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(54) **METHOD AND DEVICE FOR OBTAINING OR AMPLIFYING NUCLEIC ACID FROM A CELL USING A NONPLANAR SOLID SUBSTRATE**

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(57) **ABSTRACT**

The present invention relates to a method of isolating a nucleic acid from a microorganism cell. The method comprises contacting a nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0 so that the microorganism cell is bound to the nonplanar solid substrate, and lysing the microorganism cell bound to the nonplanar solid substrate. The invention also relates a device including the nonplanar solid substrate for isolating and amplifying a nucleic acid.

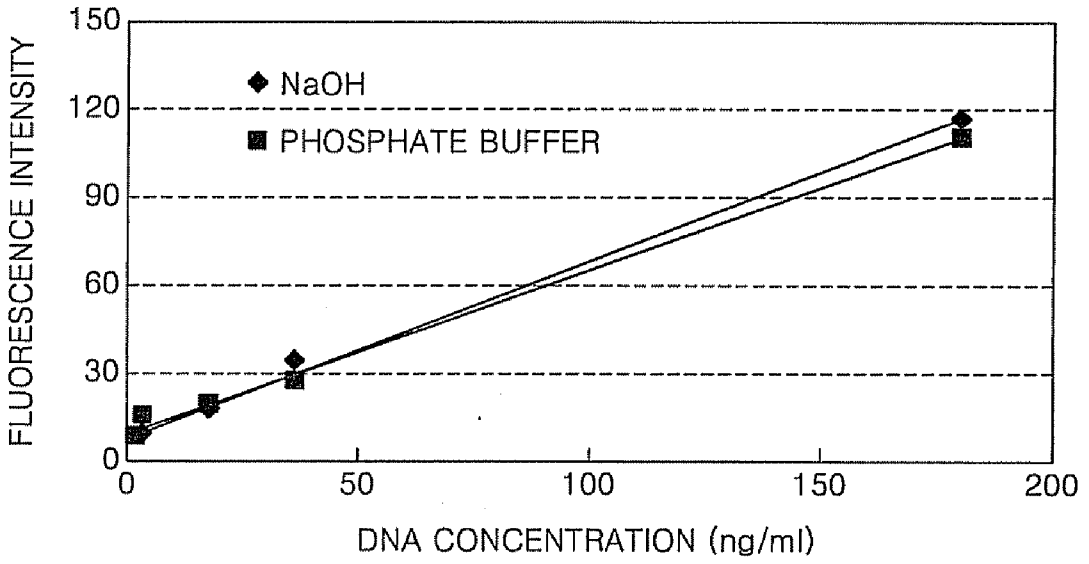


FIG. 1

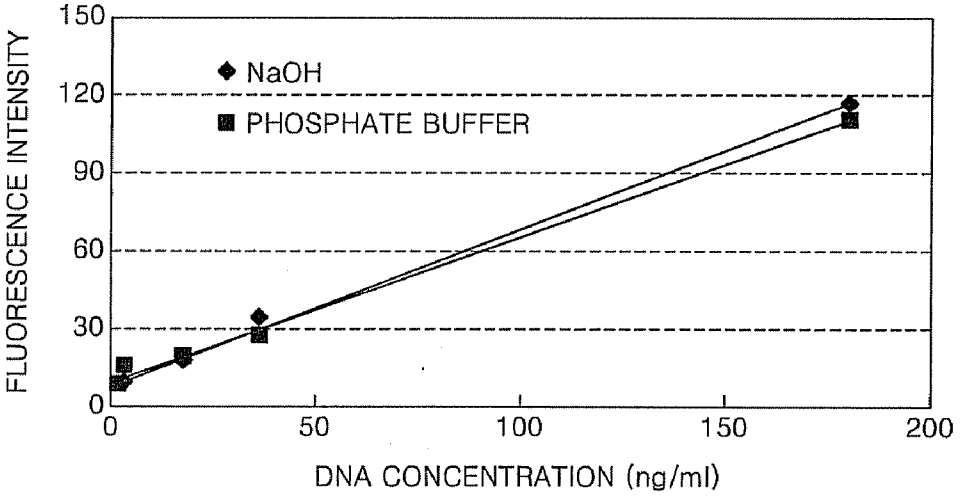

150
120
90
60
30
0
FLUORESCENCE INTENSITY
NaOH
PHOSPHATE BUFFER
0
50
100
150
200
DNA CONCENTRATION (ng/ml)

FIG. 2

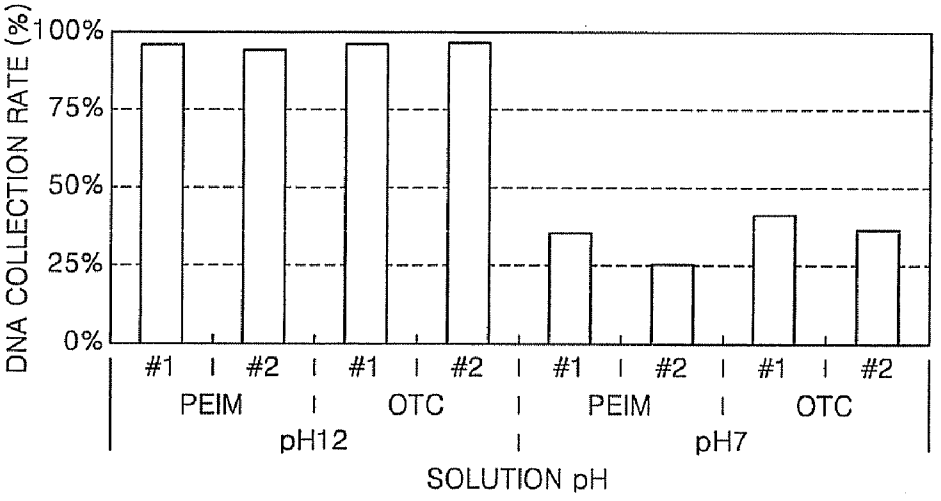

DNA COLLECTION RATE (%)
100%
75%
50%
25%
0%
#1
#2
#1
#2
#1
#2
#1
#2
PEIM
OTC
PEIM
OTC
pH12
pH7
SOLUTION pH

FIG. 3

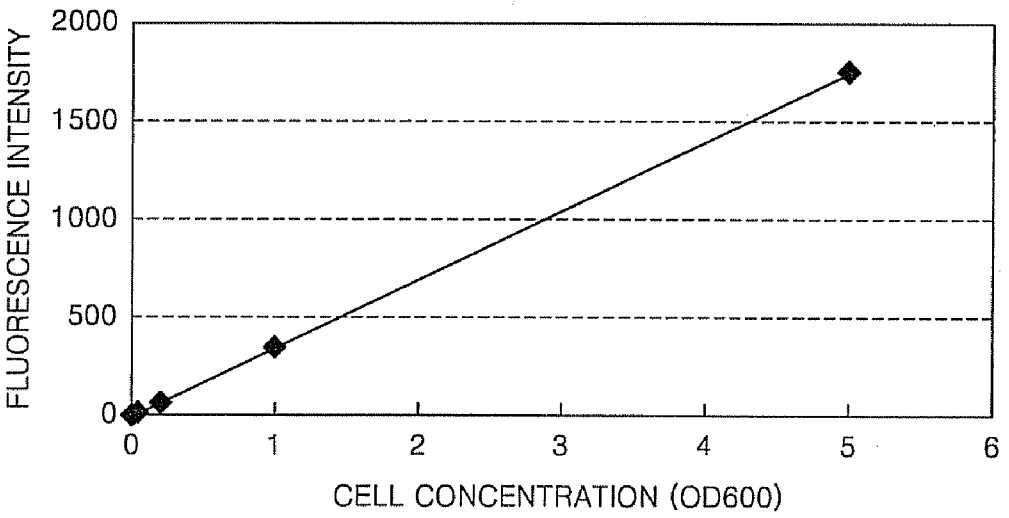

FLUORESCENCE INTENSITY
2000
1500
1000
500
0
0
1
2
3
4
5
6
CELL CONCENTRATION (OD600)

FIG. 4

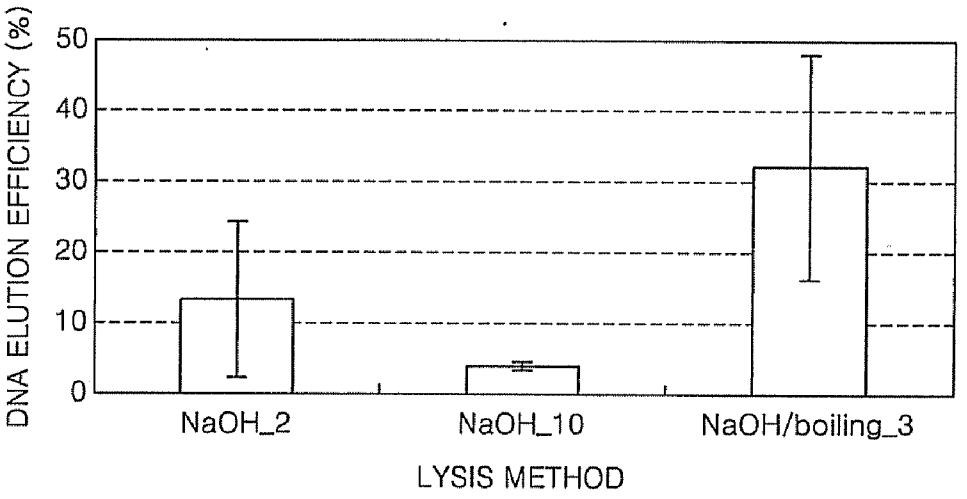

DNA ELUTION EFFICIENCY (%)
50
40
30
20
10
0
NaOH_2
NaOH_10
NaOH/boiling_3
LYSIS METHOD

FIG. 5

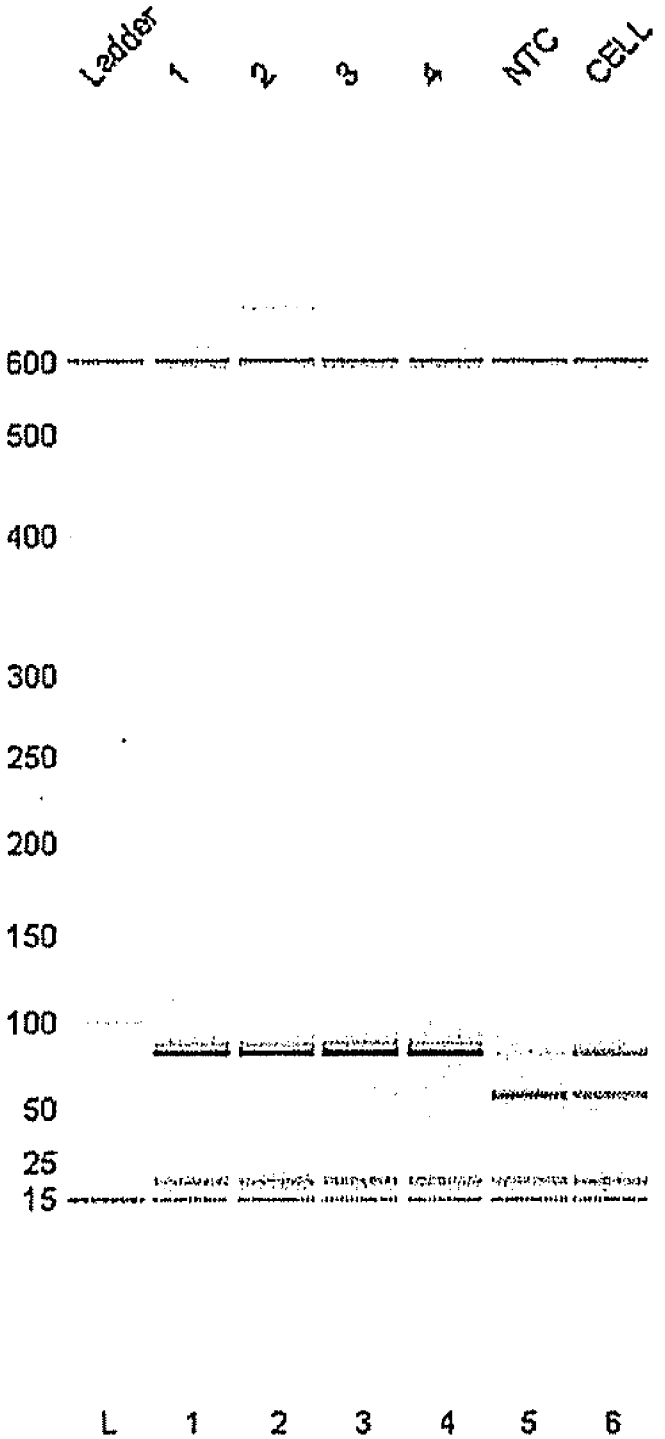

Ladder
1
2
3
4
NTC
CELL
600
500
400
300
250
200
150
100
50
25
15
L
1
2
3
4
5
6

FIG. 6

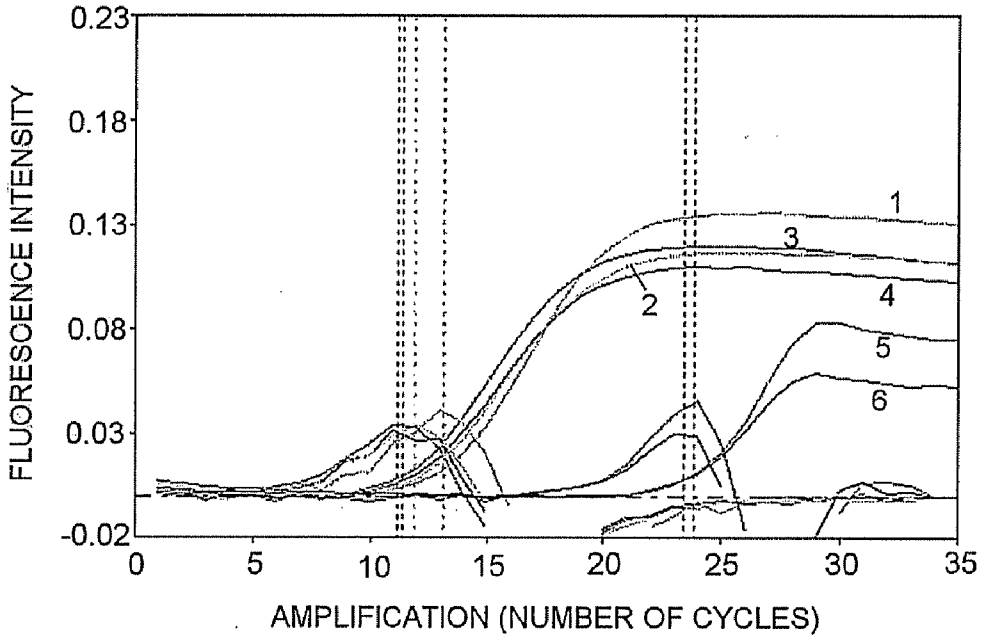

0.23
0.18
0.13
0.08
0.03
-0.02
FLUORESCENCE INTENSITY
0
5
10
15
20
25
30
35
AMPLIFICATION (NUMBER OF CYCLES)
1
2
3
4
5
6

FIG. 7

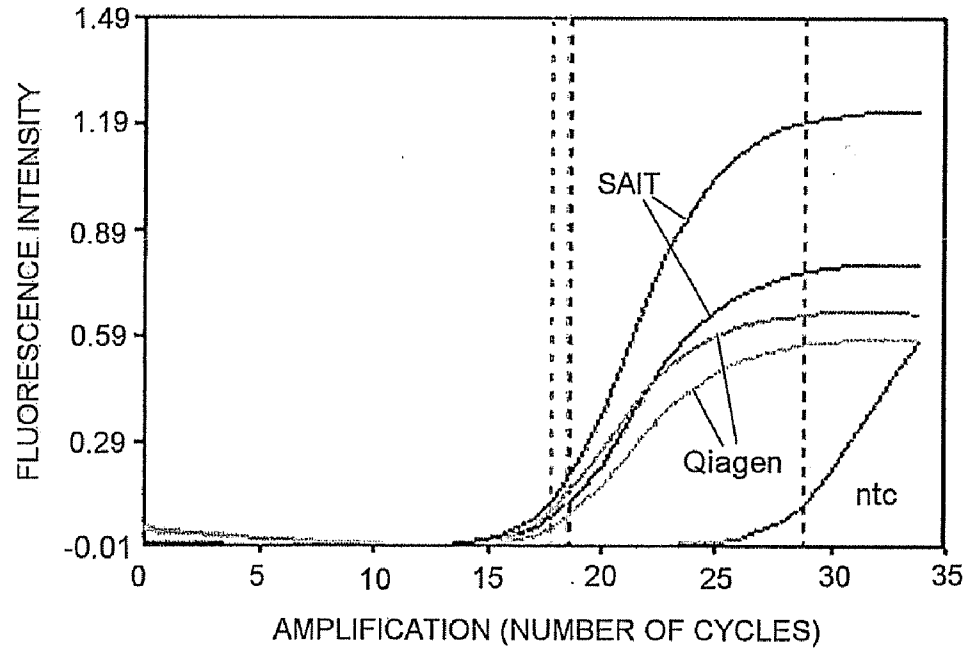
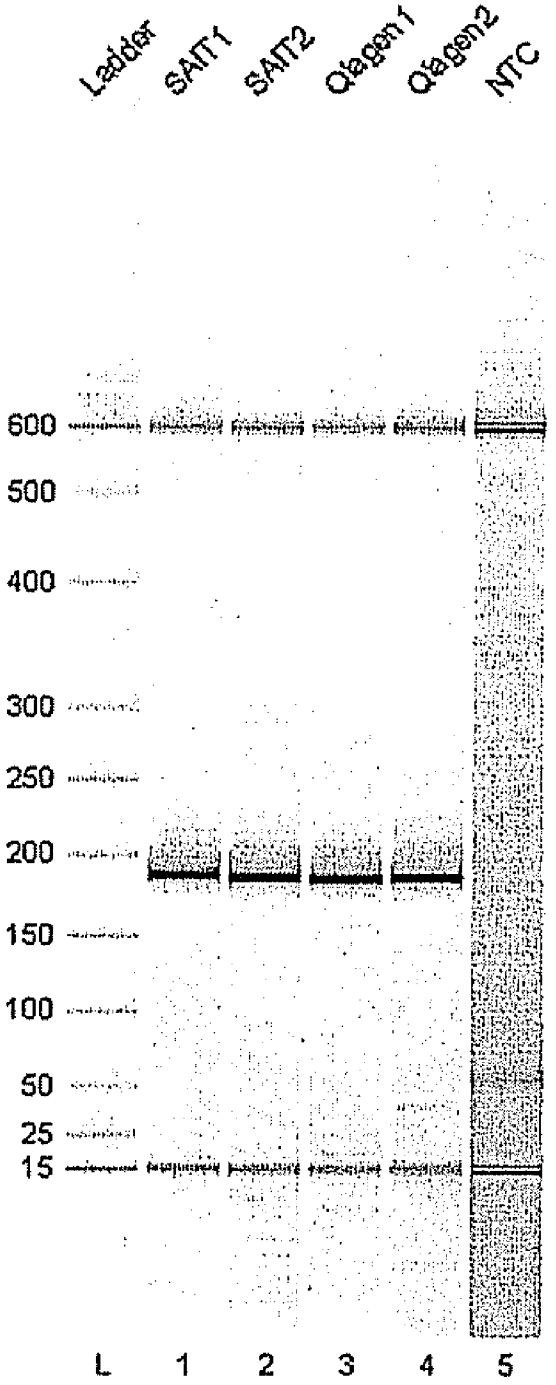

1.49
1.19
0.89
0.59
0.29
-0.01
FLUORESCENCE INTENSITY
0
5
10
15
20
25
30
35
AMPLIFICATION (NUMBER OF CYCLES)
SAIT
Qiagen
ntc

FIG. 8


Ladder
SAT1
SAT2
Qiagen1
Qiagen2
NTC
600
500
400
300
250
200
150
100
50
25
15
L
1
2
3
4
5

METHOD AND DEVICE FOR OBTAINING OR AMPLIFYING NUCLEIC ACID FROM A CELL USING A NONPLANAR SOLID SUBSTRATE

[0001] This application claims priority to Korean Patent Application Nos. 10-2006-0079056, 10-2006-0079053, 10-2006-0079054, and 10-2006-0079055, each filed on Aug. 21, 2006, and all the benefits accruing therefrom under 35 U.S.C. § 119, the disclosure of each is incorporated herein in its entirety by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to a method of isolating a nucleic acid from a cell using a nonplanar solid substrate, a method of amplifying the isolated nucleic acid as a template, and a device comprising the nonplanar solid substrate for isolating and amplifying a nucleic acid.

[0004] 2. Description of the Related Art

[0005] Several conventional methods of purifying a nucleic acid using a solid phase are known. For example, U.S. Pat. No. 5,234,809 discloses a method of purifying a nucleic acid using a solid phase to which the nucleic acid is bound. This method, however, is time-consuming and complex, and thus, is unsuitable for a lab-on-a-chip (LOC). In addition, this method requires use of a chaotropic substance. That is, when a chaotropic substance is not used, a nucleic acid does not bind to the solid phase.

[0006] U.S. Pat. No. 6,291,166 discloses a method of archiving a nucleic acid using a solid phase matrix. According to this method, the nucleic acid is irreversibly bound to the solid phase matrix. Such irreversible binding enables delayed analysis or repeated analysis after a nucleic acid-solid phase matrix composite is generated and stored. However, in this method, a substance having a positively charged surface, such as alumina, is activated by a base substance, such as NaOH, and then the nucleic acid is irreversibly bound to the activated alumina. As a result, the bound nucleic acid cannot be isolated from the alumina.

[0007] U.S. Pat. No. 5,705,628 discloses a method of reversibly and non-specifically binding DNA in a DNA-containing solution containing a salt and polyethylene glycol to a magnetic microparticle having a carboxyl group-coated surface. This method uses a magnetic microparticle having a carboxyl group-coated surface, a salt, and polyethylene glycol, in order to isolate DNA.

[0008] In the conventional methods of isolating and purifying a nucleic acid described above, addition of a high-concentration reagent is required for DNA binding. However, such addition can affect a subsequent process, such as a polymerase chain reaction (PCR) and cannot be used on a lab-on-a-chip (LOC). In addition, these conventional methods of isolating and purifying a nucleic acid are performed independently from a method of purifying or concentrating a cell. Furthermore, a method of purifying or concentrating a cell and then, in the same reaction vessel, isolating a nucleic acid from the resulting purified or concentrated cell is not known.

[0009] Accordingly, there is a need to develop a method in which a cell is isolated or concentrated by binding the cell to a solid surface, such as a substrate, and then, in the same reaction vessel, a nucleic acid derived from the cell can be purified, isolated and concentrated due to high affinity with respect to the nucleic acid.

BRIEF SUMMARY OF THE INVENTION

[0010] In one embodiment, the invention is directed to a method of obtaining a nucleic acid from a cell, the method comprising contacting a nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0 so that the cell is bound to the nonplanar solid substrate, wherein the nonplanar solid substrate is hydrophobic and has a water contact angle of 70° to 95° or the nonplanar solid substrate has at least one amine-based functional group at its surface; and lysing the cell bound to the nonplanar solid substrate to obtain a nucleic acid from the lysed cell.

[0011] In another embodiment, the invention is directed to a device for isolating and amplifying a nucleic acid, the device comprising a reaction chamber comprising a nonplanar solid substrate, wherein the nonplanar solid substrate is hydrophobic and has a water contact angle of 70° to 95° or the nonplanar solid substrate has at least one amine-based functional group at its surface; a heating unit which heats the reaction chamber; and a temperature controlling unit which controls the heating unit.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The above and other features and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in which:

[0013] FIG. **1** is a graph showing concentration of DNAs, indicated by fluorescence intensity, in a NaOH solution of pH 12.0 and in a phosphate buffer of pH 7.0.

[0014] FIG. **2** is a graph showing the degree of DNA collection with respect to pH of a DNA sample after the DNA sample is pumped through a fluidic device with a pillar array.

[0015] FIG. **3** is a graph showing the concentration of a cell with respect to the concentration of DNA, indicated by fluorescence intensity, present in a cell lysate obtained by cell lysis.

[0016] FIG. **4** is a graph illustrating DNA elution efficiency measured according to a method of the present invention in which an *E. coli*-containing sample is pumped through a fluidic device including a chamber having an octadecyldimethyl (3-trimethoxysilyl propyl)ammonium (OTC)-coated surface and a pillar array, the bound cell is lysed, and then the resultant lysate is collected to measure the DNA elution efficiency.

[0017] FIG. **5** is a graph showing the concentration of amplified DNA measured according to a method in which the *E. coli*-containing sample is pumped through a chamber having a surface with a pillar array of a fluidic device, undergoes cell lysis, cell washing, and real time PCR amplification, and electrophoresis.

[0018] FIG. **6** is a graph illustrating results of real time PCR amplification after an *E. coli*-containing urine sample is pumped through a chamber having a pillar array of a fluidic device and undergoes cell lysis and cell washing.

[0019] FIG. **7** is a graph illustrating results of real time PCR amplification after an *E. coli*-containing whole blood sample is pumped through a chamber having a pillar array of a fluidic device and undergoes cell lysis and DNA.

[0020] FIG. **8** is a graph illustrating results of electrophoresis after an *E. coli*-containing whole blood sample is pumped through a chamber having a pillar array of a fluidic device and undergoes cell lysis, DNA extraction, and real time PCR amplification.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The invention will now be describe more fully hereinafter with reference to the accompanying drawings, in which embodiments of the invention are shown. The invention may, however, be embodied n may different forms and should not be construed as limited to embodiments set forth herein. Rather these embodiments are provided so that this disclosure will be through and complete, and will fully convey the scope of the invention to those skilled in the art.

[0022] In one embodiment, the invention provides a method of obtaining a nucleic acid from a cell. The method comprises contacting a nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0 so that the cell is bound to the nonplanar solid substrate; and lysing the cell bound to the nonplanar solid substrate to obtain a nucleic acid from the lysed cell.

[0023] The method comprises contacting a nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0 so that the cell is bound to the nonplanar solid substrate. The cell binds to the nonplanar solid substrate by the contacting.

[0024] As used herein, the term "cell" means a prokaryotic or eukaryotic cell, a plant cell, a bacteria cell, a pathogenic cell, a yeast cell, an aggregate of cells, a virus, a fungus, or other nucleic acid containing biological material, such as, for example, an organelle.

[0025] As used herein, the term "nucleic acid" means DNA or RNA, or a combination of both. The DNA or RNA can be in any possible configuration, i.e., in the form of double-stranded (ds) nucleic acid, or in the form of single-stranded (ss) nucleic acid, or as a combination thereof (in part ds or ss).

[0026] As used herein, the term "cell-binding" means the ability to bind a cell or other biomaterial, such as, for example a nucleic acid.

[0027] In a liquid medium containing a solid substrate, a cell, such as, for example, a bacteria cell, can exist in the liquid medium or can be bound to the solid substrate. Whether the cell exists in the liquid medium or is bound to the solid substrate is determined by a difference in surface tensions of the liquid medium and the cell. For example, when the liquid medium has greater surface tension than the cell, the cell may be easily bound to a solid substrate having low surface tension, that is, to a hydrophobic solid substrate. When the surface tension of the liquid medium is less than the surface tension of the cell, the cell may be easily bound to a solid substrate having greater surface tension, that is, to a hydrophilic solid substrate. When the liquid medium and cell have the same surface tension, the surface tension does not affect the binding of cell to the solid substrate and other interaction factors, such as electrostatic interaction, may affect such binding (see Applied and Environmental Microbiology, July 1983, p. 90-97). In addition, it is known that cell can be bound to the solid substrate by electrostatic attraction as well as by a thermodynamic approach based on the surface tension. However, such bindings occur very slowly and its bound quantity was minimal.

[0028] In an attempt to address the problems described above, the inventors of the present invention found that a large amount of cells could be bound to a nonplanar solid substrate by contacting the nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0. The use of a nonplanar solid substrate provides increased surface area upon which the cells bind, relative to a planar surface. Therefore, without being held to theory, it is believed that a large amount of cells can be bound because the surface area of a nonplanar solid substrate is increased, relative to a planar surface. Furthermore, by using a liquid medium having a pH 3.0 to 6.0, the cell membrane of the cell is denatured and thus the cell is less soluble with respect to a solution and therefore relatively more cells can be bound to the solid surface. However, the present invention is not limited to such a technique.

[0029] During the contacting step, the sample can be any sample containing a cell. For example, the sample can be a biological sample containing a cell, a clinical sample containing a cell, or a lab sample containing a cell.

[0030] As used herein, the term "biological sample" a sample that comprises or is formed of a cell or tissue, such as a cell or biological liquid isolated from an animal or plant. In one advantageous embodiment, the animal can be a human. The biological sample can be saliva, sputum, blood, blood cells (for example, red blood cells or white blood cells), amniotic fluid, serum, semen, bone marrow, tissue or a micro needle biopsy sample, urine, peritoneum fluid, pleura fluid, or cell cultures. In addition, the biological sample can be a tissue section, such as a frozen section taken for a histological object. Preferably, the biological sample is a clinical sample obtained from a human patient. More preferably, the biological sample is blood, urine, saliva, or sputum. Furthermore, the term "biological sample" means a sample that is formed comprising an organism, group of organisms from the same or different species, cells or tissues, obtained from the environment, such as from a body of water, from the soil, or from a food source or an industrial source.

[0031] In one embodiment, the method comprises, during the contacting step, the biological sample can be diluted with a solution or buffer that may buffer the cell with a low pH. The buffer can be, for example, a phosphate buffer, such as sodium phosphate of pH 3.0 to 6.0, or an acetate buffer, such as sodium acetate of pH 3.0 to 6.0. The degree of dilution is not limited, and, for example, the biological sample can be diluted in a range of 1:1 to 1:1,000, and preferably, 1:1 to 1:10.

[0032] In another embodiment, the method comprises, during the contacting step, the sample may have a salt concentration of 10 mM to 500 mM, and preferably, 50 mM to 300 mM. That is, the sample may have an acetate or phosphate ion concentration of 10 mM to 500 mM, preferably 50 mM to 300 mM.

[0033] In one embodiment, during the contacting process, the solid substrate contacted with the cell-containing sample has a nonplanar shape such that the surface area of the nonplanar solid substrate can be increased compared to a planar surface. For example, the nonplanar solid substrate may have a corrugated surface. As used herein, the term "corrugated surface" refers to a non-level surface having grooves and ridges. The corrugated surface can be a surface having a plurality of pillars or a sieve-shaped surface having a plurality of pores. However, the corrugated surface is not limited thereto, and may comprise other shapes.

[0034] For example, the nonplanar solid substrate can be a solid substrate having a surface comprising a plurality of pillars, a bead-shaped solid substrate, and a sieve-shaped solid substrate having a plurality of pores in its surface. The solid substrate can be a single solid substrate or a combination of one or more solid substrates, such as a solid substrate assembly that fills a tube or container.

[0035] In one embodiment, the nonplanar solid substrate may form an inner wall of a microchannel or microchamber of a microfluidic device. Accordingly, the method of obtaining a nucleic acid from a cell according the present invention can be used in a fluidic device or microfluidic device having at least one inlet and outlet connected through a channel or microchannel.

[0036] As used herein, the term "microfluidic device" incorporates the concept of a microfluidic device that comprises microfluidic elements such as, e.g., microfluidic channels (also called microchannels or microscale channels). As used herein, the term "microfluidic" refers to a device component, e.g., chamber, channel, reservoir, or the like, that includes at least one cross-sectional dimension, such as depth, width, length, diameter, etc. of from about 0.1 micrometer to about 1000 micrometer. Thus, the term "microchamber" and "microchannel" refer to a channel and a chamber that includes at least one cross-sectional dimension, such as depth, width, and diameter of from about 0.1 micrometer to about 1000 micrometer, respectively.

[0037] According to the current embodiment, during the contacting process, the nonplanar solid substrate used in the contacting step may have a surface having a plurality of pillars. Methods of forming pillars on a solid substrate is well known in the art. For example, micro pillars can be formed in a high density structure using a photolithography process used in a semiconductor manufacturing process. The micro pillars can have an aspect ratio of 1:1 to 20:1. However, the aspect ratio of the micro pillars is not limited thereto. As used herein, the term "aspect ratio" refers to a ratio of a cross-sectional diameter to height of a pillar. In the pillar structure, a ratio of the height of the pillars to a distance between adjacent pillars may be in the range of 1:1 to 25:1. The distance between adjacent pillars may be in the range of 5 μm to 100 μm.

[0038] In another embodiment, cell during the contacting process, the nonplanar solid substrate used during the contacting step can be hydrophobic and can have a water contact angle of 70° to 95°. In one embodiment, the hydrophobic property of the nonplanar solid substrate having a water contact angle of 70° to 95° can be obtained by coating octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS) on a solid substrate. More specifically, the surface of a solid substrate having a water contact angle of 70° to 95° can be obtained by self-assembled molecule (SAM) coating octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS) on a SiO_2 layer of the solid substrate.

[0039] In this application, the term "water contact angle" refers to water contact angle measured by a Kruss prop Shape Analysis System type DSA 10 Mk2. A droplet of 1.5 μl deionized water is automatically placed on the sample. The droplet was monitored every 0.2 seconds for a period of 10 seconds by a CCD-camera and analyzed by prop Shape Analysis software (DSA version 1.7, Kruss). The complete profile of the droplet was fitted by the tangent method to a general conic section equation. The angles were determined both at the right and left side. An average value is calculated for each drop and a total of five drops per sample are measured. The average of the five drops is taken the contact angle.

[0040] According to one embodiment, the nonplanar solid substrate used during the contacting step can have at least one amine-based functional group at its surface. The surface with the amine-based functional group may be obtained by coating polyethyleneiminetrimethoxysilane (PEIM) on a solid substrate. For example, the coated surface can be obtained by self-assembled molecule (SAM) coating polyethyleneiminetrimethoxysilane (PEIM) on a SiO_2 layer of the solid substrate.

[0041] In one embodiment of the method of obtaining a nucleic acid from a cell, the nonplanar solid substrate, during the contacting step, the nonplanar solid substrate can be a substrate formed of any kind of material that has the water contact angle described above, or has at least one amine-based functional group at its surface. For example, the nonplanar solid substrate can be formed of glass, silicon water, plastic, or the like, but is not limited thereto. When a nonplanar solid substrate with a surface having a water contact angle of 70° to 95° or a surface having at least one amine-based functional group is contacted with a sample containing a cell, it is assumed that the cell is bound to the nonplanar solid substrate. However, the present invention is not limited to such a specific mechanism.

[0042] According to the current embodiment, the method of obtaining a nucleic acid from a cell may further include, after the contacting step, washing the cells bound to the nonplanar solid substrate by introducing a washing solution to the nonplaner solid substrate to wash other materials which are not bound to the nonplanar solid substrate, whereby the washing step does not remove the target cell or target nucleic acid bound to the nonplaner solid substrate. During the washing step, any solution that does not liberate the target cell bound to the nonplanar solid substrate from the nonplanar solid substrate and removes impurities that may adversely affect subsequent processes can be used. For example, the washing solution can be an acetate buffer or phosphate buffer, which can also used as a binding buffer, can be used as the washing solution. In one embodiment, the washing solution can have a pH of 3.0 to 6.0.

[0043] As used herein, "isolation of a microorganism cell" means concentrating the cell in the sample as well as purely separating the cell.

[0044] According to the current embodiment the method comprises lysing the cell bound to the nonplanar solid substrate.

[0045] The step of lysing of the microorganism cell can be performed using any lysing method known in the art. For example, the lysis method can be boiling lysis, laser lysis, lysis using a chemical material, or electrochemical lysis, such as electrolysis, but is not limited thereto.

[0046] In the current embodiment, the cell can be lysed in any liquid medium having a pH of 3.0 to 6.0 to bind a nucleic acid derived from the cell to the nonplanar solid substrate. In one embodiment, the lysis can be performed in a phosphate buffer or an acetate buffer.

[0047] In the current embodiment, the nucleic acid derived from the lysed microorganism cell is bound to the nonplanar solid substrate. The bound nucleic acid can be isolated by removing cell debris, and the like, which are not bound to the nonplanar solid substrate.

[0048] Accordingly, the method according to the current embodiment further comprises, after the lysing step, washing the nonplanar solid substrate with a washing buffer to remove materials which are not bound to the nonplanar solid substrate.

[0049] During the washing step, the washing buffer can be any solution which does not liberate the bound nucleic acid from the nonplanar solid substrate and removes materials which are not bound to the nonplanar solid substrate. More specifically, the washing solution can be a solution having these properties described above and which removes impurities that may adversely affect subsequent processes. Therefore, the washing solution used to wash bound nucleic acid can be the same as the wash buffer used to wash the cells bound to the nonplaner solid substrate, but is not limited thereto. In one embodiment, the washing buffer can be an acetate buffer or phosphate buffer which can also be used as the binding buffer. The washing buffer can be a buffer having a pH of 3.0 to 6.0.

[0050] In the current embodiment, the nucleic acid bound to the nonplanar solid substrate can be used in its bound form, or the bound nucleic acid can be extracted from the nonplanar solid substrate.

[0051] Accordingly, the according to the current embodiment, the method may further comprise extracting the nucleic acid bound to the nonplanar solid substrate.

[0052] During the extracting step, the extracting solution may be any solution known in the art which can liberate the nucleic acid bound to the nonplanar solid substrate from the nonplanar solid substrate. For example, the extracting solution can be a solution having a high pH. Without being bound to theory, it is believed that by using a solution having a high pH, hydroxide ions (OH—) make the surface of the nonplanar solid substrate anionic such that anionic DNA can be liberated from the nonplanar solid substrate. In one embodiment, the extracting solution can be a solution having a pH 11 or more, such as, for example, a NaOH solution.

[0053] According to another embodiment of the invention, the step of lysing the cell in a solution of pH 11 to 14. Lysis of the cell in a solution having a high pH can minimize the binding of DNA to a nonplanar solid substrate so that DNA can be easily extracted. By using a solution having a high pH, hydroxide ions (OH—) make the surface of the nonplanar solid substrate anionic such that anionic DNA can be liberated after the lysis is not bound to the nonplanar solid substrate. That is, if the object is to bind a cell to a nonplanar solid substrate and to extract DNA derived from the cell, it is desired that the cell is lysed in a solution having a high pH, followed by an extraction step performed using the same solution having a high pH. Therefore, according to the current embodiment, the method of isolating a nucleic acid from a cell can further comprise purifying the nucleic acid from a lysate obtained during the lysing step. The purifying method can be any method known in the art.

[0054] In another embodiment, the present invention also provides a method of amplifying a nucleic acid using as a template the nucleic acid that is isolated using the method of isolating a nucleic acid from a cell according to an embodiment of the present invention.

[0055] The amplifying of a nucleic acid step can be performed using any amplifying method known in the art, for example, using PCR. The method of isolating a nucleic acid from a microorganism cell is described above.

[0056] In the method of amplifying a nucleic acid, the nucleic acid can be DNA or RNA, or a combination of both, and preferably DNA.

[0057] According to the current embodiment, the nucleic acid can be isolated and amplified in the same container that includes the nonplanar solid substrate. The container can be a microchannel, a microchamber, or a tube, but is not limited thereto. For example, the container may be a microchamber of a microfluidic device which is equipped with a PCR device. In one embodiment, the PCR device includes a heater and a cooler. Therefore, in the method of amplifying a nucleic acid according to the current embodiment, a nucleic acid is extracted in a microchamber and the extracted nucleic acid is amplified in the same microchamber.

[0058] In another embodiment of the method of amplifying a nucleic acid, the isolation and amplification of the nucleic acid can be performed in different containers. For example, the isolation of the nucleic acid can be performed in a container that includes the nonplanar solid substrate, and the amplification of the nucleic acid can be performed in a different container that may or may not in fluid communication with the container that includes the nonplanar solid substrate. The different container can be a microchannel, a microchamber, or a tube, but is not limited thereto. For example, the isolation of the nucleic acid can be performed in a microchannel or microchamber of a microfluidic device, and the isolated nucleic acid is then transported to a different microchannel or microchamber and extracted for amplification.

[0059] In one embodiment, the invention comprises a device for isolating and amplifying a nucleic acid The device for isolating and amplifying nucleic acid comprises: a reaction chamber including a nonplanar solid substrate; a heating unit which heats the reaction chamber; and a temperature controlling unit which controls the heating unit.

[0060] According to one embodiment, the device for isolating and amplifying a nucleic acid comprises a solid substrate having a nonplanar surface that has a greater surface area than a planar solid substrate. The nonplanar solid substrate may have a corrugated surface. In the present specification, the corrugated surface refers to a non-level surface having grooves and ridges. The corrugated surface

can be a surface with a plurality of pillars or a sieve-shaped surface with a plurality of pores. However, the corrugated surface is not limited thereto, and may have other shapes.

[0061] In one embodiment, the device for isolating and amplifying a nucleic acid comprises a nonplanar solid substrate, which can have various shapes. For example, the nonplanar solid substrate can be selected from a solid substrate having a surface with a plurality of pillars, a bead-shaped solid substrate, and a sieve-shaped solid substrate having a plurality of pores in its surface. The solid substrate can be a single solid substrate or a combination of solid substrates, such as a solid substrate assembly which fills a tube or container.

[0062] In one embodiment, the device for isolating and amplifying a nucleic acid, the nonplanar solid substrate of the device for isolating and amplifying a nucleic acid can form an inner wall of a microchannel or microchamber of a microfluidic device. Accordingly, the device for isolating and amplifying a nucleic can be a fluidic device or microfluidic device having at least one inlet and outlet connected through a channel or microchannel. That is, the device for isolating and amplifying a nucleic acid according to the current embodiment can be a fluidic device or microfluidic device in which the isolation and PCR of a nucleic acid can be performed in the same chamber.

[0063] According to the current embodiment of the present invention, the nonplanar solid substrate can have a surface having a plurality of pillars. Methods of forming pillars on a solid substrate is well known in the art. For example, micro pillars can be formed in a high density structure using a photolithography process used in a semiconductor manufacturing process. The micro pillars can have an aspect ratio of 1:1 to 20:1. However, the aspect ratio of the micro pillars is not limited thereto. As used herein, the term "aspect ratio" refers to a ratio of a cross-sectional diameter to height of a pillar. In the pillar structure, a ratio of the height of the pillars to a distance between adjacent pillars may be in the range of 1:1 to 25:1. The distance between adjacent pillars may be in the range of 5 μm to 100 μm.

[0064] According to the current embodiment, the nonplanar solid substrate may be hydrophobic, having a water contact angle of 70° to 95°. In one embodiment, the hydrophobic property of the nonplanar solid substrate having a water contact angle of 70° to 95° can be obtained by coating octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS) on the nonplanar solid substrate. More specifically, the nonplanar solid substrate having a water contact angle of 70° to 95° can be obtained by self-assembled molecule (SAM) coating octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS) on a SiO_2 layer of the non planar solid substrate.

[0065] According to the current embodiment of the present invention, the nonplanar solid substrate can have at least one amine-based functional group at its surface. The surface with the amine-based functional group may be obtained by coating polyethyleneiminetrimethoxysilane (PEIM) on the nonplanar solid substrate. For example, the coated surface can be obtained by self-assembled molecule (SAM) coating polyethyleneiminetrimethoxysilane (PEIM) on a SiO_2 layer of the nonplanar solid substrate.

[0066] In the device for isolating and amplifying a nucleic acid according to the current embodiment, the heating unit can be any device known in the art which can be used to heat the chamber. The heating unit can be a heater or a micro heater.

[0067] In the device for isolating and amplifying a nucleic acid according to the current embodiment, the temperature controlling unit can be any controller known in the art which can control the heating unit to generate a temperature cycle used in PCR. The temperature controlling unit can include a temperature sensor that senses the temperature of the chamber and a device that controls on/off operation of the heating unit.

[0068] The present invention will now be described in further detail with reference to the following examples. These examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

Binding Properties of DNA to Solid Substrate Having Pillar Structure

[0069] In the current example, binding properties of DNA were determined by loading a DNA sample into a fluidic device including an inlet, an outlet, and a chamber having a pillar array on a 10 mm×23 mm substrate. In the pillar array, the distance between adjacent pillars was 12 μm, the height of each pillar was 100 μm, and a sectional surface of each pillar was a regular square with sides of 25 μm.

[0070] In the pillar array, each pillar had a surface coated with PEIM having at least one amine-based functional group, or a surface coated with OTC having a water contact angle of 80°. Coatings of OTC having a water contact angle of 70° to 95° give similar results.

[0071] DNA in 0.01N NaOH (pH 12) or DNA in 100 mM NaH_2PO_4 (pH 7.0) was used as a DNA sample. A volume of 200 μl of the DNA sample was pumped through a fluidic device at a flow rate of 200 μl/minute.

[0072] The DNA bound to the pillar array in the fluidic device was measured using a PICOGREEN® kit (obtained from Molecular Probes Inc) and a spectroscope according to the instructions provided by the manufacturer.

[0073] FIG. **1** is a graph showing fluorescence intensity as a function of the concentration of DNA in NaOH solution of pH 12.0 or in a phosphate buffer of pH 7.0. Referring to FIG. **1**, the graph demonstrates that the concentration of DNA is proportional to the fluorescence intensity, and thus it was found that the concentration of DNA could be determined using an equation based on the proportional relation between a fluorescence intensity and DNA concentration.

[0074] FIG. **2** is a graph showing the degree of DNA collection as a function of pH of the DNA sample after the DNA sample was pumped through a fluidic device with a pillar array coated with OTC having a water contact angle of 80°. Referring to FIG. **2**, when DNA was in NaOH solution of pH 12.0, 95% or more of the DNA was re-collected, that is, only 5% or less of the DNA in the sample bound to the substrate surface. On the other hand, when DNA was in a

phosphate buffer of pH 7.0, about 40% or less of the DNA was re-collected, with 60% or more of the DNA bound to the substrate surface. These results show that when the pH of the sample solution is high, such as in the range of 11 to 14, DNA does not bind efficiently to the solid substrate, but when pH of the sample solution is relatively low, such as in the range of 3 to 8, preferably 3 to 6, DNA is efficiently bound to the solid substrate. Accordingly, it was determined that by using solutions having different pH values, DNA can be easily bound to or eluted from a solid substrate having a pillar structure coated with PEIM having at least one amine-based functional group or coated with OTC having a water contact angle of 70° to 95°. That is, binding of DNA to a solid substrate should be performed in low pH, and eluting of DNA from the solid substrate should be performed in high pH.

Example 2

Binding of a Cell to a Solid Substrate with a Pillar Array in the Fluidic Device, Cell Lysis, and DNA Elution

[0075] In this example, a cell sample was loaded into a fluidic device including an inlet, an outlet, and a chamber having a pillar array formed on a 10 mm×23 mm substrate, and then the cell was lysed to elute DNA. In the pillar array, a distance between adjacent pillars was 12 μm, the height of each pillar was 100 μm, and a sectional surface of each pillar was a regular square having sides of 25 μm.

[0076] The pillar array had a surface coated with OTC having a water contact angle of 80°.

[0077] The cell sample was *E. coli* in LB medium (pH 7.2), with an OD_{600} value of 0.01. 250 μl of the cell sample was pumped through the fluidic device at a flow rate of 200 μl/minute.

[0078] Subsequently, *E. coli* that was bound to the surface of the pillar array was lysed. The cell lysis was performed in one of three ways: (1) by pumping through 50 μl of 0.01N NaOH aqueous solution (pH 12.0) to the chamber (chamber volume 5 ul) at a flow rate of 25 μl/minute for 2 minutes (hereinafter referred to as 0.01N NaOH 2), (2) by pumping 50 μl of 0.01N NaOH solution to the chamber at a flow rate of 5 μl/minute for 10 minutes (hereinafter referred to as 0.01N NaOH_10), or (3) by loading 3.5 μl of 0.01N NaOH to the chamber at a flow rate of 200 μl/minute, and then raising the temperature to 100° C. and maintained for 2 minutes, and then pumping through 46.5 μl of 0.01N NaOH solution at a flow rate of 200 μl/minute (hereinafter referred to as 0.01N NaOH/boiling 3).

[0079] 40 μl of the resulting lysate obtained by the cell lysis was collected and the concentration of DNA therein was measured using a PICOGREEN® kit (obtained from Molecular Probes Inc) and a spectroscope. The DNA elution efficiency was measured using the following formula: (fluorescence intensity of the sample/fluorescence intensity when 100% of the cells are lysed)×100.

[0080] A reference plot for determining DNA concentration in the cell lysates was obtained by lysing samples containing *E. coli* having a known concentration using NaOH in a tube, measuring the fluorescence intensity of the lysed sample, and generating a standard curve of the fluorescence intensity as a function of the cell concentration.

[0081] FIG. **3** is a graph showing fluorescence intensity of DNA present in a cell lysate as a function of the concentration of the cells lysed. Referring to FIG. **3**, the fluorescence intensity of the cell lysate is proportional to the concentration of the cells.

[0082] The expected DNA concentration when 100% of the cells were lysed was obtained by following conversion process. The amount of input cells=10^7 cells/ml (assuming 1.0 D_{600} as 10^9 cell/ml)×0.25 ml=2.5×10^6 cells, (assuming that the binding efficiency was 100%). Therefore, the expected maximum amount of eluted DNA=2.5×10^6 cells×5 fg DNA/*E. coli* cell=12.5 ng. Then, the amount of eluted DNA was divided by about 50 μl, which was the entire volume of NaOH used as the eluting solution. As a result, the concentration of DNA obtained from the total cells was 0.25 ng/μl.

[0083] FIG. **4** is a graph illustrating DNA elution efficiency measured according to a method in which an *E. coli*-containing sample was pumped through a fluidic device including a chamber having a octadecyldimethyl (3-trimethoxysilyl propyl)ammonium (OTC)-coated surface and a pillar array, the bound cells from the sample were lysed by one of the three methods described above, and then the resultant cell lysate was collected. As illustrated in FIG. **4**, DNA elution efficiency was highest when the DNA was eluted after using the method designated NaOH/boiling 3. When 0.01N NaOH/boiling_3 was performed, 32% of DNA was collected, assuming 1.0 D_{600} represents 10^9 cell/ml. As such, it was found that the DNA collection rate after the cell lysis was in the range of 30-60% based on the total DNA present in the cells, even if considering a cell concentration correspond to 1.0 OD600 value varies, generally 5×10^8 to 10^9 cell/ml.

Example 3

Binding of Cells to a Solid Substrate with a Pillar Array in a Fluidic Device, Cell Lysis, and DNA Purification and Amplification

[0084] In the current example, a cell sample was pumped through a fluidic device including an inlet and an outlet and having a pillar array on a 7.5 mm×15 mm substrate, the cells were lysed to bind DNA derived from the cells to the substrate, materials that were not bound to the substrate were removed by washing, and DNA amplification was performed using the DNA bound to the substrate as a template. In the pillar array, a distance between adjacent pillars was 15 μm, the height of each pillar was 100 μm, and a sectional surface of each pillar was a regular square having sides of 25 μm.

[0085] The pillar array had a surface coated with OTC having a water contact angle of 80°.

[0086] The cell sample was an *E. coli*-containing sample of 0.01 OD_{600} in a LB medium. The *E. coli*-containing sample was adjusted to have a pH of 4.0 by using 100 mM sodium acetate buffer, and was pumped into a fluidic device from the inlet to the outlet through a chamber at a flow rate of 100 μl/minute for five minutes.

[0087] Then, *E. coli* bound to the surface of the pillar array was lysed. More specifically, the chamber was filled with 100 mM phosphate buffer (pH 4.0), treated at 95° for 2

minutes, and then the temperature was decreased to room temperature. This lysis step was repeated five times. After cell lysis, impurities which were not bound to the surface of the pillar array were washed using 200 μl of 100 mM phosphate buffer (pH 4.0) at a flow rate of 200 μl/minute.

[0088] Subsequently, PCR was performed using DNA bound to the surface of the pillar array of the solid substrate as a template for a TAQMAN® probe.

[0089] Table 1 shows a Ct value of real time PCR amplification of DNA obtained from an *E. coli*-containing sample which was pumped through the chamber having a pillar array of a fluidic device.

TABLE 1

Sample No.	Treatment	Ct
1	dPCR1	14.14
2	dPCR2	14.13
3	Purified PCR1	13.29
4	Purified PCR2	14.63
5	0.01 OD cell	25.72
6	Negative Control group	25.23-

[0090] In Table 1, purified PCR refers to a PCR using an *E. coli-containing* sample that was lysed and washed (samples 3 and 4 of Table 1 or Lanes 3 and 4 of the electrophoresis gel depicted in FIG. **5**), while dPCR refers to a PCR using an *E. coli*-containing sample that was not lysed and washed (samples 1 and 2 of Table 1 or Lanes of 1 and 2 of the electrophoresis gel shown in FIG. **5**). 0.01 OD cell refers to a PCR using 0.01 OD *E. coli* cells in a LB medium that was not flew the microfluidic device The threshold cycle (Ct) is the cycle number in the PCR at which the reporter dye emission intensity rises above background noise. The Ct is inversely proportional to the copy number of the target template; the higher the template concentration, the lower the threshold cycle measured. In the present invention, the Ct is practically defined as a cycle number at the reflection point of real time PCR product curve.

[0091] As shown in Table 1, in the case when the *E. coli-containing* sample (at OD600=0.01) was pumped through the chamber, underwent cell lysis, washing, and subsequent real time PCR amplification (samples 3 and 4), an increase in fluorescence intensity above the baseline was detected around 15 PCR cycles, while when a 0.01 OD600 reference *E. coli*-containing sample was used in a PCR as template, Ct was observed at around 25 (sample 5), thus DNA was concentrated about 1,000 times (that is, $\times 2^{10}$) by the isolation process. As shown in Table 1, the *E. coli* cells which underwent binding, cell lysis, washing, and PCR (samples 3 and 4) showed similar Ct value to the *E. coli* which was bound and underwent PCR without a separate cell lysis process (samples 1 and 2). Such results may result from high concentration and purification effect at a cell level. That is, it is assumed that the purification effect at a DNA level may be negligible. The negative control (sample 6) was sample without containing *E. coli* cell.

[0092] FIG. **5** is an electrophoresis gel showing amplified DNA obtained according to a method in which the *E. coli*-containing sample was pumped through the chamber, and subsequently underwent cell lysis, cell washing, real time PCR amplification, and electrophoresis. Referring to FIG. **5**, Lanes 1 and 2 show results obtained when the *E. coli* was bound and then underwent PCR without a separate cell lysis process (samples 1 and 2 of Table 1), Lanes 3 and 4 shows results obtained when *E. coli* was bound and underwent cell lysis and PCR (samples 3-4 of Table 1), and Lane 6 shows results obtained from PCR using a 0.01 OD_{600} *E. coli*-containing sample as a template (sample 5 of Table 1). Lane 5 presents sample without containing *E. coli* cells. The concentration of the target PCR product is shown in Table 2. The Lapchip instrument (Agilent) was used to measure the DNA concentration automatically. The concentration of the target PCR product increased by 70% or more when cell lysis was performed on the bound *E. coli* sample even though the two types of samples showed similar Ct values.

TABLE 2

	Cell binding/ Cell Lysis/ Washing/PCR	Cell Binding/PCR	PCR using 0.01 OD *E. coli*-containing sample
DNA concentration (ng/μl)	24.45	17.35	7.5
Increase rate compared to when PCR using 0.01 OD *E. coli*-containing sample was performed	226%	131%	1

Example 4

Binding of Cells from a Clinical Mimic Sample to a Solid Substrate with a Pillar Array in a Fluidic Device, Followed by Cell Lysis, DNA Purification, and Amplification

[0093] In the current example, a urine sample containing E-coli was loaded into a fluidic device including an inlet and an outlet and having an pillar array on a 7.5 mm×15 mm substrate, the cell was lysed and DNA derived from the cell was bound to the substrate, materials that were not bound to the substrate were removed by washing, and DNA was amplified using the DNA bound to the substrate as a template. In the pillar array, a distance between adjacent pillars was 15 μm, the height of each pillar was 100 μm, and a sectional surface of each pillar was a regular square having sides of 25 μm.

[0094] The pillar array had a surface coated with OTC having a water contact angle of 80°.

[0095] The cell sample was a urine sample which was diluted with a sodium acetate buffer (pH 4.0) in a 4:1 ratio and contained *E. coli* at a concentration yielding a measured value of 0.01 OD_{600}. The *E. coli*-containing urine sample was pumped into the fluidic device from the inlet to the outlet through a chamber at a flow rate of 100 μl/minute for five minutes. The reference sample used was a 0.01 OD_{600} *E. coli*-containing sample in sodium acetate buffer (pH 4).

[0096] Then, *E. coli* bound to the surface of the pillar array was lysed. More specifically, the chamber was filled with 100 mM phosphate buffer (pH 4.0), treated at 95° C. for 2 minutes, and then the temperature was decreased to room temperature. The lysis process was repeated five times. After the cell lysis, impurities which were not bound to the surface of the pillar array were washed using 200 μl of 100 mM

phosphate buffer (pH 4.0) at a flow rate of 200 μl/minute. Subsequently, PCR was performed using DNA bound to the surface of the pillar array of the solid substrate as a template and SYBR® Green.

[0097] FIG. **6** is a graph illustrating results of the real time PCR amplification of various *E. coli*-containing urine samples pumped through the chamber. Referring to FIG. **7**, samples according to Table 3 were used.

TABLE 3

Sample No.	Treatment	Ct
1	Purified PCR1	11.43
2	Purified PCR2	11.21
3	dPCR1	11.94
4	dPCR2	13.16
5	Negative Control group 1	23.76
6	Negative Control group 2	23.38

[0098] In Table 3, purified PCR refers to a PCR using a lysed/washed sample and dPCR refers to a PCR using a sample that was not lysed or washed. In the Negative Control groups 1 and 2, PCR was conducted using the 0.01 OD *E. coli*-containing sample as template, without loading into the fluidic device and undergoing the process of concentrating and purifying the cells in the urine sample).

[0099] Referring to FIG. **6**, the Ct value was about one cycle lower (1.23) when an *E. coli*-containing urine sample was loaded through the chamber, and subsequently underwent cell lysis, cell washing, and real time PCR than when an *E. coli*-containing urine sample was loaded through the chamber, and subsequently underwent real time PCR without cell lysis or washing.

Comparative Example 4

Binding of Cells in a Clinical Mimic Sample to a Solid Substrate with a Pillar Array in a Fluidic Device, Cell Lysis, and DNA Elution and Amplification

Comparative Example with Respect to Commercially Available DNA Purification Kit

[0100] In the current example, an *E. coli*-containing whole blood sample was loaded into a fluidic device including a 10 mm×23 mm substrate with a pillar array, and then subsequently underwent cell lysis to elute DNA from the substrate. Then, the eluted DNA was used as a template for amplification. DNA purified by the disclosed method was compared to DNA purified using a commercially available DNA purification kit produced by Qiagen Inc.

[0101] In the pillar array, the distance between adjacent pillars was 12 μm, the height of each pillar was 100 μm, and the sectional surface of each pillar was a regular square having sides of 25 μm. The pillar array had a surface coated with OTC having a water contact angle of 80°.

[0102] A clinical mimic cell sample containing *E. coli* was prepared by adding 10 μl of *E. coli* (1.0 OD_{600}) to 1 ml of whole blood. 200 μl of the clinical mimic cell sample containing *E. coli* was diluted with sodium acetate buffer (pH 3.0, 100 mM) in a 1:1 ratio and then pumped into the chamber of the fluidic device at a flow rate of 200 μl/minute. *E. coli* cells bound to the surface of the pillar array was lysed. In order to perform the cell lysis, 5 μl of 0.01N NaOH was added to the chamber and then boiled for 2 minutes. Subsequently, 45 μl of 0.01N NaOH was added to the chamber at a flow rate of 200 μl/min to elute the DNA from the lysate.

[0103] As a control sample, 200 μl of the clinical mimic sample was prepared using a *Blood & Cell Culture DNA Mini Kit* (Qiagen Inc., Cat 13323) according to the protocol provided by the manufacturer.

[0104] Then, DNA purified from the clinical mimic cell sample containing *E. coli* using the method of the invention ("SAIT") or purified using the commercial control ("Qiagen") were subjected to PCR using SYBRO® Green to detect amplification. The amplification results for the samples are summarized in Table 4. The concentration of the PCR product was determined from the electrophoresis analysis illustrated in FIG. **8**. The target bands are located between ladder 150 and 200. As shown in Table 4, the two samples (SAIT and Qiagen samples) showed similar Ct values and PCR product concentration. That is, it was found that the quality of DNA prepared according to the method of the present invention was high enough to undergo PCR, not requiring additional DNA purification. These methods, however required different times to prepare DNA. The method of the present invention required about 10 minutes, while the Qiagen method required about 45 minutes. In addition, the method of the present invention can be easily automated because cell capture and DNA elution can be performed in a single chip. Therefore, the method of the present invention can be implemented on a lap-on-a-chip (LOC). In Table 4, the sample labeled "Negative Control Group" represents a sample for which PCR was conducted using the *E. coli*-containing whole blood sample as the template, without undergoing any purification process.

TABLE 4

Chip	Ct	Concentration (ng/μl)
SAIT1	18.7	11.8
SAIT2	18.9	13.1
Qiagen1	18.4	13.0
Qiagen2	17.8	11.5
Negative Control Group	28.8	—

[0105] FIG. **7** is a graph illustrating the results of real time PCR amplification for the samples listed in Table 4.

[0106] FIG. **8** shows the g results of electrophoresis of PCR products generated with the samples listed in Table 4.

[0107] In summary, in the method of isolating a nucleic acid from a cell according to the present invention, isolation of a cell and isolation of a nucleic acid can be efficiently performed at the same time. In addition, a nucleic acid separator, such as a chaotropic substance, is not required for isolation of the nucleic acid. Furthermore, the method can be usefully realized using a small device, such as lap-on-a-chip (LOC).

[0108] Further, in the method of amplifying DNA according to the present invention, isolation of a cell and isolation of a nucleic acid derived from the cell can be performed in rapid succession so that the nucleic acid can be effectively amplified using a small device, such as a LOC.

[0109] In addition, by using a device for isolating and amplifying a nucleic acid according to the present invention, isolation of a cell, isolation of a nucleic acid derived from the cell, and amplification of the nucleic acid derived from the cell can be performed in the same container or in different containers.

[0110] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. The terms "a" and "an" do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. The term "or" means "and/or". The terms "comprising", "having", "including", and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to").

[0111] Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. The endpoints of all ranges are included within the range and independently combinable.

[0112] All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention as used herein. Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

[0113] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context. While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

What is claimed is:

1. A method of obtaining a nucleic acid from a cell, the method comprising:

contacting a nonplanar solid substrate with a cell-containing sample in a liquid medium having a pH of 3.0 to 6.0 so that the cell is bound to the nonplanar solid substrate, wherein the nonplanar solid substrate is hydrophobic and has a water contact angle of 70° to 95° or the nonplanar solid substrate has at least one amine-based functional group at its surface; and

lysing the cell bound to the nonplanar solid substrate to obtain a nucleic acid from the lysed cell.

2. The method of claim 1, wherein the cell is a bacteria, a fungus, or a virus.

3. The method of claim 1, wherein the cell-containing sample is a biological sample.

4. The method of claim 3, wherein the cell-containing sample is blood, urine, or saliva.

5. The method of claim 1, wherein the cell-containing sample is a solution diluted with a phosphate buffer or an acetate buffer.

6. The method of claim 5, wherein the cell-containing sample is diluted in a ratio of 1:1 to 1:10.

7. The method of claim 5, wherein the cell-containing sample has a salt concentration of 10 mM to 500 mM.

8. The method of claim 7, wherein the cell-containing sample has a salt concentration of 50 mM to 300 mM.

9. The method of claim 1, wherein the nonplanar solid substrate is selected from the group consisting of

a solid substrate having a surface comprising a pillar structure formed of a plurality of pillars,

a bead-shaped solid substrate, and

a sieve-shaped solid substrate having a surface comprising pores.

10. The method of claim 9, wherein each of the pillars has an aspect ratio of 1:1 to 20:1.

11. The method of claim 9, wherein, in the pillar structure, a ratio of a height of the pillars to a distance between adjacent pillars is in the range of 1:1 to 25:1.

12. The method of claim 9, wherein, in the pillar structure, a distance between adjacent pillars is in the range of 5 μm to 100 μm.

13. The method of claim 1, wherein the hydrophobic nonplanar solid substrate is obtained by coating the nonplanar solid substrate with octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS).

14. The method of claim 1, wherein the nonplanar solid substrate having at least one amine-based functional group is prepared by coating the nonplanar solid substrate with polyethyleneiminetrimethoxysilane (PEIM).

15. The method of claim 1, further comprising

washing the nonplanar solid substrate with a wash buffer to remove materials in the cell-containing sample which are not bound to the nonplanar solid substrate.

16. The method of claim 1, wherein lysing the cell is performed by boiling lysis, laser lysis, lysis using a chemical material, or electrochemical lysis.

17. The method of claim 1, wherein lysing is performed in a liquid medium having a pH of 3.0 to 8.0 so that the nucleic acid obtained from the cell binds to the nonplanar solid substrate.

18. The method of claim 17, further comprising

washing the nonplanar solid substrate to remove materials which are not bound to the nonplanar solid substrate.

19. The method of claim 17, further comprising

eluting the nucleic acid bound to the nonplanar solid substrate.

20. The method of claim 1, wherein lysing the cell is performed in a liquid medium having a pH of 11 to 14.

21. A method of amplifying a target nucleic acid comprising

amplifying a target nucleic acid using the nucleic acid obtained according to the method of claim 1 as a template.

22. The method of claim 21, wherein the nucleic acid is DNA or RNA.

23. The method of claim 21, wherein amplifying the target nucleic acid is performed in a first chamber comprising the nonplanar solid substrate or in a second chamber in fluid communication with the first chamber.

24. The method of claim 21, wherein amplifying is performed through PCR.

25. A device for isolating and amplifying a nucleic acid, the device comprising:

a reaction chamber comprising a nonplanar solid substrate, wherein the nonplanar solid substrate is hydrophobic and has a water contact angle of 70° to 95° or the nonplanar solid substrate has at least one amine-based functional group at its surface;

a heating unit which heats the reaction chamber; and

a temperature controlling unit which controls the heating unit.

26. The device of claim 25, further comprising

a nucleic acid amplification chamber in fluid communication with the reaction chamber comprising the nonplanar solid substrate.

27. The device of claim 25, wherein the nonplanar solid substrate is selected from the group consisting of a solid substrate having a surface comprising a pillar structure formed of a plurality of pillars, a bead-shaped solid substrate, and a sieve-shaped solid substrate having a surface comprising pores.

28. The device of claim 27, wherein each of the pillars has an aspect ratio of 1:1 to 20:1.

29. The device of claim 27, wherein, in the pillar structure, a ratio of a height of the pillars to a distance between adjacent pillars is in the range of 1:1 to 25:1.

30. The device of claim 27, wherein, in the pillar structure, a distance between adjacent pillars is in the range of 5 μm to 100 μm.

31. The device of claim 25, wherein the nonplanar solid substrate is hydrophobic and has a water contact angle of 70° to 95°.

32. The device of claim 31, wherein the hydrophobic nonplanar solid substrate is obtained by coating the nonplanar solid substrate with octadecyldimethyl(3-trimethoxysilyl propyl)ammonium (OTC) or tridecafluorotetrahydrooctyltrimethoxysilane (DFS).

33. The device of claim 25, wherein the nonplanar solid substrate has at least one amine-based functional group at its surface.

34. The device of claim 33, wherein the nonplanar solid substrate having at least one amine-based functional group is prepared by coating the nonplanar solid substrate with polyethyleneiminetrimethoxysilane (PEIM).

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