Current BIO301 FILE 2022 BY ASMAT KHAN NIAZI 15-03-22



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This is 1st edition so do not totally depend upon this file

I hope k ap sb final term enjoy kr rhy hongy

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1. Which techniques are used for protein?

There are three major protein analysis techniques:

- Protein separation
- Western blotting
- Protein identification

2. Common recessive disorders.

Autosomal recessive disorders include

- Cystic fibrosis (CF) Cystic fibrosis is one of the most common inherited single gene disorders in Caucasians.
- Sickle cell anemia (SC)
- Tay Sachs disease.

3. PCR (polymerase chain reaction) tests are a fast, highly accurate way **to diagnose certain infectious diseases and genetic changes**. The tests work by finding the DNA or RNA of a pathogen (disease-causing organism) or abnormal cells in a sample.

Types

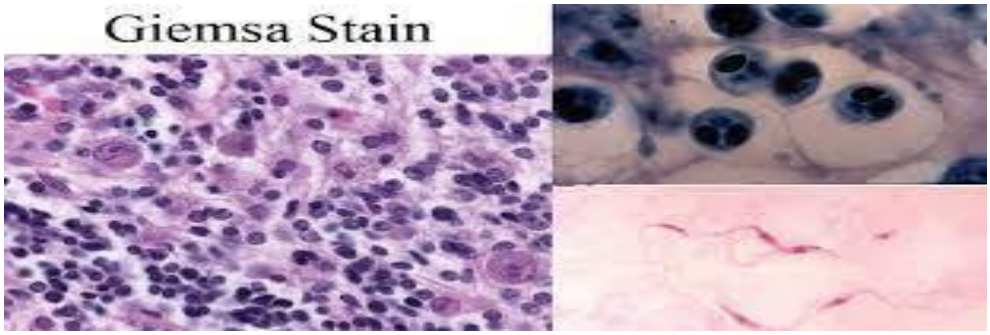
Nested PCR

- Two pairs (instead of one pair) of PCR primers are used to amplify a fragment.
- First pair amplify a fragment similar to a standard PCR.

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4. Used of giemsa staining.

Giemsa stain is a gold standard staining technique that is used for both thin and thick smears **to examine blood for malaria parasites, a routine check-up for other blood parasites and to morphologically differentiate the nuclear and cytoplasm of Erythrocytes, leucocytes and Platelets and parasites**.



5. Western blotting

A western blot is **a laboratory method used to detect specific protein molecules from among a mixture of proteins**. This mixture can include all of the proteins associated with a particular tissue or cell type.

6. Different between chromosome in dicot and monocot

Dicots in general, have a higher number of chromosomes than monocots. Chromosomes are longer in monocot than dicots.

7. Effects of mutations.

The majority of mutations have neither negative nor positive effects on the organism in which they occur.

Harmful mutations **may cause genetic disorders or cancer**. A genetic disorder is a disease caused by a mutation in one or a few genes. A human example is cystic fibrosis. A mutation in a single gene causes the body to produce thick, sticky mucus that clogs the lungs and blocks ducts in digestive organs.

8. Different between western and southern blotting.

Western blotting is the counterpart which is used to detect proteins. The difference lies in the visualization process. **In Western blotting, this is made possible by primary and secondary antibodies, whereas in Southern blotting, a radiolabeled (fluorescent) probe or dye that binds to the DNA is used**..

9. Base of analogs chromosomes.

Chemicals with structures similar to that of any of the four standard bases of DNA**.** DNA polymerases cannot distinguish these analogs. They may be incorporated into newly synthesized DNA molecules

10. Causes of non disjunction .

Nondisjunction during meiosis is the most common cause of the aneuploidies, which can also occasionally result from a chromosomal rearrangement (e.g., Robertsonian translocation or a balanced sex chromosome translocation)

11. What is spontaneous mutation and its agents

Spontaneous mutations are **"the net result of all that can go wrong with DNA during the life cycle of an organism"** (Glickman et al., 1986).

In genetics, a mutagen is **a physical or chemical agent that permanently changes genetic material, usually DNA, in an organism and thus increases the frequency of mutations above the natural background level**.

12. Different between DNA and cDNA.

The main difference between DNA and cDNA is that **DNA is composed of both coding and non-coding sequences whereas cDNA only contains the coding sequences**. The coding sequences are the exons of a gene, which codes for a functional protein. The non-coding sequences are the remaining DNA sequences of the genome.

13. Define telophase 1.

During telophase I, **the chromosomes are enclosed in nuclei**. The cell now undergoes a process called cytokinesis that divides the cytoplasm of the original cell into two daughter cells. Each daughter cell is haploid and has only one set of chromosomes, or half the total number of chromosomes of the original cell.

14. Western blotting principle.

In Western blotting (WB), **target proteins are transferred to a hydrophobic membrane after SDS-PAGE and detected using specific antibodies**. After SDS-PAGE, a membrane is placed on

the gel, to which the separated proteins in the gel are electrophoretically transferred.

15. Types of mutation

The types of mutations include:

- **Silent mutation:** Silent mutations cause a change in the sequence of bases in a DNA molecule, but do not result in a change in the amino acid sequence of a protein.
- **Missense mutation:** This type of mutation is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene .
- **Nonsense mutation:** A nonsense mutation is also a change in one DNA base pair. Instead of substituting one amino acid for another, however, the altered DNA sequence prematurely signals the cell to stop building a protein . This type of mutation results in a shortened protein that may function improperly or not at all.
- **Insertion or Deletion:** An insertion changes the number of DNA bases in a gene by adding a piece of DNA. A deletion removes a piece of DNA. Insertions or deletions may be small (one or a few base pairs within a gene) or large (an entire gene, several genes, or a large section of a chromosome). In any of these cases, the protein made by the gene may not function properly.
- **Duplication:** A duplication consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein.
- **Frameshift mutation:** This type of mutation occurs when the addition or loss of DNA bases changes a gene's reading frame. A reading frame consists of groups of 3 bases that each code for one amino acid. A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frameshift mutations.

16. Silent mutation

Silent mutations cause a change in the sequence of bases in a DNA molecule, but do not result in a change in the amino acid sequence of a protein.

17. Principle of southern blotting .

Southern blotting is based on the principle of **separation of DNA fragments by gel electrophoresis followed by the identification by labeled probe hybridization**. The DNA fragments are separated based on their size and charge during electrophoresis.

18. Different between organic and inorganic method and steps of DNA extraction . (long important)

Organic method

Properties

Yields relatively pure, high molecular weight DNA.

DNA is double stranded – good for RFLP and other molecular techniques.

Drawbacks

1. Time consuming.
2. Requires sample to the transferred to multiple tubes
3. Involves use of hazards and smelly chemical

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Non -organic method

- No use of organic reagents such as phenol or chloroform.
- Digested proteins are removed by salting out with high concentrations of LiCl or NaCl.

Salts should be removed

However, if salts are not adequately removed, problems could occur with the RFLP/PCR procedures due to alteration of DNA mobility.

procedure

Step 1: Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei.

Step 2: Resuspend nuclei in protein lysis buffer containing a high concentration of proteinase K.

Step 3: Lyse nuclear membrane and digest protein at 65 oC for 2-3 hours or 37 oC for over night.

Step 4: Temperature helps denature proteins, and digestion of proteins by Proteinase K.

Step 5: To remove proteinaceous material, LiCl or NaCl is added, and incubated on ice.

Step 6: Proteins precipitate out and are pelleted by centrifugation.

Step 7: DNA remains in solution. Transfer supernatant to a new tube, care must be taken not to take any of protein pellet to next tube.

Step 8: DNA is precipitated by the addition of room temperature isopropanol. LiCl or NaCl will not precipitate with DNA.

Step 9: Precipitated DNA is washed with 70% ethanol, dried under vacuum and re-suspended in TE buffer.

19. Properties of mitochondrial DNA.

- More than one copy of mtDNA is passed.
- Mutations are common.
- More than one type of mtDNA genome is passed

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20. Nomenclature of mutation .

Standard nomenclature based on genomic DNA reference sequence sand RNA reference sequences is not shown. “Genomic DNA reference sequence” simply indicates any human DNA sequence in the database that is not based on a cDNA sequence. Example of standard mutation nomenclature based on a coding DNA sequence.