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DNA Sequencing and its application in Animal Improvement

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Abstract: ***The development of various molecular techniques to extract genome and the use of bioinformatics for processing the obtained results have gradually assisted in establishing molecular biology for improvement of phenotype of several species. Sequencing is the first step in many of such experiments. Genetic makeup of domesticated animals is profoundly related to the level of their performances, through phenotypic expressions. The special qualities like adaptability to local climatic conditions and farming situations, resistance to diseases, quantity and quality of produce ,etc are basically governed by the relevant genes located inside the genome of the species. The present study deals with the various techniques of DNA sequencing, and their subsequent use in animal improvement programmes. DNA sequencing begun with the development of sequencing technique by Sanger and et al. Later, Sanger developed a method (later named as Sanger Sequencing), which is widely used even at present. The advent of DNA sequencing methods as first, second, third and next generation tecnologies aided in more rapid, easy and economic sequencing in the subsequent years. Among them, Next Generation Sequencing (NGS) is widely used nowadays specially in the case of whole-genome sequencing. The application of NGS in various fields of genome mapping, detection of linkages and mutant alleles, etc. have revolutionalised the field of genomics. Nowadays, animal breeders use thousands of genetic markers to select and improve animals. These all have been possible due to discovery of sophisticated DNA sequencing techniques that have eventually set the milestone in animal breeding and animal genome projects.***
Keywords: Genome, DNA sequencing, Next Generation Sequencing (NGS), genetic markers, animal breeding.

I. INTRODUCTION

Modern biology is particularly associated with the study of infinitesimal particles that regulate the metabolism, inheritance and variance in living system. Molecular biology is one of the compelling fields of modern biology, under which lots of research activities are being carried out globally. The inception of molecular biology dates back to late 1860s when DNA (basic element of heredity and variance) was first identified by Swiss chemist Friedrich Miescher, and later, the discovery of double helix structure of DNA in 1953 by Watson and Crick created the foundation for instigating research works in molecular biology (Pray, 2008). The development of various molecular techniques to extract genome and the use of bioinformatics for processing the obtained results have gradually assisted in establishing molecular biology for improvement of phenotype of several species. Sequencing is the first step in many of such experiments (Dunislawska et al., 2017).

Genetic makeup of domesticated animals is profoundly related to the level of their performances, through phenotypic expressions. The special qualities like adaptability to local climatic conditions and farming situations, resistance to diseases, quantity and quality of produce ,etc are basically governed by the relevant genes located inside the genome of the species. The genome mapping is essentially carried out to seek for the location of candidate genes, linked genes, mutant genes etc. for the evaluation of their usages in animal breeding, and this subsequently leads to the improvement of performances of farm animals. Various breeding methods have been developed for this purpose. However, almost all those methods rely on DNA seuqences of the given animal species to conduct the further researches of trait improvement. The advent of DNA sequencing techniques adds a new dimension to animal breeding (Bhat et al., 2017).

II. OBJECTIVES OF THE STUDY

This review paper is formatted with the objectives of exploring different techniques of DNA sequencing and their application in various areas such as animal breeding, genome mapping, animal genome projects etc., eventually aiming at the animal improvement.

III. RESEARCH METHODOLOGY

The present study is conducted analytically with the exploration of data, theories, facts and hypotheses from variable sources. These sources include book, book section, journal article, conference proceedings, report and other published web based resources.

IV. GENERATION SEQUENCING TECHNIQUES

A. First Generation Sequencing

DNA Sequence is the first determinant of chromatin organization and cross-talks between the DNA sequence, the protein complexes involved in the chromatin architecture and the structural components of the nucleus provide a proper subnuclear environment that ensures correct spatial and temporal gene expression (Schluth-Bolard etal., 2011). The first method for sequencing DNA was developed by a team led by Fred Sanger in the middle 1970s (Miller, 2001). Subsequently, Sanger developed a new method popularly known as Sanger Sequencing, and as a result, Sanger was named the father of sequencing. Sanger sequencing involves the use of di-deoxy nucleotides in DNA synthesis reactions, which is based on the partial digestion of radiolabeled fragments after two-dimensional fractionation (Sanger et al., 1965). This method has been widely used for its great advantage of potential for automation. Another efficient method ,known as pyrosequencing was developed a decade later with the major advantage of real time sequencing (Ronaghi, 2001).

The development of new technologies enabled the first attempts of sequencing whole genome of an organism. *Mycoplasma genitalium* (Bacterium) was the first cellular organsim whose whole genome was sequenced successfully for the first time (Fraser, 1995). Whole genome random sequencing method was applied in this process of sequencing the bacterial genome (580,000 base pairs long). The process of DNA sequencing got the step forward with the commencement of Human Genome Project in 1990. The work was led by the US Department of Energy and the US National Institutes of Health. The full sequence of human genome was given by Venter et al., (2001).

The team was able to discover 90% of the total human genome, the highest coverage possible at that time (Dunislawska et al., 2017). The additional information presented in 2003 extended the coverage of human genome to 99%. The suceess of this project boosted the feasibility of DNA sequencing in farm animals ,and its subsequent utilisation in animal breeding.

B. Second Generation Sequencing

Second generation sequencing is based on sequencing of amplified DNA molecules. Solexa and Pyrosequencing are based on it (Hetather and Chain, 2016).

The principle of second generation sequencing is to generate millions of relatively short reads from amplified single DNA fragments using repeative cycles of nucleotide extensions (Bayes et al., 2012). Solexa sequencing (Illumina dye sequencing) is a four-color sequencing-by-synthesis approach where incorporation of a reversible terminator nucleotide generates a flourescence signal detected by a high-sensitivity camera for A, C, G, and T during each cycle (Lefrancois et al., 2010). Another methodology used in this generation sequencing is pyrosequencing, which relies on the luminometric detection of pyrophosphate released during nucleotide incorporation. Pyrosequencing has been succesful for both confirmatory and *de novo* sequencing (Ronaghi, 2001).

C. Third Generation Sequencing

The introduction of first and second generation sequencing techniques greatly assisted the researchers to sequence the genome of different animal species. Since these techniques involve the multiple manipulation steps, the goal of achieving accuracy with unbiasness in sequencing remain unaccomplished. In addition to this, there was also a need of a novel technique to sequence cellular RNA directly without reverse transcription or the multiple manipulations. Hence, third generation sequencing techinque has been developed to effectuate the shortcomings of first and second generation techinques. Third generation sequencing include the methods such as tSMS (True Single Molecular Sequencing) and SMRTS (Single Molecule Real Time Sequencing), which involves the no prior pre-amplification of sequencing matrix (Schadt, et al., 2010).

D. Next generation sequencing

Sanger sequencing technology, which was used to decipher the human genome, required over a decade to deliver the final draft (Behjati, 2013). Next generation sequencing (NGS) or massive parallel or deep sequencing is defined as technology allowing one to determine in a single experiment the sequence of a DNA molecule(s) with total size significantly larger than 1 million base pairs (1 Mb) (Ploski, 2016). NGS works with two major paradigms: short-read sequencing and long-read sequencing , the former dealing with sequencing approaches with lower cost and higher accuracy, and the later providing the read lengths that are well suited for *de novo* genome assembly applications and full-length isoform sequencing (Goodwin, 2016). The sequencing method takes place in three stages: (I) Isolation and creation of the DNA library, (II) Amplification of the template, and (III) massive parallel sequencing (Mardis, 2008). The application of NGS in various fields of genome mapping, detection of linkages and mutant alleles, etc. have revolutionalised the field of genomics.

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V. APPLICATIONS OF DNA SEQUENCING IN ANIMAL IMPROVEMENT

A. Genome Mapping

The sequencing of genome of an animal species helps in determining the underlying genes that code for the traits of economic value. Hence, genome mapping can potentially boost up the breeding related works for fostering the performances of farm animals. The experimental strategies developed for the identification of underlying genes can be broadly categorized into two approaches: linkage studies and candidate gene approach (Jeffreys, 1985).

Linkage studies helps in the identification of chromosome genes that are expected to affect a trait by their simultaneous inheritance. Generally, Quantitative Loci (QTL) mapping is performed at the sites of linkage maps by pedigree analysis and, through the study of segregation patterns of genetic markers. Candidate gene approach is based on the study of relationship between the traits and known genes that may be associated with the physiological pathways underlying the trait (Liu etal., 2008). In simple words, candidate gene approach uses the 'Genome to Phenome' outlook i.e. it is based on the variability within gene that codes a specific protein involving a metabolic pathway, and subsequently, it results in the expression of an economic trait. Evaluating the role play, growth hormone (GH) and myostatin (MSTN) genes can be considered candidate genes for meat production traits, and casein genes in milk production traits (Rothschild, 1997).

B. Genomics of Endangered Species

The sophisticated techniques of DNA sequencing can be applied in sequencing the genome of vulnerable and endangered species. Li etal., (2010) announced that they had sequenced 94% of total genome of giant panda, and this study was aimed to find the potential genes that determine the specific bamboo diet and allow for the exclusion of diet dependance affecting the gut microbiome. The similar techinques can also be adopted to sequence the genome of indigenous breeds of cattles, goats, sheeps, poultry, pigs, etc that are likely to get endangered in near future.

C. Evolutionary Approach

Since evolution is a continously occuring process, majorily due to variability in genetic makeup of the species; genomic analysis of past and present races of animals can largely determine the evolutionary history. The domestication of farm animals started about 12,000-15,000 years ago.The availability of accurate and reliable DNA analysis now allows a more cost- effective determination of a genomic breeding value (GBV) for individual animals (Niemann, 2018). GBV of related species can be studied, and the obtained data can be analyzed for determining the evolutionary signifance, and its subsequent use in animal improvement.

D. Microbiological approach

The sophisticated technique(s) like NGS provide microorganism sequencing without need for prior culture (Garza, 2015). This faciliates in the identification of new metabolites produced by microorganisms that may be of potential use in human or veterinary medicine (Bryan et al., 2012). The important achievement in this approach has been made by deciphering the complete sequence of the bacteriophage with antimicrobial properties that inhabits chicken intestins and which could be used to fight bacterial diseases (Diaz-Sanchez et al., 2013). Hence, this approach can potentially foster the use of microorganisms in veterinary science that are difficult to culture and manipulate in laboratory.

E. Animal Breeding

After the complete genome sequencing of human in 2001 and cattle in 2004, various research activities were conducted for the identification of potential genetic markers in many animals, and their subsequent use in animal breeding programmes. In the past decades, the breeding activities were carried out majorily with selection by visualizing the phenotypic markers and through pedigree records. Nowadays, animal breeders use thousands of genetic markers to select and improve animals.

Some practical use cases of DNA sequencing in animal breeding are discussed below:

1) The International Goat Genome Consortium (IGGC) was founded in 2010 in China ,and the initial activities of de novo goat genome assembly and resequencing; development of a Goat Radiation Hybrid Panel and Mapping; and production of a high-density Single Nucleotide Polymorphism (SNP) chip (Zhang, 2011).

2) Chickens have thousands of SNPs in their genome. The phenotypic information for feed conversion and growth rate in Broilers and egg production, egg quality and life span of birds in layers are gathered with the help of pedigree records, and this is correlated with the individual's SNPs (Raj).

3) A number of gene and marker tests such as CAST (meat quality), ESR and EPOR (litter size), FUT1 (E. coli resistance), MC4R (growth and fat) etc. are now available comercially from genotyping service companies for pigs (Walters, 2011).

F. Animal Genome Projects

After the advent of various techniques in DNA sequencing, many resesrch activities are being carried out in animal genome sequencing and animal breeding projects. In March 2004, the release of the first draft of the chicken genome spawned the current boom in chicken genomic research (Antin, 2005).

The pig genome sequencing projects have been found instrumental in the improvement of human health. Clinical studies in areas such as organ transplantation, physiology, infectious disease, pharmacology, obesity and cardiovascular disease have used pig models (Rothschild M. , 2004).

The identification of several SNPs has assisted the geneticists to find associations between certain genes and cow traits that will eventually lead to the production of superior-quality beef (Adam, 2002). Similarly, many other projects related to endangered species and farm animals are being conducted in genome sequencing, and its application in conservation and improvement of those animals.

VI. CONCLUSIONS

DNA sequencing has been an indespensable tool for various activities in the field of animal breeding. Several molecular techniques are being used in the field of animal breeding and biotechnology. These techniques are majorily related with QTL mapping, marker-assisted selection, precision breeding, etc. for animal improvement. All of these require a fundamental process of DNA sequencing at the beginning.

The sequenced genome is passed through Agarose Gel Electrophoresis (AGE), and other relevant techniques to reveal the marker-sites constituting the traits of economic value.

The field of molecular science is vast and vague. Hence, these techniques are always in the transitional stages for further improvements, and this eventually results in the development of more sophisticated techniques for rapid, easy and economic animal improvement.

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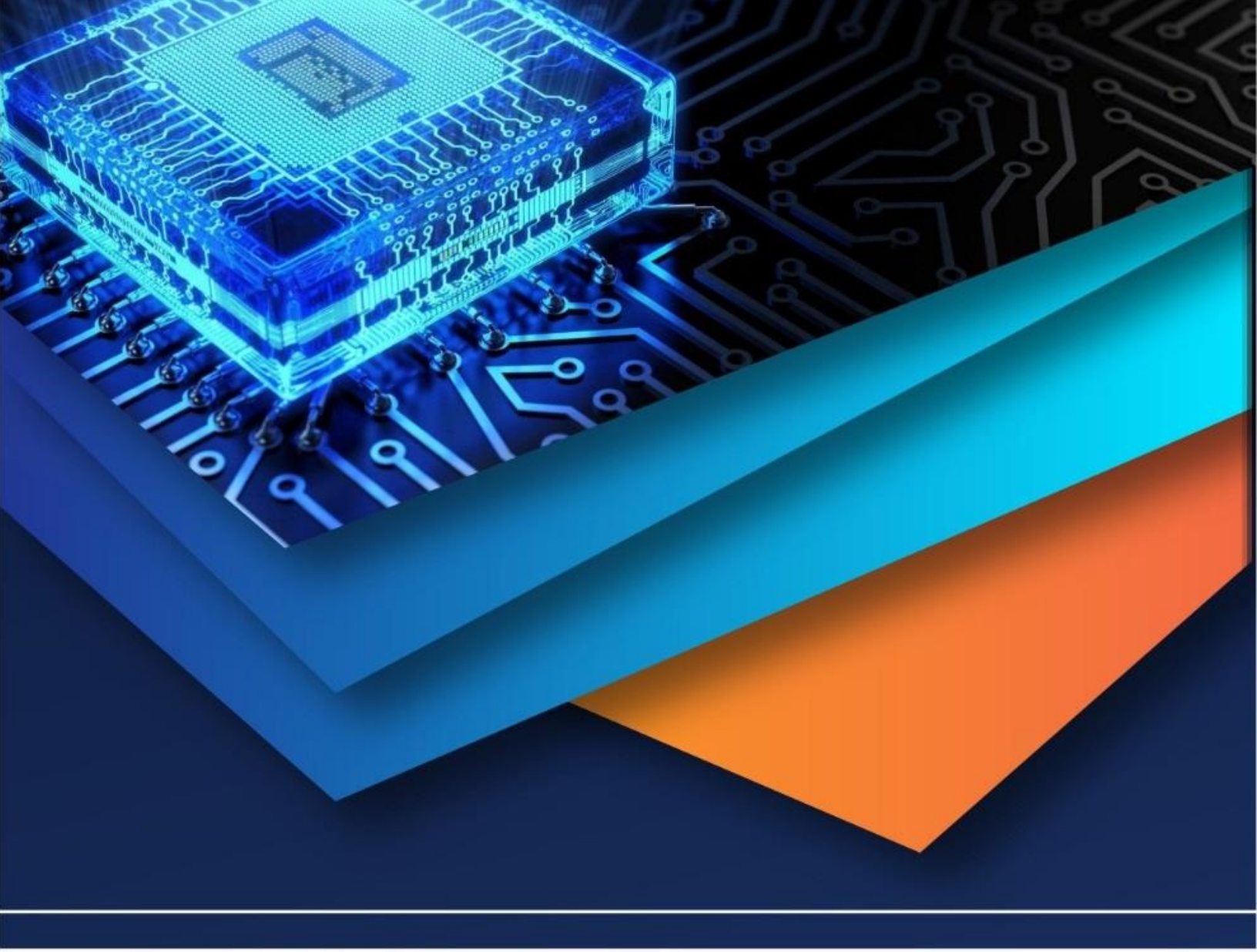

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