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(54) **Title**: METHODS AND COMPOSITIONS FOR EXTRACTION AND STORAGE OF NUCLEIC ACIDS

(57) **Abstract**: A solid matrix for the extraction, stabilization, and storage of nucleic acids is provided. At least one protein denaturant, and at least one acid or acid-titrated buffer reagent are impregnated in a dry state therein the matrix; and the matrix is configured to provide an acidic pH on hydration. The matrix is configured to extract nucleic acids from a sample and stabilize the extracted nucleic acids, particularly RNA, in a dry format under ambient conditions for a prolonged period of time. Methods for collecting and recovering the nucleic acids stored in the dry solid matrix are also described.

METHODS AND COMPOSITIONS FOR EXTRACTION AND STORAGE OF NUCLEIC ACIDS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of US Patent Application No.13/460076 entitled "Methods and compositions for extraction and storage of nucleic acids", filed April 30, 2012; and US Patent Application No. 13/721948 entitled "Formulations for nucleic acid stabilization on solid substrates", filed December 20, 2012; which are herein incorporated by reference.

FIELD

[0002] The invention relates to solid substrates and methods for ambient extraction and stabilization of nucleic acids from a biological sample in a dry format. Methods for collecting, extracting, preserving, and recovering nucleic acids from the dry solid substrates are also described.

BACKGROUND

[0003] Preserving the structural and functional integrity of biomolecules during isolation or purification from a biological sample is essential for various downstream applications. The downstream applications of purified biomolecules may include analyte detection, sensing, forensic, diagnostic or therapeutic applications, sequencing, amplification, and the like. The success of these downstream applications may depend on maintaining the integral structure and function of target biomolecules. Various factors, such as temperature, pressure, pH, chemical or

enzymatic hydrolysis, or the presence of contaminants may cause degradation of biomolecules such as DNA, RNA or protein.

[0004] RNA is one of the most unstable biomolecules due to chemical self-hydrolysis and enzyme-mediated degradation. The extraction and stabilization of RNA derived from a biological sample is sensitive to a number of environmental factors including, but not limited to, the buffer used to extract or collect the RNA, solution pH, temperature, and particularly the ubiquitous presence of robust ribonucleases (RNases). RNA is typically stored under refrigeration (e.g. 4°C, -20°C, or -80°C) in both purified and unpurified forms to prevent hydrolysis and enzymatic degradation and thus preserve the integrity of the RNA sample. The methods and articles for extraction and stabilization of RNA under ambient temperatures are desirable in order to avoid the costs and space requirements associated with refrigeration for maintaining the integrity of the RNA samples.

[0005] Current methodologies for stabilizing RNA under ambient temperature have focused on deactivating RNases in excess liquid solutions of, for example, detergents, chaotropic compounds, reducing agents, transitional metals, organic solvents, chelating agents, proteases, RNase peptide inhibitors, and anti-RNase antibodies. Additional efforts have focused on chemical modification of RNA to restrict trans-esterification and self-hydrolysis. Dry-state technologies claiming successful collection and preservation of RNA in dry formats typically require that RNA be "pre-purified" and concentrated from a sample prior to storage of the RNA. Other dry-state technologies for the preservation of RNA in dry formats require additional drying facilities (e.g. forced air flow, lyophilization, or heat treatment).

These methods are therefore not conducive to direct RNA collection from a sample (e.g., a biological sample) without significant sample processing.

[0006] Accordingly, compositions and methods that enable dry-state RNA extraction and stabilization from a biological sample under ambient conditions within a single process-step are needed. Moreover, the ability to store a dried biological sample for a substantial period at ambient temperature and recover intact RNA thereafter for further analysis is highly desirable.

BRIEF DESCRIPTION

[0007] One embodiment of a solid matrix comprises at least one protein denaturant, and at least one acid or acid-titrated buffer reagent impregnated therein in a dry state; wherein the matrix is configured to provide a pH in a range of 2 to 7 upon hydration, extract nucleic acids from a sample and preserve the nucleic acids in a dry state at ambient temperature.

[0008] In another embodiment, an RNA extraction matrix comprises a protein denaturant comprising a chaotropic agent, a detergent or combination thereof; and an acid or acid-titrated buffer reagent impregnated therein in a dry state, wherein the matrix is a porous non-dissolvable dry material configured to provide a pH of 2 and 7 upon hydration for extracting RNA and stabilizing the extracted RNA with an RNA Integrity Number (RIN) of at least 4.

[0009] In one embodiment, an RNA extraction matrix comprises a protein denaturant comprising a chaotropic agent, a detergent or combination thereof; an acid or acid-titrated buffer reagent; and an RNase inhibitor comprising a triphosphate salt or pyrophosphate salt, impregnated therein in a dry state, wherein the matrix comprises a porous non-dissolvable dry material configured to provide a pH of 2 and 7 upon hydration and stabilize RNA with an RNA Integrity Number (RIN) of at least 4.

[0010] One example of a method for extracting and storing nucleic acids from a sample comprises the steps of providing the sample to a dry solid matrix comprising a protein denaturant and an acid or acid titrated buffer reagent; generating a pH in a range of 2 to 7

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upon hydration for extraction of nucleic acids from the sample; drying the matrix comprising the extracted nucleic acids; and storing the extracted nucleic acids on the matrix in a dry state at ambient temperature.

DRAWINGS

[0011] These and other features, aspects, and advantages of the present invention will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein:

[0012] FIG. 1 is a P^{31} NMR profile showing the oxidation of Tris(2-carboxyethyl) phosphine (TCEP) and preparation of TCEP Oxide (TCEP-O).

[0013] FIG. 2 shows a bar graph derived from a 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) colorimetric assay for TCEP and TCEP-O reducing activity on cellulose samples.

[0014] FIG. 3 shows RNA Integrity Numbers (RIN) for dried blood spots collected on chemically treated cellulose paper containing TCEP or TCEP-O.

[0015] FIG. 4 shows RNA Integrity Numbers (RIN) for dried blood spots collected on various chemically-treated cellulose matrix and stored at ambient temperature for 5, 6, or 12 days prior to RNA analysis on an Agilent 2100 Bioanalyzer.

DETAILED DESCRIPTION

[0016] The embodiments of present invention provide suitable matrices and methods for ambient extraction and preservation of nucleic acids, such as RNA. RNA is generally known as an unstable molecule which is difficult to preserve in an intact form. One or more embodiments of the invention relate to a nucleic acid extraction matrix, wherein the matrix is configured to collect, extract and store nucleic acids from a biological sample for a prolonged period within a single process step, followed by use in various applications. The matrix is configured to store nucleic acids in a substantially dry-state at ambient temperature and substantially retain the integrity of the nucleic acids.

[0017] To more clearly and concisely describe the subject matter of the claimed invention, the following definitions are provided for specific terms, which are used in the following description and the appended claims. Throughout the specification, exemplification of specific terms should be considered as non-limiting examples.

[0018] The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Approximating language, as used herein throughout the specification and claims, may be applied to modify any quantitative representation that could permissibly vary without resulting in a change in the basic function to which it is related. Accordingly, a value modified by a term such as

"about" is not to be limited to the precise value specified. In some instances, the approximating language may correspond to the precision of an instrument for measuring the value. Where necessary, ranges have been supplied, and those ranges are inclusive of all sub-ranges there between.

[0019] The term "nucleic acid" as referred to herein comprises all forms of RNA (e.g., mRNA, miRNA, rRNA, tRNA, piRNA, ncRNA), DNA (e.g. genomic DNA, mtDNA), as well as recombinant RNA and DNA molecules or analogues of DNA or RNA generated using nucleotide analogues. The nucleic acids may be single stranded or double stranded. The nucleic acids may include the coding or non-coding strands. The term also comprises fragments of nucleic acids, such as naturally occurring RNA or DNA which may be recovered using the extraction methods disclosed. "Fragment" refers to a portion of a nucleic acid (e.g., RNA or DNA).

[0020] The term "biological sample" as referred to herein includes, but is not limited to, blood, serum, tissue, and saliva obtained from any organism, including a human. Biological samples may be obtained by an individual undergoing a self-diagnostic test (e.g., blood glucose monitoring) or by a trained medical professional through a variety of techniques including, for example, aspirating blood using a needle or scraping or swabbing a particular area, such as a lesion on a patient's skin. Methods for collecting various biological samples are well known in the art. The term "sample" includes biological samples as defined above, but also includes, for example, tissue cultured cells and purified nucleic acids.

[0021] The term, "reducing agents" as referred to herein include any chemical species that provides electrons to another chemical species. A variety of reducing

agents are known in the art. Exemplary reducing agents include dithiothreitol (DTT), 2-mercaptoethanol (2-ME), and tris(2-carboxyethyl)phosphine (TCEP). Moreover, any combination of these or other reducing agents may be used. In particular embodiments, the reducing agent is TCEP.

[0022] The term "buffer" as used herein includes, for example, 2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris), 2-(*N*-morpholino) ethanesulfonic acid (MES), 3-(*N*-morpholino)propanesulfonic acid (MOPS), citrate buffers, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and phosphate buffers. This list of potential buffers is for illustrative purposes only. The pH of the buffer selected for use in the compositions and methods disclosed herein is typically acid-titrated in the range of 2 to 7.

[0023] One or more embodiments of a solid matrix comprise at least one protein denaturant and at least one acid or acid-titrated buffer reagent impregnated in a dry state therein, wherein the matrix is configured to provide an acidic pH upon hydration. The matrix is also configured to extract nucleic acids from a sample and preserve the nucleic acids in a substantially dry state at ambient temperature. As used herein, the term "substantially dry state" refers to further drying the sample to have approximately less than 2% of water content.

[0024] Solid matrices for the extraction and storage of nucleic acids from a sample comprise at least one acid or acid-titrated buffer and a protein denaturant in a dry state. The term "matrix" is interchangeably used herein as "extraction matrix". The term "solid matrix" as used herein refers to a non-dissolvable matrix. The matrix enables collection, extraction and storage of nucleic acids without solubilizing the

matrix material. The solid matrix includes, but is not limited to, materials such as cellulose, cellulose acetate, nitrocellulose, glass fibers or combinations thereof. "Incorporation" of the compositions into the matrix includes, but is not limited to, the "dipping" procedure described below. In some embodiments, such methods accomplish incorporation of the composition into the dry solid matrix. Following incorporation of the composition into the dry solid matrix, the solid matrix is dried using any appropriate method.

[0025] As noted, the solid matrix comprises the composition in a dry state and also preserves the extracted nucleic acids under dry conditions. The use of a dry solid matrix for extraction and storage is advantageous over liquid-based extraction, because the dry matrix ensures minimal volumetric dilution of the sample applied to the matrix. One of skill in the art would appreciate that liquid-based extraction dilutes the concentration of the sample in an excess volume of stabilizing reagent. Use of dry solid matrix for collecting, extracting, and preserving a sample maintains the concentration of the sample and eliminates issues, such as sample degradation, that are related to improper dilution of sample in a liquid preservative. In addition, the solid matrix comprises a fixed composition of the dry reagents, which enables efficient extraction of nucleic acids, such as RNA, upon hydration, followed by stabilization of the extracted RNA at ambient temperature.

[0026] The terms "ambient condition" or "ambient temperature" are interchangeably used hereinafter. As used herein, the term "ambient temperature" refers to a temperature in a range between 0° C to 60°C. In one or more embodiments, the ambient temperature is room temperature. In some embodiments,

the matrix is configured to store or preserve nucleic acids under ambient temperature in a dried state.

[0027] As noted, the solid matrix is configured to store or preserve nucleic acids under dry-state for prolonged period. The term "configured to" or "configured for" is referred to herein as the structure or composition of the matrix that enables the matrix to extract and store nucleic acids for periods of time at ambient temperature. The terms "storage" or "preservation" may be interchangeably used herein with respect to maintaining the extracted nucleic acids in a format suitable for further analysis. More specifically, the nucleic acids may be stored or preserved in a solid nucleic acid extraction matrix, wherein the matrix ensures maintaining the integrity of the molecules.

[0028] In some embodiments, the nucleic acid extraction matrix is a solid phase extraction matrix. A matrix, where the solid phase extraction method is used, is referred to herein as a solid phase extraction matrix. Solid-phase extraction (SPE) technology has been leveraged to reduce the extraction times of high purity nucleic acids for sequencing and other applications. The solid phase extraction is an extraction method that uses a solid phase and a liquid phase to isolate one or more molecules of the same type, or different types, from a material. The solid phase extraction matrix is used, for example, to purify a sample upstream of a chromatographic or other analytical method. tone example of the method comprises loading a sample (e.g. a biological sample) onto the solid phase extraction matrix, storing the matrix at ambient temperature to achieve a substantially dry state, and

rehydrating the matrix with a suitable buffer to differentially extract RNA from the matrix.

[0029] The term "extraction" refers to any method for separating or isolating the nucleic acids from a sample, more particularly from a biological sample. Nucleic acids such as RNA and DNA may be released, for example, by cell-lysis. In one embodiment, nucleic acids may be released during evaporative cell-lysis. In another embodiment, the cells are lysed upon contact with the matrix comprising cell lysis reagents. Contacting a biological sample comprising cells to the matrix results in cell lysis which releases nucleic acids, for example by using FTA™ Elute cellulose papers.

[0030] The solid matrix may be porous. In one embodiment, the solid matrix is a porous cellulose paper, such as a cellulose matrix from Whatman™. In one example, the cellulose matrix from Whatman™ comprises 903-cellulose, FTA™ or FTA™ Elute.

[0031] In one or more examples, the extraction matrix is impregnated with one or more reagents. As noted, in an example embodiment, the matrix comprises one or more protein denaturants impregnated in a dry state. In one embodiment, the matrix further comprises one or more acids or acid-titrated buffer reagents. In another embodiment, the matrix further comprises one or more reducing agents. In some embodiments, the impregnated reagents comprise lytic reagents, nucleic acid-stabilizing reagents, nucleic acid storage chemicals and combinations thereof.

[0032] In some embodiments, the dried reagents impregnated in the matrix are hydrated by adding a buffer, water or a sample. In one embodiment, the impregnated dried reagents are hydrated by a sample, more specifically a biological sample, which is disposed on the matrix for extraction or storage of nucleic acids. In some other embodiments, in addition of a sample, water or buffer is added to hydrate the matrix and reconstitute or activate the reagents embedded in the matrix. In some embodiments, the hydration of the matrix generates an acidic pH on the matrix. In some embodiments, the hydration further results in reconstituting the reagents, such as protein denaturant, acid or acid titrated buffer reagents that are present in a dried form in the matrix.

[0033] In one or more embodiments, the matrix comprises a protein denaturant. The protein denaturant may comprise a chaotropic agent or detergent. Without intending to be limited to a particular denaturant, protein denaturants may be categorized as either weak denaturants or strong denaturants depending on their biophysical properties and ability to completely inhibit biological enzyme activity (e.g. RNases). In some embodiments, weak protein denaturants (e.g. detergent) may be used for lysing cells and disrupting protein-protein interactions without denaturing nucleic acids. In further embodiments, use of strong protein denaturants (e.g. chaotropic salts) may also denature nucleic acid secondary structure in addition to denaturing cells and proteins. Numerous protein denaturants are known in the art and may be selected for use in the compositions and methods described herein. Without intending to be limited to a particular protein denaturant, exemplary protein denaturants include guanidinium thiocyanate, guanidinium hydrochloride, sodium thiocyanate, potassium thiocyanate, arginine, sodium dodecyl sulfate (SDS), urea or a

combination thereof. Exemplary detergents may be categorized as ionic detergents, non-ionic detergents, or zwitterionic detergents. The ionic detergent may comprise anionic detergent such as, sodium dodecylsulphate (SDS) or cationic detergent, such as ethyl trimethyl ammonium bromide. Non-limiting examples of non-ionic detergent for cell lysis include TritonX-100, NP-40, Brij 35, Tween 20, Octyl glucoside, Octyl thioglucoside or digitonin. Some zwitterionic detergents may comprise 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and 3-[(3-Cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO).

[0034] In one or more embodiments, the protein denaturant comprises a thiocyanate salt. One or more embodiments of the matrix comprises an acid-titrated thiocyanate salt impregnated in a dry state. Exemplary thiocyanate salts include, but are not limited to, guanidinium thiocyanate, sodium thiocyanate, potassium thiocyanate or combinations thereof.

[0035] The extraction matrix maintains the stability and integrity of RNA at a desired level after RNA extraction from a biological sample. In one embodiment, the matrix is impregnated with nucleic acid stabilizing reagents. These stabilizing reagents may include RNAse inhibitors, acid-titrated buffer, or chelating agents (e.g EDTA). The composition may further comprise an ultraviolet (UV) inhibitor or a free-radical scavenger.

[0036] As noted, the matrix further comprises an RNase inhibitor, wherein the RNase inhibitor comprises vanadyl ribonucleoside complex (VRC), a nucleotide analogue, or a commercially available RNase inhibitor (e.g., SUPERase-In™). The RNAse inhibitor may further comprise pyrophosphate compounds. In one

embodiment, sodium pyrophosphate dibasic may be used as an RNase-inhibitor. One or more embodiments of the RNAse inhibitor may further comprise triphosphate salts, such as sodium triphosphate. In one example, addition of sodium pyrophosphate to acid-titrated buffer enhances RNA stability in both liquid state and dry-formats.

[0037] Embodiments of the matrix comprise acid or acid-titrated buffer reagents in a dry-state, which may be re-hydrated during extraction of nucleic acids. Examples of the acid include, but are not limited to, acetic acid, citric acid, tartaric acid, phosphoric acid, hydrochloric acid, Tris(2-carboxyethyl) phosphine- hydrochloric acid (TCEP-HCl), oxidized Tris(2-carboxyethyl) phosphine- hydrochloric acid (TCEP-O-HCl), sulfuric acid, nitric acid, vanillic acid, 3-(N-morpholino)propanesulfonic acid, or combinations thereof. As noted, the matrix provides an acidic pH on hydration which extracts and stabilizes the extracted nucleic acids, wherein the hydration may be achieved by adding a sample, water or any other solution (e.g. a buffer solution). One or more embodiments of the matrix provide a pH in a range from 2 to 7 on hydration. In some embodiments, the matrix provides a pH in a range from 3 to 6 on hydration.

[0038] The extracted nucleic acids, particularly RNA, are stabilized under acidic condition, as shown in Table IV. In one embodiment, the acid-titrated buffer comprises guanidine thiocyanate. At acidic pH from 2 to 7, more particularly at a pH from 3 to 6, a dry-state mixture of guanidine thiocyanate and sodium pyrophosphate in the acidic range on a dry solid matrix stabilizes high-quality RNA in dried blood spots at ambient temperature, as shown by RIN score in FIG. 4. In one embodiment, the acid-titrated buffer comprises guanidine thiocyanate, wherein at acidic pH from 2

to 7, more particularly at pH from 3 to 6, the presence of sodium triphosphate in a dry solid matrix stabilizes high quality RNA, as shown in FIG. 4 by RIN score.

[0039] As noted, in some embodiments, the matrix further comprises a UV protectant, a free-radical scavenger, a chelator or combinations thereof. Without intending to be limited to any specific UV protect, an exemplary antioxidants include, for example, hydroquinone monomethyl ether (MEHQ), hydroquinone (HQ), toluhydroquinone (THQ), and ascorbic acid. In some embodiments, the antioxidant is THQ.

[0040] In some embodiments, the matrix further comprises at least one reducing agent, wherein the reducing agent is selected from the group consisting of dithiothreitol (DTT), 2-mercaptoethanol (2-ME), tris(2-carboxyethyl) phosphine (TCEP) and combinations thereof.

[0041] The extracted nucleic acids comprise ribonucleicacids (RNA), deoxy ribonucleicacids (DNA) or a combination thereof. In one embodiment, the extracted nucleic acids comprise RNA. The RNA may be mRNA, tRNA, rRNA, small RNA, siRNA, miRNA, non-coding RNA, animal RNA, plant RNA, viral RNA or bacterial RNA.

[0042] The matrix is configured to store nucleic acids in a dry format at ambient temperature under substantially intact condition. The condition of the RNA refers to the quality of the RNA or integrity of the RNA. The stability and quality of RNA may be assessed on the basis of: quantitative RT-PCR amplification of mRNA targets; the ratio of 28s:18s ribosomal RNA (rRNA), which compromises the bulk of total

cellular RNA, and RIN analysis on an Agilent 2100 Bioanalyzer. As noted, RNA quality is determined as a ratio of 28S and 18S ribosomal RNA intensity values, wherein the ratio is calculated by obtaining the intensity of 28S and 18S rRNA by gel electrophoresis of the extracted rRNA followed by ethidium bromide staining. High-quality cellular RNA generally exhibits a 28s:18s rRNA ratio greater than 1. Moreover, high-quality cellular RNA supports efficient amplification of both low-abundance and large (e.g., greater than1 kB) mRNAs. For the purposes of convenience, rRNA signal intensity and the ratio of 28s:18s rRNA are frequently used to rapidly screen and identify samples with robust RNA storage properties by gel electrophoresis.

[0043] As noted, in one embodiment, the RNA quality is determined by capillary electrophoresis of the extracted RNA through a bioanalyzer. As is customary, the RNA quality is quantified as a RIN, wherein the RIN is calculated by an algorithmic assessment of the amounts of various RNAs present within the extracted RNA. High-quality cellular RNA generally exhibits a RIN value approaching 10. In one or more embodiments, the RNA extracted from the dry matrix has a RIN value of at least 4. In some embodiments, the matrix provides for ambient extraction and stabilization of a biosample and produces intact, high quality RNA with a RIN value in a range from 4 to 10, or in one embodiment, the RIN value is in a range from 5 to 8.

[0044] An example of a method for extracting and storing nucleic acids from a sample comprises the steps of providing the sample onto a solid matrix comprising a protein denaturant and acid or acid-titrated buffer reagent, generating an acidic pH for extraction of the nucleic acids from the sample upon hydration of the solid matrix

with the sample or any externally added liquid, drying the matrix comprising extracted nucleic acids, and storing the extracted nucleic acids on the matrix in a substantially dry state under ambient temperature. Non-limiting examples of the term "providing a sample" include, applying a sample or disposing a sample on the extraction matrix using a pipet, catheter, syringe or conduit. The sample may be poured on the matrix.

[0045] The method comprises storing the extracted nucleic acids on the matrix in a dry state at ambient temperature. In some embodiments, the nucleic acids may be stored for more than a one month time period. In some embodiments, the nucleic acids may be stored for more than a six months period. As RNA is generally prone to degradation, the extraction and preservation of RNA using the matrix is useful and may further be used for various downstream applications.

[0046] One or more embodiments of the method comprise recovering nucleic acids from the matrix by solid phase extraction technique. In one or more embodiments, the nucleic acids are recovered from the solid matrix by rehydrating the matrix in an aqueous solution, a buffer, or an organic solution, and wherein the nucleic acids are subjected to further analysis. Any method that results in the extraction of nucleic acids, particularly RNA from a sample (e.g., an unpurified biological sample) may be employed. The method delineated above may optionally include a step of washing the matrix before recovering the nucleic acids from the solid matrix for further analysis. For example, the matrix may be washed for one or more times with a suitable buffer or water prior to recovery of the nucleic acids. The nucleic acids may be recovered by rehydrating the solid matrix (e.g., cellulose paper) in an aqueous

solution, a buffer solution, as defined above, or an organic solution. In some embodiments, the nucleic acids are recovered from the solid matrix by electroelution.

[0047] In one embodiment, a method for extracting and preserving the nucleic acids (e.g., RNA, DNA, or a combination thereof) comprises the steps of: providing a solid matrix, wherein a composition comprises at least one protein denaturant, an acid or acid-titrated buffer reagent, and optionally a free-radical scavenger incorporated into the solid matrix in a dried format; applying a sample (e.g., a biological sample) to the solid matrix to extract the nucleic acids under acidic pH; drying the solid matrix; and storing the nucleic acids on the solid matrix in a substantially dry state at ambient temperature.

[0048] In certain examples of the method, the matrix permits the storage of nucleic acids, particularly RNA which is widely known to be an unstable biomolecule to store, in a dry format (e.g., on a solid matrix) at ambient temperatures. The samples utilized in this method include, but are not limited to, biological samples such as blood, serum, tissue, and saliva obtained from any organism, including a human.

EXAMPLE

Reagents: 31-ETF was from GE Healthcare. TCEP was from Soltec Bio Science (Beverly MA, USA), MOPS was purchased from Aldrich (MO, USA).

EXAMPLE 1. *Preparation of TCEP-O by oxidation of TCEP*

[0049] TCEP was oxidized to TCEP-O to analyze the contribution of reducing activity to RNA preservation in dried biosamples. Approximately 1 gram of TCEP was dissolved in 25mL of 30% hydrogen peroxide and solution pH was adjusted to

8.0 using sodium hydroxide. The reaction mixture was incubated for 3 hours to complete the oxidation reaction and the products were dried in an oven for subsequent analysis. P^{31} NMR confirmed loss of TCEP in the reaction product relative to a TCEP reference. This oxidation reaction was repeated in the presence of the antioxidant THQ with similar results. The results are set forth in FIG. 1.

EXAMPLE 2 *Confirmation of loss of reducing activity on dry matrices coated with TCEP-O*

[0050] Paper samples were prepared in solutions containing TCEP or TCEP-O using a simple dip-coating process. Briefly, coating solutions were prepared as described in Table I. Since the control sample, 25-1, resulted in a final solution having pH of 3.5, all other samples were adjusted to pH 3.5 with HCl. 31-ETF cellulose paper was dipped into each coating solution, and after complete saturation, the paper was passed through a nip roller to remove excess solution. Paper samples were then dried in an oven, packaged in Mylar foil bags with desiccant, and stored under 4°C until use.

Table I. Preparation of paper samples containing TCEP or TCEP-O

Sample	MOPS (mg/mL)	GuSCN (mg/mL)	TCEP (mg/mL)	TCEP-O (mg/mL)	THQ (mg/mL)	Process
25-1	20	300	10		5	Prepare MOPS buffer pH 7, add other components, final pH is 3.5
25-3	20	300		10	5	Prepare MOPS buffer pH 7, add other components, adjust pH to 3.5 with HCl
27-5		300	10			Dissolve GuSCN and TCEP in water, adjust pH to 3.5 with HCl

27-6		300		10		Dissolve GuSCN and TCEP-O in water, adjust pH to 3.5 with HCl

[0051] Following sample preparation, the reducing activity of the paper was analyzed using a DTNB colometric assay. A 1 mM DTNB working solution was prepared in PBS from a 2.5 mM stock solution in water. Sample punches (with 3 mm diameter) were cored from each paper described in Table I, submerged into 5 mL of DTNB working solution, and shaken for 30 minutes. TNB (thiobis-(2-nitrobenzoic acid) in the resulting solutions were then measured by UV absorbance at 412 nm, which are set forth in Fig. 2. Samples 25-3 and 27-6, containing TCEP-O, showed no reduction of DTNB to TNB, indicating loss of reducing power. Samples 25-1 and 27-5 containing TCEP show strong reducing activity by converting DTNB to TNB using the DTNB colorimetric assay. These results confirmed the prior NMR analyses in Example 1.

EXAMPLE 3- *RNA Stability Analysis from Dried Blood Spots*

[0052] Samples from Example 2, as described in Table I, were spotted with whole blood and tested for the ability to stabilize RNA at room temperature. 50 μL of rat whole blood was collected from the tail vein of a test animal and spotted onto samples 25-1, 25-3, 27-5, and 27-6. Blood spots were air-dried and stored at ambient room temperature under controlled humidity (~20% RH) for 5 days (25-1, 25-3) or 12 days (27-5, 27-6). RNA was extracted from a 7 mm center punch using RLT lysis buffer (Qiagen) fortified with beta-mercaptoethanol, and purified using conventional silica-membrane spin columns in accordance with protocols known in the art (e.g. Qiagen

QIAamp RNA blood kit). Purified RNA was eluted from spin columns with nuclease-free water, and RIN for each of the samples were measured on an Agilent 2100 Bioanalyzer using RNA 6000 Pico LabChips. By convention, RIN > 6 is indicative of high quality RNA and is highly desirable for quantitative downstream analyses such as RT-PCR or microarray applications.

[0053] As noted, the RIN value was determined by an Agilent 2100 Bioanalyzer using RNA 6000 Pico Lab Chips for each composition listed in Table 1, and the data is shown in FIG. 3. Unexpectedly the samples containing TCEP-O provided comparable RNA integrity to those containing TCEP. RIN scores were only slightly higher in the presence of TCEP (samples 25-1, 27-5) than that of fully oxidized TCEP (samples 25-3, 27-6). This phenomenon may be dependent on acidic pH, since all samples were prepared from coating solutions titrated to at a final pH of 3.5, in order to replicate the natural pH end-point of the control formulation, 25-1, containing TCEP-HCl.

EXAMPLE 4- *Substrate preparation of alternative chemistries at acidic or basic pH profiles*

[0054] Example 4 was designed to investigate the effects of different mixtures of acid, antioxidant, chaotropic salt, detergent, and pyrophosphate or polyphosphate salts at different solution pH. Paper samples were prepared using the simple dip-coating process described above. Briefly, coating solutions were prepared as described in Table II. 31-ETF cellulose paper was dipped into each coating solution, and after complete saturation the paper was passed through a nip roller to remove excess

solution. Paper samples were then dried in an oven and packaged in Mylar foil bags with desiccant until use.

Table II. Preparation of paper samples at acidic or basic pH profiles: *Acids*

Sample	MOPS (mg/ml)	GuSCN (mg/ml)	SDS (mg/ml)	p-coumaric acid (mg/ml)	Vanillic acid (mg/ml)	Acetic acid (mg/ml)	Citric acid (mg/ml)	Tartaric acid (mg/ml)	Phophoric acid (mg/ml)	Process
26-8	20	300			5.5					Prepare MOPS buffer pH 7.0, add other components, adjust pH to 3.5 with HCl
26-7	20	300		3.5						Prepare MOPS buffer pH 7.0, add other components, adjust pH to 3.5 with HCl
27-2	20	300								Prepare MOPS buffer pH 7.0, add other components, adjust pH to 3.5 with HCl
28-9	20		20		5.5					Prepare MOPS buffer pH 7.0, add other components, adjust pH to 3.5 with HCl
27-8		300			5.5					Dissolve GuSCN and vanillic acid in water, adjust pH to 3.5 with HCl

27-7		300		3.5						Dissolve GuSCN and p-coumaric acid in water, adjust pH to 3.5 with HCl
28-2		300				20				Dissolve GuSCN and acetic acid in water, adjust pH to 3.5 with NaOH
28-5		300						20		Dissolve GuSCN and tartaric acid in water, adjust pH to 3.5 with NaOH
28-4		300					20			Dissolve GuSCN and citric acid in water, adjust pH to 3.5 with NaOH
28-3		300							20	Dissolve GuSCN and phosphoric acid in water, adjust pH to 3.5 with NaOH

Table III. Preparation of paper samples at acidic or basic pH profiles :*Poly- and pyrophosphate salts*

Sample	GuSCN (mg/ml)	NaSCN (mg/ml)	Sodium triphosphate (mg/ml)	Sodium pyrophosphate (mg/ml)	Process

27-10	300		20		Dissolve GuSCN and sodium triphosphate in water, adjust pH to 3.5 with HCl
27-11	300			20	Dissolve GuSCN and sodium pyrophosphate in water, adjust pH to 3.5 with HCl
26-11	300		20		Dissolve GuSCN and sodium triphosphate in water, adjust pH to 7.2 with HCl
26-12	300			20	Dissolve GuSCN and sodium pyrophosphate in water, adjust pH to 7.2 with HCl
28-6		206	20		Dissolve NaSCN and sodium triphosphate in water, adjust pH to 3.5 with HCl
28-7		206	20		Dissolve NaSCN and sodium triphosphate in water, adjust pH to 7.2 with HCl

EXAMPLE 5- *RNA Stability Analysis from Dried Blood Spots on alternative chemistries*

[0055] Samples from Example 4, described in Table II and Table III, were spotted with whole blood and tested for the ability to stabilize RNA at ambient temperature. 50 μL of rat whole blood was collected from the tail vein of a test animal and spotted directly onto paper samples. Blood spots were dried and stored at ambient

temperature but controlled humidity (~20% RH) for 5, 6 or 12 days. RNA was extracted from a 7 mm center punch into lysis buffer and purified through silica-membrane spin columns in accordance with protocols known in the art. Following purification and elution, RIN were measured on an Agilent 2100 Bioanalyzer using RNA 6000 Pico Lab Chips. A RIN > 6 implies high quality RNA and desirable for quantitative downstream analyses such as RT-PCR or microarray applications.

[0056] The results of Example 5 are set forth in FIG. 4. It was discovered that acid-titrated chaotropic salt or detergent compositions yielded RNA of reasonable quality from dried blood spots, although certain formulations are preferable over others based on RIN score. For example, samples 28-2 and 28-5 contain guanidium thiocyanate (GuSCN) and showed RIN values of 7.8 and 7.0 at pH 3.5 in acetic acid and in tartaric acid, respectively. The RIN values for acetic acid (7.8) and tartaric acid (7.0) are higher than the same composition in citric acid (sample 28-4, RIN 5.8) and phosphoric acid (sample 28-3, RIN 4.9) at pH 3.5. In particular, the efficacy of pyrophosphate or triphosphate salts showed a clear pH-dependence for stabilizing RNA in the presence of chaotropic agent, with an overall acidic pH providing very high RIN scores. Identical formulations titrated to neutral pH resulted in severe RNA degradation. For example, samples 27-10 and 28-6, coated with either guanidinium thiocyanate (GuSCN) or sodium thiocyanate (NaSCN) and sodium triphosphate at pH 3.5, showed high RIN values of 7.1 and 7.0, respectively, compared to samples 26-11 and 28-7, which contain the same reagents at pH 7.2. Similarly, samples 27-11 and 26-12 contain GuSCN and sodium pyrophosphate and showed RIN values of 6.7 at pH 3.5 and 1.6 at pH 7.2 respectively. The pyrophosphate and triphosphate moieties

are generally understood to be small-molecule RNase inhibitors, for which a pH-dependent mechanism of action in dry-states is not intuitive.

EXAMPLE 6- *Correlation of RIN performance to solid-matrix pH*

[0057] The pH of the solid-matrix using samples from Example 1 (described in Table I), and Example 4 (described in Table II and Table III), were measured and compared to biological RIN performance. To measure solid-matrix pH, 9 punches (7mm round) were cored from each paper and submerged into 1mL water. The punches were homogenized into pulp using a high shear lab homogenizer, and the pH of the aqueous phase was determined with pH test strips.

[0058] The results of Example 6 are set forth in Table IV. The pH of each dry solid matrix is generally maintained from the original pH of the solution, by which the solid matrices were coated, although certain formulations are preferable over others. Without limiting to a particular theory, the results confirm that solid matrices bearing acidic pH yield RNA of reasonable quality from dried blood spots, as RIN values for RNA samples derived from compositions under acidic pH are greater than or equal to 4 after several days of ambient storage.

Table IV: RIN values for different matrix compositions at acidic or basic pH and under ambient conditions

Sample code	Composition					pH		RIN	Ambient storage
	denaturant	buffer	acid	antioxidant	Phosphate salt	Dipping solution	Paper		

25-1	GuSCN	MOPS	TCEP-HCL	THQ		3.6	4	6.5	5 days
25-3	GuSCN	MOPS	TCEP-O-HCL	THQ		3.6	4.5	6.3	5 days
27-5	GuSCN		TCEP-HCL			3.5	4	5.3	12 days
27-6	GuSCN		TCEP-O-HCL			3.5	4	5.3	12 days
26-8	GuSCN	MOPS	Vanillic acid			3.3	4	5.7	5 days
26-7	GuSCN	MOPS	p-coumeric acid			3.5	4.5	5.3	5 days
27-2	GuSCN	MOPS	HCl			3.5	5.5	4.9	5 days
28-9	SDS	MOPS	Vanillic acid			3.3	4	4.3	6 days
27-8	GuSCN		Vanillic acid			3.5	4	4.1	5 days
27-7	GuSCN		p-coumeric acid			3.5	4.5	3.3	5 days
28-2	GuSCN		Acetic acid			3.5	5.5	7.8	6 days
28-5	GuSCN		Tartaric acid			3.5	4.5	7	6 days
28-4	GuSCN		Citric acid			3.4	4.5	5.8	6 days
28-3	GuSCN		Phosphoric acid			3.6	5	4.9	6 days

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27-10	GuSCN		HCl		Sodium triphosphate	3.5	5	7.1	12 days
27-11	GuSCN		HCl		Sodium pyrophosphate	3.5	5	6.7	12 days
26-11	GuSCN		HCl		Sodium triphosphate	7.2	8	1.9	6 days
26-12	GuSCN		HCl		Sodium pyrophosphate	7.2	8	1.6	6 days
28-6	GuSCN		HCl		Sodium triphosphate	3.5	5	7	6 days
28-7	GuSCN		HCl		Sodium triphosphate	7.3	8.5	2.6	6 days

[0059] While only certain features of the invention have been illustrated and described herein, many modifications and changes will occur to those skilled in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the scope of the invention.

[0060] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0061] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A solid matrix, comprising:

at least one protein denaturant, and

at least one acid or acid-titrated buffer reagent impregnated therein in a dry state;

wherein the matrix is configured to provide a pH in a range of 2 to 7 upon hydration, extract nucleic acids from a sample, and preserve the nucleic acids in a dry state at ambient temperature.

2. The matrix of claim 1, wherein the extracted and preserved nucleic acids comprise ribonucleic acids (RNA), deoxy ribonucleic acids (DNA) or a combination thereof.

3. The matrix of either claim 1 or claim 2, wherein the acid comprises acetic acid, citric acid, tartaric acid, phosphoric acid, hydrochloric acid, Tris(2-carboxyethyl) phosphine- hydrochloric acid (TCEP-HCl), oxidized Tris(2-carboxyethyl) phosphine- hydrochloric acid (TCEP-O-HCl), sulfuric acid, nitric acid, vanillic acid, 3-(N-morpholino)propanesulfonic acid or combinations thereof.

4. The matrix of any one of claims 1 to 3, further comprising a UV protectant, a free-radical scavenger, a chelator or combinations thereof.

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5. The matrix of claim 4, wherein the UV protectant or free-radical scavenger is selected from the group consisting of hydroquinone monomethyl ether (MEHQ), hydroquinone (HQ), toluhydroquinone (THQ), and ascorbic acid.

6. The matrix of any one of claims 1 to 5, further comprising an RNase inhibitor.

7. The matrix of claim 6, wherein the RNase inhibitor comprises a triphosphate salt, pyrophosphate salt or combinations thereof.

8. The matrix of claim 6, wherein the RNase inhibitor comprises vanadyl ribonucleoside complex (VRC), sodium pyrophosphate, nucleotide analogues, or a commercially available RNase inhibitor.

9. The matrix of claim 6, wherein the RNase inhibitor comprises sodium triphosphate.

10. The matrix of any one of claims 1 to 10, further comprising at least one reducing agent.

11. The matrix of claim 10, wherein the reducing agent is selected from the group consisting of dithiothreitol (DTT), 2-mercaptoethanol (2-ME), tris(2-carboxyethyl)phosphine (TCEP), tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) and a combination thereof.

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12. The matrix of any one of claims 1 to 11, wherein the matrix comprises cellulose, cellulose acetate, nitrocellulose, glass fiber or any combination thereof.

13. The matrix of any one of claims 1 to 12, wherein the matrix is porous.

14. The matrix of any one of claims 1 to 13, wherein the protein denaturant is selected from a group consisting of guanidinium hydrochloride, guanidinium thiocyanate, sodium thiocyanate, potassium thiocyanate, arginine, sodium dodecyl sulfate (SDS), urea and combinations thereof.

15. An RNA extraction matrix comprising:

a protein denaturant comprising a chaotropic agent, a detergent or combinations thereof; and

an acid or acid-titrated buffer reagent impregnated therein in a dry state,

wherein the matrix is a porous non-dissolvable dry material configured to provide a pH in a range of 2 to 7 upon hydration for extracting RNA and stabilizing the extracted RNA with a RIN of at least 4.

16. The matrix of claim 15 further comprising a UV protectant or free-radical scavenger selected from the group consisting of MEHQ, HQ, THQ, ascorbic acid and combinations thereof.

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17. An RNA extraction matrix comprising:

a protein denaturant comprising a chaotropic agent, a detergent or combinations thereof;

an acid or acid-titrated buffer reagent; and

an RNase inhibitor comprising a triphosphate salt or pyrophosphate salt impregnated therein in a dry state, wherein the matrix comprises a porous non-dissolvable dry material configured to provide a pH in a range of 2 to 7 upon hydration and stabilize RNA with a RIN value of at least 4.

18. A method for extracting and storing nucleic acids from a sample, comprising:

providing the sample on a dry solid matrix comprising a protein denaturant and an acid or acid-titrated buffer reagent;

generating a pH in a range of 2 to 7 upon hydration for extraction of nucleic acids from the sample;

drying the matrix comprising the extracted nucleic acids; and

storing the extracted nucleic acids on the matrix in a dry state at ambient temperature.

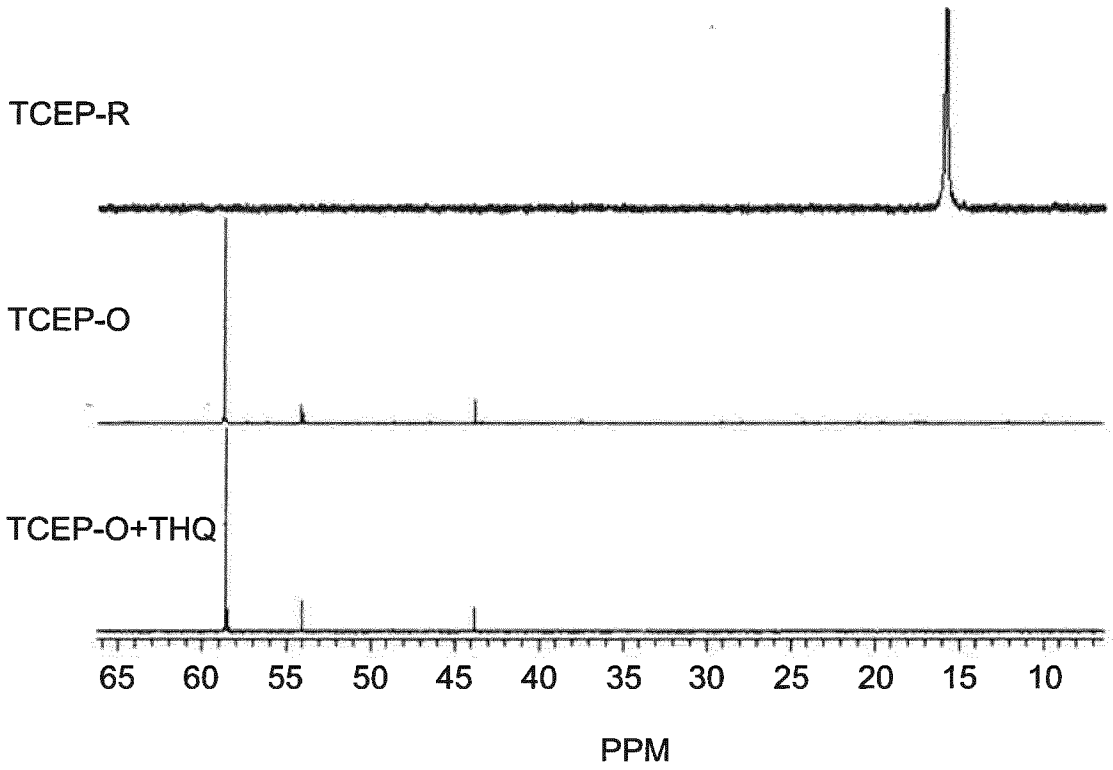

FIG. 1

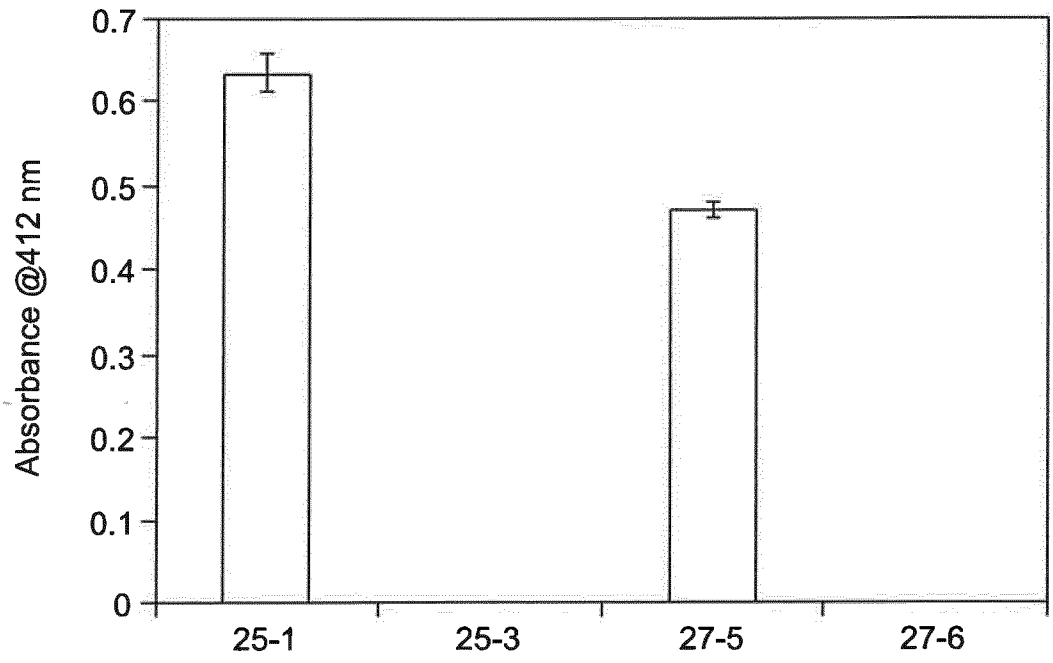

FIG. 2

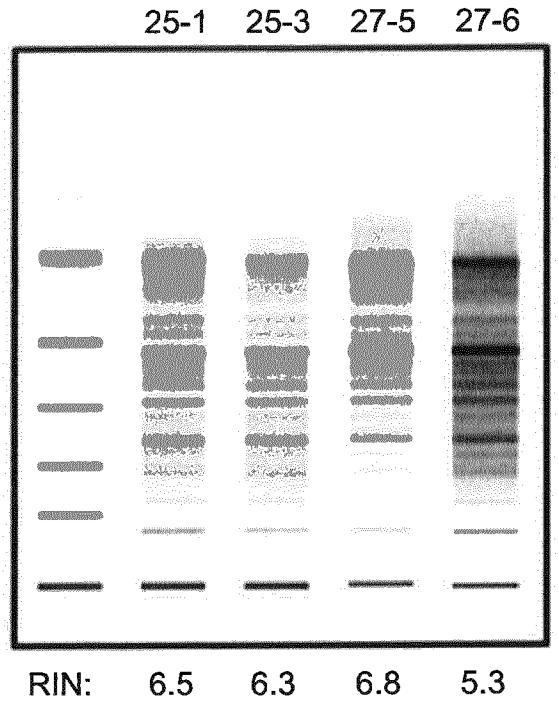

FIG. 3

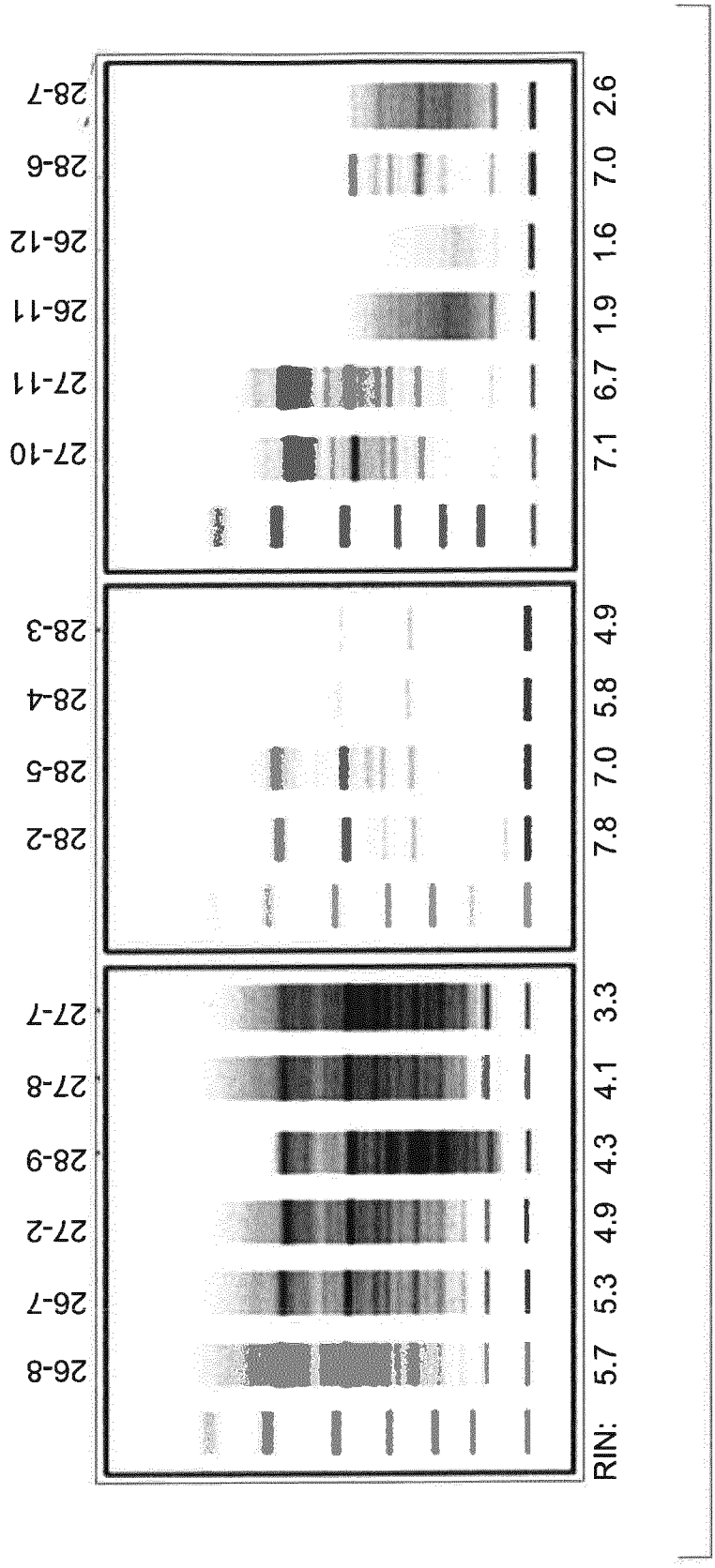
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FIG. 4