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Molecular Biology

Final-Term Assignment (Spring-2020) (BS-MLT 4th)

Course Title: Molecular Biology **Instructor: Mr. Fazli Zahir Mian**

Q1: Fill in the Blanks.

Answers

1. **Denaturation, annealing, extension**
2. **Variolea vaccine**
3. **Yeast**
4. **Molecular scissors**
5. **Restriction map**
6. **Dna finger printing**
7. **Restriction endonuclease, methylase enzyme**

Q2: Write short notes on the following

1) Vaccines and its types

A vaccine may be a biological preparation that improves immunity to a specific disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is usually made up of weakened or killed sorts of the microbe.

The agent stimulates the body's system to acknowledge the agent as foreign, destroy it, and keep a record of it. So that the system can more easily recognize and destroy any of those microorganisms that it later encounters.

Types of vaccines:

1. Live, attenuated vaccines
2. Inactivated vaccines
3. Subunit vaccines
4. Toxoid vaccines
5. Conjugate vaccines
6. DNA vaccines
7. Recombinant vector vaccines

Biotechnology and its scope

Biotechnology is "the application of biological organisms, system or methods to developed and repair industries"(British Biotechnologist). Biotechnology is that the use of living organisms and their components in agriculture, food and other industrial processes.

Concept

The origin of biotechnology is as old as human civilization. As a science it's little quite 100 years old. Fermentation is that the oldest biotechnological process discovered by the people by prolonged soaking of grains or by sorting juices of fruits or palms. Nowadays biotechnology has achieved greatest advancement in the field of Recombinant DNA technology, PCR, Cell-culture and fusion, gene cloning, DNA fingerprinting, environmental engineering, Immunology etc.

Scope of biotechnology

Biotechnology is a multidisciplinary pursuit that has emerged as a demanding industry during the recent past. Besides being a branch of advance biological sciences, it's attracted many multinational companies including those are concerned with: the assembly of pharmaceutical products for the cure or control of many human diseases. These products include antibiotics, vaccines, lifesaving drugs and gene therapy. Improvement of clinical testing and diagnostic tools. Production of novel sorts of crop plants and animals. Production of wide range of food products, fertilizers, pesticides and beverages.

Waste treatment, bioremediation and energy production. Production of reagents including enzymes, DNA/RNA, etc. Biotechnology has also enhanced the sale of the many biological products within the world market by improving their varieties and increasing their large scale production. These items contain alcohol, antibiotics (penicillins, tetracyclines etc.), amino acids, enzymes, vaccines, steroids, vitamins, insulin, human growth hormones, microbial pesticides, acid, improved variety seeds, etc.

Q3: Explain in detail the Restriction modification system.

Restriction-modification systems

Restriction-modification (R-M) systems are significant constituents of prokaryotic protection tools against attacking 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 established by bacteria to safeguard themselves from these attacks.
- The system they have for this defense is that the restriction-modification system.
- This system is composed of
- Restriction endonuclease
- Methylase enzyme

Every bacterial species and strain has their individual grouping of restriction and methylating enzymes.

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

Methylase - an enzyme that adds a methyl to a molecule; in restriction-modification systems of bacteria a methyl is added to DNA at a selected site to guard the site from restriction endonuclease cleavage

Nucleases

• The enzyme that cleaves nucleic acids.

• Nucleases, which fit to the class of enzymes are termed hydrolases.

• Nucleases are further described by the addition of the prefix ‘endo’ or ‘exo’ to the name.

- **Endonuclease**: Break the nucleic-acid chains somewhere in the interior, rather than at the ends, of the molecule. Also Called Restriction Endonucleases
- **Exonucleases**: removing nucleotides from the ends of the molecule.

Restriction Endonucleases

• 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 endonucleases.

Type I, type II, and type III,

• Each type is characterized by a slightly different mode of action on DNA.

• Type II restriction endonucleases are used in recombinant DNA methods as they can identify definite DNA sequences and cleave at a site that comes within that sequences.

• These recognition sequences are called restriction sites.

Type I and III restriction endonucleases:

They have both endonuclease and methylase activities on one protein.

Type I REs cleave the DNA at a random site located at 1,000 base pairs from the popularity site.

Type III RE does an equivalent at 24 to 24 bp far away from the popularity sites.

Type II RE:

They cleave DNA at specific site within the popularity sequence.

Restriction Sites

• The restriction sites are typically four to eight nucleotides in length and are palindromic.

• The palindromic sequences read the same on both the strands of DNA in a 5′–3′ direction.

For example,

The restriction site of EcoRI is 5′GGATCC3′ and the cleave site is between the G and A on the complementary strands, which is demonstrated in Figure below

• The restriction-site recognition sequence for different restriction enzymes is unique and therefore different endonucleases yield different sets of cuts or DNA fragments.

• But one endonuclease will constantly cut a specific base sequence the same way, no matter what DNA molecule it is acting on.

Type II restriction enzymes cut DNA and produce two sorts of fragments.

Some restriction enzymes produce fragments with blunt ends, whereas others produce fragments with sticky (overhanging or staggered) ends.

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

A restriction endonuclease (or restriction enzyme) is an enzyme that cuts double-stranded or single stranded DNA at specific recognition nucleotide sequences referred to as restriction sites.

Restriction endonucleases are categorized into three general groups.

- Type I
- Type II
- Type III

These types are categorization based on: Their composition. Enzyme co-factor requirement. The nature of their target sequence.

TYPE I

Type I restriction enzymes were the primary to be identified and are characteristic of two different strains (K-12 and B) of E. coli. The recognition site is irregular and consists of two portions – one containing 3-4 nucleotides, and another containing 4-5 nucleotides – detached by a piece of about 6-8 nucleotides.

COFACTORS OF TYPE I

Several enzyme cofactors include: S-Adenosyl methionine. Hydrolyzed adenosine triphosphate (ATP). Magnesium (Mg^{2+}).

SUBUNITS OF TYPE I

Type I restriction enzymes own three subunits: HsdR: is necessary for restriction. HsdM: necessary for adding methyl groups to host DNA (methyltransferase activity). HsdS: significant for specificity of cut site recognition furthermore to its methyltransferase activity.

TYPE II

These are the foremost commonly available and used restriction enzymes they're composed of just one subunit. Their recognition sites are usually undivided and palindromic and 4-8 nucleotides long, they recognize and cleave DNA at an equivalent site. They do not use ATP or Ado Met for his or her activity – they typically require only Mg^{2+} as a cofactor.

CUTS OF TYPE II

Type II restriction enzymes can generate two differing types of cuts counting on whether or not they cut both strands at the middle of the popularity sequence: the previous cut will generate “blunt ends” with no nucleotide overhangs. The latter, generates “sticky” or “cohesive” ends

SUBGROUPS OF TYPE II

These subgroups are defined using a letter suffix.

Type IIB restriction enzymes.

Type IIE restriction endonucleases.

Type IIM restriction endonucleases.

Type IIT restriction enzymes

TYPE III

Type III restriction enzymes distinguish two separate non-palindromic sequences that are inversely focused on. They cut DNA about 20-30 base pairs after the attractiveness site. These enzymes encompass more than one subunit. And require Ado Met and ATP cofactors for their roles in DNA methylation and restriction

Recombinant DNA (rDNA):

Production of a singular DNA molecule by joining together two or more DNA fragments not normally related to one another. Recombinant DNA (rDNA) is a series of procedures used to recombine DNA segments. Under certain conditions, a recombinant deoxyribonucleic acid molecule can enter a cell and replicate.

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

Using recombinant deoxyribonucleic acid technology, we will 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 Applications of rDNA Tech

- Quantitative preparation of biomolecules
- Recombinant Vaccines
- Antenatal diagnosis of genetic diseases
- Monoclonal antibodies
- Cell/tissue culture
- To identify mutations in genes
- Xenotransplantation
- To detect activation of oncogenes
- Production of next generation antibiotics
- Forensics
- Biosensors
- Genetically modified crops
- Bioterrorism detection

1. Quantitative Preparation of Biomolecules

If molecules are isolated from higher organisms, the supply are going to be greatly limited. For eg. - To get 1 unit of growth hormone, more than 1000 pituitaries from cadavers are required.

2. Risk of contamination is eliminated

It is now possible to supply a biological substance with none contamination. Hepatitis, caused by HBV, is highly contagious. Preparations of vaccines or clotting factors are free from contaminants such as hepatitis B particles. RD-Technology provides the solution to supply safe antigens for vaccine production.

3. Specific probes for Diagnosis of Diseases

Diseases Specific probes are useful for:

- Antenatal diagnosis of genetic diseases. For e.g. - Many of the only gene defects like cystic fibrosis, phenyl ketonuria etc. Could be identified by taking cell samples from fetus.
- ii. To identify viral particles or bacterial DNA in suspected blood and tissue samples.

- iii. To demonstrate virus integration in transformed cells. iv. To detect activation of oncogenes in cancer. v. To pinpoint the location of a gene in a chromosome.
- iv. To identify mutations in genes.

4. Gene Therapy it's a crucial applications of RD-Technology. Normal genes might be introduced into the patient in order that genetic diseases are often cured.

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

In DNA cloning, researches make many copies of a piece of DNA, such as a gene. In many cases, cloning involves inserting the gene into a bit of circular DNA called a plasmid, which can be copied in bacteria.

How can pieces of DNA from different sources like a person's gene and a bacterial plasmid). Be joined together to form one DNA molecule One common method is predicated on restriction

Restriction Enzymes and DNA ligase

A restriction enzyme is a DNA-cutting enzyme that recognize specific sites in dna. Many restriction enzymes make staggered cuts at or near their recognition sites, producing ends with one stranded overhang

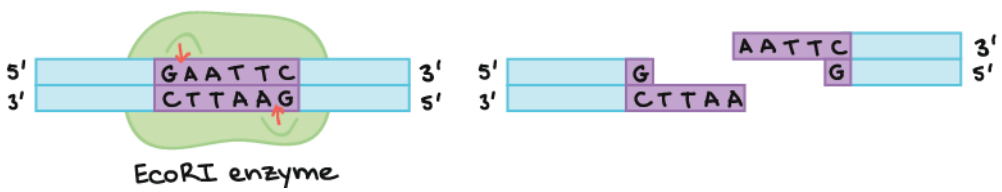
It two DNA molecules have matching ends they can be joined by the enzyme

DNA ligase

Seals the gap between the molecules, forming one piece of DNA. Restriction enzymes and DNA ligase are often wont to insert genes and other pieces of DNA into plasmids during DNA cloning

Restriction enzymes

Restriction enzymes are found in bacteria (and other prokaryotes) they recognize and bind to specific sequences of DNA called restriction sites each restriction enzyme recognize just one or a few restriction. When it finds it target sequence a restriction enzyme will make a double stranded cut in the DNA molecules. Typically, the cut is at or near the site and occurs during a tidy predictable pattern. As an example of how a restriction enzymes recognize and cuts at a DNA sequence let's consider EcoR1 a common restriction enzyme used in labs EcoR1 cuts at the following site



When EcoR1 recognize and cuts this site it always does in a very specific pattern that produce ends with single stranded DNA exchange

If another piece of DNA has matching exchange for instance because it has also been cut by EcoR1.