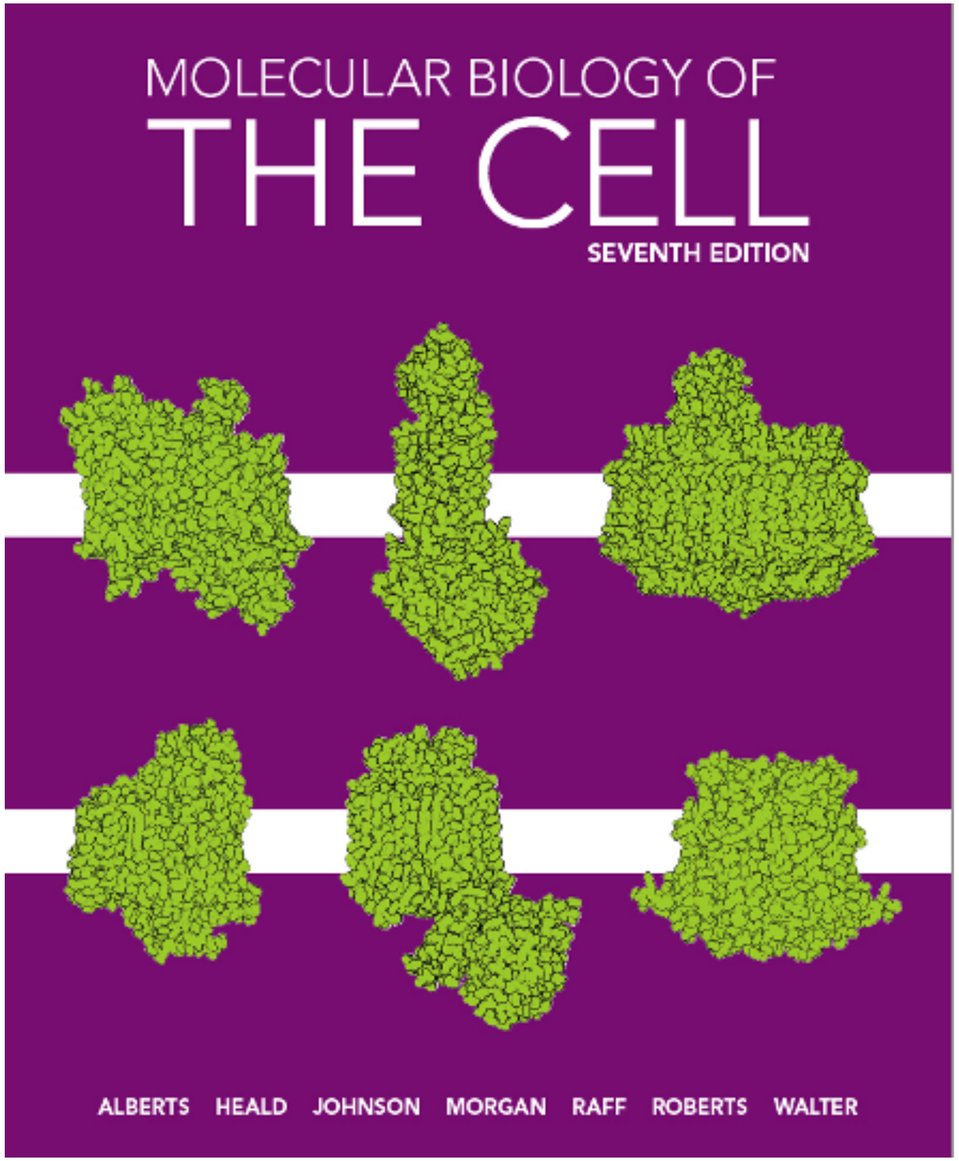
Cellular and Molecular Biology I

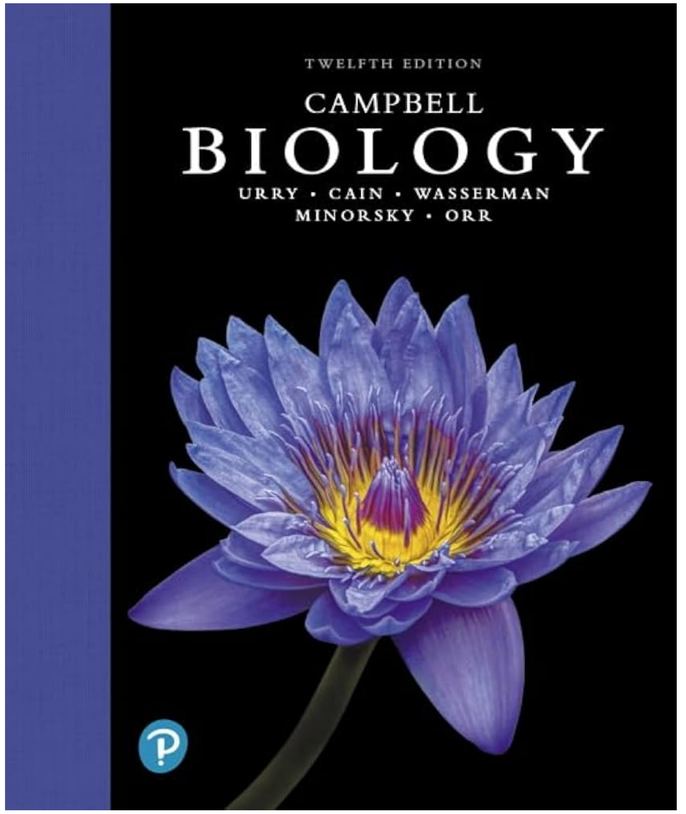
BIO-205-10

Camille Goemans - 2024



Chapter 8

Analyzing Cells, Molecules, and Systems



Summary

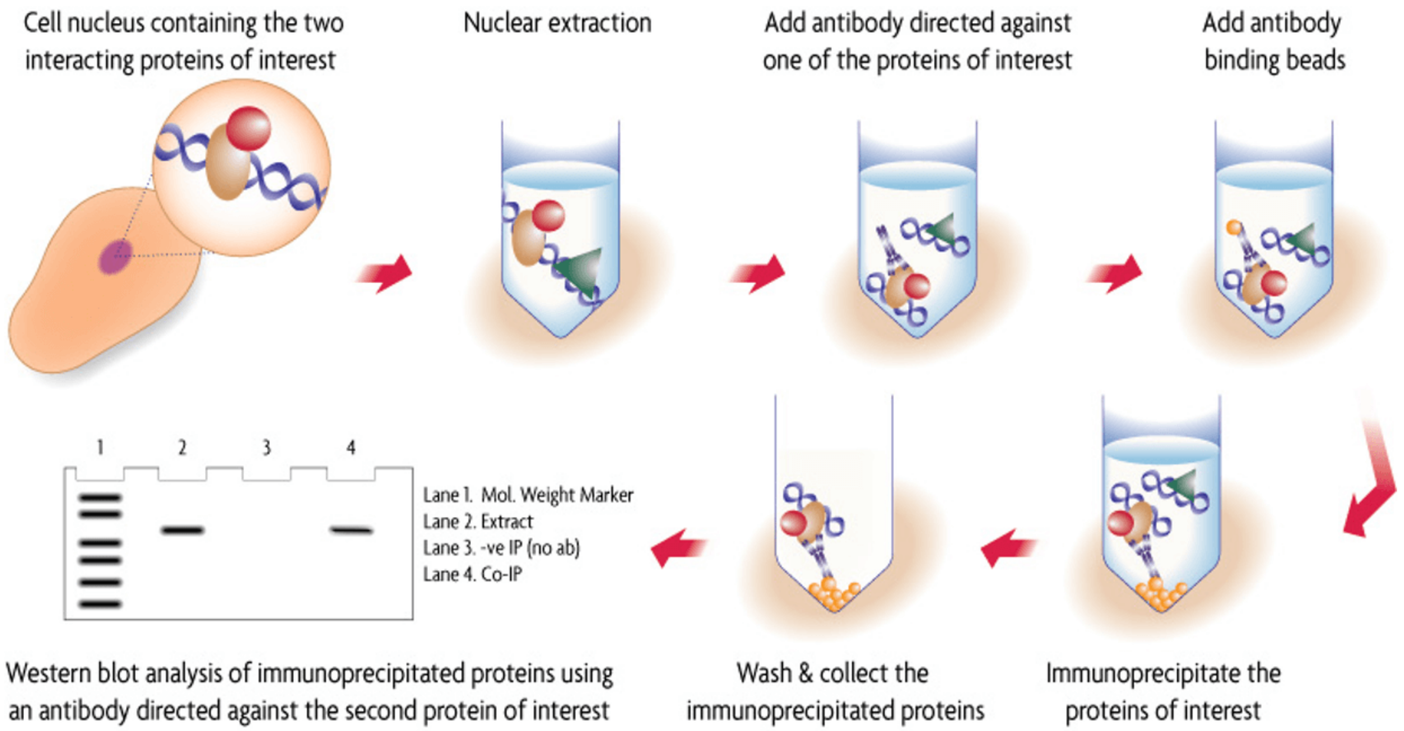
- Model organisms
- Isolating cells and growing them in culture
- Studying proteins
    - Protein sequence
    - Protein purification
    - Protein structure
    - Protein visualization
    - Mass spectrometry

Plan

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

Identifying interacting proteins

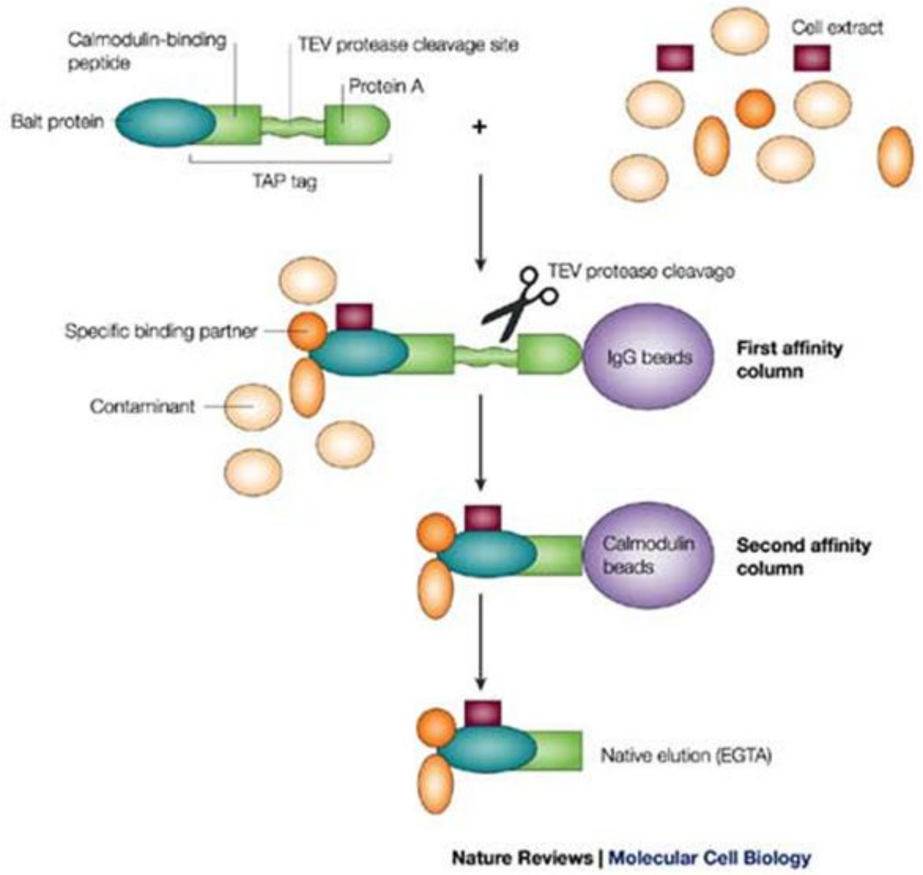
- **co-immunoprecipitation** (or co-IP)



https://www.activemotif.com/catalog/25/nuclear-complex-co-ip-kit

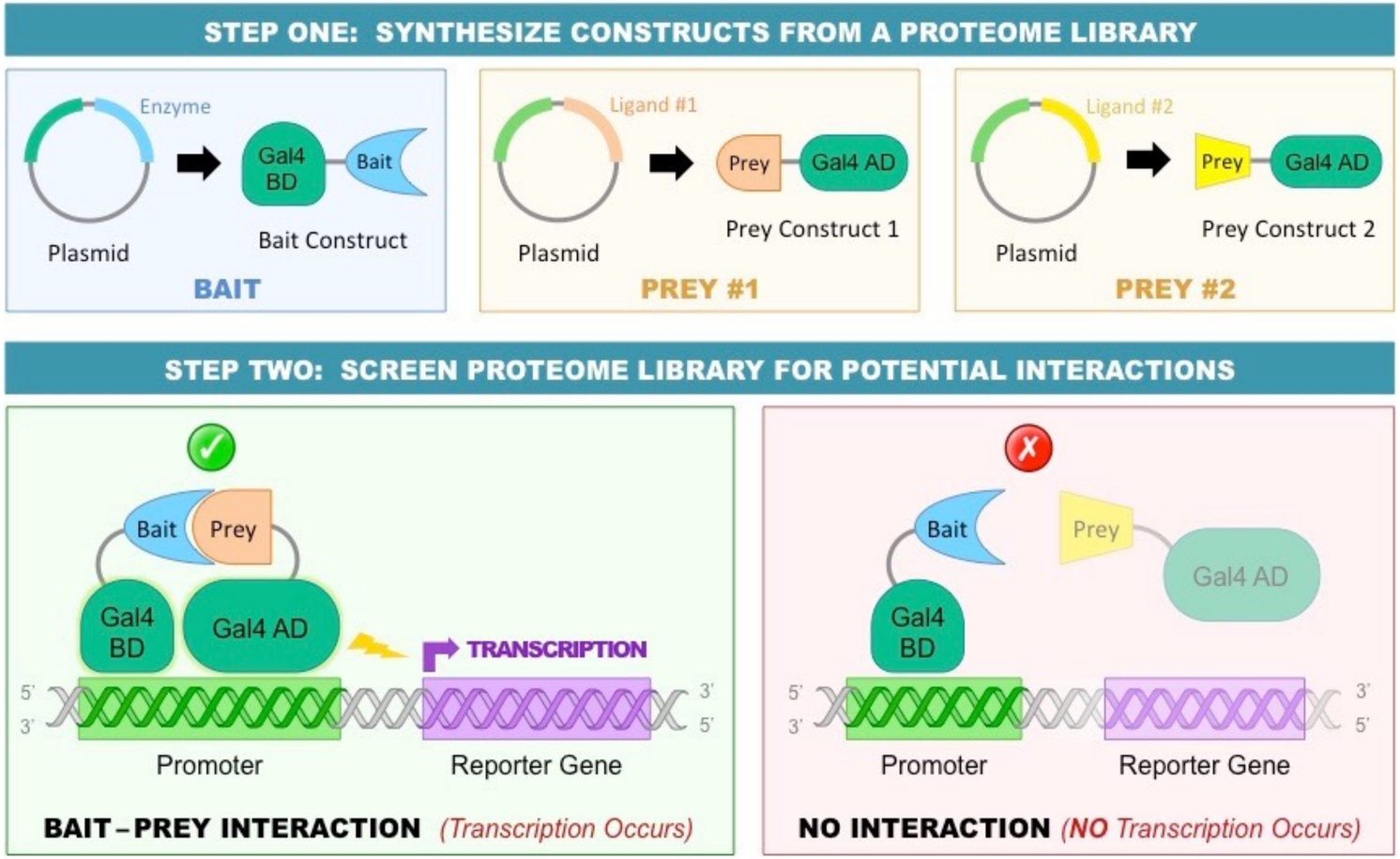
Identifying interacting proteins

- tandem affinity purification (TAP-TAG)



Identifying interacting proteins

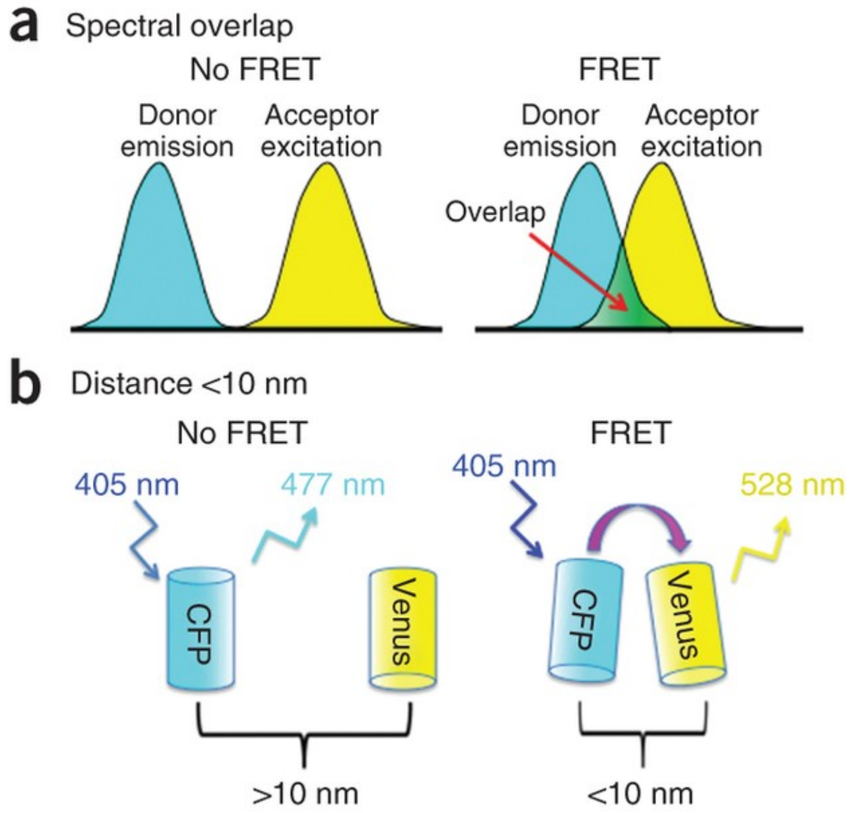
- Yeast/Bacterial two-hybrid



https://old-ib.bioninja.com.au/options/untitled/b4-medicine/yeast-2-hybrid-system.html

Identifying interacting proteins

- FRET = fluorescence resonance energy transfer

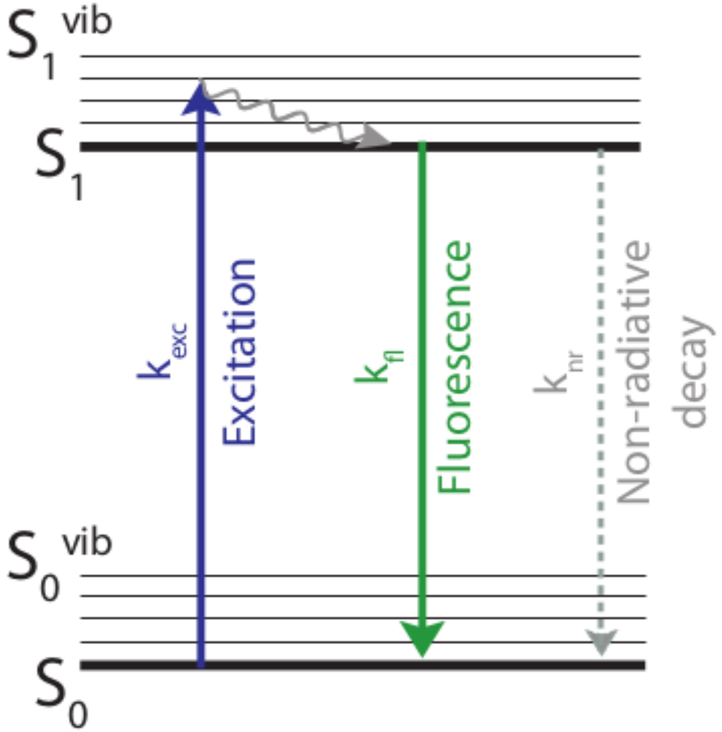


https://www.photometrics.com/learn/physics-and-biophysics/fret

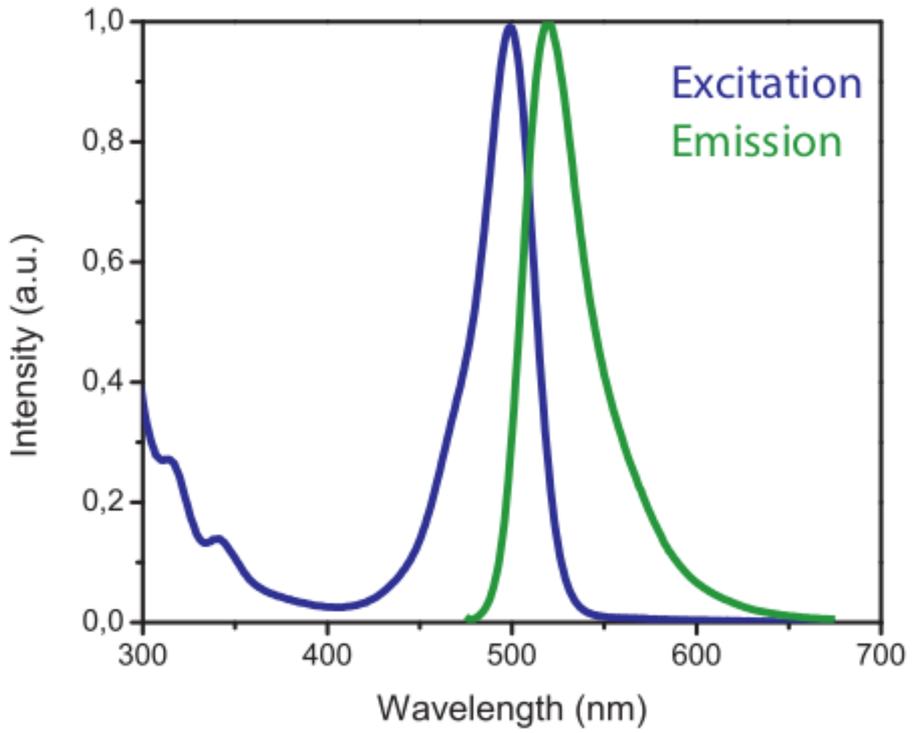
Fluorescence, phosphorescence, luminescence

- 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

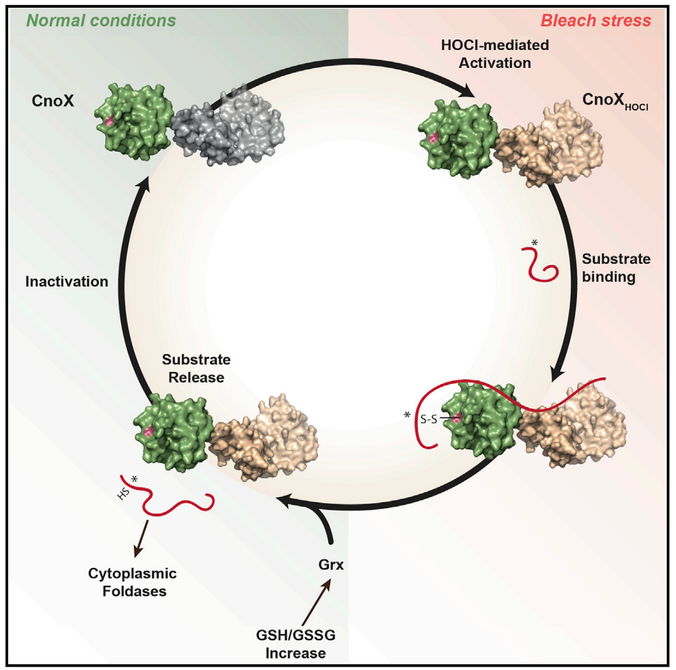
Real-life example

Article

Molecular Cell

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

Graphical Abstract



Authors

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In Brief

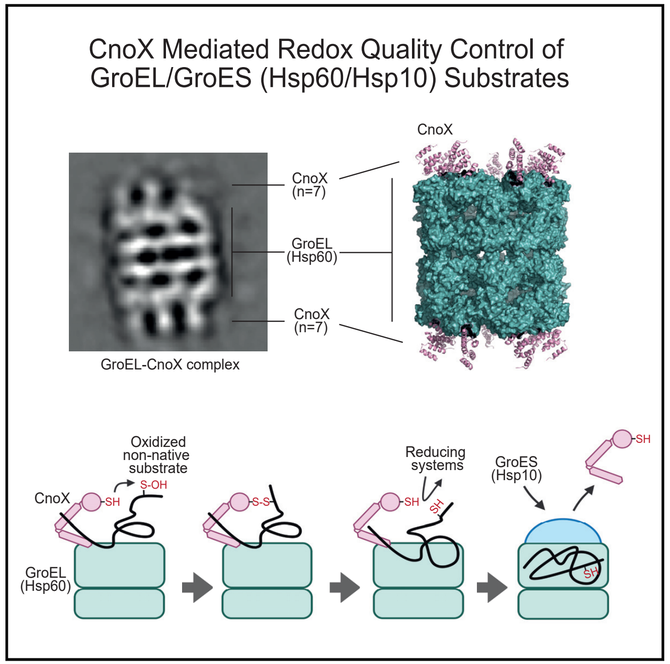
Bleach is a powerful oxidant that kills bacteria by causing protein aggregation. Goemans et al. identified *Escherichia coli* CnoX (YbbN) as a bleach-activated chaperone that uniquely combines holdase activity with the ability to protect its substrates from irreversible oxidation. After bleach stress,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

Article

Cell

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

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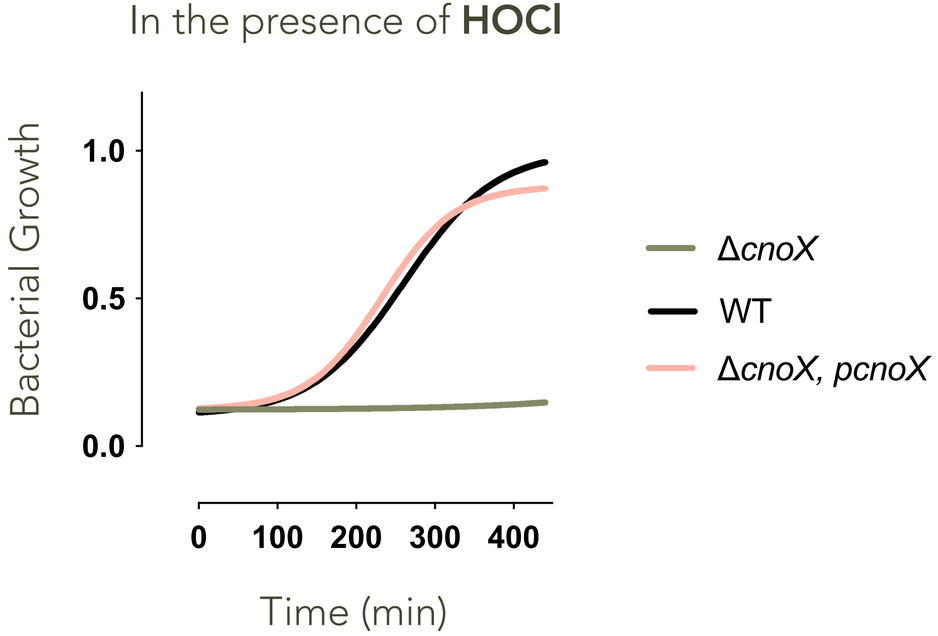
In brief

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

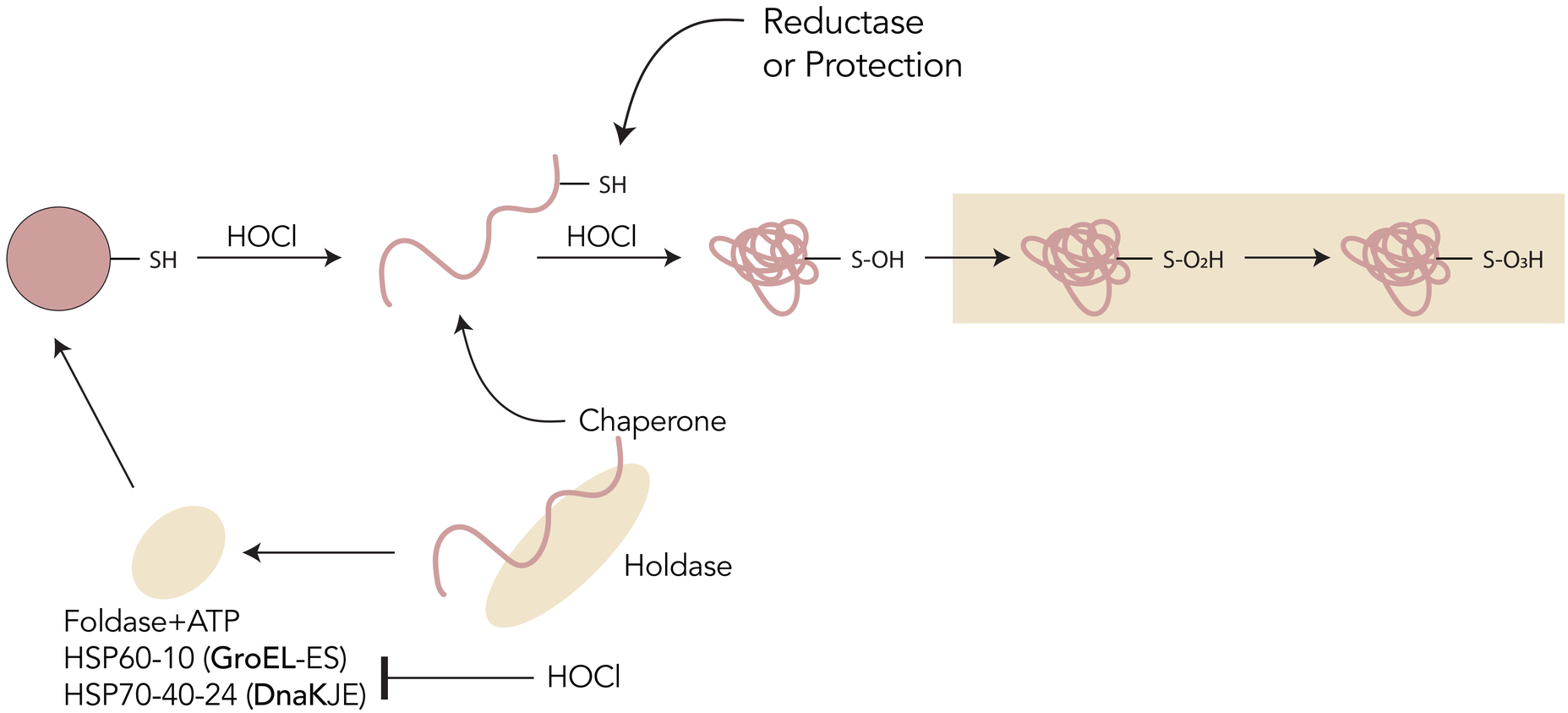
Real-life example



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

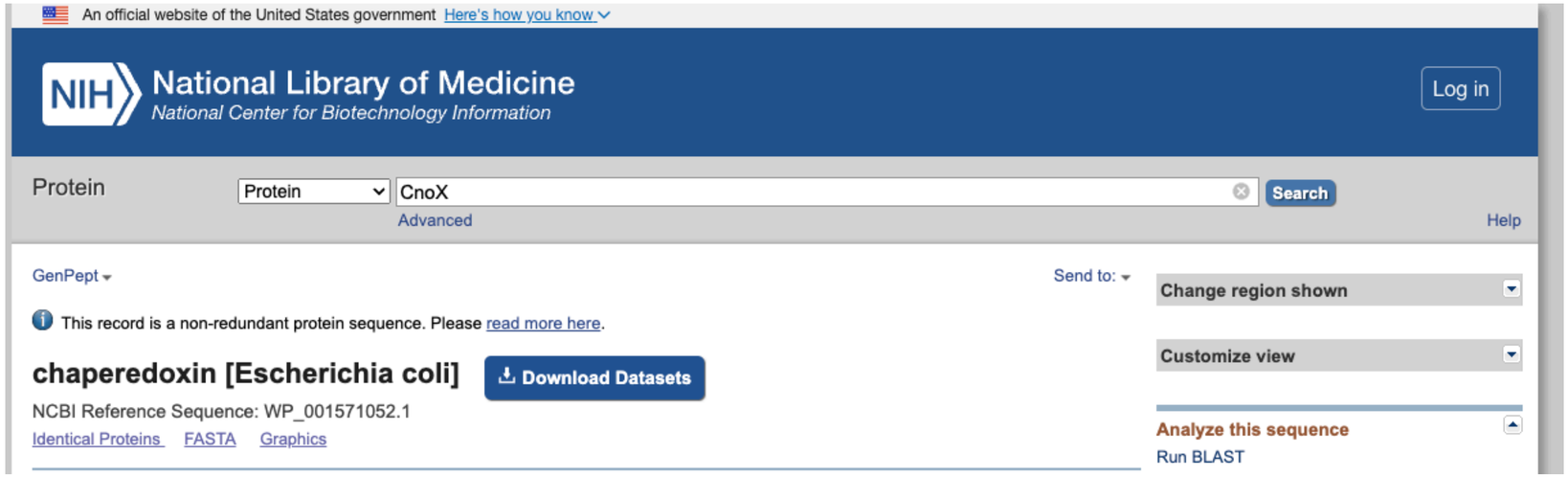
How does this work?

Real-life example



Real-life example

NCBI

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ORIGIN
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      121 amqlmqegny tdalpllkda wqlsnqngei glllaetlia lnrsedaeav lktiplqdqd
      181 tryqglvaqi ellkqaadtp eiqqlqqqva enpedaalat qlalqlhqvg rneealellf
      241 ghlrkdltaa dgqtrktfqe ilaalgtgda laskyrrqly ally
//
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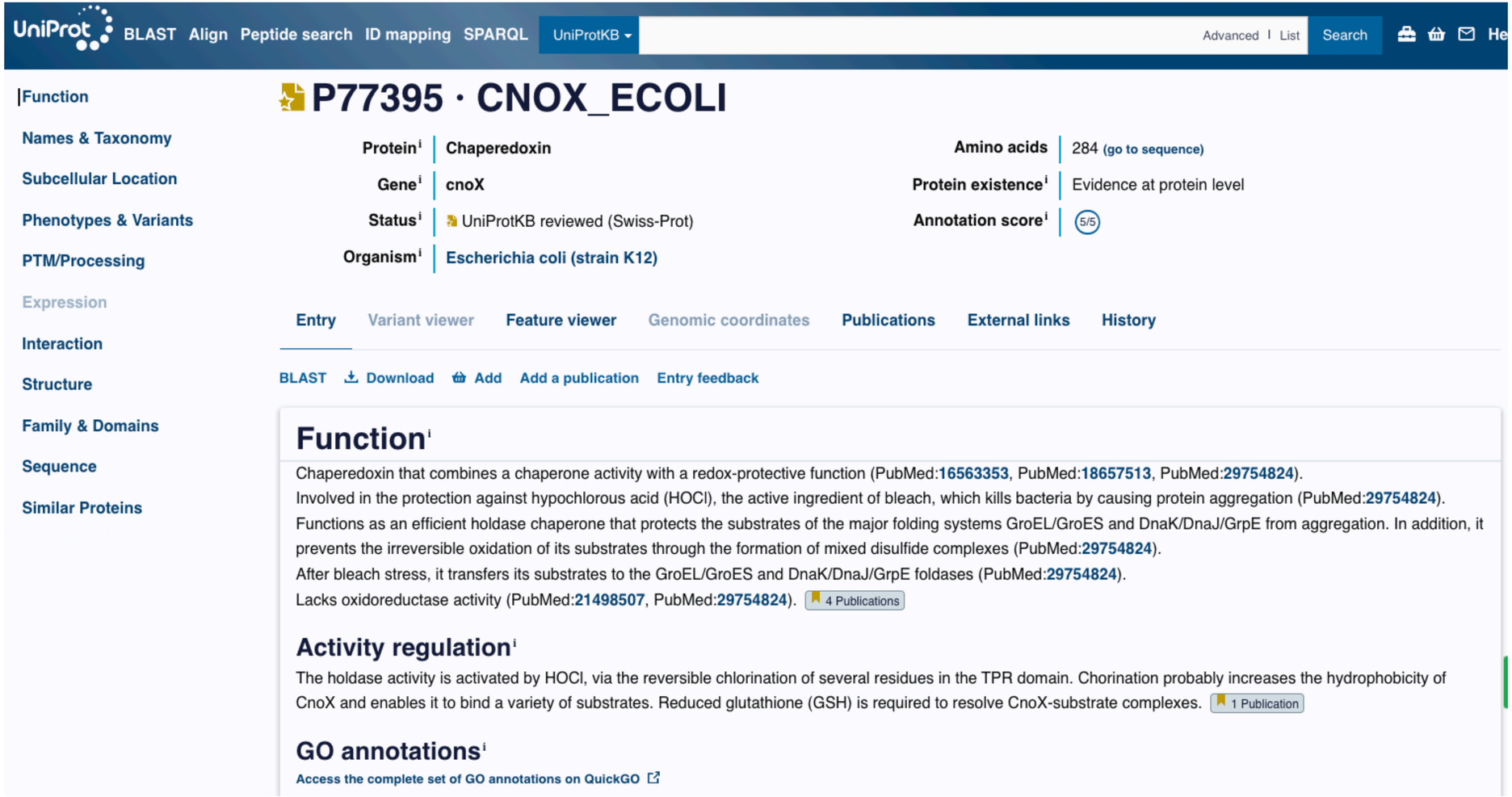
Protein sequence

Real-life example

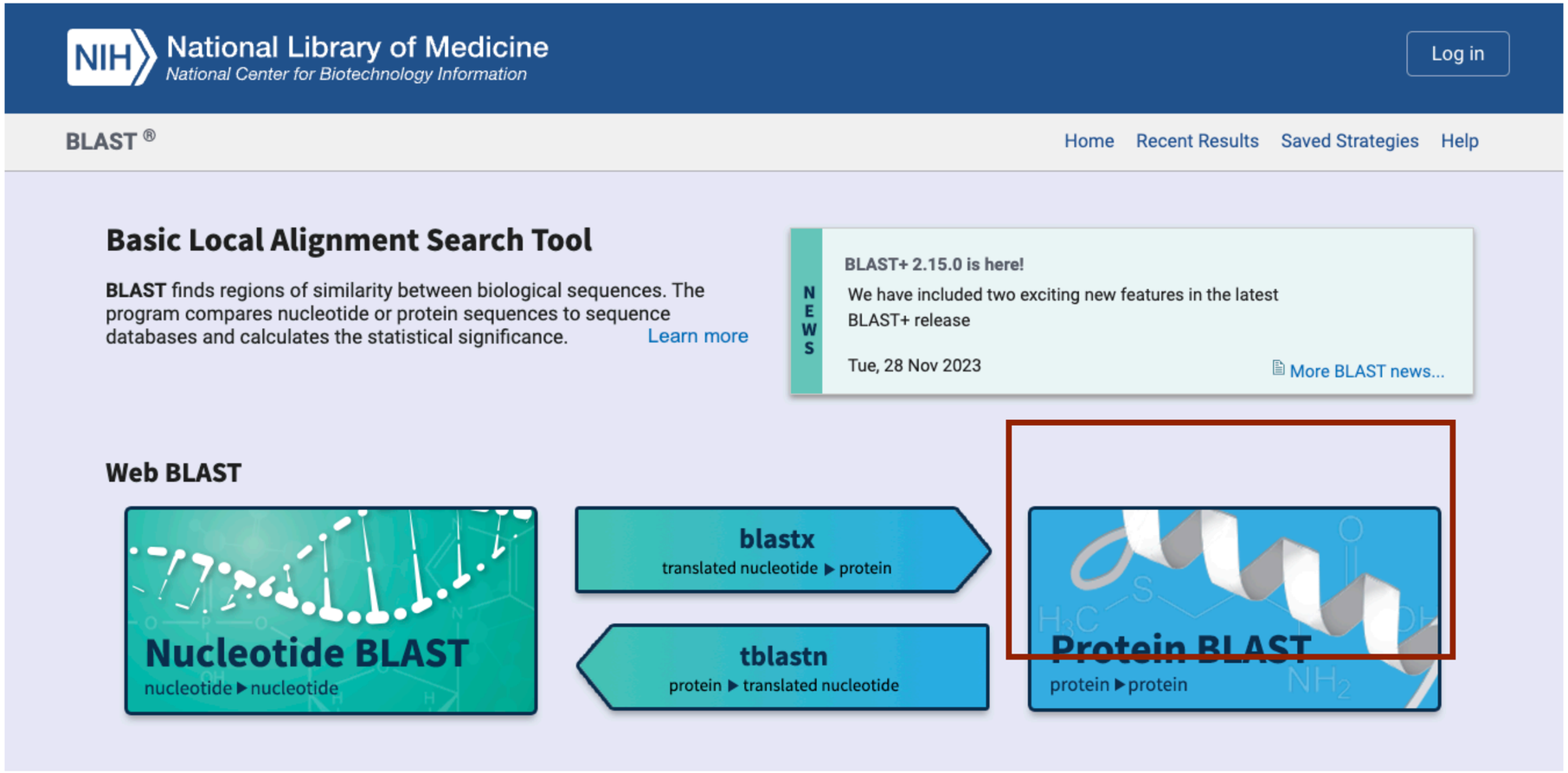
Uniprot



https://www.uniprot.org/uniprotkb/P77395/entry

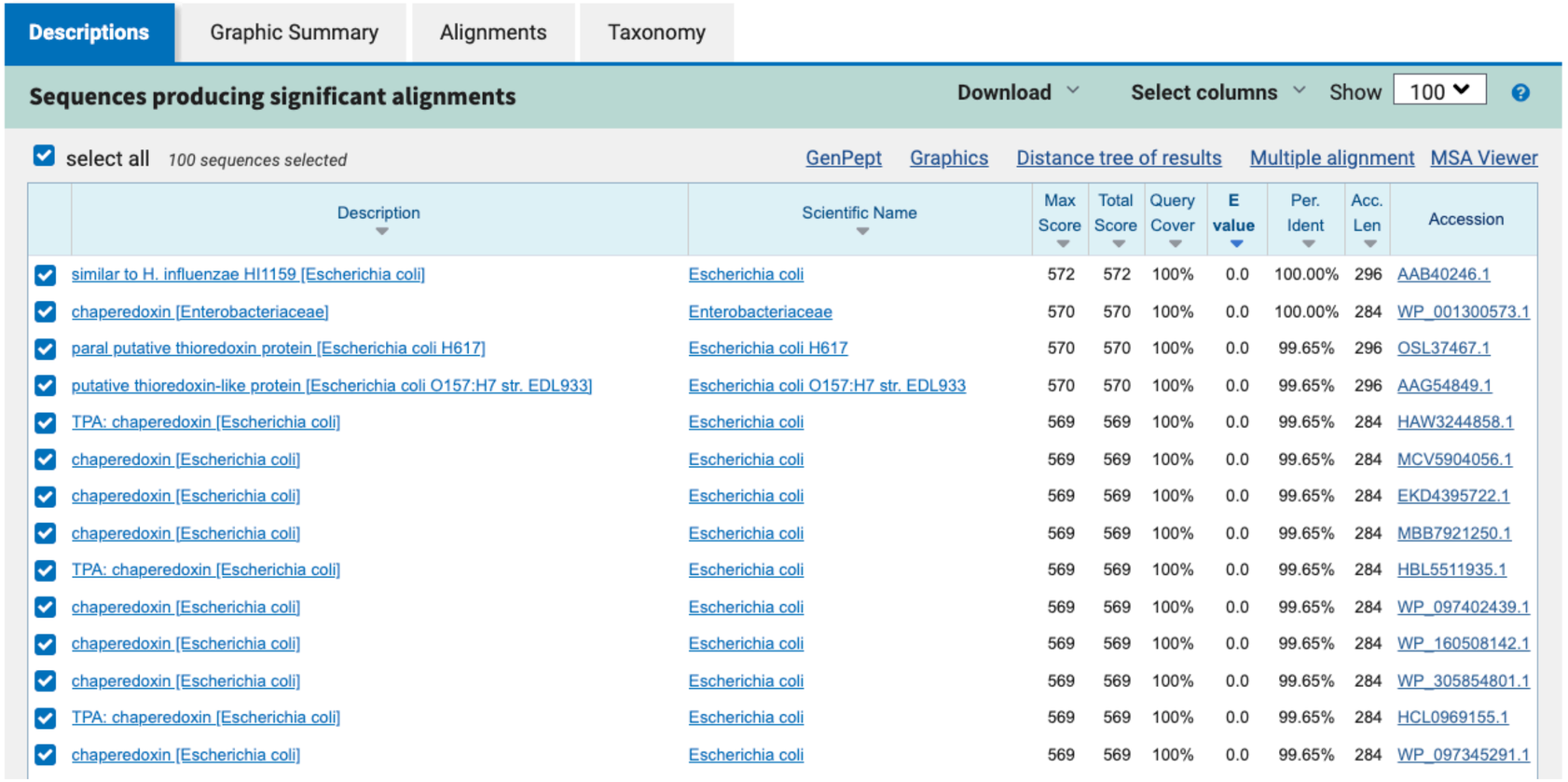
Real-life example

BLAST



Real-life example

BLAST



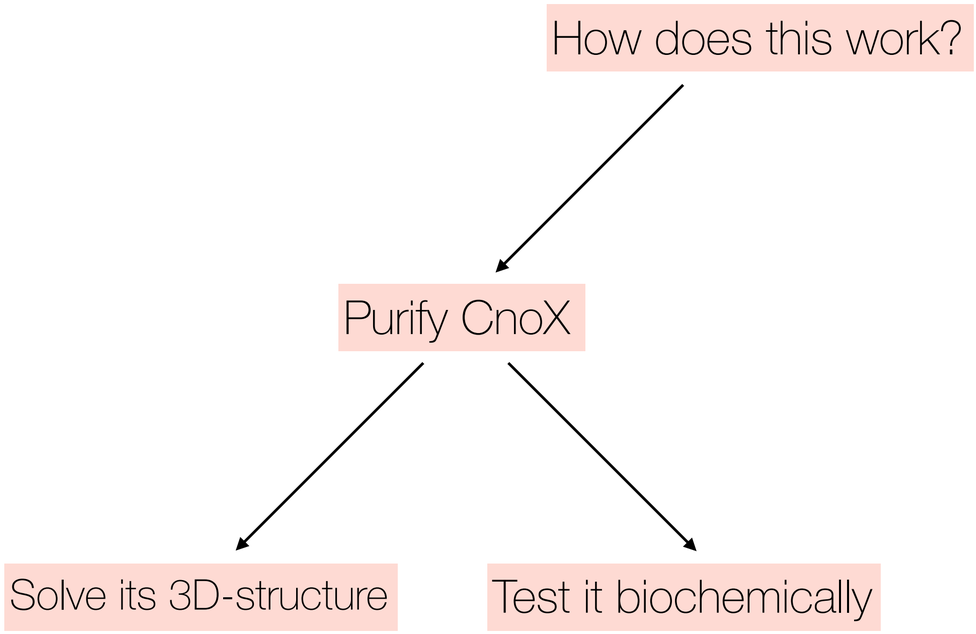
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all *100 sequences selected* — GenPept — Graphics — Distance tree of results — Multiple alignment — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
similar to H. influenzae HI1159 [Escherichia coli]	Escherichia coli	572	572	100%	0.0	100.00%	296	AAB40246.1
chaperedoxin [Enterobacteriaceae]	Enterobacteriaceae	570	570	100%	0.0	100.00%	284	WP_001300573.1
paral putative thioredoxin protein [Escherichia coli H617]	Escherichia coli H617	570	570	100%	0.0	99.65%	296	OSL37467.1
putative thioredoxin-like protein [Escherichia coli O157:H7 str. EDL933]	Escherichia coli O157:H7 str. EDL933	570	570	100%	0.0	99.65%	296	AAG54849.1
TPA: chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	HAW3244858.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	MCV5904056.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	EKD4395722.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	MBB7921250.1
TPA: chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	HBL5511935.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	WP_097402439.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	WP_160508142.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	WP_305854801.1
TPA: chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	HCL0969155.1
chaperedoxin [Escherichia coli]	Escherichia coli	569	569	100%	0.0	99.65%	284	WP_097345291.1

Real-life example



Real-life example

Solve its 3D-structure with alpha fold

Chaperedoxin

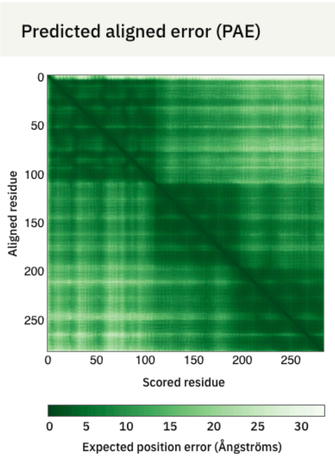
AlphaFold structure prediction

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) go to search
UniProt	P77395 go to UniProt
Experimental structures	2 structures in PDB for P77395 go to PDBe-KB
Biological function	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 ... [show more] go to UniProt

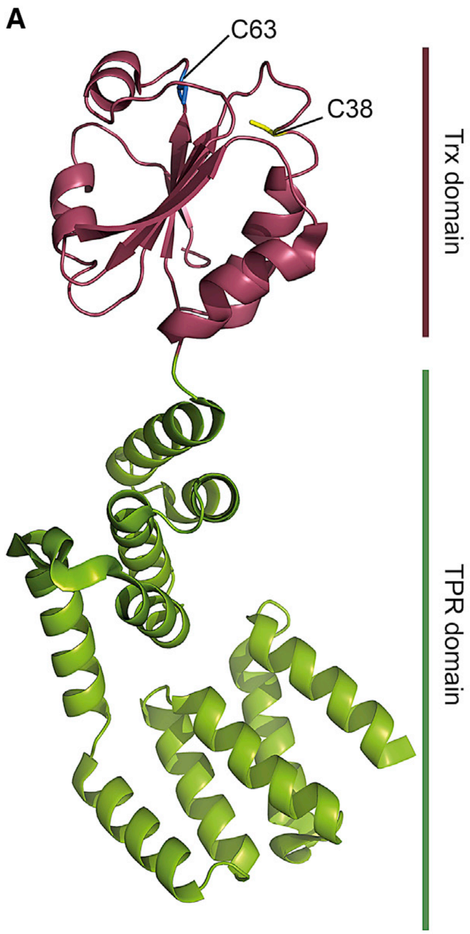


Click and drag a box on the PAE viewer to select regions of the structure and highlight them on the 3D viewer.

PAE data is useful for assessing inter-domain accuracy – go to Help section below for more information.

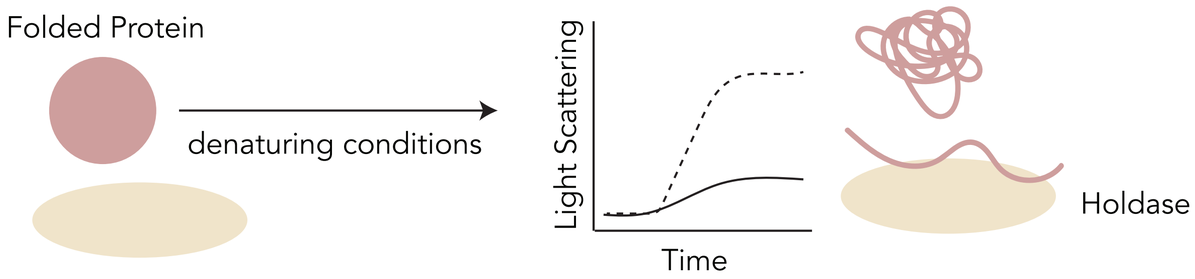
Real-life example

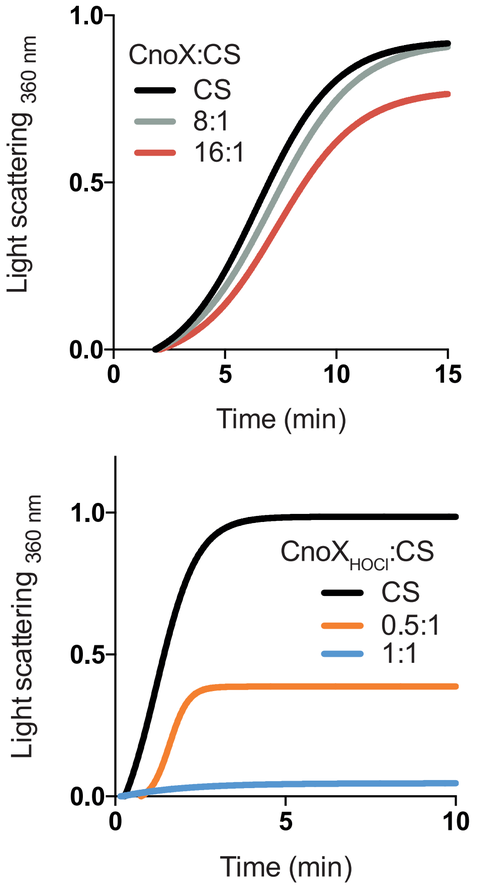
Solve its 3D-structure (cristallography)



Real-life example

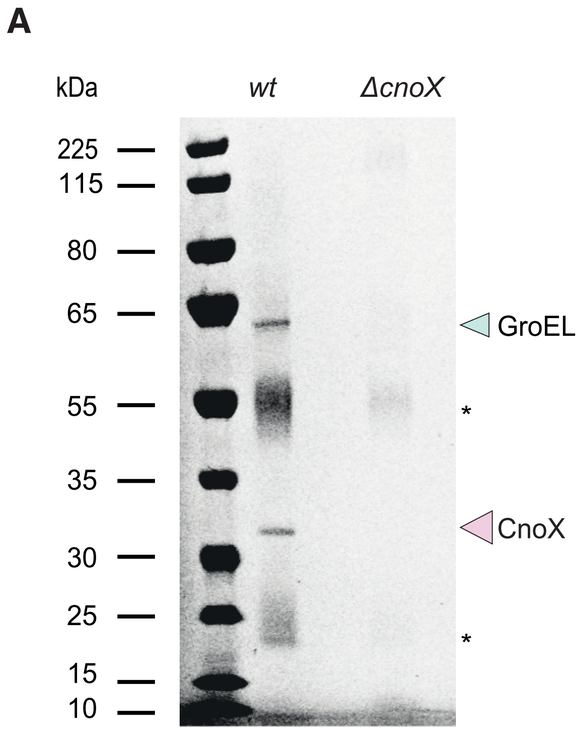
Test it biochemically





Real-life example

Find the partners: Co-IP +SDS-PAGE

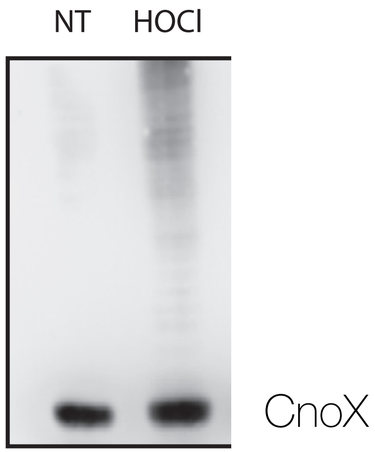


- co-IP using a-CnoX antibodies
- SDS-PAGE: we only detect one other band
- Mass spectrometry: this band is GroEL

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
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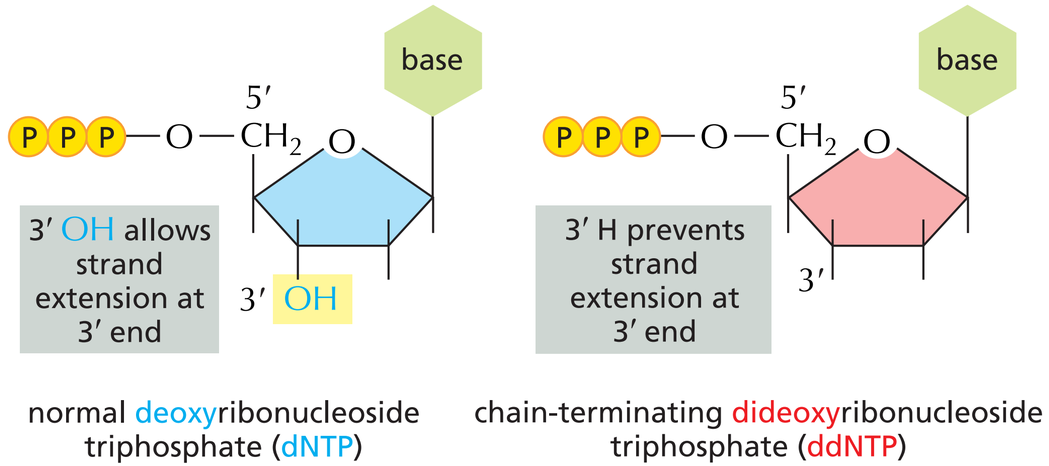
- Studying DNA
    - **DNA sequencing**
    - DNA extraction
    - DNA amplification

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 synthetized, 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)

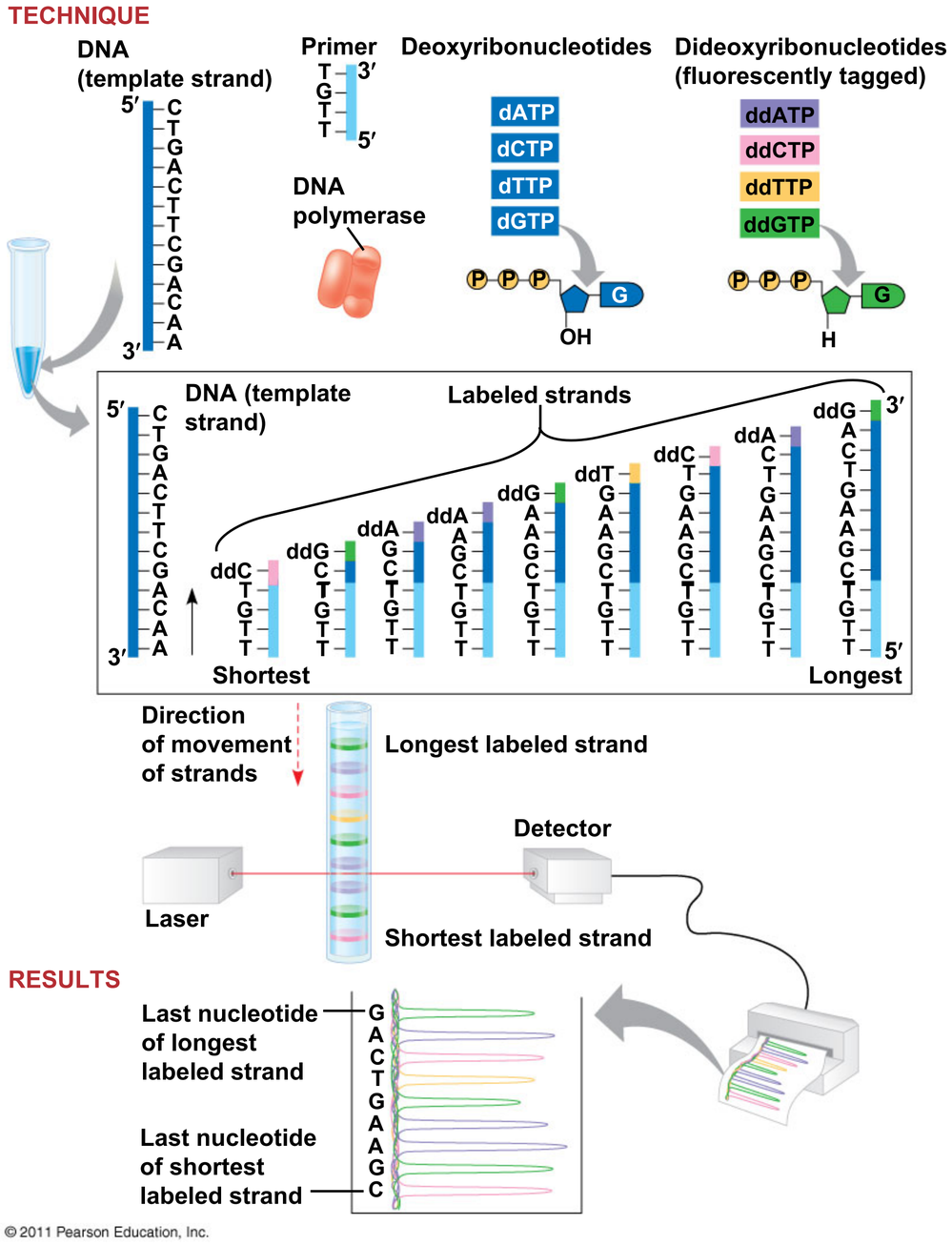
DNA sequencing

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



DNA sequencing

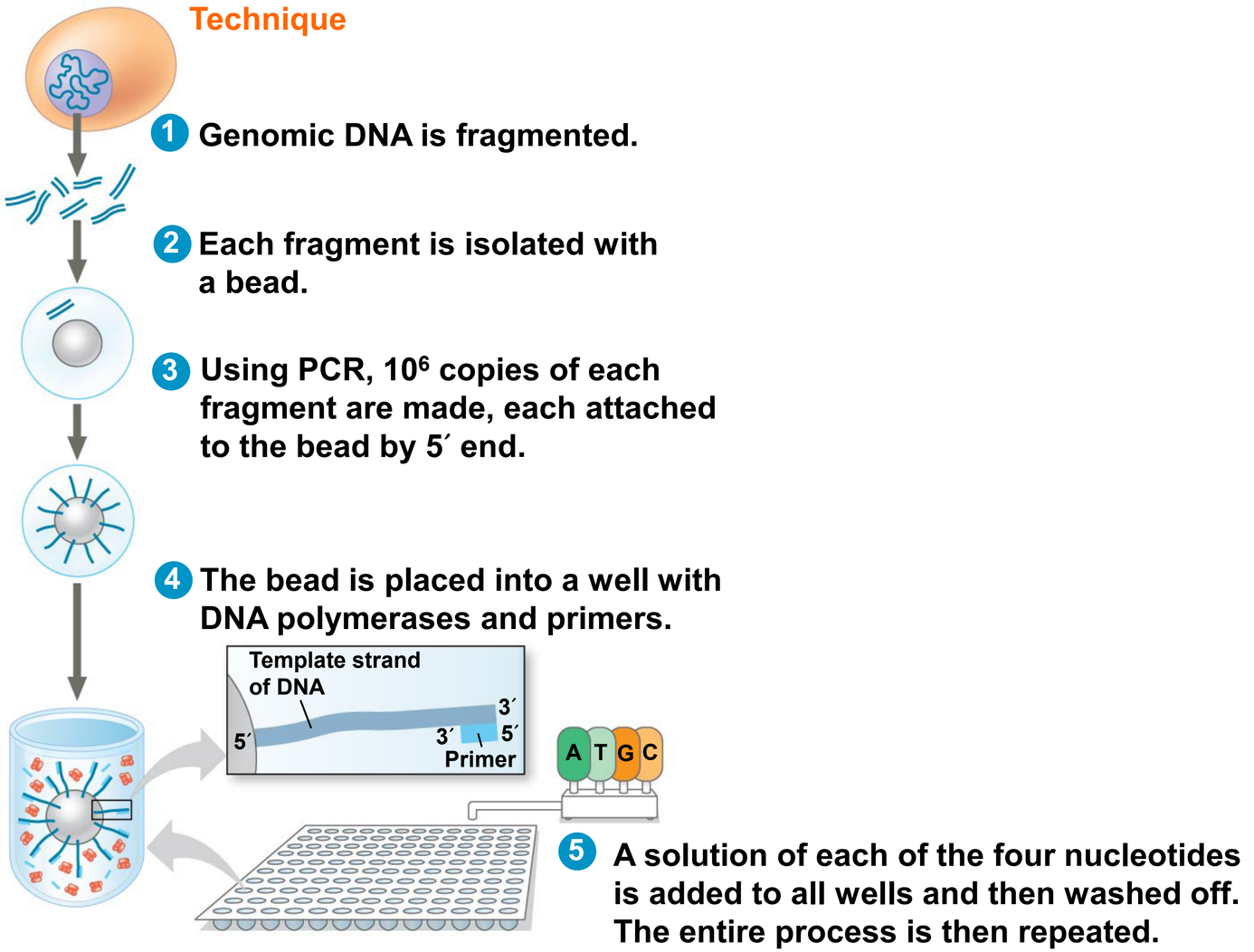
- **Sanger or dideoxy sequencing** relies on dideoxy nucleotides that terminate elongation
  - low cost, small scale (short DNA fragments)



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

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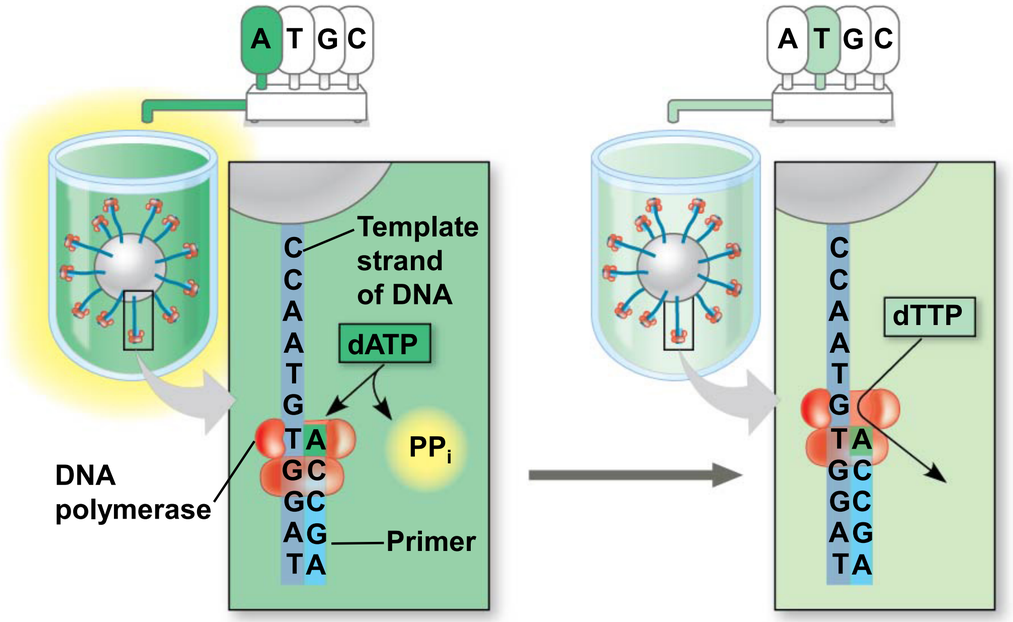
Technique



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)
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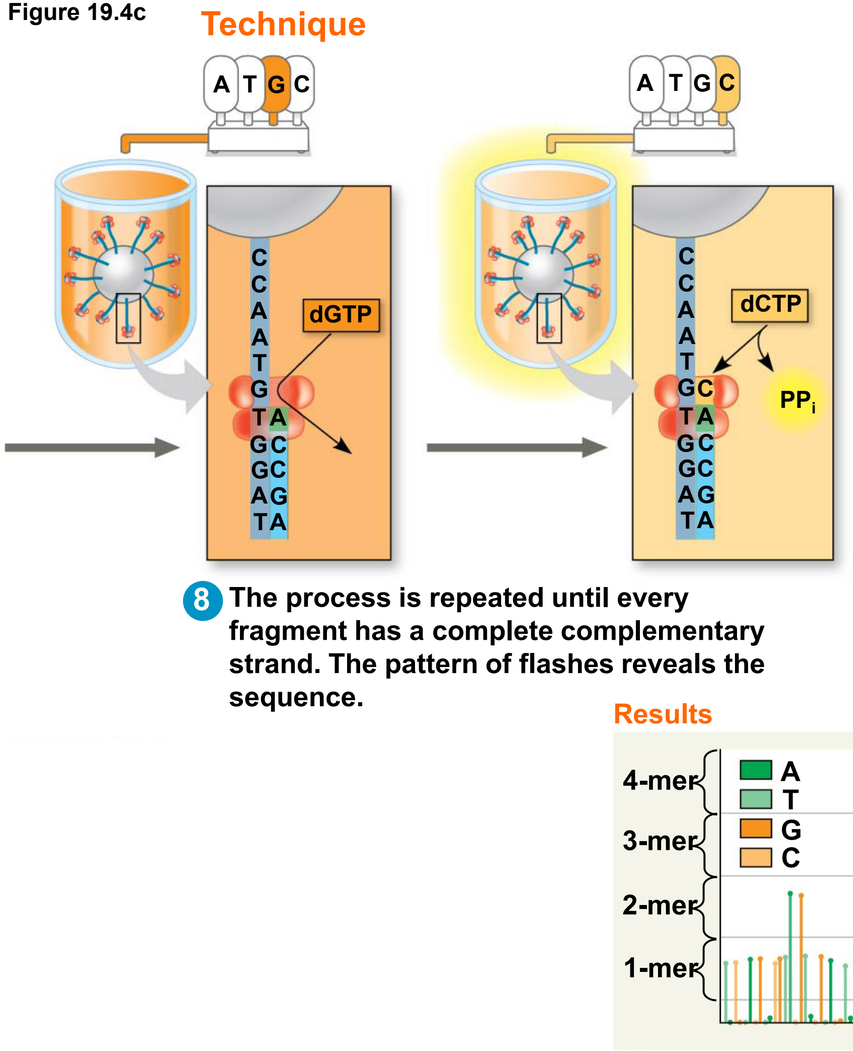
Figure 19.4c



DNA sequencing

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

Gene annotations

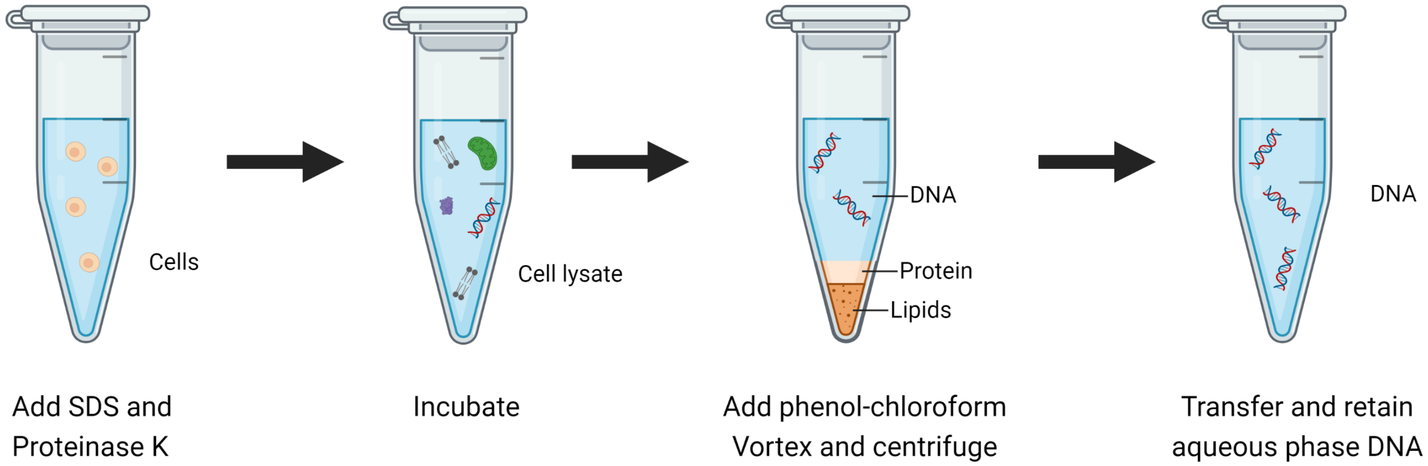
- **Mark** the genes
- Assign possible **roles**

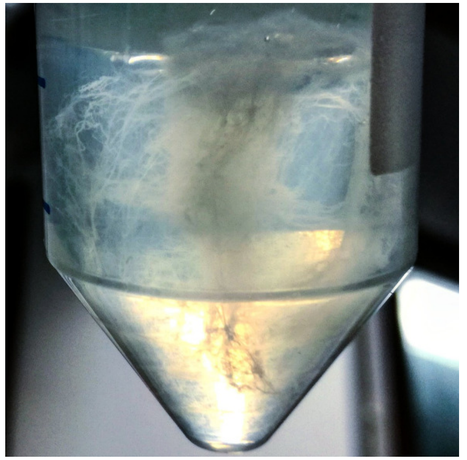
Plan

- 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

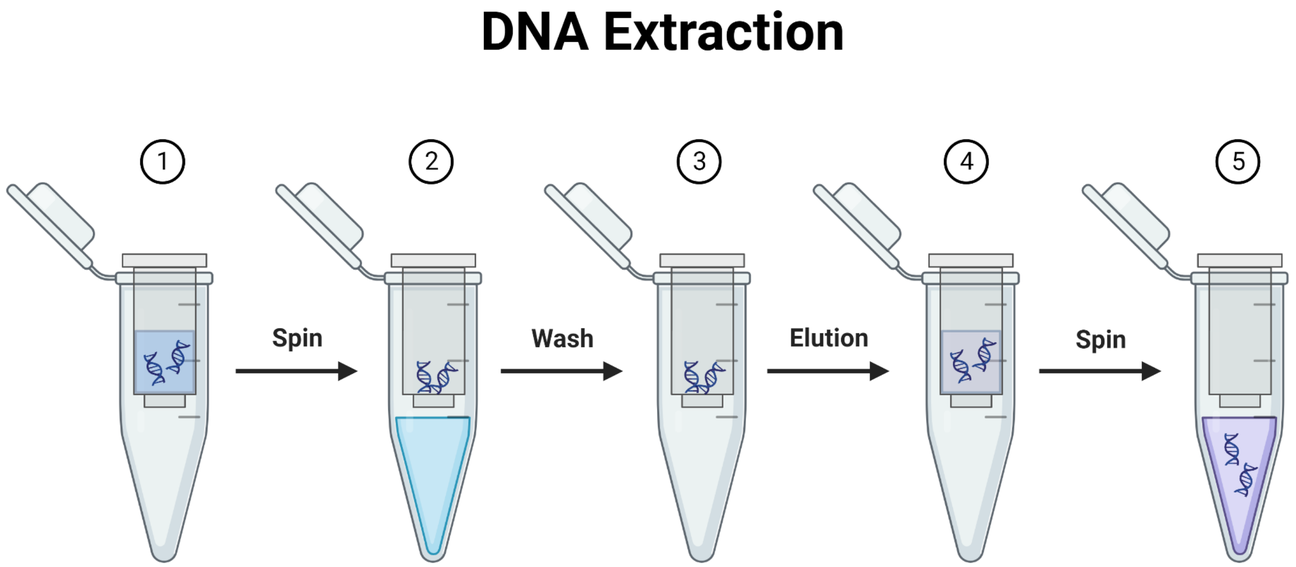




- Phenol-chloroform is less polar than water and induces protein aggregation
- DNA is further precipitated with ethanol

But how is DNA extracted?

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



Plan

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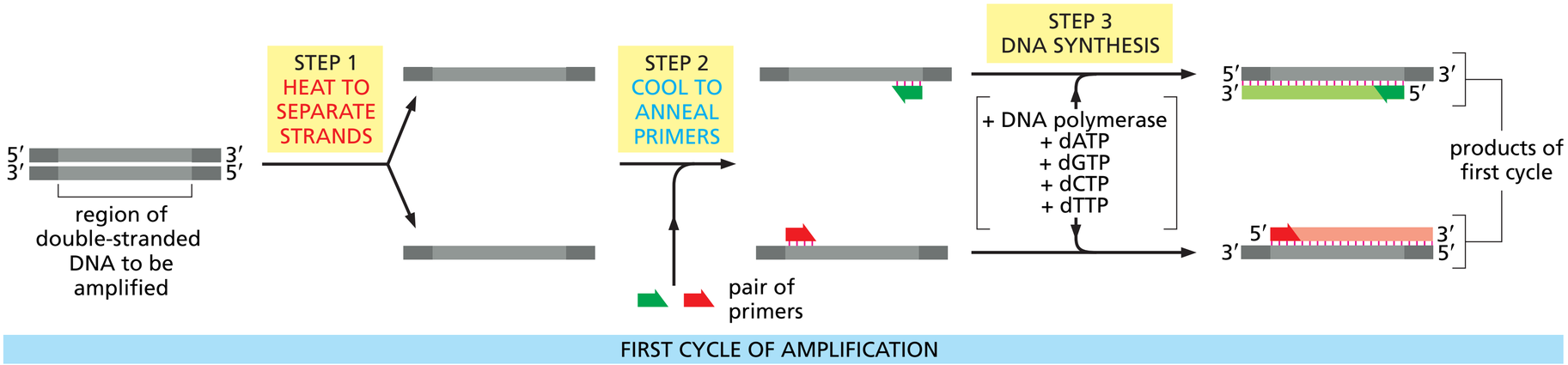
    - Real-life examples
- Studying DNA
    - DNA sequencing
    - DNA extraction
    - **DNA amplification**

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

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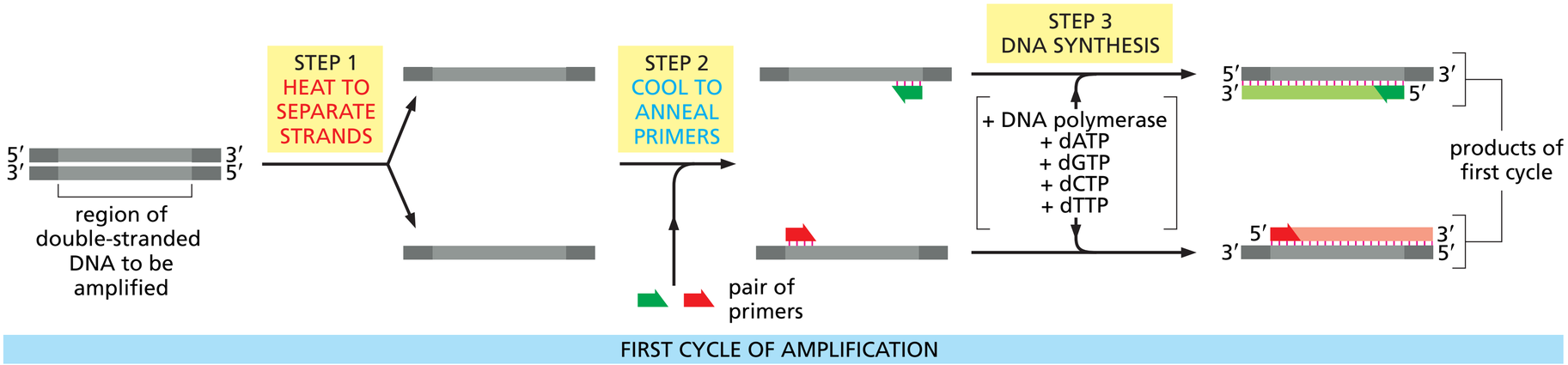
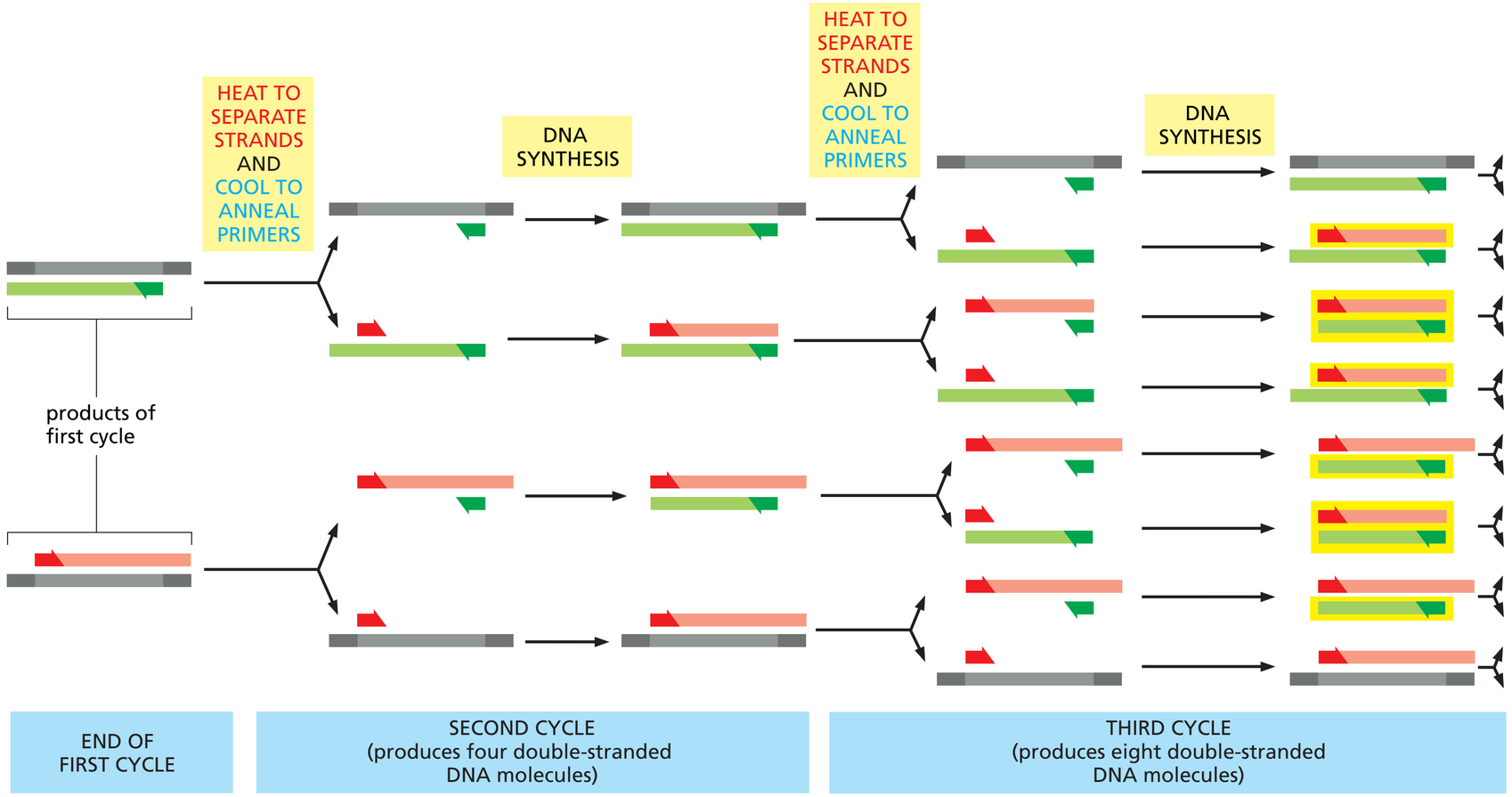


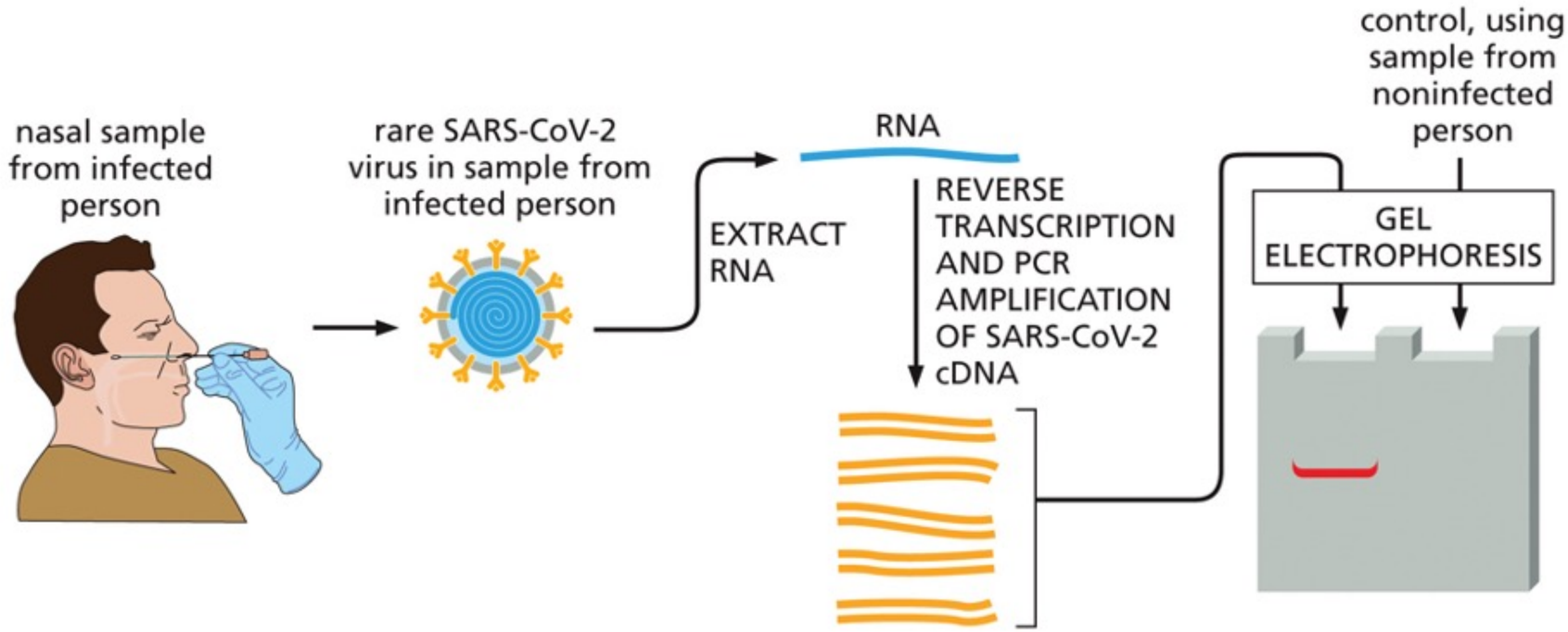
Amplifying specific regions of DNA

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



Amplifying specific regions of DNA

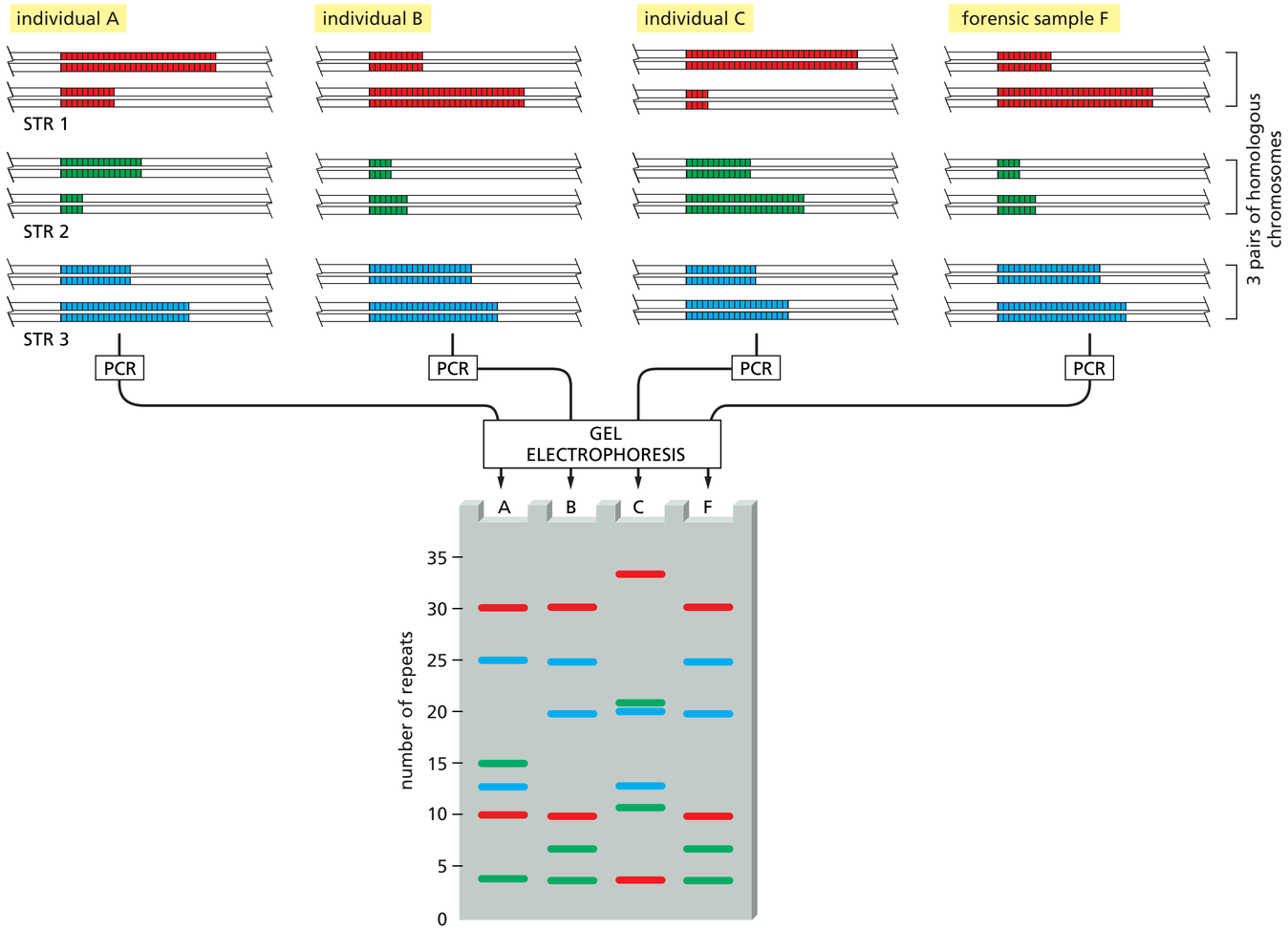
- Polymerase Chain Reaction (**PCR**) for specific **DNA region amplification - diagnostic or forensics**



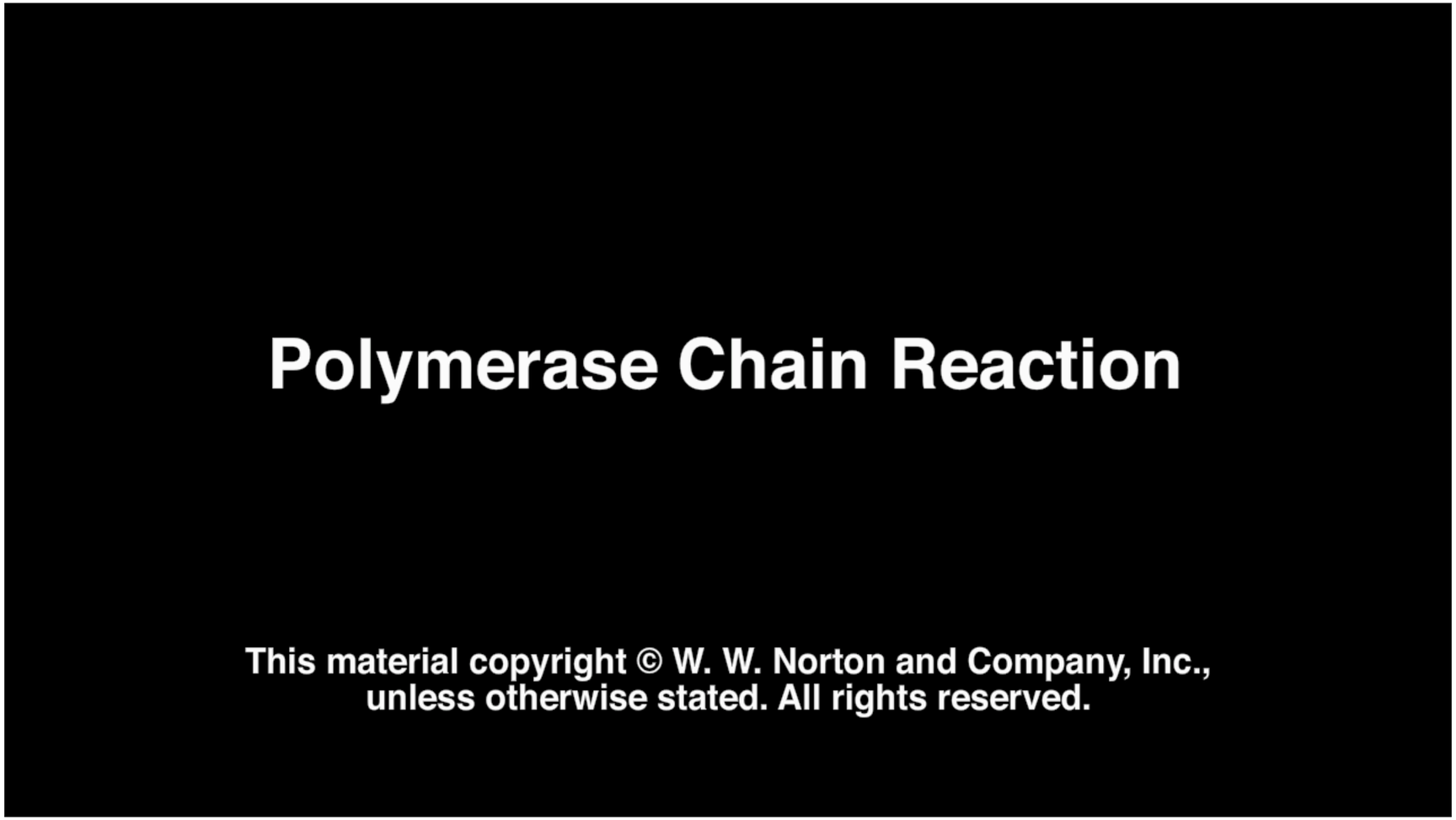
Amplifying specific regions of DNA

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

STR = short tandem repeats



Amplifying specific regions of DNA



Summary

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

Have a nice day!