The cattle BACs covering the region from *FAM13A1 to MLR1*

E0152P21, E0375J15, E0259M14, E0101G10, E0181A19, E0303P06, E0274F22, E0098H02 E0445L10, E0060K13, E0367N10, E0174N17, E0049M05, E0331I16, E0338G15, E0263K19, E0351N06, E0039I05, E0062M13, E0351N06, E0308O12, E0393F21, E0417A15.

BAC clone selection, subcloning and shotgun sequencing

Filters from RPCI-42 bovine library (http://bacpac.chori.org/mbovine42.htm) 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 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 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 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 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 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 we sequenced contains 66.1 Kbp of the bovine *ABCG2* gene. Following an intergenic region of 10.3 Kbp and encoded on the complementary strand, we observed the last exon of a gene orthologous to the human polycystic kidney disease 2 (*PKD2*). Using *BLASTN*, we found 20 ESTs that matched the 3' end of the 4941 bp putative cDNA transcript deposited with this BAC. We predicted the 5' end of this transcript 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, and we did not find any other genes 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). We identified three sites of variation in this intron sequence and genotyped the polymorphism at position 151 (Table 1, Supplemental Table 2).

PPM1K

The human protein phosphatase 1K (*PPM1K*) is a member of the *PP2C* family of Ser/Thr protein phosphatases. We cloned the bovine *PPM1K* ortholog that maps to critical region of the QTL on BTA6. We observed two splice variants *PPM1K_v1* and *PPM1K_v2* that were capable of encoding 372 and 324 amino acids, respectively. The 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). We identified in this exon a di-nucleotide variation that is capable of encoding an amino acid substitution (R26H) and we used it as a genetic marker (Table 1, Supplemental Table 2). We annotated two other SNPs in exon 2 and 5 (GenBank accession AJ871967).

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, Supplemental Table 2). In exon 6 (position 33437), a 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). 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, Supplemental Table 2).

PKD2

We designed PCR primers for amplification of coding regions in the 15 exons of *PKD2* (#252 to #261). We cloned the promoter and the first exon of *PKD2*, 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 GC-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, Supplemental Table 2).

SPP1

We sequenced the products amplified by PCR primers (#121 to #142) of secreted phosphoprotein 1 (*SPP1*), including 0.8 Kbp upstream to the initiation site in the promoter region, and all seven exons, and seven introns. We genotyped 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, Supplemental Table 2). In addition we sequenced 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.

IBSP

Bovine integrin binding sialoprotein gene (*IBSP*) has been previously cloned (GenBank accession NM_174084, Chenu et al. 1994). We used this sequence to design PCR primers for amplification of exon 7 (#801 and #802). We detected and genotyped a SNP that was capable of encoding an amino acid substitution (T252A) (Table 1, Supplemental Table 2).

LAP3

Bovine leucine amino peptidase 3 gene (*LAP3*) has been partially cloned (GenBank accession S65367, Wallner et al. 1993). We used this sequence to design PCR primers (#400 and #401) for amplification of intron 12 and the adjacent exons. We detected three polymorphic sites in intron 12 and a sense mutation in exon 12 (Table 1). We genotyped the polymorphism at exon 12 (Supplemental Table 2).

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). We used this sequence to design PCR primers for amplification of exon 4 (#500 and #501), and detected four polymorphic sites 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, Supplemental Table 2). 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 (http://cowry.agri.huji.ac.il/web/) 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^{o}C. Chloroform was added (200 μl/ml) to the supernatant and the samples were centrifuged at 12,000 g for 15 min at 4^{o}C. Acid-phenol: chloroform (600 μl/ml) was added to the aqueous supernatant. Samples were votexed and centrifuged at 12,000 g for 15 min at 4^{o}C and the upper phase was discarded. Isopropanol (500 μl/ml) was added to samples and following an overnight incubation at -20^{o}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^{o}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*. Supplemental Table 1 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 ng oligo $dT_{(18)}$ primer, 4 µl 5X first strand buffer, 2 µl 0.1M DTT, 40 U RNasin and 1µl 10 mM dNTPs. Specific primers were synthesized for all genes in 3' UTR non-coding region of the last exon (Supplemental Table 1). All reactions were performed on ABI PRISM 7700 sequence detection system using 2X Syber Green PCR Mastermix (Applied Biosystems, Foster City, CA), 1µl RT product, 10 pmol forward and reverse primer in 25µl reaction volume. PCR thermal cycling conditions were as followed: initial denaturation step 95^0C, 10 min, followed by 40 cycles of denaturation for 15 seconds at seconds at 95^0C, annealing and extension for 60 seconds at 60^0C.

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, *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. χ^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), 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 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 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 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 the unknown parent. The estimated allelic frequencies as a function of birth year were computed for the entire population of cows.

Supplementary Table 1. Primers for physical mapping and real-time PCR analysis.

Gene	Primer	Sequence	Number of BAC clone[1]
BM143	BM143_F	TET-ACCTGGGAAGCCTCCATATC	E0199P19
	BM143_R	CTGCAGGCAGATTCTTTATCG	
SLIT2	SLIT2_3'UTR_f	GTCAGAATGGAGCTCAATGC	E0380G22
	SLIT2_3'UTR_r	GATGTTTGTTTGAGGCCGGA	
MED28	MED28_3'UTR_f	TAAGACATTGGCAGCAGGTG	E0060K13
	MED28_3'UTR_r	CTAGTGTTCGGGTGCCTTTC	
LAP3	LAP3_3'UTR_f	TGCCTTGATTTTTCATTTTATGC	E0060K13
	LAP3_3'UTR_r	CTGACAATCGCACAGCAACT	
IBSP	IBSP_3'UTR_f	GCAGCAACAGCACAGAGGTA	E0393F21
	IBSP_3'UTR_R	TGGTGTGGGGTTGTAGGTTT	
SPP1	SPP1_3'UTR_f	CATTAAAGCAGGGTGGGAGA	H0005K14; E0049M05
	SPP1_3'UTR_r	ATGCTGTGATGGTTTGCATT	
PKD2	PKD2_3'UTR_f	TGGGACCAACCATTTCACTT	H0005K14; E0049M05
	PKD2_3'UTR_r	AGCCACACGAAAAGACT	
ABCG2	ABCG2_3'UTR_f	CCCCCAATTAAAAAGGGACT	H0005K14; E0049M05
	ABCG2_3'UTR_r	GAGGCAAGTGAAAAGAAGACAA	
PPM1K	PPM1K_3'UTR_f	TGCCTGGGGAAAATACAAGA	E0331I16; E0412B12
	PPM1K_3'UTR_r	GGGTCACCACTTACAGTTCACTT	
HERC6	HERC6_3'UTR_f	GAAATTTCAGGGGGATT	E0417A15
	HERC6_3'UTR_r	TTCATCAAGACTCGGTGCTG	
FAM13A1	FAM13A1_3'UTR_f	CATCCATCACCTCAGTGTGC	E308O12
	FAM13A1_3'UTR_r	AAAGGCAGAGCTGCAGAAAC	
18SrRNA	18S_f	GATCCATTGGAGGGCAAGTCT	
	18S_r	AACTGCAGCAACTTTAATATACGCTATT	

[1]E0380G22 and E0199P19 in contig 8342 and all other BAC in contig 503.

Supplementary Table 2. Primers for SNP genotyping

Genotyping platform	Gene	Location	Primer	Sequence
Mass Spec	*FAM13A1*	Exon 12	Fam13A1_ex12F	ACGTTGGATGCCACGCCCAAATCTTTTCTC
			Fam13A1_ex12R	ACGTTGGATGTTCAAGTTGGGAGCCGAAAC
			Fam13A1_ex12E	GAAGATATCAGAGGAGGAC
	SPP1	Exon 7	SPP1_ex 6F	ACGTTGGATGTCTCCCACCCTGCTTTAATG
			SPP1_ex 6R	ACGTTGGATGGCCTCTTCTGAGGTCAATTG
			SPP1_ex 6E	CTGCTTTAATGTATCCTTTTC
	IBSP	Exon 7	IBSP_ex 7F	ACGTTGGATGTAAACCTACAACCCCACACC
			IBSP_ex 7R	ACGTTGGATGGCCTGTTTGTTCATACTCCC
			IBSP_ex 7E	ACCGTTTGGGAAAATCACC
	PPMIK	Exon 2	PPM1K_ex 2F	ACGTTGGATGATTTCGGCTCTGAAGTGGAG
			PPM1K_ex 2R	ACGTTGGATGTAAGAAGTGGTGGGAACCAG
			PPM1K_ex 2E	CCTGTCATCCTGCAGACC
	ABCG2	Intron 3	ABCG2F	ACGTTGGATGGATTGTGTCCTGAGGAAGTC
			ABCG2R	ACGTTGGATGCAAGTCATAGCTGACAGCTG
			ABCG2E	CTGAGGAAGTCTTATTAGGT
	ABCG2	Exon 14	ABCG2ex14F	ACGTTGGATGAATCTCAAAACCGTCGTGCC
			ABCG2ex14R	ACGTTGGATGCGGTGACAGATAAGGAGAAC
			ABCG2ex14E	GAGCATTCCTCGATACGGCT
	MED28	Exon 4	MED28F	ACGTTGGATGGCTTCTCACTTTGTAGGATG
			MED28R	ACGTTGGATGTTGTCAAGTGCTTCTGGACC
			MED28E	TTCGCTGTAATTCATTCCTTA
	LAP3	Exon 12	LAP3_ex12F	ACGTTGGATGCAAGACAGGTTATAGATTGCC
			LAP3_ex12R	ACGTTGGATGCTGAAAATGCTCATTTTGGC
			LAP3_ex12E	GTTATAGATTGCCAACTTGC
ABI377	*HERC6*	Intron 5	HERC6F	HEX-CTGAGTCCCAACCACTGGAC
			HERC6R	TGTATGCTGAATGGGTATCTTCA
	PKD2	Intergenic	PKD2F	TGCTATGGATCAAATACTATCCAAGTT
			PKD2R	FAM-CCCCGTCCTCTAAAGAATGC
	MLR1	Exon 21	MLR1F	FAM-TGTGCGATTCCACATTGTTT
			MLR1 R	AAAGCAAGCAGCCGCTAAT
ABI7000	*SPP1*	Intron 5	SPP1int5_365F	CTCTGATCCCCTGAGAATTTTCA
			SPP1int5_486R	CACTGTTTTTCCTTGTTCATAATAAACAC
			SPP1int5_486P1	FAM-ATCTGTATTTA**c**TGGATCAT
			SPP1int5_486P2	VIC-CTGTATTTA**t**TGGATCATT
	FAM13A1	Intron 9	FAM13A1int9F	AACTTTAAAAGGGAGAGGAATGTTACC
			FAM13A1int9R	TGCACTTGGATGGAGATATACTTACAA
			FAM13A1int9P1	VIC-ATGGCTAGTCAT**a**TTT
			FAM13A1int9P2	FAM-ATGGCTAGTCAT**g**TTT

Supplementary Table 3. Primers for sequencing in the critical region of the QTL

Primer code	Gene	Primer Name	Sequence
1102	*MLR1*	MLR1ex21F	AAACAATGTGGAATCGCACA
1103		MLR1ex21R	AAAGCAAGCAGCCGCTAAT
500	*MED28*	MED28ex4F	CCTGGATATTGCAAGACA
501		MED28ex5R	TAAGACATTGGCAGCAGGTG
502		MED28ex4Fnes	TCTGTCCAGAAACCAGAGCA
503		MED28ex5Rnes	GAAAGGATGCTCTGGTCCAG
400	*LAP3*	LAP3ex12F	CATTGAAACAGGAGACCGTGT
401		LAP3ex13R	TGTGACTCATCCTAAGTGGGC
801	*IBSP*	IBSPex7F	CTGGGGCTACAGGAAAGAAG
802		IBSPex7R	ATTCTGGGATTTTGTGTGGC
155	*SPP1*	SPP1prom_1602F	AGATCCCACATGCACCTAGC
156		SPP1prom_1147R	CCCGGCCCTCCAAGGCATGC
121		SPP1prom_771F	CAGTAACCCTGCTCGGTCAT
122		SPP1prom_28R	TCTGGGAGATCCTGGTTGTC
123		SPP1ex1aF	CACAGGGGACTGGACTCTTC
124		SPP1ex1aR	TTGCTGTCTCCATTTTCCAA
125		SPP1ex1bF	CCCTTTCTGAATATTTTCACCTC
126		SPP1ex1bR	GATTTGCTTCTGCCTCTTGG
111		SPP1ex1F	AGCATCTGGAGCAGCCTTTA
112		SPP1int2R	ACTCCTGTCCTCTCTGTGCG
113		SPP1int1F	TGGAGTGTTTCCACACAAAA
114		SPP1int3R	TTGTGTGCCTGCTATGCTTC
115		SPP1int3F	TCACTTAGAGACCCCTGTTT
116		SPP1int4R	TTTGGGCTGGTTAAATGGAT
127		SPP1int3aF	TGCAACTTCTGCAAGATGTACT
128		SPP1int3aR	TGCTCAATGAAGATGTTAGGAGA
129		SPP1int3bF	CAAACGGGTATTGTCCCAAG
130		SPP1int3bR	GAAGAAAACCCTTCTTTCAGC
131		SPP1int3cF	GAACCTTTGAACTCATCTACAGC
132		SPP1int3cR	GCTAATTAAGGGCACCTCTGC
133		SPP1int3dF	TCTTCCATAGAGGAAGGAAAA
134		SPP1int3dR	AAATACCCAGATGCTGTAGCC
117		SPP1int4F	AAATTCTCACAATTAAAGAACAACCA
118		SPP1int5R	TTCAAATTCCGGCAAAATTC
135		SPP1int4aF	AAATTCTCACAATTAAAGAACAACCA
136		SPP1int4aR	TCTGAGGAAACTGATGACAACAA
109		SPP1ex5F	CCTCTGAGGAAACTGATGACAA
110		SPP1ex5R	CGTTAGATCGGCGGAACTTCT
137		SPP1int5aF	TCTGATGTCTGTTGTGCCTTAGA
138		SPP1int5aR	GCACTGTAAAGCCTAAGGGACA
139		SPP1int5bF	GCCATTAAGTGCTTTGTTGTGA
140		SPP1int5bR	GTTTTTGCGCTCAAGTCCAT
119		SPP1int6F	CCCTTCCTAGCTGTTCGTTG
120		SPP1int7R	AAGCAGGGTGGGAGACAATA
141		SPP1int6aF	CGTACGTGTTCATTCAGCA
142		SPP1int6aR	CAGAGTCCAGATGCCACAGA
261	*PKD2*	PKD2_ex1_365812F	GGCCCAAGGAAGAAACGAAC
262		PKD2_ex1_370002R	GGAATGGTGGTGGAGATGGA
212		PKD2ex1F	CGAGGAGGAAGAGGAGGAAG
255		PKD2ex1R	CGACCTCCTCTTCCTCCTCT
221		PKD2int1F	AACAGGAGAGCCTCCCTTAAA
222		PKD2int2R	TTGCATATTTGCCCTGTCAA
245		PKD2int2Fe	GTGCGGTCTGTAAGGGTCAG
246		PKD2int3Re	TATGGGAAGGGAATTTGGAG

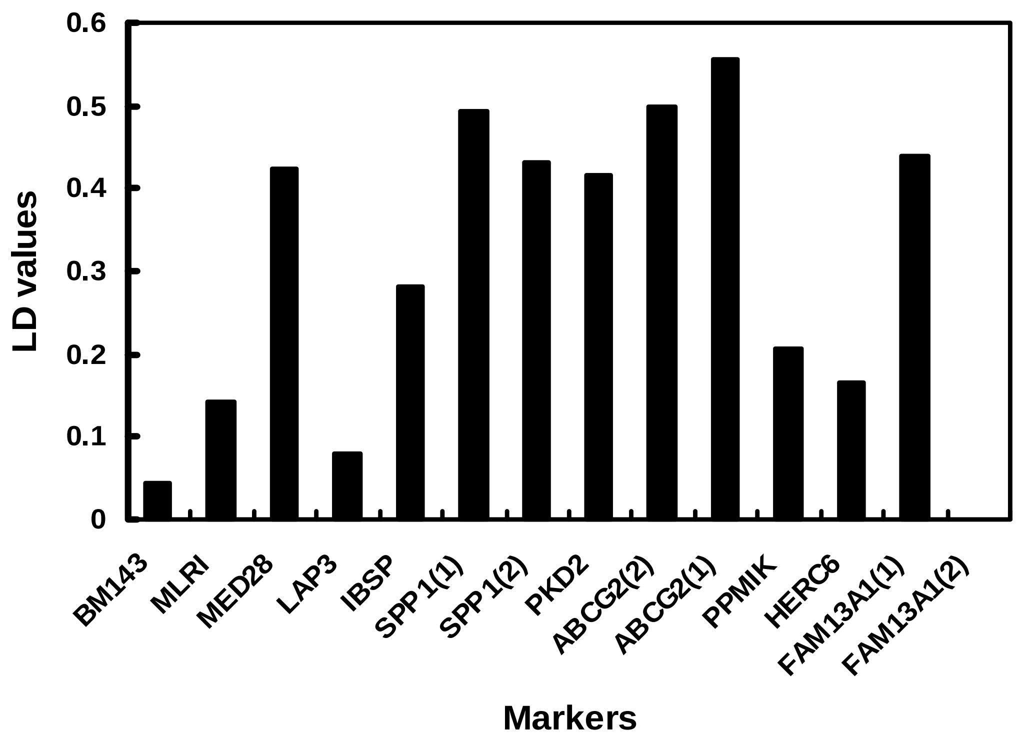
247		PKD2int2F	TTGGCTTGTTCTGTCTTCCA
248		PKD2int3R	GCTGTGCACTTAACACTGGG
223		PKD2int3F	AAAATGTTGCCTTTGCTTTCA
224		PKD2int4R	AAGTGTCTGTGGCTTGTGGA
267		PKD2int4F	TCAGGAACCAGTTGTCTCTGTAA
268		PKD2int5R	AAACTGCAGGCAATGGTTTT
227		PKD2int5F	CCTGACTGCATCCATGTGTT
228		PKD2int6R	AGGTTGGAGAACAACACCAAA
229		PKD2int6F	TCTTCATTTAATCTTTTGTTTTCCA
230		PKD2int7R	TGTTGAAGGACCTGAATTTGCT
231		PKD2int7F	ATTTCCCCTCTCTTTTGCAG
232		PKD2int8R	GAAACCTTCATGGTGGCTGT
233		PKD2int8F	TGTCAAAAGAATGCTGGACA
234		PKD2int9R	CATCATCTCTTCTTTTCTTCCACA
235		PKD2int9F	TTTTCCCAAAGAATTTGGTAGC
236		PKD2int10R	GTTGTTTCAGCCAGATTGCC
237		PKD2int10F	GGCAGAACAAACGAAAAAGG
238		PKD2int11R	AAGAATCTCAATTTGCCCGT
239		PKD2int11F	GATCGTGTGCATGGATGAGT
240		PKD2int12R	GATTGGTTCAACACCTGCAA
241		PKD2int12F	CAGTGATCCCGTGTTCTTCA
242		PKD2int13R	TTCGAGTTGACAAGGGGC
263		PKD2int13F	CACAAGATGTTTTTGTCCCTC
264		PKD2int14R	TGTTTTCCCCATACATGCAA
265		PKD2int14F	TTCCGAAGGCAATTCCTAAA
266		PKD2int15R	ATATGGTGGTCAGGGCACAT
214		PKD2ex15F	TGGAAAAGAATCCCAAACCA
215		PKD2ex15R	GCTCACCAAATTTATGGGGA
251		PKD2_ex15_31525F	ACCAACCGTACTTTGGCTTG
252		PKD2_ex15_32487R	GATTCAGCTTGCCTACCTGC
603	*ABCG2*	ABCG2_63223F	CCTCTTGATTGCCAGGAAAA
604		ABCG2_63906R	GATTCCTGTGAGCTCAACCC
605		ABCG2_65770F	CACACACCACAAAAACCCTC
606		ABCG2_66373R	TTCATCTTGTCAGATGGTAACCA
615		ABCG2int1F	TGTTTACAGTCTCATTTACCTGGA
616		ABCG2int2R	ATGCAGATTTTGGCAGGTTT
617		ABCG2int2F	AACTGGCTTTAAACTGGGTCA
618		ABCG2int3R	TTTCTTTGTAGTTTTCATGTGTGG
642		ABCG2ex3F	CATGAAACCTGGCCTCAATG
643		ABCG2ex4R	TCCATGTGGATCCTTCCTTG
619		ABCG2int3F	AAGAGGTAAAGCCTGATTTGG
620		ABCG2int4R	TTCATATGGGCAAGTGCCTT
621		ABCG2int4F	GAGTGATGGTATTAGAAAAGACCTG
622		ABCG2int5R	TAGGACCTCACCTGTGTGGA
613		ABCG2int5F	CAACAAATGATAGTGGCAGAGG
614		ABCG2int6R	TCCTGAAGAGGTAAATGCCATG
623		ABCG2int6F	CCAAGAAATGTAAGTTTCAGATGTTT
624		ABCG2int7R	ACAAAGGAGTCACTTGGAGCA
625		ABCG2int7F	TTTACCAGGACTATCAATTTTTGTG
626		ABCG2int8R	TAAACCACGGCTGTTTGAATT
627		ABCG2int8F	AAAGGGGTTGTAGAAAAATGGA
628		ABCG2int9R	CATTTGGGGGACATTATGCT
629		ABCG2int9F	GGAGAGATTTGATTAAGTAGCCAGA
630		ABCG2int10R	GAATTTGAAACAAGCACAGGG
631		ABCG2int10F	TTGGGGAAAGAATTTTGCAG
632		ABCG2int11R	GGTCAGACTGGTCACATCCA
644		ABCG2int11F	GCAAATGGTTTAATCTCCTGGT
645		ABCG2int12R	ACAGAAAGTCCCCTCCCATC

633		ABCG2int12F	TTGGATTAACCCCCTCTTTG
634		ABCG2int13R	ATTCCTACCCCCAAACTTGC
635		ABCG2int13F	ATTTGCTAGACGGCACCAGA
636		ABCG2int14R	TATCCTTGGCCATGAGCTGT
637		ABCG2int14F	TTTCTTTATCCTGCTCCCACTT
638		ABCG2int15R	ACTGGGCTGAGGAATCCTTT
1000	*PPM1K*	PPM1Kex2F	GGCATCCCATTATTGTTCCA
1001		PPM1Kex2R	TACCCACATGGAGAAATGCA
705	*HERC6*	HERC6ex5F	TGAAGACTCTCGGTGTGGTT
706		HERC6ex6R	GAATTGAAGGCCTCGTCTCA

Supplementary Table 4: Number of animals included in the variance components analyses

	Number of:		
Markers analyzed	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

Supplementary Figure 1: Linkage disequilibrium values for adjacent markers computed from 411 Israeli Holstein bulls.



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