**Paper …molecular biology**

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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 endonucleases are 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 DNAsequences is known as \_\_\_DNA figure printing\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.
7. Restriction modification system is mainly composed of \_\_\_\_restriction endonuclease\_\_\_\_\_\_\_\_ and \_\_\_\_methylase enzyme\_\_\_\_\_\_\_\_\_\_

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***Q NO 02:***

***(A)***

***Vaccine:*** it is the substance used to stimulate the production of antibodies and provide immunity against disease, prepared from the causative agent of a disease

***Action of vaccine:*** when inactivated are weakened causing microorganisms enter the body which can initiate an immune response. This response decrease the body natural response to infection. This antigens triggers the production of antibodies by immune system, antibodies bind to corresponding antigens and induce their destruction by other immune cells.

***Types of vaccine: there are four basic types of vaccine which are given below briefly:***

1. ***Live vaccine;***

These vaccine are composed of live attenuated microorganisms that cause a limited infection on their host sufficient to induce an immune response but insufficient to cause disease.

1. ***Killed vaccine:***

Insisting of virus particle bacteria or other pathogens that have been grown in culture and then lose disease capacity. E.g. inactivated poliovirus vaccine.

1. ***Subunit vaccine:***

It is the fragment of pathogens which is typically a surface protein which used to trigger an immune response and stimulated acquired immunity against the pathogens from which it is derived.

1. ***Toxoid vaccine:***

Vaccine made from a poisons that has been made harmless but that elicits an immune response against the toxin.

***B)***

***Technology:*** *it is the technology that utilizes biological systems, living organisms or parts of this to develop or create different products****.***

***Scope of biotechnology:***

1. ***Plant science;***

*Productivity:* resistance to biotech stress viruses, pathogen, plan soil interaction, nutrient absorption metabolism improvement etc.

*Nutrition improvement:* vitamin enrichment, flavor enhancement.

*Bio factories:* biopolymers, biodegradable plastics etc.

1. ***Animal science:***

* Cattle shapes, pigs, cats, rates, have been cloned.
* A transgenic the transferal of a specific gene from one organisms to another.
* Scientist used reproductive cloning technique to produce multiple copies of mammal that are nearly identical copies of other animal.

1. ***Environmental science:***

*Bioremediation:* it is used for natural organisms to clean contaminants.

*Ammunoassaytests:* it is 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.

1. ***Health agriculture medicine:***

* Plants and animals are capable of producing medical substance.
* DNA analysis testing has emerge is a technique to test the genetic ancestry of animal.

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***Q NO.03:***

***Restriction modification system;***

Virus (or phage) invade all type of cell ***.****Bacteria are favorite target.*

Bacteria develop a defense mechanism against invasion to defend themselves.

This defense mechanism is called restriction modification system.

Restriction modification system composed of:

*1:* Restriction *endonuclease.*

*2:* Methylase *enzyme.*

***Restriction enzyme:***

That cuts DNA at internal phosphodiester bonds.

Molecular biology (type2) cleaves at a specific DNA sequence.

***Methylase:***

That adds methyl group to a molecule.in R.M.S of bacteria the methyl group is added to DNA at a specific site to protect the site from restriction endonuclease cleavage.

***Endonuclease:***

Break the nucleic acid chains in the interior. Also called restriction endonuclease.

***Exonuclease:***

Removing nucleotides from the ends of the molecule.

***Restriction endonuclease:***

Three classes of endonuclease.

*1:*type1

2:type2

3:type3.

Type2 restriction endonuclease used in rDNA techniques which recognizes specific DNA sequence.

The recognition sequence are called restriction site.

Type1 restriction endonuclease cleaves the DNA at a random site located at 1000 base pair from the recognition site.

Type3 RE does not the same at 24to 24 base pair from the recognition site.

Type2 RE they cleaves DNA at specific site within the recognition sequence*.*

***Restriction site:***

The restriction site are usually 4 to 8 nucleotides in length and are palindromic. The palindromic sequences read the same on both the strand of DNA in a 5”3”direction.

e.g.: the restriction site of EcoR1 is 5 GGATCC3 and cleaves site is between the G and A on the complementary strand.

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***Q.NO:4***

***Restriction enzyme:***

An enzyme produced by certain bacteria that has the property of cleaving DNA molecule at or near a specific sequence of bases*.*

***Types of restriction enzyme:***

***TYPE*** *1:*

***Cleavage site:***random around 1000 base pairs away from recognition site.

***Location***: endonuclease and methylase located on a single protein molecule.

***Example:*** EcoKI, CfrAI.

***TYPE2****:*

***Cleavage site****:* specific within the recognition site.

***Location:***endonuclease and methylase are separate entities.

***Example****:* EcoRI, Hind3

***TYPE3:***

***Cleavage:***random 24 to 26 base pair away from recognition site.

***Location:*** endonuclease and methylase located on a single protein molecule*.*

***Example****: Ecop1.*

***Recombinant DNA technology:***

These are DNA molecule formed by lab methods of genetic recombination to bring together genetic materials from multiple sources, creating sequences that should not otherwise be found in the genome.

***Applications:***

Recombinant DNA technology has application in Health and Nutrition.

***Medicine****:* production of insulin

***Agriculture****:* to increase their yield and improve nutritional content of plant.

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***Q NO 05:***

***Practical uses of restriction endonuclease:*** there are three main uses which has given below.

***Construction of restriction maps:***

* Take the sample of DNA in X in number of vials
* X is the number of restriction enzyme that we have
* Treat the sample DNA with the specific R.E separately
* After the restriction digestion run the DNA sample on a 2% aggarose gel with DNA marker for the separation of DNA
* Point out the size of each fragments and draw a map according to the size of fragment and the total size of the intact DNA.

***Construction of DNA finger print:*** restriction enzyme also used to construct DNA finger print. The region of DNA in an organisms that are highly variable on restriction digestion generate unique DNA finger printing for the identification of mutation or variation within in the genome of population.

***Construction:***

* In a rape case we use DNA sample from a forensic investigation.
* DNA is isolated from

1. The victim
2. The evidence such as from semen blood or hair
3. The suspect

* then the DNA sample is digested with a specific restriction enzyme
* Fragment after restriction digestion are separated on agarose gel.
* After electrophoreses the DNA fragment are transfer to a nylon membrane by southern blotting techniques
* The bound DNA is then denatured and then treated with radioactively label probes.
* The label DNA probes are hybridized specifically to the restriction fragment on the nylon membrane that are derived from the result it is clear the suspect A is the actual criminal in the investigation.

***Steps in recombinant DNA technology:***

* The first step is to extraction of DNA organisms which contain the desired gene of interest
* Next is the generation of fragment of the above DNA with the restriction enzyme
* The cloning vector can be a plasmid bacteriophage or virus
* After cloning introduce the recombine net vector into a host cell
* The product can be extracted from the medium using suitable downstream processing technique
* Then induced the express the gene of interest in the host cell to produce desired products.

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