Understanding the Common Techniques in Molecular Biology

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Objectives

• Compare Molecular testing to other testing commonly seen in an Anatomic laboratory.

• Understanding basic DNA structure is essential to understanding molecular assays.

• Understanding the relationship between DNA, RNA, Chromosomes, Genes and Proteins.

• Listing some molecular tests and with a brief explanation of the method.
Histology

Histology is the study of the microscopic anatomy of cells and tissues of plants and animals.

It is commonly performed by examining cells and tissues by sectioning and staining, followed by examination under a light microscope or electron microscope.

The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine.
Histopathology

- The microscopic study of diseased tissue, is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples.

- The trained scientists who perform the preparation of histological sections are histotechnicians, histology technicians (HT), histology technologists (HTL), medical scientists, medical laboratory technicians, or biomedical scientists. Their field of study is called histotechnology.
Histology

Microscope

HPV cervix

HPV cervix
Histology

- All laboratory specimens are accessioned prior to testing
- Fixation (*all tissue must be properly fixed*)
- Gross (*macroscopic description and dissection*)
- Processing (*removing water from cell and replacing with paraffin*)
- Embedding (*orient specimen in paraffin*)
- Cutting (*microtomy*)
- Staining (*H&E routine stain used to visualize the nucleus & cytoplasm*)
- Special Stains (*used to visualize particular cellular components or microorganisms*)
- IHC stains (*used to visualize the antigen / antibody reaction*)
- ISH stains (*used to visualize the DNA or RNA probe reaction, this is actually a molecular test*)
Routine Histology
Routine Histology
Routine Histology
Routine Histology
Cytology

- Cytology means "the study of cells". Cytology is that branch of life science, which deals with the study of cells in terms of structure, function and chemistry.

Staining slides

HPV
Cytology Processing
IHC

• Immunohistochemistry or IHC refers to the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

• Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis).

• Visualising an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine.
IHC

Ventana IHC stainer

Antigen
Antibody

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Flow Cytometry

• In cell biology, flow cytometry is a laser based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

• Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in basic research, clinical practice and clinical trials. A common variation is to physically sort particles based on their properties, so as to purify populations of interest.
Histogram

A histogram is a graphical representation of the distribution of data.
Organ System

• In biology, a biological system (or organ system or body system) is a group of organs that work together to perform a certain task.

Common systems, such as those present in mammals and other animals, seen in human anatomy, are those such as the circulatory system, the respiratory system, the nervous system, etc.

• A group of systems composes an organism, e.g. the human body.
Organ

- In biology, an organ is a collection of tissues joined in a structural unit to serve a common function.

- Organs exist in all higher biological organisms, in particular they are not restricted to animals, but can also be identified in plants.

In single-cell organisms like bacteria, the functional analogues of organs are called organelles.
Tissue

- Tissue is a cellular organizational level intermediate between cells and a complete organism.

A tissue is an ensemble of similar cells from the same origin that together carry out a specific function. Organs are then formed by the functional grouping together of multiple tissues.

- The study of tissue is known as histology or, in connection with disease, histopathology.
Cells

The cell is the basic structural, functional and biological unit of all known living organisms. It is the smallest unit of life that is classified as a living thing (except virus, which consists only of DNA/RNA covered by protein and lipids), and is often called the "building block of life".
Cells

- Schematic of typical animal cell, showing subcellular components.
- Organelles:
  1. Nucleolus
  2. Nucleus
  3. Ribosomes *(little dots)*
  4. Vesicle
  5. Rough endoplasmic reticulum
  6. Golgi apparatus
  7. Cytoskeleton
  8. Smooth endoplasmic reticulum
  9. Mitochondria
  10. Vacuole
  11. Cytosol
  12. Lysosome
  13. Centrioles within Centrosome
  14. Cell membrane
In cell biology, the nucleus is a membrane-enclosed organelle found in eukaryotic cells. It contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes.

The genes within these chromosomes are the cell's nuclear genome.

The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression — the nucleus is, therefore, the control center of the cell.
DNA

- Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. Along with RNA and proteins, DNA is one of the three major macromolecules essential for all known forms of life.

- Most DNA molecules are double-stranded helices, consisting of two long biopolymers of simpler units called nucleotides—each nucleotide is composed of a nucleobase (guanine, adenine, thymine, and cytosine), recorded using the letters G, A, T, and C, as well as a backbone made of alternating sugars (deoxyribose) and phosphate groups (related to phosphoric acid), with the nucleobases (G, A, T, C) attached to the sugars.

- DNA is well-suited for biological information storage, since the DNA backbone is resistant to cleavage and the double-stranded structure provides the molecule with a built-in duplicate of the encoded information.
DNA

The two strands of DNA run in opposite directions to each other and are therefore anti-parallel, one backbone being 3’ (three prime) and the other 5’ (five prime).

This refers to the direction the 3rd and 5th carbon on the sugar molecule is facing. Attached to each sugar is one of four types of molecules called nucleobases (informally, bases).

It is the sequence of these four nucleobases along the backbone that encodes genetic information.

This information is read using the genetic code, which specifies the sequence of the amino acids within proteins.

The code is read by copying stretches of DNA into the related nucleic acid RNA in a process called transcription.
Within cells, DNA is organized into long structures called chromosomes.

During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes.

Eukaryotic organisms (animals, plants, fungi, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts.

In contrast, prokaryotes (bacteria & archaea) store their DNA only in the cytoplasm.

Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.
DNA

- Minor groove
- Major groove

- Hydrogen
- Oxygen
- Nitrogen
- Carbon
- Phosphorus

Pyrimidines
Purines

T
C
A
G
DNA
DNA
DNA to Protein
Proteins are large biological molecules consisting of one or more chains of amino acids.

Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another.

Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity.
Chromosomes

- A chromosome is an organized structure of DNA, protein, and RNA found in cells.
- It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences.
- Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions.
- Chromosomal DNA encodes most or all of an organism's genetic information; some species also contain plasmids or other extrachromosomal genetic elements.
- Chromosomes vary widely between different organisms. The DNA molecule may be circular or linear, and can be composed of 100,000 to over 3,750,000,000 nucleotides in a long chain.
- Typically, eukaryotic cells (cells with nuclei) have large linear chromosomes and prokaryotic cells (cells without defined nuclei) have smaller circular chromosomes, although there are many exceptions to this rule.

Also, cells may contain more than one type of chromosome; for example, mitochondria in most eukaryotes and chloroplasts in plants have their own small chromosomes.
Human Chromosomes

- Chromosomes in humans can be divided into two types: autosomes and sex chromosomes.
- Certain genetic traits are linked to a person's sex and are passed on through the sex chromosomes.
- The autosomes contain the rest of the genetic hereditary information.

All act in the same way during cell division. Human cells have 23 pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), giving a total of 46 per cell.

In addition to these, human cells have many hundreds of copies of the mitochondrial genome. Sequencing of the human genome has provided a great deal of information about each of the chromosomes.
Chromosomes

This table compiling statistics for the chromosomes, based on the Sanger Institute's human genome information in the Vertebrate Genome Annotation (VEGA) database.

Number of genes is an estimate as it is in part based on gene predictions.

Total chromosome length is an estimate as well, based on the estimated size of unsequenced heterochromatin regions.
Genes

A gene is a molecular unit of heredity of a living organism.

Living beings depend on genes, as they specify all proteins and functional RNA chains.

Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring.

All organisms have many genes corresponding to various biological traits, some of which are immediately visible, such as eye color or number of limbs, and some of which are not, such as blood type, increased risk for specific diseases, or the thousands of basic biochemical processes that comprise life.
Genes

This stylistic diagram shows a gene in relation to the double helix structure of DNA and to a chromosome (right). The chromosome is X-shaped because it is dividing.

Introns are regions often found in eukaryote genes that are removed in the splicing process (after the DNA is transcribed into RNA): Only the exons encode the protein. This diagram labels a region of only 55 or so bases as a gene. In reality, most genes are hundreds of times larger.
Genome

In modern molecular biology and genetics, the genome is the entirety of an organism's hereditary information. It is encoded either in DNA or, for many types of viruses, in RNA. The genome includes both the genes and the non-coding sequences of the DNA/RNA.
Molecular Basic Steps

Each molecular assay requires three basic steps:

1. The extraction and purification of nucleic acid.

2. The amplification or making copies of the nucleic acid of interest (target) or attaching multiple copies of a dye to a single target copy.

3. The detection of the amplified target using real time polymerase chain reaction (PCR) or end product detection including microarrays, Luminex (similar to flow cytometry), or sequencing.

These three steps can be performed by three separate devices or combined into one device.
Molecular Diagnostics Methods

- Cytogenetics
- Fluorescence In Situ Hybridization (FISH)
- Derivatives of FISH
- Polymerase Chain Reaction (PCR)
- Derivatives of PCR
- Direct Sequence Analysis (DNA Sequencing)
- Southern Blot Analysis (SBA)
- Variable Number Tandem Repeats (VNTR)
- Mutation Scanning
- DNA Microarrays (Gene Expression Profiling)
- Proteomics
- Loss of Heterozygosity (LOH)
- Methylation Assays
Common Techniques in Molecular Biology

- Nucleic Acid Extraction Methods
- Resolution and Detection of Nucleic Acids
- Analysis & Characterization of Nucleic Acids & Proteins
- Nucleic Acid Amplification Target Amplification
- Chromosomal Structure and Chromosomal Mutations
- Gene Mutations types of Gene Mutations
- DNA Sequencing Direct Sequencing
The purpose of nucleic acid extraction is to release the nucleic acid from the cell for use in subsequent procedures.

Ideally, the target nucleic acid should be free of contamination with proteins, carbohydrate, lipids, or other nucleic acid that is, DNA free of RNA or RNA free of DNA.

The initial release of the cellular material is achieved by breaking the cell and nuclear membranes (cell lysis).

Lysis must take place in conditions that will not damage the nucleic acid.

Following lysis, the target material is purified and then the concentration and purity of the sample can be determined.
Isolation of DNA

• Preparing the Sample
• Organic Isolation Methods
• Inorganic Isolation Methods
• Solid-Phase Isolation
• Crude Lysis
• Isolation of Mitochondrial DNA
Isolation of RNA

- Total RNA
- Specimen Collection
- Extraction of Total RNA
- Isolation of polyA (messenger) RNA
Measurement of Nucleic acid quality and quantity

- Electrophoresis
- Spectrophotometry
- Fluorometry
- Microfluidics
Resolution and Detection of Nucleic Acids

- Electrophoresis
- Gel systems
  - Agrose Gels
  - Polyacrylamide Gels
  - Capillary Electrophoresis
- Buffer Systems
- Detection systems
Analysis and Characterization of Nucleic Acids and Proteins

- Restriction Enzyme Mapping
- Hybridization Technologies
  - Southern Blots
  - Northern Blots
  - Western Blots
- Probes
  - DNA Probes
  - RNA Probes
  - Other Nucleic Acid Probe Types
  - Protein Probes
  - Probe Labeling
- Nucleic Acid Probe Design
- Hybridization Conditions, Stringency
- Detection Systems
- Interpretation of Results
- Array-Based Hybridization
  - Dot/slot blots
  - Genomic Array Technology
- Solution Hybridization
Nucleic Acid Amplification

- Target Amplification
  - Polymerase Chain Reaction
  - Transcription-Based Amplification systems
  - Probe Amplification
  - Ligase Chain Reaction
  - Strand Displacement amplification
  - Replicase
- Signal Amplification
  - Branched DNA Amplification
  - Hybrid Capture Assays
  - Cleavage-Based Amplification
  - Cycling Probe
CPT Codes

88302 – Level II- Surgical pathology, gross and microscopic examination. (Appendix, Vas)

88312- Special stain – microorganisms

88342 – IHC stain, each antibody

88142 – thin prep pap

87621 – molecular hpv testing

88121 - ISH
Conclusion

Nucleic acids offer several characteristics that support their use for clinical purposes. Highly specific analyses can be carried out without requiring extensive physical or chemical selection of target molecules or organisms, allowing specific and rapid analysis from limiting specimens.