General Introduction to Biology

Prof. Sebastian Maerkl

Purpose of the laboratory course

- Introduce engineers to basic concepts in biology, biological engineering, biotechnology, and synthetic biology
- Provide hands on laboratory experience to acquire the necessary skills to conduct biological experiments

- No prior knowledge in biology is required…

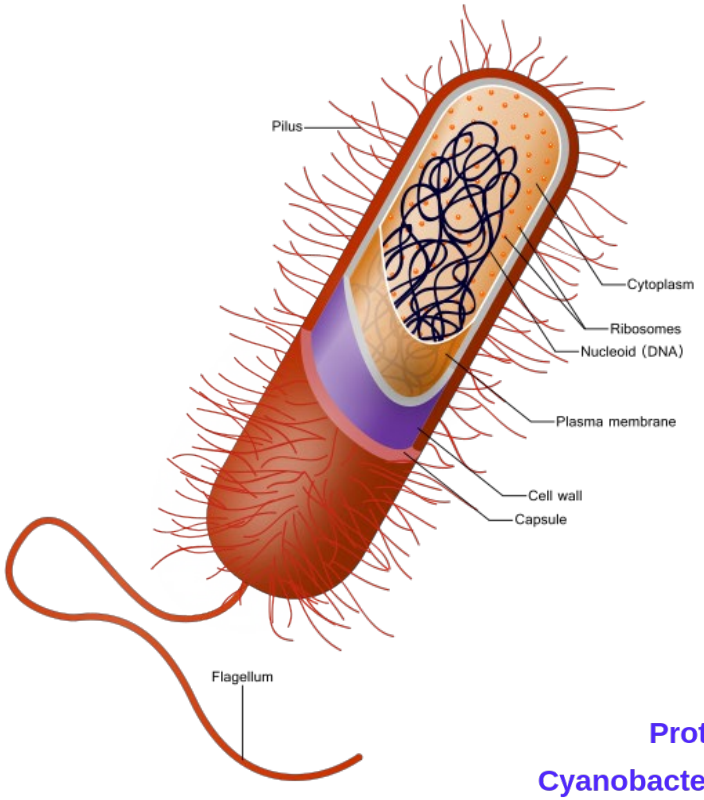
Course Content

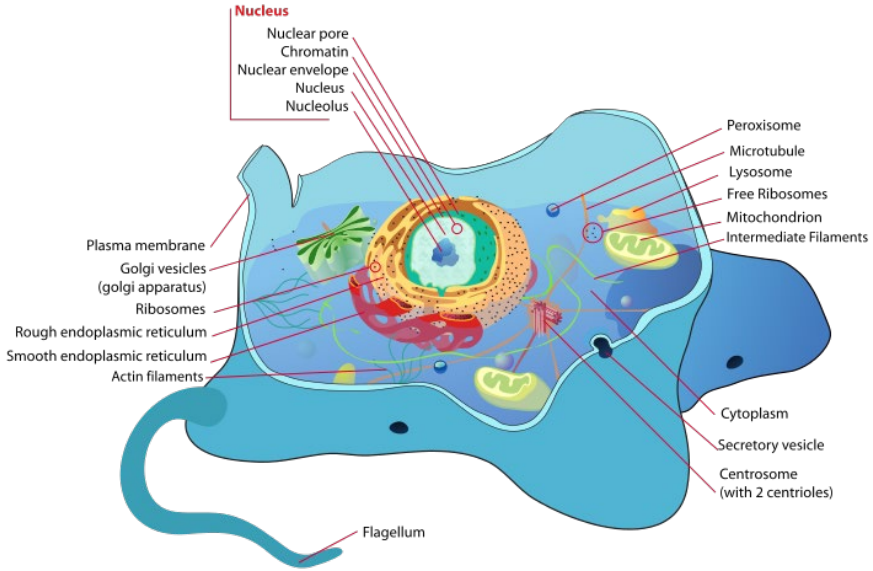
We will cover these main topics:

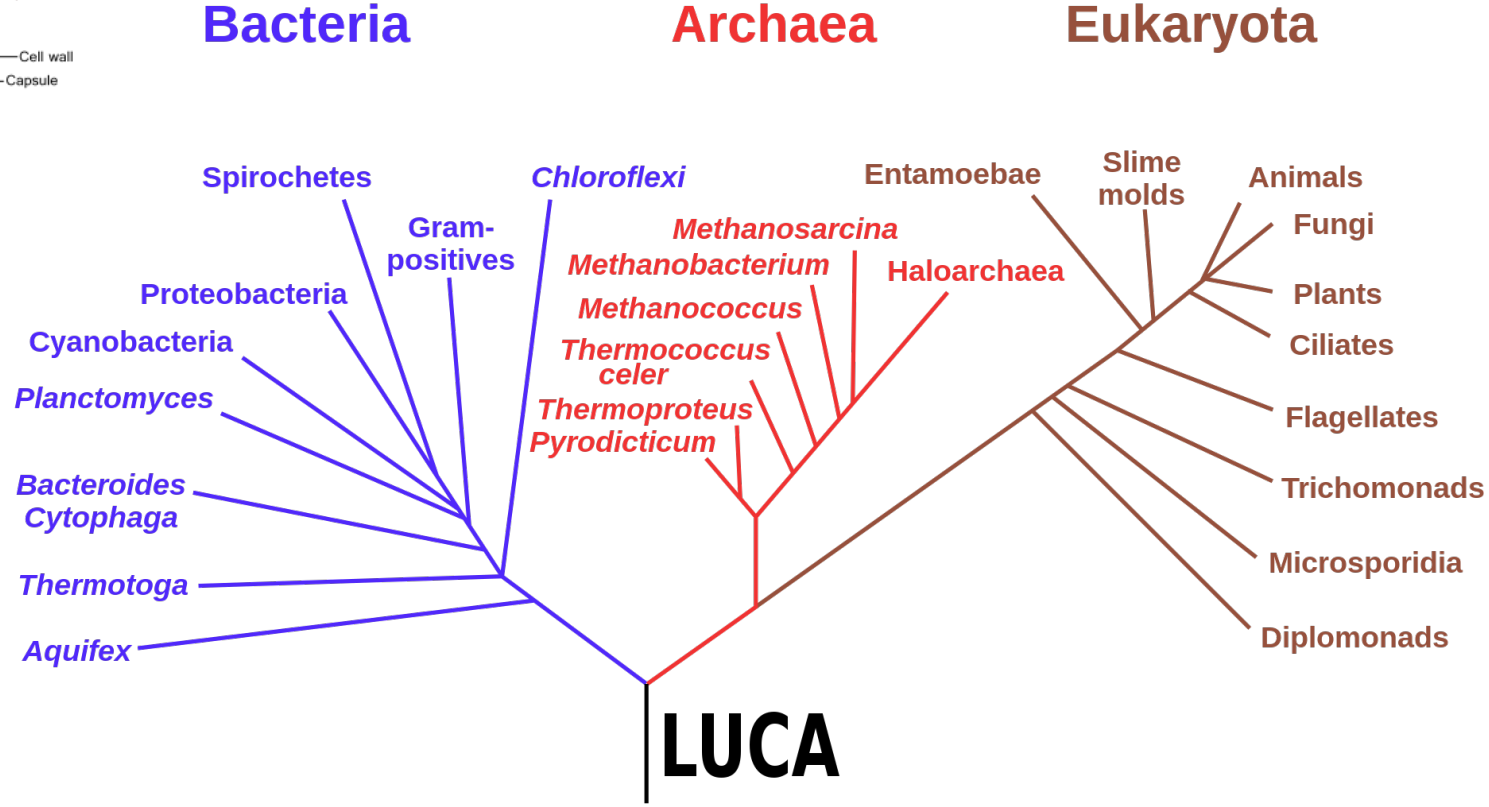
- Cell culturing techniques
- Molecular Cloning
- PCR
- Protein expression (fermentation)
- Protein purification
- Cell-free transcription-translation (cell-free synthetic biology)

A brief introduction to biology

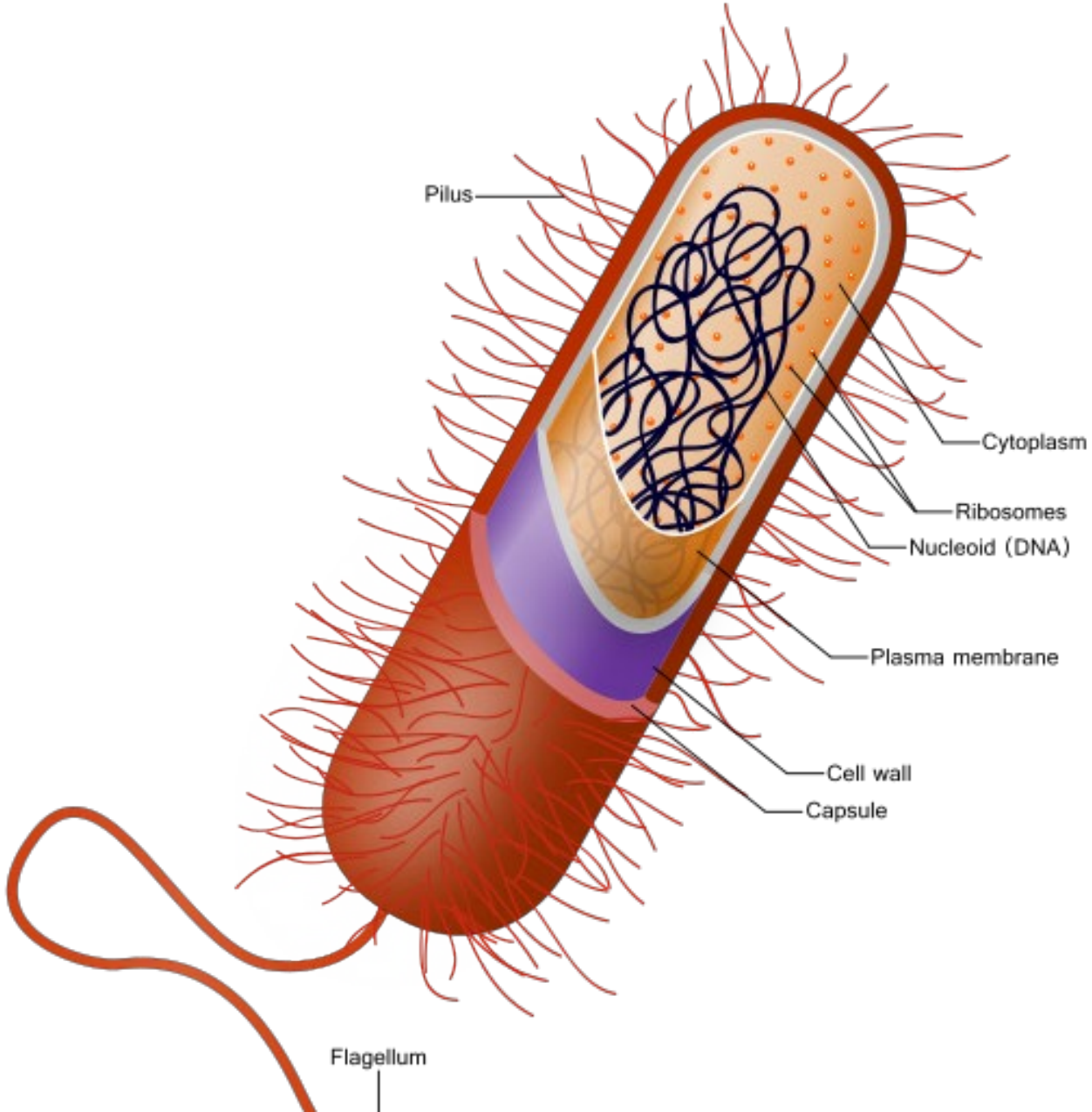
The tree of life



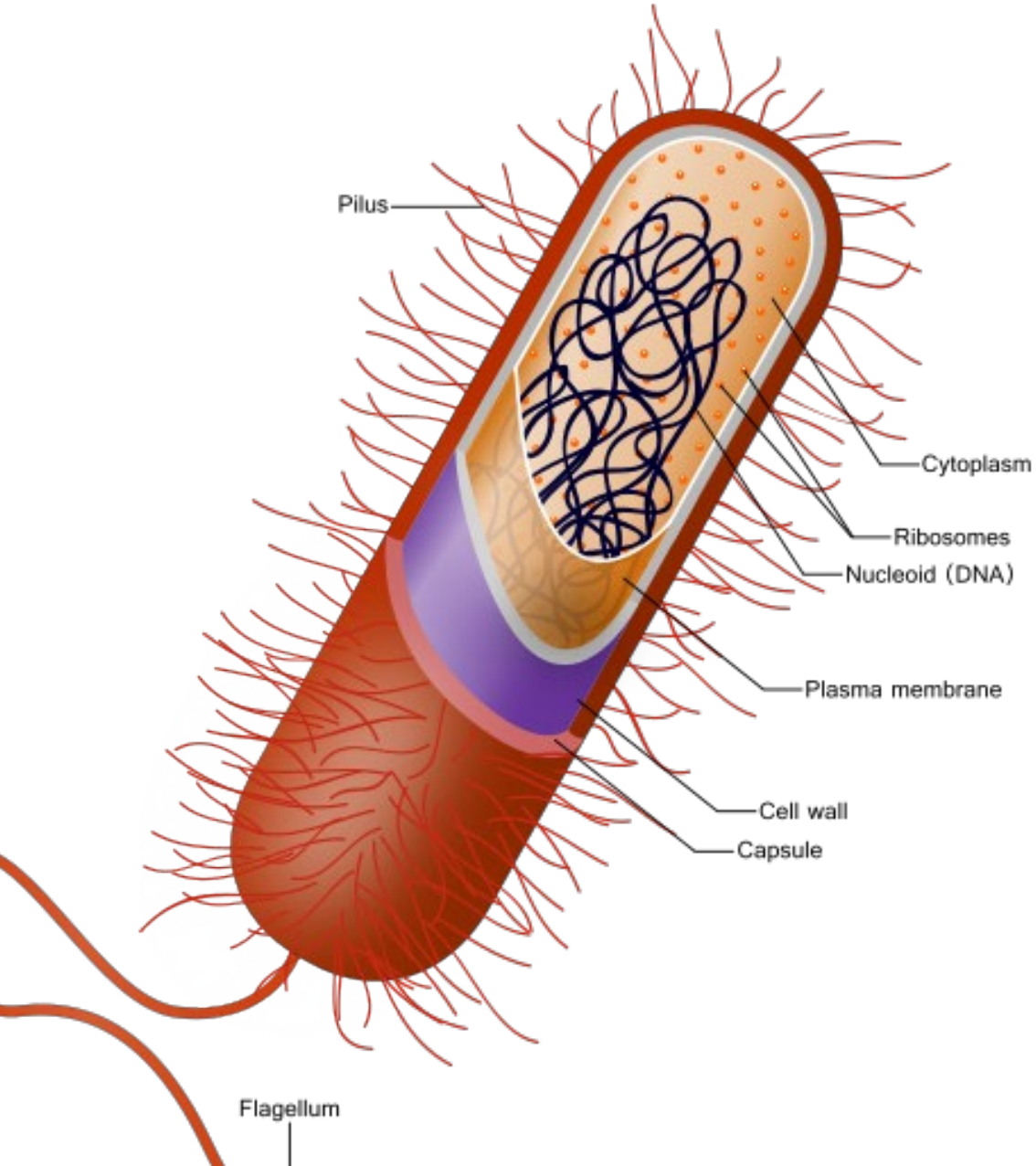


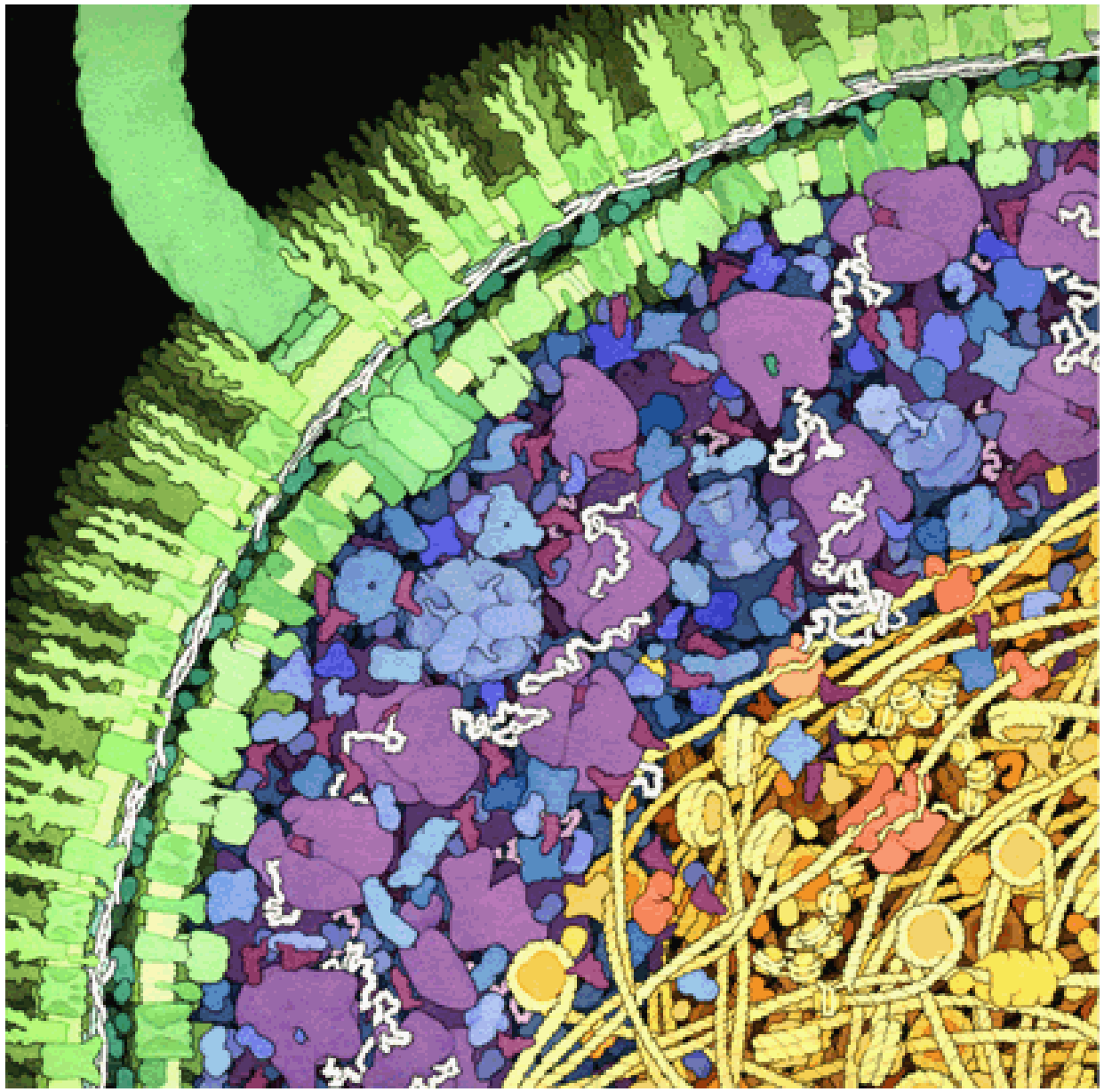


Cell Physiology Prokaryotes

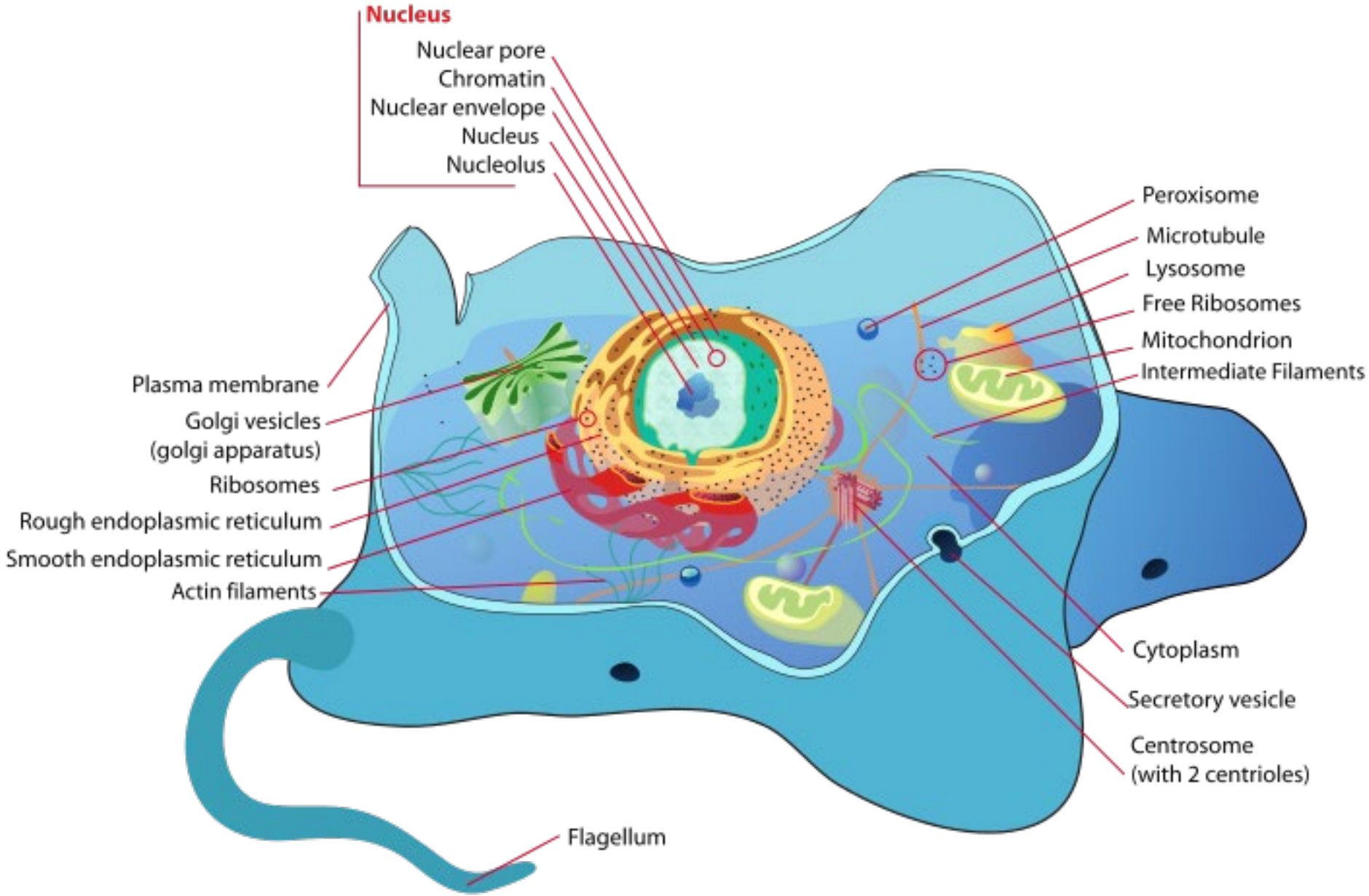


Cell Physiology Prokaryotes



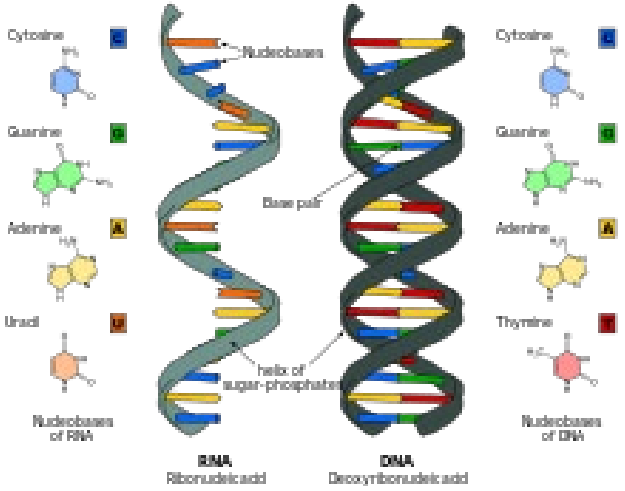


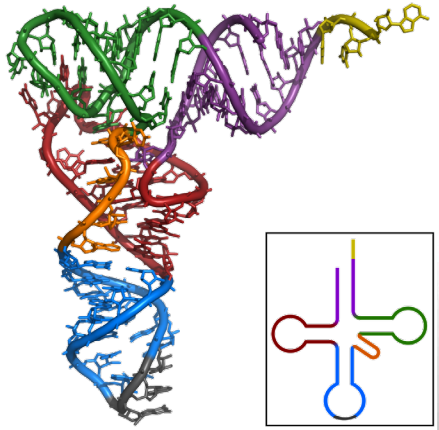
Cell Physiology Eukaryotes

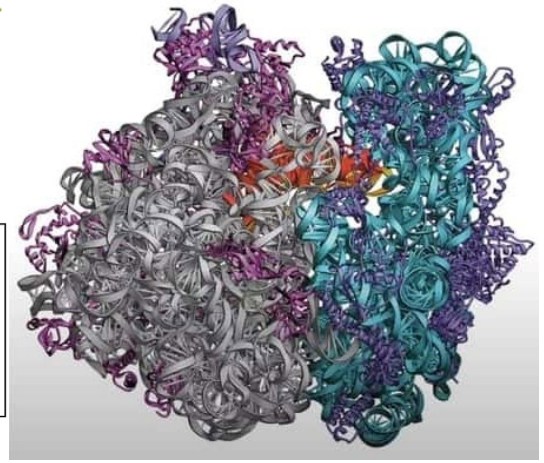


Macromolecular Building Blocks

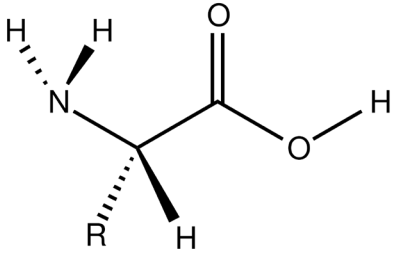
Nucleic Acids



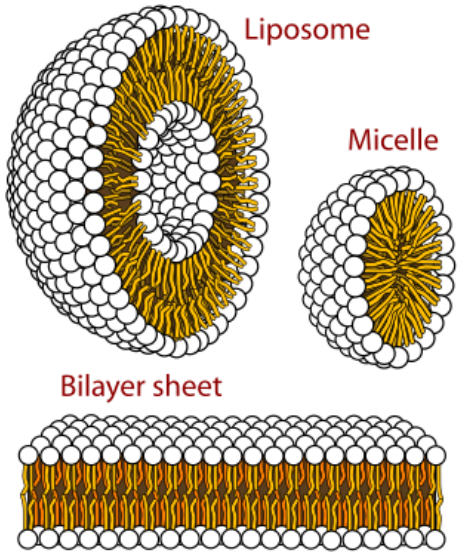


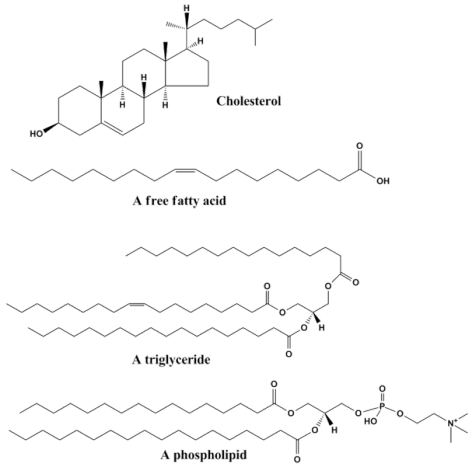


Proteins

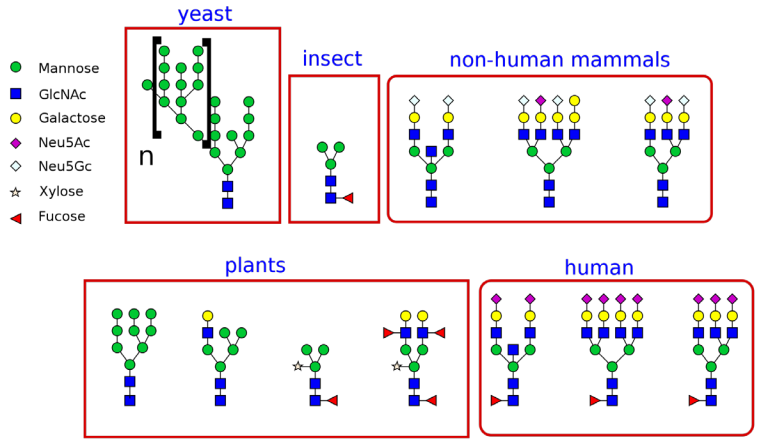


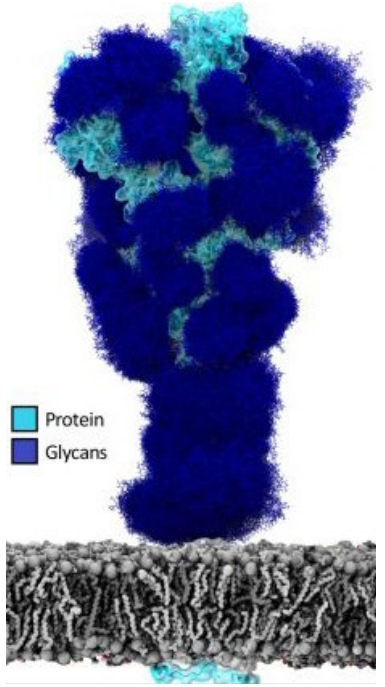
Lipids



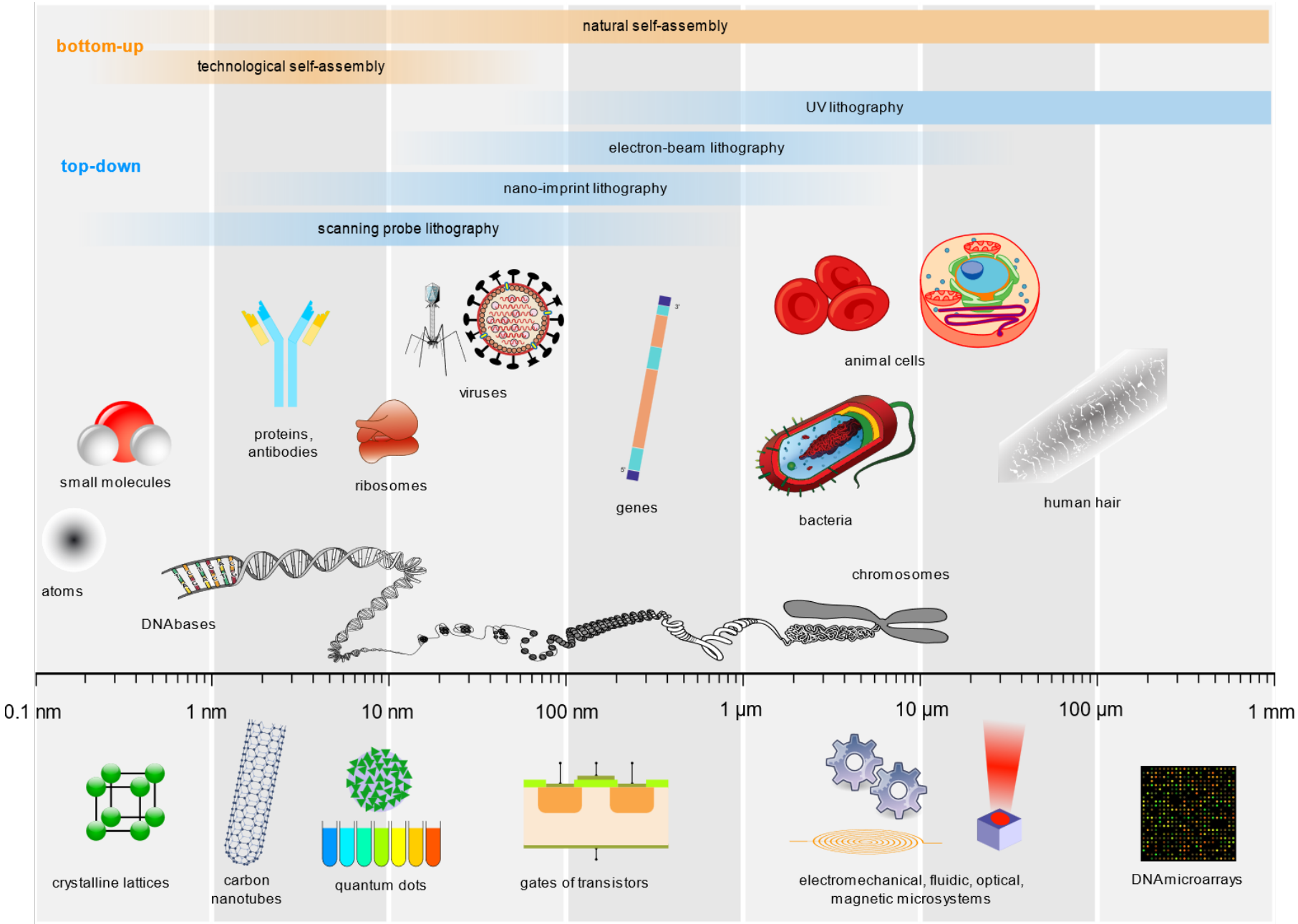


Glycans

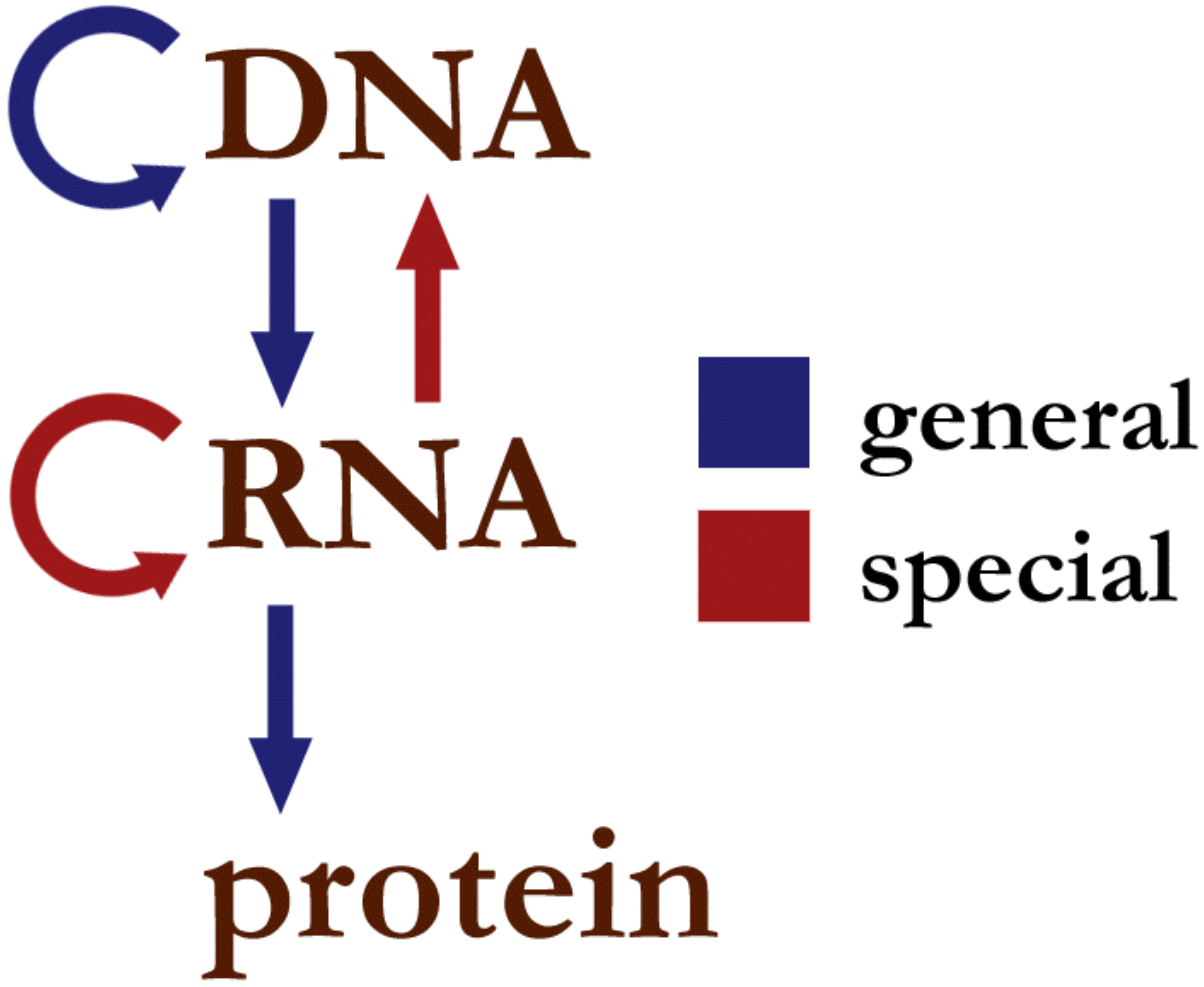


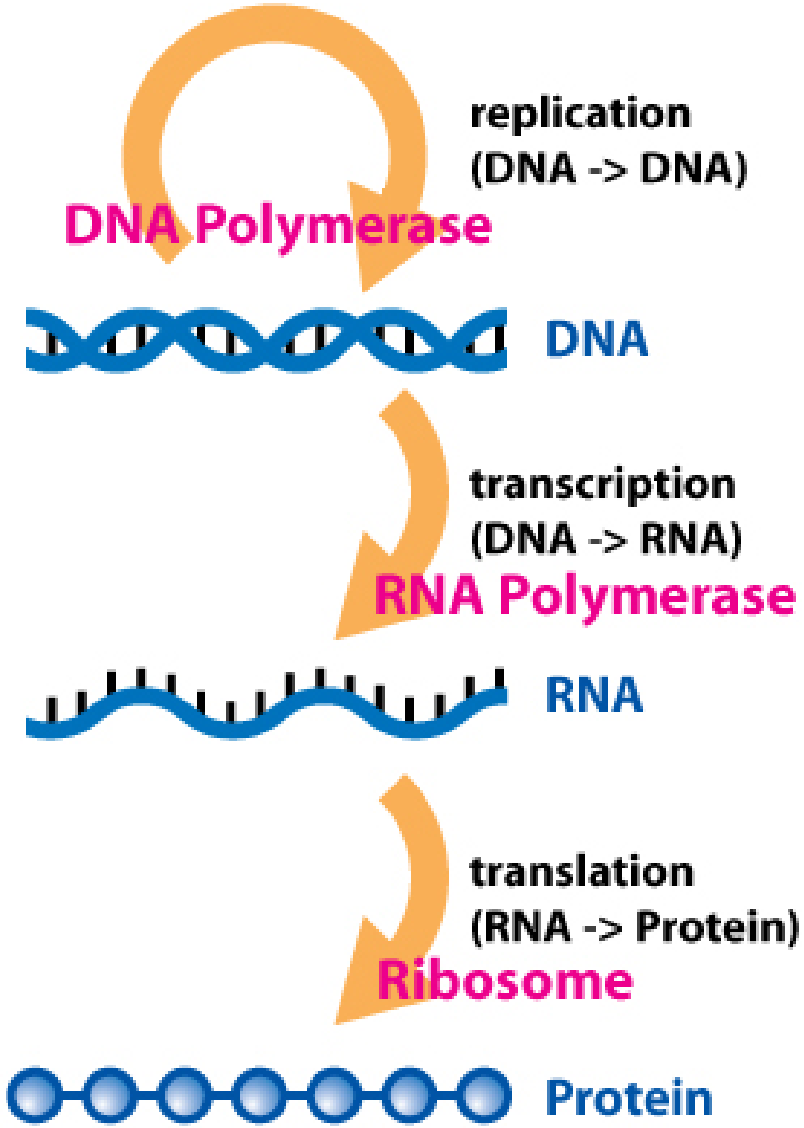


Biological Length Scales

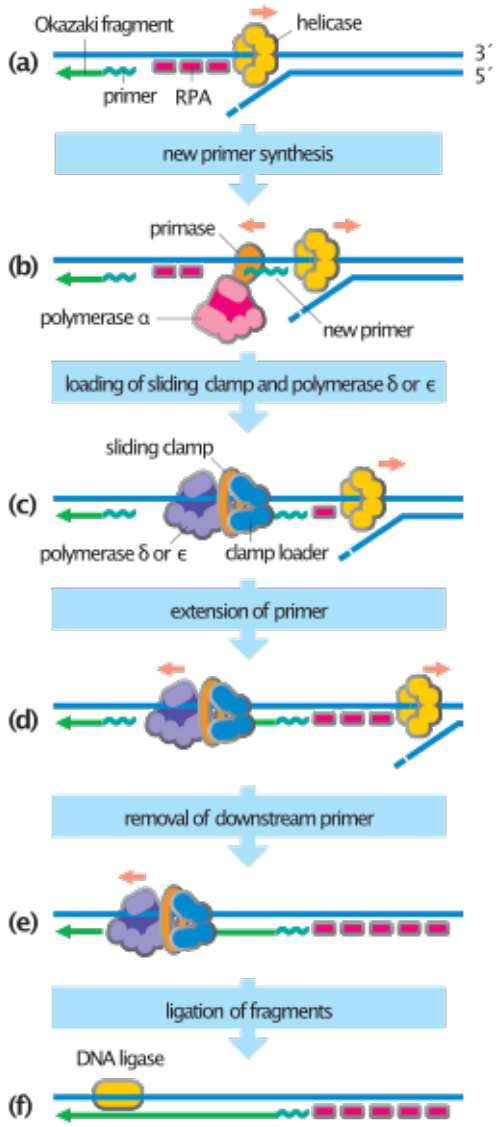


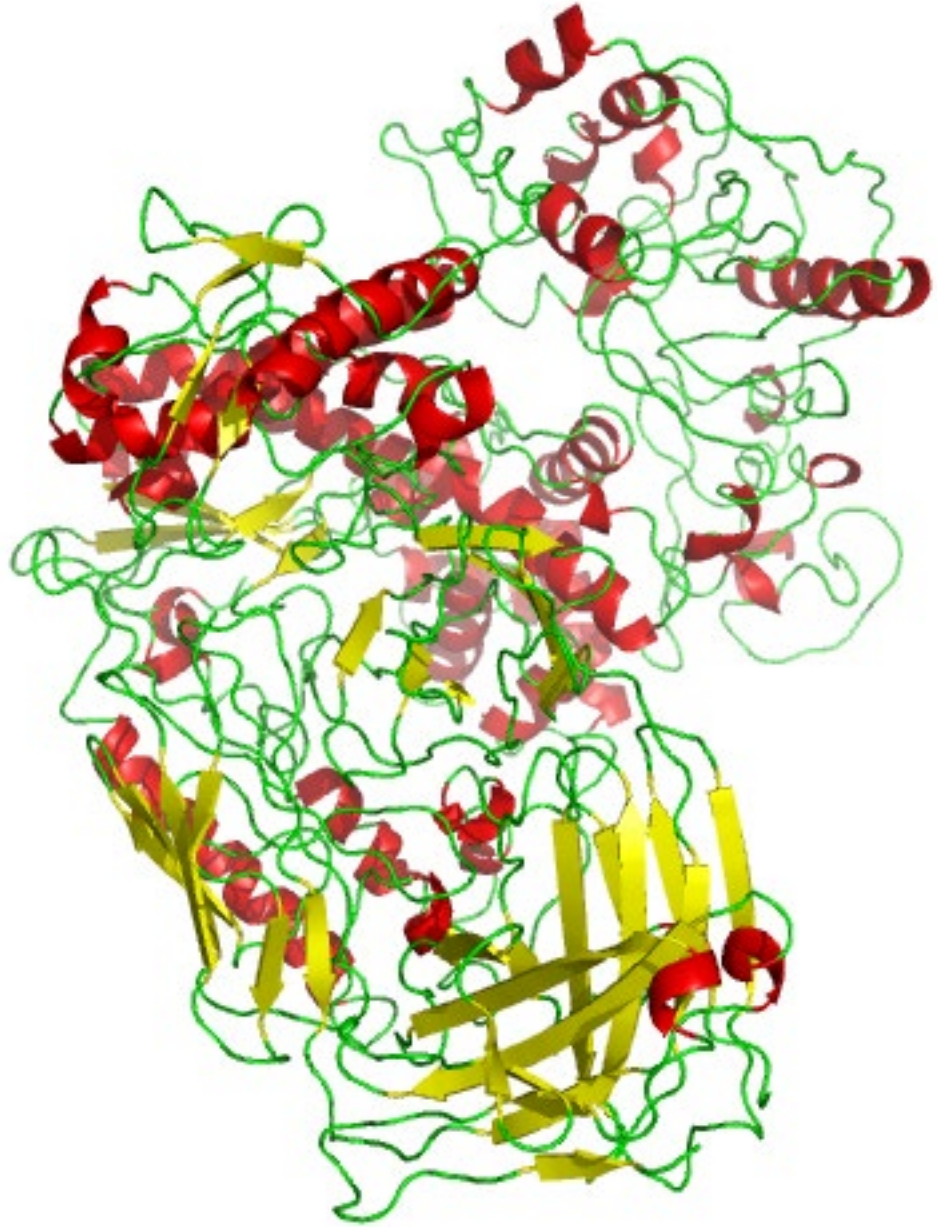
Central Dogma



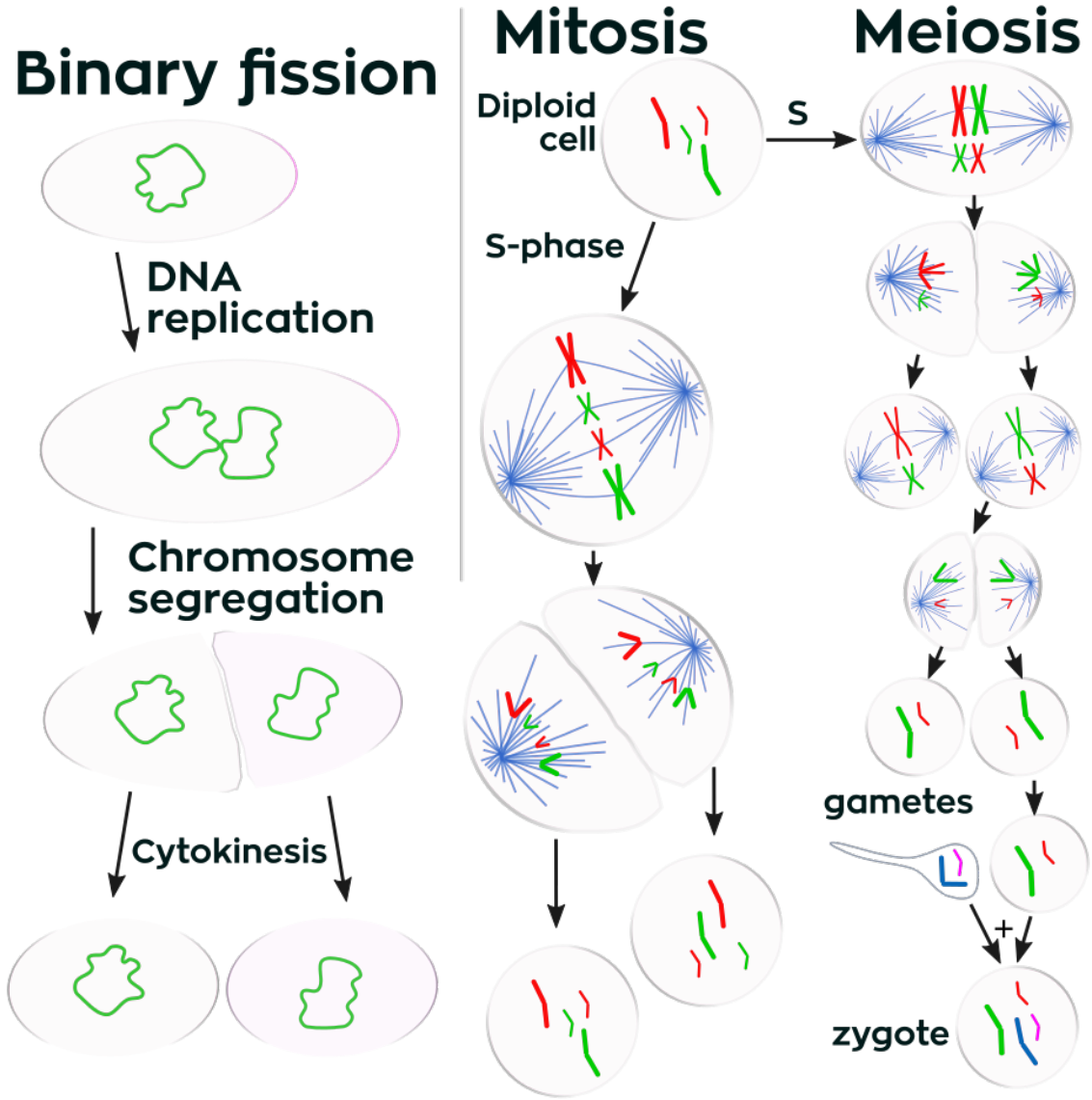


DNA Replication (make DNA)

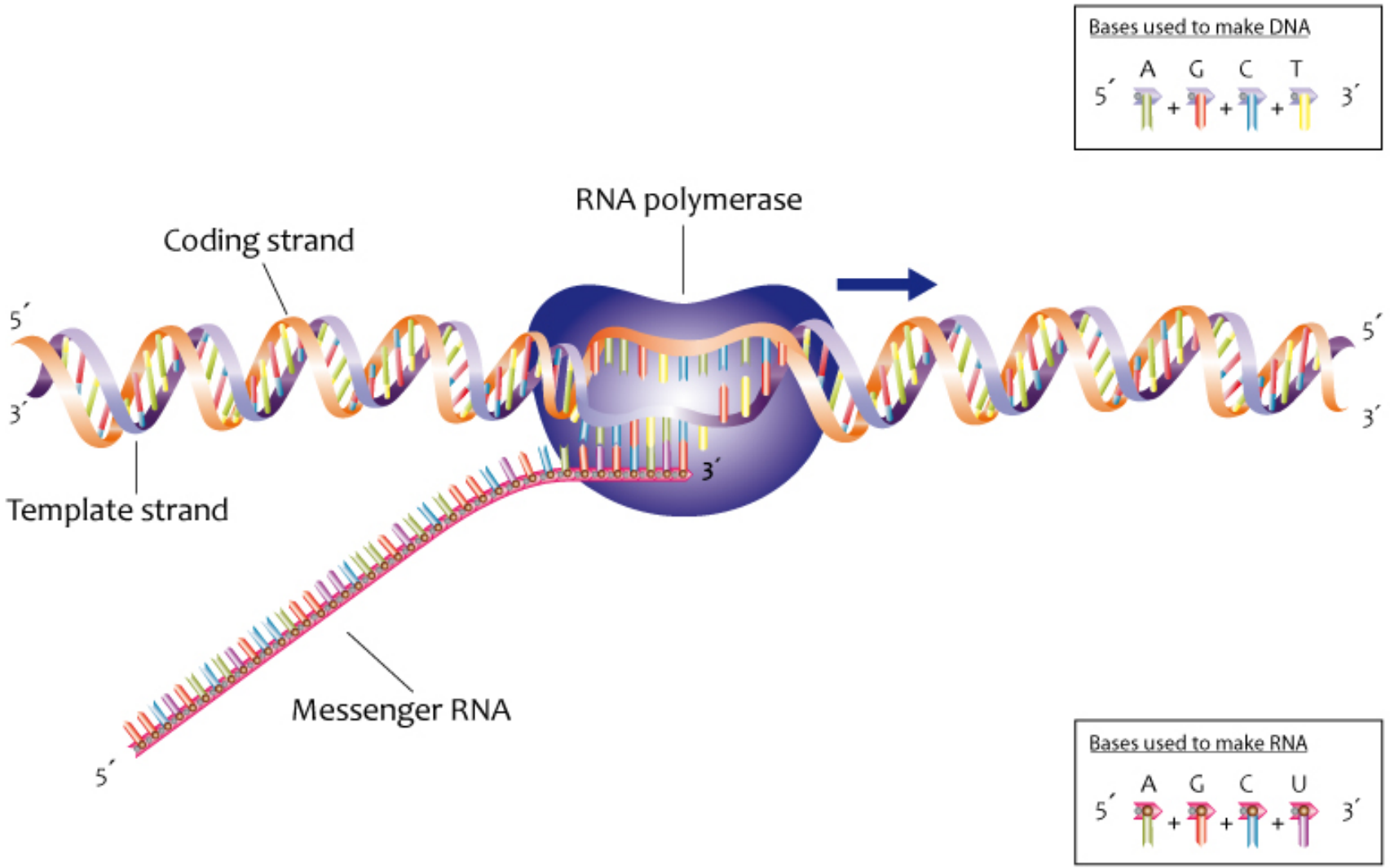




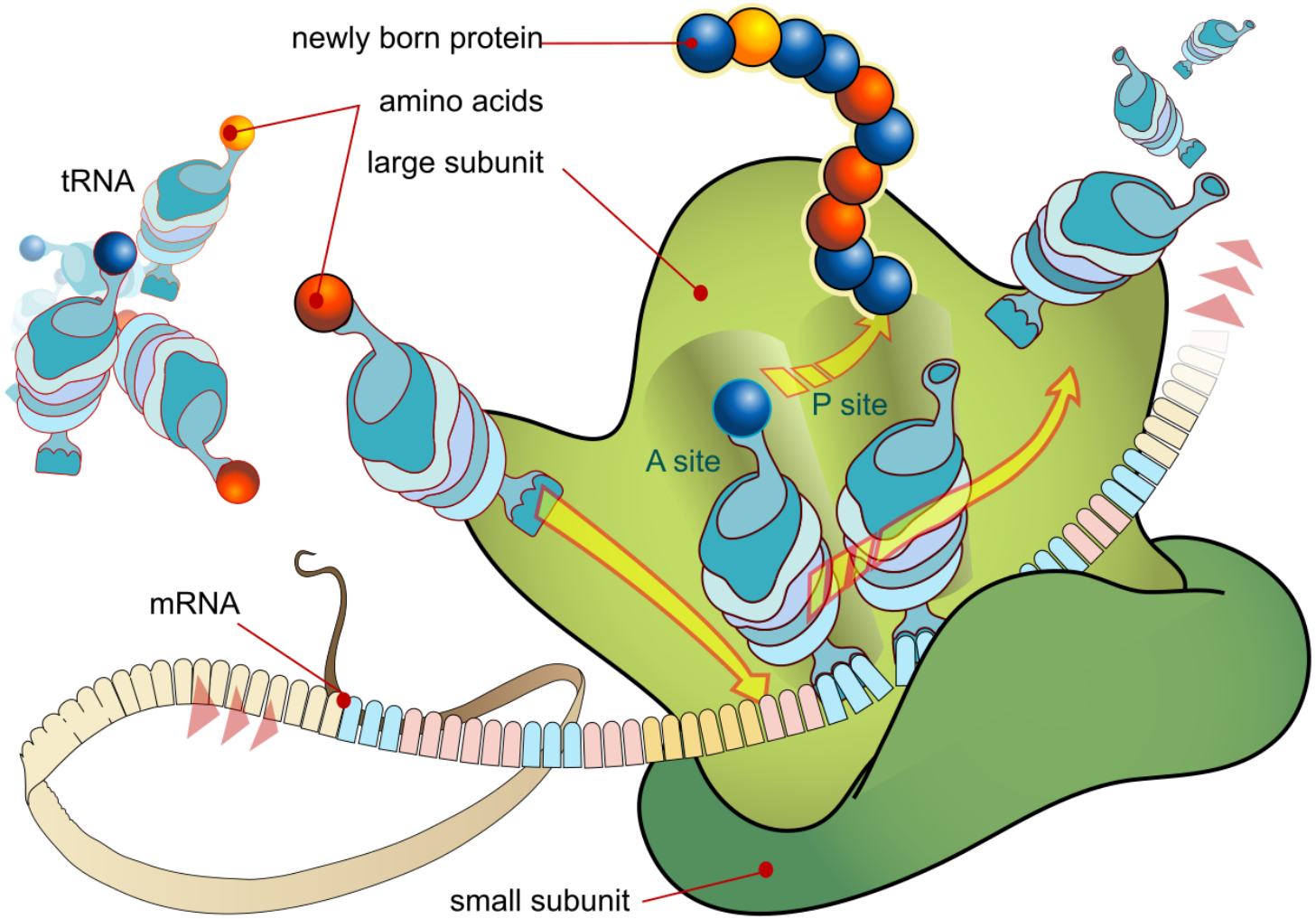
Cell Division

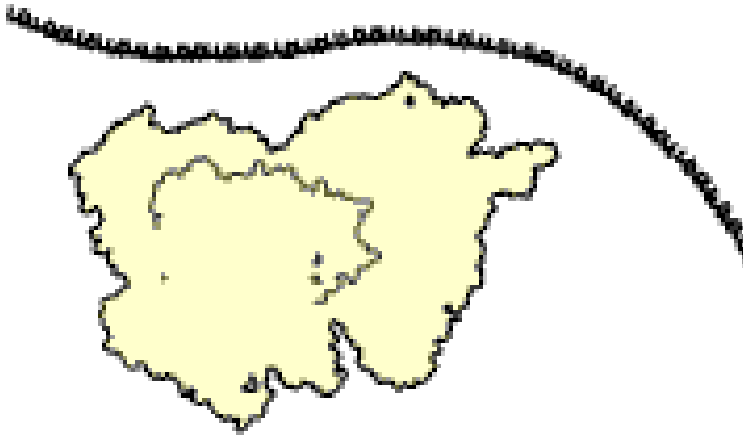


Transcription (make mRNA from DNA)

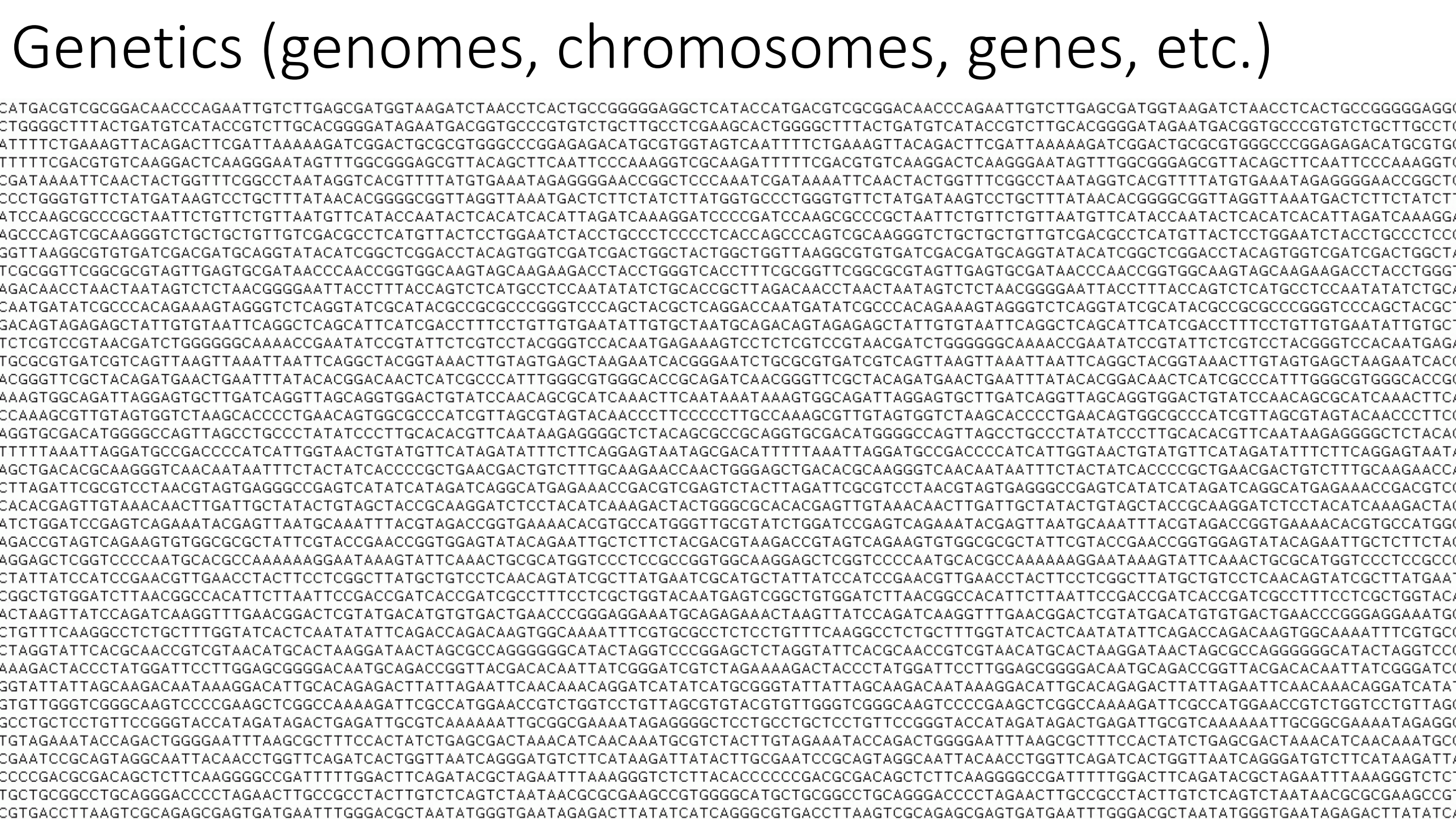


Translation (make protein from mRNA)

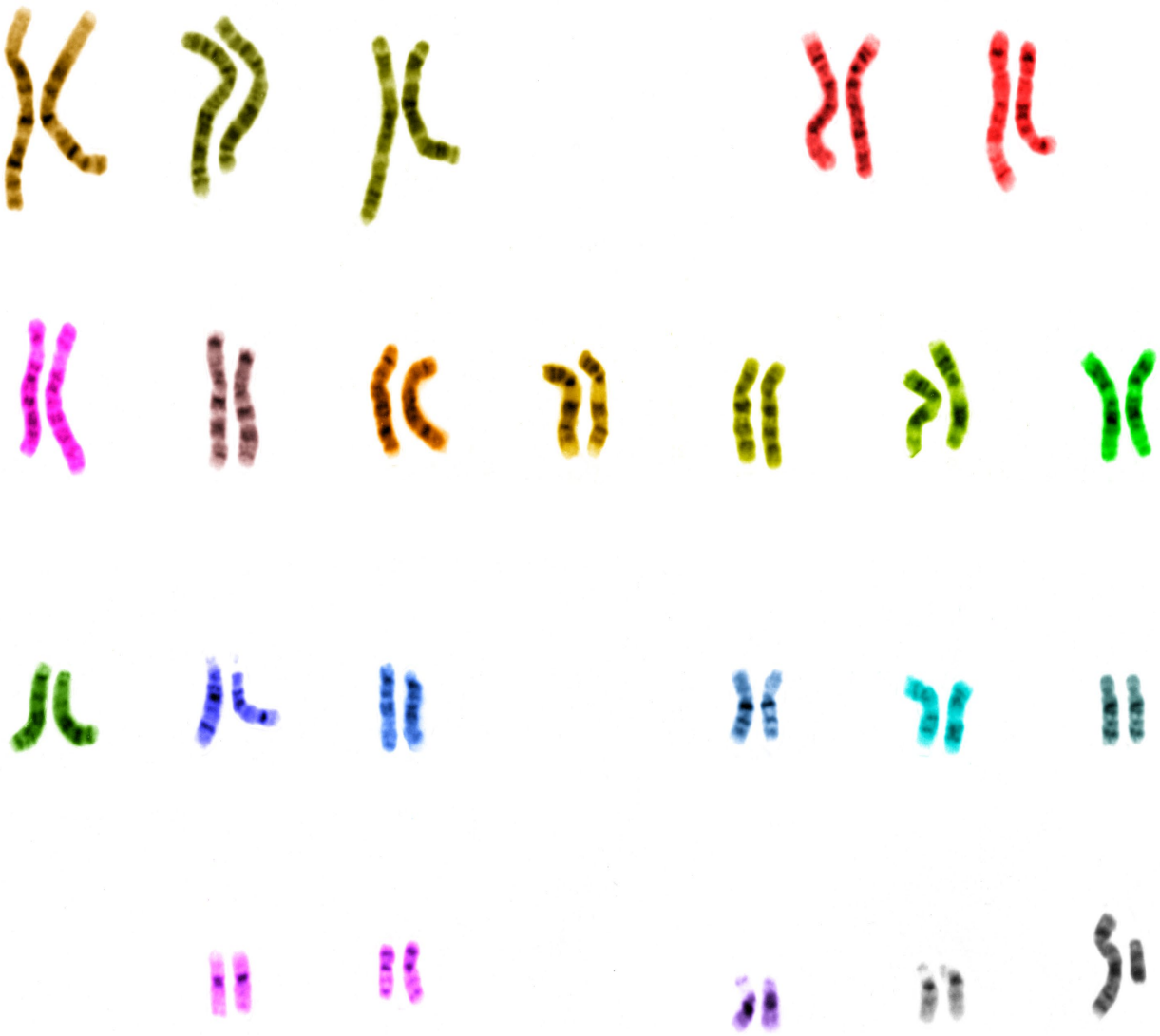


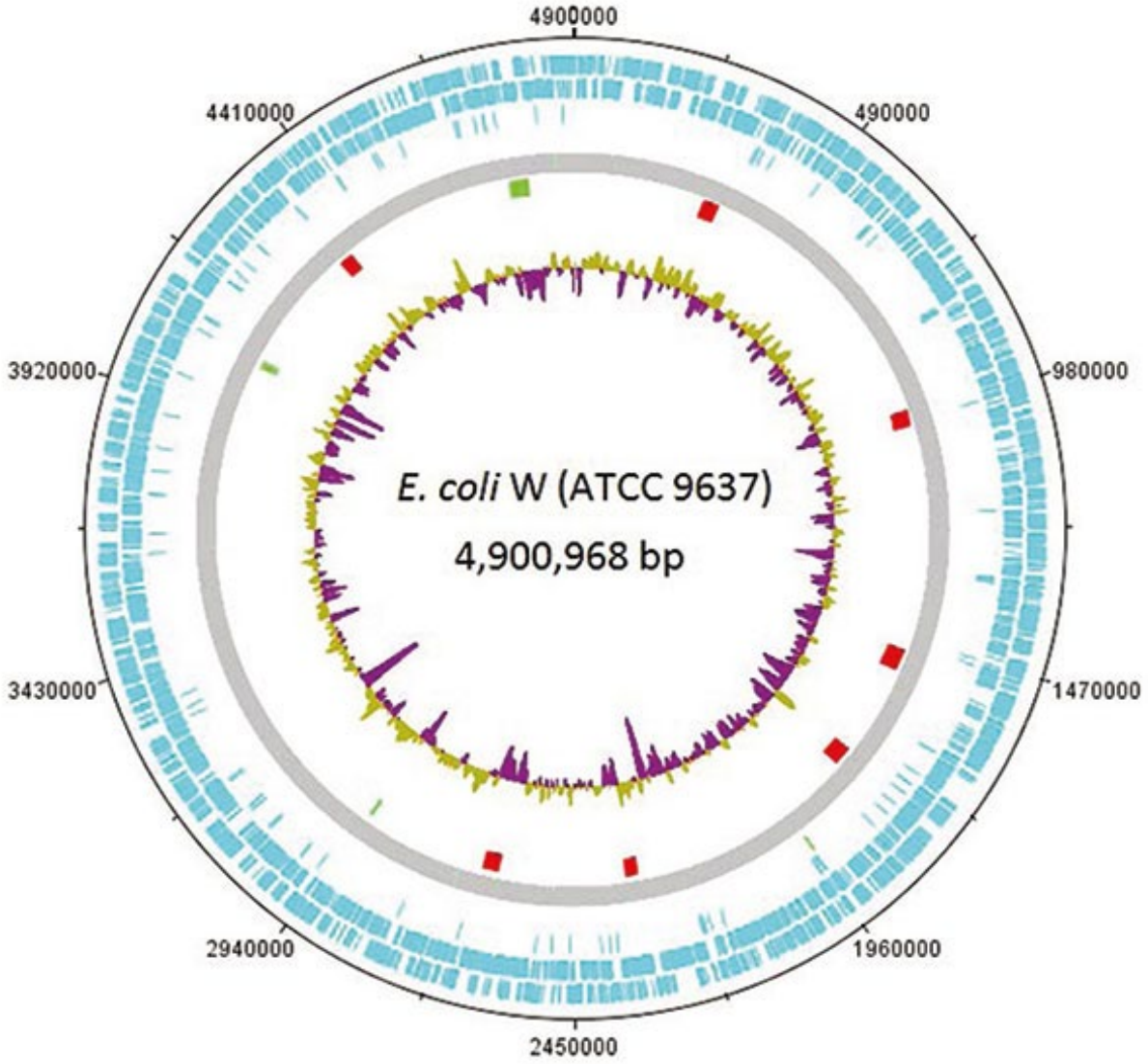


Genetics (genomes, chromosomes, genes, etc.)

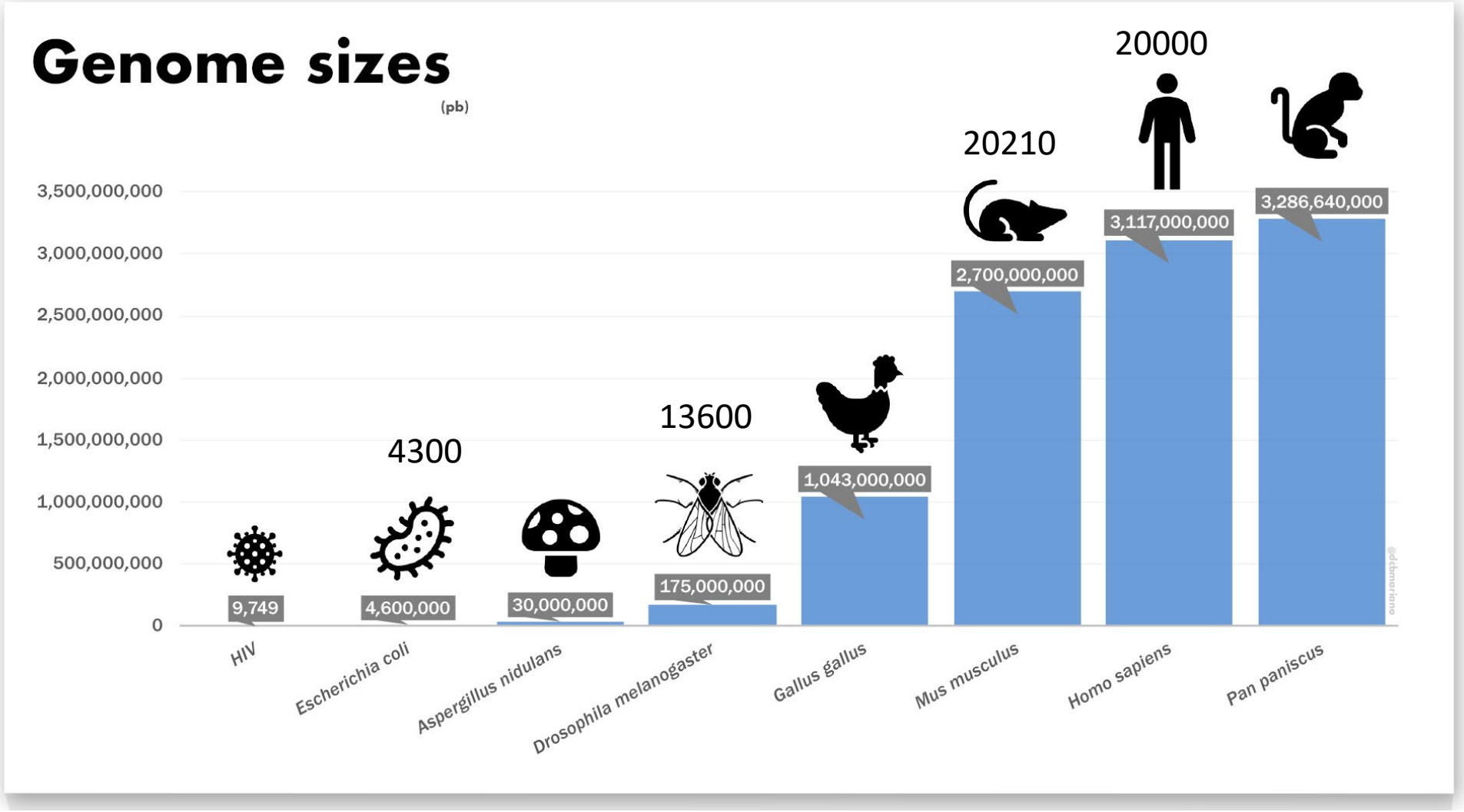


Genomes and Chromosomes

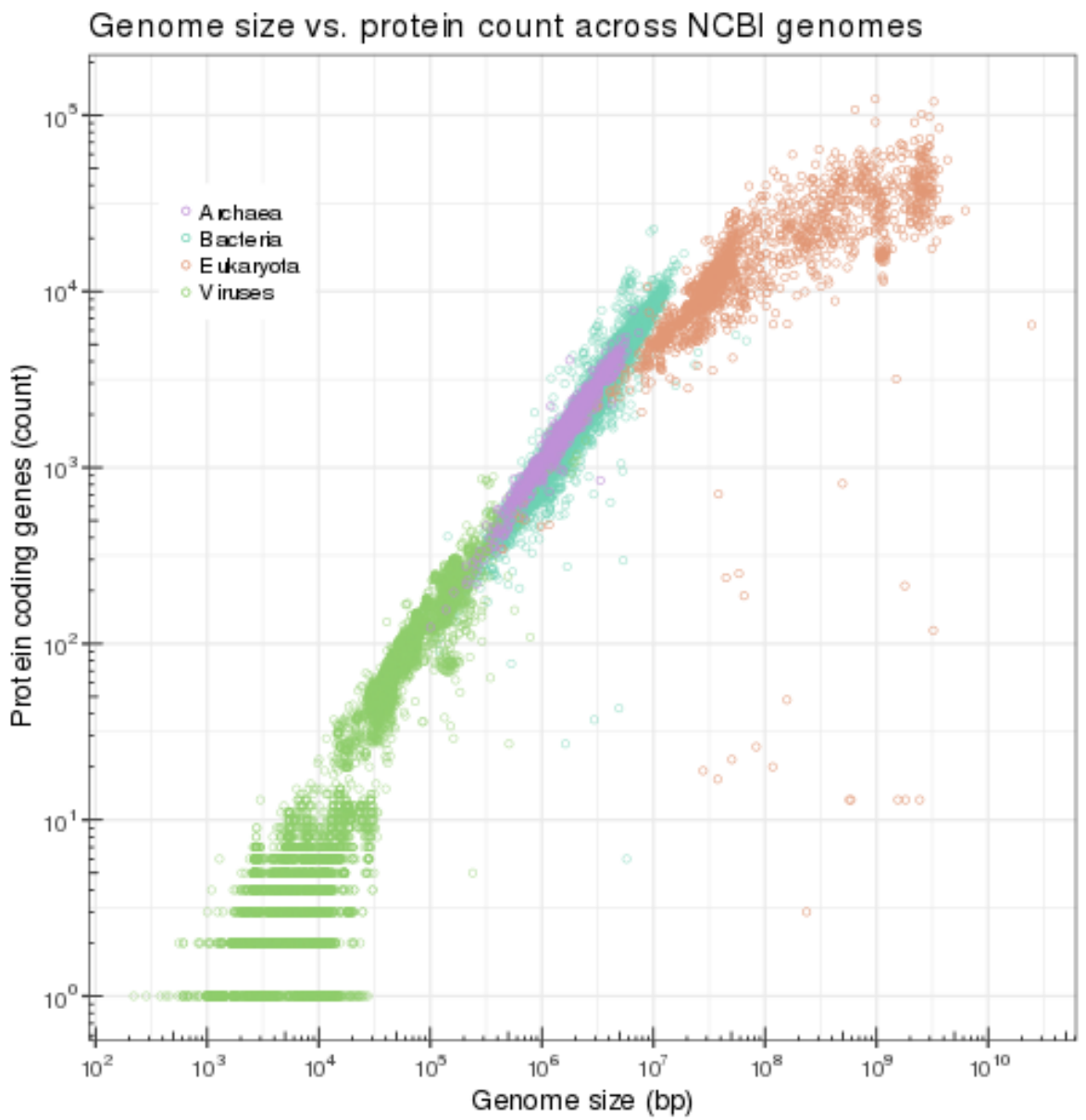




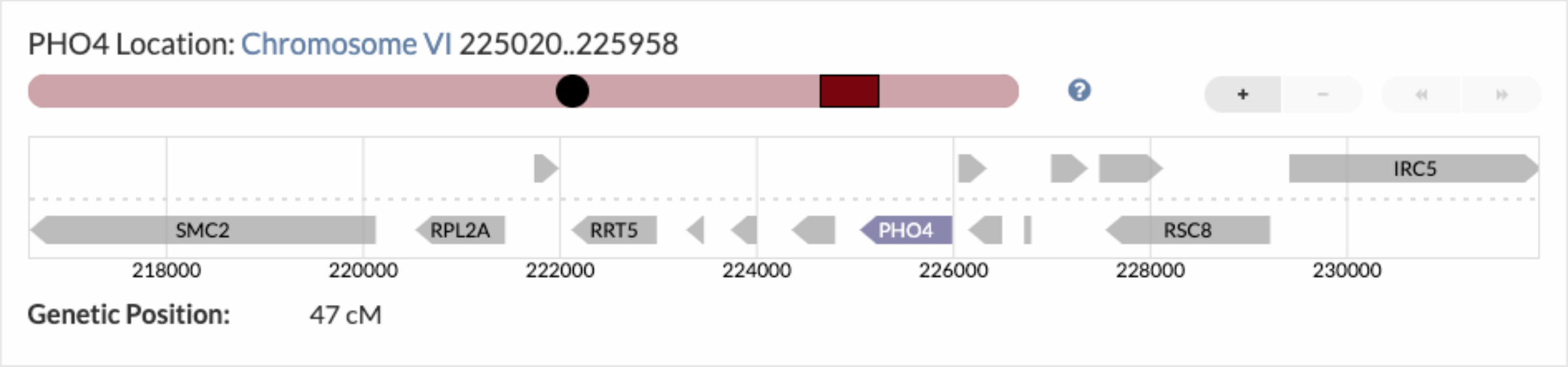
Genome sizes



Genome sizes



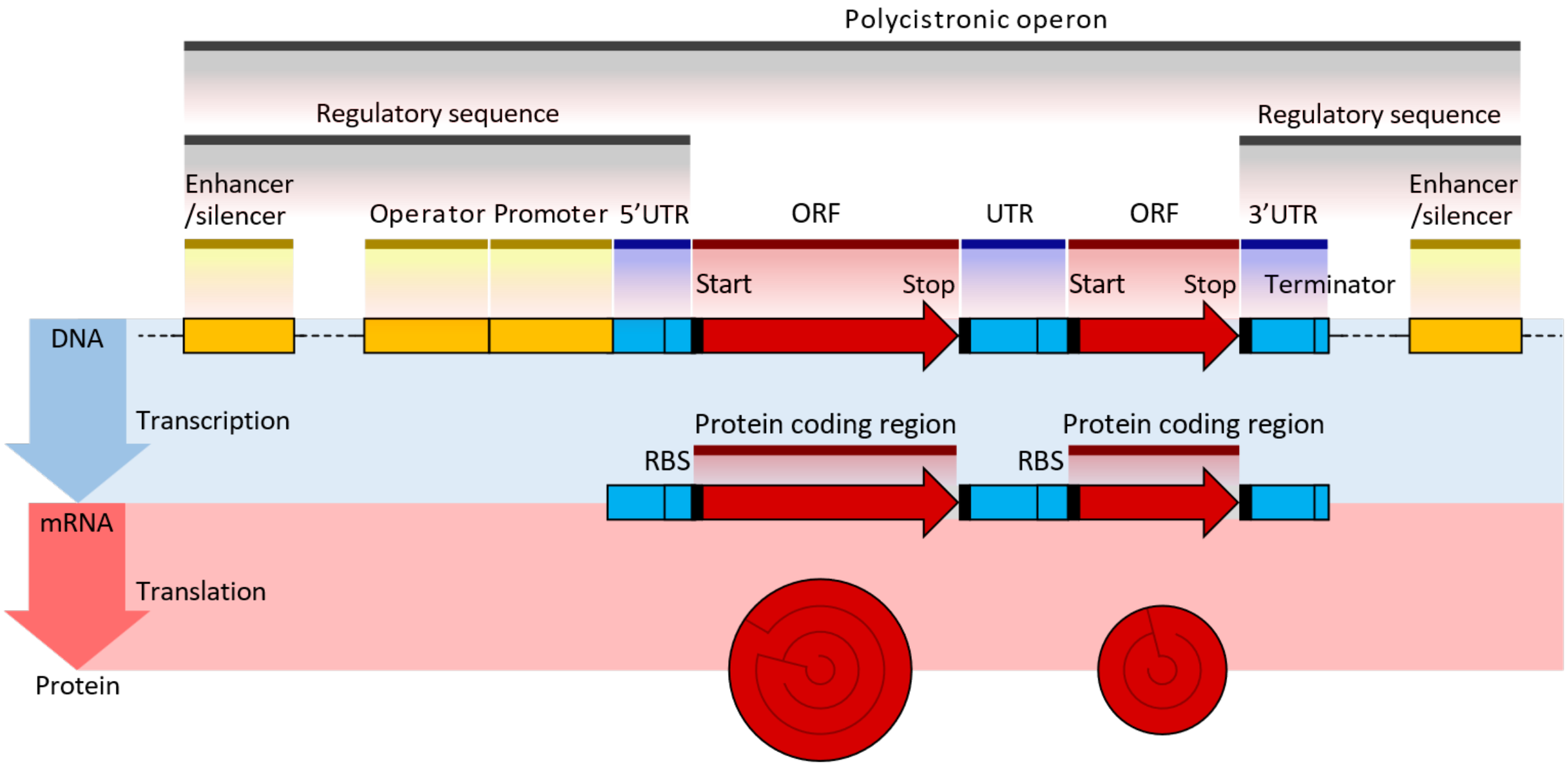
Genes, Promoters, Terminators, Gene regulation



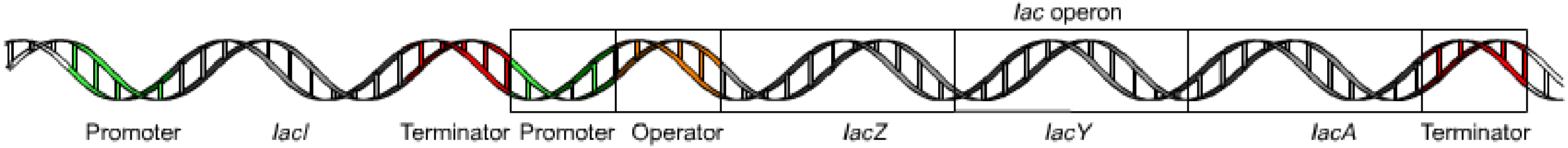
"A gene is a DNA sequence that codes for a diffusible product. This product may be protein (as is the case in the majority of genes) or may be RNA (as is the case of genes that code for tRNA and rRNA). The crucial feature is that the product diffuses away from its site of synthesis to act elsewhere."

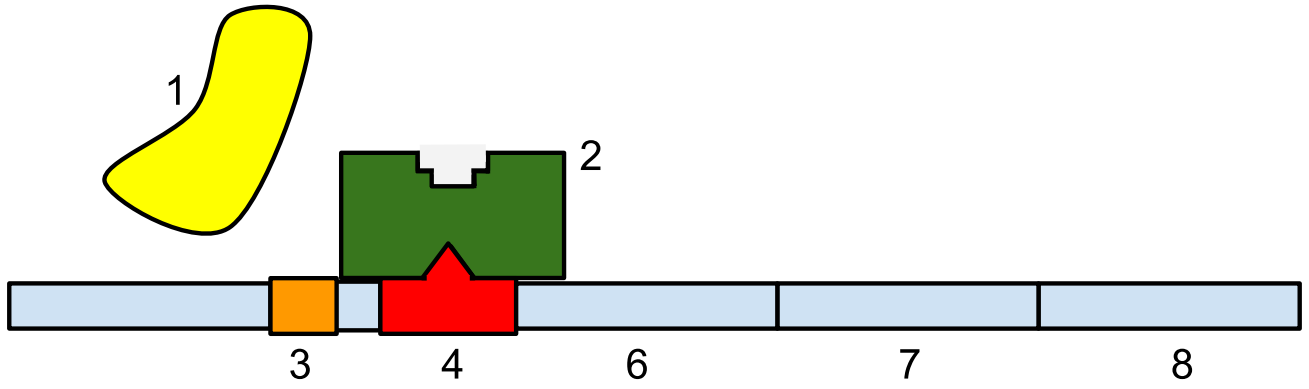
Lewin B (2004). *Genes VIII*. Upper Saddle River, NJ, USA: Pearson/Prentice Hall.

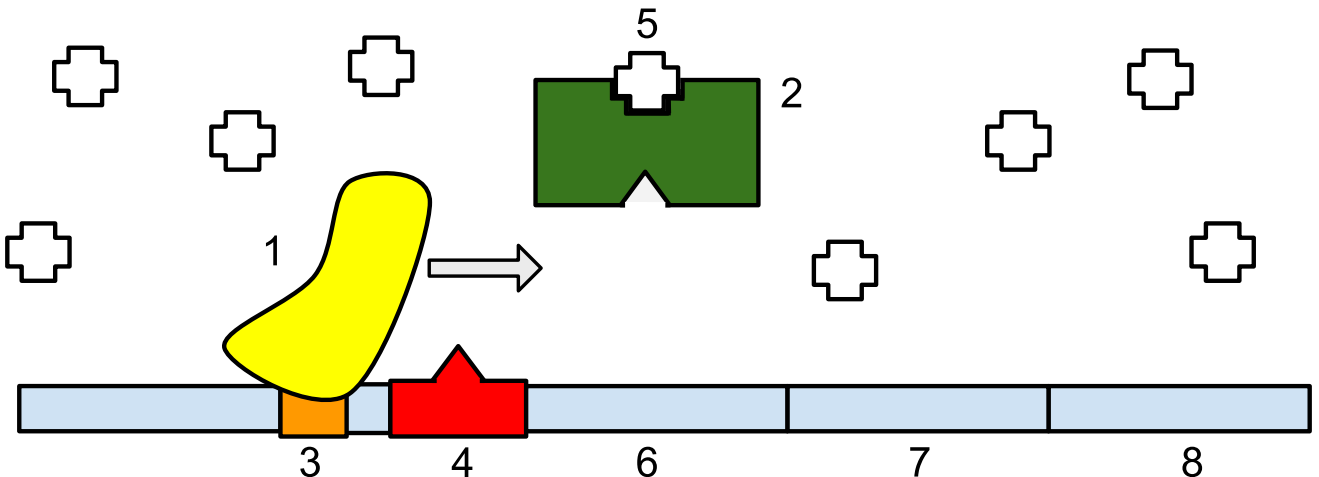
Genes, **Promoters, Terminators**, Gene regulation

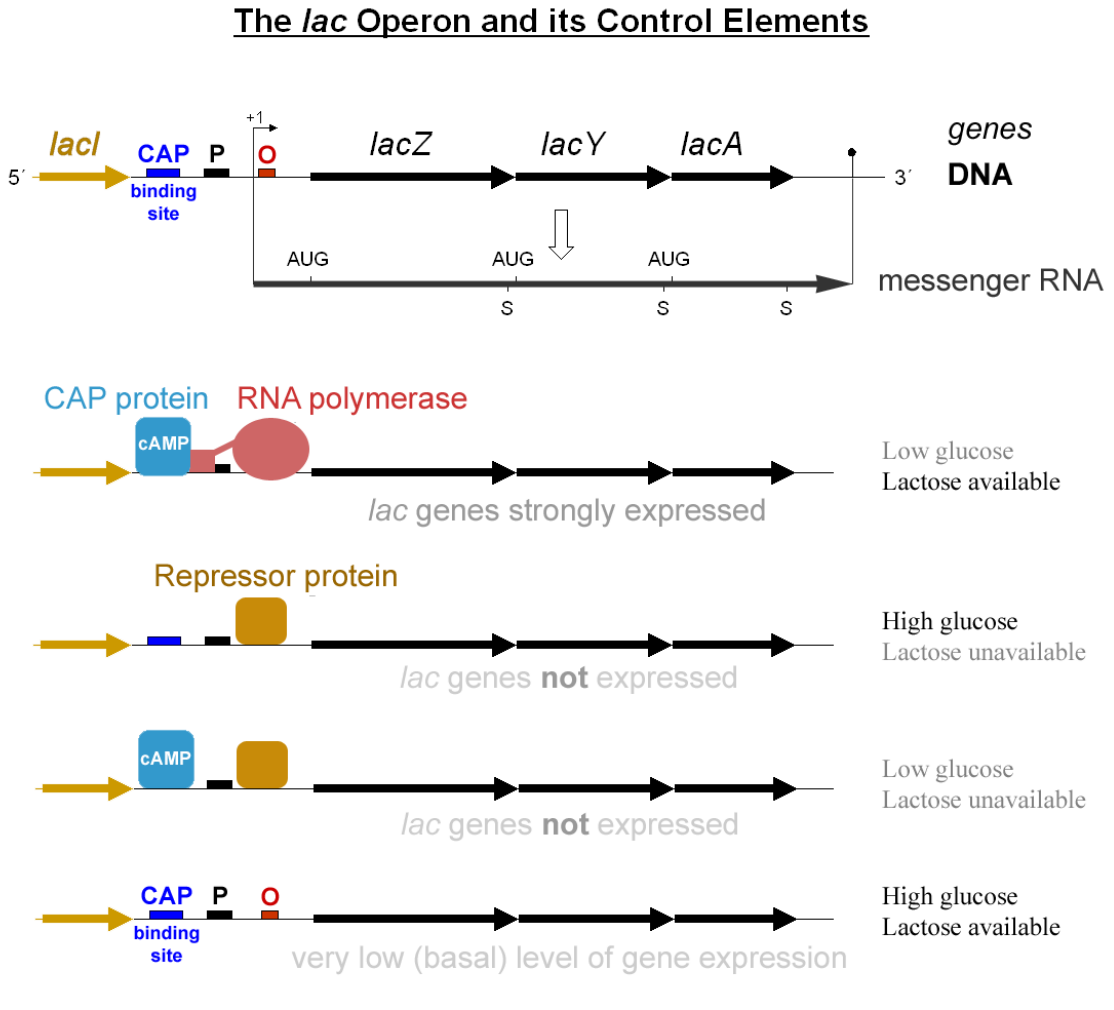


Genes, Promoters, Terminators, **Gene regulation**

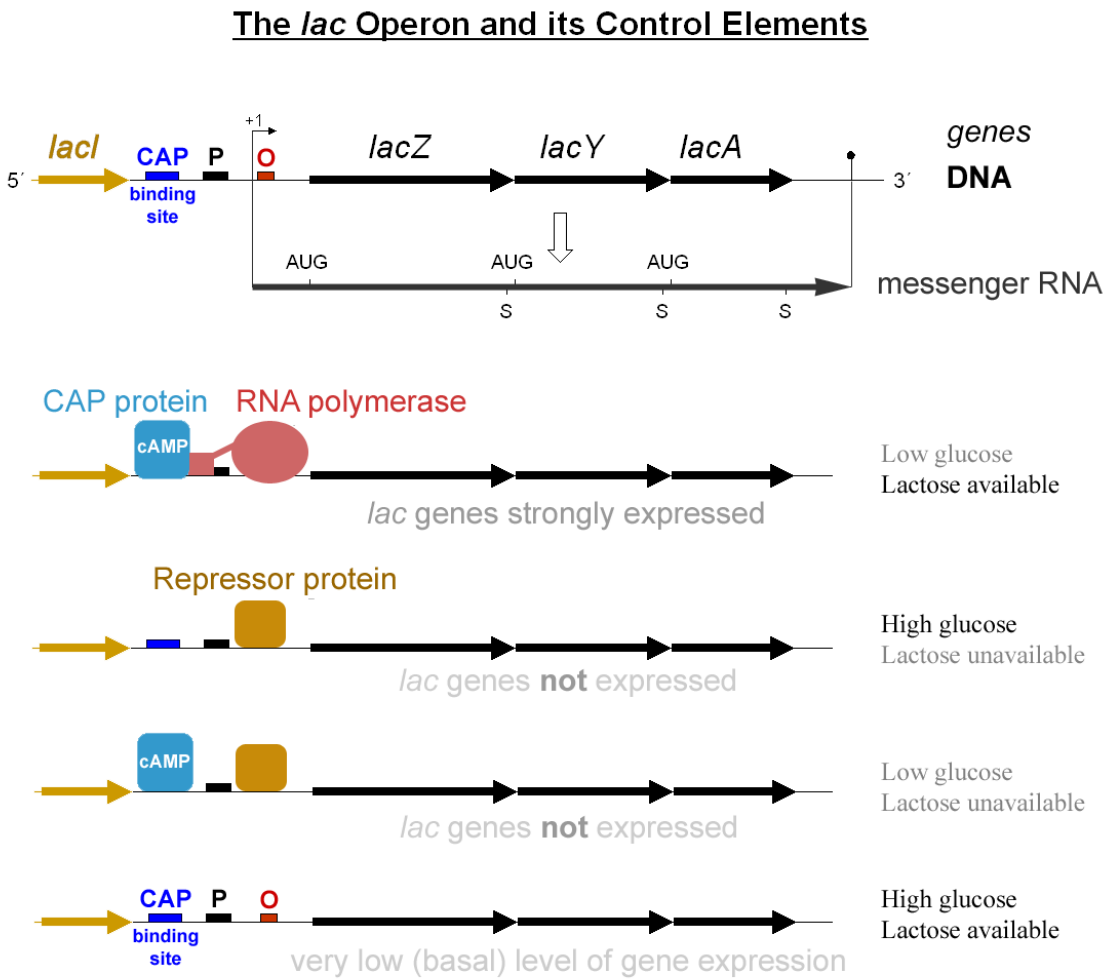


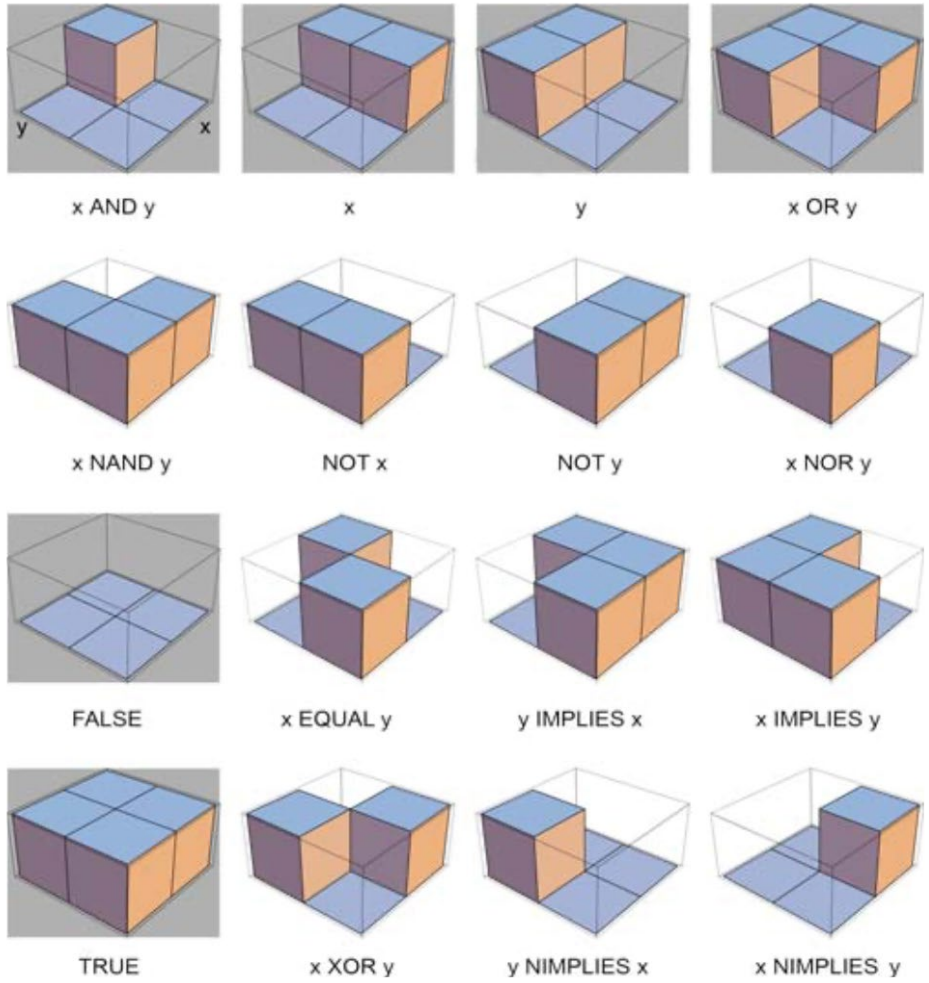




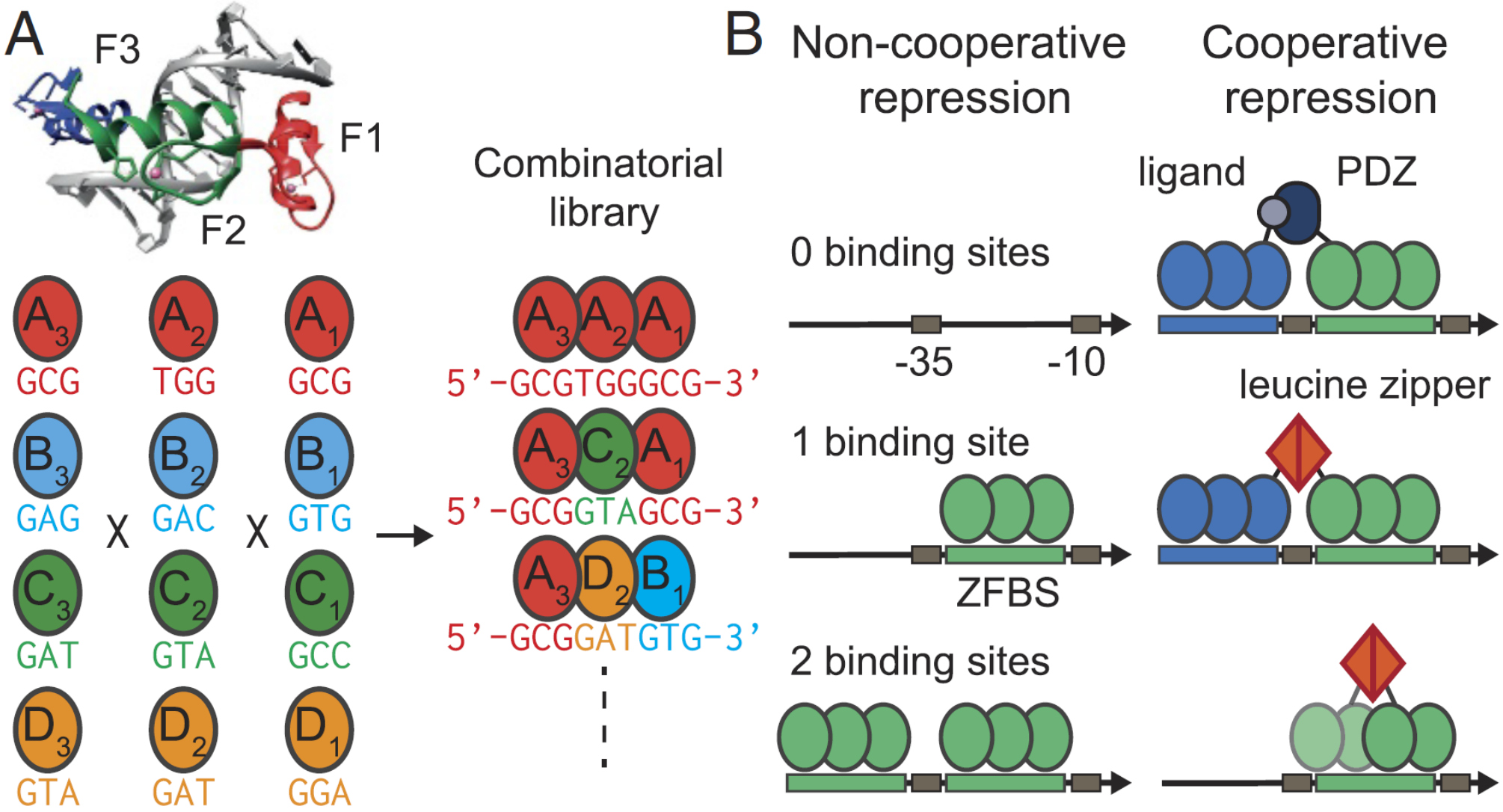


Genes, Promoters, Terminators, **Gene regulation**





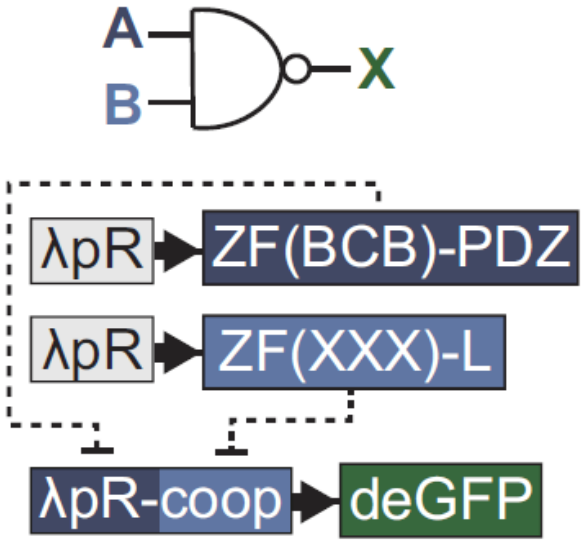
Genes, Promoters, Terminators, **Gene regulation**



Genes, Promoters, Terminators, **Gene regulation**

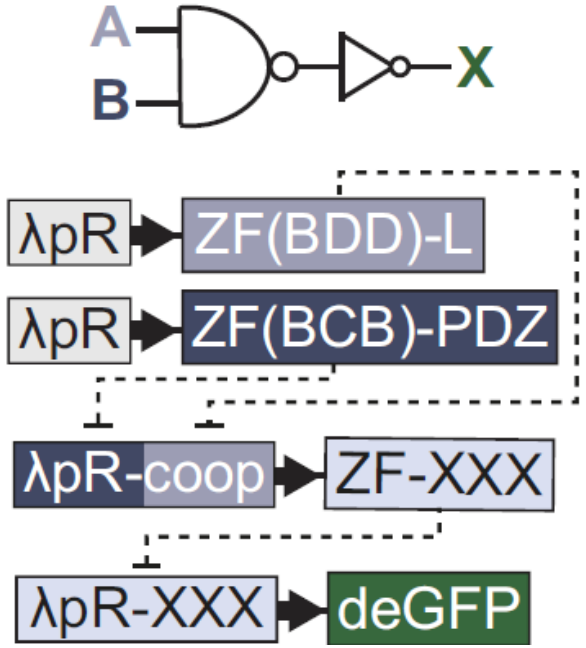
A **NAND**

Inputs		Output
ZF-BCB	ZF-XXX	deGFP
0	0	1
0	1	1
1	0	1
1	1	0

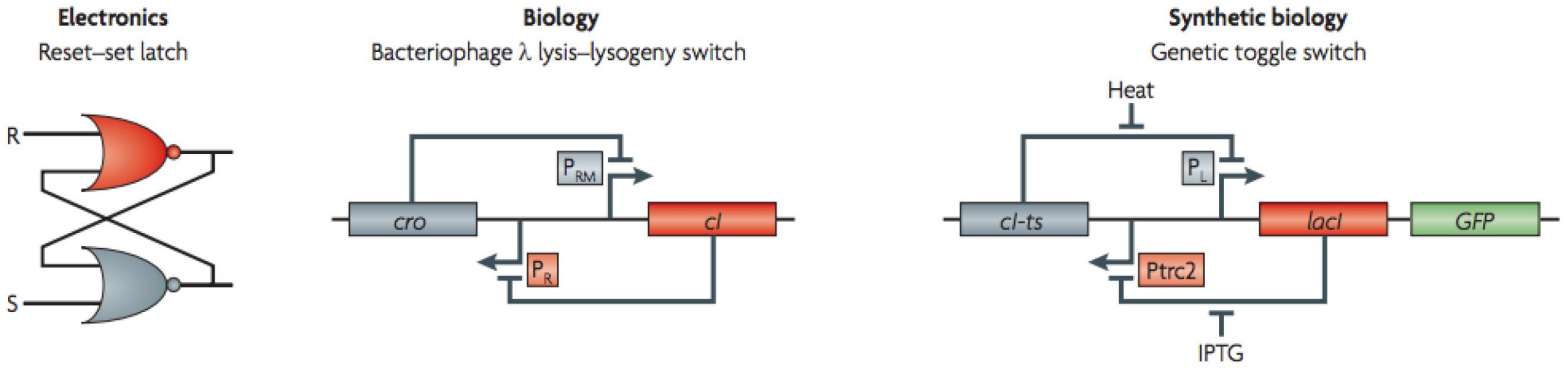


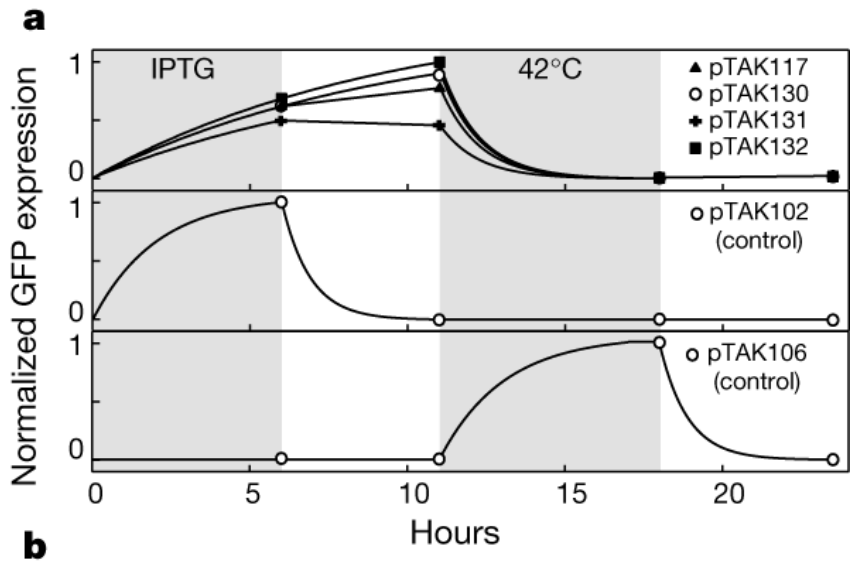
D **AND**

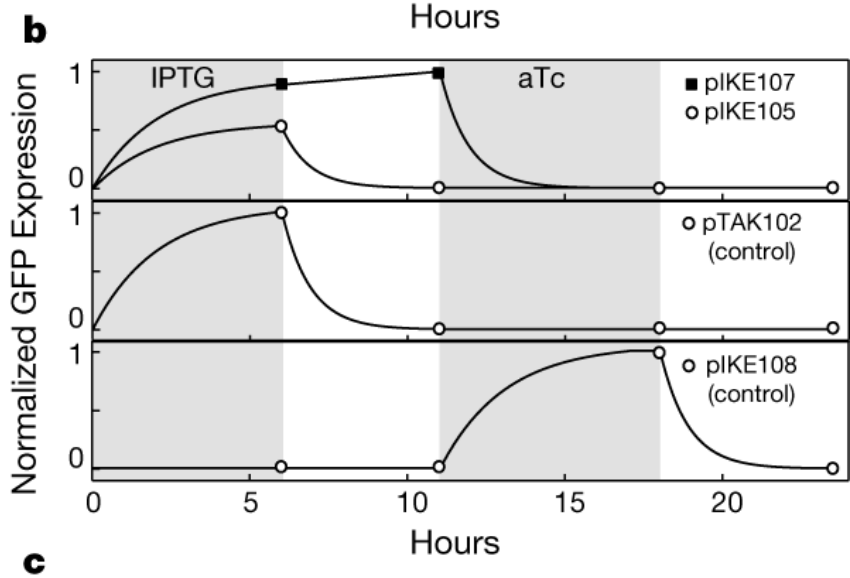
Inputs		Output
ZF-L	ZF-PDZ	deGFP
0	0	0
0	1	0
1	0	0
1	1	1

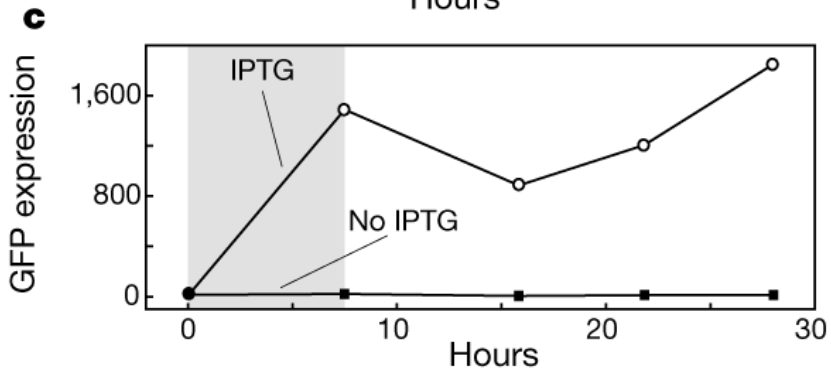


Systems

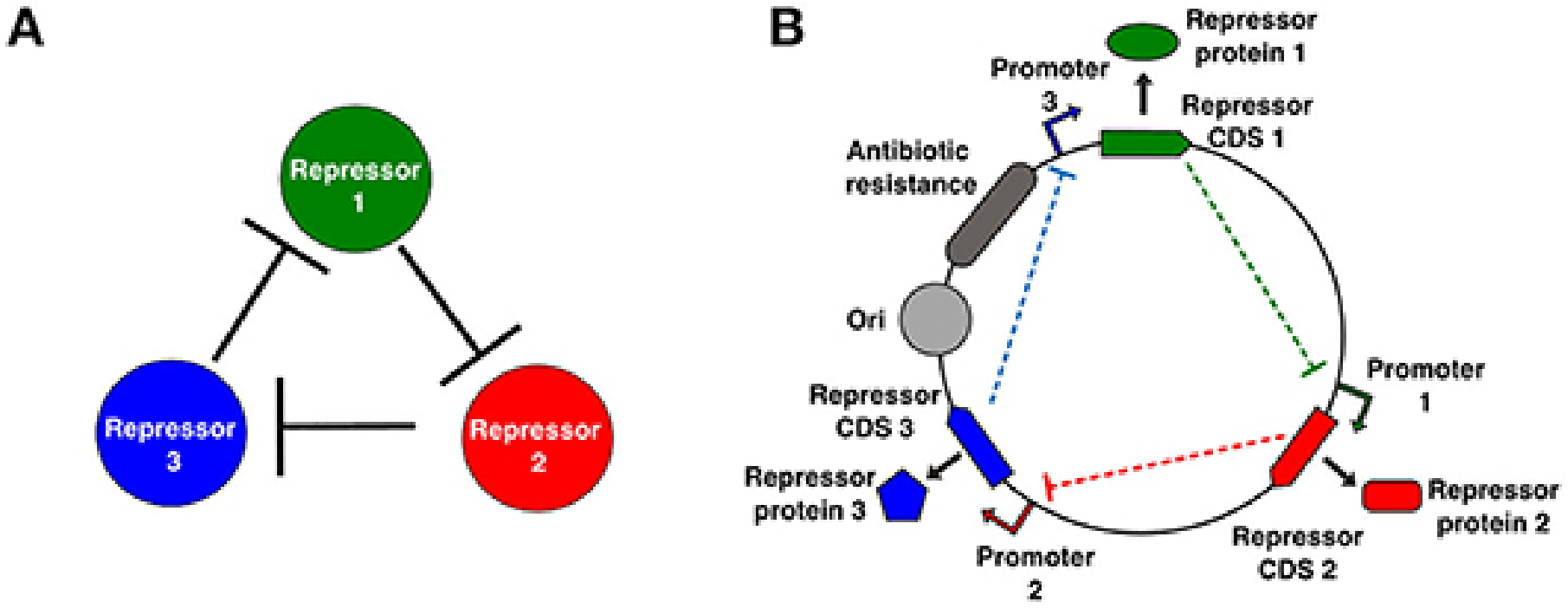

Electronics
Reset–set latch
R
S
Biology
Bacteriophage λ lysis–lysogeny switch
P RM
cro
cI
P R
Synthetic biology
Genetic toggle switch
Heat
P L
cI-ts
lacI
GFP
Ptrc2
IPTG


a
IPTG
42°C
pTAK117
pTAK130
pTAK131
pTAK132
pTAK102 (control)
pTAK106 (control)
Normalized GFP expression
Hours


b
IPTG
aTc
pIKE107
pIKE105
pTAK102 (control)
pIKE108 (control)
Normalized GFP Expression
Hours


c
IPTG
No IPTG
GFP expression
Hours

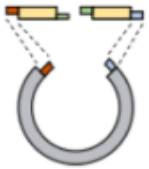
Systems


A
Repressor 1
Repressor 3
Repressor 2
B
Repressor protein 1
Promoter 3
Repressor CDS 1
Antibiotic resistance
Ori
Repressor CDS 3
Repressor protein 3
Promoter 2
Promoter 1
Repressor CDS 2
Repressor protein 2
C
Time

How do biological engineers make things?

Programable nucleic acid interactions

Professional DNA part libraries



Golden Gate cloning toolkits

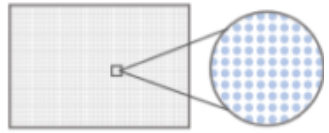
Cheap synthetic genes

Computer aided design

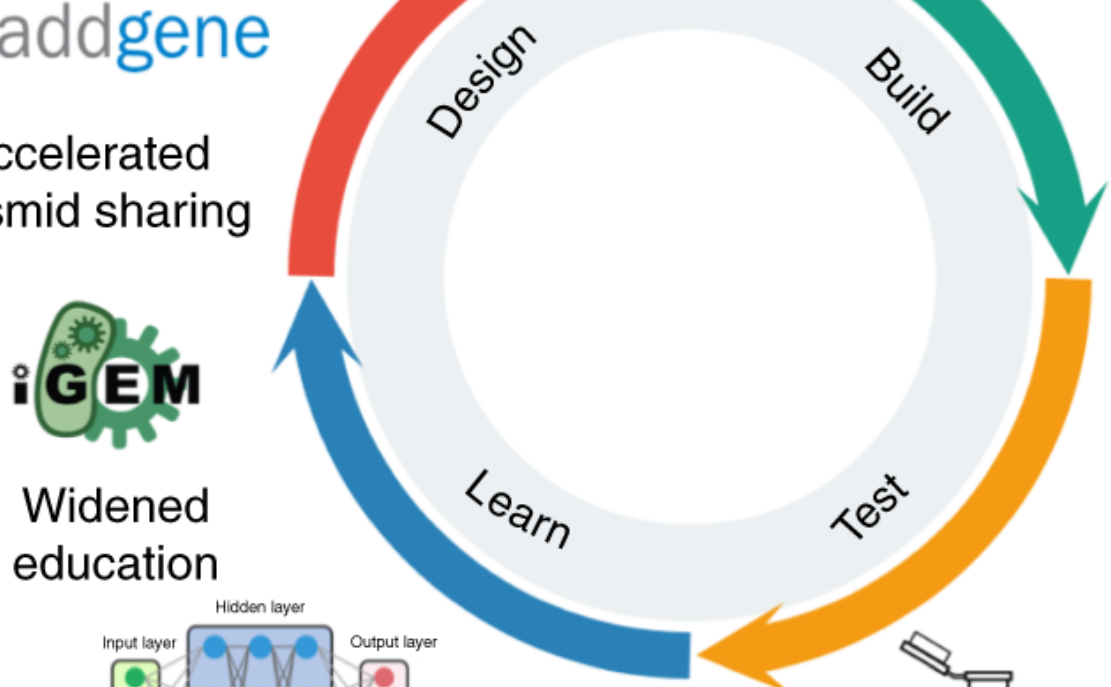
addgene

Accelerated plasmid sharing

CRISPR, CRISPRi and base editing



Pooled oligo synthesis



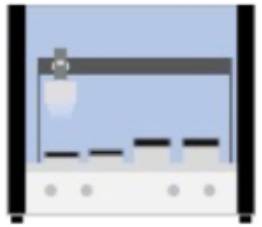
bioRχiv

Manuscript preprinting

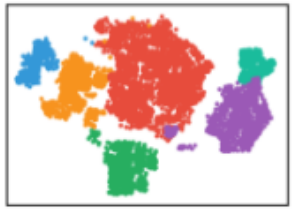
iGEM

Widened education

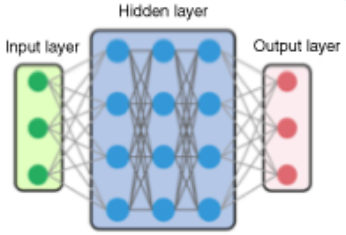
NGS-based measurement



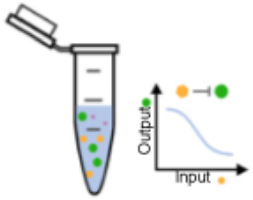
Acoustic liquid handling



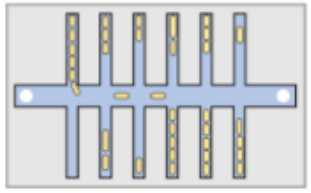
Dimension reduction



Machine learning

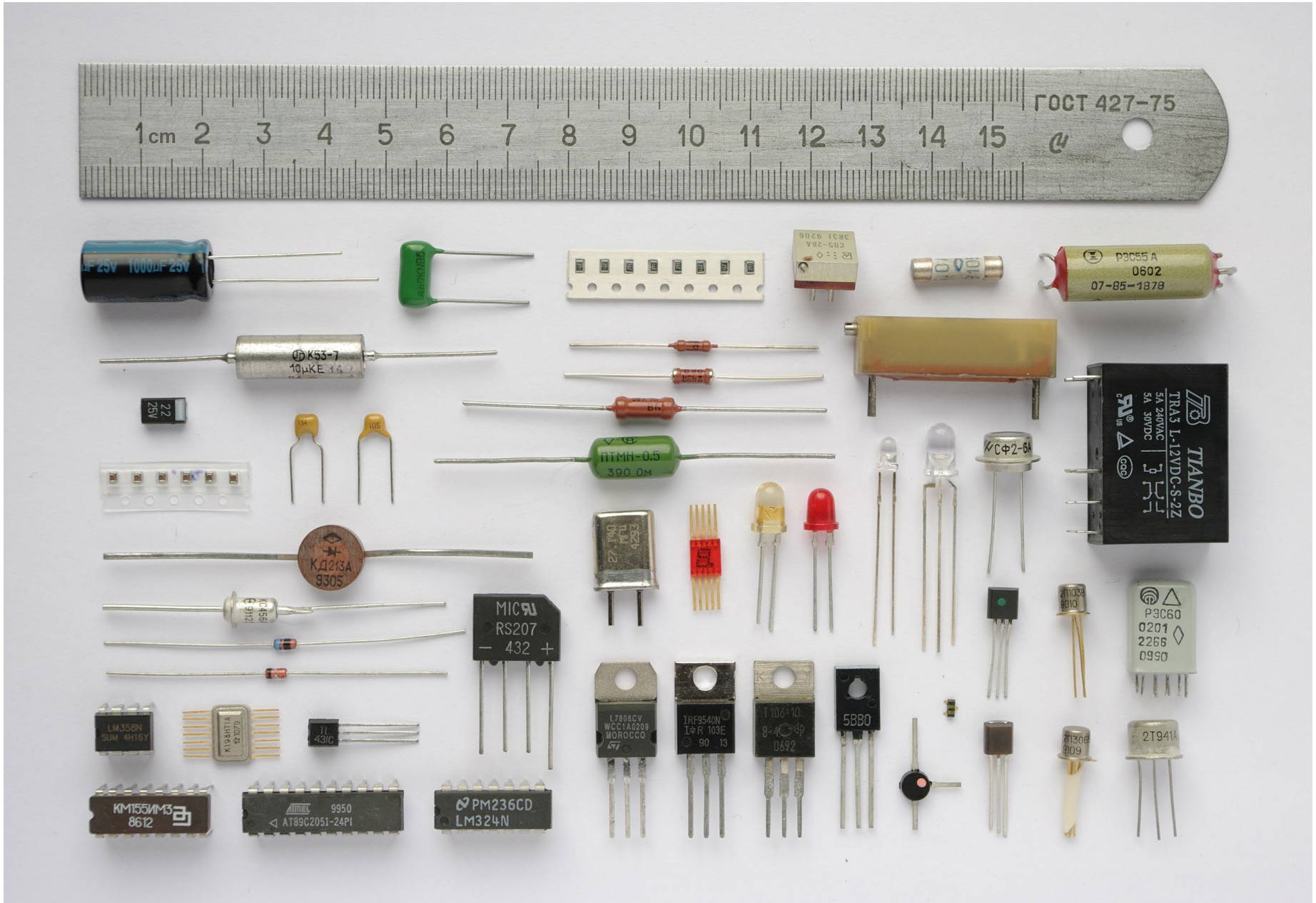


Cell-free systems



Microfluidic devices

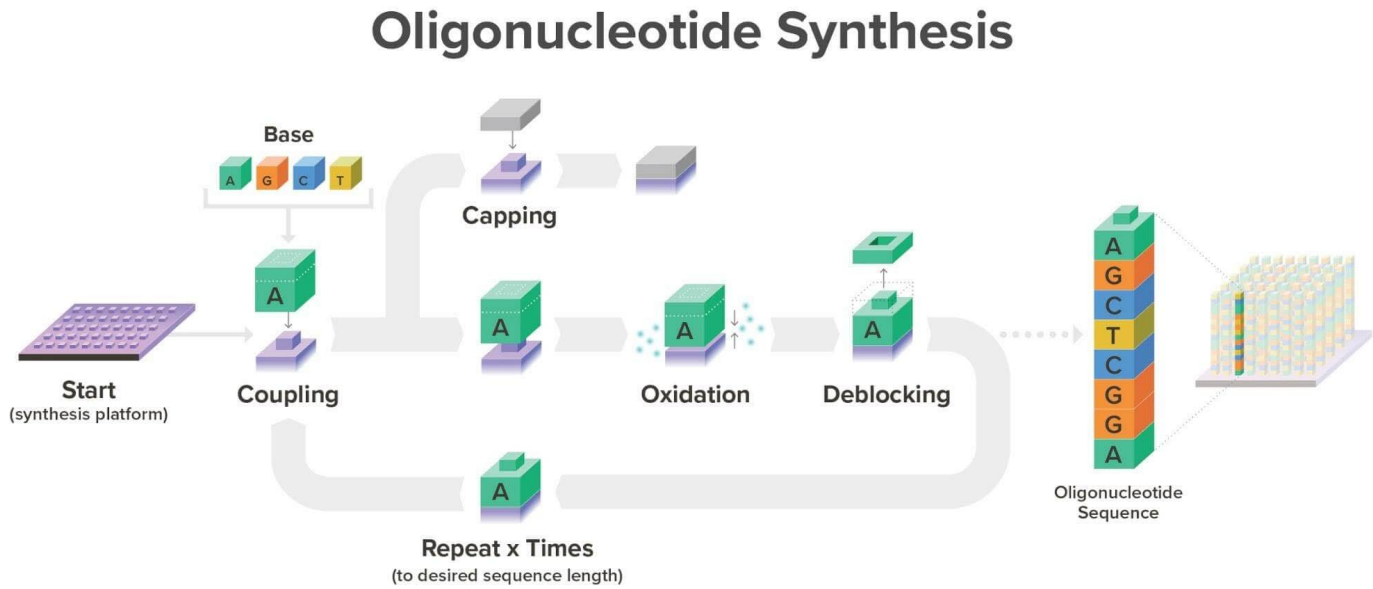
One needs parts to build things…



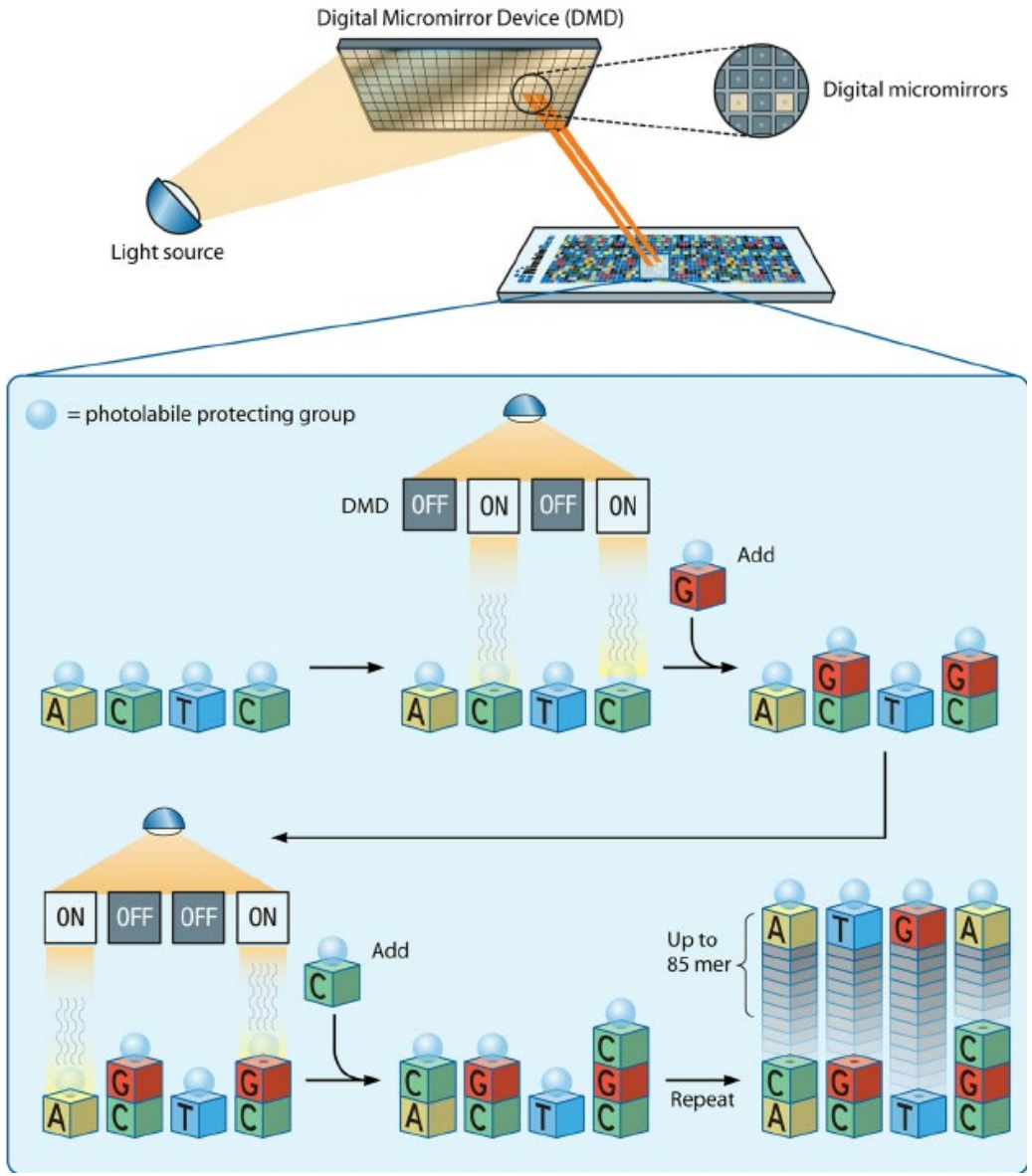
Oligos, Synthetic DNA, PCR

Oligonucleotides:

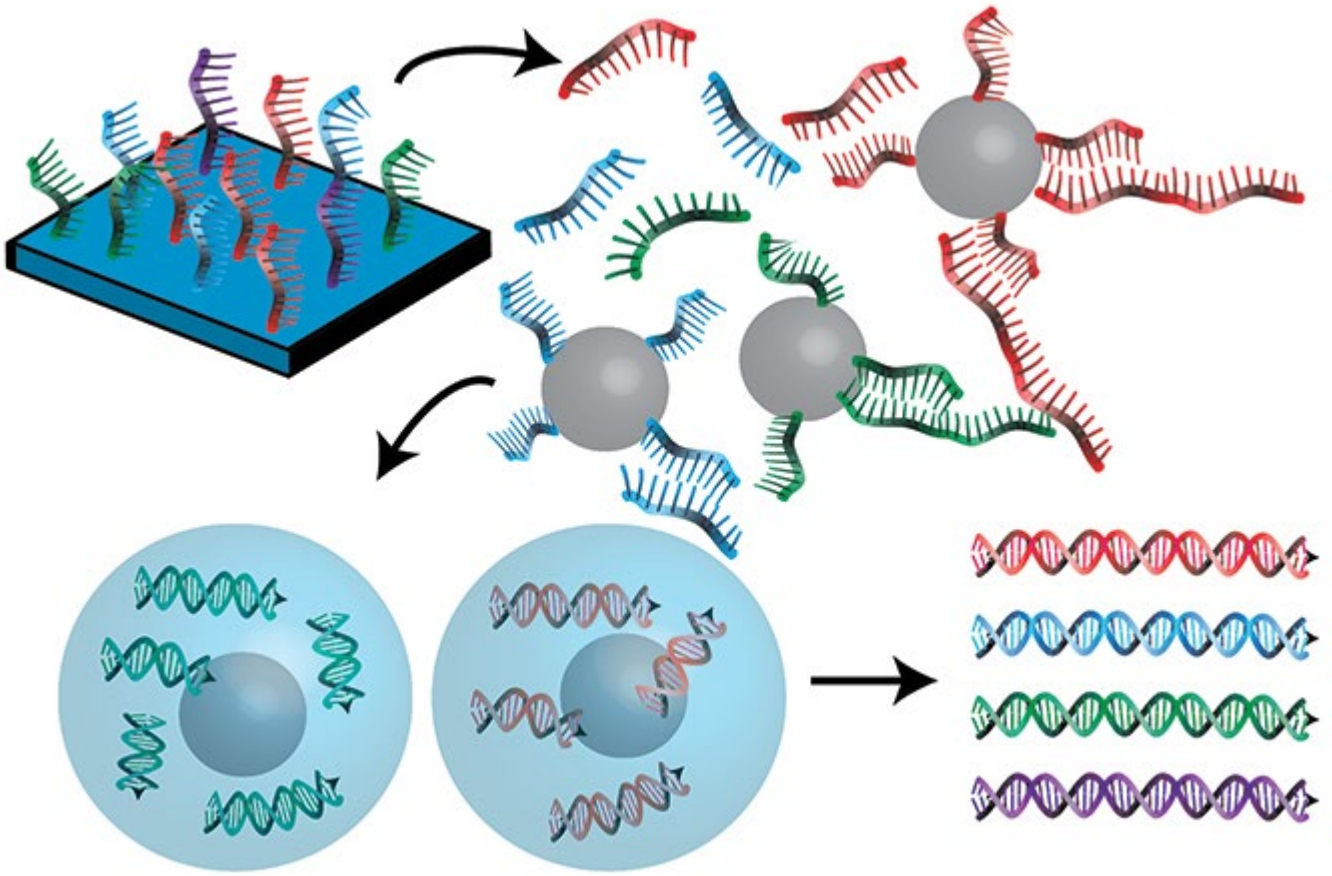
- Single stranded DNA
- Up to 200 bases in length
- Made by chemical synthesis
- Custom sequences can be ordered
- Oligos can be used in PCR and assembled into longer double stranded DNA



Oligos, Synthetic DNA, PCR



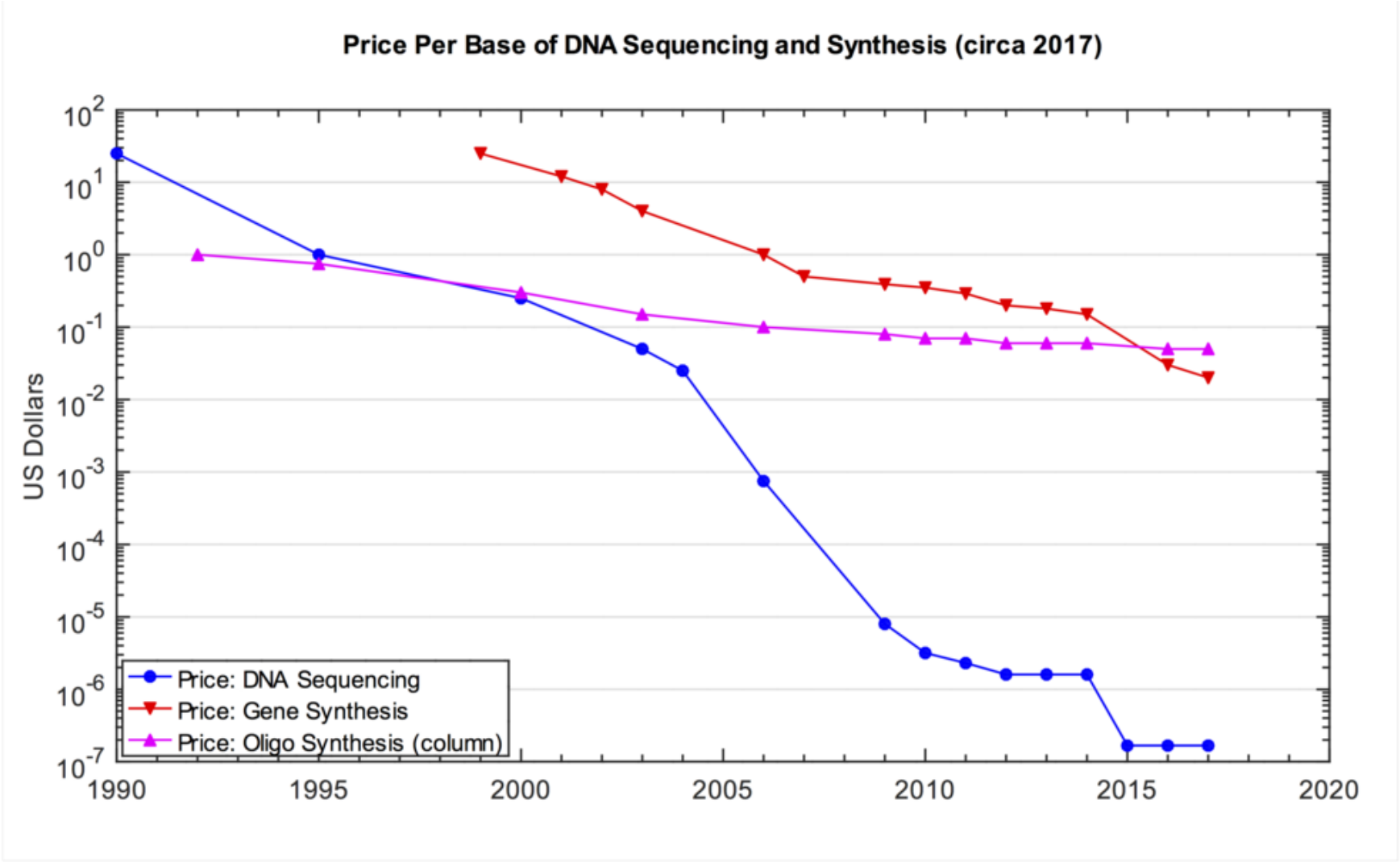
Oligos, **Synthetic DNA**, PCR



Oligonucleotides:

- Double stranded DNA up to ~5kb in length
- Available as linear templates or cloned into a plasmid

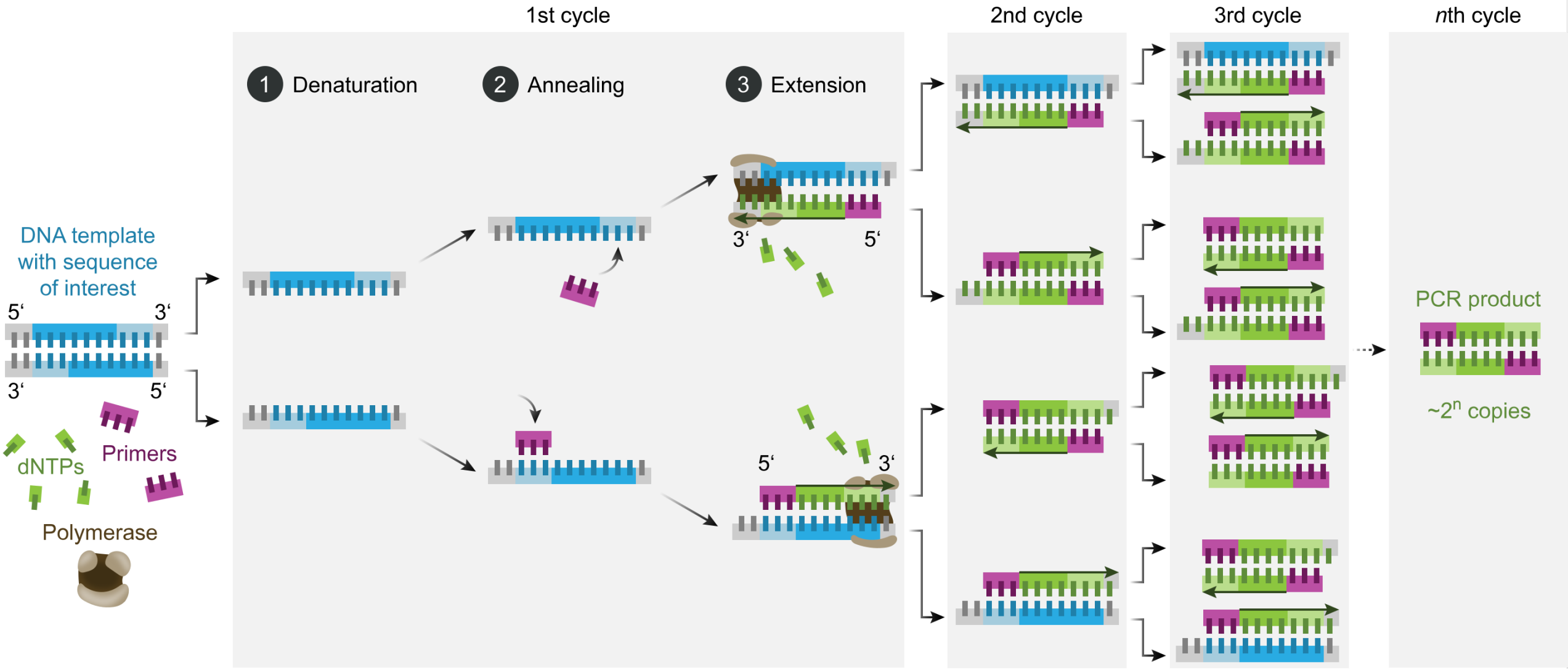
Oligos, **Synthetic DNA**, PCR



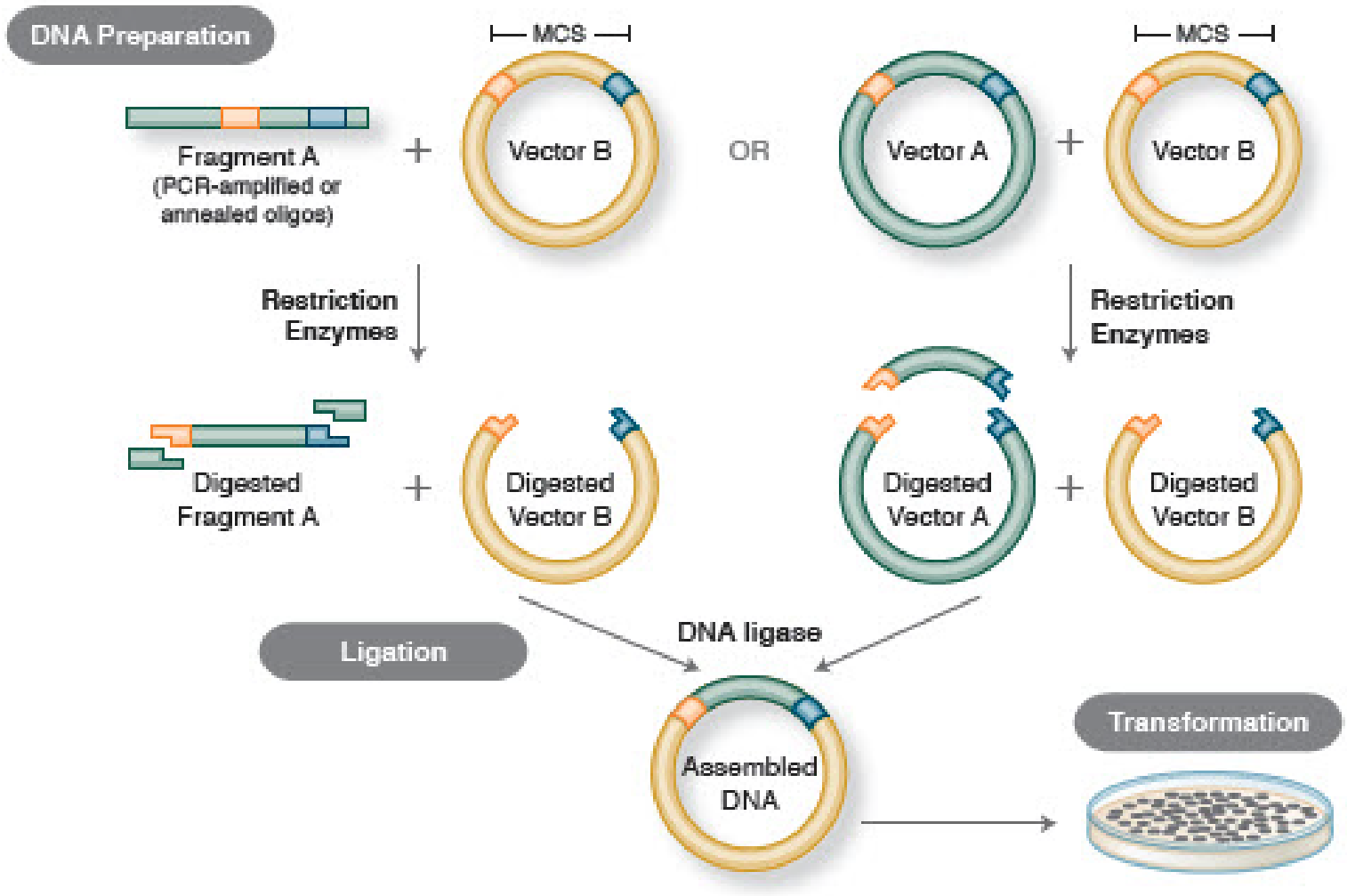
Bioeconomy CAPITAL

20 January, 2018

Oligos, Synthetic DNA, **PCR**



Cloning



Restriction Digest

- Use a restriction enzyme that cuts dsDNA at specific sequences

De-Phosphorylation

- Use a phosphorylase to remove phosphate group from the cut sites, this prevents re-ligation of the cut vector

Ligation

- Use an enzyme called a ligase to link two pieces of DNA to one another

Transformation

- The cloned vector is then put into E. coli cells which amplify the vector

Protein Expression and Purification

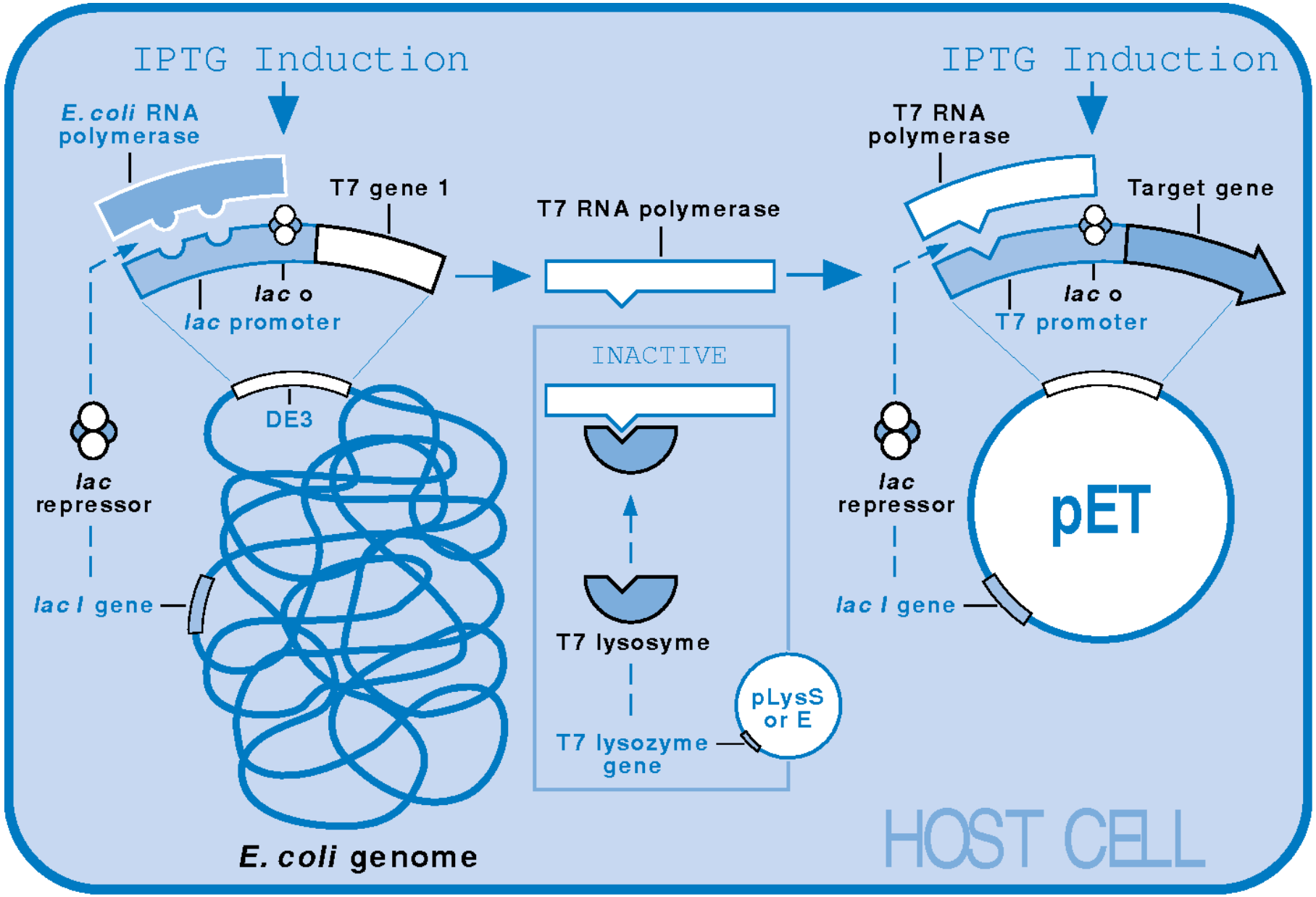
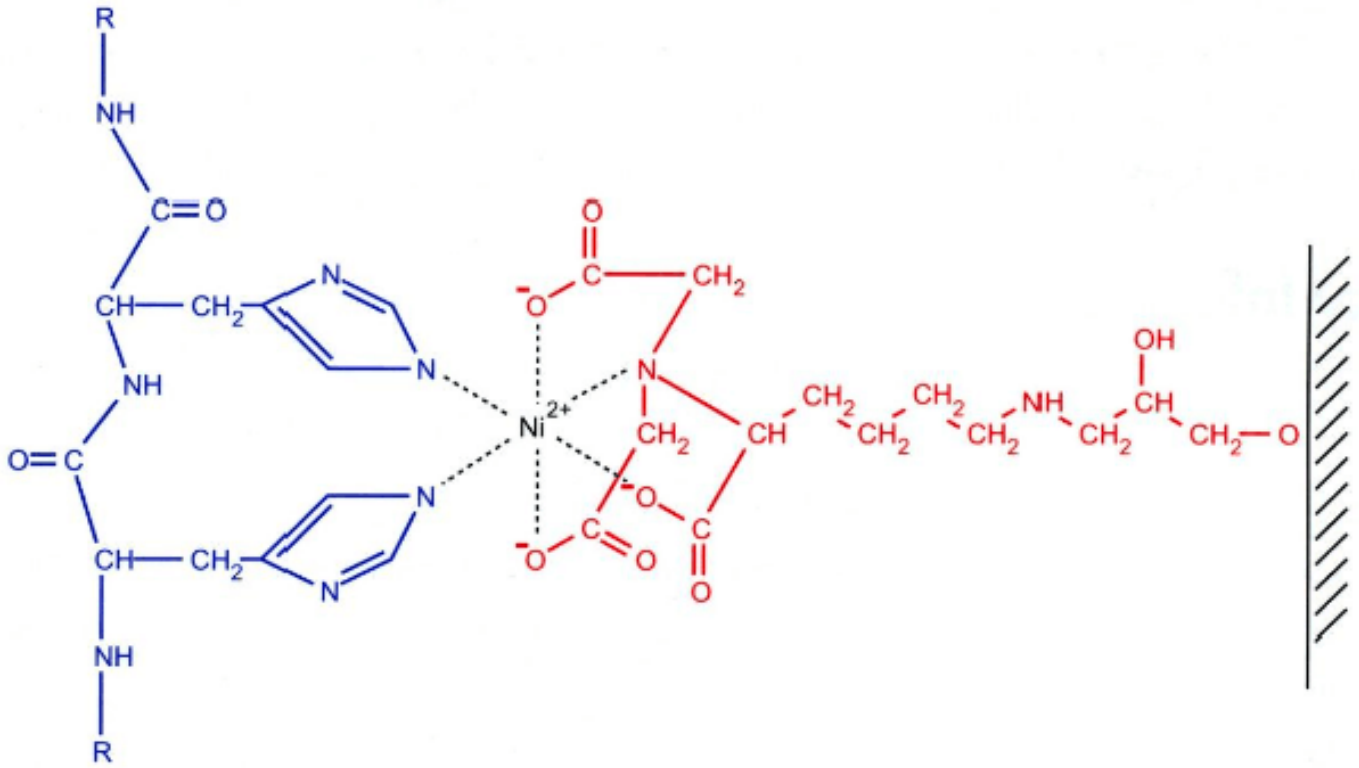
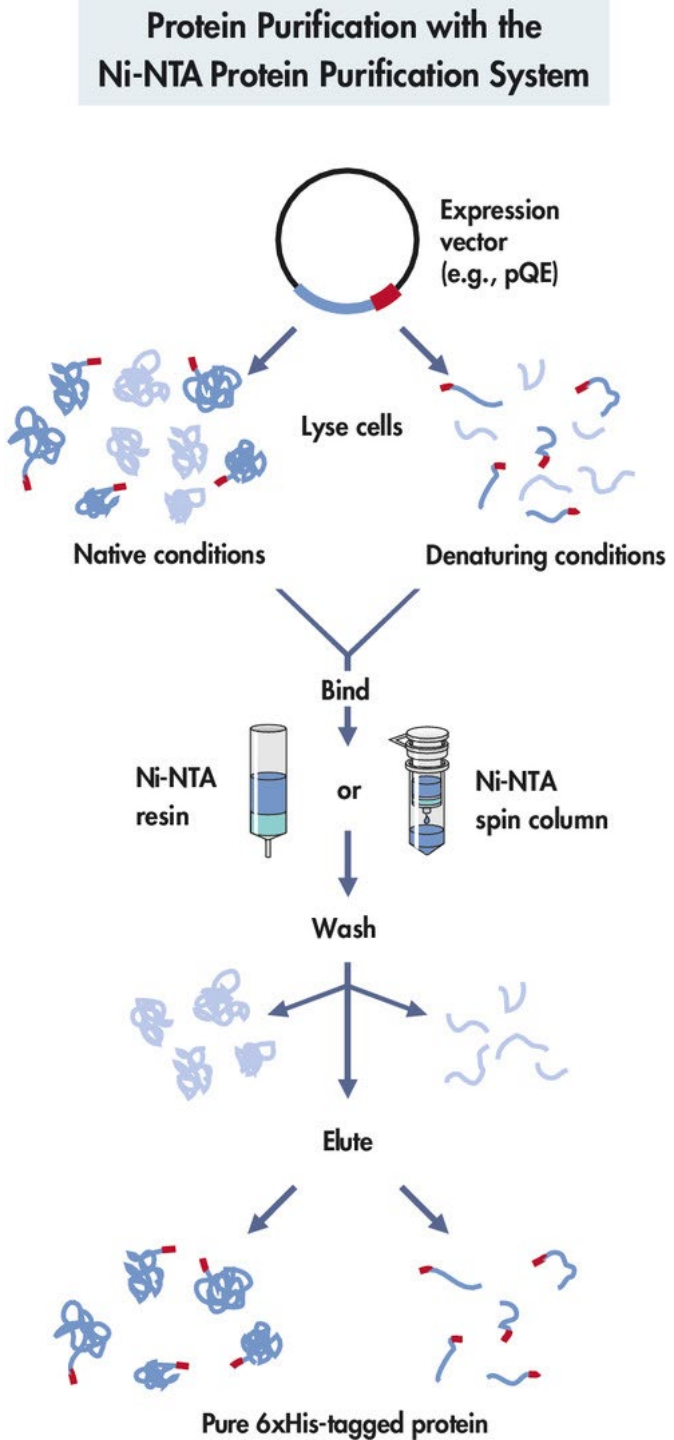


Figure 1. Control elements of the pET System

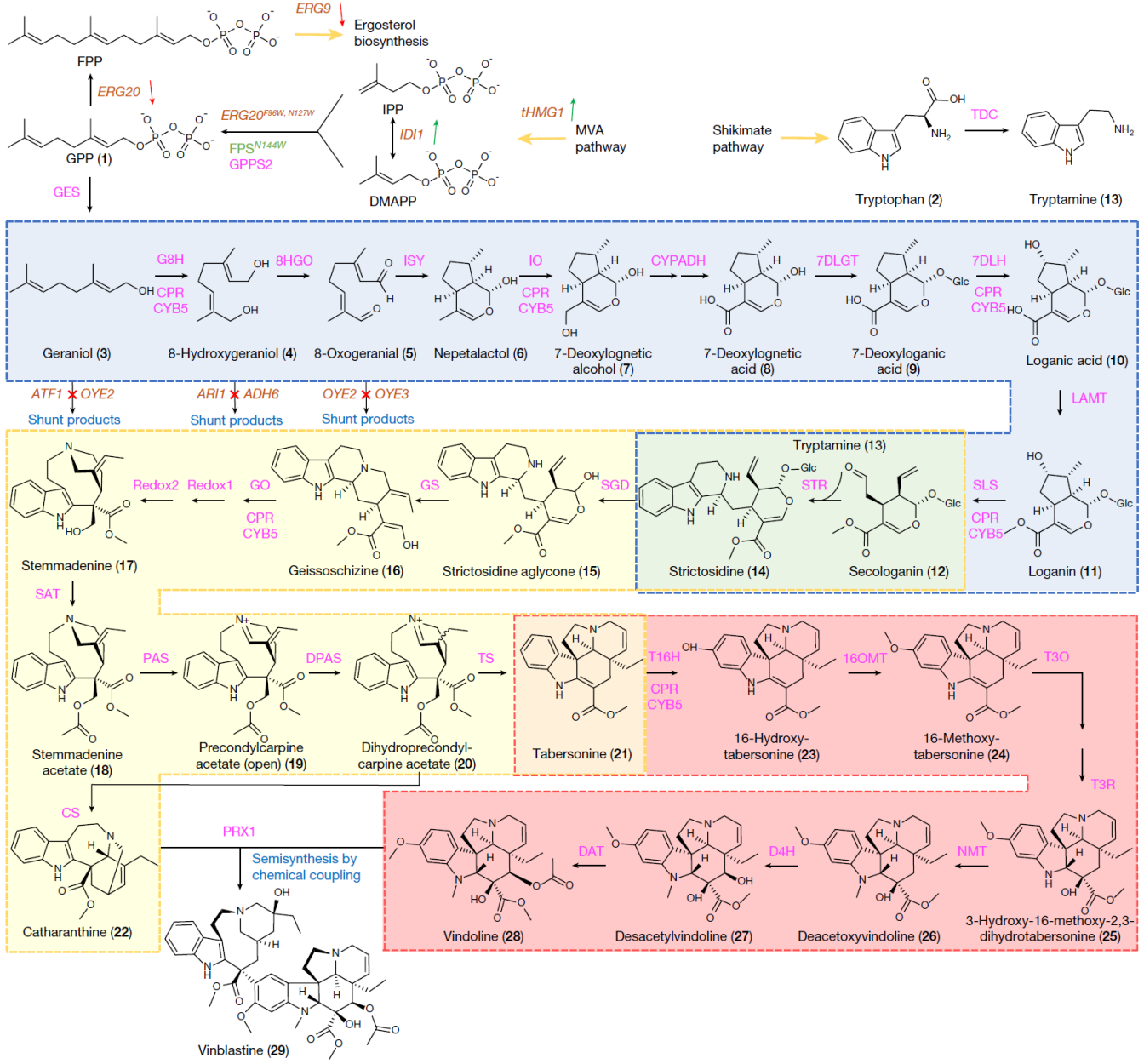
Protein Expression and Purification

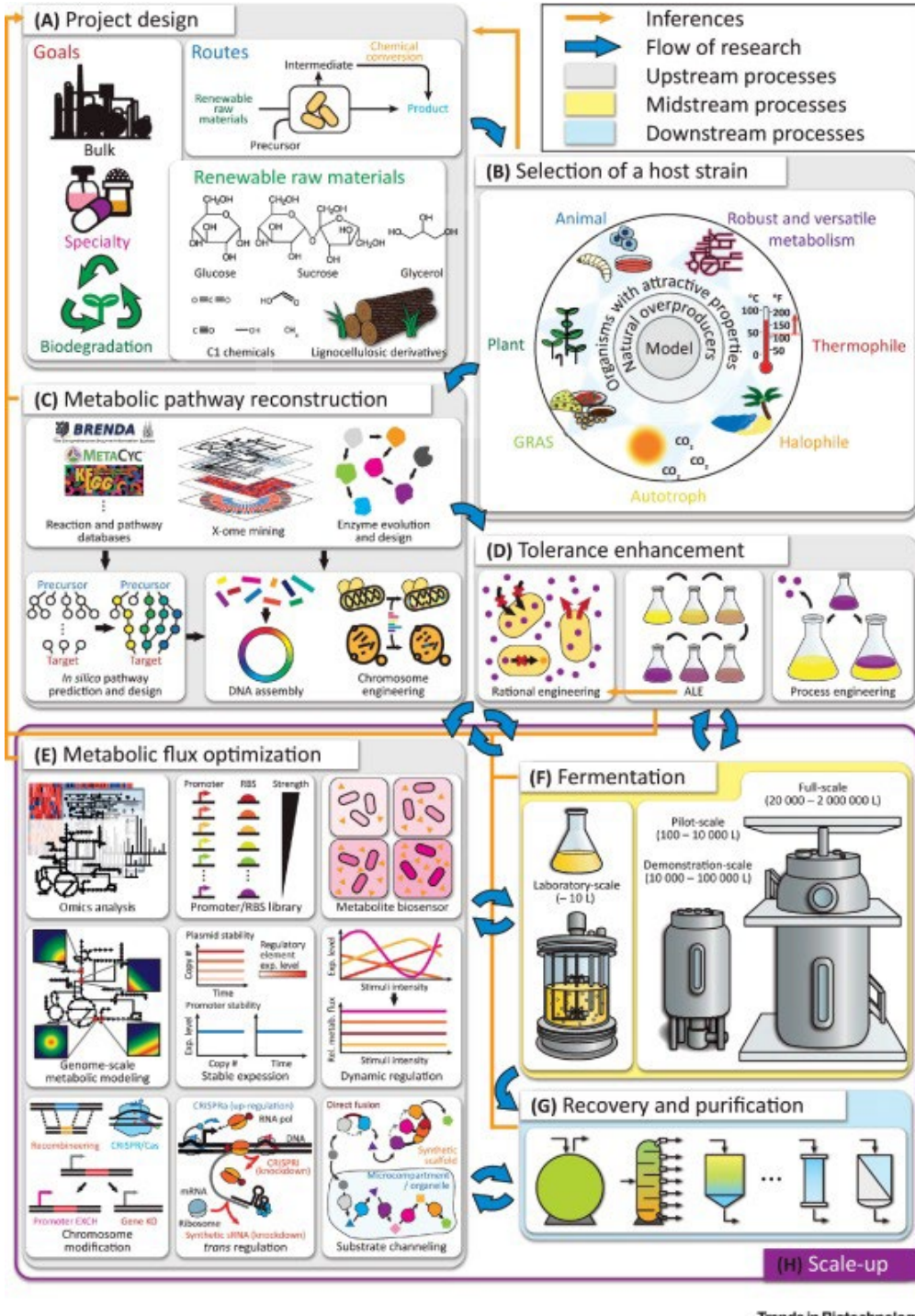




What is Biological Engineering good for?

Metabolic Engineering





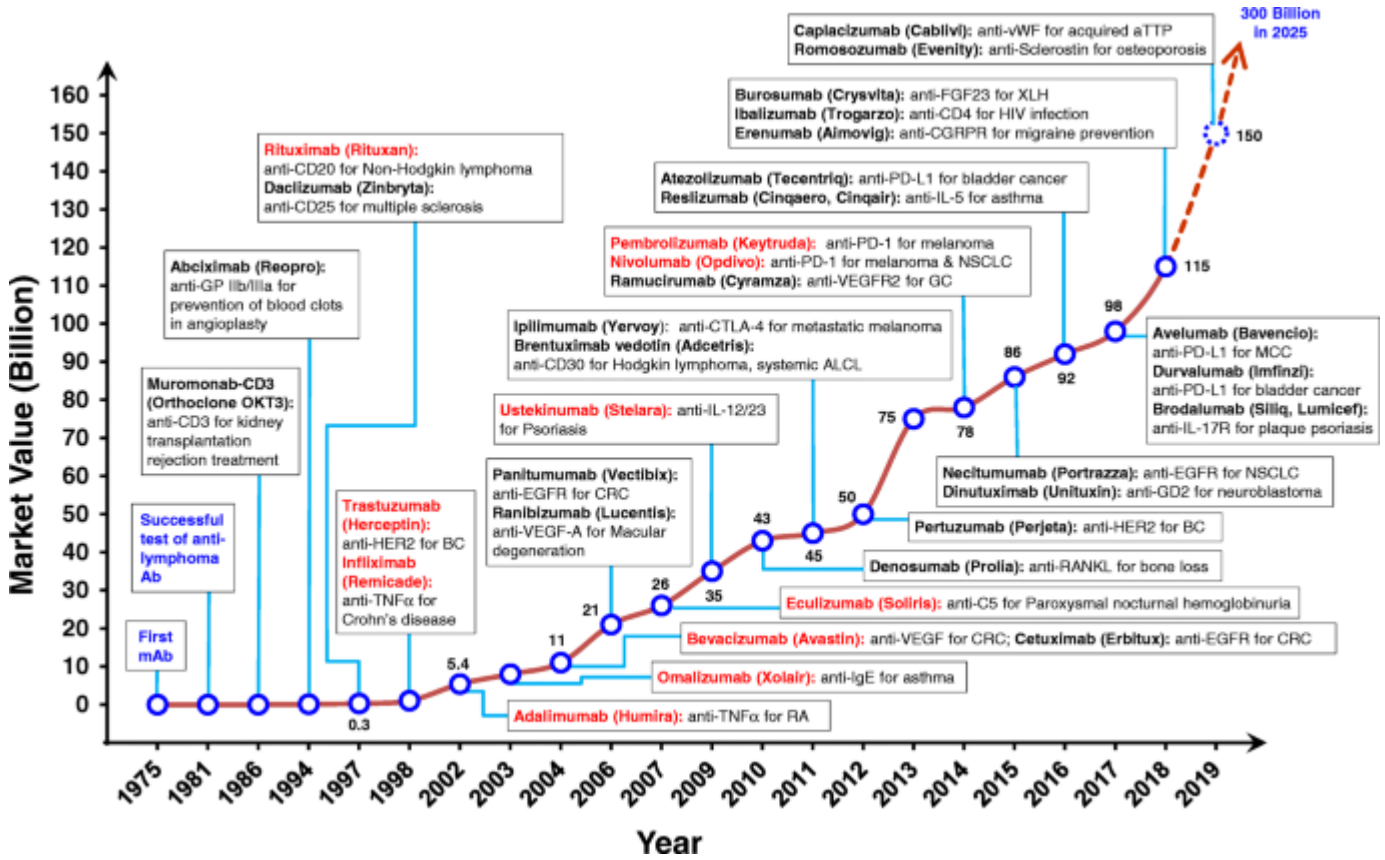
Antibody Therapeutics

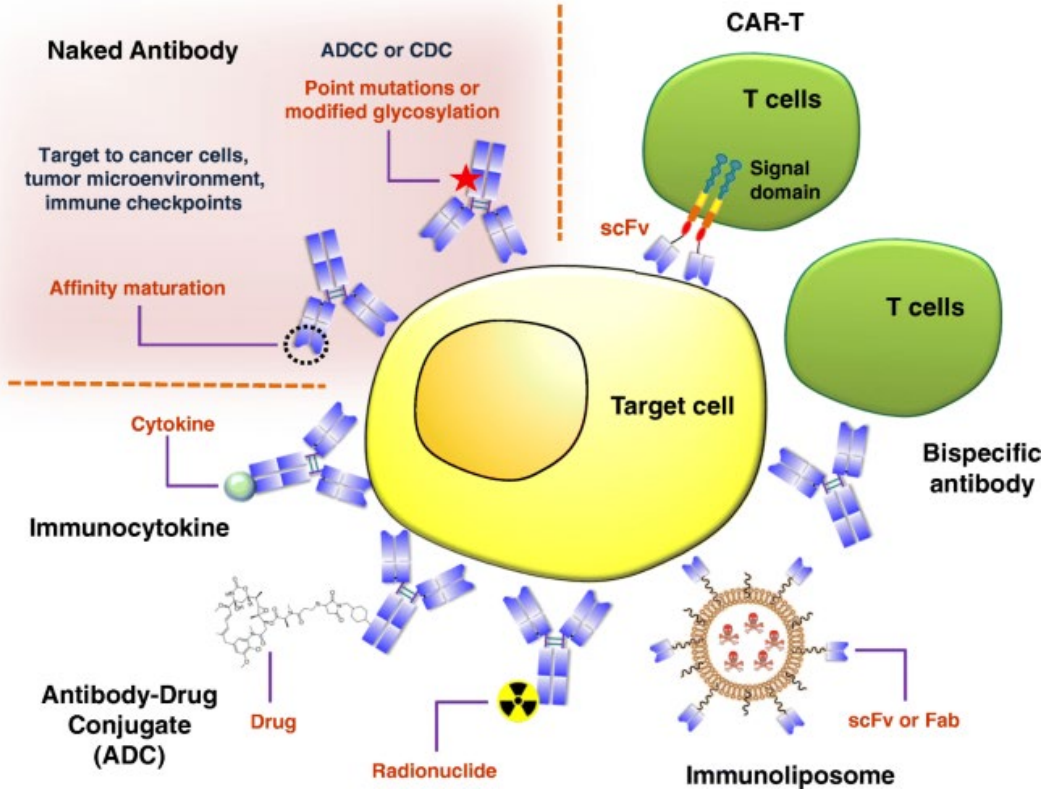
$188.18 billion

The global monoclonal antibodies (MAbs) market size is expected to grow from $168.70 billion in 2021 to **$188.18 billion** in 2022 at a compound annual growth rate (CAGR) of 11.5%.

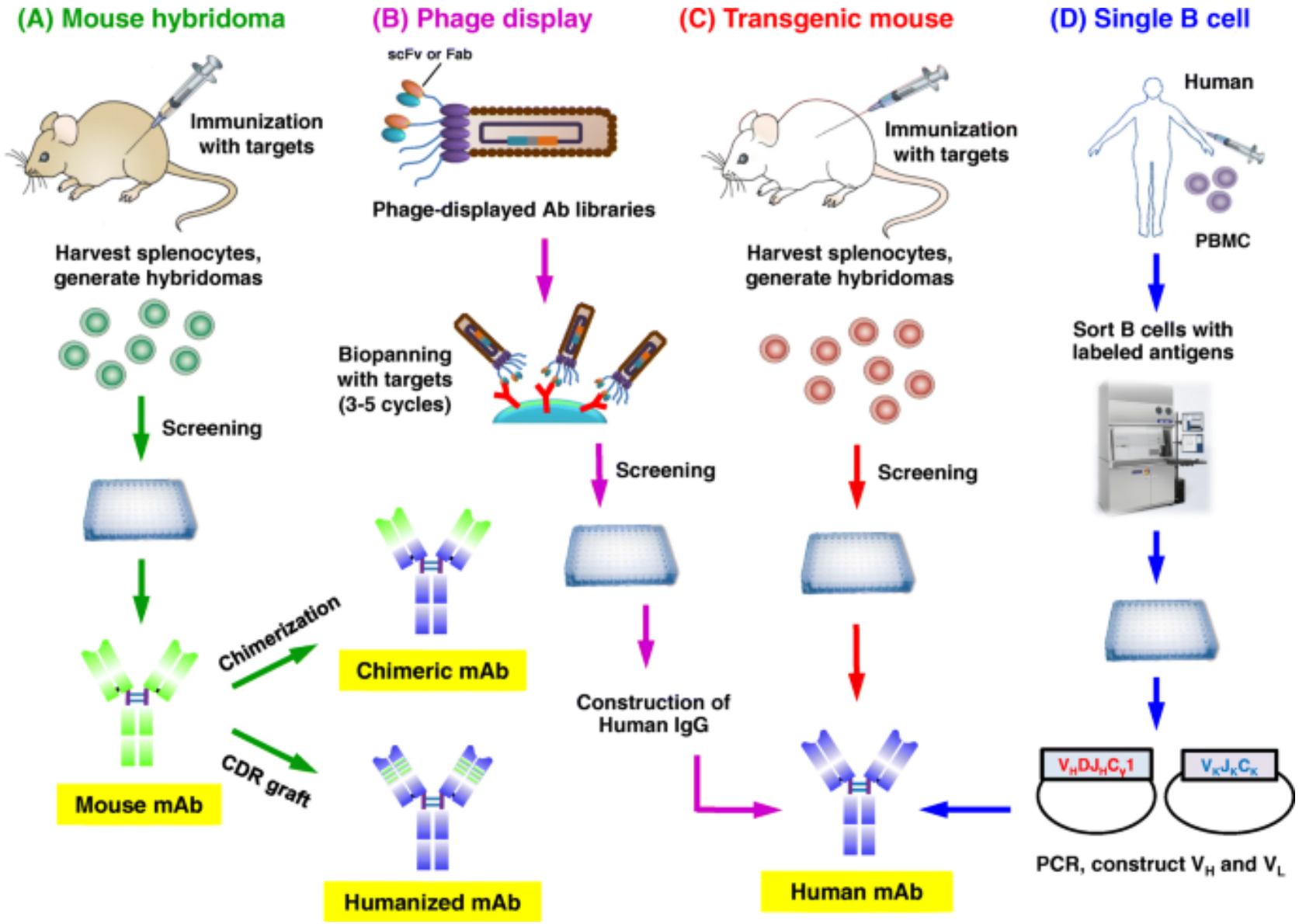
https://www.thebusinessresearchcompany.com › report

Monoclonal Antibodies (MAbs) Global Market Report 2022

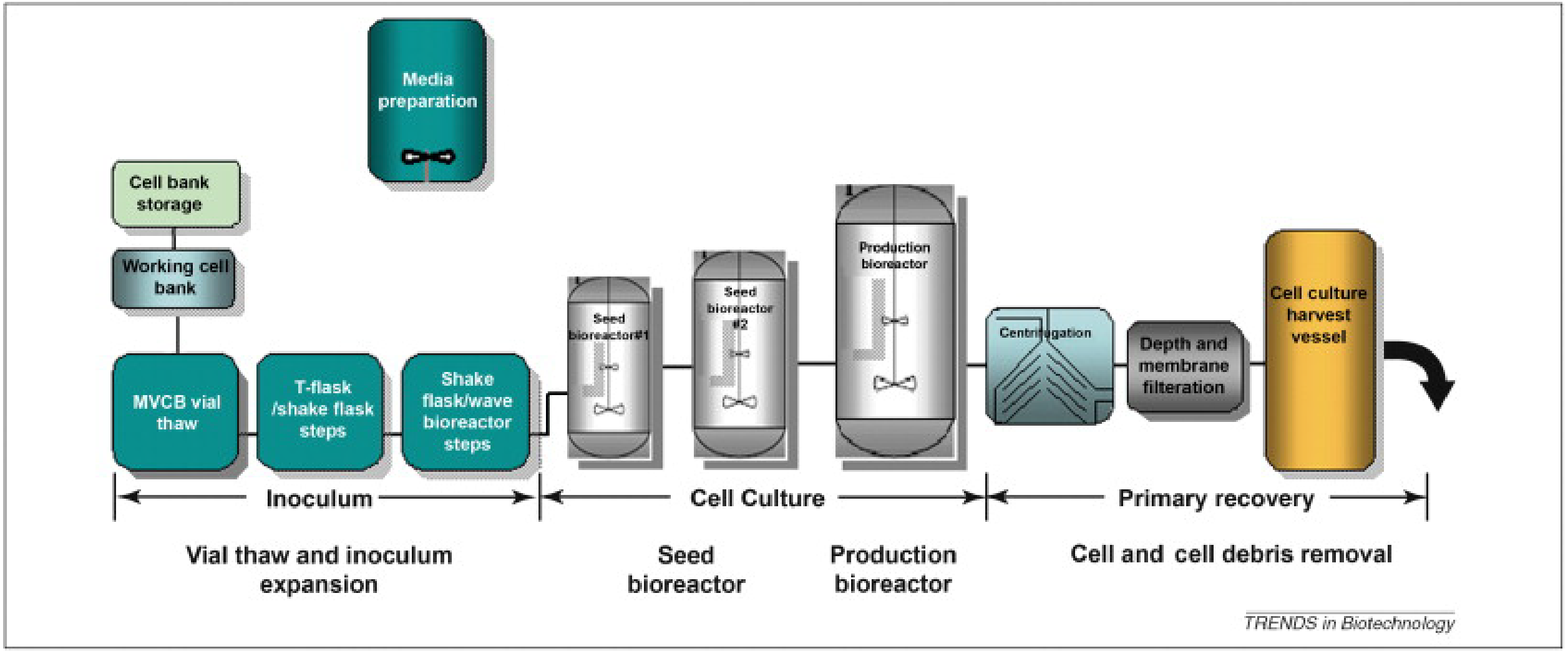




Antibody Discovery

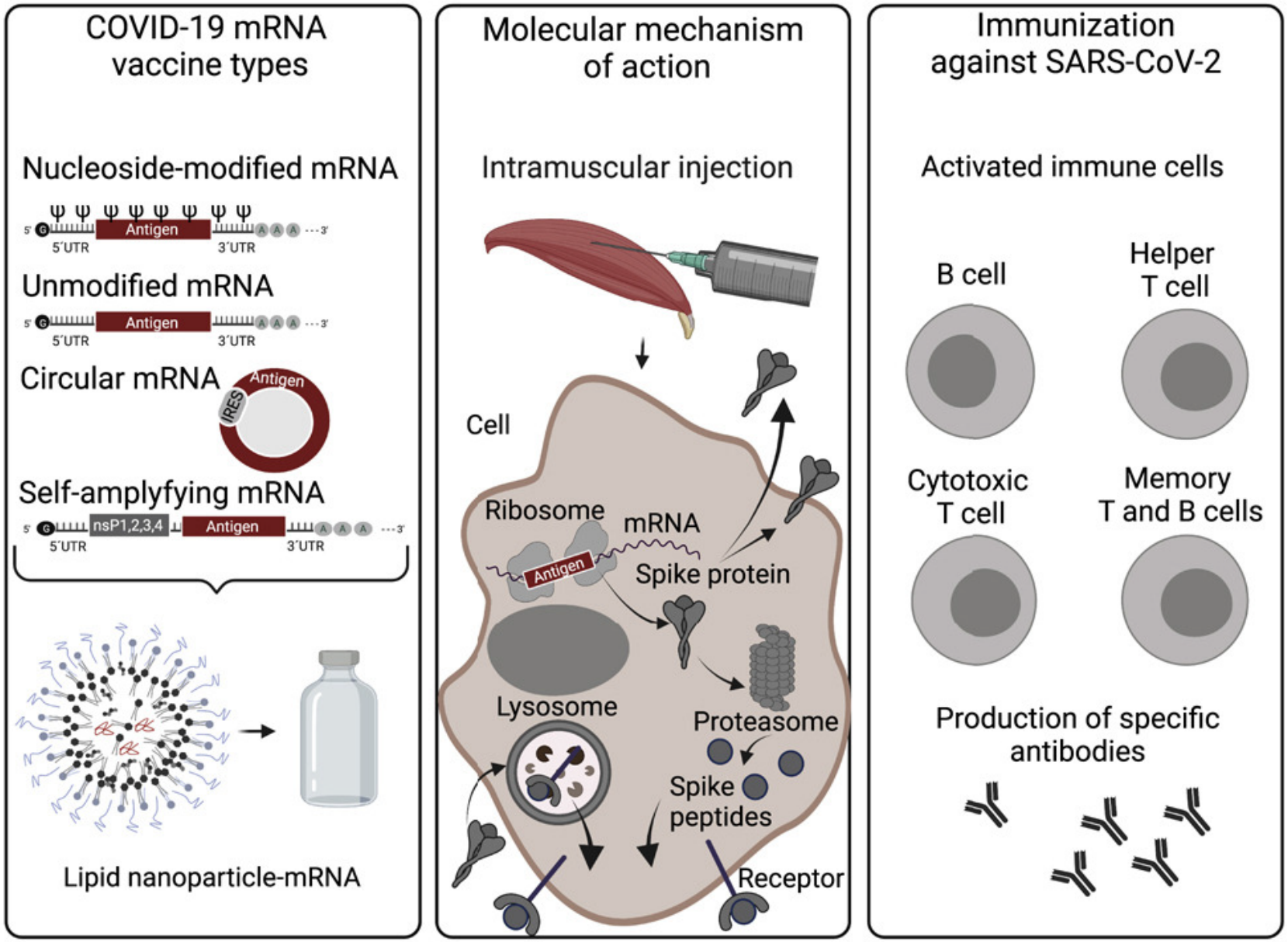


Antibody Production

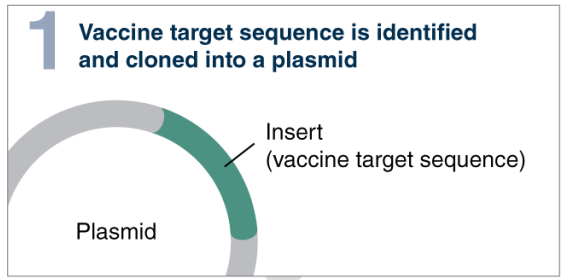


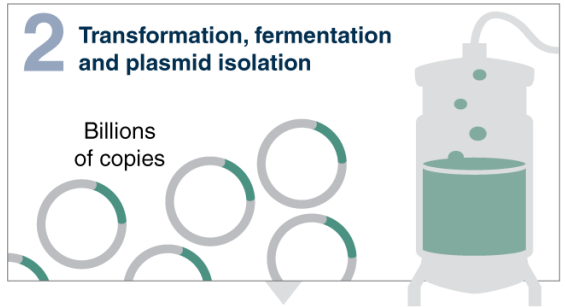
Antibody Production

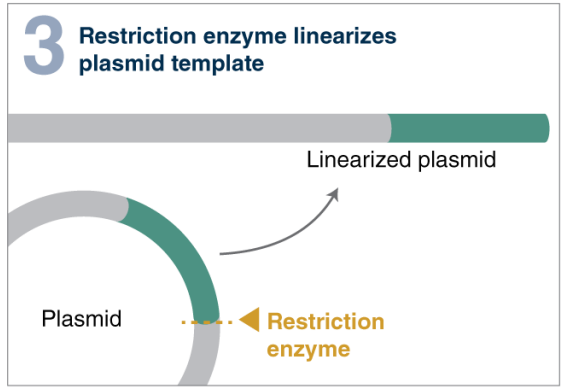
mRNA Vaccines

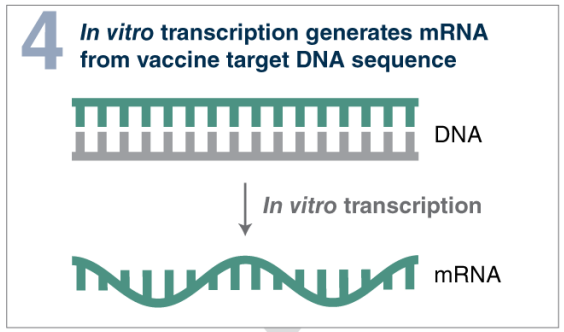


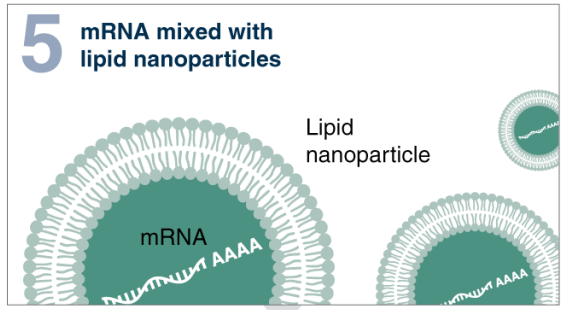
mRNA Vaccines how do you make them?

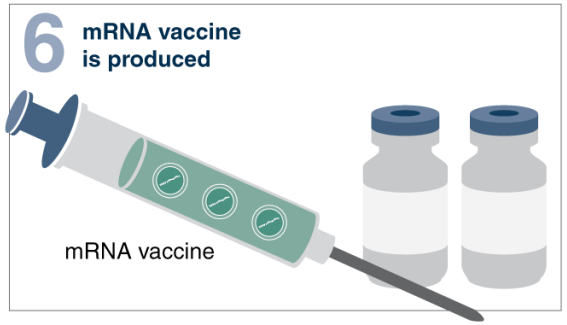












mRNA

LNP-mRNA

Lab-scale
(in microliters
or milliliters)

Scale-up
(in liters)