Innovation, Sciences et
Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and
Economic Development Canada
Canadian Intellectual Property Office

CA 2823638 C 2021/04/06

(11)(21) **2 823 638**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

---

(22) **Date de dépôt/Filing Date:** 2013/08/09

(41) **Mise à la disp. pub./Open to Public Insp.:** 2014/02/09

(45) **Date de délivrance/Issue Date:** 2021/04/06

(30) **Priorité/Priority:** 2012/08/09 (US61/681,152)

(51) **Cl.Int./Int.Cl.** *C12N 15/12* (2006.01),
A01K 67/00 (2006.01), *C07H 21/04* (2006.01),
C40B 30/00 (2006.01), *C40B 40/06* (2006.01)

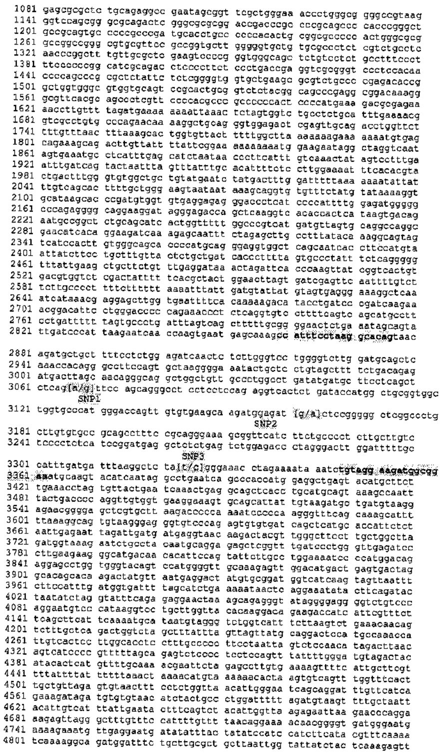
(72) **Inventeur/Inventor:**
KHATIB, HASAN, US

(73) **Propriétaire/Owner:**
WISCONSIN ALUMNI RESEARCH FOUNDATION, US

(74) **Agent:** MCCARTHY TETRAULT LLP

---

(54) Titre : POLYMORPHISMES DE NUCLEOTIDE SIMPLE ASSOCIES A LA FERTILITE DES TAUREAUX
(54) Title: SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BULL FERTILITY



(57) **Abrégé/Abstract:**

Single nucleotide polymorphic sites of the bovine MAP1B, PPP1R11, and DDX4 genes are associated with improved bull fertility as measured by e.g. sire conception rates. Nucleic acid molecules, arrays, kits, methods of genotyping and marker-assisted bovine breeding methods based on these SNPs are disclosed.

ABSTRACT OF THE DISCLOSURE

Single nucleotide polymorphic sites of the bovine MAP1B, PPP1R11, and DDX4 genes are associated with improved bull fertility as measured by e.g. sire conception rates. Nucleic acid molecules, arrays, kits, methods of genotyping and marker-assisted bovine breeding methods based on these SNPs are disclosed.

SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BULL FERTILITY

FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods for testing and selecting cattle using molecular genetic methods by assaying for the presence of at least one genetic marker indicative of increased bull fertility. Specifically, genetic variations in the *MAPJB* and *PPPlRl 1* genes are tested and used for selecting cattle animals with improved blastocyst or fertilization rates, or both.

BACKGROUND OF THE INVENTION

[0002] The dairy cattle genome has been significantly restructured over the past 30 years due to intensive breeding effort selecting for production traits, including high quality milk and high and sustained productivity. However, while those efforts led to dramatic improvement of productivity, there has been significant reproductive deterioration in high-producing dairy cows, which in turn has caused substantial economic loss in the dairy cattle industry (Lucy, 2007, Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. *Society of Reproduction and Fertility Supplement.* **64** 237-254). Key factors contributing to decreasing fertility of dairy cow are low fertilization rates and decreased embryonic survival.

[0003] Fertility is a complex trait that comprises developmental stages such as combining sperm and egg to form a zygote, compaction of embryo cells to form a morula, establishment of the blastocyst, attachment of the embryo to the uterus, and fetal development (Amann and Delarnette, 2012). This complexity makes accurate prediction of successful pregnancy difficult, as aberrant development of sperm, oocyte, embryo, or fetus all would lead to conception failure. Conception rate in dairy cattle is about 40%, and only 50% of the fertilized eggs produce viable embryos (Santos et al., 2004). The decline in reproductive performance in cattle over the past few decades (Dobson et al., 2007) has been ascribed primarily to fertilization failure and early embryonic loss (Santos et al., 2004).

[0004] Previous studies have shown that genetic makeup of an individual plays crucial roles in embryonic development and reproductive success (Weigel, 2006; Shook, 2006). Although a male and female parent each contributes half of its genetic material to the new zygote and both are necessary for embryo development, it is not obvious whether or not this contribution is equally important to pregnancy success. For example, it is well established that the paternal genome supports growth of extra-embryonic tissues while the maternal genome fosters development of the embryo proper (Barton et al., 1984). After fertilization, the development of an embryo is controlled by maternal genomic information that is accumulated during oogenesis (Telford et al., 1990). It is only at the 8-cell stage in the bovine embryo that the embryonic genome activates and the embryo switches to transcribing its own RNA (Memili and First, 2000).

[0005) Despite that most breeding schemes in cattle are focused on the selection of elite bulls using progeny testing or genomic selection, and that some semen traits (e.g., sperm motility and percentage of abnormal sperm) show moderate to high heritabilities (Druet et al., 2009), most fertility studies in cattle have focused on the maternal contribution, and the paternal contribution to reproductive performance has not been thoroughly investigated, and only a few studies have been reported in the literature (Feugang et al., 2009; Khatib et al., 201O; Pei'lagaricano et al., 2012). Therefore, characterization of bull fertility markers is both feasible and highly desirable, and the deployment of these markers in cattle breeding would lead to improved reproductive performance in cattle.

[0006] A recent comparative genomics study has characterized many genes involved in the control of spermatogenesis that were highly conserved from fly to human (Bonilla and Xu, 2008). Some of these genes were reported to be crucial for human fertility. However, it is not known whether or not these spermatogenesis genes play important roles in the fertility of bulls.

SUMMARY OF THE INVENTION

[0007) The present inventor carried out an association analysis between highly conserved spermatogenesis genes and sire conception rate (SCR) as a measure of bull fertility, with the objective that significant polymorphisms associated with bull fertility can be used as genetic markers in breeding programs aimed at improving reproductive performance in cattle.

[0008) Specifically, an association analysis is performed between highly conserved spermatogenesis genes and SCR in US Holstein populations as a measure of bull fertility. Sequence analysis revealed 24 single nucleotide polymorphisms (SNPs) in 9 genes in the bull population using the pooled DNA sequencing approach. These 9 genes were selected for their high level of sequence conservation between flies and humans. Overall, the 24 SNPs were tested for association with SCR in a population of 1,988 bulls. Three SNPs located in the MAPlB gene, one SNP in the PPPI R I I gene and one SNP in the DDX4 gene showed significant associations with SCR. Nucleotide probes based upon these SNPs are found to be useful for genetic testing of bull animals for improved fertilization rate.

[0009] Accordingly, in one embodiment, the present invention provides an isolated oligo- or poly-nucleotide molecule consisting of

> 1) Position 1986 of SEQ ID NO: 1 (Position 3066 of Fig. I) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID NO: 1 adjacent to position 1986;
>
> 2) Position 2243 of SEQ ID NO: 1 (Position 3323 of Fig. 1) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID NO: 1 adjacent to position 2243;
>
> 3) Position 1991 of SEQ ID N0:2 (Position 87071 of Fig. 3) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID NO: 2 adjacent to position 1991,
>
> (4) Position 232 of SEQ ID N0:3 (Position 1 12 of Fig. 4) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID N0:3 adjacent to position 232,
>
> (5) Position 2006 of SEQ ID N0:4 (Position 61646 of Fig 6.) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID NO: 4 adjacent to position 2006, and
>
> 6) Position 2139 of SEQ ID N0:5 (Position 34239 of Fig. 5) and at least 12, but not more than 200 contiguous nucleotides of SEQ ID NO: 5 adjacent to position 2139.

[0010] In one embodiment, the nucleotide molecule of the present invention comprises at least about 15 contiguous nucleotides adjacent to its respective position (hereinafter the "SNP position") of the respective figure. In one embodiment, the nucleic acid molecule of the present invention comprises at least about 20 contiguous nucleotides adjacent to the respective SNP position. In one embodiment, the oligonucleotide molecule of the present invention consists of not more than about 1 00 nucleotides. In one embodiment, the oligonucleotide molecule of the present invention consists ofnot more than about 50 nucleotides. In one embodiment, the SNP position of the nucleotide molecule of the present invention near or at the center of the molecule; alternatively, the SNP position is at the 3'-end of the oligonucleotide molecule

[0011] Also provided herein is an array of nucleic acid molecules, comprising the isolated oligonucleotide molecule of the present invention, supported on a substrate. The substrate may be any suitable medium, known and readily available to one of ordinary skills in the art, and the array may be addressable.

[0012] The present invention further provides a kit comprising an isolated oligonucleotide molecule of the present invention, and a suitable container.

(0013] In another embodiment, the present invention provides a method for detecting single nucleotide polymorphism (SNP) in a gene listed in Table I below in a bovine cell, the method comprising optionally isolating an DNA from the bovine cell, determining the identity of a nucleotide on the gene of the cell at a SNP position identified in Table I below, and comparing the identity to the preferred nucleotide identity at a corresponding position in Table I.

[0014] In one embodiment, the bovine cell may be an adult cell, an embryo cell, a sperm, an egg, a fertilized egg, or a zygote. The identity of the nucleotide may be determined by many methods known and readily available to those ordinarily skilled in the art, such as but not limited to sequencing a nucleic acid molecule comprising a suitable portion of the gene of the cell comprising a respective SNP position, or by hybridizing a suitable probe to a nucleic acid preparation from the cell, which probe may be suitably labeled e.g. fluorescently or radioactively.

(0015] The nucleic acid molecule may be isolated from the cell via a large variety of methods, known and readily available to an ordinarily skilled artisan, such as amplification by the polymerase chain reaction (PCR) of genomic DNA of the cell, or when appropriate, by RT-PCR of the mRNA of the cell.

[0016] In preferred embodiment, both copies of the gene in a diploid genome are genotyped according to the method of the present invention.

[0017) The identity of the nucleotide may be determined based on genotypes of the parent of the cell, genotypes of the daughter of the cell, or both, through genetic analysis methods well-known to those skilled in the art.

(0018] A method is further provided for determining whether an individual bovine animal is suitable as a gamete donor for natural mating, artificial insemination or *in vitro* fertilization, the method comprising detecting the SNP according to the above method of the present invention, and excluding as gamete donor an individual which does not have the preferred allele identity at the respective SNP position as described in Table I .

[0019) In one embodiment, the individual is excluded as a gamete donor if the individual, whose genotype is not homozygous of the preferred allele with regard to the respective SNP position.

[0020) The present invention additionally provides a method of selecting a bovine embryo for planting in a uterus, the method comprising genotyping the embryo according to the present invention, while preserving the viability of the embryo, and excluding from planting an embryo which does not have the preferred allele identity at the respective SNP position as described in Table 1.

(0021] In another embodiment, the present invention further provides a method for selectively breeding 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 male animal which has at least one preferred allele identity at the respective SNP position as described in Table I, implanting said fertilized eggs into other females allowing for an embryo to develop.

[0022] In another embodiment, the method for selectively breeding cattle using MOET may further comprise a step of genotyping the male animal according to the method of the present invention.

(0023] In another embodiment, the method for selectively breeding cattle using MOET may further comprise a step of genotyping the developing embryo, and allowing pregnancy to proceed only if the genotype of the embryo comprises at least one preferred allele identity at the respective SNP position as described in Table 1.

DESCRIPTION OF THE ORAWINGS

[0024] **FIG. 1** depicts a partial genomic sequence (SEQ ID NO: 1) of MAPIB, showing the locations of SNPs 1, 2 and 3 on the MAPIB gene and the locations of the primers (IF and IR, corresponding to positions 2859-2876, and positions 3346-3362, respectively) used to amplify the region comprising the SNP sites. The numbering of the sequences is according to that of GenBank Accession No. (Gene ID: 514739, updated on 12-Jul-2012).

[0025] FIG. 2 is the partial genomic sequence (SEQ ID NO: 6) of the MAPlB gene showing the locations of SNPs 4 and 6 on the MAP1B gene and the locations of the primers 2F and 2R (corresponding to positions 51447-51466, and positions 51931-51948, respectively used to amplify the region comprising the SNP sites. The numbering of the sequence is according to that of GenBank Accession No. (Gene ID: 514739, updated on 12- Jul-2012).

[0026] FIG. 3 is the partial genomic sequence (SEQ ID NO: 2) of the MAP**1**B gene showing the location of SNP 5 on the MAPIB gene and the locations of the primers 3F and 3R (corresponding to positions 86634-86651, and positions 87260-87278, respectively) used to amplify the region comprising the SNP sites. The numbering of the sequence is per that of GenBank Accession No. (Gene ID: 514739).

[0027] FIG 4. is the partial genomic sequence (SEQ ID NO: 3) of the PPP!R11 gene showing the locations of SNPs 1-4 on the PPPiR11 gene and the locations of the primers 1F and 1R (corresponding to positions -102 to -85, and positions 470-489, respectively) used to amplify the region comprising the SNP sites. The numbering of the sequences is according to GenBank Accession No. (Gene ID: 504846).

(0028) FIG. 5 is the partial genomic sequence (SEQ ID NO: 5) of the DDX4 gene, showing the location of SNP 2 on the DDX4 gene and the locations of the primers 1F and 1R (corresponding to positions 34014-34031, and positions 34398-34417, respectively) used to amplify the region comprising the SNP site. The numbering of the sequences is according to that of GenBank Accession No. (Gene ID: 493725).

[0029] FIG. 6 is the partial genomic sequence (SEQ ID NO: 4) of the DDX4 showing the location of SNP **1** on the DDX4 gene and the locations of the primers 2F and 2R (corresponding to positions 61531-61549 , and positions 61867-61884, respectively) used to amplify the region comprising the SNP site. The number of the sequences is per that of GenBank Accession No. (Gene ID: 493725).

DETAILED DESCRIPTION OF THE INVENTION

[0030) The present inventor has found that the spermatogenesis genes *MAP 1B, PPP 1Rl 1* and *DDX4* showed significant associations with SCR. Table 1 below summarizes the SNPs demonstrated to be significantly associated with sire conception rates according to the present invention.

Table 1. Genetic markers significantly associated with sire conception rate

SNP ID	Location/Position	Nucleotide Identity of Preferred Allele	Nucleotide Identity of Polymor h in GenBank
SNPl.MAPlB	Position 3066 of Fig. 1	A	A
SNP3.MAP1B	Position 3323 of Fig. 1	T	T
SNP5.MAP1B	Position 87071 of Fig. 3	C	C
SNPl.PPPIRl 1	Position 112 of Fig. 4	G	T
SNP1.DDX4	Position 61646 of Fig. 6	A	A
SNP2.DDX4	Position 34239 of Fig. 5	G	G

[0031) Three SNPs in *MAP JB,* in low to moderate linkage disequilibrium (LD), were significantly associated with SCR. After correction for multiple testing, only one SNP in intron 5, SNP 5, showed the most significant association with SCR.

[0032) The *MAP 1B* gene belongs to the microtubule-associated protein family and is known to affect neuronal development such as axon growth (Tymanskyj et al., 2012), development of dendritic spine and synaptic maturation (Tortosa et al., 2011), and regulation of the interaction between microtubules and actin microfilaments for axonal development (Montenegro-Venegas et al., 2010). Recent reports on the expression of *MAP 1B* in the male reproductive tract in both rat and human (Queir6z et al., 2006) and in testis of fruit fly and mouse (Bonilla and Xu, 2008) suggest important functions of this gene in the regulation of male fertility. The finding by the present inventor, disclosed herein, that the *MAP JB* gene is associated with SCR, supports the conclusion that *MAP 1B* plays a role in male fertility across a wide range of species.

[0033) The present inventor also found that a SNP in the 5'UTR of *PPP 1Rl 1* was associated with SCR in the bull population examined in this study. This is the first report of association between male fertility in cattle and *PPP 1Rl 1,* which is consistent with previous reports on the roles of this gene in spermatogenesis in mouse and human. For example, the different isoforms of *PPP 1Rl 1* (also known as *TCTEX5)* were found to be expressed in most mouse tissues with high expression in testis, epididymis, and in the head and tail regions of spermatozoa (Han et al., 2007). In a subsequent study, it was shown that mutations in the long transcript of *PPP 1Rl 1* were associated with normal sperm function (Han et al., 2008).

The authors concluded that *PPPJRJ 1* plays important roles in sperm motility and spermatogenesis. A recent study reported that an isoform of protein phosphatase 1 (PPly 2), which has an essential role in spermatogenesis, forms a complex with PPPIR 11 in the testis (Cheng et al., 2009). Given that PPly 2 is regulated by *PPP IRI I,* these results further support the idea that *PPPIRI 1* has important functions in spermatogenesis.

[0034) The spermatogenesis genes investigated in this study were selected from a pool of genes whose expression is highly conserved in testis of both fruit fly and mouse (Bonilla and Xu, 2008). The protein sequence identities between cattle and human and between cattle and fly are 91% and 32%, respectively for *MAP1*B and 99% and 47%, respectively for *PPP IRI I*. As such, the association of these genes with bull fertility testifies to the usefulness of the comparative genomics approach in selecting candidate male fertility genes.

[0035) To further explore involvement of male fertility genes identified in this study in female fertility, we tested the association of the SNPs in *MAPIB,* with fertilization and embryo survival rates using data from the IVF system. *MAP IB* genotypes of the cows, from which oocytes were extracted and used for fertilization and embryo culture, were significantly associated with differential fertilization rate and embryo survival rate. Recently, the expression of *MAP IB* was found to be downregulated in follicular cystic follicles compared to normal follicles, suggesting that alteration in *MAP IB* expression may be involved in reproduction failure in cattle (Choe et al., 2010). The instant disclosure again demonstrates the significance of both parental genomes to embryonic development and fertility.

[0036] Accordingly, the present invention provides nucleic acid-based genetic markers for identifying bovine animals, especially bulls, with superior fertility, specifically, sire conception rate as a measure of male fertility. In general, for use as markers, isolated oligonucleotide or polynucleotide molecules, or isolated nucleic acid fragments, preferably DNA fragments, as used. Such markers will be of at least 10 nucleotides (nt), preferably at least 11, 12, or 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.

[0037) In one embodiment, the isolated nucleic acid molecule comprises not more than 1,500 nt, or not more than 1000 nt, or not more than 900 nt, or not more than 800 nt, or not more than 700 nt, or not more than 600 nt, or not more than 500 nt, or not more than 400 nt, or not more than 300 nt, or not more than 200 nt., or not more than 150 nt., or not more than 100 nt., or not more than 75 nt.

[0038] In the context of the present invention, the tenn "isolated" refers to a nucleic acid molecule purified to some degree from endogenous materials with which the nucleic acid molecule may naturally occur or exist. At the least, the term "isolated" refers to a nucleic acid molecule separated from chromatin or other protein or components of the genomic DNA. Preferably, the isolated oligonucleic acid molecule or polynucleic acid molecule of the present invention comprises a fragment that is shorter than that which is naturally occurring.

(0039) In the context of the present invention, the provided sequences also encompass the complementary sequence corresponding to any of the provided polymorphisms. Where appropriate, and in order to provide an unambiguous identification of the specific site of a polymorphism, the numbering of the original nucleic sequences in the GenBank may be used; alternatively, the numbering may simply refer to the specific sequence in the Sequence Listing accompanying this dislcosure.

[0040] 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 fonn 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. One of these two primers is often referred to as the "forward primer," while the other the "reverse primer."

[0041] 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.

[0042] 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 G. 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.

(0043] 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 gene of interest. 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.

[0044] 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.

[0045) 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. 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. (1996) Nature Biotechnol. 14:1675-1680; and De Risi et al. (1996) Nature Genetics 14:457-460.

[0046) 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).

[0047) 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 (W092/ 1 5712) and the ligase/polymerase mediated genetic bit analysis (U.S. Pat. No. 5,679,524). Related methods are disclosed in W091/02087, W090/09455, W095/1 7676, 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).

[0048) 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] Alternatively, an invasive signal amplification assay, as described in e.g. U.S. Pat. No. 5,422,253 and Lyamichev et al., 2000, Biochemistry 39:9523-9532, may be used for detecting the SNP of interest. This assay takes advantage of enzymes such as the 5' nuclease activity of a DNA polymerase or the gene 6 product from bacteriophage T7 in their ability to cleave polynucleotide molecules by recognizing specific structures instead of specific sequences. A single-stranded target molecule is annealed to a pilot oligonucleotide such that the 5' end of the pilot forms a duplex with the target molecule. If the 3' end of the pilot oligonucleotide does not pair with the target, a 3' arm is formed. When exposed to a cleavage agent such as a DNA polymerase having a 5' nuclease activity or the gene 6 product from bacteriophage T7, the target molecule is cleaved in the 5' region, one nucleotide into the duplex adjacent to the unpaired region of the target. If a cut in a double-stranded molecule is required, the double-stranded molecule is denatured. Because this unpaired 3' ann can be as short as one nucleotide, this assay can be used for detecting a single-nucleotide difference, e.g. in the context of SNP detection. The pilot oligonucleotide is designed such that it pairs perfectly with one allele, but has a 3', single nucleotide mismatch with another allele. Cleavage only occurs ifthere is a mismatch between the target molecule and the pilot. To achieve signal amplification, the above invasive reaction is modified such that cleavage occurs on the pilot oligonucleotide. Two oligonucleotides are annealed in an adjacent manner to the target molecule. The resulting adjacent duplexes overlaps by at least one nucleotide to create an efficient substrate, called the overlapping substrate, for the *5'* nucleases. The *5'* end of the downstream oligonucleotide, also called the probe, contains an unpaired region termed the 5' arm (Lyamichev et al., 1993, *Science* 260:778-783.) or flap (Harrington and Lieber, 1994, *EMBO J* 13: 1235-1246) that is not required for the enzyme activity; however, very long arms can inhibit cleavage (Lyamichev et al., 1993, *Science* 260:778-783). Specific cleavage of the probe, tenned invasive cleavage (Lyamichev et al., 1999, *Nat. Biotechnol.* 17 292-296; Kwiatkowski et al., 1999, *Mol. Diagn.* 4, 353-364.), occurs at the position defined by the 3' end of the upstream oligonucleotide, which displaces or "invades" the probe. Ifthe overlap between the adjacent oligonucleotides is only one nucleotide, cleavage takes place between the first two base pairs of the probe, thus releasing its 5' arm and one nucleotide of the base paired region (Lyamichev et al., 1999, *Proc. Natl. Acad Sci. USA.* 96: 6143-6148, and Kaiser et al., 1999, *J Biol. Chem.* 274:21387-21394). Ifthe upstream oligonucleotide and the probe are present in large molar excess over the target nucleic acid, and invasive cleavage is carried out near the melting temperature of the probe, a cut probe can rapidly dissociate, and an intact probe will anneal to the target more frequently than will a cut probe, thus initiating a new cycle of cleavage. This allows multiple probes to be cut for each target molecule under isothennal conditions, resulting in linear signal amplification with respect to target concentration and time (Lyamichev et al., 1999, *Nat. Biotechnol.* 17:292-296).

(0053] 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.

[0054] In one embodiment of the invention an assay is provided for detection of presence of a desirable genotype using the markers.

[0055) 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.

[0056] 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 reproduction traits.

[0057) Further provided is a method for genotyping the bovine genes identified in Table 1, comprising determining for the two copies of the gene in a diploid genome present the identity of the nucleotide pair at the relevant SNP position (see below).

[0058) 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.

[0059] 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.

[0060] In one embodiment the present invention provides a breeding method whereby genotyping as described above is conducted on a bovine animal, especially a bull, and based on the results, certain cattle are either selected or dropped out of the breeding program.

[0061] 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).

[0062] A method is further provided for determining whether an individual bovine animal is suitable as a gamete donor for natural mating, artificial insemination or in vitro fertilization, the method comprising determining the identity of one or more SNPs according to the present invention using a method of the present invention, and excluding as gamete donor an individual which does not have the preferred allele identity at the respective SNP position as described in Table 1.

[0063] Specifically, an individual bovine animal, especially a bull, is excluded as a gamete donor, if its genome does not have at least:

1) adenine (A) at the position of its MAP1B gene corresponding to position 3066 of Fig. 1;

2) thymine (T) at the position of its MAP1B gene corresponding to position 3323 of Fig. 1;

3) cytosine (C) at the position of its MAP1B gene corresponding to position 87071 of Fig. 3;

4) guanine (G) at the position ofits PPPIRl1 gene corresponding to position 112 of Fig. 4;

5) adenine (A) at the positon of its DDX4 gene corresponding to position 61646 of Fig. 6, or

6) G at the position of its DDX4 gene corresponding to position 34239 of Fig. 5.

[0064] In one embodiment, the individual is excluded as a gamete donor if the genotype of the individual is not homozygous of the preferred allele with regard to the respective SNP position.

[0065) The present invention additionally provides a method of selecting a bovine embryo for planting in a uterus, the method comprising genotyping the embryo according to the present invention, while preserving the viability of the embryo, and excluding from planting an embryo which does not have the preferred allele identity at the respective SNP position as described in Table I.

[0066] In another embodiment, the present invention further provides a method for selectively breeding 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 male animal which has at least one preferred allele identity at the respective SNP position as described in Table land above, implanting said fertilized eggs into other females allowing for an embryo to develop.

[0067] In another embodiment, the method for selectively breeding cattle using MOET may further comprise a step of genotyping the male animal according to the method of the present invention.

[0068] In another embodiment, the method for selectively breeding cattle using MOET may further comprise a step of genotyping the developing embryo, and allowing pregnancy to proceed only if the genotype of the embryo comprises at least one preferred allele identity at the respective SNP position as described in Table 1.

[0069) 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.

EXAMPLES

(0070] Materials and methods

[0071] Associations of candidate fertility genes examined were carried out in two experiments. In the first part, single nucleotide polymorphisms (SNPs) in the spermatogenesis genes were tested for associations with sire conception rate (SCR) in a large bull population. In the second part, genes found significantly associated with SCR were tested for association with female fertility traits (fertilization and blastocyst rates). Male fertility genes that play roles in female fertility can be used to improve reproductive performance in cattle using genetic information from both males and females in breeding schemes.

[0072] *Gene Selection, SNP Identification, and Genotypingfor Bull Fertility*

[0073] A total of 58 spermatogenesis genes, with conserved testicular expression from fly to human, were reported in Bonilla and Xu (2008). Of those genes, only 22 were annotated in the bovine genome. For SNP identification and genotyping, genomic DNA was extracted from semen samples of 268 Holstein bulls (Genex Cooperative/CR!, Shawano, WI) using standard phenol/chloroform protocols. One DNA pool was constructed from 20 random semen samples with equal amounts of DNA. The DNA pool was amplified using primers designed in the 22 candidate genes to amplify 5' untranslated regions (UTRs), exons, intrans, and 3' UTRs. The PCR products were sequenced, and SNPs were identified by visually inspecting sequence traces. PCR amplification and sequencing were performed as described in Khatib et al. (2008). Table 2 shows the primer sets used to amplify the nine candidate spermatogenesis genes found to be polymorphic in the bull population.

Table 2. Primers used to amplify the nine spermatogenic genes

Gene	Forward Primer	SEQ ID NO:	Reverse Primer	SEQ ID NO:	Product Size
DC UNJD/	ATACCC1TAGGCAG1TAG	7	AA1TGTAAACCCTGAGAC	8	536
DDX4(1)	AAACACGGAACAGAGGGT	9	AGGCAGGATfAGCAAGTATG	10	404
DDX4(2)	AACCAAGTGGCTGGGATG	11	CAGACTCAAATGCGACAA	12	354
DNAJJ(J)	CGGTAAGTGAGCAGCATC	13	ACTGAAGCCTfTGCCCTA	14	495
DNA11(2)	CCCAGTGCTCCAAATCCT	15	ATGGCTCATCTTGTCTTCAGTA	16	413
DNAJJ(J)	CGTGACTGGGTITAGGAT	17	CTGGTGGCTGCTGTCTAT	18	602
GAPDHS(l)	CCAGGAAACGGCATCACC	19	ACACGCAGCAGGGCAACT	20	414
GAPDHS(2)	GTGAAGGCCAGGGACTATGA	21	ACATGAACAAGAGGGCTGCT	22	541
GSTMJ	'ITCTCTTCCCTGCAAGTCGT	23	TGAGAACAGCTGCCATCATC	24	664
MAPJ B(I)	CCATTTCCTAAGGCACAG	25	TTCCGCCATCTTCCTACA	26	504
MAPJ B(2)	CTTATGGTCGTGATTATGAA	27	AAGGCTAACACTGCTGGT	28	502
MAPJB(3)	GGCTGTGACATACCTACC	29	CAGACCTTCCCTACTTATT	30	645
PPPIRl1(l)	CACATTACGGCGGAACTA	31	ATCCCAAGCAGTATCACCTA	32	591
PP1'IR/1(2)	ACCTGTTCTATCTCCTCCCA	33	GTCACCTACCCACcrfGC	34	543
SPATA20	'ITGGAGAAGAAACCCACCAG	35	CCTCACAAGCAAGGCTAAGG	36	459
UBC	TCGCTCAGTCGTGTCTrAC	37	TCAACCAACGCCTAATGT	38	420

[0074] A total of 24 SNPs located in the nine spennatogenic genes were genotyped in the Genex population (268 animals) by MALDI-TOF MS (GeneSeek Inc., Lincoln, NE).

[0075] Imputation of SNPs for validation of significant SNPs found in Genex population in a larger bull population obtained from the USDA

[0076] Solely for the purpose of validating the conclusions drawn from the results using the Genex population, and to increase the sample size and improve the statistical power of the study, the 24 SNPs identified in the 9 candidate genes and genotyped in 268 bulls (from here forward, the reference population) were imputed in a total of 1,720 bulls (from here forward, the imputed population) so that a final dataset of 1,988 bulls with genotypic data was generated for subsequent statistical analyses. Bulls in the reference and imputed populations have been previously genotyped with the Illumina BovineSNPSO Bead Chip, and hence shared SNP were used to infer the genotypes of the unshared SNP in the imputed population. Genotypes of the reference (n = 268) and the imputed populations (n = 1,720) for the SOK SNP Chip were provided by Genex Cooperative/CR! (Shawano, WI) and the Animal Improvement Programs Laboratory of the United States Department of Agriculture (AIPL, USDA; Beltsville, MD), respectively. SNP with minor allele frequencies below 5% were removed. After data editing, 38,265 SNP spanning the entire bovine genome were available in both populations for the imputation process.

[0077] Imputation of SNPs was carried out for each candidate gene separately. In each case, a total of 100 SNP on each side of the gene were used to infer the genotypes of the ungenotyped SNP. Imputation was performed using the population-based haplotype clustering algorithm of Scheet and Stephens (2006), which was implemented via the fastPHASE version 1.2 software using the default settings for all parameters (University of Washington TechTransfer Digital Ventures Program, Seattle, WA).

[0078) Phenotypic Data for Bull Fertility

[0079] The 1,988 bulls genotyped with the SOK SNP Chip were evaluated for sire conception rate (SCR), a phenotypic evaluation of bull fertility provided to dairy producers by AIPL-USDA as described in Pei'iagaricano et al. (2012). Briefly, SCR is the expected difference in conception rate of a sire compared with the mean of all other evaluated sires (Kuhn and Hutchison, 2008; Kuhn et al., 2008).

[0080] In this study, SCR values ranged from -10.66% to +6.80%, and the number of breedings per bull ranged from 303 to 111,402. SCR data were obtained from seven consecutive evaluations provided by AIPL-USDA between August 2008 and December 2010. For bulls with multiple evaluations, the most recent SCR evaluation was used in the analysis.

[0081) Statistical Analysis for **Bull** Fertility

[0082] The association between each SNP and SCR was evaluated using the following mixed linear model,

$$SCRiikl = \mu + EVALi + PSNPk + sire, + eijkt$$

where μ is the general mean, EVALi is the fixed effect of the/h AIPL-USDA SCR evaluation (*j* = I, 2, ..., 7), *SNPk* is the number of copies of one allele of the SNP (corresponding to 0, I or 2 copies) carried by the *lh* animal (i = I, 2,..., 1988), *p* is the regression coefficient for the SNP considered (also known as the allele substitution effect), sire$_l$ represents the random additive genetic effect of the l^{1}_{h}sire (/ = 1, 2, ..., 246) of the *lh* animal, and eiik/ represents the random residual for each observation. To detect possible deviations from the additive model, associations between genotype and SCR were evaluated using SNP as a categorical variable.

[0083) Random effects were assumed to follow the multivariate normal distribution,

$$\left(\begin{matrix}\mathbf{s}\\ \boldsymbol{\varepsilon}\end{matrix}\middle| \sigma_s^2,\sigma_\varepsilon^2\right) \sim N\left[\mathbf{0},\begin{pmatrix}\mathbf{A}\sigma_s^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}^{-1}\sigma_\varepsilon^2\end{pmatrix}\right]$$

where *s* and & are the vectors of sire and residual effects, respectively; *a;* and *a* are the sire and residual effect variances, respectively; A represents the matrix of additive relationships between sires in the pedigree (1,558 x 1,558) and W is a diagonal matrix of order 1,988 with its elements representing reliabilities of SCR values. The A matrix was calculated based on a five-generation pedigree of sires downloaded from AIPL-USDA. The association between each SNP and SCR was tested using a likelihood ratio test by comparing to a reduced model without the SNP effect.

(0084) Phenotypic and Genotypic Data for Cow Fertility

[00851 The most significant SNP for SCR (rsI09423562 located in *MAP1B)* was further investigated for association analysis with fertilization and blastocyst rates-the main cow fertility traits-using an IVF system. The procedures of in vitro fertilization and subsequent embryo culture were described in Khatib et al. (2008). To generate fertilization and blastocyst rate data, a total of 6,282 in vitro fertilizations were performed, and a total of 4,207 embryos were produced using oocytes from 359 ovaries collected from 359 Holstein cows and semen samples from 12 Holstein bulls. For 74 ovaries, oocytes were fertilized by two different bulls each. Fertilization rate was calculated as the number of cleaved embryos at Day 2 post-fertilization divided by the total number of fertilized oocytes collected from one ovary. Blastocyst rate was calculated as the number of embryos that reached the blastocyst stage (Day 8) and appeared normal out of the total number of embryos produced.

[0086] The 359 ovaries were genotyped for SNP rsl09423562 *(GIA)* using PCR – RFLP. A 171 bp fragment was amplified using the primers 5'-GCAGCTCTTITAGGAGTGTTAGCGTCTGAT-3' (SEQ JD NO: 39) (forward) and 5'-CTCACAGAGGGCATTTGACA-3' (SEQ ID NO: 40) (reverse). The PCR product was then digested by the restriction enzyme *Hinjl* and electrophoresed on a 2.0% agarose gel. Allele G was cut while allele A was uncut.

[0087] Statistical Analysis for Cow Fertility

[0088] Association between SNP rsl09423562 (G/A) in *MAPJB* and fertilization and blastocyst rates were analyzed using the following mixed linear model,

$$Yijk = \mu + \text{ovary}; + \text{sirej} + SNPy\cdot k + ey'k$$

where *Yifk* represents the fertilization or blastocyst rate of oocyte *k* from ovary *i* fertilized with semen from bull j, *μ* represents a general mean for the trait considered, ovary; represents the random effect of the individual ovary from which oocytes were harvested, sire$_{1}$ represents the random effect of the sire used in the fertilization, SNP*ifk* represents the fixed effect of the ovary genotype for the SNP considered, and *eifk* represents the residuals, assumed normal, independent and identically distributed with mean 0 an variance IO}. Ovaries and bulls were assumed uncorrelated with variance structures Ia; and Ia;, respectively. Association between the SNP and fertilization or blastocyst rate was tested again using a likelihood ratio test by comparing with a reduced model without the SNP effect. All the statistical analyses were performed using the pedigreemm package (Vazquez et al. 2010) of the R language/environment (R Development Core Team 2009).

RESULTS

[0089] SNP Identification and Association of Candidate Genes with SCR

[0090] Sequencing analysis revealed 24 SNPs in 9 spermatogenesis genes *(DCUNJDJ, DNA/l , DDX4, GAPDHS, GSTM3, MAPJB, PPPJRll, SPATA20* and *UBC)* in the bull population using the pooled DNA sequencing approach. All 24 SNPs located in 9 candidate genes were tested for association with sire conception rate first in Genex population and then in a larger population of 1,988 bulls for validation.

(0091] Association analysis in Genex population

(0092] SNPs in the genes MAPIB, PPPlR11, and DDX4 are associated with SCR

[0093) For MAP18 gene, a SNP *C/T* in intron 5 at position 9331992 (University of Maryland bovine version 3.1; UMD3.1) showed significant association with sire conception rate. Primers used to amplify the gene and SNP location are shown in Figure 1. Frequency of allele T was 25% and frequency of allele C was 75%. The allele substitution effect was - 0.15 ± 0.01 (p-value = 0.01) so that allele C is favorable for SCR.

[0094] For PPPlR11, one SNP (TIG) in in the 5'UTR region at position 28710268 (UMD3. 1) was significantly associated with SCR in the Genex population, with allele G associated with increased SCR. FIG. 4 shows SNP location and primers used to identify the SNP.

[0095] For DDX4, two SNPs located at positions 23382814 (SNPI; *AIG)* and 23410221 (SNP2; *GIA)* were identified in the gene. For SNP locations see Figure 3. The two SNPs were in almost complete linkage disequilibrium, so they have the same allele frequencies in the bull population examined. Genotype AA of SNPJ has 0.783 units of SCR versus -0.214 SCR units for GG genotype (P-value = 0.05). Similarly, GG genotype of SNP2 has 0.749 units of SCR versus -0.494 for AA genotype.

[0096] Association of spermatogenesis genes in the combined Genex and USDA populations

[0097) Three SNPs located in *MAPJB* and one SNP in *PPPJRJJ* showed significant associations with SCR (Table 3). The SNP with the most significant association with SCR is SNPS.MAPlB located in intron *5* with an allele substitution effect of -0.24 and a P-value of 0.001. The other two significant SNPs were SNP1.MAP1B and SNP3.MAP1B, both located in intron 1 with an allele substitution effect of 0.15 and P-values of 0.025 and 0.039, respectively. After Bonferroni correction for multiple testing, only SNP5.MAP1B remained significant (P-value = 0.024). Pair-wise linkage disequilibrium (LD) tests of *MAPJB* SNPs showed a moderate LD (r = 0.38) between SNPl.MAPlB and SNP3.MAP1B and SNP5.MAP1B. The LD between SNP3.MAP1B and SNP5.MAP1B was relatively low (r^2 = 0.14).

[0098] SNPI.PPPl R 1 1 located in the 5'UTR region of *PPPJRJ 1* showed significant association with SCR with an allele substitution effect of 0.15 and a P-value of 0.046.

Table 3. Genetic markers significantly associated with sire conception rate

SNP ID	Gene	Genotype (N)	Allele substitution effect ± SE	P-value
SNP1.MAP1B	*MAPJB*	GO (463) GA (987) AA (538)	0.15 ± O.ol	0.025
SNP3.MAP1B	*MAPJB*	**CC** (972) CT (847) TI (169)	0.15 ± 0.01	0.039
SNPS.MAPlB	*MAPJB*	**CC** (1081) TC (763) TI(144)	-0.24 ± 0.01	0.001
SNP1.PPPIR1 I	*PPPJRJJ*	TT (1033) TG (821) GG (134)	0.15 ± 0.01	0.046

[0099] Association of MAPlB with Fertilization Rate and Embryo Survival Rate

[00100] SNP5.MAP1B located in intron 5 of *MAPJB* showed the most significant association with sire conception rate in the bull population analyzed above. To characterize its impact on female fertility, we tested the association of this SNP with fertilization rate and blastocyst rate in the IVF system. SNPS.MAPl B showed significant associations with both fertilization rate (P-value = 0.027) and blastocyst rate (P-value = 0.029) (Table 4). Oocytes collected from genotype CT cows showed the lowest fertilization rate (59.9%) compared with that from CC (66.4%) and TT (66.3%) cows (Table 4). For blastocyst rate, the CT genotype

again showed the lowest rate (27.1%) while homozygous CC and IT individuals showed blastocyst rates of 31.0% and 41.8%, respectively (Table 4).

Table 4. Association between SNP5 of *MAP1B* gene and fertilization rate and blastocyst rate

Genotype (N)	Fertilization rate		Blastocyst rate	
	Estimate ± SE	P-value	Estimate ± SE	P-value
CC (321)	0.664 ± 0.03	0.027	0.310 ± 0.02	0.029
CT (84)	0.599 ± 0.03		0.271 ± 0.03	
IT (28)	0.663 ± 0.04		0.418 ± 0.05	

REFERENCES

Amann, R.P., J. M. Dejarnette. 2012. Impact of genomic selection of AI dairy sires on their likely utilization and methods to estimate fertility: A paradigm shift. Theriogenology 77:795-817.

Barton, S. C., M. A. Surani, and M. L. Norris. 1984. Role of paternal and maternal genomes in mouse development. Nature. 311:374-376.

Bonilla, E., and E. Y. Xu. 2008. Identification and characterization of novel mammalian spermatogenic genes conserved from fly to human. Mot. Hum. Reprod. 14:137-142.

Cheng, L., S. Pilder, A. C. Nairn, S. Ramdas, and S. Vijayaraghavan. 2009. PP1gamma2 and PPP1R 1 1 are parts of a multimeric complex in developing testicular germ cells in which their steady state levels are reciprocally related. PLoS One. 4:e4861.

Choe, C., Y. W. Cho, C. W. Kim, D. S. Son, J. Han, and D. Kang. 2010. Identification of differentially expressed genes in bovine follicular cystic ovaries. Korean J. Physiol. Pharmacol. 14:265-272.

Dobson, H., R. Smith, M. Royal, Ch. Knight, and I. Sheldon. 2007. The high-producing dairy cow and its reproductive performance. Reprod Domest Anim. 42 Suppl 2:17-23.

Druet, T., S. Fritz, E. Sellem, B. Basso, O. Gerard, L. Salas-Cortes, P. Humblot, X. Druart, and A. Eggen. 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. J. Anim. Breed. Genet. 126:269-277.

Feugang, J. M., A. Kaya, G. P. Page, L. Chen, T. Mehta, K. Hirani, L. Nazareth, E. Topper, R. Gibbs, and E. Memili. 2009. Two-stage genome-wide association study identifies integrin beta 5 as having potential role in bull fertility. BMC Genomics. 10:176.

Han, Y. B., H. L. Feng, C. K. Cheung, P. M. Lam, C. C. Wang, and C. J. Haines. 2007. Expression of a novel T-complex testis expressed 5 (TctexS) in mouse testis, epididymis, and spermatozoa. Mo!. Reprod. Dev. 74:1 132-1140.

Han, Y., X. X. Song, H. L. Feng, C. K. Cheung, P. M. Lam, C. C. Wang, and C. J. Haines. 2008. Mutations of t-complex testis expressed gene 5 transcripts in the testis of sterile t-haplotype mutant mouse. Asian J Androl. 10:219-226.

Imumorin, I.G., E. H. Kim, Y. M. Lee, D. J. De Koning, J. A. van Arendonk, M. De Donato, J. F. Taylor, and J. J. Kim. 2011. Genome Scan for Parent-of-Origin QTL Effects on Bovine Growth and Carcass Traits. Front. Genet. 2:44.

Khatib, H., R. L. Monson, W. Huang, R. Khatib, V. Schutzkus, H. Khateeb, and J. J. Parrish. 2010. Short communication: Validation of in vitro fertility genes in a Holstein bull population. J. Dairy. Sci. 93:2244-2249.

Khatib, H., W. Huang, X. Wang, A. H. Tran, A. B. Bindrim, V. Schutzkus, R. L. Monson, and B. S. Yandell. 2009. Single gene and gene interaction effects on fertilization and embryonic survival rates in cattle. J. Dairy Sci. 92:2238-2247.

Khatib, H., R. L. Monson, V. Schutzkus, D. M. Kohl, G. J. M. Rosa, and J. J. Rutledge. 2008. Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. J. Dairy Sci. 91:784-793.

Kuhn, M. T., and J. L. Hutchison. 2008. Prediction of dairy bull fertility from field data: use of multiple services and identification and utilization of factors affecting bull fertility. J. Dairy Sci. 91:2481-2492.

Kuhn, M. T., J. L. Hutchison, and H. D. Norman. 2008. Modeling nuisance variables for prediction of service sire fertility. J. Dairy Sci. 91:2823-2835.

Magee, D. A., D. P. Berry, E. W. Berkowicz, K. M. Sikora, D. J. Howard, M. P. Mullen, R. D. Evans, C. Spillane, and D. E. MacHugh. 2011. Single nucleotide polymorphisms within the bovine DLK1-0103 imprinted domain are associated with economically important production traits in cattle. J. Hered. 102:94-101.

Magee, D. A., K. M. Sikora, E. W. Berkowicz, D. P. Berry, D. J. Howard, M. P. Mullen, R. D. Evans, C. Spillane, and D. E. MacHugh. 2010. DNA sequence polymorphisms in a panel of eight candidate bovine imprinted genes and their association with performance traits in Irish Holstein-Friesian cattle. BMC Genet. 11:93.

Memili, E., and N. L. First. 2000. Zygotic and embryonic gene expression in cow: A review of timing and mechanisms of early gene expression as compared with other species. Zygote. 8:87-96.

Montenegro-Venegas, C., E. Tortosa, S. Rosso, D. Peretti, F. Bollati, M. Bisbal, I. Jausoro, J. Avila, A. Caceres, and C. Gonzalez-Billault. 2010. MAPIB regulates axonal development by modulating Rho-GTPase Rael activity. Mol. Biol. Cell. 21:3518-3528.

Pefiagaricano, F., K. A. Weigel, and K. Khatib. 2012. Genome-wide association study identifies candidate markers for bull fertility in Holstein dairy cattle. Anim. Genet. (In press).

Queir6z, D. B., A. M. Silva, G. Gutierrez-Ospina, C. S. Porto, G. Grossman, P. Petrusz, and M. C. Avellar. 2006. Cells positive for microtubule-associated protein IB (MAP IB) are present along rat and human efferent ductules and epididymis. Cell Tissue Res. 325:125-133.

R Development Core Team. 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvao. 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 83:513-535.

Scheet, P., and M. Stephens. 2006. A fast and flexible statistical model for large-scale population genotype data: Applications to inferring missing genotypes and haplotypic phase. Am. J, Hum. Genet. 78:629-644.

Shook G. E. 2006. Major advances in determining appropriate selection goals. J. Dairy Sci. 89:1349-1361.

Telford, N. A., A. J. Watson, and G. A. Schultz. 1990. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. Mot. Reprod. Dev. 26:90-100.

Tortosa. E., C. Montenegro-Venegas, M. Benoist, S. Hartel, C. Gonzalez-Billault, J.A. Esteban, and J. Avila. 2011. Microtubule-associated protein I B (MAPIB) is required for dendritic spine development and synaptic maturation. J. Biol. Chem. 286:40638-40648.

Tymanskyj, S. R., T. M. Scales, and P. R. Gordon-Weeks. 2012. MAPIB enhances microtubule assembly rates and axon extension rates in developing neurons. Mo!. Cell Neurosci. 49:110-119.

Vazquez, A. I., D. M. Bates, G. J. M. Rosa, D. Gianola, and K. A. Weigel. 2010. Technical Note: An R package for fitting generalized linear mixed models in animal breeding. J. Anim. Sci. 88:497-504.

Weigel, K. A. 2006. Prospects for improving reproductive performance through genetic selection. Anim. Reprod. Sci. 96: 323-330.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising at least one nucleotide selected from the group consisting of:

i) a nucleotide at position 1986 or position 2243 of SEQ ID NO:1, and at least 10 but not more than 200 contiguous nucleotides of SEQ ID NO:1 adjacent to position 1986 or position 2243, wherein position 1986 is adenine, and position 2243 is thymine; and

ii) a nucleotide at position 1991 of SEQ ID NO:2, and at least 10 but not more than 200 contiguous nucleotides of SEQ ID NO:2 adjacent to position 1991, wherein position 1991 is cytosine.

2. A nucleic acid molecule according to Claim 1, which comprises at least 13, at least 14, or at least 15 contiguous nucleotides of:

i) SEQ ID NO:1 adjacent to position 1986 or position 2243; or

ii) SEQ ID NO:2 adjacent to position 1991.

3. A nucleic acid molecule according to Claims 1 or 2, which comprises at least 20 contiguous nucleotides of:

i) SEQ ID NO:1 adjacent to position 1986 or position 2243; or

ii) SEQ ID NO:2 adjacent to position 1991.

4. An isolated nucleic acid molecule according to any one of Claims 1 to 3, which comprises not more than 150 contiguous nucleotides of:

i) SEQ ID NO:1 adjacent to position 1986 or position 2243; or

ii) SEQ ID NO:2 adjacent to position 1991.

5. An isolated nucleic acid molecule according to any one of Claims 1 to 4, which comprises not more than 100 contiguous nucleotides of:

i) SEQ ID NO:1 adjacent to position 1986 or position 2243; or

ii) SEQ ID NO:2 adjacent to position 1991.

6. An isolated nucleic acid molecule according to any one of Claims 1 to 5, which comprises not more than 50 contiguous nucleotides of:

i) SEQ ID NO:1 adjacent to position 1986 or position 2243; or

ii) SEQ ID NO:2 adjacent to position 1991.

7. A nucleic acid molecule according to any one of Claims 1 to 6, wherein the at least one nucleotide is within 4 nucleotides of the center of the nucleic acid molecule.

8. A nucleic acid molecule according to Claim 7, wherein the at least one nucleotide is at the center of the nucleic acid molecule.

9. A nucleic acid molecule according to any one of Claims 1 to 6, wherein the nucleotide is at the 3'-end of the nucleic acid molecule.

10. An array of nucleic acid molecules comprising at least two nucleic acid molecules according to any one of Claims 1 to 9, wherein the nucleic acid molecules are affixed on a medium.

11. A kit comprising a nucleic acid molecule of any one of Claims 1 to 9, and a suitable container.

12. A method for detecting single nucleotide polymorphism in a bovine MAP1B gene whose partial nucleotide sequence is shown in SEQ ID NO:1 and SEQ ID NO:2, the method comprising obtaining a sample of the respective bovine gene from a bovine animal, determining the identity of a nucleotide of the gene at a position corresponding to
i) position 1986 or position 2243 of SEQ ID NO:1, wherein position 1986 is adenine and position 2243 is thymine; or
ii) position 1991 of SEQ ID NO:2, wherein position 1991 is cytosine,
and comparing the nucleotide identity of the position respectively to the nucleotide identity at a corresponding position of the sequences shown in the respective SEQ ID NO:.

13. A method for genotyping a bovine cell, comprising obtaining a nucleic acid sample from said cell and determining the single nucleotide polymorphism of the MAP1B gene according to claim 12.

14. A method according to Claim 13, wherein the bovine cell is an adult cell, an embryo cell, a sperm, an egg, a fertilized egg, or a zygote.

15. A method according to Claims 13 or 14, wherein the identity of the nucleotide is determined by sequencing the MAP1B gene, or a relevant fragment thereof, isolated from the cell.

16. A method according to any one of Claims 13 to 15, wherein the gene or a relevant fragment thereof 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.

17. A method according to Claims 15 or 16, wherein both copies of the gene in the cell are genotyped.

18. 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 any one of Claims 13 to 17.

19. A method for selectively breeding 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 any one of Claims 13 to 17, and terminating pregnancy if the developing embryo does not have at least one polymorph selected from the group consisting of:
adenine at a position of the MAP1B gene corresponding to position 1986 of SEQ ID NO:1;
thymine at a position of the MAP1B gene corresponding to position 2243 of SEQ ID NO:1;
and cytosine at a position of the MAP1B gene corresponding to position 1991 of SEQ ID NO:2.

20. The method according to Claim 19, wherein pregnancy is terminated if the developing embryo does not have at least two of the polymorphs.

21. A method for selecting a bull bovine animal as a breeder, wherein the animal is genotyped according to Claim 13, and is only selected for breeding purpose if it comprises at least one polymorph selected from the group consisting of
adenine at a position of the MAP1B gene corresponding to position 1986 of SEQ ID NO:1; thymine at a position of the MAP1B gene corresponding to position 2243 of SEQ ID NO:1; and cytosine at a position of the MAP1B gene corresponding to position 1991 of SEQ ID NO:2.

22. The method according to claim 21, wherein the cattle animal is selected only if it comprises at least two of the polymorphs.

1081 gagcgcgctc tgcagaggcc gaatagcggt tcgctgggaa accctgggcg gggccgtaag
1141 ggtccagcgg gcgcagactc gggcgcgcgg accgacccgc cccgcagccc cacccgggct
1201 gccgcagtgc cccgcccga tgcacctgcc ccccacactg cggcgccccc actgggcgcg
1261 gccggccggg cgtgcgttcc gccggtgctt gggggtgctg tgcgccctct cgtctgcctc
1321 aacccggctt tgttgcgctc gaagtccccg ggtgggcagc tctgtcctct gcctttccct
1381 ttcccccgg catcgcagac ctccccttct ccctgaccga ggtcgcgggt ccctccacaa
1441 ccccagcccg cgctctattc tctcggggtg gtgctgaagc gcgtctgccc cgagacaccg
1501 gctggtgggc gtggtgcagt ccgcactgcg gtctctacgg cagcccgagg cggacaaagg
1561 gcgttcacgc agccctcgtt ccccacgccc gcccccact ccccatgaaa gacgcgagaa
1621 aaccttgttt tagatgaaaa aaaattaaac tctagtggtc tgcctctgca tttgaaaacg
1681 gtcgcctgtg cccagaacaa aaggctgcag ggtggagact cgagttgcag acctggttct
1741 tttgtttaac tttaaagcac tggtgttact ttttggctta aaaaaagaaa aaaatgtgag
1801 cagaaagcag acttgttatt ttattcggaa aaaaaaaatg gaagaatagg ctaggtcaat
1861 agtgaaatgc ctcatttgag catctaataa cccttcattt gtcaaactat agtcctttga
1921 atttgatcag tactaattta gtttatttgc acattttctc cttggaaaat ttcacacgta
1981 ctgactttgg gtgtggctgc tgtatgaatc tatgactttg gatttttaaa aaaatattat
2041 ttgtcagcac ttttgctggg aagtaataat aaagcaggtg tgtttctatg tataaaaggt
2101 gcataagcac ccgatgtggt gtgaggagag ggaccctcat ccccattttg gagatggggg
2161 cccagagggg caggaaggat agggagacca gctcaaggtc acaccactca taagtgacag
2221 aatgccggct ctgcagcatc actggttttt ggcccgtcat gatgttagtg caggccaggc
2281 gaacatcaca ggaagatcaa agagcaattt ctagagcttg cctttataca aaggcagtag
2341 tcatccactt gtgggcagca ccccatgcag ggaggtggct cagcaatcac cttccatgta
2401 attatcttcc tgctttgtta ctctgctgat caccctttta gtgccctatt tctcaggggg
2461 tttattgaag ctgcttctgt ttgaggataa actagattca cccaagttat cggtcactgt
2521 gacgtggtct cgactatttt tcacgctact ggaacttagt gatcgagttc aattttgtct
2581 tcttgcccct tttctttttt aaaatttatt gatgtattat gtagtgaggg aaaggctcaa
2641 atcataaacg aggagcttgg tgaattttca caaaaagaca tacctgatcc cgatcaagaa
2701 acggacattc ctgggacccc cagaaaccct ctcaggtgtc cttttcagtc agcatgcctt
2761 cctgattttt tagtgccctg atttagtcag ctttttgcgg ggaactctga aatagcagta
2821 ttgatcccat taagaatcaa ccaagtgaat gagcaaag**cc atttcctaag gcacag**taac

2881 agatgctgct tttcctctgg agatcaactc tcttgggtcc tggggtcttg gatgcagctc
2941 aaaccacagg gccttccagt gctaagggga aatactgctc ctgtagcttt tctgacagag
3001 atgacttagc aacagggcag gctggctgtt gccctggcct gatatgatgc ttcctcagct
3061 ctcag[a/g]ttcc agcagggcct cctcctccag aggtcactct gataccatgg ctgcggtggc
SNP1
3121 tggtgcccat gggaccagtt gtgtgaagca agatggagat [g/a]ctccggggg ctcggccctg
SNP2
3181 cttgtgtgcc gcagcctttc cgcagggaaa gcggttcatc ttctgcccct cttgcttgtc
3241 tcccctctca tccggatgag gctctctgag tctggagacc ctagggactt ggatttttgc
SNP3
3301 catttgatga tttaaggctc ta[t/c]gggaaac ctagaaaata aatc**tgtagg aagatggcgg**
3361 **aa**atgcaagt acatcaatag gcctgaatca gcccaccatg gaggctgagt acatgcttct
3421 tgaaacctag tgttactgaa tcaaactgag gcagctcacc tgcatgcagt aaagccaatt
3481 tactgacccc aggttgtggt gaaggaaagt gcagcattat tgtaagatgc tgatgtaagg
3541 agaacgggga gctcgtgctt aagaccccca aaatccccca agggtttcag caaagcattt
3601 ttaaaggcag tgtaagggag ggtgtcccag agtgtgtgat cagctcatgc accattctct
3661 aattgagaat tagattgatg atgaggtaac aagactacgt tgggcttcct tgctggctta
3721 gatggtaaag aatctgccta caatgcagga gagctcggtt tgatccctgg gttgagatcc
3781 cttgaagaag ggcatgacaa cacattccag tattcttgcc tggaaaatcc ccatggacag
3841 aggagcctgg tgggtacagt ccatggggtt gcaaagagtt ggacatgact gagtgactag
3901 gcacagcaca agactatgtt aatgaggact atgtgcggat ggtcatcaag tagttaattt
3961 cttccatttg atggtgattt tagcatctga aaaataactc aggaaatata cttcagatac
4021 taatatctag gtatttcaga gaggaactaa agcagagggt atagggaggg ggtctgtccc
4081 aggaatgtcc cataaggtcc tgcttggtta cacaaggaca gaagaccatc attcgtttct
4141 tcagcttcat tcaaaatgca taatgtaggg tctggtcatt tcttaagtct gaaacaacag
4201 tctttgctca gactggtcta gctttattta gttagttatg caggactcca tgccaaacca
4261 ttgtcactcc ttggcacctc ctttgccccc ttcctaatta gtctccaaca tagacttaac
4321 agtcatcccc gtttttagca gagtctcccc tcctccagtt tattttggga tgtagactac
4381 atacactcat gttttgcaaa acgaattcta gagccttgtg aaaagtttc attgcttcgt
4441 tttattttat tttttaaact aaaacatgta aaaaacacta agtgtcagtt tggtttcact
4501 tgctgttaga gtgtaacttt cctctggtta acattgggaa tcagcaggat ttgttcatca
4561 gaaagataga tgtgtgtaac atctactgcc ctggattttt agatgtaagt tttgctaatt
4621 acattgtcat ttattgaata ctttcagtct acattggtta agagaattaa gaacccagga
4681 aagagttagg gctttgtttc cattttgttt taacaggaaa acaacggggt gatgggaatg
4741 aaaagaaatg ttgaggaatg atatatttac tatatccatc catcttcata cgtttcaaaa
4801 tcaaaaggca gatggatttc tgcttgcgct gcttaattgg tattatctac tcaaagagtt

FIGURE 1

FIGURE 1

Partial genomic sequence of Genomic sequence of MAP1B showing the location of SNPs 1, 2 and 3 on the MAP1B gene and the locations of the primers (1F and 1R, corresponding to positions 2859-2876, and positions 3346-3362, respectively) used to amplify the region comprising the SNP sites. The number of the sequences is per that of GenBank Accession No. (514739)

49681 tccagcctgt tctgtggatg tttgaacttg agaagtgggg tctttgtcca gaggaaacac
49741 tgcttttcgc ctggtagagg atgggctcca tccgaatcat acccagtttg ttcctttgct
49801 acttcttcat cttcccgtgg tttcatgtcg agtcagaatg taaggactgt ttagcttttg
49861 tgaggggcaa aaatgtgttt ttgaactgga caaggtaagg tttgaaccca ttcttttgtc
49921 tttcttgtat acttccattt tcactttgag cacaaagcag gttggggaag caggaggggg
49981 gaagatgtta ttgtggatta gagacagagg aaaaggcagg tgggggttg gaactgaacc
50041 ccacttcctg cagccgtttc ccagccggtt ttgaaaagac tctgaaagga gaataacgtc
50101 tttaatcaag agcaatagta ttagctcctt tactataagt aatacttttc tttgagccta
50161 tatttatttt accgggctag aaatagctga agttattcca gcagccatga ctattgtcta
50221 ggagttggat gtgggctggc aatagactgg ctgattacac tgtttagaaa taaacccctt
50281 tgttggcagt ctcttctggt gagaatggtt cataaaggtc cctgtggctg gttgttccat
50341 ggtgcttgct tttatatcag ttcagtaccc tcataatgag gtggtcttct agaatatatt
50401 attataactc tgttgcagag ggcgtagggc tcgtcagtta tgcaggcaac atcacaaagc
50461 tttggaagaa atctcttaat taagtgctag ggctggtgct gcagtgaagg gatgaatgga
50521 ctgaaatgct tccatctctg agcgtctttt caaactaaac gggccctttg ccgcatcata
50581 gccaggagtc cagcagacgg acacactgag aaagtggtgg tggatgtgat tggtgatgtt
50641 cctgacttcc tctgacctac ccctggggat ttctgtactt cacgtcacac gtggctcctt
50701 gttgatggta tggtgaaaac ataggtgttg aaagaaccag aattgacagc agttcagcga
50761 ccttttgggt cttcaggtct gagaccatac cctaggcagc atcagtccct ccacggtaga
50821 tggcactgga ctttctgtgg cgtttaagac ctaacgttct gtgactgaga atgtggcctg
50881 tcttggccac agctggtacg atgacaagat gactatctga gttaggaaga aaaagtgaag
50941 tgaaagtcat ttagttgtgt ccgactccgt gacctgcagc tcttctaggc tcctctaggc
51001 tcctctgtcc acgggatttt ccaggcaaga ataatggagt gggttgccat ttcctcctcc
51061 agatcttccc aacccaggtc tcctgctttg caggcagatt cgttatgtgt ctacaatgaa
51121 agaaagggta ggagcaaata cagaggcaga agtttgttcc ctcctaggaa ggttattctt
51181 gatctggcca ttcaaagacc ttttcatttc ctctcagatc ttttcaaagt gactaacctg
51241 aaaaatcttt gatgtgtggg ccaggacatg atggaggaag gcatcttttt ctttcctttt
51301 actccttgga gaagagatga aactaaaagg gctctaaggg aaaaaaaaaa attcttaaaa
51361 aaaaaaagtt aataaaaaaa caaaaggtaa taaatagctc tttgtaaaca gcttaccacc
51421 ttacttcctg tggttacatg cattac**ctta** **tggtcgtgat** **tatgaa**agat ttctagagaa
51481 acgttaggat gatcacataa ctccctccta aggcgccagt ggagcccaaa gtctttgctg
51541 ttcacgtgcc ttgtgagtgg cccaacacag tggggacttt ataaatatca aatcattgtc
51601 gttaaaaaac acttcccgct tcactctgag acccttcctt ttaaggagtg catgtggtgg
51661 [g/a]ggaggatta atgacagcac agcgagtgtg gcttgaaggt ggtgacatca cccggcttga
SNP6
51721 acccttcagt gccgggtgag aggattttca tctcatccat cctcctgagt ttgcca[c/t]gag
SNP4
51781 gggtctccaa gaacaggaaa agaggagtct gaggagagga gacttctgga cattctgtga
51841 tagtcccctg gctctgtgcc gtattgtttt gtaaataagg cagttatggt ttctagtctg
51901 ttgtttttct acaaaaatgg aggacgtgtg **accagcagtg** **ttagcctt**cg tgaatgagat
51961 tctgtgtttc ggccatcact ggttcaagta ggtaacctaa gagctgagct taagttgctt
52021 ctcttgcagc ccatctttgg ctttcagtaa ggaatctgag caacattaga ctgagaatca
52081 gacaccttta ccatcacttt acgggatgct tccatttgct gtgtgatagg acgcaggtgc
52141 aggagggagg cgtctggacc ccagagtcgt ggcgtcagga ggtcccgtgg gagcatcctc
52201 agcctctgca gtggtcctac caggagagga aggtgcttgg gtgtcgggat acccatgctc
52261 aactcctggt tctcccgtta tcatgccctg tgactctgga caagtcactt agcctctctg
52321 aaccttaatg tttgtatctg ttaagacaag ggtttggaat agatccgtcc aaattcacat
52381 ttctgcagac ctgggttact ggctgtaggg ctctaattgg actggatctc ctcatccctg
52441 ataacctatt tccagggcgt ggggccccct gctgtacagc gtcttgcttc attttcccac
52501 cgtcttttag ctcccatctg gatgccatct tctgtgtagt atggaagcct tcctaaacca
52561 tccagtgact cccttgagcc cttcctctcc ctgctgaggt gtgtgtccag gagccagggg
52621 ccatcctgcc ctttttgctg gccactgccc catggttctg gtctcatgcc agggtcggca
52681 ctctgtcagg atgtggtggg ttgagtttat acctgatctt gatgtaaaca catggcctct
52741 gcccagtcat ttgttcctgt tcccacactg gcttccagct cttttgtgga ctctgactct
52801 ctgctctcct ggcctcctga tggctggaca tcttttcttt tccttctaga atgccacccc
52861 ttttttgtt gtctaacttg taaaagcccc atagatcatc tcccatttca aacccttaga
52921 gatgactatc ttgatatgtt gataagaggt gaactttctc agaggagttt cttgttacag
52981 tgtcaaatgt ggttataaat cactggaact taaggatctg tctgccaagc agtagacacg
53041 agtttgatcc ctgggtcagg aagatccgct gaagaaggga atggctatcc atgccagtat
53101 tcttgcctgg agaatcccat ggacagagga acctggaggc tacagtctat ggtgtcacca
53161 aaaaattgga catgacttag tgactaaaca acaacaataa gagaagcttt aagggaggtc
53221 agccctctct ccaccccagc actagaacag tccctagagc agagtctccc aaatctgcct
53281 ggtgagcaga atcatcaccc agtgctttta ttattatttt aatatttatt aaaaaatttt
53341 ttttgtttgg ctatattagg tcttagttgt ggcatgaggg atctttagtt gcaacatgtg

FIGURE 2

FIGURE 2

Partial genomic sequence of Genomic sequence of MAP1B showing the location of SNPs 4 and 6 on the MAP1B gene and the locations of the primers 2F and 2R (corresponding to positions 51447-51466, and positions 51931-51948, respectively used to amplify the region comprising the SNP sites. The number of the sequences is per that of GenBank Accession No. (514739)

85081 agtgctctgg ttaatcaacc agtgaggtca gctgaccact gggtacccag tacagattga
85141 gaaaagagca tccaagactc tacttattcc attctgacca cactgcctca ctaatacaga
85201 ctcaacatct tgttttaggt cgaaattcag cttggcaaga gcaagcatcc ctgacttcat
85261 gttcttagac ttatcaagtt ctcggcagtg atctgggttt ttgatgaatc tgggtttctg
85321 atgatccagc ttcattttgt tgcttcaaaa caatcacagg gatttgaatt catatatttt
85381 atttgcttta catagttact tgaaggtttt agatcacagt tcacaaacat gtaaagcaaa
85441 aaataagcaa cactttcttg atttattatg gaaaaattca gtatttagta ctttaggaag
85501 tactagttac aggtacaagt ttttactttt aggcaacgtg aagcagcaat ttcaagactc
85561 atatcagatt tcctcttttt atttgcacat agaaaacaaa ctgaatttgt tcatgcttag
85621 aatttgtata gagccaccag ataataaatc ttgatctaaa ggacttaaca gtgaccatgc
85681 acttaggaga aaacatgaaa tcaattcaaa cagataaaaa cccaactgaa atttgctgcc
85741 aaactcatga aacttacact atagcccaca caattgattt tatcactttt tttttttttg
85801 gtcatttaaa gataattttg agggaaagtg agttaatttg aatttacatt gaggatgctt
85861 tcccaacaga tttttttaaa agacaaaact gcactattaa ttaattttaa aacaaacctg
85921 ggtcaacttc cagtggttct atcagtttgg gcttctatga gcaatgttca tttggtgtca
85981 acgggagtga ttcaaggtgc aagtggaaac tgcaggcatt taaaaatatt agatgatctg
86041 taactcacaa acctctgcta caagtcagaa ttctttggga gatttacaca tgaatatgtt
86101 taggactttt agcttaggtt cattataatg gctggttaat ctattcatga aatgattcag
86161 tttatccaaa taccagtttg gctgattctt actacccccct gccctccaaa ataaaaataa
86221 accagttcat agctgatttt gactgtggga tggcagtctc tatacatccc atggagaaag
86281 gcaagagaat taaatttagg ggatcttgct agtattttaa gtggtttcac agcagtggtc
86341 tcaaaccaga tacacattag cattggctgg gatgctttta aaaagtgatg gtaccctggt
86401 cagtgaagcc ttaccatagc cattgaagcc agggcatctg tattaagcat gctaagtgat
86461 tctaatcatg tggccaggag gaagaaccac tgccttacaa tgctagttct gttaatgttt
86521 caaccttctg attagaacaa atcagaaagc caattctaga aacaaggtag ccagaaactg
86581 agattaatct gaaccttcat tttgcccagg ctttctgact tggggggaat ttt**ggctgtg**
86641 acatacctac cccttacctc agtccggtat gttctgattg gctagagaaa gcagagtctt
86701 tctgaacctt cctgttgcta aagtttggta tctagtcttg tctaaggaga gacgtctacc
86761 atttagagga ctgtcctaag gagagaatac agtgttttca tcagtttatg catgaggctg
86821 aggtgctgag ggtcttggag atcatatgac attaagatct gactactggc tagatcaaat
86881 gtgaggggat aatattcagc tgtgggccaa actgctttta aatgaaatcc taacatgaat
86941 tactaagatg gcttaactat gctttaccaa atgcagatgc tttcctttgt cctttaaaat
87001 ctatttctta gatcacattt caaattaaaa gacacactag cagctctttt aggagtgtta
87061 gcgtctagtt [c/t]tatctttgg ggaaagcctt ggcaactctt cttaattgct aatgtgttta
SNP5
87121 agggaaacgc cccattcttc atttctcctg agatggtaaa cagtcaagtg atgctgtctc
87181 agactgccag tgtcaaatgc cctctgtgag agaggggagt gccaacaccc actcccatgt
87241 cccagagcgc cttctgggg**a ataagtaggg aaggtctg**ct ggacagatga gtctctttgc
87301 atttttgtga ccctggcctt ctctttgttt ttatttgttt acaaagggcc aggaaccacc
87361 aagacgtcca agccctcagc tgtgccccca ggcccccctg tgtacctgga cctatgctat
87421 attcccaacc atagcaatag taagaatgtc gacgttgaat ttttcaagag agtgagatcg
87481 tcctactacg tggtgagtgg gaacgaccct gctgctgagg agcccagccg ggctgtcttg
87541 gatgccttgc tggaagggaa agcccagtgg ggcagcaaca tgcaggtaag agttccagga
87601 cggtgtttgc acaacacgtg gagctgtgtc cagaggcagc aggaagggat cgtgtttaat
87661 gaggcaccac cgtggatccc catgaggtgc ccacagggcc tgctgcactt ggacaaagtg
87721 gatttcacac acacaagctg gtctaaaagc attcgcgcca ccagccacca tggacttgga
87781 ggaaggccac tttaccaccc taaagtataa tctgcagagt gggcccaaga ttacacaccg
87841 ttcatatacc aagaaaatta accagcgtaa ccaagtgtca tatttccatg tgagatggat
87901 aaagattagc ctttacttgt ctttcccaag tagacaaaag ctagagatat ggccatttag
87961 aaaatcagct gtccacatga gattctgcag gagcactgct gaaaatggtc ctcagcagga
88021 cactcccaac acccaaacat cgtaatgagc cacaaaccac tcattatttc agttatggat
88081 tttatctaag ttttacttac ggttttgtat agtgatctag taaactgtat ttgcataacg
88141 ttaaatagaa atcctggtta tttcattata tgaaatctaa tgcactcagt ggcctcttac
88201 tgaatactag gtagaattta agctagtaat cacttaccca ccccactcct ctgtccccaa
88261 acacacacac aaagacataa atctttgctc tcatgatgaa atgttagtta acatgcaatt
88321 agaaggtttt cggctgcatt aataactaaa gcccctttgt tttaaatatg caatatcttt
88381 aatgtaaaac atcagttgtg ttaaagaaaa tacaagaaat tccaccttaa ctgaagaact
88441 tctcataatg ctaaagaatt gaaaactgat atagatgaac taactggcta gtcatgactt
88501 gcttttggtt ctagtcttca actgccccag aaaaactaat tttttagcag ctttattctg
88561 gttcctagaa aatgtaagtt ggaaagtcct atggattttc taaggacaat agaatatttt
88621 tctctttccc tttctttttc taatggtcta attaatacct tactgctgtt ctatttttcc
88681 ccaccccatt tctggttctg ctcttcagta gctgttttct ctctccctgc aggtgaccct
88741 gatcccgact catgactcag aagtgatgag ggaatggtac caggagaccc atgagaaaca
88801 gcaggacctc aacatcatgg ttttagcaag cagcagcaca gtggttatgc aagatgaatc
88861 cttccctgca tgcaagatcg aactgtaaca accaaggtca gccgcaccac aggatttgaa
88921 ctttgtttcc agaaattctt cgatttgaaa ccaccttttc taaaaaaaaa gtcaattcat

FIGURE 3.

FIGURE 3

Partial genomic sequence of Genomic sequence of MAP1B showing the location of SNP 5 on the MAP1B gene and the locations of the primers 3F and 3R (corresponding to positions 86634-86651, and positions 87260-87278, respectively) used to amplify the region comprising the SNP sites. The nu mber of the sequences is per that of GenBank Accession No. (514739).

-120 gcggcgcctg cgcactgt**ca cattacggcg gaacta**atcc ggcgacccag cgctttgacg
-60 catttagtac caggaaggga aaagggggac cacagaacgc gtcacacccg gaagtaggga
1 gccggaactg gggttggaca ggttatcccc aggggtgggg cagcggaggc ccaggaggag
61 ggggaaaaaa gaaggtggag gatcctggct gctaatctga atcgataccg a[t/g]tctcttag
SNP1
121 acctcagaga cacagaaaag acagaagggt gcctcatccc ctttcctccg cttctctctc
181 tcctcagcct tagccatggc ggaggcaggg gccgggctga gtgagaccgt cactgagaca
241 acggttaccg tgacaacgga gcccgtgaga aaggctgggg gcggtgctgt ttaggggtct
301 gagagatacc gggagggaag ggataaggct ttggagagtt gctggatggg ctgggcctgg
361 ggatatggga ggaagtgggt ttgggagaat cgcagagtat tagggatttt ttggtgtgtc
421 agagttggtg cagaaggctg gtcaagtgac atgcaataga gttaagatg**t aggtgatact**
481 gcttgggatg gtggtgtctg taagtattga aagactggga acttggcgat taatgagcaa
541 gggatgtact gggggaaatg aagggttgtg tgagaaagca tggttggaag ctcgctgtag
601 ggaaacttga cactaagcat gcttatcaat aaatatttct tgaagagatt attgcaaacg
661 gaagcagagg gaatgaggga acaagaaaag ggagatgatg ggagtatttt gaaaaatcag
721 agatgtagag aaaaacagcg tttttgcaaa aacattgctt tcaataggag atgttcctgt
781 cgggcttaat aaccctttga ttaagggagt ttagagtaat agttactaga gatgccagga
841 tgctggagaa taggtggata acagattggg agggctgggc ttgaggatga gagatgtgag
901 aacagagtca tttctttaat gggaaaaaga ataggcgttc tgggaaaaga aagggagatc
961 aaagtttagg cattggtgac tgaaaaaata attttcatgt attaatacca ccaaagatga
1021 tttggggagg aagatggagg aacagcgagg attatatttt cctttgaaga tttgctggga
1081 ctttccctag gttaggaatt gtatcttctc tgtatactag tggttactaa gaatactaag
1141 aacagaattc ctcaagggac tccttgaggt caaaa**acctg ttctatctcc tccca**gcatc
1201 agctcctctg t[a/t]gctgtgtt tgtgatcctg attgaactgg gaaagggaag aaaggaggcc
SNP4
1261 ccagggagga cgcaggaaga gttagtagga ggggactagc taggtatgcc tatccttctt
1321 aaccttccag gagaaccgga gcctaaccat caaacttcgg aaacggaagc cagagaaaaa
1381 ggtggaatgg acgagtgaca ctgtggacaa tgaacacatg ggccgccgct catcaaaatg
1441 tgagtaattg ttgccccaca gtaacgctgg agtcctggct cccct[a/c]agca tatcttttgc
SNP2
1501 cttcaggcat tcactggcct tcccaaagcc cccagatg[c/t]t cacagtcctg tggctgcctt
SNP3
1561 ggtggttctc tgttatcagg gagaggaggt taaagttaga gggaaagagg tagggagggg
1621 cttcaatttc catgtgcaag gcctaaagtc aaaggtatct gaggtgggag aagaggagct
1681 ttggattccc ggctggaaag **gcaaggtggg taggtgac**ag agtcccagag tgtaggcctg
1741 gggaagctgg atctggaagg tagaaggaga aaatggtggg aagtaggaat tttgactgag
1801 atccagtggg aatggaactg acactacatc tgaactcttc ctcctttttc actgggctcc
1861 tccatccaaa tccaggctgc tgtatttatg agaaacctcg ggcctttggc gagagctcca
1921 cagagagtga tgacgaggaa gaggagggct gtggtcacac acactgtgtg cggggccacc
1981 gcaaaggacg gcgtcatgca accccgggac caagccccac cagccctccc cagcctcctg
2041 acccctccca gccccctcca gggccaatgc agcactaaat tcctcgctcc cccaccattc
2101 ctgtgtctgt ctggccctga atgtattcat gtggctactc ggggactaaa cccacgattt
2161 gatcccttct ccagccccct cctcccctct cctctgcctg acagagggaa gagggagagg
2221 aaggtggaca gagatcctgg aattctgact tgctgctatt ccagaaccta ggcttctggg
2281 ttcccccagc cctcatttct ccttacaata cccagcctcc tctctccagg gatccaggca
2341 tcttgatccc aatctttttc ctttgttctc actgccaaac tgcctgtcct gggatccagt
2401 tatcttggcc ccttgcactc tctacttgag ttccaaacag ctaaattggg tttccagcag
2461 ccccagcttt cactgccagg gtcctagtca gattccaggc aatcttgctc cagctatgct
2521 tgttaatcct ggcttagagc tcttccactt atgtatttat gtcatcctaa ctcttagtcg
2581 ttgcctgtgg gatgtgaggt cttctgtgag acctcagggc tcctagccct ttcccttctc
2641 tcctgcccac ttcccccaag cccttaagag gagttaggag agagggaggt ctttgtcctt
2701 ctcaccttta atgagaaatg gaaaaaagaa atgggcatgt cctctctcct caccgttctc
2761 atgtgactag ggtttctgac aaaactggct ccaagactag tcacttagag cccactatct
2821 cctcagcctt tggtcttcca acttaggaga cagatccgac ccaggggcct gggtccctgg
2881 gagaggatgg aaaagggagg gagccaagag atgcaatctc acccccttcct tccaaggcct

FIGURE 4

FIGURE 4

Partial genomic sequence of Genomic sequence of PPP1R11 gene showing the location of SNPs 1-4 on the PPPiR11 gene and the locations of the primers 1F and 1R (corresponding to positions -102 to -85, and positions 470-489, respectively) used to amplify the region comprising the SNP sites. The number of the sequences is per that of GenBank Accession No. (504846).

32101 gagtgaggcc ctggatcctg tatttaaaag cctccctgct gactctgatg cctctttcat
32161 ttggtaacca ctgatctatg gagtctttat aacttctctg cacacagatg tatagtgtat
32221 tttgtggtta tttttcccgt atcttgtgtc ccttagctgg tccagtatag taataaggag
32281 ctcagatttt ggaatcagac acccgggatt tgagacccag atccttctct tcctgtcagt
32341 tgtattattt taaaaaagca tgtttaactt tgtatttctt tttttctgtt tataaattga
32401 ggataatacc cacctcataa ggtttttgtg aggatttaaa aagttaaaat agaattcatt
32461 tagaagagtg tcagatggat actgttttat gtgttattat tacattattt ttctataatt
32521 agtagattat aactgatctt gggatcatta tctcattttt gtttgtgctt aactttattt
32581 ttaactctac agagattatt ttttaataac tttttatttt gaaatcattt gactcaagaa
32641 gtttcaaaaa tagtacagaa gatttccttc agcttgcctc taatgtaatt gtactcctca
32701 cccagtttct cctaatgtca ttagcctatt ttaattccca gtgtggtcaa aataactgta
32761 agtttgtttg aggagaacag ttggccaaag gttatgtgag gtgggttttc tttcatatat
32821 gacaataaat gttagctacc atcatcatta ttccagatga tgatgaatta ttatatcatc
32881 aaataataat tccattattc cagattattc taagaatctt tgcagaattc tttctttcct
32941 cttcaacccc cttgtatgaa atctttgctt ctgagaaggt tgtcttgatg ttaaatgatt
33001 ctttaggaat attgtcaatt gttgatgtca gctcaaatag gagcctgcaa caagagctgt
33061 gggtcattgt ttattataaa tcaatattaa ttgagtagat tagtactttt gtacataaac
33121 aactgatagc ttttaatctg tcgagccaca tatgtcatca ctgggaccta gttctctggc
33181 actaaatgtt agtgatatgt acaaagattc cttgaaacca tcttggtatt ttccaaatat
33241 gggtttattg gaatcttcta gaaagcttaa agttattact gaaagttata tcaataaggt
33301 ataatttttt aatttagaaa aattgttcat tcctggatat cactctgcac attcaaaatg
33361 aatctctcta gggtgggttc tgataattta tttttaacca actttcctga taatttttag
33421 tcatccttca gtgtagtgat cctcatacta ctatgtataa actctggtaa tgttacttaa
33481 taatgtcact aaggacagaa agccgggatg tccccaaatg cttccattag gatggatagg
33541 gaaaagtttg catatattaa aaaaacacta taatgcctca ggtttattaa gaaagacaat
33601 ttacagatta atgatgacat tataaataca atagttatgc atttctgaga tccgtttgac
33661 tactcaactg ttcagatatt tctgaaactg tttcgtgaca tttatgaaat tcttattttt
33721 tggctgtgct cagaacttga cagataacat gcttaacatt tagtatttag gtatattagg
33781 tgatttttaa aaagaattga ctgaataatg tgtttgtatt ttgttgttat ggtgatttta
33841 aaatttaaaa ttttgttcat atgttagctt atgaatatat tttttctcag taatttctct
33901 tgtgatagta ctatttagat actacagtaa tataaatact gcaataatta tttagatgct
33961 attcacatgt taaattttta ttcaagaatc tagtattgac tgtgaagata atc**aaacacg**
34021 gaacagaggg ttttccaaga gaggcggtaa ggaccatgtt ttggaacaac ttgtacttaa
34081 gacagaaatt aaactgaaaa attgattttg gaagagctga aagaaaaatt ctggtggtga
34141 aaacctttca agaaaaatac tttggcatat cctttatgct gttaatattt gagttaatat
34201 tcagtaggtg tctctccttc tgctttctga tgtcactc[g/a]a tttgtctttt cctaagacct
SNP2
34261 ccagagtgtt ctatgaacta caaaaggtgg gactgtgtga atcttggtca ttcacagtat
34321 agataaactg ggatgtcttt gtctctgagt aggaacattg gagatatggg ggaagggaga
34381 agttgtagat taattac**cat acttgctaat cctgcct**ctg cttgaggtga gatggtataa
34441 aaattatagt gctcagttct ggattatcta taggcagaca tgttaaaata gcaacaatat
34501 ccacgaaaaa ccacagtgaa cttataaaat tgctacaagt gtgcaaatat atttatgata
34561 gaactttagt gtttggagct gcactagata catcatagtt tttgctgcaa cttggagata
34621 tcgttttccc ttgcctatta gatgattggc tcattgaata gatcattgaa tagcaggcct
34681 tcctagtgaa gctgagactt gctgtggatt tcactatagc cttggatgag ttgtgagggg
34741 cggtgggtag gaatttggtg gtgaatcagt tcagtcgctc agttgtgtct gactctttgc
34801 gaccccatga attgcagcat gccaggcctc cctgtccatc accgactcct ggagttcatt
34861 caaactcaag tccatcgagt cggtgatgcc atccaaccat ctcatcctct gttgtcccct
34921 tctcctcctg cccccaatcc ctcccagcat cacagtcttt tccaatgagt cagctcttcg
34981 catgaggtgg ccaaagtact ggagtttcag ctttagcatc attccttcca aagaacaccc
35041 aggactgatc tcctttagaa tggactagtt ggatctcctt gcagtccaag ggactctcaa
35101 gagtcttttc caacaccaca gttcaaaagc atcaattctt cggcgctcag ctttcttcac
35161 agtccaactc tcacatccat atatgaccac tggaaaaacc atagccttga ctatatggac
35221 ctttgttggc aaagtaatgt ctctgctttt cagtatgcta tctaggttgg tcataacttt
35281 ccttccaagg agtaagcgtc ttttaatttc acagctgcag tcaccatctg cagtgatttt
35341 ggagcccaga aaaataaagt ctgccactgt ttccactgtt tccccatcta tttcccatga
35401 agtgatggga ccagatgcca tgatctttgt tttctgaatg ttgagcttta agccaacttt
35461 ttcactctcc tctttcactt tcatcaagag gctttttagt tcctcttcac tttctgcata
35521 agctgagtct ttaatggcaa ttgtaggggg ccctgcaatg atggggcgga catttagtta
35581 agaaatagac tcgtctttta acattgctct ccttccccct ttaacaagga gttttgacac
35641 taatgttcct aaaacatagc tcttttggtt ttctgcagaa cagtggctat ctttcttact
35701 attcagtttt ctttaaatca ttttaattca tatttaagtg cagcaatgaa aagccagttg
35761 cagctctttg tgcttgatcc tgacttattg actagtgtag gttgtctagt agggtgtccc
35821 tgattgctat tttctttaga tgtatacatt tgaaggtaga aaacttgtgt gtgacacggt
35881 ggtcactcag taaatacaga tgtgtgtgta aatagaacct tatctaagtt tatgttgaag
35941 tatcatgtca tacaggaaca ggttggtgca tatgtcataa atgtatacag ctcagtgatt

FIGURE 5

Figure 5 shows the partial genomic sequence of DDX4 showing the location of SNP 2 on the DDX4 gene and the locations of the primers 1F and 1R (corresponding to positions 34014-34031, and positions 34398-34417, respectively) used to amplify the region comprising the SNP site. The number of the sequences is per that of GenBank Accession No. (493725).

59641 ctgatatctg agattaaata tcacctctaa aaccttcctt ttttgcagtc ctactatctt
59701 acttaggaaa ttccattcag tatcttgccg ggagaattcc agggacagag gtgcctggtg
59761 ggctatagtc catggggtca caaagaatca atcggacatg actgagcgac tgacactcat
59821 tccagttgtt cagacacaaa aatatgaggc atctttgatt tctttctcca gtgtacatat
59881 ctgatctgtc agcagagtcc tcttgtttct gtctagcatc aaactatatc agaacccatc
59941 agttgctaag ttaaaacaaa gatcagatct acttcttgcc tccaaaccca atggcttccc
60001 tcctcaaagt aaagatcttt cagtggattt caaggcactt catgaaatgg cttccatcat
60061 ccctctccaa atcctgctgc tctccattgc ttaccctatt ctagctgcac tttgctcatt
60121 actcatcccc acacgtacca gacatggttc tacctcattg cctttgttct tgatattccc
60181 tctgcctaga acgtaaaaac cagatgggga cctcttttct ggtttgtttg tttgtttgtg
60241 agcacgtaaa gaacacctat accttagaac ctttatattt gttcttacct ctgacctata
60301 atattgtttc ttcagatatc tgaatagttc acttgtttag taatatctgt cctcagatgt
60361 catctaatta gagaaggtgc ataaacccat ttggcccttt attctgctaa attttttttct
60421 tttttagact taatgcatca tatttgttat ttacctctcc acaatgagtg cagatgcttt
60481 gttttatttg ctgtgttttc agtatctaga atagatactg gcatctactg ggtgctaaat
60541 gtttgttaat aaatggagac agctgatagg gacatggagg agtgggatta caaaaagtaa
60601 caccctgtta cttctattca ggctatagat tattatcttt cctctctaac attttataga
60661 aaatttcttg gtgtggaaga gtctttggct catgcttata tttaaaccaa ttaacaaagc
60721 taacaagtaa atattcttta atgtgttaag accctgagat aatatcttat atatctatat
60781 atataagata ctttggggat taaggggttt gaattgagat aatgttgaaa catagaatat
60841 tgtagagttt gtcagccctg ggttgaatgc tattattatg aatgcctctg gcagtcaagg
60901 taaaataata gattctataa ccatgggaag aacaaaggaa tttatttata gagagggaaa
60961 tgaagggctg gagccaaatt tggaactgct aagagttggc aggtagatat catttatctt
61021 tccctaagtc tctacttatt ggttggccta tgaaggcaag gggcaagcgg tcgtttttct
61081 tttttttag tcataaagta ggaaaaagtc cttgtctctc tatctttgac atttatccac
61141 tcaggaaata tttgaattgc tcttacctat caggcctgag tttaggtgct gggaatacag
61201 tggtgaataa gacagtttcc tactcaccct atgctttttt ttcttttcct aattttgtag
61261 tttgaatgtt tcattgtctt gtaggtgatg aaagaactat ggtctttgtt gaaactaaga
61321 aaaaagcaga ttttattgcc acttttcttt gtcaagaaaa aatatcaaca acaagtattc
61381 atgggtgagt agattattat gatttcctag taaggaggta acttctattt gtcatttgtt
61441 aagaaatgtt ggtatattta actagtaaaa aatcctggaa ttaggatctc aagatctagc
61501 tattattctt catctttaag tttttataag **aaccaagtgg ctgggatg**ga tgagttagaa
61561 gtatcttaag ttcctcatca tgattatcaa gcagtctcac ttagtctctc taatccttag
61621 *atctaggtgc ttattcagct aatct[a/g]tcca gtgcttggta ttttttcac tgggcctcaa*
SNP1
61681 aaatgtcata gtaacacaac ttcattggca ctttactagg agatctaaaa tattaattgg
61741 tgaatatgta gaattccgag attatacttt taaaaaatca ggaatttttg agaaaggatt
61801 tgatcaacta gttgtgtatt ttttgtcaaa actagaaaca gtttattagt tggtaagatt
61861 tgatgt**ttgt cgcatttgag tctg**tatttt ggctgtaggt attagctgtc accttcatct
61921 gcaaatggag ttaattgtcc taacctcaaa gtattattgt gagaattaaa tgatgactta
61981 caagaaatta gtataatact ttgtatgtaa gaagtagaca ttaatgttaa ctgttgctat
62041 ttttgtttta ctgttatcca ttgaacttat ccaaaaagaa atctatccat tgaactttaa
62101 tggttgtctt taatagtttt cttaagggct attctaatac atgctttgtt ttctttcaag
62161 tgatcgtgaa cagagagaaa gagagcaagc tcttggagat ttccgctgtg gaaagtgccc
62221 tgttcttgtt gctacttcag tagctgcccg agggctggat attgaaaatg ttcagcatgt
62281 tattaatttt gatcttcctt ctaccattga tgaatatgtt *catcgaattg ggcgtactgg*
62341 tcgttgtgga aatactggca gagctatttc ctttttttgat ctggaatcag atagccagtt
62401 agcacagcct ctagtgaaag tgctatcaga tgtaagtttt taatttttaa aactgaatgg
62461 atagtgttct taccttgtca ttgaaagcag acattttata tgtatggatt ttcagttcag
62521 ttcatttgct cagtcgtgtc tgactctttg caaccccatg gactgcagta tgccaggctt
62581 ccctgtccat caccaactcc cagagcttac tcgaactcat gtccatcgag ttggtgatgc
62641 catccaaccg tctcatcttc tgtcatcccc ttctcctcct gccttcaatc tttgccagca
62701 tcagggtctt ttccaatgag tcagttcttt gcatcaggtg gccagagtat tggagcttca
62761 gcttcagcat cagtccttgc gatgaatatt caggactgat ttcctttagg gttgactggt
62821 ttgatctcct tgtagtccag gagactcaag agtcttctcc aacatcacag ttcaaaagca
62881 tcaattcttc agcactcagt tttctttgaa gtccacttct cacatccata catgactact
62941 gaagaaacca tagctttgac cagacagacc tttggtggca aaataatgtc tctggttttt
63001 aatatgctgt ctaggttggt catagctttt ctgcttttct tccaaggagc aagcatcttt
63061 taatttcatg gctgtagtca ccatctgtag tgatttcgga gcccccaaaa ataaagtctc
63121 tcactgtttc cattgtttct ccatctgttt gccctgaatt gatgggaccg gatgccatga
63181 tcttagtttt ctgaatgttg agctttaagc caacttttc actctcttct ttcactttca
63241 tcaagaggct ctttagttct tcgctttctg ccataagggt ggtgtcgtct gcgtatctca
63301 ggttattgat atttctccag gcagtcttga ttctaccttg tgcttcatcc agcccagcat
63361 ttcacatgat gtactctaaa atcccacggg cggaggagcc tggtaggctg *cagtccatgg*
63421 ggtcgctaag agtcggacac gactgagtga cttcactttc acttttcact ttcatgcatt
63481 tgaggaggaa atggcaaccc actccagtgt tcttgcctgg agaatcccag ggacggggga
63541 gcctggtggg ctgccgtcta tggggttgca cagagtcgga cacgactcaa gcgacttagc
63601 agcagcagca tataaattaa ataagcaggg tgacaatata cggccttgac atactccttt
63661 ccctatttgg aaccagtctg ttgttccatg tccagttcta actgttgctt cttgatctgc

FIGURE 6

FIGURE 6 shows the partial genomic sequence of DDX4 showing the location of SNP 1 on the DDX4 gene and the locations of the primers 2F and 2R (corresponding to positions 61531-61549 , and positions 61867-61884, respectively) used to amplify the region comprising the SNP site. The number of the sequences is per that of GenBank Accession No. (493725).

```
1081 gagcgcgctc tgcagaggcc gaatagcggt tcgctgggaa accctgggcg gggccgtaag
1141 ggtccagcgg gcgcagactc gggcgcgcgg accgacccgc cccgcagccc cacccgggct
1201 gccgcagtgc ccccgcccga tgcacctgcc ccccacactg cggcgccccc actgggcgcg
1261 gccggccggg cgtgcgttcc gccggtgctt gggggtgctg tgcgccctct cgtctgcctc
1321 aacccggctt tgttgcgctc gaagtccccg ggtgggcagc tctgtcctct gcctttccct
1381 ttcccccggg catcgcagac ctccccttct ccctgaccga ggtcgcgggt ccctccacaa
1441 ccccagcccg cgctctattc tctcggggtg gtgctgaagc gcgtctgccc cgagacaccg
1501 gctggtgggc gtggtgcagt ccgcactgcg gtctctacgg cagcccgagg cggacaaagg
1561 gcgttcacgc agccctcgtt ccccacgccc gccccccact ccccatgaaa gacgcgagaa
1621 aaccttgttt tagatgaaaa aaaattaaac tctagtggtc tgcctctgca tttgaaaacg
1681 gtcgcctgtg cccagaacaa aaggctgcag ggtggagact cgagttgcag acctggttct
1741 tttgtttaac tttaaagcac tggtgttact ttttggctta aaaaaagaaa aaaatgtgag
1801 cagaaagcag acttgttatt ttattcggaa aaaaaaaatg gaagaatagg ctaggtcaat
1861 agtgaaatgc ctcatttgag catctaataa cccttcattt gtcaaactat agtcctttga
1921 atttgatcag tactaattta gtttatttgc acattttctc cttggaaaat ttcacacgta
1981 ctgactttgg gtgtggctgc tgtatgaatc tatgactttg gatttttaaa aaaatattat
2041 ttgtcagcac ttttgctggg aagtaataat aaagcaggtg tgtttctatg tataaaaggt
2101 gcataagcac ccgatgtggt gtgaggagag ggaccctcat ccccattttg gagatggggg
2161 cccagagggg caggaaggat agggagacca gctcaaggtc acaccactca taagtgacag
2221 aatgccggct ctgcagcatc actggttttt ggcccgtcat gatgttagtg caggccaggc
2281 gaacatcaca ggaagatcaa agagcaattt ctagagcttg cctttataca aaggcagtag
2341 tcatccactt gtgggcagca ccccatgcag ggaggtggct cagcaatcac cttccatgta
2401 attatcttcc tgctttgtta ctctgctgat caccctttta gtgccctatt tctcaggggg
2461 tttattgaag ctgcttctgt ttgaggataa actagattca cccaagttat cggtcactgt
2521 gacgtggtct cgactatttt tcacgctact ggaacttagt gatcgagttc aattttgtct
2581 tcttgcccct tttctttttt aaaatttatt gatgtattat gtagtgaggg aaaggctcaa
2641 atcataaacg aggagcttgg tgaattttca caaaaagaca tacctgatcc cgatcaagaa
2701 acggacattc ctgggacccc cagaacccct ctcaggtgtc cttttcagtc agcatgcctt
2761 cctgattttt tagtgccctg atttagtcag ctttttgcgg ggaactctga aatagcagta
2821 ttgatcccat taagaatcaa ccaagtgaat gagcaaagcc atttcctaag gcacagtaac

2881 agatgctgct tttcctctgg agatcaactc tcttgggtcc tggggtcttg gatgcagctc
2941 aaaccacagg gccttccagt gctaagggga aatactgctc ctgtagcttt tctgacagag
3001 atgacttagc aacagggcag gctggctgtt gccctggcct gatatgatgc ttcctcagct
3061 ctcag[a/g]ttcc agcagggcct cctcctccag aggtcactct gataccatgg ctgcggtggc
          SNP1
3121 tggtgcccat gggaccagtt gtgtgaagca agatggagat [g/a]ctccggggg ctcggccctg
                                             SNP2
3181 cttgtgtgcc gcagcctttc cgcagggaaa gcggttcatc ttctgcccct cttgcttgtc
3241 tcccctctca tccggatgag gctctctgag tctggagacc ctagggactt ggatttttgc
                        SNP3
3301 catttgatga tttaaggctc ta[t/c]gggaaac ctagaaaata aatctgtagg aagatggcgg
3361 aaatgcaagt acatcaatag gcctgaatca gcccaccatg gaggctgagt acatgcttct
3421 tgaaacctag tgttactgaa tcaaactgag gcagctcacc tgcatgcagt aaagccaatt
3481 tactgacccc aggttgtggt gaaggaaagt gcagcattat tgtaagatgc tgatgtaagg
3541 agaacgggga gctcgtgctt aagaccccca aaatccccca agggtttcag caaagcattt
3601 ttaaaggcag tgtaagggag ggtgtcccag agtgtgtgat cagctcatgc accattctct
3661 aattgagaat tagattgatg atgaggtaac aagactacgt tgggcttcct tgctggctta
3721 gatggtaaag aatctgccta caatgcagga gagctcggtt tgatccctgg gttgagatcc
3781 cttgaagaag ggcatgacaa cacattccag tattcttgcc tggaaaatcc ccatggacag
3841 aggagcctgg tgggtacagt ccatggggtt gcaaagagtt ggacatgact gagtgactag
3901 gcacagcaca agactatgtt aatgaggact atgtgcggat ggtcatcaag tagttaattt
3961 cttccatttg atggtgattt tagcatctga aaaataactc aggaaatata cttcagatac
4021 taatatctag gtatttcaga gaggaactaa agcagagggt ataggggagg ggtctgtccc
4081 aggaatgtcc cataaggtcc tgcttggtta cacaaggaca gaagaccatc attcgtttct
4141 tcagcttcat tcaaaatgca taatgtaggg tctggtcatt tcttaagtct gaaacaacag
4201 tctttgctca gactggtcta gctttattta gttagttatg caggactcca tgccaaacca
4261 ttgtcactcc ttggcacctc ctttgccccc ttcctaatta gtctccaacc tagacttaac
4321 agtcatcccc gtttttagca gagtctcccc tcctccagtt tattttggga tgtagactac
4381 atacactcat gttttgcaaa acgaattcta gagccttgtg aaaagttttc attgcttcgt
4441 tttattttat tttttaaact aaaacatgta aaaaacacta agtgtcagtt tggtttcact
4501 tgctgttaga gtgtaacttt cctctggtta acattgggaa tcagcaggat ttgttcatca
4561 gaaagataga tgtgtgtaac atctactgcc ctggattttt agatgtaagt tttgctaatt
4621 acattgtcat ttattgaata ctttcagtct acattggtta agagaattaa gaacccagga
4681 aagagttagg gctttgtttc cattttgttt taacaggaaa acaacggggt gatgggaatg
4741 aaaagaaatg ttgaggaatg atatatttac tatatccatc catcttcata cgtttcaaaa
4801 tcaaaaggca gatggatttc tgcttgcgct gcttaattgg tattatctac tcaaagagtt
```