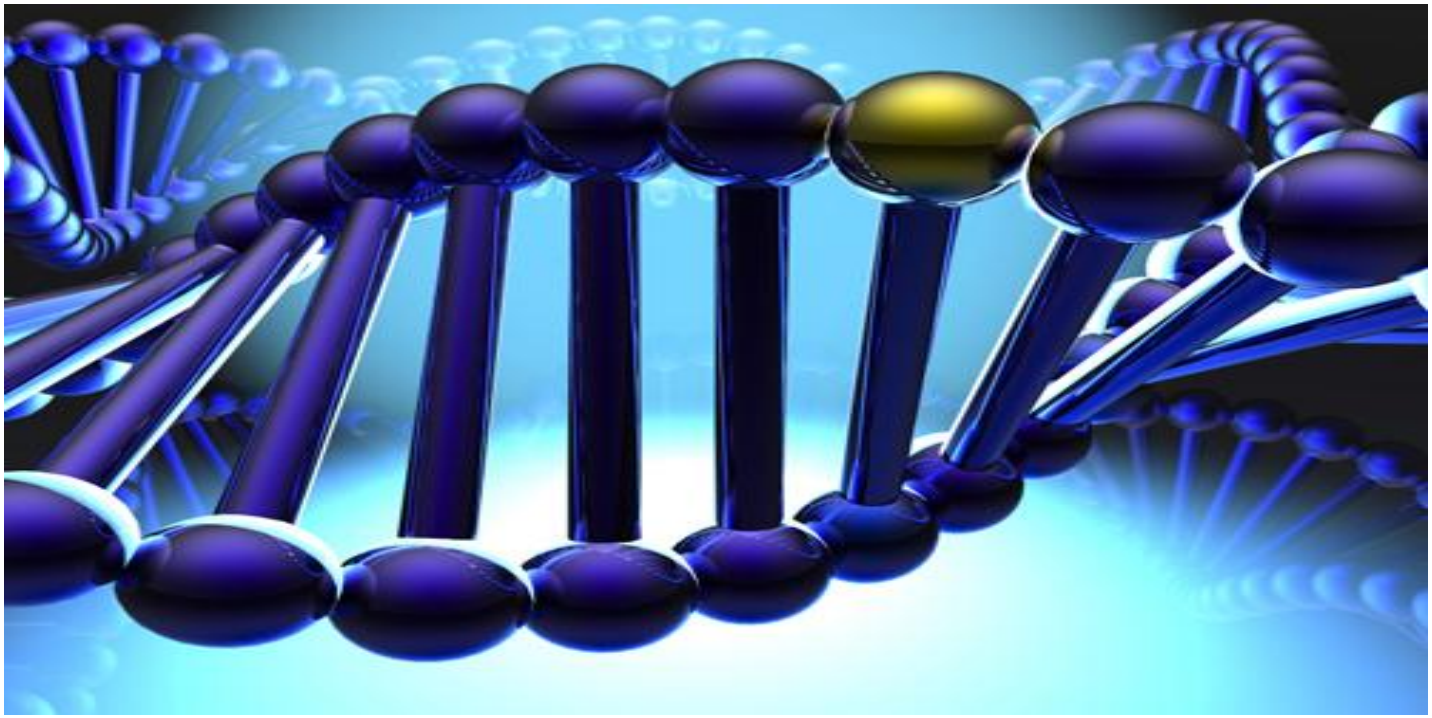
# DNA Amplification



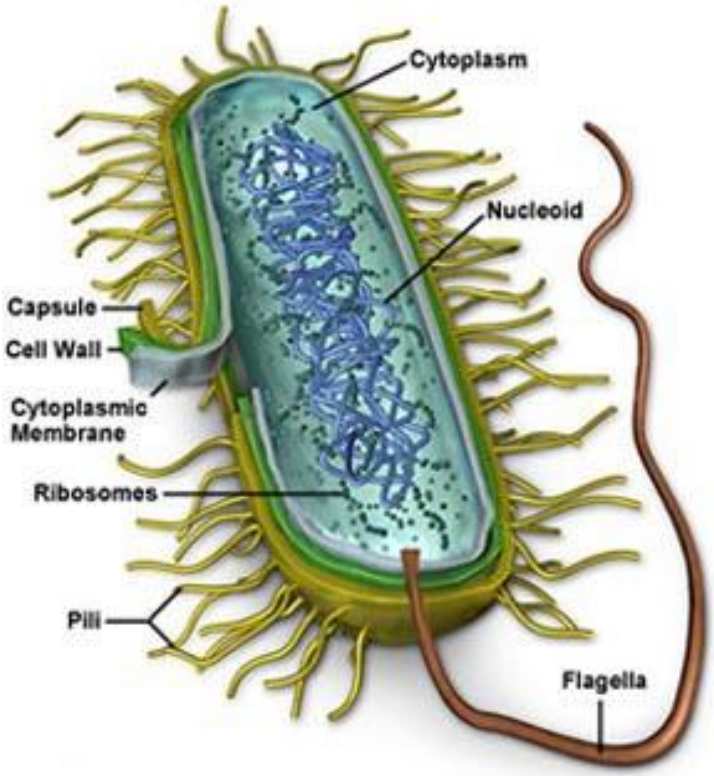
**Mohamed N. Seleem**

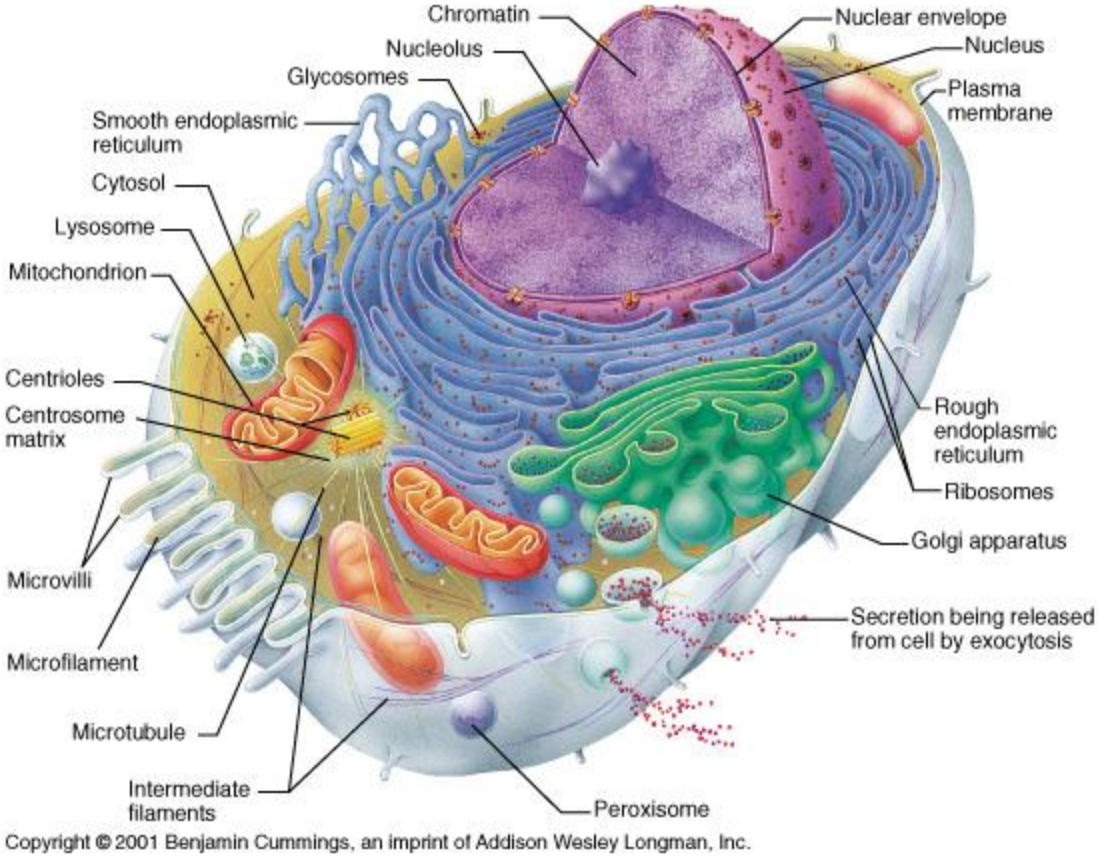
Soil
Water
Air

Helmont

# From a seed to a tree

# Basic structure of the cell




Prokaryotic cell

Eukaryotic cell

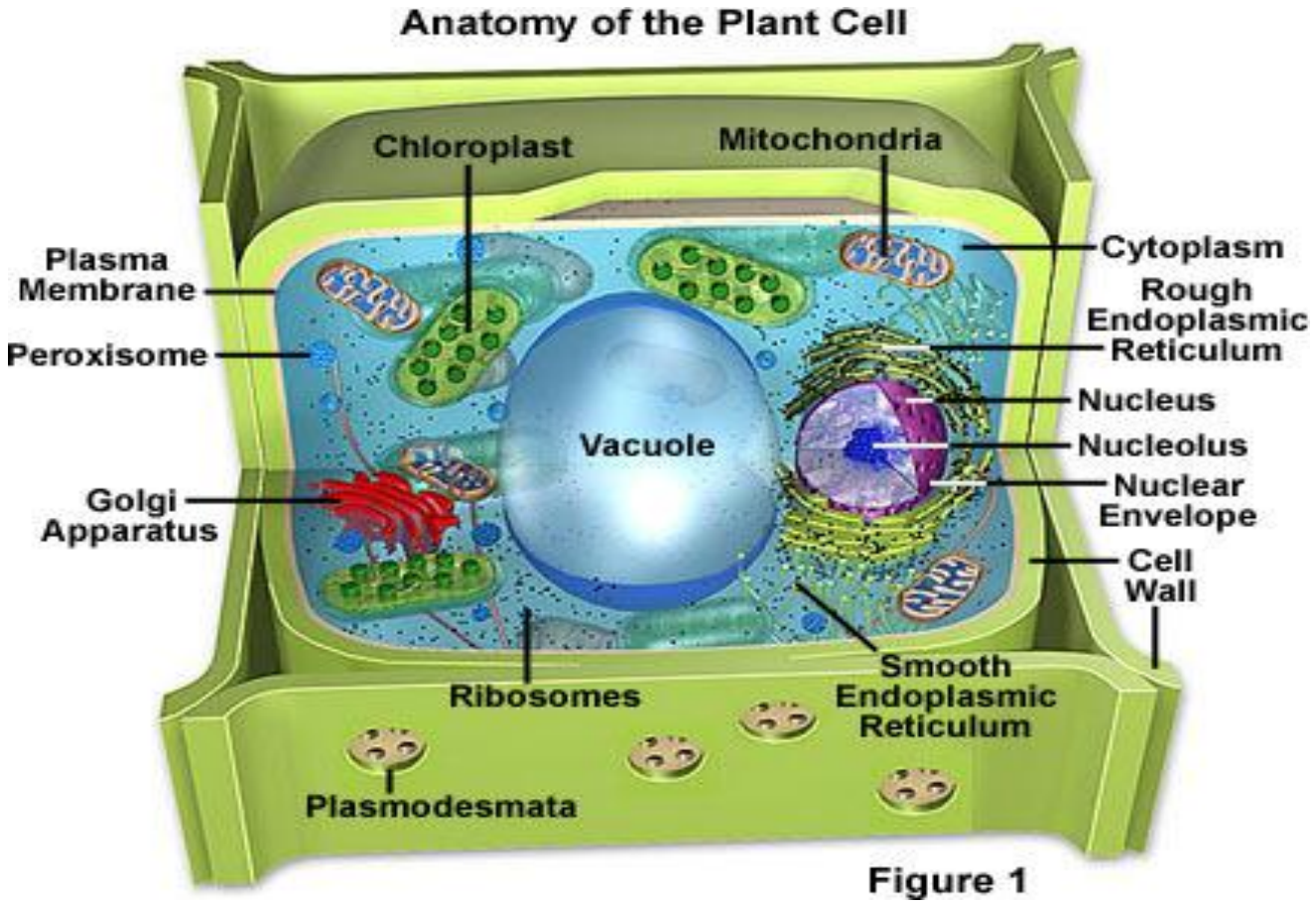



Figure 1

Exceptions, RBCs, …..etc

# How to amplify DNA in a tube?

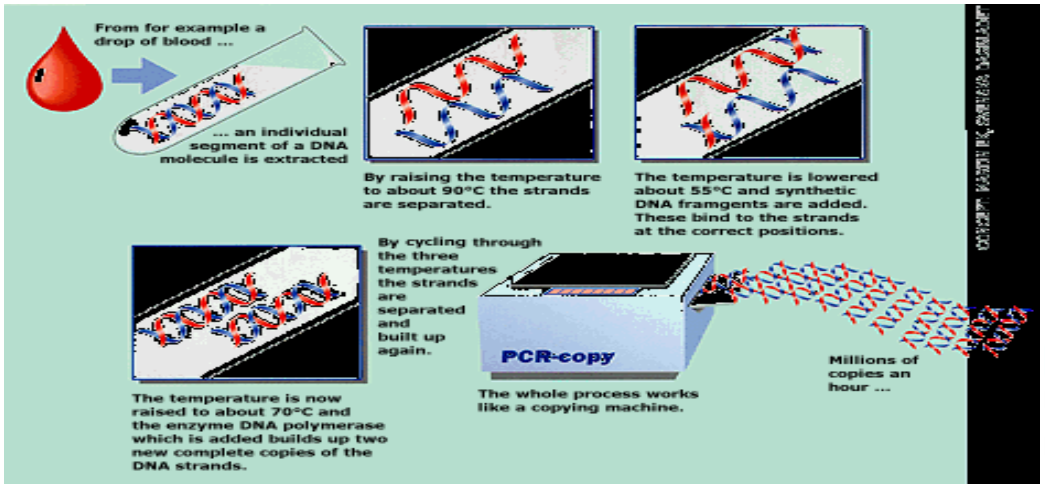
## Polymerase Chain Reaction

- Selectively amplifying a particular segment of DNA.

e.g. a specific gene or certain area in the DNA

1 copy can produce billions of copies

- It can be described as a molecular photocopier.




# Set up PCR reaction

***Reagents in a tube***

- Sample (DNA: Virus , bacteria, cells)
- Primers (forward and reverse)
- Enzyme (DNA polymerase)
- dNTPs (A, T, G, C )

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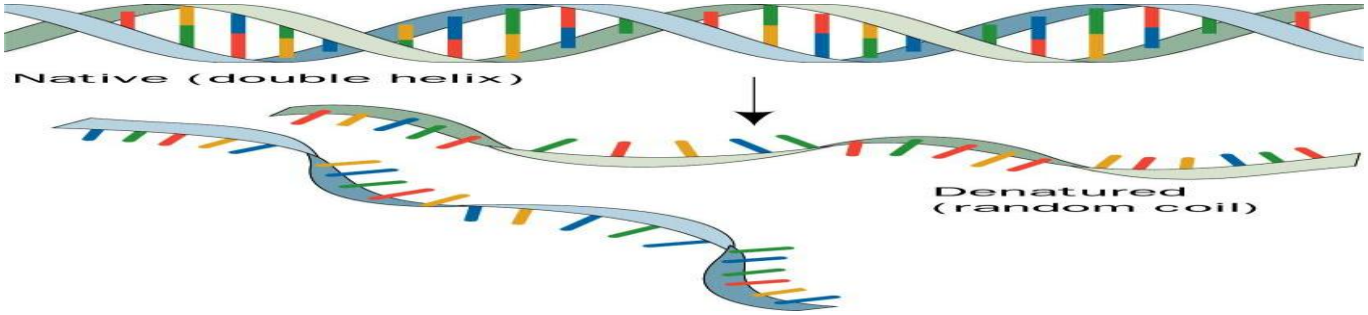
Invent the Future

# PCR Cycles

- **Denature** **94 °C for 30 sec**




ACCATCGGACTGCATCAGTACCATCGGCTGCATCAGTACCATCGGACTGCATCAGA
TGGTAGCCTGACGTAGTCATGGTAGCCTGACGTAGTCATGGTAGCCTGACGTAGTCT

↓

ACCATCGGACTGCATCAGTACCATCGGCTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCCTGACGTAGTCATGGTAGCCTGACGTAGTCT

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# PCR Cycles

- **Primer annealing** **55 °C for 30 sec**

ACCATCGGACTGCATCAGTACCATCGG---------ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGA

*oligonucleotide*=Primers

*oligonucleotide*=Primers

CTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC---------TGACGTAGTCATGGTAGCCTGACGTAGTCT

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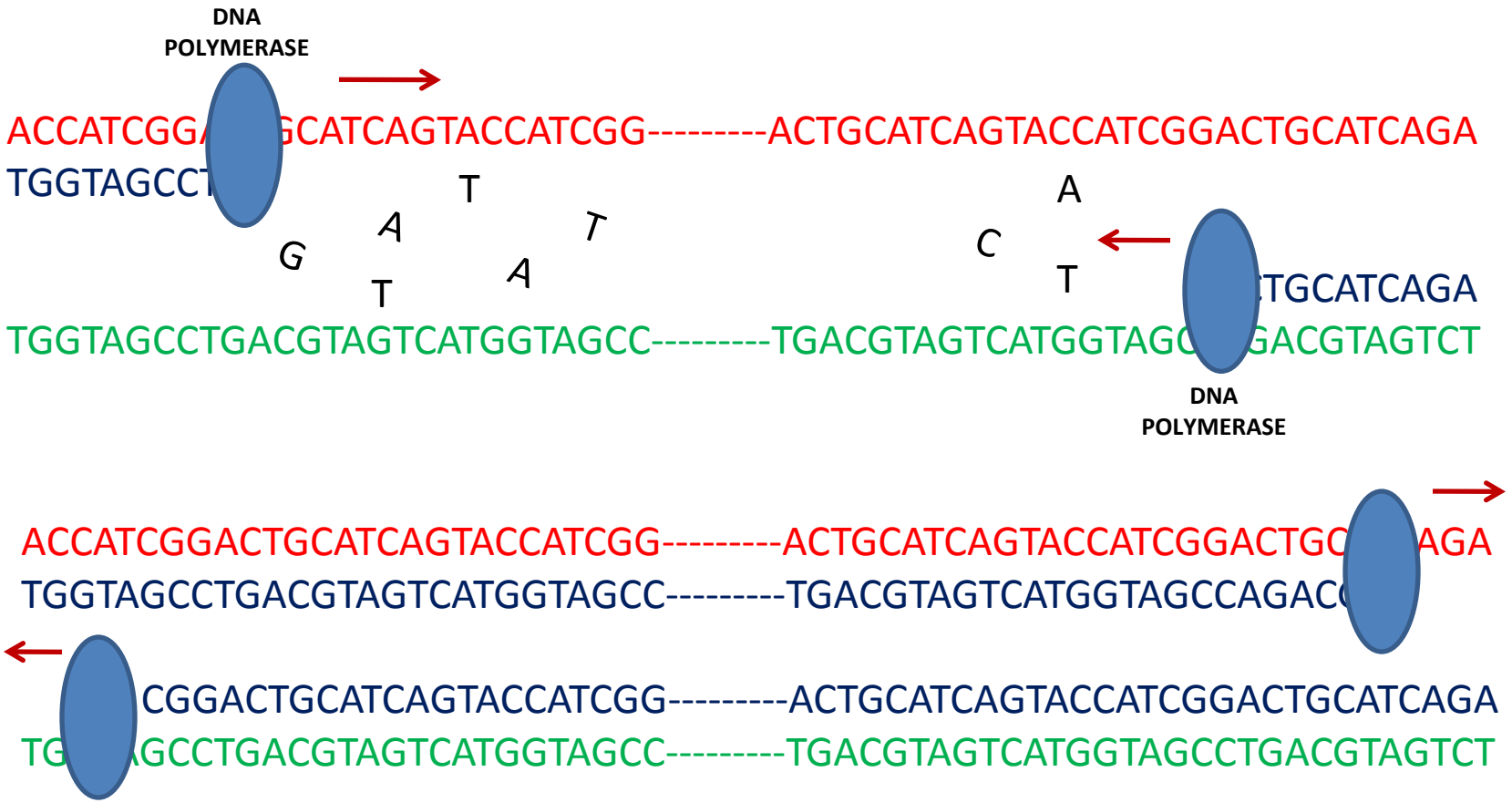
# PCR Cycles

- **Extension** — 72 °C for XXX sec



# 2nd cycle

- **Denature**

**94 °C for 30 sec**

ACCATCGGACTGCATCAGTACCATCGG---------ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC---------TGACGTAGTCATGGTAGCCAGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG---------ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC---------TGACGTAGTCATGGTAGCCTGACGTAGTCT

# 2nd cycle

- **Primer annealing**

**55 °C for 30 sec**

ACCATCGGACTGCATCAGTACCATCGG---------ACTGCATCAGTACCATCGGACTGCATCAGA
TGGTAGCCTGA

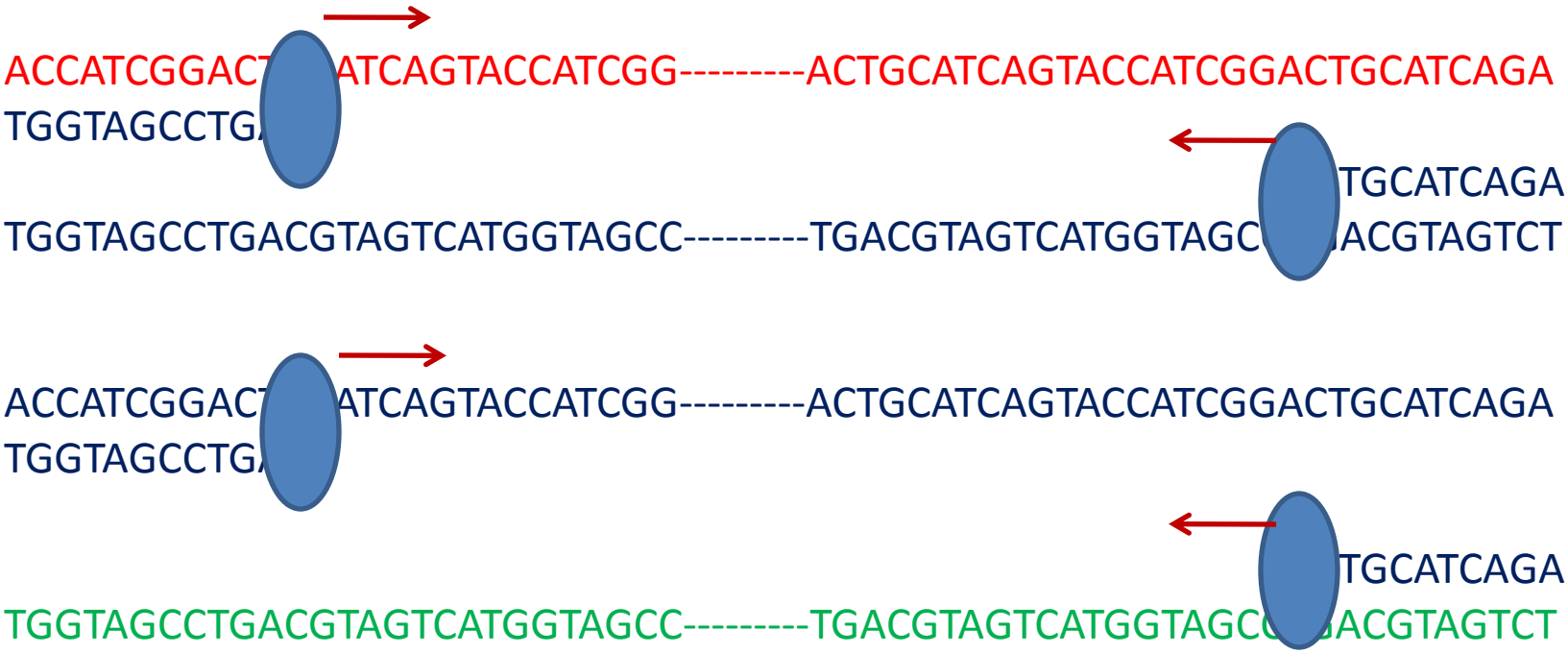
CTGCATCAGA
TGGTAGCCTGACGTAGTCATGGTAGCC---------TGACGTAGTCATGGTAGCCAGACGTAGTCT

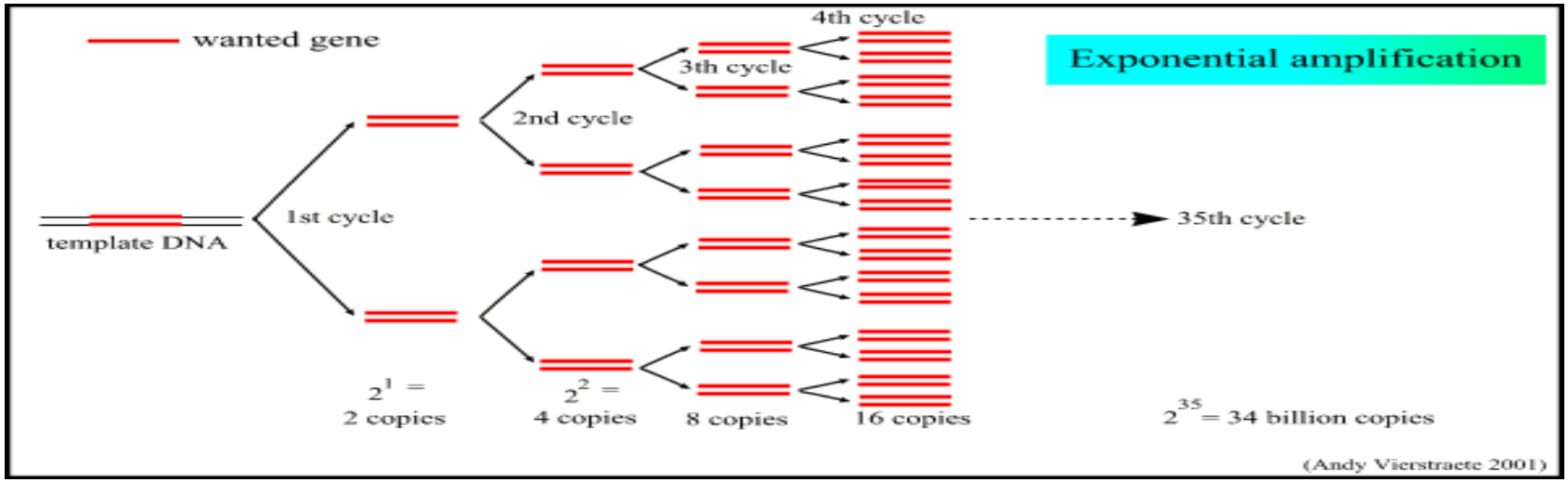
ACCATCGGACTGCATCAGTACCATCGG---------ACTGCATCAGTACCATCGGACTGCATCAGA
TGGTAGCCTGA

CTGCATCAGA
TGGTAGCCTGACGTAGTCATGGTAGCC---------TGACGTAGTCATGGTAGCCTGACGTAGTCT

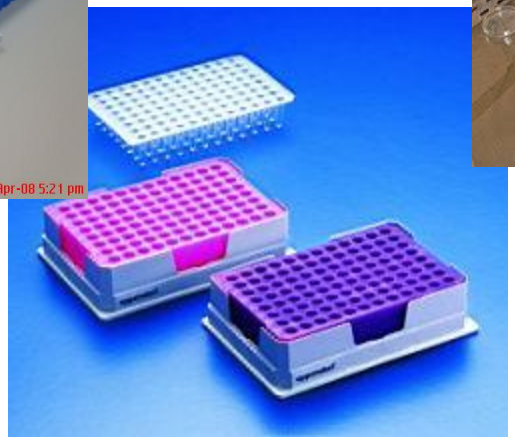
# 2nd cycle

- **Extension** — 72 °C for XXX sec



# Set up PCR reaction

# Thermocycler

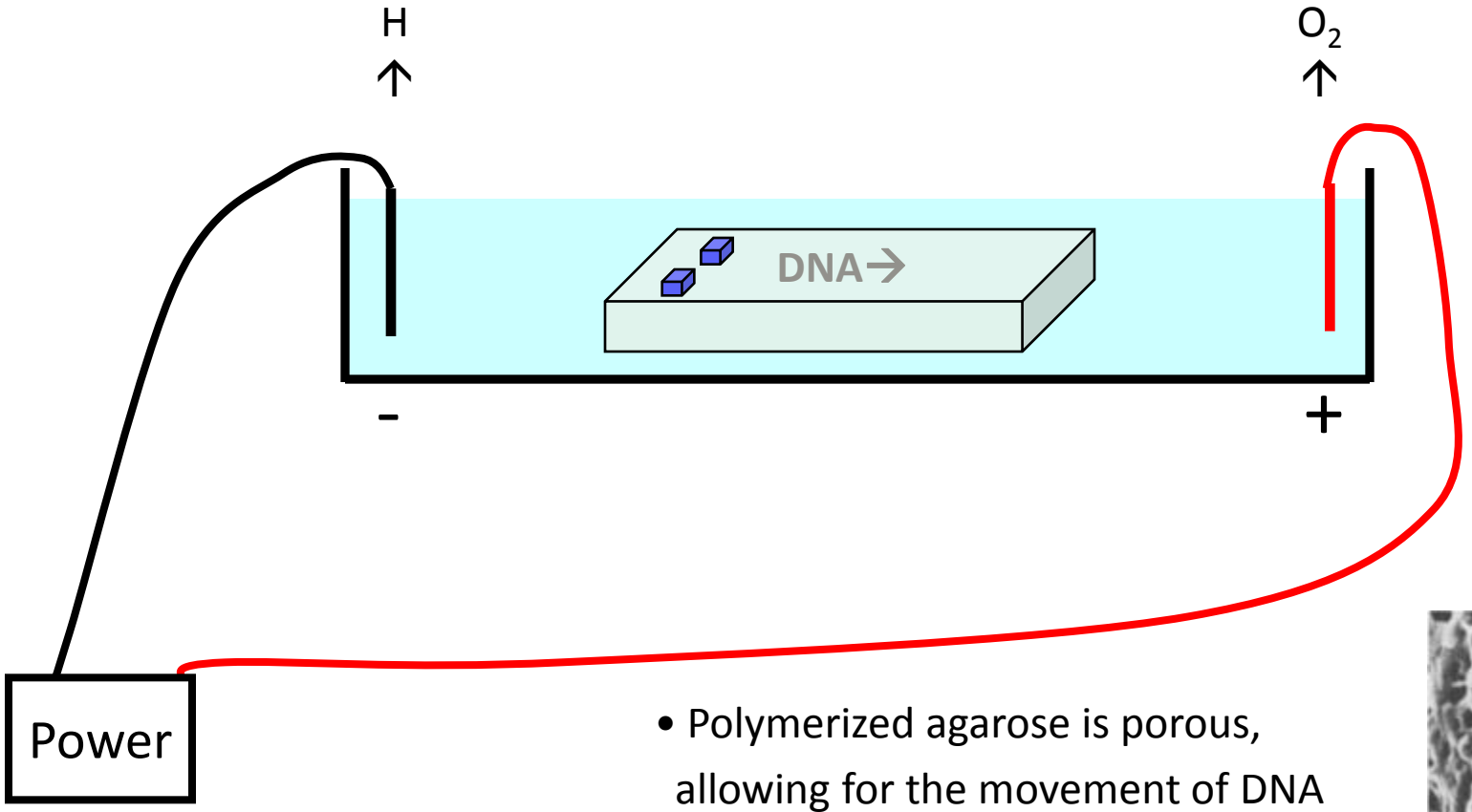


# PCR reaction

***Reagents in a tube***

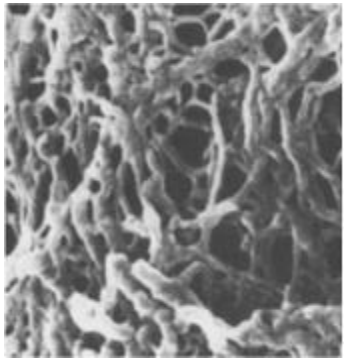
- Sample (DNA: Virus , bacteria, cells)
- Primers (forward and reverse)
- Enzyme (DNA polymerase)
- dNTPs (A, T, G, C )
- Gene of interest 35 billion copies

- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel is used to slow the movement of DNA and separate by size.




- Polymerized agarose is porous, allowing for the movement of DNA

Scanning Electron Micrograph of Agarose Gel (1×1 µm) →



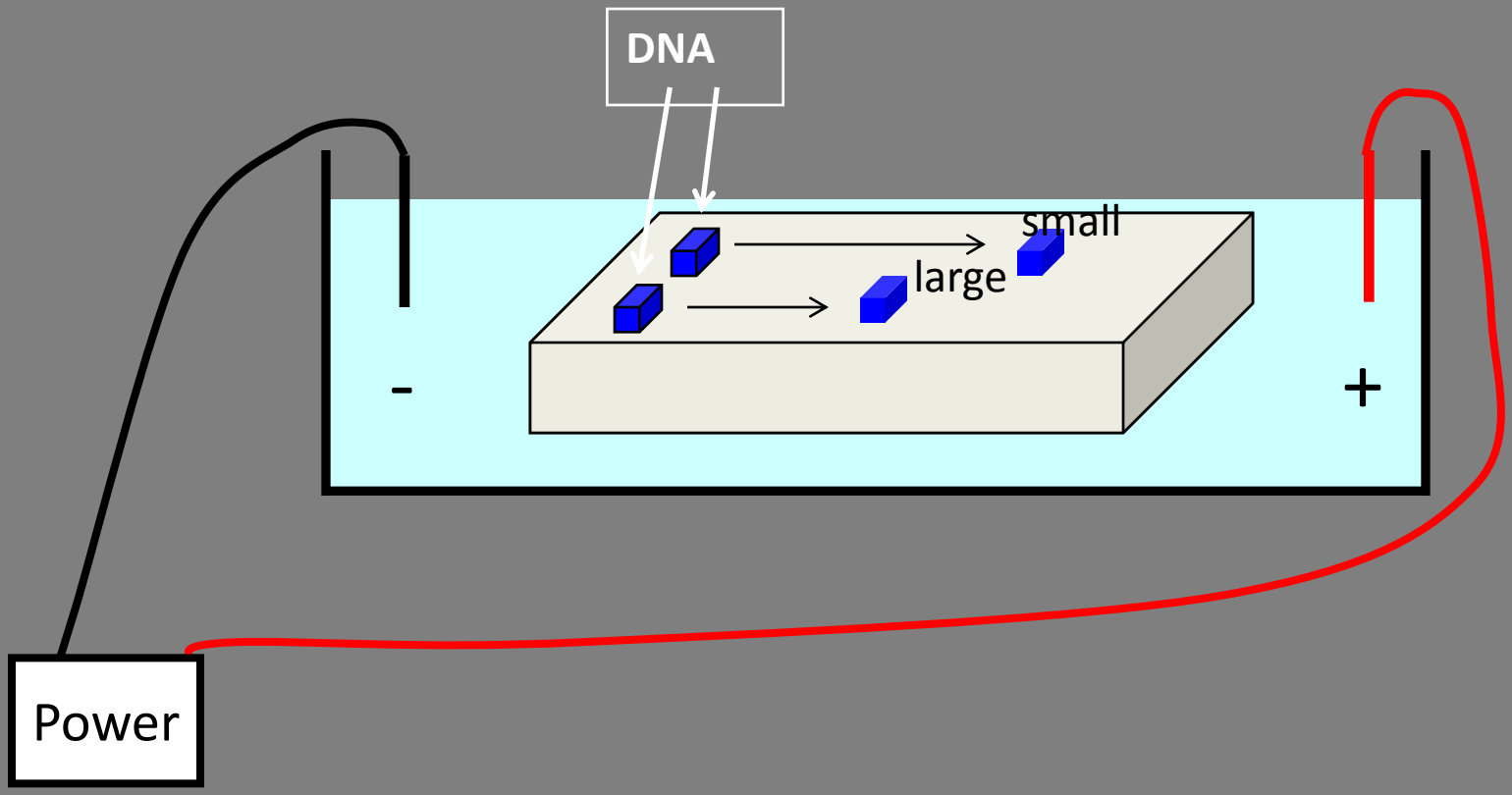
# How fast will the DNA migrate?

strength of the electrical field, buffer, density of agarose gel…

Size of the DNA!

*Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to size



# Agarose Gel under UV light