Biyani's Think Tank

Concept based notes

Biotechnology

Class -XII

Science Department
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Biyani's
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Sector-3, Vidhyadhar Nagar,
Jaipur-302 023 (Rajasthan)
Ph : 0141-2338371, 2338591-95 Fax : 0141-2338007
E-mail : acad@biyanicolleges.org
Website :www.gurukpo.com; www.biyanicolleges.org

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Author

Protein structure & engineering

Q.1 What is the consequence if a protein is incorrectly folded? Explain giving example?

Ans. Proteins are the polymers of amino acids and find expression and product of a gene. Any deformity in gene protein results in deformity of proteins and finally to the host metabolism resulting into a disease. Proteins that are folded incorrectly cause diseases like mad cow disease. These proteins are c/a priors. These proteins can cause normal proteins to turn diseased result to into alteration of protein structure.

Q.2 Distinguish b/w chymotrypsinogen and chymotrypsis?

Ans. Chymotrypsin is on enzyme responsible for hydrolysis of protein peptide found in pancreas. These protealytic enzymes are generated and folded into structures which are functionally inactive called zymogens. These are then activated when required only in the duodenum.

Chymotrypsiongen distinguish from chymotrypsim in that chymotrypoinogen is inactive form while the chymotrupsin is active form the enzyme. Chymotrypsis is made up of a linear chain of 245 amino acid residues interrupted into 3 peptides A, B, C. The protein folds forming a slobular structure. The correctly folded structure consist of amino acid residues his 57, asp 102 and ser 195 close together in space in a definite sequence which forms a charge relay system, asp form H-bond with adjacent his. His attract from ser by –on group finally a negative charge is produced over serine. Serine in normal comformation does not possess any negative charge. The significance of this charge or Serine to the enzyme is in peptide bond hydrolysis where it acts as a nucleophile and

attack on carbonyl carbon of peptide bond of the substrate so that water can hydrolysis the bond.

The specificity site of chymotrypsis is lined by hydropholic residues which allow only bulky aromatic, hydropholic amino acid residues to bind. Which in chymotrypsinogen. The substrate binding site is blocked and so the enzyme is inactive. During activation chymotrypsis unclergo a photolytic at exposing the specific site.

Q.3 What is IEF? Describe the principle techniques?

Ans Soft Copy.

Q.4 What is proteomics? Describe various types of proteome.

Ans **Definition**:-

The large scale characterization of the entire protein complement of cells, tissues and organisms is called as proteomics.

It mainly deeds with the studies of protein – proteome interaction, protein function and protein localization

Types of Proteomics:-

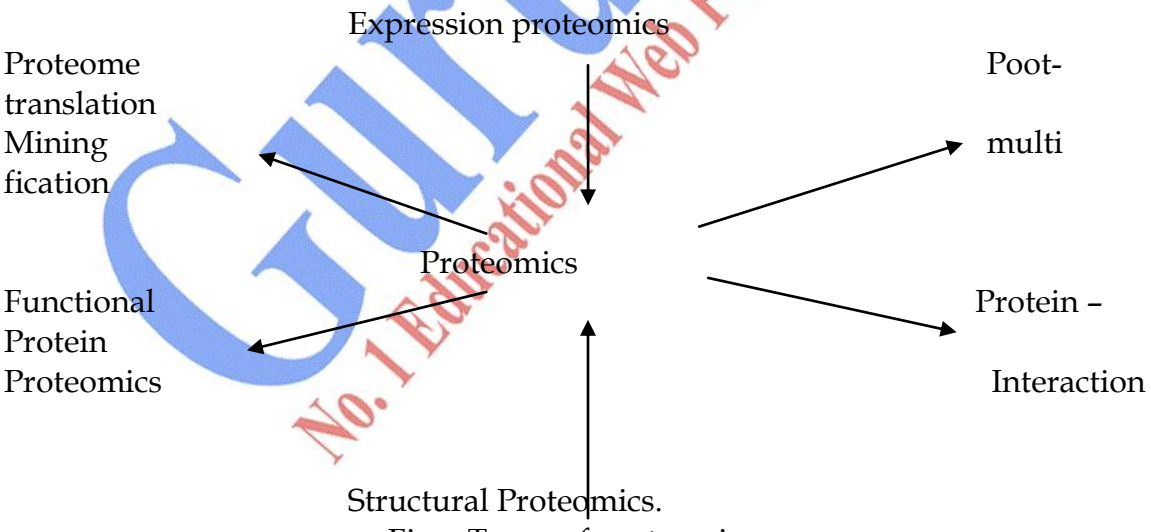


Fig: - Types of proteomics

Proteomics have been divided into three main type:-

Expression Proteomics:-

Definition:-

The quantitative study of protein expression between samples that differ by some variable is k/a expression proteomics.
By the help of expression proteomics protein expression of the entire proteome or of subfroteomes between samples can be prepared. This can further be used for identification of disease specific proteins.
e.g. Tumor samples from a cancer patient and a similar tissue sample from a normal individual could be analyzed for differential protein expressions. By making the use of advanced techniques like PAGE, Mass spectrometry, micro-array techniques, expression of proteins low / high or normal can be identified in a normal / cancer patient. This could help in understanding the development of cancer in an individual.

(II) **Structural Proteomics**:-
Definition:- This type of proteomics deals with the structure and nature of protein complexes present in a and characterization of all protein-protein interactions occurring b/w proteins present in these cells.
The advantage of the s. proteomics is in identification on of cell architecture and characteristics which can be accomplished by isolating specific sub-cellular organelles or by purifying proteins.

(III) **Functional Proteomics**:-
Definition:- use of proteomics to analyze the functional properties of molecular networks involved in a living cell.
The main objective of functional proteomics is to identify molecules that participate in these networks.
Functional proteomics has been applied for identification and analysis of protein networks involved in the nuclear pore – complex. This further can be used to translate important molecules from the cytoplasm of a cell to the nucleus and vice versa.

Q.5 Describe the use of designing a protein for any product?

Ans. Bimolecular are effective under certain environmental or physiological condition, exposure to any extremity of any such factor (like temp., ph,

chemicals etc. may result in either their inactivation or destruction. To stabilize biomoleculs like protein to make them more effective and useful in their characteristic function their clesiging in done through genetic engineering by making certain essential modifications.
One such example of protein designing includes:-

Subtilisin:-
It is a protease produced by bacteria that has a broad range for proteins that commonly soil clothing. Its enzymatic activity is the result of 3 amino acids namely ser 221, His 64 and Asps similar to chmotrtypsin.
It represents largest industrial market for any enzyme
To improve efficiency of laundry detergents, detergent manufacture supplement subtilisin in their products,
But native subtilisin is easily inactivated by bleach (up to 90 %).
A small synthetic DNA C/e oligonucletide is synthesized chemically such that its sequence is complementary to the nucleotide sequence of the region of the gene to be mutated. This oligonucleotide also incorporates the desired mutation in its sequence. This mutagenic oligonucletide is allowed to anneal to the single – stranded target gene and oct as a primer to synthesize the complementary DNA strand in-Vitro. The duplex DNA molecule thus obtained will contain wild type parent strand $ the mutant sequence in the newly synthesized DNA strand. This duplex DNA molecule is then introduced into bacterial cells by transformation. Further to screen the mutants plasmid DNA is isolated sequenced.

Q.6 What is genomics? Describe its various types?

Ans. **Genomics**:-
The term genomics was coined in 1986 by Thomas Roderick to describe the scientific discipline of mapping, sequencing and analyzing genomes.
Genomics is the branch of biotech that deals with the complete study of genome.

Types of genomics:-
There are two main types of genomics:-

(1) **Structural genomics**:-

This type of genomics mainly deals with high through put DNA sequencing followed by assembly organization and management of DNA sequences. It represents initial phase of genome analysis which involves

the construction of high-resolution genetic, physical, or transcripts map of the organize.

(2) **Functional genomics**:-

It represents the functional aspect of genome analysis and deals with the reconstruction of the genome to determine the biological function of genes and gene interactions.

Q.7 What are the IUPAC codes for (i) 'G' or 'c' (ii) A or T (iii) A or C (IV) C or T

(v) A or G

	IUPAC code
Ans. (1) Gorc	- 'S'
(2) A or T	- 'W'
(3) A or C	- 'M'
(4) C or T	- M
(5) A or G	- 'R'

Q.8 What are the single letter IUPAC codes for alaninly glycogen, tryptophan, tyrosine, serine, methioxine?

Ans. Single letter codes for the given amino acids Are :-

Amino acids	IUPAC Codes
Alanine	A
Glycine	G
Tryptophan	W
Tyrosine	Y
Serine	S
Methionine	M

Q.9 Name some of the database retrieval tools. What is their purpose?

Ans. The database retrieval tools include ENTREZ, TAXONOMY BROWSER, LOCUS LINK etc.

Their main objective is the retrieval of data which has been assigned under the respective name of database:-

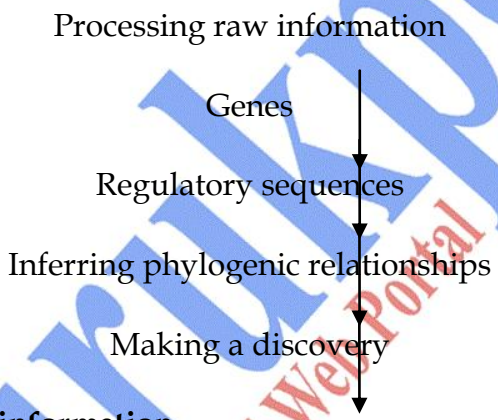
ENTREZ: - It is an integrated database retrieval system and used to access comprehensive information. On a given biological question.

TAXONOMY: - It provides information on taxonomic classification of various species. This database consist of information on over 79,000 organisms.

Locus LIMK – It is used to access information on homologous genes.

Suggest one possible way for going about analyzing a given sequence using bioin farmatics?

Ans. For analyzing a given sequence using biois formatting tools can be done as follow:-



Processing raw information

The experimentally determined sequence (raw information) is processed using bioinformatics tools into genes, the proteins encoded and their function the regulatory sequences and infringe phylogenetic relationships.

Genes:-

Gene prediction can be done by using computer programs like Gene mark for bacterial genomes and GENSCAN for eukaryotes:-

Proteins:-

Protein sequence can be inferred from the predicted genes by using simple computer programs.

Regulatory Sequences:-

These can also be identified and analyses lay using bioinformatics tools.

Inferring phylogenetic relationships.
Information regarding the relationships between organisms can be obtained by aligning multiple sequences, calculating evolutionary distance and constructing Physiogenetic trees.

Making a discovery:-
Using the bioinformcs tools and database, the functions of unknown gene can be predicted.

Q.7 What are the essential requirements for the growth of microbial culture?

Ans. The essential requirements for the growth of microbial culture includes the following?

1. **Carbon Source**:-
   It includes cereal grains, starch, cane molasses, glucose, sucrose, lactose and whey which act as a good carbon source.
2. **Nitrogen sources**:-
   Ammonium salts, urea, corn steep liquor slaughter house wastes are used as nitrogen source.
3. **Trace dements**:-
   Includes elements required in less quantity:-
   Fe, W, Zn, Mn, Mo
4. **Growth factors**:-
   Some micro organisms cannot synthesize specific nutrients like amino acids or vitamins.
5. **Antifoams** :-
   Foaming is one of the problems with microbiocultures which is formed as a result of computer of medium. To overcome this problem antifoams like fatty acids (olive oil/ sun flower oil), silicones are used.
6. **Energy sources** :-
   The carbon sources duch as carbohydrate lipids and proteins in the culture medium are major sources which provide energy for the growth of microbes.
7. **Water**

Major component of a culture media. Single or double distilled water is adequate – for large- scale microbial cultures in industry PH and dissolved salts of the water are need to be considered.

Q.8 What are the different types of microbial culture?

Ans. There are three major types of microbial cultures:-

- Batch Culture
- Fed- batch culture
- Continuous culture

1. **Batch Culture**

   It is a closed culture system, which contains limited amount of nutrients.

   After the medium is insulated with bacterial inoculums, the organism grows and shows normal growth phases – lag, log (exponential). Stationary and decline.

   Finally the growth results in the consumption of nutrients and growing cells are exposed to a changing environment continuously due to accumulation of metabolites and nutrient consumption. When microbial growth limits due to exhaustion of nutrients, one batch is complete.

2. **Fed-batch culture**:-

   If a batch culture is continuously or sequentially fact with fresh medium without removing the growing culture, the culture is c/a Fed-batch culture this type of culture is preferred when high substrate concentration causes growth inhibition. To avoid this substrate is fect at concentrations below toxic level to achieve cell growth.

   It is an ideal process for maximum production of intracellular metabolites from the same volume of the reactor.

3. **Continuous culture**:-

   Cultures used for getting continuous define of microbial growth and or products.

   Such cultures work on the principle of limiting an essential component of medium to control microbial growth.

   They are again divided into 2 subs – types:-

1. **Chemostat**:-
   In chemostat a particular nutrient essential for microbial growth is added in a les or limited amount compared to other company when growth of microbes takes place, consumption of nutrients increases but the grow is controlled due to the limiting nutrient.
2. **Turbid stat**:-
   Instead of limiting a nutrient as in chemostat in turbidostat cell concentration is maintained by fixing turbidity of cell cultural.
   So, in a continuous culture at steady state, the cell growth and substrate consumption takes place at fixed rate,

Q.9 Describe any two methods for strain preservation?

Ans. For the preservation of useful strains certain methods have been developed which includes:-

1. **Storage on agar**:-
   Cultures in this process are grown on agar slants or stabs and stored at 5 to – 20^0c. These must be sub-cultured at every 6 month intervals.
   The time of sub-culture can be extended to 1 year if cultures are covered with sterile mineral oil.
2. **Storage in liquid nitrogen**:-

   The culture is grown and a cryoprotective agent like glycerol (10-30%) is added. These are despised into sealed ampoules and frozen in liquid Nitrogen. (-176 to – 196^0c)

Q.10 Describe the growth finetics of microbes during microbial culture?

Ans. A microbial culture undergoing balanced growth is similar to a chemical reaction where substrate is getting converted into product that is cell mass in this case instead of a chemical component.
Microbial growth behaves like a auto- catalytic reaction and the rate of growth will be proportional to the cell mass present at that time. During this period the rate of increase in cell mass with time dx/dt is equivdent to the product of specific growth rate and the biomass concentration:

$Dx/dt = uX$

Where x is the cell concentration in gm/h
T is time in hour
ll " specific growth rate in hour
In an microbial culture environment the index of rate of growth will be:-

$$\left\{\frac{dx}{dt}\right\}$$

Specific growth rate can be calculated by plotting dx/dt Vs x and determining the slope of the straight line, For determining concentration of cell mass Xo at the beginning of the exponential growth, the time required for doubling the biomass could be calculated by using eqn – 1

$1n\ x/x_o = ut$, assume x_o double of x

Thus, $\ln 2 = ut$

So $t = 0.639 / ll$ thus, $t = 1n\ 2/ll$ - 2

Where is the doubling time of the culture? By calculating and substituting it in equation 2 we can calculate the doubling time of the culture. This shows that the doubling time and specific growth rates are inversely related. Higher the doubling time, lower will be the specific growth rate and vice versa. The microbial culture having high specific growth rate have low doubling times.
Using equation 1 and counting the cell number at different time points, the specific growth rate of microbial culture can be calculated as
$\ln x / x_o = llt$ or $\ln x - x_o = ll\ (t - to)$ converting natural log to log to the base 10

$$\text{Log}_{10} x - \text{Log}_{10} x_o = ll / (2.303)\ (t - to)$$

Where x and x_o are concentration of bacteria cells at time t and to

Q.11 Define the following?
1. Plasmid 2. Restriction 3. Transformation 4. Mutation 5. YAC 6. BAC 7. Cosmids 8. DNA probes
9. Transfection

Ans

1. **Plasmids**: - Plasmids are extra chromosomal, self – replicating circular, double stranded DNA molecules, which are found naturally in many bacteria and also in some yeast.

They are not essential for cell survival but rather import some characteristic features to cell in which found like- antibiotic resistance, virulence.
PBR322, PUC family etc.

2. **Restriction site**: - it is a specific DNA sequence recognized by a restriction enzyme uses it to induce cut at this particular sequence only.
   The restriction site of a enzyme varies with particular type of DNA found in a organism i-e. Prokaryotic or eukaryotic and is also specific for particular enzyme.

3. **Transformation**:- It refers to the process wherein a cell take up DNA from the surrounding environment and is said to be transformed when this DNA adjoin the DNA of the cell and function and replicate within the host cell resulting into certain changes in that particular cell genome.

4. **Mutation**: - It is an alteration in a base of a DNA sequence which is heritable to further generations.
   It may be beneficial or lethal and may result into a defective protein or premature termination of non-functional protein.

5. **YAC**: - Yeast artificial chromosome it is a type of vector which is used as to clone DNA fragments of more than Mb in size. These consist of telemetric sequence, centromere. And autonomously replicating sequence from yeast chromosome.

6. **BAC**: - Bacterial Artificial chromosomes these are vectors based on the natural, extra chromosomal plasmid of E.coli – the fertility or F- plasmid. It consist of genes or replication and maintenance of the F-factor a selectable marker coning sites.
   These vectors can accommodate up to 300-350 kb of foreign DNA.

7. **Cosmids** :- 'cos + plasmid'

Vectors which are formed by combining certain features of plasmid and 'cos' sites of phage lambda. It consist of an ori, a selectable marker, suitable restriction enzyme sites and the lambda 'cos' site used to clone fragments up to 45kb in length.

8. **DNA probe**: - It is a relatively small sequence of DNA that recognizes and blinds to into complementary sequence.

9. **Transfect ion**:- It is a process of introducing foreign DNA into cultured cells. Generally the term used for transformation in animals.

Q.12 Explain 'Intentional Inactivation'?

Ans. After transformation to select transformants certain methods have been developed one such method is called as intentional inactivation. For intentional inactivation generally the procedure that is followed involves antibiotic resistance e.g. E. coli plasmid pBR322 consist of two resistance genes for and tetracycline. If the target DNA fragment is inserted into a restriction enzyme site located in one of these two resistance genes, the one to which it is inserted say ampicillin resistance gene then the respective gene is inactivated. Thus, the host cells which sensitive to ampicillin and resistant to tetracycline. 50 they will be able to grow on a tetracycline containing medium, but not on a medium containing ampicillin while self – ligated or non-recombinants will be resistant to both amplicillin and tetracycline and thus be able to grow on a medium containing both antibiotics.

Q.13 Describe 'Site-directed mutageneses?

Ans. A mutation is an alternation in a base of a DNA sequence. These mutation are generally harmful but if can be used in a way to produce beneficial effects. One such application of mutation is site directed mutagenesis.

In site – directed mutagenesis modification is done at a particular point i.e. introducing point mutation at specific base which is pre- selected to increase beneficial characters of proteins like stability.

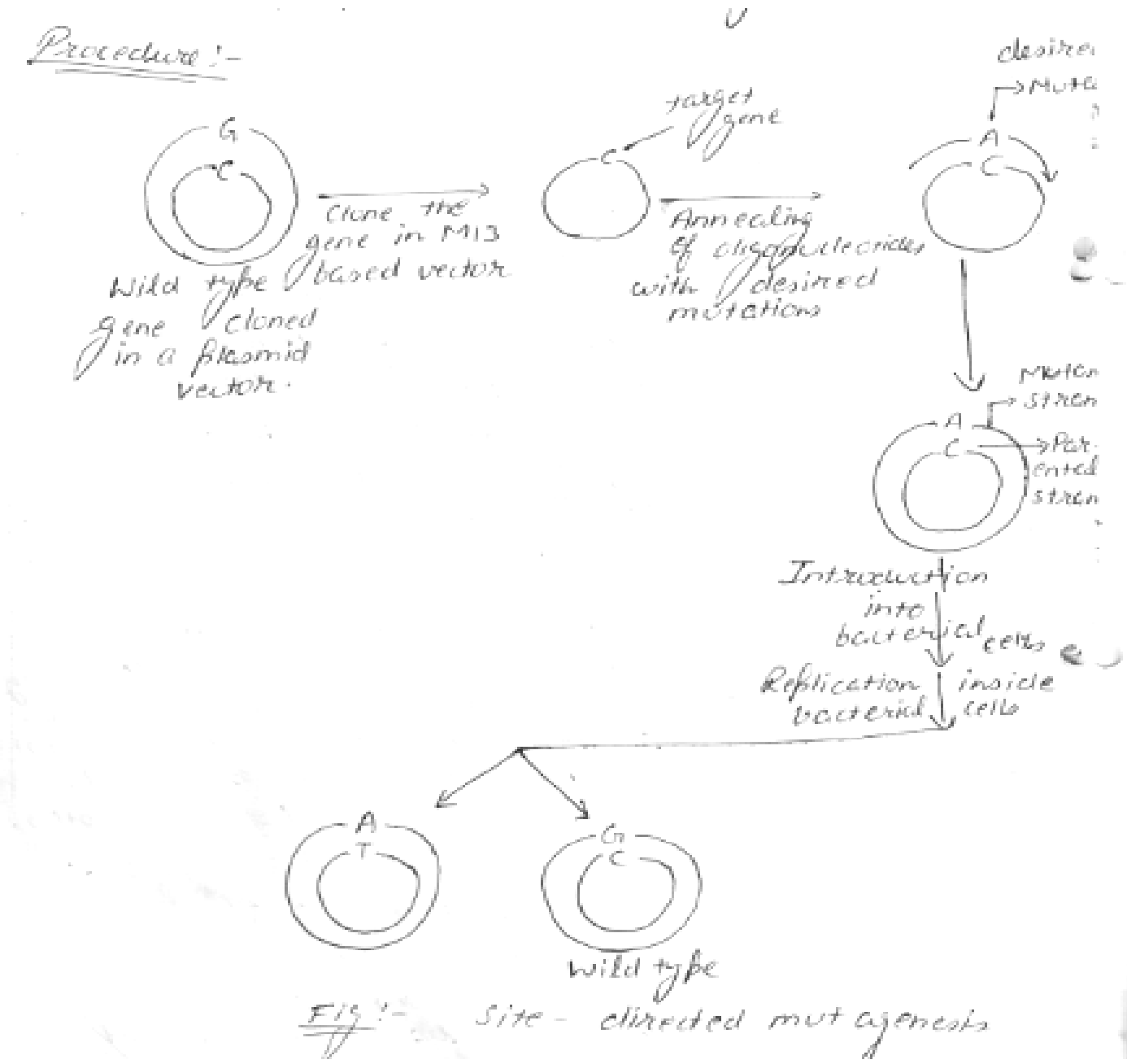


Fig :- Site - directed mutagenesis

The technique involves the cloning of gene which needs to be mutated in a M13 based vector so that this target gene is present as a single-stranded DNA.

It has been observed that nutritional proteins also have some therapeutic functions. Such proteins are categorized under neutraceutical proteins.
e.g. Milk powder – Formulated such that it have a similar composition as mother's milk from buffalo and cow milk (Amul, Lactogen)