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**SUBJECT: molecular biology**

**MODULE : MLT 4TH**

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**Q1: Fill in the blinks:**

**ANS:** 1) the three main steps PCR are denaturing, annealing and extension.

2) The word “vaccine” originates from the Latin word Variolae vaccinae .

3) Yeast is the oldest microbes exploited by humans for their benefit.

4) Restriction endonucleases are also called as Molecular scissor

5) Restrictions map is a diagram or map of DNA molecule of an organism that shows specific sites of cleavage restriction sites.

6) A forensic technique used to identify individuals based on the variations in their DNA sequences is known as DNA fingerprinting .

7) Restriction Modification system is mainly composed of restricting endonucleases and methylase enzyme

**Q2: vaccines and its types:**

**Answer :**

**(a): Vaccine:**

The word “vaccine” originates from the Latin Variolae vaccinae (cowpox), which Edward Jenner demonstrated in 1798 could prevent smallpox in humans.

Today the term ‘vaccine’ applies to all biological preparations, produced from living organisms, that enhance immunity against disease and either prevent (prophylactic vaccines) or, in some cases, treat disease (therapeutic vaccines).

**What vaccine do**:

When inactivated or weakened disease-causing microorganisms enter the body, they initiate an immune response.

This response mimics the body’s natural response to infection.

These antigens trigger the production of “antibodies” by the immune system.

Antibodies bind to corresponding antigens and induce their destruction by other immune cells.

**Types of vaccines :**

* Live
* Dead
* Subunit
* Genetically engineered

**Live attenuated vaccine :**

These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease.

To make an attenuated vaccine, the pathogen is grown in foreign host such as animals, embryonated eggs or tissue culture, under conditions that make it less virulent.

Killed vaccine

When it is unsafe to use live microorganisms to prepare vaccines, they are killed or inactivated

These are preparations of the normal (wild type) infectious pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat so that they cannot replicate at all.

Subunit vaccine

A subunit vaccine is a fragment of a pathogen, typically a surface protein , that is used to trigger an immune response and stimulate acquired immunity against the pathogen from which it is derived.

Recombinant vaccine

The vaccines are produced using recombinant DNA technology or genetic engineering.

Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.

Different strategies are: Using the engineered vector that is expressing desired antigen as a vaccine The engineered vector (e.g., yeast) is made to express the antigen, such is vector is grown and the antigen is purified and injected as a subunit vaccine.

(b): **Biotechnology and it’s scope:**

Biotechnology is the manipulation of living organisms and organic material to serve human needs. The science of using living organisms or the products of living organisms for the benefit of humans and their surroundings. Example

Yeast in bread making and alcohol production Use of beneficial bacteria (penicillin) to kill harmful organisms Cloning of plants and animals Artificial insemination.

There has been increased activity and research between different agricultural areas with common research techniques and goals Plant Science Animal Science Environmental Science Health/Agri-Medicine

Plant sciences.Improvement of varieties according to relevant agronomic features: Productivity (resistance to biotic stress: pests, viruses, pathogens, abiotic stress tolerance to drought, salinity ... herbicide tolerance. Plant-soil interaction, nutrient absorption, metabolism improvement, etc.) Nutrition improvement: vitamin enrichment, flavour enhancement.

**Animal sciences :**

Increased use of methods of in vitro fertilization and artificial insemination improve selected breed programs Transgenic (also known as recombinant DNA) is the transferal of a specific gene from one organism to another. Scientists use reproductive cloning techniques to produce multiple copies of mammals that are nearly identical copies of other animals, including transgenic animals, genetically superior animals and animals that produce high quantities of milk or have some other desirable trait.

**Environmental sciences :**

Use of biotechnology techniques in environmental science for cleaning contaminants and protecting endangered species Bioremediation-use of natural organisms to clean contaminants Immunoassay tests are used to test for the presence of contaminants in soil, water and even blood Installation of biological barriers to prevent the transfer of harmful microorganisms between production facilities Example: Tire wash channels

Health / agri-medicine

Pharming-the creation of plants and animals capable of producing medical substances The use of biological barriers to prevent the spread of harmful microorganisms that could contaminate food sources DNA analysis/paternity testing has emerged as a technique to test the genetic ancestry of animals

**Q.3:**

**Answer**: **Restrictions modification system:**

Traditionally, four types of restriction enzymes are recognized, designated I, II, III, and IV, which differ primarily in structure, cleavage site, specificity, and cofactors.

**Type I:** enzymes cleave at sites remote from a recognition site; require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction and methylate activities.

**Type II:** enzymes cleave within or at short specific distances from a recognition site; most require magnesium; single function (restriction) enzymes independent of methylase.

**Type III**:enzymes cleave at sites a short distance from a recognition site; require ATP (but do not hydrolyze it); S-adenosyl-L-methionine stimulates the reaction but is not required; it exists as part of a complex with a modification methylase.

**Type IV:** enzymes target modified DNA, e.g. methylated, hydroxymethylated and glucosyl-hydroxymethylated DNA.

Restriction-modification (R-M) systems are important components of prokaryotic defense mechanisms against invading genomes. They occur in a wide variety of unicellular organisms, including bacteria and archaea

They comprise two contrasting enzymatic activities:

* Restriction endonuclease(REase)
* Methyltransferase (MTase).

Phage (or viruses) invade all types of cells.

Bacteria are one favorite target.

Defense mechanisms have been developed by bacteria to defend themselves from these invasions.

The system they possess for this defense is the **restriction:** modification system.

This system is composed of a

1) Restriction endonuclease

2) Methylase enzyme

Each bacterial species and strain has their own combination of restriction and methylating enzymes.

Restriction enzyme - an enzyme that cuts DNA at internal phosphodiester bonds; different types exist and the most useful ones for molecular biology (Type II) are those which cleave at a specific DNA sequence

**Methylase:** an enzyme that adds a methyl group to a molecule; in restriction-modification systems of bacteria a methyl group is added to DNA at a specific site to protect the site from restriction endonuclease cleavage.

**Q.4:**

**Answer** : Different type of restricted enzymes There are six key types of enzymes in organic chemistry. They are organized according to the way they work on a molecular level.

* **Oxidoreductases**: Transfer of O and H atoms between substances involved – oxidation and reduction reactions
* **Transferase** :Transfer of a chemical group(e.g. Amino, carboxyl, methyl, phosphoryl or acyl groups) fromone substrate to another
* **Isomerase**: The rearrangement of groups within a molecule.
* **Ligases Formation**: of bonds between two molecules using energy derived from the breakdown of ATP
* **lyase enzymes**: Addition or removal of a chemical group (e.g. H2O, CO2and NH2) other than by hydrolysis to form a double bond
* 6**. Hydrolases bring about hydrolysis**: this is the breaking of chemical bonds with the addition water. Exohydrolase enzymes cut the molecules at the end of the chain, and endohydrolase enzymes do so in the middle of the chain.

**Recombinant DNA**

DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources This is DNA that has been formed artificially by combining constituents from different organisms.

Recombinant DNA technology

Using Recombinant DNA technology, we can isolate and clone single copy of a gene or a DNA segment into an indefinite number of copies, all identical. Simply defined, it is the art of cutting and pasting genes.

Major application of recombinant DNA

* DNA sequencing
* Mutation studies
* Transformation
* Genetic engineering
* Recombinant DNA libraries
* Restriction enzyme site analysis
* Polymerase chain reaction (PCR)

**Q. 5:** In the laboratory restriction enzymes (o restriction endonuclease) are used to cut DNA into Smaller fragments.

* The cuts are always made at specific nucleotide sequences
* Different restriction enzymes recognized and cut different DNA sequences
* Resonation enzymes are found in bacteria
* Bacteria use restriction enzyme to kill viruses
* Different restriction enzymes recognized and cut different DNA sequences.
* Restriction enzymes are found in bacteria
* Bacteria use restriction enzymes to kill viruses
* Like all enzymes, a restriction enzyme works by shape-to-shape matching.When it comes into contact with a DNA sequence that makes a part of the enzyme called recognition site.
* It wraps around the DNA and causes a break in both strands of the DNA molecules
* Each restriction enzyme recognizes a different and specific recognition site, or DNA sequence.
* The enzymes attack the viral DNA and break it into useless fragments.
* Restriction enzyme is a basic tool for biotechnology research
* They are used for DNA cloning and DNA fingerprinting.

THANK YOU