

## Final-Term Assignment (Spring-2020) (BS-MLT 4<sup>th</sup>)

Course Title: Molecular Biology

Instructor: Mr. Fazli Zahir Mian

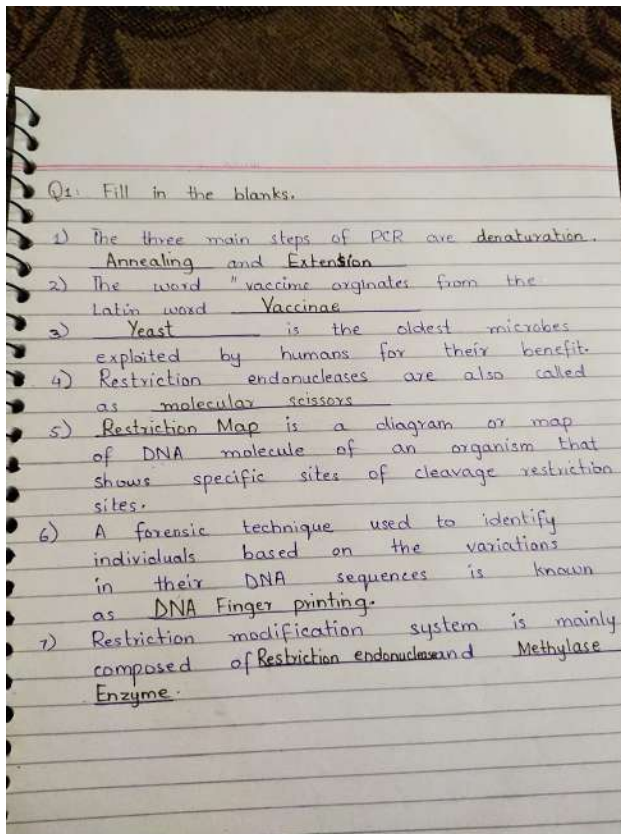
Time: 6 Hours

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### Q1: Fill in the Blanks.

- 1) The three main steps of PCR are \_\_\_\_\_, \_\_\_\_\_ and \_\_\_\_\_.
- 2) The word "vaccine" originates from the \_\_\_\_\_ Latin word \_\_\_\_\_.
- 3) \_\_\_\_\_ is the oldest microbes exploited by humans for their benefit.
- 4) Restriction endonucleases are also called as \_\_\_\_\_.
- 5) \_\_\_\_\_ 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 \_\_\_\_\_.
- 7) Restriction modification system is mainly composed of \_\_\_\_\_ and \_\_\_\_\_.



**Q2: Write short notes on the following**

1) Vaccines and its types

①

## MICRO BIOLOGY

QUESTION NO: 2

Write short note on following?

### ① Vaccines and its types:-

- 1) The word vaccine is derived from latin word Variolae vaccinae (cowpox).
- 2) The vaccine is applied to all biological preparations which are produced from living organisms.
- 3) It enhance immunity against diseases.
- 4) When microorganism enter the body they initiate an immune system.
- 5) These antigens trigger the production of "antibodies" by immune system.

### Types of Vaccines:-

There are four types of vaccines.

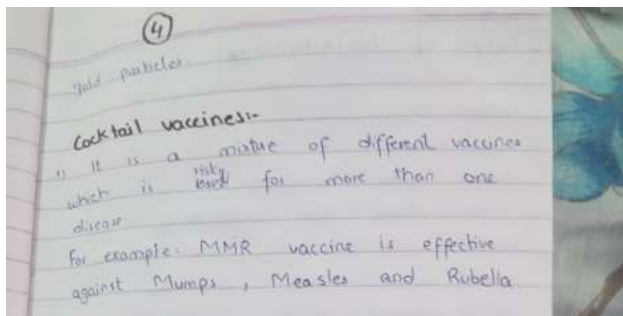
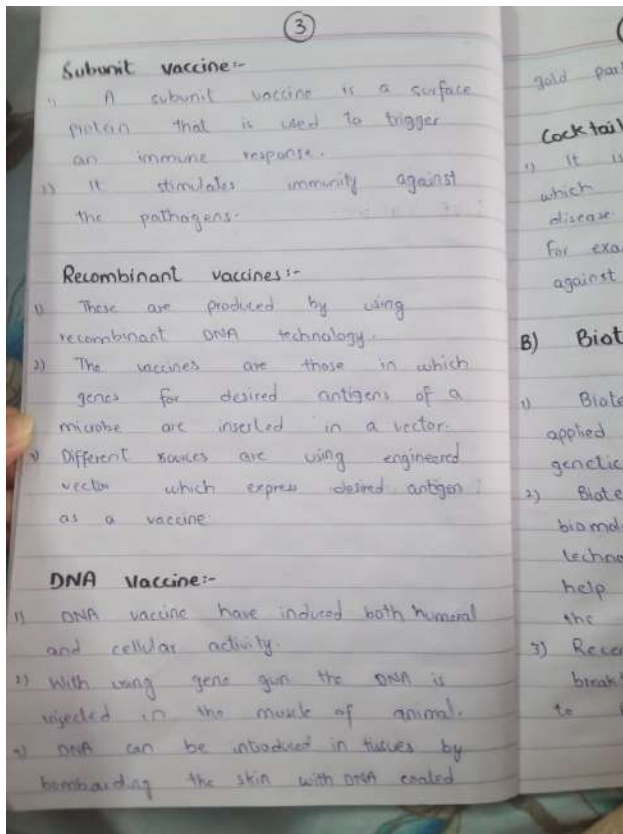
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### LIVE Vaccine:-

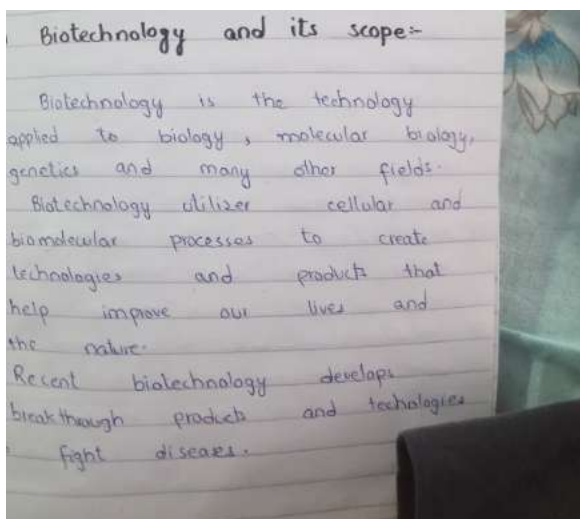
- 1) The vaccines are composed of live, microorganisms that cause an attenuated infection to induce an immune response.
- 2) To make an attenuated vaccine the pathogen is grown in foreign host such as animals, tissue culture that make it less virulent.

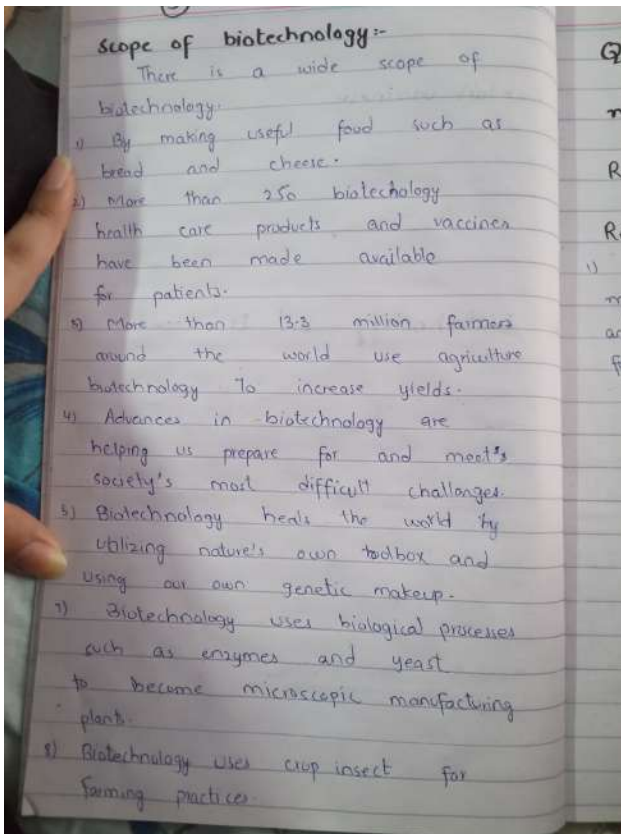
### Dead vaccines:-

- 1) They are killed when it is unsafe to use live microorganism to prepare vaccines.
- 2) These are preparations of the normal infectious pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat.

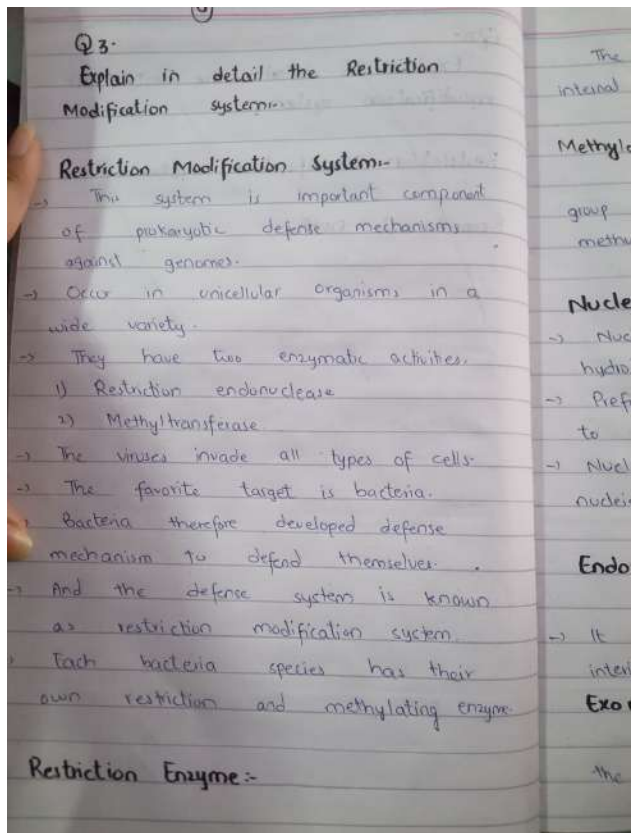


## 2) Biotechnology and its scope





**Q3: Explain in detail the Restriction modification system.**



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The enzyme that cut the DNA at internal phosphodiester bonds.

### Methylase:-

An enzyme that adds a methyl group to a molecule. In RMs a methyl group is added to DNA.

### Nucleases:-

- > Nucleases belong to a class called hydrolases.
- > Prefix 'endo' or 'exo' are added to the name.
- > Nucleases are enzymes that cleaves nucleic acid.

### Endonucleases:-

It is also called restriction endonucleases

- > It breaks the nucleic acid chains in the interior of the molecule.

### Exonucleases:-

It removes nucleotides from the end of molecule.

②

### Restriction Endonucleases:-

- > It consist of three classes: type I, type II and type III.
- > Each class has different mode of action.
- > Type II is used in recombinant DNA techniques because they recognize specific DNA sequences.
- > The sequences are called restriction sites.
- > Type I and III both have endonuclease and methylase.
- > Type I cleave DNA at random site.
- > Type III does the same at 24 to 24pp away.
- > Type II cleave DNA at specific site within recognition sequence.

### Restriction sites:-

- > They are four to eight nucleotides in length.
- > They read the same on both strands of DNA in 5' - 3' direction.

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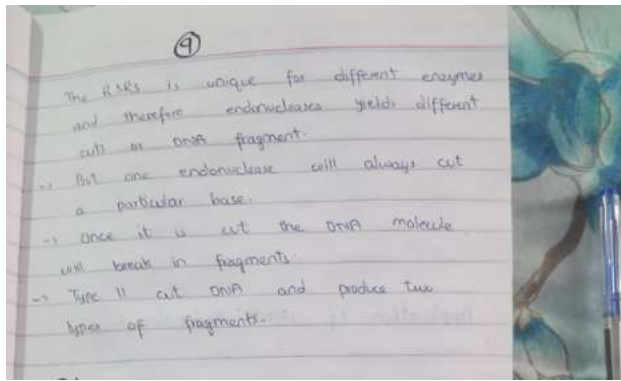
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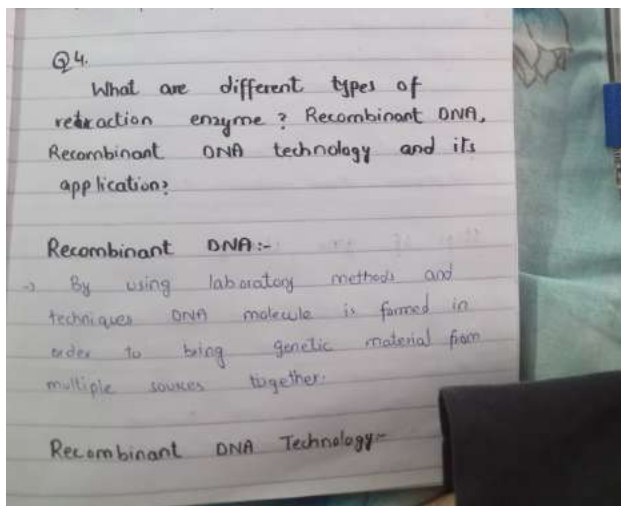
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**Q4: What are Different types of retraction enzymes? Recombinant DNA, Recombinant DNA technology and its application**



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By using recombinant DNA Technology we can clone single copy of DNA segment into diff. several number of copies.

→ These copies are all identical  
→ In simple words it is the art of cutting and pasting genes.

### Application of rDNA Technology:-

- Transformation
- DNA sequencing
- Genetic engineering
- Mutation studies
- Recombinant DNA libraries
- Polymerase chain reaction

### Steps of gene cloning:-

1. Firstly, to isolate DNA from organism
2. Then cut DNA with restriction enzymes.
3. Place pieces of DNA in a cloning vector to create rDNA molecule.
4. Then transform recombinant DNA into a host.

### Tools of

Tools

- The DNA
  - A vector
- for cutting and stitching DNA a number of molecular tools required.

Types of

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### 1. Oxidore

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reductive

### 2. Transfer

Amino

### 3. Isomerase

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### 4. Ligase

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### Tools of rDNA Technology:-

Tools are required for rDNA molecules.

- The DNA fragment is cloned
- A vector or vector DNA for cutting and stitching DNA a number of molecular tools required.

### Types of Enzymes:-

The six types of enzymes are

#### 1. Oxidoreductases:-

It transfer O and H atoms between substances involving oxidation and reduction reaction.

#### 2. Transferase:-

Transfer of chemical group i.e. Amino, carboxyl etc to another.

#### 3. Isomerase:-

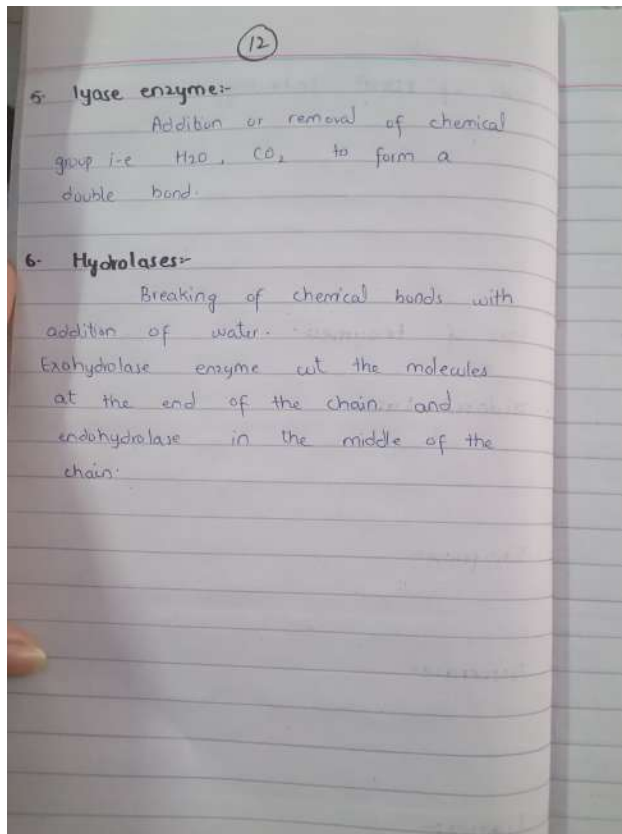
Rearrangement of groups within a molecule.

#### 4. Ligases:-

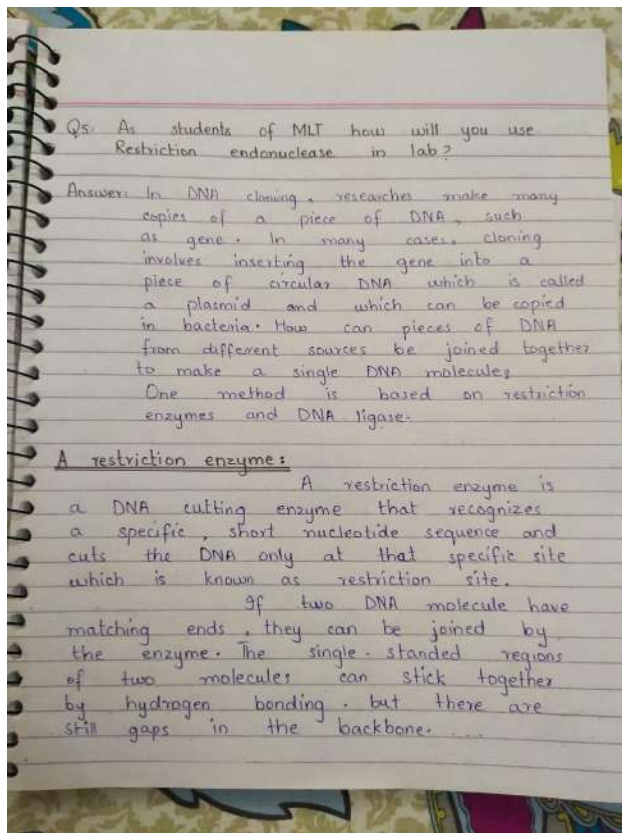
Formation of bonds between molecules using energy for breakdown of ATP.







Q5: As students of MLT how will you use Restriction endonuclease in lab?



Restriction enzymes are found in bacteria. They recognize and bind to specific sequence of DNA called restriction sites. Each restriction enzyme recognize just one or a few restriction sites. When it finds its target sequence, a restriction enzyme will make a double-strand cut in the DNA molecule. Typically, the cut is at or near the restriction site and occurs in a tidy, predictable pattern.

As an example:

how a restriction enzyme recognizes and cuts at a DNA sequence. Let's consider EcoRI, a common restriction enzyme used in labs. EcoRI cut at the following site

- When EcoRI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA.

DNA ligase:

DNA ligase is a specific type of enzyme. In DNA replication, ligase's job is to join together fragments of newly synthesized DNA to form a seamless strand.

DNA ligase seals the gap between the molecules, forming a single piece of

DNA into plasmids during DNA cloning.

The single-stranded regions of the two molecules can stick together by hydrogen bonding, but there are still gaps in the backbone.

When EcoRI recognizes and cuts this site it always does so in a very specific pattern that produces ends with single-stranded DNA "overhangs"

Not all restriction enzymes produce sticky ends. Some are "blunt cutters" which cut straight down the middle of a target sequence and leave no overhang. The restriction enzyme SmaI is an example of a blunt cutter.

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Good luck.