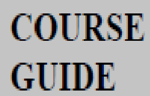

ANP 504
BIOTECHNOLOGY IN ANIMAL PRODUCTION

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INTRODUCTION

ANP 504: Biotechnology in Animal Production. The course is a two (2) credit unit course designed for 500 level undergraduate students pursuing a degree in Agricultural Science. The course is expected to provide a good knowledge base for the production of healthy animal/ improvement of Nigerian livestock resources towards a sustainable production of livestock. It explains the following: Definition of biotechnology / recombinant DNA technology / genetic engineering. Tools: cloning, marker-assisted selection, QTL mapping, physical and genetic mapping, transgenes, transgenesis, gene banks, gene sequencing, DNA fingerprinting, bio- informatics. Application of biotechnology in animal and feed production, nutrition, reproduction, genetics and breeding, disease and metabolic control, pollution abatement and environmental management. Use of DNA tests for meat quality genotype identification, paternity testing, disease diagnostics. Conservation of animal genetic resources, ex *situ* and *in situ*. The course will provide a basic foundation for students intending to take up Biotechnology in Animal Production as a Career in the future. The course is divided into units. Each unit begins with a clear introduction and statement of objectives followed by the main content. The conclusion, summary and references (for further reading) were also provided for each unit. Tutor marked assignments were provided for each unit to enable you attempt some questions on the topics treated for onward submission to your tutor. The Course Guide provides you with access to brief information and overview of the course content, course duration, what you are expected to know in each unit, what course material you need to use and how you can systematically go through the course materials. Thus, we intend to achieve the above through the following broad aim and other specific objectives.

MAIN COURSE

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UNIT 1: DEFINITION OF BIOTECHNOLOGY / RECOMBINANT DNA TECHNOLOGY / GENETIC ENGINEERING.

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1.1 Introduction

People have been harnessing biological processes to improve their quality of life for some

10,000 years, beginning with the first agricultural communities. Approximately 6,000 years ago, humans began to tap the biological processes of microorganisms in order to make bread, alcoholic beverages, and cheese and to preserve dairy products. But such processes are not what is meant today by biotechnology, a term first widely applied to the molecular and cellular technologies that began to emerge in the 1960s and '70s. A fledgling ―biotech‖ industry began to coalesce in the mid- to late 1970s, led by Genentech, a pharmaceutical company established in 1976 by Robert A. Swanson and Herbert W. Boyer to commercialize the recombinant DNA technology pioneered by Boyer, Paul Berg, and Stanley N. Cohen. Early companies such as Genentech, Amgen, Biogen Cetus, and Genex began by manufacturing genetically engineered substances primarily for medical and environmental uses.

For more than a decade, the biotechnology industry was dominated by recombinant DNA technology, or genetic engineering. This technique consists of splicing the gene for a useful protein (often a human protein) into production cells—such as yeast, bacteria, or mammalian cells in culture—which then begin to produce the protein in volume. In the process of splicing a gene into a production cell, a new organism is created. At first, biotechnology investors and researchers were uncertain about whether the courts would permit them to acquire patents on organisms; after all, patents were not allowed on new organisms that happened to be discovered and identified in nature. But,

in 1980, the U.S. Supreme Court, in the case of Diamond v. Chakrabarty, resolved the matter by ruling that ―a live human-made microorganism is patentable subject matter.‖ This decision spawned a wave of new biotechnology firms and the infant industry's first investment boom. In 1982 recombinant insulin became the first product made through genetic engineering to secure approval from the U.S. Food and Drug Administration (FDA). Since then, dozens of genetically engineered protein medications have been commercialized around the world, including recombinant versions of growth hormone, clotting factors, proteins for stimulating the production of red and white blood cells, interferons, and clot- dissolving agents.

1.2 Objectives

- You will understand biotechnology / recombinant DNA technology / genetic engineering.

1.3 Main content

1.3.1 Biotechnology

Biotechnology is the use of biological systems found in organisms or the use of the living organisms themselves to make technological advances and adapt those technologies to various different fields. These include applications in various fields from agricultural practice to the medical sector. It does not only include applications in fields that involve the living, but any other field where the information obtained from the biological aspect of an organism can be applied.

"Biotechnology is technology based on biology – biotechnology harnesses cellular and biomolecular processes to develop technologies and products that help improve our lives and the health of our planet. We have used the biological processes of microorganisms for more than 6,000 years to make useful food products, such as bread and cheese, and to preserve dairy products."

Biotechnology can be applied in nutrient supplementation, a biotic stress resistance, industry, strength fibres, biofuels and healthcare

1.3.2 Recombinant DNA technology

Recombinant DNA technology is defined as joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry. Since the focus of all

genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes. Although it is relatively easy to isolate a sample of DNA from a collection of cells, finding a specific gene within this DNA sample can be compared to finding a needle in a haystack. Consider the fact that each human cell contains approximately 2 metres (6 feet) of DNA. Therefore, a small tissue sample will contain many kilometres of DNA. However, recombinant DNA technology has made it possible to isolate one gene or any other segment of DNA, enabling researchers to determine its nucleotide sequence, study its transcripts, mutate it in highly specific ways, and reinsert the modified sequence into a living organism.

1.3.3 Genetic engineering

Genetic engineering is the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms.

The term genetic engineering initially referred to various techniques used for the modification or manipulation of organisms through the processes of heredity and reproduction. As such, the term embraced both artificial selection and all the interventions of biomedical techniques, among them artificial insemination, in vitro fertilization (e.g., ―test-tube‖ babies), cloning, and gene manipulation. In the latter part of the 20th century, however, the term came to refer more specifically to methods of recombinant DNA technology (or gene cloning), in which DNA molecules from two or more sources are combined either within cells or in vitro and are then inserted into host organisms in which they are able to propagate.

1.4 Conclusion

Decades of documented evidence demonstrates that agricultural biotechnology is a safe and beneficial technology that contributes to both environmental and economic sustainability. Farmers choose biotech crops and animals because they increase yield and lower production costs. Farmers get a greater financial return while using more environmentally friendly farming practices through the use of agricultural biotechnology, genetic engineering and recombinant DNA technology

1.5 Summary

- Biotechnology is the use of biological systems found in organisms or the use of the living organisms themselves to make technological advances and adapt those technologies to various

different fields

- Recombinant DNA technology is defined as joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry
- Genetic engineering is the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms

1.6 Tutor marked assignment

i. Define biotechnology and give example of how it is being used
ii. Define genetic engineering
iii. Define recombinant DNA technology and comment on its importance

1.7 References/ Further Reading

https://www.britannica.com/science/recombinant-DNA-technology
https://www.britannica.com/technology/biotechnology

UNIT 2: GENETIC ENGINEERING TOOLS: CLONING, MARKER-ASSISTED SELECTION, QTL MAPPING, PHYSICAL AND GENETIC MAPPING, TRANSGENES, TRANSGENESIS, GENE BANKS, GENE SEQUENCING, DNA FINGERPRINTING, BIO-INFORMATICS.

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- 2.3.9 DNA fingerprinting
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2.1 Introduction

Genetic engineering can be accomplished using multiple techniques. There are a number of steps that are followed before a genetically modified organism (GMO) is created. Genetic engineers must first choose what gene they wish to insert, modify or delete. The gene must then be isolated and incorporated, along with other genetic elements, into a suitable vector. This vector is then used to insert the gene into the host organism, creating the GMO. The ability to genetically engineer organisms is built on years of research and discovery on how genes function and how we can manipulate them. Following the discovery of genes by Gregor Mendel and the proof that they were involved in inheritance, tools were developed that allowed their direct manipulation.

Important advances included the discovery of restriction enzymes and DNA ligases and the development of polymerase chain reaction and sequencing.

However, in breeding programmes much work has been done on alteration of nucleotides (changing of genes) by several parasexual or conjugational methods in different organisms. Now, a large number of mutagenic chemicals are available which mutate the genes. It is likely that the changed genes may be beneficial, neutral or lethal. In recent years, protoplast fusion and somatic hybridization have also become a tool in breeding programmes for conferring genes of beneficial properties. The conventional breeding techniques take a long time to assure the changed gene in progeny and its role for human beings.

Genetic engineering has been applied for the production of valuable polypeptides, insulin, interferon, growth hormones, and in the transfer of Nit genes, control of genetic diseases, etc. There are various biological tools which are used to carry out manipulation of genetic material and cells as well, for example, enzymes, foreign or passenger DNA, vector or vehicle DNA, DNA bank and gene bank.

2.2 Objectives

- You will understand, identify, discuss and differentiate between the various genetic engineering tools

2.3 Main content

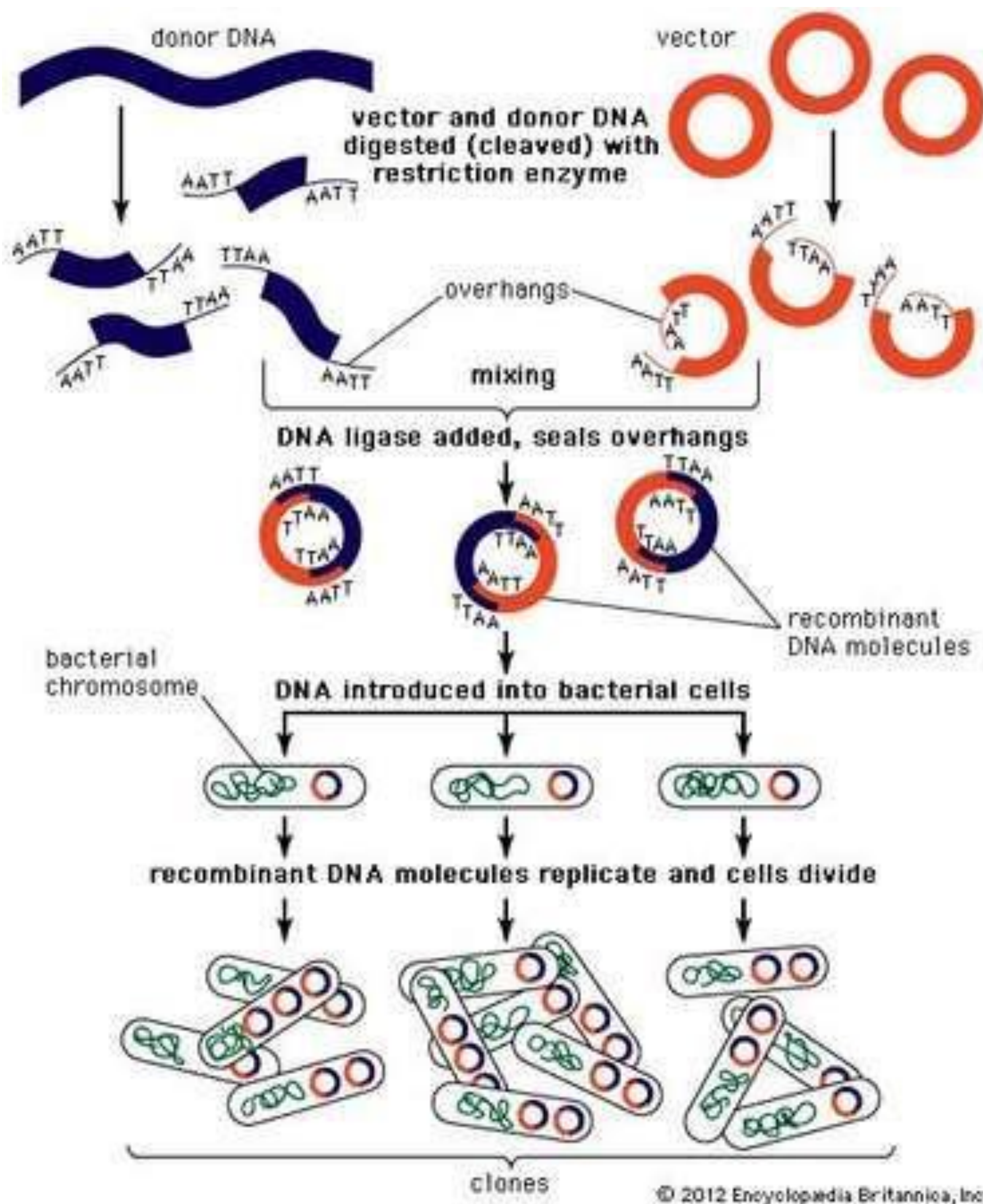
2.3.1 Cloning

In biology, a clone is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. Molecular cloning refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic fingerprinting to large scale protein production. The use of the word clone has been extended to recombinant DNA technology, which has provided scientists with the ability to produce many copies of a single fragment of DNA, such as a gene, creating identical copies that constitute a DNA clone. In practice the procedure is carried out by inserting a DNA fragment into a small DNA molecule and then allowing this molecule to replicate inside a simple living cell such as a bacterium. The small replicating molecule is called a DNA vector (carrier). The most commonly used vectors are plasmids (circular DNA molecules that originated from bacteria), viruses, and yeast cells. Plasmids are not a part of the main cellular genome, but they can

carry genes that provide the host cell with useful properties, such as drug resistance, mating ability, and toxin production. They are small enough to be conveniently manipulated experimentally, and, furthermore, they will carry extra DNA that is spliced into them Cloning of any DNA fragment essentially involves four steps namely

1. fragmentation - breaking apart a strand of DNA
2. ligation - gluing together pieces of DNA in a desired sequence
3. transfection – inserting the newly formed pieces of DNA into cells
4. screening/selection – selecting out the cells that were successfully transfected with the new DNA

The process is as shown in the following figure.



2.3.2 Marker-assisted selection

Marker assisted selection or marker aided selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself. This process has been extensively researched and proposed for plant and animal breeding, nevertheless, as of 2013 "breeding programs based on DNA markers for improving quantitative traits in plants are rare". Marker Assisted Selection [MAS] refers to indirect selection for a desired plant phenotype based on the banding pattern of linked molecular (DNA) markers. MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker which is tightly linked to the gene of interest. If the marker and the gene are located far apart then the possibility of their transmission together to the progeny individuals will be reduced due to double crossover recombination events.

For example, using MAS to select individuals with disease resistance involves identifying a marker allele that is linked with disease resistance rather than the level of disease resistance. The assumption is that the marker associates at high frequency with the gene or quantitative trait locus (QTL) of interest, due to genetic linkage (close proximity, on the chromosome, of the marker locus and the disease resistance-determining locus). MAS can be useful to select for traits that are difficult or expensive to measure, exhibit low heritability and/or are expressed late in development. At certain points in the breeding process the specimens are examined to ensure that they express the desired trait. MAS is applicable for genetic improvement of plants as well as animals. In plants, it is equally applicable in both self-pollinated and cross pollinated species.

MAS makes use of various types of molecular markers. The most commonly used molecular markers include amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) or micro satellites, single nucleotide polymorphisms (SNP), etc. The use of molecular markers differs from species to species also.

The marker aided selection consists of five important steps, viz: (i) Selection of parents, (ii) Development of breeding population, (iii) Isolation of DNA from each plant, (iv) Scoring RFLPs, and (v) Correlation with morphological traits.

Important applications of MAS in plant or animal breeding are briefly presented below:

i. MAS are very effective, efficient and rapid method of transferring resistance to biotic and abiotic stresses in crop plants.
ii. It is useful in gene pyramiding for disease and insect resistance.
iii. It is being used for transfer of male sterility and photo period insensitivity into cultivated genotypes from different sources.
iv. MAS are being used for improvement of quality characters in different crops such as for protein quality in maize, fatty acid (linolenic acid) content in soybean and storage quality in vegetables and fruit crops.
v. MAS can be successfully used for transferring desirable transgene (such as Bt gene) from one cultivar to another.
vi. MAS is very effective in introgression of desirable genes from wild into cultivated genotypes.
vii. MAS are equally effective in genetic improvement of plants and animals.

viii. MAS are useful in genetic improvement of tree species where fruiting takes very long time (say 20 years) because for application of phenotypic selection we have to wait for such a long time.
ix. MAS have wide application for genetic improvement of oligogenic traits as compared to polygenic traits.

2.3.3 QTL mapping

A quantitative trait locus (QTL) is a locus (section of DNA) which correlates with variation of a quantitative trait in the phenotype of a population of organisms. QTLs are mapped by identifying which molecular markers (such as SNPs or AFLPs) correlate with an observed trait. A QTL is defined as “a region of the genome or locus of gene that is associated with an effect on a quantitative trait’’. A quantitative trait which is controlled by several genes, all the genes having small effects, additive in nature and is affected by environment. The phenotypes of quantitative trait are typically depicted by a bell curve.

QTL mapping is process of locating genes with effects on quantitative traits using molecular markers. A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s.

The basic Principle is the co- segregation of marker locus and QTL together. Co segregation is due to linkage between marker and QTL.QTL analysis depends on linkage disequilibrium which is the non-random association of alleles at different loci in a given population.

The QTL mapping strategies are choose divergent parents, Screen for marker loci polymorphism create mapping populations for generating saturated linkage map, phenotype screening and Contrast the mean of the marker attached and pair of gene of interest at every marker locus.

Methods for QTL mapping

Single marker approach (SMA): This is also called as Single factor analysis of variance or single point analysis. It is widely used method for quick scanning of whole genome to determine best QTLs. It is used for each marker locus which is free from other loci. This technique is unable to determine QTL position.

Some major limitations of this approach

1. The method cannot determine whether the markers are associated with one or more QTLs.
2. Chance of QTL detection decreases with distance between marker and QTL.
3. An effect of QTL is underestimated of confounding with recombination frequencies.
4. Its accuracy is less compare to other methods

Simple interval mapping (SIM): SIM was first proposed by Lander and Botstein (1989) based on linkage map and also called as two marker approach. QTL is determined in interval generated between two markers at various points. It gives more accurate results compare to single marker approach but less than CIM and MIM technique. In this technique, likelihood ratio test is used to determine every QTL position in interval created by both markers. SIM is mostly preferred as it can be easily performed through statistical packages such as MAPMAKER (Lander *et al*., 1987) and JOIN MAP (Stam, 1993). Lander and Botstein (1989) developed formulae for significance levels appropriate for interval mapping when the genome size, number of chromosomes, number of marker intervals, and the overall false positive rate desired are given.

Composite interval mapping (CIM) CIM techniques are developed by Zeng, 1994.It is used to minimize effects of various linked QTLs. It is based on one QTL and other markers used as covariates. This technique gives more precise results and used to exclude bias due to another QTLs (nontarget QTLs) linked to target QTL It used to fit the parameters for a single QTL in one interval. The partial regression coefficient is used to

determine genetic variance due to non-target QTLs.

Merits of CIM are as follows:

1. Mapping of multiple QTLs can be carried out by the search in one dimension.
2. By using linked markers as covariates, the test is not affected by QTL out of region, thereby increasing the precision of QTL mapping and by eliminating as much as the genetic variance produced by other QTL, the residual variance is reduced, thereby the efficiency of determination of QTL is increased.
3. CIM is more efficient than SIM, but not widely used in QTL mapping as in SIM.

Multiple intervals mapping (MIM): It is recent method of QTL Mapping.MIM techniques are developed by Jansen and Stam (1994). Multiple Interval Mapping (MIM) is the extension of interval mapping to multiple QTLs, just as multiple regression extends analysis of variance. It is used to map multiple QTLs. This method is potential tool for detection of QTL X QTL interaction.

Inclusive composite interval mapping (ICIM): An approach to QTL (quantitative trait locus) mapping for populations derived from bi parental crosses. QTL mapping is based on genetic linkage map and phenotypic data.To locate individual genetic factors on chromosomes and to estimate their genetic effects.

ICIM has been successfully used in Wild and cultivated soybeans in mapping conserved salt tolerance QTL. Rice mapping tiller angle QTL and grain length QTL Wheat mapping flour and noodle, color components and yellow pigment content and adult-plant resistance to stripe rust QTL.

ICIM extended to map Maize Nested Association Mapping design recently proposed by the

Buckler laboratory at Cornell University.

2.3.4 Physical and genetic mapping

Genetic and **physical maps** illustrate the arrangement of **genes** and DNA markers on a chromosome. The relative distances between positions on a **genetic map** are calculated using recombination frequencies, whereas a **physical map** is based on the actual number of nucleotide pairs between loci.

Mapping of genes allows us to understand the arrangement of genes, to identify genes responsible for the expression of a particular phenotype and to identify the mutated gene responsible for a specific variant. They are useful in the field of molecular biology for performing map-based cloning and marker-aided selection.

Genetic mapping gives us an idea on relative location of a gene on a chromosome which is calculated based on the recombination frequencies. The distance on genetic maps is denoted in centimorgans (cM). Physical map indicates the physical distance of the loci based on the number of base pairs present between the loci.

High resolution physical and genetic maps serve as valuable tools in whole genome alignment and assembly.

Genetic maps

Genetic maps are constructed based on information derived from recombination frequencies between genetic markers (unique DNA sequences on a gene).

Genetic mapping demonstrates the arrangement of genes and the distance between the genes on a chromosome. Gene mapping gives us firm evidence on genetic disorders that are associated with one or more genes. It helps us to identify the chromosome on which the gene is present and the exact location of the gene on the chromosome.

Genetic mapping has helped the scientists to identify single gene inherited disorders such as cystic fibrosis and muscular dystrophy. They have also played an important role in identifying genes responsible for common diseases like diabetes, cancer, and asthma.

Physical maps

Physical maps are simply an alignment of DNA sequences. Physical maps give an idea of the actual distance of the genetic marker and the distance of the base pairs on a gene. They are constructed using three methods: cytogenetic mapping, radiation hybrid mapping, and sequence mapping. Identifying the location of the gene helps us understand the nature of disease, whether the disease is genetically inherited or is caused by just random mutation on the gene.

2.3.5 Transgenes

A transgene is a gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another. The introduction of a transgene has the potential to change the phenotype of an organism. Transgenes are pieces

of genetic material that are used to modify the genome of a certain organism. The modification of the organism's phenotype is also possible through the use of transgenes. In order to function properly, transgenes require several key components. The promoter is a regulatory sequence that determines where and when the transgene will be activated, while the exon is in charge of protein-coding sequence and the stop sequence. A third element is the bacterial plasmid that is used to deliver these components to the host genome.

Transgenic organisms have the important role of expressing various genes, which may make them vulnerable to specific disorders or conditions that researchers aim to study. The valuable research gathered from the development of humanized transgenic mice, for example, has been used to develop new treatments for cancer and other, equally debilitating disorders. Advancements in molecular biology allow for new models to be developed, helping scientists study the human genome more closely and conditions such as aging, diabetes, infertility and immune response. Although there are many examples, perhaps the most significant is the use of transgenes in plants and food. Corn, cotton and rapeseed are just a few of the plants that have been genetically modified for the purpose of increasing the yield and the health of crops as much as possible. New mouse models are also being developed on a regular basis, the uncommon use being an entirely new genetically modified mouse species developed for the study of cancer. Researchers also point to potential applications like the xenotransplantation of organs, the development of artificial protein-based foods and a cure for fertility-related genetic disorders

2.3.6 Transgenesis

Transgenesis is the process of introducing an exogenous gene – called a transgene – into a living organism so that the organism will exhibit a new property and transmit that property to its offspring.

Why use transgenesis instead of selective breeding?

1. It is more specific — scientists can choose with greater accuracy the trait they want to establish. The number of additional unwanted traits can be kept to a minimum.
2. It is faster — establishing the trait takes only one generation compared with the many generations often needed for traditional selective breeding, where much is left to chance.
3. It is more flexible — traits that would otherwise be unavailable in some animals or plants may be achievable using transgenic methods.
4. It is less costly — much of the cost and labour involved

in administering feed supplements and chemical treatments to animals and crops could be avoided.

Uses of transgenesis

1. In toxicology: as responsive test animals (detection of toxicants)
2. In mammalian developmental genetics;
3. To introduce human genes into other organisms (particularly human) for the study of disease processes;
4. In molecular biology, the analysis of the regulation of gene expression;
5. In the pharmaceutical industry, the production of human pharmaceuticals in farm animals ("pharming"); targeted production of pharmaceutical proteins, drug production and product efficacy testing;
6. In biotechnology: as producers of specific proteins;
7. Genetically engineered hormones to increase milk yield, meat production; genetic engineering of livestock in agriculture affecting modification of animal physiology and/or anatomy; cloning procedures to reproduce specific blood lines;
8. To speed up the introduction of existing characters into a strain/breed for improvement and modification;
9. Developing animals specially created for use in xenografting, ie. modify the antigenic make-up of animals so that their tissues and organs can be used in transfusions and transplants.

2.3.7 Gene banks

Gene banks are a type of biorepository which preserve **genetic** material. For plants, this could be by in vitro storage, freezing cuttings from the plant, or stocking the seeds (e.g. in a seed bank). For animals, this is the freezing of sperm and eggs in zoological freezers until further need. In addition to preserving animal and crop diversity useful to future agriculture, gene banks can also contribute directly to improve the livelihoods of farming communities. For example, several gene banks have restored lost and forgotten landraces and farmer varieties to communities from which they were collected. Many are also working directly with national breeding programs and farmers in participatory breeding and selection activities to help them adapt their farming systems to the challenges of climate change. Gene banks and seed banks can be setup at a local level, or they can be setup on a national or even international level. One famous seed bank is located in the Arctic Circle called the Svalbard Global Seed Vault. This international seed bank, located between Norway and the North Pole, can hold a maximum of 2.5 billion seeds representing 4.5 million crop varieties (500 seeds from each crop). The

seeds at Svalbard are kept at -18 degrees Celsius to keep them viable for a long time. This global seed bank was built as a backup to the world's more than 1,700 gene banks located in different countries. If something were to happen to the other seed banks such as an economic or natural disaster, then the world would still have the seeds stored at Svalbard to use if needed.

2.3.8 Gene sequencing

DNA sequencing is the process of determining the exact sequence of nucleotides within a DNA molecule. This means that by sequencing a stretch of DNA, it will be possible to know the order in which the four nucleotide bases – adenine, guanine, cytosine and thymine – occur within that nucleic acid molecule. The necessity of DNA sequencing was first made obvious by Francis Crick‘s theory that the sequence of nucleotides within a DNA molecule directly influenced the amino acid sequences of proteins. At the time, the belief was that a completely sequenced genome would lead to a quantum leap in understanding the biochemistry of cells and organisms. The first DNA fragment to be sequenced belonged to a small virus called T4 bacteriophage that specifically infects Escherichia coli bacteria. An important gene in this organism codes for the enzyme, lysozyme. The amino acid sequence of this enzyme had been elucidated earlier through sequential digestion with the enzyme trypsin, and the DNA sequence was identified later.

In the mid-1970s, Frederick Sanger improved this initial method by using a plus-minus system for running a sequencing reaction. In this modified method, DNA polymerization initially occurred using radiolabeled nucleotides. After that, a short two-second pulse of polymerization was done by adding or omitting a single nucleotide in each reaction mixture. This created a set of eight reactions to give a definitive picture of the nucleotide sequence within a DNA molecule. The completed reactions were run on a polyacrylamide gel for analysis. In this manner, the first complete genome was sequenced, that of bacteriophage ϕX174.

DNA Sequencing Methods

There are two main types of DNA sequencing. The older, classical chain termination method is also called the Sanger method. Newer methods that can process a large number of DNA molecules quickly are collectively called High-Throughput Sequencing (HTS) techniques or Next-Generation Sequencing (NGS) methods.

2.3.9 DNA fingerprinting

DNA profiling (also called DNA fingerprinting) is the process of determining an individual's DNA characteristics, which are as unique as fingerprints. DNA analysis intended to identify a species, rather than an individual, is called DNA bar-coding.

DNA fingerprinting was invented in 1984 by Professor Sir Alec Jeffreys after he realized you could detect variations in human DNA, in the form of these minisatellites.

DNA fingerprinting is a technique that simultaneously detects lots of minisatellites in the genome to produce a pattern unique to an individual. This is a DNA fingerprint. The probability of having two people with the same DNA fingerprint that are not identical twins is very small. Just like your actual fingerprint, your DNA fingerprint is something you are born with, it is unique to you.

Modern-day DNA profiling is also called STR analysis and relies on microsatellites rather than the minisatellites used in DNA fingerprinting. Microsatellites, or short tandem repeats (STRs), are the shorter relatives of minisatellites usually two to five base pairs long. Like minisatellites they are repeated many times throughout the human genome, for example ‗TATATATATATA‘.

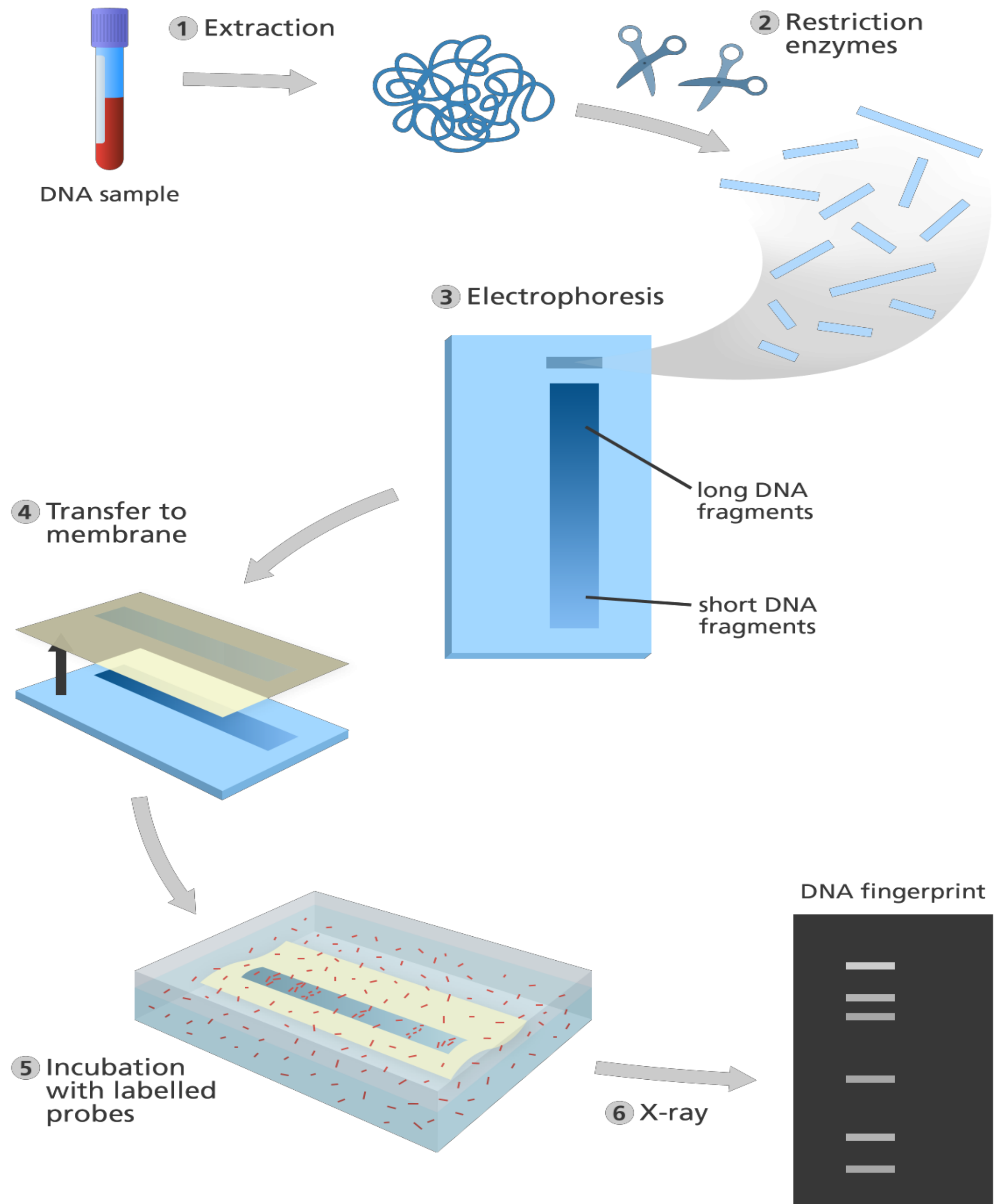
DNA profiles are very useful in forensics because only a tiny sample of human material left behind after a crime may be sufficient to identify someone. The police may use this DNA evidence to support other evidence to help prosecute someone for a crime. Complete DNA profiles give very reliable matches and may provide strong evidence that a suspect is guilty or innocent of a crime.

Steps in DNA fingerprint

- The first step of DNA fingerprinting was to extract DNA from a sample of human material, usually blood.
- Molecular ‗scissors‘, called restriction enzymes were used to cut the DNA. This resulted in thousands of pieces of DNA with a variety of different lengths.
- These pieces of DNA were then separated according to size by a process called gel electrophoresis
- The DNA was loaded into wells at one end of a porous gel, which acted a bit like a sieve.
- An electric current was applied which pulled the negatively-charged DNA through the gel.
- The shorter pieces of DNA moved through the gel easiest and therefore fastest. It is more difficult for the longer pieces of DNA

to move through the gel so they travelled slower.

- As a result, by the time the electric current was switched off, the DNA pieces had been separated in order of size. The smallest DNA molecules were furthest away from where the original sample was loaded on to the gel.
- Once the DNA had been sorted, the pieces of DNA were transferred or _blotted‘ out of the fragile gel on to a robust piece of nylon membrane and then _unzipped‘ to produce single strands of DNA.
- Next the nylon membrane was incubated with radioactive probes.
- Probes are small fragments of minisatellite DNA tagged with radioactive phosphorous.
- The probes only attach to the pieces of DNA that they are complementary? to – in this case they attach to the minisatellites in the genome.
- The minisatellites that the probes have attached to were then visualised by exposing the nylon membrane to X-ray film.
- When exposed to radioactivity a pattern of more than 30 dark bands appeared on the film where the labelled DNA was. This pattern was the DNA fingerprint.
- To compare two or more different DNA fingerprints the different DNA samples were run side-by-side on the same electrophoresis gel.
- The following figure is that of processes involved in DNA fingerprinting


1 Extraction
DNA sample
2 Restriction enzymes
3 Electrophoresis
long DNA fragments
short DNA fragments
4 Transfer to membrane
5 Incubation with labelled probes
6 X-ray
DNA fingerprint

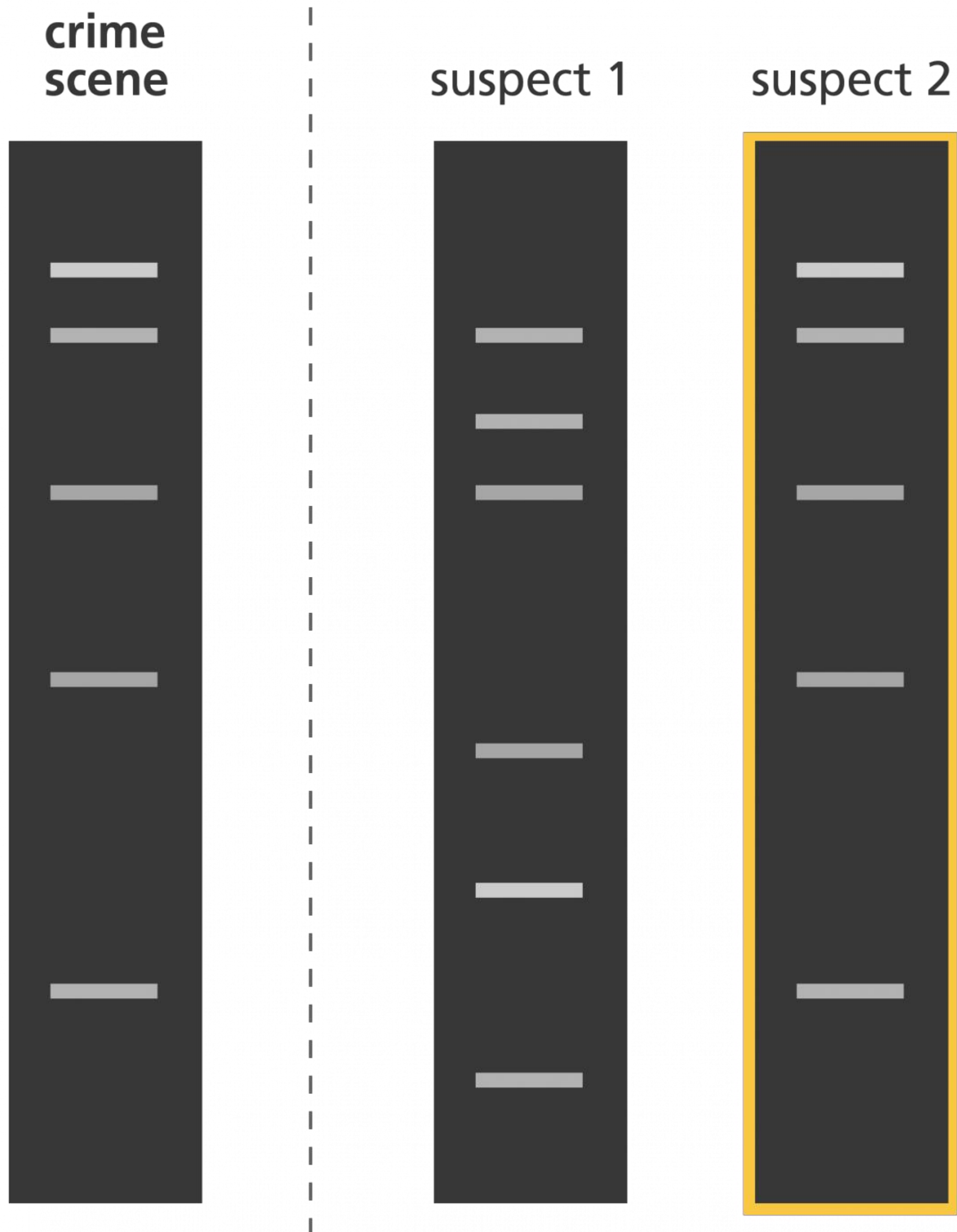


Illustration showing acomparison of a DNA fingerprint from a crime scene and DNA fingerprints from two suspects. The DNA fingerprint from suspect 2 matches that taken from the crime scene. Image credit: Genome Research Limited

2.3.10 Bio-informatics

Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines biology, computer science,

information engineering, mathematics and statistics to analyze and interpret biological data. Various biological analyses result in exponential amounts of biological data and it becomes very hard to analyze them using manual means. This is where Computer Science comes to the rescue. Various computational techniques are used to analyze hunks of biological data more accurately and efficiently by means of automated processes. Hence, bioinformatics can be considered as a field of data science for solving problems in biology and medicine. Bioinformatics has proven to possess great potential to identify diseases beforehand, determine treatment and help make human lives better. With the inspiration and knowledge of computer science, fields such as gene technology, medicine and healthcare can evolve from curing individual patients to healing entire populations. Researche are being carried out to identify genetic alterations in patients genes, DNA, RNA, protein structures, various synthesis processes etc. allowing scientists to come up with better treatments and even possible measures of prevention. Certain types of cancer, being caused by such genetic alterations can be identified beforehand and can be treated before the conditions get worse.

2.4 Conclusion

Genetic engineering is the process of using gene cloning and other genetic manipulations to achieve isolate specific genes and use it for research and other purposes. Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and microorganisms. Bacteria, the first organisms to be genetically modified, can have plasmid DNA inserted containing new genes that code for medicines or enzymes that process food and other substrates. Plants have been modified for insect protection, herbicide resistance, virus resistance, enhanced nutrition, tolerance to environmental pressures and the production of edible vaccines. Most commercialized GMOs are insect resistant or herbicide tolerant crop plants. Genetically modified animals have been used for research, model animals and the production of agricultural or pharmaceutical products. The genetically modified animals include animals with genes knocked out, increased susceptibility to disease, hormones for extra growth and the ability to express proteins in their mil

2.5 Summary

2.6 Tutor marked assignment

i. With the aid of a diagram, discuss the process of cloning
ii. What is DNA fingerprinting?

iii. Differentiate between Physical and genetic mapping
iv. Differentiate between transgene, transgenic and transgenesis
v. What are genetic engineering tools?
vi. What is marker assisted selection? Outline the importance in plant or animal breeding

2.7 References/ Further Reading

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UNIT 3: APPLICATION OF BIOTECHNOLOGY IN ANIMAL AND FEED PRODUCTION, NUTRITION, REPRODUCTION, GENETICS AND BREEDING, DISEASE AND METABOLIC CONTROL, POLLUTION ABATEMENT AND ENVIRONMENTAL MANAGEMENT.

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3.1 Introduction

Biotechnology applies the knowledge of biology to enhance and improve the environment, health, and food supply. Using biotechnology, scientists work to develop environment- friendly alternatives to fossil fuels and plastics; new medicines, vaccines and disease diagnostic tools; and higher yielding and more nutrient-rich crop plants. Biotechnology has application in four major industrial areas, including health care (medical), crop production and agriculture, non-food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses.

3.2 Objectives

- You will understand the application of biotechnology in animal and feed production
- You will understand the application of biotechnology in reproduction
- You will understand the application of biotechnology genetics

and breeding
- You will understand the application of biotechnology in disease and metabolic control
- You will understand the application of biotechnology in pollution abatement and environmental management.

3.3 Main content

3.3.1 Application of biotechnology in animal, feed production and nutrition

Animal nutrition is keys to any animal husbandry production system. Feed is the largest single cost item for livestock and poultry production, accounting for 60%–70% of the total cost in most years. Many research interventions are in progress to reduce the feeding cost either by utilization of unconventional feed or by applications of biotechnology tool to enhance the nutritional availability and digestibility of animal feed. Biotechnology in animal nutrition utilizes mainly recombinant DNA technology and fermentation technologies. These techniques are often based on the use of micro-organisms for the production of nutrients such as particular essential amino acids or complete proteins or to improve the digestibility of animal feeds. These technologies are being used, to increase the quality of silage, to produce probiotics, to produce specific enzymes and hormones (somatotropin, phytase), to degrade fiber content in feed and fodder and, to alter ruminal flora. Recently, there are wide potential application of biotechnology in the field of animal production to increase the productivity of animals through better plane of nutrition, better production potential and improved health conditions. Nutrients like protein, amino acids and fats can be produced or protected according the need at different physiological states of the animals. Enzymes can be used to improve the availability of nutrients from feed and to reduce the wastage of the feed and fodder. Prebiotics and probiotics or immune supplements can be useful to inhibit enteric pathogenic bacteria. Along with these use of plant biotechnology to produce feed and fodder with good nutritive values can be done with ease. Addition of vaccines or antibodies in feeds can be used to protect the animals from the disease. Genetic manipulation of rumen microbes can be done to improve the animal health. However, it is very difficult to accept the role of biotechnology in animal nutrition and it mainly depends on social and cultural aspects and economic importance to consumers and society. Various ways to improve feed production, nutrition and animal production includes:

PROTECTION OF PROTEIN, AMINOACIDS AND FAT: Rumen degrades the protein to form ammonia and then the ruminal microbes

use this non-proteinous nitrogen to synthesize the microbial protein. To increase the efficient utilization of degradable protein, it should be protected from ruminal degradation through chemical treatments such as formaldehyde and physical treatments like heat treatment and extrusion cooking. Some researchers have reported 33% reduction in protein degradability by formaldehyde treatment of groundnut cake. Fat can be protected using the saponification of fat with calcium salts. Feeding Ca soaps of fatty acids, which are inert in rumen, to negative energy balance animals enhances dietary energy density and thus, energy intake in early without compromising the activity of rumen micro flora. Thus, the deleterious effect of negative energy balance on animals can be alleviated.

USE OF ENZYMES: There is an increasing trend of use of enzymes to enhance the feed utilization. It can also be helpful by reducing the methane production thus help in reducing the carbon foot print. Most of enzymes are cocktail of various enzymes (pentosanase, pectinase and a-galactosidase activity and phytase) of bacterial or fungal origin. Enzymes can be used for removal of ant nutritional factors (e.g., 13-glucans and arabino-xylans in barley grain cell-wail), increasing the digestibility of nutrients (e.g., Phytate phosphorus in grains) and non-starch polysaccharides (e.g., xylose and arabinose in plants). Improvement in weight gain and feed efficiency with supplementation of enzymes such as cellulose and hemicelluloses‘ in diet have been reported.

PREBIOTICS AND PROBIOTICS: Prebiotics are some oligosaccharide like fructo-, gluco- and galacto-oligosaccharides resist attack by the digestive enzymes of animals and thus are not metabolized directly by the host and act as bed for growth of beneficial microbes. Probiotics are live microbial feed supplements which beneficially affect the host animal by improving the intestinal microbial balance. The most common probiotics are lactic acid producing bacteria.

ADDITION OF VACCINES OR ANTIBODIES IN FEEDS: Now a day‘s many feeds and fodder are from crop plants that have been modified for characteristics such as disease or pest resistance and their nutritive value remain unaffected. Secondly, plants are used as bioreactors for the production of recombinant biopharmaceuticals like cytokines, hormones, monoclonal antibodies, bulk enzymes and vaccines.

METABOLIC MODIFIERS: Metabolic modifiers like recombinant bovine somatotropin (rBST) have been used to increase efficiency of production such as weight gain or milk yield per feed unit), improve carcass composition (meat-fat ratio). In developed countries like USA,

its use increases 10-15% of milk yield. Similarly, researchers have also developed porcine somatotropin that increases muscle growth and reduces body-fat deposition, resulting in pigs that are leaner and of greater market value.

GENETIC MANIPULATION OF MICROBES: The rumen microbes can be altered genetically to increase their cellulolytic ability and reduction in methanogenesis to improve the overall utilization of feed and fodder. This can be done to eliminate the ant nutritional factors in feeds and also increase the essential amino acid specially limiting amino acids synthesis by rumen microbes. Attempts are being made to introduce the lignin breakdown property into ruminal microbes. Depolarization of lignin by lignase enzyme which is produced by the soft-rot fungus (Phanerochaete chrysosporium) (Tien and Kirk, 1983) can be useful for the animals. Efficiency and stability of lignase gene has been modified by Recombinant DNA technology (Tien and Tu, 1987).

USE OF HORMONES: For example, Porcine Growth Hormone (PSt.). Undoubtedly porcine growth hormone injected in controlled amounts into pigs on grain based concentrates increase their growth rate, efficiency of growth and reduce fat deposition. The place of PSt in pig production, as it pertains to developing countries, is unknown. The small production units, the likely high cost of injections and the ―scavenger system‖ generally operated at the small farmer level in developing countries suggests that PSt is unlikely to be used. The exception may be in grain-based feeding systems for pigs aimed at the markets provided by the higher income groups and tourists.

3.3.2 Application of biotechnology in animal reproduction

Biotechnology has great impact on breed improvement, reproductive rate, and animal production. The most common reproductive applications that are integrated with biotechnology are artificial insemination (AI), semen preservation, fertilization capacity of sperms, sperm sexing, synchronization and fixed-time insemination, super ovulation, embryo transfer (ET), and in vitro embryo production (IVEP).

Artificial Insemination: Artificial insemination has been practiced on many domestic animals for hundreds of years. It is one of the earliest reproductive biotechnologies and permits the use of superior males for breeding purposes. This technique involves semen collection from superior males, its dilution, freezing, and deposition in the female reproductive tract.

Advantages of Artificial Insemination

1. Artificial insemination not only increases the use of superior male animals but also makes their use more efficient. More people can be benefited from superior male. Use of the proven sires in dairy herds markedly increases milk production up to 30 % compared to natural breeding
2. Artificial insemination helps in great genetic improvement of farm animals. The selection and efficient use of superior bulls improves production.
3. Artificial insemination helps in controlling different venereal and other diseases like trichomoniasis, Vibriosis, brucellosis, etc.
4. The danger and expenses of keeping and handling bulls that prove to be inferior males can be eliminated.
5. It is easier to transport semen doses over long distances than to transport male animals.
6. Artificial insemination makes it possible to use the semen even after the death of a male.
7. Widespread use of artificial insemination in the dairy industry helps in proper breeding records.
8. Artificial insemination makes possible breeding of animals with size differences without injuries.
9. Artificial insemination made the use of those sires that are not capable of copulating, like aged or crippled sires.
10. It is a pre-requisite for embryo transfer.

Disadvantages of Artificial Insemination

1. Artificial insemination is an advanced and sophisticated technique, so well-trained personnel are required to supervise semen collection, examination, extension, freezing, shipping, and insemination of females.
2. Widespread use of artificial insemination increases the possibility of transmission of genetic abnormalities, for example COD, spastic syndrome, poor conformation especially of feet and limbs, and lack of libido.
3. Artificial insemination uses a limited number of elite bulls. This limited gene pool may improve milk production but it has a reverse effect due to increased inbreeding, which results in genetic abnormalities because of expression of recessive genes.

Sex Sorted Semen

Sex of the fetus is determined by the sperm because the sperm may carry either X or Y sex chromosome. Sperm having X sex chromosome when fertilizes an oocyte will result in a female and a sperm having Y

sex chromosome when fertilizes an oocytes will result in a male offspring. The desire to separate X and Y bearing sperms is driven by the fact that one sex has more economic importance than the other for certain species. As in the dairy industry, the female calves are more important than the males because of maximum utilization of AI. As the major income of a dairy farm comes from milk, so it is advantageous to have more female calves that will become future producer.

Controlled Breeding and Synchronization

Early detection of estrus is becoming a major concern with the extension of the number of cows reared, the improvement of milking cows with high milk production, and the changes in the circumstances of feeding and management of cows. Estrus synchronization or controlled breeding is grouping of females for parturition at the same time. It is used at commercial dairy farms for uniform milk production throughout the year. It is closely linked with AI and is also a pre-requisite for embryo transfer, or is the first step of embryo transfer. Estrus detection is a major problem but by use of synchronization, we can reduce the time required for estrus detection with timed insemination

Multiple ovulation and embryo transfer (MOET): By increasing the number of offspring that can be obtained from monotocous species in particular, MOET has the potential to increase genetic improvement by enhancing the selection intensity on the female side. In cattle, however - the species in which this technology is again the most widely disseminated - the major impact of MOET might result from the reduction in generation interval vis-à-vis the conventional progeny-testing scheme, if sires are selected based on the performances of their MOET produced full- sisters rather than the performances of their female progeny: the so-called MOET nucleus scheme. Despite associated technical hurdles, MOET has the potential to play an important role in developing countries where the implementation of a large-scale AI based progeny-testing scheme would be difficult to implement.

Oocyte harvesting (OPU), in vitro oocyte maturation (IVM), in vitro fertilization (IVF): While the number of embryos that can be obtained from a cow / year using MOET is on an average limited to the order of 20 or less, the development of OPU in conjunction with IVM and IVF increases this number by a factor of at least 5. Moreover, OPU can be applied to pregnant animals as well as prepubic animals. The impact of these methodologies on genetic response operates through the same channels as MOET, i.e. increase of selection intensity on the female side and increase of selection accuracy on the male and female side.

Nuclear transfer or embryo cloning: The transfer of totipotent nuclei in enucleated oocytes theoretically allows for the production of large numbers of identical twins or "clones". This opened the prospective to affect genetic response in a variety of ways including selection intensity, selection accuracy and generation interval. Initially, the source of tot potent nuclei were blastomeric. Despite the potential use of first as well as higher order generation blastocysts as nuclei donors, the size of the clones has remained very small. The recent generation of totipotent embryonic stem –ES‖-like cells in sheep which will likely be followed by similar developments in other species, might lead to a considerable increase in the efficiency of embryo cloning

In Vitro-Fertilization (IVF)

In vitro-fertilization is the collection of oocytes from a donor female that are matured in the lab and fertilization of that matured oocyte in a laboratory dish. The embryo resulting from that fertilization is cultured in a specific media for a few days and ultimately transferred into a female recipient. The eggs after collection are placed in CO2 incubators in the IVF laboratory. Most viable spermatozoa are recovered after processing for inseminating the eggs. Because of the thick layer of zona pellucida and thousands of follicular cells around the ova, embryologists usually add approximately 100,000 spermatozoa for an egg. The addition of large number of viable spermatozoa to each ova will disperse the follicular cells and also ensure fertilization of egg by one spermatozoa. IVF has been used to treat many infertility issues, i.e., when both fallopian tubes are blocked, fertilization of the egg cell has to take place outside the body

Advantages of In Vitro Embryo Production

1. Circumvention of the problem of timing ovulation for AI.
2. Potential for producing more embryos.
3. Make possible the use of animals suffering from certain issues of infertility such as tubal obstruction, Endometritis, etc.
4. A reduced number of viable sperm needed for IVF compared to AI or natural breeding.
5. By using sperm microinjection techniques, the potential of using nonviable and testicular or epididymal-derived sperm for assisted fertilization
6. Potential of salvaging genetic material from female animals after death

3.3.3 Application of biotechnology in genetics and breeding

Animal breeding is a field related to a whole range of biotechnologies. The impact of a biotechnology can be measured by the influence it has on genetic progress. According to the type of biotechnology considered, different component of genetic progress may be affected: accuracy of prediction, generation interval, intensity of selection and genetic variance. The first type of biotechnologies affects the efficiency of male and female reproduction: artificial insemination, multiple ovulation, in-vitro-fertilization, ova pick-up, embryo-transfer, twining, sexing of semen and embryos cloning and selfing. The impact of these technologies is mainly in the enhanced distribution of superior germplasm and the selection intensity, but also in the accuracy obtained when testing animals. In the past, artificial insemination has been a very successful biotechnology, enhancing greatly the genetic progress. A secondary, negative, impact is that these biotechnologies affect indirectly genetic diversity and therefore reduce genetic variance. A second group of biotechnologies can improve determination of the genetic merit of animals. These are all the techniques relate to quantitative or economical trait loci (QTL/ETL), their detection and use. Their main feature is the early availability in life, therefore allowing an earlier and more accurate selection. Two direction of research exists: detection of markers for the unknown QTL and direct use of a potential candidate genes as QTL/ETL. QTL/ETL will have a major impact on animal breeding especially if their use in future breeding programs can be optimized. A last type of biotechnologies with a large potential to affect animal breeding in the future are those with the ability to transform artificially DNA. The impact of these technologies is however still not very clear especially as gene expression and other issues remain unsolved. Biotechnology had, has and will have a major impact on animal breeding and genetic progress. To a certain extend animal breeding is a very promising field to use biotechnology as the past has already proven. Biotechnology contributes to animal production by improving the environmental component of the production systems as well as by improving the genetic make- up of livestock. Biotechnology is being applied to enhance genetic progress through these four factors: increase genetic variation (or the molecular substrate of breeding programmes), increase the accuracy of selection, reduce the generation interval and to increase the selection intensity.

3.3.4 Application of biotechnology in disease and metabolic control

Metabolism is the set of chemical reactions that occur in living organisms to maintain life. There are diseases caused by defects in

metabolism, such as diabetes, which will emphasis the importance of metabolic control. Genomics has opened new possibilities for applications in biotechnology and medicine. For these to be realized, one must not forget that most gene products are proteins, most proteins are enzymes, and most enzymes operate as components of metabolism. It is therefore important to put genetic information in the context of what has long been known about how enzymes behave and how they are regulated in metabolism. Without such a framework, there is little chance of predicting the effects of mutations, deletions, or insertions of genes.

Many areas of microbiology and biotechnology are directly concerned with the isolation, study or engineering of cells capable of (over)producing metabolites of commercial significance. Yet the study, production or improvement of such strains has often been at best semi-empirical. The metabolic control theory developed by Kacser, Burns, Heinrich and Rapoport can provide a rational and quantitative basis for the description and improvement of such processes. Metabolic control analysis (MCA) provides a quantitative description of substrate flux in response to changes in system parameters of complex enzyme systems. Medical applications of the approach include the following: understanding the threshold effect in the manifestation of metabolic diseases; investigating the gene dose effect of aneuploidy in inducing phenotypic transformation in cancer; correlating the contributions of individual genes and phenotypic characteristics in metabolic disease (e.g., diabetes); identifying candidate enzymes in pathways suitable as targets for cancer therapy; and elucidating the function of "silent" genes by identifying metabolic features shared with genes of known pathways. MCA complements current studies of genomics and proteomics, providing a link between biochemistry and functional genomics that relates the expression of genes and gene products to cellular biochemical and physiological events. Thus, it is an important tool for the study of genotype-phenotype correlations. It allows genes to be ranked according to their importance in controlling and regulating cellular metabolic networks. We can expect that MCA will have an increasing impact on the choice of targets for intervention in drug discovery.

Metabolic control analysis developed from observations of this kind. Although it remains little known by many active biotechnologists, it may well provide the key to understanding why modern drug design is not noticeably more efficient or successful than it was 30 years ago, and why the impact of genetic manipulation on the production of commercial metabolites has been likewise disappointing. More optimistically, the study of metabolic control analysis may provide pointers toward greater success in the future.

Taking cancer as an example of how a better understanding of metabolism may lead to progress, can control analysis shed light on its causes? Can it open the door to better approaches to treatment? Perhaps surprisingly, the answer to both questions may be yes.

3.3.5 Application of biotechnology in pollution abatement and environmental management.

The use of Biotechnology for solving environmental problems and ecosystem is known as Environmental Biotechnology. It is applied and is used to study the natural environment.

According to the international Society for environmental Biotechnology the environmental Biotechnology is defined as "an environment that helps to develop, efficiently use and regulate the biological systems and prevent the environment from pollution or from contamination of land, air and water".

There are four major different types of applications of Environmental Biotechnology.

They are as follows:

Bio-marker: This type of Application of environmental Biotechnology gives response to a chemical that helps to measure the level of damage caused or the exposure of the toxic or the pollution effect caused. In other word, Biomarker can also be called as the Biological markers the major use of this applications helps to relate the connection between the oils and its sources.

Bio-energy: The collective purport of Biogas, biomass, fuels, and hydrogen are called the Bioenergy. The use of this application of Environment Biotechnology is in the industrial, domestic and space sectors. As per the recent need it is concluded that the need of clean energy out of these fuels and alternative ways of finding clean energy is the need of the hour. One of the pioneer examples of green energy are the wastes collected from the organic and biomass wastes; these wastes help use to over the pollution issues caused in the environment. The Biomass energy supply has become a prominent importance in every country.

Bioremediation: The process of cleaning up the hazardous substances into non-toxic compounds is called the Bioremediation process. This process is majorly used for any kind of technology clean up that uses

the natural microorganisms.

Biotransformation: The changes that take place in the biology of the environment which are changes of the complex compound to simple non-toxic to toxic or the other way round is called the biotransformation process. It is used in the Manufacturing sector where toxic substances are converted to Bi-products.

Benefits:

- The major benefits of environmental biotechnology are it helps to keep our environment safe and clean for the use of the future generations. It helps the organisms and the engineers to find useful ways of getting adapted to the changes in the environment and keep the environment clean and green.

- The benefit of environmental biotechnology helps us to avoid the use of hazardous pollutants and wastes that affect the natural resources and the environment. The development of the society should be done in such a way that it helps to protect our environment and also helps us to develop it.

- The environmental biotechnology has a role to play in the removal of the pollutants. It is becoming an advantage for the scientists and the environmentalists to find ways to convert the waste to re-useable products.

- The applications of environmental biotechnology are becoming a benefiting factor for the environment; the applications includes â€" genomics, proteomics, bioinformatics, sequencing and imaging processes are providing large amounts of information and new ways to improvise the environment and protect the environment.

3.4 Conclusion

Applications of Biotechnology. Biotechnology has application in four major industrial areas, including health care (medical), crop production and agriculture, non-food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses.

3.5 Summary

- The use of Biotechnology for solving environmental problems and ecosystem is known as Environmental Biotechnology
- Biotechnology principles is also being used to study metabolites and their alteration in disease and metabolic control
- The impact of these technologies is mainly in the enhanced distribution of superior germplasm and the selection intensity, but also in the accuracy obtained when testing animals. In the past, artificial insemination has been a very successful biotechnology, enhancing greatly the genetic progress in animal breeding and genetics
- The most common reproductive applications that are integrated with biotechnology are artificial insemination (AI), semen preservation, fertilization capacity of sperms, sperm sexing, synchronization and fixed-time insemination, superovulation, embryo transfer (ET), and in vitro embryo production (IVEP).
- Using biotechnology, scientists work to develop environment- friendly alternatives to fossil fuels and plastics; new medicines, vaccines and disease diagnostic tools; and higher yielding and more nutrient-rich crop plants.

3.6 Tutor marked assignment

- How is biotechnology being applied in animal, feed and nutrition
- Discuss the various ways being used to improve reproduction in animals
- What are the advantages of AI?
- What is bioremediation?
- Describe MOET
- What are the different types of applications of environmental Biotechnology

3.7 References/ Further Reading

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UNIT 4: USE OF DNA TESTS FOR MEAT QUALITY, GENOTYPE IDENTIFICATION, PATERNITY TESTING, DISEASE DIAGNOSTICS.

Content

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4.1 Introduction

Genetic testing, also known as DNA testing, allows the determination of bloodlines and the genetic diagnosis of vulnerabilities to inherited diseases and the quality of life. In agriculture, a form of genetic testing known as progeny testing can be used to evaluate the quality of breeding stock.

DNA testing involves a set of procedures in which DNA (the genetic material) extracted from a patient's cells (usually from a blood sample) is tested in the laboratory for *changes*. Although these DNA changes are usually suspects for causing a disease, DNA testing can also be used to gather other information important for proper healthcare, quality of life and contamination of meat by foreign DNA. It can also be used to confirm the identity of a particular species or individual. DNA testing plays an important role in research. It forms a core basis of other fields such as medicine, agriculture, genealogy, forensic science and more. Most DNA tests are tests for *constitutional* mutations. That type of mutation is inherited, so it is present at the moment of conception and therefore occurs in all cells in the body. Any readily available source of living cells can be tested. Most commonly, blood cells are used. The blood is drawn from a vein into a glass Vacutainer tube containing a preservative, such as liquid EDTA or acid-citrate solution. The blood specimen is usually drawn at a local laboratory and mailed to a reference laboratory. The reference laboratory usually waits until it receives many specimens and then processes them all at once in order to conserve labor and chemicals. After the test is completed, the report is returned to the local laboratory.

In some diseases, the mutation is *focal*, which means that it is present only in certain body cells (*tissues*). An example of a focal mutation is a mutation that causes a tumor. The DNA change that causes a tumor only occurs in cells that make up the tumor and not in other cells of the body. In this case, additional steps are required in the testing process. A biopsy (*piece*) of the tissue must be obtained surgically. Simple liquid solutions may not suffice for preservation of this tissue, so more exotic mechanisms can be required such as freezing in liquid nitrogen. This makes mailing difficult. The added steps make the process expensive.

There are several reasons, or *indications*, why DNA testing of an individual or a family be it human or animal or plant may be needed.

4.2 Objectives

- You will understand the use of DNA tests for meat quality
- You will understand genotype identification
- You will understand paternity testing
- You will understand disease diagnostics

4.3 Main content

4.3.1 Use of DNA tests for meat quality

DNA (Deoxyribonucleic acid) is the unique hereditary material in all cells, and is found in animals and plants, and therefore in our food. With advancements in molecular biology, DNA testing has become a useful instrument to assess the safety, quality and integrity of the food chain. It has numerous applications including the identification of allergenic material, the detection of adulteration (e.g. species replacement as a result of commercial fraud), and the identification of microbes that cause food-borne diseases. It can also be used for traceability purposes throughout the supply chain. DNA testing can be used in different contexts by different operators including researchers, food business operators and regulatory authorities. The latter may use this methodology to assess the safety and authenticity of foodstuffs, and uncover any breaches of labelling legislation.

In the aftermath of the globalized horsemeat scandal, control of processed meat products became a must do in all countries and particularly in countries that heavily rely on imported goods and those with a meat consumption pattern based on cultural preferences and religious beliefs. DNA testing for meat adulteration performed on a total of 105 samples of imported raw and processed food products marketed

in the Arabian Gulf region showed the presence of horse and pork DNA in respectively 7 and 26% of tested samples. Of the pork-DNA-positive samples, few revealed higher than the 1% threshold for adulteration; while most of these products showed traces of pork)<1%(. The 1% limit rate does not warrant active meat adulteration by international standards; however, it is rejected by communities such as Muslims, Jewish, and others for whom any level of pork consumption is not acceptable, even in minute amounts. The presence of trace DNA from species other than the one on the product label (up to 1%) is commonly attributed to carryover resulting from successive manufacturing processes using the same industrial equipment. Because DNA testing allows the detection of infinitesimal amounts of any meat species including undesirable ones, it extends the halal/kosher issues to the current industrial manufacturing processes involving meat even in the absence of active fraud.

How the test is conducted?

Three methods can be used:

(i) Polymerase Chain Reaction [PCR] sequencing
(ii) RFLP (Restriction Fragment Length Polymorphisms)
(iii) DNA barcoding.

(i) The most common method, PCR (Polymerase Chain Reaction), involves extracting DNA from the food followed by amplification of specific pieces of DNA through an enzymatic process. The amplified DNA fragments are separated by size using a technique called agarose gel electrophoresis and are compared with DNA fragments of known size to enable their identification.

(ii) In RFLP analysis, the DNA sample is broken into pieces by restriction enzymes i.e. enzymes that can recognize specific base sequences in DNA and cut the DNA at that site (the restriction site). The resulting restriction fragments are separated according to their size using gel electrophoresis. RFLP analysis was the first DNA profiling technique inexpensive enough for widespread application.

(iii) DNA barcoding is a molecular based system, which is based on the analysis of a short genetic marker called the "DNA barcode" in an organism's DNA. By comparing the DNA barcode to a compiled database of barcodes it can be identified as belonging to a particular species. The success of the technique depends on (a) the molecular variability between species and (b) the availability of high quality repositories of reference sequences (i.e. DNA sequences of known species).

4.3.2 Use of DNA tests for genotype identification

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. Traditionally genotyping is the use of DNA sequences to define biological populations by use of molecular tools. It does not usually involve defining the genes of an individual.

Current methods of genotyping include restriction fragment length polymorphism identification (RFLPI) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, amplified fragment length polymorphism detection (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allele specific oligonucleotide (ASO) probes, and hybridization to DNA microarrays or beads. Genotyping is important in research of genes and gene variants associated with disease. Due to current technological limitations, almost all genotyping is partial. That is, only a small fraction of an individual's genotype is determined, such as with (epi)GBS (Genotyping by sequencing) or RADseq. New mass-sequencing technologies promise to provide whole-genome genotyping (or whole genome sequencing) in the future.

Genotyping applies to a broad range of individuals, including microorganisms. For example, viruses and bacteria can be genotyped. Genotyping in this context may help in controlling the spreading of pathogens, by tracing the origin of outbreaks. This area is often referred to as molecular epidemiology or forensic microbiology.

Humans can also be genotyped. For example, when testing fatherhood or motherhood, scientists typically only need to examine 10 or 20 genomic regions (like single-nucleotide polymorphism (SNPs)), which represent a tiny fraction of the human genome. Human being can also be genotyped to know which allele of blood they have inherited and carrying. For example, a blood test is used to determine whether the A and/or B characteristics are present in a blood sample. It is not possible to determine the exact genotype from a blood test result of either type A or type B. If someone has blood type A, they must have at least one copy of the A allele, but they could have two copies. Their genotype is either AA or AO. Similarly, someone who is blood type B could have a genotype of either BB or BO. A blood test of either type AB or type O is more informative. Someone with blood type AB must have both the A and B alleles. The genotype must be AB. Someone with blood type O has neither the A nor the B allele. The genotype must be OO.

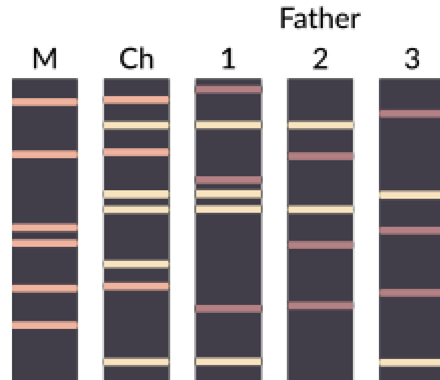
When genotyping transgenic organisms, a single genomic region may be all that needs to be examined to determine the genotype. A single PCR assay is typically enough to genotype a transgenic mouse; the mouse is the mammalian model of choice for much of medical research today.

4.3.3 Use of DNA for paternity testing

DNA paternity testing is the use of DNA profiling (known as genetic fingerprinting) to determine whether two individuals are biologically parent and child. A test establishes genetic proof whether a man is the biological father of an individual, and a maternity test establishes whether a woman is the biological mother of an individual. Tests can also determine the likelihood of someone being a biological grandparent to a grandchild. Though genetic testing is the most reliable standard, older methods also exist, including ABO blood group typing, analysis of various other proteins and enzymes, or using human leukocyte antigen antigens. The current techniques for paternity testing are using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Paternity testing can now also be performed while the woman is still pregnant from a blood draw.

DNA testing is currently the most advanced and accurate technology to determine parentage. In a DNA parentage test, the result (called the 'probability of parentage) is 0% when the alleged parent is not biologically related to the child and the probability of parentage is typically 99.99% when the alleged parent is biologically related to the child. However, while almost all individuals have a single and distinct set of genes, rare individuals, known as "chimeras", have at least two different sets of genes, which can result in a false negative result if their reproductive tissue has a different genetic make-up from the tissue sampled for the test. Over time, the use of additional blood antigens, such as those associated with the MN and Rh systems, refined the use of blood- typing for both paternity and forensics. However, such blood groups were only about 40% effective in ruling out a man as a child's father. Then, in the 1970s, testing for human leukocyte antigens (HLAs) added a distinguishing feature that made it possible to rule out men as fathers with 80% effectiveness. The genes responsible for the HLA system are involved in antigen presentation to T cells. The HLA system is highly polymorphic, with more than 3,200 different alleles identified so far. Although this vast number of alleles causes headaches for cell and organ transplants, the multiplicity of genotypes the HLA system provides—in the tens of millions— makes it ideal for consideration in identity and paternity testing.

The testing is performed by collecting buccal cells found on the inside of a person's cheek using a buccal swab or cheek swab. These swabs have wooden or plastic stick handles with a cotton on synthetic tip. The collector rubs the inside of a person's cheek to collect as many buccal cells as possible. The buccal cells are then sent to a laboratory for testing. For paternity testing, samples from the alleged father and child would be needed. For maternity testing, samples from the alleged mother and child would be needed.



Example of DNA profiling in order to determine the father of a child (Ch). Child's DNA sample should contain a mixture of different size DNA bands of both parents. In this case person #1 is likely the father

4.3.4 Use of DNA tests for disease diagnostics.

Genetic testing, also known as DNA testing, involves obtaining DNA from a sample of cells in your body to identify specific genes, chromosomes, or proteins, including those that are faulty (known as mutations). The test can be used to help identify genetic lineage, confirm or rule out an inherited genetic disorder, assess your risk of developing or passing on a genetic disorder, and select which drugs may be most effective based on your genetic profile. Several hundred genetic tests are currently available, with many more being developed. The tests can be performed on blood, urine, saliva, body tissues, bone, or hair. Several reasons related to diagnosis, prevention, and treatment can warrant DNA test. Genetic testing can also be used in legal investigations Diagnostic testing is used to identify or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical signs and symptoms. Diagnostic testing can be performed before birth or at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disorder. Diagnostic testing is used to identify or confirm the diagnosis of a disease or condition in a person or a family. Diagnostic testing gives a

"yes" or "no" answer in most cases. It is sometimes helpful in determining the course of a disease and the choice of treatment. Examples of diagnostic testing include chromosome studies, direct DNA studies, and biochemical genetic testing.

Among some of the genetic tests currently used for diagnosis:

Becker/Duchenne associated with muscular dystrophy

BRCA1 and BRCA2 mutations associated with breast, ovarian, and prostate cancers

Cell-free DNA screening used to diagnose Down syndrome

CTFR mutations associated with cystic fibrosis

FH mutations associated with familial hypercholesterolemia **FMR1 mutations** linked to autism and intellectual disability **HD mutations** associated with Huntington's disease

HEXA mutations associated with Tay-Sachs disease

HBB mutations associated with sickle cell anemia

IBD1 mutations linked to inflammatory bowel disease (IBD)

LCT mutations associated with lactose intolerance

MEN2A and MEN2B mutations associated with thyroid cancer

NOD2 mutations associated with Crohn‘s disease

PAI-1 mutations, predictive of coronary artery disease (CAD) and stroke

4.4 Conclusion

Genetic testing is a type of medical test that identifies changes in chromosomes, genes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder. It can also be used to confirm the identity of an individual or contamination of the DNA.

4.5 Summary

- DNA paternity testing is the use of DNA profiling (known as genetic fingerprinting) to determine whether two individuals are biologically parent and child
- DNA test can be used to help identify genetic lineage, confirm or rule out an inherited genetic disorder, assess your risk of developing or passing on a genetic disorder, and select which drugs may be most effective based on your genetic profile
- Genotype identification 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
- DNA testing has become a useful instrument to assess the safety, quality and integrity of the food chain. It has numerous applications including the identification of allergenic material, the detection of adulteration (e.g. species replacement as a result of commercial fraud), and the identification of microbes that cause food-borne diseases

4.6 Tutor marked assignment

i. Describe the use of DNA test in meat quality test
ii. Discuss the use of DNA tests for paternity testing
iii. What are the examples of DNA test in disease diagnostics

4.7 References/ Further Reading

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UNIT 5: CONSERVATION OF ANIMAL GENETIC RESOURCES, *EX SITU* AND *IN SITU*.

Content

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5.1 Introduction

The term animal genetic resources (AnGR) is used to include all animal species, breeds and strains that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or the future. Another equivalent term increasingly used is farm animal genetic resources. Animal genetic resources for food and agriculture (AnGR) are a subset of genetic resources (defined by the Convention on Biological Diversity as "genetic material of actual or potential value") and a specific element of agricultural biodiversity. The term animal genetic resources refers specifically to the genetic resources of avian and mammalian species, which are used for food and agriculture purposes. Further terms referring to AnGR are "farm animal genetic resources" or "livestock diversity".

AnGR can be embodied in live populations or in conserved genetic materials such as cryoconserved semen or embryos. The diversity of animal genetic resources includes diversity at species, breed and within-breed level. Known are currently 8,800 different breeds of birds and mammals within 38 species used for food and agriculture. The main animal species used for food and agriculture production are cattle, sheep, goats, chickens and pigs. In the livestock world, these species are often referred to as "the big five". Some less-utilized species include the dromedary, donkey, bactrian camel, buffalo, guinea pig, horse, rabbit, yak, goose, duck, ostrich,partridge, pheasant, pigeon, and turkey. The wide number of livestock breeds and the genetic diversity within them mean that animal genetic resources have a substantial value to society. The different breeds provide a wide range of animal products and services for the benefit of humankind. The diversity of animal

genetic resources allows livestock to be raised successfully in a diverse range of different environments and underpins the supply of a range of different products and services: from meat, milk and eggs to fuel, manure and draught power.

5.2 Objectives

- You will understand animal genetic resources
- You will understand conservation of animal genetic resources
- You will understand *ex situ* and *in situ*

5.3 Main content

5.3.1 Conservation of animal genetic resources, *ex situ* and *in situ*.

For some breeds, opportunities for sustainable use are limited. For such breeds, to ensure that their critical genetic diversity is not lost, conservation programs are required. Several approaches for conservation can be applied, including in situ conservation with live animal populations, and ex situ conservation or cryoconservation involving the freezing of genetic materials. In many instances, both of these approached are used in a complementary manner. In order to establish and strengthen these programs, more research on methods and technologies must be undertaken, especially for less common livestock species, and greater financial investment is required.

Many countries are currently operating conservation programs for their animal genetic resources, at least for some species and breeds. In situ conservation programs are the most commonly used approach

5.3.2 *ex situ* Conservation of animal genetic resources

Ex situ conservation is the preservation and propagation of species and populations, their germ cell lines, or somatic cell lines outside the natural habitat where they occur. This method maintains the genetic diversity extant in the population in a manner that makes samples of the preserved material readily available. It literally means, "off-site conservation". It is the process of protecting an endangered species, variety or breed, of plant or animal outside its natural habitat; for example, by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans. The degree to which humans control or modify the natural dynamics of the managed population varies widely, and this may include alteration of living environments, reproductive patterns, access to resources, and protection from predation and mortality. *Ex*

situ management can occur within or outside a species' natural geographic range. Individuals maintained *ex situ* exist outside an ecological niche. This means that they are not under the same selection pressures as wild populations, and they may undergo artificial selection if maintained *ex situ* for multiple generations. Agricultural biodiversity is also conserved in ex situ collections. This is primarily in the form of gene banks where samples are stored in order to conserve the genetic resources of major crop plants and their wild relatives. Examples Botanical gardens, zoos, and aquariums. Others are Cryopreservation, Seed banking, Tissue culture (storage and propagation), Field gene banking.

5.3.3 *in situ* Conservation of animal genetic resources

In situ conservation is the preservation of species and populations of living organisms in a natural state in the habitat where they naturally occur. This method preserves both the population and the evolutionary processes that enable the population to adapt by managing organisms in their natural state or within their normal range. For example, large ecosystems may be left intact as protected reserve areas with minimal intrusion or alteration by humans.

In-situ conservation is the on-site conservation or the conservation of genetic resources in natural populations of plant or animal species, such as forest genetic resources in natural populations of Teagan species. It is the process of protecting an endangered plant or animal species in its natural habitat, either by protecting or restoring the habitat itself, or by defending the species from predators. It is applied to conservation of agricultural biodiversity in agro ecosystems by farmers, especially those using unconventional farming practices. This can be carried out in biosphere reserve, national parks, wildlife sanctuaries, biodiversity hotspots, gene sanctuary, community reserves, sacred groves.

In situ conservation is an important component of the conservation and management of genetic resources. It supplements the ex situ conservation efforts of local, national, and international collections and provides some important advantages. In situ conservation sites preserve potentially important and useful genes, many of which may be unrecognized today. Their existence enables the selective and adaptive processes that give rise to new genetic traits to continue in response to environmental stresses. These areas can be sources of genetic traits not already captured in ex situ collections. In situ reserves can also provide living laboratories for studying the genetic diversity of the wild species that are the progenitors of modern crops.

5.4 Conclusion

Genetic resources must be an integral part of the objectives of existing conservation efforts. In situ conservation provides the capacity to protect a wide range of genetic and species diversity and the adaptive processes that shape them. The scientific understanding necessary to achieve the most effective in situ conservation is, however, only just beginning to emerge. Cultural and economic factors likely to impede or promote long-term, in situ conservation of domesticated genetic resources often still need to be identified. The protected areas would benefit from serving as wild genetic resources areas since it would increase their value to society. The alternative— a separate network of genetic resources conservation areas, developed more or less independently from conventional protected areas— is unlikely, since competition for land is so intense that it is increasingly difficult to establish new protected areas, particularly if their purpose is narrow, such as the protection of a single species

5.5 Summary

- AnGR can be embodied in live populations or in conserved genetic materials such as cryoconserved semen or embryos. The diversity of animal genetic resources includes diversity at species, breed and within-breed level
- AnGR conservation is the need to reduce the lost and degradation of farm animal genetic resources and establish programmes for their maintenance and sustainable use both for the present and the future
- In-situ conservation is the on-site conservation or the conservation of genetic resources in natural populations of plant or animal species, such as forest genetic resources in natural populations
- Ex situ conservation is the preservation and propagation of species and populations, their germ cell lines, or somatic cell lines outside the natural habitat where they occur.

5.6 Tutor marked assignment

i. What do you understand by animal genetic resource?
ii. Using specific examples, differentiate between in-situ and ex-situ system of animal genetic resource conservation
iii. Why is it necessary to conserve animal genetic resource?

5.7 References/ Further Reading

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