2026 iGEM Team

COLLABORATE · DESIGN · BUILD · COMPETE

The 2026 iGEM EPFL team application deadline is 12:00 noon CET on the 3rd of November 2024.

https://go.epfl.ch/igem

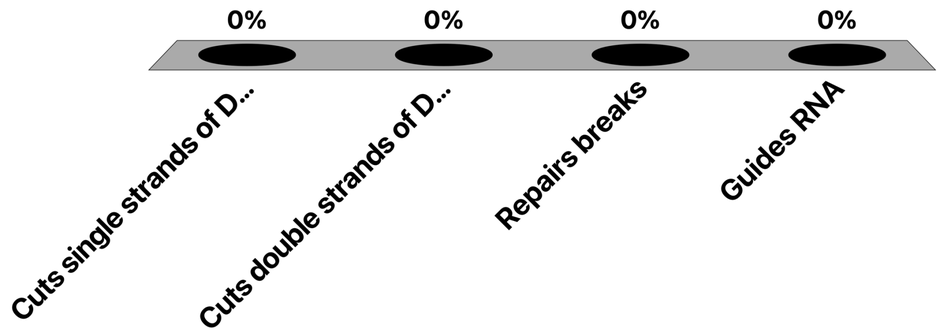
https://participant.turningtechnologies.eu/en/join
https://go.epfl.ch/TurningPointPoll

Session ID: **bio411a**

GENOME TO FUNCTION
LIFE SCIENCES ENGINEERING

Cas9…

A. Cuts single strands of DNA
B. Cuts double strands of DNA
C. Repairs breaks
D. Guides RNA



miRNAs

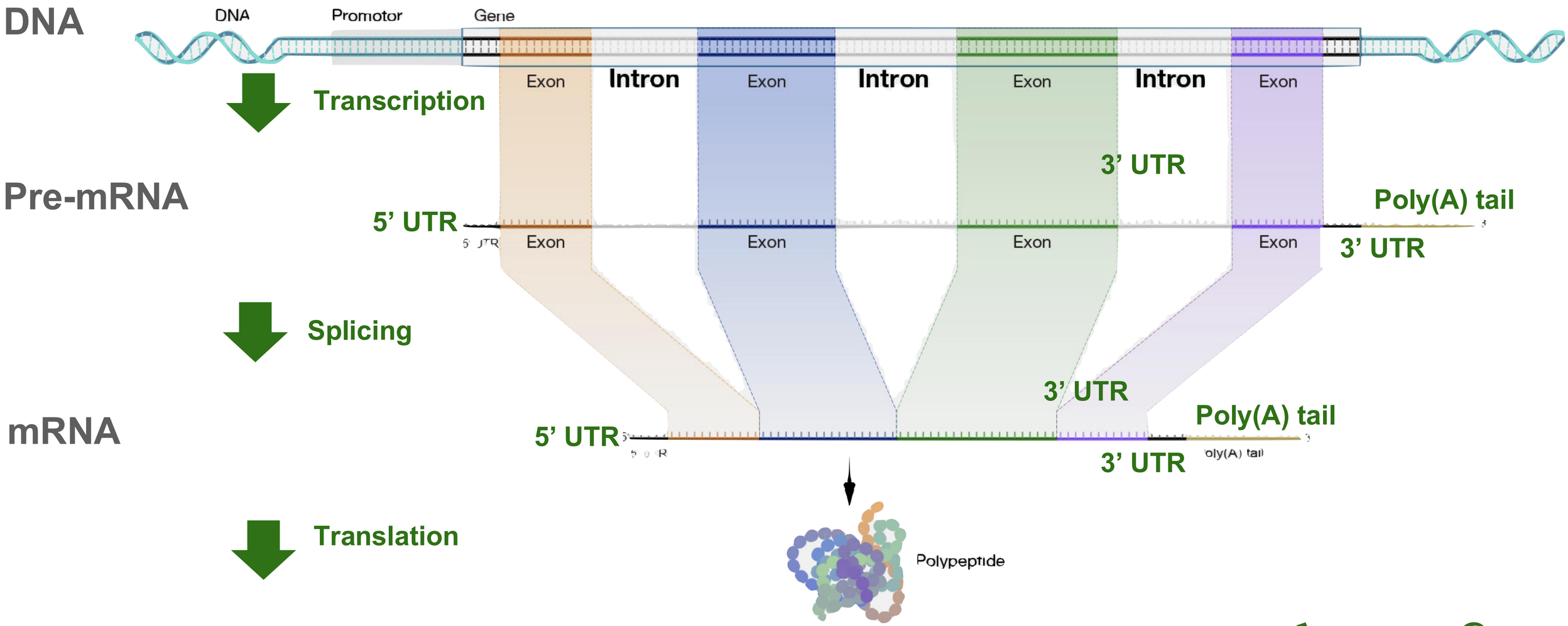
A. Usually bind in the 5’ UTR of mRNAs
B. Usually bind in the 3’ UTR of genes
C. Cannot bind in the coding region
D. Usually regulate the expression of only one gene

The RISC complex

A. Enables mRNA degradation
B. Is unique for shRNA
C. Requires perfect sequence complementarity to work
D. Is when you only read course materials the night before

Finding Targets

mRNA expression



GENOME TO FUNCTION
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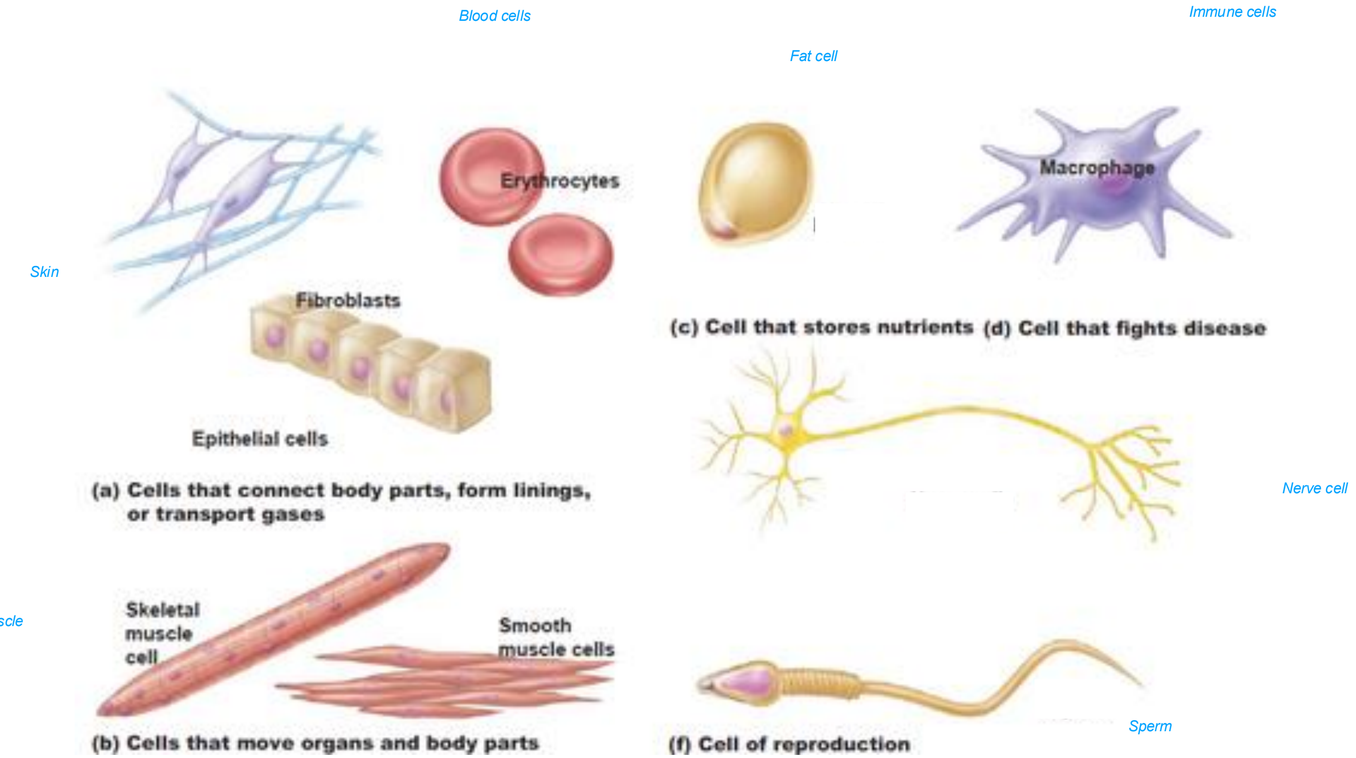
One genome, many outputs

In human body there are:

~ 100 trillion cells
~ 200 types of cells

Individual cell, tissue and organ outputs from the genome are very complex



GENOME TO FUNCTION
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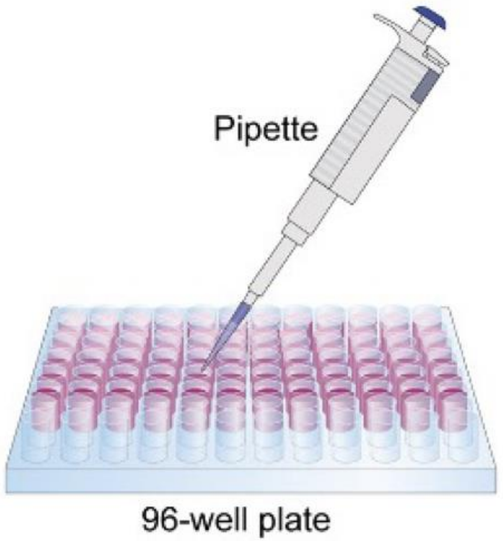
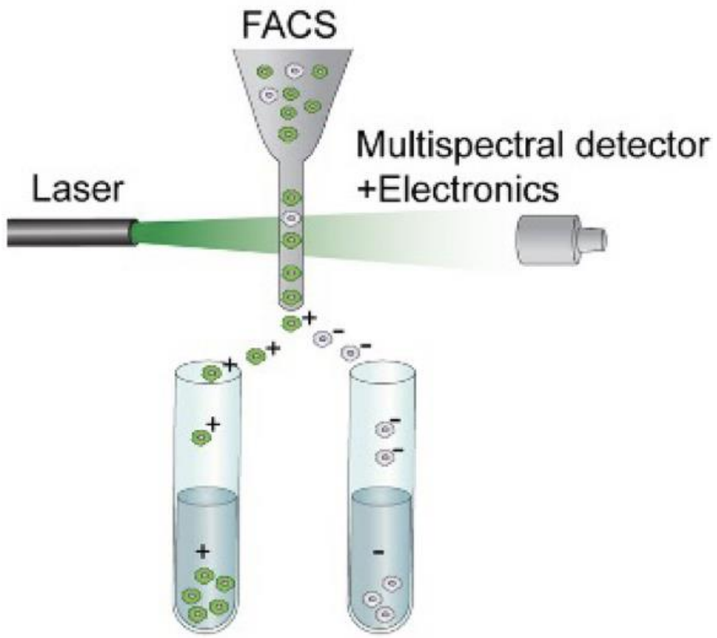
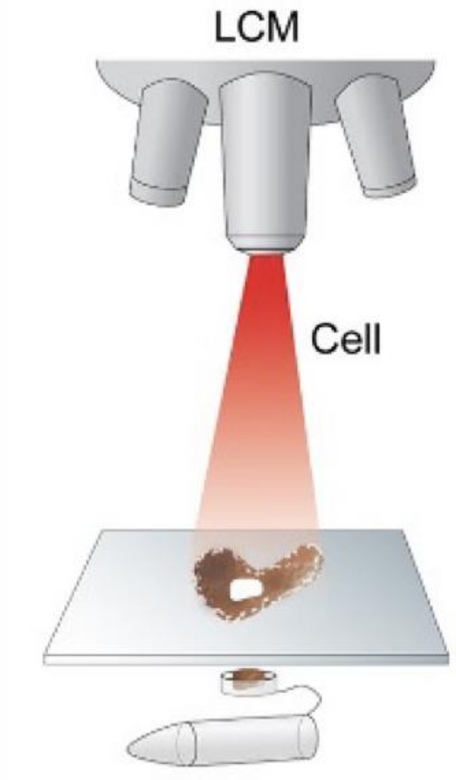
Getting Samples to Sequence



Cell lines



Fluorescence-activated cell sorting



Laser Capture Microdissection

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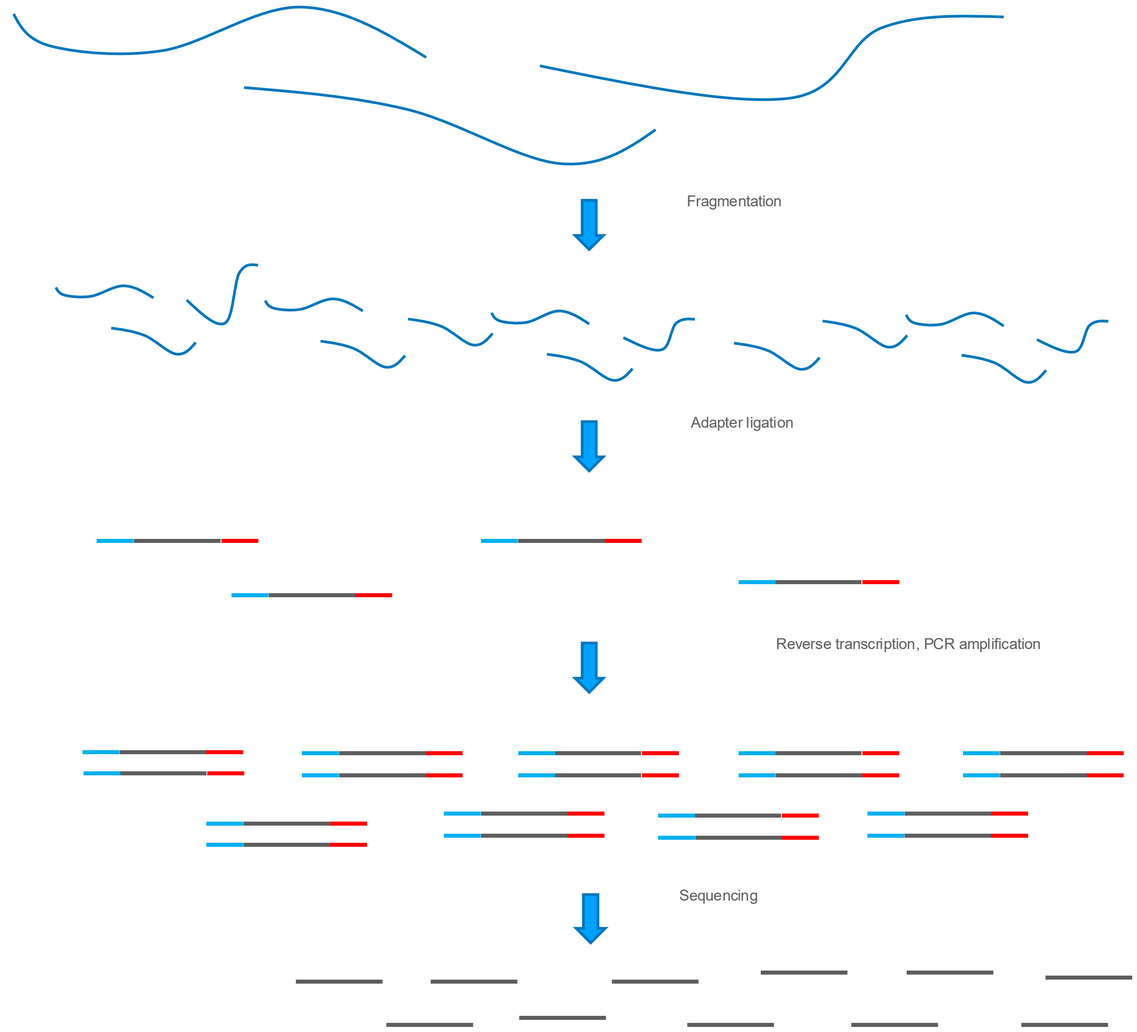
Bulk RNA sequencing



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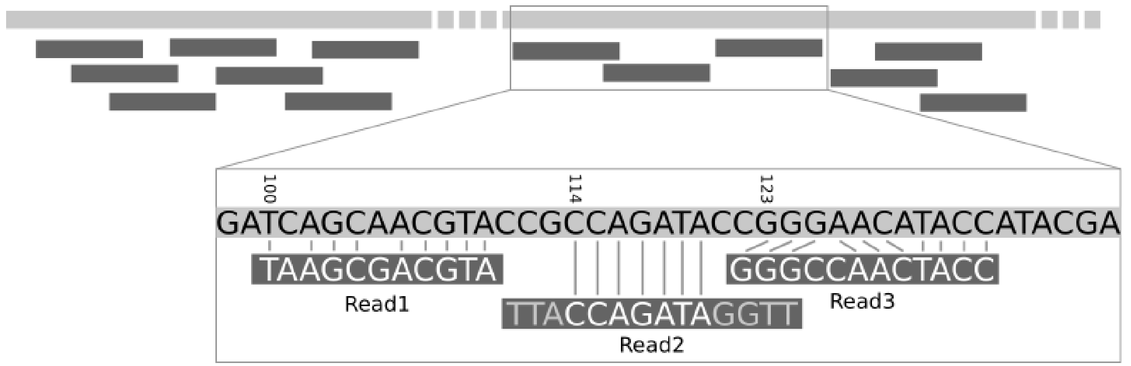
RNA sequencing

RNA-seq raw reads

Mapping to reference genome



Mapped reads

Quantify gene expression as read abundance, per gene

Read count matrix

	Sample 1
Gene A	5
Gene B	2
Gene C	6

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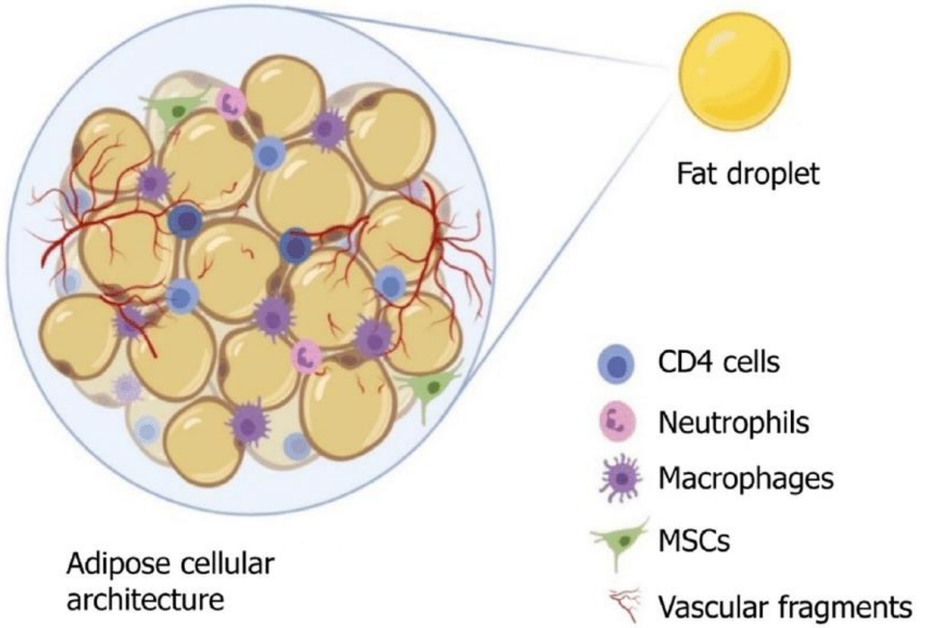
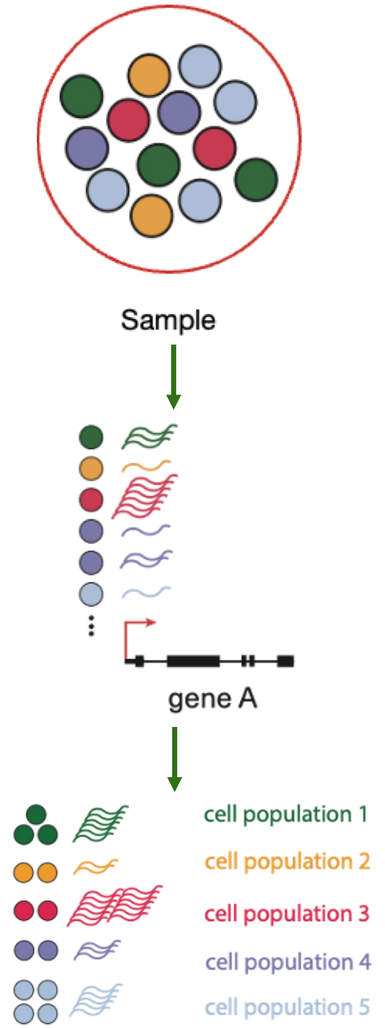
Limits of Bulk RNA sequencing

- Requires usually **thousands or millions of cells**
- Useful for **tissues or cell lines**.
- **Rare cell types and states cannot** be analyzed
- Tissues are generally **heterogeneous**
- Most often **multiple cell types** in a tissue
- Bulk RNA-seq sample is an **average** transcriptome of many cells
- No information about expression levels or heterogeneity of **individual cells**

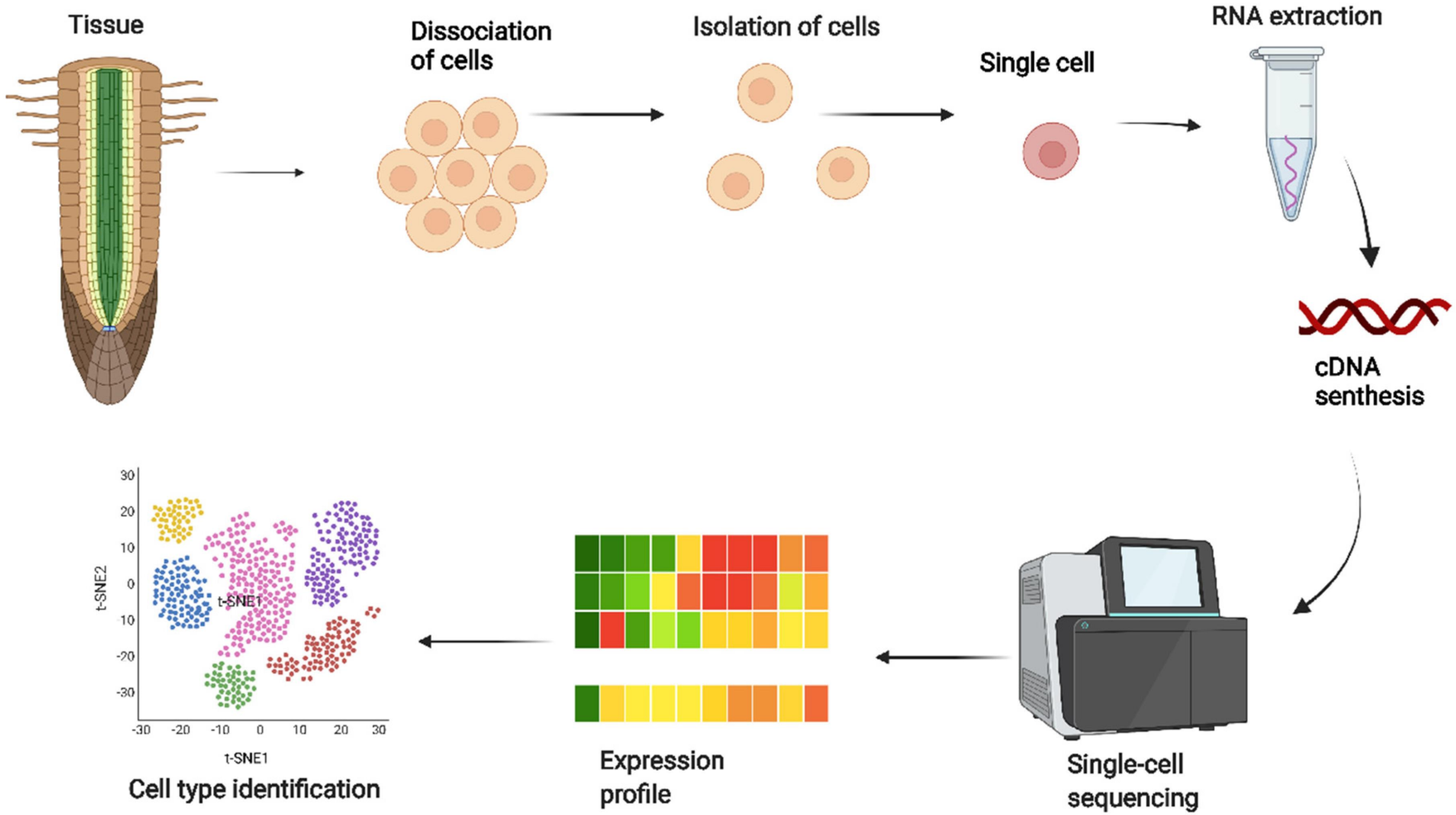


Single cell (sc) RNA sequencing

- **scRNA-seq enables:**
- Identification of **rare cell types** (e.g. early development, stem cells, circulating tumour cells)
- Elucidating **gene regulatory networks**
- Studying **heterogeneity** (e.g. tissue composition, cancer, temporal processes)
- Exploring changes in expression while **incorporating** other information e.g spatial, regulatory or protein information
- **Single cell phenomena** (gene expression stochasticity, mono-allelic expression)



scRNA sequencing overview



Bawa et al, IJMS, 2022

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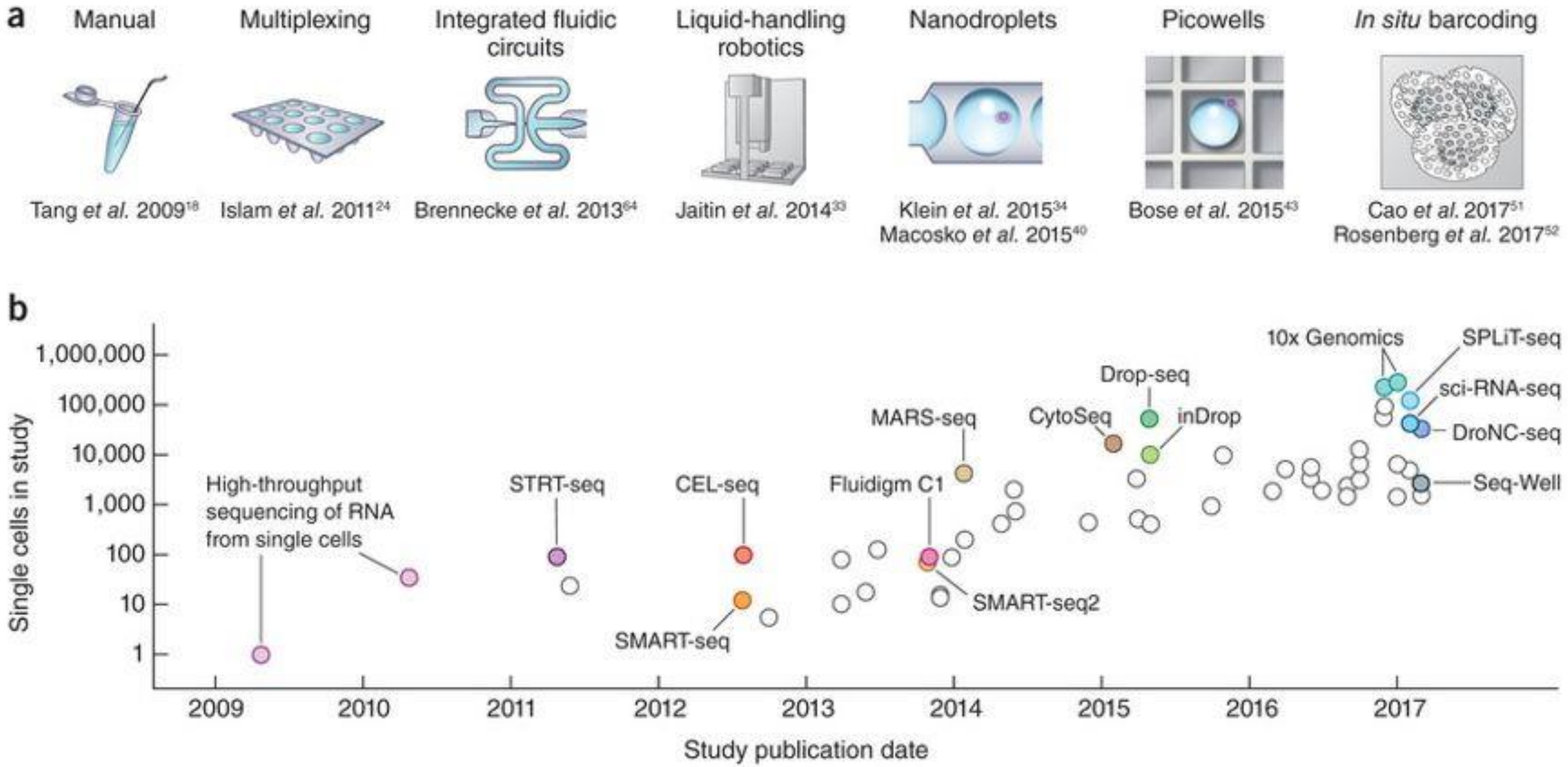
scRNA sequencing technology



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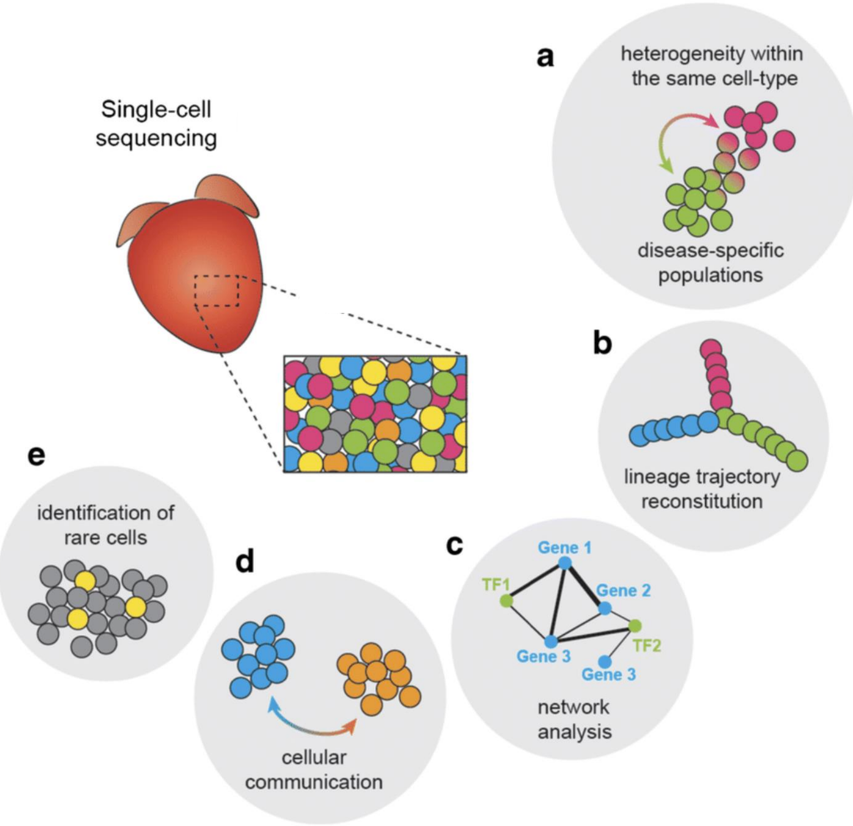
Drop sequencing

- Most commonly used current methods

  10x genomics - commercial

  Drop-seq - cheaper, more complicated to set-up



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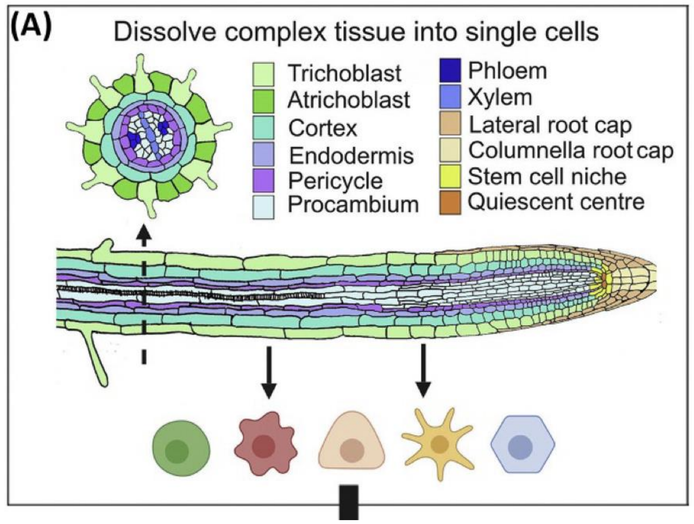
scRNA sequencing

scRNA-seq procedure

- Disassociate tissue into **single cells**



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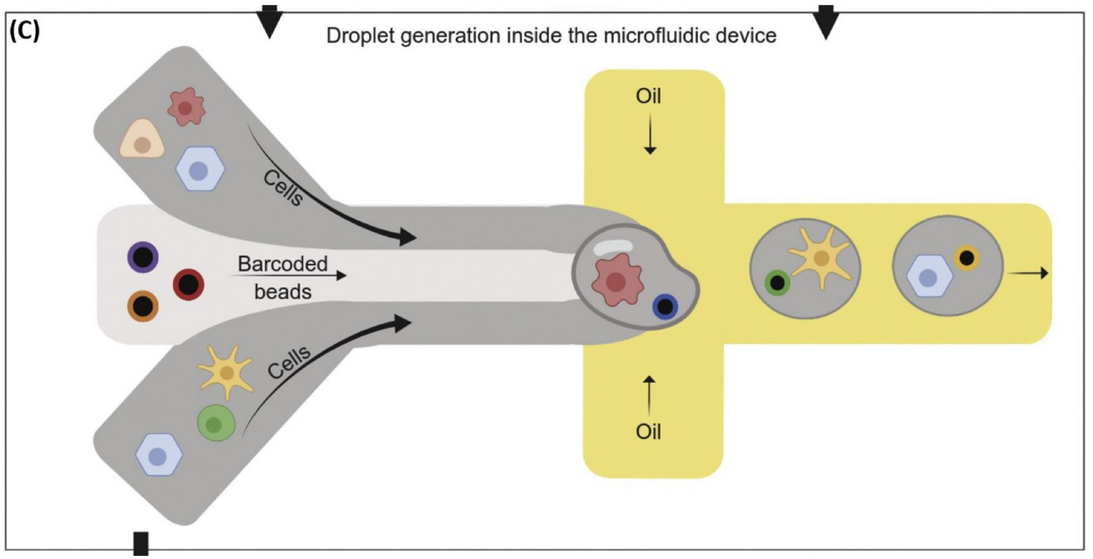
scRNA sequencing

scRNA-seq procedure

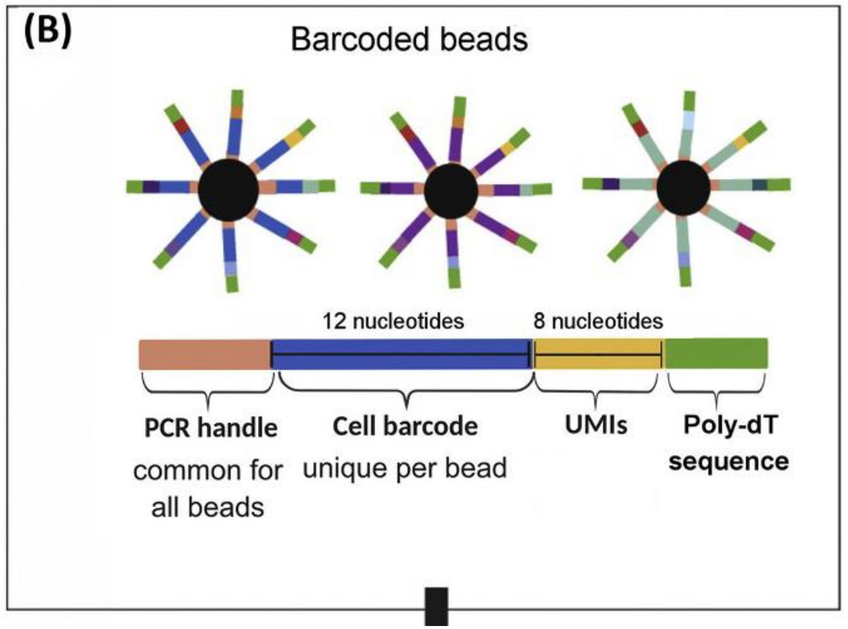
- Disassociate tissue into **single cells**
- Introduce cells into **microfluidic** device



scRNA sequencing

scRNA-seq procedure

- Disassociate tissue into **single cells**
- Introduce cells into **microfluidic** device
- Combine cells with **barcoded beads** into a droplet



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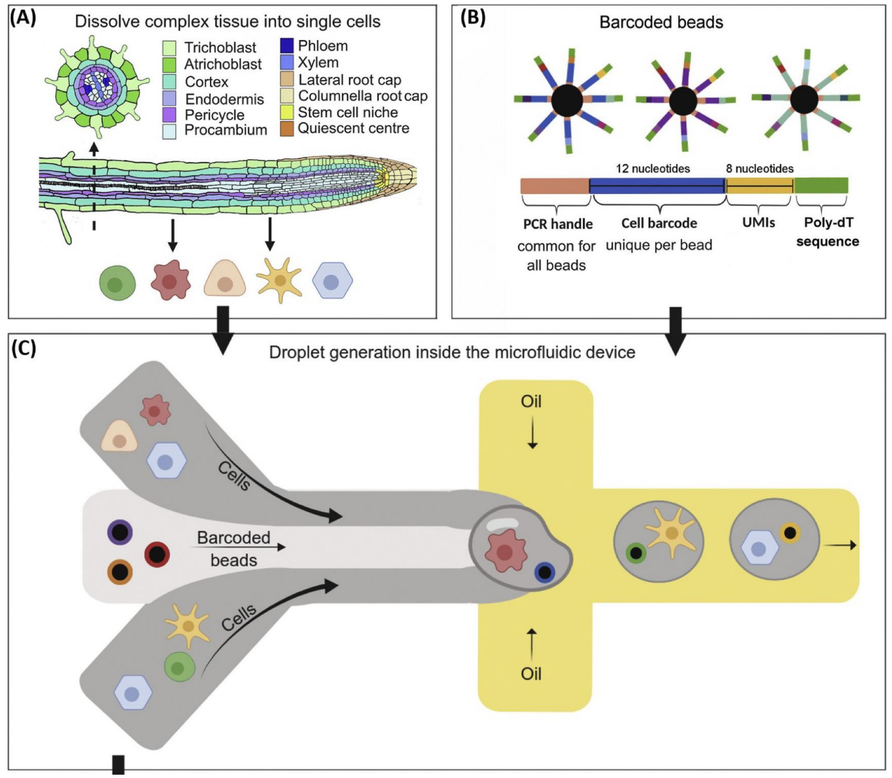

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scRNA sequencing

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- Disassociate tissue into **single cells**
- Introduce cells into **microfluidic** device
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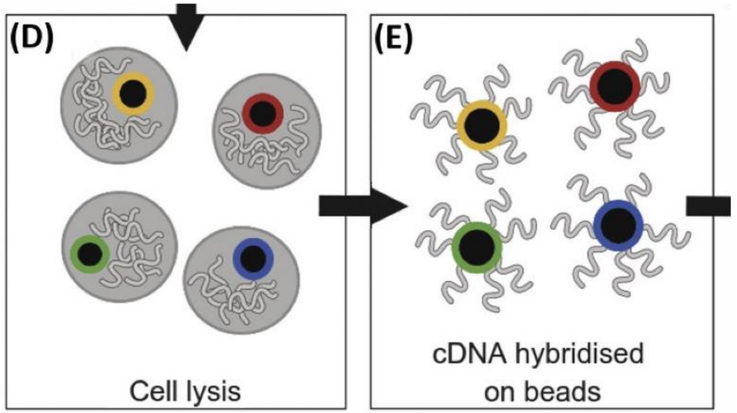
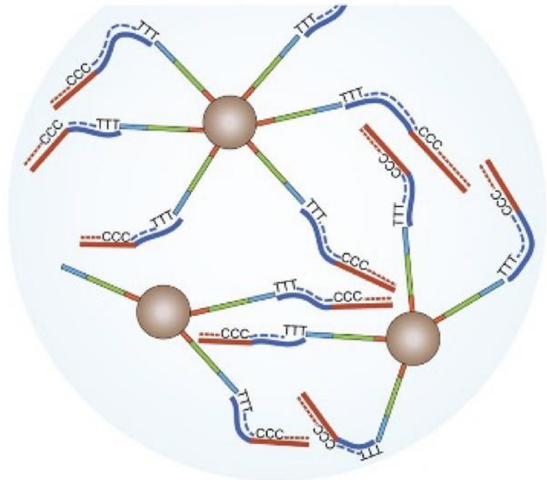
- **Lyse** cells to release RNA and **reverse transcribe** to cDNA
- cDNA derived from cell **hybridises** to beads





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scRNA sequencing

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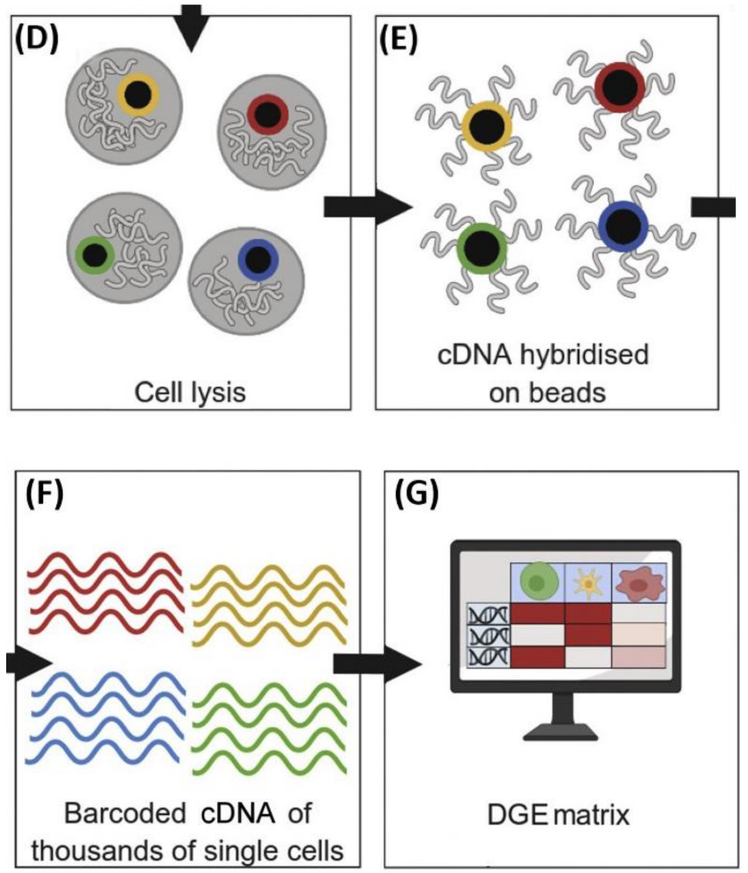

GENOME TO FUNCTION
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scRNA sequencing

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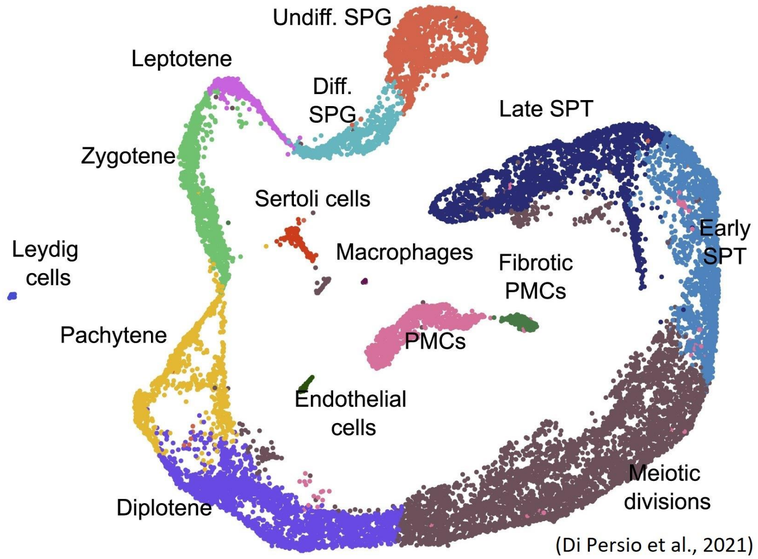
- cDNA derived from cell **hybridises** to beads
- **Barcoded cDNAs** then sequenced
- **Analysis** of data is complex



(Di Persio et al., 2021)

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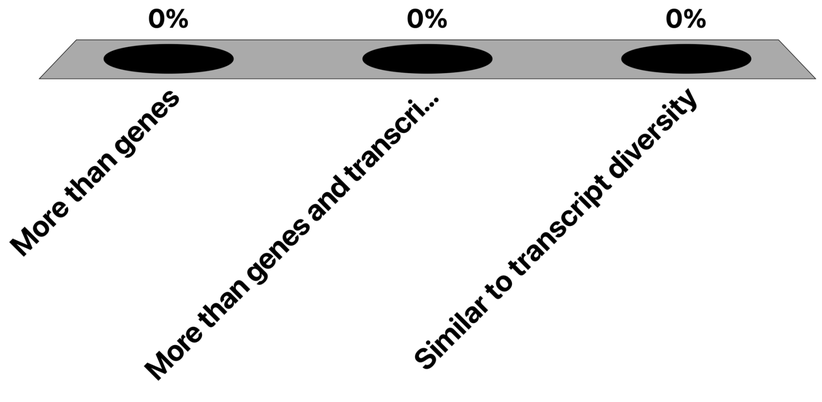
Manipulating Proteins

The Diversity of proteins is…

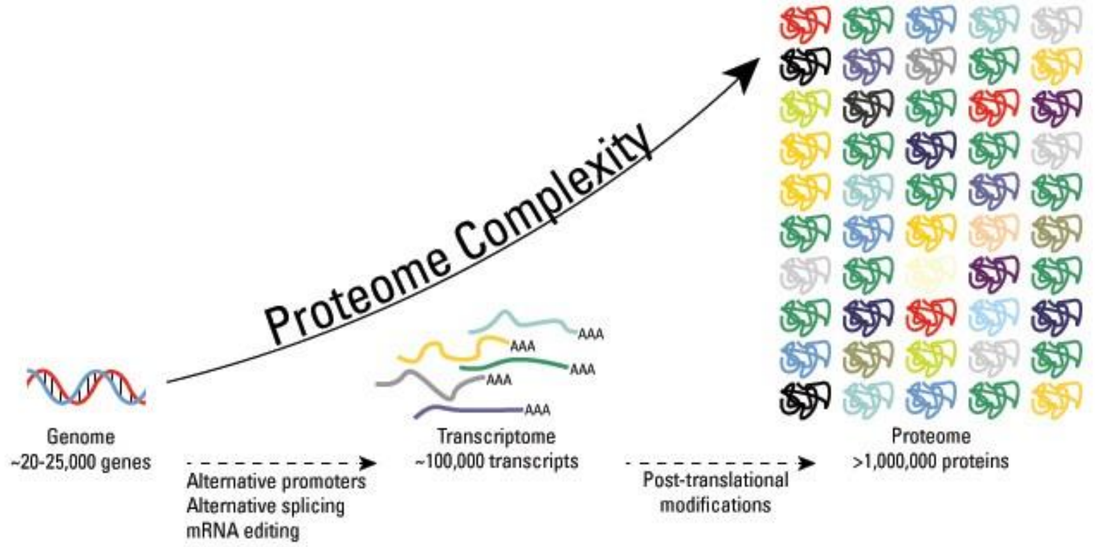
A. More than genes
B. More than genes and transcripts
C. Similar to transcript diversity



Proteome vs Genome

Human protein coding

- **~20,500** human genes
- **> 100,000s** transcript variants
- Many proteins undergo **Post-translational modification (PTM)**
- **Millions of protein** variants per cell type



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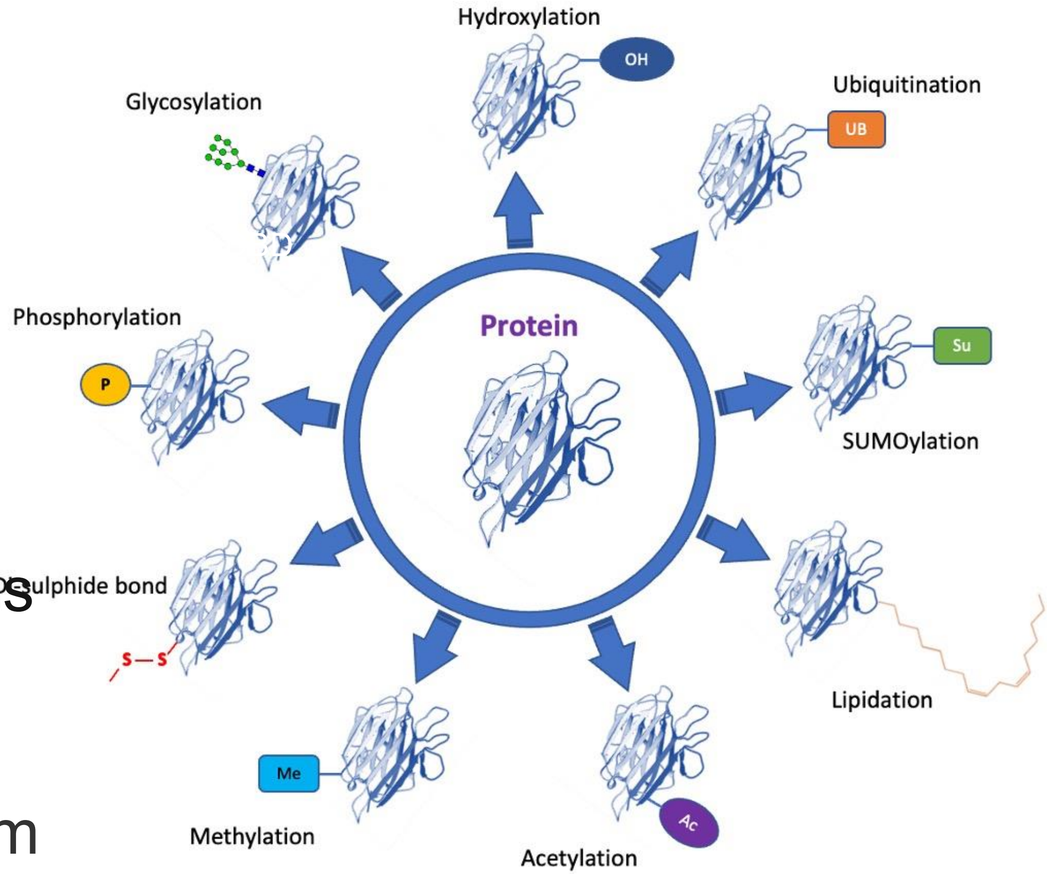
Post-translational modifications

PTMs

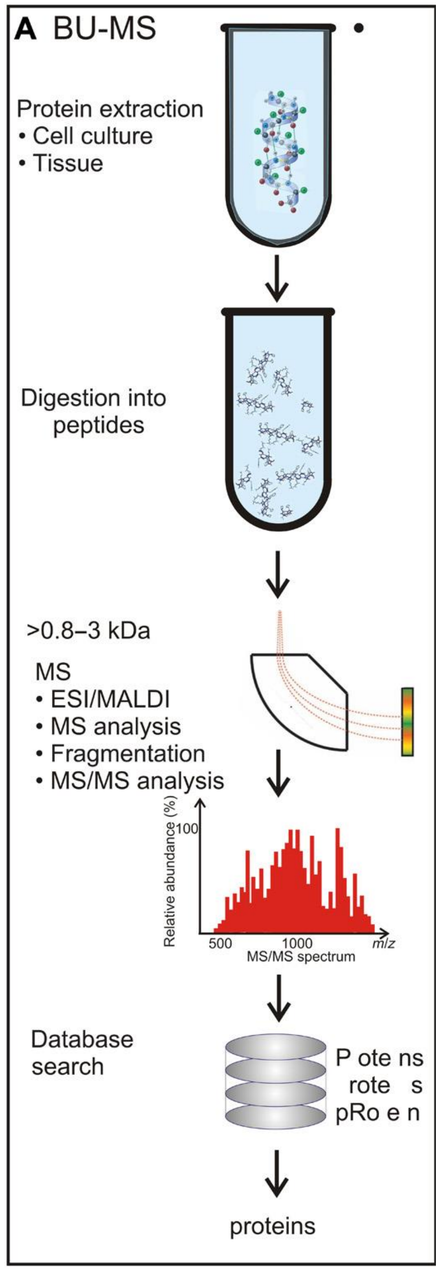
- PTMs increase the **functional diversity** of the proteome
- Include the covalent addition of **functional groups**, **proteolytic cleavage** of regulatory subunits, or **degradation** of entire proteins.
- PTMs can regulate **activity, localization, and interaction** with other molecules such as other proteins, nucleic acids, sugars, lipids and other cofactors
- 5% of the proteome are enzymes that perform **> 200 types of PTMs** to other proteins



Protein Identification

Mass Spectrometry (MS)

- **BU-MS** (Bottom UP MS)
- **Enzymatic digestion** of the proteins (Trypsin) into short peptides (5 -20 Aas)
- Ionized peptides are then analysed by **electrospray ionization** or matrix-assisted laser desorption/ionization (**MALDI**)
- Analyse their masses, **compare to database**
- Does capture **PTMs**
- **Short peptide sequences** can be similar complicating identification, low sensitivity, short reads means ‘novel’ peptides or PTMs can be hard to identify.



https://doi.org/10.1126/sciadv.aax8978

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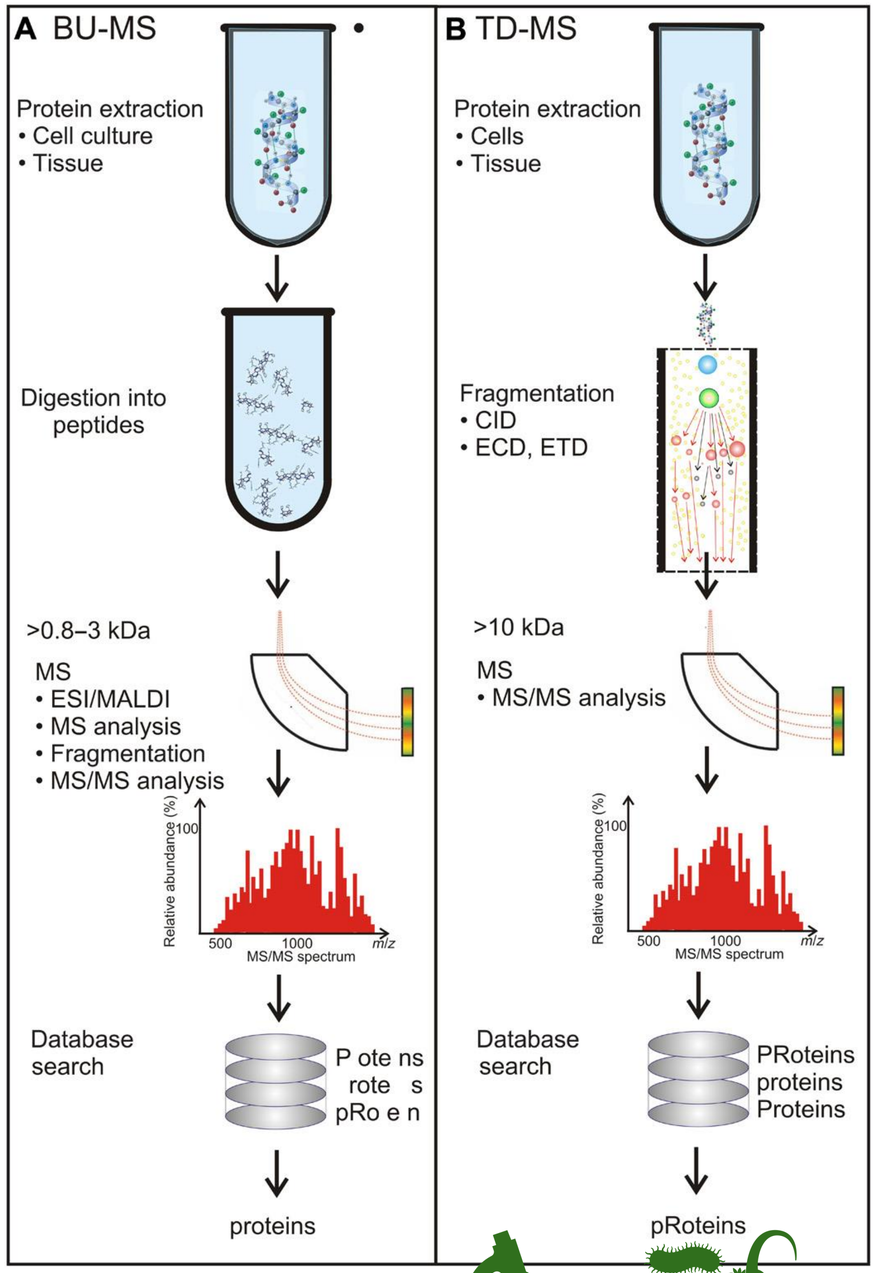
Protein Identification

Mass Spectrometry (MS)

- **TD-MS** (Top Down UP MS)
- Introduce **intact proteins** into the gas phase by electrospray ionization that are **subsequently fragmented**
- Can reveal **primary structure** of the protein with PTMs
- **Limited in size** to 70kDa
- **Sensitivity is poor** vs short peptides in BU-MS
- Much **less frequently** used



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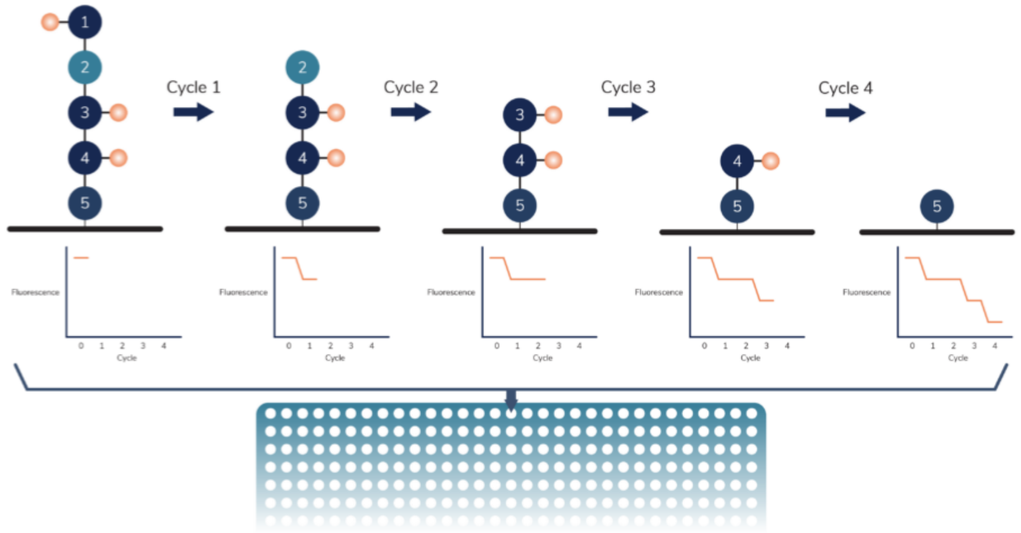
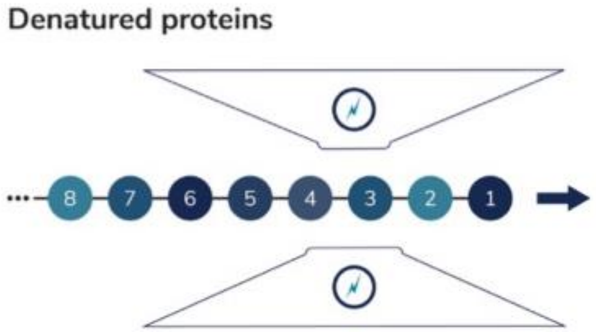
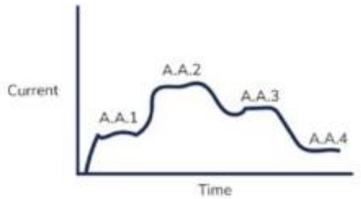
Next Generation Peptide sequencing

Edman Degradation based



Pore based





Changes in current indicate the identity of individual amino acids (A.A.s) or sets of amino acids

- **Not database** dependent
- **Single molecule** protein measurements possible
- **Slower,** must be **parallelised**
- **more expensive,** but technology is evolving rapidly

GENOME TO FUNCTION
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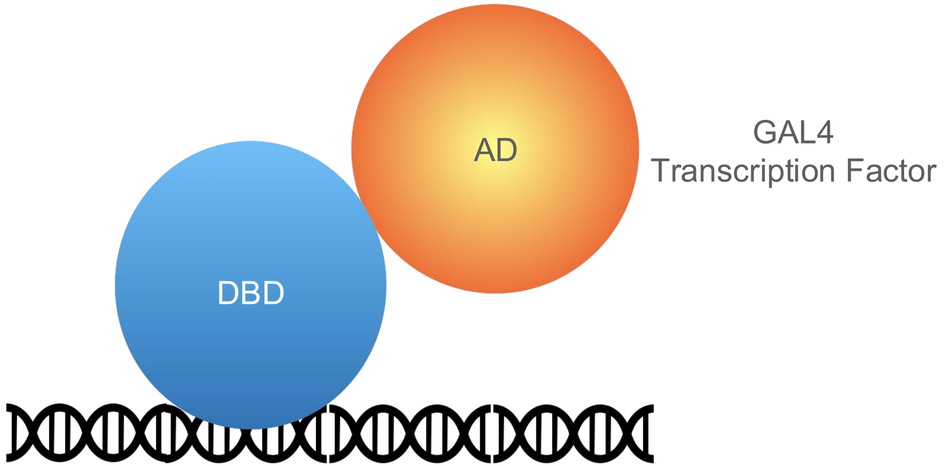
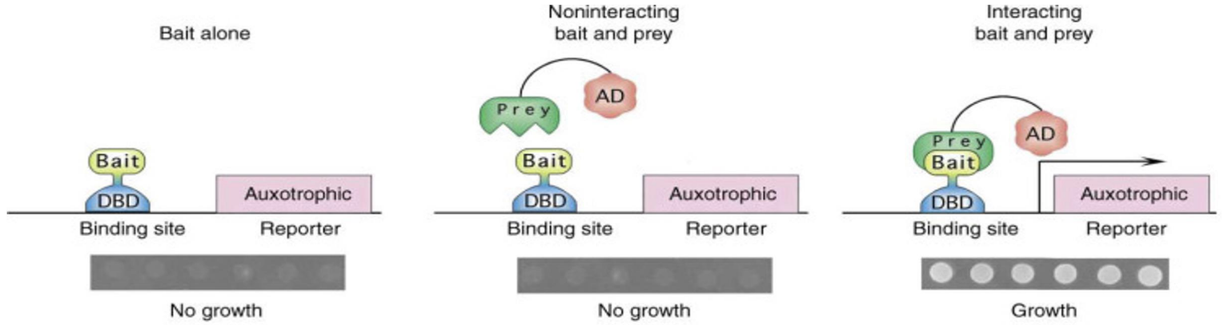
Finding Protein interactions

Yeast 2-hybrid system

- Key concept creation of **hybrid fusion proteins**
- Transcription factors (e.g. GAL4) have **DNA binding domain** (DBD) and **Activation domain** protein regions (AD) that can be split apart
- DBD region can be **fused to your protein of interest** [POI] (bait)
- This can be screened against a **library** of other proteins (prey) that are fused to the AD of the transcription factor
- When the POI interacts with a prey protein, this brings the DBD and AD together **enabling transcription**.
- This is used to express a **reporter gene**





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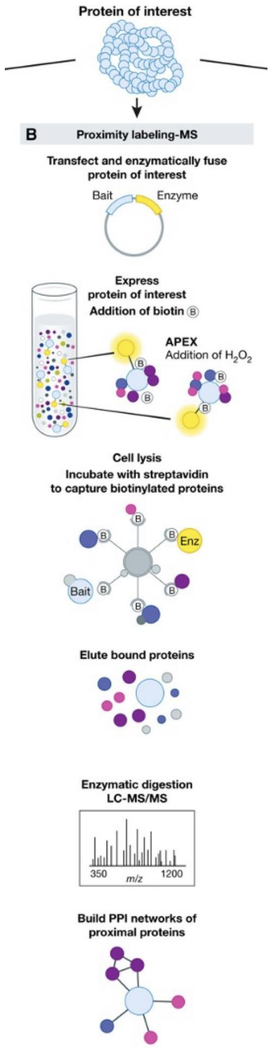
Finding Protein interactions

Proximity labelling methods

- **Protein of interest [POI]** is fused to an enzyme (Apex) that biotinylates other proteins
- This hybrid protein is expressed in cells and **biotin** is provided
- Proteins that interact with the POI are **biotinylated** by Apex activity
- These can be purified from all other proteins using **streptavidin** which binds to biotin with high affinity
- Interacting proteins are then **identified** e.g. by mass spectrometry



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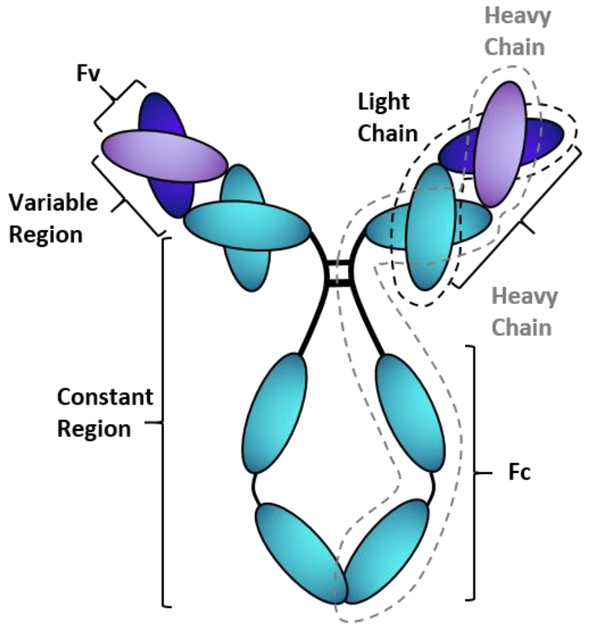
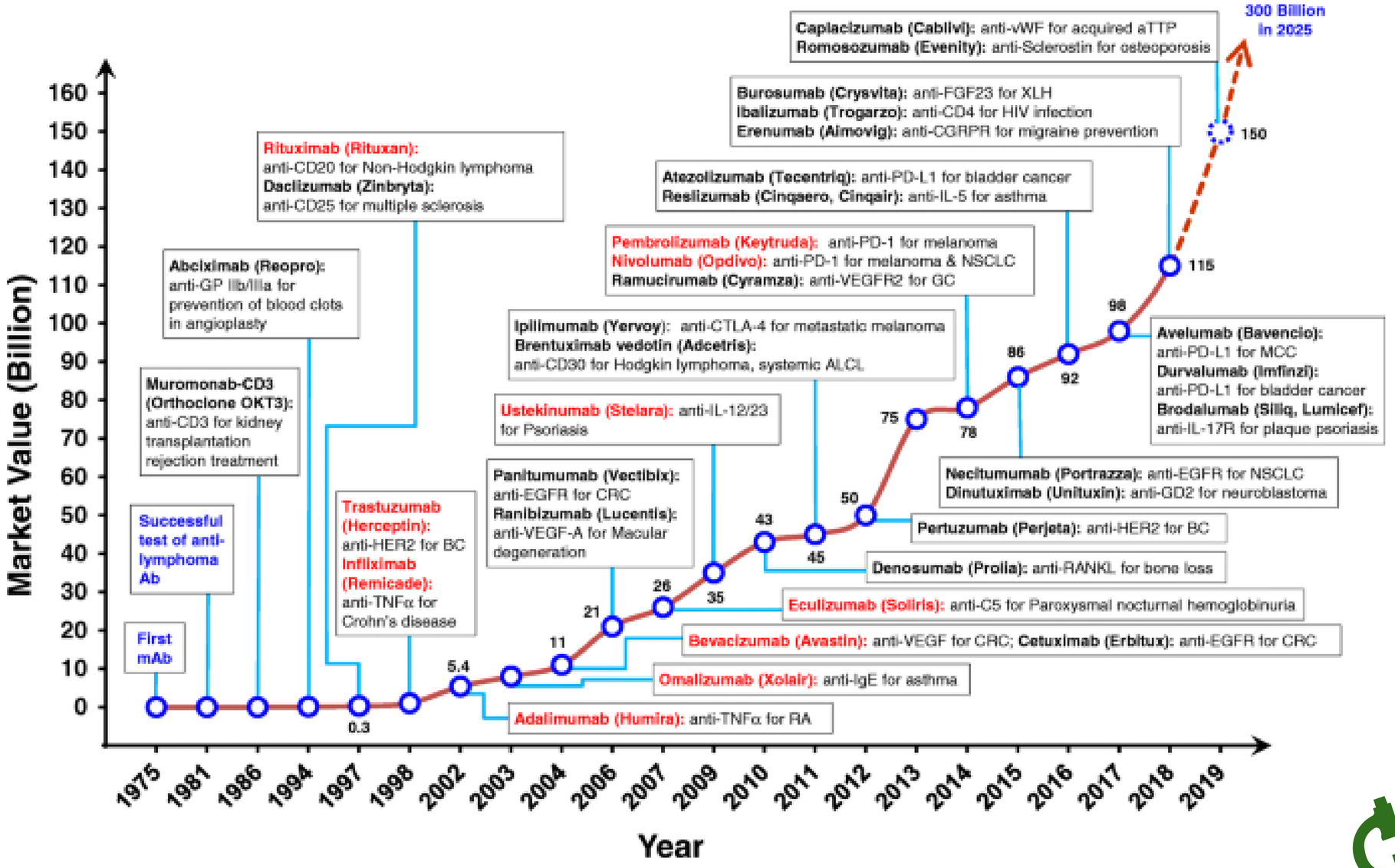
Changing Protein Activity

- **Drugs** small molecules
- **Antibodies** focus on monoclonal antibodies (mAbs) and nanobodies.



mAbs in medicine



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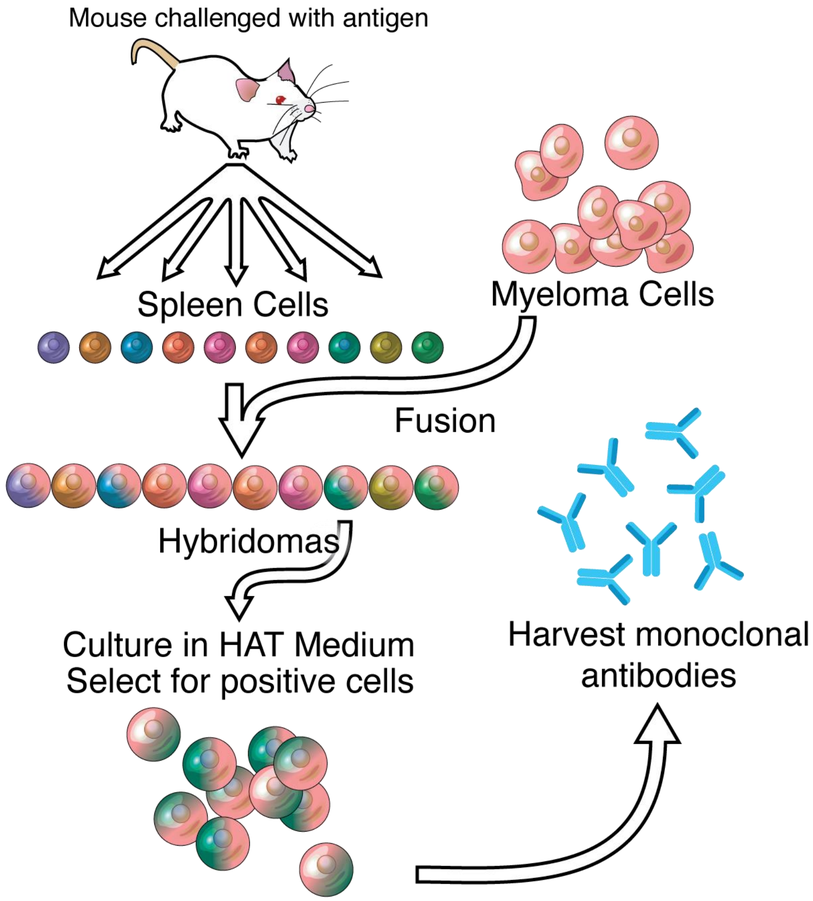
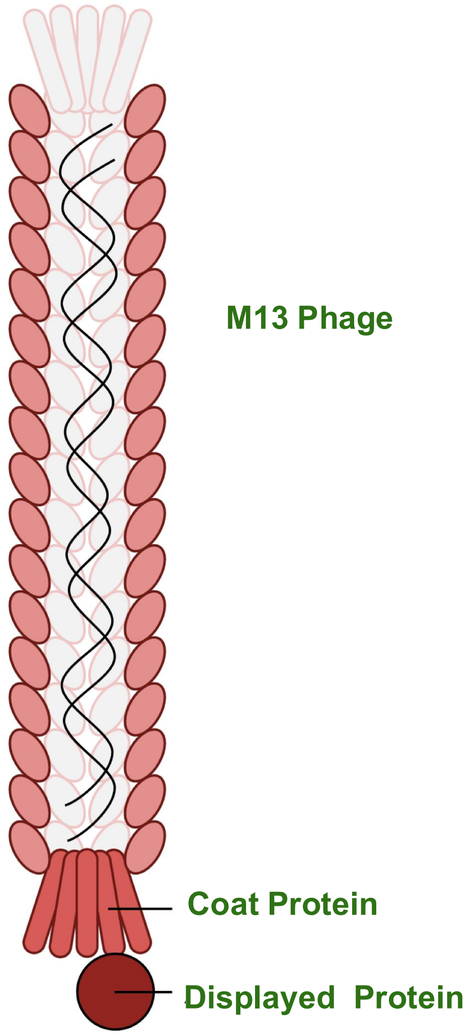
Monoclonal antibodies

- **Monoclonal antibodies (mAbs)** are produced from a hybridoma cell lines made from spleen derived B cells
- mAbs usually have **monovalent affinity** i.e. bind only to one epitope (different to polyclonal antibodies)
- Once a cell line is established Mabs can be produced in **unlimited quantities**
- Can be used to **detect and visualise proteins** in tissues or for biochemistry assays
- For human therapeutics, mAbs are often '**humanized**' to avoid immunogenicity against rodent sequences.



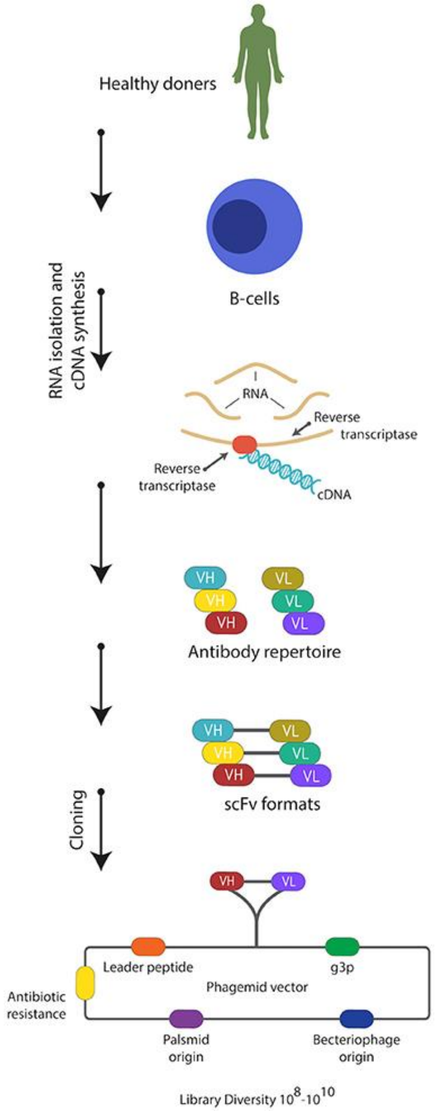
Phage Display

- **Genes** encoding protein sequences are inserted into a bacteria phage such as M13
- The inserted protein is then **displayed on the outside** of the phage.



Phage Display

- **Genes** encoding protein sequences are inserted into a bacteria phage such as M13
- The inserted protein is then **displayed on the outside** of the phage.
- A **library of phages** can be made that express antigen binding sequences on their surface
- These can then be screened in vitro to **select antigen binding** sequences with high affinity
- Once identified they can be engineered to **make therapeutic antibodies**



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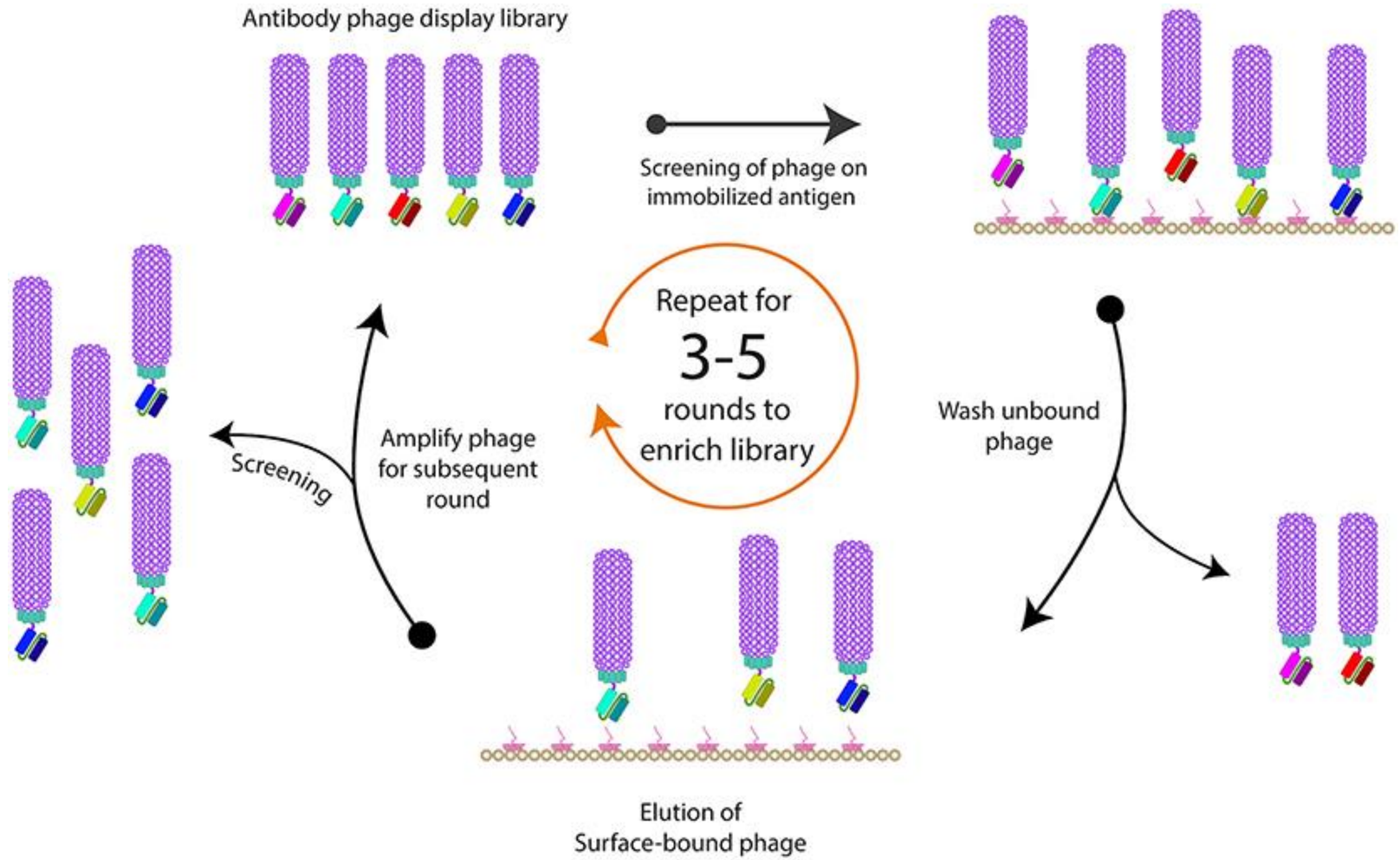
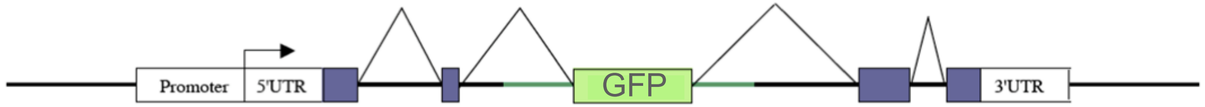
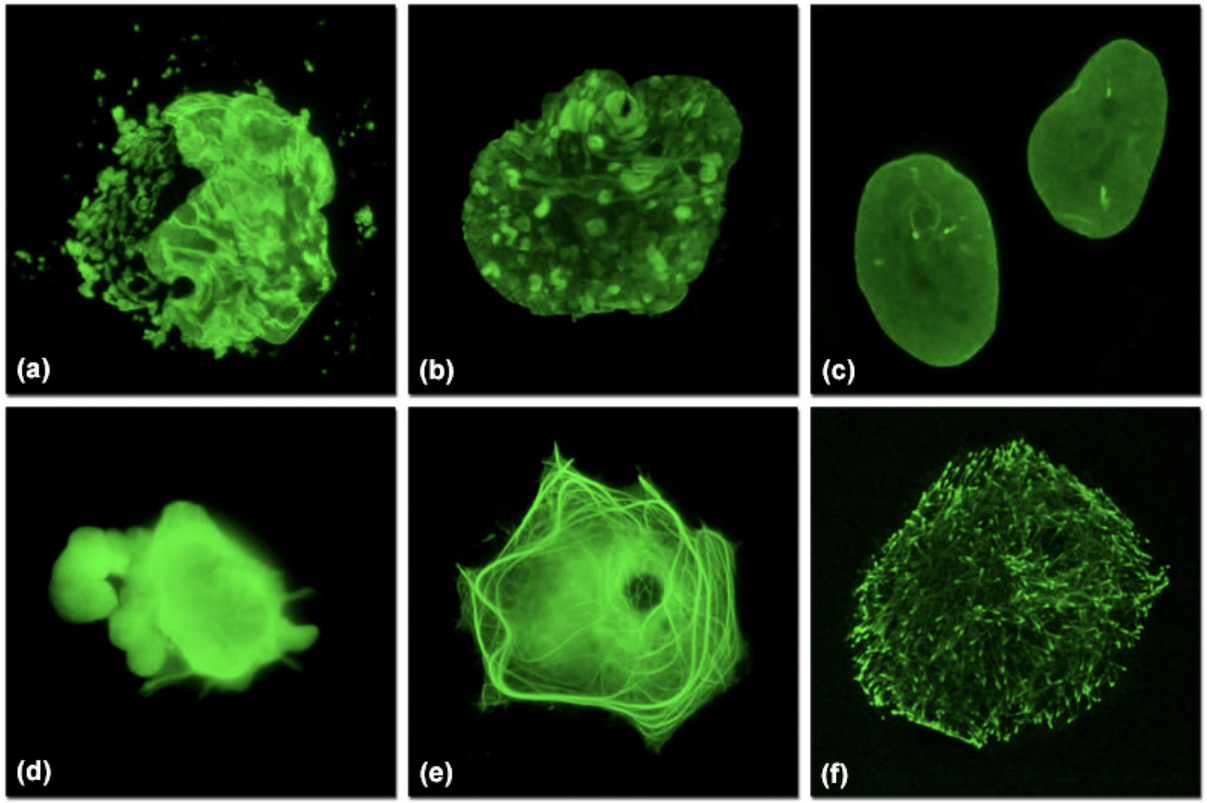
Phage Display



Tagging proteins

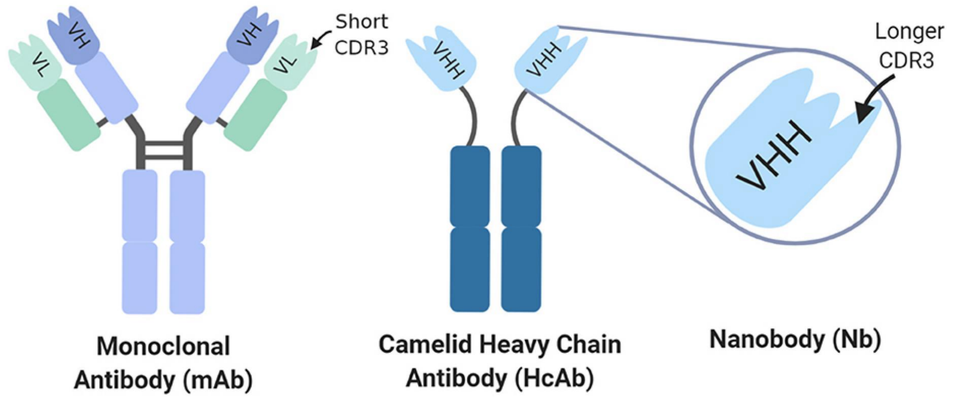
- **Protein tags** are peptide sequences genetically introduced into proteins
- Can be added to either **transgenic or endogenous** proteins
- Can be used to visualise or isolate proteins e.g. **epitope tags** (HA, V5 etc.) which are recognised by mAbs
- **Fluorescent protein tags** (e.g. GFP) allow protein dynamics to be observed in vivo



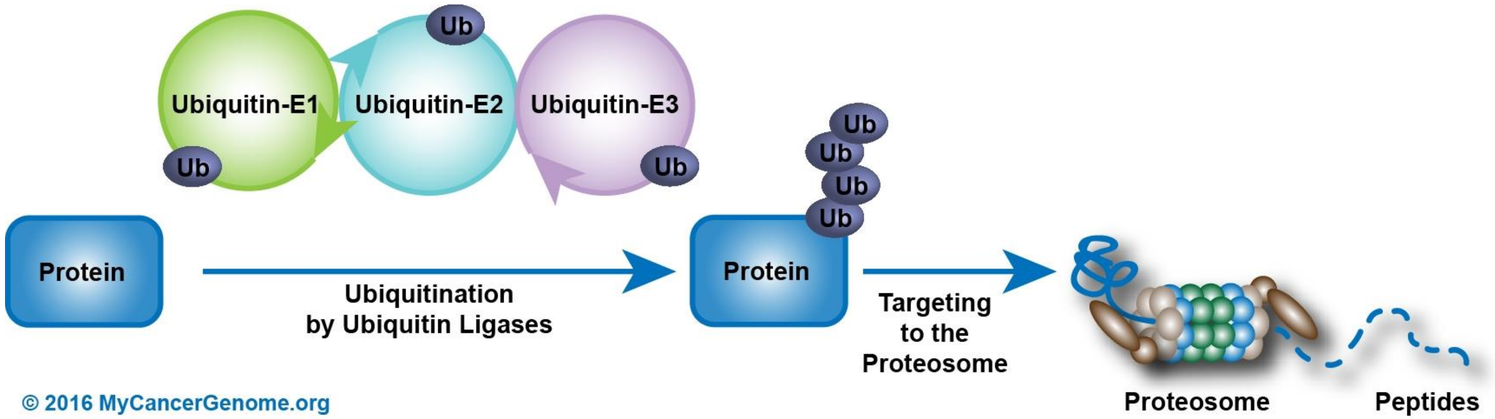


Nanobodies

- **Nanobodies** are the recombinant variable domains of heavy-chain-only antibodies
- Heavy-chain-only antibodies were first identified in **camels** but also found in cartilaginous fish.
- Nanobodies have a **small size**, excellent solubility, superior stability
- Can be cloned, engineered and **produced in bacteria**.

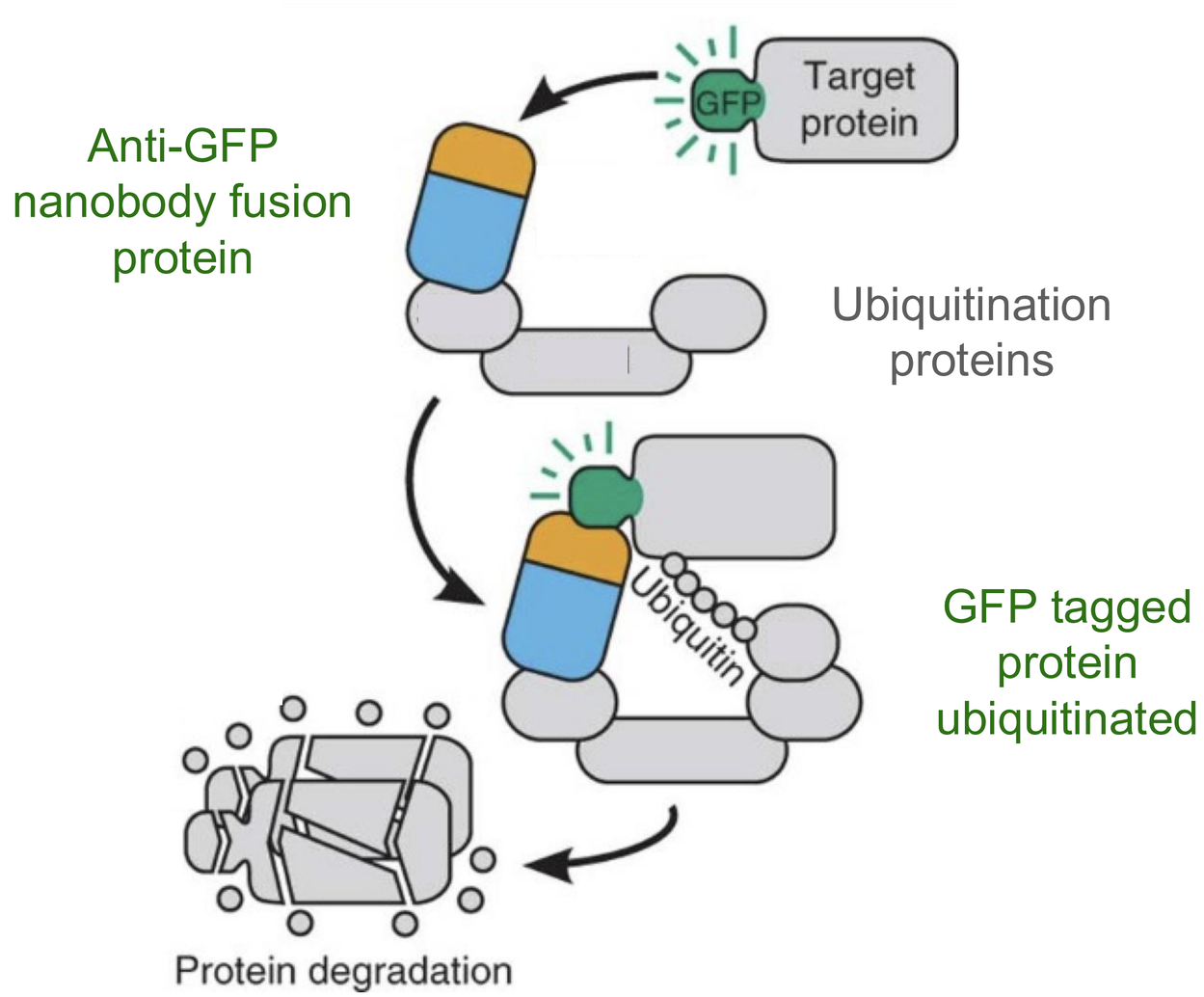


Ubiquitination degrades proteins



- **Ubiquitination** is the process in which proteins are labelled with ubiquitin in a post-translational modification.
- **Ubiquitin** is small protein which is attached in chains to Lysine residues in the ubiquitinated protein via the action of ubiquitin ligases
- Ubiquitination most often targets a protein for **degradation** by the proteasome.

Nanobody promoted Ubiquitination



How Brian

Why Pierre

What Felix