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ANIMAL GENOTYPING METHOD

5 This invention relates to a method for identifying a physical trait of an animal by genotyping in the region of a gene which determines variation in the physical trait and determining whether the animal possesses a specific variant of a gene by reference to the DNA in the region of the gene, rather than by genotyping for the gene itself. In particular, the invention relates to a method for determining whether a bovine animal possesses a gene for the β -casein A¹ protein or a gene
10 for the β -casein A² protein and therefore the ability of bovine cows to produce β -casein A¹ or β -casein A² in their milk.

BACKGROUND

15 Consumers demand high quality and low price when purchasing goods such as dairy and meat products. However, many consumers also want assurance that the products and production systems are managed to minimise risk and maximise benefits to human health, while also being non-detrimental to the
20 environment and to the welfare of animals.

It is well known that the genetics of an animal has a substantial impact on production and product quality, and on health, environmental and animal welfare issues. The ability to determine a phenotype of an animal by using a genetic test
25 is a valuable tool for achieving rapid identification of animals and animal products with beneficial characteristics and for forming a group of animals having enhanced production and/or product quality. Animals can be grouped based on genetic differences that relate to animal or animal product traits that are of economic interest. In the case of the dairy industry, examples of important traits
30 are milk production, milk protein content, fat production, and specific components of milk that are associated with health, for example the absence of the β -casein A¹ protein or the percentage of saturated fats.

In a typical genetic test, the DNA sequence of a gene encoding a protein or group of proteins related to a physical trait of interest will be known. A DNA sample is obtained from an animal and a combination of polymerase chain reaction (PCR) amplification, DNA fragment analysis, and data processing is used to identify the DNA present at the known location for the gene in the animal's genome. Highly automated testing enables the presence of a gene or gene variant, and therefore the ability to exhibit a physical trait, to be determined for a large number of animals comparatively quickly and efficiently.

The gene that is responsible for a particular physical trait of an animal may be identifiable by a single nucleotide polymorphism (SNP). An SNP is a DNA sequence at a location in an animal's genome which is different to the DNA sequence at the same location in the genome of another animal by virtue of only one nucleotide. Even a difference as small as this can mean an animal exhibits a particular physical trait whereas another animal does not.

One example of the significance of an SNP is the genetic makeup of a bovine cow that enables the production of β -casein proteins in its milk. Typically, a cow will produce β -caseins in its milk. However, several β -casein variants are known including A¹, A², A³, B, C, D, E, and F. One difference between the A², A³, D, E, and F variants on the one hand and the A¹, B, and C variants on the other hand is that the former group has a proline residue at position 67 of the β -casein protein whereas the latter group has a histidine residue at position 67. This difference is determined by substitution of the nucleotide adenine with the nucleotide cytosine at position 200 of the coding region of the β -casein gene. It is therefore possible to distinguish between the two groups of β -casein variants by identifying and testing for the SNP that encodes for the β -casein protein of an animal.

There are a number of reports indicating that the presence of β -casein A¹ in the human diet is linked with the incidence of certain diseases, specifically diabetes (Elliott, R. B., Harris, D. P., Hill, J. P., Bibby, N. J., Wasmuth, H. E., Type I (Insulin-Dependent) Diabetes Mellitus and Cow Milk: Casein Variant Consumption, Diabetologia 1999; 42:292-6; Wasmuth, H. E., Rosenbauer, J.,

Elliot, R. B., McLachlan, C., Erhardt, G., Giani, G., Kolb, H., β -Casein A1 Consumption and Incidence of Type 1 Diabetes in Germany. Kongress der Europäischen Diabetesgesellschaft vom 28.-30.09.1999 in Brüssels/Belgium. Proceedings published in Diabetologia 42 (Suppl.1): A88; 1999) and coronary heart disease (McLachlan, C.N. (2001) β -Casein A1, Ischaemic Heart Disease Mortality, and Other Illnesses. *Med. Hypotheses* 56(2):262-72).

In addition to phenotyping a cow by identifying the particular β -casein variant or variants produced in the cow's milk, it is well known to genotype a cow by identifying the SNP it possesses to gain knowledge of whether the cow has the ability to produce a certain β -casein variant. A method of selecting bovine cows on the basis of such genotyping to form milking herds which will produce milk free of the β -casein A¹ variant, and preferably solely the β -casein A² variant, is described in PCT/NZ96/00039 (published as WO 96/36239).

Studies have shown that SNPs, or other DNA variants (tandem repeats, insertion-deletions) are valuable in predicting a disease, the quality of an animal product, or a production benefit. However, in cases where genetic selection is used to sort, cull or mate animals, a selection strategy based on the use of a single SNP or trait is sub-optimal. This is because an SNP, such as an SNP within the β -casein gene, is not randomly associated with the surrounding DNA. The region of DNA that surrounds the genotyped SNP may encode one or more functions which also influence a physical trait. Selection based on a single SNP may therefore inadvertently select for traits additional to the trait of interest.

The inventors have now found that it is possible to determine whether a bovine animal possesses a gene for the β -casein A¹ protein or a gene for the β -casein A² protein, not by identifying the DNA of that gene, but by identifying SNPs or haplotypes (combinations of SNPs) in the region of the animal's genome where the gene for β -casein is located.

It is therefore an object of the invention to provide a novel method of genotyping an animal for its β -casein gene, or to at least provide a useful alternative to known methods.

STATEMENTS OF INVENTION

5 In a first aspect of the invention there is provided a method for determining whether a bovine animal possesses a gene encoding for the protein β -casein A¹ or a gene encoding for the protein β -casein A² by testing the DNA of the animal for the presence of at least one DNA marker for either of the β -casein A¹ or β -casein A² genes, where the at least one DNA marker is located in a gene encoding for the protein α_{s2} -casein.

10 In a preferred embodiment of the invention each of the at least one DNA markers represents a single nucleotide polymorphism (SNP), a tandem repeat, or an insertion-deletion. Preferably the at least one DNA markers represents an SNP.

Although the method may be used to determine the β -casein genotype for bovine bulls or cows, the bovine animal of the invention is preferably a cow.

15 In a preferred embodiment of the invention the at least one DNA marker is linked with the presence or absence of β -casein A² in the milk of a bovine cow. In an alternative embodiment, the at least one DNA marker is linked with the presence or absence of β -casein A¹ in the milk of the cow.

20 In a preferred embodiment of the invention the at least one DNA marker is a marker for the gene encoding for β -casein A¹. In an alternative embodiment of the invention the at least one DNA marker is a marker for the gene encoding for β -casein A².

25 The DNA of the animal tested may be obtained from any tissue of the animal which contains or contained nucleated cells, preferably blood, sperm, hair, or milk of the animal.

30 In a second aspect of the invention there is provided a method of producing milk substantially free of β -casein A¹ including the steps:

- a) determining whether one or more bovine cows possesses a gene encoding for the protein β -casein A¹ or a gene encoding for the protein β -casein A² according to the first aspect of the invention;

- b) selecting those cows that do not have a gene encoding for the protein β -casein A¹; and
- c) milking the selected cows.

5 Preferably the milk produced by this method is milk containing β -casein which is substantially all β -casein A².

10 In another aspect of the invention there is provided a method of forming a herd of bovine cows by testing the DNA of each cow in accordance with the method of the first aspect of the invention and selecting those cows that possess a gene encoding for β -casein A² and do not possess a gene encoding for β -casein A¹.

15 In another aspect there is provided milk obtained from a bovine cow that has been tested in accordance with the method of the first aspect of the invention.

In a further aspect of the invention there is provided a food or food product containing, or processed from, the milk of this invention.

20 In yet another aspect of the invention there is provided semen or an embryo from an animal that has or have been tested in accordance with the method of this

invention. Preferably the semen or embryo is used for producing offspring using any artificial reproduction technique.

DETAILED DESCRIPTION

This invention enables a user to group a population of cattle, or tissue derived from an animal (such as blood, sperm, hair, or milk) based on genetic differences. In a preferred embodiment of the invention these genetic differences are SNPs or combinations of SNPs. The use of such SNPs, as well as enabling a user to catalogue populations of animals, can also be used to predict an animal phenotype.

Examples of traits relating to milk production include milk yield, milk protein composition, amount of milk fat, and more specific traits such as the presence of β -casein variants, the proportion of saturated and unsaturated fat, and the number of somatic cells present in the milk.

The variant caseins are distinguished by a small number of amino acid changes in the overall sequence of these proteins. In bovine cattle, the difference between the A^2 and A^1 variant of β -casein is a single change in amino acid from a proline to histidine at position 67 of this protein. The relevant SNPs occur on chromosome 6 of *Bos taurus* bovines.

While other β -casein variants have histidine as opposed to proline at position 67, they are usually minor variants and for the purposes of describing this invention are to be considered generally as β -casein A^1 . Similarly, those β -casein variants, in addition to β -casein A^2 , which have proline at position 67 are usually minor variants and are to be considered generally as β -casein A^2 .

A determination can be made as to whether cattle, or tissue derived from cattle, possesses genes that code exclusively for the A^1 variant of β -casein (A^1 homozygous), exclusively for the A^2 variant (A^2 homozygous), or for a mixture of

the A¹ and A² variants (A¹/A² heterozygous). This allows cattle to be identified which produce milk containing solely β -casein A¹, solely β -casein A², or a mixture of both of these casein variants. It also allows cattle to be identified which may produce offspring with the ability to produce milk containing solely β -casein A¹, solely β -casein A², or a mixture of both of these casein variants.

In this way, herds of milking cows can be formed which produce milk having a particular physical trait, for example the absence of the β -casein A¹ protein or the presence of only β -casein A² of the β -casein variants.

An additional feature of the invention is that once animals with a particular genotype or haplotype have been selected and milk is produced from them, the origin of the milk, or other products such as milk powder and processed milk products, can be verified as being produced from the selected animals. This has the benefit of providing consumers with confidence that the milk is indeed from animals of the desired genotype or haplotype group.

Specifically, the inventors have shown that SNPs can be readily identified in regions outside the β -casein gene which are predictive of β -casein protein type. These SNPs are therefore also predictive, or potentially causative or partially causative, of health risks associated with β -casein alleles. A population of cattle with eight SNPs has been identified and analysed. The SNPs are derived from the casein gene cluster of cattle which includes the gene encoding β -casein. This region of DNA is estimated to consist of about 200-300 kilobases of DNA. Of these eight SNPs associated randomly in different individuals, a theoretical number of possible combinations (or haplotypes) of 2 to the power of eight (2^8) would be expected. Surprisingly, the number of haplotypes observed with these SNPs and subsets of these SNPs is much less than what would be expected randomly. It is therefore possible, using the discovery of the specific associations between the SNPs, to correctly class a cattle population into haplotype groups using only a small number of SNPs. More importantly, these haplotypes can be used to predict specific phenotypes in individuals or populations, such as the identity of the α -S1, α -S2, β -, and κ -casein milk protein variants.

The associations between SNP haplotype(s) and production and product quality traits were examined. These traits, specific to bovine milk, include characteristics associated with β - or κ -casein variants, whey %, protein %, fat %, fatty acid profiles (C4 to C22), conjugated linoleic acid content (CLA), melting point, β -lactoglobulin content, lactoferrin content, somatic cell count, daily milk yield, fat yield, protein yield, full-lactation yields of milk, fat and protein.

It was found that significant associations exist between casein region haplotype and production and product quality traits, such as somatic cell count, fat %, protein %, β -casein yield, and fatty acid profile.

Information about the non-random association between adjacent DNA markers and combinations of these markers (i.e. haplotypes) in a population, and the relationship between those markers and haplotypes and physical traits has the following potential benefits:

1. Selection procedures for selecting animals based on a single marker can be modified to avoid or minimise the inadvertent amplification of undesirable physical traits, which may result from co-selection of linked genetic information with known or unknown effects on phenotype.
2. Identification of specific combinations of SNP alleles within a region of the genome (haplotypes) which provide an equal or better predictor of product quality than any single SNP within the region.
3. Identification of a subset of SNP alleles which efficiently predict variation in a larger group of known or unknown SNPs.
4. Prediction, with a useful degree of accuracy, of the identity of an SNP which for any reason cannot itself be genotyped.

5. Identification of the major association groups occurring within a region of a genome into which new, as yet undiscovered, DNA variations in that region will typically fall.

5 This invention enables the identification of SNPs, and the use of SNPs and SNP haplotypes to:

1. Provide the means to efficiently select, sort or group animals producing specific α -casein and/or β -casein and/or κ -casein variants using a minimum of SNP tests. In particular, genotypes and phenotypes related to casein variants can be inferred by genotyping a small number of associated SNPs. The use of associated markers that are linked to multiple genotypes or phenotypes, or to genotypes that are unable to be tested, can provide economic and technological advantages.
2. Predict the performance of dairy cattle and therefore product quality and health effects of the milk from the cattle, or the progeny of the cattle, as well as or better than any single SNP from the casein region.
3. Catalogue much of the variation in the casein genes in the cattle population with a minimum number of markers therefore providing an efficient means to class or group cattle according to predicted performance or product quality.
4. Provide an efficient means of selecting specific casein types (e.g. β -casein A²) while minimising the alteration of the frequency of variants in related caseins.
5. Provide a means to select for other casein genes or associated traits, while at the same time increasing the proportion of β -casein A² animals in a specific herd.

The invention is described in further detail by reference to the following examples that demonstrate the procedure for discovering SNPs and how these can be used. It is to be appreciated that these examples do not limit the scope of this invention. Any person skilled in this area of technology will recognise that these procedures may be used generally to discover useful SNPs in the genome of an animal.

EXAMPLES

Example 1: Identification of SNPs in a population

The DNA sequences in the α s1, α s2 and β -casein genes from a number of individual cattle were determined and analysed to identify SNPs. A comparison of these sequences enabled the identification of polymorphic sequences.

The oligonucleotide primers SEQ ID NO:1 to SEQ ID NO:14 were designed and synthesised. The sequences are based on the published sequences of the *Bos taurus* α s1 (ACCESSION X59856) and α s2 (ACCESSION M94327) sequences. Each primer pair was also designed so that any resulting PCR product would have an Xho1 and EcoR1 restriction endonuclease site at its flanking ends. The conditions for PCR amplification were optimised for the appropriate primer pairs:

SEQ ID NO:1, SEQ ID NO:2
SEQ ID NO:3, SEQ ID NO:4
SEQ ID NO:5, SEQ ID NO:6
SEQ ID NO:7, SEQ ID NO:8
SEQ ID NO:9, SEQ ID NO:10
SEQ ID NO:11, SEQ ID NO:12
SEQ ID NO:13, SEQ ID NO:14

From these results it was determined that regions of DNA corresponding to exons 1-2 and exons 3-6 of the α s1 gene and exons 1-2 and 17-18 of the α s2 gene gave PCR products of the anticipated sizes.

Seminal genomic DNA was purified from cattle that were previously genotyped and shown to be either carriers of the A¹ or A² variant of the β -casein protein. DNA was amplified using genomic DNA mixed in equimolar concentrations from five A¹ homozygous (A¹ DNA) and five A² (A² DNA) homozygous animals. The PCR products of amplification from A¹ DNA and A² DNA were purified with a High Pure PCR Product Purification Kit, digested with *EcoR*1 and *Xho*1 restriction endonucleases, and the enzymes then heat inactivated by heating for 20 min at 70 °C. A portion of each of these samples was ligated into the plasmid pBluescript KS (*Stratagene*) that was previously digested with *Xho*1 and *EcoR*1 restriction enzyme, treated with shrimp alkaline phosphatase (*Amersham Pharmacia Biotech Inc.*) and finally heat inactivated for 20 min at 70 °C.

The ligated plasmids were used to transform chemically competent *E.coli* strain XL1-Blue. Individual colonies containing either inserts derived from A¹ or A² DNA were analysed by colony PCR. For the α s1 gene region, plasmids containing DNA from exons 3-6 (3 each from the A¹ and A² DNA) and exons 1-2 (5 derived from A¹ DNA and 5 from A² DNA) were sequenced. In the α s2 region plasmids from the region spanning exon 1-2 (5 each from the A¹ and A² DNA) and exon 17-18 (5 each from the A¹ and A² DNA) were sequenced. A comparison of these sequences enabled identification of polymorphic sequences and nucleotides. Examples of such polymorphic sequences are given in SEQ. ID. NOs:15-28.

Example 2: The use of SNPs as predictive tools

The sequences were analysed by alignment using programs from the *Wisconsin Package Version 10.2, Genetics Computer Group (GCG), Madison, Wisc.* Surprisingly, the analyses revealed that a number of the SNPs could be readily used to distinguish an A¹ animal from an A² animal. The alignment of the sequences derived from the α s2 genes of A¹ homozygous and A² homozygous animals revealed that a number of SNPs in these sequences were linked directly with either the A¹ or the A² animal. Therefore, as shown in Figure 1, a total of 10

plasmids containing inserts derived from the α S2 genes of 5 pooled DNAs from A¹ animals and from 5 A² animals were sequenced bi-directionally from the t7 and t3 primer sites in the pbluescript plasmid.

5 The sequences were all aligned using the programs Gelstart, Gelenter, Gelmerge and Gelassemble. The output from Gelassemble is given in Figure 1 where the sequences from P1 to P5-82_t3 or t7 are derived from A¹ homozygous cattle and the sequences P6 to P10-82_t3 or t7 are from A² homozygous cattle. A
10 consensus sequence is also given in this alignment. Multiple differences or polymorphisms occur between the sequences from different individuals. Many of these differences are single deletions or are single nucleotide base differences (SNPs) occurring between individuals. In the sequences three SNPs at positions 664, 926 and 1377, reading consecutively from the first base of the consensus
15 sequence, have a guanine (G), thymine (T) and T respectively in only the A¹ animal sequences. Conversely, in the A² animal sequences an adenine (A), G, and cytosine (C) occur respectively at these positions of the sequence. The three SNPs at positions 664, 926 and 1377 are shown in bold and are underlined. The *Eco*R1 (GAATTC) and *Xho*1 (CTCGAG) restriction endonuclease recognition site are also shown in bold.

20 The identity of these nucleotides can also be determined by methods other than by sequencing, especially those methods used in combination with genomic sequence-specific amplification technologies, such as the polymerase chain reaction (Mullis, K. et al., Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Erlich H. et al., EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. et al., US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. et al., US 4,683,194)). Examples of such methods include an exonuclease resistance method (US 4,656,127), primer-guided nucleotide
25 incorporation procedures for assaying polymorphic sites in DNA (Komher, J. S. et al., Nucl. Acids. Res. 17:7779-7784 (1989); Sokolov, B. P., Nucl. Acids Res. 18:3671 (1990); Syvanen, A.-C., et al., Genomics 8:684-692 (1990); Kuppaswamy, M. N. et al., Proc. Natl. Acad. Sci. (U.S.A.) 88:1143-1147 (1991); Prezant, T. R. et al., Hum. Mutat. 1:159-164 (1992); Ugozzoli, L. et al., GATA 9:107-112 (1992); Nyren, P. et al., Anal. Biochem. 208:171-175 (1993)), the
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"Oligonucleotide Ligation Assay" ("OLA") (Landegren, U. et al., Science 241:1077-1080 (1988)), pyrosequencing (Kittles et al. Cancer Epidemiol Biomarkers Prev 2001 Sep;10(9):943-7), and MassARRAY (Sequenom Corp.).

5 **Example 3: The use of SNPs to predict a genotype or phenotype by using an alternative genotyping method**

10 This example shows that the identity of SNPs, in a larger population than used in Example 2, can be determined with methods other than DNA sequencing and that these SNPs can be used to predict a genotype or phenotype. Specifically, a method based on PCR amplification, primer extension and finally analysis of the primer extension product with a *Sequenom Mass Spectroscopy* apparatus was used to predict whether an animal, or tissue derived from an animal, contains either one or two copies of the gene encoding the A¹ (or conversely the A²) variant of the milk β -casein gene.

20 The semen from a known heterozygous A¹A² animal was used to artificially inseminate cows. DNA samples (from milk) were isolated from the sire and from the progeny of these matings. The identity of the progeny with respect to them being phenotypically or genotypically A¹ or A² was determined independently. A total of 71 progeny were analysed for SNPs associated with AC00069, AC00070, AC00055, AC00057, AC00058, AC00059, AC00060, AC00061, AC00063, and AC00064 (see SEQ ID. listing) with a standard procedure from *Sequenom Inc.* In addition to these SNPs, markers AC000069 and AC000070, that determine the identity of the milk protein variant β -casein A or B, were also genotyped.

30 The genotypes from individuals with complete genotypes were analysed to derive their haplotypes (i.e. the combination and organisation of the markers as they occur physically their chromosomes). Initially, the package *Crimap* was used to check the SNPs for anomalies whereupon the maternal and two paternal haplotypes were derived with the program *Simwalk*. The haplotypes derived from analyses of these SNPs, including the SNP determining the A¹ or A² β -casein milk phenotype, are given in Table 1.

Table 1: Derived haplotypes of cattle individuals

<i>animal</i>	<i>haplotype</i>										
<i>ID</i>	<i>ac69</i>	<i>ac70</i>	<i>ac55</i>	<i>ac57</i>	<i>A1 or 2</i>	<i>ac58</i>	<i>ac59</i>	<i>ac60</i>	<i>ac61</i>	<i>ac63</i>	<i>ac64</i>
87203a	C	A	C	C	A1	C	G	T	G	T	T
87203b	C	A	T	T	A2	C	A	C	A	G	C
5001	T	C	C	T	A1	T	G	T	A	T	C
5002	T	C	C	T	A2	T	G	C	A	G	C
5003	C	A	C	T	A2	T	A	T	A	T	C
5004	C	A	T	T	A2	T	G	C	A	G	C
5005	T	C	C	T	A2	T	G	C	A	G	C
5006	C	A	C	T	A1	T	A	T	G	T	T
5019	C	A	T	T	A1	C	G	T	G	T	T
5022	C	A	C	T	A1	T	A	T	G	T	T
5023	C	A	T	C	A2	C	G	C	A	G	C
5025	T	C	C	T	A2	C	A	C	A	G	C
5027	C	A	C	T	A1	T	G	T	G	T	T
5029	C	A	C	C	A1	T	G	T	G	T	T
5032	C	A	T	T	A2	C	A	C	A	G	C
5034	C	A	T	T	A2	T	A	C	A	G	C
5035	C	A	C	T	A1	T	A	T	G	T	T
5036	T	C	T	T	A2	T	A	T	A	T	C
5037	C	A	C	C	A1	T	G	T	G	T	T
5038	T	C	C	T	A2	C	A	C	A	G	C
5040	T	C	C	C	A2	T	G	C	A	G	C
5044	T	C	C	T	A2	T	G	C	A	G	C
5045	T	C	T	T	A2	T	G	C	A	G	C
5048	C	A	T	T	A1	T	G	T	G	T	C
5049	C	A	C	T	A1	C	G	T	G	T	T
5050	T	C	C	T	A1	T	G	T	G	T	T
5053	C	A	T	T	A2	T	G	C	A	G	C
5054	C	A	C	T	A1	T	G	T	G	T	T
5055	C	A	C	T	A1	T	G	T	G	T	T
5056	C	A	T	T	A2	T	A	C	A	G	C
5057	C	A	T	T	A2	T	A	C	A	G	C
5061	C	A	C	T	A1	T	G	C	G	T	T
5062	C	A	C	T	A1	T	G	T	G	T	T
5064	C	A	C	T	A2	T	A	T	A	T	C
5065	T	C	C	T	A1	T	G	T	G	T	T
5066	C	A	C	T	A1	C	G	T	G	T	T
5069	C	A	C	T	A1	C	G	T	G	T	T
5070	C	A	T	T	A2	C	G	C	A	G	C

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There are a total of 38 haplotypes derived that consist two paternal and 36 maternal haplotypes. However, with closer observation there are only 18 unique haplotypes associated with these animals. More importantly these SNPS, or

more specifically a small subset of these SNPs, can be used to discriminate between animals having the A¹ or A² genotypes. This study showed that in the 36 animals and the sire it would require the genotyping of only one of the SNPs (AC00061) to infer successfully all but one of the identities of these animals (i.e. 97% accuracy). The genotyping of a further two SNPs (AC00059 and AC00063) allows all the animals in this population to be successfully identified in terms of being of A¹ or A² genotype and phenotype (see Table 2).

Table 2: Prediction of β -casein genotype with a haplotype of markers outside the β -casein gene

Haplotype of markers outside β -casein gene			β -casein nucleotide 200 (A1/A2)	
AC61	AC59	AC63	nC (A2)	nA (A1)
A	A	G	7	
A	A	T	3	
A	G	G	9	
A	G	T		1
G	A	G		3
G	G	T		15

Although the invention has been described by way of example, it should be appreciated that variations and modifications may be made without departing from the scope of the invention. Furthermore, where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in this specification.

CLAIMS

1. A method for determining whether a bovine animal possesses a gene encoding for the protein β -casein A¹ or a gene encoding for the protein β -casein A² by testing the DNA of the animal for the presence of at least one DNA marker for either of the β -casein A¹ or β -casein A² genes, where the at least one DNA marker is located in a gene encoding for the protein α_{s2} -casein.
2. A method as claimed in claim 1, where the at least one DNA marker is located in exon 17 or exon 18 of the α_{s2} -casein gene.
3. A method as claimed in claim 1 or claim 2, where each of the at least one DNA markers is an SNP.
4. A method as claimed in claim 3, where the SNP is AC000061 of SEQ ID No:21.
5. A method as claimed in claim 4 which further comprises testing for the presence of the SNPs AC000059 of SEQ ID No:19 and AC000063 of SEQ ID No:23.
6. A method as claimed in any one of claims 1 to 5 where the bovine animal is a cow.
7. A method as claimed in claim 6, where the presence of the at least one DNA marker is linked with the presence or absence of β -casein A¹ in milk produced by the cow.
8. A method as claimed in claim 6, where the presence of the at least one DNA marker is linked with the presence or absence of β -casein A² in milk produced by the cow.
9. A method as claimed in any one of claims 1 to 8, where the at least one DNA marker is a marker for the gene encoding for β -casein A¹.
10. A method as claimed in any one of claims 1 to 8, where the at least one DNA marker is a marker for the gene encoding for β -casein A².
11. A method as claimed in any one of claims 1 to 10, where the DNA of the bovine animal is obtained from any tissue of the bovine animal which contains or contained nucleated cells.

12. A method as claimed in claim 11, where the DNA of the animal is obtained from blood, sperm, hair, or milk of the animal.
13. A method of producing milk substantially free of β -casein A¹ including the steps:
- a) determining whether one or more bovine cows possesses a gene encoding for the protein β -casein A¹ or a gene encoding for the protein β -casein A² according to claim 1;
 - b) selecting those cows that do not have a gene encoding for the protein β -casein A¹; and
 - c) milking the selected cows.
14. A method as claimed in claim 13, where the milk contains β -casein which is substantially all β -casein A².
15. Milk obtained by the method of claim 13 or claim 14.
16. A food or food product containing, or processed from, milk as claimed in claim 15.
17. A method of forming a herd of bovine cows by testing the DNA of each cow in accordance with the method of claim 1 and selecting those cows that possess a gene encoding for β -casein A² and do not possess a gene encoding for β -casein A¹.
18. Semen or an embryo from a bovine animal that has been tested in accordance with the method of claim 1.
19. Semen or an embryo as claimed in claim 18 which is used for producing offspring using any artificial reproduction technique.