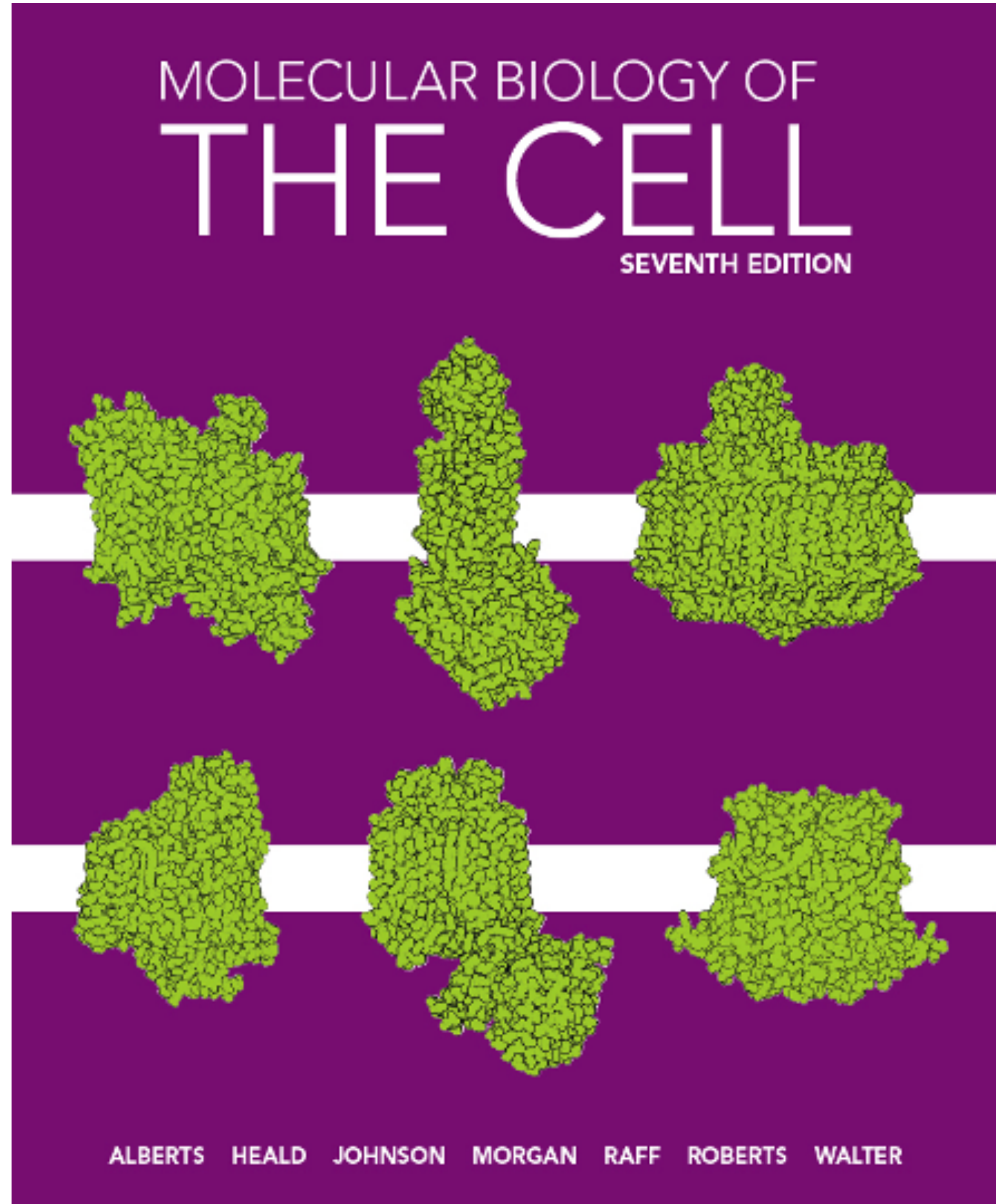


# **Cellular and Molecular Biology I**

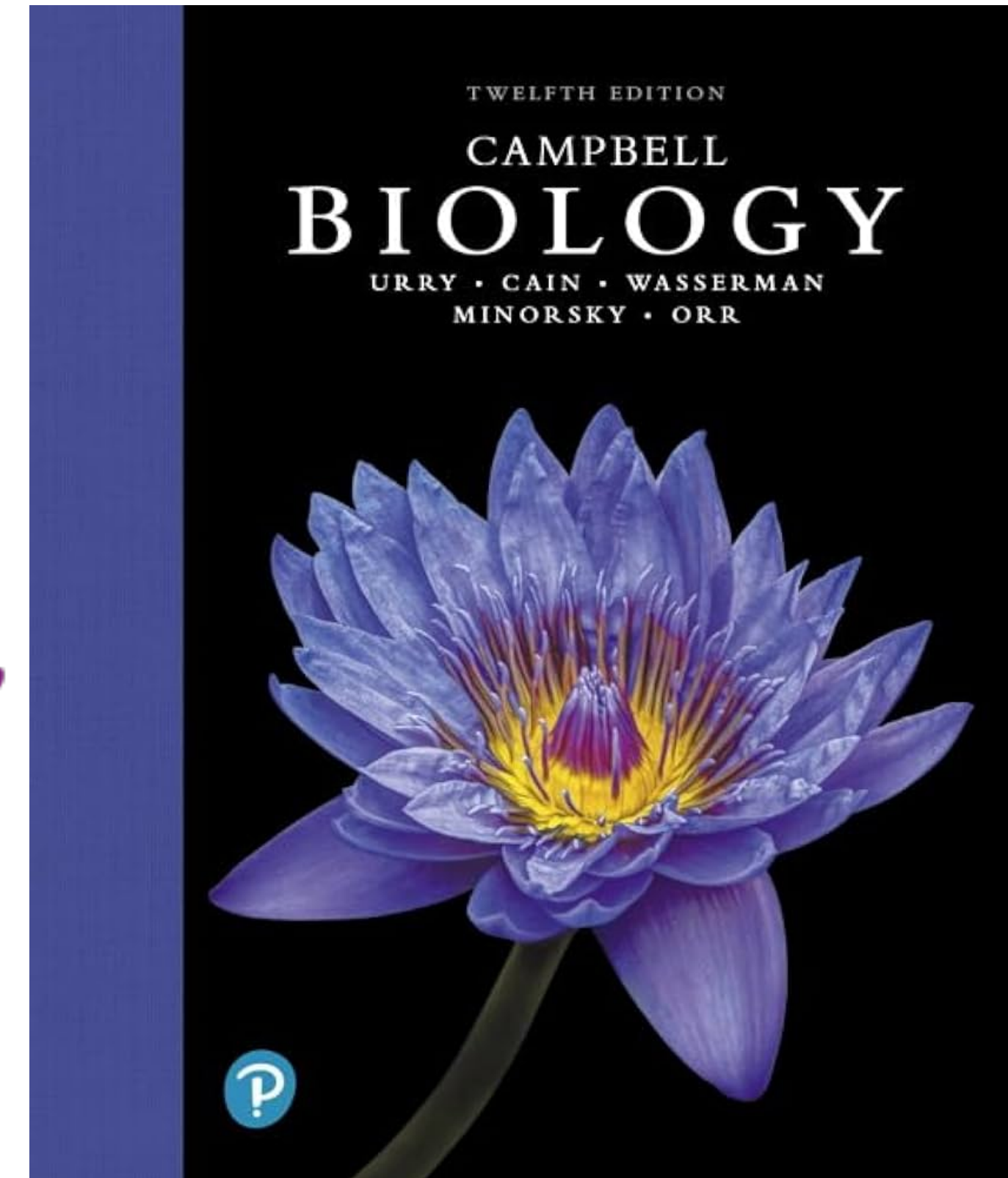
**BIO-205-10**

**Camille Goemans - 2024**



## Chapter 8

### Analyzing Cells, Molecules, and Systems



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# Summary

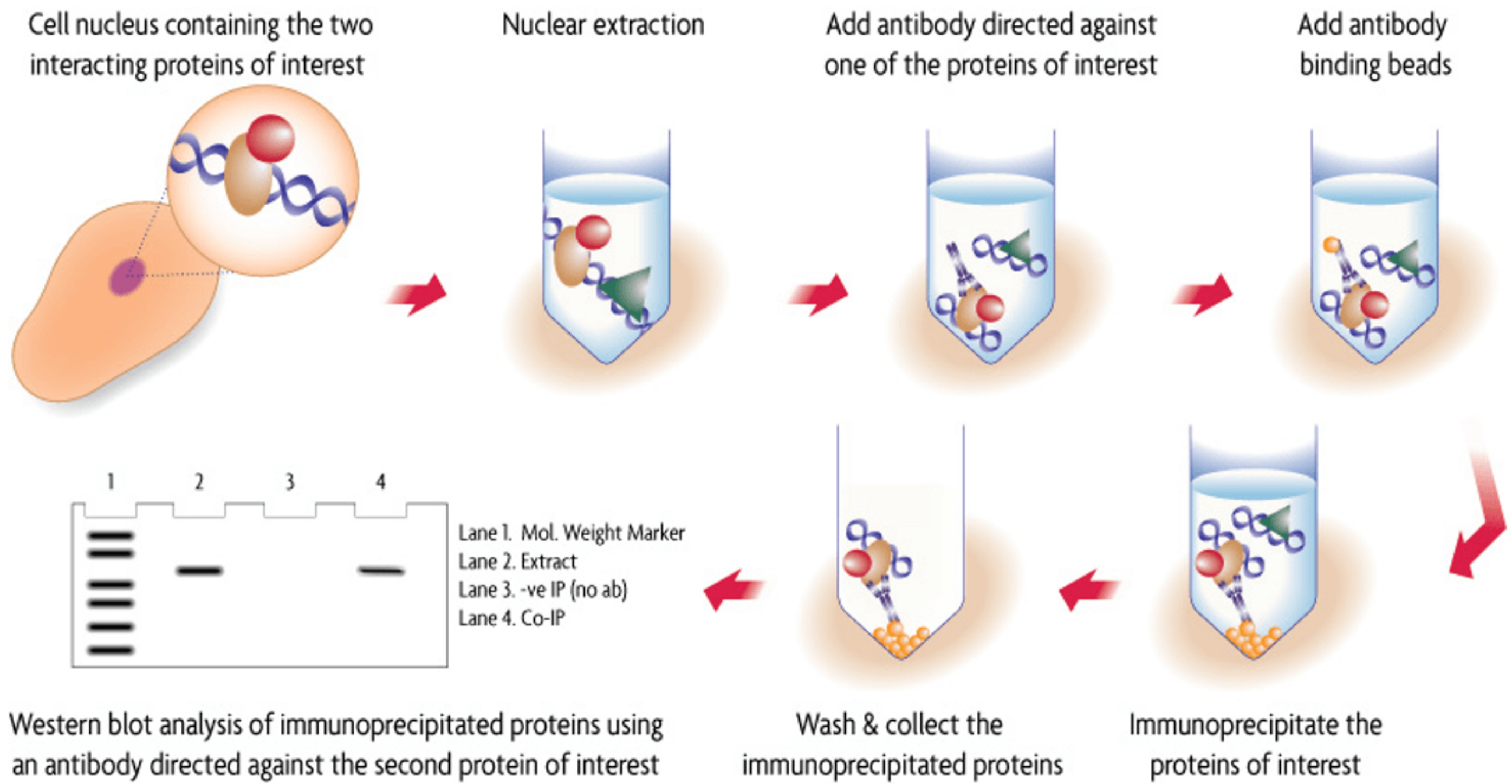
- Model organisms
- Isolating cells and growing them in culture
- Studying proteins
  - Protein sequence
  - Protein purification
  - Protein structure
  - Protein visualization
  - Mass spectrometry

# Plan

- Studying proteins
  - **Protein interactions**
  - Real-life example
- Studying DNA
  - DNA sequencing
  - DNA extraction
  - DNA amplification

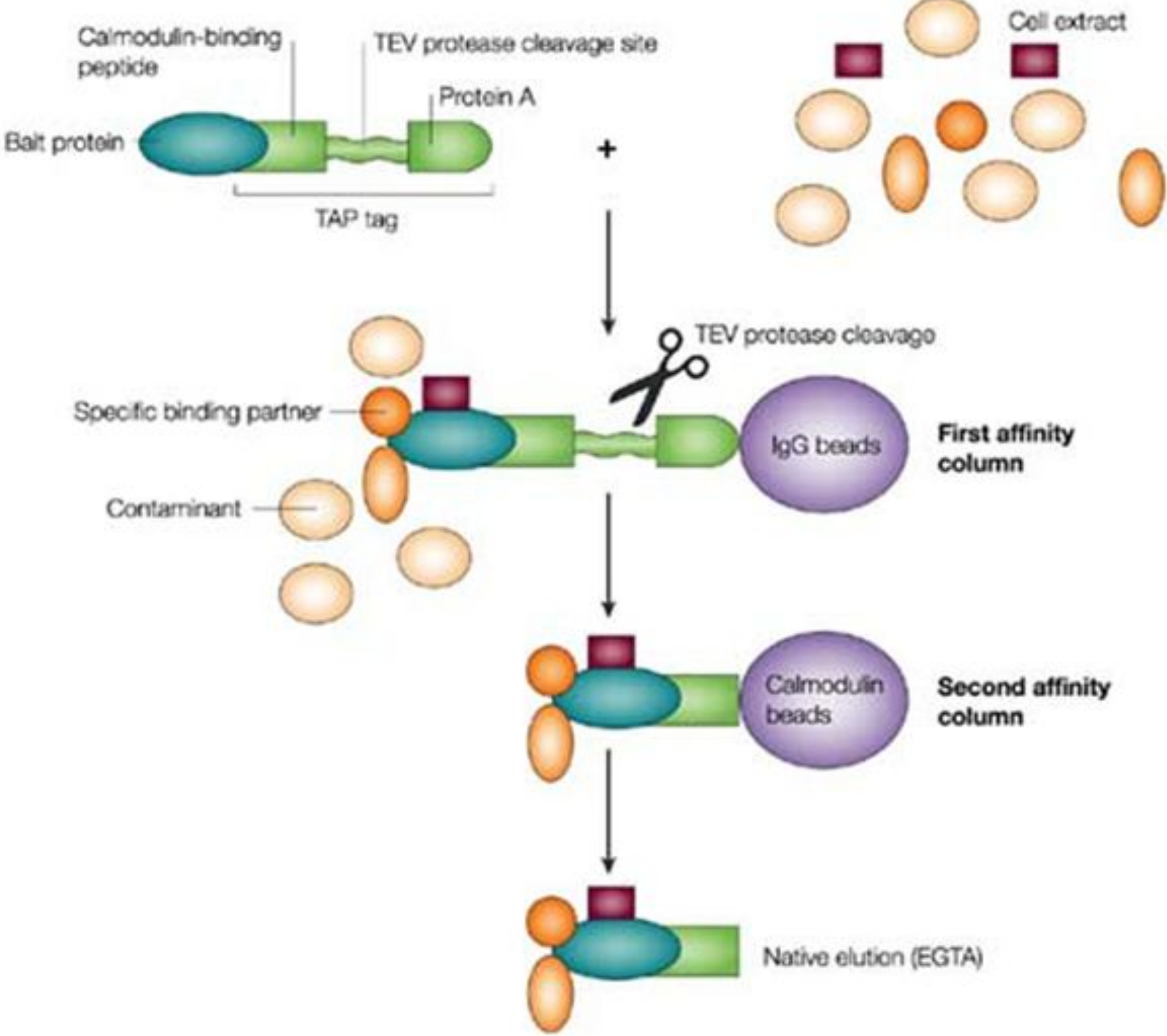
# Identifying interacting proteins

- **co-immunoprecipitation** (or co-IP)



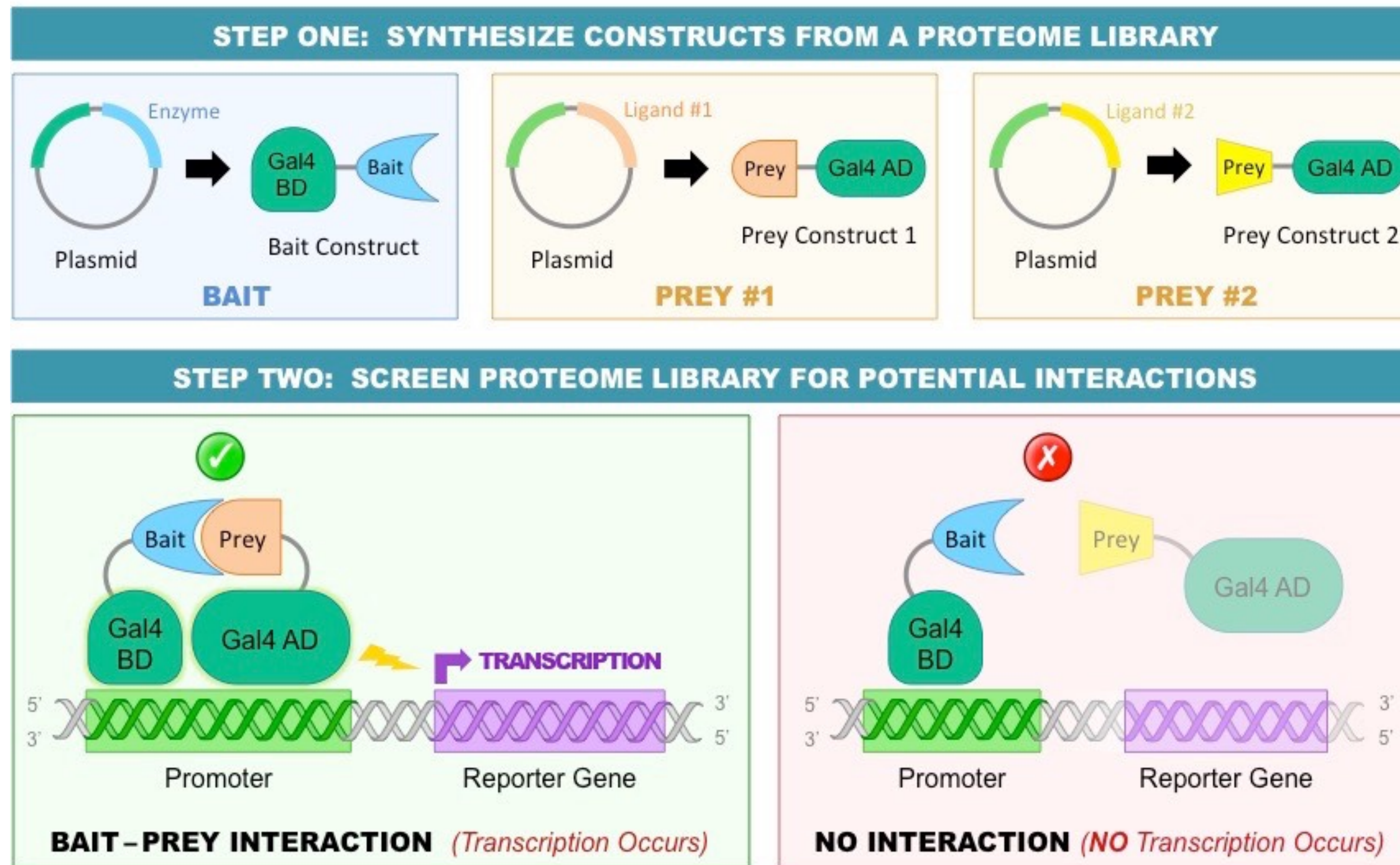
# Identifying interacting proteins

- tandem affinity purification (TAP-TAG)



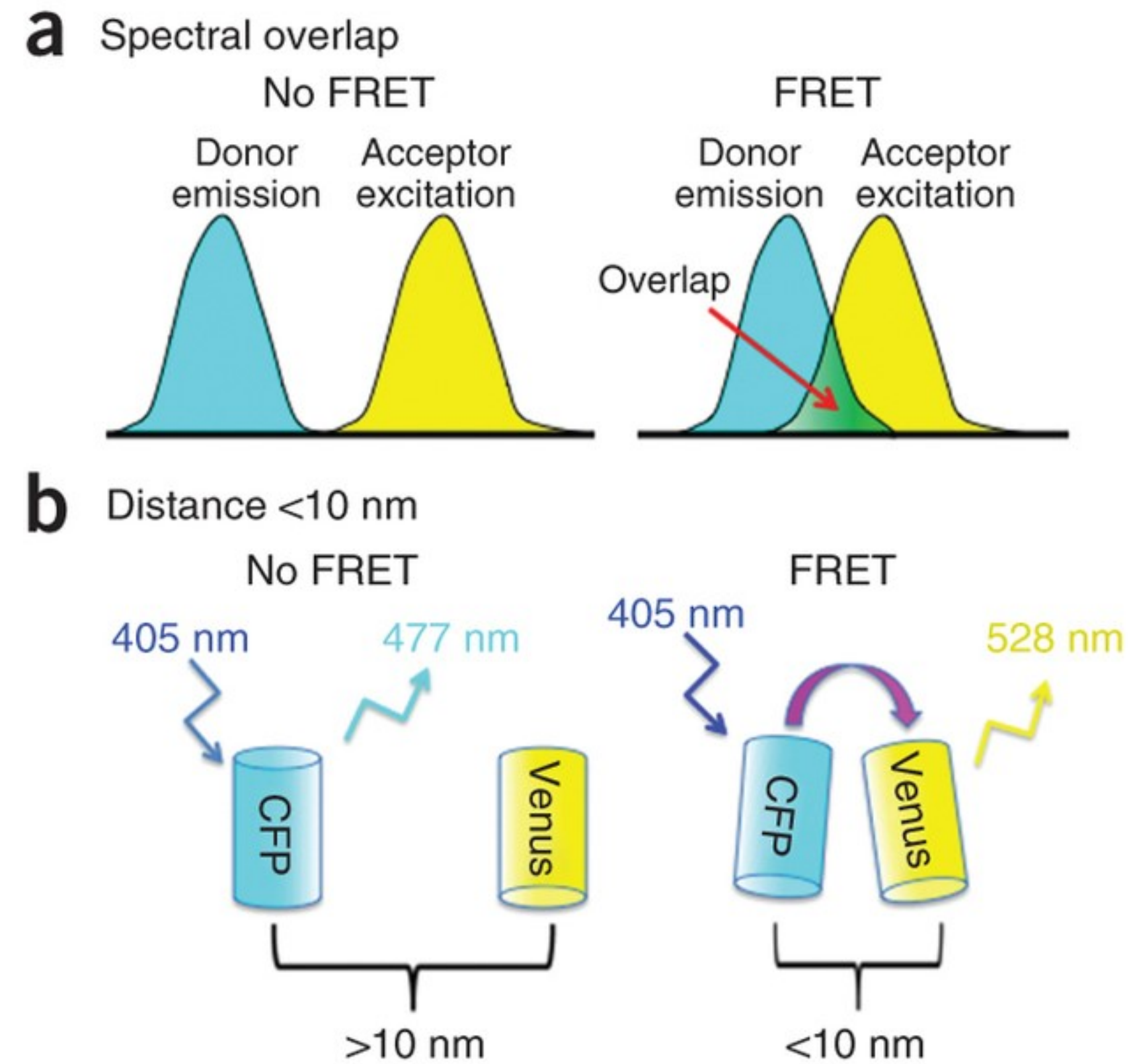
# Identifying interacting proteins

- Yeast/Bacterial two-hybrid



# Identifying interacting proteins

- FRET = fluorescence resonance energy transfer

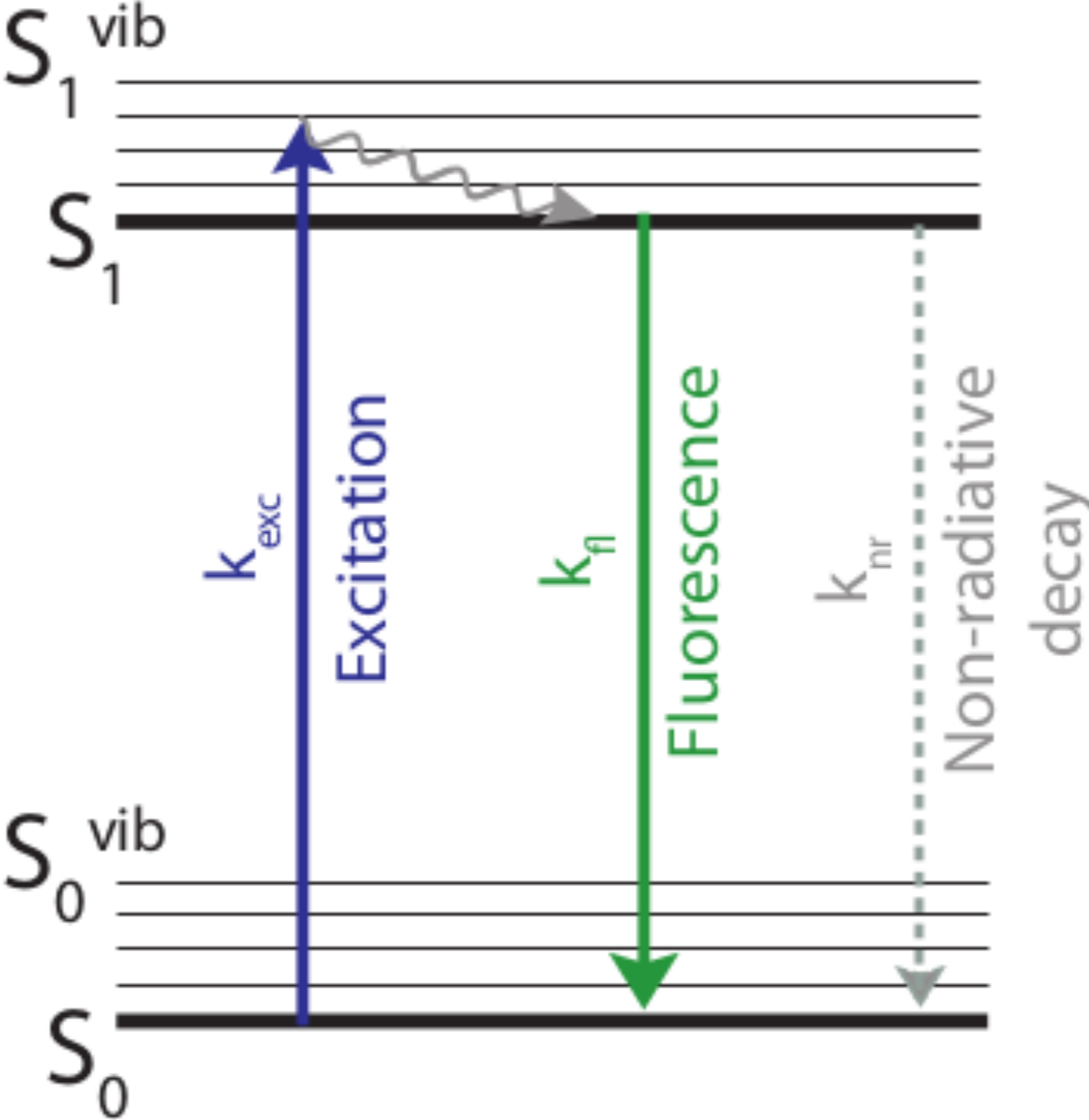




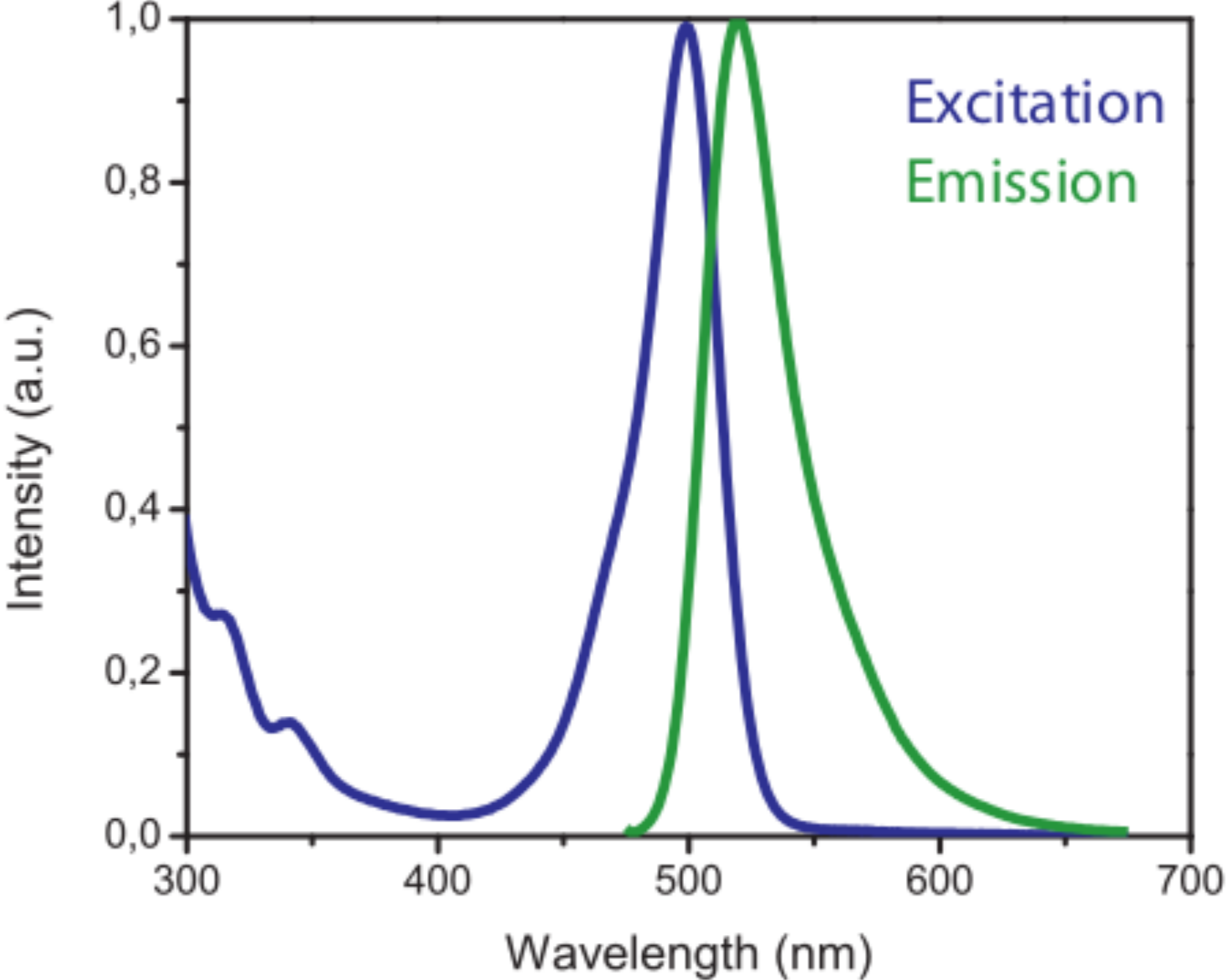
# Fluorescence, phosphorescence, luminescence

- Fluorescence and phosphorescence are both **photoluminescence** (i.e. glow is triggered by light) whereas in chemiluminescence, glow is triggered by a **chemical reaction**
- Fluorescence and phosphorescence both **absorb light and emit light of a longer wavelength (and lower energy)**
- Fluorescence is **immediate**
- In phosphorescence, absorbed light can be **stored and emitted later on**

# Fluorescence



(a)



(b)

# Plan

- Studying proteins
  - Protein interactions
  - **Real-life example**
- Studying DNA
  - DNA sequencing
  - DNA extraction
  - DNA amplification

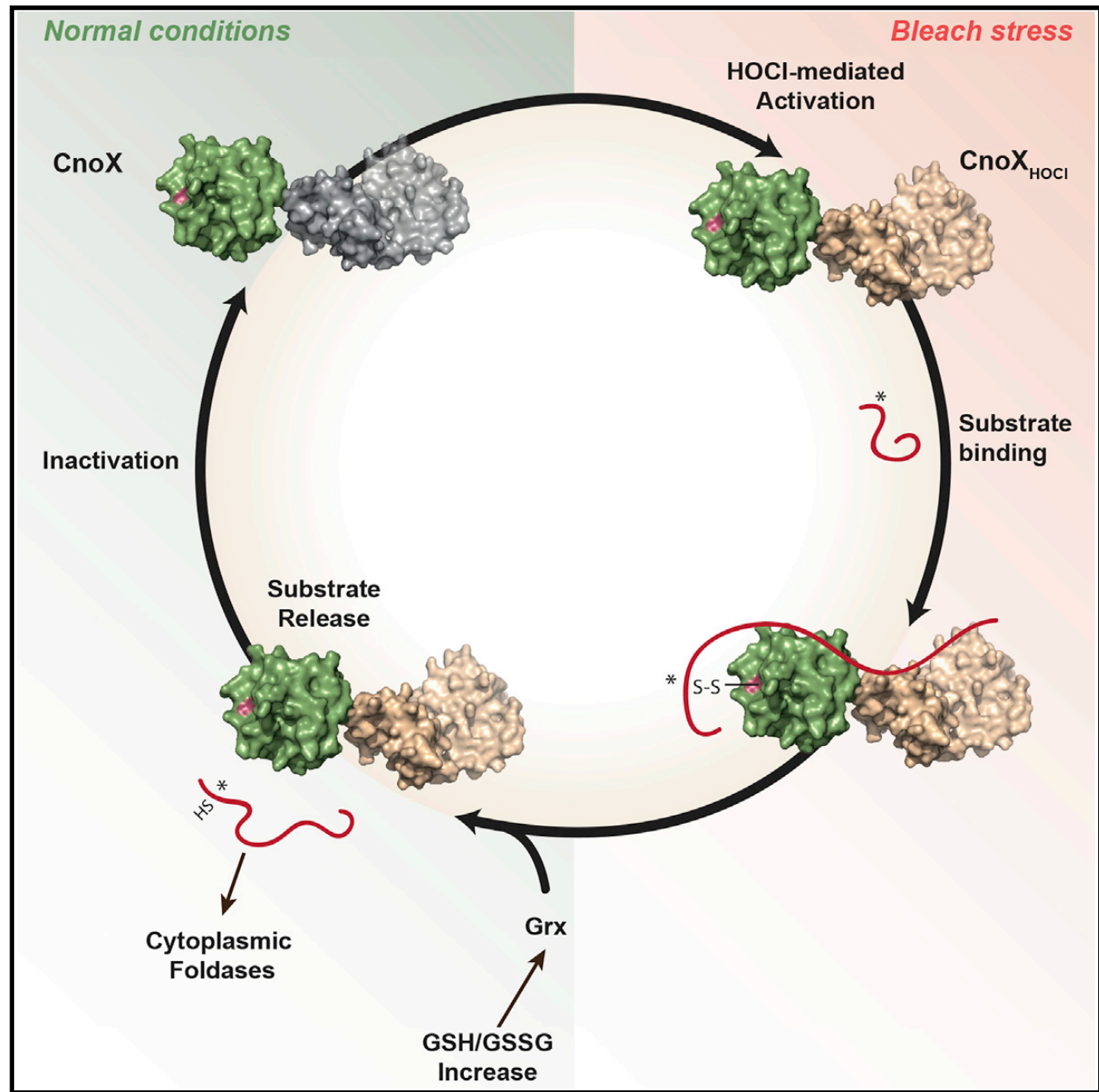
# Real-life example

## Molecular Cell

Article

### CnoX Is a Chaperedoxin: A Holdase that Protects Its Substrates from Irreversible Oxidation

Graphical Abstract



Authors

Camille V. Goemans, Didier Vertommen, Rym Agrebi, Jean-François Collet

Correspondence

jfcollet@uclouvain.be

In Brief

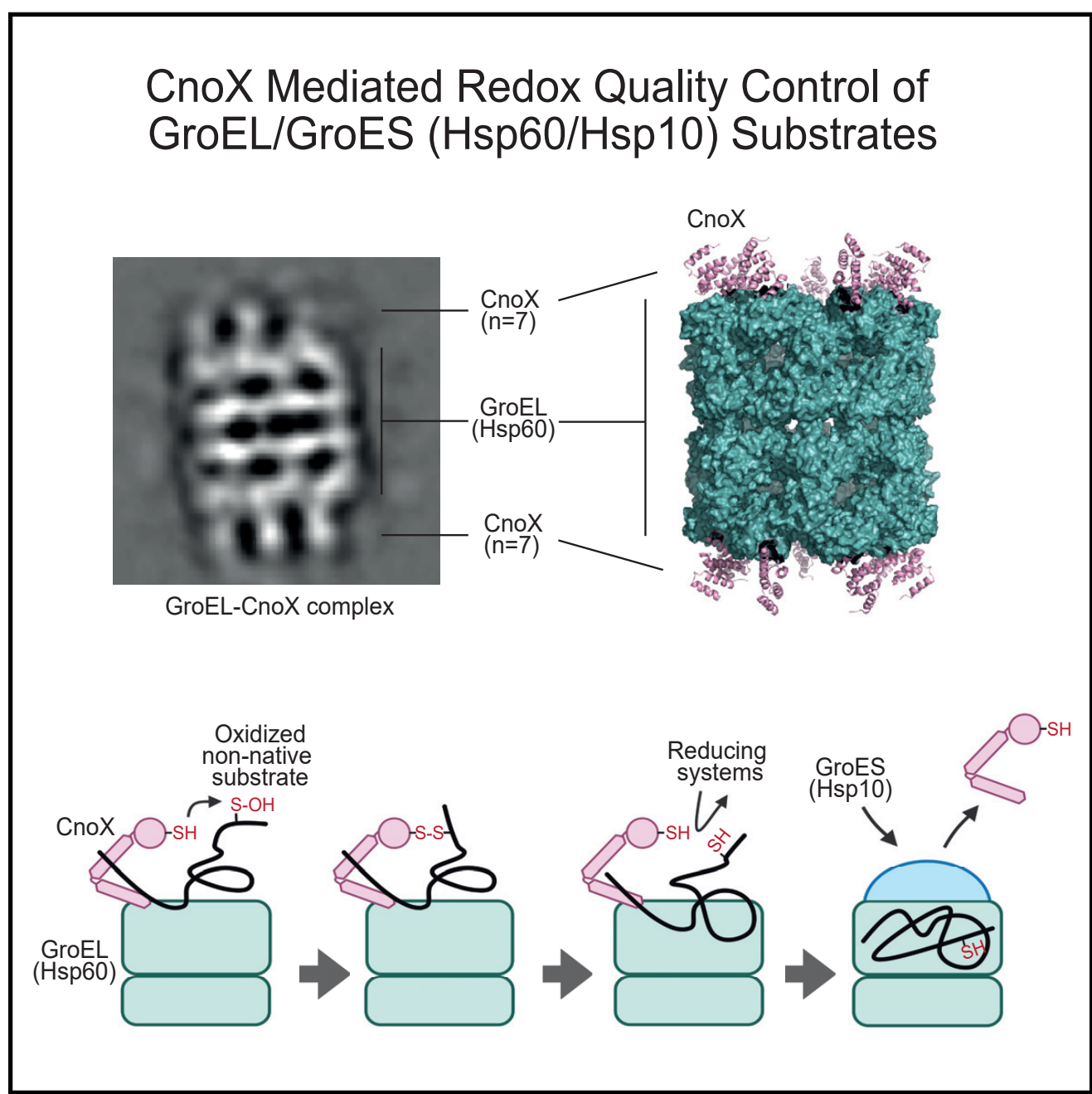
Bleach is a powerful oxidant that kills bacteria by causing protein aggregation. Goemans et al. identified *Escherichia coli* CnoX (YbbN) as a bleach-activated chaperone that uniquely combines holdase activity with the ability to protect its substrates from irreversible oxidation. After bleach stress, CnoX transfers its client proteins to GroEL/ES and DnaK/J/GrpE.

## Cell

Article

### A molecular device for the redox quality control of GroEL/ES substrates

Graphical abstract



Authors

Emile Dupuy, Sander Egbert Van der Verren, Jiusheng Lin, ..., Camille Véronique Goemans, Han Remaut, Jean-François Collet

Correspondence

camille.goemans@embl.de (C.V.G.), han.remaut@vub.be (H.R.), jfcollet@uclouvain.be (J.-F.C.)

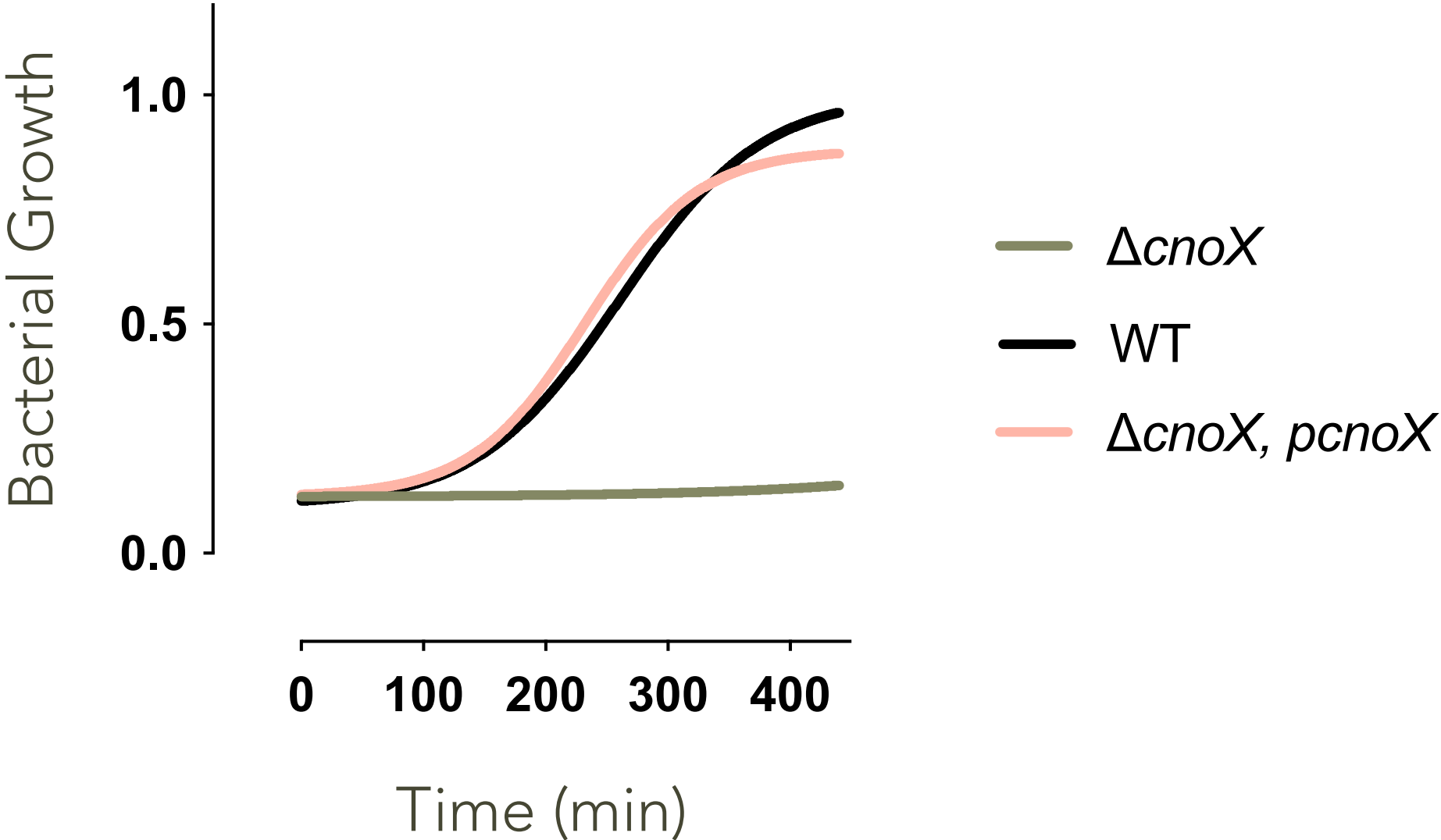
In brief

CnoX is a redox quality-control molecular plugin for an evolutionarily conserved Hsp60 chaperonin complex crucial for protein folding in all living cells.

# Real-life example



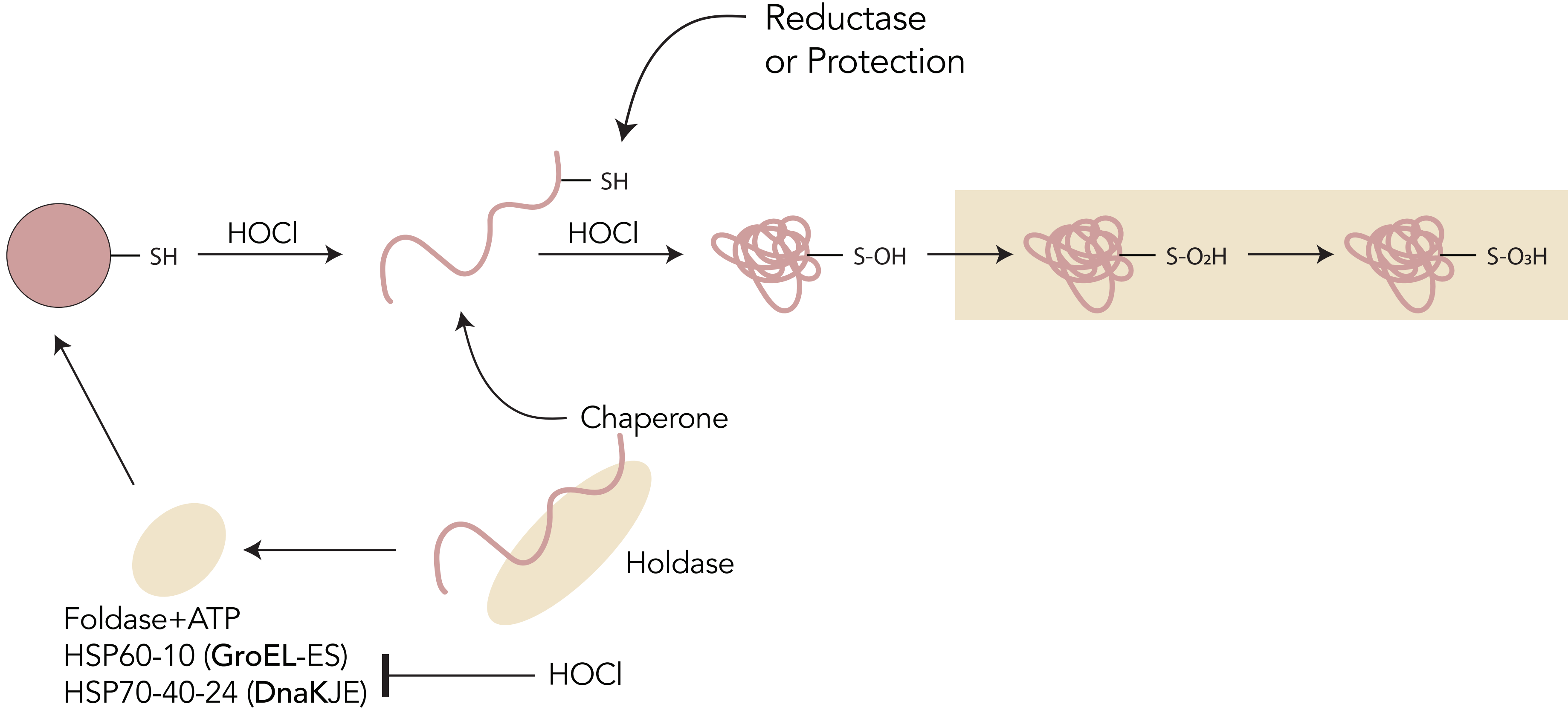
In the presence of HOCl



- the *cnoX* gene is important for bacteria to survive oxidative stress (HOCl)


How does this work?

# Real-life example



# Real-life example

NCBI

An official website of the United States government [Here's how you know](#)  **National Library of Medicine**  
*National Center for Biotechnology Information* [Log in](#)

Protein    [Help](#)

GenPept     [Run BLAST](#)

**chaperedoxin [Escherichia coli]**

NCBI Reference Sequence: WP\_001571052.1  
[Identical Proteins](#) [FASTA](#) [Graphics](#)

```
ORIGIN
  1 msvenivnin esnlqqvleq smttpvlfyf wtersqhclq ltpilesaa qyngqfilak
  61 ldcdaeqmia aqfglraipt vylfngqpv dgfgppqpee airalldkvl predelkaqq
 121 amqlmqegny tdalplkda wqlsnqgei glllaetlia lnrsedaeav lktiqlqdd
 181 tryqglvaqi ellkqaadtp eiqlqqqva enpedaalat qlalqlhqvq rneaelellf
 241 ghlrkdltaa dgqtrktfge ilaalgtgda laskyrrqly ally
//
```

Protein sequence

# Real-life example

Uniprot

**UniProt** BLAST Align Peptide search ID mapping SPARQL UniProtKB Advanced | List Search

## P77395 · CNOX\_ECOLI

<b>Protein<sup>i</sup></b>	Chaperedoxin	<b>Amino acids</b>	284 (go to sequence)
<b>Gene<sup>i</sup></b>	cnoX	<b>Protein existence<sup>i</sup></b>	Evidence at protein level
<b>Status<sup>i</sup></b>	UniProtKB reviewed (Swiss-Prot)	<b>Annotation score<sup>i</sup></b>	5/5
<b>Organism<sup>i</sup></b>	Escherichia coli (strain K12)		

[Entry](#) [Variant viewer](#) [Feature viewer](#) [Genomic coordinates](#) [Publications](#) [External links](#) [History](#)

[BLAST](#) [Download](#) [Add](#) [Add a publication](#) [Entry feedback](#)

### Function<sup>i</sup>

Chaperedoxin that combines a chaperone activity with a redox-protective function (PubMed:[16563353](#), PubMed:[18657513](#), PubMed:[29754824](#)). Involved in the protection against hypochlorous acid (HOCl), the active ingredient of bleach, which kills bacteria by causing protein aggregation (PubMed:[29754824](#)). Functions as an efficient holdase chaperone that protects the substrates of the major folding systems GroEL/GroES and DnaK/DnaJ/GrpE from aggregation. In addition, it prevents the irreversible oxidation of its substrates through the formation of mixed disulfide complexes (PubMed:[29754824](#)). After bleach stress, it transfers its substrates to the GroEL/GroES and DnaK/DnaJ/GrpE foldases (PubMed:[29754824](#)). Lacks oxidoreductase activity (PubMed:[21498507](#), PubMed:[29754824](#)). 4 Publications

### Activity regulation<sup>i</sup>

The holdase activity is activated by HOCl, via the reversible chlorination of several residues in the TPR domain. Chlorination probably increases the hydrophobicity of CnoX and enables it to bind a variety of substrates. Reduced glutathione (GSH) is required to resolve CnoX-substrate complexes. 1 Publication

### GO annotations<sup>i</sup>

[Access the complete set of GO annotations on QuickGO](#)



# Real-life example

BLAST

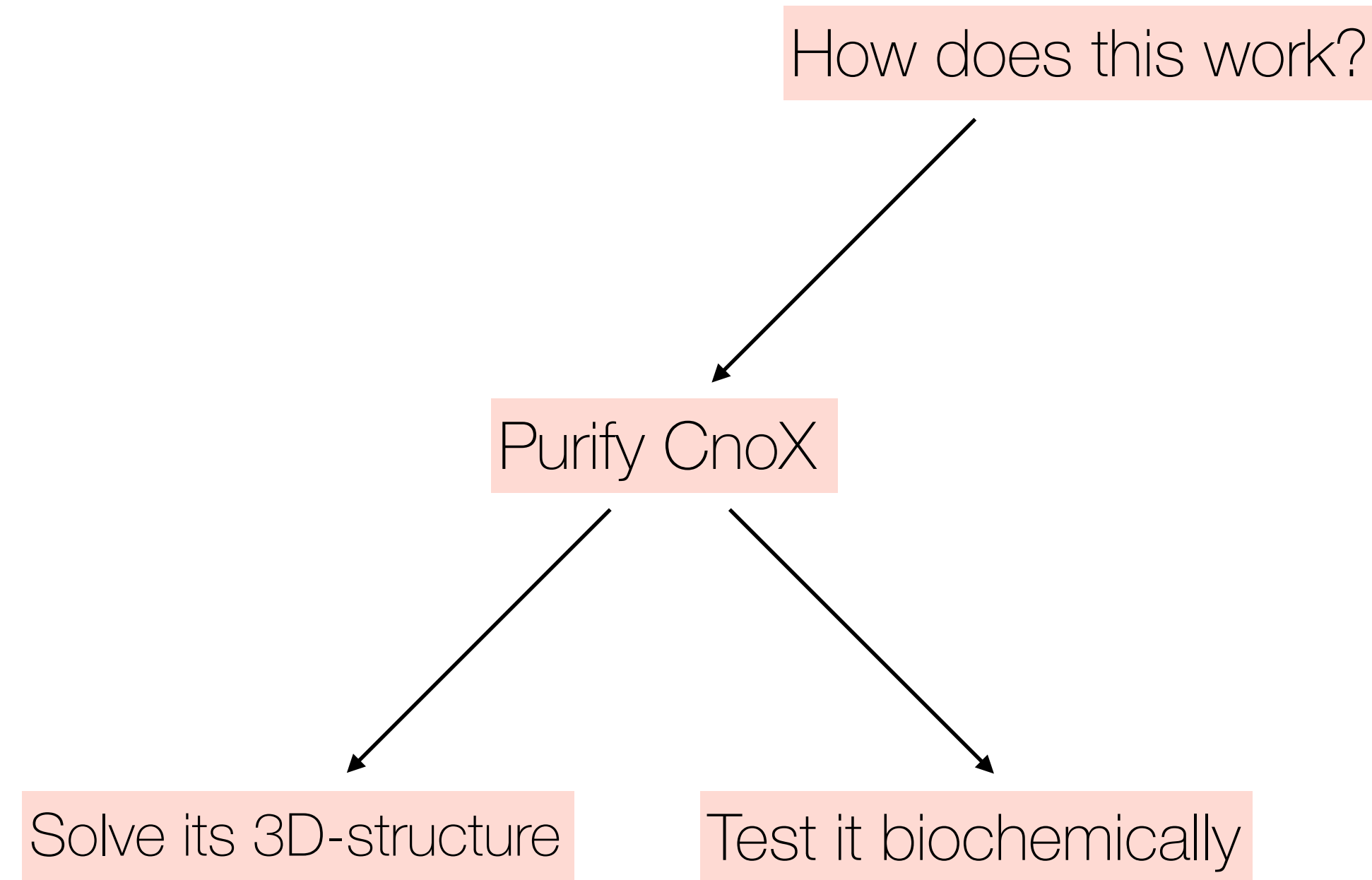
The screenshot shows the BLAST website interface. At the top left is the NIH logo and the text "National Library of Medicine National Center for Biotechnology Information". A "Log in" button is in the top right. Below the header is a navigation bar with "BLAST®" on the left and "Home Recent Results Saved Strategies Help" on the right. The main content area features a "Basic Local Alignment Search Tool" section with a description and a "Learn more" link. To the right is a "NEWS" alert box for "BLAST+ 2.15.0 is here!" dated "Tue, 28 Nov 2023" with a "More BLAST news..." link. Below this is the "Web BLAST" section, which contains three tool options: "Nucleotide BLAST" (nucleotide to nucleotide), "blastx" (translated nucleotide to protein), and "tblastn" (protein to translated nucleotide). The "Protein BLAST" option (protein to protein) is highlighted with a red rectangular box.

# Real-life example

BLAST

Descriptions		Graphic Summary	Alignments	Taxonomy						Download	Select columns	Show 100	?
Sequences producing significant alignments													
<input checked="" type="checkbox"/> select all 100 sequences selected				<a href="#">GenPept</a>	<a href="#">Graphics</a>	<a href="#">Distance tree of results</a>	<a href="#">Multiple alignment</a>	<a href="#">MSA Viewer</a>					
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession				
<input checked="" type="checkbox"/>	<a href="#">similar to H. influenzae HI1159 [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	572	572	100%	0.0	100.00%	296	<a href="#">AAB40246.1</a>				
<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Enterobacteriaceae]</a>	<a href="#">Enterobacteriaceae</a>	570	570	100%	0.0	100.00%	284	<a href="#">WP_001300573.1</a>				
<input checked="" type="checkbox"/>	<a href="#">paral. putative thioredoxin protein [Escherichia coli H617]</a>	<a href="#">Escherichia coli H617</a>	570	570	100%	0.0	99.65%	296	<a href="#">OSL37467.1</a>				
<input checked="" type="checkbox"/>	<a href="#">putative thioredoxin-like protein [Escherichia coli O157:H7 str. EDL933]</a>	<a href="#">Escherichia coli O157:H7 str. EDL933</a>	570	570	100%	0.0	99.65%	296	<a href="#">AAG54849.1</a>				
<input checked="" type="checkbox"/>	<a href="#">TPA: chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">HAW3244858.1</a>				
<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">MCV5904056.1</a>				
<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">EKD4395722.1</a>				
<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">MBB7921250.1</a>				
<input checked="" type="checkbox"/>	<a href="#">TPA: chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">HBL5511935.1</a>				
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<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">WP_305854801.1</a>				
<input checked="" type="checkbox"/>	<a href="#">TPA: chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">HCL0969155.1</a>				
<input checked="" type="checkbox"/>	<a href="#">chaperedoxin [Escherichia coli]</a>	<a href="#">Escherichia coli</a>	569	569	100%	0.0	99.65%	284	<a href="#">WP_097345291.1</a>				

# Real-life example



# Real-life example

Solve its 3D-structure with alpha fold

## Chaperedoxin

AlphaFold structure prediction

Download [PDB file](#) [mmCIF file](#) [Predicted aligned error](#)

Share your feedback on structure with Google DeepMind [Looks great](#) [Could be improved](#)

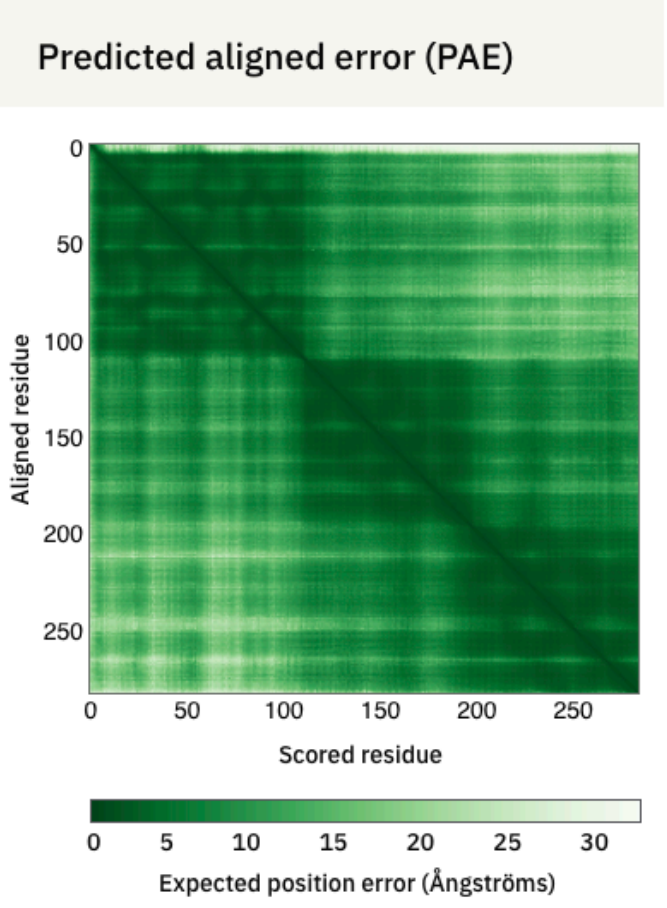
### Information

Protein	Chaperedoxin
Gene	cnoX
Source organism	Escherichia coli (strain K12) <a href="#">go to search</a>
UniProt	P77395 <a href="#">go to UniProt</a>
Experimental structures	2 structures in PDB for P77395 <a href="#">go to PDBe-KB</a>
Biological function	Chaperedoxin that combines a chaperone activity with a redox-protective function ( <a href="#">PubMed:16563353</a> , <a href="#">PubMed:18657513</a> , <a href="#">PubMed:29754824</a> ). Involved in the protection against hypochlorous acid (HOCl), the active ingredient of bleach, which kills bacteria by causing protein aggregation ( <a href="#">PubMed:29754824</a> ). Functions as an efficient holdase chaperone that protects the substrates of the major folding systems GroEL/GroES and DnaK/DnaJ/GrpE from aggregation. In addition, it prevents the irreversible oxidation of ... <a href="#">+ [show more]</a> <a href="#">go to UniProt</a>

**Model Confidence**

- Very high (pLDDT > 90)
- High (90 > pLDDT > 70)
- Low (70 > pLDDT > 50)
- Very low (pLDDT < 50)

AlphaFold produces a per-residue model confidence score (pLDDT) between 0 and 100. Some regions below 50 pLDDT may be unstructured in isolation.

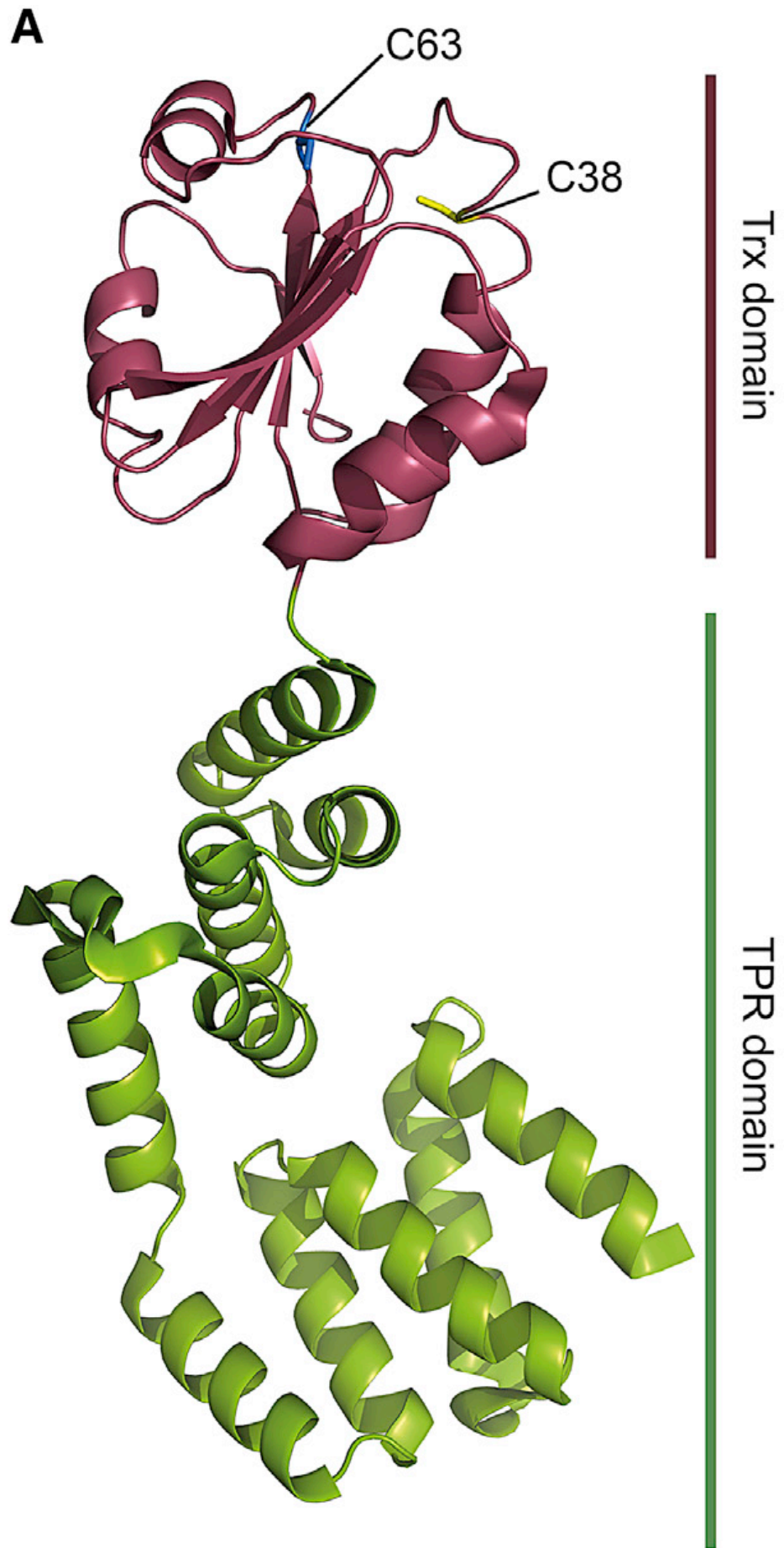


Click and drag a box on the PAE viewer to select regions of the structure and highlight them on the 3D viewer.

PAE data is useful for assessing inter-domain accuracy – [go to Help section below](#) for more information.

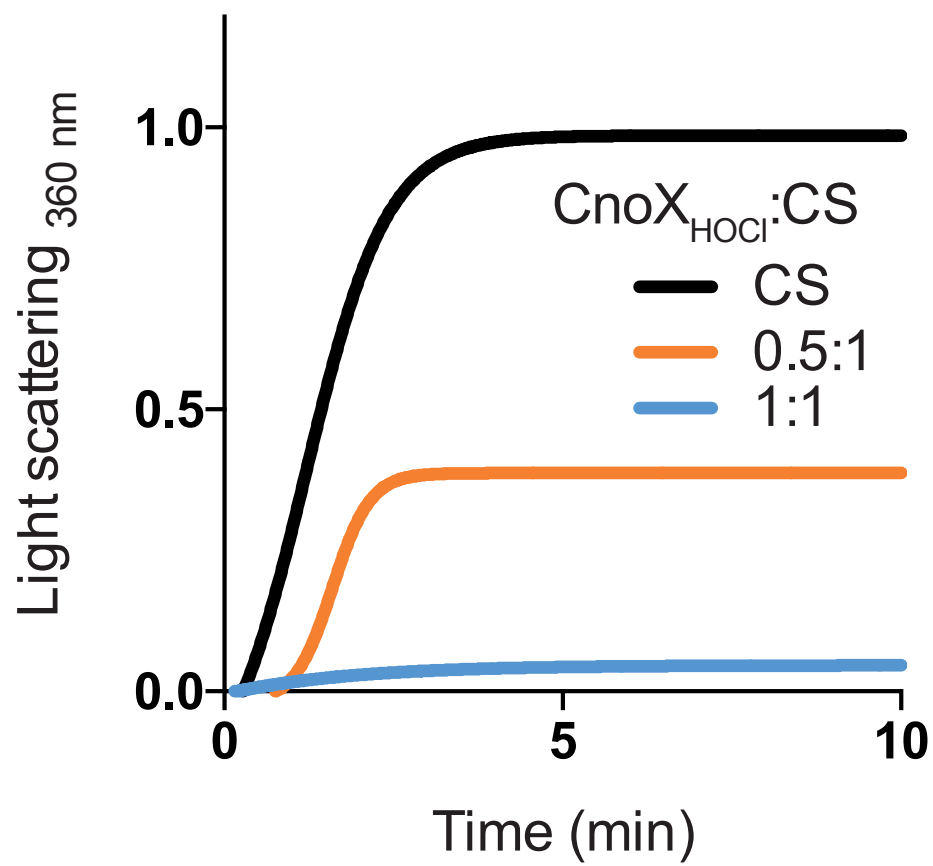
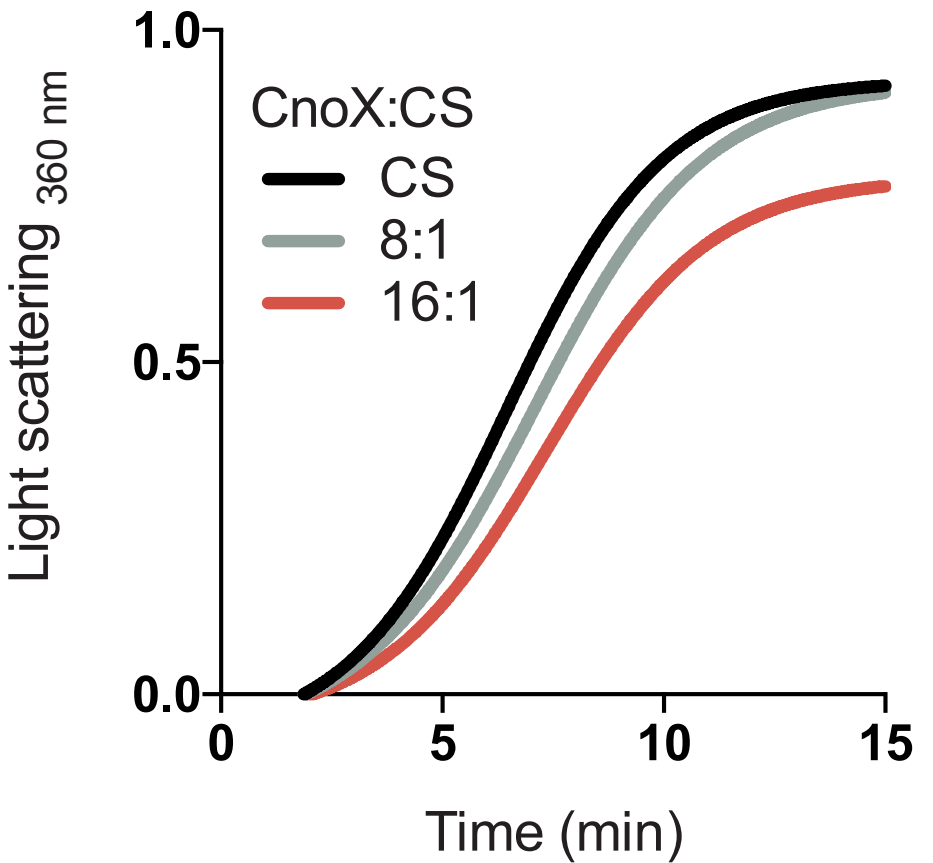
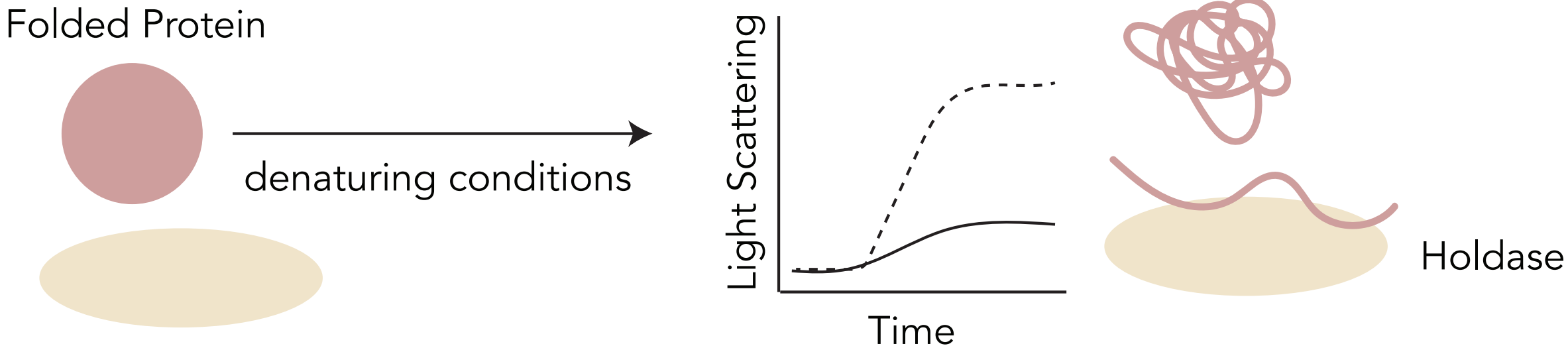
# Real-life example

Solve its 3D-structure (cristallography)



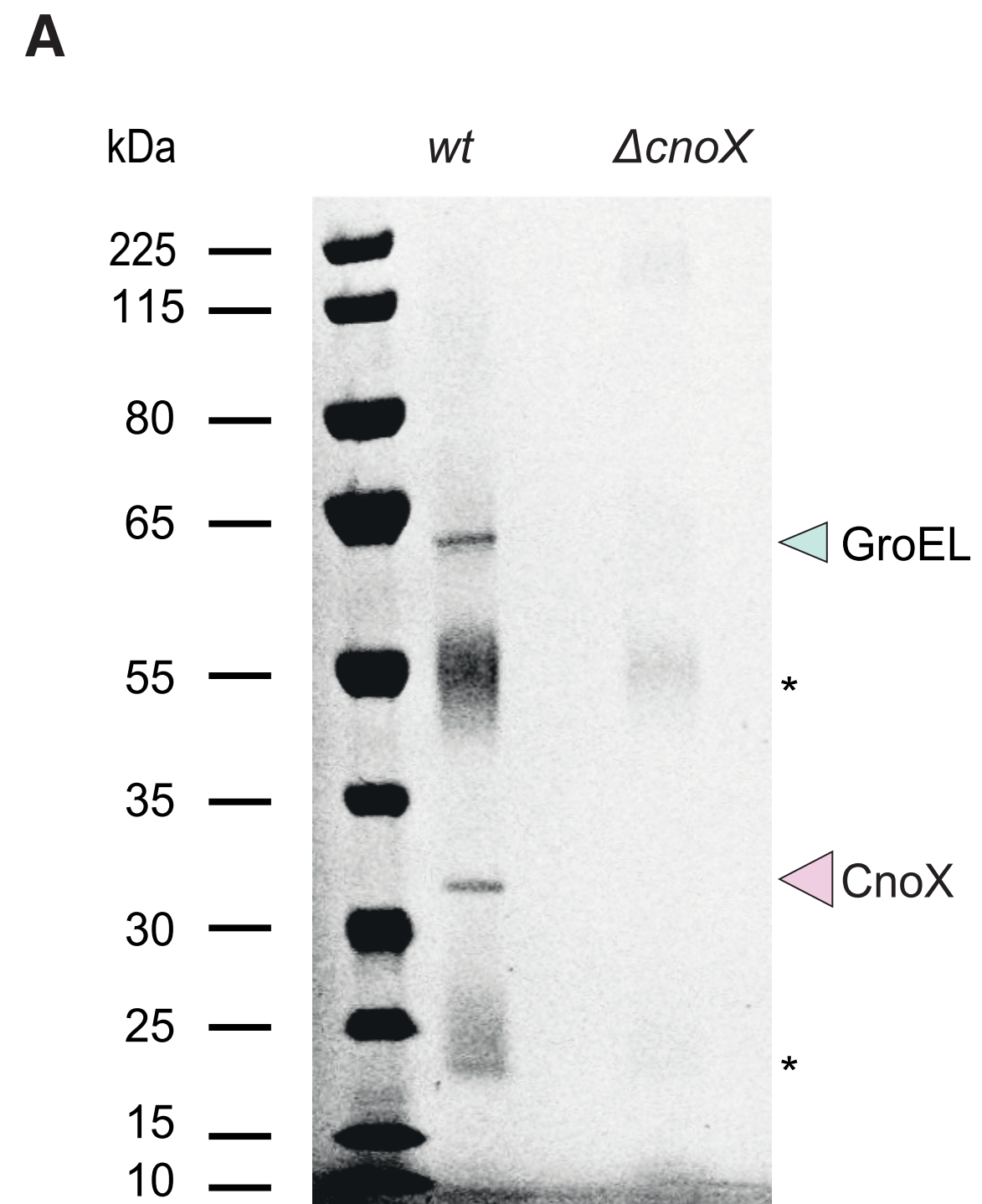
# Real-life example

Test it biochemically



# Real-life example

Find the partners: Co-IP + SDS-PAGE

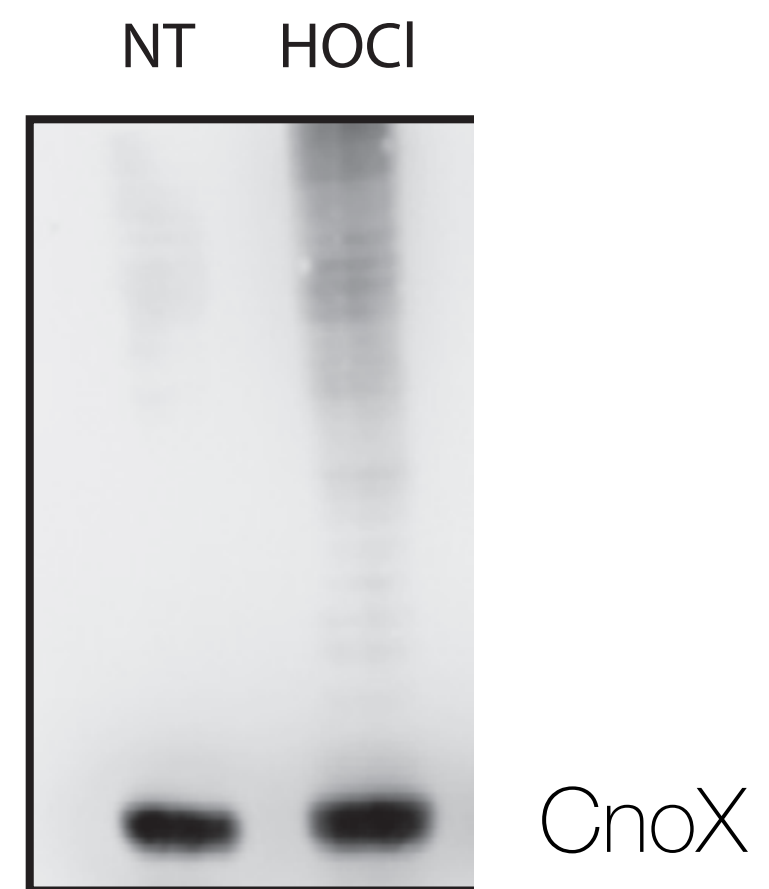


- co-IP using  $\alpha$ -CnoX antibodies
- SDS-PAGE: we only detect one other band
- Mass spectrometry: this band is GroEL

Why don't we do a western blot here?

# Real-life example

On a western blot, CnoX looks like this



What could be those bands above CnoX upon HOCl treatment ?



# Plan

- Studying proteins
  - Protein interactions
  - Real-life example
- **Studying DNA**
  - DNA sequencing
  - DNA extraction
  - DNA amplification

# Building a DNA toolbox

- Based on **DNA sequencing**
- DNA sequencing has allowed advances in **technology**
- Use of **recombinant DNA**, i.e DNA from different sources that is combined
- This is useful for **genetic engineering**, i.e. manipulating genes for practical purposes
- DNA technologies have an **impact** on research, medicine, forensics, agriculture, ...

# Plan

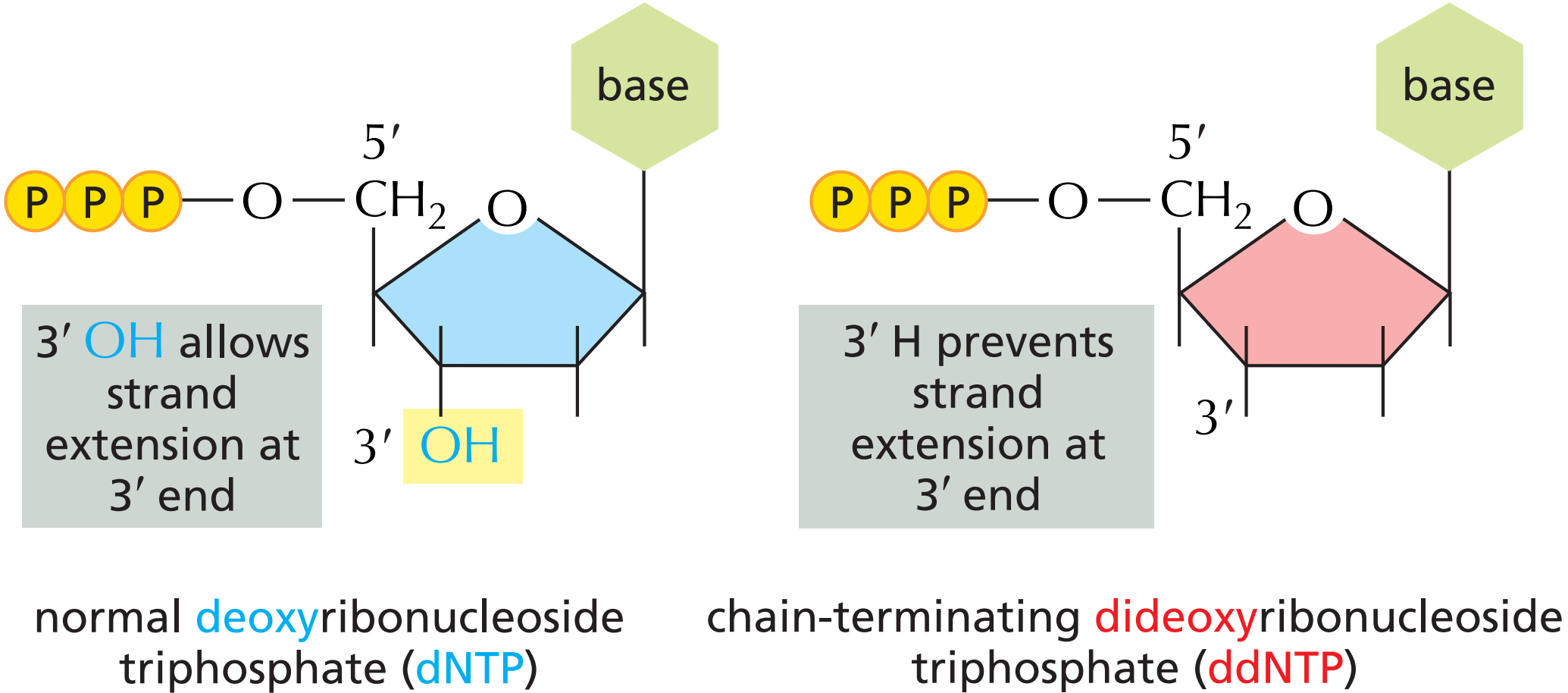
- Studying proteins
  - Protein interactions
  - Real-life examples
- Studying DNA
  - **DNA sequencing**
  - DNA extraction
  - DNA amplification

# DNA sequencing

- exploits **complementary base pairing**
- developed in the **1970s by Sanger** (Nobel Prize in 1980)
- in 2000s, development of **next-generation sequencing**, which is faster and cheaper: the DNA fragments are amplified, then one strand is immobilized and the complementary strand is synthesized, one nucleotide at a time —  
> real-time identification of the added nucleotide
- recently, development of **third-generation sequencing**. In some methods, long stretches of DNA are sequenced without cutting or amplifying (e.g. nanopore)

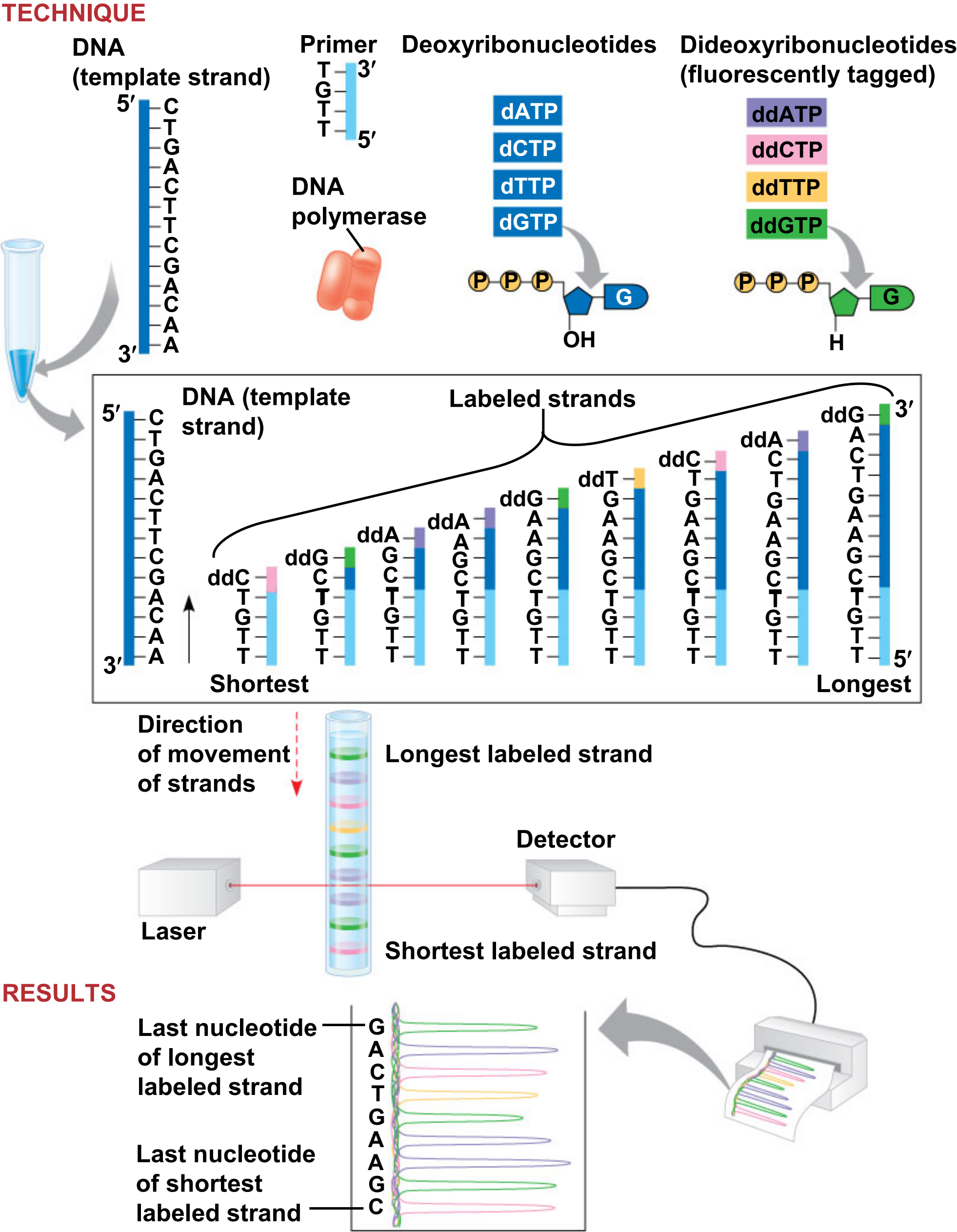
# DNA sequencing

- **Sanger or dideoxy sequencing** relies on dideoxy nucleotides that terminate elongation



# DNA sequencing

- Sanger or dideoxy sequencing relies on dideoxy nucleotides that terminate elongation
- low cost, small scale (short DNA fragments)

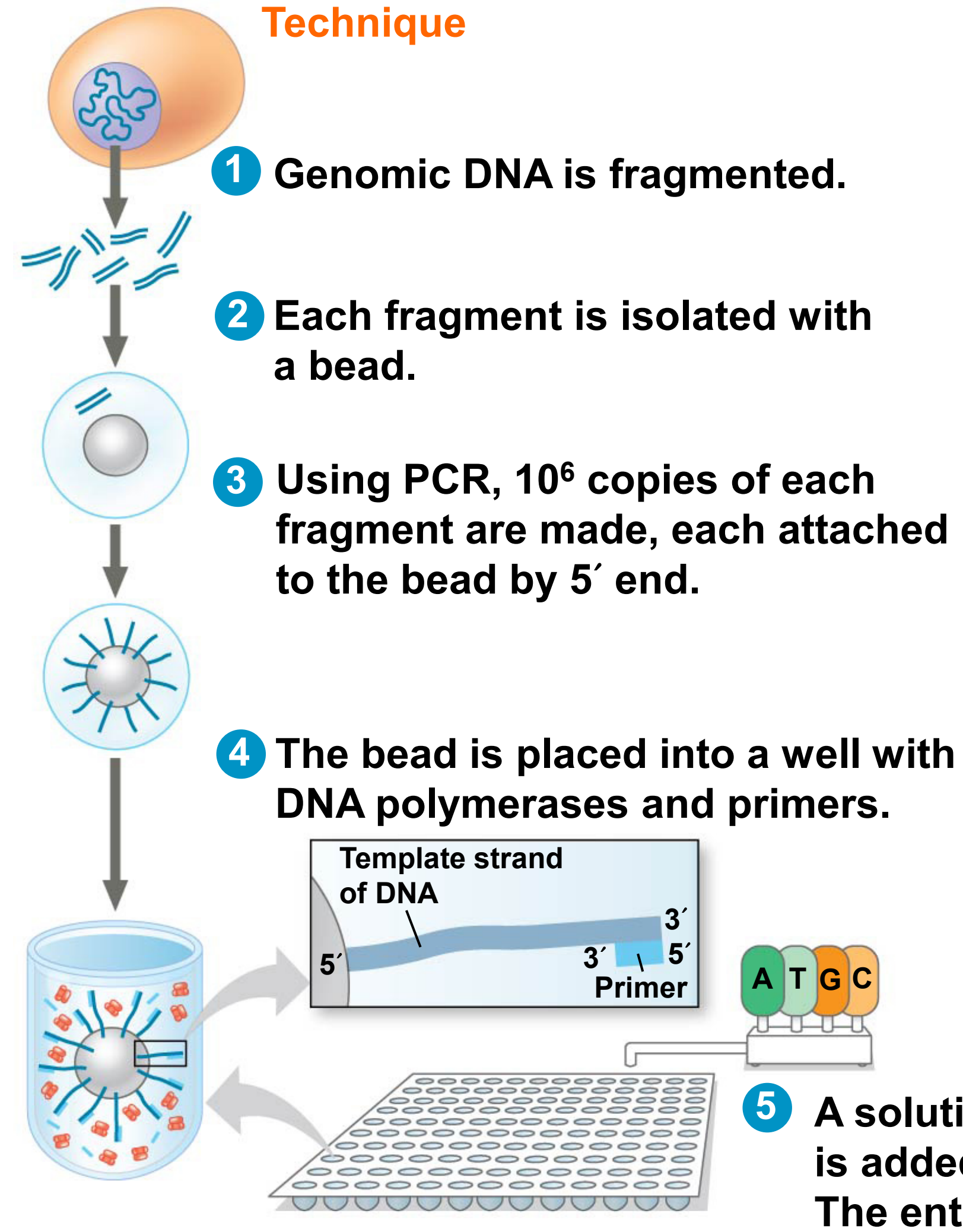


# DNA sequencing

- Next-generation sequencing

- since 2005
- allow large-scale sequencing
- most common is **Illumina Sequencing**
- **short** DNA sequences (few hundreds nt)
- **bioinformatic** analysis

Figure 19.4a

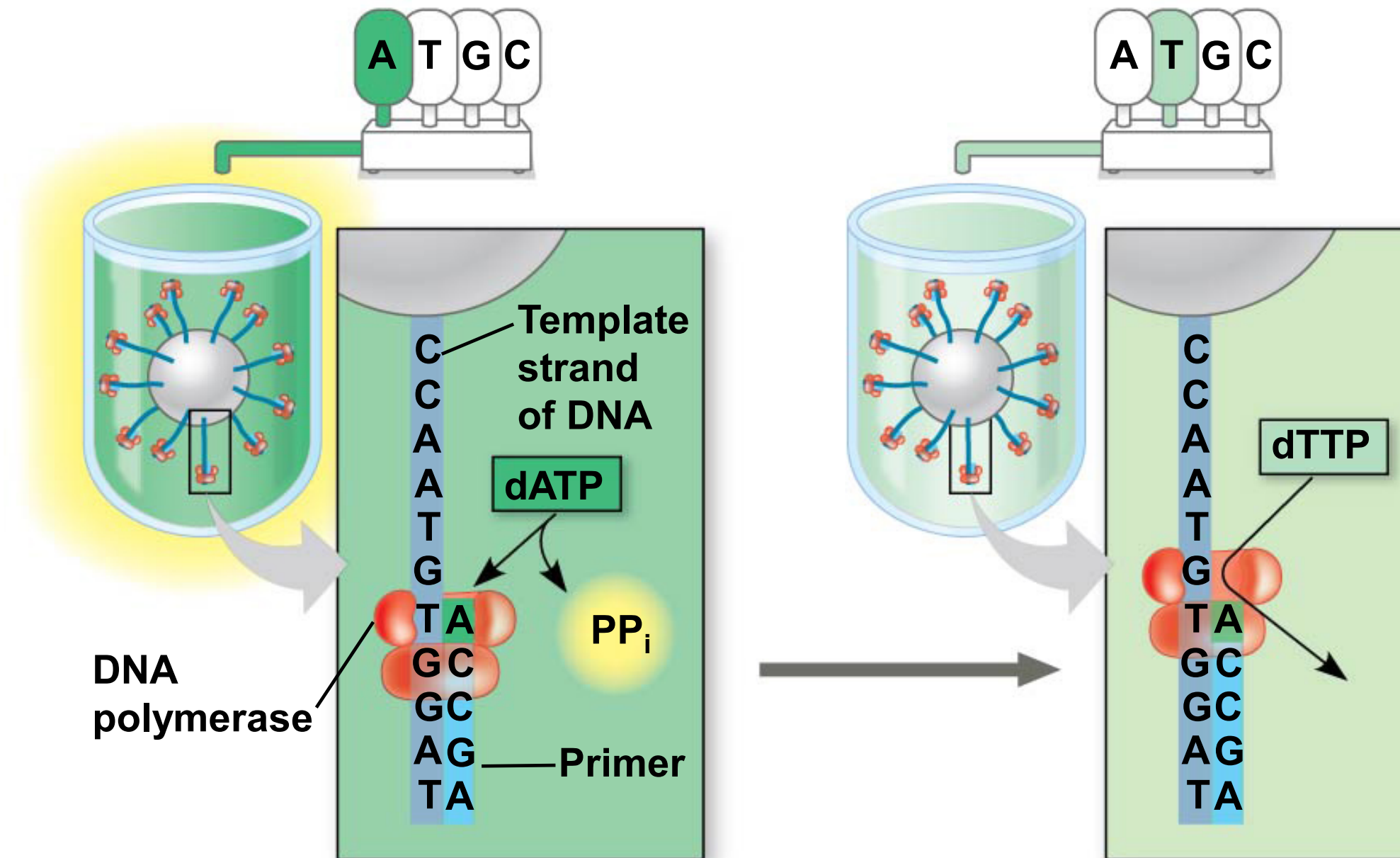


# DNA sequencing

- Next-generation sequencing

- since 2005
- allow large-scale sequencing
- most common is **Illumina Sequencing**
- **short** DNA sequences (few hundreds nt)
- **bioinformatic** analysis

## Technique



**6** If a nucleotide is joined to a growing strand,  $PP_i$  is released, causing a flash of light that is recorded.

**7** If a nucleotide is not complementary to the next template base, no  $PP_i$  is released, and no flash of light is recorded.

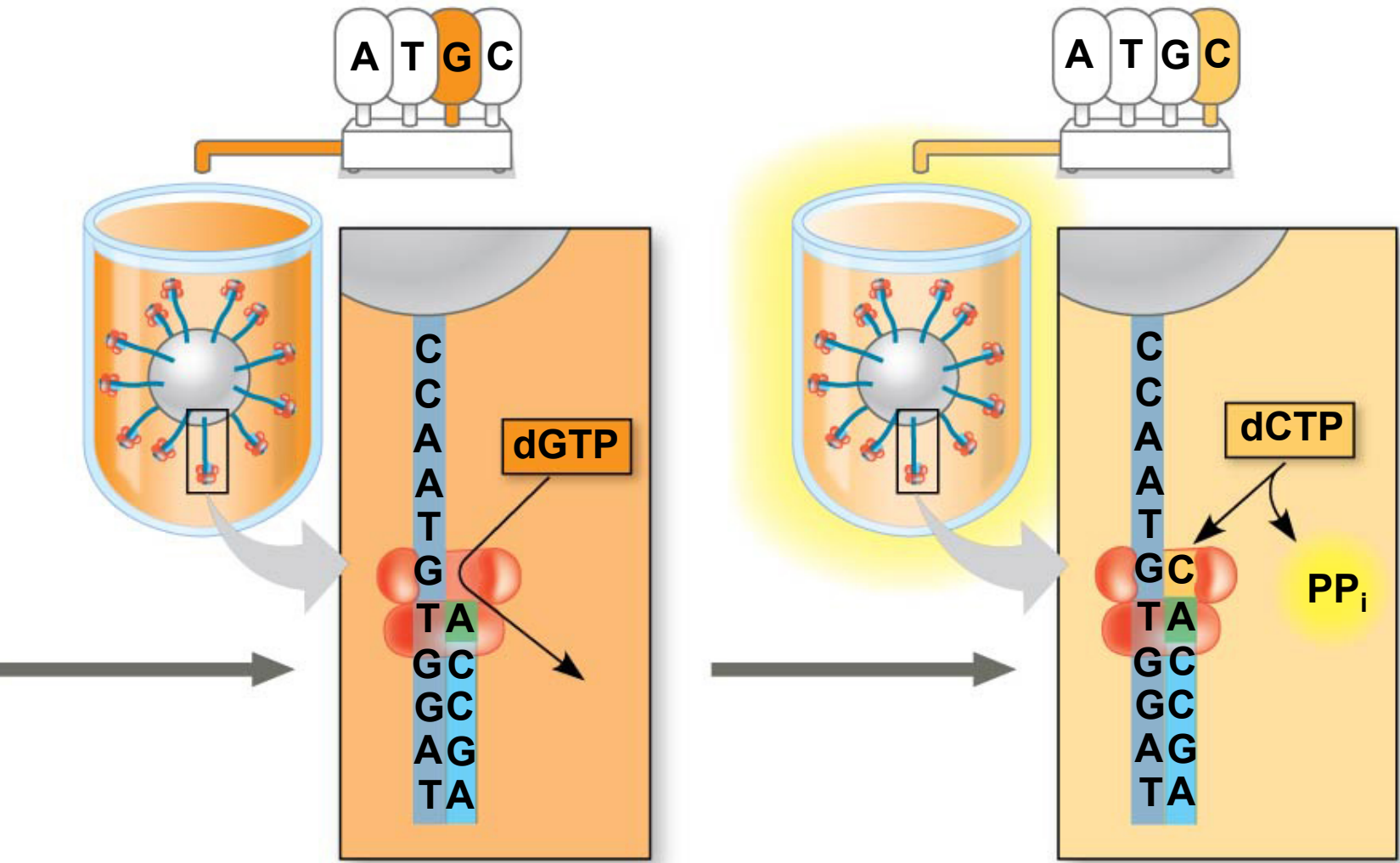


# DNA sequencing

- Next-generation sequencing

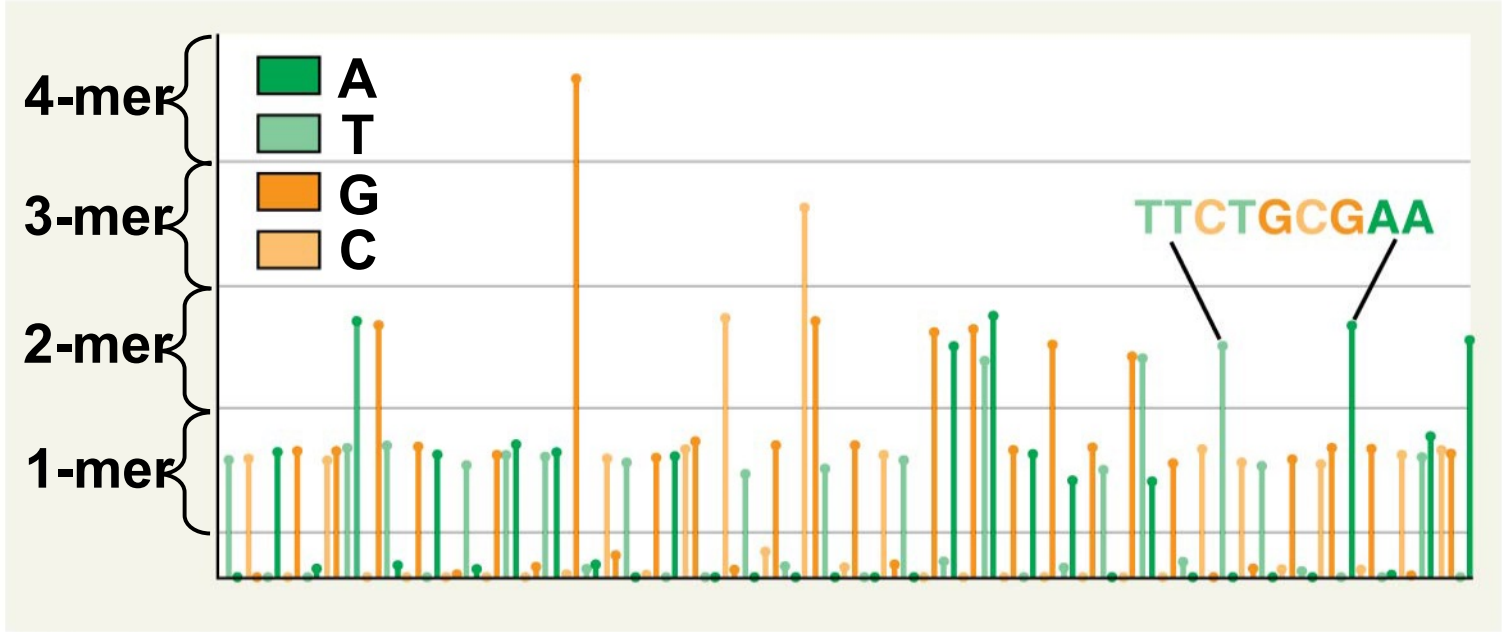
- since 2005
- allow large-scale sequencing
- most common is Illumina Sequencing
- short DNA sequences (few hundreds nt)
- bioinformatic analysis

Figure 19.4c **Technique**



8 The process is repeated until every fragment has a complete complementary strand. The pattern of flashes reveals the sequence.

**Results**

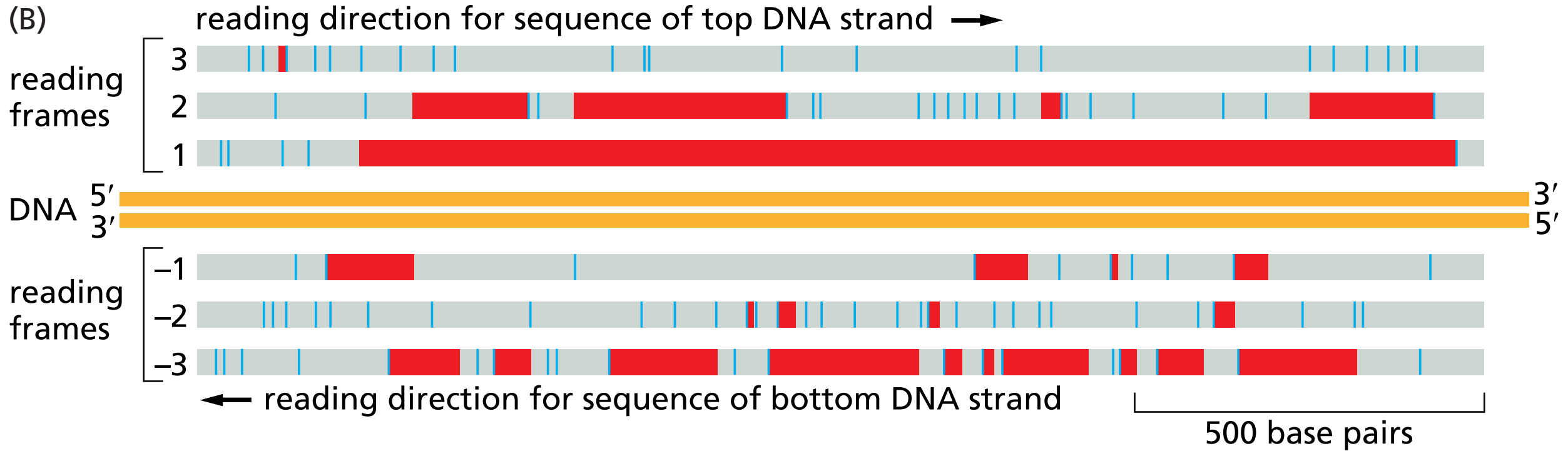
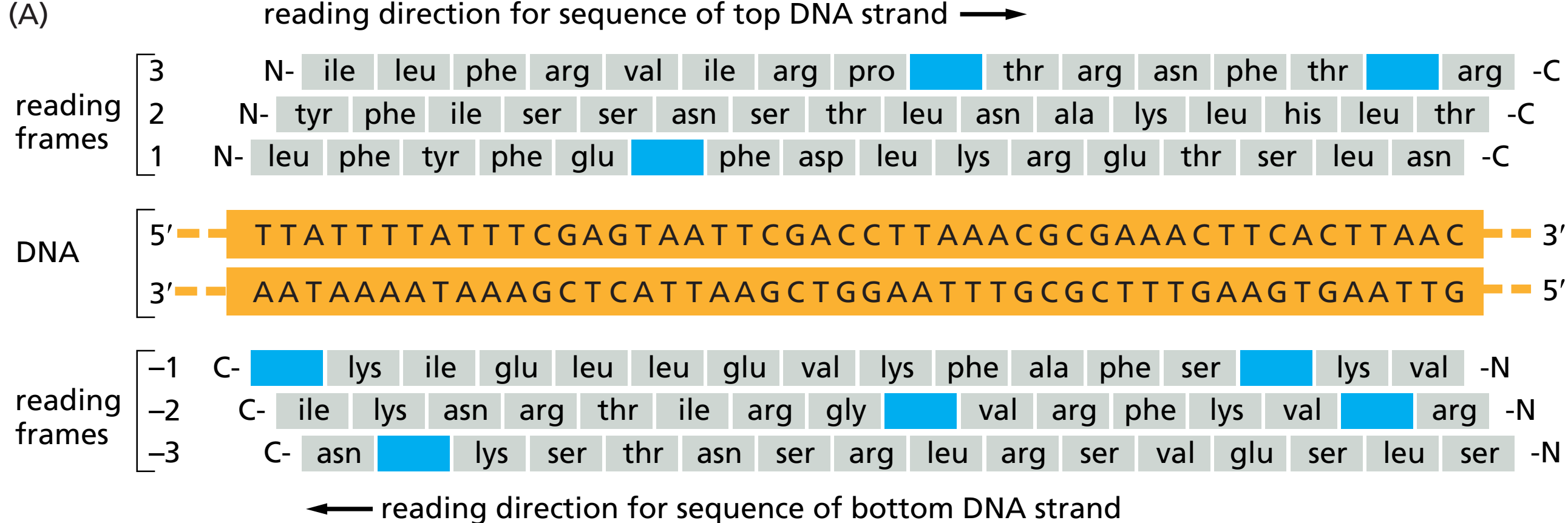


# DNA sequencing

- Third-generation sequencing
  - **longer** DNA molecules - better to **reconstitute genomes**
  - **more expensive**

# Gene annotations

- **Mark** the genes
- Assign possible **roles**

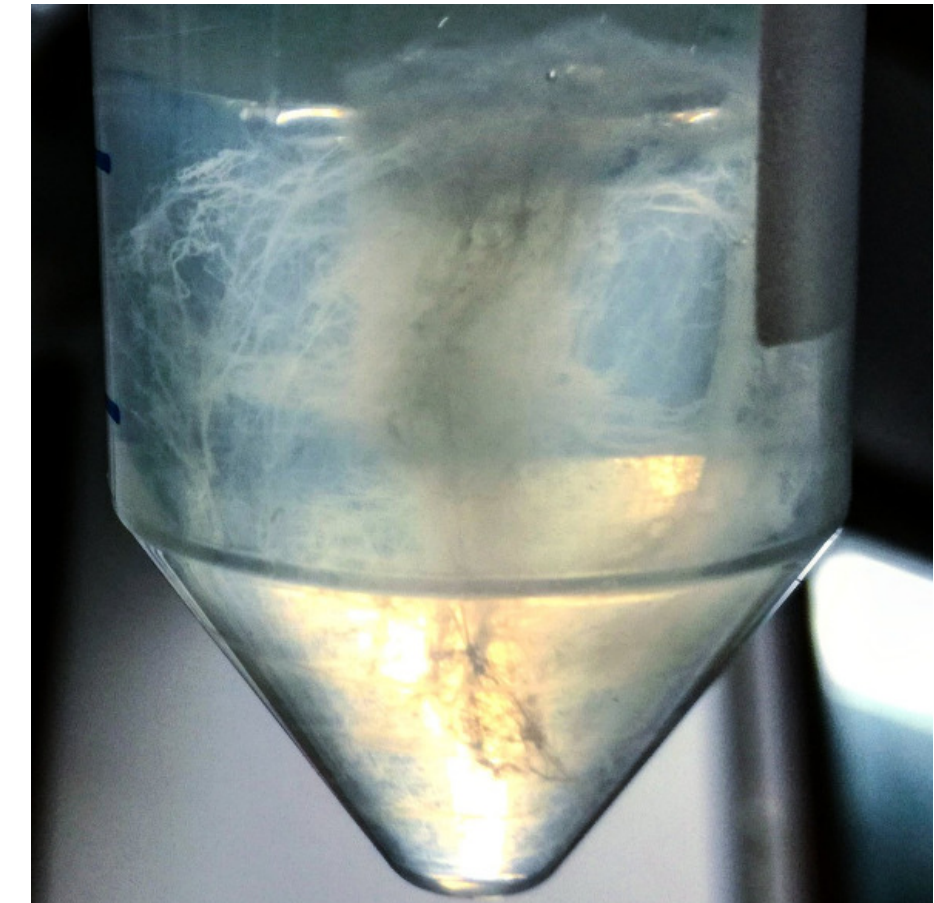
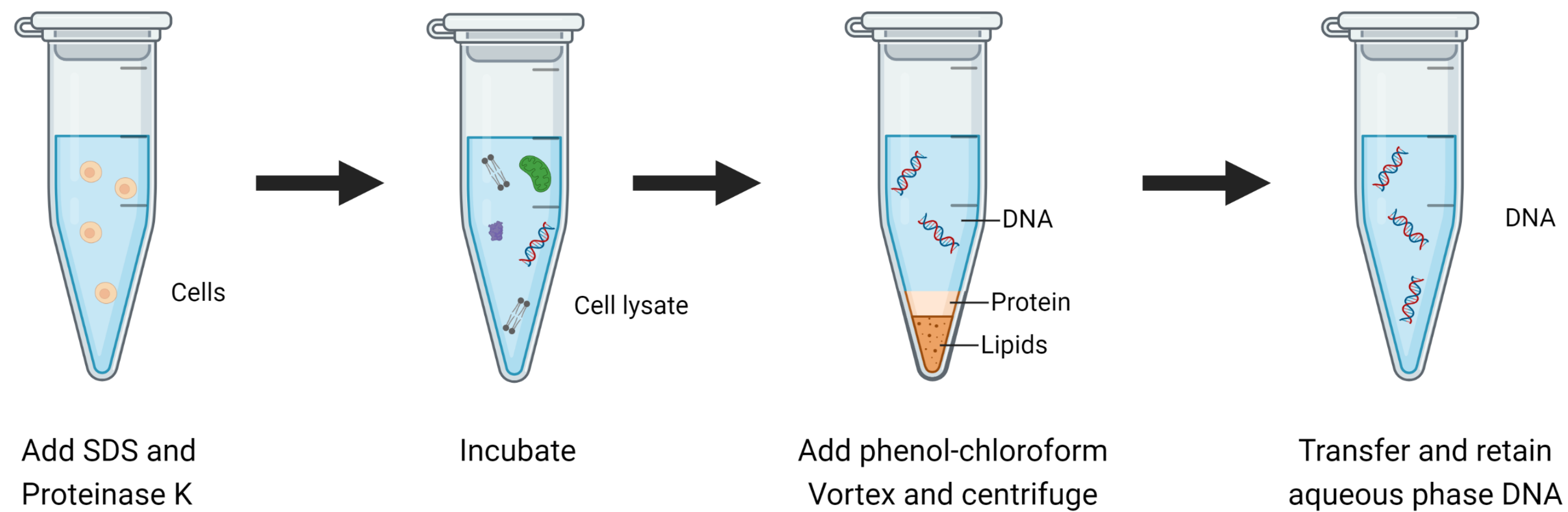


# Plan

- Studying proteins
  - Protein interactions
  - Real-life example
- Studying DNA
  - DNA sequencing
  - **DNA extraction**
  - DNA amplification

# But how is DNA extracted?

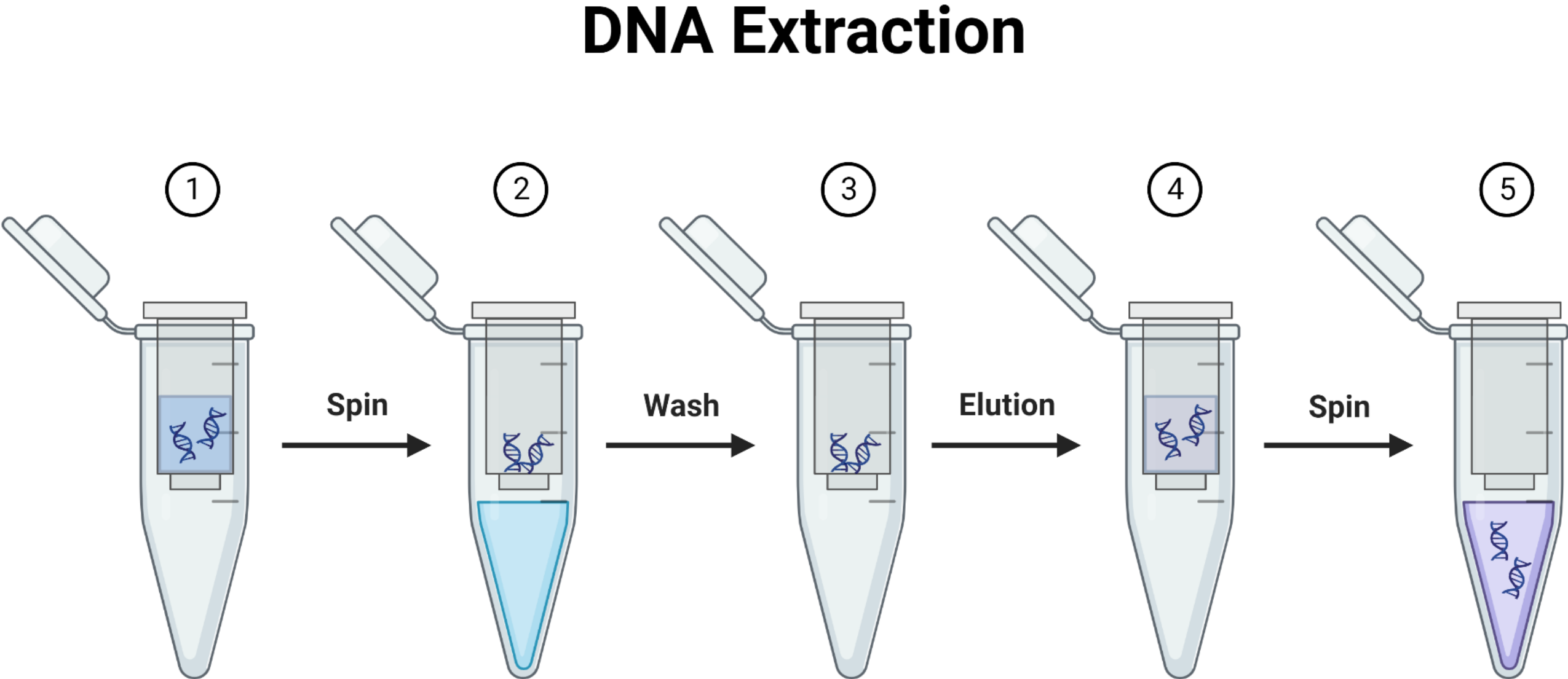
- **Goal:** isolate the DNA without its associated proteins



- Phenol-chloroform is less polar than water and induces protein aggregation
- DNA is further precipitated with ethanol

# But how is DNA extracted?

- **Goal:** isolate the DNA without its associated proteins

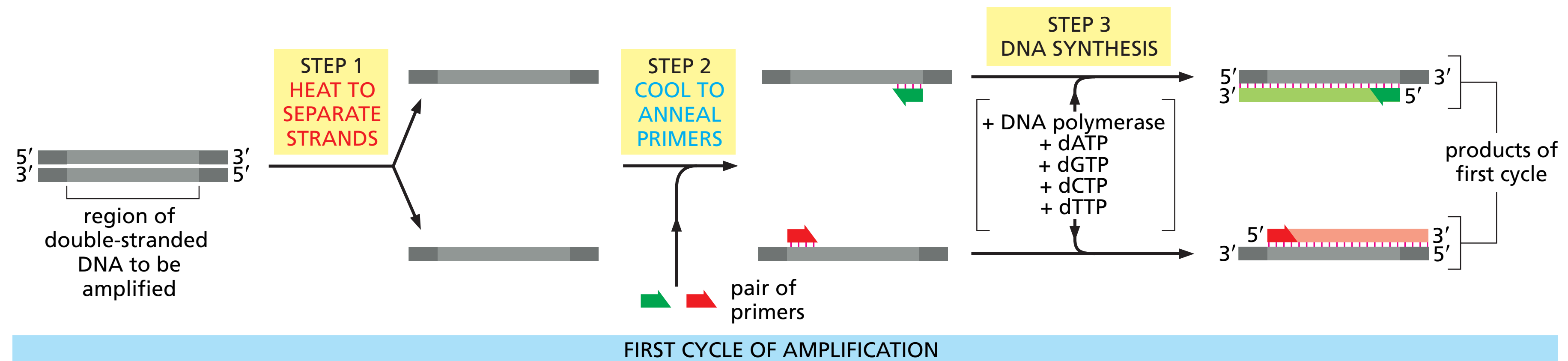


# Plan

- Studying proteins
  - Protein interactions
  - Real-life examples
- Studying DNA
  - DNA sequencing
  - DNA extraction
  - **DNA amplification**

# Amplifying specific regions of DNA

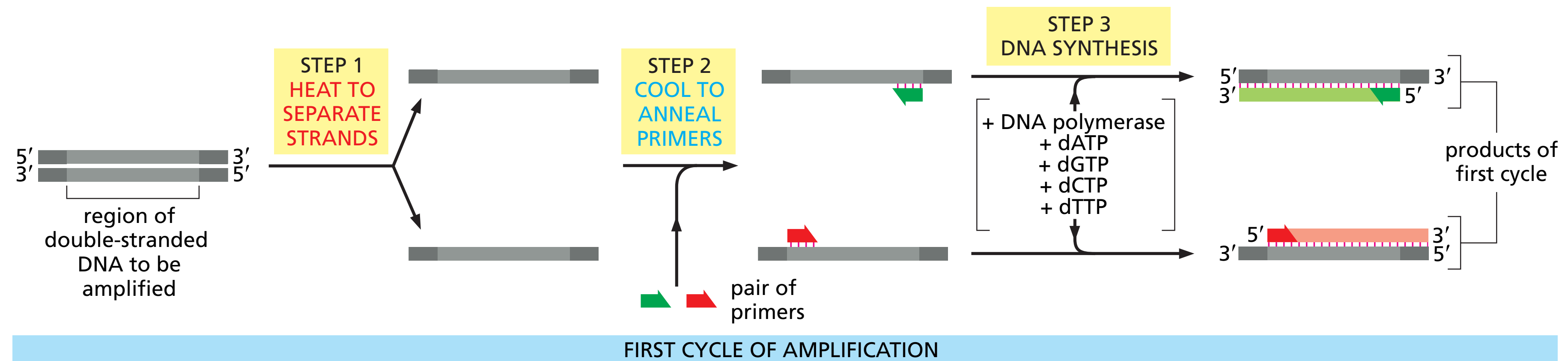
- To work directly on **specific regions/genes**, we amplify these regions to obtain multiple identical copies
- Polymerase Chain Reaction (**PCR**) for specific **DNA region amplification**
  - design the **DNA primers** needed by the **DNA polymerase**
  - need **nucleotides**
  - get **billions of copies** of the original sequences after **20-30 cycles**





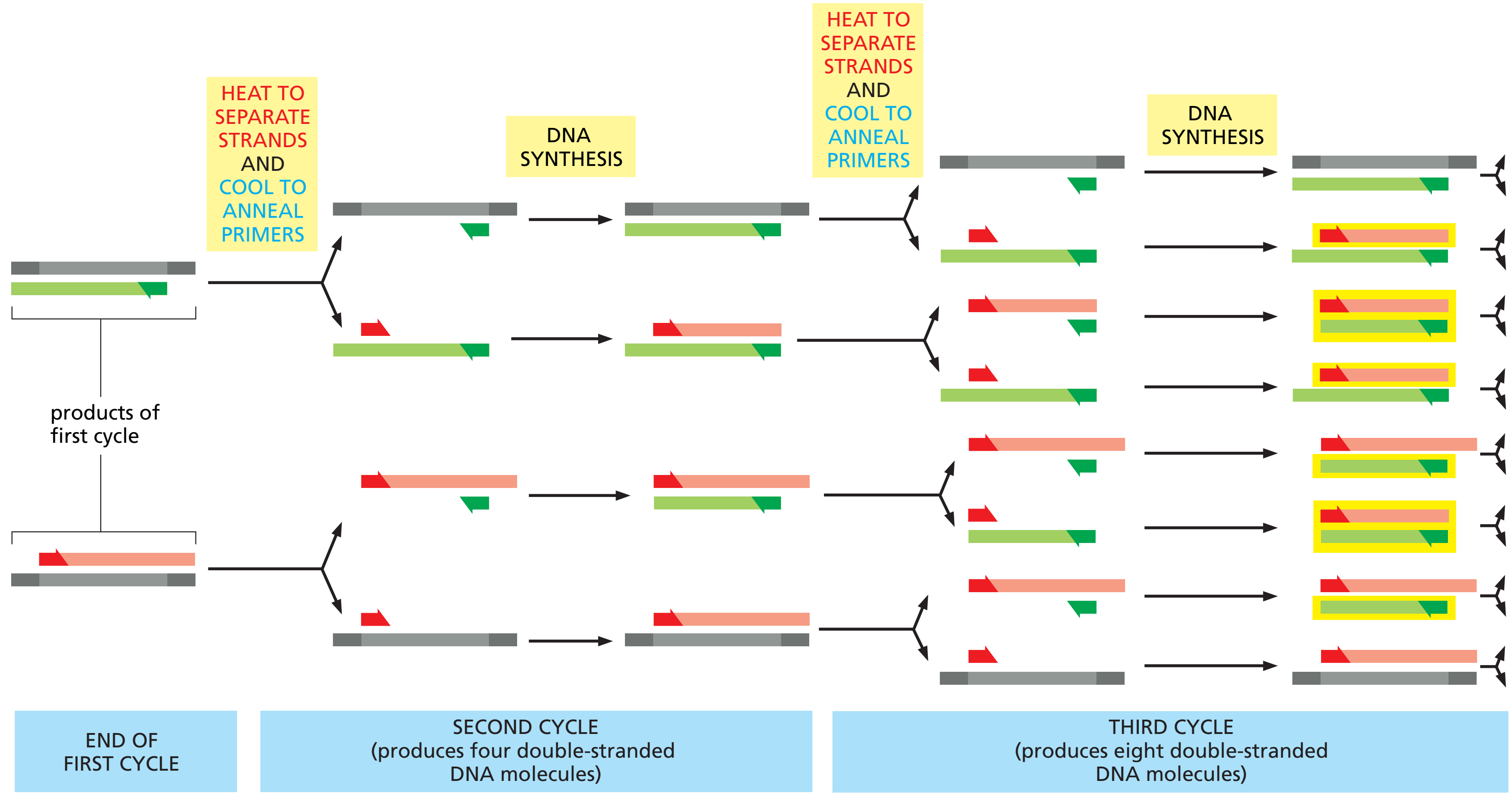
# Amplifying specific regions of DNA

- Polymerase Chain Reaction (**PCR**) for specific **DNA region amplification**
  - design the **DNA primers** needed by the **DNA polymerase**
  - need **nucleotides**
  - get **billions of copies** of the original sequences after **20-30 cycles**



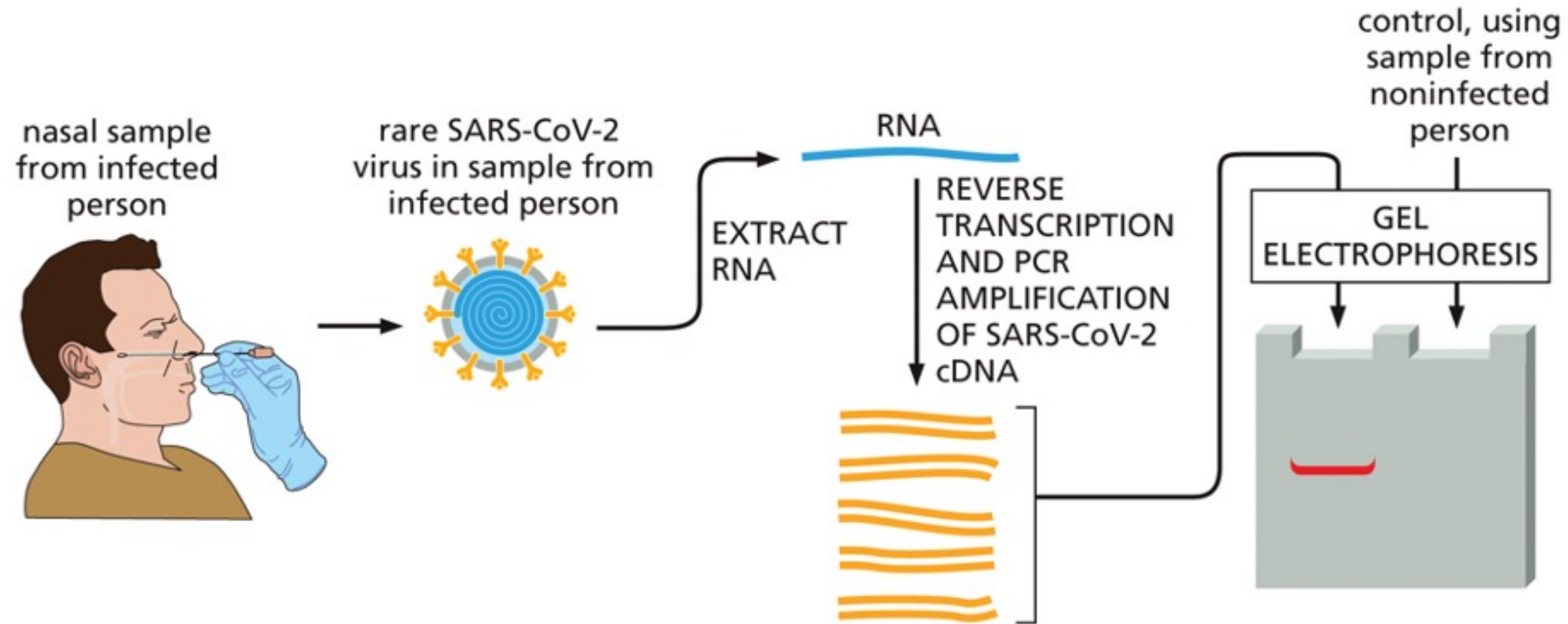
# Amplifying specific regions of DNA

- Polymerase Chain Reaction (**PCR**) for specific **DNA region amplification**



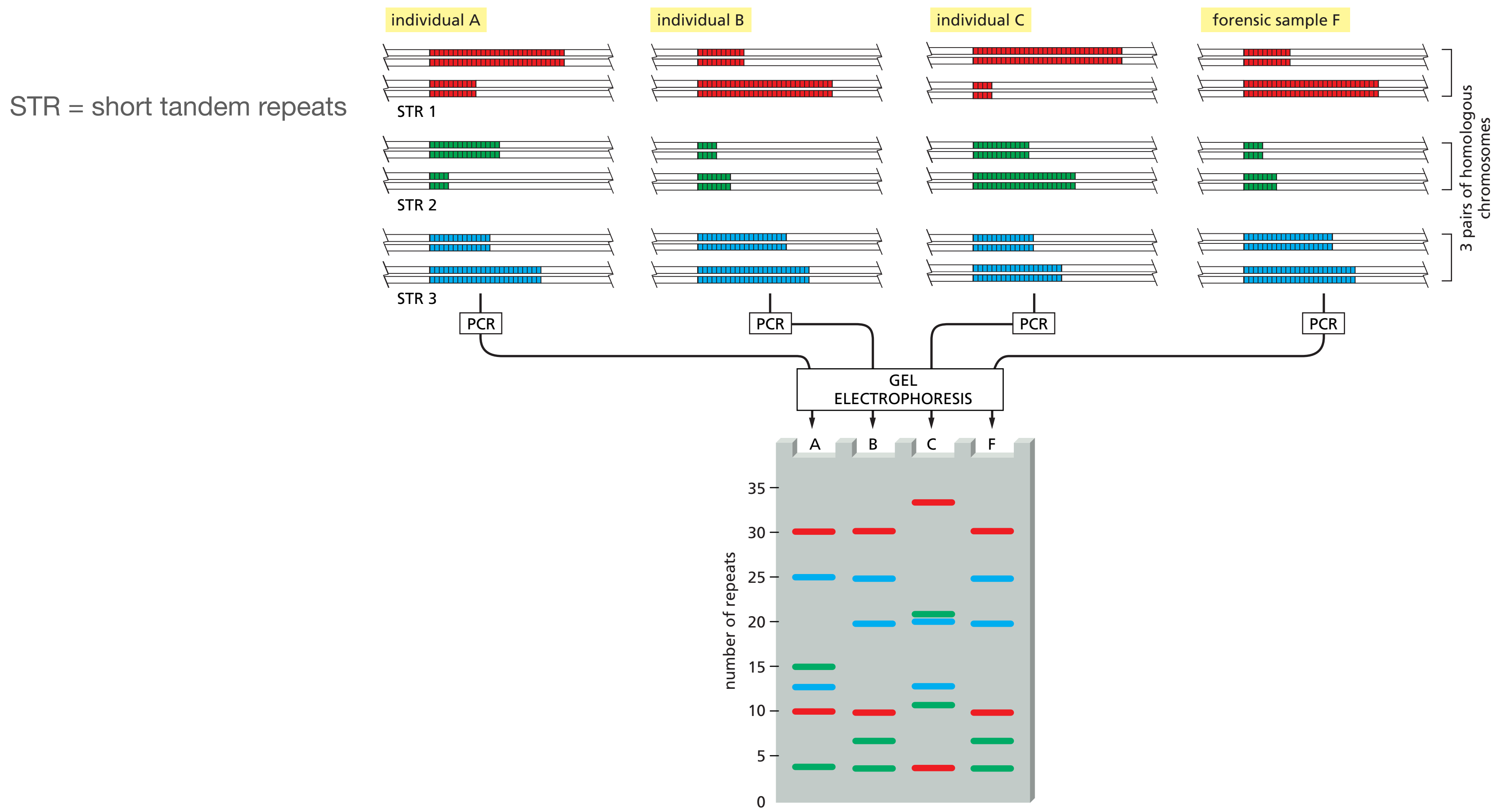
# Amplifying specific regions of DNA

- Polymerase Chain Reaction (**PCR**) for specific **DNA** region amplification - diagnostic or forensics



# Amplifying specific regions of DNA

- Polymerase Chain Reaction (**PCR**) for specific **DNA region amplification** - diagnostic or forensics



# Amplifying specific regions of DNA

## Polymerase Chain Reaction

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# Summary

- Studying proteins
  - Protein interactions
  - Real-life examples
- Studying DNA
  - DNA sequencing
  - DNA extraction
  - DNA amplification

**Have a nice day!**