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(54) Title: POLYMORPHISMS IN MITOCHONDRIAL TRANSCRIPTION FACTOR A (TFAM) GENE AND THEIR ASSOCIATIONS WITH CARCASS TRAITS

(57) Abstract: The physiological regulation of intake, growth and energy partitioning in animals is under the control of multiple genes, which may be important candidates for unraveling the genetic variation in economically relevant traits in beef production. The present invention relates to the identification of single nucleotide polymorphisms (SNPs) within the bovine gene encoding mitochondrial transcription factor A ("TFAM") and their associations with economically relevant traits in beef production. The invention further encompasses methods and systems, including network-based processes, to manage the SNP data and other data relating to specific animals and herds of animals, veterinarian care, diagnostic and quality control data and management of livestock which, based on genotyping, have predictable meat quality traits, husbandry conditions, animal welfare, food safety information, audit of existing processes and data from field locations.

TITLE OF THE INVENTION

Polymorphisms in Mitochondrial Transcription Factor A ("TFAM") Gene and their Associations with Carcass Traits

INCORPORATION BY REFERENCE

All documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

FIELD OF THE INVENTION

The present invention relates to the identification of single nucleotide polymorphisms (SNPs) within the bovine genes encoding mitochondrial transcription factor A ("*TFAM*") and their associations with economically relevant traits in beef production. The invention further relates to methods and systems, including network-based processes, to manage the SNP data and other data relating to specific animals and herds of animals, veterinarian care, diagnostic and quality control data and management of livestock which, based on genotyping, have predictable meat quality traits, husbandry conditions, animal welfare, food safety information, audit of existing processes and data from field locations.

BACKGROUND OF THE INVENTION

Significant improvements in animal performance, efficiency and carcass and meat quality have been made over the years through the application of standard animal breeding and selection techniques. However, such classical animal breeding techniques require several years of genetic evaluation of performance records on individual animals and their relatives and are therefore very expensive. Other efforts have been made to improve productivity and quality through the application of such management practices as the use of feed additives, animal hormonal implants and chemotherapeutics. However, there is significant political and regulatory resistance to the introduction and use of such methodologies. Such methodologies are also non-inheritable and need to be applied differently in every production system.

There is a need for methods that allow relatively easy and more efficient selection and breeding of farm animals with an advantage for an inheritable trait of circulating leptin levels,

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TITLE OF THE INVENTION

Polymorphisms in Mitochondrial Transcription Factor A ("TFAM") Gene and their Associations with Carcass Traits

INCORPORATION BY REFERENCE

This application claims benefit of U.S. provisional patent application Serial No. 60/685,213 filed May 27, 2005.

The foregoing applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

FIELD OF THE INVENTION

The present invention relates to the identification of single nucleotide polymorphisms (SNPs) within the bovine genes encoding mitochondrial transcription factor A ("*TFAM*") and their associations with economically relevant traits in beef production. The invention further relates to methods and systems, including network-based processes, to manage the SNP data and other data relating to specific animals and herds of animals, veterinarian care, diagnostic and quality control data and management of livestock which, based on genotyping, have predictable meat quality traits, husbandry conditions, animal welfare, food safety information, audit of existing processes and data from field locations.

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There is a need for methods that allow relatively easy and more efficient selection and breeding of farm animals with an advantage for an inheritable trait of circulating leptin levels,

feed intake, growth rate, body weight, carcass merit and carcass composition. The economic significance of the use of genetic markers that are associated with specific economically important traits (especially traits with low heritability) in livestock through marker-assisted selection cannot therefore be over-emphasized.

The physiological regulation of intake, growth and energy partitioning in animals is under the control of multiple genes, which may be important candidates for unraveling the genetic variation in economically relevant traits (ERT) in beef production. Polymorphisms in these candidate genes that show association with specific ERT are useful quantitative trait nucleotides for marker-assisted selection

Mitochondrial transcription factor A ("TFAM"), a member of a high mobility group protein family and the first-identified mitochondrial transcription factor (Fisher and Clayton, Mol Cell Biol. 1988; 8:3496-509), is essential for maintenance and biogenesis of mitochondrial DNA (mtDNA). First, TFAM plays a histone-like role in mitochondria, as it is tightly associated with mtDNA as a main component of the nucleoid (Kanki et al. Mol Cell Biol. 2004; 24:9823-34). Evidence has shown that one molecule of mtDNA is packed with ~900 molecules of TFAM on average (Alam et al. Nucleic Acids Res. 2003; 31:1640-5), which makes mtDNA no longer naked. Second, TFAM regulates mtDNA copy number in mammals. Investigation using a combination of mice with TFAM overexpression and TFAM knockout demonstrated that mtDNA copy number is directly proportional to the total TFAM protein level in mouse embryos (Ekstrand et al. Hum Mol Genet. 2004; 13:935-44). RNA interference of the endogenous TFAM expression in HeLa cells also indicated that the mtDNA amount is correlated in parallel with the amount of TFAM (Kanki et al. Ann N Y Acad Sci. 2004; 1011:61-8). Third, TFAM stimulates transcription of mtDNA. The TFAM protein possesses two tandem high mobility group domains, which makes TFAM bind, unwind and bend DNA without sequence specificity and thus facilitate transcription initiation of mtDNA (Gaspari et al. 2004; 1659:148-52). Evidence has shown that import of wt-TFAM into liver mitochondria from hypothyroid rats increased RNA synthesis significantly up to 4-fold (Garstka et al. Nucleic Acids Res. 2003; 31:5039-47).

It has been known for many years that adipose tissue plays a central rule in regulation and manipulation of energy metabolisms through the storage and turnover of triglycerides and through the secretion of factors that affect satiety and fuel utilization. However, many key aspects of adipogenesis are accompanied by stimulation of mitochondrial biogenesis (Wilson-Fritch et al. Mol Cell Biol. 2003; 23:1085-94). For example, the major site of fatty acid ß-oxidation occurs in mitochondria (Reichert and Neupert, Trends Genet. 2004; 20:555-

62), which may provide key intermediates for the synthesis of triglycerides via the action of pyruvate carboxylase (Owen et al. J Biol Chem. 2002; 277:30409-12). In addition, a relatively large mitochondrial mass are needed to generate acetyl-CoA for fatty acid activation prior to esterification into triglycerides. All these studies demonstrated the essential role and function of mitochondria in lipid metabolism.

To further explore the mechanism of mitochondria involved in adipogenesis, Wilson-Fritch and colleagues (Wilson-Fritch et al. Mol Cell Biol. 2003; 23:1085-94 and Wilson-Fritch et al. J Clin Invest. 2004; 114:1281-9) studied the 3T3-L1 cell (representative of white adipocytes) differentiation by using both proteomic and genomic approaches. Proteomic analysis revealed a 20- to 30-fold increase in the concentration of numerous mitochondrial proteins, while genomic analysis with gene expression profiling using Affymetrix GeneChips detected a statistically significant increase in the expression of many nucleus-encoded mitochondrial genes during adipogenesis. In particular, the authors found a profound decrease of approximately 50% in the levels of transcripts for nuclear-encoded mitochondrial genes accompanying the onset of obesity (Wilson-Fritch et al. J Clin Invest. 2004; 114:1281-9).

It remains advantageous to provide further SNPs that may more accurately predict the meat quality phenotype of an animal and also a business method that provides for increased production efficiencies in livestock cattle, as well as providing access to various records of the animals and allows comparisons with expected or desired goals with regard to the quality and quantity of animals produced.

Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

The present invention relates to the identification of single nucleotide polymorphisms (SNPs) within the bovine genes encoding mitochondrial transcription factor A ("*TFAM*") and their associations with economically relevant traits in beef production.

The invention encompasses a method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar polymorphism in a *TFAM* gene which may comprise determining the genotype of each animal to be sub-grouped by determining the presence of a single nucleotide polymorphism in the *TFAM* gene, and segregating individual animals into sub-groups wherein each animal in a sub-group has a similar polymorphism in the *TFAM* gene.

The invention also encompasses a method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar genotype in the *TFAM* gene which may comprise determining the genotype of each animal to be sub-grouped by determining the presence of a single nucleotide polymorphism(s) of interest in the *TFAM* gene, and segregating individual animals into sub-groups depending on whether the animals have, or do not have, the single nucleotide polymorphism(s) of interest in the *TFAM* gene.

The single nucleotide polymorphism(s) of interest may be selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene.

The invention further relates to a method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar genotype in the *TFAM* gene which may comprise determining the genotype of each animal to be sub-grouped by determining the presence of any one of the above SNPs, and segregating individual animals into sub-groups depending on whether the animals have, or do not have, any one of the above SNPs in the *TFAM* gene.

The invention also relates to method for identifying an animal having a desirable phenotype as compared to the general population of animals of that species, which may comprise determining the presence of a single nucleotide polymorphism in the *TFAM* gene of the animal, wherein the presence of the SNP is indicative of a desirable phenotype.

In an advantageous embodiment, the animal may be a bovine. In another advantageous embodiment, the *TFAM* gene may be a bovine *TFAM* gene.

The invention also encompasses computer-assisted methods and systems for improving the production efficiency for livestock having marketable tender meat using multiple data, and in particular the genotype of the animals as it relates to *TFAM* SNPs. Methods of the invention encompass obtaining a genetic sample from each animal in a herd of livestock, determining the genotype of each animal with respect to specific quality traits as defined by a panel of at least two single polynucleotide polymorphisms (SNPs), grouping animals with like genotypes, and optionally, further sub-grouping animals based on like phenotypes. Methods of the invention may also encompass obtaining and maintaining data relating to the animals or to herds, their husbandry conditions, health and veterinary care and condition, genetic history or parentage, and providing this data to others through systems that are web-based, contained in a database, or attached to the animal itself such as by an implanted microchip. An advantageous aspect of the present invention, therefore, is directed

to a computer system and computer-assisted methods for tracking quality traits for livestock possessing specific genetic predispositions.

The present invention advantageously encompasses computer-assisted methods and systems for acquiring genetic data, particularly genetic data as defined by the absence or presence of a SNP within the *TFAM* gene related to meat quality traits of the breed of animal and associating those data with other data about the animal or its herd, and maintaining those data in ways that are accessible. Another aspect of the invention encompasses a computer-assisted method for predicting which livestock animals possess a biological difference in meat quality, and which may include the steps of using a computer system, e.g., a programmed computer comprising a processor, a data storage system, an input device and an output device, the steps of: (a) inputting into the programmed computer through the input device data that includes a genotype of an animal as it relates to any one of the *TFAM* SNPs described herein, (b) correlating meat quality predicted by the *TFAM* genotype using the processor and the data storage system and (c) outputting to the output device the meat quality correlated to the *TFAM* genotype, thereby predicting which livestock animals possess a particular meat quality.

Yet another aspect of the invention relates to a method of doing business for managing livestock comprising providing to a user computer system for managing livestock comprising physical characteristics and genotypes corresponding to one or more animals or a computer readable media for managing livestock comprising physical characteristics and genotypes corresponding to one or more animals or physical characteristics and genotypes corresponding to one or more animals, wherein a physical characteristic intake, growth or carcass merit in beef cattle and the genotype is a *TFAM* genotype.

It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, in which:

FIG. 1 provides a schematic annotation of cDNA and genomic DNA sequences of the bovine *TFAM* gene using a combination of in silico approach with PCR target region amplification.

FIG. 2 provides a nucleotide sequence of the upstream region of the bovine *TFAM* gene (SEQ ID NO: 1). This sequence corresponds to the 5' flanking region and exon 1. Coding sequence is shadowed. The putative transcription site was numbered as +1. Consensus sequences for potential SP1, NRF1 and transcription repressor are shown by arrows. Many potential mCpG loci are underlined. An extra AUG codon upstream from normal translation site is bold and marked. Both C/A and C/T substitutions are marked by arrows and numbers.

FIG. 3 provides a demonstration of a C/A and a C/T SNP in the bovine *TFAM* promoter region. Left: a homozygote with CC and CC; Right: a homozygote with AA and TT at two positions apart by 9 bp inclusive.

FIG. 4 provides PCR-RFLP genotyping of two SNPs in the bovine *TFAM* promoter. Lanes 1 and 8: 100 bp ladders. Lanes 2 – 7: a 801 bp fragment was digested with restriction enzyme *Dpn*II. Lanes 2 and 3, TT animals (55+68+135+241+302bp); lanes 4 and 5, CT animals (55+68+135+241+302+543bp); and lanes 6 and 7, CC animals (55+68+135+543bp). Lanes 9 – 14: a 801 bp fragment was digested with restriction enzyme *Hae*III. Lanes 9 and 10, AA animals (152+187+462bp); lanes 11 and 12, CA animals (83+104+152+187+462bp); and lanes 13 and 14, CC animals (83+104+152+462bp).

FIG. 5 identifies genetic polymorphisms in the bovine *TFAM*, *TFB1M* and *TFB2M* genes. A. A third mutation of C/T substitution in *TFAM* promoter region. B. Two mutations detected in the bovine *TFB1*M gene using DNA pools. C. Five mutations developed in the bovine *TFB2M* gene using DNA pools.

FIG. 6A provides a cattle *TFAM* cDNA sequence (2259bp) (SEQ ID NO: 2).

FIG. 6B provides a cattle *TFAM* genomic DNA sequence (16666bp) (SEQ ID NO: 3). Exons are shaded, as well as mutation sites. See, e.g., GenBank Accession Nos. AAFC02110692 and AAFC02019444.

FIG. 7 illustrates a flowchart of the input of data and the output of results from the analysis and correlation of the data pertaining to the breeding, veterinarian histories and

performance requirements of a group of animals such as from a herd of cows and the interactive flow of data from the computer-assisted device to a body of students learning the use of the method of the invention.

FIG. 8 illustrates potential relationships between the data elements to be entered into the system. Unidirectional arrows indicate, for example, that a barn is typically owned by only one farm, whereas a farm may own several barns. Similarly, a prescription may include veterinarian products.

FIG. 9A illustrates the flow of events in the use of the portable computer-based system for data entry on the breeding and rearing of a herd of cows.

FIG. 9B illustrates the flow of events through the sub-routines related to data entry concerning farm management.

FIG. 9C illustrates the flow of events through the sub-routines related to data entry concerning data specific to a company.

FIG. 10 illustrates a flow chart of the input of data and the output of results from the analysis and the correlation of the data pertaining to the breeding, veterinarian histories, and performance requirements of a group of animals.

DETAILED DESCRIPTION

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press; DNA Cloning, Vols. I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Animal Cell Culture (R. K. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL press, 1986); Perbal, B., A Practical Guide to Molecular Cloning (1984); the series, Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); and Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell eds., 1986, Blackwell Scientific Publications).

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular DNA, polypeptide sequences or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

In describing the present invention, the following terms will be employed and are intended to be defined as indicated below.

The term "cow" or "cattle" is used generally to refer to an animal of bovine origin of any age. Interchangeable terms include "bovine", "calf", "steer", "bull", "heifer" and the like. It also includes an individual animal in all stages of development, including embryonic and fetal stages. The animals as referred to herein may also include individuals or groups of individuals that are raised for other than food production such as, but not limited to, transgenic animals for the production of biopharmaceuticals including antibodies and other proteins or protein products.

By the term "complementarity" or "complementary" is meant, for the purposes of the specification or claims, a sufficient number in the oligonucleotide of complementary base pairs in its sequence to interact specifically (hybridize) with a target nucleic acid sequence of the gene polymorphism to be amplified or detected. As known to those skilled in the art, a very high degree of complementarity is needed for specificity and sensitivity involving hybridization, although it need not be 100%. Thus, for example, an oligonucleotide that is identical in nucleotide sequence to an oligonucleotide disclosed herein, except for one base change or substitution, may function equivalently to the disclosed oligonucleotides. A "complementary DNA" or "cDNA" gene includes recombinant genes synthesized by reverse transcription of messenger RNA ("mRNA").

A "cyclic polymerase-mediated reaction" refers to a biochemical reaction in which a template molecule or a population of template molecules is periodically and repeatedly copied to create a complementary template molecule or complementary template molecules, thereby increasing the number of the template molecules over time.

By the term "detectable moiety" is meant, for the purposes of the specification or claims, a label molecule (isotopic or non-isotopic) which is incorporated indirectly or directly into an oligonucleotide, wherein the label molecule facilitates the detection of the oligonucleotide in which it is incorporated, for example when the oligonucleotide is hybridized to amplified gene polymorphic sequences. Thus, "detectable moiety" is used synonymously with "label molecule". Synthesis of oligonucleotides can be accomplished by

any one of several methods known to those skilled in the art. Label molecules, known to those skilled in the art as being useful for detection, include chemiluminescent, fluorescent or luminescent molecules. Various fluorescent molecules are known in the art which are suitable for use to label a nucleic acid for the method of the present invention. The protocol for such incorporation may vary depending upon the fluorescent molecule used. Such protocols are known in the art for the respective fluorescent molecule.

"DNA amplification" as used herein refers to any process that increases the number of copies of a specific DNA sequence by enzymatically amplifying the nucleic acid sequence. A variety of processes are known. One of the most commonly used is the polymerase chain reaction (PCR) process of Mullis as described in U.S. Pat. Nos. 4,683,195 and 4,683,202. Methods, devices and reagents as described in U.S. Patent Nos. 6,951,726; 6,927,024; 6,924,127; 6,893,863; 6,887,664; 6,881,559; 6,855,522; 6,855,521; 6,849,430; 6,849,404; 6,846,631; 6,844,158; 6,844,155; 6,818,437; 6,818,402; 6,794,177; 6,794,133; 6,790,952; 6,783,940; 6,773,901; 6,770,440; 6,767,724; 6,750,022; 6,744,789; 6,733,999; 6,733,972; 6,703,236; 6,699,713; 6,696,277; 6,664,080; 6,664,064; 6,664,044; RE38,352; 6,650,719; 6,645,758; 6,645,720; 6,642,000; 6,638,716; 6,632,653; 6,617,107; 6,613,560; 6,610,487; 6,596,492; 6,586,250; 6,586,233; 6,569,678; 6,569,627; 6,566,103; 6,566,067; 6,566,052; 6,558,929; 6,558,909; 6,551,783; 6,544,782; 6,537,752; 6,524,830; 6,518,020; 6,514,750; 6,514,706; 6,503,750; 6,503,705; 6,493,640; 6,492,114; 6,485,907; 6,485,903; 6,482,588; 6,475,729; 6,468,743; 6,465,638; 6,465,637; 6,465,171; 6,448,014; 6,432,646; 6,428,987; 6,426,215; 6,423,499; 6,410,223; 6,403,341; 6,399,320; 6,395,518; 6,391,559; 6,383,755; 6,379,932; 6,372,484; 6,368,834; 6,365,375; 6,358,680; 6,355,422; 6,348,336; 6,346,384; 6,319,673; 6,316,195; 6,316,192; 6,312,930; 6,309,840; 6,309,837; 6,303,343; 6,300,073; 6,300,072; 6,287,781; 6,284,455; 6,277,605; 6,270,977; 6,270,966; 6,268,153; 6,268,143; D445,907; 6,261,431; 6,258,570; 6,258,567; 6,258,537; 6,258,529; 6,251,607; 6,248,567; 6,235,468; 6,232,079; 6,225,093; 6,221,595; D441,091; 6,218,153; 6,207,425; 6,183,999; 6,183,963; 6,180,372; 6,180,349 ; 6,174,670; 6,153,412; 6,146,834; 6,143,496; 6,140,613; 6,140,110; 6,103,468; 6,087,097; 6,072,369; 6,068,974; 6,063,563; 6,048,688; 6,046,039; 6,037,129; 6,033,854; 6,031,960; 6,017,699; 6,015,664; 6,015,534; 6,004,747; 6,001,612; 6,001,572; 5,985,619; 5,976,842; 5,972,602; 5,968,730; 5,958,686; 5,955,274; 5,952,200; 5,936,968; 5,909,468; 5,905,732; 5,888,740; 5,883,924; 5,876,978; 5,876,977; 5,874,221; 5,869,318; 5,863,772; 5,863,731; 5,861,251; 5,861,245; 5,858,725; 5,858,718; 5,856,086; 5,853,991; 5,849,497; 5,837,468; 5,830,663; 5,827,695; 5,827,661; 5,827,657; 5,824,516; 5,824,479; 5,817,797; 5,814,489; 5,814,453; 5,811,296; 5,804,383; 5,800,997; 5,780,271 ;

5,780,222; 5,776,686; 5,774,497; 5,766,889; 5,759,822; 5,750,347; 5,747,251; 5,741,656; 5,716,784; 5,712,125; 5,712,090; 5,710,381; 5,705,627; 5,702,884; 5,693,467; 5,691,146; 5,681,741; 5,674,717; 5,665,572; 5,665,539; 5,656,493; 5,656,461; 5,654,144; 5,652,102; 5,650,268; 5,643,765; 5,639,871; 5,639,611; 5,639,606; 5,631,128; 5,629,178; 5,627,054; 5,618,703; 5,618,702; 5,614,388; 5,610,017; 5,602,756; 5,599,674; 5,589,333; 5,585,238; 5,576,197; 5,565,340; 5,565,339; 5,556,774; 5,556,773; 5,538,871; 5,527,898; 5,527,510; 5,514,568; 5,512,463; 5,512,462; 5,501,947; 5,494,795; 5,491,225; 5,487,993; 5,487,985; 5,484,699; 5,476,774; 5,475,610; 5,447,839; 5,437,975; 5,436,144; 5,426,026; 5,420,009; 5,411,876; 5,393,657; 5,389,512; 5,364,790; 5,364,758; 5,340,728; 5,283,171; 5,279,952; 5,254,469; 5,241,363; 5,232,829; 5,231,015; 5,229,297; 5,224,778; 5,219,727; 5,213,961; 5,198,337; 5,187,060; 5,142,033; 5,091,310; 5,082,780; 5,066,584; 5,023,171 and 5,008,182 may also be employed in the practice of the present invention. PCR involves the use of a thermostable DNA polymerase, known sequences as primers, and heating cycles, which separate the replicating deoxyribonucleic acid (DNA), strands and exponentially amplify a gene of interest. Any type of PCR, such as quantitative PCR, RT-PCR, hot start PCR, LAPCR, multiplex PCR, touchdown PCR, etc., may be used. Advantageously, real-time PCR is used. In general, the PCR amplification process involves a cyclic enzymatic chain reaction for preparing exponential quantities of a specific nucleic acid sequence. It requires a small amount of a sequence to initiate the chain reaction and oligonucleotide primers that will hybridize to the sequence. In PCR the primers are annealed to denatured nucleic acid followed by extension with an inducing agent (enzyme) and nucleotides. This results in newly synthesized extension products. Since these newly synthesized sequences become templates for the primers, repeated cycles of denaturing, primer annealing, and extension results in exponential accumulation of the specific sequence being amplified. The extension product of the chain reaction will be a discrete nucleic acid duplex with a termini corresponding to the ends of the specific primers employed.

By the terms "enzymatically amplify" or "amplify" is meant, for the purposes of the specification or claims, DNA amplification, i.e., a process by which nucleic acid sequences are amplified in number. There are several means for enzymatically amplifying nucleic acid sequences. Currently the most commonly used method is the polymerase chain reaction (PCR). Other amplification methods include LCR (ligase chain reaction) which utilizes DNA ligase, and a probe consisting of two halves of a DNA segment that is complementary to the sequence of the DNA to be amplified, enzyme QB replicase and a ribonucleic acid (RNA) sequence template attached to a probe complementary to the DNA to be copied which is used

to make a DNA template for exponential production of complementary RNA; strand displacement amplification (SDA); Qß replicase amplification (QßRA); self-sustained replication (3SR); and NASBA (nucleic acid sequence-based amplification), which can be performed on RNA or DNA as the nucleic acid sequence to be amplified.

A "fragment" of a molecule such as a protein or nucleic acid is meant to refer to any portion of the amino acid or nucleotide genetic sequence.

As used herein, the term "genome" refers to all the genetic material in the chromosomes of a particular organism. Its size is generally given as its total number of base pairs. Within the genome, the term "gene" refers to an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (e.g., a protein or RNA molecule). In general, an animal's genetic characteristics, as defined by the nucleotide sequence of its genome, are known as its "genotype," while the animal's physical traits are described as its "phenotype."

By "heterozygous" or "heterozygous polymorphism" is meant that the two alleles of a diploid cell or organism at a given locus are different, that is, that they have a different nucleotide exchanged for the same nucleotide at the same place in their sequences.

By "homozygous" or "homozygous polymorphism" is meant that the two alleles of a diploid cell or organism at a given locus are identical, that is, that they have the same nucleotide for nucleotide exchange at the same place in their sequences.

By "hybridization" or "hybridizing," as used herein, is meant the formation of A-T and C-G base pairs between the nucleotide sequence of a fragment of a segment of a polynucleotide and a complementary nucleotide sequence of an oligonucleotide. By complementary is meant that at the locus of each A, C, G or T (or U in a ribonucleotide) in the fragment sequence, the oligonucleotide sequenced has a T, G, C or A, respectively. The hybridized fragment/ oligonucleotide is called a "duplex."

A "hybridization complex", such as in a sandwich assay, means a complex of nucleic acid molecules including at least the target nucleic acid and a sensor probe. It may also include an anchor probe.

As used herein, the term "locus" or "loci" refers to the site of a gene on a chromosome. Pairs of genes, known as "alleles" control the hereditary trait produced by a gene locus. Each animal's particular combination of alleles is referred to as its "genotype". Where both alleles are identical the individual is said to be homozygous for the trait controlled by that gene pair; where the alleles are different, the individual is said to be heterozygous for the trait.

A "melting temperature" is meant the temperature at which hybridized duplexes dehybridize and return to their single-stranded state. Likewise, hybridization will not occur in the first place between two oligonucleotides, or, herein, an oligonucleotide and a fragment, at temperatures above the melting temperature of the resulting duplex. It is presently advantageous that the difference in melting point temperatures of oligonucleotide-fragment duplexes of this invention be from about 1°C to about 10°C so as to be readily detectable.

As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but advantageously is double-stranded DNA. "DNA" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid.

A "nucleoside" refers to a base linked to a sugar. The base may be adenine (A), guanine (G) (or its substitute, inosine (I)), cytosine (C), or thymine (T) (or its substitute, uracil (U)). The sugar may be ribose (the sugar of a natural nucleotide in RNA) or 2-deoxyribose (the sugar of a natural nucleotide in DNA). A "nucleotide" refers to a nucleoside linked to a single phosphate group.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides may be chemically synthesized and may be used as primers or probes. Oligonucleotide means any nucleotide of more than 3 bases in length used to facilitate detection or identification of a target nucleic acid, including probes and primers.

A "polymerase" is an enzyme that catalyzes the sequential addition of monomeric units to a polymeric chain, or links two or more monomeric units to initiate a polymeric chain. The "polymerase" will work by adding monomeric units whose identity is determined by and which is complementary to a template molecule of a specific sequence. For example, DNA polymerases such as DNA pol 1 and Taq polymerase add deoxyribonucleotides to the 3' end of a polynucleotide chain in a template-dependent manner, thereby synthesizing a nucleic acid that is complementary to the template molecule. Polymerases may be used either to extend a primer once or repetitively or to amplify a polynucleotide by repetitive priming of two complementary strands using two primers. A "thermostable polymerase" refers to a DNA or RNA polymerase enzyme that can withstand extremely high temperatures, such as those approaching 100°C. Often, thermostable polymerases are derived from organisms that live in extreme temperatures, such as Thermus aquaticus. Examples of thermostable polymerases include Taq, Tth, Pfu, Vent, deep vent, UlTma, and variations and derivatives thereof.

A "polynucleotide" refers to a linear chain of nucleotides connected by a phosphodiester linkage between the 3'-hydroxyl group of one nucleoside and the 5'-hydroxyl group of a second nucleoside which in turn is linked through its 3'-hydroxyl group to the 5'-hydroxyl group of a third nucleoside and so on to form a polymer comprised of nucleosides linked by a phosphodiester backbone. A "modified polynucleotide" refers to a polynucleotide in which one or more natural nucleotides have been partially,substantially, or completely replaced with modified nucleotides.

A "primer" is an oligonucleotide, the sequence of at least of portion of which is complementary to a segment of a template DNA which is to be amplified or replicated. Typically primers are used in performing the polymerase chain reaction (PCR). A primer hybridizes with (or "anneals" to) the template DNA and is used by the polymerase enzyme uses as the starting point for the replication/amplification process. The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the

sequence of the strand to hybridize therewith and thereby form the template for the synthesis of the extension product.

"Probes" refer to oligonucleotides nucleic acid sequences of variable length, used in the detection of identical, similar, or complementary nucleic acid sequences by hybridization. An oligonucleotide sequence used as a detection probe may be labeled with a detectable moiety.

The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracil, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as by conjugation, with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides or solid support.

An "isolated" polynucleotide or polypeptide is one that is substantially pure of the materials with which it is associated in its native environment. By substantially free, is meant at least 50%, at least 55%, at least 60%, at least 65%, at advantageously at least 70%, at least 75%, more advantageously at least 80%, at least 85%, even more advantageously at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, most advantageously at least 98%, at least 99%, at least 99.5%, at least 99.9% free of these materials.

An "isolated" nucleic acid molecule is a nucleic acid molecule separate and discrete from the whole organism with which the molecule is found in nature; or a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences (as defined below) in association therewith.

The term "polynucleotide encoding a protein" as used herein refers to a DNA fragment or isolated DNA molecule encoding a protein, or the complementary strand thereto; but, RNA is not excluded, as it is understood in the art that thymidine (T) in a DNA sequence is considered equal to uracil (U) in an RNA sequence. Thus, RNA sequences for use in the

invention, e.g., for use in RNA vectors, can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

A DNA "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory elements. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

"Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, preferably at least about 90%, 91%, 92%, 93%, 94% and most preferably at least about 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity (100% sequence identity) to the specified DNA or polypeptide sequence.

Homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al. supra; DNA Cloning, supra; Nucleic Acid Hybridization, supra.

Two nucleic acid fragments are considered to be "selectively hybridizable" to a polynucleotide if they are capable of specifically hybridizing to a nucleic acid or a variant thereof or specifically priming a polymerase chain reaction: (i) under typical hybridization and wash conditions, as described, for example, in Sambrook et al. supra and Nucleic Acid Hybridization, supra, (ii) using reduced stringency wash conditions that allow at most about 25-30% basepair mismatches, for example: 2x SSC, 0.1% SDS, room temperature twice, 30 minutes each; then 2x SSC, 0.1% SDS, 37°C once, 30 minutes; then 2 x SSC room temperature twice, 10 minutes each, or (iii) selecting primers for use in typical polymerase

chain reactions (PCR) under standard conditions (described for example, in Saiki, et al. (1988) Science 239:487-491).

The term "capable of hybridizing under stringent conditions" as used herein refers to annealing a first nucleic acid to a second nucleic acid under stringent conditions as defined below. Stringent hybridization conditions typically permit the hybridization of nucleic acid molecules having at least 70% nucleic acid sequence identity with the nucleic acid molecule being used as a probe in the hybridization reaction. For example, the first nucleic acid may be a test sample or probe, and the second nucleic acid may be the sense or antisense strand of a nucleic acid or a fragment thereof. Hybridization of the first and second nucleic acids may be conducted under stringent conditions, e.g., high temperature and/or low salt content that tend to disfavor hybridization of dissimilar nucleotide sequences. Alternatively, hybridization of the first and second nucleic acid may be conducted under reduced stringency conditions, e.g. low temperature and/or high salt content that tend to favor hybridization of dissimilar nucleotide sequences. Low stringency hybridization conditions may be followed by high stringency conditions or intermediate medium stringency conditions to increase the selectivity of the binding of the first and second nucleic acids. The hybridization conditions may further include reagents such as, but not limited to, dimethyl sulfoxide (DMSO) or formamide to disfavor still further the hybridization of dissimilar nucleotide sequences. A suitable hybridization protocol may, for example, involve hybridization in 6 x SSC (wherein 1 x SSC comprises 0.015 M sodium citrate and 0.15 M sodium chloride), at 65° Celsius in an aqueous solution, followed by washing with 1 x SSC at 65° C. Formulae to calculate appropriate hybridization and wash conditions to achieve hybridization permitting 30% or less mismatch between two nucleic acid molecules are disclosed, for example, in Meinkoth et al. (1984) Anal. Biochem. 138: 267-284; the content of which is herein incorporated by reference in its entirety. Protocols for hybridization techniques are well known to those of skill in the art and standard molecular biology manuals may be consulted to select a suitable hybridization protocol without undue experimentation. See, for example, Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, the contents of which are herein incorporated by reference in their entirety.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M sodium ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) from about pH 7.0 to about pH 8.3 and the temperature is at least about 30° Celsius for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of

destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37° Celsius, and a wash in 1-2 x SSC at 50 to 55° Celsius. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37° Celsius, and a wash in 0.5-1 x SSC at 55 to 60° Celsius. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37° Celsius, and a wash in 0.1 x SSC at 60 to 65° Celsius.

Methods and materials of the invention may be used more generally to evaluate a DNA sample from an animal, genetically type an individual animal, and detect genetic differences in animals. In particular, a sample of genomic DNA from an animal may be evaluated by reference to one or more controls to determine if a SNP, or group of SNPs, in a gene is present. Any method for determining genotype can be used for determining the genotype in the present invention. Such methods include, but are not limited to, amplimer sequencing, DNA sequencing, fluorescence spectroscopy, fluorescence resonance energy transfer (or "FRET")-based hybridization analysis, high throughput screening, mass spectroscopy, microsatellite analysis, nucleic acid hybridization, polymerase chain reaction (PCR), RFLP analysis and size chromatography (e.g., capillary or gel chromatography), all of which are well known to one of skill in the art. In particular, methods for determining nucleotide polymorphisms, particularly single nucleotide polymorphisms, are described in U.S. Patent Nos. 6,514,700; 6,503,710; 6,468,742; 6,448,407; 6,410,231; 6,383,756; 6,358,679; 6,322,980; 6,316,230; and 6,287,766 and reviewed by Chen and Sullivan, Pharmacogenomics J 2003;3(2):77-96, the disclosures of which are incorporated by reference in their entireties. Genotypic data useful in the methods of the invention and methods for the identification and selection of animal traits are based on the presence of SNPs.

A "restriction fragment" refers to a fragment of a polynucleotide generated by a restriction endonuclease (an enzyme that cleaves phosphodiester bonds within a polynucleotide chain) that cleaves DNA in response to a recognition site on the DNA. The recognition site (restriction site) consists of a specific sequence of nucleotides typically about 4-8 nucleotides long.

A "single nucleotide polymorphism" or "SNP" refers to a variation in the nucleotide sequence of a polynucleotide that differs from another polynucleotide by a single nucleotide difference. For example, without limitation, exchanging one A for one C, G or T in the entire sequence of polynucleotide constitutes a SNP. It is possible to have more than one SNP in a particular polynucleotide. For example, at one position in a polynucleotide, a C may be

exchanged for a T, at another position a G may be exchanged for an A and so on. When referring to SNPs, the polynucleotide is most often DNA.

As used herein, a "template" refers to a target polynucleotide strand, for example, without limitation, an unmodified naturally-occurring DNA strand, which a polymerase uses as a means of recognizing which nucleotide it should next incorporate into a growing strand to polymerize the complement of the naturally-occurring strand. Such a DNA strand may be single-stranded or it may be part of a double-stranded DNA template. In applications of the present invention requiring repeated cycles of polymerization, e.g., the polymerase chain reaction (PCR), the template strand itself may become modified by incorporation of modified nucleotides, yet still serve as a template for a polymerase to synthesize additional polynucleotides.

A "thermocyclic reaction" is a multi-step reaction wherein at least two steps are accomplished by changing the temperature of the reaction.

A "variance" is a difference in the nucleotide sequence among related polynucleotides. The difference may be the deletion of one or more nucleotides from the sequence of one polynucleotide compared to the sequence of a related polynucleotide, the addition of one or more nucleotides or the substitution of one nucleotide for another. The terms "mutation," "polymorphism" and "variance" are used interchangeably herein. As used herein, the term "variance" in the singular is to be construed to include multiple variances; i.e., two or more nucleotide additions, deletions and/or substitutions in the same polynucleotide. A "point mutation" refers to a single substitution of one nucleotide for another.

As used herein, the terms "traits", "quality traits" or "physical characteristics" or "phenotypes" refer to advantageous properties of the animal resulting from genetics. Quality traits include, but are not limited to, the animal's genetic ability to efficiently metabolize energy, produce meat or milk, put on intramuscular fat. Physical characteristics include, but are not limited to, marbled, tender or lean meats. The terms may be used interchangeably.

A "computer system" refers to the hardware means, software means and data storage means used to compile the data of the present invention. The minimum hardware means of computer-based systems of the invention may comprise a central processing unit (CPU), input means, output means, and data storage means. Desirably, a monitor is provided to visualize structure data. The data storage means may be RAM or other means for accessing computer readable media of the invention. Examples of such systems are microcomputer

workstations available from Silicon Graphics Incorporated and Sun Microsystems running Unix based, Linux, Windows NT, XP or IBM OS/2 operating systems.

"Computer readable media" refers to any media which can be read and accessed directly by a computer, and includes, but is not limited to: magnetic storage media such as floppy discs, hard storage medium and magnetic tape; optical storage media such as optical discs or CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories, such as magnetic/optical media. By providing such computer readable media, the data compiled on a particular animal can be routinely accessed by a user, e.g., a feedlot operator.

The term "data analysis module" is defined herein to include any person or machine, individually or working together, which analyzes the sample and determines the genetic information contained therein. The term may include a person or machine within a laboratory setting.

As used herein, the term "data collection module" refers to any person, object or system obtaining a tissue sample from an animal or embryo. By example and without limitation, the term may define, individually or collectively, the person or machine in physical contact with the animal as the sample is taken, the containers holding the tissue samples, the packaging used for transporting the samples, and the like. Advantageously, the data collector is a person. More advantageously, the data collector is a livestock farmer, a breeder or a veterinarian

The term "network interface" is defined herein to include any person or computer system capable of accessing data, depositing data, combining data, analyzing data, searching data, transmitting data or storing data. The term is broadly defined to be a person analyzing the data, the electronic hardware and software systems used in the analysis, the databases storing the data analysis, and any storage media capable of storing the data. Non-limiting examples of network interfaces include people, automated laboratory equipment, computers and computer networks, data storage devices such as, but not limited to, disks, hard drives or memory chips.

The term "breeding history" as used herein refers to a record of the life of an animal or group of animals including, but not limited to, the location, breed, period of housing, as well as a genetic history of the animals, including parentage and descent therefrom, genotype, phenotype, transgenic history if relevant and the like.

The term "husbandry conditions" as used herein refers to parameters relating to the maintenance of animals including, but not limited to, shed or housing temperature, weekly

mortality of a herd, water consumption, feed consumption, ventilation rate and quality, litter condition and the like.

The term "veterinary history" as used herein refers to vaccination data of an animal or group of animals, including, but not limited to, vaccine type(s), vaccine batch serial number(s), administered dose, target antigen, method of administering of the vaccine to the recipient animal(s), number of vaccinated animals, age of the animals and the vaccinator. Data relating to a serological or immunological response induced by the vaccine may also be included. "Veterinary history" as used herein is also intended to include the medication histories of the target animal(s) including, but not limited to drug and/or antibiotics administered to the animals including type of administered medication, quantity and dose rates, by whom and when administered, by what route, e.g., oral, subcutaneously and the like, and the response to the medication including desired and undesirable effects thereof.

The term "diagnostic data" as used herein refers to data relating to the health of the animal(s) other than data detailing the vaccination or medication history of the animal(s). For example, the diagnostic data may be a record of the infections experienced by the animal(s) and the response thereof to medications provided to treat such medications. Serological data including antibody or protein composition of the serum or other biofluids may also be diagnostic data useful to input in the methods of the invention. Surgical data pertaining to the animal(s) may be included, such as the type of surgical manipulation, outcome of the surgery and complications arising from the surgical procedure. "Diagnostic data" may also include measurements of such parameters as weight, morbidity, and other characteristics noted by a veterinary service such as the condition of the skin, feet, etc.

The term "welfare data" as used herein refers to the collective accumulation of data pertaining to an animal or group of animals including, but not limited to, a breeding history, a veterinary history, a welfare profile, diagnostic data, quality control data, or any combination thereof.

The term "welfare profile" as used herein refers to parameters such as weight, meat density, crowding levels in breeding or rearing enclosures, psychological behavior of the animal, growth rate and quality and the like.

The term "quality control" as used herein refers to the desired characteristics of the animal(s). For non-poultry animals such as cattle and sheep for example, such parameters include muscle quantity and density, fat content, meat tenderness, milk yield and quality, breeding ability, and the like.

The term "performance parameters" as used herein refers to such factors as meat yield, breeding yield, dairy form, meat quality and yield, productive life and the like that may be the desired goals from the breeding and rearing of the animal(s). Performance parameters may be either generated from the animals themselves, or those parameters desired by a customer or the market.

The term "nutritional data" as used herein refers to the composition, quantity and frequency of delivery of feed, including water, provided to the animal(s).

The term "food safety" as used herein refers to the quality of the meat from a livestock animal, including, but not limited to, preparation time, place and manner, storage of the food product, transportation route, inspection records, texture, color, taste, odor, bacterial content, parasitic content and the like.

It will be apparent to those of skill in the art that the data relating to the health and maintenance of the animals may be variously grouped depending upon the source or intention of the data collector and any one grouping herein is not therefore intended to be limiting.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of molecular biology. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

In an embodiment wherein the gene of interest is bovine *TFAM*, the bovine *TFAM* nucleotide sequence can be selected from, but is not limited to, the sequence corresponding to GenBank Accession Nos. AAFC0211069 or AAFC02019444 (SEQ ID NO: 3) or a fragment thereof or a region of the bovine genome that comprises this sequence.

The present invention, therefore, provides isolated nucleic acids that may specifically hybridize to the nucleotide sequence corresponding to GenBank Accession No. AFC0211069 or AAFC02019444 (SEQ ID NO: 3), or the complement thereof, and which comprises the polymorphic site corresponding to nucleotide positions -1220, -1212 or -995.

The single nucleotide polymorphism(s) of interest may be selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene.

The SNP advantageous in the present invention is associated with certain economically valuable and heritable traits relating to meat quality in bovines. Therefore, it is an object of the present invention to determine the genotype of a given animal of interest as

defined by the *TFAM* locus SNP according to the present invention. It is also contemplated that the genotype of the animal(s) may be defined by additional SNPs within the *TFAM* gene or within other genes identified with desirable traits or other characteristics, and in particular by a panel or panels of SNPs.

There are many methods known in the art for determining the sequence of DNA in a sample, and for identifying whether a given DNA sample contains a particular SNP. Any such technique known in the art may be used in performance of the methods of the present invention.

The methods of the present invention allow animals with certain economically valuable heritable traits to be identified based on the presence of SNPs in their genomes and particularly with a SNP located within the promoter of the *TFAM* gene. The methods further allow, by computer-assisted methods of the invention, to correlate the SNP-associated traits with other data pertinent to the well-being and productive capacity of the animals, or group of animals.

To determine the genotype of a given animal according to the methods of the present invention, it is necessary to obtain a sample of genomic DNA from that animal. Typically, that sample of genomic DNA will be obtained from a sample of tissue or cells taken from that animal. A tissue or cell sample may be taken from an animal at any time in the lifetime of an animal but before the carcass identity is lost. The tissue sample can comprise hair, including roots, hide, bone, buccal swabs, blood, saliva, milk, semen, embryos, muscle or any internal organs. In the methods of the present invention, the source of the tissue sample, and thus also the source of the test nucleic acid sample, is not critical. For example, the test nucleic acid can be obtained from cells within a body fluid of the animal, or from cells constituting a body tissue of the animal. The particular body fluid from which cells are obtained is also not critical to the present invention. For example, the body fluid may be selected from the group consisting of blood, ascites, pleural fluid and spinal fluid. Furthermore, the particular body tissue from which cells are obtained is also not critical to the present invention. For example, the body tissue may be selected from the group consisting of skin, endometrial, uterine and cervical tissue. Both normal and tumor tissues can be used.

Typically, the tissue sample is marked with an identifying number or other indicia that relates the sample to the individual animal from which the sample was taken. The identity of the sample advantageously remains constant throughout the methods and systems of the invention thereby guaranteeing the integrity and continuity of the sample during extraction and analysis. Alternatively, the indicia may be changed in a regular fashion that ensures that

the data, and any other associated data, can be related back to the animal from which the data was obtained.

The amount/size of sample required is known to those skilled in the art and for example, can be determined by the subsequent steps used in the method and system of the invention and the specific methods of analysis used. Ideally, the size/volume of the tissue sample retrieved should be as consistent as possible within the type of sample and the species of animal. For example, for cattle, non-limiting examples of sample sizes/methods include non-fatty meat: 0.0002 gm-10.0 gm; hide: 0.0004 gm-10.0 gm; hair roots: at least one and advantageously greater than five; buccal swabs: 15 to 20 seconds of rubbing with modest pressure in the area between outer lip and gum using, for example, a cytology brush; bone: 0.0002 gm-10.0 gm; blood: 30 µl to 50 ml.

Generally, the tissue sample is placed in a container that is labeled using a numbering system bearing a code corresponding to the animal, for example, to the animal's ear tag. Accordingly, the genotype of a particular animal is easily traceable at all times. The sampling device and/or container may be supplied to the farmer, a slaughterhouse or retailer. The sampling device advantageously takes a consistent and reproducible sample from individual animals while simultaneously avoiding any cross-contamination of tissue. Accordingly, the size and volume of sample tissues derived from individual animals would be consistent.

DNA can be isolated from the tissue/cells by techniques known to those skilled in the art (see, e.g., U.S. Patent Nos. 6,548,256 and 5,989,431; Hirota et al. (1989) Jinrui Idengaku Zasshi. 34: 217-23 and John et al. (1991) Nucleic Acids Res. 19:408, the disclosures of which are incorporated by reference in their entireties). For example, high molecular weight DNA may be purified from cells or tissue using proteinase K extraction and ethanol precipitation. DNA, however, may be extracted from an animal specimen using any other suitable methods known in the art.

In one embodiment, the presence or absence of the SNP of any of the genes of the present invention may be determined by sequencing the region of the genomic DNA sample that spans the polymorphic locus. Many methods of sequencing genomic DNA are known in the art, and any such method can be used, see for example Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press. For example, as described below, a DNA fragment spanning the location of the SNP of interest can be amplified using the polymerase chain reaction. The amplified region of DNA form can then be sequenced using any method known in the art, for example using an automatic nucleic

acid sequencer. The detection of a given SNP can then be performed using hybridization of probes and or using PCR-based amplification methods. Such methods are described in more detail below.

The methods of the present invention may use oligonucleotides useful as primers to amplify specific nucleic acid sequences of the *TFAM* gene, advantageously of the region encompassing a *TFAM* SNP. Such fragments should be of sufficient length to enable specific annealing or hybridization to the nucleic acid sample. The sequences typically will be about 8 to about 44 nucleotides in length. Longer sequences, e.g., from about 14 to about 50, may be advantageous for certain embodiments. The design of primers is well known to one of ordinary skill in the art.

Inventive nucleic acid molecules include nucleic acid molecules having at least 70% identity or homology or similarity with a *TFAM* gene or probes or primers derived therefrom such as at least 75% identity or homology or similarity, preferably at least 80% identity or homology or similarity, more preferably at least 85% identity or homology or similarity such as at least 90% identity or homology or similarity, more preferably at least 95% identity or homology or similarity such as at least 97% identity or homology or similarity. The nucleotide sequence similarity or homology or identity can be determined using the "Align" program of Myers and Miller, ("Optimal Alignments in Linear Space", CABIOS *4*, 11-17, 1988) and available at NCBI. Alternatively or additionally, the terms "similarity" or "identity" or "homology", for instance, with respect to a nucleotide sequence, is intended to indicate a quantitative measure of homology between two sequences. The percent sequence similarity can be calculated as $(N_{ref} - N_{dif})*100/N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence similarity of 75% with the sequence AATCAATC (N_{ref} = 8; N_{dif}=2). Alternatively or additionally, "similarity" with respect to sequences refers to the number of positions with identical nucleotides divided by the number of nucleotides in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman, 1983 PNAS USA 80:726), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics ™ Suite, Intelligenetics Inc. CA).. When RNA sequences are said to be similar, or have a degree of

sequence identity with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence.

A probe or primer can be any stretch of at least 8, preferably at least 10, more preferably at least 12, 13, 14, or 15, such as at least 20, e.g., at least 23 or 25, for instance at least 27 or 30 nucleotides in a *TFAM* gene which are unique to a *TFAM* gene. As to PCR or hybridization primers or probes and optimal lengths therefor, reference is also made to Kajimura et al., GATA 7(4):71-79 (1990).

RNA sequences within the scope of the invention are derived from the DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

The oligonucleotides can be produced by a conventional production process for general oligonucleotides. They can be produced, for example, by a chemical synthesis process or by a microbial process that makes use of a plasmid vector, a phage vector or the like. Further, it is suitable to use a nucleic acid synthesizer.

To label an oligonucleotide with the fluorescent dye, one of conventionally known labeling methods can be used (Tyagi & Kramer (1996) Nature Biotechnology 14: 303-308; Schofield et al. (1997) Appl. and Environ. Microbiol. 63: 1143-1147; Proudnikov & Mirzabekov (1996) Nucl. Acids Res. 24: 4532-4535). Alternatively, the oligonucleotide may be labeled with a radiolabel e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, etc. Well-known labeling methods are described, for example, in Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press. The label is coupled directly or indirectly to a component of the oligonucleotide according to methods well known in the art. Reversed phase chromatography or the like used to provide a nucleic acid probe for use in the present invention can purify the synthesized oligonucleotide labeled with a marker. An advantageous probe form is one labeled with a fluorescent dye at the 3'- or 5'-end and containing G or C as the base at the labeled end. If the 5'-end is labeled and the 3'-end is not labeled, the OH group on the C atom at the 3'-position of the 3'-end ribose or deoxyribose may be modified with a phosphate group or the like although no limitation is imposed in this respect.

During the hybridization of the nucleic acid target with the probes, stringent conditions may be utilized, advantageously along with other stringency affecting conditions, to aid in the hybridization. Detection by differential disruption is particularly advantageous to reduce or eliminate slippage hybridization among probes and target, and to promote more effective hybridization. In yet another aspect, stringency conditions may be varied during the

hybridization complex stability determination so as to more accurately or quickly determine whether a SNP is present in the target sequence.

One method for determining the genotype at the polymorphic gene locus encompasses obtaining a nucleic acid sample, hybridizing the nucleic acid sample with a probe, and disrupting the hybridization to determine the level of disruption energy required wherein the probe has a different disruption energy for one allele as compared to another allele. In one example, there can be a lower disruption energy, e.g., melting temperature, for an allele that harbors a cytosine residue at a polymorphic locus, and a higher required energy for an allele with a different residue at that polymorphic locus. This can be achieved where the probe has 100% homology with one allele (a perfectly matched probe), but has a single mismatch with the alternative allele. Since the perfectly matched probe is bound more tightly to the target DNA than the mis-matched probe, it requires more energy to cause the hybridized probe to dissociate.

In a further step of the above method, a second ("anchor") probe may be used. Generally, the anchor probe is not specific to either allele, but hybridizes regardless of what nucleotide is present at the polymorphic locus. The anchor probe does not affect the disruption energy required to disassociate the hybridization complex but, instead, contains a complementary label for using with the first ("sensor") probe.

Hybridization stability may be influenced by numerous factors, including thermoregulation, chemical regulation, as well as electronic stringency control, either alone or in combination with the other listed factors. Through the use of stringency conditions, in either or both of the target hybridization step or the sensor oligonucleotide stringency step, rapid completion of the process may be achieved. This is desirable to achieve properly indexed hybridization of the target DNA to attain the maximum number of molecules at a test site with an accurate hybridization complex. By way of example, with the use of stringency, the initial hybridization step may be completed in ten minutes or less, more advantageously five minutes or less, and most advantageously two minutes or less. Overall, the analytical process may be completed in less than half an hour.

In one mode, the hybridization complex is labeled and the step of determining the amount of hybridization includes detecting the amounts of labeled hybridization complex at the test sites. The detection device and method may include, but is not limited to, optical imaging, electronic imaging, imaging with a CCD camera, integrated optical imaging, and mass spectrometry. Further, the amount of labeled or unlabeled probe bound to the target may be quantified. Such quantification may include statistical analysis. The labeled portion

of the complex may be the target, the stabilizer, the probe or the hybridization complex in toto. Labeling may be by fluorescent labeling selected from the group of, but not limited to, Cy3, Cy5, Bodipy Texas Red, Bodipy Far Red, Lucifer Yellow, Bodipy 630/650-X, Bodipy R6G-X and 5-CR 6G. Colormetric labeling, bioluminescent labeling and/or chemiluminescent labeling may further accomplish labeling. Labeling further may include energy transfer between molecules in the hybridization complex by perturbation analysis, quenching, electron transport between donor and acceptor molecules, the latter of which may be facilitated by double stranded match hybridization complexes. Optionally, if the hybridization complex is unlabeled, detection may be accomplished by measurement of conductance differential between double stranded and non-double stranded DNA. Further, direct detection may be achieved by porous silicon-based optical interferometry or by mass spectrometry. In using mass spectrometry no fluorescent or other label is necessary. Rather detection is obtained by extremely high levels of mass resolution achieved by direct measurement, for example, by time of flight (TOF) or by electron spray ionization (ESI). Where mass spectrometry is contemplated, probes having a nucleic acid sequence of 50 bases or less are advantageous.

The label may be amplified, and may include, for example, branched or dendritic DNA. If the target DNA is purified, it may be un-amplified or amplified. Further, if the purified target is amplified and the amplification is an exponential method, it may be, for example, PCR amplified DNA or strand displacement amplification (SDA) amplified DNA. Linear methods of DNA amplification such as rolling circle or transcriptional runoff may also be used.

Where it is desired to amplify a fragment of DNA that comprises a SNP according to the present invention, the forward and reverse primers may have contiguous stretches of about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or any other length up to and including about 50 nucleotides in length. The sequences to which the forward and reverse primers anneal are advantageously located on either side of the particular nucleotide position that is substituted in the SNP to be amplified.

A detectable label can be incorporated into a nucleic acid during at least one cycle of an amplification reaction. Spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means can detect such labels. Useful labels in the present invention include fluorescent dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, etc.), enzymes (e.g. horseradish peroxidase, alkaline phosphatase etc.) colorimetric labels such as colloidal gold or colored

glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads. The label is coupled directly or indirectly to a component of the assay according to methods well known in the art. As indicated above, a wide variety of labels are used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions. Non-radioactive labels are often attached by indirect means. Polymerases can also incorporate fluorescent nucleotides during synthesis of nucleic acids.

Reagents allowing the sequencing of reaction products can be utilized herein. For example, chain-terminating nucleotides will often be incorporated into a reaction product during one or more cycles of a reaction. Commercial kits containing the reagents most typically used for these methods of DNA sequencing are available and widely used. PCR exonuclease digestion methods for DNA sequencing can also be used. Many methods of sequencing genomic DNA are known in the art, and any such method can be used, see for example Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press. For example, as described below, a DNA fragment spanning the location of the SNP of interest can amplified using the polymerase chain reaction or some other cyclic polymerase mediated amplification reaction. The amplified region of DNA can then be sequenced using any method known in the art. Advantageously, the nucleic acid sequencing is by automated methods (reviewed by Meldrum, (2000) Genome Res. 10: 1288-303, the disclosure of which is incorporated by reference in its entirety), for example using a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter Instruments, Inc.). Methods for sequencing nucleic acids include, but are not limited to, automated fluorescent DNA sequencing (see, e.g., Watts & MacBeath, (2001) Methods Mol Biol. 167: 153-70 and MacBeath et al. (2001) Methods Mol Biol. 167:119-52), capillary electrophoresis (see, e.g., Bosserhoff et al. (2000) Comb Chem High Throughput Screen. 3: 455-66), DNA sequencing chips (see, e.g., Jain, (2000) Pharmacogenomics. 1: 289-307), mass spectrometry (see, e.g., Yates, (2000) Trends Genet. 16: 5-8), pyrosequencing (see, e.g., Ronaghi, (2001) Genome Res. 11: 3-11), and ultrathin-layer gel electrophoresis (see, e.g., Guttman & Ronai, (2000) Electrophoresis. 21: 3952-64), the disclosures of which are hereby incorporated by reference in their entireties. The sequencing can also be done by a commercial company. Examples of such companies include, but are not limited to, the University of Georgia Molecular Genetics Instrumentation Facility (Athens, Georgia) or SeqWright DNA Technologies Services (Houston, Texas).

A SNP-specific probe can also be used in the detection of the SNP in amplified specific nucleic acid sequences of the target gene, such as the amplified PCR products generated using the primers described above. In certain embodiments, these SNP-specific probes consist of oligonucleotide fragments. Advantageously, the fragments are of sufficient length to provide specific hybridization to the nucleic acid sample. The use of a hybridization probe of between 10 and 50 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 12 bases in length are generally advantageous, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of particular hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having stretches of 16 to 24 nucleotides, or even longer where desired. A tag nucleotide region may be included, as at the 5' end of the primer that may provide a site to which an oligonucleotide sequencing primer may hybridize to facilitate the sequencing of multiple PCR samples.

The probe sequence must span the particular nucleotide position that may be substituted in the particular SNP to be detected. Advantageously, two or more different "allele-specific probes" may be used for analysis of a SNP, a first allele-specific probe for detection of one allele, and a second allele-specific probe for the detection of the alternative allele.

It will be understood that this invention is not limited to the particular primers and probes disclosed herein and is intended to encompass at least nucleic acid sequences that are hybridizable to the nucleotide sequence disclosed herein, the complement or a fragment thereof, or are functional sequence analogs of these sequences. It is also contemplated that a particular trait of an animal may be determined by using a panel of SNPs associated with that trait. Several economically relevant traits may be characterized by the presence or absence of one or more SNPs and by a plurality of SNPs in different genes. One or more panels of SNPs may be used in the methods of the invention to define the phenotypic profile of the subject animal.

Homologs (i.e., nucleic acids derived from other species) or other related sequences (e.g., paralogs) can be obtained under conditions of standard or stringent hybridization conditions with all or a portion of the particular sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

The genetic markers, probes thereof, methods, and kits of the invention are also useful in a breeding program to select for breeding those animals having desirable phenotypes for

various economically important traits, such as improved meat quality and yield, in particular meat tenderness. Continuous selection and breeding of animals, such as livestock, that are at least heterozygous and advantageously homozygous for desirable alleles of the *TFAM* gene polymorphic sites associated with economically relevant traits of growth, feed intake, efficiency and/or carcass merit, would lead to a breed, line, or population having higher numbers of offspring with economically relevant traits of growth, feed intake, efficiency and carcass merit. Thus, the *TFAM* SNPs of the present invention can be used as a selection tool.

Desirable phenotypes include, but are not limited to, feed intake, growth rate, body weight, carcass merit and composition, and milk yield. Specific carcass traits with desirable phenotypes include, but are not limited to, additional carcass value (additional carc value, $), average daily gain (ADG, lb/d), backfat thickness (BFAT, in), calculated live weight (Calc Lv Wt, lb), calculated yield grade (cYG), days on feed (DOF, d), dressing percentage (DP, %), dry matter intake (DMI, lb), dry matter intake per day on feed (DMI per DOF, lb/d), hot carcass weight (HCW, lb), hot carcass weight value (HCW value, $), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), quality grade, less than or equal to select versus greater than or equal to choice (QG, < Se vs, > Ch), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW) and subcutaneous fat depth (SFD).

One aspect of the present invention provides for grouping animals and methods for managing livestock production comprising grouping livestock animals such as cattle according the genotype as defined by panels of SNPs, each panel comprising at least one SNP, one or more of which are in the *TFAM* gene of the present invention. Other SNPs that may be included in panels of SNPs include, but not limited to, SNPs found in the calpastatin gene, *GHR* gene, *FABP4* gene, ghrelin gene, leptin gene, *NPY* gene, *ob* gene, *UASMS1* gene, *UASMS2* gene, *UASMS3* gene and/or the *UCP2* gene. The genetic selection and grouping methods of the present invention can be used in conjunction with other conventional phenotypic grouping methods such as grouping animals by visible characteristics such as weight, frame size, breed traits, and the like. The methods of the present invention provide for producing cattle having improved heritable traits, and can be used to optimize the performance of livestock herds in areas such as breeding, feed intake, carcass/meat quality and milk production. The present invention provides methods of screening livestock to determine those more likely to develop a desired body condition by identifying the presence or absence of one or more gene polymorphisms correlated with meat quality.

As described above, and in the Examples, there are various phenotypic traits with which the SNPs of the present invention may be associated. Each of the phenotypic and genetic traits can be tested using the methods described in the Examples, or using any suitable methods known in the art. Using the methods of the invention, a farmer, or feedlot operator, or the like, can group cattle according to each animal's genetic propensity for a desired trait such as growth rate, feed intake or feeding behavior, as determined by SNP genotype. The cattle are tested to determine homozygosity or heterozygosity with respect to the SNP alleles of one or more genes so that they can be grouped such that each pen contains cattle with like genotypes. Each pen of animals is then fed and otherwise maintained in a manner and for a time determined by the feedlot operator, and then slaughtered.

The individual genotypic data derived from a panel or panels of SNPs for each animal or a herd of animals can be recorded and associated with various other data of the animal, e.g. health information, parentage, husbandry conditions, vaccination history, herd records, subsequent food safety data and the like. Such information can be forwarded to a government agency to provide traceability of an animal or meat product, or it may serve as the basis for breeding, feeding and marketing information. Once the data has or has not been associated with other data, the data is stored in an accessible database, such as, but not limited to, a computer database or a microchip implanted in the animal. The methods of the invention may provide an analysis of the input data that may be compared with parameters desired by the operator. These parameters include, but are not limited to, such as breeding goals, egg laying targets, vaccination levels of a herd. If the performance or properties of the animals deviates from the desired goals, the computer-based methods may trigger an alert to allow the operator to adjust vaccination doses, medications, feed etc accordingly.

The results of the analysis provide data that are associated with the individual animal or to the herd, in whole or in part, from which the sample was taken. The data are then kept in an accessible database, and may or may not be associated with other data from that particular individual or from other animals.

Data obtained from individual animals may be stored in a database that can be integrated or associated with and/or cross-matched to other databases. The database along with the associated data allows information about the individual animal to be known through every stage of the animal's life, i.e., from conception to consumption of the animal product.

The accumulated data and the combination of the genetic data with other types of data of the animal provides access to information about parentage, identification of herd, health information including vaccinations, exposure to diseases, feedlot location, diet and ownership

changes. Information such as dates and results of diagnostic or routine tests are easily stored and attainable. Such information would be especially valuable to companies, particularly those who seek superior breeding lines.

Each animal may be provided with a unique identifier. The animal can be tagged, as in traditional tracing programs or have implant computer chips providing stored and readable data or provided with any other identification method which associates the animal with its unique identifier.

The database containing the SNP-based genotype results for each animal or the data for each animal can be associated or linked to other databases containing data, for example, which may be helpful in selecting traits for grouping or sub-grouping of an animal. For example, and not for limitation, data pertaining to animals having particular vaccination or medication protocols, can optionally be further linked with data pertaining to animals having food from certain food sources. The ability to refine a group of animals is limited only by the traits sought and the databases containing information related to those traits.

Databases that can usefully be associated with the methods of the invention include, but are not limited to, specific or general scientific data. Specific data includes, but is not limited to, breeding lines, sires, dames, and the like, other animals' genotypes, including whether or not other specific animals possess specific genes, including transgenic genetic elements, location of animals which share similar or identical genetic characteristics, and the like. General data includes, but is not limited to, scientific data such as which genes encode for specific quality characteristics, breed association data, feed data, breeding trends, and the like.

One method of the present invention includes providing the animal owner or customer with sample collection equipment, such as swabs and tags useful for collecting samples from which genetic data may be obtained. Advantageously, the packaging is encoded with a bar code label. The tags are encoded with the same identifying indicia, advantageously with a matching bar code label. Optionally, the packaging contains means for sending the tags to a laboratory for analysis. The optional packaging is also encoded with identifying indicia, advantageously with a bar code label.

The method optionally includes a system wherein a database account is established upon ordering the sampling equipment. The database account identifier corresponds to the identifying indicia of the tags and the packaging. Upon shipment of the sampling equipment in fulfillment of the order, the identifying indicia are recorded in a database. Advantageously, the identifier is a bar code label which is scanned when the tags are sent.

When the tags are returned to the testing facility, the identifier is again recorded and matched to the information previously recorded in the database upon shipment of the vial to the customer. Once the genotyping is completed, the information is recorded in the database and coded with the unique identifier. Test results are also provided to the customer or animal owner.

The data stored in the genotype database can be integrated with or compared to other data or databases for the purpose of identifying animals based on genetic propensities. Other data or databases include, but are not limited to, those containing information related to SNP-based DNA testing, vaccination, Sure Health pre-conditioning program, estrus and pregnancy results, hormone levels, food safety/contamination, somatic cell counts, mastitis occurrence, diagnostic test results, milk protein levels, milk fat, vaccine status, health records, mineral levels, trace mineral levels, herd performance, and the like.

The present invention, therefore, encompasses computer-assisted methods for tracking the breeding and veterinary histories of livestock animals encompassing using a computer-based system comprising a programmed computer comprising a processor, a data storage system, an input device and an output device, and comprising the steps of generating a profile of a livestock animal by inputting into the programmed computer through the input device genotype data of the animal, wherein the genotype may be defined by a panel of at least two single nucleotide polymorphisms that predict at least one physical trait of the animal, inputting into the programmed computer through the input device welfare data of the animal, correlating the inputted welfare data with the phenotypic profile of the animal using the processor and the data storage system, and outputting a profile of the animal or group of animals to the output device.

The databases and the analysis thereof will be accessible to those to whom access has been provided. Access can be provided through rights to access or by subscription to specific portions of the data. For example, the database can be accessed by owners of the animal, the test site, the entity providing the sample to the test site, feedlot personnel, and veterinarians. The data can be provided in any form such as by accessing a website, fax, email, mailed correspondence, automated telephone, or other methods for communication. These data can also be encoded on a portable storage device, such as a microchip, that can be implanted in the animal. Advantageously, information can be read and new information added without removing the microchip from the animal.

The present invention comprises systems for performing the methods disclosed herein. Such systems comprise devices, such as computers, internet connections, servers, and

storage devices for data. The present invention also provides for a method of transmitting data comprising transmission of information from such methods herein discussed or steps thereof, e.g., via telecommunication, telephone, video conference, mass communication, e.g., presentation such as a computer presentation (e.g., POWERPOINT), internet, email, documentary communication such as computer programs (e.g., WORD) and the like.

Systems of the present invention may comprise a data collection module, which includes a data collector to collect data from an animal or embryo and transmit the data to a data analysis module, a network interface for receiving data from the data analysis module, and optionally further adapted to combine multiple data from one or more individual animals, and to transmit the data via a network to other sites, or to a storage device.

More particularly, systems of the present invention comprise a data collection module, a data analysis module, a network interface for receiving data from the data analysis module, and optionally further adapted to combine multiple data from one or more individual animals, and to transmit the data via a network to other sites, and/or a storage device. For example, the data collected by the data collection module leads to a determination of the absence or presence of a SNP of a gene in the animal or embryo, and for example, such data is transmitted when the feeding regimen of the animal is planned.

In one embodiment where the data is implanted on a microchip on a particular animal, the farmer can optimize the efficiency of managing the herd because the farmer is able to identify the genetic predispositions of an individual animal as well as past, present and future treatments (e.g., vaccinations and veterinarian visits). The invention, therefore also provides for accessing other databases, e.g., herd data relating to genetic tests and data performed by others, by datalinks to other sites. Therefore, data from other databases can be transmitted to the central database of the present invention via a network interface for receiving data from the data analysis module of the other databases.

The invention relates to a computer system and a computer readable media for compiling data on an animal, the system containing inputted data on that animal, such as but not limited to, vaccination and medication histories, DNA testing, thyroglobulin testing, leptin, MMI (Meta Morphix Inc.), bovine spongiform encephalopathy (BSE) diagnosis, brucellosis vaccination, FMD (foot and mouth disease) vaccination, BVD (bovine viral diarrhea) vaccination, Sure Health pre-conditioning program, estrus and pregnancy results, tuberculosis, hormone levels, food safety/contamination, somatic cell counts, mastitis occurrence, diagnostic test results, milk protein levels, milk fat, vaccine status, health records, mineral levels, trace mineral levels, herd performance, and the like. The data of the animal

can also include prior treatments as well as suggested tailored treatment depending on the genetic predisposition of that animal toward a particular disease.

The invention also provides for a computer-assisted method for improving animal production comprising using a computer system, e.g., a programmed computer comprising a processor, a data storage system, an input device and an output device, the steps of inputting into the programmed computer through the input device data comprising a breeding, veterinary, medication, diagnostic data and the like of an animal, correlating a physical characteristic predicted by the genotype using the processor and the data storage system, outputting to the output device the physical characteristic correlated to the genotype, and feeding the animal a diet based upon the physical characteristic, thereby improving livestock production.

The invention further provides for a computer-assisted method for optimizing efficiency of feedlots for livestock comprising using a computer system, e.g., a programmed computer comprising a processor, a data storage system, an input device and an output device, and the steps of inputting into the programmed computer through the input device data comprising a breeding, veterinary history of an animal, correlating the breeding, veterinary histories using the processor and the data storage system, outputting to the output device the physical characteristic correlated to the genotype, and feeding the animal a diet based upon the physical characteristic, thereby optimizing efficiency of feedlots for livestock.

The invention further comprehends methods of doing business by providing access to such computer readable media and/or computer systems and/or data collected from animals to users; e.g., the media and/or sequence data can be accessible to a user, for instance on a subscription basis, via the Internet or a global communication/computer network; or, the computer system can be available to a user, on a subscription basis.

In one embodiment, the invention provides for a computer system for managing livestock comprising physical characteristics and databases corresponding to one or more animals. In another embodiment, the invention provides for computer readable media for managing livestock comprising physical characteristics and veterinary histories corresponding to one or more animals. The invention further provides methods of doing business for managing livestock comprising providing to a user the computer system and media described above or physical characteristics and veterinary histories corresponding to one or more animals. The invention further encompasses methods of transmitting information obtained in any method or step thereof described herein or any information

described herein, e.g., via telecommunications, telephone, mass communications, mass media, presentations, internet, email, etc.

The invention further encompasses kits useful for screening nucleic acid isolated from one or more bovine individuals for allelic variation of any one of the mitochondrial transcription factor genes, and in particular for any of the SNPs described herein, wherein the kits may comprise at least one oligonucleotide selectively hybridizing to a nucleic acid comprising any one of the one or more of which are *TFAM* sequences described herein and instructions for using the oligonucleotide to detect variation in the nucleotide corresponding to the SNP of the isolated nucleic acid.

One embodiment of this aspect of the invention provides an oligonucleotide that specifically hybridizes to the isolated nucleic acid molecule of this aspect of the invention, and wherein the oligonucleotide hybridizes to a portion of the isolated nucleic acid molecule comprising any one of the polymorphic sites in the *TFAM* sequences described herein.

Another embodiment of the invention is an oligonucleotide that specifically hybridizes under high stringency conditions to any one of the polymorphic sites of the *TFAM* gene, wherein the oligonucleotide is between about 18 nucleotides and about 50 nucleotides.

In another embodiment of the invention, the oligonucleotide comprises a central nucleotide specifically hybridizing with a *TFAM* gene polymorphic site of the portion of the nucleic acid molecule.

Another aspect of the invention is a method of identifying a *TFAM* polymorphism in a nucleic acid sample comprising isolating a nucleic acid molecule encoding *TFAM* or a fragment thereof and determining the nucleotide at the polymorphic site.

Another aspect of the invention is a method of screening cattle to determine those bovines more likely to exhibit a biological difference in meat quality comprising the steps of obtaining a sample of genetic material from a bovine; and assaying for the presence of a genotype in the bovine which is associated with meat quality, the genotype characterized by a polymorphism in any one of the mitochondrial transcription factor genes.

In other embodiments of this aspect of the invention, the step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, minisequencing, MALD-TOF, SINE, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).

In various embodiments of the invention, the method may further comprise the step of amplifying a region of the *TFAM* gene or a portion thereof that contains the polymorphism.

In other embodiments of the invention, the amplification may include the step of selecting a forward and a reverse sequence primer capable of amplifying a region of the *TFAM* gene.

Another aspect of the invention is a computer-assisted method for predicting which livestock animals possess a biological difference in meat quality comprising: using a computer system, e.g., a programmed computer comprising a processor, a data storage system, an input device and an output device, the steps of: (a) inputting into the programmed computer through the input device data comprising a *TFAM* genotype of an animal, (b) correlating growth, feed intake, efficiency or carcass merit quality predicted by the *TFAM* genotype using the processor and the data storage system and (c) outputting to the output device the meat quality correlated to the *TFAM* genotype, thereby predicting which livestock animals possess a particular growth level, feed intake, efficiency or carcass merit quality.

Yet another aspect of the invention is a method of doing business for managing livestock comprising providing to a user computer system for managing livestock comprising physical characteristics and genotypes corresponding to one or more animals or a computer readable media for managing livestock comprising physical characteristics and genotypes corresponding to one or more animals or physical characteristics and genotypes corresponding to one or more animals.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Example 1

This Example provides DNA sequences, genetic polymorphisms and significant associations with marbling and subcutaneous fat depth in Wagyu x Limousin F_2 crosses for the bovine mitochondrial transcription factor A (*TFAM*) gene.

Mitochondrial transcription factor A (*TFAM*), a nucleus-encoded protein plays an important role in initiation of transcription and replication of mitochondrial DNA (mtDNA). Decreased expression in nuclear-encoded mitochondrial genes has been associated with onset of obesity in mice. Therefore, it was hypothesized that genetic variants in *TFAM* gene influence mitochondrial biogenesis consequently affecting body fat deposition and energy metabolism. In the present study, both cDNA (2259 bp) and genomic DNA (16,666 bp) sequences were generated for the bovine *TFAM* gene using a combination of *in silico* cloning with targeted region PCR amplification. Alignment of both cDNA and genomic sequences led to the determination of genomic organization and characterization of the promoter region of the bovine *TFAM* gene. Unfortunately, no polymorphisms were detected in the coding

region, but two closely linked A/C and C/T single nucleotide polymorphisms (SNPs) were found in the bovine *TFAM* promoter. These two SNPs were genotyped on 237 F_2 Wagyu x Limousin animals with recorded phenotypes for marbling and subcutaneous fat depth (SFD). Statistical analysis demonstrated that both SNPs were associated with marbling ($P = 0.0153$ for A/C and $P = 0.0026$ for C/T) and SFD ($P = 0.0200$ for A/C and $P = 0.0039$ for C/T), respectively. A search for transcriptional regulatory elements using MatInspector indicated that both SNPs lead to a gain/loss of six putative binding sites for genes relevant to fat deposition and energy metabolism. Compared with previous reports on thyroglobulin, leptin and diacylglycerol O-acyltranferase genes, the *TFAM* gene had the greatest effects on both marbling and SFD in this population, indicating its potential as a new target for marker-assisted selection in the beef industry.

Mitochondrial transcription factor A (TFAM), a member of a high mobility group protein family and the first-identified mitochondrial transcription factor (Fisher and Clayton, 1988), is essential for maintenance and biogenesis of mtDNA. First, TFAM plays a histone-like role in mitochondria, as it is tightly associated with mtDNA as a main component of the nucleoid (Kanki et al., 2004a). Evidence has shown that one molecule of mtDNA is packed with ~900 molecules of TFAM on average (Alam et al., 2003), which makes mtDNA no longer naked. Second, TFAM regulates mtDNA copy number in mammals. Investigation using a combination of mice with TFAM overexpression and TFAM knockout demonstrated that mtDNA copy number is directly proportional to the total TFAM protein level in mouse embryos (Ekstrand et al., 2004). RNA interference of the endogenous TFAM expression in HeLa cells also indicated that the mtDNA amount is correlated in parallel with the amount of TFAM (Kanki et al., 2004b). Third, TFAM stimulates transcription of mtDNA. The TFAM protein possesses two tandem high mobility group domains, which makes TFAM bind, unwind and bend DNA without sequence specificity and thus facilitate transcription initiation of mtDNA (Gaspari et al., 2004). Evidence has shown that import of wt-TFAM into liver mitochondria from hypothyroid rats increased RNA synthesis significantly up to 4-fold (Garstka et al., 2003).

It has been known for many years that adipose tissue plays a central rule in regulation and manipulation of energy metabolisms through the storage and turnover of triglycerides and through the secretion of factors that affect satiety and fuel utilization. However, many key aspects of adipogenesis are accompanied by stimulation of mitochondrial biogenesis (Wilson-Fritch et al., 2003). For example, the major site of fatty acid ß-oxidation occurs in mitochondria (Reichert and Neupert, 2004), which may provide key intermediates for the

synthesis of triglycerides via the action of pyruvate carboxylase (Owen et al., 2002). In addition, a relatively large mitochondrial mass are needed to generate acetyl-CoA for fatty acid activation prior to esterification into triglycerides. All these studies demonstrated the essential role and function of mitochondria in lipid metabolism.

To further explore the mechanism of mitochondria involved in adipogenesis, Wilson-Fritch and colleagues (2003 and 2004) studied the 3T3-L1 cell (representative of white adipocytes) differentiation by using both proteomic and genomic approaches. Proteomic analysis revealed a 20- to 30-fold increase in the concentration of numerous mitochondrial proteins, while genomic analysis with gene expression profiling using Affymetrix GeneChips detected a statistically significant increase in the expression of many nucleus-encoded mitochondrial genes during adipogenesis. In particular, the authors found a profound decrease of approximately 50% in the levels of transcripts for nuclear-encoded mitochondrial genes accompanying the onset of obesity (Wilson-Fritch et al., 2004). As *TFAM* is one of the nuclear-encoded mitochondrial genes, it was hypothesized that it plays an important role in lipogenesis or fat deposition via its role in mitochondrial biogenesis. Here, evidence is presented to support the hypothesis by reporting significant associations of bovine *TFAM* promoter polymorphisms with marbling scores and SFD measurements in Waygu x Limousin crossbreds.

A F_1 generation of a Wagyu x Limousin cross was developed at Washington State University and transferred to the Fort Keogh Livestock and Range Research Laboratory, ARS, USDA in the autumn of 1998, including 6 F_1 bulls and 113 dams. Inter se mating of these F_1 animals produced 71 F_2 progeny in 2000, 90 in 2001 and 109 in 2003, respectively. Each calf was weighed within 24 h after birth and again at weaning when the calves averaged approximately 180 d of age. After weaning, the calves were returned to native range pastures and were supplemented with 0.7 kg per calf per day of both barley cake and alfalfa pellets. In mid-January, the calves were moved from the range and were fed silage and chopped hay to achieve anticipated gains of 0.5 to 0.8 kg per day. They were then placed on finishing diet for approximately 150 days followed by slaughter. Growth rate and carcass and meat quality data were collected on all F_2 calves. Marbling scores varied from 4 = Slight^0 to 9.5 = Moderately Abundant^{50} (SD = 1.00) and SFD measurements ranged from 0.1 to 1.3 inches (SD = 0.18) in this F_2 population. Marbling was a subjective measure of the amount of intramuscular fat in the *longissimus* muscle based on USDA standards (http://www.ams.usda.gov/). SFD was measured at the 12-13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine

bone end. DNA was extracted from blood samples. Based on the availability of both data and DNA samples, 246 observations were used in the current study.

Unfortunately, both cDNA and genomic DNA sequences were not available for the bovine *TFAM* gene when the project was started. However, the bovine genome mapping project has advanced significantly in recent years. In particular, more than 500,000 bovine ESTs (expressed sequence tags) (http://www.ncbi.nlm.nih.gov/) and 3X bovine genome sequences (http://www.hgsc.bcm.tmc.edu/projects/bovine/) have been released to the public databases. Therefore, a combination of an *in silico* comparative cloning with a PCR target cloning approach was developed and used to determine both cDNA and genomic DNA sequences of the bovine gene (FIG. 1). The procedure included three steps: 1), BLAST searches against the public databases using a full-length cDNA sequence of the human *TFAM* gene as a reference to retrieve all bovine sequences that are orthologous to the human gene; 2), annotation of both ESTs and genomic DNA sequences in order to design primers for the target region amplification to close gaps if there are any; and 3); alignment of cDNA sequences and genomic DNA sequences to determine the full-length cDNA sequence and genomic organization of the bovine *TFAM* gene.

Two pairs of primers were designed to close two gaps for the genomic DNA sequence of the bovine *TFAM* gene (Table 1). PCR reactions were performed using 25 ng of bovine genomic DNA as template in a final volume of 10 μL containing 12.5 ng of each primer, 200 μM dNTPs, 1.5 - 3 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl and 0.2U of Platinum Taq polymerase (Invitrogen, Carlsbad, CA). The PCR conditions were carried out as follows: 94°C for 2 min, 32 cycles of 94°C for 30 sec, 63°C for 30 sec and 72°C for 30 sec, followed by a further 5 min extension at 72°C. PCR products were then examined by electrophoresis through a 1.5% agarose gel with 1X TBE buffer to determine the quality and quantity for DNA sequencing. Sequencing was performed on ABI 3730 sequencer in the Laboratory for Biotechnology and Bioanalysis (Washington State University). Sequences of these two PCR-amplified products spanning the gap regions and three contigs of genome sequences derived from the cattle genome sequence project were then assembled to form a complete genomic DNA sequence for the bovine *TFAM* gene.

Table 1. Primers designed for genomic gap closing and mutation detection in the bovine *TFAM* gene.

Target region	Primer sequences (5'-3')	Size in bp	Annealing Tm	SEQ ID NO:
Promoter	Forward: GTTGTTGCAGAAATCAGCTAAAATG	801	61°C	4
	Reverse: CATCCACTGAGACTATCGCTGACCT			5
Exon 1	Forward: CGCCTCCTAGCTAATCGGAAGTTAG	405	61°C	6
	Reverse: GTCGGAATCACAGGGCTAAGTCAGT			7
Exon 2	Forward: TTCCCCTGGATAGGACAGGATTTTA	421	61°C	8
	Reverse: TACAGGCCATCACACAGAATGGTTA			9
Exon 3	Forward: GAGCTAATGGATTATTCTTTCCTGA	407	57°C	10
	Reverse: ATGTGTTATCCAAGGTGAAGGTCTA			11
Exon 4	Forward: TTATAAGTGGGATTTCAGAGTGCAT	459	57°C	12
	Reverse: AACTGAAGTCATTCTCTACCACGTC			13
Exon 5	Forward: AACAATCGCATACTCATAATGTTCA	392	57°C	14
	Reverse: TGGTAAGAAAAAGGATTTTTAGGTC			15
Intron 5 gap	Forward: GCACAAACAAAGGAACCATCAA	222	57°C	16
	Reverse: TTCCCTGACAATGATGTTGAGC			17
Exon 6	Forward: TACAGCTCAGAGTTTTGAGGAGTCT	408	57°C	18
	Reverse: CACTAAGTTACGAGGGACACTGTTT			19
Intron 6 gap& Exon 7	Forward: TGAAAACTGGAAAAATCTCTCTA	736	57°C	20
	Reverse: AACAGCTTCCGGTATTGAGACCT			21

Primers were designed to target the promoter region and all coding regions in order to screen genetic polymorphisms in the bovine *TFAM* gene (Table 1). Four DNA pools were formed, one from all six F_1 bulls, one from 30 randomly selected F_1 dams, one from 30 F_2 high marbling progeny and one from 30 F_2 low marbling progeny. PCR products for each pair of primers were amplified on these four DNA pools and directly sequenced on ABI 3730 sequencer as described above. Nucleotide polymorphisms were identified by comparison of

sequence patterns among these four DNA pools. Unfortunately, no polymorphisms were detected in the coding sequences, but two SNPs, i.e., C/A substitution and C/T substitution were found in the bovine *TFAM* promoter region.

These two SNPs in the bovine promoter region were then genotyped in Wagyu X Limousin F_2 animals that have both DNA samples and performance data for marbling score and SFD measurements. Using the PCR-RFLP (restriction fragment length polymorphism) approach, these two mutations were revealed by digestion at 37°C for three hours of PCR amplicons with 2U of *Hae*III for the C/A substitution and 2U of *Dpn*I for the C/T substitution, followed by analysis on 4% agarose gels. The phenotypic data for marbling scores and SFD measurements were adjusted for effects of year, gender, and age at harvest (linear) before assessing the effects of the genotypes using the GLM (general linear model) procedure of SAS v9.1 (SAS institute Inc., Gary, NC) to assess the effects of contemporary group and genotype at the *TFAM* gene locus.

The BLAST searches using the human *TFAM* cDNA (NM_003201) as a reference retrieved eight bovine orthologous EST sequences from the ESTs_others database at National Center for Biotechnology Informatics (NCBI) and three genomic contigs from the cattle 3X genome assembly at Baylor College Medicine. Three ESTs (DN286575, DN285251 and CN793484) were chosen to form an initial consensus cDNA sequence of the bovine *TFAM* gene, but they left a gap in the 3'UTR (untranslated region) (FIG. 1). However, initial alignment of these EST sequences with the genomic DNA sequence revealed that the cDNA sequence gap could be easily closed using the genomic DNA sequence corresponding to the 3'UTR region. The total length of the assembled mRNA sequence is 2,259 bp for the bovine *TFAM* gene. Three genomic DNA contigs (contig45319, contig729099 and contig138856) apparently had no overlap. Orientating these three genomic contigs to a 5' – 3' direction corresponding to the mRNA sequence made it possible to design primers to close two gaps among them (FIG. 1). Two PCR products of 222 bp and 736 bp (Table 1) were amplified and sequenced. Assembling of the three genomic contigs and these two PCR products made it possible to form a 16,666 bp genomic DNA sequence for the bovine *TFAM* gene (FIG. 1).

All eight bovine ESTs that are orthologous to the human *TFAM* gene are 99 - 100% identical to the assembled consensus mRNA sequence. The putative complete coding sequence of the bovine *TFAM* gene is 741 bp in length, which is identical to that in human (NM_003201, D'Errico et al., Gene 362, 125-132 (2005)), but 6 bp and 9 bp longer than that in rat (NM_031326, Piantadosi and Suliman, J. Biol. Chem. 281 (1), 324-333 (2006)) and mouse (NM_009360, Noack et al., Biochim. Biophys. Acta 1760 (2), 141-150 (2006)), and

48 bp shorter than that in chicken (NM_204100, Caldwell et al., Genome Biol. 6 (1), R6 (2005)), respectively. The translated amino acid sequence encoded by the bovine *TFAM* gene showed 91% identity with pig (AY923074), 71% with human (NM_003201), 65% with rat (NM_031326), 63% with mouse (NM_009360), and 43% with chicken (NM_204100), respectively. The overall structure of the bovine *TFAM* gene was determined by comparing the genomic DNA sequence with the complete cDNA sequence determined in the study (FIG. 1). Like that in human, mouse, rat and chicken, the genomic organization of the bovine *TFAM* gene consists of seven exons and six introns (FIG. 1).

It is estimated that the bovine genome is similar in size to the genomes of humans and other mammals, containing approximately 3 billion DNA base pairs. Sequencing of the bovine genome began in December 2003 and the initial assembly based on 3.3-fold coverage of the bovine genome was released on October 6, 2004, which can now be accessed through GenBank (www.ncbi.nih.gov/Genbank) at NCBI. In addition, more than 500,000 bovine ESTs have also been released to the public and can be accessed through GenBank at NCBI. Both bovine ESTs and genome sequences provide us valuable resources to revolutionize genome research in cattle. In the current study, a tool that combines an *in silico* comparative cloning with a PCR target cloning approach was developed and used to clone both cDNA and genomic DNA sequences of the bovine *TFAM* gene. This approach is very straightforward, simple, fast and inexpensive. Therefore, this approach may serve as one of the model tools in identifying, mapping, and understanding the function of genes in cattle, which will further advance basic biology research.

FIG. 2 shows the nucleotide sequences for the 5'-up-stream region and the entire exon 1 of the bovine *TFAM* gene. Analysis using MatInspector program (Quandt et al., 1995) revealed one potential nuclear respiratory factor 1 (NRF1) and one stimulating protein 1 (SP1) binding site in the bovine *TFAM* promoter region (FIG. 2). However, the bovine *TFAM* promoter lacks the putative binding sites for nuclear respiratory factor 2 (NRF2). In the human *TFAM* promoter region, both NRF1 and NRF2 binding sites were found, while only NRF2 binding sites existed in the rat and mouse *TFAM* promoters (Scarpulla, 2002). All *TFAM* promoters in human, rat and mouse have the SP1 binding sites. NRF1, NRF2 and SP1 are the most prevalent factors associated with respiratory genes (Scarpulla, 2002). In addition to the NRF1 and SP1 binding sites, the bovine *TFAM* promoter contains a provisional transcriptional repressor binding site, which binds to elements found predominantly in genes that participate in lipid metabolism (FIG. 2). Although it is unclear

whether the CpG islands are methylated in vivo, many potential methylated CpG loci are present in the bovine *TFAM* promoter (FIG. 2).

Very interestingly, the bovine *TFAM* promoter may be one of the few promoters that contain naturally occurring AUG codon upstream from the normal translational start site (FIG. 2). This extra AUG codon was also confirmed by sequencing with primers spanning partial promoter, entire exon 1 and partial intron 1 (see description below) in the study. The Kozak's general rule is that in most cases the AUG codon nearest the 5'end is the unique site of initiation of translation, because this "position effect" is seen in cases where a mutation creates an AUG codon upstream from the normal start site and translation shifts to the upstream site (Kozak, 2002). However, this first rule can be ruled out when the 5' proximal AUG triplet is followed shortly by a terminator codon, which makes the reinitiation at a downstream AUG codon possible (Kozak, 1995). It is observed that this extra AUG codon in the bovine *TFAM* promoter is not in frame (FIG. 2). If it did translate, it would just generate a peptide of 12 amino acids as MQWRFSGAYGAC (SEQ ID NO: 22). Whether and how this 5' proximal AUG triplet interferes with normal translation remains unknown. Therefore, the bovine *TFAM* gene could be a natural model gene for investigation of mechanisms involved in translation initiation of mammalian genes.

A total of eight pairs of primers were designed and used to screen genetic polymorphisms in the bovine *TFAM* gene. One pair of primers targets the promoter region, and the remaining primers amplify seven exons with a pair of primers per exon. However, the last pair of primers was used for both gap closing and exon 7 amplification (Table 1). In order to have each exon region completely amplified and sequenced, at least 100 bp of sequences from each flanking side were included in the products. No polymorphisms were found in all coding sequences of the bovine *TFAM* gene, even though the reference population includes two divergently bred breeds of cattle: an Asian origin Wagyu and a European origin Limousin, which have characteristics that are quite different from each other. However, two SNPs were detected in the promoter region (FIG. 2 and FIG. 3). These two SNPs are located just 9 bp apart, one C/A substitution and another C/T substitution. Both SNPs were revealed by digestion with *Hae*III and *Dpn*II, respectively on a fragment of 801 bp (FIG. 4).

As the fragment possesses three *Hae*III restriction sites, digestion yields two invariable bands of 152 bp and 462 bp, and one 187 bp band, which can be, depending on the nucleotide at position -1220 (FIG. 2), further cleaved in two 83 bp and 104 bp bands (FIG. 4). Therefore, homozygous animals with A allele have two *Hae*III sites, and reveal after

complete digestion three bands: 152 bp, 187 bp and 462 bp, homozygous animals with C allele have gained an additional *Hae*III site at this position and result in four bands (83 bp, 104 bp, 152 bp and 462 bp) after complete digestion. However, heterozygous animals showed five bands after *Hae*III digestion (FIG. 4). These two common *Hae*III sites were considered as internal controls of the enzyme digestion.

In comparison, the fragment contains four *Dpn*II restriction sites, including one polymorphic site. Therefore, digestion with *DpnII* yields three invariable bands of 55 bp, 68 bp and 135 bp, and three polymorphic bands of 241 bp, 302 bp and 543 bp, respectively (FIG. 4). Homozygous animals with T allele have all four *Dpn*II sites, and reveal after complete digestion five bands: 55 bp, 68bp, 135 bp, 241 bp and 302 bp, while homozygous animals with C allele have lost a *Dpn*II site at position -1212 (FIG. 2) and result in four bands (55 bp, 68 bp, 135 bp and 543 bp) after complete digestion. However, heterozygous animals showed six bands after *Dpn*II digestion (FIG. 4). These three common *Dpn*II sites also served as internal controls of the enzyme digestion.

Genotyping 237 F_2 animals for both C/A and C/T SNPs, revealed 75 homozygous CC animals, 45 homozygous AA and 117 heterozygous CA animals for the former SNP and 84 homozygous CC animals, 33 homozygous TT and 120 heterozygous CT animals for the latter SNP (Table 2). For C/A substitution, the frequencies of allele C and allele A in the population were 0.56 and 0.44, respectively. The frequency of allele C slightly increased to 0.61 for the C/T substitution. However, both genotype distributions were in Hardy-Weinberg equilibrium.

General linear model analysis clearly indicated that the effect of genotype at either SNP reached statistical significance (for C/A substitution, P=0.0019 for marbling score and P=0.0200 for SFD measurement; and for C/T substitution, P=0.0011 for marbling score and P=0.0039 for SFD measurement) (Table 2). For C/A substitution, the cattle with the homozygote (CC) genotype had an additional 0.047 inches of subcutaneous fat and 0.482 score of marbling compared to the AA homozygotes ($P<0.05$). However, the differences between two homozygotes CC and TT were further enlarged for the C/T substitution. Subcutaneous fat thickness was 0.073 inches thicker and marbling score was 0.634 higher in cattle with the homozygote (CC) genotype than the TT homozygotes ($P<0.05$) (Table 2).

Only five haplotypes between these two promoter polymorphisms were observed in 237 Wagyu x Limousin F_2 animals, including 75 CCCC, 108 CACT, 33 AATT, 12 AACT and 9 CACC, respectively. Due to relatively few samples, both haplotypes AACT and CACC were excluded in further statistical analysis. As indicated in Table 2, haplotype had

significant effects on both marbling and SFD in the reference population (P = 0.0004 for marbling and P = 0.0029 for SFD). Marbling score was 0.655 different between CCCC and AATT animals and 0.518 different between CCCC and CACT animals (P < 0.05). For SFD measurements, the cattle with CCCC haplotype had an additional 0.079 and 0.073 inches of subcutaneous fat compared to the CACT and AATT animals (P < 0.05), respectively. The CCCC haplotype seems to be associated with an increase of the whole body fat deposition in cattle.

Table 2. Associations of the bovine *TFAM* promoter SNPs with marbling and SFD in Waygu x Limousin F_2 crosses*.

SNP	Genotype	No. of animals	Marbling (score)		SFD (in inch)	
			Mean ± S.E.	P value	Mean ± S.E.	P value
C/A	CC	75	6.245 ± 0.115^a	0.0019	0.441 ± 0.018^a	0.0200
	CA	117	5.748 ± 0.094^b		0.376 ± 0.015^b	
	AA	45	5.763 ± 0.147^b		0.394 ± 0.023^b	
C/T	CC	84	6.220 ± 0.108^a	0.0011	0.447 ± 0.017^a	0.0039
	CT	120	5.822 ± 0.091^b		0.375 ± 0.015^b	
	TT	33	5.586 ± 0.172^b		0.374 ± 0.027^b	
Both	CCCC	75	6.250 ± 0.113^a	0.0004	0.444 ± 0.018^a	0.0029
	CACT	108	5.732 ± 0.097^b		0.365 ± 0.015^b	
	AATT	33	5.595 ± 0.171^b		0.371 ± 0.027^b	

* Means within a column with different superscripts are significantly different (P<0.05).

Previous efforts have identified candidate genes responsible for marbling and/or SFD in beef. Barendse and colleagues (1997) identified a TG5 polymorphism that occurs in the 5' promotor region of the thyroglobulin (*TG*) gene. This marker had a genotypic association with marbling score in long-fed cattle. Leptin is a 16-kilodalton protein produced by the obesity (*ob*) gene. Mutations in the leptin (*LEP*) gene cause beef cattle to reach slaughter weight sooner and develop more marbling in the carcass (Buchanan et al., 2002). A nonconservative K232A substitution in the *DGAT1* (diacylglycerol O-acyltransferase) gene has been shown to affect intramuscular fat deposition (marbling) in beef (Thaller *et al.* 2003). Genotyping a C/T SNP in the *TG* gene, a C/T mutation in the *LEP* gene and an A/C polymorphism in the *DGAT1* gene were also performed in this Waygu x Limousin cross population (De et al., 2004 and Wu et al., 2005, in press). Analysis of variance using a generalized linear model did not show any significant differences among genotypes in *LEP* gene. However, the *DGAT1* gene had a significant additive effect on SFD ($P = 0.036$), while the *TG* gene showed a dominant effect on marbling that approached significance ($P = 0.061$).

De and colleagues (2004) observed that in the *TG* gene, the genotype differences between homozygotes CC and TT were -0.074±0.093 score for marbling and -0.002±0.015 inches for SFD (P>0.05 for both traits), while in the *DGAT1* gene, AA homozygous animals

were superior to CC homozygous animals by 0.092±0.095 score for marbling (P>0.05) and 0.032±0.015 inches for SFD (P<0.05), respectively. In the *LEP* gene, the same FIGs for genotype differences between CC and TT animals were 0.075±0.116 score for marbling and 0.019±0.018 inches for SFD (P>0.05 for both traits), respectively. Obviously, the current study on the bovine *TFAM* gene indicated that the genotype differences between two homozygotes at either position exceeded any differences observed in the bovine *TG*, *DGAT1* and *LEP* gene, respectively. In particular, the genotype differences between homozygotes CC and TT in the bovine *TFAM* gene accounted for 0.634 standard deviation in marbling and 0.402 standard deviation in SFD as this Wagyu X Limousin F_2 animals had a standard deviation of 1 score for marbling and a standard deviation of 0.18 inches for SFD. Therefore, among these four candidate genes studied so far in the reference population, the results showed that the *TFAM* gene had the greatest effects on both marbling and SFD, indicating a major gene for both traits.

A search for transcriptional regulatory elements using MatInspector (http://www.gsf.de/) indicated that both SNPs in the bovine *TFAM* promoter jointly or separately lead to a gain/loss of six putative binding sites for 1), tal-1alpha/E47 heterodimer; 2), cAMP-responsive element binding protein 1; 3), heterodimers of the bHLH transcription factors HAND2 (Thing2) and E12; 4), nuclear factor 1; 5), RAR-related orphan receptor alpha1 and 6), Zinc finger protein RP58 (ZNF238), which is associated preferentially with heterochromatin. Reusch and Klemm (2002) reported that the transcription factor cAMP-response element-binding protein (CREB) participates in adipogenesis, with constitutively active forms of CREB inducing adipocyte differentiation and dominant negative forms of CREB blocking this process. Evidence has shown that nuclear factor 1 is essential for the expression of stearoyl-CoA desaturase 1 gene during preadipocyte differentiation (Singh and Ntambi, 1998). The RAR-related orphan receptor alpha1, or RORα1 forms a part of the multifactorial regulatory mechanisms that control expression of the PPARgamma gene, which has been extensively studied for the past decade mainly due to its central role in promoting and maintaining the adipocyte phenotype (Sundvold and Lien, 2001). However, how these two SNPs in the bovine *TFAM* promoter affect binding efficiency for these genes, how these binding alterations regulate the subsequent *TFAM* gene expression patterns and how these expression patterns stimulate mitochondrial biogenesis differently and thus lead to the differences in fat deposition and energy metabolisms, need to be further explored.

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Example 2

This Example describes basal nucleus-encoded mitochondrial transcription genes and meat quality in beef cattle.

Evidence has shown that the basal mitochondrial transcription machinery directs the mitochondrial biogenesis and gene expression, and thus it may play an important role in body fat deposition and energy metabolism. Here we report sequence compilation, genetic marker development and association analysis of *TFAM, TFB1M* and *TFB2M* genes with marbling and subcutaneous fat depth (SFD) in cattle using a reference population of Wagyu x Limousin F_2 crosses. Statistical analysis revealed that the bovine *TFAM* gene was significantly associated with marbling (F=3.84, P=0.0229) and SFD (F=3.56, P=0.0301). The

genetic markers developed in the study can be used to further determine how this mitochondrial complex is important to improve meat quality in the beef industry.

Due to its limited protein coding capacity, the initiation and regulation of gene expression in mitochondrial DNA (mtDNA) rely heavily on a relatively small set of nuclear encoded mitochondrial regulatory proteins (Gleyzer et al., 2005). The basic mitochondrial transcription machinery consists of mitochondrial RNA polymerase (*POLRMT*) and mitochondrial transcription factor A (*TFAM*), B1 (*TFB1M*) and B2 (*TFB2M*). TFAM, a member of a high mobility protein family group and the first-identified mitochondrial transcription factor, is essential for maintenance and biogenesis of mtDNA (Fisher and Clayton, 1988). Both TFB1M and TFB2M are newly identified mitochondrial transcription factors and they interact directly with POLRMT to form a heterodimer (Falkenberg et al., 2002). On the other hand, mitochondria perform a large number of reactions in eukaryotic cells, including the β-oxidation of fatty acids, which provides key intermediates for the synthesis of triglycerides via the action of pyruvate carboxylase (Owen et al., 2002). As the basic mitochondrial transcription machinery directs the mitochondrial biogenesis and gene expression, it has been envisaged that the machinery may play an important role in body fat deposition and energy metabolism. Here sequence compilation, genetic marker development and association analysis of *TFAM TFB1M* and *TFB2M* genes with marbling and subcutaneous fat depth (SFD) using a reference population of Wagyu x Limousin F_2 crosses are reported.

The bioinformatics procedures used for retrieving both cDNA and genomic DNA sequences of these three bovine genes employed a three-step approach. First, cDNA sequences of the human orthologs were used as references to retrieve the ortholgous ESTs against the GenBank database "est_others" with a species option limited to *Bos taurus*. Second, several ESTs were chosen and assembled to form a primary cDNA sequence for each cattle gene, which was then used to perform a species-specific ESTs search against the same database in order to expand the primary sequence to a full-length cDNA sequence. Finally, the full-length cDNA sequence was used to search for genomic DNA sequences of the same gene against the 6X bovine genome sequence database and thus determine its genomic organization.

Primers were designed to target promoter regions and all coding exons for all three bovine genes based on the genomic DNA sequences. To ensure each exon region was completely amplified and sequenced, at least 100 bp of flanking sequences were included in the products. To facilitate discovery of genetic polymorphisms in these genes, two DNA

pools were formed: one from 6 Wagyu x Limousin F_1 sires and one from 113 Wagyu x Limousin F_1 dams. PCR reactions were performed on these two DNA pools and sequenced on an ABI 3730 sequencer in the Laboratory for Biotechnology and Bioanalysis (Washington State University) using a standard protocol. Nucleotide polymorphisms were identified by comparison of sequence patterns between these DNA pools. A total of ten single nucleotide polymorphisms (SNPs) were detected, including 3 in *TFAM*, 2 in *TFB1M* and 5 in *TFB2M* gene.

Only one SNP in *TFAM*, two SNPs in *TFB1M* and one SNP in the bovine *TFB2M* gene were chosen for genotyping using PCR-RFLP and Bi-PASA techniques. Animals used in the study were F_2 progeny from inter se mating of 6 Wagyu x Limousin F_1 sires and 113 Wagyu x Limousin F_1 dams as described above. Marbling scores varied from 4 = Slight^0 to 9.5 = Moderately Abundant^{50} (SD = 1.00). SFD was measured at the 12-13th rib interface perpendicular to the outside surface at a point three-fourths the length of the longissimus muscle from its chine bone end, which ranged from 0.1 to 1.3 inches (SD = 0.18) in this F_2 population. The phenotypic data for marbling scores and SFD were analyzed with a mixed linear model using the PROC MIXED module in SAS v9.1. Source of variation included year of birth, gender, age at harvest and genotype of each gene marker as fixed effects and a random effect to account for polygene background. The covariance structure of the polygene effect was defined by a numerical relationship calculated from pedigree using SAS macro LORG. The residual effect was assumed to have identical independent distribution with unknown variance. The additive genetic variance and residual variance components were estimated using the ridge stabilized Newton-Raphson algorithm for restricted maximum likelihood (REML) estimation. Tests of marker effects were performed using the Kenward-Roger method for calculating denominator degrees of freedom. This method uses an adjusted estimator of covariance matrix to reduce small sample bias. Pair-wise comparisons of least-squares means were performed using Fisher's protected least significant difference (LSD) t-test procedure.

The human ortholog-based BLAST search retrieved more than 20 ESTs for each of the bovine *TFAM*, *TFB1M* and *TFB2M* from the GenBank "est_others" database. Several overlapping ESTs were chosen and assembled to form primary cDNA sequences for these genes. The primary cDNA sequence was then used as a reference to search for ESTs of the same gene, in particular for its 5' and 3' flanking sequence expansion, which were missed by the human ortholog search due to low sequence similarity in these regions. The final assembly produced a full-length cDNA sequence of 2,259 bp for the bovine *TFAM* gene,

2,617 bp for the bovine *TFB1M* gene and 1,991 bp for the bovine *TFB2M* gene, respectively. Using these full-length cDNA sequences as references, BLAST searches against the 6x cattle genome sequence database retrieved three genomic contigs of 16,666 bp for *TFAM*, four genomic contigs of 108,966 bp for *TFB1M* and one genomic contig of 53,542 bp for *TFB2M*, respectively. Like in human, dog, mouse and rat, both bovine *TFAM* and *TFB1M* genes consist of seven exons, while the bovine *TFB2M* contain eight exons.

In addition to two closely linked A/C and C/T SNPs described in Example 1, a third mutation with a C/T transition was also detected in the bovine *TFAM* promoter region (FIG. 5A). A Bi-PASA assay was developed to genotype this marker on individuals. Direct sequencing of PCR products on two DNA pools revealed two mutations in the *TFB1M* gene (FIG. 5B) and five mutations in the *TFB2M* gene (FIG. 5C), respectively. The PCR amplicons were digested with 2U of *Msp*I and *Ban*I for genotyping SNPs of T/G and G/C in the bovine *TFB1M* gene. Initial genotyping of 48 samples using three of the five polymorphisms in the bovine *TFB2M* gene revealed they are fixed in two haplotypes. Therefore, only one SNP was chosen for genotyping by digestion with restriction enzyme *Aci*I.

Statistical analysis revealed that the bovine *TFAM* gene was significantly associated with marbling ($F=3.84$, $P=0.0229$) and SFD ($F=3.56$, $P=0.0301$). However, none of the markers in either *TFB1M* and *TFB2M* affected the measured traits significantly ($F<1.70$, $P>0.1842$). The additive and dominance effects of each marker were estimated and are listed in Table 3. Only the additive effect of the bovine *TFAM* gene on marbling reached a significant level ($P=0.0059$) and the additive effects of the *TFAM* and *TFB2M* on SFD approached significance ($P=0.0651$ for *TFAM* and $P=0.1118$ for *TFB2M*) (Table 3). The results indicate that involvement of these three genes in promoting transcription initiation of the mitochondrial genome may be tissue-specific or relevant. That is, *TFAM* contributed significantly to both marbling and SFD, while *TFB1M* had no effect on either trait. However, *TFB2M* contributed more to SFD, but almost nothing to marbling.

Table 3. Additive and dominance effects of the bovine *TFAM*, *TFB1M* and *TFB2M* markers on marbling and SFD.

Genetic effect	Marbling			SFD (in inches)		
	Estimate±S.E.	t	Pr > |t|	Estimate±S.E.	t	Pr > |t|
C/T in the bovine *TFAM* gene						
Additive	-0.384±0.138	-2.78	0.0059	-0.036±0.019	-1.85	0.0651
Dominance	0.117±0.088	1.27	0.2066	-0.007±0.013	-0.56	0.5780
G/T in the bovine *TFB1M* gene						

Genetic effect	Marbling			SFD (in inches)		
	Estimate±S.E.	t	Pr > |t|	Estimate±S.E.	t	Pr > |t|
Additive	-0.206±0.160	-1.28	0.2003	-0.007±0.022	-0.33	0.7451
Dominance	-0.017±0.100	0.17	0.8646	-0.003±0.014	-0.23	0.8209
C/G in the bovine *TFB1M* gene						
Additive	0.017±0.186	0.09	0.9273	-0.005±0.026	-0.19	0.8528
Dominance	0.074±0.118	0.63	0.5288	-0.023±0.017	1.41	0.1612
C/T in the bovine *TFB2M* gene						
Additive	0.121±0.120	1.00	0.3188	-0.028±0.018	1.60	0.1118
Dominance	-0.112±0.079	-1.41	0.1588	-0.000±0.011	-0.03	0.9798

Both marbling and SFD have attracted a great deal of publicity and interest for many years, since they are two of the major quantitative traits that affect carcass quality and production efficiency in beef cattle. The genetic markers developed in the study can be used to further determine how this mitochondrial complex is important to improve meat quality in the beef industry.

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Example 3

This Example provides associations between TFAM-1, TFAM-2, and FABP4 markers and carcass traits in commercial feedlot steers and heifers.

The following markers were evaluated: (1) a C to A substitution at the 1220 nucleotide position in the mitochondrial transcription factor A gene (TFAM-1) promoter, (2) a C to T substitution at the 1212 nucleotide position in the TFAM-2 promoter and (3) a G to C substitution at the 7516 nucleotide position of the fatty acid binding protein 4 gene (FABP4). Previous results indicate that the markers affect Markers affect marbling and backfat.

Initially, there were 1,589 records initially from steers and heifers. The target endpoint was 12.2 mm backfat. Harvest date was predicted optimal economic endpoint by animal. Contemporary groups included source and sex. It was assumed that the breed type confounded with the source. The final data set included the number of records based on available phenotypes and genotypes for each trait.

The tested traits are: hot carcass weight (HCW, lb), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW), hot

carcass weight value (HCW value, $), calculated live weight (Calc Lv Wt, lb), dry matter intake (DMI, lb), days on feed (DOF, d), dry matter intake per day on feed (DMI per DOF, lb/d), average daily gain (ADG, lb/d), dressing percentage (DP, %), backfat thickness (BFAT, in), calculated yield grade (cYG), quality grade, less than or equal to select versus greater than or equal to choice (QG, < Se vs, > Ch), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), additional carcass value (additional carc value, $), adjusted net return--all costs removed (adj. net return--all costs removed, $) and adjusted net return--initial animal value not removed (adj. net return--initial animal value not removed, $).

The analysis models were genotype, wherein genotypes were fit as fixed effects and additive or allele substitution, which showed regression on allele number (0, 1, 2). Both models fit with 2-marker combinations. Another analysis model is haplotype, which shows regression on (expected) haplotype when fitting multiple TFAM markers. Significant single marker associations are presented in Table 4 and significant 2-marker combinations are presented in Table 5.

Table 4: Single Marker Analyses for TFAM-1, TFAM-2, & FABP4

		Model										
			Allele Substitution			Fixed genotype			Genotype			
Marker	Trait	N	Estimate	SE	P-value	P-value	Add. est.	SE	Add. P-Value	Dom. est.	SE	Dom. P-value
FABP4	QG Ch(1,2,5,9,20)	1528	-.065	.019	.0007	.0027	-.057	.023	.0156	.020	.030	.5161
	REA/cwt HCW	1528	.00016	.00009	.0558	.0345	.00027	.00010	.0102	.00023	.00013	.0795
TFAM-1	Calc Lv Wt	1539	10.629	3.578	.0030	.0122	10.794	3.731	.0039	.816	5.178	.8748
	Hot Carcass Wt	1539	5.929	2.402	.0137	.0478	5.894	2.504	.0187	-.172	3.476	.9606
	HCW Value	1539	.274	.192	.1535	.0073	.432	.200	.0308	.776	.277	.0052
	Marbling score	1125	.906	.358	.0115	.0354	.962	.373	.0099	.281	.516	.5865
	MBS/DOF	1125	.006	.003	.0452	.0892	.007	.003	.0296	.004	.004	.3641
TFAM-2	Calc Lv Wt	1503	-9.607	3.590	.0075	.0099	-10.145	3.608	.0050	-7.321	5.070	.1490
	Hot Carcass Wt	1503	-5.931	2.418	.0143	.0270	-6.208	2.431	.0107	-3.775	3.416	.2693
	HCW Value	1503	-.453	.193	.0191	.0104	-.415	.194	.0326	.520	.273	.0567
	Marbling score	1097	-.845	.366	.0210	.0557	-.872	.368	.0179	-.345	.512	.5005
	Ribeye Area	1503	-.142	.058	.0139	.0439	-.139	.058	.0165	.037	.082	.6503

Markers fit for allele substitution: A for TFAM1; C for TFAM2 & FABP4

Marbling scores range from 10 to 99; 10 = PD0 = Std, 99 = A90 = Prime

QG Ch(1,2,5,9,20) implies Ch or better - includes Prime, Ch, CAB, Sterling Silver, & Angus Pride;

Alternate included Se, No Roll, Dark cutter, & Hard bone

Table 5: Two Marker Analyses for TFAM-1, TFAM-2, & FABP4

				Model											
				Allele Substitution						Genotype					
				Marker 1			Marker 2			Markers 1*2			Marker 1	Marker 2	Markers 1*2
Marker 1	Marker 2	Trait	N	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	P-value	P-value	P-value
TFAM-1	FABP4	Adj NR w/o init val	1526	-1.383	4.007	.7300	-.416	3.786	.9124	-.621	5.003	.9013	.4996	.2159	.0404
		Calc Lv Wt	1526	12.076	4.388	.0060	-4.194	4.146	.3119	-3.120	5.478	.5691	.0153	.2933	.6639
		Hot Carcass Wt	1526	7.092	2.948	.0163	-2.672	2.785	.3374	-2.237	3.680	.5435	.0393	.1629	.5571
		IMF%	377	-.046	.078	.5526	-.056	.072	.4345	.042	.095	.6598	.4765	.9997	.0372
		MBS/DOF	1117	.007	.003	.0299	-.001	.003	.8048	-.004	.004	.4059	.0426	.8568	.6798
		Marbling score	1117	1.069	.429	.0128	-.334	.408	.4140	-.317	.543	.5594	.0282	.4454	.8402
		QG Ch(1,2,5,9,20)	1526	-.004	.021	.8403	-.072	.020	.0003	.034	.026	.2028	.6912	.0016	.6534
TFAM-1	TFAM-2	Adj NR w/o init val	1501	8.151	5.602	.1459	11.913	5.791	.0398	-2.564	5.312	.6294	.1528	.0868	.3998
		DMI	1501	22.819	25.853	.3776	-2.520	26.725	.9249	-12.727	24.514	.6037	.0152	.0230	.0388
		DMI/DOF	1501	.208	.179	.2457	.034	.185	.8555	-.022	.170	.8989	.0142	.0171	.0479
		HCW Value	1501	-.466	.328	.1561	-.938	.339	.0058	.650	.311	.0368	.3637	.9102	.6043
		QG Ch(1,2,5,9,20)	1501	-.042	.030	.1558	-.066	.031	.0302	.033	.028	.2388	.2871	.5127	.2949
		YG	1434	.080	.039	.0419	.059	.041	.1481	-.040	.037	.2760	.4031	.5462	.7033
TFAM-2	FABP4	Calc Lv Wt	1495	-11.350	4.367	.0094	-5.556	4.050	.1703	4.329	5.306	.4147	.0113	.1017	.6175
		DOF	1495	-.181	.475	.7030	-.672	.441	.1274	.209	.577	.7175	.9786	.0189	.0398
		Hot Carcass Wt	1495	-6.424	2.943	.0292	-3.431	2.729	.2090	1.235	3.576	.7299	.0530	.0614	.8073
		Marbling score	1093	-.990	.433	.0223	-.372	.403	.3568	.311	.529	.5568	.0726	.8003	.8134
		QG Ch(1,2,5,9,20)	1495	-.017	.021	.4248	-.063	.020	.0014	-.025	.026	.3308	.8411	.0051	.2986
		REA/cwt HCW	1495	-.00002	.00009	.8546	.00018	.00009	.0385	-.00011	.00011	.3377	.8702	.0523	.2467

Markers fit for allele substitution: A for TFAM1; C for TFAM2 & FABP4

Marbling scores range from 10 to 99; 10 = PD0 = Std, 99 = A90 = Prime

QG Ch(1,2,5,9,20) implies Ch or better - includes Prime, Ch, CAB, Sterling Silver, & Angus Pride;

Alternate included Se, No Roll, Dark cutter, & Hard bone

Example 4

FIG. 7 shows a flowchart of the input of data and the output of results from the analysis and correlation of the data pertaining to the breeding, veterinarian histories and performance requirements of a group of animals such as from bovines. The flowchart illustrated in FIG. 7 further indicates the interactive flow of data from the computer-assisted device to a body of students learning the use of the method of the invention and the correlation of such interactive data to present an output as a pie-chart indicating the progress of the class. The flowchart further indicates modifications of the method of the invention in accordance with the information received from the students to advance the teaching process or optimize the method to satisfy the needs of the students.

FIG. 8 illustrates potential relationships between the data elements to be entered into the system. Unidirectional arrows indicate, for example, that a barn is typically owned by only one farm, whereas a farm may own several barns. Similarly, a prescription may include veterinarian products.

FIG. 9A illustrates the flow of events in the use of the portable computer-based system for data entry on the breeding and rearing of a herd of cows. FIG. 9B illustrates the flow of events through the sub-routines related to data entry concerning farm management. FIG. 9C illustrates the flow of events through the sub-routines related to data entry concerning data specific to a company.

FIG. 10 illustrates a flow chart of the input of data and the output of results from the analysis and the correlation of the data pertaining to the breeding, veterinarian histories, and performance requirements of a group of animals.

The invention is further described by the following numbered paragraphs:

1. A method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar polymorphism in a mitochondrial transcription factor A ("*TFAM*") gene comprising:

(a) determining the genotype of each animal to be sub-grouped by determining the presence of a single nucleotide polymorphism in the *TFAM* gene, and

(b) segregating individual animals into sub-groups wherein each animal in a sub-group has a similar polymorphism in the *TFAM* gene.

2. A method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar genotype in the *TFAM* gene comprising:

(a) determining the genotype of each animal to be sub-grouped by determining the presence of a single nucleotide polymorphism(s) of interest in the *TFAM* gene,

(b) segregating individual animals into sub-groups depending on whether the animals have, or do not have, the single nucleotide polymorphism(s) of interest in the *TFAM* gene.

3. The method of paragraphs 1 or 2, wherein the single nucleotide polymorphism(s) of interest is selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene.

4. A method for sub-grouping animals according to genotype wherein the animals of each sub-group have a similar genotype in the *TFAM* gene comprising:

(a) determining the genotype of each animal to be sub-grouped by determining the presence of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene, and

(b) segregating individual animals into sub-groups depending on whether the animals have, or do not have, an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene.

5. A method for identifying an animal having a desirable phenotype as compared to the general population of animals of that species, comprising determining the presence of a single nucleotide polymorphism in the *TFAM* gene of the animal, wherein the polymorphism is selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene single nucleotide polymorphism is indicative of a desirable phenotype.

6. The method of paragraph 5, wherein the desirable phenotype is feed intake, growth rate, body weight, carcass merit and composition, milk yield or any combination thereof.

7. The method of paragraph 5 or 6, wherein the desirable phenotype is additional carcass value (additional carc value, $), average daily gain (ADG, lb/d), backfat thickness (BFAT, in), calculated live weight (Calc Lv Wt, lb), calculated yield grade (cYG), days on feed (DOF, d), dressing percentage (DP, %), dry matter intake (DMI, lb), dry matter intake per day on feed (DMI per DOF, lb/d), hot carcass weight (HCW, lb), hot carcass weight value (HCW value, $), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), quality grade, less than or equal to

select versus greater than or equal to choice (QG, < Se vs, > Ch), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW), subcutaneous fat depth (SFD) or any combination thereof.

8. The method of any one of paragraphs 1 to 7 wherein the animal is a bovine.

9. The method of any one of paragraphs 1 to 8 wherein the *TFAM* gene is a bovine *TFAM* gene.

10. An interactive computer-assisted method for tracking the rearing of livestock bovines comprising, using a computer system comprising a programmed computer comprising a processor, a data storage system, an input device, an output device, and an interactive device, the steps of: (a) inputting into the programmed computer through the input device data comprising a breeding history of a bovine or herd of bovines, (b) inputting into the programmed computer through the input device data comprising a veterinary history of a bovine or herd of bovines, (c) correlating the veterinary data with the breeding history of the bovine or herd of bovines using the processor and the data storage system, and (d) outputting to the output device the breeding history and the veterinary history of the bovine or herd of bovines.

11. The method according to paragraph 10, wherein the computer system is an interactive system whereby modifications to the output of the computer-assisted method may be correlated according to the input from the interactive device.

12. The method according to paragraph 10 or 11, further comprising the steps of inputting into the programmed computer diagnostic data related to the health of the cow or herd of cows; and correlating the diagnostic data to the breeding and veterinary histories of the cow or herd of cows.

13. The method according to any one of paragraphs 10 to 12, wherein the veterinary data comprises a vaccination record for a cow or herd of cows.

14. The method according to any one of paragraphs 10 to 13 wherein the health data is selected from the group consisting of husbandry condition data, herd history, and food safety data.

15. The method according to any one of paragraphs 10 to 14, further comprising at least one further step selected from the group consisting of inputting into the programmed computer data related to the quality control of the bovine or herd of bovines and correlating the quality control data to the breeding and veterinary histories of the cow or herd of cows, inputting into the programmed computer performance parameters of the cow or herd of cows; and correlating the required performance parameters of the bovine or herd of bovines to a

specific performance requirement of a customer, correlating the vaccine data to the performance parameters of the bovine or herd of bovines, correlating herd to the performance parameters of the bovine or herd of bovines, correlating the food safety data to the performance parameters of the bovine or herd of bovines, correlating the husbandry condition data to the performance parameters of the bovine or herd of bovines, inputting into the programmed computer data related to the nutritional data of the bovine or herd of bovines; and correlating the nutritional data to the performance parameters of the bovine or herd of bovines, and alerting to undesirable changes in the performance parameters of the bovine or herd of bovines.

16. The method according to any one of paragraphs 10 to 15, further comprising the steps of inputting into the programmed computer through the input device data comprising a genotype of a bovine; correlating a physical characteristic predicted by the genotype using the processor and the data storage system; and outputting to the output device the physical characteristic correlated to the genotype for a bovine or population of bovines, and feeding the animal(s) a diet based upon the physical characteristic, thereby improving bovine production.

17. The computer-assisted method according to any one of paragraphs 10 to 16 for optimizing efficiency of feedlots for livestock comprising outputting to the output device the breeding and veterinary history of the bovine or herd of bovines and feeding the animal(s) a diet based upon their breeding and veterinary histories, thereby optimizing efficiency of feedlots for the bovine or herd of bovines.

18. A method of transmitting data comprising transmission of information from such methods according to any one of paragraphs 10 to 16, selected from the group consisting of telecommunication, telephone, video conference, mass communication, a presentation, a computer presentation, a POWERPOINT™ presentation, internet, email, and documentary communication.

19. An interactive computer system according to any one of paragraphs 10 to 16 for tracking breeding and welfare histories of cows comprising breeding and veterinarian data corresponding to a bovine or herd of bovines, and wherein the computer system is configured to allow the operator thereof to exchange data with the device or a remote database.

20. The interactive computer system according to paragraph 19, wherein the input and output devices are a personal digital assistant or a pocket computer.

21. A method of doing business for tracking breeding and welfare histories of livestock comprising breeding and veterinarian data corresponding to one or more livestock animals comprising providing to a user the computer system of paragraph 19.

22. A method of doing business for tracking breeding and welfare histories of livestock comprising breeding and veterinarian data corresponding to one or more livestock animals comprising providing to a user the computer system of paragraph 20.

23. The method of doing business according to paragraph 21, further comprising providing the animal owner or customer with sample collection equipment, such as swabs and tags useful for collecting samples from which genetic data may be obtained, and wherein the tags are optionally packaged in a container which is encoded with identifying indicia.

24. The method of doing business according any one of paragraphs 10 to 16, wherein the computer system further comprises a plurality of interactive devices and wherein the method further comprises the steps of a receiving data from the interactive devices, compiling the data , outputting the data to indicate the response of a student or class of students to a question relating to the operation of the computer-assisted method, and optionally modifying the operation of the computer-assisted method in accordance with the indication of the response.

25. The method of any one of paragraphs 8 to 24 wherein the data comprises presence or absence of one or more of a single nucleotide polymorphism(s) of interest in the *TFAM* gene.

26. The method of paragraph 25 wherein the single nucleotide polymorphism(s) of interest is selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the TFAM gene.

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A method for identifying an animal having desirable calculated live weight (Calc Lv Wt, lb), calculated yield grade (cYG), days on feed (DOF, d), dry matter intake (DMI, lb), dry matter intake per day on feed (DMI per DOF, lb/d), hot carcass weight (HCW, lb), hot carcass weight value (HCW value, $), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), quality grade, less than or equal to select versus greater than or equal to choice (QG, < Se vs, > Ch), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW), or a combination thereof, as compared to the general population of animals of that species, comprising determining the presence of a single nucleotide polymorphism in a mitochondrial transcription factor A ("*TFAM*") gene the presence of a single nucleotide polymorphism in the *TFAM* gene of the animal, wherein the single nucleotide polymorphism is indicative of desirable calculated live weight (Calc Lv Wt, lb), calculated yield grade (cYG), days on feed (DOF, d), dry matter intake (DMI, lb), dry matter intake per day on feed (DMI per DOF, lb/d), hot carcass weight (HCW, lb), hot carcass weight value (HCW value, $), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), quality grade, less than or equal to select versus greater than or equal to choice (QG, < Se vs, > Ch), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW), or a combination thereof.

2. The method of claim 1 further comprising sub-grouping animals according to genotype, wherein the animals of each sub-group have a similar polymorphism in the *TFAM* gene, said method comprising:

(a) determining the genotype of each animal to be sub-grouped by determining the presence of a single nucleotide polymorphism in the *TFAM* gene, and

(b) segregating individual animals into sub-groups depending on whether the animals have, or do not have, the single nucleotide polymorphism of interest in the *TFAM* gene.

3. The method of claim 1, wherein the single nucleotide polymorphism(s) of interest is selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene.

4. The method of claim 1 wherein the animal is a bovine.

5. The method of claim 1 wherein the *TFAM* gene is a bovine *TFAM* gene.

6. An interactive computer-assisted method for tracking the rearing of livestock bovines comprising, using a computer system comprising a programmed computer comprising a processor, a data storage system, an input device, an output device, and an interactive device, the steps of: (a) inputting into the programmed computer through the input device data comprising a breeding history of a bovine or herd of bovines and a genotype of a bovine; correlating a physical characteristic predicted by the genotype using the processor and the data storage system, (b) inputting into the programmed computer through the input device data comprising a veterinary history of a bovine or herd of bovines, (c) correlating the veterinary data with the breeding history of the bovine or herd of bovines using the processor and the data storage system, and (d) outputting to the output device the breeding history, the veterinary history of the bovine or herd of bovines and the physical characteristic correlated to the genotype for a bovine or population of bovines,

wherein the physical characteristic is desirable calculated live weight (Calc Lv Wt, lb), calculated yield grade (cYG), days on feed (DOF, d), dry matter intake (DMI, lb), dry matter intake per day on feed (DMI per DOF, lb/d), hot carcass weight (HCW, lb), hot carcass weight value (HCW value, $), intramuscular fat content (IMF%, %), marbling score (MBS, 10 to 99), marbling score divided by days on feed (MBS/DOF), quality grade, less than or equal to select versus greater than or equal to choice (QG, < Se vs, > Ch), ribeye area (REA, in^2), ribeye area per hundred weight HCW (REA/cwt HCW, in^2/100 lb hot carcass weight (HCW), or a combination thereof, as compared to the general population of bovines, and the genotype is a single nucleotide polymorphism in a *TFAM* gene.

7. The method according to claim 6, wherein the computer system is an interactive system whereby modifications to the output of the computer-assisted method may be correlated according to the input from the interactive device.

8. The method according to claim 6, further comprising the steps of inputting into the programmed computer diagnostic data related to the health of the cow or herd of cows; and correlating the diagnostic data to the breeding and veterinary histories of the cow or herd of cows.

9. The method according to claim 6, wherein the veterinary data comprises a vaccination record for a cow or herd of cows.

10. The method according to claim 6 wherein the health data is selected from the group consisting of husbandry condition data, herd history, and food safety data.

11. The method according to claim 6, further comprising at least one further step selected from the group consisting of inputting into the programmed computer data related to

the quality control of the bovine or herd of bovines and correlating the quality control data to the breeding and veterinary histories of the cow or herd of cows, inputting into the programmed computer performance parameters of the cow or herd of cows; and correlating the required performance parameters of the bovine or herd of bovines to a specific performance requirement of a customer, correlating the vaccine data to the performance parameters of the bovine or herd of bovines, correlating herd to the performance parameters of the bovine or herd of bovines, correlating the food safety data to the performance parameters of the bovine or herd of bovines, correlating the husbandry condition data to the performance parameters of the bovine or herd of bovines, inputting into the programmed computer data related to the nutritional data of the bovine or herd of bovines; and correlating the nutritional data to the performance parameters of the bovine or herd of bovines, and alerting to undesirable changes in the performance parameters of the bovine or herd of bovines.

12. The method according to claim 6, wherein the single nucleotide polymorphism(s) of interest is selected from the group consisting of an A to C substitution at the -1220 nucleotide position in the promoter of the *TFAM* gene, a T to C substitution at position -1212 in the promoter of the *TFAM* gene and a T to C substitution at position -995 in the promoter of the *TFAM* gene

13. A method of transmitting data comprising transmission of information from such methods according to claim 6, selected from the group consisting of telecommunication, telephone, video conference, mass communication, a presentation, a computer presentation, a POWERPOINT™ presentation, internet, email, and documentary communication.

14. An interactive computer system according to claim 6 for tracking breeding and welfare histories of cows comprising breeding and veterinarian data corresponding to a bovine or herd of bovines, and wherein the computer system is configured to allow the operator thereof to exchange data with the device or a remote database.

15. The interactive computer system according to claim 14, wherein the input and output devices are a personal digital assistant or a pocket computer.

16. A method of doing business for tracking breeding and welfare histories of livestock comprising breeding and veterinarian data corresponding to one or more livestock animals comprising providing to a user the computer system of claim 14.

17. A method of doing business for tracking breeding and welfare histories of livestock comprising breeding and veterinarian data corresponding to one or more livestock animals comprising providing to a user the computer system of claim 14.

18. The method of doing business according to claim 16, further comprising providing the animal owner or customer with sample collection equipment, such as swabs and tags useful for collecting samples from which genetic data may be obtained, and wherein the tags are optionally packaged in a container which is encoded with identifying indicia.

19. The method of doing business according to claim 16, wherein the computer system further comprises a plurality of interactive devices and wherein the method further comprises the steps of a receiving data from the interactive devices, compiling the data , outputting the data to indicate the response of a student or class of students to a question relating to the operation of the computer-assisted method, and optionally modifying the operation of the computer-assisted method in accordance with the indication of the response.

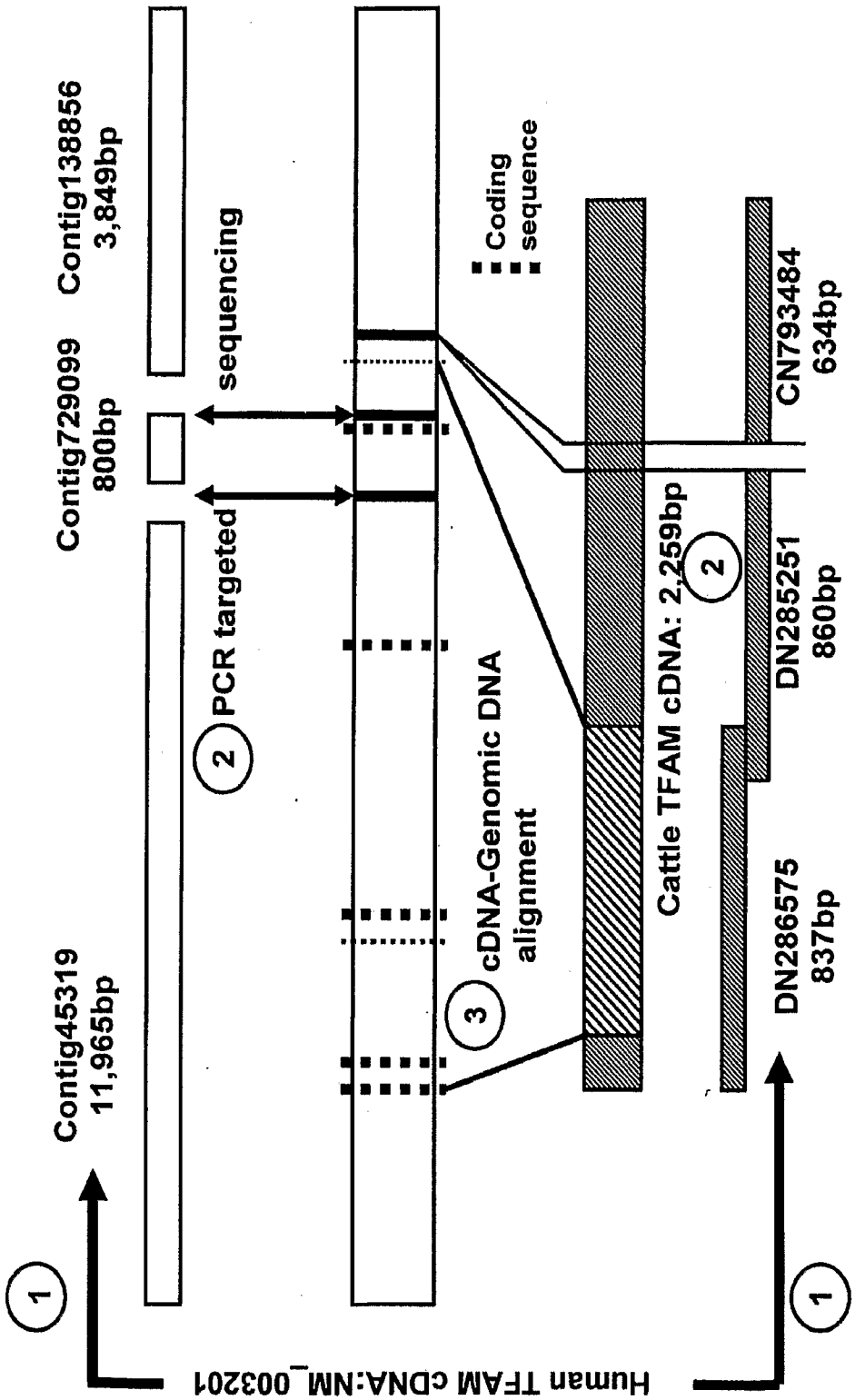

Contig138856
3,849bp
Contig729099
800bp
Contig45319
11,965bp
sequencing
PCR targeted
2
cDNA-Genomic DNA
alignment
3
Coding
sequence
Cattle TFAM cDNA: 2,259bp
2
CN793484
634bp
DN285251
860bp
DN286575
837bp
1
1
Human TFAM cDNA:NM_003201

Fig. 1

```
-1699 TAGATCAGATTTATAAGAACTTTATAGCAAGGCTTATCAAAATGACACTTTAAAACAACA
-1539 AAGACTTTAAATATTCAAAAAAATTTTTTATATCTATCAAACGTCTGAAAGAATAAAAAC
-1479 ACTCTAGAAAAATGTAAAGAAAAGTTGTTGCAGAAATCAGCTAAAATGTAATTATAAGGA
-1419 ATTCTATCCCATACTTTTAGCTTGACATTGTAGTGTAACTGTTCATTTCTTTGCCTCTGC
-1359 CACTAGACTTCATGGGCGGTGAAAAGAAGGCTGTGTTTTTTATCTAAGTTTGTGGCCTTG
-1299 TACATAATGTGTACTCAGAAAATAATCAAACAAACAAAATCCAACCCTGTTTCTTCCATG
                     -1220   -1212

-1239 ACAGAGTGAGAGATTTGGACAGATGATCTAAAAGGTCCCTTTCAATACTGAGAATTTTTG
                           C       C
-1179 AAAAGGAAGTGGTGATAGTTGCTTACCACTTAGAAGTGGGCTTGGTGGTCGCACTCAACG
-1119 GGCCAGTGAAAGACTCTCAGCTCAATATGACACTAGTAAGAATTTTCTTACAAACCTATC
-1059 CAACAGAGAAAAAGCTGTTTCAGGAAGTGGCTCTTTGAAATAAATGGAACCTGAGAGCTA
- 999 GAAGTGTCCTTAGATAGCTTTAGATAAGGTTATCCTAATCTTTTTTCATGCCATTGTCCA
- 939 CATGGAAAATGGCGGTACTGCACATCGATCAATATCTCAAGGCATAATTGTCACAGGTTC
- 879 TCAGAGCTTTGGTTGGGAAATGATCTCGTCCAACATCCTCGTCTGCAGTGGGGGAAATTA
- 819 AATCTTACAGCGGGGAAGTGACATTTCCAGATCAAGACGTTAATGCAAATGATGGAGCTA
- 759 GAACTCTAGTCTCCGGACAGATAACTAGTCAGTCTCCTCCCCACAAAGTAAGCAGGGTTG
- 699 GATGTCAAGCTTGCCGATTAGGTCAGCGATAGTCTCAGTGGATGGGGCTATGGTCTGGAT
- 639 CTGTGTTGCTTTAAATGTGGTCCCATAACCACTGGCATCAGAGCTTGTTAAACCTTGAGG
- 579 AACTTGTTAAGATAGCTGAGACATGGGACCCACACAAACACGGAATCAGGCTCTGCGGCA
- 519 AGGCCCGGGAATCTGCATTTTAAGGTTAGAAACCGTGATCCATCCCACCTCCTCTACGGT
- 459 AATTTTGGACTACGCGCTCCGACTTGAACTGAGGGACTCTGCTGGGCGCATATCGCCCAG
- 399 CAACCTGACCTCAGAGCAGCGTGGGCGGCACGGCTACCGCCGTTCTAGCCGTAAACTGTC
- 339 TGTTACGTACTAGCCCTTCCAGTCGTCACAAACCAGCCAGGGCCCCATCCGTGTGAGGAC
                                                              SP1

- 279 ACCGCCGTGCCTCCAATCAGCCTCCTGGCCGGGCGGCTGAGCAGGCTGCCGGGGGGGTGG

- 219 GGGGGTGGGGTTGGGACAGAGGCGGCTCAGCTTCGCCCCCTCGAGGCCAGGTCCCTCCGC

     Transcription repressor
- 159 AGGCTAGCCGGGTTGCAGTTTCCCTTCTCCGCCTCCGAGCGCCTTTTTCTTCGTAGTCCC
                                NRF1

-  99 GCCCACCAAGGAAGCCAGTACAGCGCCTGCGCCTTCTACCTTGCCCCGCCTCCTAGCTAA

                                                    +1(putative)
-  39 TCGGAAGTTAGCCGATTTCCCATAGTGCCCCGCGAGTGGCGGGCATGATAGTAAATCCGG
+  22 TAGGCTCTCTGGCGGGTTTAAGTCGGCTTTGTTGCGGTTTCTCTTGTGCCAGGGCGCTGT
                                              ATG  Exon 1
+  82 AGGGAATTGGGGCCTGGTCAGTGCTTTGTCTGCGGATGCAATGGCGCTTCTCCGGGGCGT
+ 142 ATGGGGCGTGCTGAATGCGCTGGGAAAGTCAGGAGCGGATCTCTGCGCGGGCTGTGGCAG
+ 202 TCGACTGCGCTCTCCCTTTAG
```

Fig. 2

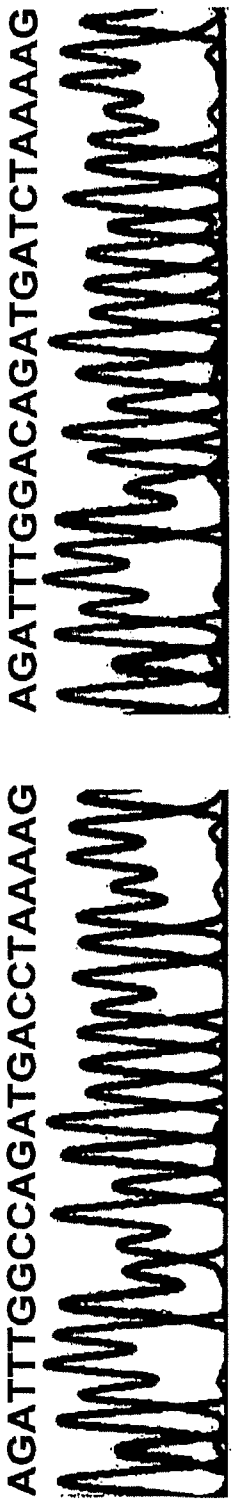

AGATTTGGCCAGATGACCTAAAAG
AGATTTGGACAGATGATCTAAAAG

Fig. 3

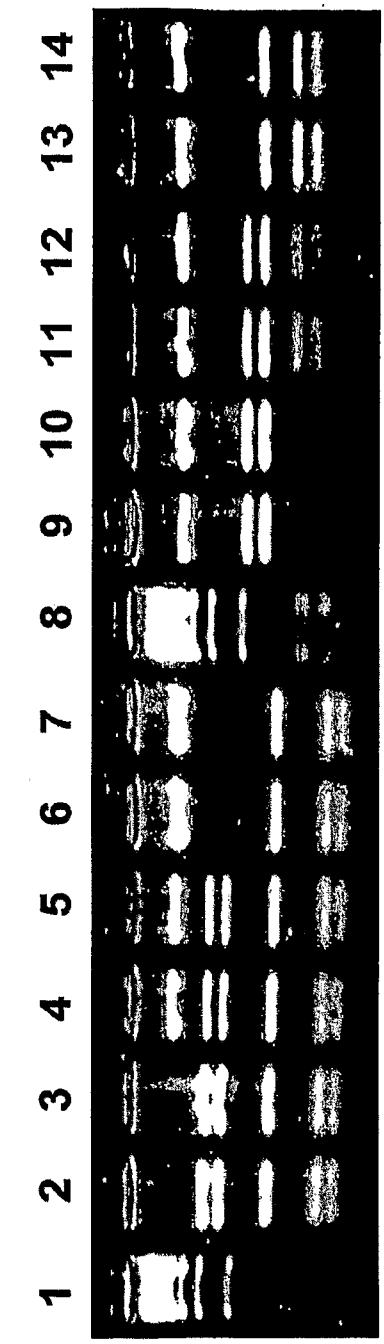

1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 4

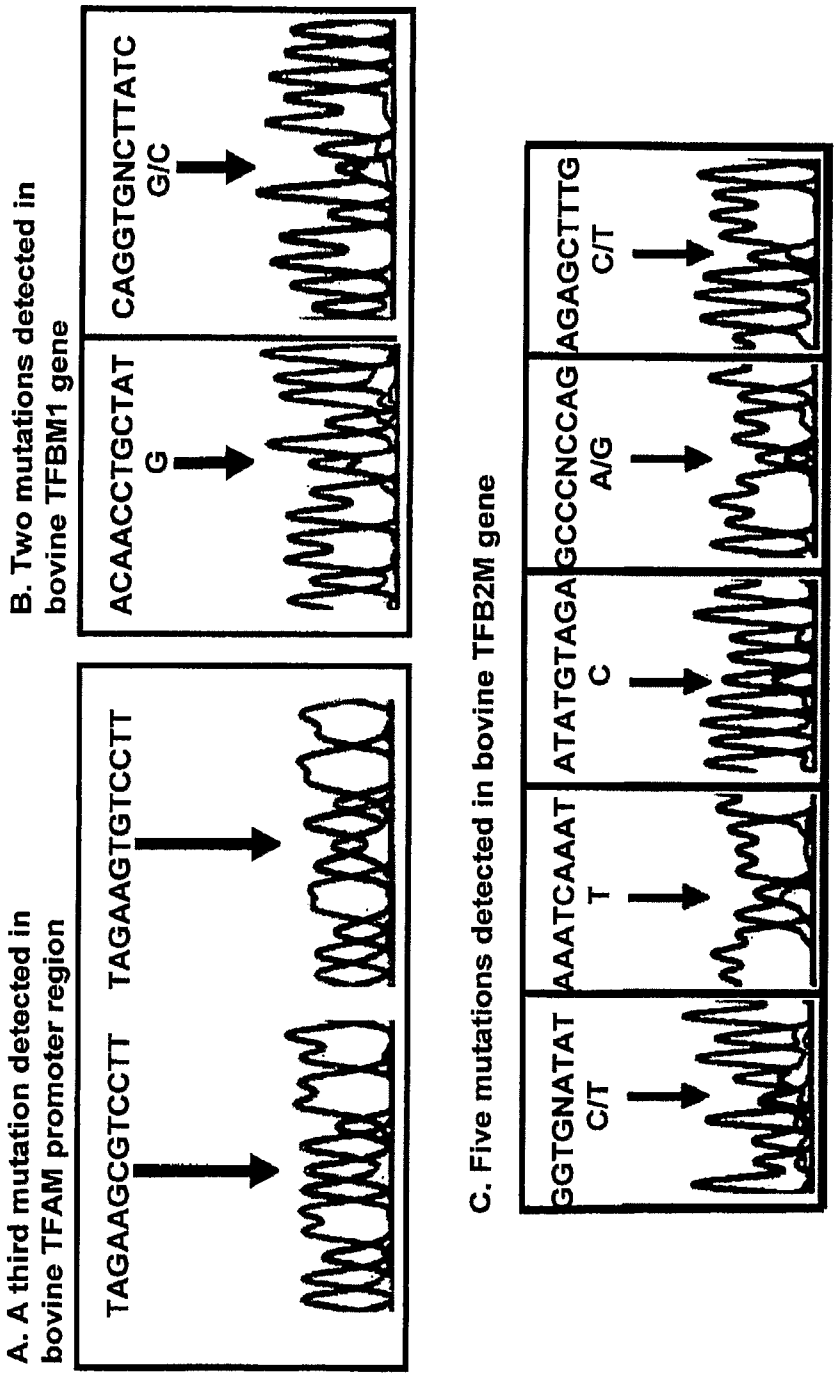

A. A third mutation detected in bovine TFAM promoter region
TAGAAGCGTCCTT TAGAAGTGTCCTT
B. Two mutations detected in bovine TFBM1 gene
ACAACCTGCTAT G CAGGTGNCTTATC G/C
C. Five mutations detected in bovine TFB2M gene
GGTGNATAT C/T
AAATCAAAT T
ATATGTAGA C
GCCCNCCAG A/G
AGAGCTTTG C/T

Fig. 5

CGGGCATGATAGTAAATCCGGTAGGCTCTCTGGCGGGTTTAAGTCGGCTTTGTTGCGGGTTCTCTTGT
GCCAGGGCGCTGTAGGGAATTGGGGCCTGGTCAGTGCTTTGTCTGCGGATGCAATGGCGCTTCTCCGG
GGCGTATGGGGCGTGCTGAATGCGCTGGGAAAGTCAGGAGCGGATCTCTGCGCGGGCTGTGGCAGTCG
ACTGCGCTCTCCCTTTAGTTTTGCGTATGTTCCAAAATGGTTTTCATCCAGCTTGAGTGGTTATCCAA
AGAAGCCCATGACTTCATACGTTCGATTTTCTAAAGAACAGCTGCCCATATTTAAAGCTCAGAACCCA
GATGCAAAAAATTCAGAACTAATTAAAAAAATTGCCAAGCTATGGAGGGAACTTCCTGATTCAGAGAA
AAAGATATATGAAGATGCTTACAGGGCAGACTGGCAGGTATACAAGGAAGAGATAAACAGAATTCAAG
AACAACTAACTCCAAGTCAAATGGTATCTTTGGAAAAAGAAATCATGCAGAAACGTTTAAAAAAGAAA
GCGTTAATAAAAAAGAGAGAGTTAACAATGCTTGGAAAACCGAAAAGACCTCGCTCAGCTTATAACAT
TTTTATAGCTGAACGTTTTCAGGAAGCTAGGGATGGCACATCACAGGTAAAGCTGAAAGCTATAAATG
AAAACTGGAAAAATCTCTCTAATTCTCAAAAGCAAGTATATATTCAGCTTGCTAAAGATGATAAAATT
CGTTATTATAACGAAATGAAATCTTGGGAGGAACAAATGATGGAAGTTGGACGAGAAGATCTTATACG
TCGCTCAATTAAATACCCAGCAAAAAATGACCCTGAGAAGTTTTAAAATAGAAGATTGAGTTATGTTC
ATAATGGATAGACATAAGAAACCAACTAGGTCTCAATACCGGAAGCTGTTGTAAAATTAGAATGGATA
AAGTTGGTGAACATTTATATTTAATTCCTTTTCTGTAGCCCATGGACTTCTGCCAGCCAATTCAATAC
ATTTTGTATTGGTGTCTTGCTTTTGAAAACCCAAACAGATAAGACTTCATGTGGAATTTATTTTGTGT
TTAGGAACTACTGAGCATCAAAATAATCCATGAAATGTAGCAGTGAATCATTTTACCTTTGATAAAGG
TAAATCAGACTGTGAAGTTTTTTTATACTTGGTGATTATGGAAAAAATATTCTTGTTTCCTTATATTA
TGGAAGCAGGAGTTTCCTATTCATAAGTATCTCAAAGTTTGTAGAAGCCATAGTGTTCTATGATATAA
CTGCATTTTTAAAAGAGCATCCAGAACTCATGCTGGTAAATTCCAAATCCTGGGTATAATTCATATTA
TAATCAGACTTGATGGTTGTACATGTGAGGAATTTCTGGTATCAGTTGCAGCTTTTATAAAAGGTATA
GATTTATAAACCTTTTCCTCATTATCTTTTTCCTAAATTAAAAACTAAAAAATTATGTACCAAATCTA
TGCATATTGTTTTATATTGCATAGAATAAAAATTATGTGTTTCCTAATTATGTTTTAAGGTGAAACAT
TCATTTTATAGCTTTCTGGGATTTTTGTTTGTTTTTTTAAACAAAGTAGGAGTTTGTATACTGAATTA
TTTTTTCTCTTATGTAAATATATTTATCCAGAAAGTAGAGAACTTATTTGGTGTAAGTTTTAAAATGA
GAGATCTAAAAAAATCATGTCTCCAAAGTCTCTCAAATTGGAACCTATAATTTTTAAACATTTGCATA
ACATATAAAAGCCTGTATAATAATTAATAGCCAGTTCTAGCCTGATGCCCACATCCAGCCCACTGCCT
GCTTTTCTTGGCCTTGTCAACTAAGAATTGTTCACAGTTTTAAATAGTTAAAAAAAACTAAGCATACT
TCAAGACACATGAAAATTAATATGAAATTCAGTGTCCATAAAAAAATGTTTTATTGGAACACAGCCAC
ACTCATCGATTATGGCTGTTTTTGTACAATAGTGCAGTTGACTTGGTTGCTACAGAGAGGTGCAGCCT
GCAAAGACTGACCCGTATAGTGTGGAATGAGATGCTCTTAAGTTACTTGCTGTGGCTCAGTAAAATAC
ATCTTAACAGTTTTCCAGTTAGCATTTAAACCTTACCCCTTCTAGCATTTGATTATCTTCTTAACAAG
AGTCAAAGGTTTTTCAAAATCCACACCACCTTTTTGAGCAAAATATTCAACTGTCATCATCAGTTCAC
ATGAATTTATAACTC

FIG. 6A

TAGATCAGATTTATAAGAACTTTATAGCAAGGCTTATCAAAATGACACTTTAAAACAACAAAGACTTT
AAATATTCAAAAAAATTTTTTATATCTATCAAACGTCTGAAAGAATAAAAACACTCTAGAAAAATGTA
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CCAACCCTGTTTCTTCCATGACAGAGTGAGAGATTTGGACAGATGATCTAAAAGGTCCCTTTCAATAC
TGAGAATTTTTGAAAAGGAAGTGGTGATAGTTGCTTACCACTTAGAAGTGGGCTTGGTGGTCGCACTC
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AGAGAAAAAGCTGTTTCAGGAAGTGGCTCTTTGAAATAAATGGAACCTGAGAGCTAGAAGTGTCCTTA
GATAGCTTTAGATAAGGTTATCCTAATCTTTTTTCATGCCATTGTCCACATGGAAAATGGCGGTACTG
CACATCGATCAATATCTCAAGGCATAATTGTCACAGGTTCTCAGAGCTTTGGTTGGGAAATGATCTCG
TCCAACATCCTCGTCTGCAGTGGGGGAAATTAAATCTTACAGCGGGGAAGTGACATTTCCAGATCAAG
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GTCTGGATCTGTGTTGCTTTAAATGTGGTCCCATAACCACTGGCATCAGAGCTTGTTAAACCTTGAGG
AACTTGTTAAGATAGCTGAGACATGGGACCCACACAAACACGGAATCAGGCTCTGCGGCAAGGCCCGG
GAATCTGCATTTTAAGGTTAGAAACCGTGATCCATCCCACCTCCTCTACGGTAATTTTGGACTACGCG
CTCCGACTTGAACTGAGGGACTCTGCTGGGCGCATATCGCCCAGCAACCTGACCTCAGAGCAGCGTGG
GCGGCACGGCTACCGCCGTTCTAGCCGTAAACTGTCTGTTACGTACTAGCCCTTCCAGTCGTCACAAA
CCAGCCAGGGCCCCATCCGTGTGAGGACACCGCCGTGCCTCCAATCAGCCTCCTGGCCGGGCGGCTGA
GCAGGCTGCCGGGGGGGGTGGGGGGGTGGGGTTGGGACAGAGGCGGCTCAGCTTCGCCCCCTCGAGGCC
AGGTCCCTCCGCAGGCTAGCCGGGTTGCAGTTTCCCTTCTCCGCCTCCGAGCGCCTTTTTCTTCGTAG
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CGGGTTTAAGTCGGCTTTGTTGCGGTTTCTCTTGTGCCAGGGCGCTGTAGGGAATTGGGGCCTGGTCA
GTGCTTTGTCTGCGGATGCAA*TGGCGCTTCTCCGGGGCGTATGGGGCGTGCTGAATGCGCTGGGAAAG*
*TCAGGAGCGGATCTCTGCGCGGGCTGTGGCAGTCGACTGCGCTCTCCCTTTAGGT*AAGCCTGCTTGCC
TGCACCTTCGGAGGCGGAAGGGTTCAGGACGCCTTGGGGTTAGAATGTAGACGCTGTTCTCCGCTTTC
TGACCCCCCTGAAATGGTCAGGACTGACTTAGCCCTGTGATTCCGACCTCGACCTTGCCAAGGGGATG
ATAGCTTTGAGACGAGGCCTTACTGCTTCAGTACCCCTTGCCTTCCTTCCGGTCACCTTTCCCCTCCT
CCCGTCCCTCCTCATTCTCGTACCTTGGAGCACCGGGTTCGTTGTCCCCTGCGAAGTCGCCGCAAAGT
CTCCTGCCTTCATCTAGCCATTGAGGCGAAGCCCAGGAGCTGGAGATACATGCTTTCCTTCACTCTCT
GCCCTTGCTAGGGGGTTAGGGCTTTTGAGACTTCAGTTTTGCAAGCTCTTTCCCCTGGATAGGACAGG
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TATTTATATGTA*GTTTTGCGTATGTTCC*AAAAT*GGTTTTCATCCAGCTTGAGTGGTTATCCAAAGAAG*
*CCCATGACTTCATACGTTCGATTTTCTAAAGAACAGCTGCCCATATTTAAAGCTCAGAACCCAGGT*AA
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ATTTTCAGTATCAAGGCATCTGTTGAATGCAGAAACATAACCATTCTGTGTGATGGCCTGTAAAATAG
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TGGGTAGGGAAGATCCCCTAGAAAAGGGAATGGTGACCCGCTCCTTTATTCTGGCCTGGAAAATTATG
GACAGAGGAGCCTGGCAGGCCACAGTCCATGGATTTGCAGAATCAGACACGACTGAAGCGACCTAGCA
GGCATGCATAAGGAATCAGTGTAAGCACAGCAGTAAGGTAATTTTACCAAATAAAAATGCTTTAAATG
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GCTTAGCTCCTGCTTCTCCCACTGCAAATTATTAATAAAGTTGCAGTGAAATAATTTATGTGGATGTG
TTGCATAAACTTGAAAGCTGAATACAGGTTATTGTTAATTTTAAGAGCTTTTTGACTGAAAGCCAGTT
GAATTTCTGAAAGATTTCCAGTTAGGTTTGTTGACTCCCTAGAAAGACTGGGTCCTTATTGGGGCTGT
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GCATTATATCACAGGGCACTTATGAAAATCATGTGAGCTGTAATAGTCACGTAAAGAACTGGTTTAAG

Fig. 6B

AGACATGCTTCAGTACTCACATCTTTTGAAATGTAGGAGTTGAAACTAACAAGTAGTTTGTTAAATCA
TTTATACCTTGTCATTCCAGTTAAGATGGGAAGGAAATGGAGAAAAATTATTTGGTTTTAGAATAATT
TAGCTTGTTTGCAAACAATATTTCTGTAATTTAGATACAGGTGGTGTTATATGAATCAAGACTATATT
TTGTTGTTTAGTCAGGTTTTATGAACTTGGTCATTCCTGAAAGACTTGAAAAAAATGTTTTTGATTGA
AATATGAAGTAGAAAACTTGTAGACACTGATTCCCCAATTGCTTTAACTTAGTAATCAATTCCTGAGG
ACCATTTTGGTGGTAAGTGGTACTATTCCTTAATTTAATCATTAGATAATAGACTTTAATAATGTTAG
ATAAAATATTAAATTATTTATTGTACTTAAATTAGTTGGTTAAAGAAAAACTAAATAATTTTAAGTTG
TGTGTGGTAAAATGTCTGTAAAAATACTTAATTTTAGAGCTAATGGATTATTCTTTCCTGAAAGTCTT
TAGAAATTGATCCAGAGATGTAAAATGGTGATACCAATTTTTATTTCATTTCATAGATGCAAAAAATT
CAGAACTAATTAAAAAAATTGCCAAGCTATGGAGGGAACTTCCTGATTCAGAGAAAAAGGTAAGCATA
TAAATTTTTAACATTGCTGACCAGTTACTTTGCAGGTAAGGACAACTGTCATGTCCTTTAAGGAAAAA
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AATGATGAGAATATGGATAAATGGCAGTGGTGATGAAGCGTTGGTTTTTGAAGCAATTTGAAGTTGGA
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GATGCTTACAGGGCAGACTGGCAGGTATACAAGGAAGAGATAAACAGAATTCAAGAACAACTAACTCC
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GACCACGTGCCTTTGAGTATGTTGTAAACCGTTTTTGCTCCTTTTGGATGTGTCTGAGTTCGAGATAC
ATAATTAGGACTGGTGTGAAAGCTGTAGGAACAGAGTTAGGAAACAGGAAGATCAGGAATAGCAAAAG
TCTTGATTCTCTGTCATTTCTAGAGTACCTTACTGATTTGAATTGTTCTATAGGTGATGGTGAATTTG
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AATAAAATTAAAGTGCCTTTAGGATGAAATTCAATTTTTCCTCATTAAAAATCCCACATGGTTCAAAA
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AAAGCTTACTTCTCTGAAAAAGTAGATTCCAGCCTTTAGTATGAAGTGATGGAATCTCTCTTCCTGAT
CGCTGTGTCTTAAGCCCTCCTAGCTCTTGGGTTGAGGACTCACCCAATTTCAAGTCAATGATAGTAAT
CGGAGACTGCTAATCCAATCAGTAAAAGGAAGCCAGAAAAAATAGGGATGGTATTTGGAGGGTGGAAA
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TTGTATTCCAGTTCAGTTCAGTTGCTCAGTTGTGTCTGACTCTTTGGTACCCCATGGACTGCAGCATG
GCAGGCCTCCCTGTCCATTAGCAACTTCTGGAGGTTACTCAAACTCATGTCCATTGAGTTGGTGATGC
CATCCAACCATCTCATCCTCTGTCATCCCCTTCTCCTCCTACCTTCAATCAGGGTCTTTTCCAGTGAG
TCAGTTCTTCGTACCAGATGGCCAAAGTATTGGAGTTTCAGCTTCAGCATCAGTCCTTCCAGTGAATA
TTCAGGACTGATTTCTTTTAGGATGGACTGGTTGAATCTTCTTGGAGTCCAAGGGGCTCTCAAGAGTC
TTCTCCAACACCACAATTCAAGACATCAATTCTTGAGCGCTCAGCTTTGTTTATGATCCAACTCAACA
TCCATACATGACTACTGGAAAAACCATAGCCTTGACTAGAGAGACCTTTGTTAGCAAAGTAATGTCTC
TGGCTTTTTAATATGCTGTCTAGGTTGGTCATAACTTTCCTTCCAAGGAGCAAGCATCTTTTAATTTC
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TAAGGGTGGTCTCATTGGCATATCTGAGGTTATTGATATTTCTCCCAGCAGTCTTGATTCCAGCTTGT
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ATGTTCTTTTTTTTTCCCTCTTCAGTCTTTGTTCTCTTTACTTTCGGATTTTCGAGGATTTTTTTTTT
TTTTTAGCCATGCTGTGTGGCTTTTGGGATCTTAGTCCCTTGACCAGGGATTGAATGTGTGCCCCCTG
CAGTGGAAGGTTGGAGTCCTAACCACTGGACTGCCAGGGAATTCCCTTTATCTCCAGCACTTCTTTTG
GGTTCTTTCTTATGATTTCCCTCTGCTTACAATGCCTGTTCTTGCAGGCTGTCTAGAATCCTTACCAT

Fig. 6B

ATTAATCACAGTTGTTTTAAAATCCTGGTCTGATAATTTTGTATCCCTGCCAGTTCTGATGCTTGTTT
TGTTTCTTTATGTTAGGCTTTTTGTCTTTTAGTATGCTTTGGGATTTTTTTTCTTGATAGTTAGACAT
ATGATGTGCTGGGTAAAAGGAACTGCTGTATATGGGCCTTCGTAATGTGGTAAGGCATGGGGCAGGGG
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AAGAATACACCTGCCAATGCAGAAGACCAGTCGGGAAGATCCCCTGGACAAGGAAACGGCTGCCCACT
CTAGTATTCTTGCCTGGGAAATCCCGTAGACAGAGGAGCCTGGTGGGCTACAGTCCCTGGGGTTGCAA
AAGAGTCAGATATGAGTTAGCAGTTAAAACACAAAAAACCCTTTAATGAGCCTGTAGCTTTGTGCTTA
GGTGGTACAGGATGGCTAAAGTGAGCTGCAGTCTAGTATTTCCTTTCATTGAGGTCTTTTAGGCTCTT
GATAGTATACCAGCAGGTTAGGTTCTTAATGTTTATTTGTGTGTTTATTTTTTATTTGACTGCACCAG
ATCATTAGTTGCAACACGTGGGATCTAGTGTCCCAGCCATAGATTGAATCCCAACCCCCTGCATTGGG
AGCACAGTCTTAACCGGTAAACCACCAGGGAAGTCCCAGCAGATTAGGTTCTGATTAACTAGTTTCCC
TTGAGGGCAGGCCTTGTTAAGAATAATAAAGTACTCTGGCATTTCGAAATGATTCCTTTTCTTCCTGC
TGAAAGCAAGAGGGGTTTTTTTTCCCTTGATATTTACTGTGGGAATCTGGTCAAGTTCCTGGAGAAA
AACCTCACAGTATTATGAGGCTTTCCTATGAGTGGTGGTAGCTCTCAGAGCTGTCCACATGGAGCTTC
CAGCAGTTAGTCAGCTGTGGTTCAGTTTCCTCCACACCACTGGTTTCCATTCCTCTATCTGTTCCAGT
AAGTCTCGACTCCCTGTGTTCACCTGTCTGTGTCTGTCTCCAATCTTGAGAGCAGCACTTCACCCTGT
GTCCCCTCTTCAGTGGATCCAAGAGAGTTGATTTTGCAGTCTGATCAGCTTTATACTTATTGGATGGA
GTGCTGACTTCCAAACTCCTTACATGTAGAACCAGAAACTTAGAAGTCTGAGTGACTTTTCCGTAAGA
CTTCCATTTGCTCTCCCTGTTGGAATTTTTCTTCCCATTAGATTCCCAGTTCCAGCTACTTGCAGTTT
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CAGAGGAGCCCTGCTTATGAATGAAAGTTGGGTTCGTTATTGACTGTAAACCAAATTGCCATTAAAAC
CCCCATCGTTTTATAAATTTCTATCTAAGTTCAGAAACTCAGTAGGTTAGGATGAGGCAGTGGCCTTC
ATTAGACAATGCAGCAAATGCGCCAATGTTCTTGAGTTGTCAAGTGGGAGTACGTAGTGCTTTGTCCA
AGGAGAAGTGGTGCCTTACAAGGATAAAACGGGTTTTTTTTTAATGTTTAAAAAGATTTTGTACATCC
TGCTCATTTATTCAGTTCATGGAGACCATAAACATAATATGTAAAGGAAATGTTTCTGTATGAAAAGT
AACTTATAAAATCTATTAGGATTTTCAAAACAGTACTAATCAAAAATTCTGATCCCAAGAGGGAACAA
TCGCATACTCATAATGTTCATTTTAAAGAATATTCTGTAATTCATGGTAGTGAATGAAGTTTCTCTAG
GTAAATTTAAGTATCATAGAGTTTAAAACTTTATCTCACCTTTCATTTTTTTTACTC*AGGAGTTAACA*
ATGCTTGGAAAACCGAAAAGACCTCGCTCAGCTTATAACATTTTTATAGCTGAACGTTTTCAGGAAGC
*TAGGGATGGCACATCACAGGT*AAAACAAAACTCTGTATGTTTTAAAAATGTTTACTCTAATTTTTAAA
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GCCCTTTAAACATTTTTGGTTATCCCATGGGAATGTATTATATTACACATATGAATGGTATCTATGTC
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GCTCCAAATTGTTAGTTGTACTATTCCTCTTTTAATATGCCATGGTAAGAAAAGTTGTCTGCTCGGAT
TCCTAATTCTTGTCAGTGTGAGATGGAGGTTTTTGCCACTCTGATTCTTTGGGTTATTTAGATCTGTA
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CAGTAGTTCTCAACTCTGACTGCATCTTGGAATCACCTCAGAAGCTTCCTGGCTGGCCTTTATCTCTG
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ATCAAATGAGGCTTGTTATGAAAGACACAAGTTTCCCTGTGCCGTCATGTTCCTCACTCCAGAAGCAA

Fig. 6B

ATGCATTCTGTTTTAGTTGATTCTTGTAGTATTTACCCCCATGTCTCTAAGTAATGTTCCTCCATTGT
CACGTCCTGATTTTTCTAAAATTCTAAGCGTTACCTATATTTTAGGCATTCCCTCTCACTGTGTAAGG
TGAGCGTTAACTCTGTCTCATCGCACTGTTCACTCTTCTGTCAGTCTGCCTCCCAATATAGTTACATA
ATTTGGGTTAGATCAGTATTGAGTACTTAATACTATGATGTGTGCATGCTAATTTGCTTTAGTCATAT
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AATACTGGAGTGGGTTGCCATGCCCTCCTCCAGGGGATCTTCCTGACCCAGGGATCAAACCAGTGTCT
CTTAAATCTCCTGCATTGGCAGGTGGATTCTTTACCACTAACGCCATCTGGGAAGCTCAGTGGCTCAG
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GGCACCTTCTCTCTCTGTGTCCTCACATGGTGTGAGTGCTCATTTAATGTGATTTTAGATAGCACCAG
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TCGCTCAATTAAATACCCAGCAAAAAATGACCCTGAGAAGTTTTAAAATAGAAGATTGAGTTATGTTC
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TTAGGAACTACTGAGCATCAAAATAATCCATGAAATGTAGCAGTGAATCATTTTACCTTTGATAAAGG

Fig. 6B

TAAATCAGACTGTGAAGTTTTTTTATACTTGGTGATTATGGAAAAAATATTCTTGTTTCCTTATATTA
TGGAAGCAGGAGTTTCCTATTCATAAGTATCTCAAAGTTTGTAGAAGCCATAGTGTTCTATGATATAA
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GATTTATAAACCTTTTCCTCATTATCTTTTTCCTAAATTAAAAACTAAAAAATTATGTACCAAATCTA
TGCATATTGTTTTATATTGCATAGAATAAAAATTATGTGTTTCCTAATTATGTTTTAAGGTGAAACAT
TCATTTTATAGCTTTCTGGGATTTTTGTTTGTTTTTTTAAACAAAGTAGGAGTTTGTATACTGAATTA
TTTTTTCTCTTATGTAAATATATTTATCCAGAAAGTAGAGAACTTATTTGGTGTAAGTTTTAAAATGA
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TAATCTGATTTCACACCTTTCTTAATAAACTTCAGTACATGGCATACCATGTATTAAATGCCATCCAT
TTGTATATACAAATACTCTGTTATGAAGAATGAGAGCGTATATCAATAACCAGTGGCTAGGTGTGTAA
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TCTGAATTAAAATGCAGTTAAAGTAACTGGCAAGTTTTATTGATATTTGTGACATTTACTGTACCTGA
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TATGGC

Fig. 6B

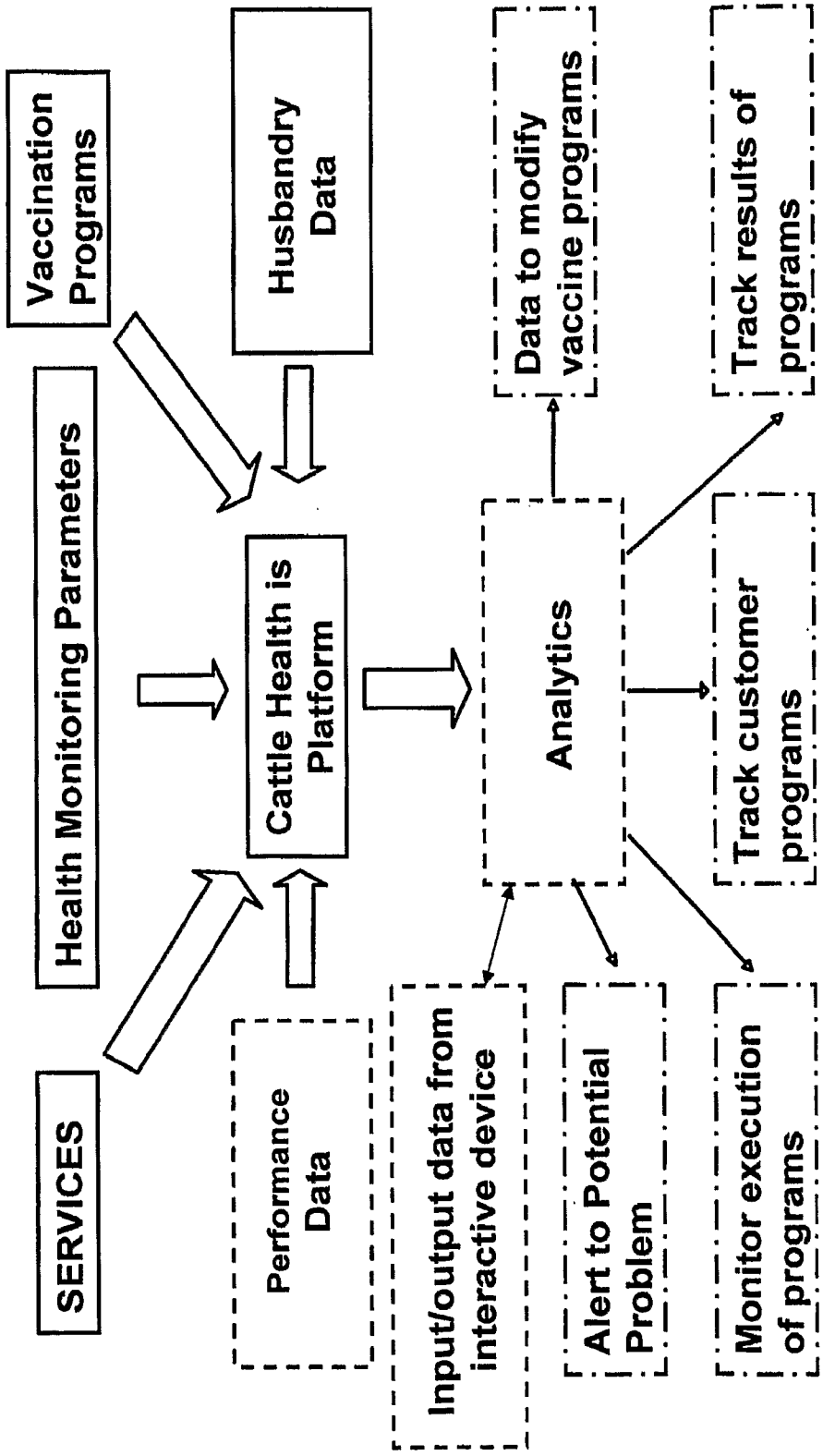

Vaccination Programs
Husbandry Data
Health Monitoring Parameters
Cattle Health is Platform
Analytics
Data to modify vaccine programs
Track results of programs
Track customer programs
SERVICES
Performance Data
Input/output data from interactive device
Alert to Potential Problem
Monitor execution of programs

Fig. 7

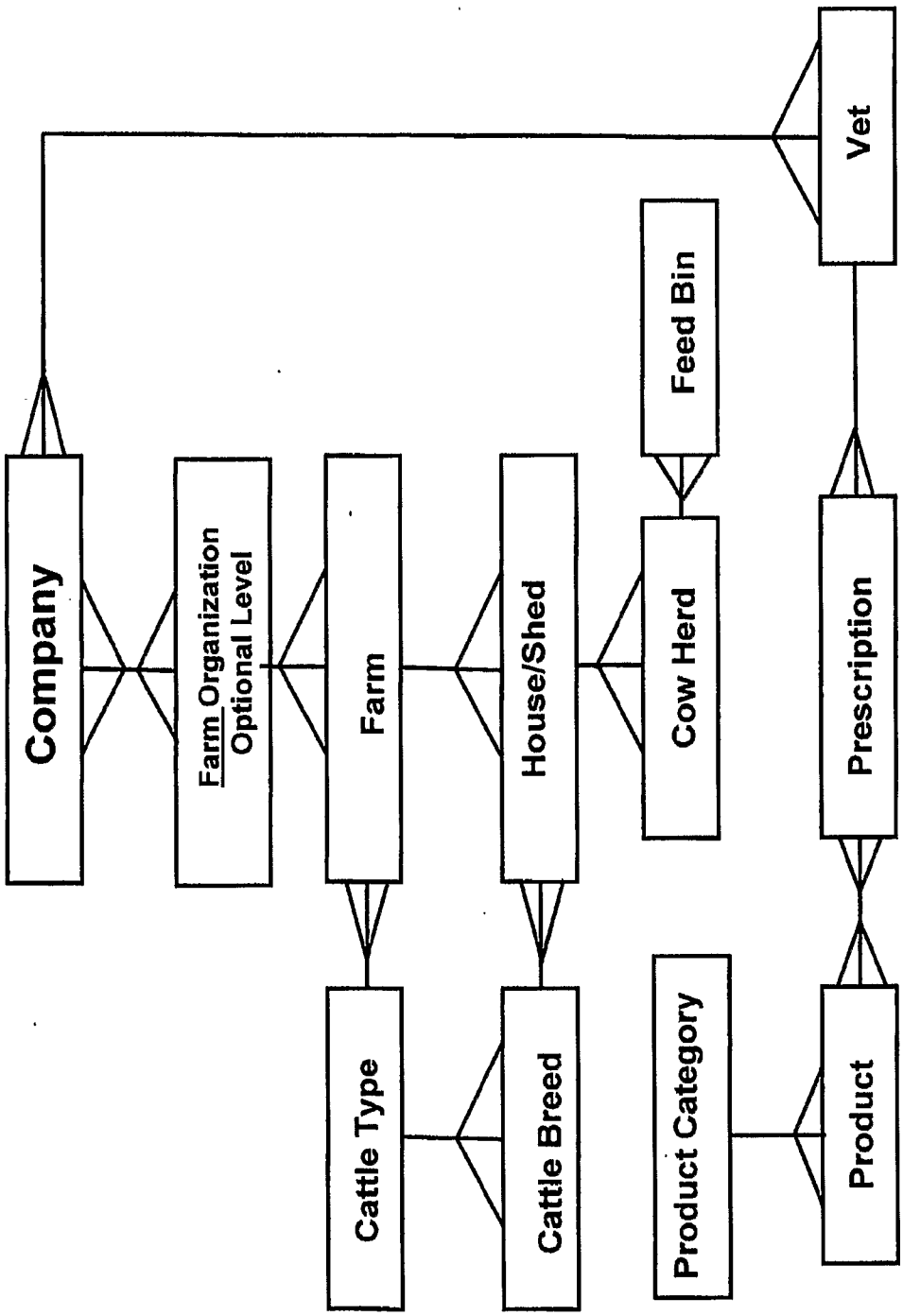

Company
Farm Organization
Optional Level
Farm
House/Shed
Cow Herd
Feed Bin
Vet
Prescription
Cattle Type
Cattle Breed
Product Category
Product

Fig. 8

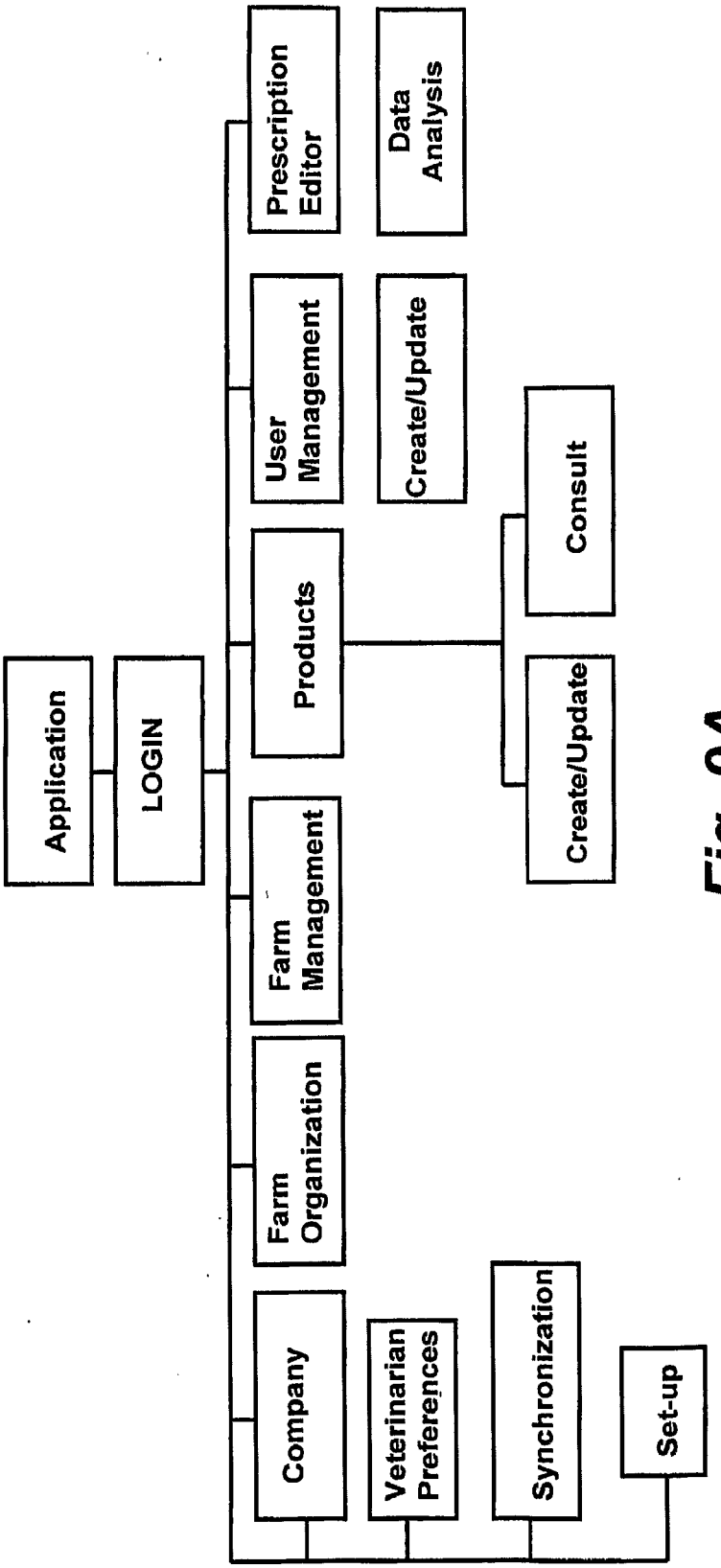

Application
LOGIN
Products
Prescription Editor
Data Analysis
User Management
Create/Update
Consult
Create/Update
Farm Management
Farm Organization
Company
Veterinarian Preferences
Synchronization
Set-up

Fig. 9A

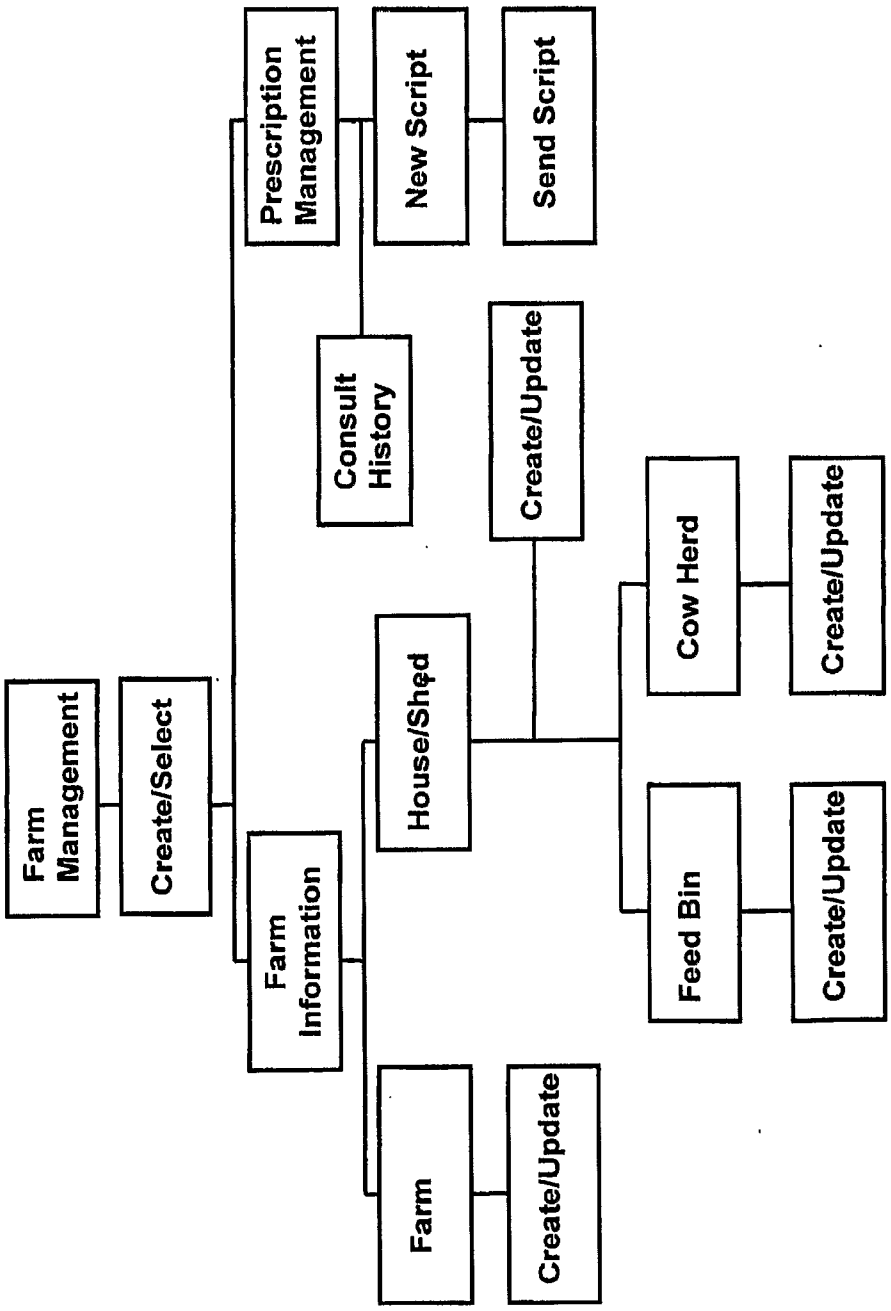

Farm Management
Create/Select
Farm Information
Prescription Management
New Script
Send Script
Consult History
House/Shed
Create/Update
Cow Herd
Create/Update
Feed Bin
Create/Update
Farm
Create/Update

Fig. 9B

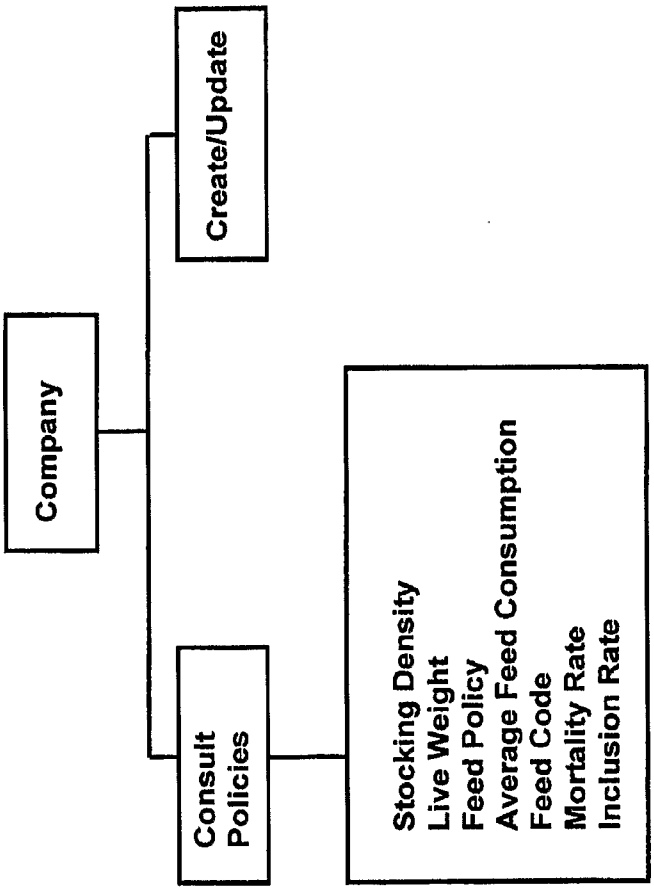

Company
Create/Update
Consult Policies
Stocking Density
Live Weight
Feed Policy
Average Feed Consumption
Feed Code
Mortality Rate
Inclusion Rate

Fig. 9C

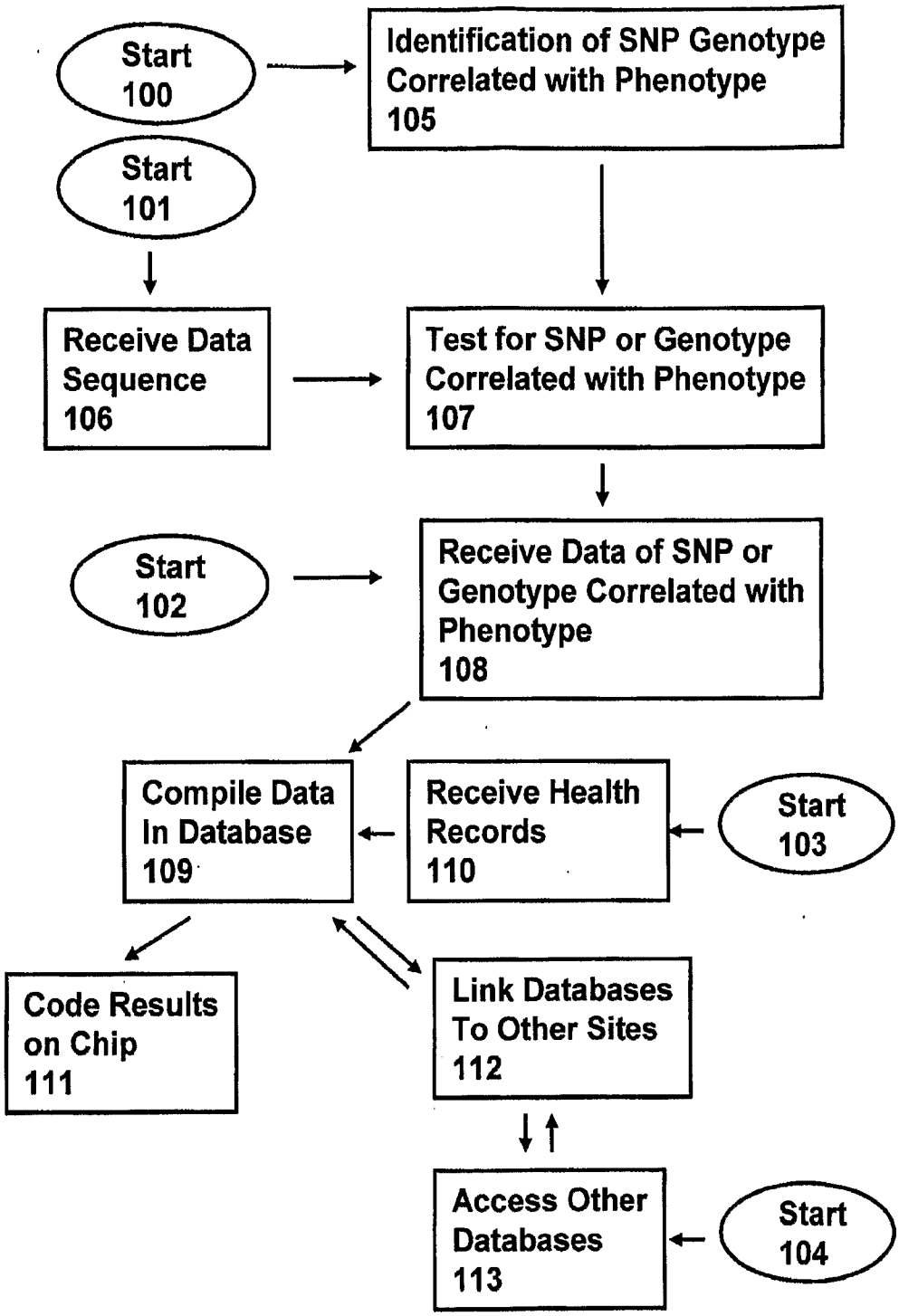

Start
100
Identification of SNP Genotype Correlated with Phenotype
105
Start
101
Receive Data Sequence
106
Test for SNP or Genotype Correlated with Phenotype
107
Start
102
Receive Data of SNP or Genotype Correlated with Phenotype
108
Compile Data In Database
109
Receive Health Records
110
Start
103
Code Results on Chip
111
Link Databases To Other Sites
112
Access Other Databases
113
Start
104

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/20775

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/20775

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12Q 1/68**(2006.01);**C12P 19/34**(2006.01);**C07H 21/02**(2006.01),**21/04**(2006.01);**G06F 17/00**(2006.01),**15/00**(2006.01)

USPC: 435/6,91.2;536/23.1,24.3;705/500;709/218
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6,91.2;536/23.1,24.3;705/500;709/218

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed, Agricola, Biosis, CABA, CAplus. Keywords: TFAM, mitochondira transcription, bovine, SNP, marbling

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JIANG, Z. et al. Significant associations of the mitochondrial transcription factor A promoter polymorphisms with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. Biochem Biophys Res Commun. 2005 Aug 26;334(2):516-23. Entire Reference.	1-5
A	MANNEN, H. et al. Identification of mitochondrial DNA substitutions related to meat quality in Japanese Black cattle. J Anim Sci. 2003 Jan;81(1):68-73.	1-5
A	STEINBORN, R. et al. Genetic variation in functionally important domains of the bovine mtDNA control region. Biochim Biophys Acta. 1998 May 11;1397(3):295-304.	1-5
A	CHOI, Y.S. et al. Mitochondrial transcription factor A (mtTFA) and diabetes. Diabetes Res Clin Pract. 2001 Dec;54 Suppl 2:S3-9.	1-5

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
29 September 2006 (29.09.2006)

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/20775

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1-5, drawn to methods for identifying animals having particular traits comprising detection of a polymorphism in a TFAM gene.

Group 2, claim(s) 6-12, drawn to an interactive computer-assisted method for tracking livestock.

Group 3, claim(s) 13, drawn to a method of transmitting data.

Group 4, claim(s) 14-15, drawn to a computer system.

Group 5, claim(s) 16-19, drawn to a method of doing business.

The inventions listed as Groups 1-5 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The common technical feature of the listed groups is determining the presence of a single nucleotide polymorphisms in the TFAM gene of an animal. However, this is not an improvement over the prior art of Choi et al ('Mitochondrial transcription factor A (mtTFA) and diabetes (2001) Diabetes Research and Clinical Practice 54 Suppl 2, pages S3-S9). Choi et al teaches the analysis of single nucleotide polymorphisms of the mtTFA (which is a synonym of TFAM) promoter region in Korean humans.

Additionally, the animal identification method of claim 1 is not required for the computer-assisted method for tracking and rearing of livestock bovines of group 2, the data transmission methods of group 3, and the business method of group 5, and the computer system of group 4 is not required for the method of claim 1.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

The different species related to different physical characteristics are:
a) Calc Lv Wt, Lb
b) cYG
c) DOF, d
d) DMI, lb
e) DMI per DOF, lb/d
f) HCW, lb
g) HCW value, $
h) IMF%, %
i) MBS, 10-99

j) MBS/DOF
k) QG, <Se vs, >Ch
l) REA, in^2
m) REA/cwt HCW, in^2/100 lb HCW

The different species related to different specific polymorphisms in the promoter of the TFAM gene are:
i) A to C at the -1220 position
ii) T to C at the -1212 position
iii) T to C at the -995 position

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The different physical characteristics (a-m) represent distinct traits that are measured using different techniques and compared using different criteria.

The different nucleotide sequences represented by the nucleotide content at the different polymorphic positions (i-iii) are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences.

The first named invention is a method for identifying an animal having a desirable calculated live weight (Calc Lv Wt, lb) by determining the presence of a an A to C substitution at the -1220 nucleotide position in the promoter of the TFAM gene.

Continuation of Box III Item 3:
1-5 as they relate to Calc Lv Wt, IMF%, and MBS, and the polymorphisms at -1220, -1212, and -995