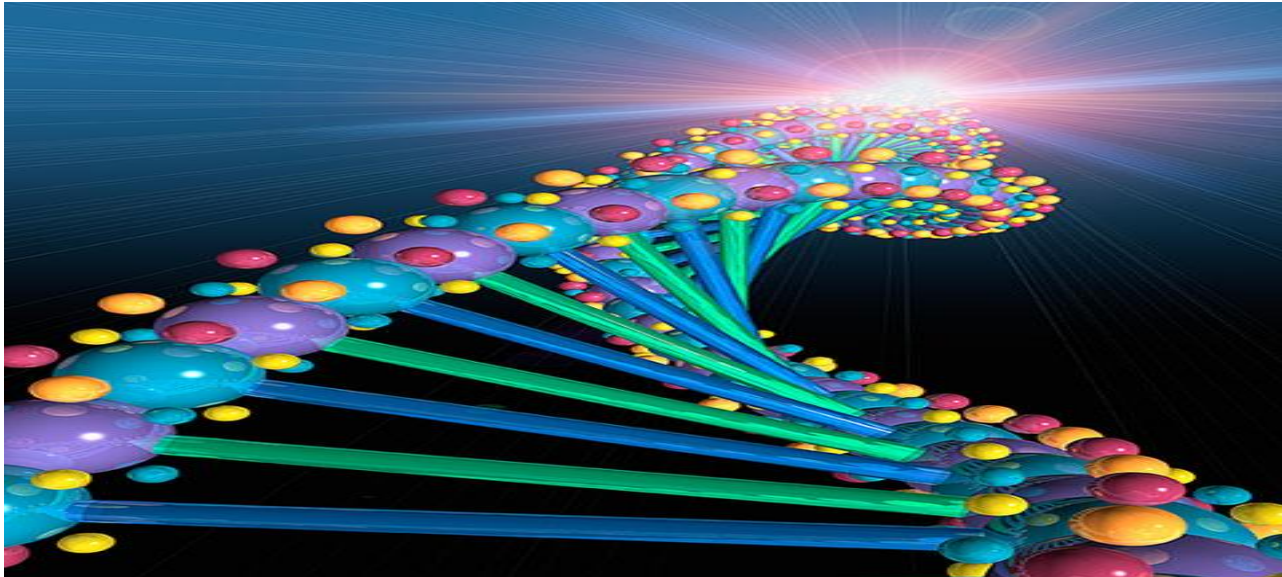


# PCR

## Introduction



Mohamed N. Seleem



# Aims

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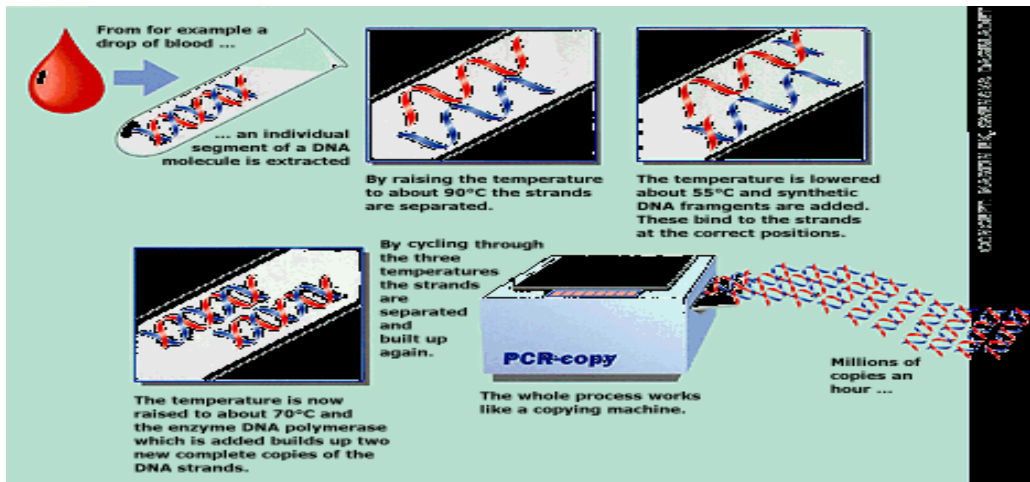


Understand the principles and applications  
Of:

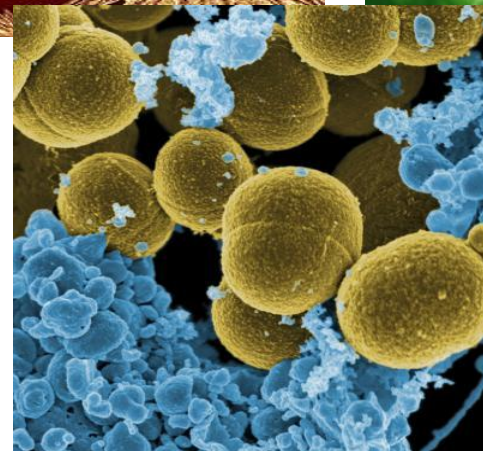
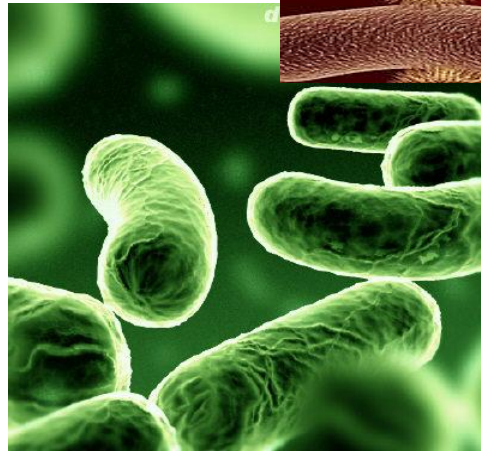
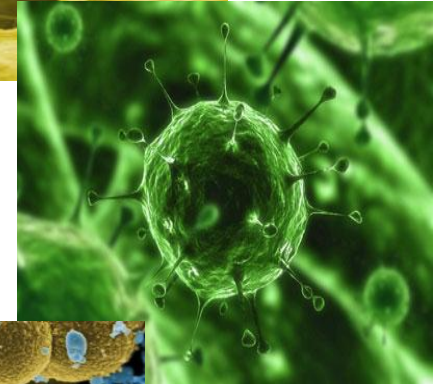
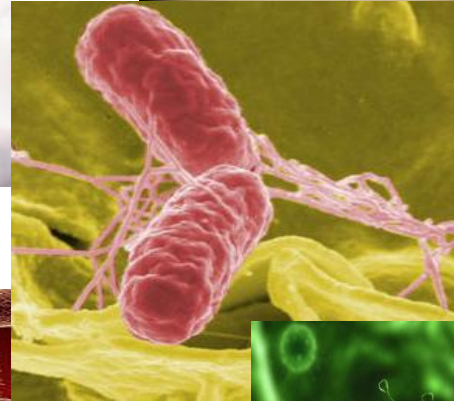
- **P**olymerase **C**hain **R**eaction
- DNA extraction
- Primers design
- Set up a PCR

# What is the Polymerase Chain Reaction?

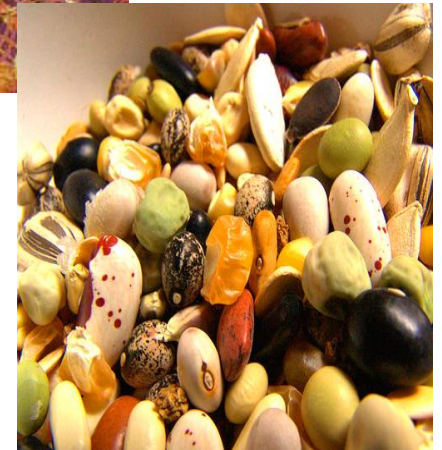
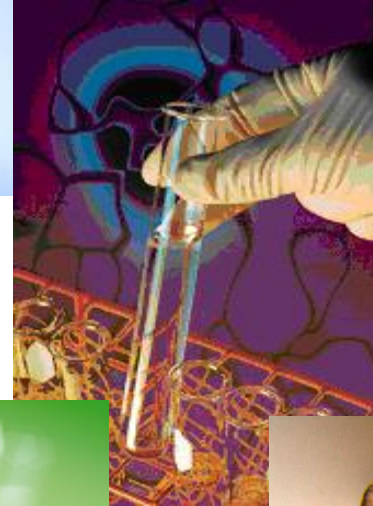
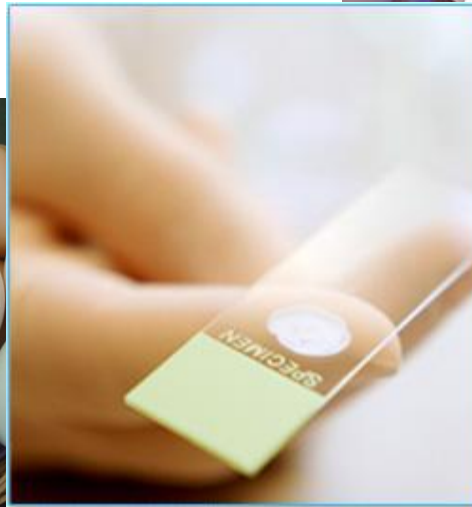
- Selectively amplifying a particular segment of DNA.  
e.g. a specific gene or certain area in the DNA  
1 copy can produce billions of copies
- It can be described as a molecular photocopier.



# Diagnosis



# Samples

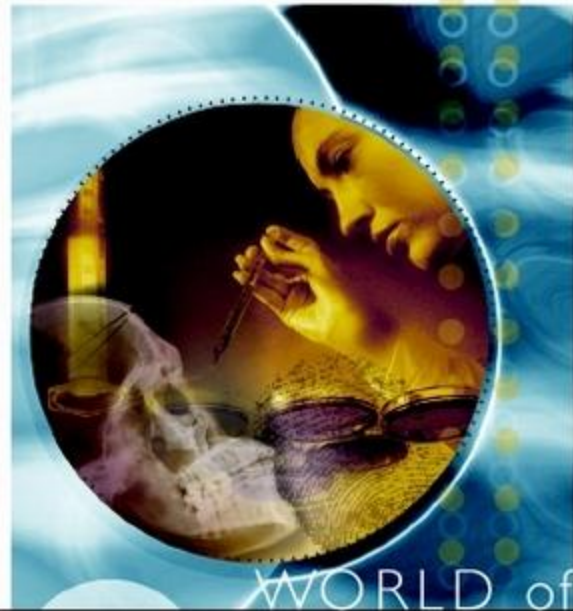


How  
to  
pick  
up



1 out of thousands genes

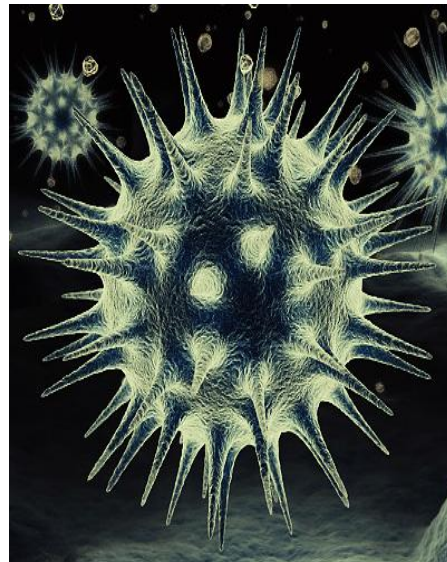
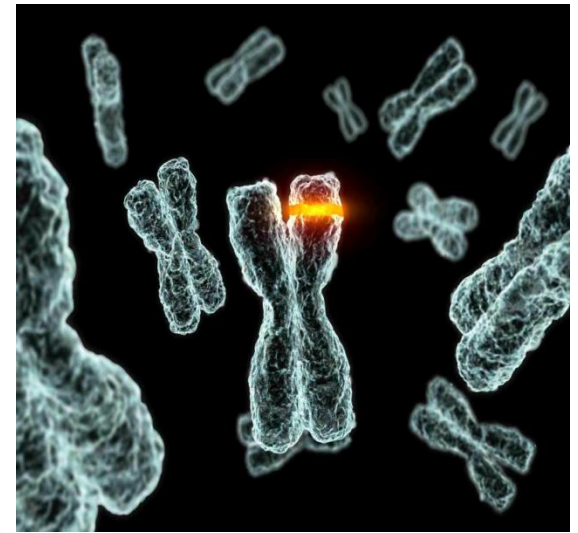
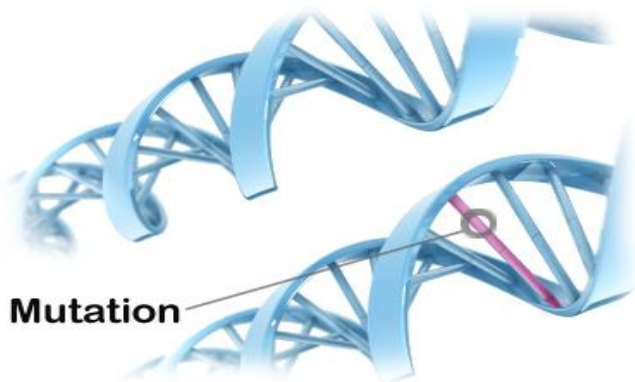
# Forensic



WORLD of  
FORENSIC SCIENCE



# Mutation







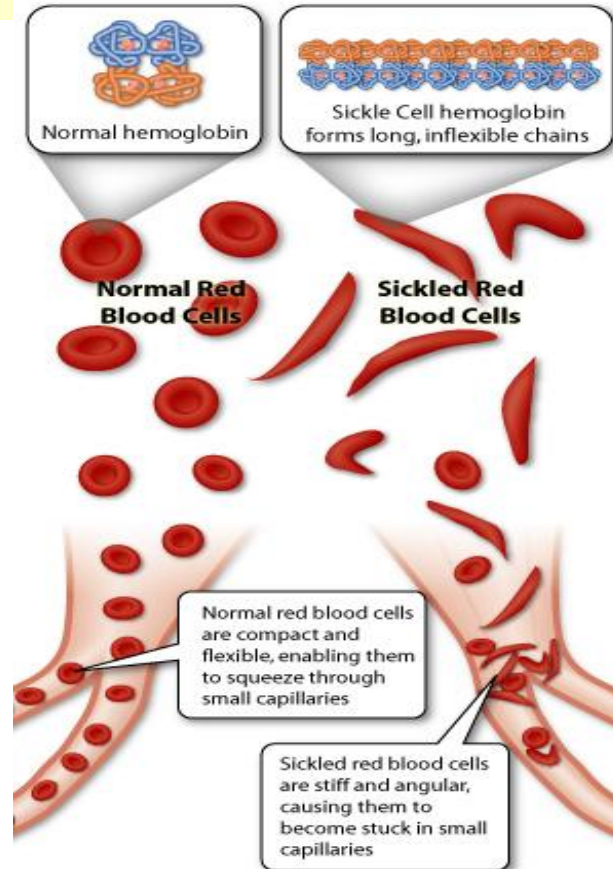
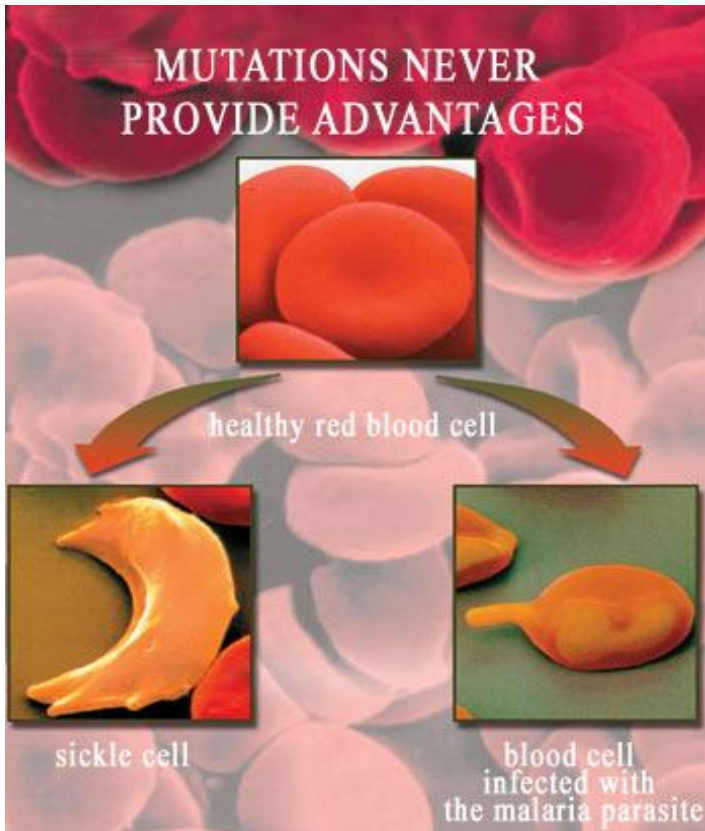
# Sickle Cell Anemia

## HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

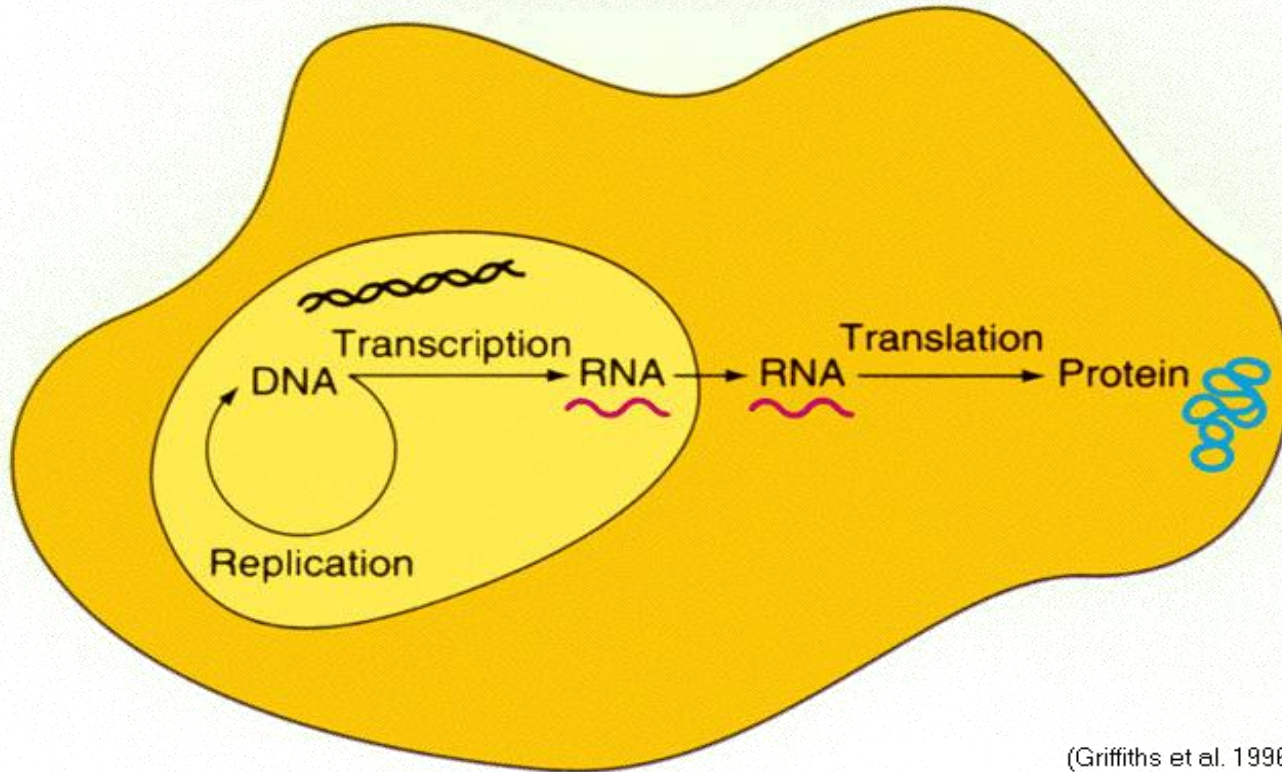
## HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9



A  
T  
Glutamic  
-Valine

# Measuring Gene Expression



**Idea:** measure the amount of **mRNA** to see which **genes** are being **expressed** in (used by) the cell different time different conditions.

# History of PCR

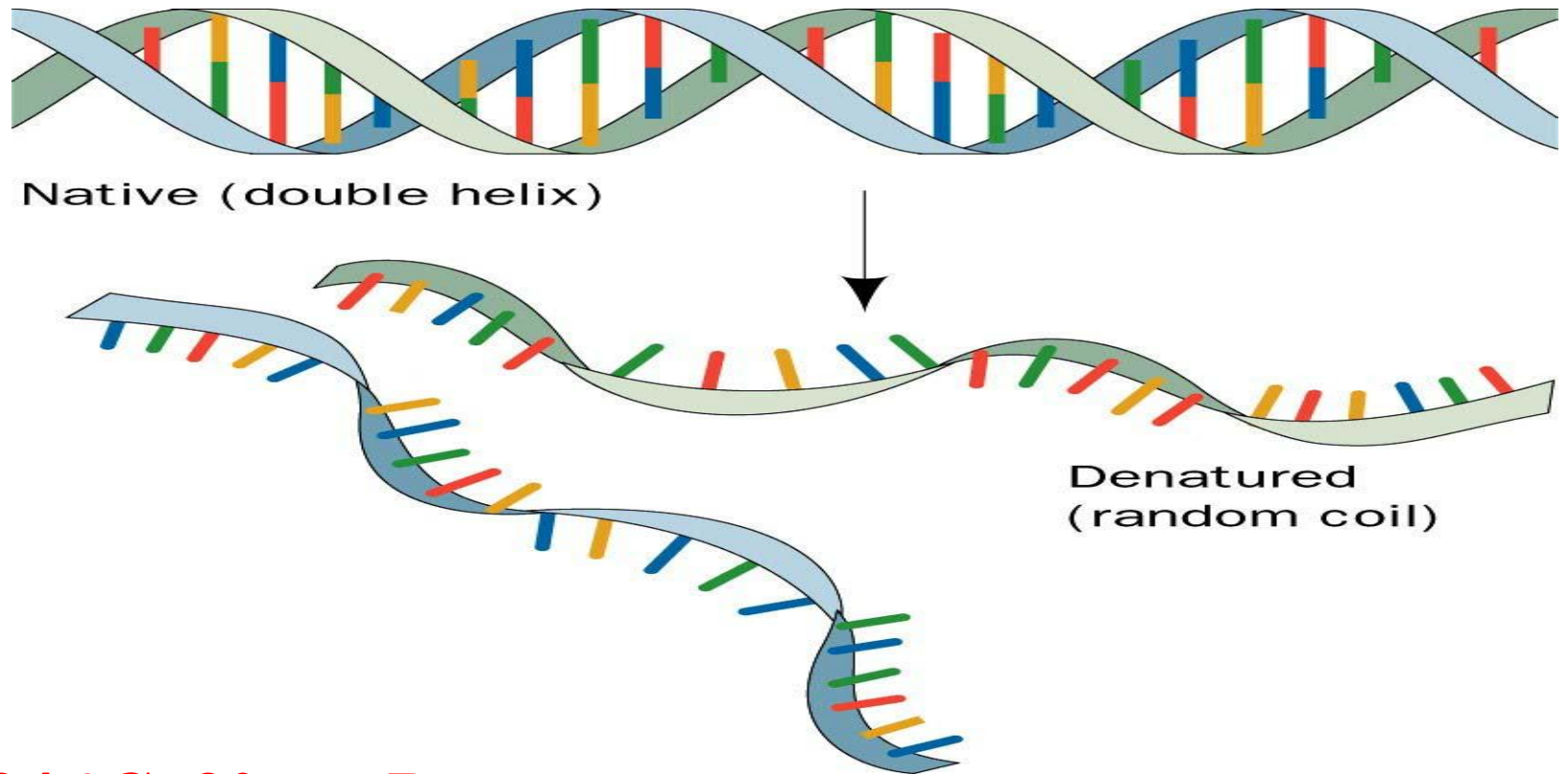
- Invented by Kary Mullis in 1983.
- First published account appeared in 1985.
- Awarded Nobel Prize for Chemistry in 1993.

One of the fastest Nobel Prize  
In Nobel terms, that's a pretty  
short turnaround.



- Challenges:

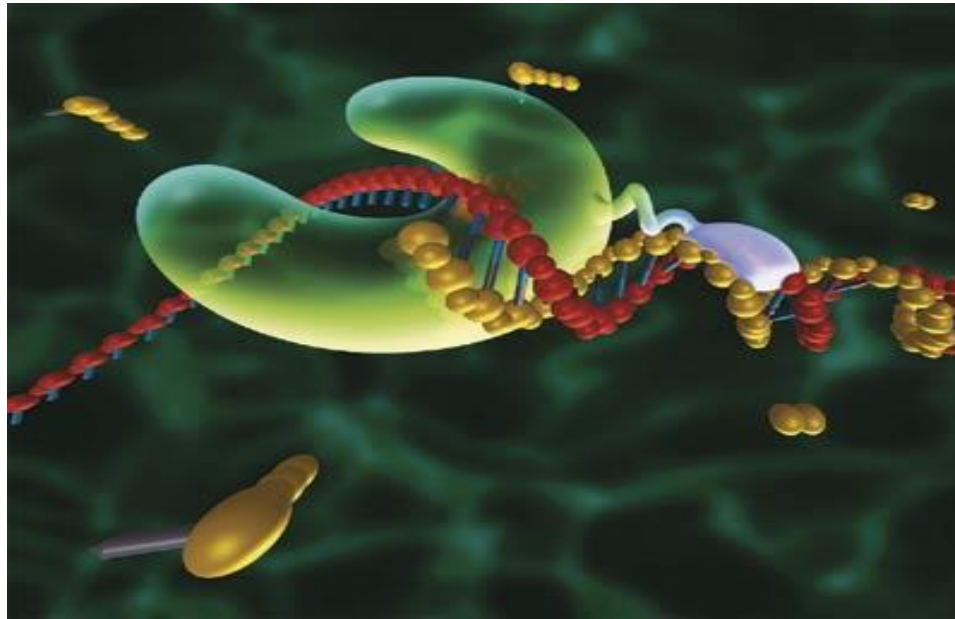
How to separate double stranded DNA?



**94 °C 30 sec Denature**

- Challenges:

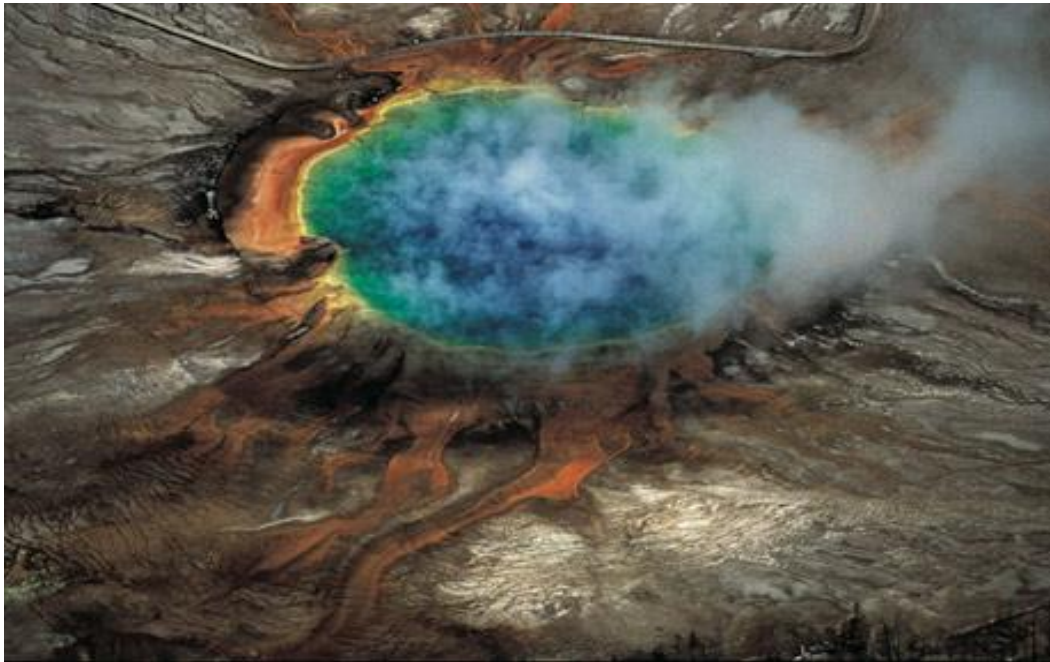
How to make copies?



**DNA Polymerase enzyme**

- Challenges:

Enzyme sensitive to heat



**Hot springs bacteria**

*Thermus aquaticus*

*Taq* enzyme

Heat stable

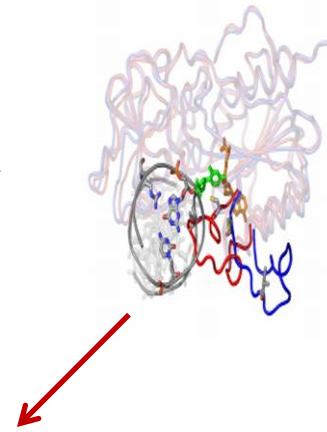
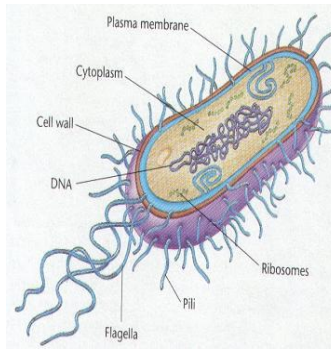
1988

- Challenges:

How to select the gene or the area in DNA

Primers





ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGA  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCTGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGA

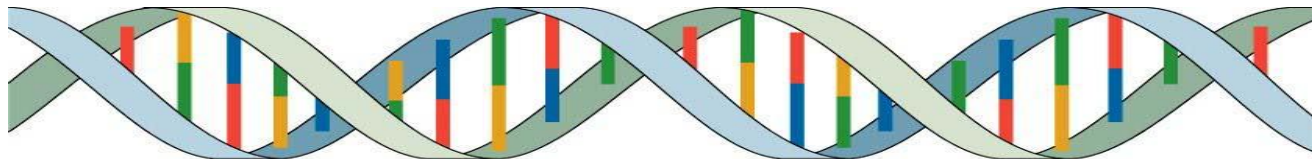
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCTGACGTAGTCT



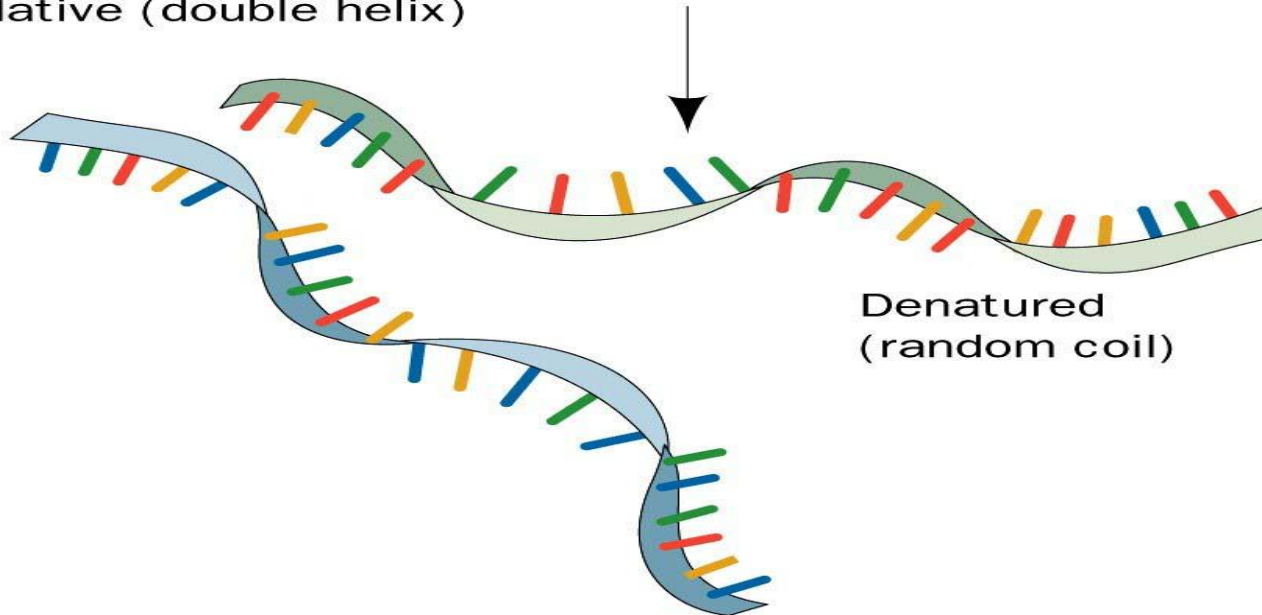
# PCR Cycles

- **Denature**

94 °C for 30 sec



Native (double helix)



Denatured  
(random coil)

## •Primer annealing

55 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGA

GACGTAGTCT

*Forward Primer*

*Reverse Primer*

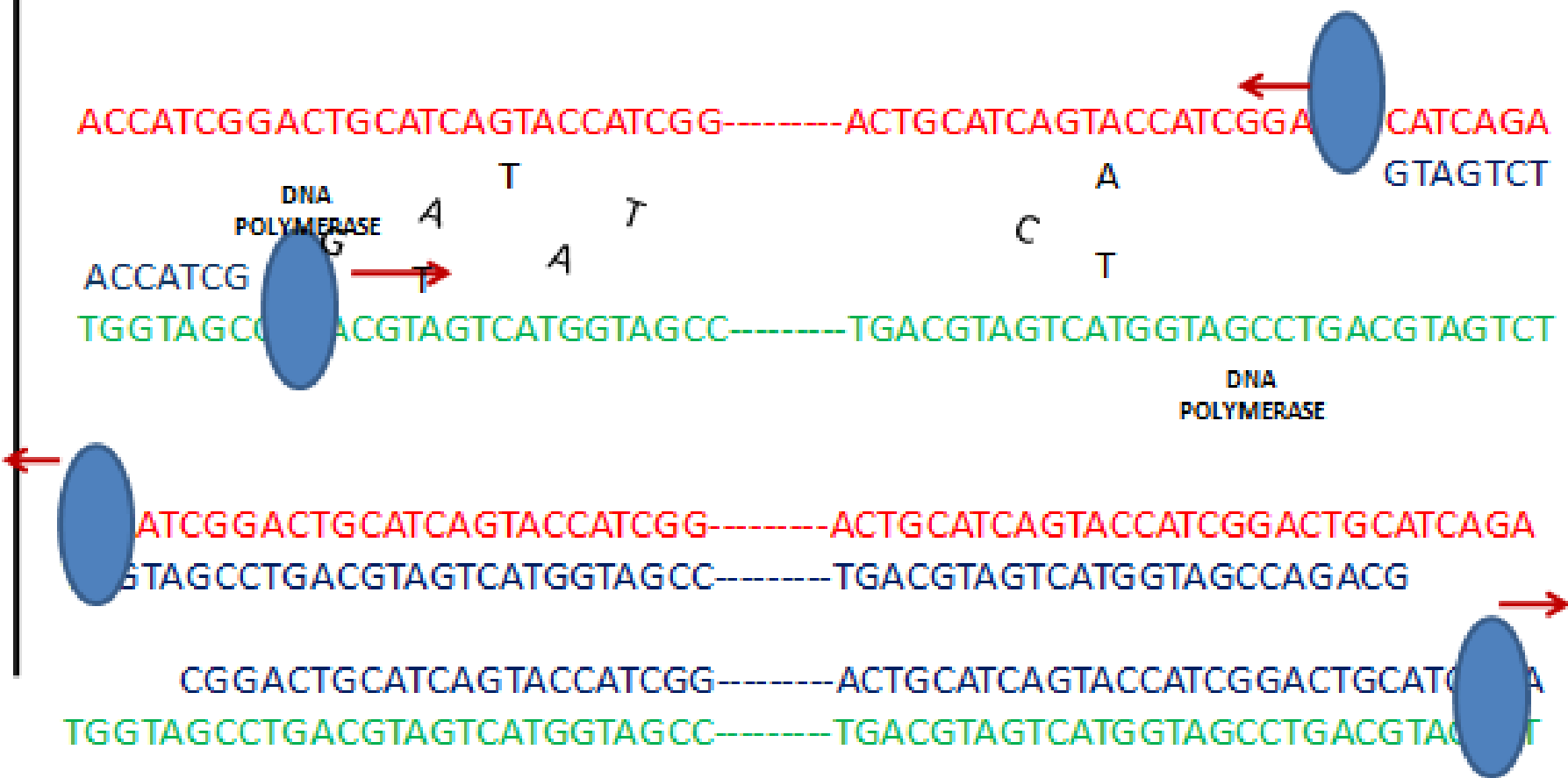
ACCATCGGACT

TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCTGACGTAGTCT

# PCR Cycles

## •Extension

72 °C for **XXX** sec



# 2<sup>nd</sup> cycle

## •Denature

94 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG——ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC——TGACGTAGTCATGGTAGCCAGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG——ACTGCATCAGTACCATCGGACTGCATCAGA

TGGTAGCCTGACGTAGTCATGGTAGCC——TGACGTAGTCATGGTAGCCTGACGTAGTCT

# 2<sup>nd</sup> cycle

- Primer annealing

55 °C for 30 sec

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGA  
GACGTAGTCT

ACCATCGGACT  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCTGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGA  
GACGTAGTCT

ACCATCGGACT  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCTGACGTAGTCT

# •Extension

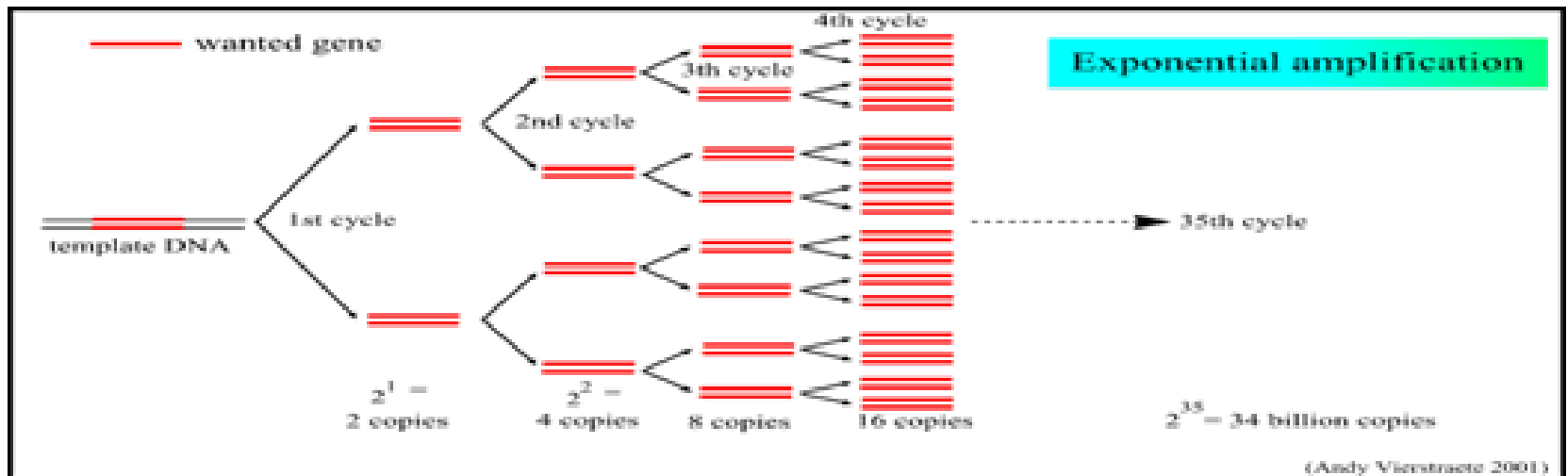
# 2<sup>nd</sup> cycle

72 °C for **XXX** sec

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGTACA  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCAGACCTGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGTACA  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCAGACCTGACGTAGTCT

ACCATCGGACTGCATCAGTACCATCGG-----ACTGCATCAGTACCATCGGACTGCATCAGTACA  
TGGTAGCCTGACGTAGTCATGGTAGCC-----TGACGTAGTCATGGTAGCCAGACCTGACGTAGTCT



# PCR Cycles

## Time and Temperature

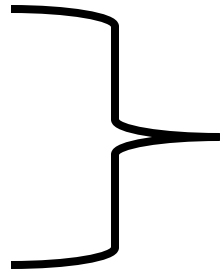
• Initial denature

94 °C for 2 minuets

• denature

• annealing

• Extension



25-40 cycles

94 °C for 30 sec

55 °C for 30 sec

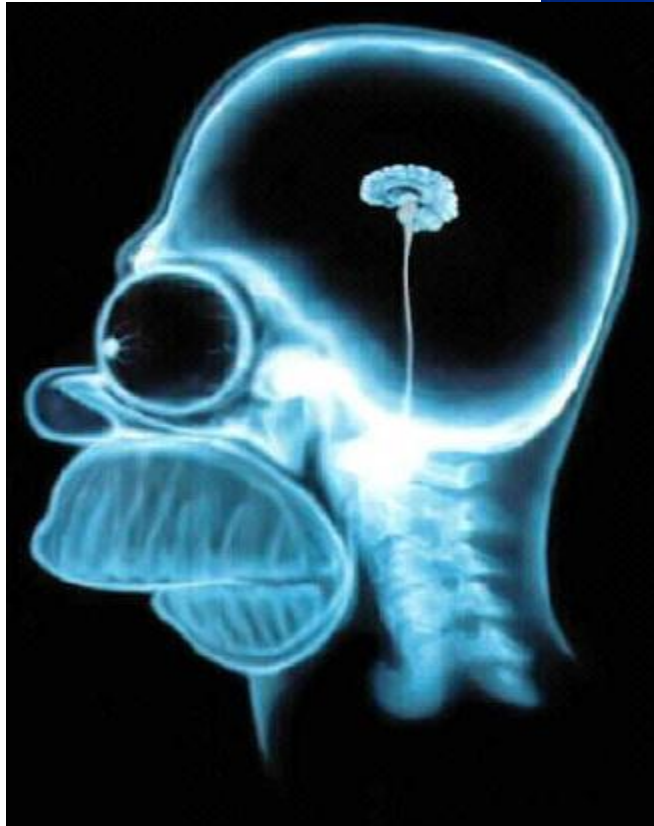
68-72 °C for xxx sec

• Final extension

72 °C for 5 minuets

# PCR is just a tool

Reaching Beyond Horizons



**How to  
use it, is  
up to you**