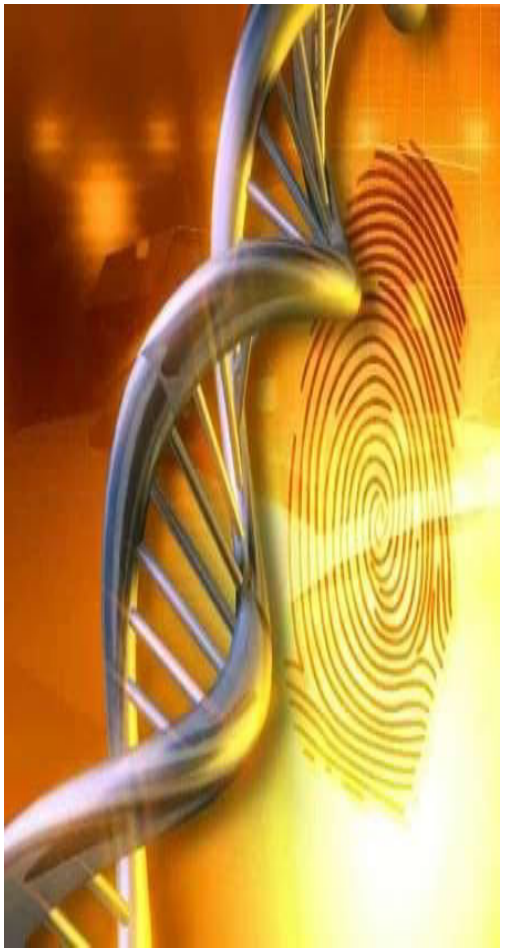
FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

DNA FINGER PRINTING

Developed by Dr. Alec Jaffrey in 1984

- A small set of DNA variation that is very likely to be different in all unrelated individual, thereby being as
- unique to individuals as are fingerprint.
  - "DNA fingerprinting is a quick way to compare the DNA sequence of any two individual"
  - Used especially for identification by extracting and identifying the base pair pattern of an individual's
    - DNA
  - DNA typing, DNA profiling, DNA testing and Genetic finger printing

1. Sample collection
2. Isolation of DNA
3. Restriction digestion
4. Separation of DNA sequence
5. Southern blotting
6. Hybridization
7. Autoradiography



Sample collection

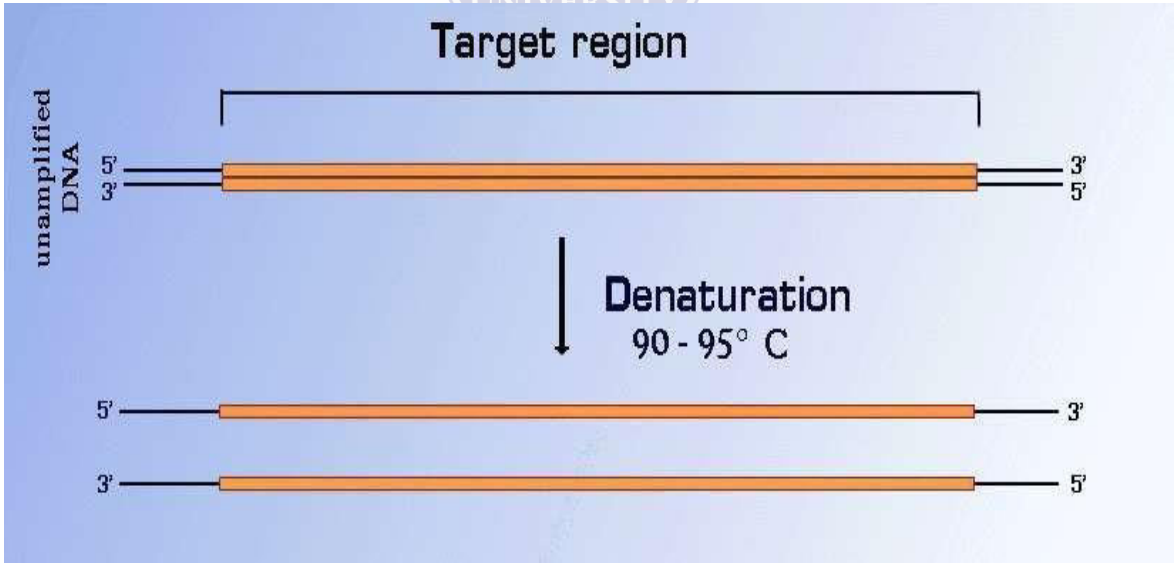
1. 1gm of leaf is taken and powdered using liquid nitrogen.
2. Transfer to a centrifuge tube containing extraction buffer(Cetyl Trimethyl Ammonium Bromide (CTAB) , NaCl, EDTA,TrisHCl , β mercapto ethanol)
3. Centrifuge
4. Treated with chloroform : Isoamyl alcohol(24:1) & centrifuge
5. Supernatant is taken and treated with cold isopropanol to precipitate DNA.
6. The precipitate further treated with 70%alcohol to further precipitate the DNA
7. Stored at 4° c

III. <u>AMPLIFICATION</u>

- Done by PCR
- Steps involved in PCR:-
    1. Denaturation
    2. Annealing
    3. Extension/ Elongation

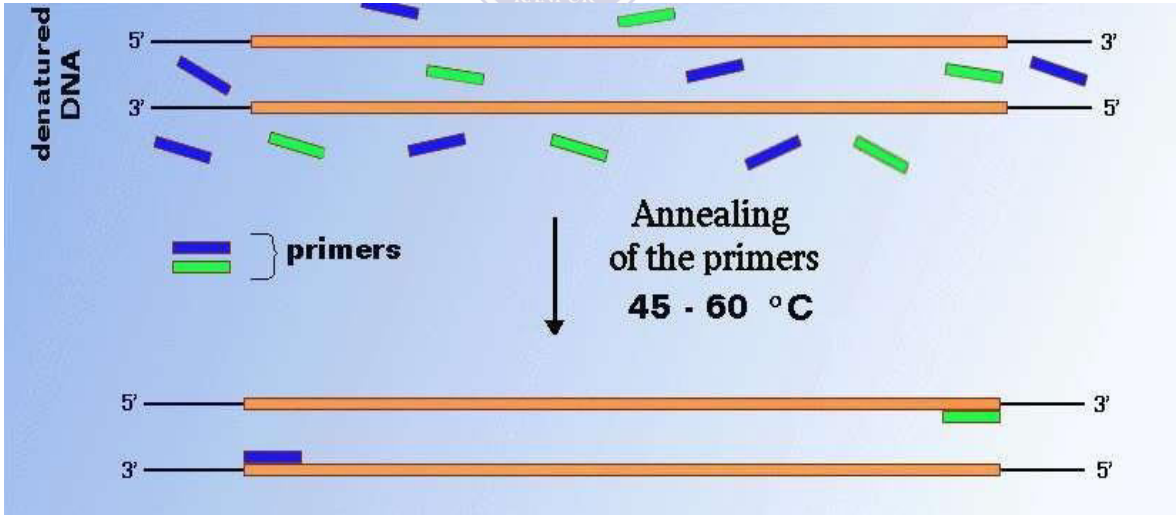
1. DENATURATION

When a DNA solution is *heated* enough, The double-stranded DNA *unwinds*, and the Hydrogen bonds that hold the two strands together *weaken* and finally *break*. The process of breaking a double-stranded **DNA** into single strands is known as *DNA denaturation*, or *DNA melting*.



2. ANNEALING

- Temperature of reaction mixture is cooled to 45-60˚C
- Primers base pairs with complementary sequence in the DNA
- Hydrogen bond reforms
- Annealing is fancy word of renaturing



3. EXTENSION

•Temperature shifted to 72˚C- ideal for polymerase

•Primers are extended by joining the base complementary to DNA strands

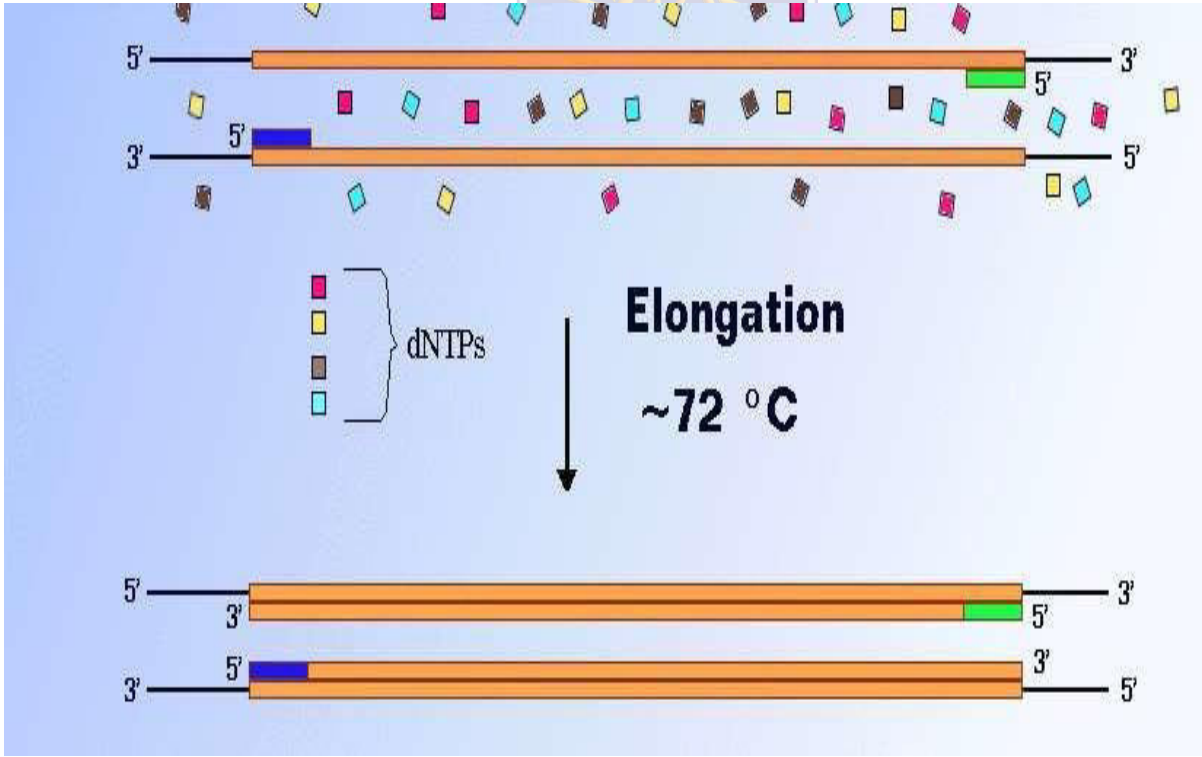
•Elongation continues by the polymerase which add dNTPs from 5'-3' side

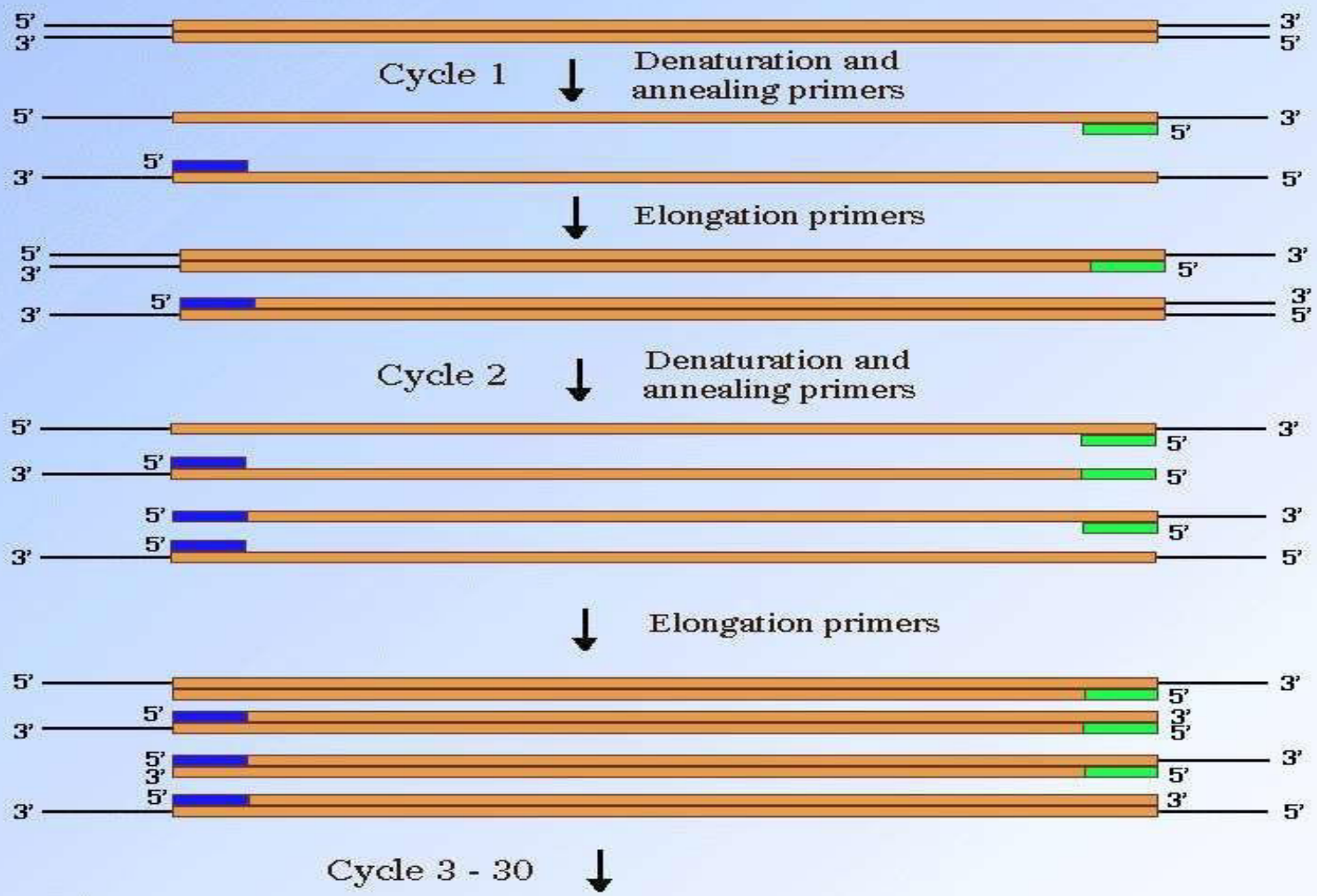
•Deoxynucleosides triphosphates (dNTPs) required for the synthesis of DNA are present in large excess

•synthesis step can be repeated a lot of times.

•To withstand the repeated exposure to high temperatures, a thermostable DNA polymerase is used for PCR - usually *Taq* polymerase.

- *Taq* polymerase works best at around 75 degrees centigrade.
- The time required for this stage depends on the length of the target sequence (for eg; the rate of primer elongation by *Taq* polymerase is about 50 - 100 nucleotides/sec).




5'
3'
3'
5'
Cycle 1
Denaturation and annealing primers
5'
3'
5'
3'
5'
Elongation primers
5'
3'
5'
3'
5'
3'
5'
Cycle 2
Denaturation and annealing primers
5'
3'
5'
3'
5'
5'
3'
5'
5'
3'
5'
Elongation primers
5'
3'
5'
3'
5'
3'
5'
3'
5'
3'
5'
3'
5'
Cycle 3 - 30