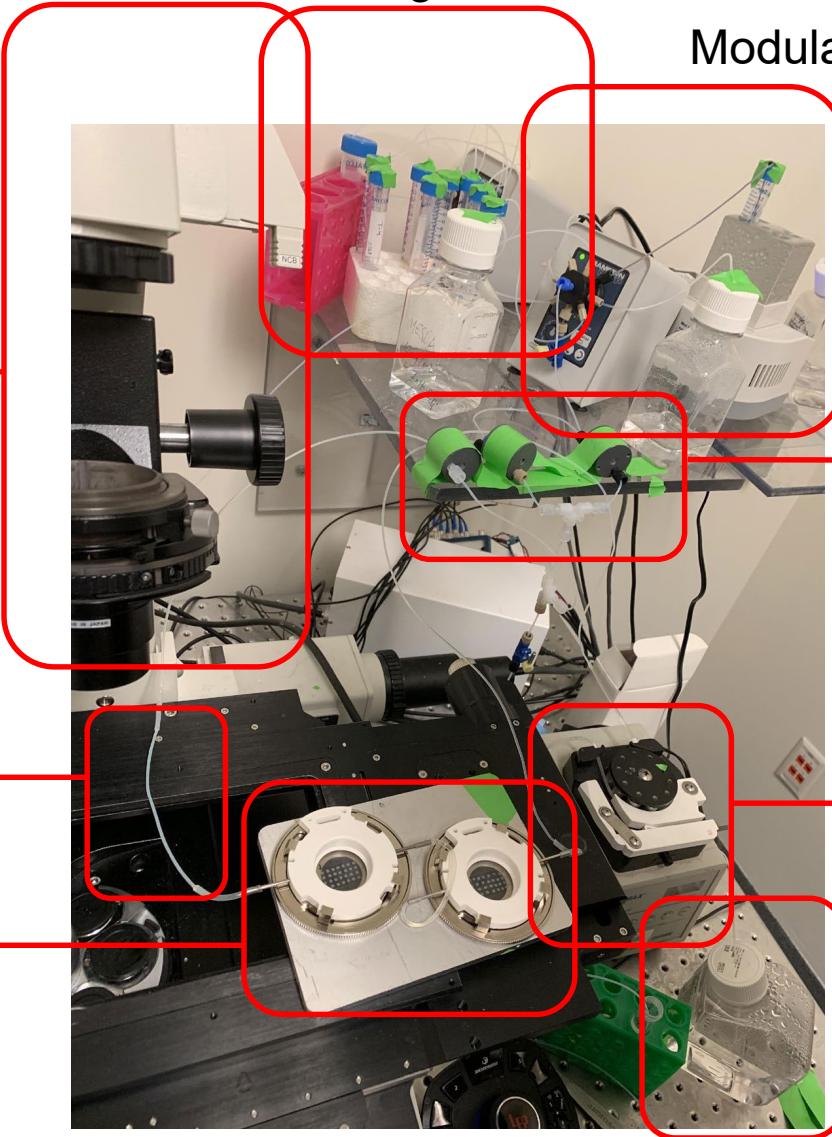
Bubble traps

Tubing

Peristaltic pump

Flow cells

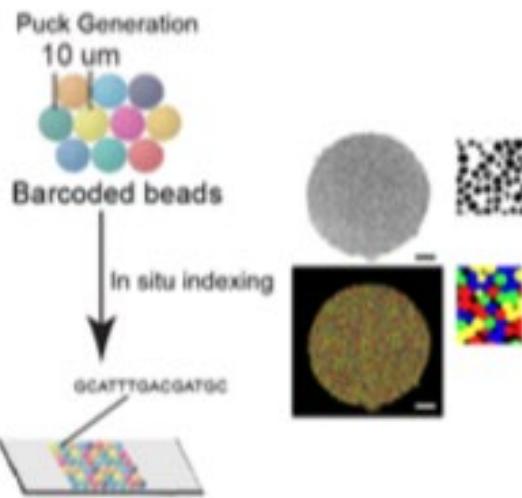
Waste



# Slide-seq

- each bead barcode has known spatial location

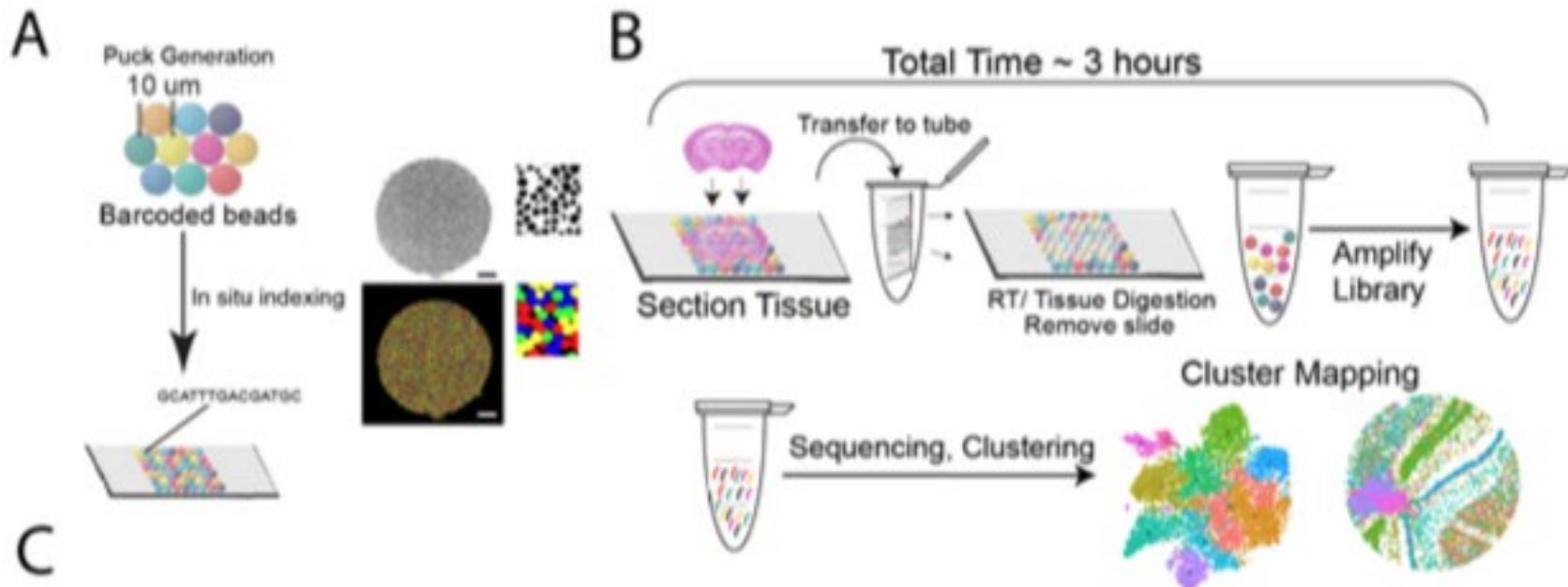
A



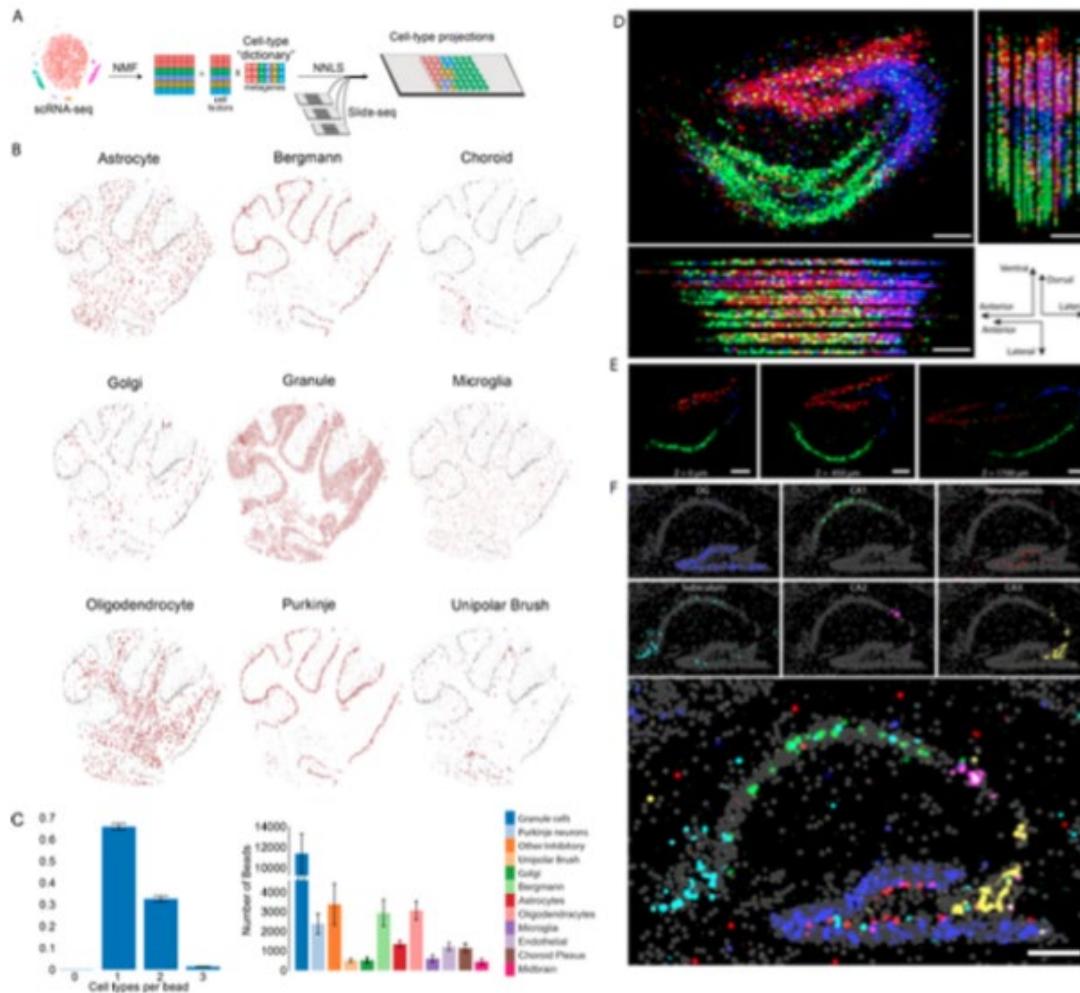
C

# 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!  
:)

# Stuff for applying for placements or jobs\*

\*Lia's short unofficial guide :)

# Lia's tips for finding science opportunities

- Nuffield Foundation fund summer lab placements  
(<https://www.stem.org.uk/placements> )
- I also had one funded by UK Medical Research council (now part of UKRI)
- Look for keywords like “summer placement” or “summer studentship”
- If not advertised, drop a friendly email to a nearby lab and ask if they have any opportunities - worst case scenario here is no reply or an email saying no :)

# Lia's tips for emailing a scientist you don't know yet

- Most scientists love to hear that someone is interested in what they work on!
- Just make sure that it looks like an email to them specifically rather than spam!
- See next slide....

# An email most scientists I know would reply to:

“Dear Dr Chappell,

I saw your talk (read about your work on LinkedIn/found the page of your team on the Sanger website) and found your work on Single Cell technologies in Cancer (or whatever they work on) really interesting!

I’m an student studying (A-Levels/T-Levels/my first year of biology degree) and would be interested to visit your lab for a day (or apply for a summer placement/ lab project as part of year in industry for my degree programme). I’ve included a PDF of my CV as an attachment and here’s my LinkedIn profile [link].

Thanks for taking the time to ready my email, I hope to hear back from you soon.

Best wishes,  
Lia”

# The kind of reply you might get

1. “Hi Lia, thanks for getting in touch! We’d love to host you, but unfortunately the team is away that week/ we don’t have capacity this summer. But feel free to email back next year and we might be able to host you then! Best wishes, Lia”
1. “Hi Lia, thanks for getting in touch! We were actually about to post an ad for a summer student, so your timing is great! I’ll send a link, please do apply! Best wishes, Lia”
1. “Hi Lia, great to hear from you! We hadn’t planned to have a student this year, but we could host you if you’d be willing to apply for funding with this scheme, here’s a link [link]. Happy to do a video call this Friday if you wanted to talk a bit more about it. Best wishes, Lia”

# Writing a CV

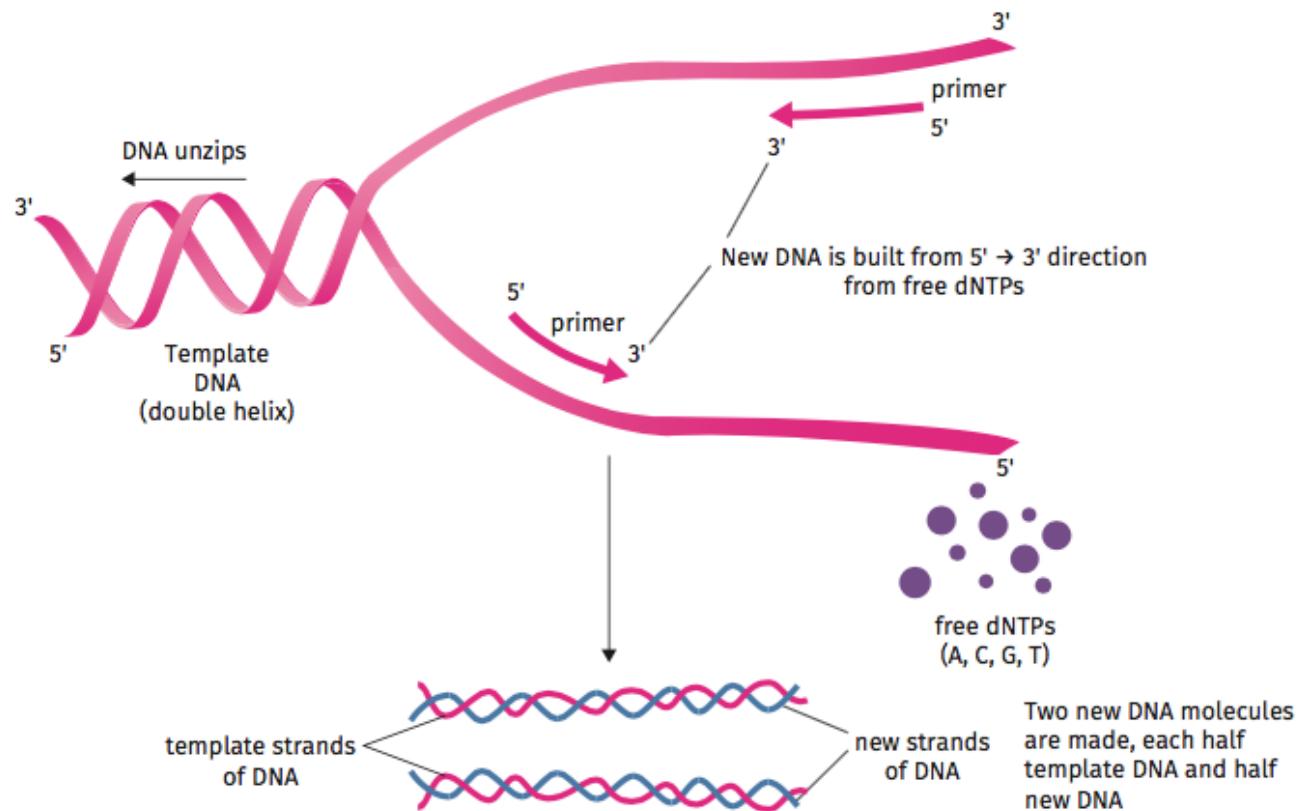
- A guide from UCAS  
(<https://www.ucas.com/careers/help-and-advice/how-write-cv>)
- This PDF is aimed at undergraduate students, but near the end has lots of nice annotation on examples of nice CV layouts that you'd be able to make in Google Docs or other free programs  
(<https://www.careers.cam.ac.uk/files/cambridgecvsandapplications.pdf>)
- Get a boring email address (something based on your name probably) for sending emails about placements. E.g. [alex.smith.1234@gmail.com](mailto:alex.smith.1234@gmail.com) is better than [alex.has.smelly.feet.1234@gmail.com](mailto:alex.has.smelly.feet.1234@gmail.com)
- You can set up the emails to auto-forward to an account you check more, but just remember to reply from the sensible one :)

# LinkedIn

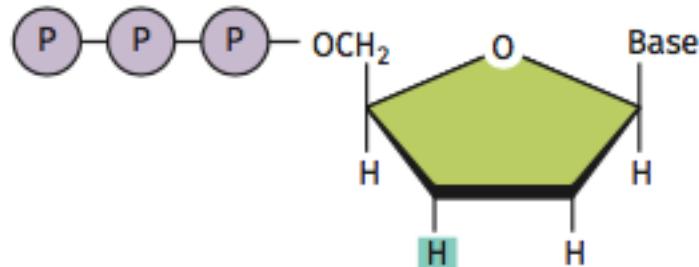
- A bit like an online CV
- Use a photo that's sensible (don't worry too much about clothes etc, but more like a passport photo than a social media profile photo that's just for your friends)
- Here's Sanger people's work photos for a rough idea:  
<https://www.sanger.ac.uk/people/people-directory/>
- Can add people as contacts - start building your network (okay to add people you meet at work events - only work stuff on profile)
- People post mostly good news ("we published a thing!") or adverts for jobs.
- UCAS has a guide (<https://www.ucas.com/careers-advice/how-use-linkedin-student-or-apprentice> )

Extra science slides in  
case I needed them to  
answer questions...

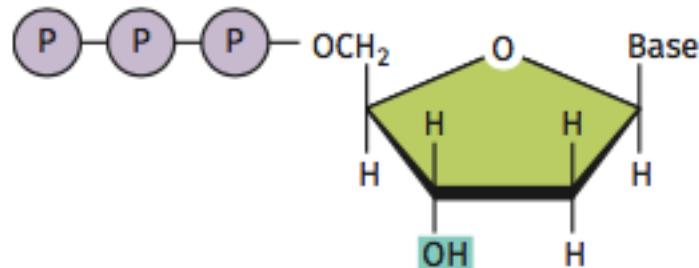
# DNA replication: the key principles that underlie the tech



# Nucleotide building blocks

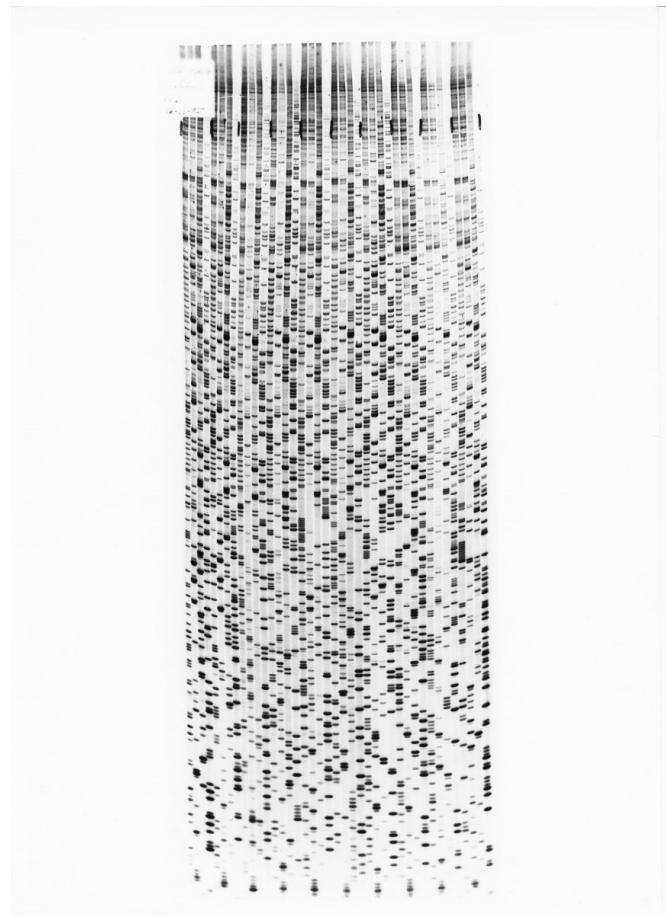


Dideoxynucleotide (ddNTP)

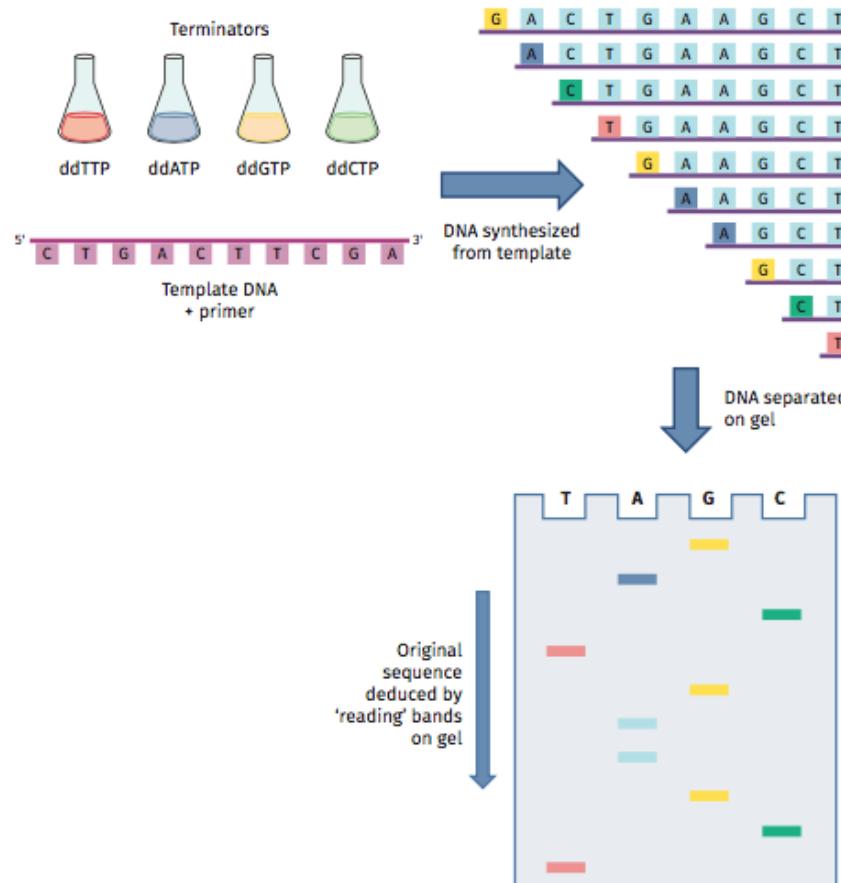


Deoxynucleotide (dNTP)

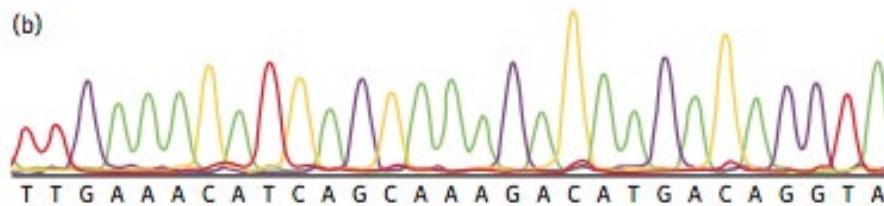
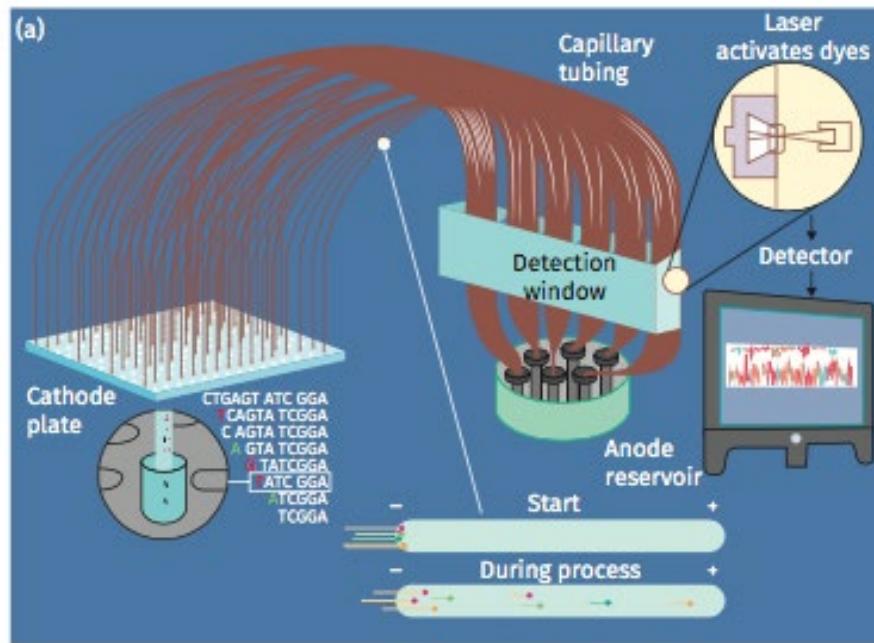
# Sanger sequencing



# Sanger sequencing



# Capillary sequencing



# “Next gen” sequencing: Illumina

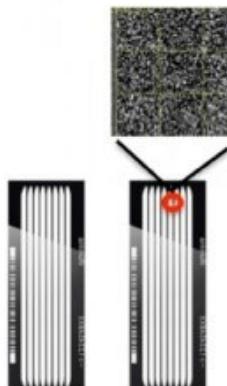


# Illumina “flow cells”

HiSeq 2000  
*New flow cell design*

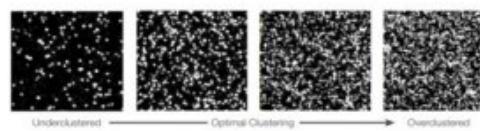
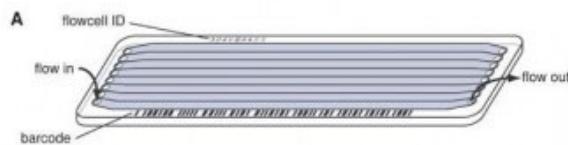
LARGER, DUAL-SURFACE ENABLED  
>5x increase in imaging area  
Retains 8 lane format

Compatible with cBot



Cluster density  
750-850/mm<sup>2</sup>

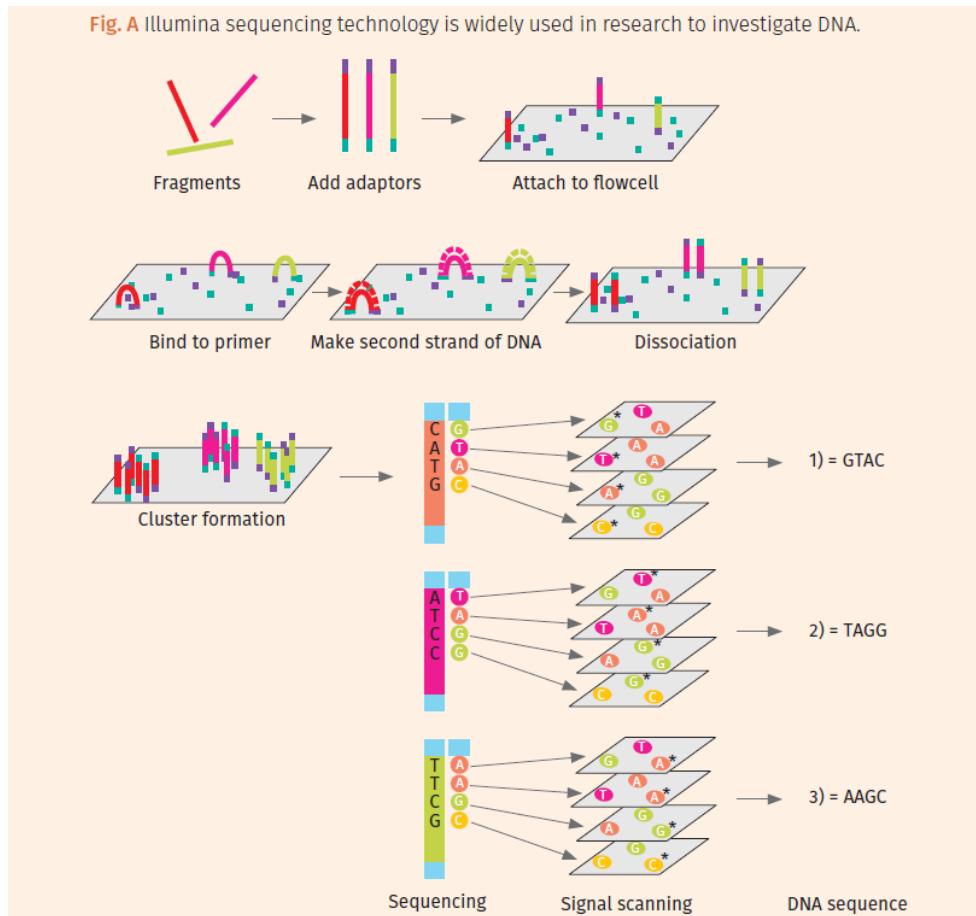
**HiSeq Flow Cells**



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

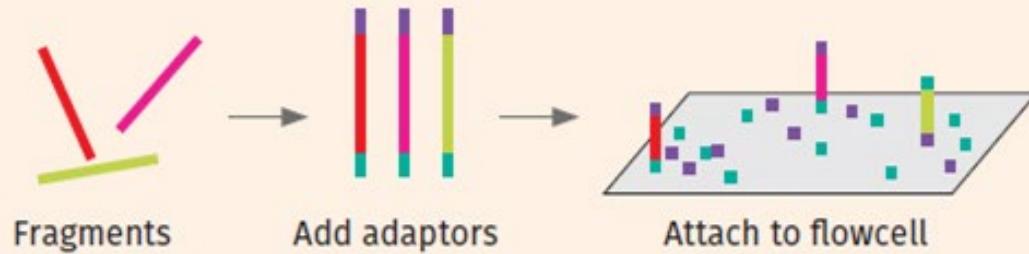
The HiSeq instrument scans both upper and lower surfaces of each flowcell lane.

# Illumina sequencing: overview

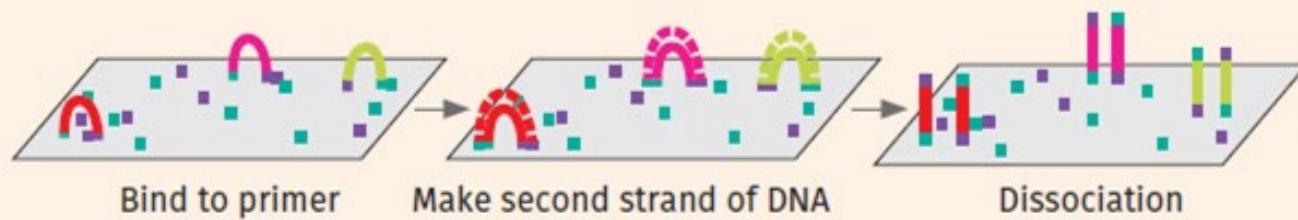


# “Seeding” the flow cell

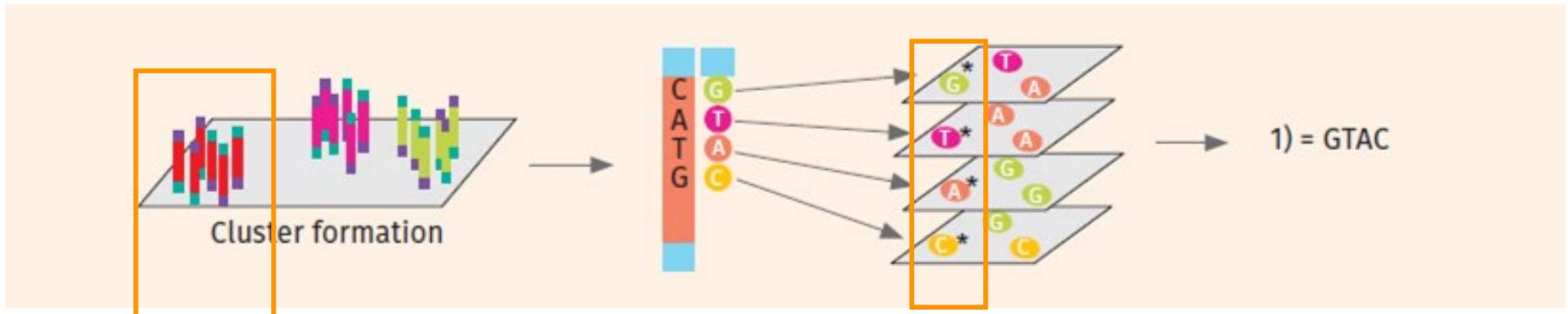
**Fig. A** Illumina sequencing technology is widely used in research to investigate DNA.



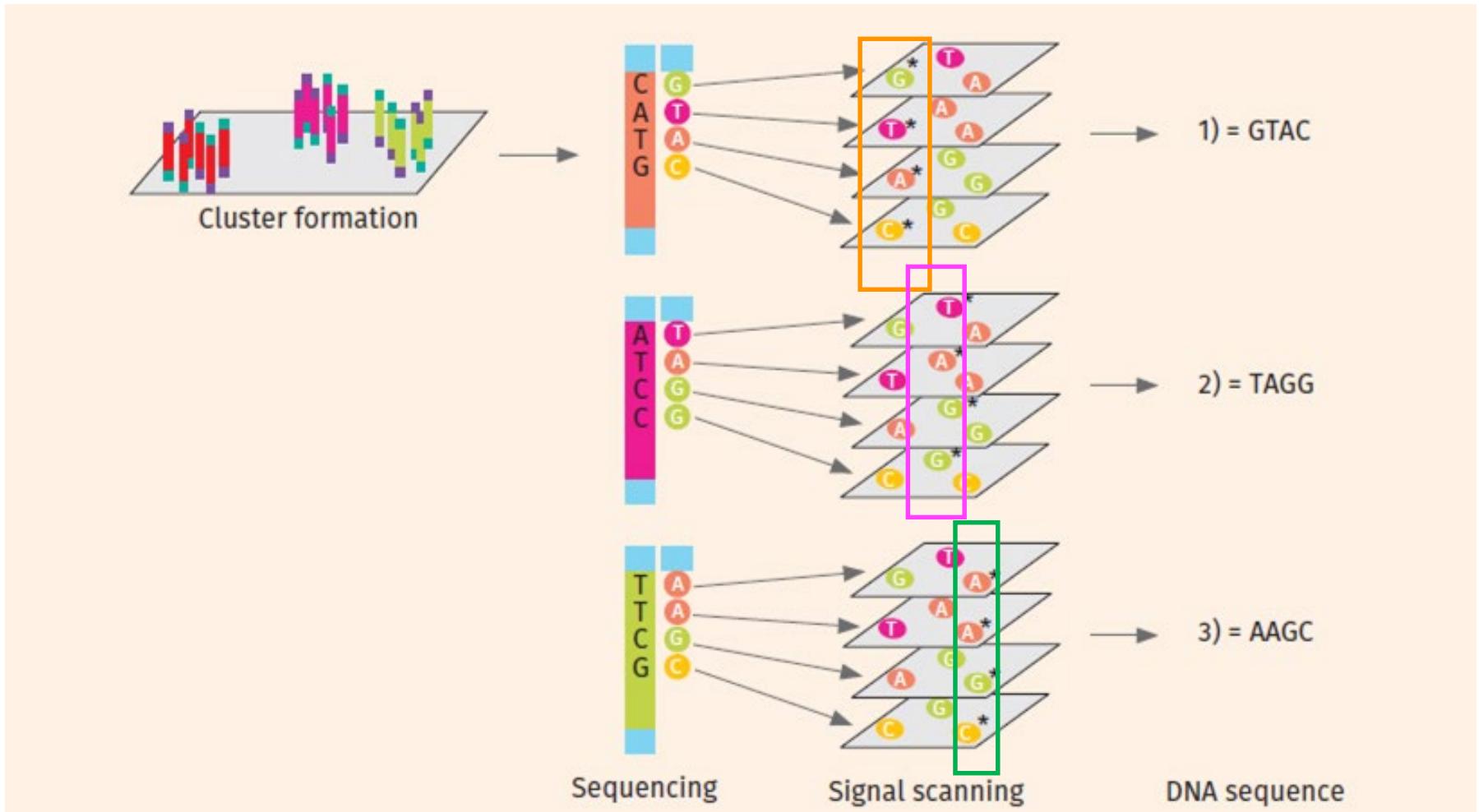
# Forming clusters



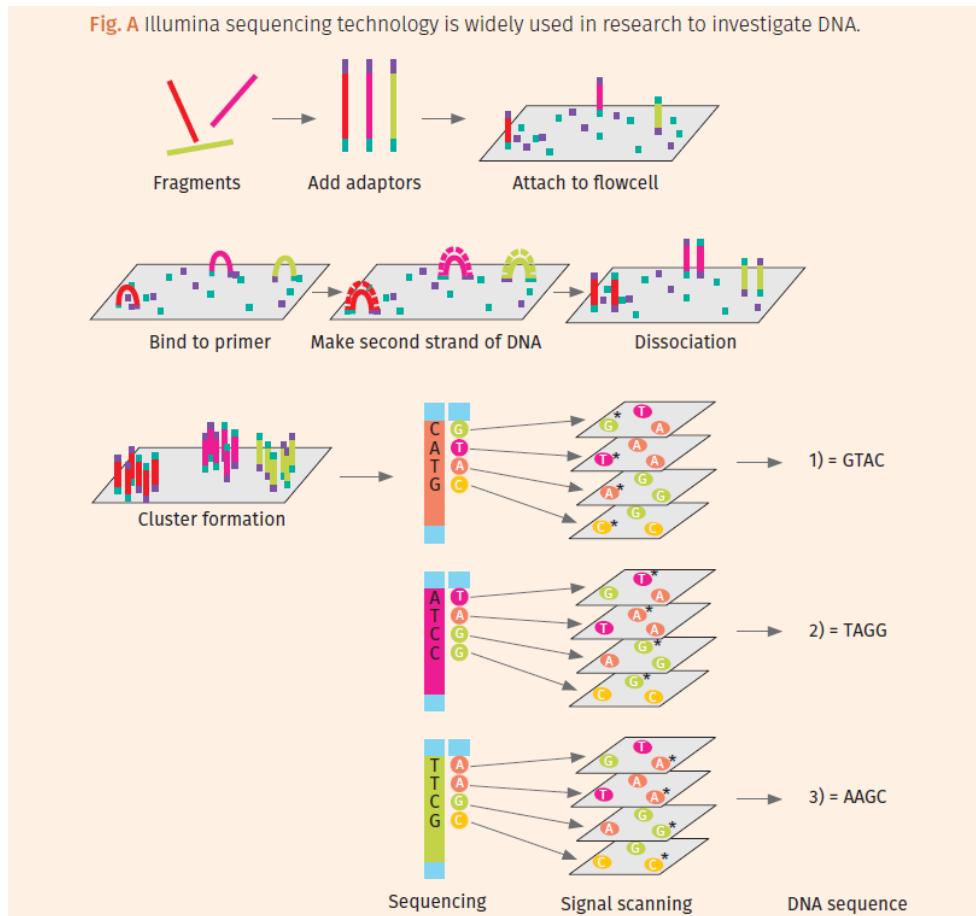
# Reading each cluster



# Reading each cluster



# Illumina sequencing: overview



# Data analysis: lots of data!



# Sequencing in remote environments: possible?



# Oxford Nanopore



# Oxford Nanopore

