Chemical Bond Energy

Table 7.1 Average Bond Dissociation Energies

	Bond Dissociation Energy		Bond Dissociation Energy		Bond Dissociation Energy
Bond	kcal/mol (kJ/mol)	Bond	kcal/mol (kJ/mol)	Bond	kcal/mol (kJ/mol)
с—н	99 (413)	N — Н	93 (391)	c=c	147 (614)
c—c	83 (347)	N — N	38 (160)	C≡C	201 (839)
C-N	73 (305)	N-CI	48 (200)	C=0*	178 (745)
C-0	86 (358)	N-0	48 (201)	0=0	119 (498)
c—cı	81 (339)	н—н	103 (432)	N=0	145 (607)
ci—ci	58 (243)	0-н	112 (467)	0=N	213 (891)
н—сі	102 (427)	0-CI	49 (203)	$N \equiv N$	226 (946)

*The C = 0 bond dissociation energies in CO₂ are 191 kcal/mol (799 kJ/mol).

Hydrogen Bond Energy

Table 3.1	H-bond	and	its	bond	strength.
-----------	--------	-----	-----	------	-----------

H-bond	Bond Strength (kcal/mol)		
F–H·····F	7		
0–НО	4.5-7.6		
0–HN	4–7		
C-H·····pi electrons	2–4		
С–НО	2–3		
N–H·····O	2–3		
N–H·····N	1.3		

Strong hydrogen bonds of 20-40 kcal/mole

Weak hydrogen bonds of 1-5 kcal/mole

Normal hydrogen bond 3 - 12 kcal/mole

Disulfide Bond Energy

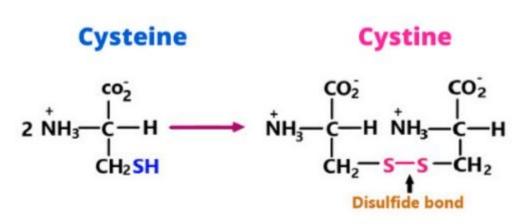


Fig: Disulfide bond in protein

This bond length is 2.2 Å and bond energy is 60 kcal/mol.

Chemical Bonds & Interactions

NAME	BASIS OF INTERACTION	STRUCTURE	BOND ENERGY [®] (KCAL/MOL)
Covalent bond	Sharing of electron pairs		50-110
lonic bond	Attraction of opposite charges	H H H H H	37
Hydrogen bond	Sharing of H atom	H 8* 5 -N-H-0-C-	37
Hydrophobic Interaction	Interaction of nonpolar substances in the presence of polar substances (especially water)		1–2
van der Waals interaction	Interaction of electrons of nonpolar substances	н—н 🥐 Сн	1

"Bond energy is the amount of energy needed to separate two bonded or interacting atoms under physiological conditions.

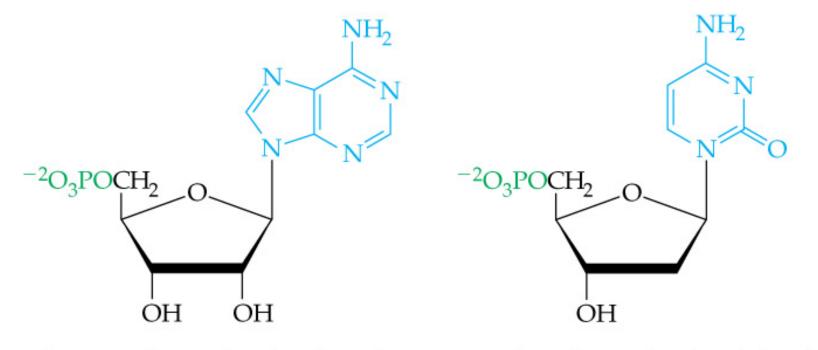
Thermal Energy

E=3/2 RT R= 1.987 cal/mol/K

E=3/2 1.987 x 300 ~ 0.9 Kcal

reaction	Gibbs energy for hydrolysis reaction ⊿G ^{,0} (kJ/mol)
phospho-anhydride (acid-acid) bond hydrolysis	
$ATP + H_2O \rightarrow ADP + phosphate$	-31 (-13 k _B T)
ADP + H ₂ O -> AMP + phosphate	-31 (-13 k _B T)
$ATP + H_2O \rightarrow AMP + PPi$	-38 (-16 k _B T)
PPi + H ₂ O -> 2 phosphate	-24 (-10 k _B T)
phospho-ester (alcohol-acid) bond hydrolysis	
glucose 6-phosphate + H ₂ O -> glucose + phosphate	-12 (-5 k _B T)
3-phosphoserine + H ₂ O -> serine + phosphate	-10 (-4 k _B T)
AMP + H ₂ O -> adenosine + phosphate	-14 (-6 k _B T)
DHAP + H ₂ O -> dihydroxyacetone + phosphate	-15 (-6 k _B T)
fructose 1,6-bisphosphate + H ₂ O -> F6P + phosphate	-16 (-6 k _B T)
glyceraldehyde 3-phosphate + H ₂ O -> glyceraldehyde + phosphate	-17 (-7 k _B T)
threonine phosphate + H ₂ O -> threonine + phosphate	-19 (-8 k _B T)

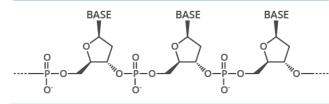
In RNA, the sugar is ribose.In DNA, the sugar is deoxyribose.



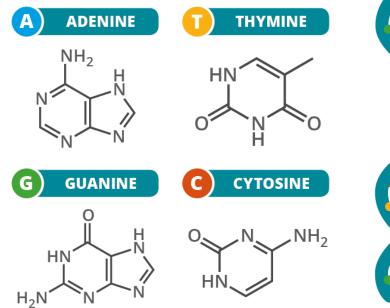
Adenosine 5'-monophosphate (AMP) (a ribonucleotide) Deoxycytidine 5'-monophosphate (dCMP) (a deoxyribonucleotide)

THE CHEMICAL STRUCTURE OF DNA





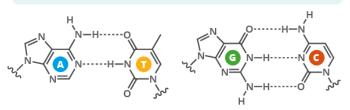
DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.





WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



FROM DNA TO PROTEINS

The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.



An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

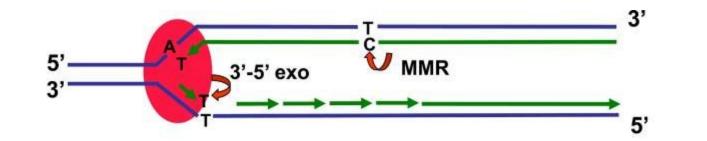


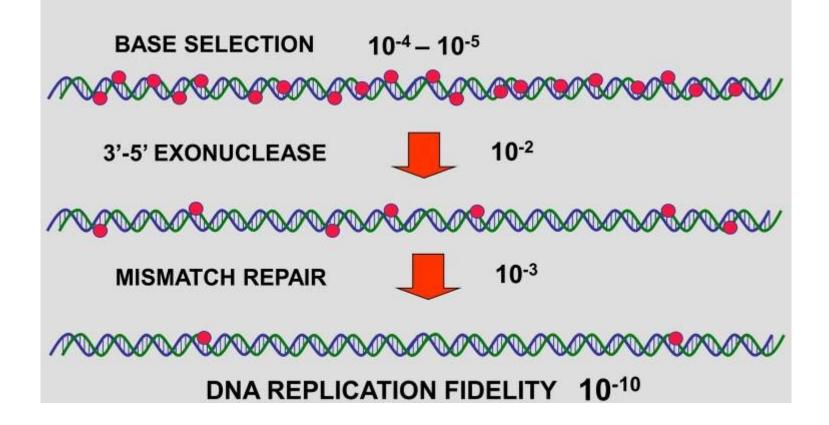
In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

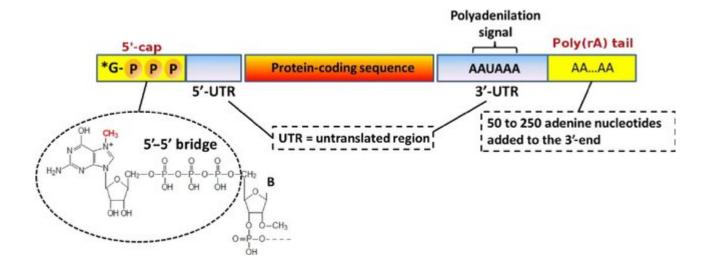


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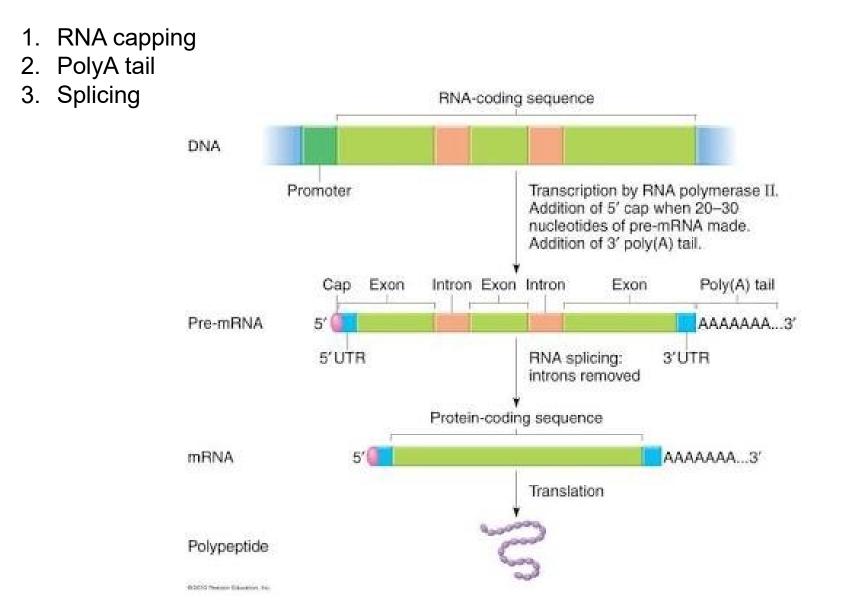


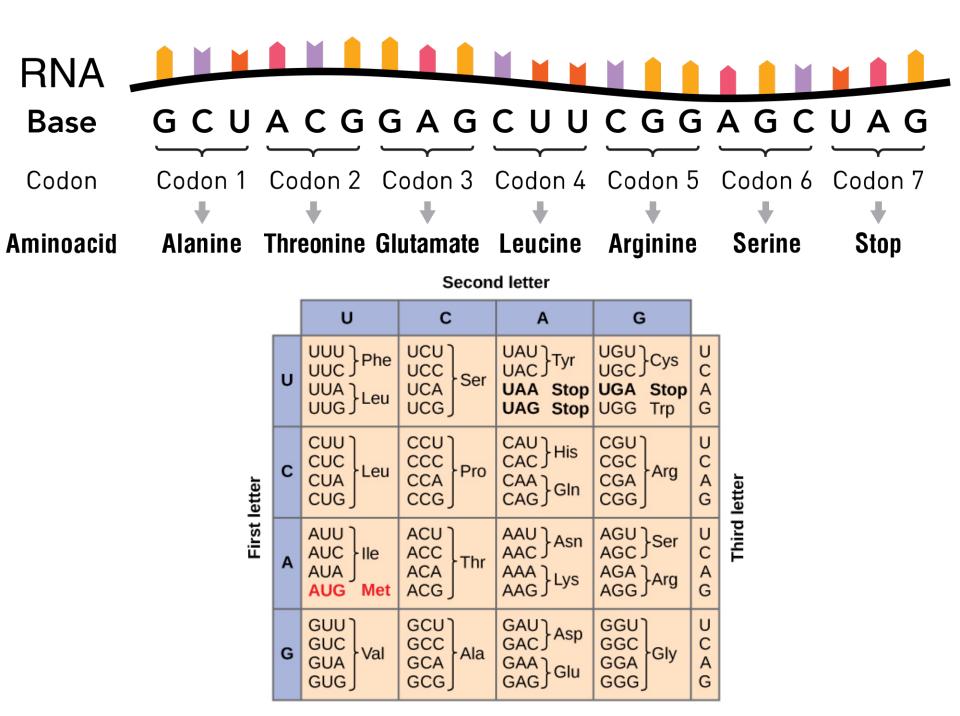


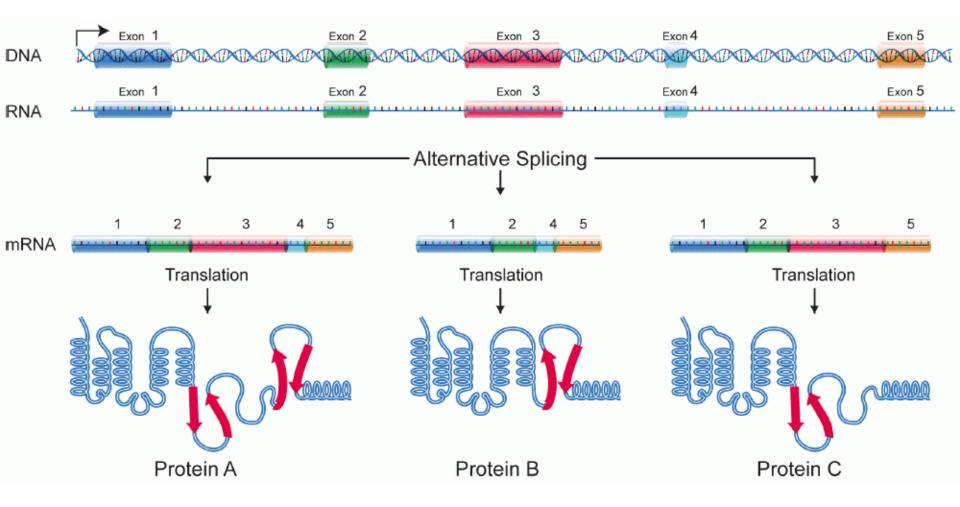


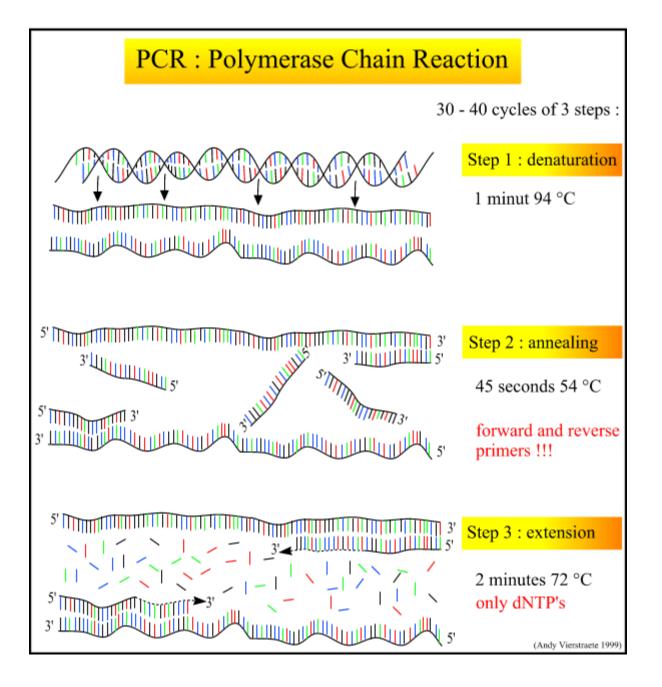


Post Transcription Modification of RNA





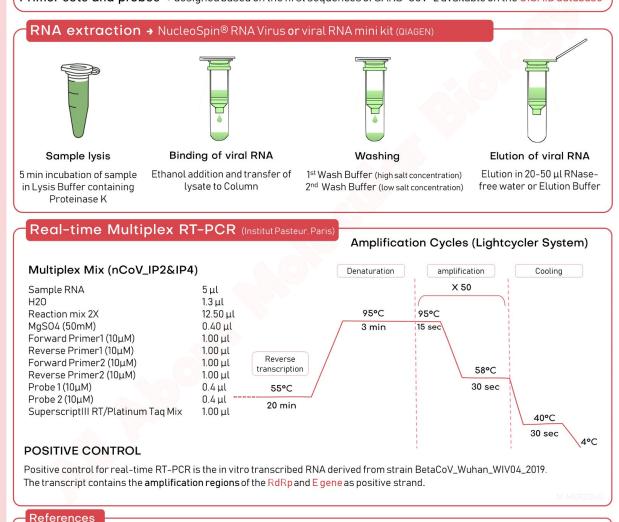




PROTOCOL OF SARS-COV-2 DETECTION USING REAL-TIME RT-PCR

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

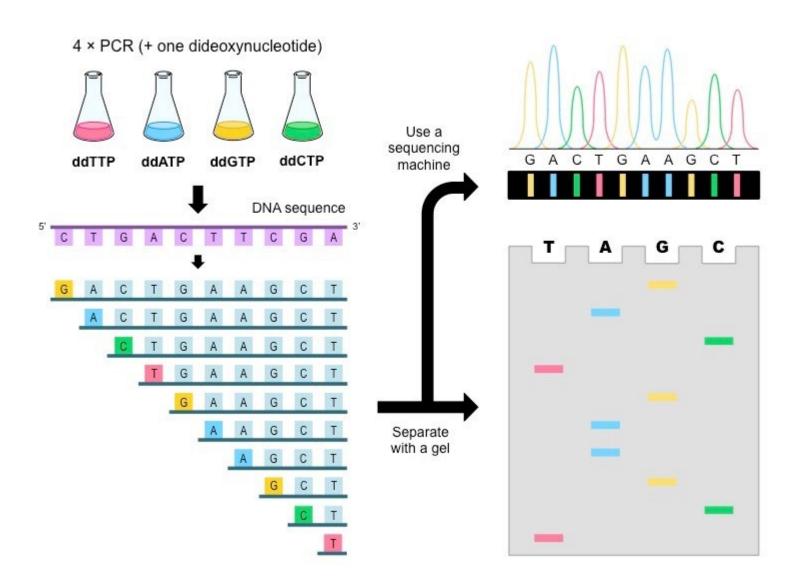
PCR amplification regions \rightarrow nCoV_IP2/12621-12727 and nCoV_IP4/14010-14116 (Institut Pasteur, Paris) Primer sets and probes \rightarrow designed based on the first sequences of SARS-CoV-2 available on the GISAID database



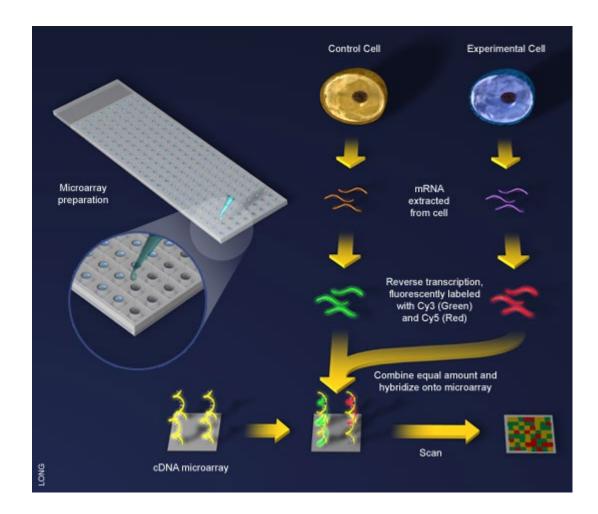
1. Institut Pasteur, Paris, « Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2 », OMS, 2 mars 2020

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.

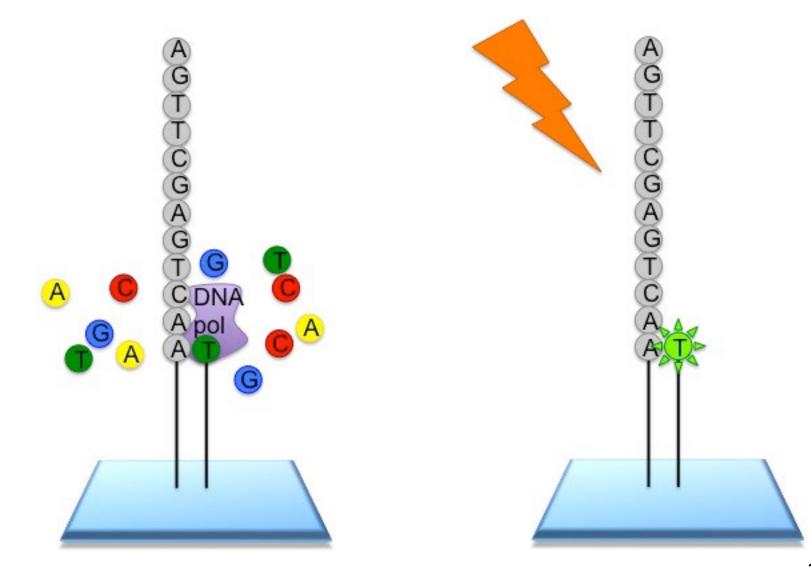
DNA Sequencing



cDNA Microarray



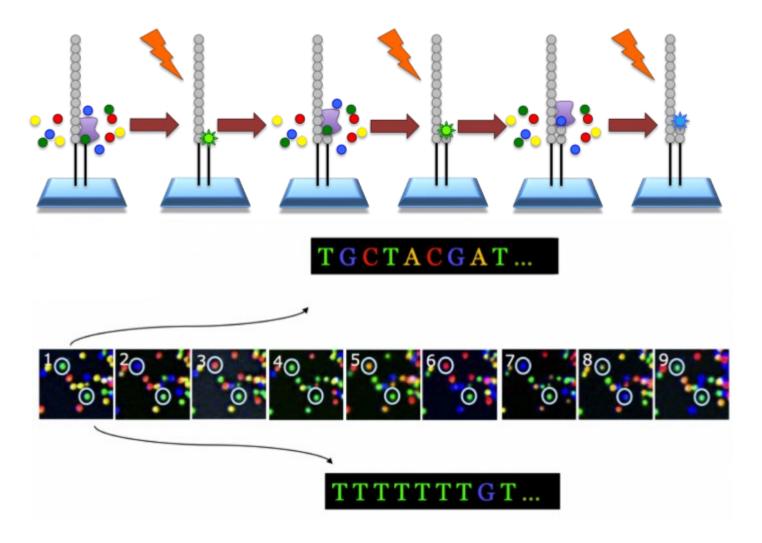
NGS Illumina

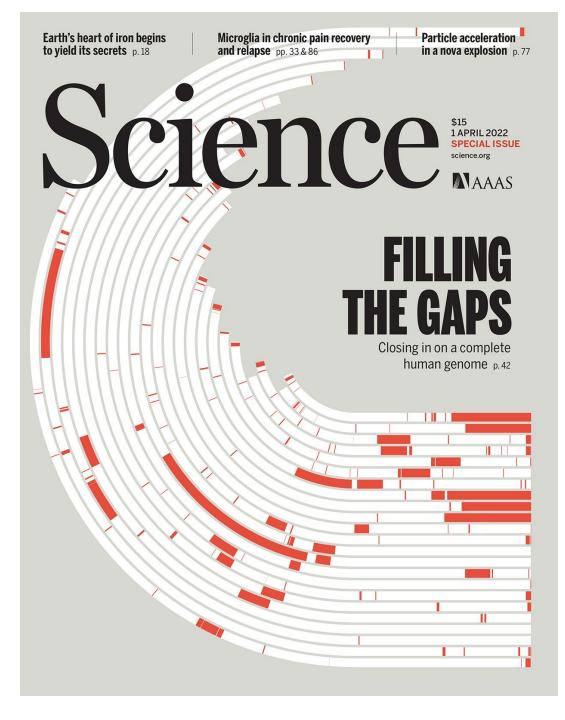


100-150 bp

NGS Illumina

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





The current version of the human genome reference assembly, GRCh38.p14 (GRCh38), has millions of bases represented by the letter "N," which means that the actual base residing at that location is unknown.

There are also 169 sequences that cannot confidently be ordered or oriented within the assembly, typically owing to their repetitive nature

Until recently, limitations of sequencing technology, primarily that the sequencers could read no more than about 1000 bases at a time,

The HGP opted for a more structured approach. This involved cloning genomic DNA into pieces that could be grown in bacteria (clones) and indexed in 96-well plates. Clones from these libraries were first mapped to chromosome regions, then individually sequenced.

A more complete reference

The new human genome assembly, T2T-CHM13 from the Telomere-to-Telomere Consortium, includes complex and repetitive regions of chromosomes that had not been included in previous versions of the human genome assembly (GRCh38). Although the Y chromosome remains to be completed, this new reference could be annotated with regulatory regions, variants, and sequence diversity to give a fuller picture of human genomic variation.



Chromosome 1 GRCh38 T2T-CHM13 Sample 1 Sample 2 Sample 3 This individual has This individual This individual has a sequence an extra copy of a is **missing** a sequence that is sequence that is that is not in in the reference. in the reference. the reference.

An important attribute of the human reference assembly is that the source DNA was derived from multiple individuals.

when two clones from different haplotypes of an individual are adjacent in the reference assembly, this can create sequence representations that are not normally found in the population

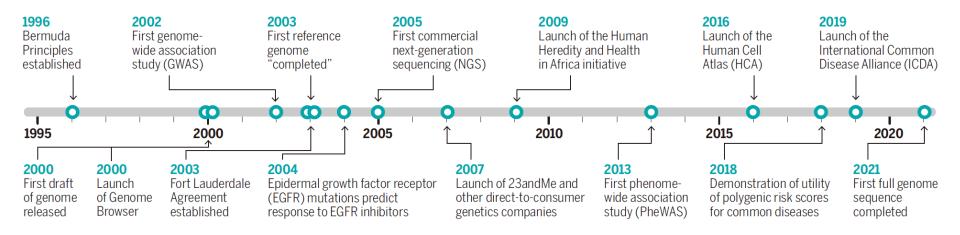
T2T Consortium, eliminated the problem of allelic diversity by sequencing the genome of a cell line derived from a complete hydatidiform mole (CHM).

This is duplicated so that the cell contains two copies of the same parental genome

Genome sequencing

February 2001 - Publication of the first draft of the human genome

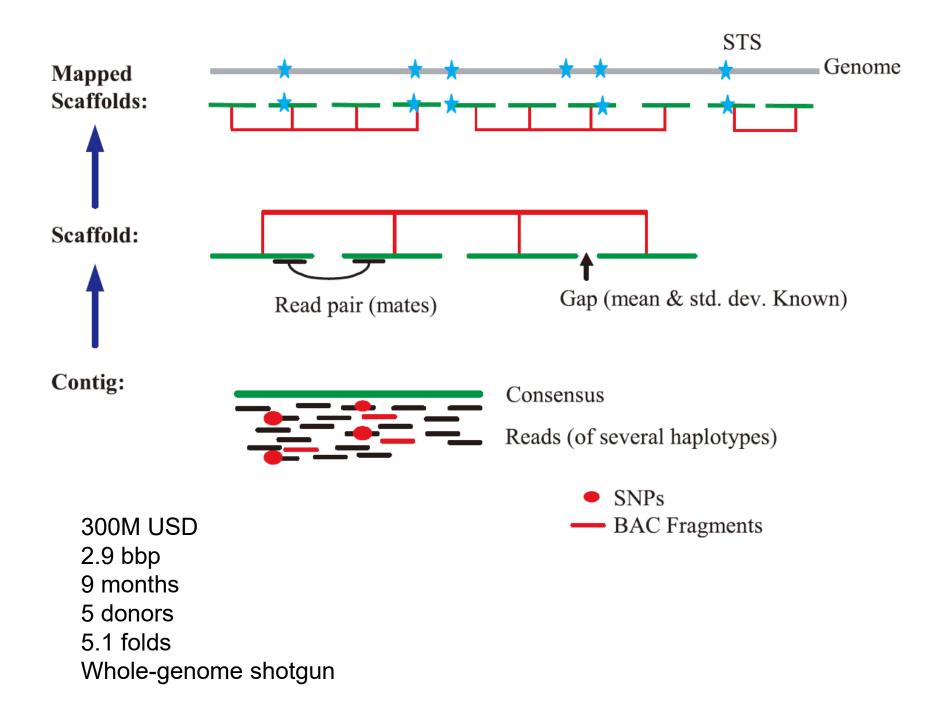




Bermuda Principles

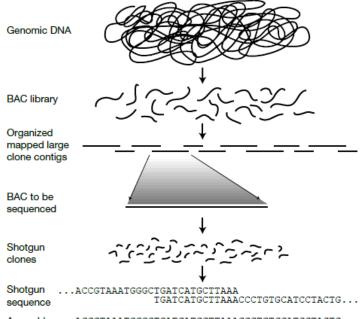
•Automatic release of sequence assemblies larger than 1 kb (preferably within 24 hours).

Immediate publication of finished annotated sequences.
Aim to make the entire sequence freely available in the public domain for both research and development in order to maximise benefits to society.



Human Genome Project

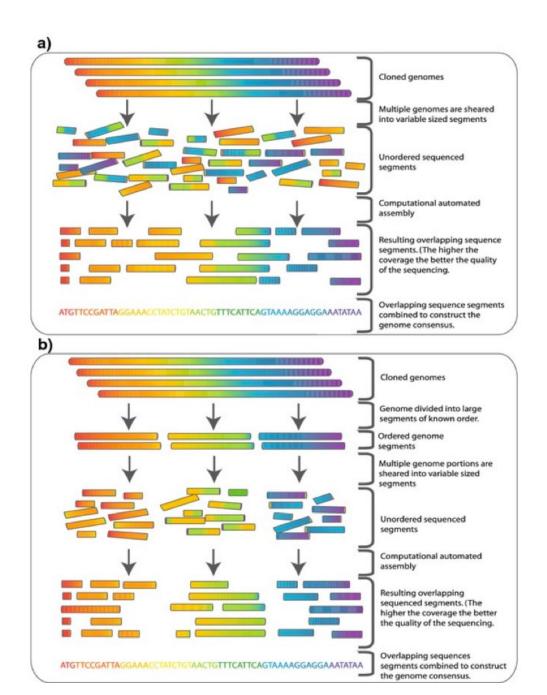


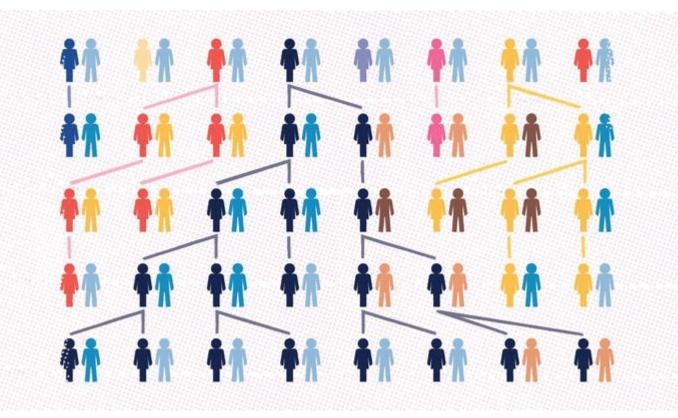






1990 15-year project 3B USD 20 groups 15 months





How are we all related to one woman/female ancestor?

The piece of Eve's DNA that has been traced comes from something called the mitochondria – it's in all human cells. There is DNA inside the mitochondria, and it's this DNA that has been traced back all the way to Eve - some 150-200,000 years ago, and is of African origin (sub-Sahara), probably from Ethiopia or Kenya.

How come our direct maternal lineage does not go back further than Eve?

The species Homo sapiens is older than 200,000 years but - this is the hard part – 'Eve' is dated back to Africa about 150 to 200 thousand years ago.

We know this because analysis of variation in living humans, plus the mutation rate in mitochondrial DNA, permits us to calculate when the ancestor of all living mitochondrial DNA chromosomes was alive.

Prior to 'Eve', there were actually still many females living, but all those lines (other than Eve's own direct ancestor) have died out. Some will have died after a hundred, some thousands, some tens of thousands of years.

Remember, for the lineage to survive to today, a female must have a fertile daughter, who in turn has a daughter over 8,000 generations.

The average mutation rate was estimated to be approximately 2.5 x 10(-8) mutations per nucleotide site or 175 mutations per diploid genome per generation. Rates of mutation for both transitions and transversions at CpG dinucleotides are one order of magnitude higher than mutation rates at other sites. Single nucleotide substitutions are 10 times more frequent than length mutations. Comparison of rates of evolution for X-linked and autosomal pseudogenes suggests that the male mutation rate is 4 times the female mutation rate, but provides no evidence for a reduction in mutation rate that is specific to the X chromosome.

What are "mutation markers" and how do they trace back to Eve through our DNA? A mutation is any change in the DNA sequence. These alterations or mutations occur by chance and are, therefore, random.

If a mutation arises on the Y chromosome, or on our mitochondrial DNA, and it does not kill us, it will be passed to the offspring.

- So these mutations can be traced back through the generations by analysing the Y chromosome ancestry of an individual's direct father's line, and therefore can only be performed on males.
- Mitochondrial DNA is the equivalent for tracking the direct maternal ancestry as it is passed through females. In this way, we can trace the direct maternal and paternal ancestries of males.
- When a new mutation occurs and 'branches off' from the line, it's called a branch. A mutation, which became known as the M branch, arose around 70,000 years ago and there have been very many further mutations of people belonging to the M branch over time, so that today there are many, many sub-branches within the ancient M branch.
- All these sub branches share the common M ancestor: a woman in who lived approximately 70 thousand years ago.

How we can trace Genghis Khan's DNA through the Y Chromosome?

Analysis of Y chromosomes in Asian men showed the spread of a Y lineage that can be explained by a historical fact – the spread of the Mongol empire under Genghis Khan and his descendants.

This particular Y lineage was found from the Caspian Sea to the Pacific and accounted for approximately 8% of the Y chromosomes in the region.

The time to the most recent common ancestor of these Y chromosomes was estimated to be approximately 1000 years and Mongolia was the clear source of the Y chromosome. This data is consistent with the hypothesis that they represent the Y chromosome of Genghis Khan, his immediate male relatives, and all their descendants. 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



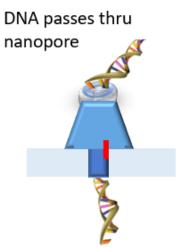
Raw output is fluorescent signal of the nucleotide incorporation, specific to each nucleotide

Intensity

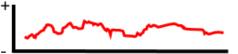
A,C,T,G have known pulse durations, which are used to infer methylated nucleotides

Т	С	G	Α	4mC
5		R		

Oxford Nanopore



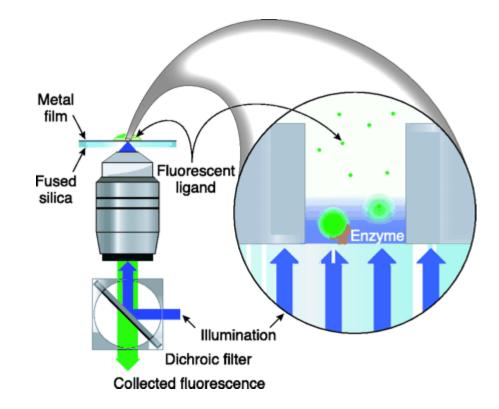
Raw output is electrical signal caused by nucleotide blocking ion flow in nanopore Each nucleotide has a specific electric "signature"



4mC А

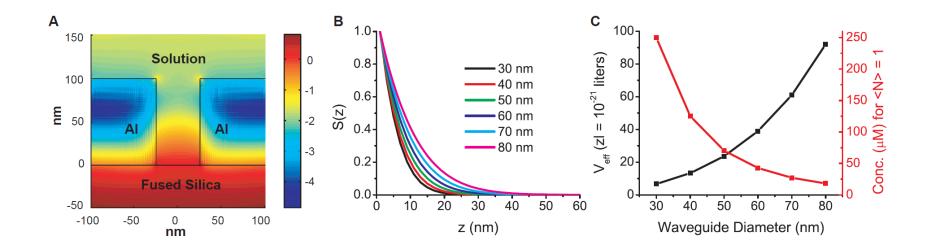
Zero Mode Waveguide

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

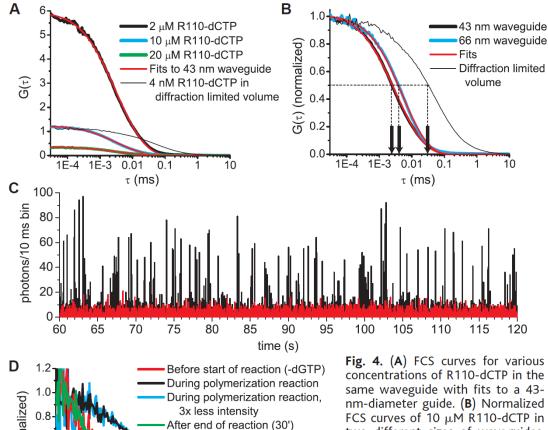


SCIENCE VOL 299 31 JANUARY 2003

Zero Mode Waveguide



50 x 50 x 10 nm³ = 2.5 x 10⁴ x 10⁻²¹ cc = 2.5 x 10⁻²⁰ L= 25 zeptoliter



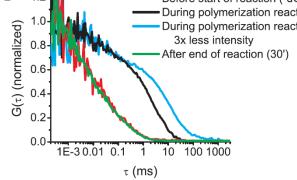
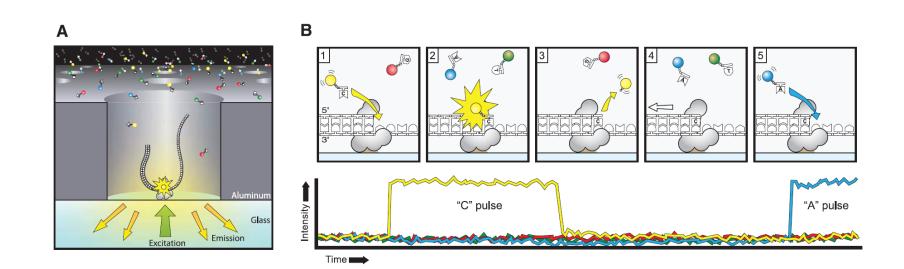


Fig. 4. (A) FCS curves for various concentrations of R110-dCTP in the same waveguide with fits to a 43nm-diameter guide. (B) Normalized FCS curves of 10 μ M R110-dCTP in two different sizes of waveguides. Arrows indicate the average residence times for molecules in the observation volumes. Curves from 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. Incorpora-

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

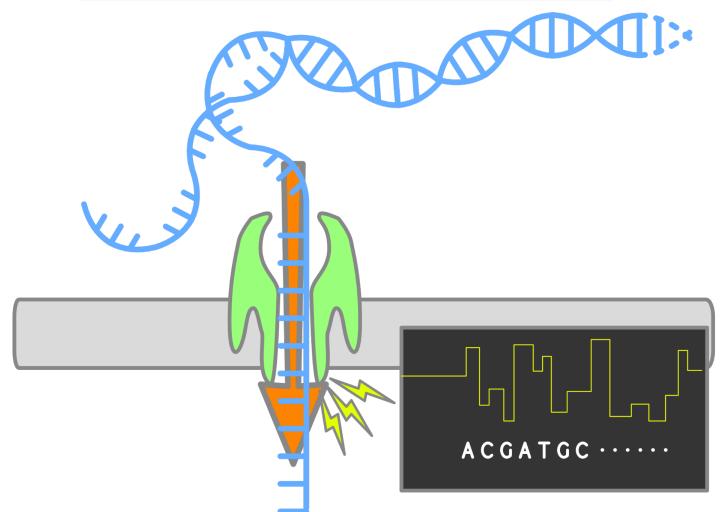


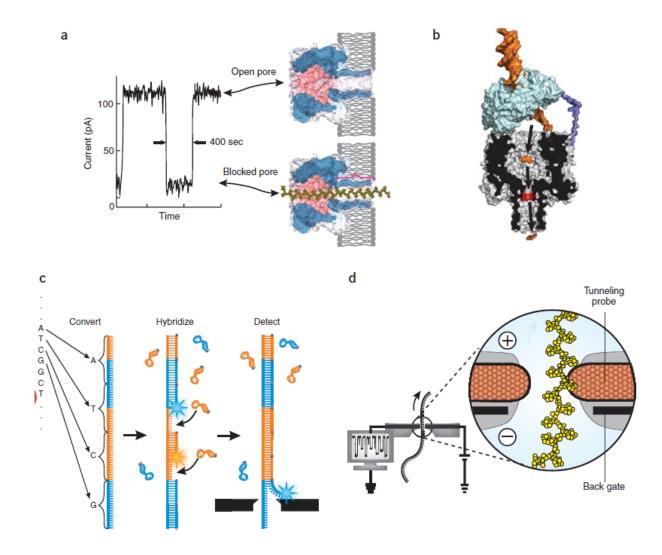
SCIENCE VOL 323 2 JANUARY 2009

Nanopore Sequencing

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

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





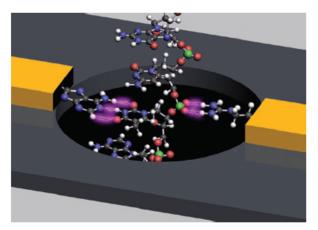
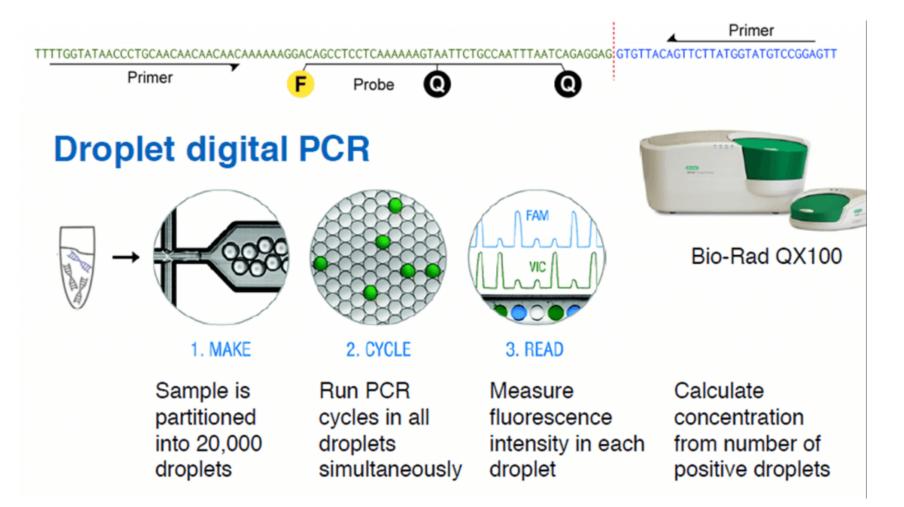
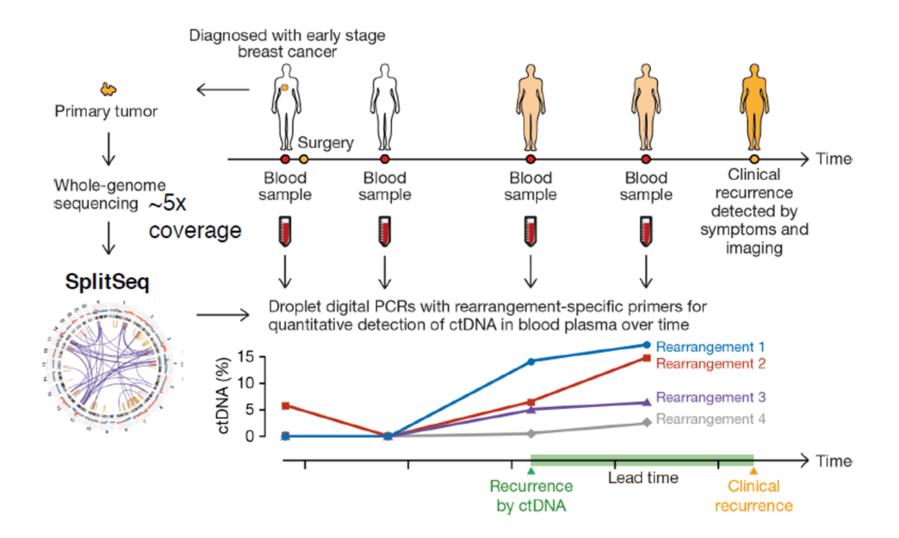


Figure 2 A nanopore reader with chemically functionalized probes. As a strand of DNA emerges from a nanopore, a 'phosphate grabber' on one functionalized electrode and a 'base reader' on the other electrode form hydrogen bonds (light blue ovals) to complete a transverse electrical circuit through each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

Digital PCR





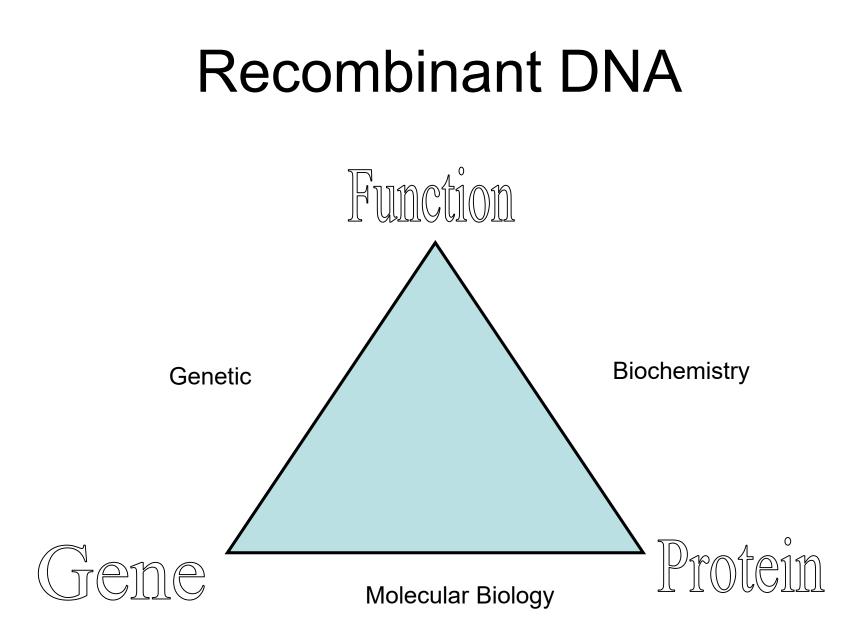
Cell Free DNA (cfDNA)

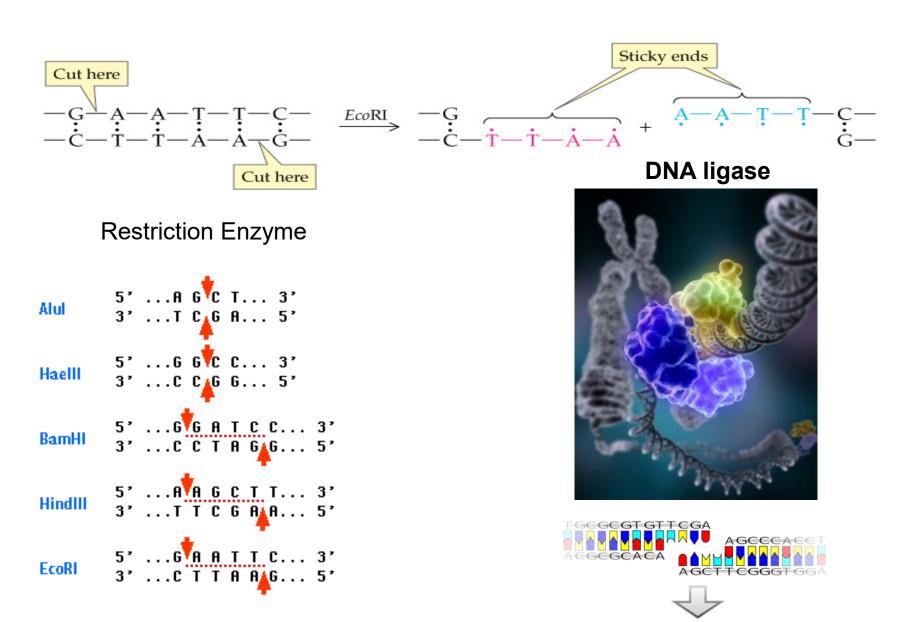
TABLE 2 Cell-free (cf)DNA concentrations and tumour response according to response evaluation criteria in solid tumours (RECIST) criteria

	Progressive disease	Stable disease	Partial response	p-value
Baseline concentration ng∙mL ⁻¹	23.88 (35.84)	32.83 (37.32)	26.79 (28.98)	0.358
Post-chemotherapy concentration ng⋅mL ⁻¹	24.16 (21.66)	28.61 (37.92)	30.72 (61.33)	0.358
Difference between post-chemotherapy and baseline concentration ng·mL ⁻¹	-0.22 (27.52)	-2.01 (28.63)	-0.56 (41.95)	0.473
Variation in concentration %	-0.01 (1.04)	-0.08 (0.92)	-0.02 (1.80)	0.402

Data are presented as median (interquartile range), unless otherwise stated.

0.01%-90% circulating tumor DNA (ctDNA)

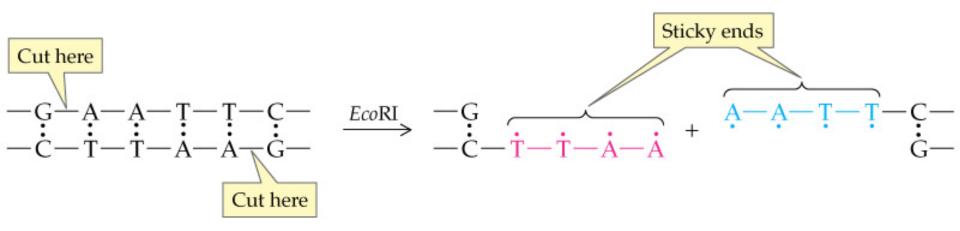


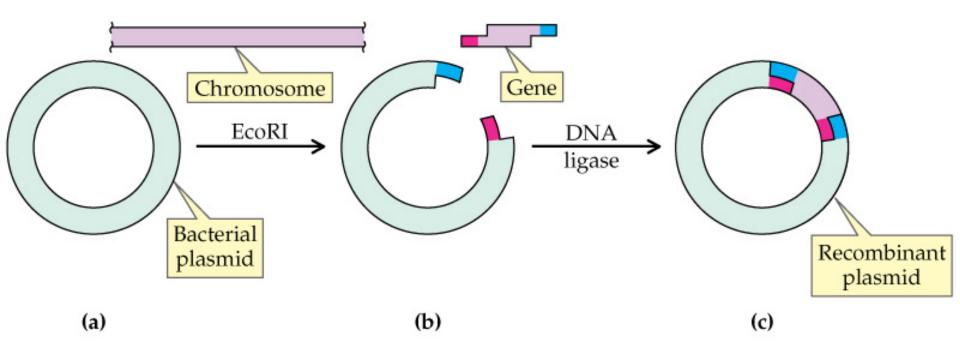


Alul and Haelli produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

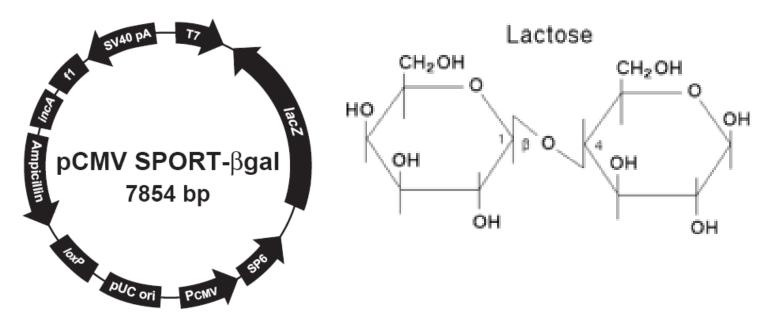






β-Galactosidase

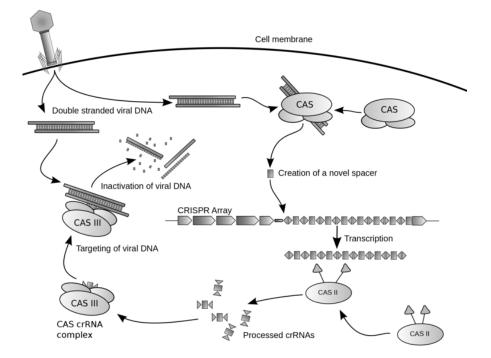
The enzyme that splits lactose into glucose and galactose. Coded by a gene (lacZ) in the lac operon of Escherichia coli.



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 β - galactosidase. Bacterial colonies in which β - 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.

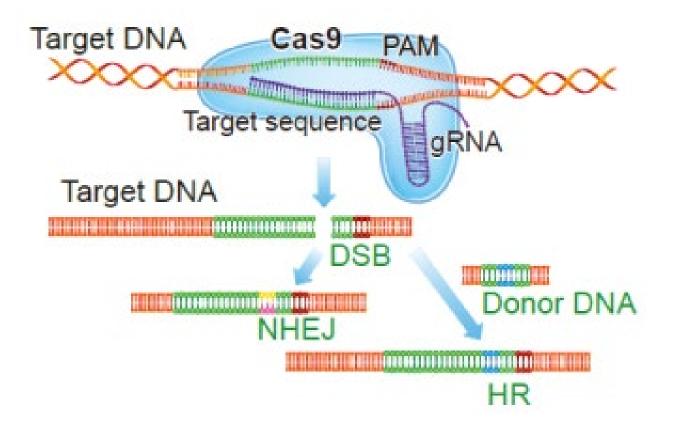
CRISPR CAS9

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



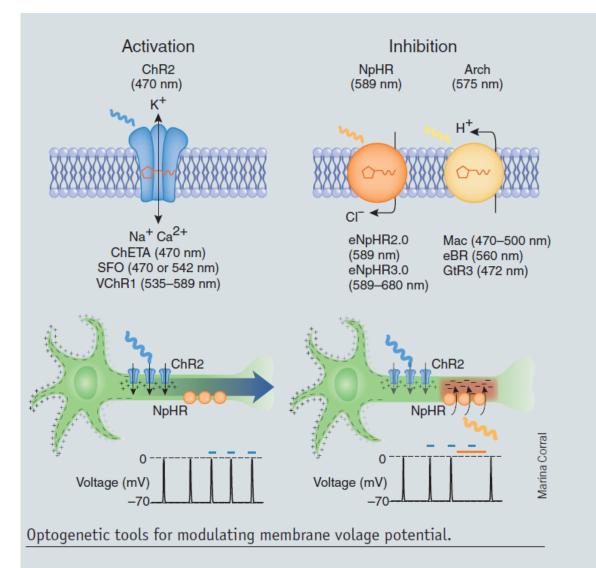
Movie

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



Optogenetics

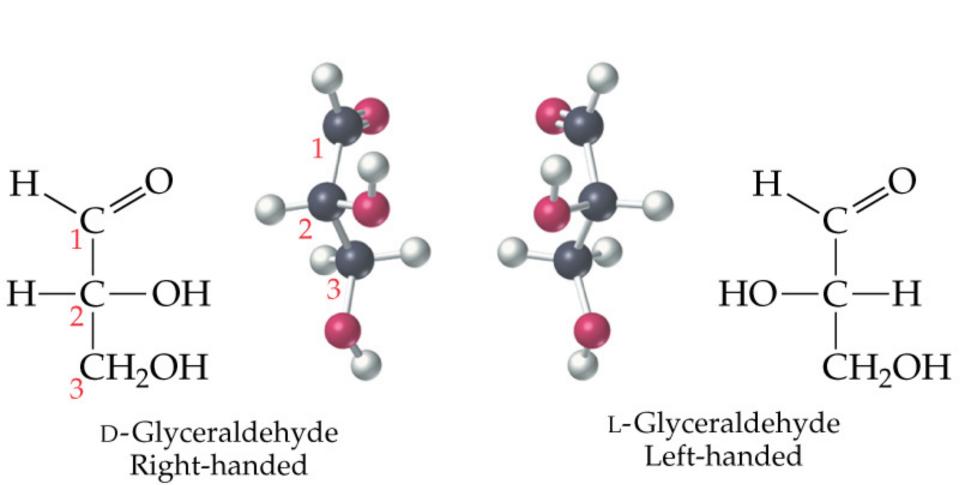
https://www.youtube.com/watch?v=I64X7vHSHOE

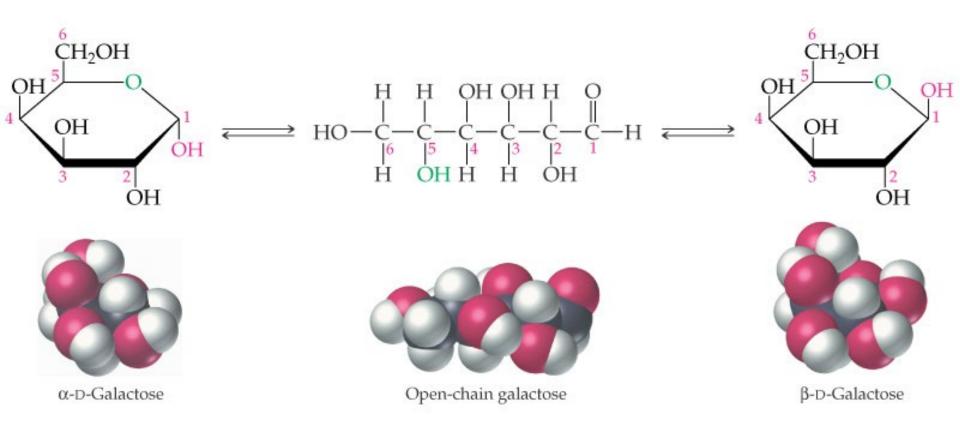


An Introduction to Carbohydrates

- 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.

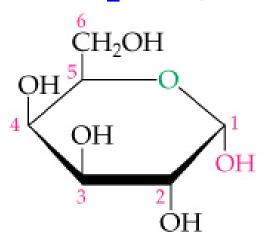
- The number of carbon atoms in an aldose or ketose may be specified as by tri, tetr, pent, hex, or hept. For example, glucose is aldohexose and fructose is ketohexose.
- Monosaccharides react with each other to form disaccharides and polysaccharides.
- Monosaccharides are chiral molecules and exist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



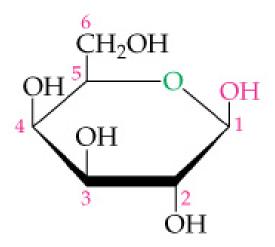




• Anomers: Cyclic sugars that differs only in positions of substituents at the hemiacetal carbon; the α -form has the –OH group on the opposite side from the –CH₂OH; the β -form the –OH group on the same side as the –CH₂OH group.



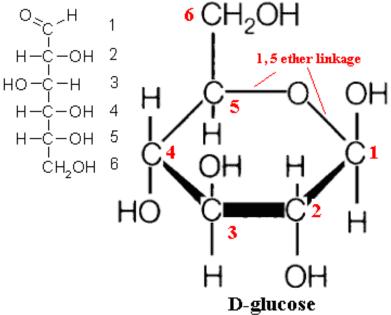
 α -D-Galactose



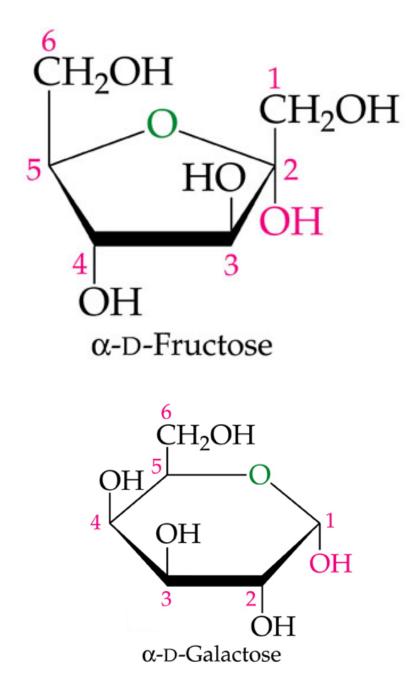
β-D-Galactose

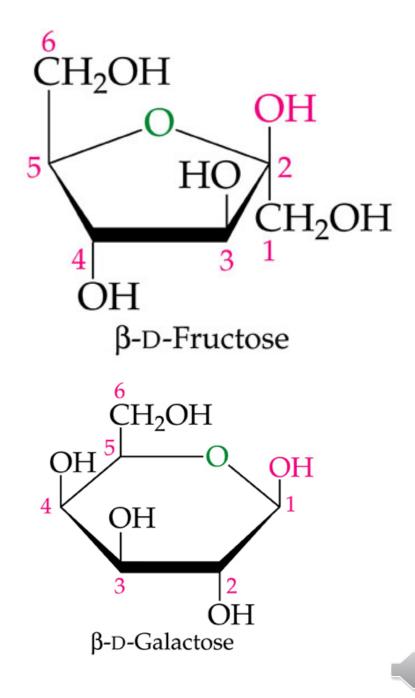
Some Important Monosaccharides

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.

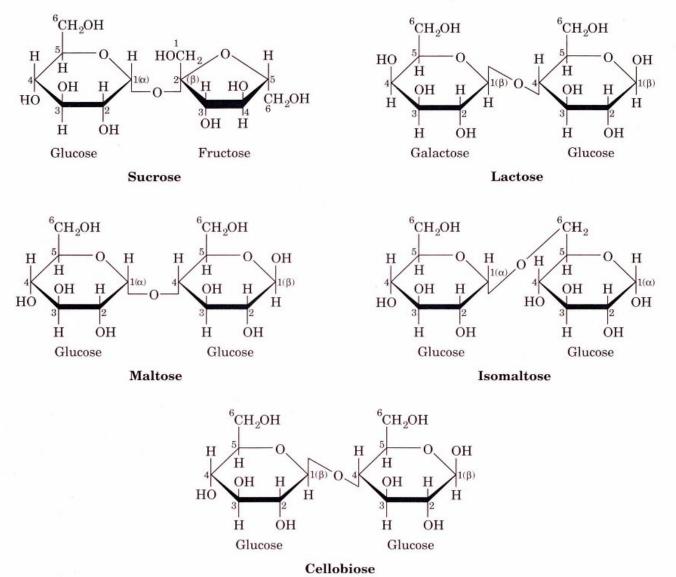






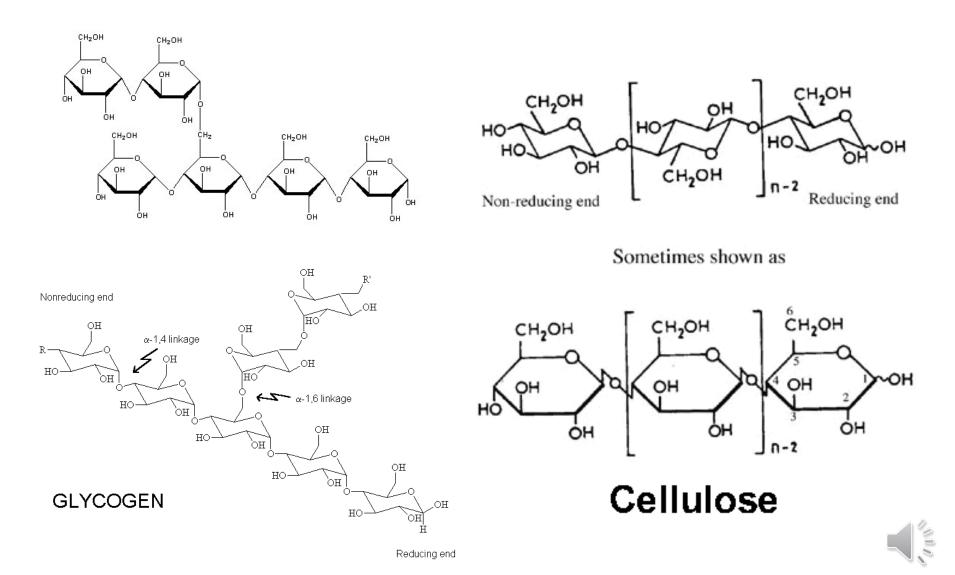


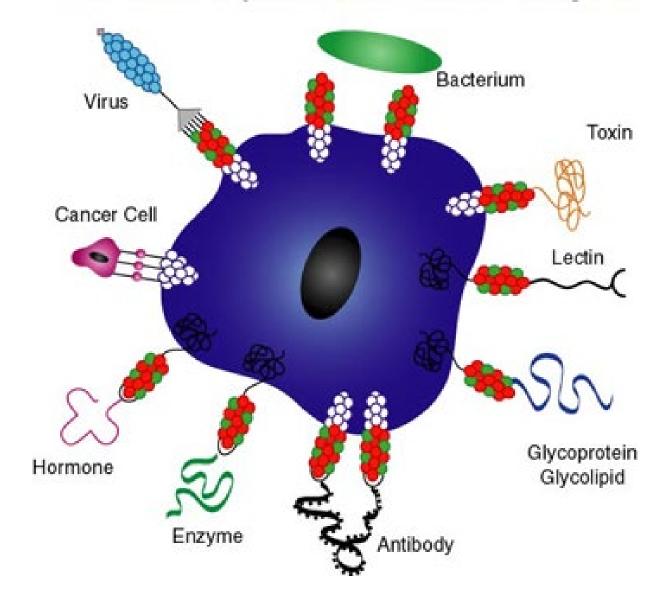
Some Common Disaccharides





Polysaccharides



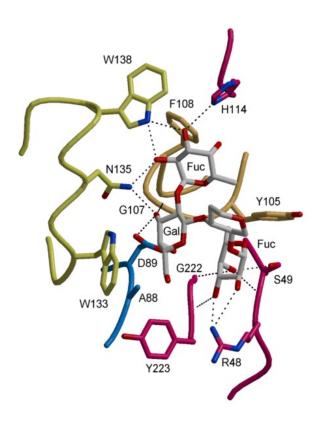


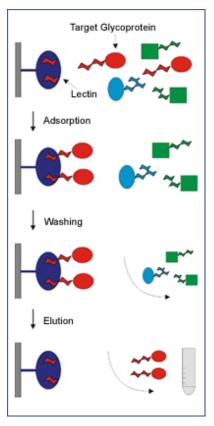
Cell-Surface Carbohydrates Involved in Molecular Recognition



Lectin

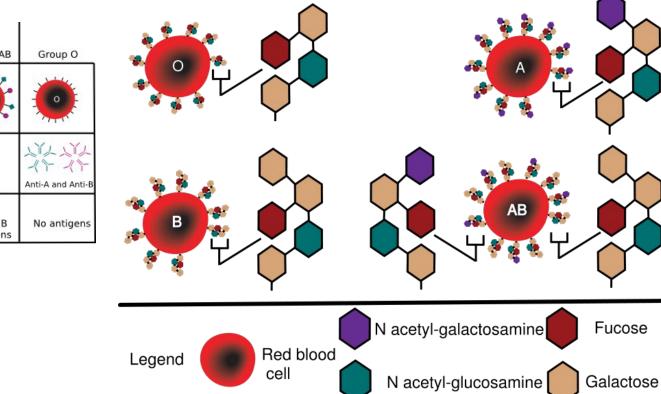
Lectins are sugar-binding proteins which are highly specific for their sugar moieties. They typically play a role in biological recognition phenomena involving cells and proteins. For example, some bacteria use lectins to attach themselves to the cells of the host organism during infection.

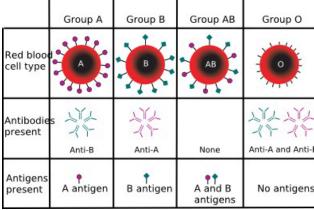






Blood Type



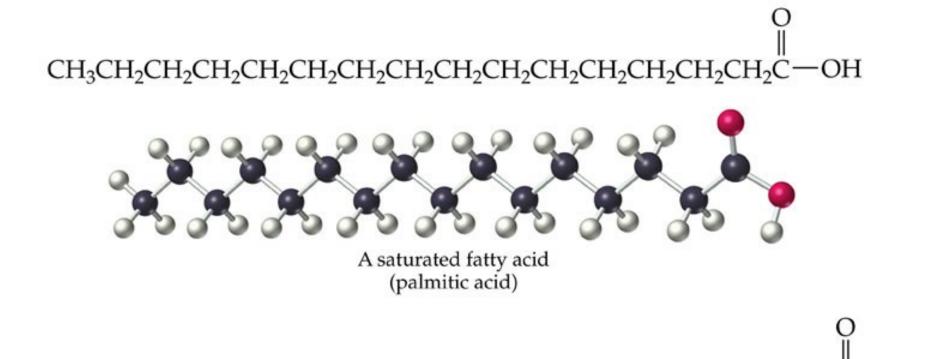


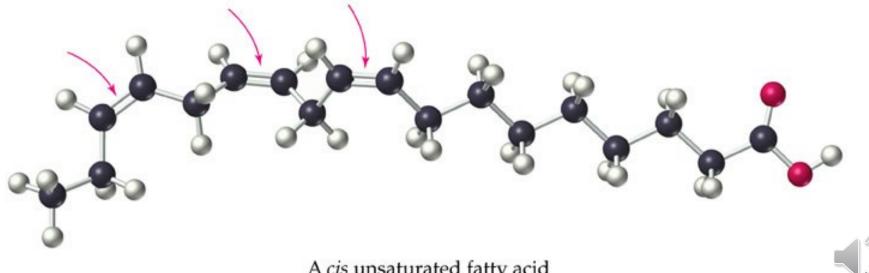


Lipid

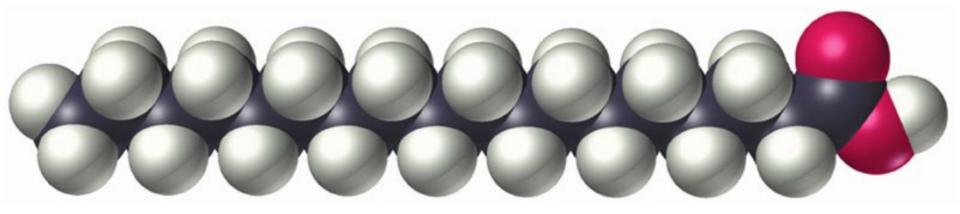
- Lipids are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents.
- Lipid molecules contain large hydrocarbon portion and not many polar functional group, which accounts for their solubility behavior.





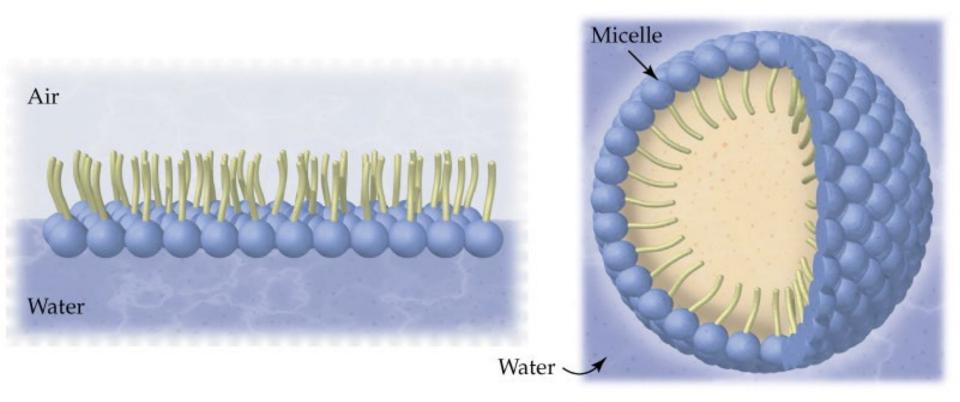


A cis unsaturated fatty acid (linolenic acid)

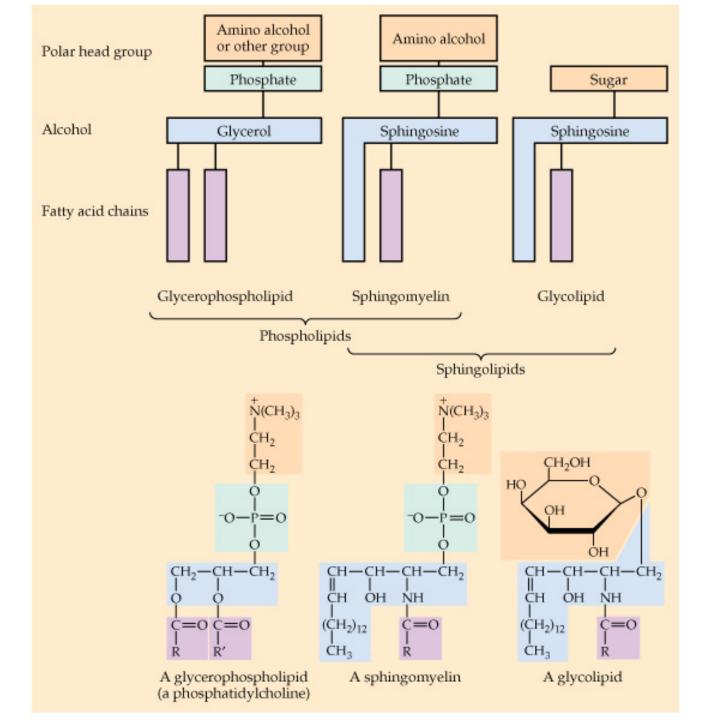


Stearic acid, an 18-carbon saturated fatty acid

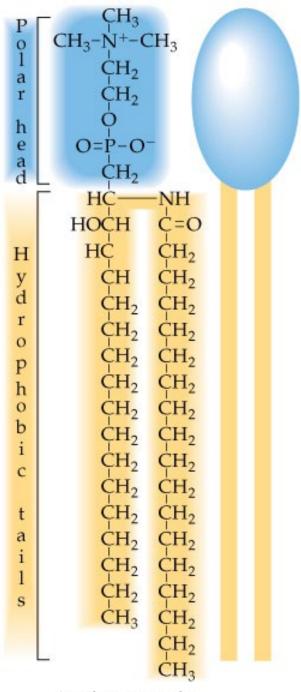






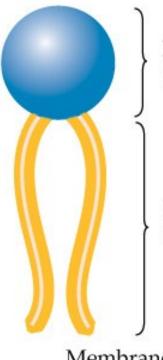






A sphingomyelin

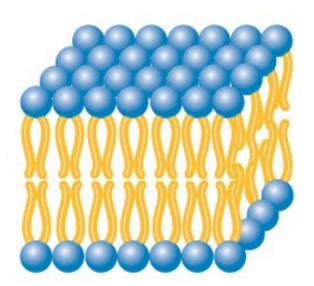




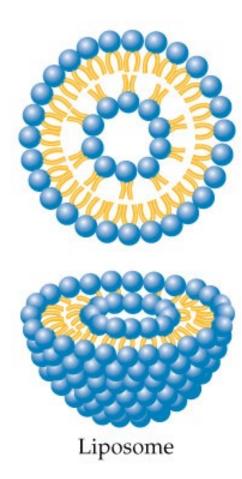
Polar head (hydrophilic)

Nonpolar tail (hydrophobic)

Membrane lipid



Lipid bilayer

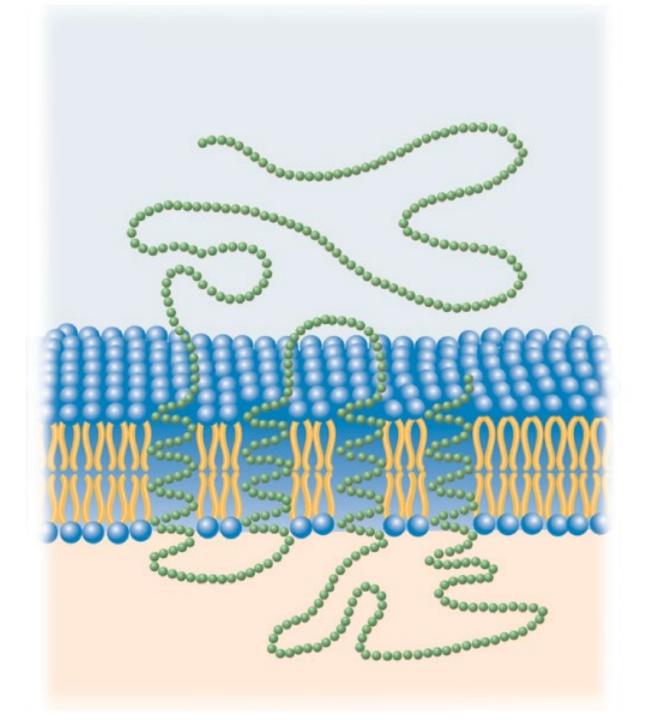




Properties of cell membranes:

- 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.
- Na⁺, 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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EXTRACELLULAR FLUID

