INTRODUCTION TO MOLECULAR BIOLOGY (DNA,RNA, Principles of PCR)

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AFRICA CENTRE OF EXCELLENCE IN PHYTOMEDICINE RESEARCH
UNIVERSITY OF JOS

(21ST JUNE 2021)

My opening words

1. Not your fault that you do not know
2. Be encouraged to seek for knowledge (My story)
3. It may be expensive and difficult but try to follow it
4. It is rewarding, put you on global knowledge stream !

Goals of this lecture

1. Overview of DNA,
2. Introduction to RNA
3. Introduction to PCR basics:
    a. Extraction,
    b. Amplification,
    c. Gel electrophoresis

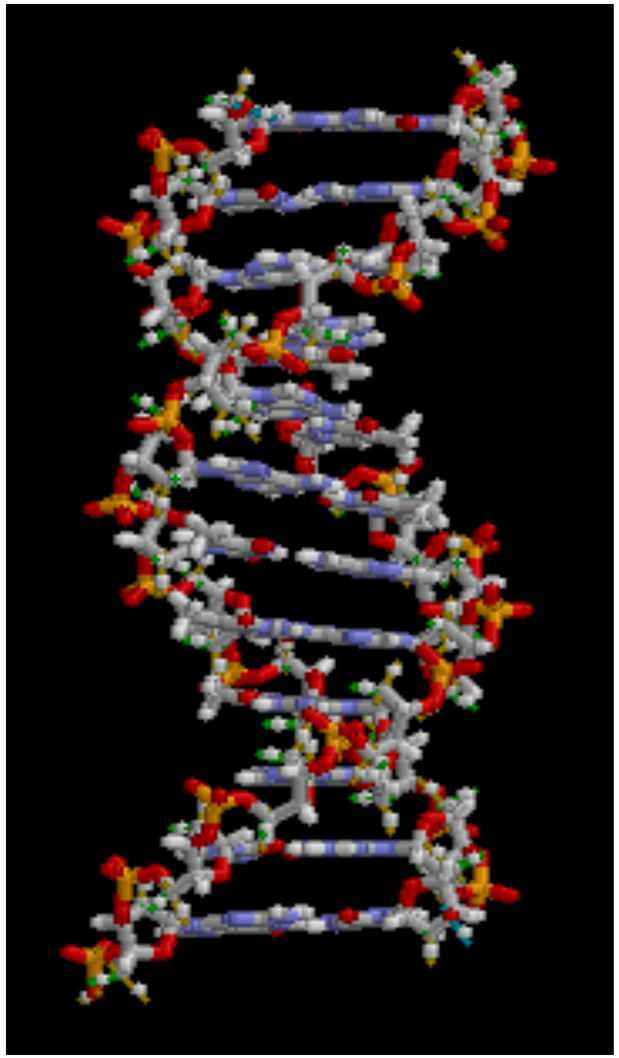
What to do to learn

1. Be attentive
2. Read extra, browse, videos, make google your friend
3. Keep learning, we are all students
4. Practice, practice and practice !

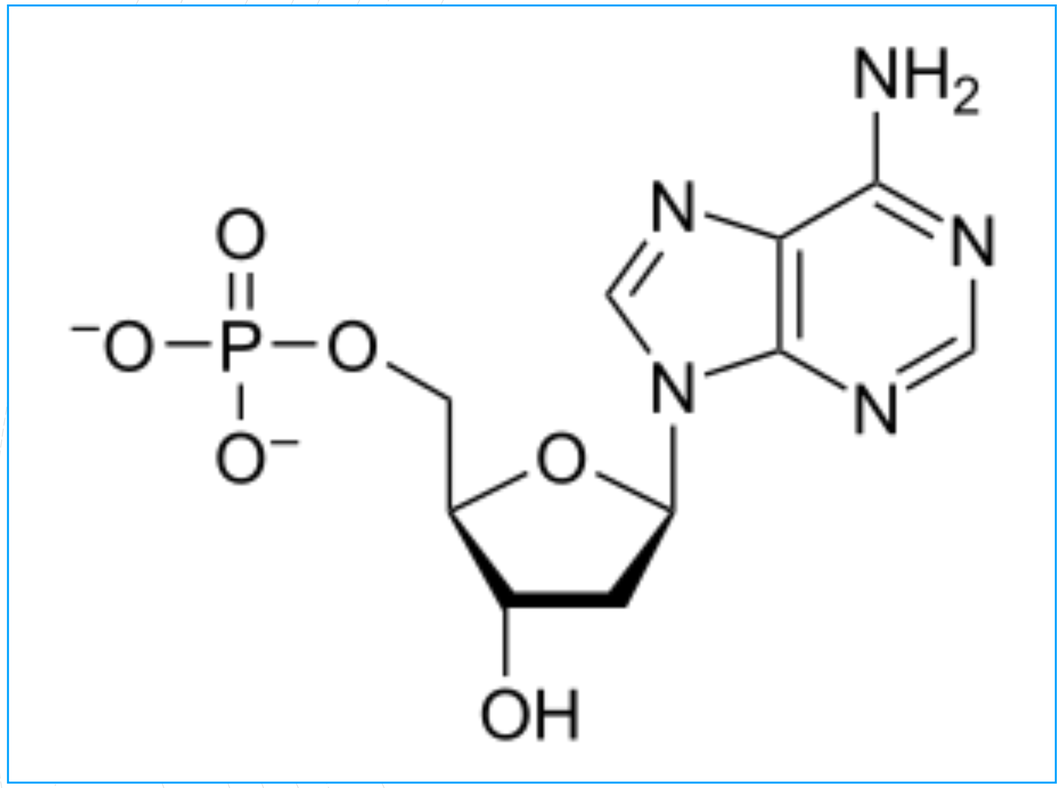
What is DNA

- **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



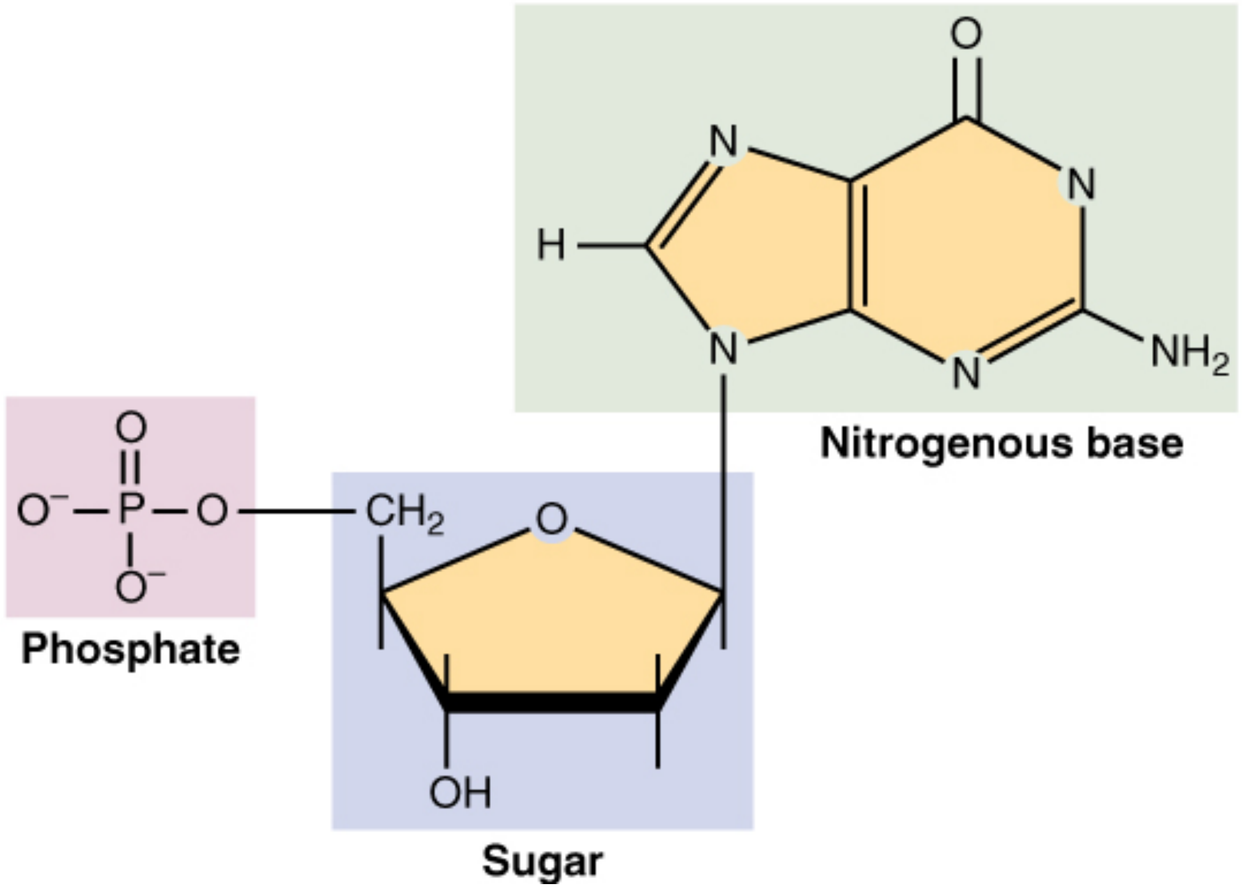
Components of DNA



- Monomeric units called **Nucleotides**

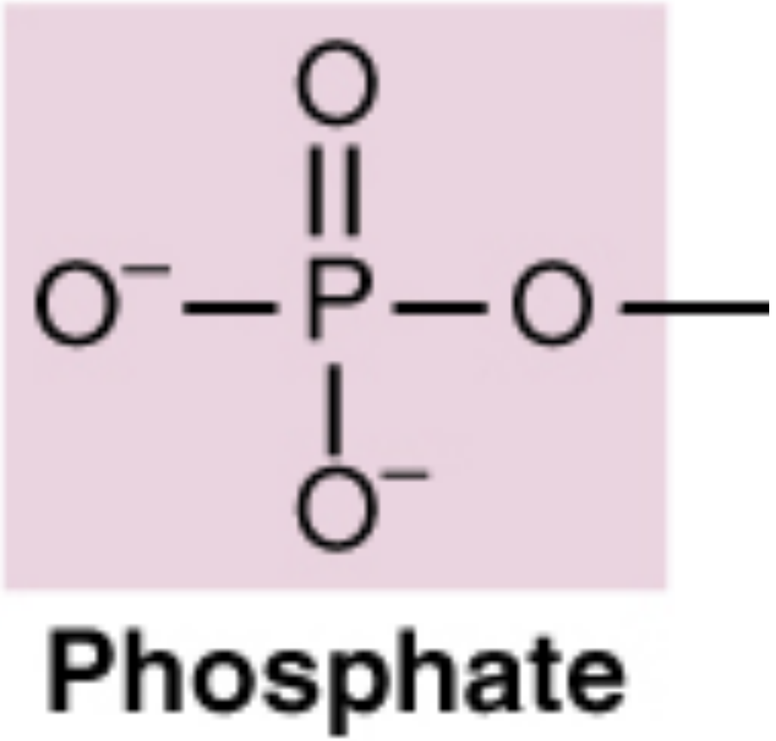
Components of a nucleotide

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



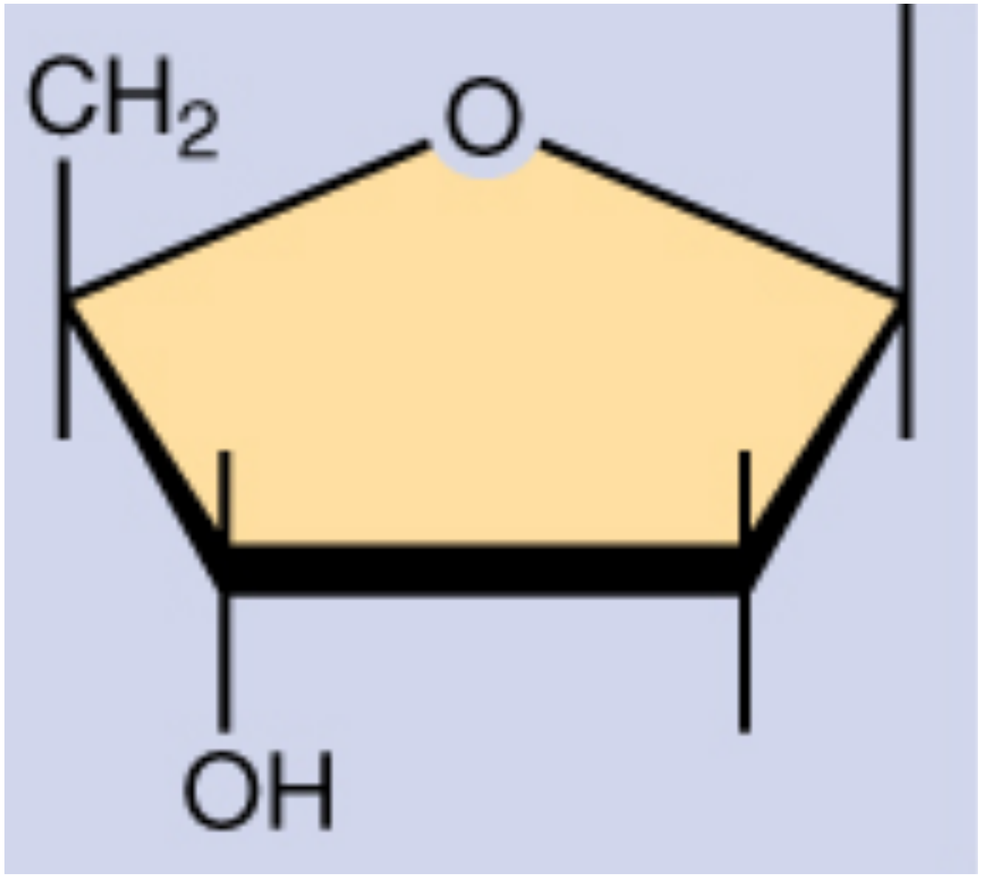
Phosphate group

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

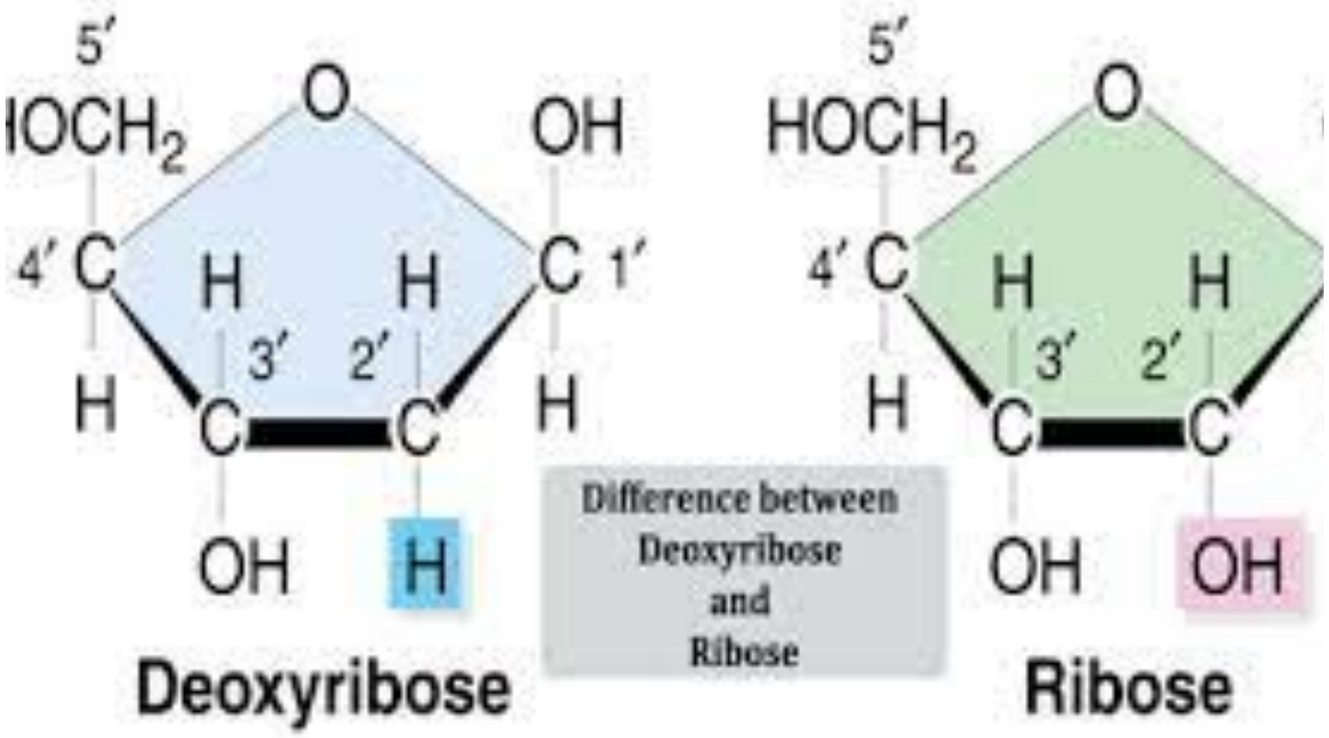


Components of a nucleotide

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



Ribose vs Deoxyribose



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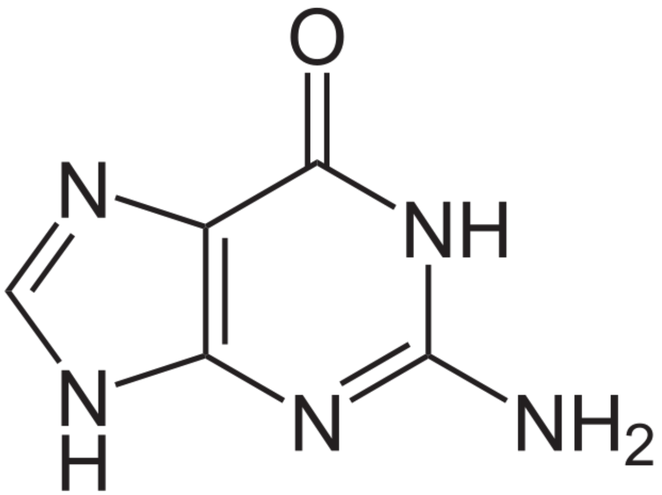
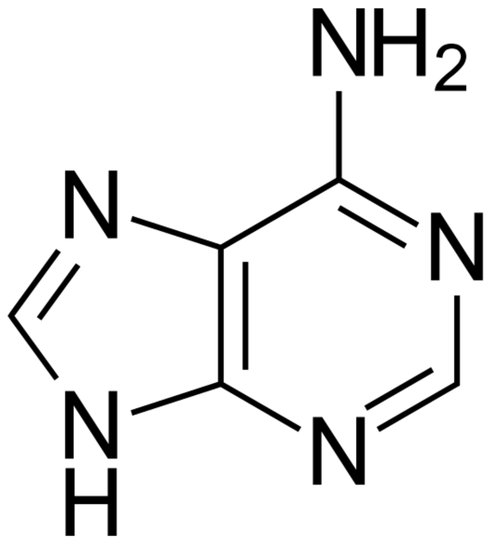
Nitrogenous bases

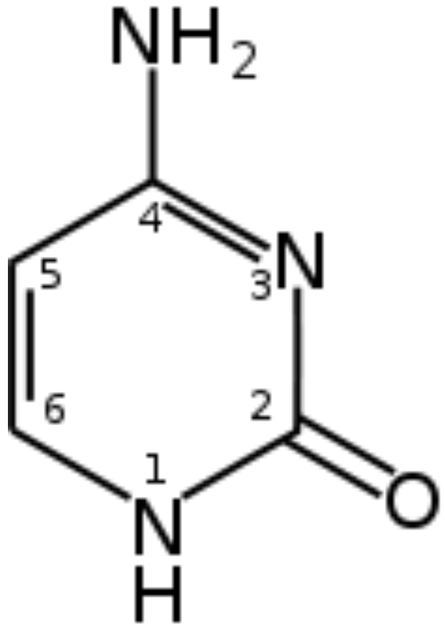


Guanine

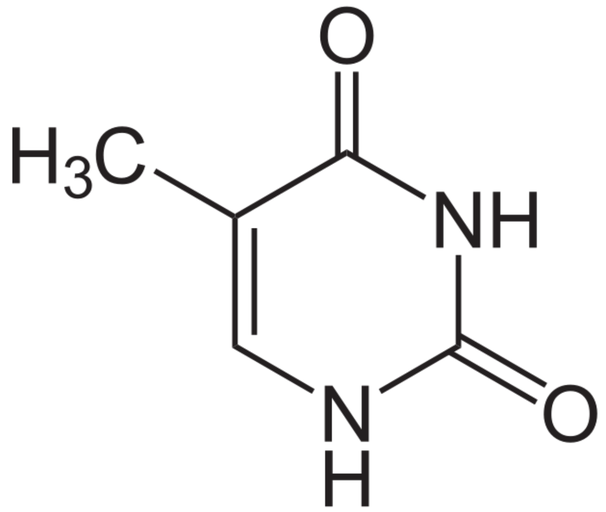


Adenine

Purines



Cytosine

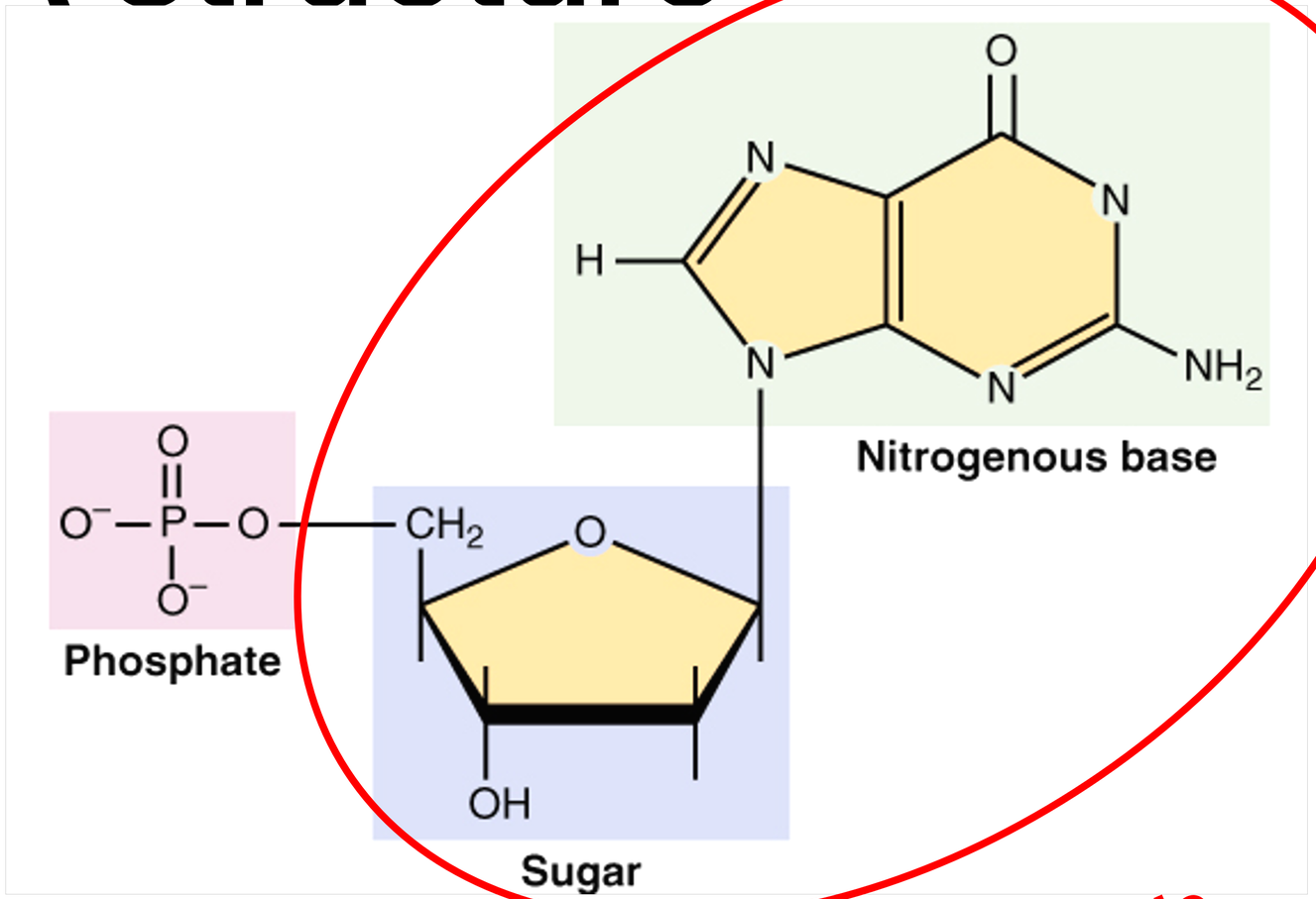


Thymine

Pyrimidines

DNA structure

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



DNA structure

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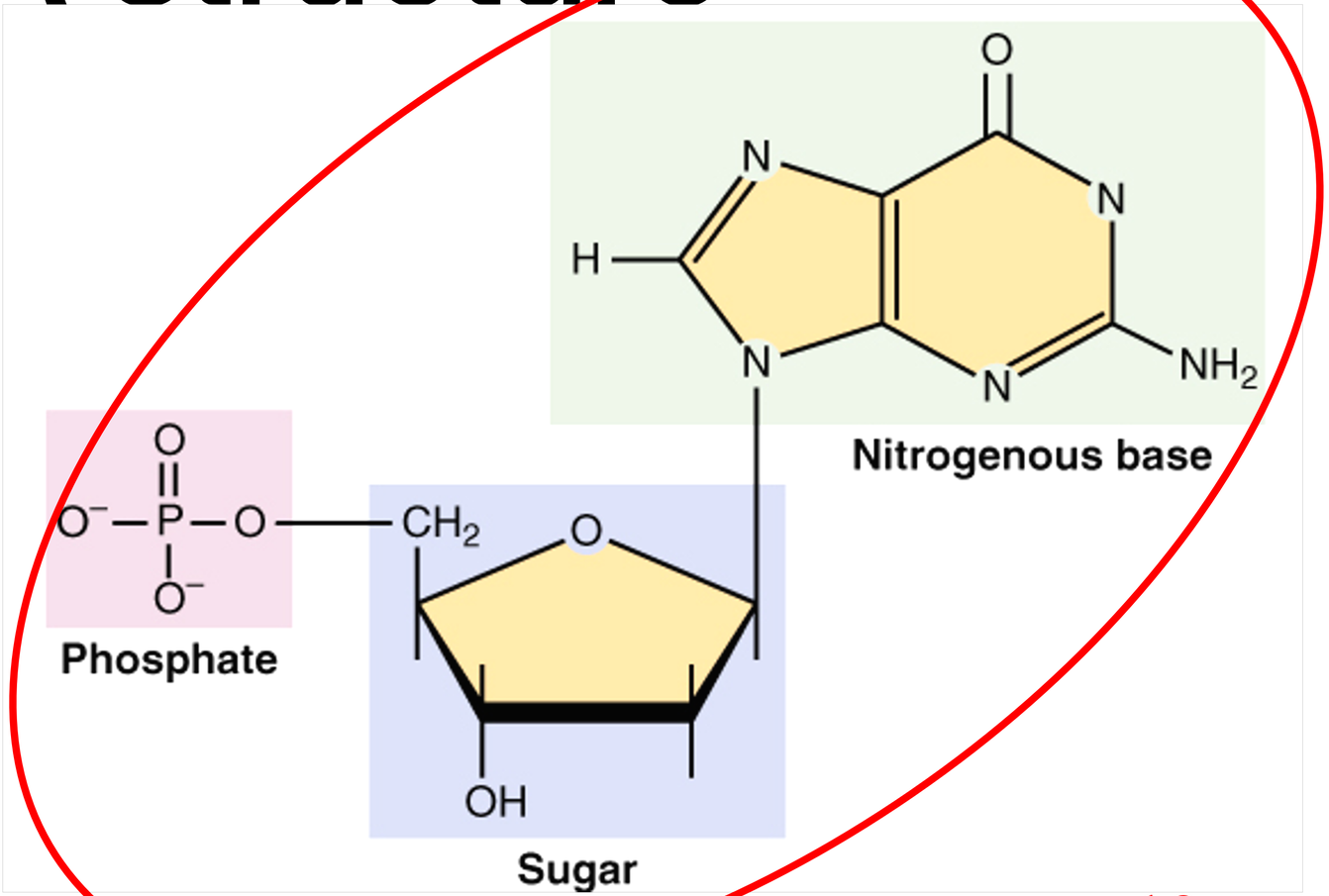
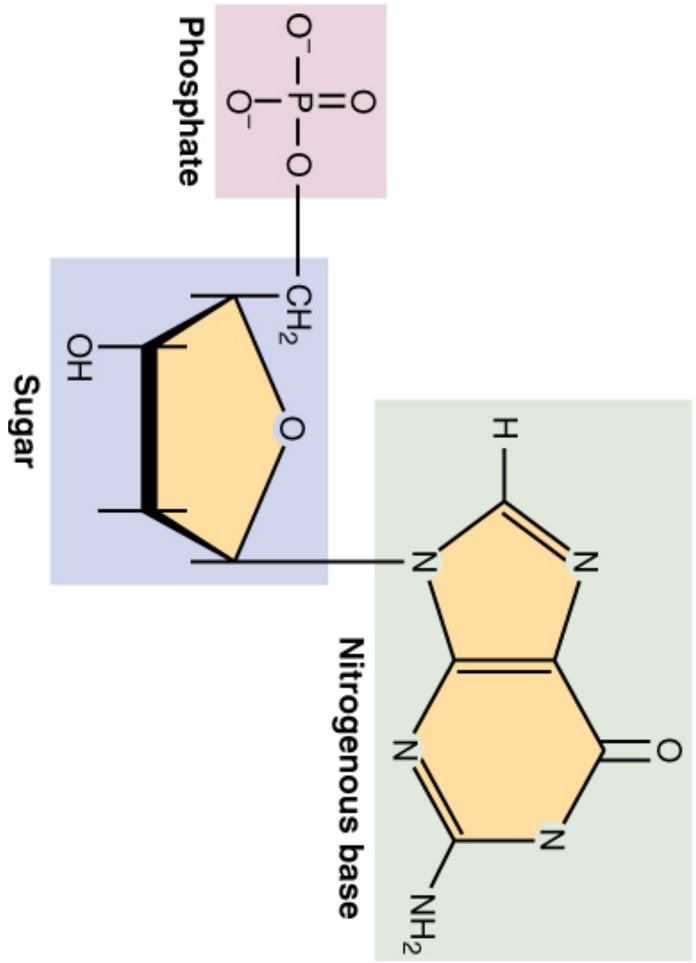
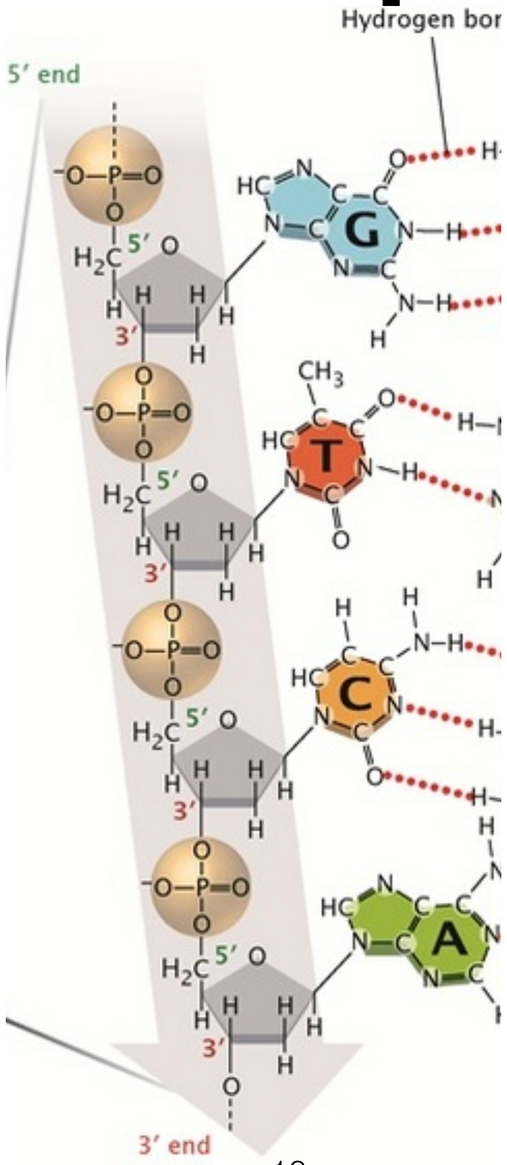


DNA structure

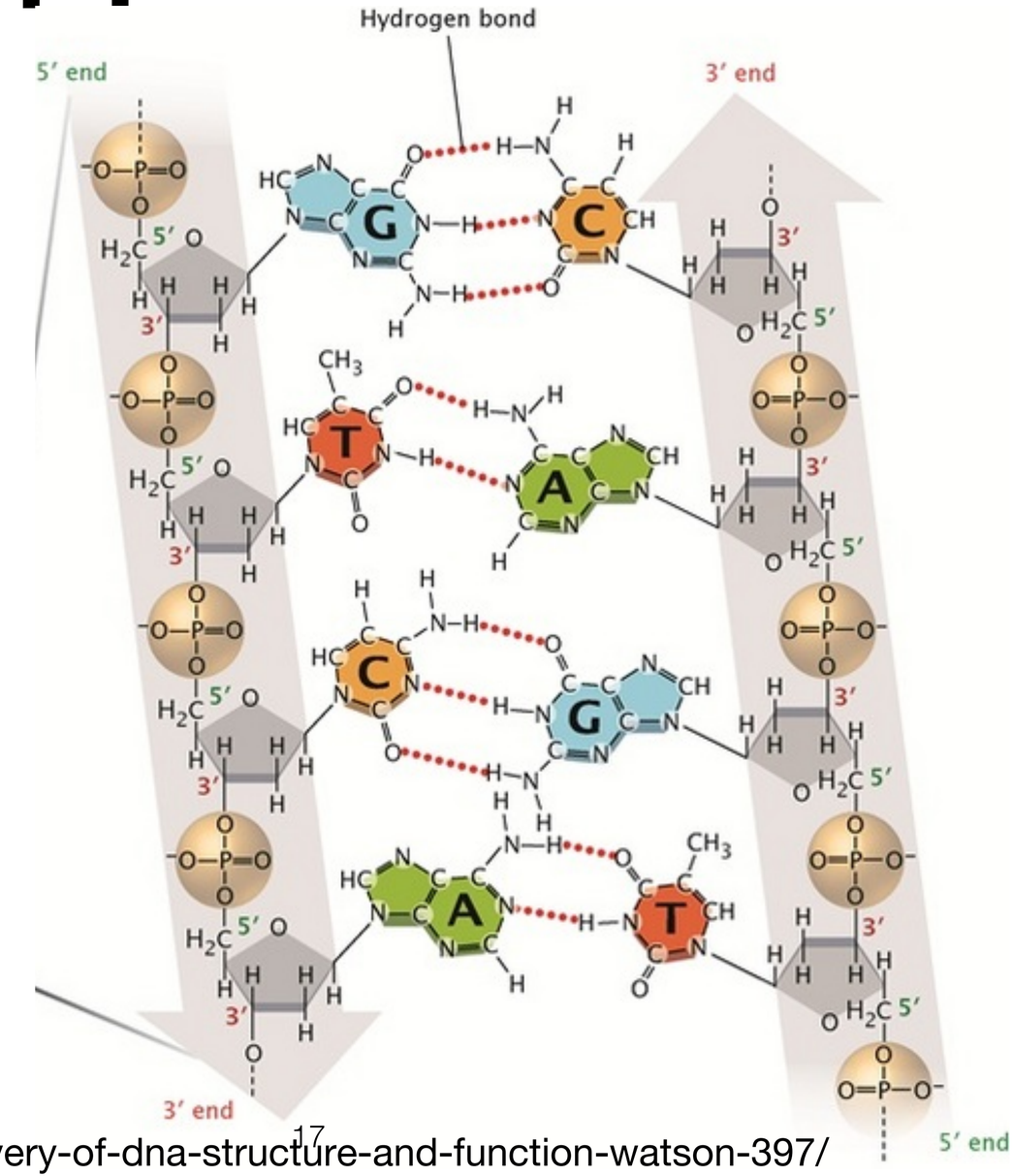


5 ‘prime’ vs 3 ‘prime’ ends



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

2 strands, opposite directions



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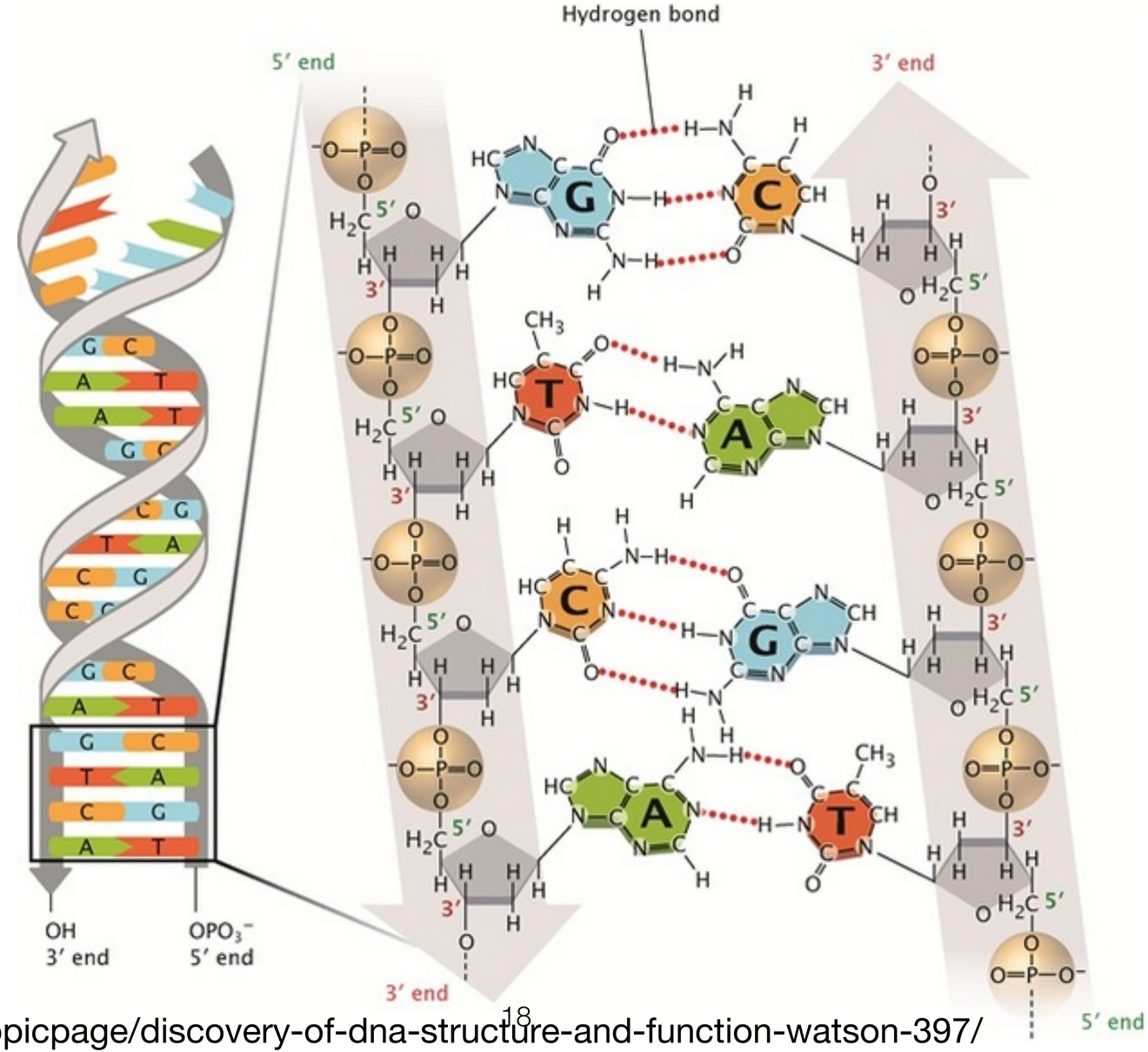
Double helix



James watson
and Francis Crick

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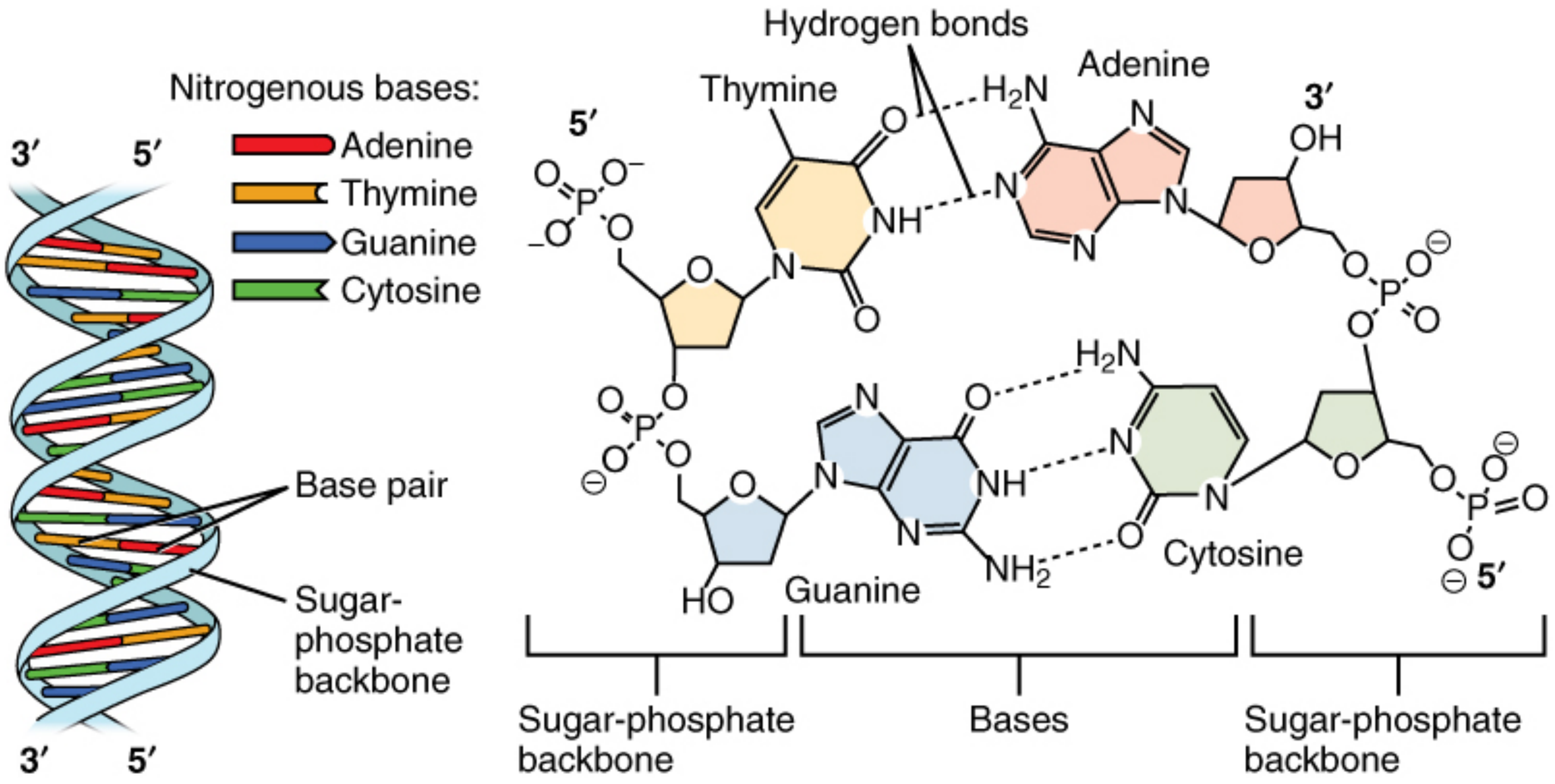
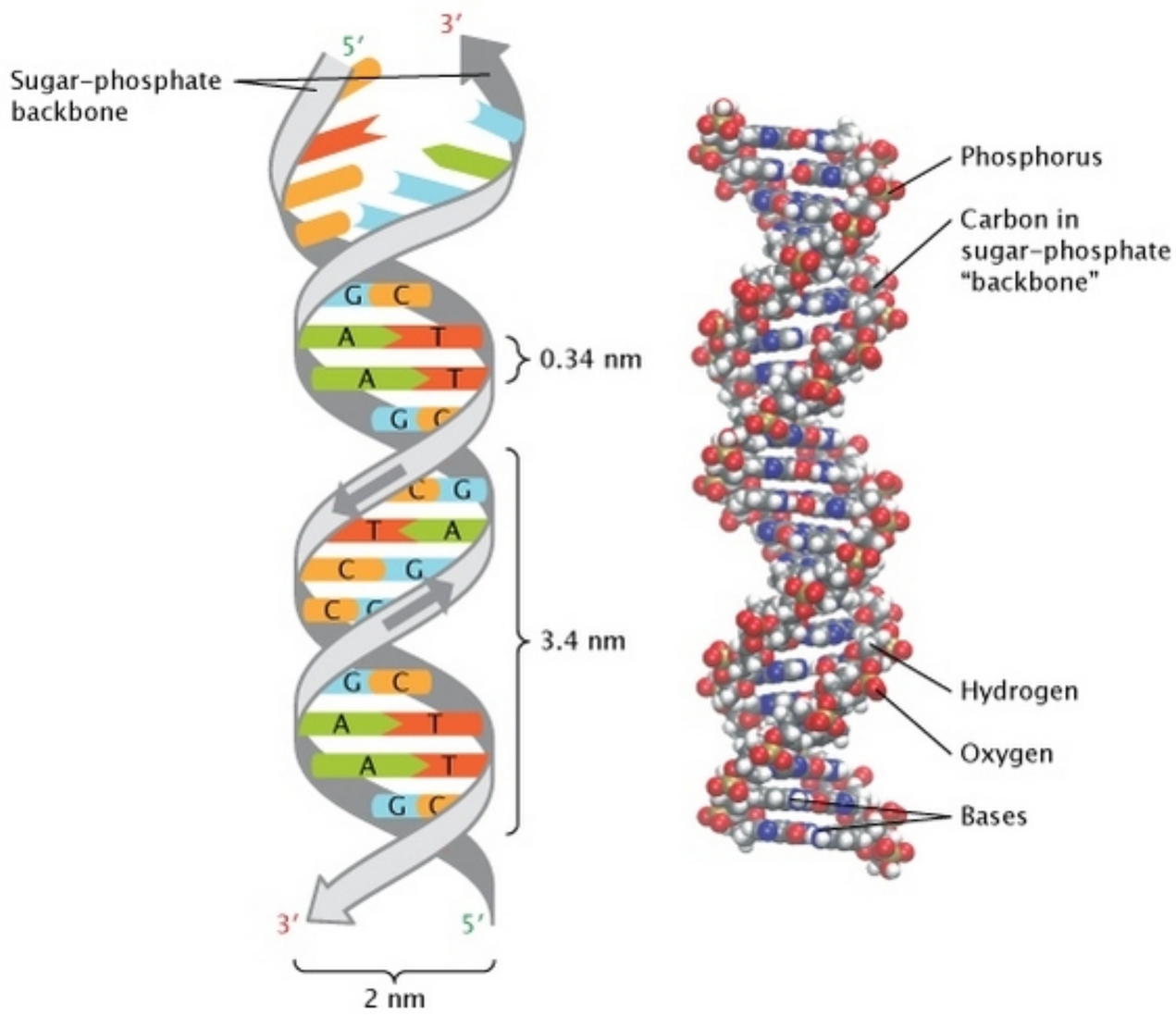
Double helix



Double helix-major and minor groves



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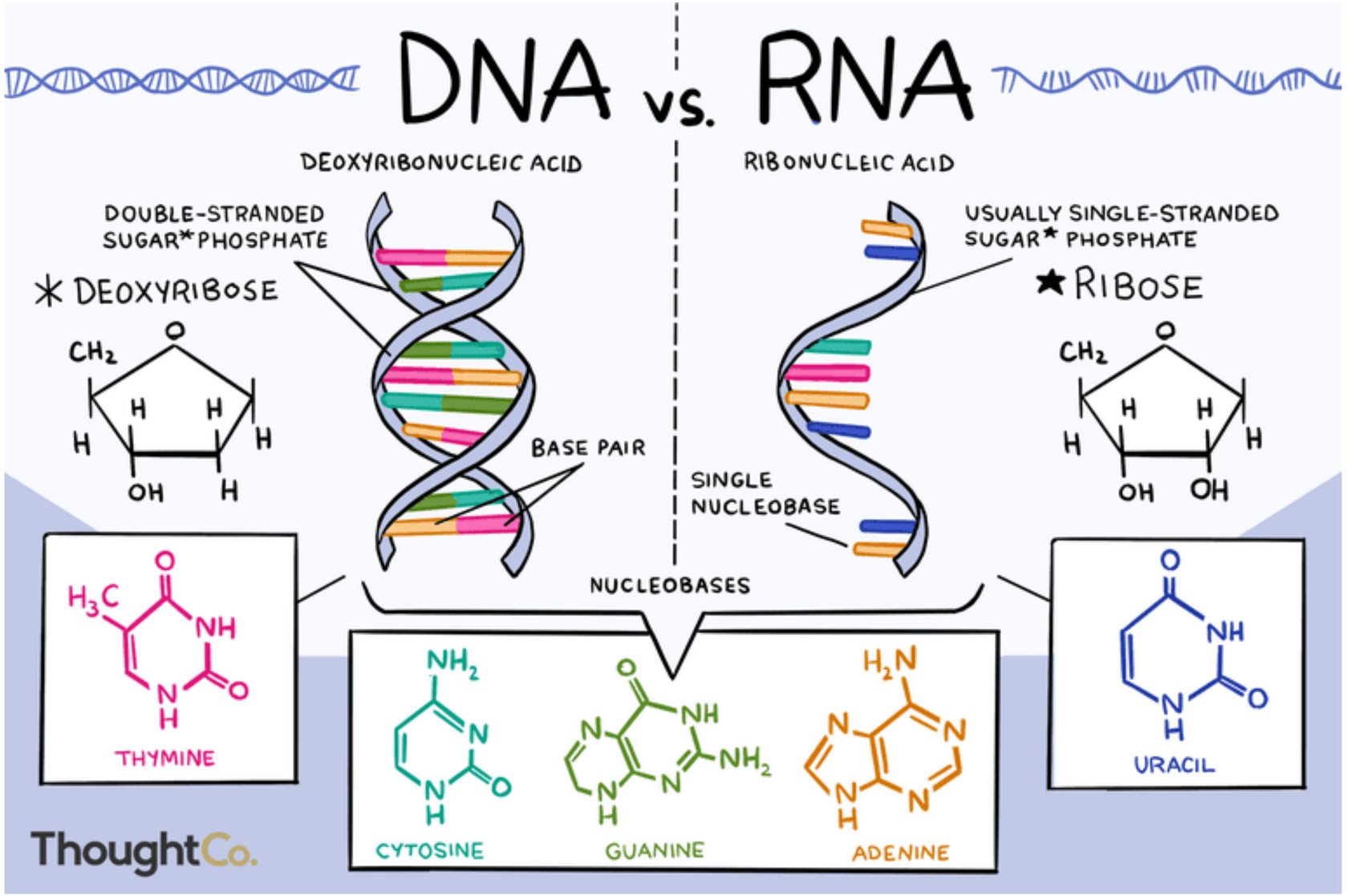
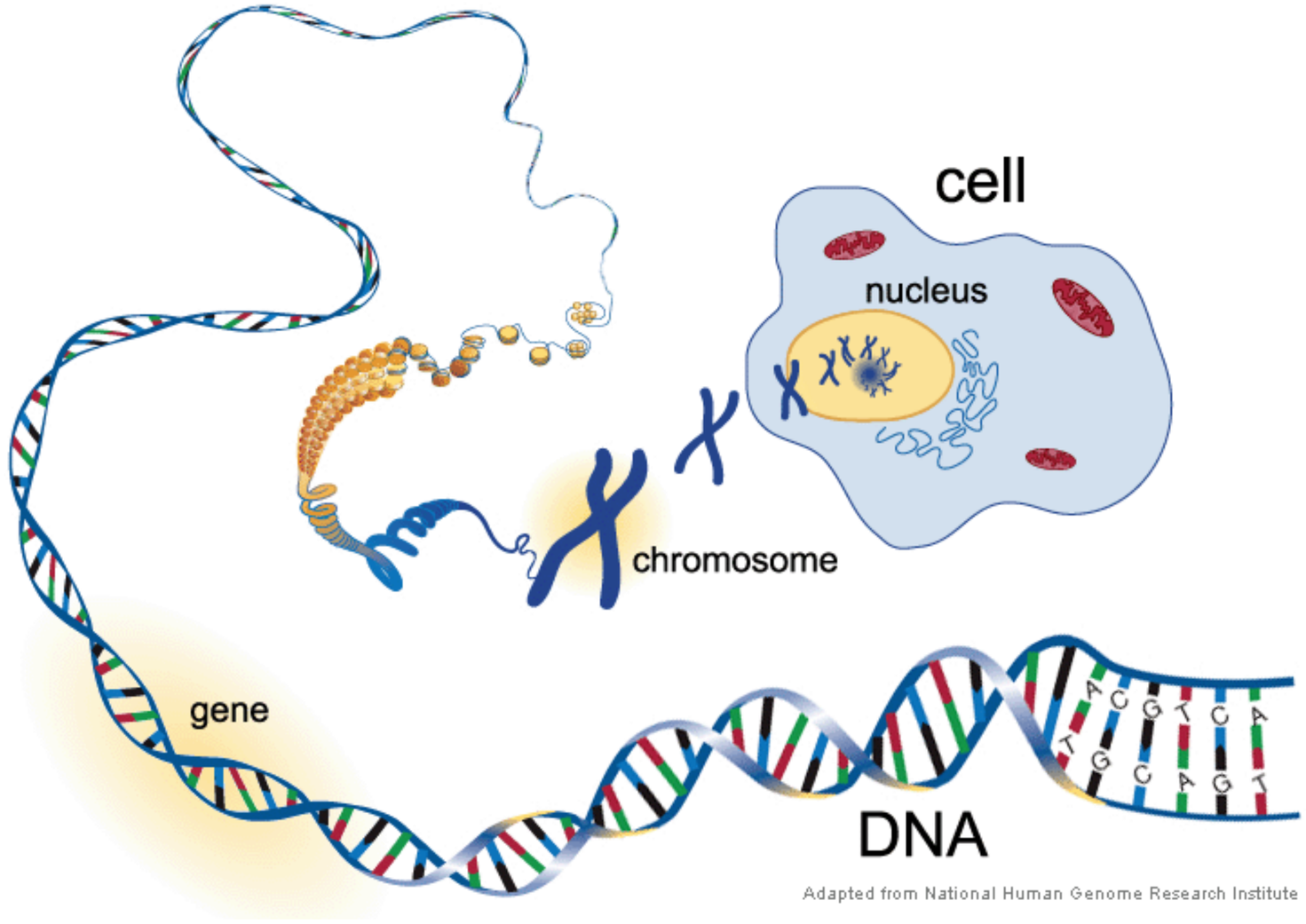
Ribonucleic acid (RNA)

DNA vs RNA



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

DNA in Eukaryotic cells

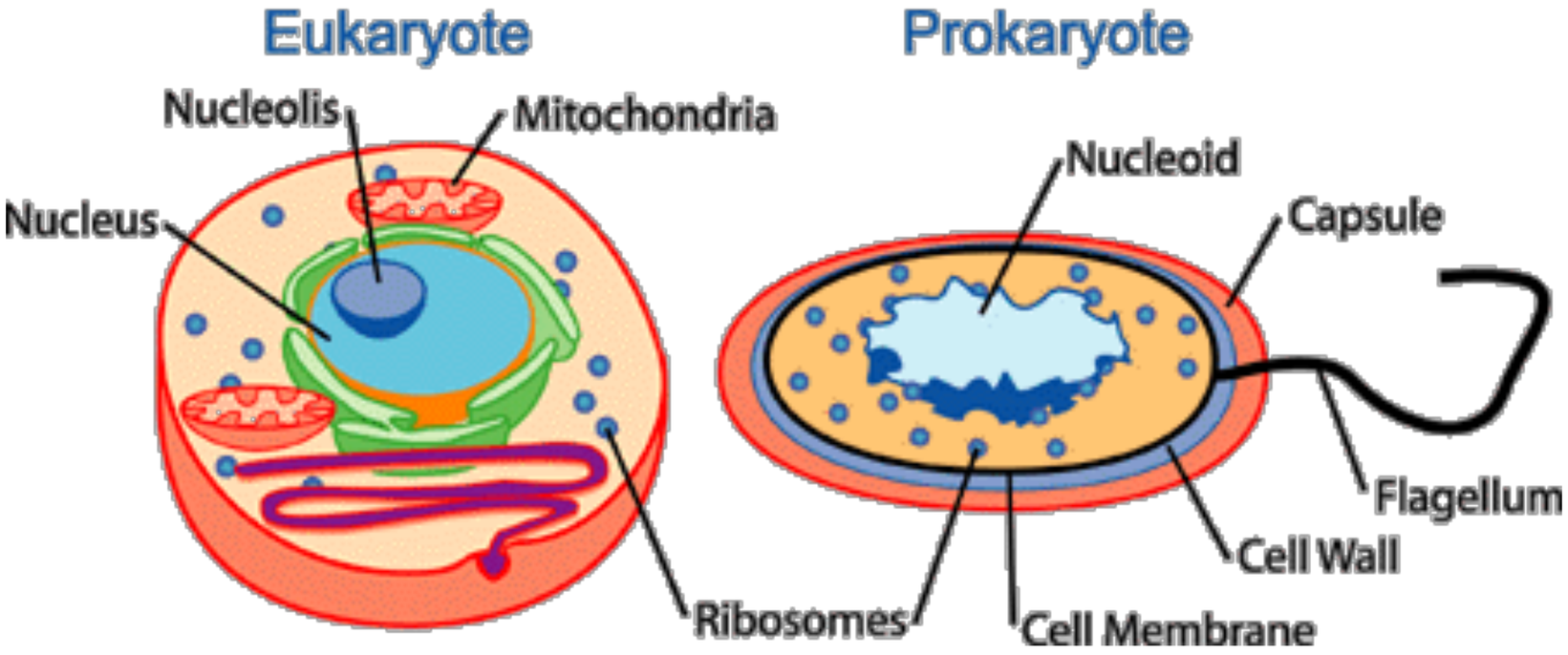


https://www.blendspace.com/lessons/mGZszATpjnNdOw/chromosomes

DNA in Prokaryotic cells

- The DNA in prokaryotes is contained in a central area of the cell called the nucleoid,
- Not surrounded by a nuclear membrane
- Many prokaryotes also carry small, circular DNA molecules called plasmids
- Plasmids are distinct from the chromosomal DNA and can provide genetic advantages in specific environments.

DNA in Eukaryotic vs Prokaryotic cells



https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/pro_and_eukaryotic.html

Questions/comments
?

What is PCR?

Polymerase chain reaction (**PCR**) is a method widely used to make billions of copies of a specific DNA sample by DNA amplification

It helps scientists to take a very small sample of DNA and amplify it to a large amount for detailed studies.

PCR was invented in 1983 by the American biochemist Kary Mullis at Cetus Corporation.

Steps in PCR

- Sample collection, preparation and storage
- Nucleic acid extraction (DNA/RNA extraction)
- Nucleic acid quantification
- DNA amplification
- PCR product qualitative and quantitative analysis (gel electrophoresis e.t.c)
- Result analysis and trouble shooting

Sample collection, preparation and storage

Sample colection, preparation and storage

- Collect tissues, cells, body fluids, cell cultures and broths. Minute quantities
- Avoid contamination with positive control material
- Keep wet samples at -20 degree centigrades, at -80 if longer than months, -196 (liquid nitrogen if longer)
- Wear gloves at all times

Nucleic acid (DNA/RNA) extraction and storage

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

Cell lysis methods

- Physical distruptions: “Freeze and thaw, Freeze and grind, bead beating and sonication
- Chemical methods: such as detergents (Sodium dodecyl suphate, triton X) and chaotropes (e.g., guanidine salts and alkaline solutions).
- Enzymatic methods for more structured samples such as tissues, plant cells, bacteria and yeast. Enzymes such as lysozyme, zymolase and, proteinase K, collagenase and lipase

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

2. Removal of cellular debris

This is to reduce carryover of unwanted materials (proteins, lipids and saccharides from cellular structures) into the purification reaction, which can clog membranes or interfere with downstream applications.

Usually clearing is accomplished by centrifugation, filtration or bead-based methods.

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

3. DNA isolation

This is the isolation and purification of DNA of interest by binding to matrices (silica, cellulose and ion exchange) or by solution based chemistries (e.g isopropanol)

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

4. Washing

Wash buffers generally contain alcohols and can be used to remove proteins, salts and other contaminants from the sample or the upstream binding buffers.

Alcohols additionally help associate nucleic acid with the matrix

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

5. Elution

DNA is soluble in low-ionic-strength solution such as TE buffer or nuclease-free water.

When such an aqueous buffer is applied to a silica membrane,

the DNA is released from the silica, and the eluate is collected.

5 steps in DNA extraction

1. Cell lysis
2. Removal of cellular debris
3. DNA isolation
4. DNA washing
5. DNA elution
6. DNA measurements

6. DNA measurements

DNA can be measured by different methods such as spectrophotometry.

This is done by the use of common spectrophotometer or different types of

Nanodrop machines.

Practical case study of extraction protocol

ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA™ Fungal/Bacterial Miniprep Kit

Catalog No. **D6005**

Highlights

- Simple, efficient isolation of DNA (up to 25 µg/prep) from all types of tough-to-lyse fungi (e.g., yeast) and bacteria in as little as 15 minutes.
- State-of-the-art, ultra-high density ***BashingBeads***™ are fracture resistant and chemically inert.
- Omits the use of organic denaturants as well as proteinases.

Practical case study of extraction protocol

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents

***Quick*-DNA™ Fungal/Bacterial Miniprep Kit** (Kit Size)	**D6005** (50 preps.)	**Storage Temperature**
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
BashingBead™ Buffer	40 ml	Room Temp.
Genomic Lysis Buffer[1]	100 ml	Room Temp.
DNA Pre-Wash Buffer[2]	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer	10 ml	Room Temp.
Zymo-Spin™ III-F Filters	50	Room Temp.
Zymo-Spin™ IICR Columns	50	Room Temp.
Collection Tubes	150	Room Temp.
Instruction Manual	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

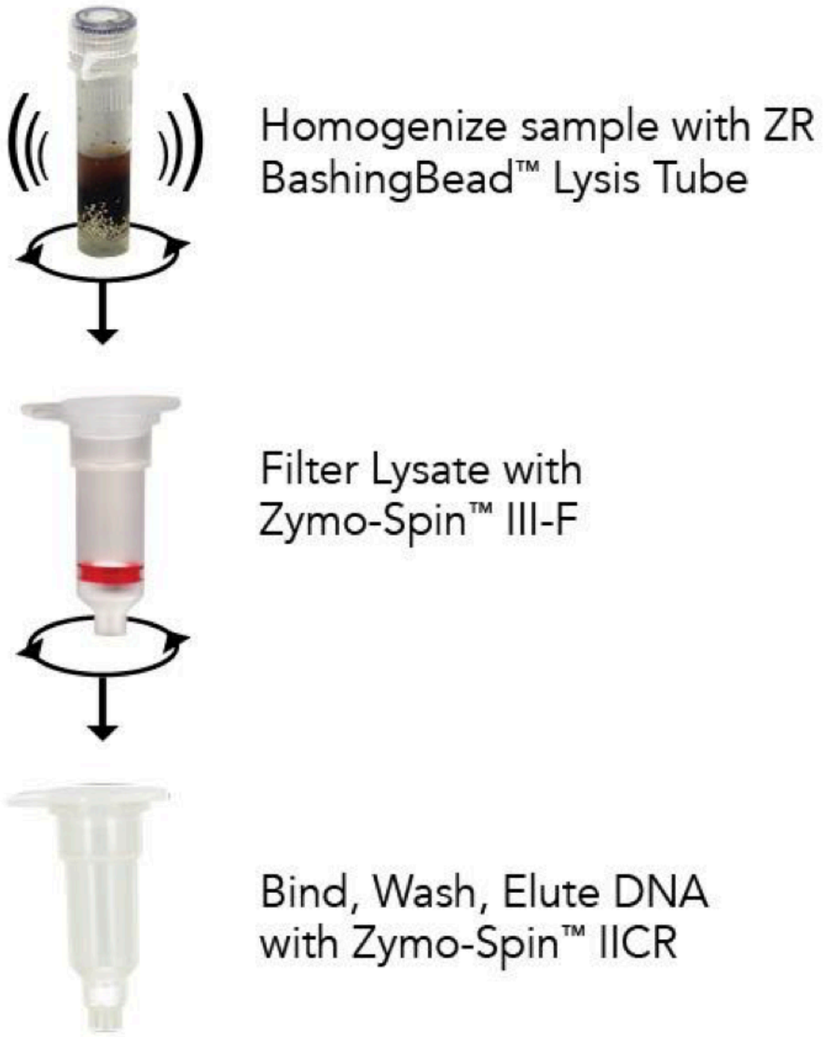
[1] For optimal performance, add beta-mercaptoethanol to 0.5%(v/v) i.e., 500 µl per 100 ml.

2 A precipitate may have formed in the DNA Pre-Wash Buffer during shipping. To completely resuspend the buffer, incubate the bottle at 30-37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Specifications

- **Format** – Bead Beating, Spin Column Purification
- **Sample Sources** – 50 - 100 mg (wet weight) fungi or bacteria; this equates to approximately 10^9 bacterial cells and 10^8 yeast cells. Spores, pollen, nematodes, as well as other microorganisms can also be sampled.

Practical case study of extraction protocol



Homogenize sample with ZR BashingBead™ Lysis Tube

Filter Lysate with Zymo-Spin™ III-F

Bind, Wash, Elute DNA with Zymo-Spin™ IICR

Practical case study of extraction protocol

Protocol

For optimal performance, add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5 %(v/v) *i.e.*, 500 µl per 100 ml.

1. Add 50 – 100 mg (wet weight) fungal or bacterial cells[1] that have been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) to a **ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm)**. Add 750 µl **BashingBead™ Buffer** to the tube[2].

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.

   Note: *Required processing time will vary depending on the device and application and therefore should be evaluated on a case by case basis.*

   For example, processing times may be as little as 3 minutes when using high-speed cell disrupters (e.g., the portable TerraLyzer™Sample Processor, FastPrep® -24, or similar) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie™, or standard benchtop vortexes). See manufacturer's literature for operating information.

3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge at 10,000 x *g* for 1 minute.

4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ III-F Filter** in a **Collection Tube** and centrifuge at 8,000 x *g* for 1 minute.

5. Add 1,200 µl of **Genomic Lysis Buffer** to the filtrate in the Collection Tube from Step 4.

Practical case study of extraction protocol

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IICR Column**[3] in a Collection Tube and centrifuge at 10,000 x *g* for 1 minute.

7. Discard the flow through from the Collection Tube and repeat Step 6.

8. Add 200 µl **DNA Pre-Wash Buffer** to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuge at 10,000 x *g* for 1 minute.

9. Add 500 µl **g-DNA Wash Buffer** to the Zymo-Spin™ IICR Column and centrifuge at 10,000 x *g* for 1 minute.

10. Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (35 µl minimum) **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x *g* for 30 seconds to elute the DNA.

Ultra-pure DNA is now ready for use in your experiments.

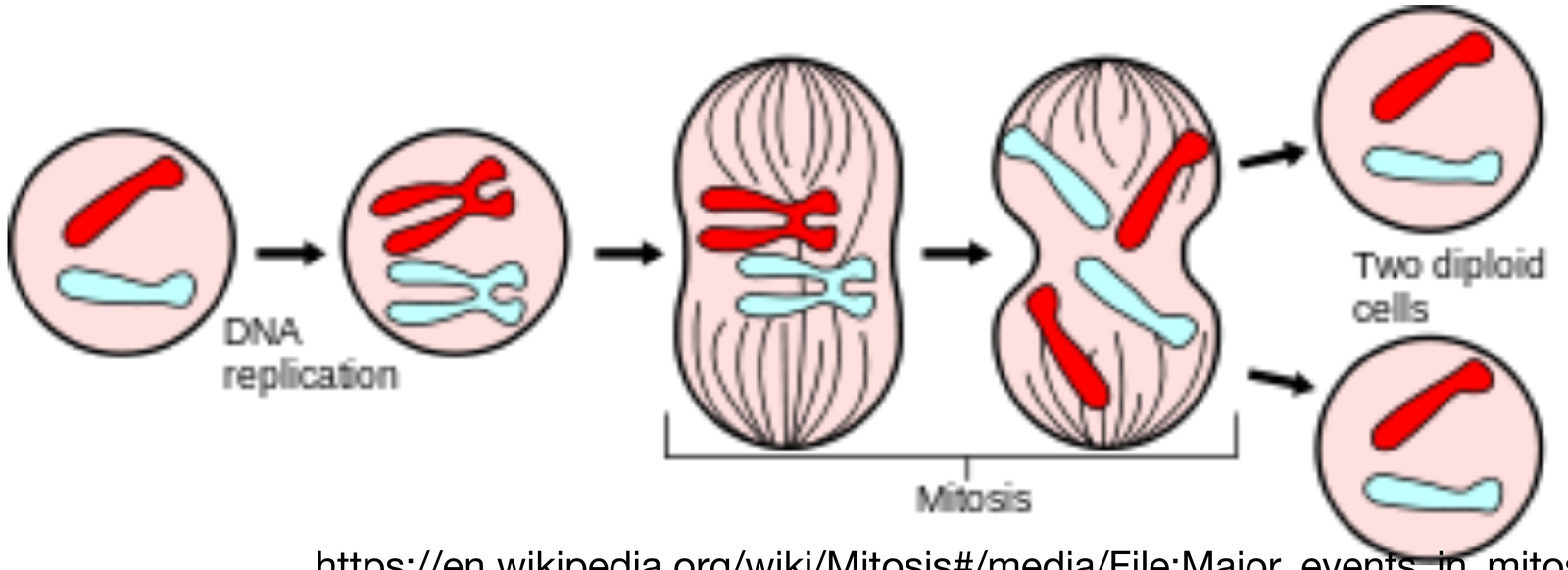
Questions or comments?

DNA replication and amplification

- DNA replication occurs in nature
- DNA amplification (copying) is done by scientists *in-vitro*
- *In-vitro* DNA amplification simulates DNA replication in nature

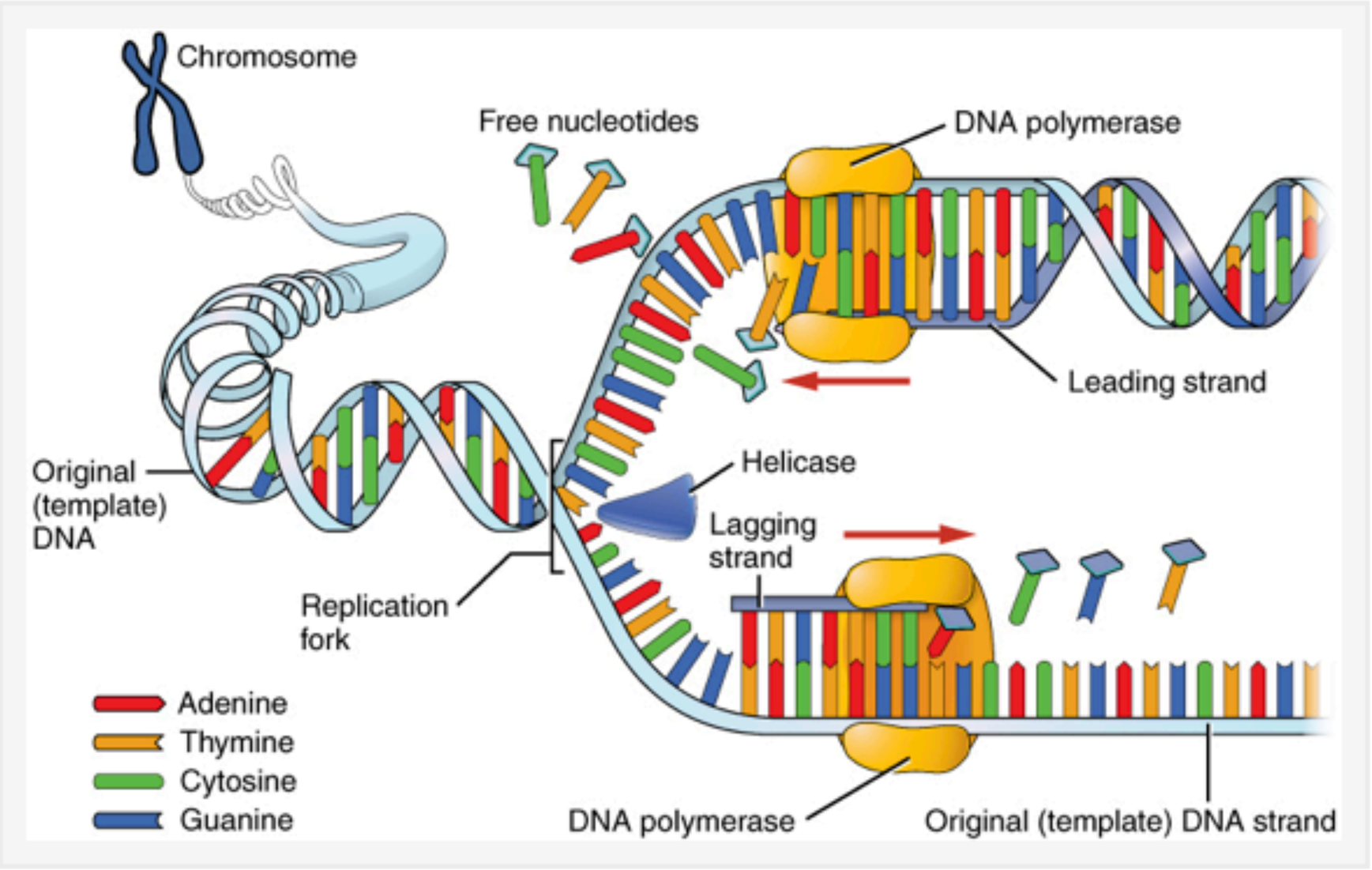
DNA replication and amplification

- DNA replication is needed for chromosome doubling
- Chromosome doubling is needed for cell doubling
- Remember mitosis
- DNA replication occurs during S phase of mitosis

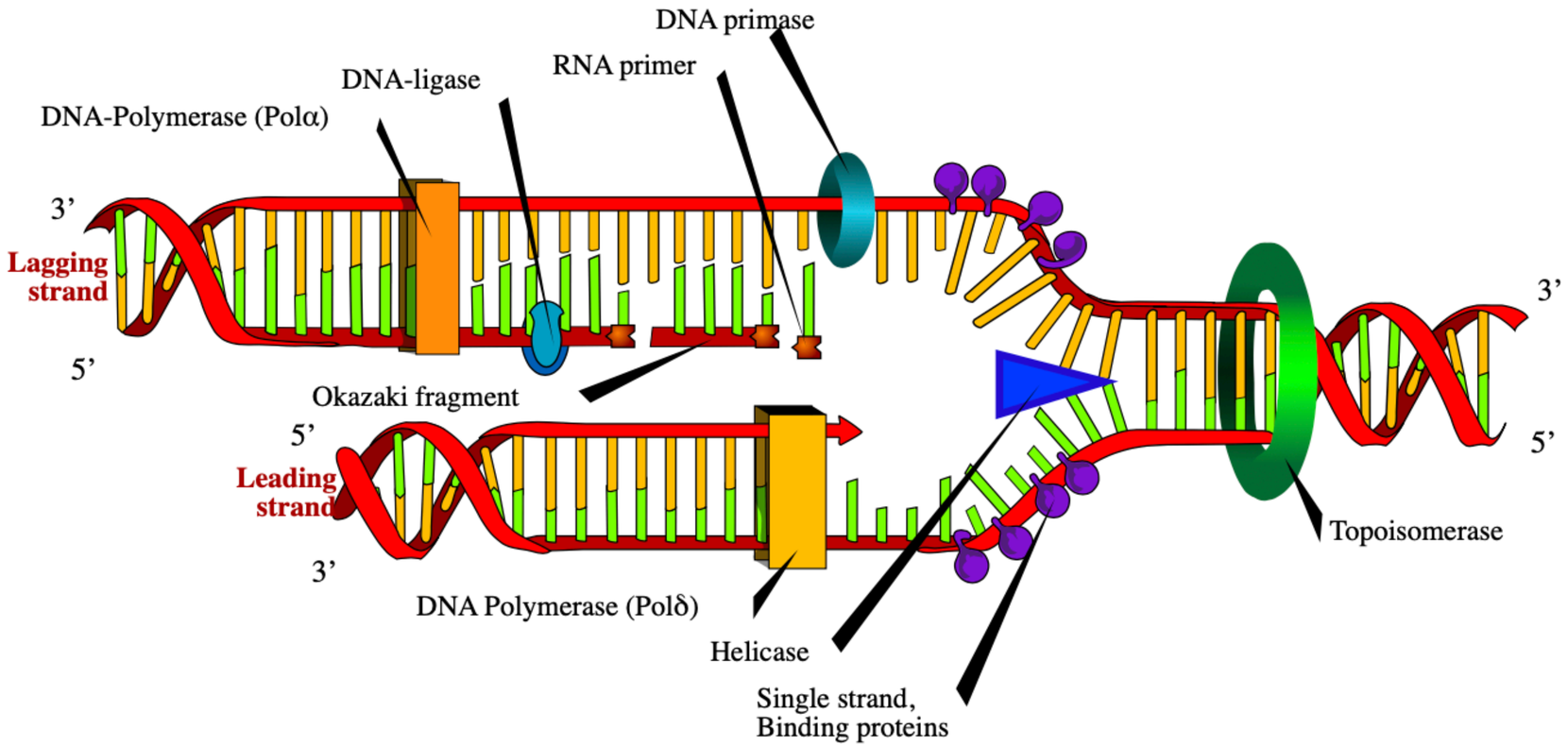


https://en.wikipedia.org/wiki/Mitosis#/media/File:Major_events_in_mitosis.svg

How DNA replication occurs in nature (Eukaryotes)



How DNA replication occurs in nature (prokaryotes)



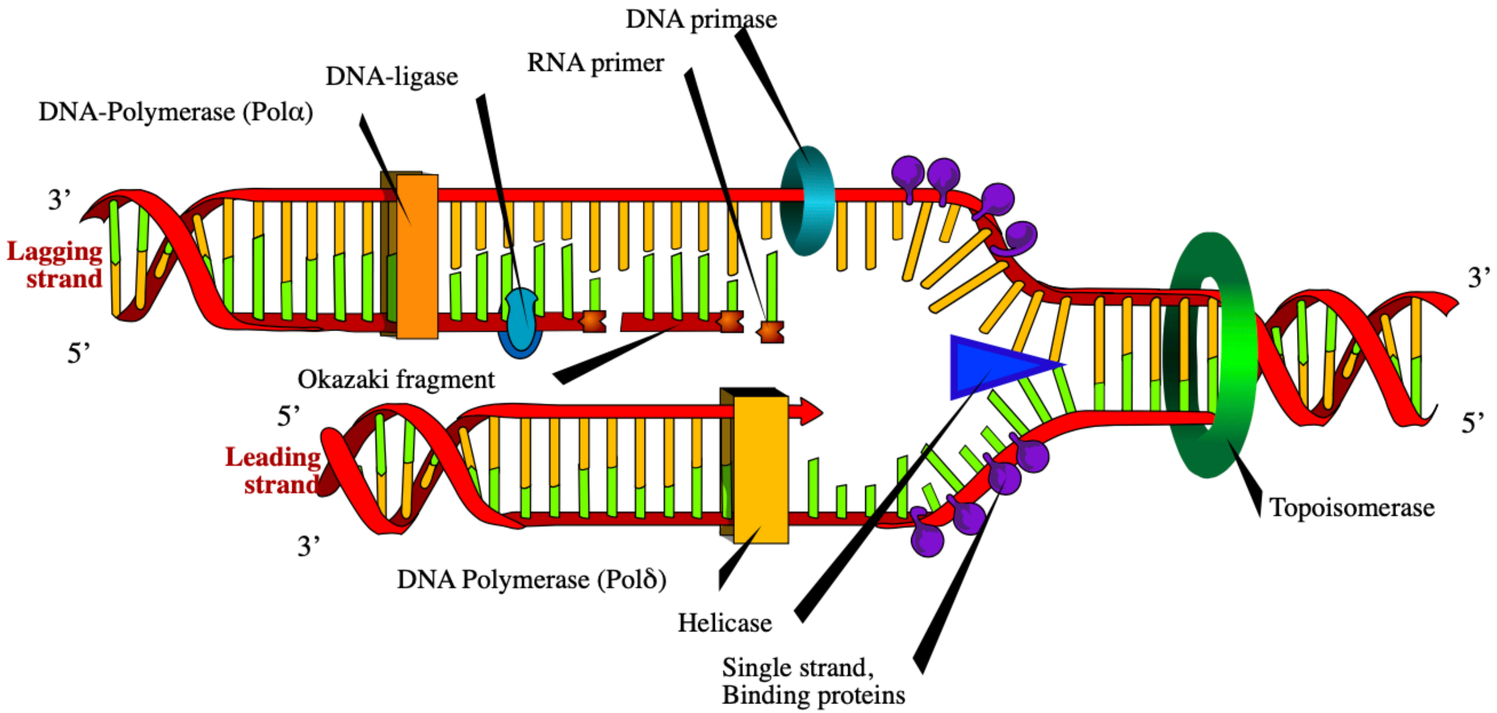
A replication fork is formed

Helicase separates the DNA strands at the origin of replication

Topoisomerase breaks and reforms DNA’s phosphate backbone ahead of the replication fork,

https://courses.lumenlearning.com/boundless-biology/chapter/dna-replication/

How DNA replication occurs in nature (prokaryotes)



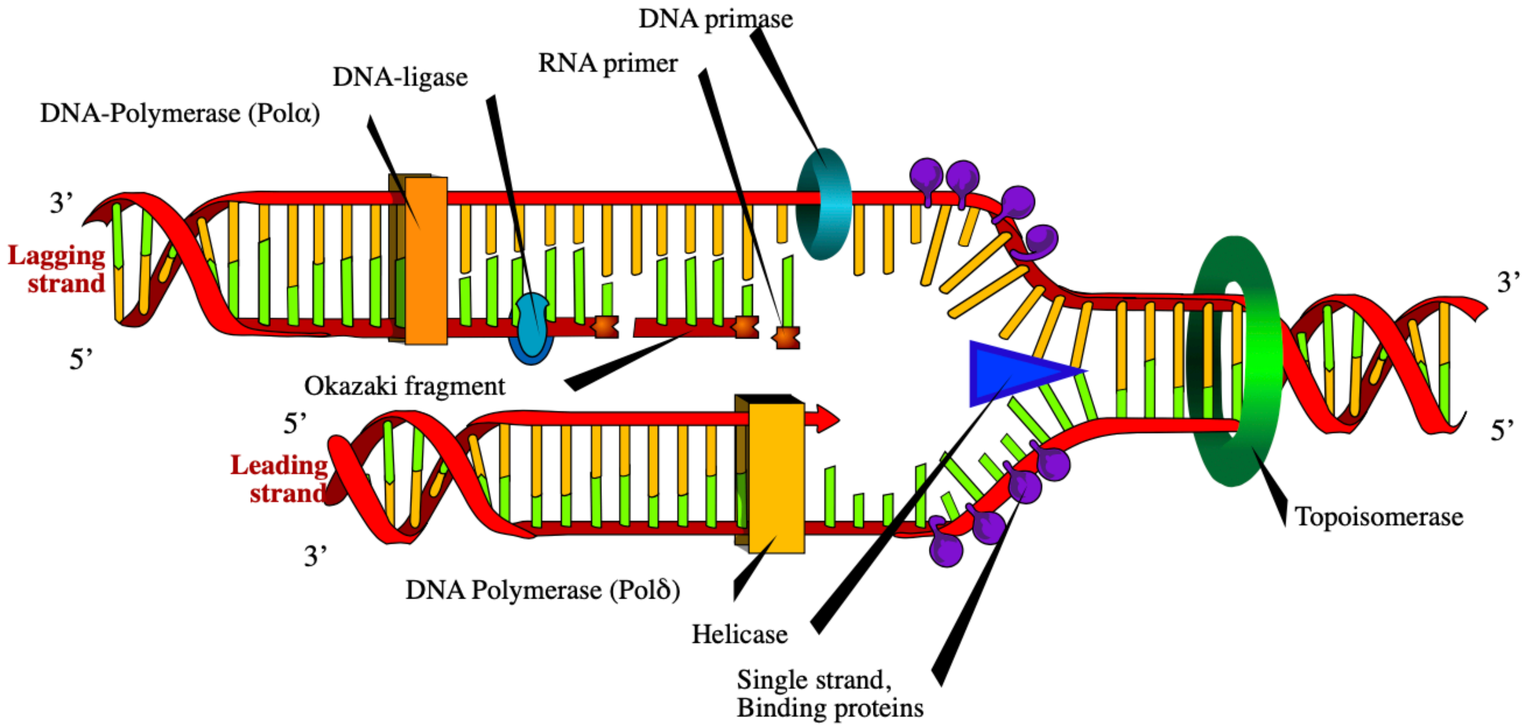
Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming

Primase synthesizes an RNA primer.

DNA polymerase III uses this primer to synthesize the daughter DNA strand.

https://courses.lumenlearning.com/boundless-biology/chapter/dna-replication/

How DNA replication occurs in nature (prokaryotes)



On the leading strand, DNA is synthesized continuously, whereas on the lagging strand,

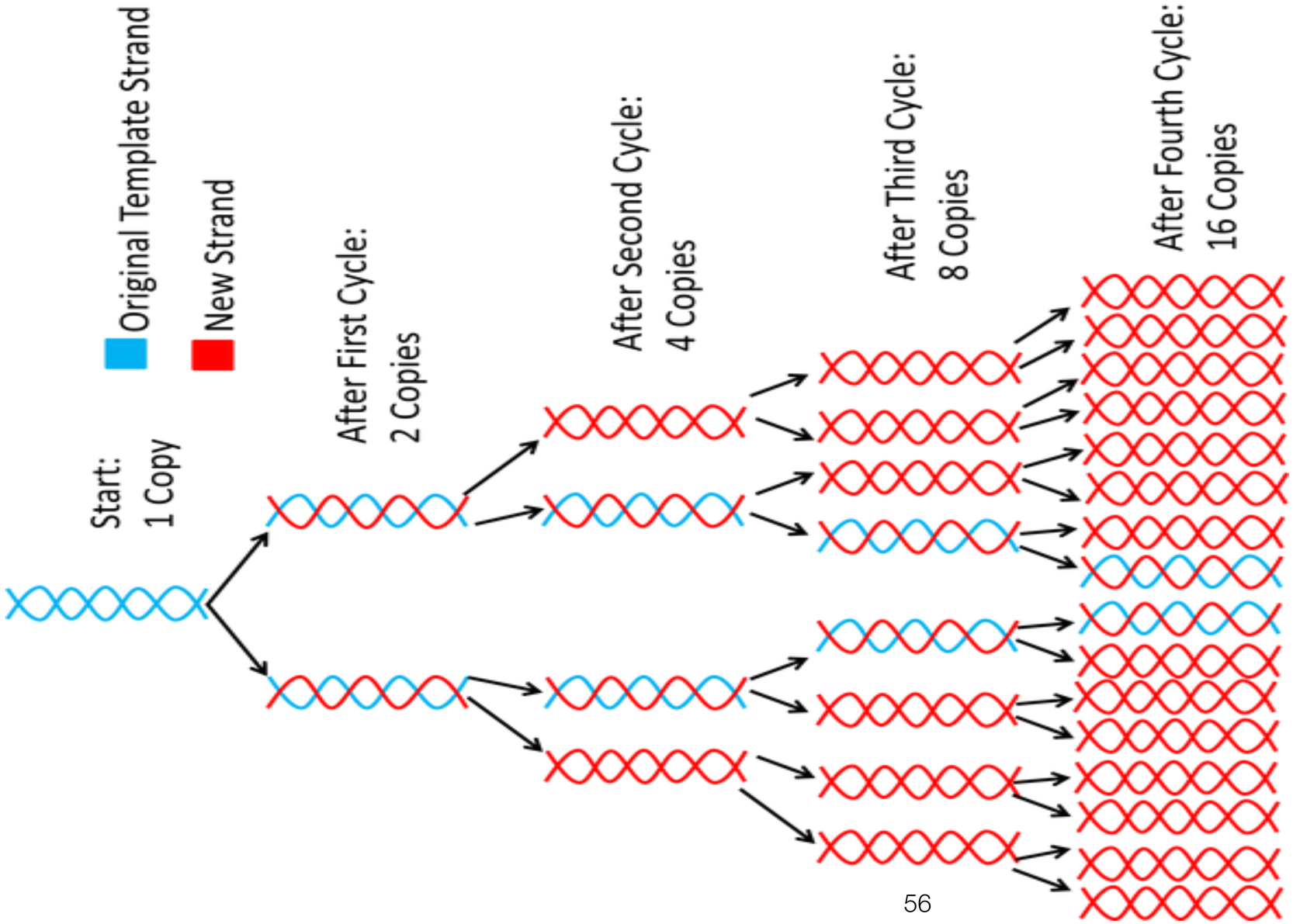
DNA is synthesized in short stretches called Okazaki fragments.

DNA polymerase I replaces the RNA primer with DNA.

DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule.

https://courses.lumenlearning.com/boundless-biology/chapter/dna-replication/

DNA amplification *in-vitro*



It is the production of multiple copies of a sequence of DNA.

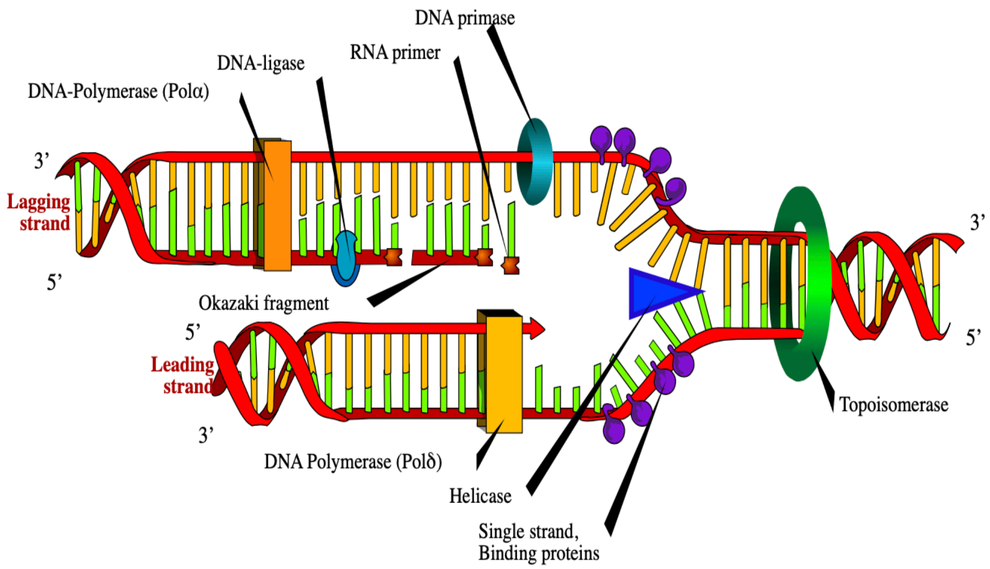
Repeated copying of a piece of DNA.

It is called Polymerase Chain Reaction, PCR

How DNA amplification *in-vitro* works

It is similar to the in-vivo replication mechanism, you need:

1. A template DNA (product of your extraction)
2. Heat source to open the DNA helix (or with enzyme)
3. Primers to begin the replication
4. **Polymerase enzyme**
5. Nucleotide units
6. Buffers containing vital elements ($Mg^{2}+$, Cl^{-})
7. To repeat the process many times



How DNA amplification *in-vitro* works

2. Heat source to open the DNA helix e.g PCR machine

How DNA amplification *in-vitro* works

1. A template DNA (product of your extraction)
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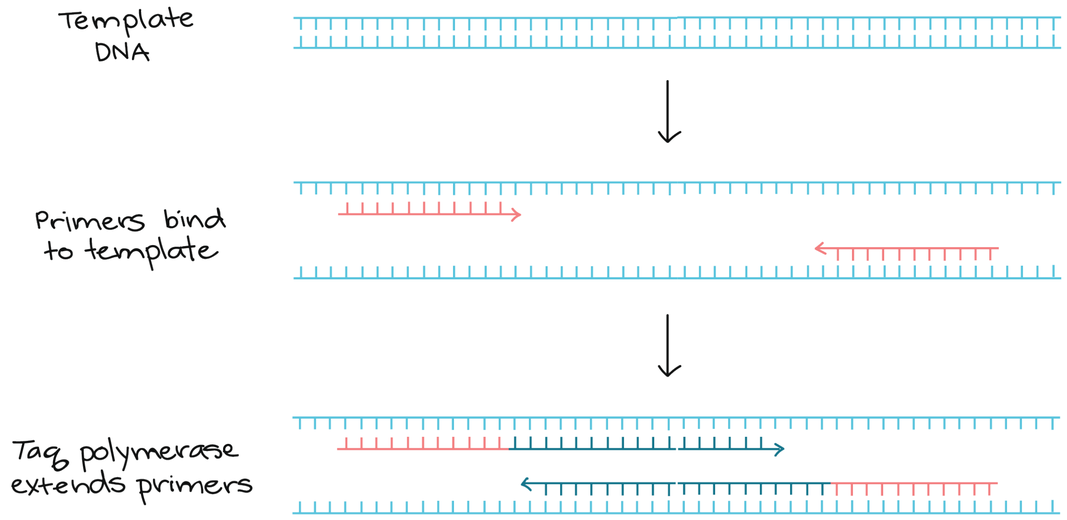
3. Primers to begin the replication



How DNA amplification *in-vitro* works

- A primer is a short, single-stranded DNA sequence used in the PCR technique.
- In the PCR method, a pair of primers is used to hybridize with the sample DNA and define the region of the DNA that will be amplified.
- Primers are also referred to as oligonucleotides.
- They are artificially sysnthesized
- There are **Forward and reverse primers**
- **Written from 5’ to 3’ always**

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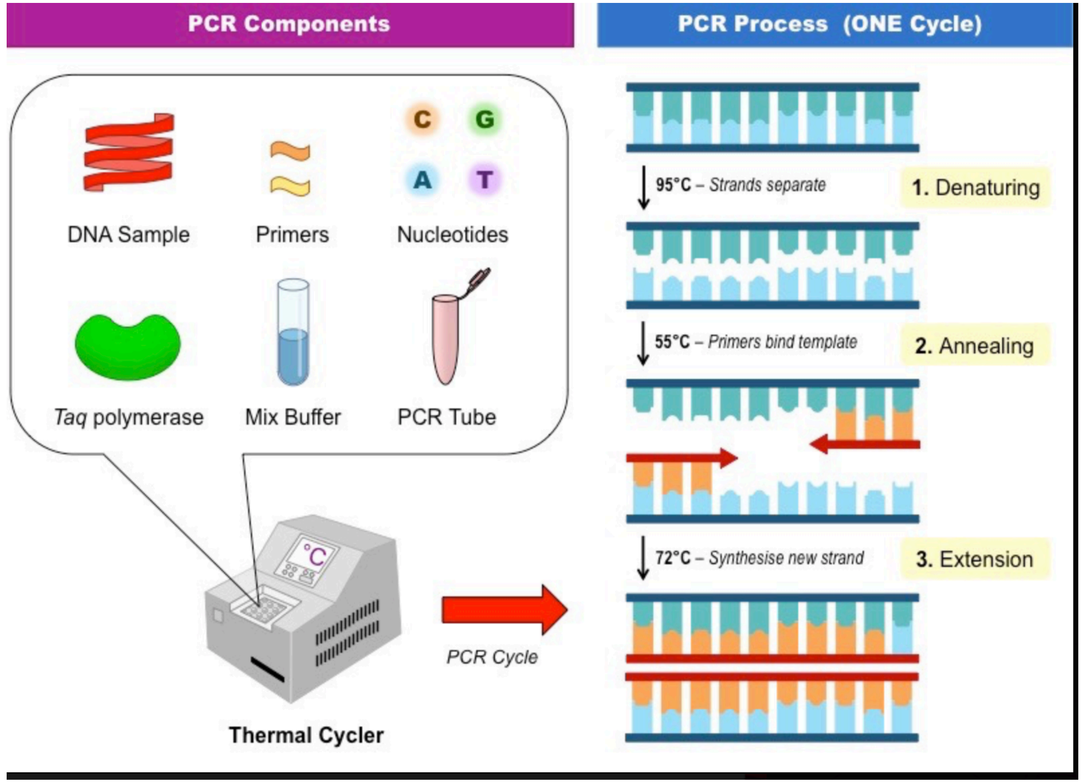
6. To repeat the process many times



How DNA amplification *in-vitro* works

1. A template DNA (product of your extraction)
2. Heat source to open the DNA helix (or with enzyme)
3. Primers to begin the replication
4. **Polymerase enzyme**
5. Nucleotide units
6. Buffers containing vital elements ($Mg^{2}+$, Cl^{-})
7. To repeat the process many times

Taq polymerase

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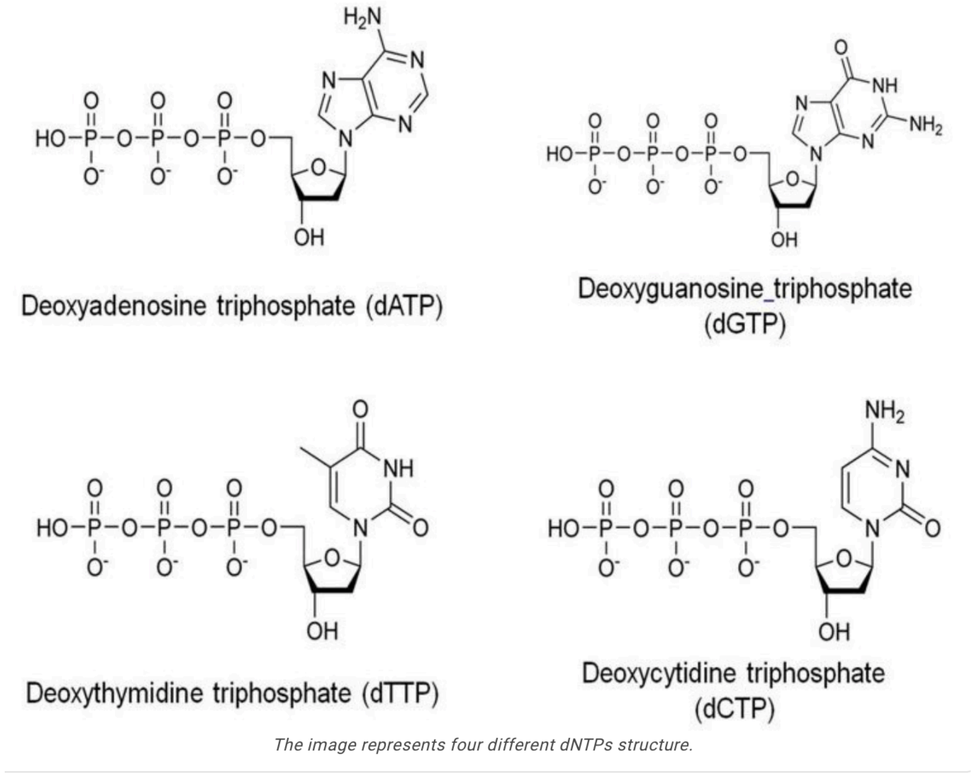
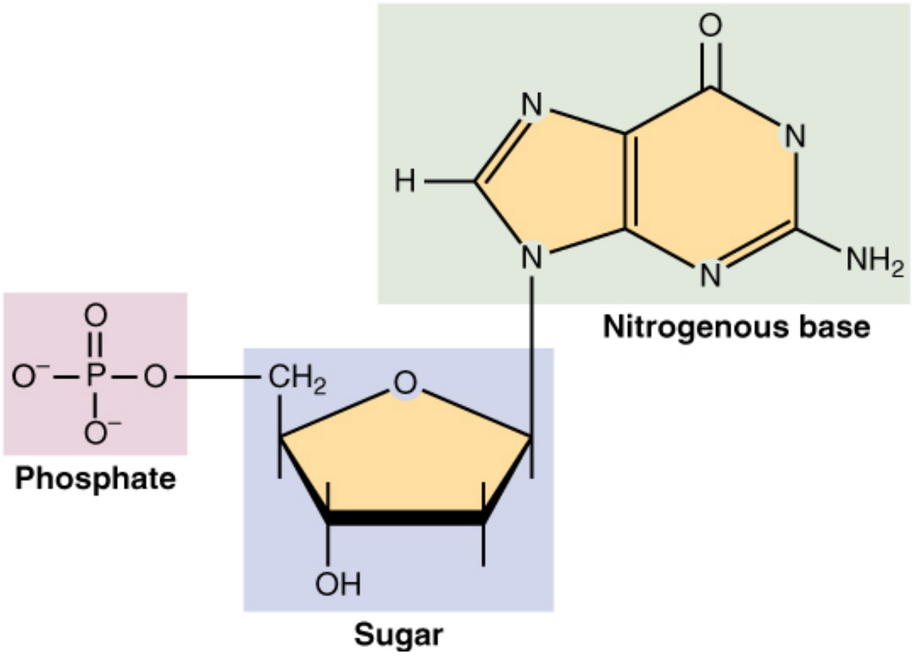
Nucleotide phosphate vs Nucleotide Triphosphates



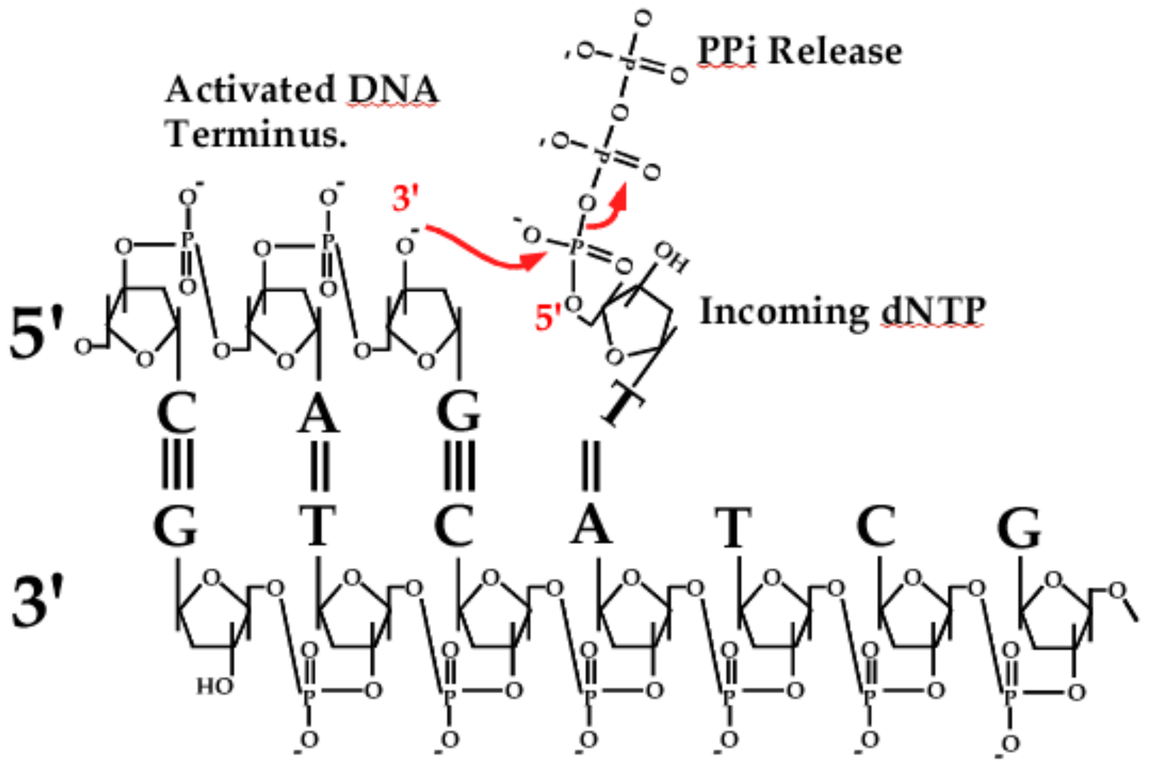
The image represents four different dNTPs structure.



Triphosphates

Mono-phosphates

Chemistry of DNA polymerases, how it works



Polymerase matches the dNTP to DNA (in this case, incoming dT to template dA), then activates the 3′ OH of one DNA strand and attaches it to the 5′ triphosphate of the incoming dNTP. The reaction releases two phosphates and adds one nucleotide (dNMP) to the DNA.

How DNA amplification *in-vitro* works

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Steps in PCR experiment

1. DNA denaturation
2. Primer annealing
3. Extention

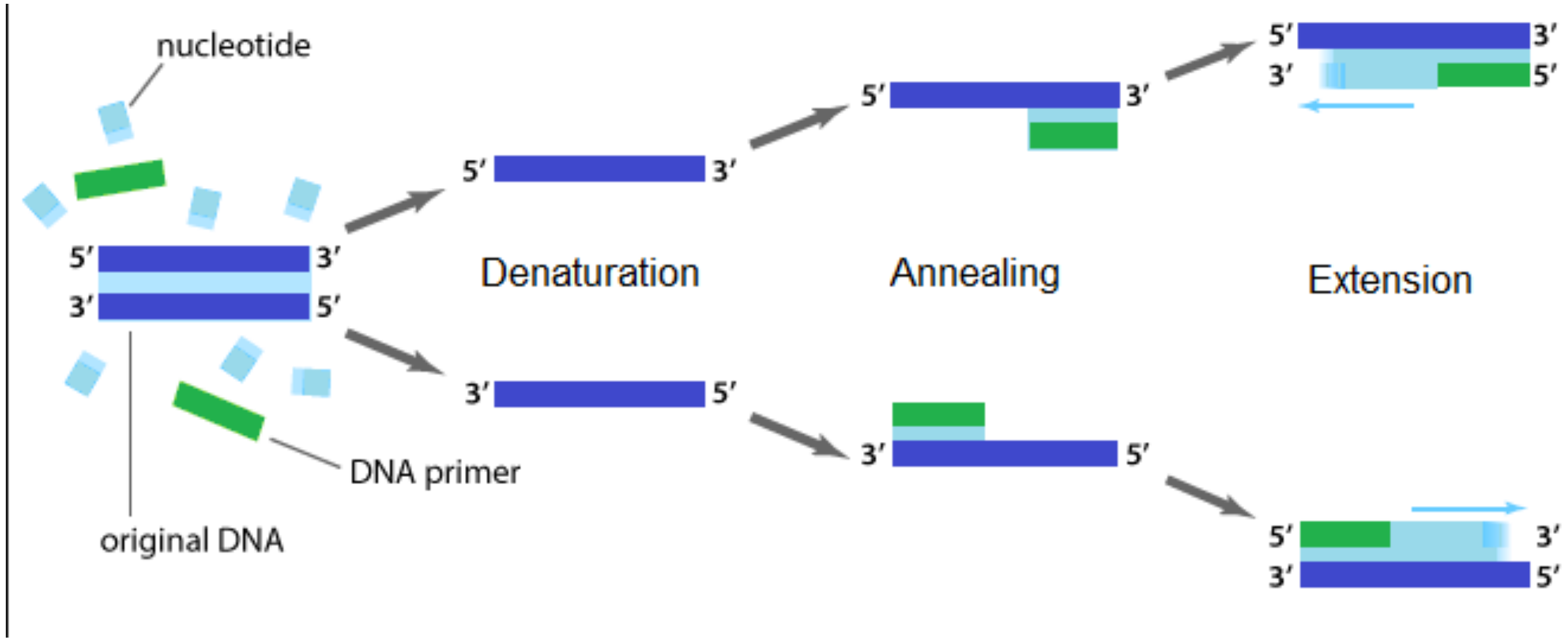
Steps in PCR experiment

(1) **denaturation**, in which double-stranded DNA templates are heated to separate the strands;

(2) **annealing**, in which short DNA molecules called primers bind to flanking regions of the target DNA

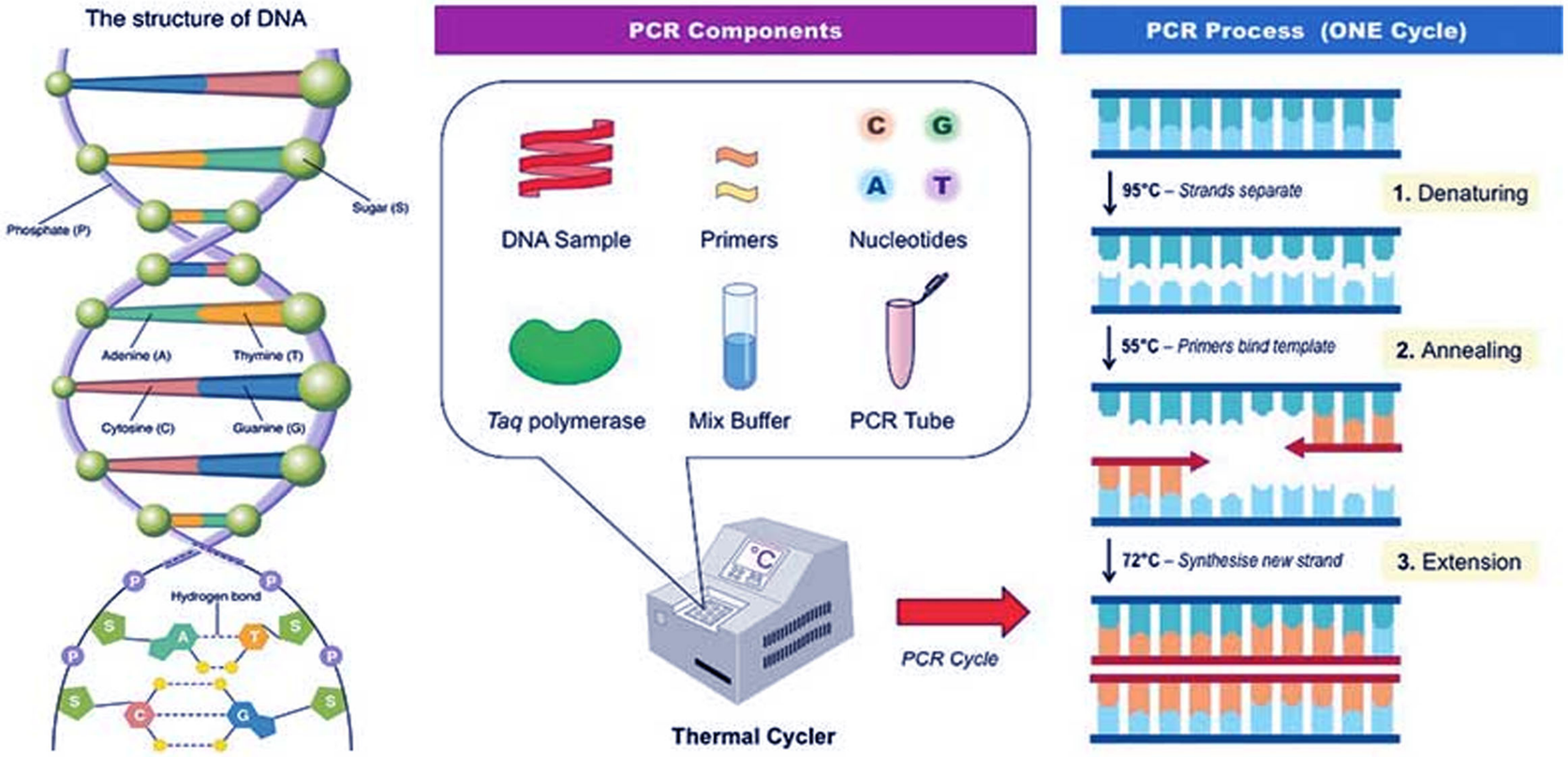
(3) **extension**, in which DNA polymerase extends the 3′ end of each primer along the template strands. These steps are repeated (“cycled”) 25–35 times to exponentially produce exact copies of the target DNA

Steps in PCR experiment



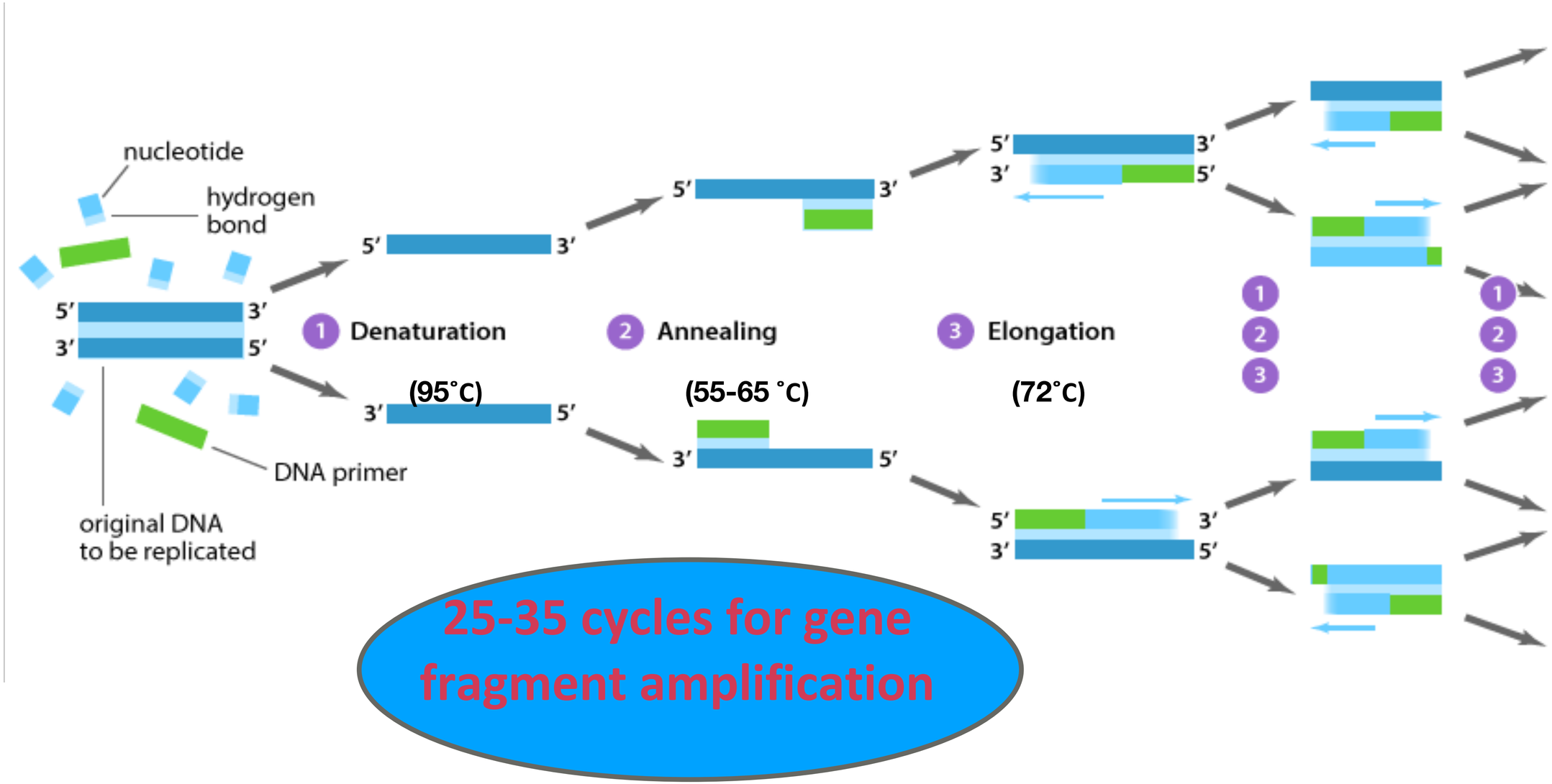
https://www.toppr.com/ask/question/name-the-three-important-steps-involved-in-pcr-process/

Steps in PCR experiment



https://rajusbiology.com/polymerase-chain-reaction-notes/

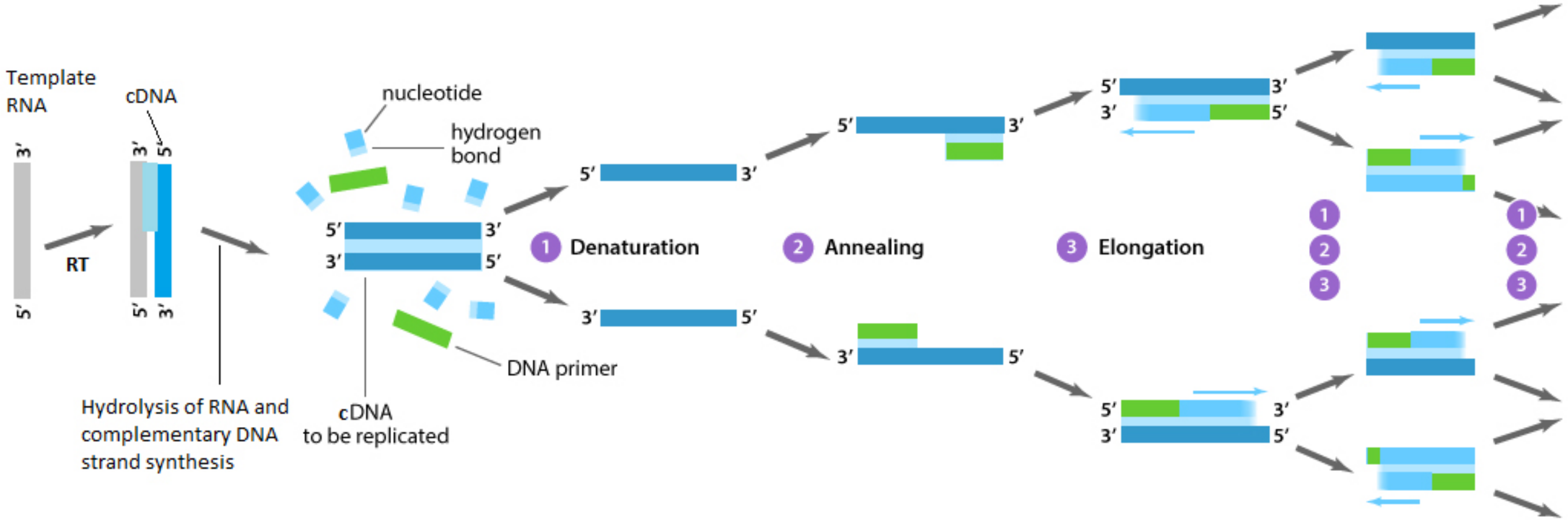
Basic steps in a PCR reaction



25-35 cycles for gene fragment amplification

https://benchling.com/protocols/GP1fQ08o/pcr-amplification-of-gfp/sbs

PCR vs Reverse transcription PCR



REVERSE TRANSCRIPTION PCR

http://laboratorytests.org/covid-19-rt-pcr/

Review questions

1. What are the components of DNA
2. Why is DNA negatively charged
3. Why is DNA acidic
4. What is the meaning of 3 prime and 5 prime ends
5. What is the correct orientation of DNA
6. What enzyme is needed for PCR in vitro
7. What are the stages of PCR
8. What is a thermocycler
9. What is a primer, what is the difference between forward and reverse primer

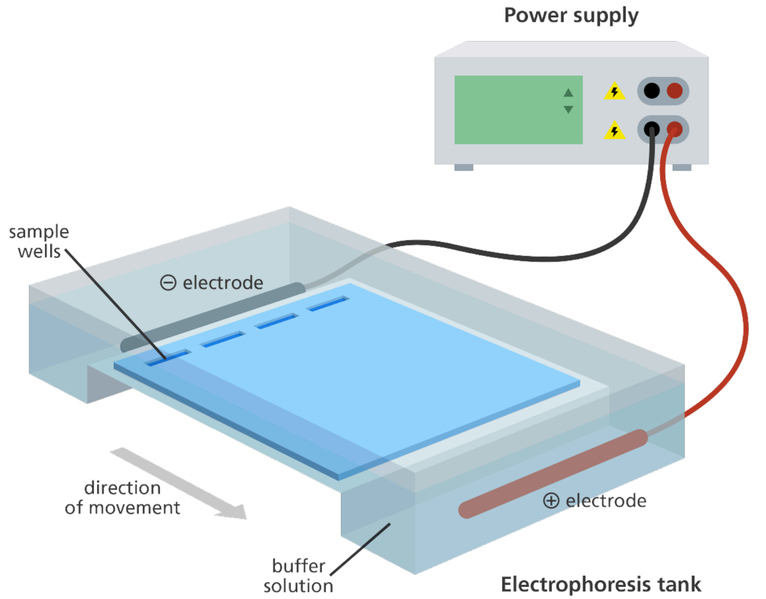
Qualitative analysis of PCR
(Gel electrophoresis)

What is gel electrophoresis

‘Electro’=electricity. ‘Phoresis’ =movement

Electrophoresis= movement in an elctric field

- Agarose gel electrophoresis is a method to separate DNA or RNA molecules by size.
- This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis).



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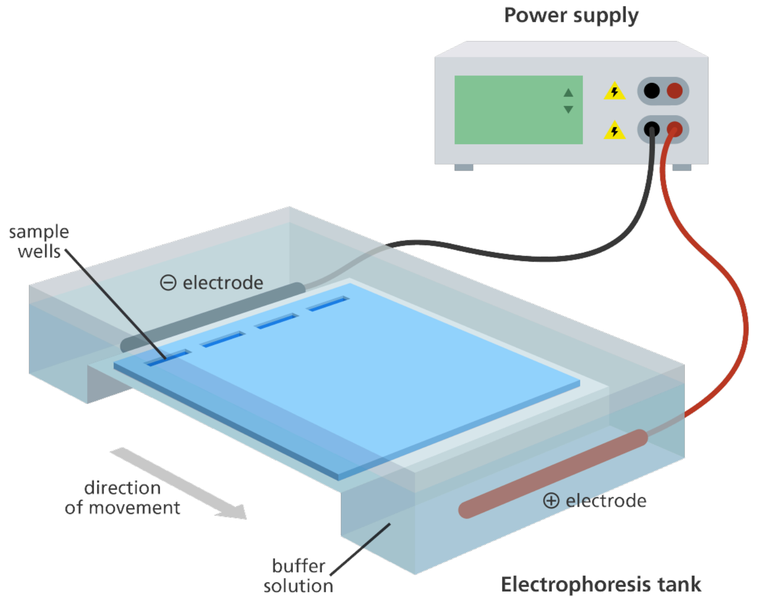


What is DNA gel electrophoresis

Gel Electrophoresis is a process where an electric current is applied to DNA samples creating fragments that can be used for comparison between DNA samples.

Things needed for DNA Gel electrophoresis?

1) DNA amplicons

2) Agarose gel and casting trays/comb

3) Electrolyte buffers

4) Electric current power source

5) DNA ladder/ ruler

6) DNA loading dye

Steps in Gel electrophoresis

1) DNA is extracted.

2) Isolation and amplification of DNA is performed.

3) DNA is added to the gel wells .

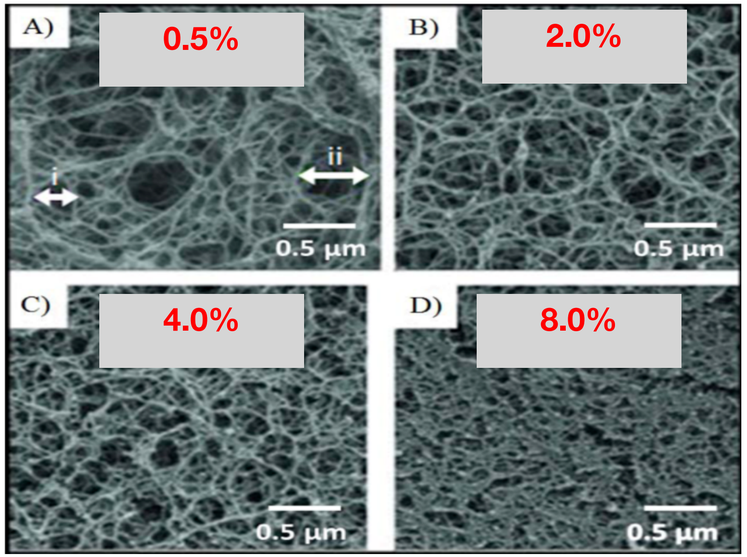
4) Electric current applied to the gel.

5) DNA bands are separated by size.

6) DNA bands are stained

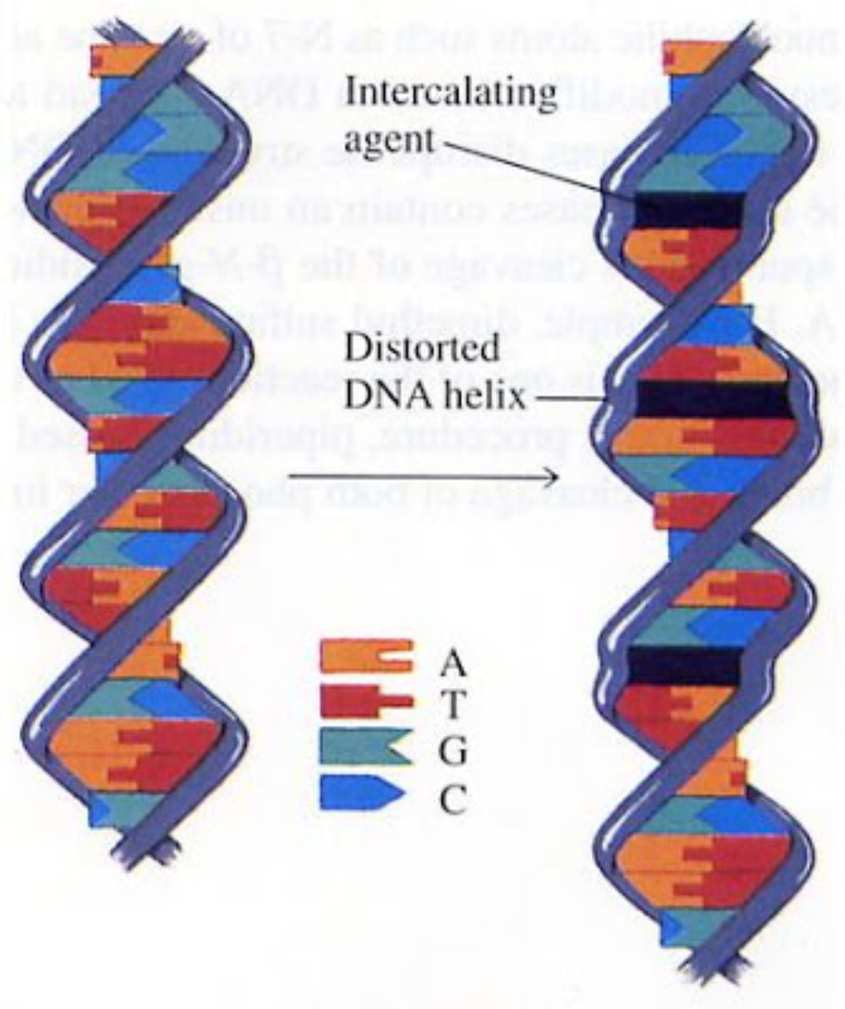
Principles of agarose gel electrophoresis

- a polysaccharide derived from red algae
- polymerizes to form a gel matrix
- act as a sieve for separating DNA molecules
- gel concentration is inversely proportional to pore size



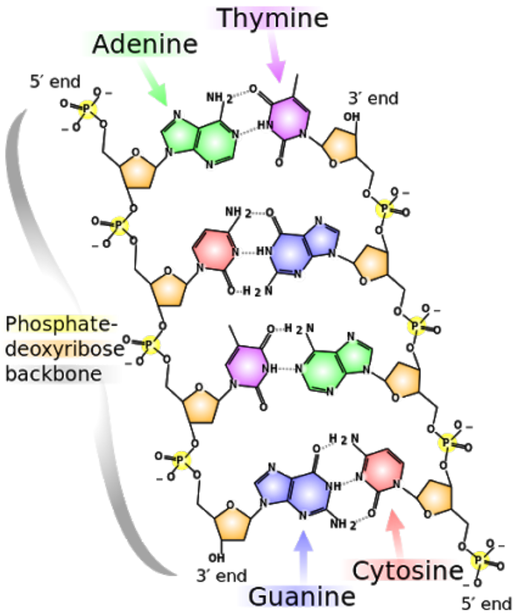
Principles of agarose gel electrophoresis

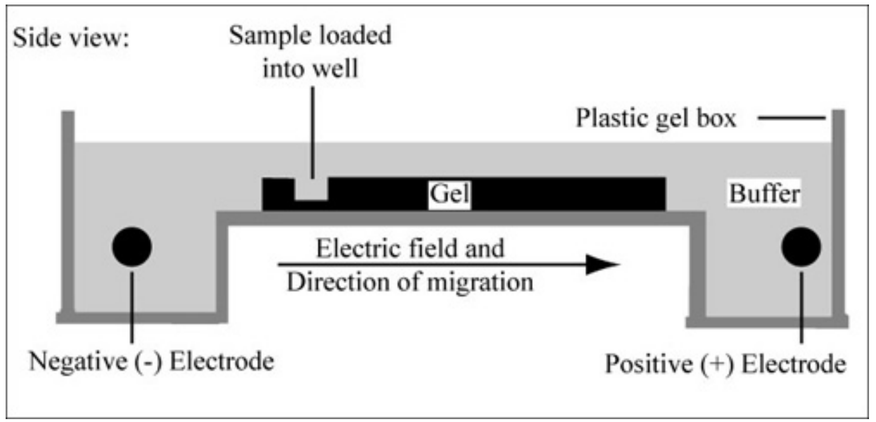
- The agarose gel is pre or post stained stained with a fluorescent dye that has affinity for DNA helix
- The dye will intercalates within the gel matrix
- e.g ethidium bromide, Gelred stain, sybergreen etc.
- The dyes can be viewed under ultraviolet light rays
- They can be viewed under the UV Transilluminator
- The DNA only will be visible



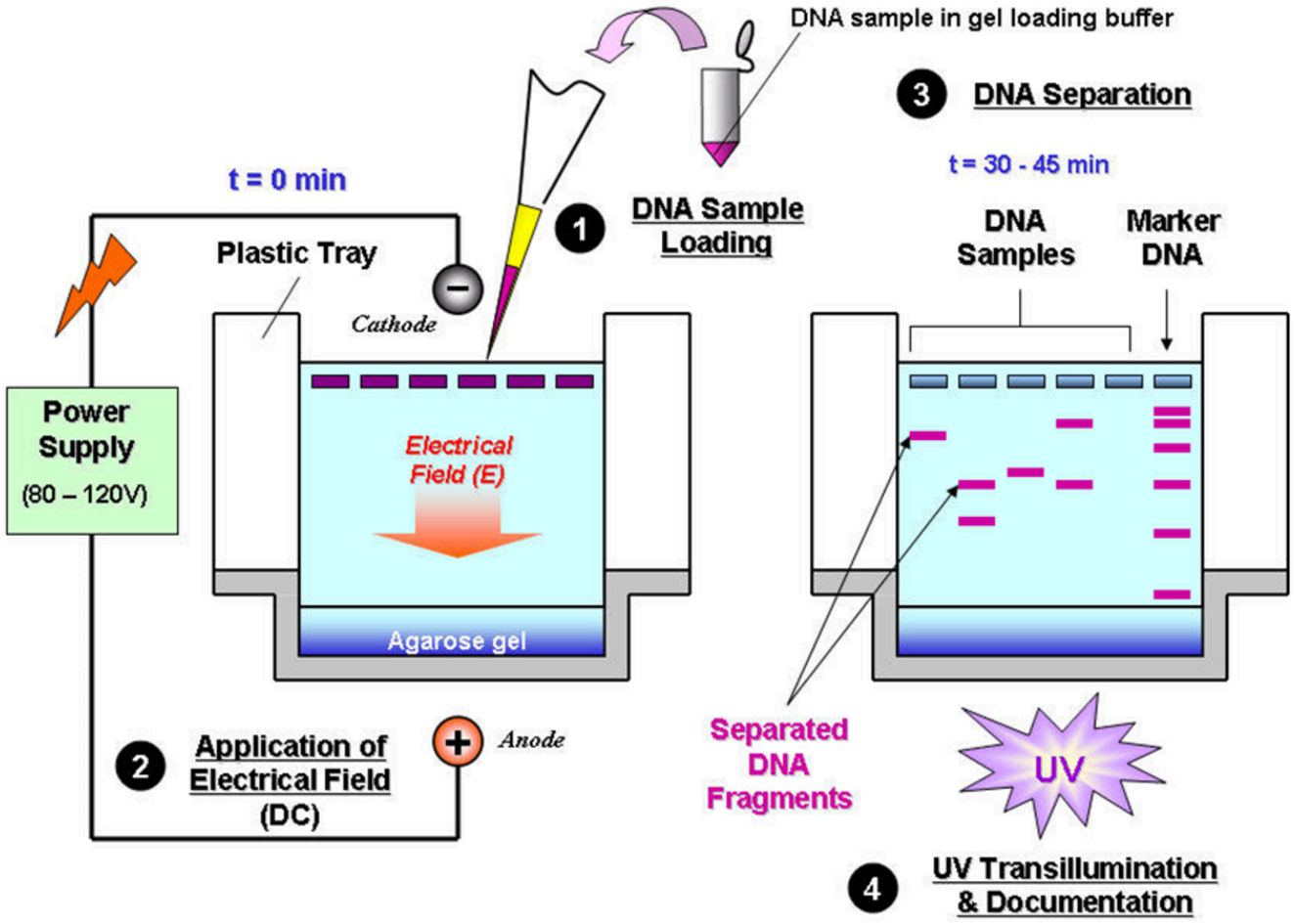
Principles of agarose gel electrophoresis

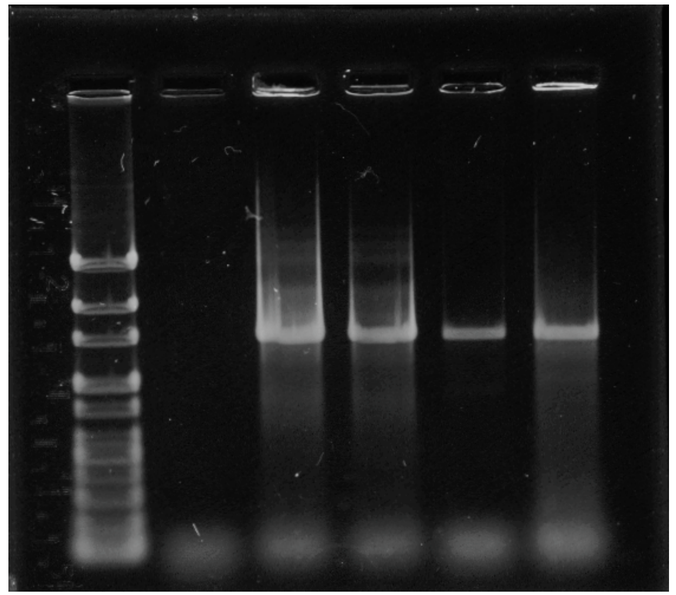
- DNA is a negatively charged molecule
- When placed in an electric field, DNA will migrate from the negative electrode (cathode) towards the positive electrode (anode)
- small molecules can move faster than large molecules



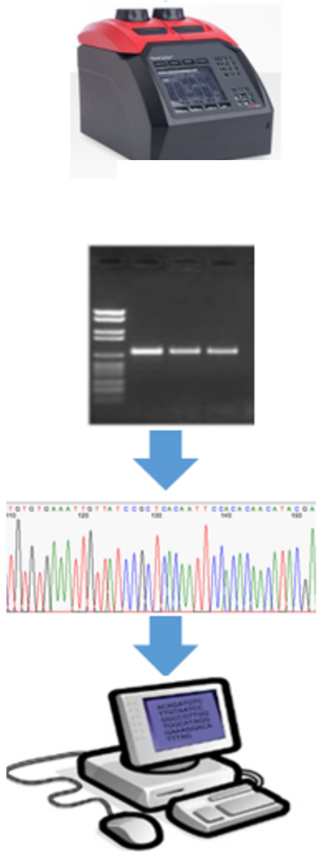


Principles of agarose gel electrophoresis

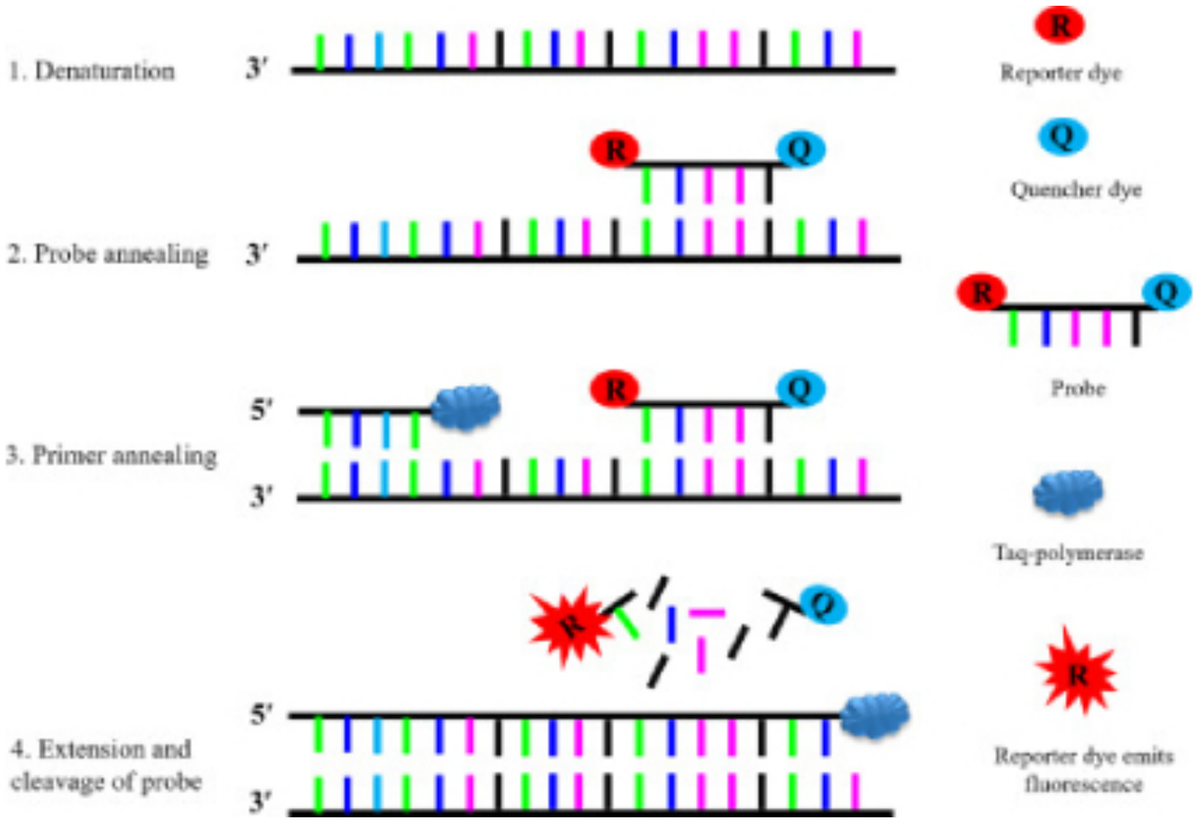




Further analysis (sequencing and bioinformatic analysis)



Real time PCR



Some videos, please search for more videos

https://www.youtube.com/watch?v=_p6t5WSKt_o&t=170s

https://www.youtube.com/results?search_query=pcr

Questions or comments?

THANK YOU!