INTRODUCTION TO DNA SEQUENCING

(sanger & next generation)

24th June, 2021

Dr. Obishakin Emmanuel Tumininu (DVM, PhD)

Head, Biotech center, NVRI, Vom

AFRICA CENTRE OF EXCELLENCE IN PHYTOMEDICINE RESEARCH

Goals of this lecture

1. Overview of DNA, RNA and PCR basics
2. To understand the principles of sequencing
3. Types of sequencing and their advantages
4. Introduction to sequence analysis

Review notes on the nucleic acids

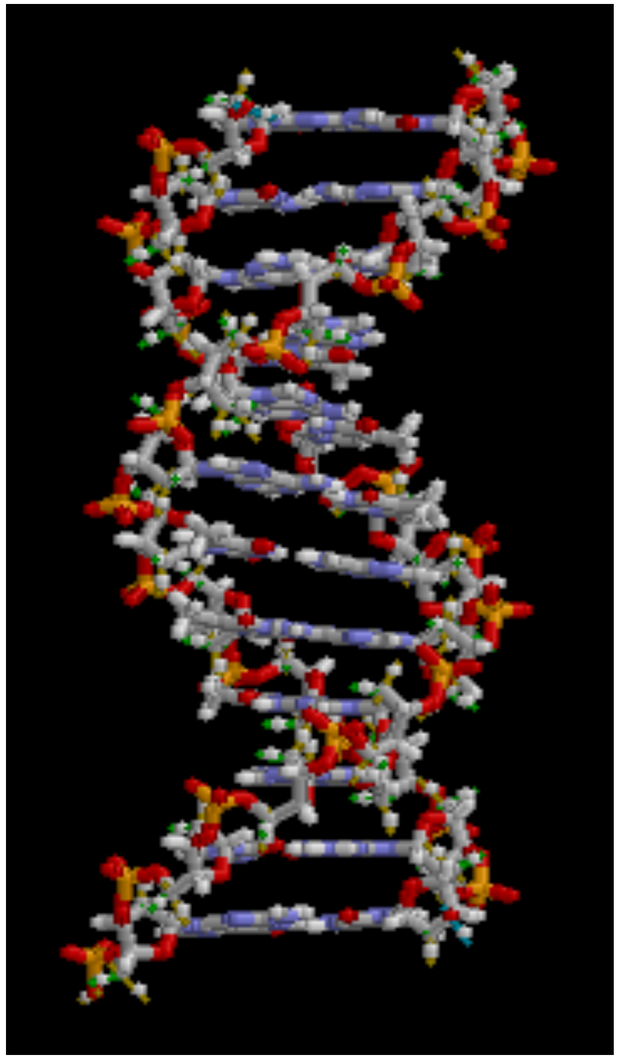
1. The Deoxyribonucleic acid

2. The Ribonucleic acid

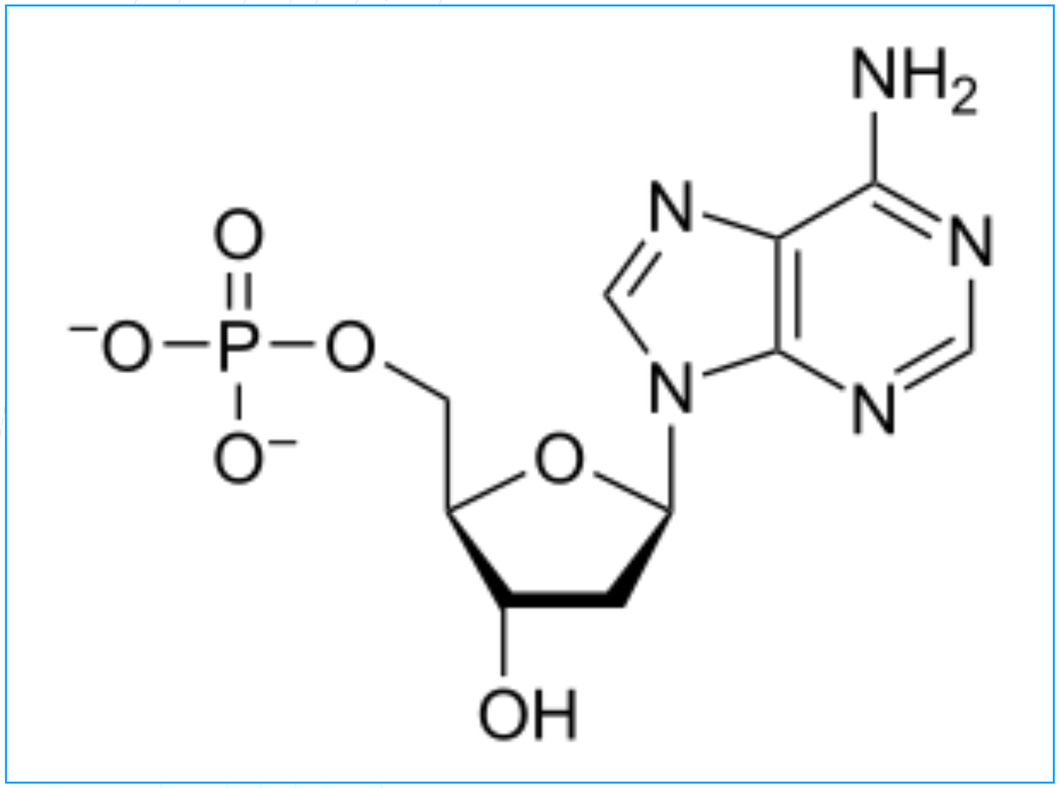
What is DNA

- **Deoxyribonucleic acid** (**DNA**) is a complex molecule
- composed of two polynucleotide chains
- The chains coil around each other to form a double helix
- It contains genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses.

What is DNA



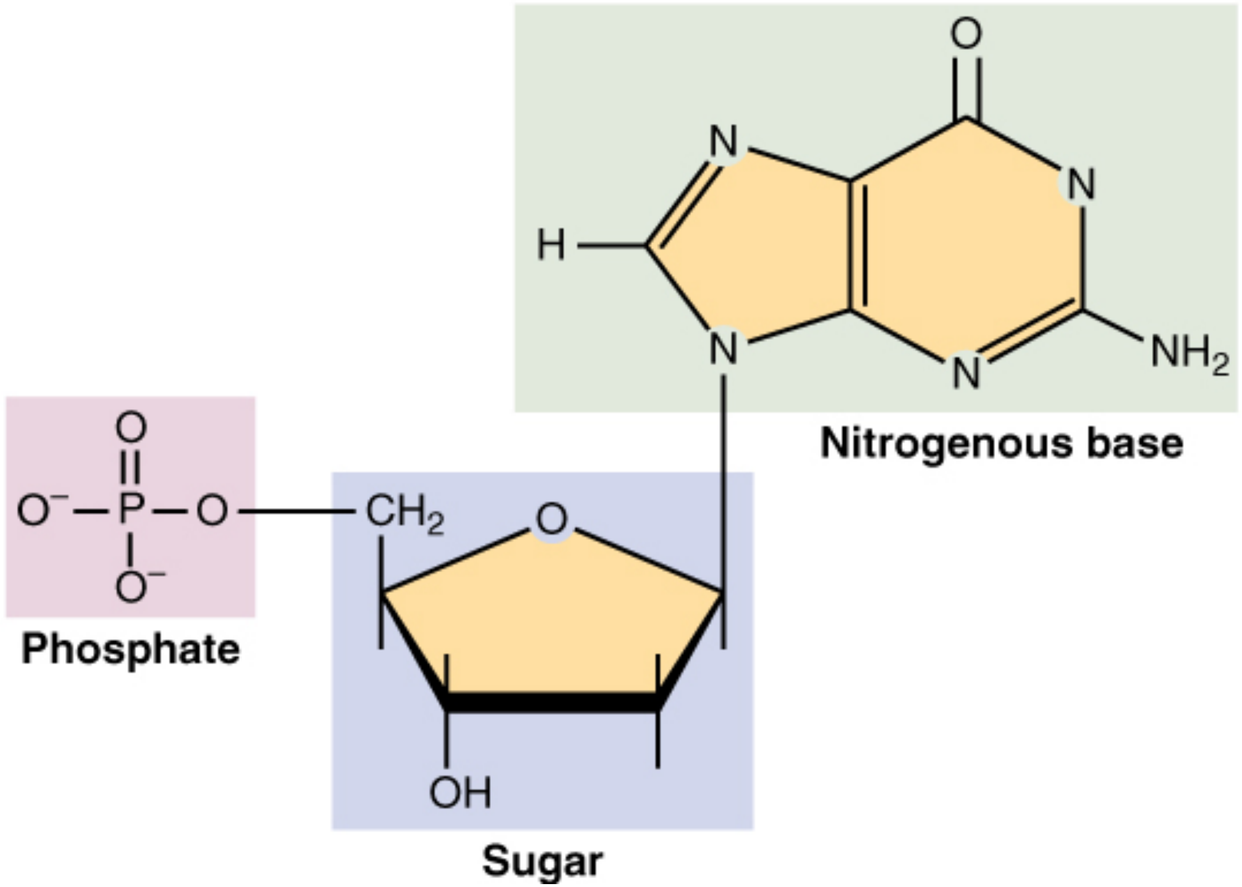
Components of DNA



- Monomeric units called **Nucleotides**

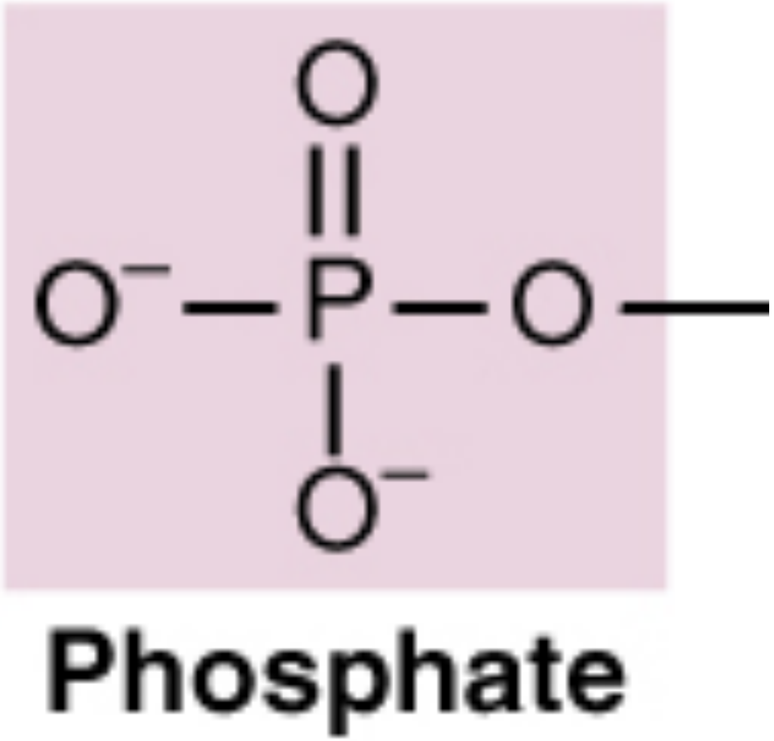
Components of a nucleotide

- A phosphate group
- A deoxyribose sugar
- A nitrogenous base



Phosphate group

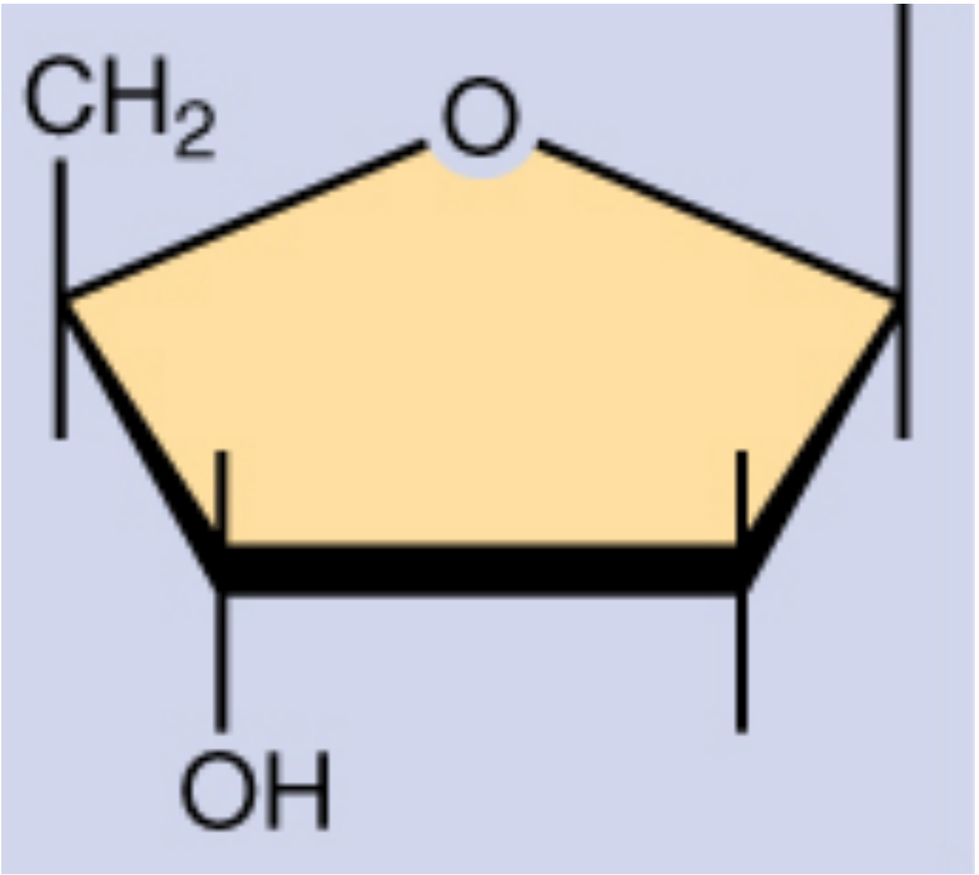
- Strongly negatively charged (anion)
- Gives the DNA its overall negative charge
- Makes the DNA acidic
- Phosphorus “expanded octet”
- can make 5 covalent bonds



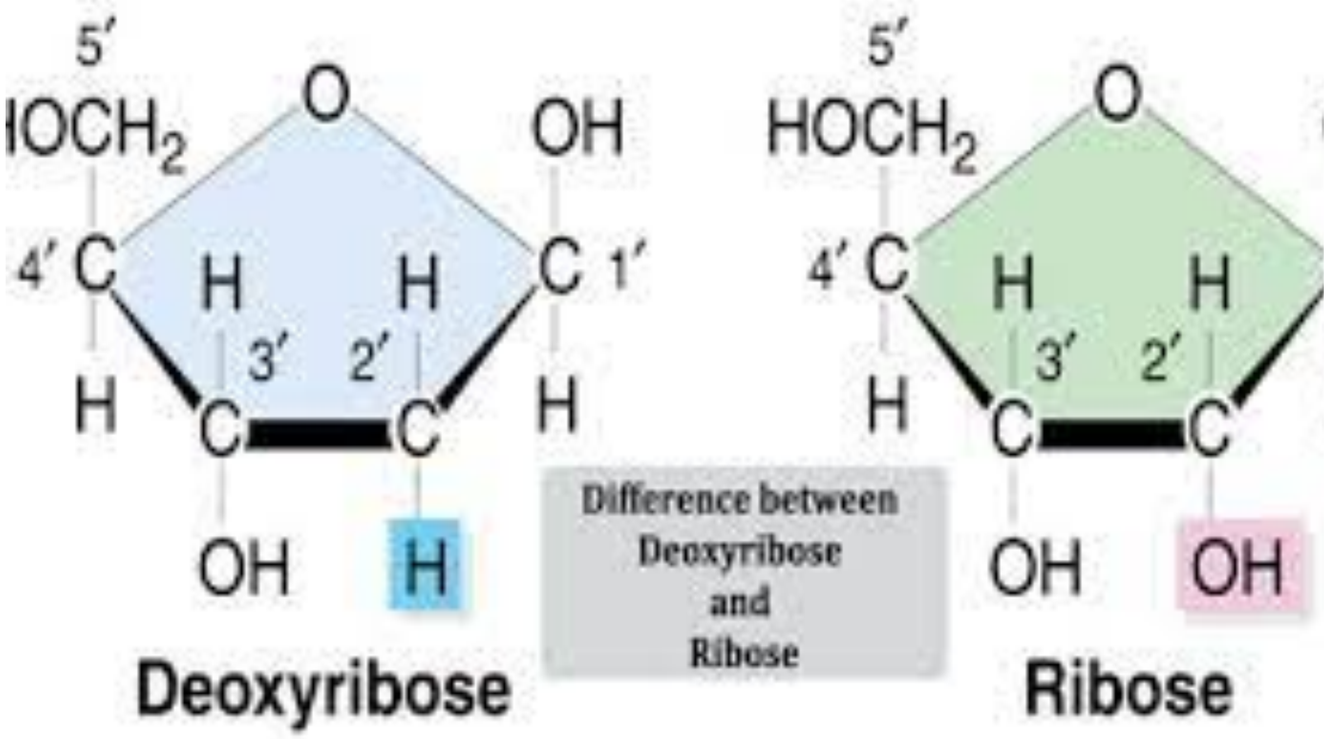
Phosphate

Components of a nucleotide

- A ribose monosaccharide
- 5 carbon
- 2-deoxyribose sugar
- Ribose with 1 less oxygen

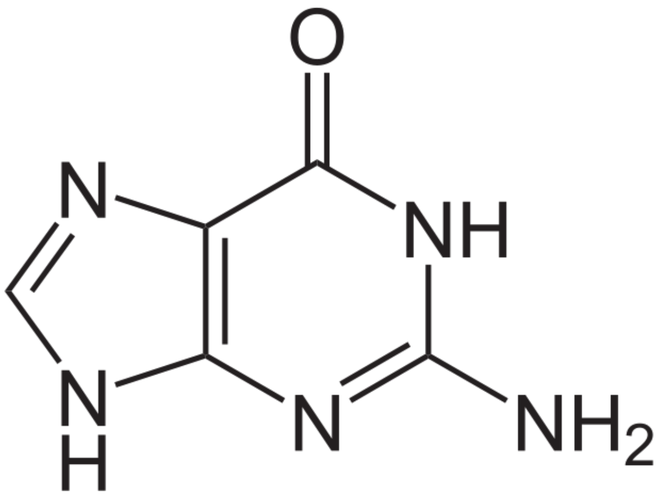


Ribose vs Deoxyribose

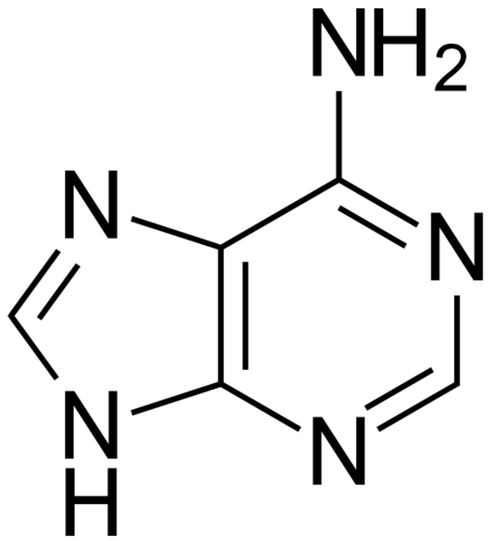


- A ribose monosaccharide
- 5 carbon
- 2-deoxyribose sugar
- Ribose with 1 less oxygen

Nitrogenous bases

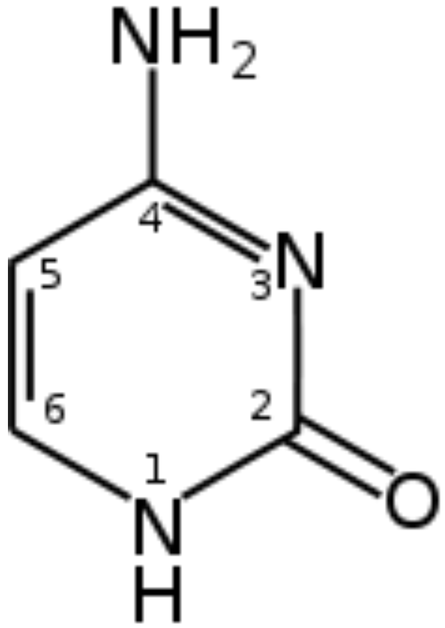


Guanine

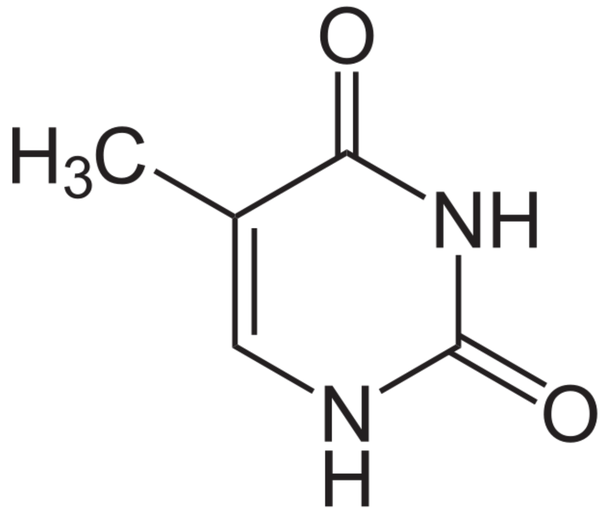


Adenine

Purines



Cytosine

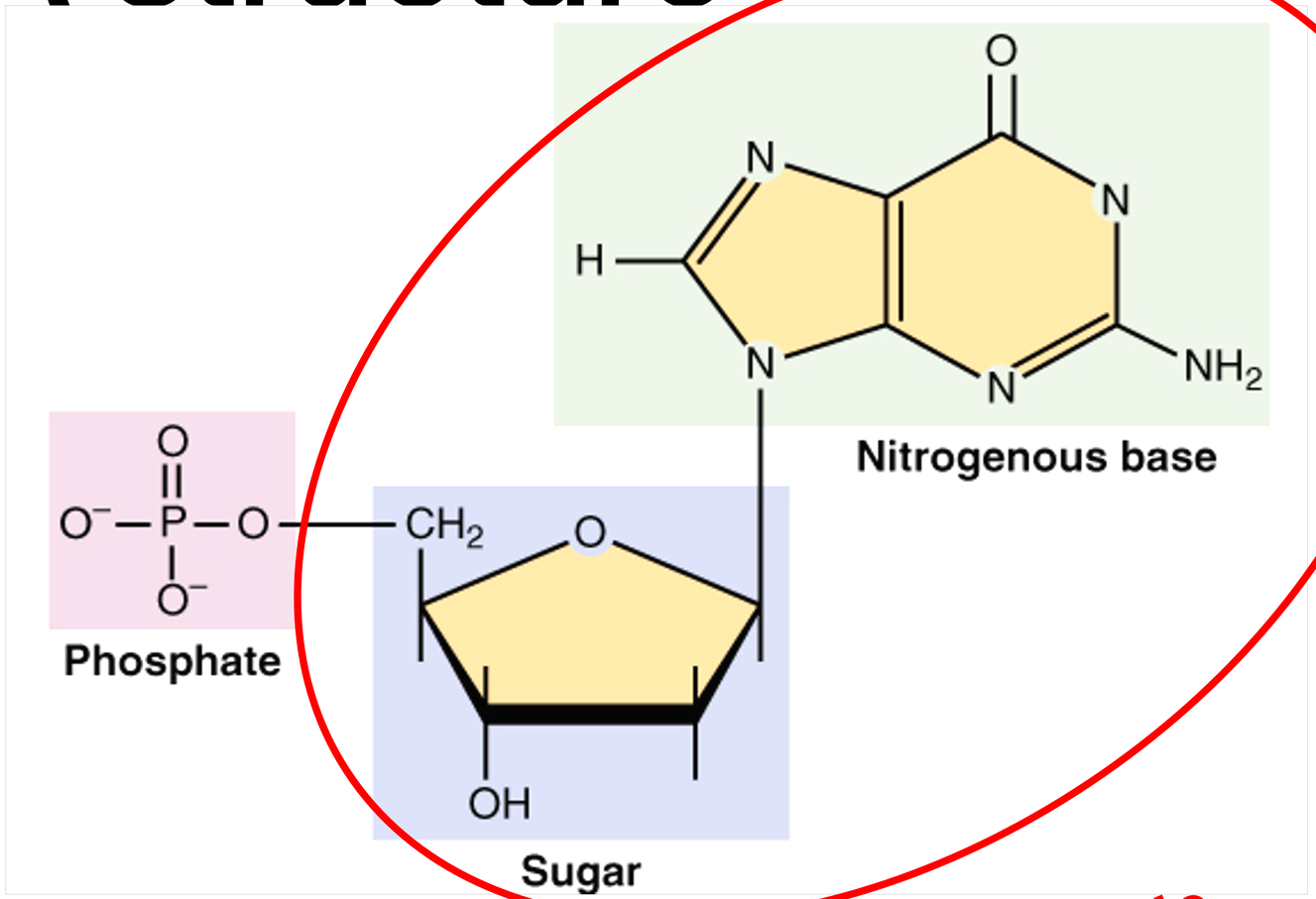


Thymine

Pyrimidines

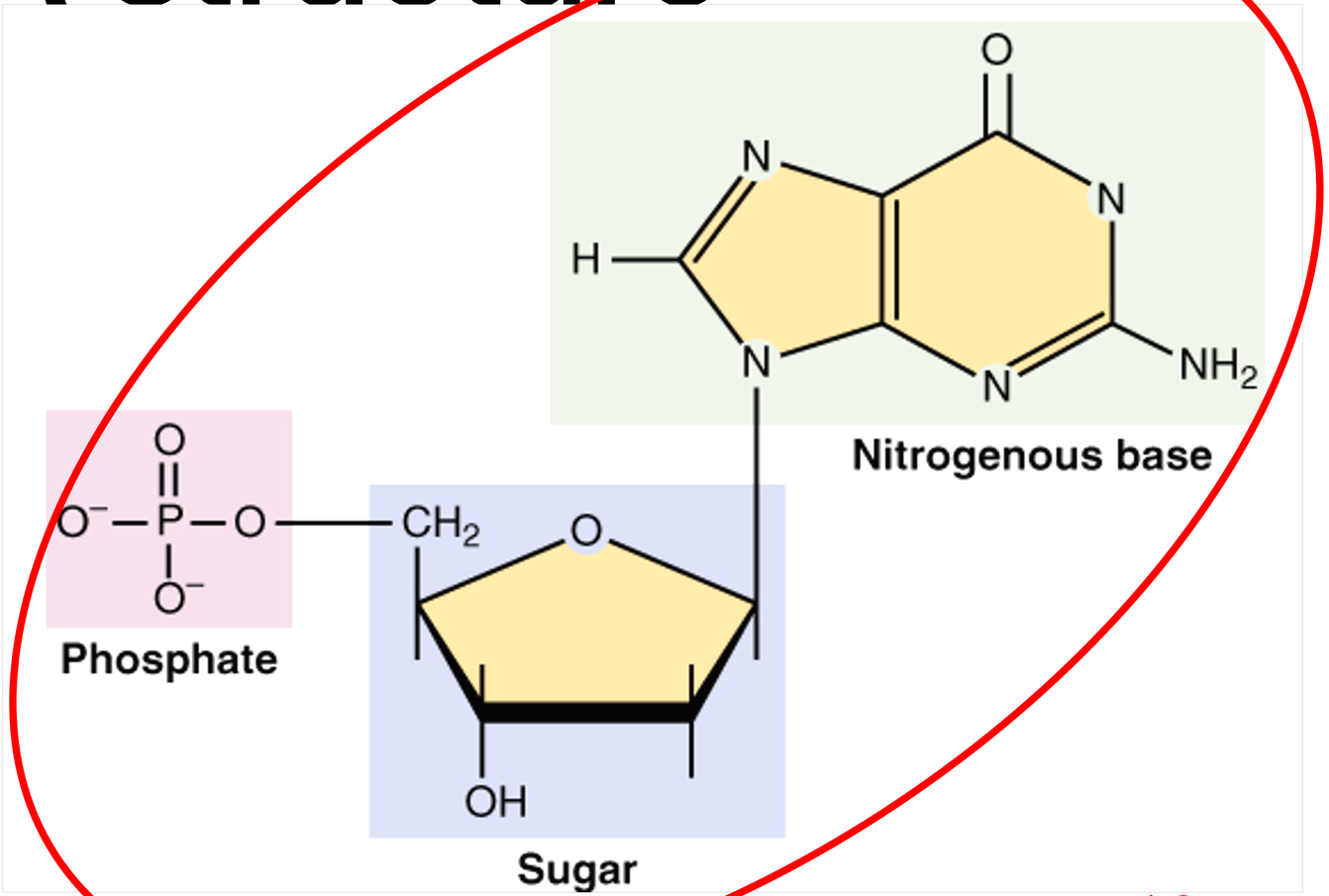
DNA structure

- A phosphate group
- A deoxyribose sugar
- A nitrogenous base
- Sugar +base=nucleoside
- Sugar+ base + phosphate= nucleotide

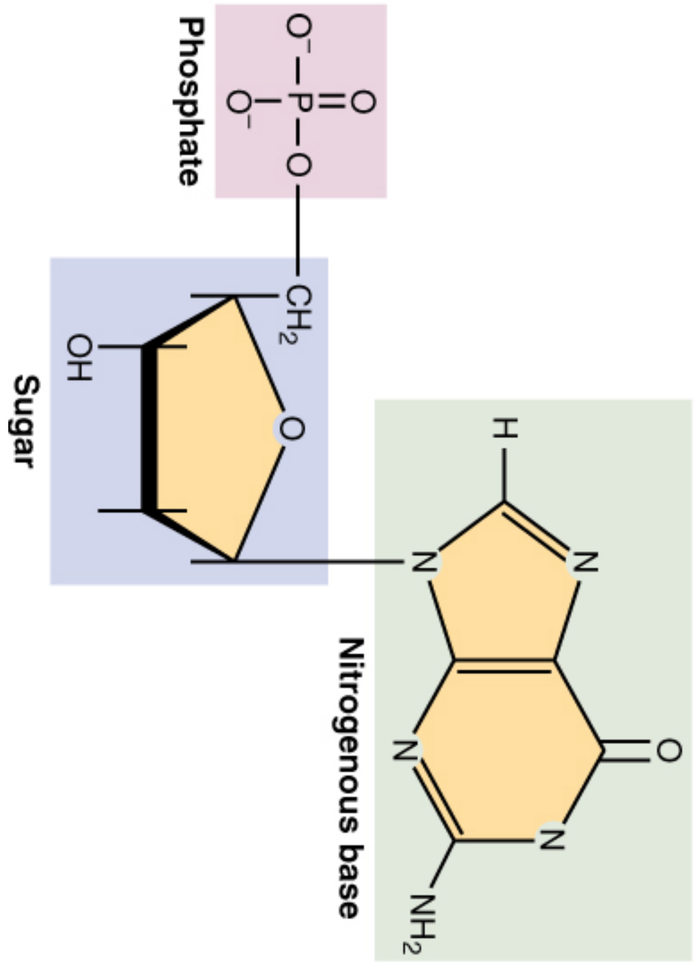


DNA structure

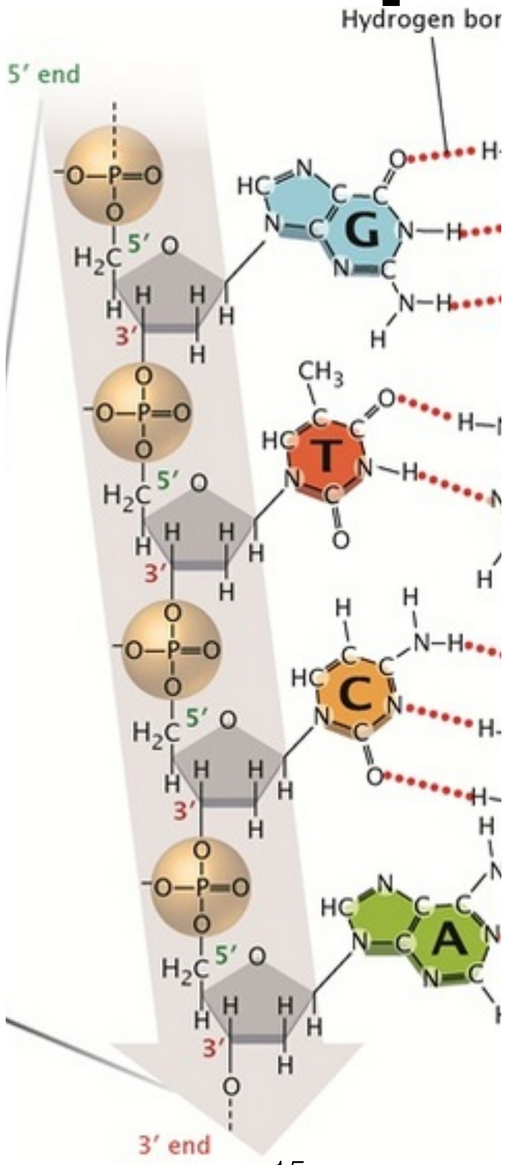
- A phosphate group
- A deoxyribose sugar
- A nitrogenous base
- Sugar +base=nucleoside
- Sugar+ base + phosphate= nucleotide



DNA structure

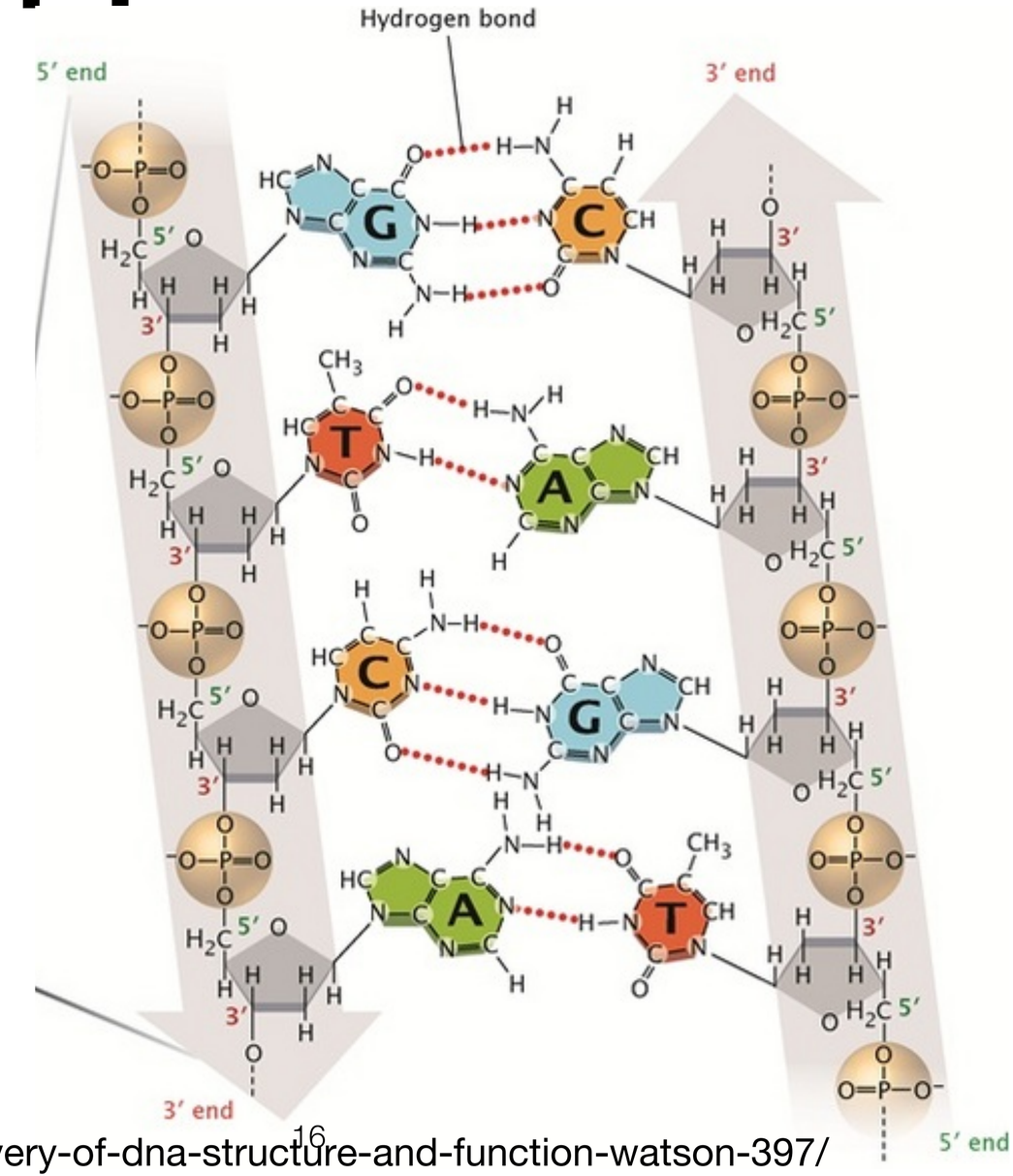


5 ‘prime’ vs 3 ‘prime’ ends



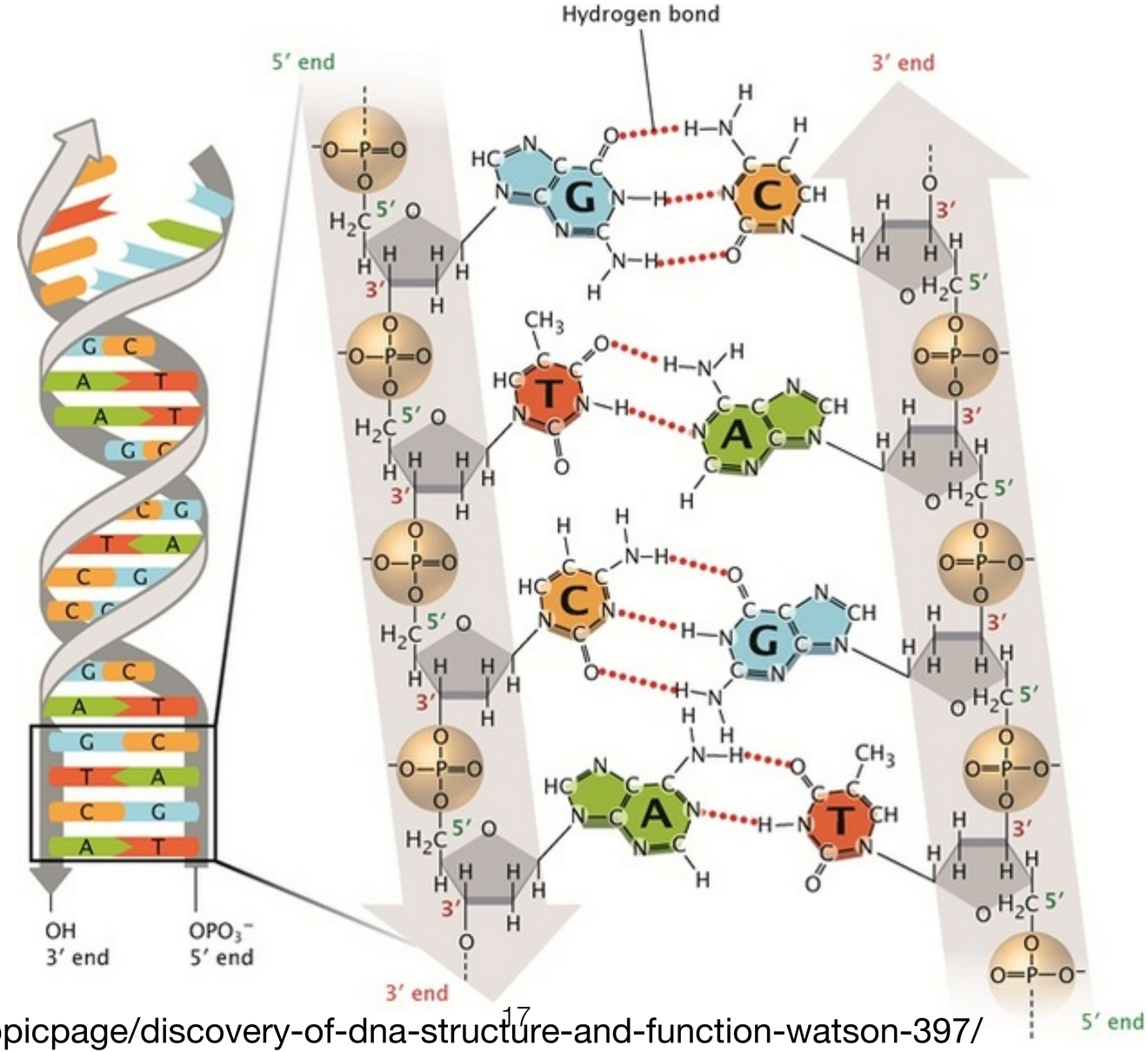
https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/

2 strands, opposite directions



https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/

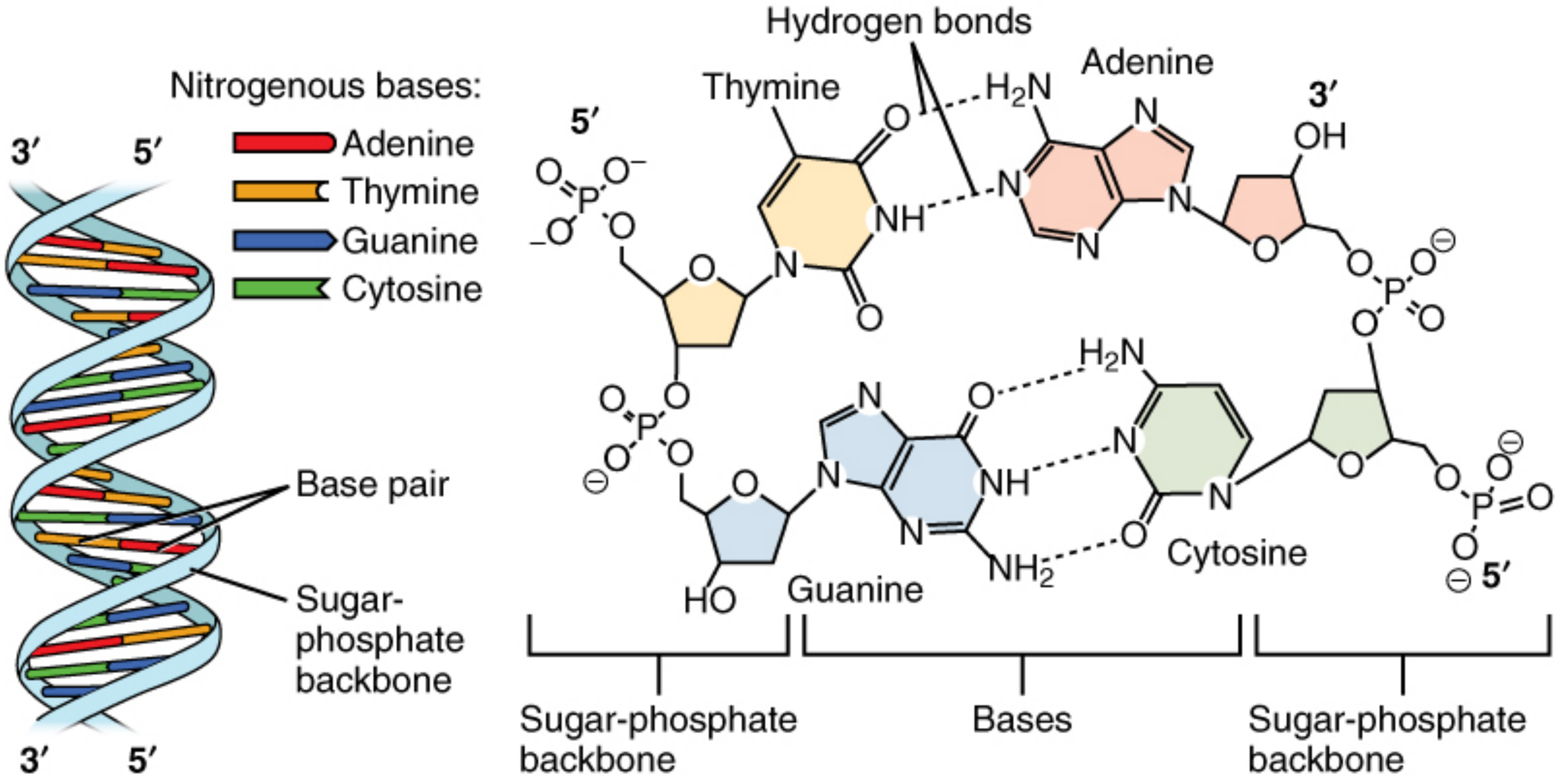
Double helix



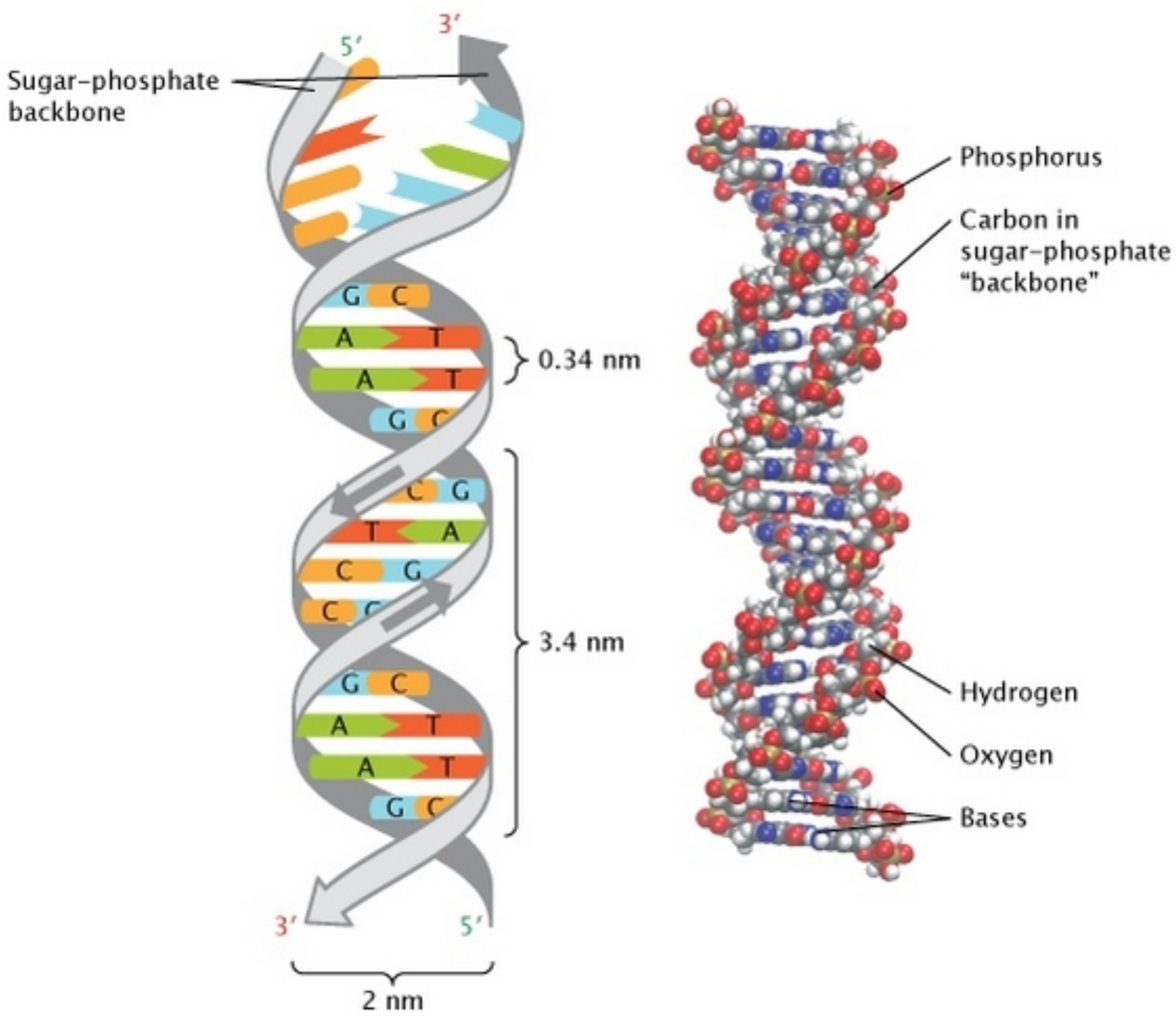
James watson
and Francis Crick

https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/

Double helix



Double helix-major and minor groves

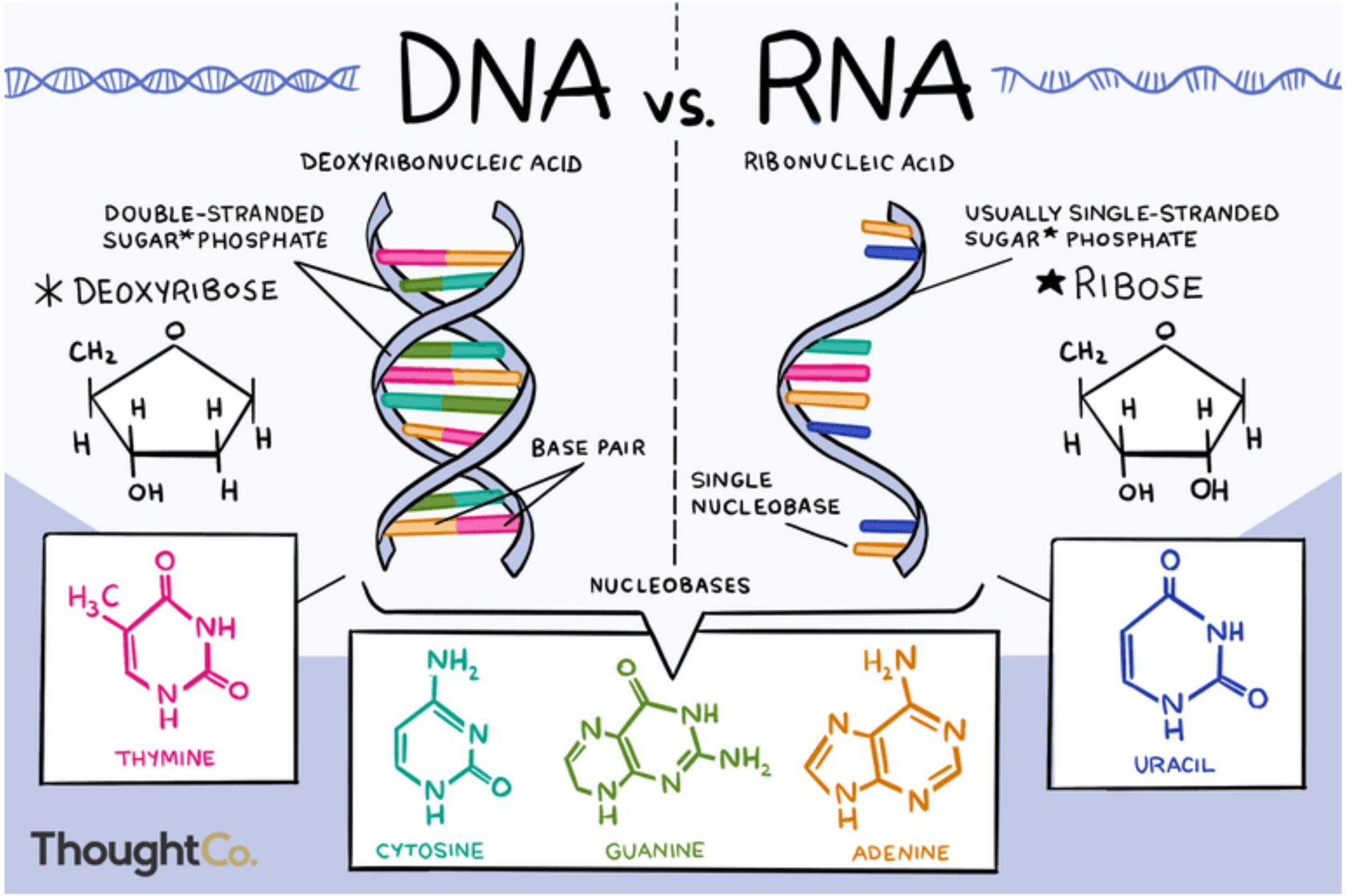


James watson
and Francis Crick

https://www.nature.com/scitable/topicpage/discovery-of-dna-structure-and-function-watson-397/

Ribonucleic acid (RNA)

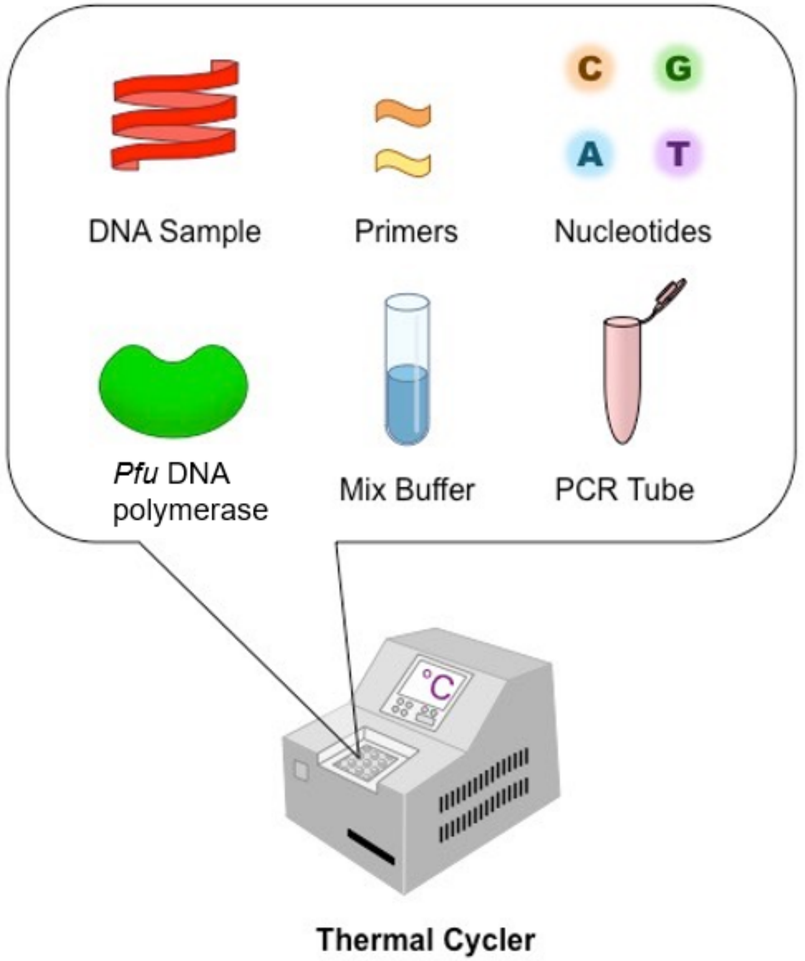
DNA vs RNA



https://www.thoughtco.com/dna-versus-rna-608191

Polymerase Chain Reaction (PCR)

- ❑ **Molecular biology technique**
- ❑ **Amplification of gene fragments**
- ❑ **Components of a PCR reaction**
  - ***Template DNA***
  - ***Primers***
  - ***Deoxy Nucleoside Triphosphates (dNTPs)***
  - ***Thermostable DNA polymerase***



Polymerase Chain Reaction (PCR)

❑ **Components of a PCR reaction**

1. Template DNA

- ***Contains the gene sequence information amplified by PCR;***
  - ***e.g. genomic DNA (gDNA)***

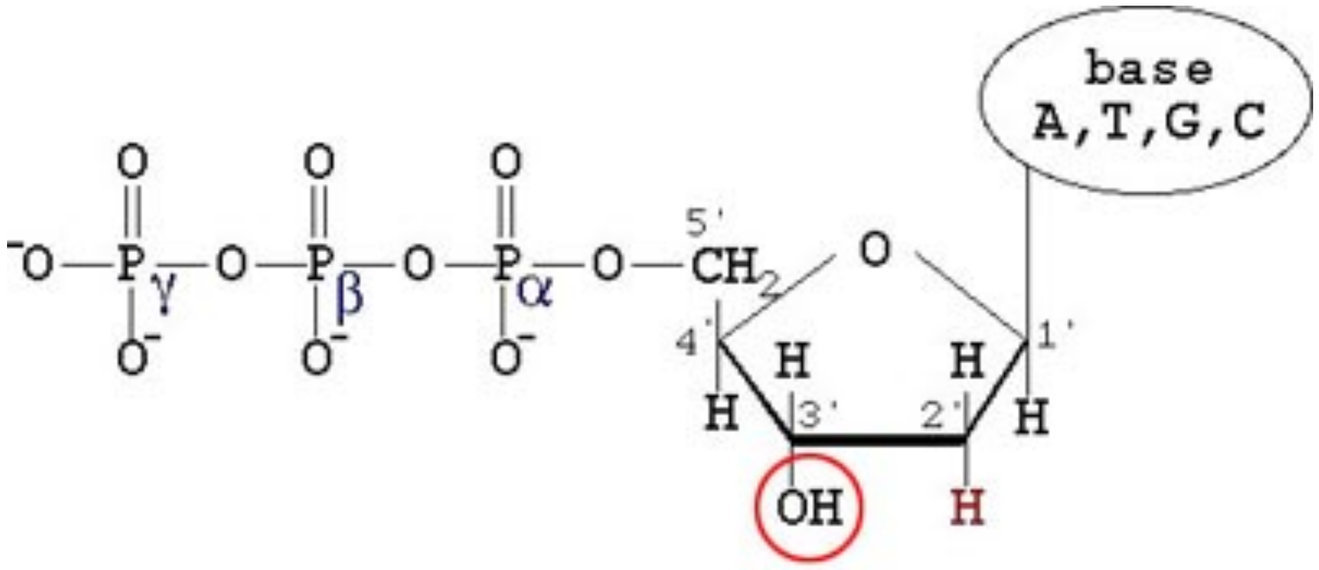
2. Primers

- ***Single-stranded DNA sequences;***
  - ***18-24 bp in length;***
- ***Anneal with the template DNA to amplify specific gene fragment;***
  - ***"Universal" primers used for 16S rRNA gene sequence amplification.***

Polymerase Chain Reaction (PCR)

❑ **Components of a PCR reaction**

3. Deoxy nucleotide triphosphates (dNTPs)
(dATP, dTTP, dGTP, dCTP)

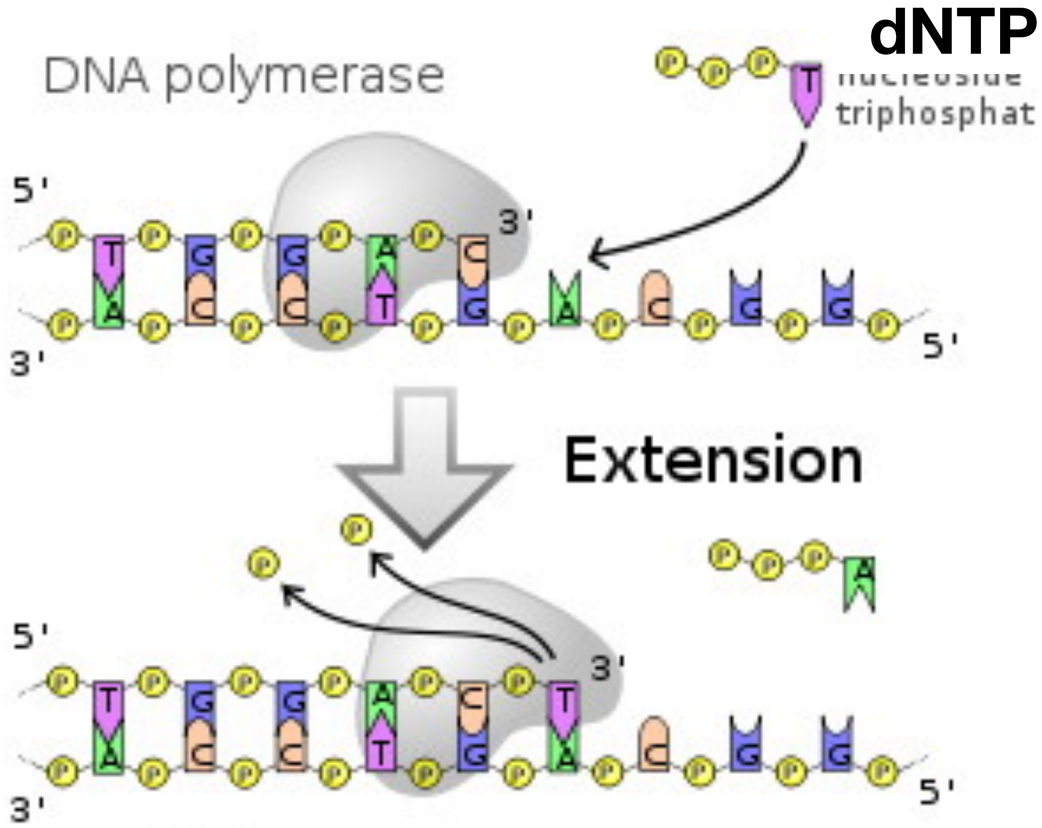


Polymerase Chain Reaction (PCR)

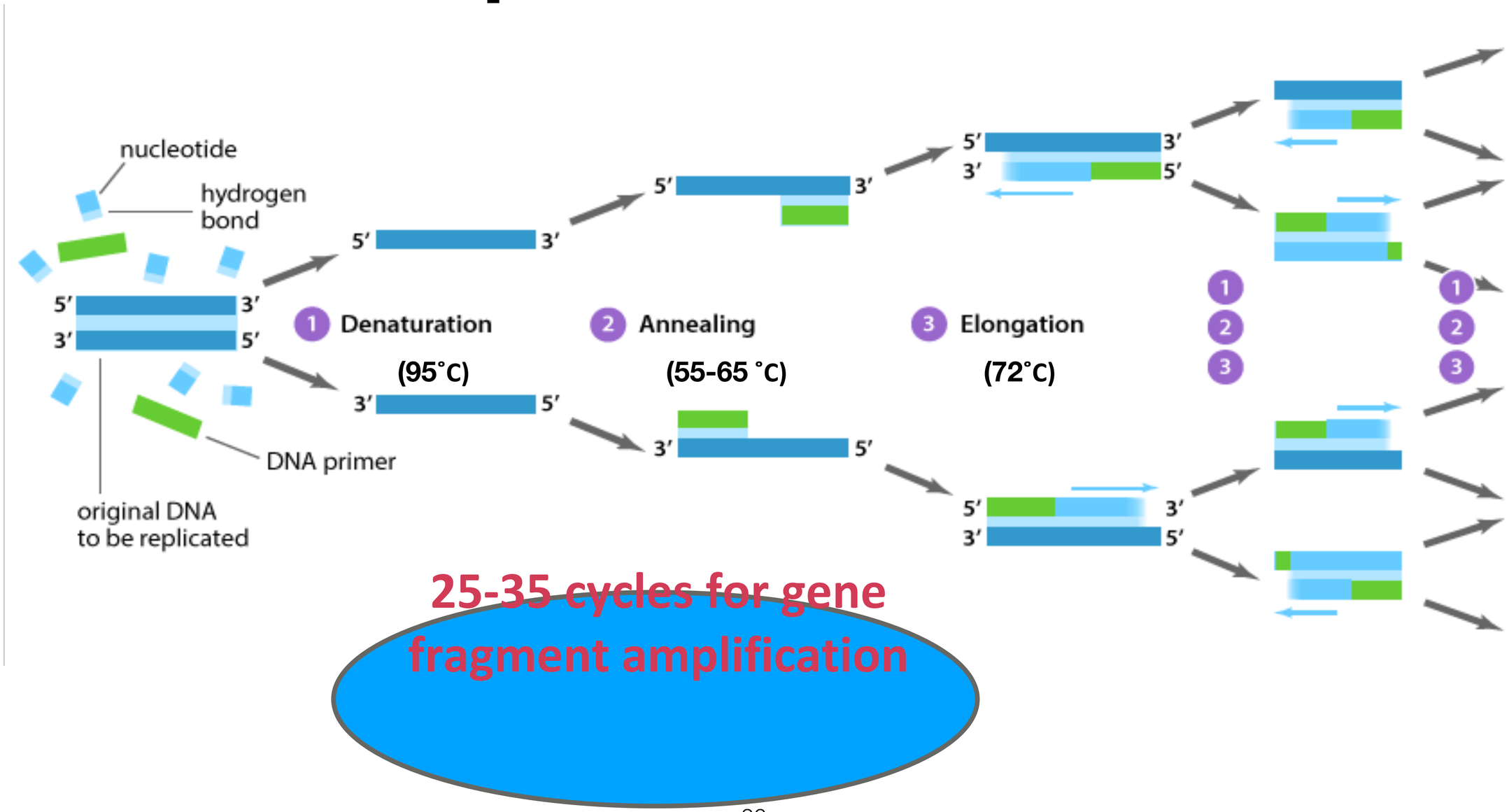
❑ **Components of a PCR reaction**

4. Thermostable DNA polymerase

- ***Extends the annealed primer by adding complementary dNTPs;***
- ***e.g. Pfu DNA polymerase purified from Pyrococcus furiosus.***



Basic steps in a PCR reaction



QUESTIONS?

THE PRINCIPLES OF SEQUENCING

Sequencing:

to determine the primary structure of an unbranched biopolymer. Sequencing results in a linear depiction known as a sequence which summarizes the atomic-level structure of the sequenced molecule.

THE TYPES OF SEQUENCING

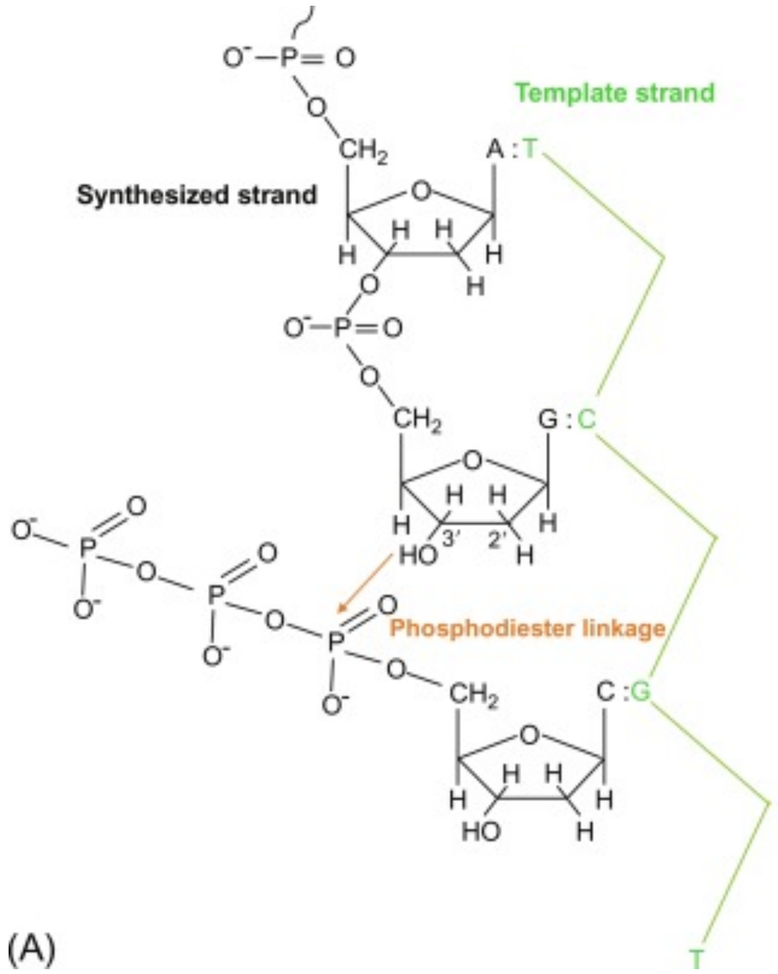
1. SANGERS Sequencing (chain termination sequencing, di-deoxynucleotide sequencing)
2. Next generation sequencing

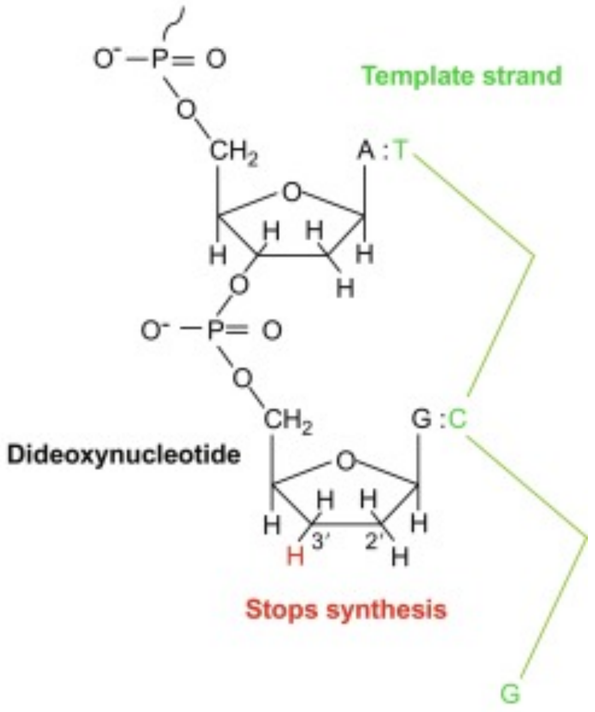
Sangers sequencing

It is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymeraseduring in vitro DNA replication.

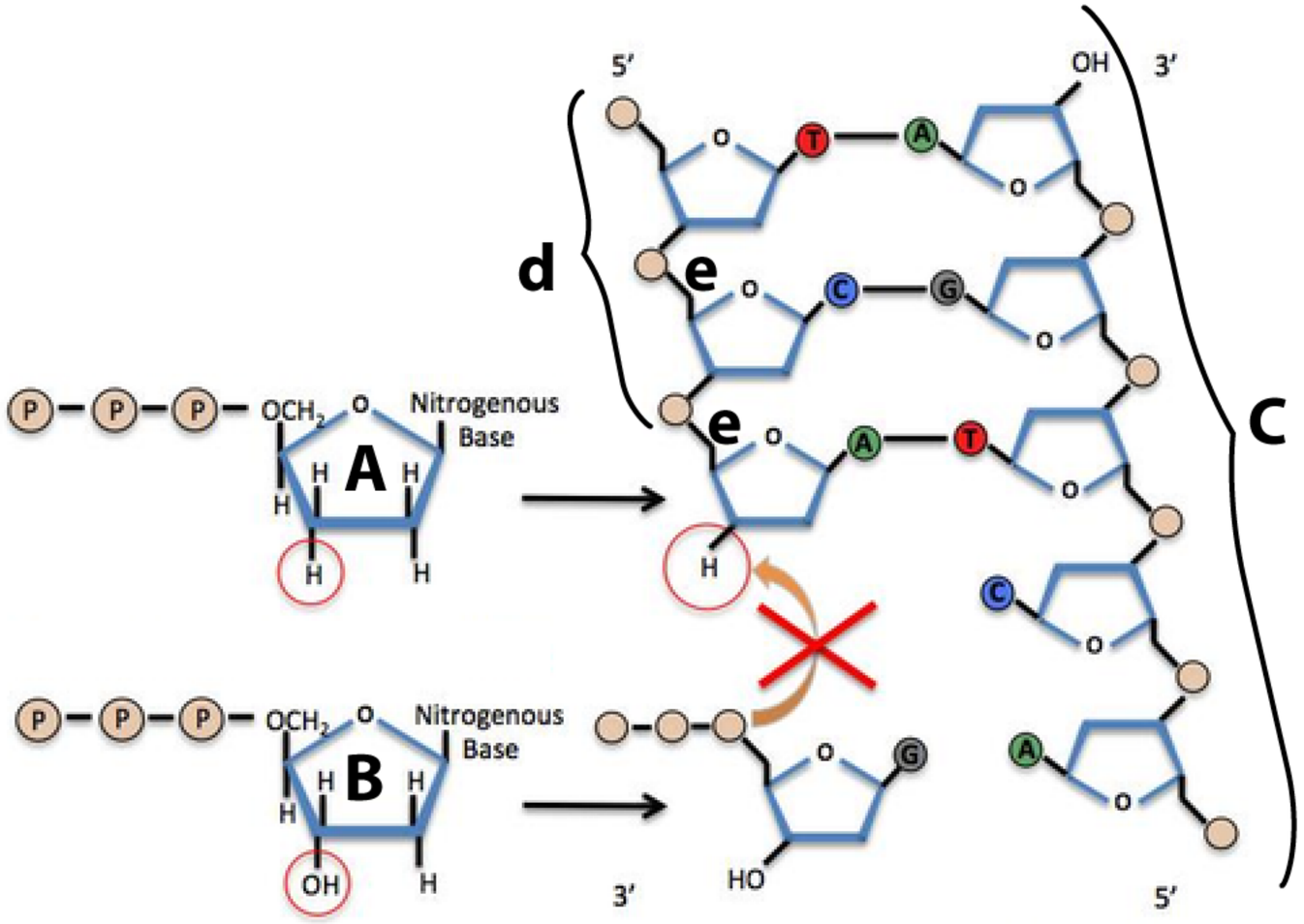
Developed by Frederick Sanger and colleagues in 1977

Sangers sequencing principle

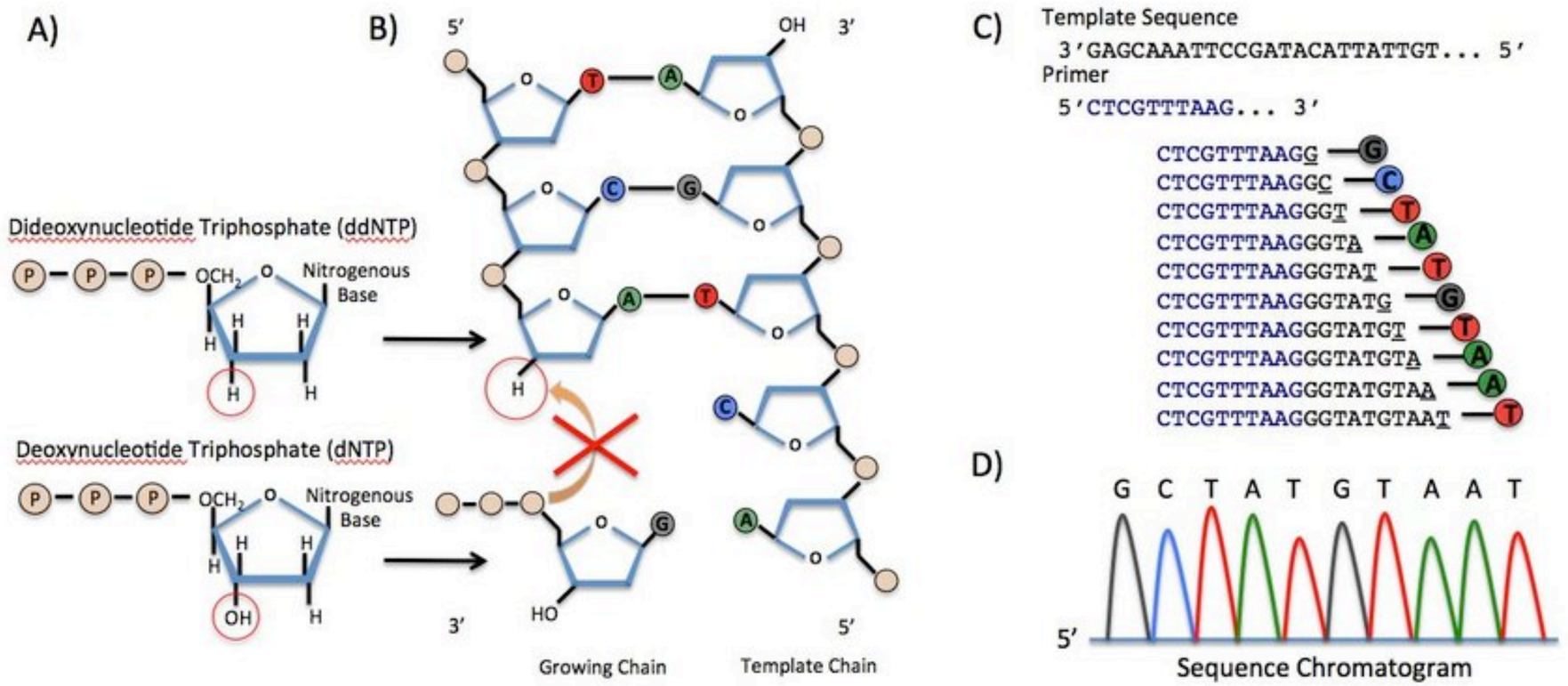




Sangers sequencing principle

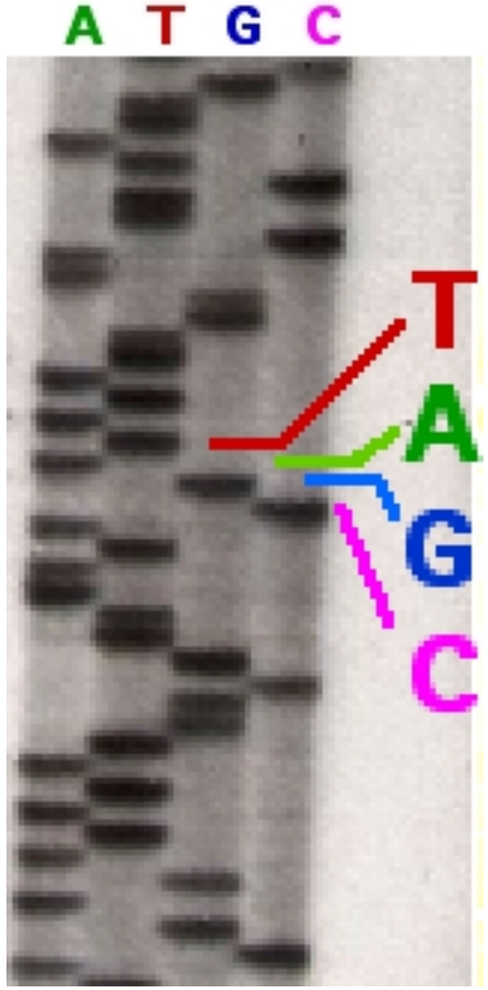


Sangers sequencing principle



https://www.researchgate.net/publication/303565848_The_Use_of_Genetic_Sequencing_Technologies_to_Determine_HIV-1_Viral_Tropism_and_to_Evaluate_the_Effects_of_Maraviroc_on_Patient_Viral_Populations/figures?lo=1

Sangers sequencing

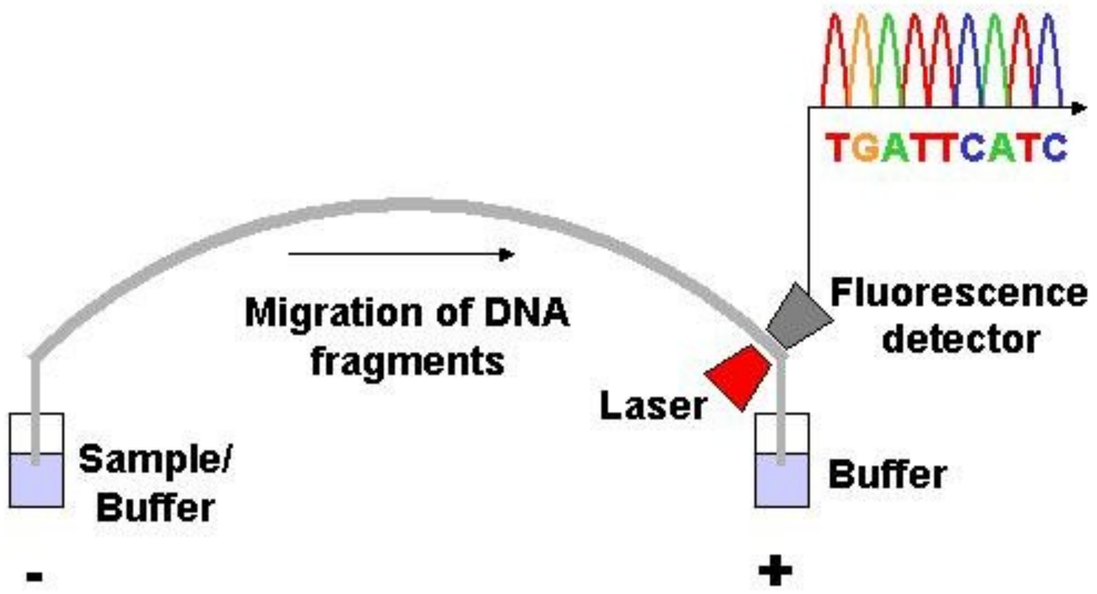


https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Sangers sequencing

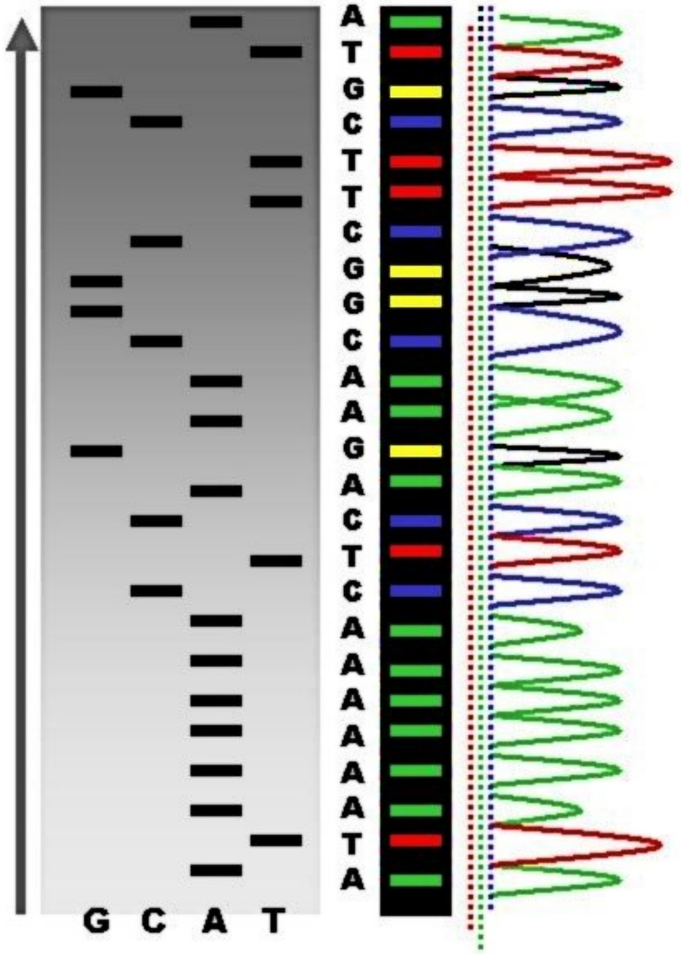
https://letstalkscience.ca/educational-resources/backgrounders/sanger-sequencing

Sangers sequencing



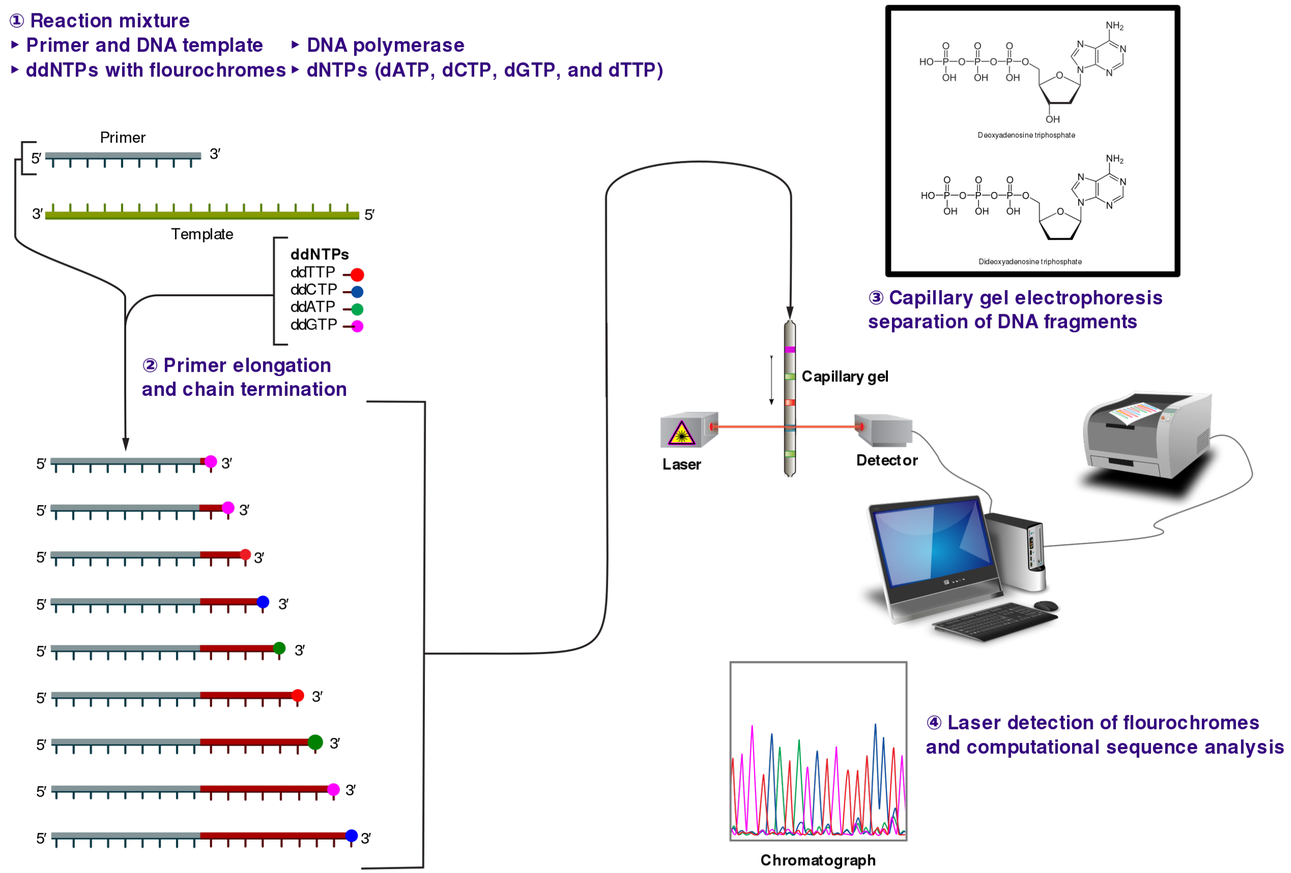
https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Sangers sequencing



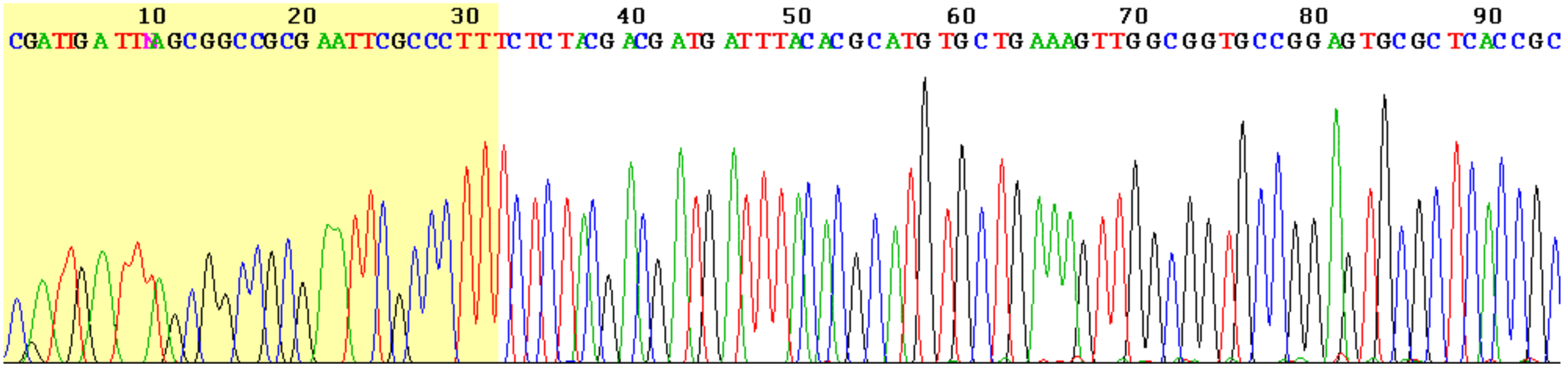
https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Sangers sequencing



https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Sangers sequencing



https://en.wikipedia.org/wiki/Sanger_sequencing#/media/File:Sanger-sequencing.svg

Next generation sequencing (NGS)

1. The generation of many millions of short reads in parallel
2. The speed up of sequencing the process compared to the first generation
3. The low cost of sequencing and
4. The sequencing output is directly detected without the need for electrophoresis.

Next generation sequencing (NGS)

Platform	Instrument	Reads per run	Avg Read length (pb)	Read Type	Error Type	Error Rate (%)	Data Generated per run (Gb)	Year
First Generation								
ABI Sanger	3730xl	96	400 – 900*	SE	NA	0.3	0.00069 to 0.0021	2002
Second Generation								
454	GS20	200	100	SE, PE	indel	1	0.02	2005
454	GS FLX	400	250	SE, PE	indel	1	0.1	2007
454	GS FLX Titanium	1 M	450	SE, PE	indel	1	0.45	2009
454	GS FLX Titanium+	1 M	700	SE, PE	indel	1	0.7	2011
454	GS Junior	100	400	SE, PE	indel	1	0.04	2010
454	GS Junior+	100	700	SE, PE	indel	1	0.07	2014
Illumina	MiniSeq	25M (maximum)	150	SE, PE	mismatch	1	7.5 (maximum)	2013
Illumina	MiSeq	25M (maximum)	300	SE, PE	mismatch	0.1	15 (maximum)	2011
Illumina	NextSeq	400M (maximum)	150	SE, PE	mismatch	1	120 (maximum)	2014
Illumina	HiSeq	5B (maximum)	150	SE, PE	mismatch	0.1	1.5Tb (maximum)	2012
Illumina	HiSeq X	6B (maximum)	150	SE, PE	mismatch	0.1	1.8Tb (maximum)	2014
SOLiD	5500 W	3B	75	SE	mismatch	~0.1	160	2011
SOLiD	5500xl W	6B	75	SE	mismatch	~0.1	320	2013
Ion Torrent	PGM 314 chip v2	400.000-550.000	400	SE	indel	1	0.06 to 0.1	2011
Ion Torrent	PGM 316 chip v2	2M - 3M	200	SE	indel	1	0.6 to 1	2011
Ion Torrent	PGM 318 chip v2	4M - 5.5M	400	SE	indel	1	1.2 to 2	2013

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

Third Generation								
PacBio	RS C1	432	1300	SE	indel	15	0.54	2011
PacBio	RS C2	432	2500	SE	indel	15	0.5 to 1	2012
PacBio	RS C2 XL	432	4300	SE	indel	15	0.5 to 1	2012
PacBio	RS II C2 XL	564	4600	SE	indel	15	0.5 to 1	2013
PacBio	RS II P5 C3	528	8500	SE	indel	13	0.5 to 1	2014
PacBio	RS II P6 C4	660	13500	SE	indel	12	0.5 to 1	2014
PacBio	Sequel	350	10000	SE	NA	NA	7	2016
Oxford Nanopore	MinION Mk	100	9545	1D,2D	indel/mismatch	12	1.5	2015
Oxford Nanopore	PromethION	NA	9846	1D,2D	NA	NA	2Tb to 4Tb	2016
*depending on run module; NA: Not available; SE: Single End; PE: Paired End; M: Million; B: Billion; Gb: Gigabytes; Tb: Terabytes								

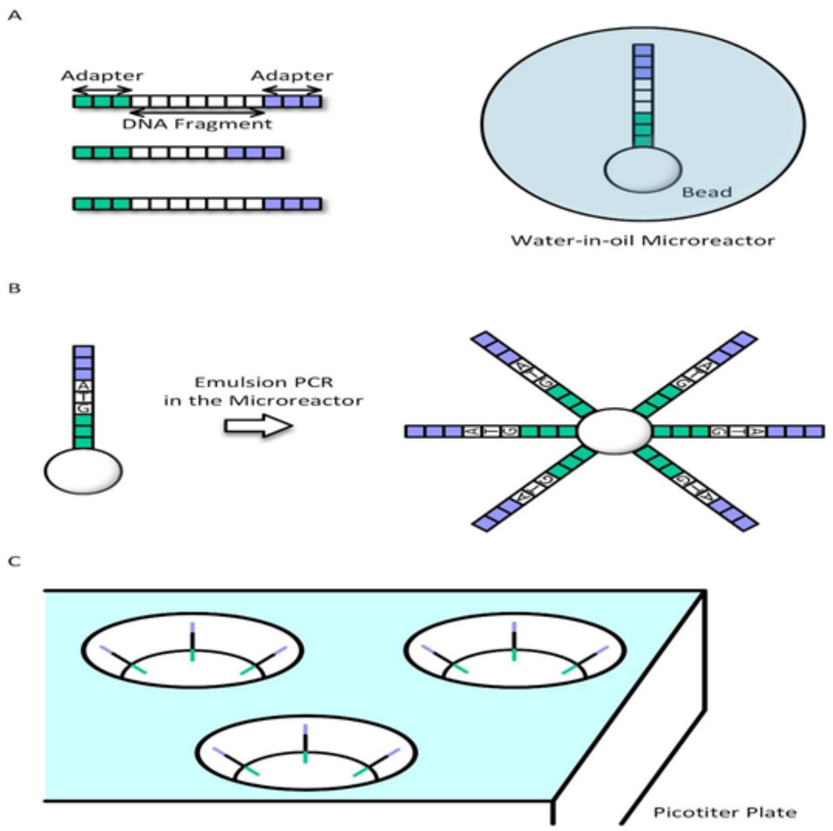
https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

Third Generation								
PacBio	RS C1	432	1300	SE	indel	15	0.54	2011
PacBio	RS C2	432	2500	SE	indel	15	0.5 to 1	2012
PacBio	RS C2 XL	432	4300	SE	indel	15	0.5 to 1	2012
PacBio	RS II C2 XL	564	4600	SE	indel	15	0.5 to 1	2013
PacBio	RS II P5 C3	528	8500	SE	indel	13	0.5 to 1	2014
PacBio	RS II P6 C4	660	13500	SE	indel	12	0.5 to 1	2014
PacBio	Sequel	350	10000	SE	NA	NA	7	2016
Oxford Nanopore	MinION Mk	100	9545	1D,2D	indel/mismatch	12	1.5	2015
Oxford Nanopore	PromethION	NA	9846	1D,2D	NA	NA	2Tb to 4Tb	2016
*depending on run module; NA: Not available; SE: Single End; PE: Paired End; M: Million; B: Billion; Gb: Gigabytes; Tb: Terabytes								

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

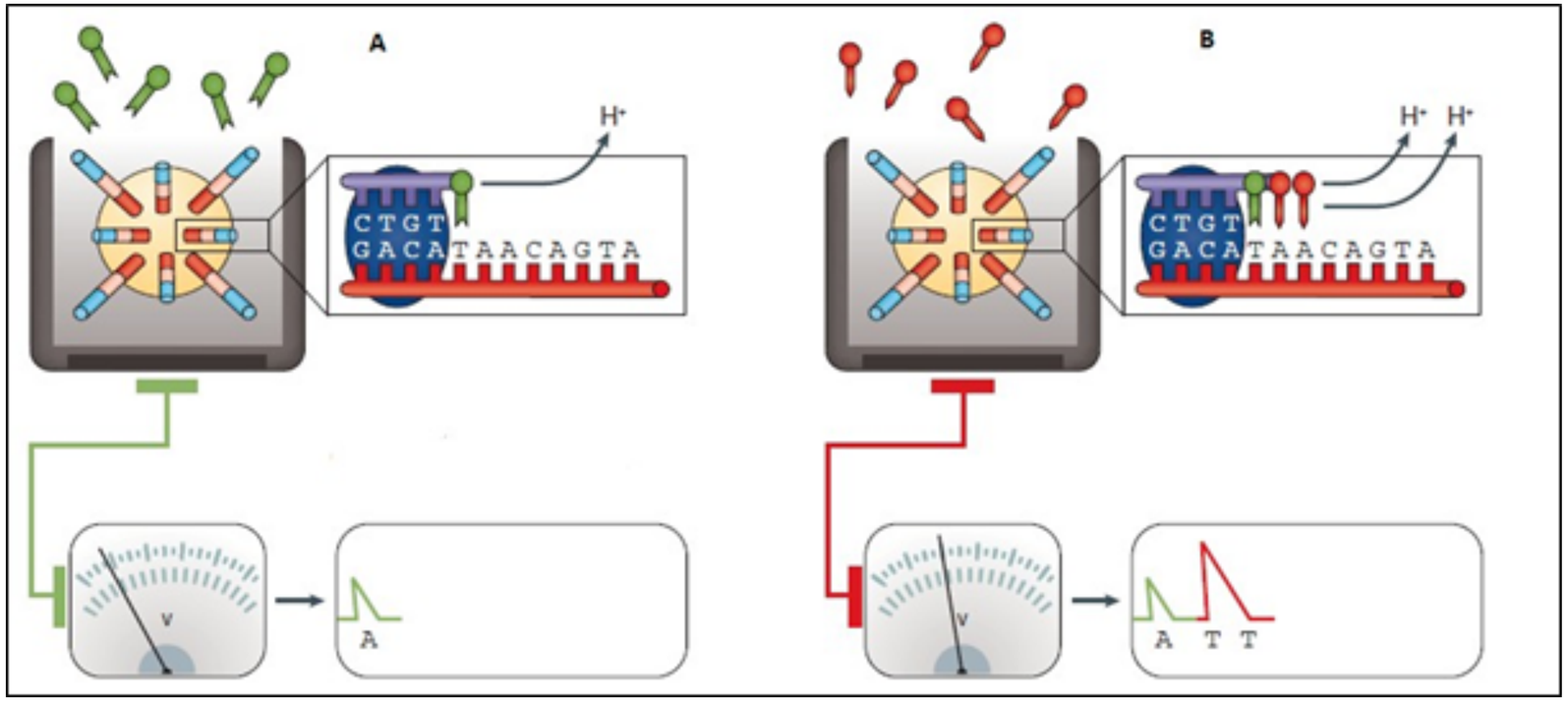
Next generation sequencing (NGS)



Roche/454 sequencing technology uses pyrosequencing methods using pyrosequencing technique which is based on the detection of pyrophosphate released after each nucleotide incorporation in the new synthetic DNA strand

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

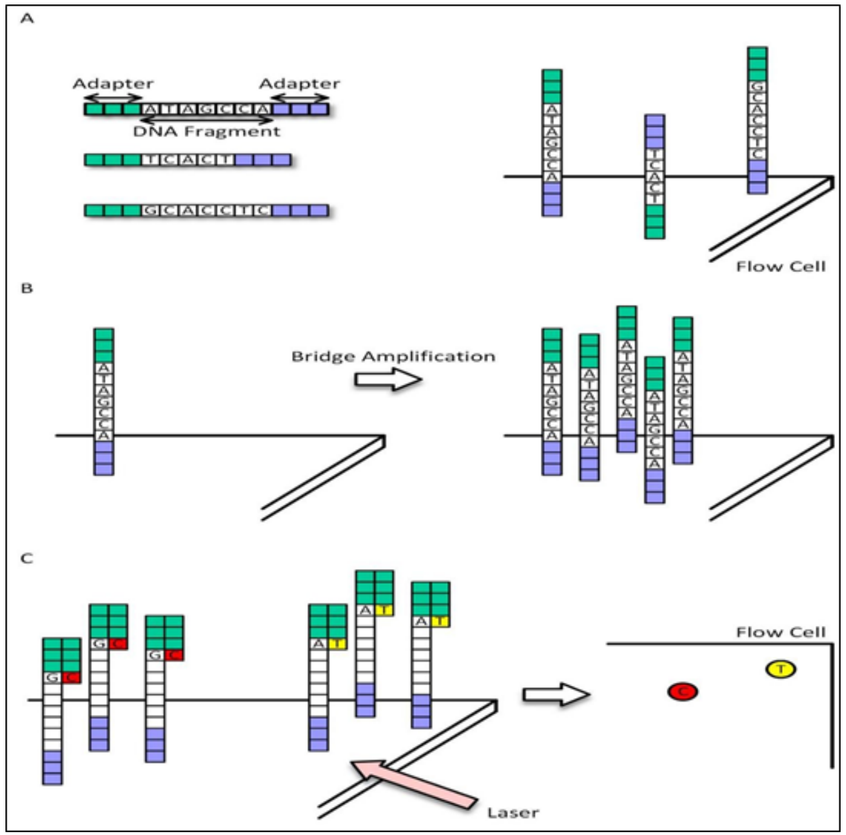
Next generation sequencing (NGS)



Ion torrent sequencing technology based on the detection of the hydrogen ion released during the sequencing process

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

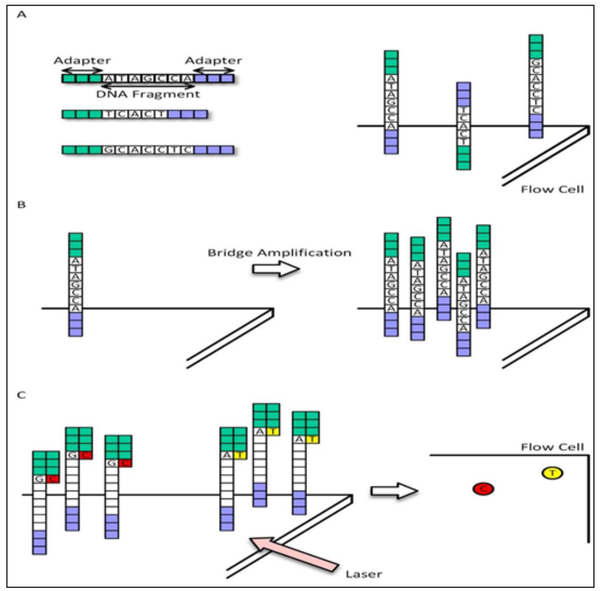


Illumina sequencing technology uses adapters, it is the most common method

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

1. DNA samples are randomly fragmented into sequences
2. Adapters are ligated to both ends of each sequence.
3. Adapters are fixed themselves to the respective complementary adapters,
4. Adapters are hooked on a slide with many variants of adapters (complementary) placed on a solid plate .



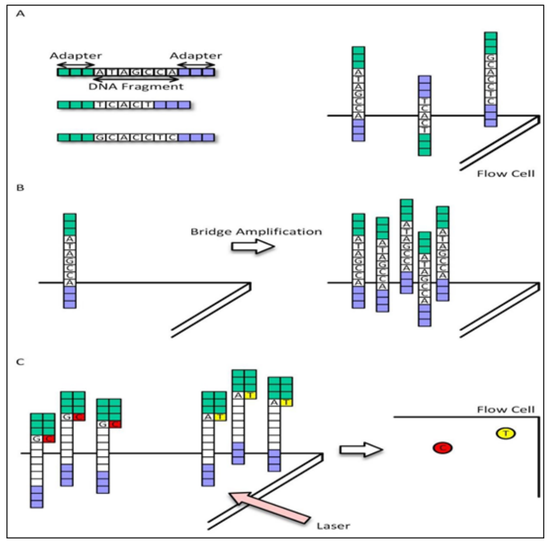
https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

5. During the second step, each attached sequence to the solid plate is amplified by “PCR bridge amplification” that creates several identical copies of each sequence;

6. Set of sequences made from the same original sequence is a cluster.

7. Each cluster contains approximately one million copies of the same original sequence



https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

8. The last step is to determine each nucleotide in the sequences, Illumina uses the sequencing by synthesis approach that employs reversible terminators in which the four modified nucleotides, sequencing primers and DNA polymerases are added as a mix, and the primers are hybridized to the sequences

9. Then, polymerases are used to extend the primers using the modified nucleotides. Each type of nucleotide is labeled with a fluorescent specific in order for each type to be unique.

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

Next generation sequencing (NGS)

10. The nucleotides have an inactive 3’-hydroxyl group which ensures that only one nucleotide is incorporated. Clusters are excited by laser for emitting a light signal specific to each nucleotide, which will be detected by a coupled-charge device (CCD) camera and Computer programs will translate these signals into a nucleotide sequence

11. The process continues with the elimination of the terminator with the fluorescent label and the starting of a new cycle with a new incorporation

https://www.longdom.org/open-access/generations-of-sequencing-technologies-from-first-to-next-generation-0974-8369-1000395.pdf

NGS Video

https://www.illumina.com/science/technology/next-generation-sequencing/beginners.html

https://www.youtube.com/watch?v=CZeN-IgjYCo

Questions?

Sequencing data analysis and Phylogenetic analysis

Softwares

MEGA : https://www.megasoftware.net/download_form
BioEdit : http://www.mbio.ncsu.edu/BioEdit/bioedit.html

GENEIOUS: https://www.geneious.com

e.t.c

Sequencing data analysis and Phylogenetic analysis

1. First import your sequence into a software e.g bioedit or Geneious
2. Crosscheck the sequence chromogram to be sure that the peaks are correct
3. Edit sequence by trimming, correcting and save as FASTA file
4. Download other similar sequences from the gene bank (NCBI)
5. Align the sequences on MEGA or Bioedit
6. Compose a phylogeny on bioedit or MEGA
7. Observe and read your phylogeny

Sequencing data analysis and Phylogenetic analysis

Download your softwares and start with practical.

Good luck.