

## DESCRIPTION

Title of Invention: FLOW PATH DEVICE, NUCLEIC ACID PURIFICATION METHOD, AND SAMPLE PRETREATMENT SYSTEM

### Technical Field

[0001]

The present disclosure relates to a flow path device, a nucleic acid purification method, and a sample pretreatment system.

### Background Art

[0002]

Gene analysis technologies have been utilized in various fields such as in-vitro diagnosis, forensic medicine, and environmental measurement along with rapid technological progress such as an improvement in analysis accuracy and generalization of technologies. Although there are various genetic testing methods and analysis targets, typical analysis processing is configured of a step of collecting a specimen, an extraction step of preparing a solution (sample) obtained by extracting a gene from the specimen, a purification step of removing a reaction inhibitory substance contained in the sample, a step of amplifying a target gene through a gene amplification reaction, a step of detecting an amplification product, and a step of analyzing a detection signal. Generally, the collected specimen is transported to a specialized facility equipped with a bench-top type gene analysis device and specialized instruments, and the subsequent treatment is performed manually by skilled specialized technicians (laboratory method).

[0003]

In recent years, there has been an increasing demand for a sample-to-answer-type gene analysis system allowing fully automatic operations from sample introduction to measurement and data analysis using a gene pretreatment/detection device and a disposable dedicated cartridge for the purpose of simplification, speed-up, cost reduction, and the like of operations.

For example, PTL 1 proposes a fully automatic DNA typing system including a sample introduction port, a nucleic acid extraction portion, a purification portion, a PCR portion, and a detection portion in a cartridge.

[0004]

As a method of purifying a nucleic acid, an organic phase solidification method or a solid phase extraction method using a porous membrane or magnetic beads is used. In particular, the solid phase extraction method has widely been used because many kits are commercially available and purification efficiency is satisfactory. The solid phase extraction method is a method of collecting a nucleic acid by binding the nucleic acid to a solid carrier. As the carrier, a porous membrane made of silica or glass fiber, magnetic beads coated with silica, or the like is used. In general, in a case where the purification treatment is manually performed, a spin column method in which a solution is placed on a porous membrane installed on a spin column as shown in PTL 2 and the solution is conveyed by a centrifugal operation is often employed. This is because the spin column method can be implemented with general laboratory equipment and operations thereof are relatively simple.

[0005]

On the other hand, in a case of an application to an automated system, a magnetic bead method by which solution exchange can be achieved without a centrifugal operation through a treatment of accumulating magnetic beads on a magnet is often employed. For example, NPL 1 discloses a method of automating a gene extraction and purification step by the magnetic bead method. In addition, NPL 2 discloses not only the magnetic bead method but also a nucleic acid purification method using a flow path chip. In particular, the Boom method has widely been used as a method capable of performing highly efficient and highly sample-robust nucleic acid purification by a simple treatment. This Boom method is a DNA purification technology to which an action of silica strongly binding to DNA in the presence of a chaotropic salt is applied. The Boom method has been applied to many lysis purification kits of the spin column method and the magnetic bead method, and nucleic acid purification on flow path chips.

Citation List

## Patent Literature

[0006]

PTL 1: JP 5815572

PTL 2: JP 2014-030397 A

PTL 3: JP 2002-512688 T

PTL 4: JP 2005-257647 A

PTL 5: JP 2016-63475 A

PTL 6: JP 2007-111653 A

PTL 7: JP 2019-144164 A

PTL 8: JP 2010-257691 A

PTL 9: JP 2002-39984 A

PTL 10: WO 2022/030605 A

## Non-Patent Literature

[0007]

NPL 1: “Microfluidic Devices for Nucleic Acid (NA) Isolation, Isothermal NA Amplification, and Real-Time Detection,” *Methods Mol. Biol.* 1256, 15-40(2015).

NPL 2: “Magnetic particles for integrated nucleic acid purification, amplification and detection without pipetting,” *Trends Analyt. Chem.* 127:115912(2020).

NPL 3: “Purification of Nucleic Acids in Microfluidic Devices,” *Anal. Chem.* 80, 6472-6479(2008)

## Summary of Invention

### Technical Problem

[0008]

The magnetic bead method requires, in addition to a magnet for collecting beads and a drive circuit for the magnet, a mechanism that conveys the beads with high accuracy for implementing a multi-stage analysis flow. For this reason, there is a problem that the configurations of the device and the flow path chip become complicated, which leads to an increase in costs. In a case where a micropillar or a monolith structure serving as a binding

carrier is provided on a flow path as described in NPL 1, for example, the flow path chip requires special processing, which leads to an increase in manufacturing costs of the flow path chip.

[0009]

PTL 1, PTL 3, and NPL 3 described above disclose implementation examples of nucleic acid purification in flow path chips that house membrane-like carriers. However, applications to high sensitivity analysis of a level (for example, a standard that can be used for inspection of forensic samples (such as items left at a scene of a case)) at which even a small amount of sample can be inspected with high sensitivity have not even been considered in any of these documents. This is also supported by the absence of any known examples referring to purification efficiency of these technologies, regardless of the fact that it is generally known that the amount and purity of nucleic acids collected from a sample significantly affect the accuracy and sensitivity of analysis.

[0010]

One of the problems of implementing highly efficient nucleic acid purification using a membrane-like carrier on a flow path chip is that purification efficiency is lower than those achieved by a laboratory scheme and a flow path chip purification scheme using a carrier other than a membrane. This is a new problem that has been clarified this time for the first time through studies of the inventors of the present application. In addition, it has become apparent for the first time from the studies of the inventors of the present application that a main cause of the problem is leakage of a solution to the outside of the membrane. Note that the process and details leading to the discovery of this problem will be clarified by the description in the embodiment and the accompanying drawings as will be described later.

[0011]

As one solution to the above problem, seal-fixing a purification membrane to prevent a solution from leaking, for example, is conceivable. Not only for the purpose of purifying a nucleic acid, but as a method of holding a thin film-shaped object in a predetermined storage portion in a contact manner with no liquid or gas leaking therefrom, membrane fixing methods using gaskets such as an O-ring or a C-ring as exemplified in PTL 4 to PTL 8 are conceivable.

[0012]

PTL 4 discloses a structure in which a reinforcing plate, a gas permeable membrane, and an O-ring (rubber packing) are housed in this order in an outer container, and the O-ring is pressed in a membrane direction by fitting an inner container from an O-ring upper portion. If the configuration of PTL 4 is applied to an application of high sensitivity analysis (for example, inspection of forensic samples) as described above, a solution may be sucked into a gap between the inner container and the outer container or a gap between the reinforcing plate and the outer container to cause liquid leakage, and the solution absorbed in the gap cannot be collected. This is because PTL 4 expects passage of gas components and does not take passage of a liquid in the membrane direction into consideration. At the same time, if it is attempted to provide the configuration of PTL 4 on a substrate of a flow path chip, substrates of at least four layers, namely an outer container storing a membrane and a ring, an inner container pressing the ring, a lower lid provided with a flow path, and an upper lid pressing the lower lid have to be assembled, which leads to increases in size and cost of the chip.

[0013]

PTL 5 shows a configuration in which a C-ring is fitted into a groove of an inner fitting member and is then fitted into an outer container, to thereby continuously feed a liquid to a separation membrane without causing a solution to leak out of the containers. This is also a common gasket ring utilization method. If the configuration of PTL 5 is applied to an application of high sensitivity analysis (for example, inspection of forensic samples) as described above, liquid leakage to the outside of the containers can be prevented, but the liquid will enter the gap between the outer container and the ring, and the solution absorbed in the gap cannot be collected. This is because PTL 5 expects continuous liquid feeding and does not take causing a small amount of solution to flow and collecting it in a later stage into consideration. In addition, if it is attempted to apply the configuration of PTL 5 to a substrate-shape chip device, chip components become complicated, which still leads to increases in size and cost, similarly to PTL 4.

[0014]

PTL 6 discloses a configuration in which a filter is housed between two chip substrates with flow path and filter storage portions formed thereon and the chip substrates are bonded to

each other to thereby fix the filter. It is essential for the configuration of PTL 6 to be a structure in which flow paths are provided in two chip substrates. Therefore, it cannot be applied to a flow path chip formed by attaching or bonding a thin and soft material such as a film to one chip substrate. Therefore, configuring an on-chip diaphragm valve or the like as exemplified in PTL 7 is conceivable. However, in this case, it is necessary to further provide another bonding layer in order to provide the valve, and the layer structure of the chip thus becomes complicated. At the same time, in order to control the bonding state with accuracy, it is necessary to strictly control bonding conditions and positioning accuracy. For this reason, restrictions on the flow path shape that can be formed and the chip material are great, which leads to an increase in manufacturing costs.

[0015]

PTL 8 and PTL 9 disclose configurations to prevent electrolytes accommodated inside devices from leaking to outside by housing gas permeable membranes, seal rings, and fixing members in this order and press-deforming the rings. These can fill the devices without leakage of small amounts of solutions. However, since the configurations do not expect liquid feeding operations, it is not possible to provide flow paths. In addition, even if flow paths are provided in order to apply the technologies to an application of high sensitivity analysis (for example, inspection of forensic samples) as described above, it is obvious that liquid residues that cannot be collected still occur.

[0016]

PTL 10 shows a configuration in which a nucleic acid extraction membrane is installed in a membrane storage container formed on a flow path chip, a rubber O-ring provided with a notch not to block the flow path is pressed to thereby fix the membrane, and a specific amount of liquid is caused to pass through the membrane. It has become apparent through the studies of the present inventors that if the configuration of PTL 10 is applied to an application of high sensitivity analysis (for example, inspection of forensic samples) as described above, purification efficiency is low and the amount of collected liquid significantly decreases. In addition, it has become apparent from the studies of the present inventors that a seal fixing force of the membrane in the configuration of PTL 10 is insufficient for the application of high

sensitivity analysis and that the phenomenon in regard to an amount of collected liquid is a phenomenon derived from generation of a liquid residue due to the notch. Since the O-ring of PTL 8 is elastically deformed, the notch portion is more easily deformed than the periphery thereof and is ununiformly deformed. At this time, if the notch is small, the flow path becomes narrow, the flow path resistance increases, and liquid feeding controllability is thus damaged. On the other hand, if the notch is large, the notch portion is larger and is deformed so as to collapse, and the flow path is further narrowed. At the same time, a liquid residue is likely to occur in the notch portion, and the liquid is likely to enter the gap between the ring and the membrane storage portion due to the O sectional shape of the ring, which leads to an increase in amount of solution that cannot be collected. At least several hundreds of  $\mu\text{L}$  of liquid is assumed to be conveyed in the configuration example exemplified in PTL 10, while it is necessary to cause a small amount of a liquid as small as several tens of  $\mu\text{L}$  to pass through the membrane and then collect the liquid for the application of high sensitivity analysis described above. Therefore, also the configuration exemplified in PTL 10 does not assume the application of high sensitivity analysis described above, and it is possible to state that expected effects cannot be obtained.

[0017]

Although a large number of membrane sealing and fixing methods are present in the related art as described above, all of them are limited to methods assuming gas flow, continuous liquid feeding, or liquid collection and do not assume collection of a small amount of liquid. In other words, the technical significance of all the known membrane fixing methods is to avoid gas or liquid leaking from flow paths or containers and entering parts where the gas or the liquid inherently should not enter. On the other hand, for the application of high sensitivity analysis, it is necessary for a small amount of liquid that has flowed in to pass through a membrane without any loss and to be able to be collected at a high yield in a later stage.

[0018]

Furthermore, as another method of holding a thin film-shaped object in a predetermined storage portion in a contact manner without leakage of liquid or gas, there is a membrane fixing method based on press-fitting using a hard ring as exemplified in PTL 2. PTL

2 shows a configuration in which a circular porous member, a membrane, and a resin hollow member are press-fitted in this order into a resin spin column to fix the membrane. This is a general configuration of a spin column, causes a small amount of liquid to pass through the membrane without any loss, and can collect the liquid at a high yield. However, it has become apparent from the studies of the present inventors that if it is attempted to apply the configuration of PTL 2 to an application of high sensitivity analysis, the press-fitted ring or the flow path chip is damaged or deformed and the configuration cannot be applied. It was discovered that the reason therefor was that the rigidity of the flow path chip was high unlike the spin column and strain at the time of ring press-fitting was not able to be absorbed by both the ring and the flow path chip.

[0019]

Another problem when the configuration of PTL 2 is applied to the application of high sensitivity analysis is that a solution remains inside the circular porous holding member serving as a membrane holding table and a yield decreases. It has been discovered that this is an event that occurs due to a small liquid feeding conveyance pressure for the application in a case where a centrifugal operation is not performed as compared with the solution conveyance based on a centrifugal operation as a precondition for implementing PTL 2. In addition, yet another problem when the configuration of PTL 2 is applied to the purpose of high sensitivity analysis is that purification efficiency is still low or a PCR reaction in a later stage is inhibited even if the Boom method, which is a standard technology, is employed as a highly efficient nucleic acid purification method after taking measures against the above problems. It has been discovered that this is caused by clogging of a sample and an inhibitor remaining due to an insufficient liquid feeding pressure. In a case where a series of purification flows are performed by a chip with a purification membrane firmly fixed thereto to cause a sample solution to pass through the purification membrane without any loss, a high liquid feeding pressure (about >200 kPa) is required. On the other hand, in a case of a general sample-to-answer-type flow path chip, it is often difficult to obtain a sufficient liquid feeding pressure from the viewpoint of a flow path pressure loss and a pressure resistance of a valve inside the flow path. In order to obtain a liquid feeding pressure at a level similar to that for solution conveyance by a centrifugal

operation by combining known technologies, it is necessary to improve chip pressure resistance and to increase an output of a liquid feeding pressure source. Therefore, it is not possible to avoid increases in costs of the chip and the system and size of the system, and it is not possible to realize a fully automatic and highly sensitive gene analysis system based on a device and a flow path chip with simple configurations achieved at low cost.

[0020]

In view of such circumstances, the present disclosure proposes a technology enabling a treatment of removal of impurities from a sample containing a nucleic acid and a treatment of purifying and collecting the nucleic acid by a device to be performed efficiently, and a flow path chip with a simple configuration achieved at low cost.

#### Solution to Problem

[0021]

In order to solve the above problem, the present disclosure proposes a flow path device with a flow path formed on a substrate, the flow path device including: a purification membrane that is able to collect the nucleic acid; a membrane storage portion that is a space formed on a substrate, is connected to a flow path, and houses the purification membrane; and an annular resin ring that is press-fitted to the membrane storage portion, the membrane storage portion including a pedestal surface formed between an upper surface and a lower surface of the substrate such that the purification membrane is installed thereon, the membrane storage portion being divided into a cylindrical membrane storage portion upper portion and a membrane storage portion lower portion having a smaller diameter than the membrane storage portion upper portion with the pedestal surface sandwiched as a boundary, the flow path including a first flow path that is connected to at least one valve mechanism, is formed on the upper surface of the substrate, and is connected to a side wall of the membrane storage portion upper portion and a second flow path that is connected to at least one valve mechanism, is formed on the lower surface of the substrate, and is connected to the membrane storage portion lower portion, the resin ring having an outer diameter that is equal to or greater than an inner diameter of the membrane storage portion upper portion, the purification membrane being fixed between the

pedestal surface and the resin ring, the outer diameter of the press-fitted resin ring being smaller than the outer diameter of the resin ring in a state where the resin ring is not housed in the membrane storage portion, and the inner diameter of the membrane storage portion upper portion being substantially the same as the inner diameter of the membrane storage portion upper portion in a state where the resin ring is not housed in the membrane storage portion, and the inner diameter of the membrane storage portion upper portion and the outer diameter of the press-fitted resin ring are the same at least at a position where the side wall of the membrane storage portion upper portion and the press-fitted resin ring are closest to each other.

[0022]

Further features related to the present disclosure will become apparent from the description in the specification and the accompanying drawings. Also, aspects of the present disclosure will be achieved and realized by elements, combinations of various elements, and aspects of the following detailed description and appended claims.

The description in the specification shows merely typical illustrative examples and is not intended to limit the claims of the present disclosure or application examples in any sense.

#### Advantageous Effects of Invention

[0023]

According to the technology of the present disclosure, it is possible to efficiently perform a treatment of removal of impurities from a sample containing a nucleic acid and a treatment of purifying and collecting the nucleic acid by a device and a flow path chip with simple configurations achieved at low costs.

#### Brief Description of Drawings

[0024]

[FIG. 1] FIG. 1 is a diagram showing a treatment process of a nucleic acid purification method based on the standard Boom method.

[FIG. 2A] FIG. 2A is a diagram showing the treatment process shown in FIG. 1 and a composition of a sample solution used in consideration.

[FIG. 2B] FIG. 2B is a diagram showing a reagent and a solution conveying method used in the purification treatment process shown in FIG. 1.

[FIG. 3A] FIG. 3A is a conceptual diagram showing a configuration example of a sample pretreatment system 301 that performs the treatment process in FIG. 1 by a flow path device.

[FIG. 3B] FIG. 3B is a diagram showing a configuration along the section A-A in FIG. 3A in a state where the flow path device is installed.

[FIG. 4A] FIG. 4A is a top view of a flow path device (Example 1) 101 to which a known membrane installation method is applied.

[FIG. 4B] FIG. 4B is a diagram showing an A-A sectional configuration in FIG. 4A.

[FIG. 5A] FIG. 5A is a top view of a flow path device (Example 2) 101 to which a known membrane installation method is applied.

[FIG. 5B] FIG. 5B is a diagram showing an A-A sectional configuration in FIG. 5A.

[FIG. 6A] FIG. 6A is a top view of a flow path device (Example 3) 101 to which a known membrane installation method is applied.

[FIG. 6B] FIG. 6B is a diagram showing an A-A sectional configuration in FIG. 6A.

[FIG. 7A] FIG. 7A is a diagram showing quantitative evaluation results of nucleic acid purification efficiency and the amounts of collected liquid in a case where a spin column and a flow path device having a known configuration are used.

[FIG. 7B] FIG. 7B is a diagram showing purification efficiency calculated by quantifying the amount of DNA contained in an eluent collected in FIG. 7A by real-time PCR (DNA collection efficiency: the dark-color (left-side) bar graph of each method) and DNA leakage rate calculated by quantifying the amount of DNA contained in a sample solution (hereinafter, referred to as a waste liquid) that has passed through a purification membrane (light- color (right-side) bar graph of each method).

[FIG. 8] FIG. 8 is a view (photograph) showing a phenomenon in which a liquid pool of an eluent 403 is formed in the vicinity of a notch portion (a liquid seems to be trapped on the notch) in a flow path device 101 (one example) having a membrane fixing structure based on an O-ring after collection of the eluent.

[FIG. 9A] FIG. 9A is a top view of a flow path device 101 according to the present embodiment.

[FIG. 9B] FIG. 9B is a diagram showing a sectional configuration example along A-A in FIG. 9A.

[FIG. 10A] FIG. 10A is a diagram showing a state in which a purification membrane 201 is installed on a pedestal surface 109 from a membrane storage portion upper portion 110.

[FIG. 10B] FIG. 10B is a diagram showing a state in which a resin ring 204 is press-fitted to the membrane storage portion upper portion 110.

[FIG. 10C] FIG. 10C is a diagram showing a purification membrane 201 fixed between the pedestal surface 109 and the resin ring.

[FIG. 11A] FIG. 11A is a diagram showing results of performing a nucleic acid purification experiment using the sample pretreatment system shown in FIG. 3A and the flow path device 101 of FIG. 9 and quantitatively evaluating the amount of collected eluent.

[FIG. 11B] FIG. 11B is a diagram showing results of performing a nucleic acid purification experiment using the sample pretreatment system shown in FIG. 3A and the flow path device 101 in FIG. 9 and quantitatively evaluating DNA purification efficiency.

[FIG. 12A] FIG. 12A is a diagram showing an upper surface configuration of a flow path device 101 to which a spin column configuration is applied.

[FIG. 12B] FIG. 12B is a diagram showing an A-A sectional configuration in FIG. 12A.

[FIG. 13A] FIG. 13A is a diagram (enlarged photograph) showing an example of the resin ring 204 which has failed to be press-fitted and fixed and has been deformed on the membrane storage portion 104.

[FIG. 13B] FIG. 13B is a diagram (photograph) showing an example of the resin ring 204 which has failed to be press-fitted and fixed and has been deformed on the membrane storage portion 104.

[FIG. 13C] FIG. 13C is a table summarizing examples of outer diameter dimensions and success/failure of press-fitting and fixing of the resin ring 204.

[FIG. 14A] FIG. 14A is a diagram showing an internal configuration example of a liquid feeding mechanism 302.

[FIG. 14B] FIG. 14B is a diagram showing an internal configuration example of the liquid feeding mechanism 302 including a multi-way valve mechanism (V0) capable of opening to atmosphere.

[FIG. 15] FIG. 15 is a diagram showing a configuration example for achieving a function equivalent to that of the multi-way valve mechanism 3006 by combining a plurality of on-off valve mechanisms 3005.

[FIG. 16] FIG. 16 is a diagram showing an internal configuration example of a reagent holding portion 303.

[FIG. 17] FIG. 17 is a flowchart for explaining a nucleic acid purification treatment in the nucleic acid purification system shown in FIG. 16.

[FIG. 18] FIG. 18 is a diagram showing another internal configuration example (modification example) of the reagent holding portion 303 in the nucleic acid purification system.

[FIG. 19] FIG. 19 is a flowchart for explaining a nucleic acid purification treatment in the nucleic acid purification system shown in FIG. 18.

[FIG. 20] FIG. 20 is a diagram showing a system configuration example of a sample pretreatment system 301 in which a reagent tank integrated flow path device (nucleic acid purification device) 101 is installed.

[FIG. 21A] FIG. 21A is a diagram showing an upper surface configuration example in the vicinity of a reagent tank 3105.

[FIG. 21B] FIG. 21B is a diagram showing a sectional configuration example along A-A in FIG. 21A.

[FIG. 22] FIG. 22 is a flowchart (example) for explaining a nucleic acid purification treatment executed by the nucleic acid purification system shown in FIG. 20.

[FIG. 23] FIG. 23 is a diagram showing an overall configuration example of a sample-to-answer-type DNA analysis system configured of a flow path device 101 and a sample pretreatment system 301 capable of automatically performing nucleic acid extraction, nucleic acid purification, and nucleic acid amplification/detection from a biological sample.

[FIG. 24] FIG. 24 is a diagram showing a configuration example of a nucleic acid

extraction portion 1201 in the sample-to-answer-type DNA analysis system.

[FIG. 25] FIG. 25 is a flowchart for explaining a nucleic acid extraction treatment by the sample-to-answer-type DNA analysis system.

[FIG. 26] FIG. 26 is a diagram showing a configuration example of a periphery of a nucleic acid purification portion 1101 in the sample-to-answer-type DNA analysis system.

[FIG. 27A] FIG. 27A is a diagram showing a configuration example of a periphery of a nucleic acid amplification portion 1301.

[FIG. 27B] FIG. 27B is a diagram showing a sectional configuration example along A-A in FIG. 27A.

[FIG. 28] FIG. 28 is a flowchart for explaining a nucleic acid amplification treatment by the sample-to-answer-type DNA analysis system.

[FIG. 29A] FIG. 29A is a diagram showing a configuration example of a periphery of a membrane storage portion 104 from above in each modification example.

[FIG. 29B] FIG. 29B is a diagram showing a sectional configuration example along A-A in FIG. 29A in a first modification example.

[FIG. 29C] FIG. 29C is a diagram showing a sectional configuration example along A-A in FIG. 29A in a second modification example.

[FIG. 29D] FIG. 29D is a diagram showing a sectional configuration example along A-A in FIG. 29A in a third modification example.

[FIG. 29E] FIG. 29E is a diagram showing a sectional configuration example along A-A in FIG. 29A in a fourth modification example.

[FIG. 30A] FIG. 30A is a diagram showing a state where a sample solution 401 is conveyed by a continuous conveying method.

[FIG. 30B] FIG. 30B is a diagram showing a state where a cleaning liquid 402 is conveyed by the continuous conveying method.

## Description of Embodiments

[0025]

An embodiment of the present disclosure proposes a technology that enables highly

efficient nucleic acid purification by allowing a small amount of reaction solution to pass through a purification membrane without leakage in a flow path device (flow path chip) and to be collected with high efficiency. In the present specification, problems that have become apparent for the first time in the examination according to the present disclosure will be described first, and features of a sample pretreatment system (sample pretreatment apparatus) according to an embodiment will be then described. Next, description will move on to modifications of the present embodiment. In the drawings in the present specification, functionally the same elements may be denoted by the same numbers. Note that although the accompanying drawings show specific embodiments and implementation examples in accordance with principles of the present disclosure, these are for understanding of the present disclosure and are not intended to be used to interpret the present disclosure in a limited manner. Furthermore, although the description will be made in detail sufficiently for those skilled in the art to implement the present disclosure, it should be understood that other implementations and modes are also possible and changes in configurations and structures and replacement of various elements can be made without departing from the scope and the spirit of the technical idea of the present disclosure. Therefore, the following description should not be interpreted as being limited thereto.

[0026]

#### (1) Further Discussion on Problems in Nucleic Acid Purification (Detailed Examination)

##### <Treatment Process of Nucleic Acid Purification>

FIG. 1 is a diagram showing a treatment process of a standard nucleic acid purification method. First, a sample solution containing a sample DNA is caused to pass through a purification membrane to cause the DNA to bond to the purification membrane (process I). In a case where a nucleic acid purification method based on the Boom method is employed, a chaotropic agent such as a guanidinium salt is added to a sample solution obtained by subjecting a biological sample to a cell lysis treatment. Next, a cleaning liquid is caused to pass through the purification membrane to wash away impurities adhering to the purification membrane (process II). In general, a solution containing ethanol is used as the cleaning liquid, and the cleaning may be performed multiple times. Furthermore, the purification membrane is dried to

remove a cleaning liquid component (process III). Finally, the DNA is bonded through the above flow, and a DNA eluent is caused to pass through the purification membrane, from which the impurities and the cleaning liquid component have been removed, for collection, thereby collecting the target DNA (process IV).

[0027]

FIG. 2A is a diagram showing the treatment process shown in FIG. 1 and a composition of a sample solution used in the consideration. Also, FIG. 2B is a diagram showing a reagent and a solution conveying method used in the purification treatment process shown in FIG. 1. Here, a human genome sample (2800M) is used as a sample, and QIAamp DNA Investigator Kit (Qiagen), which is a standard kit for a laboratory method, is used as a lysis and purification reagent. In a case of a spin column method performed in a laboratory, the conveyance of each solution is achieved by a centrifugal operation.

[0028]

<Sample Pretreatment System for Nucleic Acid Purification>

FIG. 3A is a conceptual diagram showing a configuration example of a sample pretreatment system 301 that performs the treatment process in FIG. 1 by a flow path device. The sample pretreatment system 301 includes a liquid feeding mechanism 302, a reagent holding portion 303, a chip holding portion 304 made of polyether ether ketone (PEEK), a cover 305 made of PEEK having a fitting 306, and a piping tube 307. The liquid feeding mechanism 302 is configured of a liquid feeding power source and a pressure control mechanism capable of applying a solution conveyance pressure at an arbitrary timing, and the liquid feeding mechanism 302 is connected to the reagent holding portion 303 via the piping tube 307. The reagent holding portion 303 includes a region for holding a solution therein and a region for collecting the solution after conveyance, each of which is connected to the flow path device 101 via the piping tube 307, and can convey and collect an arbitrary solution in response to a pressurizing or depressurizing operation controlled by the liquid feeding mechanism 302. Specific internal system configuration examples of the liquid feeding mechanism 302 and the reagent holding portion 303 will be described later.

[0029]

FIG. 3B is a diagram showing a configuration along the section A-A in FIG. 3A in a state where the flow path device is installed. The flow path device 101 is installed such that the flow path device 101 can be held at a predetermined position between the chip holding portion 304 and the cover 305. When the flow path device 101 is installed, a first vent 107 and a second vent 108 provided in the flow path device 101 are connected to the fitting 306 provided in the cover 305.

[0030]

The liquid feeding power source of the liquid feeding mechanism 302 can apply a pressure sufficient to convey the solution and does not exceed chip pressure resistance. For example, a diaphragm pump or a syringe pump capable of controlling the pressure to be applied within a range of 0.1 kPa to 250 kPa can be used. Although an example of a liquid feeding pressure source using an air pressure is shown here, mechanical compression, a centrifugal force, or the like may be used.

[0031]

The material of the chip holding portion 304 and the cover 305 may not be PEEK and is not particularly limited as long as the material has rigidity sufficient to hold the flow path device 101. As the material, a resin material such as polycarbonate (PC) or acrylic (PMMA), a metal material such as aluminum or stainless steel, or the like can be selected as well as PEEK. In consideration of a case where a solution such as a surfactant or a salt contained in the sample solution or ethanol contained in the cleaning liquid leaks, the material preferably has chemical resistance against these solutions.

<Flow Path Device for Nucleic Acid Purification>

[0032]

Next, the flow path device 101 used here (for the consideration) will be described. FIGS. 4A, 5A, and 6A are top views of the flow path device (Examples 1 to 3) 101 to which a known membrane installation method is applied. FIGS. 4B, 5B, and 6B are views showing A-A sectional configurations of FIGS. 4A, 5A, and 6A, respectively.

[0033]

Each flow path device 101 commonly includes a substrate 102 having a flow path

structure, sealing tapes 103 made of polyolefin (PO) and attached to upper and lower surfaces of the substrate 102, and a purification membrane 201 installed in a membrane storage portion 104. First, a membrane storage portion 104 with an upper portion having a diameter of 6 mm  $\times$  a depth of 1 mm and a lower portion having a diameter of 4 mm  $\times$  a depth of 2 mm, which are coaxial and different dimensions, a first flow path 105 and a second flow path 106 with a width of 1 mm  $\times$  a depth of 1 mm, and a first vent 107 and a second vent 108 with a diameter of 1 mm are formed on the substrate 102 made of PC and having a vertical length of 20 mm  $\times$  a horizontal length of 50 mm  $\times$  a thickness of 4 mm by cutting. The first vent 107 is located at an end point of the first flow path 105, the second vent 108 is located at an end point of the second flow path 106, and the first vent 107 and the second vent 108 are processed as through-holes on the substrate 102 with upper surfaces thereof opened. The first flow path 105 is formed on the upper surface of the substrate 102 and is connected to a side wall of a membrane storage portion upper portion 110. The second flow path 106 is formed on the lower surface of the substrate 102 and is connected to a side wall of a membrane storage portion lower portion 111. [0034]

FIGS. 4A and 4B show a configuration of the flow path device 101 having no membrane fixing structure, that is, a configuration in which the purification membrane 201 is left to stand on a pedestal surface 109. Before the sealing tape 103 is attached to the upper surface of the substrate 102, the purification membrane 201 (silica membrane filter, GF/F, Whatman) having a diameter of 6 mm is installed by being pressed in the direction of the pedestal surface 109 from the side of the membrane storage portion upper portion 110. After confirming that it has been able to achieve the installation without an outer peripheral portion of the purification membrane 201 floating, the sealing tape 103 is attached to the upper surface of the substrate 102, thereby forming the flow path device 101 with no membrane fixing structure.

[0035]

FIGS. 5A and 5B show a configuration of a flow path device 101 with a membrane fixing structure based on an O-ring provided with a notch. Before the sealing tape 103 is attached to the upper surface of the substrate 102, the purification membrane 201 (silica

membrane filter, GF/F, Whatman) having a diameter of 6 mm is installed by being pressed in the direction of the pedestal surface 109 from the side of the membrane storage portion upper portion 110. Furthermore, a rubber O-ring 202 (outer diameter of 6 mm  $\times$  inner diameter of 4 mm) provided with a notch is inserted such that the notch portion coincides with the first flow path 105 with the first flow path 105 not blocked. After confirming the purification membrane 201 and the O-ring 202 have been able to be installed without floating, a sealing tape 103 is attached to the upper surface of the substrate 102, and a 10 mm square PMMA block is attached to the sealing tape 103 at the upper portion of the membrane storage portion 104 to collapse the O-ring. As described above, the flow path device 101 having a membrane fixing structure based on the O-ring is formed.

[0036]

FIGS. 6A and 6B show a configuration of a flow path device 101 having a membrane fixing structure based on an adhesive. Before the sealing tape 103 is attached to the upper surface of the substrate 102, a double-sided tape (#9969, 3M) is attached to the pedestal surface 109. The purification membrane 201 (silica membrane filter, GF/F, Whatman) having a diameter of 6 mm is installed by being pressed in the direction of the pedestal surface 109 from the side of the membrane storage portion upper portion 110 to thereby bond the purification membrane 201 to the pedestal surface 109. After confirming that the purification membrane 201 has been able to be installed without the outer peripheral portion floating, the sealing tape 103 is attached to the upper surface of the substrate 102, thereby forming the flow path device 101 having the membrane fixing structure based on the adhesive.

[0037]

#### <Experimental Results>

A nucleic acid purification experiment was performed using the sample pretreatment systems shown in FIGS. 3A and 3B and the flow path devices 101 in FIGS. 3A, 3B, 5A, and 5B. FIGS. 7A and 7B are diagrams showing results of quantitatively evaluating amounts of collected eluent and DNA purification efficiency by the nucleic acid purification experiment. As shown in FIG. 7A, the average amount of eluent finally collected by the spin column method was 17  $\mu$ L, while the average amount of the eluent collected from the chip devices was 10  $\mu$ L

or less. In particular, in the case of the flow path device 101 having the membrane fixing structure based on the O-ring provided with the notch, the amount of collected liquid was reduced to 1/10 or less the amount of input liquid. FIG. 8 is a view (photograph) showing a phenomenon in which a liquid pool of an eluent 403 is formed in the vicinity of the notch portion (a liquid seems to be trapped on the notch) in the flow path device 101 (one example) having the membrane fixing structure based on the O-ring after collection of the eluent.

[0038]

FIG. 7B shows purification efficiency (DNA collection efficiency) calculated by quantifying the amount of DNA contained in the eluent collected in FIG. 7A by real-time PCR using a dark (left) bar graph of each method. In addition, FIG. 7B shows the DNA leakage rate calculated by quantifying the amount of DNA contained in the sample solution (hereinafter, referred to as a waste liquid) that has passed through the purification membrane by a light (right) bar graph of each method. The DNA leakage rate was evaluated by putting the waste liquid into a new spin column, performing the purification treatment of the present embodiment, and quantifying the content of DNA by the real-time PCR. As shown in FIG. 7B, it was found that for the spin column method, the purification efficiency was about 50% on average and the DNA leakage was 0% in all the six tried cases, and for the chip device, the purification efficiency was half or less the purification efficiency of the spin column method in all the cases and the DNA leakage rate was high. In addition, an example in which mixing of a PCR inhibitor in the collected eluent was suspected was observed in the flow path device 101 having the membrane fixing structure based on the adhesive.

[0039]

The reason that the amount of collected liquid in the chip device was smaller than that in the spin column method is considered to be because of differences in liquid feeding methods in addition to liquid residue on the ring observed in the flow path device 101 having the membrane fixing structure based on the O-ring (which is just insertion of an elastic body unlike press-fitting by the technology of the present disclosure, which will be described later). Since the purification membrane is a porous material and has a certain water holding power, the amount of eluent remaining in the membrane depends on the material and density of the

membrane and a liquid feeding pressure. The purification membrane 201 used in the present study is a silica membrane having substantially the same density both in the spin column method and in the chip device. Therefore, it is considered that the purification membrane 201 did not contribute to differences in amounts of collected liquid observed in this study. On the other hand, the liquid feeding pressure differed between the spin column method and the chip device since solution conveying methods differed. In the spin column method, the solution input to the column was caused to pass through the purification membrane by a centrifugal force and was conveyed to the collection tube. At this time, 20,000 g (corresponding to a conveying pressure of a maximum of about 100 kPa to 150 kPa under the conditions of this study) was constantly applied to the solution. On the other hand, the chip device of this study employed air pressure liquid feeding, and the feeding pressure of the eluent is as low as a maximum of about 50 kPa. Therefore, it is considered that in the flow path device scheme, the sample solution held in the membrane became unlikely to be discharged to the flow path and the amount of collected liquid decreased.

[0040]

In regard to purification efficiency, the waste liquid contained substantially no DNA in the case of the spin column method. This indicates that the sample solution had passed through the purification membrane without leakage, and that binding efficiency between the purification membrane and DNA was extremely high. On the other hand, there was no mechanism for fixing the purification membrane in the case of the flow path device 101 having no membrane fixing structure. For this reason, it is considered that membrane floating or oozing from a peripheral edge portion occurred. Since membrane floating was suppressed in the flow path device 101 having the membrane fixing structure based on the O-ring as compared with the flow path device 101 having no membrane fixing structure, it is considered that the DNA leakage rate decreased and there was a trend of an improvement in purification efficiency. However, the purification efficiency was lower than that of the spin column method, indicating that a DNA leakage suppression force, that is, a membrane fixing force was insufficient.

[0041]

A high DNA leakage rate and remarkably low purification efficiency were observed in

the flow path device 101 having the membrane fixing structure based on the adhesive as well. This is considered to be because the adhesion was insufficient, and an unintended gap was thus generated, or the purification membrane was clogged due to the adhesive. PCR inhibition did not occur in cases other than the flow path devices using the adhesive 203. From this point, contamination by the adhesive 203 was suspected as a cause of the PCR inhibition. It has been confirmed that the adhesive 203 used in this study has chemical resistance to a purification reagent. However, the adhesive 203 was not a product, biocompatibility of which was guaranteed. Therefore, a minute amount of component elution that was negligible for other purposes may have served as an inhibition factor of the PCR analysis. In addition, it is considered that since the assembly of the flow path device 101 of this study was manually performed, the adhesion state was not able to be controlled with satisfactory reproducibility, the adhesive component was likely to stick out from the pedestal or ununiformly attached thereto, and a PCR inhibiting substance was likely to be mixed into the solution by coming into contact with the solution. In order to solve this problem, it is necessary to more precisely manage the device manufacturing process and to use an adhesive component that does not contain any PCR inhibiting substance. However, considering the sizes of the membrane storage portion 104 and the pedestal surface 109 (a portion serving as an attachment margin) of the flow path device 101 according to this study, it is expected to be difficult to prototype this in a laboratory in a stable manner. At the same time, the fact that the purification reagent of this study includes a highly concentrated solution of ethanol, which is an organic solvent, and the types of adhesives and double-sided tapes that can be selected are considerably limited is also an important matter of concern.

[0042]

## (2) Embodiment of Present Disclosure

A sample pretreatment system 301 and a flow path device 101 according to the present embodiment, which are solutions to the problems discovered in the above consideration (studies), will be described.

[0043]

<System and Flow Path Device>

The sample pretreatment system 301 is identical to the system shown in FIG. 3A. FIG. 9A is a top view of the flow path device 101 according to the present embodiment. Also, FIG. 9B is a diagram showing a sectional configuration along A-A in FIG. 9A. The flow path device 101 includes a membrane storage portion 104 formed on a substrate 102, a first flow path 105, a second flow path 106, a first vent 107, a second vent 108, sealing tapes (film sheets) 103 attached to upper and lower surfaces of the substrate 102, and a purification membrane 201 installed in the membrane storage portion 104. The membrane storage portion 104 is configured of a membrane storage portion upper portion 110 and a membrane storage portion lower portion 111 with different inner diameters with a pedestal surface 109 sandwiched as a boundary such that the pedestal surface 109 where the purification membrane 201 can be installed is formed in the middle between the upper surface and the lower surface of the substrate 102. The inner diameter of the membrane storage portion upper portion 110 is formed to be substantially the same as the diameter of the purification membrane 201, and the inner diameter of the membrane storage portion lower portion 111 is formed to be smaller than the diameters of the purification membrane 201 and the membrane storage portion upper portion 110. The first vent 107 is located at an end point of the first flow path 105. The second vent 108 is located at an end point of the second flow path 106. Both the first vent 107 and the second vent 108 are processed as through-holes on the substrate 102, for example, and upper surfaces thereof are opened. The first flow path 105 is formed on the upper surface of the substrate 102 and is connected to a side wall of a membrane storage portion upper portion 110. The second flow path 106 is formed on the lower surface of the substrate 102 and is connected to a side wall of a membrane storage portion lower portion 111.

[0044]

Specifically, the flow path device 101 according to the present embodiment is formed by cutting the membrane storage portion 104 with an upper portion having a diameter of 6 mm  $\times$  a depth of 1 mm and a lower portion having a diameter of 4 mm  $\times$  a depth of 2 mm, which are coaxial and different dimensions, a first flow path 105 and a second flow path 106 with a width of 1 mm  $\times$  a depth of 1 mm, and a first vent 107 and a second vent 108 with a diameter of 1 mm on the substrate 102 made of PC and having a vertical length of 20 mm  $\times$  a horizontal

length of 50 mm, and a thickness of 4 mm.

[0045]

Materials of the substrate 102 and the sealing tapes 103 are not particularly limited as long as the materials are generally used in the technical field. For example, PC, polypropylene (PP), a cyclic olefin polymer (COP), a cyclic olefin copolymer (COC), polyethylene terephthalate, and polyurethane can be used as a material with a small amount of DNA adsorption. In addition, the amount of adsorption can also be suppressed by modifying the surface to be negatively charged. Other examples of the material include glass materials such as glass, quartz glass, fused quartz, synthetic quartz, alumina, sapphire, ceramics, forsterite, and photosensitive glass, plastics such as polyester resins, polystyrene, polyethylene resins, dimethylpolysiloxane (PDMS), nylon, acrylic resins, fluororesins, polycarbonate resins, polyurethane resins, methylpentene resins, phenol resins, melamine resins, epoxy resins, and vinyl chloride resins, and any combinations thereof. The flow path device 101 can be configured by attaching the sealing tapes (film sheet) 103 made of the material as described above to the upper and lower sides of the substrate 102 with the first flow path 105, the second flow path 106, and the membrane storage portion 104 formed thereon (causing the sealing tapes 103 to adhere thereto with an adhesive that does not inhibit PCR) and at least partially sealing openings formed by the flow paths and the like (at least performing sealing except for the parts corresponding to the first vent 107 and the second vent 108).

[0046]

<Method of Fixing Purification Membrane>

FIGS. 10A to 10C are diagrams for explaining a method of installing the purification membrane 201. Before the sealing tape 103 is attached to the upper surface of the substrate 102, the purification membrane 201 having a diameter P and a thickness Q is installed on the pedestal surface 109 from the membrane storage portion upper portion 110 having a diameter R and a depth D (FIG. 10A). Furthermore, the resin ring 204 having an outer diameter a, an inner diameter b, and a height c (where  $a > R$ ) is press-fitted to fix the purification membrane 201 between the resin ring 204 and the pedestal surface 109 (FIGS. 10B and 10C). After confirming that the inner diameter of the membrane storage portion upper portion 110 and the

outer diameter of the resin ring 204 are the same ( $R' = a'$ :  $R'$  represents the diameter of the membrane storage portion upper portion 110 after the resin ring 204 is press-fitted;  $a'$  represents the outer diameter of the resin ring 204 after press-fitting to the membrane storage portion upper portion 110) and that the purification membrane 201 and the resin ring 204 were able to be installed without floating, the sealing tape 103 is attached to the upper surface of the substrate 102 to thereby form the flow path device 101. Note that the height of the resin ring 204 is preferably a height with which the first flow path 105 is not blocked in an assembled state (FIG. 10C). More preferably, it is desirable that the upper surface (an end surface on the side on which the resin ring 204 is not in contact with the purification membrane 201) of the resin ring 204 be located on a side further downward than the bottom surface of the first flow path 105 at least in the vicinity of the connecting portion between the membrane storage portion upper portion 110 and the first flow path 105 (the thickness  $T$  of the chip - (the depth of the first flow path 105)  $\geq Q + c$ ). In other words, it is desirable that the upper surface of the resin ring 204 be located on the side further downward than the bottom surface portion of the first flow path 105. However, it is desirable that a side surface of the resin ring 204 be in contact with a side surface of the membrane storage portion 104 in an area that is as large as possible. Therefore, it is only necessary to determine the height of the resin ring 204 such that the upper surface of the resin ring 204 corresponds to the height of the bottom surface of the first flow path 105. In this manner, it is possible to prevent the conveyed solution from remaining in the gap between the resin ring 204 and the membrane storage portion 104, and it is also possible to reduce the flow path resistance and to collect a small amount of solution without any loss.

[0047]

In the technology of the present disclosure, “press-fitting” means a fitting state corresponding to a mechanical fitting accuracy criterion from pushing to press-fitting. In other words, the membrane storage portion 104 and the resin ring 204 cannot be moved relative to each other after completion of the press-fitting, and are in a state where it is possible to remove them from each other if a strong force is applied thereto or it is not possible to disassemble them without breaking parts. In the case of the membrane fixing structure based on the O-ring shown in PTL 10 or the above discussion (consideration of the problems), the O-ring 202 is elastically

deformed (the shape is recovered if an external force is removed). Therefore, after attachment to the membrane storage portion upper portion 110, the O-ring returns in the direction away from the membrane to repel the external force of the attachment. At the same time, a frictional force acts between the outer periphery of the O-ring 202 and the side wall of the membrane storage portion upper portion 110. Therefore, although the O-ring 202 once housed does not jump out, its force to push down the purification membrane is weak. Therefore, this does not correspond to the “press-fitted” state in the technology of the present disclosure.

[0048]

On the other hand, in a case where the resin ring 204 is inserted (press-fitted) into the membrane storage portion upper portion 110, the resin ring 204 experiences plastic deformation (the deformation remains even after the external force is removed, and the shape is not recovered to the original one) and is held at the place with a frictional force with the side wall of the membrane storage portion upper portion 110. Therefore, a force in a direction of pushing down the purification membrane 201 is maintained in the membrane storage portion 104. At this time, the resin ring 204 can be held in the fitted state from a pushed state to a press-fitted state by appropriately setting the dimension range relative to the membrane storage portion upper portion 110. It is thus possible to realize a high membrane fixing force. Whether or not the membrane fixing structure based on the press-fitting of the resin ring 204 has been realized in the flow path device 101 can be confirmed at least by the fact that  $R' = a'$  at the position where the side wall of the membrane storage portion upper portion 110 and the resin ring 204 are closest to each other in the assembled state, more strictly, at least by precisely observing the flow path device 101 in the assembled state and observing  $|R' - a'| < 1 \mu\text{m}$  (not more than the resolution of the optical microscope) at the position where the side wall of the membrane storage portion upper portion 110 and the resin ring 204 are closest to each other. Implementation of the technology of the present disclosure can also be confirmed by measuring the inner diameter of the membrane storage portion upper portion 110 and the outer diameter of the resin ring 204 in the non-assembled state (FIG. 10B) and the assembled state (FIG. 10C), and confirming that  $R/R' (\approx 1) > a'/a$  is satisfied. In other words, the feature that the membrane storage portion upper portion 110 is hardly deformed due to high rigidity in the non-assembled

state and the assembled state and the resin ring 204 with the larger outer diameter than the inner diameter R of the membrane storage portion upper portion 110 is installed such that it contracts is an important feature of the technology of the present disclosure. Note that although known examples of a membrane fixing method based on press-fitting using a resin ring include the configuration of the spin column as exemplified in PTL 2 as well, when the inner diameters of the spin column in the non-assembled state and the assembled state are defined as S and S', respectively, and the outer diameters of the ring are defined as A and A', respectively,  $S/S' < A'/A (\approx 1)$  is satisfied, and there is a relationship opposite to that in the case of the technology of the present disclosure. This is attributable to the fact that in the case of the configuration of the spin column, the spin column is a thin container with low rigidity and can be deformed by press-fitting of the ring. In a case of a spin column included in the QIAamp DNA Investigator Kit (Qiagen), for example,  $P = 7$  mm,  $S =$  about 6.4 mm to about 6.7 mm,  $A =$  about 7 mm to about 7.2 mm, and  $S' = A' \approx A$  are satisfied.

[0049]

Specifically, in the present embodiment, the purification membrane 201 (Silica membrane filter, GF/F, Whatman) having a diameter of 6 mm  $\times$  a thickness of 0.4 mm and the resin ring 204 made of PE and having an outer diameter of 6.2 mm  $\times$  an inner diameter of 4 mm  $\times$  a height of 0.5 mm are installed in the membrane storage portion 104 in the flow path device 101.

[0050]

It is only necessary for the purification membrane 201 to be a membrane that can hold particles of equal to or greater than 100  $\mu\text{m}$ . The film thickness is preferably equal to or greater than 1  $\mu\text{m}$ . Furthermore, since DNA can be more efficiently collected as the pore size is finer, it is desirable that the purification membrane 201 can hold particles of equal to or greater than 10  $\mu\text{m}$ , more preferably equal to or greater than 1  $\mu\text{m}$ , and yet more preferably equal to or greater than 0.1  $\mu\text{m}$ . The type of the membrane may not be a silica membrane, and a purification membrane made of a solid base material containing cellulose capable of adsorbing DNA as a main component, carboxylated particles, or an ion exchange resin may be used. If the volume of the purification membrane is excessively small, the amount of biomolecules that can be

adsorbed decreases. On the other hand, if the volume is excessively large, there is a concern of a probability that unintended molecular adsorption occurs in purification or a later step or deterioration of conveyance efficiency of the solution, and it is thus necessary to appropriately set the volume although the size is not particularly limited.

[0051]

The material of the resin ring 204 may not be PE, and it is desirable to use a material that is not elastically deformed, such as a polyester resin, polystyrene, a polyethylene resin, nylon, an acrylic resin, a fluororesin, a polycarbonate resin, a polyurethane resin, a methylpentene resin, a phenol resin, a melamine resin, an epoxy resin, or a vinyl chloride resin, and the resin ring 204 may not be made of a resin. Specific physical properties include a property that the Young's modulus of the material is equal to or greater than 5 MPa and equal to or less than 1 GPa, that the elastic modulus is equal to or greater than 100 kPa, and that the elastic strain limit value is less than 100%.

[0052]

The outer diameter  $a$  of the resin ring 204 may be any dimension as long as the resin ring 204 can be fitted to the membrane storage portion upper portion 110 and can achieve fixing through press-fitting with the dimension. Specifically, it has become apparent from the studies of the present inventors that the outer diameter  $a$  of the resin ring 204 is preferably designed to be larger than the inner diameter  $R$  of the membrane storage portion upper portion 110 and within a range satisfying  $0 \leq a - R < 400 \text{ } \mu\text{m}$  or  $(a - R)/R \leq 0.2$ . Details of the outer diameter  $a$  of the resin ring 204 will be described later.

[0053]

Although it is only necessary for the inner diameter  $b$  of the resin ring 204 to be a diameter with which the solution can pass, it is desirable that the inner diameter  $b$  be substantially the same as the inner diameter  $r$  of the membrane storage portion lower portion. In this manner, it is possible to cause the solution in contact with the purification membrane 201 to efficiently pass through the membrane storage portion lower portion 111 and to maximize the amount of solution that can be collected. Also, it is desirable that the difference between the outer diameter  $a$  and the inner diameter  $b$  of the resin ring 204 be as small as

possible. Specifically, it is desirable that the difference is equal to or less than about 2.5 mm. In this manner, it is possible to prevent the solution from being captured and remaining on the upper surface of the resin ring 204 and to more efficiently collect the solution.

[0054]

#### <Experimental Results>

FIGS. 11A and 11B are diagrams showing results of performing a nucleic acid purification experiment using the sample pretreatment system shown in FIG. 3A and the flow path device 101 in FIG. 9 and quantitatively evaluating the amount of collected eluent and DNA purification efficiency. Note that the operation was performed by the sample solution and the treatment process shown in FIG. 1 in the present embodiment as in above “Further Consideration of Problems in Nucleic Acid Purification”.

[0055]

As shown in FIG. 11A, the amount of collected eluent of the chip device of the technology of the present disclosure was 13  $\mu$ L on average. Although the amount of the collected eluent was slightly smaller as compared with the spin column method, satisfactory collection efficiency (equal to or greater than 50%) was obtained as compared with the conventional chip device. The dark (left-side) bar graph of each scheme in FIG. 11B is a diagram showing purification efficiency (DNA collection efficiency) calculated by quantifying the amount of DNA contained in the eluent collected in FIG. 11A by real-time PCR. The light (right-side) bar graph of each scheme in FIG. 11B shows the DNA leakage rate calculated by quantifying the amount of DNA contained in the sample solution (hereinafter, referred to as a waste liquid) having passed through the purification membrane. As shown in FIG. 11B, in the case where the chip device according to the present embodiment was used, a high DNA leakage rate, which had been a problem of the conventional chip device, was solved, and purification efficiency at a level equivalent to that of the spin column method was obtained. Note that in the purification treatment process according to the present embodiment, the solution conveyance pressure required is 65 kPa at the maximum, which is the same level as in the case of the conventional chip device. As described above, according to the present embodiment, it is possible to achieve strong membrane fixation without sample leakage and stable feeding of

a small amount of solution by using the chip device and the small-sized device with simple configurations and to realize high efficiency purification with performance at a level equivalent to that of the spin column method. The above technical effects are similar in embodiments described below and derivatives thereof.

[0056]

### (3) Consideration Regarding Problems of Flow Path Device to Which Spin Column Configuration Is Applied

As shown in FIG. 11B, high efficiency purification can be realized even in a case where the spin column configuration is used. Here, problems of the flow path device 101 to which the configuration of the spin column that is the only known example for achieving membrane fixation by press-fitting of a resin ring is applied will be examined and considered, and advantages of the flow path device 101 (configuration not using the porous holding member 205) according to the present disclosure shown in FIG. 9 will be further clarified.

[0057]

FIG. 12A is a diagram showing an upper surface configuration of the flow path device 101 to which the spin column configuration is applied. FIG. 12B is a diagram showing A-A sectional configuration in FIG. 12A. The flow path device 101 used for the consideration includes a porous holding member 205 having a diameter of 6.7 mm  $\times$  a thickness of 2 mm, a purification membrane 201 having a diameter of 7.0 mm  $\times$  a thickness of 0.4 mm, and a resin ring 204 made of PE and having an outer diameter a (seven types of a = 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and 7.1)  $\times$  an inner diameter of 5 mm  $\times$  a height of 0.5 mm in the membrane storage portion 104. The flow path device 101 to which the spin column configuration is applied is formed by cutting a tapered membrane storage portion 104 in which the diameter is continuously changed from 7.1 mm at the upper portion to 5 mm at the lower portion, the first flow path 105 and the second flow path 106 with a width of 1 mm  $\times$  a depth of 1 mm, and the first vent 107 and the second vent 108 with a diameter of 1 mm on the substrate 102 made of PC and a vertical length of 20 mm  $\times$  a horizontal length of 50 mm  $\times$  a thickness of 5 mm. The first vent 107 is located at an end point of the first flow path 105. The second vent 108 is located at an end point of the second flow path 106. Both the first vent 107 and the second vent 108 are processed as through-

holes on the substrate 102, and upper surfaces thereof are opened. The first flow path 105 is formed on the upper surface of the substrate 102 and is connected to the side wall of the membrane storage portion 104. Also, the second flow path 106 is formed on the lower surface of the substrate 102 and is connected to the side wall of the membrane storage portion 104.

[0058]

The porous holding member 205 has a function of holding the purification membrane 201 at a specific position in the membrane storage portion 104 and is deformed to collapse in the centripetal direction when press-fitted into the spin column and the flow path device 101. First, the porous holding member 205 was press-fitted and fixed to the membrane storage portion 104 such that the porous holding member 205 was horizontal to the surface direction (procedure 1). The purification membrane 201 is placed on the fixed porous holding member 205 (procedure 2), and the resin ring 204 was press-fitted and fixed to be horizontal to the surface direction (procedure 3). It has been discovered through the processes of the purification membrane fixing procedures 1 to 3 that there are multiple problems below in practice.

[0059]

(i) The first problem in the purification membrane fixing procedure in the case of using the flow path device 101 based on the spin column configuration including the porous holding member 205 is that it is difficult to control the fixation position of the porous holding member 205 in the procedure 1. The porous holding member 205 is easily deformed in response to a stress from the side wall of the membrane storage portion 104 through press-fitting. Therefore, if pressurization from the upper portion is continued even after reaching the predetermined fixation position, the porous holding member 205 extends over the connecting portion between the membrane storage portion 104 and the second flow path 106, and the flow path resistance thus increases. Finally, the porous holding member 205 reaches the bottom portion of the membrane storage portion 104 and completely blocks the second flow path 106. If the sample solution 401 is introduced from the first flow path 105 in this state, it is not possible to efficiently collect the sample solution 401 due to the flow path resistance. On the contrary, if the pressurization from the upper portion is stopped before reaching the predetermined fixation position, the resin ring 204 extends over the connecting portion between the membrane storage

portion 104 and the first flow path 105 when the purification membrane 201 and the resin ring 204 are fixed, and the flow path resistance thus increases. If the sample solution 401 is introduced from the first flow path 105 in this state, the sample solution 401 may not efficiently come into contact with the purification membrane 201, or unintended routing of the sample solution 401 to a gap may occur.

[0060]

In the case where the flow path is provided on the substrate 102 and the purification membrane 201 is installed therein as in the flow path device 101 (see FIG. 9) according to the present disclosure, it is generally possible to expect further simplification, size reduction, and cost reduction of the flow path device as the thickness of the substrate 102 is thinner, and it is thus preferable to provide the flow path in the surface direction of the purification membrane 201. On the other hand, in the case of the spin column method, there is no motivation to provide the flow path in the surface direction of the purification membrane, and therefore, the disadvantage indicated as the first problem does not occur. In other words, in the case of the spin column method, no disadvantages of using the porous holding member 205 are present.

[0061]

(ii) The second problem in the purification membrane fixing procedure in the case of using the flow path device 101 having the spin column configuration including the porous holding member 205 is that it is not possible to press-fit and fix the resin ring 204 to the membrane storage portion 104 by dimension control at the same level as that of the spin column method in the above procedure 3. As described above in the configuration of the flow path device 101 of the present embodiment (FIG. 9), the resin ring 204 is deformed in response to a stress from the side wall of the membrane storage portion 104 when press-fitted to the membrane storage portion 104. At this time, since the resin ring 204 is not an elastic material like rubber, the resin ring 204 collapses without entering the membrane storage portion 104, or cracking occurs on the substrate 102 in the vicinity of the membrane storage portion 104, which leads to breakage, if the resin ring 204 is excessively large relative to the membrane storage portion 104.

[0062]

FIGS. 13A and 13B are views (photographs) showing an example of the resin ring 204 that has failed to be press-fitted and fixed and has been deformed on the membrane storage portion 104. FIG. 13C is a table summarizing examples of outer diameter dimensions and success/failure of press-fitting and fixing of the resin ring 204. A press-fitting failure as shown in FIG. 13A was observed when the inner diameter R of the membrane fixing portion 104 at the fixation position of the purification membrane 201 was about 6.5 mm and the outer diameter a of the resin ring 204 was equal to or greater than 6.9 mm, that is,  $(a - R)$  was equal to or greater than at least 400  $\mu\text{m}$ . In regard to this relationship, the same results were obtained in a case where the inner diameter b and the thickness (height) c of the resin ring 204 were changed and in a case of the membrane storage portion 104 (FIG. 9) including a pedestal portion instead of the porous holding member 205 as well. At the same time, an event in which press-fitting and fixing failed within a range satisfying  $a - R \geq 400 \mu\text{m}$  was observed at least in a case of  $1 \text{ mm} \leq R \leq 7 \text{ mm}$ . The main reason for the failure of the press-fitting and fixing is considered to be because rigidity of the substrate 102 is large as compared with the spin column method, and the strain when the resin ring 204 is press-fitted cannot be absorbed by both the resin ring 204 and the substrate 102.

[0063]

In the case of the spin column method, the container storing the purification membrane 201 is thin and has low rigidity, and it is thus not necessary to consider any dimensional range like that for the flow path device 101 according to the present disclosure. As described above, the flow path device 101 of the present disclosure can be stably manufactured at a practical level by designing the inner diameter R of the membrane storage portion 104 and the resin ring 204 to satisfy, for example,  $0 \leq a - R < 400 \mu\text{m}$ . This dimensional range can be selected depending on physical properties such as materials, rigidity, and bending moment of the substrate 102 and the resin ring 204, and is preferably set in an appropriate manner.

[0064]

(iii) Another problem in the case of using the flow path device 101 based on the spin column configuration including the porous holding member 205 is that the eluent 403 is

captured by the porous holding member 205 and the amount of the collected liquid decreases. The porous holding member 205 is made of a porous material in order to enable the sample solution 401 having passed through the purification membrane 201 to be collected while holding the purification membrane 201 at a specific position in the membrane storage portion 104. The porous holding member 205 has a water holding capacity in accordance with a material, a pore diameter, a volume, and the like. In the case of the spin column method, since the eluent 403 input to the column is caused to pass through the purification membrane 201 by a centrifugal force (for example, 20,000 g), most of the eluent 403 retained inside the porous holding member 205 can also be collected, and this problem does not occur.

[0065]

On the other hand, when the liquid is fed by a small general-purpose pneumatic pump as in the technology of the present disclosure, for example, without using a centrifugal force, the eluent 403 captured by the porous holding member 205 cannot be collected, and the amount of collected liquid considerably decreases. Furthermore, in the case of performing the purification flow of the Boom method shown as an example in FIGS. 1, 2A, and 2B, the sample solution 401 and the cleaning liquid 402 may remain in the porous holding member 205, which may lead to a likelihood that these components are mixed into the eluent 403 after the collection and the eluent 403 is contaminated. Although this event was improved (in the case of the Boom method) to a certain extent by using a hydrophobic porous holding member 205 made of PTFE, for example, increasing the pore size, or reducing the thickness and thus volume of the porous holding member 205, it was discovered that a sufficient improvement was not obtained by such a measure in the case of the configuration according to the present disclosure. Therefore, it is preferable to adopt a configuration not using the porous holding member 205 in implementing the flow path device 101 according to the present disclosure.

[0066]

(iv) As described above, it is possible to ascertain that the application of the spin column configuration to the flow path device 101 is not appropriate and the configuration like the flow path device 101 according to the present embodiment described above is preferably employed.

[0067]

### (3) Details of Liquid Feeding Mechanism and Reagent Holding Portion

Here, detailed configurations of the liquid feeding mechanism 302 and the reagent holding portion 303 in the sample pretreatment system 301 of the present embodiment will be described.

[0068]

#### <Configuration of Liquid Feeding Mechanism 302>

FIG. 14A is a diagram showing an internal configuration example of the liquid feeding mechanism 302. FIG. 14B is a diagram showing an internal configuration example of the liquid feeding mechanism 302 including a multi-way valve mechanism (V0) capable of opening to atmosphere.

[0069]

According to FIG. 14A, the liquid feeding mechanism 302 includes a first connection port 3001 and a second connection port 3002 connected to the reagent holding portion 303, a liquid feeding power source 3003 connected to the first connection port 3001 to provide a liquid feeding pressure, and a pressure control mechanism 3004 that adjusts the liquid feeding pressure provided from the liquid feeding power source 3003. For example, the liquid feeding power source 3003 and the pressure control mechanism 3004 can be configured by combining a diaphragm pump or a DC fan having a specific output (for example, 100 kPa) and a vacuum regulator, or may be integrated using a syringe pump or a DC fan capable of controlling the amount of ejection/suction.

[0070]

According to FIG. 14B, the liquid feeding mechanism 302 includes a multi-way valve mechanism 3006 (V0) that can be opened to the atmosphere between the pressure control mechanism 3004 and the first connection port 3001 in addition to the configuration shown in FIG. 14A. In the case of the configuration of FIG. 14B, the connection between the first connection port 3001 and the liquid feeding power source 3003 can be instantaneously disconnected by operating the multi-way valve mechanism 3006 at the moment at which it is desired to stop the liquid feeding operation, and it is thus possible to obtain high-speed

responsiveness. Furthermore, since the liquid feeding power source 3003 can be freely operated in the atmosphere open state, an operation of starting liquid feeding after the outputs of the liquid feeding power source 3003 and the pressure control mechanism 3004 are stabilized and an operation of returning a plunger of a syringe to a predetermined position in a case where a syringe pump is used as the liquid feeding power source 3003 can be performed with the piping tube 307 connected to the reagent holding portion 303. It is also possible to operate such that the timing at which the pressure is applied to the first connection port 3001 is controlled by an operation of the multi-way valve mechanism 3006 with the liquid feeding power source 3003 constantly driven. The configuration of FIG. 14B can be used in a case where it is desired to minimize the number of times the liquid feeding power source 3003 and the pressure control mechanism 3004 are switched, such as a case where the liquid feeding power source 3003 with large power consumption immediately after current application is used. Note that the multi-way valve mechanism 3006 can also achieve an equivalent function by combining a plurality of on-off valve mechanisms 3005 as shown in FIG. 15.

[0071]

The liquid feeding power source 3003, the pressure control mechanism 3004, and the multi-way valve mechanism 3006 may be connected to the second connection port 3002. In this case, an equivalent operation can be achieved by performing pressurization and depressurization operations opposite to those in the case where they are connected to the first connection port 3001. It is also possible to use pairs of liquid feeding power sources 3003, pressure control mechanisms 3004, and multi-way valve mechanisms 3006 and to connect each of them to both the first connection port 3001 and the second connection port 3002. It is thus possible to establish access from both the connection ports to the flow path device 101 via the reagent holding portion 303 and to select a liquid feeding route that minimizes influences of a liquid feeding pressure loss due to an increase in piping length. Similarly, a multi-way valve mechanism 3006 having a function of a three-way valve that can switch a pair of liquid feeding power source 3003 and the pressure control mechanism 3004, the first connection port 3001, the second connection port 3002, or the atmosphere opening flow path 3007 can be used as a set of the liquid feeding power source 3003 and the pressure control mechanism 3004. In this

manner, it is possible to reduce the size of the device while enabling the pressure to be applied to both the first connection port 3001 and the second connection port 3002.

[0072]

<Configuration of Reagent Holding Portion 303>

FIG. 16 is a diagram showing an internal configuration example of the reagent holding portion 303. The reagent holding portion 303 includes, for example, a first liquid feeding port 3101 connected to the first connection port 3001 of the liquid feeding mechanism 302, a second liquid feeding port 3102 connected to the second connection port 3002, a first device connection port 3103 connected to the first vent 107 of the flow path device 101, a second device connection port 3104 connected to the second vent 108, at least one or more reagent tanks 3105 (T1) attachable and detachable between the first liquid feeding port 3101 and the first device connection port 3103 via the on-off valve mechanism 3005, and a waste liquid tank 3106/solution collection tank 3107 (T2) attachable and detachable between the second liquid feeding port 3102 and the second device connection port 3104. Note that the tank T2 is denoted as 3106/3107 in FIG. 16 because the waste liquid tank 3106 is attached first and is then replaced with the solution collection tank 3107 after performing an operation in the middle.

[0073]

The liquid feeding mechanism 302 and the reagent holding portion 303, and the reagent holding portion 303 and the flow path device 101 may be connected via the piping tube 307, or may be directly connected by a piping joint. The first liquid feeding port 3101 and the second liquid feeding port 3102 are pressurized or depressurized by the control of the liquid feeding mechanism 302, and the reagent stored in the reagent tank 3105 can be conveyed in order to the flow path device 101 and can be collected in the waste liquid tank 3106 or the solution collection tank 3107.

[0074]

FIG. 17 is a flowchart for explaining a nucleic acid purification treatment in the nucleic acid purification system shown in FIG. 16. First, the reagent tank 3105 containing the sample solution 401 and the waste liquid tank 3106 are connected at the position of T1 and the position of T2 in FIG. 16, respectively (S1701). The connection may be automatically performed by a

robot (robot arm) in the system, or may be performed by an operator. The liquid feeding mechanism 302 introduces the sample solution 401 (sample) held in the reagent tank 3105 into the first vent 107 of the flow path device 101 and collects, in the waste liquid tank 3106, the sample solution 401 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1702). Next, the operator or the robot removes the empty tank from T1 in FIG. 16 and replaces the empty tank with the reagent tank 3105 containing a first cleaning liquid 402 (S1703). The liquid feeding mechanism 302 introduces the first cleaning liquid 402 (cleaning liquid 1) held in the reagent tank 3105 into the first vent 107 of the flow path device 101 and collects, in the waste liquid tank 3106, the first cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1704). Next, the operator or the robot removes the empty tank from T1 in FIG. 16 and replaces the empty tank with the reagent tank 3105 containing a second cleaning liquid 402 (S1705). The liquid feeding mechanism 302 introduces the second cleaning liquid 402 (cleaning liquid 2) held in the reagent tank 3105 into the first vent 107 of the flow path device 101 and collects, in the waste liquid tank 3106, the second cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1706). Similarly, the operator or the robot removes the empty tank from T1 in FIG. 16 and replaces the empty tank with the reagent tank 3105 containing a third cleaning liquid 402 (S1707). The liquid feeding mechanism 302 introduces the third cleaning liquid 402 (cleaning liquid 3) held in the reagent tank 3105 into the first vent 107 of the flow path device 101 and collects, in the waste liquid tank 3106, the third cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1708). Following the conveyance of the third cleaning liquid 402, the liquid feeding mechanism 302 sends dry air to the flow path device 101 for three minutes (S1709). Finally, the operator or the robot removes the empty tank from T1 in FIG. 16 and replaces the empty tank with the reagent tank 3105 containing the eluent 403. At the same time, the operator or the robot removes the waste liquid tank 3106 from

T2 and replaces the waste liquid tank 3106 with the solution collection tank 3107 (S1710). The liquid feeding mechanism 302 introduces the eluent 403 held in the reagent tank 3105 into the first vent 107 of the flow path device 101 and collects, in the solution collection tank 3107, the eluent 403 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1711).

[0075]

Note that the reagent tank 3105, the waste liquid tank 3106, and the solution collection tank 3107 are not particularly limited as long as these are generally used in the technical field. For all of these, microtubes, conical tubes, blister packs, pouch packs, or the like that have chemical resistance to the reagent to be used and can be connected to the liquid feeding mechanism 302 and the flow path device 101 with an appropriate connector may be adopted. For example, combinations of Microfluidic Reservoirs (Elveflow) and a microtube can be used. In addition, the reagent tank 3105 may perform the treatment in FIG. 17 by replacing tanks storing reagents in advance in order at the position of T1 in FIG. 16. Alternatively, reagents may be input in order to the empty reagent tank 3105 after each process in FIG. 17 ends, and the treatment may proceed to the next process in FIG. 17. As described above, the operation of replacing the tank or inputting the reagents may be manually performed or can be automatically performed using a robot arm or the like.

[0076]

Also, the first liquid feeding port 3101 may be connected to the second connection port 3002, and the second liquid feeding port 3102 may be connected to the first connection port 3001. Similarly, the first device connection port 3103 may be connected to the second vent 108, and the second device connection port 3104 may be connected to the first vent 107.

[0077]

#### <Modification Example of Reagent Holding Portion 303>

A configuration example of a nucleic acid purification system (reagent holding portion 303) more suitable for automation will be described with reference to FIGS. 18 and 19. FIG. 18 is a diagram showing another internal configuration example (modification example) of the reagent holding portion 303 in the nucleic acid purification system. FIG. 19 is a flowchart for

explaining a nucleic acid purification treatment in the nucleic acid purification system shown in FIG. 18.

[0078]

In FIG. 18, the reagent holding portion 303 includes a first liquid feeding port 3101 connected to the first connection port 3001 of the liquid feeding mechanism 302, the second liquid feeding port 3102 connected to the second connection port 3002, the first device connection port 3103 connected to the first vent 107 of the flow path device 101, the second device connection port 3104 connected to the second vent 108, at least one or more reagent tanks 3105 disposed between the first liquid feeding port 3101 and the first device connection port 3103 via the on-off valve mechanism 3005 and holding a reagent therein, and at least one or more waste liquid tanks 3106 and solution collection tanks 3107 that are disposed between the second liquid feeding port 3102 and the second device connection port 3104 via the multi-way valve mechanism 3006 and collect a solution discharged from the flow path device. The liquid feeding mechanism 302 and the reagent holding portion 303, and the reagent holding portion 303 and the flow path device 101 may be connected via the piping tube 307, or may be directly connected by a piping joint. The first liquid feeding port 3101 and the second liquid feeding port 3102 are pressurized or depressurized by the control of the liquid feeding mechanism 302, and the reagents can be conveyed in order to the flow path device 101 by switching the flow paths by the on-off valve mechanism 3005 and the multi-way valve mechanism 3006. Therefore, an operation of replacing the reagent tank 3105, the waste liquid tank 3106, and the solution collection tank 3107 is not needed, and it is possible to achieve automation of the nucleic acid purification treatment by a simpler system as compared with the configuration in FIG. 16.

[0079]

In FIG. 19, the control device (not shown: configured of a computer connected to the system, for example) operates the on-off valve mechanism 3005 first to bring V1 and V6 into an open state and bring V2 to V5 and V7 to V10 into a closed state. At the same time, the control device operates the multi-way valve mechanism 3006 (V11 and V12) and connects the second liquid feeding port 3102 and the second device connection port 3104 to the waste liquid

tank 3106 (S1901). The control device causes the liquid feeding mechanism 302 to operate, introduces the sample solution 401 (sample) held in the first reagent tank 3105 to the first vent 107 of the flow path device 101 and collects, in the waste liquid tank 3106, the sample solution 401 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1902). Next, the control device operates the on-off valve mechanism 3005 to bring V2 and V7 into an open state and V1, V3 to V6, and V8 to V10 into a closed state (S1903). The control device causes the liquid feeding mechanism 302 to operate, introduces the first cleaning liquid 402 (AW1) held in the second reagent tank 3105 to the first vent 107 of the flow path device 101, and collects, in the waste liquid tank 3106, the first cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1904). After the conveyance of the first cleaning liquid 402 is ended, the control device operates the on-off valve mechanism 3005 to bring V3 and V8 into an open state and bring V1, V2, V4 to V7, V9, and V10 into a closed state (S1905). The control device causes the liquid feeding mechanism 302 to operate, introduces the second cleaning liquid 402 (AW2) held in the third reagent tank 3105 to the first vent 107 of the flow path device 101, and collects, in the waste liquid tank 3106, the second cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1906). The control device operates the on-off valve mechanism 3005 to bring V4 and V9 into an open state and bring V1 to V3, V5 to V8, and V10 into a closed state (S1907). The control device causes the liquid feeding mechanism 302 to operate, introduces the third cleaning liquid 402 (EtOH) held in the fourth reagent tank 3105 to the first vent 107 of the flow path device 101, and collects, in the waste liquid tank 3106, the third cleaning liquid 402 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1908). Following the conveyance of the third cleaning liquid 402, the control device causes the liquid feeding mechanism 302 to operate to send dry air to the flow path device 101 for three minutes (S1909). Finally, the control device operates the on-off valve mechanism 3005 to bring V5 and V10 into an open state and bring V1 to V4

and V6 to V9 into a closed state, and operates the multi-way valve mechanism 3006 (V11 and V12) to connect the second liquid feeding port 3102 and the second device connection port 3104 to the solution collection tank 3107 (S1910). The control device causes the liquid feeding mechanism 302 to operate, introduces the eluent 403 (ATE) held in the fifth reagent tank 3105 to the first vent 107 of the flow path device 101, and collects, in the solution collection tank 3107, the eluent 403 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second vent 108 (S1911). Note that the solution collected in the waste liquid tank 3106 or the solution collection tank 3107 is preferably held in the waste liquid tank 3106 and the solution collection tank 3107 without leaking from the second liquid feeding port 3102 or returning to the flow path device 101. Therefore, it is only necessary for the waste liquid tank 3106 and the solution collection tank 3107 to be designed to have sufficient capacity with respect to the total amount of liquid to be conveyed thereto. Alternatively, this can also be achieved by providing a filter vent mechanism that allows passage of gas and does not allow passage of liquid in the waste liquid tank 3106 and the solution collection tank 3107 and bringing the connection to the second liquid feeding port 3102 into a closed state in a system performing the nucleic acid purification flow in FIG. 17 by a pressurizing operation from the first connection port 3001.

[0080]

The on-off valve mechanism 3005 and the multi-way valve mechanism 3006 can be replaced with each other as shown in FIG 15. If the multi-way valve mechanism 3006(V11 and V12) in FIG. 16 is replaced with the on-off valve mechanism 3005, the same valve mechanisms can be aligned in parallel, which is advantageous in terms of controllability, expansibility, and the like. On the other hand, if the on-off valve mechanism 3005 (V1 to V10) in FIG. 16 is replaced with the multi-way valve mechanism 3006, the valve mechanisms can be integrated, and size reduction of the system, and sharing of the control mechanism may become easy. Also, the valve mechanism may not necessarily be provided at each of both ends of the reagent tank 3105 and may be disposed only on the upstream side (V1 to V5) or only on the downstream side (V6 to V10). As shown in FIG. 16, if the valve mechanism is provided at each of both ends of the reagent tank 3105, it is possible to reduce the likelihood that unintended leakage of

the reagent occurs due to a capillary phenomenon, a change in flow path inner pressure, or the like and to realize more stable liquid feeding. On the other hand, if the valve mechanism is provided only on one side of the reagent tank, it is possible to reduce the number of valve mechanisms and to construct a simpler system with a smaller size. The on-off valve mechanism 3005 and the multi-way valve mechanism 3006 may be any mechanisms as long as the mechanisms have chemical resistance to the reagent used for the nucleic acid purification treatment and can pass or stop a fluid or adjust a flow rate, such as diaphragm valves, needle valves, bellows valves, or gate valves, for example, and the operation may be performed manually or may be controlled by an electric signal or the like.

[0081]

### (3) Reagent Tank Integrated Nucleic Acid Purification System to Which Configuration of Flow Path Device 101 of Present Disclosure Is Applied

A sample pretreatment system and a nucleic acid purification method according to a reagent tank integrated nucleic acid purification device will be described with reference to FIGS. 20 to 22.

[0082]

#### <Configuration Example of Reagent Tank Integrated Nucleic Acid Purification System>

FIG. 20 is a diagram showing a system configuration example of a sample pretreatment system 301 in which a reagent tank integrated flow path device (nucleic acid purification device) 101 is installed. The flow path device (nucleic acid purification device) 101 includes a membrane storage portion 104 formed on a substrate 102, a first flow path 105, a second flow path 106, a first vent 107, a second vent 108, sealing tapes 103 attached to upper and lower surfaces of the substrate 102, a purification membrane 201 fixed to the membrane storage portion 104 by press-fitting of a resin ring 204, at least one or more reagent tanks 3105 installed between the first vent 107 and the first flow path 105 via an on-off valve mechanism 3005, a waste liquid tank 3106 connected to the second flow path 106 via the on-off valve mechanism 3005 and including a vent filter 3109, and a solution collection flow path 3108 connected to the second flow path 106 via the on-off valve mechanism 3005 and connected to the second vent 108. The configuration in the vicinity of the membrane storage portion 104 is similar to that in

FIGS. 9A, 9B, 14A, and 14B. The second vent 108 is connected to the solution collection tank 3107 provided in the reagent holding portion 303.

[0083]

FIG. 21A is a diagram showing an upper surface configuration example in the vicinity of the reagent tank 3105. FIG. 21B is a diagram showing a sectional configuration example along A-A in FIG. 21A. In the reagent tank 3105, the reagent solution is sealed in a hollow container having an internal volume slightly larger than amount of reagent liquid (for example, 800  $\mu\text{L}$  of sample solution, 1000  $\mu\text{L}$  of cleaning liquid, and 20  $\mu\text{L}$  of eluent), and the reagent tank 3105 is attached to the substrate 102 at the upper portion of the connection flow path 1001 provided with a protruding portion 1004. Once the reagent tank 3105 is pushed by a reagent tank actuator 3110 and collapsed, the protruding portion 1004 comes into contact with a sealing member 1003 at the bottom portion of the reagent tank 3105 and breaks the sealing member 1003. In this manner, the reagent can flow into the connection flow path 1001. In the reagent tank integrated nucleic acid purification device, the reagent tank 3105 is formed by molding an aluminum sheet having a thickness of 50  $\mu\text{m}$  by bending while providing the reagent filling port 1005 and the vent 1006 therein and welding an aluminum sheet having a thickness of 10  $\mu\text{m}$  as the sealing member 1003 to the bottom portion. Then, a micropipette is inserted from the reagent filling port 1005, the inside of the reagent tank 3105 is filled with the reagent, and the reagent filling port 1005 and the vent 1006 are then sealed with a sealing tape. Finally, a double-sided tape is attached to an upper portion of the connection flow path 1001 provided on the substrate 102. As the reagent tank actuator 3110, it is possible to use a small linear actuator having a propulsive force of 10 N to which a tapered tip jig is connected.

[0084]

The on-off valve mechanism 3005 is configured of a valve portion 1002 provided on the flow path device 101 and a valve actuator 3111. The valve portion 1002 has a shape that matches the tip of the valve actuator 3111. It is possible to seal the connection flow path 1001 by pushing the valve portion 1002 by the valve actuator 3111. The reagent tank integrated nucleic acid purification device includes the dome-shaped valve portion 1002 having a curvature of  $\phi 1\text{ mm}$  in the connection flow path 1001 with a width of  $1\text{ mm} \times$  a depth of  $1\text{ mm}$ .

As the valve actuator 3111, a small linear actuator having a propulsion force of 5 N and having a spherical stainless steel tip with a curvature of  $\phi 1$  mm is used.

[0085]

The material, shape, and the like of the reagent tank 3105 are not particularly limited as long as they are generally used in the technical field, and it is possible to use a blister pack, a pouch pack, or the like. Also, the reagent tank 3105 may be any reagent tank as long as it has chemical resistance to a reagent to be used, can hold the reagent at a predetermined position in the flow path device 101, and can convey the reagent at an arbitrary timing. Preferably, the flow path device 101 is configured such that the reagent can be held at the predetermined position even in a state where the flow path device is not installed in the sample pretreatment system 301 and the reagent tank 3105 is opened by an external force applied from the sample pretreatment system 301 after the flow path device 101 is installed in the sample pretreatment system 301. However, the flow path device 101 may have a configuration in which the reagent cannot be held at the predetermined position in the state where the flow path device 101 is not installed in the sample pretreatment system 301. In this case, it is only necessary to introduce the reagent from the reagent filling port 1005 and to seal the reagent filling port 1005 and the vent 1006 after performing the installation in the sample pretreatment system 301 and bringing all the on-chip valves into a closed state, for example. Note that the reagent tank 3105 may not necessarily include the reagent filling port 1005 and the vent 1006. The sealing member 1003 may be assembled after the filling with the reagent. Also, the shape of the reagent tank 3105 may not be a hexagon, and may be, for example, a quadrangle, a triangle, a circle, or the like. However, a shape in which the liquid feeding direction is diagonally arranged can minimize the liquid remaining inside the reagent tank 3105. Moreover, the external force applied from the sample pretreatment system 301 to the reagent tank 3105 may not be a mechanical pressing force that deforms the reagent tank 3105, like that of the reagent tank actuator 3110. For example, a pressure inside the flow path that breaks the sealed portion of the reagent tank 3105 via the flow path connected to the reagent tank 3105 may be used. In particular, in the case of the configuration in which the reagent tank 3105 is opened by the pressure inside the flow path, the liquid feeding mechanism 302 and the piping flow path necessary to perform the nucleic

acid purification flow can be shared, which is advantageous in terms of simplification and size reduction of the mechanism of the system.

[0086]

The on-off valve mechanism 3005 is not particularly limited as long as it is generally used in the technical field, and may be any on-off valve mechanism as long as it has chemical resistance to a reagent to be used and a pressure resistance of equal to or greater than the liquid feeding pressure (100 kPa, for example), and can be installed on the flow path device 101 and control the liquid feeding direction at an arbitrary timing. Specifically, examples of the on-off valve mechanism 3005 include an on-chip diaphragm valve, a valve using magnetic beads, a heat deformation valve, a piezo valve, and the like, in addition to the film deformation valve that achieves a closed state by causing a flow path lid material attached to the surface of the substrate 102 to be deformed by a mechanical pressing force. Alternatively, a rotary on-chip valve may be used to replace the multi-way valve mechanism 3006. An advantage of the film deformation valve is that it can operate with a relatively simple structure and can stably operate at low cost. Therefore, the film deformation valve is a particularly promising option in a high-performance sample pretreatment system that inevitably requires a large number of valve mechanisms because a complicated treatment flow is required and that is required to be small and low cost. On the other hand, in a case of a technology using a rubber O-ring or a gasket ring which is one of conventional membrane fixing technologies, pressurization in the fixing direction (the surface direction of the membrane) is essential. Therefore, it is necessary for the flow path lid material to be formed of a material with high rigidity that is not deformed, or in a case where a film sheet that is easily deformable is attached (see FIG. 21B), it is necessary to provide a pressurizing actuator at the upper portion. Addition of the pressurizing actuator leads to increases in size and cost of the device while the flow path lid material formed of a rigid material does not allow the film deformation valve to be employed, and there is thus still a concern of increases in size and cost of the system. The purification membrane fixing method according to the present disclosure does not require application of an external force from the upper surface after completion of press-fitting of the resin ring 204, and it is thus possible to easily combine it with the film deformation valve, which is one of the effects achieved by the

technology of the present disclosure.

[0087]

The reagent tank actuator 3110 and the valve actuator 3111 may not be linear actuators, and for example, solenoid locks, XYZ stages, stepping motors, DC motors, or the like may be used, or the reagent tank actuator 3110 and the valve actuator 3111 may be operated by an actuator of a plurality of integrated valve mechanisms. The external power source and the acting portion may be separated using an arm, a wire coil, or the like. In this manner, it is possible to reduce the size of the mechanisms to be disposed on the flow path device 101 and to reduce the size of the flow path device 101.

[0088]

<Nucleic Acid Purification Treatment by Reagent Tank Integrated Nucleic Acid Purification System>

FIG. 22 is a flowchart (example) for explaining a nucleic acid purification treatment executed by the nucleic acid purification system shown in FIG. 20. First, the control device (not shown: configured of a computer connected to the system, for example) operates the valve actuator 3111 to bring V1 into an open state and bring V2 to V10 into a closed state, and operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 (sample) containing the sample solution 401 (S2201). Next, the control device operates the valve actuator 3111 to bring V1, V4, and V8 into an open state and bring V2, V3, and V5 to V7 into a closed state. Also, the control device causes the liquid feeding mechanism 302 to operate, introduces the sample solution 401 to the first flow path 105 of the flow path device 101, and collects, in the waste liquid tank 3106, the sample solution 401 passing through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second flow path 106 (S2202). Next, the control device operates the valve actuator 3111 to bring V2 and V8 into an open state and bring V1 and V3 to V7 into a closed state, and further operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 (wash buffer) containing the cleaning liquid 402 (S2203). Thereafter, the control device operates the valve actuator 3111 to bring V2, V5, and V8 into an open state and bring V1, V2 to V4, V6, and V8 into a closed state, further causes the liquid feeding mechanism 302 to operate, introduces

the cleaning liquid 402 held in the second reagent tank 3105 to the first flow path 105 of the flow path device 101, and collects, in the waste liquid tank 3106, the sample solution 401 caused to pass through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second flow path 106 (S2204). Following the conveyance of the cleaning liquid 402, the control device causes the liquid feeding mechanism 302 to operate and sends dry air to the flow path device 101 for three minutes (S2205). Next, the control device operates the valve actuator 3111 to bring V3 into an open state and bring V1, V2, and V4 to V8 into a closed state, and further operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 (elution buffer) containing the eluent 403 (S2206). Finally, the control device operates the valve actuator 3111 to bring V3, V6, and V7 into an open state and bring V1, V2, V4, V5, and V8 into a closed state, further causes the liquid feeding mechanism 302 to operate, introduces the eluent 403 to the first flow path 105 of the flow path device 101, feeds the sample solution 401 caused to pass through the purification membrane 201 installed in the membrane storage portion 104 of the flow path device 101 and discharged from the second flow path 106 to the solution collection flow path 3108, and collects the sample solution 401 in the solution collection tank 3107 connected to the second vent 108 (S2207).

[0089]

Note that although FIG. 22 shows the nucleic acid purification treatment flow using one kind of cleaning liquid for the purification membrane 201, it is possible to address various treatment flows including the nucleic acid purification flow shown in FIG. 2 by changing the disposition and the combination of the reagent tank 3105 and the on-off valve mechanism 3005.

[0090]

(4) Sample-to-Answer-type DNA Analysis System to Which Configuration of Flow Path Device 101 of Present Disclosure Is Applied

[0091]

A sample pretreatment system and a flow path device according to a sample-to-answer-type DNA analysis system will be described with reference to FIGS. 23 to 28. Here, a configuration and content of processing of the system will be described on the basis of an

example in which a person is identified from a swab sample with several  $\mu\text{L}$  of human blood adhering thereto through short tandem repeat (STR) analysis using a capillary electrophoresis device.

[0092]

<Configuration Example of Sample-to-Answer-type DNA Analysis System>

FIG. 23 is a diagram showing an overall configuration example of a sample-to-answer-type DNA analysis system configured of a flow path device 101 and a sample pretreatment system 301 capable of automatically performing nucleic acid extraction, nucleic acid purification, and nucleic acid amplification/detection from a biological sample. The configuration of the sample pretreatment system 301 is substantially similar to the configuration of the system shown in FIG. 3A and includes a liquid feeding mechanism 302, a reagent holding portion 303, and a chip holding portion 304. The first vent 107 and the second vent 108 of the flow path device 101 fixed to the chip holding portion 304 are connected to the reagent holding portion 303 via the piping tube 307. The flow path device 101 includes a nucleic acid extraction portion 1201 that has a sample introduction port 1202 and dissolves a sample to extract a nucleic acid contained in the sample, a nucleic acid purification portion 1101 that is connected to the nucleic acid extraction portion 1201 and removes impurities from the sample solution 401 discharged from the nucleic acid extraction portion 1201 to collect a target nucleic acid, and a nucleic acid amplification portion 1301 that is connected to the nucleic acid purification portion 1101 and amplifies and detects the target nucleic acid contained in the eluent 403 discharged from the nucleic acid purification portion 1101.

[0093]

<Nucleic Acid Extraction Treatment of Nucleic Acid Extraction Portion 1201>

FIG. 24 is a diagram showing a configuration example of the nucleic acid extraction portion 1201 in the sample-to-answer-type DNA analysis system. The nucleic acid extraction portion 1201 includes, on the substrate 102, a sample introduction port 1202, a nucleic acid extraction chamber 1203 connected to the sample introduction port, at least one or more reagent tanks 3105 that hold reagents for performing sample dissolution and a nucleic acid purification reaction, at least one or more on-off valve mechanisms 3005, a purification portion connection

flow path 1204 that connects the nucleic acid extraction chamber 1203 and the nucleic acid purification portion 1101, and a liquid feeding flow path 1205 that is connected to the first vent 107 or the second vent 108 and enables liquid feeding by an operation of the liquid feeding mechanism 302.

[0094]

A sample to be analyzed (for example, a swab sample (sample 400): for example, a swab sample obtained by wiping off a sample (blood) with a tip of a swab) is introduced into the sample introduction port 1202. Thereafter, an introduction port sealing member 1206 made of polyurethane is attached to the sample introduction port 1202. In this manner, it is possible to prevent the sample and the reagent from leaking out of the flow path device 101 while ensuring air permeability inside the nucleic acid extraction chamber 1203.

[0095]

In addition, the nucleic acid extraction chamber 1203 is designed to have, for example, an internal volume of 4 mL. A temperature control mechanism 3202 can control a nucleic acid extraction portion heat source 3201 and maintain, at a predetermined temperature, the nucleic acid extraction chamber 1203 in contact with the nucleic acid extraction portion heat source 3201 with an adjusted temperature. Note that as the on-off valve mechanism 3005 and the reagent tank 3105, it is possible to employ mechanisms similar to those shown in FIGS. 21A and 21B.

[0096]

The introduction port sealing member 1206 may not be made of polyurethane, and can be configured using a member that allows passage of air to some extent and can prevent leakage of a sample and a solution inside the nucleic acid extraction chamber 1203, such as a vent filter, a porous resin sintered filter, or a sealing tape with holes, for example. It is possible to reduce the internal pressure of the nucleic acid extraction chamber 1203 during the nucleic acid extraction reaction by adopting the structure in which the nucleic acid extraction chamber 1203 is not tightly sealed. Therefore, it is possible to use even the flow path device 101 having low pressure resistance without breakage and to realize cost reduction of the device. In addition, the introduction port sealing member 1206 may be sealed using a material having no air

permeability (for example, a rubber member). In that case, it is possible to obtain effects similar to those of the introduction port sealing member 1206 by providing a filter vent mechanism in the nucleic acid extraction chamber 1203.

[0097]

<Nucleic Acid Extraction Treatment>

FIG. 25 is a flowchart for explaining the nucleic acid extraction treatment by the sample-to-answer-type DNA analysis system.

[0098]

First, an operator inserts a swab sample (sample 400) with blood (for example, 0.5  $\mu\text{L}$ ) adhering to the tip thereof into the nucleic acid extraction chamber 1203 from the sample introduction port 1202, and seals the sample introduction port 1202 with the introduction port sealing member 1206 (S2501). Next, the control device (not shown: configured of a computer connected to the system, for example) operates the valve actuator 3111 (not shown in FIG. 23) of the on-off valve mechanism 3005 to bring V2 into an open state and bring V1 and V3 to V5 into a closed state, and operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 containing a nucleic acid extract (lysis buffer; 300  $\mu\text{L}$ , for example), in response to an operator's instruction to start the nucleic acid extraction treatment. At the same time, the control device operates the temperature control mechanism 3202 to set the surface temperature of the nucleic acid extraction portion heat source 3201 to 56°C (S2502). Once the control device confirms (detects) that the temperature of the nucleic acid extraction chamber 1203 has become 56°C, the control device operates the valve actuator 3111 to bring V1 and V2 into an open state and bring V3 to V5 into a closed state, further causes the liquid feeding mechanism 302 to operate, and introduces the nucleic acid extract to the nucleic acid extraction chamber 1203 (S2503). Following the conveyance of the nucleic acid extract, the control device controls the system to send air into the nucleic acid extraction chamber 1203, and stirs the nucleic acid extract and the sample 400 by air bubbles and heat convection for thirty minutes (S2504). In this manner, cell membranes of the blood cells contained in the sample 400 are lysed, and the nucleic acid in the cells is released into the nucleic acid extract. Next, the control device operates the valve actuator 3111 of the on-off valve mechanism 3005 to bring V3 into an open

state and bring V1, V2, V4, and V5 into a closed state, and further operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 containing the purification reagent (sample buffer, 500  $\mu$ L, for example). At the same time, the control device operates the temperature control mechanism 3202 to set the surface temperature of the nucleic acid extraction portion heat source 3201 to 72°C (S2505). Once the control device confirms (detects) that the temperature of the nucleic acid extraction chamber 1203 has become 72°C, the control device operates the valve actuator 3111 to bring V3 and V4 into an open state and bring V1, V2, and V5 into a closed state, further causes the liquid feeding mechanism 302 to operate, and introduces the purification reagent to the nucleic acid extraction chamber 1203 (S2506). Following the conveyance of the purification reagent in S2506, the control device controls the system to send air into the nucleic acid extraction chamber 1203 and stirs the sample 400 and the solution (the nucleic acid extract and the purification reagent) by air bubbles and heat convection for ten minutes (S2507). The purification reagent is a solution containing a chaotropic salt and ethanol and enables highly efficient DNA adsorption to a silica membrane. The blood cells remaining in S2504 are completely dissolved, and at the same time, the preparation of the sample solution 401 (a mixed solution of the human genomic DNA, the nucleic acid extract, and the purification reagent in the sample) capable of performing the Boom method in the nucleic acid purification portion 1101 in a later stage can be completed, by the operation in S2507. Finally, the control device brings V3 to V5 into an open state, brings V1 and V2 into a closed state, and conveys the sample solution 401 from the purification portion connection flow path 1204 to the nucleic acid purification portion 1101 (S2508).

[0099]

<Nucleic Acid Purification Treatment>

FIG. 26 is a diagram showing a configuration example of the periphery of the nucleic acid purification portion 1101 in the sample-to-answer-type DNA analysis system. The nucleic acid purification portion 1101 has a configuration that is substantially similar to that of the reagent tank integrated flow path device (nucleic acid purification device) 101 (see FIG. 20). Main differences from the reagent tank integrated flow path device (nucleic acid purification device) 101 are that the sample solution 401 is conveyed from the purification portion

connection flow path 1204 connected to the nucleic acid extraction portion 1201 and that the solution collection flow path 3108 is connected to the nucleic acid amplification portion 1301. The nucleic acid purification portion 1101 can also realize highly efficient nucleic acid purification through the nucleic acid purification treatment shown in FIG. 22.

[0100]

<Configuration Example of Nucleic Acid Amplification Portion 1301>

FIG. 27A is a diagram showing a configuration example of the periphery of the nucleic acid amplification portion 1301. FIG. 27B is a diagram showing a sectional configuration example along A-A in FIG. 27A. The nucleic acid amplification portion 1301 includes, on the substrate 102, a nucleic acid amplification chamber 1302 connected to the solution collection flow path 3108 of the nucleic acid purification portion 1101, at least one or more reagent tanks 3105 that hold the reagent to perform a nucleic acid amplification reaction, at least one or more on-off valve mechanisms 3005, an amplification product collection flow path 1303 that is connected to the nucleic acid amplification chamber 1302 and the first vent 107 or the second vent 108 and discharges the sample solution after the nucleic acid amplification reaction, and a liquid feeding flow path 1205 that is connected to at least either the first vent 107 or the second vent 108 and enables liquid feeding by an operation of the liquid feeding mechanism 302.

[0101]

The nucleic acid amplification chamber 1302 can be configured, for example, in a linear shape with a width of 5 mm  $\times$  a length of 8 mm  $\times$  a thickness of 0.5 mm. In addition, the substrate 102 on the lower surface of the nucleic acid amplification chamber 1302 is processed to be thinner than the peripheral portion (for example, a thickness of 0.5 mm). In this manner, it is possible to bring the upper and lower surfaces of the nucleic acid amplification chamber 1302 into contact with the nucleic acid amplification portion heat source 3301 and to give a temperature change to the sample solution inside the nucleic acid amplification chamber at a high speed. Note that one temperature control mechanism 3302 may be installed in each of the upper and lower nucleic acid amplification portion heat sources 3301. In this manner, it is possible to perform feedback control of the temperature adjustment from temperature data of each of the upper and lower surfaces and to thereby more precisely perform temperature control.

On the other hand, the temperature control mechanism 3302 may be shared also as that (temperature control mechanism 3202) of the nucleic acid extraction portion heat source 3201 (see FIG. 24). In this manner, it is possible to reduce the size of the device. Note that as the on-off valve mechanism 3005 and the reagent tank 3105, it is possible to employ mechanisms similar to those shown in FIG. 20.

[0102]

#### <Nucleic Acid Amplification Treatment>

FIG. 28 is a flowchart for explaining a nucleic acid amplification treatment by the sample-to-answer-type DNA analysis system. The above control device (not shown) operates the valve actuator 3111 first to bring V1 and V2 into an open state and bring V3 and V4 into a closed state, and introduces a part (10  $\mu$ L, for example) of the eluent 403 from the solution collection flow path 3108 to the nucleic acid amplification chamber (S2801). Next, the control device operates the valve actuator 3111 to bring V3 into an open state and bring V1, V2, and V4 into a closed state, further operates the reagent tank actuator 3110 to break the sealing of the reagent tank 3105 containing the nucleic acid amplification reagent (PCR buffer; 10  $\mu$ L of Globalfiler PCR kit reagent, for example), and introduces the nucleic acid amplification reagent to the nucleic acid amplification chamber 1302 (S2802). Furthermore, the control device operates the valve actuator 3111, brings all V1 to V4 into an open state once to pull out air bubbles inside the nucleic acid amplification chamber 1302, brings all V1 to V4 into a closed state, and stores 20  $\mu$ L of reaction solution (2  $\mu$ L of eluent and 18  $\mu$ L of nucleic acid amplification reagent) in the nucleic acid amplification chamber 1302 (S2803). Here, the control device operates the temperature control mechanism 3302 to set the surface temperature of the nucleic acid amplification portion heat source 3301 to 95°C, and maintains the temperature for one minute when the nucleic acid extraction chamber 1203 reaches (detects) 95°C (S2804). In this manner, polymerase contained in the nucleic acid amplification reagent is activated, and the nucleic acid amplification reaction can be started. Next, the control device operates the temperature control mechanism 3302 to change the surface temperature of the nucleic acid amplification portion heat source 3301 to 94°C, maintains the temperature for ten seconds when the nucleic acid extraction chamber 1203 reaches 94°C, and then performs a

DNA denaturation reaction (S2805). Subsequently, the control device operates the temperature control mechanism 3302 to change the surface temperature of the nucleic acid amplification portion heat source 3301 to 59°C, maintains the temperature for ninety seconds when the nucleic acid extraction chamber 1203 reaches (detects) 59°C, and an annealing/DNA extension reaction is then performed (S2806). The control device repeats the treatments in S2805 and S2806 thirty cycles and amplifies the target nucleic acid (STR) to achieve a product concentration that enables detection by a capillary electrophoresis device. The control device performs the treatments in S2805 and S2806 thirty cycles, then subsequently maintains the temperature at 59°C for ten minutes, and performs adenylation (S2807). Finally, the control device operates the temperature control mechanism 3302 to change the surface temperature of the nucleic acid amplification portion heat source 3301 to 4°C and stops the nucleic acid amplification reaction (S2808). At the same time, the control device operates the valve actuator 3111 to bring V1, V2, and V4 into an open state and bring V3 into a closed state, further causes the liquid feeding mechanism 302 to operate, and conveys the reaction solution from the nucleic acid amplification chamber 1302 to the amplification product collection flow path 1303 (S2809).

[0103]

#### <STR Analysis Treatment by Electrophoresis>

The reaction solution conveyed to the amplification product collection flow path 1303 is discharged to the outside via the second vent 108 and is mixed with an electrophoresis reagent (for example, a formamide solution) at a certain ratio (for example, 200 µL of electrophoresis reagent with respect to 20 µL of reaction solution). This mixed solution can be used and installed as a sample solution in an electrophoresis apparatus to perform STR analysis.

[0104]

Note that it is also possible to store the electrophoresis reagent in the reagent tank 3105, to install it on the substrate 102, and to mix the reaction solution and the electrophoresis reagent by the flow path device 101 similarly to the nucleic acid extraction portion 1201 to the nucleic acid amplification portion 1301. Furthermore, the electrophoresis portion may be configured on the flow path device 101, and the introduction of the sample to the electrophoresis analysis

may be fully automatically performed.

[0105]

#### (5) Modification Examples of Membrane Storage Portion 104 and Resin Ring 204

FIGS. 29A to 29E are diagrams illustrating modification examples of the membrane storage portion 104 and the resin ring 204 according to the present embodiment. FIG. 29A is a diagram showing a configuration example of the periphery of the membrane storage portion 104 from above in each modification example. FIGS. 29B to 29E are diagrams showing sectional configuration examples along A-A in FIG. 29A in the first to fourth modification examples.

[0106]

As shown in FIG. 29A, the resin ring 204 preferably has an annular seamless shape when seen from the upper surface in each modification example. In this manner, a state where the peripheral edge portion of the purification membrane 201 is fixed to the pedestal surface 109 without any missing over the entire periphery is achieved, and it is possible to minimize leakage of the solution. Although the pedestal surface 109 also has an annular seamless shape for a similar reason, it has become apparent through an experiment that the effects of the technology of the present disclosure can be sufficiently obtained even with a C-type pedestal surface 109 with a notch corresponding to about equal to or less than 1/5 the circumference.

[0107]

In the modification examples illustrated in FIGS. 29B to 29E, a resin ring 204 with a circular sectional shape in the ring vertical direction (a donut sectional shape in the horizontal direction) like a general O-ring may be used. However, in this case, contact between the resin ring 204 and the purification membrane 201 and between the resin ring 204 and the side wall of the membrane storage portion upper portion 110 is preferably achieved in surfaces that are as large as possible. This is because floating of the purification membrane 201 can be further suppressed as the contact area between the resin ring 204 and the purification membrane 201 increases, and because a frictional force working between the resin ring 204 and the membrane storage portion upper portion 110 increases in proportion to the contact area.

[0108]

As illustrated in FIG. 29B, one preferable modification example is a resin ring 204 with a section in the ring vertical direction inclined inward. In the shape of the resin ring 204 shown in FIG. 29B, the contact areas between the resin ring 204 and the purification membrane 201 and between the resin ring and the membrane storage portion upper portion 110 are the same as those of the resin ring 204 (FIG. 9) according to the aforementioned embodiment. Therefore, it is possible to obtain a high purification membrane fixing effect, to minimize a liquid remaining on the upper surface of the resin ring 204, and to thereby obtain a higher yield.

[0109]

FIG. 29C shows a modification example using a resin ring 204 having tapered shapes on upper and lower sides. As shown in FIG. 29C, an upper taper 2001 is provided to be inclined inward in the center direction of the purification membrane 201 in the resin ring 204. In this manner, it is possible to expect a liquid residue suppression and yield improving effects similarly to the modification in FIG. 29B. A lower taper 2002 is provided on the outer peripheral side of the purification membrane 201. In this manner, it is possible to obtain an effect that the resin ring 204 can be easily inserted into the membrane storage portion upper portion 110 at the time of assembly. The resin ring 204 (the embodiment and each modification example) according to the present disclosure is characterized by having an inner diameter of equal to or greater than that of the membrane storage portion upper portion 110. Therefore, it may be difficult to position the resin ring 204 at the time of assembly and to fit the resin ring 204. Therefore, since the outer diameter of the bottom surface of the resin ring 204 decreases by providing the lower taper 2002, it becomes easy for the resin ring 204 to be accommodated in the membrane storage portion upper portion 110 at the time of starting the fitting, which facilitates more accurate press-fitting and fixing.

[0110]

Another modification example shown in FIG. 29D relates to a resin ring 204 with a notch portion 2003 provided at a connecting portion between the first flow path 105 and the membrane storage portion upper portion 110. The notch portion 2003 is preferably designed and disposed such that the upper surface of the notch portion 2003 is located at a position lower than the bottom surface of the first flow path 105 and the side wall of the notch portion 2003 is

located on the side further outward than the side wall surface of the first flow path 105 not to block the first flow path 105. According to the resin ring 204 of an upper surface C type (a predetermined rate of the upper portion of the resin ring 204; a part connected to the first flow path 105 is provided with a notch portion)/a bottom surface O type (a predetermined rate of the lower portion of the resin ring 204) as in FIG. 29D, it is possible to reduce the effective volume of the membrane storage portion upper portion 110. Therefore, the amount of air serving as a damper at the time of liquid feeding can be minimized, and the liquid feeding pressure can be more effectively raised. In this manner, it is possible to reduce the size of the liquid feeding mechanism 302 by reducing a necessary pressure, to enhance liquid feeding controllability, and to automatically realize a stabler nucleic acid purification treatment.

[0111]

Another modification example shown in FIG. 29E relates to the membrane storage portion 104 in which the membrane storage portion upper portion 110 and the membrane storage portion lower portion 111 have tapered shapes. In this case, the resin ring 204 also preferably has a tapered shape with which the resin ring 204 can be press-fitted to the membrane storage portion upper portion 110. The tapered shapes of the membrane storage portion upper portion 110 and the resin ring 204 have an effect that the resin ring 204 can be stably fitted at the time of assembly similarly to the lower taper 2002 shown in FIG. 29C. At the same time, the contact area between the resin ring 204 and the membrane storage portion upper portion 110 increases as compared with a case where the side wall of the membrane storage portion upper portion 110 is nearly vertical. Therefore, a higher press-fitting effect can be obtained. On the other hand, if the membrane storage portion lower portion 111 is formed into a tapered shape, it is possible to reduce the amount of solution remaining at the membrane storage portion lower portion 111 while securing the effective area of the purification membrane 201, to efficiently convey the solution passing through the purification membrane to the second flow path 106, and to further enhance collection efficiency.

[0112]

Note that the characteristic configurations of the modification examples shown in FIGS. 29B to 29E can be combined. It is possible to obtain a high purification membrane fixing

force and collection efficiency by appropriate combinations. For example, it is preferable to determine an appropriate shape in consideration of the materials and working strains of the substrate 102 and the resin ring 204, the material and size of the purification membrane 201, and the like. Moreover, the membrane fixing method can also be used in combination with a conventional membrane fixing method. For example, a slight gap between the press-fitted resin ring 204 and the side wall of the membrane storage portion upper portion 110 may be filled with an adhesive. In this manner, it is possible to minimize defects due to a working error and surface roughness caused by material working or the like and to achieve high-performance nucleic acid purification at a practical level in accordance with applications.

[0113]

#### (6) Application of Continuous Conveying Method in System to Which Configuration of Flow Path Device 101 of Present Disclosure Is Applied

(i) Here, a nucleic acid purification treatment and a sample pretreatment system to which the continuous conveying method is applied will be described. The composition of the sample solution 401 used in the continuous conveying method is as in FIG. 2A, and pure ethanol is used as the cleaning liquid 402. In addition, the sample pretreatment system 301 and the flow path device 101 have configurations that are substantially the same as those shown in FIG. 20. FIG. 30A is a diagram showing a state where the sample solution 401 is conveyed by the continuous conveying method. FIG. 30B is a diagram showing a state where the cleaning liquid 402 is conveyed by the continuous conveying method.

[0114]

In the operation of the continuous conveying method, a tail end of the sample solution 401 stops at a liquid feeding stop location 1104 located in the middle of the sample solution flow path 1102 without completely conveying the sample solution 401 to the second flow path 106 as shown in FIGS. 30A and 30B. Such liquid feeding control is different from that in FIG. 20. Note that it is only necessary for the liquid feeding stop location 1104 to be a location at which it is possible to detect that the tail end of the sample solution 401 has reached a predetermined position, and it is possible to dispose a liquid surface detection sensor or a camera on the upper surface at the liquid feeding stop location 1104, for example.

[0115]

Next, the nucleic acid purification treatment based on the continuous conveying method will be briefly described with reference to FIGS. 30A and 30B. First, the control device (not shown) conveys the sample solution 401 from the first reagent tank 3105 to the second flow path 106 through the purification membrane 201 installed in the membrane storage portion 104 and stops the liquid feeding at the moment at which the tail end of the sample solution 401 reaches the liquid feeding stop location 1104 (FIG. 30A). Next, the control device discharges the cleaning liquid 402 from the second reagent tank 3105, causes the sample solution 401 to continuously pass through the purification membrane 201, and conveys the sample solution 401 to the second flow path 106 (FIG. 30B). The subsequent nucleic acid purification treatment is the same as that in the process described in FIG. 22.

[0116]

(ii) If the purification membrane fixing method using the resin ring 204 according to the present disclosure is applied, the gap between the purification membrane 201 and the membrane storage portion 104 becomes considerably small, and escaping of liquid and gas is suppressed. Therefore, it has been discovered through an experiment that the liquid feeding pressure necessary to convey the solution in the technology of the present disclosure increases as compared with a case where the technology of the present disclosure is not performed. The maximum liquid feeding pressure is 20 kPa in the case where the configuration shown in FIGS. 4A and 4B is applied, and the maximum liquid feeding pressure is about 120 kPa in the case where the configuration in FIGS. 9A and 9B is applied, for example. At this time, the highest liquid feeding pressure is needed in the process of starting to cause the cleaning liquid 402 to flow after causing the sample solution 401 to completely flow. This is because when air reaches the vicinity of the purification membrane 201 in a state where the purification membrane 201 is wet with the sample solution 401, the air cannot pass through the purification membrane 201 until the Laplace pressure is exceeded, and a high pressure is needed until the cleaning liquid 402 is caused to reach the purification membrane 201.

[0117]

In order to suppress such a high liquid feeding pressure, it is possible to apply the

continuous conveying method of reducing the liquid feeding pressure by continuously conveying the two solutions satisfying specific conditions without air sandwiched therebetween in the technology of the present disclosure. It has been found by experiments that since the main component of the sample solution 401 is water and the main component of the cleaning liquid 402 is ethanol, the liquid feeding pressure reducing effect can be easily obtained by applying the continuous conveying method. In addition, it is known from experiments that it is preferable that “the evaporation rate of the second liquid (cleaning liquid 402) is higher than that of the first liquid (sample solution 401), and the surface tension of the second liquid is lower than that of the first liquid” from the experiments. When the nucleic acid purification treatment shown in FIGS. 30A and 30B was actually performed, it was confirmed that the maximum liquid feeding pressure was about 40 kPa and high purification efficiency was able to be achieved even by the flow path device 101 of the present disclosure. As described above, it is possible to enable low-pressure liquid feeding by the continuous conveying method and to realize high-performance nucleic acid purification with a small-sized and low-cost mechanism while achieving high-yield and high-robust nucleic acid purification by the purification membrane fixing method using the resin ring 204 according to the present disclosure.

[0118]

#### (7) Summary

(i) The flow path device 101 of the present embodiment includes the purification membrane 201 that is formed on the substrate 102 and is able to collect a nucleic acid, the membrane storage portion 104 that is a space formed on the substrate 102, is connected to the first flow path 105 and the second flow path 106 formed on the substrate, and houses the purification membrane 201, and the annular resin ring 204 that is press-fitted to the membrane storage portion 104. Also, the membrane storage portion 104 includes a pedestal surface 109 formed between the upper surface and the lower surface of the substrate 102 such that the purification membrane 201 can be installed thereon, and is divided into a cylindrical membrane storage portion upper portion 110 and the membrane storage portion lower portion 111 with a smaller diameter than that of the membrane storage portion upper portion 110 with the pedestal surface 109 sandwiched as a boundary. The first flow path 105 and the second flow path 106

include the first flow path 105 that is connected to at least one valve mechanism, is formed on the upper surface of the substrate 102, and is connected to the side wall of the membrane storage portion upper portion 110 and the second flow path 106 that is connected to at least one valve mechanism, is formed on the lower surface of the substrate 102, and is connected to the membrane storage portion lower portion 111. The resin ring 204 has an outer diameter equal to or greater than the inner diameter of the membrane storage portion upper portion 110. Also, the purification membrane 201 is fixed between the pedestal surface 109 and the resin ring 204. Furthermore, the outer diameter of the press-fitted resin ring 204 is smaller than the outer diameter of the resin ring 204 in a state where the resin ring 204 is not housed in the membrane storage portion 104, and the inner diameter of the membrane storage portion upper portion 110 is substantially the same as the inner diameter of the membrane storage portion upper portion 110 in a state where the resin ring 204 is not press-fitted to the membrane storage portion. Moreover, the inner diameter of the membrane storage portion upper portion 110 and the outer diameter of the press-fitted resin ring 204 are the same at least at a position where the side wall of the membrane storage portion upper portion 110 and the press-fitted resin ring 204 are closest to each other. Here, when the resin ring 204 is press-fitted to the membrane storage portion 104, the resin ring 204 is plastically deformed and is held at the press-fitted location due to a frictional force with the side wall of the membrane storage portion upper portion 110. In other words, if the resin ring 204 is press-fitted, the resin ring 204 is not elastically deformed like a known O-ring. The resin ring 204 is configured of a material with a Young's modulus of equal to or greater than 5 MPa and equal to or less than 1 GPa. Note that the Young's modulus of the material constituting the resin ring 204 may be less than the Young's modulus of the material constituting the substrate 102. Also, the elastic modulus of the resin ring is equal to or greater than 100 kPa. On the other hand, the elastic strain limit value of the resin ring 204 is less than 100%. More specifically, when the inner diameter of the membrane storage portion upper portion 110 in a state where the flow path device 101 has been assembled is defined as  $R'$ , and the outer diameter of the press-fitted resin ring 204 in the state where the flow path device 101 has been assembled is defined as  $a'$ ,  $|R' - a'| < 1 \mu\text{m}$  is satisfied at the position where the side wall of the membrane storage portion upper portion 110 and the press-fitted resin ring 204 are

closest to each other. On the other hand, when the inner diameter of the membrane storage portion upper portion 110 in a state where the flow path device 101 has not been assembled (the state before the resin ring is press-fitted) is defined as R, and the outer diameter of the resin ring (before press-fitting) 204 in the state where the flow path device 101 has not been assembled is defined as a,  $0 \leq a - R < 400 \mu\text{m}$  is satisfied. It is possible to realize the flow path device (flow path chip) 101 at low cost with a simple configuration by configuring the flow path device 101 in this manner. Also, it is possible to fully automatically achieve highly efficient purification equivalent to the laboratory scheme and to perform gene analysis with high sensitivity by using the flow path device 101.

[0119]

The inner diameter of the pedestal surface 109 can be substantially the same as the inner diameter of the resin ring 204. In this manner, it is possible to cause the solution in contact with the purification membrane 201 to efficiently pass through the membrane storage portion lower portion 111 and to maximize the amount of solution that can be collected.

[0120]

A difference between the inner diameter of the membrane storage portion upper portion 110 and the inner diameter of the membrane storage portion lower portion 111 and/or a difference between the outer diameter and the inner diameter of the resin ring 204 is preferably set to be as small as possible, and it is desirable that the difference be equal to or less than 2.5 mm. In this manner, it is possible to prevent the solution from being captured and remaining on the upper surface of the resin ring 204 and to more efficiently collect the solution. Note that the difference between the outer diameter and the inner diameter of the resin ring 204 may be equal to or less than 1 mm.

[0121]

Furthermore, the press-fitted resin ring 204 and the side wall of the membrane storage portion upper portion 110, and the press-fitted resin ring 204 and the purification membrane 201 are in surface contact. These are preferably in contact on surfaces that are as large as possible. In this manner, it is possible to further suppress floating of the purification membrane 201 as the contact area between the resin ring 204 and the purification membrane 201 increases,

and to obtain a higher press-fitting effect.

[0122]

Furthermore, the press-fitted resin ring 204 is configured such that an end surface on the side opposite to the surface on which the press-fitted resin ring 204 is in contact with the purification membrane 201 is located on the side further downward than the connecting portion between the first flow path 105 and the side wall of the membrane storage portion upper portion 110. In this manner, it is possible to prevent the solution to be conveyed from remaining in the gap between the resin ring 204 and the membrane storage portion 104. Also, it is possible to reduce the flow path resistance and to collect a small amount of solution without any loss.

[0123]

(ii) The present embodiment also proposes a nucleic acid purification method of generating a nucleic acid using the flow path device 101. As shown in FIG. 17, for example, the method includes: a process of connecting the sample tank (reagent tank 3105) that accommodates the sample solution to the first flow path 105 of the flow path device 101; a process of connecting the second flow path 106 to the waste liquid tank 3106; a process of conveying the sample solution from the sample tank to the waste liquid tank 3106 via the first flow path 105, the purification membrane 201, and the second flow path 106 by operating the liquid feeding mechanism 302; a process of connecting the cleaning liquid tank that accommodates the cleaning liquid to clean the purification membrane 201 (causes the reagent tank 3105 to hold the cleaning liquid 402) to the first flow path 105; a process of conveying the cleaning liquid 402 from the cleaning liquid tank to the waste liquid tank 3106 via the first flow path 105, the purification membrane 201, and the second flow path 106 by operating the valve mechanism; a process of sending dry air in a direction from the first flow path 105 to the second flow path 106 via the purification membrane 201 by operating the liquid feeding mechanism 302; a process of connecting the first flow path 105 to the eluent tank that accommodates the eluent to elute the nucleic acid (causes the reagent tank 3105 to hold the eluent) via the valve mechanism; a process of connecting the second flow path 106 to the solution collection tank 3107; and a process of conveying the eluent from the eluent tank to the solution collection tank 3107 via the first flow path 105, the purification membrane 201, and the second flow path 106

by operating the liquid feeding mechanism 302. In this manner, it is possible to efficiently purify a nucleic acid and to perform gene analysis with high sensitivity.

[0124]

(iii) The present embodiment also proposes another nucleic acid purification method. As shown in FIGS. 30A, 30B, and 22, the method includes at least: a process of preparing a first liquid tank (the reagent tank 3105) that accommodates a first liquid (sample solution 401) and a second liquid tank (cleaning liquid tank) that accommodates a second liquid (cleaning liquid 402); a process of connecting the first flow path 105 of the flow path device 101 to the first liquid sending flow path (sample solution flow path 1102) connected to the first liquid tank; a process of connecting the first flow path 105 of the flow path device 101 to the second liquid sending flow path (cleaning liquid flow path) connected to the second liquid tank; a process of connecting the second flow path 106 of the flow path device 101 to the waste liquid tank 3106; a process of conveying the first liquid from the first liquid feeding tank to the waste liquid tank 3106 via the first flow path 105, the purification membrane 201, and the second flow path 106 by operating the liquid feeding mechanism 302; a process of stopping conveyance of the first liquid before the tail end of the first liquid reaches the meeting point between the first liquid sending flow path and the first flow path (at the liquid feeding stop location 1104) to achieve a state where the first liquid is retained in the first sending flow path by operating the liquid feeding mechanism 302; and a process of start sending of the second liquid from the second liquid tank to the second liquid sending flow path and conveying the second liquid to the waste liquid tank 3106 via the meeting point between the second liquid sending flow path and the first flow path, the first flow path 105, the purification membrane 201, and the second flow path 106 by operating the liquid feeding mechanism 302 in the state where the first liquid is retained in the first sending flow path, the flowing into the membrane storage portion 104 being continuously executed without air sandwiched between the first liquid and the second liquid.

[0125]

Furthermore, the preparing process in this method further includes: a process of preparing a third liquid tank (reagent tank (elution buffer) 3105) that accommodates the eluent to elute a nucleic acid, and further includes: a process of sending dry air in a direction from the

first flow path 105 to the second flow path 106 via the purification membrane 201 by operating the liquid feeding mechanism 302; and a process of conveying the eluent from the eluent tank to the solution collection tank 3107 via the first flow path 105, the purification membrane 201, and the second flow path 106 by operating the liquid feeding mechanism 302. In this manner, it is possible to reduce a liquid feeding pressure. Therefore, breakage of the flow path device 101 due to conveyance of the solution does not occur.

[0126]

(iv) The present embodiment proposes the sample pretreatment system 301 including the flow path device 101 according to the present embodiment, the nucleic acid extraction portion 1201 that is connected to the first flow path 105 of the flow path device 101 and extracts a nucleic acid from a biological sample, and the nucleic acid amplification portion 1301 that is connected to the second flow path 106 of the flow path device 101 and amplifies the nucleic acid (see FIGS. 23 to 27A, and 27B).

[0127]

(v) The technology according to the present disclosure can also form various functions by appropriately combining a plurality of components disclosed in the present embodiment. For example, some of all the components described in the embodiment may be deleted, and the components in different specific examples may be appropriately combined.

[0128]

Although the present disclosure has been described with specific examples as described above, this is not for limitation in all aspects but for explanation (understanding of the technology of the present disclosure). Furthermore, those ordinarily skilled in the technical field can clarify other implementations of the present disclosure from consideration of the present embodiment. Note that the specification and specific examples are merely typical examples and the scope and the spirit of the technology of the present disclosure are indicated by the following claims.

#### Reference Signs List

[0129]

101 flow path device  
102 substrate  
103 sealing tape  
104 membrane storage portion  
105 first flow path  
106 second flow path  
107 first vent  
108 second vent  
109 pedestal surface  
110 membrane storage portion upper portion (diameter R, depth D)  
111 membrane storage portion lower portion (diameter r, depth d)  
201 purification membrane (diameter P, thickness Q)  
202 o-ring  
203 adhesive  
204 resin ring (outer diameter a, inner diameter b, height c)  
205 porous holding member  
301 sample pretreatment system  
302 liquid feeding mechanism  
303 reagent holding portion  
304 chip holding portion  
305 cover  
306 fitting  
307 piping tube  
400 sample  
401 sample solution  
402 cleaning liquid  
403 eluent  
1001 connection flow path  
1002 valve portion

1003 sealing member  
1004 protruding portion  
1005 reagent filling port  
1006 vent  
1101 nucleic acid purification portion  
1102 sample solution flow path  
1103 cleaning liquid flow path  
1104 liquid feeding stop location  
1105 nucleic acid purification and amplification portion  
1201 nucleic acid extraction portion  
1202 sample inlet  
1203 nucleic acid extraction chamber  
1204 purification portion collection flow path  
1205 liquid feeding flow path  
1206 inlet sealing member  
1301 nucleic acid amplification portion  
1302 nucleic acid amplification chamber  
1303 amplification product collection flow path  
2001 upper taper  
2002 lower taper  
2003 notch portion  
3001 first connection port  
3002 second connection port  
3003 liquid feeding power source  
3004 pressure control mechanism  
3005 on-off valve mechanism  
3006 multi-way valve mechanism  
3007 atmosphere opening flow path  
3101 first liquid feeding port

3102 second liquid feeding port  
3103 first device connection port  
3104 second device connection port  
3105 reagent tank  
3106 waste liquid tank  
3107 solution collection tank  
3108 solution collection flow path  
3109 vent filter  
3110 reagent tank actuator  
3111 valve actuator  
3201 nucleic acid extraction portion heat source  
3202, 3302 temperature control mechanism  
3301 nucleic acid amplification portion heat source

## CLAIMS

### [Claim 1]

A flow path device with a flow path formed on a substrate comprising:

- a purification membrane that is able to collect a nucleic acid;
- a membrane storage portion that is a space formed on the substrate, is connected to the flow path, and houses the purification membrane; and
- an annular resin ring that is press-fitted to the membrane storage portion,

wherein the membrane storage portion includes a pedestal surface formed between an upper surface and a lower surface of the substrate such that the purification membrane is able to be installed thereon, the membrane storage portion being divided into a cylindrical membrane storage portion upper portion and a membrane storage portion lower portion having a smaller diameter than the membrane storage portion upper portion with the pedestal surface sandwiched therebetween as a boundary,

the flow path including a first flow path that is connected to at least one valve mechanism, is formed on the upper surface of the substrate, and is connected to a side wall of the membrane storage portion upper portion and a second flow path that is connected to at least one valve mechanism, is formed on the lower surface of the substrate, and is connected to the membrane storage portion lower portion,

the resin ring has an outer diameter that is equal to or greater than an inner diameter of the membrane storage portion upper portion,

the purification membrane is fixed between the pedestal surface and the resin ring,

an outer diameter of the press-fitted resin ring is smaller than an outer diameter of the resin ring in a state where the resin ring is not housed in the membrane storage portion, and an inner diameter of the membrane storage portion upper portion is substantially the same as the inner diameter of the membrane storage portion upper portion in the state where the resin ring is not press-fitted in the membrane storage portion, and

the inner diameter of the membrane storage portion upper portion and the outer diameter of the press-fitted resin ring are the same at least at a position at which the side wall of the membrane storage portion upper portion and the press-fitted resin ring are closest to each

other.

[Claim 2]

The flow path device according to claim 1, wherein  
the resin ring is configured of a material having a Young's modulus of equal to or greater than 5 MPa.

[Claim 3]

The flow path device according to claim 2, wherein  
the resin ring is configured of a material having a Young's modulus of equal to or less than 1 GPa.

[Claim 4]

The flow path device according to claim 1, wherein  
an elastic modulus of the resin ring is equal to or greater than 100 kPa.

[Claim 5]

The flow path device according to claim 1, wherein  
an elastic strain limit value of the resin ring is less than 100%.

[Claim 6]

The flow path device according to claim 1, wherein  
when the inner diameter of the membrane storage portion upper portion in a state where the flow path device has been assembled is defined as  $R'$ , and the outer diameter of the press-fitted resin ring in the state where the flow path device has been assembled is defined as  $a'$ ,  $|R' - a'| < 1 \mu\text{m}$  is satisfied at the position where the side wall of the membrane storage portion upper portion and the press-fitted resin ring are closest to each other.

[Claim 7]

The flow path device according to claim 1, wherein  
when the inner diameter of the membrane storage portion upper portion in a state where the flow path device has not been assembled is defined as  $R$ , and the outer diameter of the resin ring in the state where the flow path device has not been assembled is defined as  $a$ ,  $0 \leq a - R < 400 \mu\text{m}$  is satisfied.

[Claim 8]

The flow path device according to claim 1, wherein  
an inner diameter of the pedestal surface is substantially the same as the inner diameter  
of the resin ring.

[Claim 9]

The flow path device according to claim 1, wherein  
a difference between the inner diameter of the membrane storage portion upper portion  
and an inner diameter of the membrane storage portion lower portion is equal to or less than  
2.5 mm.

[Claim 10]

The flow path device according to claim 9, wherein  
a difference between the outer diameter and the inner diameter of the resin ring is equal  
to or less than 1 mm.

[Claim 11]

The flow path device according to claim 1, wherein  
the press-fitted resin ring and the side wall of the membrane storage portion upper  
portion, and the resin ring and the purification membrane are in surface contact.

[Claim 12]

The flow path device according to claim 1, wherein  
an end surface of the press-fitted resin ring on a side opposite to a surface on which  
the press-fitted resin ring and the purification membrane are in contact with each other is located  
on a side further downward than a connecting portion between the first flow path and the side  
wall of the membrane storage portion upper portion.

[Claim 13]

The flow path device according to claim 1, wherein  
a Young's modulus of a material constituting the resin ring is less than a Young's  
modulus of a material constituting the substrate.

[Claim 14]

The flow path device according to claim 1, wherein  
film sheets are attached to the upper surface and the lower surface of the substrate,

such that the first flow path, the second flow path, and at least a part of an opening in the upper surface or the lower surface of the substrate that the membrane storage portion has are blocked.

[Claim 15]

The flow path device according to claim 1, wherein

the press-fitted resin ring is fixed to the pedestal surface of the membrane storage portion with an adhesive.

[Claim 16]

A flow path device with a flow path formed on a substrate comprising:

a purification membrane that is able to collect a nucleic acid;

a membrane storage portion that is a space formed on the substrate, is connected to the flow path, and houses the purification membrane; and

an annular resin ring that is press-fitted to the membrane storage portion,

wherein the membrane storage portion includes a pedestal surface formed between an upper surface and a lower surface of the substrate such that the purification membrane is able to be installed thereon, the membrane storage portion being divided into a cylindrical membrane storage portion upper portion and a membrane storage portion lower portion having a smaller diameter than the membrane storage portion upper portion with the pedestal surface sandwiched as a boundary,

the flow path includes a first flow path that is formed on the upper surface of the substrate and is connected to a side wall of the membrane storage portion upper portion and a second flow path that is formed on the lower surface of the substrate and is connected to the membrane storage portion lower portion,

the resin ring has an outer diameter that is equal to or greater than an inner diameter of the membrane storage portion upper portion,

the purification membrane is fixed between the pedestal surface and the resin ring,

the outer diameter of the press-fitted resin ring is smaller than the outer diameter of the resin ring in a state where the resin ring is not housed in the membrane storage portion, and the inner diameter of the membrane storage portion upper portion is substantially the same as the inner diameter of the membrane storage portion upper portion in a state where the resin ring is

not press-fitted to the membrane storage portion, and

the inner diameter of the membrane storage portion upper portion and the outer diameter of the press-fitted resin ring are the same at least at a position where the side wall of the membrane storage portion upper portion and the press-fitted resin ring are closest to each other.

[Claim 17]

A nucleic acid purification method of generating a nucleic acid using the flow path device according to claim 1, the method comprising:

connecting the first flow path to a sample tank that accommodates a sample solution;

connecting the second flow path to a waste liquid tank;

by operating a liquid feeding mechanism, conveying the sample solution from the sample tank to the waste liquid tank via the first flow path, the purification membrane, and the second flow path;

connecting the first flow path to a cleaning liquid tank that accommodates a cleaning liquid to clean the purification membrane via the liquid feeding mechanism;

by operating the liquid feeding mechanism, conveying the cleaning liquid from the cleaning liquid tank to the waste liquid tank via the first flow path, the purification membrane, and the second flow path;

by operating the liquid feeding mechanism, sending dry air in a direction from the first flow path to the second flow path via the purification membrane;

connecting an eluent tank that accommodates an eluent to elute the nucleic acid to the first flow path;

connecting the second flow path to a collection tank; and

by operating the liquid feeding mechanism, conveying the eluent from the eluent tank to the collection tank via the first flow path, the purification membrane, and the second flow path.

[Claim 18]

A nucleic acid purification method of generating a nucleic acid using the flow path device according to claim 1, the method comprising:

preparing at least a first liquid tank that accommodates a first liquid and a second liquid tank that accommodates a second liquid;

connecting the first flow path of the flow path device to a first liquid sending flow path connected to the first liquid tank;

connecting the first flow path of the flow path device to a second liquid sending flow path connected to the second liquid tank;

connecting the second flow path of the flow path device to a waste liquid tank;

by operating a liquid feeding mechanism, conveying the first liquid from the first liquid tank to the waste liquid tank via the first flow path, the purification membrane, and the second flow path;

by operating the liquid feeding mechanism, stopping conveyance of the first liquid before a tail end of the first liquid reaches a meeting point between the first liquid sending flow path and the first flow path to achieve a state where the first liquid is retained in the first liquid sending flow path; and

by operating the liquid feeding mechanism in the state where the first liquid is retained in the first liquid sending flow path, starting to feed the second liquid from the second liquid tank to the second liquid sending flow path to convey the second liquid to the waste liquid tank via a meeting point between the second liquid sending flow path and the first flow path, the first flow path, the purification membrane, and the second flow path,

wherein flowing into the membrane storage portion is continuously executed with no air sandwiched between the first liquid and the second liquid.

[Claim 19]

The nucleic acid purification method according to claim 18, wherein

the first liquid is a sample solution,

the second liquid is a cleaning liquid, and

the preparing at least the first liquid tank and the second liquid tank further includes preparing a third liquid tank that accommodates an eluent to elute the nucleic acid, and

the method further comprises:

by operating the liquid feeding mechanism, sending dry air in a direction from the first

flow path to the second flow path via the purification membrane;

connecting the second flow path of the flow path device to a collection tank for collecting the eluent; and

by operating the liquid feeding mechanism, conveying the eluent from the third liquid tank to the collection tank via the first flow path, the purification membrane, and the second flow path.

[Claim 20]

A sample pretreatment system comprising:

the flow path device according to claim 1;

a nucleic acid extraction portion that is connected to the first flow path of the flow path device and extracts a nucleic acid from a biological sample; and

a nucleic acid amplification portion that is connected to the second flow path of the flow path device and amplifies the nucleic acid.