AGR 311

INTRODUCTION TO BIOTECHNOLOGY

LECTURE NOTE

BIOTECHNOLOGY IN ANIMAL BREEDING

In 1919, Kari Ereky, a Hungarian engineer, introduced the term "biotechnology," defining it as using living organisms to produce goods from raw materials. Biotechnology, according to the FAO, encompasses any technology involving biological systems or their products for human benefit. While biotech is not new—people have used it for ages to produce things like beer and bread—the field has advanced significantly since the 1970s. Breakthroughs like recombinant DNA and embryo manipulation have expanded our ability to use living organisms for human purposes. So today, biotechnology includes cutting-edge techniques like DNA manipulation, embryo manipulation, and more, all aimed at improving human life.

Animal breeding focuses on improving specific traits in animal populations that are economically valuable. One key aspect of animal improvement is selecting the best individuals to be parents of the next generation. The effectiveness of this selection for traits like size or milk production which is termed genetic gain depends on four main factors namely:

Accuracy of selection: Accuracy of selection refers to how closely the chosen individuals for breeding represent the desired traits. Higher accuracy means that the chosen individuals possess the desired traits to a greater degree, increasing the likelihood that those traits will be passed on to their offspring.

Selection intensity: The intensity of selection refers to the degree to which individuals are chosen for breeding based on certain desirable traits. High intensity of selection means that only individuals with the most desirable traits are chosen as parents for the next generation, while those with less desirable traits are not selected.

Genetic standard deviation: The genetic standard deviation of a trait, is a measure of the genetic variability within a population for a specific trait. It indicates the extent to which individuals within the population differ from the population average for that trait due to genetic factors. A larger genetic standard deviation suggests greater genetic diversity within the population for that trait, while a smaller genetic standard deviation indicates less variability.

Generation interval: The generation interval is the average age difference among parents and their offspring in a population. A short generation interval allows for more rapid turnover of generations and faster genetic progress within a population.

The relationship among these four factors (Genetic gain) is defined as:

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

Therefore, from an animal breeding perspective, any biotechnology method that can boost the rate of genetic improvement by altering any of the factors affecting genetic gain is valuable.

There are two main types of biotechnology interventions that play significant roles in enhancing genetic progress.

Reproductive Technologies

Reproduction physiology and animal breeding are closely connected because for superior genetic traits to continue, animals must have satisfactory reproductive capacity because reproduction ensures genes are passed from one generation to the next.

Thus, maintaining a high reproductive rate is crucial for achieving genetic improvement through selection because:

i. Reproductive rate affects the percentage of animals needed to maintain a constant herd size, directly impacting the intensity of selection.
ii. The accuracy of selection is influenced by the number of offspring per parent, as larger family sizes offer more data for evaluation.
iii. A low reproductive rate extends the generation interval, slowing down the pace of genetic progress.

Reproductive rate varies considerably among various species of domestic animals. Typical figures for the different components of the reproductive rate are given in the Table 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	1-3	1-3	1-2
Swine	7-9 months	130 days	12-30	6-15	12-30
Chicken	5-6 months	21 days	1	-	150-200*

** Using artificial incubation*

Poultry and pigs reproduce faster than dairy cattle and buffaloes, enabling quicker genetic changes in them. Dairy cattle and buffaloes have economically important traits measured later in life, which, combined with their slower reproduction, hinders genetic improvement compared to poultry and pigs.

Enhancing ruminant reproduction through technology could benefit both breeders and livestock producers, leading to faster genetic progress and better economic outcomes. Several reproductive technologies can enhance the rate the genetic gain, the commonest of these are:

a. Artificial Insemination

Artificial insemination (AI) is a reproductive technology where semen from a male is introduced into a female in heat. It involves collecting, evaluating, diluting, freezing, and delivering semen to the female's reproductive tract. AI has been a practical technology for over 60 years, primarily in dairy cattle. It is most commonly adopted in dairy cattle populations of developed countries but less in beef cattle

Benefits:

i. Enhanced Genetic Gain: AI boosts genetic progress by improving male selection accuracy through the production of numerous offspring across different herds. It also intensifies selection among males, reducing the generation interval.
ii. Rapid Dissemination of Genetic Improvement: AI enables the widespread use of high quality sires across various herds or regions, promoting genetic improvement.
iii. Establishment of Genetic Links: AI allows progeny of a sire to be produced in different herds, fostering genetic connectivity and facilitating large-scale genetic evaluation.
iv. AI enables large-scale progeny testing. Technical advancements like semen extenders and freezing techniques have made it successful.

Constraints:

i. AI increased inbreeding due to enhanced relatedness among individuals. This can be mitigated by semen exchange between cooperative herds or breeders.
ii. its usage in beef populations is limited due to factors like heat detection challenges and high costs.
iii. In sheep and goat breeding, the lack of simple insemination procedures hinders AI adoption, although new techniques are emerging.
iv. In developing countries, AI adoption is limited by high costs relative to output value, environmental stresses, and inefficient transport.

b. Embryo Transfer Technologies

Embryo transfer (ET) involves collecting embryos from donor females and transferring them to recipient females.

There are two main procedures for embryo production:

i. Super-ovulation of the donor, followed by embryo recovery and transfer to recipients.
ii. In vitro fertilization (IVF), where eggs are collected from ovaries, fertilized in a lab, and then transferred to recipients. IVF allows for the use of thousands of eggs that do not develop to ovulation, increasing offspring per female and potentially decreasing generation interval. Although IVF is expensive and with a low success rate, but it facilitates the recovery of a large number of embryos from a single female at a reduced cost thus making ET techniques economically feasible on a

large scale. Advancements in ET techniques have made it possible to recover embryos non-surgically and freeze them for later use.

Multiple ovulation and embryo transfer (MOET) offer several benefits:

i. **Increased Genetic Gain**: MOET boosts genetic progress by producing multiple progeny from a single female, enhancing female selection intensity and accuracy.
ii. **International Trading:** Shipping embryos reduces the cost and risk of disease transmission compared to live animal transport.
iii. **Transmission of Genetic Improvement**: MOET facilitates the dissemination of genetic improvement from elite to commercial sectors of livestock industries.
iv. **Genetic Conservation**: MOET allows for the rapid expansion and preservation of rare or endangered breeds.
v. MOET is a valuable tool for genetic improvement and conservation in livestock breeding.

Constraints

i. MOET can lead to increased inbreeding, especially in small populations.
ii. more expensive and less effective than artificial insemination (AI),

c. Semen and Embryo Sexing

Efforts to sex semen date back to the early 1920s, with Prof Lush's unsuccessful attempts noted. Traditional methods relied on physical differences between X- and Y-chromosome-bearing sperm, but success rates were low. Flow cytometry (a technique used to detect and measure the physical and chemical characteristics of a population of cells or particles), developed later, and has proven more accurate due to its ability to differentiate sperm based on slight DNA content differences. Sexing embryos involves removing cells at later developmental stages and confirming sex through karyotyping (a test to examine chromosomes in a sample of cells) or detecting sex-specific DNA sequences.

Benefits:

i. **Increased Selection Intensity:** Sexing can increase selection intensity for females in species with traits limited to females, like milk or egg production. However, it may reduce male selection intensity, especially when performance records exist for both sexes.
ii. **Faster Dissemination of Genetic Improvement:** Cost-effective sexing techniques for semen could greatly improve genetic improvement dissemination through conventional AI.
iii. Producing large numbers of inexpensive, sexed embryos could enhance genetic improvement dissemination.

In summary, advancements in sexing techniques hold promise for accelerating genetic gain and dissemination in animal breeding programs.

d. Embryo Cloning

Embryo cloning, or embryo multiplication, involves producing groups of genetically identical embryos from a single original embryo. This is done in two main ways:

i. Embryo splitting creates twin embryos from one original, which are then transferred to recipient females. However, splitting into more than two parts is not usually successful due to limited cells available for normal development.
ii. Nuclear transplantation involves transferring nuclei from early embryos to unfertilized eggs, resulting in the development of a new embryo as if newly fertilized. This technique allows for the production of a greater number of identical animals from elite parents.

Until the mid-1990s, nuclear transfer for cloning used early embryos. Later, scientists were able to create viable cloned embryos and live cloned animals by transferring cells cultured in labs, originally from embryos. This method allows for the production of more identical animals from a single pair of elite parents. In 1997, a viable lamb was successfully cloned using a cultured cell from an adult sheep's mammary tissue. These advancements could lead to higher success rates in cloning.

Benefits

i. Evaluation of individuals through their clones.
ii. Cloning elite individuals for industrial use (e.g., beef bulls for natural mating).
iii. Potential improvement in genetic evaluation accuracy, particularly on the female side, though the impact is modest.

Constraints

i. Risk of producing a large number of animals that may later prove susceptible to disease or unsuitable for new production methods. Common issues with animals from nuclear transfer embryos include abnormalities like extended gestation, increased stillbirth rates, and prenatal deaths.
ii. Cloning can reduce genetic variation as the population size decreases and inbreeding becomes more likely.
iii. The process of cloning is expensive and time-consuming.

Molecular Genetic Technologies

In the 1970s, molecular biology took a big leap forward. Scientists found ways to pinpoint important genes using tools like DNA markers.

Discovery of restriction enzymes: Bacteria have special enzymes called restriction enzymes that can cut DNA at specific spots. Each enzyme recognizes a different sequence of DNA letters. By using these enzymes, scientists can chop DNA into pieces of specific sizes. This helps in tasks like identifying genes and comparing DNA from different organisms.

Separating and detecting DNA fragments: After cutting DNA into pieces, scientists need to separate & see these fragments. They do this using a technique called electrophoresis. In this process, DNA pieces move through a gel at different speeds based on their size. Stained gels show bands representing DNA pieces of various sizes. By comparing these bands, scientists can spot similarities and differences in DNA samples.

Multiplying DNA sequences: Sometimes, scientists need many copies of a specific DNA piece for study. They achieve this through methods like molecular cloning and PCR (polymerase chain reaction). In cloning, DNA fragments are inserted into bacterial DNA, which then reproduces, making multiple copies of the original DNA. PCR, on the other hand, multiplies DNA in a test tube, quickly producing lots of copies from a tiny starting amount.

Advancements in DNA sequencing: Sequencing means figuring out the exact order of DNA letters (A, T, C, and G) in a strand. Initially, a method called Sanger sequencing was used, which produced fragments of varying lengths. These fragments were then analyzed to determine the DNA sequence. Later, "next generation sequencing" took over. This method fragments the genome and sequences many pieces at once, speeding up the process significantly. It's like reading many books at once, making DNA sequencing faster & more efficient.

GENETIC MATERIALS

Genetic material, like DNA and RNA, is essential for all living things. It carries instructions from one generation to the next. Human DNA is made of billions of nucleotides, arranged like ladder steps, determining traits. Along chromosomes, specific locations called loci contain sequences that guide protein building. Proteins, synthesized in the cell's cytoplasm, form every part of a living body. Genes, through their sequences, make proteins, which then create bodies. Genetic material is passed from parent to offspring, ensuring resemblance. Mutations, small errors in gene copying, drive evolution. DNA, found in the nucleus of eukaryotic cells and cytoplasm of prokaryotic cells, determines an organism's makeup. RNA, another genetic material, is found in cells and viruses.

Structure of Genetic materials

Genetic materials like DNA and RNA are made of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T) in DNA (or uracil (U) in RNA). These bases pair up: A with T (or U in RNA), and C with G. DNA is double-stranded, forming a spiral ladder-like structure called a double helix, with nucleotides as the ladder rungs and sugar-phosphate as the sides. DNA wraps around proteins called histones and forms chromosomes. RNA is structurally similar to DNA but single-stranded, with uracil replacing thymine. In RNA, A pairs with U, and C pairs with G.

Levels of organization in DNA structure replication, and technology

DNA, with its organized structure, serves as a handy tool for living organisms. At its core, it consists of four nucleotide bases—adenine, guanine, cytosine, and thymine—connected to a sugar and phosphate backbone. These nucleotides arrange in specific sequences, crucial for carrying genetic information. In organisms, these sequences form double-stranded DNA.

However, the sheer amount of DNA in eukaryotic cells exceeds their capacity. To manage this, DNA wraps around histone proteins, forming nucleosomes, which further coil into chromatin fibers. During cell division (mitosis and meiosis), this compaction ensures accurate distribution of genetic material to daughter cells.

Beyond division, in interphase, DNA packaging varies. Some regions tightly wrap to suppress gene activity, while others remain open for gene expression. This dynamic packaging regulates RNA transcription, essential for controlling gene functions.

Eukaryotic cells also feature a nucleus housing DNA, while protein-making machinery resides in the cytoplasm. This spatial separation adds another layer of regulation, ensuring precise gene expression.

Gene Organization and Expression

Eukaryotic cells and bacteria exhibit several key differences in gene arrangement, expression, and regulation:

i. **Genetic information quantity:** Eukaryotic cells, being more complex, contain significantly imore genetic material. For instance, a human cell holds around 1000 times more DNA than an E. coli cell. To accommodate this abundance,

eukaryotic DNA, stretching several centimeters in length, is tightly packed into a few micrometers, facilitated by histone proteins.

ii. **Spatial separation of processes:** Eukaryotic chromosomes reside within a nucleus enclosed by a nuclear membrane. Since protein synthesis occurs in the cytoplasm, transcription and translation sites are physically segregated. This spatial separation results in less direct coupling of these processes compared to bacteria.

iii. **RNA processing:** In eukaryotic cells, primary RNA transcripts undergo extensive modifications, cleavage, and splicing within the nucleus before being transported as mature mRNA to the cytoplasm

iv. **Gene expression regulation complexity:** Eukaryotes feature a more intricate and diverse regulation of gene expression. Multiple levels of control exist, including transcriptional and post-transcriptional mechanisms. Moreover, post-translational modifications further modulate the primary translation products.

Gene Expression

Gene expression is the process where information stored in a gene is utilized to produce a functional gene product, which can be a protein or a functional RNA in genes like tRNA or snRNA. This process is vital for all forms of life, as it creates the necessary machinery for life functions. It involves various steps such as transcription, RNA splicing, translation, and post-translational modification. Gene regulation, which controls these steps, is essential for structuring and functioning of cells, and it underpins processes like cellular differentiation, morphogenesis, and an organism's adaptability.

Moreover, gene regulation can influence evolutionary changes by controlling when, where, and how much a gene is expressed, thereby affecting its function in cells or organisms. In genetics, gene expression is pivotal as it links the genotype (genetic code stored in DNA) to the phenotype (observable traits). The way genes are expressed shapes an organism's phenotype, often through protein synthesis that influences its shape or catalyzes metabolic pathways. Therefore, regulating gene expression is crucial for an organism's development and survival.

Transcription

Transcription, performed by RNA polymerase in the nucleus, creates an RNA copy of DNA. This RNA is made one nucleotide at a time, following the complementarity rule with the DNA template. The resulting RNA is identical to the coding DNA strand, except that thymine (T) is replaced by uracil (U). For example, "ATG" in DNA becomes "AUG" in RNA. In prokaryotes, this RNA is ready for translation into protein, but in eukaryotes, it becomes a pre-mRNA that needs modifications to become mature mRNA. One crucial modification is RNA splicing, where introns are removed, and exons are joined together by the spliceosome complex. This alternative splicing can produce various mRNA versions from a single gene, increasing gene expression complexity. Most mature RNA in eukaryotes is exported from the nucleus to the cytoplasm, including RNA involved in protein synthesis. During translation, tRNA brings amino acids to the ribosome, aligning them with the correct mRNA triplet, and the ribosome links them to form a protein chain.

Folding

Proteins undergo a transformation from a disordered, linear chain of amino acids, known as a random coil, into their functional, three-dimensional structures. This process is crucial for their proper function. The sequence of amino acids dictates how the protein folds into its characteristic shape, called the native state. This folding is driven by interactions between amino acids. While the correct three-dimensional structure is vital for function, some parts of functional proteins may remain unfolded. If a protein fails to fold correctly, it can become inactive or even harmful, as seen in toxic prions and various diseases and allergies caused by misfolded proteins.

Translocation

Both eukaryotic and prokaryotic cells require secretory proteins to enter the secretory pathway. In eukaryotes, signal peptides guide newly synthesized proteins to the translocation channel, crucial for efficient protein secretion. Different proteins have diverse destinations within the cell, and various signaling sequences direct them accordingly. While prokaryotes have a simpler process due to less cell compartmentalization, eukaryotes employ various targeting mechanisms to ensure proteins reach the correct organelles. Many proteins are exported from the cell, and eukaryotes have a well-developed export pathway. Proteins first move to the endoplasmic reticulum and then through the Golgi apparatus. This process ensures accurate protein delivery, essential for cellular function.

Gene Regulation

Gene regulation controls the activation and deactivation of genes. It influences various stages of gene expression, from the beginning of transcription to the final protein modifications. Cells employ diverse mechanisms to adjust the production of specific gene products, termed gene regulation. This regulation is crucial for several biological processes:

i. Initiating developmental pathways.
ii. Timing the expression of genes appropriately
iii. Responding to changes in the environment.
iv. Adapting to new nutritional sources.
v. Enhancing an organism's versatility and adaptability by enabling protein expression as needed.

Driving cellular differentiation and morphogenesis, creating different cell types with unique gene expression profiles and structures tailored to their functions.

Gene expression regulation occurs at multiple stages:

i. Chromatin domain organization
ii. Transcriptional control.
iii. Post-transcriptional modifications.
iv. RNA transportation.
v. Translation.
vi. mRNA degradation.

These stages allow cells to finely tune gene expression, ensuring proper cellular function and response to internal and external signals.

BASIC PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY

When scientists combine nucleic acid sequences in a lab, they create recombinant DNA. This DNA can either contain sequences from the same species (cisgenic) or from different species that wouldn't naturally breed (transgenic). The process involves several steps:

i. Gene Cloning and Recombinant DNA Formation: DNA from a source is cut and joined with other DNA, like a cloning vector, to create recombinant DNA.

ii. Vector Transfer into Host: The recombinant DNA is transferred into a host cell, often done through a process called transformation in bacteria.

iii. Selection of Transformed Cells: Cells that take up the recombinant DNA are identified and selected.

iv. Transcription and Translation of Inserted Gene: The inserted gene is transcribed into RNA and translated into a protein.

Genetic engineering does not always involve creating recombinant DNA; advancements in gene transfer allow DNA to be transferred directly to hosts without vectors. These manipulations create individuals with new inherited traits, achieved through cellular or molecular methods. Cellular manipulation involves cell culturing and somatic cell hybridization, while molecular manipulation constructs recombinant DNA molecules inserted into vectors and established in host cells. This approach is often referred to as recombinant DNA (rDNA) technology. The terms "genetic engineering" and "rDNA technology" are often used interchangeably, but they both involve manipulating genes or transferring constructed genes.

Gene Isolation/ Gene Cloning

Genes are fundamental units within cells that carry traits from parents to offspring. Traits are heritable characteristics in organisms. Genes consist of sequences of bases on a DNA strand, with many genes found along chromosomes. Each gene codes for a single polypeptide, which is a chain of amino acids linked together. A polypeptide can form the primary structure of a simple protein, or multiple polypeptides can combine to create more complex proteins.

Gene cloning involves locating and copying a specific gene from DNA extracted from an organism. This process is commonly used in molecular biology labs to produce multiple copies of a gene for various applications, such as sequencing, mutagenesis, genotyping, or expressing a protein in different organisms.

Sequencing involves putting things in order, like arranging steps in a process. In molecular biology, it refers to determining the order of nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA oligonucleotide, which is a small chain of nucleic acid residues. Each nucleotide consists of a phosphate group, a sugar (ribose in RNA or deoxyribose in DNA), and one of four nucleobases.

Mutagenesis is a process that changes an organism's genetic information in a stable way, leading to a mutation. This can happen naturally or due to exposure to mutagens.

Genotyping involves analyzing an individual's DNA sequence to determine differences in their genetic makeup compared to others or a reference sequence. It helps identify the alleles inherited from parents.

Heterologous expression refers to expressing a gene or part of a gene in a host organism that doesn't naturally possess it. This is done using recombinant DNA technology to insert the gene into the host's genome.

Cloning

Cloning involves creating exact genetic copies of organisms or cells, whether naturally occurring (like identical twins) or done artificially in a lab. It can replicate genes, cells, tissues, or entire organisms.

There are two main methods for cloning genes:

i. In vivo: This method uses restriction enzymes and ligases to cut DNA fragments containing genes and insert them into vectors, which can then amplify the genes in host cells.
ii. In vitro: This involves using the polymerase chain reaction (PCR) to create copies of DNA fragments in a lab setting.

There are three types of cloning:

i. Molecular cloning (or gene cloning): This focuses on making identical copies of DNA molecules.
ii. Organism cloning (or reproductive cloning): This creates identical copies of entire organisms.
iii. Therapeutic cloning: This involves cloning human embryos to produce stem cells, but the embryos are ultimately destroyed in the process.

Methods for gene isolation and cloning include:

i. Cloning by complementation.

Cloning by complementation is a common method for isolating genes. It involves cloning a dominant allele of the gene of interest into a host strain carrying a recessive allele. Transformants are then screened for the phenotype associated with the dominant allele.

ii. Positional cloning.

Positional cloning relies on the physical proximity of a cloned DNA fragment to isolate the gene of interest. This method involves moving step-by-step from the cloned fragment toward the gene of interest using a process called chromosome walking. Primer walking, a sequencing method, is often used for this purpose.

iii. Cloning by sequence.

Cloning by sequence homology utilizes shared ancestry between genes in different species. This method isolates genes based on their evolutionary conservation or similarity to known sequences. It can be used to isolate homologous genes from different species or members of a gene family from a particular genome.

Vectors

In traditional medicine, a vector refers to an organism that spreads infection without causing disease itself. In gene therapy, a virus can serve as a vector, delivering a gene to a target cell after being re-engineered. In this context, a vector acts as a carrier for genetic material like DNA. It is a section of DNA capable of incorporating another DNA fragment while still being able to replicate itself. When a vector carries an additional DNA fragment, it is called a hybrid vector.

Characteristics of a cloning vector include:

i. being relatively small for easy manipulation,
ii. capable of replication in living cells to amplify the inserted DNA,

iii. containing convenient restriction sites for DNA insertion,
iv. preferably with unique sites for targeted insertion, and
v. having mechanisms for easy identification and recovery of the recombinant molecule.

The choice of vector depends on factors like the size of the DNA sample being cloned and the ease of manipulating the vector.

There are four main types of vectors:

i. Plasmid: Small circular DNA molecules found in bacteria, replicating independently from the bacterial chromosome.
ii. Bacteriophage (phage): Viruses that infect and replicate within bacteria.
iii. Cosmids and fosmids: Specialized plasmids able to carry larger DNA fragments.
iv. Expression vectors: Used to detect protein expression in bacterial cells, requiring the ability to transcribe and translate the gene into protein. These vectors are necessary for cloning genes for expression in bacteria, but cloned sequences must be devoid of introns since bacteria cannot process them.

Splicing of introns

In genetics, splicing is a process where introns (non-coding regions) are removed from the nascent pre-messenger RNA (pre-mRNA) transcript, and the exons (coding regions) are joined together. This modification ensures that the final RNA molecule contains only the coding regions necessary for protein synthesis.

RNA splicing involves retaining the exons while removing the introns from the RNA strand. Sometimes, exons can be recombined to create alternative splicings, which can have different functional effects.

Introns are regions within a gene that are removed during RNA processing, and they can be found in genes across various organisms and viruses. They are removed from the pre-mRNA transcript by RNA splicing, either during or shortly after transcription.

Exons, on the other hand, are nucleotide sequences within a gene that remain present in the final mature RNA product after introns have been removed. They contain the coding information needed to produce proteins. During RNA splicing, introns are removed, and exons are joined together to generate the mature messenger RNA.

TRANSGENSIS (GENE TRANSFER)

Transgenesis, or gene transfer, is a technique where specific genes from one species are permanently added to the genome of another species. This involves inserting DNA coding for these genes into the DNA of the recipient species, usually animals. The added genes, known as transgenes, differ from the organism's own genes. Animals containing these introduced genes are called transgenic animals. The tools commonly used in inserting the foreign DNA into embryos are viruses, plasmids, or gene guns. As the embryo develops, the inserted DNA becomes part of its genome. The resulting organism expresses the added genes, and these modifications can be passed on to its offspring through breeding.

A genetically modified organism (GMO) is any organism whose genetic makeup has been altered using genetic engineering techniques, including transgenesis. This alteration results in organisms with novel combinations of genetic material, achieved through modern biotechnology methods.

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 are single genes exerting major effect on trait- phenotype.

(ii). Production of valuable pharmaceutical products: There are a number of proteins 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.

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

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 process where a cell naturally takes in new genetic material, merging it with its own DNA to express the received genes. This can happen naturally or through artificial means. In the natural process, foreign DNA binds to the cell's receptors and is ushered inside by DNA translocase proteins. Enzymes called nucleases help control the entry, ensuring only one DNA strand gets in and integrates with the host's DNA.

Artificial transformation occurs in controlled settings, often using chemicals or electricity. Chemical methods involve treating cells with substances like calcium chloride and subjecting them to temperature changes to make their membranes more permeable to foreign DNA. Electroporation, on the other hand, employs brief electric pulses to create temporary pores in the cell membrane, through which the new DNA can enter. Once inside, the cell repairs these pores, sealing itself back up.

b. Transduction

There are two types of transduction: generalized, where any bacterial gene can be transferred,

and specialized, which involves the transfer of specific genes. In transduction, genes are transferred between bacterial cells using viruses as messengers. These viruses serve as tools to insert foreign DNA into target organisms. Transduction happens in two phases: lysogenic and lytic.

During the lysogenic phase, viral DNA joins with bacterial DNA and remains dormant across generations. External factors like UV light can trigger the switch to the lytic phase. In the lytic phase, viral DNA operates independently within the host cell, causing it to produce numerous viruses. Eventually, the host cell bursts open, releasing the viruses to infect other cells. This process can lead to gene exchange between viral and bacterial DNA. Newly formed viruses leaving the cell may carry bacterial genes to infect other cells. Some viral genes may also integrate into the host cell's genome.

d. Transfection

In transfection, 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 mechanical 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

d. Other methods of gene transfer techniques include:

i. Conjugation: This process involves direct cell-to-cell contact, facilitating the transfer of plasmids from donor to recipient cells. Bacterial conjugation includes pairing of cells, synthesis of conjugal DNA, DNA transfer, and maturation.

ii. **DNA Microinjection:** A desired gene construct is injected into the pronucleus of a reproductive cell using a tiny glass needle. The manipulated cell is then cultured in vitro to a specific embryonic stage before being transferred to a recipient female.

iii. **Retrovirus-mediated Gene Transfer**: Retroviruses carry genetic material in RNA form and are used as vectors to transfer genes into host cells. This results in chimeras, organisms composed of tissues or parts with different genetic constitutions caused by fusion of embryos in the womb or artificially through genetic engineering techniques.

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).