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(54) Title: METHODS AND COMPOSITIONS FOR TESTING AND BREEDING CATTLE FOR IMPROVED FERTILITY AND EMBRYONIC SURVIVAL

(57) Abstract: Disclosed are arrays of nucleic acid molecules, kits, methods of genotyping and marker assisted bovine breeding methods using SNPs on genes of the bovine interferon tau signaling pathway for improved bovine fertilization rate, or embryo survival, or both.

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METHODS AND COMPOSITIONS FOR TESTING AND BREEDING CATTLE FOR IMPROVED FERTILITY AND EMBRYONIC SURVIVAL

FIELD OF THE INVENTION

[0001] The present invention relates to methods of genetic testing of cattle using molecular genetic methods by assaying for the presence of at least one genetic marker which is indicative of fertility or embryonic survival.

BACKGROUND OF THE INVENTION

[0002] Dairy cows are significant investments for dairy farmers, and enormous efforts, such as animal breeding and artificial insemination, have been and continue to be invested in ensuring that the animals have high and sustained productivity, and that the milk produced is of high quality. About 50 quantitative trait loci (QTL) affecting milk production traits have been identified (Bagnato et al., 2008; Lipkin et al., 2008). The dairy cattle genome has been significantly restructured over the past 30 years due to intense selection for production traits.

[0003] Such restructuring of the dairy cattle genome over the past 30 years due to intense selection for production traits may have resulted in a hitchhiking effect on a large number of loci adversely affecting fertilization rate and embryo survival, leading to dairy cattle genotypes that are suboptimal for reproductive competence (Royal et al., 2000; Lucy, 2001). The decrease in dairy cattle fertility is a worldwide problem and a major cause of economic loss and cow culling in the global dairy herd.

[0004] Many reasons account for this reduced reproductive efficiency, but the most important component seems to be a reduction in embryo survival rate from over 80% twenty years ago to less than 50% today. There appears to be an important genetic basis for this decline (Veerkamp and Beerda, 2007); so genetic approaches may help alleviate this problem. As such, there is an urgent need to identify the genetic factors responsible for the decline in embryo survival rate.

[0005] Previously the present inventor has demonstrated the effectiveness of the candidate pathway approach in choosing candidate genes affecting milk production traits (Leonard et al., 2005; Cobanoglu et al., 2006; Khatib et al., 2007a,b; Khatib et al., 2008a; Wang et al.,

2008). Recently an *in vitro* fertilization (IVF) experimental system in cattle has been demonstrated that enables the association of single nucleotide polymorphisms (SNPs) in candidate genes with fertilization rate and embryo survival. Using this system, two genes: fibroblast growth factor 2 (*FGF2*) and signal transducer and activator of transcription 5 (*STAT5A*) were found to be significantly associated with variation in fertilization and embryo survival rates (Khatib et al., 2008a,b). These two genes were chosen from the interferon-tau (IFNT) and placental lactogen (PL) signal transduction pathway.

[0006] Interferon-τ (IFNT) is a major product of ovine and bovine conceptuses during the period before the trophoblast makes firm attachment to the uterine wall and begins to form a placenta. Its primary function is in preventing a return to ovarian cyclicity and hence ensuring the pregnancy to continue, although it undoubtedly has other roles in ensuring receptivity of the maternal endometrium.

[0007] IFNT is a member of the Type I IFN family, and signals through the Type I IFN receptor and Janus Kinase (JAK)-signal transducer and activator of transcription (STAT) signal transduction pathway (Stewart et al., Endocrinology 142:98-107 (2001)). IFNT activates multiple STATs and has differential effects on IFN-stimulated response element- (ISRE) and γ-activated sequence (GAS) element-driven gene transcription. It is known to induce a number of genes in the ovine uterus including 2',5'-oligoadenylate synthetase (Johnson et al., Biol. Reprod. 64:1392-1399 (2001)), β 2-microglobulin (Vallet et al., J. Endocrinol. 130:R1-4 (1991)), IFN regulatory factor 1 (Spencer et al., 1998), ubiquitin cross-reactive protein (Johnson et al., Biol. Reprod. 62:622-627(2000)), and Mx protein (Charleston and Stewart, Gene 137:327-331(1993); Ott et al., Biol. Reprod. 59:784-794 (1998)). Many of these proteins are known to function in the antiviral response as well as in early pregnancy of ungulates especially ruminant animals (see e.g. U.S. Pat. App. No. 20070009969). The aforementioned data most likely apply to cattle as well.

[0008] Identifying additional genetic factors that show association with fertilization rate or embryo survival rate would enable selection or breeding programs that reduce the frequency of deleterious alleles at these loci by marker- or gene-assisted selection, preventing further decline or even improving reproductive status of the global dairy herd.

[0009] Furthermore, a plurality of or multiple genes are likely more reliable than a single gene or SNP in predicting high fertility or enhanced embryo survival.

SUMMARY OF THE INVENTION

[0010] The present inventor investigated the effects of various genes of the IFNT signaling pathway and discovered that several of these genes comprise SNPs that are correlated with increased fertilization rate, or embryo survival rate, or both, and these SNPs may be used in breeding programs or other cattle testing or selection programs for cattle with improved fertility, more specifically for increased pregnancy rate in cattle. Accordingly, in one embodiment, the present invention provides a collection, or an array, of at least two of isolated polynucleotide molecule species selected from the group consisting of (1) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 1296 of SEQ ID NO:1; (2) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 213 of SEQ ID NO:2; (3) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 8504 of SEQ ID NO:3; (4) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 154963 of SEQ ID NO:4; (5) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 577 of SEQ ID NO:5; (6) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 23 of SEQ ID NO:6; (7) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 11646 of SEQ ID NO:6; and (8) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 12195 of SEQ ID NO:7. Preferably, the collection comprises at least three, at least four, at least five, at least six, or at least seven species described above. More preferably, the collection comprises all eight species.

[0011] In another embodiment, the present invention provides a method for genotyping a bovine cell, comprising obtaining a nucleic acid sample from said cell and determining the identity of the nucleotide of eight SNP positions in the cell, wherein the eight SNP positions are (1) position 1296 of SEQ ID NO:1; (2) position 213 of SEQ ID NO:2; (3) position 8504 of SEQ ID NO:3; (4) position 154963 of SEQ ID NO:4; (5) position 577 of SEQ ID NO:5; (6) position of 23 SEQ ID NO:6; (7) position 11646 of SEQ ID NO:6; and (8) position 12195 of SEQ ID NO:7, the method, comprising (1) determining the identity of a nucleotide at each

of the eight SNP positions, and (2) comparing the identity to the nucleotide identity at a corresponding position of in SEQ ID NOs: 1-7, respectively. In preferred embodiments, the method according to the present invention is used to test an adult bovine cell, an embryonic bovine cell, a bovine sperm, a bovine egg, a fertilized bovine egg, or a bovine zygote. In one embodiment, both copies of the respective gene in the cell are genotyped.

[0012] In another embodiment, the present invention provides a method for selectively breeding of cattle using a multiple ovulation and embryo transfer procedure (MOET), the method comprising super-ovulating a female animal, collecting eggs from said superovulated female, in vitro fertilizing said eggs from a suitable male animal, implanting said fertilized eggs into other females allowing for an embryo to develop, and genotyping said developing embryo as described above, and terminating pregnancy if said developing embryo does not all have a corresponding desired polymorphic nucleotide as shown in Table 1A.

DESCRIPTION OF THE DRAWINGS

[0013] **Figure 1** shows the partial sequence of the UTMP gene (SEQ ID NO:1) where the relevant SNP position is noted.

[0014] **Figure 2** shows the partial sequence of the STAT1 gene (SEQ ID NO:2) where the relevant SNP position is noted.

[0015] **Figure 3** shows the partial sequence of the OPN gene (SEQ ID NO:3) where the relevant SNP position is noted.

[0016] **Figure 4** shows the partial sequence of the GHR gene (SEQ ID NO:4) where the relevant SNP position is noted.

[0017] **Figure 5** shows the partial sequence of the POU1F1 gene (SEQ ID NO:5) where the relevant SNP position is noted.

[0018] **Figure 6** shows the partial sequence of the FGF2 gene (SEQ ID NO:6) where the two relevant SNP positions at positions 23 and 11646 are noted.

[0019] **Figure 7** shows the partial sequence of the STAT5A gene (SEQ ID NO:7) where the relevant SNP position is noted.

DETAILED DESCRIPTION OF THE INVENTION

[0020] It has now been found that many genes encoding proteins of the IFNT signaling pathway contain single nucleotide polymorphisms (SNPs), and certain of these alleles correspond to increased fertilization rate, or embryonic survival rate, or both, in dairy cattle, and the beneficial effects of these alleles are additive. Specifically, it has been discovered that SNPs exist in the following genes: growth hormone receptor (GHR), osteopontin (OPN/SPP1), POU1F1, signal transducer and activator of transcription (STAT1), signal transducer and activator of transcription (STAT5A), bovine uterine milk protein (UTMP), and fibroblast growth factor 2 (FGF2).

[0021] These SNPs are summarized in the Table 1 below.

Table 1A Gene Names, SNP Locations, and Polymorphisms

Gene	**SNP Position**	**Originally Reported Nucleotide**	**Polymorphic Nucleotide**	**Desired Nucleotide**
UTMP	1296	A	G	A
STAT1	213	T	C	C
OPN	8504	T	C	T
GHR	154,963	T	A	A
POU1F1	577	C	A	A
FGF2 SNP23	23	G	T	G
FGF2 SNP11646	11646	A	G	G
STAT5A	12195	C	G	C

Table 1B. Gene Names, Chromosomal Locations, and References

Gene	Chromosome	SNP (location)	Reference
POU class 1 homeobox 1 (*POU1F1*)	1	A/C (exon 3)	Huang et al. 2008
Growth hormone receptor (*GHR*)	20	A/T (exon 8)	Blott et al. 2003
Signal transducer and activator 5A (*STAT5A*)	19	C/G (exon 8)	Khatib et al. 2008
Osteopontin (*OPN*)	6	C/T (intron 4)	Leonard et al. 2005
Uterine milk protein (*UTMP*)	21	A/G (exon 4)	Khatib et al. 2007
STAT1	2	C/T (3`UTR)	Cobanoglu et al. 2006
FGF2 SNP23	6	G/T (5`UTR)	Khatib et al. 2008
FGF2 SNP 11646	6	A/G (intron 1)	Khatib et al. 2008

[0022] Aside from FGF2 SNP23, the SNPs listed in Table 1 above have been previously reported. Specifically, U.S. Pat. Apps. No. 11/179,581 discloses UTMP SNP 1296. (*see* Figure 1 of the present invention). This same patent application also discloses STAT1 SNP213 (see Figure 2) and OPN SNP8504 (see Figure 3).

[0023] GHR SNP 154963 was reported by Blott et al. 2003 (Genetics 163:253-266) (see Figure 4).

[0024] U.S. Pat. App. No. 12/267,104 discloses POU1F1 SNP 577 (see Figure 5).

[0025] U.S. Pat. App. No. 61/046,253, filed on April 18, 2008, discloses FGF2 SNP11646 (see Figure 6). Figure 6 further depicts FGF2 SNP23.

[0026] U.S. Pat. App. No. 12/267,076 discloses STAT5A SNP 12195 (See Figure 7).

[0027] These and other references cited herein are all incorporated by reference in their entirety.

[0028] POU1F1 is a member of the tissue specific POU (Pit, Oct, Unc) homeobox transcription factor DNA binding protein family that is found in all mammals studied so far (Bastos et al., 2006; Ingraham et al., 1988; Ingraham et al., 1990). The pituitary specific expression of POU1F1 is required for the activation of growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH) (Li et al., 1990). These genes are involved in a variety of signaling pathways that are important for many developmental and physiological processes, including pituitary gland development (Li et al., 1990, Mullis, 2007), mammary gland development and growth (Svennersten-Sjaunja and Olsson, 2005), milk protein expression (Akers, 2006), and milk production and secretion (Svennersten-Sjaunja and Olsson, 2005). Moreover, binding of GH and PRL to their receptors on the cell membrane triggers a cascade of signaling events including the JAK/STAT pathway, which has been shown to be required for adult mammary gland development and lactogenesis (Liu et al., 1997).

[0029] Several genes in the same pathway of POU1F1 have been reported to be associated with different milk production and health traits. For example, growth hormone receptor (*GHR*) has shown associations with milk yield and composition (Viitala et al., 2006). Also, the signal transducer and activator of transcription 1 (*STAT1*) and osteopontin (*OPN*) genes have been

shown to have significant effects on milk yield and milk protein and fat yields in Holstein dairy cattle (Cobanoglu et al., 2006; Leonard et al., 2005; Schnabel et al., 2005). The uterine milk protein (*UTMP*) is another gene in the pathway of POU1F1 that has been found to be associated with productive life in dairy cattle (Khatib et al., 2007b).

[0030] The *FGF2* regulates the trophectoderm expression of interferon-τ, a key member of the signal transduction pathway involved in milk production (Ocon-Grove et al., 2007). Bovine FGF2 is mapped to chromosome 17, with 3 exons and a total length of over 55 kb; it is expressed by the endometrium throughout the estrous cycle and early pregnancy (Michael et al., 2006).

[0031] The signal transducer and activator (STAT) proteins are known to play an important role in cytokine signaling pathways. STAT proteins are transcription factors that are specifically activated to regulate gene transcription when cells encounter cytokines and growth factors, hence they act as signal transducers in the cytoplasm and transcription activators in the nucleus (Kisseleva et al., 2002). In mammals, STATs comprise a family of seven structurally and functionally related proteins: STAT1, STAT2, STAT3, STAT4, STAT5a and STAT5b, STAT6 (Darnell, 1997). The seven mammalian STAT proteins range in size from 750 to 850 amino acids. The chromosomal distribution of these STATs, as well as the identification of STATs in more primitive eukaryotes, suggest that this family arose from a single primordial gene (Chen et al., 1998). In addition, STATs share a number of structurally and functionally conserved domains.

[0032] The STAT5 protein is also known as the mammary gland factor. This protein was initially identified in the mammary gland as a regulator of milk protein gene expression (Watson, 2001). *STAT5A* is a member of the interferon-tau (IFN-tau) and placental lactogen (PL) signaling pathway, which is involved in signal transduction within a variety of cells, including the uterus and mammary epithelial cells. The uterus is exposed to IFN-tau and PL, as well as many others hormones including estrogen, progesterone, and placental growth hormone. The PL stimulates the formation of STAT5 homodimers, which in turn induce the transcription of the bovine uterine milk protein (*UTMP*) and osteopontin (*OPN*) genes (Spencer and Bazer, 2002; Stewart et al., 2002; Spencer and Bazer, 2004). In previous studies, the present inventor showed that the *UTMP* (Khatib et al., 2007a) and *OPN* (Leonard et al. 2005; Khatib et al. 2007b) genes have surprisingly strong effects on milk production

and health traits in cattle. Furthermore, the present inventor showed that *STAT1*—also a member of the IFN-tau and PL signal transduction pathway—is associated with milk composition and health traits (Cobanoglu et al., 2006).

[0033] Studies in mouse have shown that *STAT5A* is involved in both milk production and fertility; *Stat5* knockout female mice fail to lactate (Miyoshi et al., 2001). Also, it has been shown that disruption of *Stat5* leads to infertility in females as a result of small-sized or a lack of corpora lutea (Teglund et al., 1998). Because the primary source of progesterone is the corpora lutea of the ovary, lack of development of corpora lutea would have significant effects on the establishment of pregnancy.

[0034] Polymorphisms at the nucleic acid level may provide functional differences in the genetic sequence, through changes in the encoded polypeptide, changes in mRNA stability, binding of transcriptional and translation factors to the DNA or RNA, and the like. Polymorphisms are also used to detect genetic linkage to phenotypic variation.

[0035] One type of polymorphism, single nucleotide polymorphisms (SNPs), has gained wide use for the detection of genetic linkage recently. SNPs are generally biallelic systems, that is, there are two alleles that an individual may have for any particular SNP marker. In the instant case, the SNPs are used for determining the genotypes of the POU1F1 gene, which are found to have strong correlation to longevity and milk production traits.

[0036] Through the following testing and analysis, it has been established that certain alleles of the SNPs shown in Table 1 correspond to increased fertilization rate, or embryonic survival rate, or both, in dairy cattle, and the beneficial effects of these alleles are additive.

[0037] **Gene Selection and Genotyping.** The genes *POU1F1*, *GHR*, *STAT5A*, *OPN*, *UTMP, STAT1, and FGF2* were chosen for association tests with fertility traits because they are members of the IFNT and PL/POU1F1 pathway. Genotyping of these genes was performed as described in the literature (Table 1) except for *GHR*, for which primers, GHR-F CTTTGGAATACTTGGGCTAGCAGTGACA"A"TAT (SEQ ID NO:8) and GHR-R GTCTCTCTGTGGACACAACA (SEQ ID NO:9) were used to amplify a 230-bp genomic fragment. The original T nucleotide at position -4 of the SNP was mutated to an A nucleotide in the forward primer to create an Ssp*I* recognition site. Restriction enzyme digestions were carried out according to the manufacturer's instructions.

[0038] Fertility Data Collection. Ovaries from mature cows were collected from a local abattoir and immediately used in the IVF experiments as described in Khatib et al. (2008a,b). Briefly, oocytes were aspirated from antral follicles (> 2-6 mm) and immediately incubated in maturation medium. On average, 12 oocytes were aspirated from each ovary. On day 2 (d 2), oocytes were fertilized with frozen-thawed percoll-separated semen that had been adjusted to a final concentration of 1 million sperm/ml. Fertilization rate was calculated as the number of cleaved embryos at 48 h post fertilization out of total number of oocytes exposed to sperm. Survival rate of embryos was calculated as the number of blastocysts on d 7 of development out of the number of total embryos cultured. Viability was determined as a function of the embryo's ability to attain the morphological stage of blastocyst on d 7 of development. Embryos that failed to show cellular compaction (morula stage) on d 5 or d 6 were considered nonviable. Therefore, only embryos exhibiting adequate compaction followed by the formation of a blastocoele on d 7 were considered viable. Ovaries from which fewer than 4 oocytes were harvested were discarded and not further analyzed. A total of 7,413 fertilizations were performed using oocytes from a total of 504 ovaries and semen from 10 different bulls.

[0039] Association of Individual Genes with Fertilization and Survival Rates. Associations of individual genes with fertilization and survival rates were analyzed using the following logistic regression model:

$$\log(\frac{p}{1-p})_i = \beta_0 + \beta_{1j} Bull_j + \beta_{2k} Genotype_k \qquad (1)$$

where $\log(\frac{p}{1-p})_i$ (i = 1, 2, … n) is the natural logarithm of odds of survival rate or fertilization rate, β_0 is a general constant, β_{1j} is the fixed effect associated with the j^{th} bull ($Bull_j$); and β_{2k} is the genotype effect associated with the k^{th} genotype ($Genotype_k$) of the gene analyzed. This model was fitted by Maximum Likelihood approach. Association between the gene and survival/fertilization rate was tested using a Likelihood Ratio Test (LRT).

[0040] Association of Candidate Genes with Embryonic Survival. The *GHR*, *STAT5A*, UTMP, FGF2 SNP11646, FGF2 SNP23, and STAT1 genes showed considerable associations

with embryonic survival rate (Table 2). For *GHR*, the survival rate of embryos produced from AA ovaries was 9% higher than that of embryos produced from TT ovaries. For *STAT5A*, CC ovaries showed 9% and 8% higher survival rates than that of GG and GC ovaries, respectively. The *UTMP* gene showed 6% survival rate differences between AA and GG genotypes (Table 2). SNP11646 and SNP23 of FGF2 showed differences of 7% each between genotypes GG and AA and between GG and TT, respectively. For STAT1, although not statistically significant, TT genotype was associated with a 4% increase in survival rate compared to GG genotype.

[0041] Association of Individual Genes with Fertilization Rate. The POU1F1, GHR, STAT5A, OPN, STAT1, and FGF2 SNP23 showed association of with fertilization rate (Table 3). The CC genotype of POU1F1 was showed 71.4% fertilization rate vs. 67.7% for AC genotype. Also, AA genotype of GHR showed 70% fertilization rate compared to 66% for AT genotype. Ovaries carrying the TT genotype of *OPN* showed a 70% fertilization rate vs. a 62% rate for ovaries carrying the CC genotype. The CC genotype of *STAT5A* showed significant association with fertilization rate (71%) vs. the GC (69%) and GG (66%) genotypes. The genotypes of STAT1 genes (CC vs. TT) showed 3% difference in fertilization rate. Similarly, although less statistically significant, FGF2 SNP23 also showed associations with fertilization rate; fertilization rate of oocytes obtained from TT cows was 63% vs. 68% for GT and GG cows. FGF2 SNP11646 did not show significant association with fertilization rate. However, interestingly, two way interaction between SNP23 and SNP11646 showed significant effects on fertilization rate (P = 4.90E-03). The genotype combination of TT(SNP23) and AA(SNP11646) was associated with the lowest fertilization rate (62%) compared to all other genotype combinations.

Table 2. Association tests (*P* values) between individual genes and embryo survival rate, genotypes of ovaries, number of embryos, and observed survival rates

Gene	*P* value	Genotype	Ovaries	Embryos	Survival rate
GHR		AA	256	3131	0.37
	3.80E-06	AT	125	1426	0.29
		TT	17	153	0.28
STAT5A		GG	87	902	0.31
	1.37E-07	GC	232	2762	0.33
		CC	85	1113	0.40

UTMP		GG	140	1735	0.30
	0.00039	GA	167	1924	0.36
		AA	112	1266	0.36
STAT1		CC	189	2235	0.34
	0.115	CT	180	2216	0.34
		TT	33	356	0.38
FGF2 SNP		GG	130	1424	0.38
11646	3.69E-04	AG	207	2343	0.32
		AA	107	1281	0.32
FGF2		GG	263	3080	0.36
SNP23	6.87E-04	GT	121	1370	0.30
		TT	22	221	0.29

Table 3. Association tests (*P* values) between individual genes and fertilization rate, genotypes of ovaries, number of fertilizations, and observed fertilization rate

Gene	*P* value	Genotype	Ovaries	Fertilizations	Fertilization Rate
		CC	279	4821	0.714
POU1F1	0.0516	AC	51	918	0.677
		AA	1	19	0.74
		AA	256	4473	0.70
GHR	0.0647	AT	125	2154	0.66
		TT	17	223	0.69
		GG	87	1360	0.66
STAT5A	0.00371	GC	232	4028	0.69
		CC	85	1574	0.71
		TT	142	2481	0.70
OPN	0.00529	TC	204	3601	0.70
		CC	48	739	0.62
		CC	189	3176	0.70
STAT1	0.0298	CT	180	3261	0.68

		TT	33	525	0.67
		GG	263	4547	0.68
FGF2 SNP23	0.172	GT	121	2015	0.68
		TT	22	352	0.63

[0042] In the context of the present invention, the provided sequences also encompass the complementary sequence corresponding to any of the provided polymorphisms. In order to provide an unambiguous identification of the specific site of a polymorphism, the numbering of the original nucleic sequences in the GenBank is shown in the figures and is used.

[0043] The present invention provides nucleic acid based genetic markers for identifying bovine animals with superior fertility and survival traits. In general, for use as markers, nucleic acid fragments, preferably DNA fragments, will be of at least 12 nucleotides (nt), preferably at least 15 nt, usually at least 20 nt, often at least 50 nt. Such small DNA fragments are useful as primers for the polymerase chain reaction (PCR), and probes for hybridization screening, etc.

[0044] The term primer refers to a single-stranded oligonucleotide capable of acting as a point of initiation of template-directed DNA synthesis under appropriate conditions (i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically ranges from 15 to 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with a template. The term primer site, or priming site, refers to the area of the target DNA to which a primer hybridizes. The term primer pair means a set of primers including a 5' upstream primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3', downstream primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

[0045] The term "probe" or "hybridization probe" denotes a defined nucleic acid segment (or nucleotide analog segment) which can be used to identify by hybridization a specific

polynucleotide sequence present in samples, said nucleic acid segment comprising a nucleotide sequence complementary of the specific polynucleotide sequence to be identified. "Probes" or "hybridization probes" are nucleic acids capable of binding in a base-specific manner to a complementary strand of nucleic acid.

[0046] An objective of the present invention is to determine which embodiment of the polymorphisms a specific sample of DNA has. For example, it is desirable to determine whether the nucleotide at a particular position is A or C. An oligonucleotide probe can be used for such purpose. Preferably, the oligonucleotide probe will have a detectable label, and contains an A at the corresponding position. Experimental conditions can be chosen such that if the sample DNA contains an A, they hybridization signal can be detected because the probe hybridizes to the corresponding complementary DNA strand in the sample, while if the sample DNA contains a G, no hybridization signal is detected.

[0047] Similarly, PCR primers and conditions can be devised, whereby the oligonucleotide is used as one of the PCR primers, for analyzing nucleic acids for the presence of a specific sequence. These may be direct amplification of the genomic DNA, or RT-PCR amplification of the mRNA transcript of the POU1F1 gene. The use of the polymerase chain reaction is described in Saiki et al. (1985) Science 230:1350-1354. Amplification may be used to determine whether a polymorphism is present, by using a primer that is specific for the polymorphism. Alternatively, various methods are known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms, for examples see Riley et al (1990) Nucleic Acids Res. 18:2887-2890; and Delahunty et al (1996) Am. J. Hum. Genet. 58:1239-1246. The detection method may also be based on direct DNA sequencing, or hybridization, or a combination thereof. Where large amounts of DNA are available, genomic DNA is used directly. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. The nucleic acid may be amplified by PCR, to provide sufficient amounts for analysis.

[0048] Hybridization may be performed in solution, or such hybridization may be performed when either the oligonucleotide probe or the target polynucleotide is covalently or noncovalently affixed to a solid support. Attachment may be mediated, for example, by antibody-antigen interactions, poly-L-Lys, streptavidin or avidin-biotin, salt bridges, hydrophobic interactions, chemical linkages, UV cross-linking baking, etc. Oligonucleotides

may be synthesized directly on the solid support or attached to the solid support subsequent to synthesis. Solid-supports suitable for use in detection methods of the invention include substrates made of silicon, glass, plastic, paper and the like, which may be formed, for example, into wells (as in 96-well plates), slides, sheets, membranes, fibers, chips, dishes, and beads. The solid support may be treated, coated or derivatized to facilitate the immobilization of the allele-specific oligonucleotide or target nucleic acid. For screening purposes, hybridization probes of the polymorphic sequences may be used where both forms are present, either in separate reactions, spatially separated on a solid phase matrix, or labeled such that they can be distinguished from each other.

[0049] Hybridization may also be performed with nucleic acid arrays and subarrays such as described in WO 95/11995. The arrays would contain a battery of allele-specific oligonucleotides representing each of the polymorphic sites. One or both polymorphic forms may be present in the array, for example the polymorphism of position 1296 may be represented by either, or both, of the listed nucleotides. Usually such an array will include at least 2 different polymorphic sequences, i.e. polymorphisms located at unique positions within the locus, and may include all of the provided polymorphisms. Arrays of interest may further comprise sequences, including polymorphisms, of other genetic sequences, particularly other sequences of interest. The oligonucleotide sequence on the array will usually be at least about 12 nt in length, may be the length of the provided polymorphic sequences, or may extend into the flanking regions to generate fragments of 100 to 200 nt in length. For examples of arrays, see Ramsay (1998) Nat. Biotech. 16:4044; Hacia et al. (1996) Nature Genetics 14:441-447; Lockhart et al. (1 996) Nature Biotechnol. 14:1675-1680; and De Risi et al. (1996) Nature Genetics 14:457-460.

[0050] The identity of polymorphisms may also be determined using a mismatch detection technique, including but not limited to the RNase protection method using riboprobes (Winter et al., Proc. Natl. Acad. Sci. USA 82:7575, 1985; Meyers et al., Science 230:1242, 1985) and proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, P. Ann. Rev. Genet. 25:229-253, 1991). Alternatively, variant alleles can be identified by single strand conformation polymorphism (SSCP) analysis (Orita et al., Genomics 5:874-879, 1989; Humphries et al., in Molecular Diagnosis of Genetic Diseases, R. Elles, ed., pp. 321-

340, 1996) or denaturing gradient gel electrophoresis (DGGE) (Wartell et al., Nucl. Acids Res. 18:2699-2706, 1990; Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232-236, 1989).

[0051] A polymerase-mediated primer extension method may also be used to identify the polymorphism(s). Several such methods have been described in the patent and scientific literature and include the "Genetic Bit Analysis" method (WO92/15712) and the ligase/polymerase mediated genetic bit analysis (U.S. Pat. No. 5,679,524). Related methods are disclosed in WO91/02087, WO90/09455, WO95/17676, U.S. Pat. Nos. 5,302,509, and 5,945,283. Extended primers containing a polymorphism may be detected by mass spectrometry as described in U.S. Pat. No. 5,605,798. Another primer extension method is allele-specific PCR (Ruao et al., Nucl. Acids Res. 17:8392, 1989; Ruao et al., Nucl. Acids Res. 19, 6877-6882, 1991; WO 93/22456; Turki et al., J. Clin. Invest. 95:1635-1641, 1995). In addition, multiple polymorphic sites may be investigated by simultaneously amplifying multiple regions of the nucleic acid using sets of allele-specific primers as described in Wallace et al. (WO 89/10414).

[0052] A detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, e.g. fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, e.g. ^{32}P, ^{35}S, ^{3}H; etc. The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, etc. having a high affinity binding partner, e.g. avidin, specific antibodies, etc., where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

[0053] It is readily recognized by those ordinarily skilled in the art that in order to maximize the signal to noise ratio, in probe hybridization detection procedure, the polymorphic site should at the center of the probe fragment used, whereby a mismatch has a maximum effect on destabilizing the hybrid molecule; and in a PCR detection procedure, the polymorphic site should be placed at the very 3'-end of the primer, whereby a mismatch has the maximum effect on preventing a chain elongation reaction by the DNA polymerase. The

location of nucleotides in a polynucleotide with respect to the center of the polynucleotide are described herein in the following manner. When a polynucleotide has an odd number of nucleotides, the nucleotide at an equal distance from the 3' and 5' ends of the polynucleotide is considered to be "at the center" of the polynucleotide, and any nucleotide immediately adjacent to the nucleotide at the center, or the nucleotide at the center itself is considered to be "within 1 nucleotide of the center." With an odd number of nucleotides in a polynucleotide any of the five nucleotides positions in the middle of the polynucleotide would be considered to be within 2 nucleotides of the center, and so on. When a polynucleotide has an even number of nucleotides, there would be a bond and not a nucleotide at the center of the polynucleotide. Thus, either of the two central nucleotides would be considered to be "within 1 nucleotide of the center" and any of the four nucleotides in the middle of the polynucleotide would be considered to be "within 2 nucleotides of the center," and so on.

[0054] In some embodiments, a composition contains two or more differently labeled oligonucleotides for simultaneously probing the identity of nucleotides or nucleotide pairs at two or more polymorphic sites. It is also contemplated that primer compositions may contain two or more sets of allele-specific primer pairs to allow simultaneous targeting and amplification of two or more regions containing a polymorphic site.

[0055] Alternatively, the relevant portion of the gene of the sample of interest may be amplified via PCR and directly sequenced, and the sequence be compared to the wild type sequence shown in the figures. It is readily recognized that, other than those disclosed specifically herein, numerous primers can be devised to achieve the objectives. PCR and sequencing techniques are well known in the art and reagents and equipments are readily available commercially.

[0056] DNA markers have several advantages; segregation is easy to measure and is unambiguous, and DNA markers are co-dominant, i.e., heterozygous and homozygous animals can be distinctively identified. Once a marker system is established selection decisions could be made very easily, since DNA markers can be assayed any time after a blood sample can be collected from the individual infant animal, or even earlier by testing embryos *in vitro* if very early embryos are collected. The use of marker assisted genetic selection will greatly facilitate and speed up cattle breeding problems. For example, a modification of the multiple ovulation and embryo transfer (MOET) procedure can be used

with genetic marker technology. Specifically, females are superovulated, eggs are collected, *in vitro* fertilized using semen from superior males and implanted into other females allowing for use of the superior genetics of the female (as well as the male) without having to wait for her to give birth to one calf at a time. Developing blastomeres at the 4-8 cell stage may be assayed for presence of the marker, and selection decisions made accordingly.

[0057] In one embodiment of the invention an assay is provided for detection of presence of a desirable genotype using the markers.

[0058] The term "genotype" as used herein refers to the identity of the alleles present in an individual or a sample. In the context of the present invention a genotype preferably refers to the description of the polymorphic alleles present in an individual or a sample. The term "genotyping" a sample or an individual for a polymorphic marker refers to determining the specific allele or the specific nucleotide carried by an individual at a polymorphic marker.

[0059] The present invention is suitable for identifying a bovine, including a young or adult bovine animal, an embryo, a semen sample, an egg, a fertilized egg, or a zygote, or other cell or tissue sample therefrom, to determine whether said bovine possesses the desired genotypes of the present invention, some of which are indicative of improved milk production traits.

[0060] Further provided is a method for genotyping one of the bovine genes listed in Table 1, comprising determining for the two copies of the gene present the identity of the nucleotide pair at the relevant SNP position.

[0061] One embodiment of a genotyping method of the invention involves examining both copies of the gene, or a fragment thereof, to identify the nucleotide pair at the polymorphic site in the two copies to assign a genotype to the individual. In some embodiments, "examining a gene" may include examining one or more of: DNA containing the gene, mRNA transcripts thereof, or cDNA copies thereof. As will be readily understood by the skilled artisan, the two "copies" of a gene, mRNA or cDNA, or fragment thereof in an individual may be the same allele or may be different alleles. In another embodiment, a genotyping method of the invention comprises determining the identity of the nucleotide pair at the polymorphic site.

[0062] The present invention further provides a kit for genotyping a bovine sample, the kit comprising in a container a nucleic acid molecule, as described above, designed for detecting the polymorphism, and optionally at least another component for carrying out such detection. Preferably, a kit comprises at least two oligonucleotides packaged in the same or separate containers. The kit may also contain other components such as hybridization buffer (where the oligonucleotides are to be used as a probe) packaged in a separate container. Alternatively, where the oligonucleotides are to be used to amplify a target region, the kit may contain, preferably packaged in separate containers, a polymerase and a reaction buffer optimized for primer extension mediated by the polymerase, such as PCR.

[0063] In one embodiment the present invention provides a breeding method whereby genotyping as described above is conducted on bovine embryos, and based on the results, certain cattle are either selected or dropped out of the breeding program.

[0064] Through use of the linked marker loci, procedures termed "marker assisted selection" (MAS) may be used for genetic improvement within a breeding nucleus; or "marker assisted introgression" for transferring useful alleles from a resource population to a breeding nucleus (Soller 1990; Soller 1994).

[0065] The present invention discloses the association between the genes listed in Table 1 and fertilization rate or embryonic survival.

[0066] The following examples are intended to illustrate preferred embodiments of the invention and should not be interpreted to limit the scope of the invention as defined in the claims.

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WHAT IS CLAIMED IS:

1. A collection of at least two of isolated polynucleotide molecule species selected from the group consisting of (1) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 1296 of SEQ ID NO:1; (2) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 213 of SEQ ID NO:2; (3) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 8504 of SEQ ID NO:3; (4) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 154963 of SEQ ID NO:4; (5) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 577 of SEQ ID NO:5; (6) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 23 of SEQ ID NO:6; (7) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 11646 of SEQ ID NO:6; and (8) an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 12195 of SEQ ID NO:7.

2. The collection according to claim 1, comprising at least three species.

3. The collection of claim 2, comprising all eight species.

4. The collection of Claim 1, wherein the nucleotide species are on a solid support.

5. The collection of Claim 1, wherein the nucleotide species are arranged in an addressable array.

6. The collection of Claim 4, wherein the nucleotide species are arranged in an array on a solid support.

7. The collection of Claim 6, wherein the array is made of silicon, glass, plastic, or paper.

8. The method of Claim 6, wherein the array is formed into wells on plates, slides, sheets, membranes, fibers, chips, dishes, and beads.

9. The collection of Claim 6, wherein array is treated, coated or derivatized to facilitate the immobilization of the nucleotide molecules.

10. A method for genotyping a bovine cell, comprising obtaining a nucleic acid sample from said cell and determining the identity of the nucleotide of eight SNP positions in the cell, wherein the eight SNP positions are (1) position 1296 of SEQ ID NO:1; (2) position 213 of SEQ ID NO:2; (3) position 8504 of SEQ ID NO:3; (4) position 154963 of SEQ ID NO:4; (5) position 577 of SEQ ID NO:5; (6) position of 23 SEQ ID NO:6; (7) position 11646 of SEQ ID NO:6; and (8) position 12195 of SEQ ID NO:7, the method, comprising

(1) Determining the identity of a nucleotide at each of the eight SNP positions, and

(2) comparing the identity to the nucleotide identity at a corresponding position of in SEQ ID NOs: 1-7, respectively.

11. The method according to Claim 10, wherein the bovine cell is an adult cell, an embryo cell, a sperm, an egg, a fertilized egg, or a zygote.

12. The method according to Claim 10, wherein the identity of the nucleotide is determined by sequencing or a relevant fragment of the respective gene isolated from the cell.

13. A method according to Claim 12, wherein relevant fragment of the respective gene is isolated from the cell via amplification by the polymerase chain reaction (PCR) of genomic DNA of the cell, or by RT-PCR of the mRNA of the cell.

14. A method according to Claim 10, wherein both copies of the respective gene in the cell are genotyped.

15. A method for progeny testing of cattle, the method comprising collecting a nucleic acid sample from said progeny, and genotyping said nucleic sample according to Claim 10.

16. A method for selectively breeding of cattle using a multiple ovulation and embryo transfer procedure (MOET), the method comprising superovulating a female animal, collecting eggs from said superovulated female, in vitro fertilizing said eggs from a suitable male animal, implanting said fertilized eggs into other females allowing for an embryo to

develop, and genotyping said developing embryo according to Claim 10, and terminating pregnancy if said developing embryo does not all have a corresponding desired polymorphic nucleotide as shown in Table 1A.

17. A method according to Claim 16, wherein pregnancy is terminated if the embryo is not homozygous with regard to all of the corresponding desired polymorphic nucleotide.

18. A method for selectively breeding dairy cattle, comprising selecting a bull that is homozygous with regard to all desired polymorphic nucleotides as shown in Table 1A and using its semen for fertilizing a female animal.

19. A method according to Claim 18, wherein the female animal is in vitro fertilized.

20. A method according to Claim 18, wherein MOET procedure is used.

21. A method according to Claim 18, wherein said female animal is also homozygous with regard to all desired polymorphic nucleotides as shown in Table 1A.

22. A method for testing a dairy cattle for its fertility, comprising genotyping its cells according to Claim 13, wherein a cattle homozygous with regard to all desired polymorphic nucleotides as shown in Table 1A indicates that the cattle has fertility rate.

Figure 1

Coding sequence for Bovine Uterine milk protein (UTMP)

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1     ggctggattg ccgcagaaat gtcccacggg agaatgaatc tggccctgtc tctggtcttc
61    atcctctgtg gcctgtttaa tagcatcttc tgtgaaaagc aacaacactc tcaaaagcac
121   atgaacctag tcttattaaa gaaaatttca gctctctccc agaagatgga agctcaccct
181   aaggattttg cccaagaatt gttcaaggct ttgataattg aggatcccag aaagaatatc
241   atcttctccc ccatggccat gaccaccacc ctggccaccc tctccctggg gatcaagtct
301   acaatgagaa cccaccaccc tgaggacctg aaacttgagc ccaaactgtt ggatgtgcac
361   aagtacttac agcctctggt ccacgtgggg cgtgagctag tgaagcagaa ggtactgaag
421   caccagcaca ttctctttat caacagaaaa atgatggtca accagatgct tctacagcag
481   ataagcaagc tgcagggaat ggacatccag atgattgact ttacagatat agaaaaagcc
541   aagaagacca tcagccacca tgtggctgaa aaaacacata cgaaaatcac aaacttaatc
601   accgacctga accctgagac catcctgtgt cttgttaacc acattttctt caaaggcatc
661   ttgaaaagag cttttcagcc caaactcacc cagaaggagg tcttctttgt gaatgaccaa
721   accaaagtgc aggtggacat gatgagaaag acagaacgga tgctttacag ccggtcagag
781   gagctacatg ctacgatggt taagatgcct tgcaaaggaa atgtgtccct aactctcatg
841   cttccagatg ccggacaatt tgacactgat cttaaaaaga tgactgctaa gcgagctaaa
901   cttcagaaaa tcagtgactt cagactggtg cgcttaattt tgcccaagtt gaagatctcc
961   ttcaagataa actttaagca tctgcttccc aagattgacc ccaaacatat actgactgcc
1021  acagcaatct cacaggccat cacatcgaag gctcccctgc ctaatttgga ggccctacat
1081  caagctgaga tagagctgag cgagcacgcc ttaaccgtgg acacagccat tcacacagat
1141  aatctgttga aagtcccagt gaaggcaaag gaggtcccgg cggtcgtgaa agtcccaatg
1201  aaggcaaagg aggtcccggc ggtcgtgaaa gtcccaatga acacaaagga ggtcccagtg
1261 gtcgtgaaag tcccaatgaa cacaaaggag gtccc(a/g)gtgg tcgtgaaggt caacagaccc
1321  ttcttgctgt ttgtggagga tgagaagact caaagagacc tctttgtggg caaagtcctc
1381  aacccccaag ttgagtagag ccagggccac actgtgcagc acaggaactt agcaggccat
1441  gaataaaaag agtacaattc acc
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Notes: The SNP at position 1296 is A/G. A is the nucleotide reported by the original submitter. The SNP is in the coding sequence, but does not change the amino acid sequence of the encoded polypeptide. Primers are designed to be positioned at positions 1071-1090 and positions 1379-1398 (underlined).

Figure 2

Coding sequence for bovine signal transducer and activator of transcription (STAT1)

1 ctttaaatat **agcctcaagt ttgccagtgg c**ttgcctgtg aaatagtgca aagctgtcct
61 gtatctgggc agaggataaa agttatgtgt gttattatat tttccacact ggccattgaa
121 aactaaagat tctctttctt gggagaatta gcttttggta tggctttatg atgctggcta
181 atatcaatag aaggaagtaa actttacaaa tt(c/t)atgagta gtatcttcca tttcagcttt
241 aataccaaag ttgaatatat tctgccttca tcatgaaatt gaagttagta aatgaaactg
301 tcttc**acagt tctatcaagg gagcc**aaact attaacagct ctcttaaggc aaatcctatt
361 atttttcaa aaagttgaaa ttaattgtag atgtaaacaa actcagaaat ttaatgcatg
421 tttcataagt gggttcactt gtctttattg tttagtaaaa attttaaaat tgagaagaaa
481 aactagtaat tgacaaatca ttaggtggag attatgagaa tccaataatt tgaaaactca
541 tcctgtgtaa ctgccttgag aattgggtaa ttttcactgg caaatgtgta tctctcacaa
601 atacattaca gatggttcca ctaaaa

Notes: SNP is at position 213 (C/T), with C being the nucleotide reported by the original submitter. Primers are designed to be positioned at positions 11-31 and positions 306-325.

Figure 3

Partial genomic sequence of the region encoding bovine osteopontin (OPN/SPP1) position 8504

7561 taattaactc taaatattaa aattctcaca attaaagaac aaccactcca aaaaatagcc
7621 accaagcagg ccatttgggc tggttaaatg gatcttccct gcctgttggg cttccctgat
7681 agctcagttg gtaaagcatc tgcctgcaac ttggaagacc cgggttcagt ccctgggtcg
7741 ggaagactcc ctggagaagg aaatggcaac cccctctagt actcttgcct ggaaaatttc
7801 catggactga ggaccctggt aggctaagag tcagacagaa ctgagcaact tcacttcact
7861 ttcctgcctg tttgtaaaag tgagcttagg acaccaattg atctgtcagg ttgtcttccg
7921 gcttaatcct tccacaatga ggctagaaaa ataagacctg ctttggatgg aaacagctaa
7981 cttttgaata aaaaagttac gttgtatgat gtgcactgat ttgtgtcttt tcttcttcag
8041 aattctgtgt cctctgagga aactgatgac aacaaacaaa atgtgagtct ttgctttgat
8101 tctgatgtct gttgtgcctt agactcagga aggcactctt tctcctaatg acattgccca
8161 ggttcaaatt ccggcaaaat tccactagca aacccttcag gaactacttt ttattgggac
8221 tattaatagg gataagttaa atttgctttc cttaagattc tatttgaaga tgctgagaat
8281 ctataagaga agttagataa atgacccagg atatttgcaa atcagaagtg tgatagacat
8341 taactgagct atagtttcta cacatggata agagagtcac cttttgatta tccaggctaa
8401 tagggaggtg attttagttt tgggggtgtg cattaataca tggattctct gatcccctga
8461 gaatttttcat ttcaaataga aaaggtagtc tcacaattat gta(t/c)ctgtat ttattggatc
8521 attgaaattt ggtaaattag tgtttattat gaacaaggaa aaacagtgtc attgatacaa
8581 atattataac tcatacgttt ggcttgaaaa tatctgtgaa aatcgttttt atgagaaacc
8641 aagaaaaatg ccttagaata ggattccatt tacccttgtg ttaaagggga aattggaata
8701 agctcatttt agcatttaaa agccattaag tgctttgttg tgaatacaaa gattctaaaa
8761 ctaaataaag atagtaaaat actaatgcac tgtaaagcct aagggacagt aaaaaccctg
8821 acacccattt ttctggccat cttgatttct agaccctccc aagtaagtcc aatgaaagcc
8881 ctgagcaaac agacgatcta gatgacgatg atgataacag ccaggacgtc aactctaatg
8941 actccgacga cgctgaaacc actgatgacc ctgaccattc cgacgagtct caccattctg
9001 atgaatctga tgaagttgat tttcccactg atattccaac aatcgcagtt ttcactccgt
9061 ttatccctac ggaaagcgca aatgatggcc gaggtgatag tgtggcttac ggactgaagt

Notes: SNP is at position 8504 (C/T), with T being the nucleotide reported by the original submitter. Primers are designed to be positioned at positions 8316-8338 and positions 8588-8606/7. Positions are numbered according to the GenBank.

Figure 4 Coding sequence for GHR gene (NC_007318)

154261 cagataatga aggataagga aggctggcat gctgcagttc atggggttgc aaagagtcag
154321 acatgactta gcaactgaac agcattctaa aatctgagag tcctagtact gatcttctgt
154381 caaacagtac tttttacgct gtaaaaatgt acaccctgca tatctaagaa gttttaataa
154441 tgattcaaaa atacaacttg gcccccatct ttttgatgga tcctcagtct agatcagatc
154501 tagatctaaa gatcacatta aaaaaaaaaa agaattggac attatttagg taaagtagta
154561 tattaacaag catcactttt ccctcaagct aaagcctttt aatgacacac cctgaacaca
154621 taagatgttt aaagcaggtt gtttatataa taaacatgga ttgtgcttaa attgtatgct
154681 gttactcttt ttttttggt atacaaaagg atctgaagaa gtggatagag gtgttcttag
154741 aaaatactaa gtaattgcat tctatttcag tggctatcaa gtgaaatcat tgactttact
154801 agatgaatac aaattaggaa gttttatgtg gaacaggaga atgagatata aacttcaact
154861 gttcatagtt ctgtgagata ttatttttgt gtttttcaga tttccagttt ccatggttct
154921 taattattat ctttggaata cttgggctag cagtgacatt at(**t/a**)tttactc atattttcta
154981 aacagcaaag gtaagtgtga tataacctac tctgatatgt tttgccagtt atttagcaaa
155041 tgtccatgtt tccatttttt gtttgatgtt ttcttttgtg aatcctgagt gaagtgtttc
155101 atcaacccag tgaaacgtta tcgctctaca tttacatctt tgttgtgtcc acagagagac
155161 aacacaggtc tcagttttat ctggaaagtt gcataggatg ttaagagggt gaggctagtg
155221 actacatacc atgtgacatg caccttaaag ttccgcactg atatttattc caggacccag
155281 aggtagcttt gagcaaaaat ttaagtggtg aactaaagct actagataat tcagtctaat
155341 aaaacctttc tttagacttc atatgatacc aatcttaagt aaatttgggt ttatttaaat
155401 tggttggcta cttacagttt ggtattttac cttcttttgt cagagataaa attctaagtt
155461 tgaggacacc atcctgcatc ctcttgcagc cagaaggcag gtttcagtta ttattctgcc
155521 actgttgttt gagttcattt gagtcccttt atctctagga ctccacgttc tcatgggtaa
155581 tttgagggtg gtggattgta tgatgtttaa gtttccctta agctgtaagg accattattc

Notes: SNP is at position position 154,963 (exon 8), with A being the nucleotide reported by the original submitter. Positions are numbered according to the GenBank.

Figure 5 Nucleotide coding sequence of POU1F1

```
1 gcaaatactg tgatttgaag ctaaccaaat aaactaattt ctattttggc tggagaagag
61 aaaggaatga aagtagaaac actcgctatt acacatagga gagcctatct gaattcgaga
121 tgctccttag aaatagtaaa taaactctga ttcaggcttg tcttcacccg tttttctctc
181 tgcttcggtt acaaaaccaa accctcacca cttctttctc caggtttagt tcttcagcca
241 tccgcaggat ctcctgagag gaaggcttat tctgttctcc aaagtgtctc tccagggcgt
301 ctttagcagc aatactgatt gttgttctcc gtttctattc ttttgtggga atgagttgcc
361 aacctttac ttcgactgat acctttatac ctctgaattc tgagtcttct gcaactctgc
421 ctctgataat gcatcccagt gctgcggagt gcctaccggt ctccaaccac gccaccaacg
481 tgatgtccac agcaacagga cttcattatt ctgttccttt ctgtcattat ggaaaccagt
541 catcgaccta tggcgtgatg gcagggagct taaccc(c/a)ttg tctttataag tttcctgacc
601 acacgttgag tcatggtttt cctcccatgc atcagcctct cctttcagag gaccccactg
661 ccgctgattt caagcaggag ctcaggcgga aaagcaaatt ggttgaagag ccaatagaca
721 tggattctcc agaaatccga gaacttgaaa agtttgccaa tgagtttaaa gtgagaagaa
781 ttaagctagg atacacccag acaaatgttg gggaagctct ggcagctgtg catggctctg
841 aattcagtca aacaactatc tgccgatttg aaaacctgca gctcagcttc aaaaatgcat
901 gcaaactaaa agcaatatta tccaaatggc tggaggaagc cgagcaagta ggagctttat
961 acaatgagaa agttggtgca aatgaaagaa aaaggaaacg gagaacaaca atcagtattg
1021 ctgctaaaga cgcgctggag agacactttg gagaacagaa taagccttcc tctcaggaga
1081 tcctgcggat ggctgaagaa ctaaacctgg agaaagaagt ggtgagggtt tggttttgta
1141 accgaaggca gagagaaaaa cgggtgaaga caagcctaaa tcagagttta tttactattt
1201 ctaaggagca tctcgaatgc agataggctc tcctattgtg taatagcgat tctacttttc
1261 attcctttct cttctcagcc aaaatagaaa ttagttattt ggttagcnnn aaaaatcaca
1321 tcagtaattt ttgncagaag tgtttctttt ctactttaaa aataaataca atttaaatta
1381 tgttgatgaa ntattctcag aaggannnnn tcantgtaca ntttaagcca aagactaata
1441 ggattaaaac aatgattctg tccctttcac tatatctttc cctctatctc tcccnggaat
1501 tc
```

Notes: SNP is at position 577, with C being the nucleotide orginally reported.

Figure 6: Coding Seqeunce for FGF2

1 ccggggccgc gccgcggagc gc(G/T)tcggagg ccggggccgg ggcgcggcgg ctccccgcgc
61 ggctccaggg gctcggggac cccgccaggg ccttggtggg gccatggccg ccgggagcat
121 caccacgctg ccagtccctg ccggaggacg gcggcagcgg cgctttcccg ccgggccact
181 tcaaggaccc caagcggctg tactgcaaga acgggggctt cttcctgcgc atccaccccg
241 acggccgagt ggacggggtc cgcgagaaga gcgacccaca cagtgagtgc tccccaggtc
301 ttccccggtg ccgtcttcgt cccctgcggt tctctccccc gcccctgcct tccagcctcc
361 gcgctccttc ttcctcttca ctgtgacccc ggtgggactt gtggtttctc tccgctcggc
421 cctcggcggt ttcgggctca ccactcgccc ccctcctgcc ccgagctgcg gtggcggtag
481 acgctcctcc aggctttgga gtgtgccggc tgctcagcaa agccagtccc ctgggccccg
541 agcccccggc gcccgggctt tgcgggcggc tccctgggcg cagacaacct gtcgcgtcgg
601 gggtgcccgg cggctgagca gaggtgagcg gctcagcgag gtgccgcccg cgccccgagc
661 ctgaagttcc gaccgcttct atgggatgcc cgttgtctcc ggggcaaagc caggagggac
721 cgcagaccaa ctaaaaggtc cttgttggaa agataccttg catcaggttt gaggatcaaa
781 tgagaatttg aagtgcgcag aggactcaat ttactagtct acagttgcat tttctgtaaa
841 aataataatg atgtatctgt ggtaatagca ataagattgg tctgaggcgt tggttgtcaa
901 actaagagtg cataagaatc acctggaagg tgtgtgtgtt gtgtgattca tggctgaacc
961 atctcccaga gtttcagatc gcttaggtct ggagtggggc ctcatttgca tttctaacta
1021 cttcccaggt gatgctgatc tgggagcaca gtttgagaac ccgctggtct agagaaagag
1081 gaaggaaaga ggtataaaat gggctgataa aaatagatga gtttgaagtg agacaaagag
1141 atcagatatt tttaaactgt catcctgtaa gtgtaggtaa aacatgtttt gaaagctgtt
1201 tgttctgcca ttccttccat aatggttttc aggtggaaaa cttgatcctc ttttttttt
261 tttttttttg cccaagttcc gcaagaggcc ttctttacct tgtgatgcta atagtgcgtc
1321 ctttggggct ctccaggtgg tactggtggt aaagatccca cctgccagtg cctgggatgt
1381 aagaggtgtg gattggatcc ctgtgttgga aagatcccct ggagaaggaa atggcacccc
1441 gctccagtat tcttgcctgg agactcccca tggacagagg agcctagtgg gctaccgtcc
1501 ctagggtccc aaagagtcgg acactgaagg aatttagcaa gctctcactc cgggatgaga
1561 cttaggaaga ggagaaaact ctgcagccaa acctagctga caaattcagt aatgggaaat
1621 gtcccttcat aagaattggt ctttattgat ttcaaaatag caacaagcaa aggattcagg
1681 tctgtaactt ttttccggcc tgccataatt aaacaatttt cttaaccact tacattatcc
1741 agtaaaactg aaaagatgct tgtagcccaa tatatcggtt agtgctcttt ctctattttg
1801 gtaactaggt ttcacaaaat tatctttctg tgtggggttt attctgtgct tgtctgccag
1861 ggtagcccag ctgaacacgg caaggtgcac atatgtccca attaattttg ctcttttcta
1921 gtatcacaaa aagtagtttg ttctttgacg agaagacaga actcttcccc cagattaggt
1981 ttatactgga gcttccttta gtacattttc ttccagacat tttatgagtt gcagtatttt
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2281 cagacgtgac tgagcacaca ggcatgtatg ggagttagta aggataattc tgaattgcat
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6961 tattaaaaag ctggctatta gcaattttgg tgtagtctat aaaaagatga atcattgctc
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8641 atgagctaga actttaaata aataagcata tacacttcag catgaagtgc gcagagcaga
8701 taagttgaac gtagcatcac tgtggttcct tctgtggaca cctttcacat tattcaaagg
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8881 ttttaattaa aaaccataga tctgagagtt catacttgag tcatatttaa tccttaaatc
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9001 ggattaagaa ctgtctggta ccattgttaa tatccctttt ggctgtgagg aaatggcaca
9061 aataatttca tcctaatatt cattcagatt tatgagccag tattcatcac aacaatctta
9121 aaactcttga aaggatagaa actgtatgac aaagtgagtc atagtctttg tgatgtgatg
9181 tctgcaaggg tgggtgggag agactactga atgaccagtc tcattctgct ttctgagctg
9241 aatcattttg caaatagaaa acaattcata gatttataga tggtcataac ataaatagga
9301 aatggaagag agtaacagag tcaaataata cctctattta aaaaattact ttttgaaaca
9361 taacacttga tgaacagtct ttattttgaa ttagaaatga aattaatcta ctgcctaata
9421 atacatatat tttgatactt gctgtatgtc tttatatatt ttcatttttt cctattggtt
9481 atgtttcttt taaaaaattc tctcatgtaa tttgaccttt atcttcatag ctatttctag
9541 ctttggcttg tttgacaatt gcgtgtgtgt ttgtgtgtgg agaaggaggg gactacttgt
9601 atggaaactt gagagaagaa ggtccctttc ctcttgaaaa ttcttaatag tataatcctg
9661 cattttgcat ggtcggtctc cttttgttac ttttcatctt actagaatta gcaatatgga
9721 gagtctttct ctgagtcaga tttaaccttt aatctttaaa tgtaagattg atttaccttа
9781 tttccttatt ttctttgaga caaagtattt gtcaaaacaa ttatatgaaa agtaaactat
9841 tctagtttga gtgtgtttct tgagttttag aactttagga ctcttcttac attcttatat
9901 ttatccatta aactcaacaa tttagtaagg gggatataat acaaataaaa ttgggaagct
9961 aatttttcta actggtttag tagaggacag tagtatatga agaagacata tattacattt
10021 aatacaacgt gttggattaa aaaatagtta cagcaatacc ttcagctgtt acaaggtggg
10081 aaaagtaagg cgcagattat tttggaggga aggtattaaa accatgacgt gttgatggga

10141 tctgtccagc ctgagccaga caccaaagca ggttccatgg caacttggcc acgtccctgc
10201 gcctttaaag aggaagggcc tattgtttgg ccttcaccaa atgacttcac ctgggatctt
10261 gttatttact gaatgttttt tgaatggatg gatgaaattc ctgagaacat gctctgggcc
10321 agctttatga acagtatgtt taatcttatt gtagtcttat gaaagaagtg ttattttcat
10381 cttacagata gggatagagt ttttgctatt ggcttttcaa accatggtct ctttgtgatt
10441 gtaagtaatt aattgtgtct tccagatttg ttagtgttta gaatacagtt catggccaga
10501 atttcagatg gacggtgtgg cataaatttg aacagaaata gtgattttta aaaatagttt
10561 aaacttccca gagcctttac tgtgctcagc aaagttagtc tctcatcttt tcttctaccc
10621 ctttattgca tccttttttа tttagaaaat atttgtcatg aattaatacg aaacaattct
10681 ttaatatttt agggattgct ttctgaagaa ctcaaagatt tttaaaaggc atatttaaaa
10741 attaagagca ggacataatt aagaataaat accatataag aatgggataa acctcaaaga
10801 tagagtctgt aaagatgcag aataagctaa ggcatgcaga aaatacaaag agaatgatta
10861 aaaggatgtt taaaaagtta gttaggccct ttcaaggaaa tttgagatag gctcactatt
10921 taaggacata gtgtaagatg aaaagaaaaa aatttagaaa aaaaagcaga tggacctggg
10981 cctattttat gttaatgtta atcttcttct ccaagtgaga ttgtcaatca ataattgtct
11041 gagtgtctca ttgagaaaat aaagaccaag gtagacaaag agatacaaag aaagcactta
11101 gccagacaca tctagaaatg tgtttataat gaaactcctc tttccttgaa atcacttgtc
11161 ccccttttt gacccccctgt attttaaaat ataaaatatt taactttgta aatttcttgc
11221 caaccagccc atctcgcaga gtacatttct actcttcatc ccctcagtct tcacatccgt
11281 ctcaggctct gtgttttcag ttctgctgtg tccttcatac tcacgggggt ctctgcattg
11341 ttgccacagc tgctctcgtt cggtccctga ctgttgcaac tgccttctac ctgatcccat
11401 ctgtatcagt ttgctagggc tgccataaca gattaccgta gactgagtgg ctcaaacaac
11461 agaaattgat tttctcatag ttctgtagac tagaagtcca agatacagct gtctgcatgt
11521 ctggtctttc tgcggcctct tcggggtttg cagcagccac cttacacatg gtcacctctc
11581 tgtgcacaca tcctgatctc ttcttcttgt aagggcacca ttcagatttg gttagggccc
11641 actct(**A/G**)taac agccccattt tgacttaatc cttctttaga ggccccatct ccaaatagta
11701 attttctgag gtactggggc ttcaggcttc agtgtatgaa tttggggtgg gggtacagtt
11761 cagcccacag caccagtgag tcaactggat attgttcctt ggcagagtat ctttccagag
11821 agcagctctg atcttgttat ccctctattt agaaaaactt catggacagt ctagtcccct
11881 ggttcccaca ttgcttacag atgtgggcac tgtagaaagt ctatgagaat
11941 aggaagttac cagcagatga gtgattgtct tatatatcag aaagtgggat aaaggtattt
12001 tctggaaact ctagatagct aggaagcctg atgtaggtcc ttgaaaaaaa tccaagggac
12061 ttgagaatac ggagaaaaga agataacata gaaaatagta aataggctcg

Notes: **SNP at position 23 (G/T) position 11646 (A/G)(**NC_007304)

Figure 7 Coding sequence for STAT5A

```
11221 ggctcagcgt ccctcccctc ccgcaggggc agtgacaccc tgagctgtcc tggggaccct
11281 gagggaggca gagagccagg aggagagcgg gacccagcag agcaggaggc ccgggccttc
11341 ttcctcatgg ggcctgaggg ggagagtcgg tctgggagga ggaggccctg cagggctgtt
11401 ctgagagccc agaagggccg gctgagccac cgcccgaccc tcaggagctg gccgagaagc
11461 accagaagac cctgcagctg ctgcggaagc agcagaccat catcctggat gacgagctga
11521 tccagtggaa gcggcggcag cagctggcgg ggaacggagg gccccccgag ggcagcctgg
11581 atgtgctaca gtcctggtac caggggtggg gggcggggag gggcaggcag cagagtggtg
11641 ctgccagctg ctgtttgcgc ccacgtctac atgagcagct ggctccctct gtctgggcgc
11701 gggtcttatc ccaccagtgg tgtgtttggt gctgacaccg gtgtcccttt ctgtgccccc
11761 tcccctggga ggatgctggg gtggggccag gtggcaaagt ggcgctcagg ctggttggac
11821 cccagtcagt gtcgctcctc ctgggtgttt ctctggtttt tttggaaggc agggcatctc
11881 tgctgtgccc agtgcacagg cgaggtggct cgggcaccag gccttcctgg gggtggagct
11941 gggtgtgggc cttgtccccg cctgggcgcc tgccagcttc tggcctggag gacgggggtg
12001 aagcccgtgt ccttcccttg ggccctgggg ctcgggttca ggtgtgagaa gttggcggag
12061 attatctggc agaaccggca gcagatccgc agagccgagc acctctgcca gcagctgccc
12121 atccccggcc ccgtggagga gatgctggct gaggtcaacg ccaccatcac tgacatcatc
12181 tcagccctgg tgac(C/G)aggtg actcctggcc acgccccgct cccatctggt tgccctgggt
12241 tgggggcagc agggtctttg cagatgggga gctctggctt aaatccttca gtttctgcct
12301 cacaccctcc tcccatccct ctccatcccc tgttgctatg gcctcttgct gtcgacctca
12361 cccagtattt ctcgtggaca ctacacgggc atttgtctcc tgcaactcct ttcagctgct
12421 gagttccttt tactgcctcc cttcccgcca gctcccctga ctcacagtgg ccccagggag
12481 ggtggactgt ccgcaaaccc tcccttcacc tgctcagcct ggtgcaaggc agcctcccca
12541 cgtggaaggt ggggccagag tcctgtcccc tgaagtgtct cctgtccctt gtgtctccgc
12601 agcaccttca tcatcgagaa gcagcccccc caggtcctga agacccagac caagttcgcg
12661 gccaccgtgc gcctgctggt gggtgggaag ctgaacgtgc acatgaaccc cccccaggtg
12721 aaggccacca tcatcagcga gcagcaggcc aagtcactgc tcaagaacga gaacacccgc
12781 aagtatgctg cccgctcctt catctgccct cccccagctc agcctctgct ctgtagctgg
```

Note: SNP G/C AT POSITION 12195

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/067948

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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)
C12Q

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 practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KHATIB H ET AL: "The fibroblast growth factor 2 gene is associated with embryonic mortality in cattle" JOURNAL OF ANIMAL SCIENCE, AMERICAN SOCIETY OF ANIMAL SCIENCE, US LNKD-DOI:10.2527/JAS.2007-0791, vol. 86, no. 9, 1 September 2008 (2008-09-01), pages 2063-2067, XP002544674 ISSN: 0021-8812 page 2067, last paragraph	1,2,4-22
X	US 2007/015164 A1 (KHATIB HASAN [US]) 18 January 2007 (2007-01-18) claims 1-10,12	1,2,4-22

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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 document but 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	Date of mailing of the international search report
27 April 2010	12/05/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gabriels, Jan

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/067948

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [X] on paper

   [X] in electronic form

   b. (time)

   [ ] in the international application as filed

   [ ] together with the international application in electronic form

   [X] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/067948

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/307535 A1 (KHATIB HASAN [US]) 11 December 2008 (2008-12-11) paragraph [0008]; claims 1-27	1,4-22
X	KHATIB H ET AL: "Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle" JOURNAL OF DAIRY SCIENCE, vol. 91, no. 2, February 2008 (2008-02), pages 784-793, XP002579838 ISSN: 0022-0302 the whole document	1-22
X	KHATIB H ET AL: "Pattern of expression of the uterine milk protein gene and its association with productive life in dairy cattle." JOURNAL OF DAIRY SCIENCE MAY 2007 LNKD-PUBMED:17430947, vol. 90, no. 5, May 2007 (2007-05), pages 2427-2433, XP002579839 ISSN: 1525-3198 page 2427, left-hand column page 2432, left-hand column	1-22
X	HUANG W ET AL: "A proline-to-histidine mutation in POU1F1 is associated with production traits in dairy cattle." ANIMAL GENETICS OCT 2008 LNKD-PUBMED:18557974, vol. 39, no. 5, October 2008 (2008-10), pages 554-557, XP002579840 ISSN: 1365-2052 cited in the application the whole document	1-21
X	BLOTT SARAH ET AL: "Molecular dissection of a quantitative trait locus: A phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition." GENETICS, vol. 163, no. 1, January 2003 (2003-01), pages 253-266, XP002579841 ISSN: 0016-6731 the whole document	1-22
X	WO 2007/050735 A2 (INNOVATIVE DAIRY PRODUCTS PTY [AU]; RAADSMA HERMANUS WILLEM [AU]; CAVA) 3 May 2007 (2007-05-03) claims 14,18	1-22

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/067948

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/109514 A2 (WISCONSIN ALUMNI RES FOUND [US]; KHATIB HASAN [US]) 27 September 2007 (2007-09-27) paragraph [0030] - paragraph [0032]; claims 1-39	1-22
X	LEONARD S ET AL: "Effects of the osteopontin gene variants on milk production traits in dairy cattle." JOURNAL OF DAIRY SCIENCE NOV 2005 LNKD-PUBMED:16230712, vol. 88, no. 11, November 2005 (2005-11), pages 4083-4086, XP002579842 ISSN: 1525-3198 the whole document	1-22
X,P	WO 2009/062008 A2 (WISCONSIN ALUMNI RES FOUND [US]; KHATIB HASAN [US]; MONSON RICKY L [US]) 14 May 2009 (2009-05-14) the whole document	1-22
X,P	WO 2009/146203 A1 (WISCONSIN ALUMNI RES FOUND; KHATIB HASAN [US]) 3 December 2009 (2009-12-03) the whole document	1-22
X,P	WO 2009/062042 A2 (WISCONSIN ALUMNI RES FOUND [US]; KHATIB HASAN [US]; WEN HUANG [US]) 14 May 2009 (2009-05-14) the whole document	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/067948

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:

see additional 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 an additional fees, this Authority did not invite payment of 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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 1296 of SEQ ID NO:1 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

---

2. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 213 of SEQ ID NO:2 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

---

3. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 8504 of SEQ ID NO:3 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

---

4. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 154963 of SEQ ID NO:4 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

---

5. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 577 of SEQ ID NO:5 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

---

6. claims: 1-22(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 23 of SEQ ID NO:6 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

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7. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 11646 of SEQ ID NO:6 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

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8. claims: 1-22(partially)

Collection of isolated polynucleotide molecule species containing an isolated polynucleotide comprising at least 12 consecutive nucleotides surrounding position of 12195 of SEQ ID NO:7 for use in methods for genotyping a bovine cell, for selectively breeding cattle and for testing the fertility of a dairy cattle.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/067948

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007015164 A1	18-01-2007	CA 2551173 A1 NZ 548517 A	13-01-2007 30-04-2008
US 2008307535 A1	11-12-2008	NONE	
WO 2007050735 A2	03-05-2007	NONE	
WO 2007109514 A2	27-09-2007	AU 2007227082 A1 CA 2645861 A1 EP 1999279 A2	27-09-2007 27-09-2007 10-12-2008
WO 2009062008 A2	14-05-2009	US 2009253952 A1	08-10-2009
WO 2009146203 A1	03-12-2009	US 2009299130 A1	03-12-2009
WO 2009062042 A2	14-05-2009	US 2009216074 A1	27-08-2009