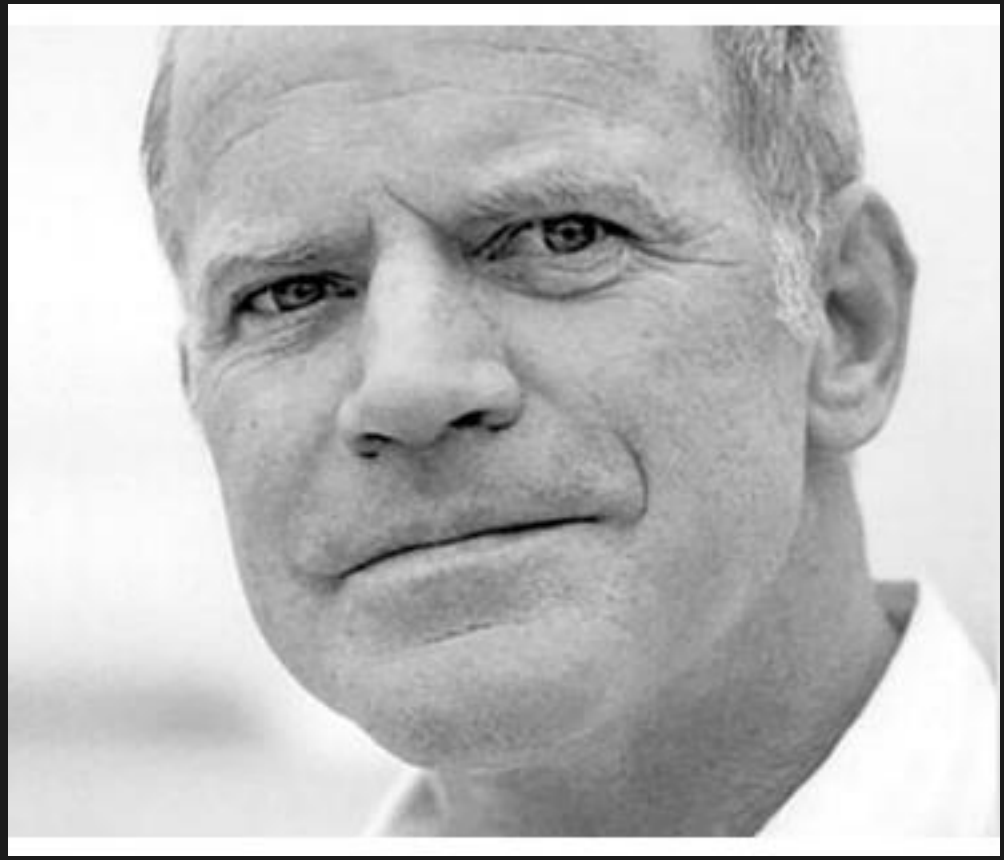
Polymerase Chain Reaction: PCR

PCR

- Conceived by Kary B. Mullis in 1983
- Wide range of applications in biological and medical fields.
- Nobel Prize in Chemistry 1993



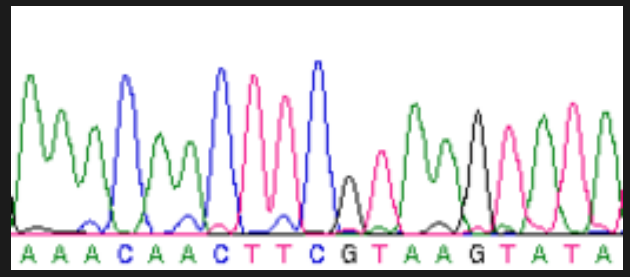
What do we get after a PCR?

- The PCR allows the amplification of a selected region of the DNA generating a very high number of copies.

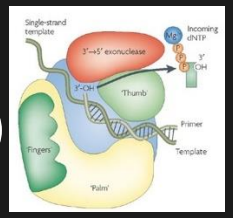
The PCR is a serie of an *in vitro* replication reactions of a specific DNA region

Components

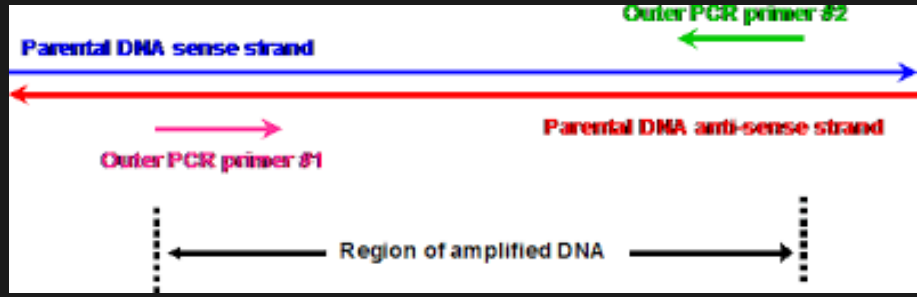
- **DNA sequence of interest (to amplify)**: we should know at least the flanking DNA sequences of the region that we want to amplify



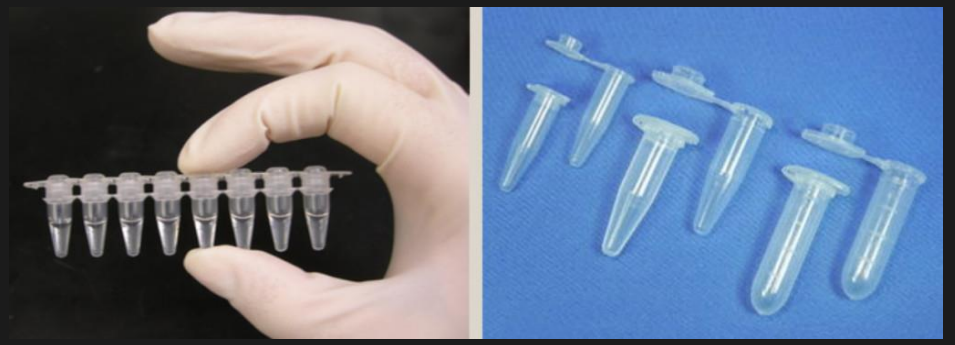
- **DNA polimerase** (Taq polimerase)



- Nucleotides: dNTPs



- **Oligonucleotides 20-30 nt** long (2, one for each filament, 5'-→3'). They are the primers needed for the DNA polimerase to initiate replication. They must complement the flanking sequences.



- Thermocycler

PCR STEPS and CYCLES

Initial DENATURATION

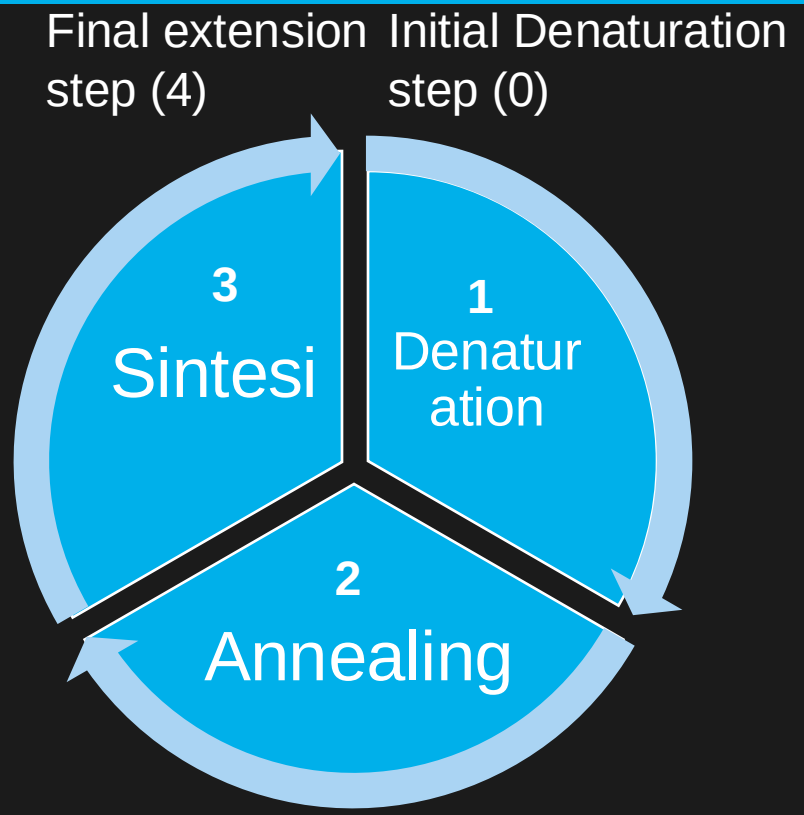
Step 0

1. DNA DENATURATION
2. ANNEALING: Primer association by complementation to the ssDNA
3. EXTENSION: Synthesis of the new DNA filament by the polimerase

X 35-40 cycles

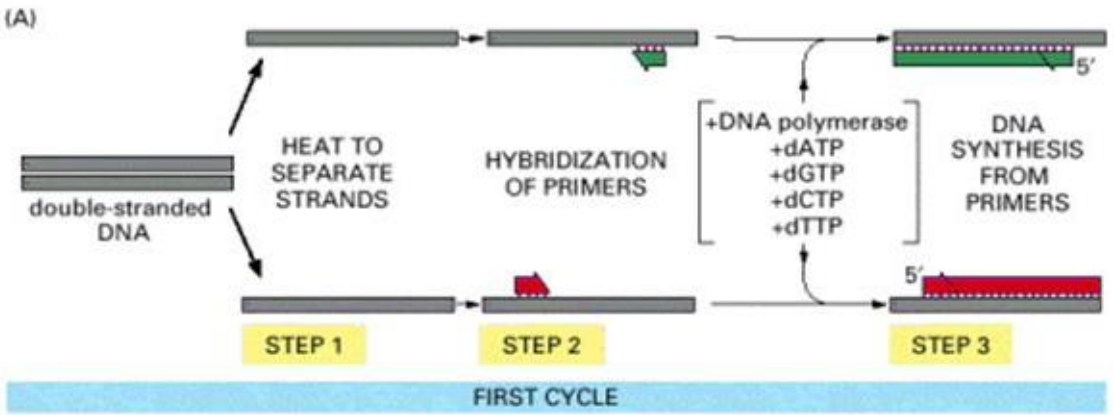
Final DNA extension

Step 4

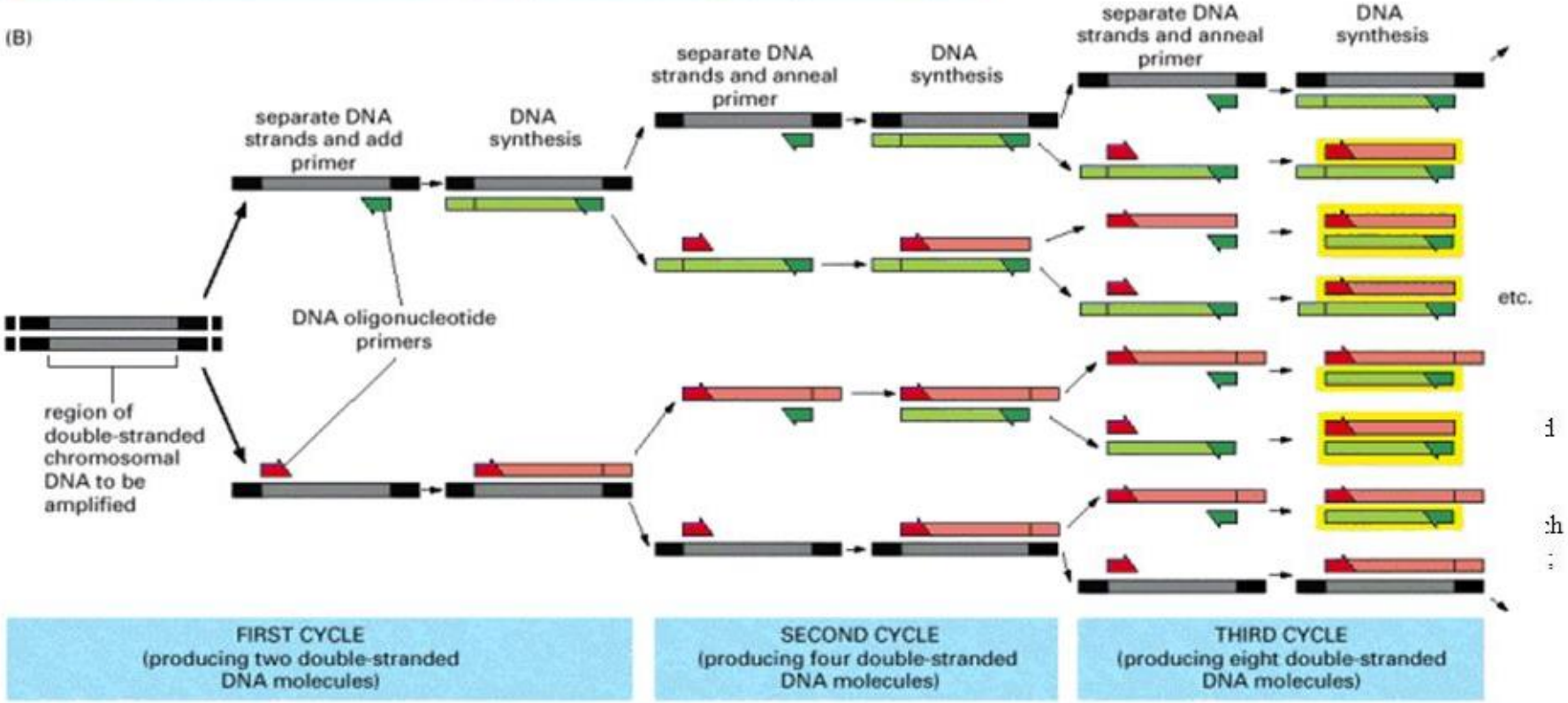


Cycle step	Temp.	Time	Number of cycles
Initial denaturation	98°C	30 s	1
Denaturation Annealing Extension	98°C 65–72°C 72°C	5–10 s 10–30 s 15–30 s/kb	25
Final extension	72°C 4°C	5–10 min hold	1

Amplification of DNA using the PCR technique



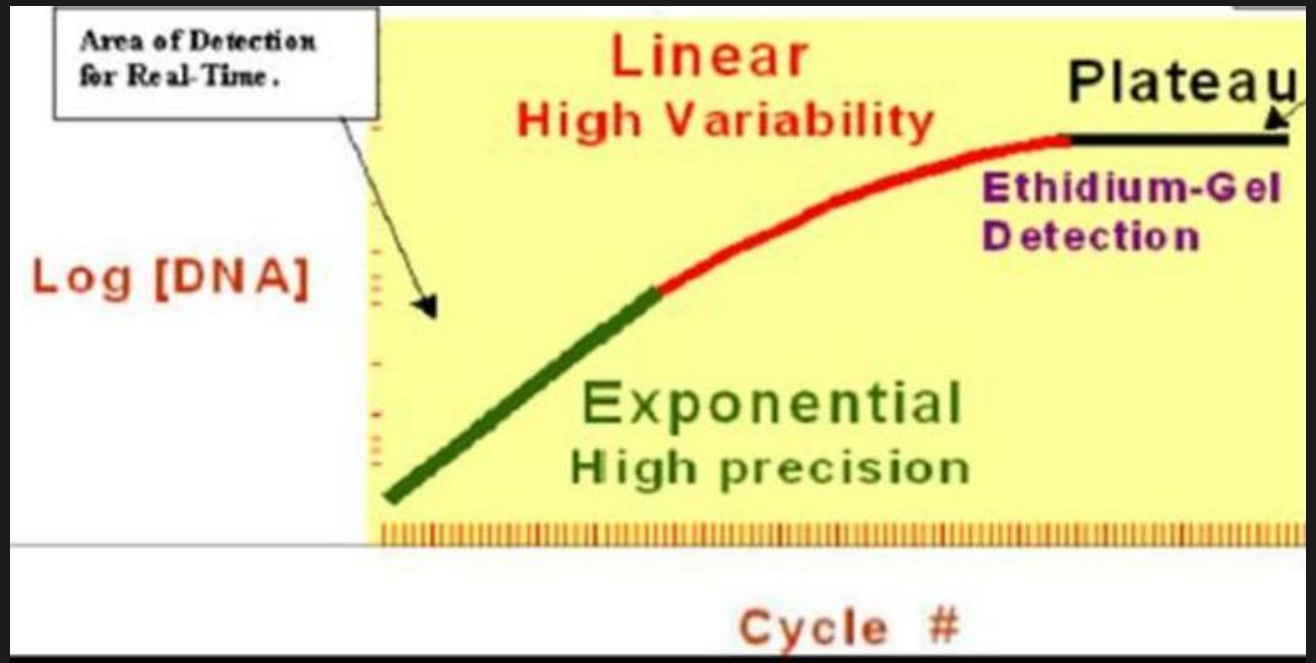
PCR



PCR Kinetics

The PCR reaction is composed of 3 phases that are dictated by the reagents depletion during the PCR cycles:

1. **Exponential**: The reaction products accumulate exponentially, doubling at each cycle. In this phase, the reaction is very specific and precise.;
2. **Linear:** In this phase the reagents concentration is not optimal since they have been consumed in the exponential phase. As consequence, the reactions slow down and the generation of products is not exponential. This phase is characterized by high variability because of the diverse kinetics between samples.
3. **Plateau:** this is the final phase in which there is no replication because the DNA pol is busy by replicating the existing amplicons. The DNA pol concentration limitation and the amplicons self-anneling determine the end of the linearity.



DNA polimerase: Taq polymerase

Advantages of using Taq polimerasi:

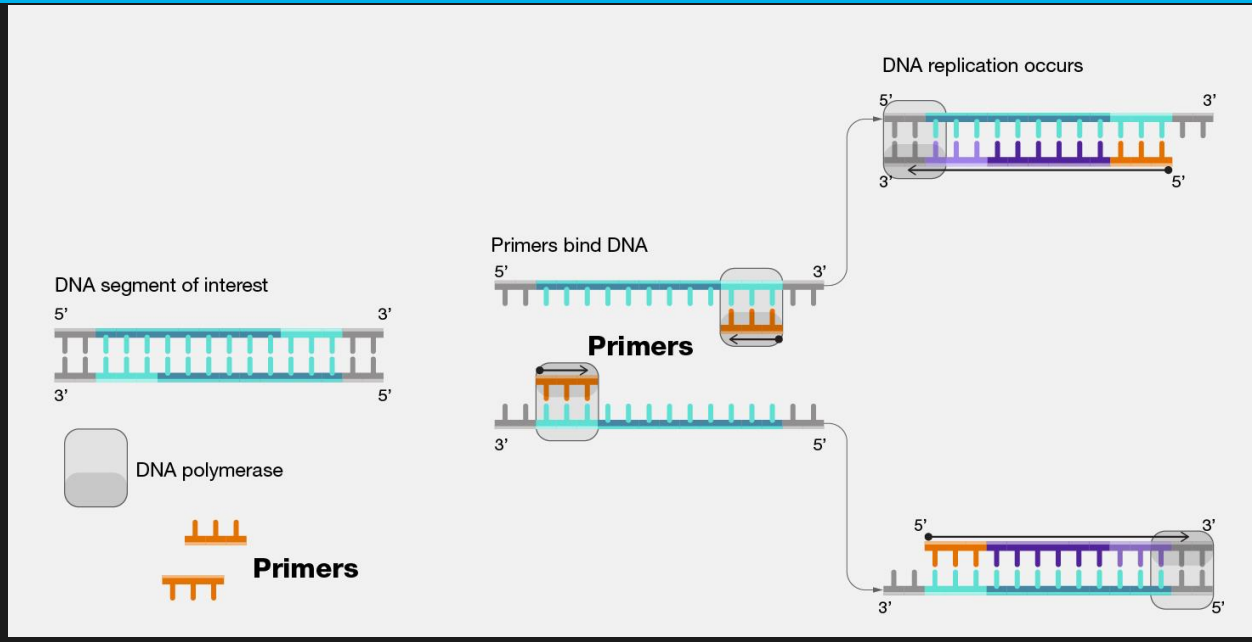
1. Thermoresistant: The enzyme must be added only once at the beginning of the PCR reaction and must remain active during all the PCR cycles (high temperature and variations)
2. By remaining active, the PCR can be automatized by using specific thermocyclers.
3. The Taq polimerase increases the PCR specificity and sensitivity.

Primers design:

They must complement the flanking sequences of the DNA sequence to amplify.

Important factors:

1. Lenght : Longer they are, better they will anneal -> higher Tm
2. % G-C: more abundant they are, better the primers anneal-> higher Tm

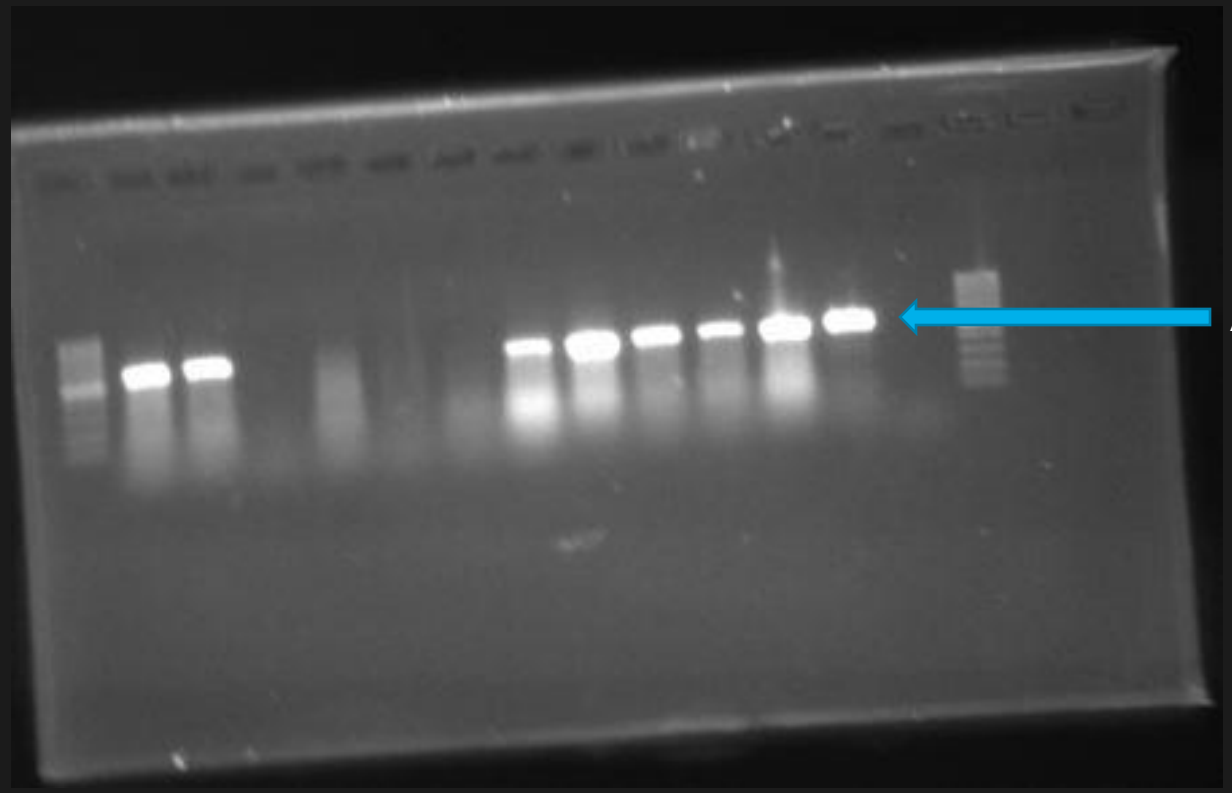


PCR Annealing Temperature, Ta= Tm -5 °C

The optimal Annealing temperature is between 60-64°C.

Ideally, the primers Tm should be the same or not differ more than 2°C. In this condition the primers anneal simultaneously increasing the amplification efficiency.

PCR result after analysis in an agarose gel



Polymerase Chain Reaction

PCR Utilities

•Genetic matching
•Detection of pathogens
•Pre-natal diagnosis
•DNA fingerprinting •Gene therapy
•Mutation screening •Drug discovery •Classification of organisms •Genotyping •Molecular Archaeology
•Molecular Epidemiology •Molecular Ecology
•Bioinformatics •Genomic cloning •Site-directed mutagenesis •Gene expression studies
Molecular IdentificationSequencingGenetic Engineering
•Molecular Archaeology •Molecular Epidemiology
•Molecular Ecology •DNA fingerprinting
•Classification of organisms •Genotyping •Pre-natal diagnosis •Mutation screening •Drug discovery
•Genetic matching •Detection of pathogens
•Bioinformatics •Genomic cloning •Human Genome Project
•Site-directed mutagenesis •Gene expression studies