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Importance of Molecular Characterization in Animal Genetics

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Abstract
Characterization of animal genetic resources encompasses all activities associated with identifying, quantitative and qualitative description and documentation of breed populations, their natural habitats and production systems to which they are or are not adapted. The process is fundamental in various fields, including medicine, agriculture, and evolutionary biology. This process encompasses identifying genes, their loci, functions, and interactions within the genome. Advanced techniques such as whole-genome sequencing, polymerase chain reaction (PCR), and bioinformatics tools play crucial roles in this endeavor. The continuous advancements in genetic characterization techniques promise to accelerate discoveries in genomics, driving forward our understanding of genetic information and its applications across various scientific disciplines. Molecular characterization to identify variation in desired traits through sequencing will provide an avenue for selection, upgrading or crossbreeding to improve performance. Using marker-assisted selection will enhance selection accuracy and the rate of genetic gain.

Molecular characterization:

A broad phrase that relates to the use of molecular markers such as DNA, RNA, and proteins to determine the genetic makeup of cells or tissues. The assessment of livestock populations and production conditions decides on their current status. It identifies strengths that can be improved and deficiencies that must be overcome, such as through a genetic improvement project. It can also help to guide conservation efforts. The invention of polymerase chain reaction (PCR) for amplifying deoxyribonucleic acid (DNA) and DNA sequencing and data processing has resulted in strong techniques for screening, characterizing, and assessing variation. The identification, quantitative and qualitative description, and documentation of breed populations, their natural habitats, and the production techniques to which they are or are not suitable are all included in the characterization of animal genetic resources (FAO, 2007). Characterization aims to increase understanding of Animal Genetic Resources (AnGR), their prevalence, and potential applications in broader contexts (FAO, 1984; Rege, 1992). In order to compare the potential performance of different types of AnGR within the various production systems present in a country or region, characterization activities should help to provide an objective and trustworthy prediction of animal performance in defined contexts (FAO, 2015). Phenotypic and molecular characterizations are the two main categories into which characterization is usually divided.

Genetic characterization of animal genetic resources:

Molecular characterization or genetic characterization can be defined as the complementary procedures used to unravel the genetic basis of phenotypes, their patterns of inheritance from one generation to the following, within-breed genetic structure and levels of variability, and relationships between breeds (FAO, 2015). Any livestock breed must be defined by its genetic variability. To maintain the most significant possible genetic diversity, it is critical to identify various breeds to determine how distinct or different they are from other local populations. It is the next step in providing answers to queries about taxonomy, evolution, domestication procedures, genetic resource management, and creating conservation plans for their efficient use is genetic characterization. Improved management of genetic variability and a more vital ability to respond to selection is being made possible by genomics. Additionally, this will make connecting phenotypes to epigenetic events and non-coding RNA molecules easier. Because there are so many different genes, traits, and breeds, genomic analysis has grown to be a broad field of study.

Why is animal genetics essential?

Livestock populations provide various products and services in multiple habitats, including meat, milk, eggs, fibre, and draught power. This range of function is only feasible due to the diversity of their genetic makeup. Genetic variability among animal populations also acts as the foundation for evolution through natural selection in response to changing environments and human-managed genetic improvement efforts. It is essential for raising output and adapting livestock populations to climate change, new diseases, and feed and water scarcity. Animal genetics is one of the fundamentals of livestock production. It covers a wide range of local, national, regional, and global activities, as well as characterization, conservation, and genetic enhancement.

Objectives of genetic characterization:

- Increase knowledge of Animal Genetic Resources (AnGR), their abundance, and potential for future uses in more comprehensive environments.
- Reliable prediction of animal performance in defined

environments allows a comparison of the potential performance of different types of AnGR within the various production systems in a country or region.

- Identifying variation in desired traits through sequencing will provide avenues for selection, upgrading or crossbreeding to improve performance.
- Using marker-assisted selection will enhance selection accuracy and the rate of genetic gain.
- Animal breeders in developing countries would be able to develop improved breeds and conserve local genetic resources for optimum use in the future.
- Assessing functional and neutral genetic variability within and between populations.
- Assessing the current state of a population regarding risks related to inbreeding and genetic drift using estimators such as effective population size.
- The establishment of whole genome sequences for various livestock species.
- The development of technologies for measuring polymorphisms at loci spread across the entire genome.

Techniques for Molecular Characterization:

- DNA Sequencing
- Conventional Sequencing Technique
- Next-Generation Sequencing Techniques
- Microsatellites or Simple Sequence Repeats (SSRs)
- Single-nucleotide polymorphism (SNP)
- Random Amplified Polymorphic DNA (RAPD)
- Amplified Fragment Length Polymorphism (AFLP)

Methodology of genetic characterization by DNA sequencing:

- **Experimental animals:** The animals for molecular characterization, genetic polymorphism and association study should be selected for the experiment.
- **Collection of blood samples:** About 10 ml of blood samples are collected aseptically through jugular vein puncture into anticoagulant EDTA-containing vacutainer tube from the animals under study. The samples were then stored at - 20°C temperature till genomic DNA isolation. Approval from the Institutional Animal Ethics Committee (IAEC) should be obtained to collect blood from selected animals.
- **Isolation of Genomic DNA:** Genomic DNA will be isolated from the blood samples by the 'Phenol: Chloroform extraction' method, as described by Sambrook et al. (2001).
- **Quantity and Quality check of Isolated Genomic DNA:** The integrity and the quality of extracted DNA should be checked on 0.8 % agarose under horizontal gel electrophoresis and visualized under a gel documentation system. The concentration and purity of DNA can be estimated through a NanoDrop Spectrophotometer. Only the DNA samples with a concentration of 50ng/µl- 100ng/µl with an optical density of 1.8 should be used further to amplify the desired DNA fragment in the PCR technique.
- **Primer Designing:** Primers are designed for gene amplification based on reference sequence available at NCBI based on the species of animal selected.
- **PCR Amplification of the IGF-1 gene for the detection of SNPs:** Polymerase Chain Reaction (PCR) is carried out to amplify the genomic DNA. Gradient PCR is performed to standardize the annealing temperature for optimum amplification. The quality of the PCR product was then checked on 1% agarose in horizontal gel electrophoresis.
- **Detection of Genetic Variation (SNPS) and Genotyping of the PCR Amplicons:** Purified PCR amplicons are then used for bidirectional sequencing using Forward and Reverse primers by 'Sanger's dideoxy chain termination method' (Sanger et al., 1977). The forward and reverse sequences obtained for each animal are aligned and analyzed using different Software to generate different sequence patterns.

Challenges in molecular characterization of animal genetic resources:

- Poor infrastructure
- Reference genomes
- Biological background information
- Population genotyping data are not available
- Inadequate funding
- Poor laboratory services, and
- Inadequate technical human resources.

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