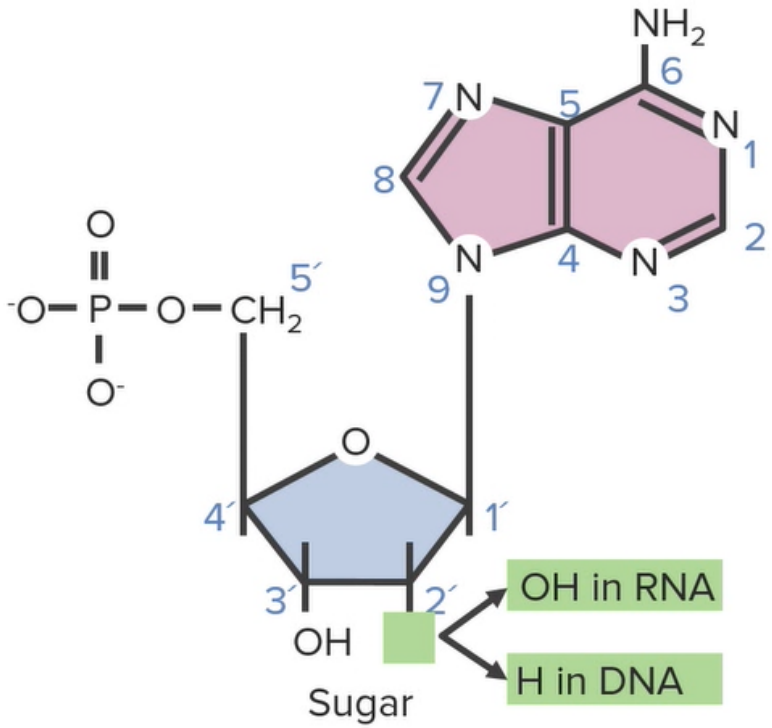
PCR

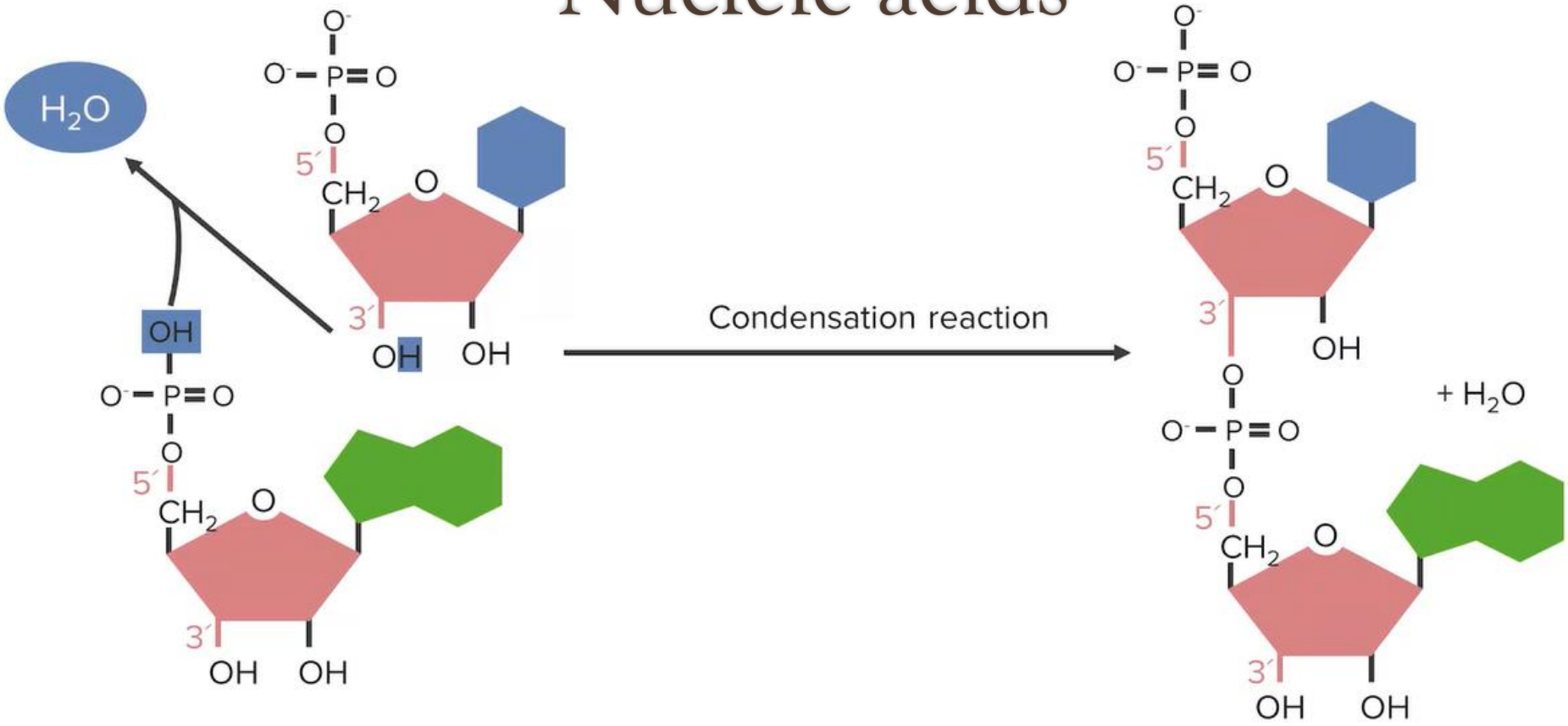
By Dr. Mwaba MH (PhD)

Nucleic acids

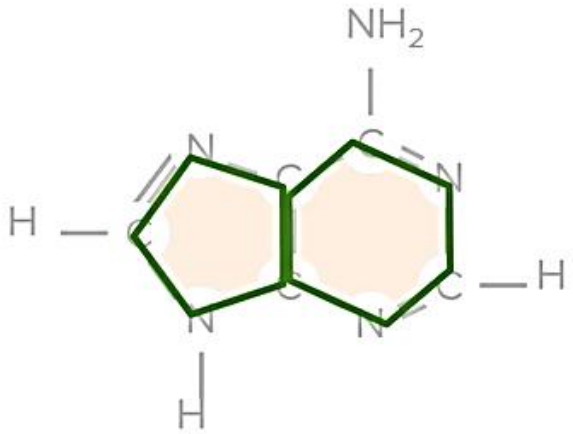
The **monomers** of nucleic acids are **nucleotides.**



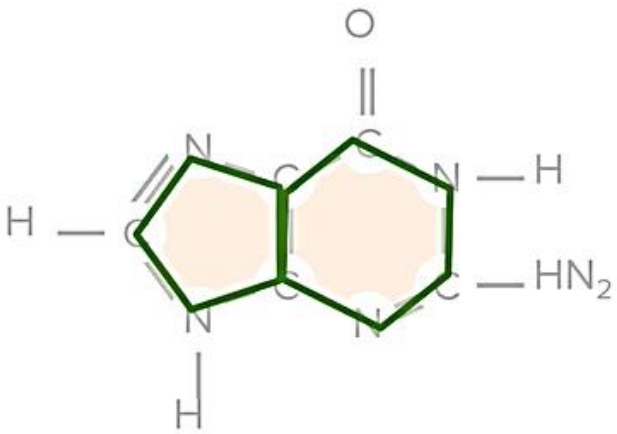
Nucleic acids



Purines



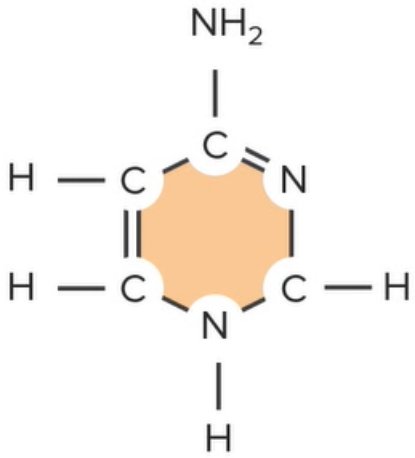
Adenine



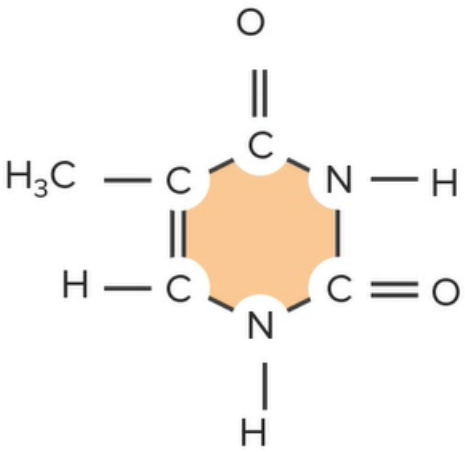
Guanine

Purines: double ring structure

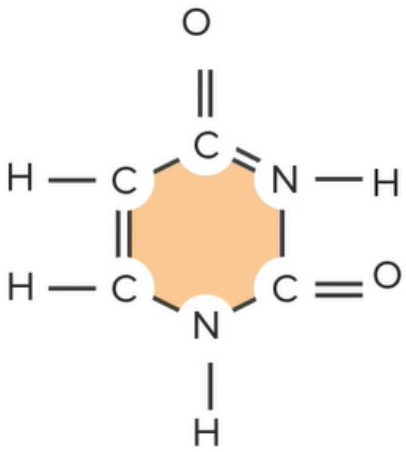
Pyrimidines



Cytosine
(both DNA and RNA)

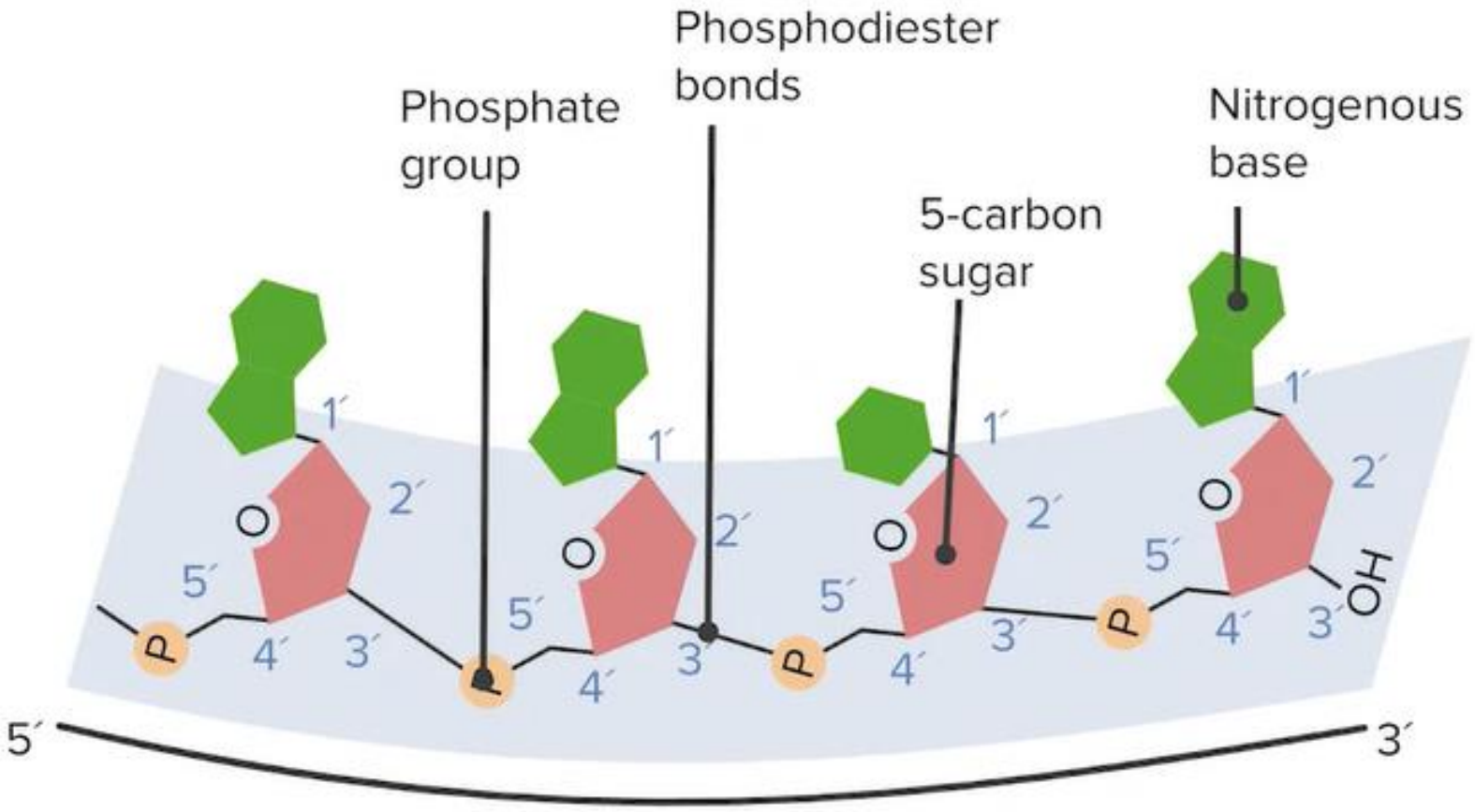


Thymine
(DNA only)

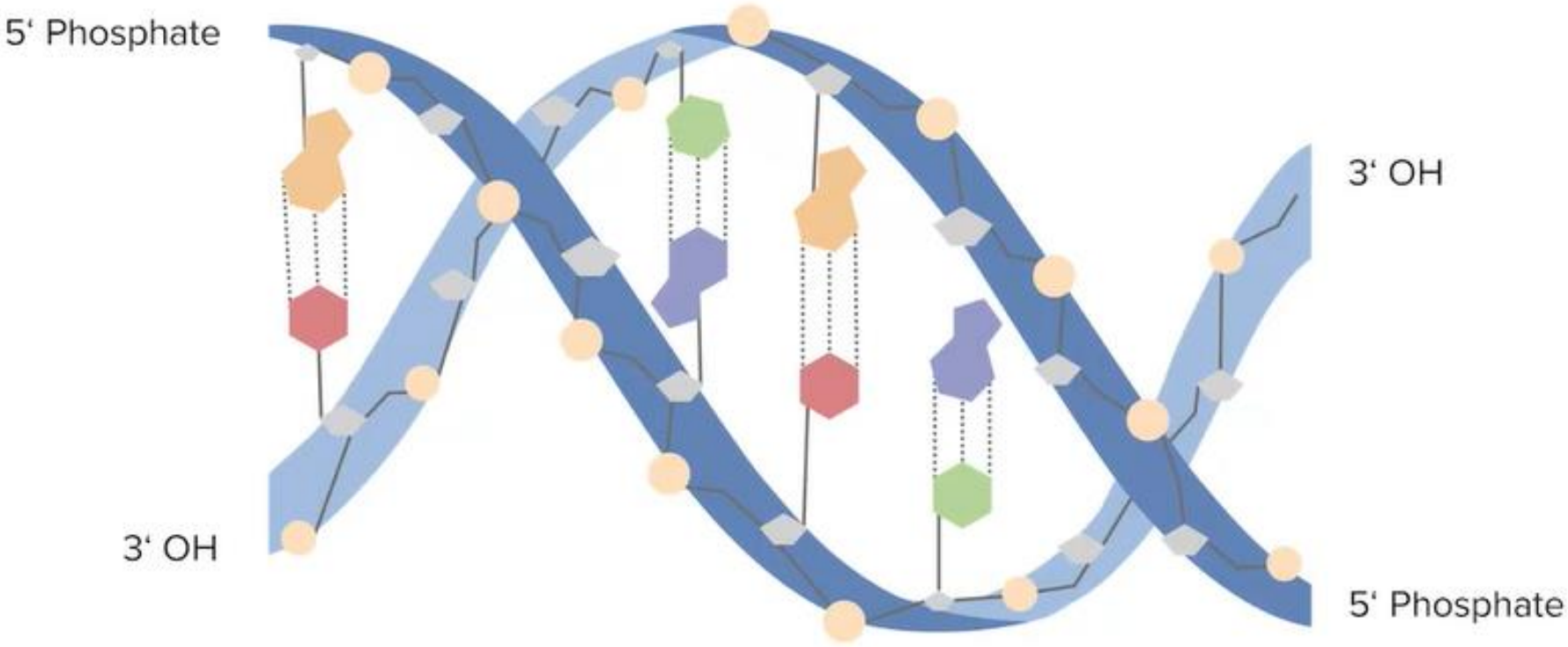


Uracil
(RNA only)

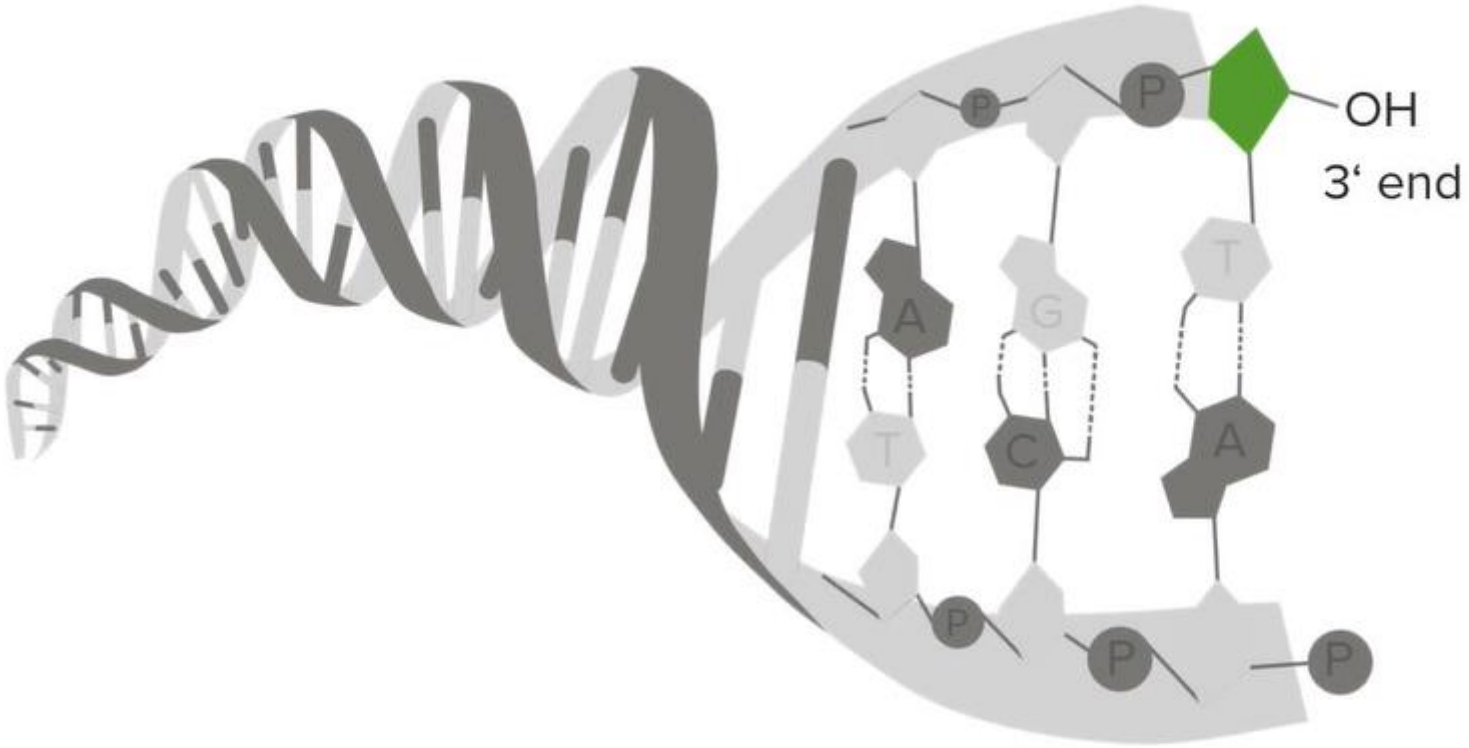
Pyrimidines: cytosine, thymine, uracil ring structure



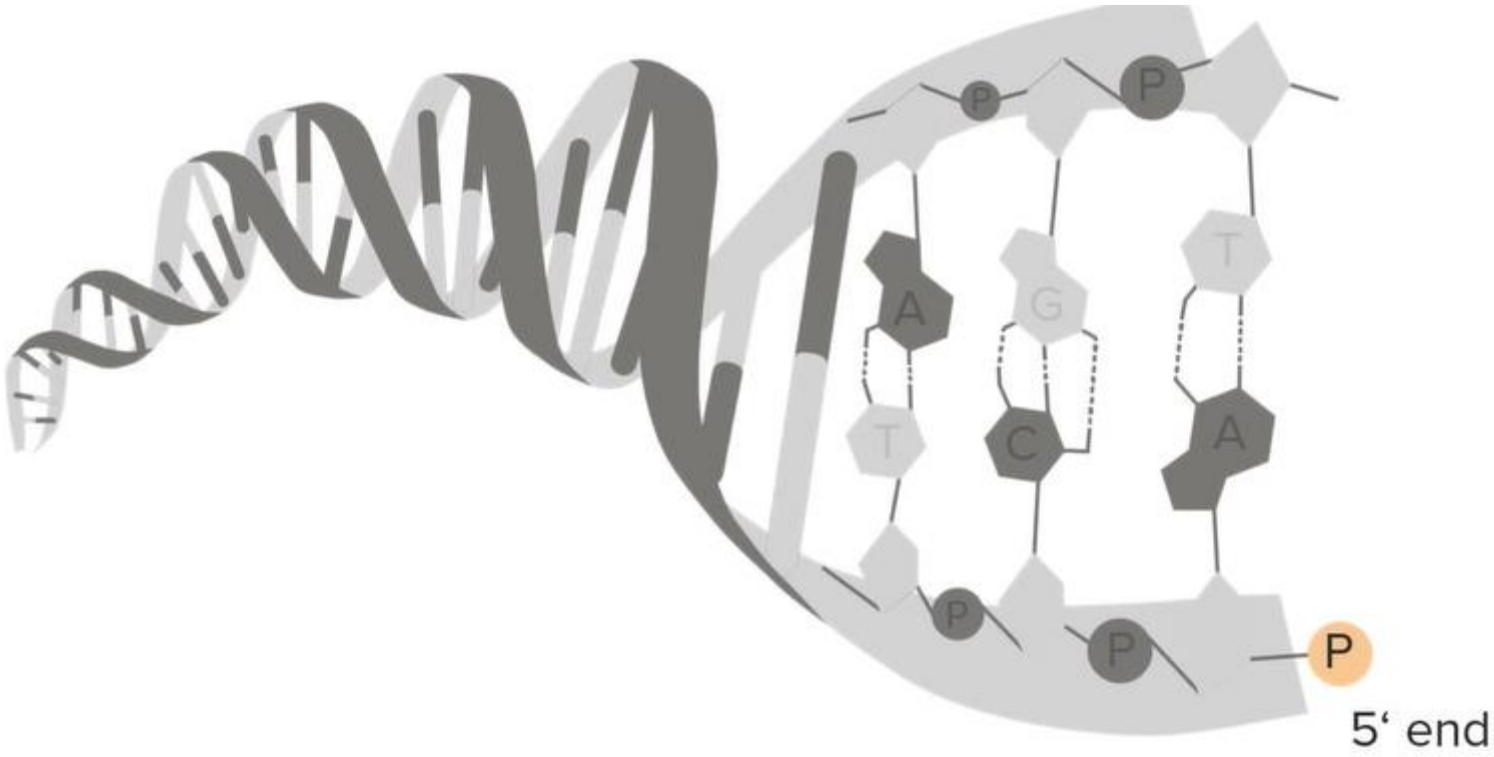
Polymers: nucleic acids
(DNA & RNA)



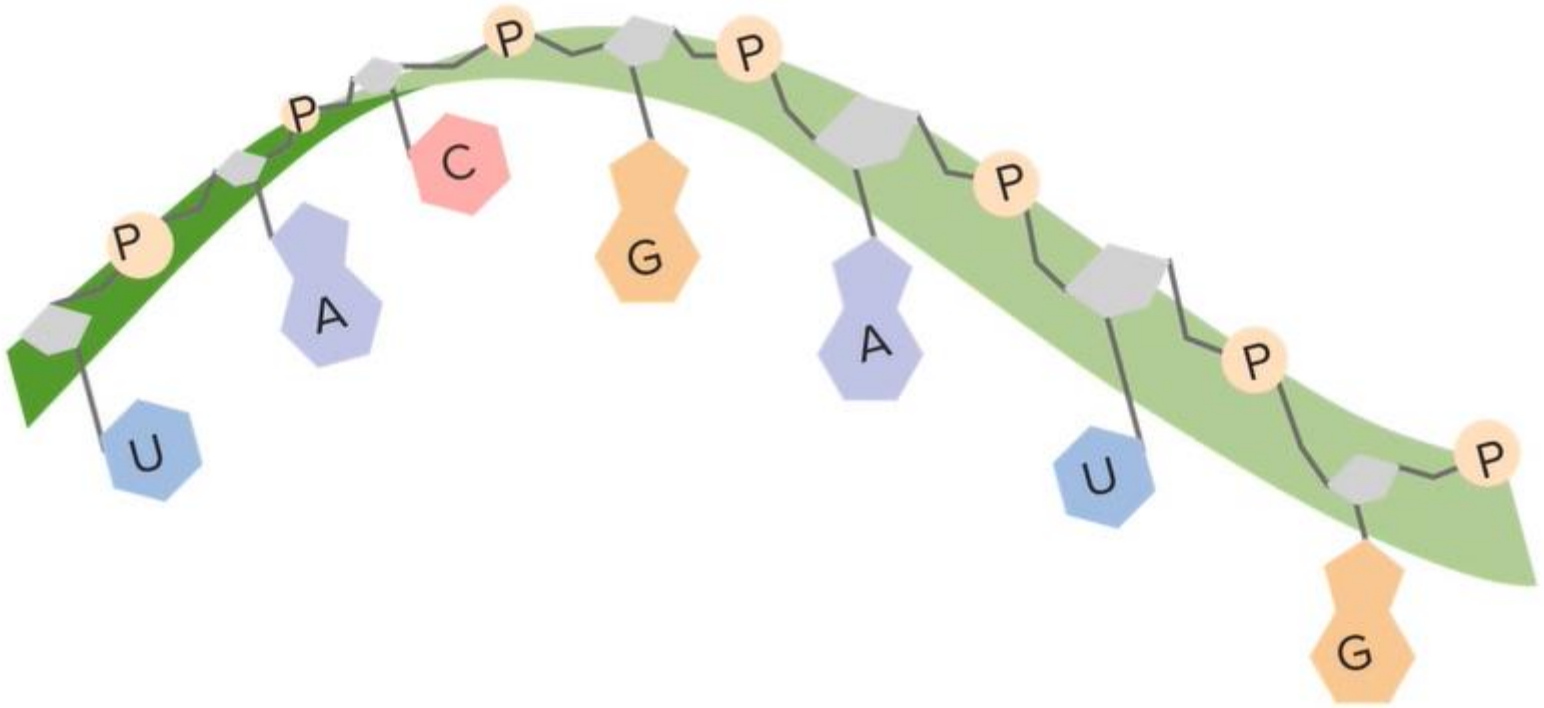
Antiparallel orientation



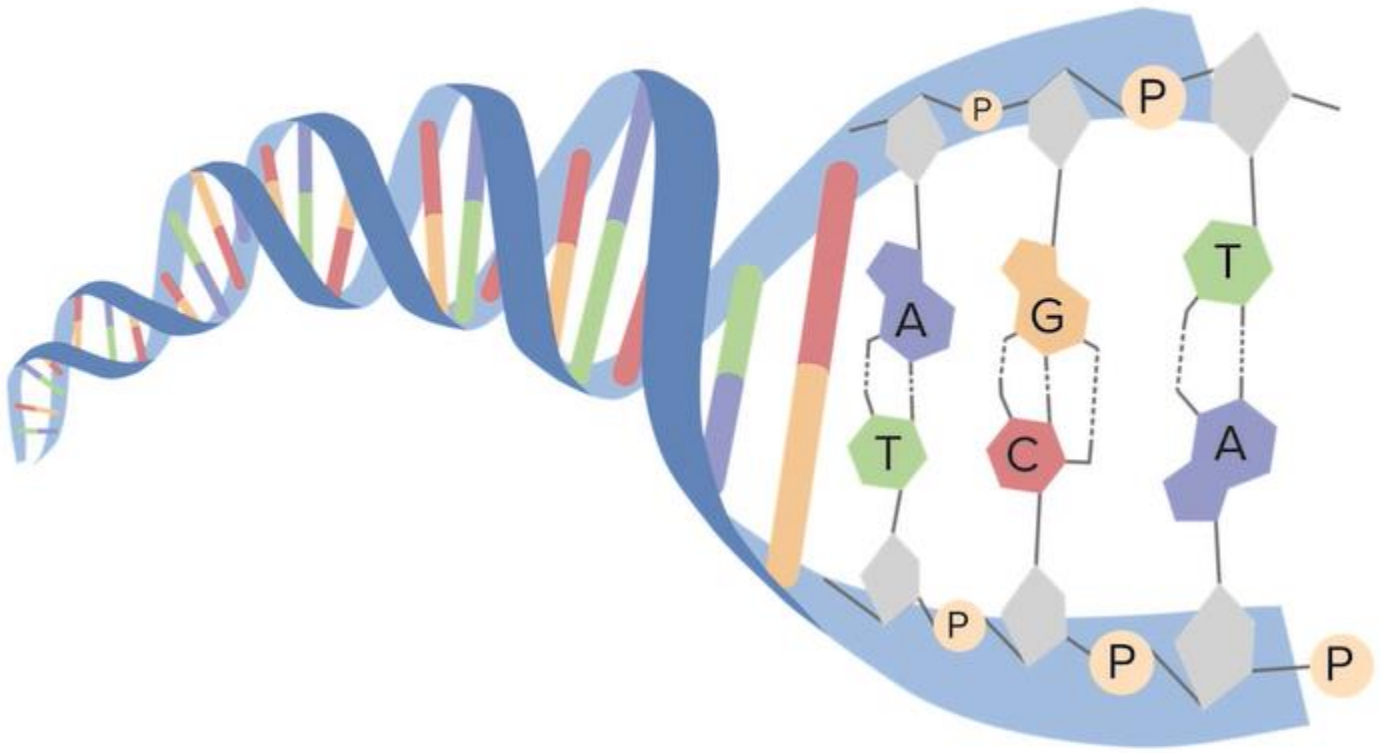
3’ OH end



5’ phosphate end

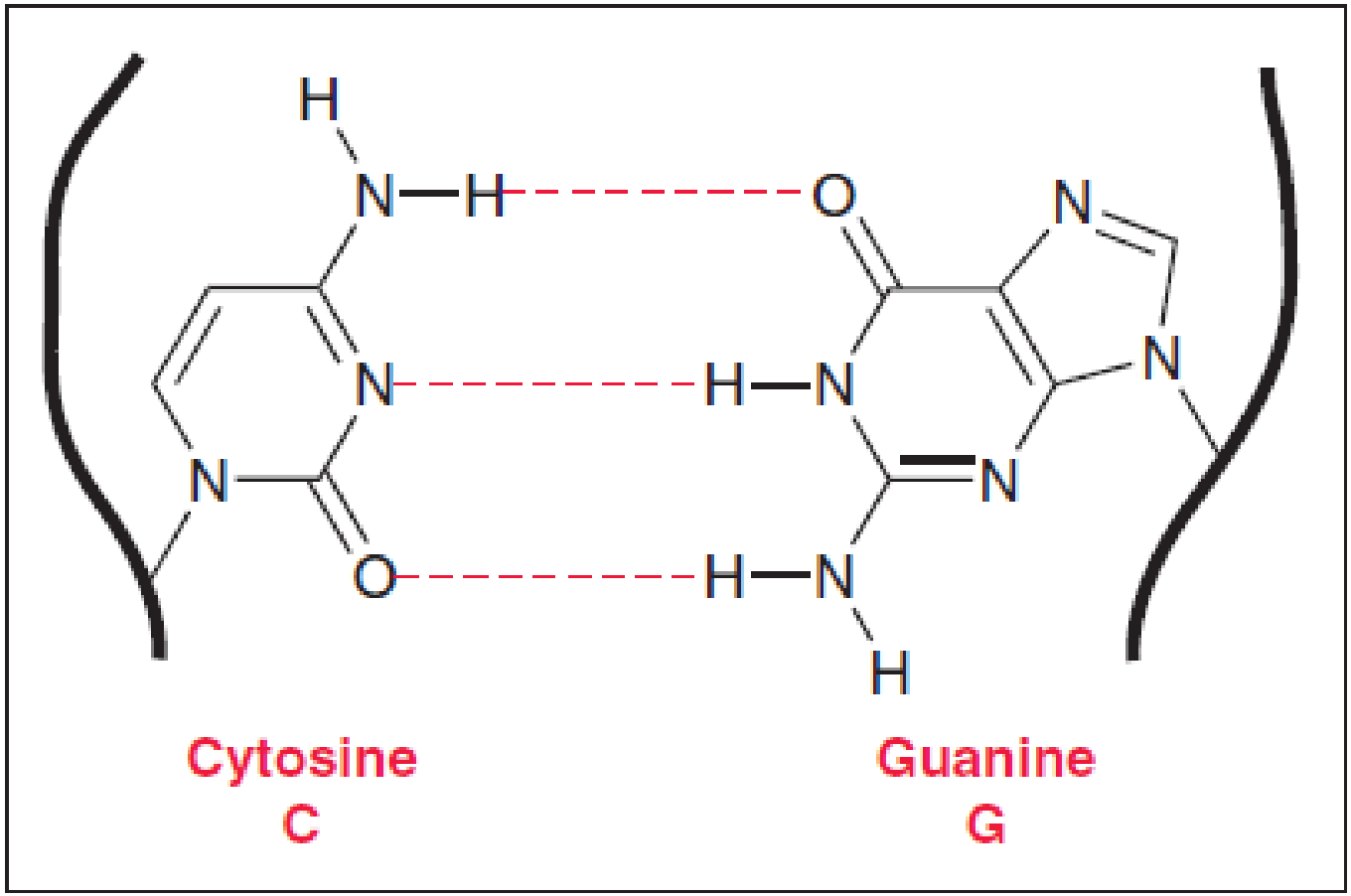


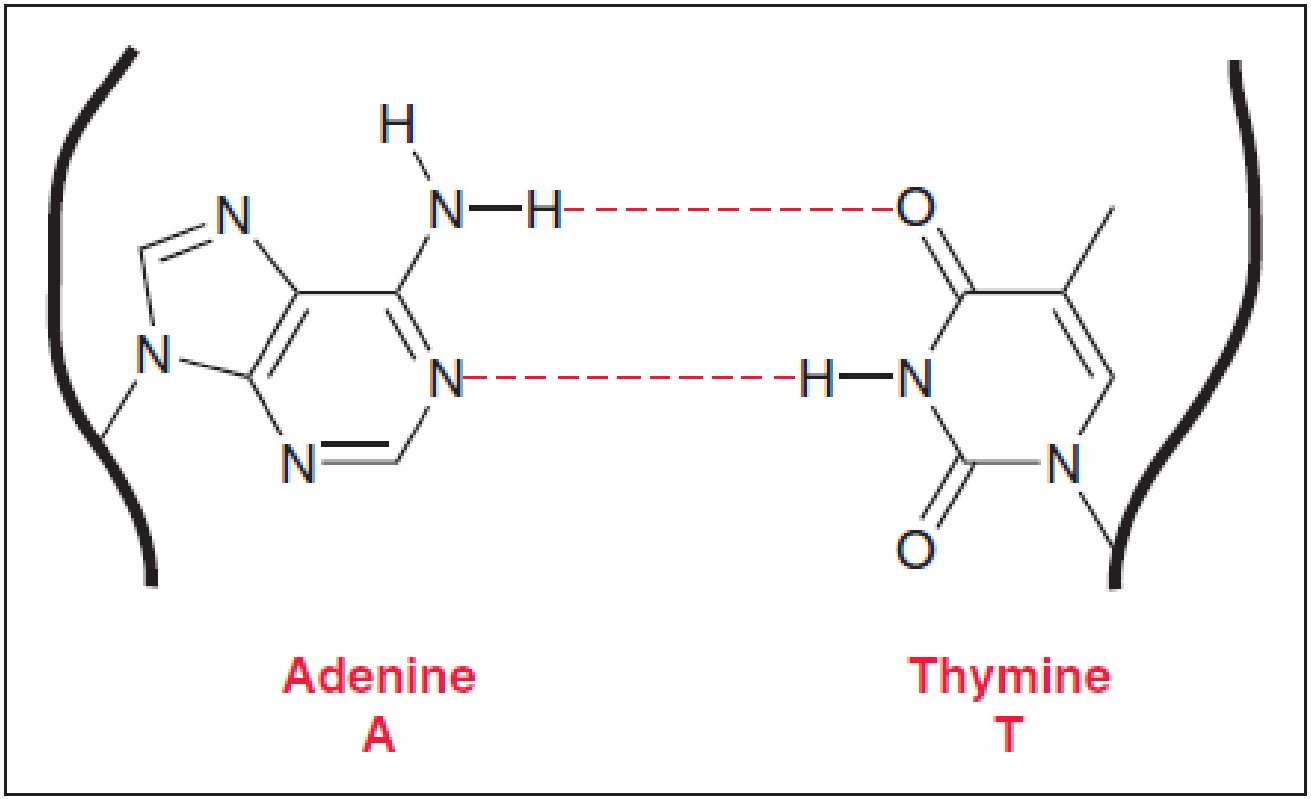
RNA is single stranded.



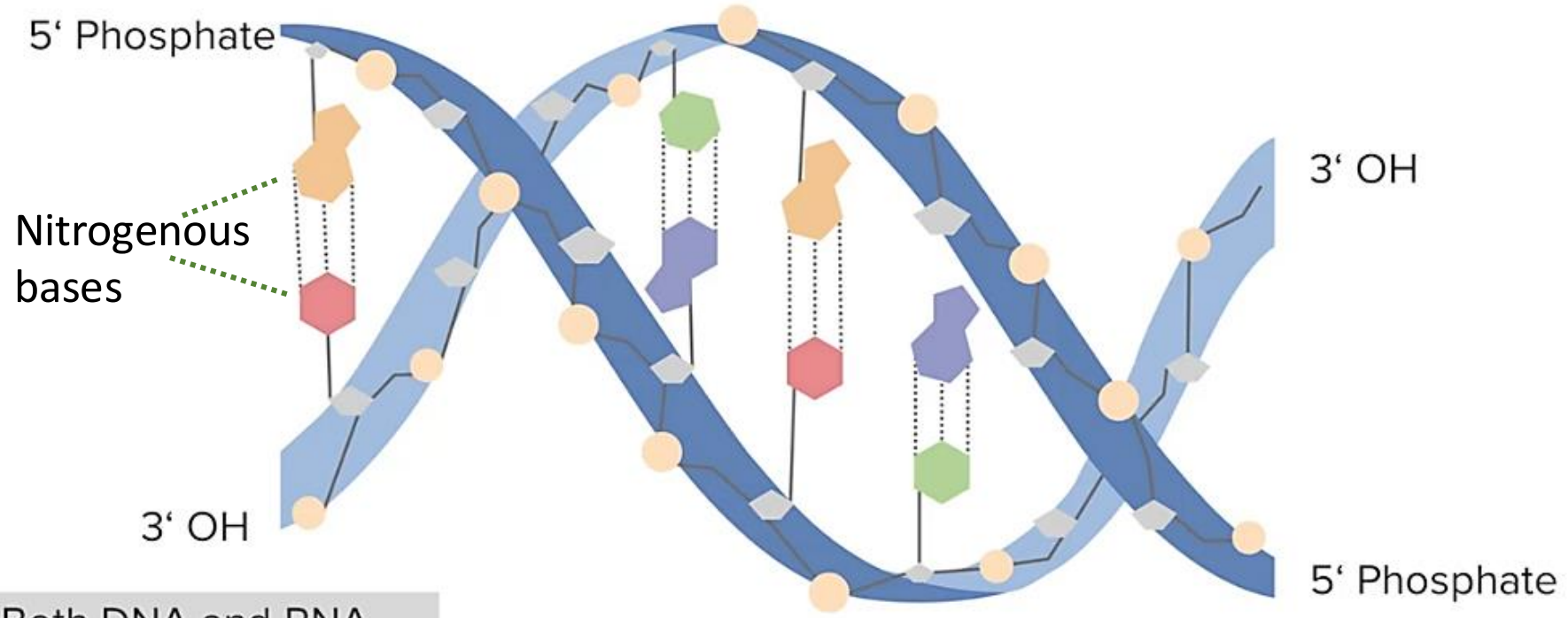
Nitrogenous bases make the steps.

The Two DNA Strands Are Held Together by a Hydrogen Bond Zipper


H
N
H
O
N
N
H
N
N
N
N
O
H
N
H
Cytosine
C
Guanine
G


H
N
N
H
O
N
N
N
H
N
N
O
Adenine
A
Thymine
T

Phosphate – Sugar backbone

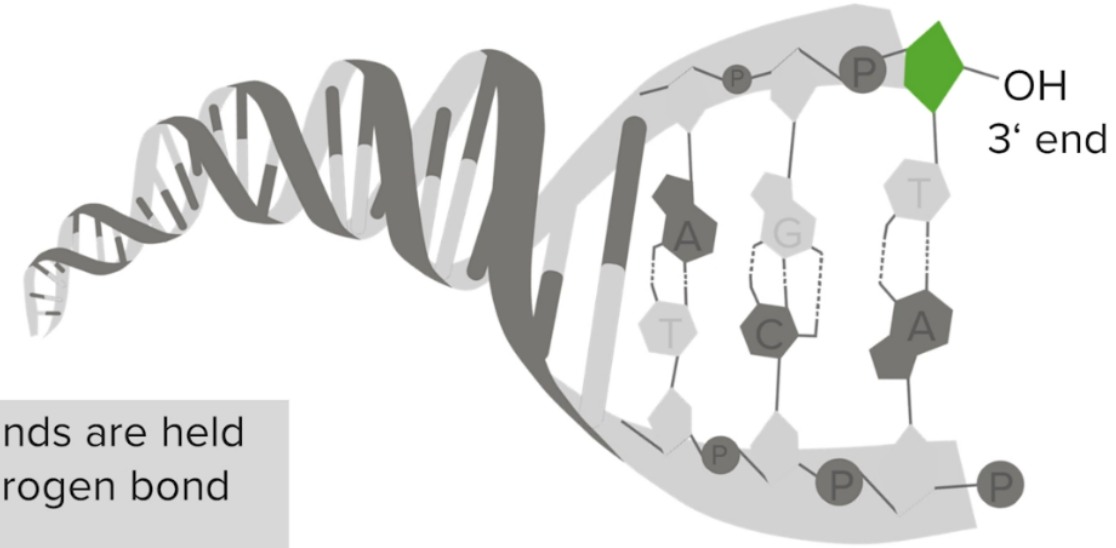


Both DNA and RNA have phosphate sugar backbones.

Antiparallel orientation

Hydrogen – Bond Zipper

Nucleic Acids: Information Storage



The two DNA strands are held together by a hydrogen bond zipper.

3’ OH end

Hydrogen – Bond Zipper

Nucleic Acids: Information Storage

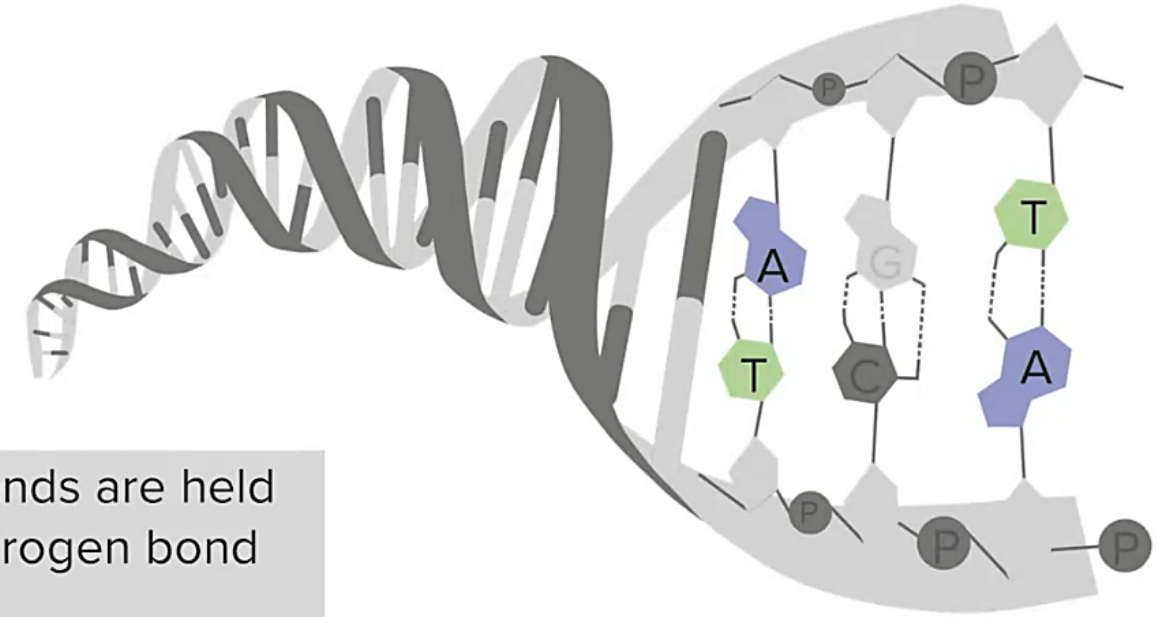
The two DNA strands are held together by a hydrogen bond zipper.

5’ phosphate end

DNA polymerase can only read strands from the 3’ to 5’ direction

Hydrogen – Bond Zipper

Nucleic Acids: Information Storage



The two DNA strands are held together by a hydrogen bond zipper.

Adenine pairs with thymine.

Hydrogen – Bond Zipper

Nucleic Acids: Information Storage

The two DNA strands are held together by a hydrogen bond zipper.

Guanine pairs with cytosine.

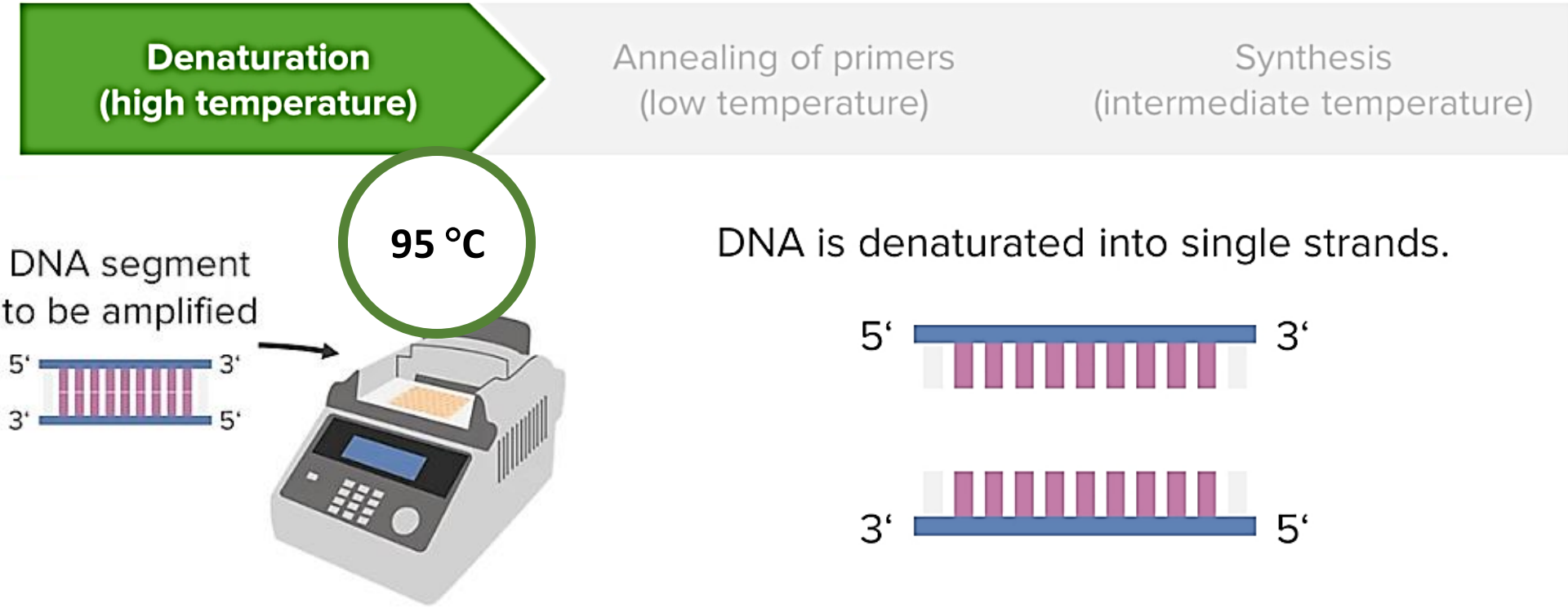
Polymerase Chain Reaction (PCR)

PCR machine

Thermocycler

Amplifying DNA quickly without using vectors.

PCR- Denaturation

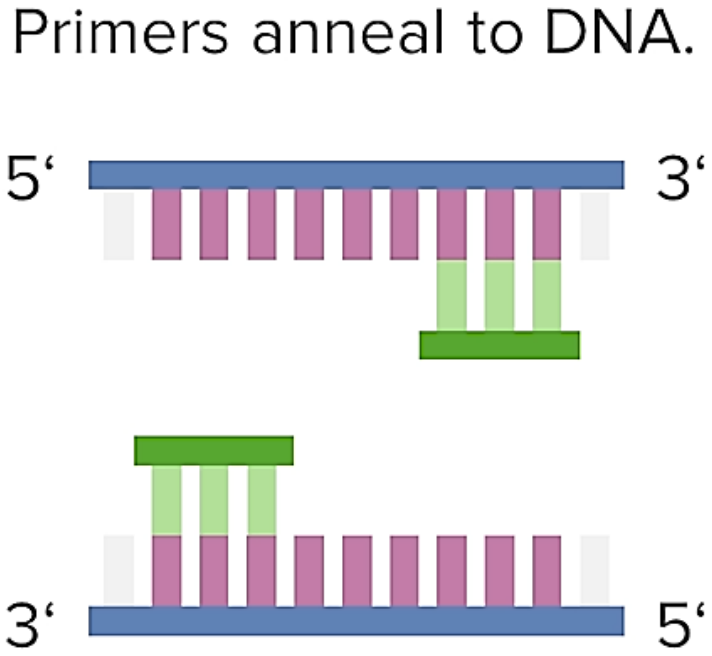


Sample is first heated to denature DNA

PCR- Annealing

Denaturation (high temperature) → **Annealing of primers (low temperature)** → Synthesis (intermediate temperature)

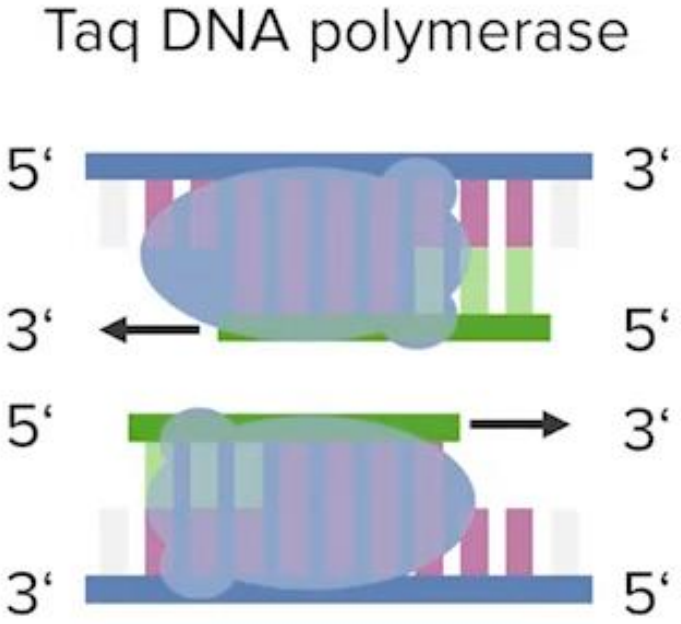
DNA is cooled to a lower temerature to allow annealing of primers.



PCR- Extension/Synthesis

Denaturation (high temperature) | Annealing of primers (low temperature) | **Synthesis (intermediate temperature)**

DNA is heated to 72°C, the optimal temperature for Taq DNA polymerase to extend primers.

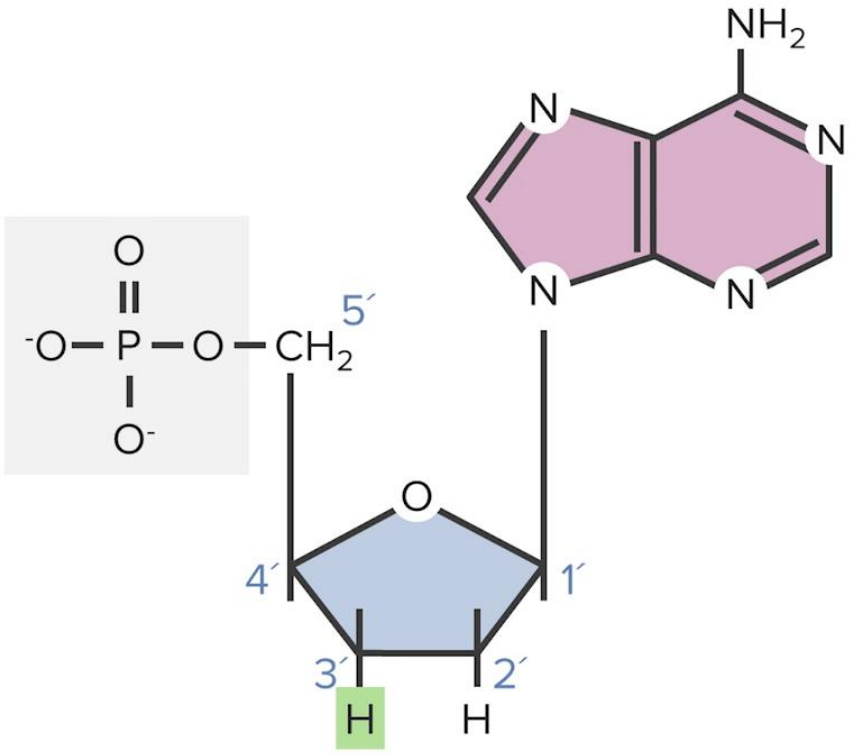


Sanger Sequencing

Dideoxynucleotides (ddNTPs) are a critical component

Recal 3'OH needed for DNA polymerase to add new nucleotides

- In vitro DNA replication
- Termination of replication



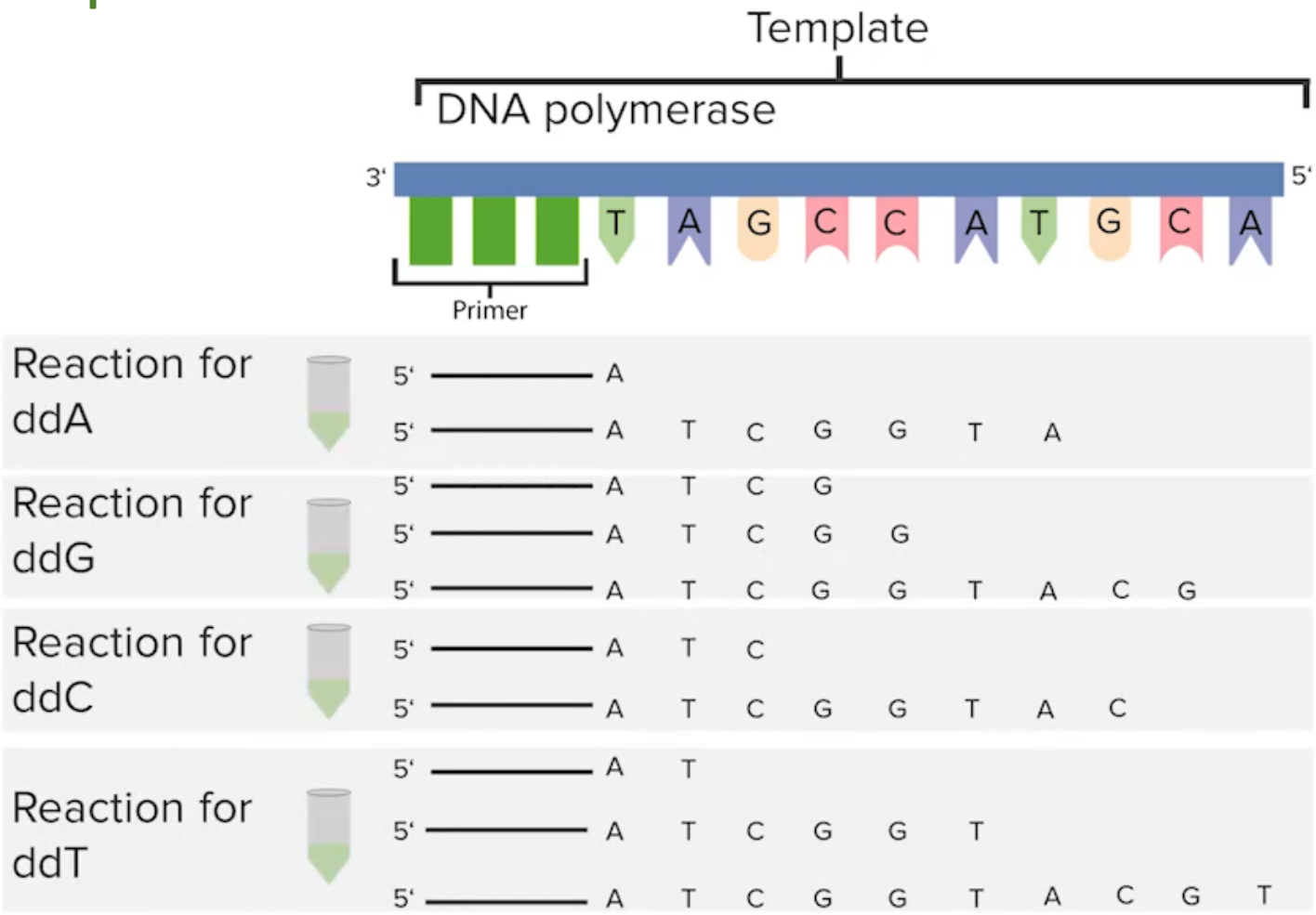
Sanger Sequencing

Manual method

- Template strand
- Primers
- DNA Polymerase
- ddNTPs

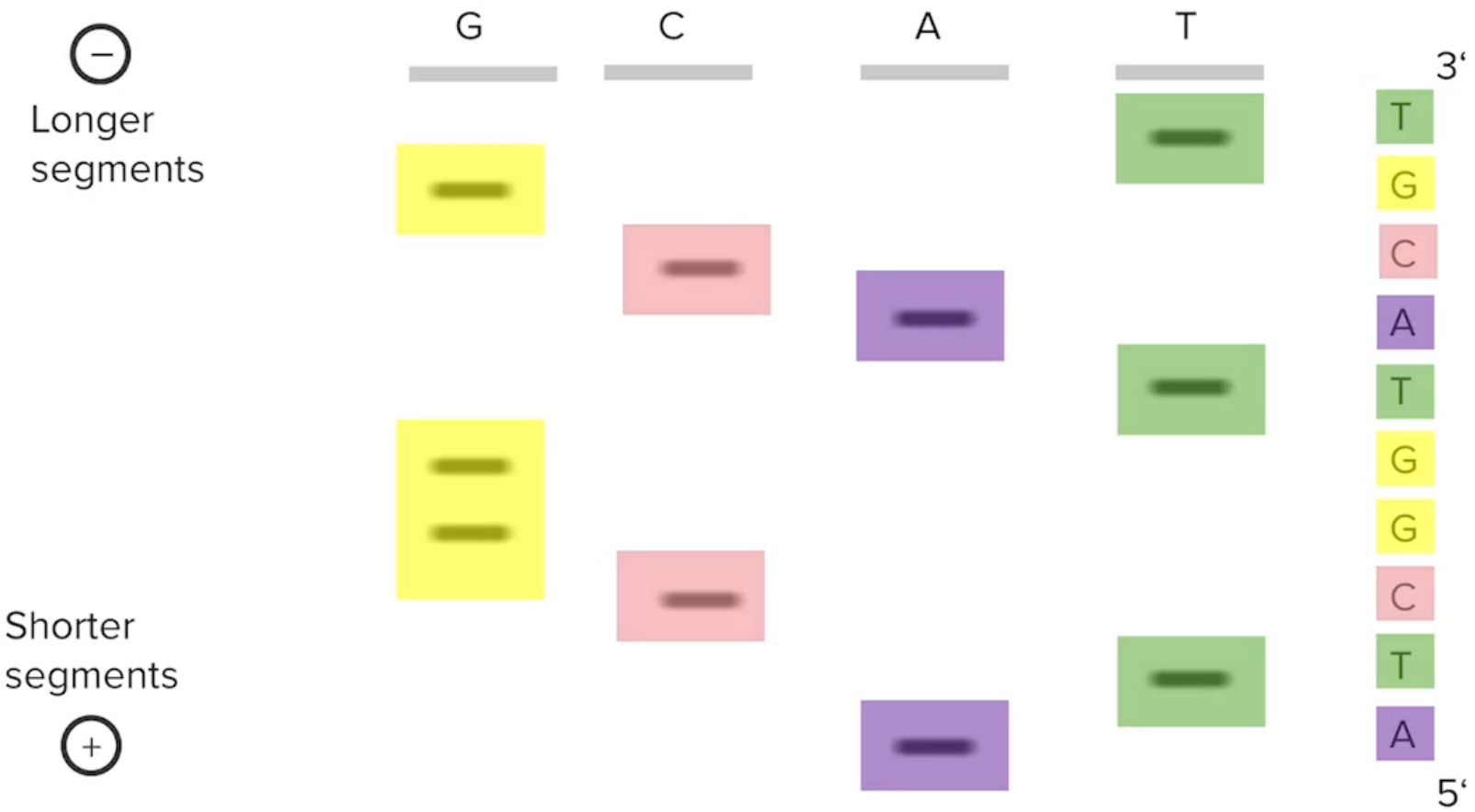
Sanger Sequencing

4 separate reactions



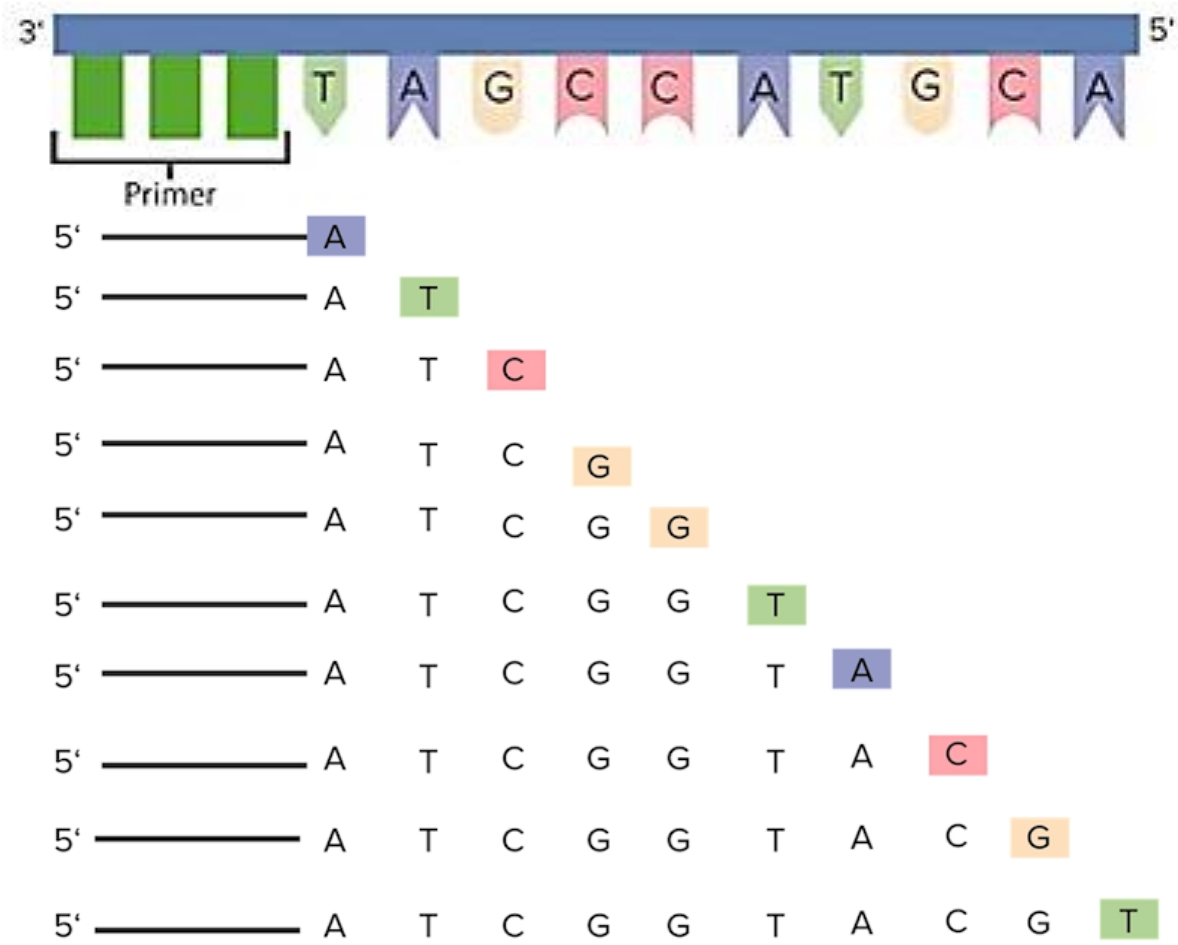
Sanger Sequencing

Electrophoresis



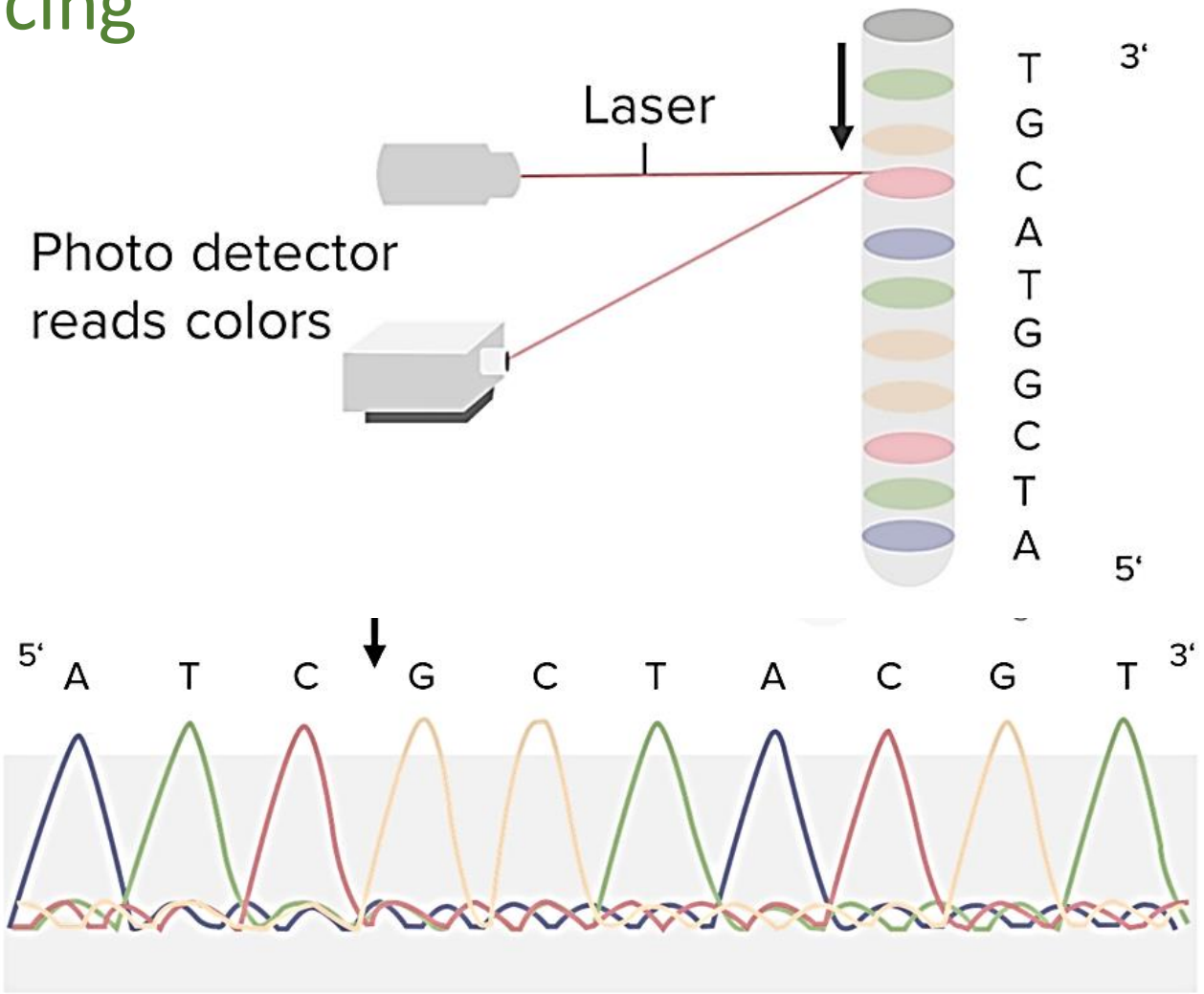
Sanger Sequencing

Automation allowed for **Rapid Sequencing**



Sanger Sequencing

Rapid sequencing



Sanger Sequencing

- Each dNTP labelled with a different colour fluorescent dye
- Reaction done in a single tube
- Computer determines the sequence