Cellular and Molecular Biology I

Answers to questions

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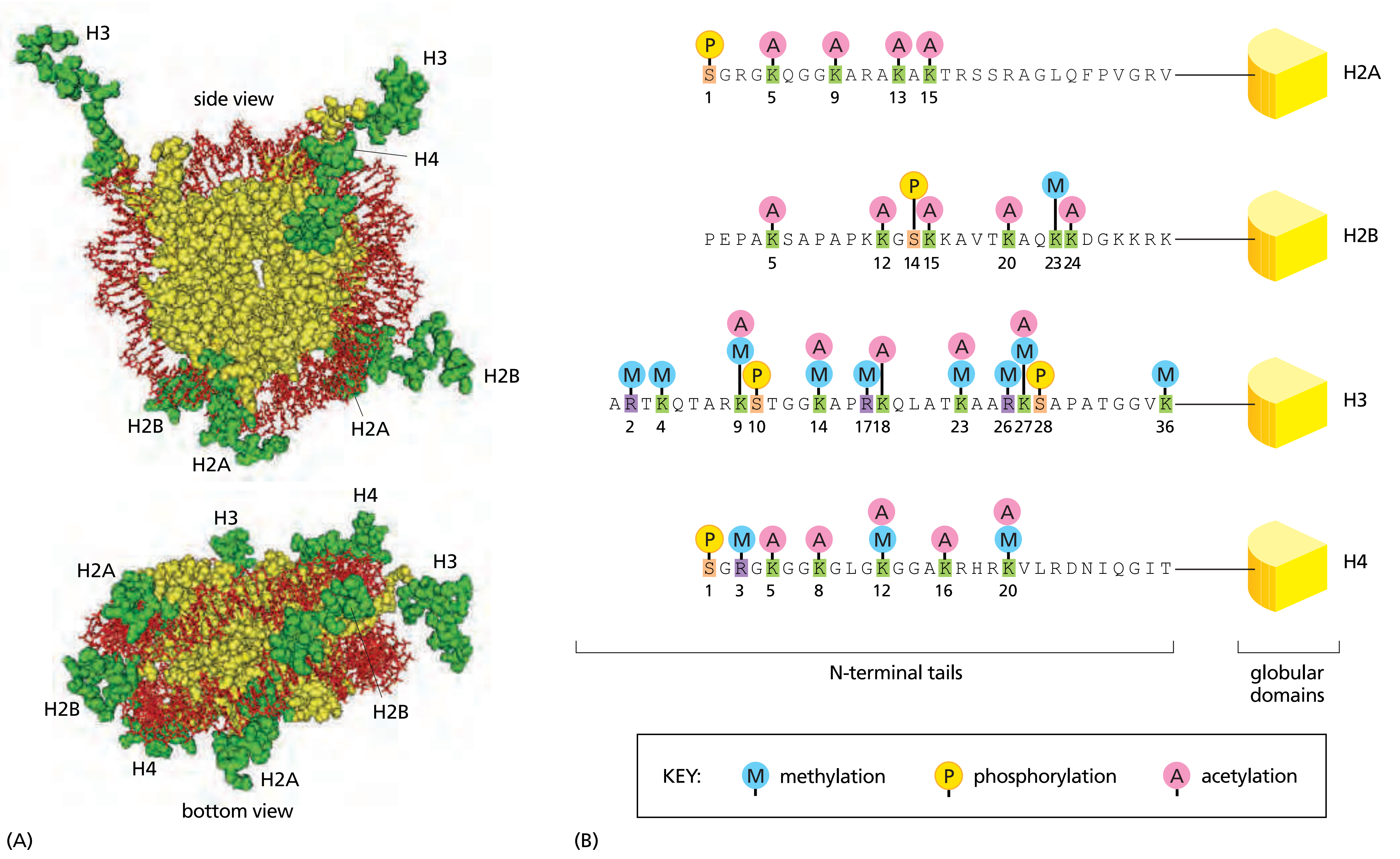
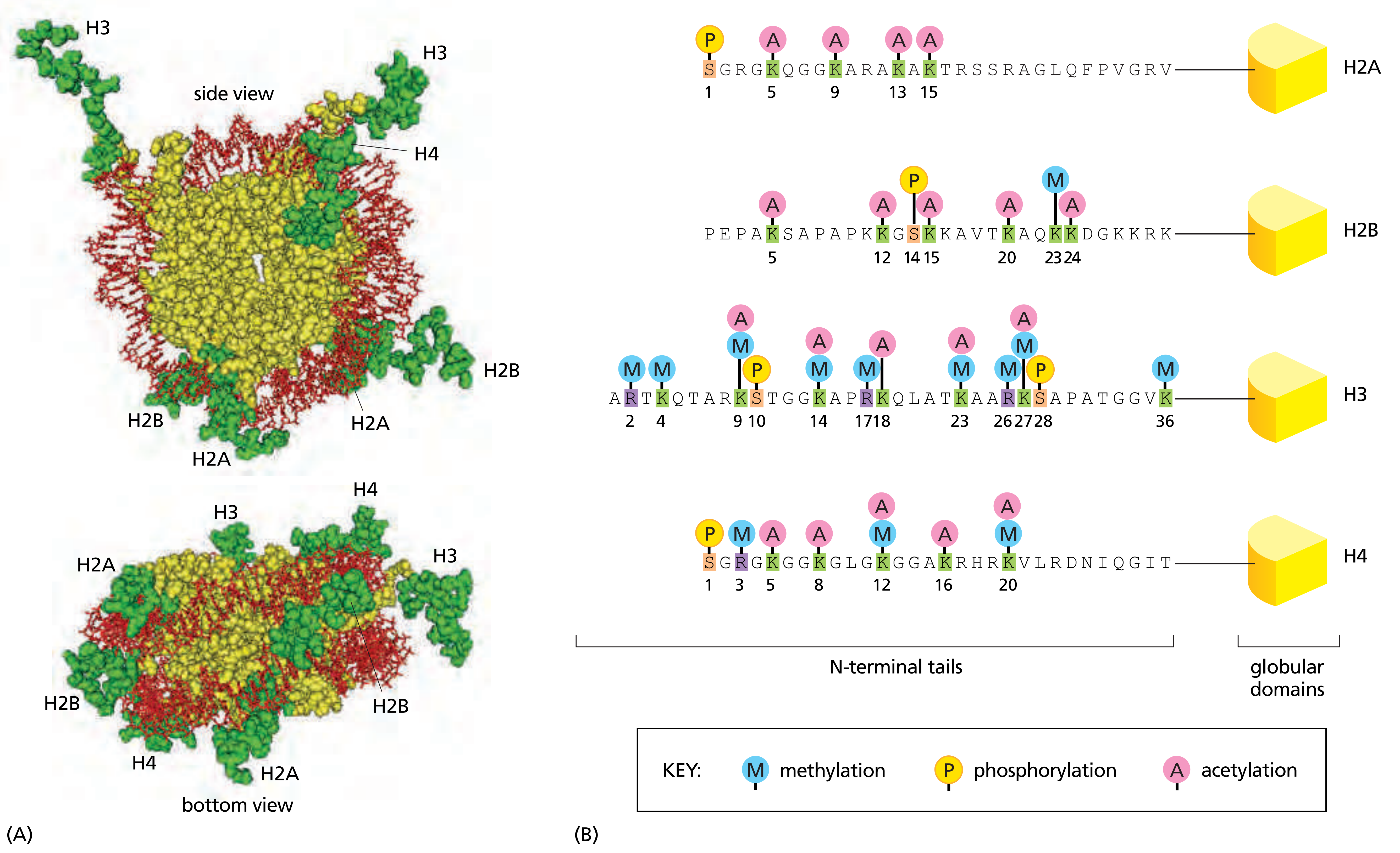
Histone modifications

- The **amino-acid side-chain of the <u>core</u> histones** are subjected to many covalent mutations (acetylation of lysines, methylation of lysines or phosphorylation of serines)
- Primarily on **histone tails** but also on **nucleosome** core
- Modifications are reversible with **dedicated enzymes** to add or remove them (e.g. HATs and HDACs)
- Result in change in **chromatin organization**
- Are referred to as the **"histone code"**



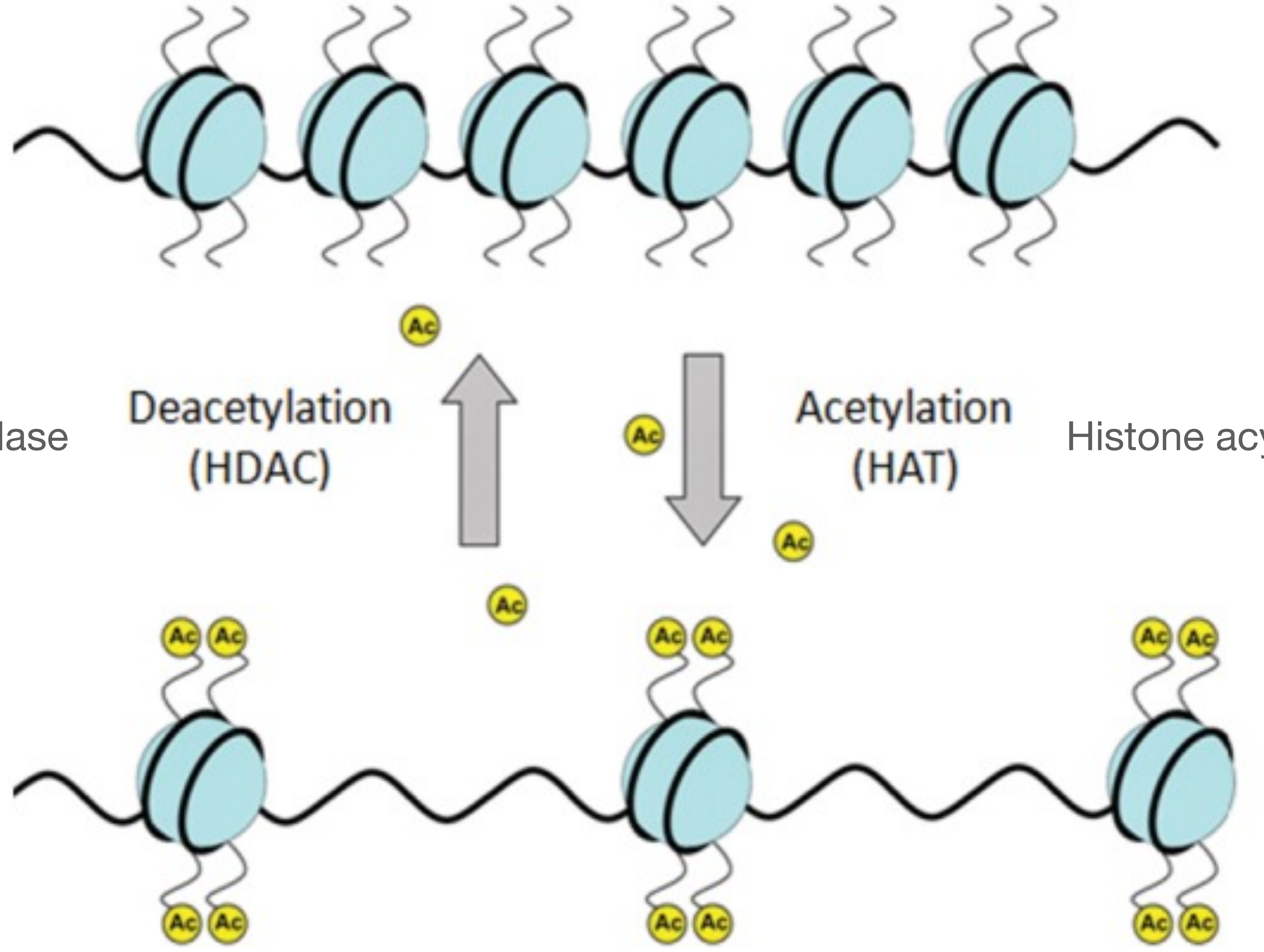
Histone modifications

- Modification enzymes bind to **DNA sequences**, so DNA determines **how histones are modified**
- Some modifications **persist in time**



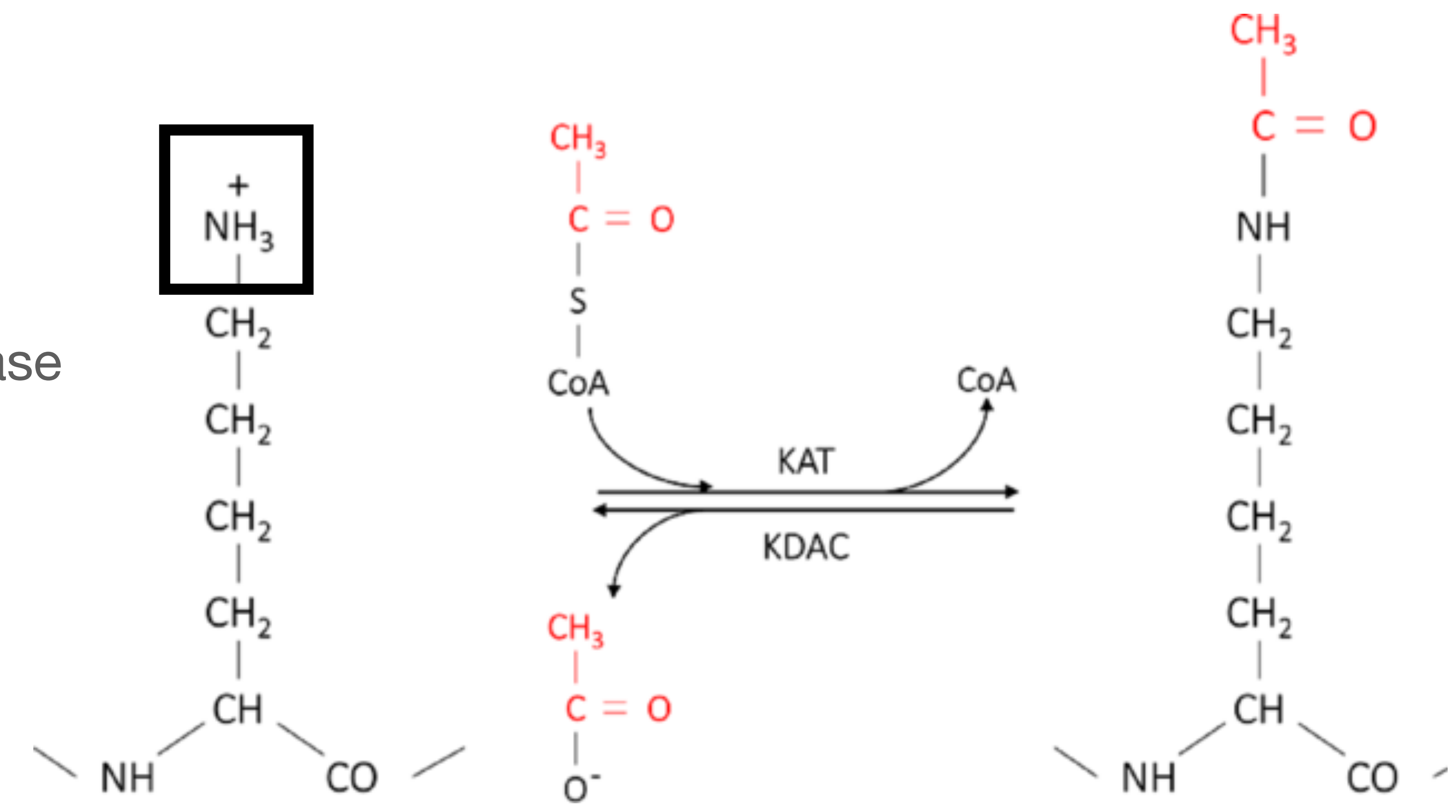
Example: Histone acetylation

Histone deacetylase



Histone acyltransferase

Masks the positive charge

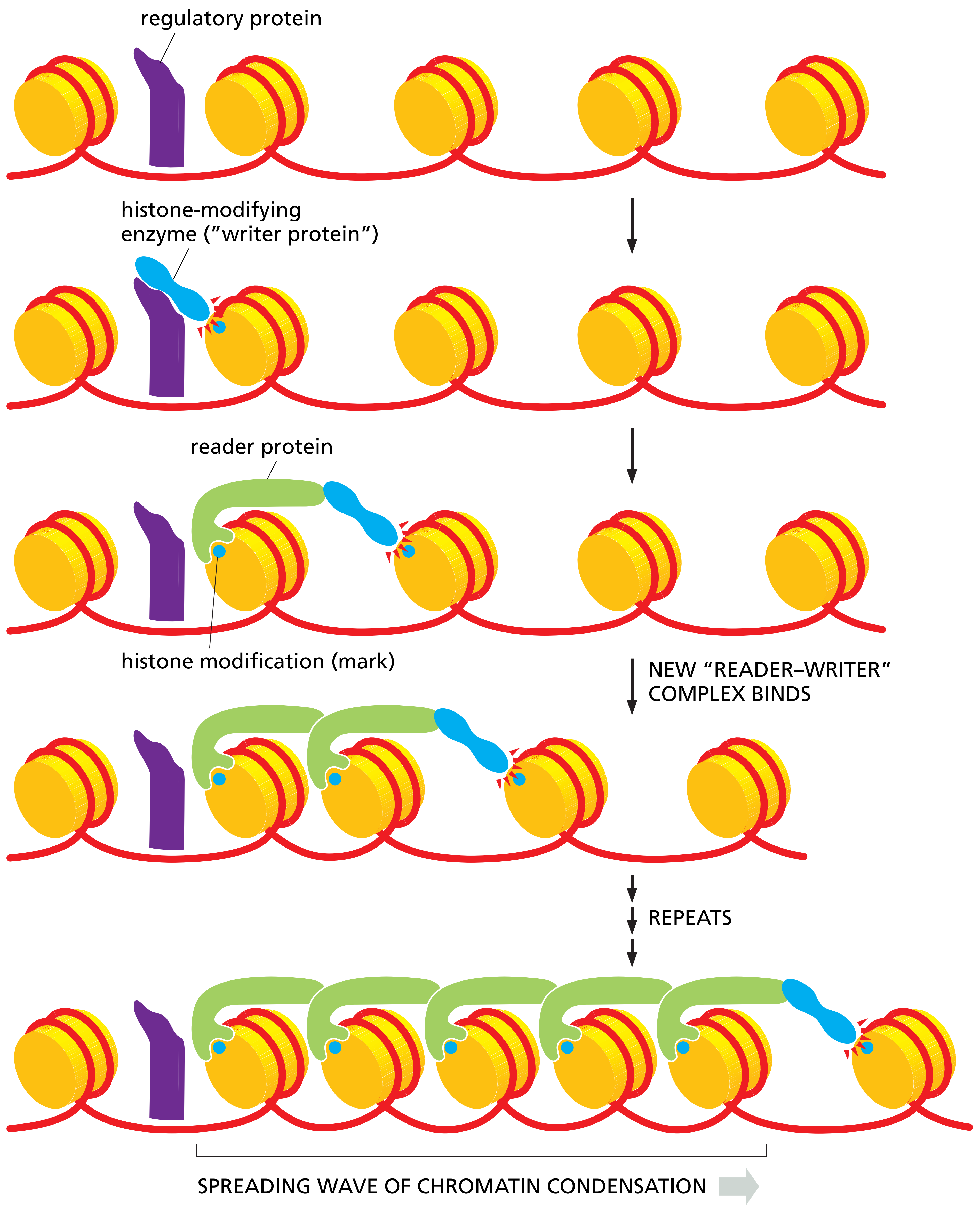


Lysine

PMCID: PMC3660019

The spread of heterochromatin (position effect)

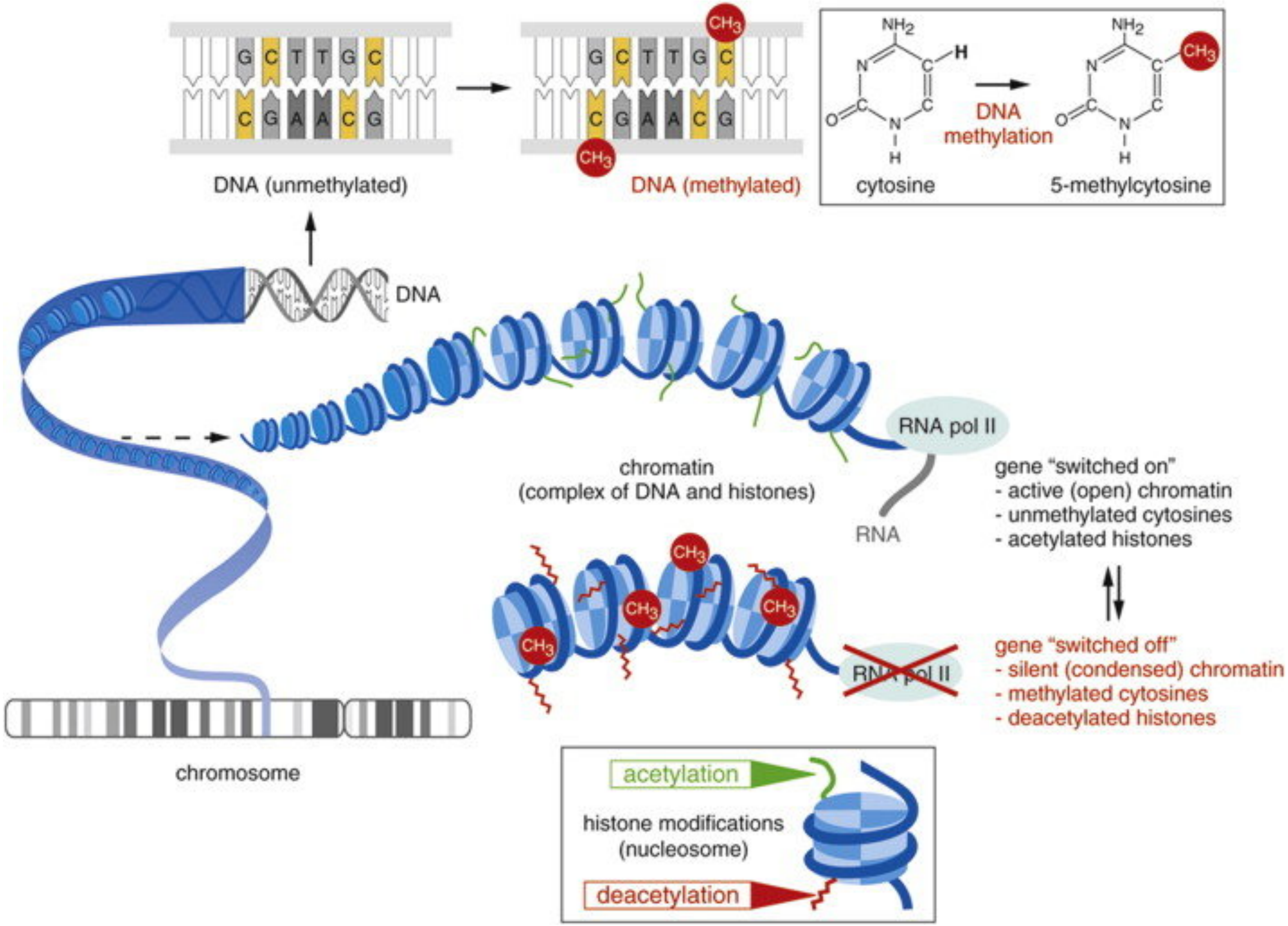
Position effect is the effect on the expression of a gene when its location in a chromosome is changed, often by translocation.



In addition to histones, is DNA also modified?

- **DNA methylation** on **cytosine**

- DNA methylation influences **gene expression** but not the chromatin organisation (hetero- and euchromatin)



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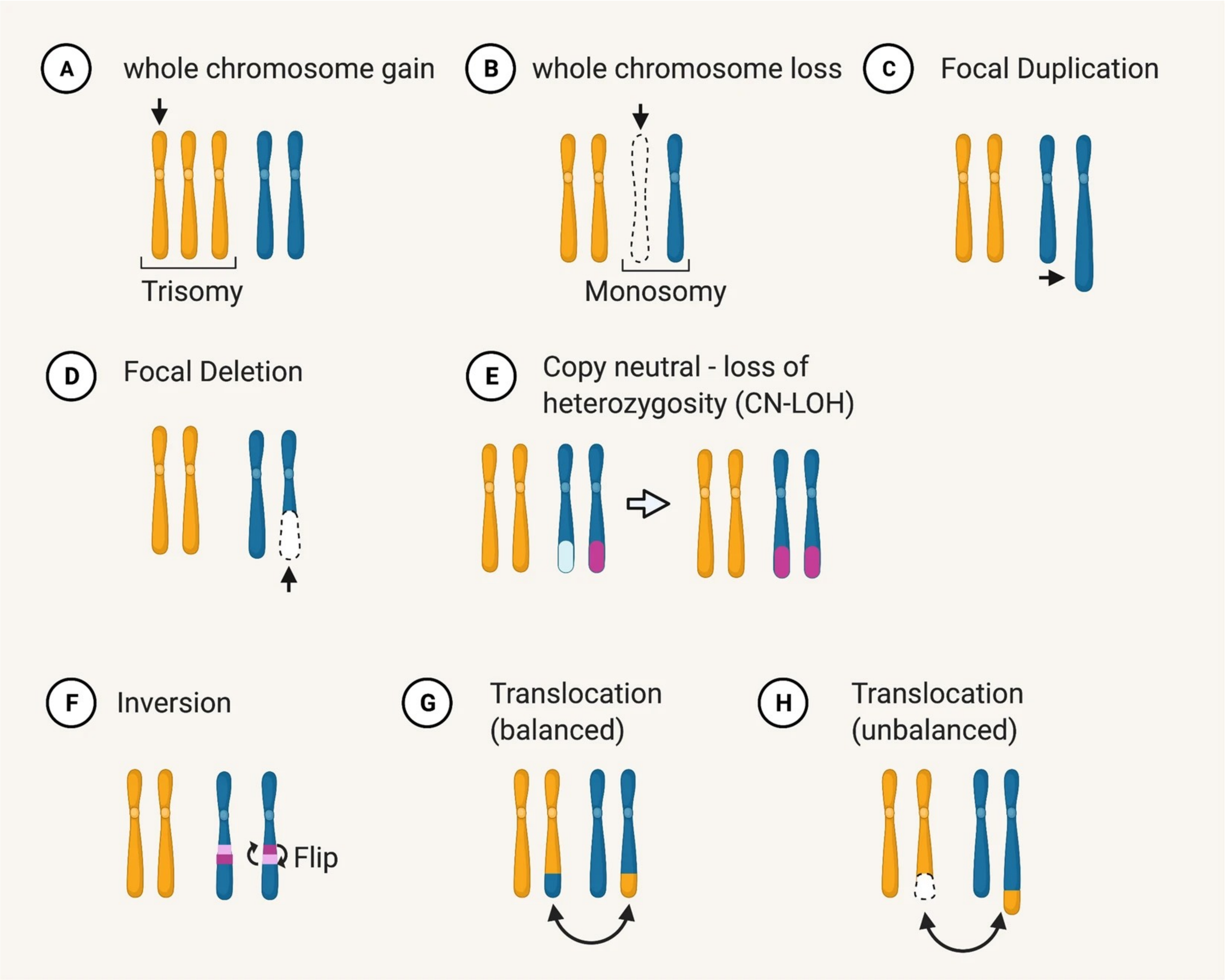
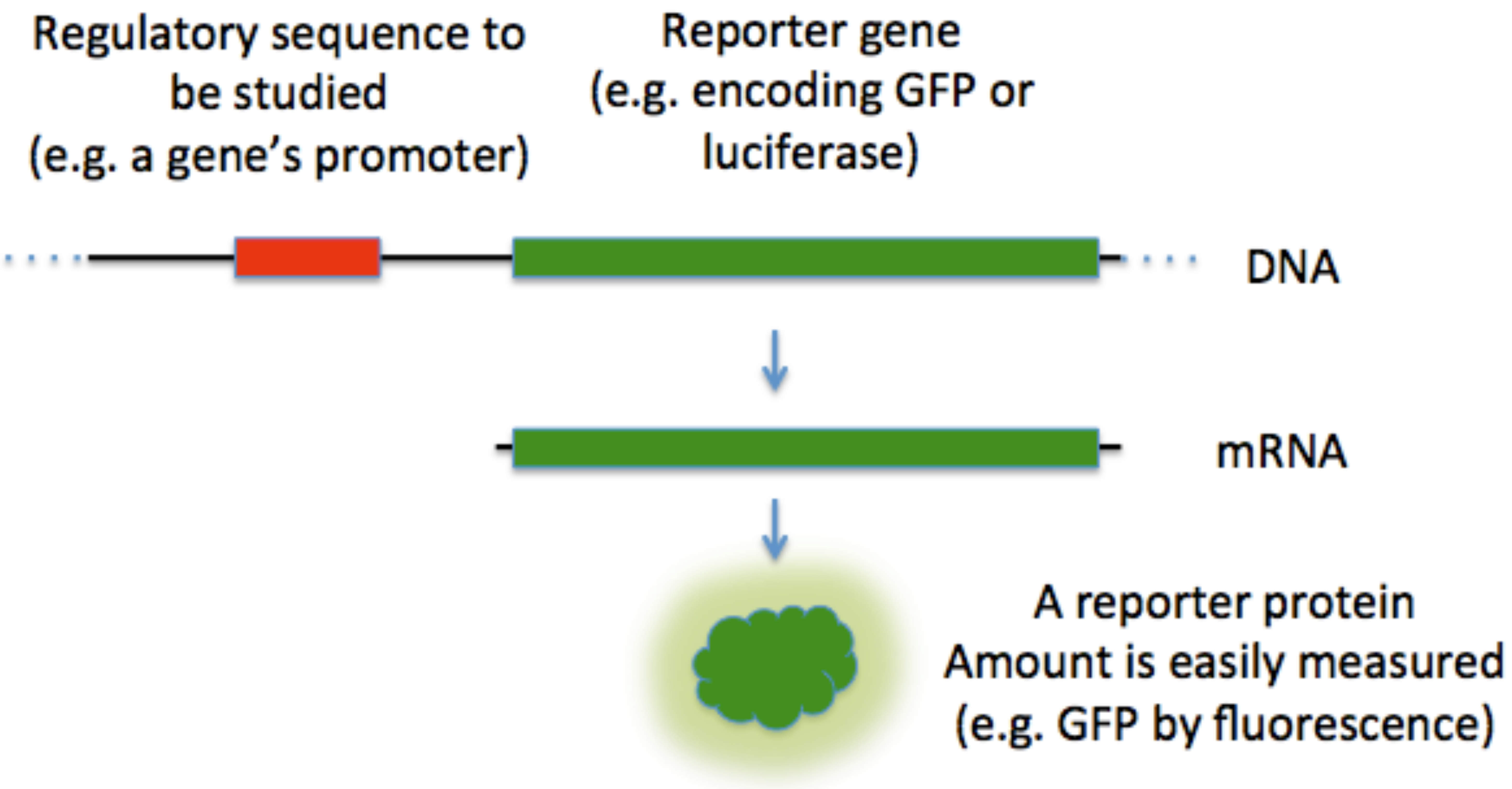
Balanced vs. unbalanced translocation



Reporter gene



a **reporter gene** (often simply **reporter**) is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals or plants. Such genes are called reporters because the characteristics they confer on organisms expressing them are easily identified and measured, or because they are selectable markers.

Homologous genes vs. homologous chromosomes

Homologous genes are genetic sequences inherited from a common ancestor (=**gene**). They are similar in sequence and can perform similar functions

- Human cells (except gametes and highly-specialised cells like red-blood cells) have **two copies of each chromosome** (one from the father, one from the mother)
- The maternal and paternal chromosomes are called **homologous chromosomes**
- The **sex** chromosomes in males are **non homologous**
- Each human cell comprises **46 chromosomes: 22 pairs of autosomes and 2 sex chromosomes**

Does the reverse transcriptase need a primer?

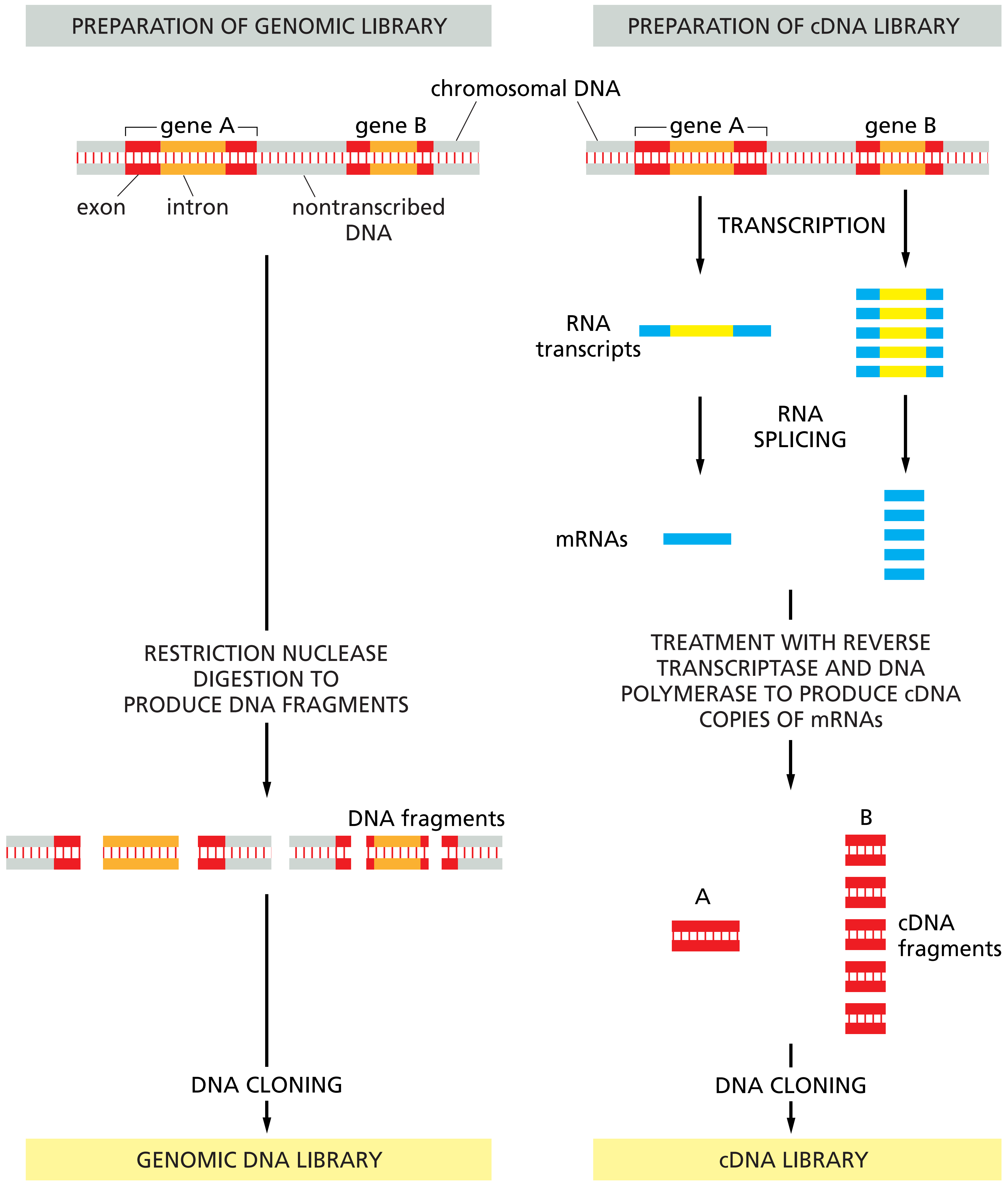
Yes, reverse transcriptase (RT) requires primers to initiate DNA synthesis. Like other DNA polymerases, reverse transcriptase cannot start DNA synthesis de novo; it needs a pre-existing primer with a free 3'-OH group to extend. Here are the key points:

1. Primer Types:
   - The primer can be RNA or DNA.
   - Commonly, in laboratory applications, researchers use short synthetic DNA primers (oligonucleotides) complementary to a specific RNA sequence.
   - In retroviruses, the primer is typically a specific cellular tRNA molecule that hybridizes to the viral RNA genome.

2. Function of the Primer:
   - The primer hybridizes to the complementary sequence on the RNA template.
   - Reverse transcriptase extends the primer by adding deoxynucleotides complementary to the RNA template, synthesizing the first strand of DNA.

3. Applications in Molecular Biology:
   - In techniques like reverse transcription-PCR (RT-PCR), oligo(dT) primers (binding to poly-A tails of mRNA), random hexamers, or sequence-specific primers are used to facilitate cDNA synthesis from RNA templates.

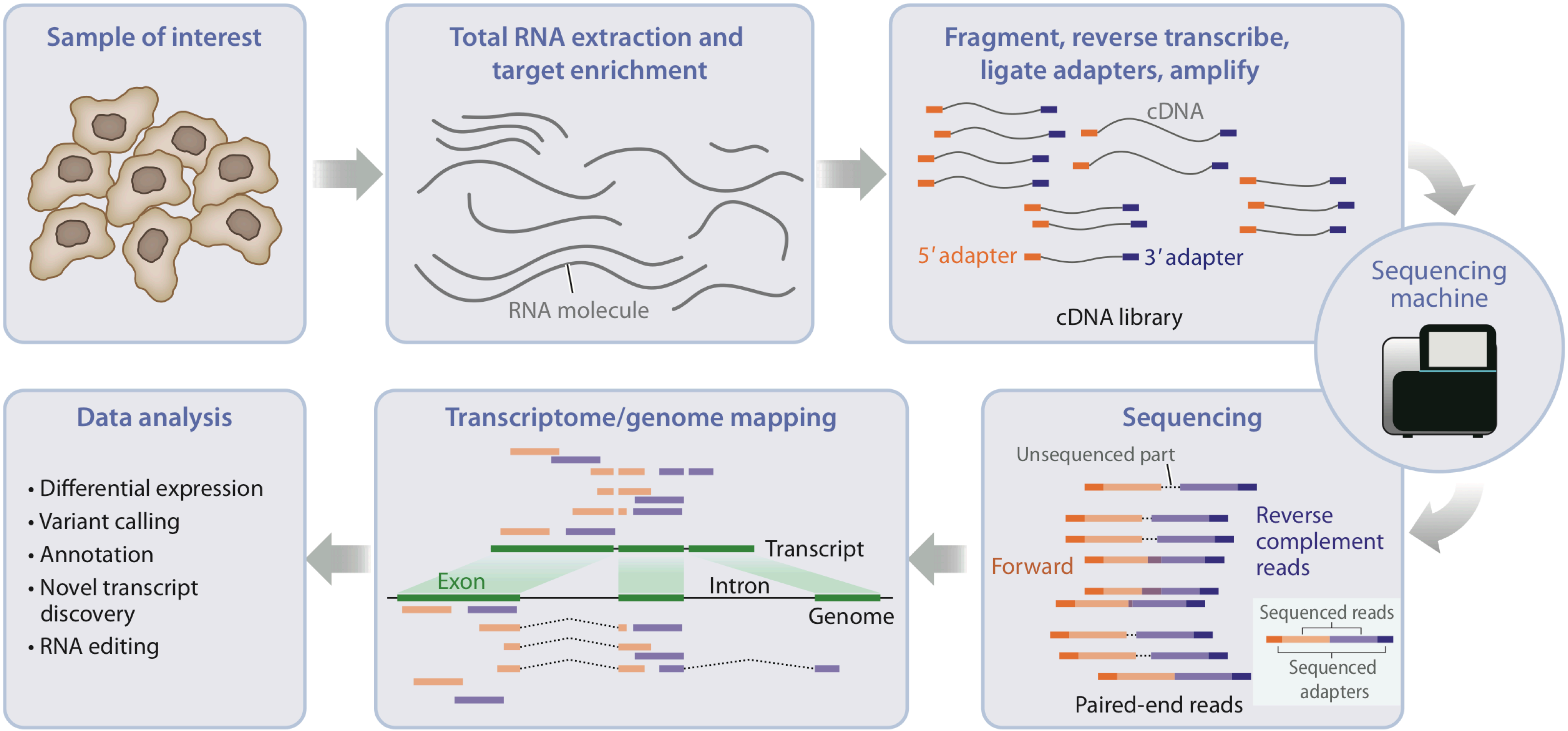
RNA-seq vs. single cell RNA-seq



RNA-seq vs. single cell RNA-seq

- using **RNA sequencing (RNA-seq)**
  - Measures **which genes are being transcribed** at a given time under given conditions
  - Uses **reverse transcriptase** that to copy all RNAs into cDNAs
  - cDNAs are then **fragmented** and **sequenced** by **next-generation sequencing**
  - Abundant **RNAs** have more **cDNA** copies and therefore more sequencing **reads**

RNA-seq vs. single cell RNA-seq



Studying gene expression

- using **RNA sequencing (RNA-seq)**

➡ **Identification** of RNAs present and **quantification**

➡ Using **cluster analysis** (computational approach), one can identify genes that are regulated together

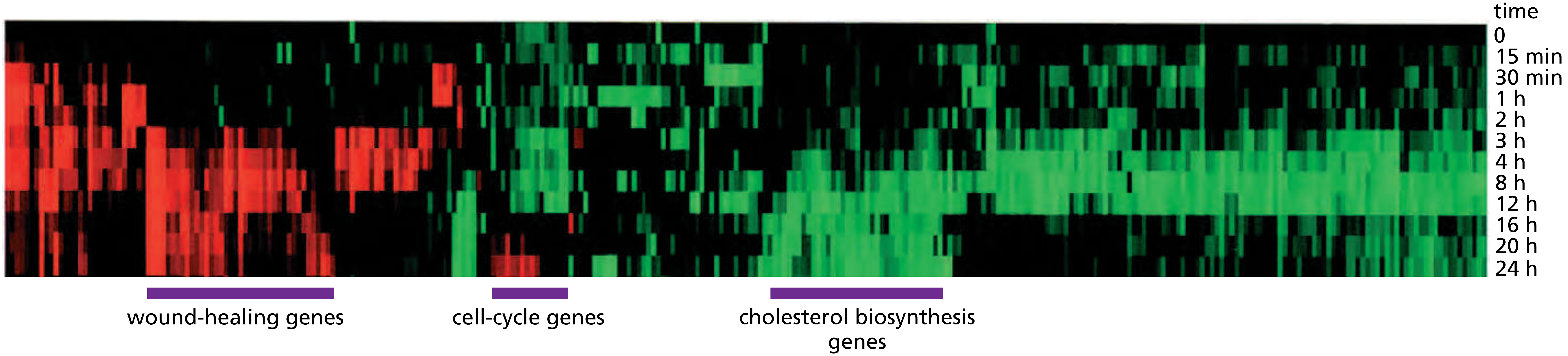
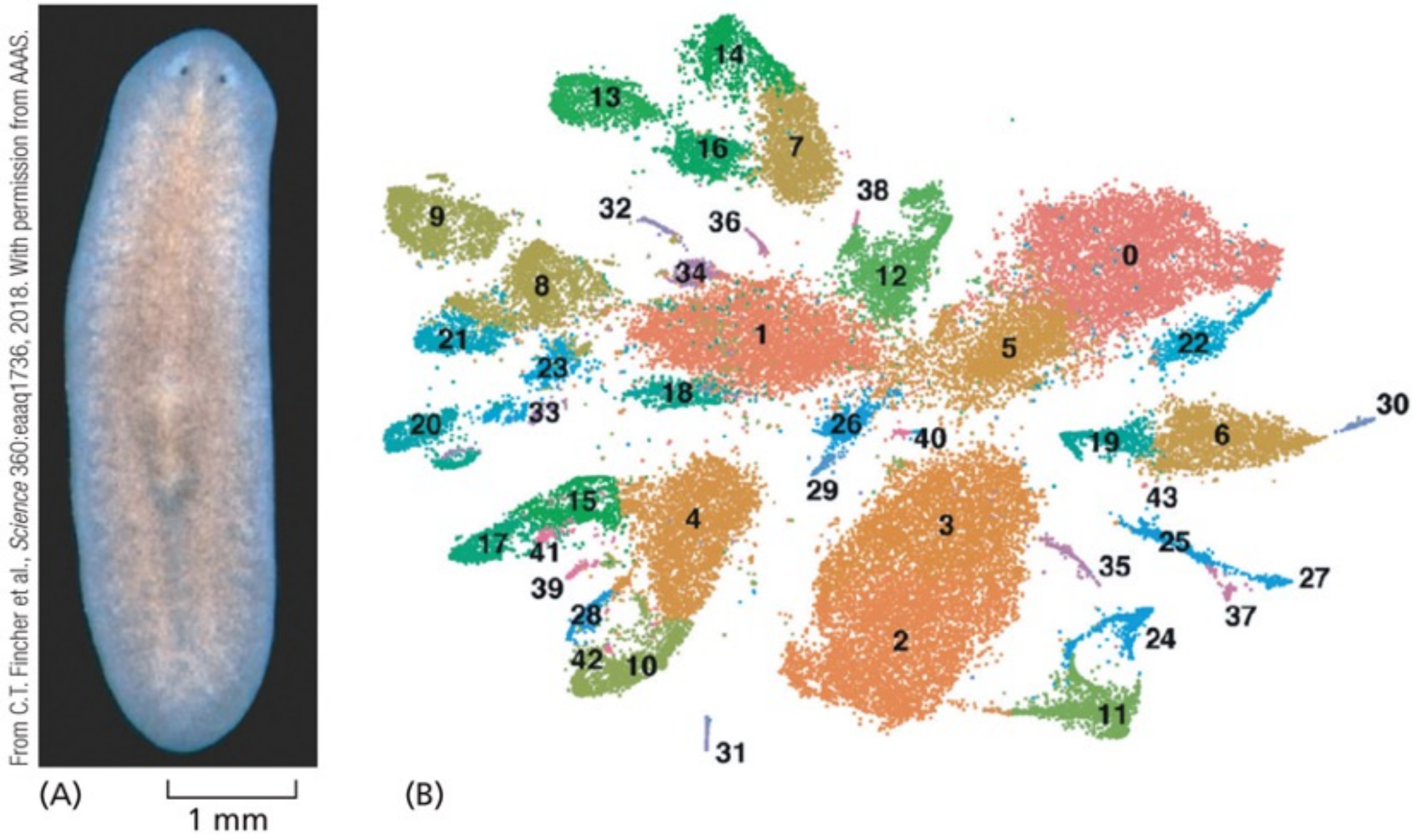


Figure 8–65 Using cluster analysis to identify sets of genes that are coordinately regulated. Genes that have the same expression pattern are likely to be involved in common pathways or processes. To perform a cluster analysis, RNA-seq or microarray data are obtained from cell samples exposed to a variety of different conditions, and genes that show coordinate changes in their expression pattern are grouped together. In this experiment, human fibroblasts were deprived of serum for 48 hours; serum was then added back to the cultures at time 0 and the cells were harvested for microarray analysis at different time points. Of the 8600 genes depicted here (each represented by a thin, vertical line), just over 300 showed threefold or greater variation in their expression patterns in response to serum reintroduction. Here, *red* indicates an increase in expression; *green* is a decrease in expression. On the basis of the results of many other experiments, the 8600 genes have been grouped in clusters based on similar patterns of expression. The results of this analysis show that genes involved in wound healing are turned on in response to serum, while genes involved in regulating cell-cycle progression and cholesterol biosynthesis are shut down. (From M.B. Eisen et al., *Proc. Natl Acad. Sci. USA* 94:14863–14868, 1998. With permission from National Academy of Sciences.)

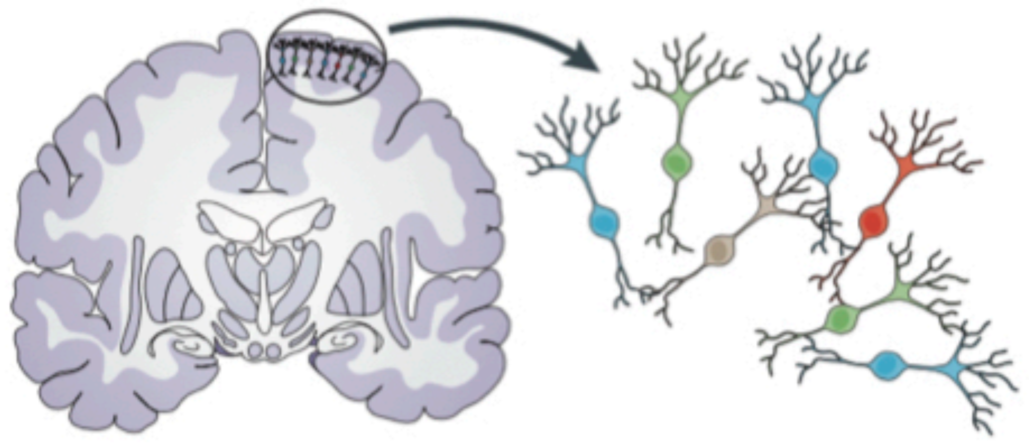
Studying gene expression

- using single cell **RNA sequencing (RNA-seq)**
    - Tissue dissociated into **single cells**
    - **Microfluidics** to separate single cells
    - Each cell is processed for **RNA-seq**
    - **Cluster analysis** algorithm that group cells with similar gene expression patterns

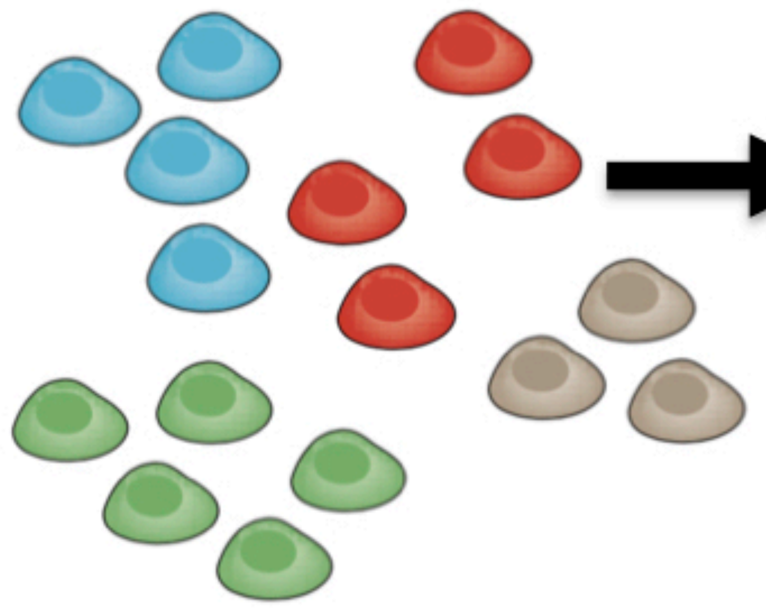


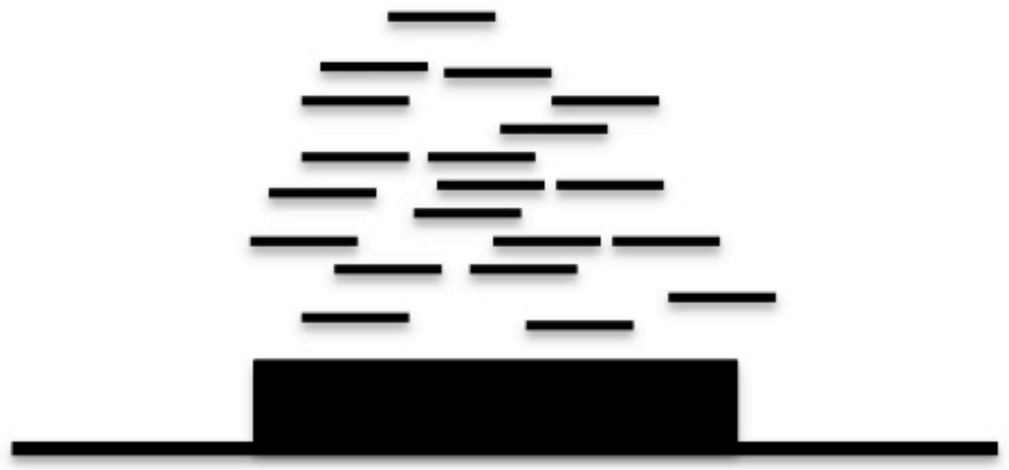
Single-cell RNA-Seq (scRNA-Seq)

Tissue (e.g. tumor)



Isolate and sequence individual cells



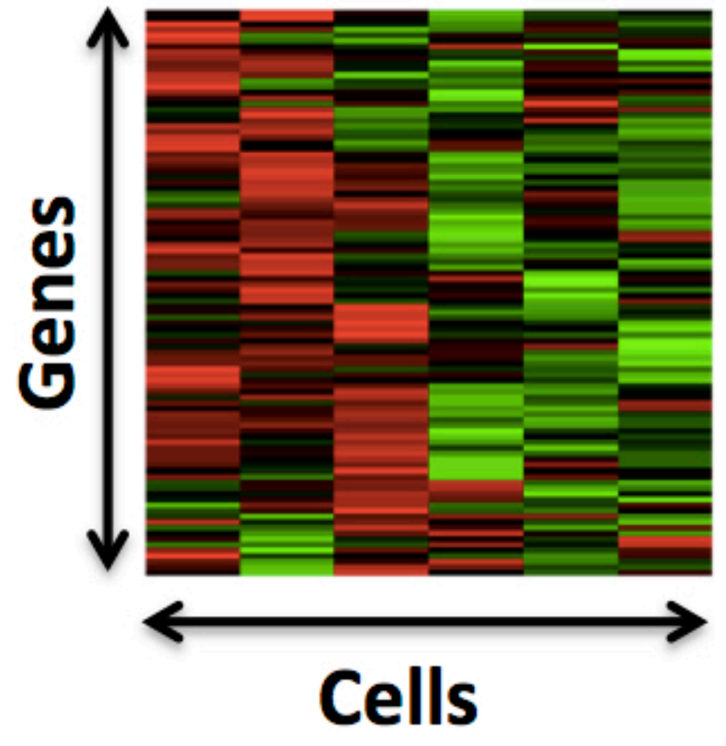


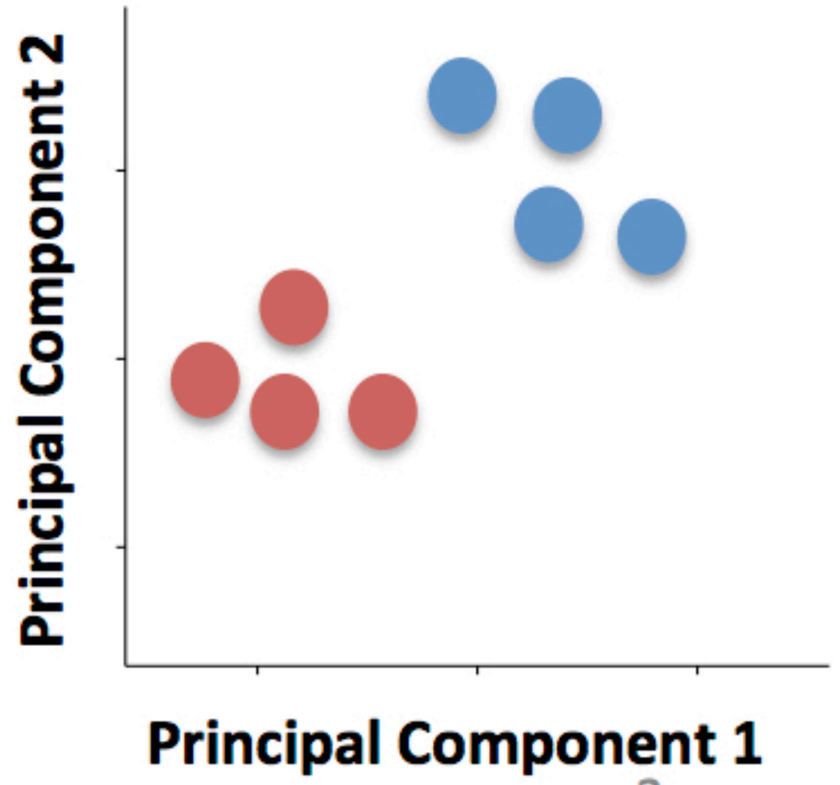
Gene 1
Cell 1

Read Counts

	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

Compare gene expression profiles of single cells





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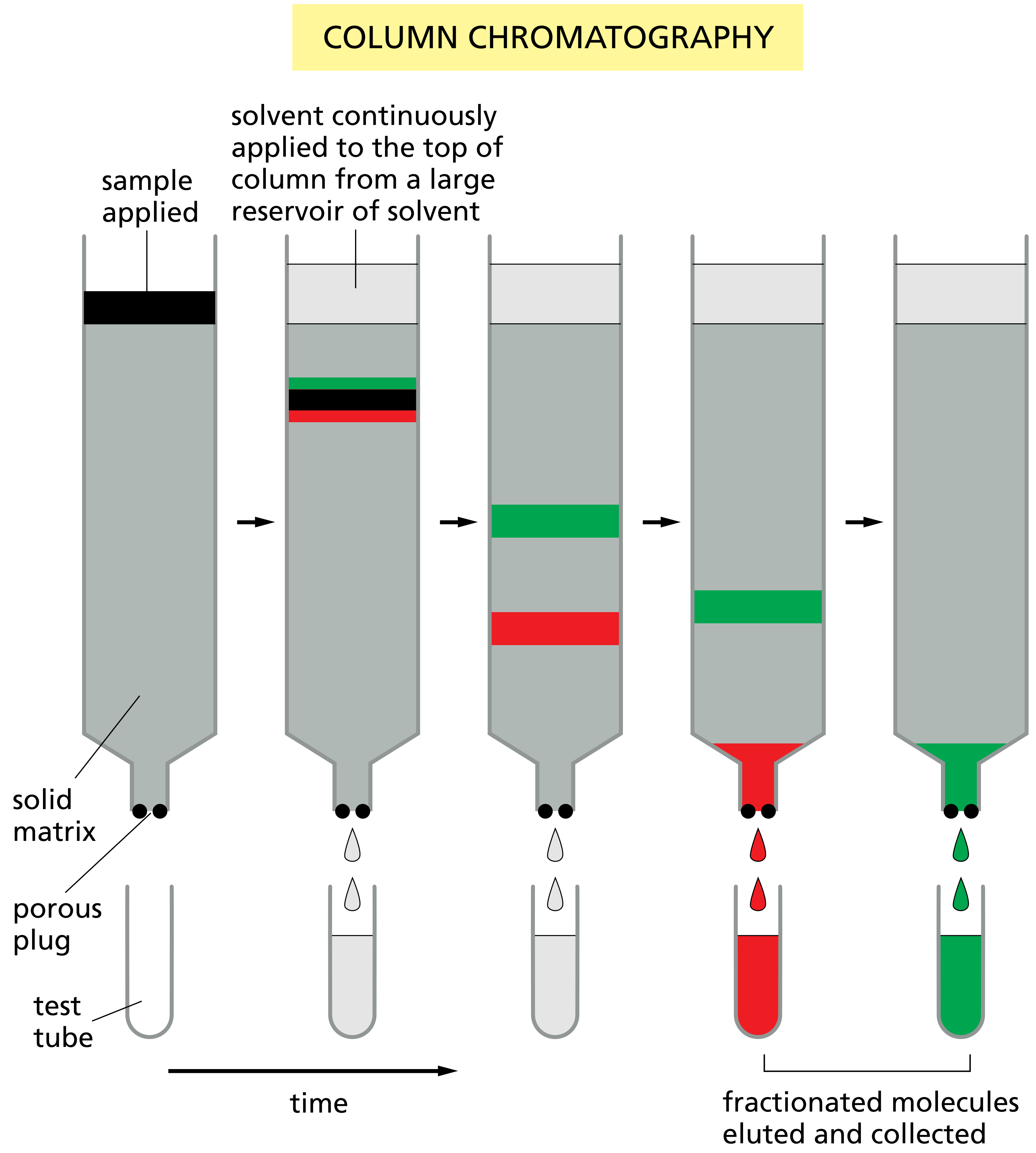
Protein isolation

Why do we need to purify proteins?

- To **separate** them from the other thousands of proteins in each cell
- To **study** them *in vitro* (perform biochemical assays)
- To get their **3D structure**

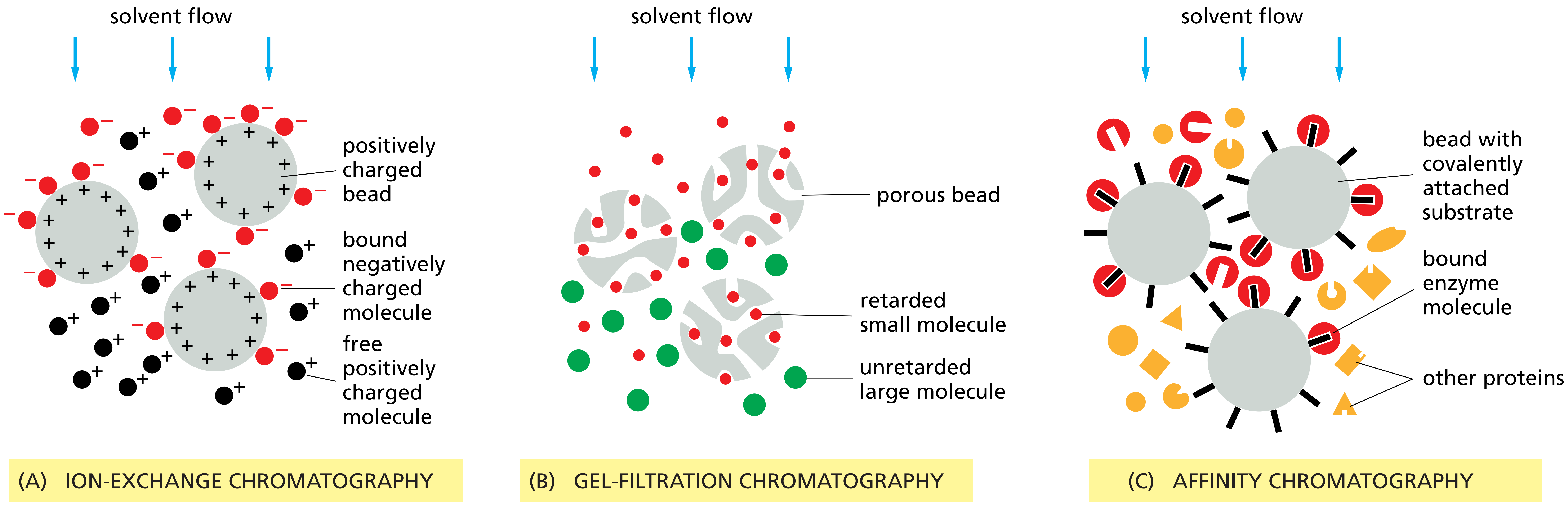
Chromatography - big volumes

- Using **column chromatography**
- A mixture of proteins is passed through a **porous gel matrix**
- Different proteins are **retarded to different extents** depending on their **interaction with the matrix**
- They are **collected separately as they come out** of the column



Chromatography - big volumes

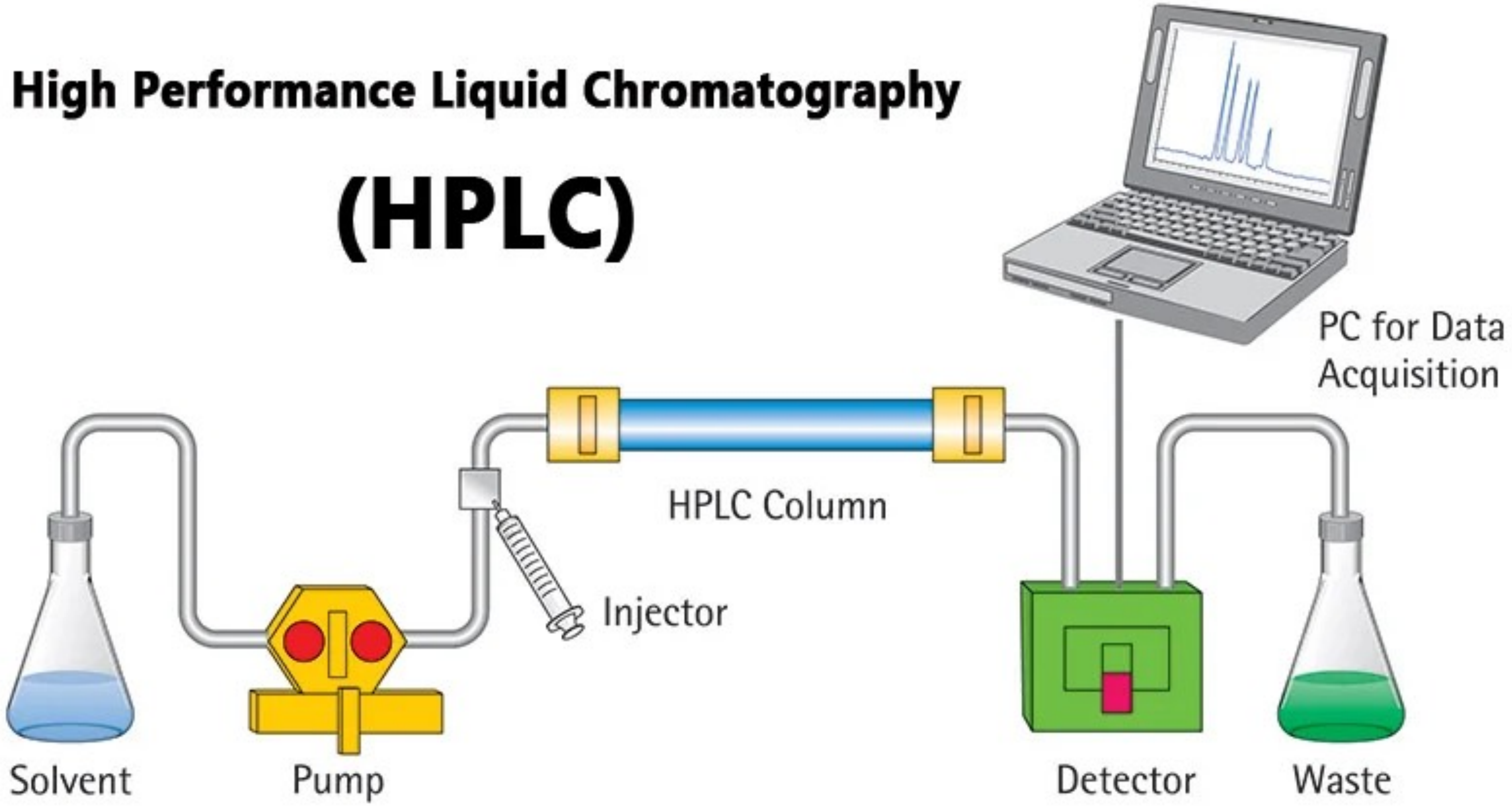
- Depending on the **matrix**, proteins can be separated according to
    - their **charge** (ion-exchange chromatography),
    - their **hydrophobicity** (hydrophobic chromatography)
    - their **molecular weight** (gel filtration = size-exclusion chromatography)
    - their **ability to bind small molecules** (affinity chromatography)



HPLC - increased resolution (=separation)

- The **resolution** of classical chromatography columns is **limited**

- Special resins (silica-based) composed of tiny spheres (3-10 um) can be packed to form a uniform column bed to attain a high degree of resolution = **high-performance liquid chromatography (HPLC)**

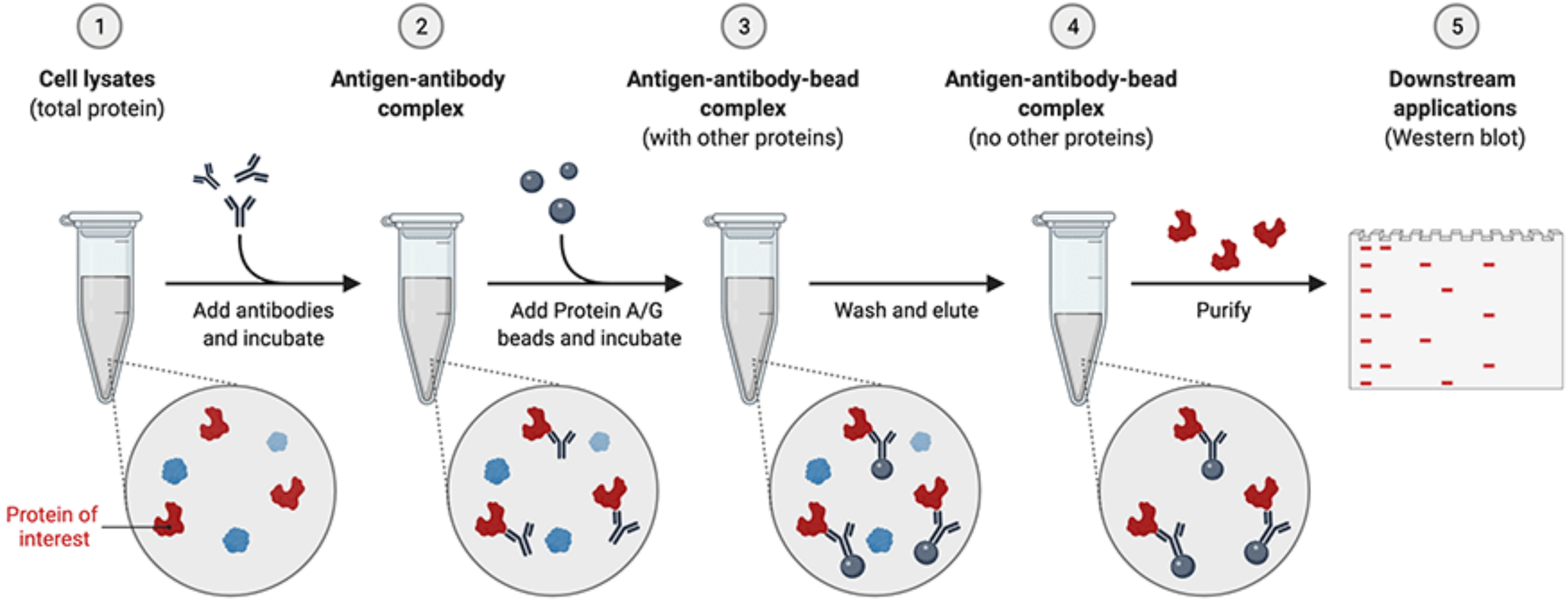


https://noguchi.ug.edu.gh/services/hplc-analysis/

https://microbenotes.com/high-performance-liquid-chromatography-hplc/

IP - small volumes/yield

- **Immunoprecipitation (IP)** as a rapid affinity purification

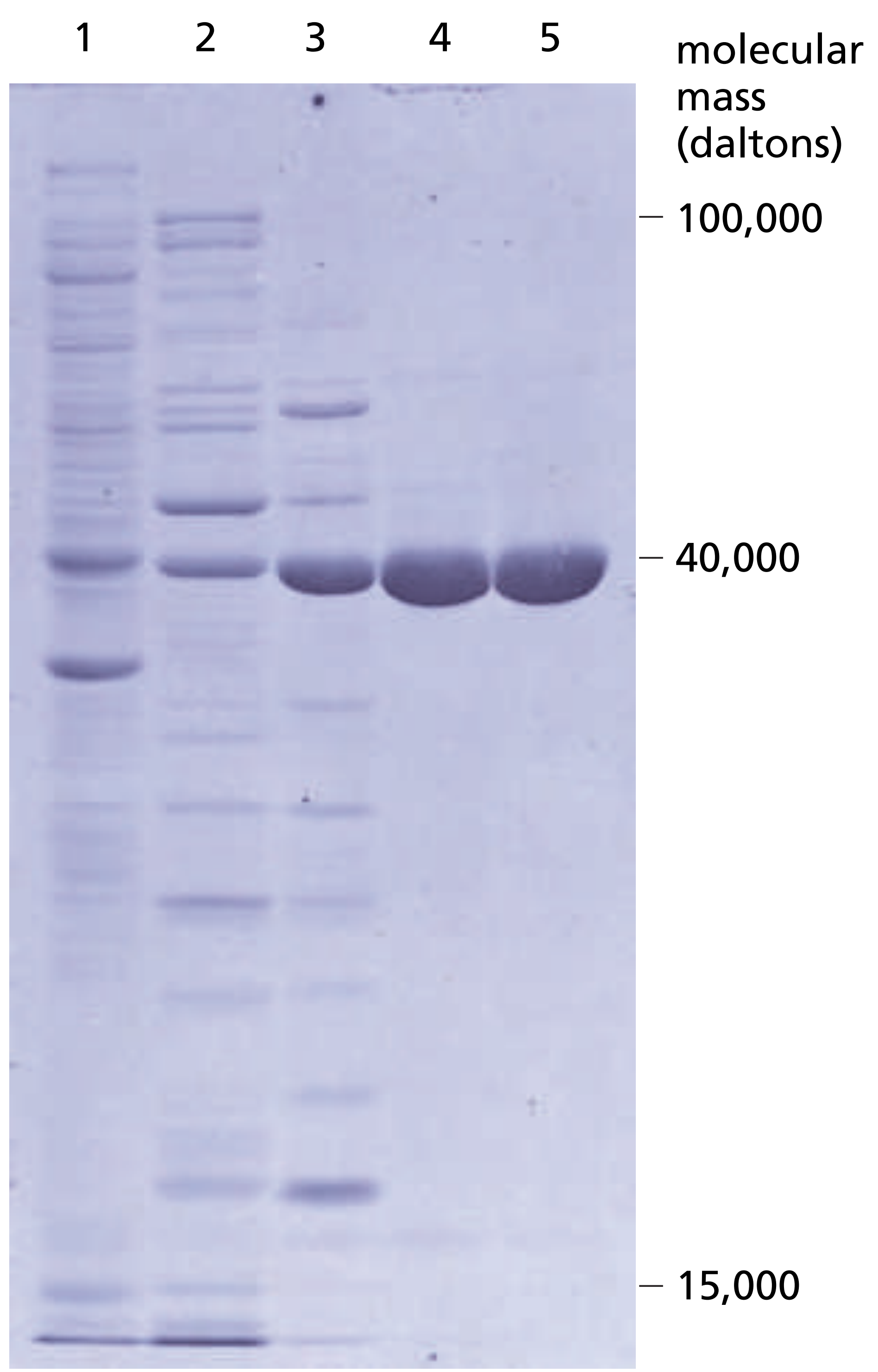


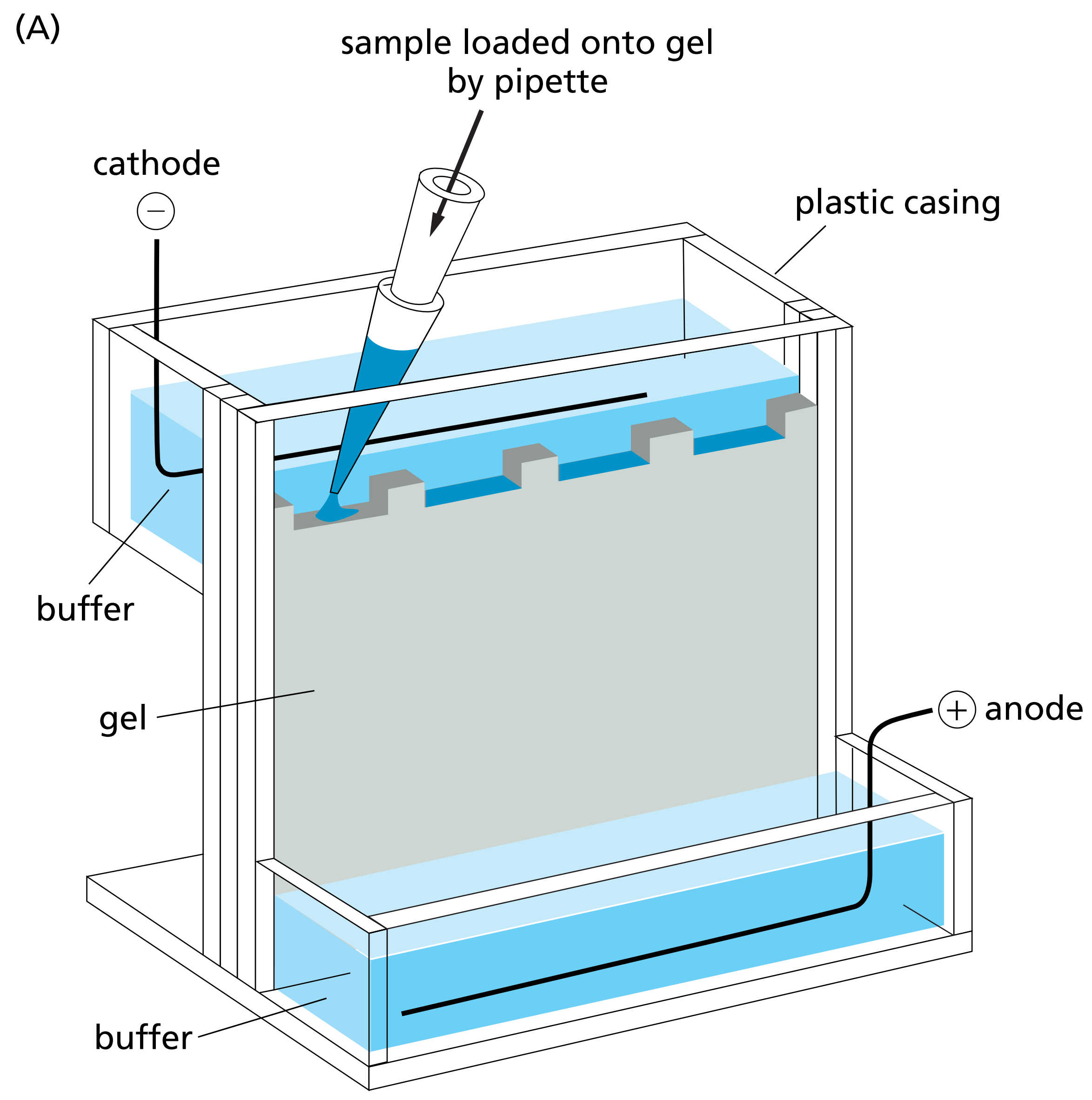
https://www.rockland.com/resources/immunoprecipitation-technique/

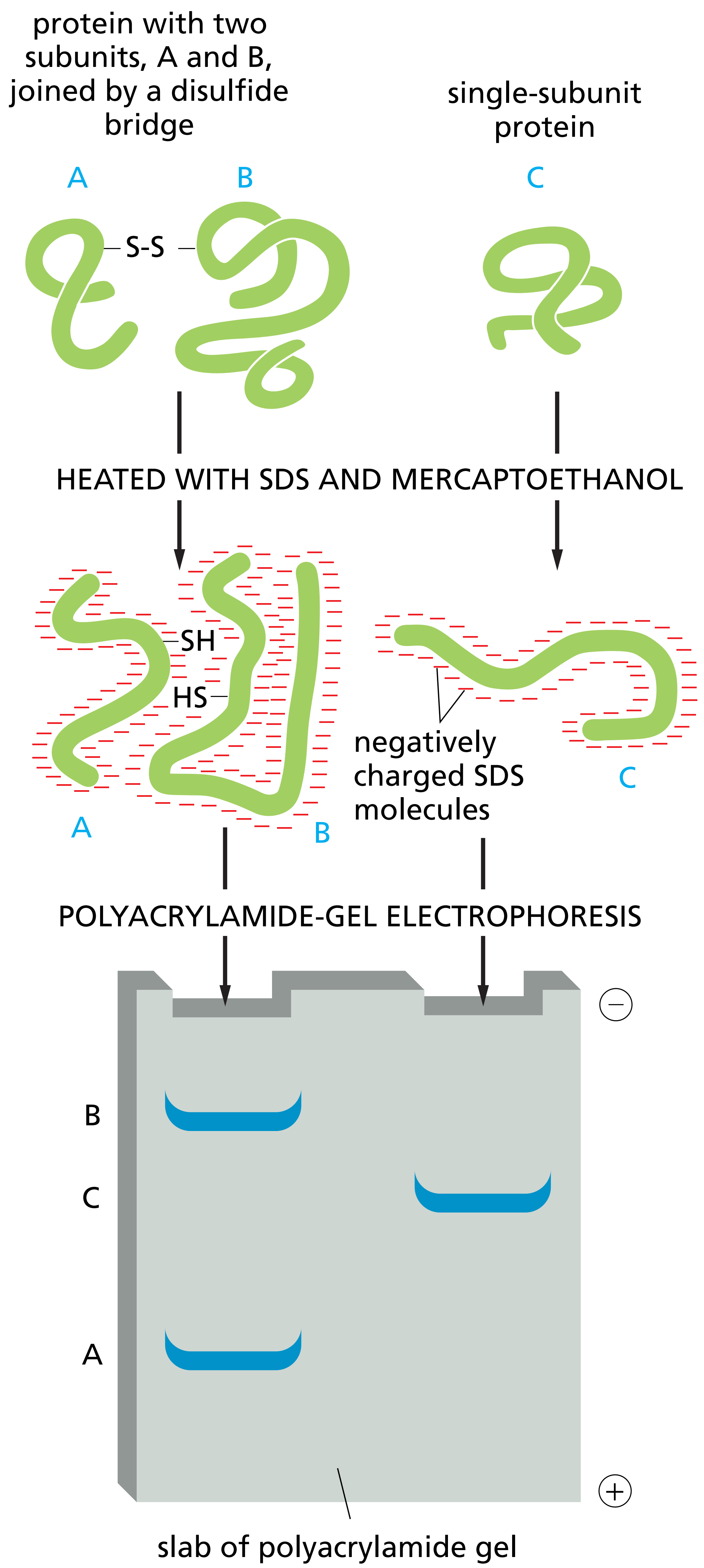
SDS-PAGE - visualization

- **Larger proteins are retarded** more than small ones in the acrylamide mesh

- Proteins can be stained (for example using **Coomassie blue)**



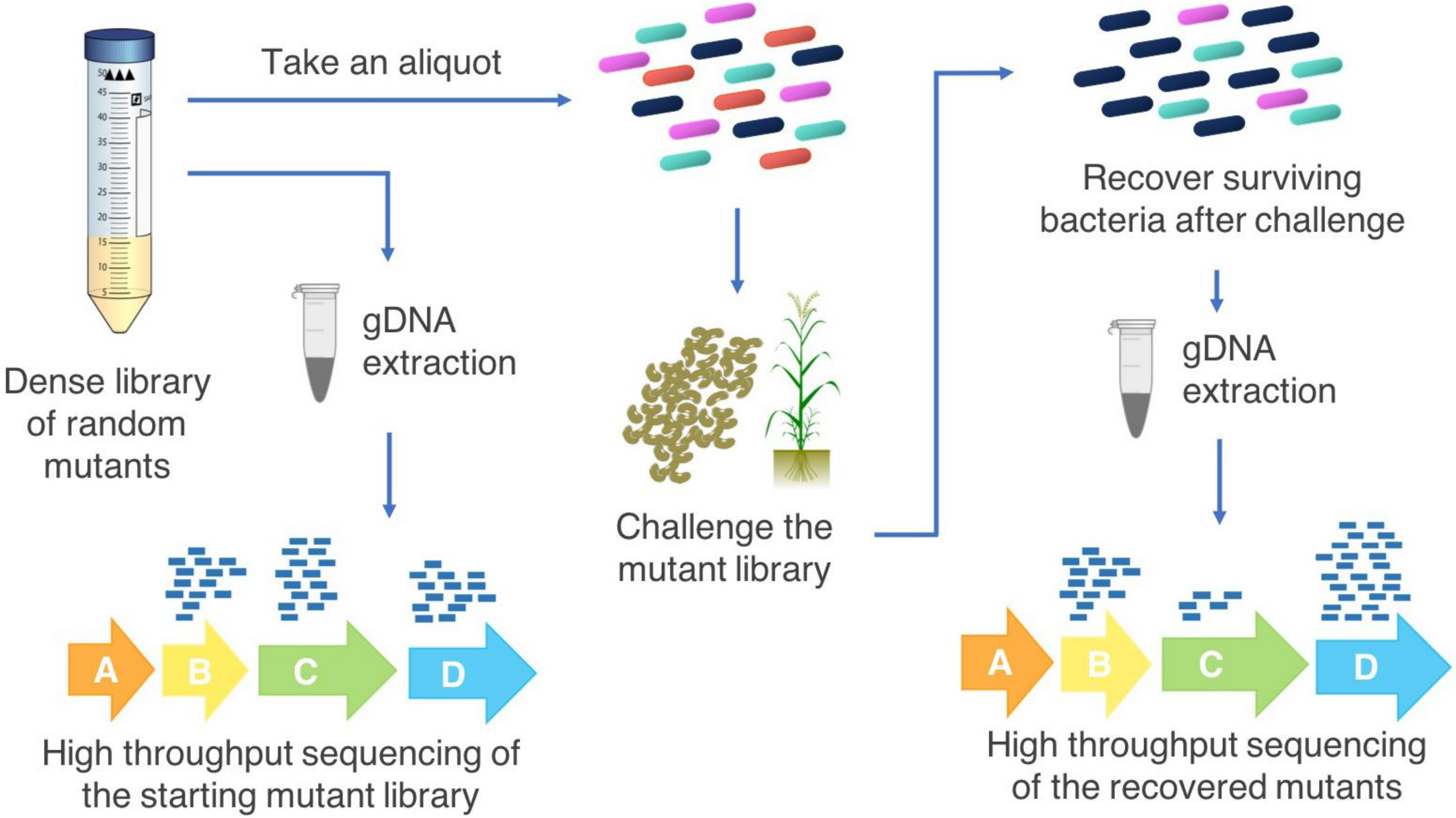




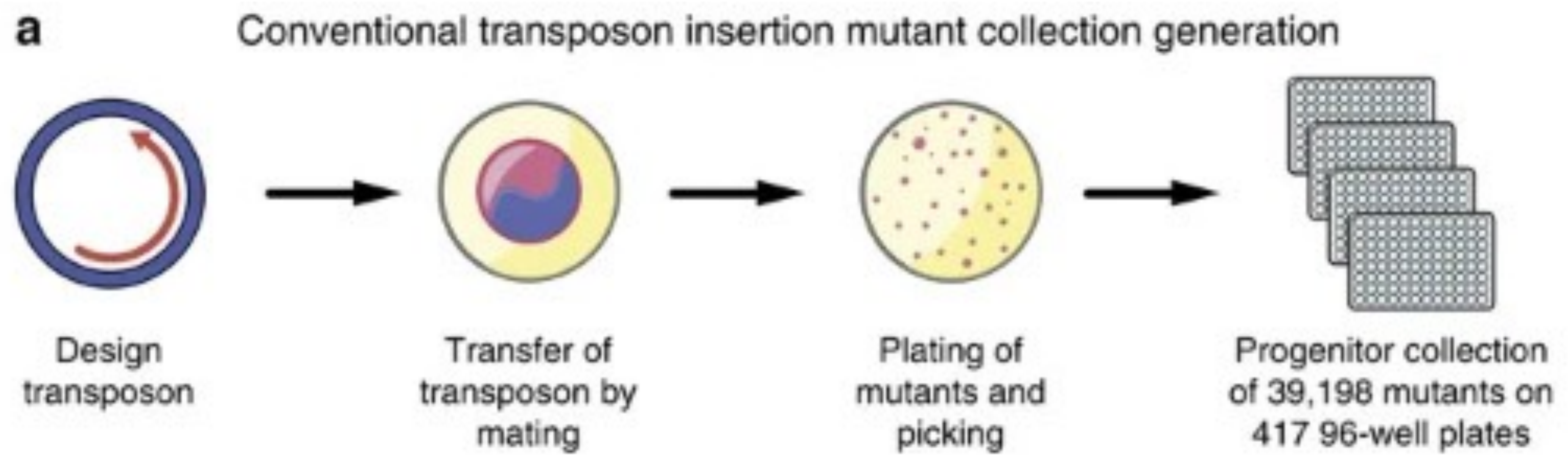
Studying gene expression = RNAs

- Observing - RNA FISH
- Detecting a precise RNA - Northern Blotting or RT-PCR
- Quantifying a precise RNA - qRT-PCR
- Global idea on RNA presence - microarrays, RNAseq

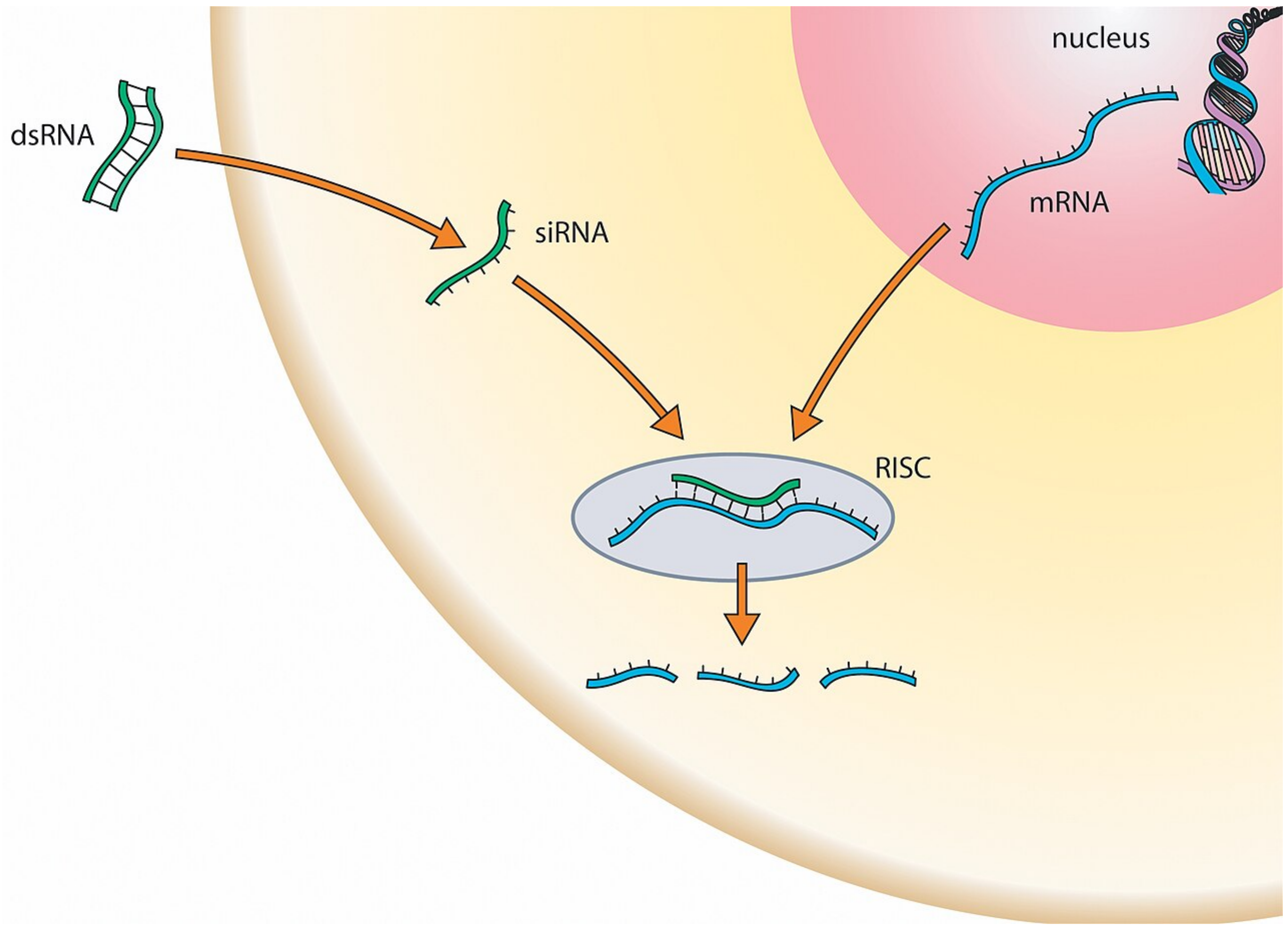
Mutants libraries - pooled library



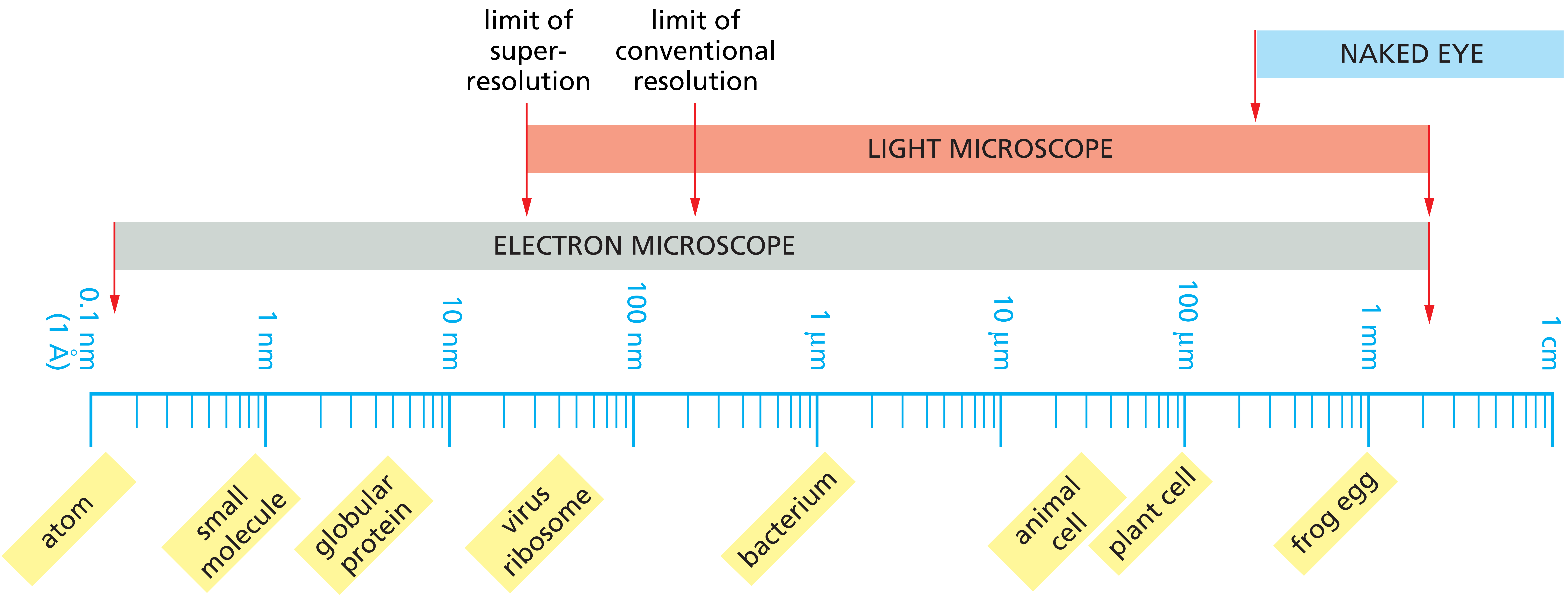
Mutants libraries - arrayed library



RNA interference

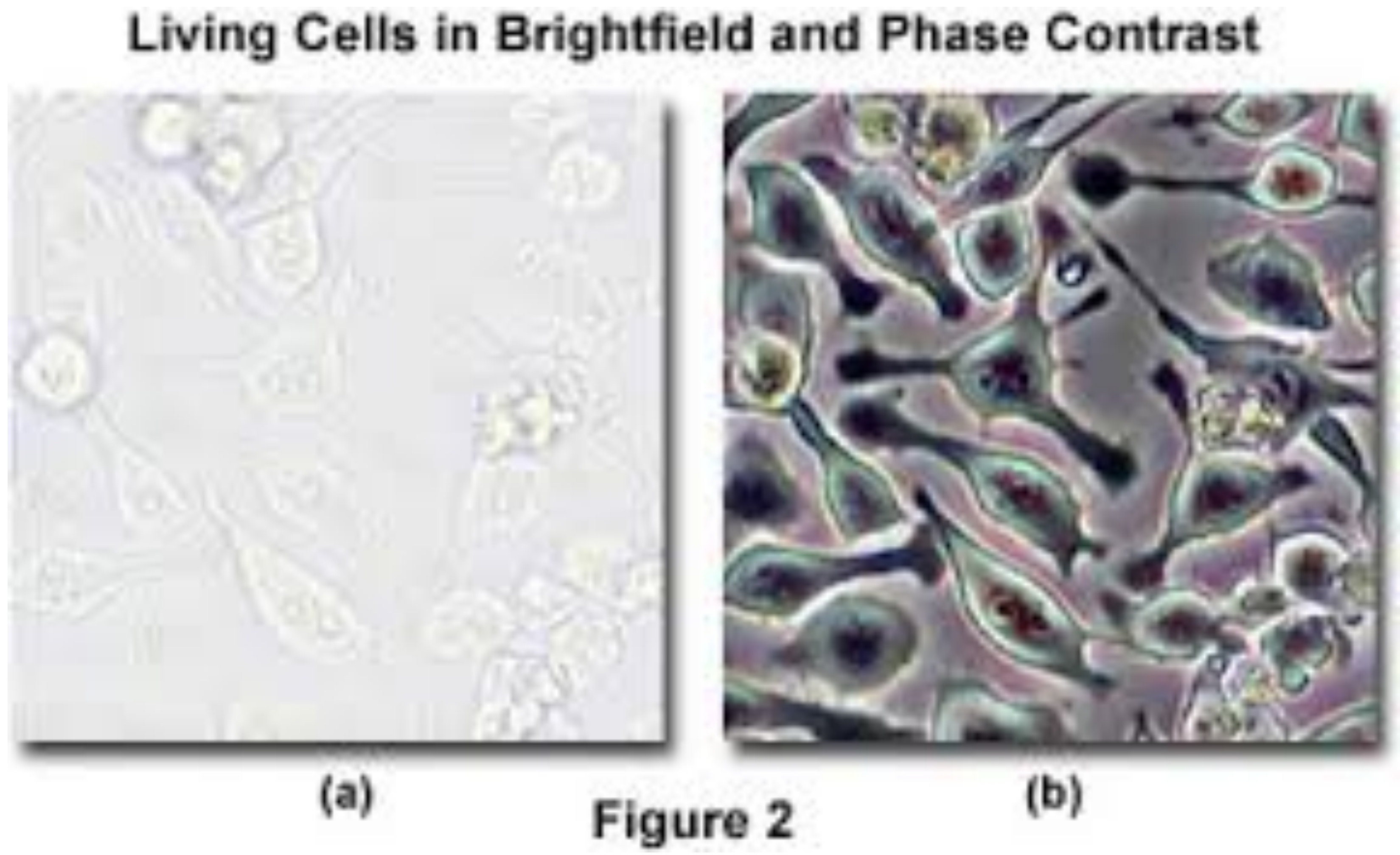


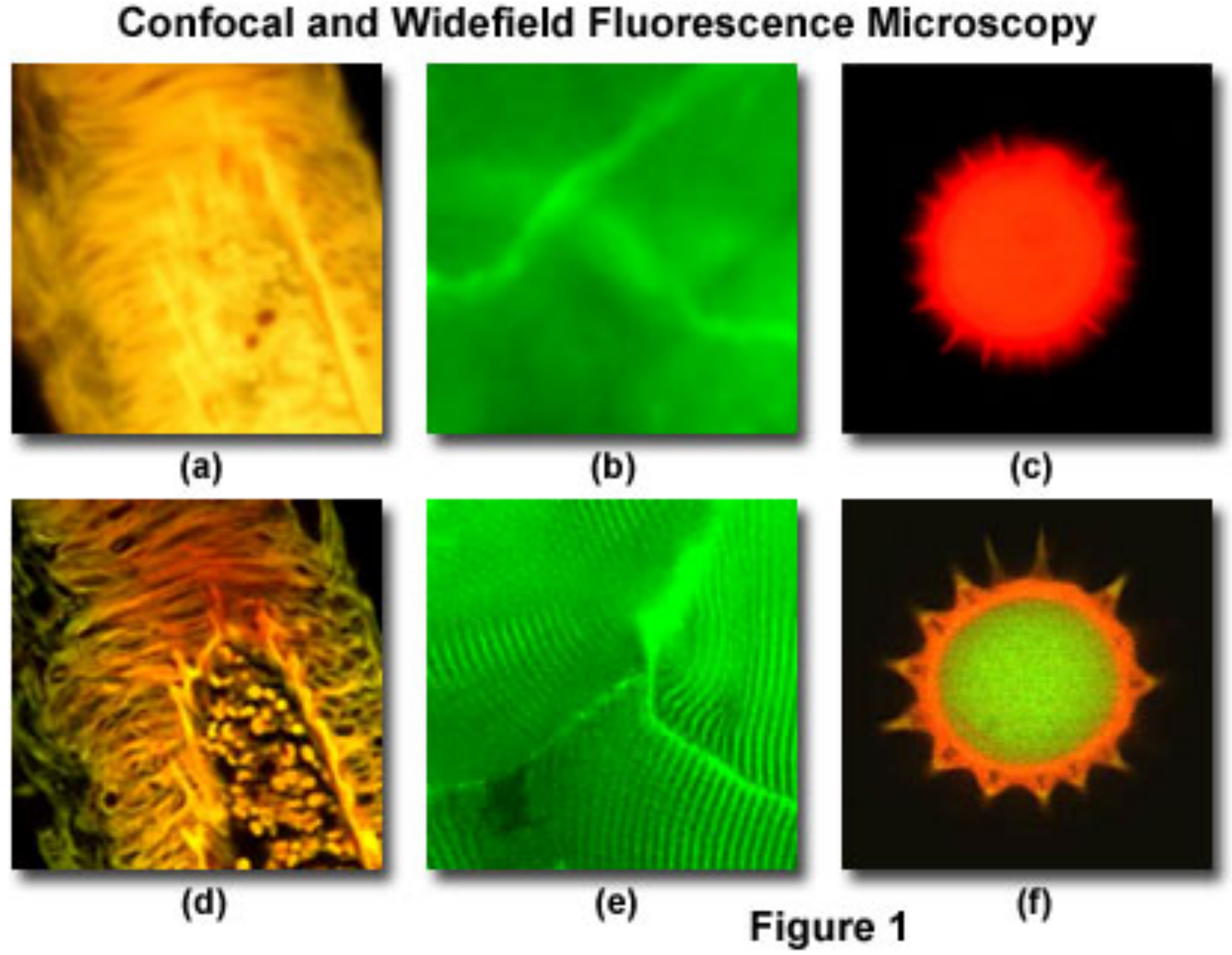
Microscopy


limit of super-resolution
limit of conventional resolution
NAKED EYE
LIGHT MICROSCOPE
ELECTRON MICROSCOPE
0.1 nm (1 Å)
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
1 cm
atom
small molecule
globular protein
virus ribosome
bacterium
animal cell
plant cell
frog egg

Microscopy

- Light Microscope
  - Classical “brightfield”
  - Phase contrast
  - Staining/fluorescence
  - Confocal microscope
  - Super-resolution





Microscopy

- Light Microscope
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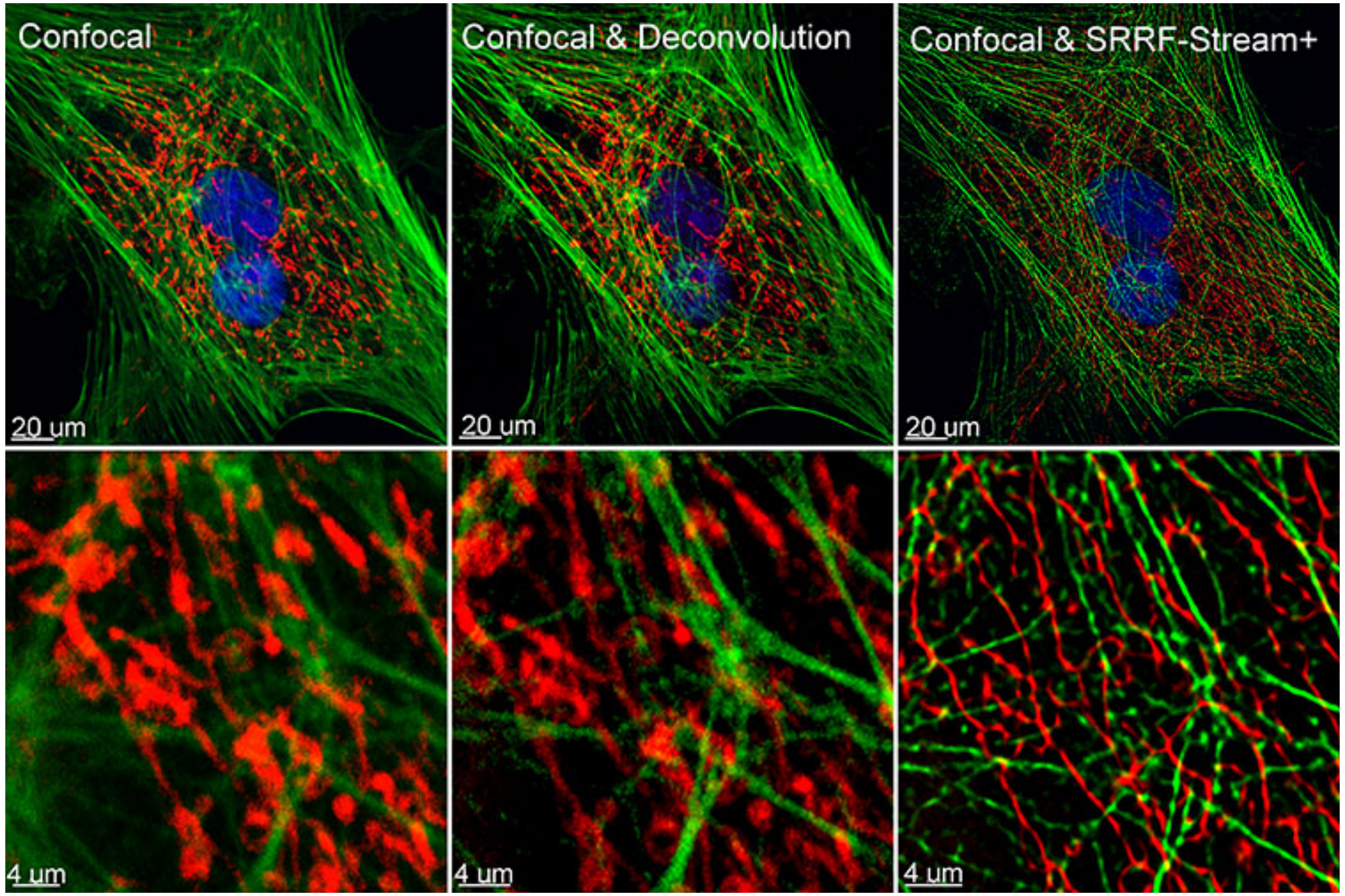
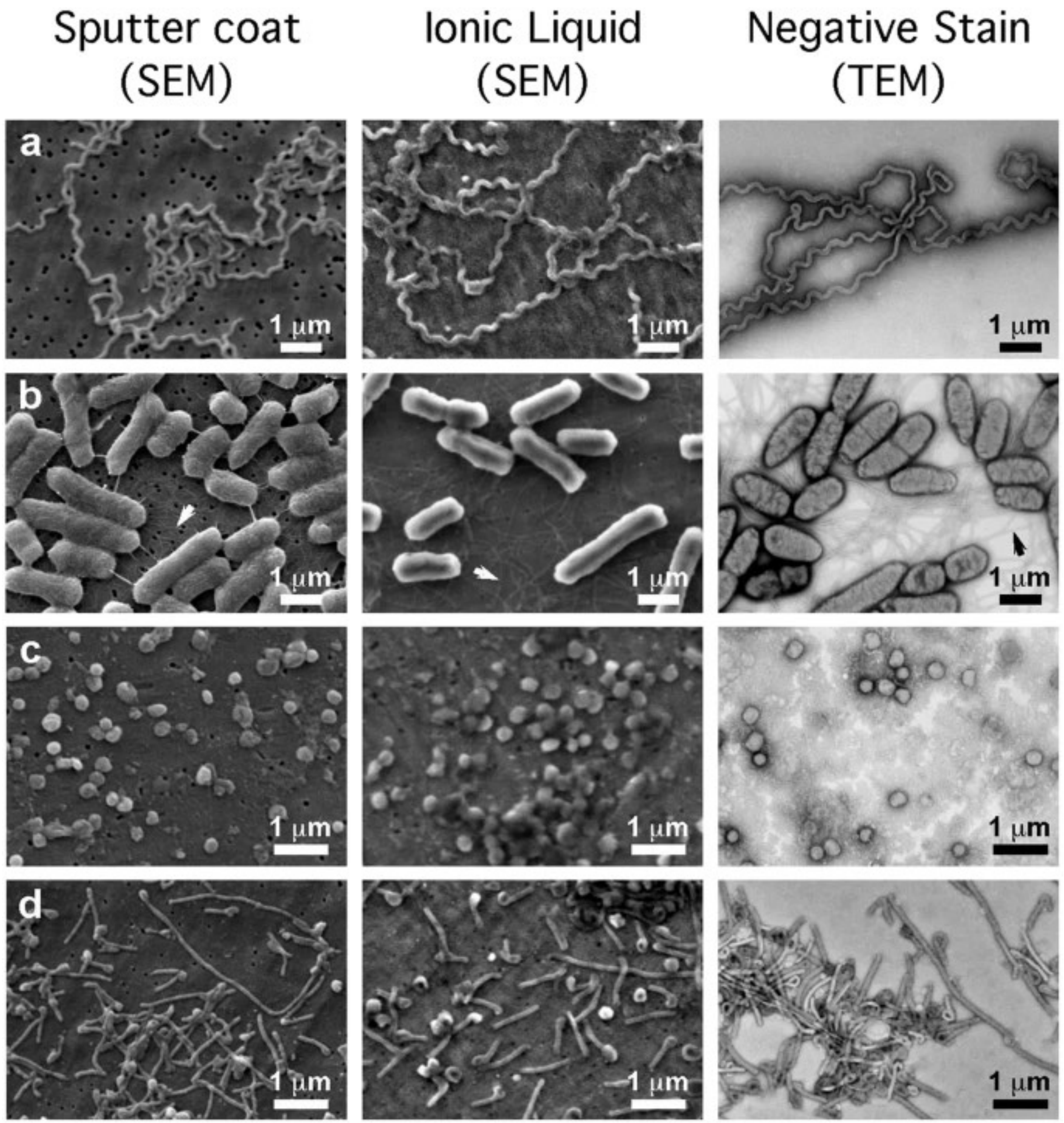
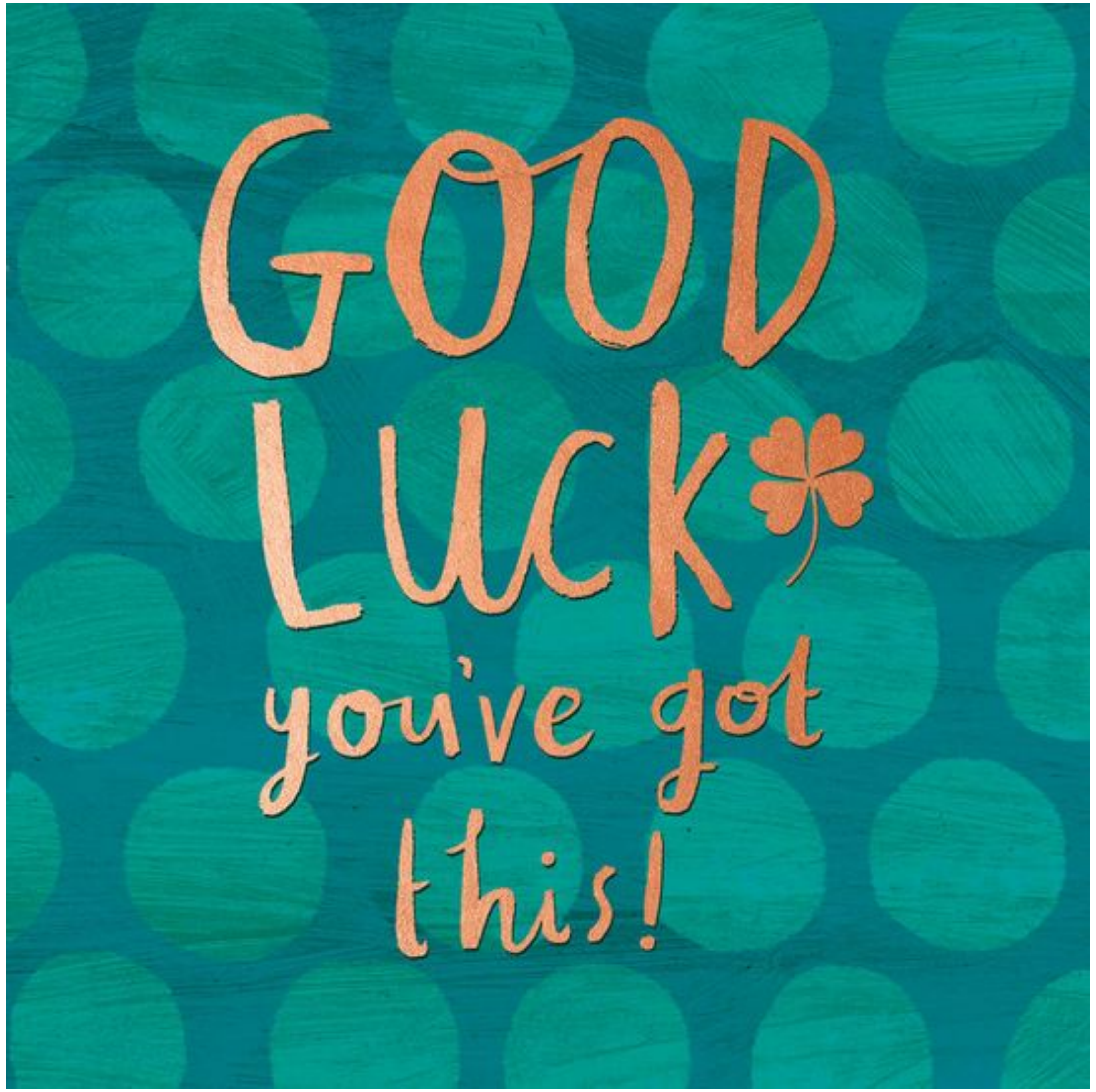


Microscopy

- Electron Microscope





Have a nice holiday/exam prep !