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(54) **BOVINE ABCG2 GENE MISSENSE MUTATIONS AND USES THEREOF**

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See application file for complete search history.

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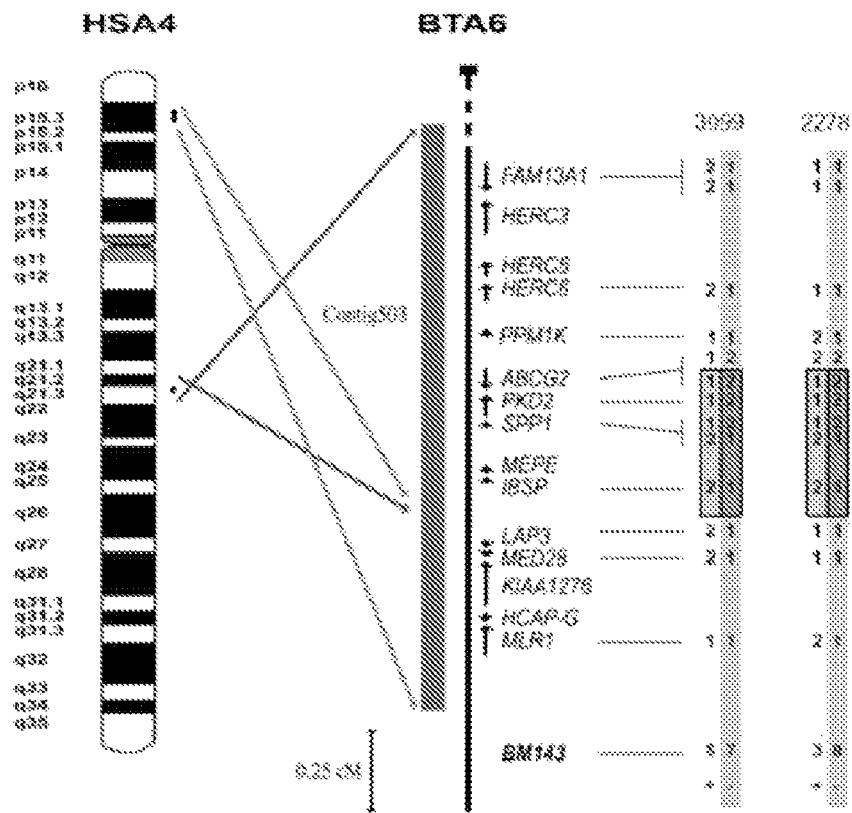
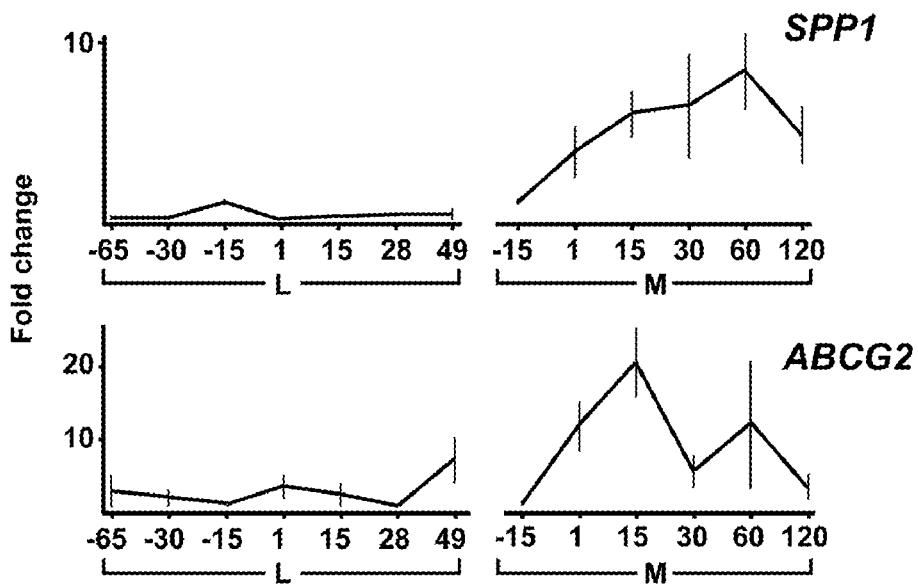
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(57) **ABSTRACT**

A quantitative trait locus (QTL) affecting milk fat and protein concentration was localized to a 4cM confidence interval on chromosome 6 centered on the microsatellite BM143. The genes and sequence variation in this region were characterized, and common haplotypes spanning five polymorphic sites in the genes IBSP, SPP1, PKD2, and ABCG2 for two sires heterozygous for this QTL were localized. Expression of SPP1 and ABCG2 in the bovine mammary gland increased from parturition through lactation. SPP1 was sequenced, and all the coding exons of ABCG2 and PKD2 were sequenced for these two sires. The single nucleotide change capable of encoding a substitution of tyrosine-581 to serine (Y581S) in the ABCG2 transporter was the only polymorphism corresponding to the segregation status of all three heterozygous and 15 homozygous sires for the QTL in the Israeli and US Holstein populations.

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**FIG. 1****FIG. 2**

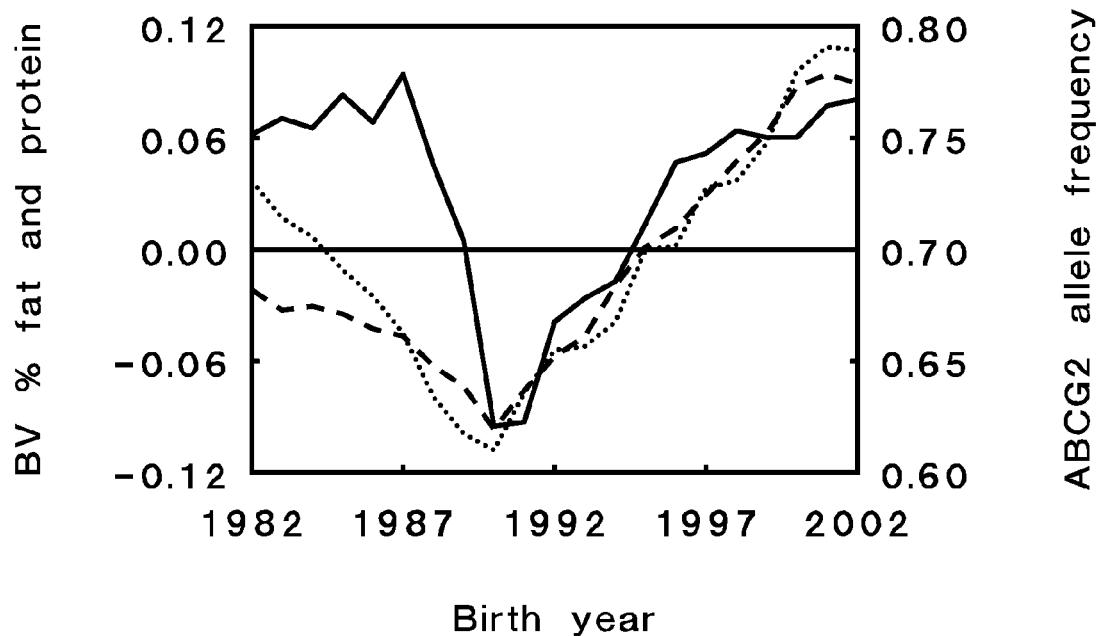


FIG. 3

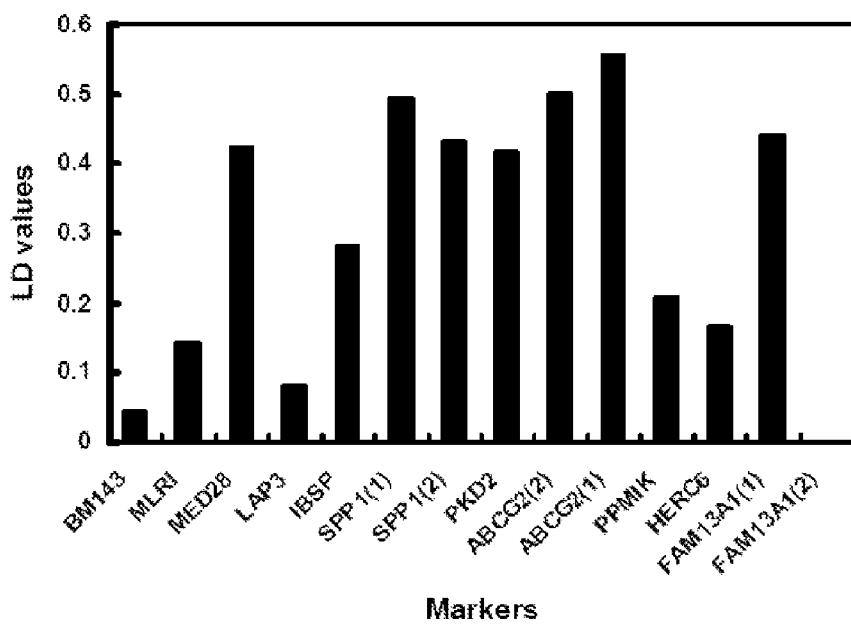


FIG. 5

Bos taurus
Canis familiaris
Sus scrofa
Macaca mulatta
Pongo pygmaeus
Homo sapiens
Pan troglodytes
Mus musculus
Rattus norvegicus

NLKTVV PWLSWLIQYLSIPRGYAA LQHNEFLGQNF CPGGLNVTTNNNTCS-- YAICTGEEELTNQGIDD SPWGGLWKNH
 NEKTVV CPWLISWLIQYLSIPRGYAA LQHNEFLGQNF CPGGLNVTTNNNTCS-- YAICTGEEELTNQGIDD SPWGGLWKNH
 NLKTVV PWLSWLIQYLSIPRGF SALQHNEFLGQNF CPGGLNVTTNNNTCS-- YAICTGAEYLNGISLSA NGMWNH
 NLTTAS SWLQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGEELYLA KQGIDDLSPWGGLWKNH
 NLTTAS SWLQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGEELYLA RQGIDDLSPWGGLWKNH
 NLTTAS SWLQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGEELYLVQGIDDLSPWGGLWKNH
 NLTTAS SWLQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGEELYLVQGIDDLSPWGGLWKNH
 NLTTAS SWLQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGEELYLVQGIDDLSPWGGLWKNH
 NLRTIGCPWLISWLIQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGNDYLINQGIDDLSPWGGLWKNH
 NLRTIGCPWLISWLIQYLSIPRGF TALQHNEFLGQNF CPGGLNATVNNTCN-- YATCTGNSYIITC GNTDNSTCVNSYIACTGNEYLINQGIDDLSPWGGLWKNH

FIG. 4

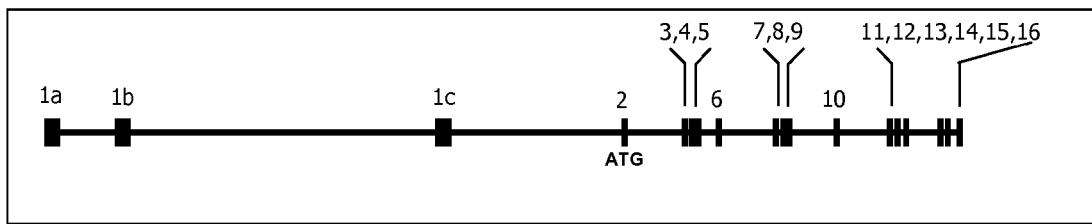


FIG. 6

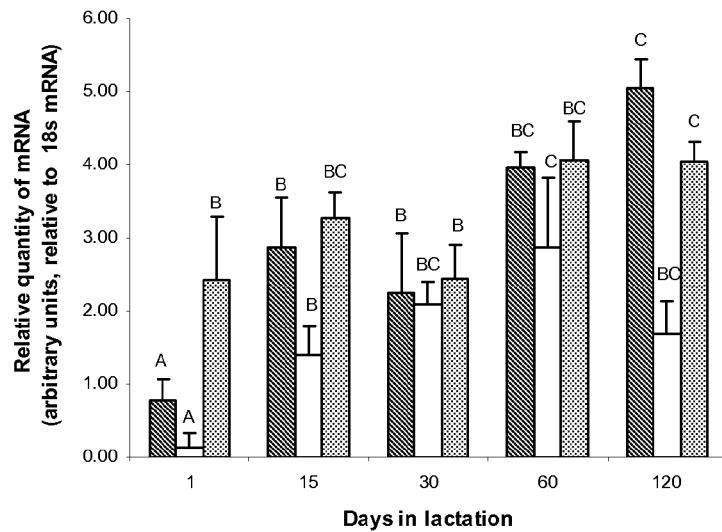
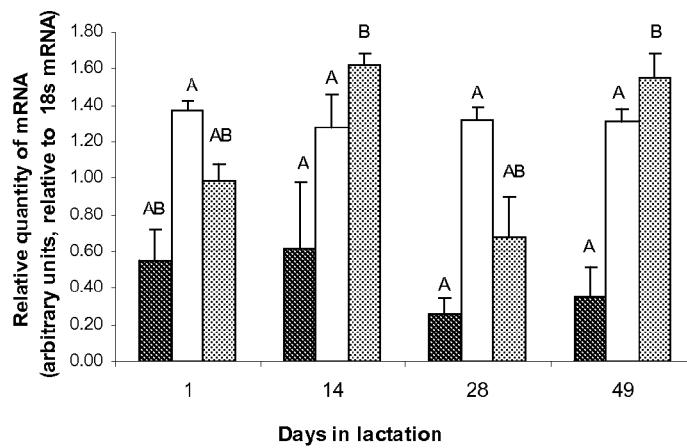
A**B**

FIG. 7 (A-B)

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**BOVINE ABCG2 GENE MISSENSE
MUTATIONS AND USES THEREOF**

This application claims priority to Application Ser. Nos. 60/694,430 filed Jun. 28, 2005 and 60/696,294 filed Jul. 1, 2005.

This invention was made with Government support under Contract No. IS-3103-99CR between the U.S. Israel Bi-national Agricultural Research and Development; and U.S. Department of Agriculture (USDA)—CSREES Livestock Genome Sequencing Initiative. The Government has certain rights in this invention.

BACKGROUND

Although many studies have demonstrated linkage between genetic markers and quantitative trait loci (QTL) in commercial animal populations, the actual DNA polymorphisms responsible for the observed effects—a quantitative trait nucleotide (QTN), has been identified in only a single case in dairy cattle (a polymorphism in exon 8 of the gene encoding acylCoA:diacylglycerol acyltransferase DGAT1) on *Bos taurus* chromosome 14 (BTA 14), which was associated with increased fat yield, fat and protein percent, as well as decreased milk and protein production. This gene was identified using bioinformatics, comparative mapping, and functional analysis.

Various studies have proposed candidate genes for the QTL on BTA6 based on their putative physiological role on the trait of interest. PPARGC1A (peroxisome proliferator activated receptor gamma, coactivator 1, alpha) was suggested as a positional and functional candidate gene for the QTL on BTA6, due to its key role in energy, fat, and glucose metabolism. The function of PKD2 corresponds with the QTL effect. This gene encodes an integral membrane protein involved in intracellular calcium homeostasis and other signal transduction pathways. SPP1 was set forth as having an essential role in mammary gland differentiation and branching of the mammary epithelial ductal system, and is therefore a prime candidate. Furthermore, anti-sense SPP1 transgenic mice displayed abnormal mammary gland differentiation and milk secretion.

Segregating quantitative trait loci (QTL) for milk production traits on chromosome BTA6 were reported in U.S. Holsteins, British black and white cattle, Norwegian cattle, and Finnish Ayrshires. Three QTLs affecting milk, fat, and protein production, as well as fat and protein concentration are segregating on BTA6 in the Israeli Holstein population. The QTL with the greatest significance was located near the middle of the chromosome, with a confidence interval of 4 cM for protein percentage centered on microsatellite BM143. Two unrelated Israeli sires were found to be heterozygous for this QTL, whereas seven other sires were homozygous for the QTL.

The QTL confidence interval on BTA6 is orthologous to two regions on both arms of human chromosome 4 (HSA4) that contain the following annotated genes: FAM13A1, HERC3, HERC5, HERC6, PPM1K, ABCG2, PKD2, SPP1, MEPE, IBSP, LAP3, MED28, KIAA1276, HCAP-G, MLR1, and SLIT2. Physical mapping and combined linkage and linkage disequilibrium mapping determined that this QTL is located within a 420 Kbp region between genes ABCG2 and LAP3.

ABCG2, a member of the ATP binding cassette (ABC) superfamily, is a “halftransporter,” with only one ATP binding cassette in the N-terminus and one C-terminal transmembrane domain. In an ATP dependent process, ABCG2 transports various xenobiotics and cytostatic drugs across the

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plasma membrane. Analysis of different stages of mammary development by immunohistochemistry and western analysis revealed that ABCG2 was not expressed in virgin mice, but was greatly induced during late pregnancy and especially during lactation. ABCG2 expression is confined to the apical membrane of alveolar, but not ductal mammary epithelial cells of mice, cows, and humans; and is responsible for the active secretion of clinically and toxicologically important substrates into mouse milk. Mice homozygous for an ABCG2 knock-out mutation lack this function. However, $-/-$ mice and their suckling progeny showed no adverse effects. ABCG2 is thought to be a drug transporter, but it is induced by estrogen. Related genes i.e. ABCG1, 5, and 8 are sterol transporters. It is therefore reasonable to propose that ABCG2 might transport cholesterol into milk.

Whereas in other tissues ABCG2 generally has a xenotoxin-protective function, transfer of xenotoxins from the mother to the suckling infant or young via milk is difficult to reconcile with a protective role.

As compared to other agricultural species, dairy cattle are unique in the value of each animal, the long generation interval, and the very limited fertility of females. Thus unlike plant and poultry breeding, most dairy cattle breeding programs are based on selection within the commercial population. Similarly, detection of quantitative trait loci (QTL) and marker assisted selection (MAS) programs are generally based on analysis of existing populations. The specific requirements of dairy cattle breeding has led to the generation of very large data banks in most developed countries, which are available for analysis.

SUMMARY

An isolated polynucleotide includes a coding region of the ABCG2 gene having a missense mutation. The ABCG2 gene includes three splice variants. A promoter region for expression of ABCG2 and its variants are disclosed. An expression construct that includes the ABCG2 gene or its variants or a functional fragment thereof, is disclosed.

A positional cloning of a QTL in an outbred cattle population is described herein. A single nucleotide polymorphism (SNP) capable of encoding a substitution of tyrosine-581 to serine (Y581S) in ABCG2 is responsible for a major QTL affecting milk yield and composition.

Sequences designated by GenBank accession nos. AJ871966, AJ871964, AJ871963, AJ871176, AJ871967, AJ871968, AJ871965, AJ877268 are incorporated herein by reference.

A functional role for ABCG2 gene in natural milk secretion is disclosed.

A method of determining whether a mammal has a ABCG2 gene that includes a missense mutation as described herein, includes obtaining a suitable sample from the animal and determining the presence or absence of a missense mutation in ABCG2 locus. At least three such missense mutations are disclosed in ABCG2 locus.

Methods for cattle breeding and cattle selection for increased milk production based on ABCG2 missense mutation analysis are disclosed.

A cattle herd in which the individuals carry the ABCG2 gene having a missense mutation as described herein in a homozygous or heterozygous form, is disclosed.

A kit includes reagents for executing the methods disclosed herein. Small molecules or drugs are used to control expression of ABCG2.

A single nucleotide change (A/C) in exon 14 capable of encoding a substitution of tyrosine-581 to serine (Y581S) in

the ABCG2 gene affects milk production traits. A polymorphism that is in linkage disequilibrium or in allelic association with the ABCG2 polymorphisms disclosed herein are within the scope of this disclosure. Closely linked or tightly associated polymorphisms with the ABCG2 locus are useful in marker assisted selection programs for increased milk production and other desirable traits such as time to weaning.

Table 10 presents terminology used herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Genes within the critical region of the QTL on BTA6 proximal to BM143 were ordered based on the cattle-human genome comparative map, 23 bovine BAC clones representing contig 503; with SPP1, IBSP, and LAP3 as anchors for the orthologous regions on HSA4. BM143 is indicated (in bold type) as the most informative marker for the QTL in cattle. Polymorphism is displayed at the respective gene positions for the two sires 2278 and 3099, heterozygous for the QTL (+/-). The alleles of the diallelic markers are denoted as either 1 or 2, with the more frequent allele denoted 1. BM143 alleles were numbered consecutively for shortest to longest based on all alleles detected in the population. Shared haplotypes in concordance with the segregation status of the two sires for the QTL are displayed.

FIG. 2. Expression data for SPP1 and ABCG2 in bovine mammary (M) and liver (L) tissues. Fold-change values are normalized intensity during pregnancy (-65, -30 and -15 d to calving date) and lactation (1, 15, 30, 60 and 120 d postpartum) using day -15 d as a base for comparison.

FIG. 3: Genetic trends for protein and fat concentration and frequency of the ABCG2 581 Y allele in the Israeli Holstein cow population by birth year. —, ABCG2 581Y allele frequency, ***, mean yearly breeding values for % fat; - -, mean yearly breeding values for % protein.

FIG. 4: Conservation of the 5th extracellular domain of ABCG2 protein in mammals. The ClustalW (Thompson et al., 1994) alignment of predicted amino acid sequences of nine orthologous ABCG2 genes is shown (SEQ ID NOS: 190-198, respectively in order of appearance). Identity and similarity between the amino acid sequences are indicated by black and grey boxes, respectively. White boxes indicate non-conservative amino acid changes between the proteins. Dashes indicate gaps introduced by the alignment program. The position of 581Y in *Bos taurus* for which the sires heterozygous for the QTL were 581Y/581S is indicated by an arrow. A conserved phenylalanine residue is located in this position for most of the other mammals.

FIG. 5: Linkage disequilibrium values for adjacent markers computed from 411 Israeli Holstein bulls.

FIG. 6 is a schematic representation of the bovine ABCG2 gene, including the three alternative first exons (variants 1a, 1b and 1c). Black boxes and numbers from 2 to 16. First ATG is located in exon number 2.

FIG. 7 shows expression data for variants 1a (dark), 1b (dark grey) and 1c (light grey) in the first exon of bovine ABCG2 gene: (A) in the mammary gland (B) in the liver, during lactation, comparing to day 15 on dry period. Expression levels were analyzed using real-time PCR analysis.

morphic sites in the genes IBSP, SPP1, PKD2, and ABCG2 for two sires heterozygous for this QTL were localized. Expression of SPP1 and ABCG2 in the bovine mammary gland increased from parturition through lactation. SPP1 was sequenced, and all the coding exons of ABCG2 and PKD2 were sequenced for these two sires. The single nucleotide change capable of encoding a substitution of tyrosine-581 to serine (Y581S) in the ABCG2 transporter was the only polymorphism corresponding to the segregation status of all three 10 heterozygous and 15 homozygous sires for the QTL in the Israeli and US Holstein populations. The allele substitution fixed effects on the genetic evaluations of 335 Israeli sires were -341 kg milk, +0.16% fat, and +0.13% protein (F-value=200). No other polymorphism gave significant 15 effects for fat and protein concentration in models that also included Y581S. The allele substitution effects on the genetic evaluations of 670 cows, daughters of two heterozygous sires, were -226 kg milk, 0.09% fat, and 0.08% protein (F-value=394), with partial dominance towards the 581S 20 homozygotes. Y581S in ABCG2 is likely the causative site for this QTL.

The variation in SPP1 (OPN3907) is an indel (insertion and deletion) in poly-T tract ~1240 bp upstream of SPP1 transcription initiation site. Accurate genotyping of such region 25 would require a tedious subcloning of the PCR products to allow separation between the homologous chromosomes present in each heterozygous individual. In several instances OPN3907 region was sequenced using cloned DNA or homozygous individuals revealing three distinct alleles that 30 are present in this locus. Interestingly all cloned sequences deposited in GenBank (AJ871176, AC185945, NW_931635) were of the allele with nine thymines (T9) described as rare (frequency 0.05). The latter also sequenced an allele (T10, AY878328) from a homozygous individual. 35 Sequencing of sire 3208 revealed the third allele with nine thymines followed by three adenines. Hence this locus displayed length variation typical of a microsatellite with different numbers of repeats of either thymines or adenines. These alleles were designated SPP1M1-M3, respectively. Sequencing of heterozygous individuals resulted in superimpositions, which were traced as follows: SPP1M1 and SPP1M2; SPP1M2 and SPP1M3; SPP1M1 and SPP1M3. Using this 40 scheme a sample of genotypes of sires that segregate (Y) and do not segregate for the QTL (N) and sire homozygous for the ABCG2 581 S allele were found. While the status of the ABCG2 mutation was in concordance with the QTL status, concordance was observed with neither the length of the T track nor the allele status of the SPP1 microsatellite. For example, the traces of the three sires segregating for the QTL 45 were all of the type M1/M2 and were indistinguishable from that of the non-segregating sire 3241. This indicates that the variation in ABCG2 is probably responsible for the QTL. Sequencing of sires homozygous for the Y581S haplotype (2182; 2227; 3573; 3396; 3094) associates it with SPP1M1 50 (T9). The results indicate that sire 2176 that has one of lowest protein % ever recorded in Israel is homozygous for Y581 S but heterozygous for SPP1M. Moreover within the BAC clone of Holstein breed (AJ871176) the SPP1M1(T9) is associated with the ABCG2 Y581 plus allele, and thus demonstrating that there are Holsteins available for such a linkage 55 disequilibrium study. Sire 3028 has one of the highest protein % and therefore is unlikely to be homozygous for the minus QTL allele. This sire is indeed homozygous the ABCG2 581 S allele but also for SPP1M3(T9) and would have been considered to be homozygous for the minus QTL allele. Sire 5117, segregating for the QTL is Carlin-M Ivanhoe Bell that was used heavily in global breeding programs. 60

DETAILED DESCRIPTION

A quantitative trait locus (QTL) affecting milk fat and protein concentration was localized to a 4cM confidence interval on chromosome 6 centered on the microsatellite BM143. The genes and sequence variation in this region were characterized, and common haplotypes spanning five poly-

Tests for concordance of the zygosity state between the QTL segregation status and the candidate polymorphism is a powerful tool for identifying the functional mutation underlying the QTL.

A polymorphism that is in linkage disequilibrium or in allelic association with the ABCG2 polymorphisms disclosed herein are within the scope of this disclosure. Closely linked or tightly associated polymorphisms with the ABCG2 locus are useful in marker assisted selection programs for increased milk production and other desirable traits such as time to weaning, meat quality and quantity. For example, a person of ordinary skill in the art can readily identify polymorphisms that are closely linked to the Y581S and other polymorphisms disclosed herein. Thus, the Y581S polymorphism serves as an anchor polymorphism to find other closely linked polymorphisms.

Comparative and Physical Mapping of the Critical Region for the BTA6 QTL

By combining comparative genomics and in-silico gene cloning, a map was produced of genes and sequence variation in the critical region of the QTL (FIG. 1). Gene order was confirmed by physical mapping of PCR probes in BAC clones that are part of genomic contigs 503 and 8342 disclosed herein. BM143 and SLIT2 were identified within contig 8342. Fifteen genes within 2 cM centromeric to BM143 were identified within contig 503 orthologous to two different regions on HSA4. FIG. 1 shows the predicted order, size, and orientation of transcription of the genes within contig 503, based on their corresponding features in the human genome.

Polymorphism Detection, LD Mapping, and Haplotype Analysis

A total of 31,655 bp was sequenced in intergenic, exonic, and intronic regions of 10 genes within the critical region of the QTL using DNA of two sires (2278 and 3099) heterozygous for the QTL (Table 1). Thirteen sites heterozygous in at least one of the two sires were selected as markers and genotyped for 411 sires. A single polymorphic site was genotyped in seven genes, and two polymorphic sites were genotyped in each of the three genes SPP1, ABCG2, and FAM13A1. Henceforth, the polymorphisms will be denoted by gene symbols for seven single gene polymorphisms, and by the gene symbol followed by either (1) or (2) for the genes with two polymorphisms. All sites of polymorphism were in highly significant LD ($P<0.0001$) with at least one other site. LD values of adjacent markers are plotted in FIG. 5. Generally LD values between adjacent markers were >0.2 . Exceptions were the BM143-MRL1-MED28 segment, LAP3-IBSP, and HERC6—FAM13A1. The two sires heterozygous for the QTL share common haplotypes for the polymorphic sites at IBSP, SPP1, PKD2, and ABCG2 (FIG. 1). For both sires the same haplotype was associated with increased protein concentration.

Cloning of Bovine ABCG2, PKD2, and SPP1 Genes

A bovine BAC clone containing the three genes, SPP1, PKD2, and ABCG2 (GenBank accession AJ871176) was shotgun sequenced. By aligning this sequence with bovine ESTs and human orthologous genes in this BAC the last 15 exons of the bovine ABCG2 gene were identified in this BAC, which included the whole putative polypeptide sequence of the ABCG2 transporter (protein CAI38796.1). In the opposite orientation on the BAC 15 exons of the gene orthologous to human PKD2 (CAI38797.1), and seven exons of bovine SPP1 (CAI38798.1) were annotated. The entire description of the cloning procedure is presented in the Materials and Methods.

Expression of Candidate Genes in the Bovine Mammary Gland

Of the eight genes analyzed, three genes; SPP1, ABCG2, and MED28 showed significant differential expression in the 5 mammary gland during lactation, as compared to the dry period ($p<0.02$). Significant differential expression was not found in liver tissue. Expression of SPP1 and ABCG2 in the mammary gland and liver during lactation and the dry period is shown in FIG. 2. The increase in the mammary gland was 10 8- and 20-fold for the two genes respectively.

The ABCG2 Missense Mutation Y581S

Using this BAC data, the exons, introns, and part of the regulatory region of SPP1, and all the coding exons of PKD2 and ABCG2 for the two Israeli sires heterozygous for the 15 QTL were sequenced. The single nucleotide change, A to C, denoted ABCG2(2), capable of encoding a tyrosine to serine substitution at position 581 (Y581S) in the 5th extra-cellular region of the ABCG2 protein, was detected. Henceforth, the 20 A allele, capable of encoding tyrosine, which was the more frequent allele in the population, will be denoted the +QTL allele. The +QTL allele decreases milk yield, and thus increases fat and protein concentration. Of the 341 sires with valid genotypes, 12 were homozygotes $-/-$, 109 were heterozygotes, and 220 were homozygotes $+/+$. The +QTL allele 25 frequency was 0.805 and the genotype frequencies corresponded nearly exactly to the expected Hardy-Weinberg frequencies. ABCG2(2) was the only polymorphism corresponding to the segregation status of all three heterozygous and 30 15 homozygous sires for the QTL in the Israeli and US Holstein populations. The probability of concordance by chance, computed as described in the Materials and Methods $= (0.68^{15})(0.16^2) = 0.00008$.

Allele Substitution Effects and Dominance

The Model 1 effects of the markers on the quantitative traits 35 are given in Table 2. This model estimated the effects associated with the polymorphisms on the sire evaluations for the milk production traits, with each polymorphism-trait combination analyzed separately (Cohen et al, 2004a). The number of bulls with valid genotypes and the frequency of the more 40 common allele for each marker are also given. Most of the markers had highly significant effects on protein concentration, but the effect associated with ABCG2(2) was more than double the next largest effect. LAP3, MED28, ABCG2(2), and HERC6 had significant effects on fat and protein yield, 45 while ABCG2(2), SPP1(1), SPP1(2), and PKD2 were associated with milk yield. The effect associated with ABCG2(2) on milk was double the next largest effect, and the effect associated with % fat was triple the next largest effect observed.

The effects on the quantitative traits associated with 500 670 daughters of the two sires heterozygous for the QTL are given in Table 3, both as class effects, and as regression effects. The class effects are given relative to the 581S homozygote ($-/-$). Dominance was estimated from the class effects, relative to 55 the 581S homozygote. The regression effects estimated from the animal model analyses of the entire Israeli Holstein population are also given. Israel and Weller (1998) demonstrated that QTL effects will be underestimated by the analysis of genetic evaluations, especially genetic evaluations of cows, 60 which have relatively low heritability, while estimates derived from animal model analyses of the entire population will be unbiased. The effects derived from the animal model for milk, percentage fat, and percentage protein were more than double the regression effects from the analyses of the 65 genetic evaluations. This was not the case for fat and protein yield, but these effects were only marginally significant in the analyses of the genetic evaluations. For all five traits, the

heterozygous effect was within the range of the two homozygous effects. Significant partial dominance was obtained for both percentage fat and percentage protein towards the 581S homozygote, which was also the less frequent allele among the daughters of the heterozygous sires.

Variance Components and Marker Substitution Effects from REML Analysis

The numbers of genotyped bulls and ancestors included in the variance component analyses are provided herein for the analyses of ABCG2(2) alone, and the analyses of ABCG2(2) with SPP1(2), HERC6, and LAP3. These analyses are presented because these markers gave the greatest Model 1 effects on the production after ABCG2(2). In each analysis the number of ancestors was slightly greater than the numbers of genotyped bulls. The total number of bulls included in each analysis ranged from 641 to 758.

The variance components are presented herein for all four analyses. The residual effects were generally low, because genetic evaluations were analyzed. In all four analyses, the variance components and the substitution effects associated with ABCG2(2) for fat and protein percentage were quite similar. The substitution effects were close to 0.21% for both traits in all analyses. These values are also close to the values of 0.22 and 0.19 for fat and protein percentage obtained from the animal model analysis. The variance components for all the markers other than ABCG2(2) were near zero for fat and protein percentage. The variance components associated with SPP1(2) were near zero for all five traits. These results correspond to the hypothesis that ABCG2(2) is the causative mutation for the QTL affecting fat and protein concentration.

The variance component associated with ABCG2(2) for milk was similar in all analyses, except for the analysis that included HERC6. In this analysis the variance component for ABCG2(2) increased to 160,000. This can be explained by postulating that two QTL are segregating on this chromosome that affects milk production, and that in general these two QTL are in repulsion throughout the population. Thus a greater effect was observed associated with ABCG2(2) with HERC6 included in the model, because the “masking” effect was removed. Sire 2278 was also segregating for the QTL proximate to the centromere, but the effects on milk were in repulsion for this sire. This QTL affects milk, fat, and protein production, but not fat or protein concentration. The effects associated with LAP3 affected milk and fat yield and protein concentration. Unlike the analyses including ABCG2(2) and HERC6, in the analyses including ABCG2(2) and LAP3, the variance components associated with both markers were positive for fat and protein yield. This corresponds to the hypothesis that neither of these markers are in complete linkage for the QTL responsible for fat and protein yield.

Genetic Trend

The genetic trend for the 581 Y of ABCG2 (2) in the entire cow population is shown in FIG. 3. The mean annual breeding values for fat and protein percent are also given. The frequency of 581Y allele by birth date of cows decreased from 0.75 in 1982 to 0.62 in 1990, and then increased to 0.77 in 2002. These trends correspond to the change in the Israeli breeding index, which was based chiefly on milk production until 1990. Since then the index has been based chiefly on protein with a negative weight for milk yield.

Conservation of ABCG2 581 in Mammals

Comparison of this protein domain across mammals is presented in FIG. 4 for the region spanning amino acid 557 to 630. The arrow indicates position 581 for which tyrosine and serine were found for the three sires heterozygous for the QTL. Phenylalanine is the conserved amino acid in the mammals analyzed, except for *Canis familiaris* and *Bos taurus*

with tyrosine at this position. Both tyrosine and phenylalanine are aromatic acids, while serine is a nucleophilic acid.

Proof for identification of a gene underlying a QTL in commercial animal populations results from multiple pieces of evidence, no single one of which is convincing, but which together consistently point to a candidate gene.

Diverse pieces of evidence support the conclusion that ABCG2 is the segregating QTL on BTA6:

1. The shared haplotypes of the two sires segregating for the QTL spanned five sites of polymorphism in the genes IBSP, SPP1, PKD2, and ABCG2. This is equivalent to the 420 Kbp region found in the Norwegian cattle (Olsen et al., 2005), except that it is shorter on the 5' end of ABCG2 (exons 1 to 3) and the 3' end of LAP3 (exons 12 and 13). The same haplotype was associated with the +QTL allele in both sires.
2. The two genes within the shared haplotype, ABCG2 and SPP1, were preferentially expressed in the bovine mammary gland at the onset of lactation. Furthermore, large scale analysis of human and mouse transcriptomes revealed that ABCG2 had the highest expression in the mammary among 61 organs and tissues tested.
3. Of the polymorphisms genotyped only ABCG2(2) was in concordance with the segregation status of all three heterozygous and 15 homozygous sires for the QTL in the Israeli and US Holstein populations. The probability that this would occur by chance is 0.00008.
4. ABCG2(2) is capable of encoding a non-conservative amino acid change (Y581S) that may affect this gene transporter function.
5. The highest population-wide substitution effects on milk yield and fat and protein concentration were obtained for the Y581S polymorphism in ABCG2, and these effects were more than double the next largest effects associated with any of the other polymorphisms.
6. In the analysis of over 300 genotyped bulls, none of the other polymorphisms gave significant effects for fat and protein concentration in models that also included Y581S.
7. The high Y581S allele substitution effects on the genetic evaluations of 670 cows, daughters of two heterozygous sires, represent the joint effects of both paternal and maternal alleles. The F-value was 394 for % protein.
8. Protein and fat concentration for cows homozygous for the 581S allele was lower than the heterozygotes, even though the second 581S allele was of maternal origin, and therefore unrelated to the daughter design effects.
9. The frequency of 581Y allele by birth date of cows decreased from 0.75 in 1982 to 0.62 in 1990, and then increased to 0.77 in 2002, in correspondence with the changes in the Israeli Holstein selection index. The close correspondence between the two analyses supports the conclusion that ABCG2(2) is the QTN, although it could also be due to a “hitch-hiker” effect.

10. Weller et al. estimated the frequency of the +QTL allele in the Israeli Holstein population as 0.69 and 0.63, relative to fat and protein percent, by the modified granddaughter design for cows born between 1992 and 1996. This corresponds closely to the frequency of 0.69 for 581Y as estimated in the current study for cows born in 1994.

11. All 18 Israeli and US sires with known QTL genotypes were sequenced and shown that this chromosomal segment is hyper-variable. At least four single nucleotide changes were found within the 20 bp region centered on the poly-A sequence. All sires except one were heterozygous for at least one of these polymorphisms. The conclusion was that OPN3907 is not the QTN. However, as long as the entire chromosomal segment within the confidence interval of the QTL has not been sequenced in the sires with known QTL

genotypes, it is not possible to completely eliminate the possibility that the QTN may be some other polymorphism in strong LD with Y581S.

This is the first example of a functional role for the ABCG2 gene in natural milk secretion.

Identification of Three Promoters for the Bovine ABCG2 Gene

The existence of three different promoters for three different 16-exon transcripts of ABCG2 gene is reported in GenBank accessions BE480042 and CK838023. The 5' region of this gene is assembled and the sequence is disclosed herein.

The current sequence of the bovine genome is based on sequence derived from a Hereford cow. The WGS trace files were BLAST searched with the cow genome database using the sequences of the three different variants. All the trace files were downloaded and their corresponding mates and assembled them using the GAP4 computer program, monitoring the consistency of the mate-pair data and adding or removing trace files accordingly. The contigs of each of the three variations were expanded using additional trace files that were found by searching against the contig end sequences. Eventually all the contigs were merged into one assembly, confirming the existence of three alternative first exons of ABCG2 including the GT motives for splice donors at their ends. The final assembly spanned 627 sequence reads in a length of 235,109 bp (FIG. 6). Following confirmation of the existence of the three promoters, their expression was verified in lactating cow mammary gland.

The promoters of ABCG2 gene and its splice variants are useful in increasing expression of a gene of interest in a suitable tissue such as, mammary gland, and during a specific period, e.g., during lactation.

Expression of the Three Splice Variants of ABCG2 Gene in Bovine Mammary Gland

All three variants showed significant expression in the cow mammary gland during lactation, as compared with the dry period ($p < 0.0002$), using real-time PCR analysis. Significant differential expression was not found in liver tissue which was used as a control. Expression of the three variants in mammary gland and liver tissues is shown in FIG. 7A-B. Variant 1c showed the highest expression, of 5-fold in the mammary gland on day 120 in lactation. Variants 1a and 1b showed an expression of 3 and 4 fold respectively on day 60 in lactation.

Materials and Methods

PCR primers and their corresponding numbers are presented in Table 7. All GenBank and other publicly available database accession numbers disclosed herein are incorporated by reference.

Physical mapping and bioinformatics. The order and location of the genes in the QTL region were determined in the bovine bacterial artificial chromosomes (BACs) from the CHORI-240 BAC library (Warren et al., 2000). Repeat-masked end sequences from CHORI-240 clones obtained from the GenBank for BLASTN search against the human genome sequence (NCBI build 33) were used. The cattle fingerprint contigs (BCCRC, Vancouver, Canada) were identified that contain clones anchored to the human genome by sequence similarity. Cattle fingerprint contig 503, which covers the confidence interval region of the QTL upstream to BM143 in HSA4, is diagramed in FIG. 1. The contig is represented on the axis of HSA4 in the following positions: 89,077,921-90,827,214 and 17,255,215-17,699,645 available at website (genome.ucsc.edu/goldenPath/hgTracks.html). A minimum tiling path of 23 cattle BACs between these positions covering the region of the QTL from FAM13A1 to MLR1 were selected. The exact position of each gene in the

human genome was identified using the UCSC Genome Browser database. Bovine BAC clones presumably containing the same gene in cattle were identified by their end sequence similarity to the human genome and presented in Table 5. When there was no BAC clone with both ends covering the whole interval of the candidate gene, several overlapping BACs with single ends matching the upper and lower boundaries of the gene interval and covering the whole region were selected for PCR analysis. The BAC templates were prepared by picking colonies grown overnight and boiling them in 200 μ l of ddH₂O for 10 minutes. Bioinformatics procedures, management of DNA sequences and EST assembly were done as previously described (Cohen et al., 2004a).

Identification of polymorphism in genes within the critical region of the QTL. To search for relevant informative genomic variation in the critical region of the QTL the genomic DNA of the two sires heterozygous for the QTL served as a template. PCR amplified genomic fragments of the bovine orthologs of the human genes are listed in Table 1. In most cases the bovine sequence required for the design of PCR primers was obtained from bovine ESTs of the orthologous genes. The PCR products were sequenced for polymorphism detection. Nucleotide substitution was detected by double peaks for the specific nucleotides, and insertion was detected by sequence overlap that was analyzed using Shift-Detector (Seroussi et al., 2002).

Experimental design and haplotype analysis. The search for the QTN was based on genotyping of the following samples:

1. Two sires heterozygous for the QTL (2278 and 3070), and seven sires homozygous for the QTL in the Israeli population as determined using a daughter design (Ron et al., 2001).

2. A single sire heterozygous for the QTL (DBDR family 9), and eight sires homozygous for the QTL in the US population (DBDR family 1 to 8) as determined using a granddaughter design analysis (Ashwell et al., 2004).

3. Six-hundred-and-seventy daughters of two Israeli sires heterozygous for the QTL with genetic evaluations for production traits (Ron et al., 2001).

4. Four-hundred-and-eleven progeny-tested Israeli sires with genetic evaluations for production traits (Cohen et al., 2004a).

5. Eight cows with mammary biopsies and five cows with liver biopsies.

The 411 Israeli Holstein sires with genetic evaluations for all five milk production traits were genotyped for the 13 markers listed in Table 1 and BM143. Eleven markers were SNPs, one was a two-base polymorphism, and two were microsatellites (BM143, and the polymorphic site in MLR1). Twenty daughters of each of the two Israeli sires heterozygous for the QTL were also genotyped for all 14 markers to determine the haplotypes of the two sires. Genotyping of polymorphism was performed following Cohen et al., (2004a). The genotyping platform and specific assay for each site are presented in Table 6.

Statistical analysis. LD parameters values were computed between each pair of markers as described by Hedrick (1987). Probability of concordance by chance between the QTL and a polymorphism was computed only for ABCG2(2), which was the only marker in complete concordance with the 18 sires with known QTL genotype (Ron et al., 2001; Ashwell et al., 2004). Since only polymorphisms heterozygous in at least one of the sires heterozygous for the QTL were genotyped on the complete sample of bulls, the probability of concordance with the QTL only considered the remaining 17 sires. This is computed as the probability that all 15 sires homozygous for

the QTL should also be homozygous for the polymorphism, and that the two remaining sires heterozygous for the QTL should also be heterozygous for the polymorphism, and that in all three heterozygous sires the same QTL allele should be associated with the same marker allele. Thus probability of concordance = $p_1^{15}(p_2/2)^2$, where p_1 = probability of homozygotes, and p_2 = probability of heterozygotes. P_2 was divided by two, because for concordance to be complete, the two additional heterozygous sires must have the same ABCG2(2) allele associated with the +QTL allele as the original genotyped sire.

Genetic evaluations for milk, fat, and protein were computed by a multtrait animal model analysis of the entire Israeli Holstein population (Weller and Ezra, 2004). Evaluations for fat and protein percent were derived from the evaluations for the production traits. The following fixed linear model, denoted Model 1, was used to estimate the effect associated with each one of the polymorphisms for each of five traits analyzed (Cohen et al., 2004a):

$$Y_{ijkl} = a_i J + b_i K + c_i (K)^2 + e_{ijkl}$$

where, Y_{ijkl} is the genetic evaluation of sire 1 with marker genotype j and birth year k for trait i; J is the number of "+" alleles ($j=0, 1$ or 2); K is the sire's birth year; a_i , b_i and c_i are regression coefficients for trait i; and e_{ijkl} is the random residual for each sire for trait i. The "+" allele for ABCG2(2) was the allele associated with increased protein concentration. For all the other markers, the allele in LD association with the "+" for ABCG2(2) was denoted the "+" allele. BM143 was analyzed as a diallelic marker, as described herein. The linear and quadratic effects of the sires' birth year were included to account for genetic trends in the population. The effects of the markers were also analyzed with three marker genotypes as class effects. Linear and quadratic birth year trends of the markers were also estimated.

Model 1 does not account for the relationships among sires or linkage among markers. Thus the genetic evaluations were also analyzed for a subset of the markers with the greatest effects by the following model, denoted Model 2:

$$Y_{ijk} = a_i J + g_{ik} + e_{ijk}$$

Where, g_{ik} is the additive polygenic effect for animal k on trait i, and the other terms are as defined previously. This model differed from the previous model in that all three effects were considered random, and the numerator relationship matrix was used to compute the variance matrix for the polygenic effect. In order to obtain a more complete relationship structure, all known parents and maternal grandsires of the genotyped bulls were included in the analysis. The numbers of animals in each analysis are given in Table 6. REML variance components were computed for the "a" and "g" effects by the MTC program University of Georgia, Department of Animal and Dairy Science, Athens, Ga.). Marker substitution effects were derived as: $[(\text{Var } a)/(2pq)]^{1/2}$ where "Var a" is the marker variance component, and p and q are the frequencies of the two QTL alleles, as derived from the sample of 411 genotyped sires (Weller, 2001). This model was also used to analyze marker pairs with highly significant effects on the quantitative traits as determined by Model 1.

Dominance of the QTL effect can only be estimated by comparison of cows that are heterozygous for the QTL to cows that are homozygous for the two alternative alleles (Weller et al., 2003). The genetic evaluations for the five milk production traits of 670 daughters of two Israeli sires heterozygous for the QTL were analyzed by a model that also included the sire effect. The QTL was considered a class effect and significance of dominance was estimated by sig-

nificance of the difference between the midpoint of the two homozygote effects and the mean of the heterozygote effect. The dominance effect was estimated as the ratio of the difference between the heterozygote effect and the mid point of the homozygote effects, divided by half the difference between the homozygote effects. Cow genetic evaluations are based on relatively few records, and are therefore highly regressed. Thus the QTL effects estimated from this model will also be underestimated (Israel and Weller 1998). However, this should not have a major effect on the estimate of dominance, which was derived as a ratio of the estimated effects.

Genotype probabilities for ABCG2(2) were determined for the entire Israeli Holstein milk-recorded population, including 600,478 cows and 1670 bulls, using the segregation analysis algorithm of Kerr and Kinghorn (1996), based on the 335 bulls with valid genotypes. Finally, the QTL effects for milk, fat, and protein yield were estimated from the entire Israeli Holstein milk-recorded population based on the genotyped cows, as proposed by Israel and Weller (1998). These QTL estimates should be unbiased, unlike the estimates derived from analysis of the genetic evaluations. The effects for fat and protein percent were derived from the estimated effects for the yield traits as described by Weller et al., (2003).

The detailed procedures for biopsy procedures, RNA extraction, BAC clone selection, subcloning and shotgun sequencing, real-time PCR, and computation of LD parameter values and ABCG2(2) genotype probabilities for the entire Israeli Holstein population are presented herein.

The cattle BACs covering the region from FAM13A1 to 30 MLR1: E0152P21, E0375J15, E0259M14, E0101G10, E0181A19, E0303P06, E0274F22, E0098H02, E0445L10, E0060K13, E0367N10, E0174N17, E0049M05, E0331116, E0338G15, E0263K19, E0351N06, E0039I05, E0062M13, E0351N06, E0308O12, E0393F21, and E0417A15.

BAC clone selection, subcloning and shotgun sequencing. Filters from RPCI-42 bovine library (Children's Hospital Oakland Research Institute, Oakland, Calif.) were hybridized with ^{32}P -labeled PCR primers specific for SPP1 gene (Rediprime II Random Prime Labelling Kit, Amersham Biosciences). Three clones positive for SPP1 were identified. The clones were PCR-screened for the presence of SPP1, PKD2, and ABCG2 genes. A clone H005K14 positive for all three genes was identified and selected for the shotgun sequencing. The H005K14 clone was grown and its DNA was purified 45 using the Large-Construct kit (Qiagen, CA) following the manufacturer's instructions. To separate the genomic DNA insert from the BAC vector, the purified DNA was digested with NotI and applied to a 0.8% low melting point SeaPlaque agarose gel (Cambrex, Me.) as previously described (Kaname and Huxley, 2001). The isolated insert fragment was sheared with a nebulizer. Blunt-ended fragments 1.6 to 5 Kbp were purified from a 0.8% low melting point agarose gel and cloned into the pCR®4Blunt-TOPO vector using the TOPO® Shotgun Subcloning kit (Invitrogen, CA) according 50 to the manufacturer's instructions. Individual transformed bacterial colonies were robotically picked and racked as glycerol stocks in 384 well plates. After overnight growth of the glycerol stocks, bacteria were inoculated into 96 well deep cultures and grown overnight. Plasmid DNA was purified 55 with Qiagen 8000 and 9600 BioRobots (Qiagen, CA). Sequencing of the 5' and 3' ends was performed using standard primers M13 forward and reverse and ABI BigDye terminator chemistry on ABI 3700 capillary systems (Applied Biosystems, CA). All 384- and 96-well format plates were 60 labeled with a barcode and a laboratory information management system (HTLims) was used to track sample flow. The shotgun sequences were trimmed of vector sequences and 65

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stored in a local Oracle database. To assemble the shotgun sequences into contings, Contig Express software (Vector NTI v 7.0 package, InforMax Inc.) was employed.

Cloning of bovine ABCG2 and PKD2 genes. BLASTN search of bovine dbEST using the sequence of these 15 exons of ABCG2 revealed 31 ESTs. Two ESTs indicated alternative splicing of 5' non-translated first exons suggesting existence of three different promoters for three different 16-exon transcripts of this gene (GenBank accessions BE480042 and CK838023). Twenty-three of the ESTs were assembled into a tentative consensus 2198 bp cDNA transcript (TIGR tentative consensus TC264405) capable of encoding a polypeptide of 658 aa (protein CAI38796.1) with a predicted molecular mass of 73 kDa. Alignment of the ABCG2 orthologs (partially displayed in FIG. 4) indicated that the homology between the bovine ABCG2 predicted protein and its putative porcine ortholog (GenBank accession NP_999175, 87% identity, 94% similarity) was higher than to the human and murine orthologs (GenBank accessions AAQ92942, 84% identity, 91% similarity; AAH53730, 79% identity, 91% similarity, respectively). All orthologs shared sequence motifs that included cytoplasmic ATP binding cassette and six putative transmembrane domains typical of a half transporter structure. The BAC sequenced contains 66.1 Kbp of the bovine ABCG2 gene. Following an intergenic region of 10.3 Kbp and encoded on the complementary strand, the last exon of a gene orthologous to the human polycystic kidney disease 2 (PKD2) was observed. Using BLASTN, 20 ESTs that matched the 3' end of the 4941 bp putative cDNA transcript deposited with this BAC were found. 5' end of this transcript was predicted using orthology to the human mRNA. This transcript is capable of encoding a polypeptide of 970 aa (protein CAI38797.1) with the predicted molecular mass of 110 kDa. Alignment of the PKD2 orthologs indicated that the homology between the bovine PKD2 putative protein and its human ortholog (GenBank accession NP_000288, 94% identity, 97% similarity) was higher than to the murine ortholog (GenBank accession NP_032887, 88% identity, 93% similarity). All orthologs shared sequence motifs that included: a. ion transport domain that typically contains six transmembrane helices in which the last two helices flank a loop that determines ion selectivity; b. EF-hand; a calcium binding motif associated with calcium sensors and calcium signal modulators.

PKD2 spanned 58.7 Kbp of the bovine BAC. Following an intergenic region (21 Kbp), and in the same orientation, we detected seven exons of the previously characterized bovine SPP1 mRNA (GenBank accession NM_174187, Kerr et al. 1991). The length of this gene was 7 Kbp. No other genes were found in the region upstream to SPP1 with a length of 9.7 Kbp.

Identification of polymorphism in genes within the critical region of the QTL HERC6. The region orthologous to the human intron 5 of hect domain and RLD 6 gene (HERC6) was PCR amplified with PCR primers (#705 and #706) that were designed according to the sequence of a bovine EST (GenBank accession BE664068) which was highly similar (86%) to human HECR6 (GenBank accession NM_017912). Three sites of variation in this intron sequence were identified and the polymorphism at position 151 (Table 1, Table 6) was genotype.

PPM1K. The human protein phosphatase 1K (PPM1K) is a member of the PP2C family of Ser/Thr protein phosphatases. The bovine PPM1K ortholog that maps to critical region of the QTL on BTA6 was cloned. Two splice variants PPM1K_v1 and PPM1K_v2 that were capable of encoding 372 and 324 amino acids, respectively were observed. The

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orthologous protein in humans mostly resembles the putative protein encoded by the first variant (GenBank accession AAR06213-92% identity, 98% similarity). As in other gene family members the second exon was large and encoded most of the catalytic domain (Seroussi et al. 2001). A di-nucleotide in this exon variation was identified that is capable of encoding an amino acid substitution (R26H) and we used it as a genetic marker (Table 1, 6). Two other SNPs in exon 2 and 5 were identified (GenBank accession AJ871967).

- 10 ABCG2. PCR primers for amplification of 15 coding exons of ABCG2 were designed (#615 to #638). Three SNPs in intron 3 were annotated (GenBank accession AJ871176), and the SNP on 29183 position, designated as ABCG2(1) was genotyped (Table 1, Table 6). In exon 6 (position 33437), a
- 15 SNP (G or T) that was capable of encoding an amino acid substitution (D219Y) was identified. The two Israeli Holstein sires that were heterozygous for the QTL were homozygous for 219D. The 219Y allele was detected in Hereford genomic sequence and Holstein (GenBank accession BE480678).
- 20 Within the translated region, a SNP (A or C) that was capable of encoding an amino acid substitution (Y581S) was revealed in exon 14 (position 62569 in AJ871176). This polymorphism, designated as ABCG2(2) was genotyped (Table 1, Table 6).
- 25 PKD2. PCR primers were designed for amplification of coding regions in the 15 exons of PKD2 (#252 to #261). The promoter and the first exon of PKD were cloned 2, but no polymorphism was detected, even though this segment included a highly repetitive GC rich region, and was therefore considered as hot spot mutation (Stekrova et al., 2004). For PCR amplification in exon 1 region, 0.5M G-Melt additive (Clontech Laboratories, Inc.) was added. Using primers (#261 and #262) we PCR amplified a region upstream this gene promoter, and observed a length variation within a stretch of adenine residues which was used as genetic marker (Table 1, Table 6).

30 SPP1. The products amplified by PCR primers (#121 to #142) of secreted phosphoprotein 1 (SPP1) were sequenced, including 0.8 Kbp upstream to the initiation site in the promoter region, and all seven exons, and seven introns. The two SNP detected in intron 5 and the 3' non-translated region of exon 7 and designated them as SPP1(1) and SPP1(2), respectively (Table 1, 6). The three segregating and 15 non-segregating Israeli sires for the QTL, for the OPN3907 poly-T polymorphism at 1240 bp upstream of the SPP1 transcription initiation site (Schnabel et al., 2005) using primers #155 and #156.

35 IBSP. Bovine integrin binding sialoprotein gene (IBSP) has been previously cloned (GenBank accession NM_174084, Chenu et al. 1994). This sequence was used to design PCR primers for amplification of exon 7 (#801 and #802). A SNP that was capable of encoding an amino acid substitution (T252A) was identified and genotype (Table 1, 6).

40 LAP3. Bovine leucine amino peptidase 3 gene (LAP3) has been partially cloned (GenBank accession S65367, Wallner et al. 1993). This sequence was used to design PCR primers (#400 and #401) for amplification of intron 12 and the adjacent exons. Three polymorphic sites in intron 12 and a sense mutation in exon 12 (Table 1) were detected. We genotyped the polymorphism at exon 12 (Table 6).

45 MED28. The bovine gene (TIGR tentative consensus TC274468) is 91% similar to the human mediator of RNA polymerase II transcription, subunit 28 homolog (yeast) (MED28, GenBank accession NM_025205). This sequence was used to design PCR primers for amplification of exon 4

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(#500 and #501). Four polymorphic sites were detected in this exon and genotyped the site at position 1345 (Table 1).

MLR1. The human chromosomal region that encodes the last exon of transcription factor MLR1 gene (MLR1) also encodes on the opposite strand the last exon of chromosome condensation protein G (HCAP-G). We sequenced the orthologous genomic region in cattle. There was 93% identity between the coding regions of bovine and human HCAP-G genes. Using primers #500 and #501 we detected a polymorphic repetitive four base sequence (TGAT)n (Table 1, 6). We annotated it as part of the last exon of MLR1, on the basis of its orthologous position in the 3' non-translated end of the human gene. Bovine ESTs (GenBank accessions CK831694 and CO883952) confirm the expression of the bovine MLR1 ortholog.

Biopsy procedures and RNA extraction. Biopsies were collected from mammary and liver tissues of Holstein cows in the herd at the University of Illinois Dairy Research Facility (Dept. of Quantitative and Molecular Genetics, Institute of Animal Science, Volcani Center, Israel) as previously described (Drackley et al. 1991; Farr, 1996; Veenhuizen, 1991). Biopsies of mammary gland and liver were collected from eight cows at six time points relative to parturition (-15d, 1d, 15d, 30d, 60d, 120d), and five cows at seven time points relative to parturition (-65d, -30d, -15d, 1d, 15d, 30d, 50d), respectively during the dry period and lactation. Tissue samples were put in TRIZOL and RNA was extracted immediately using RNase-free vessels. Mammary and liver tissues (0.5 to 2 grams) were homogenized and centrifuged at 12,000 g for 15 min at 4° C. Chloroform was added (200 µl/ml) to the supernatant and the samples were centrifuged at 12,000 g for 15 min at 4° C. Acid-phenol: chloroform (600 µl/ml) was added to the aqueous supernatant. Samples were vortexed and centrifuged at 12,000 g for 15 min at 4° C. and the upper phase was discarded. Isopropanol (500 µl/ml) was added to samples and following an overnight incubation at -20° C. the supernatant was aspirated and washed with 75% ethanol (1 ml 75% ethanol/ml Trizol). Samples were centrifuged at 7,500 g for 5 min at 4° C. Supernatant was aspirated. Tubes were air-dried at room temperature for 10 minutes. RNA pellet was resuspended in a suitable volume (20-400 µl) of RNA storage solution. Concentration of RNA was 2-5 µg RNA/µl buffer.

Quantitative Real-time PCR analysis for gene expression. Quantitative Real-Time PCR was carried out for the following genes: SPP1, ABCG2, PKD2, LAP3, MED28, PPM1K, HERC6 and FAM13A1. Table 5 shows the list of primers designed for Q-PCR analysis. The 18S ribosomal RNA gene was used as control.

One µg mRNA was transcribed in a total volume of 20 µl using 200 U Superscript II (Invitrogen), 500 µg oligo dT(18) primer, 4 µl 5x first strand buffer, 2 µl 0.1M DTT, 40 U RNasin and 1 µl 10 mM dNTPs. Specific primers were synthesized for all genes in 3' UTR non-coding region of the last exon (Table 5). All reactions were performed on ABI PRISM 7700 sequence detection system using 2x Syber Green PCR Mastermix (Applied Biosystems, Foster City, Calif.), 1 µl RT product, 10 pmol forward and reverse primer in 25 µl reaction volume. PCR thermal cycling conditions were as followed: initial denaturation step 95° C., 10 min, followed by 40 cycles of denaturation for 15 seconds at 95° C., annealing and extension for 60 seconds at 60° C.

Computation of LD parameter values. LD parameters values were computed between each pair of markers as described by Hedrick (1987). The microsatellite BM143 had 13 alleles ranging in fragment length from 90 to 118 bp. Most of allele frequencies were quite low, and the distribution of the allelic frequencies was strongly bimodal. Thus, for estimating LD,

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BM143 was converted to a "diallelic" marker by assigning all alleles <108 the value of 1, and all allele >108 the value of 2. For individuals that were heterozygous for both markers, computation of the LD value requires that phase be known, which was not the case. For these individuals both phases were considered to be equally likely, and the LD value was computed accordingly. Thus, the LD values presented slightly underestimate the true values. X 2 values for independent association between each marker pair were also computed.

Computation of ABCG2(2) genotype probabilities. Genotype probabilities for ABCG2(2) were determined for the entire Israeli Holstein milk-recorded population, using the segregation analysis algorithm of Kerr and Kinghorn (1996), 15 The number of animals analyzed by the segregation analysis algorithm was reduced to 44,135 by four "pruning" steps (Weller et al. 2003). At each step, animals that were not genotyped, and were not listed as parents of animals remaining in the data file were deleted. The pruning did not affect the 20 segregating analysis, because these animals by definition include no information with respect to the allelic frequencies. The algorithm requires an estimate of the allelic frequencies in the base population. The initial estimate was derived from the frequencies of the 335 genotyped bulls. After application 25 of the algorithm this estimate was revised, based on the allelic frequencies of all animals with unknown parents. The segregation analysis algorithm was rerun with the updated base population allelic frequencies until convergence for the base population allelic frequencies was obtained at a frequency of 30 0.75 for the A allele. The genotype probabilities for the "pruned" cows were then regenerated from the genotype probabilities of their parents, assuming random distribution of alleles. For cows with either one or two unknown parents, the allelic frequencies of the base population were used for 35 the unknown parent. The estimated allelic frequencies as a function of birth year were computed for the entire population of cows.

T

Dairy cattle breeding programs. In most developed countries, dairy cattle breeding programs are based on the "progeny test" (PT) design. The PT is the design of choice for moderate to large dairy cattle populations, including the US Holsteins, which include over 10,000,000 animals. This population consists of approximately 120,000 cows of which 40 90% are milk recorded. Approximately 20 bulls are used for general service. Each year about 300 elite cows are selected as bull dams. These are mated to the two to four best local bulls and an equal number of foreign bulls, to produce approximately 50 bull calves for progeny testing. At the age of one year, the bull calves reach sexual maturity, and approximately 1000 semen samples are collected for each young bull. These bulls are mated to 30,000 first parity cows to produce about 5000 daughters, or 100 daughters per young bull. Gestation length for cattle is nine months. Thus the young bulls are 45 approximately two years old when their daughters are born, and are close to four when their daughters calve and begin their first lactation. At the completion of their daughters' first lactations, most of the young bulls are culled. Only four to five are returned to general service, and a similar number of the old proven sires are culled. By this time the selected bulls are 55 approximately five years old.

Dairy cattle breeding in developing countries. The genus *Bos* includes five to seven species, of which *Bos Taurus* and *Bos indicus* are the most widespread and economically 60 important. *Bos Taurus* is the main dairy cattle species, and is found generally in temperate climates. Several tropical and subtropical cattle breeds are the result of crosses between

taurus and *indicus*, which interbreed freely. In the tropics, cows need at least some degree of tolerance to environmental stress due to poor nutrition, heat, and disease challenge to sustain relatively high production levels. Tropical breeds are adapted to these stressors but have low milk yield, whereas higher productive temperate breeds cannot withstand the harsh tropical conditions, to the point of not being able to sustain their numbers. Furthermore, most topical countries are developing countries, which lack systematic large-scale milk and pedigree recording.

Methods and theory for marker assisted selection (MAS) in dairy cattle. Considering the long generation interval, the high value of each individual, the very limited female fertility, and the fact that nearly all economic traits are expressed only in females, dairy cattle should be a nearly ideal species for application of MAS. As noted by Weller (2001), MAS can potentially increase annual genetic gain by increasing the accuracy of evaluation, increasing the selection intensity, decreasing the generation interval.

The following dairy cattle breeding schemes that incorporate MAS have been proposed:

1. A standard progeny test system, with information from genetic markers used to increase the accuracy of sire evaluations in addition to phenotypic information from daughter records (Meuwissen and van Arendonk 1992).

2. A multiple ovulation and embryo transfer (MOET) nucleus breeding scheme in which marker information is used to select sires for service in the MOET population, in addition to phenotypic information on half-sisters (Meuwissen and van Arendonk 1992).

3. Progeny test schemes, in which information on genetic markers is used to preselect young sires for entrance into the progeny test (Kashi et al. 1990; Mackinnon and Georges 1998).

4. Selection of bull sires without a progeny test, based on half-sib records and genetic markers (Spelman et al. 1999).

5. Selection of sires in a half-sib scheme, based on half-sib records and genetic markers (Spelman et al. 1999).

6. Use of genetic markers to reduce errors in parentage determination (Israel and Weller 2000).

Spelman et al. (1999) considered three different breeding schemes by deterministic simulation:

1. A standard progeny test with the inclusion of QTL data.

2. The same scheme with the change that young bulls without progeny test could also be used as bull sires based on QTL information.

3. A scheme in which young sires could be used as both bull sires and cow sires in the general population, based on QTL information.

They assumed that only bulls were genotyped, but once genotyped, the information on QTL genotype and effect was known without error. It was then possible to conduct a completely deterministic analysis. They varied the fraction of the genetic variance controlled by known QTL from zero to 100%. Even without MAS, a slight gain is obtained by allowing young sires to be used as bull sires, and a genetic gain of 9% is obtained if young sires with superior evaluations are also used directly as both sires of sires and in general service. As noted previously, genetic gain with MAS used only to increase the accuracy of young bull evaluations for a standard progeny test scheme is limited, because the accuracy of the bull evaluations are already high. Thus, even if all the genetic variance is accounted for by QTL, the genetic gain is less than 25%. However, if young sires are selected for general service based on known QTL, the rate of genetic progress can be doubled. The maximum rate of genetic gain that can be obtained in scheme 3, the "all bulls" scheme, is 2.2 times the

rate of genetic gain in a standard progeny test. Theoretically, with half of the genetic variance due to known QTL, the rate of genetic gain obtained is greater than that possible with nucleus breeding schemes.

- 5 The final scheme, with use of genetic markers to reduce parentage errors, is the most certain to produce gains, since it does not rely on QTL genotype determination, which may be erroneous. Weller et al. (2004) genotyped 6,040 Israeli Holstein cows from 181 Kibbutz herds for 104 microsatellites.
 10 The frequency of rejected paternity was 11.7%, and most errors were due to inseminator mistakes. Most advanced breeding schemes already use genetic markers to confirm parentage of young sires.

The current status of MAS in dairy cattle. Two ongoing 15 MAS programs in dairy cattle have been reported so far, in German and French Holsteins (Bennewitz et al. 2004; Boichard et al. 2002). Currently in the German program markers on three chromosomes are used. The MA-BLUP evaluations (Fernando and Grossman 1989) are computed at 20 the VIT-computing center in Verden, and are distributed to the Holstein breeders, who can use these evaluations for selection of bull dams and preselection of sires for progeny testing. The MA-BLUP algorithm only includes equations for bulls and bull dams, and the dependent variable is the bull's DYD 25 (Bennewitz et al. 2003). Linkage equilibrium throughout the population is assumed. To close the gap between the grandsire families analyzed in the German granddaughter design, and the current generation of bulls, 3600 bulls were genotyped in 2002. Only bulls and bull dams are genotyped, because tissue samples are already collected for paternity testing. Thus additional costs due to MAS are low. Thus even a very modest 30 genetic gain can be economically justified. This scheme is similar to the "top-down" scheme of Mackinnon and Georges (1998) in that the sons' evaluations are used to determine which grandsires are heterozygous for the QTL and their linkage phase, and this information is then used to select 35 grandsons, based on which haplotype was passed from their sires. It differs from the scheme of Mackinnon and Georges (1998) in that the grandsons are preselected for progeny test 40 based on MA-BLUP evaluations, which include general pedigree information, in addition to genotypes.

The French MAS program includes elements of both the "top-down" and "bottom-up" MAS designs. Similar to the 45 German program, genetic evaluations including marker information were computed by a variant of MA-BLUP, and only genotyped animals and non-genotyped connecting ancestors were included in the algorithm. Genotyped females were characterized by their average performance based on precorrected records (with the appropriate weight), whereas 50 males were characterized by twice the yield deviation of their ungenotyped daughters. Twelve chromosomal segments, ranging in length from 5 to 30 cM are analyzed. Regions with putative QTL affecting milk production or composition are located on BTA 3, 6, 7, 14, 19, 20, and 26; segments affecting 55 mastitis resistance are located on BTA 10, 15, and 21; and chromosomal segments affecting fertility are located on BTA 1 and 7. Each region was found to affect one to four traits, and on the average three regions with segregating QTL were found for each trait. Each region is monitored by 2 to 4 evenly spaced microsatellites, and each animal included in the MAS 60 program is genotyped for at least 33 markers. Sires and dams of candidates for selection, all male AI ancestors, up to 60 AI uncles of candidates, and sampling daughters of bull sires and their dams are genotyped. The number of genotyped animals was 8000 in 2001, and is intended to reach 10,000 per year, with equal proportions of candidates for selection and historical animals.

TABLE 1

Polymorphism detection in the course of positional cloning to the QTL on BTA6							
Gene	Number of exons		Sequencing size (bp)		Polymorphism		
	total	sequenced	exons	introns	promoter	type ^a	location
MLR1	7	2	482	228		Insertion TGAT	Exon 7 (AJ871966)
MED28	5	2	133	1,268		C to T	Exon 4 (AJ871964)
LAP3	13	2	147	450		C to T	Exon 12 (AJ871963)
IBSP	7	1	560			A to G	Exon 7 (NM_174084 ^b)
SPP1(1)	7	7	1,362	5,633	1,205	A to G	Intron 5 (AJ871176)
SPP1(2)						T to G	Exon 7 (AJ871176)
PKD2	15	15	3,023	2,485	2,931	Insertion A	Promoter (AJ871176)
ABCG2(1)	16	15 ^c	2,029	3,416		A to T	Intron 3 (AJ871176)
ABCG2(2)						A to C ^d	Exon 14 (AJ871176)
PPMIK	7	1	490			GC to AT	Exon 2 (AJ871967, AJ871968)
HERC6	23			330		Insertion C	Intron 5 (AJ877268)
FAM13A1(1)	18	18	2,580	2,190		A to G	Intron 9 (Cohen et al., 2004a)
FAM13A1(2)						C to A	Exon 12 (Cohen et al., 2004a)
Total			10,806	16,713	4,136		

^aThe more frequent allele is listed first.^bAt position 802.^cCoding region of this gene starts in exon 2.^dY581S

TABLE 2

Effects of the polymorphisms on the bulls' breeding values for the quantitative traits with each marker analyzed separately.							
Marker	Number of bulls	Frequency of the more common allele	Quantitative traits				
			Milk	Fat	Protein	% fat	% protein
BM143 ^b	346	55.1	-34	0.7	3.5**	0.019	-0.022*
MLR1	298	50.5	-67	-2.8	.7	-0.005	0.025*
MED28	316	57.2	80	6.0***	.4***	0.031	0.018*
LAP3	341	57.3	13	6.1**	.7***	0.053**	0.039****
IBSP	336	61.3	-35	1.1	.6	0.021	0.015
SPP1(1)	366	57.0	-123**	-0.1	.8	0.039*	0.043****
SPP1(2)	309	72.9	-171**	-0.7	.4	0.048*	0.061****
PKD2	326	67.1	-141**	0.6	.9	0.046*	0.048****
ABCG2(2)	335	80.5	-341****	5.3*	.1**	0.159****	0.135****
ABCG2(1)	282	55.4	-67	0.8	.4	0.029	0.042****
PPMIK	369	73.6	-58	-1.7	.8	0.001	0.033**
HERC6	328	67.9	-14	4.9**	.6***	0.049**	0.056****
FAM13A1(1)	381	81.8	-64	0.3	.1	0.023	0.028*
FAM13A1(2)	370	41.1	-107*	2.0	.2	0.053**	0.042****

^a For ABCG2(2) effects were computed relative to the Y581 allele. This allele, denoted the +allele was associated with increased protein concentration. For all the other markers, the effects were computed relative to the allele in LD association with the +allele for ABCG2(2).^bThis microsatellite was analyzed as a diallelic marker as described herein. Significance: *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001

TABLE 3

Effect of ABCG2(2) on the breeding values of the daughters of the heterozygous sires, and QTL effects derived from the animal model analyses.							
Quantitative traits							
Analysis	Genotype ^a	Number of cows	Kg Milk	Kg Fat	Kg Protein	% fat	% protein
Class effects ^b	-/-	78	0	0	0	0	0
	+/-	328	-185	0.4	1.2	0.059	0.065
	++	264	-432****	4.2**	3.3**	0.169****	0.145****
% dominance ^c			14.3	80.9	27.3	30.2*	17.2*

TABLE 3-continued

Effect of ABCG2(2) on the breeding values of the daughters of the heterozygous sires, and QTL effects derived from the animal model analyses.

Analysis	Genotype ^a	Number of cows	Quantitative traits				
			Kg Milk	Kg Fat	Kg Protein	% fat	% protein
Regression ^d		670	-226****	2.6**	1.8**	0.093****	0.076****
Animal model ^d		-597	2.2	1.3	0.225	0.193	

^aY581S was denoted the “-” QTL allele, and Y581 the “+” QTL allele.

^bSignificance of the class effect is indicated in the +/+ row. Effects are computed relative to the -/- homozygote.

^cRelative to the -/- homozygote.

^dAllele substitution effects assuming additivity.

Significance: *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001

TABLE 4

	Trait				
	Kg milk	Kg fat	Kg protein	% fat	% protein
<u>Variance components</u>					
ABCG2(2)	86,640	13.0	12.9	0.0145	0.0128
Polygenic	272,720	553.0	286.4	0.0481	0.0101
Residual	84,504	1.2	0.1	0.0005	0.0001
<u>Substitution effects^a</u>					
ABCG2(2)	520	6.4	6.3	0.213	0.200
<u>Variance components</u>					
ABCG2(2)	103,080	2.71	5.1	0.0135	0.0129
SPP1(2)	0	0.0	0.0	0.0000	0.0000
Polygenic	270,550	563.0	289.1	0.0480	0.0096
Residual	77,542	1.6	0.0	0.0000	0.0007
<u>Substitution effects</u>					
ABCG2(2)	568	2.9	4.0	0.213	0.201
SPP1(2)	0	0.0	0.0	0.000	0.000
<u>Variance components</u>					
ABCG2(2)	161,952	0	0	0.0158	0.0153
HERC6	15,178	20.6	22.6	0	0
Polygenic	267,670	521.3	282.3	0.0456	0.0093
Residual	86,103	1.0	0.1	0	0.0002
<u>Substitution effects</u>					
ABCG2(2)	711	0	0	0.222	0.219
<u>Variance components</u>					
HERC6	218	8.0	8.4	0	0
<u>Variance components</u>					
ABCG2(2)	85,277	4.7	8.4	0.0133	0.0134
LAP3	2,697	9.2	7.1	0	0
Polygenic	291,069	556.9	286.0	0.0493	0.0094
Residual	77,829	1.0	0	0	0
<u>Substitution effects</u>					
ABCG2(2)	516	3.8	5.1	0.204	0.205
LAP3	92	5.4	4.7	0	0

^aComputed as described in the Materials and Methods section.

TABLE 5

Primers for physical mapping and real-time PCR analysis (SEQ ID NOS: 1-24), respectively in order of appearance).			
Gene	Primer	Sequence	Number of BAC clone ¹
BM143	BM143_F	TET-ACCTGGGAAGCCTCCATATC	E0199P19
	BM143_R	CTGCAGGCAGATTCTTATCG	
SLIT2	SLIT2_3'UTR_f	GTCAGAAATGGAGCTCAATGC	E0380G22
	SLIT2_3'UTR_r	GATGTTGTTTGAGGCCGA	
MED28	MED28_3'UTR_f	TAAGACATTGGCAGCAGGTG	E0060K13
	MED28_3'UTR_r	CTAGTGTGCGGTGCCTTC	
LAP3	LAP3_3'UTR_f	TGCCTTGATTTTCATTTATGC	E0060K13
	LAP3_3'UTR_r	CTGACAATCGCACAGCACT	
IBSP	IBSP_3'UTR_f	GCAGCAACAGCACAGAGGTA	E0393F21
	IBSP_3'UTR_R	TGGTGTGGGTTGTAGGTTT	
SPP1	SPP1_3'UTR_f	CATTAAAGCAGGGTGGGAGA	H0005K14;
	SPP1_3'UTR_r	ATGCTGTGATGGTTGCATT	E0049M05
PKD2	PKD2_3'UTR_f	TGGGACCAACCATTTCACTT	H0005K14;
	PKD2_3'UTR_r	AGCCACACGAAAAGACT	E0049M05
ABCG2	ABCG2_3'UTR_f	CCCCCAATTAAAAAGGGACT	H0005K14;
	ABCG2_3'UTR_r	GAGGCAGTAAAAAGAACAA	E0049M05
PPM1K	PPM1K_3'UTR_f	TGCCTGGGAAATAACAAGA	E0331116;
	PPM1K_3'UTR_r	GGGTACCACTTACAGTCATT	E0412B12
HERC6	HERC6_3'UTR_f	GAAATTCAGGGGGATT	E0417A15
	HERC6_3'UTR_r	TTCATCAAGACTCGGTGCTG	
FAM13A1	FAM13A1_3'UTR_f	CATCCATCACCTCAGTGTGC	E308012
	FAM13A1_3'UTR_r	AAAGGCAGAGCTGCAGAAC	
18SrRNA	18S_f	GATCCATTGGAGGGCAAGTCT	
	18S_r	AACTGCAGCAACTTTAATATACGCTATT	

¹E0380G22 and E0199P19 in contig 8 42 and all other BAC in contig 503

TABLE 6

Primers for SNP genotyping (SEQ ID NOS: 25-59), respectively in order of appearance)				
Genotyping platform	Gene	Location	Primer	Sequence
Mass Spec	FAM13A1	Exon 12	Fam13A1_ex12F	ACGTTGGATGCCACGCCAAATCTTCTC
			Fam13A1_ex12R	ACGTTGGATGTTCAAGTTGGAGGCCAAC
			Fam13A1_ex12E	GAAGATATCAGAGGAGGAC
SPP1		Exon 7	SPP1_ex 6F	ACGTTGGATGTCCTCCACCTGCTTAAATG
			SPP1_ex 6R	ACGTTGGATGCCCTCTCTGAGGTCATTG
			SPP1_ex 6E	CTGCTTAAATGATCTTTC
IBSP		Exon 7	IBSP_ex 7F	ACGTTGGATGTAACCTACAAACCCACACC
			IBSP_ex 7R	ACGTTGGATGCCCTGTTGTTCATACTCCC
			IBSP_ex 7E	ACCGTTGGAAAATCACC
PPM1K		Exon 2	PPM1K_ex 2F	ACGTTGGATGATTCGGCTCTGAAGTGGAG
			PPM1K_ex 2R	ACGTTGGATGTAAGAAGTGGTGGGAAACAG
			PPM1K_ex 2E	CCTGTCATCTGCAGAC
ABCG2		Intron 3	ABCG2F	ACGTTGGATGGATTGTCCTGAGGAAGTC
			ABCG2R	ACGTTGGATGCAAGTCATAGCTGACAGCTG
			ABCG2E	CTGAGGAAGTCATTAGGT
ABCG2		Exon 14	ABCG2ex14F	ACGTTGGATGAATCTAAACCGTCGTGCC
			ABCG2ex14R	ACGTTGGATGGGTCACAGATAAGGAGAAC
			ABCG2ex14E	GAGCATTCCTCGATACGGCT
MED28		Exon 4	MED28F	ACGTTGGATGGCTCTCACTTGTAGGATG
			MED28R	ACGTTGGATGTTGCAAGTGCTTCTGGACC
			MED28E	TTCGCTGTAATTCAATTCTTA
ABI377	LAP3	Exon 12	LAP3_ex12F	ACGTTGGATGCAAGACAGGTATAGATTGCC
			LAP3_ex12R	ACGTTGGATGCTGAAAATGCTCATTTGGC
			LAP3_ex12E	GTTATAGATTGCCAACTTGC

TABLE 6-continued

Primers for SNP genotyping (SEQ ID NOS: 25-59), respectively in order of appearance)				
Genotyping platform	Gene	Location	Primer	Sequence
ABI7000	HERC6	Intron 5	HERC6F	HEX-CTGAGTCCCAACCACTGGAC
			HERC6R	TGTATGCTGAATGGGTATCTTC
	PKD2	Intergenic	PKD2F	TGCTATGGATCAAATACTATCCAAGTT
			PKD2R	FAM-CCCCGTCCTCTAAAGAATGC
MLR1	MLR1	Intron 5	MLR1F	FAM-TGTGCGATTCCACATTGTTT
			MLR1R	AAAGCAAGCAGCCGCTAAT
			SPP1int5_365F	CTCTGATCCCTGAGAATTTC
			SPP1int5_486R	CACTGTTTTCTGTTCATATAAACAC
			SPP1int5_486P1	FAM-ATCTGTATTTAcTGGATCAT
			SPP1int5_486P2	VIC-CTGTATTATGGATCATT
			FAM13A1	AACTTTAAAAGGGAGAGGAATGTTACC
			FAM13A1int9F	

TABLE 7

Primers for sequencing in the critical region of the QTL (SEQ ID NOS: 60-181, respectively in order of appearance)			
Primer code	Gene	Primer Name	Sequence
1102	MLR1	MLR1ex21F	AAACAAATGTGGAATCGCACA
1103		MLR1ex21R	AAAGCAAGCAGCCGCTAAT
500	MED28	MED28ex4F	CCTGGATATTGCAAGACA
501		MED28ex5R	TAAGACATTGGCAGCAGGTG
502		MED28ex4Fnes	TCTGTCCAGAACCCAGAGCA
503		MED28ex5Rnes	GAAAGGATGCTCTGGTCCAG
400	LAP3	LAP3ex12F	CATTGAAACAGGGAGACCGTGT
401		LAP3ex1 3R	TGTGACTCATCCTAAAGTGGG
801	IBSP	IBSPex7F	CTGGGGCTACAGGAAAGAAG
802		IBSPex7R	ATTCTGGGATTTGTGTGGC
155	SPP1	SPP1prom 1602F	AGATCCCACATGCACCTAGC
156		SPP1prom1147R	CCCGGCCCTCCAAGGCATGC
121		SPP1prom771F	CAGTAACCCCTGCTCGGTCAT
122		SPP1prom28R	TCTGGGAGATCCTGGTTGTC
123		SPP1lex1aF	CACAGGGGACTGGACTCTTC
124		SPP1lex1aR	TTGCTGTCCTCATTTCCAA
125		SPP1lex1bF	CCCTTTCTGAATATTTCACCTC
126		SPP1lex1bR	GAATTGCTCTGCCTCTTGG
111		SPP1lex1F	AGCATCTGGAGCAGCCTTA
112		SPP1int2R	ACTCCTGTCCTCTGTGCG
113		SPP1int1F	TGGAGTGTCCACACAAAAA
114		SPP1int3R	TTGTGTGCCTGCTATGCTTC
115		SPP1int3F	TCACCTAGAGACCCCTGTTT
116		SPP1int4R	TTTGGGCTGGTTAAATGGAT
127		SPP1int3aF	TGCAACTCTGCAAGATGTACT

TABLE 7-continued

Primers for sequencing in the critical region of the QTL
(SEQ_ID_NOS: 60-181, respectively in order of appearance)

Primer code	Gene	Primer Name	Sequence
128		SPP1int3aR	TGCTCAATGAAGATGTTAGGAGA
129		SPP1int3bF	CAAACGGTATTGTCCCAAG
130		SPP1int3bR	GAAGAAAACCCTTCTTCAGC
131		SPP1int3cF	GAACCTTGAACTCATCTACAGC
132		SPP1int3cR	GCTAATTAAGGGCACCTCTGC
133		SPP1int3dF	TCTTCCATAGAGGAAGGAAAA
134		SPP1int3dR	AAATACCCAGATGCTGTAGCC
117		SPP1int4F	AAATTCTCACAATTAAAGAACAAACCA
118		SPP1int5R	UCAAATTCCGGCAAAATTC
135		SPP1int4aF	AAATTCTCACAATTAAAGAACAAACCA
136		SPP1int4aR	TCTGAGGAAACTGATGACAACAA
109		SPP1ex5F	CCTCTGAGGAAACTGATGACAA
110		SPP1ex5R	CGTTAGATCGCGGAACTTCT
137		SPP1int5aF	TCTGATGTCCTGTTGCGCTTAGA
138		SPP1int5aR	GCACTGTAAAGCTAAGGGACA
139		SPP1int5bF	GCCATTAAGTGCTTGTGTGA
140		SPP1int5bR	GTTTTGCGCTCAAGTCCAT
119		SPP1int6F	CCCTTCCTAGCTGTTCTTG
120		SPP1int7R	AAGCAGGGTGGGAGACAATA
141		SPP1int6aF	CGTACGTGTTCATTCAGCA
142		SPP1int6aR	CAGAGTCCAGATGCCACAGA
261	PKD2	PKD2ex1365812F	GGCCAAGGAAGAACGAAC
262		PKD2ex1370002R	GGAATGGTGGTGGAGATGGA
212		PKD2ex1F	CGAGGAGGAAGAGGGAGGAAG
255		PKD2ex1R	CGACCTCTCTTCCTCCCTCT
221		PKD2int1F	AACAGGAGAGCCTCCCTAAA
222		PKD2int2R	TTGCATATTTGCCCTGTCAA
245		PKD2int2Fe	GTGCGGCTGTAAAGGTCAG
246		PKD2int3Re	TATGGGAAGGAAATTGGAG
247		PKD2int2F	TTGGCTTGTCTGTCTTCCA
248		PKD2int3R	GCTGTGCACTTAACACTGGG
223		PKD2int3F	AAAATGTTGCCTTGCTTTCA
224		PKD2int4R	AAGTGTCTGTGGCTTGAGA
267		PKD2int4F	TCAGGAACCAGTTGTCTGTAA
268		PKD2intSR	AAACTGCAGGCAATGGTTT
227		PKD2intSF	CCTGACTGCATCCATGTGTT
228		PKD2int6R	AGGTTGGAGAACACACCAAA

TABLE 7-continued

Primers for sequencing in the critical region of the QTL
(SEQ ID NOS: 60-181, respectively in order of appearance)

Primer code	Gene	Primer Name	Sequence
229		PKD2int6F	TCTTCATTTAACCTTTGTTTCCA
230		PKD2int7R	TGTTGAAGGACCTGAATTGCT
231		PKD2int7F	ATTTCCCCCTCTCTTTGCAG
232		PKD2int8R	GAAACCTTCATGGTGGCTGT
233		PKD2int8F	TGTCAAAAGAATGCTGGACA
234		PKD2int9R	CATCATCTCTTCTTTCTTCACA
235		PKD2int9F	TTTTCCCAAAGAATTGGTAGC
236		PKD2int10R	GTTGTTTCAGCCAGATTGCC
237		PKD2int10F	GGCAGAACAAACGAAAAAGG
238		PKD2int11R	AAGAATCTCAATTGCCCGT
239		PKD2int11F	GATCGTGTGCATGGATGAGT
240		PKD2int12R	GATTGGTTAACACACTGCAA
241		PKD2int12F	CAGTGATCCGTGTTCTCA
242		PKD2int13R	TTCGAGTTGACAAGGGGC
263		PKD2int13F	CACAAGATGTTTGTCCCTC
264		PKD2int14R	TGTTTCCCCATACTGCAA
265		PKD2int14F	TTCCGAAGGCAATTCTAAA
266		PKD2int15R	ATATGGTGGTCAGGGCACAT
214		PKD2ex1SF	TGGAAAAGAATCCAAACCA
215		PKD2ex1SR	GCTCACCAAATTATGGGGA
251		PKD2ex153152SF	ACCAACCGTACTTGGCTTG
252		PKD2ex1532487R	GATTCAAGCTTGCCTACCTGC
603	ABCG2	ABCG263223F	CCTCTTGATTGCCAGGAAAA
604		ABCG263906R	GATTCCCTGTGAGCTAACCC
605		ABCG2 65770F	CACACACCACAAAAACCTC
606		ABCG2 66373R	TTCATCTTGTCAAGATGGTAACCA
615		ABCG2int1F	TGTTTACAGTCTCATTACCTGGA
616		ABCG2int2R	ATGCAGATTGGCAGGTTT
617		ABCG2int2F	AACTGGCTTAAACTGGGTCA
618		ABCG2int3R	TTCTTTGTAGTTTCATGTGTGG
642		ABCG2ex3F	CATGAAACCTGGCCTCAATG
643		ABCG2ex4R	TCCATGTGGATCCTTCCTTG
619		ABCG2int3F	AAGAGGTAAGCCTGATTGG
620		ABCG2int4R	TTCATATGGCAAGTGCCTT

TABLE 7-continued

<u>Primers for sequencing in the critical region of the QTL (SEQ_ID NOS: 60-181, respectively in order of appearance)</u>			
Primer code	Gene	Primer Name	Sequence
621		ABCG2int4F	GAGTGATGGTATTAGAAAAGACCTG
622		ABCG2int5R	TAGGACCTCACCTGTGTGGA
613		ABCG2int5F	CAACAAATGATAGTGGCAGAGG
614		ABCG2int6R	TCCTGAAGAGGTAAATGCCATG
623		ABCG2int6F	CCAAGAAATGTAAGTTTCAGATGTTT
624		ABCG2int7R	ACAAAGGAGTCACTGGAGCA
625		ABCG2int7F	TTTACCAAGGACTATCAATTGGTG
626		ABCG2int8R	TAAACCACGGCTGTTGAATT
627		ABCG2int8F	AAAGGGGTTGTAGAAAAATGGA
628		ABCG2int9R	CATTTGGGGGACATTATGCT
629		ABCG2int9F	GGAGAGATTTGATTAAGTAGCCAGA
630		ABCG2int10R	GAATTGAAACAAGCACAGGG
631		ABCG2int10F	TTGGGGAAAGAATTTCAG
632		ABCG2int11R	GGTCAGACTGGTCACATCCA
644		ABCG2int11F	GCAAATGGTTAACCTGGT
645		ABCG2int12R	ACAGAAAGTCCCCTCCATC
633		ABCG2int12F	TTGGATTAACCCCTCTTG
634		ABCG2int13R	ATTCCTACCCCCAACTTGC
635		ABCG2int13F	ATTTGCTAGACGGCACCAGA
636		ABCG2int14R	TATCCTTGGCCATGAGCTGT
637		ABCG2int14F	TTTCTTATCCTGCTCCACTT
638		ABCG2int15R	ACTGGGCTGAGGAATCCTT
1000	PPM1K	PPM1Kex2F	GGCATCCCATTATTGTTCCA
1001		PPM1Kex2R	TACCCACATGGAGAAATGCA
705	HERC6	HERC6ex5F	TGAAGACTCTCGGTGTGGTT
706		HERC6ex6R	GAATTGAAGGCCTCGTCTCA

TABLE 8

Number of animals included in the variance components analyses for ABCG2 polymorphism.			
Markers analyzed	Number of:		
	Genotyped bulls	Ancestors	Total
ABCG2(2)	336	422	758
ABCG2(2), SPP1(2)	274	367	641
ABCG2(2), HERC6	298	396	694
ABCG2(2), LAP3	308	399	707

TABLE 9

Breed ¹	Animals genotyped	Allele frequencies		
		ABCG2 ^A	ABCG2 ^C	SE
60 Aberdeen Angus	25	1.00	0.00	
Anatolian Black	31	1.00	0.00	
Angler	30	1.00	0.00	
Asturian Mountain	43	1.00	0.00	
Ayrshire	32	1.00	0.00	
Banyo Gudali	67	1.00	0.00	
65 Belgian Blue (beef)	28	0.95	0.05	±0.041
Belgian Blue mix	8	0.94	0.06	±0.084

TABLE 9-continued

Number of animals genotyped per breed and allele frequencies of the ABCG2 gene with standard errors (SE).

Breed ¹	Animals genotyped	Allele frequencies		
		ABCG2 ^A	ABCG2 ^C	SE
Bohemian Red	35	0.99	0.01	±0.017
British Friesian	37	0.93	0.07	±0.042
Casta Navarra	19	1.00	0.00	
Charolais	10	1.00	0.00	
Chianina	36	1.00	0.00	
East Anatolian Red	28	0.88	0.12	±0.061
Gelbvieh	6	1.00	0.00	
German Angus	18	0.92	0.08	±0.064
German Black Pied	22	0.93	0.07	±0.054
German Brown	22	0.95	0.05	±0.046
German Brown Swiss	21	1.00	0.00	
German Holstein	27	1.00	0.00	
German Simmental	22	0.86	0.14	±0.074
Hereford	39	1.00	0.00	
Israeli Holstein ²	341	0.80	0.20	±0.022
Menorquina	15	0.97	0.03	±0.044
N'Dama	7	1.00	0.00	
<i>Nellore</i>	8	1.00	0.00	
Pezzata Rossa	18	1.00	0.00	
Pinzgauer	9	1.00	0.00	
Polish Red	11	1.00	0.00	
Santa Gertrudis	11	1.00	0.00	
South Anatolian Red	17	1.00	0.00	
Toro de Lydia	13	1.00	0.00	
Turkish Grey Steppe	9	1.00	0.00	

TABLE 9-continued

Number of animals genotyped per breed and allele frequencies of the ABCG2 gene with standard errors (SE).				
Breed ¹	Animals	Allele frequencies		
	genotyped	ABCG2 ^A	ABCG2 ^C	SE
US Holstein ²	9	0.95	0.05	±0.073
White Fulani	9	1.00	0.00	

Bos indicus breeds are in italics; other breeds are *Bos taurus*.

Cohen-Zinder et al. (2005)

TABLE 10

Notation of alleles, polymorphisms and QTL status

Nucleotide allelic designation	ABCG2 allelic designation	Amino acid allelic designation	QTL status
A (adenine) allele	ABCG2(1)	Y581 allele (tyrosine)	+QTL, decreases milk yield
C (cytosine) allele	ABCG2(2)	581S allele allele (serine)	-QTL, increases milk yield
A->C	Y581S, denotes that at position 581 of amino acid sequence of ABCG2 protein, a serine (S) is present instead of a tyrosine (Y).		

TABLE 10

Notation of alleles, polymorphisms and QTL status

	Nucleotide allelic designation	ABCG2 allelic designation	Amino acid allelic designation	QTL status
25	A (adenine) allele	ABCG2(1)	Y581 allele (tyrosine)	+QTL, decreases milk yield
30	C (cytosine) allele	ABCG2(2)	581S allele (serine)	-QTL, increases milk yield
	A-->C	Y581S, denotes that at position 581 of amino acid sequence of ABCG2 protein, a serine (S) is present instead of a tyrosine (Y).		

ABCG2 splice variants of first exon and exon 2 are highlighted in bold.
(SEQ ID NO: 182)
AGGAGAGACT CCATCTTGA^G GCCTGTCATC CGTCTAAAG ACAGGATGTG AACTGGGCG
GAACCCTGCT TAAGAGTGA^G GAAACAGTTG CTAGTAAAAA CCAGGCTCTCC TGGAGACTTC
ACTCCCTACA GATGGCAAA^C GGAGATTGTA GTTGTGGTCA GGCTGCCCT GTTAGATTAA
TCATGGAGAC ATCCTCCCTT GATGTATAAT CATTGTTCCC CCCTCCCGC CCCACCTCCC
CCGTTAACCT TAATTGTTT TTCTCCTAGC ACCTACTTGT AAAACTCA^T CATATACAA^C
AAAAAGATTG TTAACATGTA ACCAGTCACG TGTGTGTGT^G TGTGTGTGT^G TGTGTGTGT^G
TGTGTAAAAC TGGGCCTCTC AAAAACATCA GGGCCTTGT^G TGGGAACTGA TTCCCCCTGG
ACCTGCTGGC ATAATAAAACT GTACTCCAGT CTTGAGTGTC CCCTGAGGTG TGTTTGCAA
CTCAGGATTC CACAA^AACATT CCAGAAGGAC ATCAGTGTG^T ACCTAGACAG GTGAAGC^A
AATGTTGGA GCCAA^CACAGAG ATCTAACCA^G TGAA^GGTCA^T GAA^CCTGT^T CACAA^A
GGGTAGATTC TTTCAAGGAC CAGGTGACTA GGAGGCAAGC GACCA^AAGGC AGGACTGGGT
ACATATTCG TGACAGTGT^G GGTCGCTCAG TCCTGTC^CCGA CTCTGTGCAA TCCCATGGGC

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TGTAGCCTT CAGGCTCCTC TGTCCAAGGG ATTCTTCAG CAAGAATACT GGAGTGGTT
 GCCATACCCCT CGGCCAGGG ATCTTCCCCA CCCAGGGACT GAACCTAGGT CTCTCGCATT
 GTAGGCAGAT TCTTTACCAT CTGAGTCACC AGCTGGGTCC TGTGCAGCTG TACAGGTCGT
 ACCCCCCGTAT CGCGAGGGGA AATACTTTCA AAGCAAACGC GGCAAGTTAA TGCAAGCAC
 GGGAAAAAGT AGGGCGCCCA TTCACTGCAT CTCAAGGCCT TCCAGCACTG AACAAAGTAGC
 ACTGTGGGTG GTGCCCTGGCC CCAGGTGGTG ACTGAGGCTG CTGCCCTCGGA TTCCCCAAC
 AGGTACACCC GGAGCAGCTC GCATCCTGGC TTCATAGGCA GAGACGAGAA TAGCGGTGTG
 GGGCGCTCTG CTCACTCTCA GGAAGGGGGC GAGAGGCTGC GCCCAGACCC TGTAACCCCC
 GCCCCCGCGCC CCTCCATCCC CGGCCCGGAG CCCCTGTATC CCCGGCCCGG CGCCCCCTCCG
 GCCCCCTGCTC CACTGGTCTA GCGGCTGCGC CTCGGGAGGG CCTGGCGGAG CCCGGGACCT
 GCGCCAGAAA ACGGTCCGAA CAGCTAGCTG CCCTTCCGGT CCTCCTTTTC CGCTTTGTT
 CTTCTCGGTT TCCATCCACC CTAAGTCCTT TTCTCCTCTC CTCTCCCCGC CCCGCGGTGT
 CAATCTCCC GGATTGACAG AGAACGTAGC CTAATACTA AAGCTGAGAG AATCGCGCC
 GGAGGCGCTC GCTGGTCCCG CCTCCTGCCG GCTTTCTTTT CTCTGTGCGC CCCGGGTGGG
 CTTGGCGGAA CTGGCCTCTA CACCCGACA TCCTCCATCG ACTGCCGGGG GCCGACTGTT
 TGGAAAAGAGG ATGGGGCTGG TGGCGGGCGGG GAAGCGCTCA TCTGCCCGGG AAAATAGCTG
 GAGAGGAGTG CGGGATTAGA GCTATGCCCG TGATAGTGTG CCCGCAACCA GCGAGACCC
 GTAGTTCTC GGTCTGGAG GTATGTTCTG GGCAGCACAA CACAGCAACT GCTATGTATT
 AACTGTCTT GCAGATAATA CTGAAGAGAT GAAAGGACTT GTCTGAGGTT TCAGACAAAT
 CCTCATCCCC AGGAACTGCC CTGTTCCCTAG CTCTTGCTTA AATGGTGGGC ATGAGTGGCT
 ATGTGTGTCC AAATGACAC ATTTTGCTG TTTGGATGGC AGGATCCTGA AGAGAACCAT
 TCCTTAGCTA GTCAGAGACC AAAGTCTATA CTAAGGAAG GATCAGCTCT CTAACGTAT
 AATGGGAGGA GCTGGTTTG AGAGATTGTG TCAGCTGGCA TGGCCATTTC TAGATAATAC
 ACACACTTTT GACTTTGGAG AGAGGAGATA CTTCCCCAGA GTGTGACAGG CAAATGGAGG
 GAACAGCTGC CTCTGCCGTG TTGTGTGTGT GTGTGTGTGT GTGTGTGTGT
 GTCTCAGTC GCCTCTGACT CTTAACACC CCATGGATTG TAGCTACCA GGGTCTCTG
 CCCATGGAAT TTTCAGGCA AGGAACTGG CATGAGTTGC CATTCCCTTC TCCAGGGGAT
 CTTCCAGCCT AGGGATAGAA CCCTCATCTC CTGCGTCTCT TGCAATTGGCA GGTGGATTCT
 TTACCAACCGC ACCACCTGGG AAGCCGCTC CACCCCTATGA GAGTCAGT TCCAACCCAT
 GGCTCGTTG ATAGGACTTC TGCACAGGCC TAAACTCCTG CAGGTAACAA AATACAAAAA
 GTTACTGCCT AAGGGTGCAG CTAGGGATTA AAACACAGCC CTATTACTGC AAATTTTCC
 ACAACAGAAAG TCAGGTAAGG TTAATAAGCA CTTATATATT AAGAATTAGG TGGGAAATA
 TTTCAGAAGG AACTGAGAAT GCTGCAGTTG TTCATTGAAA GCCAGGAGGA ATAATCGGG
 AATGTGTAG GCTCCCTCTG TCCATTCTCC ACATGCTGAT CACCAACACAC TCATGTTGC
 ATTCTTCTAA TCTCACCTCC CAGATAATT AAAACACTTT AGCATTGCT AAAAAAAAAAA
 AAAAAGCCCT TCCTTCCTGG TCTATTCCCT GCCTCTACTC CCTTGTCATT TTTCTAACT
 TCCCTCTTG AACTTATCC CAGCCTGTGT ACGTTCTCT CTCTCCCTGT AACACAATCC
 CACTCTTTC CCAGGTAAAC TTCAAGTTCA GATGTCATGT CCCATCGGAT GTTTTATTCT
 GCCATTCCCT CAGTCTAAAT GTCCCTTCCA TTTAGTCTC TGCCATCCAA TATTTACTTC
 TATTCTAAACA CCTGTTACCC TGTGTCAGAA CTCTTTGTTT CCTTCCCTT CTTCAACCTT

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AGGGTGAATT GTTGAGGGC AGGGGCTAGG TCTCTTCCT AAATAATCCT AACAGCACAG
 TAGGCATTTG GTAAAGTTG GAATGCATGA ATGACATGCT TAAAATAGAG AAGTTATTAT
 CTCATTCTG AACCTTATCT TAGTGCTTGA GTGTACACCG TTCCAAAATG ATGAATCATG
 GAAAAGATAA AAATGCACTG TGTTACTAAG AAATGAAGCC TTAAGGTTTC TAAAATTACA
 ACCAAAAGTGG GCAGGTGGGC CCAGCACCAT TGTATGAAGA TCTTATTCAAG TCAGTTCTAG
 CAAGCTAGGA TGGCATGGCT GAGGAAGTAC GGCAGTGGTA CTTGAAGTAA GAAACAATGA
 TAATGTAAGA ATATCCAAGT CTAAGGGTT TTGTAGGTCC TGCAACGTCT TTACACTGTG
 ATATTCCTG GATGCTAAC ATAGGAACTA AAAAGCCTCT TGATGAGGGT GAAAGAGGAG
 AGTGAAAAG CTGGCTTAA ACTCAACATT CAGAAAACTA AGATCAAAAA CAAACAAAGA
 TCATTGAATC TGTTCCATC ACTTCATGGC AGATTGATGG GGAAAAAGTG GAAACAGTGA
 CAGATTTAT TTTCTTGGC TCCAAAATCA CTGCATATGA TGACAGCAGT CATGAAATTA
 AAGGCACCTG CTCCCTGGAA GAAAACGAT GGCAAATCTA GACAATATAT TCAAAAGCAG
 AGATATCACT TTGATGAGGG TAAGAGGAGG AAGGTGTGGC AGAGGATGAG ATGGCTGGAT
 GACATCACCA ACTTAATGGA CATGAGTTG AACAAACTCC GGGAGATAAT GAAGGACAGG
 GAAGCCTGGA GTGCTGCAGT TAATGGGTCA CAGAGTCAGA CATGATTTAG CGACTGAACG
 ACAATAAAC ATAATGAGAA GCTTGTCTAC TGCCAAAGCC TAAAACCAAG TTCATTGAAG
 AGAATCCCTG CCTCAAGGTT TCAATTGGA AAGTCAGAGA ACAGTAGAAT TTGGTTTCT
 AATAGTTAAC CTCTTACTTT CAAGGTACA CAGTTTATTA GGTGTTAACATC CAGAAATTGT
 TCCAAGCTGT ACCCCATGGG GCTTCCCCAG TGGCTCAGCG GGTAAAGACT CCTGCAATGC
 AGGAGACACA AGAGAGGGGG GTTCGATCCC TGAGTTGGGA AGATCCCCTG GAGGAGGGCA
 TGGCAACCCA CTCCAGTATT CTTGCCTGGA GAATCCGATG GACAGAGGAT CCCGGCGGTC
 TACGGTCCAT ATGGTCACAA AAGAGTCAGA CATGACTGAA GTGACTGAGT GTATACCGT
 AGGTCACTGT GCAGTTTTG AGGACAGGGC CTAGGTGGTT TTACTCAGTC ATGCACACAC
 ACAGTACCTG TTGCAAGACCT GCCACAGTGG GTACTCAGCT TGCTGAATGA AGGAAGAAAT
 GAATAATGT GCTCTACCCT AGGGGTGTAG ATGAGAGGGGA AAGGCACTGT CATTCTCCA
 AAGATGGAAG GCTTTAGAAT CTGGGGAAA ATAAATATTT ACTTTGAAAA TAAACTTATC
 AAAGTAAAGG CAAAAAACTA TTTTAGATGT CACAAAGATC TATGTTAAGT TGCTGAATCA
 GTTGTACTA TTTTAGAGGA TGATGGAAAC TTATCTTCTG AAATGTTGGC TTGCTGCGCT
 AAGAGGGTC AAAGCAAAAT GGTCAGTCT GGAGTTCCCT GAATCCTGAC CTCCTTACCT
 GAAAAACTGA GCAGTTATTT GGCCCAGTTA TTTAACAGAT GACTCAGTT TGTCATTTGT
 AAAATGGGG TAATTATACC ACATGGGTG TTGAGAGACA TTAAATAGTT AATACACAC
 CTATGAAGTA ATTGTATCC CATTTCCTGC CACTATTCC TATTCTCTA GGTGTCATT
 TGCCTTCAC TGTGGCATAA AACATTCTG TTTTTCTCG GTCCACTTCT GTGCTTTTT
 CCTCTCTACT ACCTTCTGC TTTTTCTTT TTTACTATCT CTCTCCCTAA CCAAATTCTT
 CTTTTTTTTT TTTTTCTCT TGGCCATAT GAGGAATGTT AGTCCCTGA CCAGGGATGC
 ATTCCCTCTCC CTATGCAGTG GAAGCACAGG ATCTTAACCA CCAGATGCC AGGAAAGTGC
 CAATTCTTC TTAACGTTCT CATAGTTTT CCTCACTCAC CTAAAAAAAT GACTGAGGGC
 TATGAACCTC AGTAAACTTA TAGAATAAGA AAGTTAATAA TGACTATTAA AACACTATT
 TCTTTCCCC AACTGATTCT CTCATCTCG CGTGCTTAT GCATACTTT TTTGTATTG
 AAAATCAGTG AATACGTTCA GGCTAATTAA GCTCTGATT TCTTCACCTA ATATAACTTT

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ATACTGAAAG GGTCAAGGATA TGTCCCTCCC CAATATGCCA CTTGGCATG AGGATTAATT
 TGAGCTGAAT GCAATTAAGA ATCAACAGAT ACAGAAAGAA GCCTTCTAG CATTCCCTT
 ATCTTATTAA AAAGCAGAAA CTTTGAGAA ATGAGGCTGT CATAAATTCC CTCTTCAGGA
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These are incorporated by reference to the extent they relate to materials or methods disclosed herein.

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primer

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 66

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cattgaaaca ggagaccgtg t

21

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<210> SEQ ID NO 67
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 67

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tgtgactcat cctaagtggg c

21

```

<210> SEQ ID NO 68
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 68

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ctggggctac aggaagaag

20

```

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 69

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attctggat ttgtgtggc

20

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<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 70

agatccccaca tgcaccttagc

20

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<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 71

cccgccctc caaggcatgc

20

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<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 72

cagtaaccct gtcgggtcat

20

```

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 73

tctgggagat cctgggtgtc

20

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<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 74

cacaggggac tggactcttc

20

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<210> SEQ ID NO 75
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 75

ttgctgtctc cattttccaa

20

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<210> SEQ ID NO 76
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 76

ccctttctga atatttcac ctc

23

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<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 77

gatttgcttc tgccttgg

20

```
<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 78

agcatctgga gcagcctta

20

```
<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 79

actcctgtcc tctctgtgcg

20

```
<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 80

tggagtgttt ccacacaaaa

20

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<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 81

tttgtgtgcct gctatgcttc

20

<210> SEQ ID NO 82

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 82

tcacttagag acccctgttt

20

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<210> SEQ ID NO 83
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 83

tttgggctgg ttaaatggat

20

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<210> SEQ ID NO 84
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 84

tgcaacttct gcaagatgta ct

22

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<210> SEQ ID NO 85
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 85

tgctcaatga agatgttagg aga

23

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<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 86

caaacgggta ttgtcccaag

20

```
<210> SEQ ID NO 87
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 87

gaagaaaacc cttcttcag c

21

<210> SEQ ID NO 88

<211> LENGTH: 23

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<212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 88

gaacccttga actcatctac agc

23

<210> SEQ ID NO 89
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 89

gctaattaag ggcacctctg c

21

<210> SEQ ID NO 90
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 90

tttccatag aggaaggaaa a

21

<210> SEQ ID NO 91
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 91

aaatacccaag atgctgttagc c

21

<210> SEQ ID NO 92
 <211> LENGTH: 26
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 92

aaattctcac aattaaagaa caacca

26

<210> SEQ ID NO 93
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 93

ttcaaattcc ggcaaattc

20

<210> SEQ ID NO 94
 <211> LENGTH: 26
 <212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 94

aaatttcac aattaaagaa caacca

26

<210> SEQ ID NO 95

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 95

tctgaggaaa ctgatgacaa caa

23

<210> SEQ ID NO 96

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 96

cctctgagga aactgtatgac aa

22

<210> SEQ ID NO 97

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 97

cgttagatcg gcggaaacttc t

21

<210> SEQ ID NO 98

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 98

tctgatgtct gttgtgcctt aga

23

<210> SEQ ID NO 99

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 99

gcactgtaaa gcctaaggga ca

22

<210> SEQ ID NO 100

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 100

gccatttaagt gctttgttgtt ga

22

<210> SEQ ID NO 101
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 101

gtttttgcgc tcaagtccat

20

<210> SEQ ID NO 102
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 102

cccttccttag ctgttcgttg

20

<210> SEQ ID NO 103
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 103

aagcagggtg ggagacaata

20

<210> SEQ ID NO 104
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 104

cgtacgtgtt cattcagca

19

<210> SEQ ID NO 105
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 105

cagagtccag atgccacaga

20

<210> SEQ ID NO 106
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 106

ggcccaagga agaaaacgaaac

20

<210> SEQ ID NO 107

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 107

ggaatgggtgg tggagatgga

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<210> SEQ ID NO 108

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 108

cgaggaggaa gaggaggaag

20

<210> SEQ ID NO 109

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 109

cgacctcttc ttccctcctct

20

<210> SEQ ID NO 110

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 110

aacaggagag cctcccttaa a

21

<210> SEQ ID NO 111

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 111

ttgcataattt gccctgtcaa

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<210> SEQ ID NO 112

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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primer

<400> SEQUENCE: 112

gtgcggtctg taagggtcag

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<210> SEQ ID NO 113

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 113

tatgggaagg gaatttggag

20

<210> SEQ ID NO 114

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 114

ttggcttggtt ctgtcttcca

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<210> SEQ ID NO 115

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 115

gctgtgact taacactggg

20

<210> SEQ ID NO 116

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 116

aaaaatgttgc ctttgcttc a

21

<210> SEQ ID NO 117

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 117

aagtgtctgt ggcttggaa

20

<210> SEQ ID NO 118

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<400> SEQUENCE: 118
tcaggaacca gttgtctctg taa                                23

<210> SEQ ID NO 119
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 119
aaactgcagg caatggttt                                20

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 120
cctgactgca tccatgtgtt                                20

<210> SEQ ID NO 121
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 121
aggttggaga acaacaccaa a                                21

<210> SEQ ID NO 122
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 122
tttcatatca atctttgtt ttcca                                25

<210> SEQ ID NO 123
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 123
tggtgaagga cctgaatttg ct                                22

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 124

atttccccctc tctttgcag

20

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 125

gaaacacctca tggtggttgt

20

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 126

tgtcaaaaga atgctggaca

20

<210> SEQ ID NO 127
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 127

catcatctct tctttcttc caca

24

<210> SEQ ID NO 128
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 128

tttcccaaa gaatttggta gc

22

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 129

gttgtttcag ccagattgcc

20

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 130

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ggcagaacaa acgaaaaagg	20
<210> SEQ ID NO 131	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 131	
aagaatctca atttgccgt	20
<210> SEQ ID NO 132	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 132	
gatcggtgc atggatgagt	20
<210> SEQ ID NO 133	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 133	
gattggttca acacctgcaa	20
<210> SEQ ID NO 134	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 134	
cagtgatccc gtgttcttca	20
<210> SEQ ID NO 135	
<211> LENGTH: 18	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 135	
ttcgagttga caagggc	18
<210> SEQ ID NO 136	
<211> LENGTH: 21	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 136	

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cacaagatgt ttttgtccct c	21
<210> SEQ ID NO 137	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 137	
tgttttcccc atacatgcaa	20
<210> SEQ ID NO 138	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 138	
ttccgaaggc aattcctaaa	20
<210> SEQ ID NO 139	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 139	
atatggtggt cagggcacat	20
<210> SEQ ID NO 140	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 140	
tggaaaagaa tcccaaacc	20
<210> SEQ ID NO 141	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 141	
gctcacaaaa ttatgggg	20
<210> SEQ ID NO 142	
<211> LENGTH: 20	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer	
<400> SEQUENCE: 142	
accaaccgta ctttggcttg	20

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<210> SEQ ID NO 143
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 143

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gattcagctt gcctacactgc 20

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<210> SEQ ID NO 144
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 144

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cctcttgatt gccaggaaaa 20

```

<210> SEQ ID NO 145
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 145

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gattccctgtg agctcaaccc 20

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<210> SEQ ID NO 146
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 146

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cacacaccac aaaaaccctc 20

```

<210> SEQ ID NO 147
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 147

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ttcatcttgt cagatggtaa cca 23

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<210> SEQ ID NO 148
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 148

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tgtttacagt ctcatttacc tgga 24

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<210> SEQ ID NO 149
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

```

<400> SEQUENCE: 149

atgcagattt tggcaggttt

20

```

<210> SEQ ID NO 150
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 150

aactggcttt aaactgggtc a

21

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<210> SEQ ID NO 151
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 151

tttccttgta gttttcatgt gtgg

24

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<210> SEQ ID NO 152
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 152

catgaaacct ggcctcaatg

20

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<210> SEQ ID NO 153
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

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<400> SEQUENCE: 153

tccatgtgga tccttccttg

20

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<210> SEQ ID NO 154
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 154

aagaggtaaa gcctgatttg g

21

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<210> SEQ ID NO 155
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 155

ttcatatatggg caagtgccctt

20

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<210> SEQ ID NO 156
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 156

gagtgtatggt attagaaaaag acctg

25

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<210> SEQ ID NO 157
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 157

taggaccta cctgtgtgga

20

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<210> SEQ ID NO 158
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 158

caacaaatga tagtggcaga gg

22

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<210> SEQ ID NO 159
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 159

tcctgaagag gtaaaatgcc a tg

22

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<210> SEQ ID NO 160
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 160

ccaagaaaatg taagtttcag atgttt

26

<210> SEQ ID NO 161

159**160**

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<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 161

acaaaaggagt cacttggagc a

21

<210> SEQ ID NO 162
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 162

tttaccagga ctatcaattt ttgttg

25

<210> SEQ ID NO 163
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 163

taaaccacgg ctgtttgaat t

21

<210> SEQ ID NO 164
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 164

aaagggggttg tagaaaaatg ga

22

<210> SEQ ID NO 165
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 165

catttggggg acattatgct

20

<210> SEQ ID NO 166
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 166

ggagagatgg gattaatgtt ccaga

25

<210> SEQ ID NO 167
<211> LENGTH: 21

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<212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 167

gaatttgaaa caagcacagg g

21

<210> SEQ ID NO 168
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 168

ttggggaaag aattttgcag

20

<210> SEQ ID NO 169
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 169

ggtcagactg gtcacatcca

20

<210> SEQ ID NO 170
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 170

gcaaatggtt taatctcctg gt

22

<210> SEQ ID NO 171
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 171

acagaaagtc ccctcccatc

20

<210> SEQ ID NO 172
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 172

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20

<210> SEQ ID NO 173
 <211> LENGTH: 20
 <212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 173

atccctaccc ccaaacttgc

20

<210> SEQ ID NO 174
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 174

atttgctaga cggcaccaga

20

<210> SEQ ID NO 175
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 175

tatccttggc catgagctgt

20

<210> SEQ ID NO 176
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 176

tttctttatc ctgctccac tt

22

<210> SEQ ID NO 177
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 177

actgggctga ggaatccttt

20

<210> SEQ ID NO 178
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 178

ggcatccat tattgttcca

20

<210> SEQ ID NO 179
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 179

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<210> SEQ ID NO 180
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 180

tgaagactct cggtgtggtt                                20

<210> SEQ ID NO 181
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 181

gaattgaagg cctcgtctca                                20

<210> SEQ ID NO 182
<211> LENGTH: 84480
<212> TYPE: DNA
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 182

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actccctaca gatggcaaac ggagattgtt gttgtggta ggctgeccct gtttagattaa      180
tcatggagac atcccccctt gatgtataat cattgttccc ccctccggc cccaccccc      240
ccgttaacct taattgtttt ttcccttgc acctacttgt aaaactcaat catatacacac      300
aaaaagattt ttaacatgtt accagtcacg tgtgtgtgtg tgtgtgtgtg tgtgtgtgt      360
tgtgtaaaac tgggcctctc aaaaacatca gggcccttgg tgggaactga ttcccttgg      420
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<213> ORGANISM: Bos taurus

<400> SEQUENCE: 183

atgctcaaaa tgtcttccaa tagttacgag gtttctatcc caatgtcaaaa aaaactcaac 60
 ggcattccag agacaacctc taaggacctg cagacattaa ctgaaggagc tgggttaagt 120
 ttccataaca tctgctatcg agttaaaatgt aagactggct ttctactttc tcggaaaaca 180
 attgagaaag aaatacttagc aaatatacat ggagtcatga aacctggct caatgccatt 240
 ctggggaccca caggtggagg caaatctcg ttggtagata tcttagctgc aaggaaggat 300
 ccacatggat tatctggaga tggttgatc aatggagcac ctgcacctgc caatttaaa 360
 tgtaactcg gttatgtgg acaagatgtat gttgtgatgg gaactctgac agtggagaa 420
 aacttacagt tctcaggcgc cttcggctt ccaacaacta tgacaagttt cggaaaaat 480
 gaacggattt acaaggatca tcaagagttt ggtctggata aagtggcaga ttccaaagggtt 540
 ggaactcagt ttatccgtgg tgggtctggaa ggagaaagaa aaaggacttag tattgcaatg 600
 gagcttattt ctgatccatc catcttggtc ctggatgagc ccacaactgg ctttagattca 660
 agcacagcaa atgctgttctt tttgtctgtt aagaggatgtt cttaaacaagg acggacaatc 720
 atcttcttcca ttcatcagcc tctgttatcc atcttcaagt tggttgatag cttccaccc 780
 ttggccctgg gaagactcat gttccacggg cttgctcagg aggccttggg gtactttgg 840
 gccataggtt tccgtgtga gcccataat aaccctgcag acttcttccctt ggacatcatt 900
 aatggagatt ttctgtgtt ggtgttaat agagaagaca taggtgtatgtt agctaacgg 960
 accgaagagc cttccaaaaa agatactcca ctcatagaaa aatttagctgtt gttttatgtc 1020
 aactcccttctt tttcaagga aacaaaatgtt gaatttagata aattctcagg ggatcagaga 1080
 aggaagaagc ttccatccta caaggaggc acttgcata cttcccttgc tcatcagtc 1140
 aatggattt ccaggcgttc attcaaaaat ttactgggtt atccccaggc ttctatagct 1200
 cagcttaattt tgacagtctt cctggactg gttataggtt ccattttcttca tgatctaaaa 1260
 aatgatcctt caggaatcca gaacagagcc ggggtgtct tttccctgac gaccaaccag 1320
 tgtttcagca gtgtgtcgc cgtggagtc ctgggtgggg agaagaagct gttttatcat 1380
 gaatataatca gtggatacta tagagtgtca ttctacttctt ttggaaaact gttatctgt 1440
 ttactcccca tgaggatgtt accaagtattt atatcttactt gtataacata cttcttggta 1500
 ggactgaagc caaagggtggaa ggccttcttc atcatgtgc ttaccctgtat gatgggtggct 1560
 tattcagctt gttccatggc actggctata gcagcaggc agagtgtggat atctatagca 1620
 actctgtca tgaccatctc tttgtgtttt atgatgtat tttcagggtt gttggtaat 1680
 ctcaaaaaccg tcgtgcctt gttgtcatgg cttcaataact tgacgattcc tcgatacggc 1740

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tatgcggctt tgcagcataa tgaatttttg ggacaaaaact tctgcccagg actcaatgt	1800
acaacaaaaca atacgtgtag ctatgccata tgtactggcg aagaatttct gaccaaccag	1860
ggcatacata ttcacatgg gggcctgtgg aagaatcacg tagccttggc atgcattgatt	1920
gttatcttcc ttacaattgc ctacctgaaa ttgttattcc ttaaaaaaatt ttcttaa	1977

<210> SEQ ID NO 184

<211> LENGTH: 11

<212> TYPE: DNA

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 184

cggtctmtgcg g 11

<210> SEQ ID NO 185

<211> LENGTH: 658

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 185

Met Leu Lys Met Ser Ser Asn Ser Tyr Glu Val Ser Ile Pro Met Ser	
1 5 10 15	

Lys Lys Leu Asn Gly Ile Pro Glu Thr Thr Ser Lys Asp Leu Gln Thr	
20 25 30	

Leu Thr Glu Gly Ala Val Leu Ser Phe His Asn Ile Cys Tyr Arg Val	
35 40 45	

Lys Val Lys Thr Gly Phe Leu Leu Cys Arg Lys Thr Ile Glu Lys Glu	
50 55 60	

Ile Leu Ala Asn Ile Asn Gly Val Met Lys Pro Gly Leu Asn Ala Ile	
65 70 75 80	

Leu Gly Pro Thr Gly Gly Lys Ser Ser Leu Leu Asp Ile Leu Ala	
85 90 95	

Ala Arg Lys Asp Pro His Gly Leu Ser Gly Asp Val Leu Ile Asn Gly	
100 105 110	

Ala Pro Arg Pro Ala Asn Phe Lys Cys Asn Ser Gly Tyr Val Val Gln	
115 120 125	

Asp Asp Val Val Met Gly Thr Leu Thr Val Arg Glu Asn Leu Gln Phe	
130 135 140	

Ser Ala Ala Leu Arg Leu Pro Thr Thr Met Thr Ser Tyr Glu Lys Asn	
145 150 155 160	

Glu Arg Ile Asn Lys Val Ile Gln Glu Leu Gly Leu Asp Lys Val Ala	
165 170 175	

Asp Ser Lys Val Gly Thr Gln Phe Ile Arg Gly Val Ser Gly Gly Glu	
180 185 190	

Arg Lys Arg Thr Ser Ile Ala Met Glu Leu Ile Thr Asp Pro Ser Ile	
195 200 205	

Leu Phe Leu Asp Glu Pro Thr Thr Gly Leu Asp Ser Ser Thr Ala Asn	
210 215 220	

Ala Val Leu Leu Leu Lys Arg Met Ser Lys Gln Gly Arg Thr Ile	
225 230 235 240	

Ile Phe Ser Ile His Gln Pro Arg Tyr Ser Ile Phe Lys Leu Phe Asp	
245 250 255	

Ser Leu Thr Leu Leu Ala Ser Gly Arg Leu Met Phe His Gly Pro Ala	
260 265 270	

Gln Glu Ala Leu Gly Tyr Phe Gly Ala Ile Gly Phe Arg Cys Glu Pro

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275	280	285
Tyr Asn Asn Pro Ala Asp Phe Phe Leu Asp Ile Ile Asn Gly Asp Ser		
290	295	300
Ser Ala Val Val Leu Asn Arg Glu Asp Ile Gly Asp Glu Ala Asn Glu		
305	310	315
320		
Thr Glu Glu Pro Ser Lys Lys Asp Thr Pro Leu Ile Glu Lys Leu Ala		
325	330	335
Glu Phe Tyr Val Asn Ser Ser Phe Phe Lys Glu Thr Lys Val Glu Leu		
340	345	350
Asp Lys Phe Ser Gly Asp Gln Arg Arg Lys Lys Leu Pro Ser Tyr Lys		
355	360	365
Glu Val Thr Tyr Ala Thr Ser Phe Cys His Gln Leu Lys Trp Ile Ser		
370	375	380
Arg Arg Ser Phe Lys Asn Leu Leu Gly Asn Pro Gln Ala Ser Ile Ala		
385	390	395
400		
Gln Leu Ile Val Thr Val Phe Leu Gly Leu Val Ile Gly Ala Ile Phe		
405	410	415
Tyr Asp Leu Lys Asn Asp Pro Ala Gly Ile Gln Asn Arg Ala Gly Val		
420	425	430
Leu Phe Phe Leu Thr Thr Asn Gln Cys Phe Ser Ser Val Ser Ala Val		
435	440	445
Glu Leu Leu Val Val Glu Lys Lys Leu Phe Ile His Glu Tyr Ile Ser		
450	455	460
Gly Tyr Tyr Arg Val Ser Ser Tyr Phe Phe Gly Lys Leu Leu Ser Asp		
465	470	475
480		
Leu Leu Pro Met Arg Met Leu Pro Ser Ile Ile Phe Thr Cys Ile Thr		
485	490	495
Tyr Phe Leu Leu Gly Leu Lys Pro Lys Val Glu Ala Phe Phe Ile Met		
500	505	510
Met Leu Thr Leu Met Met Val Ala Tyr Ser Ala Ser Ser Met Ala Leu		
515	520	525
Ala Ile Ala Ala Gly Gln Ser Val Val Ser Ile Ala Thr Leu Leu Met		
530	535	540
Thr Ile Ser Phe Val Phe Met Met Ile Phe Ser Gly Leu Leu Val Asn		
545	550	555
560		
Leu Lys Thr Val Val Pro Trp Leu Ser Trp Leu Gln Tyr Leu Ser Ile		
565	570	575
Pro Arg Tyr Gly Tyr Ala Ala Leu Gln His Asn Glu Phe Leu Gly Gln		
580	585	590
Asn Phe Cys Pro Gly Leu Asn Val Thr Thr Asn Asn Thr Cys Ser Tyr		
595	600	605
Ala Ile Cys Thr Gly Glu Glu Phe Leu Thr Asn Gln Gly Ile Asp Ile		
610	615	620
Ser Pro Trp Gly Leu Trp Lys Asn His Val Ala Leu Ala Cys Met Ile		
625	630	635
640		
Val Ile Phe Leu Thr Ile Ala Tyr Leu Lys Leu Leu Phe Leu Lys Lys		
645	650	655
Phe Ser		

<210> SEQ ID NO 186

<211> LENGTH: 208

<212> TYPE: DNA

<213> ORGANISM: Bos taurus

-continued

<400> SEQUENCE: 186

cgggtgggct tggcggaaact ggcctctaca ccccgacatc ctccatcgac tgccggggc	60
cgactgttg gaaagaggat ggggctggtg gcggcgaaaa agcgctcatc tgcccgaa	120
aatacgcttga gaggagtgcg ggattagac tatccccctg atagtgtccc cgcaaccagc	180
gagaccctgt agttccctcg tcctggag	208

<210> SEQ ID NO 187

<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 187

gtgatggaga aggaactgtg gttataacc agctaacagt ggagaaaaaa ggaagtcaat	60
tagatatgag aactggacat ttcccaaga ctatgttgg tggaaagcct cagtcttct	120
ggtagttgcg gggggctgtat aaggttcctc tctggtaact tctttgcgc cttgaaagct	180
ggcaggaagg gaagctcctg gactgttaat agatgcggct ctgtttgaa gtttctatga	240
gaaagccgac aagagtgcgaa atcttctctg tatccccact gcctctctac agaggtttgg	300
gtgtttcc ttccaaacatc acagatcata actgag	336

<210> SEQ ID NO 188

<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 188

tgcgctcgct cgtcgccctt cgaccggccgg ctgcggccccc gtcgtcttcg acgtgacgg	60
aaaccggggc cagtgccttc ccaggtcagc cgctgcgc	100

<210> SEQ ID NO 189

<211> LENGTH: 1552
<212> TYPE: DNA
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 189

aggagagact ccatcttgcgaa gcctgtcata cgtcttaaag acaggatgtg aactggggcg	60
gaaccctgct taagagttag gaaacagttt ctatgtaaaa ccaggcttcc tggagacttc	120
actccctaca gatggcaaac ggagattgtt gttgtggca ggctggccct gtttagattaa	180
tcatggagac atccctccctt gatgtataat cattgttccc ccctccggc cccacccccc	240
ccgttaacct taatttttg ttcccttagc acctactgtt aaaactcaat catatacaac	300
aaaaagattt ttaacatgtt accagtcacg tttgtgtgtg tttgtgtgtg tttgtgtgt	360
tgtgtaaaac tggcccttc aaaaacatca gggccctgtt tggaaactga ttcccttgg	420
acctgtggc ataataaaact gtactccagt cttgagtttc ccctgagggtg tttttgca	480
ctcaggattt cacaacattt ccagaaggac atcagtttgc accttagacag gtgaagcaaa	540
aatgtttgga gccaaacagag atctaaccag tgaagtcaat gaaaccttgc cacaatcaa	600
gggttagattt ttccaggac caggtgacta ggaggcaagc gaccaaaaggc aggactggtt	660
acatatttcg tgacagtgtt ggtcgctcag tttgtgtccgc ctctgtgc aaatccatggc	720
tgttagccctt caggctccctc tgtccaagg attcttcaag caagaataact ggagtgggtt	780
gccataccct ccggccaggaa atcttccca cccaggact gaaaccttaggt ctctcgatt	840
gtaggcagat tctttaccat ctgagtcacc agctgggtcc tttgtgcagctg tacaggctg	900

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accccccgtat ccggagggga aatactttca aagcaaacgc ggcaagttaa tgcagagcac      960
ggaaaaaaagt agggegcccc ttcactgcat ctcaaggcct tccagcactg aacaagttagc    1020
actgtgggtg gtgcctggcc ccaggtggtg actgaggctg ctgcctcgga ttccccaacc    1080
aggtacaccc ggagcagctc gcattctggc ttcataggca gagacgagaa tagcggtgtg     1140
ggcgctctg ctcactctca ggaagggggc gagaggctgc gcccgaccc tgtaacccccc    1200
cccccgccccc cctccatccc ccgccccggag cccctgtatc cccggccccc cgccctccg    1260
gccctgtctc cactgtctca gccggctgccc ctccggggag cctggggag cccggacccct    1320
gccccagaaa acggtccgaa cagctagctg cccttccggc cctccctttc cgctttttt     1380
cttctcggtt tccatccacc ctaagtccctt ttctcccttc ctctcccccc cccgggggtgt    1440
caatctcccc ggattgacag agaacgttagc ctaaatacta aagctgagag aatcgccgc     1500
ggagggcgtc gctggtcccc cctctgtcccg gctttctttt ctctgtgcgc cc           1552

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<210> SEQ ID NO 190

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Bos taurus

<400> SEQUENCE: 190

Asn	Leu	Lys	Thr	Val	Val	Pro	Trp	Leu	Ser	Trp	Leu	Gln	Tyr	Leu	Ser
1				5				10				15			

Ile	Pro	Arg	Tyr	Gly	Tyr	Ala	Ala	Leu	Gln	His	Asn	Glu	Phe	Leu	Gly
			20					25				30			

Gln	Asn	Phe	Cys	Pro	Gly	Leu	Asn	Val	Thr	Thr	Asn	Asn	Thr	Cys	Ser
			35				40				45				

Tyr	Ala	Ile	Cys	Thr	Gly	Glu	Glu	Phe	Leu	Thr	Asn	Gln	Gly	Ile	Asp
				50		55		60							

Ile	Ser	Pro	Trp	Gly	Leu	Trp	Lys	Asn	His						
65					70										

<210> SEQ ID NO 191

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 191

Asn	Phe	Lys	Thr	Val	Gly	Pro	Trp	Leu	Ser	Trp	Leu	Gln	Asn	Leu	Ser
1				5				10				15			

Ile	Pro	Arg	Tyr	Gly	Tyr	Ala	Leu	Gln	His	Asn	Glu	Phe	Leu	Gly
			20				25				30			

Gln	Asn	Phe	Cys	Pro	Gly	Leu	Asn	Val	Thr	Thr	Asn	Lys	Thr	Gly	Ser
			35				40				45				

Tyr	Ala	Ile	Cys	Thr	Gly	Glu	Glu	Phe	Leu	Thr	Asn	Gln	Gly	Ile	Asp
				50		55		60							

Ile	Ser	Pro	Trp	Gly	Leu	Trp	Lys	Asn	His						
65					70										

<210> SEQ ID NO 192

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Sus scrofa

<400> SEQUENCE: 192

Asn	Leu	Lys	Thr	Val	Val	Pro	Trp	Leu	Ser	Trp	Leu	Gln	Tyr	Phe	Ser
1				5				10				15			

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Ile Pro Arg Tyr Gly Phe Ser Ala Leu Gln Tyr Asn Glu Phe Leu Gly
20 25 30

Gln Asn Phe Cys Pro Gly Leu Asn Val Thr Thr Asn Asn Thr Cys Ser
35 40 45

Phe Ala Ile Cys Thr Gly Ala Glu Tyr Leu Glu Asn Gln Gly Ile Ser
50 55 60

Leu Ser Ala Trp Gly Leu Trp Gln Asn His
65 70

<210> SEQ ID NO 193

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Macaca mulatta

<400> SEQUENCE: 193

Asn Leu Thr Thr Ile Ala Ser Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln His Asn Glu Phe Leu Gly
20 25 30

Gln Asn Phe Cys Pro Gly Leu Asn Ala Thr Val Asn Asn Thr Cys Asn
35 40 45

Tyr Ala Thr Cys Thr Gly Glu Glu Tyr Leu Ala Lys Gln Gly Ile Asp
50 55 60

Leu Ser Pro Trp Gly Leu Trp Lys Asn His
65 70

<210> SEQ ID NO 194

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Pongo pygmaeus

<400> SEQUENCE: 194

Asn Leu Thr Thr Ile Ala Ser Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln His Asn Glu Phe Leu Gly
20 25 30

Gln Asn Phe Cys Pro Gly Leu Asn Ala Thr Ala Asn Asn Thr Cys Asn
35 40 45

Tyr Ala Thr Cys Thr Gly Glu Glu Tyr Leu Ala Arg Gln Gly Ile Asp
50 55 60

Leu Ser Pro Trp Gly Leu Trp Lys Asn His
65 70

<210> SEQ ID NO 195

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 195

Asn Leu Thr Thr Ile Ala Ser Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln His Asn Glu Phe Leu Gly
20 25 30

Gln Asn Phe Cys Pro Gly Leu Asn Ala Thr Gly Asn Asn Pro Cys Asn
35 40 45

Tyr Ala Thr Cys Thr Gly Glu Glu Tyr Leu Val Lys Gln Gly Ile Asp
50 55 60

-continued

Leu Ser Pro Trp Gly Leu Trp Lys Asn His
65 70

<210> SEQ ID NO 196

<211> LENGTH: 74

<212> TYPE: PRT

<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 196

Asn Leu Thr Thr Ile Ala Ser Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln His Asn Glu Phe Leu Gly
20 25 30

Gln Asn Phe Cys Pro Gly Leu Asn Ala Thr Gly Asn Asn Pro Cys Asn
35 40 45

Tyr Ala Thr Cys Thr Gly Glu Glu Tyr Leu Val Lys Gln Gly Ile Asp
50 55 60

Leu Ser Pro Trp Gly Leu Trp Lys Asn His
65 70

<210> SEQ ID NO 197

<211> LENGTH: 76

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 197

Asn Leu Arg Thr Ile Gly Pro Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln His Asn Glu Phe Leu Gly
20 25 30

Gln Glu Phe Cys Pro Gly Leu Asn Val Thr Met Asn Ser Thr Cys Val
35 40 45

Asn Ser Tyr Thr Ile Cys Thr Gly Asn Asp Tyr Leu Ile Asn Gln Gly
50 55 60

Ile Asp Leu Ser Pro Trp Gly Leu Trp Arg Asn His
65 70 75

<210> SEQ ID NO 198

<211> LENGTH: 76

<212> TYPE: PRT

<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 198

Asn Leu Arg Thr Ile Gly Pro Trp Leu Ser Trp Leu Gln Tyr Phe Ser
1 5 10 15

Ile Pro Arg Tyr Gly Phe Thr Ala Leu Gln Tyr Asn Glu Phe Leu Gly
20 25 30

Gln Glu Phe Cys Pro Gly Phe Asn Val Thr Asp Asn Ser Thr Cys Val
35 40 45

Asn Ser Tyr Ala Ile Cys Thr Gly Asn Glu Tyr Leu Ile Asn Gln Gly
50 55 60

Ile Glu Leu Ser Pro Trp Gly Leu Trp Lys Asn His
65 70 75

251

The invention claimed is:

1. An isolated polynucleotide comprising the bovine ABCG2 nucleotide sequence shown in SEQ ID NO: 183, wherein the nucleotide sequence comprises a missense mutation that encodes a substitution of tyrosine-581 to serine 5 (Y581S).

252

2. An isolated polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 183, wherein at position 1742 the adenine (A) is replaced by a cytosine (C), resulting in the missense mutation Y581S.

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