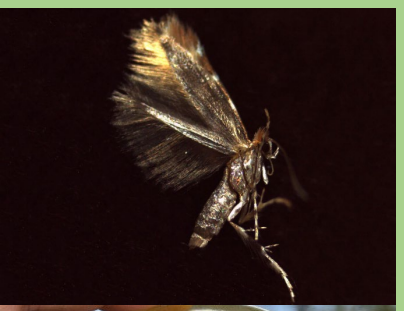
universität freiburg

FOREST PATHOLOGY



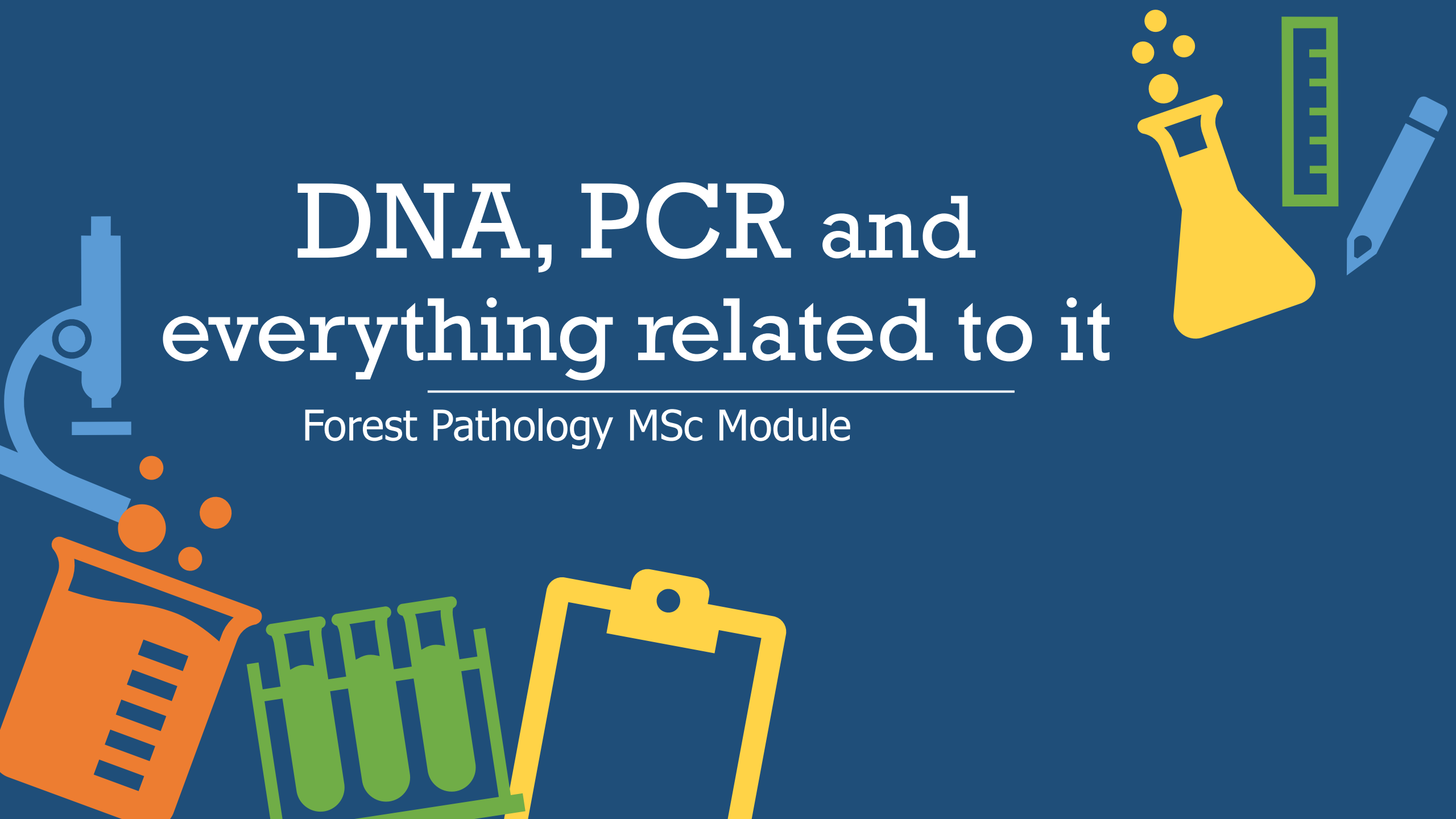
02.06.25 – 27.06.25

at the Chair of Pathology of Trees

Lecturers: Yasin Korkmaz and Kathrin Blumenstein

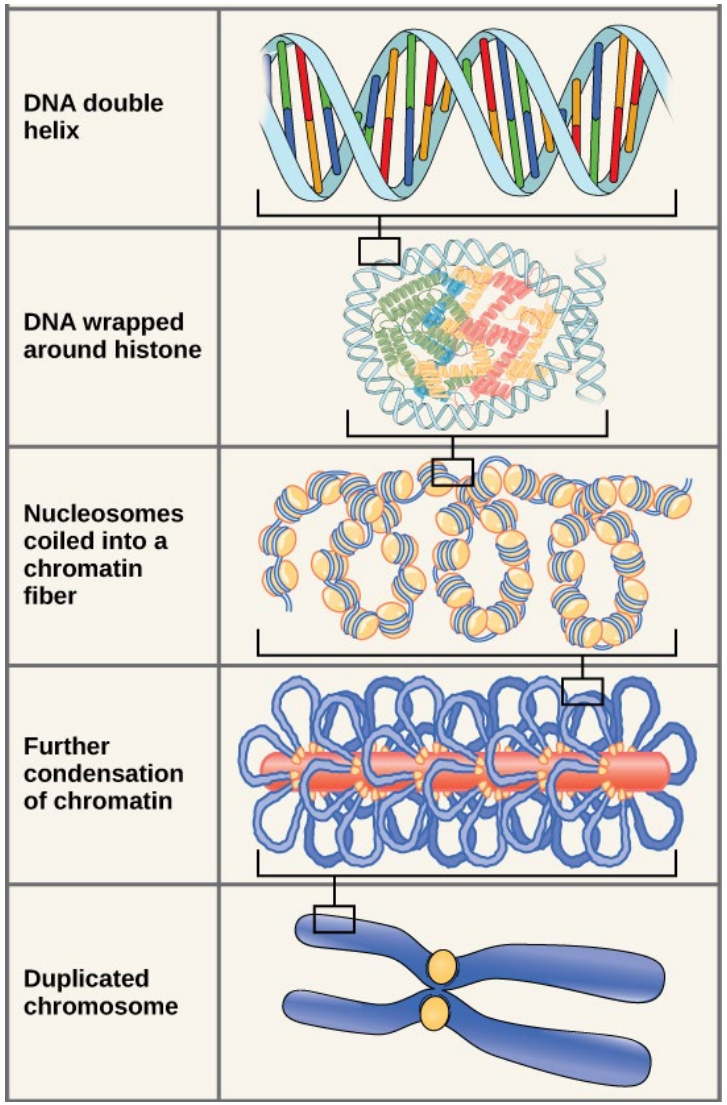
DNA, PCR and everything related to it

Forest Pathology MSc Module



https://img.digitaltrends.com/image/dna-double-helix-2-1000x667.jpg

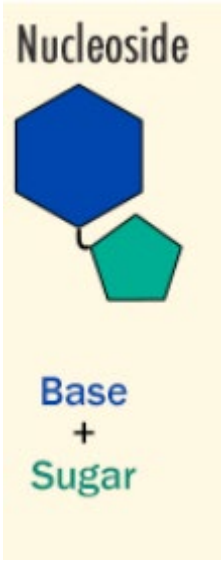
Organization of the compaction of the eukaryotic chromosome



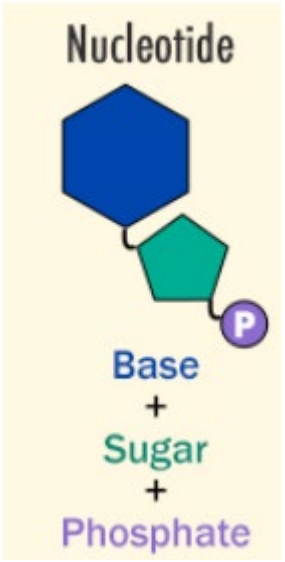
https://archive.cnx.org/contents/f2fd97cd-d728-4e03-b336-259c8d9b87da@5/the-structure-of-dna

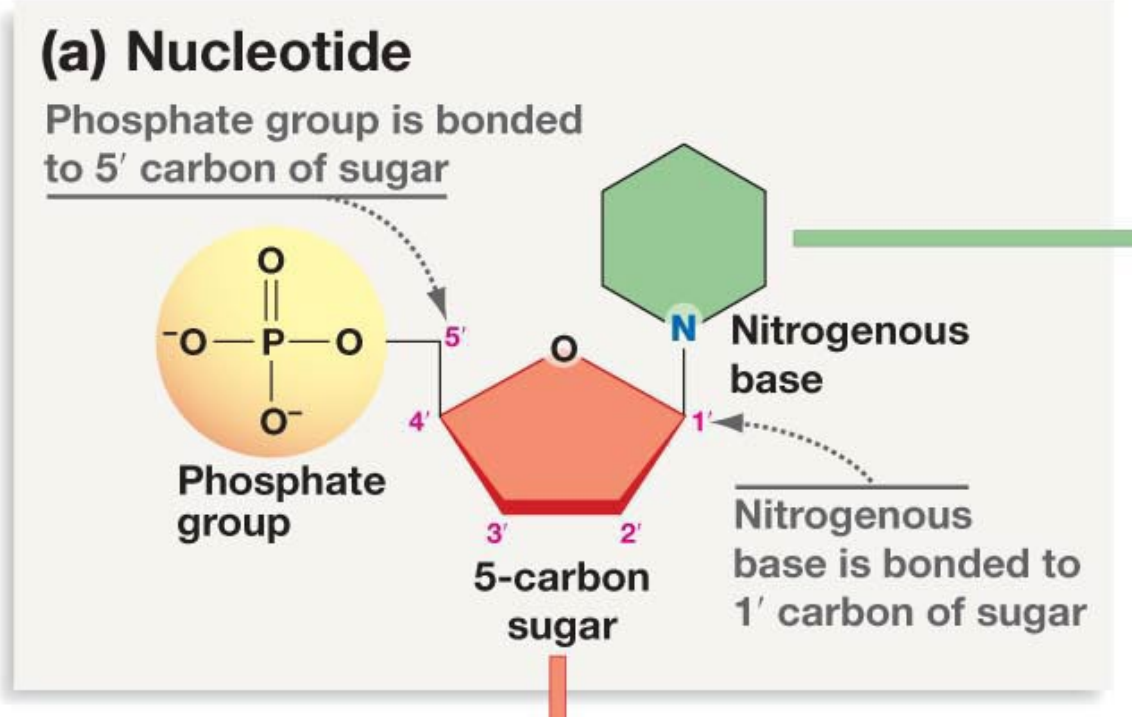
Nucleosides and Nucleotides

- Nucleosides are made of:
  - Nitrogenous base
  - pentose sugar



- Nucleotides are made of:
  - nitrogenous base
  - pentose sugar
  - phosphate group





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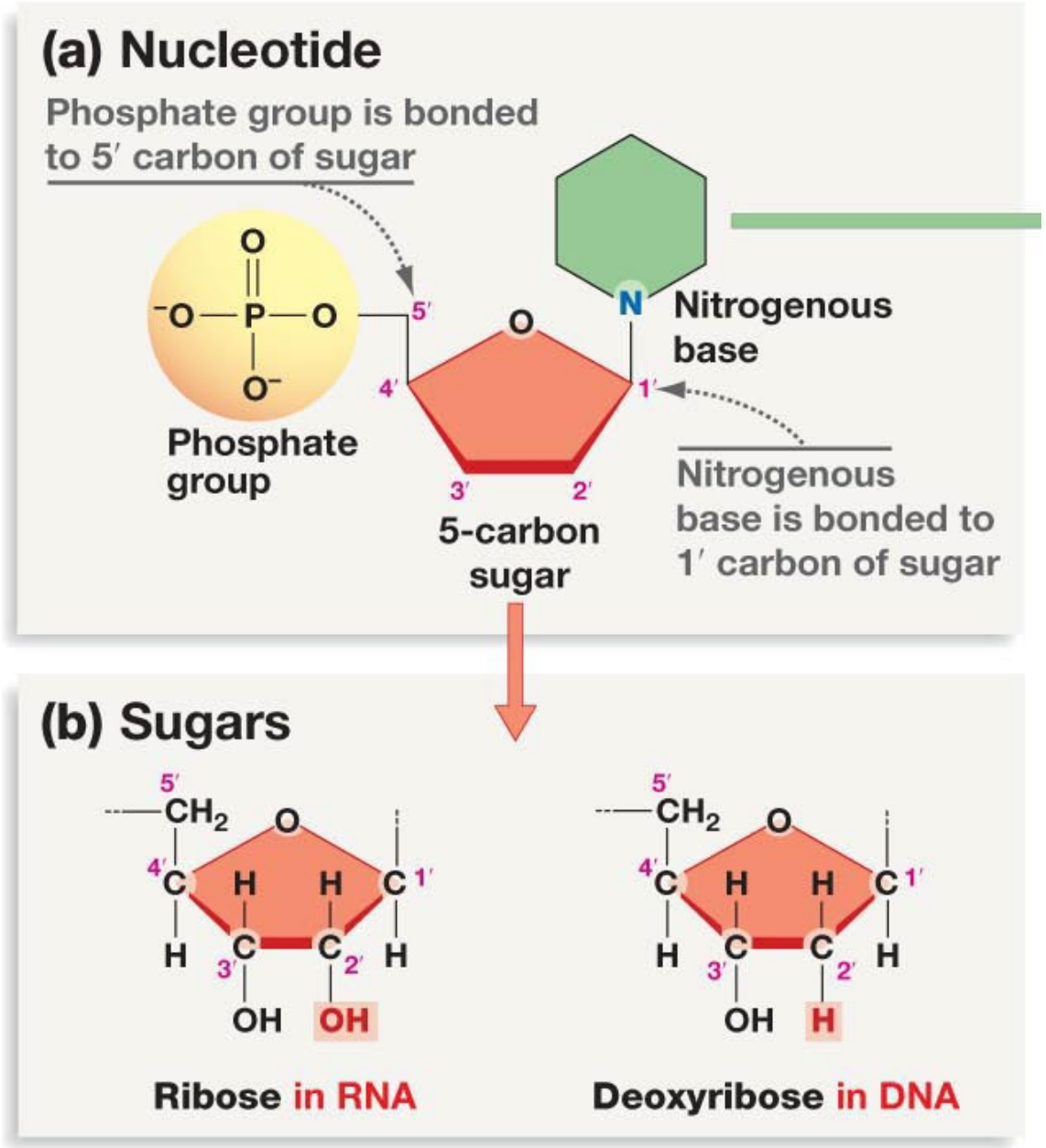

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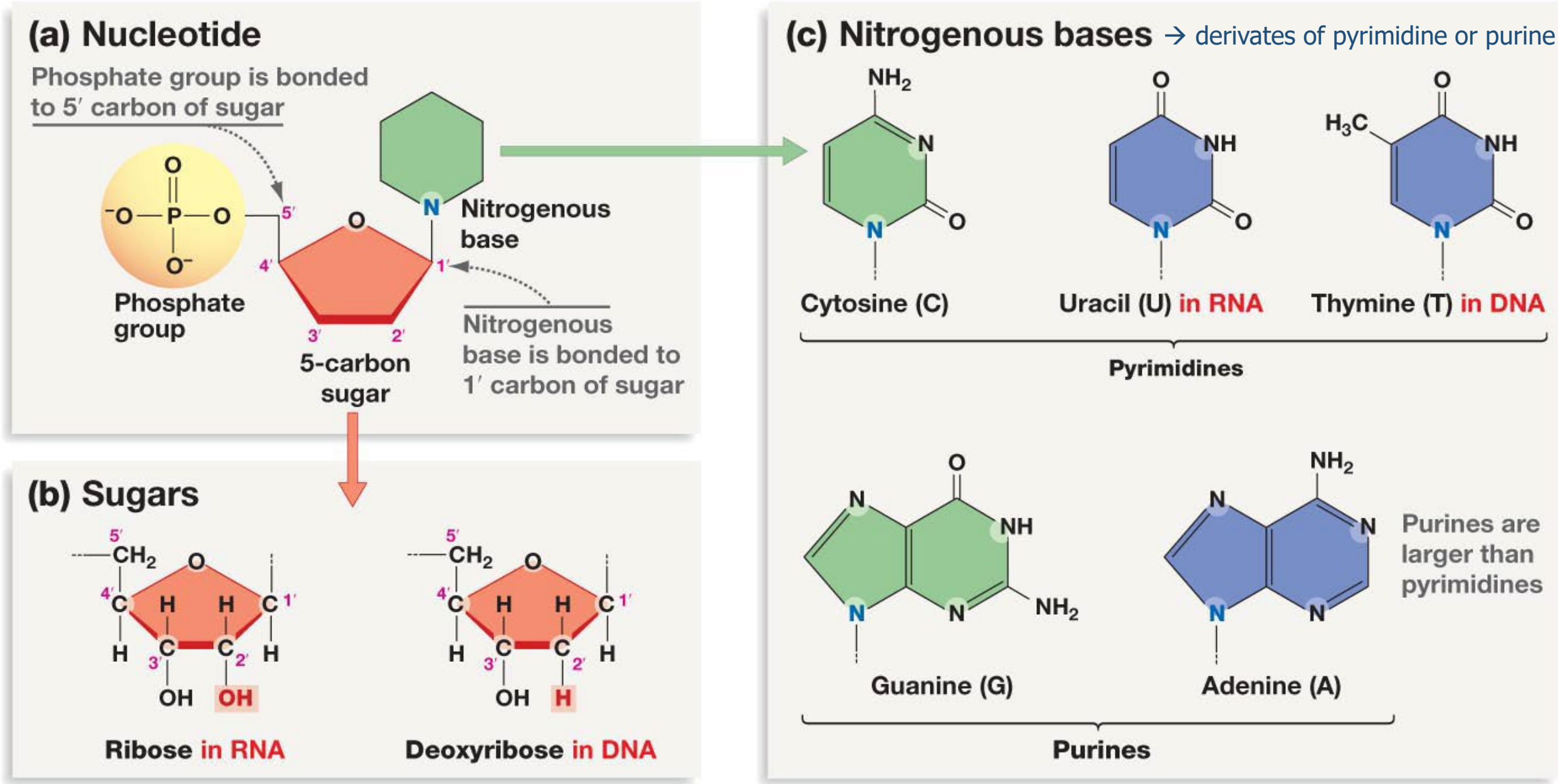
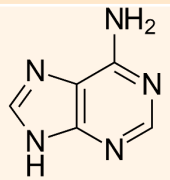
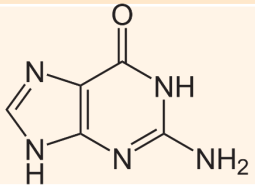
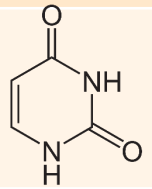
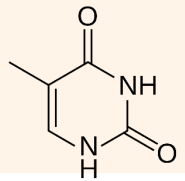
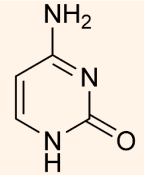


https://biochemneverland.files.wordpress.com/2014/03/04_01_nucleotide_structure.jpg

Nucleic acid constituents

(table shows only a selection of acids relevant in this lecture)

Nucleobase (Nitrogenous base)		Adenine	Guanine	Uracil	Thymine	Cytosine
						

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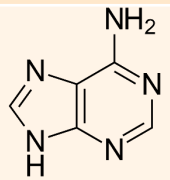
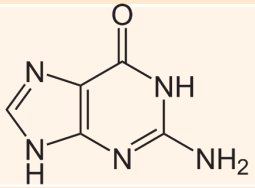
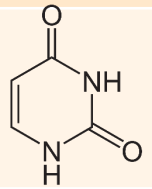
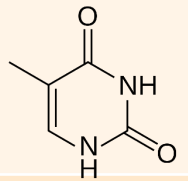
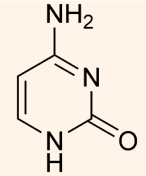
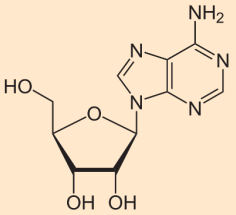
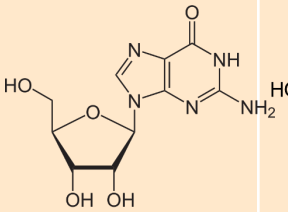
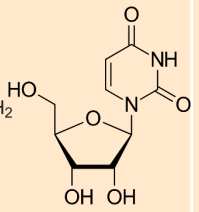
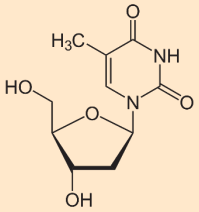
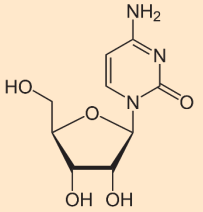
						
Nucleoside	**Ribonucleoside**	**Adenosine**	**Guanosine**	**Uridine**		**Cytidine**
	Deoxyribonucleoside				**Thymidine**	
						

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(table shows only a selection of acids relevant in this lecture)

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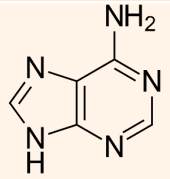
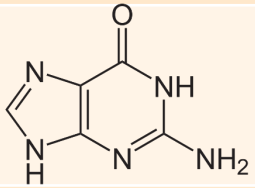
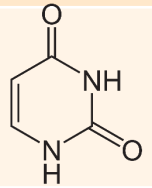
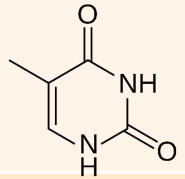
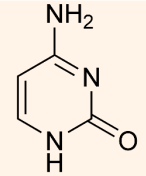
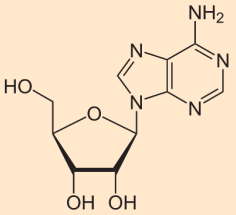
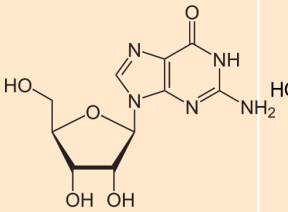
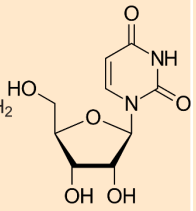
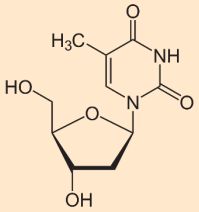
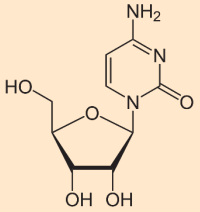
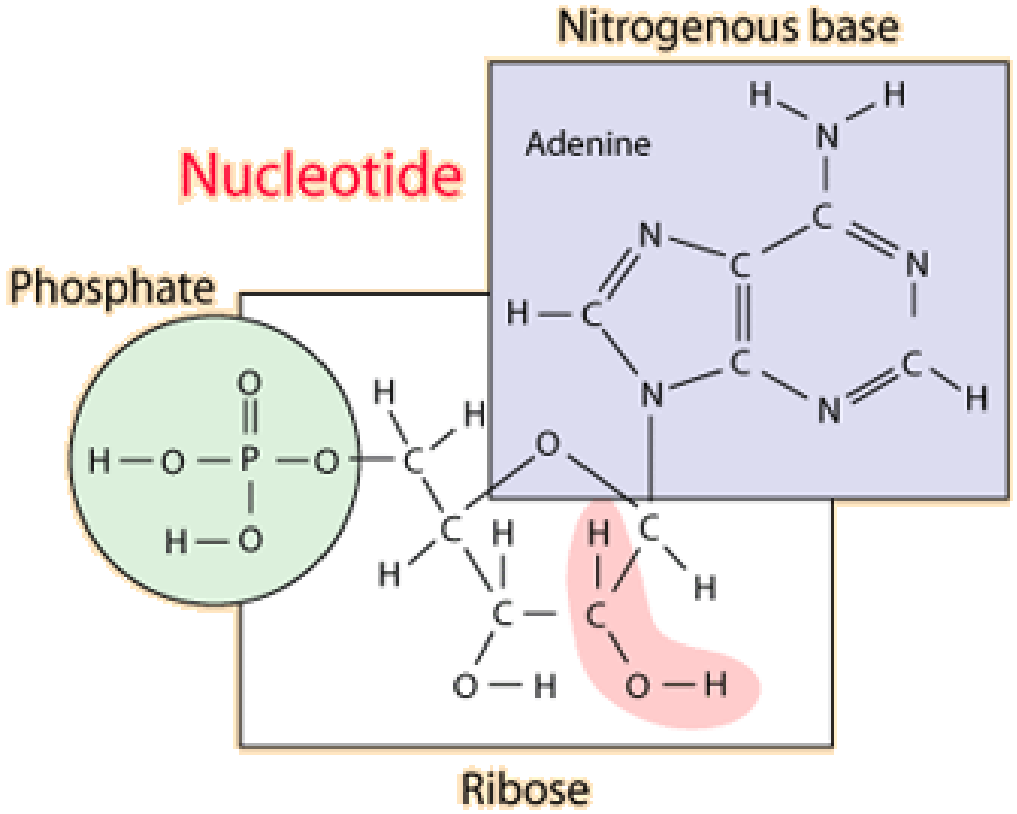
						
Nucleoside	Ribonucleoside	Adenosine	Guanosine	Uridine		Cytidine
	Deoxyribonucleoside				Thymidine	
						
Nucleotide (Nucleoside monophosphate)	Ribonucleotide	AMP	GMP	UMP		CMP
	Deoxyribonucleotide	dAMP	dGMP	dUMP	dTMP	dCMP
	Cyclic nucleotide	cAMP	cGMP			
Nucleoside diphosphate		ADP dADP	GDP dGDP	UDP dUDP	dTDP	CDP dCDP
Nucleoside triphosphate		ATP dATP	GTP dGTP	UTP dUTP	dTTP	CTP dCTP

Naming nucleotides

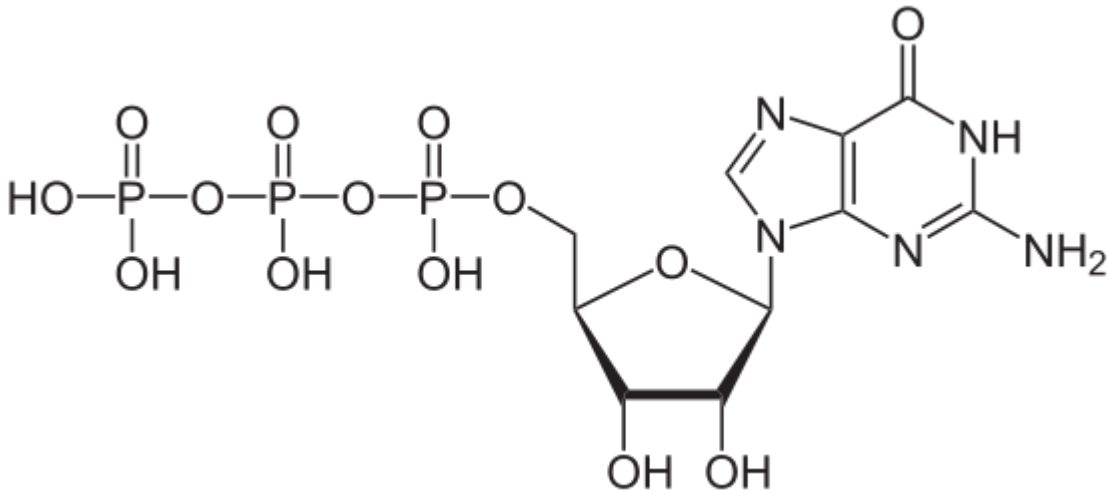
Notice: when naming nucleotides, we want to start off by naming the nucleoside, followed by the type of linkage between the sugar and phosphate(s), followed by the number of phosphate groups.

Adenosine 5'-monophosphate
The phosphate group is esterified at the fifth carbon atom of the ribose.

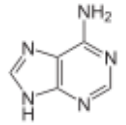
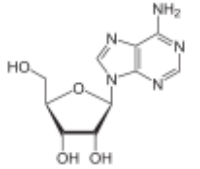
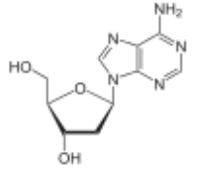
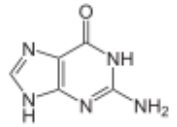
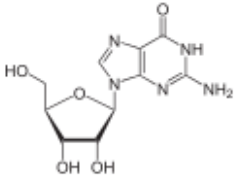
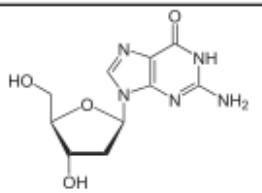
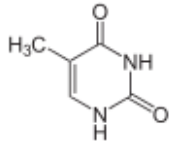
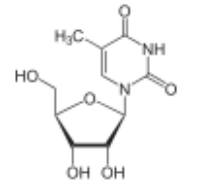
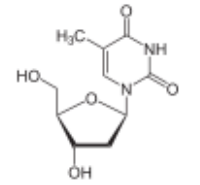
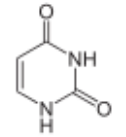
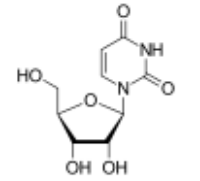
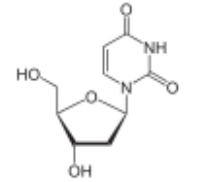
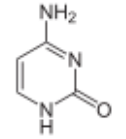
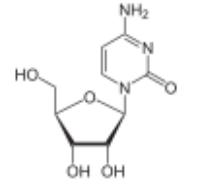
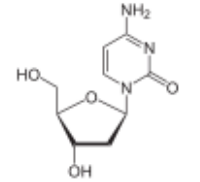


http://hyperphysics.phy-astr.gsu.edu/hbase/Organic/pensug.html

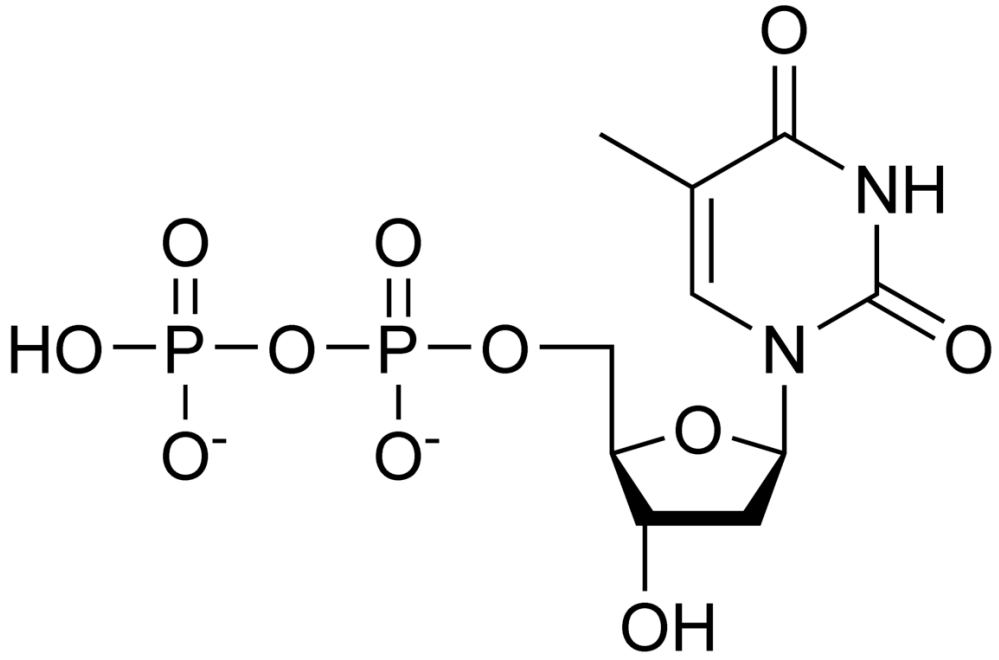
Name this nucleotide!



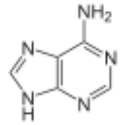
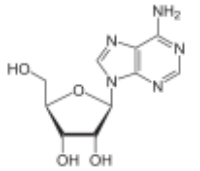
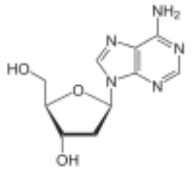
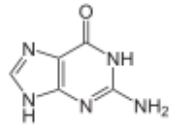
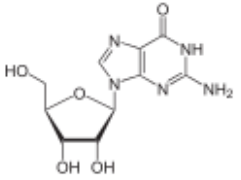
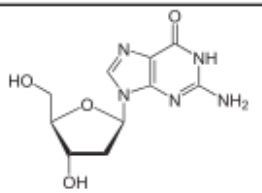
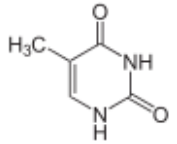
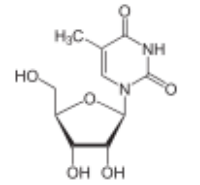
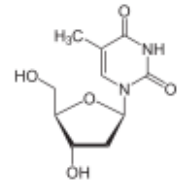
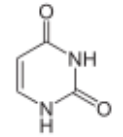
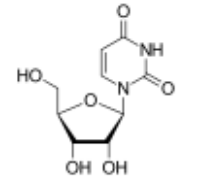
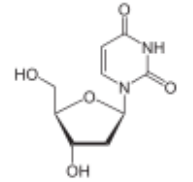
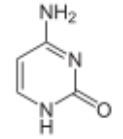
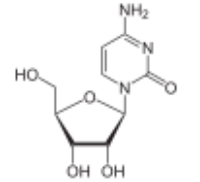
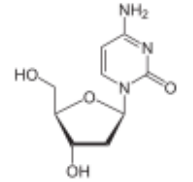
Guanosine triphosphate (GTP)
Guanosine-5'-triphosphate (GTP)

Nitrogenous base	Ribonucleoside	Deoxyribonucleoside
 Adenine	 Adenosine A	 Deoxyadenosine dA
 Guanine	 Guanosine G	 Deoxyguanosine dG
 Thymine	 5-Methyluridine m^5U	 Thymidine dT
 Uracil	 Uridine U	 Deoxyuridine dU
 Cytosine	 Cytidine C	 Deoxycytidine dC

Name this nucleotide!

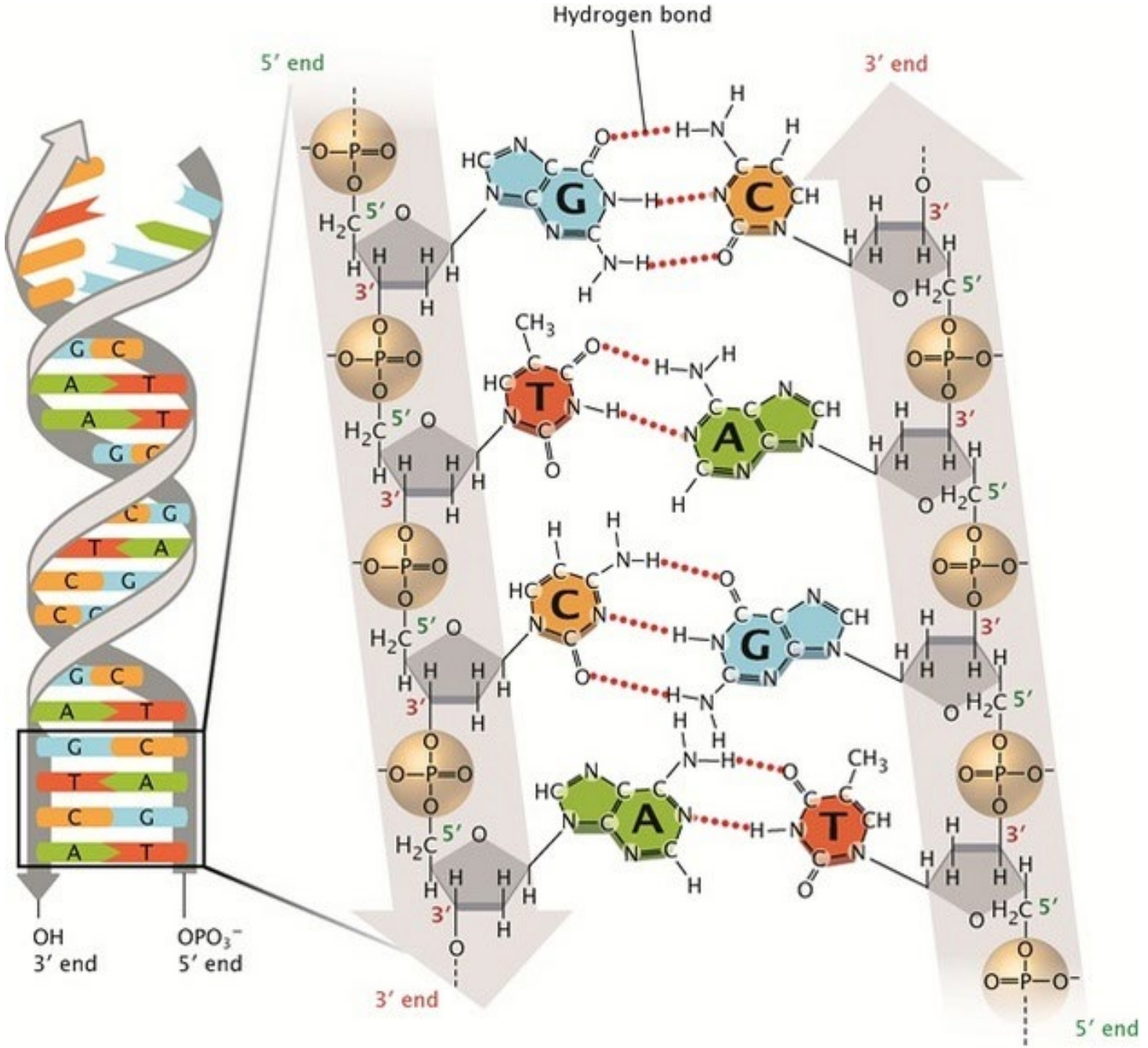


Thymidine diphosphate
Thymidine 5’-diphosphate

Nitrogenous base	Ribonucleoside	Deoxyribonucleoside
 Adenine	 Adenosine A	 Deoxyadenosine dA
 Guanine	 Guanosine G	 Deoxyguanosine dG
 Thymine	 5-Methyluridine m^5U	 Thymidine dT
 Uracil	 Uridine U	 Deoxyuridine dU
 Cytosine	 Cytidine C	 Deoxycytidine dC

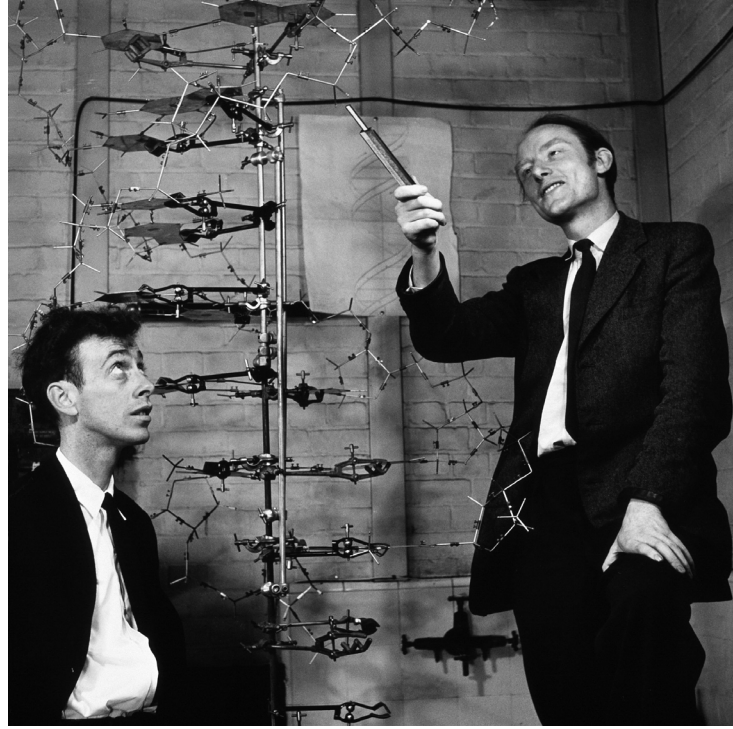
Nucleic acids

DNA is a polynucleotide → the monomers are nucleotides



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

Watson-Crick Model of DNA



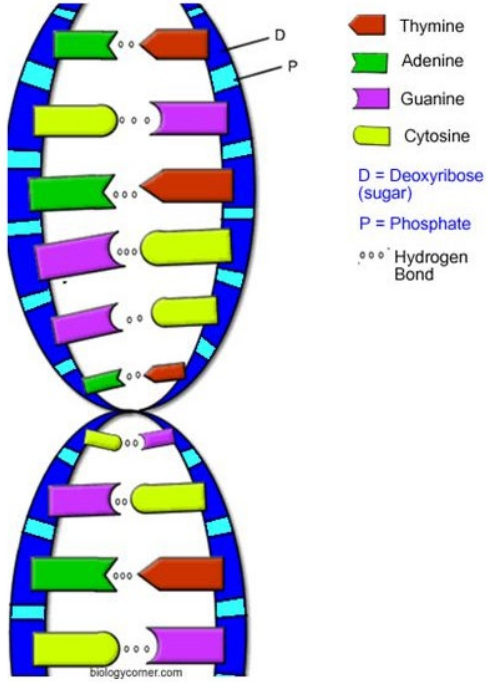
http://dataphys.org/list/watson-and-cricks-3d-model-of-dna/

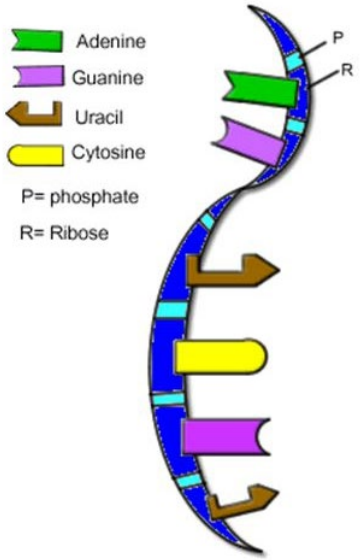
- 1953 James Watson and Francis Crick (Cambridge, UK) were able to show the three-dimensional structure of DNA (together with Rosalind Franklin and Maurice Wilkins)
- One of the most significant accomplishments
- Ability to understand the gene function in molecular term
- Two polynucleotide chains are coiled around a common axis (these two strands of DNA run in a parallel but opposite direction)
- Bases are inside the helix, whereas the phosphate and deoxyribose units are outside.
- Strands are held together by hydrogen bonds between pairs of bases
- The precise sequence of bases carries the genetic information

Nucleic acids

DNA

- deoxyribonucleic acid
- deoxyribose sugar
- typically exists in a double-helix form
- carries genetic information

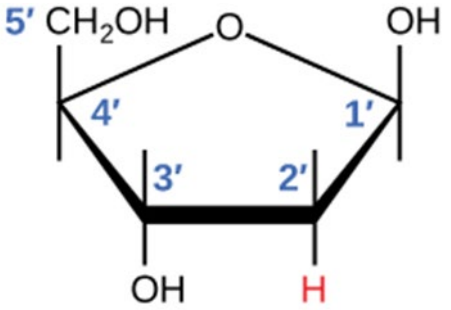




RNA

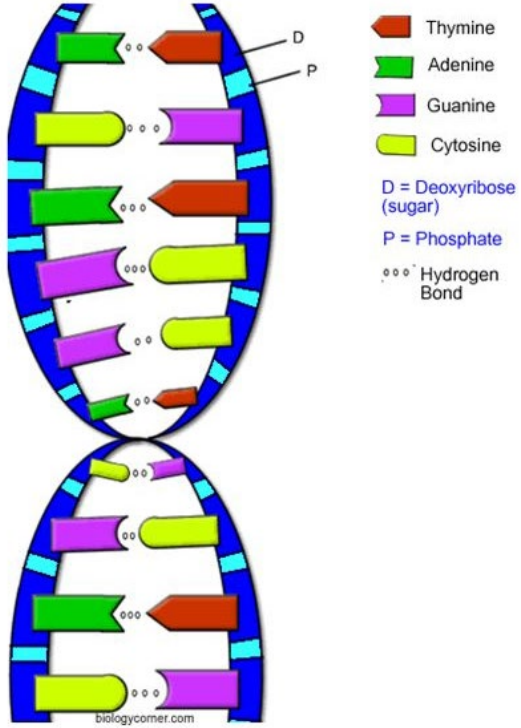
- ribonucleic acid
- ribose sugar
- exist predominately as single-strand molecules
- transcribes the genetic info

Structure and composition of DNA

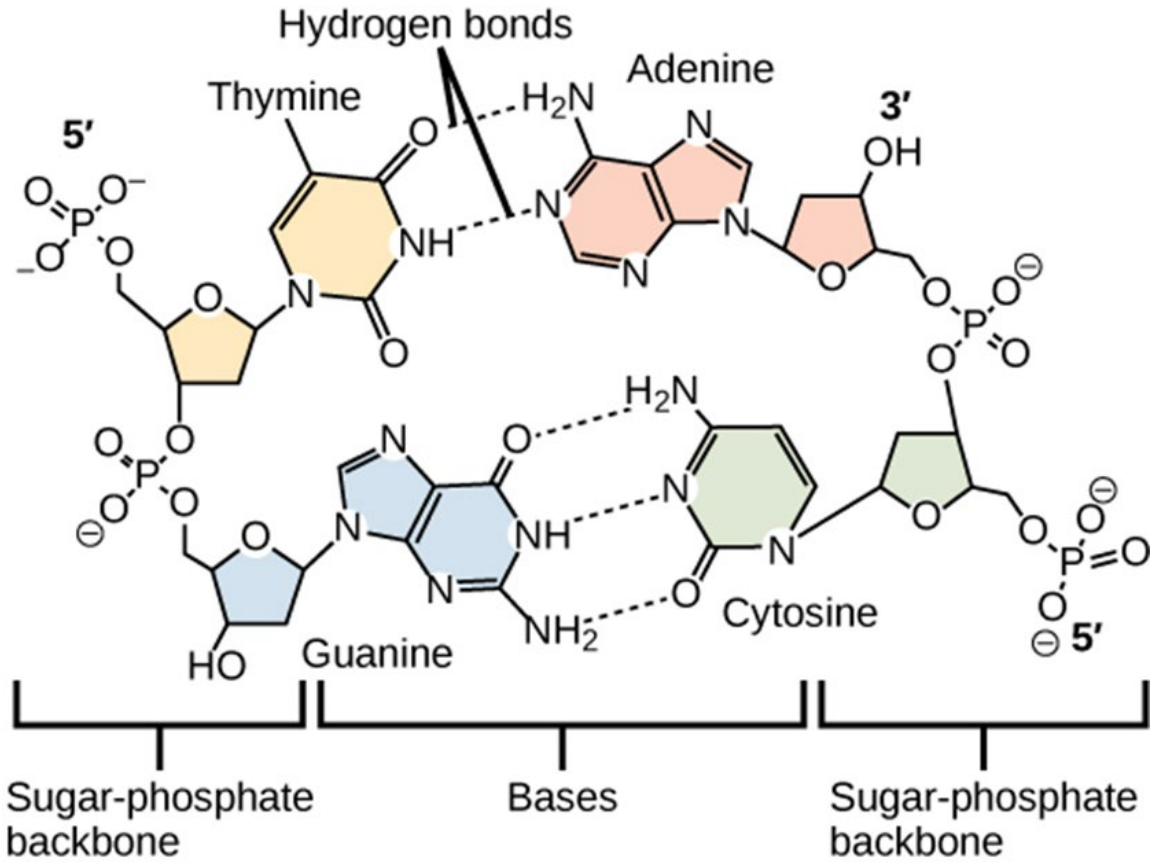


Deoxyribose

- Nucleic acids are linear polymers → beginning and an end
- Backbone: repeating sugar-phosphate groups that are connected by phosphodiester bonds
- A phosphodiester linkage is a connection between the 3' carbon on one sugar molecule and the 5' carbon on adjacent sugar molecule.
- The presence of a negative charge on the phosphate makes the backbone of the nucleic acid negatively charged.
- The sugar molecule is also attached onto the nitrogenous base via the 1' carbon atom.



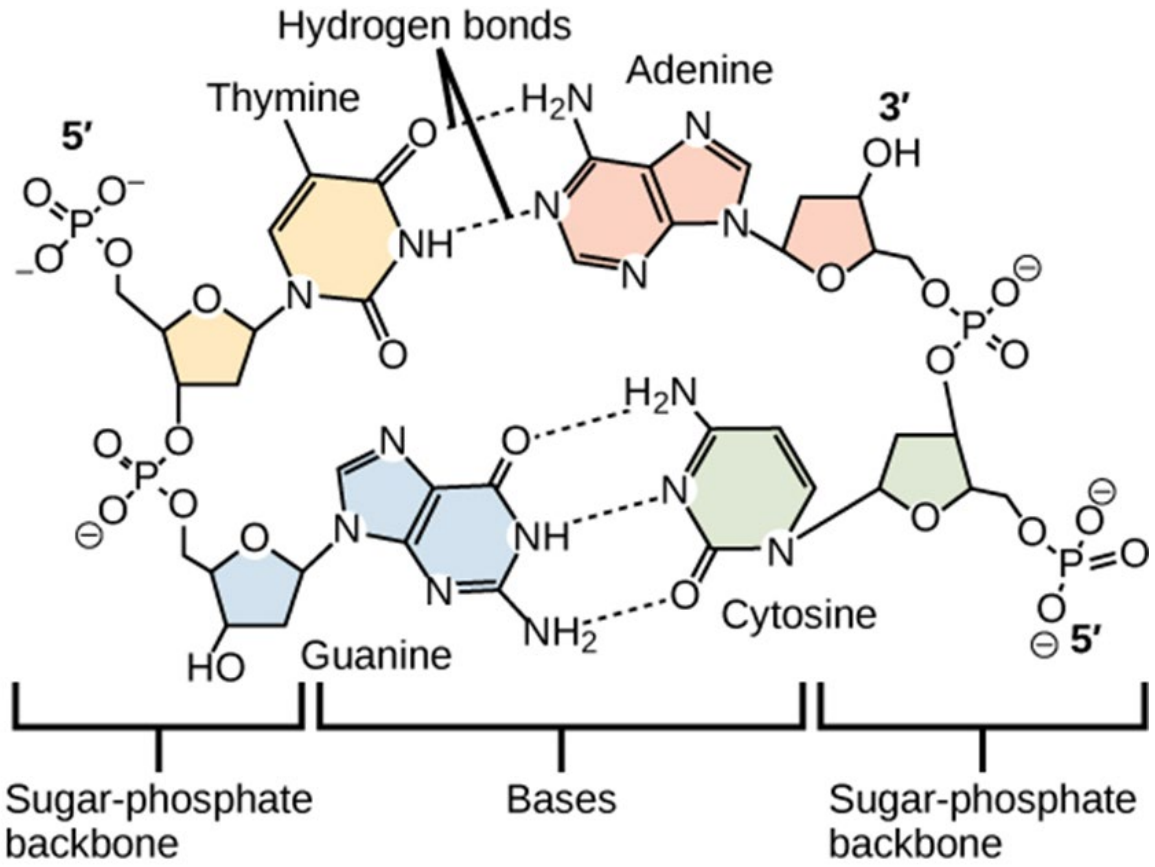
Structure and composition of DNA

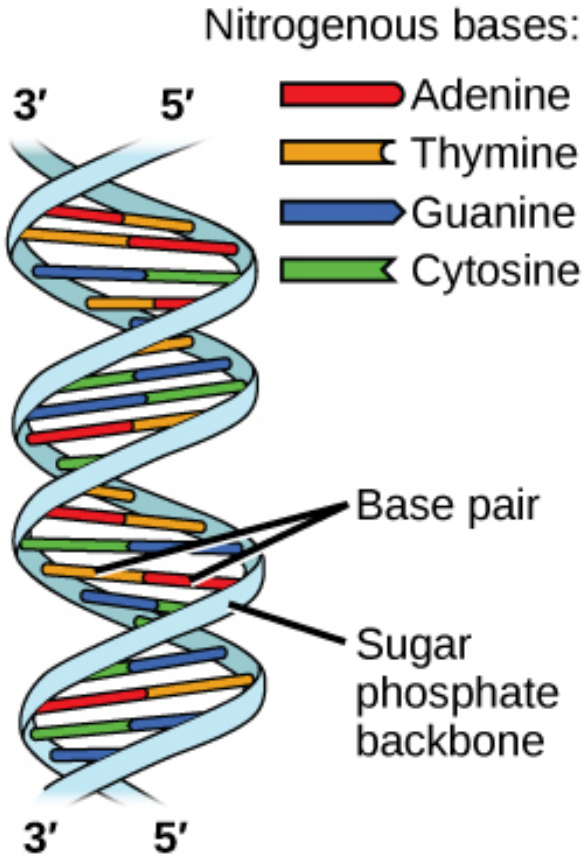


- The bases of DNA molecules carry genetic information, whereas their sugar and phosphate groups perform a structural role.

- Base pairs are connected via hydrogen bonds: the two strands of DNA stay together by H bonds that occur between complementary nucleotide base pairs
- A & T
- G & C
- These particular pairs fit exactly to form very effective hydrogen bonds with each other.

DNA is to be read from 5´ to 3´direction





Practical excersise

1. Name and complete your assigned nucleotides
2. In which direction leads the DNA strand?
3. Place them in the right position on the board!
4. Draw the correct bonds (covalend / hydrogen / how many?)?

Functions of DNA: Sequence

- Length of the sequence depends on the organism
- Except for viruses, all life uses DNA to store information
- Sequence is obtained through sequencing methods
- Sequence can be uploaded in NCBI database

DNA extraction – Part 1

Take photos of samples front and back sides	
Prepare fresh plates for backups	
Collect gently **hyphae** with a scalpel	1.5 ml tubes
Add **PVP Extraction buffer**	500 µl
Vortex	20 seconds
Incubate at 65°C in a heat block	5 minutes
Vortex	20 seconds
Microwave	5 seconds (3 times)
Ultrasonic homogenizer	2 seconds (2 times)
Incubate at 65°C in a heat block	15 minutes
Vortex	20 seconds
Centrifuge - 5000 rpm	10 minutes
Transfer the supernatant into 1.5 ml tubes	250 µl

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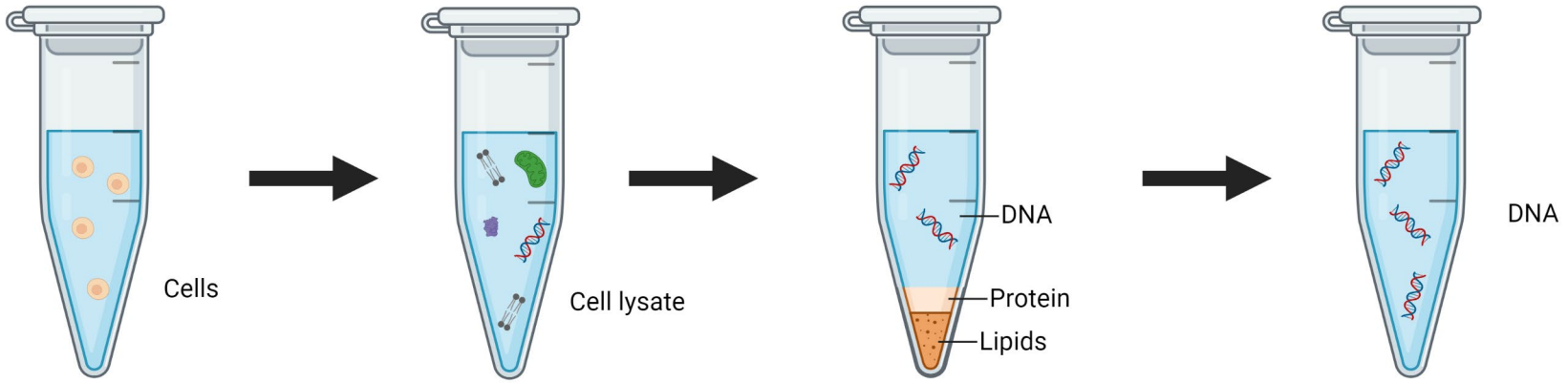
DNA extraction - PVP extraction buffer

- Polyvinylpyrrolidone
- Plants are rich in phenolic compounds
- Polyphenols bind DNA right after cells are lysed
- The buffer removes the phenols by forming hydrogen bonds



https://images.aatbio.com/universal/newsletter/volume-11-1/Organic%20Extraction%20workflow.png

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DNA extraction
- ultrasonic homogenizer

- How do we reach the DNA that is inside the cell?

→ we need to break the cell membrane

DNA extraction – Part 2

Add **SDS wash buffer**	250 µl
Vortex	20 seconds
Centrifuge - 13300 rpm	15 minutes
Transfer the supernatant into 1.5 ml tubes	300 µl
Add **Isopropanol**	255 µl
Mix with inversion	20 times
Centrifuge - 13300 rpm	15 minutes
Remove the supernatant	
Wash the pellet with **70% cold Ethanol**	200 µl
Centrifuge - 13300 rpm	10 minutes
Remove Ethanol	
Dry the pellets in heatblock at 37°C (Caps open)	15 minutes
Resuspend the pellets in **Nuclease free water**	50 µl

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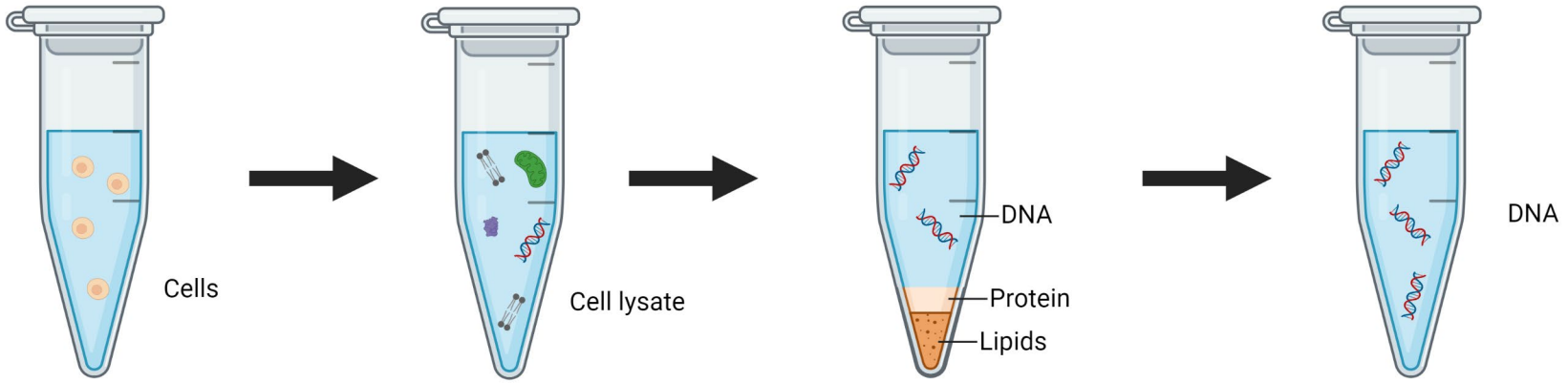
DNA extraction - SDS wash buffer

- To remove membrane barriers
- SDS releases the DNA from histones and other DNA binding proteins by denaturing them



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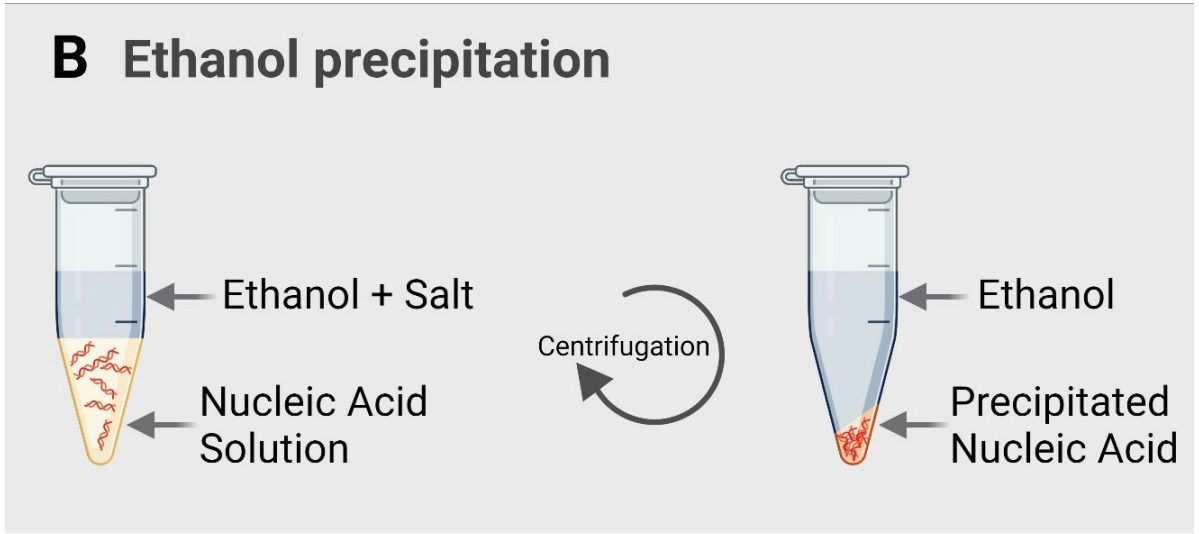
DNA extraction

Isopropanol

- Precipitates the available proteins
- Washes remaining salt

Ethanol

- Eliminates the solvation shell surrounding the DNA
- Helps DNA to precipitate in pellet form
- Helps to promote DNA aggregation



https://bioecho-prod-media-hp.s3.sbg.perf.cloud.ovh.net/media/22/5a/a3/1664538632/Cleanup%20methods%20(1).png

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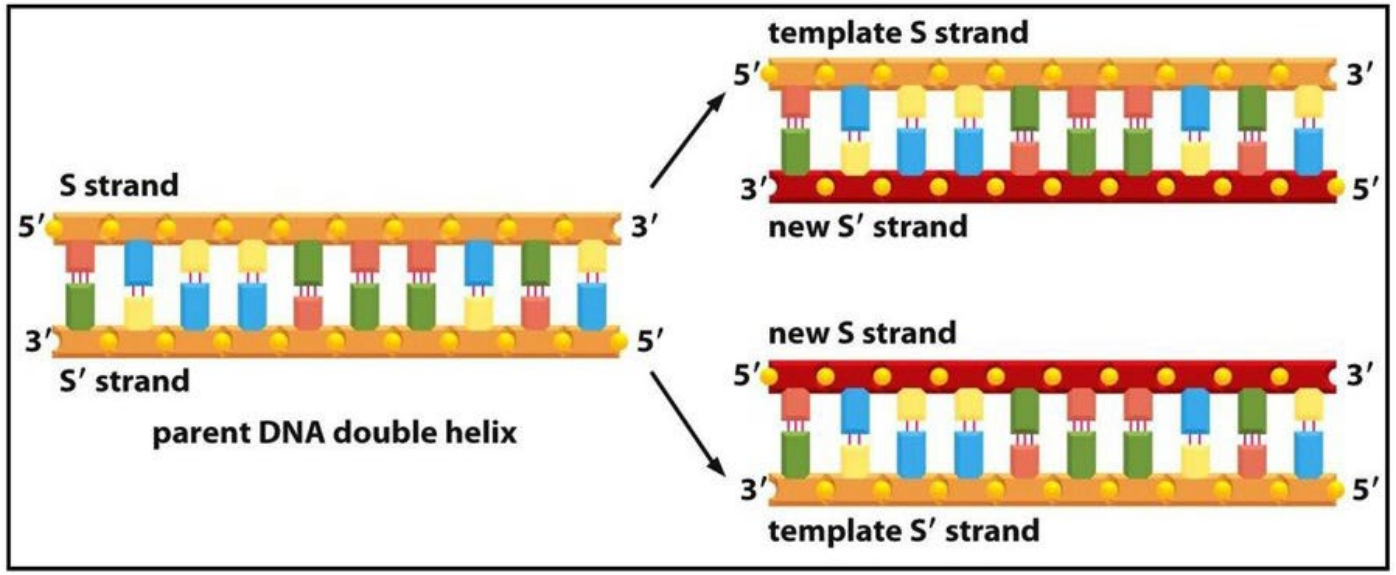
Functions of DNA
DNA as a template for its own duplication

- Duplication of the genetic information occurs by the use of one DNA strand as a template for the formation of a complementary strand.

- The copying process is performed by a cluster of proteins that form a replication machine.



Cell Biology

DNA Replication, Repair, and Recombination

Denaturation (Melting) and Annealing

- The hydrogen bonds are based on attraction between opposite charges.

→the two strands of DNA aren't physically connected to each other

- Instead, the nitrogenous base pairs have an electrostatic attraction. When DNA is copied, the strands have to separate.

- "unzipping" → denaturation (no longer in its natural state)

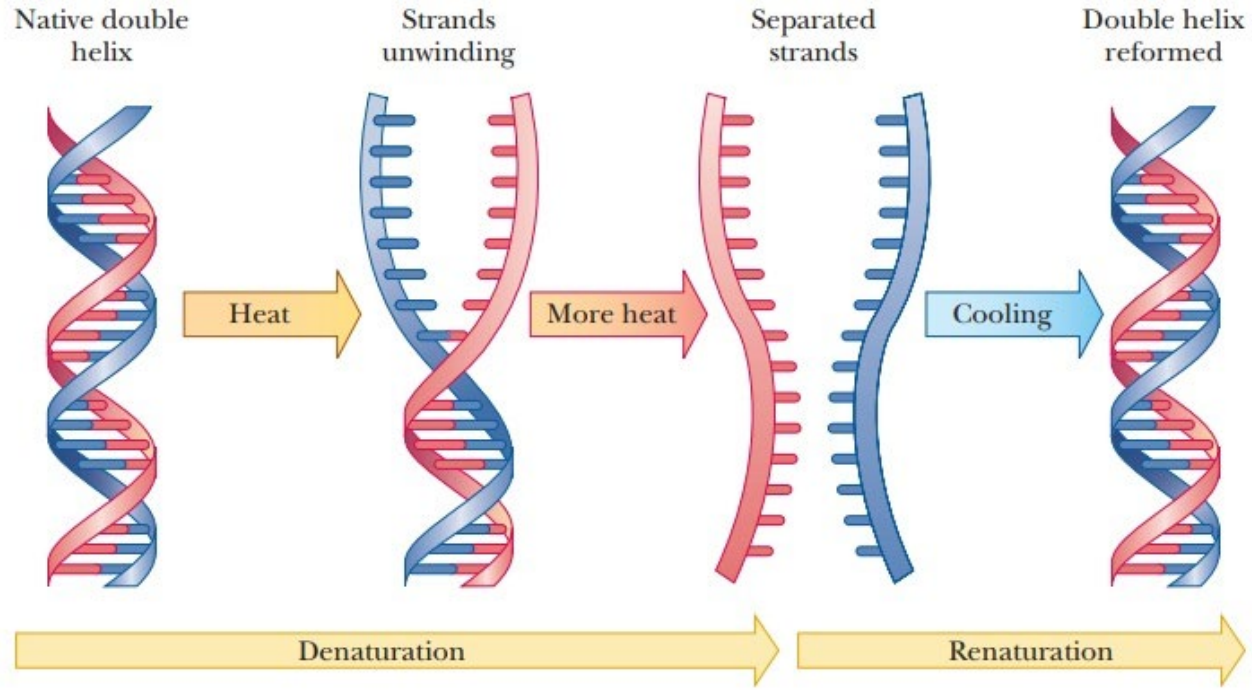
- Heat can disrupt the DNA's hydrogen bonds and lead to denaturation.

- The process of two strands of DNA re-joining is called annealing. Annealing happens when temperatures drop or return to a level where DNA can be in its natural state.

Melting and Annealing of DNA

(1) increase or decrease the pH of the solution, thereby ionizing the nitrogenous bases and breaking the hydrogen bonds

(2) Heating the solution to increase the temperature and break the hydrogen bonds.



- The dissociation of the double helix structure is called melting.

https://www.brainkart.com/article/Denaturation-of-DNA_27539/

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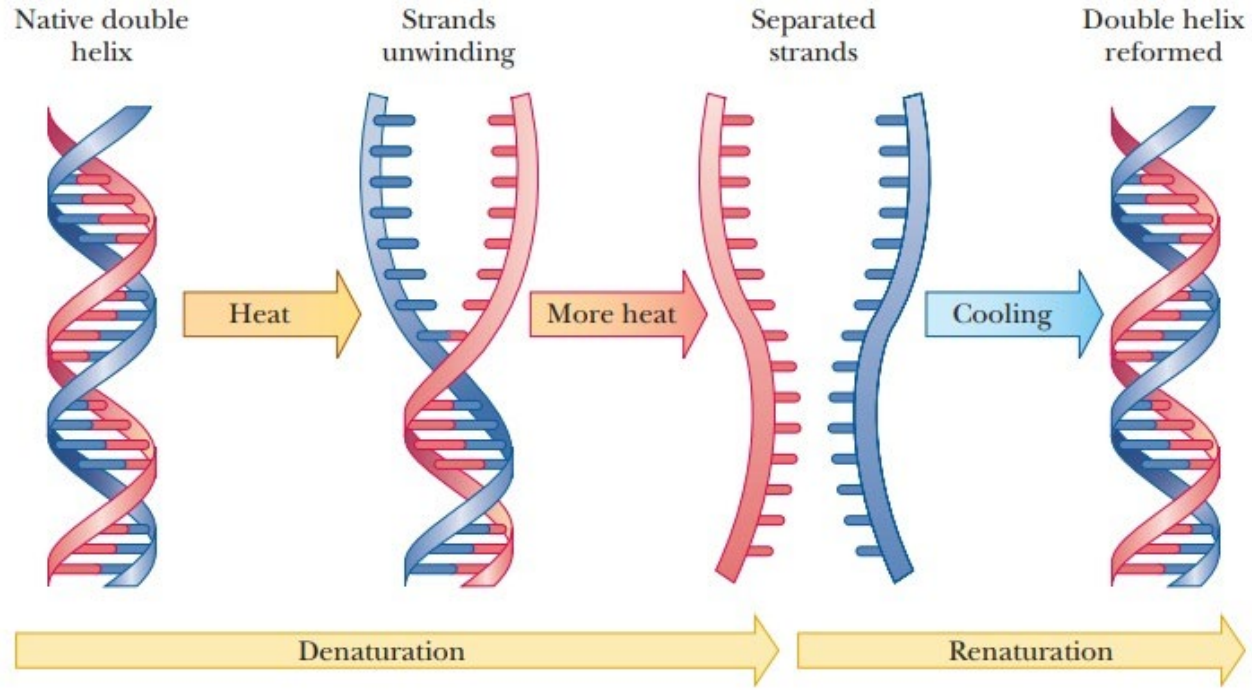
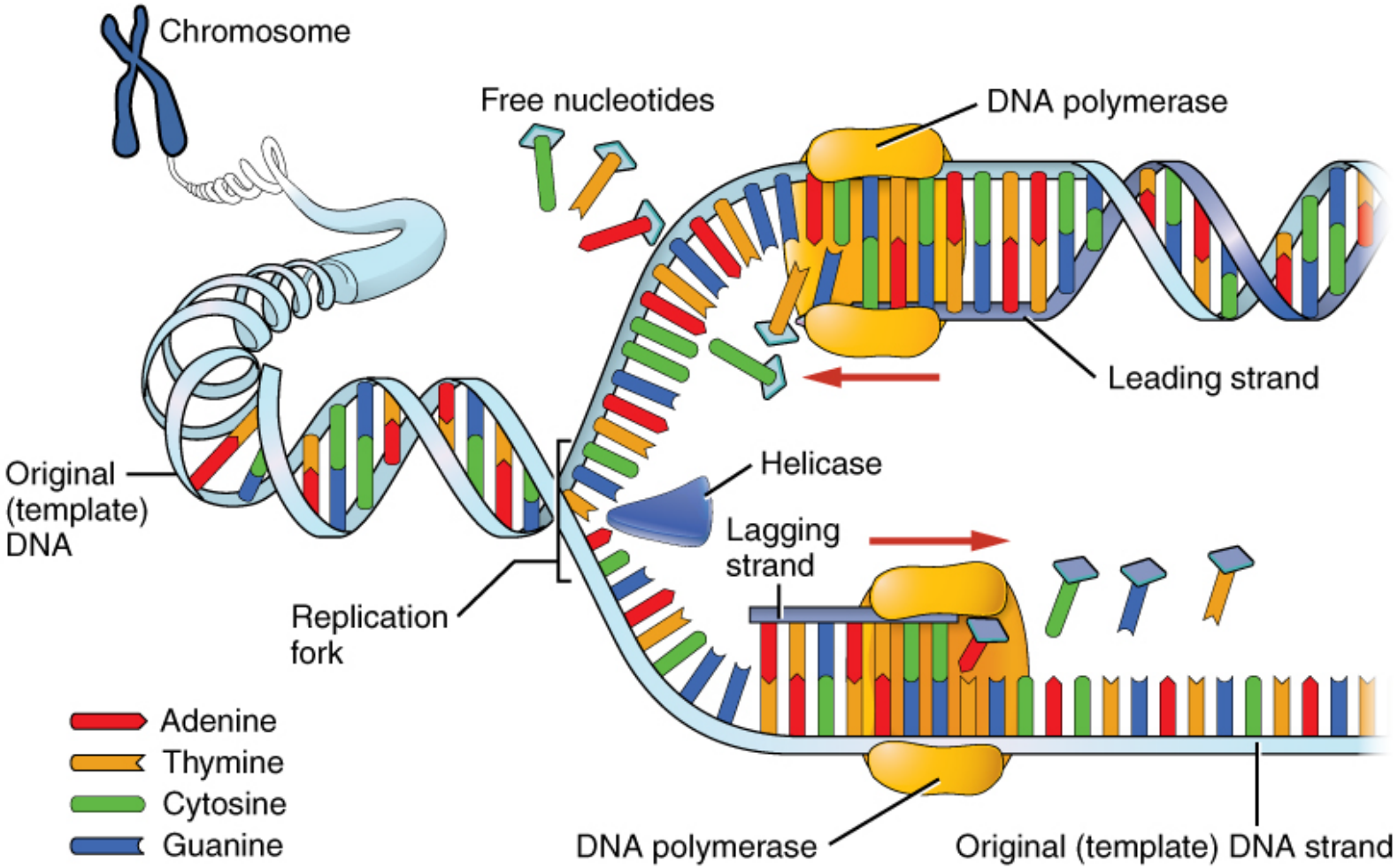


- The dissociation of the double helix structure is called melting.
- Inside our cells, the temperature remains constant. How do our cells unravel the DNA molecule? → Our cells use a special enzyme called DNA helicase to break the hydrogen bonds and unwind the DNA.

https://www.brainkart.com/article/Denaturation-of-DNA_27539/

DNA Replication → duplicates the entire genome of the cell



https://opentextbc.ca/anatomyandphysiology/chapter/3-3-the-nucleus-and-dna-replication/

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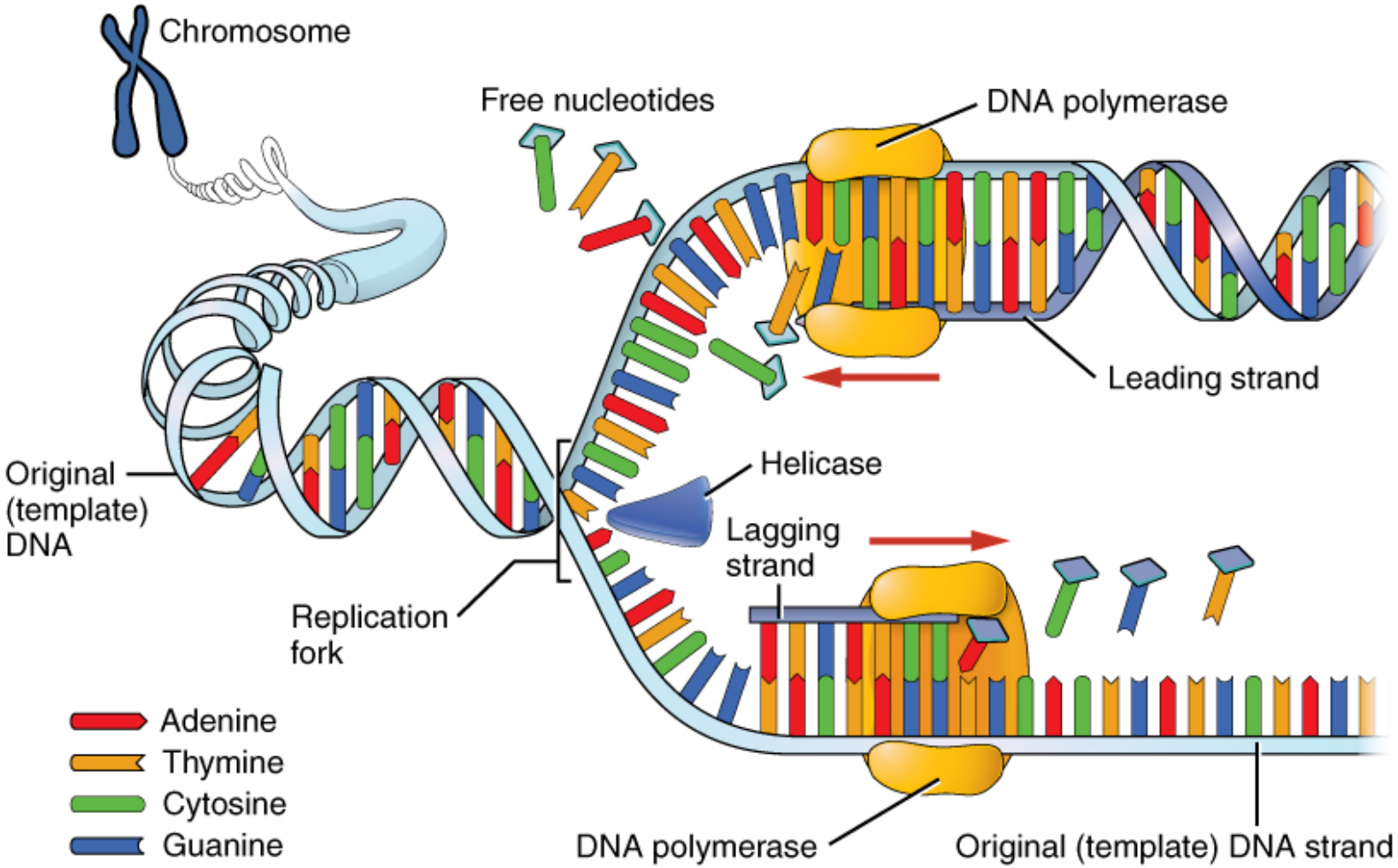


- The two strands are used as a template to synthesize new complementary strands.

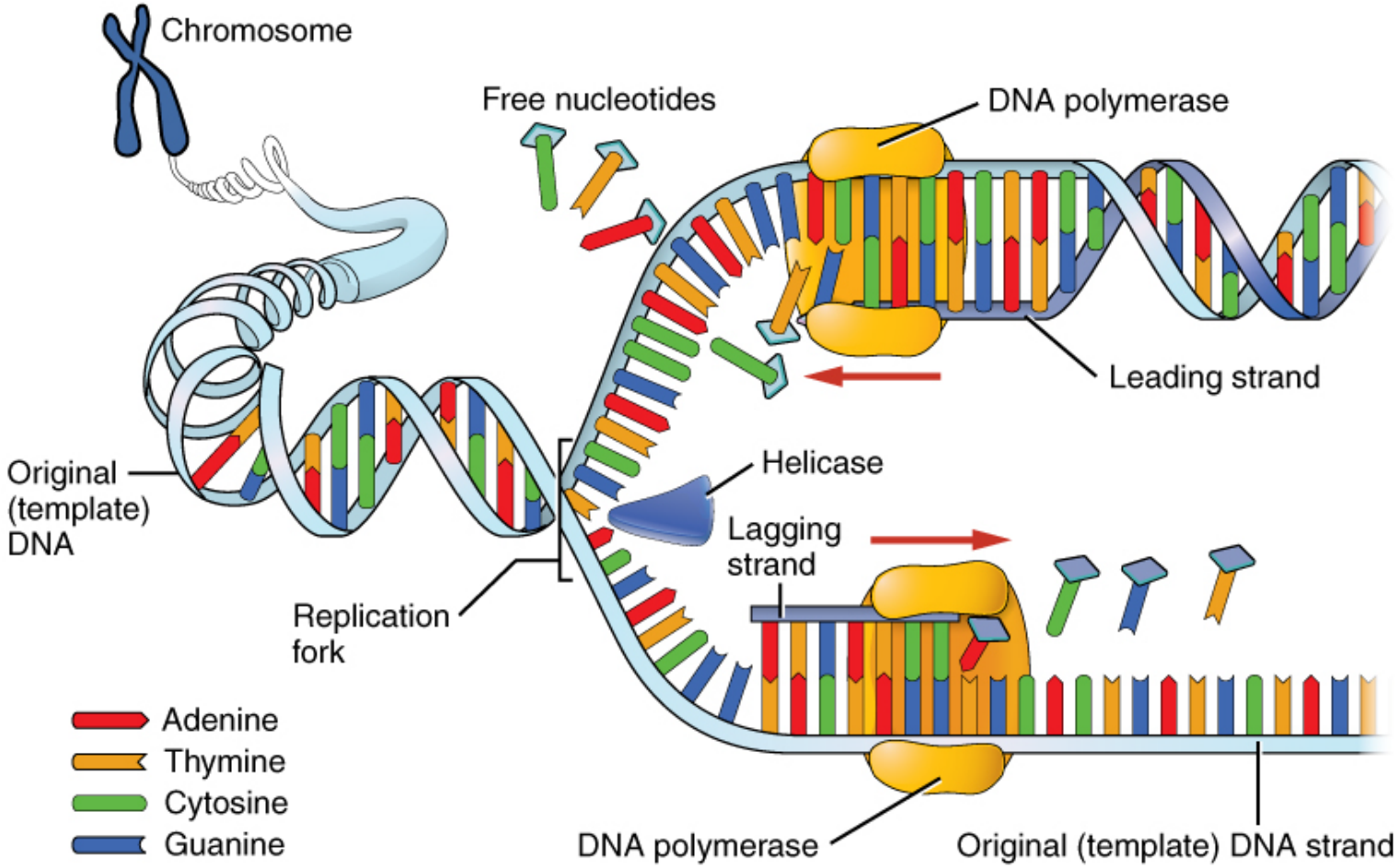
- DNA polymerase catalyses the formation of the phosphodiester bond by adding the deoxynucleoside triphosphate (dNTPs) onto the growing polynucleotide chain.

https://opentextbc.ca/anatomyandphysiology/chapter/3-3-the-nucleus-and-dna-replication/

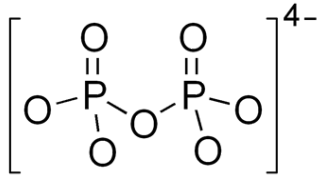
DNA Replication – the polymerase

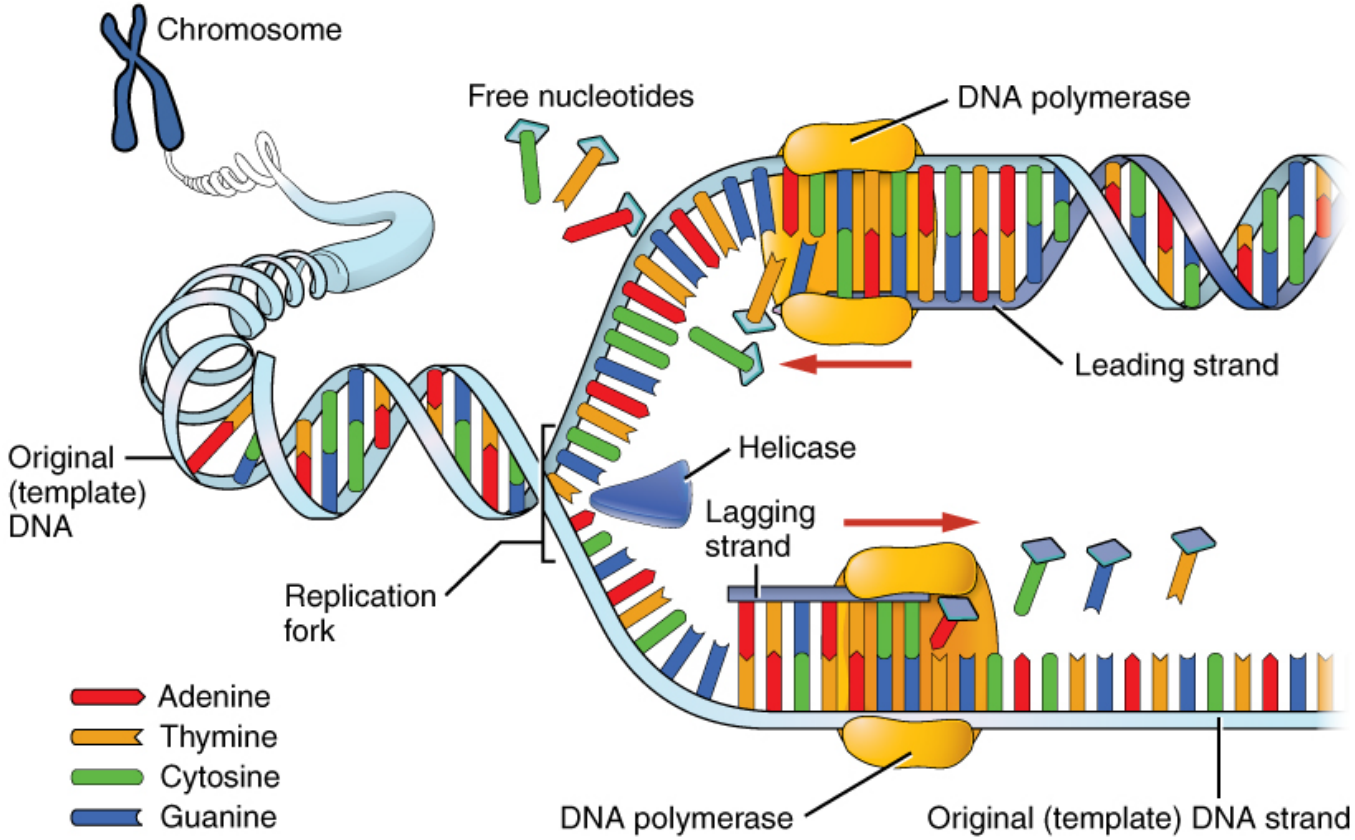
DNA polymerase requires

- the four types of deoxynucleoside triphosphates (dNTPs: dCTP, dTTP, dGTP and dATP)
- the DNA template and
- a primer to synthesize the polynucleotide chain



DNA Replication – the polymerase





- a single pyrophosphate molecule is released every time the chain is elongated by one deoxyribonucleotide

- DNA polymerase also displays nuclease activity, which means it has the ability to remove mismatched bases and insert the correct complementary bases.

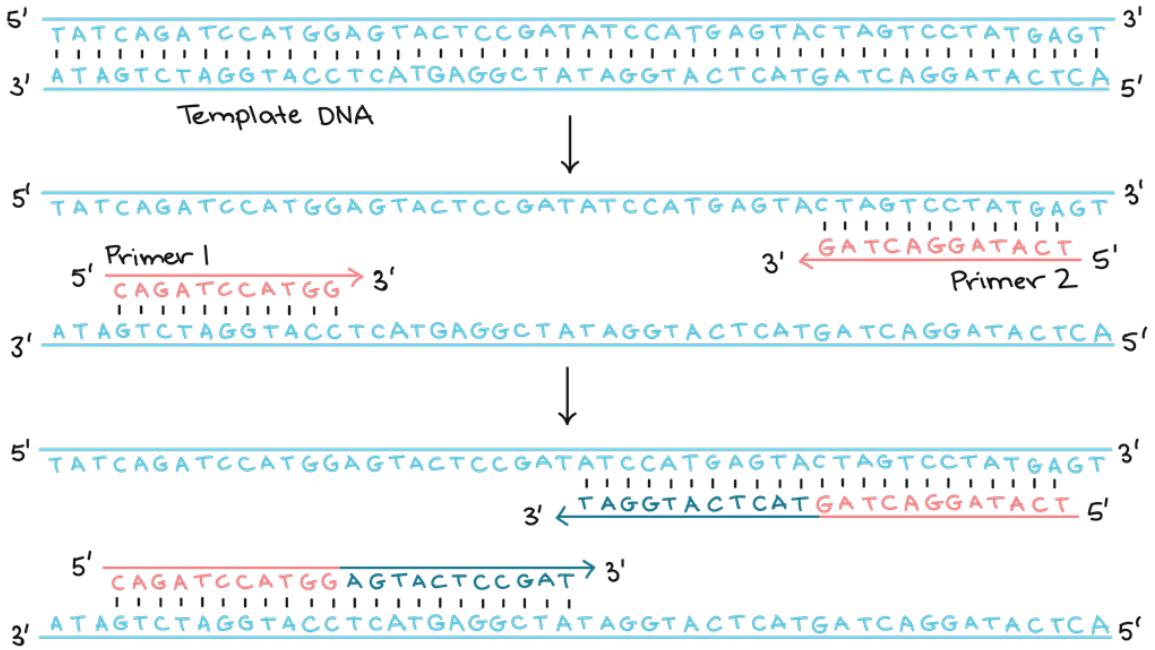
Primers

- short single-stranded nucleic acid (oligonucleotide)

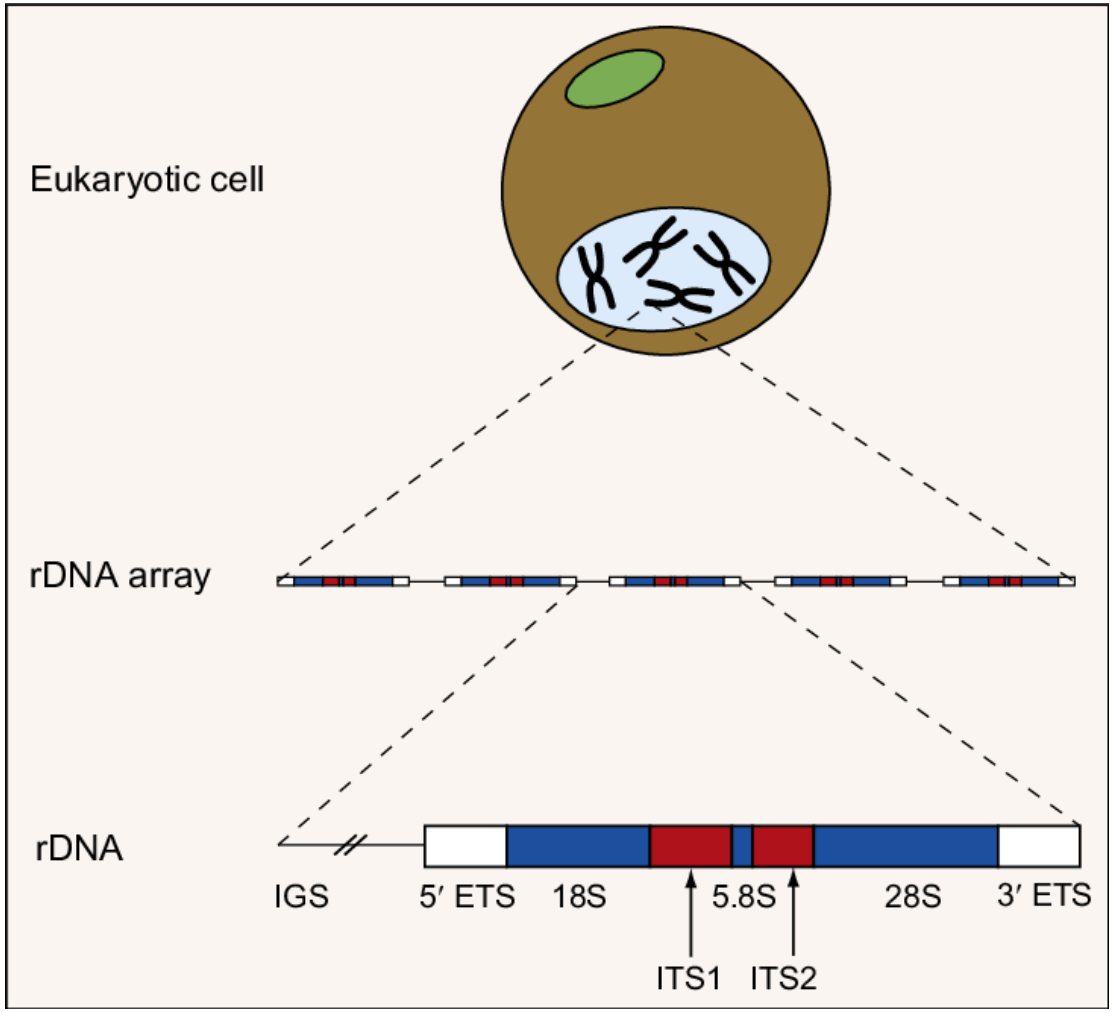
-necessary for the initiation of DNA synthesis

DNA polymerase adds nucleotides to the 3’-end of an existing nucleic acid

→requiring a primer to be bound to the template



Primers used for fungal identifications - the ITS region = the universal fungal barcode sequence



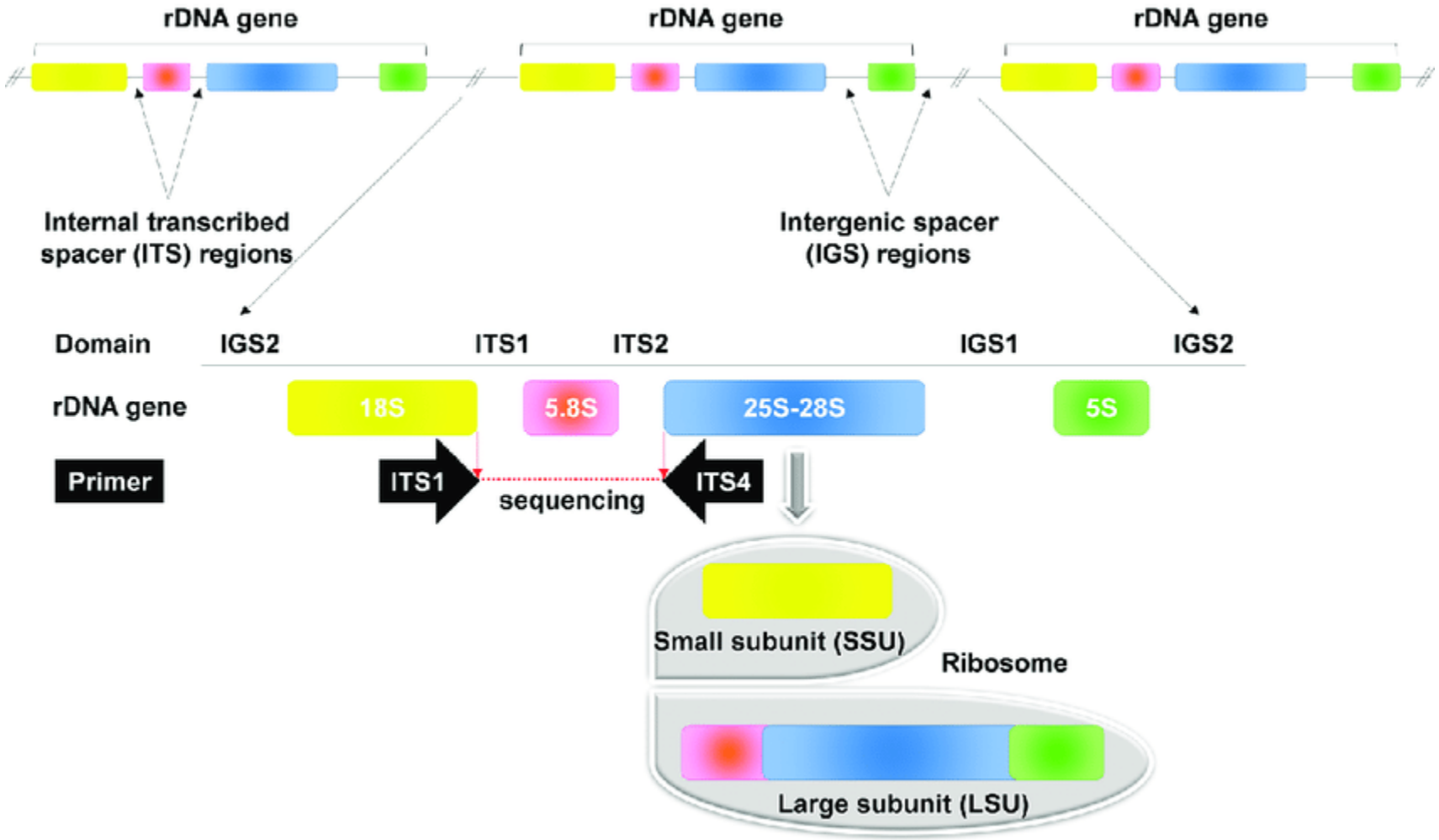
The internal transcribed spacer regions and rDNA in eukaryotes.

ITS regions 1 and 2 are coloured red and functional rRNA elements involved in protein structure are shown in blue.

IGS: intergenic spacer region, ETS: external transcribed spacer region.

https://www.researchgate.net/figure/The-internal-transcribed-spacer-regions-and-rDNA-in-eukaryotes-ITS-regions-1-and-2-are_fig3_230644270

Primers used for fungal identifications - the ITS region

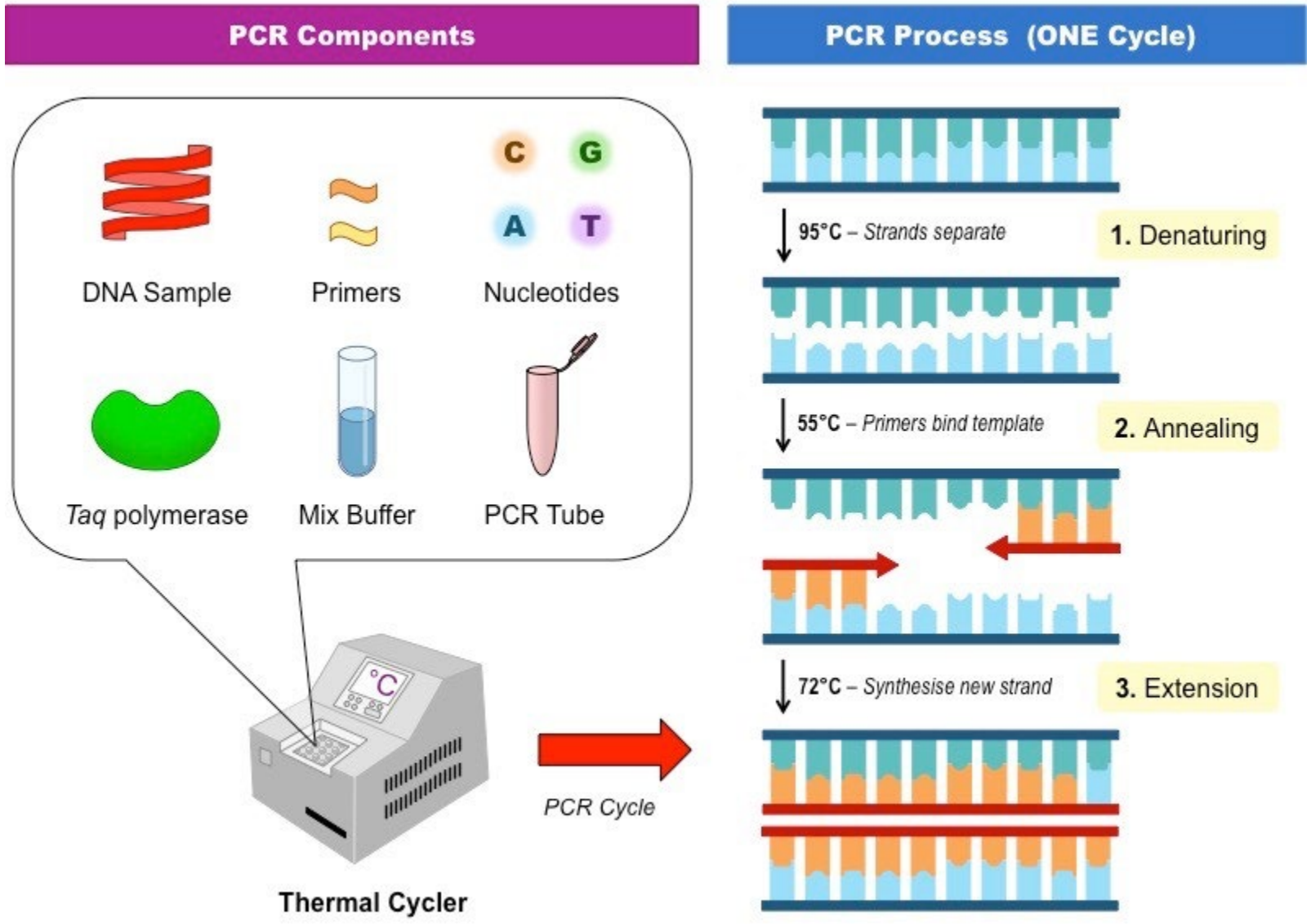


DOI:10.21608/ejbo.2019.7575.1283

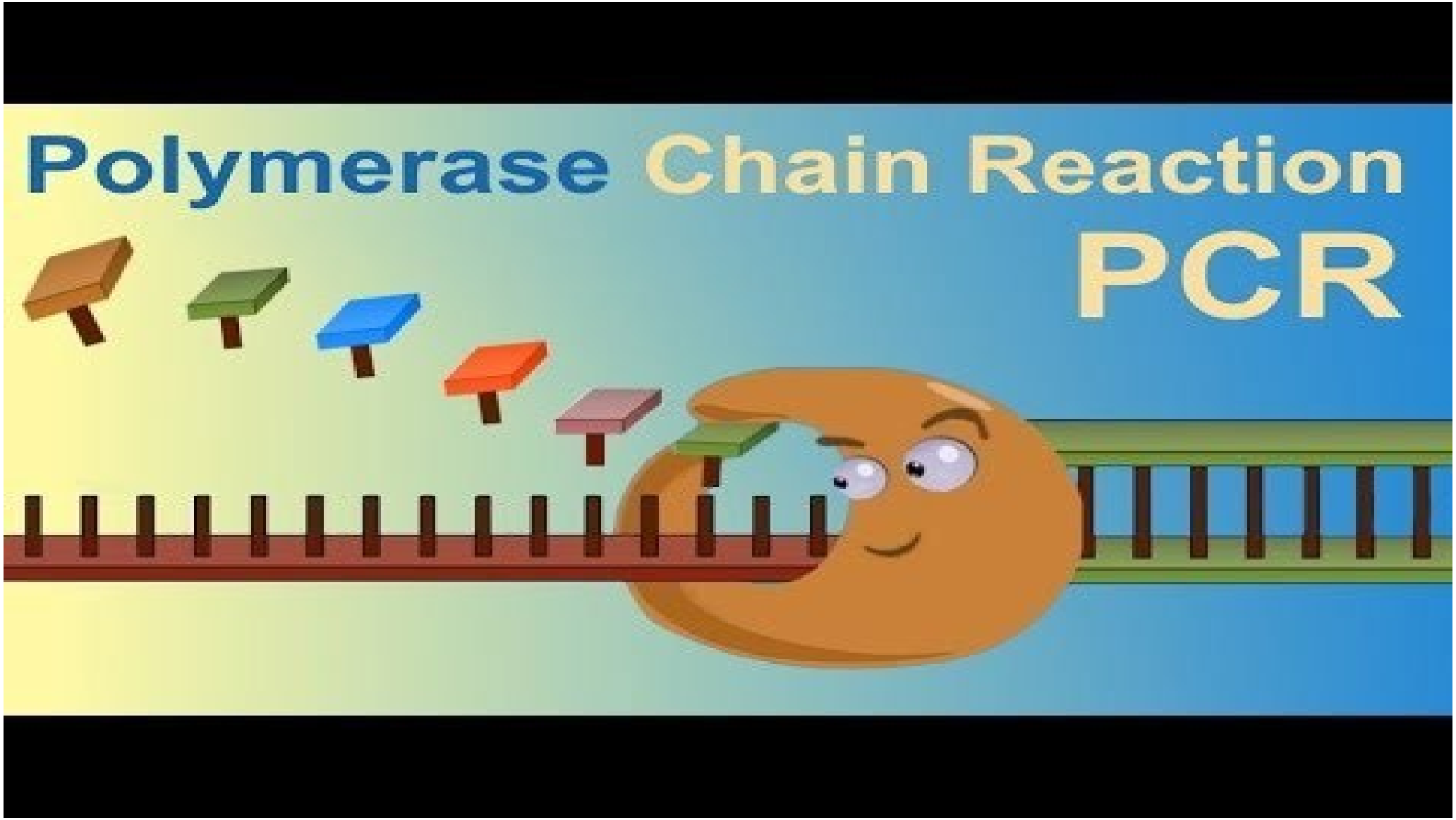
Polymerase Chain Reaction

- PCR uses variations in temperature to control the replication process via three steps:

- Denaturation – DNA sample is heated to separate it into two single strands (~95ºC for 1 min)
- Annealing – DNA primers attach to the 3’ ends of the target sequence (~55ºC for 1 min)
- Elongation –Taq- polymerase binds to the primer and copies the strand (~72ºC for 2 min)

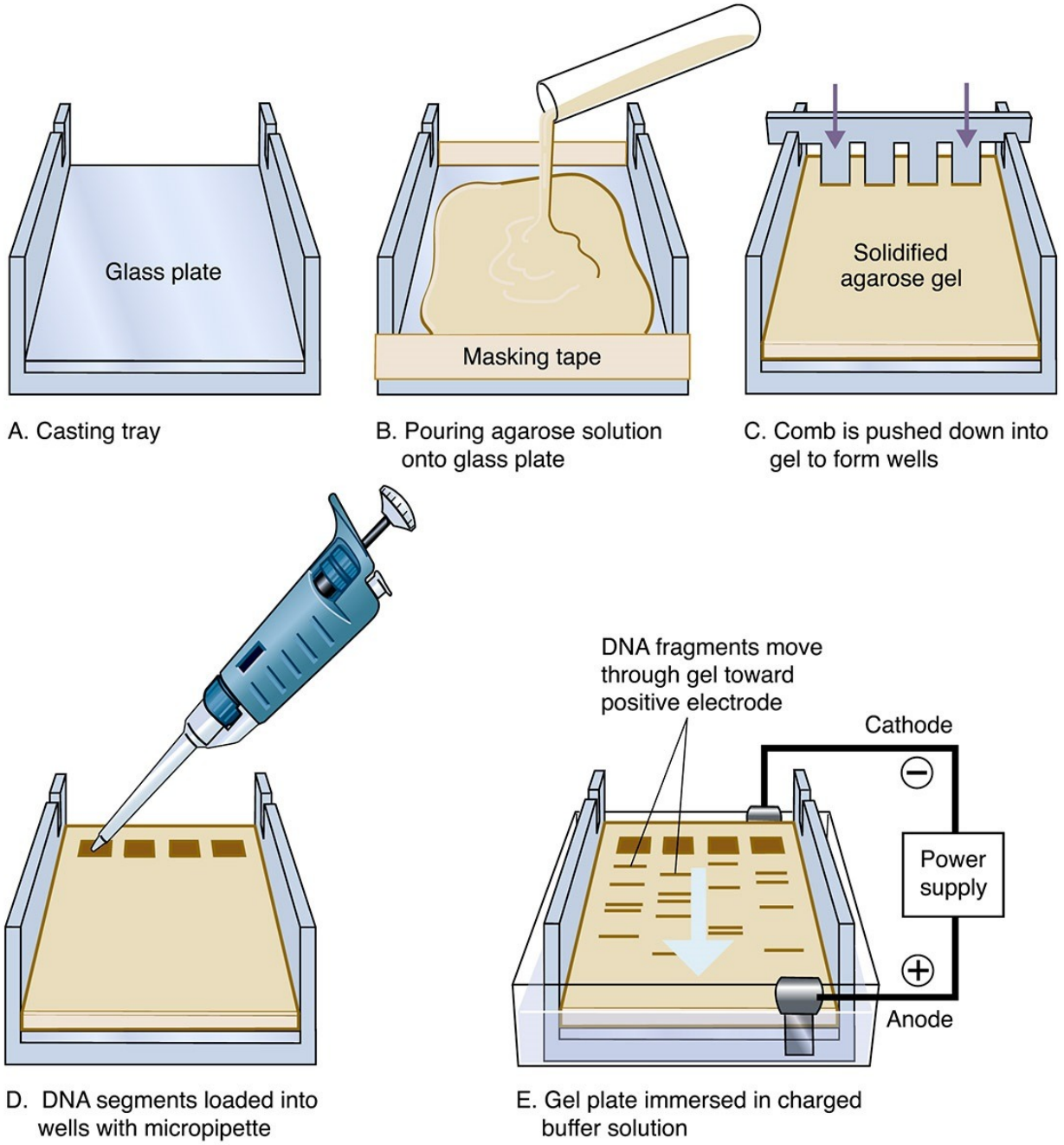


https://ib.bioninja.com.au/standard-level/topic-3-genetics/35-genetic-modification-and/pcr.html

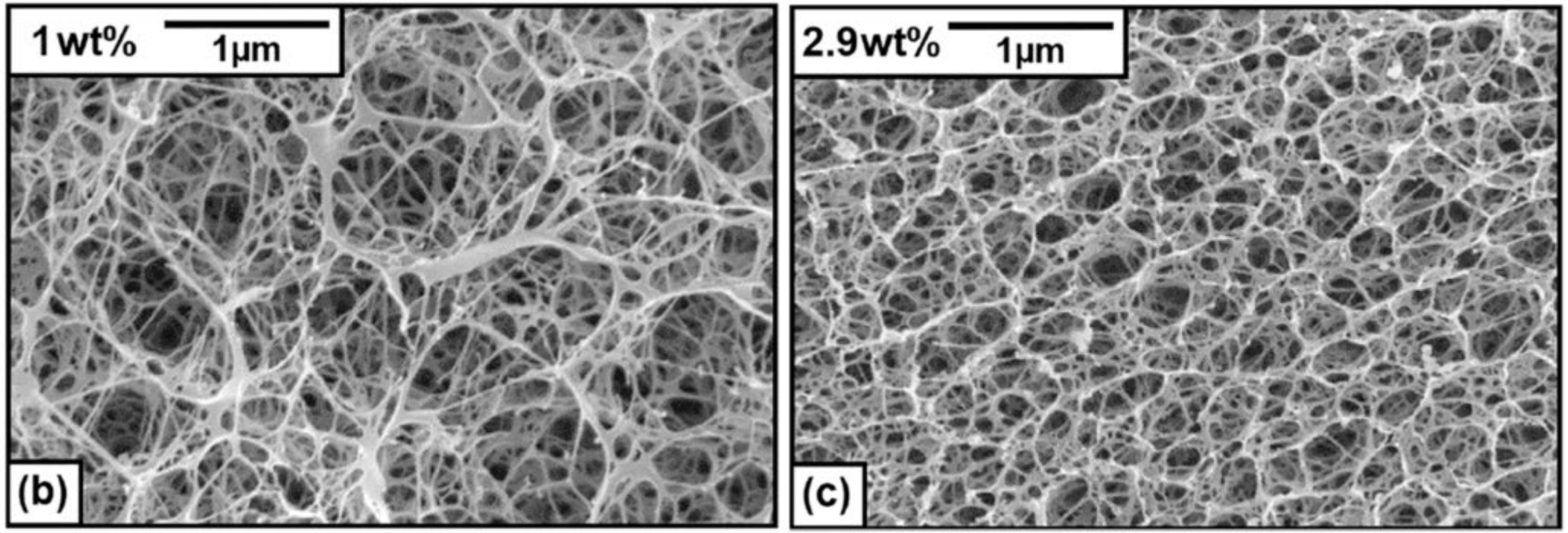


https://www.youtube.com/watch?v=iQsu3Kz9NYo

Electrophoresis gel with Agarose



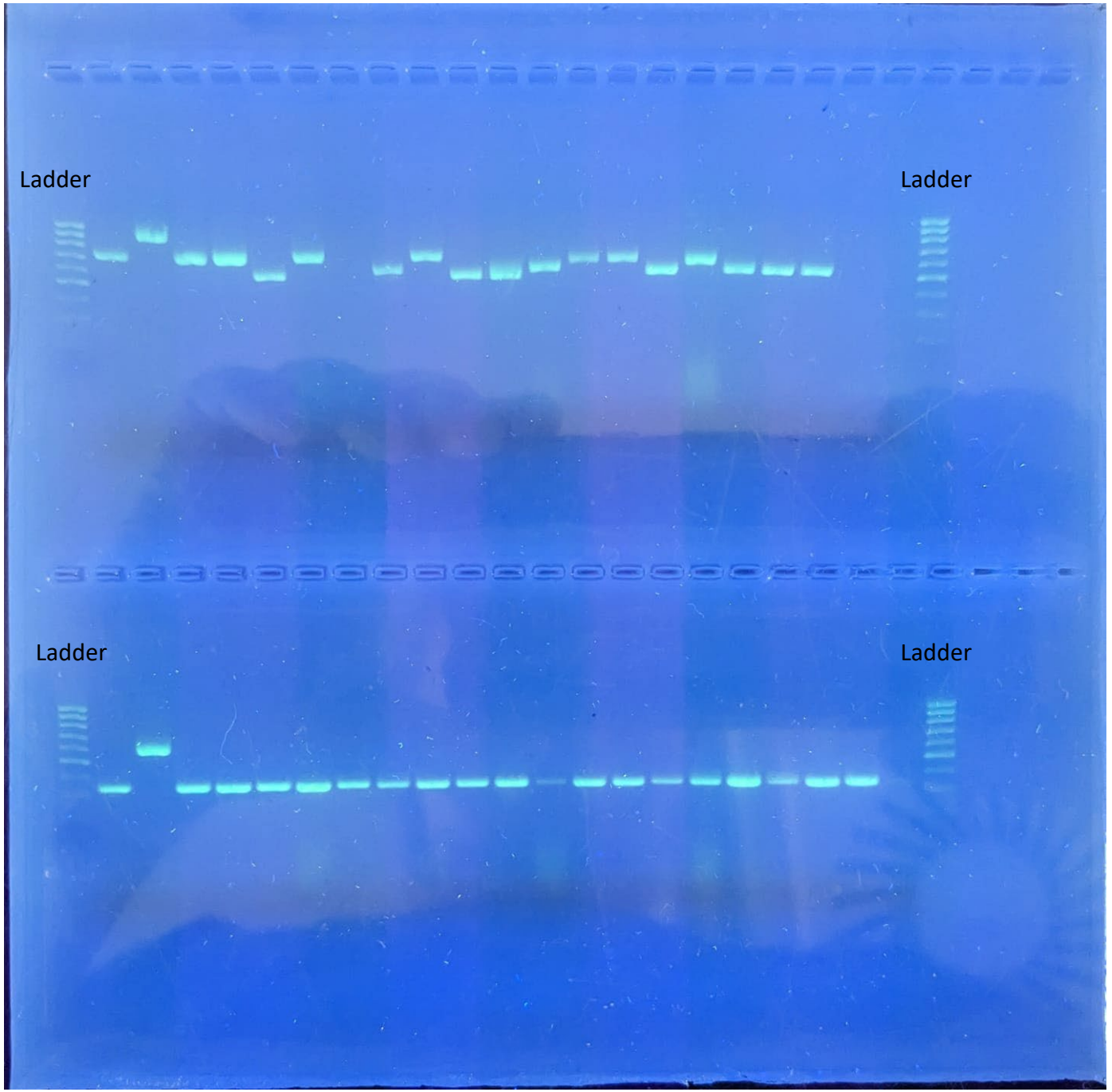
Agarose gel structure



https://www.minipcr.com/wp-content/uploads/agarose-comparison_ed-daoui-and-Snabre-2021-horizontal-1030x356.png

ITSu1 – ITSu4

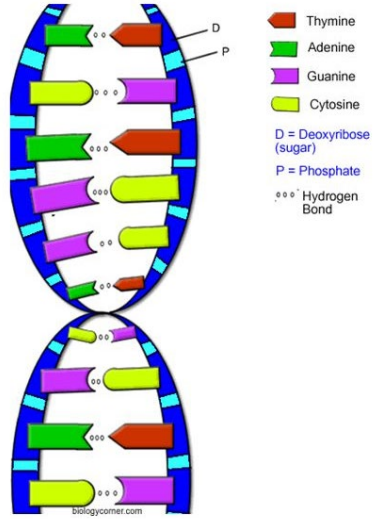
LSU-LR6



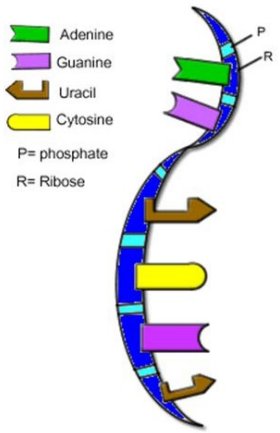
DNA

- The genetic information stored in an organism's DNA contains the instructions for all the proteins the organism will ever synthesize.

- Passes down genetic information to offspring during reproduction.



Function



RNA

- Transcribes the genetic information into a form that is easy to understand and read by the cell
- Assists in protein synthesis.

DNA Sequencing

- NCBI Blast