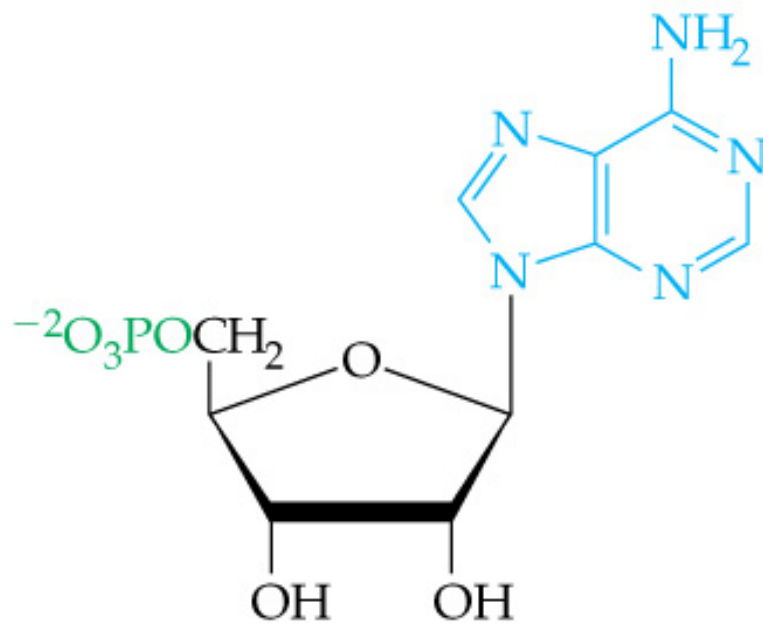
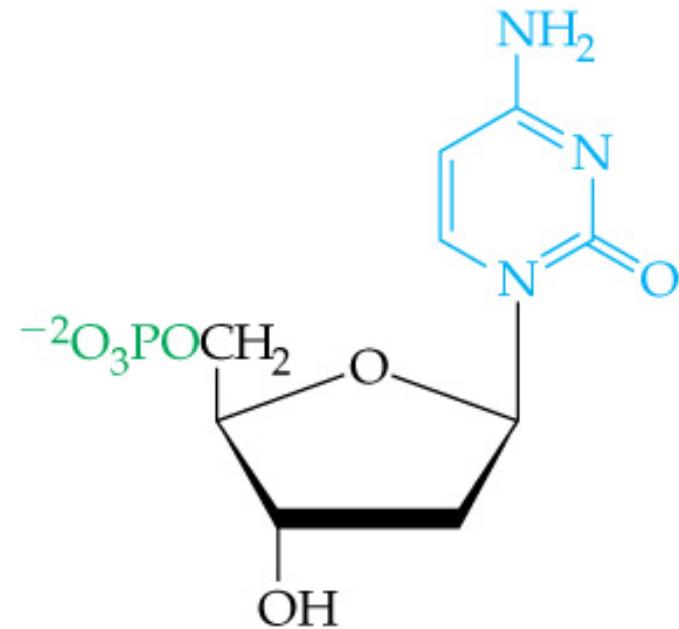


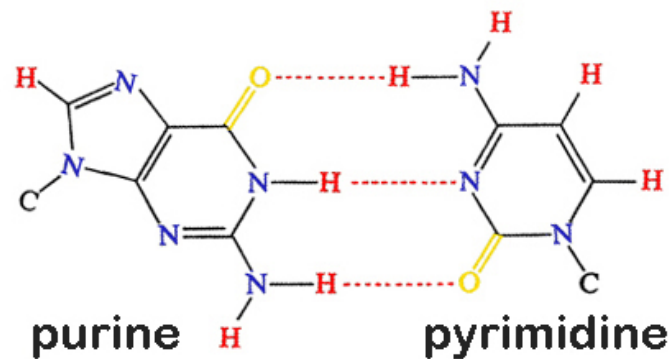
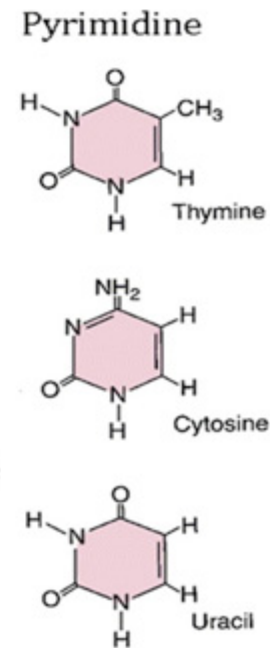
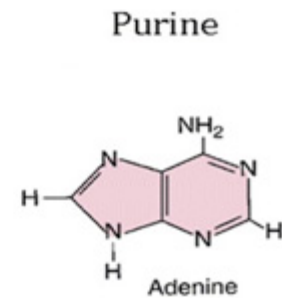
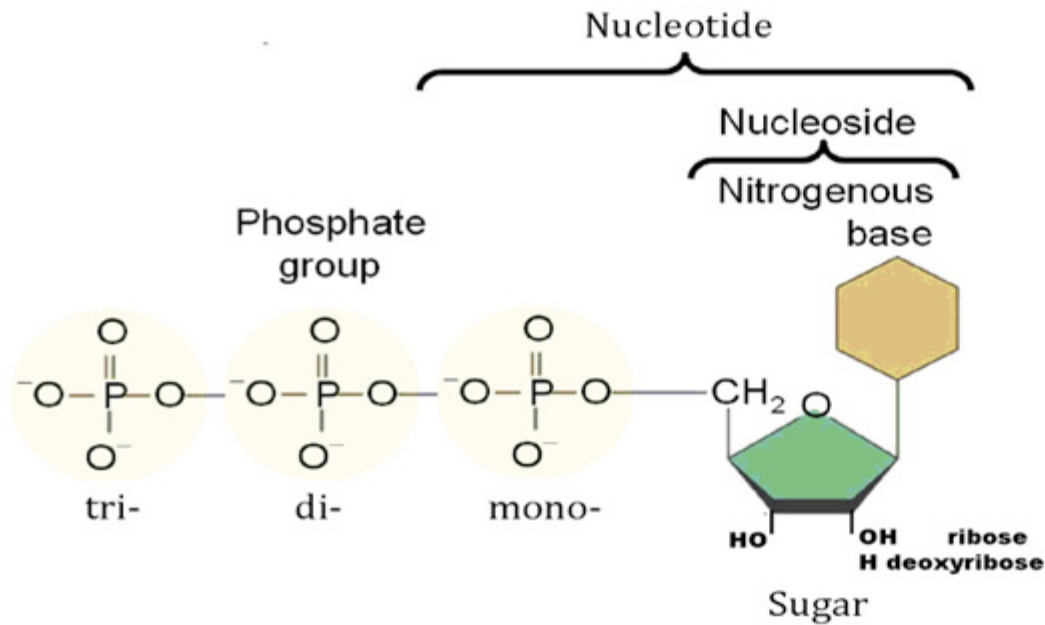
- In RNA, the sugar is ribose.
- In DNA, the sugar is deoxyribose.



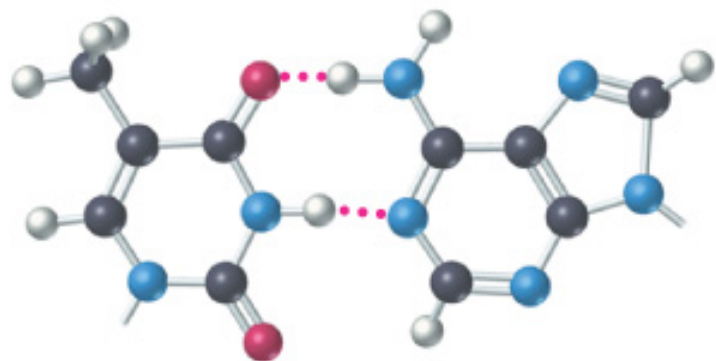
Adenosine 5'-monophosphate (AMP)  
(a ribonucleotide)



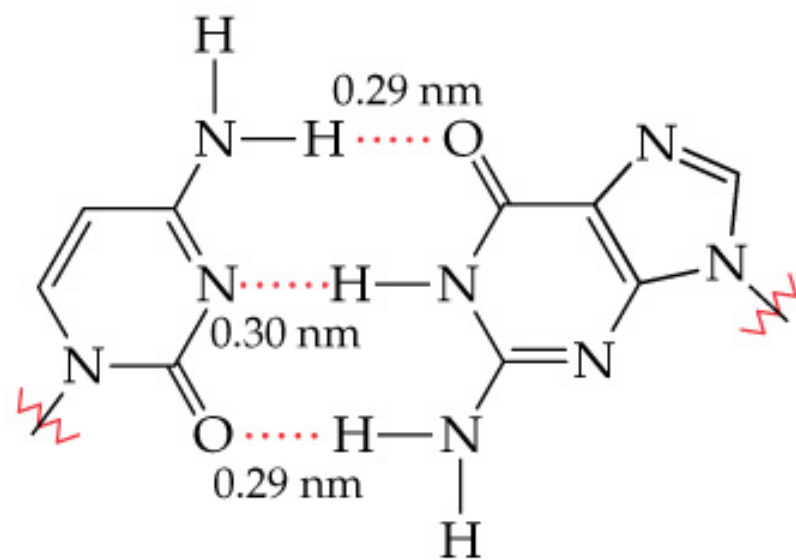
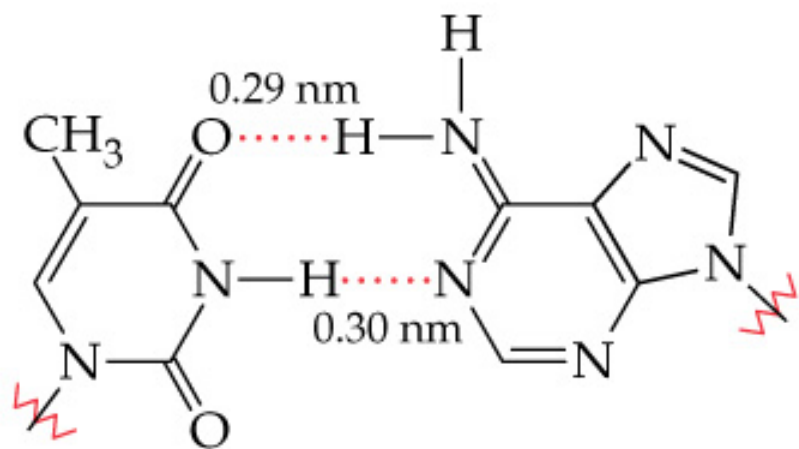
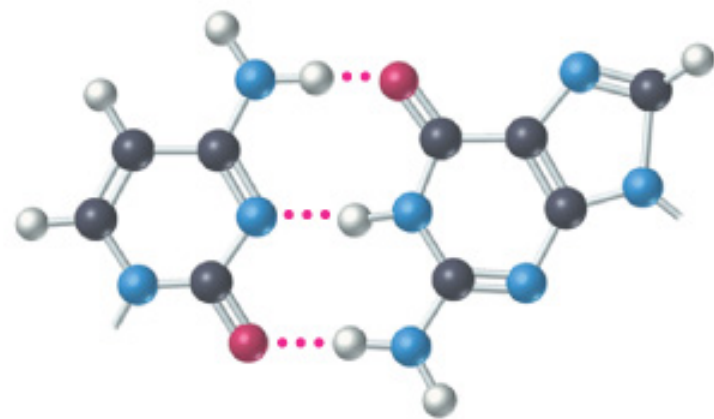
Deoxycytidine 5'-monophosphate (dCMP)  
(a deoxyribonucleotide)

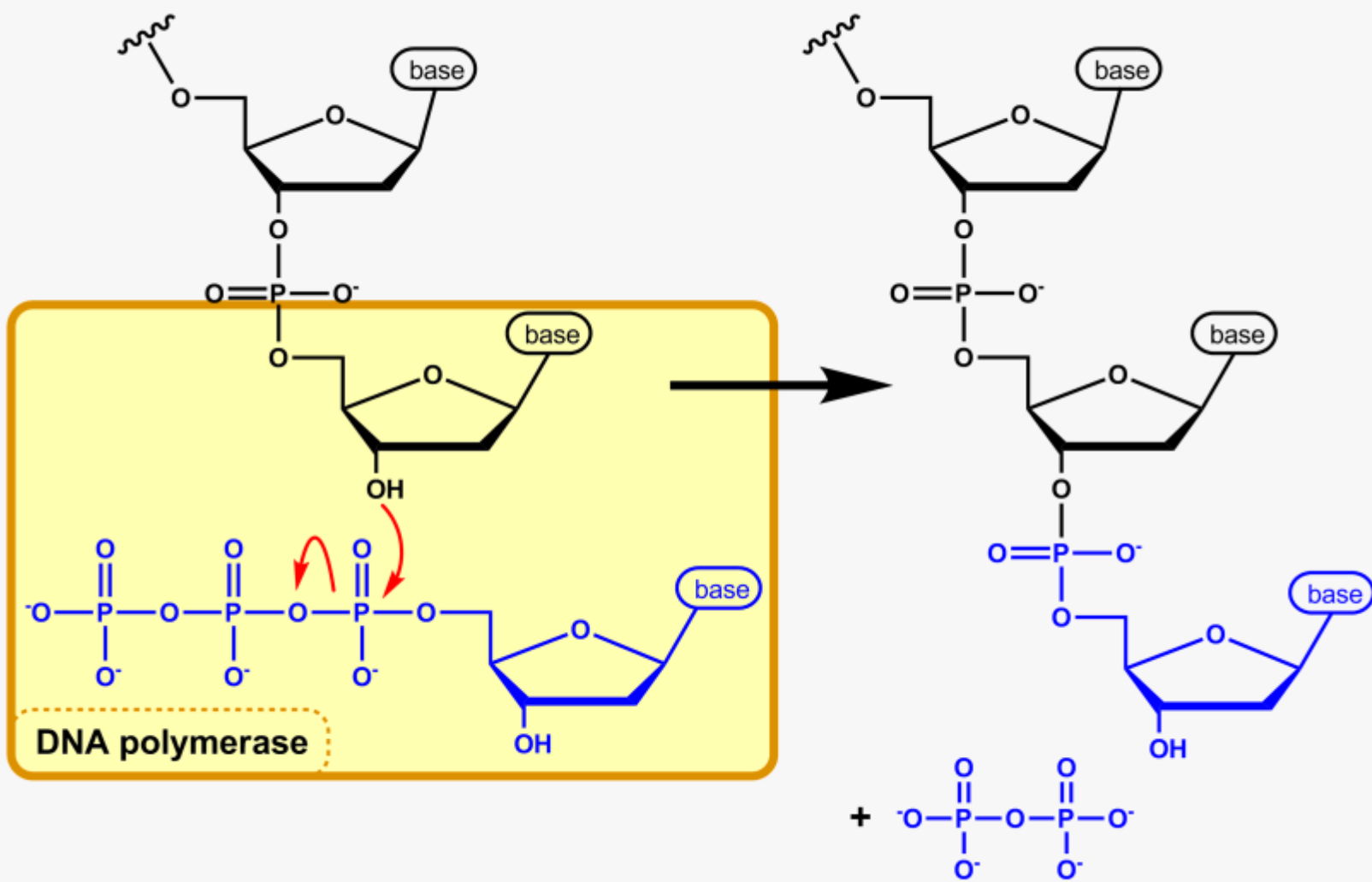


Thymine-Adenine



Cytosine-Guanine







# RNA

## Base

**G C U A C G G A G C U U C G G A G C U A G**

## Codon

Codon 1 Codon 2 Codon 3 Codon 4 Codon 5 Codon 6 Codon 7

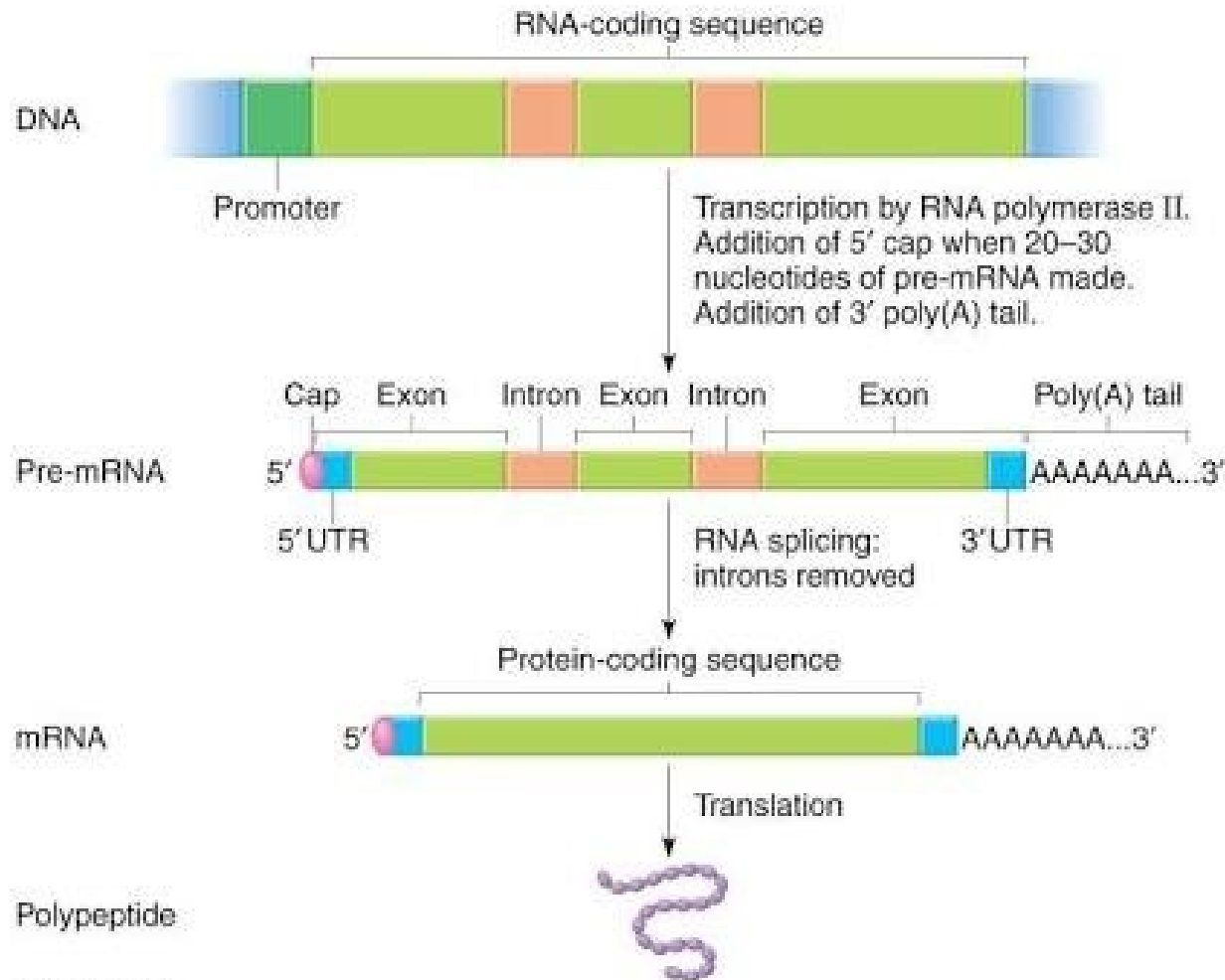
## Aminoacid

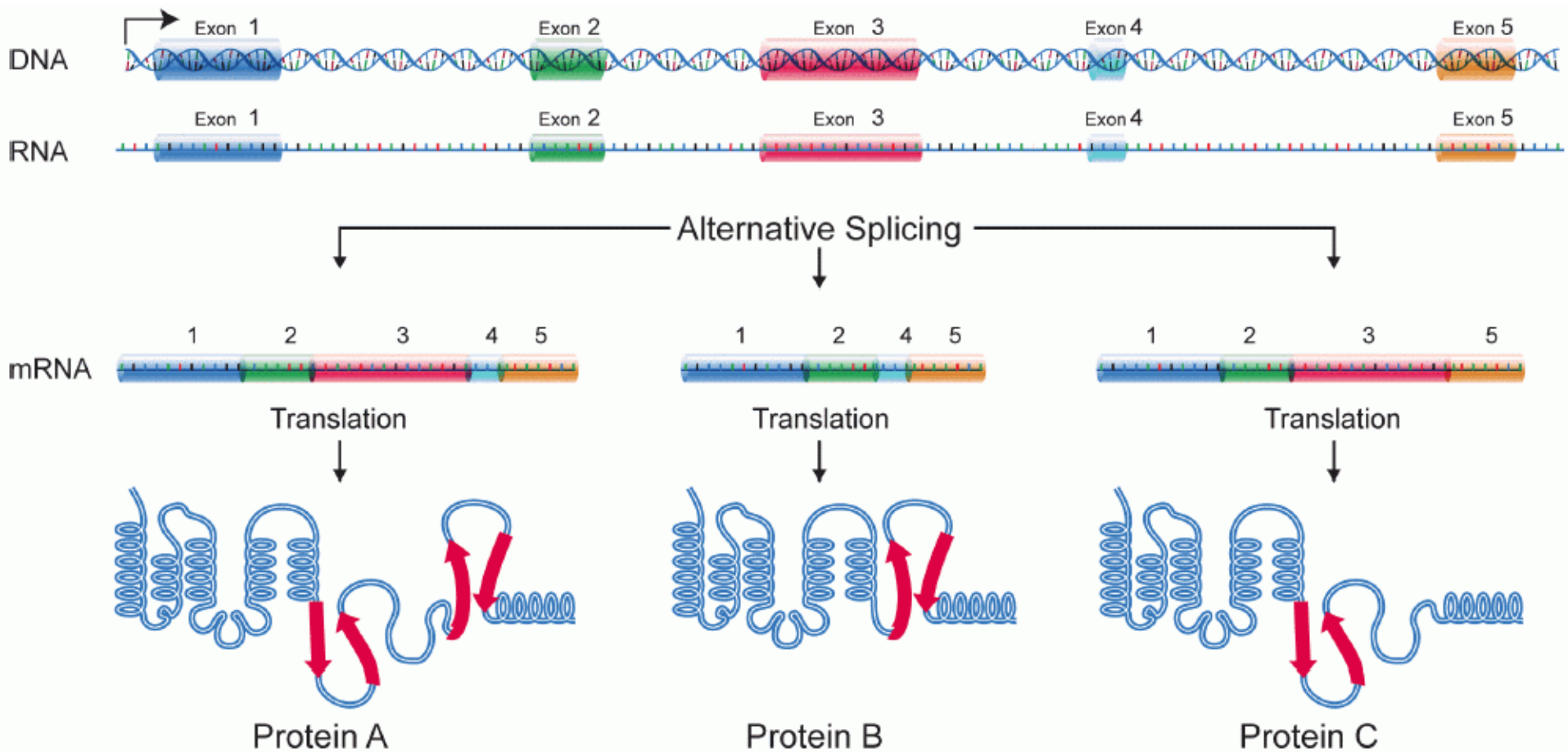
**Alanine Threonine Glutamate Leucine Arginine Serine Stop**

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } <b>UAA Stop</b> <b>UAG Stop</b>	UGU } Cys UGC } <b>UGA Stop</b> UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } <b>AUG Met</b>	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

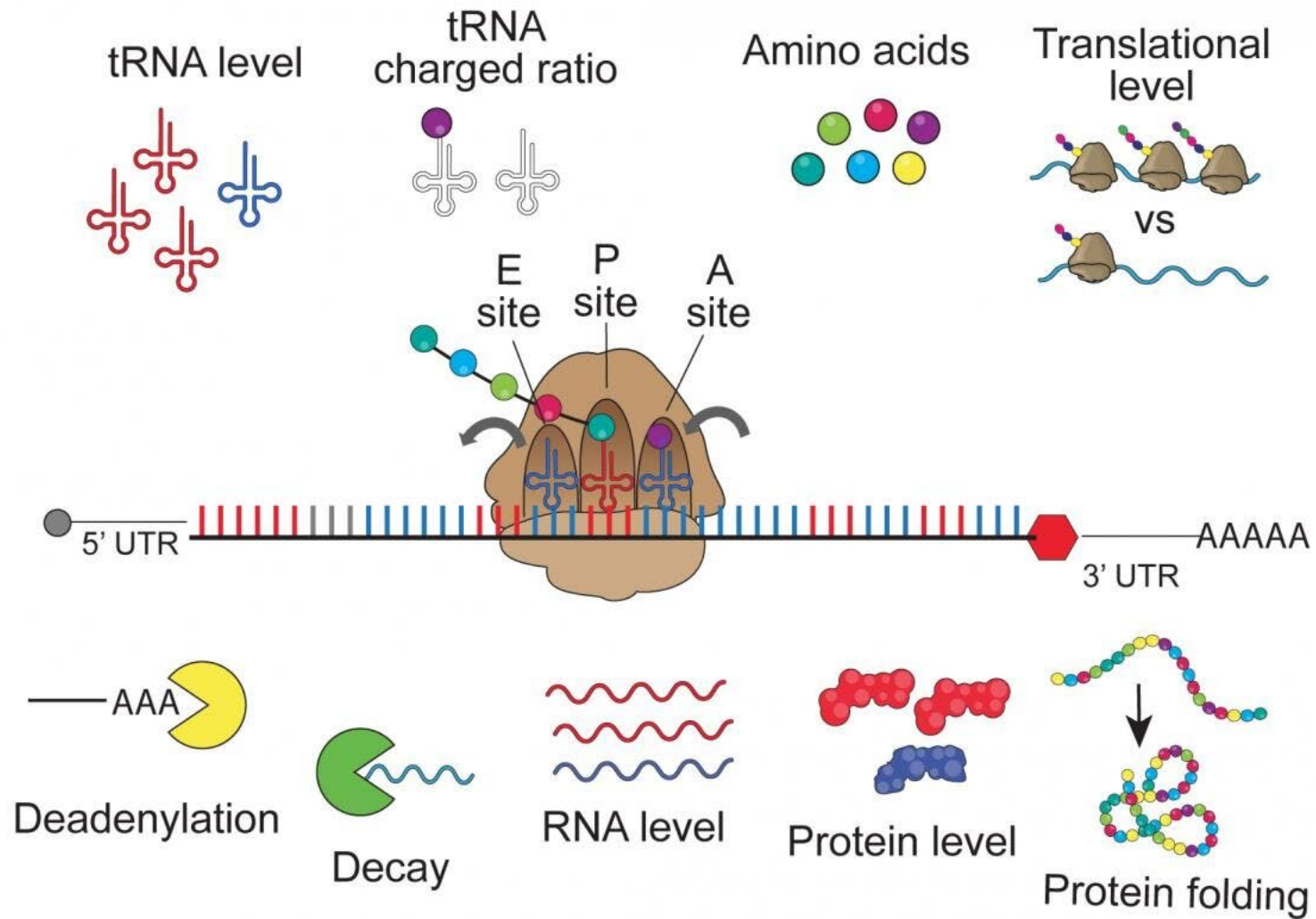
# Post Transcription Modification of RNA

1. RNA capping
2. PolyA tail
3. Splicing



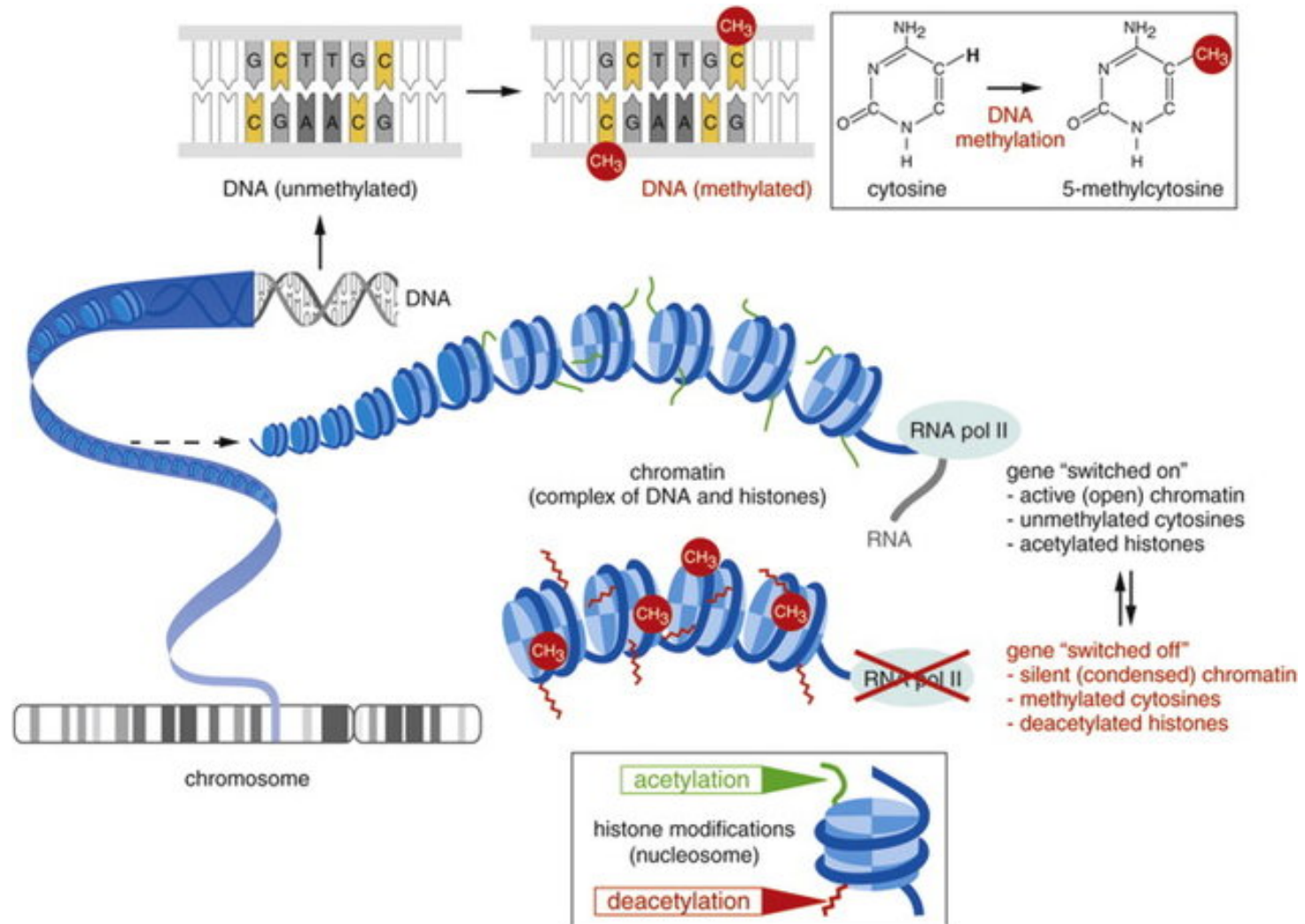


# Upstream regulator



Downstream effects

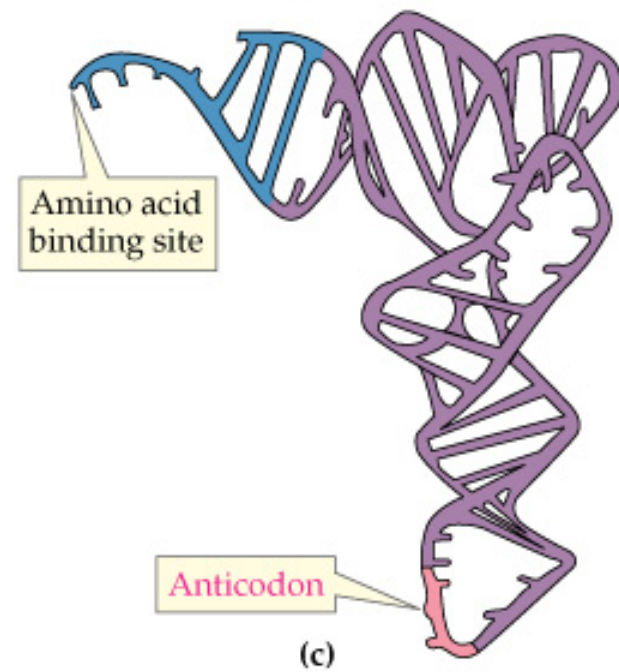
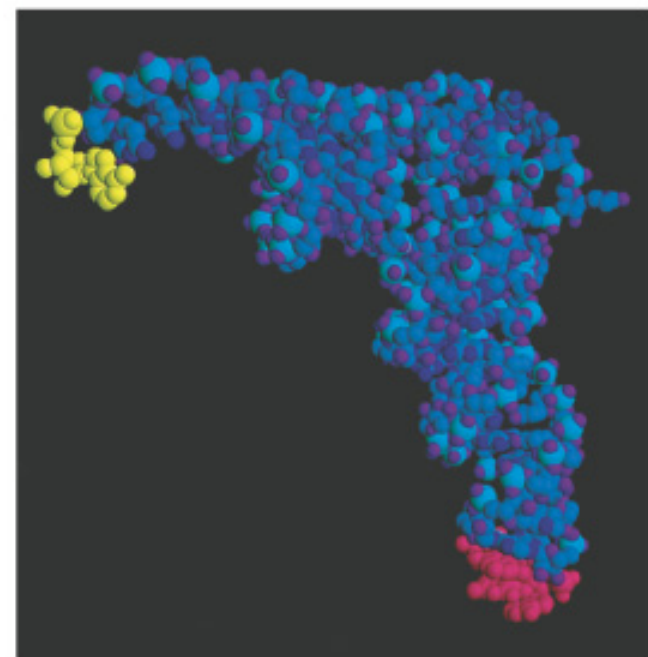
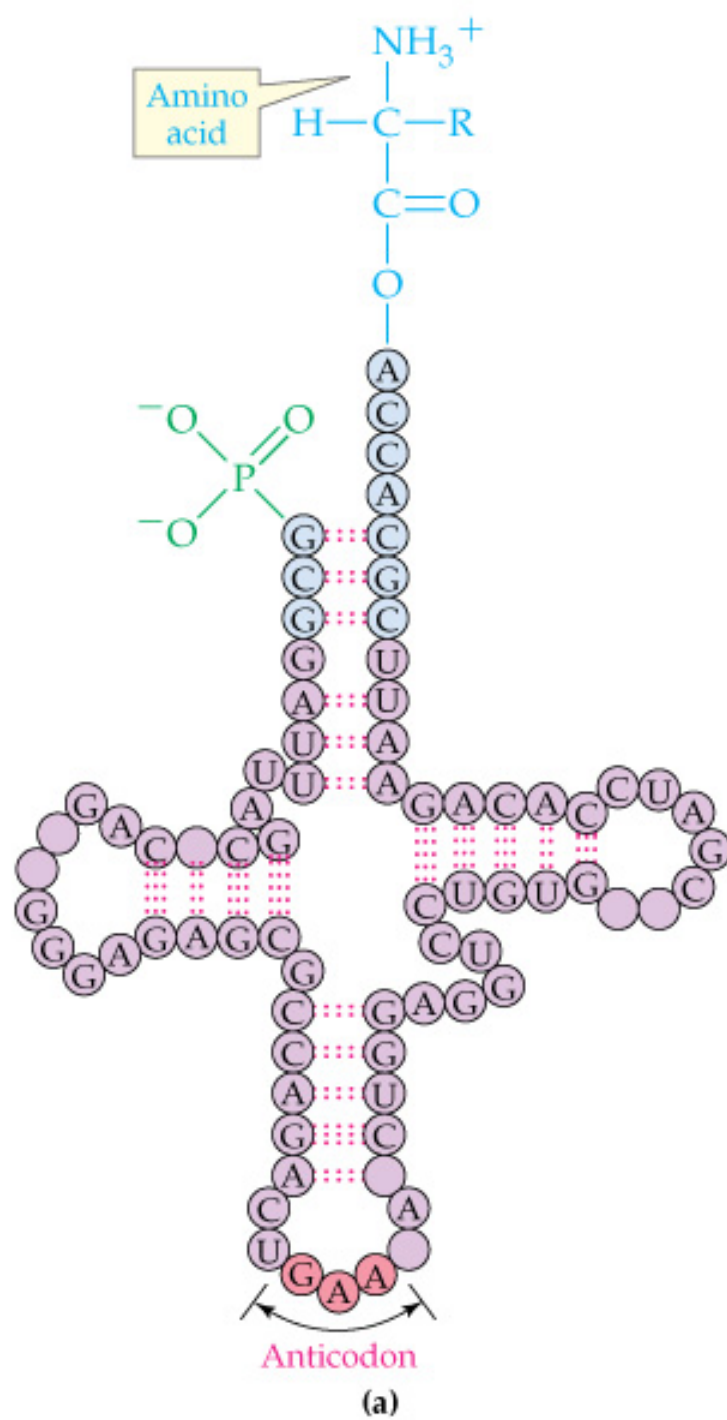
# DNA Methylation and Histone Acetylation



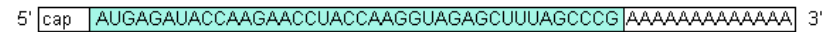
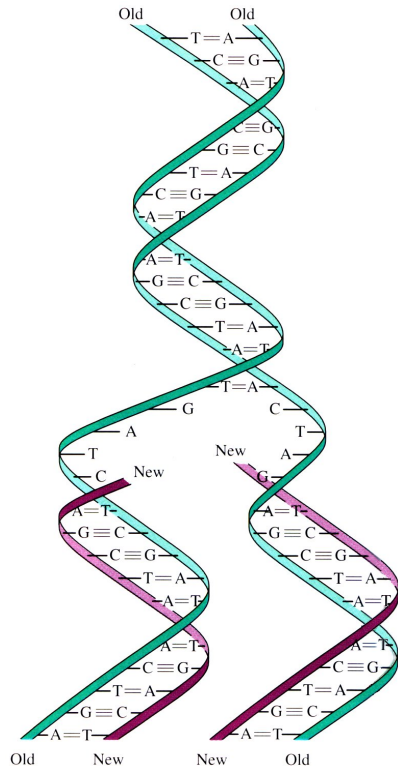


# DNA Sequence

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G



# Self-Assembly Process in Nature

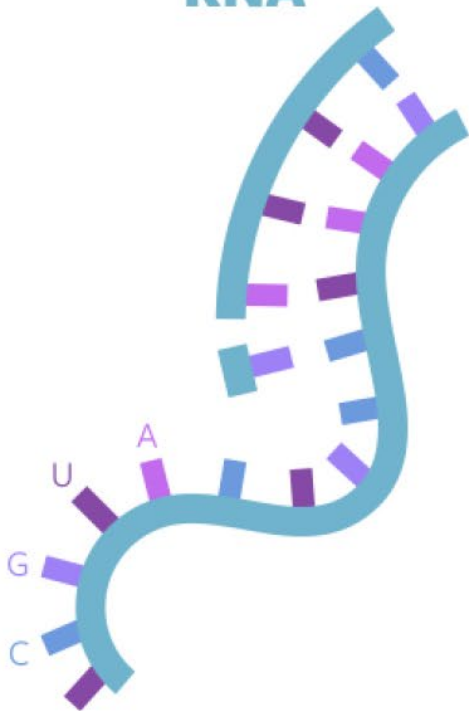




# Definition of Life

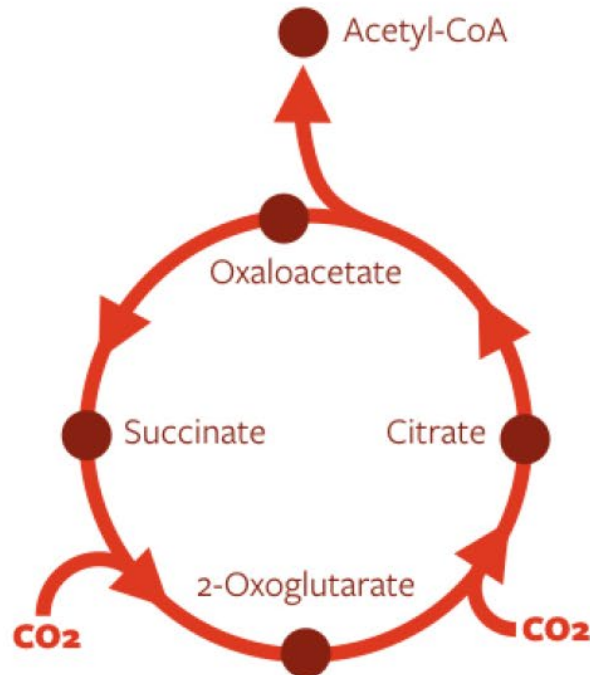
## REPLICATION

### RNA



## METABOLISM

### rTCA CYCLE



## COMPARTMENTS

### LIPOSOME



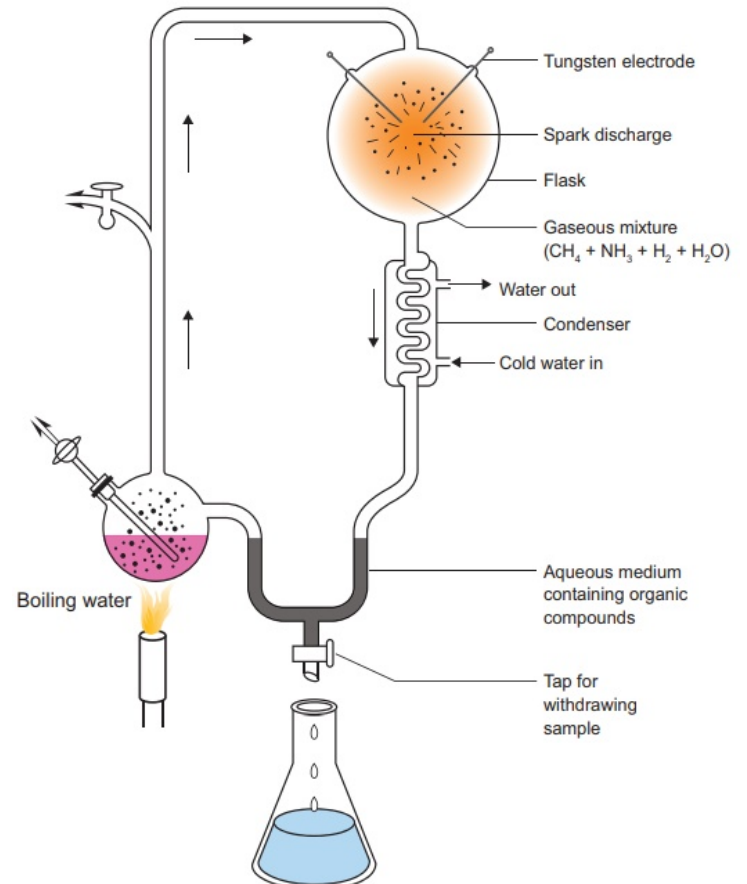
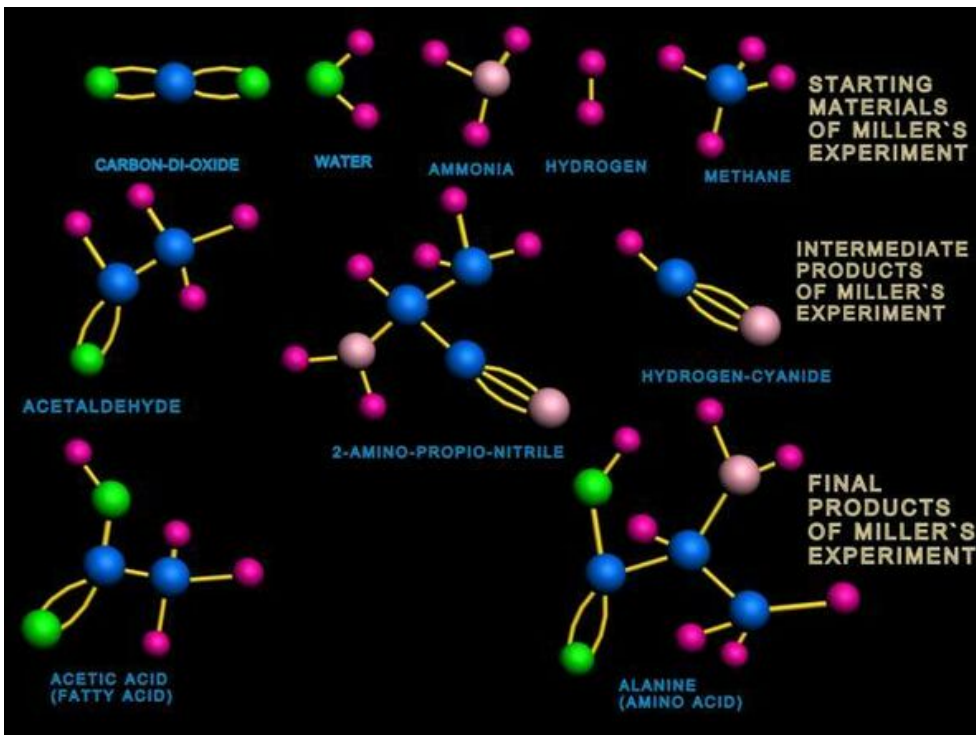
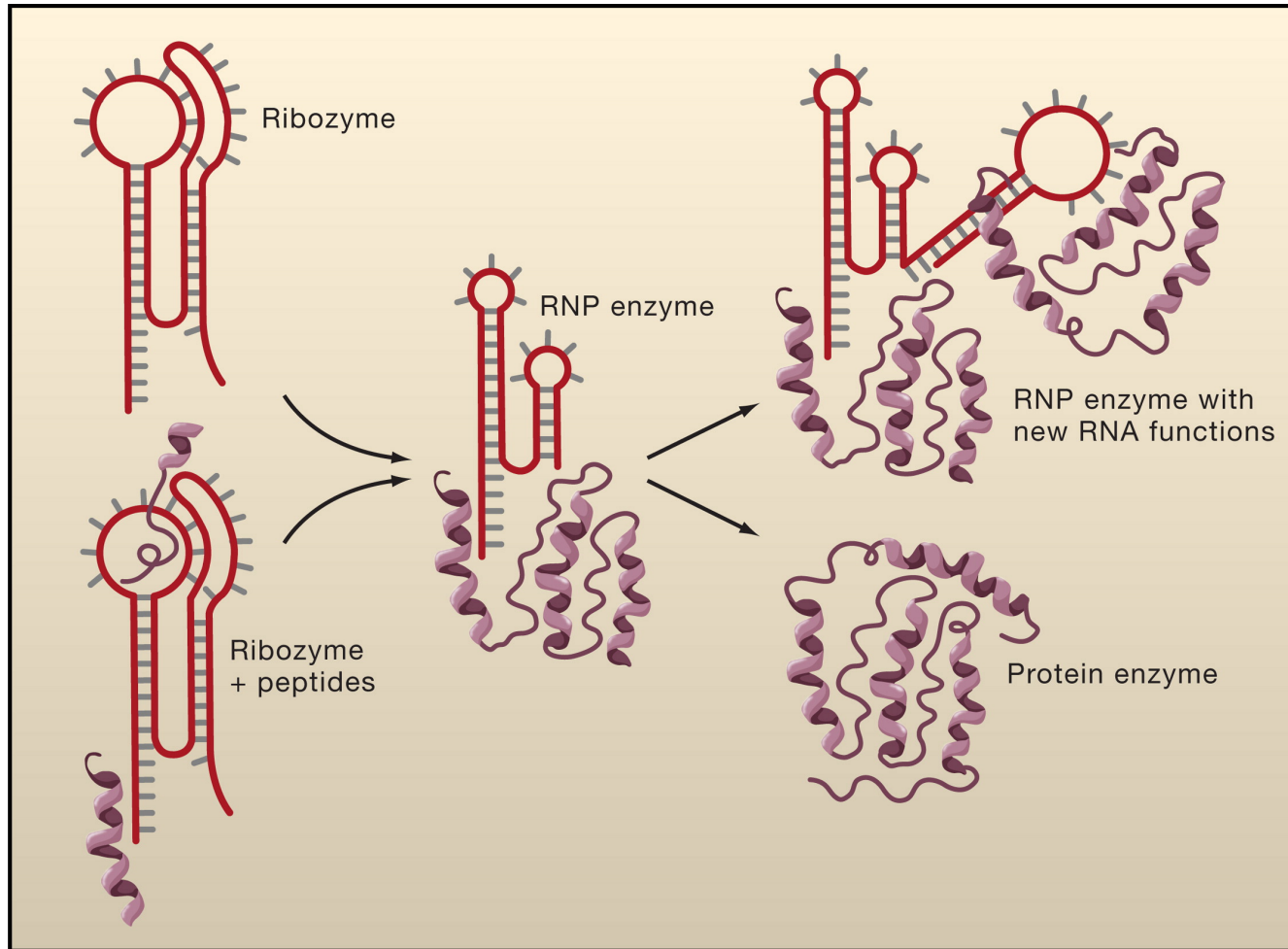
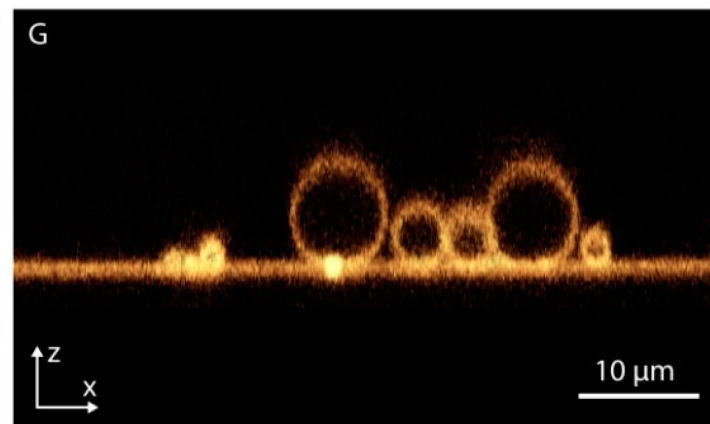
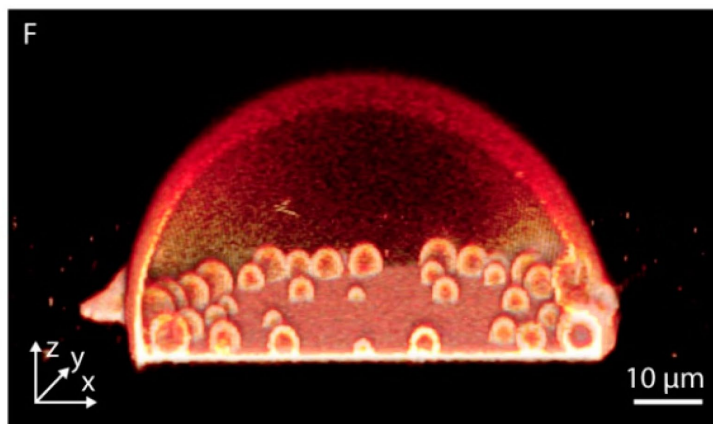
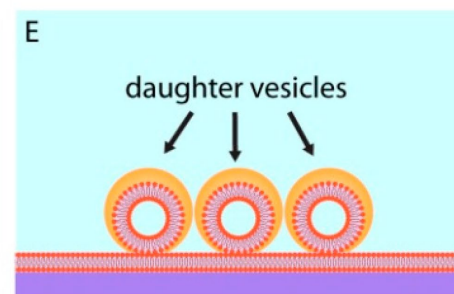
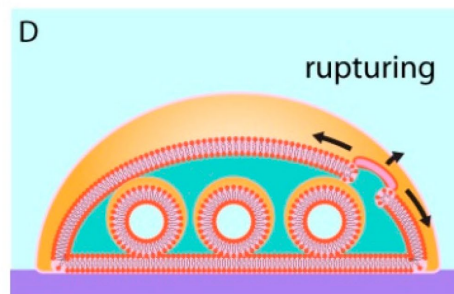
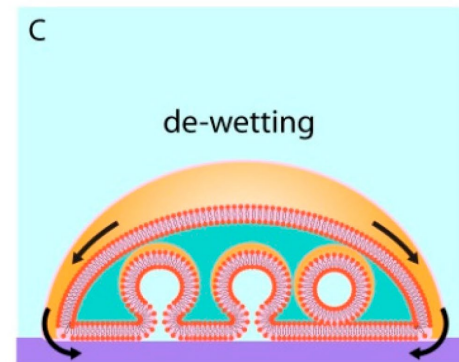
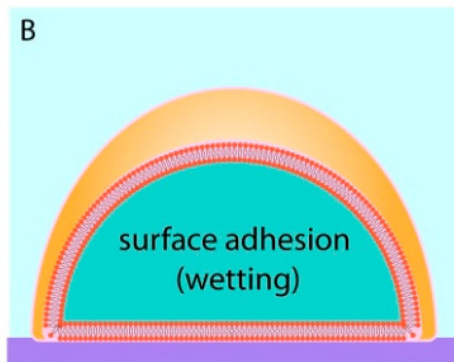


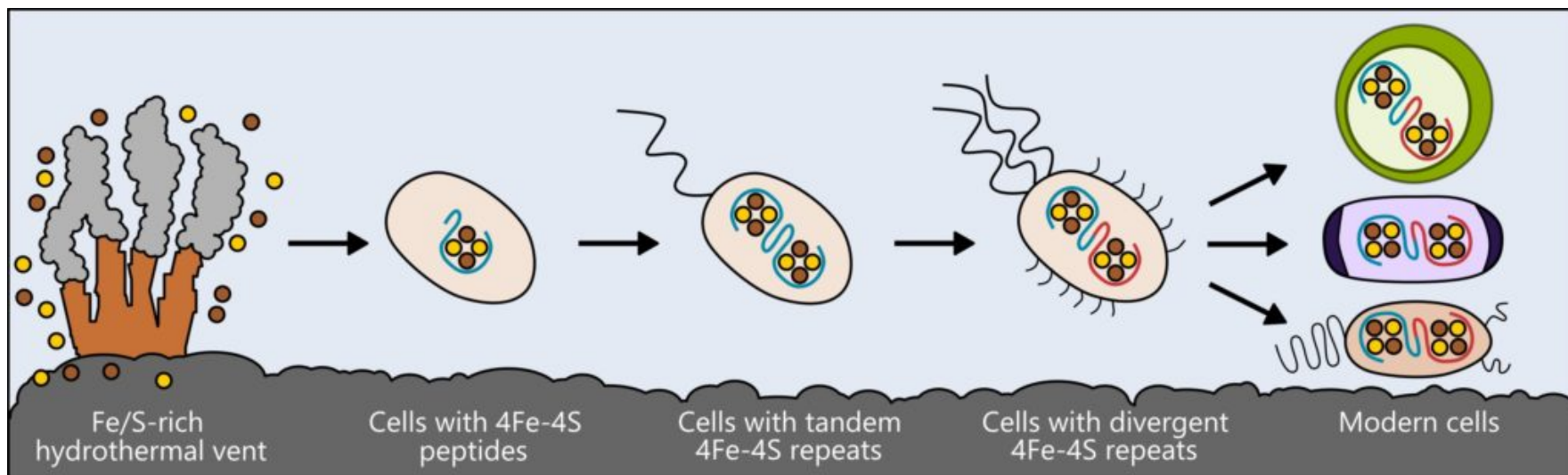
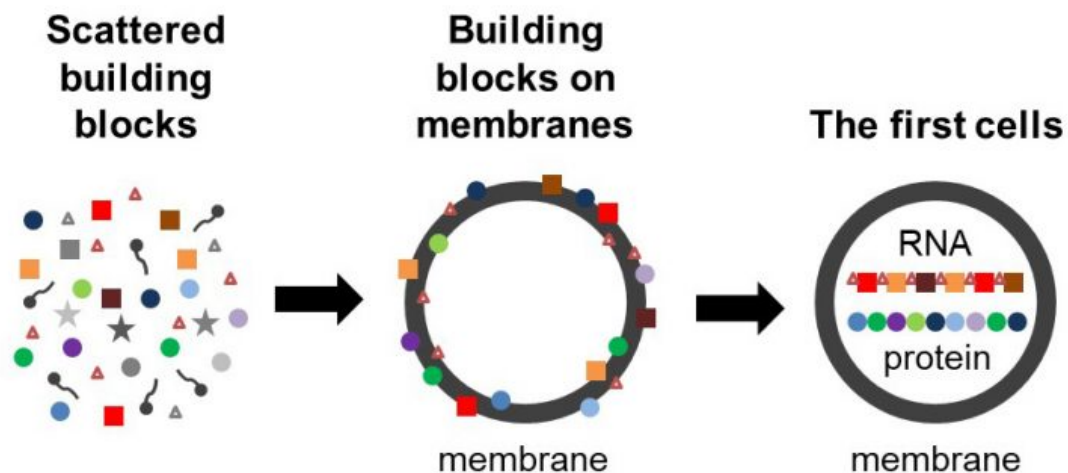
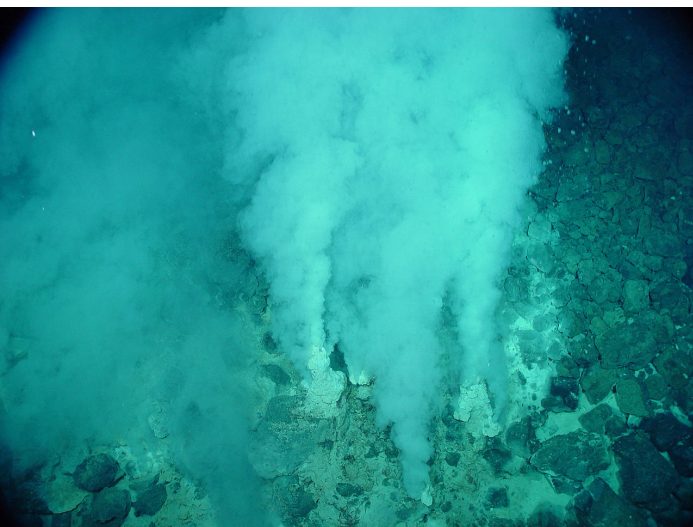
Fig. 6.1 Diagrammatic representation of Urey-Miller's experiment

# RNA World

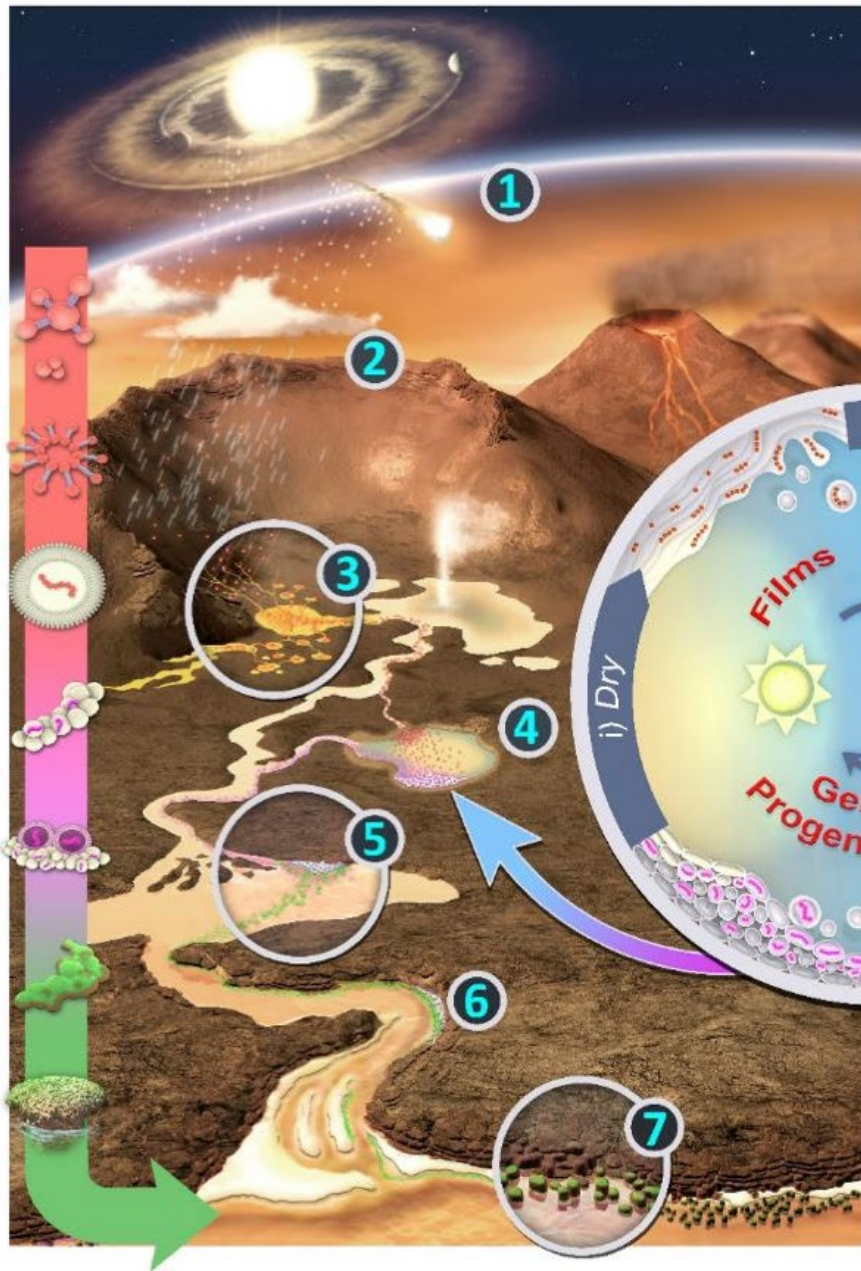








a-Organics    b-Pre-Life ~ c-Early Life    d-Global Life



1. Synthesis

2. Accumulation

3. Concentration

5. Distribution

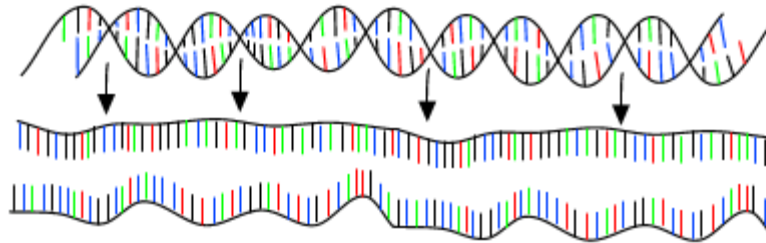
6. Adaptation

7. Colonization

A. Cycling

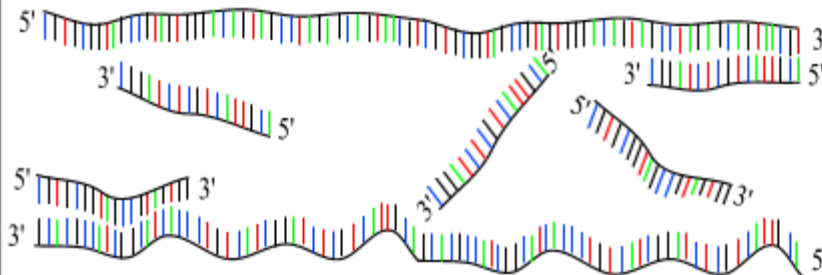
# PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

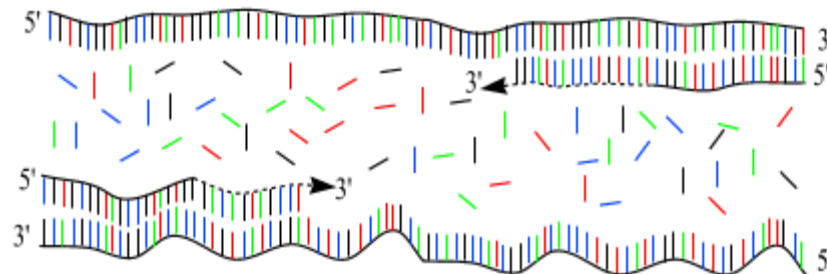
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse  
primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

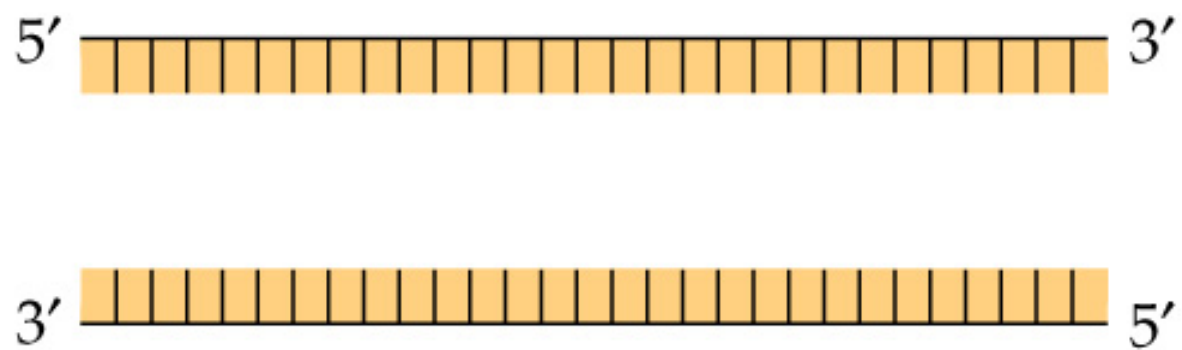
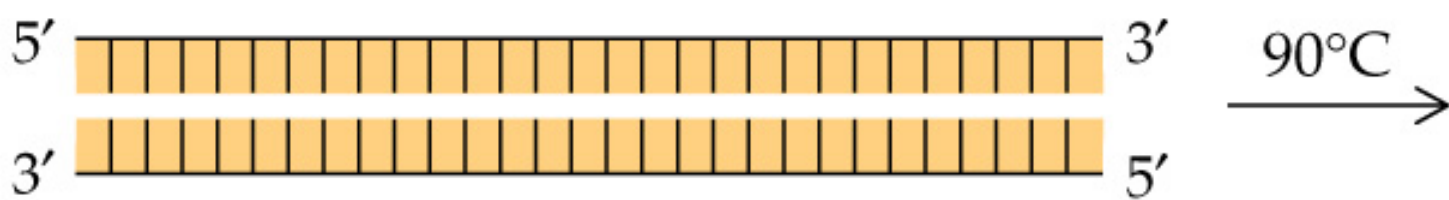
(Andy Viersraete 1999)

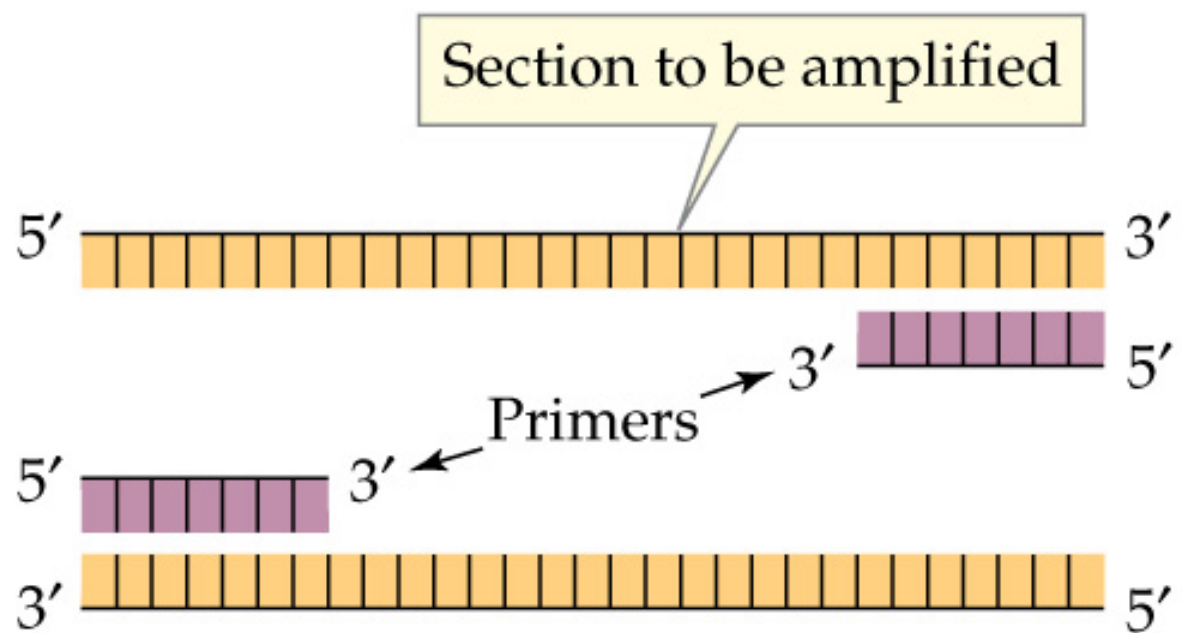
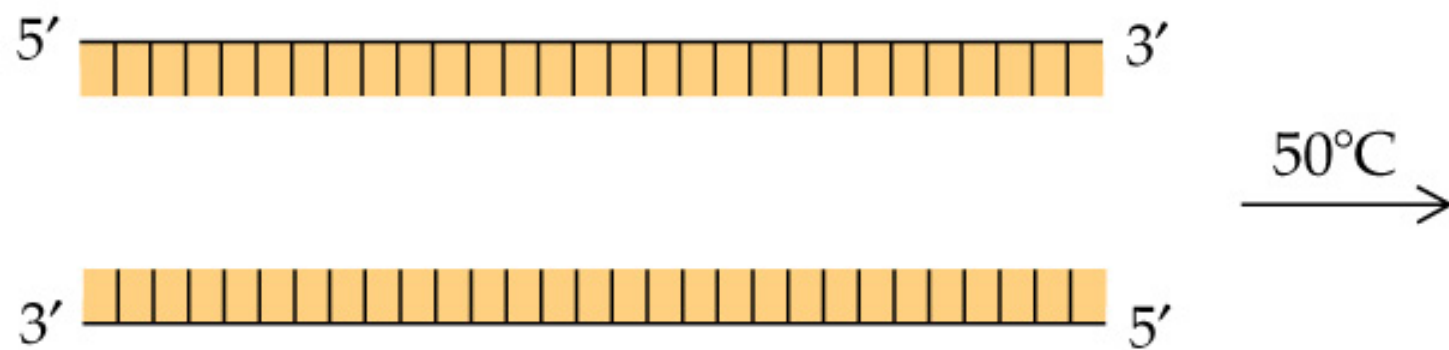
# PCR

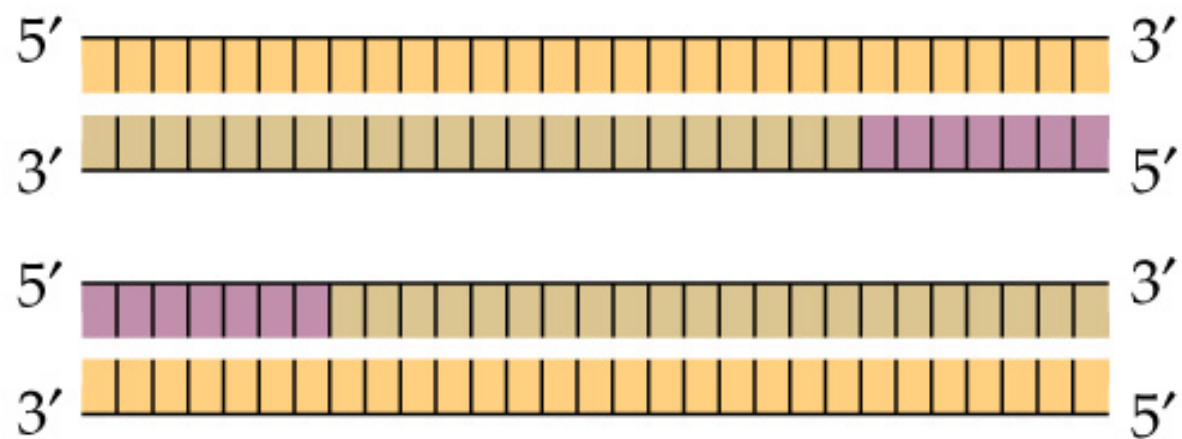
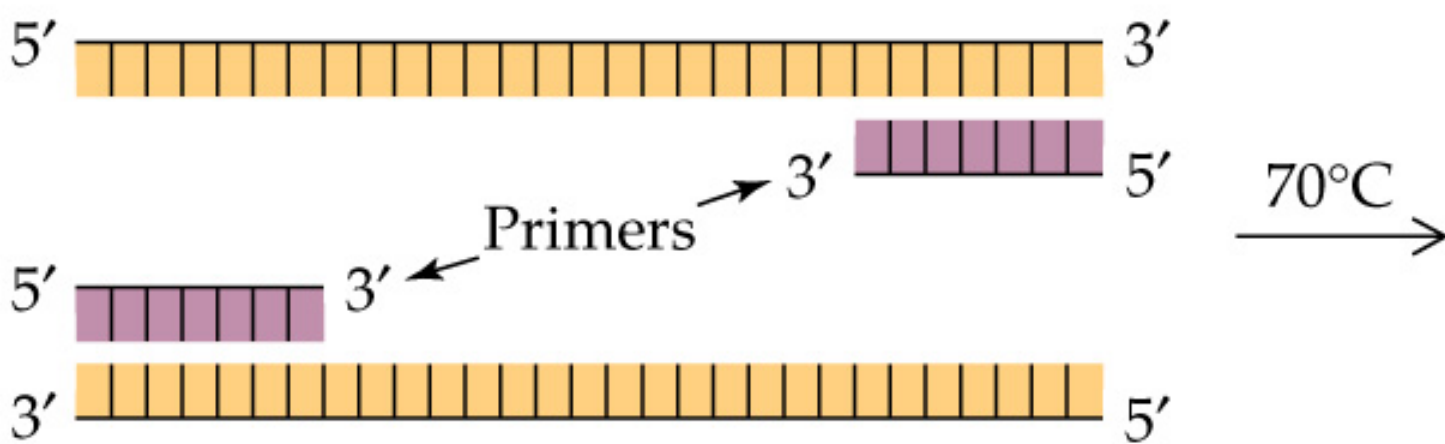
<https://www.youtube.com/watch?v=2KoLnIwoZKU>

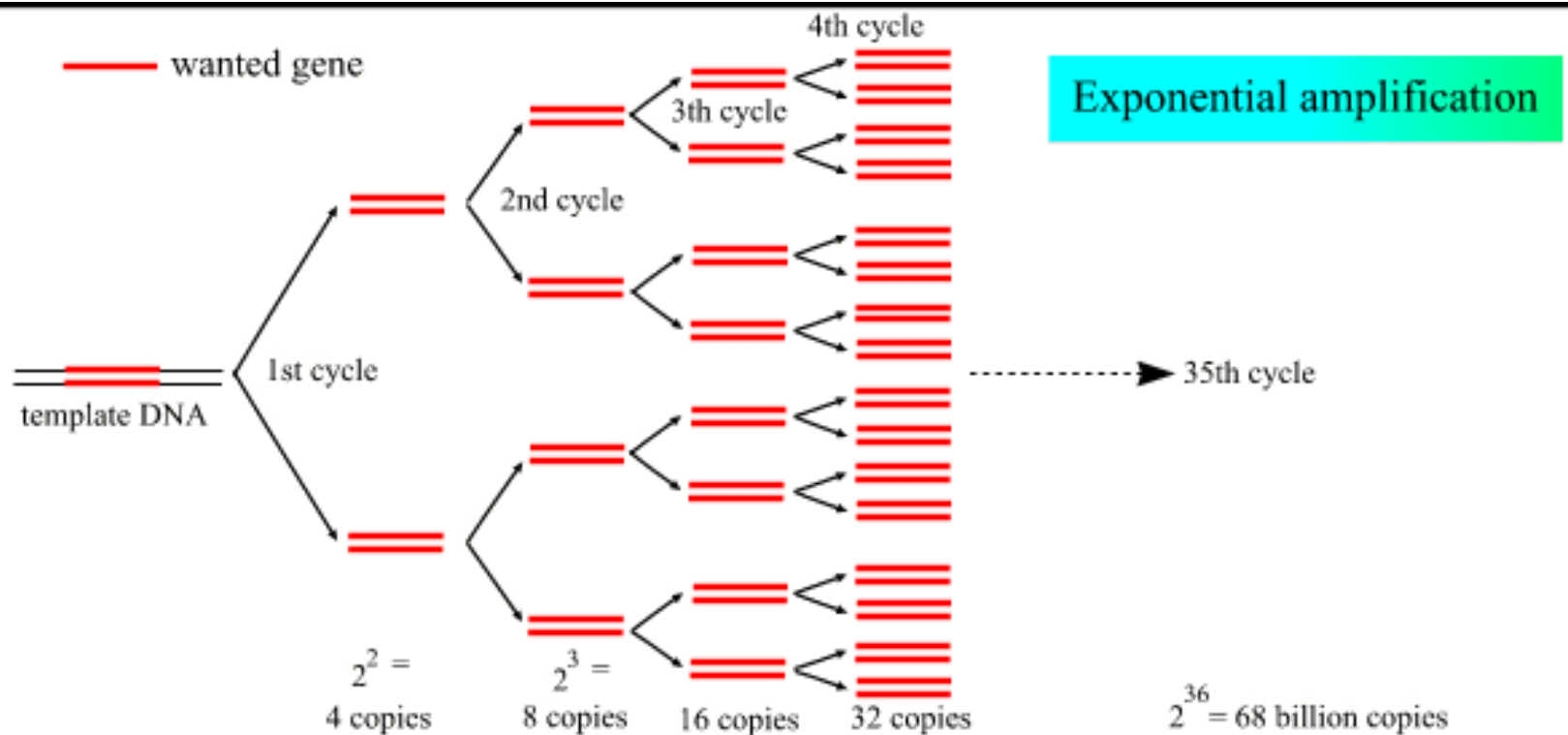
<https://www.youtube.com/watch?v=iQsu3Kz9NYo>







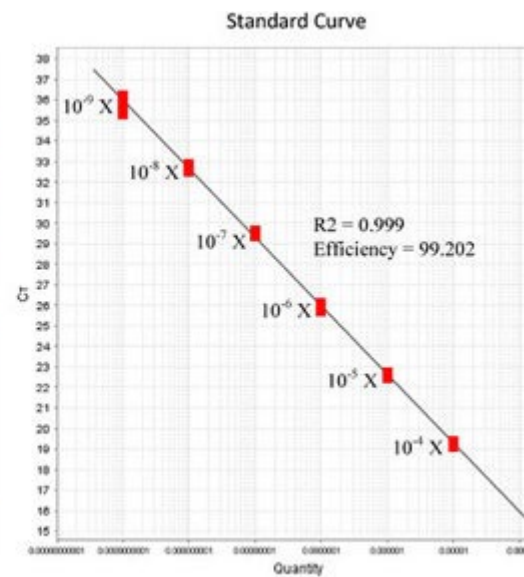
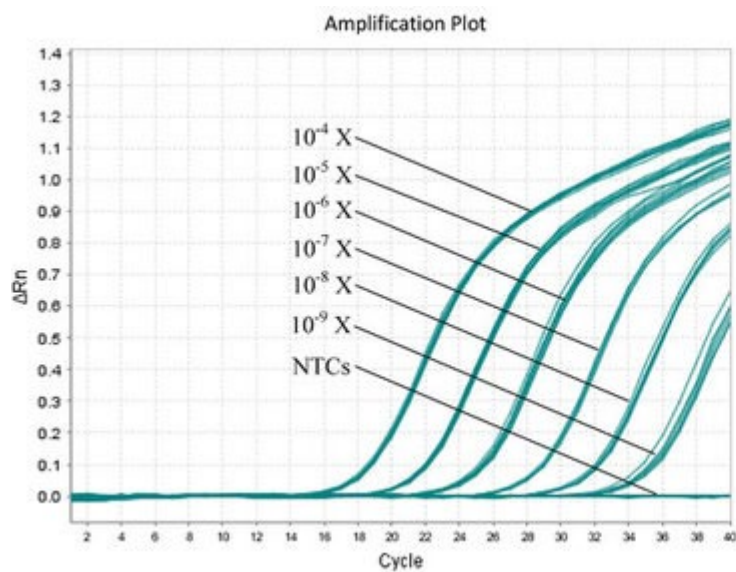
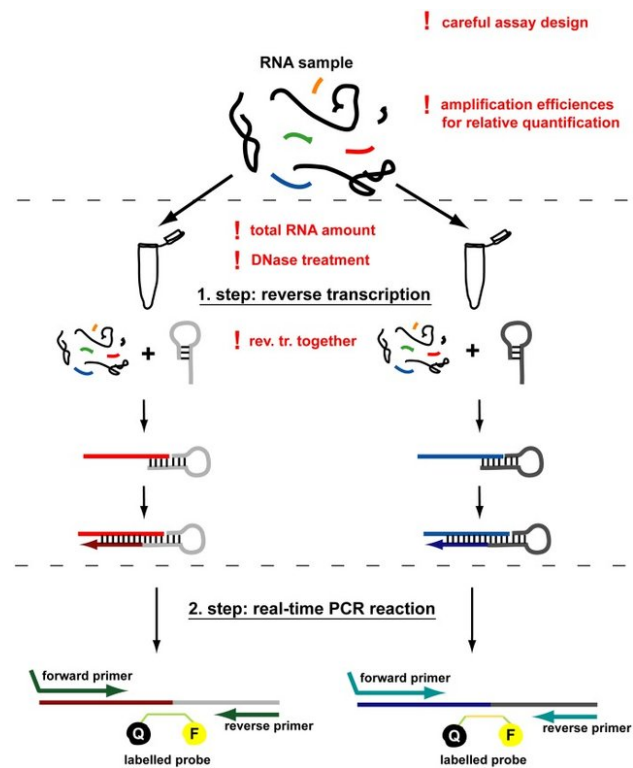




(Andy Vierstraete 1999)

## Real-time PCR

<https://www.youtube.com/watch?v=1kvy17ugl4w>



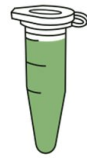
# PROTOCOL OF SARS-COV-2 DETECTION USING REAL-TIME RT-PCR

**Target gene** → RdRp gene (Corman *et al.* 2020)

**PCR amplification regions** → nCoV\_IP2/12621-12727 and nCoV\_IP4/14010-14116 (Institut Pasteur, Paris)

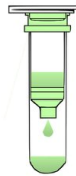
**Primer sets and probes** → designed based on the first sequences of SARS-CoV-2 available on the [GISAID database](#)

**RNA extraction** → NucleoSpin® RNA Virus or viral RNA mini kit (QIAGEN)



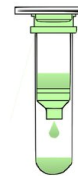
**Sample lysis**

5 min incubation of sample in Lysis Buffer containing Proteinase K



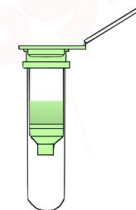
**Binding of viral RNA**

Ethanol addition and transfer of lysate to Column



**Washing**

1<sup>st</sup> Wash Buffer (high salt concentration)  
2<sup>nd</sup> Wash Buffer (low salt concentration)



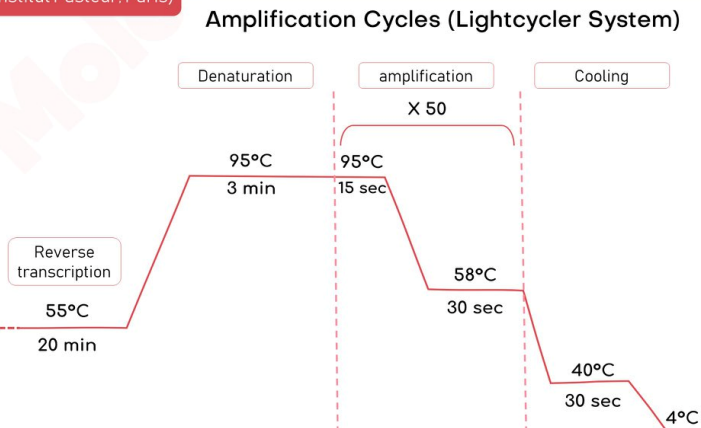
**Elution of viral RNA**

Elution in 20–50 µl RNase-free water or Elution Buffer

**Real-time Multiplex RT-PCR** (Institut Pasteur, Paris)

## Multiplex Mix (nCoV\_IP2&IP4)

Sample RNA	5 µl
H2O	1.3 µl
Reaction mix 2X	12.50 µl
MgSO4 (50mM)	0.40 µl
Forward Primer1 (10µM)	1.00 µl
Reverse Primer1 (10µM)	1.00 µl
Forward Primer2 (10µM)	1.00 µl
Reverse Primer2 (10µM)	1.00 µl
Probe 1 (10µM)	0.4 µl
Probe 2 (10µM)	0.4 µl
SuperscriptIII RT/Platinum Taq Mix	1.00 µl



## POSITIVE CONTROL

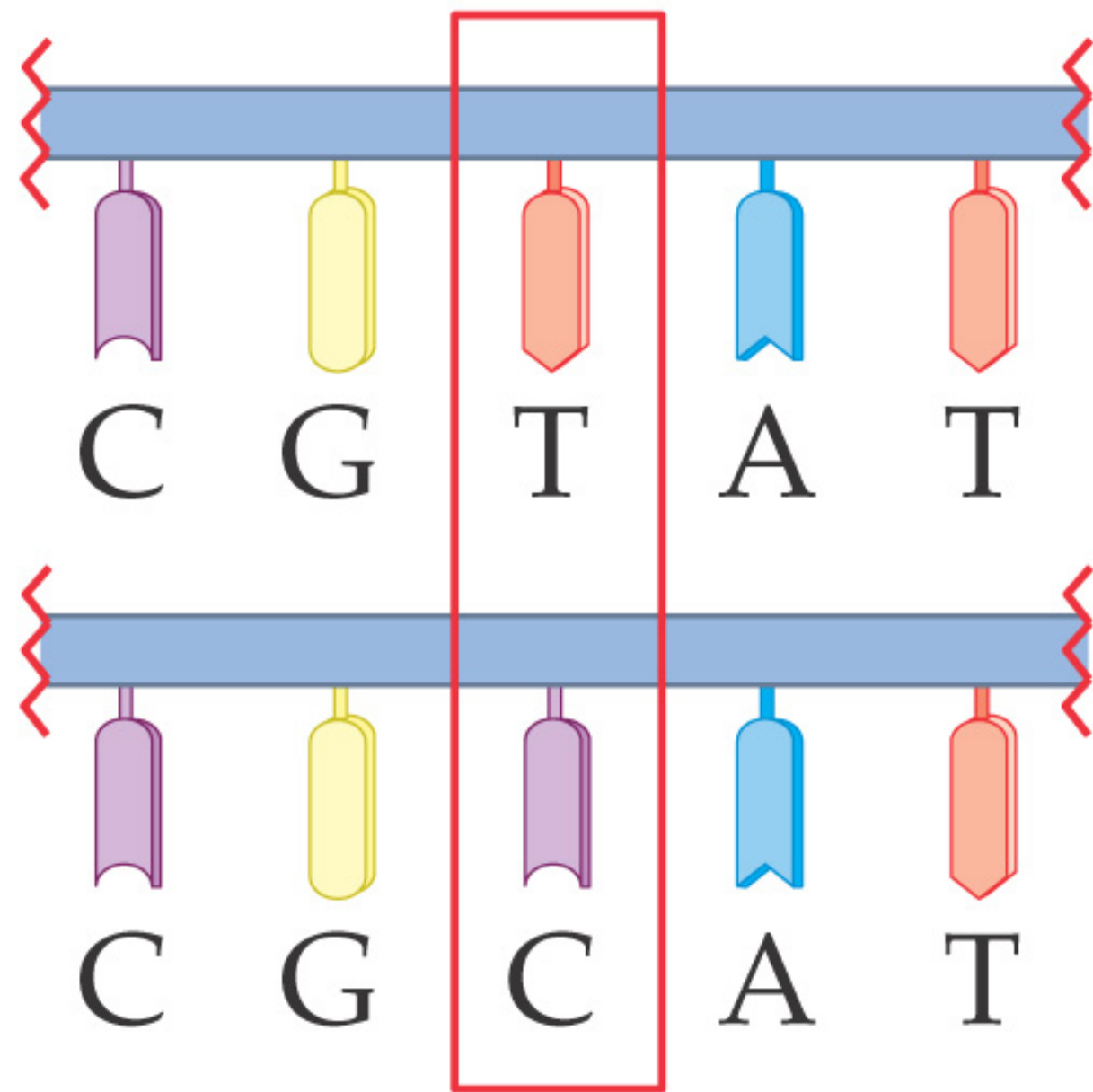
Positive control for real-time RT-PCR is the in vitro transcribed RNA derived from strain BetaCoV\_Wuhan\_WIV04\_2019. The transcript contains the amplification regions of the RdRp and E gene as positive strand.

M. MERZOUG

## References

1. Institut Pasteur, Paris. « Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2 », OMS, 2 mars 2020.
2. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25.

# A SNP

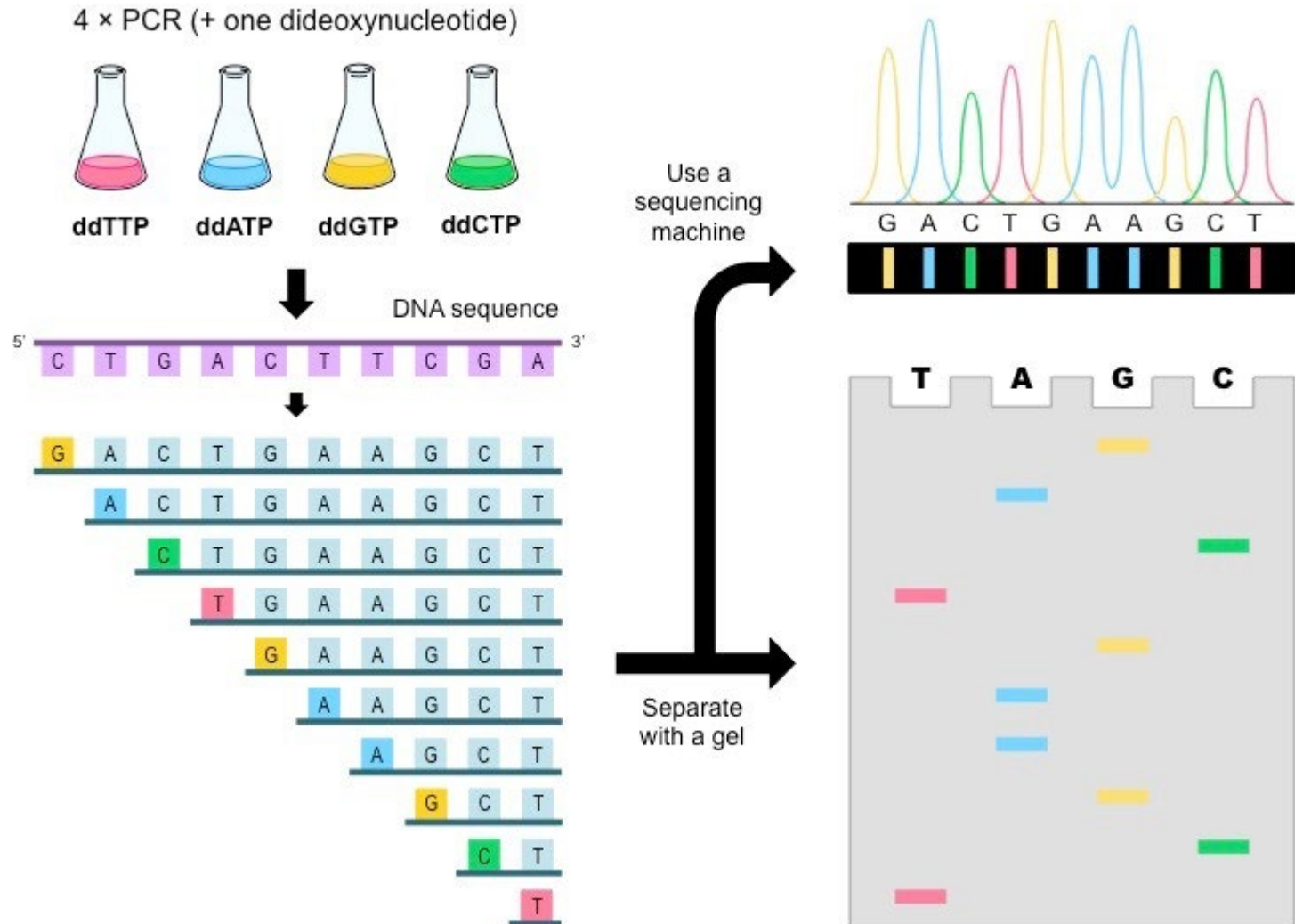


DNA  
sample 1

DNA  
sample 2

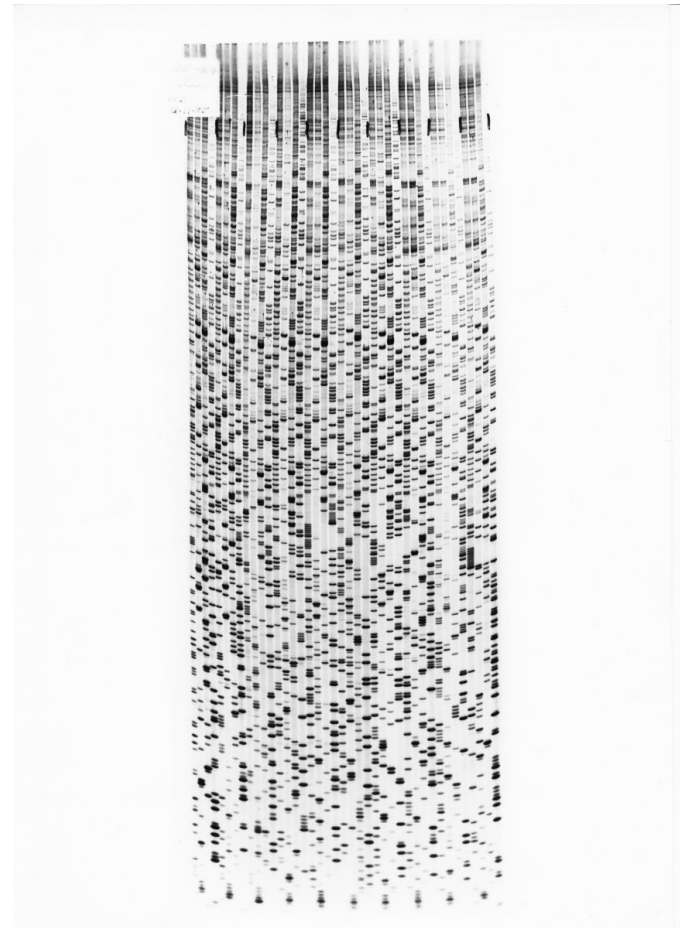
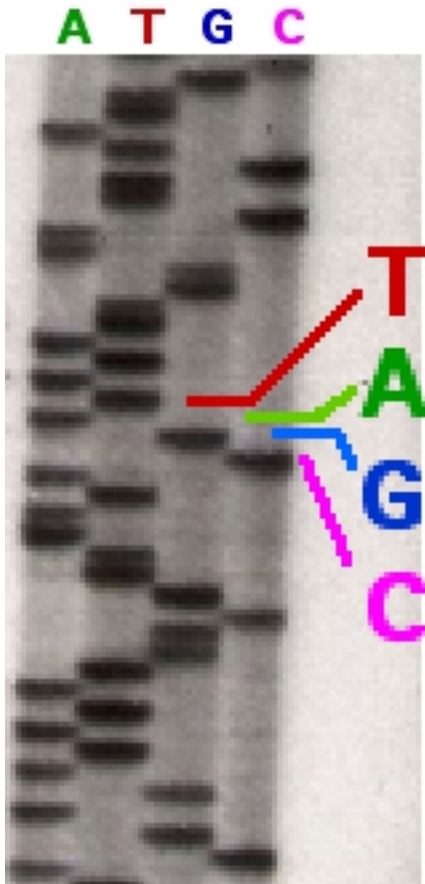


# DNA Sequencing

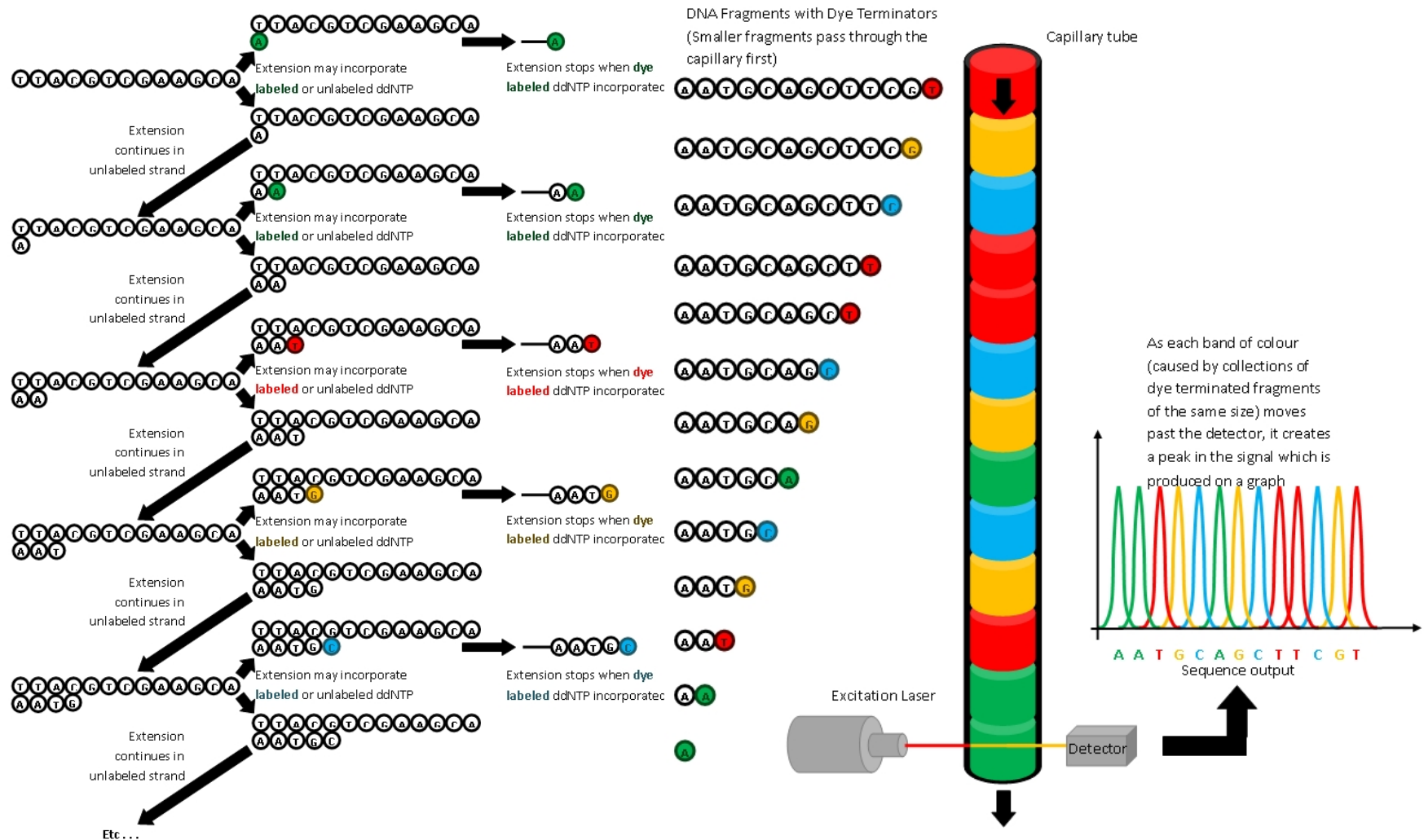


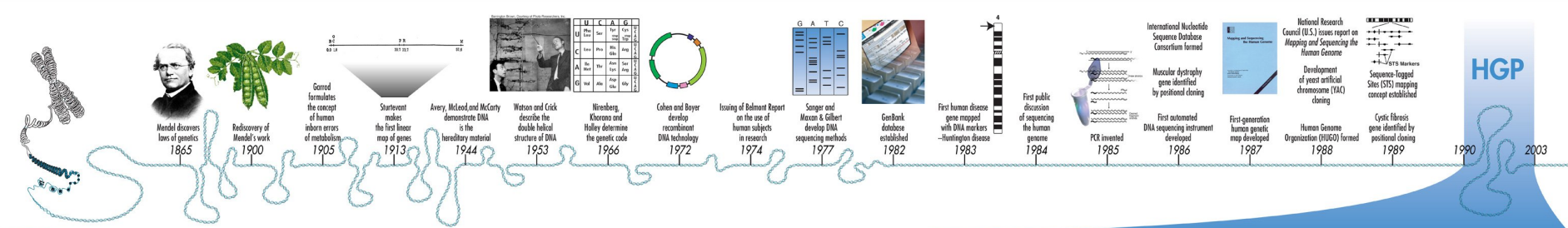
# DNA Sequencing

<https://www.youtube.com/watch?v=vK-HIMaitnE>

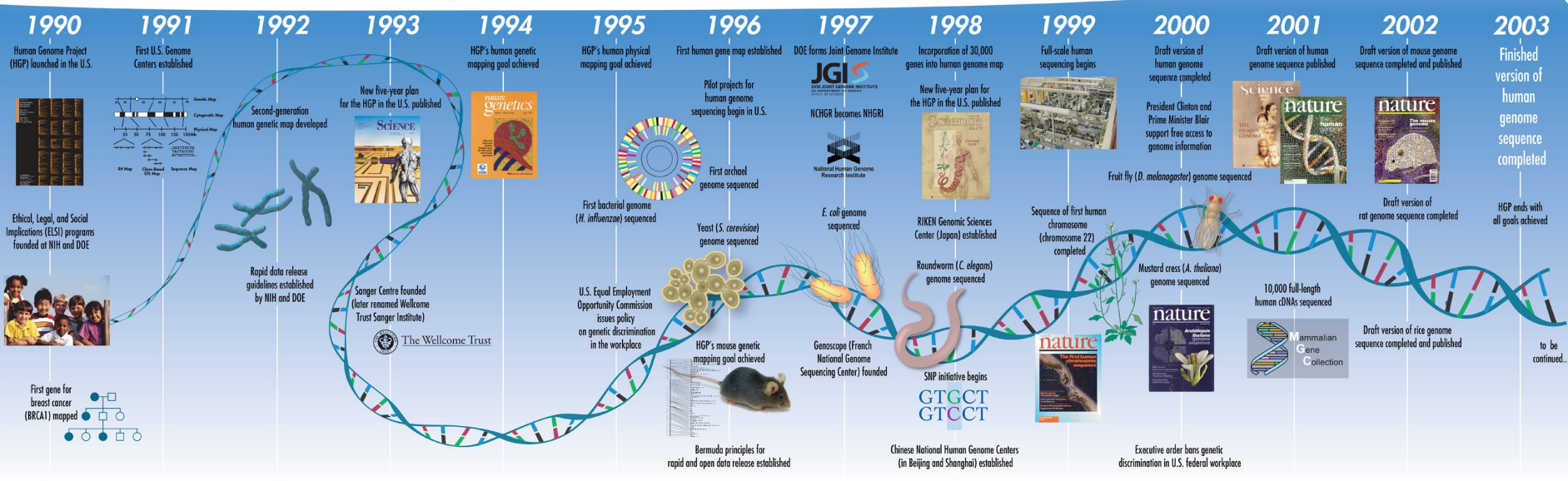


# Dye Terminations

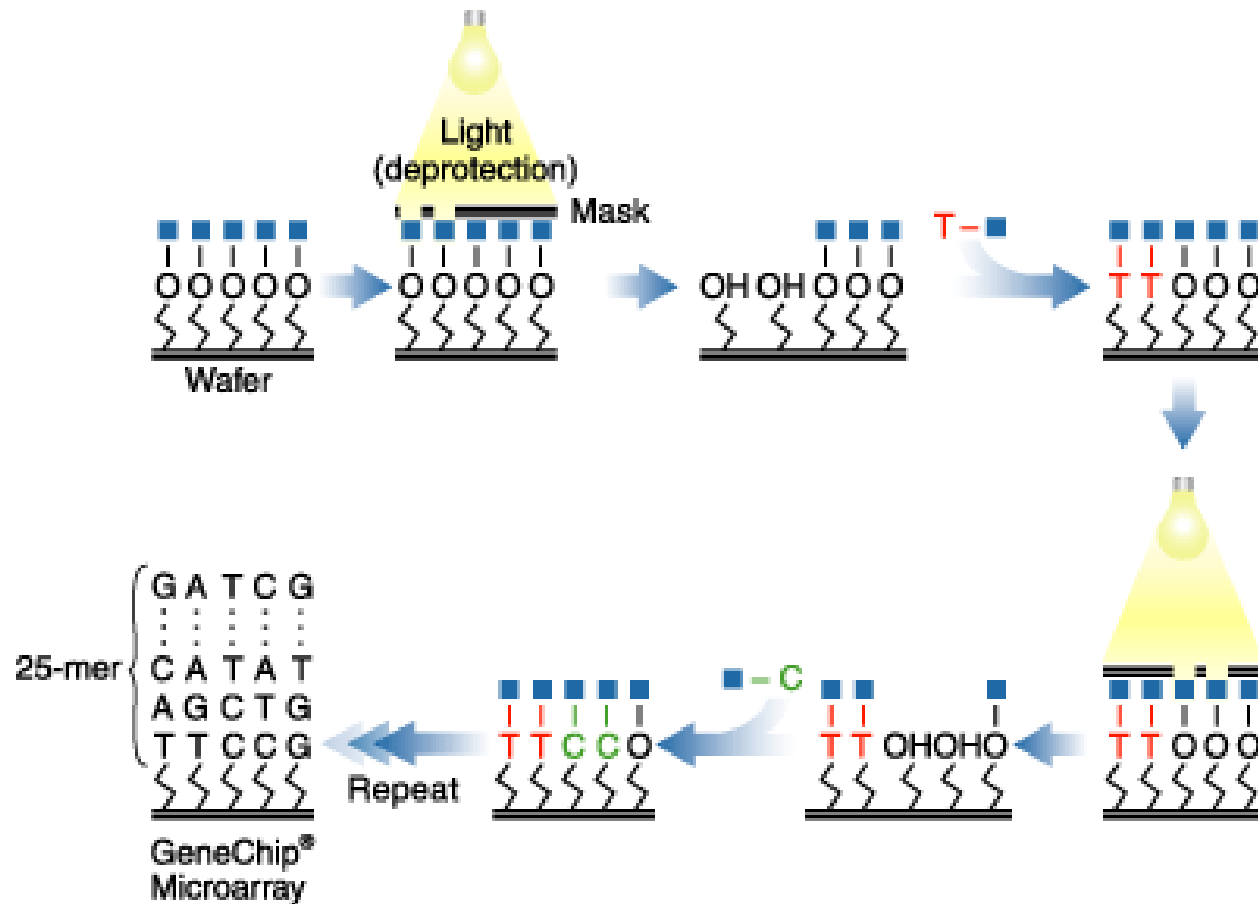




# HGP

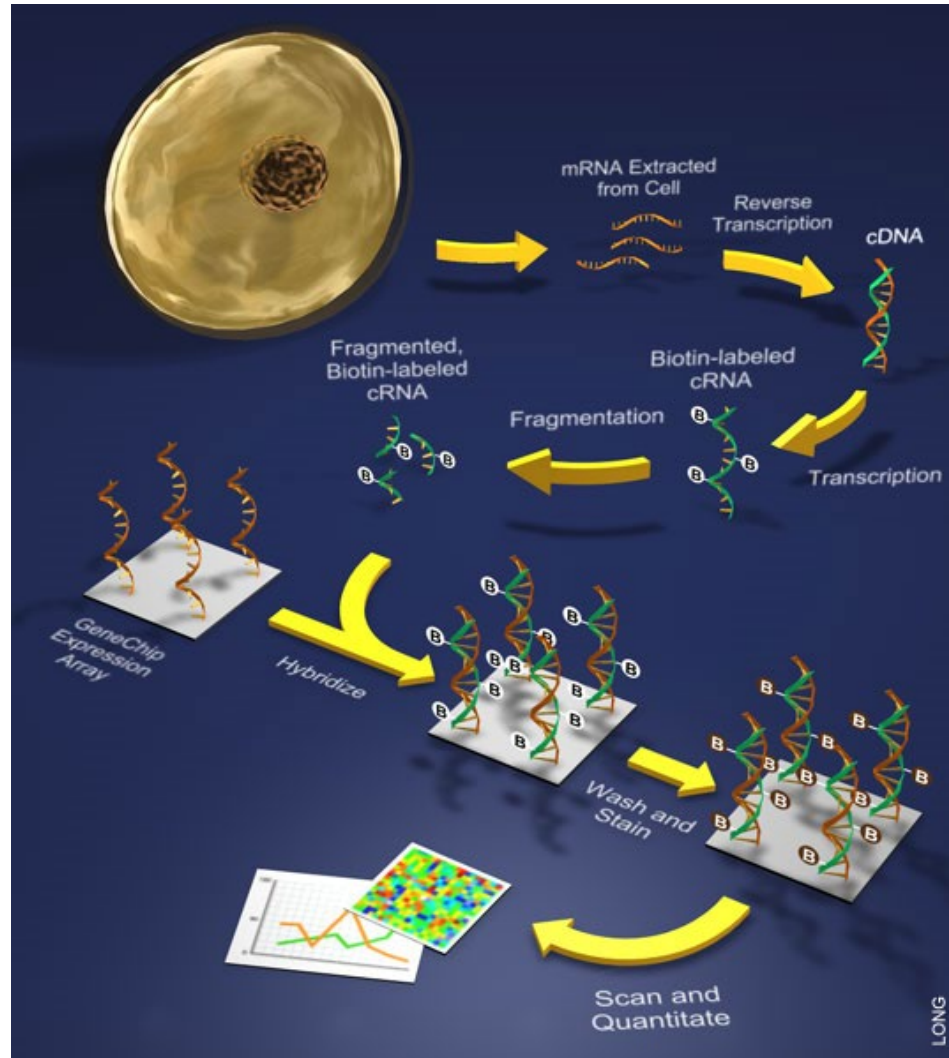


# GeneChip

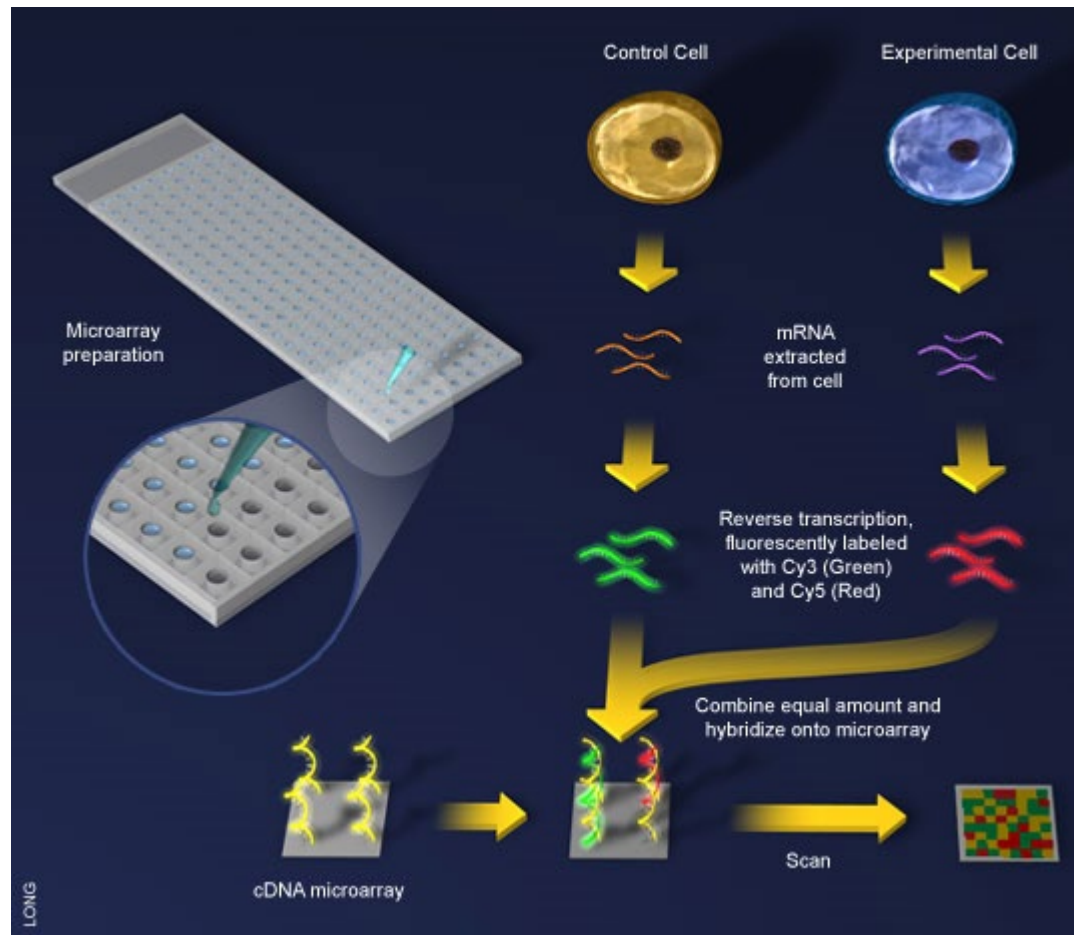




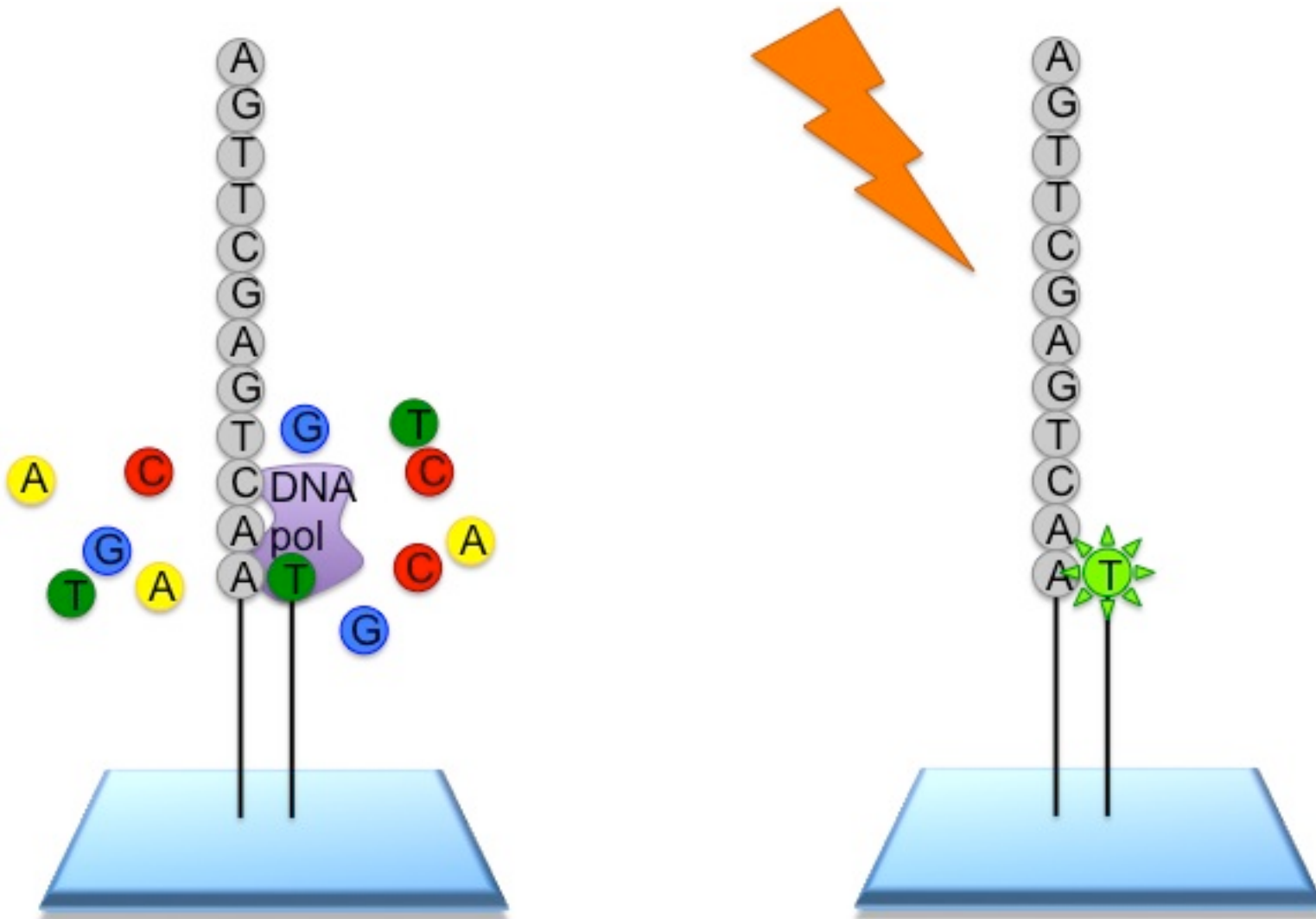
# Scheme



# cDNA Microarray



# NGS Illumina

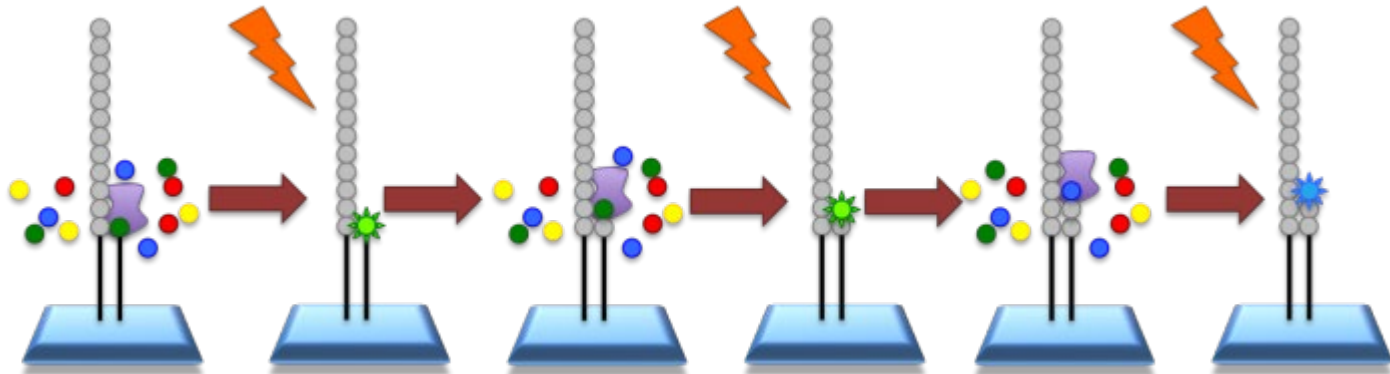


100-150 bp

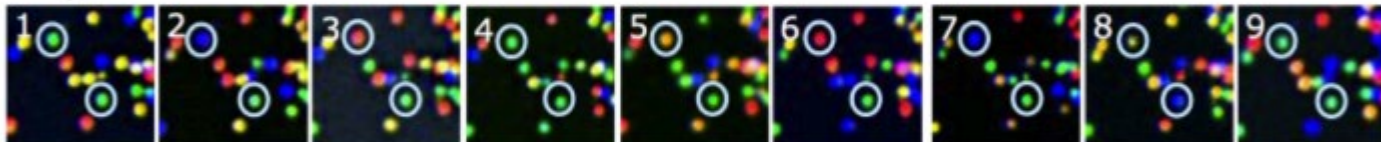


# NGS Illumina

<https://www.youtube.com/watch?v=fCd6B5HRaZ8>

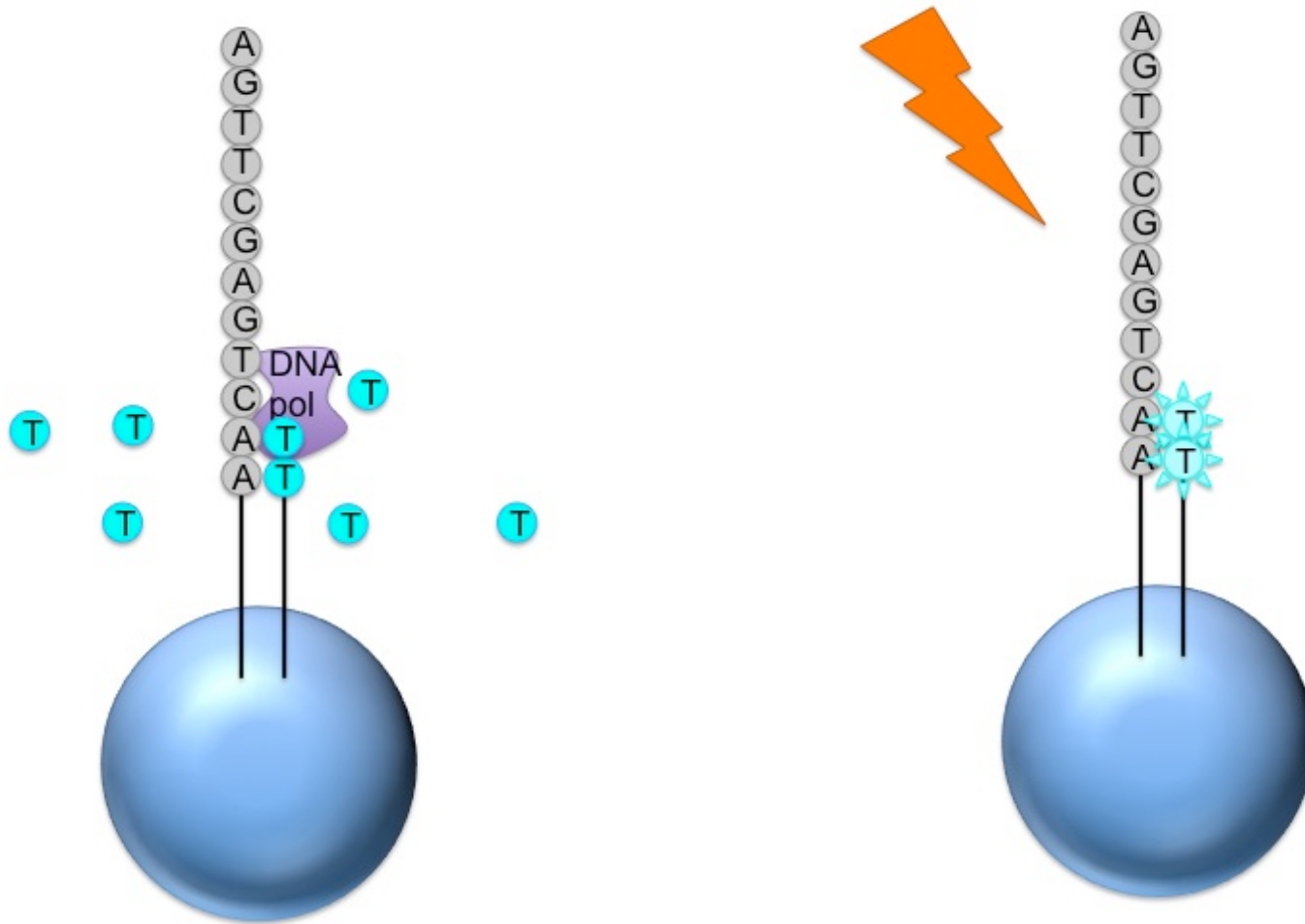


TGCTACGAT...



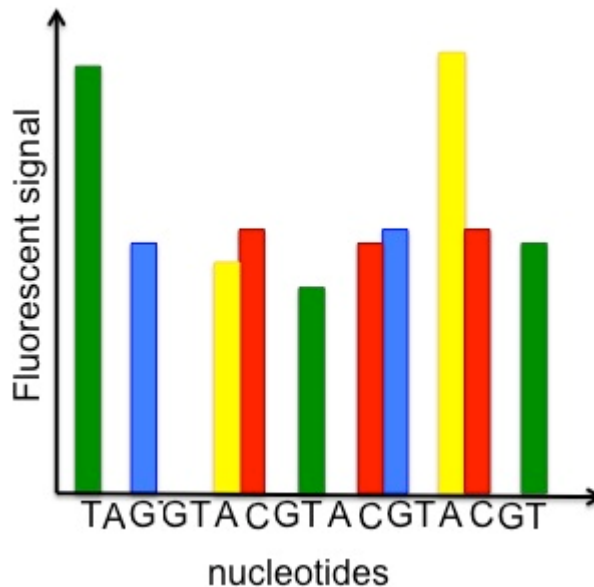
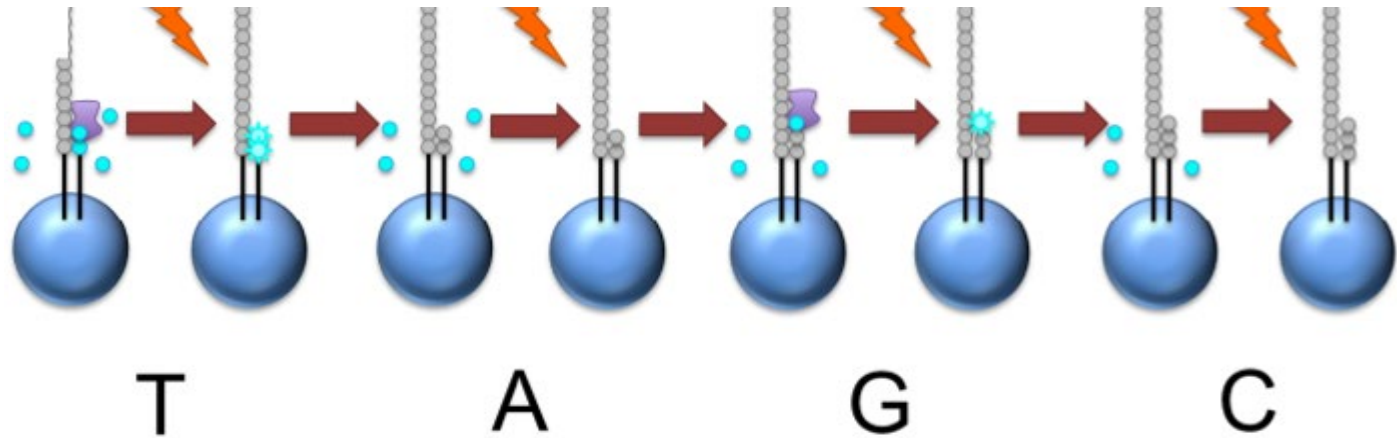
TTTTTTTGT...

# Roche 454 sequencing



1000 bp

# Roche 454 sequencing



The four main advantages of NGS over classical Sanger sequencing are:

**speed**

**cost**

**sample size**

**accuracy**

NGS is significantly cheaper, quicker, needs significantly less DNA and is more accurate and reliable than Sanger sequencing.

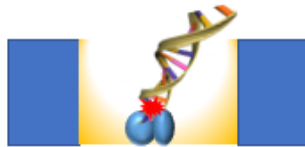
NGS is quicker than Sanger sequencing in two ways. Firstly, the chemical reaction may be combined with the signal detection in some versions of NGS, whereas in Sanger sequencing these are two separate processes. Secondly and more significantly, only one read (maximum ~1kb) can be taken at a time in Sanger sequencing, whereas NGS is massively parallel, allowing 300Gb of DNA to be read on a single run on a single chip.

The first human genome sequence cost in the region of £300M. Using modern Sanger sequencing methods, aided by data from the known sequence, a full human genome would still cost £6M. Sequencing a human genome with Illumina today would cost only £6,000.

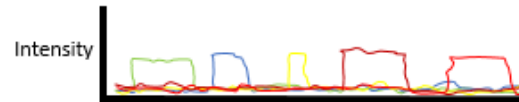
# Third Generation Sequencing

## PacBio SMRT seq

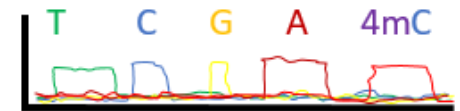
DNA passes thru  
polymerase in an  
illuminated volume



Raw output is fluorescent signal  
of the nucleotide incorporation,  
specific to each nucleotide

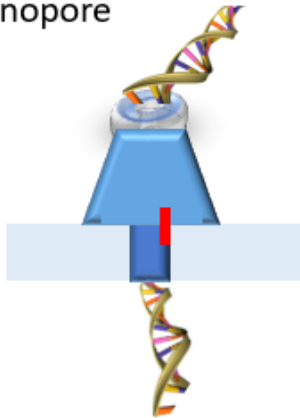


A,C,T,G have known pulse  
durations, which are used to  
infer methylated nucleotides

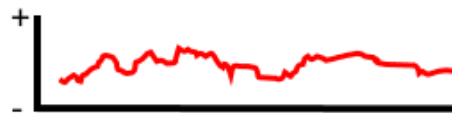


## Oxford Nanopore

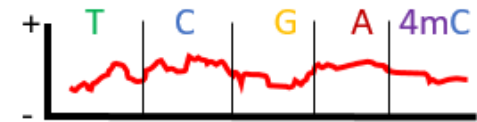
DNA passes thru  
nanopore



Raw output is electrical signal  
caused by nucleotide blocking  
ion flow in nanopore

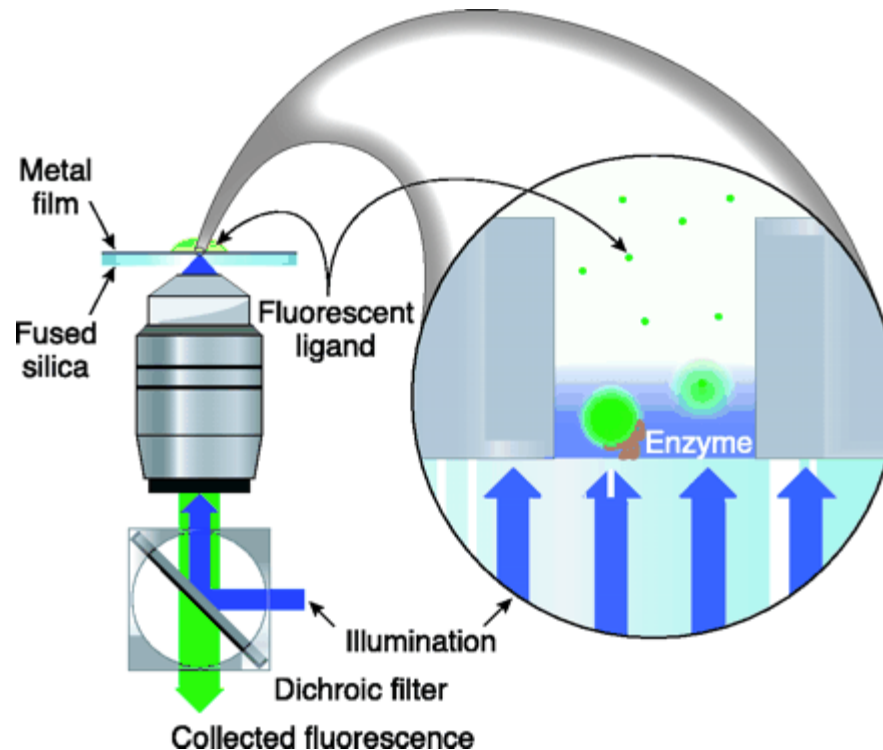


Each nucleotide has a specific  
electric "signature"



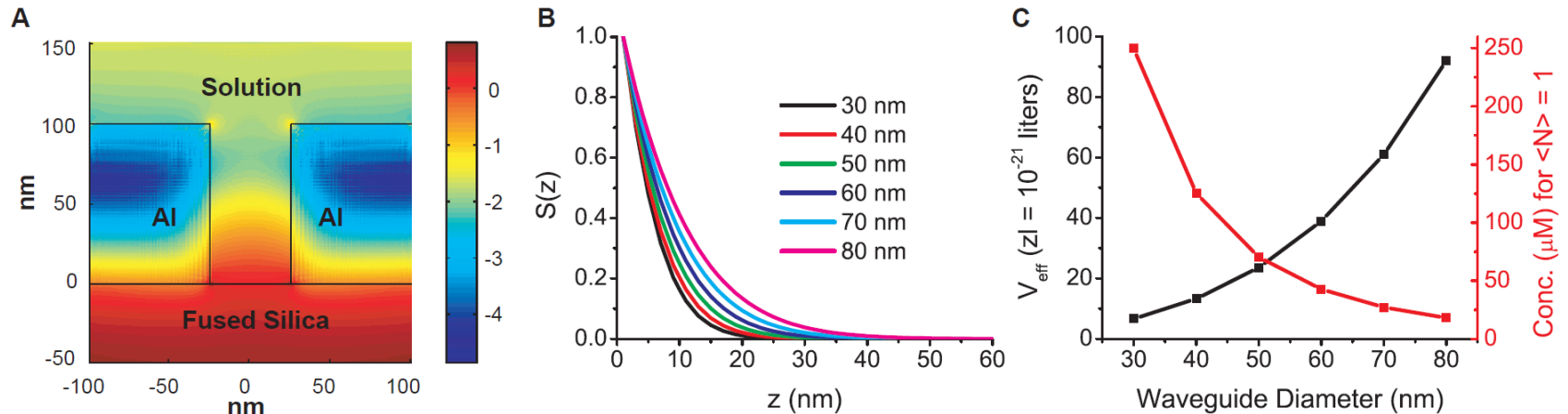
# Zero Mode Waveguide

<https://www.youtube.com/watch?v=NHCJ8PtYCFc>

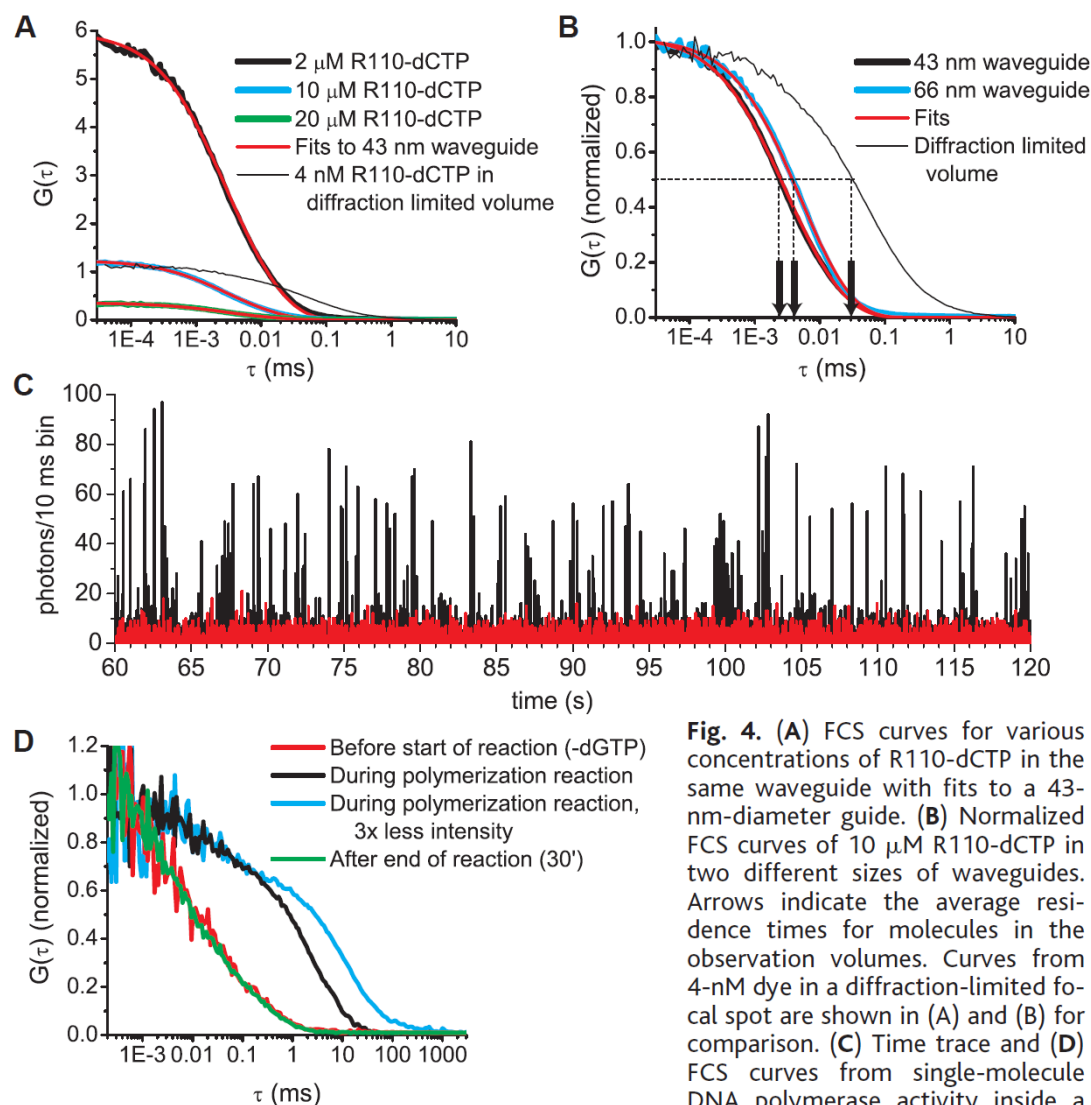




# Zero Mode Waveguide



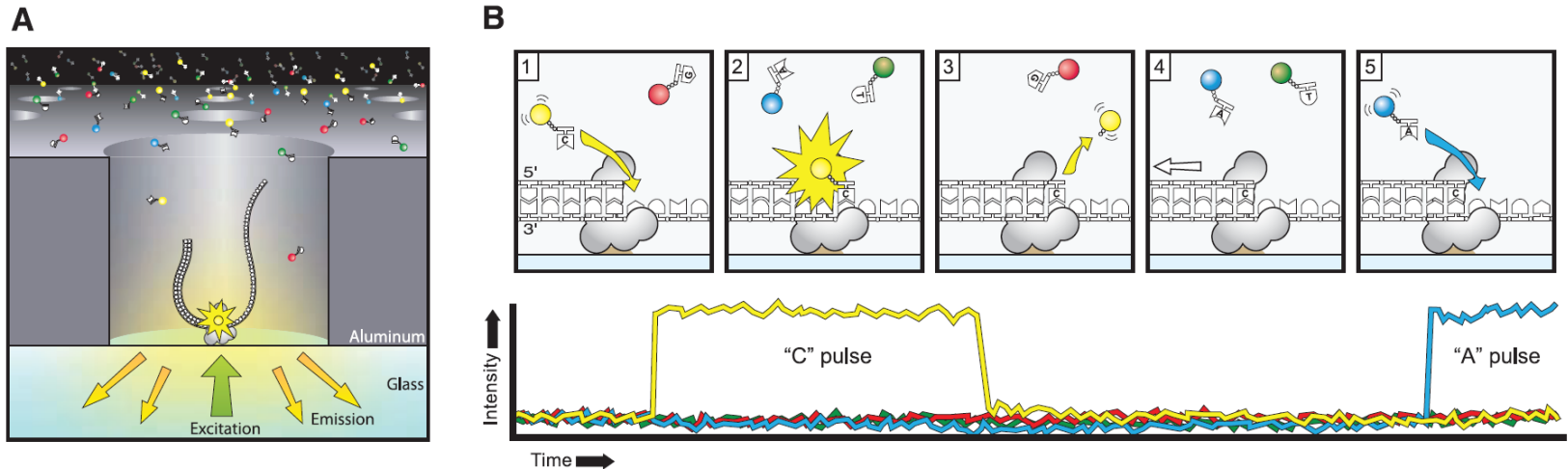
$$50 \times 50 \times 10 \text{ nm}^3 = 2.5 \times 10^4 \times 10^{-21} \text{ cc} = 2.5 \times 10^{-20} \text{ L} = 25 \text{ zeptoliter}$$



**Fig. 4.** (A) FCS curves for various concentrations of R110-dCTP in the same waveguide with fits to a 43-nm-diameter guide. (B) Normalized FCS curves of 10  $\mu$ M R110-dCTP in two different sizes of waveguides. Arrows indicate the average residence times for molecules in the observation volumes. Curves from 4-nM dye in a diffraction-limited focal spot are shown in (A) and (B) for comparison. (C) Time trace and (D) FCS curves from single-molecule DNA polymerase activity inside a zero-mode waveguide. Incorporation events and subsequent photobleaching of coumarin-dCTP appear as distinct fluorescence bursts in the black time trace (10-ms time bins). This results in a long-time shoulder in the corresponding FCS curves during polymerization (black and blue curves) in (D). Decreasing the intensity results in slower photobleaching as seen by the longer residence time in the blue curve. The red curves in (C) and (D) are the corresponding negative controls (absence of one native nucleotide) in the same waveguide before initiation of the reaction. The green curve in (D) is the control after the reaction has stopped.

tion events and subsequent photobleaching of coumarin-dCTP appear as distinct fluorescence bursts in the black time trace (10-ms time bins). This results in a long-time shoulder in the corresponding FCS curves during polymerization (black and blue curves) in (D). Decreasing the intensity results in slower photobleaching as seen by the longer residence time in the blue curve. The red curves in (C) and (D) are the corresponding negative controls (absence of one native nucleotide) in the same waveguide before initiation of the reaction. The green curve in (D) is the control after the reaction has stopped.

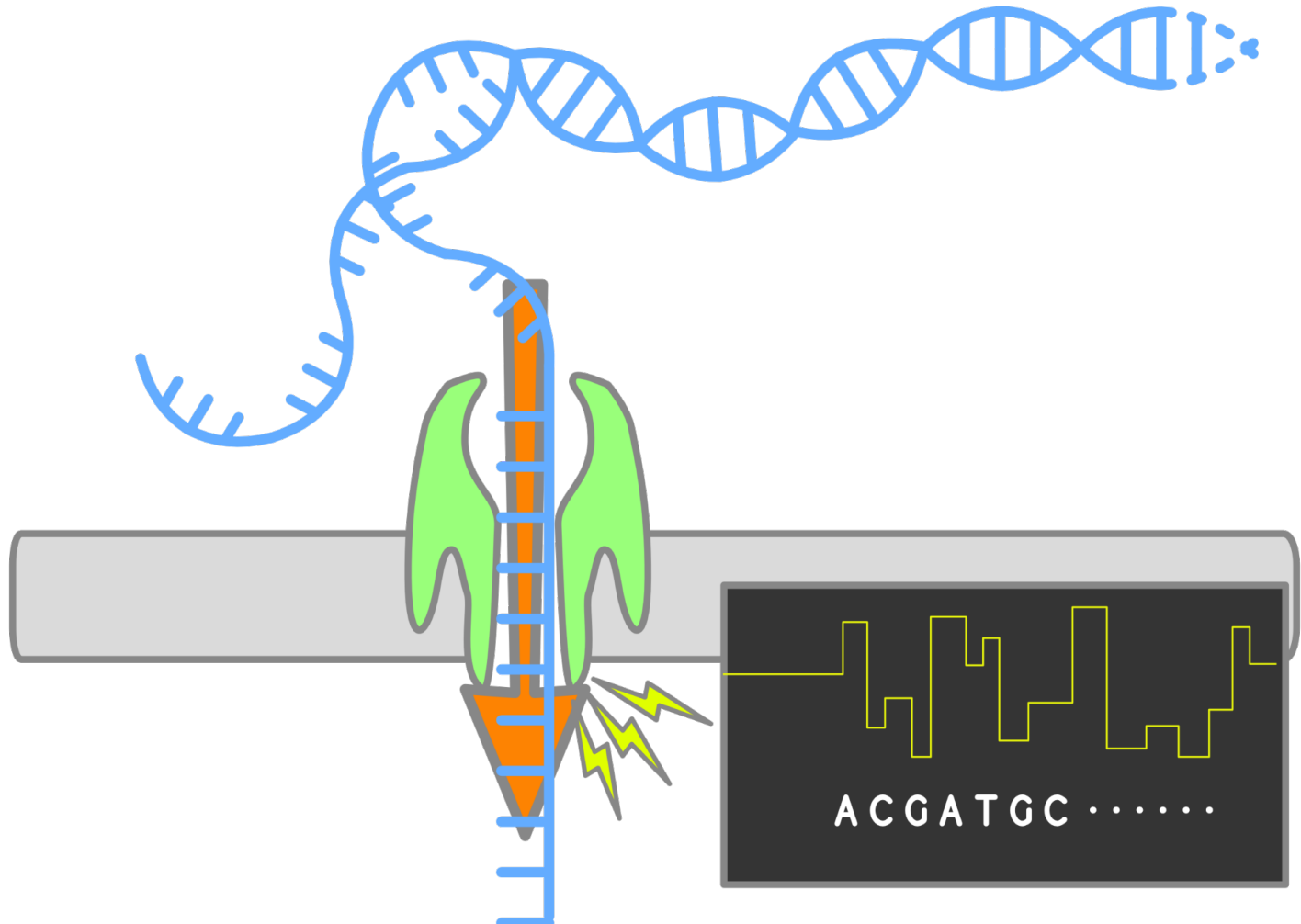
# Real-Time DNA Sequencing from Single Polymerase Molecules

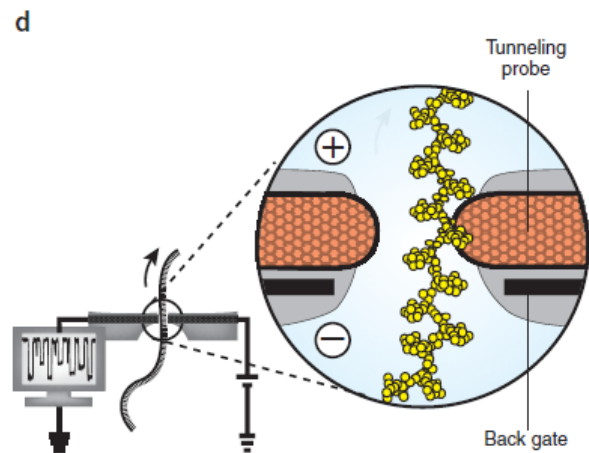
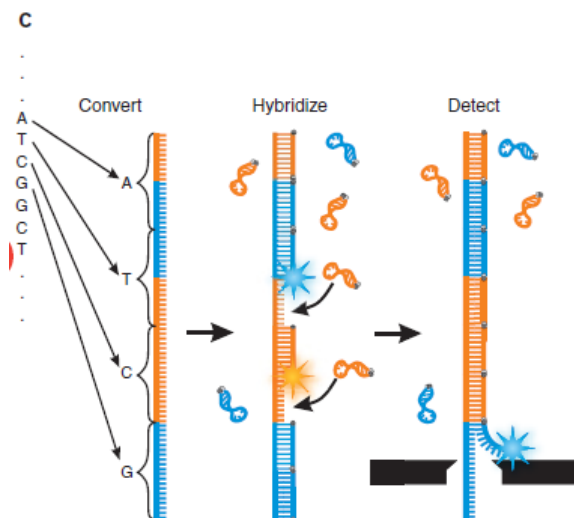
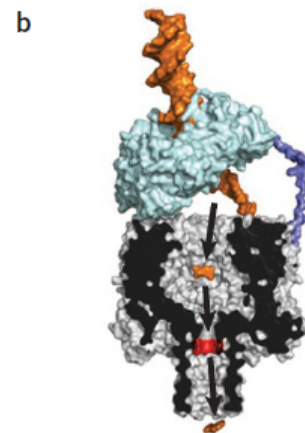
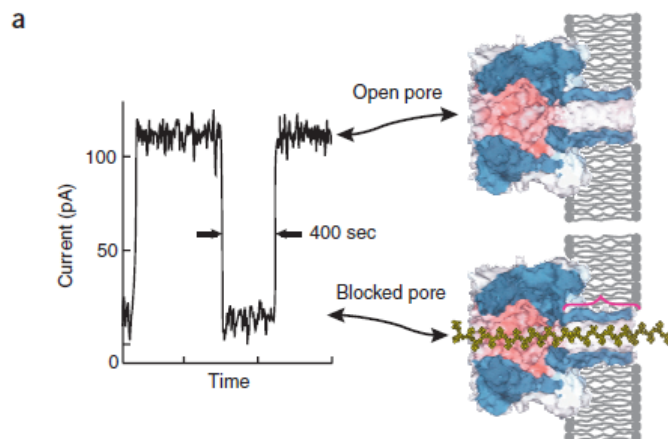


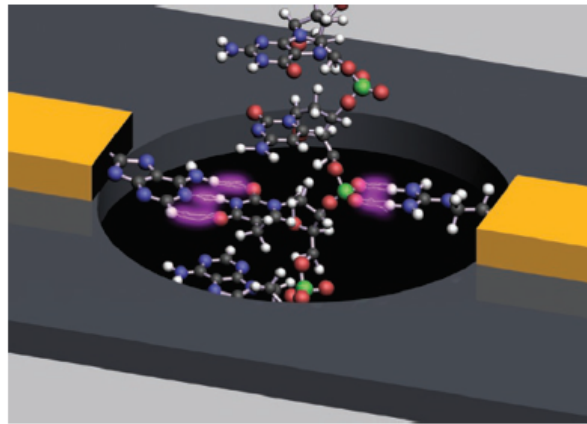
# Nanopore Sequencing

<https://www.youtube.com/watch?v=RcP85JHLmnl>

<https://www.youtube.com/watch?v=qzusVw4Dp8w>







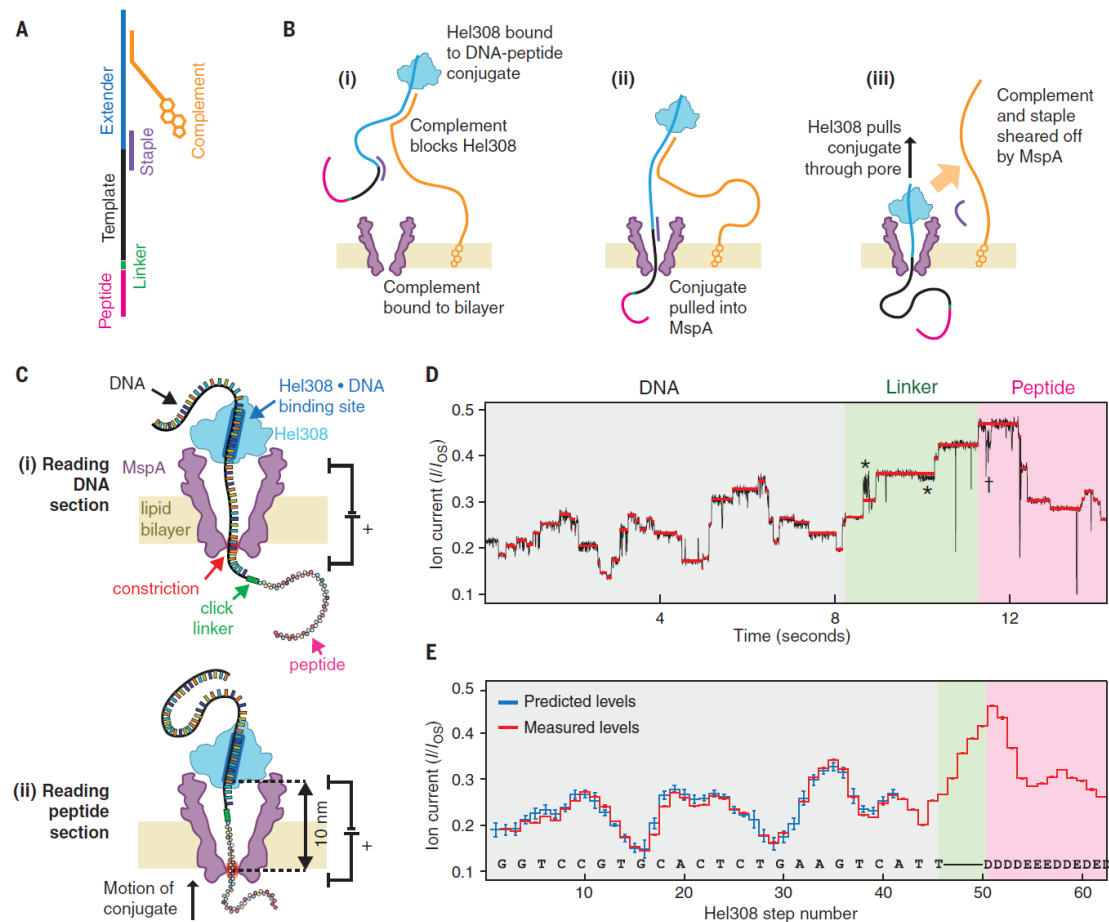
**Figure 2** A nanopore reader with chemically functionalized probes. As a strand of DNA emerges from a nanopore, a 'phosphate grabber' on one functionalized electrode and a 'base reader' on the other electrode form hydrogen bonds (light blue ovals) to complete a transverse electrical circuit through each nucleotide as it is translocated through the nanopore.

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# Multiple rereads of single proteins at single-amino acid resolution using nanopores

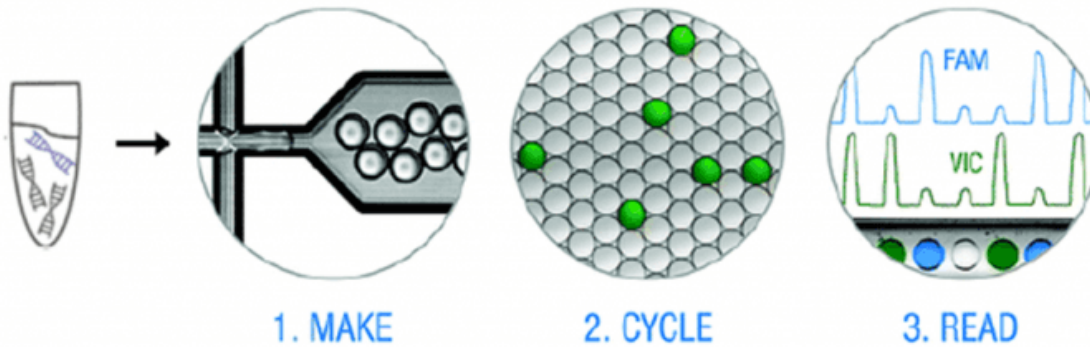
Henry Brinkerhoff<sup>1</sup>, Albert S. W. Kang<sup>1</sup>, Jingqian Liu<sup>2</sup>, Aleksei Aksimentiev<sup>2</sup>, Cees Dekker<sup>1\*</sup>



# Digital PCR



## Droplet digital PCR



Sample is partitioned into 20,000 droplets

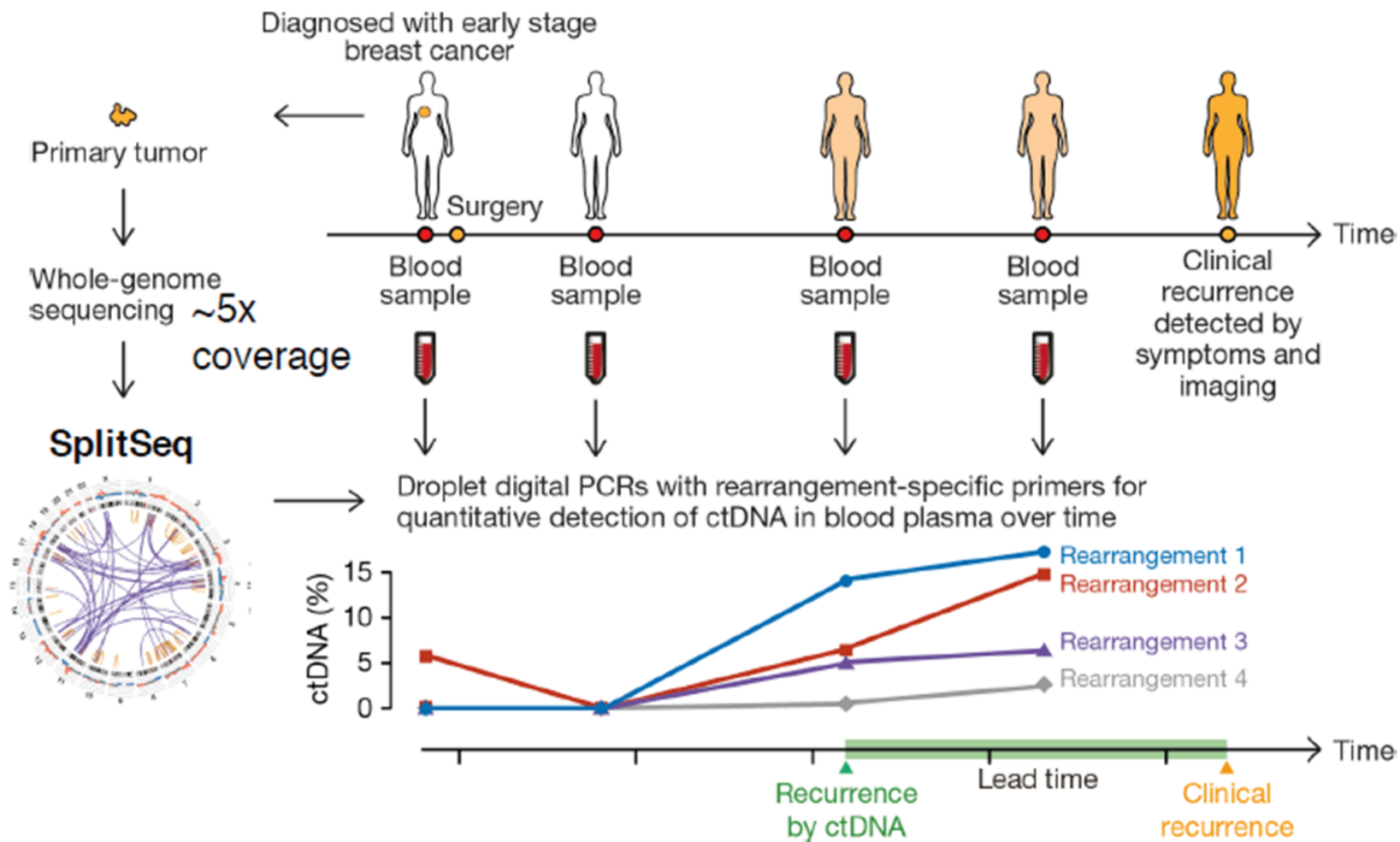
Run PCR cycles in all droplets simultaneously

Measure fluorescence intensity in each droplet

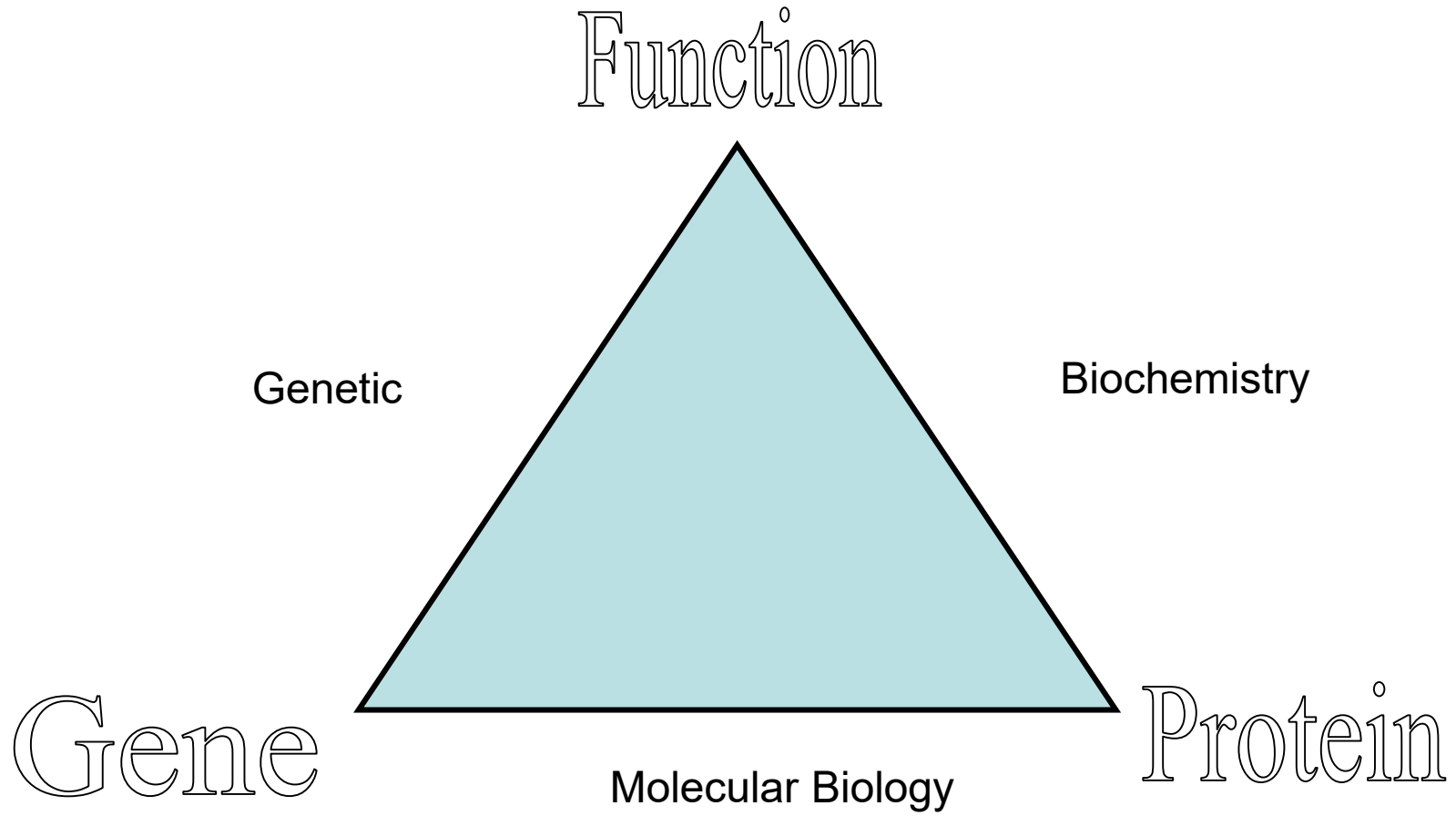
Calculate concentration from number of positive droplets

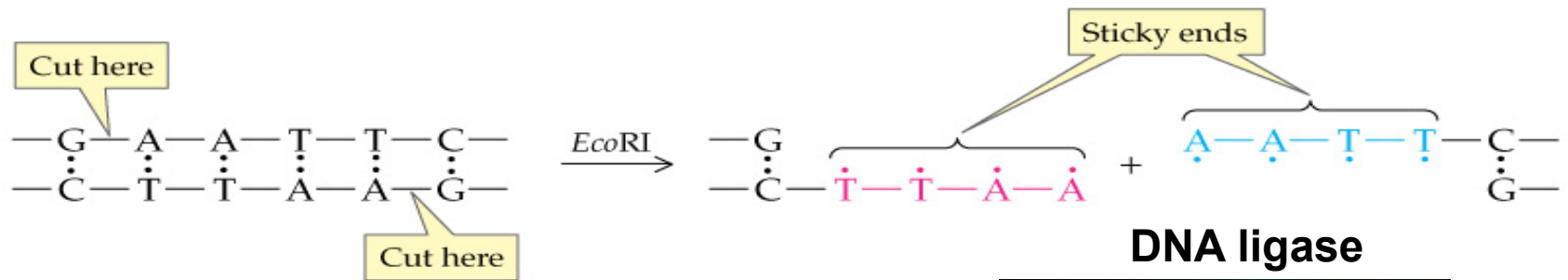


Bio-Rad QX100

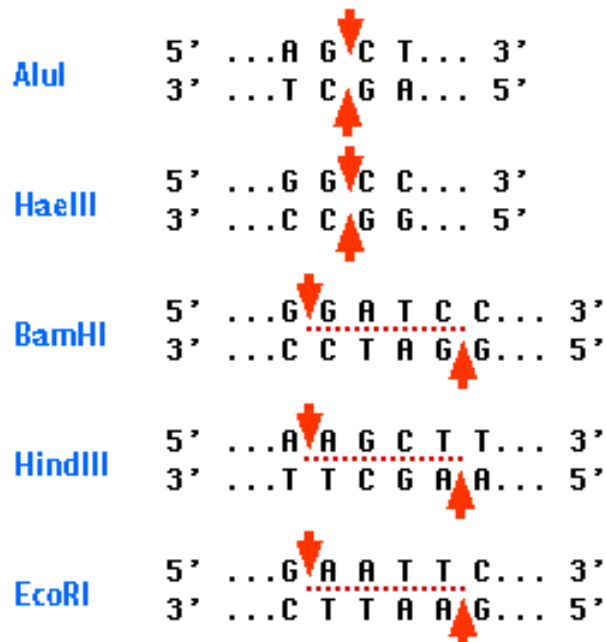


# Recombinant DNA



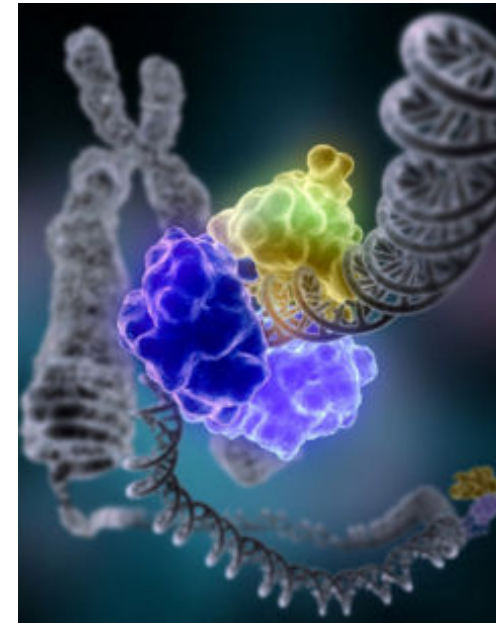


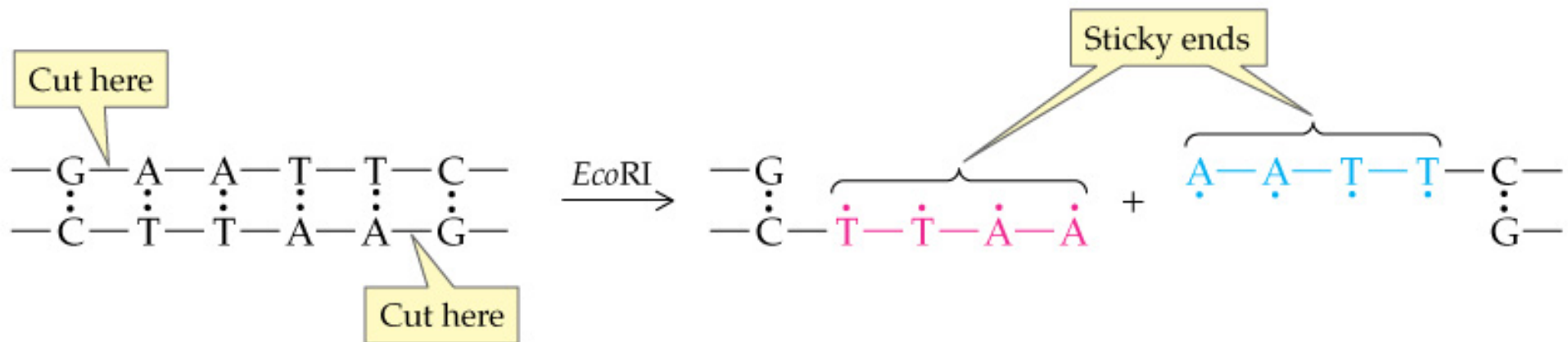
## Restriction Enzyme



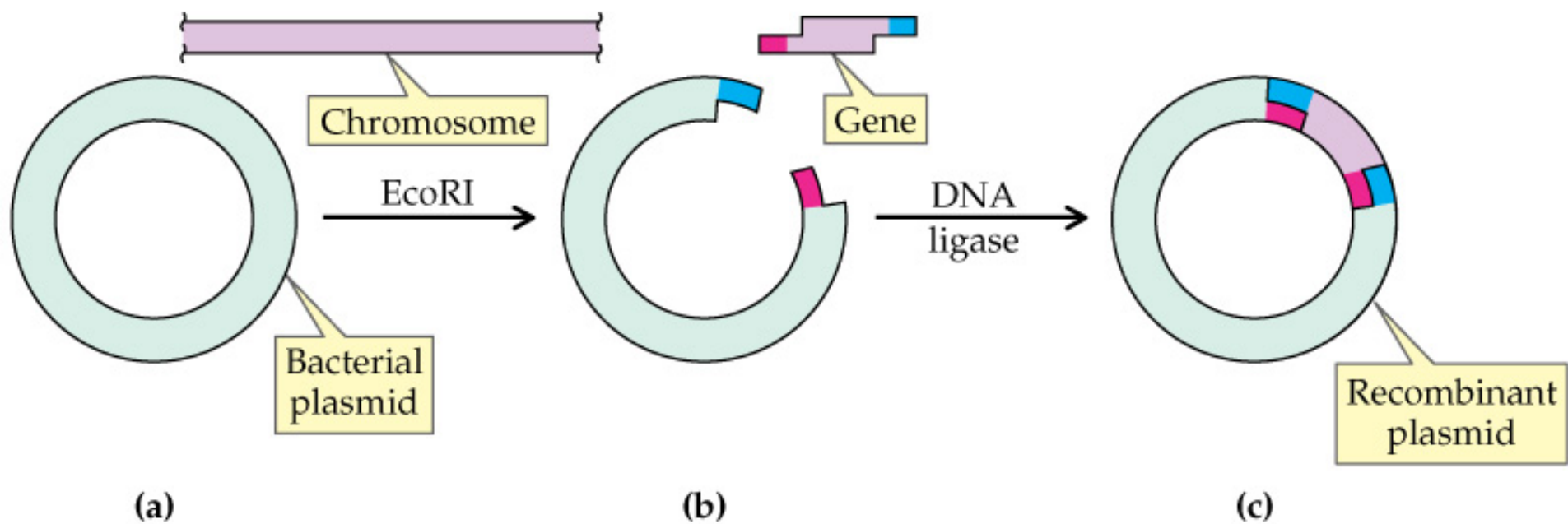
**AluI** and **HaeIII** produce blunt ends

**BamHI** **HindIII** and **EcoRI** produce "sticky" ends



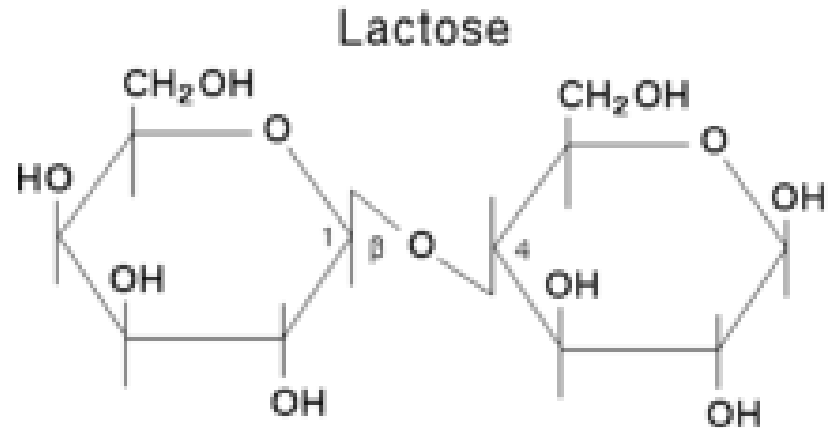
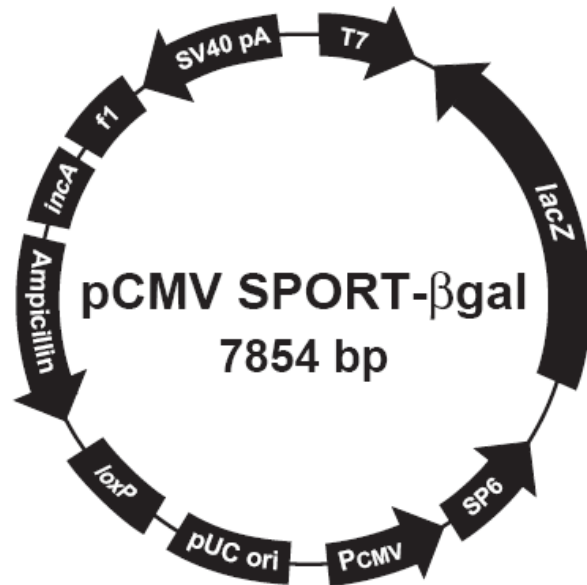






# $\beta$ -Galactosidase

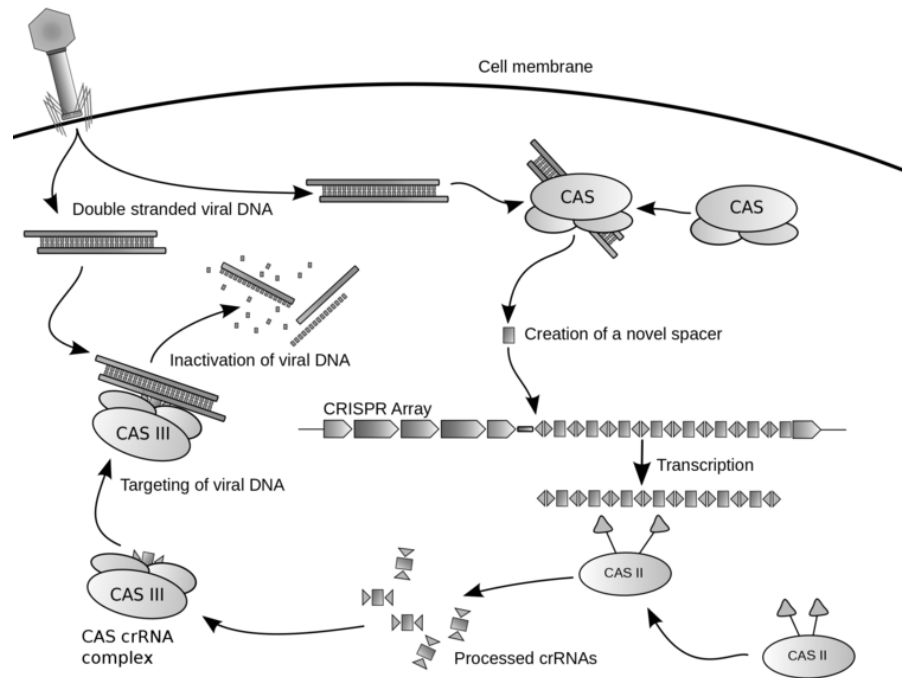
The enzyme that splits lactose into glucose and galactose. Coded by a gene ([lacZ](#)) in the [lac operon](#) of Escherichia coli.



PUC is a family of plasmids that have an ampicillin resistance gene and more importantly a *lacZ* gene. A functional *lacZ* gene will produce the protein  $\beta$  - galactosidase. Bacterial colonies in which  $\beta$  - galactosidase is produced, will form blue colonies in the presence of the substrate 5 - bromo - 4 - chloro - 3 - indolyl - b - D - galactoside or as it is more commonly referred to, X-gal.

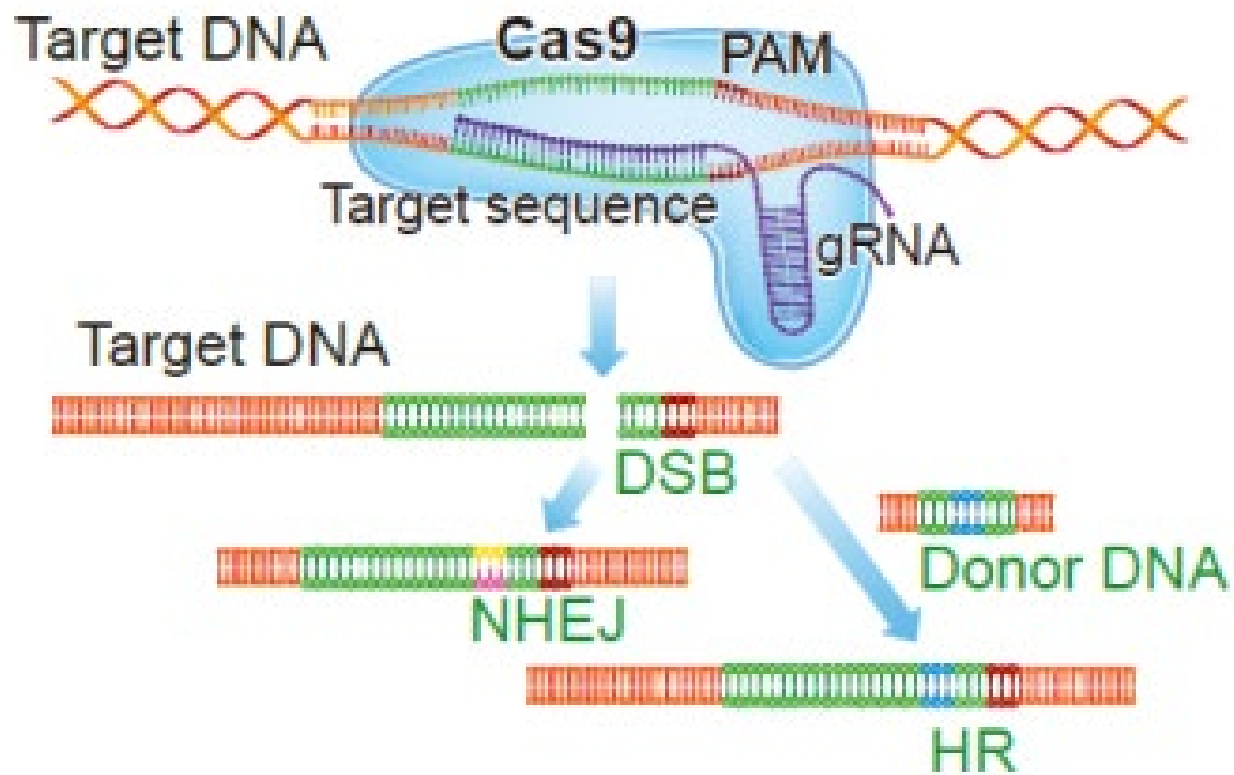
# CRISPR CAS9

**CRISPRs (clustered regularly interspaced short palindromic repeats)** are segments of prokaryotic DNA containing short repetitions of base sequences. Each repetition is followed by short segments of "spacer DNA" from previous exposures to a bacterial virus or



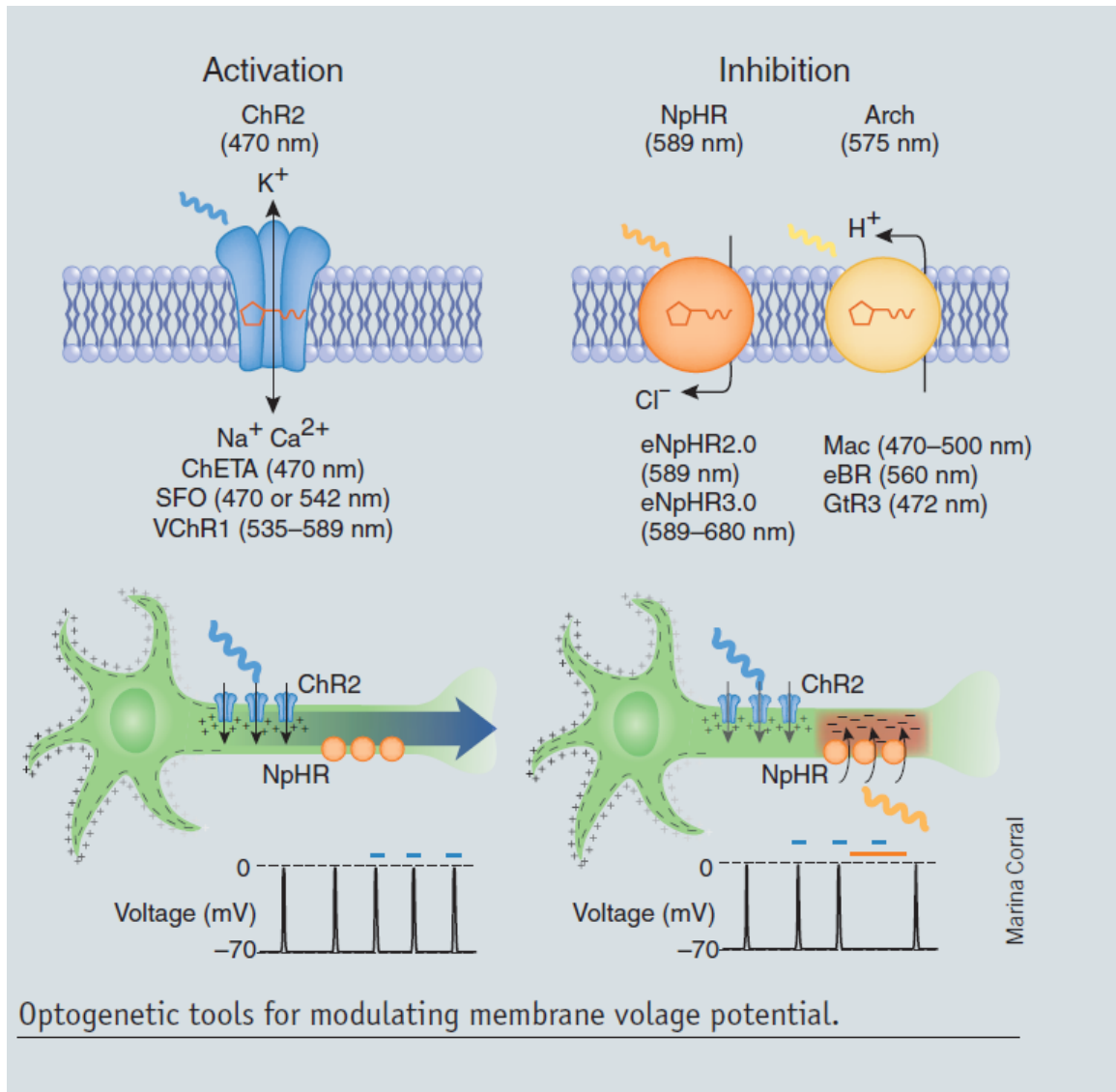
# Movie

- <https://youtu.be/2pp17E4E-O8>



# Optogenetics

<https://www.youtube.com/watch?v=I64X7vHSHOE>

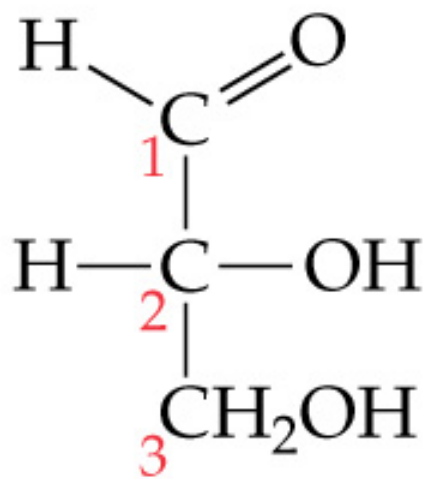




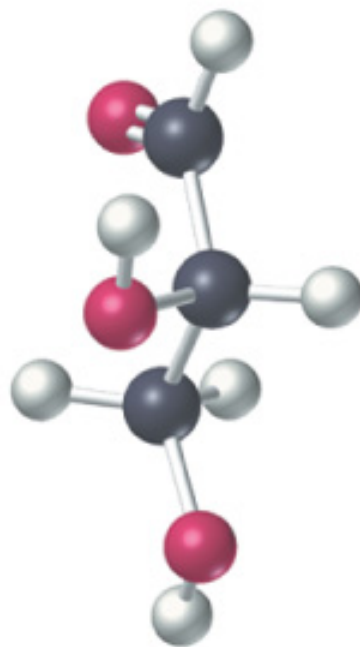
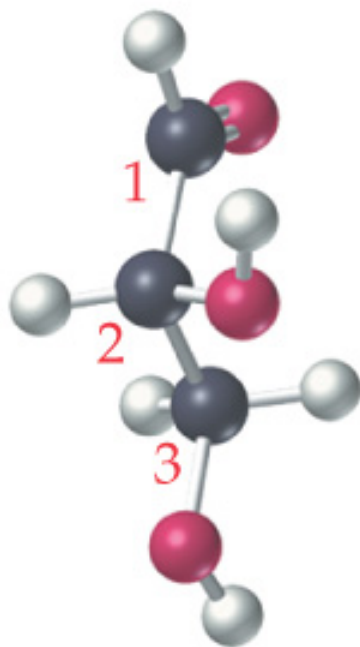
# An Introduction to Carbohydrates

- *Carbohydrates* are a large class of naturally occurring polyhydroxy aldehydes and ketones.
- Monosaccharides also known as simple sugars, are the simplest carbohydrates containing 3-7 carbon atoms.
- sugar containing an aldehydes is known as an aldose.
- sugar containing a ketones is known as a ketose.

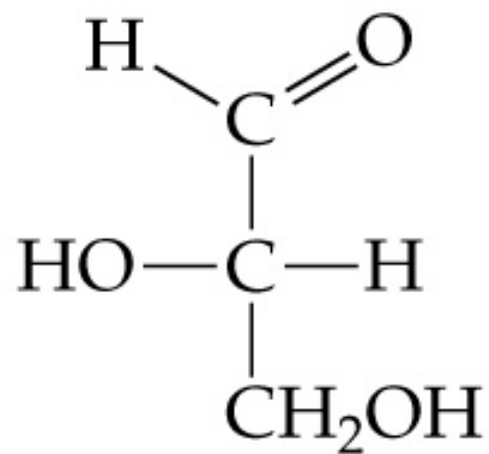
- The number of carbon atoms in an aldose or ketose may be specified as by tri, tetr, pent, hex, or hept. For example, glucose is aldohexose and fructose is ketohexose.
- Monosaccharides react with each other to form disaccharides and polysaccharides.
- Monosaccharides are chiral molecules and exist mainly in cyclic forms rather than the straight chain.

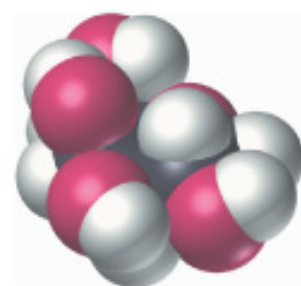
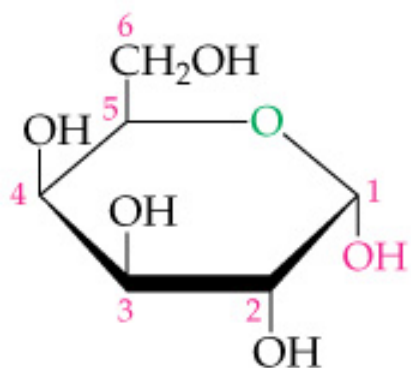


D-Glyceraldehyde  
Right-handed

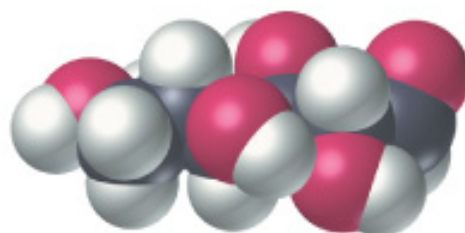
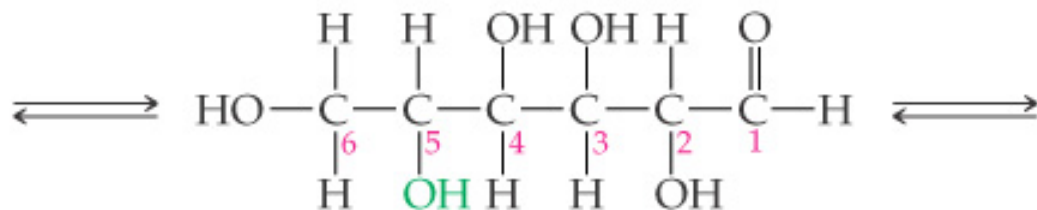


L-Glyceraldehyde  
Left-handed

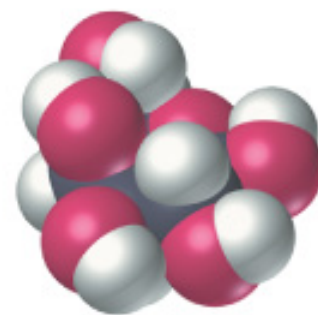
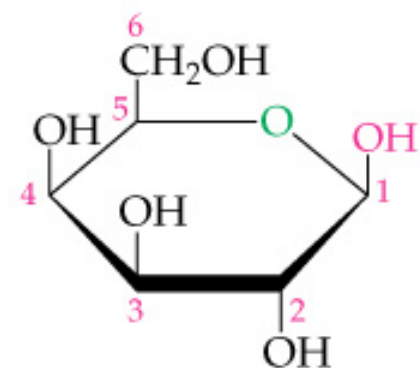




$\alpha$ -D-Galactose

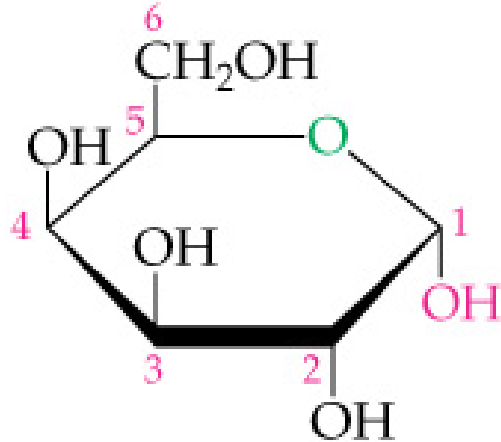


Open-chain galactose

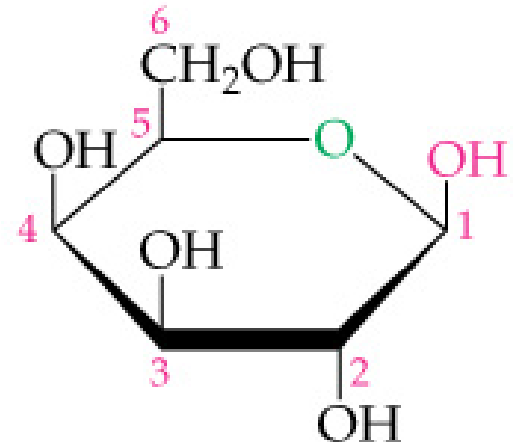


$\beta$ -D-Galactose

- *Anomers:* Cyclic sugars that differ only in the positions of substituents at the hemiacetal carbon; the  $\alpha$ -form has the  $\text{-OH}$  group on the opposite side from the  $\text{-CH}_2\text{OH}$ ; the  $\beta$ -form has the  $\text{-OH}$  group on the same side as the  $\text{-CH}_2\text{OH}$  group.



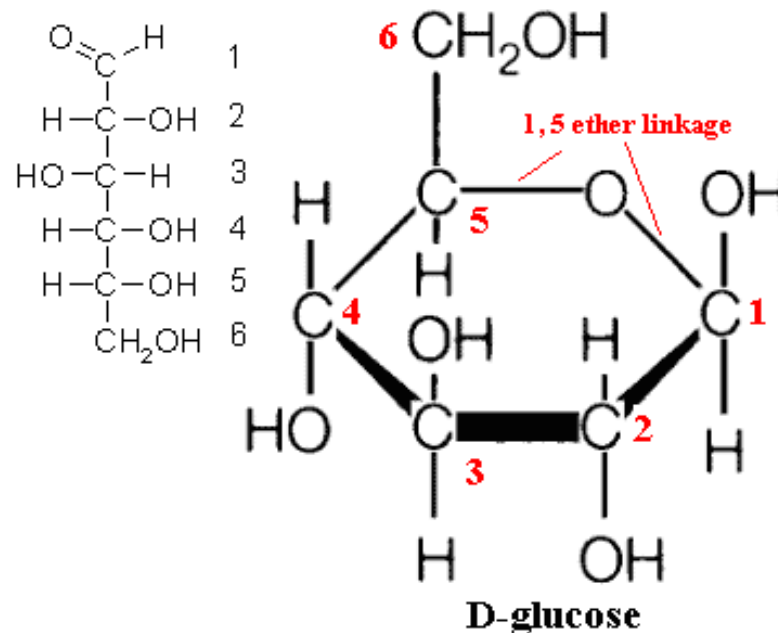
$\alpha$ -D-Galactose



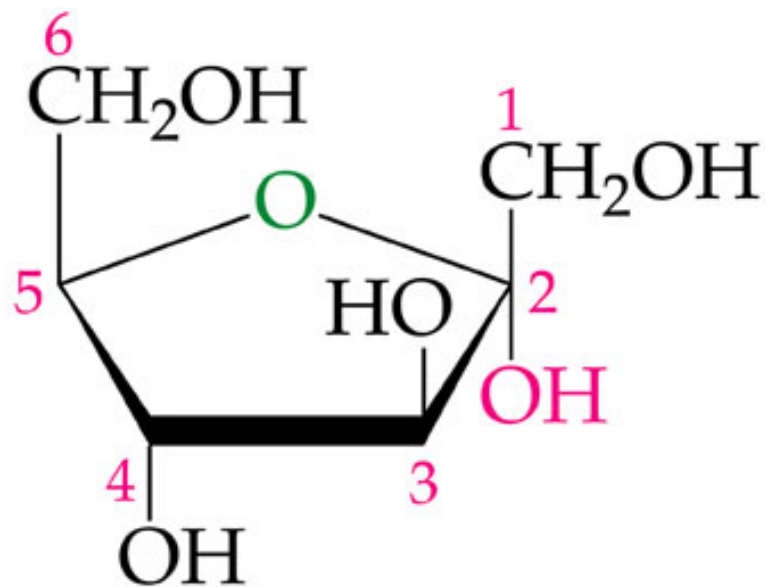
$\beta$ -D-Galactose

# Some Important Monosaccharides

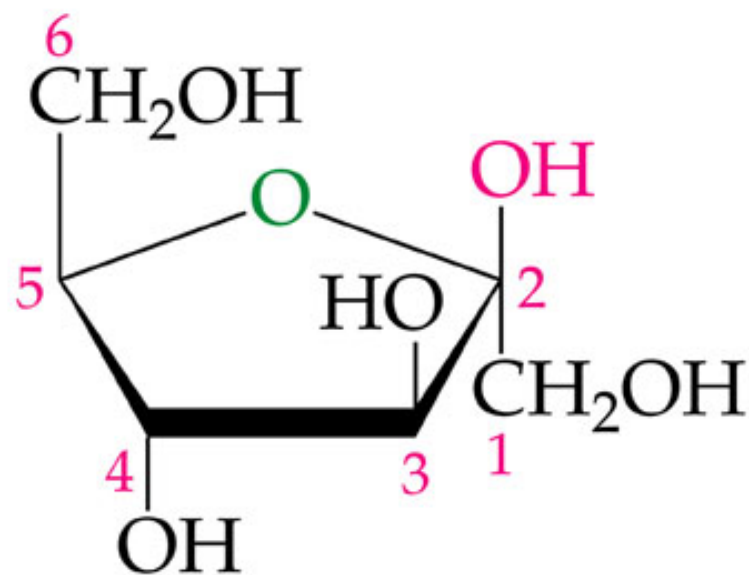
Monosaccharides are generally high-melting, white, crystalline solids that are soluble in water and insoluble in nonpolar solvents. Most monosaccharides are sweet tasting, digestible, and nontoxic.



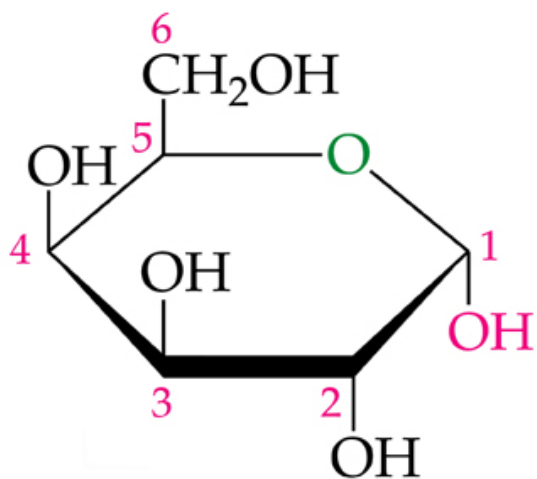




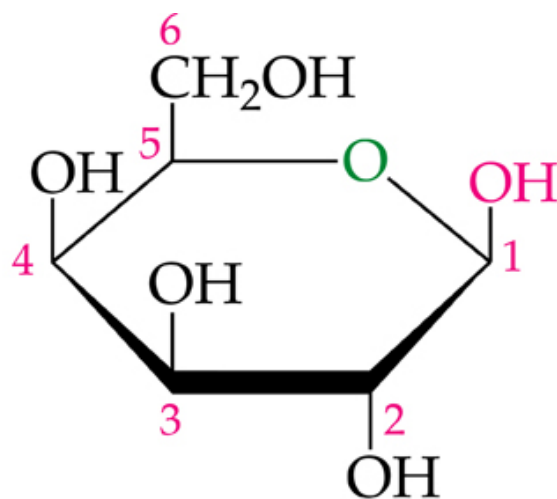
$\alpha$ -D-Fructose



$\beta$ -D-Fructose

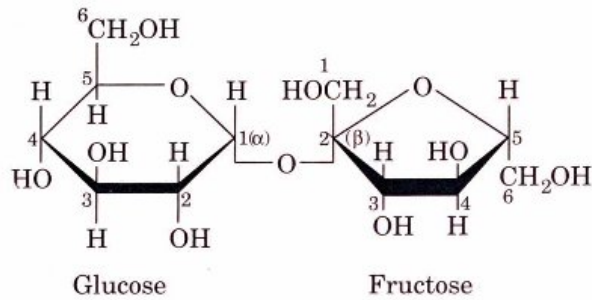


$\alpha$ -D-Galactose

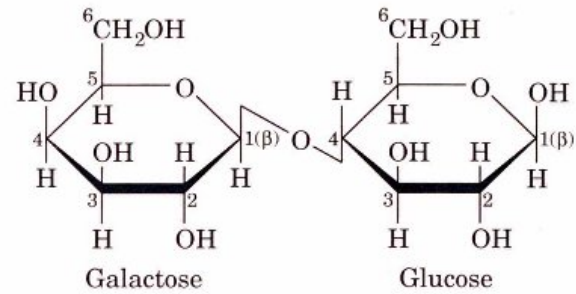


$\beta$ -D-Galactose

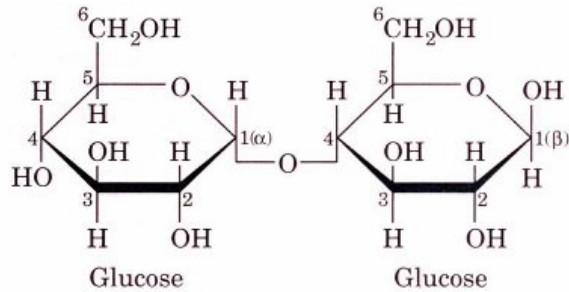
# Some Common Disaccharides



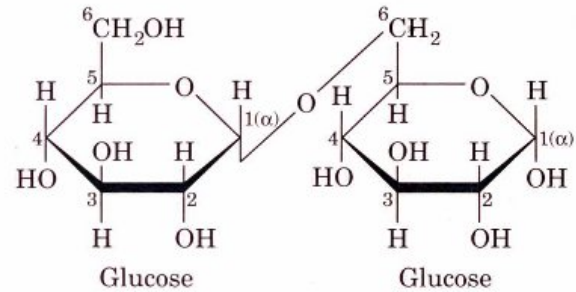
**Sucrose**



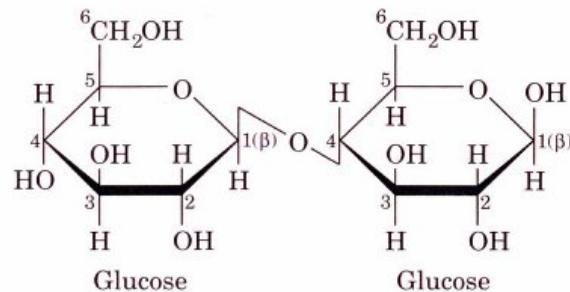
**Lactose**



**Maltose**

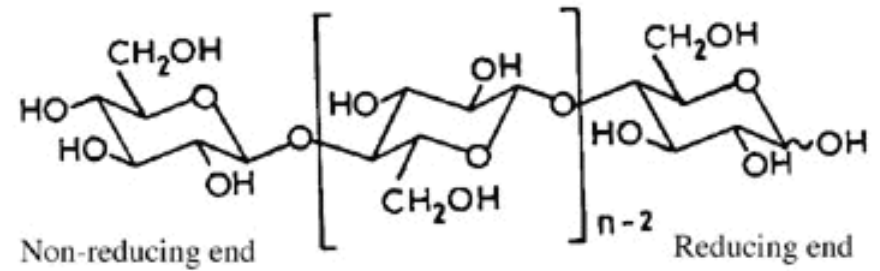
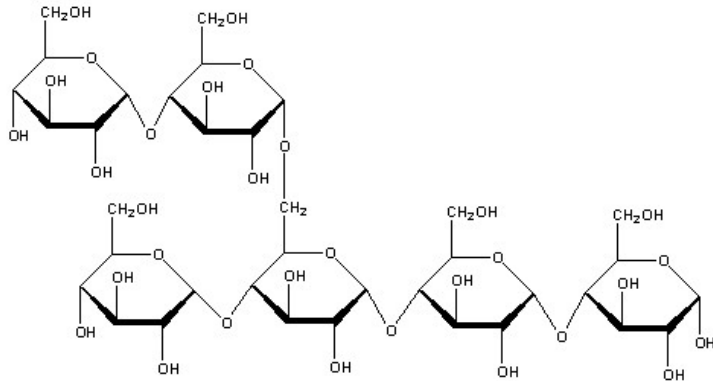


**Isomaltose**

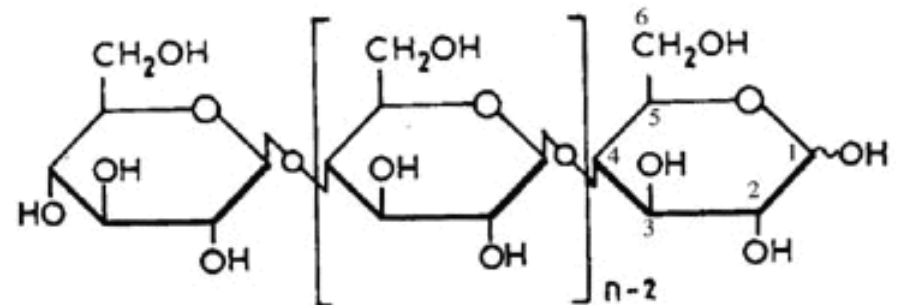
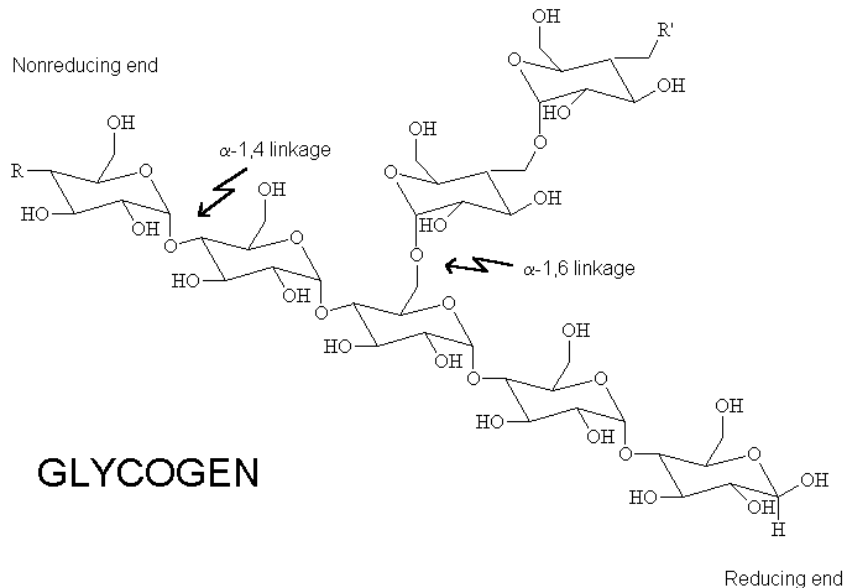


**Cellobiose**

# Polysaccharides

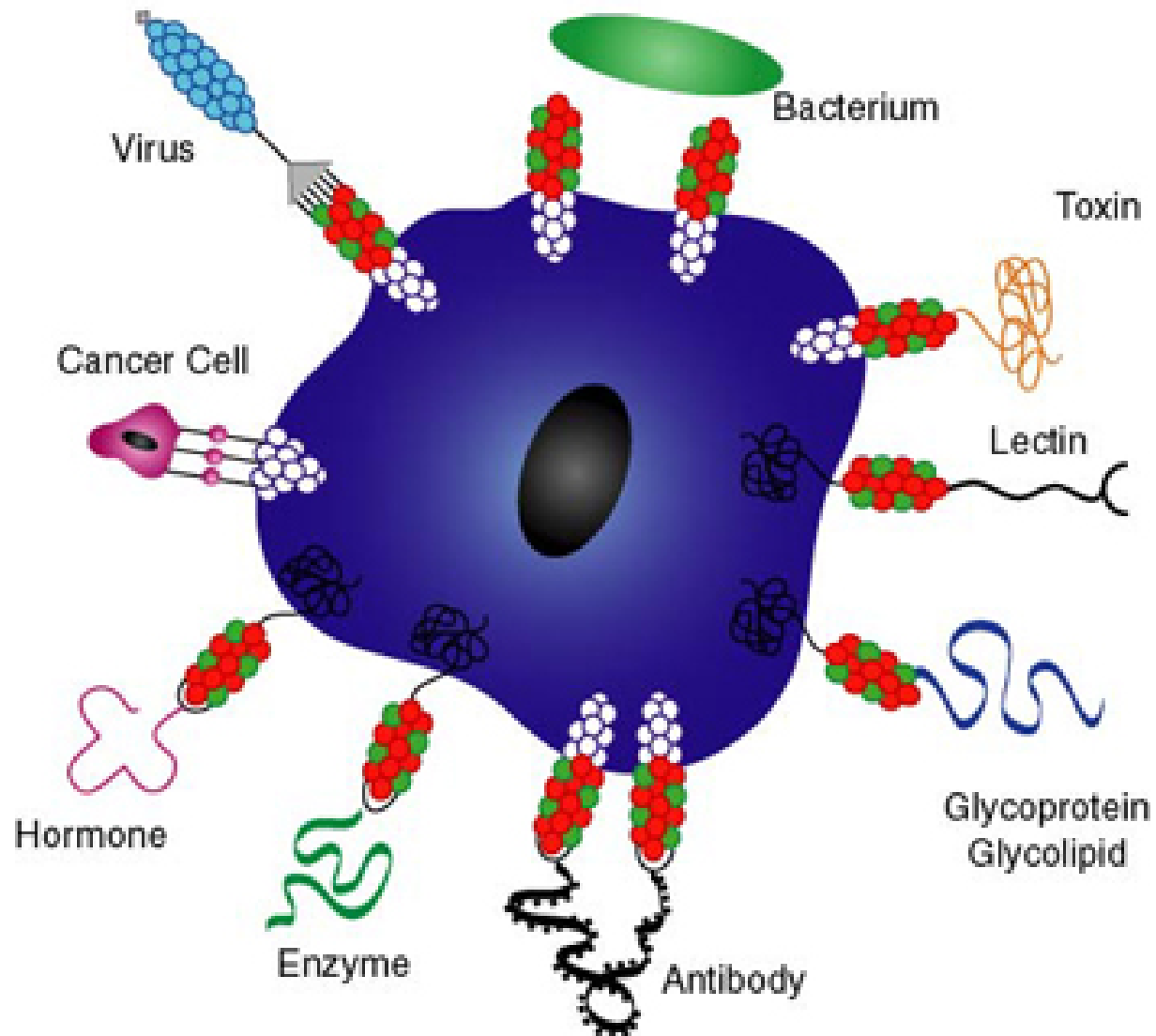


Sometimes shown as



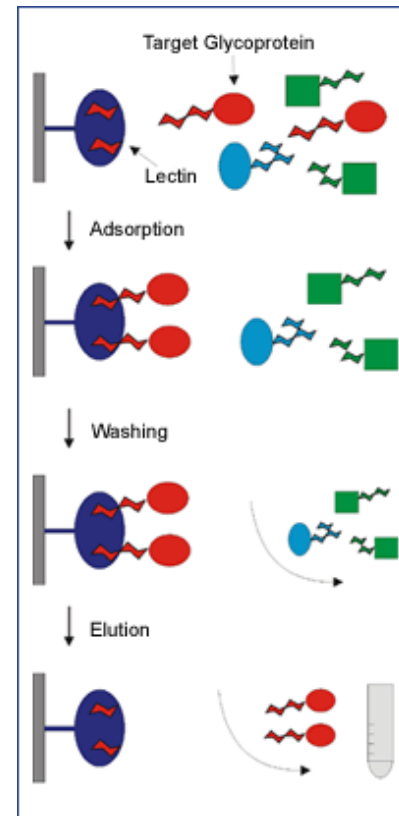
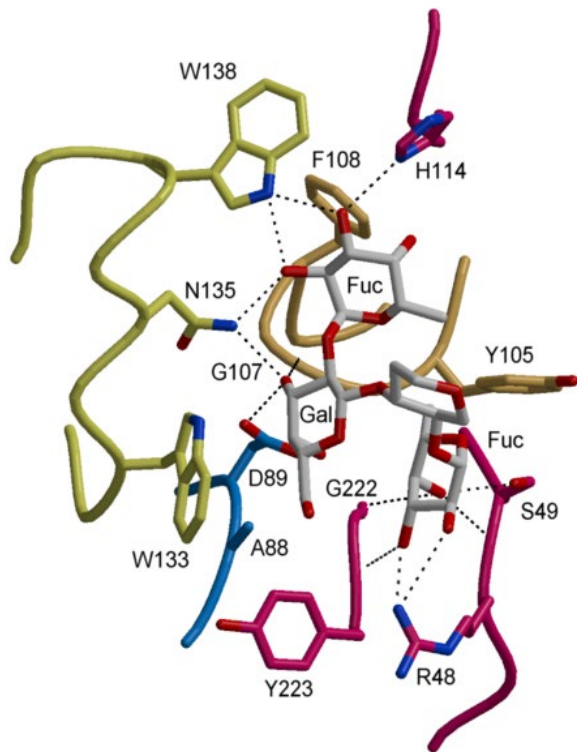
**Cellulose**

## Cell-Surface Carbohydrates Involved in Molecular Recognition

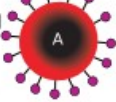
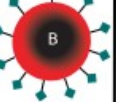
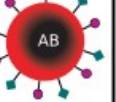










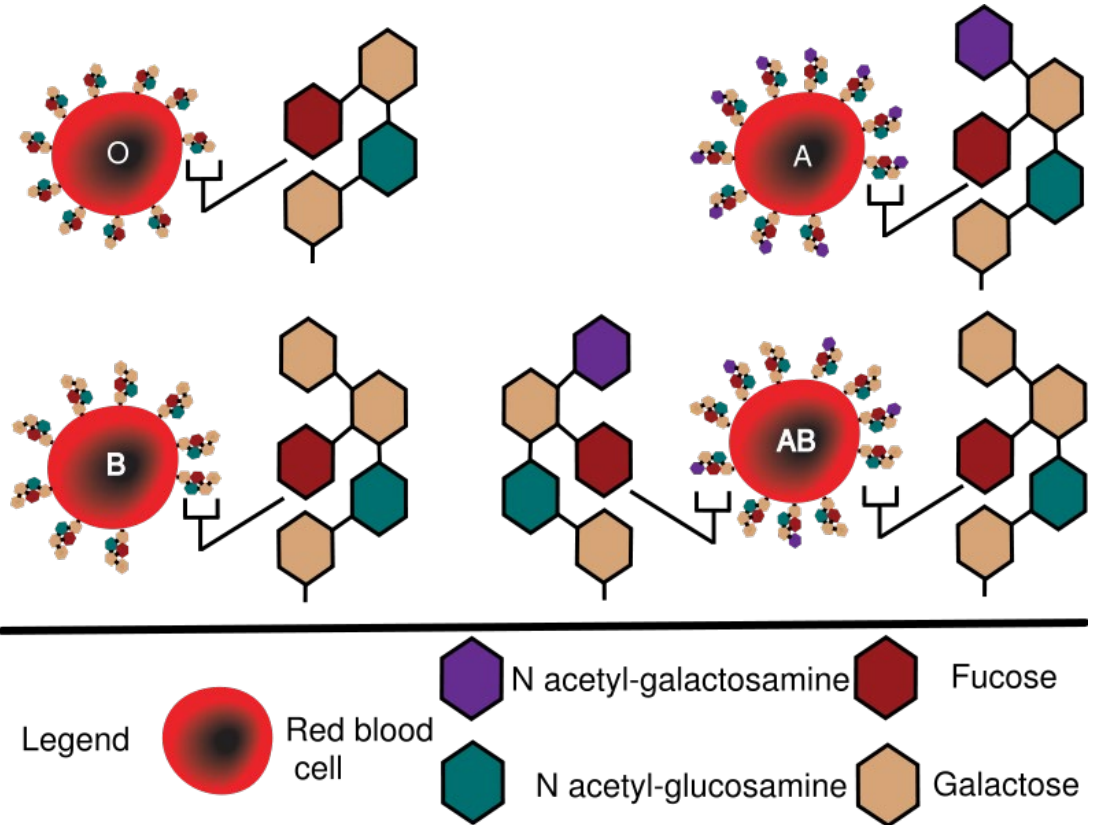
# Lectin

**Lectins** are sugar-binding proteins which are highly specific for their sugar moieties. They typically play a role in biological recognition phenomena involving cells and proteins. For example, some bacteria use lectins to attach themselves to the cells of the host organism during infection.



# Blood Type

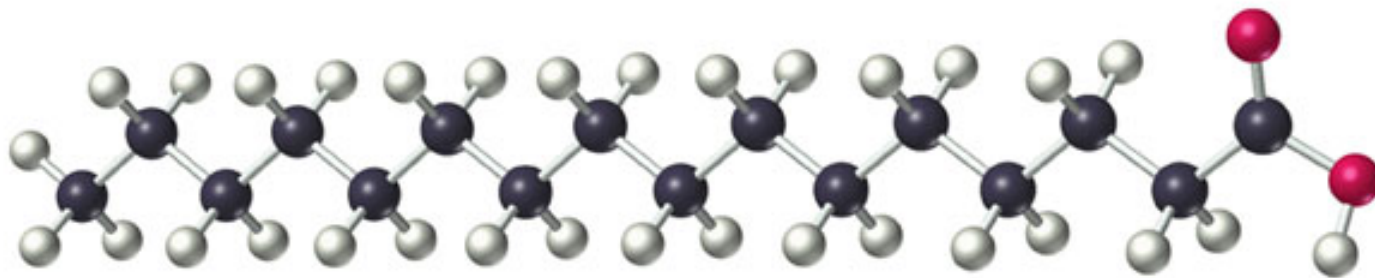
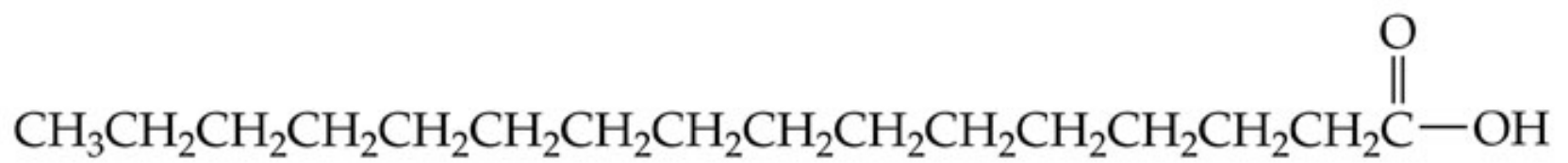
	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	A antigen 	B antigen 	A and B antigens  	No antigens



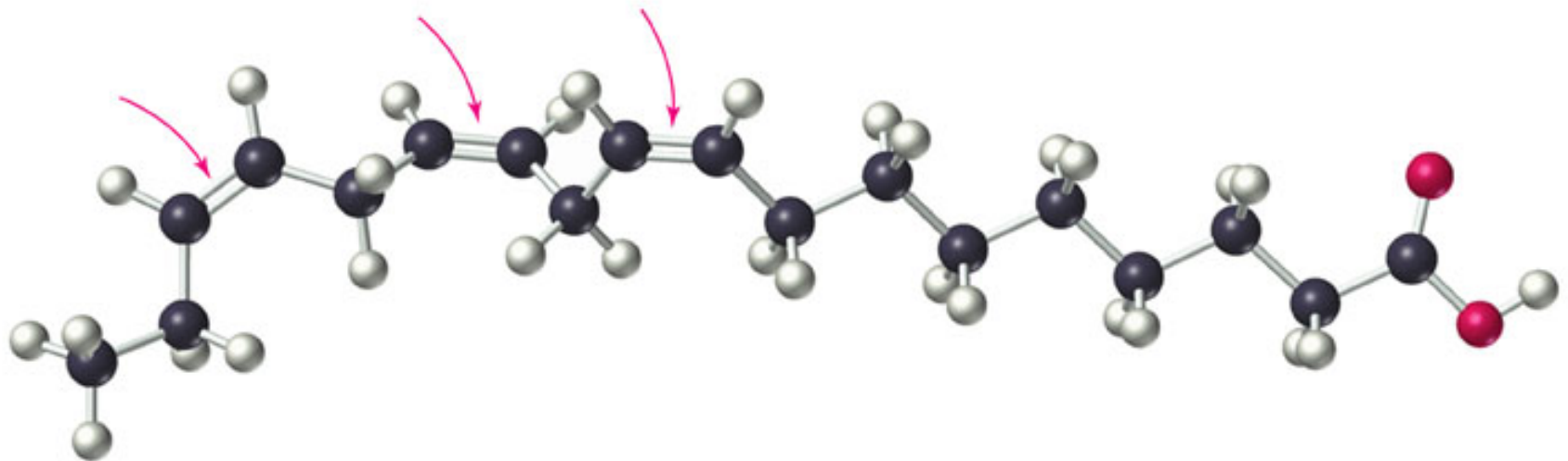
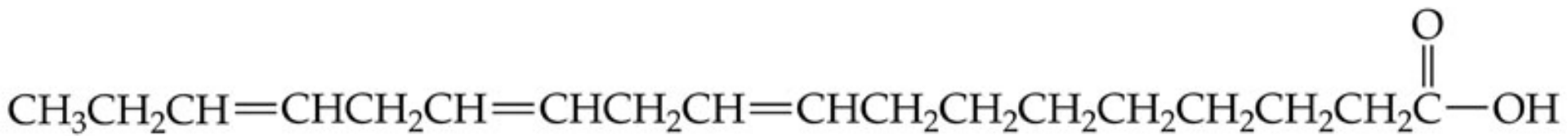
# Lipid

- ***Lipids*** are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.

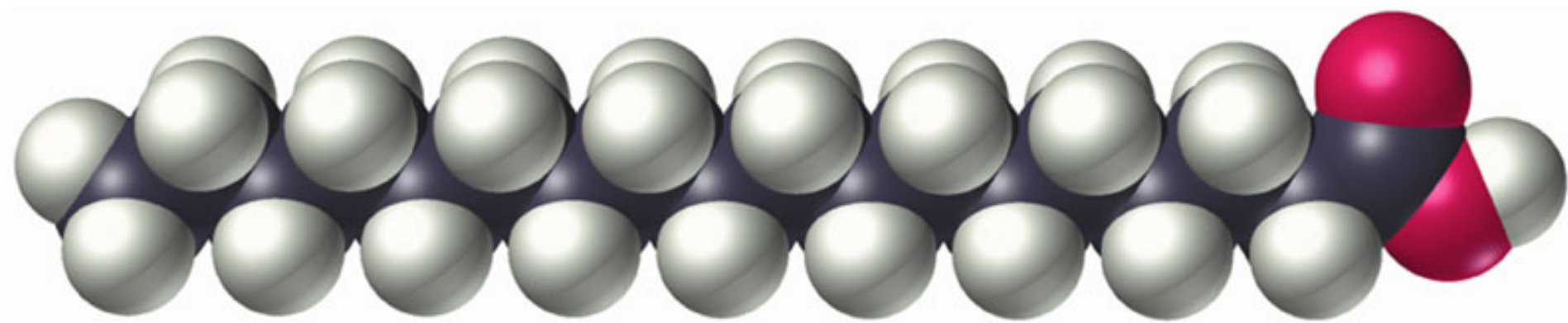




A saturated fatty acid  
(palmitic acid)



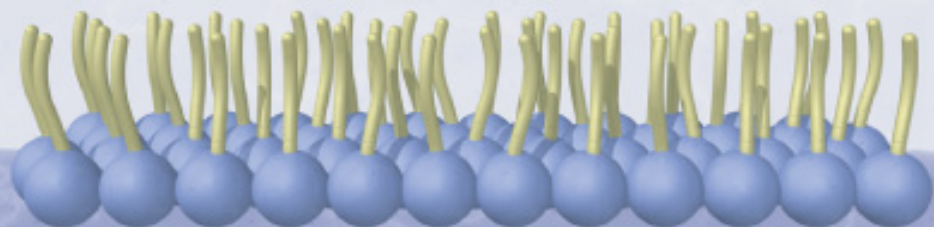
A *cis* unsaturated fatty acid  
(linolenic acid)



Stearic acid, an 18-carbon saturated fatty acid

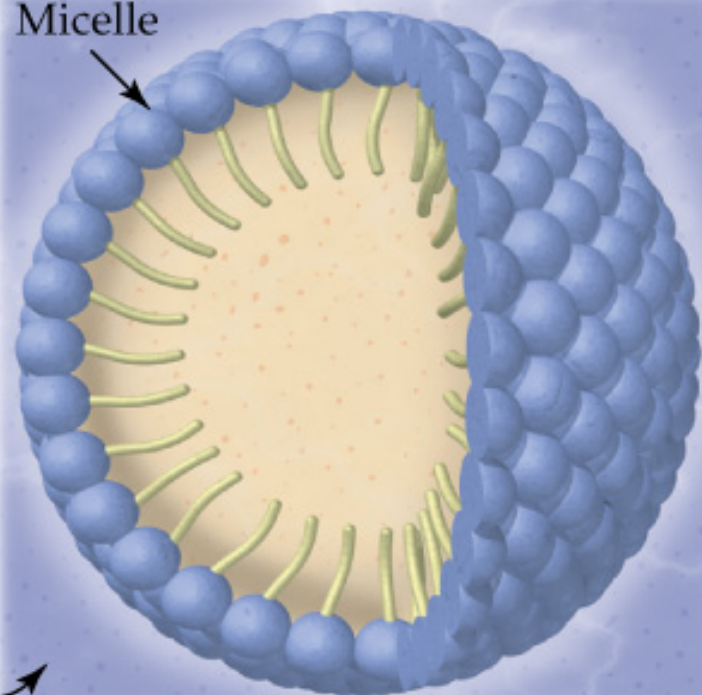
Air

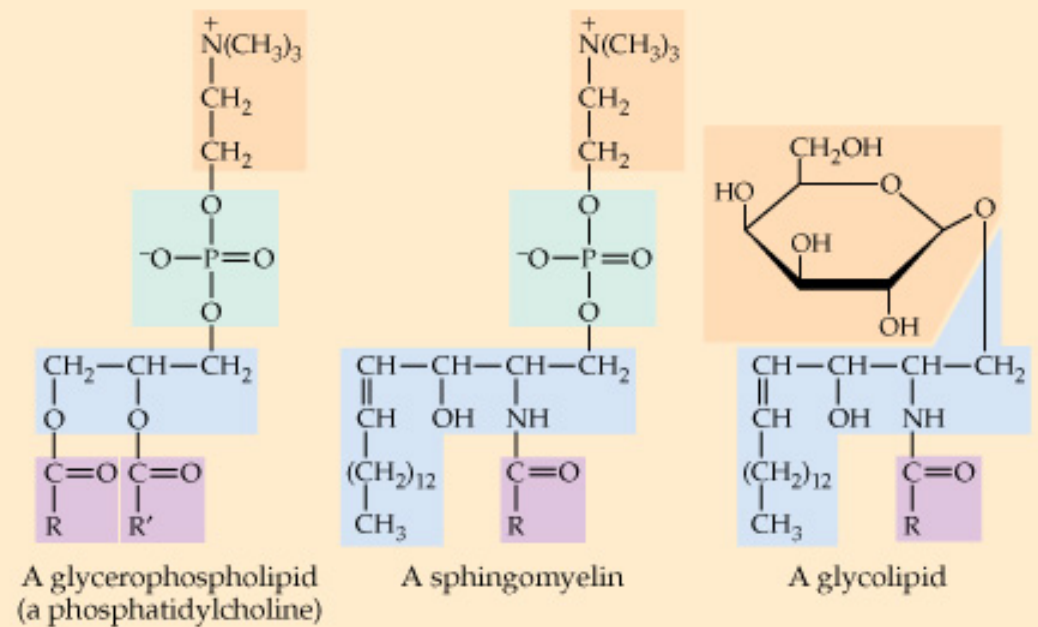
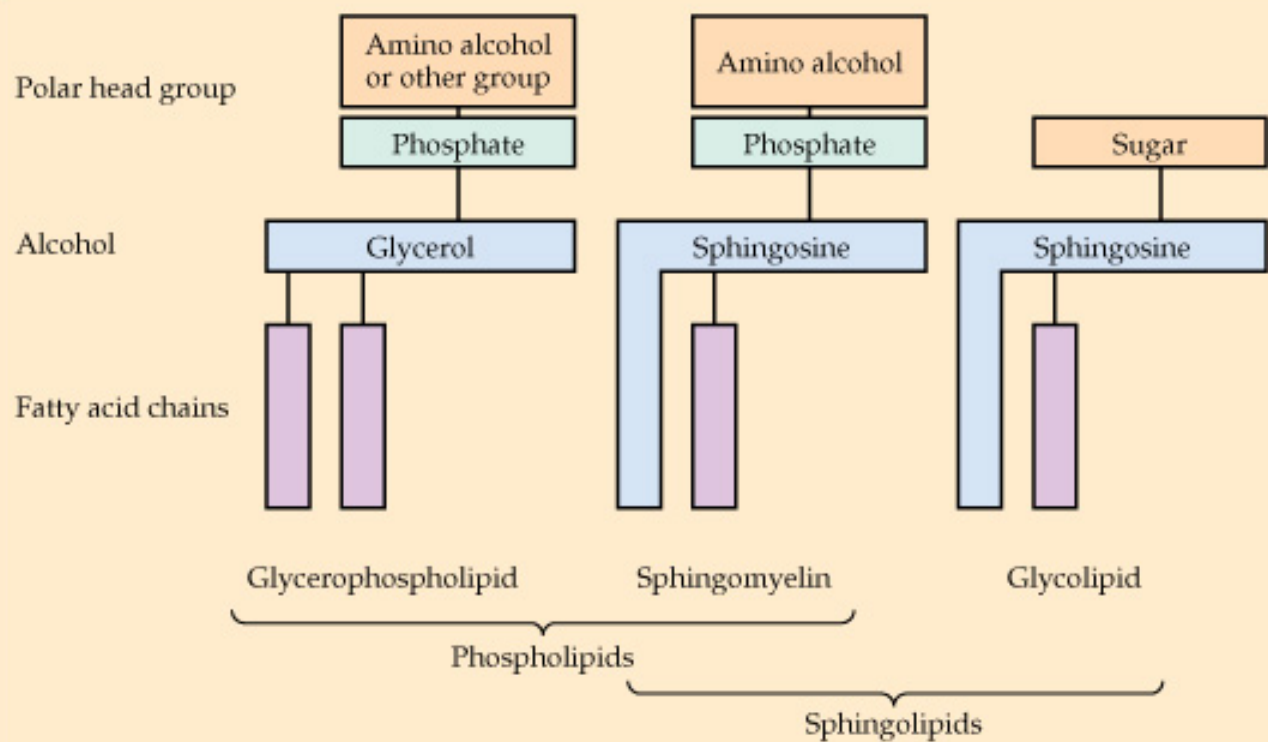
Water

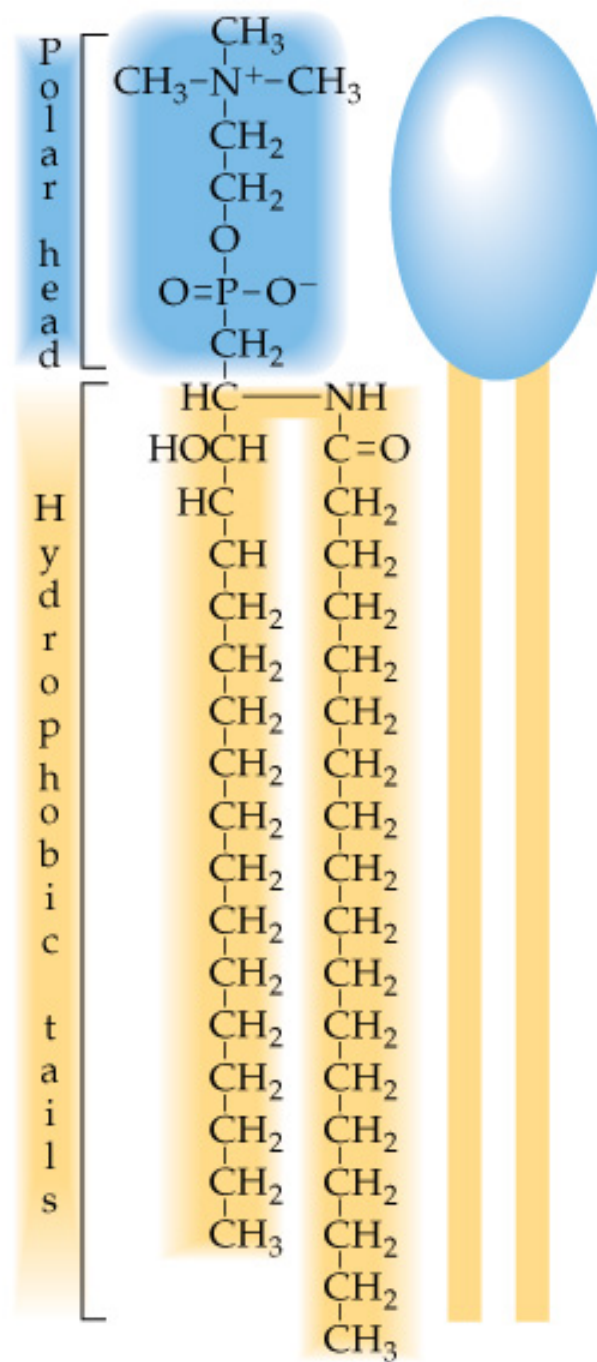


Micelle

Water

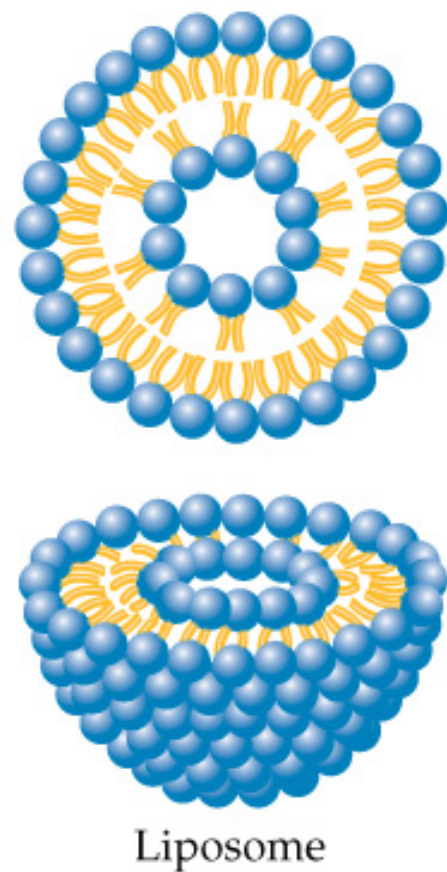
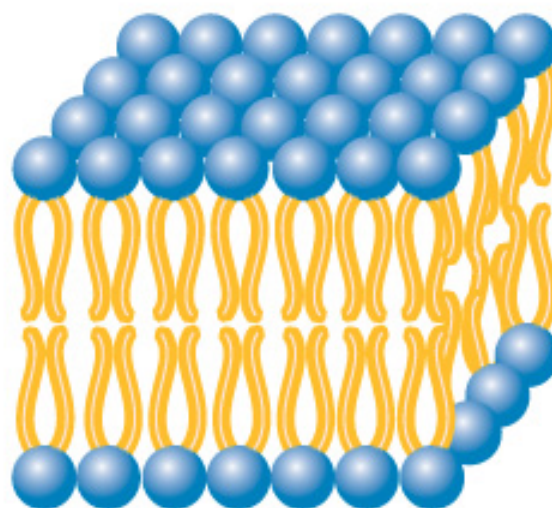
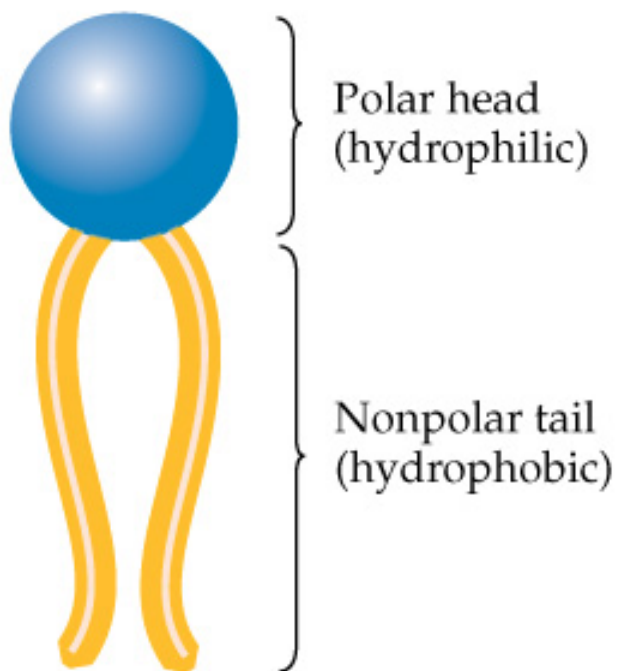






A sphingomyelin



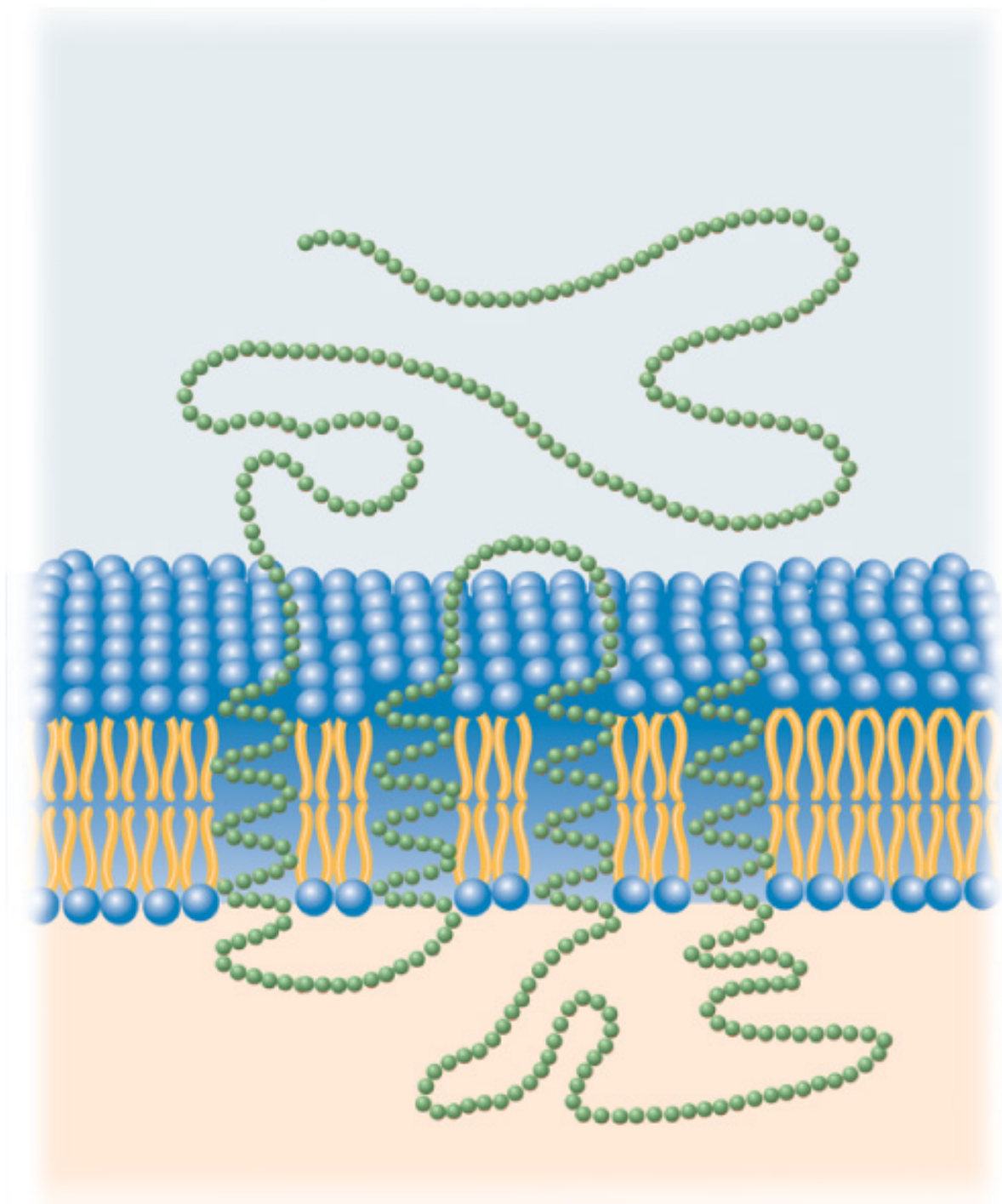


# Properties of cell membranes:

- Cell membranes are composed of a fluid like phospholipid bilayer.
- The bilayer incorporates cholesterol, proteins, and glycolipids.
- Small nonpolar molecules cross by diffusion through the lipid bilayer.
- Small ions and polar molecules diffuse through the aqueous media in protein pores.
- Glucose and certain other substances cross with the aid of proteins without energy input.
- $\text{Na}^+$ ,  $\text{K}^+$ , and other substances that maintain concentration gradients inside and outside the cell cross with expenditure of energy and the aid of proteins.



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- Glucose and certain other substances cross with the aid of proteins without energy input.
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## EXTRACELLULAR FLUID

