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(54) Title: BUFFER COMPOSITION FOR ONE STEP NUCLEIC ACID SAMPLE PREPARATION AND STORAGE FROM BIOLOGICAL SAMPLES

(57) Abstract: Buffer composition for one step nucleic acid sample preparation and storage from biological samples Disclosed are buffer compositions for extraction of nucleic acids from micro-volumes of various biological samples including from plants, animals and microbial cells, blood, semen and forensic samples at an ultra-rapid speed of 5 seconds or less, without need of any equipment in a single-step extraction method. The said buffers are comprised of optimized concentrations of buffering chemicals, cell lysis chemicals, stabilizing chemicals and protein denaturation chemicals and processes of preparation thereof. The nucleic acids thus extracted can directly be used for downstream processes and can be stored and transported without any further processing step. The shelf-life of the extracted nucleic acid samples are 7 to 14 days at 4oC and longer at -20oC. The invention provides for affordable and efficient diagnostic solutions and kits for point-of-care genetic diagnostics, other scientific, clinical and industrial usage.

Buffer composition for one step nucleic acid sample preparation and storage from biological samples

FIELD OF INVENTION

The present invention pertains to the field of buffers for extraction and storage of nucleic acids. More particularly, the invention relates to optimized functional single-buffer compositions capable of extraction of nucleic acid from micro-volumes of various biological samples at ultra-rapid speed, without need of any equipment. The nucleic acids thus extracted can be directly used for downstream processes, and can be stored for at least 7 to 14 days and transported without any further processing steps.

BACKGROUND OF THE INVENTION

Nucleic acids play central role in routine procedures in molecular biology, forensic analysis and diagnostics. Nucleic acid extraction and purification are important steps that determine efficiency and accuracy of nucleic acid based diagnostic tests. Each of the steps in nucleic acid extraction, further require several other processes, buffers and equipment, that needs to be skillfully operated in order to obtain good quality nucleic acids for further down-streaming processes.

Conventionally, nucleic acid extraction from biological samples is the first step in any molecular diagnostics. Basic procedure of nucleic acid extraction and purification involves the steps of cell lysis, precipitation and purification.

Several methods have been proposed so far for isolating nucleic acids. One of the biggest challenges in nucleic acid extraction is that biological samples like blood, sputum, saliva contains inhibitors that restrict direct amplification of nucleic acid present in them and hence it has to be purified for further analysis. Some research has shown that blood sample can be used directly in a PCR (Polymerase Chain Reaction) mixture with an additional preheating step or by adding PCR enhancing chemicals like gelatine, betaine, etc. It is also shown that the same can be performed with high fidelity polymerases like HotStart Polymerases or GoldTaq Polymerases.

The main disadvantages of these direct PCR techniques or buffers are that the nucleic acids present in the sample cannot be verified unless the amplification happens and that the nucleic acids samples cannot be stored for future purposes. In diagnostic setup, it is mandatory to confirm the presence of the nucleic acids before amplifying the desired region

of interest, secondly it is recommended to store the sample for future reference. These disadvantages remain unaddressed in the technical field of the art.

The most commonly used method for DNA isolation is silica column (spin column) based, followed by magnetic bead-based isolation. However, these methods have multiple processing steps and are time consuming, using laboratory equipment. Few rapid methods are known, with two or more steps, with or without equipment use.

Zou et al. (PMCID: PMC5697807), teaches an ultra-rapid DNA isolation using a cellulose-paper-based dipstick that can bind, wash, and elute purified nucleic acids in under 30 seconds without requiring any pipetting or electrical equipment. This method uses cellulose-paper-based dipstick for DNA isolation, requiring additionally use of lysis buffer, washing buffer and extraction buffer for the complete process. The over-all extraction process involves more than one step in its processing.

Patent publication CN104404030A, teaches a rapid extracting plant genome kit comprises different combinations of reagents and DNA adsorption column for separating and purifying DNA with different combination of silicon matrix, which is capable of DNA extraction within 15 minutes. This publication is limited to plant genome extraction, and uses toxic chemicals such as CTAB. This method involves several steps and is limited to plant genome, taking several minutes to carry out the DNA extraction process.

Different buffer based nucleic acid extraction methods are available but they have several drawbacks including low yield, compromised quality, cost, time consumption, laborious process, use of toxic organic solvents / enzymes, and many more. No technology is available till date to isolate nucleic acids in less than five (05) seconds with zero processing steps and without use of any minor or major equipment.

The present invention addresses the problems in the existing prior art of nucleic acid extraction technology. It provides for ultra-rapid nucleic acid extraction functional single-buffer compositions. The present invention is an optimized cocktail of reagents which extracts nucleic acids from micro volume of various biological plant and animal samples including but not limited to yeast, blood, cell culture, saliva, forensic samples, bacterial cell, cheek cells, sperm cells, plant leaves, root, seeds, pollen grains, insect tissues in less than five (05) seconds without need of any equipment and zero processing steps.

The present invention also has added advantage of storing and transporting the extracted nucleic acid samples at room temperature, 4°C for a period of 7 to 14 days and at

-20°C for long-term storage, without any additional processing. Extracted nucleic acid samples can be used directly for downstream applications like PCR and qPCR. The present invention is extremely useful in the development of point-of-care genetic diagnostics among other scientific, clinical and industrial usage.

OBJECT OF THE INVENTION

The main object of this invention is to provide functional single-buffer compositions for ultra-rapid and single-step nucleic acid extraction.

Another object of the invention is to provide a single-buffer technology, wherein an optimized cocktail of chemicals is used for the entire process of nucleic acids extraction, storage and transportation.

Yet another object of the invention is to provide a method of nucleic acid extraction in a single-step using the single-buffer technology.

Yet another object of the invention is to provide a nucleic acid extraction technology wherein the nucleic acid can be either RNA or DNA.

Yet another object of this invention is to provide a nucleic acid extraction technology wherein the extraction sample is plant based or animal based.

Yet another object of this invention is to provide a nucleic acid extraction technology wherein the extraction samples are yeast, blood, cell culture, saliva, forensic samples, bacterial cell, cheek cells, sperm cells, plant leaves, root, seeds, pollen grains, insect tissues.

Yet another object of the invention is to provide a technology wherein the nucleic acids extraction is possible from a micro-volume of sample.

Yet another object of the invention is to provide an ultra-rapid or instantaneous method of nucleic acid extraction.

Yet another object of the invention is to provide the single-step nucleic acid extraction buffer compositions that does not require additional processing for storing and transportation of the extracted nucleic acid samples.

Yet another object of the invention is to provide functional single-buffer compositions having a shelf life of up to 6 months at room temperature, and more than 12 months at 4°C and -20 °C.

Yet another object of the invention is to provide functional single-buffer compositions that does not require further processing steps, wherein the extracted nucleic acid samples can be directly used for down-stream processing.

Yet another object of the invention is to provide nucleic acid samples, extracted with the functional single-buffer compositions that have a shelf life of 7 to 14 days at 4°C and long-term storage at -20°C.

Yet another object of the invention is to eliminate the use of laboratory infrastructure and equipment for nucleic acid extraction.

Yet another object of the invention is to provide ultra-rapid, single-step nucleic acid extraction method with a single buffer technology using functional single-buffer compositions for point-of-care genetic diagnostics.

Yet another object of the invention is to provide affordable and efficient diagnostic kits for the point-of-care genetic diagnostics.

Yet another object of the invention is to provide ultra-rapid, single-step nucleic acid extraction method with a single buffer technology using functional single-buffer compositions for scientific, clinical and industrial usage.

SUMMARY OF THE INVENTION

The present invention pertains to the field of buffers for extraction and storage of nucleic acids using micro-volumes of various biological samples at ultra-rapid speed, without need of any equipment in a single-step process. The compositions disclosed herein comprises Tris Hydrochloride, Magnesium chloride hexahydrate, Potassium chloride, Sodium chloride, Ammonium chloride, Sodium bicarbonate, Sodium dodecyl sulphate, and water. The nucleic acids thus extracted can be directly used for downstream processes, and can be stored and transported without any further processing steps. The extracted nucleic acid samples can be stored at 4°C for 7 to 14 days and -20°C for long-term storage.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Gel bands of PCR amplified DNA extracted through Buffer A to Buffer G

Figure 2A: Gel bands of PCR amplified DNA extracted through Buffer H, and Buffer I

Figure 2B: Gel bands of PCR amplified DNA extracted through Buffer J and Buffer K

Figure 2C: Gel bands of PCR amplified DNA extracted through Buffer L and Buffer M

Figure 2D: Gel bands of PCR amplified DNA extracted through Buffer N and Buffer O

Figure 2E: Gel bands of PCR amplified DNA extracted through Buffer P

Figure 3A: Gel bands for optimized sample volume

Figure 3B: Gel bands of PCR products amplified from DNA extracted with optimized Buffer 1 at 200 µL buffer and 5µL blood ratio

Figure 3C: Gel bands of PCR products amplified from DNA extracted with different optimized buffer compositions, Buffer 1, Buffer 2, Buffer 3 and Buffer 4 at 200 µL buffer and 5µL blood ratio

Figure 3D: Gel bands of PCR products amplified from DNA extracted with different optimized buffer compositions, Buffer 1, Buffer 2, Buffer 3 and Buffer 4 at 300 µL buffer and 5µL blood ratio

Figure 4: Gel bands of optimized template volume of buffer extracted sample

Figure 5A: Gel bands of genomic DNA extracted from animal cells, yeast cells and bacterial plasmid

Figure 5B: Gel bands of genomic DNA and RNA extracted from insect tissues

Figure 5C: Gel bands of genomic DNA and RNA extracted from plant seeds

Figure 5D: Gel bands of genomic DNA extracted from plant roots, pollen and leaves

Figure 5E: Gel bands of genomic DNA extracted from plant leaves with different optimized buffer compositions

Figure 5F: Gel bands of genomic DNA extracted from insect tissue with different optimized buffer compositions

Figure 6: Gel bands of comparative DNA yield obtained from manual method and functional single-buffer compositions

Figure 7A: Gel bands of DNA extracted with Buffer 1 and the corresponding PCR amplified product validated by CIDRF (Central Inter-Disciplinary Research Facility, Puducherry)

Figure 7B: Gel bands of DNA extracted with Buffer 1 and the corresponding PCR amplified product validated by School of Lifesciences, Manipal University, Manipal

Figure 7C: Gel bands of DNA extracted with Buffer 1 and the corresponding PCR amplified product validated by Elango Genetics, Chennai

Figure 8A: Gel bands of PCR amplified DNA extracted from buffers stored for one month at different temperature

Figure 8B: Gel bands of PCR amplified DNA extracted from buffers stored for three months at different temperature

Figure 8C: Gel bands of PCR amplified DNA extracted from buffers stored for four months at different temperature

Figure 8D: Gel bands of PCR amplified DNA extracted from buffers stored for six months at different temperature

Figure 8E: Gel bands of PCR from buffer extracted DNA stored at 4°C at day 2, day 4, day 7

Figure 8F: Gel bands of PCR from buffer extracted DNA stored at day 10, day 12, day 14 and day 16

DEFINITIONS

Embodiments described herein can be understood more readily by reference to the following detailed description, examples, and drawings. Elements, apparatus and methods described herein are merely illustrative of the principles of the present invention and are not limited to the specific embodiments presented in the detailed description, examples, and drawings. Numerous modifications and adaptations will be readily apparent to those of skill in the art without departing from the spirit and scope of the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods belong. Although any compositions or methods similar or equivalent to those described herein can also be used in the practice or testing of the embodiments of the present invention, representative illustrative methods and compositions are now described.

Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within by the methods and compositions. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within by the methods and compositions, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both

of the limits, ranges excluding either or both of those included limits are also included in the methods and compositions.

It is appreciated that certain features of the methods, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the methods and compositions, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other embodiments without departing from the scope or spirit of the present methods. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

The term “functional single-buffer compositions” or “buffer composition” mean a cocktail of buffers and chemicals comprising of buffering chemicals, cell lysis chemicals, stabilizing chemicals and protein denaturation chemicals, and particularly referred herein as Buffer 1, Buffer 2, Buffer 3 and/or Buffer 4, and its other optimized variants.

The term “DBS” mean dried blood sample.

The term “Gel bands” means gel-electrophoresis bands.

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses functional single-buffer compositions for extraction and storage of nucleic acids. More particularly, the invention relates to optimized single-buffer compositions capable of extraction of nucleic acid from micro-volumes of various biological samples at ultra-rapid speed in a single-step method, without need of any equipment. The nucleic acids thus extracted can be directly used for downstream processes, and can be stored and transported without any further processing steps.

The single-buffer technology using functional single-buffer compositions of the present invention is characterized by the following advantages:

a. The ultra-rapid nucleic acid extraction method reduces the turn-around time for the process to less than five (05) seconds, which is an instantaneous method.
b. Low amount of a sample is required for this method, thus provides ease of sample handling and transportation.
c. A single cocktail of optimized buffers/chemicals is used, and therefore it reduces the cost of reagents being used for the extraction process.
d. No further processing is required, and therefore the nucleic acids so obtained by the method can be directly used for downstream processes.
e. The storage and transportation of the nucleic acids so obtained by this method, does not require any further processing and can be stored and transported as it is.
f. This method eliminates the requirement of laboratory infrastructure and equipment for the nucleic acid extraction.
g. The present invention is extremely useful for preparing affordable and efficient diagnostic kits for the point-of-care genetic diagnostics. The present invention will reduce the problems of skilled manpower for nucleic acid sampling/collection, reduce the cost of sample extraction, preparation, storage and transportation. The present invention will be extremely useful for large scale screening and sampling and/or point-of-care genetic diagnostics.

Before the buffer compositions, processes and methods of the present disclosure are described in detail, it is to be understood that the invention is not limited to particular embodiments and may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

The buffer cocktail contains chemicals that lyse the cells, degrade the proteins present and protect the nucleic acids. A small quantity of biological sample has to be added with the cocktail buffers and mixed properly for 5 seconds. This lysed mixture can be used as the starting sample for any molecular biology research or diagnostics. This mixture can be stored at room temperature, 4°C or -20 °C for any future reference or studies.

The invention provides for the said functional single-buffer compositions comprising of buffering chemicals, cell lysis chemicals, stabilizing chemicals and protein denaturation chemicals.

The compositions comprise of Tris Hydrochloride, Magnesium chloride hexahydrate, Potassium chloride, Sodium chloride, Ammonium chloride, Sodium bicarbonate, Sodium dodecyl sulphate, and water.

In one embodiment, the invention provides for the said functional single-buffer compositions comprising of buffering chemicals, cell lysis chemicals, stabilizing chemicals and protein denaturation chemicals in water solution.

In another embodiment, the invention provides for functional single-buffer compositions comprising Tris Hydrochloride and Potassium chloride as buffering agents.

In another embodiment, the invention provides for functional single-buffer compositions comprising Sodium dodecyl sulphate as cell lysis agents.

In another embodiment, the invention provides for functional single-buffer compositions comprising Ammonium chloride, Magnesium chloride hexahydrate and Sodium bicarbonate as stabilizing agents.

In another embodiment, the invention provides for functional single-buffer compositions comprising Sodium chloride as protein denaturation agents.

In one embodiment, the invention provides for functional single-buffer compositions comprising Tris Hydrochloride at pH 7.0 to 9.0 in concentration range of 10 mM to 50 mM or in concentration range of 10 mM to 30 mM, preferably at 20 mM.

In another embodiment, the invention provides for functional single-buffer compositions comprising Magnesium chloride hexahydrate in the concentration range of 0 mM to 5 mM or in concentration of 1 mM to 3 mM, preferably at 0 mM and 2.5 mM.

In another embodiment, the invention provides for functional single-buffer compositions comprising Potassium chloride in the concentration range of 10 mM to 100 mM or in concentration range of 15 mM to 40 mM, preferably at 25 mM.

In certain embodiment, the invention provides for functional single-buffer compositions comprising Sodium dodecyl sulphate in the concentration range of 0.01% to 0.1% w/v in the composition, preferably at 0.05% w/v and at 0.02% w/v.

In a further embodiment, the invention provides for functional single-buffer compositions comprising Sodium chloride in the concentration range of 50 mM to 250 mM, preferably at 200 mM and 100 mM.

In certain embodiments, the invention provides for functional single-buffer compositions comprising Ammonium chloride in the concentration range of 2 mM to 20 mM or in concentration range of 2 mM to 10 mM, preferably at 5 mM.

In certain embodiments, the invention provides for functional single-buffer compositions comprising Sodium bicarbonate in the concentration of 1 mM to 5 mM or in concentration range of 1.5 mM to 3.5 mM, preferably at 2.5 mM.

In certain embodiment, the invention provides for functional single-buffer compositions dissolved in nuclease-free water, wherein the water is undistilled, distilled, double distilled, high pure, ultra-high pure or ultra-filtered.

In certain embodiment, the invention provides for functional single-buffer compositions dissolved in nuclease-free water, wherein the water is sterilized by autoclave or UV rays exposure.

In certain embodiment, the invention provides for functional single-buffer compositions dissolved in nuclease free water, wherein the water is either sterilized or unsterilized.

In one embodiment, the invention provides for a method of preparing the functional single-buffer compositions, comprising the following steps:

a. preparation of stock solutions of all chemicals in respective concentration ranges: 2 M Tris Hydrochloride, 1 M Magnesium chloride, 1 M Potassium chloride, 5 M Sodium chloride, 1 M Ammonium chloride, 0.5 M Sodium bicarbonate and 10% w/v Sodium dodecyl sulphate.
b. addition of chemicals in the aforementioned concentration and the final required volume is adjusted with water.
c. The cloudy precipitate, if any, can be dissolved by the heating the composition at 37°C for 10 minutes, wherein the final buffer compositions are maintained between pH 7.0 to 9.0, preferably between pH 7.5 to 8.5.

In one embodiment, liquid or solid biological sample is added to required volume of buffer and mixed for complete lysis of cells and isolation of the nucleic acids.

In another embodiment, the liquid sample is mixed with the functional single-buffer compositions by pipetting or tapping.

In yet another embodiment, the solid tissues samples mixed with the functional single-buffer compositions require complete or mild homogenization.

In one embodiment, the functional single-buffer compositions have a shelf life of up to 6 months at room temperature, and more than 6 months at 4°C and -20 °C.

In another embodiment, the functional single-buffer compositions do not require further processing steps such that the extracted nucleic acid samples can be directly used for down-stream processing.

In yet another embodiment, the nucleic acid samples, extracted with the functional single-buffer compositions, have a shelf life of up to 16 days at 4°C and long-term storage at -20°C.

The functional single-buffer compositions comprise of buffering chemicals, which maintains the pH of the solution during the nucleic acid extraction. The cell lysis chemicals break the cell wall and cell membranes of the cell and releases the nucleic acids, cell organelles and proteins. The stabilizing chemicals stabilizes the nucleic acid in the solution. The protein denaturation chemicals denature the proteins present in the solution.

These buffers and chemicals are added together in an optimized concentration to make a cocktail composition that is finally diluted with nuclease-free water to obtain the functional buffer composition for nucleic acid sample extraction, preparation and storage.

The compositions comprising buffers and chemicals of the present invention may further comprise a pharmaceutically acceptable carrier or excipient. The carriers include, but are not limited to sterile aqueous media, solid diluents or fillers, excipients, and various non-toxic organic solvents.

All the biological materials used herein this invention were solely for the purposes of testing the effectivity and efficacy of the functional buffer compositions. None of the biological material is part of any of the compositions or the invention, and is merely used as a source of nucleic acids to demonstrate the effectiveness of the functional single-buffer compositions.

The blood samples, cheek cells and sperm cells were obtained from the first named inventor of this present invention. Plant leaves, roots and pollen were obtained from plants in and around the School of Life Sciences at University of Hyderabad. Insect tissues were obtained from dead honeybees and ants in and around the School of Life Sciences at University of Hyderabad. The cultured cells, which were used-up, completely matured and waste to be discarded, were obtained from Oncoseek Private Limited, Hyderabad. Watermelon seeds and pulses were purchased from local market/store. Yeast and bacterial cells were obtained from a startup from Albus Eco Projects incubated at BioNEST.

EXAMPLES

Before the functional single-buffer compositions, processes and methods of the present disclosure are described in greater detail, it is to be understood that the invention is not limited to particular embodiments and may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

EXAMPLE 1: Optimization of chemical constituents and concentration of the buffer

The functional single-buffer compositions were prepared after optimization of various chemical constituents that are generally present in an isolation/extraction buffer for nucleic acid extraction. Table 1 elucidates a complete extraction buffer, used as control, selected from a range of concentrations of various constituents, for optimal nucleic acid extraction. The complete extraction buffer, so prepared, was then was tested for its significance on the nucleic acid extraction with and without one or more constituent.

The complete extraction-buffer was prepared in different compositions used as control, and other buffers were prepared by keeping all the constituents concentration constant and by modifying concentrations and/or removing one constituent at a time. The new buffer compositions were then compared for its nucleic acid extraction effectiveness against that of the complete extraction-buffer, which is presented in Table 2.

Table 1 details the complete-buffer composition with respective concentration which was used for the optimization process.

Complete extraction-buffer (CEB)	Concentration	Concentration range
Tris Hydrochloride (pH 8.0)	20 mM	10 mM to 50 mM
Magnesium chloride hexahydrate	2.5 mM	1 mM to 5 mM

Potassium chloride	25 mM	10 mM to 100 mM
Sodium chloride	100 mM	50 mM to 250 mM
Ammonium chloride	5 mM	1 mM to 20 mM
Sodium bicarbonate	2.5 mM	0.5 mM to 5 mM
Sodium dodecyl sulphate	0.02% w/v	0.005 to 0.05% w/v
EDTA	0.4 mM	0.1 mM to 1.0 mM
Triton X100	0.6% w/v	0.01% to 1.0% w/v
Water	Based on final volume required	Based on final volume required

Table 1: Optimization of complete extraction-buffer composition

Buffer A was prepared by removing EDTA from the complete extraction-buffer and then this Buffer A was used to extract DNA from blood sample, followed by PCR analysis. Buffer B was prepared from complete extraction-buffer without Triton X100 and used to extract DNA from blood sample, followed by PCR analysis. Similarly, other iterations of the new buffers, i.e. Buffer A to Buffer G, from complete extraction-buffer with modification and/or without individual components were also prepared and checked for DNA extraction efficiency. Table 2 below enlists such various new buffer compositions prepared from the complete extraction-buffer and their corresponding effect on DNA extraction. Thereafter, PCR amplification was carried out and the gel-electrophoresis results corresponding to Table 2 are depicted in Figure 1.

In the present invention, quantitative and qualitative analysis of the extracted nucleic acid is done through agarose gel electrophoresis, in comparison with nucleic acid purified with other methods. In the present invention, absorbance ratio method was not used since it cannot determine the quality and quantity of the extracted nucleic acid, since the solution is a crude extract containing other cell components.

Name	**New buffer composition**	**Concentration**	**Effect on DNA extraction / PCR amplification**
Buffer A	CEB without Tris	No change	Present
Buffer B	CEB without Potassium chloride	No change	Present
Buffer C	CEB without Sodium bicarbonate	No change	Present

Buffer D	CEB without Ammonium chloride	No change	Present
Buffer E	CEB without EDTA	No change	Insignificant
Buffer F	CEB without Magnesium chloride hexahydrate	No change	Present
Buffer G	CEB without Triton X100	No change	Insignificant

Table 2: Effect of buffer constituents

Based on the results, further optimization of the new buffer compositions was done and are referred to here as Buffer H to Buffer Q, as represented in Table 3. These buffers were tested in absence of individual constituent, without any change in concentration of those individual constituent, in half the concentration (0.5x) and in double the concentration (2x). The effects of concentration variations in the resultant buffer were identified. Thereafter, PCR amplification was carried out and the results are depicted in Figures 2A to 2E.

Name	**New buffer composition with variable concentration constituent**	**Concentration**	**Effect on DNA extraction / PCR amplification**
Buffer H	CEB with variable Sodium bicarbonate concentration	No Sodium bicarbonate	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant
Buffer I	CEB with variable Triton X-100 concentration	No Triton X-100	Present
		No change	Insignificant
		0.5x	Insignificant
		2x	Insignificant
Buffer J	CEB with variable magnesium chloride hexahydrate concentration	No magnesium chloride hexahydrate	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant

Buffer K	CEB with variable ammonium chloride concentration	No ammonium chloride	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant
Buffer L	CEB with variable sodium dodecyl sulphate concentration	No sodium dodecyl sulphate	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant
Buffer M	CEB with variable potassium chloride concentration	No potassium chloride	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant
Buffer N	CEB with variable Tris Hydrochloride concentration	No Tris Hydrochloride	Insignificant
		No change	Insignificant
		2x	Present
		3x	Insignificant
Buffer O	CEB with variable Sodium chloride concentration	No Sodium chloride	Insignificant
		No change	Insignificant
		0.5x	Present
		2x	Insignificant

Table 3: Effect of concentration variations of buffer constituents

Figure 2A shows the gel electrophoresis results of PCR amplified DNA extracted through Buffer H, Buffer I. The insignificance role of Triton X-100 was confirmed and Sodium bicarbonate concentration was optimized.

Figure 2B shows the gel electrophoresis results of PCR amplified DNA extracted through Buffer J and Buffer K. Lower concentration of Magnesium chloride was found to be efficient and ammonium chloride concentration is optimized.

Figure 2C shows the gel electrophoresis results of PCR amplified DNA extracted through Buffer L and Buffer M. Sodium dodecyl sulphate was found to highly significant for the buffer composition.

Figure 2D shows the gel electrophoresis results of PCR amplified DNA extracted through Buffer N and Buffer O. Sodium chloride was found to be important for the buffer composition.

Figure 2E shows the gel electrophoresis results of PCR amplified DNA extracted through Buffer P. The role of EDTA and Triton X-100 were found to be insignificant. Role of other constituents and their concentrations were measured and are reported in Table 4.

Name	**New buffer composition with variable concentration constituent**	**Constituents with variable concentration**	**Effect on DNA extraction / PCR amplification**
Buffer P	CEB without EDTA and without Triton X-100, with other constituents in variable concentration	No SDS	Insignificant
		No Sodium chloride	Insignificant
		No Potassium chloride	Insignificant
		No EDTA	Insignificant
		2x Magnesium chloride hexahydrate	Insignificant
		2x all constituents of CEB	Insignificant
		0.5x all constituents of CEB	Present

Table 4: Effect of concentration variations of buffer constituents in absence of EDTA and Triton X-100

The buffer compositions were checked without EDTA and Trito X-100 and by removing other selected ingredients like SDS, NaCl, KCl and $MgCl_2$, to confirm their significance in

the absence of EDTA and Triton X-100. The concentration of all ingredients were checked at 2 times and 0.5 times the earlier concentration. The 0.5 times concentrations of all ingredients were found to be good (without EDTA and Triton X-100), which was finally optimized.

Based on the optimizations carried out in Example 1 and Example 2, funtional single-buffer compositions were prepared which is represented in Table 5, Table 6 Table 7 and Table 8.

EXAMPLE 2: Preparation of various functional single-buffer compositions

The process of selection involved various constituents, and thereafter the selected sets of the buffer constituents were further optimized to a particular sub-set of concentration range. Further, the composition constituents so selected were optimized from its sub-set concentration range to a particular set of concentration. Four buffers, namely Buffer 1, Buffer 2, Buffer 3 and Buffer 4 were found to be the most effective in the nucleic acid extraction from various sets of samples. These four functional single-buffer compositions are elaborated in Table 5, Table 6 Table 7 and Table 8.

The water used for preparation of all the functional single-buffer compositions can be nuclease-free water, wherein the water is undistilled, distilled, double distilled, high pure, ultra-high pure or ultra-filtered, unsterilized or sterilized by using autoclave or sterilized by using UV rays.

Table 5 provides the sub-set of concentration range of the individual concentration of the Buffer 1, along with the particular concentration of the individual constituents in which the Buffer 1 was prepared and tested for nucleic acid extraction.

Buffer 1	**Concentration**	**Concentration range**
Tris Hydrochloride (pH 8.0)	20 mM	10 mM to 50 mM
Magnesium chloride hexahydrate	2.5 mM	1 mM to 5 mM
Potassium chloride	25 mM	10 mM to 100 mM
Sodium chloride	100 mM	50 mM to 250 mM
Ammonium chloride	5 mM	2 mM to 20 mM
Sodium bicarbonate	2.5 mM	1 mM to 5 mM
Sodium dodecyl sulphate	0.02% w/v	0.01 to 0.05% w/v
Water	As per required volume	As per required volume

Table 5: Composition of Buffer 1

Table 6 provides the sub-set of concentration range of the individual concentration of the Buffer 2, along with the particular concentration of the individual constituents in which the Buffer 2 was prepared and tested for nucleic acid extraction. In the Buffer 2, all the constituents of Buffer 1 were maintained as it is, except that Sodium chloride was used in the concentration range of 50 mM to 250 mM and preferably at 200 mM concentration.

Buffer 2	**Concentration**	**Conc. range**
Tris Hydrochloride (pH 8.0)	20 mM	10 mM to 50 mM
Magnesium chloride hexahydrate	2.5 mM	1 mM to 5 mM
Potassium chloride	25 mM	10 mM to 100 mM
Sodium chloride	200 mM	50 mM to 250 mM
Ammonium chloride	5 mM	2 mM to 20 mM
Sodium bicarbonate	2.5 mM	1 mM to 5 mM
Sodium dodecyl sulphate	0.02% w/v	0.01 to 0.05% w/v
Double distilled water	As per required volume	As per required volume

Table 6: Composition of Buffer 2

Table 7 provides the sub-set of concentration range of the individual concentration of the Buffer 3, along with the particular concentration of the individual constituents in which the Buffer 3 was prepared and tested for nucleic acid extraction. In the Buffer 3, all the constituents of Buffer 1 were maintained as it is, except that Sodium dodecyl sulphate was used in the concentration range of 0.01% to 0.1% w/v, and preferably at 0.05% concentration.

Buffer 3	**Concentration**	**Conc. range**
Tris Hydrochloride (pH 8.0)	20 mM	10 mM to 50 mM
Magnesium chloride hexahydrate	2.5 mM	1 mM to 5 mM
Potassium chloride	25 mM	10 mM to 100 mM
Sodium chloride	100 mM	50 mM to 250 mM
Ammonium chloride	5 mM	2 mM to 20 mM
Sodium bicarbonate	2.5 mM	1 mM to 5 mM
Sodium dodecyl sulphate	0.05%	0.01 to 0.1% w/v
Double distilled water	As per required volume	As per required volume

Table 7: Composition of Buffer 3

Table 8 provides the sub-set of concentration range of the individual concentration of the Buffer 4, along with the particular concentration of the individual constituents in which the Buffer 4 was prepared and tested for nucleic acid extraction. In the Buffer 4, all the constituents of Buffer 1 were maintained as it is, except that Magnesium chloride hexahydrate was used in the concentration range of 0mM to 5 mM, and preferably at 0 mM concentration.

Buffer 4	**Concentration**	**Conc. range**
Tris Hydrochloride (pH 8.0)	20 mM	10 mM to 50 mM
Magnesium chloride hexahydrate	0 mM	0 mM to 5 mM
Potassium chloride	25 mM	10 mM to 100 mM
Sodium chloride	100 mM	50 mM to 250 mM
Ammonium chloride	5 mM	2 mM to 20 mM
Sodium bicarbonate	2.5 mM	1 mM to 5 mM
Sodium dodecyl sulphate	0.02% w/v	0.01 to 0.05% w/v
Double distilled water	As per required volume	As per required volume

Table 8: Composition of Buffer 4

EXAMPLE 3: Optimization of sample/buffer ratio

The sample/buffer ratio was checked with the optimized i.e. Buffer 1, Buffer 2, Buffer 3 and Buffer 4. Commercial kits and manual methods use a minimum of 50 µL of blood sample for DNA extraction. The functional single-buffer compositions of the present invention are optimized to reduces the sample volume requirement to 1 to 10 µL, and most preferably 5 µL for 200 µL of the functional single-buffer compositions. Figure 3A elucidates the optimized sample volume.

The optimized functional single-buffer compositions were tested in concentrations of 200 µL, 300 µL, 400 µL and 500 µL. Functional single-buffer compositions of higher volumes were not used, as the higher volumes were diluting the salt concentration present in the buffers, making it unfit for the intended purpose. Figure 3B elucidates gel-electrophoresis bands of PCR products amplified from DNA extracted with optimized Buffer 1 at 200 µL buffer and 5µL blood ratio.

Figure 3C elucidates gel-electrophoresis bands of PCR products amplified from DNA extracted with different optimized buffer compositions, Buffer 1, Buffer 2, Buffer 3 and Buffer 4 at 200 µL buffer and 5µL blood ratio.

Figure 3D elucidates gel-electrophoresis bands of PCR products amplified from DNA extracted with different optimized buffer compositions, Buffer 1, Buffer 2, Buffer 3 and Buffer 4 at 300 µL buffer and 5µL blood ratio.

It was found that 200 µL of the functional single-buffer compositions were best suited for 5 µL of sample (based on blood sample) for nucleic acid extractions.

EXAMPLE 4: DNA quantification by Qubit assay

Qubit assay utilizes target-selective dyes that emit fluorescence when bound to DNA, RNA or protein, unlike UV absorbance which can overestimate sample concentration due to contaminants in the sample. Fluorescence measurements are much more sensitive than UV absorbance, giving accurate measurements with significantly less noise.

Two standards were used, Standard 1 at 98.15 concentration and Standard 2 at 342.49 ng/µL. 5 µL blood samples were mixed in 200 µL Buffer 1 composition and Qubit assay was carried out, results of which are presented in Table 9.

Samples	Concentration (ng/µL)
1	1.21
2	1.59
3	0.868
4	0.522
5	0.382
6	0.781
7	1.62
8	0.717
9	1.88
10	Too low
11	0.717
12	0.396

Table 9: Qubit Assay Results

Average concentration was found to be 0.972 ng/µL (972 pg/µL), which is about 97 times higher than minimum required DNA concentration of 0.01 ng/µL (10 pg/µL) for performing PCR reactions.

EXAMPLE 5: Optimization of template volume for downstream PCR applications

The template volume of buffer extracted sample was optimized and it was found that the optimal volume of prepared sample for PCR applications is in the volume range of 1 to 5 µL, and more particularly in the volume range of 2 to 3 µL. Figure 4 depicts the results corresponding to optimized template volume of buffer extracted sample for PCR applications/downstream processes.

EXAMPLE 6: Method of nucleic acid (DNA/RNA) isolation from various biological samples

Different biological samples like dried blood samples (dried in floor, cloth, tissue paper, cotton), cheek cells, sperm cells, culture mammalian cells, old lysed blood, yeast cells, bacterial plasmids, plant leaves, root, seeds, pollen grains, insect tissues were collected from various sources.

All the liquid samples can be used as it is, whereas the solid samples have to be pre-homogenized, either mildly or completely. Blood sample of 1 to 10µL is collected by finger prick and the sample was directly added to buffer. For frozen samples, the blood was stored at -20 °C for more than 6 months. Fresh blood with / without anticoagulant was used.

For DBS tissue samples, a few drops of blood sample were dropped on commercial tissue paper and dried at room temperature. For DBS cotton samples, a few drops of blood sample were dropped on cotton and dried at room temperature. For DBS cloth samples, few drops of blood sample were dropped on a piece of cloth and dried at room temperature. For DBS floor samples, a few drops of blood sample were dropped on laboratory floor and dried at room temperature.

The functional single-buffer compositions were prepared by using a method comprising the following steps:

a. preparation of stock solutions of all chemicals in respective concentration ranges of 2 M Tris Hydrochloride, 1 M Magnesium chloride, 1 M Potassium chloride, 5 M Sodium chloride, 1 M Ammonium chloride, 0.5 M Sodium bicarbonate and 10% w/v Sodium dodecyl sulphate,
b. addition of chemicals in the aforementioned concentration and the final required volume of 200 µL of the functional single-buffer compositions are prepared by diluting with water,

c. The cloudy precipitate, if any, can be dissolved by the heating the composition at 37°C for 10 minutes, wherein the final buffer compositions are maintained at pH 7.0 to 9.0, preferably at pH 7.5 to 8.5, more preferably at pH 8.0.

Liquid or solid biological sample is added to 200 µL of the functional single-buffer compositions (Buffer 1, Buffer 2, Buffer 3 or Buffer 4) and mixed for complete lysis of cells and isolation of the nucleic acids. The liquid sample is mixed with the functional single-buffer compositions by pipetting or tapping. The solid tissues samples mixed with the functional single-buffer compositions require complete or mild homogenization.

The liquid samples were directly mixed with the optimized buffer compositions, while solid tissue samples were homogenized with pestle and added to the buffers. The samples were gently mixed. After lysis, the buffer extracted samples were directly loaded in 0.8% agarose gel and visualized under UV light.

Figure 5A shows the gel electrophoresis results of DNA extracted through Buffer 1 using various samples, namely DBS tissue, DBS cotton, DBS cloth, DBS floor, cheek cells, culture mammalian cells, sperm cells, old lysed blood, yeast cells and bacterial cells.

Figure 5B shows the gel electrophoresis results of genomic DNA, RNA and degraded RNA extracted through Buffer 1using various samples, namely insect tissues.

Figure 5C shows the gel electrophoresis results of genomic DNA and degraded RNA extracted through Buffer 1using seeds.

Figure 5D shows the gel electrophoresis results of genomic DNA extracted through Buffer 1using various samples, namely plant roots, pollens and leaves.

Figure 5E shows the gel-electrophoresis bands of genomic DNA extracted from plant leaves with different optimized buffer compositions.

Figure 5F shows the gel-electrophoresis bands of genomic DNA extracted from insect tissue with different optimized buffer compositions.

All the results, as elucidated in Figures 5A to 5D, confirm that the yield of extracted nucleic acids (DNA/RNA) were good. The gel-electrophoresis bands were bright and clear, indicating that the functional single-buffer compositions of the present invention were able to yield nucleic acids (DNA/RNA) in good quantity and quality from all biological samples, whether it be a plant sample, an animal sample or a microbial sample.

EXAMPLE 7: Comparison of DNA yield extracted with -buffer compositions and commercial kit/manual method

Commercial kits and manual methods use a minimum of 50 µL blood, however, the buffer compositions of the present invention extract nucleic acid (DNA/RNA) from 1 µL to 10 µL blood. To compare and establish the enhanced efficacy of Buffer 1, the DNA extracted with manual method was diluted equalizing the final volume of blood sample and then compared with the corresponding results of the present invention.

The undiluted DNA from manual method was 450.2 ng/µL and was diluted 300 times to match with the buffer extracted DNA. To calculate the equalization factor, the concentration multiple of manual method has to be multiplied with that of the buffer. The concentration multiple of the manual method is 7.5 (=300/40), as 300 µL blood gave 40 µL of final concentrated DNA. The concentration multiple of the buffer is 40 (=200/5), as 200 µL of the buffer is mixed in 5 µL blood sample. Hence, the Equalization Factor comes to 300 times (40 x 7.5).

Manual method of DNA extraction (alkaline lysis method) uses 300 µL blood sample and DNA eluted to final concentrated volume of 40 µL, while the buffer use only 5 µL blood and mixed with 200 µL of buffer resulting in further dilution. Hence, the final extracted DNA using manual method was diluted to equalize the initial blood volume and then loaded in 0.8% agarose gel. The yield of DNA (diluted) from manual method was lesser compared to DNA extracted using the buffer.

Figure 6 elucidates the comparative DNA yields wherein the gel-electrophoresis band obtained from the buffer are much brighter than those obtained from manual method, establishing the superiority of the buffer of present invention.

EXAMPLE 8: Third party validation of the buffer compositions

To further validate the functional efficacy and effectiveness of the buffer compositions of the present invention, the inventors got third party validations done through three separate independent organizations of repute, including both private and government recognized public organizations.

Figure 7A depicts the gel-electrophoresis bands of the DNA extracted from Buffer 1 and the corresponding PCR amplified products, which was validated by Central Inter-Disciplinary Research Facility, Puducherry (CIDRF), which is a scientific and industrial

research organization (SIRO) as recognized by the Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India. PCR was performed to amplify product of 692bp size. In the Figure 7A, L indicates DNA ladder to identify the product size, E is empty lane, F is blood sample collected through finger prick, V is blood collected from veins, 3F & 2V are PCR products amplified from buffer extracted DNA, C is PCR product amplified from DNA extracted through commercial kit, 3V & 2F are buffer extracted genomic DNA, 3 & 2 are random sample numbers. Blood sample of 5µL was mixed with 200 µL of Buffer 1.

Figure 7B depicts the gel-electrophoresis bands of the DNA extracted from Buffer 1and the corresponding PCR amplified products, which was validated by School of Lifesciences, Manipal University, Manipal, India. PCR was performed using Buffer 1 extracted DNA. Blood sample of 5µL was mixed with 200 µL of Buffer 1.

Figure 7C depicts the gel-electrophoresis bands of the DNA extracted from Buffer 1and the corresponding PCR amplified products, which was validated by Elango Genetics, Chennai. The samples were used with and without coagulants in addition to the lysed blood. It was observed that no PCR amplification took place in heparin added blood. PCR was performed using Buffer 1 extracted DNA. Blood sample of 5µL was mixed with 200 µL of Buffer 1. Lane 1 is PCR product of EDTA anticoagulated blood, lane 2 and 3 are blood sample collected by finger prick, lane 4 is Heparin anticoagulated blood, lane 5 & 6 are lysed blood samples and lane 7 is DNA ladder.

EXAMPLE 9: Storage and shelf life of buffer:

The buffer composition vials were stored at room temperature (RT), 4°C and -20°C separately and checked for its storage parameters and shelf life. The buffers were used to extract DNA and thereafter PCR analysis was performed at different time intervals.

Figure 8A elucidates gel-electrophoresis bands of PCR amplified DNA extracted from the buffer compositions, wherein the buffers were stored for one month at room temperature (RT), 4°C and -20°C. No significant difference was observed in the gel-electrophoresis bands of PCR amplified DNA at various temperature conditions for a time period of one months.

Figure 8B elucidates gel-electrophoresis bands of PCR amplified DNA extracted from the buffer compositions, wherein the buffers were stored for three months at room temperature (RT), 4°C and -20°C. No significant difference was observed in the gel-

electrophoresis bands of PCR amplified DNA at various temperature conditions for a time period of three months.

Figure 8C elucidates gel-electrophoresis bands of PCR amplified DNA extracted from the buffer compositions, wherein the buffers were stored for four months at room temperature (RT), 4°C and -20°C. No significant difference was observed in the gel-electrophoresis bands of PCR amplified DNA at various temperature conditions for a time period of four months.

Figure 8D elucidates gel-electrophoresis bands of PCR amplified DNA extracted from the buffer compositions, wherein the buffers were stored for six months at room temperature (RT), 4°C and -20°C. No significant difference was observed in the gel-electrophoresis bands of PCR amplified DNA at various temperature conditions for a time period of six months. Thereby, the shelf life of buffer compositions is at least six months.

Furthermore, the buffer extracted DNA samples were stored at 4°C for period of 7 to 16 days and its integrity were observed with PCR amplification. The DNA was found to be intact which produces good PCR bands. In one of the preferred embodiments of this present invention, it was established that the PCR amplified DNA extracted using the buffer compositions have high integrity at 4°C for period of 16 days. Thereby, the shelf life of buffer extracted DNA samples is at least 7 to 16 days with high integrity. The shelf life of the buffer extracted DNA samples is higher than 16 days at 4°C, and at -20°C, the shelf-life of the same is for a longer period of time.

Figure 8E elucidates gel-electrophoresis bands of PCR of Buffer 1 extracted DNA stored at 4°C after 2 days, 4 days and 7 days. No significant difference was observed in the gel-electrophoresis bands of PCR amplified DNA its indicating high integrity.

Figure 8F elucidates gel-electrophoresis bands of PCR of Buffer 1 extracted DNA stored at 4°C after 10 days, 12 days, 14 days and 16 days. No significant difference was observed in the gel-electrophoresis bands of PCR amplified DNA indicating its high integrity.

I/We claim:

1. A buffer composition for nucleic acid extraction from a sample comprising:
   a. at least one buffering chemical selected from a group comprising tris hydrochloride, magnesium chloride hexahydrate and potassium chloride, wherein tris hydrochloride is present at concentration range of 10 mM to 50 mM, magnesium chloride hexahydrate is present at a concentration range of 0 mM to 5 mM and potassium chloride is present at a concentration range of 10 mM to 100 mM;
   b. at least one cell lysis chemical selected from a group comprising sodium dodecyl sulphate and sodium chloride, wherein sodium dodecyl sulphate is present at concentration range of 0.01% w/v to 0.1% w/v and sodium chloride is present at a concentration range of 50 mM to 250 mM;
   c. at least one stabilizing chemical selected from a group comprising ammonium chloride and sodium bicarbonate, wherein ammonium chloride is present at concentration range of 2 mM to 20 mM and sodium bicarbonate is present at a concentration range of 1 mM to 5 mM;
   d. nuclease-free water;

   wherein, the buffer composition is capable of nucleic acid extraction at ultra-rapid speed in a single-step method.
2. The buffer composition as claimed in claim 1, wherein the nuclease-free water comprised of undistilled, distilled, double distilled, high pure, ultra-high pure or ultra-filtered, unsterilized or sterilized by using autoclave or sterilized by UV rays.
3. The buffer composition as claimed in claim 1, wherein the nucleic acid extraction is carried out from genomic samples derived from animals, plants and microbes.
4. The buffer composition as claimed in claim 1, wherein 200 µL of buffer composition is optimized for 5 µL of genomic sample.
5. The buffer composition as claimed in claim 1, wherein the ultra-rapid speed is five seconds or less.
6. The buffer composition as claimed in claim 1, wherein the single-step method is carried out without any equipment or further processing steps.
7. The buffer composition as claimed in claim 6, wherein the extracted nucleic acid material can be stored, transported and used in PCR applications and down-streaming processes.

8. A method of preparation of buffer composition as claimed in claim 1, wherein the composition is prepared comprising following steps:
   a. preparation of stock solutions of all chemicals in respective concentration ranges of 2 M Tris Hydrochloride, 1 M Magnesium chloride, 1 M Potassium chloride, 5 M Sodium chloride, 1 M Ammonium chloride, 0.5 M Sodium bicarbonate and 10% w/v Sodium dodecyl sulphate;
   b. addition of chemicals in the aforementioned concentration and the final required volume of 200 µL of the buffer compositions are prepared by diluting with nuclease-free water; and
   c. cloudy precipitates are dissolved by the heating the composition at 37°C for 10 minutes, wherein the buffer composition is maintained between pH 7.0 to 9.0.
9. The buffer composition as claimed in claim 1, wherein buffer composition has shelf-life of six months at room temperature, and more than six months at 4°C and -20°C.
10. The buffer composition as claimed in claim 1, wherein the buffer extracted DNA sample have shelf-life of more than 16 days at 4°C and for longer term at -20°C.
11. A kit for ultra-rapid nucleic acid extraction comprising buffer composition as claimed in claim 1, wherein the pH of the buffer composition is between 7.0 to 9.0.

Figure 1

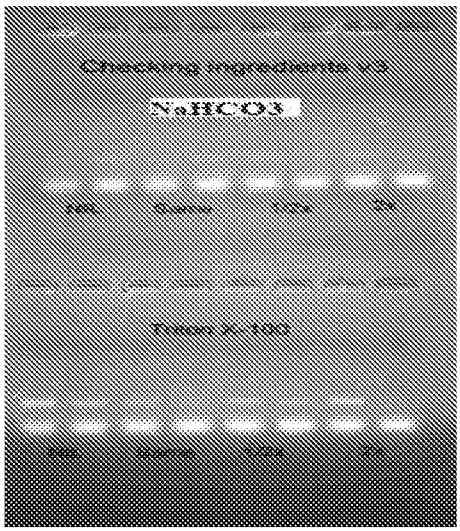


Figure 2A

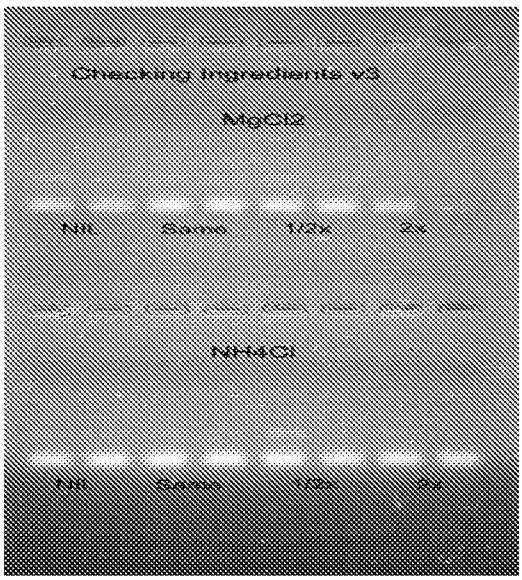


Figure 2B

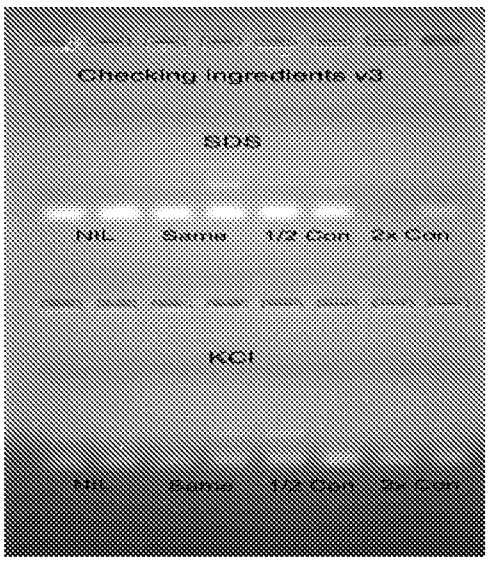


Figure 2C

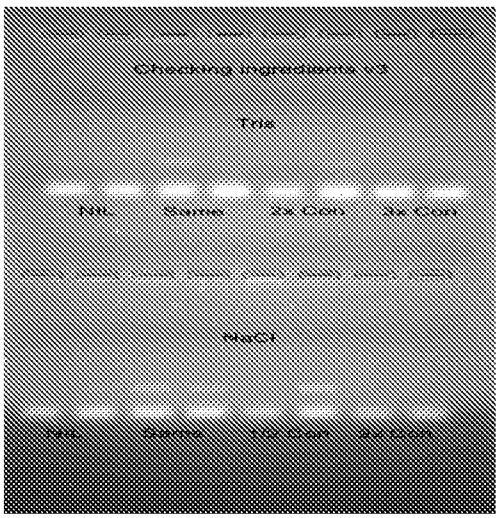


Figure 2D

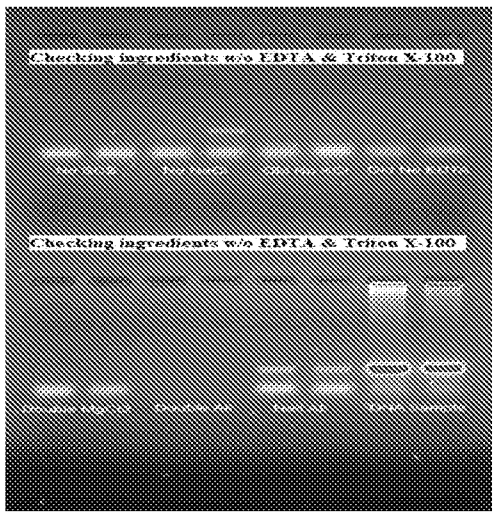


Figure 2E

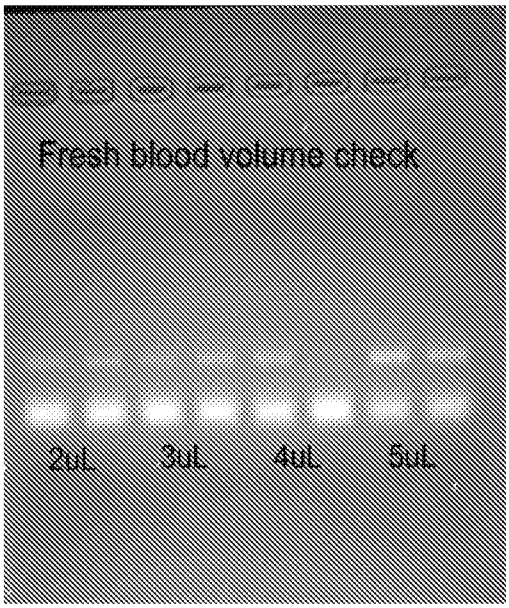


Figure 3A

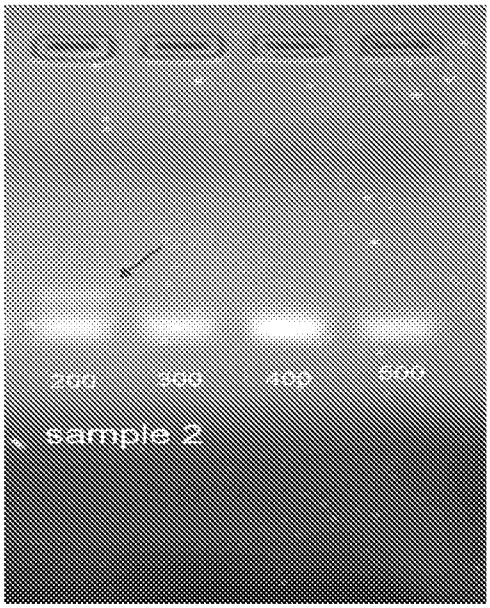
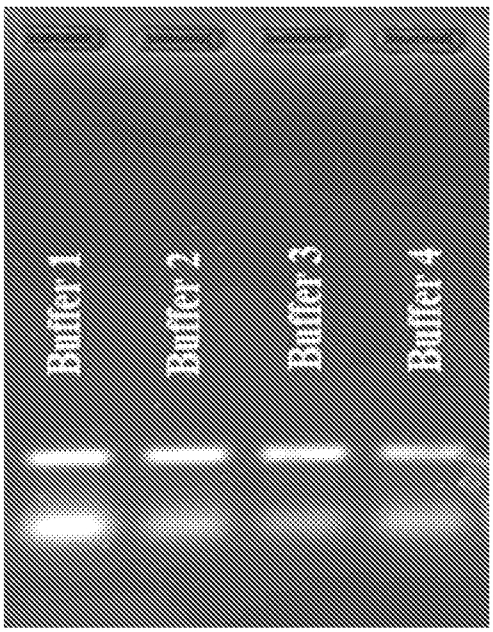


Figure 3B



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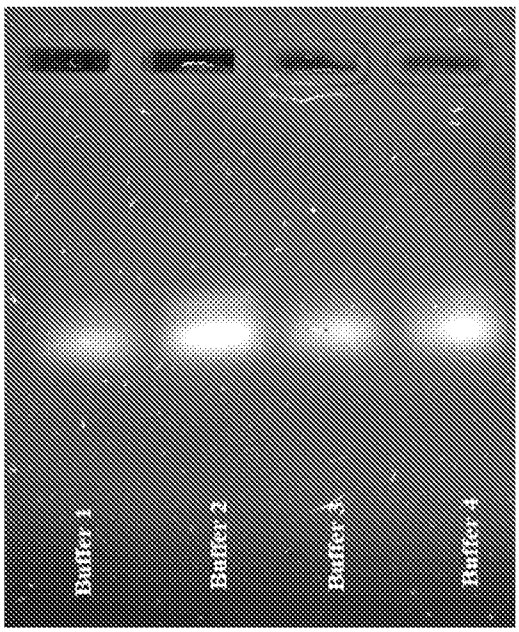

Figure 3D

Figure 4

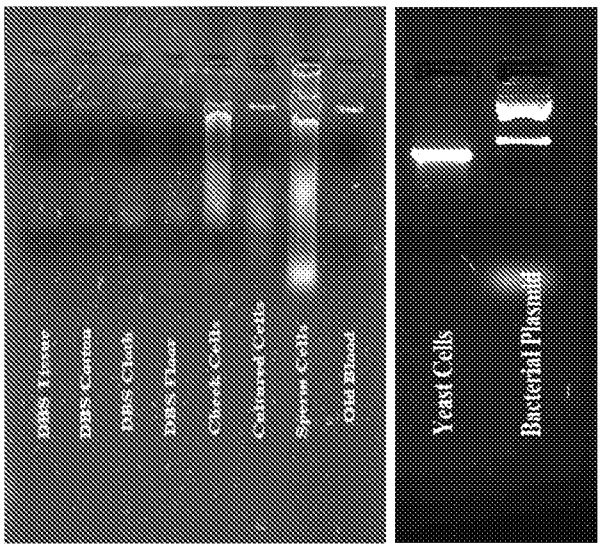

Figure 5A

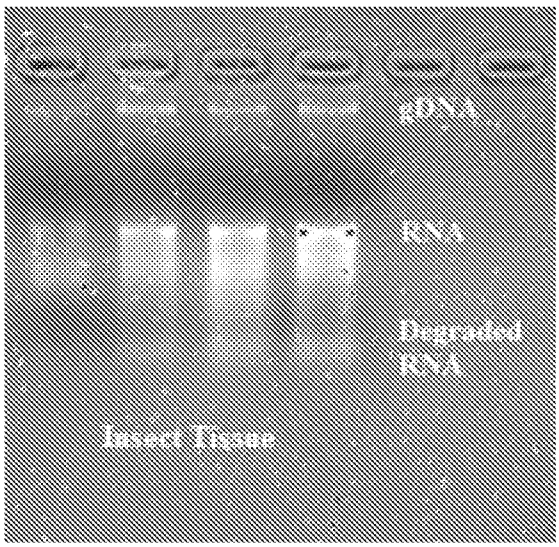

Figure 5B

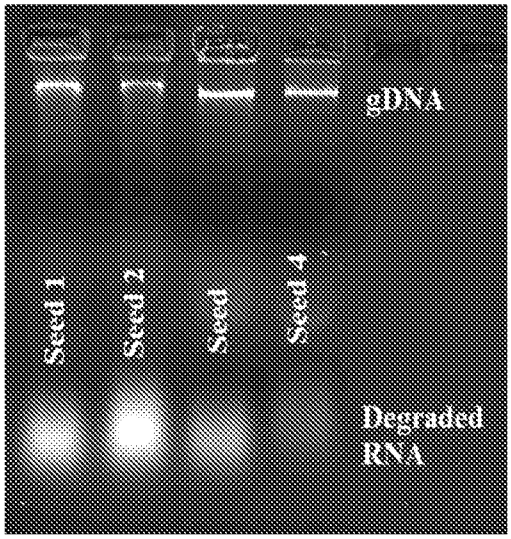

Figure 5C

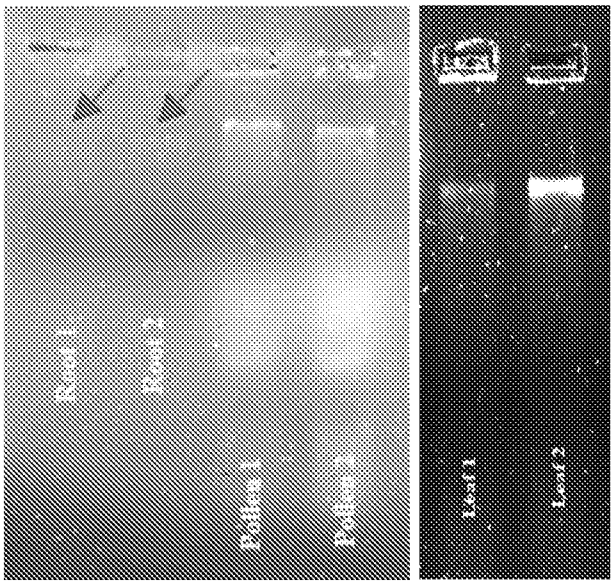

Figure 5D

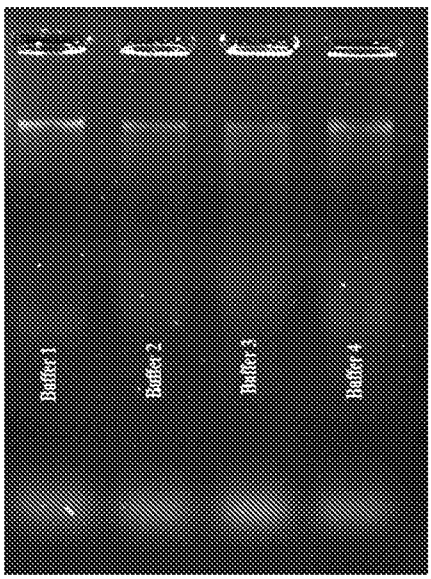

Figure 5E

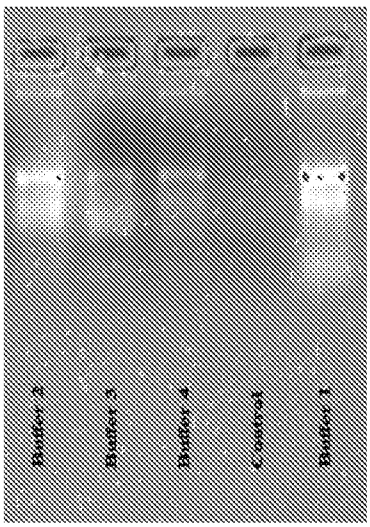

Figure 5F

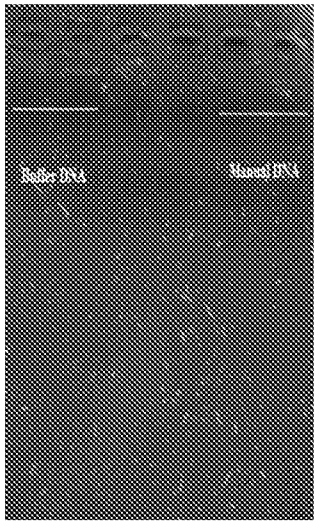

Figure 6

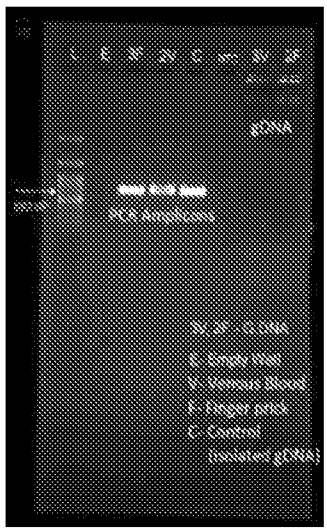

Figure 7A

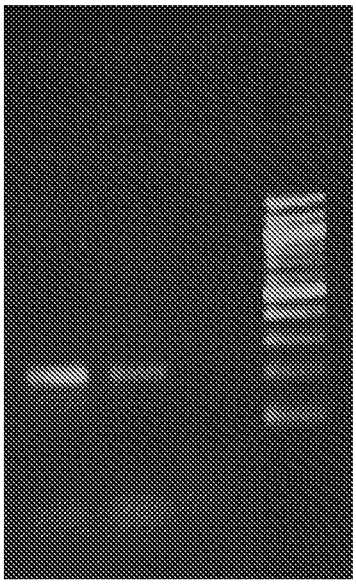

Figure 7B

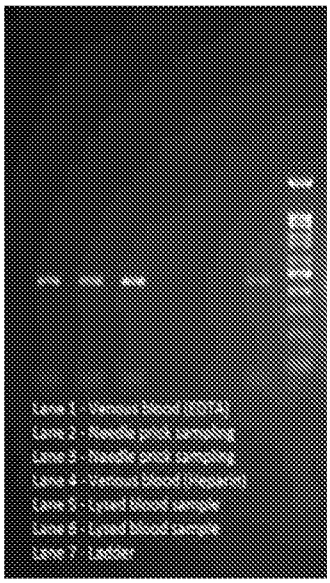

Figure 7C

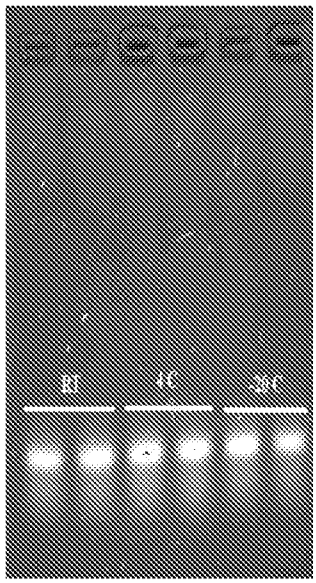

Figure 8A

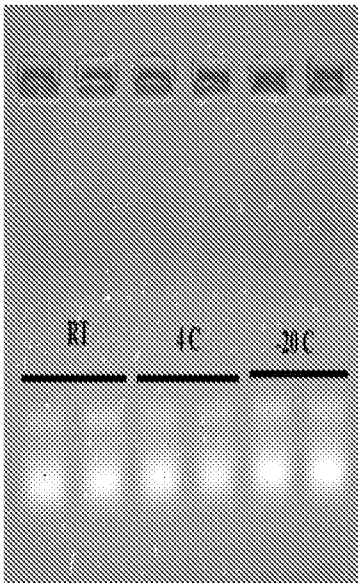

Figure 8B

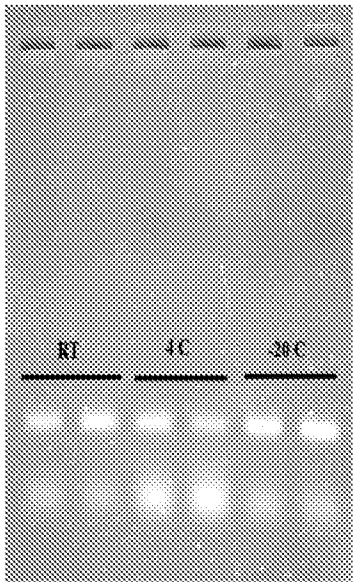

Figure 8C

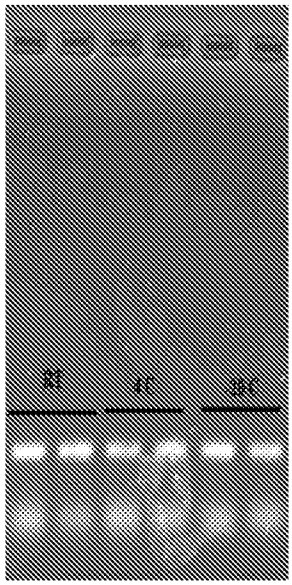

Figure 8D

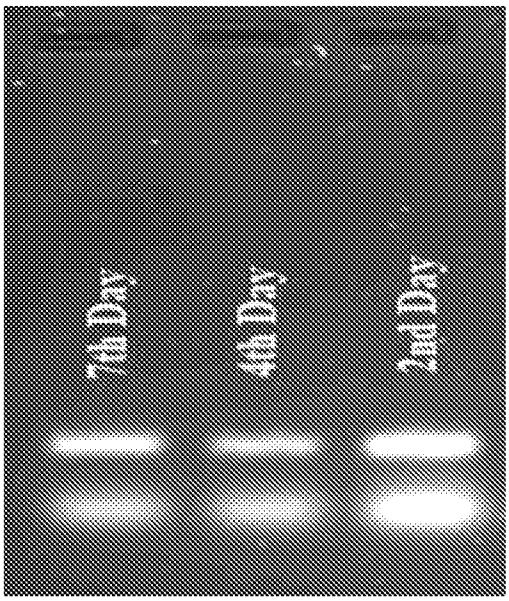

Figure 8E

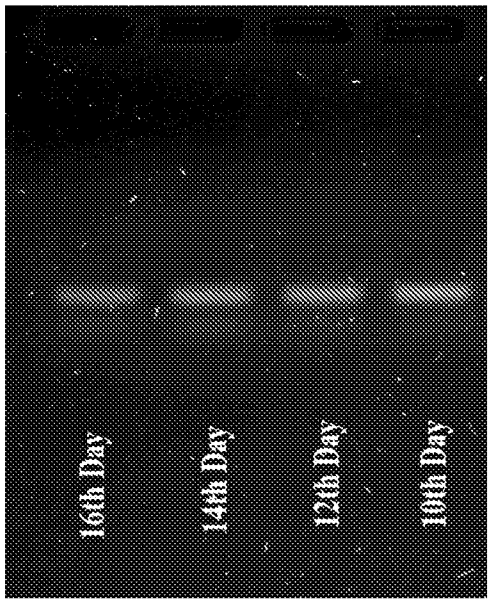

Figure 8F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2021/050335

A. CLASSIFICATION OF SUBJECT MATTER
C12N15/10 Version=2021.01

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

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)

PatSeer, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 20190062806 A1 (SPECTRUM SOLUTIONS LLC) 28 FEBRUARY 2019 (28.02.2019) *para 9; claim 1; para 67; para 82; claim 8; para 55; paras 61,68,71,84; paras 111-120; para 131*	1-11
Y	CA3042088A1 (SPECTRUM SOLUTIONS LLC) 19 JULY 2018 (19.07.2018) *claims 10-11; para 66; para 81; claim 13; paras 59,67-68; paras 95-101; para 130*	1-11
Y	JP 2007068452 A (FUJIFILM CORPORATION) 22 MARCH 2007 (22.03.2007) *claim 6; para 24; para 25; para 40* FAMILY:[NONE]	1-11
Y	HILL, V. R., NARAYANAN, J., GALLEN, R. R., FERDINAND, K. L., CROMEANS, T., & VINJÉ, J. (2015), DEVELOPMENT OF A NUCLEIC ACID EXTRACTION PROCEDURE FOR SIMULTANEOUS RECOVERY OF DNA AND RNA FROM DIVERSE MICROBES IN WATER. PATHOGENS, 4(2), 335-354.	1-11

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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	Date of mailing of the international search report
11-08-2021	11-08-2021
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Vishakha Gupta Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2021/050335

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	pg. 344, point 4.2; pg. 345, point 4.3; pg. 337	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2021/050335

Citation	Pub.Date	Family	Pub.Date
US 20190062806 A1	28-02-2019	EP 3568475 A1	20-11-2019
		WO 2018132827 A1	19-07-2018
CA 3042088 A1	19-07-2018	US 20180201977 A1	19-07-2018
		EP 3568475 A1	20-11-2019