Molecular Methods for Microorganisms Identification using Biotechnology approaches

طرق التشخيص الجزيئي للاحياء المجهرية
باستخدام اساليب التقنيات الاحيائية

Common Tools of Molecular Biology

- Nucleic acid fractionation
- Polymerase chain reaction
- Probes, Hybridization
- Vector, Molecular cloning
- Nucleic acid enzymes
- Microarray
- DNA sequencing
- Electrophoretic separation of nucleic acid
- Detection of genes:

  DNA: Southern blotting; inSitu hybridization; FISH Technique

  RNA: Northern blotting

  Protein: Western blotting, immunohistochemistry

Polymerase Chain Reaction

- The polymerase chain reaction, or PCR, is one of the most well-known techniques in molecular biology. Replication of single-stranded DNA from a template using synthetic primers and a DNA polymerase was first reported as early as the 1970s. Nevertheless, the PCR method as we know it today to amplify target DNA was not developed as a research tool until 1983, by Kary Mullis.Since then, PCR has become an integral part of molecular biology, with applications ranging from basic research to disease diagnostics, agricultural testing, and forensic investigation. For his invention, Kary Mullis was awardedNobal Prize in Chemistry in 1993

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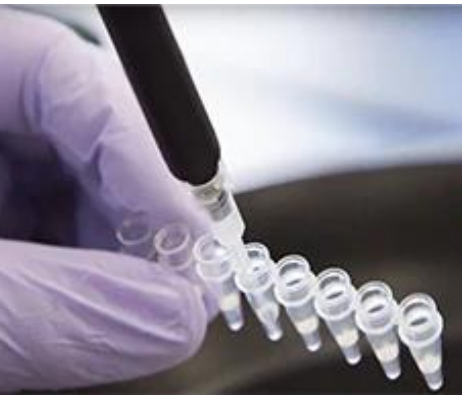
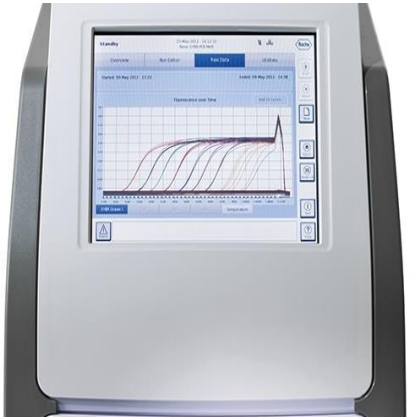
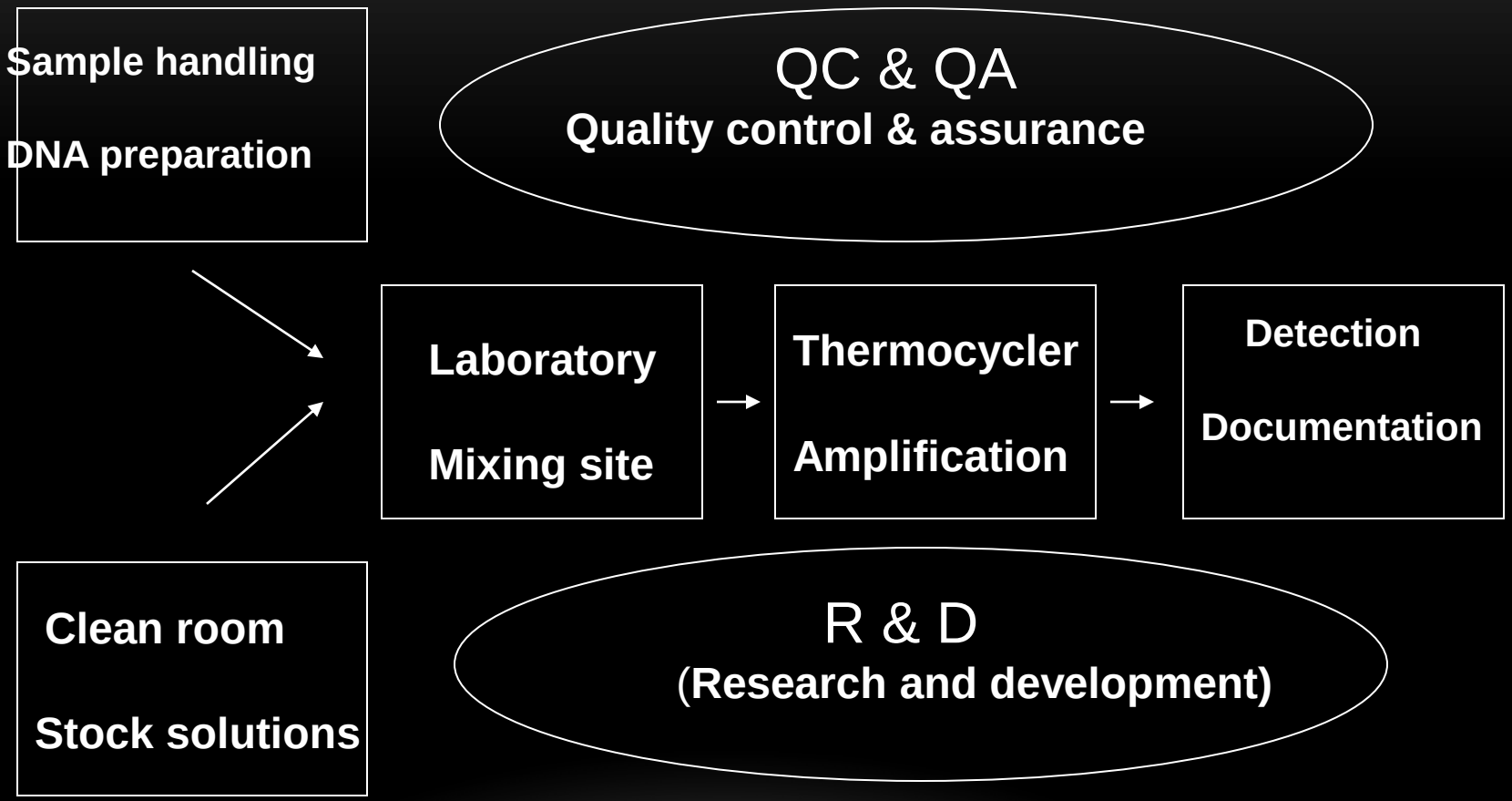


PCR Applications

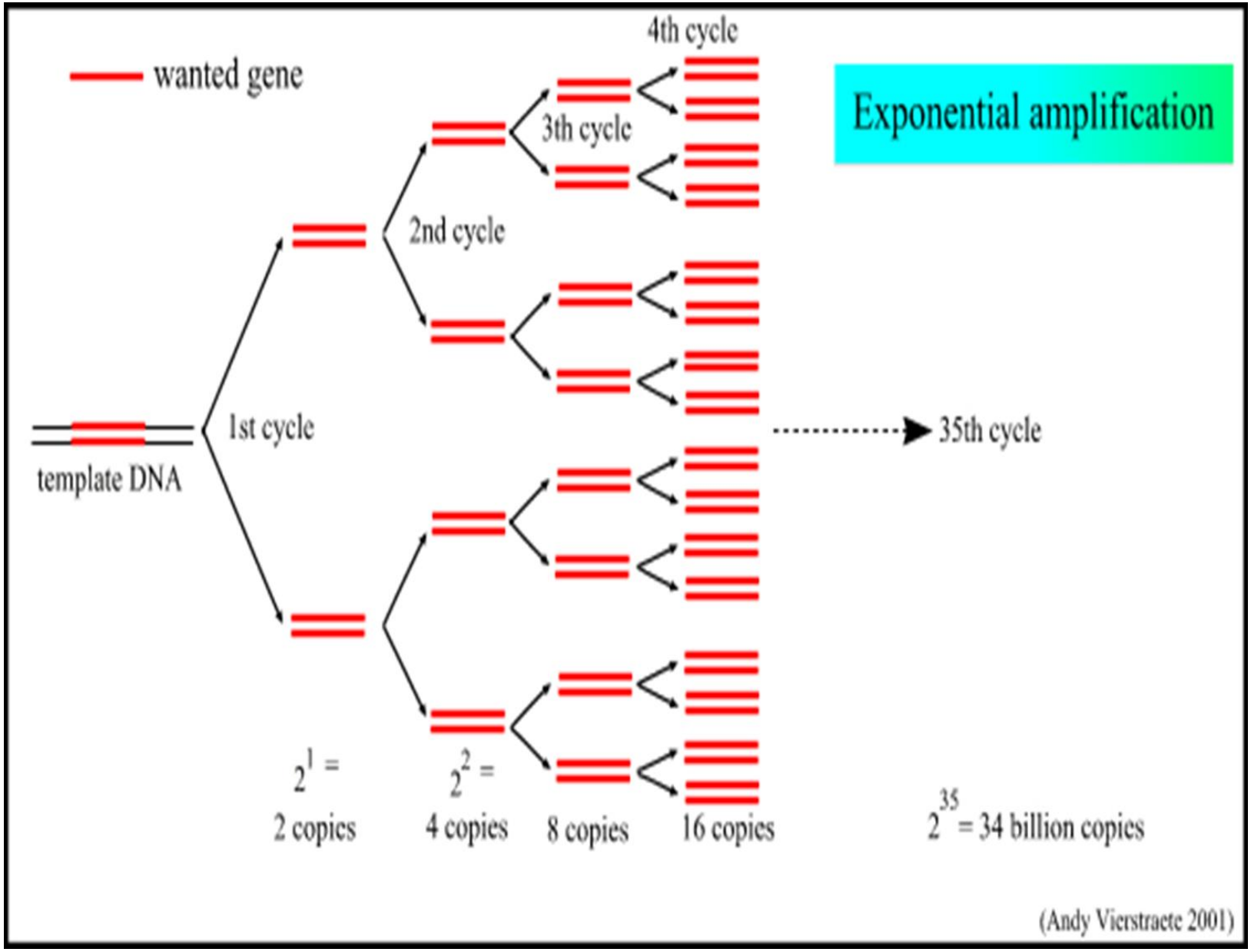
- PCR has a broad range of applications, not only in basic research but also in the areas of medical diagnostics, forensics, and agriculture.



PCR laboratory



Alternatives: robots + kits


wanted gene
4th cycle
Exponential amplification
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^1 =
2 copies
2^2 =
4 copies
8 copies
16 copies
2^{35} = 34 billion copies
(Andy Vierstraete 2001)

USES AND ADVANTAGES IN TESTING BY PCR METHODS

- Clinical diagnostics: detection and quantification of infectious microorganisms, cancer cells and genetic disorders
- Capable of amplifying long targets, up to 6.0 kb
- One-tube system allows rapid, sensitive and reproducible analysis of RNA with minimal risk of sample contamination
- Amplifies products from a wide variety of total RNA or mRNA sources

Molecular identification using sequence method of DNA

•Amplification (synthesis) of species specific sequences

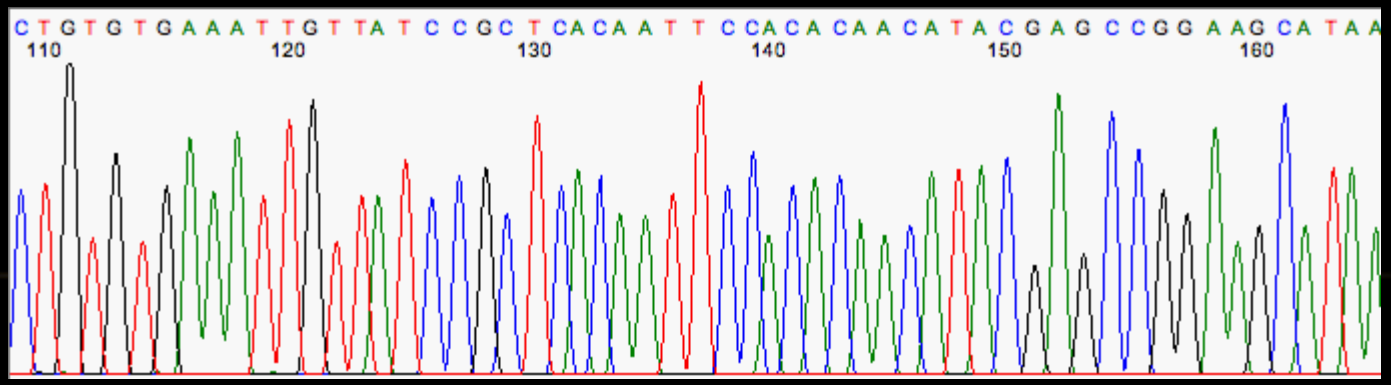
PCR – polymerase chain reaction

-A-A-T-T-C-G-C-G-A-T-G-

• complementary sequences

-A-A-T-T-C-G-C-G-A-T-G-

- T-T-A-A-G-C-G-C-T-A-C-



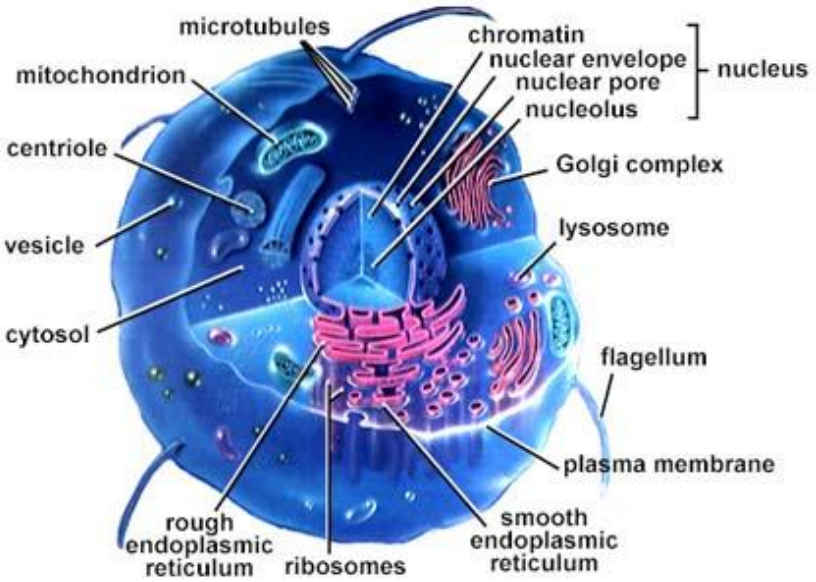
Three Domain of Life

All living things are grouped into three domain:

- Eukaryotes;
- Prokaryotes and
- Archaea.

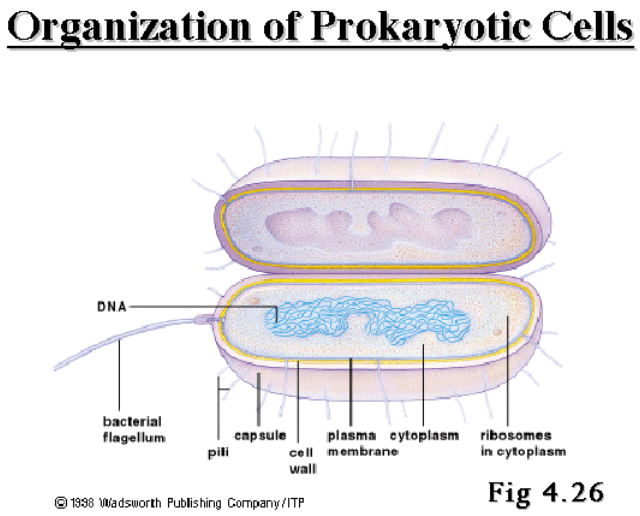
Eukaryotic Cell

- Cell with a true nucleus, where the genetic material is surrounded by a membrane;
- Eukaryotic genome is more complex than that of prokaryotes and distributed among multiple chromosomes;
- Eukaryotic DNA is linear;
- Eukaryotic DNA is complexed with proteins called "histones;
- Numerous membrane-bound organelles;
- Complex internal structure;
- Cell division by mitosis.



Prokaryotic Cell

- Unicellular organisms, found in all environments. These include bacteria and archaea.
- Without a nucleus; no nuclear membrane (genetic material dispersed throughout cytoplasm ;
- No membrane-bound organelles;
- Cell contains only one circular DNA molecule contained in the cytoplasm;
- DNA is naked (no histone);
- Simple internal structure; and
- Cell division by simple binary fission.

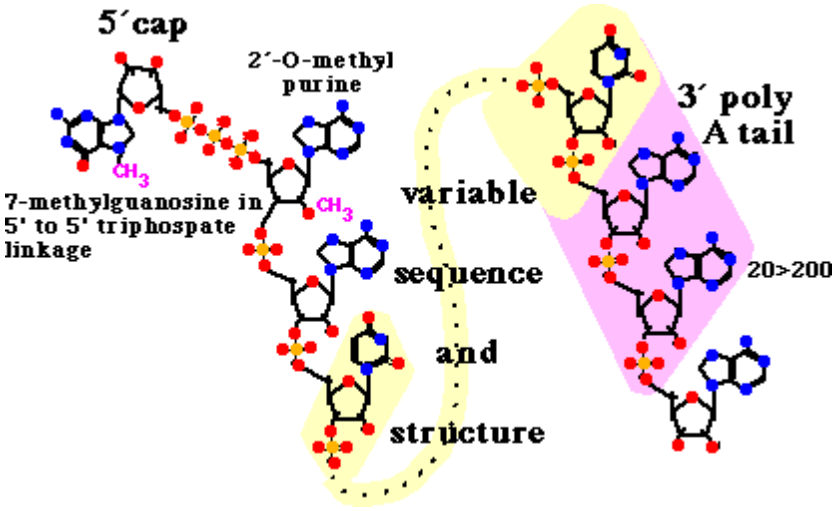


The RNA

- **Three major classes of RNA: messenger (mRNA), transfer (tRNA) and ribosomal (rRNA). Minor classes of RNA include small nuclear RNA ; small nucleolar RNA;……..**

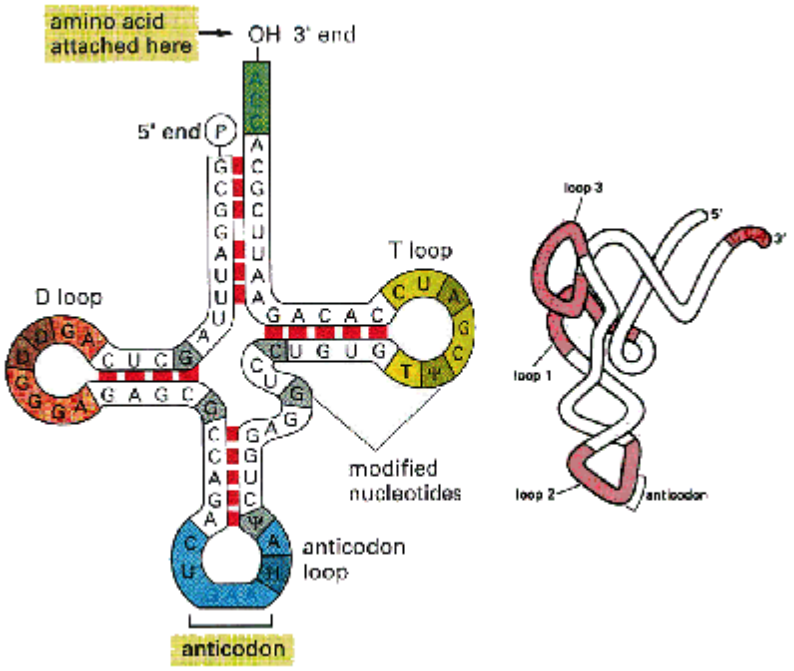
Messenger RNA

- **mRNA** molecules represent transcripts of structural genes that encode all the information necessary for the synthesis of a single type polypeptide of protein.
- **mRNA;** intermediate carrier of genetic information; deliver genetic information to the cytoplasm where protein synthesis take place.
- The **mRNA** also contains regions that are not translated: in eukaryotes this includes the 5' untranslated region, 3' untranslated region, 5' capand poly-A tail.



Transfer RNA(tRNA)

- All **tRNAs** share a common secondary structure represented by a coverleaf. They have four-paired stems defining three stem loops (the D loop, anticodon loop, and T loop) and the acceptor stem to which amino acids are added in the charging step.
- RNA molecules that carry amino acids to the growing polypeptide.

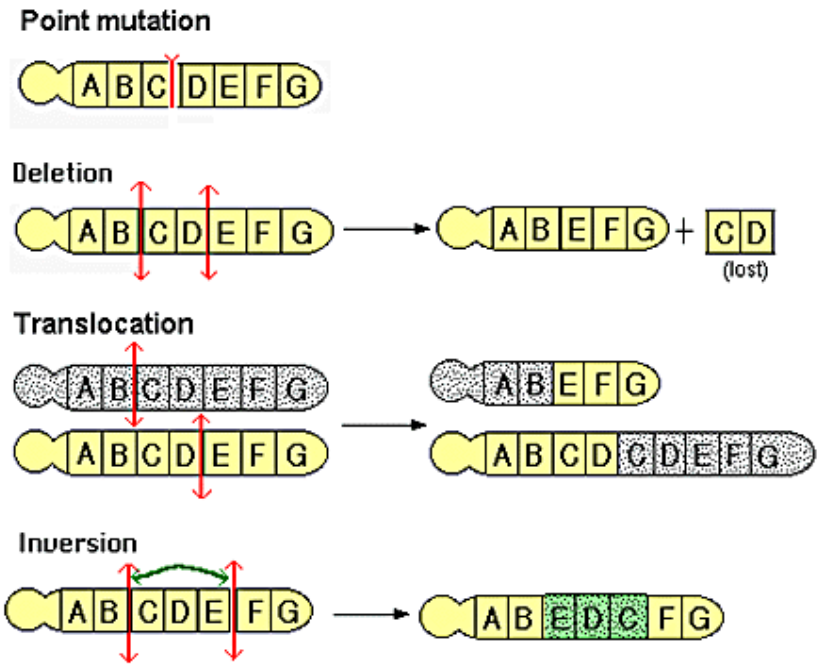


Ribosomal RNA (rRNA

Ribosomal RNA (rRNA) is the central component of the ribosome, the function of the rRNA is to provide a mechanism for decoding mRNA into amino acids and to interact with the tRNAs during translation by providing peptidyl transferase activity.

Mutation

Mutations can have harmful, beneficial, neutral, or uncertain effects on health and may be inherited as autosomal dominant, autosomal recessive, or X-linked traits. Mutations that cause serious disability early in life are usually rare because of their adverse effect on life expectancy and reproduction.



Mutations of Chromosomes