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(54) Title: ISOLATION OF DNA AND RNA FROM A SINGLE SAMPLE

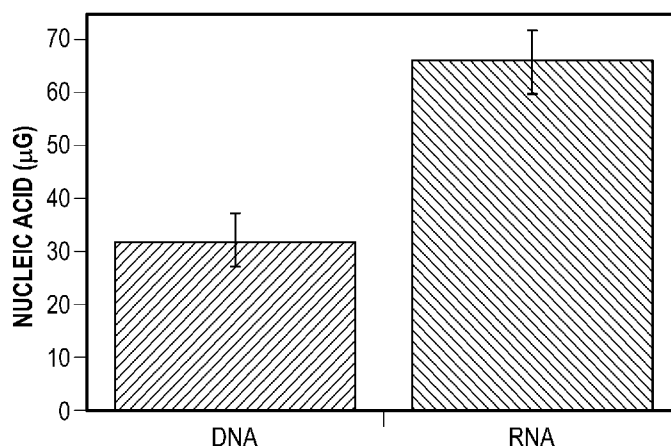


FIG. 2A

(57) Abstract: Various methods for isolating DNA and RNA from a biological sample are described.



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ISOLATION OF DNA AND RNA FROM A SINGLE SAMPLE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Appl. Ser. No. 62/738,829, filed September 28, 2019, which is incorporated by reference as if fully set forth herein.

BACKGROUND

[0002] Extraction and isolation of DNA, RNA, and protein is the basic method used in molecular biology. These biomolecules can be isolated from any biological material for subsequent downstream processes, analytical or preparative purposes. Processes for the extraction and purification of nucleic acids are generally complicated, time-consuming, labor-intensive, and limited in terms of overall throughput. Currently, there are many specialized methods that can be used to extract pure biomolecules, such as solution-based and column-based protocols. Manual method has certainly improved over time with various commercial offerings that include complete kits containing most of the components needed to isolate nucleic acid, but most of them require repeated centrifugation steps, followed by removal of supernatants depending on the type of specimen and additional mechanical treatment. Although automated systems designed for medium-to-large laboratories have grown in demand over recent years, they still are not optimal in terms of yield, purity, reproducibility, scalability, speed, accuracy, and reliability, not to mention cross-contamination. There are currently no commercially available kits for the separate isolation of DNA and RNA from a whole sample.

SUMMARY

[0003] The ability to isolate DNA and RNA from a whole sample (e.g., a single sample comprising completely lysed cells) would greatly simplify methods of isolating nucleic acids from biological samples. The instant disclosure addresses these and other needs. This disclosure provides at least two methods for separately isolating DNA and RNA from a single sample. The methods described herein rely on the selective binding of DNA and RNA to solid supports (e.g., magnetic particles) based on size (e.g., DNA and RNA molecules having greater than 10,000 bps). The methods described herein take advantage of the ability of DNA to non-selectively bind to magnetic particles (e.g., paramagnetic particles comprising a functional group that non-selectively binds nucleic acids) under relatively mild precipitation conditions (e.g., using solution comprising relatively lower concentrations of crowding agents, salts, and C₂-C₉-alkanols), while RNA, which is typically smaller in size, remains in solution.

Alternatively, the RNA may also be induced to bind the beads by increasing the concentration of the crowding agent.

[0004] As used herein, C₂-C₉ alkanols include (C₂-C₉)alkanols and mixtures of (C₂-C₉)alkanols. By “(C₂-C₉)alkanol” is meant an alcohol of the formula (C₂-C₉)alkyl-OH, wherein “alkyl” refers to straight chain and branched alkyl groups having from 2 to 9 carbon atoms such as ethyl, n-propyl, n-butyl, isopropyl, iso-butyl, sec-butyl, and t-butyl, such that the resulting (C₂-C₉)alkanol can be methanol, ethanol, n-propanol, n-butanol, isopropanol, iso-butanol, sec-butanol, t-butanol, and the like.

[0005] In some examples, both RNA and DNA are bound to magnetic particles using conditions that cause both DNA and RNA to non-selectively bind the magnetic particles. Substantially all the RNA is then selectively eluted from the magnetic particles using precipitation conditions that can be relatively mild. The supernatant comprising the RNA is separated from the magnetic particles comprising at least substantially all the DNA. RNA is then isolated from the supernatant and the DNA isolated from the magnetic particles.

[0006] In other examples, at least substantially all the DNA is bound to the magnetic particles using precipitation conditions that can be relatively mild and that cause the DNA to bind the magnetic particles, while substantially all the RNA remains in solution. The supernatant comprising the RNA is separated from the magnetic particles comprising the DNA. RNA is then isolated from the supernatant and the DNA isolated from the magnetic particles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 is a plot of nucleic acid yield from single blood sample (EDTA, heparin, PaxGene). The methods started with 400 uL PaxGene blood, 150 uL EDTA blood, or 200 uL heparin blood. DNA was quantified with picogreen, and RNA was quantified with Ribogreen by correcting for DNA fluorescence in each sample.

[0008] FIG. 2A is a plot showing the amount of DNA and RNA isolated from a 10 mg mouse liver tissue sample. DNA yield averaged 32 µg, RNA yield averaged 66 µg from three technical replicates.

[0009] FIG 2B is a size exclusion chromatogram of a mouse liver tissue sample showing DNA sizes averaged >60,000 bp.

[0010] FIG. 2C is a size exclusion chromatogram of a mouse liver tissue sample showing two prominent rRNA peaks were observed in the RNA fraction.

[0011] FIG. 3A is a plot showing the amount of DNA and RNA isolated from a 10 mg mouse brain tissue sample. DNA yield averaged 5.5 µg and RNA yield averaged 1.8 µg from three biological replicates.

[0012] FIG 3B is a size exclusion chromatogram of a mouse liver tissue sample showing DNA sizes averaged 20,000 bp or larger.

[0013] FIG 3C is a size exclusion chromatogram of a mouse liver tissue sample showing RNA integrity number (RIN) values were 9.1 – 9.7, showing good RNA integrity.

5 [0014] FIG. 4A is a plot showing the yield of DNA and RNA. DNA and RNA were isolated from increasing amounts of cells. Both DNA and RNA yield increased linearly with cell input.

[0015] FIGS. 4B-1-4B-3 and FIGS. 4C-1-4C-3 are size exclusion chromatograms showing that the DNA integrity number (DIN) and RIN scores for DNA and RNA isolated from 50,000, 100,000 and 500,000 cells were consistent and above 9, indicating that DNA and RNA is intact.

10 The concentration of nucleic acids from 10,000 was too low for an accurate measurement.

[0016] FIG. 5 is a plot of nucleic acid yield from nasal swabs from a human subject.

DESCRIPTION

[0017] The instant disclosure relates generally to a method of isolating DNA and RNA from a biological sample. The instant disclosure also includes kits for isolating DNA and RNA from
15 a biological sample.

[0018] The methods for isolating DNA and RNA from a biological sample described herein comprise: a) contacting the DNA and RNA from the sample with a first buffer comprising magnetic particles and binding at least substantially all of the DNA to the magnetic particles, while at least substantially all of the RNA remains unbound; b) separating the magnetic
20 particles of step a) from a first supernatant comprising the RNA; c) isolating the RNA from the first supernatant; and d) isolating the DNA from the magnetic particles. The first buffer can be any suitable buffer, such as a Tris-HCl buffer. The first buffer can be a bind buffer that promotes the binding of DNA and/or RNA molecules to the magnetic particles described herein. Example of bind buffers include Agencourt AMPure XP available from Beckman
25 Coulter, Inc., Brea, CA, such as a bind buffer comprising about 3% to about 7% PEG with about 0.35 M to about 0.7 M NaCl (final concentration). The bind buffer can be a 0.5X bind buffer. The first buffer can further comprise salts, such as any Group (I) or Group (II) salt, including NaCl and KCl. The first buffer can further comprise a suitable amount of at least one crowding agent, such as those known in the art.

30 [0019] The methods described herein can further comprise, before step a), the steps of contacting the biological sample with a lysis buffer. A lysis buffer is a buffer solution used for the purpose of breaking open cells for use in molecular biology experiments that analyze, among other things, the amount of DNA and RNA present. A non-limiting example of a lysis

buffer is a buffer comprising 0.8 N NaOH/8% SDS lysis buffer, which can be prepared by combining 160 mL of 2 N NaOH, 160 mL of 20% SDS, and 80 mL of distilled water.

[0020] The isolating of the RNA from the first supernatant can comprise: contacting the first supernatant with the first buffer comprising magnetic particles and binding the RNA to the magnetic particles; replacing the first buffer with a solution to release the RNA from the magnetic particles; and separating the magnetic particles from a second supernatant comprising the isolated RNA. Suitable solutions to release the RNA from the magnetic particles can be at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water. The RNA released from the magnetic particles can optionally be treated with a DNase.

[0021] The isolating the DNA from the magnetic particles comprises: contacting the magnetic particles bound to the DNA with a solution to release the DNA from the magnetic particles; and separating the magnetic particles from a third supernatant comprising the isolated DNA. Suitable solutions to release the DNA from the magnetic particles can be at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water. The magnetic particles bound to the DNA or the DNA released from the magnetic particles can optionally be treated with an RNase.

[0022] The methods encompassed by the instant disclosure also include a method of isolating DNA and RNA from a biological sample comprising cells, the method comprising: i) contacting the biological sample with a lysis buffer and releasing DNA and RNA from the cells; ii) contacting the DNA and RNA with a first buffer comprising magnetic particles, thereby binding the DNA and the RNA to the magnetic particles; iii) contacting the magnetic particles of step ii) with a second buffer releasing at least substantially all of the RNA from the magnetic particles while at least substantially all of the DNA remains bound to the magnetic particles; iv) separating the magnetic particles of step iii) from a first supernatant comprising the RNA; v) isolating the RNA from the first supernatant; and vi) isolating the DNA from the magnetic particles.

[0023] The isolating the RNA from the first supernatant (step v) can comprise contacting the first supernatant with the first buffer comprising magnetic particles and binding the RNA to the magnetic particles; replacing the buffer with a solution to release the RNA from the magnetic particles; and separating the magnetic particles from a second supernatant comprising the isolated RNA. Suitable solutions to release the RNA from the magnetic particles can be at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water. The RNA released from the magnetic particles can optionally be treated with a DNase.

[0024] The isolating the DNA from the magnetic particles can comprise contacting the magnetic particles bound to the DNA with a solution to release the DNA from the magnetic particles; and

separating the magnetic particles from a third supernatant comprising the isolated DNA.

5 Suitable solutions to release the DNA from the magnetic particles can be at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water. The magnetic particles bound to the DNA or the DNA released from the magnetic particles can optionally be treated with an RNase.

[0025] As used herein, the term “crowding agent” generally refers to any material that allows
10 for, enhances, or facilitates molecular crowding. Without wishing to be bound by any specific theory, it is believed that a crowding agent allows for molecular crowding and reduces the amount of water that comes into contact with the DNA and RNA molecules in a solution, allowing components of the solution to come into closer contact with one another. For example, DNA and/or RNA molecules can condense more quickly in the presence of the crowding agent,
15 which facilitates methods described herein for separating DNA from RNA.

[0026] A variety of suitable crowding agents will be evident to the skilled worker. These include a variety of well-known macromolecules, such as polymers, e.g., polyethylene glycol (PEG) (e.g., PEG that ranges in weight average molecular weight from about 500 g/mol to about 20,000 g/mol); Ficoll, such as Ficoll 70; dextran, such as dextran 70; or the like. Any
20 suitable size of PEG can be used, e.g., ranging from about PEG-200 (e.g., PEG-4000, PEG-6000, or PEG-8000) to about PEG-20,000, or even higher. Much of the discussion in this application is directed to PEG. In the Examples herein, PEG-8000 is used. However, the discussion is meant also to apply to other suitable crowding agents. A skilled worker will recognize how to implement routine changes in the method in order to accommodate the use
25 of other crowding agents. In general, the amount of the crowding agent used will be determined by the molecular weight of the crowding agent. Thus, for example, when PEG-8000 is used, a concentration of about 5% (weight/volume) can be used. However, the amount of crowding agent can range, e.g., from about 3% to about 7% or higher (e.g., 8%).

[0027] The first buffer can comprise components in addition to salts. For example, the
30 first buffer can comprise magnetic particles. The magnetic particles can be present in the buffer in any suitable amount, such as 1% (w/v) magnetic particles. The magnetic particles can be any suitable magnetic particles. For example, the magnetic particles can be ferrimagnetic particles, ferromagnetic particles, paramagnetic particles, superparamagnetic particles, or mixtures of various classes of magnetic particles can also be used. Therefore, any specific recitation of a

magnetic particle can be equally applied to a ferrimagnetic particle, ferromagnetic particle, paramagnetic particle, superparamagnetic particle, or mixtures thereof.

[0028] Suitable magnetic particles for use in the methods described herein include, but are not limited to AMPure XP beads available from Beckman Coulter, Inc., Brea, CA. Suitable magnetic particles also include those described in U.S. Patent Nos. 5,705,628; 5,898,071; and 6,534,262, and in PCT/US2019/042628, filed July 19, 2019, all of which are incorporated by reference as if fully set forth herein.

[0029] As used herein, “ferrimagnetic particles” refers to particles comprising a ferrimagnetic material. Ferrimagnetic particles can respond to an external magnetic field (e.g., a changing magnetic field), but can demagnetize when the external magnetic field is removed. Thus, the ferrimagnetic particles are efficiently mixed through a sample by external magnetic fields as well as efficiently separated from a sample using a magnet or electromagnet, but can remain suspended without magnetically induced aggregation occurring.

[0030] The magnetic particles described herein are sufficiently responsive to magnetic fields such that they can be efficiently moved through a sample. In general, the range of the field intensity could be the same range as any electromagnet as long as it is able to move the particles. For example, the magnetic field has an intensity of between about 10 mT and about 100 mT, between about 20 mT and about 80 mT, and between about 30 mT and about 50 mT. In some examples, more powerful electromagnets can be used to mix less responsive microparticles. In some examples, the magnetic field can be focused into the sample as much as possible. Also, the electromagnets can be as close to the sample as possible since the strength of the magnetic field decreases as the square of the distance.

[0031] The magnetic particles can be a variety of shapes, which can be regular or irregular; In some examples, the shape maximizes the surface areas of the particles. For example, the magnetic particles can be spherical, bar shaped, elliptical, or any other suitable shape. The magnetic particles can be a variety of densities, which can be determined by the composition of the core. In some examples, the density of the magnetic particles can be adjusted with a coating, as described herein.

[0032] In some examples, the magnetic particles have sufficient surface area to permit efficient binding of a target analyte and are further characterized by having surfaces which are capable of reversibly or irreversibly binding the target analyte (e.g., biological molecules, such as DNA and RNA). In some examples, a surface area of the magnetic particles can be in a range of from about 0.1 m²/g to about 500 m²/g, about 50 m²/g to about 200 m²/g, or about 150 m²/g to about 175 m²/g.

[0033] Suitable magnetic particles can be of a size that their separation from solution is not difficult, for example by magnetic means or by filtration. In addition, magnetic particles should not be so large that their surface area is minimized or that they are not suitable for nanoscale to microscale manipulation.

5 **[0034]** Suitable sizes range from about 1 nm mean diameter to about 1 mm mean diameter, about 5 nm to about 50 μm , or between about 100 nm and about 100 μm . A suitable is between about 1 μm and about 10 μm . For example, in some examples, the magnetic particles can be nanoparticles (e.g., particles having a mean diameter less than 1 μm , but greater than 1 nm). In other examples, the magnetic particles can be microparticles (e.g., particles having a
10 mean diameter greater than 1 μm , but less than 100 μm). In general, larger magnetic particles (that is about 1 mm in size) are useful in cellular fractionation, tissue digestion, liquid mixing, and the like.

[0035] The magnetic particles can be substantially solid or can have some degree of porosity. Where the magnetic particles do include some degree of porosity, a pore size of the
15 individual pores can be in a range of from about 5 Å to about 1000 Å, about 50 Å to about 500 Å. At least a plurality of the pores can be through pores (e.g., extending fully between opposed surfaces). The pore sizes or total porosity of the magnetic particles can be determined according to many suitable methods. For example, the bulk volume of an ideal (e.g., non-porous) magnetic particle can be determined and then the volume of the actual porous skeletal material
20 can be determined. The porosity is then calculated by subtracting the volume of the actual porous skeletal material from the ideal magnetic particle. The porosity of the magnetic particle or individual pore size can also be determined through optical measurements using a microscope and processing the images to measure the individual pores.

[0036] The magnetic particles described herein can include several different materials.
25 To the extent that mixtures of materials are present, the total magnetic content of the magnetic particles can constitute at least 50 wt% of the magnetic particle, at least 70 wt% of the magnetic particle, or even 100 wt% of the magnetic particle. The magnetic particles can include any of those described herein. The non-magnetic material constituting the balance of the magnetic particles can include any of the coating materials described herein, for example. Non-magnetic
30 material can be used as a coating to encapsulate the magnetic portion of the magnetic particle, they can also be used as a functional component to interact with and bind an analyte of interest. Non-magnetic material can also act as a filler component.

[0037] According to various examples, the magnetic strength of the magnetic particles can be greater than or equal to about 20 emu/g, about 25 emu/g, about 30 emu/g, about 35

emu/g, about 40 emu/g, about 45 emu/g, about 50 emu/g, about 75 emu/g, about 100 emu/g, about 150 emu/g, about 175 emu/g, about 200 emu/g, about 225 emu/g, about 250 emu/g, in a range of from about 20 emu/g to about 250 emu/g, or about 35 emu/g to about 100 emu/g. This value can be considered to be the maximum field strength of the particle, which is a measure of the magnetic strength generated by the particle upon exposure to a magnetic field. In combination with the magnetic strength of the magnetic particles, the permeability of the magnetic particle should be sufficient to generate an induced magnetic field greater than or equal to about 10 emu/g, 15 emu/g, 20 emu/g, about 25 emu/g, about 30 emu/g, about 35 emu/g, about 40 emu/g, about 45 emu/g, about 50 emu/g, about 75 emu/g, about 100 emu/g, about 150 emu/g, about 175 emu/g, about 200 emu/g, about 225 emu/g, about 250 emu/g, in a range of from about 10 emu/g to about 250 emu/g, or about 35 emu/g to about 100 emu/g. The magnetic field to which the magnetic particles are exposed, can have a strength of about 700 Oersted to about 800 Oersted, about 725 Oersted to about 775 Oersted, less than, equal to, or greater than about 700 Oersted, 725, 750, 775, or about 800 Oersted.

[0038] According to various examples, the remanence of the magnetic materials can be in a range of from about 0 emu/g to about 30 emu/g, about 0 emu/g to about 10 emu/g, about 1 emu/g to about 8 emu/g, about 3 emu/g to about 5 emu/g, less than, equal to, or greater than about 0 emu/g, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or about 30 emu/g.

[0039] The magnetic components of the particles can be magnetic nanoparticles, magnetic sub-micrometer particles, or magnetic micrometer particles. The magnetic particles described herein can have many different structures. For example, the magnetic particles can be magnetic nanoparticles incorporated in polymer matrix or silica matrix, magnetic beads encapsulated in silica shell or polymer shell, magnetic nanoparticles or magnetic beads functionalized with organic ligands, bare magnetic nanoparticles or beads. In examples where the magnetic particles are core-shell particles, the shell can include a coating as described herein.

[0040] The magnetic particles of the disclosure can comprise a magnetic core, surrounded by a coating. In an example, the magnetic particles are coated with one or more layers of a non-magnetic material. The use of coated magnetic particles, having no exposed iron, on their surfaces, can eliminate the possibility of iron interfering with certain downstream manipulations of the sample. The coating can be, for example, a polymer layer, or a silica layer.

[0041] Example polymer layers can include polyethylene, polystyrene, poly methyl methacrylate, polyvinyl alcohol, or any other suitable polymer. Example silica layers can

include silicon dioxide, borosilicate, soda lime, barium titanate, and other types of glass. The polymer or silica layer can be for adjusting the density of the magnetic particles. For example, the polymer or silica layer can adjust the density of the magnetic particles to be close to the density of the sample, for example, an aqueous sample (e.g., approximately 1 g/cm³).

5 [0042] In other examples, other types of coating can include metal plating such as aluminum, gold, zinc oxide, or any of the other coatings mentioned herein, etc. Furthermore, any of the coatings described herein can have a fluorescent or colored dye included.

[0043] The coating can also comprise a ligand such as capture reagent or a functional group, including those mentioned herein, for selectively or non-selectively binding target
10 analytes. The functional group can be for adsorbing biomolecules, such as nucleic acids, which can non-sequence-specifically and reversibly bind to the functional group coating the magnetic particles. The polynucleotides can be DNA, RNA, or polyamide nucleic acids (PNAs). In an example, the functional group is a carboxyl group. Various coatings comprising functional groups suitable for these purposes are described in U.S. Patent No. 5,705,628, U.S. Patent No.
15 5,898,071, and U.S. Patent No. 6,534,262, the teachings of which are hereby incorporated by reference into this application in their entirety. Any of the coatings described herein can be functionalized with surface chemicals as described herein, for example, with carboxylic acid, streptavidin, amine, hydrazide, silanol, azide. And those can be further functionalized with biological molecules such as antibodies, enzymes, DNA or RNA fragments, catalysts, etc.

20 [0044] In some examples, the coating can comprise a capture reagent. The capture reagent can be for capturing an analyte in a sample. The surface of the magnetic particles can be coated with a capture reagent that is a suitable ligand or receptor (e.g., antibodies, lectins, oligonucleotides, other affinity groups, or any of the other capture reagents mentioned herein), which can selectively bind a target analyte or a group of analytes in a mixture. In some
25 examples, the capture reagent can be an antibody.

[0045] Those of skill will recognize that any number of capture reagents can be used for this purpose, e.g. aptamers, nanoparticles, binding proteins, and the like. The capture reagent can be designed to capture a specific analyte or a specific panel of analytes, e.g., drug panel or endocrine panel, etc.

30 [0046] Alternatively, the ligand can include an enzyme. In some embodiments the enzyme can be linked to the coating in order to selectively interact with a substrate of that enzyme. Upon interacting with the substrate, the enzyme can function to degrade or digest the substrate. This can lead to generation of a substance of interest through enzyme's action or to

remove a substrate from a sample. According to various embodiments, the enzyme can be trypsin.

[0047] While only a single layer of coating is described it is understood that some examples can include multiple layers of coatings. For example, some examples can include a base metal coating with a polymer coating or functional group disposed thereon. In some examples, a layer of coating can function to sufficiently hold an external coating to the magnetic particle.

[0048] The magnetic particles can be manufactured using any suitable method of manufacturing nanoscale to microscale magnetic particles. As an example, U.S. Patent No. 5,648,124 discloses a process for preparing magnetically responsive microparticles, and is hereby incorporated by reference herein in its entirety. The magnetic particles can be manufactured using any suitable magnetic material, as described herein.

[0049] For example, a magnetic particle can be manufactured by first adding magnetic nanoparticles to a chemical bath. The nanoparticles can be encapsulated in an inorganic silica matrix, thus producing a microparticle that contains many magnetic particles. Sonication can then be used to help produce these particles in a monodispersed fashion. Although a silica matrix is mentioned above, it is also possible for individual magnetic nanoparticles or microparticles to be encapsulated in other inorganic or organic materials. For example, the magnetic nanoparticles can be encapsulated in SiO_2 , TiO_2 , ZnO_2 , Al_2O_3 , CeO_2 , or any suitable ceramic material. As a further example, the magnetic nanoparticles can be encapsulated in an organic material such as polyacrylic acid (PAA), poly(methyl acrylate) (PMA), polystyrene (PS), divinylbenzene (DVB), polyvinylpyrrolidone (PVP), or polyvinyl alcohol (PVA).

[0050] In another example, a ferromagnetic material can be used to manufacture magnetic particles. The magnetic properties can be altered by changing the structure of the ferromagnetic material. Hematite (Fe_2O_3) is naturally ferromagnetic when allowed to crystalize in its pure form. However, if impurities like nickel and zinc are added, then the nickel and zinc can take the place of some of the iron in the crystalline structure, thus turning the naturally ferromagnetic material into a magnetic particle. Or, in a different example, ferromagnetic hematite can be ground down to less than 50 nm in size such that each particle contains a single magnetic domain. In this form, the particle can be a superparamagnetic particle. An exemplary magnetic particle can be made from magnetic magnetite nanoparticles 50-100 nm in size joined together in silica or polymer. These nanoparticles are too large to be superparamagnetic.

[0051] A sample used in the present disclosure can be a fluid sample and can be, for example, a biological sample. As used herein, "biological samples" can comprise biological

fluids and may include, but are not limited to, blood, plasma, serum, or other bodily fluids or excretions, such as but not limited to saliva, urine, cerebrospinal fluid, lacrimal fluid, perspiration, gastrointestinal fluid, amniotic fluid, mucosal fluid, pleural fluid, sebaceous oil, exhaled breath, and the like. The biological sample can be bodily fluid or a tissue sample.

5 Biological samples can also be obtained from swabs used to collect animal cells, such as at least one of forensic, anal, vaginal, oral, buccal, aural, and nasal swabs. Biological samples also include at least one of animal (e.g., mammalian and insect) tissue, animal (e.g., mammalian and insect) cells, bacteria, fungi, and yeast. Biological samples can be lysed samples, where, e.g., animal cells have been lysed using a lysing buffer, such as those described herein.

10 **[0052]** Appropriate biological samples may also include lysates prepared from cells obtained from either mammalian tissue, cell culture, or body fluids, nucleic acid samples eluted from agarose or polyacrylamide gels, solutions containing multiple species of DNA molecules resulting either from a polymerase chain reaction (PCR) amplification or from a DNA size selection procedure and solutions resulting from a post-sequencing reaction. Suitable samples
15 can be mixtures of biomolecules (e.g. proteins, polysaccharides, lipids, low molecular weight enzyme inhibitors, oligonucleotides, primers, templates) and other substances such as agarose, polyacrylamide, trace metals and organic solvents, from which the target nucleic acid molecule can be isolated.

[0053] As used herein, the terms “selective” and “selectively” refer to the ability to
20 isolate a particular biological molecule species such as a DNA molecule or molecules, on the basis of particular property, such as molecular size, from a combination which includes or is a mixture of species of molecules, such as a host cell lysate and other host cell components. In some examples, the selective isolation of a particular species is accomplished through the use of an appropriate crowding agent (e.g., polyalkylene glycol) to result in the precipitation and
25 facilitated adsorption of a particular DNA species (e.g., characterized on the basis of size) to the surfaces of magnetic particles of the disclosure.

[0054] As used herein, the term “at least substantially all” means that at least about 60%, usually at least about 75%, often at least about 90%, of a particular nucleic acid (RNA or DNA) from a sample is separated from the other nucleic acid and/or other sample components
30 in a given step of the method described herein.

[0055] The methods described herein are useful in a number of contexts in which solid supports are used to non-selectively bind nucleic acids in biological samples. For example, SPRI® paramagnetic bead-based technology available from Beckman Coulter, which is used to isolate nucleic acids from a variety of biological samples, can be conveniently used in the

methods described herein. Functionalized paramagnetic particles are known in the art. In a typical embodiment, the functional group is a carboxyl group. Various coatings comprising functional groups suitable for these purposes are described in U.S. Patent No. 5,705,628, U.S. Patent No. 5,898,071, and U.S. Patent No. 6,534,262, the teachings of which are hereby incorporated by reference into this application in their entirety.

[0056] The instant disclosure also contemplates kits for isolating DNA and RNA from a biological sample, the kit comprising: a first buffer comprising magnetic particles; optionally a lysis buffer for releasing DNA and RNA from cells in the biological sample; and optionally one or more swabs for collecting the biological sample.

EXAMPLES

[0057] The present disclosure can be further understood by reference to the following examples which are offered by way of illustration. The disclosure is not limited to the examples given herein.

[0058] Buffers used in the examples below are as follows:

[0059] Lysis buffer: Guanidine thiocyanate 3.1M; Triton X100 2% (V/V); Na citrate 400Mm; Tris Borate-EDTA 2X; Proteinase K.

[0060] 0.5X Bind Buffer: this buffer generally comprises a crowding agent and a salt. A specific, non-limiting example of a bind buffer comprises PEG 8000 10% (crowding agent); NaCl 0.875M (a salt); Tris-HCL pH:8 25 Mm; Beads: 1% (w/v).

[0061] 1X Bind Buffer: PEG 8000 21.3%; NaCl 1.75M; Tris-HCL pH:8 50 Mm; Beads: 2% (w/v).

[0062] Rebind Buffer (RBA): this buffer generally comprises a crowding agent, a C₂-C₉-alkanol such as isopropanol or ethanol, and a salt. And as the name suggests, the rebind buffer promotes rebinding of RNA to the beads described herein. A specific, non-limiting example of a rebind buffer comprises about 18% to about 22% PEG with about 1.75 M to about 2.26 M NaCl, such as PEG 8000 21.3%; NaCl 1.75M; Tris-HCL pH:8 50 Mm.

[0063] Wash Buffer: this buffer generally comprises at least one of a crowding agent or a C₂-C₉-alkanol such as isopropanol or ethanol, with other components being optional. The wash buffer is a buffer that would leave DNA and/or RNA present in a sample bound to the magnetic beads described herein and would allow for the removal of impurities present in a sample. A specific, non-limiting example of a wash buffer comprises about 5% to about 22% PEG with about 0.4 M to about 2.26 M NaCl or about 70% or higher (e.g., about 75% or higher, about 80% or higher, about 85% or higher, about 90% or higher or about 95% ethanol, such as from

about 70% to about 85%) ethanol and about 40% or higher (e.g., about 45% or higher, about 50% or higher, about 55% or higher, about 60% or higher, about 65% or higher, about 70% or higher, about 75% or higher or about 85% or higher) isopropanol will work. A specific, non-limiting example of a wash buffer is PEG 8000 12.5%; NaCl 1M; Tris-HCL pH:8 50 Mm.

5 Example 1

[0064] The following is a protocol according to the methods described herein in which DNA is first bound to magnetic particles, while RNA remains in solution. The results are presented in FIGS. 2 and 3. In this non-limiting example, lysis buffer, Proteinase K, and 10 mg tissue were combined, and the tissue was homogenized. The sample was then incubated at 37°C for 10 25 minutes. After incubation, the homogenized tissue was centrifuged at 12000 rpm for 1 minute and the supernatant was transferred to a fresh tube. Bind buffer containing magnetic beads was added in sufficient volume (0.5x) to bind the DNA to the beads but not the RNA. The beads were pelleted on a magnet, and the RNA supernatant was removed. The RNA supernatant was then combined with 1x bind buffer to bind the RNA to a fresh set of beads. 15 These beads were then pelleted and the supernatant was removed. The beads were washed with 80% ethanol. Beads were then resuspended in a DNase solution to remove any contaminating DNA and were incubated at 37°C for 20 minutes. Rebind buffer was then added to rebind the RNA to the beads. The beads were pelleted and the supernatant was removed. The beads were then washed with 80% ethanol and finally the RNA was eluted in nuclease-free water. 20

[0065] The beads with bound DNA were resuspended in wash buffer containing RNase and incubated at room temperature for 5 minutes. The beads were pelleted on a magnet, then washed with 70% ethanol. The DNA was then eluted in nuclease-free water.

Example 2

25 [0066] The protocol from Example 1 was followed, except the samples were mammalian cells. Different amounts of cells were tested and no homogenization was done.

Example 3

[0067] The protocol from Example 1 was followed, except nasal swabs were used as the sample and DTT was added to the lysis buffer.

30 Example 4

[0068] The following is a protocol according to the methods described herein in which DNA and RNA are first both bound to magnetic beads, and RNA is subsequently selectively eluted from the particles. The results are presented in Figure 4.

[0069] Blood samples (e.g., PaxGene blood, which can be used as is; heparin blood and EDTA blood need to be mixed with detergent buffer (200 μ L of each for heparin, 150 μ L EDTA blood with 250 μ L detergent buffer)). In this example, 400 μ L of blood sample are

5 **[0070]** Beads are separated on the magnet and supernatant is discarded. Samples are washed with Wash Buffer, then washed with 70% ethanol two times. Separation of RNA and DNA does not work with dirty initial blood samples.

[0071] Beads are separated on the magnet and supernatant is discarded. Samples are washed with Wash Buffer, then washed with 70% ethanol two times. Separation of RNA and DNA does not work with dirty initial blood samples.

10 **[0071]** Beads are incubated in “unbind buffer;” which is diluted wash buffer to a PEG concentration of 4-5%. This will bind the DNA and leave the RNA in the supernatant. RNA supernatant is mixed with 1 X bind buffer and isopropanol in the proportions listed in the RNAdvance Blood protocol. Beads are separated and supernatant is removed. DNase is added to samples (if needed). Samples are washed with 70% ethanol and eluted in water DNA pellet
15 is washed with 100 μ L wash buffer and 5 μ L RNase (if needed). DNA is washed with 70% ethanol and eluted in water.

[0072] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of
20 this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A method of isolating DNA and RNA from a biological sample, the method comprising:
 - a) contacting the DNA and RNA from the sample with a first buffer comprising magnetic particles and binding at least substantially all of the DNA to the magnetic particles, while at least substantially all of the RNA remains unbound;
 - b) separating the magnetic particles of step a) from a first supernatant comprising the RNA;
 - c) isolating the RNA from the first supernatant; and
 - d) isolating the DNA from the magnetic particles.
2. The method of claim 1, wherein the first buffer comprises at least one crowding agent.
3. The method of claim 2, wherein the at least one crowding agent is at least one of high-mass hydrophilic polysaccharide, a polyethylene glycol (PEG), and dextran.
4. The method of claim 2, wherein the at least one crowding agent is PEG.
5. The method of claim 4, wherein the PEG ranges in weight average molecular weight from about 500 g/mol to about 20,000 g/mol.
6. The method of claim 4, wherein the PEG is PEG8000.
7. The method of claim 2, wherein the first buffer further comprises sodium chloride, Tris-HCl, and magnetic particles.
8. The method of claim 1, wherein the first buffer comprises 1% (w/v) magnetic particles.
9. The method of claim 1, wherein the first buffer is a bind buffer.
10. The method of claim 1, wherein the first buffer is a 0.5X bind buffer.

11. The method of claim 1, wherein the biological sample comprises cells.
12. The method of claim 11, wherein the method further comprises, before step a), the steps of
contacting the biological sample with a lysis buffer.
13. The method of any of the preceding claims, wherein the isolating the RNA from the first supernatant comprises:
 contacting the first supernatant with the first buffer comprising magnetic particles and
 binding the RNA to the magnetic particles;
 replacing the buffer with a solution to release the RNA from the magnetic particles;
 and
 separating the magnetic particles from a second supernatant comprising the isolated RNA.
14. The method of claim 13, further comprising treating the RNA released from the magnetic particles with a DNase.
15. The method of claim 13, wherein the solution to release the RNA from the magnetic particles is at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water.
16. The method of any of the preceding claims, wherein the isolating the DNA from the magnetic particles comprises:
 contacting the magnetic particles bound to the DNA with a solution to release the DNA from the magnetic particles; and
 separating the magnetic particles from a third supernatant comprising the isolated DNA.
17. The method of claim 16, further comprising treating the magnetic particles bound to the DNA or the DNA released from the magnetic particles with RNase.

18. The method of claim 16, wherein the solution to release the DNA from the magnetic particles is at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water.
19. A method of isolating DNA and RNA from a biological sample comprising cells, the method comprising:
- i) contacting the biological sample with a lysis buffer and releasing DNA and RNA from the cells;
 - ii) contacting the DNA and RNA with a first buffer comprising magnetic particles, thereby binding the DNA and the RNA to the magnetic particles;
 - iii) contacting the magnetic particles of step ii) with a second buffer releasing at least substantially all of the RNA from the magnetic particles while at least substantially all of the DNA remains bound to the magnetic particles;
 - iv) separating the magnetic particles of step iii) from a first supernatant comprising the RNA;
 - v) isolating the RNA from the first supernatant; and
 - vi) isolating the DNA from the magnetic particles.
20. The method of claim 19, wherein the first buffer comprises at least one crowding agent.
21. The method of claim 20, wherein the at least one crowding agent is at least one of high-mass hydrophilic polysaccharide, a polyethylene glycol (PEG), and dextran.
22. The method of claim 20, wherein the at least one crowding agent is PEG.
23. The method of claim 22, wherein the PEG ranges in weight average molecular weight from about 500 g/mol to about 20,000 g/mol.
24. The method of claim 22, wherein the PEG is PEG8000.
25. The method of claim 20, wherein the first buffer further comprises sodium chloride, Tris-HCl, and magnetic particles.

26. The method of claim 19, wherein the buffer comprises 1% (w/v) magnetic particles.
27. The method of claim 19, wherein the buffer is a bind buffer.
28. The method of claim 19, wherein the buffer is a 0.5X bind buffer.
29. The method of claims 19-28, wherein the isolating the RNA from the first supernatant comprises:
- contacting the first supernatant with the first buffer comprising magnetic particles and binding the RNA to the magnetic particles;
 - replacing the buffer with a solution to release the RNA from the magnetic particles;
 - and
 - separating the magnetic particles from a second supernatant comprising the isolated RNA.
30. The method of claim 29, further comprising treating the RNA released from the magnetic particles with a DNase.
31. The method of claim 29, wherein the solution to release the RNA from the magnetic particles is at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water.
32. The method of claims 19-31, wherein the isolating the DNA from the magnetic particles comprises:
- contacting the magnetic particles bound to the DNA with a solution to release the DNA from the magnetic particles; and
 - separating the magnetic particles from a third supernatant comprising the isolated DNA.
33. The method of claim 32, further comprising treating the magnetic particles bound to the DNA or the DNA released from the magnetic particles with RNase.

34. The method of claim 32, wherein the solution to release the DNA from the magnetic particles is at least one of nuclease-free water, buffer comprising Tris and EDTA, and buffered water.
35. The method of any one of the preceding claims wherein the magnetic particles are paramagnetic particles comprising a functional group that non-selectively binds nucleic acids.
36. The method of claim 35, wherein the functional group is a carboxyl group.
37. The method of any one of the preceding claims, wherein the biological sample is a bodily fluid or a tissue sample.
38. The method of claim 37, wherein the bodily fluid is blood, plasma, or serum.
39. The method of claims 1-36, wherein the biological sample comprises at least one of mammalian tissue, mammalian cells, bacteria, fungi, yeast, and swabs.
40. The method of claim 39, wherein the swabs are at least one of buccal and nasal swabs.
41. A kit for isolating DNA and RNA from a biological sample, the kit comprising:
a bind buffer comprising magnetic particles;
optionally a lysis buffer;
optionally a rebind buffer; and
optionally a wash buffer.
42. The method or the kit of any preceding claim, wherein:
the bind buffer comprises from about 3% to about 7% PEG and about 0.35 M to about 0.7 M NaCl.
43. The method or the kit of any preceding claim, wherein:
the rebind buffer comprises from about 18% to about 22% PEG and about 1.75 M to about 2.26 M NaCl..

44. The method or the kit of any preceding claim, wherein:
the wash buffer comprises about 5% to about 22% PEG and about 0.4 M to about 2.26 M NaCl; or
70% or higher ethanol and 40% or higher isopropanol.

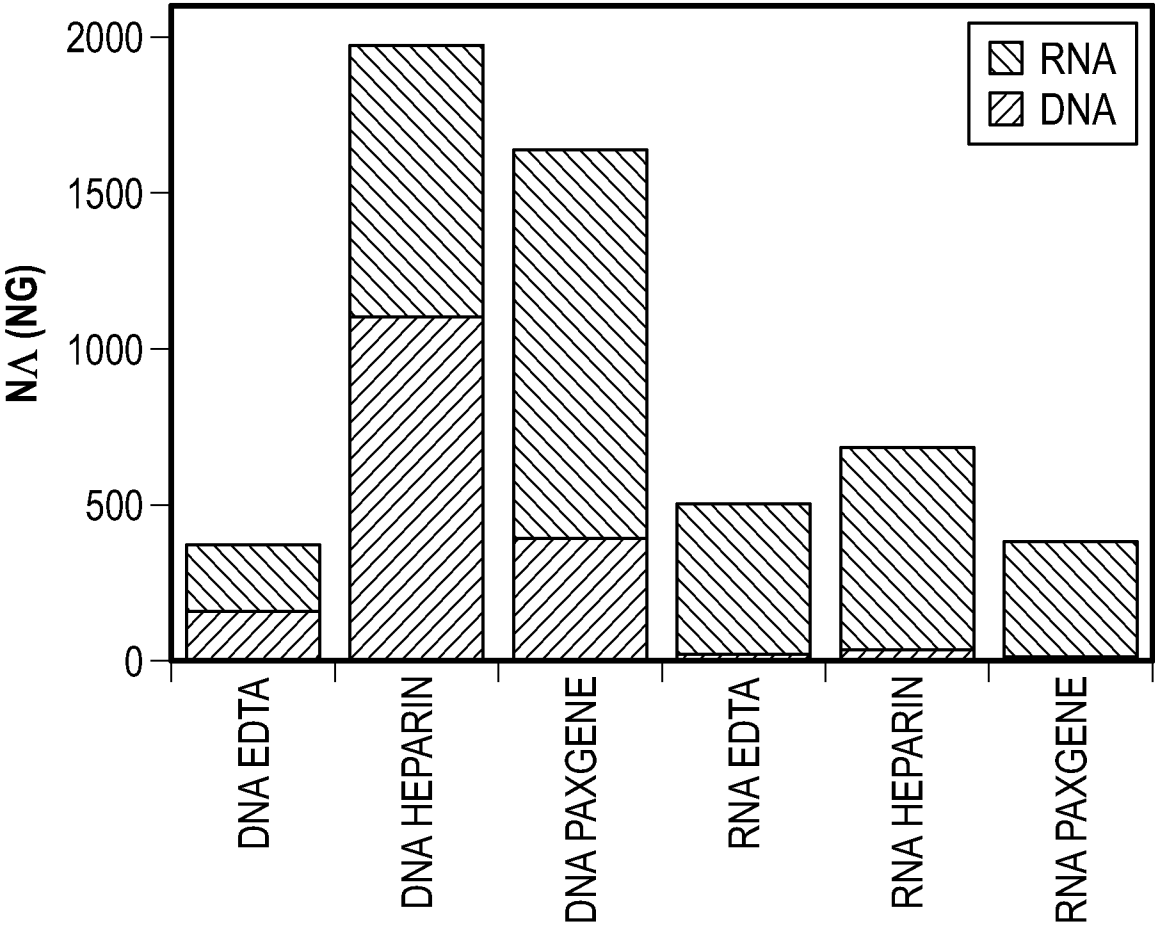


FIG. 1

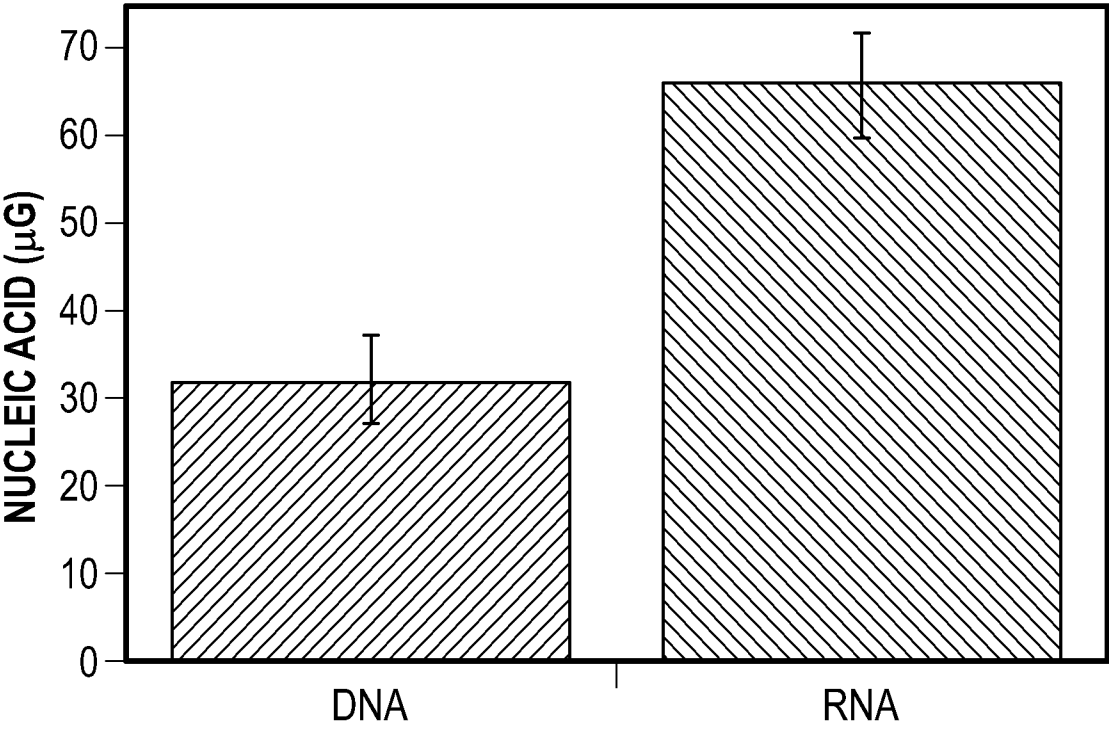


FIG. 2A

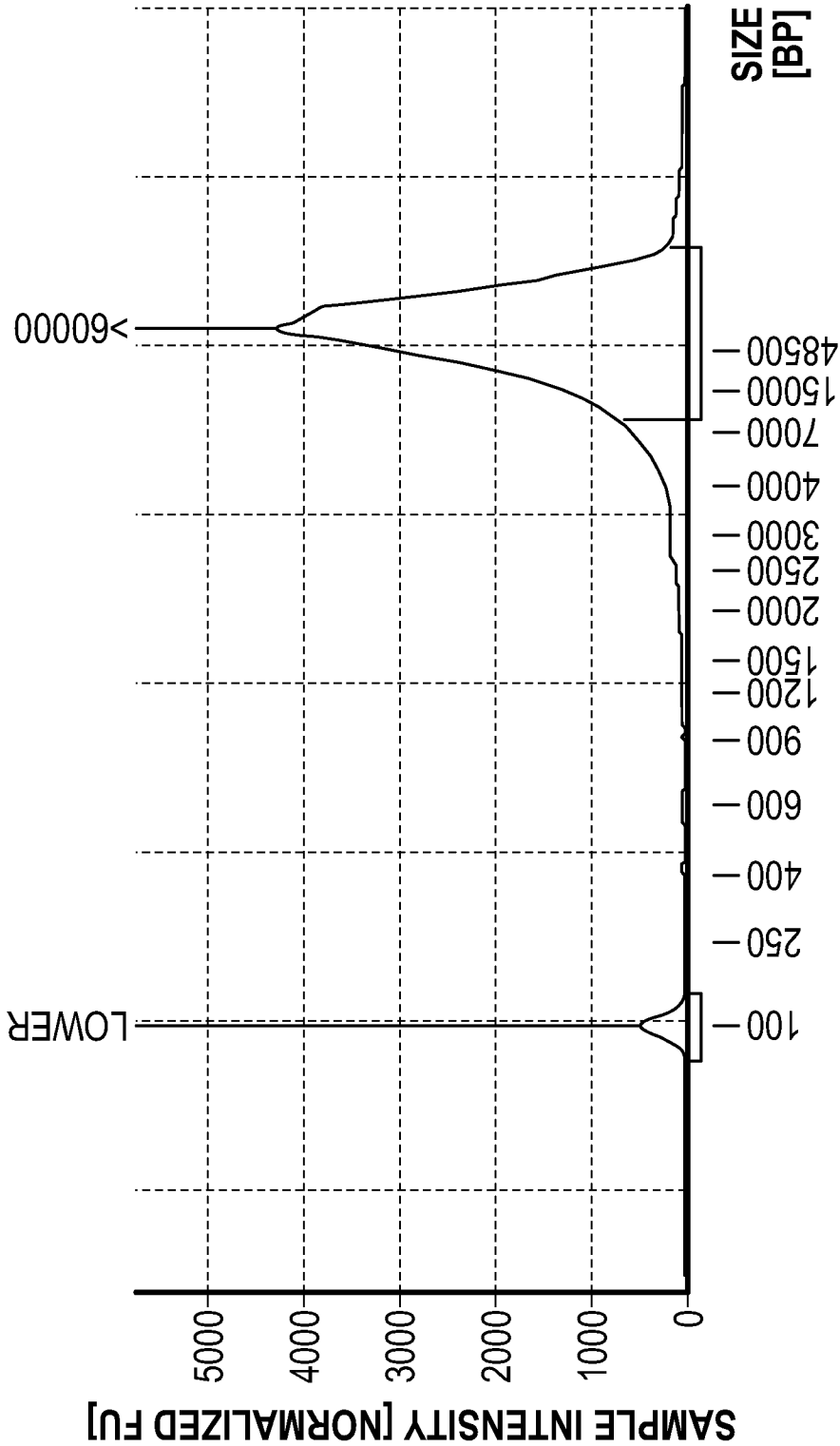


FIG. 2B

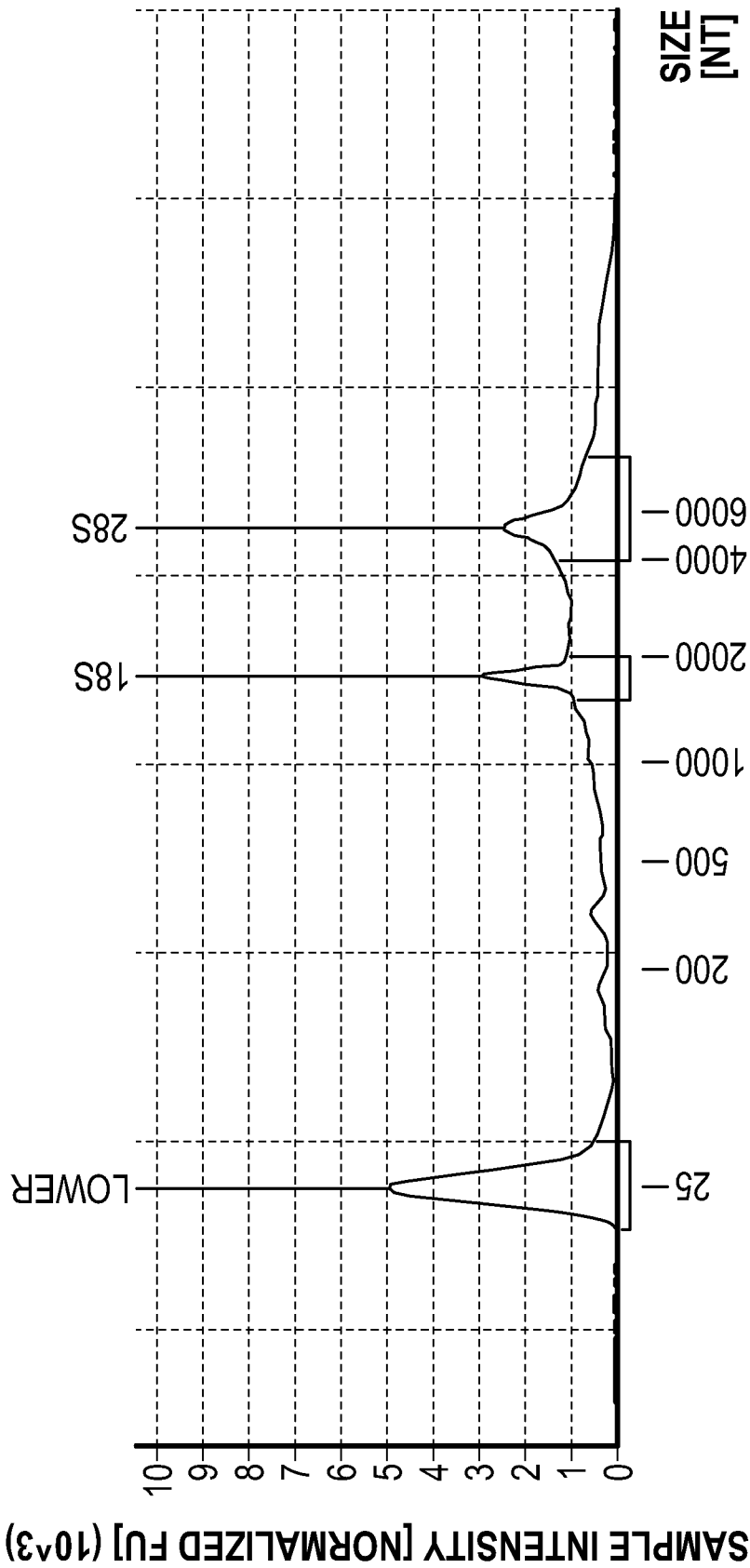


FIG. 2C

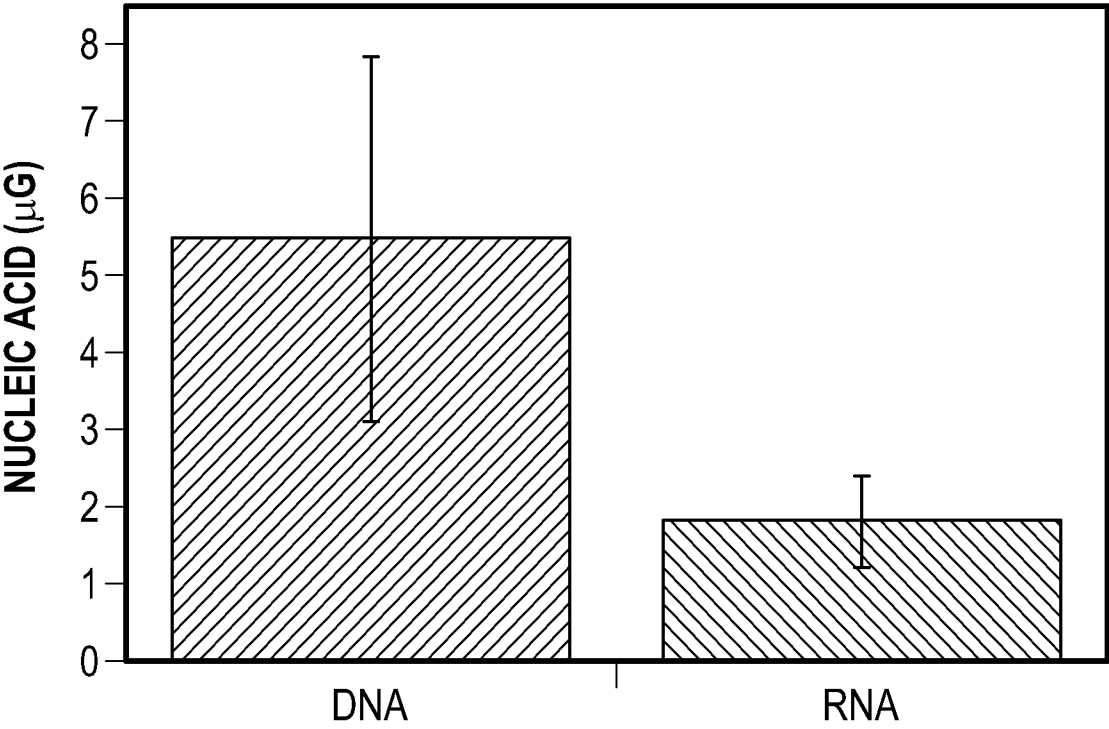


FIG. 3A

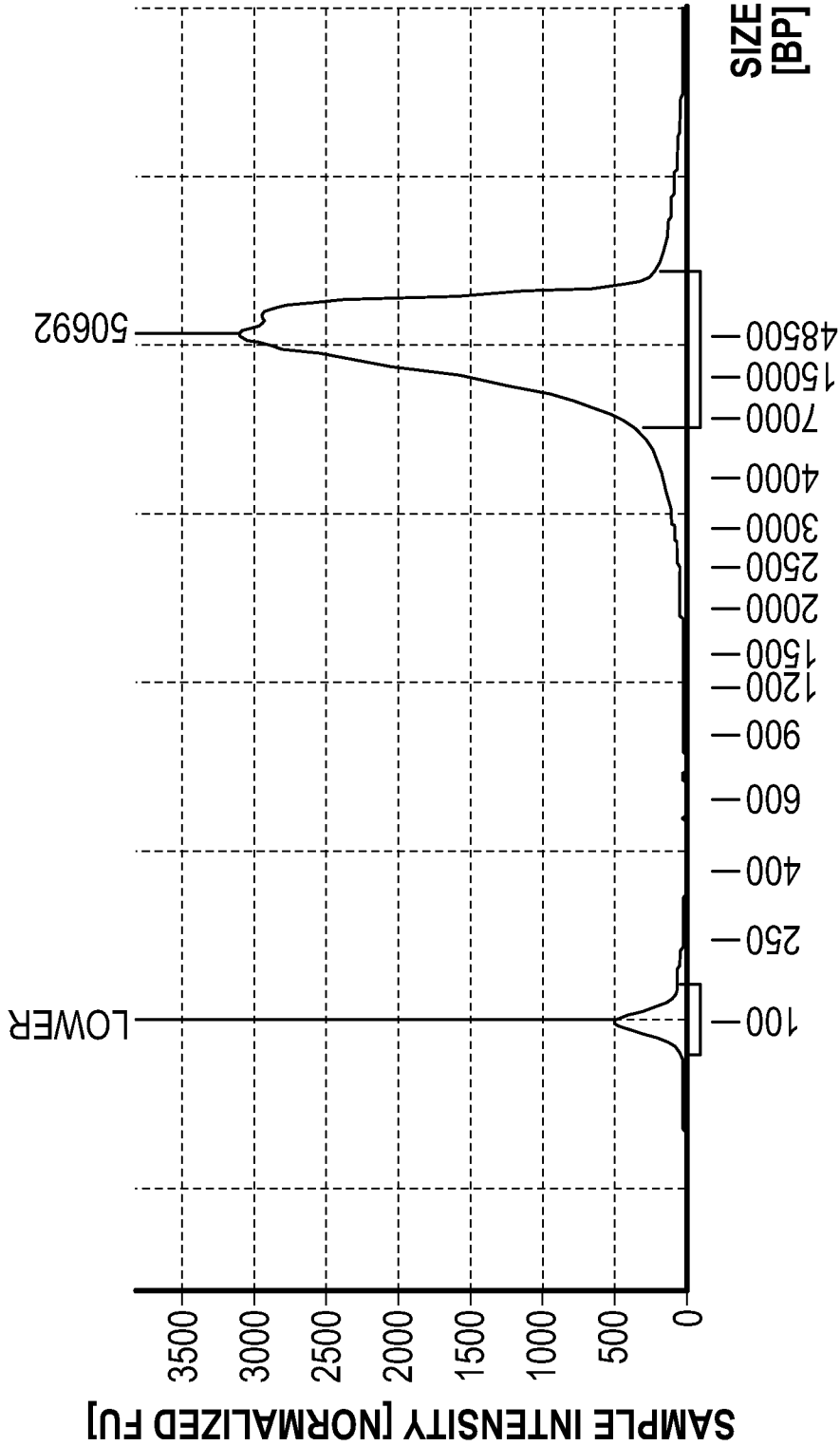
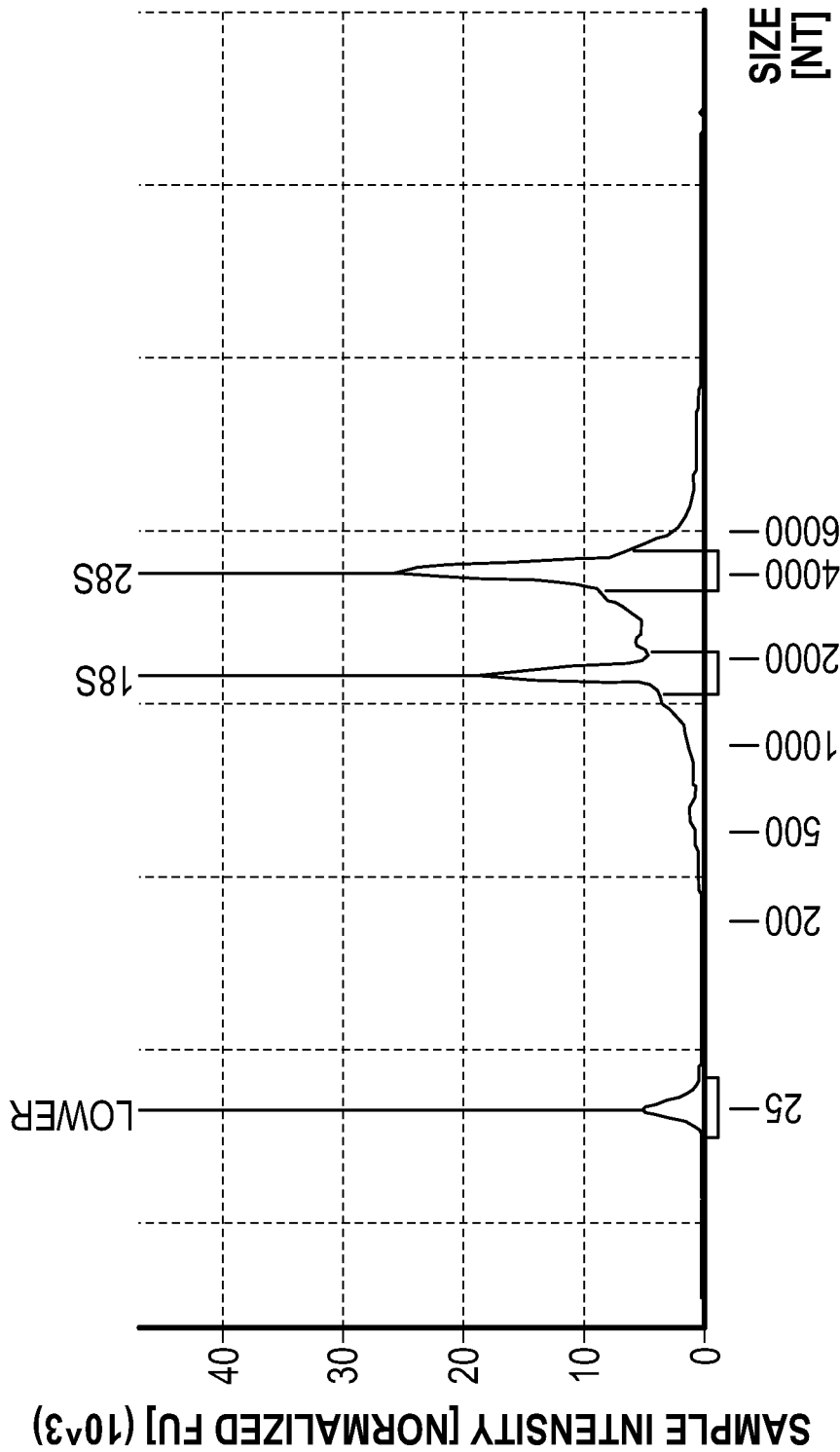


FIG. 3B



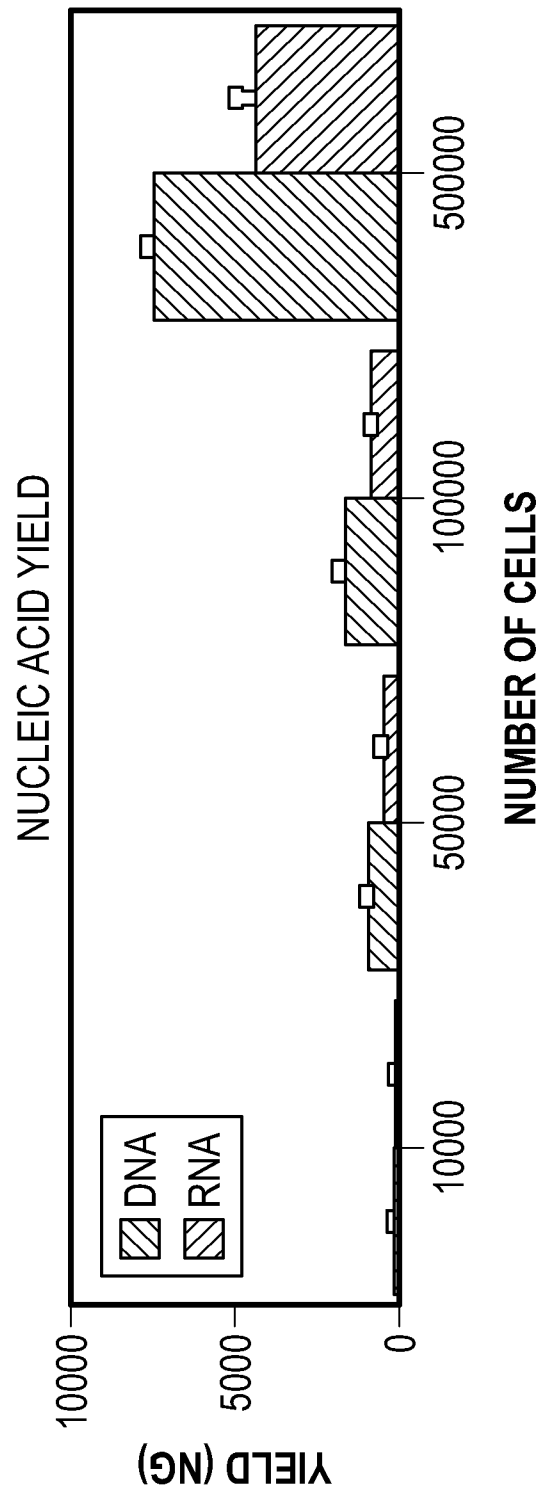


FIG. 4A

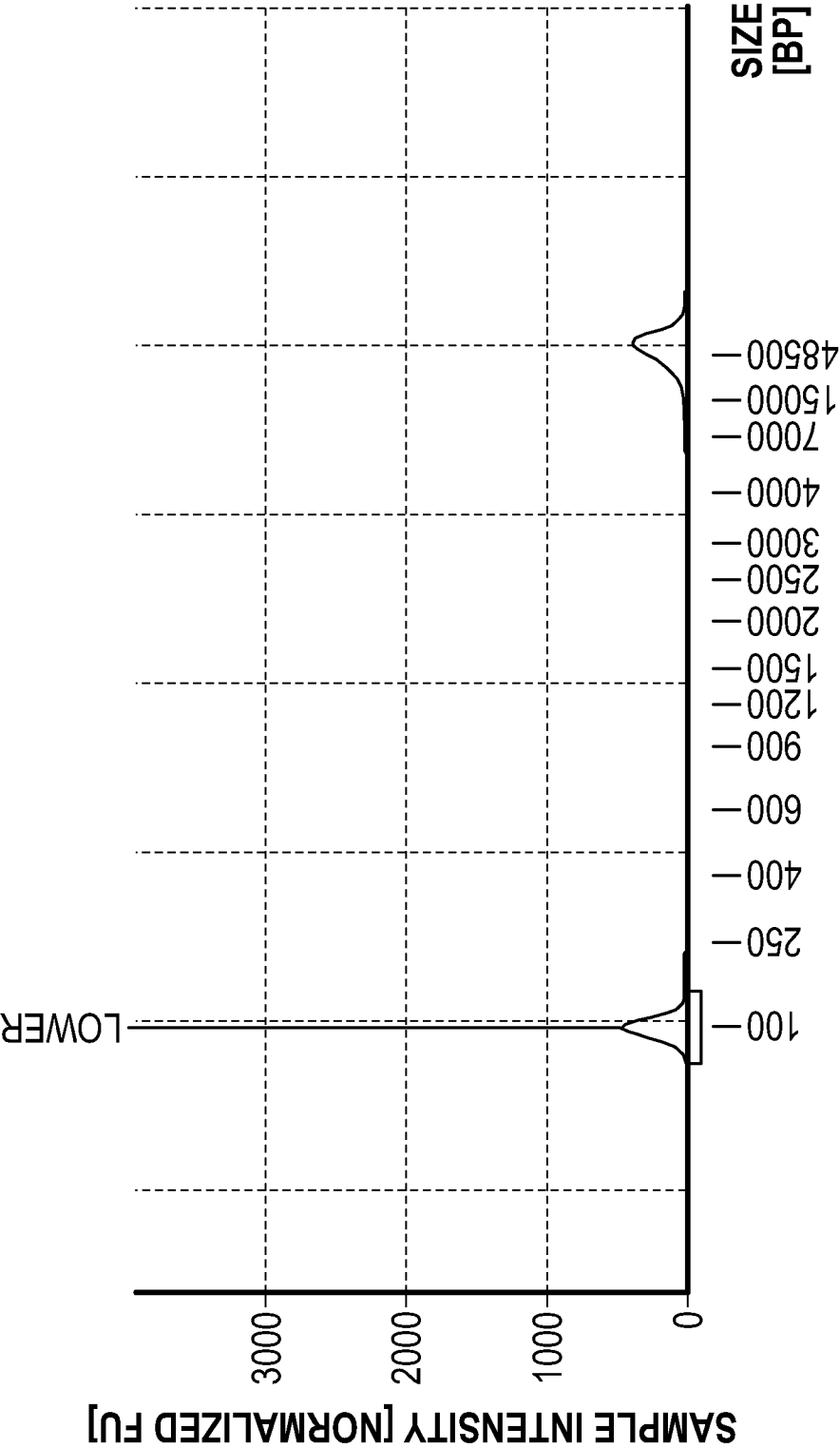


FIG. 4B-1

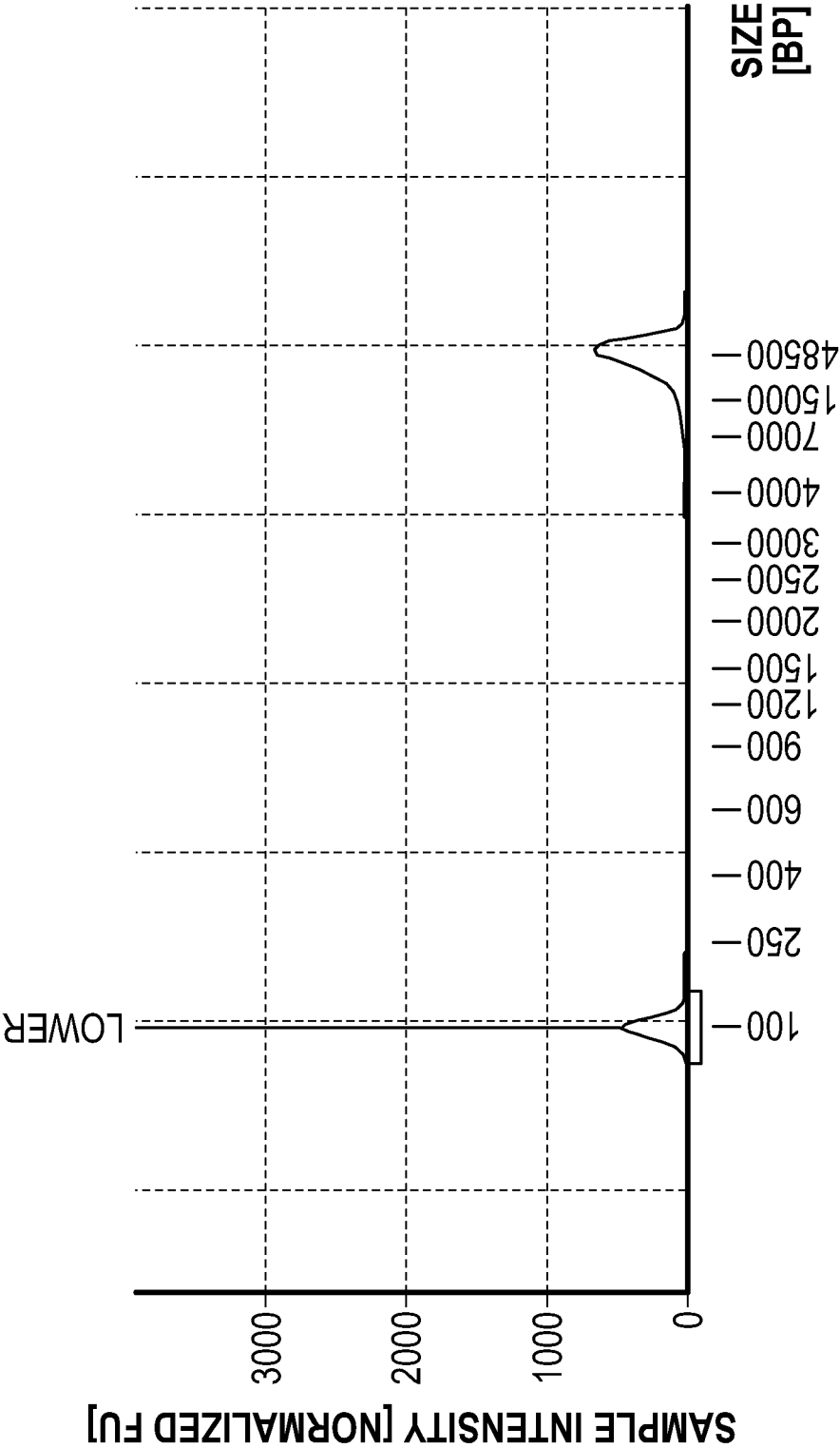


FIG. 4B-2

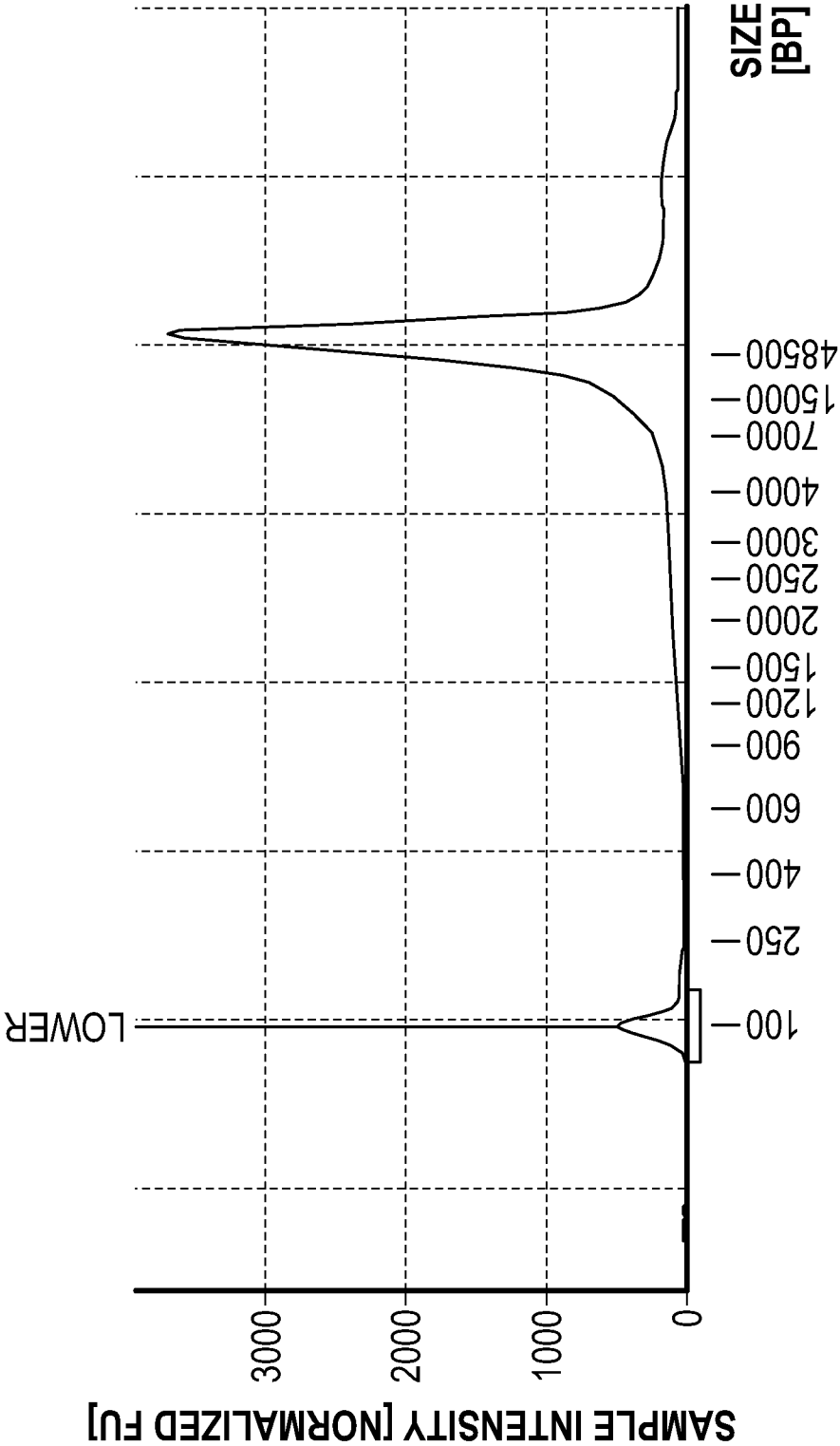


FIG. 4B-3

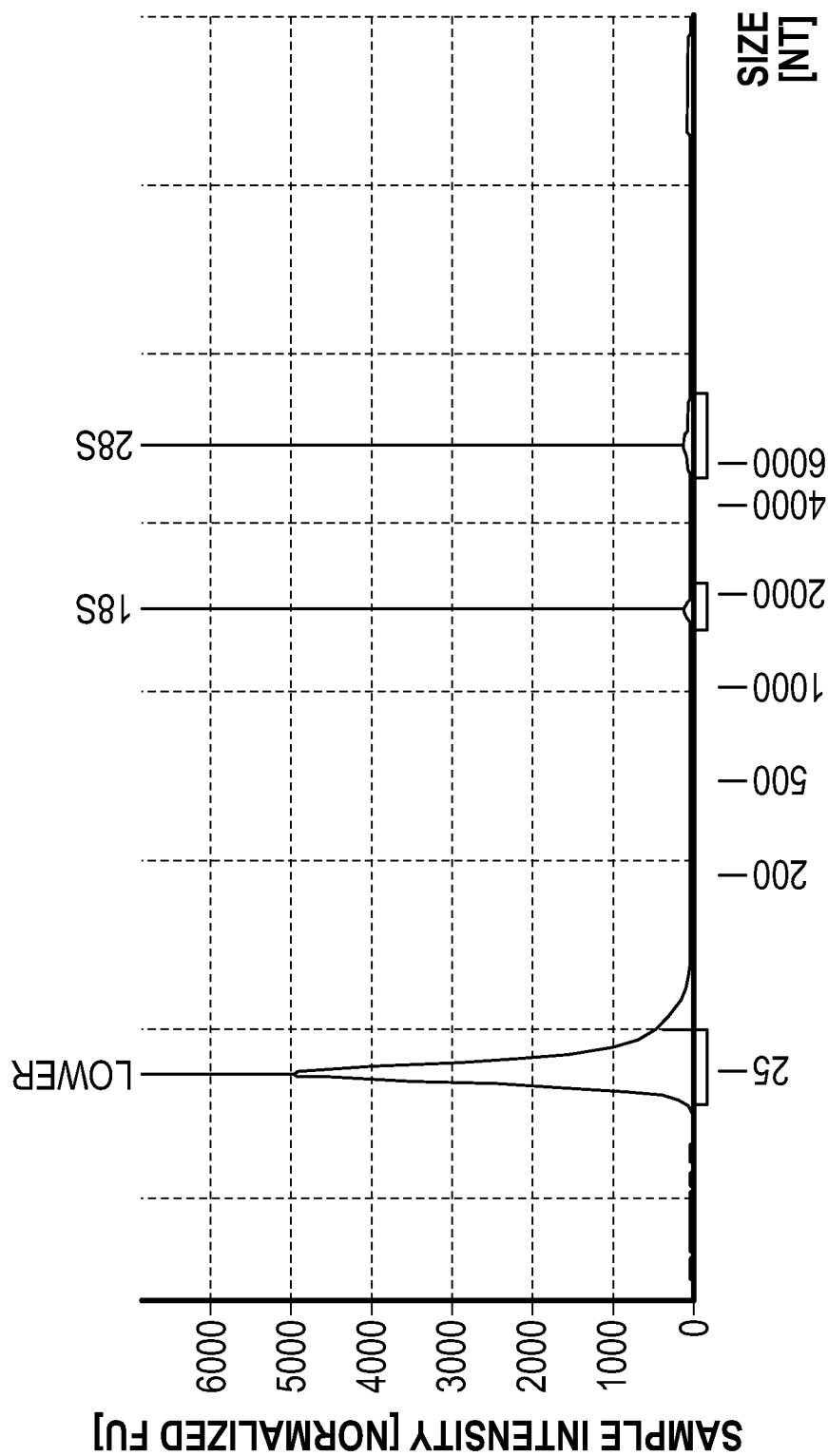


FIG. 4C-1

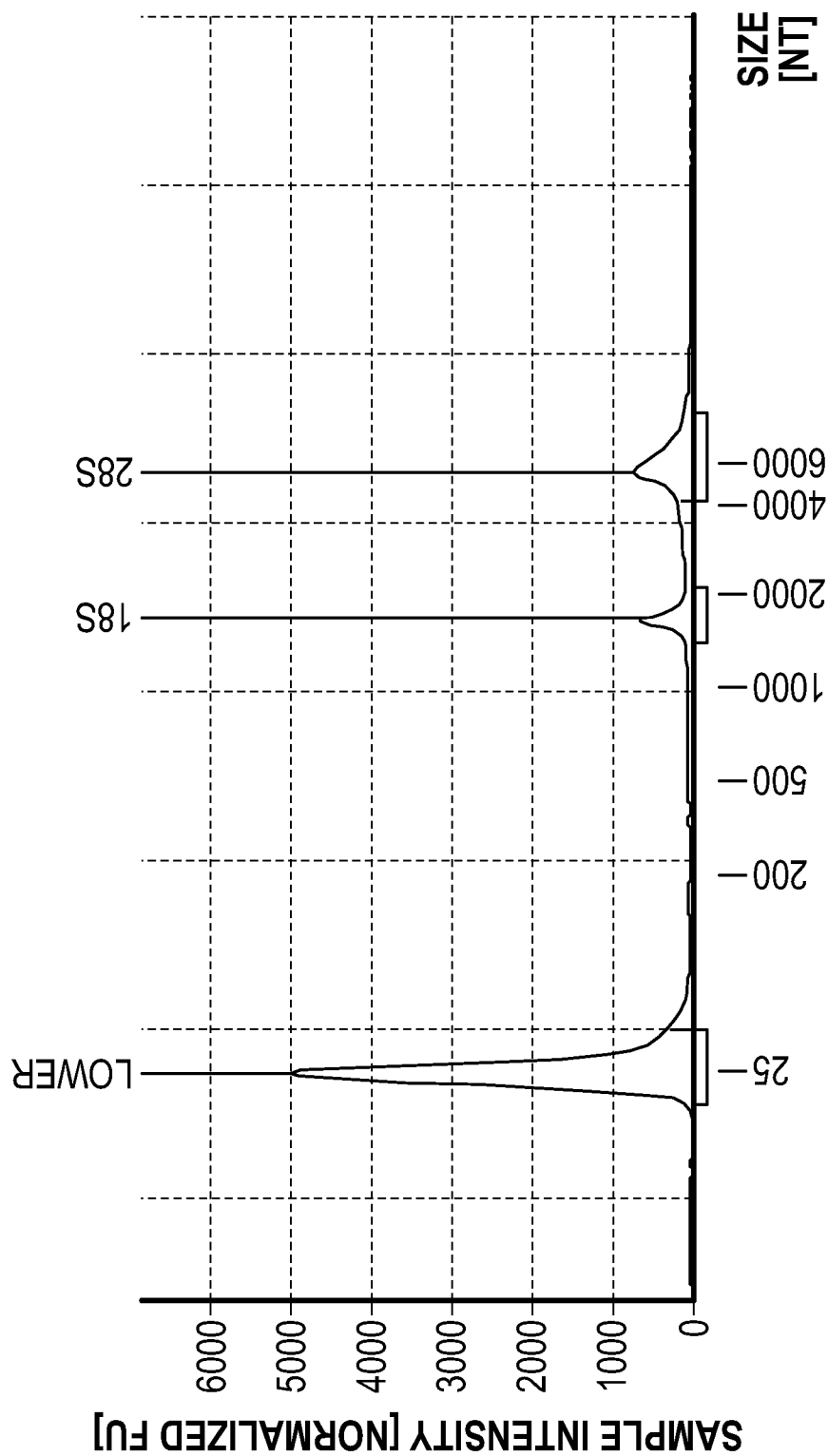


FIG. 4C-2

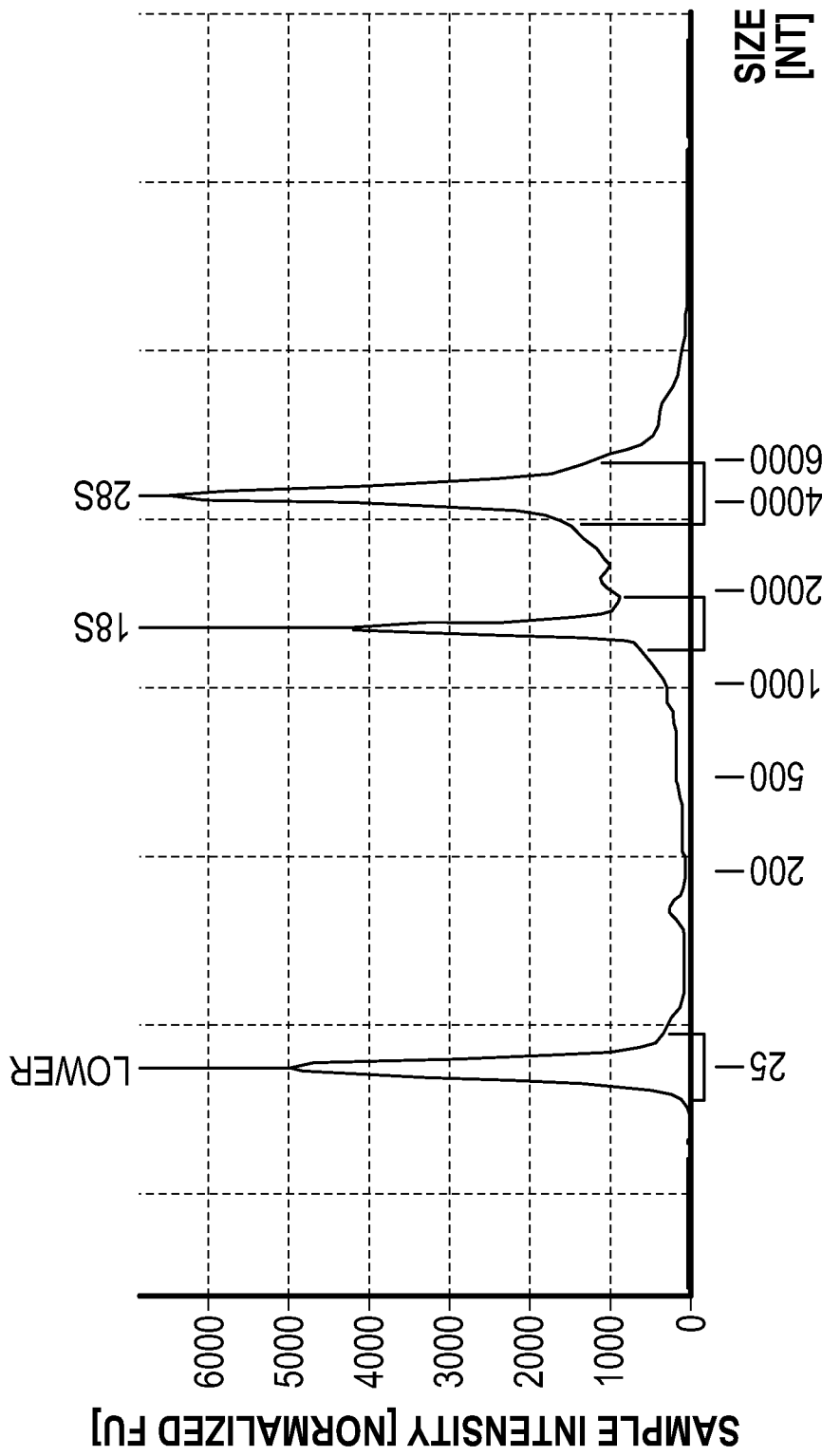


FIG. 4C-3

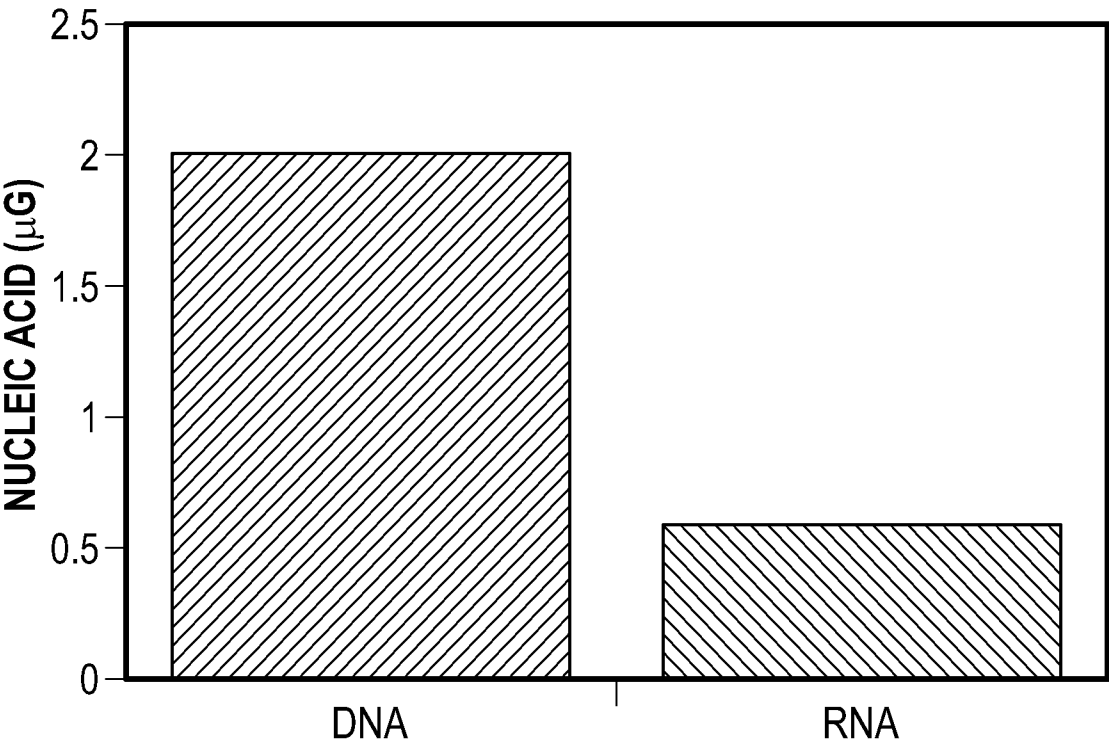


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2019/053565

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C40B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2012/002887 A1 (SJOEBLOM TOBIAS [SE]; MATHOT LUCY [SE]) 5 January 2012 (2012-01-05) abstract; claims 1-10; figure 1 page 6 - page 8	1,9-12, 14-18, 35-41 2-8,13, 19-34, 42-44
X A	----- US 2006/078923 A1 (MCKERNAN KEVIN [US] ET AL) 13 April 2006 (2006-04-13) paragraph [0013] paragraph [0065] paragraph [0075] paragraph [0225]	41-44 1-40
X	----- US 2011/172409 A1 (HAN ZHIQIANG [US]) 14 July 2011 (2011-07-14) paragraph [0011] - paragraph [0013]; claims 14-25; example 3 ----- -/-	41-44



Further documents are listed in the continuation of Box C.



See patent family annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 December 2019

Date of mailing of the international search report

18/12/2019

Name and mailing address of the ISA/

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Authorized officer

Tilkorn, A

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/053565

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	BOHUSLAV RITTICH ET AL: "SPE and purification of DNA using magnetic particles : Sample Preparation", JOURNAL OF SEPARATION SCIENCE., vol. 36, no. 15, 9 July 2013 (2013-07-09), pages 2472-2485, XP55561883, DE ISSN: 1615-9306, DOI: 10.1002/jssc.201300331 page 2477, column 2, paragraph 2 - page 2478, column 1, paragraph 2 -----	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/053565

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			NO 2588609 T3 16-06-2018
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			PT 2588609 T 02-04-2018
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