8-29-2000

*Sarcocystis Neurona* Diagnostic Primer and Its Use in Methods of Equine Protozoal Myeloencephalitis Diagnosis

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Fenger, Clara K.; Granstrom, David E.; Gajadhar, Alvin A.; and Dubey, Jitender P., "*Sarcocystis Neurona* Diagnostic Primer and Its Use in Methods of Equine Protozoal Myeloencephalitis Diagnosis" (2000).  
*Veterinary Science Faculty Patents*. 6.  
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SARCOCYSTIS NEURON DIAGNOSTIC PRIMER AND ITS USE IN METHODS OF EQUINE PROTOZOAL MYELOENCEPHALITIS DIAGNOSIS

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Assignee: University of Kentucky Research Foundation, Lexington, Ky.

Notice: This patent is subject to a terminal disclaimer.


Field of Search: 435/5, 6, 91, 2, 435/235.1, 8, 77, 78; 536/24.32


[54] Abstract

An amplification primer and probe which can be used in an in vitro diagnostic test for the presence of S. neurona in equine blood or cerebrospinal fluid. Sarcocystis neurona is responsible for the equine condition of protozoal myelitis. The amplification primer is seventeen nucleotides in length and complementary to a unique section of the small ribosomal subunit of Sarcocystis neurona. The primer encompasses nucleotide positions 1470-1487 of the small ribosomal subunit of S. neurona. The primer has the sequence 5' CCAATCGGACGCGGT SEQ ID NO:1.

10 Claims, 7 Drawing Sheets
OTHER PUBLICATIONS


Figure 1A

POS=604

S. neurona  ATAGTA-ACCGAACGGATCGCA-T-TAT  SEQ ID NO:8
S. muris    ATAGTA-ACCGAACGGATCGCA-C-TAT  SEQ ID NO:9
S. capricanis ATNTCG-CNNTCTGAGATCGCAGT-NAT  SEQ ID NO:10
S. arietcanis NTANNANTCCGNNNGGTTNANN-TNANT  SEQ ID NO:11
S. cnuazi    ATAGTA-ACCGAACGGATCGCA-AT-TAT  SEQ ID NO:12
S. gigantea  ATAGTA-NCNGAACGGATCGCATCATAT  SEQ ID NO:13
S. tenella   ATNGTNTTCCGTTCN-TTCGTCAT-TNT  SEQ ID NO:14
T. gondii    ATAGTA-ACCGAACGGGTCAGCTGTTGACT  SEQ ID NO:15
E. tenella   ATAGTA-ACCGAACGGATCGC-AN-GTT  SEQ ID NO:16
C. parvum    ATAAATA-AC'TTTNCGGATCACATTAAT  SEQ ID NO:17

POS=661

SN:    GCGATGGATCATTCA-AGTTTCT-GAC-CTATC-AGCT-TTC-GA-CGGTACTGTA  SEQ ID NO:18
SM:    GCGATAGATCATTCA-AGTTTCT-GAC-CTATC-AGCT-TTC-GA-CGGTACTGTA  SEQ ID NO:19
SCA:   GCGATNANNNTNNTCN-NGTITTCT-GNC-CTATCNGCT-TCNGN-CGGTACTGTA  SEQ ID NO:20
SA:    GCGGTTNGATTTACATTCA-TTGC-CTATCNAGCT-TTN-GANCNCGATGTN  SEQ ID NO:21
SCR:   GCGATAGATCATTCA-AGTTTCT-GAC-CTATC-AGCT-TTC-GA-CGGTACTGTA  SEQ ID NO:22
SG:    GCGATAGATCATTCA-AGTTTCT-GAC-CTATC-AGCT-TTC-NA-CGGTACTGTA  SEQ ID NO:23
ST:    GCGNNNGATNNNTNCTGANTNTCTNGACTCTATC-NGCT-T-C-G-CGGTACTGTA  SEQ ID NO:24
TG:    GCGACGGATCAATCA-AGTTTCT-GAC-CTATC-AGCTATTCA-NGCNACTGTA  SEQ ID NO:25
ET:    GCGATGGATCATTNA-AGNTTCT-GAC-CTATCNAGCT-TTC-GA-CGGTACTGTA  SEQ ID NO:26
CR:    GTGACATATCATCATTCA-AGTTTCT-GAC-CTATC-AGCT-AT-TA-GN-CGGTACTGTA  SEQ ID NO:27
Figure 1D

POS=2181
SN:    GAAAGCATTTGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
SM:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
SCA:   GAAAGCATTTGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
SA:    GAAAGCATTTGGCCAAGAGGTGTGGTCATNAATCAAGAAACGAAAAGTTAGGCCGCTC
SCR:   GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
SG:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
ST:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
TG:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
ET:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
CR:    GAAAGCATTAGGCCAAGAGGTGTGGTCATTAATCAAGAAACGAAAAGTTAGGCCGCTC
SEQ ID NO:68
SEQ ID NO:69
SEQ ID NO:70
SEQ ID NO:71
SEQ ID NO:72
SEQ ID NO:73
SEQ ID NO:74
SEQ ID NO:75
SEQ ID NO:76
SEQ ID NO:77

POS=2263
SN:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SM:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SCA:   GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SA:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SCR:   GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SG:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
ST:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
TG:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
ET:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
CR:    GAAGACGATCAATACGATACCCTCGTGCTGTTAACCTATGCGGATGCTAGGAG
SEQ ID NO:78
SEQ ID NO:79
SEQ ID NO:80
SEQ ID NO:81
SEQ ID NO:82
SEQ ID NO:83
SEQ ID NO:84
SEQ ID NO:85
SEQ ID NO:86
SEQ ID NO:87
Figure 1B

POS=2318
SN:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
SM:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
CA:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
SA:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
SCR:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
ST:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
TG:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
CT:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
CR:
ATAGGAAAACTCCTCTGCTCTCTGAGACACACCTATGAAAAT-
T
Figure 2

- T. gondii
  - S. neurna
  - S. muris
  - S. gigantea
  - S. cruzi
    - S. capracanis
      - S. arieticanis
      - S. tenella
  - C. parvum
- E. tenella
SARCOCYSTIS NEURONA DIAGNOSTIC PRIMER AND ITS USE IN METHODS OF EQUINE PROTOZOA MYELOENCEPHALITIS DIAGNOSIS

TECHNICAL FIELD

The present invention relates to an amplification primer and probe which can be used in an in vitro diagnostic test for the presence of S. neurona in equine blood or cerebrospinal fluid.

Sarcocystis neurona is responsible for the equine condition of protozoal myelitis. The amplification primer is seventeen nucleotides in length and complementary to a unique section of the small ribosomal subunit of Sarcocystis neurona, nucleotide positions 1470–1487. The primer has the sequence 5′ CCAATTCCGAGCCCGGTT-3′ (SEQ ID NO:1).

BACKGROUND

Equine protozoal myeloencephalitis (EPM) is a treatable, but often fatal, central nervous system (CNS) disease of equids. It has not been reported among horses originating outside the Western hemisphere. Several hundred cases occur in North America annually. Although EPM has been reported in ponies, donkeys and most breeds of horses, the greatest incidence has been among thoroughbreds, standardbreds, and quarter horses. (See references section below.) The disease occurs as a result of infection with Sarcocystis neurona. Merozoites multiply in neurons, leukocytes and vascular endothelial cells of the CNS resulting in perivascular mononuclear cell infiltration and necrosis of the neuropile. Antemortem diagnosis of EPM is difficult. Clinical signs vary dramatically, depending upon the location and severity of CNS lesions. The disease may mimic various neurological disorders of the horse. Clinopathologic data are frequently of little diagnostic value.

Some methods of diagnosing equine parasitic and other infections are known. For example, U.S. Pat. No. 4,740,456 to Kuhn et al. discloses immunological methods for diagnosing active human neurocysticercosis, including a serum test and a cerebrospinal fluid test. The test involves detecting an antigen or antigens of larval origin, specifically of Taenia solium larva.

U.S. Pat. No. 4,759,927 to Dutta discloses a vaccine against Potomac Horse Fever comprising deactivated E. Ristiä as the active agent. The patent also discloses an assay for detecting the presence of E. Ristiä antibodies.

U.S. Pat. No. 5,141,925 to Alroy et al. discloses a method for the prophylactic and therapeutic treatment of animals having a parasite to cause Coccidiosis.


“Evidence for Sarcocystis as the etiologic agent of equine protozoal myeloencephalitis”, Simpson et al., J Protozool (UNITED STATES) August 1980, 27 (3) p288–92, discloses the diagnosis of Equine protozoal myeloencephalitis (EPM) in 10 horses. By electron microscopy, schizonts were found in intact host cells of the spinal cords or, more frequently, free in the extracellular spaces. Developmental stages of schizonts differed morphologically, and the late stage of schizogyon was characterized by endopolygeny. These findings permitted tentative identification of the protozoon as a Sarcocystis sp. Free merozoites were present in the extra-
cellular spaces or in cells of the spinal cord. Pericytes of capillaries were most frequently parasitized by merozoites, but the cytoplasm of neurons, macrophages, intravascular and tissue neutrophils, and axons of myelinated nerve fibers also contained these organisms. The presence of parasites in the cytoplasm of tissue and circulating neutrophils suggest that this putative Sarcocystis sp. may have a hematogenous phase of infection.

“Immunohistochemical study to demonstrate Sarcocystis neurona in equine protozoal myeloencephalitis”, Hamir et al., J Vet. Diagn. Invest. (UNITED STATES) July 1993, 5 (3) p418–22; discloses a 5-year (1985–1989) retrospective immunohistochemical study was conducted using an avidin-biotin complex (ABC) immunoperoxidase method to demonstrate Sarcocystis neurona in histologically suspect cases of equine protozoal myeloencephalitis (EPM). Primary antibodies against S. neurona and S. cruzi were utilized for the ABC technique. The findings were compared with those from cases in which the organisms were detected by examination of hematoxylin and eosin (HE)-stained neuronal sections. HE-stained sections detected the presence of the organisms in 20% of the suspect cases; whereas the ABC technique confirmed the presence of S. neurona in 51% and 67% of the cases by S. neurona and S. cruzi antibodies, respectively. A review of clinical case histories showed that 21/47 (45%) of the EPM horses with parasites in the tissue sections had prior treatment with antiprotozoal drugs and/or steroids. Using the test results of S. neurona and S. cruzi as a standard reference, HE test sensitivity based on examination of up to 30 neuronal sections per case was only 25%, and test specificity was 91%.

“Equine protozoal myeloencephalitis: Antigen analysis of cultured Sarcocystis neurona merozoites", Granstrom et al., J. Vet. Diagn. Invest. (UNITED STATES) January 1993, 5(1) p 88–90; discloses antigens of cultured Sarcocystis neurona merozoites were examined using immunoblot analysis. Blotted proteins were probed with S. cruzi, S. mansoni, and S. neurona antiserum produced in rabbits, S. fayeri (pre- and post-infection) and S. neurona (pre- and post-inoculation) sera produced in horses, immune sera from 7 histologically confirmed cases of equine protozoal myeloencephalitis, and pre-suckle serum from a newborn foal. Eight proteins, 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S. neurona antiserum and/or immune serum from EPM-affected horses. Equine sera were titered by the indirect immunofluorescent antibody (IFA) method using air-dried, cultured S. neurona merozoites. Anti-Sarcocystis IFA titers were found in horses with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

“A five year (1985–1989) retrospective study of equine neurological diseases with special reference to rabies”, Hamir et al., J. Comp. Pathol. (ENGLAND) May 1992, 106 (4) p411–21; discloses a retrospective study of horses necropsied between 1985 and 1989 at a diagnostic laboratory of a veterinary school in North America is documented. In this investigation over 20 per cent of the horses had clinical neurological signs. Equine protozoal myeloencephalitis (caused by Sarcocystis neurona) and cervical stenotic myelopathy (wobbler syndrome) were the most common of these disorders. However, only four cases of equine rabies were diagnosed during the 5-year study. The gross microscopic and immunohistochemical findings from these rabies-positive horses are documented. Immunoperoxidase tests for detection of rabies antigen in another 35 horses with non-specific encephalitis/encephalopathy did not reveal any positive cases. Based on this investigation, it appears that...
immunoperoxidase is a valid method for diagnosis of rabies when fresh tissues are not available for the fluorescent antibody test.

“Characterization of Sarcoctysis neuroma from a thoroughbred with equine protozoal myeloencephalitis” by Bowman et al., appears in Cornell Vet. 1992, April 82(2):115, Cornell Vet. (UNITED STATES) January 1992, 82 (1) p41–52 and discloses morphological information for the syncytial stage of the eotrophic agent of equine protozoal myeloencephalitis, Sarcoctysis neuroma. A clinical description of the horse from which the organism was isolated and the methodology used to immunosuppress the horse in an attempt to increase parasite numbers are also given. The description includes microscopic details observed both with light and transmission electron microscopy. Mainly stages from tissue are illustrated, but information is also presented on the development of the organism after inoculation on to monolayers of bovine monocytes. It is believed that the large numbers of organisms observed in this horse were due to its having experienced antibody to a trichomycosis Sarcocystis spp. antigen which only detected antibodies to shared or cross reactive Sarcocystis spp. epitopes. Preliminary immunoblot screening of several groups of horses in central Kentucky indicated that many horses are exposed while few develop clinical signs. At present, there is no method to directly detect and differentiate S. neuroma from non-pathogenic Sarcoctysis in live animals.

There is a need in the art for a diagnostic method of detecting Equine protozoal myeloencephalitis (EPM). The primer/probe of the present invention provides the first diagnostic primer for the diagnosis of Equine protozoal myeloencephalitis (EPM) caused by the parasite Sarcoctysis neuroma.

**DISCLOSURE OF THE INVENTION**

The present invention provides an amplification primer/probe which can be used in an in vitro diagnostic test for the presence of S. neuroma in equine blood or cerebrospinal fluid. Sarcoctysis neuroma is responsible for the equine condition of protozoal myelitis.

The above and other objects of the invention will become readily apparent to those of skill in the relevant art from the following detailed description and figures, wherein only the preferred embodiments of the invention are shown and described, simply by way of illustration of the best mode of carrying out the invention. As is readily recognized the invention is capable of modifications within the skill of the relevant art without departing from the spirit and scope of the invention.

**BRIEF DESCRIPTION OF DRAWINGS**

FIGS. 1(A)–(E) shows the relatedness of a 450 nt sequence from SRSU of S. neuroma when compared to S. muriis, S. giganica, T. gondii, S. capicanis, S. araticianis, S. cruzi, S. tenella, E. tenella and C. parvum by comparison of sequences from the small ribosomal subunit of each.

FIG. 2 shows a phylogenetic tree depicting the relatedness of the complete gene sequence (1806 nt) of SRSU of S. neuroma when compared to S. muriis, S. giganica, T. gondii, S. capicanis, S. araticianis, S. cruzi, S. tenella, E. tenella and C. parvum by comparison of sequences from the small ribosomal subunit of each.

FIG. 3 shows a gel showing efficacy of the probe according to the present invention.

**DESCRIPTION OF THE INVENTION**

Sarcoctysis neuroma is the etiological agent of Equine protozoal myeloencephalitis (EPM) which was isolated and described in 1991. There are limited means available for antemortem detection of this protozoan in horses, and the life cycle and mode of transmission and pathogenesis of this disease remain unknown.

The gene sequence of the Sarcoctysis neuroma small ribosomal subunit (SRSU) was determined using polymerase chain reaction techniques and Sanger sequencing methods. The SRSU gene was found to be 1806 nucleotides (nt) in length and have a G/C content of 46%. The small ribosomal subunit gene is an ideal molecular marker because it contains both sequences which are highly conserved among species and sequences which are variable. Variable regions are used to determine genetic relatedness of protozoal organisms. For example, FIG. 1 shows the relatedness of a 450 nt sequence from SRSU of S. neuroma when compared to S. muriis, S. giganica, T. gondii, S. capicanis, S. araticianis, S. cruzi, S. tenella, E. tenella and C. parvum by comparison of sequences from the small ribosomal subunit of each. FIG. 2 shows a phylogenetic tree depicting the
relatedness of the complete gene sequence (1806 nt) of SRSU of *S. neurona* when compared to *S. muris*, *S. gigantea*, T. gondii, *S. capricornis*, *S. arrieticans*, *S. cruzi*, *S. tenella*, *E. tenella* and *C. parvum* by comparison of sequences from the small ribosomal subunit of each.

The present inventors have isolated an rDNA sequence which can be used as a species-specific diagnostic probe and primer for *Sarcocystis neurona*. The methodology for isolating the rDNA probe is set forth below.

**EXAMPLE 1**

**Parasite Isolation and DNA Preparation**

Merozoites of *S. neurona* (fifth isolate, strain SN5) were cultured in vitro from the spinal cord of an infected horse which was necropsied at the University of Kentucky Livestock Disease Diagnostic Center. The parasite was maintained in tissue culture in bovine monocyes (provided by C. A. Speer, Montana State University) supplemented with 10% neonatal bovine serum (BioWhittaker, Walkersville, Md.). Culture media were removed from the culture vials, centrifuged at 800xG for 20 min to pellet the cells, then resuspended in RPMI 1640 media (BioWhittaker, Walkersville, Md.). Culture media were removed from the culture vials, centrifuged at 800xG for 20 min to pellet the cells, then resuspended in RPMI 1640 media (BioWhittaker, Walkersville, Md.). The suspension of parasites and bovine monocyes was layered above 1 ml of Percoll (Sigma, St. Louis, Mo.) at a specific gravity of 1.070, and centrifuged at 400xG for 20 min. Merozoites were found in the pellet, while the bovine monocyes were found at the Hank's balanced salt solution-percoll interface.

Approximately 5x10⁶ merozoites were washed in 1 ml phosphate-buffered saline (PBS), then resuspended in 20 μL of PBS, and 200 μL of 50% Chelex-100 (Bio-Rad, Richmond, Calif.). *S. neurona* DNA was released by boiling this suspension for 5 min. The sample was subsequently quenched on ice for 5 min, and centrifuged. This supernatant was used in the polymerase chain reaction (PCR) for the amplification of the small ribosomal subunit gene.

**DNA Amplification**

The PCR (Saiki et al., 1988) was performed using eukaryotic specific universal amplification primers developed by Medlin et al., (1988). The described amplification protocol was modified to optimize DNA yield. The “hot start” technique (Mullis, 1991) was employed to limit mispriming at the onset of the reaction. Amplification primers, MgCl₂, and dNTP in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) were placed in 0.5 ml microcentrifuge tubes, and overlaid with 40 μl paraffin wax. Ten μl of Chelex supernatant and Taq polymerase (1.25 U, AmpliTaq, Perkin-Elmer, Norwalk, Conn.) in PCR buffer were overlaid on the wax, and the microcentrifuge tubes were placed in a thermocycler (Perkin-Elmer, Norwalk, Conn.). The final concentrations of reagents were 0.5 μM of each primer, 2 mM MgCl₂, and 500 μM of each deoxynucleotide.

Denaturation of the DNA in the reactions was accomplished by heating to 94° C. for 1 min. The annealing temperature was 55° C. for 1 min, and the elongation temperature was 72° C. for 2 min. These sequential incubations were repeated for 5 cycles, and then 30 additional cycles were performed at the same conditions, with the exception of an annealing temperature of 50° C. for 1 min. The final primer extension was continued for 7 min to permit complete elongation of all amplified product.

Cloning into a pT7Blue Vector

The PCR product was purified by ultrafiltration (Krowczynsky and Henderson, 1992) using microcon-100 microconcentrators (Amicon, Beverly, Mass.). The amplified product was directly ligated into a pT7Blue vector (Novagen, Madison, Wis.) and transformed according to the recommendations of the manufacturer. Transformed cells were selected by culturing on LB agar plates containing 50 μg/ml ampicillin and 15 μg/ml tetracycline. Colonies containing PCR product insert were selected using blue/white screening, by the addition of 40 μl of 20 mg/ml X-gal in dimethyl formamide, and 4 μl 200 mg/ml IPTG per 100 mm plate. Plates were incubated for 12 to 24 hours (Sambrook, Fritsch and Maniatis, 1989). White colonies were screened for insert by PCR. Individual colonies were scraped from the plate, and diluted in 20 μl of sterile water in 1.5 ml microcentrifuge tubes. These were boiled for 2 min to release plasmid DNA, and 10 μl of supernatant was used in the PCR protocol described above. Twelve positive clones were identified in this manner, and two were arbitrarily chosen for sequencing.

**Dideoxynucleotide Sequencing and Analysis**

Small scale plasmid purifications were performed for two positive clones, by a protocol modified from Sambrook et al. (1989). Alkaline denaturation of these plasmid solutions was performed to yield single stranded template. Sanger sequencing reactions were performed using these templates. Primers used for sequencing included primers complementary to regions of the vector flanking the insert, and also synthetic primers complementary to conserved regions of the eukaryotic small ribosomal subunit gene (Elwood et al., 1985). Additional internal primers (5′-GGAATTCCGTTCTATAATTGTTG3′ (SEQ ID NO:2) and 5′-GGTTCAGGCCCTGCGGACCAT3′ (SEQ ID NO:3) were designed from the partial sequence to determine the complete sequence.

The complete gene sequence of the small ribosomal subunit of *S. neurona* has 1806 nt. A specific 450 nt sequence fragment from SRSU of *S. neurona* was also obtained and is disclosed.

**EXAMPLE 2**

Various amplification primers for the SRSU gene which are species-specific for *Sarcocystis neurona* were tested. The amplification primer for *Sarcocystis neurona* SRSU with species specificity is seventeen nucleotides in length and complementary to a unique section of the small ribosomal subunit of *Sarcocystis neurona*. The unique amplification primer encompasses nucleotide positions 1470–1487. The primer has the sequence: 5′-CAATCCGGACCCGTT-3′ (SEQ ID NO:1). The primer/probe may be modified at the 3′ end by adding 1–5 nucleotides, such that the primer/probe maintains the function of the original probe and hybridizes specifically to *Sarcocystis neurona* small ribosomal subunit.

1470R Ampli. Primer: 5′ CCAATCCGGACCCGTT 3′ (SEQ ID NO:1) MW=5267

The 1470 R primer can be coupled to a detectable label and used as a diagnostic probe for the detection of *Sarcocystis neurona*. The detectable label may be selected from the group consisting of chromophores, fluorophores, chemiluminescent materials and radioisotopes. Alternatively the primer may be used as a PCR primer as set forth below.

**EXAMPLE 3**

The advent of DNA based diagnostic assays has facilitated rapid, accurate identification of micro-organisms through the use of species-specific nucleotide sequences. Generally, DNA probes have been developed from unique gene or intergenic sequences for DNA probes, or from
repetitive elements. In addition, development of the polymerase chain reaction (PCR) has greatly increased the speed at which new diagnostic assays can be developed. The time required to construct DNA libraries or clone specific DNA fragments has been reduced considerably by the ability to directly amplify the desired DNA region.

The methodology for the nested polymerase chain reaction (PCR) procedure used to conduct the diagnostic assay according to the present invention is set forth below.

Nested PCR Diagnostic Method

I. Required Reagents/Supplies/Equipment

A. Supplies:

Sterile 1.5 ml microfuge tubes
Sterile 0.5 ml microfuge tubes
Blue (first stage)
Yellow (second stage)
PCR wax beads (created by dropping 30 µl hot paraffin wax on tin foil)
forceps for wax beads
1.5 and 0.5 ml tube racks
styrofoam ice cooler
Ice
dedicated 1000 µl, 200 µl pipetman pipettors sterile, plugged tips for 1000 µl and 20 µl
Thermocycler (48 reactions)
Dedicated microcentrifuge
B. Reagents:

Millipore water, sterilized and in 1 ml aliquots (store in freezer)
DMSO, in 1 ml aliquots (store away from light in box)
Perkin-Elmer 10xPCR buffer
Perkin-Elmer dNTPs, 10 mM per each, mixed together to form a single dNTP mixture containing 2.5 mM each nucleotide
Perkin-Elmer Mg,Cl, 25 mM Primer 1470R (unique primer, reverse primer, first stage), 10 mM, 5' CCAATTCGGAGCCGCGGTCG-3(SEQ ID NO:1) Primer 1184F (forward primer, first stage), 10 mM
5' CCAAGGCGTGGAGCTGCG-3(SEQ ID NO:4) Primer 1055F (forward primer, second stage), 10 mM
5' GGTGTTGTTGTACATGGCCG-3(SEQ ID NO:5) Primer 1475R (reverse primer, second stage), 10 mM
5' GCCGCTGAGCCGAAC-3(SEQ ID NO:6) (Primers may be synthesized on Applied Biosystems DNA synthesizer)
Ethyridium Bromide
DNA 100 bp ladder

II. First Stage Reaction:

A. The nested PCR reaction with hot start technique is set up in several stages. First, the hot start technique requires a lower reaction including the primers and dNTPs in PCR buffer. The wax is overlaid and melted over the lower reaction mixture. The upper reaction containing the DNA template (in this case, cerebrosial fluid (CSF)) and Taq polymerase are placed in PCR buffer on top of the wax. A drop (20 to 40 µl) of sterile mineral oil is put on top to prevent excessive evaporation from the upper reaction mixture. The entire tube is then spun briefly to collect the fluid at the bottom of the tube.

B. Lower Reaction Mixture

<table>
<thead>
<tr>
<th>Reagents</th>
<th>'X 1' (µl)</th>
<th>Concentrations (µl)</th>
<th>'X 50'</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1184F</td>
<td>5.00</td>
<td>1 mM</td>
<td>250.00</td>
</tr>
<tr>
<td>P1470R</td>
<td>5.00</td>
<td>1 mM</td>
<td>250.00</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>8.00</td>
<td>4 mM</td>
<td>400.00</td>
</tr>
<tr>
<td>dNTPs</td>
<td>4.00</td>
<td>1 mM</td>
<td>200.00</td>
</tr>
<tr>
<td>10 x PCRB</td>
<td>2.50</td>
<td>1 X</td>
<td>125.00</td>
</tr>
<tr>
<td>(+MgCl₂)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>2.50</td>
<td>5%</td>
<td>125.00</td>
</tr>
<tr>
<td>mH₂O</td>
<td>3.00</td>
<td>—</td>
<td>150.00</td>
</tr>
<tr>
<td>Reaction Volume</td>
<td>30.00</td>
<td>—</td>
<td>1500.00</td>
</tr>
</tbody>
</table>

f = forward primer;  
r = reverse primer

The lower reaction mixture is made up on 50 reaction quantities in a 1.5 ml microcentrifuge tube. This mixture (labelled "#1", and dated) may be frozen for at least 1 month safely. When the nested reaction is to be performed, the tube is slowly thawed, mixed by pipetting up and down, then 30.0 µl aliquots are placed into 48 blue 0.5 ml microfuge tubes. A wax bead (PCR gem) is placed in the tube over the solution. The tubes are placed in the thermocycler at 80°C for 5 minutes to melt the wax, and then cooled to 4°C to harden the wax. Alternatively, the tubes may be placed in a water bath (>65°C) for about 5–10 minutes, then removed carefully and placed in a refrigerator for a few minutes.

C. Upper Reaction Mixture

<table>
<thead>
<tr>
<th>Reagents</th>
<th>'X 1' (µl)</th>
<th>Concentrations (µl)</th>
<th>'X 50'</th>
</tr>
</thead>
<tbody>
<tr>
<td>mH₂O</td>
<td>7.25</td>
<td>—</td>
<td>362.50</td>
</tr>
<tr>
<td>10 x PCRB</td>
<td>2.50</td>
<td>1 X</td>
<td>125.00</td>
</tr>
<tr>
<td>Taq</td>
<td>0.25</td>
<td>—</td>
<td>12.50</td>
</tr>
<tr>
<td>Template</td>
<td>10.00</td>
<td>—</td>
<td>125.00</td>
</tr>
<tr>
<td>Reaction Volume</td>
<td>20.00</td>
<td>—</td>
<td>500.00</td>
</tr>
</tbody>
</table>

The upper reaction mixture must be made up fresh, and chilled in ice throughout the procedure. The reagents must be added to the upper reaction mixture in order, so that the PCR buffer is in the solution before the Taq is added. The upper reaction mixture, not including the DNA template (CSF) is made up and then 10 µl aliquots are placed over the wax into the blue microcentrifuge tubes containing the lower reaction.

Forty clinical samples can be tested per run, with seven water controls and a positive S. neurona control. Preferably, about ten µl of each clinical cerebrospial fluid (CSF) is placed into a tube, and mixed with the upper reaction mixture by pipetting up and down. Care must be taken to avoid cross-contamination. The tubes are numbered 1 to 48, with numbers 1, 8, 15, 22, 29, 38, and 47 as water, or reagent controls. These should be done in order for internal control. Number 48 is the positive S. neurona control. The reagent controls will have the same water tube which was used for the reaction setup as the “DNA template” to control for the accidental carryover of PCR product into this water. Each reaction tube should then be overlaid with a drop of sterile mineral oil to avoid evaporation.

D. Thermal-cycle Profile

The tubes are placed in the thermal-cycler, and the amplification protocol follows:
Samples are removed, and the wax is pierced with a sterile pipette tip. Then, 10 µl of the product is used as the DNA template for the second stage reaction.

III. Second Stage Reaction

A. It is critical that no PCR carryover occur, and therefore, the entire procedure is done in a UV-clean area. Addition of the PCR product from the first reaction, is performed in a different area, so as not to risk contamination of “clean” area.

B. Lower Reaction Mixture

The lower reaction mixture is made up in 50 reaction quantities in a 1.5 ml microcentrifuge tube. This mixture (labelled “#2”, and dated) may be frozen for at least 1 month safely.

When the nested reaction is to be performed, the tube is slowly thawed, mixed by pipetting up and down, then 30.0 µl aliquots are placed into 48 yellow 0.5 ml microfuge tubes. A wax bead (PCR gen) is placed in the tube over the solution. The tubes are placed in the thermocycler at 80°C for 5 minutes to melt the wax, and then cooled to 4°C to harden the wax. Alternatively, the tubes may be placed in a water bath (>65°C) for about 5–10 minutes, then removed carefully and placed in a refrigerator for a few minutes.

C. Upper Reaction Mixture

The upper reaction mixture must be made up fresh, and chilled in ice throughout the procedure. The reagents are added to the upper reaction mixture in order, so that the PCR buffer is in the solution before the Taq is added.

FIG. 3, shows a gel showing efficacy of the probe according to the present invention. A detailed description of the gel lanes in FIG. 3 follow:

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Denaturing temp: 92°C</td>
<td>2 min</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 58°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>2-6</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 58°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>7-31</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 58°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>32</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 58°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>33</td>
<td>Soak temp: 4°C</td>
<td>Until removed</td>
</tr>
</tbody>
</table>

Ten µl of each blue tube (first stage) reaction is placed into a tube, and mixed with the upper reaction mixture by pipetting up and down. Care must be taken to avoid cross-contamination. The tubes are numbered 1 to 48, to correspond to the same tubes from which product will be used for template. Each reaction tube is then overlaid with a drop of sterile mineral oil to avoid evaporation.

D. Thermal-cycle Profile

The tubes are placed in the thermal-cycler, and the amplification protocol follows:

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Denaturing temp: 92°C</td>
<td>2 min</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 57°C</td>
<td>30 min</td>
</tr>
<tr>
<td>2-6</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 57°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>7-31</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 57°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>32</td>
<td>Denaturing temp: 92°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing temp: 57°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>33</td>
<td>Soak temp: 4°C</td>
<td>Until removed</td>
</tr>
</tbody>
</table>

Samples are removed, and the wax is pierced with a sterile pipette tip. Then, the PCR product is run out on a horizontal gel apparatus in a TAE 1% agarose gel at 100 V for about 2–3 hours. The band should be about 250 bp in length when compared to the DNA 100 bp latter. Presence of a band is determined by EtBr staining. Stained band indicates *Sarcocystis neurona* is present in specimen.

In an alternative embodiment, the conditions for PCR using the species specific primer of the present invention follow: PCR Conditions Lower reaction mix

<table>
<thead>
<tr>
<th>Reagents</th>
<th>'X 1' Concentrations (µl)</th>
<th>'X 50' Concentrations (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1055F</td>
<td>2.5</td>
<td>125.0</td>
</tr>
<tr>
<td>1470R</td>
<td>2.5</td>
<td>125.0</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>4.0</td>
<td>200.0</td>
</tr>
<tr>
<td>dNTPs</td>
<td>4.0</td>
<td>200.0</td>
</tr>
<tr>
<td>10 x PCRB</td>
<td>2.5</td>
<td>125.0</td>
</tr>
<tr>
<td>mpH₂O</td>
<td>7.25</td>
<td>362.50</td>
</tr>
<tr>
<td>10 x PCR</td>
<td>2.5</td>
<td>125.0</td>
</tr>
<tr>
<td>Taq</td>
<td>0.25</td>
<td>12.50</td>
</tr>
<tr>
<td>Template</td>
<td>10.00</td>
<td>—</td>
</tr>
<tr>
<td>Reaction Volume</td>
<td>20.00</td>
<td>500.00</td>
</tr>
</tbody>
</table>

FIG. 3, shows a gel showing efficacy of the probe according to the present invention. A detailed description of the gel lanes in FIG. 3 follow:
EXAMPLE 4

In an alternative embodiment a RAPD assay may be used to detect Sarcocystis neurona. Unlike standard PCR, the random amplified polymorphic DNA (RAPD) assay is a procedure which amplifies DNA fragments without the use of specific primers, thus circumventing the need for nucleotide sequence information and specific primer design.6,7 These random DNA fragments are often polymorphic among species and isolates, behave as dominant alleles, and are inherited in a Mendelian fashion. Therefore, not only can they be used in the same manner as restriction fragment length polymorphic (RFLP) markers to characterize an organism’s genome through genetic mapping, but also as a means to differentiate species or isolates.6,9

The present inventors utilize the RAPD assay as a means to differentiate four isolates of S. neurona from other coccidial species and have isolated a short DNA fragment which is useful as a specific DNA probe for S. neurona. Four isolates of Sarcocystis neurona from horses with equine protozoal myeloencephalitis and eight species of coccidia from the genera Sarcocystis, Toxoplasma or Eimeria were differentiated using the random amplified polymorphic DNA (RAPD) assay. A single, common 550-bp DNA fragment was amplified from the DNA of each S. neurona isolate using a 16 nucleotide universal primer (TGF): 5′-GCCAGAAGCGCCACAAA-3′ (SEQ ID NO:7).

Cross-hybridization analysis among S. neurona isolates showed that DNA fragments had at least partial sequence homology. The primer generated several DNA fragments, including a 550-bp DNA fragment, from S. cruzi, Eimeria falciformis, E. nieschulzi, E. alsatsa and E. bovis. DNA hybridization analyses indicated no sequence homology between these fragments and the 550-bp fragment generated from S. neurona. The S. neurona 550-bp DNA fragment also did not hybridize with genomic blots of various other coccidia. These results evidence that the S. neurona DNA fragment 550-bp fragment is also a species-specific probe for this parasite.

Diagnostic Assay

Four geographic isolates of S. neurona were used; SN 2 and SN 4 from California, SN 3 from Panama and SN 5 from Kentucky. Organisms were isolated from gross spinal cord lesions and grown in bovine monocyte (M617) cell cultures as originally described by Dubey et al.13 Merocysts were isolated and purified from cultures as previously described by Granstrom et al.3 DNA from S. neurona, S. cruzi, S. campestris, Toxoplasma gondii or bovine monocytes was extracted using proteinase K digestion and phenol/chloroform extraction as described by Gajdhar et al.14 DNA was extracted from Eimeria tenella, E. falciformis, E. nieschulzi, E. alsatsa or E. bovis as outlined previously.6 Each RAPD assay consisted of a 25-μl reaction volume which contained approximately 50 ng of DNA from each species or geographic isolate, 0.5 μl Taq DNA polymerase (Promega, Madison, Wis., USA) in 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 3.0 mM MgCl2, 0.01% gelatin, 0.1% Triton X-100, 0.4 μM primer (TGF; 5′-GCCAGAAGCGCCACAAA-3′ (SEQ ID NO:7) and 200 μM of each dNTP. The samples were overlaid with 25 μl of mineral oil and preincubated for 10 min at 94°C in a DNA Thermal Cycler model 480 (Perkin-Elmer Cetus Corporation, Norwalk, Conn., USA). This was followed by 40 cycles of 94°C for 30 s, 38°C for 30 s, 72°C for 45 s, as suggested by Yu and Pauls.15 After completion of the thermal cycling reaction, a final 10-min incubation at 72°C was performed.

DNA fragments generated by PCR were separated by electrophoresis on 1.2% agarose gels in borate buffer and visualized by staining with ethidium bromide.16 DNA fragments unique to S. neurona were isolated from agarose gels by band excision and purified using Quick (Qiagen, Chatsworth, Calif., USA). Putative DNA probe fragments were labelled with [α-32P]-dATP using Escherichia coli DNA polymerase I as described by Rigby et al.17 The putative DNA probe fragments were analyzed using standard hybridization conditions.18 DNA fragments were applied to Zeta probe GT membranes (BioRad, Hercules, Calif.) and baked in vacuo at 80°C for 2 h. Genomic DNA from S. cruzi, S. campestris, T. gondii, E. nieschulzi, E. alsatsa, E. vermiciformis, E. coli or bovine monocytes were digested with Pst I at 37°C for 2 h. The resulting DNA fragments were electrophoretically separated in a 0.75% agarose gel and transferred to Zeta probe GT membrane by capillary action.18

Hybridization analysis with putative S. neurona-specific DNA probes was conducted at 65°C for 12 h in 10 ml hybridization buffer (5× Denhardt's, 4× SSS, 0.1% SDS at 65°C, 30 min, and once in 0.5× SSS, 0.1% SDS for 30 min at the same temperature. The membranes were exposed to XAR-5 film at −70°C for 24 h using lighting plus intensifying screens.

Random amplified polymorphic DNA assays using primer TGF generated a single DNA fragment with an electrophoretic mobility corresponding to 550 bp in all S. neurona isolates. The amplification of only 15 minute amount of product was detectable from isolate 3. A DNA fragment with an identical electrophoretic mobility was also amplified from the DNA of S. campestris, E. falciformis, E. alsatsa and

Lanes
1 100 bp Ladder
2 1.0 μl pr, 2 mM MgCl2, 10 ng DNA Templ.
3 1.0 μl pr, 2 mM MgCl2, 40 ng DNA Templ.
4 1.0 μl pr, 2 mM MgCl2, 80 ng DNA Templ.
5 1.0 μl pr, 3 mM MgCl2, 10 DNA
6 1.0 μl pr, 3 mM MgCl2, 40 DNA
7 1.0 μl pr, 3 mM MgCl2, 80 DNA
8 2.5 μl pr, 1 mM MgCl2, 10
9 2.5 μl pr, 1 mM MgCl2, 40
*10 2.5 μl pr, 1 mM MgCl2, 80
11 2.5 μl pr, 2 mM MgCl2, 10
12 2.5 μl pr, 2 mM MgCl2, 40
13 2.5 μl pr, 2 mM MgCl2, 80
14 2.5 μl pr, 3 mM MgCl2, 10
15 2.5 μl pr, 3 mM MgCl2, 40
*16 2.5 μl pr, 3 mM MgCl2, 80
*17 3.0 μl pr, 2 mM MgCl2, 10
18 3.0 μl pr, 2 mM MgCl2, 40
19 3.0 μl pr, 2 mM MgCl2, 80
20 1.0 pr, 2 mM MgCl2, DMSO, 10
21 1.0 pr, 2 mM MgCl2, DMSO, 40
22 1.0 pr, 2 mM MgCl2, DMSO, 80
*23 2.0 pr, 3 mM MgCl2, DMSO, 10
*24 2.0 pr, 3 mM MgCl2, DMSO, 40
*25 2.0 pr, 3 mM MgCl2, DMSO, 80
*26 2.5 pr, 1 mM MgCl2, DMSO, 10
**27 2.5 pr, 1 mM MgCl2, DMSO, 40
**28 2.5 pr, 1 mM MgCl2, DMSO, 80
**29 2.5 pr, 2 mM MgCl2, DMSO, 10
**30 2.5 pr, 2 mM MgCl2, DMSO, 40
**31 2.5 pr, 2 mM MgCl2, DMSO, 80
**32 2.5 pr, 3 mM MgCl2, DMSO, 10
**33 2.5 pr, 3 mM MgCl2, DMSO, 40
**34 2.5 pr, 3 mM MgCl2, DMSO, 80.
E. bovis, but not from bovine monocyte cells. However, when the 550-bp DNA fragment from S. neurona (SN 2) was used as a probe, only those DNA fragments amplified from the S. neurona isolates hybridized.

Although 550-bp DNA fragments were also generated from other coccidia, the fragments did not share nucleotide sequence homology with S. neurona. Cross-species hybridization did not occur when the 550-bp DNA fragment from S. neurona (SN 2) was used to probe Southern transfers of genomic DNA isolated from bovine monocyte cells, Escherichia coli, E. vermiciformis, E. ahsta, E. nieschulzi, T. gondii, S. canepastris or S. cruzi.

The RAPD assay has been successfully used to differentiate among closely related organisms. It was found that it is possible to identify unique RAPD fragments which could be used as species-specific DNA probes.

In the present study, the RAPD assay differentiated S. neurona from S. cruzi and S. canepastris, as well as T. gondii and five Eimeria spp.

These data, among geographically distinct isolates of S. neurona, suggest that EPM is caused by a single organism. The 550-bp DNA fragment generated by the RAPD assay has been used to develop a species-specific probe for S. neurona. The recognition of a putative DNA probe has facilitated the development of a diagnostic assay for S. neurona based on DNA hybridization. Sarcocystis spp. have a heterogenous, predator-prey or scavenger-carrion life cycle. The definitive host(s) and true intermediate host(s) of S. neurona are not known. Sarcocystis of S. neurona, required for transmission to the definitive host, have not been observed in the horse. Consequently, the horse is considered an aberrant, dead-end host. Sarcocystis neurona probably cycles normally between two or more wildlife species. Although many species have been suggested as the true definitive host(s) of S. neurona, skunks, raccoons or opossums are the most probable. Similar to EPM, these species are broadly distributed but unique to the Western Hemisphere. Small rodents or birds are speculative but reasonable candidates for the true intermediate host(s) of S. neurona.

While the RAPD assay and resultant probes have immediate application for the differentiation of S. neurona from other equine coccidia, an equally important use is the ability to discern and solve the parasite's life cycle by testing Sarcocystis spp. from potential hosts. The availability of infective stages will permit the experiments necessary to develop effective prevention and control measures for EPM.

REFERENCES


In sum, the present invention provides a specific amplification primer/probe which can be used in an in vitro diagnostic test for the presence of S. neurona in equine blood or cerebrospinal fluid. Sarcozystis neurona is responsible for the equine condition of protozoal myeloitis. The purpose of the above description and examples is to illustrate some embodiments of the present invention without implying any limitation. It will be apparent to those of skill in the art that various modifications and variations may be made to the composition and method of the present invention without departing from the spirit or scope of the invention. All patents and publications cited herein are incorporated by reference in their entireties.
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

(2) INFORMATION FOR SEQ ID NO:3:

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

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GTCGTCGTCG CATGCGCG 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 16 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCGGTCGCG CAGAAC 16

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 18 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCACGACG CCCACAAA 18

(2) INFORMATION FOR SEQ ID NO:8:

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

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

ATAGTAAACC AACCGATGCG ATTAT 25

(2) INFORMATION FOR SEQ ID NO:9:

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

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

ATAGTAACCG AACGGATCGC ACTAT

25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 26 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

ATNTGCNNT CTNAGATCGC GATNAT

26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 27 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

NTNANTCC GNINNTGCN NNTNANT

27

(2) INFORMATION FOR SEQ ID NO:12:

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

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

ATAGTAACCG AACGGATCGC ATTAT

25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 27 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATAGTACCC AACGAGTCC ATCATAT

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGCTTCGCC GTTCCTCC CATTT

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATAGTAACCC AACGAGTCC GTGACT

(2) INFORMATION FOR SEQ ID NO:16:

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

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATAGTAACCC AACGAGTCC ANTTT

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
ATATAAACTT TACGATAC ATTAAAT

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GCATGGGAT ATTCAGGTTT CTCAGCTTAC AGCTTTGAC GUTACTCTA

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GCATAGAGAT ATTCAGGTTT CTCAGCTTAC AGCTTTGAC GUTACTCTA

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GCATNNNNNH NTNCHNNSTTT CTCHCTTATN HGCTTTCCNG HHGTAAGTGT A

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:21:
(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCCATAGCTTT CTGACCTATC AGCTTTCGAC GOTNCTUTA 49

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCCATAGCTTT CTGACCTATC AGCTTTCGAC GUTACTUTA 49

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCCNKGATN NNNTCGANTN TCTNGACTCT ATCNGGTTCG CGGNTGTA 50

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

GCCAOCCGATC ATTCAAGTTT CTGACCTATC AGCTATTGCA CGGNACTUTA 50
(2) INFORMATION FOR SEQ ID NO:26:
   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 50 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: DNA (genomic)
   (iii) HYPOTHETICAL: NO
   (iv) ANTI-SENSE: NO
   (x) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGATGAGAT ATTAAAGTTT CTGACCTATT TAGTTTGA CCGTAGNTA

(2) INFORMATION FOR SEQ ID NO:27:
   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 49 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: DNA (genomic)
   (iii) HYPOTHETICAL: NO
   (iv) ANTI-SENSE: NO
   (x) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTGACATATT TCAAGTTTT TAGTACCTAT AGCCTTACG GCCTTAGNTA

(2) INFORMATION FOR SEQ ID NO:28:
   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 51 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: DNA (genomic)
   (iii) HYPOTHETICAL: NO
   (iv) ANTI-SENSE: NO
   (x) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTGGACATCC GTGGCAAGTGA CGGCTAACGG GGAATTAGG GTCGATTCCG G

(2) INFORMATION FOR SEQ ID NO:29:
   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 51 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear
   (ii) MOLECULE TYPE: DNA (genomic)
   (iii) HYPOTHETICAL: NO
   (iv) ANTI-SENSE: NO
   (x) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTGGACATCC GTGGCAAGTGA CGGCTAACGG GGAATTAGG GTGATTCG G

(2) INFORMATION FOR SEQ ID NO:30:
   (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

TTGCTCCCG TGCGNTGNN NGGNHNNGG GNATTAGGG TGNNITCCG

50

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

TTNGGACTAGC CUGGCACTG CNGGNNNGC GGGNNATTAG GGGCNATTCC GG

52

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TTGCGTCCNC TNGCAGTGCG NCNGNNNGC GGGNNATTAGC GGGCNATTCCG C

49

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTGACTACC NTGGCAGNGA CGGGNNACCG GAATTTAGG TGNNATTCGG G

51

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(i) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TTGCTCNCHN TNGCAYTGNG GMMNNNGGCG AATCCGATG CGATCCGG

49

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TTGACTACC NGGCAANGA CCGTAACG GGAATNAGNN NNNNATTCGG N

51

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:36:

NTGCGCTACH NGNCASTGGA CGGNNACGG GGAATTAGGG TTCGATTCGG G

51

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TTGCGCTCAG NNCCCAATGA CCGTACNGG GGAATTAGGN NTGCGATTCGA G

52

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) SEQUENCE DESCRIPTION: SEQ ID NO:39:
AGAGGGACC TGAGAACG CTACCACAT TAAGGAAGGC AGCAGGCOCG CA 52

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) SEQUENCE DESCRIPTION: SEQ ID NO:39:
AGAGGGACC TGAGAACG CTACCACAT TAAGGAAGGC AGCAGGCOCG CA 52

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) SEQUENCE DESCRIPTION: SEQ ID NO:40:
AGAGGGACC TGAGNNCGG CTNCCNNTC TNNGGNNGC NGCNGGGCGGC CA 52

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) SEQUENCE DESCRIPTION: SEQ ID NO:41:
AGNNGGACC CTGAAANHGC GCTHCCACAT CTNNNNGGCS GCNGGGCCNC A 51

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
AGNGGGAGCC TGAGNNNGCGG CTCNCBNCNCT TNNNGNNNGC GNNBNNNNNB NN CA 52

(2) INFORMATION FOR SEQ ID NO:43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
AGAGGGAGCC TGAGAANNGCG CTACACATC TAAAGNGSCGA GCAGGCNCNCA A 51

(2) INFORMATION FOR SEQ ID NO:44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
AGAGGGAGCC TGAGNNNGCGG CTACACATC TNNNGNNNGCN GCNGGCNGCA A 51

(2) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
AGANMGAGNC TGAGAANCGG CTACACATC TAAGAAGGC AGAGGCNGCG CA 52

(2) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
AGAGGAAGCC TGAGAACG GCCTACNNATC TAAGGNNGGCG AGCAGCCCGC CA

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

AGAGGAAGCC TGAGAACG GCCTACACAT TAAGGGAGGC AAGCCGGGCA NCA

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACACTGGAAA TATATTCT TGTGATTGGA ATGATGGAA TCCAAACCCC TTT

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

ACACTGGAAA TTCAATTCT TGTGATTGGA ATGATGGAA TCCAAACCCC TTT

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ACACTGGAAA TTCAATTCT ATGATGGGA ATGATGGGA TTYBNCCCC TTT
(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 53 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ACNCTGGAAA TTTTNTTCTC NOTgATTTGAA ATgATgGAA ATgATgGAA TTTNNCCN C TTT 53

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 53 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ACACTGGAAA TTTTNTTCTC AGTgATTTgGT ATgANgGgNA ATgATgGAA ATgATgGAA TTTNNCCN C TTT 53

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 54 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ACgCNCTGGAAA ATgOTNTTTC TAGrGATTTGG AATgATGgGA ATgCTgNNCCg C TTg 54

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 53 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ACACTGGAAA TTTTNTTCTC AGTgATTTGAA ATgATgGAA ATgATgGAA TTTNNCCN C TTT 53

(2) INFORMATION FOR SEQ ID NO:55:

ACACTGGAAA TTTTNTTCTC AGTgATTTGAA ATgATgGAA ATgATgGAA TTTNNCCN C TTT
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDINESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:
ACACTGGAATTTGATCC ATGATGGAA ATGATGCAA TCCAAACCC TTT

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDINESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:
ATACAGGAGTTTATGCTT TTTAATGGAA ATGATGCAA TCCAAACCC TTN

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDINESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
ATACAGGAGTTTATGCTT TTTAATGGAA ATGATGCAA TCCAAACCC CTTT

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDINESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:
ATTAACTGG CATGAATGAA ATCCTGATATATTAAGGA CAAACTG TG

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single 
(D) TOPOLOGY: linear 

(ii) MOLECULE TYPE: DNA (genomic) 
(iii) HYPOTHETICAL: NO 
(iv) ANTI-SENSE: NO 
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ATTTAAGTTT CAGAGTGAAA ATCTTTAGAT TGTGAAAAC CAACTACTG C 51

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS: 
(A) LENGTH: 50 base pairs 
(B) TYPE: nucleic acid 
(C) STRANDEDNESS: single 
(D) TOPOLOGY: linear 

(ii) MOLECULE TYPE: DNA (genomic) 
(iii) HYPOTHETICAL: NO 
(iv) ANTI-SENSE: NO 
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATTTAAGTTN AGAGTGAAA ATCTTTAGATT TGTGAAAGAC GACCCTCTGC 50

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS: 
(A) LENGTH: 52 base pairs 
(B) TYPE: nucleic acid 
(C) STRANDEDNESS: single 
(D) TOPOLOGY: linear 

(ii) MOLECULE TYPE: DNA (genomic) 
(iii) HYPOTHETICAL: NO 
(iv) ANTI-SENSE: NO 
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATTTAAGTTN NAGAGTGAAA ATCTTTAGATT TGTGAAAGAC CAACTCTCTGC 52

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS: 
(A) LENGTH: 50 base pairs 
(B) TYPE: nucleic acid 
(C) STRANDEDNESS: single 
(D) TOPOLOGY: linear 

(ii) MOLECULE TYPE: DNA (genomic) 
(iii) HYPOTHETICAL: NO 
(iv) ANTI-SENSE: NO 
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATTTAAGTTN AGAGTGAAA TCTTTAGATT TGTGAAAGGC GACCCTCTGC 50

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS: 
(A) LENGTH: 51 base pairs 
(B) TYPE: nucleic acid 
(C) STRANDEDNESS: single 
(D) TOPOLOGY: linear 


(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ATTIACTGN NAGCATGGA ATTCTTAGAT TTGTAAGA CGAACACTGC

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 51 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ATTASACTGN NAGCATGGA ATTCTTAGAT TTGTAAGA CGAACACTGC

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 50 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

ATTIACTGN CAGCATGGA ATTCTTAGAT TTGTAAGA CGAACACTGC

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 52 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ATTIACTGN NAGCATGGA ATTCTTAGAT TTGTAAGA CGAACACTGC

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 51 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

ATTAAAGT CAGAGGTGAA ATTCTCTGAGT TTTAAAGGA CAAGACTAAG C

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GAAAGCATT GCCAAAGATG TTTCCATTAA TCAAGAACGA AAGTIAAGGG CTC

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GAAAGCATT GCCAAAGATG TTTCCATTAA TCAAGAACGA AAGTIAAGGG CTC

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GAAAGCATT GCCAAAGATG TTTCCATTAA TCAAGAACGA AAGNNNGGG CTC

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO
GAAAGCATT GCCAACAGTG TTTCATTAA TCAGAAACGA AAGTNNGGGN CTC

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(x) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GAAAGCATT GCCAACAGTG TTTCATTAA TCAGAAACGA AAGTNNGGGN CTC

(3) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(x) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GAAAGCATT GCCAACAGTG TTTCATTAA TCAGAAACGA AAGTNNGGGN CTC

(4) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(x) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GAAAGCATT GCCAACAGTG TTTCATTAA TCAGAAACGA AAGTNNGGGN CTC

(5) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(x) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GAAAGCATT GCCAACAGTG TTTCATTAA TCAGAAACGA AAGTNNGGGN CTC
(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRAND: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GAAAGGATTTGCCAGGATTTTCAATTAA AGATTTTAGG TTT

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRAND: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GAAAGGATTTGCCAGGATTTTCAATTAA AGATTTTAGG ATC

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRAND: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GAAAGGATCGATACGTTG TCTAATCTAA ATCCAAACTA TGCCGACTAG AG

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRAND: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GAAAGGATCGATACGTTG TCTAATCTAA ATCCAAACTA TGCCGACTAG AG

(2) INFORMATION FOR SEQ ID NO:80:


(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GAAGCCGATC AGATACTGCT GTAGCTTTAA CCATAA ACTA TCCTCCACTAG AG 52

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GAAGCCGATCA GATACTGCTC TAGCTTAAAC CCATA ACTAT GCTGACTAGA G 51

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GNAGCCGATC NGNTNNGCTC GTAGCTTCCN CCATNACTA TNCCTACTAG AG 52

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GAAGCCGATC AGATACTGCT GTAGCTTTAA CCATAA ACTA TGCGACTAG AG 52

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GNAGEGAGTH NGATGCCTGG TNGTCCTNHN CATNNHCTAT GNGCACTNG AG

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GAAGACGATC GATAACCTCG TGATCTTAAC CATAACTAT GGGAGATCA G

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GAAGACGATG GATAACCTGC GAAATCTCTTA CCATAACTTA TGCCGACTAG AG

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GAAGACGATG GATAACCTGC GTACTCTTTAA CCATAACTTA TGCCGACTAG AG

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:88:

ATAGGAAAAC GTCATTCCTG ACTTCTCCCTG CACCTTATGA GAAAT

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 45 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:89:

ATAGGAAAA A GTCATTCCTG ACTTCTCCCTG CACCTTATGA GAAAT

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 47 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:90:

ATAGGAAAAT GTCATTTCG TGCTCCTCCTC TNCACTTTAT GAGNNAT

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 47 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) SEQUENCE DESCRIPTION: SEQ ID NO:91:

ATAGGAAAAT GTCATTTCG TGCTCCTCCTC TNCACTTTAT GAGNNAT

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 47 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

ATAGGAAAT NTCAATTTCT GCCCTCTCCT NCACCCTTAAG AAAAAT

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ATAGGAAATG TCAATTTCT GCCCTCTCCA CCAATGAA AAT

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

ATAGGAAAT GYNATTTTNCC TNCTCTCCON NCNCCTTAAG AAAAAT

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

ATAGGAAATG CTCAATCTTG CCACTGACTCCTAATA AAGAAAAT

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
We claim:

1. A diagnostic primer consisting of nucleotide positions 1470-1487 of the small ribosomal subunit of Sarcocystis neurona.

2. The primer according to claim 1, wherein said primer has a sequence as shown in SEQ ID NO:1, 5'-CCATTCGGACCGGGT-3'.

3. The primer according to claim 1, wherein said primer is coupled to a detectable label.

4. The primer according to claim 3, wherein said detectable label is selected from the group consisting of chromophores, fluorophores, chemiluminescent materials and radioisotopes.

5. The primer according to claim 4, wherein said detectable label is a radioisotope.

6. A method for the diagnosis of Equine protozoal myeloencephalitis (EPM) comprising the steps of

   (a) contacting a equine cerebrospinal fluid specimen with a primer according to claim 3;

   (b) removing unreacted sample from the specimen; and

   (c) detecting the detectable label hybridized to the specimen, where the presence of a detectable label hybridized to the specimen is indicative of EPM.

7. A method for the diagnosis of Equine protozoal myeloencephalitis (EPM) comprising the steps of obtaining equine cerebrospinal fluid and testing for the presence of Sarcocystis neurona with the primer of SEQ ID NO:1, where the presence of Sarcocystis neurona is indicative of EPM.

8. A method for the diagnosis of Equine protozoal myeloencephalitis according to claim 7, wherein said step of testing for the presence of Sarcocystis neurona is testing by nested polymerase chain reaction.

9. A method for the diagnosis of Equine protozoal myeloencephalitis according to claim 8, wherein said nested polymerase chain reaction comprises two stages and employs the primer pairs a–d,

   a) primer 1470R, reverse primer, first stage,
   5' CCATTCGGACCGGGT-3(SEQ ID NO:1);

   b) primer 1184F, forward primer, first stage,
   5' CAGGCGTGAGCTGC-3(SEQ ID NO:4);

   c) primer 1055F, forward primer, second stage,
   5' GGTGGTGGTGCATGC-3(SEQ ID NO:5); and

   d) primer 1475R, reverse primer, second stage,
   5' GGGCTGCCCAGA-3(SEQ ID NO:6).

10. A method for the diagnosis of Equine protozoal myeloencephalitis (EPM) comprising the steps of obtaining equine cerebrospinal fluid and testing for the presence of Sarcocystis neurona with a ribosomal RNA primer or probe from Sarcocystis neurona a consisting of nucleotide positions 1470-1487 or derivatives thereof in which 1-5 nucleotides are added to the 3' end of the primer or probe, where the presence of Sarcocystis neurona is indicative of EPM.