AGR 311

INTRODUCTION TO BIOTECHNOLOGY

LECTURE NOTE

BIOTECHNOLOGY IN ANIMAL BREEDING

Kari Ereky coined the term 'biotechnology' in 1919 and defined it as 'the process by which products could be synthesized from raw materials with the aids of living organisms'. FAO however defined **biotechnology** in a broader sense 'as any technology (technological application) that involves the use of biological systems, living organism or their products in a way to benefit humans. In this board context, biotechnology is not a new concept as it has been used for thousands of years by man to manufacture products such as beer, wine and bread. The 'traditional' plant and animal breeding that involves selection and mating of desired individuals is a good example of age-old application of biotechnology. However, breakthroughs in molecular biology and genetics since 1970's such as recombinant DNA and embryo manipulation technologies etc have provided new and improved ways to make use of the living organism for the benefit of hu mans. The term biotechnology has, therefore, become associated with the use of all the newer biological techniques like Zygote, embryo and DNA manipulation, for the benefit of humans.

Animal breeding involves directed genetic improvement of animal populations for traits of economic importance. Selection of genetically superior individuals to become parents of next generation is one of the most important components of genetic improvement. Genetic gain attributable to selection for quantitative traits is dependent on four factors:

i. Accuracy of selection
ii. Selection intensity
iii. Genetic standard deviation
iv. Generation interval

$$\text{Genetic gain/year} = \frac{\text{Accuracy of selection x Selection intensity x Genetic standard deviation}}{\text{Generation interval}}$$

From animal breeding viewpoint, therefore, any biotechnology technique that can enhance rate of genetic progress through change in any of the components of genetic gain shall be of use in genetic improvement. Broadly, the following two categories of biotechnological interventions are important in enhancing the genetic progress:

1. Reproductive Technologies

The process of reproduction serves the mechanism of transmission of genes from one generation to the next. Reproduction physiology and animal breeding are interlinked. Genetically superior animals can not contribute their superiority to next generation unless their reproductive capacity is satisfactory. Therefore, maintaining high reproductive rate is critically important in achieving high rate of genetic improvement through selection as:

i. Reproductive rate determines the percentage of animals, which have to be retained to

keep constant herd (flock) size; therefore, intensity of selection is directly dependent on reproductive rate.

ii. Accuracy of selection is also directly related to the number of offspring produced per parent (family size)

iii. Low reproductive rate also prolong the generation interval.

Reproductive rate varies considerably among various species of domestic animals. Typical figures for the different components of the reproductive rate are given below:

Typical values for different components of reproduction in farm animals

Species	Age a 1st breeding	Gestation length	Ovulation rate	Offspring/gestation	No of offspring/yr
Buffalo	24-30 months	315days	1	1	1
Cattle	13-16 months	283 days	1	1	1
Horse	24-36 months	336 days	1	1	1
Sheep	16-20 months	150 days	1-3	1-3	1-2
Goat	10-12 months	150 days	1	1	1
Swine	7-9 months	130 days	12-30	6-15	12-30
Chicken	5-6 months	22 days	1	-	150-200*

** Using artificial incubation*

Factors that determine rate of genetic change

i. **Reproductive rate**: The higher the reproductive rate, the higher the rate of genetic change for example with the exception of chicken, and to some extent swine in the above table, the reproduction rate of other species of livestock is low, therefore, comparing poultry and pigs with ruminants, higher rate of genetic changes are possible in the later species than the former.

ii. **Nature and Time of measurement of economic trait**: In species like dairy cattle and buffaloes, the main traits of economic importance are sex-limited and measured late in life. This thus puts dairy cattle and buffaloes at a disadvantage for genetic improvement.

Methods of reproductive enhancement in ruminants

Enhancement of reproductive rate of ruminants through various reproduction technologies is expected to yield substantial benefits not only to animal breeder but to livestock producers as well. Several reproductive technologies that can enhance the rate of genetic gain in ruminants are many but the most common are:

A. Artificial Insemination

Artificial insemination (AI) is a reproductive technology in which semen is collected from the

male and is introduced into the genital tract of the female which is in heat. It consists of a number of steps viz; semen collection and its evaluation for quality; dilution of semen to increase its volume and to preserve it; semen freezing and finally delivery of the semen to the receptive female's genital tract.

Benefits of AI

Artificial insemination has been highly valuable in enhancing the reproductive rate of males in low-prolific species of livestock. From animal breeding standpoint, it offers the following benefits.

a. Increased rate of genetic gain: Al enhances genetic gain per annum through the sire path by:

i. Increasing the accuracy of male selection through production of its large number of daughters and sisters across many herds (environments).

ii Increasing the intensity of selection among males as a very small number of top sires can be used to serve large population of females.

iii. Decreasing the male generation interval by producing the required number of progeny in a shorter time compared to natural mating.

The combination of these three components of genetic gain arising from AI can lead to four-fold increase in rate of genetic improvement in dairy cattle relative to that from natural mating.

b. **Rapid dissemination of genetic improvement:** Artificial insemination permits extensive use of proven sires of high genetic merit across herds, regions or countries. It is thus most effective method of dissemination of genetic improvement form elite sector to the lower strata of breeding pyramid.

c. **Establishment of genetic links between herds:** Through AI, progeny of a sire can be produced in many different herds/flocks. This in turn makes a '**genetic connectedness'** between different herds. Such genetic connectedness makes the application of new large-scale genetic evaluation procedures, like BLUP, possible.

The advantages of AI have led to the replacement of natural service with AI in commercial dairy cattle populations of developed countries. However, in beef cattle populations its usage is much less for a number of factors which include:

i. Difficulty in detecting heat in large-sized beef herds kept on ranch

ii. Beef production traits can be measured in both sexes, progeny testing of sires thus offers less advantage than in dairy

iii. High cost of AI as proportion of output per beef cow.

In sheep and goat, the unavailability of a simple, non-surgical insemination procedure has prevented AI's extensive use. In breeding of turkeys and meat-type chicken, AI is perhaps the rule.

Constraints of AI

i. The greatest genetic problem associated with AI is the increased relationship among individuals of a population leading to increased inbreeding. This can partially be overcome by exchange of semen between the cooperative herds or breeders.

ii. AI has high cost as a proportion of output value per cow. The cost is further increased by a combination of environmental and nutritional stresses together with less efficient transport.

B. Embryo Transfer Technologies

Embryo transfer (ET) involves collection of embryos from donor females and their transfer to recipient females. There are two procedures presently available for production of embryos from donor females.

The first procedure consists of super ovulation of the donor through hormone administration, artificial insemination of embryos to recipients immediately after recovery or following freezing at a later time.

The other procedure consists of collection of eggs from ovaries, their maturation and fertilization in the laboratory (in vitro). The resulting embryos are cultured on to a certain stage at which point they are either transferred to recipients immediately or can be frozen for later transfer. The whole process is sometimes known as IVF (in vitro fertilization).

Benefits of embryo transfer

The procedure of multiple ovulation and embryo transfer, referred to as MOET, potentially offers a number of advantages.

i. **Increased genetic gain:** The principal benefit of embryo transfer is to produce several progeny from a cow just as through AI in male. In cow calves can be increased from 4 to 25 calves. This increased the female selection intensity as well as the female accuracy and thus enhancing the rate of genetic gain through female path of selection up to about two-fold. Increase in rate of genetic improvement in cattle and sheep has been predicted to be possible through MOET.

ii. **International trading:** MOET makes international trading of germplasm easy by reducing the cost of international transport by shipping embryos rather than live animals, reducing risk of importing diseases, and is also benefical from animal- welfare viewpoint. Therefore, embryo transfer is the cheapest and the safest way of import or export of germplasm.

iii. **Transmission of genetic improvement***:* MOET could be a highly effective technique for dissemination of genetic improvement from upper tier (elite sector) to the commercial sector of livestock industries. Since embryo already contain full complement of chromosomes necessary for their development, the MOET can deliver populations of commercial animals with 10% of their genes from elite sector parents.

iv. **Genetic conservation:** Rapid expansion of rare or newly introduced breeds than possible with grading up (repeated) backcrossing is possible by this technology. Also, it is useful in ex-situ preservation of endangered breeds (species)

Constraints of embryo transfer

i. The greatest disadvantage of MOET is the increased level of inbreeding since fewer females make a large contribution to genetic makeup of the next generation. This is particularly important in small and closed nucleus populations.
ii. MOET is more expensive than AI and is far less effective than AI in increasing rate of genetic improvement.

C. Semen and Embryo Sexing

Sexing of semen has been attempted since early 1920 or even before. Incidentally, the PhD dissertation of Prof Lush, father of scientific animal breeding, was on semen sexing though without success. The basis of most of the methods for separation of sperms carrying X- and Y-chromosomes has been the presence of physical differences between them and the slight difference in their DNA content.

Methods for separation of semen

These methods include:

i. Using differences in mass, surface charge, antigenic properties, buoyant density of sperms carrying X and Y bearing sperms. The success rate and repeatability of these methods was low.
ii. Flow cytometery has proved successful with higher accuracy. The procedure consists of staining of sperms with fluorescence when cell pass through a laser beam to identify and sort X- and Y- bearing sperm.

In animal breeding, sexing of semen and embryos holds the potential of enhancing genetic gain through:

i. **Increased selection intensity:** Sexing of either semen (sperms) or embryos could be useful in increasing the selection intensity applied to females by producing more of them (in species where the main traits of interest are limited to females). It will, however, be of little value when performance records are available on both sexes, and where the total number of animals tested is fixed, as an increase in the number of female implies a
ii. reduction in the number of males, and therefore, a reduction in male selection intensities. However, when the trait is sex-limited (e.g. milk and egg production), there may be benefits in creating more animals of the recorded sex. This may be beneficial in nucleus schemes if they already have access to high genetic merit males which have been accurately tested in a separate population.
iii. **Faster dissemination of genetic improvement:** The development of a cheap, reliable technique for sexing semen in large quantities for conventional AI could lead to major improvements in dissemination of genetic improvement.

D. Embryo Cloning

Production of groups of genetically identical embryos from a single original embryo is termed embryo cloning or embryo multiplication. At present embryo cloning can be achieved in two ways:

i. **Embryo splitting:** Splitting of an embryo to produce twin embryos which are then transferred to recipient females, is a relatively simple technique of production of identical offspring. Splitting into more than two parts is not successful as there are few cells available for normal development.
iv. **Nuclear transplantation:** Nuclei from cell of embryos (16-, 32-, or 64-cell) are transferred to unfertilized egg from which the nucleus containing single copy of chromosomes has been removed. And the new embryo then develops as if newly fertilized.

Until mid 1990's, nuclear transfer was accomplished using early embryos. Subsequently, viable cloned embryos as well as live cloned animals had been produced by transferring cells which were originally derived from embryos but had been cultured and multiplied in the laboratory. This procedure gives the potential for production of far greater number of identical animals to be produced from a single pair of elite parents. In 1997, a viable lamb was produced following nuclear transfer from a cultured cell originating from mammary tissue of an adult sheep. These developments have the potential to increase success rates and reduce costs.

Benefits of embryo cloning

Cloned embryos, in monotonous species like cattle, could be useful for some purposes:

i. Evaluation of individuals via their clones

ii. Cloning of elite individuals for use in industry (e.g. beef bulls for natural mating).

iii. Cloning offers the potentiality to improve the accuracy of genetic evaluation on the female side but its impact is small.

Disadvantages of embryo cloning

The potential disadvantages of embryo cloning include:

i. The risk of producing large number of animals which may subsequently turn out to be susceptible to disease or to be unsuitable for new production methods. A widely reported problem with animals derived from nuclear transfer embryos concerns a number of abnormalities viz; extended gestation, increased stillbirth rate and prenatal deaths.
ii. Cloning may reduce genetic variation as a population is reduced to small numbers and inbreeding increases.
iii. Expensive and time consuming.

2. Molecular genetic technologies

Advances in molecular biology beginning from 1970's have allowed the identification and flagging the location of functional genes using DNA markers. The most common of these are;

i. **Discovery of restriction enzymes:** Bacteria produce a large number of enzymes, called restriction enzymes, which are capable of 'cutting up' sequences of DNA at specific recognition sites. Different restriction enzymes recognize different sequences of four to six bases pairs in DNA, and make their cut whatever they come across such sequences of bases in a strand of DNA. This property of restriction enzymes has been used in Animal and plant genomics (species characterization, cloning and in vitro gene expression, to identify and use molecular markers etc). Treating or digesting identical sequenced of DNA with same enzymes produces identical sets of DNA fragments, each of a specific size. Conversely treating different sequences of DNA with the same restriction enzyme produces different sets of fragments of different sizes. Therefore, restriction enzymes are useful in detecting similarities and differences in DNA sequences of different animals.
ii. **Separation and detection of DNA fragment:** Fragments of DNA of different lengths, resulting from digestion with restriction enzymes, are separeated by applying a sample of fragments of mixed sized through electrophoresis. The movement of the fragments in the gel is inversely proportional to their size; the large fragments move slowly while the small fragments move quickly. The gel can be stained to display a series of bands, with each band representing DNA fragments of particular size. When samples of DNA from different animals are applying side by side to the same gel, a series of bands can be detected in the respective lanes representing the experimental sample. By comparing the patterns of bands across columns, it is possible to tell whether different samples contain DNA fragments of a similar length.

iii. **Multiplying sequences of DNA:** There are two methods for rapid multiplication of a target DNA sequence viz. molecular cloning and polymerase chain reaction (PCR) cells. In this case, the mammalian DNA fragments which are to be multiplied can be inserted into the DNA from a vector (such as a plasmid like, pUC, pBR322) which occurs independently in some bacterial cells). Incorporation of foreign DNA is achieved by treating the vector DNA with the same/similar restriction enzymes that was used to create fragments of mammalians DNA. The target DNA molecules can then be introduced into a rapidly reproducing host (e.g. bacterium in case of plasmid vectors). DNA fragments reproduce with the host cell DNA, and thus producing multiple copies of the original DNA fragments which can then be extracted from host cells. The other method, called polymerase chain reaction or PCR, involves DNA multiplication in a test tube rather than in living cells. PCR produces large qualities of DNA in a matter of hours, and needs only very small amounts of DNA initially e.g. from hair follicles.

iv. Development of DNA sequencing technology: DNA sequencing technology has been used to sequence a particular fragment of DNA (produced by PCR or cloning or RE digestion) or the whole genome (shotgun sequencing and next generation sequencing).. DNA sequencing is the process of determining the exact order of the bases A, T, C, and G on a strand of DNA to be sequenced by sanger sequencing method is used to generate a set of fragments that differ in length from each other by one base pair. The fragments are separated by size using electrophoresis. By reading, the gel from the bottom up, the sequencing DNA was developed by Sanger in 1977. In recent years, 'next generation sequencing' has become globally the main player in the area of genomics and molecular breeding. In this approach, the whole genome is fragmented by physical or chemical shearing and the fragments are sequenced in parallel (that's why it is also called as massive parallel sequencing) by any one of the chemistry like, pyro sequencing, reversible dye terminator method etc.

GENETIC MATERIALS

Genetic material, also known as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), plays a fundamental role in the composition of living organisms. It is the medium by which instructions are transmitted from one generation of organisms to the next. Each molecule of human DNA has billions of nucleotides arranged like steps on a ladder. It is the sequence of these nucleotides that determines the traits of the organism.

At various locations, called loci, along each chromosome between large stretches of non-coding, DNA sequences of nucleotides resolve into coherent patterns that instruct messenger proteins in how to build other proteins. These proteins are synthesized in the cytoplasm of the cell and work to build every structure of a living body. Genes, as a natural consequence of their nucleotide sequences, build proteins, and proteins build bodies.

Genetic material is passed among large organisms by vertical transmission from parent to offspring. Each offspring resembles its parent more closely than it resembles a randomly chosen member of its species because the exact sequence of genetic instructions on how to build the body have been inherited from the parent. Small errors in copying genes are known as mutations, and their proliferation throughout a gene pool drives the process of evolution. **DNA** is the hereditary material found in the nucleus of eukaryotic cells (animal and plant) and the cytoplasm of prokaryotic cells (bacteria) that determines the composition of the organism. DNA is found in the nucleus of every cell, and it is exactly the same in each cell. There is another type of genetic material found in cells and viruses known as ribonucleic acid (RNA).

Structure of Genetic materials

The basic building blocks of DNA are comprised of four chemical bases called nucleotides: adenine (A), guanine (G) which are purines; cytosine (C), and thymine (T) which are pyrimidines. These bases pair with one another, A with T and C with G. Each base is attached to a sugar (ribose) and a phosphate molecule: hence the name deoxyribonucleic acid. DNA is double stranded, and each strand is held together by the pairing of the nucleotides. The double stranded DNA is spirally coiled to form a helix. This double helix looks much like a ladder with the nucleotides representing the rungs on the ladder and the sugar and phosphate are the sides. The double helixes are then wrapped around proteins called histones and packaged into chromosomes.

The other type of genetic material called ribonucleic acid (**RNA**) is structurally similar to DNA, but there are a few differences primarily in their chemical bases. Both have a sugar, phosphate group, and four chemical bases. Unlike DNA which is double stranded, RNA is single stranded. RNA contains adenine (A), guanine (G), cytosine (C), and uracil (U). So, in RNA the base thymine is replaced with uracil. In RNA the base pairs are A pairs with U and C pairs with G.

Levels of Organization in DNA Structure, Replication, and Technology

DNA is convenient for living things to use due to its clearly defined levels of organization. At the molecular level, there are 4 nucleotide bases: adenine, guanine, cytosine, and thymine, which are all linked to a ribose sugar and phosphate backbone. These nucleotides are joined in various sequences that are highly important for determining the genetic information that they encode. These strings of double-stranded nucleotides form what we call DNA in most organisms. If left unpackaged, the amount of DNA in a eukaryotic organism would not fit into a eukaryotic cell.

Therefore, higher levels of organization are necessary, such as the wrapping of DNA around histone proteins, forming a nucleosome core. These nucleosomes are further wrapped around each other to condense into what we call a chromatin fiber. The chromatin fiber condenses into what we identify as a chromosome in eukaryotic cells. This level of condensation only exists during mitosis and meiosis, where it is important that there be no stray bits of DNA floating around so when it divides, it wants to make sure each daughter cell has the right amount and type of DNA necessary for survival.

However, most of the life of the cell is spent outside of mitosis (in interphase), so the levels of packaging are quite variable. Depending on the needs of the cell, certain regions of the chromosome are heavily packaged to silence them and prevent transcription from taking place, while other regions that make important proteins are loosely packaged and open for RNA transcription. This variable packaging process is important for regulating RNA transcription of various genes.

Another level of organization in eukaryotic cells is the presence of the nucleus. The fact that all the DNA in the cell is found only in the nucleus, though some exists in the mitochondria while all the machinery for making proteins like ribosomes are only in the cytoplasm, provides another tight level of regulation..

GENE ORGANIZATION AND EXPRESSION

There are a number of fundamental differences between the way genes are arranged, expressed, and controlled in eukaryotic cells when compared with bacteria.

1. Eukaryotes, because of their greater complexity, possess much more genetic information. For example, a human cell contains about 1000 times more DNA than an *E. coli* cell. As a result the DNA may have an overall length of several centimetres which must be packed into a few micrometres. Hence the DNA of eukaryotic cells is very highly condensed and this is aided by the presence of a class of basic proteins called *histones*.

2. Eukaryotic chromosomes are located within a nucleus bounded by a nuclear membrane and, since proteins are synthesized in the cytoplasm, the sites of transcription and translation are physically separated. Consequently these two processes are not as closely coupled as they are in bacteria.

3. Primary RNA transcripts in eukaryotic cells are extensively modified, cleaved and spliced in the nucleus before being transported to the cytoplasm in the form of mRNA.

4. The control of gene expression is much more complex and diverse in eukaryotes, with many levels of regulation. In addition to transcriptional control, post-transcriptional mechanisms also play a major role. Furthermore, the primary translation products may in turn be subjected to post-translational modification.

Gene expression

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as tRNA or small nuclear RNA (snRNA) genes, the product is a functional RNA. The process of gene expression is used by all known life to generate the macromolecular machinery for life.

Several steps in the gene expression process may be modulated, including the transcription, RNA splicing, translation and post-translational modification of a protein. Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism. Gene regulation may also serve as a substrate for evolutionary change, since control of the timing, location, and amount of gene expression can have a profound effect on the functions (actions) of the gene in a cell or in a multicellular organism.

In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype, i.e. observable trait. The genetic code stored in DNA is "interpreted" by gene expression, and the properties of the expression give rise to the organism's phenotype. Such phenotypes are often expressed by the synthesis of proteins that control the organism's shape, or that act as enzymes catalyzing specific metabolic pathways characterizing the organism. Regulation of gene expression is thus critical to an organism's development.

Transcription

The production of the RNA copy of the DNA is called transcription, and is performed in the nucleus by RNA polymerase, which adds one RNA nucleotide at a time to a growing RNA strand as per the complementarity law of the bases. This RNA is complementary to the template 3' → 5' DNA strand, which is itself complementary to the coding 5' → 3' DNA strand. Therefore, the resulting 5' → 3' RNA strand is identical to the coding DNA strand with the exception that Thymines are replaced with Uracils (U) in the RNA. A coding DNA strand reading "ATG" is indirectly transcribed through the “TAC” in the non-coding template strand as "AUG" in the mRNA.

While transcription of prokaryotic protein-coding genes creates mRNA that is ready for translation into protein, transcription of eukaryotic genes leaves a primary transcript of RNA (pre-mRNA), which first has to undergo a series of modifications to become a mature mRNA. A very important modification of eukaryotic pre-mRNA is RNA splicing. The majority of eukaryotic pre-mRNAs consist of alternating segments called exons and introns. During the process of splicing, an RNA-protein catalytical complex known as splicesome catalyzes two transesterification reactions, which remove an intron and release it in form of lariat structure, and then splice neighbouring exons together. In certain cases, some introns or exons can be either removed or retained in mature mRNA. This so-called alternative splicing creates series of different transcripts originating from a single gene. Because these transcripts can be potentially translated into different proteins, splicing extends the complexity of eukaryotic gene expression.

In eukaryotes most mature RNA must be exported to the cytoplasm from the nucleus. While some RNAs function in the nucleus, many RNAs are transported through the nuclear pores and into the cytosol. Notably this includes all RNA types involved in protein synthesis. In some cases RNAs are additionally transported to a specific part of the cytoplasm, such as a synapse; they are then towed by motor proteins that bind through linker proteins to specific sequences (called "zipcodes") on the RNA. During the translation, tRNA charged with amino acid enters the ribosome and aligns with the correct mRNA triplet. Ribosome then adds amino acid to growing protein chain.

Folding

Te polypeptide folds into its characteristic and functional three-dimensional stricture from a random coil, Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA into a linear chain of amino acids. This polypeptide lacks any developed three-dimensional structure (the left hand side of the neighboring figure). Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure) known as the native state. The resulting three-dimensional structure is determined by the amino acid sequence. The correct three-dimensional structure is essential to function, although some parts of functional proteins may remain unfolded. Failure to fold into the intended shape usually produces inactive proteins with different properties including toxic prions. Several diseases and allergies are believed to result from the accumulation of *misfolded* proteins.

Translocation

Secretory proteins of eukaryotes or prokaryotes must be translocated to enter the secretory pathway. Newly synthesized proteins are directed to the eukaryotic translocation channel by signal peptides on which the efficiency of protein secretion in eukaryotes depend. Many proteins are destined for other parts of the cell than the cytosol and a wide range of signalling sequences are used to direct proteins to where they are supposed to be. In prokaryotes this is normally a simple process due to limited compartmentalisation of the cell. However, in eukaryotes there is a great variety of different targeting processes to ensure the protein arrives at the correct organelle. Not all proteins remain within the cell and many are exported. In eukaryotes the export pathway is well developed and the main mechanism for the export of these proteins is translocation to the endoplasmic reticulum, followed by transport via the golgi apparatus.

Gene regulation

Gene regulation is the process of turning genes on and off. Virtually any step of gene expression can be modulated, from transcriptional initiation to RNA processing and to the post translation modification of protein. Regulation of gene expression includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (RNA or proteins), and is informally termed gene regulation**.** Sophisticated programs of gene expression are widely observed in biology for the following reasons:

i. To trigger developmental pathways
ii. To ensure that appropriate genes are expressed at the proper times
iii. To respond to environmental stimuli
iv. To adapt to new food sources.
v. To increase the versatility and adaptability of an organism by allowing the cell to express protein when needed.
vi. To drive the process of cellular differentiation and morphogenesis leading to the creation of different cell types that possess different gene expression profiles, and hence produce different proteins/have different ultrastructures that suit them to their functions

The following is a list of stages where gene expression is regulated:

i. Chromatin domain
ii. Transcription
iii. Post-transcriptional modification
iv. RNA transport
v. Translation
vi. mRNA degradation

BASIC PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

When nucleic acid sequences are combined in a laboratory, the resulting DNA is called recombinant DNA. Recombinant DNA may contain oligonucleotides from the same or similar species, in which case it is called cisgenic, or may contain oligonucleotides from different organisms that could not naturally interbreed, in which case it is called transgenic.

The four steps are:

i. Gene Cloning and Development of Recombinant DNA: The foreign DNA (gene of interest) from the source is enzymatically cleaved and ligated (joined) to other DNA molecule i.e. cloning vector (plasmid, phagemid etc.) to form recombinant DNA
ii. Transfer of Vector into the Host: This cloning vector with recombinant DNA is transferred into and maintained within a host cell. The introduction of rDNA into a bacterial host cell is called transformation.
iii. Selection of Transformed Cells: Those host cells that take up the rDNA are identified and selected from the pool.
iv. Transcription and Translation of Inserted Gene.

Genetic engineering may or may not have recombinant DNA (rDNA) preparation step and with the advancement of gene transfer technology, artificially DNA can be transferred to different hosts (host cells) without any vector and host organism can be engineered to carry desired properties. The genetic manipulations are used to produce individuals having a new combination of inherited properties. Such manipulations may be of two kinds**:**

i. Cellular manipulation involving culturing of cells (e.g., haploid cells) and hybridization of somatic cells (protoplast fusion)

ii. and Molecular manipulation, involving construction of artificial rDNA molecules, their insertion into a vector and their establishment in a host cell or organism.

The latter approach has been called “recombinant DNA (rDNA) technology”. Therefore, use of term “genetic engineering” and “rDNA technology” is overlapping. However, all the manipulations involving use of constructed gene or constructed gene transfer are termed as rDNA technology.

Gene isolation/Gene cloning

Genes are the basic unit of a cell that passes on the traits/characters of parents to their offspring. A trait/character is any heritable attribute in an organism. Genes are actually a small string of bases on a DNA chain. A DNA chain therefore has many genes, and these DNA are located in the chromosomes. Genes are part of a DNA molecule which codes for 1 polypeptide. A **polypeptide** is a string of amino acids linked together. A single polypeptide chain might make up the entire primary structure of a simple protein; more complex proteins are formed when two or more polypeptides link together.

Gene cloning is the process in which a gene of interest is located and copied (cloned) out of DNA extracted from an organism. It is a common practice in molecular biology laboratories that researchers use to create copies of a particular gene for downstream applications - sequencing, mutagenesis, genotyping or heterologous expression of a protein.

Sequencing means to put something in order such as first, next, and last. Sequencing uses biochemical methods to determine the order of nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA oligonucleotide. **Oligo or oligonucleotide** is a small chain of nucleic acid residues. Each nucleotide consists of three subunits: a phosphate group and a sugar (ribose in RNA and deoxyribose in DNA) make up the backbone of the nucleic acid strand, and attached to the sugar is one of a set of nucleobases (one of four chemicals: adenine, thymine/uracil, guanine, and cytosine).

Mutagenesis is a process by which the genetic information of an organism is changed in a stable manner, resulting in a mutation. It may occur spontaneously in nature or as a result of exposure to mutagens.

Genotyping is the process of determining differences in the genetic make-up (*genotype*) of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence. It reveals the alleles an individual has inherited from their parents.

Heterologous expression refers to the *expression* of a gene or part of a gene in a host organism, which does not naturally have this gene or gene fragment. Insertion of the gene in the heterologous host is performed by recombinant DNA technology

Cloning

To **clone** is to propagate an organism or cell**. Clones** are organisms or cells that have exact genetic copies. It can happen naturally e.g. identical twins or they can be made in the laboratory. **Cloning** is the process of creating genetically identical copies of biological matter. This may include genes, cells, tissues or entire organisms.

Media of cloning

There are two media for cloning a gene:

i. *In vivo* - involves the use of restriction enzymes and ligases to cut a fragment of DNA, containing a single gene or a number of genes and insert such into a **vector** that can amplify the gene(s) within another host cell..

ii. *In vitro* - involves using the polymerase chain reaction (PCR) method to create copies of fragments of DNA in a laboratory.

Types of cloning

There are actually three different types of cloning:

i. Molecular cloning/gene cloning: It focuses on making identical copies of DNA molecules.
ii. Organism cloning/ reproductive cloning: It involves making an identical copy of an entire organism.
iii. Therapeutic cloning: It involves the cloning of human embryo for the production of stem cells. The embryos are eventually destroyed in this process.

Methods involved in gene isolation/gene cloning

Broadly speaking, there are three basic methods to cloning a gene. The three approaches are:

i. Cloning by complementation
ii. Positional cloning
iii. Cloning by sequence homology

Cloning by complementation

This is the most commonly used method for gene isolation. In this method, one clones a dominant allele of the gene of interest using a host strain carrying a recessive allele and selected screens transformants for the phenotype of the dominant allele. A **phenotype** is the composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties.

Positional cloning

Positional cloning uses the physical proximity of a cloned DNA fragment (or gene) to isolate the gene of interest. The cloned fragment is the starting point from which one moves, in a step-wise fashion, toward the gene of interest by a process called chromosome walking. **Primer walking** is a sequencing method of choice for sequencing DNA fragments between 1.3 and 7 kilobases. The term "primer walking" is used where the main aim is to sequence the genome. The term "**chromosome walking**" is used instead when we know the sequence but don't have a clone of a gene.

Cloning by sequence homology

Homology is the existence of shared ancestry between a pair of structures, or genes, in different species. In its simplest form, **cloning by sequence homology** uses the evolutionary conservation of the sequence to isolate homologous genes from different species or homologous members of a repeated gene family from the genome of a particular species.

Vector

Traditionally in medicine**, a *vector*** is an organism that does not cause disease itself but which spreads infection by conveying pathogens from one host to another. In gene therapy, a virus itself may serve as a **vector**, if it has been re-engineered and is used to deliver a gene to its target cell. Therefore, a **"vector"** in this sense is a vehicle for delivering genetic material such as DNA to a cell. A vector is a section of DNA that can incorporate another DNA fragment without losing the capacity for self-replication. A vector containing an additional DNA fragment is known as a **hybrid vector**.

Characteristics of a cloning vector

i. It must be relatively small molecules for convenience of manipulation

ii. They must be capable of prolific replication in a living cell, thereby enabling the amplification of the inserted donor fragment.

iii. Presence of convenient restriction sites that can be used for insertion of the DNA to be cloned.
iv. Unique sites are most useful because there the insert can be targeted to one site in the vector.
v. It is also important that there be a mechanism for easy identification and recovery of the recombinant molecule.

The choice of vector to use depends on:
i. The size of the segment of genome (or other DNA sample) being made into the library.
ii. The ease of manipulating the vector.

Types of vectors

There are 4 different type of vectors:

i. Plasmid: Bacterial plasmids are small circular DNA molecules that are distinct from, as well as additional to, the main bacterial chromosome. They replicate their DNA independently of the bacterial chromosome.

ii. Lamda (λ) phage vector/bacteriophage/phage/bacterial eater/antibacterial agent: This is a virus that infects and replicates within a bacterium. Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures.

iii. Cosmids and fosmids vectors are specialized plasmids with X cos sites, that are able to carry 8-44 kpb DNA fragments; the fosmid form is more stable.
iv. Expression vectors detect the protein product expressed in a bacterial cell. Therefore, in these cases, it is necessary to be able to express the gene in bacteria; that is, to transcribe it and translate the mRNA into protein. Most cloning vectors do not permit expression of cloned

genes, but such expression is possible if special vectors are used. However, because bacteria cannot process introns, the cloned sequences must be spliced of introns.

Splicing of introns

In genetics, **splicing** is a modification of the nascent pre-messenger RNA (pre-mRNA) transcript in which introns are removed and exons are joined. **RNA splicing** is the modification of an RNA strand where exons (the coding regions of a transcribed gene) are retained and the introns are removed. Sometimes the exons are recombined either *in vivo* or experimentally to form alternative splicings, which have various functional effects. The word **intron** is derived from the term *intragenic region*, that is, a region inside a gene. The term intron refers to both the DNA sequence within a gene and the corresponding sequence in the unprocessed RNA transcript. As part of the RNA processing pathway, introns are removed by RNA splicing either shortly after or concurrent with transcription. Introns are found in the genes of most organisms and many viruses. They can be located in a wide range of genes, including those that generate proteins, ribosomal RNA (rRNA), and transfer RNA (tRNA). An **exon** is any nucleotide sequence encoded by a gene that remains present within the final mature RNA product of that gene after introns have been removed by RNA splicing. The term *exon* refers to both the DNA sequence within a gene and to the corresponding sequence in RNA transcripts. In RNA splicing, introns are removed and exons are covalently joined to one another as part of generating the mature messenger RNA.

TRANSGENSIS (GENE TRANSFER)

Transgenesis or gene transfer or is a procedure, which involves stable incorporation of specific gene (s) of interest from one species into the genome of another species in such a way that it functions in the receiving species and is passed on from one generation to the next. This entails physically inserting the DNA coding for specific gene (s) into the DNA of another species (animal). The foreign or introduced genes are referred to as 'transgenes' to distinguish them from endoganous genes. And the animal that contains the introduced genes (the foreign DNA) is called a **transgenic animal.**

A transgenic organism therefore is an organism which has been modified with genetic material from another species. The genetic modification is accomplished by inserting DNA into an embryo with the assistance of a virus, a plasmid, or a gene gun. The embryo is allowed to develop, and the mature organism will express the DNA which has been inserted into its genome. Transgenic organisms can also pass the modification on to future generations by breeding with other members of the same species. A genetically modified organism (GMO) is any organism whose genetic material has been altered using genetic engineering or any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.

Organisms are genetically modified for a number of reasons:

i. Making the organisms more vigorous
ii. Adding resistance to specific threats
iii. For the goal of expressing a particular trait like ease to harvest and ease to handle
iv. Transgenic organisms may grow in areas where other members of the species cannot
v. Transgenic organisms are useful in scientific research, for example, transgenic mice can be modified with human DNA for the purpose of testing medical treatments and seeing how they might behave in a human.
vi. While species cannot interbreed, as a general rule, DNA from one species can express in another. Transgenic organisms are able to express foreign genes because the genetic code is similar for all organisms. This means that a specific DNA sequence will code for the same protein in all organisms. Due to this similarity in protein sequence, scientists can cut DNA at these common protein points and add other genes. An example of this is the 'super mice' of the 1980s. These mice were able to produce the human protein tPA to treat blood clots.
vii. However, there is some controversy over the practice of genetic modification. Some advocates are concerned that interbreeding between transgenic and wild organisms could have unforeseen consequences, for this reason transgenic organisms are rendered sterile so they cannot interbreed, for the purpose of protecting patents and to prevent transgenic organisms from cross-breeding with wild relatives. Some others worry that consuming things like transgenic organisms could be dangerous.

Transfer of gene(s) allows the manipulation of individual genes rather than the entire genomes.

The premises on which transgensis is based is the high level of conservation of genes not only within species but also between species, and potentially between different classes of organism. The gene-transfer technologies are the outcome of a number of molecular genetic techniques.

The primary features of gene transfer technology are:

i. To identify, locate and multiply specific gene (s) of interest (i.e. genes with desirable effect).
ii. To remove the gene and its controlling elements from the DNA molecule in which it normally resides
iii. To insert it into a carrier DNA molecule (vector) and to have the vector transferred and integrated into the genome of the host

Two methods that are predominantly used to produce transgenic animals

i. Direct microinjection of foreign DNA into the pronuclear of fertilized eggs
ii. Infection of preimplantation embryos with retroviral vectors.

In mammals, gene insertion is mostly accomplished by the first method (e.g. microinjection of DNA in to fertilized eggs nucleus at the early embryonic stage).

The first successful gene transfer in animals was carried out in mice where the gene coding for human growth hormone was introduced into the genome of a mouse. The transgenic mouse grew to about twice the size of normal mouse. Since then, over hundreds of different genes have been transferred into recipient mouse eggs or embryos and stably maintained in laboratories throughout the world.

Since then, there have been dramatic advances in gene transfer technology. The technique has now become routine in mouse and the resulting transgenic mice are able to transmit their transgenes to offspring thereby allowing a large number of transgenic animals to be produced. Transgenic sheep, pigs, rabbits, and chickens have also been produced.

The proper expression of genes from other mammals and poultry in transgenic mice indicates that many of the regulatory sequences may not be necessary for producing transgenic.

Applications The technology for creating transgenic plants and mice has been extended to a number of domestic species of animals.

Two broad uses of this technology are relevant to animal production so as to:

(i) Improving productivity of animals: Increasing growth rate of transgenic mice which overproduced bovine or human growth hormone has prompted animal breeders for the introduction of foreign genes (either extra copies of the same hormone genes from related species) into livestock to achieve higher growth, production and disease tolerance, if available, can be incorporated from one species into another using DNA manipulation

techniques. Under natural conditions, reproductive incompatibility between species does not allow interspecific gene transfer. Some example of interspecific gene transfer are:

i. Growth hormone gene has been incorporated into pigs for producing leaner animals with improved meat quality and faster growth.

ii. Transgenic chicken carrying gene responsible for resistance against avian leucosis virus have been produced. The resistance gene has been transmitted to 5^{th} generation progenny, thus indicating its stability. This example indicated that the use of transgnic chicken may provide one approach for production of virus resistant domestic animals.

iii. Gene responsible for cysteine synthesis has been transferred into sheep with the objective of enhancing wool production.

iv. Gene responsible for cold-tolerance has been transferred from flounder into salmon.

In all these cases, the transferred genes fall in the category of qualitative genes which re single genes exerting major effect on trait- phenotype.

(ii). Production of valuable pharmaceutical products: There are a number of protein used in treatment of human diseases. These proteins could be produced in abundant quantities in milk livestock. Such novel pharamaceutical proteins are not a part of normal functioning of the animal but for these proteins a farm species is a convenient medium of production. Production of medically important proteins in mammary glands of livestock through introduction of cloned gene (i.e through recombinant DNA technology) is sometimes called **molecular pharming.**

Several human proteins have been successfully produced in milk of livestock. Compared with production of such proteins in bacteria, their production in mammas is advantageous because human protein is likely to function more properly when expressed in mammals due to their tissue-specific expression. Further milking strains of cattle, sheep and goats have some other advantages: they synthesize a range of proteins that make up about 40% of solids in milk. The relative cost of production of a transgenic protein through milk of livestock is much lesser than through recombinant bacteria. A few examples of such proteins are given below.

Constraints of transgenics:

Through the transfer of single gene has been achieved in all the major livestock species but there are still a number of constraints in its wide-scale application

(i) High cost and low success rate: the process of gene transfer between species is slow because it involves separate steps.

(ii) Side effects or consumers acceptability

Examples of some proteins that can be produced in milk of domestic animals		
Protein	Host	Uses
Lactoferrin	Cattle	as iron supplement in infant
Tissue plasminogen activator (TPA)	Goat	Dissolves blood clots
L-1-Antitrypsin	Sheep	Treatment of emphysema
Factor ix	Sheep	Treatment of some inherited forms of hemophilia
Insuline-like growth factor	Cattle	Treatment of diabetes

Gene Transfer Techniques

The three very effective modes of gene transfer observed in bacteria that fascinated the scientist leading to the development of molecular cloning are:

a. Transformation

Transformation is the naturally occurring process of gene transfer which involves absorption of the genetic material by a cell through cell membrane causing the fusion of the foreign DNA with the native DNA resulting in the genetic expression of the received DNA. Transformation is usually a natural method of gene transfer but as a result of technological advancement artificial or induced transformation evolved.

In natural transformation, the foreign DNA attaches itself to the host cell DNA receptor and with the help of the protein DNA translocase it enters the host cell. The presence of nucleases restricts the entry of two strands of the DNA, destroys a single strand thus allowing only one strand to enter the host cell. This single stranded DNA mingles with the host genetic material successfully.

The induced transformation is done under laboratory condition which is either a chemical mediated gene transfer or electroporation. In the chemical mediated gene transfer, the cold conditioned cells in calcium chloride solution are exposed to sudden heat which increases the permeability of the cell membrane allowing the foreign DNA. Electroporation involves the introduction of DNA from one organism into the cell of another by use of an electric pulse. The electroporation method indicates that pores are made in the cell by exposing it to suitable electric field, allowing the entry of the DNA. The opened up portions of the cell are sealed by the ability of the cell to repair.

b. Transduction

In transduction, a media like virus is required between two bacterial cells in transferring genes

from one cell to the other. Researchers used virus as a tool to introduce foreign DNA from the selected species to the target organism. Transduction mode of gene transfer follows either a lysogenic phase or lytic phase.

In the lysogenic phase, the viral (phage) DNA once joining the bacterial DNA through transduction stays dormant in the following generations. The induction of lysogenic cycle by an external factor like UV light results in lytic phase. In lytic phase, the viral or phage DNA exists as a separate entity in the host cell and the host cell replicates viral DNA mistaking it for its own DNA. As a result, many phages are produced within the host cell and when the number exceeds, it causes the lysis of the host cell and the phages exits and infects other cells.
As this process involves existence of both the genome of the phage and the genome of the bacteria in the same cell, it may result in exchange of some genes between the two DNA. As a result, the newly developed phage leaving the cell may carry a bacterial gene and transfer it to the other cell it infects. Also some of the phage genes may be present in the host cell. There are two types of transduction namely: generalized transduction in which any of the bacterial gene is transferred via the bacteriophage to the other bacteria and specialized transduction which involves transfer of limited or selected set of genes.

c. Transfection

Here, the genetic material is deliberately introduced into the animal cell in view of studying various functions of proteins and the gene. This mode of gene transfer involves creation of pores on the cell membrane which enables the cell to receive the foreign genetic material. The significance of creating pores and introducing the DNA into the host mammalian cell contributed to different methods in transfection.

The different methods are:

i. Chemical mediated transfection which involves use of either calcium phosphate or cationic polymers or liposomes.
ii. Non chemical transfection which involves Electroporation, sonoporation, impalefection, optical transfection, and hydro dynamic delivery.
iii. Particle based transfection which uses gene gun technique where a nanoparticle is used to transfer the DNA to host cell
iv. Magnetofection
v. Nucleofection and
vi. Use of heat shock
vii. Targeting Proteins and Peptides**:** A range of protein and peptide sequences have been utilized to target, enhance or mediate delivery of nucleic acids in a large variety of applications. The key benefits of utilizing targeting proteins and peptides are that both will enhance the transfection efficiencies and provide targeted delivery.

d. Other methods of gene transfer techniques include:

i. Conjugation: This requires a direct cell to cell contact. The conjugation process helps these plasmids to be transferred between the donor cells to a recipient one. The bacterial conjugation involves the following steps: formation of pair after mating, synthesis of the conjugal DNA, transfer of DNA followed by maturation.

ii. DNA microinjection: The desired gene construct is injected in the pronucleus of a reproductive cell using a glass needle around 0.5 to 5 micrometers in diameter. The manipulated cell is cultured *in vitro* to develop to a specific embryonic phase, which is then transferred to a recipient female. DNA microinjection does not have a high success rate (roughly 2% of all injected subjects), even if the new DNA is incorporated in the genome, if it is not accepted by the germ-line the new traits will not appear in their offspring. If DNA is injected in multiple sites the chances of over-expression increase**.**

iii. Retrovirus-mediated gene transfer: A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Retroviruses are used as vectors to transfer genetic material into the host cell. The result is a chimera, an organism consisting of tissues or parts of diverse genetic constitution. Chimeras are inbred for as many as 20 generations until homozygous genetic offspring are born.

PATENT AND SOCIETY

A patent is a temporary government-granted monopoly right on something made by an inventor. There are actually various kinds of patents:

i. utility patent (protects inventions);
ii. design patents (protect new, original and ornamental designs of articles);
iii. plant patents, which are granted to anyone who invents or discovers any asexually reproduced, distinct and new variety of plants.

Of them all, utility patient is the most powerful and most widely used. A utility patent gives a 20-year right to stop anyone from practicing the protected invention. This is not necessarily the same as having a monopoly on practicing the invention, since there may be patents held by others that also cover some aspect of the invention. For example, to build a car you need an engine, a transmission and wheels. Each of these could be protected by one or more patents held by different entities. This would mean that none of them could build a car without the permission of the others. However, any of them could stop an outsider from building a car.

Because a patent is a right to exclude others from practicing an invention, the patent system affects everyone. You cannot choose to ignore patents or decline to participate in the system. The patent holder can take infringers to court to enforce his patent, and possibly to recover damages suffered from the infringing activities. Also, unlike with copyright, independently developing an invention is not a defense. So even if you are developing something by yourself, without paying any attention to what the rest of the world is doing, you could still be stopped by someone who holds a patent on what you are developing.

Purposes of the patent system

The purposes of the patent system are to:

i. Encourage the development of new inventions
ii. Encourage the disclosure of those new inventions: Inventors are often hesitant to reveal the details of their invention; for fear that someone else might copy it. This leads to keeping inventions secret, which impedes innovation. A government-granted temporary monopoly on the commercial use of their invention provides a remedy for this fear, and so acts as an incentive to disclose the details of the invention.
iii. Give the inventor a chance to recoup the investments he made during the development of his invention. He could for instance use the patent to monopolize the market, excluding possible competitors by enforcing his patent. He could then set a high price and make a nice profit. He could also request money from others in return for a license to practice the invention.
iv. Provide opportunity for other people to learn of the existence of this invention. They might then be inspired to think of enhancements or alternatives to the patented invention. This is particularly true when the inventor refuses to license his invention, or when the licensing fee is too high. Third parties could then develop alternative technologies to

work around the patent. Presumably they would then patent these alternatives and then society benefits by having two inventions rather than one.

Reasons for patenting

i. To earn licensing money, although in some fields exclusivity is the most important reason.

ii. Another important reason to file for a patent is that it is a much stronger right than copyright. Copyright only protects your own particular expression of some idea, and does not protect against independent redevelopment or reimplementation of that idea. A patent protects the idea itself, provided the idea represents an invention. The patent holder can then go after the people who independently practice that idea, even if those people never knew of the patent or the invention.

iii. Patents can also be used for bargaining. If one company has a patent on a particular invention which another company wants to practice, the other company will have to pay. But if the other company also has patents on inventions likely to be used by the first company, the other company could negotiate a lower license fee, or perhaps even close a zero-sum deal.

iv. In the e-commerce industry, patents also served as a very important indication of the worth of a company. Many venture capitalists refused to invest in a company that did not have at least a patent application on its product(s) or service(s).