US006114118A

United States Patent [19]

Templeton et al.

[11] **Patent Number: 6,114,118**

[45] **Date of Patent: Sep. 5, 2000**

[54] **METHOD OF IDENTIFICATION OF ANIMALS RESISTANT OR SUSCEPTIBLE TO DISEASE SUCH AS RUMINANT BRUCELLOSIS, TUBERCULOSIS, PARATUBERCULOSIS AND SALMONELLOSIS**

[75] Inventors: **Joe W. Templeton**; **Jianwei Feng**; **L. Garry Adams**, all of College Station, Tex.; **Erwin Schurr**; **Philippe Gros**, both of Montreal, Canada; **Donald S. Davis**; **Roger Smith, III**, both of College Station, Tex.

[73] Assignees: **Texas A&M University System**, College Station, Tex.; **McGill University**, Montreal, Canada

[21] Appl. No.: **08/903,139**

[22] Filed: **Jul. 30, 1997**

Related U.S. Application Data

[60] Provisional application No. 60/031,443, Sep. 20, 1996.

[51] **Int. Cl.**[7] **C12Q 1/68**; C12P 19/34

[52] **U.S. Cl.** **435/6**; 435/91.2

[58] **Field of Search** 435/6, 91.2; 935/77, 935/78

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Primary Examiner—Eggerton A. Campbell
Attorney, Agent, or Firm—Fulbright & Jaworski L.L.P.

[57] **ABSTRACT**

The present invention relates to materials and methods for identifying animals that are resistant or susceptible to diseases associated with intracellular parasites such as brucellosis, tuberculosis, paratuberculosis and salmonellosis. More particularly, the present invention relates to the identification of a gene, called NRAMP1, which is associated with the susceptibility or resistance of an animal, such as an artiodactyla to diseases such as brucellosis, tuberculosis, paratuberculosis and salmonellosis. Still more particularly, the present invention relates to the identification of specific sequences of bovine NRAMP1 which associate with resistance or susceptibility to ruminant brucellosis, tuberculosis, paratuberculosis and salmonellosis, and to the method of identifying said sequences to identify animals who are susceptible or resistant to disease.

44 Claims, 21 Drawing Sheets

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Sequences of Primers used In SSCA and/or SSCP Analysis

SEQ ID NO; 1			
Fmicro1	5'	AAGGCAGCAA GACAGACAGG	3'
SEQ ID NO: 2			
3end3	5'	ATGGAACTCA CGTTGGCTG	3'

FIG. 1A

Sequences of Primers used to Clone Bovine NRAMP1

SEQ ID NO: 3			
murine 1F primer	5'	TCTCTGGCTG AAGGCTCTCC	3'
SEQ ID NO: 4			
murine 1R primer	5'	CCAAGCTCAC CTTAGGGTAG	3'
SEQ ID NO: 5			
PE2 primer	5'	CGTGGTGACA GGCAAGGAC	3'
SEQ ID NO: 6			
Mut2 primer	5'	CCAAGAAGAG GAAGAAGAAG GTGTC	3'

FIG. 1B

```
                                        pts
                                         |
SEQ ID NO. 7 NRAMP1      MtGDkGPqrl sGssYGSISS PtSPgPQQAP PreTYLSEKI PIPDTkpGTF>
SEQ ID NO. 8 Nramp1      MisDksPPrl srpsYGSISS lPgPaPQpAP creTYLSEKI PIPsadqGTF>
SEQ ID NO. 9 BovNramp1   MSGDTGPPKQ GGTRYGSISS PPSPEPQQAP PGGTYLSEKI PIPDTESGTF 50
                                                                      PKC
             NRAMP1      SLRKLWAFTG PGFLMSIAFL DPGNIESDLQ AGAVAGFKLL WVLLWATVLG>
             Nramp1      SLRKLWAFTG PGFLMSIAFL DPGNIESDLQ AGAVAGFKLL WVLLWATVLG>
             BovNramp1   SLRKLWAFTG PGFLMSIAFL DPGNIESDLQ AGAVAGFKLL WVLLWATVLG 100
                        PKC          TM1                          TM2
             NRAMP1      LLCQRLAARL GVVTGKDLGE VCHcYYPKVP RtvLWLTIEL AIVGSDMQEV>
             Nramp1      LLCQRLAARL GVVTGKDLGE VCHLYYPKVP RILLWLTIEL AIVGSDMQEV>
             BovNramp1   LLCQRLAARL GVVTGKDLGE VCHLYYPKVP RILLWLTIEL AIVGSDMQEV 150
                                                                   TM3
             NRAMP1      IGTAIAFnLL SAGRIPLWGG VLITiVDTFF FLFLDNYGLR KLEAFFGlLI>
             Nramp1      IGTAIsFnLL SAGRIPLWGG VLITiVDTFF FLFLDNYGLR KLEAFFGlLI>
             BovNramp1   IGTAIAFSLL SAGRIPLWGG VLITVVDTFF FLFLDNYGLR KLEAFFGFLI 200
                                        TM4
             NRAMP1      TIMALTFGYE YVVArPeQGA LLrGLFLPSC PGCGhPELLQ AVGIvGAIIM>
             Nramp1      TIMALTFGYE YVVAhPsQGA LLkGLvLPtC PGCGQPELLQ AVGIvGAIIM>
             BovNramp1   TIMALTFGYE YVVAQPAQGA LLQGLFLPSC PGCGQPELLQ AVGIIGAIIM 250
                        TM5
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FIG. 2A

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NRAMP1      PHNIYLHSaL VKSREiDRaR RADIREANMY FLIEATIALS VSFiINLFVM>
Nramp1      PHNIYLHSaL VKSREVDRtR RvDvREANMY FLIEATIALS VSFiINLFVM>
BovNramp1   PHNIYLHSSL VKSREVDRSR RADIREANMY FLIEATIALS VSFLINLFVM 300
              TM6                                    TM7
NRAMP1      AVFGQAFYqk TNQAAFNICA nSSLHDYAkI FPmNNaTVAV DIYQGGVILG>
Nramp1      AVFGQAFYqQ TNeeAFNICA nSSLqnYAkI FPRdNnTVsV DIYQGGVILG>
BovNramp1   AVFGQAFYKQ TNQAAFNICA DSSLHDYAPI FPRNNLTVAV DIYQGGVILG 350

NRAMP1      CLFGPaALYI WAiGLLAAGQ SSTMTGTYAG QFVMEGFLrL RWSRFARVLL>
Nramp1      CLFGPaALYI WAVGLLAAGQ SSTMTGTYAG QFVMEGFLKL RWSRFARVLL>
BovNramp1   CLFGPPALYI WAVGLLAAGQ SSTMTGTYAG QFVMEGFLKL RWSRFARVLL 400
                    TM8              Transport motif
NRAMP1      TRSCAILPTV LvAVFRDLRD LSGLNDLLNV LQSLLLPFAV LPILTFTSMP>
Nramp1      TRSCAILPTV LvAVFRDLkD LSGLNDLLNV LQSLLLPFAV LPILTFTSMP>
BovNramp1   TRSCAILPTV LLAVFRDLRD LSGLNDLLNV LQSLLLPFAV LPILTFTSMP 450
              TM9                                    TM10
NRAMP1      tLMQEFANGL lnKVvTSSIM VLVCAiNLYF VvSYLPSLPH PAYFgLaALL>
Nramp1      AvMQEFANGr mSKaITScIM aLVCAiNLYF VISYLPSLPH PAYFgLVALf>
BovNramp1   ALMQEFANGL VSKVITSSIM VLVCAVNLYF VISYLPSLPH PAYFSLVALL 500
                                   TM11
NRAMP1      AAAYLGLsTY LVWTCclahG ATpLAHSSHh hFLYGLlEED Q-KGeTSG>
Nramp1      AigYLGLTaY LaWTCcIahG ATfLtHSSHk hFLYGLPnEe QggvqgSG>
BovNramp1   AAAYLGLTTY LVWTCLITQG ATLLAHSSHQ RFLYGLPEED QEKGRTSG   548
              TM12
```

FIG. 2B

FIG. 3A

Association of bovine *NRAMP1* SSCA polymorphism with bovine resistant and susceptible phenotypes in unrelated individuals

	Resistant Phenotype	Susceptible Phenotype
Resistant Alleles (SSCPr)	9*	2
Susceptible Alleles (SSCPs)	2	9
Number of Cattle	11	11

*Significant Association - $p = 0.0089$ (Fisher's Exact Analysis), RR = 4.5±1.69% (99% CI).

FIG. 3B

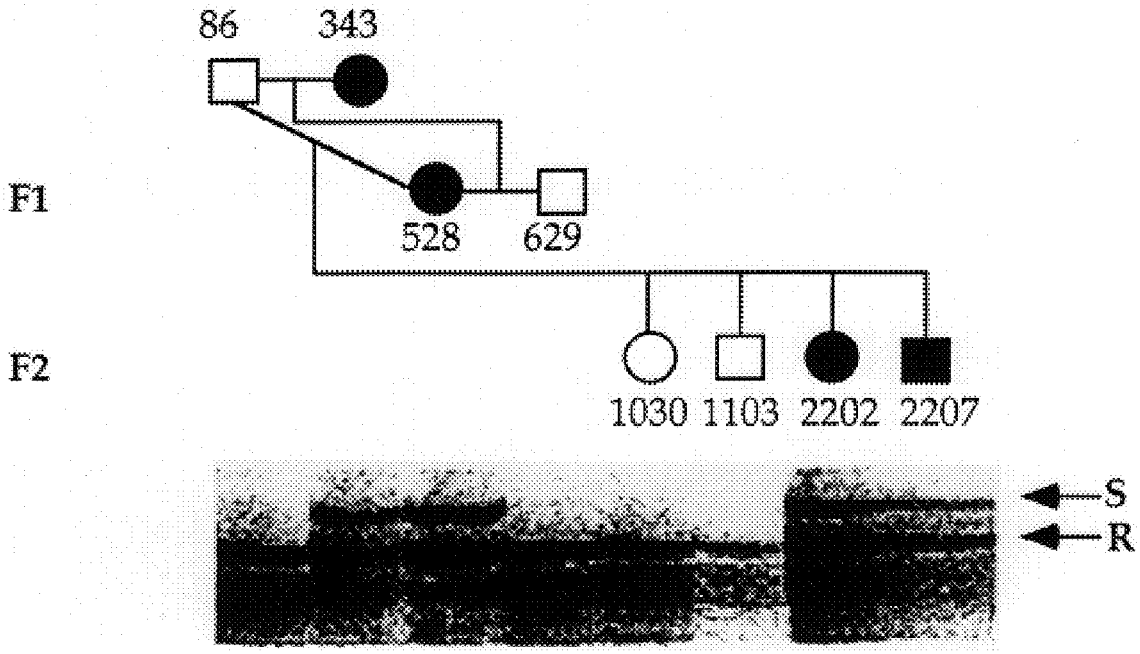
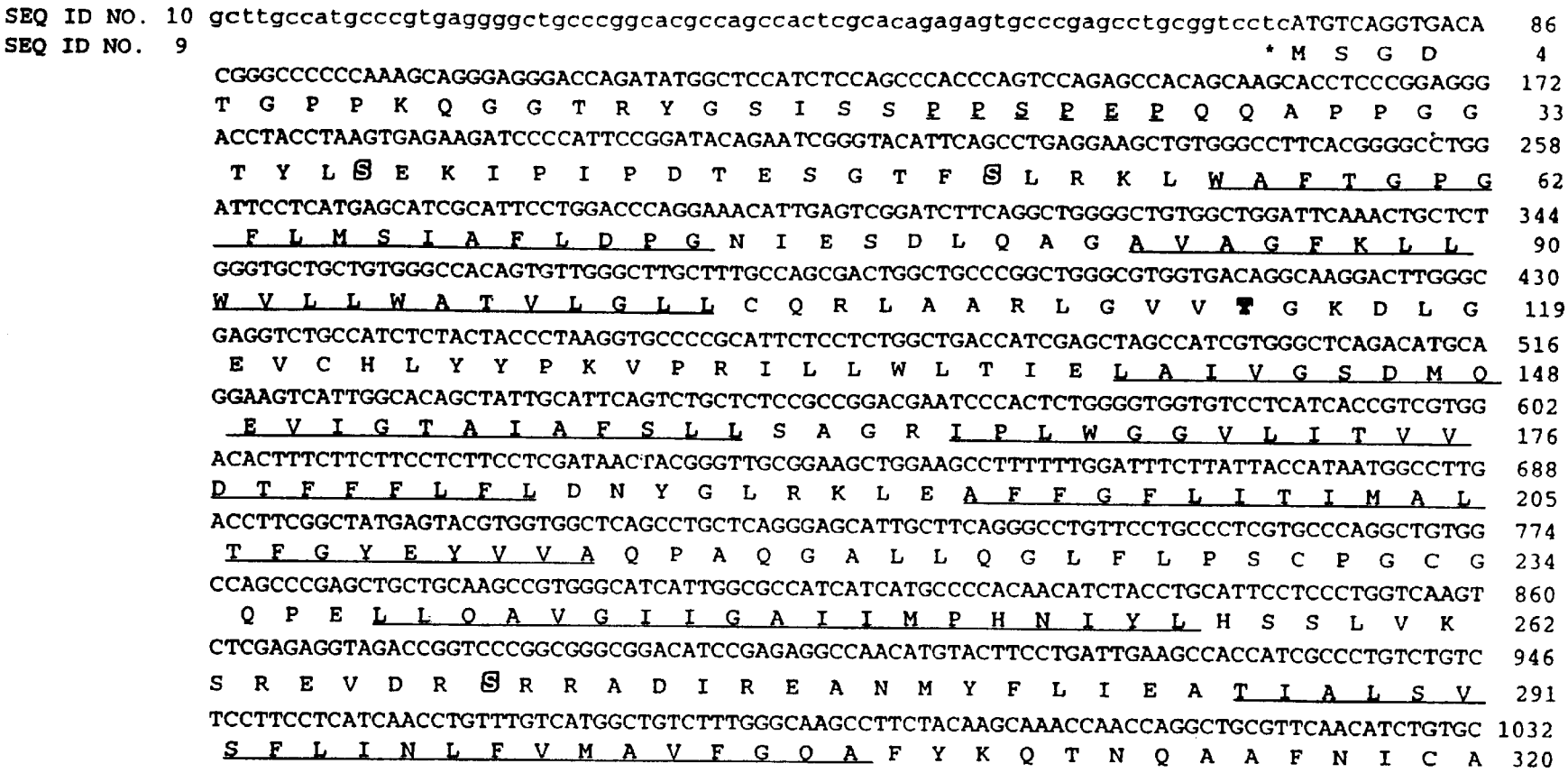


FIG. 3C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SEQ ID NO. 9 * M S G D 4
CGGGCCCCCCAAAGCAGGGAGGGACCAGATATGGCTCCATCTCCAGCCCACCCAGTCCAGAGCCACAGCAAGCACCTCCCGGAGGG 172
T G P P K Q G G T R Y G S I S S P P S P E P Q Q A P P G G 33
ACCTACCTAAGTGAGAAGATCCCCATTCCGGATACAGAATCGGGTACATTCAGCCTGAGGAAGCTGTGGGCCTTCACGGGGCCTGG 258
T Y L S E K I P I P D T E S G T F S L R K L W A F T G P G 62
ATTCCTCATGAGCATCGCATTCCTGGACCCAGGAAACATTGAGTCGGATCTTCAGGCTGGGGCTGTGGCTGGATTCAAACTGCTCT 344
F L M S I A F L D P G N I E S D L Q A G A V A G F K L L 90
GGGTGCTGCTGTGGGCCACAGTGTTGGGCTTGCTTTGCCAGCGACTGGCTGCCCGGCTGGGCGTGGTGACAGGCAAGGACTTGGGC 430
W V L L W A T V L G L L C Q R L A A R L G V V T G K D L G 119
GAGGTCTGCCATCTCTACTACCCTAAGGTGCCCCGCATTCTCCTCTGGCTGACCATCGAGCTAGCCATCGTGGGCTCAGACATGCA 516
E V C H L Y Y P K V P R I L L W L T I E L A I V G S D M Q 148
GGAAGTCATTGGCACAGCTATTGCATTCAGTCTGCTCTCCGCCGGACGAATCCCACTCTGGGGTGGTGTCCTCATCACCGTCGTGG 602
E V I G T A I A F S L L S A G R I P L W G G V L I T V V 176
ACACTTTCTTCTTCCTCTTCCTCGATAACTACGGGTTGCGGAAGCTGGAAGCCTTTTTTGGATTTCTTATTACCATAATGGCCTTG 688
D T F F F L F L D N Y G L R K L E A F F G F L I T I M A L 205
ACCTTCGGCTATGAGTACGTGGTGGCTCAGCCTGCTCAGGGAGCATTGCTTCAGGGCCTGTTCCTGCCCTCGTGCCCAGGCTGTGG 774
T F G Y E Y V V A Q P A Q G A L L Q G L F L P S C P G C G 234
CCAGCCCGAGCTGCTGCAAGCCGTGGGCATCATTGGCGCCATCATCATGCCCCACAACATCTACCTGCATTCCTCCCTGGTCAAGT 860
Q P E L L Q A V G I I G A I I M P H N I Y L H S S L V K 262
CTCGAGAGGTAGACCGGTCCCGGCGGGCGGACATCCGAGAGGCCAACATGTACTTCCTGATTGAAGCCACCATCGCCCTGTCTGTC 946
S R E V D R S R R A D I R E A N M Y F L I E A T I A L S V 291
TCCTTCCTCATCAACCTGTTTGTCATGGCTGTCTTTGGGCAAGCCTTCTACAAGCAAACCAACCAGGCTGCGTTCAACATCTGTGC 1032
S F L I N L F V M A V F G Q A F Y K Q T N Q A A F N I C A 320

FIG. 4A

```
CGACAGCAGCCTCCACGACTACGCGCCGATCTTTCCCAGGAACAACCTGACCGTGGCAGTGGACATTTACCAAGGAGGCGTGATCC 1118
  D  S  S  L  H  D  Y  A  P  I  F  P  R  N  N  L  T  V  A  V  D  I  Y  Q  G  G  V  I    348
TGGGCTGCCTCTTTGGTCCTCCAGCCCTGTACATCTGGGCCGTGGGTCTCCTGGCTGCTGGGCAGAGCTCCACCATGACCGGCACC 1204
L  G  C  L  F  G  P  P  A  L  Y  I  W  A  V  G  L  L  A  A  G  Q  S  S  T  M  T  G  T    377
TACGCGGGACAGTTTGTGATGGAGGGCTTCCTGAAGCTGCGGTGGTCACGCTTCGCCCGAGTCCTGCTCACTCGCTCCTGCGCCAT 1290
Y  A  G  Q  F  V  M  E  G  F  L  K  L  R  W  S  R  F  A  R  V  L  L  T  R  S  C  A  I    406
CCTGCCCACTGTGCTCCTGGCTGTCTTCAGGGACTTGCGGGACCTGTCAGGCCTCAACGACCTGCTCAATGTGCTGCAGAGCCTGC 1376
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TGCTTCCCTTCGCTGTGCTGCCCATCCTCACCTTCACCAGCATGCCCGCCCTGATGCAGGAGTTTGCCAATGGCCTGGTGAGCAAA 1462
L  L  P  F  A  V  L  P  I  L  T  F  T  S  M  P  A  L  M  Q  E  F  A  N  G  L  V  S  K    463
GTTATCACTTCCTCCATCATGGTGCTGGTCTGCGCCGTCAACCTTTACTTCGTGATCAGCTACTTGCCCAGCCTCCCCCACCCTGC 1548
V  I  T  S  S  I  M  V  L  V  C  A  V  N  L  Y  F  V  I  S  Y  L  P  S  L  P  H  P  A    492
CTACTTCAGCCTTGTAGCACTGCTGGCCGCAGCCTACCTGGGCCTCACCACTTACCTGGTCTGGACCTGTCTCATCACCCAGGGAG 1634
  Y  F  S  L  V  A  L  L  A  A  A  Y  L  G  L  T  T  Y  L  V  W  T  C  L  I  T  Q  G    520
CCACTCTTCTGGCCCACAGTTCCCACCAACGCTTCCTGTATGGGCTTCCTGAAGAGGATCAGGAGAAGGGGAGGACCTCGGGATGA 1720
A  T  L  L  A  H  S  S  H  Q  R  F  L  Y  G  L  P  E  E  D  Q  E  K  G  R  T  S  G  *    548
gctcccaccagggcctggccacgggtggaatgagtgggcacagtggcctgtcagacaagggtgtgtgtgtgtgtgtgtgtgtgtat 1806
gtgtgtgaaggcagcaagacagacagggagttctggaagctggccaacgtgagttccagagggacctgtgtgtgtgtgacacactg 1892
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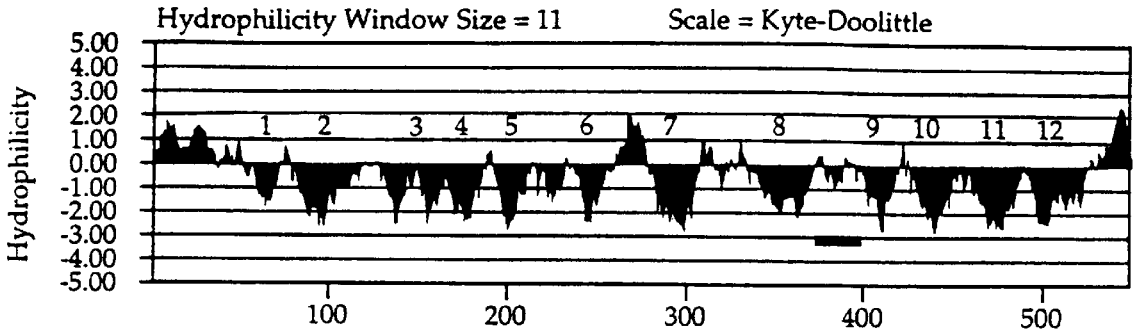
FIG. 4B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Scale = Kyte-Doolittle
Hydrophilicity
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
1 2 3 4 5 6 7 8 9 10 11 12
100
200
300
400
500

FIG. 5

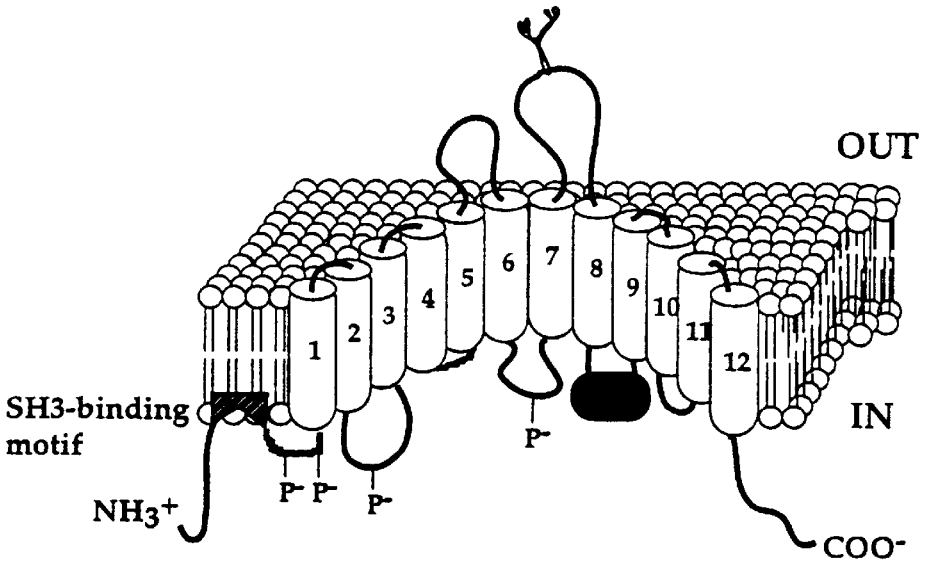

OUT
IN
SH3-binding motif
NH3+
COO-
P-
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 6

Sequence Differences in Naturally Susceptible (S) and Resistant (R) Bovine at the 3' Untranslated region of Bovine NRAMP1

Phenotype	Nucleotides (starting at position 1779)	SEQ ID NO.
R	GGGTGTGTGTGTGTGTGTGTGTGTATGTGTGT GAAGGCAGCAAGACAGACAGGGAGTTCTGGAAGC TGGCCAACGTGAGTTCCAGAGGGACCTGTGTGTG TGTGACACACTGGCCTGCCAGACAAGGGTGTGTG TGTGTGTGTGTGTGTGTGT	SEQ ID NO. 11
S	GGGGGTGTGTGTGTGTGTGTATGTGTGTGAAGGC AGCAAGACAGACAGGGAGTTCTGGAAGCTGGCCA ACGTGAGTTCCAGAGGGACCTGTGTGTGTGTGAC ACACTGGCCTGCCAGACAAGGGTGTGTGTGTGTG TGTGTGTGTGTGTGTGTGT	SEQ ID NO. 12
S	GGGGGTGTGTGTGTGTGTGTGTATGTGTGTGAAG GCAGCAAGACAGACAGGGAGTTCTGGAAGCTGGC CAACGTGAGTTCCAGAGGGACCTGTGTGTGTGTG ACACACTGGCCTGCCAGACAAGGGTGTGTGTGTG TGTGTGTGTGTGTGTGTGT	SEQ ID NO. 13
S	GGGGGTGTGTGTGTGTGTGTGTGTATGTGTGTGA AGGCAGCAAGACAGACAGGGAGTTCTGGAAGCTG GCCAACGTGAGTTCCAGAGGGACCTGTGTGTGTG TGACACACTGGCCTGCCAGACAAGGGTGTGTGTG TGTGTGTGTGTGTGTGTGT	SEQ ID NO. 14

FIG. 7A

Generalized Sequence of Bovine NRAMP1 at the 3' Untranslated region associated with resistance:

SEQ ID NO.: 15 $GGGTGT(GT)_{10}AT(GT)_3(N)_{61}(GT)_5(N)_{24}(GT)_{13}$

FIG. 7B

Generalized Sequence of Bovine NRAMP1 at the 3' Untranslated region associated with susceptiblity:

$GGGGGT(GT)_{<10}AT(GT)_3(N)_{<61}(GT)_5(N)_{<24}(GT)_{>13}$

FIG. 7C

```
                                10         20         30         40         50         60
Bos spp.                .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAG GGGAGGACCT
Bison bison             .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAG GGGAGGACCT
Odocoileus virginianus  .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAG GGGAGGACCT
Capra hirus             ....CACCAA CGCTTCCTGT ATGGGCTTCC TGGAGAGGAT CA.GGAGGAG GGGAGGACCT
Alces alces             .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAG GGGAGGACCT
Cervus canadensis       .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CAGGGAGAAT GGGAGGACCT
Cervus elaphus          .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAT GGGAGGACCT
Dama dama               .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAT GGGAGGACCT
Elaphurus davidianus    .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAAT GGGAGGACCT
Ursus spp.              .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAGG GGGAGGACCT
Sus scrofa              .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGAAGAGGAT CA.GGAGAGG GGGAGGACCT
Oreamnos americanus     .TCCCACCAA CGCTTCCTGT ATGGGCTTCC TGGAGAGGAT CA.GGAGGAG GGGAGGACCT

                                70         80         90        100        110        120
Bos spp.                CGGGATGAGC TCCCACCAGG GCCTGGCCAC GGGTGGAATG AGTGGGCACA GTGGCCTGTC
Bison bison             CGGGATGAGC TCCCACCAGG GCCTGGCCAC GGGTGGAATG AGTGGGCACA GTGGCCTGTC
Odocoileus virginianus  CGGGATGAGC TCCCACCAGG GCCTGGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGTC
Capra hirus             CGGGATGAAC TCCCACCAGG GCCTGGCCAC GGGTGGGATG AGTGACCACA GTGGCCTGCC
Alces alces             CAGGATGAGC TCCCACCAGG GCCTGGCCAC GGGTGGAATG AGTGGGCACA GTGGCCTGCC
Cervus canadensis       CAGGATGAGC TCCCACCAGG ACCCTGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGCC
Cervus elaphus          CGGGATGAGC TCCCACCAGG ACCCGGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGCC
Dama dama               CGGGATGAGC TCCCACCAGG ACCCGGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGCC
Elaphurus davidianus    CGGGATGAGC TCCCACCAGG GCCCGGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGCC
Ursus spp.              CAGGATGAGC TCCCACCAGG GCCTGGCCAC GGGTGGGATG AGTGGGCACA GTGGCCTGCC
Sus scrofa              CGGGATGAGC TCCCACCAGG GCCTGGCCAC AGGTGGGATG AGTGGGCACA GTGGCTTGCC
Oreamnos americanus     CGGGATGAAC TCCCACCAGG GCCCGGCCAC GGGTGGGATG AGTGACCACA GTGGCCTGCC
```

FIG. 8A-1

```
                          130         140         150         160         170         180
Bos spp.                  AGACAAGGGT GTGTGTGTGT GTGTGTGTGT GTATGTGTGT GAAGGCAGCA AGACAGACAG
Bison bison               AGACAAGGGT GTGTGTGTGT GTGTGTGTGT GTATGTGTGT GAAGGCAGCA AGACAGACAG
Odocoileus virginianus    AGACAAAG.. GGGTGTGTGT GTGTGTGTGT GTATGTGTGC GAAGGCAGCA AGACAGACAG
Capra hirus               AGACAAGG.. ....GTGTGT GTGTGTGTGT GTGTGTGTG. .......... ..........
Alces alces               AGACAAGGGT GTGTGTGTGT ATGTGTGTGT GTGTGTGTG. .......... ..........
Cervus canadensis         AGACAAGGGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTG. .......... ..........
Cervus elaphus            AGACAAGGGT GTGTGTGTGT GTGTGTGTGT GTGTGTGTG. .......... ..........
Dama dama                 AGACAAGG.. GTGTGTGTGT GTGTGTGTGT GTGTGTGTG. .......... ..........
Elaphurus davidianus      AGACAAGGGG GTGTGTGTGT GTGTGT.... .......... .......... ..........
Ursus spp.                AGACAAGGGT GTGTGTGT.. .......... .......... .......... ..........
Sus scrofa                AGACAAGGGT GTGTGTGT.. .......... .......... .......... ..........
Oreamnos americanus       AGACAAGGGT GTGTGTGTGT GTGTGTGTGT GTGTCTGTG. .......... ..........

                          190         200         210         220         230         240
Bos spp.                  GGAGTTCTGG AAGCTGGCCA ACGTGAGTTC CAGAGGGACC TGTGTGTGTG TGACACACTG
Bison bison               GGAGTTCTGG AAGCTGGCCA ACGTGAGTTC CAGAGGGACC TGTGTGTGTG TGACACACTG
Odocoileus virginianus    GGAGTTCTGG AAGCTGGCCA ACGTGAGTTC CAGAGGGACC TGTGTGTGTG TGACACACTG
Capra hirus               .......TGT GTGTGCATGC ACAGCAAGAT GGAGAGGGAG TTCACGGGTG GGATGAGTGG
Alces alces               .......TGT GTGTGTGC.. .......... .......... .......... ..........
Cervus canadensis         .......TGC GCGCGCGCGC GC........ .......... .......... ..........
Cervus elaphus            .......TGT GTGCGCGCGC GC........ .......... .......... ..........
Dama dama                 .......TGT GTGCGCGCGC GC........ .......... .......... ..........
Elaphurus davidianus      .......... ......GCAC GC........ .......... .......... ..........
Ursus spp.                .......... .......... .......... .......... .......... ..........
Sus scrofa                .......... .......... .......... .......... .......... ..........
Oreamnos americanus       .......TGT GTGCGCGCGC A......... .......... .......... ..........
```

FIG. 8A-2

	250	260	270	280	290	300
Bos spp.	GCCTGCCAGA	CAAGGGTGTG	TGTGTGTGTG	TGTGTGTGTG	TGCATGCACA	GCAAGACGGA
Bison bison	GCCTGCCAGA	CAAGGGTGTG	TGTGTGTGTG	TGTGTGTGTG	TGCATGCACA	GCAAGACGGA
Odocoileus virginianus	GCCTGCCAGA	CAAAG..GTG	TGTGTGTGTG	TGTGTGTGTG	TGCATGCACA	GCAAGACGGA
Capra hirus	GCACAGTGGC	CTGCCAGACA	AGG....GTG	TGTGTGTGTG	TGCACGCACA	GCAAGATGGA
Alces alces	.....	.....	.....	.....GCG	CTCACCCACA	ACAAGACGGA
Cervus canadensis	.....	.....	.....	.....GAGCG	CTCACACACA	GCAAGACAGA
Cervus elaphus	.....	.....	.....	.....GCG	CTCACACACA	GCAAGACAGA
Dama dama	.....	.....	.....	.....GCG	CTCACACACA	GCAAGACAGA
Elaphurus davidianus	.....	.....	.....	.....GCGCG	CTCACACACA	GCAAGACAGA
Ursus spp.	.....	.....	.....	.....G	GTCACCCACA	GCAAGACGGA
Sus scrofa	.....	.....	.....	.....G	GTCACCCACA	GCAAGACGGA
Oreamnos americanus	.....	.....	.....	.....	.....CACA	GCAAGATGGA

	310	320	330	340	350	360
Bos spp.	GAGGGAGTTC	TGGAAGGCAG	CCAACGTGAG	TTCCATAGGG	ACCTGCTATT	TCCTAGCTCA
Bison bison	GAGGGAGTTC	TGGAAGGCAG	CCAACGTGAG	TTCCATAGGG	ACCTGCTATT	TCCTAGCTCA
Odocoileus virginianus	GAGGGAGTTC	TGGAAGGCAG	CCAACGTGAG	TTCCATAGGG	ACCTGCTATT	TCCTAGCTCA
Capra hirus	CAGGGAATTT	TGGAAGCCGG	CCAA.....	.GCCATAGGG	ACCTGCTATT	TCCTAGCTCA
Alces alces	GAGGGAGTTC	TGGAAGCCGG	ACAACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Cervus canadensis	GAGGGAGTTC	TGGAAGCCGG	ACGACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Cervus elaphus	GAGGGAGTTC	TGGAAGCAGG	ACGACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Dama dama	GAGGGAGTTC	CGGAAGCCGG	ACGACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Elaphurus davidianus	GAGGGAGTTC	TGGAAGCAGG	ACGACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Ursus spp.	GAGGGAGTTC	TGGAAGCCGG	ACAACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Sus scrofa	GAGGGAGTTC	TGGAAGCCGG	ACAACGTGAG	TTCCATAGGG	ACCTGCTGTT	TCCTAGCTCA
Oreamnos americanus	GAGGGAATTC	TGGAAGCCGG	CCAA.....	.GCCATAGGA	GCCTGCTATT	TCCTAGCTCA

FIG. 8A-3

	370	380	390	400	410	420	
Bos spp.	GATCTCAGTG	TTCTTGACTA	TAAAATGGGG	ACACCTACCT	TGGAGTGGTT	GTAAATAAGA	SEQ ID NO. 16
Bison bison	GATCTCAGTG	TTCTTGACTA	TAAAATGGGG	ACACCTACCT	TGGAGTGGTT	GTAAATAAGA	SEQ ID NO. 17
Odocoileus virginianus	GATCTCAGTG	TTCTTGACTA	TAAAATGGGG	ACACCCACCT	TGGAGTGGTT	GTTAATAAGA	SEQ ID NO. 18
Capra hirus	GATCTCGGTA	TTCTTGAGTA	TTAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 19
Alces alces	GATCTCAGTG	TTCTTGATTA	TAAAATGGGG	ACACCTACCT	TGCAACGGTT	GTAAATAAGA	SEQ ID NO. 20
Cervus canadensis	TTCTTCACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	GTAAATAAGA	SEQ ID NO. 21
Cervus elaphus	GATCTCAGTG	TTCTTCACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 22
Dama dama	GATCTCAGTG	TTCTTCACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 23
Elaphurus davidianus	GATCTCAGTG	TTCTTCACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 24
Ursus spp.	GATCTCAGTG	TTCTTGACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 25
Sus scrofa	GATCTCAGTG	TTCTTGACTA	TAAAATGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 26
Oreamnos americanus	GATCTTGGTA	TTCTTGAGTA	TTAACTGGGG	ACACCTACCT	TGCAATGGTT	GTAAATAAGA	SEQ ID NO. 27

	430	440	450	
Bos spp.	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Bison bison	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Odocoileus virginianus	CAC..TTGAA	CGCAGAACCT	AGCACCTCAG	ATT
Capra hirus	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Alces alces	CACATTGGAA	CGCAGAGGCT	AGCACTTCAG	ATT
Cervus canadensis	CAC..TTGAA	TGCAGAGCCT	AGCACTTCAG	ATT
Cervus elaphus	CAC..TTGAA	TGCAGAGCCT	AGCACTTCAG	ATT
Dama dama	CAC..TTGAA	TGCAGAGCCT	AGCACTTCAG	ATT
Elaphurus davidianus	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Ursus spp.	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Sus scrofa	CAC..TAGAA	CGCAGAGCCT	AGCACTTCAG	ATT
Oreamnos americanus	CAC..TTGAA	CGCAGAGCCT	AGCACTTCAG	ATT

FIG. 8A-4

SEQ ID NO. 9
SEQ ID NO. 28

```
                                        pts
                                         |
BovNramp1       MSGDTGPPKQ GGTRYGSISS PPSPEPQQAP PGGTYLSEKI PIPDTESGTF
BisNramp1       MSGDTGPPKQ GGTRYGSISS PPSPEPQQAP PGGTYLSEKI PIPDTESGTF  50
                           &&&&&&&              PKC

BovNramp1       SLRKLWAFTG PGFLMSIAFL DPGNIESDLQ AGAVAGFKLL WVLLWATVLG
BisNramp1       SLRKLWAFTG PGFLMSIAFL DPGNIESDLQ AGAVAGFKLL WVLLWATVLG 100
                PKC          TM1                   TM2

BovNramp1       LLCQRLAARL GVVTGKDLGE VCHLYYPKVP RILLWLTIEL AIVGSDMQEV
BisNramp1       LLCQRLAARL GVVTGKDLGE VCHLYYPKVP RILLWLTIEL AIVGSDMQEV 150
                                                      TM3

BovNramp1       IGTAIAFSLL SAGRIPLWGG VLITVVDTFF FLFLDNYGLR KLEAFFGFLI
BisNramp1       IGTAIAFSLL SAGRIPLWGG VLITiVDaFF FLFLDNYGLR KLEAFFGFLI 200
                           TM4

BovNramp1       TIMALTFGYE YVVAQPAQGA LLQGLFLPSC PGCGQPELLQ AVGIIGAIIM
BisNramp1       TIMALTFGYE YVVAQPAQGA LLQGLFLPSC PGCGQPELLQ AVGIIGAIIM 250
                TM5
```

FIG. 8B-1

```
BovNramp1    PHNIYLHSSL VKSREVDRSR RADIREANMY FLIEATIALS VSFLINLFVM
BisNramp1    PHNIYLHSSL VKSREVDRSR RADIREANMY FLIEATIALS VSFLINLFVM 300
               TM6                                    TM7

BovNramp1    AVFGQAFYKQ TNQAAFNICA DSSLHDYAPI FPRNNLTVAV DIYQGGVILG
BisNramp1    AVFGQAFYKQ TNQAAFNICA nSSLqDYAPI FPRNNLTVAV DIYQGGVILG 350
                                                @@@

BovNramp1    CLFGPPALYI WAVGLLAAGQ SSTMTGTYAG QFVMEGFLKL RWSRFARVLL
BisNramp1    CLFGPaALYI WAVGLLAAGQ SSTMTGTYAG QFVMEGFLKL RWSRFARVLL 400
                       TM8                Transport motif

BovNramp1    TRSCAILPTV LLAVFRDLRD LSGLNDLLNV LQSLLLPFAV LPILTFTSMP
BisNramp1    TRSCAILPTV LLAVFRDLRD LSGLNDLLNV LQSLLLPFAV LPILTFTSMP 450
               TM9                                    TM10

BovNramp1    ALMQEFANGL VSKVITSSIM VLVCAVNLYF VISYLPSLPH PAYFSLVALL
BisNramp1    ALMrEFANGL VSKVITSSIM VLVCAVNLYF VISYvPSLPH PAYFSLVALL 500
                                   TM11

BovNramp1    AAAYLGLTTY LVWTCLITQG ATLLAHSSHQ RFLYGLPEED QEKGRTSG
BisNramp1    AAAYLGLTTY LVWTCLITQG ATLLAHSSHQ RFLYGLPEED QEKGRTSG   548
               TM12
```

FIG. 8B-2

Examples of the Length and Pattern of Microsatellites for Several Species of Mammals Using Bovine Nramp1 3' end Primer Pair F1655/3 - end2 for Amplification

Species of Mammals	Length (base pairs)	Number of Microsatellites	Patterns
Cattle	448bp	3	(GT)12..(GT)5..(GT)14
American Bison	448bp	3	(GT)12..(GT)5..(GT)14
White-tailed Deer	446bp	3	(GT)9..(GT)6..(GT)12
Red Deer	347bp	2	(GT)18(GC)7
Fallow Deer	345bp	2	(GT)17(GC)7
Elk	349bp	2	(GT)16(GC)7
Pere David's Deer	327bp	1	(GT)9..(CG)4
Moose	345bp	1	(GT)14TA(GT)5
Goat	402bp	2	(GT)7..(GT)16
Mountain Goat	329bp	1	(GT)12CT(GT)4
Dall Sheep	416bp	2	(GT)19..(GT)9
Caribou	309bp	1	(GT)5
Reindeer	309bp	1	(GT)5
Pig	309bp	1	(GT)5
Barasinga Cervus	309bp	1	(GT)5
Samber Cervus	309bp	1	(GT)5
Black Bear	309bp	1	(GT)5
Grizzly Bear	309bp	1	(GT)5
Polar Bear	309bp	1	(GT)5
Red Mazama	307bp	1	(GT)5
Elephant	309bp	1	(GT)5

FIG. 8C

3' Untranslated Sequence of Bison NRAMP1 in Resistant Bison

SEQ ID NO 29 Fmicro

```
     1676-GGGCTTCCTGAAGAGGATCAGGAGAAGGGGAGGACCTCG        1714
           G  L  P  E  E  D  Q  E  K  G  R  T  S
GGGATGagctcccaccagggcctggccacgggtgggatgagtgggcacag      1763
 G  @
tggcctgtcagacaagggtgtgtgtgtgtgtgtgtgtgtgtgaa          1809
                  (TG)13
Bmicro1'
ggcagcaagacagagacggagttctggaagctggccaacgtgagttccag      1859

agggacctgtgtgtgtgtgtgtgacacactggcctgccagacaaggg        1906
          (TG)8
tgtgtgtgtgtgtgtgtgtgtgtgtgtgtgcatgcacagcaag           1951
(TG)16
acagagagggagttctggaagccagccaacgtgagttccatagggacctg    2001
ctatttcctagctcagatctcagtgttcttgactataaaatggggacacc    2051
taccttggaatggttgtaaataagacacttgaacgcagagcctagcactt    2101
cagatttaaaaacaaaagaatcataattccaaaagttactgagcactatc    2151
acaggagtgacctgacagacccacccagtccagggtgggacccaggctcc    2201
aaactgatttaaaataagagtctgaaaatgctaaataaatgctgttgtgc    2251
ttagtccccg      2261
```

3' Untranslated Sequence of Bison NRAMP1 in Susceptible Bison

SEQ ID NO. 30 Fmicro

```
     1676-GGGCTTCCTGAAGAGGATCAGGAGAAGGGGAGGACCTCG        1714
           G  L  P  E  E  D  Q  E  K  G  R  T  S
GGGATGagctcccaccagggcctggccacgggtgggatgagtgggcacag      1763
 G  @
tggcctgtcagacaagggtgtgtgtgtgtgtgtgtgtgtgtgaa          1807
                  (TG)12
Bmicro1'
ggcagcaagacagagacggagttctggaagctggccaacgtgagttccag      1857
agggacctgtgtgtgtgtgtgtgacacactggcctgccagacaaggg        1904
        (TG)8
tgtgtgtgtgtgtgtgtgtgtgtgtgtgtgcatgcacagcaag           1949
(TG)16
acagagagggagttctggaagccagccaacgtgagttccatagggacctg    1999
ctatttcctagctcagatctcagtgttcttgactataaaatggggacacc    2049
taccttggaatggttgtaaataagacacttgaacgcagagcctagcactt    2099
cagatttaaaaacaaaagaatcataattccaaaagttactgagcactatc    2149
acaggagtgacctgacagacccacccagtccagggtgggacccaggctcc    2199
aaactgatttaaaataagagtctgaaaatgctaaataaatgctgttgtgc    2249
ttagtccccg      2259
```

FIG. 8D

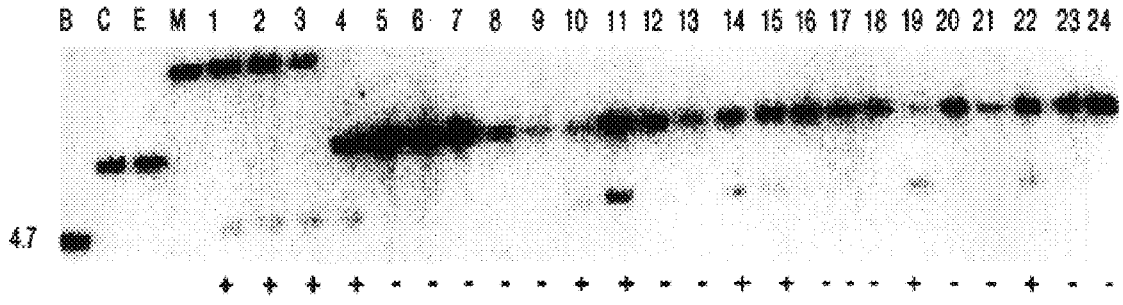
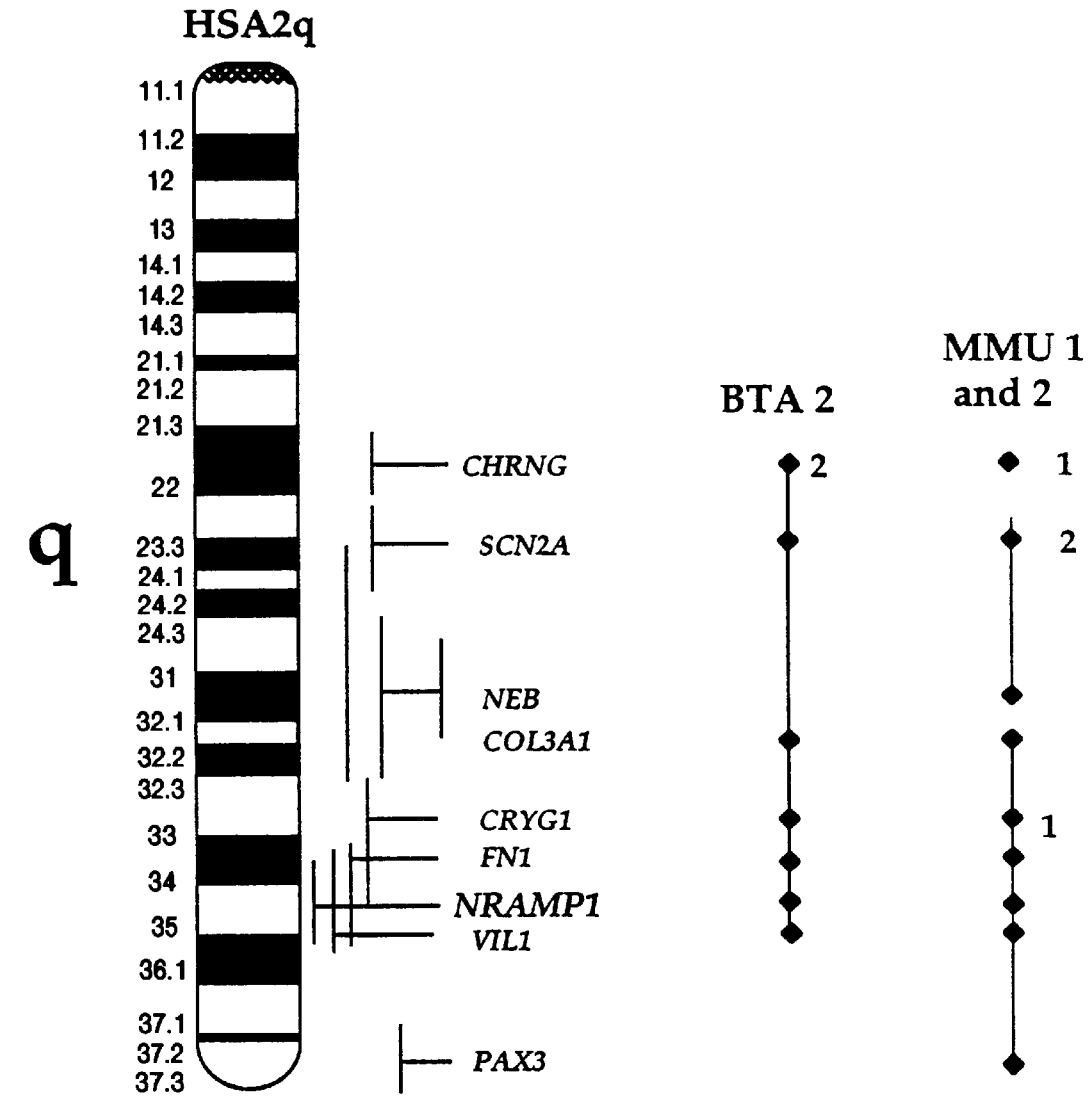


FIG. 9



Denotes mapped in three species, bars connect loci that are syntenic on indicated chromosomes in corresponding species

FIG. 10

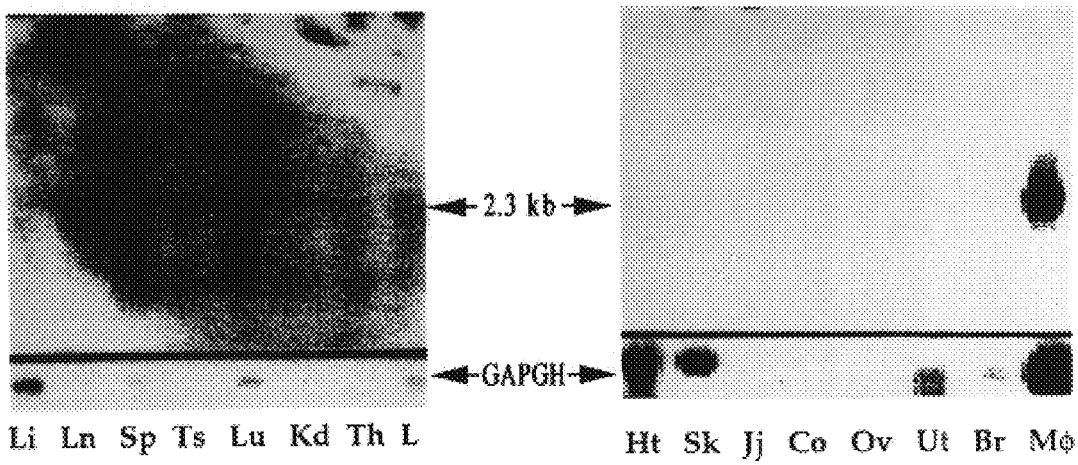


FIG. 11

METHOD OF IDENTIFICATION OF ANIMALS RESISTANT OR SUSCEPTIBLE TO DISEASE SUCH AS RUMINANT BRUCELLOSIS, TUBERCULOSIS, PARATUBERCULOSIS AND SALMONELLOSIS

This application claims priority of U.S. Provisional Application Ser. No. 60/031,443, filed Sep. 20, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method for identifying animals that are resistant or susceptible to diseases associated with intracellular parasites. More particularly, the present invention relates to the identification of a gene, called NRAMP1, associated with the susceptibility or resistance of an animal, such as an artiodactyla, to diseases such as brucellosis, tuberculosis, paratuberculosis and salmonellosis. Still more particularly, the present invention relates to the identification of specific sequences of the 3' untranslated region (3' UTR) of bovine NRAMP1 which associate with resistance or susceptibility to bovine brucellosis, tuberculosis, paratuberculosis and salmonellosis, and to the use of the general sequence patterns to identify artiodactyl animals containing those sequences in situ, allowing therefore the identification of animals predicted to be either resistant or susceptible to diseases associated with intracellular parasites.

2. General Background

Intracellular zoonotic bacterial diseases like brucellosis and tuberculosis cause significant losses in livestock industries despite widespread application of antimicrobials, vaccination, isolation and quarantine, test and slaughter, or a combination of these. The lack of success in eradicating infectious diseases of animals using these approaches indicates a need for a different strategy, such as the development of a means to identify genetic sequences associated with resistance and/or susceptibility, where such means could allow the identification of animals that are resistant or susceptible to disease. This could then allow the treatment, prophylactic or therapeutic, or elimination of susceptible animals, and the use of and/or selective breeding of resistant animals (see, for example, Templeton et al. 1988).

Diseases such as ruminant brucellosis, tuberculosis, paratuberculosis and salmonellosis cause an estimated $250,000,000 loss annually to the U.S.A. beef and dairy industry. Further, tuberculosis especially is a health threat to all ungulates including rare and endangered mammals. These are diseases for which the usual eradication programs have been long-term, expensive, and somewhat unsuccessful. For example, bovine tuberculosis was thought to be a disease of antiquity in 1970 but has re-emerged as an endemic disease in the El Paso, Tex. dairy herds. Outbreaks of bovine tuberculosis have been reported in the past 5 years in California, Idaho, Indiana, Louisiana, Missouri, Montana, Nebraska, New Mexico, New York, North Carolina, Pennsylvania, South Carolina, Texas, Wisconsin, and Virginia (Essey and Koller 1994; and Essey M. A. 1991).

Further, each of these specific diseases are zoonotic diseases which continually threaten the U.S. population. The benefit of cattle naturally resistant to these, and other diseases would be a key component of the preharvest pathogen reduction programs like the National Hazard Analysis Critical Control Point (HACCP) program proposed for farm use (Pierson, M. D. and Corlett, D. A., 1992; and Vanderzant, C., 1985). Further, it is desired that the approach used to control these diseases use natural resistance since it is environmentally compatible.

The only method currently available for the detection of artiodactyla resistant to brucellosis or tuberculosis is by a potent in vivo challenge with virulent *Brucella abortus, Salmonella dublin, Mycobacterium paratuberculosis,* or *Mycobacterium bovis* (Templeton and Adams 1996). Unfortunately, for this assay, the tested ungulates have to be euthanized in order to culture for the specific pathogen. Males challenged with *B. abortus* or *M. bovis* must be necropsied and cultured to determine if the bacterium has been cleared (resistant) or persists (susceptible). Nonpregnant females challenged with *M. bovis* must be necropsied and cultured to determine resistance or susceptibility. Although the gametes from both males and females can be stored frozen and used in a breeding-selection program to produce naturally resistant progeny with some success, this is both extremely expensive, and inefficient. The viability of frozen gametes and embryos is variable and a much lower birth rate occurs than with natural matings. Additionally, the breeding-selection program would be based on phenotypic selection (so-called mass selection) which is not as efficient as determining genotypes and selecting resistance associated with genetic sequences directly. (See, for example, Martin et al. 1994; and Dietrich et al. 1986).

The present invention solves these prior art problems by providing an efficient and reliable method for determining whether an animal, such as an artiodactyla, is susceptible or resistant to diseases such as brucellosis, tuberculosis, paratuberculosis and salmonellosis.

SUMMARY OF THE PRESENT INVENTION

In this invention, we identify homologs of murine NRAMP1 from bovine, bison, and other artiodactyla and show that particular sequences of the 3' UTR of these NRAMP1 homologs have a highly significant association with resistance or susceptibility to diseases associated with bacterial pathogens.

More specifically, this invention relates to the discovery of distinct, naturally occurring sequences of bovine NRAMP1, where the presence of a particular sequence strongly correlates (P=0.0089) with either resistance or susceptibility to, inter alia, brucellosis, tuberculosis, paratuberculosis and salmonellosis in unrelated cattle.

The genetic sequences associated with artiodactyla NRAMP1 that statistically associate with either susceptibility or resistance involve a transversion at position 1782 of the NRAMP1 complementary (c) DNA and a polymorphic DNA microsatellite sequence difference; both of which are located in the 3' UTR. The sequence associated with resistance contains a thymine at position 1782 and a polymorphic microsatellite sequence beginning at position 1785 characterized by:

SEQ ID NO. 31: 5' $(GT)_{10}AT(GT)_3(N)_{61}(GT)_5(N)_{24}(GT)_{13}3'$

where "N" symbolizes any one of the four nucleotide bases A, C, G or T. In contrast, the sequences associated with susceptibility contain a guanine at position 1782 and a polymorphic DNA microsatellite region characterized by:

SEQ ID NO: 32: 5' $(GT)_{<10}AT(GT)_3(N)_{>61}(GT)_5(N)_{<24}(GT)_{>13}3'$

where "N" again symbolizes any one of the four nucleotide bases A, C, C or T.

These sequence differences in the 3' UTR of the NRAMP1 gene can be used to detect whether animals are

susceptible or resistant to disease. For example, by screening animals for the presence of sequences associated with susceptibility or resistance, one can easily and accurately predict the susceptibility or resistance of an animal to diseases such as brucellosis, tuberculosis, paratuberculosis, salmonellosis and other diseases associated with infections of macrophages.

Once identified, susceptible animals can be segregated, prophylactically or therapeutically treated, or sacrificed. Resistant animals, on the other hand, can be safely handled, used to produce food stuffs, and/or bred to produce disease resistant animals.

BRIEF DESCRIPTION OF THE DRAWINGS

For a further understanding of the nature and objects of the present invention, reference should be had to the following detailed description, taken in conjunction with the accompanying drawings, wherein:

FIG. **1A** shows the sequence of PCR primers useful in the detection of bovine NRAMP1 sequences associated with susceptibility and resistance to disease (SEQ ID NO.1 and SEQ ID NO. 2);

FIG. **1B** shows the sequences of primers used to clone bovine NRAMP1 (SEQ ID NO. 3, SEQ ID NO.4, SEQ ID NO. 5, and SEQ ID NO.6);

FIG. **2** shows the predicted amino acid sequence of bovine Nramp1 (SEQ ID NO. 7), and human (SEQ ID NO. 8), and murine (SEQ ID NO. 9) Nramp1 homologs and their alignment with each other;

FIG. **3A** shows the SSCP analysis of 22 unrelated cattle, phenotypically determined (by in vivo challenge and/or by an in vitro macrophage killing assay) to be either naturally resistant or susceptible to disease;

FIG. **3B** shows the tabular results of the experiment performed in FIG. **3A**;

FIG. **3C** shows the SSCP analysis and pedigree of naturally resistant bull sired to a naturally susceptible cattle and their progeny;

FIG. **4** shows the nucleotide sequence (SEQ ID NO. 10) and predicted amino acid sequence (SEQ ID NO. 9) of bovine NRAMP1;

FIG. **5** shows the hydrophobicity profile of the predicted amino acid sequence of bovine NRAMP1;

FIG. **6** shows a schematic representation of the putative structure of the bovine Nramp1 protein;

FIG. **7A** shows the sequences of bovine NRAMP1 associated with susceptibility and resistance to disease (SEQ ID NO. 11, 12, 13 and 14);

FIG. **7B** shows the generalized sequence of bovine NRAMP1 associated with resistance (SEQ ID NO. 15);

FIG. **7C** shows the generalized sequence of bovine NRAMP1 associated with susceptibility;

FIGS. **8A1** and **8A2** (SEQ ID NO.s 16–27) show the conserved amino acid sequence alignment at the 3' UTR of various ungulates;

FIG. **8B** shows an alignment of the amino acid sequences encoded by NRAMP1 of bovine (BovNramp1, SEQ ID NO. 9) and bison (BisNramp1, SEQ ID NO. 28);

FIG. **8C** shows the length and pattern of microsatellites for several species of mammals;

FIG. **8D** shows the 3' Untranslated Sequence of Bison NRAMP1 in Resistant (SEQ ID NO. 29) and Susceptible (SEQ ID NO. 30) bison;

FIG. **9** shows the genetic mapping of bovine NRAMP1 on BTA2;

FIG. **10** shows a representation of the conserved chromosomal segments among three species around the NRAMP1 locus; and

FIG. **11** shows a northern blot analysis of RNA isolated from bovine tissues and cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Genetic studies in mice have demonstrated that innate susceptibility to *Mycobacterium bovis* (BCG), *Leishmania donovani, Salmonella typhimurim* and several atypical mycobacteriae are controlled by a single gene on Mus Musculus (MMU) 1 autosome, called Bcg, Lsh, or Ity (Mock et al. 1990; Plant et al. 1982; Schurr et al. 1991; Goto et al. 1989; Skamene et al. 1984; de Chastellier et al. 1993; and Frelier et al. 1990). Bcg mediates antimicrobial activity of macrophages against intracellular parasites early during infection (Gros et al., 1983; Blackwell et al., 1991; Roach et al., 1994; Roach et al., 1991). Cattle which are naturally resistant (R) or susceptible (S) to brucellosis were identified by in vivo *Brucella abortus* challenge experiments (Harmon et al. 1985). Studies demonstrated that macrophages from resistant cattle were better able to control intracellular replication of *B. abortus* in an in vitro assay (Harmon et al. 1989; Price et al. 1990; Campbell et al. 1992). These observations were comparable to the differences in macrophage function between mice resistant and susceptible to *M. bovis*-BCG, *Salmonella typhimurium* and *L. donovani* controlled by the Bcg/Lsh/Ity gene(s) (Radzioch et al. 1991; Kramnik et al. 1994; Blackwell et al. 1994; Gros et al. 1983; Blackwell et al. 1991; Blackwell et al. 1994; Roach et al. 1991).

In mice, an approximately 30 cM segment on MMU1 (Mock et al. 1990; Skow et al. 1987; Malo et al. 1993) including Bcg was reported to be conserved on Homo sapiens autosome (HSA) 2q (Cellier et al. 1994; White et al. 1994) and Bos taurus autosome (BTA) 2 (Womack et al. 1986; Fries et al. 1993; Adkinson et al. 1988; Beever et al. 1994). Vidal and coworkers (Vidal et al. 1993) isolated a murine Bcg candidate gene, designated natural resistance associated macrophage protein (NRAMP1), that apparently encodes a polytopic integral membrane protein that has structural features similar to prokaryotic and eukaryotic transporters. Recent studies using knock-out mice have shown that NRAMP1 is the Bcg/Lsh/Ity gene. It is suggested that the murine Nramp1 protein might function in phagolysosomal membranes as a concentrator of oxidation products of nitric oxide, mediating cytocidal activity against the ingested parasites of infected macrophage (Vidal et al. 1993; Malo et al. 1994a; Cellier et al. 1994; Malo et al. 1994b).

In the present invention, a study was undertaken to determine if a bovine homolog of the murine NRAMP1 gene was expressed in bovine macrophages and involved in susceptibility of cattle to, for example, *B. Abortus*. Comparison of human, murine and bovine homologs of the bovine NRAMP1 gene product indicates a remarkable degree of homology (see, FIG. **2**). The bovine NRAMP1 cDNA encodes a protein with an overall predicted amino acid sequence homology of 86.9% and 88.6% to the human NRAMP1 and murine NRAMP1 gene products, respectively. Northern blot and RT-PCR analysis indicate that similar to the human and murine gene products, bovine NRAMP1 is principally expressed in the reticuloendothelia (RE) organs and macrophages (Vidal et al. 1993; Cellier et al. 1994; Gruenheid et al. 1995). All three homologs contain 12 potential membrane-spanning helical domains and sev-

eral functional sequence motifs including an N-terminal SH3-binding PNNP motif, a 20 amino acid transport motif, also known as the "binding-protein-dependent transport system inner membrane component signature" motif within the transmembrane (TM) 8–9 segment (Vidal et al., 1993; Malo et al., 1994a; Cellier et al., 1994; Malo et al., 1994b) four Protein Kinase C (PKC) phosphorylation sites; and one predicted N-linked glycosylation site (FIG. **2**). Additionally, very few substitutions in the Nramp1 protein appear to be tolerated in the membrane-spanning regions.

The bovine NRAMP1 has been mapped to BTA 2 within a group of syntenic loci conserved on HSA 2q and murine chromosome 1 overlapping the Lsh/Ity/Bcg locus (Adkinson et al. 1988; Beever et al. 1994; Cellier et al. 1994; White et al. 1994). Additionally, the interleukin-8 receptor is linked to bovine NRAMP1. The data presented herein further extends the large conserved synteny of bovine, human, and murine genes on these chromosomes. Taken altogether, these findings indicate that the observed collective properties have important structural and mechanistic roles in mediating Nramp1 function.

SSCA (single stranded conformational analysis) and SSCP (single stranded conformational polymorphism) analysis are two very similar techniques commonly used to detect differences in DNA sequences. SSCP tends to be slightly more sensitive; it can be used to detect single nucleotide differences between two sequences. SSCA is often used when detecting multiple sequence differences such as those occurring in microsatellite DNA sequence regions. In the present invention, both SSCA and SSCP analysis, along with direct DNA sequencing, were used to show that different sequences of bovine NRAMP1 associate with susceptibility or resistance to infection. The significant association of the bovine NRAMP1 conformational polymorphisms (i.e. the sequence variations) associated with natural resistance or susceptibility to bovine brucellosis, inter alia, strongly suggests that, although the inventors do not wish to be bound by theory, bovine NRAMP1 is the bovine Bcg homolog or is equally important as Bcg in regulating natural resistance to the intracellular parasites. In fact, the finding that sequence variants of bovine NRAMP1 associate with resistance or susceptibility strengthens the case for the proposed role of NRAMP1 in controlling natural resistance to brucellosis, salmonellosis, and tuberculosis in all artiodactyla.

Potential mechanisms for bovine NRAMP1 control over, or association with, resistance/susceptibility have been reviewed by others, which are incorporated herein by reference (Vidal et al. 1993; Cellier et al. 1994; Blackwell et al. 1994; Ivanyi et al. 1994; Vidal et al. 1995; Blackwell et al. 1995; Nathan 1995). Given the conservation of NRAMP1 genes in at least three species, it is likely that the fundamental function of the NRAMP1 homologs against the different intracellular pathogens, such as, but not limited to, Mycobacteriae, Brucellae, Salmonellae, and Leishmania is conserved and may be related to the level of killing by macrophages. The exact mechanism may vary with different pathogens and can include: transportation and production of nitrogen oxide; production of reactive nitrogen and oxygen intermediates; respiratory bursts and the hexose monophosphate shunt; SH3 and tyrosine kinase signal transduction; upregulation of MHC Class II expression; and interleulkin-1 production.

Given the complex structure and conservation of the predicted Nramp1 protein in three species, it would not be surprising if the proposed signaling and bactericidal mechanisms are involved in macrophage antimicrobial/parasite activity. While it is possible that the regulation of Nramp1 activity may be different in the various species, with the high degree of similarity between the species, it is more likely that the fundamental function of Nramp1 is conserved against the different intracellular pathogens, i.e. Mycobacteriae, Brucellae, Salmonellae, and Leishmania. Thus, the present invention which relates to the use of the discovered genetic variation in the NRAMP1 gene in selecting and breeding domestic and free-ranging artiodactyla that are naturally resistant to these important diseases could play a key role in preharvest pathogen reduction in the National Hazard Critical Control Point (HACCP) program for farm use (Pierson, M. D. and Corlett, D. A., 1992; and Vanderzant, C., 1985).

The mechanisms by which the sequence variations in the 3' UTR of NRAMP1 contribute to susceptibility or resistance to disease caused by infection by intracellular parasites is not precisely known. However, and while not intending to be bound by or to a particular theory, applicants suggest that the variations in the 3' UTR sequence could affect the translation of the bovine NRAMP1 message, with one sequence being transcribed more or less than the other. One possible mechanism by which this could occur could be selective ribosome instability on either the resistance-associated or susceptible-associated mRNA. This instability may result in a translation complex that is more likely to fall off the message of one sequence type than the other.

An embodiment of the instant invention therefore involves the identification, cloning and use of an artiodactyla gene associated with resistance and susceptibility to disease(s) involving intracellular parasites, such as brucellosis, tuberculosis, paratuberculosis and salmonellosis. More particularly, the present invention relates to the discovery that artiodactyla, and specifically ungulates, and more specifically, cattle, have a homolog (bovine NRAMP1) of the human and murine NRAMP1 gene.

The present invention discloses that this NRAMP1 gene has at least two differing sequences in the 3' untranslated region of the gene that significantly (P=0.0089) associate with either the resistance or susceptibility of an animal containing the sequence to at least the diseases brucellosis, tuberculosis, paratuberculosis and salmonellosis.

Still more particularly, the present invention shows that at least these two different NRAMP1 sequences can be readily differentiated by SSCA or SSCP analysis or any other technique suitable to detect a particular genetic sequence, for example, but not limited to direct sequencing, so that one can easily screen animals for the presence of either a resistance associated sequence or a susceptible associated sequence of NRAMP1.

This information as to whether an animal contains either a resistant associated sequence or a susceptible associated sequence can then be used to predict whether the screened animal is likely to be susceptible or resistant to diseases caused by intracellular parasites such as brucellosis, tuberculosis, paratuberculosis and salmonellosis. According to the screening results of the instant invention, susceptible animals can then be segregated, treated prophylactically or therapeutically, or sacrificed. Resistant animals, on the other hand, can then be safely raised, harvested and/or bred to create disease resistant animals.

Further, according to the present invention, animal breeding for disease resistance can be easily monitored by practicing the genetic screening methods of the invention in order to assay for the transmission of resistance to disease. Further still, the method of the present invention allows for

the selective breeding of disease resistant animals based upon the selective tracking of only a single genetic trait and the assaying of that that via genetic analysis, rather than phenotypic selection. This allows for the favorable trait to segregate and be traced independently, allowing for the selective tracing of the favorable genetic sequence, which can avoid unnecessary selection of unwanted traits and allow the simultaneous tracing of other favorable traits.

The invention further relates to the use of the discovered sequences of bovine NRAMP1 as indicated, or "genetic markers," of disease susceptibility or resistance in artiodactyla. Specifically, the invention includes the detection and identification of these specific gene sequences via conventional molecular biological techniques such as, but not limited to, SSCA and SSCP. Still more specifically, the present invention illustrates specific methods and materials (such as, for example, specific PCR primers) for identifying and distinguishing these sequences via SSCA, SSCP or direct sequencing. This allows one to screen an animal and detect which sequences of NRAMP1 the animal possesses, which then allows the accurate prediction of the animal's susceptibility or resistance to disease.

Still further, the present invention relates to the use of these predictive genetic markers in animal husbandry including in food production, and selective breeding of disease resistant animals, including cattle.

The conception of this invention was based, in part, on a series of published reports on genetic selection of swine naturally resistant to swine brucellosis in the 1930's and 1940's. These publications reported that swine which did not produce antibodies to an oral challenge of virulent *Brucella suis* produced offspring that did not produce an antibody to a similar challenge approximately 70% of the time compared to a frequency of approximately 20% for progeny from unselected control groups. Id.

The observation that approximately 20% of unvaccinated control cattle challenged with a virulent strain of *Brucella abortus* S2308 did not exhibit any signs of brucellosis (infection with *Brucella abortus*) and the lack of production of antibodies post-challenge led the inventors to hypothesize that this was a natural resistance to bovine brucellosis. The inventors then began breeding studies to determine if this natural resistance was heritable and to search for genes that could control this natural resistance, if it was heritable. The natural resistance was shown to be heritable as it responded to selection; a greater percentage of offspring were naturally resistant to brucellosis (57% compared to 37%) when a naturally resistant sire was bred to naturally resistant dams than when a naturally resistant sire was bred to naturally susceptible dams.

Genetic studies in other animals indicated a major gene termed Bcg might control this natural resistance to *Mycobacterium bovis*-BCG. A candidate gene in mice was reported (Vidal, et al., 1993). However, unlike the field of the present invention, this report was not in an artiodactyla animal. The inventors then proposed that cattle might possess a conserved homolog of the murine gene and that this conserved homolog might have a major controlling effect over the natural resistance to brucellosis in the cattle the inventors had been breeding.

The inventors then tested the above hypothesis and cloned, sequenced, and genetically mapped bovine NRAMP1 in the bovine. The bovine NRAMP1 was mapped to BTA 2, within syntenic loci conserved on HSA 2q and MMU1. Bovine NRAMP1 is expressed primarily in macrophages and tissues of the reticuloendothelial system, and is predicted to encode a 548 amino acid protein that has 12 transmembrane segments with one hydrophilic N-terminal region containing a Src homology 3 (SH3)-binding motif located at the cytoplasmic surface, and a conserved consensus transport motif. The gene is designated as bovine NRAMP1 because of conserved genetic linkage, tissue expression, and amino acid sequence homology with murine NRAMP1.

The inventors discovered macrophage restricted expression of the bovine NRAMP1 gene, and importantly, discovered sequence and conformational differences in the bovine NRAMP1 gene which significantly associate with natural resistance or susceptibility to brucellosis in cattle. Testing was also conducted to determine if bovine NRAMP1 is conserved in other artiodactyla. Significantly, swine, goats, sheep bison (American Buffalo), llamas, elk (wapiti), red deer, sika deer, water buffalo, follow deer, and white-tailed deer, indeed all artiodactyla (for a definition of artiodactyla, see Nowak, R. M. et al., 1983) analyzed thus far, have a conserved NRAMP1 gene.

The present invention has also shown that cattle whose phenotypes have been ascertained to be resistant to a challenge of virulent *B. abortus* are significantly different in their ability to control the intracellular replication of *Brucella abortus, Mycobacterium bovis*-BCG, and *Salmonella dublin* in an in vitro macrophage killing assay than cattle whose phenotypes have been ascertained to be susceptible (85% correlation with challenge phenotype) (Qureshi, T., Templeton, J. W., and Adams, L. G. 1996).

These cattle were phenotyped both by an in vivo challenge with *Brucella abortus* Strain 2308 and by an in vitro macrophage killing assay of *Brucella abortus* Strain 2308, *Mycobacterium bovis*-BCG strain and *Salmonella dublin* to determine their resistance or susceptibility to bovine brucellosis and tuberculosis. Using SSCA or SSCP, a genetic polymorphism was discovered in the 3' UTR of the gene. This polymorphism has two different forms which significantly associate (p=0.0089) with naturally resistant and naturally susceptible phenotypes to bovine brucellosis, tuberculosis, salmonellosis, and paratuberculosis in unrelated cattle.

By screening for the particular polymorphism and/or sequence that a given animal has, one can accurately and efficiently predict the susceptibility, or resistance of that animal to ruminant brucellosis, tuberculosis, paratuberculosis and salmonellosis and other diseases involving intracellular parasites of macrophages.

The bovine NRAMP1 gene is conserved in Bos spp. Bison bison, *Odocoileus virginianus, Capra hirus, Alces alces, Cervus canadensis, Cervus elaphus, Dama dama, Elaphurus davidianus,* Ursus spp. Sus scrofa, and *Oreamnos americanus* (SEQ ID NOs. 16–27, respectively) and most likely all domestic and wild artiodactyla (see FIGS. **8A1** and **8A2** SEQ ID Nos. 16–27, FIG. **8**C, and FIG. **8**D). In addition, the Nramp1 protein is also highly conserved (see, for example, FIG. **8**B SEQ ID Nos 9 and 28). The discovered genetic variation in the 3' UTR of the NRAMP1 gene of artiodactyla can be used, inter alia, in selecting and breeding domestic and free-ranging artiodactyla that are resistant to, inter alia, brucellosis, tuberculosis, paratuberculosis and salmonellosis (see FIG. **8**D, SEQ ID Nos. 29 and 30).

The bovine NRAMP1 polymorphism results from a transversion at position 1782 of the bovine NRAMP1 cDNA; thymine in the resistant sequence to guanine in the susceptible sequence. Additionally, there is a polymorphic DNA microsatellite sequence difference between resistant and

susceptible cattle involving the number of (GT) dinucleotide repeats and spacing in the 3' UTR of bovine Nramp1. This sequence in resistant animals, beginning at position 1779, is:

SEQ ID NO 15: $GGGTGT(GT)_{10}AT(GT)_3(N)_{61}(GT)_5(N)_{24}(GT)_{13}$

where "N" symbolizes any one of the four nucleotide bases A, C, G or T. In contrast, the DNA sequences associated with susceptible cattle follow the form:

SEQ ID NO: 32: $(GT)_{<10}AT(GT)_3(N)_{>61}(GT)_5(N)_{<24}(GT)_{>13}$

where "N" again symbolizes any one of the four nucleotide bases A, C, G or T

The detection of the resistance associated sequence or the susceptible associated sequence can be done by SSCA, SSCP, polymerase chain reaction (PCR) followed by direct DNA sequencing or any other technique known to those of skill in the art capable of detecting genetic sequence differences. The sequence of PCR primers used to detect the genomic DNA sequence of the bovine NRAMP1 which contains the polymorphic DNA sequences associated with resistance or susceptibility are indicated in FIG. **1**A, SEQ ID Nos 1 and 2. These PCR primers will amplify the resistant and susceptible allelic sequences in genomic (g) or cDNA. However, it should be stated that any PCR primers that will amplify the polymorphic region can also be used in this invention.

In one screening trial (see FIG. **3**, for example), the bovine NRAMP1 sequences correctly identified animals as being either resistant or susceptible in 18 of 22 cattle naturally resistant or susceptible to brucellosis (FIG. **3**A and FIG. **3**B) (Significant association, p=0.0089, Fisher's exact analysis). Importantly, these 22 cattle were all unrelated animals.

The bovine NRAMP1 sequences can be detected in gDNA isolated from any tissue including gDNA isolated from, but not limited to, peripheral blood samples, semen, mucosal scrapings, etc. using PCR amplification.

As shown in Example 9, for example, approximately 82% of the cattle naturally resistant or susceptible to brucellosis and tuberculosis can be identified by typing them for the bovine NRAMP1 resistant or susceptible polymorphism by using SSCP (or SSCA). The zygosity of cattle for the resistant associated polymorphism (heterozygote or homozygote genotype) can be determined and a breeding program can be practiced to efficiently produce cattle naturally resistant to brucellosis and tuberculosis. Additionally, the bovine NRAMP1 gene is a good candidate gene for production of transgenic animals which possess genes for outstanding production traits and by transgene action are naturally resistant to, inter alia, brucellosis, salmonellosis, paratuberculosis, and tuberculosis.

The genetic selection of breeding animals for a single locus is not detrimental to overall animal production i.e. beef, muscle, grain or milk production, as long as a breeding plan is constructed to buffer this effect. All of the other chromosomes will segregate by independent assortment and will perpetuate heterozygosity. Additionally, with the current availability of microsatellite markers spaced throughout the bovine genome, selection for disease resistant genotypes can now be achieved without compromising other desirable production traits while maximizing heterozygosity at approximately 100 microsatellite loci. With the development of the bovine gene map and the identification of major genes controlling economically important traits in cattle and other livestock, the ability to identify a prized genotype of disease resistance and high quality production will be possible in the near future. Tremendous progress in cattle breeding will be realized when it is possible to select for superior genotypes directly by identifying important genes. The SSCP or SSCA based detection of NRAMP1 polymorphic sequences can be conducted on gDNA isolated from antemortem or postmortem tissues, provided the postmortem tissue has been reasonably protected from a DNA degrading environment where autolysis of the tissue would occur.

In a preferred embodiment, one mode for the detection of the artiodactyla NRAMP1 sequences is in a laboratory with routine DNA isolation, DNA PCR amplification, electrophoresis technique, SSCA, SSCP analysis, direct DNA sequencing or any other technique suitable for detecting differences in genetic sequences.

In a preferred embodiment, one mode for identification of the artiodactyla NRAMP1 resistant and susceptible associated sequences is by specific PCR amplification of gDNA isolated from an individual animal's peripheral blood collected in an anticoagulant. The PCR amplification can performed in an ordinary laboratory with capabilities of performing the polymerase chain reaction and ordinary expertise in molecular biology. With hand-held thermal cyclers it is also possible to perform the PCR amplification of the alleles outside the laboratory in a so-called "chute-side" assay shortly after the blood is collected.

In a preferred embodiment, one mode for utilizing the present invention in detecting the NRAMP1 resistant and susceptible sequences is by a PCR amplification of gDNA isolated from peripheral blood of individual artiodactyla animals followed by SSCA or SSCP analysis of the PCR product. The isolation of the gDNA from blood cells can be done by standard methods suitable for subsequent PCR amplification. As shown in Example 8 and FIG. **1**A, PCR primer sequences can be used to amplify the polymorphic DNA region of both resistant and susceptible animals, namely cattle. PCR products can be specifically labeled, either by using radioactive nucleotides in the PCR reaction (as in the case of SSCA) or by using a specifically end-labeled primer (as in the case of SSCP analysis) in the PCR reaction. It should be stated that other radioactive (i.e. ^{32}P, ^{33}P, etc.) or non-radioactive alternatives (for example, but not limited to DIG-labeling) can be used to specifically label the PCR products. These amplicons can then be run on a polyacrylamide gel and the migration of the amplicons visualized by standard autoradiographic techniques. It should be noted that if, for example, non-radioactive labeling techniques are used, alternative detection methods can also be employed. Susceptible-associated and resistance-associated DNA sequences can be readily distinguished (FIG. **3**A). The banding patterns of amplicons from resistance-associated and susceptible-associated DNA are quite different; the amplicons from resistance-associated DNA sequences show faster migration through the gel as is expected from their smaller size amplicons (175 bp for resistance associated amplicons vs. >175 bp for susceptible associated amplicons).

One primary advantage of a diagnostic test using SSCA or SSCP analysis is that this technology is readily available. It is relatively simple compared to many techniques used to identify DNA sequences; it is relatively inexpensive to equip a laboratory With the necessary equipment; it is conducive technology for mass through-put of large numbers of samples; and the relatively simple technology yields in minimal false (positive or negative) test results when properly controlled. Further, because the resistance and susceptibility associated sequences are genetic, they are transferable, meaning, for example, resistance can be a heritable trait. Since the transmission of these diseases is

dependent on a susceptible host, resistant animals offer an excellent opportunity to break the cycle of disease spread and begin eradication.

There are no particular unique disadvantages to the proposed SSCA or SSCP analysis based assay compared to other molecular biologic diagnostic tests. All such tests require some specialized equipment, a laboratory utilizing basic good laboratory practices and at least currently, tissue (blood) collection and transportation to a laboratory. The occasional stress associated with restraining an animal for blood collection will not effect the test results.

A major purpose of this invention is to identify sequences of NRAMP1 associated with resistance or susceptibility to disease, using SSCA or SSCP-based techniques which results in correctly identifying artiodactyla that are naturally resistant to brucellosis, tuberculosis, salmonellosis, and paratuberculosis with a high degree, for example 82%, of accuracy.

There are several possibilities which could account for the lack of 100% SSCP association with resistant phenotypes. It is possible that Bcg is not a single gene but a gene complex and NRAMP1 is one of the Bcg genes, or there are two or more genes controlling natural resistance to bovine brucellosis. Alternatively, NRAMP1 may be in linkage disequilibrium Faith the Bcg gene and is a marker gene for the $Bcg^{r\ or\ s}$ alleles. The lack of 100% association of the bovine NRAMP1 SSCP with the natural resistant or susceptible phenotypes could also be due to incorrect ascertainment of some of the true cattle R or S phenotypes caused by genetic heterogeneity, phenocopies, lack of penetrance, or error in the challenge procedure. We attempted to minimize effects of phenocopies by a uniform, potent challenge (conjunctival instillation of 10^7 colony forming units of virulent *B. abortus*). An indication that lack of penetrance is not a major problem is the fact that the phenotypes of the founders respond to genetic selection in breeding studies (Templeton et al. 1990a; Templeton et al. 1990b). The fact that the cattle used in these studies are derived from four different breeds implies genetic heterogeneity does not confound the phenotypes and provides strong, direct evidence that NRAMP1 is a likely candidate for a major gene controlling natural resistance to, inter alia, brucellosis, and in particular bovine NRAMP1 is a likely candidate for a major gene controlling natural resistance to, inter alia, bovine brucellosis.

The following Examples are intended to illustrate the embodiments of the present invention and are not in any way intended to limit the scope of the invention in any manner.

EXAMPLE 1

Cloning of Bovine NRAMP1 and Isolation of Bovine NRAMP1 cDNA

The bovine NRAMP1 gene was cloned as follows. Based on the genomic sequence of murine NRAMP-1, oligonucleotide primers SEQ ID NO. 3 and SEQ ID NO. 4 (also designated as 1F and 1R) were used to amplify a 155 bp segment in bovine genomic DNA (FIG. **1**B).

From this 155 bp bovine sequence, RT-PCR was performed on bovine macrophage mRNA, using a bovine specific forward primer designated SEQ ID NO. 5 (PE2): 5' CGTGGTGACAGGCAAGGACT3' and a reverse primer, SEQ ID NO. 6 (MUT2): 5' CCAAGAAGAGGAAGAAGAAGG-TGTC3' from the murine NRAMP1 cDNA sequence (Vidal et al. 1993).

Reverse transcription was performed as follows. Total RNA was extracted from bovine macrophage, spleen, lung, or heart as described (Chirgwin et al. 1979). 0.5 μg total RNA was transcribed in 25 μl reaction at 37° C. for 60 min. with MMLV reverse transcriptase (Gibco-BRL). cDNA amplification was performed at 95° C. (5 min) followed by 32 cycles of 94° C. (1 min), 58° C. (1 min), and 72° C. (1 min) with 1 mM $MgCl_2$, 2 μl 10×PCR buffer, 2 Units Taq polymerase (Perkin-Elmer) and 4 μl RT template in a final volume of 25 μl.

A 222 bp product was amplified from reverse transcribed bovine macrophage total RNA. Sequence analysis showed that this PCR product contained 90% nucleotide identity with the third exon (nucleotide positions 338–458) of the murine homolog. The 222 bp product was generated to screen a bovine splenic λgt11 cDNA library (Clontech). A total of 1×10^6 clones were screened by in situ plaque hybridization with radiolabeled [^{32}P]-α-dCTP (3000 Ci/mmol) (Dupont, NEN Research Products) by hexamer priming ($1–3\times10^9$ cpm/μg)(Feinberg et al., 1983). Filters were washed under conditions of increasing stringency up to 1×SSC, 0.1% SDS at 65° C. for 30 min. Positive clones were verified using PCR with primers SEQ ID NO. 5 (PE2) and SEQ ID NO. 6 (MUT-2) and subsequently PCR amplified to obtain a 2.3 kb insert with λgt11 insert screening amplimers. This PCR product was gel purified and ligated into pT7BlueT-Vector (Novagen). Both strands of plasmid DNA were sequenced by the dideoxy method of Sanger et al. (Sanger et al. 1977) using modified T7 DNA polymerase (USB) and [^{35}S]-α-dATP (3000 Ci/mmol) (NEN Research Products, Boston, Mass.). All sequence data were compiled and analyzed using MacVector 4.1 software (Eastman Kodak Comp. New Haven, Conn.).

Twenty potentially full-length NRAMP1 clones(≈2.3 kb) were obtained, eight of which were sequenced and used to construct the complete sequence. As shown in FIG. **4**, the in-frame initiator codon ATG is located at nucleotide position 73 from the 5'-end, and is followed by a segment of 1644 nucleotides, forming a single open reading frame (ORF) encoding a protein of 548 residues with a calculated molecular weight of 59.6 KDa. A TGA termination codon located immediately dowvnstream from glycine 548 (nucleotide pst 1717) is followed by an intact AATAAA polyadenylation signal, position 2257.

EXAMPLE 2

Analysis of the Predicted Bovine NRAMP1 Structure

Note that as used herein, it is understood that the term "NRAMP1" includes the coding sequence and at least the 3' UTR of the gene. The first 64 N-terminal amino acids of Bovine NRAMP1 are rich in proline (11/64), glycine (10/64), serine (8/64), and charged amino acids (10/64), and include two putative PKC phosphorylation sites at amino acid positions 37 and 51 (FIG. **2**). Because SH3 domains interact specifically with proline-rich peptides, we compared the proline-rich coding fragment PPSPEP (positions 21–26) to several identified SH3- binding sequences (Lim et al. 1994). The analysis revealed that the "PNNP" binding motif (Musacchio et al. 1994) is conserved in bovine Nramp1, which indicates that bovine Nramp1 contains an N-terminal SH3 binding domain. Kyte-Doolittle hydrophilicity analysis (FIG. **5**) disclosed that the surface probability of peptide PPSPEP is from 50.3% to 67.6%, which indicates that the bovine Nramp1 SH3-binding motif is most likely located at the inner membrane surface.

Analysis of the remaining bovine NRAMP1 indicates the predicted protein to be highly hydrophobic with 12 putative

transmembrane domains (FIG. **6**) in agreement with the murine and human Nramp1 putative structure (Vidal et al. 1993; Barton et al. 1994; Cellier et al. 1994). The bovine NRAMP1 gene product contains one potential N-linked glycosylation site at position 335, within a highly hydrophilic region between predicted transmembrane (TM) domains 7 and 8, and three PKC phosphorylation sites on serine (positions 37, 51, and 269, respectively). A 20 amino acid transport motif is located between the predicted TM domains 8 and 9 and conserved in murine and human Nramp1 (FIG. **2**). This conserved motif is known as the "binding-protein-dependent transport system inner membrane component signature" (Vidal et al. 1993; Malo et al. 1994a; Cellier et al. 1994; Malo et al. 1994b). Based on the hydropathic analysis and conversed transport motif, we propose, but not in a limiting sense, that the membrane-associated topography of bovine Nramp1 (FIG. **6**) is as follows: the NH2-terminus is located in the cytoplasm, and the following 12 TM domains result in 5 consecutive transmembrane loops. This arrangement would place the SH3-binding motif on the cytoplasmic membrane surface; SH3-binding domain with two potential phosphorylation sites and the transmembrane (TM) loops 2 and 3 and the TM 6 and TM 7 loops containing one phosphorylation site each, all projecting into the cytoplasm; the TM 7 and TM 8 loop containing one predicted N-linked extra-cellular glycosylation site; and the carboxyl terminus in the cytoplasm.

EXAMPLE 3

Homology Among Human, Murine, and Bovine Nramp Proteins

Comparison of human, murine and bovine predicted Nramp protein sequences (FIG. **2**) indicates a remarkable degree of homology (86.9% amino acid sequence identity between murine and bovine; 88.6% amino acid sequence identity between human and bovine). The predicted TM segments 1–8 are highly conserved hydrophobic membrane associated domains in the three species, with 99% identity between human and bovine, and 96% identity between murine and bovine. The most conserved consecutive region is from TM 8–9 with 100% identity from position 346 to 456 between human and bovine; 98.2% identity between murine and bovine. Within the TM 8–9 segment, the bovine "binding protein dependent transport system inner membrane component signature" was identical with murine and human Nramp with one exception (substitution of lysine to arginine at position 392 in the human (FIG. **2**). Also among these three species, one predicted N-linked glycosylation site was conserved within the fourth putative extracellular loop between TM 7 and 8; and one consensus PKC phosphorylation site was conserved in the predicted intracytoplasmic loop between TM 6 and 7 at position 37 (FIG. **2**) (Vidal et al. 1993; Cellier et al. 1994; Gruenheid et al. 1995).

Amino acid substitutions were not randomly distributed along the sequence of the protein but were significantly clustered within certain regions. The most striking differences were located at extreme ends of the proteins, NH2 terminus (57.4% identity of positions 1–47 between murine and bovine; 66% identity of positions 1–50 between human and bovine) and COOH-terminus (57.6% identity of positions 516–548 between murine and bovine; 69.6% identity between human and bovine). The predicted third and fourth extracellular loops at positions 215–237 and positions 307–346 were less conserved in amino acid sequences than the TM domains. Identity was 78.2% between murine and bovine and 82.0% between human and bovine for the predicted third extracellular loop, respectively, and 75.0% identity between murine and bovine and 85% identity between the human and bovine, respectively, for the predicted fourth extracellular loop.

EXAMPLE 4

Genetic Mapping of Bovine NRAMP1

Genetic Mapping was performed as follows. Bovine-hamster hybrid somatic cell panel blots (Womack et al. 1986; Adkinson et al. 1988; Beever et al. 1994) were hybridized with the 1F/1R PCR generated probe (4–8×10^8 cpm/μg) (Feinberg et al. 1983). Hybridization was performed at 43° C. for 18 hrs in 20 ml of 50% formamide, 5×SSC, 1×Denhardt's solution, and 20 mM $NaPO_4$ (pH-6.8), followed by washing once in 2×SSC, 0.5% SDS at room temperature for 15 min., two successive washes in 1×SSC, 0.1% SDS at 65° C. for 30 min (Adkinson et al. 1988). All gene probes were labeled with the random primed DNA labeling method is α-[^{32}P] dCTP (3000 Ci/mmol) (NEN Research Products, Boston, Mass. (Feinberg et al. 1983). Synteny was ascertained by analysis of concordancy of the probe with known marker genes as described (Womack et al. 1986; Adkinson et al. 1988; Womack et al. 1994).

The syntenic arrangement of bovine NRAMP1 was determined using somatic cell hybrid segregation analysis (Womack et al. 1986; White et al. 1994). DNA from 87 bovine/rodent somatic hybrid cells was digested with Hind III and hybridized to a PCR generated probe using SEQ ID NO. 3 and SEQ ID NO. 4 (1F and 1R) primers (FIG. **1**B). The bovine specific Hind III restriction fragment of 4.7 kb was easily discriminated from fragments representing the hamster and mouse homologs, permitting detection of bovine-specific fragments in each cell line. A pairwise concordancy analysis indicated that bovine NRAMP1 segregated 100% concordantly with Cry-γ, which has been assigned to BTA 2 (FIG. **9**). An analysis of 87 somatic hybrids revealed that 28 were positive and 59 were negative for both Cry-γ and bovine NRAMP1. A group of bovine syntenic loci, villin, Cry-γ (Adkinson et al. 1988; Beever et al. 1994) and Interleukin-8 receptor has been mapped to a region of BTA 2 and conserved on HSA 2q (White et al. 1994) and proximal MMU 1 (Cerretti et al. 1993), which were closely linked to the Lsh/Ity/Bcg locus in the mouse (FIG. **10**). These results further support the homology among human, bovine and murine NRAMP.

EXAMPLE 5

Single-stranded Conformational Analysis (SSCA)

We have identified cattle phenotypically resistant and susceptible to brucellosis by in vivo challenge (Harmon et al. 1985). Screening of 22 outbred, unrelated individuals by SSCA revealed the existence of two general single stranded polymorphic forms of bovine NRAMP1 (FIG. **3**A). Sequencing analyses of the PCR-amplified fragments showed a microsatellite length polymorphism starting at position 1785 of two types; one being SEQ ID NO. 31: SEQ ID NO: 32: 5' $(GT)_{10}AT(GT)_3(N)_{61}(GT)_5(N)_{24}(GT)_{13}$3'. The other being $(GT)_{<10}AT(GT)_3(N)_{>61}(GT)_5(N)_{<24}(GT)_{>13}$ where "N" symbolizes any one of the four nucleotide bases A, C, G or T (FIG. **7**A). These polymorphisms correlate both with their distinctive patterns analyzed by SSCA or SSCP and with their respective in vivo phenotypes [p=0.0089, Fisher's Exact Analysis]. We will designate the

former DNA sequence as $SSCP^r$ and the latter sequence as $SSCP^s$. The relative risk (RR) of susceptibility, if an animal possess the $SSCP^s$ is 4.5.

EXAMPLE 6

Cell Specific Expression of Bovine Nramp1-1 mRNA

To test whether bovine NRAMP1 was expressed primarily in macrophage populations, we analyzed total RNA prepared from 15 different bovine tissues (peripheral blood lymphocytes, liver, lymph node, spleen, tonsil, lung, kidney, thymus, heart, skeletal muscle, jejunum, colon, ovary, uterus, brain and cultured macrophages) by northern blot analysis using a SEQ ID NO. 3 and SEQ ID NO. 4 PCR generated bovine DNA probe (FIG. **11**).

Northern blot analysis was performed as follows. Monocyte-derived macrophages were harvested and cultured as described (Campbell and Adams 1993). Total RNA was isolated from these macrophages and lymphocytes using standard techniques (Chirgwin et al. 1979). 10 μg of total RNA from macrophages and lymphocytes were separated on 1% formaldehyde agarose gels, transferred to Nytran plus membranes (Schleicher & Schuell). Blots were prehybridized in 20 ml of 50% formamide, 10% dextran sulfate, 4.7×SSPE (1×SSPE is 10 mM sodium phosphate, 1 mM EDTA, 150 mM NaCl), 0.47×Denhardt's solution, 0.1% SDS, 0.18 mg/ml heat-denatured salmon sperm DNA, and 0.34% fat free milk for 4 hrs at 42° C. Hybridization at 42° C. for 18 hrs was performed in the same solution containing 2×10^8 cpm/ml [^{32}P]-radiolabeled probe SEQ ID NO. 3 and SEQ ID NO. 4 fragment. Final wash conditions were 0.2×SSC, 0.1% SDS at 68° C. for 30 min.

A band of approximately 2.3 kb was detected in macrophage, spleen and lung RNA, but was absent in the RNA analyzed from other tissues. These results indicate that bovine Nramp1 is principally expressed in the macrophage and the reticuloendothelial (RE) system.

EXAMPLE 7

Identification of Animals Naturally Resistant or Susceptible to Disease (traditional method)

Cattle were phenotyped for resistance or susceptibility by both in vivo challenge experiments with *Brucella abortus* Strain 2308 and by an in vitro macrophage killing assay.

In vivo assays: Unvaccinated control cattle were challenged with a virulent strain of *Brucella abortus* S2308. Those not exhibiting any signs of brucellosis, bacteriologically culture negative for *B. abortus*, and lacking production of anti-lipopolysaccharide *B. abortus* antibodies post-challenge were considered naturally resistant. Those exhibiting signs of brucellosis, bacteriologically *B. abortus* culture positive, or producing anti-lipopolysaccharide *B. abortus* antibodies post-challenge wvere considered naturally susceptible (Harmon et al. 1985).

In vitro assays: Macrophages from unvaccinated control cattle were isolated according to standard techniques and subsequently challenged weith *B. abortus* in an in vitro assay (Harmon et al. 1989; Price et al. 1990; Campbell et al. 1992). The macrophages from those cattle that were able to control intracellular replication of *B. abortus* in vitro were considered resistant, while the macrophages from cattle that were not as good at controlling intracellular replication of *B. abortus* in vitro were considered susceptible.

EXAMPLE 8

Single Stranded Conformational Analysis (SSCA):

In a total PCR reaction of 15 μl used for SSCA, inventors mixed 0.2 mM each dGTP, dGTP, dCTP, 0.07 mM dATP, 1 mM $MgCl_2$, 10×M PCR buffer, 0.2 Units of Taq DNA polymerase (Cetus-Perkin Elmer), and 8 μCi of [^{35}S]-dATP with 50 ng genomic DNA. Parameters for PCR amplification were a single denaturation step of 5 min at 94° C. followed by 32 cycles of denaturation (1 min at 94° C.), annealing (40 sec at 60°) and extension (1 min at 72° C.), and a final extension step of 7 min at 72° C. The oligonucleotide primers designed from 3'-UT were SEQ ID NO. 1 (Fmicrol)=5' AAGGCAGCAAGACAGACAGG3', nucleotide positions 1814–1833 and SEQ ID NO. 2 (3end3)=5' ATGGAACTCACGTTGGCTG3', nucleotide positions 1970–198). The SSCA polymorphisms of 22 unrelated cattle were detected by separating these 175 bp PCR fragments on 6% polyacrylamide gels with or without urea at room temperature at 90 W for 2.5 hr.

SSCP Analysis:

SSCP was performed essentially the same as SSCA with the following two exceptions. First, for SSCP analysis, either SEQ ID NO. 1 or SEQ ID NO. 2 was end-labeled prior to the PCR reaction rather than having [^{35}S]-dATP in the PCR reaction mix. Second, for SSCP analysis, the polyacrylamide gels always contained urea.

EXAMPLE 9

Specific Genetic Sequences Associated with NRAMP1 in situ

Significantly Associate with Resistance or Susceptibility to Ruminant Brucellosis, Tuberculosis, Paratuberculosis and Salmonellosis

The cattle used in these experiments are all unrelated. One is a purebred Angus (*Bos taurus*) and other twenty two are cross bred cattle produced by a three-way cross—F_1 [Jersey (*Bos taurus*)×American Brahman (*Bos indicus*)] (Harmon et al. 1985). All animals used in experiments were housed in USDA-approved facilities with daily supplemental feeding. All experimental protocols are University reviewed and approved.

PCR amplification of DNA isolated from susceptible or resistance cattle was performed as follows. In a total PCR reaction of 15 μl used for SSCA, 0.2 mM dGTP, dTTP and dCTP, 0.07 mM dATP, 1 mM $MgCl_2$, 1.5 μl 10×PCR buffer, 0.2 Units of Taq DNA polymerase (Cetus-Perkin Elmer), and 8 μCi of [^{35}S]-dATP were added to 50 ng of genomic DNA. Parameters for PCR amplification were a single denaturation step of 5 min at 94° C. followed by 32 cycles of denaturation (1 min at 94° C.), annealing (40 sec at 60° C.) and extension (1 min at 72° C.), and a final extension step of 7 min at 72° C. The oligonucelotide primers designed from the 3' UTR were SEQ ID NO. 1 and SEQ ID NO. 2 (FIG. **1A**). This amplified DNA was then subjected to gel electrophoresis under SSCA or SSCP conditions. Namely, the PCR fragments were run on a 6% polyacrylamide gel (National Diagnostics, G.A.) at room temperature at 90 W for 2.5 hrs. The different migration patterns of the amplified DNA under these gel conditions correlated with the phenotype of the cattle previously determined by in vivo or in vitro challenge experiments with *B. abortus* (Harmon et al., 1985). PCR amplification of DNA (using SEQ ID NO. 1 and SEQ ID NO. 2 as primers) from cattle previously determined to be resistant produced an amplicon that migrated faster than the DNA isolated and amplified from cattle determined to be sensitive when said amplicons were run on a 6% polyacrylamide gel under said conditions (FIG. **3**).

Subsequent sequencing of the amplicons from some of these cattle revealed two significant sequence differences

(FIG. 7A). These sequence differences correlated with in vivo determined resistance/susceptibility and can be categorized into two general groups. The DNA amplified from resistant cattle corresponds to DNA sequences of the type (SEQ ID NO. 15):

$GGGTGT(GT)_{10}AT(GT)_3(N)_{61}(GT)_5(N)_{24}(GT)_{13}$

whereas the DNA amplified from susceptible cattle corresponds to DNA sequences of the type,

SEQ ID NO: 33: $GGGGGT(GT)_{<10}AT(GT)_3(N)_{>61}(GT)_5(N)_{<24}(GT)_{>13}$

Although SSCA and SSCP cannot determine the exact DNA sequence of the cattle in this region, the differences in migration patterns of the susceptible and resistant-associated amplicons allow us to define said amplicons as being $SSCP^r$ or $SSCP^s$ for single stranded conformation polymorphism of the resistant or susceptible type, respectively.

In addition, because of the conserved nucleotide sequence among swine, goats, sheep, bison (American buffalo), llamas, elk (wapiti), red deer, silka deer, water buffalo, fallow deer, white-tailed deer, and most likely all domestic and wild artiodactyla in this region, we can expect these same sequence differences and their relationship to disease susceptibility to be applicable to all artiodactyla (see, FIGS. **8**A1, **8**A2, **8**C, and **8**D). In this regard, the intracellular survival of *Brucella abortus* and *Mycobacterium bovis* BCG was determined in an in vitro monocyte-derived macrophage killing assay using macrophages from Bison phenotypically determined to be naturally resistant or susceptible to *Brucella abortus* infection, see Table 1.

TABLE 1

Intracellular Survival of *Brucella abortus* and *Mycobacterium bovis* BCG in an in vitro Monocyte-derived Macrophage Killing Assay Using Macrophages from Bison Phenotypically determined to be naturally resistant or susceptible to *Brucella abortus* Infection

	% Survival[2]	
Bison Number	*B. abortus*	*M. bovis* BCG
1-R[1]	81%	75%
2-R	84%	84%
3-R	64%	77%
4-R	86%	76%
5-R	93%	87%
6-R	59%	87%
7-S[1]	123%	121%
8-S	120%	125%
9-S	110%	123%
10-S	150%	200%

[1]The R and S designates bison phenotypically determined to be naturally resistant (R) or susceptible (S) to *Brucella abortus*. This was determined by a challenge of a not previously exposed, either by natural exposure or vaccination, pregnant bison at mid-gestation with 1×10^7 virulent *B. abortus* organisms.

[2]Percent survival refers to the number of *B. abortus* organisms that survive after being phagocytosed by the macrophages compared to the numbers of bacteria at Time 0 after 3 days of culture for the *B. abortus* and 14 days of culture for the *M. bovis* BCG.

In addition, these in vitro phenotypically determined resistant/susceptibility profiles of bison were compared with the genotypically determined resistant/susceptibility profiles determined from the 3' UTR of the bison NRAMP1 gene. Similar to the bovine studies, a correlation between the sequence at the 3' UTR of bison NRAMP1 and the resistance/susceptibility phenotype exists. The bison phenotypically determined to be resistant, had a resistance-associated gene sequence at the 3' UTR, while the bison phenotypically determined to be susceptible, had a susceptible-associated gene sequence at the 3' UTR (FIG. **8**D).

FIG. **8**D shows the NRAMP1 cDNA sequences of the 3' UTR in naturally susceptible and resistant bison. Nucleotides are numbered positively in the 5' to 3' orientation to the right of each lane, starting with the coding nucleotide G at 1676 and ending with the last nucleotide. Stop code ATG is indicated by (@). Three TG repeats including (TG)13, (TG)8, and (TG)16 are bolded, separately. The differences between the R and S nucleotide sequences are the S sequence has one less TG in the first repeat (TG)12 versus (TG)13 and is therefore two bases shorter overall (2259) versus (2261) for this area of the NRAMP1 3' UT sequence. the Polymorphisms containing the first (TG) 13 were detected by SSCA using primers Fmicro and Bmicro1' as indicated.

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Because many varying and different embodiments may be made within the scope of the inventive concept herein taught, and because many modifications may be made in the embodiments herein detailed in accordance with the descriptive requirement of the law, it is to be understood that the details herein are to be interpreted as illustrative and not in a limiting sense.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 31

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGGCAGCAA GACAGACAGG 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGAACTCA CGTTGGCTG 19

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

-continued

TCTCTGGCTG AAGGCTCTCC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCAAGCTCAC CTTAGGGTAG 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGTGGTGACA GGCAAGGAC 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCAAGAAGAG GAAGAAGAAG GTGTC 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 547 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Thr Gly Asp Lys Gly Pro Gln Arg Leu Ser Gly
5 10

Ser Ser Tyr Gly Ser Ile Ser Ser Pro Thr Ser Pro
15 20

Gly Pro Gln Gln Ala Pro Pro Arg Glu Thr Tyr Leu
25 30 35

Ser Glu Lys Ile Pro Ile Pro Asp Thr Lys Pro Gly
40 45

Thr Phe Ser Leu Arg Lys Leu Trp Ala Phe Thr Gly
50 55 60

Pro Gly Phe Leu Met Ser Ile Ala Phe Leu Asp Pro
65 70

Gly Asn Ile Glu Ser Asp Leu Gln Ala Gly Ala Val

-continued

```
          75                  80

Ala Gly Phe Lys Leu Leu Trp Val Leu Leu Trp Ala
85                  90                  95

Thr Val Leu Gly Leu Leu Cys Gln Arg Leu Ala Ala
            100                 105

Arg Leu Gly Val Val Thr Gly Lys Asp Leu Gly Glu
    110                 115                 120

Val Cys His Cys Tyr Tyr Pro Lys Val Pro Arg Thr
                125                 130

Val Leu Trp Leu Thr Ile Glu Leu Ala Ile Val Gly
        135                 140

Ser Asp Met Gln Glu Val Ile Gly Thr Ala Ile Ala
145                 150                 155

Phe Asn Leu Leu Ser Ala Gly Arg Ile Pro Leu Trp
            160                 165

Gly Gly Val Leu Ile Thr Ile Val Asp Thr Phe Phe
    170                 175                 180

Phe Leu Phe Leu Asp Asn Tyr Gly Leu Arg Lys Leu
                185                 190

Glu Ala Phe Phe Gly Leu Leu Ile Thr Ile Met Ala
        195                 200

Leu Thr Phe Gly Tyr Glu Tyr Val Val Ala Arg Pro
205                 210                 215

Glu Gln Gly Ala Leu Leu Arg Gly Leu Phe Leu Pro
            220                 225

Ser Cys Pro Gly Cys Gly His Pro Glu Leu Leu Gln
    230                 235                 240

Ala Val Gly Ile Val Gly Ala Ile Ile Met Pro His
                245                 250

Asn Ile Tyr Leu His Ser Ala Leu Val Lys Ser Arg
        255                 260

Glu Ile Asp Arg Ala Arg Arg Ala Asp Ile Arg Glu
265                 270                 275

Ala Asn Met Tyr Phe Leu Ile Glu Ala Thr Ile Ala
            280                 285

Leu Ser Val Ser Phe Ile Ile Asn Leu Phe Val Met
    290                 295                 300

Ala Val Phe Gly Gln Ala Phe Tyr Gln Lys Thr Asn
                305                 310

Gln Ala Ala Phe Asn Ile Cys Ala Asn Ser Ser Leu
        315                 320

His Asp Tyr Ala Lys Ile Phe Pro Met Asn Asn Ala
325                 330                 335

Thr Val Ala Val Asp Ile Tyr Gln Gly Gly Val Ile
            340                 345

Leu Gly Cys Leu Phe Gly Pro Ala Ala Leu Tyr Ile
    350                 355                 360

Trp Ala Ile Gly Leu Leu Ala Ala Gly Gln Ser Ser
                365                 370

Thr Met Thr Gly Thr Tyr Ala Gly Gln Phe Val Met
        375                 380

Glu Gly Phe Leu Arg Leu Arg Trp Ser Arg Phe Ala
385                 390                 395
```

-continued

```
Arg  Val  Leu  Leu  Thr  Arg  Ser  Cys  Ala  Ile  Leu  Pro
                400                    405

Thr  Val  Leu  Val  Ala  Val  Phe  Arg  Asp  Leu  Arg  Asp
     410                    415                    420

Leu  Ser  Gly  Leu  Asn  Asp  Leu  Leu  Asn  Val  Leu  Gln
                425                    430

Ser  Leu  Leu  Leu  Pro  Phe  Ala  Val  Leu  Pro  Ile  Leu
          435                    440

Thr  Phe  Thr  Ser  Met  Pro  Thr  Leu  Met  Gln  Glu  Phe
445                    450                    455

Ala  Asn  Gly  Leu  Leu  Asn  Lys  Val  Val  Thr  Ser  Ser
                460                    465

Ile  Met  Val  Leu  Val  Cys  Ala  Ile  Asn  Leu  Tyr  Phe
     470                    475                    480

Val  Val  Ser  Tyr  Leu  Pro  Ser  Leu  Pro  His  Pro  Ala
                485                    490

Tyr  Phe  Gly  Leu  Ala  Ala  Leu  Leu  Ala  Ala  Ala  Tyr
          495                    500

Leu  Gly  Leu  Ser  Thr  Tyr  Leu  Val  Trp  Thr  Cys  Cys
505                    510                    515

Leu  Ala  His  Gly  Ala  Thr  Pro  Leu  Ala  His  Ser  Ser
                520                    525

His  His  His  Phe  Leu  Tyr  Gly  Leu  Leu  Glu  Glu  Asp
     530                    535                    540

Gln  Lys  Gly  Glu  Thr  Ser  Gly
                545
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 548 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met  Ile  Ser  Asp  Lys  Ser  Pro  Pro  Arg  Leu  Ser  Arg
                5                      10

Pro  Ser  Tyr  Gly  Ser  Ile  Ser  Ser  Leu  Pro  Gly  Pro
          15                     20

Ala  Pro  Gln  Pro  Ala  Pro  Cys  Arg  Glu  Thr  Tyr  Leu
25                     30                     35

Ser  Glu  Lys  Ile  Pro  Ile  Pro  Ser  Ala  Asp  Gln  Gly
                40                     45

Thr  Phe  Ser  Leu  Arg  Lys  Leu  Trp  Ala  Phe  Thr  Gly
     50                     55                     60

Pro  Gly  Phe  Leu  Met  Ser  Ile  Ala  Phe  Leu  Asp  Pro
                65                     70

Gly  Asn  Ile  Glu  Ser  Asp  Leu  Gln  Ala  Gly  Ala  Val
          75                     80

Ala  Gly  Phe  Lys  Leu  Leu  Trp  Val  Leu  Leu  Trp  Ala
85                     90                     95

Thr  Val  Leu  Gly  Leu  Leu  Cys  Gln  Arg  Leu  Ala  Ala
                100                    105
```

-continued

```
Arg  Leu  Gly  Val  Val  Thr  Gly  Lys  Asp  Leu  Gly  Glu
     110                    115                    120

Val  Cys  His  Leu  Tyr  Tyr  Pro  Lys  Val  Pro  Arg  Ile
                125                    130

Leu  Leu  Trp  Leu  Thr  Ile  Glu  Leu  Ala  Ile  Val  Gly
          135                    140

Ser  Asp  Met  Gln  Glu  Val  Ile  Gly  Thr  Ala  Ile  Ser
145                    150                    155

Phe  Asn  Leu  Leu  Ser  Ala  Gly  Arg  Ile  Pro  Leu  Trp
               160                    165

Gly  Gly  Val  Leu  Ile  Thr  Ile  Val  Asp  Thr  Phe  Phe
     170                    175                    180

Phe  Leu  Phe  Leu  Asp  Asn  Tyr  Gly  Leu  Arg  Lys  Leu
                185                    190

Glu  Ala  Phe  Phe  Gly  Leu  Leu  Ile  Thr  Ile  Met  Ala
          195                    200

Leu  Thr  Phe  Gly  Tyr  Glu  Tyr  Val  Val  Ala  His  Pro
205                    210                    215

Ser  Gln  Gly  Ala  Leu  Leu  Lys  Gly  Leu  Val  Leu  Pro
               220                    225

Thr  Cys  Pro  Gly  Cys  Gly  Gln  Pro  Glu  Leu  Leu  Gln
     230                    235                    240

Ala  Val  Gly  Ile  Val  Gly  Ala  Ile  Ile  Met  Pro  His
                245                    250

Asn  Ile  Tyr  Leu  His  Ser  Ala  Leu  Val  Lys  Ser  Arg
          255                    260

Glu  Val  Asp  Arg  Thr  Arg  Arg  Val  Asp  Val  Arg  Glu
265                    270                    275

Ala  Asn  Met  Tyr  Phe  Leu  Ile  Glu  Ala  Thr  Ile  Ala
               280                    285

Leu  Ser  Val  Ser  Phe  Ile  Ile  Asn  Leu  Phe  Val  Met
     290                    295                    300

Ala  Val  Phe  Gly  Gln  Ala  Phe  Tyr  Gln  Gln  Thr  Asn
                305                    310

Glu  Glu  Ala  Phe  Asn  Ile  Cys  Ala  Asn  Ser  Ser  Leu
          315                    320

Gln  Asn  Tyr  Ala  Lys  Ile  Phe  Pro  Arg  Asp  Asn  Asn
325                    330                    335

Thr  Val  Ser  Val  Asp  Ile  Tyr  Gln  Gly  Gly  Val  Ile
               340                    345

Leu  Gly  Cys  Leu  Phe  Gly  Pro  Ala  Ala  Leu  Tyr  Ile
     350                    355                    360

Trp  Ala  Val  Gly  Leu  Leu  Ala  Ala  Gly  Gln  Ser  Ser
                365                    370

Thr  Met  Thr  Gly  Thr  Tyr  Ala  Gly  Gln  Phe  Val  Met
          375                    380

Glu  Gly  Phe  Leu  Lys  Leu  Arg  Trp  Ser  Arg  Phe  Ala
385                    390                    395

Arg  Val  Leu  Leu  Thr  Arg  Ser  Cys  Ala  Ile  Leu  Pro
               400                    405

Thr  Val  Leu  Val  Ala  Val  Phe  Arg  Asp  Leu  Lys  Asp
     410                    415                    420

Leu  Ser  Gly  Leu  Asn  Asp  Leu  Leu  Asn  Val  Leu  Gln
```

-continued

```
                  425                 430

Ser Leu Leu Leu Pro Phe Ala Val Leu Pro Ile Leu
        435                 440

Thr Phe Thr Ser Met Pro Ala Val Met Gln Glu Phe
445                 450                 455

Ala Asn Gly Arg Met Ser Lys Ala Ile Thr Ser Cys
              460                 465

Ile Met Ala Leu Val Cys Ala Ile Asn Leu Tyr Phe
     470                 475                 480

Val Ile Ser Tyr Leu Pro Ser Leu Pro His Pro Ala
                  485                 490

Tyr Phe Gly Leu Val Ala Leu Phe Ala Ile Gly Tyr
        495                 500

Leu Gly Leu Thr Ala Tyr Leu Ala Trp Thr Cys Cys
505                 510                 515

Ile Ala His Gly Ala Thr Phe Leu Thr His Ser Ser
              520                 525

His Lys His Phe Leu Tyr Gly Leu Pro Asn Glu Glu
     530                 535                 540

Gln Gly Gly Val Gln Gly Ser Gly
                  545
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 548 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Ser Gly Asp Thr Gly Pro Pro Lys Gln Gly Gly
                  5                  10

Thr Arg Tyr Gly Ser Ile Ser Ser Pro Pro Ser Pro
        15                  20

Glu Pro Gln Gln Ala Pro Pro Gly Gly Thr Tyr Leu
25                  30                  35

Ser Glu Lys Ile Pro Ile Pro Asp Thr Glu Ser Gly
              40                  45

Thr Phe Ser Leu Arg Lys Leu Trp Ala Phe Thr Gly
     50                  55                  60

Pro Gly Phe Leu Met Ser Ile Ala Phe Leu Asp Pro
                  65                  70

Gly Asn Ile Glu Ser Asp Leu Gln Ala Gly Ala Val
        75                  80

Ala Gly Phe Lys Leu Leu Trp Val Leu Leu Trp Ala
85                  90                  95

Thr Val Leu Gly Leu Leu Cys Gln Arg Leu Ala Ala
              100                 105

Arg Leu Gly Val Val Thr Gly Lys Asp Leu Gly Glu
     110                 115                 120

Val Cys His Leu Tyr Tyr Pro Lys Val Pro Arg Ile
                  125                 130

Leu Leu Trp Leu Thr Ile Glu Leu Ala Ile Val Gly
        135                 140
```

-continued

```
Ser  Asp  Met  Gln  Glu  Val  Ile  Gly  Thr  Ala  Ile  Ala
145                     150                     155

Phe  Ser  Leu  Leu  Ser  Ala  Gly  Arg  Ile  Pro  Leu  Trp
                160                     165

Gly  Gly  Val  Leu  Ile  Thr  Val  Val  Asp  Thr  Phe  Phe
     170                     175                     180

Phe  Leu  Phe  Leu  Asp  Asn  Tyr  Gly  Leu  Arg  Lys  Leu
                185                     190

Glu  Ala  Phe  Phe  Gly  Phe  Leu  Ile  Thr  Ile  Met  Ala
          195                     200

Leu  Thr  Phe  Gly  Tyr  Glu  Tyr  Val  Val  Ala  Gln  Pro
205                     210                     215

Ala  Gln  Gly  Ala  Leu  Leu  Gln  Gly  Leu  Phe  Leu  Pro
                220                     225

Ser  Cys  Pro  Gly  Cys  Gly  Gln  Pro  Glu  Leu  Leu  Gln
     230                     235                     240

Ala  Val  Gly  Ile  Ile  Gly  Ala  Ile  Ile  Met  Pro  His
                245                     250

Asn  Ile  Tyr  Leu  His  Ser  Ser  Leu  Val  Lys  Ser  Arg
          255                     260

Glu  Val  Asp  Arg  Ser  Arg  Arg  Ala  Asp  Ile  Arg  Glu
265                     270                     275

Ala  Asn  Met  Tyr  Phe  Leu  Ile  Glu  Ala  Thr  Ile  Ala
                280                     285

Leu  Ser  Val  Ser  Phe  Leu  Ile  Asn  Leu  Phe  Val  Met
     290                     295                     300

Ala  Val  Phe  Gly  Gln  Ala  Phe  Tyr  Lys  Gln  Thr  Asn
                305                     310

Gln  Ala  Ala  Phe  Asn  Ile  Cys  Ala  Asp  Ser  Ser  Leu
          315                     320

His  Asp  Tyr  Ala  Pro  Ile  Phe  Pro  Arg  Asn  Asn  Leu
325                     330                     335

Thr  Val  Ala  Val  Asp  Ile  Tyr  Gln  Gly  Gly  Val  Ile
                340                     345

Leu  Gly  Cys  Leu  Phe  Gly  Pro  Pro  Ala  Leu  Tyr  Ile
     350                     355                     360

Trp  Ala  Val  Gly  Leu  Leu  Ala  Ala  Gly  Gln  Ser  Ser
                365                     370

Thr  Met  Thr  Gly  Thr  Tyr  Ala  Gly  Gln  Phe  Val  Met
          375                     380

Glu  Gly  Phe  Leu  Lys  Leu  Arg  Trp  Ser  Arg  Phe  Ala
385                     390                     395

Arg  Val  Leu  Leu  Thr  Arg  Ser  Cys  Ala  Ile  Leu  Pro
                400                     405

Thr  Val  Leu  Leu  Ala  Val  Phe  Arg  Asp  Leu  Arg  Asp
     410                     415                     420

Leu  Ser  Gly  Leu  Asn  Asp  Leu  Leu  Asn  Val  Leu  Gln
                425                     430

Ser  Leu  Leu  Leu  Pro  Phe  Ala  Val  Leu  Pro  Ile  Leu
          435                     440

Thr  Phe  Thr  Ser  Met  Pro  Ala  Leu  Met  Gln  Glu  Phe
445                     450                     455
```

-continued

```
Ala  Asn  Gly  Leu  Val  Ser  Lys  Val  Ile  Thr  Ser  Ser
                460                     465

Ile  Met  Val  Leu  Val  Cys  Ala  Val  Asn  Leu  Tyr  Phe
     470                     475                     480

Val  Ile  Ser  Tyr  Leu  Pro  Ser  Leu  Pro  His  Pro  Ala
                    485                     490

Tyr  Phe  Ser  Leu  Val  Ala  Leu  Leu  Ala  Ala  Ala  Tyr
          495                     500

Leu  Gly  Leu  Thr  Thr  Tyr  Leu  Val  Trp  Thr  Cys  Leu
505                     510                     515

Ile  Thr  Gln  Gly  Ala  Thr  Leu  Leu  Ala  His  Ser  Ser
                520                     525

His  Gln  Arg  Phe  Leu  Tyr  Gly  Leu  Pro  Glu  Glu  Asp
     530                     535                     540

Gln  Glu  Lys  Gly  Arg  Thr  Ser  Gly
                    545
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2271 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
GCTTGCCATG CCCGTGAGGG GCTGCCCGGC ACGCCAGCCA CTCGCACAGA               50

GAGTGCCCGA GCCTGCGGTC CTCATGTCAG GTGACACGGG CCCCCCAAAG              100

CAGGGAGGGA CCAGATATGG CTCCATCTCC AGCCCACCCA GTCCAGAGCC              150

ACAGCAAGCA CCTCCCGGAG GGACCTACCT AAGTGAGAAG ATCCCCATTC              200

CGGATACAGA ATCGGGTACA TTCAGCCTGA GGAAGCTGTG GGCCTTCACG              250

GGGCCTGGAT TCCTCATGAG CATCGCATTC CTGGACCCAG GAAACATTGA              300

GTCGGATCTT CAGGCTGGGG CTGTGGCTGG ATTCAAACTG CTCTGGGTGC              350

TGCTGTGGGC CACAGTGTTG GGCTTGCTTT GCCAGCGACT GGCTGCCCGG              400

CTGGGCGTGG TGACAGGCAA GGACTTGGGC GAGGTCTGCC ATCTCTACTA              450

CCCTAAGGTG CCCCGCATTC TCCTCTGGCT GACCATCGAG CTAGCCATCG              500

TGGGCTCAGA CATGCAGGAA GTCATTGGCA CAGCTATTGC ATTCAGTCTG              550

CTCTCCGCCG GACGAATCCC ACTCTGGGGT GGTGTCCTCA TCACCGTCGT              600

GGACACTTTC TTCTTCCTCT TCCTCGATAA CTACGGGTTG CGGAAGCTGG              650

AAGCCTTTTT TGGATTTCTT ATTACCATAA TGGCCTTGAC CTTCGGCTAT              700

GAGTACGTGG TGGCTCAGCC TGCTCAGGGA GCATTGCTTC AGGGCCTGTT              750

CCTGCCCTCG TGCCCAGGCT GTGGCCAGCC CGAGCTGCTG CAAGCCGTGG              800

GCATCATTGG CGCCATCATC ATGCCCCACA ACATCTACCT GCATTCCTCC              850

CTGGTCAAGT CTCGAGAGGT AGACCGGTCC CGGCGGGCGG ACATCCGAGA              900

GGCCAACATG TACTTCCTGA TTGAAGCCAC CATCGCCCTG TCTGTCTCCT              950

TCCTCATCAA CCTGTTTGTC ATGGCTGTCT TTGGGCAAGC CTTCTACAAG             1000

CAAACCAACC AGGCTGCGTT CAACATCTGT GCCGACAGCA GCCTCCACGA             1050
```

-continued

CTACGCGCCG ATCTTTCCCA GGAACAACCT GACCGTGGCA GTGGACATTT 1100

ACCAAGGAGG CGTGATCCTG GGCTGCCTCT TTGGTCCTCC AGCCCTGTAC 1150

ATCTGGGCCG TGGGTCTCCT GGCTGCTGGG CAGAGCTCCA CCATGACCGG 1200

CACCTACGCG GGACAGTTTG TGATGGAGGG CTTCCTGAAG CTGCGGTGGT 1250

CACGCTTCGC CCGAGTCCTG CTCACTCGCT CCTGCGCCAT CCTGCCCACT 1300

GTGCTCCTGG CTGTCTTCAG GGACTTGCGG GACCTGTCAG GCCTCAACGA 1350

CCTGCTCAAT GTGCTGCAGA GCCTGCTGCT TCCCTTCGCT GTGCTGCCCA 1400

TCCTCACCTT CACCAGCATG CCCGCCCTGA TGCAGGAGTT TGCCAATGGC 1450

CTGGTGAGCA AAGTTATCAC TTCCTCCATC ATGGTGCTGG TCTGCGCCGT 1500

CAACCTTTAC TTCGTGATCA GCTACTTGCC CAGCCTCCCC CACCCTGCCT 1550

ACTTCAGCCT TGTAGCACTG CTGGCCGCAG CCTACCTGGG CCTCACCACT 1600

TACCTGGTCT GGACCTGTCT CATCACCCAG GGAGCCACTC TTCTGGCCCA 1650

CAGTTCCCAC CAACGCTTCC TGTATGGGCT TCCTGAAGAG GATCAGGAGA 1700

AGGGGAGGAC CTCGGGATGA GCTCCCACCA GGGCCTGGCC ACGGGTGGAA 1750

TGAGTGGGCA CAGTGGCCTG TCAGACAAGG GTGTGTGTGT GTGTGTGTGT 1800

GTGTATGTGT GTGAAGGCAG CAAGACAGAC AGGGAGTTCT GGAAGCTGGC 1850

CAACGTGAGT TCCAGAGGGA CCTGTGTGTG TGTGACACAC TGGCCTGCCA 1900

GACAAGGGTG TGTGTGTGTG TGTGTGTGTG TGTGCATGCA CAGCAAGACG 1950

GAGAGGGAGT TCTGGAAGGC AGCCAACGTG AGTTCCATAG GGACCTGCTA 2000

TTTCCTAGCT CAGATCTCAG TGTTCTTGAC TATAAAATGG GGACACCTAC 2050

CTTGGAGTGG TTGTAAATAA GACACTTGAA CGCAGAGCCT AGCACTTCAG 2100

ATTTAAAAAC AAAAGAATCA TAATTCCAAA AGTTACTGAG CACTATCACA 2150

GGAGTGACCT GACAGACCCA CCCAGTCTAG GGTGGGACCC AGGCTCCAAA 2200

CTGATTTAAA ATAAGAGTCT GAAAATGCTA AATAAATGCT GTTGTGCTTA 2250

GTCCCCGAGA AAAAAAAAAA A 2271

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGTGTGTGT GTGTGTGTGT GTGTGTATGT GTGTGAAGGC AGCAAGACAG 50

ACAGGGAGTT CTGGAAGCTG GCCAACGTGA GTTCCAGAGG GACCTGTGTG 100

TGTGTGACAC ACTGGCCTGC CAGACAAGGG TGTGTGTGTG TGTGTGTGTG 150

TGTGT 155

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGGGTGTGT GTGTGTGTGT ATGTGTGTGA AGGCAGCAAG ACAGACAGGG 50

AGTTCTGGAA GCTGGCCAAC GTGAGTTCCA GAGGGACCTG TGTGTGTGTG 100

ACACACTGGC CTGCCAGACA AGGGTGTGTG TGTGTGTGTG TGTGTGTGTG 150

TGTGT 155

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGGGTGTGT GTGTGTGTGT GTATGTGTGT GAAGGCAGCA AGACAGACAG 50

GGAGTTCTGG AAGCTGGCCA ACGTGAGTTC CAGAGGGACC TGTGTGTGTG 100

TGACACACTG GCCTGCCAGA CAAGGGTGTG TGTGTGTGTG TGTGTGTGTG 150

TGTGT 155

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGGGGTGTGT GTGTGTGTGT GTGTATGTGT GTGAAGGCAG CAAGACAGAC 50

AGGGAGTTCT GGAAGCTGGC CAACGTGAGT TCCAGAGGGA CCTGTGTGTG 100

TGTGACACAC TGGCCTGCCA GACAAGGGTG TGTGTGTGTG TGTGTGTGTG 150

TGTGT 155

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGTGTGTGT GTGTGTGTGT GTGTGTATGT GTGTNNNNNN NNNNNNNNNN 50

NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNGTGTG 100

TGTGTNNNNN NNNNNNNNNN NNNNNNNNNG TGTGTGTGTG TGTGTGTGTG 150

TGTGT 155

(2) INFORMATION FOR SEQ ID NO:16:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAAGGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGGC CTGGCCACGG GTGGAATGAG 100

TGGGCACAGT GGCCTGTCAG ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT 150

ATGTGTGTGA AGGCAGCAAG ACAGACAGGG AGTTCTGGAA GCTGGCCAAC 200

GTGAGTTCCA GAGGGACCTG TGTGTGTGTG ACACACTGGC CTGCCAGACA 250

AGGGTGTGTG TGTGTGTGTG TGTGTGTGTG CATGCACAGC AAGACGGAGA 300

GGGAGTTCTG GAAGGCAGCC AACGTGAGTT CCATAGGGAC CTGCTATTTC 350

CTAGCTCAGA TCTCAGTGTT CTTGACTATA AAATGGGGAC ACCTACCTTG 400

GAGTGGTTGT AAATAAGACA CTTGAACGCA GAGCCTAGCA CTTCAGATT 449

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAAGGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGGC CTGGCCACGG GTGGAATGAG 100

TGGGCACAGT GGCCTGTCAG ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT 150

GTGAAGGCAG CAAGACAGAC AGGGAGTTCT GGAAGCTGGC CAACGTGAGT 200

TCCAGAGGGA CCTGTGTGTG TGTGTGTGTC TGGCCTGCCA GACAAGGGTG 250

TGTGTGTGTG TGTGTGTGTG TGTGTGTGTA CAGCAAGACG GAGAGGGAGT 300

TCTGGAAGGC AGCCAACGTG AGTTCCATAG GGACCTGCTA TTTCCTAGCT 350

CAGATCTCAG TGTTCTTGAC TATAAAATGG GGACACCTAC CTTGGAGTGG 400

TTGTAAATAA GACACTTGAA CGCAGAGCCT AGCACTTCAG ATT 443

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 445 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAAGGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGGC CTGGCCACGG GTGGGATGAG 100

TGGGCACAGT GGCCTGTCAG ACAAAGGGGT GTGTGTGTGT GTGTGTGTAT 150

GTGTGCGAAG GCAGCAAGAC AGACAGGGAG TTCTGGAAGC TGGCCAACGT 200

-continued

GAGTTCCAGA GGGACCTGTG TGTGTGTGAC ACACTGGCCT GCCAGACAAA 250

GGTGTGTGTG TGTGTGTGTG TGTGTGCATG CACAGCAAGA CGGAGAGGGA 300

GTTCTGGAAG GCAGCCAACG TGAGTTCCAT AGGGACCTGC TATTTCCTAG 350

CTCAGATCTC AGTGTTCTTG ACTATAAAAT GGGGACACCC ACCTTGGAGT 400

GGTTGTTAAT AAGACACTTG AACGCAGAAC CTAGCACCTC AGATT 445

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 401 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CACCAACGCT TCCTGTATGG GCTTCCTGGA GAGGATCAGG AGGAGGGGAG 50

GACCTCGGGA TGAACTCCCA CCAGGGCCTG GCCACGGGTG GGATGAGTGA 100

CCACAGTGGC CTGCCAGACA AGGGTGTGTG TGTGTGTGTG TGTGTGTGTG 150

TGTGTGCATG CACAGCAAGA TGGAGAGGGA GTTCACGGGT GGGATGAGTG 200

GGCACAGTGG CCTGCCAGAC AAGGGTGTGT GTGTGTGTGC ACGCACAGCA 250

AGATGGACAG GGAATTTTGG AAGCCGGCCA AGCCATAGGG ACCTGCTATT 300

TCCTAGCTCA GATCTCGGTA TTCTTGAGTA TTAAATGGGG ACACCTACCT 350

TGCAATGGTT GTAAATAAGA CACTTGAACG CAGAGCCTAG CACTTCAGAT 400

T 401

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 344 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAAGGG 50

GAGGACCTCA GGATGAGCTC CCACCAGGGC CTGGCCACGG GTGGAATGAG 100

TGGGCACAGT GGCCTGCCAG ACAAGGGTGT GTGTGTGTAT GTGTGTGTGT 150

GTGTGTGTGT GTGTGTGCGC GCTCACCCAC AACAAGACGG AGAGGGAGTT 200

CTGGAAGCCG GACAACGTGA GTTCCATAGG GACCTGCTGT TTCCTAGCTC 250

AGATCTCAGT GTTCTTGATT ATAAAATGGG GACACCTACC TTGCAACGGT 300

TGTAAATAAG ACACATTGGA ACGCAGAGGC TAGCACTTCA GATT 344

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 349 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGGAGAATG 50

GGAGGACCTC AGGATGAGCT CCCACCAGGA CCCTGCCACG GGTGGGATGA 100

GTGGGCACAG TGGCCTGCCA GACAAGGGTG TGTGTGTGTG TGTGTGTGTG 150

TGTGTGTGTG CGCGCGCGCG CGCGAGCGCT CACACACAGC AAGACAGAGA 200

GGGAGTTCTG GAAGCCGGAC GACGTGAGTT CCATAGGGAC CTGCTGTTTC 250

CTAGCTCATT CTTCACTATA AAATGGGGAC ACCTACCTTG CAATGGTTGT 300

AAATAAGAGT AAATAAGACA CTTGAATGCA GAGCCTAGCA CTTCAGATT 349

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAATGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGAC CCGGCCACGG GTGGGATGAG 100

TGGGCACAGT GGCCTGCCAG ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT 150

GTGTGTGTGT GTGTGCGCGC GCGCGCGCTC ACACACAGCA AGACAGAGAG 200

GGAGTTCTGG AAGCAGGACG ACGTGAGTTC CATAGGGACC TGCTGTTTCC 250

TAGCTCAGAT CTCAGTGTTC TTCACTATAA AATGGGGACA CCTACCTTGC 300

AATGGTTGTA AATAAGACAC TTGAATGCAG AGCCTAGCAC TTCAGATT 348

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 344 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAATGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGAC CCGGCCACGG GTGGGATGAG 100

TGGGCACAGT GGCCTGCCAG ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT 150

GTGTGTGTGT GCGCGCGCGC GCGCTCACAC ACAGCAAGAC AGAGAGGGAG 200

TTCCGGAAGC CGGACGACGT GAGTTCCATA GGGACCTGCT GTTTCCTAGC 250

TCAGATCTCA GTGTTCTTCA CTATAAAATG GGGACACCTA CCTTGCAATG 300

GTTGTAAATA AGACACTTGA ATGCAGAGCC TAGCACTTCA GATT 344

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 326 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAATGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGGC CCGGCCACGG GTGGGATGAG 100

TGGGCACAGT GGCCTGCCAG ACAAGGGGGT GTGTGTGTGT GTGTGCACGC 150

GCGCGCTCAC ACACAGCAAG ACAGAGAGGG AGTTCTGGAA GCAGGACGAC 200

GTGAGTTCCA TAGGGACCTG CTGTTTCCTA GCTCAGATCT CAGTGTTCTT 250

CACTATAAAA TGGGGACACC TACCTTGCAA TGGTTGTAAA TAAGACACTT 300

GAACGCAGAG CCTAGCACTT CAGATT 326

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 308 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAGGGG 50

GAGGACCTCA GGATGAGCTC CCACCAGGGC CTGGCCACGG GTGGGATGAG 100

TGGGCACAGT GGCCTGCCAG ACAAGGGTGT GTGTGTGGTC ACCCACAGCA 150

AGACGGAGAG GGAGTTCTGG AAGCCGGACA ACGTGAGTTC CATAGGGACC 200

TGCTGTTTCC TAGCTCAGAT CTCAGTGTTC TTGACTATAA AATGGGGACA 250

CCTACCTTGC AATGGTTGTA AATAAGACAC TTGAACGCAG AGCCTAGCAC 300

TTCAGATT 308

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 308 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GAAGAGGATC AGGAGAGGGG 50

GAGGACCTCG GGATGAGCTC CCACCAGGGC CTGGCCACAG GTGGGATGAG 100

TGGGCACAGT GGCTTGCCAG ACAAGGGTGT GTGTGTGGTC ACCCACAGCA 150

AGACGGAGAG GGAGTTCTGG AAGCCGGACA ACGTGAGTTC CATAGGGACC 200

TGCTGTTTCC TAGCTCAGAT CTCAGTGTTC TTGACTATAA AATGGGGACA 250

CCTACCTTGC AATGGTTGTA AATAAGACAC TAGAACGCAG AGCCTAGCAC 300

TTCAGATT 308

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 329 base pairs
(B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCCCACCAAC GCTTCCTGTA TGGGCTTCCT GGAGAGGATC AGGAGGAGGG 50

GAGGACCTCG GGATGAACTC CCACCAGGGC CCGGCCACGG GTGGGATGAG 100

TGACCACAGT GGCCTGCCAG ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT 150

GTCTGTGTGT GTGCGCGCGC ACACAGCAAG ATGGAGAGGG AATTCTGGAA 200

GCCGGCCAAG CCATAGGAGC CTGCTATTTC CTAGCTCAGA TCTTGGTATT 250

CTTGAGTATT AACTGGGGAC ACCTACCTTG CAATGGTTGT AAATAAGACA 300

CTTGAACGCA GAGCCTAGCA CTTCAGATT 329

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 548 amino acids
(B) TYPE:AMINO
(B) TYPE:AMINO
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Ser Gly Asp Thr Gly Pro Pro Lys Gln
5 10

Gly Gly Thr Arg Tyr Gly Ser Ile Ser Ser
15 20

Pro Pro Ser Pro Glu Pro Gln Gln Ala Pro
25 30

Pro Gly Gly Thr Tyr Leu Ser Glu Lys Ile
35 40

Pro Ile Pro Asp Thr Glu Ser Gly Thr Phe
45 50

Ser Leu Arg Lys Leu Trp Ala Phe Thr Gly
55 60

Pro Gly Phe Leu Met Ser Ile Ala Phe Leu
65 70

Asp Pro Gly Asn Ile Glu Ser Asp Leu Gln
75 80

Ala Gly Ala Val Ala Gly Phe Lys Leu Leu
85 90

Trp Val Leu Leu Trp Ala Thr Val Leu Gly
95 100

Leu Leu Cys Gln Arg Leu Ala Ala Arg Leu
105 110

Gly Val Val Thr Gly Lys Asp Leu Gly Glu
115 120

Val Cys His Leu Tyr Tyr Pro Lys Val Pro
125 130

Arg Ile Leu Leu Trp Leu Thr Ile Glu Leu
135 140

Ala Ile Val Gly Ser Asp Met Gln Glu Val
145 150

-continued

```
Ile  Gly  Thr  Ala  Ile  Ala  Phe  Ser  Leu  Leu
                  155                    160

Ser  Ala  Gly  Arg  Ile  Pro  Leu  Trp  Gly  Gly
                  165                    170

Val  Leu  Ile  Thr  Ile  Val  Asp  Ala  Phe  Phe
                  175                    180

Phe  Leu  Phe  Leu  Asp  Asn  Tyr  Gly  Leu  Arg
                  185                    190

Lys  Leu  Glu  Ala  Phe  Phe  Gly  Phe  Leu  Ile
                  195                    200

Thr  Ile  Met  Ala  Leu  Thr  Phe  Gly  Tyr  Glu
                  205                    210

Tyr  Val  Val  Ala  Gln  Pro  Ala  Gln  Gly  Ala
                  215                    220

Leu  Leu  Gln  Gly  Leu  Phe  Leu  Pro  Ser  Cys
                  225                    230

Pro  Gly  Cys  Gly  Gln  Pro  Glu  Leu  Leu  Gln
                  235                    240

Ala  Val  Gly  Ile  Ile  Gly  Ala  Ile  Ile  Met
                  245                    250

Pro  His  Asn  Ile  Tyr  Leu  His  Ser  Ser  Leu
                  255                    260

Val  Lys  Ser  Arg  Glu  Val  Asp  Arg  Ser  Arg
                  265                    270

Arg  Ala  Asp  Ile  Arg  Glu  Ala  Asn  Met  Tyr
                  275                    280

Phe  Leu  Ile  Glu  Ala  Thr  Ile  Ala  Leu  Ser
                  285                    290

Val  Ser  Phe  Leu  Ile  Asn  Leu  Phe  Val  Met
                  295                    300

Ala  Val  Phe  Gly  Gln  Ala  Phe  Tyr  Lys  Gln
                  305                    310

Thr  Asn  Gln  Ala  Ala  Phe  Asn  Ile  Cys  Ala
                  315                    320

Asn  Ser  Ser  Leu  Gln  Asp  Tyr  Ala  Pro  Ile
                  325                    330

Phe  Pro  Arg  Asn  Asn  Leu  Thr  Val  Ala  Val
                  335                    340

Asp  Ile  Tyr  Gln  Gly  Gly  Val  Ile  Leu  Gly
                  345                    350

Cys  Leu  Phe  Gly  Pro  Ala  Ala  Leu  Tyr  Ile
                  355                    360

Trp  Ala  Val  Gly  Leu  Leu  Ala  Ala  Gly  Gln
                  365                    370

Ser  Ser  Thr  Met  Thr  Gly  Thr  Tyr  Ala  Gly
                  375                    380

Gln  Phe  Val  Met  Glu  Gly  Phe  Leu  Lys  Leu
                  385                    390

Arg  Trp  Ser  Arg  Phe  Ala  Arg  Val  Leu  Leu
                  395                    400

Thr  Arg  Ser  Cys  Ala  Ile  Leu  Pro  Thr  Val
                  405                    410
```

-continued

```
Leu  Leu  Ala  Val  Phe  Arg  Asp  Leu  Arg  Asp
                   415                  420

Leu  Ser  Gly  Leu  Asn  Asp  Leu  Leu  Asn  Val
                   425                  430

Leu  Gln  Ser  Leu  Leu  Leu  Pro  Phe  Ala  Val
                   435                  440

Leu  Pro  Ile  Leu  Thr  Phe  Thr  Ser  Met  Pro
                   445                  450

Ala  Leu  Met  Arg  Glu  Phe  Ala  Asn  Gly  Leu
                   455                  460

Val  Ser  Lys  Val  Ile  Thr  Ser  Ser  Ile  Met
                   465                  470

Val  Leu  Val  Cys  Ala  Val  Asn  Leu  Tyr  Phe
                   475                  480

Val  Ile  Ser  Tyr  Val  Pro  Ser  Leu  Pro  His
                   485                  490

Pro  Ala  Tyr  Phe  Ser  Leu  Val  Ala  Leu  Leu
                   495                  500

Ala  Ala  Ala  Tyr  Leu  Gly  Leu  Thr  Thr  Tyr
                   505                  510

Leu  Val  Trp  Thr  Cys  Leu  Ile  Thr  Gln  Gly
                   515                  520

Ala  Thr  Leu  Leu  Ala  His  Ser  Ser  His  Gln
                   525                  530

Arg  Phe  Leu  Tyr  Gly  Leu  Pro  Glu  Glu  Asp
                   535                  540

Gln  Glu  Lys  Gly  Arg  Thr  Ser  Gly
                   545
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 587 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
GGGCTTCCTG AAGAGGATCA GGAGAAGGGG AGGACCTCGG GGATGAGCTC                50

CCACCAGGGC CTGGCCACGG GTGGGATGAG TGGGCACAGT GGCCTGTCAG               100

ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT GTGAAGGCAG CAAGACAGAG               150

ACGGAGTTCT GGAAGCTGGC CAACGTGAGT TCCAGAGGGA CCTGTGTGTG               200

TGTGTGTGAC ACACTGGCCT GCCAGACAAG GGTGTGTGTG TGTGTGTGTG               250

TGTGTGTGTG TGTGCATGCA CAGCAAGACA GAGAGGGAGT TCTGGAAGCC               300

AGCCAACGTG AGTTCCATAG GGACCTGCTA TTTCCTAGCT CAGATCTCAG               350

TGTTCTTGAC TATAAAATGG GGACACCTAC CTTGGAATGG TTGTAAATAA               400

GACACTTGAA CGCAGAGCCT AGCACTTCAG ATTTAAAAAC AAAAGAATCA               450

TAATTCCAAA AGTTACTGAG CACTATCACA GGAGTGACCT GACAGACCCA               500

CCCAGTCCAG GGTGGGACCC AGGCTCCAAA CTGATTTAAA ATAAGAGTCT               550

GAAAATGCTA AATAAATGCT GTTGTGCTTA GTCCCCG                             587
```

-continued

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 585 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGGCTTCCTG AAGAGGATCA GGAGAAGGGG AGGACCTCGG GGATGAGCTC 50

CCACCAGGGC CTGGCCACGG GTGGGATGAG TGGGCACAGT GGCCTGTCAG 100

ACAAGGGTGT GTGTGTGTGT GTGTGTGTGT GAAGGCAGCA AGACAGAGAC 150

GGAGTTCTGG AAGCTGGCCA ACGTGAGTTC CAGAGGGACC TGTGTGTGTG 200

TGTGTGACAC ACTGGCCTGC CAGACAAGGG TGTGTGTGTG TGTGTGTGTG 250

TGTGTGTGTG TGCATGCACA GCAAGACAGA GAGGGAGTTC TGGAAGCCAG 300

CCAACGTGAG TTCCATAGGG ACCTGCTATT TCCTAGCTCA GATCTCAGTG 350

TTCTTGACTA TAAAATGGGG ACACCTACCT TGGAATGGTT GTAAATAAGA 400

CACTTGAACG CAGAGCCTAG CACTTCAGAT TTAAAAACAA AAGAATCATA 450

ATTCCAAAAG TTACTGAGCA CTATCACAGG AGTGACCTGA CAGACCCACC 500

CAGTCCAGGG TGGGACCCAG GCTCCAAACT GATTTAAAAT AAGAGTCTGA 550

AAATGCTAAA TAAATGCTGT TGTGCTTAGT CCCCG 585

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTGTGTGTGT GTGTGTGTGT ATGTGTGTNN NNNNNNNNNN NNNNNNNNNN 50

NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNG TGTGTGTGTN 100

NNNNNNNNNN NNNNNNNNNN NNNGTGTGTG TGTGTGTGTG TGTGTGTGT 149

What is claimed is:

1. A method of detecting polymorphisms in the genetic material of an artiodactyla animal being resistant to disease caused by intracellular parasites comprising:

utilizing analytical methods to identify sequences homologous to the polymorphism of SEQ ID NO: 11 wherein said polymorphism corresponds to resistance to disease caused by said intracellular parasites.

2. The method of claim 1 wherein said analytical method is sequence analysis.

3. The method of claim 1 wherein said analytical method is nucleic acid hybridization.

4. The method of claim 1 wherein said analytical method is PCR.

5. The method of claim 1 wherein said artiodactyla is an ungulate.

6. The method of claim 5 wherein said ungulate is a ruminant.

7. The method of claim 1 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

8. A method of detecting polymorphisms in the genetic material of an artiodactyla animal being susceptible to disease caused by intracellular parasites comprising:

utilizing analytical methods to identify sequences homologous to the polymorphism from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14 wherein said polymorphism corresponds to susceptibility to disease caused by said intracellular parasites.

9. The method of claim 8 wherein said analytical method is sequence analysis.

10. The method of claim 8 wherein said analytical method is nucleic acid hybridization.

11. The method of claim 8 wherein said analytical method is PCR.

12. The method of claim 8 wherein said artiodactyla is an ungulate.

13. The method of claim 12 wherein said ungulate is a ruminant.

14. The method of claim 8 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

15. A method of detecting polymorphisms in the genetic material of a bovine animal being resistant to disease caused by intracellular parasites comprising:

utilizing analytical methods to identify the polymorphism from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 31 wherein said polymorphism corresponds to resistance to disease caused by said intracellular parasites.

16. The method of claim 15 wherein said analytical method is PCR.

17. The method of claim 15 wherein said analytical method is PCR and utilizes at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO:1 and SEQ ID NO: 2.

18. The method of claim 17 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO:2.

19. The method of claim 15 wherein said analytical method is restriction digestion analysis.

20. The method of claim 15 wherein said analytical method is sequence analysis.

21. The method of claim 15 wherein said analytical method is single stranded conformational analysis.

22. The method of claim 15 wherein said analytical method is single stranded conformational polymorphism.

23. The method of claim 15 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

24. A method of detecting polymorphisms in the genetic material of a bovine animal being susceptible to disease caused by intracellular parasites comprising:

utilizing analytical methods to identify the polymorphism from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 32, and SEQ ID NO: 33 wherein said polymorphism corresponds to susceptibility to disease caused by said intracellular parasites.

25. The method of claim 24 wherein said analytical method is PCR.

26. The method of claim 24 wherein said analytical method is PCR and utilizes at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO:1 and SEQ ID NO: 2.

27. The method of claim 26 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO:2.

28. The method of claim 24 wherein said analytical method is restriction digestion analysis.

29. The method of claim 24 wherein said analytical method is sequence analysis.

30. The method of claim 24 wherein said analytical method is single stranded conformational analysis.

31. The method of claim 24 wherein said analytical method is single stranded conformational polymorphism.

32. The method of claim 24 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

33. A method for predicting the likelihood of a bovine animal being susceptible to disease caused by intracellular parasites comprising:

analyzing said bovine animal's genetic material by PCR for the presence of the genetic sequence of Nramp1 wherein the PCR utilizes at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2, the presence of said sequence of Nramp1 in situ being associated with susceptibility of said bovine animal to disease caused by intracellular parasites.

34. The method of claim 33 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO: 2.

35. The method of claim 33 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

36. A method for predicting the likelihood of a bovine animal being resistant to disease caused by intracellular parasites comprising:

analyzing said bovine animal's genetic material by PCR for the presence of the genetic sequence of Nramp1 wherein the PCR utilizes at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2, the presence of said sequence of Nramp1 in situ being associated with resistance of said bovine animal to disease caused by intracellular parasites.

37. The method of claim 36 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO:2.

38. The method of claim 36 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

39. A method of predicting the likelihood of a bovine animal being resistant to disease caused by intracellular parasites by identifying polymorphisms in the genetic material of a bovine animal comprising:

amplification of nucleic acid sequences with PCR utilizing at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2; and

analyzing said amplified nucleic acid sequences, wherein said amplified nucleic acid sequences which contain a sequence from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 31 correspond to a bovine animal resistant to said intracellular parasites.

40. The method of claim 39 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO:2.

41. The method of claim 39 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

42. A method of predicting the likelihood of a bovine animal being susceptible to disease caused by intracellular parasites by identifying polymorphisms in the genetic material of a bovine animal comprising:

amplification of nucleic acid sequences with PCR utilizing at least one primer comprising a sequence selected from the group consisting of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2; and

analyzing said amplified nucleic acid sequences, wherein said amplified nucleic acid sequences which contain a sequence from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 32 and SEQ ID NO: 33 corresponds to abovine animal susceptible to said intracellular parasites.

43. The method of claim 42 wherein the PCR utilizes both SEQ ID NO: 1 and SEQ ID NO:2.

44. The method of claim 42 wherein said disease caused by intracellular parasites is selected from the group consisting of brucellosis, tuberculosis, paratuberculosis and salmonellosis.

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