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DNA Markers and Their Application in Animal Genetics: An overview

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ملخص البحث

أحدثت تقنيات دراسة الحامض النووي منزوع الأكسجين (DNA) ثورة في علم الوراثة في الحيوان. في هذا الاستعراض تم شرح أربع تقنيات لذلك الحامض النووي والتي تستعمل لتوضيح التباين الجيني وهي الحامض النووي منزوع الأكسجين متعدد الشكل والمضخم عشوائياً [Random Amplified Polymorphic DNA (RAPD)] والتوابع بالغة الصغر (Micro-satellites) والتوابع الصغيرة (Mini-satellites) والحصر متعدد الشكل بطول الشظية [Restriction Fragment Length polymorphism (RFLP)] تم استعراض التصنيف المختلف لهذه الواسمات والطرق المعملية التى تمكن من معرفة كل واسمه وكما أوضح هذا الاستعراض مساوئ ومحاسن كل من هذه التقنيات الأربع. وكذلك المجال الذي يحكم التطبيق والاستفادة من كل واسمة.

Summary

The DNA techniques have a revolutionary impact on the field of animal genetics. This overview describes the most extensively used DNA markers in the field of animal genetics, including Restriction Fragment Length polymorphism (RFLP), Micro-satellites or Simple Sequence Repeat (SSR), Mini-satellites and Random Amplified polymorphic DNA (RAPD). The different groups of these DNA markers are described. The techniques used for the identification of each marker system are discussed; the advantages and disadvantages of each marker are stated. Moreover, the practical utilization of each marker is considered.

DNA Markers

Two classes of molecular markers are well known: biochemical markers and DNA markers. The biochemical markers are chemical products of genes, whereas the DNA markers are derived from analysis of polymorphisms that occur in DNA sequences (Strauss *et al.*, 1992). DNA techniques have been revolutionized by the Polymerase Chain Reaction (PCR) adopted by Kary Mullin in 1985. These techniques require small amounts of biological samples that are not only restricted to fresh blood, but also other biological samples, e.g. hair, semen, and skin. DNA techniques are highly automated, simple and cheap.

Eukaryotic genomes show considerable DNA sequence variations (polymorphisms) between species and among individuals within the same species. Many different molecular techniques can be used to detect polymorphism at DNA level and provide markers for

diversity. According to O'Brien (1991), DNA markers can be grouped into two types:

Type I markers are associated with a gene of known function whereas type II markers are associated with anonymous gene segments of one sort or another. Furthermore, Dodgson *et al*. (1997) classified these DNA markers as fingerprint (FP) markers. These markers do not require a prior knowledge of the sequence of the polymorphic region or isolation of a cloned DNA fragment. They include random amplified polymorphic DNA (RAPD), mini-satellites, amplified fragment length polymorphisms (AFLP), restriction landmark genome scanning (RLGS) among others. The finger print (FP) markers are inherently type II markers. They are particularly useful to characterize individuals' genetic lines, gene flow or parentage questions. They are quick, cost- effective and useful for scanning of genetic diversity between two individuals or lines.

In terms of the type of information that DNA markers can provide at a single locus, Vignal *et al*. (2002) described three main categories:

(a) Biallelic dominant, e.g. RAPD., (b) Biallelic codominant, e.g. RFLPs and (c) multiallelic codominant, e.g. micro-satellites. The codominant markers, e.g. micro-satellites and RFLPs can identify both alleles at a locus. They are preferable to the dominant markers (e.g. RAPD) which identify multiple loci, but are not able to distinguish alleles (Morgan, 1998).

Some DNA markers techniques

1) Restriction Fragment Length Polymorphism (RFLP):

Restriction endonuclease enzymes cut DNA molecule at specific sequence patterns known as restriction endonuclease recognition sites. When a point mutation occurs at a restriction enzyme site, the DNA molecule loses the ability to be cleaved by the particular enzyme. RFLP is a technique used for the identification of variable lengths of DNA fragments that result from digestion of DNA molecules with a specific restriction endonuclease enzyme.

The presence or absence of certain recognition site in the DNA molecule will lead to formation of variable length of DNA fragments (Beuzen *et al*., 2000).

This technique is easy because it can be typed by PCR. Although many enzymes need to be tested in the initial phase to be able to identify the polymorphism, it is easy, reliable and relatively cheap (Strachan and Read, 2001). The potential disadvantage of RFLP

technique is its dimorphic nature, since it can only verify the presence or absence of a cleavage site; therefore, it does not provide a great deal of genotypic information.

2/) Micro-satellites:

Eukaryotic genomic DNA consists of non-coding and coding DNA, i.e. some parts of the genome encode for synthesis of proteins through the translation of RNA that transcribed from the DNA template (Coding DNA). However, non-coding DNA makes up the large proportion of the genomes of eukaryotes and contains regulatory elements such as promoters and enhancers, but in many cases can contain repetitive elements (Turner *et al*., 1998). These elements include satellite DNA, which are thousands of tandem repeats in one site as well as mini- and micro-satellite DNA, depending on the number of the repeats.

Micro-satellites are also called simple sequence repeats (SSR), consist of tandem repeats between one and six bp up to 60 times, which are interspersed throughout the DNA of animal genomes (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989). Hamada and his colleagues (1982) demonstrated these domains for the first time during the early eighties. Repeat units may consist of $(A)_{n,}$ $(CA)_{n}$, or $(AAT)_{n}$ repeat. Microsatellites are highly polymorphic due to variation in the number of repeats. It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small number of samples (Goldstein and Pollock, 1997). Slippage of DNA polymerase and mismatch repair during replication appear to be the mechanisms generating diversity of the micro-satellite length. Micro-satellite length variation is easy to be detected by PCR using unique flanking primer sequences. Initially, each micro-satellite region must be cloned and the surrounding region being determined, but once this is done, these markers can usually be employed in many different resource populations due to their high level of polymorphism (Moore *et al*., 1991; Rubinsztein *et al*., 1995).

Micro-satellites have the advantages of being multi allelic, highly polymorphic, codominant and assayable by PCR. Therefore, these elements have become the most valuable markers in genetic variability studies and parentage verifications. They are the workhorse of animal gene mapping projects (Dodgson *et al*., 1997). A large number of micro-satellite markers have been mapped for various animal species, including humans, mice, fruit flies and farm animals (cattle, sheep, swine and chickens). Micro-satellites display great

variation than protein polymorphisms when studying genetic diversity in domestic animals, (Sanchez *et al*., 1996; Barker *et al*., 1997). They are useful and have a powerful discriminating ability at the level of genetic relationships, especially among closely related populations and breeds.

3) Mini-satellites:

Mini-satellites are firstly discovered by Jeffreys *et al*. (1985) in human genome. They were being described as hypervariable tandem repeats and with certain hybridization probes for repetitive sequences, can generate complex banding patterns that contain heritable polymorphisms. They have longer repeats than micro-satellites, consisting of up to 2000 bp. This led to the use of the term of Variable Number of Tandem Repeats (VNTRs), and this term is also used for repetitive units that include microatellites.

Mini-satellite patterns are highly polymorphic. This is attributed to the number of bands observed at one time and the fact that repetitive regions of the genome are inherently variable. Accordingly, fingerprint analysis has been used in several studies of Quantitative Trait Loci (QTL) in many animal species (Haberfeld and Hillel, 1991; Haberfeld *et al*., 1991; Plotsky *et al*., 1995; Lamont *et al*., 1996; Zhu *et al.,* 1996).

Mini-satellite markers have some disadvantages. Firstly, they detect repetitive sequences that primarily reside in heterochromatic regions near telomeres and centromeres of chromosomes, and thus they do not uniformly mark the genome. Secondly, the marker fragment is difficult, if not possible; to be cloned for more detailed analysis. Thirdly, they are dominant markers, which reduce the potential information for genotyping (Dodgson *et al*., 1997**).**

4) Random Amplified Polymorphic DNA (RAPD):

Random amplified polymorphic DNA (RAPD) has been proposed by Williams *et al.* (1990) as a DNA analysis technique for evaluating genetic variation and relatedness within and among species. The assay uses only one short oligonucleotide primer of arbitrary sequence of about 10 mer in PCR reaction with a low annealing temperature. This generates a spectrum of amplified fragments from almost any template DNA and these can be adequately resolved and visualized using polyacrylamide gel electrophoresis (PAGE), with silver staining. Agarose gel electrophoresis with ethidium bromide staining is also often used, but it only detects the major fragments. Polymorphisms are detected as the presence or

absence of a particular fragment between individual animals. The variation is due to the differences in spacing between primer binding sites as well as point mutation, which allows or abolishes primer binding. Relaxed PCR conditions allow multiple unspecific priming sites on opposite DNA strands to occur even if the match is imperfect. Wide variation in band intensity can be reproducible between experiments, which could be the result of multiple copies of the amplified regions in the template DNA or the efficiency with which particular regions are amplified.

RAPD markers have the advantage of being obtained at a reasonable cost, will generally amplify a range of fragments of most DNA and show polymorphism. The RAPD generated polymorphic bands can also be cloned for further analysis. One of the most advantages of RAPD markers is that no prior knowledge of sequence information is needed. RAPD markers are also fairly evenly distributed throughout the genome (Levin *et al*., 1994; Cheng *et al*., 1995). However, the major problem with RAPD patterns is their dependence on the exact PCR conditions employed, which can lead to poor reproducibility. (Meunier and Grimont, 1993; Romanov and Weigend, 2001). Another limitation of RAPD markers is that they are dominant markers, i.e. heterozygotes cannot be distinguished from homozygotes.

Application of genetic Markers

DNA markers could be applied in many fields of animal breeding and genetics.

1- Genome Mapping:

Genome mapping is a reconstruction of the entire set of chromosomes for a certain species, which shows the relative position of every gene. Two main types of DNA maps can be identified:

a) Genetic map:

It is also known as linkage map and the chromosome maps of its genes or markers are relative to each other.

b) Physical maps:

This type of maps gives the distances in base pairs from a landmark to another. The landmarks can be restriction enzymes sites, genes etc.

Genome mapping have many applications in the field of animal science. It can be used in finding functional genes and identification of genes associated with disease resistance. For evolutionary studies, comparative maps can be made between

different species, e.g. humans and chicken (Groenen *et al*., 1999). Comparative maps have several potential advantages; they are used for the identification of conserved regions between species, e.g. Yonash *et al.* (1999) identified the location of Marek's disease resistance quantitative trait loci (QTL) in chicken genome map. The mapping of these regions showed their conservation with portions of three human chromosomes (Suchyta *et al.*, 2001). Also they contribute to the search for QTLs and provide valuable information for gene expression studies.

The first genetic linkage map was constructed for chickens (Bumstead and Playga, 1992). Micro-satellites markers have revolutionized the construction of genetic maps of many animal species. Micro-satellites based maps are available for many animal species, e.g. pig (Rohrer *et al.*, 1996), goat (Vaiman *et al.*, 1996), cow (Kappes *et al.*, 1997), horse (Swinburne *et al.*, 2000) and sheep (Maddox *et al.*, 2001).

2- Quantitative Trait Loci:

Genes that influence quantitative traits are known as QTL. If the trait is well understood, there may be one or more genes that are strongly suspected of contributing to variation in the trait; these are called candidate genes (Beuzen *et al*., 2000). Several factors influence the chance of detecting a QTL. These include the size of the effect, frequency of the alleles, density of the genetic map, heritability of the trait, variation among the animals, number of animals studied and the method of analysis (Weller *et al.*, 1990; Haley, 1995). Once a QTL has been provisionally located, the next step is to identify the specific gene that is responsible for the phenotype of interest. One strategy is the positional cloning (Knott *et al.*, 1998). Other options include the comparison of different populations or even a comparison of genes maps in different species to identify candidate genes (Beuzen *et al.*, 2000).

3- Conservation Genetics:

Genetic variation is critical in maintaining the biodiversity found in species, populations, and ecosystems. Habitat destruction, hunting and other factors have resulted in the extinction of a number of species and the near extinction of many others. All of these factors have stimulated attempts to maintain genetic variation in many different species by a discipline known as conservation genetics. It is concerned with maintaining genetic variation in animal breeds and in rare endangered species. The primary goals of the conservation

genetics are to avoid inbreeding depression and loss of genetic variation. (Weaver and Hedrick, 1992). Genetic conservation is based on a thorough understanding of population genetic fundamentals, including genetic drift, selection and inbreeding. Molecular markers in this field may serve as an important initial guide to evaluate breeds as genetic resources (Ruane, 1999).

Genetic variation can be defined by allelic diversity and by the percent of heterozygosity found in a population. The percent of heterozygosity in a population describes the number of genes or DNA regions found and those that have more than one allele. A population with many different alleles may be better and is capable of dealing with changes in its surroundings such as the introduction of a new disease. Genetic variation can be lost by several different events including founder effects, inbreeding, selection and various cross breeding systems, genetic drift and bottlenecks. By definition, endangered organisms have small populations and therefore they may have experienced a reduction in the level of genetic variation found in their populations. Small populations face greater demographic and genetic risks than large ones. In fragmented groups, a small number of individuals mate, thus increasing the chance of breeding among close relatives; this is being defined as inbreeding (Calcagnotto, 2001). A reduction in fitness and vigour caused by homozygosity as a result of one or more generations of inbreeding is called inbreeding depression. In small populations inbreeding depression is common because of the cumulative effects of genetic drift (Hedrick and Kalinowski, 2000). Avoidance of inbreeding has become a major goal in the management of small populations since it has been documented to be one of the causes of reduction in fitness especially in captive populations of endangered species. Inbreeding and the loss of variants in small populations can lead to a homogeneous population which is more susceptible to diseases and vulnerable to environmental changes.

4- Genetic variation:

The most widespread use of DNA markers is the identification of genetic variations, e.g. in taxonomic studies, phylogenetic relationship (Mburu *et al***.,** 2003; Aranguren-Mendez *et al*., 2001; Canon *et al*., 2000**)** and genetic fingerprinting (Plotsky *et al*., 1995; Zhu *et al*., 1996).

Diversity can be addressed at the species, population and within population (individual) levels. At the species level, molecular markers can provide information that help to define the distinctiveness

of species and their ranking according to the number of close relatives and their phylogenetic positions. They can show how many different genetic classes are present and the level of their genetic similarities, how much diversity is present in those classes and their evolutionary relationships with wild relatives. The DNA markers can provide information on distribution of populations of a given species, their genetic distinctive differences and determine the gene flow or migration between them. Within population (individuals) diversity is extremely important for establishment of identities in a breed for paternity testing and forensics (Nei, 1987). Parentage verification provides pedigree analysis and ensures the accuracy of studbooks. For parentage testing the most efficient genetic markers are the co-dominant markers.

Micro-satellite loci have been identified as particularly useful markers for discrimination among populations. Bowcock *et al.* (1994) and Mac Hugh *et al*. (1996) have found that individuals from the same population clustered together when phylogenetic trees were constructed from micro-satellite markers.

Methods used for the measurement of genetic variation

1- Gene frequency:

Gene frequencies are the frequencies of alleles at one locus in a given population. So a homozygote for an allele has a frequency equal to the square of the frequency of that allele, and a heterozygote frequency is twice the product of the two corresponding alleles frequencies (Strachan and Read, 2001).

Factors affecting allele frequency:

a) Genetic drift:

Gene frequencies are subject to the effect of statistical sampling at every generation. This effect cause random fluctuation in the allele frequencies. The magnitude of fluctuation is great, on average, on small population sizes, because of the relatively large contribution of each individual to the gene pool (Burnet, 1995). Such fluctuation is known as genetic drift. The smaller the population, the more significant the effects of drift over a finite number of generations.

b) Differential reproduction:

Differential reproduction is the most interesting factor affecting allele's frequencies; alleles favourable to reproduction will increase in frequency at the expense of alternative alleles. Difference in the reproductive success of the various genotypes is brought about

by selection. Selection means that certain individuals make greater contribution to the gene pool than others. If selection is a conscious choice of man then it is called artificial selection and if it is due to the natural circumstances of the environment it is called natural selection (Burnet, 1995).

Natural selection is the mechanism by which populations adapt to their environment. This process ensures that genotypes, which are more suited to a given environment, will increase in relative frequency over the generations. Those phenotypes that are transmitted to the progeny and confer an advantage will automatically expand within a population. The advantages that matter in terms of natural selection are the capacity to survive and to leave offspring.

c) Mutation:

Mutation is the raw fuel that drives evolution. Mutation rates are generally given low and a change in allele frequencies due to mutation would be hardly noticeable. Mutation can be induced in DNA by exposure to a variety of mutagens occurring in the external environment, e.g. radioactive materials. However, the greatest source of mutation is from endogenous mutation, notably spontaneous errors in DNA replication and repair. Many mutations are essentially randomly generated in the DNA of individuals. Coding-DNA and non-coding DNA are about equally susceptible to mutation; however, the major consequences of mutation are restricted to the coding DNA (Strachan and Read, 2001).

2- Hardly-Weinberg Equation:

The method used to calculate the expected proportions of different genotypes in a population was published in 1908 by the British mathematician G. H. Hardly and, independently, by the German physician W. Weinberg. It is known as the Hardly –Weinberg equation. This equation gives the probabilities of the three types of zygotes in a population, and the equation is

$p^2+2pq+q^2 = 1$, where: p^2 is the frequency of the homozygous dominant allele, q^2 is the frequency of the homozygous recessive allele and 2pq is the frequency of the heterozygote.

The equation is only applicable to large, randomly mating populations of sexually reproducing diploid organisms. If any one of these conditions is not fulfilled, then the Hardly-Weinberg equation cannot predict genotypes frequencies. In natural populations, alleles frequencies are normally calculated from observed genotype frequencies, whereas the Hardly–Weinberg equation gives expected

genotype frequencies. Observed and expected frequencies are not necessarily the same. A population in which the observed genotype frequencies are the same as those expected according to the prediction of the Hardly-Weinberg equation is said to be in Hardly Weinberg equilibrium, (Burnet, 1995). Deviation from the Hardly–Weinberg law equation is rare. It may be due to non-random mating or natural selection although, rarely, may be detected as a deviation from Hardly–Weinberg equilibrium (Bodmer and Cavalli-Sforza, 1976).

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References

Aranguren-Mendez, J.; Jordana, J. and Gomez, M. (2001). *Genet. Sel. Evol.*, **33**: 433-442.

Barker, J. S.; Moore, S.S.; Hetzel, D. J.; Evans, D.; Tan, S. G. and Byrne, K. (1997). *Anim. Genetics*., **28:** 103 – 115.

Beuzen, N. D.; Stear M. J. and Chang, K. C. (2000). *Vet. J.*, **160**: 42 – 52.

Bodmer, W. F. and Cavalli-Sforza, L. L. (1976). Genetics evoluation and Man. W.H. Freeman and Company, San Francisco. USA., 273 P.

Bowcock, A. M.; Ruiz –Linares, A.; Tomfohrde, J.; Minch, E.; Kidd,J.R. and Cavalli – Sforza, L. L. (1994). *Nature*, **386**: 455 – 457.

Bumstead, N. and Palyga, J. (1992). Genome, **13**: 690 - 697.

Burnet, L. (1995). Essential genetics. A course book, Cambridge University Press, Cambridge, U.K.

Calcagnotto, D. (2001). Conservation genetics sessions guides. www.cbc.amnh.org/symposia/chives/conservation-genetics/ session.

Canon, J.; Checa, M. L.; Carleos, C.; Vega-pla, J. L.; Vallejo, M. and Dunner, S. (2000). *Anim. Genetics*, **31**: 39-48.

Cheng, H. H.; Levin, I.; Vallejo, R.L.; Khatib, H.; Dodgson, J. B.; Crittenden, L. B. and Hillel. J. (1995). *Poult. Sci*., **74**: 1855-1874.

Dodgson, J.B.; Cheng, H.H. and Okimoto, R. (1997). *Poult. Sci.*, **76**: 1108-1114.

Goldstein, D. B. and Pollock, D. D. (1997). *J. Heredity*, **88**: 335-342.

Groenen, M. A. M.; Grooijmans, R. P. M. A.; Dijkof, R. J. M.; Acar, R. and Van der Poel, J. J. (1999). *Anim. Genet.,* **30**: 418 – 422.

Haberfeld, A. and Hillel, J. (1991). *Anim. Biotechnol*., **2**: 61-73.

Haberfeld, A.; Cahaner, A.; Yoffe, O.; Plotsky, Y. and Hillel, J (1991). *Anim. Genetics,* **22**: 299-305.

Haley, C.S. (1995). *Trends in Gene*t., **11**:488 -492.

Hamada, H.; Petrino, M. and Kakunga, T. (1982). *Proc. Nat. Acad. Sci.,* USA., **79**: 6465-6469.

Hedrick, P. W. and Kalinowski, S. T. (2000). *Ann. Rev. Ecol. Systemat*., **31**: 139-162.

Jeffreys, A. J.; Willson, V. and Thein, S. L. (1985). *Nature*, **316**:76-79**.**

Kappes, S. M.; Keele, J. W.; Stone, R. T.; McGraw, R. A.; Sonstegard, T.; Smith, T. P.; Lopez–Corrales, N. L. and Beattie, C. W.(1997). *Genome Res.,* **7**:235 -249.

Knott, S. A.; Marklund, L. and Haley, C. S. (1998). *Genetics,* **149**: 1069 – 1080.

Lamont, S.J.; Lakshmanan, N.: Ploltsky, Y.; Kaiser, M.G.; Kuhn, M.; Arthur, J.A.; Beck, N.J.; O. and Sullivan N.P. (1996). *Anim. Genetics,* **27:** 1-8.

Levine, J. B. I.; Santangelo, L.; Cheng, H.; Crittenden, I. B. and Dodgson, J. B. (1994). *J. Heredity,* **85**: 79-85.

Litt, M. and Luty, J. A. (1989). *Am. J. Hum. Genetics,* **44**: 397-401.

Mac Hugh, D. E.; Shriver, M. D.; Loftus, R. T.; Cunningham, E. P. and Bradley, D. G.(1996). *Genetics,* **146**: 1071- 1080.

Maddox, J. F.; Davies, K. P.; Grawford, A. M.; Huleme, D. J.; Vaiman, D.; Cribiu, E. P.; Freking, B. A.; Beh, K. J.; Cockett, N. E.; Kang, N.; Riffkin, C. D.; Drinkwater, R.; Moore, S. S.; Dodds, K. G.; Lumsden, J. M.; VanStijn, T. C.; Phua, S. H.; Adelson, D. L.; Burkin, H. R.; Broom, J. E.; Buitkamp, J.; Cambridge, L.; Cushwa, W. T.; Gerard, E.; Galloway, S. M.; Harrison, B.; Hawken, R. J.; Hiendleder, S.; Henry, H. M.; Medrano, J. F.; Paterson, K. A.; Schibler, L.; Stone, R. T. and VanHest, H. (2001). *Genome Res*., **11**:1275 -1289.

Mburu, D. N.; Ochieng, J. W.; Kuria, S. G.; Jianlin, H.; Kaufmann, B.; Rege. J. E. O. and Hanotte, O. (2003). *Anim. Genetics,* **34**: 26-32.

Meunier, J. R. and Grimont, P. A. D. (1993). *Res. Microbiol*., **144** : 373 – 379.

Moore, S. S.; Sargeant, L. L.; King, T. J.; Mattick, J. S.; Georges, M. and Hetzel, G. J. (1991). *Genom*e, **10**: 654-600.

Morgan, M. (1998). Population genetics. www.wsu,edu/mmorgan/gencb511f98/discussion5/node3.html.

Nei, M. (1987). Molecular evolutionary genetics. 2nd edn. Columbia University Press, New York. Pp. 273 – 292.

O'Brien, S. J. (1991). *Curr. Opin. Genet. Dev.*, **1**: 105-111.

Plotsky, Y.; Kaiser, M. G. and Lamont, S. J. (1995). *Anim. Genet*ics, **26**: 163-170.

Rohrer, G. A.; Alexander, L. J.; Smith, T. P. L.; Keele, J. W. and Beattie, C. W. (1996). *Genome Res.,* **6**: 371 -391.

Romanov, M. N. and Weigend, S. (2001). *Archiv für Geflügelkunde*, **65**: 1 – 4.

Ruane, J. (1999). *J. Anim. Breed. Genet.*, **116**: 317 – 323.

Rubinsztein, D. C.; Amos, W. and Leggo, J. (1995). *Nature genetics*, **10:** 337-343.

Sanchez, J. A.; Clabby, C.; Ramos, D.; Blanco, G.; Flavin, F.; Vazquez, E. and Powell, R. (1996). J. *Hered*it., **77**: 423 – 432.

Strachan, T. and Read, A. P. (2001). Human Molecular genetics. 2nd edn. Bios Scientific publishers, Oxford, U.K. Pp. 273- 883.

Strauss, S. H.; Bousquet, J.; Hipkins, V. D. and Hong, Y. P. (1992). *New Forests*, **6**: 125 – 158.

Suchyta, S. P.; Cheng, H. H.; Burnside, J. and Dodgson, J. B. (2001). *Anim. Genetics*, **32**: 12 – 18.

Swinburne, J.; Gerstenberg, C.; Breen, M.; Aldridge, V.; Lockhart, L.; Marti, E.; Antczak, D.; Eggleston-Stott, M.; Bailey, E.; Mickelson, J.; Roed, K.; Lindgren, G. and Binns, M. (2000) . *Genomics*, **66**: 123 – 134

Tautz, D. (1989). *Nucl. Acid Res.*, **17**:16.

Turner, P. C.; Mclennan, A.G.; Bates, A. D. and White, M. R. H. (1998). Instant notes on molecular biology. 1st edn. Bios Scientific publishers limited, Oxford, UK.

Weaver, R. F. and Hedrick, P.W. (1992). Genetics. 2nd edn. W.M.C. Brown publisher. Pp. 570-593

Weber, J. and May, P. E. (1989). *Am. J. Hum. Genetics*, **44**: 388-396.

Weller, J. I.; Kashi, Y. and Soller, M. (1990). *J. Dairy Sci*., **73**: 2525- 2537.

Willaims, J. G. K.; Kubelik, A. R.; Livak, K. J.; Reveals, J. A. and Tingey, S. V. (1990). *Nuc. Acids Res.*, **8**: 6531-6535.

Vaiman, D.; Schibler, L.; Bourgeois, F.; Oustry, A.; Amigues, Y. and Cribiu, E. P.; (1996). *Genetics*, **144**: 279-305.

Vignal, A.; Milan, D.; SanCristobal, M. and Eggen, A. (2002). *Genet. Sel. Evol.*, **34**: 275 – 305.

Yonash, N.; Bacon, L. D.; Witter, R. L.and Cheng, H. H. (1999). *Anim. Genetics*, **30**:126 – 135.

Zhu, J.; Nestor, K. E.; Patterson, R. A.; Jackwood, D. J. and Emmerson, D. A. (1996). *Poult. Sci*., **75**: 439-446.