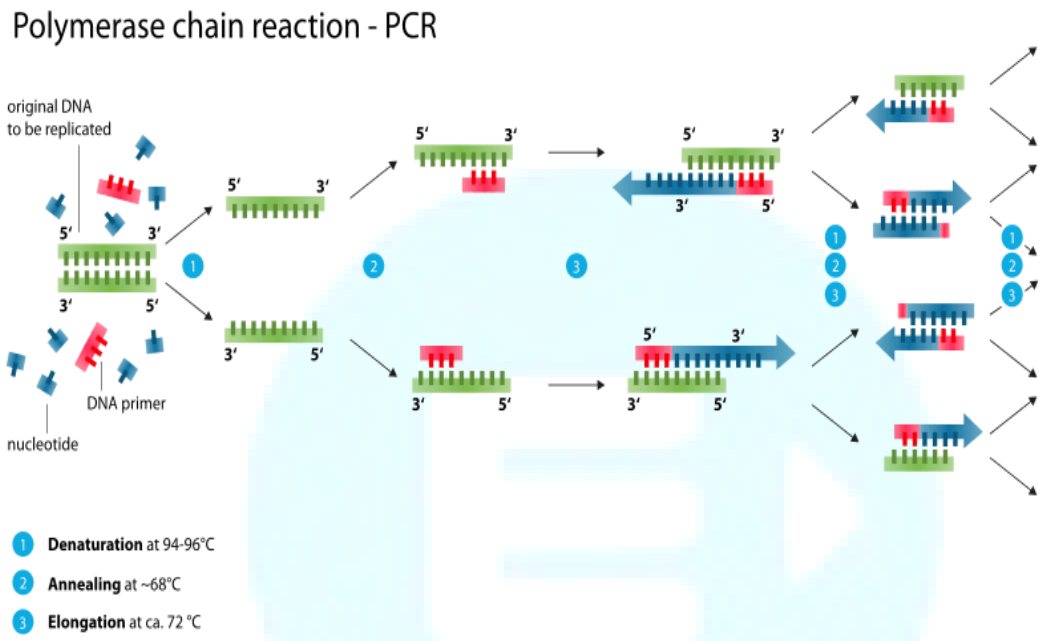
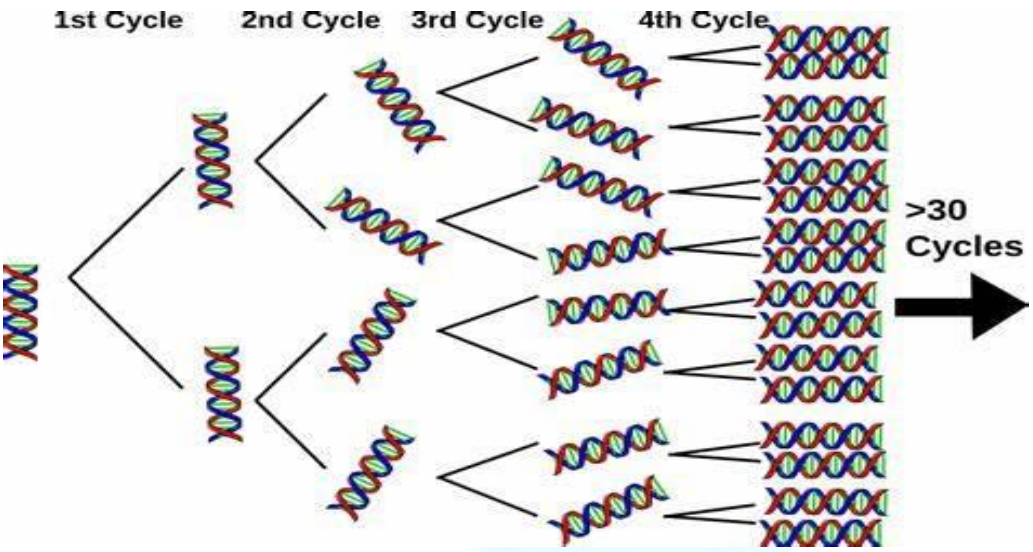
POLYMERASE CHAIN REACTION



- The polymerase chain reaction technique is carried out in vitro and is used for the amplification of DNA.
- It was developed by Kary Mullis in 1983.
- Through this technique a billion copies of the desired DNA or RNA can be made in a matter of few hours.
- The PCR reaction mix contains genomic DNA having the target sequence, two oligonucleotide primers- forward and reverse primer that are complementary to the borders of the two strands of the desired DNA segment, the four deoxynucleoside triphosphates i.e. dTTP (deoxythymidine triphosphate), dCTP

(deoxycytidine triphosphate), dATP (deoxyadenosine triphosphate) and dGTP (deoxyguanosine triphosphate) and Taq polymerase, MgCl2 and Buffer.

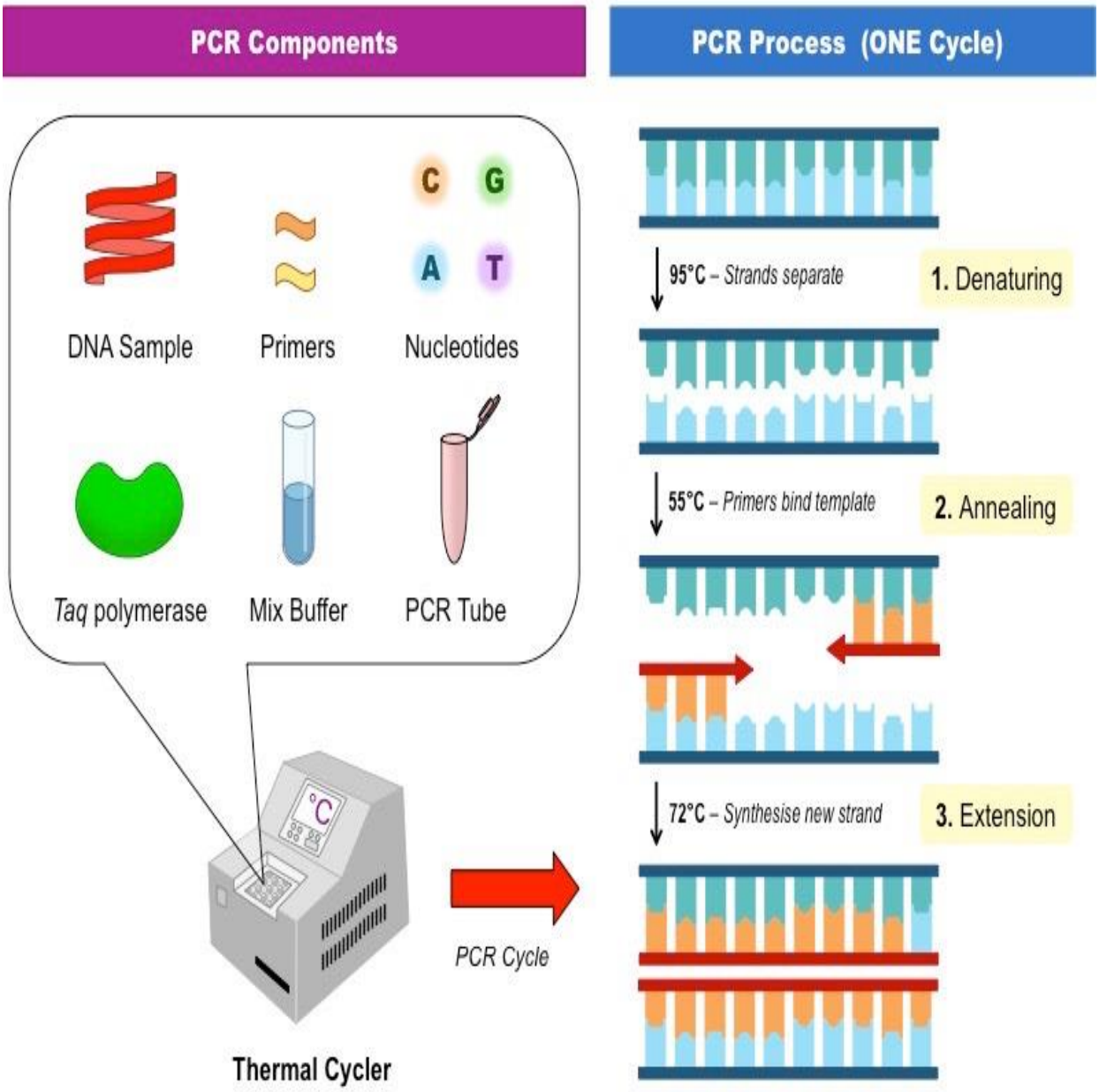
- Designing of Primer; Good primer design is essential for successful reactions.
- The important design considerations described below are a key to specific amplification with high yield.
- The preferred values indicated are built into all our products by default.
- Primers should be 17-28 bases in length.
- The base composition should be 50-60% (G+C).
- Primers should end (3′) in a G or C, or CG or GC: this prevents "breathing" of ends and increases the efficiency of priming.
- Temperature between 55-80oC is referred.
- The basic formula to calculate melting temperature is Tm = 4°C X (number of G's and Cs in the primer) + 2°C X (number of As and Ts in the primer).
- Two primers must have a similar Tm value.
- In case of several primer candidates, we have to choose primers which have the higher Tm value among them.
- There should be no base complementarities between the two primers. 3′-ends of primers should not be complementary (i.e. base pair), as otherwise primer dimers will be synthesized preferentially to any other product;
- Primer self-complementarity (ability to form secondary structures such as hairpins) should be avoided.
- Runs of three or more Cs or Gs at the 3′-ends of primers may promote mis priming at G or C-rich sequences (because of stability of annealing) and should be avoided.



Procedure of PCR

- Denaturation:
  - During denaturation step, the reaction mixture is first heated to a temperature between 90-98C that ensures DNA denaturation.
  - The duration of this step in the first cycle of PCR is usually 2 min at 94C.
- Annealing:
  - During annealing, the mixture is cooled to a temperature of 40-60oC so that annealing of primer to the complementary sequences in the DNA takes place.
  - The duration of annealing step is usually 1 minute.
  - The primer-template hybrid formation is greatly favored over reannealing of the template strand.
- Primer Extension:
  - By utilizing 3`-OH of the primers, primer extension is done.
  - The duration of primer extension is usually 2 minute at 72oC.

- The primers are extended towards each other so that the DNA segment lying between the two primers is copied.
- Taq polymerase catalyzes the extension of DNA segment.
- The optimum temperature for working of Taq polymerase is 72-74oC.
- These cycles are repeated 20-30 times to get a million copies of desired gene segment as after each cycle there is (2n) exponential increase in the copies of DNA segment.



Applications of PCR

- Infectious disease diagnosis, progression, and response to therapy
- PCR technology facilitate the detection of DNA or RNA of pathogenic organisms and as such, helps in clinical diagnostic tests for a range of infectious agents like viruses, bacteria, protozoa etc.
- These PCR-based tests have numerous advantages over conventional antibody-based diagnostic methods that determine the body's immune response to a pathogen.
- In particular, PCR-based tests are competent to detect the presence of pathogenic agent in-advance than serologically-based methods, as patients can take weeks to develop antibodies against a contagious agent.
- PCR-based tests have been developed to enumerate the amount of virus in a person's blood (viral load') thereby allowing physicians to check their patient's disease progression and response to therapy.
- This has incredible potential for improving the clinical management of diseases caused by viral infection, including AIDS and hepatitis, assessment of viral load throughout and after therapy.
- PCR technique is also used to for checking the mycoplasm contamination in mammalian cell lines.

Diagnosis of genetic diseases

- The use of PCR in diagnosing genetic diseases, whether due to innate genetic changes or as a result of natural genetic mutations, is becoming more common.

- Abnormality can be diagnosed even prior to birth. Single-strand conformation polymorphism (SSCP), or single-strand chain polymorphism, is defined as the conformational difference of single-stranded nucleotide sequences of identical length as induced by differences in the sequences under certain experimental conditions.

- These days, SSCP is most applicable as a diagnostic tool in molecular biology.

- It can be used in genotyping to detect homozygous individuals of different allelic states, as well as heterozygous individuals who inherit genetic aberrations.

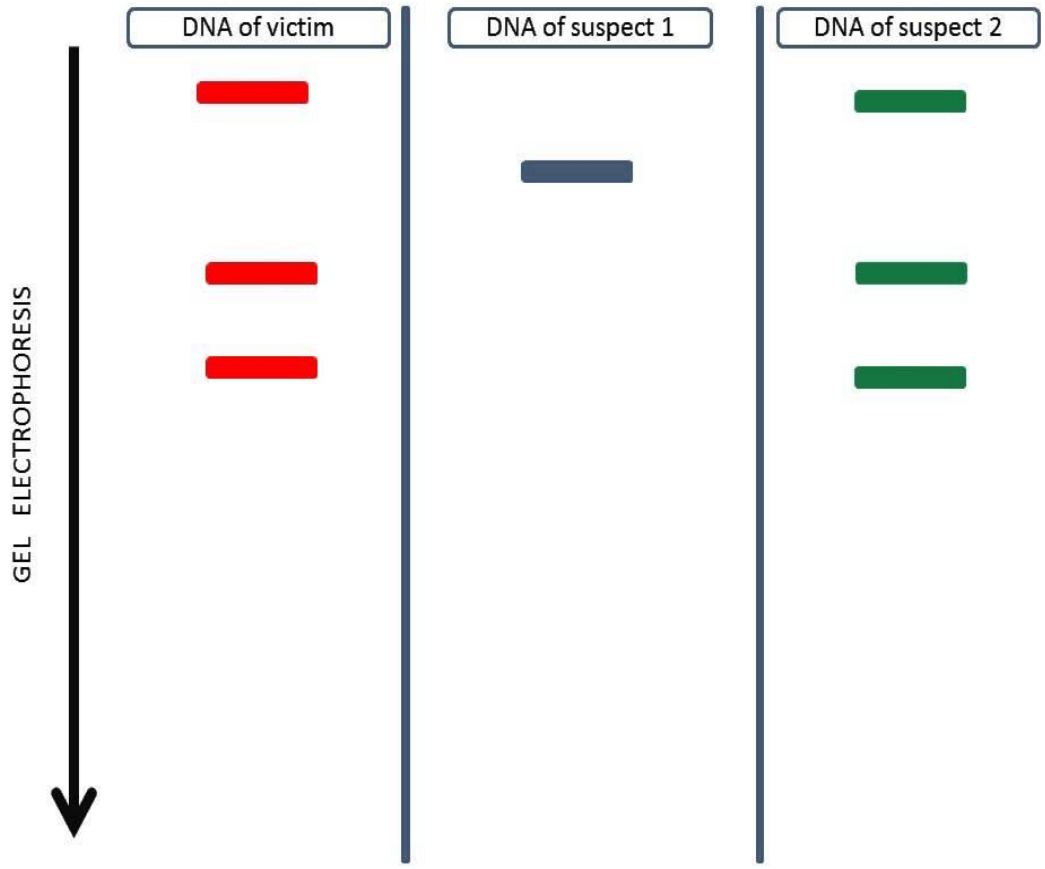
Genetic counseling

- It is done for the parents to check the account of genetic disease beforehand to make a decision on having children.

- This is of course governed by national laws and guidelines. Detection of genetic disease before implantation of an embryo in IVF (In vitro fertilization) also known as pre-implantation diagnosis can also be done exploiting PCR based method.

- Further to diagnose inherited or a spontaneous disease, either symptomatic or asymptomatic (because of family history like Duchene muscular dystrophy) PCR based method is very useful.

Forensic sciences



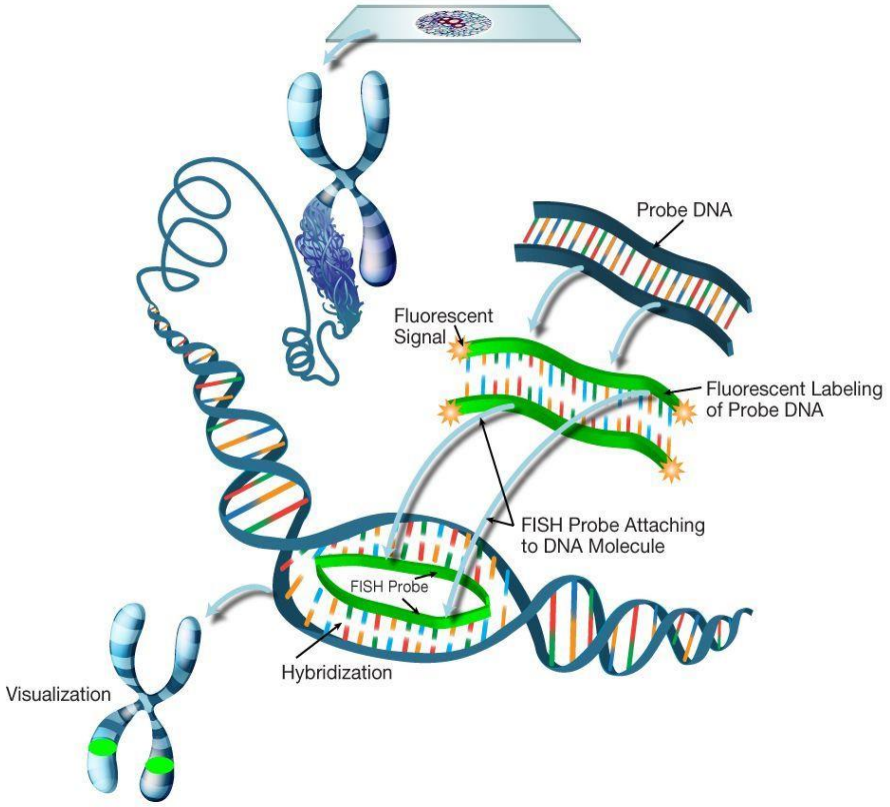
- DNA fingerprint is one of the most exploited applications of PCR (also known as DNA profiling).
- Profiles of specific stretches of DNA are used in genetic fingerprinting (generally 13 loci are compared) which differs from person to person.
- PCR also plays a role in the analysis of genomic or mitochondrial DNA, in which investigators used samples from hair shafts and bones when other samples are not accessible.

Research in Molecular Biology

- PCR is an essential technique in cloning procedure which allows generation of large amounts of pure DNA from a tiny amount of template strand and further study of a particular gene.

- Some alterations to the PCR protocol can generate mutations (general or site-directed) in a sequence either by an inserted fragment or base alteration.
- PCR is used for sequence-tagged sites (STS 's) as an indicator that a particular segment of a genome is present in a particular clone.
- A common application of Real-time PCR is the study of expression patterns of genes during different developmental stages.
- PCR can also investigate _ON or OFF of particular genes at different stages in tissue (or even in individual cells).

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)



- Fluorescence in situ hybridization (FISH) is a macromolecule recognition technique, which is considered as a new advent in the field of cytology.
- Initially, it was developed as a physical mapping tool to delineate genes within chromosomes.
- The accuracy and versatility of FISH were subsequently capitalized upon in biological and medical research.
- This visually appealing technique provides an intermediate degree of resolution between DNA analysis and chromosomal investigations.
- FISH consists of a hybridizing DNA probe, which can be labeled directly or indirectly.
- In the case of direct labeling, fluorescent nucleotides are used, while indirect labeling is incorporated with reporter molecules that are subsequently detected by fluorescent antibodies or other affinity molecules.

- FISH is applied to detect genetic abnormalities that include different characteristic gene fusions or the presence of an abnormal number of chromosomes in a cell or loss of a chromosomal region or a whole chromosome.