Laboratory methods

Molecular pathology

Molecular pathology

- Molecular pathology is the branch of laboratory medicine or clinical pathology that utilizes the techniques of molecular biology to **diagnose** disease, **predict** disease course, select **treatments**, and **monitor** the effectiveness of therapies.

Molecular pathology

- Molecular pathology shares some aspects of practice with
- **anatomic pathology**
- and **clinical pathology**,
- **molecular biology**,
- **biochemistry,**
- **proteomics** and
- **genetics,**
- and is sometimes considered a "**crossover**" discipline.

Molecular pathology

- Molecular pathology is commonly used in diagnosis of **cancer and infectious diseases**.

Molecular pathology

- **Techniques are numerous**
- - quantitative polymerase chain reaction (qPCR),
- - multiplex PCR,
- - DNA microarray,
- - *in situ* hybridization,
- - *in situ* RNA sequencing,
- - DNA sequencing,
- - molecular profiling of pathogens,
- - analysis of bacterial genes for antimicrobial resistance.

Diagnostic and Predictive Molecular Testing

- The clinical applications of molecular pathology testing can be generalized into two groups based on whether the clinical information sought is intended for **diagnostic or predictive** purposes.

Molecular genetics

- specifically entails an assessment of how the DNA sequence of a gene compares with its wild-type or normal sequence. Ultimately, protein malfunctions related to **gene mutations** lead to organ dysfunction and disease states.

Clinical benefits of a molecular diagnosis

- a preventive medicine and treatment plan based on the known natural history of a particular disorder.
- a more individualized medical approach to care with respect to severity of disease, expected age of onset for presymptomatic cases, and increased risk for certain associated complications can be determined.
- predictive testing options become available for at-risk family members.
- a negative test result directs implementation of a more appropriate, less aggressive screening strategy

Quantitative Polymerase Chain Reaction

- Quantitative Polymerase Chain Reaction (qPCR) is **a collection of methods for estimating the number of copies of a specific DNA template in a sample**.

Multiplex PCR

- Multiplex PCR is the **simultaneous detection of multiple targets in a single reaction well, with a different pair of primers for each target**.
- This technique requires two or more probes that can be distinguished from each other and detected simultaneously.

In situ hybridization (ISH)

- is a type of hybridization that uses a labeled complementary DNA, RNA or modified nucleic acids strand (i.e., probe) to localize a **specific DNA or RNA sequence** in a portion or section of tissue, in cells, and in circulating tumor cells (CTCs).
- This is distinct from **immunohistochemistry**, which usually localizes proteins in tissue sections.

In situ RNA sequencing

- single-cell RNA sequencing enables transcriptomic analysis of an individual cell.
- Through the combination of high-throughput sequencing and bioinformatic tools, single-cell RNA-seq can detect more than 10,000 transcripts in one cell to distinguish cell subsets and dynamic cellular changes.

DNA sequensing

- **DNA sequencing** is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine.

The human genome

- The human genome is composed of **3 billion base pairs of DNA**.
- This is not present as one continuous piece of double-stranded DNA, but is distributed among 22 pairs of autosomal chromosomes and 2 sex chromosomes.
- The DNA is associated with a large number of proteins (histones and others) that serve regulatory functions and package the genetic material into these large chromosomal units.
- Along the length of each chromosome, DNA is organized into linear domains consisting of genes (primarily nonrepetitive DNA), repetitive elements, and apparently functionless regions, much like beads on a string

The human genome

- Repetitive DNA can be subdivided into several different categories or families.
- It occurs either in clusters of tandem repeats or as repetitive elements of various lengths dispersed throughout the genome.
- Clusters of tandem repeats can be localized to one or many locations. Such clusters are commonly referred to as satellite DNA.
- Repetitive sequences that are not localized to a particular area or areas of the genome are referred to as dispersed repetitive elements.

The human genome

- Genes are found among the **nonrepetitive DNA** in the genome.
- Genes code for specific protein chains, each with a specific function in cell physiology.
- A gene is composed of regulatory elements, which determine where, when, and how a gene is transcribed and coding regions, which are broken into segments, termed exons (promoter), which is the site where gene transcription is initiated.
- The exons are separated by noncoding regions of DNA called introns (intervening sequences).
- The size of a gene may influence the molecular diagnostic laboratory's ability to design a clinical test for a particular disorder and certainly impacts the selection of the technology used to detect mutations

MOLECULAR PATHOLOGY AND DNA REPAIR MECHANISMS

- **Mutation and Genetic Variation**
- Although the entire genome sequence from any given human is approximately 99.9% identical to the genome sequence of any other individual human, there are on the order of **3 million sequence** variations between any **two unrelated persons**.

Mutation and Genetic Variation

- A large number of genetic variations occur at measurable frequencies in the population.
- Such variations are termed **polymorphisms**.

DNA Repair

- A number of DNA repair pathways are known and can be roughly characterized into the following functional categories:
- (i) **direct reversal**,
- (ii) **excision repair**,
- (iii) **DNA double-strand break repair**

MODES OF INHERITANCE

- **Mendelian Inheritance**
- Autosomal Dominant Inheritance
- Autosomal Recessive Inheritance
- X-Linked Recessive Inheritance
- X-Linked Dominant Inheritance
- X-Linked Dominant Male Lethal Inheritance
- Y-Linked or Holandric Inheritance

- **Non-Mendelian Inheritance**
- Epigenetic Inheritance—Imprinting
- Inheritance Through Mitochondrial DNA
- Multifactorial Inheritance
- Sporadic Inheritance

- Certain factors are known to influence the gene penetrance for specific disorders.
- **Age** (age-related penetrance)
- **Gender** (gender-related penetrance)
- **Reduced** penetrance

Philadelphia chromosome

- The **Philadelphia chromosome** or **Philadelphia translocation** (**Ph**) is a specific genetic abnormality in chromosome 22 of leukemia cancer cells