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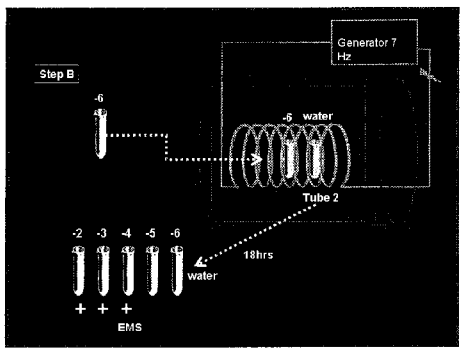
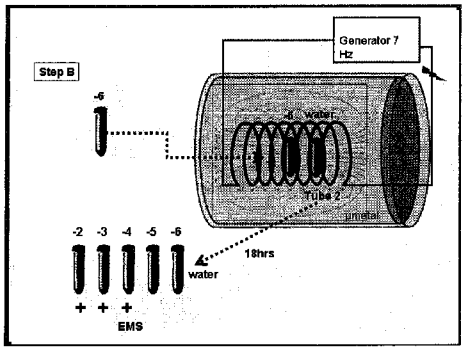
(54) Title: REMOTE TRANSMISSION OF ELECTROMAGNETIC SIGNALS INDUCING NANOSTRUCTURES AMPLIFIABLE INTO A SPECIFIC DNA SEQUENCE

Fig. 1





(57) Abstract: A general method for identifying both known and unknown DNA sequences at the origin of EMS, including DNA sequences in the plasma of patients suffering of chronic diseases such as Alzheimer, Parkinson, multiple sclerosis, rheumatoid arthritis, and other similar diseases, disorders and conditions. The invention is based on the discovery that: (1) The nanostructures induced by DNA sequences in water or other dipole solutions can faithfully reflect the information contained in these sequences at dilutions which do not contain anymore of that DNA, as evidenced by the fact that it can be retranscribed into the same DNA sequence by the polymerases and reagents used in classical polymerase chain reaction (PCR); (2) this information can be transmitted at a distance in water or other dipole solutions by EMS emitted by the nanostructures; and (3) EMS signatures of nanostructures containing this information can be detected, stored, transmitted, transduced and imprinted in water or other dipole solutions.

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TITLE

REMOTE TRANMISSION OF ELECTROMAGNETIC SIGNALS INDUCING NANOSTRUCTURES AMPLIFIABLE INTO A SPECIFIC DNA SEQUENCE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. 61/476,110, filed April 15, 2011 and to U.S. 61/476,545, filed April 18, 2011. Both of these documents are incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

Field of the Invention

Methods for remote transmission of electromagnetic signal (EMS) signatures of DNA molecules and for detecting DNA or DNA associated nanostructures (or water replicas) associated with particular diseases or disorders, especially those of unknown or incomplete etiology by detecting and/or replicating EMS signatures of DNA contained in a sample, in particular a biological sample.

Description of the Related Art

Two previous patent applications described a method for selectively detecting DNA sequences of a pathogenic microorganism in a sample by the emission of low frequency electromagnetic waves (EMS) in water dilutions of the sample. U.S.12/560,772, filed September 16, 2009, entitled "*System and Method for the Analysis of DNA sequences in Biological Fluids*" disclosed a method for detecting electromagnetic waves derived from bacterial DNA, comprising extracting and purifying nucleic acids from a sample; diluting the extracted purified nucleic acids in an aqueous solvent; measuring a low frequency electromagnetic emission over time from the diluted extracted purified nucleic acids in an

aqueous solvent; performing a signal analysis of the low frequency electromagnetic emission over time; and producing an output, based on the signal analysis, in dependence on the DNA in the sample. The products, compositions, methods and other subject matter disclosed in this patent application are expressly incorporated by reference.

Methods for detecting some low electromagnetic frequency electromagnetic signals in diluted filtrates of the culture medium of certain bacteria and viruses, as well as in diluted plasma of patients infected by the same agents are disclosed by U.S. 12/097,204 and PCT/FR2007/001042, filed June 22, 2007, and U.S. 12/797,826, filed June 10, 2010 each of which is expressly incorporated by reference. The electromagnetic signals (EMS) were believed to be produced by certain defined nanostructures induced by the microorganism in high dilutions in water, since the microorganism had been previously removed by filtration.

The background of the invention is also described by the publications listed below, which are also incorporated by reference:

U.S. 6,541,978, WO 00/17638 A (Digibio; Benveniste, Jacques; Guillonnet, Didier) filed March 30, 2000;

U.S. 09/787,781, WO 00/17637 A (Digibio; Benveniste, Jacques; Guillonnet, Didier) filed March 30, 2000;

U.S. 09/720,634 and WO 00/01412 A (Digibio; Benveniste, Jacques; Guillonnet, Didier) filed January 13, 2000;

FR 2,811,591 A (Digibio) filed January 18, 2002 (2002-01-18);

FR 2,700,628 A (Benveniste Jacques) filed July 22, 1994;

Benveniste J. et al: "Remote Detection Of Bacteria Using An Electromagnetic/Digital Procedure", Faseb Journal, Fed. Of American Soc. For Experimental Biology, Bethesda, Md, US, No. 5, Part 2, March 15, 1999, page A852, XP008059562 ISSN: 0892-6638;

Thomas et al: "Activation Of Human Neutrophils By Electronically Transmitted Phorbol-Myristate Acetate" Medical Hypotheses, Eden Press, Penrith, US, vol. 54, no. 1, January 2000 (2000-01), pages 33-39, XP008002247, ISSN: 0306-9877;

Benveniste J. et al.: "Qed And Digital Biology" Rivista Di Biologia, Universita Degli Studi, Perugia, IT, vol. 97, no. 1, January, 2004, pages 169-172, XP008059428 ISSN: 0035-6050;

Benveniste J. et al.: "A Simple And Fast Method For *In Vivo* Demonstration Of Electromagnetic Molecular Signaling (EMS) Via High Dilution Or Computer Recording" FASEB Journal, Fed. of American Soc. For Experimental Biology, Bethesda, Md, US, vol. 13, no. 4, Part 1, March 12, 1999 (1999-03-12), page A163,Abstr. No. 016209, XP008037356 ISSN: 0892-6638;

Benveniste J: "Biological effects of high dilutions and electromagnetic transmission of molecular signal" [Progress In Neonatology; 25th National Conference Of Neonatology] S. Karger Ag, P.O. Box, Allschwilerstrasse 10, CH-4009 Basel, Switzerland; S. Karger Ag, New York, New York, USA Series: Progres En Neonatologie (ISSN 0251-5601), 1995, pages 4-12, XP009070841; and 25ES Journees Nationales De Neonatologie; Paris, France; May 26-27, 1995 ISSN: 3-8055-6208-X;

Benveniste et al.: "Abstract 2392" FASEB Journal, Fed. of American Soc. for Experimental Biology, Bethesda, Md, US, 22 April 1998 (1998-04-22), page A412, XP009070843 ISSN: 0892-6638;

Benveniste et al.: "Abstract 2304" FASEB Journal, Fed. of American Soc. For Experimental Biology, Bethesda, Md, U.S., April 28, 1994, page A398, XP009070844 ISSN: 0892-6638; and

U.S. Patent Nos. 7,412,340, 7,081,747, 6,995,558, and 6,952,652.

In some instances, it was demonstrated that the EMS could originate from specific genes or even from some fragmented DNA sequences. For example, this was discovered to be the case for the adhesin gene of *Mycoplasma pirum* (U.S. 12/097,204, filed December 14, 2006) and of the LTR (Long terminal repeat), *nef* and *pol* genes of Human Immunodeficiency Virus (HIV) (U.S. 61/186,610, filed June 12, 2009 & U.S. 12/797,826, filed June 10, 2010). However, for many microbial agents or diseases of unknown origin or etiology this identification was not possible. Consequently, the inventors developed new methods, disclosed herein for detecting and identifying biological molecules, specifically DNA or other nucleic acids, associated with these other disease or disorders.

U.S. Provisional Application 61/358,282, *General Method for Specific DNA Amplification for DNA or DNA Associated Nanostructures*, which is incorporated by reference, describes a procedure for detecting DNA sequences derived from microorganisms in the plasma of patients suffering from various chronic diseases, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, rheumatoid arthritis, and other similar diseases, disorders and conditions. The detection was based on the production of electromagnetic signals (EMS) of low frequency in water dilutions upon excitation by an electromagnetic background. The inventors have found that this method may can be applied to other diseases, disorders, or conditions of unknown etiology.

U.S. Provisional Patent Application 61/476,110, *Digital Signals Allowing the Remote Transmission of a DNA Specific Sequence* as shown by PCR amplification shows that the signal coming from dilutions of an originating sample containing DNA are specific for the DNA sequences. After transmission of the EMS of the DNA to unsignalized, the DNA can be regenerated from the nanostructure induced in the receiving sample or unsignalized water;

Induction, detection and transmission of electromagnetic signals (EMS) of self-replicating molecules like DNA was also described by U.S. Patent Application 13/168.367,

General Procedure for the Identification of DNA Sequences Generating Electomagnetic Signals in Biological Fluids and Tissues. The methods and products disclosed in these prior-filed applications are hereby incorporated by reference.

BRIEF SUMMARY OF THE INVENTION

General Method of EMS Transmission

Many embodiments of the invention involve producing an EMS signature in an aqueous buffer comprising placing an originating sample in an aqueous buffer having an EMS signature and a receiving sample not having the EMS signature next to each other inside of an electromagnetically shielded container, applying an electromagnetic field for a time and under conditions sufficient to transfer the EMS signature from the originating sample to the receiving sample.

Water or other aqueous solutions, such as normal saline, phosphate buffered saline, physiologically acceptable aqueous solutions, buffered aqueous solutions, or alcohol and water mixtures, including 10, 20, 30, 40, 50, 60 and 70% or more of ethanol or other alcohol solutions or other solvents selected on a basis of their relevant properties depending on the molecule to be tested, may be employed in the methods described herein. Signalized samples or solutions producing an EMS signature should not be boiled, heated or frozen for long periods of time so as to preserve the EMS signatures or nanostructures they contain. Preferably these samples or solutions should be stored above freezing and at 40°C or less.

Control samples may constitute pure water, distilled water or pyrolyzed water, or other samples known to be nucleic acid free.

The electromagnetic field may be applied via a copper coil located inside of the electromagnetically shielded container. Coils made of other electrically conducting metals may also be employed. The electromagnetic field is applied for a time sufficient to transfer the EMS signal from the originating sample to the receiving sample. Exposure time can be

selected based on the amount of time required for transfer to occur. Representative times include > 0, 1, 2, 3, 4, 4-8, 8-12, 12-18, 18-24 and 24-48 hrs or longer.

Test samples used in various embodiments of the invention can be obtained from a subject having or suspected of having a parasitic or fungal disease or disorder, bacterial disease or disorder viral disease or disorder, an autoimmune disease, disorder or condition, diseases such as Alzheimer's Disease or Parkinson's Disease or another disease, disorder or condition of unknown or incomplete etiology. Representative test samples include blood, plasma, serum, CSF, joint fluid, saliva, mucous, semen, vaginal fluid, sweat, urine, and feces. Tissue samples and samples from other sources, including laboratory or hospital sources, foods, drinks and potable water may be used.

The invention also concerns a method for amplifying a nucleic acid from a receiving sample comprising performing a nucleic acid amplification on the receiving sample and recovering amplified nucleic acid. The nucleic acid amplification can be performed with random priming or with one or more primers for one or more specific nucleic acid sequence(s). Amplified nucleic acids produced by this method may be further characterized by cloning and/or DNA sequencing as further described below.

Detection of Unknown Target DNA Sequences

Another aspect of the invention represents a method for detecting an unknown nucleic acid or nanostructures associated with the unknown nucleic acid in a test sample. This method comprises amplifying a nucleic acid in a test sample using random primers; diluting and agitating during dilution the amplified nucleic acids in an aqueous solvent; measuring over time a low frequency electromagnetic emission from the diluted amplified nucleic acids; and identifying an EMS signature for amplified nucleic acid or its associated nanostructures by comparing the EMS of the test sample to the EMS of a control sample.

This method may further comprise performing a signal analysis of the low frequency electromagnetic emissions over time and producing an output, based on the signal analysis. Various means for signal analysis are known and are incorporated by reference to the documents mentioned above.

This method detects a nucleic acid or nanostructure derived from or associated with a nucleic acid in the test sample, preferably DNA or a nanostructure derived from DNA. The test sample can be diluted by a factor of 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 10^{10}, 10^{11}, 10^{12}, 10^{13} or more. The beginning concentration of a nucleic acid in an originating sample prior to dilution generally ranges from 1 ng/ml to 4 ng/ml.

Various forms and time periods for agitation are contemplated and are incorporated by reference to the documents mentioned above. Vortexing for a period of 15 seconds between 10-fold serial dilutions is one representative method.

Another aspect of the invention is a general procedure for the identification of any unknown polynucletide sequence (DNA, RNA) capable of producing EMS in biological fluids. The principle is shown by Figure 3. The transmission of EMS in water allows the selective transmission of only the DNA sequences which were emitting the EMS. This PCR-based method involves a combination of random and Tag primers. The random primer associated with the Tag has the following formula:

5'- GGACTGACGAATTCCAGTGACTNNNNNNNN (SEQ ID NO: 1)

in which are made all possible combinations of 8 nucleotides for the 4 possible bases (60,000). An embodiment of this aspect of the invention is shown by Example 5.

Another variation of a method for characterizing a sample containing a polynucleotide of unknown origin involves detecting a nucleic acid or nanostructures associated with an unknown nucleic acid in a test sample. This method comprises amplifying a nucleic acid in a test sample using random nucleotide sequence or polynucleotides or primers; diluting and

agitating during dilution the amplified nucleic acids in an aqueous solvent; measuring over time a low frequency electromagnetic emission from the diluted amplified nucleic acids; and optionally (i) identifying an EMS signature for amplified nucleic acid or its associated nanostructures by comparing the EMS of the test sample to the EMS of a control sample, and optionally (ii) comparing the results to relevant standard EMS signature(s). This method may further comprise performing a signal analysis of the low frequency electromagnetic emission over time, and/or producing an output, based on the signal analysis. This method may detect a biological molecule, such as a nucleic acid like DNA in a test sample and/or may detect a nanostructure derived from or associated with a nucleic acid such as DNA in the test sample. A suitable dilution of the test sample is selected for use within this method, for example, the test sample can be diluted by a factor of at least 10^4, 10^5, 10^6, 10^7, 10^8, or 10^9.

The test sample is generally derived for a subject suffering from or at risk of developing a particular disease, disorder or condition. For example, the test sample can be obtained from a subject having or suspected of having a parasitic or fungal disease or disorder, a subject having or suspected of having a bacterial disease or disorder, a subject having or suspected of having viral disease or disorder, from a subject having or suspected of having had an autoimmune disorder, a subject having or suspected of having Alzheimer's Disease or Parkinson's Disease or any other neurological disease, a subject having or suspected to have a genetic disease or a gene alteration, or a subject having a disease, disorder or condition of unknown or incomplete etiology in comparison with a noninfected subject. For instance, an EMS signature of an HIV gene sequence, such as that of *nef* or *pol,* may be detected in a sample in comparison to a sample not containing the HIV gene sequence. Verification of the presence of a gene sequence in a sample may be made by PCR.

Nucleic acids amplified from the receiving sample can be further characterized by isolating or concentrating them from the signalized sample, cloning the isolated or

concentrated nucleic acid, usually DNA, into a vector and transforming a competent cell with the vector. The transformed cell may then be used to express the cloned DNA for DNA sequencing to obtain a DNA sequence usable for bioinformatic analysis such as BLAST. Expression vectors may be used to facilitate the expression of polypeptides from the cloned DNA.

Detection of Known Target DNA Sequences. Another aspect of the invention is a method similar to that described above where specific primers are used to detect known nucleic acid sequences or nanostructures associated with known nucleic acid sequences, preferably DNA. Such a method may be performed on a diluted and agitated test sample or on a receiving sample. It comprises performing nucleic acid amplification on the diluted and agitated test sample or receiving sample and recovering amplified nucleic acid. The nucleic acid amplification can be performed with primers for one or more specific nucleic acid sequences, such as RNA or cDNA sequences derived from or known to be associated with a disease like HIV, or may be performed using random primers. Preferably, the nucleic acid amplification produces DNA.

Devices

The invention also contemplates a device for producing an EMS signature in an aqueous buffer comprising at least two containers, at least one for an EMS originating sample and at least one for an EMS receiving sample, an electrically conducting coil that can emit a variable frequency ranging from 1 to 20,000 Hz, optionally connected to an external generator of alternating current having a variable frequency from 1 to 20,000 Hz, means for electromagnetic shielding the at least two containers and the electrically conducting coil.

In one embodiment, this device represents a device for producing an EMS signature in a solvent or an aqueous buffer comprising at least two containers, at least one for an EMS originating sample and at least one for an EMS receiving sample, an electrically conducting

coil that can emit a variable frequency ranging from 1 to 20,000 Hz, optionally connected to an external generator of alternating current having a variable frequency from 1 to 20,000 Hz, means for electromagnetic shielding the at least two containers and the electrically conducting coil, and means for electromagnetic shielding the at least two containers and the electrically conducting coil. In this device the shielding means can be mu metal, the electrically conducting coil may be a copper coil with the following characteristics: bobbin with internal diameter 50 mm, length 80 mm, R = 3.6 ohms, 3 layers of 112 turns of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A.

In another embodiment the device will comprise o means for transmitting at a distance EMS emitted by a biological sample or by nanostructures contained in a sample. Such a device will contain at least two containers, at least one to contain a sample determined to produce EMS characteristic of a particular biomolecule such as DNA (*e.g.*, Tube 1), and another to receive emitted EMS and contain signalized water produced (*e.g.*, Tube 2). The device will contain an electrically conducting coil linked to an external generator of alternating current having a variable frequency from 1 to 20,000 Hz. The device will have shielding means, such as mu metal $\geq$ 1 mm in thickness, capable of isolating external ambient electromagnetic signals or noise, enclosing a space into which will accommodate the coil and the containers. Any suitable material may be used to make the coil and the elements and design of the coil are selected based on the size of the samples, shielding, and other elements of the apparatus. One example of a coil is a copper coil with the following characteristics: bobbin with internal diameter 50 mm, length 80 mm, R = 3.6 ohms, 3 layers of 112 turns of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A. An example of shielding is a cylinder of μ metal having a minimal thickness of 1 mm, closed at both ends in a manner that completely isolates the enclosed containers and coil from the external ambient electromagnetic noise.

EMS Transmission and Imprinting Receiving Sample

The invention also relates to a method for producing an EMS signature in an aqueous buffer comprising placing an originating sample in an aqueous buffer having an EMS signature and a receiving sample not having the EMS signature next to each other inside of an electromagnetically shielded container, applying an electromagnetic field for a time and under conditions sufficient to transfer the EMS signature from the originating sample to the receiving sample. The electromagnetic field is generally applied by a coil, such as a copper coil, located inside of an electromagnetically shielded container. The electromagnetic field can be applied to the sample for a time period ranging sufficient to produce an EMS signature, for example, from 12 to 24 hrs although other suitable time periods may be selected based on the nature of the sample, the sample dilution and the physical characteristics of the apparatus. Signalized samples produced by this method as well as nucleic acids like DNA amplified from a signalized sample are also contemplated.

Alternatively, an EMS signature may be imprinted in water or another aqueous buffer by contacting the one or more receiving samples with a recorded or transmitted and optionally amplified EMS signature previously obtained from an originating sample in an aqueous buffer having an EMS signature, for a time and under conditions sufficient to imprint the recorded or transmitted EMS signature of the originating sample onto the one or more receiving samples. Imprinting may be performed using means for applying an electromagnetic field, for example using a device, such as a copper coil or solenoid coil, optionally located inside of an electromagnetically shielded container. The electromagnetic field is applied to the sample for a time period sufficient to produce an EMS signature in the sample, for example for a period of 1 to 24 hrs. Other suitable time periods may be selected based on the nature of the sample, the sample dilution and the physical characteristics of the device or other means for applying

the electromagnetic field. Signalized samples produced by this method as well as nucleic acids like DNA amplified from a signalized sample are also contemplated.

Recording, Transducing, Storing and Transmitting EMS Signatures

The inventors previously discovered that an electromagnetic signal of low frequency (EMS) induced in a water dilution by the DNA of some kinds of bacteria and viruses can be transmitted at a distance into a naive or unsignalized water, aqueous medium or other dipole solution. It has also been discovered that such an EMS corresponding to a particular biomolecule like DNA (*i.e.*, an EMS signature of a particular molecule), can be recorded. This involves recording EMS from DNA fragments obtained by PCR (polymerase chain reaction) with sequence specific primers in an electromagnetic coil. The resulting amplified current is connected to a computer and stored as a file, such as an analog or digital file (*e.g.*, a digital sound file). The recorded EMS can then undergo signal processing, for example a digital sound file can be processed using computer software for storage, transmission, or use.

In another embodiment the invention involves recording, transducing, storing, and/or transmission of an EMS signature of a nucleic acid, such as that produced after serial dilution of a signalized sample. An EMS signature may be recorded by a suitable electronic device, such as a recorder, computer or computer network. The recorded EMS signature may undergo signal processing or signal transformation for example into a digital or analog signal, be transmitted by a communications device, such as via radio, telephone, modem, or Internet transmission to a receiver, such as a receiving computer, anywhere in the world.

A stored or transmitted EMS signature is then reconstituted and/or amplified and contacted with a receiving sample to imprint it with the EMS signature and produce nanostructures in the water or dipole solution of the receiving sample. Such a signal may be amplified prior to or after transmission, for example, using a commercial amplifier (e.g., Conrad). The electrical output from the amplifier containing the EMS signature is then

applied to an electrically conducting coil (*e.g.*, of copper wire) as described herein in which a plastic tube of pure non-signalized water or other dipole solution has been inserted for a time sufficient for imprinting of the EMS signature, generally for a period of at least one hour.

The production of EMS is then verified in water dilutions of the signalized water or dipole solution. The positive dilutions can be used for retrieving the DNA by PCR as described above. The DNA is then amplified by cloning and its sequence determined to be 98 - 100 % identical to the initial DNA. This development will be useful for remote diagnosis or use in other telemedicine procedures or protocols.

Reconstitution of Nucleic Acid from Signalized Sample

DNA may be reconstituted from its EMS signature. For example, the recorded or remotely transmitted EMS signature of a DNA molecule is input into a soundcard and the output from the soundcard is linked to an amplifier. Amplifier output is connected to a transducer solenoid into which an unsignalized water sample is placed. After a certain time, depending on the type of EMS signature, its intensity and the exposure time, the unsignalized water becomes signalized. In other words, the unsignalized water has memorized the EMS signature of the originating DNA molecule. By use of PCR the originating DNA molecule may be retrieved from the water signalized with its EMS signature. Verification of retrieval of the originating DNA sequence from the signalized water or verification of the fidelity of its reproduction can be verified by DNA sequencing.

In another embodiment, prior to retrieval and synthesis of the DNA molecule by PCR, the signalization of the receiving sample with a DNA EMS signature may be determined by detecting the EMS emissions of the signalized sample using dilutions of the signalized water as previously described, *e.g.*, by the device used to record the originating DNA sample's EMS signature in the first place. Only EMS positive dilutions will yield the DNA sequence. The procedure allows the transmission of DNA EMS signatures of

medical interest as well as the remote retrieval of the corresponding originating DNA. Such transmission may be made by a medium of choice, for example, a digital signal may be transmitted over the internet or by sending USB keys (*e.g.*, flashdrives) to remote laboratories or medical units.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the apparatus and process described Step B of Example 1. For clarity, Fig. 1 is shown twice, with the second version having its colors inverted.

Fig. 2A: D-4 LTR HIV DNA (104 bp) detected. Method was performed using excitation frequency 7 Hz, an 18 hr exposure followed by 35 cycles of PCR from D-2 to D-15 after 450nM and 20 nM filtration. DW denotes distilled water control. FD2-FD15, dilution to10^{-2}-10^{-15} after filtration 450 and 20 nM. For clarity, Fig. 2A is shown twice, with the second version having its colors inverted.

Fig. 2B shows transmission in water of D-4 LTR HIV DNA (104 bp). Method was performed using excitation frequency 7 Hz, an 18 hr exposure followed by 35 cycles of PCR from D-2 to D-15 after 450nM and 20 nM filtration. DW denotes distilled water control. FD2-FD15, dilution to 10^{-2}-10^{-15} after filtration 450 and 20 nM. Note that DNA band formation is up to D-8. For clarity, Fig. 2B is shown twice, with the second version having its colors inverted.

Fig. 3 illustrates a general procedure for the identification of any unknown polynucleotide sequence capable of producing EMS in biological fluids. The transmission of EMS in water allows the selective transmission of only the DNA sequences which were emitting the EMS

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a general method for identifying both known and unknown DNA sequences at the origin of EMS, including DNA sequences in the plasma of patients suffering of chronic diseases such as Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, rheumatoid arthritis, and other similar diseases, disorders and conditions. Unknown nucleic acids or structures corresponding to these nucleic acids associated with diseases or disorders of unknown origin, such as diseases caused by vector-borne microorganisms, like *Borrelia* which is transmitted by ticks, may be also be identified.

The invention is based on the discovery that:

(1) The nanostructures induced by DNA sequences in water can faithfully reflect the information contained in these sequences at dilutions which do not contain anymore of that DNA, as evidenced by the fact that it can be retranscribed into the same DNA sequence by the polymerases and reagents used in classical polymerase chain reaction (PCR)

(2) This information can be transmitted at distance in water by the EMS emitted by the nanostructures.

Definitions:

Nucleic acid: Includes RNA, DNA, single stranded DNA, double-stranded DNA. Biological samples containing DNA associated with a disease or disorder are generally isolated or recovered in double stranded form.

DNA Amplification: Methods for amplifying nucleic acids are known. Conventional methods including polymerase chain reaction (PCR) are known and are also incorporated by reference to *Current Protocols in Molecular Biology* (updated April 5, 2010), Print ISSN: 1934-3639; Online ISSN: 1934-3647.

Nanostructures: These structures of water are induced by biological molecules like nucleic acids such as single stranded or double stranded DNA. While not being bound to any

particular theory, according to the physical theory of diphasic water, filtration and mechanical agitation (succussion) are believed to induce in water a low energy potential favoring the formation of quantum coherent domains. These domains will become replicas of a DNA molecule and vibrate by resonance when properly diluted and excited; see Del Guidice, et al., *Water as a Free Electric Dipole Laser*, Phys. Rev. Lett. 61, 1085–1088 (1988). Hydrogen bonding networks in liquid water, such as those described by Cowan, et al., Nature 434 (7030): 199-202 (2005) have not been associated with nanostructures.

Serial Dilutions: Serial dilution is a well-known technique and involves the stepwise dilution of a substance, such as DNA, in a solvent, such as water, saline solution, aqueous buffer, or an aqueous alcohol solution. Generally, serial dilutions as performed herein are stepwise dilutions by a factor of 10, or dilution of 1 part of a more concentrated solution in 9 parts of a solvent.

EMS: Electromagnetic signal. EMS in the context of the methods herein generally involves those having frequencies ranging from 0 Hz to 20,000 Hz as well as all intermediate subranges and values. Components of the ambient electromagnetic field include Schumann resonances which represent a set of spectrum peaks in the extremely low frequency (ELF) portion of the Earth's electromagnetic field spectrum. Schumann resonances are global electromagnetic resonances excited by lightning discharges in the cavity formed by the Earth's surface and the ionosphere and are the principal background in the electromagnetic spectrum between 3 and 69 Hz. A representative Schumann resonance peak occurs in the Earth's electromagnetic spectrum and an ELF of about 7.83 Hz. By comparison, 60 Hz cycling of electricity is used in North America and 50 Hz elsewhere in the world.

EMS detection. Any suitable means for interrogating a sample and measuring its EMS may be employed. Exemplary systems, methods, and apparatuses for this purpose are disclosed by Butters, et al., WO 03/083439 A2, and are incorporated by reference to this

document. Generally, these procedures will involve placing a sample into a container having electromagnetic and magnetic shielding, a source of Gaussian noise for injection in to the sample, a detector for detecting an electromagnetic time-domain signal composed of sample source radiation superimposed on the injected Guassian noise, and a storage device for storing the time-domain signal and a time-domain signal separately detected from the same of a similar sample.

EMS Signature: The EMS characteristic of a particular biological molecule or a time domain signal associated with a material of interest. EMS signatures for various biological molecules are disclosed by U.S. 12/797,826, filed June 10, 2010. Such EMS signatures as well as methods for producing samples suitable for EMS detection and methods for detecting an EMS signature are incorporated by reference to this patent application.

An EMS Signature of a particular molecule can be represented by a characteristic electromagnetic time domain signal. An EMS Signature may be recorded and replayed, undergo signal transformation or processing, or be transmitted.

Excitation Frequency: A frequency used to excite a sample in which an EMS signature has been detected and induce an EMS signature in a sample previously devoid of the EMS signature, e.g., pure water. These frequencies include those of 7 Hz or above, e.g., 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 45, 50, 55, 60, 65, 70 and up to 150 Hz.

Originating Sample: A biological sample that contains an EMS signature, such as one characteristic of one or more biomolecules. An example would be a sample containing an EMS signature characteristic of DNA derived from human immunodeficiency virus.

Receiving or Signalized Sample: A sample, such as water or another aqueous buffer or dipole that has acquired or been imprinted with a nanostructure corresponding to a biological molecule, such as DNA. Methods for producing signalized water by serial dilution and agitation in water or in an aqueous solvent are disclosed herein.

Pathogenic Disease: Disease caused by or associated with a pathogen, such as a pathogenic parasite, yeast or fungus, bacterium, virus or infectious protein, such as a prion. Examples include parasitic diseases such as malaria or trypanosomiasis, fungal diseases, such as infections caused by or associated with *Aspergillus*, *Candida*, *Histoplasma*, *Pneumocystis*, *Cryptococcus*, *Stachybotrys* (black mold), bacterial infections such as Lyme Disease, sexually transmitted bacterial infections, tuberculosis, viral infections, including HIV infection, herpes virus infection, or hepatitis, and prion associated diseases such as Creutzfeldt–Jakob disease and so-called Mad Cow disease.

Autoimmune Disease, Degenerative Disease, Disorders or Conditions: These diseases, disorders or conditions may or may not have been previously associated with a particular biological molecule, such as a particular DNA molecule or its corresponding water nanostructure. Examples include allergic conditions, multiple sclerosis, rheumatoid arthritis, disorders associated with transplantation or replacement of body parts, Alzheimer's disease, Parkinson's disease and other diseases or disorders of unknown or incomplete etiology, such as Chronic Fatigue Syndrome, Gulf War Syndrome, or with exposure to particular biological, chemical or physical agents or with the *sequela* of such exposure.

Example 1: Production of Originating Samples Containing EMS Signature Characteristic of HIV DNA

Step A: Synthesis of DNA by PCR

A particular DNA sequence is first synthesized by polymerase chain reaction (PCR) on a DNA template, for example, a region of the LTR sequence present in the viral DNA extracted from the plasma of a HIV infected patient or obtained from a purified infectious DNA clone of HIV1 Lai, is amplified by PCR and nested PCR with respectively LTR-derived outer and inner primers.

Those were chosen to pick up some conserved regions of the LTR, given to several subtypes of HIV1. This amplified DNA was sequenced and found 100% identical to the known sequence of the prototype strain of HIV1 subtype B, HIV1 LAI (3). The resulting amplicon is 488 bp long and the nested-PCR amplicon is 104 bp long.

Filtration and Dilution: A sample of each amplicon is prepared at a concentration of 2 ng/ml in a final volume of 1 ml of pure water that had been previously filtered through a sterile 450 nM Millipore (Millex) filter and then to a 20 nM filter (Whatman, Anotop) to eliminate any contamination by viruses or bacteria. All manipulations are done under sterile atmosphere in a biological safety cabinet.

The DNA solution is diluted one in 100 (10^{-2}) in 2 ml of water and filtered through a 450 nM Millex filter (Millipore) and filtered again through an Anotop filter of porosity size 20 nM (Whatman).

The resulting DNA filtrate (there is practically no DNA loss through filtration, as the DNA molecules do not bind to the filters), is then diluted serially 1 in 10 (0.1 ml in 0.9 ml of water in a Eppendorf sterile tube of 2 ml from 10^{-2} to 10^{-15}.

A strong vortex agitation was performed at each dilution step for 15 seconds.

Each dilution in its stoppered plastic tube was placed on a coil under the ambient electromagnetic background at room temperature for 6 seconds, the resulting electric current is amplified 500 times and analyzed in a Sony laptop computer with specific software as previously described (1). The positive vibrating dilutions (usually between 10^{-4} to 10^{-8}) were detected not only by new peaks of frequency, but also quantitatively by the difference in amplitude and intensity of the signals measured in the software, as compared to the same parameters given by the background noise.

Table 1 shows the role of excitation frequency in inducing EMS from DNA into water.

A fragment of LTR DNA (Tar region, 104 base pairs) was amplified by PCR with specific primers from the entire genomic HIV1 LAI DNA cloned in a plasmid (pLAI2). The fragment was purified by electrophoresis on an agarose gel, the DNA band was then cut and extracted with a Qiagen kit. Time of exposure DNA tube and water tube to the exciting frequency was 18 hrs.

TABLE 1				
Content	**Frequency (Hz)**	**EMS**	**% over noise**	**Positive dilutions**
LTR DNA 104bp	2	+	33.3	D6→D8
Water		-	1.2	
DNA	3	+	39.6	D4→D7
Water		-	0.5	
DNA	4	+	43.9	D5→D8
Water		-	1.5	
DNA	5	+	41.6	D5→D8
Water		-	0	
DNA	6	+	33.5	D5→D8
Water		-	1	
DNA	7	+	40	D6→D8
Water		+	43.9	D5→D8

STEP B: Producing a Signalized Sample from the Originating Sample

A tube 1 containing one of the dilutions found positive for EMS in step A (10^{-5}) is placed in the vicinity of an identical tube 2 which has been previously filled with 1 ml of pure water under a separate safety cabinet different from the one utilized in step A for the DNA manipulation. Both tubes are placed inside a copper coil with the following characteristics: bobin with internal diameter 50 mm, length 80 mm, R = 3.6 ohms, 3 layers of 112 turns of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A, linked to an external generator of alternate electric current of variable frequency from 1 to 20,000 Hz.

The tubes and the coil are enclosed in a cylinder of thick (1 mm) μmetal closed at both ends in order to isolate the system from the external ambient electromagnetic noise. A current intensity of 100 mA is applied to the coil, so that no significant heat is generated inside the cylinder.

The tubes are kept 18 Hrs at room temperature in an oscillating magnetic field strength of 25 Oe/A generated by the coil system. Afterwards, the signalized water of tube 2 is filtered on 450 nM and 20 nM filters and diluted from 10^{-2} to 10^{-15}. As a control the tube 1 is also filtered and diluted in the same way. EMS analysis revealed positive dilutions for EMS in the same range as those found for tube 1 (10^{-2} to 10^{-5}) (Fig. 1). As shown in Table 1 a minimal frequency of 7 Hz was found necessary and sufficient to induce the EMS in the water filled tube 2.

The intensity of the signals was however was sometimes reduced in comparison to that found in tube 1 (originating sample).

The following controls suppressed EMS emission from tube 2 (receiving sample or sample to be signalized).

- Time of exposure of the two tubes less than 16-18 hrs (Table 2).
- No coil.

- Generator of magnetic field turned off.
- Frequency of excitation < 6 Hz.
- No use of DNA in tube 1.
- Tube 2 frozen at -80°C overnight and defrosted before recording the EMS.
- Tube 2 heated at 95°C for 60 minutes after the overnight exposure.

Therefore, it was concluded that excitation of tube 1 by a magnetic field of low frequency and of very low intensity has allowed the water nanostructures generated by the DNA fragment contained in this tube to be transmitted via waves to tube 2.

STEP C: Reconstitution by PCR of the LTR DNA from the nanostructures in the Receiving or Signalized Sample.

A sample volume (5 µl) of tube 2-signalized water is added to 45 µl of an amplification mixture in a propylene 200 µl PCR tube (Eppendorf).

The amplification mixture is composed of (buffer composition) 0.2 mM dNTP's, 10 µM of each specific HIV-1 LTR primer containing the ingredients for synthesizing DNA, either from a positive dilution for EMS or in a lesser dilution, starting with 10^{-2} down to 10^{-10}: and using 1 unit of Taq DNA polymerase.

Once the first cDNA strand is synthesized, cycling of denaturation, annealing and polymerization steps are performed as usually used for the PCR amplification.

The reaction (35 cycles, T° annealing 56°C) yielded a DNA band of the size (in electrophoresis migration in agarose 1.5%) of the expected 104 bp sequence. This amplicon was then cloned in a bacterial plasmid (Topo Cloning, Invitrogen) which was used to transform bacterial competent cells. Plasmid clones were purified from isolated bacterial transformants and screened for the presence of the 104 bp insert by EcoRI digestion. Positive plasmid clones are then sequenced and the sequence of the insert shown to be 98% identical (difference of 2 nucleotides) to the original DNA of tube 1.

The first step of DNA synthesis using the nanostructures as templates can also be achieved by a reverse transcriptase (RT) and other more classical DNA polymerase, at lower temperature (42°C for example for the reverse transcription step).

It has to be noted that the synthesis of the DNA (LTR band is obtained in high water dilutions (up to 10^{-9}) of the tube 2 containing the signalized water, indicating the transmission of the DNA information from tube to tube, in the presence of the ambient electromagnetic background.

The same phenomenon is also observed in high dilution of tube 2, indicating also the synthesis of DNA at dilutions containing no DNA molecule. This PCR technology can be applied to the detection of nanostructures in body fluid (plasma, urine) apparently devoid of the microorganisms from which they originate. In all cases, it is necessary to use mechanical agitation (vortex) at each water dilution in addition to the ambient or controlled electromagnetic background.

Table 2 shows the role of time of exposure to the 7 Hz frequency on EMS transmission from DNA to water. These results used the DNA LTR preparation as used for procedures reported in Table 1.

Table 2			
Content	**Time of exposure (hr)**	**EMS % over noise**	**Positive dilutions**

Control DNA tube	**2**	+	57.3	D4→D8
Water	**2**	-	0	
Water	**4**	-	0	
Water	**6**	-	0	
Water	**8**	±	6.4	D4→D8
Water	**16**	+	13.4	D5→D8
Control DNA tube	**16**	+	63	D4→D8

As shown above EMS were detected in the receiving sample after an exposure time of 8 or 16 hrs when the originating sample exhibited positive EMS at dilutions of D4 to D8 or at dilutions of 10^{-4} to 10^{-8}. No EMS was detected in water exposed for less than 8 hrs.

Example 3: Identification of Unknown DNA Sequences

It is also possible to apply this technology for identification of DNA species producing EMS, when the sequence in unknown. This is the case of the plasmas of patients suffering from neurodegenerative diseases (Alzheimer's Disease, Parkinson's Disease, multiple sclerosis, *etc.*) or from rheumatoid arthritis or ill defined neuropathies. It is then necessary to use random primers for PCR amplification of the water nanostructures induced by the DNA sequences to be determined. The procedure is similar for the steps A and B described in the Examples above. However in step C, a GenomePlex® single cell whole genome amplification commercial kit (WGA4, Sigma; or other commercially available kits) containing random primers is used following a modified procedure from the one suggested by the manufacturer and described below:

A library preparation mixture made of 10µl signalized water sample, 2 µl 1x Single cell library preparation buffer, 1 µl Library stabilization solution and 1 µl Library preparation

enzyme is incubated stepwise at 16°C for 20 min, 24 °C for 20 min, 37 °C for 20 min and 50 °C for 5 min. The following amplification reagents are then added to the library preparation mix : 7.5 µl of 10x amplification master mix, 48.5 µl of nuclease-free water (0.02 µm-filtered) and 5 µl of WGA DNA polymerase.

This amplification mix is incubated stepwise at 50°C for 5 min, 60°C for 5 min, and 65 °C for 5 min and subjected to 40 cycles of 95°C for 30 sec and 65 °C for 5 min. After cycling, the reaction sample is maintained at 4°C.

Amplification products are then analyzed by electrophoresis in 1.5% agarose gel. Since random primers were used, amplicons of different sizes are obtained.

The amplicons are then processed (purification, cloning, screening, and sequencing) as described earlier for the HIV-LTR in step C.

Example 4: Recording of EMS signatures of HIV and *Borrelia Burgdorferi*

EMS signatures of HIV DNA and *Borrelia* DNA sequences are recorded and transduced as described below.

STEP 1: Preparation of DNAs

1. A fragment of HIV DNA taken from its long terminal repeat (LTR) sequence present in the viral DNA extracted from the plasma of a HIV-infected patient or obtained from a purified infectious DNA clone of HIV1 Lai, is amplified by PCR (487 base pairs) and nested PCR (104 base pairs) using specific primers: TR InS:

5'-GCCTGTACTGGGTCTCT (SEQ ID NO: 3) and

LTR InAS:

5'-AAGCACTCAAGGCAAGCTTTA (SEQ ID NO: 4).

A longer variant (300 bp) is obtained using the following primer:

5'- TGTTAGAGTGGAGGTTTGACA (SEQ ID NO: 5)

in conjunction with the above primer InAS.

2. A DNA sequence from *Borrelia Burgdorferi*, the agent of Lyme disease, is amplified by PCR (907 base pairs) and nested PCR (499 base pairs) with respectively *Borrelia* 16S outer and inner primers.

Inner BORR16S inS:

5'-CAATCYGGACTGAGACCTGC (SEQ ID NO: 6) and

BORR16S inAS:

5'-ACGCTGTAAACGATGCACAC (SEQ ID NO: 7).

A shorter variant of 395 bp is obtained by using the following primer:

5'- GACGTCATCCTCACCTTCCT (SEQ ID NO: 8)

in conjunction with the above primer inAS.

STEP 2: Signal Recording

The resulting amplicons 104 bp and 300 bp for LTR and 499 bp and 395 bp for *Borrelia* were prepared at a concentration of 2 ng/ml in a final volume of 1 ml of DNAse/RNAse-free distilled water. The samples were read on an electromagnetic coil, connected to a Sound Blaster card (Creative Labs) itself connected to a microcomputer, (preferably Sony VGN - CS31) preferentially powered by its 12 volt battery. Each emission is recorded for 6 seconds, amplified 500 times and the digital file is saved, for example under the form of a sound file with the *.wav* format. This file can later undergo digital processing, by a specific software, Matlab (Mathworks), as for example digital amplification for calibrating the signal level, filtering for eliminating unwanted frequencies, or be analyzed by transformation into its spectrum by a discrete Fourier transform, preferably by the algorithm of FFT "Fast Fourier Transform".

STEP 3: Signal transduction in water:

For transduction, the digital signal was converted by the digital/analog converter

of the sound card into an analog signal. The output of the sound card of the microcomputer was linked to the input of a commercial amplifier (Kool Sound SX-250, www._conrad.com) having the following characteristics: passband from 10 Hz to 20 kHz, gain 1 to 20, input sensitivity 250 mV, output power RMS 140 W under 8 ohms.

The output of the amplifier was connected to a transducer solenoid which has the following characteristics: the bobbin has a length of 120 mm, an internal diameter of 25 mm, an external diameter of 28 mm, with 3 layers of 631 spirals of copper wire of 0.5 mm diameter and a resistance of 8 ohms, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A. A measurement of 4.4 milliTesla (mT) was obtained when current, voltage and resistance were respectively, 100 mA, 4V and 8 ohms.

50 ml of DNAse/RNAse-free distilled water (5-Prime Ref 2500010) are filtered first through a sterile 450 nM filter (Millex, Millipore, Cat N° SLHV033RS) and then to a 20 nM filter (Whatman, Anotop 25, Cat N° 6809-2002). For transduction, 1 ml of this filtered water in a Eppendorf sterile tube of 1.5 ml was placed at the center of the solenoid, itself installed at room temperature on an isolated (non metal) working bench. Alternatively, a sterile tube of 15 ml (Falcon–Becton Dickinson), filled with the filtered water can be used instead of the 1.5 ml Eppendorf tube.

The modulated electric current produced by the amplifier was applied to the transducer coil for 1 hr at the tension of 4 Volts. A current intensity of 100 mA was applied to the coil, so that no significant heat was generated inside the cylinder.

STEP 4: Reconstitution by PCR of the DNA from the signalized water.

The water which has received the recorded specific signal is called "signalized water". The signalized water, which was kept in the same tube, was first shaked by strong vortex for 15 seconds at room temperature and then diluted 1/100 in non signalized DNAse/RNAse-free distilled water (30µl/3ml). 1 ml was kept for control (NF, non filtered),

the 2 mls remaining of signalized water were filtered through a sterile 450 nM filter and then through a 100nM (Millex, Millipore, Cat N° SLVV033RS) for *Borrelia* DNA or 20 nM filter (Whatman, notop25) for HIV DNA. The filtrate was then diluted serially 1 in 10 (0.1 ml in 0.9 ml of DNAse/RNAse-free distilled water) in a Eppendorf sterile tube of 1.5 ml from 10-2 to 10-15 (D2 to D15). A strong vortex agitation was performed at each dilution step for 15 seconds. 5 µl of each dilution is added to 45µl of the mix.

1. Preparation of the mix for HIV LTR:

The PCR mixture (50 µl) contained 37.4 µl of DNAse/RNAse-Free distilled water, 5 µl of 10x Taq PCR buffer, 0.4 µl of 25 mM dNTPs, 1 µl of 50 µM each appropriate primer Inner [LTR InS: 5'- GCCTGTACTGGGTCTCT(SEQ ID NO: 3) and

LTR InAS: 5'- AAGCACTCAAGGCAAGCTTTA(SEQ ID NO: 4)],

0.2 µl of 5 U/µl Taq DNA Polymerase and 5µl of each dilution. The PCR was performed with the mastercycler ep (Eppendorf). The PCR mixtures were pre-heated at 68°C for 3 min (elongation step), followed by 40 PCR cycles of amplification (95°C for 30 s; 56°C for 30 s; 70°C for 30 sec). A final extension step was performed at 70°C for 10 min.

2. Preparation of the mix for *Borrelia*: The PCR mixture (50 µl) contained 37.4 µl of DNAse/RNAse-Free distilled water, 5 µl of 10x Taq PCR buffer, 0.4 µl of 25 mM dNTPs, 1 µl of 50 µM each appropriate primer Inner

[BORR16S inS: 5'-CAATCYGGACTGAGACCTGC(SEQ ID NO: 6) and

BORR16S inAS: 5'-ACGCTGTAAACGATGCACAC(SEQ ID NO: 7)],

0.2 µl of 5 U/µl Taq DNA polymerase and 5µl of each dilution. The PCR was performed with the mastercycler ep (Eppendorf). The PCR mixtures were pre-heated at 68°C for 3 min (elongation step), followed by 40 PCR cycles of amplification (95°C for 30 s; 61°C for 30 s; 70°C for 1 min). A final extension step was performed at 70°C for 10 min.

Electrophoresis of the PCR products in 1.5% agarose gel: A band of 104bp for HIV LTR and a band of 499bp Borrelia DNA should be detected at several dilutions.

3. Sequencing: The DNA bands are cut and DNA is extracted using a Qiagen kit which also describes classical conditions for cloning in *E. coli*. The amplified specific DNA is then sequenced and should be identical to the original DNA.

Example 5: General procedure for the identification of any uncharacterized polynucleotide sequence (DNA, RNA) capable of producing EMS in biological fluids.

A detailed procedure is described in the following example:

1) Purify DNA using a QiaAMP kit (Qiagen) from plasma derived from EDTA-collected human peripheral blood.

2) Filter (0.45 and 0.1μm), vortex and 10-fold serially dilute (FD2-FD15) DNA for SEM analysis. FD2-FD15 describes serial dilutions to 10^{-2} to 10^{-15}.

3) Signalize 20nm-filtered water (molecular biology grade, 5 Prime) with a EMS^{+} dilution of a patient DNA sample under an oscillating magnetic field of 7Hz, 4V (coil in mu-metal) for 18 hours.

4) Filter (0.45 and 0.1μm), vortex and 10-fold serially dilute (FD2-FD5) the signalized water sample and proceed to SEM analysis. FD2-FD5 describes serial dilutions to10^{-2} to 10^{-15}.

5) Use the FD2-FD5 diluted samples of signalized water (SEM+) as template for a standard PCR amplification using random and Tag primers, following the protocol described below:

A 49 μl PCR amplification mix containing 1x Advanced Taq buffer with Mg^{2+} (cat# 2201240, 5 Prime), 200μM dNTPs, 20nM of designed random primer Tag8N: 5'-GGACTGACGAATTCCAGTGACTNNNNNNNN (SEQ ID NO: 1),

20 μl of vortexed diluted signalized water, and 1 unit of Taq DNA polymerase

(cat#2200020, 5 Prime) is incubated stepwise at 8°C, 15°C, 20°C, 25°C, 30°C, 36°C, 42°C, and 46°C for 2 min at each temperature (or alternatively, increment the annealing temperature by 1 degree each 15 seconds, from 8°C to 50°C) to allow annealing of the random portion of the primer. An elongation step at 68°C for 2-15 min is performed to allow synthesis of DNA, followed by a denaturation step at 95°C for 3 min. One microliter of the designed primer Tag-SEUL:

5'-GGACTGACGAATTCCAGTGACT (SEQ ID NO: 2)

is then added to the mixture at a final concentration of 200nM. The resulting sample is subjected to 40 cycles of amplification (95°C/30s, 59°C/30s, and 70°C/2min), followed by an incubation at 70°C for 10 min. PCR-amplified samples are subjected to electrophoresis in 1.3% agarose gel and stained with ethidium bromide (or SYBR green, cat# S7567, In Vitrogen) to allow visualization of amplified DNA bands under UV light.

6) If needed (if faint or no DNA bands are detected), sample can be subjected to additional thermocycles (usually 10 cycles) using the same temperatures, or alternatively, can be reamplified in a second PCR reaction using only the primer Tag-SEUL, following the reamplification protocol described below:

A 50 µl PCR amplification mix containing 1x Hot Start Taq buffer with Mg^{2+} (cat#2200320, 5 Prime), 200µM dNTPs, 200nM of designed primer Tag-SEUL: 5'-GGACTGACGAATTCCAGTGACT (SEQ ID NO: 2), 1-10 µl of PCR-amplified sample as template, and 1 unit of Hot Taq DNA polymerase (cat#2200320, 5 Prime) is denatured at 95°C for 3 min and subjected to 25-40 cycles of amplification (95°C/30s, 59°C/30s, and 70°C/2min), followed by an incubation at 70°C for 10 min.

7) Resulting amplicons are gel-purified, and cloned in pCR2.1-TOPO vector (InVitrogen), followed by transformation of competent Escherichia coli cells (One Shot Mach1-T1^R, In Vitrogen), and screening for clones containing insert.

8) Cloned amplicons are sequenced using M13 universal primers (Eurofins MWG GmbH, Germany) and homologous relatives to the amplicons sequences are identified using the program BLASTN 2.2.26 (ref. Zheng Zhang, Scott Schwartz, Lukas Wagner, and Webb Miller (2000), "A greedy algorithm for aligning DNA sequences", J Comput Biol 2000; 7(1-2):203-14.) and retrieved from GenBank (http:// ncbi.nlm.nih.gov/).

Incorporation by Reference

Each document, patent, patent application or patent publication cited by or referred to in this disclosure is incorporated by reference in its entirety, especially with respect to the specific subject matter surrounding the citation of the reference in the text or with regard to the pertinent portions of the invention supported by the reference. However, no admission is made that any such reference constitutes background art and the right to challenge the accuracy and pertinence of the cited documents is reserved.

CLAIMS

1. A method for detecting an unknown nucleic acid or nanostructures associated with an unknown nucleic acid in a test sample comprising:

amplifying a nucleic acid in a test sample using random nucleotide sequence or polynucleotides or primers;

diluting and agitating during dilution the amplified nucleic acids in an aqueous solvent;

measuring over time a low frequency electromagnetic emission from the diluted amplified nucleic acids; and

optionally (i) identifying an EMS signature for amplified nucleic acid or its associated nanostructures by comparing the EMS of the test sample to the EMS of a control sample, and optionally (ii) comparing the results to relevant standard EMS signature(s).

2. The method of claim 1, further comprising:

performing a signal analysis of the low frequency electromagnetic emission over time; and producing an output, based on the signal analysis.

3. The method of claim 1, which detects a nucleic acid in the test sample.

4. The method of claim 1, which detects a nanostructure derived from or associated with a nucleic acid in the test sample.

5. The method of claim 1, which detects a nanostructure derived from or associated with DNA in the test sample.

6. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^4.

7. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^5.

8. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^6.

9. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^7.

10. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^8.

11. The method of claim 1, wherein the test sample is diluted by a factor of at least 10^9.

12. The method of claim 1, wherein the test sample is obtained from a subject having or suspected of having a parasitic or fungal disease or disorder.

13. The method of claim 1, wherein the test sample is obtained from a subject having or suspected of having a bacterial disease or disorder.

14. The method of claim 1, wherein the test sample is obtained from a subject having or suspected of having viral disease or disorder.

15. The method of claim 1, wherein the test sample is obtained from a subject having or suspected of having had an autoimmune disorder.

16. The method of claim 1, wherein the test sample is obtained from a subject having or suspected of having Alzheimer's Disease or Parkinson's Disease or any other neurological disease.

17. The method of claim 1, wherein the test sample is obtained from a subject having a disease, disorder or condition of unknown or incomplete etiology in comparison with a noninfected subject.

18. A method for producing an EMS signature in an aqueous buffer comprising:

placing an originating sample in an aqueous buffer having an EMS signature and an receiving sample not having the EMS signature next to each other inside of an electromagnetically shielded container,

applying an electromagnetic field for a time and under conditions sufficient to transfer the EMS signature from the originating sample to the receiving sample.

19. The method of claim 18, wherein the electromagnetic field is applied by a copper coil located inside of the electromagnetically shielded container.

20. The method of claim 18, wherein the electromagnetic field is applied to the sample for a time period ranging from 12 to 24 hrs.

21. A method for amplifying a nucleic acid from a receiving sample comprising:

performing a nucleic acid amplification on the receiving sample, and

recovering amplified nucleic acid.

22. The method of claim 21, wherein the nucleic acid amplification is performed with random priming.

23. The method of claim 21, wherein the nucleic acid amplification is performed with primers for a specific nucleic acid sequence.

24. The method of claim 21, wherein the nucleic acid amplification produces DNA.

25. A signalized sample produced by the method of claim 21.

26. A nucleic acid amplified from a signalized sample, such as that produced by the method of claim 21.

27. A device for producing an EMS signature in a solvent or an aqueous buffer comprising:

at least two containers, at least one for an EMS originating sample and at least one for an EMS receiving sample,

an electrically conducting coil that can emit a variable frequency ranging from 1 to 20,000 Hz, optionally connected to an external generator of alternating current having a variable frequency from 1 to 20,000 Hz,

means for electromagnetic shielding the at least two containers and the electrically conducting coil.

28. The device of claim 27, wherein the shielding means is mu metal.

29. The device of claim 27, wherein the electrically conducting coil is a copper coil with the following characteristics: bobbin with internal diameter 50 mm, length 80 mm, R = 3.6 ohms, 3 layers of 112 turns of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A.

30. The method according to claim 1, wherein the test sample is obtained from a subject having or suspected to have a genetic disease or a gene alteration.

31. A process of detecting an HIV nucleic acid sequence in a sample according to claim 1, comprising detecting an electromagnetic signal characteristic of the HIV nucleic acid sequence (EMS signature) compared to that obtained from an otherwise identical sample not containing the HIV nucleic acid.

32. A method for signalizing a sample with an EMS (electromagnetic signal) signature comprising transmitting an EMS signature from an originating sample to a receiving sample not having said EMS signature.

33. The method of claim 32, further comprising storing the EMS signature prior to transmitting it.

34. A device for transmitting at a distance an EMS signature emitted by a biological sample or by nanostructures.

35. The device of claim 34 that comprises a storage device for storing a time-domain signal of an EMS signature characteristic of a particular biomolecule.

36. A process for transmitting an EMS signature comprising:

detecting an EMS signature from a nanostructure derived from or associated with a nucleic acid,

inputting the detected EMS signature into an electronic transmitter, and

transmitting the EMS signature to one or more electronic receiver(s).

37. The process of claim 36, wherein the electronic transmitter is a computer linked to at least one receiving computer or linked to the Internet.

38. A method for detecting a DNA sequence in a biological sample obtained from a subject suffering from a chronic disease comprising:

isolating or purifying DNA from a biological sample of a subject suffering from a chronic disease, disorder or condition,

filtering the isolated or purified DNA and vortexing and diluting it (*e.g.*, FD2-FD15),

selecting a filtered, vortexed and diluted sample that is positive for EMS and signalizing a naïve water sample or aqueous solution with the EMS from said sample under an oscillating magnetic field (*e.g.*, 7 Hz, 4V for 18 hrs inside of a mu-metal coil) thus obtaining a signalized water sample,

filtering, vortexing and diluting (*e.g.*, FD2-FD5) the signalized water sample and analyzing the resulting sample for EMS,

amplifying DNA from a signalized water sample in which EMS has been detected (*e.g.*, FD2) using random and Tag primers.

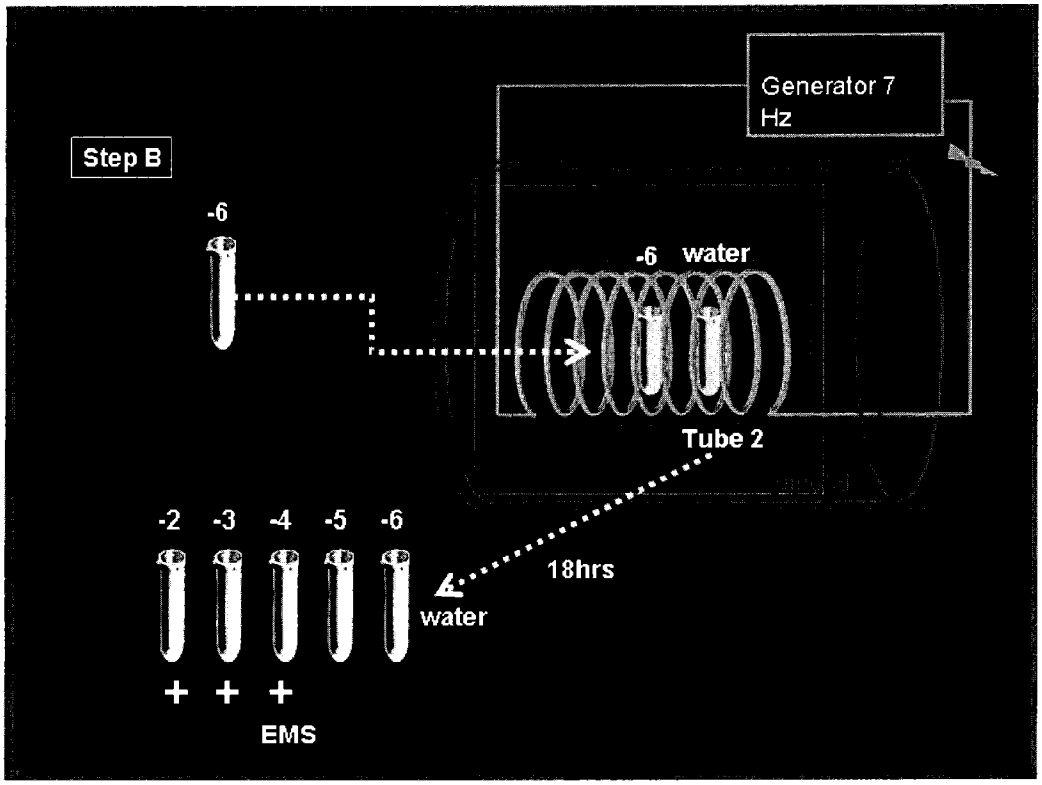
39. The method of claim 38, further comprising isolating or purifying the DNA amplified from the signalized water.

40. The method of claim 39, further comprising cloning the amplified DNA into a vector and transforming a competent cell with the vector.

41. The method of claim 39, further comprising sequencing the isolated or purified DNA an optionally performing BLAST or another kind of a bioinformatic analysis on the sequence.

42. The method of claim 41, further comprising identifying a causative agent or a pathogen whose genome or polynucleotides comprises a sequence in said isolated or purified DNA.

Fig. 1


Generator 7 Hz
Step B
-6
-6 water
Tube 2
-2 -3 -4 -5 -6
18hrs
water
+ + +
EMS

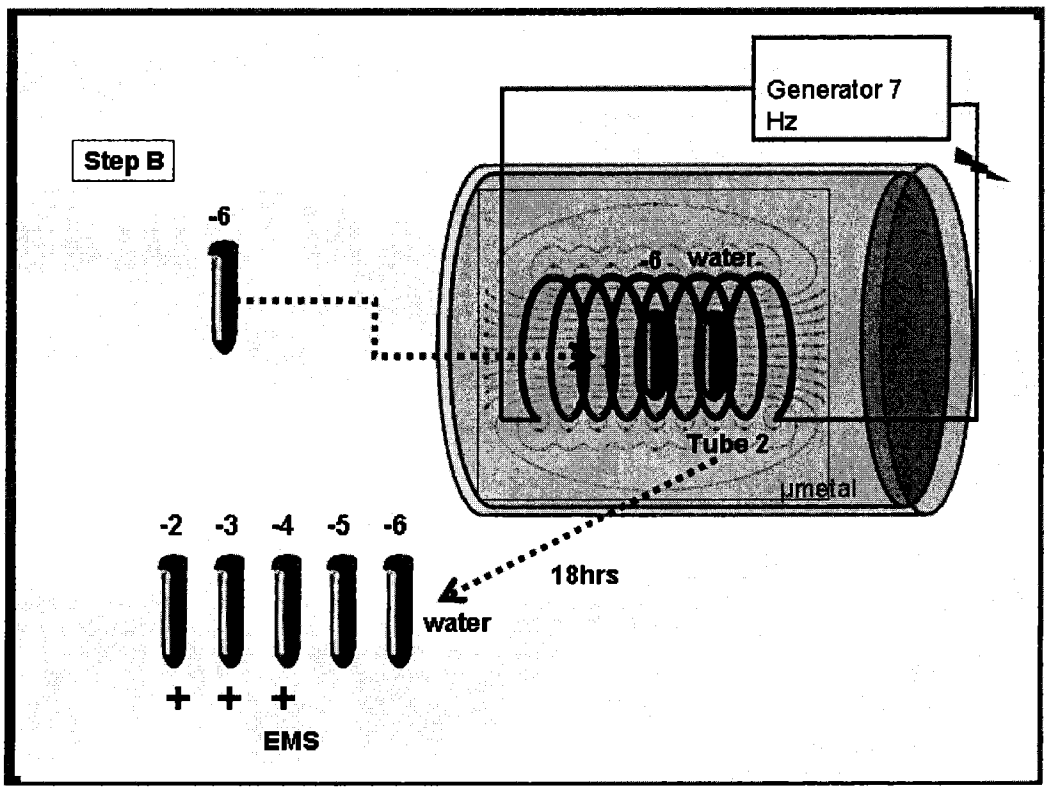
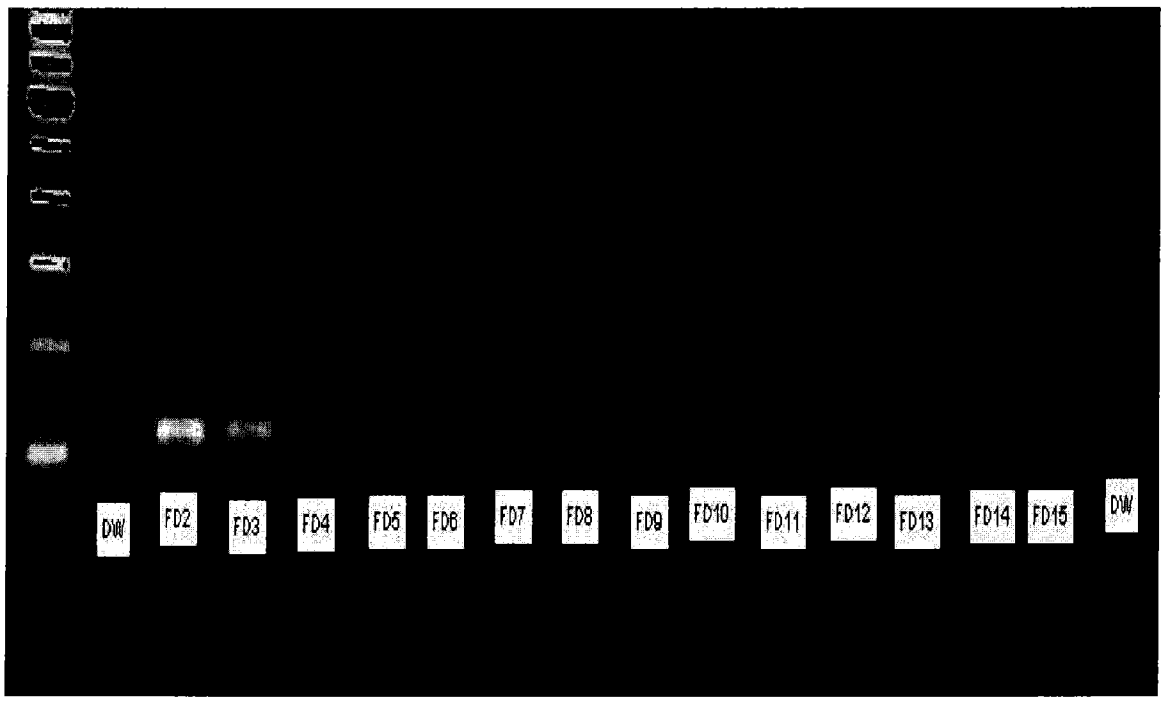

Generator 7 Hz
Step B
-6
-6 water
Tube 2
μmetal
-2 -3 -4 -5 -6
18hrs
water
+ + +
EMS

Fig. 2A

D-4 LTR HIV DNA (104bp) 7Hz, 18 Hrs and then PCR (35 cycles) from D-2 to D-15 after filtration 450 and 20 nM



DW: Distilled Water
FD2: Dilution 10^{-2} after filtration 450 and 20 nM

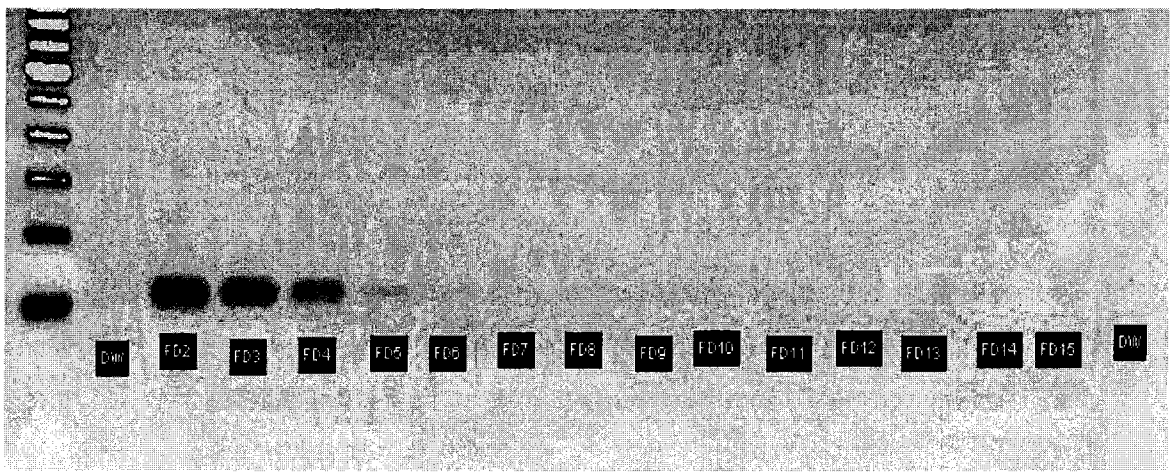
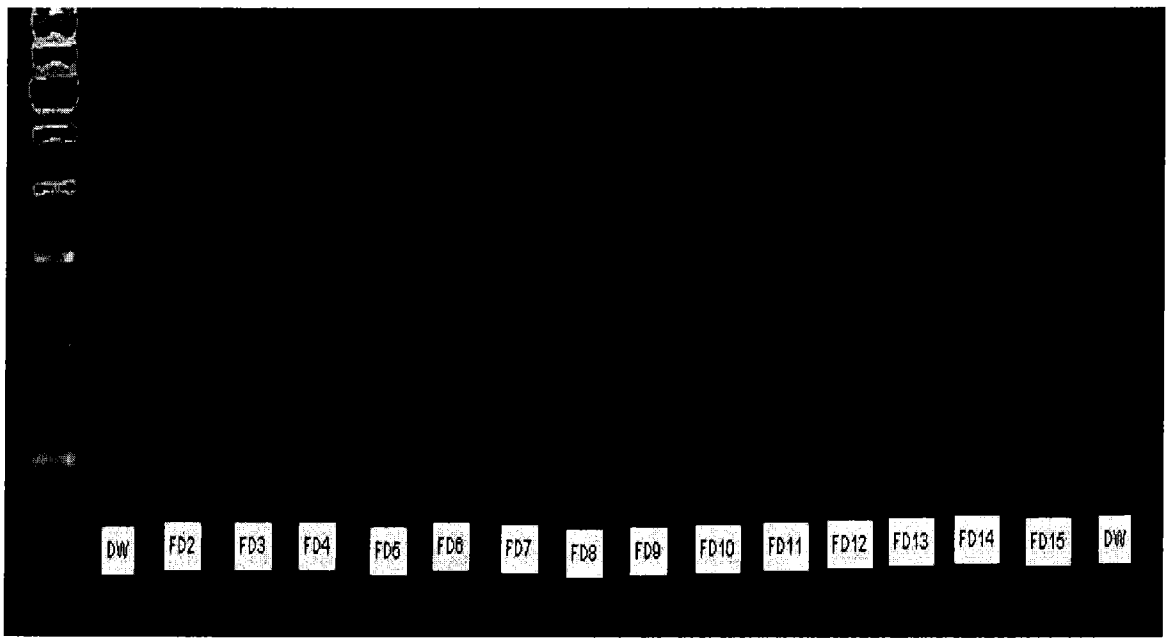


Fig. 2B

Transmission in water of D-4 LTR HIV DNA (104bp) 7Hz, 18 Hrs and then PCR (35 cycles) from D-2 to D-15 after filtration 450 and 20 nM



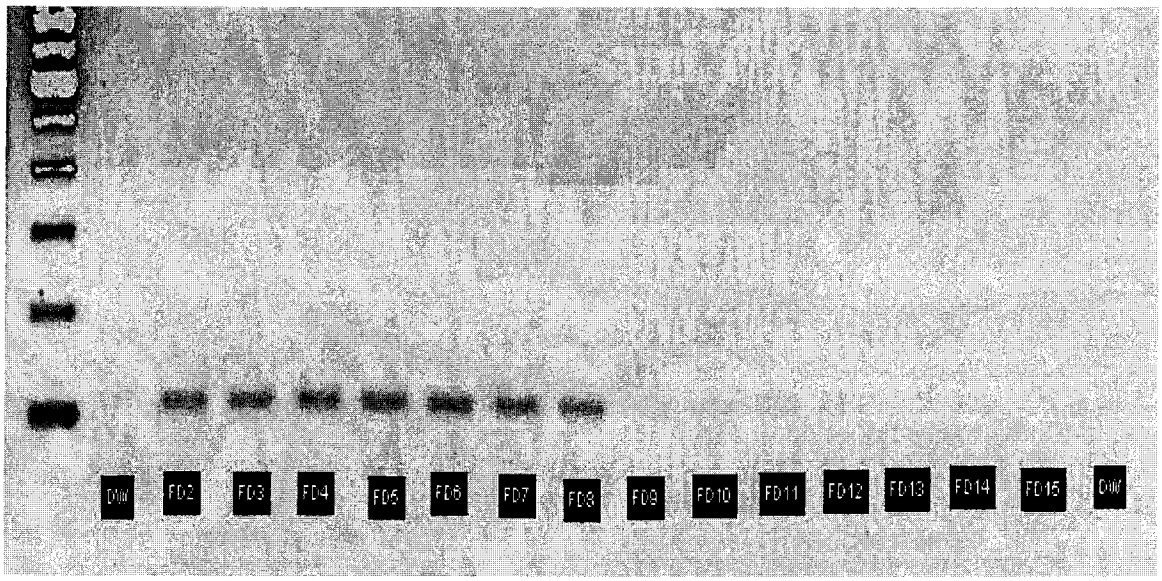
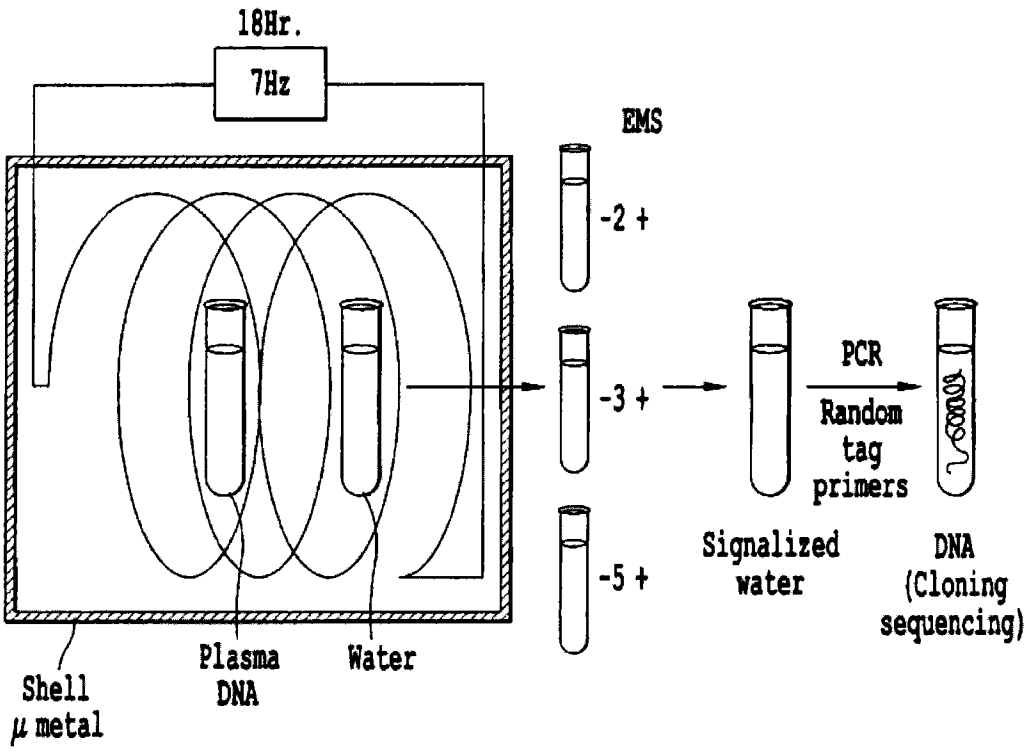


Fig. 3


18Hr.
7Hz
EMS
-2 +
-3 +
-5 +
PCR
Random tag primers
Signalized water
DNA (Cloning sequencing)
Shell μ metal
Plasma DNA
Water