

AN INTRODUCTION TO DNA TECHNOLOGIES AND THEIR ROLE IN LIVESTOCK PRODUCTION: A REVIEW

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ABSTRACT

DNA technologies have revolutionized modern science in many ways. DNA (Deoxyribonucleic acid), the hereditary material in all living organism from bacteria to bull, controls and directs all the functions of a living organism. Many techniques have been evolved to understand and manipulate the DNA for many purposes like parentage analysis, curing genetic diseases, forensics, gene therapy and to improve the livestock production. In this paper DNA technologies. Polymerase Chain Reaction (PCR), DNA sequencing, Recombinant DNA technology, DNA microarray technology, marker assisted selection (MAS), stem cell, genetic engineering and their role in order to improve livestock production are reviewed.

Key words: Deoxyribo nucleic acid (DNA), Short tandem repeat (STR), Single nucleotide polymorphism (SNP), Marker assisted selection (MAS), Polymerase chain reaction (PCR).

INTRODUCTION

Exploring the DNA means to identify the gene and mark Short Tandem Repeats (STRs) (Ciampolini *et al.*, 2006), Single Nucleotide Polymorphism (SNP) (Verschoor *et al.*, 2009) in that gene responsible for different traits. It is very much possible to discover those genes, controlling milk production and other production traits by using DNA technologies such as DNA mapping, DNA microarray, DNA sequencing and then modify them by genetic engineering or recombinant DNA technology to produce genetically modified organisms (GMO) (Holst, 2009) or Transgenic animals for more and better yield of desired products. Doing all this will enable us to select improved livestock on the basis of their genotype or genetic makeup. The first step is to understand the genetic control of the desired trait and then to uncover the involved genes.

Polymerase Chain Reaction (PCR): PCR is the basic technique to amplify the desired sequence or sequences of DNA for checking STRs, SNP in different breeds, parentage identification, genetic mutation etc. In 1980s, Biosearch manufactured automated, solid-phase DNA synthesizers, such as the SAM I, the Cyclone, Biosearch 8800 Prep and Biosearch 8700. These instruments manufactured oligonucleotides, made path for the development of revolutionary new oligo based technologies. In 1982, Kary Mullis then used a Biosearch SAM I DNA synthesizer to create oligos, which eventually resulted in the discovery of the Polymerase Chain Reaction (PCR). Kary Mullis performed his first successful experiment on December 16, 1983. The development of the PCR has also been linked to the development of the Internet. Both inventions have

emerged in the last 20 years to the point where it is difficult to imagine life without them (Bartlett and Stirling 2009).

Major components of the PCR are the Taq polymerase enzyme, DNA template, primers, and the PCR machine (Thermocycler) which maintains the optimum temperature for each step in every cycle. In PCR three steps in each cycle include Denaturation, Annealing and Extension or Elongation (Bartlett and Stirling 2003). The PCR is widely used for applications which require a high level of specificity and reliability, such as genetic testing, clinical diagnostics, blood screening, forensics and biodefense (Alexandre *et al.*, 2008). To check whether the PCR generated the anticipated DNA fragment, agarose gel electrophoresis is employed for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder (a molecular weight marker), which contains DNA fragments of known size, run on the gel alongside the PCR product.

Many types of PCR used for different purposes for example Allele-specific PCR used for selective PCR amplification of one of the alleles to detect Single Nucleotide Polymorphism (SNP) (Newton *et al.*, 1989). Hot-start PCR reduces non-specific product amplification by maintaining reaction temperature above that where non-specific annealing of primers to targets occurs (Sharkey *et al.*, 1994). Multiplex-PCR enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers (Elnifro *et al.*, 2000). Assembly PCR synthesizes long DNA sequences by performing reaction on a pool of long oligonucleotides (primers) with short overlapping segments (Stemmer *et al.*, 1995). Asymmetric PCR used to preferentially amplify one strand of the original DNA

more than the other by adding a great excess of the primers for the chosen strand (Innis *et al.*, 1988). Quantitative PCR or RT-PCR is used to amplify and simultaneously quantify a targeted DNA molecule (Higuchi, *et al.*, 1992). Single-cell PCR used to amplify a single cell. It has proven to be of enormous use to address diverse immunological, neurological and developmental questions (Khan *et al.* 2008). TAIL-PCR: Thermal asymmetric interlaced PCR is an efficient tool for the recovery of DNA fragments adjacent to known sequences (Liu and Whittier 1995). Touchdown PCR or Step-down PCR involves the use of an annealing temperature that is higher than the target optimum in early PCR cycles to avoid unspecific amplification (Don *et al.*, 1991). Miniprimer PCR use an engineered polymerase and 10-nucleotide miniprimers to expand the scope of detectable conserved sequences beyond those detected by standard methods using longer primers and Taq polymerase (Isenbarger *et al.*, 2008). Ligation-mediated PCR used to exponentially amplify segments of DNA located between two specified primer hybridization sites (Mueller and Wold 1998).

Real Time Polymerase Chain Reaction (RT-PCR):

Real-time PCR (RT-PCR) also known as Real Time Quantitative PCR (RTQ-PCR) (Van Guilder *et al.*, 2008) is a relatively new technology that has been used for research. Some of the advantages of RT-PCR are high sensitivity, high specificity, rapid time-to-result, scalability, cost, and quantitative nature (Erica and Suarez 2008). The invention of the Real Time PCR was caused by some serious limitation of the original PCR method. Such as by first amplifying the DNA sequence and then analyzing the product, quantification was exceedingly difficult since the PCR gave rise to essentially the same amount of product independently of the initial amount of DNA template molecules that were present. This limitation was resolved in 1992 by the development of Real-Time PCR (Higuchi *et al.*, 1992). In Real-Time PCR, five main chemistries used for the detection of PCR product. These are the DNA binding fluorophores, the 5' endonuclease, adjacent linear and hairpin oligoprobes and the self-fluorescing amplicons (Mackay *et al.*, 2002). By using the real time PCR it is possible to calculate the number of DNA molecules amplified in a complex sample by monitoring the cycles (checking the fluorescence of dyes) (Kubista *et al.*, 2006). Amounts of RNA or DNA are then determined by comparing the results to a standard curve produced by Real-Time PCR of serial dilutions of a known amount of RNA or DNA (Nailis *et al.*, 2006). Typical uses of real-time PCR include pathogen detection, gene expression analysis, single nucleotide polymorphism (SNP) analysis, analysis of chromosome aberrations, and most recently also protein detection by Real-Time immuno PCR (Kubista *et al.*, 2006).

Stem Cell Technology: Stem cells are the foundation for every organ, tissue and cell in a multicellular organism. In 1963 first quantitative descriptions of the stem cells were documented by Canadian researchers. Stem cells may be able to repair or replace damaged tissue, thereby reversing diseases and injuries such as cancer, diabetes, cardiovascular disease and blood diseases. Two types of Stem Cells are there, one is the embryonic stem cell and the other is called adult stem cell (Tuch 2006). Stem cells should have two basic properties i.e. self renewal and potency (the ability to change in different type of body cells). Potency wise stem cells are classified into five types. Unipotent stem cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells. Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells. Totipotent stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells. Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells (Hans, 2007). Embryonic stem cells are pluripotent and derived from blastocyte. Potential use of embryonic stem cells (ESC), especially for genetic modifications, there is great interest in establishing domestic animals-related ESCs (Munoz *et al.*, 2008). Farm animal ESCs may be useful for the generation of transgenic animals as usually have a self-renewal capacity more prolonged than normal primary cultures thus increasing the possibility to transform and select cells to be used as nucleus donors in cloning procedures. Farm animal ESCs may also be an excellent experimental model in pre-clinical trials (Brevini *et al.*, 2008). The adult stem cell (in a developed organism) has two properties: the ability to divide and create another cell like itself and also divide and create a cell more differentiated than itself. Also known as somatic stem cells and germline stem cells (Jiang *et al.*, 2002). A very vital aspect of stem cell research is stem cell therapy. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia (Gahrton and Bjorkstrand 2000). In the future stem cell research and technologies developed will enable to treat a wider variety of diseases including cancer, Parkinson's disease (Lindvall 2003), spinal cord injuries, Amyotrophic lateral sclerosis, multiple sclerosis, and muscle damage, amongst a number of other impairments and conditions (Goldman and Windrem 2006).

DNA Sequencing: DNA molecule is completely comprised four nucleotide bases known as Adenine, Guanine, cytosine and Thymine abbreviated as A, T, C and G. DNA sequencing refers to methods for determining the order of the nucleotide bases in a

molecule of DNA (Ewing and Phil 1998). Knowledge of DNA sequences of genes and other parts of the genome of organisms has become indispensable for basic research studying biological processes. The history of DNA sequencing is a brief one. Merely 30 years ago it was first described by Fred Sanger (1977). In that brief time, progress has been staggering, owing in large part to interdisciplinary innovation that has built on the fundamental and elegant concept of dideoxynucleotide termination. Development of rapid DNA sequencing methods in the early 1970s is affiliated with Frederick Sanger. In 1973, Gilbert and Maxam reported the sequence of 24 base pairs using a method known as wandering-spot analysis (Sanger and Coulson 1975). Up to now different platforms have been developed to address the demand for speed, low cost, quality, reliability, scalability, and automation. Such as capillary electrophoresis sequencing (CE sequencing). Capillary DNA sequencing played a major role in the early completion of the draft sequence of the human genome. In CE sequencing gel matrix, which contains capillaries, is used to separate the DNA fragments. Increased throughput through automation of gel preparation and sample loading have made capillary electrophoresis (CE)-based machines a mainstay not only in large genomic research laboratories but also in small core service facilities. Although CE sequencing offers advantages such as ease of operation and reduced user interaction, some factors like DNA quality and concentration are still a major issue in CE sequencing (Ernesto *et al.*, 2003). Technological advances in CE sequencing have improved resolution remarkably, enabling the use of a drastically reduced amount of DNA template in dye terminator sequencing reactions (Yoshida *et al.*, 2001). It has enabled direct sequencing of bacterial genomic DNA and plasmids of low copy number or low concentrations.

Different steps involved in sequencing, are; sub cloning, template preparation and sequencing reaction, separation and base-calling. While some other sequencing methods are also being used such as Sequencing by hybridization (Hanna *et al.* 2000), Atomic Force Microscope (AFM) or electron microscopy .

An aid in the development of livestock genome maps has been the high level of conservation of gene sequences between humans, cattle, sheep, goat, pig and mice. By such reason, once loci of particular DNA sequences has been mapped in one species, the information is frequently of help in the genome mapping in one another. Livestock species mapping had been greatly facilitated by the increasing availability of human and murine sequences (Schimenti, 1998). Once we able to identify and map the gene for milk and meat production, then it can be engineered and modified for desired results in order to improve livestock.

Genome sequence information is available at the websites below mentioned

- <http://sol.marc.usda.gov/genome/cattle/cattle.html>
- <http://sol.marc.usda.gov/genome/swine/swine.htm>

DNA Microarray: A DNA microarray is a complex technology used in molecular biology and in medicine. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles of a specific DNA sequence. This can be a short section of a gene or other DNA element that are used as probes to hybridize a cDNA or cRNA sample called target under high-stringency conditions (Kulesh *et al.*, 1987). In standard microarrays, the probes are attached to a solid chemical matrix (glass or a silicon chip) known as *gene chip*. This hybridization assay provides basics for the DNA based diagnosis of different genetic diseases.

Innovation of methods for high-density spatial synthesis of DNA oligonucleotides and production of arrays with more than 250,000 DNA oligonucleotides probes at a time has really accelerated speed the technique (Lipshutz *et al.*, 1999). DNA microarrays have made important contributions, most notably to gene-expression studies. Techniques such as transcript profiling and genotyping are becoming routine, still other techniques and applications, such as genome-wide epigenetic analysis synthesis is in the early phases of development.

DNA microarrays are used to measure changes in expression levels, to detect single nucleotide polymorphisms (SNPs), in genotyping or in resequencing mutant genomes (Kulesh *et al.*, 1987). DNA microarrays can be used to detect DNA, or detect RNA that may or may not be translated into proteins. The process of measuring gene expression via cDNA is called expression analysis or expression profiling.

The principle of DNA arrays is based on the hybridization technique. Oligonucleotides or PCR probes are immobilized on a solid support (the matrix) and due to their specificity to a target gene they will detect complementary sequences present in a mix which is to be analyzed. The hybridization signals are detected, depending upon the type of labeling by radiography or fluorescence and then quantified (Martin and Claus, 2007).

In livestock production DNA microarrays is revolutionizing detection of genes expressed in diseased versus healthy tissue and tough versus tender meat. This together with much cheaper DNA testing may allow a move towards marker assisted selection.

Genetic Engineering: Genetic engineering is manipulating and modifying genome of organisms for many beneficial purposes. For example to get the desired product (biopharmaceuticals), better breeding (recombinant DNA technology), make them more defensive against diseases, and curing genetic diseases by gene therapy.

Recombinant DNA technology: Recombinant DNA is created by combining DNA sequences that would not normally occur together in nature (Garret and Grisham 2008). In appropriate conditions a recombinant DNA molecule can enter in cell and replicate there (Jonathan and Wiley 2003). It differs from genetic recombination, in a way that it does not occur through processes within the cell, but is engineered (Garret and Grisham 2008). Recombinant DNA's directed proteins are known as recombinant proteins (Colowick and Kapanian 1980).

The Recombinant DNA technique (RDT) was first thought by Peter Lobban (Wright, 1972). The first miracle of recombinant DNA technology was the production of Human insulin. The specific gene sequence, or oligonucleotide, that code for insulin production in humans was introduced to a sample colony of *E. coli*. In 24-hour period, there may be billions of *E. coli* that are coded with the DNA sequences needed to induce insulin production (Johnson, 1983).

Recombinant DNA Technology is strongly associated with cloning. Cloning is the process of producing populations of genetically-identical individuals asexually. Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. RDT produces the initial cell from which the host organism is then expected to recapitulate when it undergoes further cell division. Recombinant DNA is first incorporated in vector or plasmid (Jeremy *et al.*, 2002) and then this plasmid is introduced in the target organism, where desired proteins are produced by this recombinant DNA.

In order to increase the livestock production RDT plays an important role. The foot-and-mouth disease virus is engineered to produce better vaccine (Roberto *et al.*, 2008). The production of tailor made crops as feedstuff and free from malnutritional or antinutritional factor is also possible through recombinant DNA technology. RDT is also involved in better breeding of the animals. The most obvious difference from conventional breeding is that genetic modification allows us to transfer genes between species. Hence producing better combinations which cannot be produced otherwise.

Gene Therapy: Gene is a structural, functional and mutational unit of DNA (Pearson, 2006). Change in natural coding property of a gene is called mutation which is often lethal (Bertram, 2000). Correcting that mutation is called gene therapy. This is a technique whereby the absent or faulty gene is replaced by a working gene, so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease (Kirm *et al.* 2002). Gene therapy has the potential to treat devastating inherited diseases.

Before applying gene therapy an accurate diagnosis of the genetic defect needs to be made. For diagnosis DNA probes are designed to stick very

specifically to certain fragments of DNA. Gene therapy may be classified into two types. One is Germ line gene therapy and the other is Somatic cell gene therapy (Brown, 1995). Four approaches are now being used in gene therapy. These are Gene augmentation therapy, Targeted killing of specific cell, Targeted mutation correction and the last is Targeted inhibition of gene expression (Cavazzana *et al.*, 2004).

In general, a gene cannot be directly inserted into an organism's cell. It must be delivered to the cell using a carrier, or vector. Vector systems can be divided into viral vectors and non-viral vectors (Laporte *et al.*, 2009). Among non viral systems Electroporation which include cell electropermeabilization, with the help of exposure to appropriate electric field pulses, is currently receiving much attention as a way to increase DNA delivery. However, the transfection efficiency of electroporation-assisted DNA delivery is still low compared to viral methods and there is a clear need to optimize this approach (Mir, 2008). Microinjection is another non viral approach for the generation of stable recombinant cell lines since it allows control of the amount and subcellular location of the DNA delivered into each cell. Microinjection of naked DNA into specific cellular compartments allows both the copy number and the location of the plasmid DNA to be controlled (Derouazi *et al.*, 2003). In gene gun system, micron-sized gold particles are coated with plasmid DNA and then accelerated at high speed toward target cells. Cells penetrated by the gold particles have high probability of being transfected by the DNA thus introduced. Micron gold metal particles are used as carrier of plasmid DNA containing desired gene (Nishizaki *et al.*, 2000).

In livestock production most important affecting factors are nutrition and disease. Animal diseases cause great reduction in their production. Use of gene therapy may significantly contribute in curing diseases and making animal healthier and more productive. Some examples of gene therapy are Gene therapy of lysosomal storage diseases (Mark and Davidson 2006), Vaccine approaches to prevent and treat prion infection (Müller-Schiffmann *et al.*, 2008), Molecular basis of pathogenesis of FMDV (Mason *et al.*, 2003), Recombinant adenovirus co-expressing capsid proteins of two serotypes of foot-and-mouth disease virus (FMDV), *in vitro* characterization and induction of neutralizing antibodies against FMDV in swine (Wu *et al.*, 2003).

Transgenesis: This technology is used to produce transgenic animals or genetically modified Organisms (GMO). Transgenic animals are produced by inserting desired foreign gene/s (Trans gene) into their genome (Hofmann *et al.*, 2003). The Federation of European Laboratory Animal Associations defines the term as an animal in which there has been a deliberate modification of its *genome*, the genetic makeup of an organism

responsible for inherited characteristics. Transgenes are produced by recombinant DNA technology. The development of methods for cell-mediated transgenesis provides a tremendous opportunity to shape the genetic make-up of livestock animals in a much more directed way than traditional animal breeding and selection methods (Laible and González 2009).

In order to produce GMO, different techniques; microinjection, retrovirus infection, embryonic stem cells, viral vectors and microinjection are used (Yao *et al.*, 2002). A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA (John., 1992). The method was successfully used in 1974 when a simian virus was inserted into mice embryos, resulting in mice carrying this DNA (Jaenisch, and Mintz 1974). Totipotent embryonic stem cells containing the desired DNA are incorporated into the host's embryo, resulting in a chimeric animal unlike the other two methods, which require live transgenic offspring to test for the presence of the desired transgene, this method allows testing for transgenes at the cell stage (Gossler *et al.*, 1986). Using DNA microinjection the mouse was the first animal to undergo successful gene transfer. This method involves transfer of a desired gene construct from same species or from a different species into the pronucleus of a reproductive cell. The manipulated cell, which first must be cultured in vitro (in a lab, not in a live animal) to develop to a specific embryonic phase, is then transferred to the recipient female (Gordon, J.W. *et al.*, 1980).

Advancements in bovine reproductive technologies such as artificial insemination, multiple ovulation and embryo transfer, as well as oocyte and embryo culture have improved the ability to increase superior genotypes in the dairy and beef industries. In combination with these reproductive technologies, the ability to insert DNA into livestock has provided limitless possibilities for agriculture (e.g., inserting genes affecting milk and beef production) as well as biomedicine (e.g., producing important proteins in milk) (Hodges and Stice 2003). Production of recombinant protein in the milk of transgenic dairy animals is currently being tested as an alternative to plasma fractionation for the manufacture of a number of blood factors (Pollock *et al.*, 2000). Possibility of genetically engineering poultry to make them resistant to avian influenza is attracting attention and has now become a real possibility with improved methods for genetic modification (Wise *et al.*, 2008).

Transgenic Calves have produced from Nonquiescent Fetal Fibroblasts which is important for agriculture, biotechnology, and human medicine (Cibelli *et al.*, 1998). Scientists are trying to clone cattle that are genetically incapable of developing "Mad Cow Disease". Thus transgenesis is playing an important role to increase livestock production by making transgenic animals which would be more disease resistant, faster growing and convert food more efficiently.

Marker Assisted Selection: Marker assisted selection (MAS) is indirect selection process where a trait of interest is selected not based on the trait itself but on a marker linked to it (Ribaut and Ragot 2007). Advances in molecular genetics have led to the identification of several genes and of genetic markers linked or associated with genes that affect traits of interest in livestock. Once Quantitative trait loci (QTL) are detected, the aim of animal breeders is to integrate linked markers for QTL into the breeding program, in so-called marker assisted selection schemes (MAS) Neuner *et al.*, 2008). DNA marker is a unique (DNA sequence), occurring in proximity to the gene or locus of interest, can be identified by a range of molecular techniques such as Restricted Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), DNA amplification fingerprinting (DAF), sequence characterized amplified regions (SCARs), microsatellites etc.

There are two main categories of genomic information that can be used in genetic improvement of livestock. They are direct markers and indirect markers. The identification of direct markers avoids the ambiguity caused by the possibility of recombination between the marker and the QTL (Montaldo and Herrera 1998). Reliable estimates for variance components in QTL models are a prerequisite in fine-mapping experiments and for marker-assisted genetic evaluations (Neuner *et al.*, 2009).

A rational use of the molecular methodologies requires the simultaneous optimization of selection on all the genes affecting important traits in the population. The maximum benefit can be obtained when these techniques are used integrated with reproductive technologies like the artificial insemination, and collection and production in vitro of embryos to accelerate the genetic change (Bishop *et al.*, 1995). French artificial insemination companies have been running a marker-assisted selection program since 2001 to determine which young bulls should be progeny tested (Guillaume *et al.*, 2008).

Single Nucleotide Polymorphism (SNP): SNP is a phenomenon in which single nucleotide may be changed either deletions or insertion in polynucleotide sequence insertion/deletion (Yue and Moulton 2006). With the help of analysis of DNA sequence variation the genes involved in both disease and in normal biological processes can be identified (Alain, *et al.*, 2002). Another approach is based on the comparison of sequences obtained from cloned fragments for investigation the SNP map of a genome (Picoult, *et al.*, 1999).

There are different techniques to investigate the SNP in given DNA such as comparison of locus-specific sequences, generated from different chromosomes. This is done by direct sequencing of genomic PCR products

obtained in different individuals (Cooper *et al.*, 2008). Scientists believe differences in SNPs reveal an individual's susceptibility to disease, meaning accurate analysis of SNPs would play a key role in diagnostics. These small SNP variations can account for as little as 0.1 per cent of a genome sequence. Information about DNA sequence variation will thus have a wide range of application in the analysis of disease and in the development of diagnostic, therapeutic, and preventative strategies.

DNA technologies and Livestock production: DNA technologies are playing a significant role in enhancing livestock production all over the world. The main targets of DNA technologies are

Better performance of Livestock: Transgenesis increase performance of livestock through improved feed intake, enhanced metabolism, better feed conversion and reduced pathogen load. It also creates novel or enhanced food and fiber products, thus allowing better agricultural products (food stuff) and reduce susceptibility to diseases. Hence improvement in food quality and security consequently decreasing antibiotic use, and also helps in maintaining resource conservation (Howard *et al.*, 2001). A significant contribution of genetically modified animals to human health are the longstanding use of bovine and porcine for production of insulin (for treatment of diabetes) as well as horse and sheep antibody against natural venoms, toxins, drugs and microbial peptides (Redwan, 2009). Transgenic cows exist that produce more milk or milk with less lactose or cholesterol, cattle that have more meat on them, and sheep that grow more wool. Transgenesis is also going to play an important role in designing of biopharmaceutical milk. Products such as insulin, growth hormone, and blood anti-clotting factors may soon be or have already been obtained from the milk of transgenic cows, sheep, or goats. Toxicity-sensitive transgenic animals have been produced for chemical safety testing. Production of such novel dairy products will really contribute in improving health and nutrition conditions.

Better Breeding: Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g., increased milk production, high growth rate). Traditional breeding is a time-consuming, difficult task. When DNA technologies using molecular biology were developed, it became possible to develop traits in animals in a shorter time and with more precision (Howard *et al.*, 2001). Marker Assisted Selection MAS can be effective in those traits which express late in the life of the animal e.g. in meat producing animals. (Ribaut and Ragot 2007). "Animal pharmings" is another milestone toward better breeding to get biopharmaceuticals. Pharming is the production of human pharmaceuticals in farm animals that is presently in the development stage with possible

commercialization by the year 2000 (Kind and Schnieke 2008). The continual advances in animal genomics towards the identification of genes that influence livestock production traits and impact on human health will increase genetic engineering's ability and versatility for the purposeful modification of livestock animals to enhance their welfare, produce superior quality food and biomedical products and reduce the environmental impact of farming (Laible, 2009). Transgenic livestock is gaining weight in order to improve the marketing practice of Livestock. It can potentially create many new niches, and some major markets, for agricultural products that will be differentiated by the presence of transgenic products. Complete genome sequences are available for a number of species, genome sequences for the chicken, cow, horse, mouse and chimpanzee are either completed or nearing completion, and single nucleotide polymorphism (SNP) libraries for these species are growing rapidly. This information will underpin most of the developments in livestock breeding and breed management during the coming two decades (Flint and Woolliams, 2008).

Disease Diagnosis: Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals. DNA sequencing is important in disease detection in livestock. PCR has enhanced the sensitivity of DNA detection tests considerably (Brandon *et al.*, 1991). DNA testing of blood samples can now identify some genetic disorders. Animals carrying defective genes and mutated genes can be identified now before they are used for breeding (Rege, 1994). Production of monoclonal antibodies and highly specific antigens through recombinant DNA technology has enabled to make the accurate and exact cure and diagnosis of Livestock diseases (Tavella *et al.*, 1985). Genetic variations increase disease susceptibility in farm animal species. The variations are in the form of single nucleotide polymorphisms. A better understanding SNP could be better harnessed to effectively identify and control, genetically, livestock diseases. Finally, genetic control of animal diseases can reduce the cost associated with diseases, improve animal welfare, and provide healthy animal products to consumers, and should be given more attention (Eveline *et al.*, 2008).

Vaccines: Conventional techniques of dealing with livestock diseases comprised of vaccination, slaughter of infected stock, chemotherapy and other management practices. But these techniques are often unaffordable for the farmer including conventional vaccines because pathogens often become resistant to them. The solution of this bottle neck is recombinant vaccines produced by recombinant DNA technology (McCullough, 1993). These vaccines are for life time with some booster after sometime. Some of them are vaccine against Orf (Reid,

1989). Orf is a zoonotic disease, caused by a parapox virus and occurring primarily in sheep and goats. Thermostable recombinant vaccines have been developed against Peste Dis Petitis ruminants (PPR) (Lefevre and Diallo, 1990), *Taenia ovis* and *Echinococcus* (Blancou, 1990). DNA vaccines against zoonotic schistosomiasis in water buffalos have greatly reduced its susceptibility for the disease (Akram *et al.*, 2008).

Animal Nutrition: Nutrition is one of the most serious limitations to livestock production. Feed resources are also inadequate in both quality and quantity, particularly during the dry seasons. Biotechnological options are available for improving digestion ability of animals and enhancing the nutritive value (Kundu and Kumar 1987). Transgenic manipulation of commensally gut or rumen microorganisms has considerable potential for improving nutrition, gut development and health in animals. Administration of recombinant somatotropin (ST) results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows (Bonneau and Laarvel. 1990).

REFERENCES

- Ashshi, A. M., R. J. Cooper and P. E. Klapper (2000). Multiplex PCR: optimization and application in diagnostic virology. *Clin Microbiol Rev.* 13(4):559-70.
- Alain, V., D. Milan, M. SanCristobal, and A. Eggen (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genet. Sel.* 34(3): 275-305.
- Andreas. H., B. Kessler, S. Ewerling, M. Weppert, B. Vogg, H. Ludwig, M. Stojkovic, M. Boelhaue, G. Brem, E. Wolf and A. Pfeifer (2003). Efficient transgenesis in farm animals by lentiviral vectors. *EMBO*, 4 (11), 1054–1058.
- Alexandre, V., Lebedev, N. Paul, J. Yee, A. Victor, Timoshchuk, J. Shum, K. Miyagi, J. Kellum, R. I. Hogrefe, and G. Zon (2008). Hot Start PCR with heat-activatable primers: a novel approach for improved PCR performance. *Nucleic Acids Res.*36(20): 131.
- Akram, A. Da'Dara, S. L. Yuesheng, T. Xiong, J. Zhou, G. M. Williams, D. P. McManus, Z. Feng, L. Xin, Yu, J. Darren. Gray and D. A. Harn (2008). DNA-based vaccines protect against zoonotic schistosomiasis in water buffalo. *Vaccine.* 4(26):3615-3625.
- Bartlett J. M. S. and D. Stirling (2009). A Short History of the Polymerase Chain Reaction, 2nd Ed: 3-6 p.
- Bartlett J. M. S., and D. Stirling (2003). PCR Protocols. 2nd Ed. Humana press (New Jersey). 226 p.
- Bonneau, M., and B. Laarvel, (1990). Biotechnology in animal nutrition, physiology and health. *Livestock Production Science* 59(2): 223-241.
- Blancou, J. (1990). Utilization and control of biotechnical procedures in veterinary science. *Revue Scientifique et Technique de l' Office International des Epizooties.* 9(3):641-659.
- Bishop, M. D., G. A. Hawkins, and C.L. Keeler (1995). Use of DNA markers in animal selection. *Theriogenology* 43(1): 61-70.
- Brandon, R. B., R. C. W. Daniel, and M. F. Lavin (1991). Early detection of bovine leukosis virus DNA in infected sheep using the polymerase chain reaction. *Research in Veterinary Science* 50(1):89-94.
- Brevini, T. A., S. Antonini, G. Pennarossa, and F. Gandolfi (2008). Recent progress in embryonic stem cell research and its application in domestic species. *Reprod Domest Anim.*43(2): 193-9.
- Brown, T. A., (1995) Gene Cloning an introduction, 3rd Ed, 87: p.
- Bertram, J., (2000). The molecular biology of cancer. *Mol. Aspects Med.* 21 (6): 167–223.
- Cavazzana-Calvo, M., A. Thrasher, and F. Mavilio (2004). The future of gene therapy. *Nature* 427(6977): 779–81.
- Cibelli, J. B., L. S. Steve, J. G. Paul, J. J. Kane, J. Jerry, C. Blackwell, F. A. P. de León, and R. M. James (1998). Cloned Transgenic Calves Produced from Nonquiescent Fetal Fibroblasts. *Science.* 280(5367): 1256 – 1258.
- Colowick, S. P., and O. N. Kapiian (1980). *Methods in Enzymology* 68; Recombinant DNA, Academic Press (London), 68 p.
- Cooper, G. M., T. Zerr, J. M. Kidd, E. E. Eichler and D. A. Nickerson (2008). Systematic assessment of copy-number variant detection via genome-wide single nucleotide polymorphism genotyping. *Nat Genet.* 40(10):1199–1203.
- Ciampolini, R., V. Cetica, E. Ciani, E. Mazzanti, X. Fosella, F. Marroni, M. Biagetti, C. Sebastiani, P. Papa, G. Filippini, D. Cianci, S. Presciuttini, (2006). Statistical analysis of individual assignment tests among four cattle breeds using fifteen STR loci. *J Anim Sci.*84(1):11-19.
- Derouazi, M., P. Girard, M. Jordan, C. Denoya and F. M. Wurm (2003). ESACT Proceedings, Animal Cell Technology Meets, Genomics Proceedings of the 18th ESACT Meeting Granada, Spain.
- Daniela, T., C. B. Watt, Y-Y. T. Su, K-J. Chang, S. Handlin, V. Gaskie, and D. M-K. Lam (1985). The production and characterization of monoclonal antibodies against enkephalins. *Neurochemistry International.* 7(3): 455-466.

- Don, R. H, P. T. Cox, B. J. Wainwright, K. Baker, and J. S. Mattick (1991). Touchdown PCR to circumvent spurious priming during gene amplification. *Nucl Acids Res* 19(14): 4008.
- Ewing B. and G. Phil (1998). Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res.* 8(3): 175-185
- Eveline, M., I. Awemu, P. Kgwatalala, A. E. Ibeagha, and X. Zhao (2008). A critical analysis of disease-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. *Mamm Genome*.19(4): 226–245.
- Ernesto, C., Almira, N. Panayotova, and W. G. Farmerie (2003) Capillary DNA Sequencing: Maximizing the Sequence Output. *J Biomol Tech.* 14(4): 270–277.
- Flint, A. P. F and J. A. Woolliams (2008). Precision animal breeding. *Philos Trans R Soc Lond B Biol Sci*.12(363): 573-590.
- Goldman, S. and M. Windrem (2006). Cell replacement therapy in neurological disease. *Philos Trans R Soc Lond B Biol Sci.* 361(1473):1463-75.
- Gordon, J. W., G. A. Scangos, D. J. Plotkin, A. Barbosa and F.H. Ruddle (1980). Genetic transformation of mouse embryos by microinjection. *Proceedings of National Academic Science (USA)*. 77(12): 7380-7384 p.
- Gahrton G, and B. Björkstrand (2000). Progress in haematopoietic stem cell transplantation for multiple myeloma. *J Intern Med* . 248(3):185-201.
- Gilbert, W. (1981). DNA sequencing and gene structure. *Science*: 214(1): 353-375.
- Garret, R. H and C.M. Grisham (2008). *Biochemistry*. 4th Ed. Cengage Learning, Canada. 354 p
- Gossler. A., T. Doetschman, R. Korn, E. Serfling, and R. Kemler (1986). Transgenesis by means of blastocyst-derived embryonic stem cell lines. *Proc Natl Acad Sci U S A.* 83(23): 9065–9069.
- Guillaume, F., S. Fritz, D. Boichard, T. Druet (2008). Short communication: correlations of marker-assisted breeding values with progeny-test breeding values for eight hundred Short communication: correlations of marker-assisted ninety-nine French Holstein bulls. *J Dairy Sci.* 91(6): 2520-2.
- Hans R. Schöler (2007). *The Potential of Stem Cells: An Inventory*. Nikolaus Knoepffler, Dagmar Schipanski, and Stefan Lorenz Sorgner. *Humanbiotechnology as Social Challenge*. Ashgate Publishing, Ltd (USA). 28 p.
- Higuchi, R., G. Dollinger, P. S. Walsh and R. Griffith (1992). Simultaneous amplification and detection of specific DNA sequences. *Biotechnology* 10(4):413–417.
- Hodges, C. A. and S. L. Stice (2003). Generation of bovine transgenics using somatic cell nuclear transfer. *Reprod Biol Endocrinol*.1(10): 81.
- Howard, T. H., E. J. Homan, R. D. Bremel (2001). Transgenic livestock: Regulation and science in a changing environment. *J. Anim. Sci.* 79:E1-E11.
- Hanna, J. G., A. J. Victoria, R. K. Daniel, D. R. Douglas, J. M-Picado, L. Sutton, J. D. Hazelwood, and R. T. D'Aquila (2000). Comparison of Sequencing by Hybridization and Cycle Sequencing for Genotyping of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. *Journal of Clinical Microbiology.* 38(7): 2715-2721
- Holst, J. A. (2009). Testing for genetically modified organisms (GMOs): Past, present and future perspectives. *Biotechnol Adv.*27(6):1071-82.
- Montaldo. H. H. and C. A. Meza-Herrera (1998). Use of molecular markers and major genes in the genetic improvement of livestock. *Electronic Journal of Biotechnology.*2(11): 83-89
- Innis, M. A., K. B. Myambo, D. H Gelfand, and M. A. Brow (1988). DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. *Proc Natl Acad Sci.* 85(24). 24:9436-9440.
- Isenbarger, T. A, M. Finney, C. Ríos-Velázquez, J. Handelsman and G. Ruvkun (2008). Miniprimer PCR, a new lens for viewing the microbial world. *Applied and Environmental Microbiology.* 74(3): 840-849.
- Johnson, I. S. (1983). Human insulin from recombinant DNA technology. *Science.* 219(4585): 632 – 637.
- Jiang, Y., B. N. Jahagirdar and R. L. Reinhardt (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature.* 418(6893):41-9.
- Jaenisch, R and B. Mintz (1974). Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. *Proc Nat Acad Sci.* 71(4): 1250-1254.
- John Med. Coffin (1992). *Structure and Classification of Retroviruses in Jay A. Levy. The Retroviridae* 1st Ed. Plenum Press (New York). 20 p.
- Rege, J. E. O. (1994). *Biotechnology options for improving livestock production in developing countries, with special reference to sub-Saharan Africa*. Third Biennial Conference of the African Small Ruminant Research Network.
- Jeremy M. B., L. J. L. Tymoczko, and L. Stryer (2002). *Biochemistry*. 5th Ed. W H Freeman (New York) 836 p.
- Jonathan. P. and J. Wiley (2003). *Bioinformatics and Functional Genomics*, edition 1st, 731 p.

- Hoheisel, J. D. (2006). Microarray technology: beyond transcript profiling and genotype analysis. *Nature Reviews Genetics* 7(3): 200-210.
- Kazuhiko N., O. Mazda, Y. Dohi, T. Kawata, K. Mizuguchi, S. Kitamura and S. Taniguchi (2000). In vivo gene gun-mediated transduction into rat heart with Epstein-Barr virus-based episomal vectors. *Ann Thorac Surg.* 70(40):1332-1337
- Kirn, D., D. I. Niculescu, G. Hallden, and C. J. Springer (2002). The emerging fields of suicide gene therapy and virotherapy. *Trends in Molecular Medicine.* 8(4): 68-73.
- Khan, Z., K. Poetter, and D. J. Park (2008). Enhanced solid phase PCR: mechanisms to increase priming by solid support primers. *Anal Biochem.* 375(2):391-3.
- Kundu, S. S., and V. Kumar (1987). Biotechnological processes to convert crop residues to quality feeds for livestock. *Indian Dairyman.* 39:113-114.
- Kulesh, D. A., D. R. Clive, D. R. Zarlenga, and D. S. Greene (1987). Identification of interferon-modulated proliferation-related cDNA sequences. *Proc Natl Acad Sci.* 84(23):8453-7.
- Kind, A., and A. Schnieke (2008). Animal pharming, two decades on. *Transgenic Res.* 17(6): 1025-33.
- Kubista, M., J. M. Andrade, M. Bengtsson, A. Forootan, J. Jonák, K. Lind, R. Sindelka, R. Sjöback, B. Sjögreen, L. Strömbom, A. Ståhlberg, and N. Zoric (2006). The real-time polymerase chain reaction. *Mol Aspect Med.* 27(2): 95-125.
- Laporte, L. D., C. R. Jennifer and D. S. Lonnie (2009). Design of modular non-viral gene therapy vectors. *Biomaterials.* 27(7): 947-954.
- Lindvall, O (2003). Stem cells for cell therapy in Parkinson's disease. *Pharmacol Res.* 47(4): 279-87.
- Lipshutz, R. J., S. P. Fodor, T. R. Gingeras and D. J. Lockhart (1999). High density synthetic oligonucleotides arrays. *Nat Genet.* 21(1):20-4.
- Laible, G., L. and Alonso-González (2009). Gene targeting from laboratory to livestock: current status and emerging concepts. *Biotechnol J.* 4(9):1278-92.
- Laible, G. (2009). Enhancing livestock through genetic engineering--recent advances and future prospects. *Comp Immunol Microbiol Infect Dis.* 32(2):123-37.
- Lefevre, P. C. and A. Diallo (1990). Diagnosis of viral and bacterial diseases. *Revue Scientifique et Technique de l'Office International des Epizooties.* 9:951-965.
- Liu, Y. G. and R. F. Whittier (1995). Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. *Genomics.* 25(3): 674-81.
- Mason, P. W., M. J. Grubman, and B. Baxt (2003). Molecular basis of pathogenesis of FMDV. *Virus Res.* 91(1):9-32.
- Mark S. S. and B. L. Davidson (2006). Gene Therapy for Lysosomal Storage Diseases. *Molecular Therapy.* 13: 839-849.
- Mir. L. M. (2008). Application of electroporation gene therapy: past, current, and future. *Methods Mol Biol.* 423(1):3-17.
- Mueller, P. R. and B. Wold (1988). In vivo footprinting of a muscle specific enhancer by ligation mediated PCR. *Science.* 246(4931): 780-786.
- Munoz, M., C. Diez, J. N. Caamano, A. Jouneau, I. Hue, and E. Gómez (2008). Embryonic stem cells in cattle. *Reprod Domest Anim.* 43(4):32-7.
- Martin, D. and Claus B.V (2007). *Methods in Molecular Biology Microarray.* 2nd Ed. 96-100 p.
- McCullough, K. C (1993). The application of biotechnology to the diagnosis and control of animal diseases. *Rev Sci Tech.* 12(2):325-53.
- Mackay, I. M., K. E. Arden, and A. Nitsche (2002). Real-time PCR in virology. *Nucleic Acids Res.* 30(6):1292-305.
- Nailis, H., T. Coenye, N. F. Van, D. Deforce, H. J. Nelis (2006). Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. *BMC Mol Biol.* 7:25.
- Nickerson, D. A., V. O. Tobe, and S. L. Taylor (1997). PolyPhred: Automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. *Nucleic Acids Res.* 25(14): 2745-2751.
- Neuner, S., R. Emmerling, G. Thaller, K. U. Götz, (2008). Strategies for estimating genetic parameters in marker-assisted best linear unbiased predictor models in dairy cattle. *J Dairy Sci.* 91(11): 4344-54.
- Newton, C. R., A. Graham, L. E. Heptinstall, S. J. Powell, C. Summers, N. Kalsheker, J. C. Smith, and A. F. Markham (1989). Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Research.* 17(7): 2503-2516.
- Pollock, D. P., P. Joseph, Kutzko, E. Birck-Wilson, J. L. Williams, Y. Echelard and H. M. Meade (1999). Transgenic milk as a method for the production of recombinant antibodies. *Journal of Immunological Methods* 231(1): 147-157.
- Pearson, H., (2006). Genetics: what is a gene? *Nature.* 441(7092):398-401.

- Picoult, N. L., T. E. Ideker, M. G. Pohl., S. L. Taylor, M. A. Donaldson, D. A. Nickerson, J. M. Boyce, (1999). Mining SNPs from EST databases. *Genome Res* 9(2): 167-174.
- Reid, H. W. (1989). Orf Virus Infection of Sheep. *Animal Diseases Research Association News Sheet* .
- Roberto, M., E. Luna, V. Rincón and G. M. Mateu, (2008). Engineering Viable Foot-and-Mouth Disease Viruses with Increased Thermostability as a Step in the Development of Improved Vaccines. *Journal of Virology*. 82(24):12232-12240.
- Redwan, E. R. (2009). Animal-derived pharmaceutical proteins. *J Immunoassay Immunochem*. 30(3):262-90.
- Ribaut, J. M., M. Ragot (2007). Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. *J Exp Bot*. 58(2): 351-60.
- Ribaut, J. M., C. Jiang and D. Hoisington (2002). Simulation Experiments on Efficiencies of Gene Introgression by Backcrossing. *Crop Science*. 42:557-565.
- Sharkey, D. J., E. R. Scalice, K. G. Christy Jr., S. M. Atwood, and J. L. Daiss (1994). Antibodies as Thermolabile Switches: High Temperature Triggering for the Polymerase Chain Reaction. *Bio/Technology* 12(5): 506–509.
- Spackman, E and D. L. Suarez (2008). *Methods in Molecular Biology*. 436:19.
- Sanger, F., and A. R. Coulson (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol*. 94(3): 441–8.
- Schimmenti, J., (1998). Global analysis of gene function in mammals: integration of physical, mutational and expression strategies. *Electronic Journal of Biotechnology*. 1(1):1-5.
- Stemmer, W. P., A. Cramer, K. D. Ha, T. M. Brennan, and H. L. Heyneker (1995). Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. *Gene*. 164(1): 49–53.
- Stefan, N., E. Christian, R. Emmerling, G. Thaller and K. U. Götz, (2009). Precision of genetic parameters and breeding values estimated in marker assisted BLUP genetic evaluation. *Genet Sel Evol*. 41(1):26.
- Tuch, B. E. (2006). Stem cells-a clinical update. *Australian Family Physician*. 35(9): 719–21.
- Verschoor, C. P., S. D. Pant, F. S. Schenkel, B. S. Sharma, and N. A. Karrow (2009). SNPs in the bovine IL-10 receptor are associated with somatic cell score in Canadian dairy bulls. *Mamm Genome*. 20(7):447-54.
- Van Guilder, H. D., K. E. Vrana, and W. M. Freeman, (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*. 44(5):619-26.
- Wu, Q., M. P. Moraes, and M. J. Grubman (2003). Recombinant adenovirus co-expressing capsid proteins of two serotypes of foot-and-mouth disease virus (FMDV): in vitro characterization and induction of neutralizing antibodies against FMDV in swine. *Virus Res*. 93(2):211-219.
- Wise, T. G., D. S. Schafer, J. W. Lowenthal, and T. J. Doran (2008). The use of RNAi and transgenics to develop viral disease resistant livestock. *Dev Biol*. 132:377-82.
- Wright, S. (1972). *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering*. The University of Chicago Press (Chicago). 1st Ed. 72p.
- Yao, J. Y., J. An and J. Li (2002). Techniques for producing transgenic animals and the recent development. *Academic journal of the First Medical College of PLA*. 22(1): 78-81.
- Yoshida, C., Y. Endo, and Y. Baba (2001). Enhanced throughput for DNA sequencing by capillary array electrophoresis with a gradient of electric field strength. *European Journal of Pharmaceutical Sciences*. 13(1): 99-103.
- Yue, P. and J. Moulton (2006). Identification and analysis of deleterious human SNPs. *J. Molec. Biol*. 356(5):1263–74.