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(54) **DETECTION D'ACIDE NUCLEIQUE UTILE AU DIAGNOSTIC**
(54) **DIAGNOSTIC DETECTION OF NUCLEIC ACIDS**

(57) Cette invention concerne des méthodes de dosage par hybridation d'acide nucléique sensibles qui permettent de détecter des acides nucléiques humains ciblés dans un échantillon biologique tel que des fluides acellulaires. Ces méthodes sont particulièremenet utiles dans le diagnostic précoce des maladies chroniques.

(57) This invention provides sensitive nucleic acid hybridization assay methods for the detection of target human nucleic acids in a biological sample, such as cellular fluids. The methods are particularly useful in early diagnosis of chronic illnesses.

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(54) Title: DIAGNOSTIC DETECTION OF NUCLEIC ACIDS

(57) Abstract

This invention provides sensitive nucleic acid hybridization assay methods for the detection of target human nucleic acids in a biological sample, such as cellular fluids. The methods are particularly useful in early diagnosis of chronic illnesses.

DIAGNOSTIC DETECTION OF NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation in part of USSN 60/026,762, filed October 4, 1996.

FIELD OF THE INVENTION

The invention relates to the detection of nucleic acids associated with disease states. In particular, the invention provides for the detection of nucleic acids in acellular biological fluids as diagnostic assays for chronic illnesses and infectious diseases. Also provided are therapeutic approaches to treating chronic illnesses.

BACKGROUND OF THE INVENTION

Chronic diseases such as cancer, autoimmune diseases, chronic fatigue syndrome and the like afflict millions of people throughout the world. It is known that environmental and other factors (*e.g.*, genotoxic compounds, infectious retroviruses, retroelements and the like) can directly disrupt and/or damage DNA and may play a role in the development of a number of chronic illnesses. The mechanisms by which damage to genetic material leads to the onset of these diseases is not well understood, however. It is known that certain sites in the genome (*e.g.*, fragile sites) are particularly susceptible to such modifications. For instance, it is known that the distribution of insertion sites for retroviruses and retroelements is not random and that fragile sites are often preferred (*see, e.g.*, Craigie *Trends in Genetics* 8:187 (June 1992); De Ambrosis *et al. Cancer Genet. Cytogenet.* 60:1-7 (1992); Durnam *et al.* and Romani *et al. Gene* 135:153-160 (1993)).

Fragile sites themselves are associated diseases. For instance, expansion of long of blocks of repeated CCG triplets together with methylation of CpG islands in particular fragile sites on the X chromosome have been linked to the fragile X syndrome, an inherited mental retardation (*see, e.g.*, Sutherland and *Richards, Proc. Nat. Acad. Sci. USA* 92:3636-3641 (1995).

The detection of nucleic acids from pathogens such as bacteria, parasites and viruses, is a commonly used method for diagnosis of disease. For instance, detection of viral sequences is useful in diagnosis of disease. Enteroviruses are a heterogeneous group of human pathogens and opportunistic agents responsible for a broad spectrum of diseases and make up a large genus within the family Picornaviridae. The genus includes

polioviruses, coxsackieviruses, echoviruses as well as a number of uncharacterized enteroviruses isolated from humans and other primates. For a review of taxonomy of Picornaviridae *see*, *Virus Taxonomy: Classification and Nomenclature of Viruses* Murphy *et al.*, eds (Springer Verlag, 1995).

Like other members of the picornaviridae, enteroviruses are small, single-stranded, nonenveloped RNA viruses. Enteroviruses are distinguished from other members of the picornaviridae by their stability in acid and their fecal-oral route of passage and transmission.

Polioviruses (which exist as at least three serotypes) are the most clinically significant of the enteroviruses worldwide, causing paralytic disease in children in developing countries. Non-polioenteroviruses (NPEV) are also responsible for large numbers of symptomatic infections each year. They are the most common etiologic agents of a number of illnesses including meningitis and nonspecific febrile illnesses. Recent reports have linked NPEV infection with chronic fatigue syndrome (Clements *et al. J. Med. Virol.* 45:156-161 (1995).

In developed countries, polioviruses have been controlled with the introduction of vaccines in the late 1950's. Vaccines typically contain either inactivated poliovirus, which is administered parenterally or live attenuated poliovirus, which is administered orally. The inactivated vaccines use tissue culture-derived poliovirus which has been inactivated, or killed with formaldehyde. Attenuated virus vaccines are prepared by passage of the virus in cell cultures until it loses its ability to cause the disease. Attenuated live virus replicates in the gut to induce a protective antibody response.

Virus used for these vaccines is typically cultured in African Green Monkey kidney cells. As noted above, a number of poorly characterized enteroviruses have been isolated from primates, including monkeys. Procedures are currently in place to identify monkey cells infected by other viruses (*e.g.*, SV40) before use in culturing polioviruses.

Understanding how these molecular changes lead to disease is not well understood in the art. Increased understanding of the cellular mechanisms, particularly changes in nucleic acids, that occur early in the pathogenesis of these diseases is important to development of useful therapies and diagnostic tools. In addition, identification of viruses, including enteroviruses, in polio vaccine preparations is important to ensure safety of polio vaccines. Moreover, the possibility that new viruses resulting from recombination

of poliovirus with other viruses from the monkey cells or the human gut is an obvious public health concern. The present invention addresses these and other concerns.

SUMMARY OF THE INVENTION

The present invention provides methods of screening for a disease state in a patient. The methods comprise providing a sample containing biological material (*e.g.*, biopsies) or biological fluids from the patient (*e.g.*, an acellular biological fluid such as serum or plasma) and contacting the sample with a nucleic acid which specifically hybridizes to a target nucleic acid sequence. The target nucleic acids are then detected. In some embodiments, the target nucleic acid includes sequences from a fragile site in the human genome, in particular, repetitive DNA. In some embodiments the target sequences are derived from *Alu* sequences in a fragile site. In other embodiments, the target nucleic acid may be a novel composite of microbial origin and in some cases human origin. The target nucleic acid is usually at least about 100 nucleotides in length, sometimes between about 500 and about 1500 nucleotides in length.

The methods are usually used to detect a chronic illness. Examples of chronic illnesses include cancers, such as multiple myeloma. Other diseases include autoimmune diseases, neurodegenerative diseases, heart diseases and the like..

In certain preferred embodiments, the target human nucleic acids are amplified (*e.g.*, by PCR). An exemplary target sequence is provided in SEQ ID NO:23. This sequence can be used in diagnosis of multiple myeloma.

The present invention further provides improved methods for detecting viral nucleic acids in biological samples and polio vaccine preparations. In one embodiment, the invention provides methods for detecting recombinant viral nucleic acids, which comprise nucleic acid sequences from a polio virus and a non-poliovirus, usually a non-polioenterovirus. The methods comprise contacting a biological sample suspected of containing the recombinant viral nucleic acid with a first primer which specifically hybridizes to a conserved sequence in a picornaviral genome and a second primer which specifically hybridizes to a poliovirus nucleic acid sequence. The presence of an amplified product which is a recombinant viral nucleic acid is then detected.

A number of primers may be used in the present invention. For instance, one or both the primers may be one that specifically hybridizes to a 5' nontranslated region

of an picornaviral genome. Since the 5' nontranslated region is conserved among picornaviruses, the primer will specifically hybridize to most picornaviruses, particularly enteroviruses. Primers PG01 and PG02 (as shown in SEQ. ID. No. 1 or SEQ. ID. No. 2) are conveniently used for this purpose. One or both of the primers may specifically hybridize to a P2-P3 region of a poliovirus genome. A preferred primer is one that specifically hybridizes to nucleotides 4922-4941 or nucleotides 5467-5487. Primers PG03 and PG04 (as shown in SEQ. ID. No. 3 or SEQ. ID. No. 4) are conveniently used for this purpose. One or both of the primers may also specifically hybridize to a P2 region of a poliovirus genome. A preferred primer is one that specifically hybridizes to nucleotides 4460-4478 or nucleotides 4634-4653. Primers PG07 and PG08 (as shown in SEQ. ID. No. 5 or SEQ. ID. No. 6) are conveniently used for this purpose. A preferred combination of primers is PG02 and PG03.

The methods may be carried out using a number of biological samples commonly used for clinical analysis of nucleic acids. A convenient sample is human serum, plasma, or white blood cells.

A number of methods may be used to detect the presence of the recombinant viral nucleic acid. In some embodiments, the detection is carried out using gel electrophoresis to identify an amplified fragment that is not present in a control sample known to contain only poliovirus nucleic acids. When the first primer selectively hybridizes to nucleotides 443-460 of a poliovirus genome (*e.g.*, PG02) and the second primer selectively hybridizes to nucleotides 4922-4941 of a poliovirus genome (*e.g.*, PG03) an amplified fragment of about 400 nucleotides in length can be used to detect the presence of a recombinant viral nucleic acid.

The invention also provides methods for detecting nonpoliovirus nucleic acids in a polio vaccine sample. The methods comprise contacting the vaccine sample with at least two primers which specifically hybridize to poliovirus nucleic acid sequences.

In these methods, one primer can be one that specifically hybridizes to a conserved sequence in an enteroviral genome, such as the 5' nontranslated region. Exemplary primers include those that specifically hybridize to nucleotides 163-178 or nucleotides 443-450. Such primers include PG01 and PG02 (as shown in SEQ. ID. No. 1 and SEQ. ID. No. 2).

A primer can also be one that specifically hybridizes to a sequence specific to a poliovirus genome, such as P2-P3 region of a poliovirus genome, for example, nucleotides 4922-4941 or nucleotides 5467-5487. Such primers include PG03 and PG04 (as shown in SEQ. ID. No. 3 and SEQ. ID. No. 4).

A primer can also be one that specifically hybridizes to a sequence specific to a poliovirus genome, such as the P2 region of a poliovirus genome, for example, nucleotides 4460-4478 or nucleotides 4634-4653. Such primers include PG07 and PG08 (as shown in SEQ. ID. No. 5 and SEQ. ID. No. 6).

In these methods, nonpoliovirus nucleic acids may be detected using gel electrophoresis to identify an amplified fragment that is not present in a control vaccine sample known to contain only poliovirus nucleic acids.

The invention further provides nucleic acid molecules from new, recombinant viruses identified here. The claimed molecules can be identified by their ability to hybridize to the exemplified sequences under stringent conditions, as defined below. The nucleic acids may be a complete viral genome, or fragments thereof. The nucleic acids may be isolated from a biological sample and may or may not be integrated in human chromosomal DNA.

DEFINITIONS

An "acellular biological fluid" is a biological fluid which substantially lacks cells. Typically, such fluids are fluids prepared by removal of cells from a biological fluid that normally contains cells (*e.g.*, whole blood). Exemplary processed acellular biological fluids include processed blood (serum and plasma), urine, saliva, sweat, tears, phlegm, cerebrospinal, semen, feces and the like.

An "archived nucleic acid sequence" is a chimeric sequence in human genomic DNA containing subsequences from other organisms, particularly pathogens such as bacteria (*e.g.*, members of the genera *Chlamydia, Mycoplasma, Neisseria, Treponema, Staphylococcus, Streptococcus,* and the like), parasites (*e.g.*, *Plasmodium falciparum, Pneumocystis carinii, Trichomonas, Cryptosporidium),* viruses (*e.g.*, herpes viruses, enteroviruses, polyoma viruses, poxviruses, such as Molluscum contagiosum viruses, retroviruses, such as HIV, and the like). Thus, when designing nucleic acids (*e.g.*, as probes or PCR primers) for detecting archived nucleic acids of the invention, sequences

based on the genome of these pathogens are conveniently used. Without wishing to be bound by theory, it is believed that archived nucleic acid sequences are usually inserted at fragile sites.

The term "biological sample", as used herein, refers to a sample obtained from an organism or from components (*e.g.*, cells) of an organism. The sample may be of any biological tissue or fluid. Frequently the sample will be a "clinical sample" which is a sample derived from a patient. Such samples include, but are not limited to, sputum, blood, serum, plasma, blood cells (*e.g.*, white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

A "chronic illness" is a disease, symptom, or syndrome that last for months to years. Examples of chronic illnesses include cancers (*e.g.*, multiple myeloma, leukemia, breast cancer, ovarian cancer, head and neck cancer, brain cancer, cervical cancer, testicular cancer, prostate cancer, Hodgkins Disease, and the like), precancerous conditions (*e.g.*, adenomatous polyposis coli (APC)), chronic fatigue syndrome, autoimmune diseases (*e.g.*, arthritis, multiple sclerosis, lupus, scleroderma, and the like) diabetes, asthma, heart disease, neuromuscular diseases (*e.g.*, fibromyalgia), neurodegenerative diseases (*e.g.*, ALS, Alzheimer's Disease, and Parkinson's Disease), AIDS, Persian Gulf War Related Illnesses and chronic hepatitis.

A "fragile site" is a locus within the human genome that is a frequent site of DNA strand breakage. Fragile sites are typically identified cytogenetically as gaps or discontinuities as a result of poor staining. Fragile sites are classified as common or rare and further divided according to the agents used to induce them. For a general description of fragile sites and their classification, *see,* Sutherland *GATA* 8:1961-166 (1991). Exemplified sequences disclosed here include sequences from viral genomes that have apparently been inserted into the human genome at a fragile site. Thus, fragile sites can contain "archived nucleic acid sequences" which result from a wide range of pathogens, including bacteria, parasites, and viruses.

A "target human nucleic acid" of the invention is a nucleic acid molecule derived from human genomic DNA (*e.g.*, chromosomal DNA, mitochondrial DNA, and other extrachromosomal DNA). As used herein human genomic DNA refers to germline DNA and may also include nucleic acids introduced into the individual as a result of

infection of the individual by a pathogenic microorganism (*e.g.*, exogenous viral DNA integrated into the genome after infection or through live virus infection). Thus, although target human nucleic acids of the invention are of human origin, they may nonetheless contain sequences shared by other pathogenic organisms, such as viruses. Such sequences are sometimes referred to here as human/viral chimeric sequences or "archived sequences". DNA "derived from" human genome DNA includes DNA molecules consisting of subsequences of the genomic DNA as well as RNA molecules transcribed from human genomic DNA.

The RNA molecules detected in the methods of the invention may be free, single or double stranded, molecules or complexed with protein. Such RNA molecules need not be transcribed from a gene, but can be transcribed from any sequence in the chromosomal DNA. Exemplary RNAs include small nuclear RNA (snRNA), mRNA, tRNA, and rRNA.

The terms "hybridize(s) specifically" or "specifically hybridize(s)" refer to complementary hybridization between an oligonucleotide (*e.g.*, a primer or labeled probe) and a target sequence. The term specifically embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired priming for the PCR polymerases or detection of hybridization signal.

"Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

The term "oligonucleotide" refers to a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, such as primers, probes, nucleic acid fragments to be detected, and nucleic acid controls. The exact size of an oligonucleotide depends on many factors and the ultimate function or use of the oligonucleotide.

The term "primer" refers to an oligonucleotide, whether natural or synthetic, capable of acting as a point of initiation of DNA synthesis under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand is induced, i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization (i.e., DNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. A primer is preferably a single-stranded

oligodeoxyribonucleotide sequence. The appropriate length of a primer depends on the intended use of the primer but typically ranges from about 15 to about 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to specifically hybridize with a template.

"Probe" refers to an oligonucleotide which binds through complementary base pairing to a subsequence of a target nucleic acid. It will be understood by one of skill in the art that probes will typically substantially bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes are typically directly labeled (*e.g.*, with isotopes or fluorescent moieties) or indirectly labeled such as with digoxigenin or biotin. By assaying for the presence or absence of the probe, one can detect the presence or absence of the target.

The term "regulatory sequence" refer to cis-acting sequences (either 5' or 3') necessary for efficient transcription of structural sequences (*e.g.*, open reading frames). These sequences include promoters, enhancers and other sequences important for efficient transcription and translation (*e.g.*, polyadenylation sites, mRNA stability controlling sequences and the like).

A "sequence specific to" a particular virus species or strain (*e.g.*, poliovirus) is a sequence unique to the species or strain, that is, not shared by other previously characterized species or strains. A probe or primer containing a sequence complementary to a sequence specific to a virus will typically not hybridize to the corresponding portion of the genome of other viruses under stringent conditions (e.g., washing the solid support in 2xSSC, 0.1% SDS at about 60°C, preferably 65°C and more preferably about 70°C).

The term "substantially identical" indicates that two or more nucleotide sequences share a majority of their sequence. Generally, this will be at least about 90% of their sequence and preferably about 95% of their sequence. Another indication that sequences are substantially identical is if they hybridize to the same nucleotide sequence under stringent conditions (see, e.g., Sambrook et al., *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1985). Stringent conditions are sequence-dependent and will be different in different

circumstances. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.2 molar at pH 7 and the temperature is at least about 60°C. For example, a nucleic acid of the invention or fragment thereof can be identified in standard filter hybridizations using the nucleic acids disclosed here under stringent conditions, which for purposes of this disclosure, include at least one wash (usually 2) in 0.2X SSC at a temperature of at least about 60°C, usually about 65°C, sometimes 70°C for 20 minutes, or equivalent conditions.

As used herein a "viral nucleic acid" is a nucleic acid molecule comprising nucleic acid sequences derived from viruses. Since as described below, the viral nucleic acids disclosed here are thought to be derived from recombination events, the viral nucleic acids of the invention may contain sequences derived from other microorganisms or from cellular sequences.

A nucleic acid comprising a "complete viral genome" is a nucleic acid molecule encoding all the polypeptide products required to construct a complete, infectious viral particle. For instance, in the case of enteroviruses, a complete viral genome would be a nucleic acid encoding all the protein products identified in Figure 1. As used herein a complete, infectious viral particle can be encoded by a sequence that is a full length genome, as well as a substantially full length (*e.g.*, 90%, preferably 95% complete) genome.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the genome structure and gene organization of enteroviruses. The filled circle at the 5' end is the genome-linked protein VPg (also referred to as the 3B gene product), followed by, the 5' non-translated region (5' NTR; solid line). The open box depicts the long ORF encoding the polyprotein that is followed by the 3' non-translated region (line) and a poly (A) track (angled line). The eventual cleavage products of the polyprotein are indicated by vertical lines in the boxes. The P1 region encodes the structural proteins 1A, 1B, 1C and 1D, usually referred to as VP4, VP2, VP3, and VP1, respectively.

Figure 2 is a bar graph showing the percentages of myeloma patients with active disease (either with or without the 700nt band).

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is based in part on the surprising discovery of novel human and archived nucleic acids in biological fluids. The detection of these previously undetected human nucleic acids is useful in the early diagnosis and continuous monitoring of diseases, particularly chronic illnesses. In addition, targetted destruction of cells from which these nucleic acids are being lost can be used to treat these diseases. The detection methods of the invention can also be used to monitor the success of treatment of disease.

In some embodiments of the invention the target sequences are sequences found in chromosomal fragile sites. Without wishing to be bound by theory, it is believed that nucleic acids in particular chromosomal regions (*e.g.*, fragile sites) are preferentially released from diseased or damaged cells early in or during the disease process. The nucleic acids can be released as a result of a number of events including contact with agents that create damage to cells, particular genetic material (genotoxic agents). Such events include integration and/or expression of viral DNA or retroelements, and contact with genotoxic agents such as aflatoxins, organophosphate poisons (*e.g.*, pesticides and nerve gas agents, nitrogen mustards), other chemical warfare agents, benzene, cigarette carcinogens, digoxins, dioxin, biotoxins, UV light, radioactive particles, and other cell damaging radiation exposures.

Repetitive DNA sequences are commonly associated with fragile sites. Thus, in some embodiments of the invention, repetitive sequences are detected in the invention. Exemplary repetitive sequences include *Alu* and Kpn families of repetitive DNA. Repetitive sequences can also be categorized into long interspersed elements (LINEs) and short interspersed elements (SINEs) (*see*, Wilkinson *et al.* in *The Retroviridae Vol. 3,* J.A. Levy (ed.), pp 465-535, Plenum Press, New York (1994)). Kpn elements are examples of LINEs, where as *Alu* elements are examples of SINEs. LINEs, unlike SINEs, contain open reading frames encoding proteins with reverse transcriptase activity. Both LINEs and SINEs are examples of retroposons, which are a subcategory of retroelement, that is, a transposable element in the genome that transposes via an RNA intermediate. Retroposons are distinguished from retrotransposons (also referred to as

human endogenous retroviruses or HERVs) by the absence of long terminal repeats (LTRs). The relationship between HERVs and various disease states as well as diagnostic detection of antibodies to HERV antigens is discussed in WO 95/32311.

In some embodiments of the invention, *Alu* sequences or elements are detected in the methods of the invention. *Alu* elements are present in 10^5 to 10^6 copies in the human genome. Each element is about 300 base pairs in length and includes a polyA tract at the 3' end. It is thought that the sequences are derived from a gene encoding the 7SL structural RNA, which is a component of the signal recognition particle located on the rough endoplasmic reticulum.

In some preferred embodiments, RNA molecules derived from *Alu* sequences from fragile sites are detected. In the example provided below, *Alu* sequences from a fragile site on the long arm of chromosome 22 (22q12-13) are detected. As shown below, detection of these sequences is associated with multiple myeloma. Translocations and other abnormalities have been associated this region with a number of diseases including schizophrenia (*see, e.g.*, Kalsi *et al. Am. J. Med. Genet.* 60:298-301 (1995)) and cancers (*see, e.g.*, Stenman *et al.*, *Int. J. Cancer* 62:398-402 (1995)).

As noted above, fragile sites may contain repeated sequences. Repeated sequences are known to contain sequences that bind nuclear proteins and are effective in regulating gene expression. Evidence indicates that mobile elements such as segments of repetitive DNA (*e.g.*, LTRs from retroviruses and *Alu* sequences) have inserted in various sites in the genome and have affected regulation of gene expression (*see, e.g.*, Britten *et al. Proc. Nat. Acad. Sci. USA* 93:9374-9377 (1996). Without wishing to be bound by theory it is believed that alteration of these sequences by insertion of retroelements or genotoxic agents may lead to altered expression of sequences within the genome.

The nucleic acids detected in the methods of the invention are typically from about 100 nucleotides to several thousand nucleotides in length. Usually, the nucleic acids are from about 200 to about 1500 nucleotides.

The present invention is also directed to the detection of non-poliovirus nucleic acids (NPVNA) and recombinants between polio and other viruses. In some embodiments that non-polioviruses are other members of the picornaviridae, such as non-polioenteroviruses (NPEV). In particular, the invention provides sensitive methods (*e.g.*, the polymerase chain reaction, PCR) for detecting NPVNA and recombinant viruses potentially derived from polio vaccines.

A schematic diagram of an enterovirus genome is provided in Figure 1. Enteroviruses contain one molecule of infectious, positive sense, ssRNA, typically between about 7 and about 8.5 kb in size. The genome comprises a 5' nontranslated region (5' NTR) of variable length followed by an ORF encoding the polyprotein precursor (240-250 Kd) to the structural proteins (P1) and the predominantly nonstructural proteins (P2, P3), followed by a short non-coding sequence and a poly (A) tract of variable length. Virion proteins include 60 copies each of the four capsid proteins, which are gene products of the P1 region (IA, IB, IC, ID), which are also referred to as VP4, VP2, VP3, VP1, respectively.

The complete nucleotide sequences of various enteroviruses are available in the scientific literature and in databases such as GenBank. Using this information, one of skill can design appropriate primers and probes targeting desired regions of the NPV or poliovirus genome. For instance, sequences of poliovirus types 1, 2 and 3 are available from GenBank Accession Numbers POLIOS1 (Sabin strain 1), PIPOLS2 (Sabin strain 2), POL3L12CG (Sabin strain 3). The sequences are also disclosed in Toyoda *et al.*, *J. Mol Biol* 174: 561-585, (1984).

The present invention is based in part on the surprising discovery of contaminating NPVNA in poliovirus vaccine preparations. The detection of these previously undetected viral components is clearly important to maintaining safe effective vaccines for poliomyelitis. In addition, the invention provides evidence suggesting that attenuated polioviruses in vaccine preparations may recombine with NPVNA present in the host gut or in the vaccine to produce new and potentially pathogenic viruses. Evidence provided below suggests the presence of such recombinants in Gulf War veterans diagnosed with Gulf War Syndrome. The occurrence of these recombinants is also detected in patients diagnosed with other diseases. Examples include multiple myeloma, prostate cancer, Parkinson's Disease, multiple sclerosis, and the like.

Selection of the primers used in the invention is based on what target sequences are being detected. In the case where contaminating NPEV are being detected (*e.g.*, in a poliovirus vaccine preparation) primers which specifically hybridize to any region of the enterovirus genome can be used. Typically, primers specific for conserved regions in the enterovirus genome are used. Examples of suitable target sequences are those present in the 5' nontranslated region of the genome. Exemplary primers for this purpose include primers which hybridize to nucleotides 163-178 or 443-460 of the poliovirus genome.

If NPV-poliovirus recombinants are being detected, a primer specific for poliovirus sequences is used in combination with a primer which hybridizes to sequences conserved in a picornaviral genome, for example an enteroviral genome. Polio-specific primers will typically hybridize to the genes encoding the polyprotein precursors P1, P2, and P3 in the poliovirus genome. Exemplary primers are those that hybridize to nucleotides 4460-4478, 4634-4653, 4922-4941, or 5467-5487 of the poliovirus genome.

The diagnostic methods of the invention typically rely on a method of amplifying the target nucleic acid from a biological fluid (*e.g.*, serum or plasma). PCR amplification of the target nucleic acid is typically used. One of skill will recognize, however, that amplification of target sequences in a sample may be accomplished by any known method, such as ligase chain reaction (LCR), Qβ-replicase amplification, transcription amplification, and self-sustained sequence replication, each of which provides sufficient amplification.

The PCR process is well known in the art and is thus not described in detail herein. For a review of PCR methods and protocols, *see*, *e.g.*, Innis, *et al.* eds. *PCR Protocols. A Guide to Methods and Application* (Academic Press, Inc., San Diego, CA. 1990). PCR reagents and protocols are also available from commercial vendors, such as Roche Molecular Systems.

In some embodiments of the invention, RNA molecules may be detected (*e.g.*, detection of enteroviral sequences). The detected RNA molecules may be also be RNA transcribed from genomic sequences, but which do not encode functional polypeptides. The first step in the amplification is the synthesis of a DNA copy (cDNA) of the region to be amplified. Reverse transcription can be carried out as a separate step, or in a homogeneous reverse transcription-polymerase chain reaction (RT-PCR), a

modification of the polymerase chain reaction for amplifying RNA. Methods suitable for PCR amplification of ribonucleic acids are described in Romero and Rotbart in *Diagnostic Molecular Biology: Principles and Applications* pp.401-406, Persing *et al.* eds., (Mayo Foundation, Rochester, MN 1993); Rotbart *et al.* U.S. Patent No. 5,075,212 and Egger *et al., J. Clin. Microbiol.* 33:1442-1447 (1995)).

The primers used in the methods of the invention are preferably at least about 15 nucleotides to about 50 nucleotides in length, more preferably from about 15 nucleotides to about 30 nucleotides in length.

To amplify a target nucleic acid sequence in a sample by PCR, the sequence must be accessible to the components of the amplification system. In general, this accessibility is ensured by isolating the nucleic acids from the sample. A variety of techniques for extracting nucleic acids, in particular ribonucleic acids, from biological samples are known in the art. As noted above, the samples of the invention are acellular biological fluids.

The first step of each cycle of the PCR involves the separation of the nucleic acid duplex formed by the primer extension. Once the strands are separated, the next step in PCR involves hybridizing the separated strands with primers that flank the target sequence. The primers are then extended to form complementary copies of the target strands. For successful PCR amplification, the primers are designed so that the position at which each primer hybridizes along a duplex sequence is such that an extension product synthesized from one primer, when separated from the template (complement), serves as a template for the extension of the other primer. The cycle of denaturation, hybridization, and extension is repeated as many times as necessary to obtain the desired amount of amplified nucleic acid.

In the preferred embodiment of the PCR process, strand separation is achieved by heating the reaction to a sufficiently high temperature for an sufficient time to cause the denaturation of the duplex but not to cause an irreversible denaturation of the polymerase (see U.S. Patent No. 4,965,188). Template-dependent extension of primers in PCR is catalyzed by a polymerizing agent in the presence of adequate amounts of four deoxyribonucleoside triphosphates (typically dATP, dGTP, dCTP, and dTTP) in a reaction medium comprised of the appropriate salts, metal cations, and pH buffering system. Suitable polymerizing agents are enzymes known to catalyze template-dependent DNA

synthesis. In the present invention, the initial template for primer extension is typically RNA. Reverse transcriptases (RTs) suitable for synthesizing a cDNA from the RNA template are well known.

PCR is most usually carried out as an automated process with a thermostable enzyme. In this process, the temperature of the reaction mixture is cycled through a denaturing region, a primer annealing region, and an extension reaction region automatically. Machine specifically adapted for this purpose are commercially available from Roche Molecular Systems.

The target human nucleic acids of the invention can also be detected using other standard techniques, well known to those of skill in the art. Although the detection step is typically preceded by an amplification step, amplification is not required in the methods of the invention. For instance, the nucleic acids can be identified by size fractionation (*e.g.*, gel electrophoresis). The presence of different or additional bands in the sample as compared to the control, is an indication of the presence of target nucleic acids of the invention. Alternatively, the target nucleic acids can be identified by sequencing according to well known techniques. Alternatively, oligonucleotide probes specific to the target nucleic acids can be used to detect the presence of specific fragments.

As explained in detail below, the size of the amplified fragments produced by the methods of the invention is typically sufficient to distinguish polioviruses from either NPV or poliovirus recombinants. Thus, in some embodiments of the invention, size fractionation (*e.g.*, gel electrophoresis) of the amplified fragments produced in a given sample can be used to distinguish poliovirus from other viruses of interest. This is typically carried out by amplifying a control containing known viruses (*e.g.*, isolated poliovirus) with the same primers used to amplify the sample of interest. After running the amplified sequences out in an agarose or polyacrylamide gel and labeling with ethidium bromide according to well known techniques (*see*, Sambrook *et al.*), the pattern of bands in the sample and control are compared. The presence of different or additional bands in the sample as compared to the control, is an indication of the presence of NPV or poliovirus recombinants.

Sequence-specific probe hybridization is a well known method of detecting desired nucleic acids in a sample comprising cells, biological fluid and the like. Under sufficiently stringent hybridization conditions, the probes hybridize specifically only to

substantially complementary sequences. The stringency of the hybridization conditions can be relaxed to tolerate varying amounts of sequence mismatch. If the target is first amplified, detection of the amplified product utilizes this sequence-specific hybridization to insure detection of only the correct amplified target, thereby decreasing the chance of a false positive caused by the presence of homologous sequences from related organisms or other contaminating sequences.

A number of hybridization formats well known in the art, including but not limited to, solution phase, solid phase, mixed phase, or *in situ* hybridization assays. In solution (or liquid) phase hybridizations, both the target nucleic acid and the probe or primer are free to interact in the reaction mixture. In solid phase hybridization assays, either the target or probes are linked to a solid support where they are available for hybridization with complementary nucleic acids in solution. Exemplary solid phase formats include Southern hybridizations, dot blots, and the like. *In situ* techniques are particularly useful for detecting target nucleic acids in chromosomal material (*e.g.*, in metaphase or interphase cells). The following articles provide an overview of the various hybridization assay formats: Singer *et al.*, *Biotechniques* 4:230 (1986); Haase *et al.*, METHODS IN VIROLOGY, Vol. VII, pp. 189-226 (1984); Wilkinson, *IN SITU* HYBRIDIZATION, D.G. Wilkinson ed., IRL Press, Oxford University Press, Oxford; and NUCLEIC ACID HYBRIDIZATION: A PRACTICAL APPROACH, Hames, B.D. and Higgins, S.J., eds., IRL Press (1987).

The hybridization complexes are detected according to well known techniques and is not a critical aspect of the present invention. Nucleic acid probes capable of specifically hybridizing to a target can be labeled by any one of several methods typically used to detect the presence of hybridized nucleic acids. One common method of detection is the use of autoradiography using probes labeled with 3H, 125I, 35S, 14C, or 32P, or the like. The choice of radioactive isotope depends on research preferences due to ease of synthesis, stability, and half lives of the selected isotopes. Other labels include compounds (*e.g.*, biotin and digoxigenin), which bind to antiligands or antibodies labeled with fluorophores, chemiluminescent agents, and enzymes. Alternatively, probes can be conjugated directly with labels such as fluorophores, chemiluminescent agents or enzymes. The choice of label depends on sensitivity required, ease of conjugation with the probe, stability requirements, and available instrumentation.

The probes and primers of the invention can be synthesized and labeled using well known techniques. Oligonucleotides for use as probes and primers may be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage, S.L. and Caruthers, M.H., 1981, Tetrahedron Letts., 22(20):1859-1862 using an automated synthesizer, as described in Needham-VanDevanter, D.R., *et al.* 1984, Nucleic Acids Res., 12:6159-6168. Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson, J.D. and Regnier, F.E., 1983, J. Chrom., 255:137-149.

The present invention also provide kits, multicontainer units comprising components useful for practicing the present method. A useful kit can contain probes for detecting the desired target nucleic acid, from either a recombinant virus or an NPV. In some cases, the probes may be fixed to an appropriate support membrane. The kit will also contain primers for RT-PCR. Other optional components of the kit include, for example, reverse-transcriptase or polymerase, the substrate nucleoside triphosphates, means used to label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the label is biotin), and the appropriate buffers for reverse transcription, PCR, or hybridization reactions. In addition to the above components, the kit can also contain instructions for carrying out the present method.

The invention provides methods of treating chronic illnesses. Generally, the therapeutic methods rely on therapies designed to significantly reduce the presence of acellular nucleic acids or to selectively destroy cells from which nucleic acids are being lost. In many cases, such cells are dysplastic, particularly in the case of cancers. Thus, compounds that can selectively destroy such cells can be used to inhibit the disease process. For instance, compounds that selectively induce apoptosis in target dysplastic or neoplastic cells can be used in this approach. Example of such compounds are sulindac-derived compounds such as sulindac sulfone, a non-steroidal anti-inflammatory drug. Sulindac, is a widely used arthritis drug and anti inflammatory agent which reduces the growth of colon polyps in patients with adenomatous polyposis coli (APC). The growth inhibitory effect of sulindac sulfone results from the ability of that compound to selectively augment cell death through apoptosis, rather than by arresting the cell cycle.

Any number of anti-neoplastic compounds and therapies known to those skilled in the art can be used in the present invention. Such compounds work by a number of

mechanisms including inhibition of purine or pyrimidine synthesis, inhibition of deoxyribonucleotide synthesis, cross-linkage of DNA, inhibition of microtubuke formation and the like. For a description of a variety of chemotherapeutic agents, *see*, *Principles of Internal Medicine* 12th ed. pp 1587-1599 Wilson *et al.* (eds.), McGraw-Hill, Inc. 1991)

Suitable pharmaceutical formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). A variety of pharmaceutical compositions comprising compounds and pharmaceutically acceptable carriers can be prepared.

Injectable preparations, for example, sterile injectable aqueous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectable.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The pharmaceutical compositions containing the compounds can be administered for therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to decrease and preferably cure or at least partially arrest the symptoms

of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the compound being administered, the severity of the disease, the weight and general state of the patient and the judgement of the prescribing physician.

5

Examples

Example 1

The following example provides the results of PCR studies of samples derived from Gulf War Veterans diagnosed with Gulf War Syndrome. The PCR conditions were generally those described in Egger *et al., J. Clin. Microbiol.* 33:1442-1447 (1995)). The primers used in the assays are summarized in Table 1, below. Table 1 also provides information about the map position, expected product and specificity of each primer. The 5' to 3' sequence of the primers used is as follows:

PG01 AAGCACTTCTGTTTCC (SEQ. ID. No. 1)

PG02 CATTCAGGGGCCGGAGGA (SEQ. ID. No. 2)

PG03 GAATGTGTAAGAACTGTCA (SEQ. ID. No. 3)

PG04 GTAAACAATGTTTCTTTTAGCC (SEQ. ID. No. 4)

PG07 CAGTTCAAGAGCAA(A/G)CACC (SEQ. ID. No. 5)

PG08 TC(A/G)TCCAT(A/G)AT(A/C)AC(T/C)AC(T/A)CC (SEQ. ID. No. 6)

Briefly, the amplifications were carried out used as follows. RNA from 0.25 ml of the sample (serum or plasma, preferably non-heparinized) was extracted using 0.75 ml of TRIZOL LS reagent (Gibco BRL, Gaithersburg, MD), and the RNA was precipitated with 10 μg of Rnase-free glycogen as a carrier. Both methods were performed according to the protocols of the manufacturer.

The precipitated RNA was washed once with 70% ethanol by centrifugation at 4°C, resuspended in 10μl of Rnase-free distilled water, and added to 17 μl of the RT mixture (GeneAmp RNA PCR kit; Perkin-Elmer, Norwalk, Conn.) containing $MgCl_2$(5mM), 1X PCR Buffer II, Rnase Inhibitor (2.5 U), MuLV Reverse Transcriptase (2.5 U), random hexamer primers (2.5 μM), and 1 mM each of dATP, dGTP, dCTP and dTTP. The mixture was incubated for 10 minutes at 22°C, 30 minutes at 42°C, 5 minutes at 95°C using a Perkin-Elmer Thermocycler. The RT mixture was then added to the top PCR mixture of a Hot Start PCR reaction using a melted Ampliwax bead (Perkin-Elmer, Norwalk, Conn.) as the barrier. The 70 μl top PCR mixture contains 1X PCR Buffer II and Amplitaq (2.5 U). The 30 μl bottom PCR mixture contains 1X PCR Buffer II, 2mM $MgCl_2$, and the appropriate primer pairs (15 μM). After 35 cycles (1 min at 94°C, 2 min at 48°C, and 1 min at 72°C), 8 μl of the PCR mixture was subjected to electrophoresis using a Pre-Cast 4-20% gradient or a 6% polyacrylamide gel in TBE Buffer (45 mM boric

acid, 1 mM EDTA) (NOVEX, San Diego, CA) for 45 minutes and 60 minutes, respectively, at 200 volts. After electrophoresis, the gel was stained in a 0.5μg/ml solution of ethidium bromide solution for 20 minutes and the bands were photographed under UV light.

TABLE 1 PRIMER SUMMARY

PRIMER	PRIMER REGION (Sabin genome)	MAP POSITION (NUCLEOTIDE #)	PRIMER LENGTH (# of bases)	EXPECTED PRODUCT LENGTH (base pairs)	SPECIFICITY
PG01	5'NTR	163-178	16	297 (about 300) if combined with PG02	Picornavirus
PG02	5'NTR	443-460	18	297 (about 300) when combined with PG01	Picornavirus
PG03	P2-P3 REGION	4922-4941	20	565 when combined with PG04	Polio Type 1 & 2
PG04	P2-P3 REGION	5467-5487	21	565 when combined with PG03	Polio Type 1 & 2
PG07	P2 REGION	4460-4478	19	193 (about 200) when combined with PG08	Polio Type 1, 2 & 3
PG08	P2 REGION	4634-4653	20	193 (about 200) when combined with PG07	Polio Type 1, 2 & 3

NOTE: PG04 & PG07 primer combination can produce a 1000 base pair PCR product

As can be seen in Table 2, the amplification using these primers led to a number of unexpected products. For instance, in the trivalent, oral polio vaccine (OPV) preparation (column 2), amplification using PG01 and PG02 (both specific to the 5' NTR) was expected to produce fragments of about 300 bp. Instead, a series of additional, unexpected products ranging in length from about 310 to about 460 bp were observed (lengths reported in Table 2 are lengths as determined by gel electrophoresis). Similar results were found when PG07 and PG08 were used. This result was not seen in the

inactivated polio vaccine (IPV) grown in human cells. The presence of these additional fragments are strong evidence that other contaminating viruses are present in the vaccine.

One amplified fragment of about 360 base pairs generated using PGO1 and PG02 was sequenced (SEQ. ID. No. 7). Sequence analysis revealed that the fragment may have arisen due to an inverted repeat with sequences from Sabin strain 1 and Sabin strain 2. A second fragment generated by these primers was also sequenced from four different clones (SEQ. ID. Nos. 8-11).

In addition, serum samples from Gulf War Veterans diagnosed with Persian Gulf War Related Illness (PGWRI) from one VA hospital showed unexpected bands using primers specific to the 5'NTR (Table 2, column 4). When these primers were used in combination with primers specific to poliovirus sequences a number of unexpected fragments were also seen. A control group of insurance applicants (Table 2, column 5) had a much lower occurrence and number of unexpected fragments. The occurrence of some unexpected fragments in this group indicates that some recombinants may also occur in this group, as well.

A particular 400 bp fragment, amplified by primers PG02 and PG03 was seen in 3 out of 3 serum samples from Gulf War veterans at the VA hospital in Martinez, California. This fragment was isolated and sequenced (SEQ. ID. Nos. 12-16)). The sequences in these samples showed no significant sequence identity with any known sequence. A second fragment of about 1200 basepairs was also sequenced (SEQ. ID. No. 17). A third fragment of about 750 basepairs was also found and sequenced from three different veterans (SEQ. ID. Nos. 18-20). Two other fragments have also been sequenced (SEQ. ID. Nos. 21-22). These results suggest that the amplified fragment contains sequences from an uncharacterized virus.

Unexpected bands have been observed in patients diagnosed with other diseases. For example, Table 2 shows results from patients with multiple sclerosis (MS) and prostate cancer.

TABLE 2
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		SABIN I LAB CONTROL 1 lot	OPV 5 lots	IPV 1 lot	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02	EXPECTED:	300	300	300	NONE	NONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	~310 357 ~380 ~410 463		760 (3/3) 1200 (3/3)	200 (9/10) 290 (2/10)			
PG03/PG04	EXPECTED:	565	565	NOT DONE	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	NONE		647 (1/3) 540 (3/3) ~600 (1/3) ~1500 (2/3)				
PG07/PG08	EXPECTED:	200	200	200	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER	NONE	210 290	NONE	200 (2/2) 750 (2/2) 750 (1/2) 1500 (2/2)				
PG02/PG03	EXPECTED:	NONE	NONE	NOT DONE	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	NONE		414 (3/3)				

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TABLE 2
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		SABIN I LAB CONTROL 1 lot	OPV 5 lots	IPV 1 lot	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02/ PG03/PG04	EXPECTED:	300 565	300 565	NOT DONE	NONE	NONE	300	NONE	NONE
	OTHER:	NONE	310 350 380 410 460		300 (7/23) 310 (1/23) 400 (12/23) 565 (7/23) 750 (4/23) 1200 (9/23)	200 (17/22) 290 (13/22) 350 (1/22) 310 (8/22)	210 (1/1)	100 (2/2) 200 (2/2) 300 (2/2) 310 (2/2) 350 (2/2) 400 (2/2) 650 (2/2) 750 (2/2)	200 (1/1) 350 (1/1) 380 (1/1) 400 (1/1) 450 (1/1) 500 (1/1) 800 (1/1) 300 (1/1) 560 (1/1)
PG01/PG02/ PG07/PG08	EXPECTED:	200 300 1000	NOT DONE	NOT DONE	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	NOT DONE		190 (1/1) 210 (1/1) 310 (1/1) 410 (1/1) 580 (1/1) 600 (1/1) 750 (1/1) 900 (1/1) 1500 (1/1)				

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TABLE 2
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		SABIN I LAB CONTROL 1 lot	OPV 5 lots	IPV 1 lot	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG03/PG04/ PG07/PG08	EXPECTED:	200 565	NOT DONE	NOT DONE	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	NOT DONE		190 (1/1) 210 (1/1) 310 (1/1) 410 (1/1) 250 (1/1) 550 (1/1) 580 (1/1) 750 (1/1) 1500 (1/1)				

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TABLE 2
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		SABIN I LAB CONTROL 1 lot	OPV 5 lots	IPV 1 lot	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02/ PG03/PG04/ PG07/PG08	EXPECTED:	200 300 565 (1000)	200 300 565	200 300 565	?	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	NONE	310 (5/5) 350 (5/5) 380 (5/5) 410 (5/5) 46 (5/5) 700 (5/5)	NONE	190 (1/1) 250 (1/1) 310 (1/1) 450 (1/1) 540 (1/1) 580 (1/1) 750 (1/1) 900 (1/1) 1500 (1/1)				

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Example 2

The following example provides the results of PCR studies of plasma samples derived from multiple myeloma patients. The primers used in the present studies were designed to amplify enteroviral sequence and were based on sequences of the enteroviral genome (Egger *et al., J. Clin. Microbiol.* 33:1442-1447 (1995)).

Materials and Methods

The primers used in the assays are summarized below.

PG01 AAGCACTTCTGTTTCC (SEQ. ID. No. 1)

PG02 CATTCAGGGGCCGGAGGA (SEQ. ID. No. 2)

The amplifications were carried out generally described above.

RESULTS

Amplification of nucleic acids in serum samples from four multiple myeloma patients produced the same amplicon of approximately 700 base pairs (SEQ ID NOs: 23-26). These sequences includes *Alu* sequences found at 22q12. The presence of the same nucleic acid in three different patients in different parts of the country is an indication that the detection of these sequences is important in the detection of myeloma and other diseases.

In addition, the same size band has been detected in 32 myeloma patients, 29 of whom had active disease. The band was not detected in an additional 31 myeloma patients, only 2 of whom had active disease. Finally, the band was not detected in 152 healthy controls. The results are presented graphically in Figure 2.

Example 3

As noted above, the sequences detected in myeloma patients were amplified using primers based on sequences in the enteroviral genome.

Amplifications using the primers of Example 1 were carried out as described above. The results are presented in Table 3. As can be seen in Table 3, the amplification using these primers led to a number of unexpected products.

TABLE 3
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02	EXPECTED:	NONE	NONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	760 (3/3) 1200 (3/3)	200 (9/10) 290 (2/10)			
PG03/PG04	EXPECTED:	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	647 (1/3) 540 (3/3) ~600 (1/3) ~1500 (2/3)				
PG07/PG08	EXPECTED:	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER	200 (2/2) 750 (2/2) 750 (1/2) 1500 (2/2)				

TABLE 3

PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG02/PG03 EXPECTED:	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
OTHER:	414 (3/3)				

TABLE 3
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02/PG03/PG04	EXPECTED:	NONE	NONE	300	NONE	NONE
	OTHER:	300 (7/23) 310 (1/23) 400 (12/23) 565 (7/23) 750 (4/23) 1200 (9/23)	200 (17/22) 290 (13/22) 350 (1/22) 310 (8/22)	210 (1/1)	100 (2/2) 200 (2/2) 300 (2/2) 310 (2/2) 350 (2/2) 400 (2/2) 650 (2/2) 750 (2/2)	200 (1/1) 350 (1/1) 380 (1/1) 400 (1/1) 450 (1/1) 500 (1/1) 800 (1/1) 300 (1/1) 560 (1/1)
PG01/PG02/PG07/PG08	EXPECTED:	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE

TABLE 3

PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR	VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
OTHER:	190 (1/1)				
	210 (1/1)				
	310 (1/1)				
	410 (1/1)				
	580 (1/1)				
	600 (1/1)				
	750 (1/1)				
	900 (1/1)				
	1500 (1/1)				

TABLE 3
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

PRIMER PAIR		VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG03/PG04/ PG07/PG08	EXPECTED:	NONE	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	190 (1/1) 210 (1/1) 310 (1/1) 410 (1/1) 250 (1/1) 550 (1/1) 580 (1/1) 750 (1/1) 1500 (1/1)				

TABLE 3
PRODUCT LENGTHS in base pairs (# of positive samples/total samples screened)

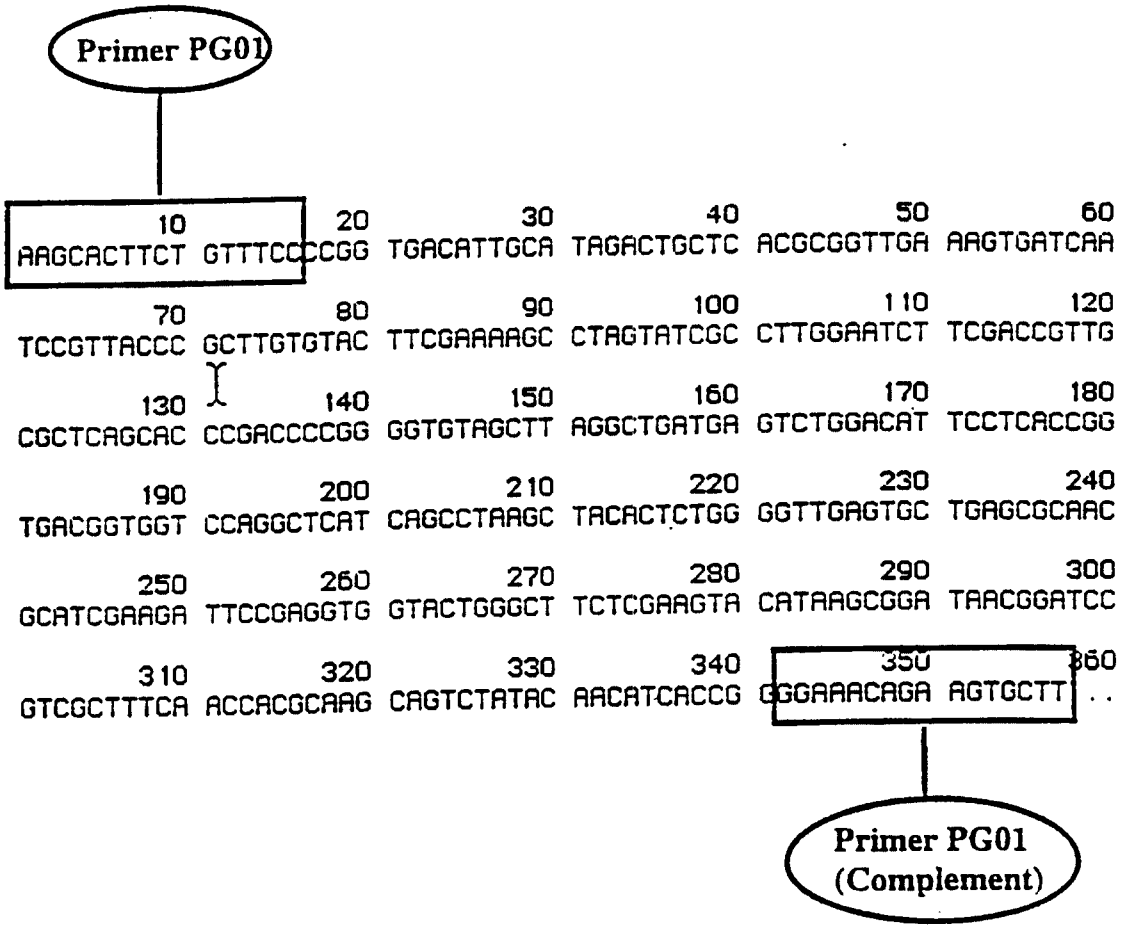
PRIMER PAIR		VA	OSBORN INSURANCE	MS	PROSTATE CANCER	MULTIPLE MYELOMA
PG01/PG02/ PG03/PG04/ PG07/PG08	EXPECTED:	?	NOT DONE	NOT DONE	NOT DONE	NOT DONE
	OTHER:	190 (1/1) 250 (1/1) 310 (1/1) 450 (1/1) 540 (1/1) 580 (1/1) 750 (1/1) 900 (1/1) 1500 (1/1)				

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

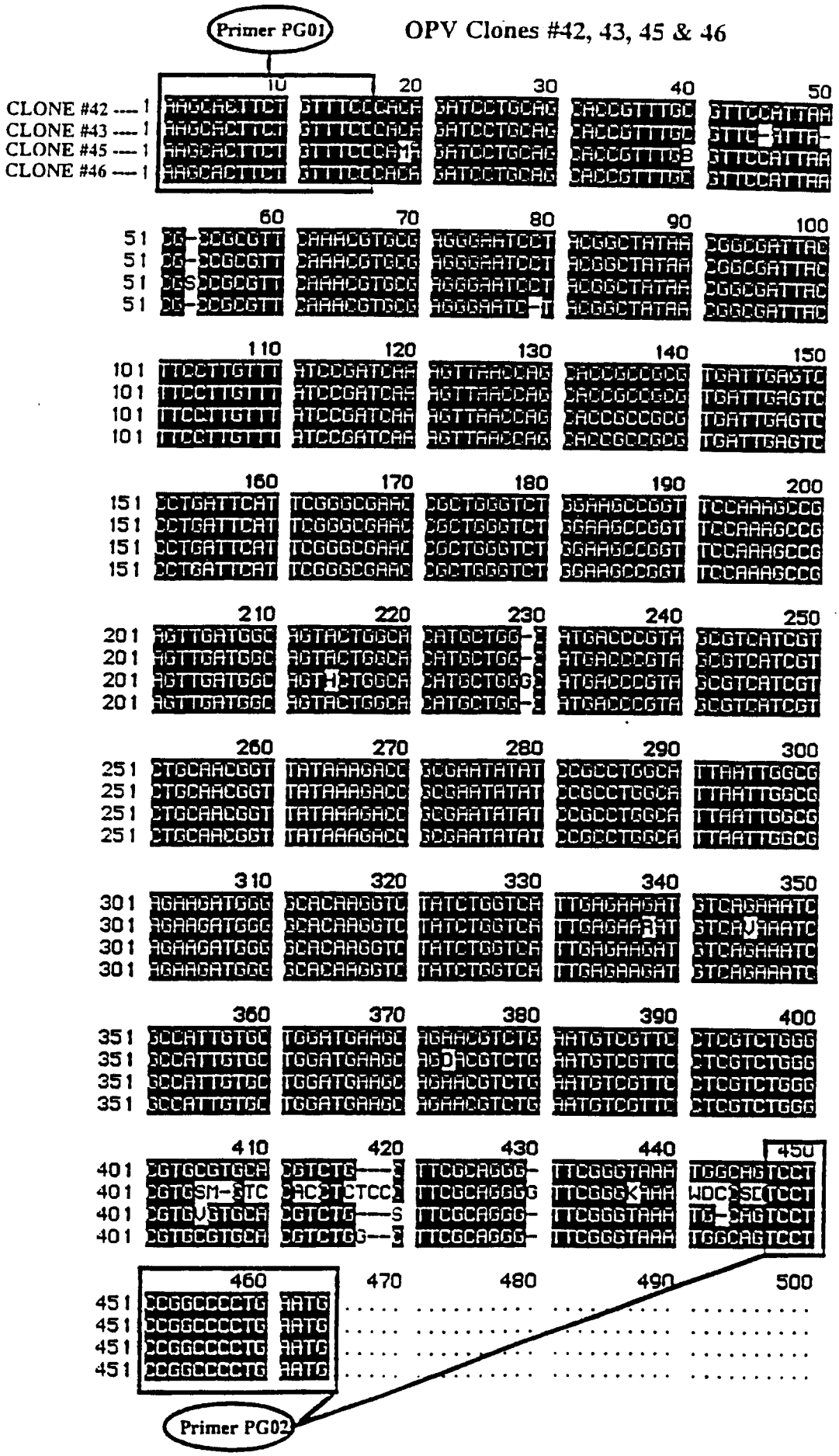
SEQUENCE LISTING

(Seq. ID No. 7)

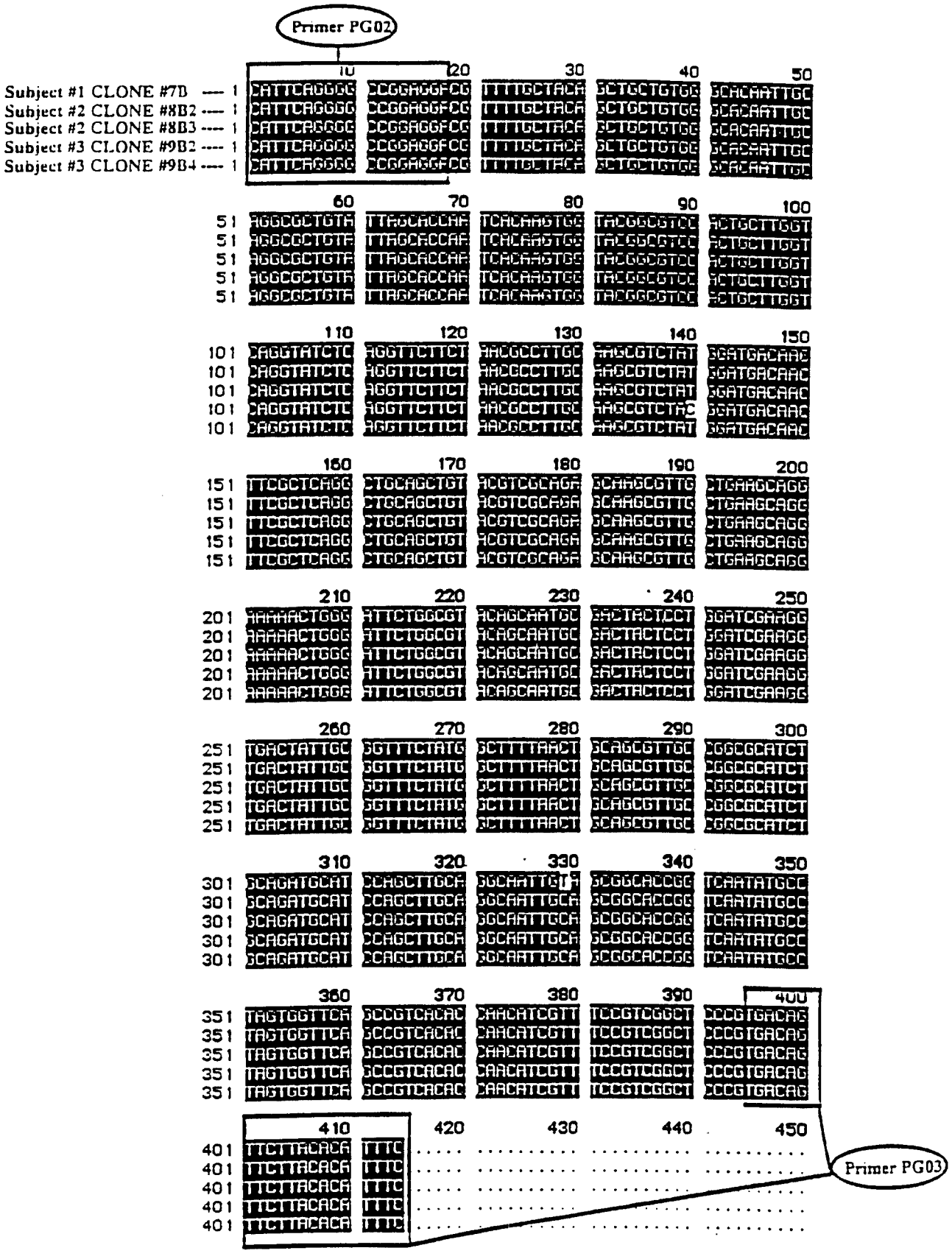
OPV CLONE #39



(Seq. ID Nos. 8-11)

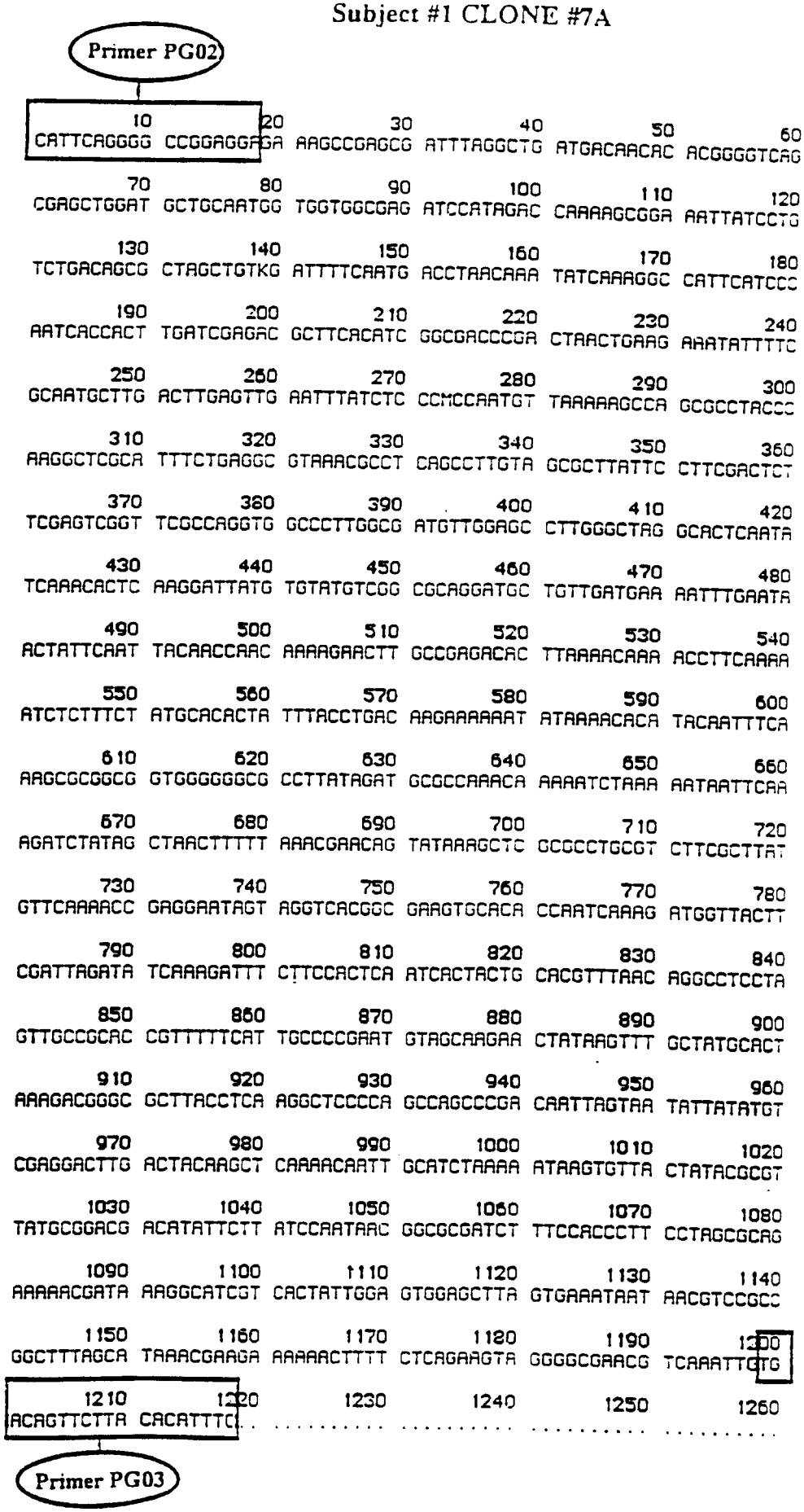


(Seq. ID Nos. 12-16)

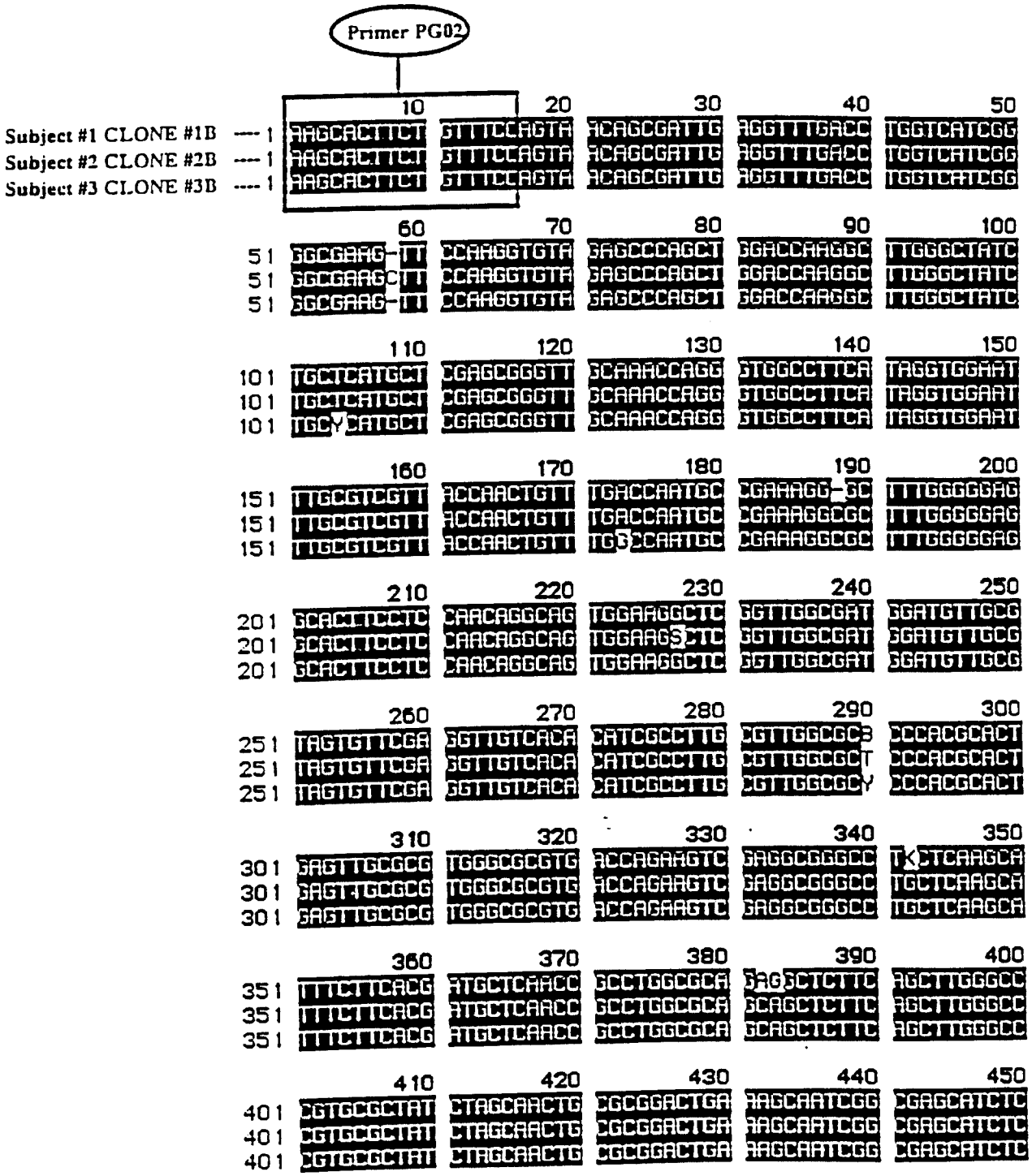


(Seq. ID No. 17)

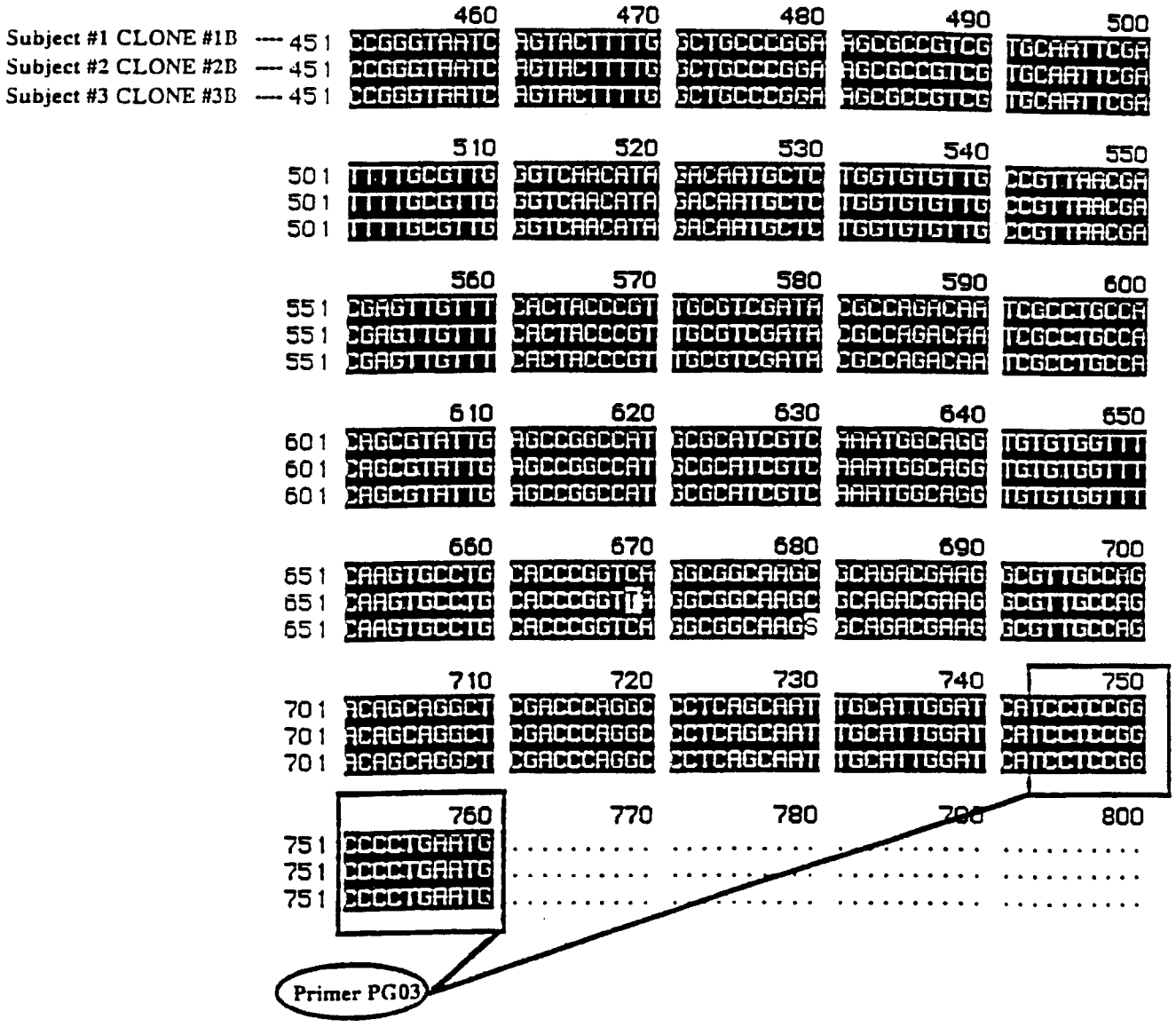
Subject #1 CLONE #7A



(Seq. ID No. 18-20)

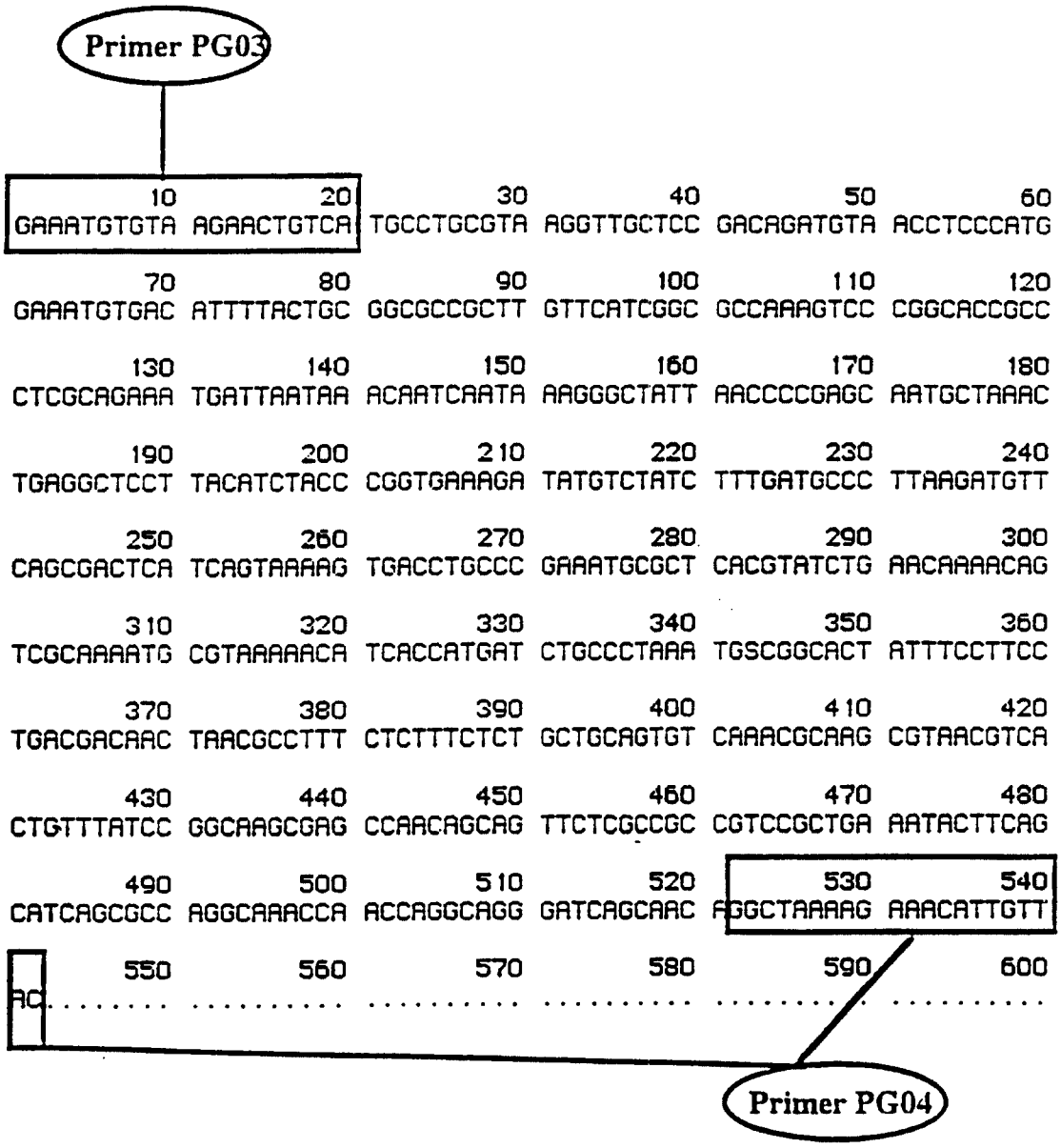


(Seq. ID No. 18-20, cont.)



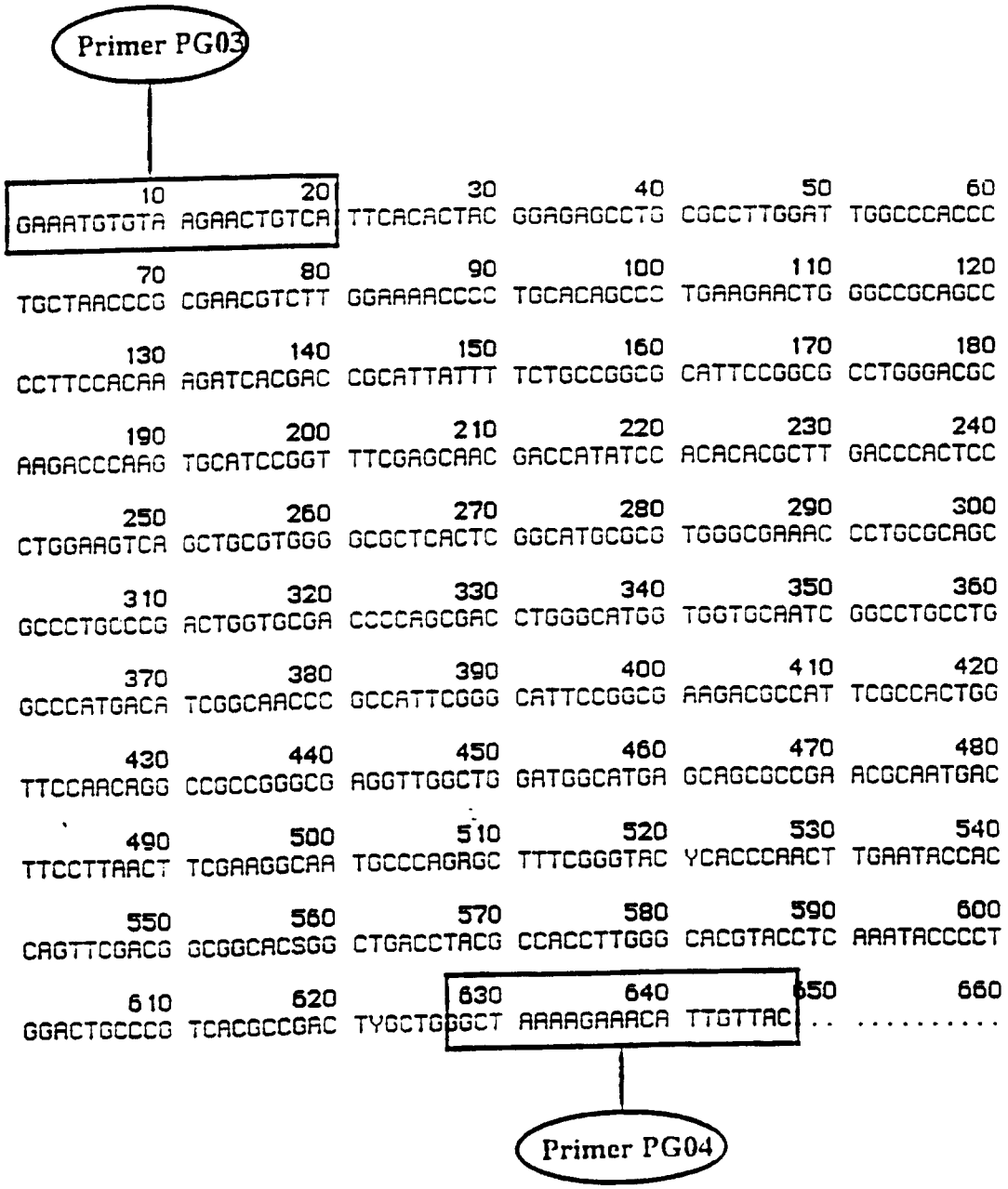
(Seq. ID No. 21)

Subject #2 CLONE #5B



(Seq. No. 22)

Subject #1 CLONE #4B



SEQUENCE LISTING

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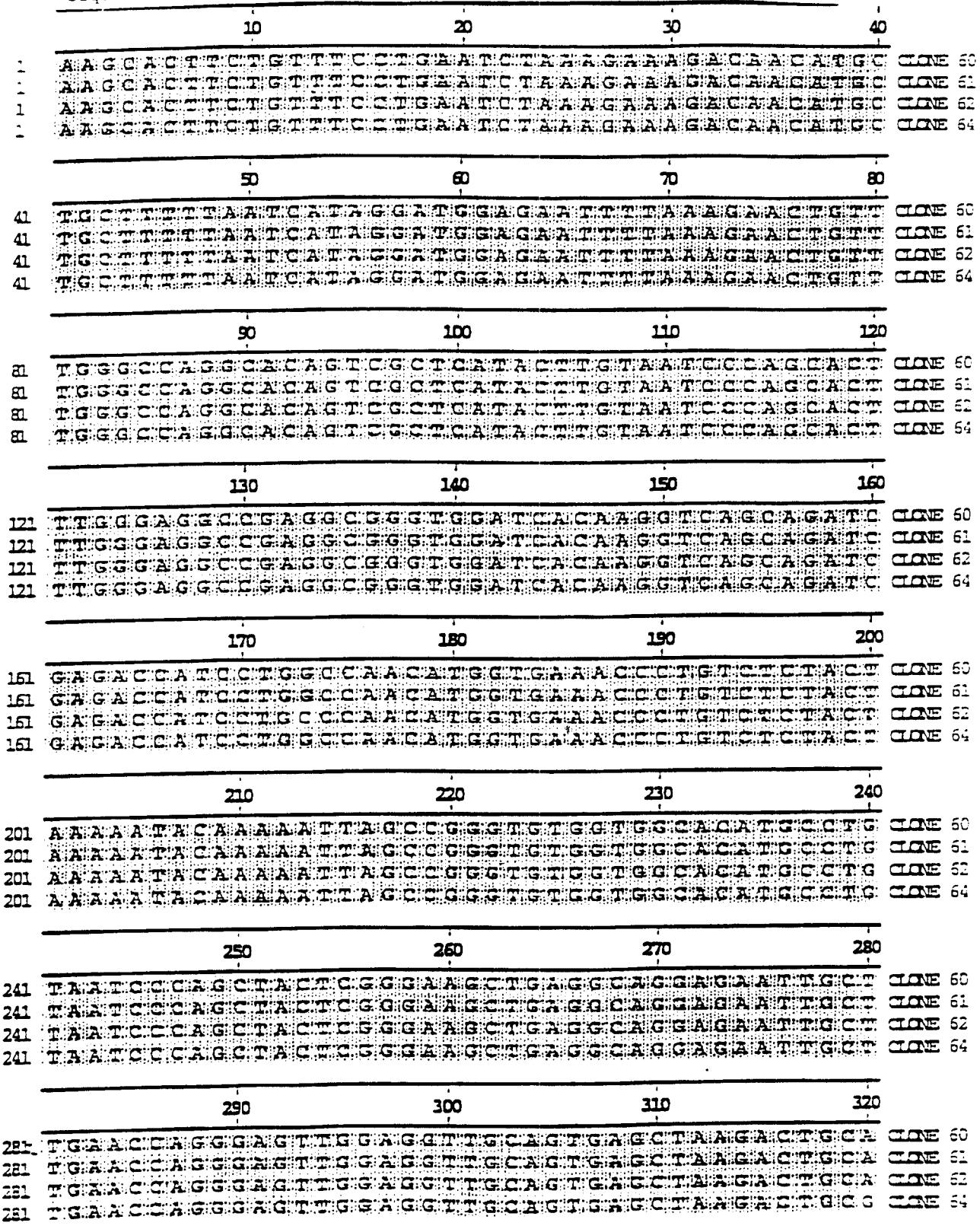
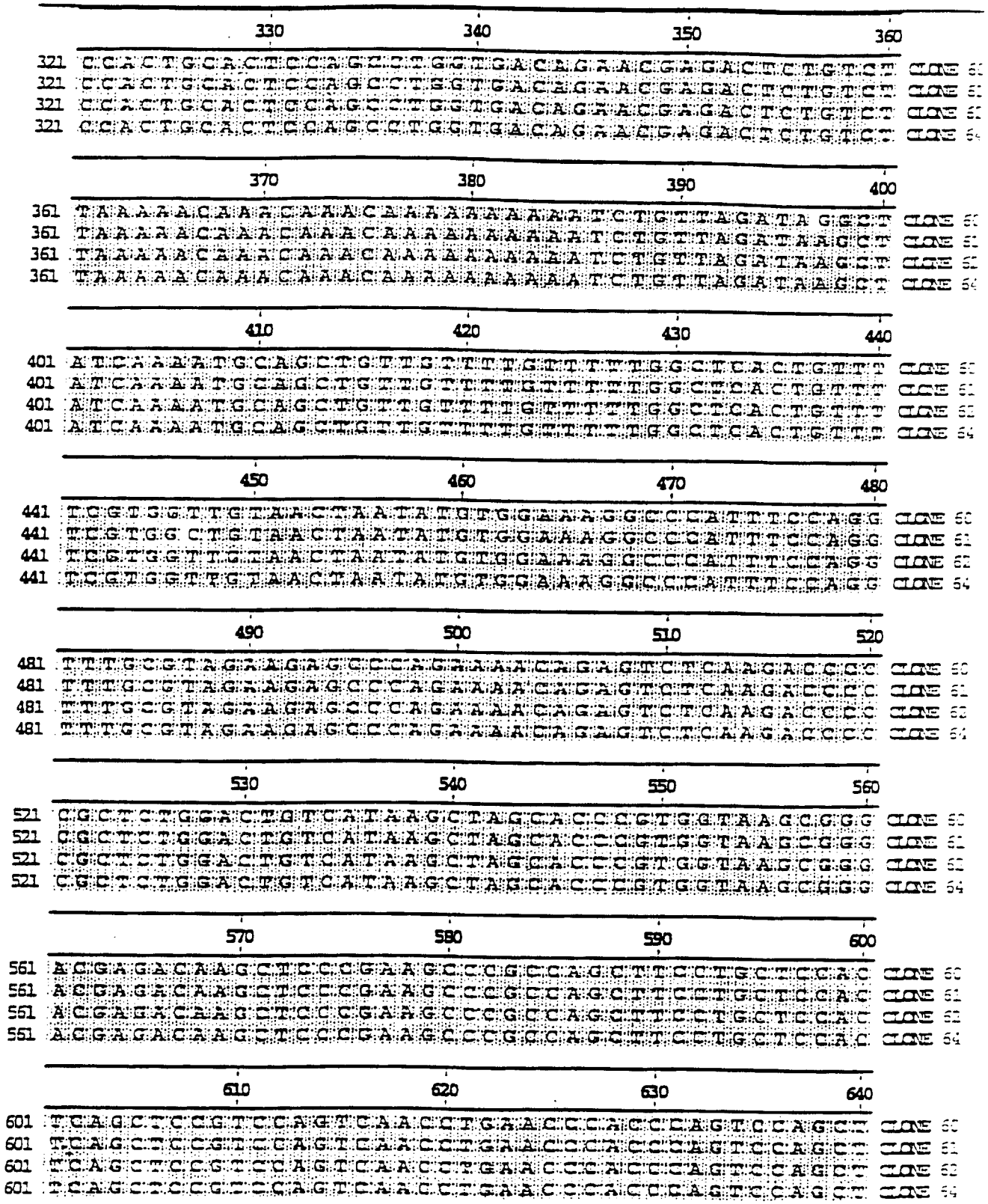
Seq.ID No. 24 (Clone 61)
Seq.ID No. 25 (Clone 62)
Seq.ID No. 26 (Clone 64)

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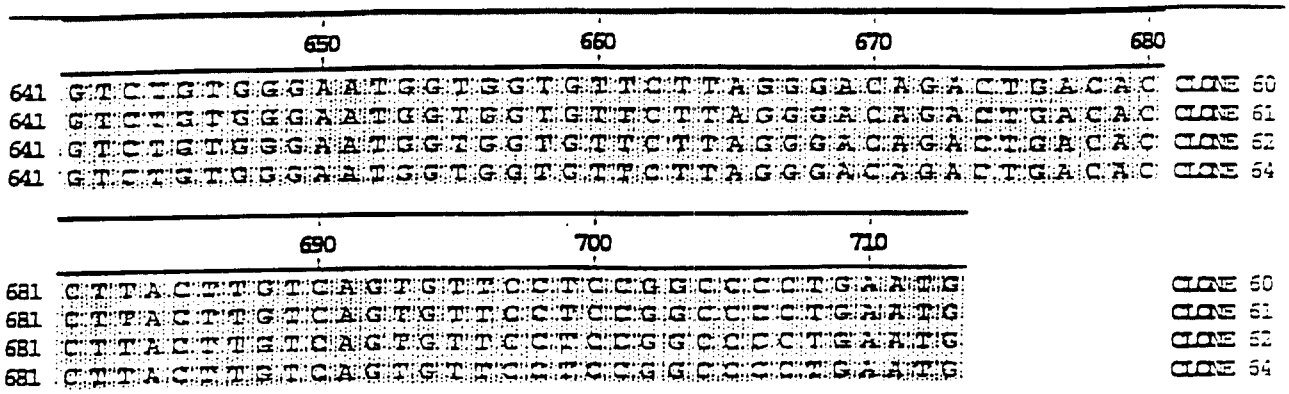
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710
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WHAT IS CLAIMED IS:

1. A method of screening for the presence of target human nucleic acids in a biological sample, the method comprising:

providing a biological sample from a patient;

contacting the sample with a nucleic acid which specifically hybridizes to a target human nucleic acid sequence; and

detecting the presence of the target human nucleic acid sequence.

2. The method of claim 1, wherein the target human nucleic acid includes sequences from a fragile site in the human genome.

3. The method of claim 1, wherein the target human nucleic acid includes sequences derived from repetitive DNA.

4. The method of claim 1, wherein the target nucleic acid includes archived nucleic acid sequences.

5. The method of claim 3, wherein the repetitive DNA comprises *Alu* sequences.

6. The method of claim 1, wherein the target human nucleic acid includes regulatory sequences.

7. The method of claim 1, wherein the target human nucleic acid is at least about 100 nucleotides in length.

8. The method of claim 7, wherein the target human nucleic acid is between about 500 and about 1500 nucleotides in length.

9. The method of claim 1, wherein the target human nucleic acid is RNA.

10. The method of claim 1, wherein the target human nucleic acid is DNA.

11. The method of claim 1, wherein the biological sample is blood plasma.

12. The method of claim 1, wherein screening for the presence of target human nucleic acids is used to monitor treatment of a disease.

13. The method of claim 1, wherein screening for the presence of human nucleic acids is used to diagnose disease.

14. The method of claim 13, wherein the disease state is a chronic illness.

15. The method of claim 14, wherein the chronic illness is cancer.

16. The method of claim 15, wherein the cancer is multiple myeloma.

17. The method of claim 14, wherein the chronic illness is an autoimmune disease.

18. The method of claim 14, wherein the chronic illness is a neurodegenerative disease.

19. The method of claim 1, wherein the target human nucleic acid is derived from a human genomic sequence having a sequence as shown in SEQ. ID. No. 1, SEQ. ID. No. 2, or SEQ. ID. No. 3.

20. The method of claim 1, wherein the step of contacting includes a step of amplifying the target human nucleic acid.

21. The method of claim 20, wherein the step of amplification is carried out using the polymerase chain reaction (PCR).

22. The method of claim 21, wherein the step of amplification includes use of a primer which is substantially identical to a primer having a sequence as shown in SEQ. ID. No. 1.

23. The method of claim 21, wherein the step of amplification includes use of a primer which is substantially identical to a primer having a sequence as shown in SEQ. ID. No. 2.

24. An isolated nucleic acid molecule having a sequence as shown in SEQ. ID. No. 1, SEQ. ID. No. 2, or SEQ. ID. No. 3.

25. A method of treating a chronic illness, the method comprising selectively destroying dysplastic cells.

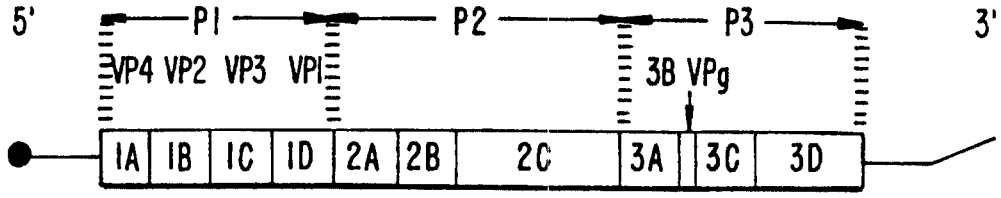
5'
P1
P2
P3
3'
VP4 VP2 VP3 VP1
3B VPg
1A 1B 1C 1D 2A 2B 2C 3A 3C 3D

FIG. 1.

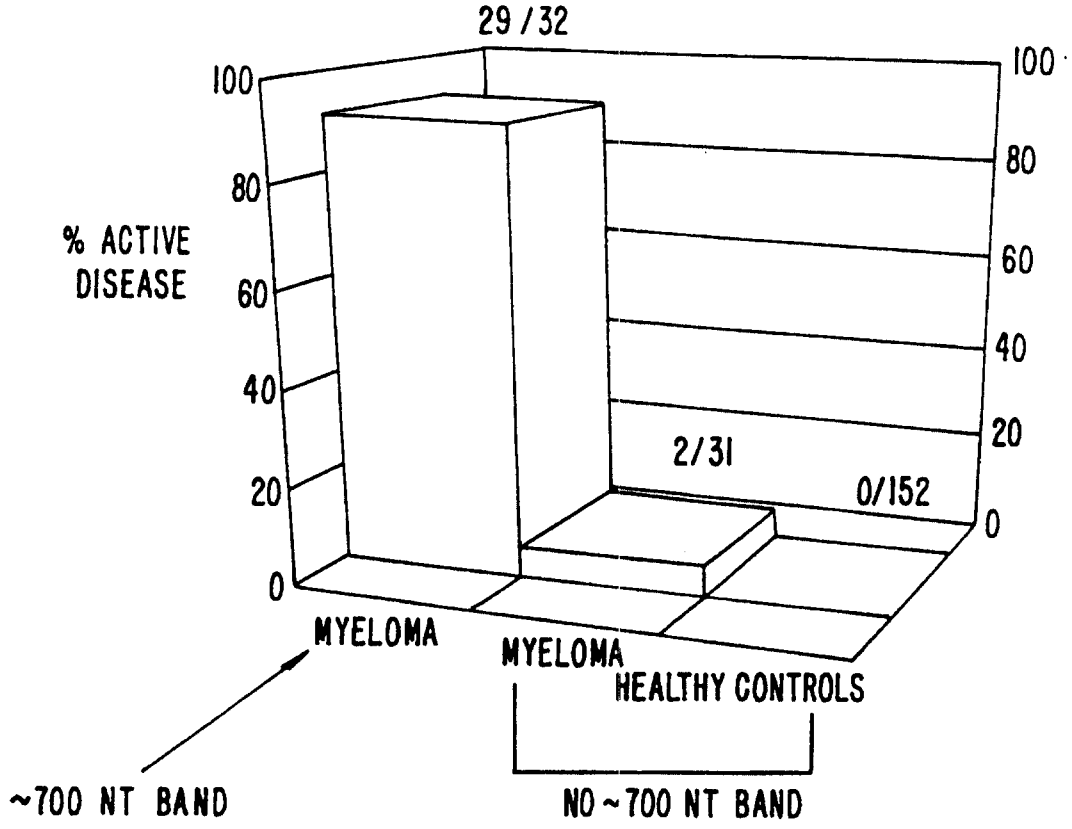
29/32
100
80
60
40
20
0
% ACTIVE DISEASE
2/31
0/152
MYELOMA
MYELOMA
HEALTHY CONTROLS
~700 NT BAND
NO ~700 NT BAND

FIG. 2.