

## Assignment (BS-MLT 4<sup>th</sup>)

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### Q1: Fill in the Blanks.

- 1) The three main steps of PCR are Denaturation, Annealing and Extension.
- 2) The word “vaccine” originates from the Latin word Vaccinae.
- 3) Yeast is the oldest microbes exploited by humans for their benefit.
- 4) Restriction endonuclease is also called as Molecular Scissors.
- 5) Restriction 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 Finger Printing.
- 7) Restriction modification system is mainly composed of Restriction Endonuclease and Methylase Enzyme.

### Q2) explain a details restrictions modification system.

#### ***Restrictions modification system,***

Restriction-modification systems are important components of prokaryotic defense mechanisms against invading genomes, they occur in a wide variety of unicellular organisms, including bacteria and Achaea, they comprise two contrasting enzymatic activities:

- Restriction endonuclease.
- Methyltransferase.

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

Restriction endonuclease

Methylase enzyme, each bacterial species and strain has their own combination of restriction and ethylating enzymes.

#### ***Restrictions endonuclease,***

In 1968 the discovery of these enzymes marked the beginning of recombinant DNA research and sequence-specific modification of DNA molecules.

There are three classes of restriction endonuclease. Type I, type II, and type III,

#### ***Methyltransferase,***

Methyltransferase is a huge group of transfers of enzymes who make all their sub-stockings, but their structural features can be divided into several sub-classes.

Methyltransferase can also be grouped as different types utilizing different substrates in methyl transfer reactions.

### ***Restriction enzyme,***

An enzyme that binds DNA to an internal phosphodiester bond, different types exist and the most useful for molecular organisms (type II) are those based on a specific DNA sequence.

*Methylase,*

An enzyme that adds the methyl group to a molecule, in a system that modifies bacterial binding, the methyl group is added to the DNA at a specific site to protect the site from binding.

### ***Nucleases,***

Enzyme that strips the nucleic acid, nucleus who belongs to the enzymes of class, which is called hydrolysis, the nucleus has been described more by adding the 'endo' or 'Exo' in the name.

*Endonuclease,*

Break the nucleic-acid chains somewhere in the interior, rather than at the ends, of the molecule. Also Called Restriction Endonuclease,

*Exonucleases,*

*Removing* nucleotides from the ends of the molecule,

**The end...**

**Q3) Write short note on the following,**

**1/vaccines and its types,**

**2/ biotechnology and its scope,**

**Vaccines and its types,**

**What is vaccine?**

Vaccine is the substance that helps to save some diseases. Vaccine is a dead or weak version of microbes. It helps you immune system and eliminate the survival of the microbes during the infection is known as vaccine.

### ***Types of vaccine,***

Vaccines can be divided into two main types.

#### ***1. Live attenuated vaccines,***

Disease virus or germs that weakened in the laboratory, so it can not cause illness, Direct tense viruses are often used as vaccine because although weaken, we can strengthen the immune response. However, due to the remote possibility that directly viruses can cause disease, so that HIV people should talk to their healthcare providers before taking the weight of vaccine, live attenuated vaccines used in the UK schedule.

#### ***2. Inactivated vaccines***

Inactivated vaccine (or killed vaccine) is a vaccine, which contains virus particles, bacteria, or other pathogens, which are in culture and then ability to lose disease. On the contrary, the vaccine uses the pathogens that are still alive (but almost always keep in mind, i.e. weaken. Pathogens of inactivated vaccines are created in controlled and infected infectivity virulence and thus preventing infection from this vaccine, this virus is hit by the method like heat or formaldehyde.

### **A. 'Whole killed' vaccines**

These vaccines are completely killed viruses; there is no use vaccine in the UK, which is available to the bacteria. Before 2004, the bacteria of the citrus cough were coughing, cough in the vaccine of the cough, but now its place has been changed from other types.

### **B. Subunit vaccines**

Sometimes called 'cellular,

A cellular' means 'not containing any whole cells'.) Instead these kind of vaccines contain polysaccharides or proteins from the surface of bacteria or viruses. These polysaccharides or proteins are the parts that our immune system recognizes as 'foreign', and they are referred to as antigens. Even though the vaccine might only contain a few out of the thousands of proteins in a bacterium, they are enough in themselves to trigger an immune response which can protect against the disease.

There are several different types of subunit vaccine which are described below.

#### ***Toxoid vaccines***

Toxoid vaccine a vaccine made from a toxin that has been made harmless but that elicits an immune response against the toxin. are based on the toxin produced by certain bacteria (e.g. tetanus or diphtheria).

#### ***Conjugate vaccines***

The conjugate vaccine is a type of vaccine that combines a weak microbial as a strong microscope so that the immune system could get a strong reaction to the weak micro, mostly, weak microscope is a psychologist that is connected to strong protein antigen.

### **Recombinant vaccines**

Recombinant vaccine is a vaccine that is developed by Rico Bantan DNA technology. It includes DNA to enter an injection (such as a bacterial surface protein) that encourages immune response in bacterial or stars, cells in the cells and then clean it.

## **Biotechnology and its scope,**

### **What is biotechnology?**

Biotechnology is a technology that involves the use of biology. Biotechnology is mainly used in agriculture, food science and medicine. In biotechnology, organisms are used to make useful chemicals and products or to perform industrial work.

An example of biotechnology is the fermentation reaction in yeast to beer and other alcoholic beverages, Another example is the use of fermented carbon dioxide to enhance bread, biotechnology is used to genetic engineering,

The definition of biotechnology can be further divided into different areas known as red biotechnology, green biotechnology blue biotechnology and white biotechnology.

### **Scope of biotechnology,**

In biotechnology, genetic engineering itself stimulated the hopes of modified human cells to treat both therapeutic, proteins, pharmaceutical, and biological organisms, such as seeds, pesticides, engineered yeast, and genetic diseases. The field of genetic engineering remains a hot topic of discussion in today's society with the advent of gene therapy, stem cell research, cloning, and genetically modified food.

Biotechnology is a practical science and has developed biochemical's in two important fields, developmentally, molecular biology and industrially important. Scientists are now turning to biotechnology companies. This has led to the development of many biotechnology industries.

In the United States alone, more than companies are established and operating successfully, such as Biogenic, Sets, Genetics, Hybrid Tech, etc. In the world, the United States, Japan, and many European countries are leaders of biotechnology researchers who have been inspired by manufacturers.

Advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological mutations. Advances in new technologies have resulted in the production of large quantities of biochemically-specified proteins of medical importance and immense potential for the pharmaceutical industry.

Biotechnology is a broad subject in itself and extends to various branches of biology, this includes plant tissue culture, production of transgenic in animals and plants, applications in medicine as tools and treatments, creation of new enzymes and their mobilization for industrial use, development of monoclonal antibodies and control of contaminants.

**The end...**

#### **Q4) what is retraction enzyme?**

##### **Restriction enzyme,**

Restriction enzyme, also called restriction endonuclease, a protein produced by bacteria that cleaves DNA at specific sites along the molecule. In the bacterial cell, restriction enzymes cleave foreign DNA, thus eliminating infecting organisms. Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensable tools of recombinant DNA technology, each restriction enzyme recognizes a short, specific sequence of nucleotide bases (the four basic chemical subunits of the linear double-stranded DNA molecule adenine, cytosine, thymine, and guanine). These regions are called recognition sequences, or recognition sites, and are randomly distributed throughout the DNA. Different bacterial species make restriction enzymes that recognize different nucleotide sequences.

##### **Varies Types of R E,**

Four types of restriction enzymes are recognized, designated I, II, III, and IV, which differ primarily in structure, cleavage site, specificity, and cofactors. Types I and III enzymes are similar in that both restriction and Methylase activities are carried out by one large enzyme complex, in contrast to the type II system, in which the restriction enzyme is independent of its Methylase. Type II restriction enzymes also differ from types I and III in that they cleave DNA at specific sites within the recognition site; the others cleave DNA randomly, sometimes hundreds of bases from the recognition sequence. Several thousand type II restriction enzymes have been identified from a variety of bacterial species. These enzymes recognize a few hundred distinct sequences, generally four to eight bases in length. Type IV restriction enzymes cleave only methylated DNA and show weak sequence specificity.

##### **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.

For the generation of recombinant DNA molecules, we require that the DNA fragment is cloned known as the insert DNA, A vehicle DNA known as the vector, since the process of making a recombinant DNA requires the precise cutting and stitching of DNA molecules, It involves a number of molecular tools the enzymes to cut and modify the DNA, finally resulting in a rDNA molecule, the first recombinant DNA was produced by Stanley N. Cohen and Herbert Boyer in 1973, in their experiment, they combined two plasmids; pSC-101 and pSC-102 and the newly created recombined DNA were incorporated into E. coli, the pSC-101 contains the gene for tetracycline resistance, the pSC-102 contains the gene for

kanamycin resistance, the transformed bacteria after recombination, show resistance to both these antibiotics, any diverse techniques are now available in recombinant DNA technology.

### **Gene cloning steps,**

- A. Isolate DNA from organism e.g. extraction,
- B. Cut DNA with restriction enzymes.
- C. Ligate or splice each piece of DNA into a cloning vector to create a recombinant DNA molecule
- D. Transform recombinant DNA /cloning vector + DNA fragment/ into a host.

### **Applications of rDNA,**

- DNA sequencing
- Mutation studies
- Transformation
- Genetic engineering
- Recombinant DNA libraries
- Restriction enzyme site analysis
- Polymerase chain reaction PCR.

His main applications/uses of restriction enzymes are:

1. Construction of Restriction Maps
2. Construction of DNA Fingerprints
3. Recombinant DNA Technology rDNA Technology,

**The end...**

### **Q5) as student of MLT how will you use restriction endonuclease in lab?**

Restriction enzymes are found in bacteria. Bacteria use restriction enzymes to kill viruses – the enzymes attack the viral DNA and break it into useless fragments.

In the laboratory, the DNA is used to cut the small pieces of restricted banners (or banners). Deduction is always on the specific nucleotide sequence. Different banned enzymes recognized various DNA continuity and cut,

When restricted enzyme use are, binding Enzymes are a basic tool for researching biochemical's, they are used to print DNA cloning and DNA fingerprints.

DNA fingerprinting is a forensic technique used to identify individuals based on the variations in their DNA sequences.

Many methods are now available for DNA fingerprinting and the most accurate one is DNA sequencing based methods.

Restriction Enzymes also can be used to construct DNA fingerprints.

The principle of DNA fingerprinting is that the different strains or species of DNA sample will have slightly different restriction maps.

This difference in the restriction maps is because of their difference in the DNA sequences.

Scientists have identified and treated hundreds of different types of restriction, His name is placed in the name of the biographies and the generations of which he was isolate and he has been given a setting indicate that the order in which they were found. For example, *ecori* was the first restriction enzyme, which was separated from *Escherichia Coli* Strain Ry13, while *Hindu* was isolated the third-angle of *Heamophilus Influenza* strain R.D.

DNA consists of two complementary strands of nucleotides that spiral around each other in a double helix. Restriction enzymes cut through both nucleotide strands, breaking the DNA into fragments, but they don't always do this in the same way.

*SmaI* is an example of a restriction enzyme that cuts straight through the DNA strands, creating DNA fragments with a flat or blunt end.

Other restriction enzymes, like *EcoRI*, cut through the DNA strands at nucleotides that are not exactly opposite each other. This creates DNA fragments with one nucleotide strand that overhangs at the end. This overhanging nucleotide strand is called a sticky end because it can easily bond with complementary DNA fragments.

**The end...**