PCR

POLYMERASE CHAIN REACTION

PCR : FIRST DESCRIBED IN MID 1980'S, KARY MULLIS ,NOBEL PRIZE IN 1993.

AN INVITRO METHOD FOR THE ENZYMATIC SYNTHESIS OF SPECIFIC DNA SEQUENCING.

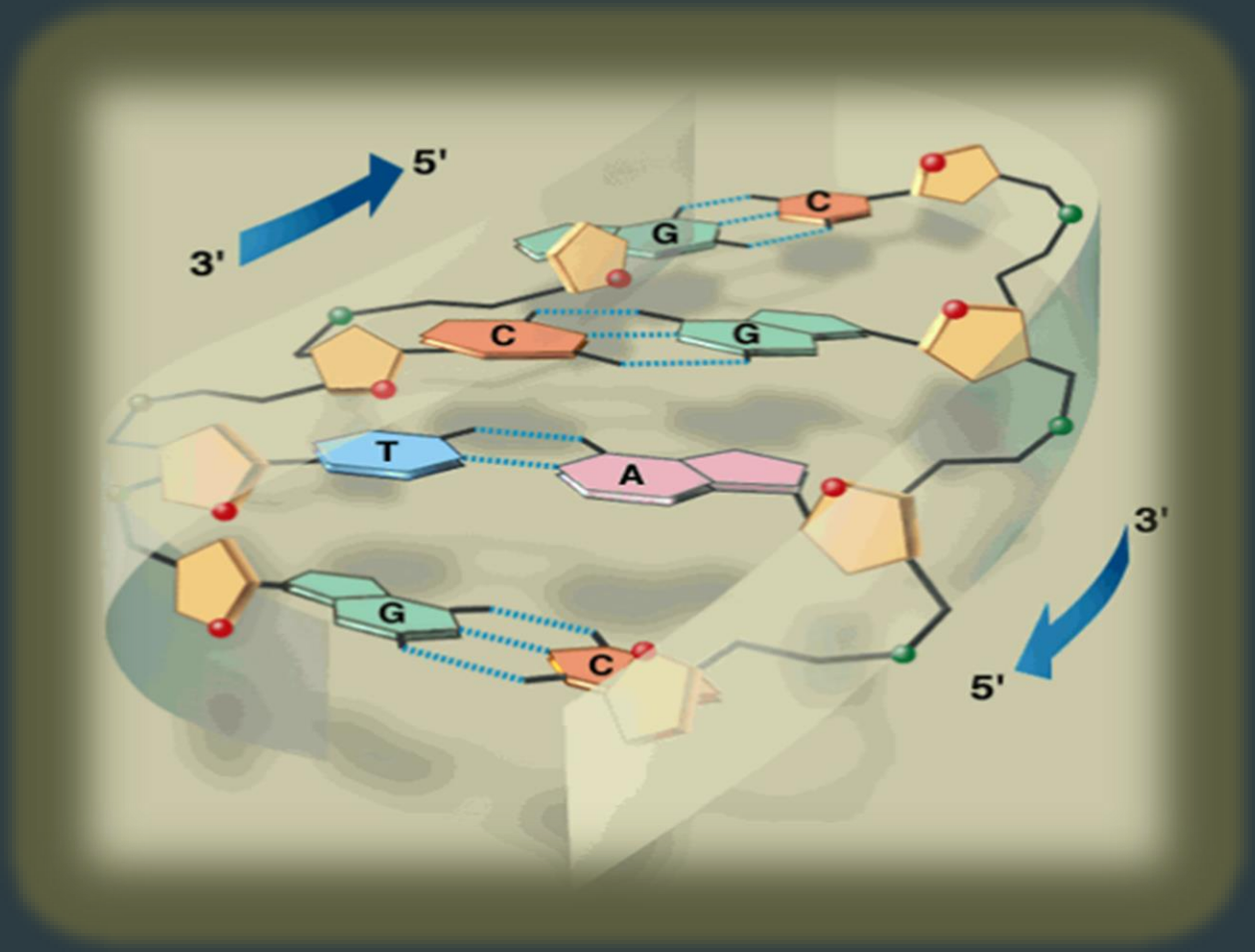
REQUIRES :

- TWO SPECIFIC OLIGONUCLEOTIDES PRIMERS
- THERMOSTABLE DNA POLYMERASE
- d NTPs
- TEMPLATE DNA
- $MgCl_2$- act as cofactor for taq polymerase.
- BUFFER

INITIALLY PCR USED THE KLENOW FRAGMENTS OF E.COLI DNA POLYMERASE- INACTIVATED BY HIGH TEMPERATURE.

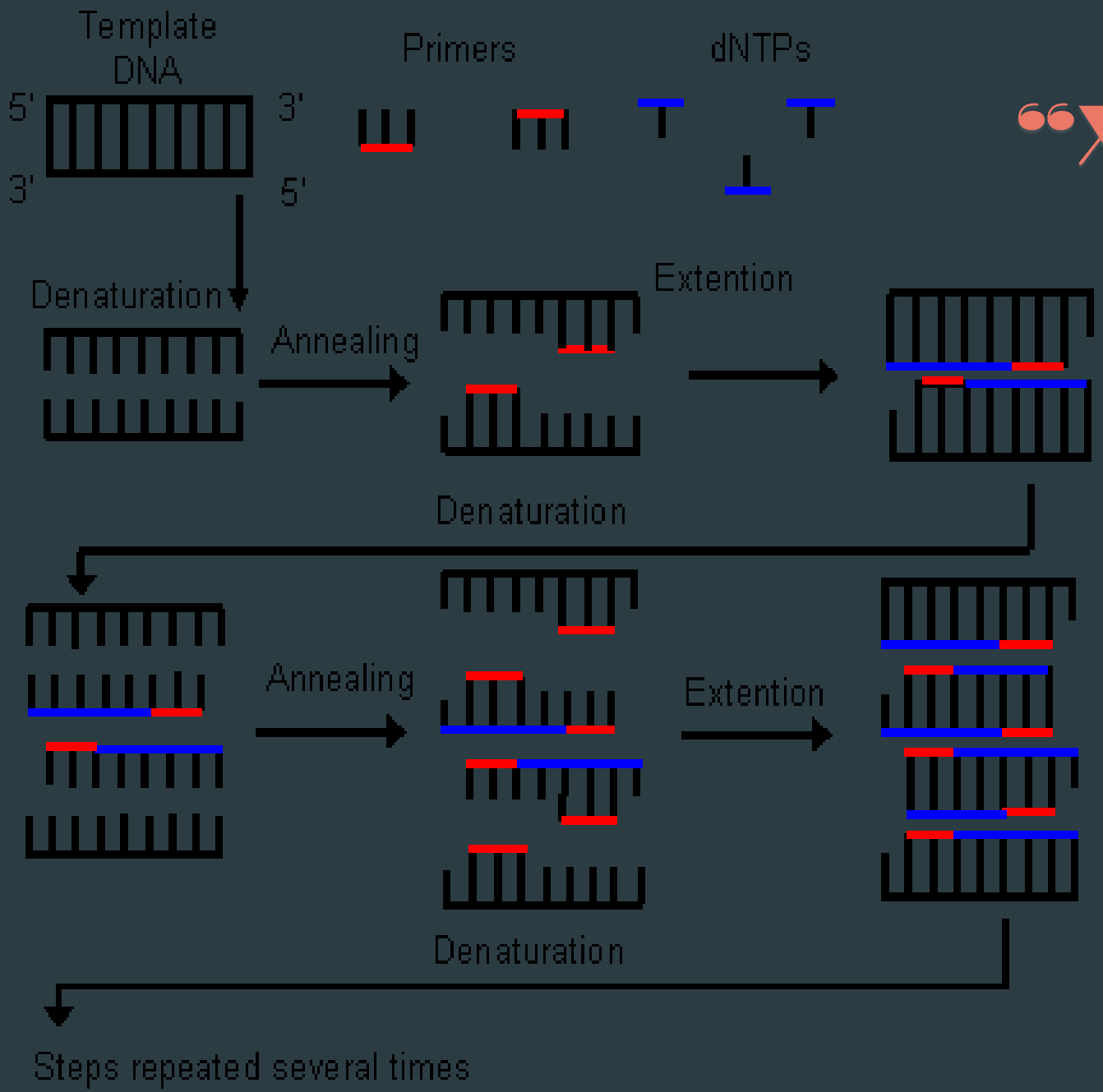
REQUIRED A THERMOSTABLE DNA POLYMERASE – TAQ POLYMERASE FROM “ THERMUS AQUATICUS” , A THERMOPHILIC EUBACTERIAL MICROORGANISM.

DIRECTIONAL BIOSYNTHESIS



CAN BE PERFORMED BY HAND OR IN A MACHINE CALLED THERMAL CYCLER

"Xeroxing" DNA



STEPS :

STEP-1

denaturation

ds DNA to ss DNA

STEP-2

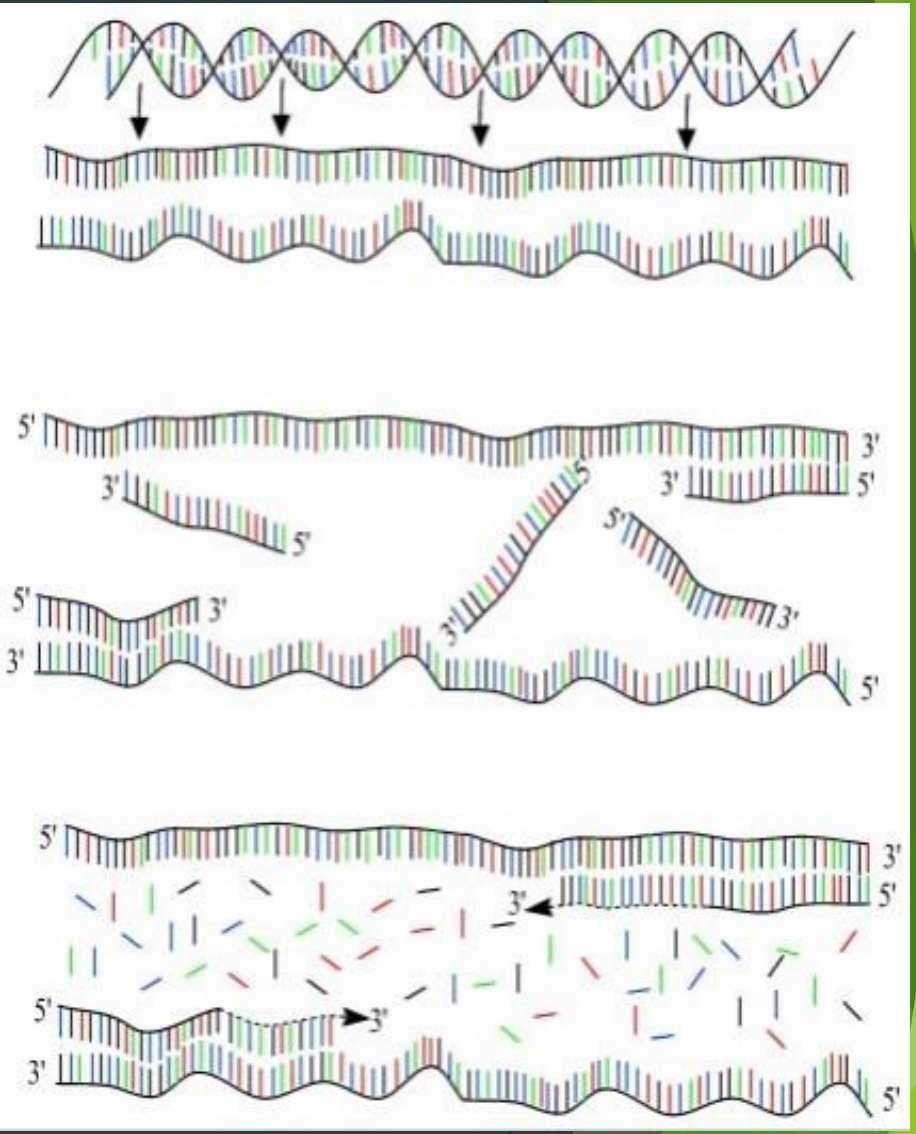
annealing

primers onto template

STEP-3

extension

d NTPs extend 2nd strand



-Extension product in one cycle serve as template in the next.

HOW PCR WORKS ?

- *BEGINS WITH DNA CONTAINING A SEQUENCE TO BE AMPLIFIED AND A PAIR OF SYNTHETIC OLIGONUCLEOTIDE PRIMERS THAT FLANK THE SEQUENCE. NEXT , DENATURE THE DNA TO SINGLE STRAND AT 94°C.*
- *RAPIDLY COOL THE DNA (37°C-65°)AND ANNEAL PRIMERS TO COMPLEMENTARY SINGLE STRAND SEQUENCES FLANKING THE TARGET DNA.*
- *EXTEND PRIMERS AT 70°C-75°C USING A HEAT RESISTANT DNA POLYMERASE SUCH AS TAG POLYMERASE DERIVED FROM THERMUS AQUATICUS.*
- *REPEAT THE CYCLE OF DENATURATION , ANNEALING AND EXTENSION 20-45 TIMES TO PRODUCE 1 MILLION TO 35 TRILLION COPIES OF THE TARGET DNA.*
- *EXTEND THE PRIMERS AT 70-75°C ONCE MORE TO ALLOW INCOMPLETE EXTENSION PRODUCTS IN THE REACTION MIXTURE TO EXTEND COMPLETELY.*
- *COOL TO 4°C AND STORE OR USE AMPLIFIED PCR PRODUCT FOR ANALYSIS.*

Polymerase chain reaction (**PCR**)

Typical PCR Temps/Times

Initial denaturation	90º – 95º C	1 – 3 mi
Denature	90º – 95º C	0.5 – 1 min
Primer annealing	45º – 65º C	0.5 – 1 min
Primer extension	70º – 75º C	0.5 – 2 min
Final extension	70º – 75º C	5 – 10 min
Stop reaction	4º C or 10 mM EDTA	hold

25 – 40 cycles

REAL – TIME QUANTITATIVE PCR

- SAME AS PCR , BUT MEASURES THE ABUNDANCE OF DNA AS IT IS AMPLIFIED.
- USEFUL FOR QUANTITATIVELY MEASURING THE LEVELS OF MRNA IN A SAMPLE.

  USES REVERSE TRANSCRIPTASE TO GENERATE c DNA FOR THE TEMPLATE.
- CAN ALSO BE USED TO QUANTITATIVELY ESTIMATE FRACTION OF DNA FROM VARIOUS ORGANISMS IN A HETEROGENEOUS SAMPLE. (e.g: can be used tomeasure abundance of different microbes in soil samples).

SUMMARY AND APPLICATIONS OF PCR

- AMPLYFY DNA FOR CLONING.
- AMPLIFY DNA FOR SEQUENCING WITHOUT CLONING (PCR).
- DNA SEQUENCING REACTION.
- MAPPING GENES AND REGULATORY SEQUENCES.
- SEX DETERMINATION.
- DIAGNOSE DISEASE.
- FORENSIC ANALYSIS.
- DETECTION OF GENETICALLY MODIFIED FOOD (GMO's).