Biotechnology

Question Paper 2010

Maximum Marks: 80
Time allowed: Three hours

- Candidates are allowed additional 15 minutes for only reading the paper. They must NOT start writing during this time.
- Answer Question 1 (Compulsory) from Part I and five questions from Part II, choosing two questions from Section A, two questions from Section B and one question from either Section A or Section B.
- The intended marks for questions or parts of questions are given in brackets [ ].
- Transactions should be recorded in the answer book.
- All calculations should be shown clearly.
- All working, including rough work, should be done on the same page as, and adjacent to the rest of the answer.

Part-I
(Compulsory)

Question 1.
(a) Mention any one significant difference between each of the following : [5]
(i) Aldoses and ketoses
(ii) Pluripotent and multipotent cell
(iii) Chemostat and turbidostat
(iv) Codon and anticodon
(v) Androgenesis and gynogenesis.

(b) Answer the following questions : [5]
(i) What are nonsense codons
(ii) What is a microprocessor?
(iii) What is the role of oligonucleotide primer during DNA replication
(iv) Name two unique bases found in the tRNA structure.
(v) Who is regarded as the father of tissue culture technique ?

(c) Write the full form of the following: [5]
(i) NBPGR
(ii) VNTR

(iii) ARS
(iv) PEG
(v) ORF

(d) Explain briefly:
(i) Chargaff's rule of equivalence
(ii) Database
(iii) Biolistic
(iv) Coenzyme
(v) Optical activity

Answer:
(a) Difference between
(i) Aldose: Aldose has one aldehyde molecule and has a double carbon and oxygen bond.
Ketose: Ketose has one ketone molecule and has the unique double carbon to carbon bond. Ketose also has no optical properties, which is one way that is being developed to detect blood sugar concentration.

(ii) Pluripotent stem cells : These are true stem cells, with the potential to make any differentiated cell in the body.

Multipotent stem cells: These are true stem cells but can only differentiate into a limited number of types. For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells.

(in) Chemostat :A type of cell culture; a component of medium is in a growth limiting concentration; fresh medium is added at regular intervals and equal volume of culture is withdrawn.

Thrbidostat : A type of suspension culture; when culture reaches a predetermined cell density, a volume of culture is replaced by fresh medium; works well at growth rates close to the maximum.

(iv) Codon is the triplet sequence in the messenger RNA (mRNA) transcript which specifies a corresponding amino acid (or a start or stop command).

Anticodon is the corresponding triplet sequence on the transfer RNA (tRNA) which brings in the specific amino acid to the ribosome dining translation. The anticodon is complementary to the codon, that is, if the codon is AUU, then the anticodon is UAA.

(v) Androgenesis is development of an embryo from a fertilised irradiated egg, involving only the male nucleus.

Gynogenesis is development of a fertilised egg through the action of the egg nucleus, without participation of the sperm nucleus.

(b) (i) Nonsense codons : The three codons, UAA (known as ochre), UAG (amber) and UGA (opal), that do not code for an amino acid but act as signals for the termination of protein synthesis are known as nonsense codons.

(ii) Microprocessor : A microprocessor or processor is the heart of the computer and it performs all the computational tasks, calculations and data processing etc. inside the computer. Microprocessor is the brain of the computer. In the computers, the most popular type of the processor is the Intel Pentium chip and the Pentium IV is the latest chip by Intel Corporation. The microprocessors can be classified based on the following features.

Instruction Set: It is the set of the instructions that the Microprocessor can execute.

Bandwidth : The number of bits processed by the processor in a single instruction.

Clock Speed : Clock speed is measured in the MHz and it determines that how many instructions a processor can processed.

(iii) Oligonucleotides : Oligonucleotides composed of oligodeoxyribonucleotides (DNA) are often used in the polymerase chain reaction (PCR), a procedure that can amplify almost any small piece of DNA. There, the oligonucleotide is referred to as a primer, allowing DNA polymerase to extend the oligonucleotide and replicate the complementary strand.

(iv) Two unique bases are :
1. dihydrouridine(DHU)
2. pseudouridine

(v) Gottlieb Haberiandt

(c) Full forms of the followings:
(i) National Bureau Of Plant Genetic Resources
(ii) Variable Number Tandem Repeat
(iii) Agricultural Research Service

(iv) Polyethylene Glycol
(v) Open Reading Frame

(d) (i) Chargaff's rule of equivalence : Deoxyribonucleic Acid (DNA), the genetic material is made up of four types of organic nitrogenous bases : adenine (A), guanine (G), thymine (T) and cytosine (C). Of these, A and G are the purines and T and C are the pyrimidines. Chargaff gave the base pairing rule or the rule of base equivalence which states that only one purine can combine with one pyrimidine. That means A can combine with T and G with C. Two purines or two pyrimidines cannot combine with each other; if they do so, there will be a sudden change in the characteristic of an organism. This sudden change is called mutation.

(ii) Database : A database is an organized collection of data for one or more multiple uses. One way of classifying databases involves the type of content, for example: bibliographic, full-text, numeric and image.

Biological databases are libraries of life sciences information, collected from scientific experiments, published literature, high-throughput experiment technology, and computational analysis. They contain information from research areas including genomics, proteomics, metabolomics, microarray gene expression, and phylogenetics. Information contained in biological databases includes gene function, structure, localization (both cellular and chromosomal), clinical effects of mutations as well as similarities of biological sequences and structures.

(iii) Biolistic : The particle gene gun is part of a method called the biolistic (also known as bioballistic) method, and under certain conditions, DNA (or RNA) become "sticky," adhering to biologically inert particles such as metal atoms (usually tungsten or gold). By accelerating this DNA-particle complex in a partial vacuum and placing the target tissue (cells/nuclei) within the acceleration path, DNA is effectively introduced for genetic recombination.

(iv) Coenzyme : A non-proteinaceous organic substance that usually contains a vitamin or mineral and combines with a specific protein, the apoenzyme, to form an active enzyme system.

(v) Optical activity : The ability of some compounds to rotate the plane of polarized light because of the asymmetry of the molecule. If the plane of light is rotated to the right, the substance is dextrorotatory and is designated by the prefix (+); if laevorotatory (rotated to the left), the prefix is (-). A mixture of the two forms is optically inactive and is termed racemic.

Patr-II
(Answer any five Questions)

Question 2.
(a) What are lipids ? Explain the following properties of lipids: [4]
(i) Amphipathic nature
(ii) Saponification
(iii) Hydrogenation
(b) Mention the chief characteristics of embryonic stem cells. Give any two uses of such cells. [4]
(c) Write the names of any four important centres for biotechnology in India. [2]

Answer:
(a) Lipids: Lipids are a broad group of naturally-occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules. Lipids are largely hydrocarbon like, and therefore do not dissolve in water but in non-polar solvents like diethyl ether and benzene.

Properties :
Amphipathic nature : Most membrane lipids are amphipathic, having a non-polar end and a polar end. The amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment.

Saponification : The hydrolysis of triacylglycerol in the presence of sufficient sodium hydroxide is called saponification. The fatty acids are released as sodium salts and the mixture of these salts of long-chain fatty acids is soap.

Hydrogenation : Vegetable oils (liquids) are generally less expensive to produce than animal fats (solids). Using hydrogenation, chemists can break some of the carbon-carbon double bonds and replace them with hydrogen to make them chemically identical to the triacylglycerols in animal fats. Breaking one of the three double bonds in the molecule above can make the product melt above room temperature.

(b) Embryonic stem cells : are derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized in vitro. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst. The blastocyst includes three structures: the trophoblast, which is the layer of cells that surrounds the blastocoel, a hollow cavity inside the blastocyst; and the inner cell mass, which is a group of cells at one end of the blastocoel that develop into the embry o proper.

Chief characteristics of stem cells : Embry onic stem cells differ from other kinds of cells in the body. Embryonic stem cells have three general properties:

1. they are capable of dividing and renewing themselves for long periods;
2. they are unspecialized; and
3. they can give rise to specialized cell types.

Embryonic stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells-which do not normally replicate themselves- Embiyonic stem cells may replicate many times, or proliferate. Embryonic stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). Embryonic stem cells, can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

Uses of Embryonic stem cells: Studies of human embryonic stem cells will yield information about the complex events that occur during human development. A primary goal of this work is to identify how undifferentiated stem cells become the differentiated cells that form the tissues and organs.

Embryonic stem cells could also be used to test new drugs. For example, new medications could be tested for safety on differentiated cells generated from human pluripotent cell lines. Perhaps the most important potential application of human embryonic stem cells is the generation of cells and tissues that could be used for cell-based therapies.

(c) Important centres for Biotechnology in India :

- Department of Biotechnology (DBT)
- Center for Cellular & Molecular Biology (CCMB)
- Center for Biochemical Technology (CBT)
- National Institute of Immunology (Nil)

Question 3.
(a) How is DNA sequencing performed using Sangar’s dideoxy technique ? Give one limitation of this technique. [4]
(b) Write short notes on : [4]
(i) Shuttle vector
(ii) YAC
(iii) PCR

(iv) Electroporation
(c) Name any two industrial enzymes and give their uses. [2]

Answer:
(a) DNA sequencing: is the determination of the precise sequence of nucleotides in a sample of DNA.

Sanger dideoxy method : The most popular method for DNA sequencing is called the dideoxy method or Sanger method (named after its inventor, Frederick Sanger, who was awarded the 1980 Nobel prize in chemistry).

The Procedure : The DNA to be sequenced is prepared as a single strand.
This template DNA is supplied with

a mixture of all four normal (deoxy) nucleotides in ample quantities

- dATP
- dGTP
- dCTP
- dTTP

a mixture of all four dideoxynucleotides, each present in limiting quantities and each labeled with a ” tag

- that fluoresces a different color:
- ddATP
- ddGTP
- ddcTP
- ddTTP

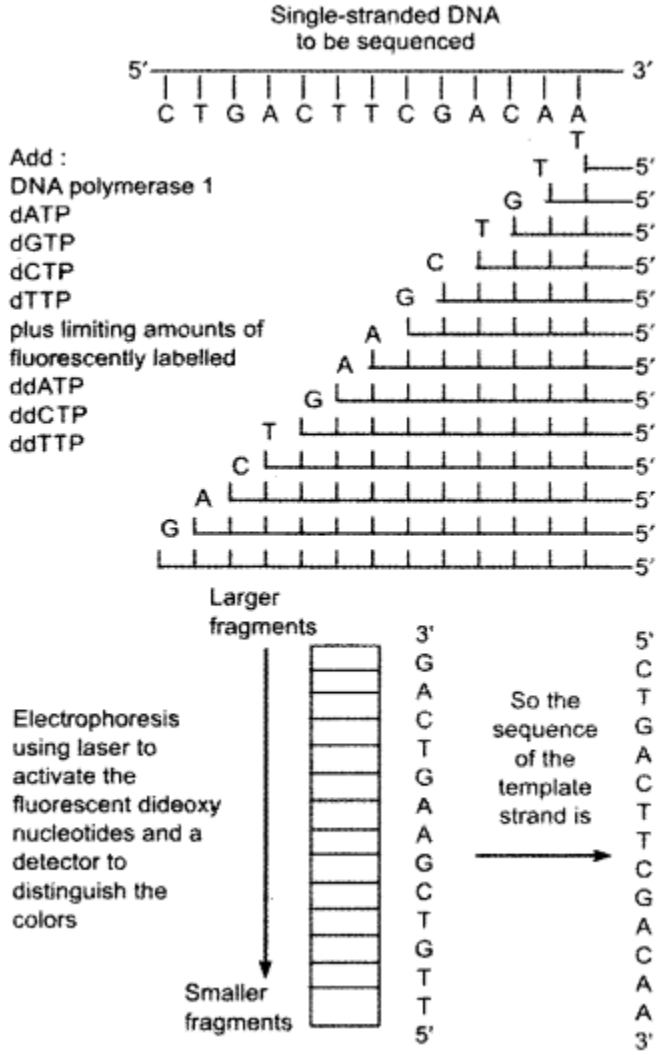
DNA polymerase I

Because all four normal nucleotides are present, chain elongation proceeds normally until, by chance, DNA polymerase inserts a dideoxy nucleotide (shown as colored letters) instead of the normal deoxynucleotide (shown as vertical lines). If the ratio of normal nucleotide to the dideoxy versions is high enough, some DNA strands will succeed in adding several hundred nucleotides before insertion of the dideoxy version halts the process.

At the end of the incubation period, the fragments are separated by length from longest to shortest. The resolution is so good that a difference of one nucleotide is enough to separate that strand from the a different color when illuminated by a laser beam and an

automatic scanner provides a next shorter and next longer strand. Each of the four dideoxynucleotides fluoresces printout of the sequence.

Limitation : Limitations include non-specific binding of the primer to the DNA, affecting accurate read-out of the DNA sequence, and DNA secondary structures affecting the fidelity of the sequence.



(b) Short notes :

Shuttle vector : A shuttle vector is a vector (usually a plasmid) constructed so that it can propagate in two different host species. Therefore, DNA inserted into a shuttle vector can be tested or manipulated in two different cell types. The main advantage of these vectors is they can be manipulated in E. coli and then used in a system which is more difficult or slower to use (e.g., yeast, other bacteria).Shuttle vectors include plasmids that can propagate in eukaryotes and prokaryotes (e.g., both Saccharomyces cerevisiae and Escherichia coli) or in different species of bacteria (e.g., both E. coli and Rhodococcus erythropolis). There are also adenovirus shuttle vectors, which can propagate in E. coli and mammals. Shuttle vectors are frequently used to quickly make

multiple copies of the gene in E. coli (amplification). They can also used for in vitro experiments and modifications (e.g., mutagenesis, PCR).

YAC : A yeast artificial chromosome (short YAC) is a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the telomeric, centromeric, and replication origin sequences needed for replication and preservation in yeast cells. Built using an initial circular plasmid, they are linearised by using restriction enzymes, and then DNA ligase can add a sequence or gene of interest within the linear molecule by the use of cohesive ends. They were first described in 1983 by Murray and Szostak.

PCR : Polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Electroporation : Electroporation, or electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA.

Electroporation is a dynamic phenomenon that depends on the local transmembrane voltage at each cell membrane point. It is generally accepted that for a given pulse duration and shape, a specific transmembrane voltage threshold exists for the manifestation of the electroporation phenomenon (from 0.5 V to 1 v). This leads to the definition of an electric field magnitude threshold for electroporation (E_{th}). That is, only the cells within areas where E E_{th} are electroporated. If a second threshold (E_{th}) is reached or surpassed, electroporation will compromise the viability of the cells, i.e.. irreversible electroporation.

(c) Two industrial enzymes with uses :
Amylases (from fungi and plants) : Production of sugars from starch, such as in making high-fructose com syrup.
Rennin (derived from the stomachs of young ruminant animals) : Manufacture of cheese, used to hydrolyze protein.

Question 4.
(a) Explain any one biochemical technique based on each of the following principles : [4]
(i) Sedimentation coefficient
(ii) Polarity
(b) What are the steps involved in the construction of an rDNA molecule for the purpose of gene cloning ? [4]
(c) How is distant hybridization technique useful in plant tissue culture ? [2]

Answer:
(a) Biochemical techniques based on
(i) Sedimentation coefficient : The sedimentation coefficient s of a particle is used to characterize its behaviour in sedimentation processes, notably centrifugation. It is defined as the ratio of a particle's sedimentation velocity to the acceleration that is applied to it (causing the sedimentation).
$s = \frac{v_t}{a}$

Sedimentation: In a sedimentation experiment, the applied force accelerates the particles to a terminal velocity v_{term} at which the applied force is exactly canceled by an opposing drag force. For small enough particles (low Reynolds number), the drag force varies linearly with the terminal velocity, i.e., $F_{drag} = fv_{term}$ (Stokes flow) where f depends only on the properties of the particle and the surrounding fluid. Similarly, the applied force generally varies linearly with some coupling constant (denoted here as q) that depends only on the properties of the particle, $F_{app} = qE_{app}$ Hence, it is generally possible to define a sedimentation coefficient $S^{def} = q/f$ that depends only on the properties of the particle and the surrounding fluid. Thus, measuring s can reveal underlying properties of the particle.

Rate zonal centrifugation : This is a type of density gradient centrifugation. The sample is applied in a thin zone at the top of the centrifuge tube. Under centrifugal force, the particles will sediment through the gradient in separate zones based on their sedimentation coefficient or'S'value.

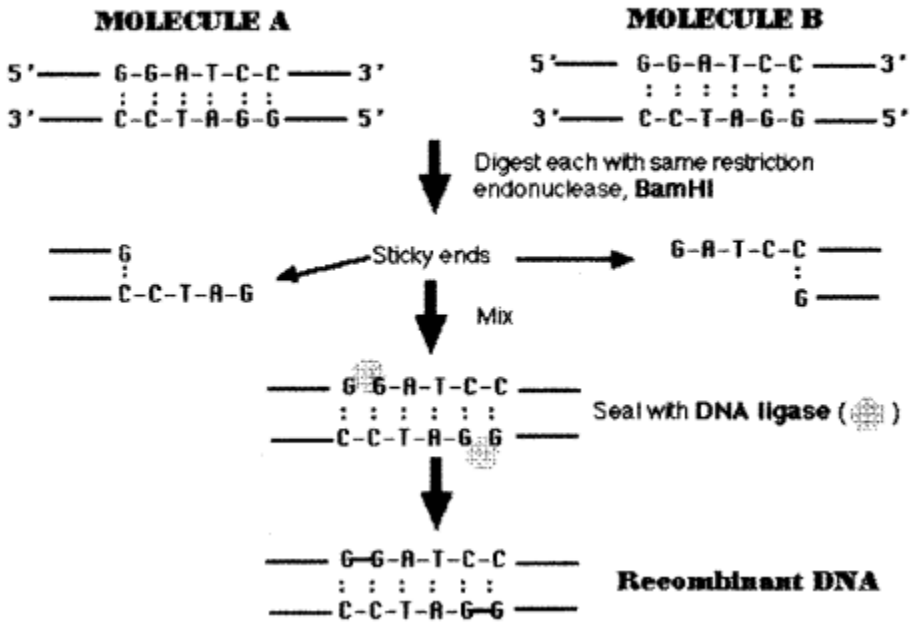
(ii) Technique used to separate biomolecules based on polarity :
Ion-exchange chromatography: It is defined as the reversible exchange of ions in solution with ions electrostatically bound to some sort of insoluble support medium. Separation is obtained since different molecules have different degree of interaction with the ion- exchanger due to difference in their charges, charge densities and distribution of charge on their surfaces. These interactions can be controlled by varying conditions such as ionic strength and PH.

An ion-exchanger consists of an insoluble matrix to which charged groups have been covalently bound. Ion exchange separations are carried out mainly in columns packed with an ion-exchanger. There are two types of ion-exchanger, namely cation and anion

exchangers. Cation exchangers possess negatively charged groups and these will attract positively charged cations. Anion exchangers have positively charged groups that will attract negatively charged anions. After the ion exchange the molecules can be eluted from the matrix by selective desorption. The selective desorption can be achieved by changes in PH and /or ionic concentration or by affinity elution, in which case an ion that has greater affinity for the exchange than has the bound ion is introduced into the system.

(b) Recombinant DNA (rDNA) : It is a form of DNA that does not exist naturally, which is created by combining DNA sequences that would not normally occur together. In terms of genetic modification, recombinant DNA is introduced through the addition of relevant DNA into an existing organismal DNA, such as the plasmids of bacteria, to code for or alter different traits for a specific purpose, such as antibiotic resistance. Recombinant DNA is DNA that has been created artificially. DNA from two or more sources is incorporated into a single recombinant molecule.

Construction of Recombinant DNA (rDNA) :



- Treat DNA from both sources with the same restriction endonuclease (BamHI in this case).
- BamHI cuts the same site on both molecules
- 5' GGATCC 3'
- 3'CCTAGG5'
- The ends of the cut have an overhanging piece of single-stranded DNA.
- These are called "sticky ends" because they are able to base pair with any DNA molecule containing the complementary sticky end.
- In this case, both DNA preparations have complementary sticky ends and thus can pair with each other when mixed.

- DNA ligase covalently links the two into a molecule of recombinant DNA.

To be useful, the recombinant molecule must be replicated many times to provide material for analysis, sequencing, etc., Producing many identical copies of the same recombinant molecule is called cloning. Cloning can be done in vitro, by a process called the polymerase chain reaction (PCR). Here, however, we shall examine how cloning is done in vivo. Cloning in vivo can be done in :

- unicellular microbes like E. coli
- unicellular Eukaryotas like yeast and
- Mammalian cells grown in tissue culture.

In every case, the recombinant DNA must be taken up by the cell in a form in which it can be replicated and expressed. This is achieved by incorporating the DNA in a vector. A number of viruses (both bacterial and of mammalian cells) can serve as vectors. But here let us examine an example of cloning using E. coli as the host and a plasmid as the vector.

(c) Distant hybridization : It implies crosses of species, subspecies, breeds and strains for the purpose of obtaining marketable hybrids of the first generation.

Importance of distant hybridization:

- Distant hybridization is a combination of valuable features of parental forms in hybrids without noticeable increase in viability and, with intermediate growth rate.
- They comprise both heterosis hybrids and hybrid forms with a favorable combination of parental features.
- Abortion of embryos at one or the other stage of development is a characteristic feature of distant hybridization, e.g., the crossing of two species or varieties often fails when using distant parents who do not share many chromosomes. Embryo rescue is the process when plant breeders rescue inherently weak, immature or hybrid embryos to prevent degeneration. Common in lily, hybridizing to create new interspecific hybrids between the various lily groups (such as Asiatic, Oriental, Trumpet, etc).

Question 5.
(a) Discuss the Cell Culture technology under the following headings [4]
(i) Different types of nutrient media used.
(ii) Sterilization of instruments and transfer area.
(b) What is a DNA library ? How is a genomic DNA library formed ? [4]
(c) Give two applications of somatic hybridization. [2]

Answer:
(a) (i) Nutrient media : It is a source of amino acids and nitrogen (e.g., beef, yeast extract). This is an undefined medium because the amino acid source contains a variety of compounds with the exact composition being unknown. Nutrient media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory culture collections. An undefined medium (also known as a basal or complex medium) is a medium that contains :

- a carbon source such as glucose for bacterial growth
- water
- various salts needed for bacterial growth

Defined media (also known as chemically defined media or synthetic media)

- all the chemicals used are known
- does not contain any yeast, animal or plant tissue

Differential medium

- some sort of indicator, typically a dye, is added, that allows for the differentiation of particular chemical reactions occurring during growth.

(ii) Sterlization of instruments : The metallic instruments (e.g., forceps, scalpels,needles,spatula etc.) are flame sterilized i.e., dipping them in 75% ethanol followed by flaming and cooling. It is called incineration.

Sterlization of transfer area : Floor and walls of culture room are first washed with detergent and then 2% sodium hypochlorite or 95% ethanol. Larger surface area is sterilized by exposure to UV light.

(b) DNA Library: DNA library is a collection of DNA fragments of one organism, each carried by a plasmid or virus and cloned in an appropriate host. A DNA probe is used to locate a specific DNA sequence in the library. A collection representing the entire genome is called a genomic (DNA) library. An assortment of DNA copies of messenger RNA produced by a cell is known as a complimentary DNA (cDNA) library.

Construction of Genomic Library: The process of subdividing genomic DNA into clonable elements and inserting them into host cells is called creating a library.

A complete library, by definition, contains the entire genomic DNA of the source organism and is called as genomic library. A genomic library is a set of cloned

fragments of genomic DNA. The process of creating a genomic library includes four steps :

In the first step the high molecular weight genomic DNA is separated and subjected to restriction enzyme digestion by using two compatible restriction enzymes.

In the second step, the fragments are then fractionated or separated by using agarose gel electrophoresis to obtain fragments of required size.

These fragments are then subjected to alkaline phosphatase treatment to remove the phosphate. In the third step, the dephosphorylated insert is ligated into vector which could be a plasmid, phage or cosmid, depending upon the interest of the researcher.

In the last step, the recombinant vector is introduced into the host by electroporation and amplified in host. In principle, all the DNA from the source organism is inserted into the host, but this is not fully possible as some DNA sequences escape the cloning procedure. Genomic library is a source of genes, and DNA sequences. A genomic library is a set of cloned fragments of genomic DNA. Prior information about the genome is not required for library construction for most organisms. In principle, the genomic DNA, after the isolation, is subjected to RE enzyme for digestion to generate inserts.

(c) Application of Somatic Hybridization:

- Somatic cell fusion appears to be the only means through which two different parental genomes can be recombined among plants that cannot reproduce sexually (asexual or sterile).
- Protoplasts of sexually sterile (haploid, triploid, and aneuploid) plants can be fused to produce fertile diploids and polyploids.
- Somatic cell fusion overcomes sexual incompatibility barriers. In some cases somatic hybrids between two incompatible plants have also found application in industry or agriculture.
- Somatic cell fusion is useful in the study of cytoplasmic genes and their activities and this information can be applied in plant-breeding experiments.

Question 6.
(a) Explain the post-transcriptional changes in mRNA and tRNA of a eukaryotic cell. [4]
(b) Classify proteins on the basis of their functions. Also give an example of each type. [4]
(c) Mention any two significant applications of bioinformatics. [2]

Answer:
(a) Post-transcriptional changes in mRN A and tRNA

RNA transcripts in eukaryotes are modified, or processed, before leaving the nucleus to yield functional mRNA.

Eukaryotic RNA transcripts can be processed in two ways:

1. Covalent alteration of both the 3' and 5' ends.
2. Removal of intervening sequences.

The pre-mRNA (primary transcript that will be processed to functional mRNA) molecule undergoes three main modifications. These modifications are 5' capping, 3' polyadenylation, and RNA splicing, which occur in the cell nucleus before the RNA is translated.

5’Capping:
Capping of the pre-mRNA involves the addition of 7-methylguanosine (m7G) to the 5' end. In order to achieve this, the terminal 5' phosphate requires removal, which is done by the aid of a phosphatase enzyme. The enzyme guanosyl transferase then catalyses the reaction which produces the diphosphate 5' end. The diphosphate 5' prime end then attacks the a phosphorus atom of a GTP molecule in order to add the guanine residue in a 5’5' triphosphate link. The enzyme S-adenosyl methionine then methylates the guanine ring at the N-7 position.

This type of cap, with just the (m7G) in position is called a cap 0 structure. The ribose of the adjacent nucleotide may also be methylated to give a cap 1. Methylation of nucleotides downstream of the RNA molecule produce cap 2, cap 3 structures and so on. In these cases the methyl groups are added to the 2' OH groups of the ribose sugar. The cap protects the 5' end of the primary RNA transcript from attack by ribonucleases that have specificity to the 3’5' phosphodiester bonds.

Cleavage and Polyadenylation :
The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 200 adenine residues to form a poly(A) tail. The cleavage and adenylation reactions occur if a polyadenylation signal sequence (5'- AAU AAA-3') is located near the 3' end of the pre-mRNA molecule, which is followed by another sequence, which is usually (5'-CA-3'). Poly(A) polymerase then adds about 200 adenine units to the new 3' end of the RNA molecule using ATP as a precursor. As the poly(A) tails is synthesised, it binds multiple copies of poly(A) binding protein, which protects the 3’end from ribonuclease digestion.

mRNA Splicing:
RNA splicing is the process by which introns, regions of RNA that do not code for protein, are removed from the pre-mRNA and the remaining exons connected to re-form

a single continuous molecule. Although most RNA splicing occurs after the complete synthesis and end-capping of the pre-mRNA, transcripts with many exons can be spliced co-transcriptionally. The splicing reaction is catalyzed by a large protein complex called the spliceosome assembled from proteins and small nuclear RNA molecules that recognize splice sites in the pre-mRNA sequence.RNA

tRNA Splicing:
tRNA splicing is another rare form of splicing that usually occurs in tRNA. The splicing reaction involves a different biochemistry than the spliceomsomal and self-splicing pathways. Ribonucleases cleave the RNA and ligases join the exons together. This form of splicing does also not require any RNA components for catalysis. In biochemistry, a ligase is an enzyme that can catalyse the joining of two molecules (ligation or glue together) by forming a new chemical bond, with concomitant hydrolysis of ATP or other similar molecules.

Most of higher eukaryotic genes coding for mRNA, tRNA and some coding for rRNA are interrupted by unrelated regions called introns. The other parts of the genes are called exons. Exons contain information that appears in the functional mRNA.
Genes for mRNA have 0 to 60 introns.
Genes for tRNA have 0 to 1 intron.

(b) Classification of proteins based on their functions
Proteins are responsible for many different functions in the living cell. Classification of proteins on the basis of functions is as follows :

Enzymes : Proteins that catalyze chemical and biochemical reactions within living cell and outside. This group of proteins probably is the biggest and most important group of the proteins. Enzymes are responsible for all metabolic reactions in the living cells. Well known and very interesting examples are : DNA and RNA-polymerases, dehydrogenases etc.

Hormones: Proteins that are responsible for the regulation of many processes in organisms. Hormones are usually quite small and can be classifies as peptides. Most

known protein hormones are : insulin, growth factor, lipotropin, prolactin etc. Many protein hormones are predecessor of peptide hormones, such as endorfme, enkephalin etc. It is possible to increase this group of proteins by adding of all protein venoms.

Transport proteins : These proteins are transporting or store some other chemical compounds and ions. Some of them are well known : cytochrome C-electron transport; haemoglobin and myoglobin – oxygen transport; albumin – fatty acid transport in the blood stream etc. It is possible to classify trans membrane protein channels as transport proteins as well.

Immunoglobulin or Antibodies : Proteins that are involved in the immune response of the organism to neutralize large foreign molecules, which can be a part of an infection. Sometimes antibodies can act as enzymes. Sometimes this group of proteins is considered as a bigger group of protective proteins with adding such proteins as lymphocyte antigen¬recognizing receptors, antiviral agents such as interferon, tumor necrosis factor (TNF). Probably the clotting of blood proteins, such as fibrin and thrombin should be classified as protective proteins as well.

Structural proteins : These proteins are maintaining structures of other biological components, like cells and tissues. Collagen, elastin, a-keratin, sklerotin, fibroin – these proteins are involved into formation of the whole organism body. Bacterial proteoglycans and virus coating proteins also belongs to this group of proteins. Currently we do not know about other functions of these proteins.

Motor proteins : These proteins can convert chemical energy into mechanical energy. Actin and myosin are responsible for muscular motion. Sometimes it is difficult to make a strict separation between structural and motion proteins.

Receptors : These proteins are responsible for signal detection and translation into other type of signals. Sometimes these proteins are active only in complex with low molecular weight compounds. Very well known member of this protein family idopsin/rhodopsin – light detecting protein. Many receptors are transmembrane proteins.

Signalling proteins: This group of proteins is involved into signalling translation process. Usually they significantly change conformation in presence of some signalling molecules. These proteins can act as enzymes. Other proteins, usually small, can interact with receptors. Classical example of this group of proteins is GTPases.

Storage proteins: These proteins contain energy, which can be released during metabolism processes in the organism. Egg ovalbumin and milk casein are such

proteins. Almost all proteins can be digested and used as a source of energy and building material by other organisms.

(c) Two significant functions of Bioinformatics :
The biological information hidden in DNA / RNA and protein sequences are processed into genes and proteins and a relationship is established between a gene and protein by bioinformatics tools.

Using bioinformatics tools such as Genmark (for bacteria) and GenScan (for eukaryotes) gene prediction is carried out in organisms. GenScan can identify introns, exons, promoter sites and polyA signals and other gene identification algorithms.

Question 7.
(a) Discuss the applications of plant tissue culture emphasizing its role in obtaining :
(i) Stress-tolerant plants
(ii) Biodegradable plastic
(b) Briefly explain the principle and application of the technique – Southern blotting similar technique for protein identification.
(c) What is DNA Micro-array technology' ?

Answer:
(a) (i) Stress-tolerant plants : Plant tissue culture develop ways of making plants better suited to site-related, environmental challenges like drought, salinity or extreme temperatures. With agriculture being relegated to marginal land and with the prospect of climate change induced drought, these traits are considered crucial ways of securing the world's food supply.

Researchers have been able to identify several plant genes that are involved with stress tolerance. Some of these genes code for antioxidants, enzymes that modify lipids in the cell membrane, stress-response transcription factors, proteins that maintain ion homeostasis, heat shock proteins, or enzymes that synthesis important stress-response compounds. Some of these factors have been used to produce transgenic plants with increased stress tolerance.

Drought tolerance : Water is crucial for all living things. Plants use water as a solvent, a transport medium, an evaporative coolant, physical support, and as a major ingredient for photosynthesis. Without sufficient water, agriculture is impossible. Therefore, drought tolerance is an extremely important agricultural trait.

One way of engineering drought tolerance is by taking genes from plants that are naturally drought tolerant and introducing them to crops. The ressurection plant (.Xerophyta viscosa), a native of dry regions of southernmost Africa, possesses a gene

for a unique protein in its cell membrane. Experiments have shown that plants given this gene are less prone to stress from drought and excess salinity.

Some genes have been found that control the production of the thin, protective cuticle found on leaves. If crops can be grown with a thickened waxy cuticle, they could be better equipped for dealing with dryness.

Salt tolerance : Irrigation has enabled the transformation of arid regions into some of the world's most productive agricultural areas. Excess salinity, however, is becoming a major problem for agriculture in dry parts of the world. In several cases, scientists have used biotechnology to develop plants with enhanced tolerance to salty conditions.

Researchers have noticed that plants with high tolerance to salt stress possess naturally high levels of a substance called glycine betaine. Further, plants with intermediate levels of salinity tolerance have intermediate levels, and plants with poor tolerance to salinity have little or none at all. Genetically modified tomatoes with enhanced glycinebetaine production have increased tolerance to salty conditions.

Another approach to engineering salt tolerance uses a protein that takes excess sodium and diverts it into a cellular compartment where it does not harm the cell. In the lab, this strategy was used to create test plants that were able to flower and produce seeds under extreme salt levels. Commercially available crops with such a modification are still several years away.

(ii) Biodegradable Plastics : PTC is currently employed in the synthesis of plastic which is biodegradable i.e., unlike other plastics; this plastic can be broken down into simpler substances by microorganisms.

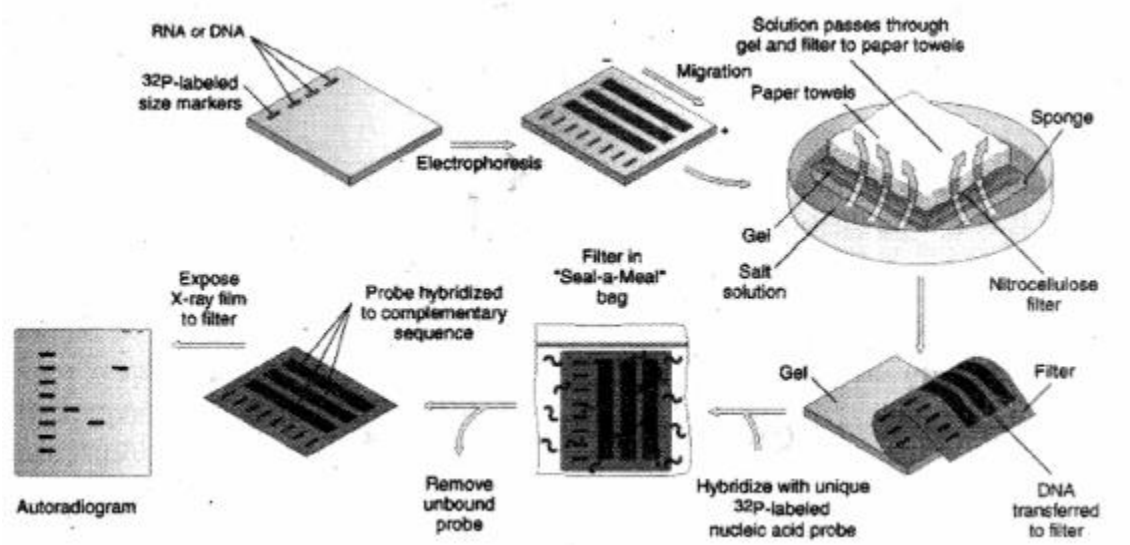
The biodegradable plastic is made from lactic acid which is produced at the time of bacterial fermentation of plant materials like discarded stalks of com. In the process, molecules of lactic Acid are chemically grouped to form the biodegradable plastic. In fact, the biodegradable plastic is a material which has most of the properties of plastic except the property of being non – biodegradable. The biodegradable plastic polyhydroxyalkanates for e.g., polyhydroxylbutyrate (PHB) are obtained commercially by fermentation with bacterium Alcaligenes eutrophus. The genetically engineered Arabidopsis plants produced polyhydroxylbutyrate (PHB) globules in their chloroplasts without effecting plant growth and development. The large scale of polyhydroxylbutyrate (PHB) can be extracted from leaves as well as from transgenic plants.

(b) Principle of Southern blotting: In this technique, DNA is usually converted into conveniently sized fragments by restriction digestion. The fragments are then separated

by migration through an agarose gel, and transferred to a nylon or nitrocellulose membrane by capillary action. The DNA of interest can be identified by hybridization to radioactive or chemiluminescent probes and visualized by autoradiography or staining.

Application of Southern blotting:

- Southern blots can easily provide a physical map of restriction sites within a gene located normally on a chromosomes.
- Southern blots reveal the number of copies of the gene in a genome.
- The degree of similarity of the gene when compared with other complementary genes. Western blotting is the technique for protein identification.



(c) DNA microarray : A DNA microarray is a multiplex technology used in molecular biology and in medicine. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing picomoles (10-12 moles) of a specific DNA sequence, known as probes. This can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (called target) under high-stringenev conditions. Probe- target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore arrays have dramatically accelerated many types of investigations.

Question 8.
(a) Give four differences between an inducible and a repressible operon. [4]
(b) Discuss the impact of the following factors on enzyme activity': [4]
(i) Substrate concentration
(ii) Temperature

(iii) Presence of a competitive inhibitor
(iv) Enzyme concentration
(c) Name any two disaccharides and give their structural units.

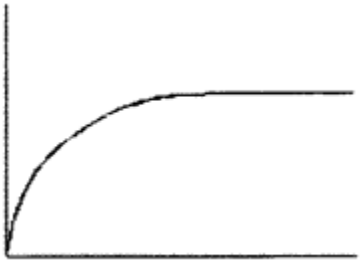
Answer:
(a) Differences between Inducible and Repressible Operon

Inducible Operon	**Repressible Operon**
1. It involves the switching on of an operon which normally remains turned off.	1. It involves turning off of an operon which normally remains switched on.
2. It is switched on in response to a new substrate which is to be handled and metabolised.	2. Repressible operon is stopped by increased formation or availability of a metabolite.
3. It is generally connected with a catabolic pathway.	3. It is mostly connected with an anabolic pathway.
4. The regulator gene of an operon produces a repressor that blocks the operator gene.	4. The regulator gene of an operon, produces a part of the repressor called aporepressor. The same cannot block the operator gene.
5. It involves the removal of the repressor of an operon by the inducer metabolite	5. It involves the blocking of the operator gene of operon through a complex repressor that is formed by union of aporepressor formed by regulator gene and corepressor which is actually a product of anabolic pathway
6. In this inducer is substrate, hormone or its by product.	6. In this repressor is a compound formed by an aporepressor and a corepressor which is commonly an end product of metabolic pathway.
7. It brings about transcription and translation.	7. Repression stops transcription and translation.

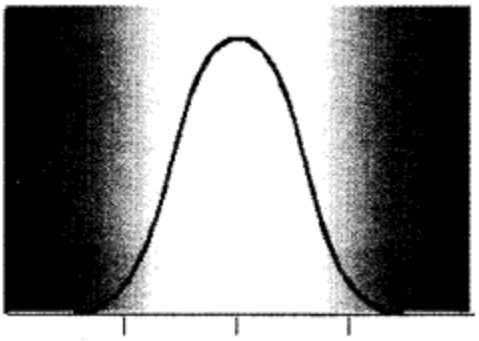
(b) Factors Affecting Enzyme Activity:
(i) Substrate concentration: It depends on the type of enzyme reaction. For a competitive enzyme reaction, the increase in substrate concentration can have an inverse effect on the activity, but for a normal enzyme reaction, as long as the substrate concentration isn't saturated, in general it will increase activity. At lower concentrations,

the active sites on most of the enzyme molecules are not filled because there is not much substrate. Higher concentrations cause more collisions between the molecules. With more molecules and collisions, enzymes are more likely to encounter molecules of reactant.

The maximum velocity' of a reaction is reached when the active sites are almost continuously filled. Increased substrate concentration after this point will not increase the rate. Reaction rate therefore increases as substrate concentration is increased but it levels off.



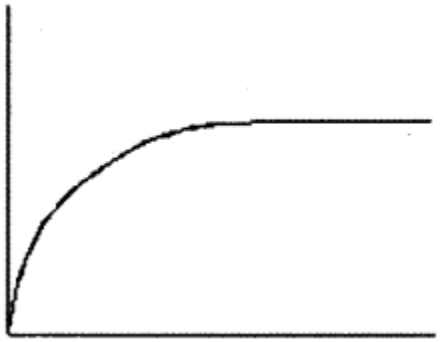
(ii) Temperature : Higher temperature generally causes more collisions among the molecules and therefore increases the rate of a reaction. More collisions increase the likelihood that substrate will collide with the active site of the enzyme, thus increasing the rate of an enzyme-catalyzed reaction. Above a certain temperature, activity begins to decline because the enzyme begins to denature. The rate of chemical reactions therefore increases with temperature but then decreases as enzymes denature.



(iii) Presence of Competitive inhibitor: A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.

(iv) Enzyme Concentration: If there is insufficient enzyme present, the reaction will not proceed as fast as it otherwise would because all of the active sites are occupied with

the reaction. Additional active sites could speed up the reaction. As the amount of enzyme is increased, the rate of reaction increases. This is so because when more enzyme molecules are present, more substrate molecules can be acted upon at the same time. This means that the total substrate molecules are broken down quickly. If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate. Reaction rate therefore increases as enzyme concentration increases but then it levels off.



(c) Disaccharides with their structural units

Disaccharide Bond	**Unit 1**	**Unit 2**	**Linkage**
Sucrose (table sugar, cane sugar)	glucose	fructose	α (1 → 2)
Lactose (milk sugar)	galactose	glucose	β (1 → 4)
Maltose	glucose	glucose	α (1 → 4)

Question 9.
(a) Explain the following methods of selection of recombinant cell : [4]
(i) Insertional inactivation
(ii) Blue-white selection
(b) Differentiate between : [4]
(i) Local and global sequence alignment
(ii) RAM and ROM
(c) Name any two protein databases and two genomic databases. [2]

Answer:
(a) (i) Insertional inactivation: Harder problem to solve is to determine w hich of the transformed colonies comprise cells that contain recombinant DNA molecules, and which contain self-ligated vector molecules. Insertional inactivation is the inactivation of a gene by inserting a fragment of DNA into the middle of its coding sequence. Any future products from the inactivated gene will not work because of the extra codes added to it. Recombinants can therefore be identified because the characteristic coded by the inactivated gene is no longer visible.

pBR322 contains genes which code for ampicillin resistance and tetracycline resistance. BamHI cuts in the middle of the gene which codes for tetracycline resistance. If a gene is inserted here, the plasmid loses it ability to code for tetracycline resistance. Thus the plasmid containing the recombinant gene is resistant to ampicillin but sensitive to tetracycline. To screen, we use replica plates.

The pUC8. plasmid is ampicillin resistant and contains a gene lac Z' which partially codes for β galactosidase. To make the plasmid capable of coding for the whole protein, we add the missing DNA along with the recombinant gene. The host which contains the plasmid pUC8 is resistant to ampicillin and is also capable of producing p galactosidase.

(ii) The blue-white screen is a molecular technique that allows for the detection of successful ligations in vector-based gene cloning. DNA of interest is ligated into a vector. The vector is then transformed into competent cell (bacteria). In this method a reporter gene lac Z is inserted in the vector. The lac Z encodes for the enzyme b-galactosidase which breaks a synthetic substrate X-gal (5-bromo-4-chloro-indolyl-b-D-galacto-pyranoside) into insoluble blue colored product. The competent cells are grown in the presence of X-gal. If the ligation was successful, the bacterial colony will be white because b- galactosidase is not synthesized due to the inactivation of lac Z; if not, the colony will be blue. This technique allows for the quick and easy detection of successful ligation, without the need to individually test each colony. An example of such a vector is the artificially reconstructed plasmid pUC19.

(b) (i) Global Vs local alignments : Illustration of global and local alignments demonstrating the 'gappy' quality of global alignments that can occur if sequences are insufficiently similar

```
Global  FTFTALILLAVAV
        F--TAL-LLA-AV

Local   FTFTALILL-AVAV
        --FTAL-LLAAV--
```

Global alignments, which attempt to align every residue in every sequence, are most useful when the sequences in the query7 set are similar and of roughly equal size (This . does not mean global alignments cannot end in gaps). A general global alignment technique is the Needleman-Wunsch algorithm, which is based on dynamic programming. Local alignments are more useful for dissimilar sequences that are suspected to contain regions of similarity or similar sequence motifs within their larger sequence context. The Smith-Waterman algorithm is a general local alignment method also based on dynamic programming. With sufficiently similar sequences, there is no difference between local and global alignments.

(ii) ROM :

- ROM is a Read only Memory.
- The contents of Rom can only be read.
- One cannot modify or write the contents of ROM.
- It is transistor based.
- It is non volatile.
- It is costly.
- Information stored in ROM is very important for computer system to work properly.

RAM :

- RAM stands for Random Access Memory.
- It is a read/write memory.
- It is capacitive based.
- It is volatile.
- It is cheaper than ROM.
- Information stored in RAM is mainly user based and has little or no link with the internal working of computer.

(c) Protein Databases :

UniProt	The Universal Protein Resource for protein sequences and is the central hub for the collection of functional information on proteins, with accurate, consistent, and rich annotation, the amino acid sequence, protein name or description, taxonomic data and citation information.
PANDIT	PANDIT – Protein and Associated Nucleotide Domains with Inferred Trees. PANDIT is a collection of multiple sequence alignments and phylogenetic trees covering many common protein domains.

Genome Databases :
Saccharomyces Genome Database (SGD) : Is a scientific database of the molecular biolog) and genetics of the yeast Saccharomyces cerevisiae, which is commonly known as baker s or budding yeast.

The Integrated Microbial Genomes (IMG): Serves as a community resource for comparative analysis and annotation of all publicly available genomes from three domains of bioinformatics