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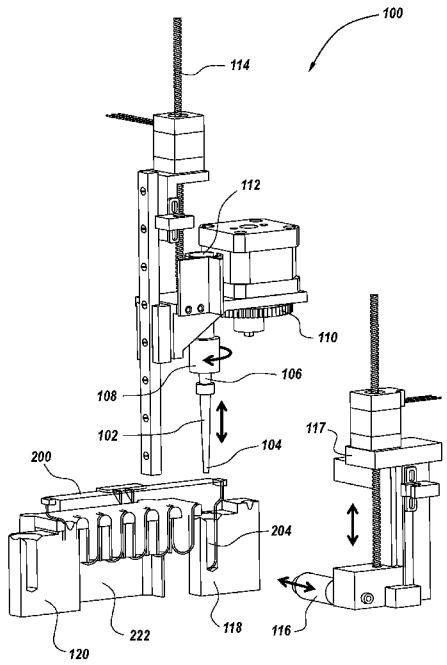

Fig. 1

(57) Abstract: An apparatus, multi-well plate and method for automated cell lysis and nucleic acid purification and processing. The plate includes a lysis well, at least one wash well, and an elution well. The apparatus includes a vertically aligned rotor mixer comprising a magnetic tip and actuators for moving the rotor mixer in a vertical and horizontal directions, to transfer magnetic beads from well to well. The rotor mixer is used to vortex lysis mixtures, wherein the vortexing speed is sufficient to overcome the magnetic attraction between the beads and mixer tip and disperse the beads in solution, to collect nucleic acids such as DNA.

WO 2021/055123 A1

IMPROVED ROTOR MIXER FOR AGITATION OF FLUIDS DURING SAMPLE PREPARATION

CROSS-REFERENCE TO RELATED APPLICATIONS

N/A

FIELD OF THE DISCLOSURE

[0001] The disclosure herein relates generally to the field of cell lysing and nucleic acid purification and isolation. More particularly, the present disclosure relates to a novel rotor mixer and multi-well tray having particular utility in the field of nucleic acid extraction in molecular diagnostics.

BACKGROUND

[0002] In a typical cell lysis and nucleic acid isolation protocol using magnetic beads, a sample is moved by a pipette system to a well within a multi-well plate along with a cell lysis buffer and by a quantity of magnetic beads. The beads are functionalized, for example with silica surfaces, to allow selective binding of nucleic acid molecules such as DNA. A succession of mixing by external vibration, magnetic bead separation, supernatant aspiration, and dilution/washing steps are repeated with respect to the well. Heating of one or more of the wells of the multi-well plate may also be employed to facilitate lysis and/or binding. The sample transfer, washing, and elution steps require separate aspiration and dispensing tips to avoid cross-contamination.

[0003] Due to the common platform for processing multiple samples, heating and time of heating is limited and not customizable. A single overhead pipetting system is typically responsible for processing all samples within the multi-well plate.

[0004] An alternative system and technique involves the use of a magnet disposed within a sealed probe. The probe is selectively disposed within a respective well to allow the magnetic beads to be attracted to the probe by the magnet located within. In one embodiment, the probe may be removed from one well and inserted into fluid within another well. The magnet may then be extracted from within the probe, thus releasing the magnetic beads to be released from the probe surface. Further processing may then follow.

[0005] In the field of molecular diagnostics, there is a need for an efficient and cost-effective system and method for lysing cells and purifying samples for amplicon detection.

SUMMARY

[0006] In order to overcome the inflexibility and expense of the prior art automated processes for cell lysis and purification, the present disclosure provides a new rotor mixer featuring a magnetic tip. The rotor generates a vortex for combining a biological sample with a lysis buffer and magnetic beads, to form a lysis mixture in a lysis well. To optimize nucleic acid absorption on the magnetic beads, the vortexing speed is sufficient to overcome the magnetic attraction between the beads and the magnetic tip of the rotor mixer and allow the beads to disperse freely in the lysis mixture. When vortexing stops, the beads reattach to the magnetic tip. As a result, the rotor tip can be used to transfer the beads from the lysis mixture to and between other wells where they undergo washing and finally elution of the nucleic acids collected from the sample lysate.

[0007] As compared to traditional laboratory vortexing equipment using external vibration of sample wells, the rotor mixer with a magnetic tip provides an efficient, easy and reliable means for transferring magnetic beads between wells, and this setup is particularly suitable to automated sample preparation techniques.

[0008] Also provided for use with the rotor mixer is a disposable multi-well plate having a series of open fluid wells. A first well serves as lysis vessel where the lysis mixture is processed by rotor-induced vortexing and the magnetic beads bind to nucleic acid molecules. Other wells serve as washing vessels where the beads are treated with wash buffer to remove undesired lysis mixture residue. In a last, elution well, the washed beads are immersed in elution buffer to collect the nucleic acid molecules from the original sample lysate. Provision is also made for selective, customizable direct heating of one or more of the lysis well and elution well to enhance lysing and/or elution, if desired.

[0009] The present system and method enable the provision of multi-well plates with wells preloaded with buffers by the manufacturer, thereby speeding up the overall process and diminishing the likelihood of operator error. Alternatively, multiple trays may be provided in bulk, in a stacked configuration, optionally with each lysis well having respective preloaded magnetic beads.

[0010] Other unique features of the presently disclosed system and method include the provision of a disposable protective sleeve to protect the magnetic tip of the rotor during vortexing and other steps of nucleic acid isolation processes. Optionally, the protective sleeve may be fitted by vortex-increasing features such as propeller-shaped projections or paddles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Illustrative embodiments of the disclosed technology are described in detail below with reference to the attached drawing figures, which are incorporated by reference herein and wherein:

[0012] Fig. 1 is a perspective view of a sample lysis and nucleic acid extraction apparatus according to the present disclosure;

[0013] Fig. 2 illustrates vortexing of a lysis mixture by the apparatus of Fig. 1 in the presence of magnetic beads;

[0014] Fig. 3 illustrates the application of an external magnetic field to the lysis well of a multi-well plate;

[0015] Fig. 4 illustrates removing the external magnetic field of Fig. 3 from the multi-well plate and lowering the magnetic tip, to collect the beads;

[0016] Fig. 5 illustrates removing the rotor mixer tip with the magnetic beads magnetically attached thereto from the lysis well;

[0017] Fig. 6 illustrates horizontally moving the rotor mixer tip of the apparatus of Fig. 1 with the magnetic beads magnetically attached thereto towards a wash well;

[0018] Fig. 7 is a perspective view of a multi-well plate according to the present disclosure;

[0019] Fig. 8 is a side section view of the multi-well plate of Fig. 7;

[0020] Fig. 9 is a top view of the multi-well plate of Fig. 7;

[0021] Fig. 10 is a rear view of the of the multi-well plate of Fig. 7;

[0022] Fig. 11 is front view of the multi-well plate of Fig. 7;

[0023] Fig. 12 is a side view of the multi-well plate of Fig. 7;

[0024] Fig. 13 is a bottom view of the multi-well plate of Fig. 7; and

[0025] Fig. 14 is a flowchart of a method of sample lysis, purification, and elution.

DETAILED DESCRIPTION

[0026] Disclosed herein is an apparatus for extracting nucleic acids such as DNA molecules from biological samples. Use of the presently disclosed and described apparatus enables simplified, easy, and reliable transfer of magnetic beads between wells in a fashion particularly suitable to automated sample preparation techniques.

[0027] Fig. 1 illustrates an exemplary embodiment of an apparatus 100 according to the present disclosure. The apparatus 100 is comprised of a rotor mixer 102 having a magnetic tip 104, such as a ferromagnetic material enclosed within an inert polymeric coating, connected to rotor hub 108 via rotor shaft 106. A disposable protective sleeve, in this instance conically-shaped transparent plastic sleeve 107 shown in Fig. 2, protects magnetic tip 104 and rotor shaft 106 from the contents of buffers and mixtures that are used in nucleic acid extraction. Rotor engine 110 powers rotor mixer 102 when vortexing mixtures of fluids and other components such as magnetic beads. When the rotor mixer 102 is not vortexing, the beads magnetically attach to magnetic tip 104. Hence, when one of the steps of a nucleic acid isolation process is completed, the beads attached to the magnetic tip 104 can be easily transferred to another vessel where the next step is carried out. To this end, the apparatus is fitted with a vertical actuator 112 configured to move rotor mixer 102 in an upwardly or downwardly direction along vertical rail 114 and with a horizontal actuator (not shown) for repositioning the rotor mixer 102 from a vessel to another. In addition, the apparatus 100 may include an external magnet 116 that may be selectively translated to and away from a side wall of a vessel in order to attract and release, respectively, magnetic beads disposed within a vessel. The external magnet may also be selectively, vertically translated relative to the vessel, as will be discussed subsequently.

[0028] The vessels may be provided in the form of process wells in a disposable multi-well plate or holder for use in cell lysing and nucleotide purification. Use of the presently disclosed and described multi-well plate enables simplified and faster cell lysis and nucleotide extraction as compared to currently practiced methods. Figs. 7-13 illustrate an embodiment of a multi-well plate 200 having a body member and a plurality of wells extending in a downwardly direction from the floor of the body member, according to the present disclosure. In this embodiment, the body member is a channel 202 and the process wells include a lysis well 204, wash wells 206, 208, 210, 212, 214, and elution well 216. The channel may help inhibit the unintended flow of working fluid off the multi-well plate.

[0029] Lysis well 204 is disposed at a first end 201 of the multi-well plate while the wash wells are disposed intermediate the first end and an opposite second end 203 where elution well 216 is located. Each well extends in a substantially orthogonal direction from the floor of the channel 202 and has an interior volume communicating with the channel via an aperture in the channel floor. The illustrated apertures are circular and coplanar with the floor surface, although embodiments of differing shapes and orientations are also

contemplated. The apertures are also substantially colinear along the floor surface and are centered about a longitudinal axis 218 of the multi-well plate.

[0030] In one embodiment, the wells are pre-filled with appropriate buffers and other components and then sealed off, for example with a peel-away layer that is removed at the time of use. In another embodiment, the wells each have a tapered lower extent. This enables multiple multi-well plates to be vertically stacked, whereby the outer surface of a lysis well of a first holder is received within the lysis well of a lower, second holder. Similarly, the outer surfaces of the wash wells of the first holder are each received within a respective wash well of the lower, second holder.

[0031] In order to optimize vortexing and bead collection performance, the lower extent of lysis well 204 may have a geometry capable of receiving the magnetic tip 104. As seen for example in the side section view of Fig. 8, lysis well 204 may have a larger volume than the wash wells in order to provide sufficient space for the biological sample, lysis buffer, and magnetic beads. Conversely, elution well 216 may have a smaller volume than the wash wells in order to minimize dilution of the final nucleic acid product and may be characterized by a conical cross-section to facilitate removal of the product with a pipettor or other devices for transferring fluids.

[0032] The lysis well 204 of the multi-well plate 200 may be subjected to heating, depending upon the characteristics of the lysis process implemented therewith. For example, the outer surface of the lower extent of the lysis well 204 may be configured to be received within a heater external to the unitary structure. Such a heater may be a heating block 118 placed beneath the holder, receiving the outer surface of the lower extent of the lysis well therewithin for a required or desired time period. Similarly, the elution well 216 of the multi-well plate 200 may be heated with another heater external to the unitary structure, such as heating block 120, depending upon the elution process implemented therewith.

[0033] The multi-well plate 200 may be provided with retention features, such as tab 220 projecting from the upper rim of channel 202 or other lateral projections extending from the multi-well plate on either side of the multi-well plate 200. During processes such as heating and vortexing, when external devices move relative to the multi-well plate 200, the retention features may be selectively engaged by external gripping mechanisms, thereby maintaining the multi-well plate in a fixed position relative to the external devices. The retention features may also be of use during the introduction of samples, buffers, beads or other components in the wells or eluted product retrieval as a pipetting system presses down on the inner surface of the elution well 216. Alternatively, the multi-well plate and

associated heating blocks and support structures, i.e., the plate holder 222, may be configured for lateral, horizontal translation relative to the rotor mixer 102, thus obviating the need for enabling horizontal translation of the rotor mixer and associated components.

[0034] A non-limiting exemplary method of using the system of Fig. 1 in combination with the multi-well plate of Figs. 7-13 is now described in conjunction with Figs. 2-6 and 14. Not all steps need be practiced in the order described below, nor be utilized at all, depending upon the embodiment. First, in step 300, a multi-well plate such as described in the foregoing is provided and placed into plate holder 222. In step 302, one or more wash buffers are loaded into the wash wells, an elution buffer is loaded into elution well 216, and lysis buffer is loaded into the lysis well 204.

[0035] Magnetic beads 402 are also introduced in the lysis well 204, as at step 304. In one example, the material of the beads may be optimized for genomic DNA extraction from blood samples, but its composition may vary to suit other types of bodily fluids or tissues or for extracting other types of nucleic acids such as RNA. A biological sample is then loaded into the lysis well (step 306), yielding a lysis mixture ready for vortexing. Typical samples include blood, sputum, hair, and other bodily fluids and tissues, optionally pretreated for example by freezing, homogenizing, or grinding. Those of skill in the art will recognize that the choice of buffers and other reactants may vary according to the type of sample and beads to provide optimal conditions for nucleic acid extraction. While this illustrated process depicts a certain order of loading the lysis well to form the lysis mixture, other orders may be employed, such as disposing the sample into the lysis well prior to adding the magnetic beads.

[0036] In step 308, the lysis mixture is vortexed by spinning the rotor mixer 102, as exemplified in Fig. 2, either continuously or intermittently. For at least a portion of the vortexing step, the rotor mixer 102 is spun at a rate sufficient to overcome attraction forces between magnetic beads 402 and magnetic tip 104, thereby freeing the beads to swirl about the lysis mixture and bind to nucleic acid molecules dispersed therein following cell lysis. In an exemplary embodiment, the rotor mixer spins at about 5,000 to about 10,000 revolutions per minute.

[0037] When vortexing ceases, magnetic beads 402 attach to magnetic tip 104. In instances where some or all the beads fail to attach and remain afloat or absorbed to the well wall, external magnet 116 may be translated to a side wall of lysis well 204 by operation of translation member 117 (Fig. 3). The rotor mixer 102 is temporarily moved upwards to a higher level of the lysis mixture, while the magnetic field exerted by external magnet 116 on

the outer surface of the well forms the beads 402 into a cluster adhering to the well wall. External magnet 116, still pressed against the side of lysis well 204, is moved by the translation member first downwards, then away from well wall (Fig. 4), thereby removing its magnetic field and leaving the beads 402 resting at the bottom of well 204 and ready for collection (step 310). The rotor mixer is then lowered into the lysis well 204 whereby the beads 402 are gathered against the magnetic tip 104.

[0038] In bead-washing step 312, vertical actuator 112 moves rotor mixer 102 in an upwardly direction, as illustrated in Fig. 5, thereby extracting tip 104 and the magnetic beads 402 attached thereto from lysis well 204. Fig. 6 shows the horizontal actuator (not shown) aligning rotor shaft 106 with one of wash wells, for example wash well 206. The external magnet 116 may also be moved horizontally in coordination with the rotor 102. Alternatively, the plate holder 222 may translate the multi-well plate 200 relative to the rotor and external magnet. Once properly aligned, the vertical actuator 112 moves the rotor mixer 102 in a downwardly direction, to immerse the magnetic tip 104 and the beads 402 attached thereto in a wash buffer contained in the wash well 206. A process similar to that executed within the lysis well 204 may then be carried out, including moving the external magnet 116 to a position adjacent the wash well, rotating the rotor to release the beads into the wash buffer and to allow the beads to gather on the wash well wall adjacent the external magnet, removing the external magnet, rotating the rotor again to resuspend the beads in the wash buffer, then ceasing rotation to allow the beads to reattach to the rotor magnetic tip 104. This process may also include vertically manipulating the external magnet to gather the beads in the bottom of the wash well prior to reintroducing the rotor and magnetic tip. This procedure can be repeated in any or all of the other wash wells 208, 210, 212, and 214. After a desired number of washing steps have been completed, the vertical and horizontal actuators immerse magnetic tip 104 and magnetic beads 402 in elution well 216, where nucleic acids elute from magnetic beads 402 into the elution buffer (step 314).

[0039] As anticipated, the contents of the lysis well 204 may be heated prior to the illustrated step 310 of applying an external magnetic field to an exterior surface of the lysis well. Following removal of the beads 402, liquid residues in the lysis well and the wash wells may be aspirated by a pipetting system and dispensed to a waste receptacle. Similarly, elution well 216 may undergo heating at any point prior to removal of the final nucleotide product solution.

[0040] The foregoing description has been directed to particular embodiments. However, other variations and modifications may be made to the described embodiments,

with the attainment of some or all of their advantages. It will be further appreciated by those of ordinary skill in the art that modifications to the above-described systems and methods may be made without departing from the concepts disclosed herein. Accordingly, the invention should not be viewed as limited by the disclosed embodiments. Furthermore, various features of the described embodiments may be used without the corresponding use of other features. Thus, this description should be read as merely illustrative of various principles, and not in limitation of the invention.

[0041] Many changes in the details, materials, and arrangement of parts and steps, herein described and illustrated, can be made by those skilled in the art in light of teachings contained hereinabove. It will be understood that certain features and sub-combinations are of utility and may be employed without reference to other features and sub combinations and are contemplated within the scope of the claims. Accordingly, it will be understood that the following claims are not to be limited to the embodiments disclosed herein and can include practices other than those specifically described, and are to be interpreted as broadly as allowed under the law. Additionally, not all steps listed in the various figures need be carried out in the specific order described.

CLAIMS

I claim:

1. A multi-well plate for performing nucleic acid isolation, comprising:

a body member extending from a first end to an opposite second end of the multi-well plate,

a series of plural process wells, comprising:

a lysis well proximate the first end,

at least one wash well intermediate the first and second end, and

an elution well proximate the second end of the body member,

each well extending from the floor of the body member and having an interior volume and an aperture in the body member, the aperture extending into the respective well interior volume.

2. The multi-well plate of claim 1, wherein each well extends substantially orthogonally from the floor of the body member.

3. The multi-well plate of claim 1, wherein each well aperture is substantially circular and coplanar with the body member floor surface.

4. The multi-well plate of claim 1, wherein an outer surface of each of the lysis well, wash well, and elution well of a first multi-well plate are capable of being received within corresponding inner surfaces of the lysis well, wash well, and elution well of a second multi-well plate.

5. The multi-well plate of claim 1, further comprising a retention feature proximate the body member for enabling selective retention of the multi-well plate by a releasable gripping mechanism external to the multi-well plate.

6. The multi-well plate of claim 1, further comprising one or more additional wash wells.

7. The multi-well plate of claim 1, wherein a lower extent of the lysis well is configured to receive at least the tip of a rotor mixer.

8. The multi-well plate of claim 1, wherein the plural wells are linearly aligned along an axis of symmetry of the body member between the first and second ends of the multi-well plate.

9. The multi-well plate of claim 1, wherein the lysis well has a larger volume than the wash well.

10. The multi-well plate of claim 1, wherein the elution well has a smaller volume than the wash well.

11. The multi-well plate of claim 1, wherein the elution well has a conical cross-section.

12. The multi-well plate of claim 1, wherein the body member is a channel and each well extends substantially orthogonally from the floor of the body member.

13. The multi-well plate of claim 1, further comprising a buffer solution in at least one well.

14. The multi-well plate of claim 13, further comprising a peelable layer sealing the buffer inside the well.

15. A sample lysis and nucleic acid extraction apparatus, comprising:

(i) a base for retaining a multi-well plate, the multi-well plate comprising: a lysis well, at least one wash well, and an elution well;

(ii) a vertically aligned rotor mixer comprising a magnetic tip;

(iii) a rotor mixer vertical actuator configured to impart elevational movement of the rotor mixer to selectively insert or remove the rotor mixer magnetic tip from a well of the multi-well plate, and

(iv) a rotor mixer horizontal actuator configured to selectively impart horizontal movement of the rotor mixer between any two wells of the multi-well plate.

16. The apparatus of claim 15, further comprising a magnetic member configured to selectively horizontally extend and retract a magnet with respect to a first position adjacent to the multi-well plate and to selectively impart elevational movement of the magnet along a surface of a multi-well plate well.

17. The apparatus of claim 15, wherein the base comprises a plurality of slots wherein each slot is configured to receive and engage with a well of a multi-well plate.

18. The apparatus of claim 15, further comprising a lysis well heating block configured to heat the lysis well of the multi-well plate.

19. The apparatus of claim 15, further comprising an elution well heating block configured to heat the elution well of the multi-well plate.

20. The apparatus of claim 15, wherein the rotor mixer magnetic tip is covered with a disposable protective sleeve.

21. The apparatus of claim 15, wherein the protective sleeve includes a vortex-increasing device selected from the group consisting of a propeller-shaped projection, a paddle, and combinations thereof.

22. A system for isolating nucleic acids from a biological sample, comprising the apparatus of claim 15 and a multi-well plate comprising a lysis well, at least one wash well, and an elution well.

23. A method of isolating nucleic acids from a biological sample, comprising:

providing a multi-well plate for performing nucleic acid isolation, the multi-well plate comprising:

a sequence of plural process wells between a first end and an opposite second end of the multi-well plate, the sequence comprising:

a lysis well proximate the first end of the multi-well plate,

at least one wash well intermediate the first and second ends of the multi-well plate,

an elution well proximate the second end of the multi-well plate;

forming a lysis mixture, in the lysis well, from ingredients comprising the biological sample, a lysis buffer, and magnetic beads;

vortexing the lysis mixture with a rotor mixer comprising a magnetic tip, wherein the vortexing speed is sufficient to disperse the magnetic beads in the lysis mixture;

removing the rotor mixer tip with the magnetic beads magnetically attached thereto from the lysis well and inserting the rotor mixer tip in the at least one wash well, wherein the at least one wash well contains a washing buffer, to wash the magnetic beads; and

removing the rotor mixer from the at least one wash well and immersing the rotor mixer tip with the magnetic beads magnetically attached thereto into the elution well, wherein the elution well contains an elution buffer, to elute the nucleic acids from the beads.

24. The method of claim 23, further comprising:

applying a magnetic field to a side wall of a process well to attract the magnetic beads to the side wall, whereby the magnetic beads are aggregated against the side wall, and

removing the magnetic field.

25. The method of claim 24, further comprising moving the magnetic field in a downward direction, whereby the magnetic beads, attracted by the magnetic field, travel down the wall of the process well, to come to rest at the bottom of the process well.

26. The method of claim 23, further comprising disposing a lysis buffer within the lysis well;

disposing the lysis buffer within the lysis well;

disposing the wash buffer within the wash well, and

disposing the elution buffer within the elution well.

27. The method of claim 23, further comprising heating the lysis well.

28. The method of claim 27, wherein the heating of the lysis well is imparted with a lysis well heating block.

29. The method of claim 23, further comprising heating the elution well.

30. The method of claim 29, wherein the heating of the elution buffer is imparted with an elution well heating block.

31. The method of claim 23, further comprising covering the rotor mixer tip with a disposable protective sleeve.

32. The method of claim 31, wherein the protective sleeve includes a vortex-increasing device selected from the group consisting of a propeller-shaped projection, a paddle, and combinations thereof.

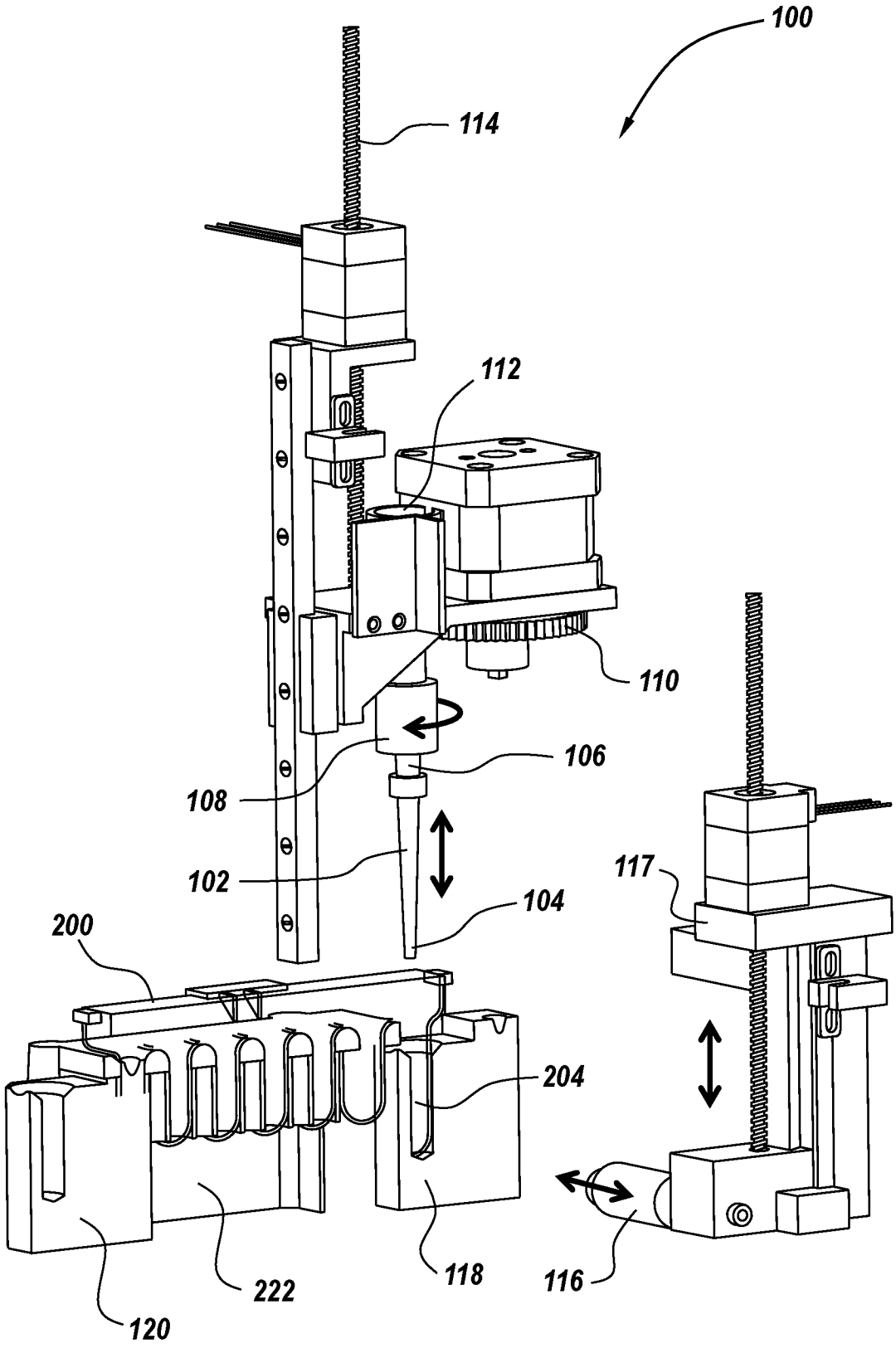

Fig. 1

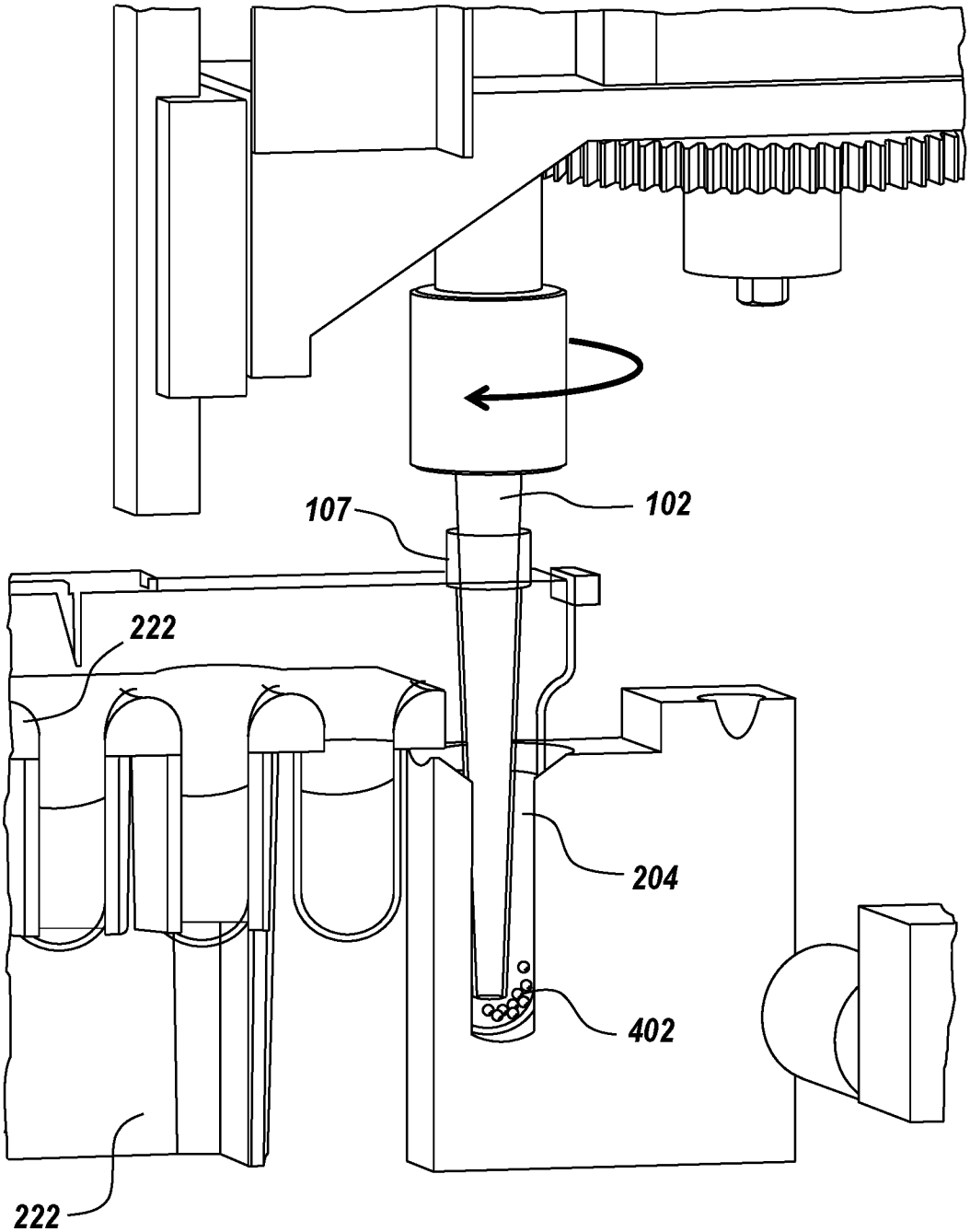

Fig. 2

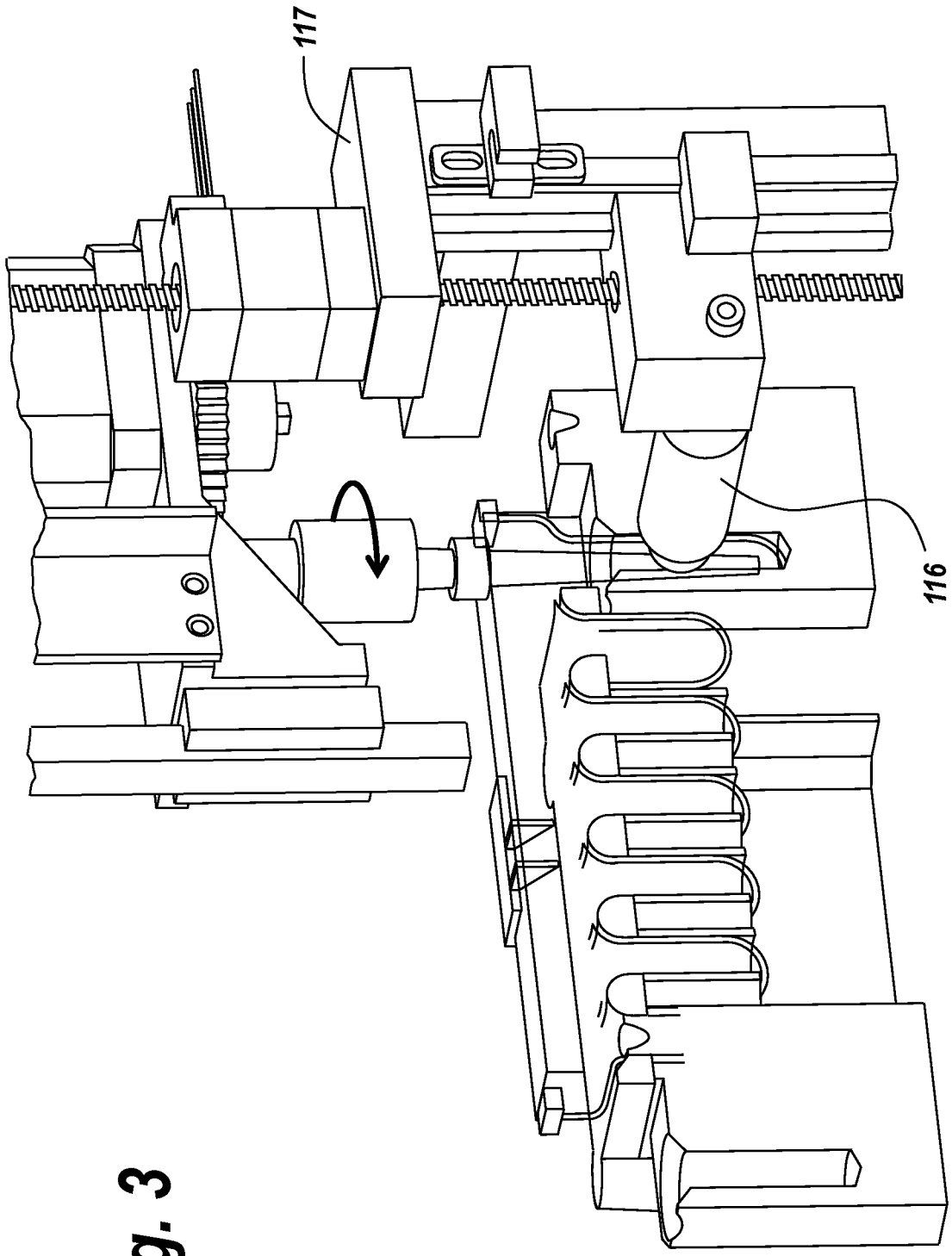

Fig. 3

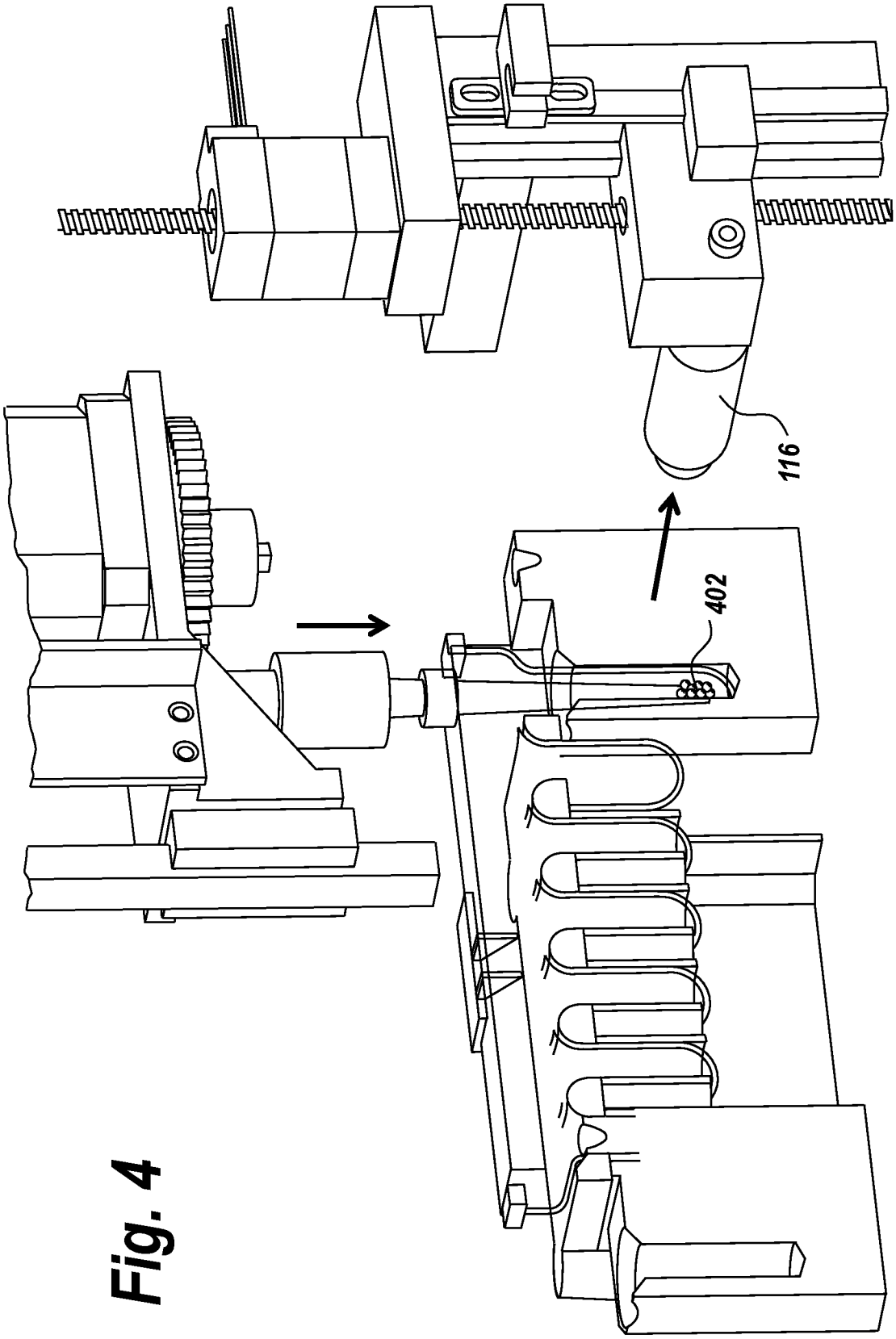

Fig. 4

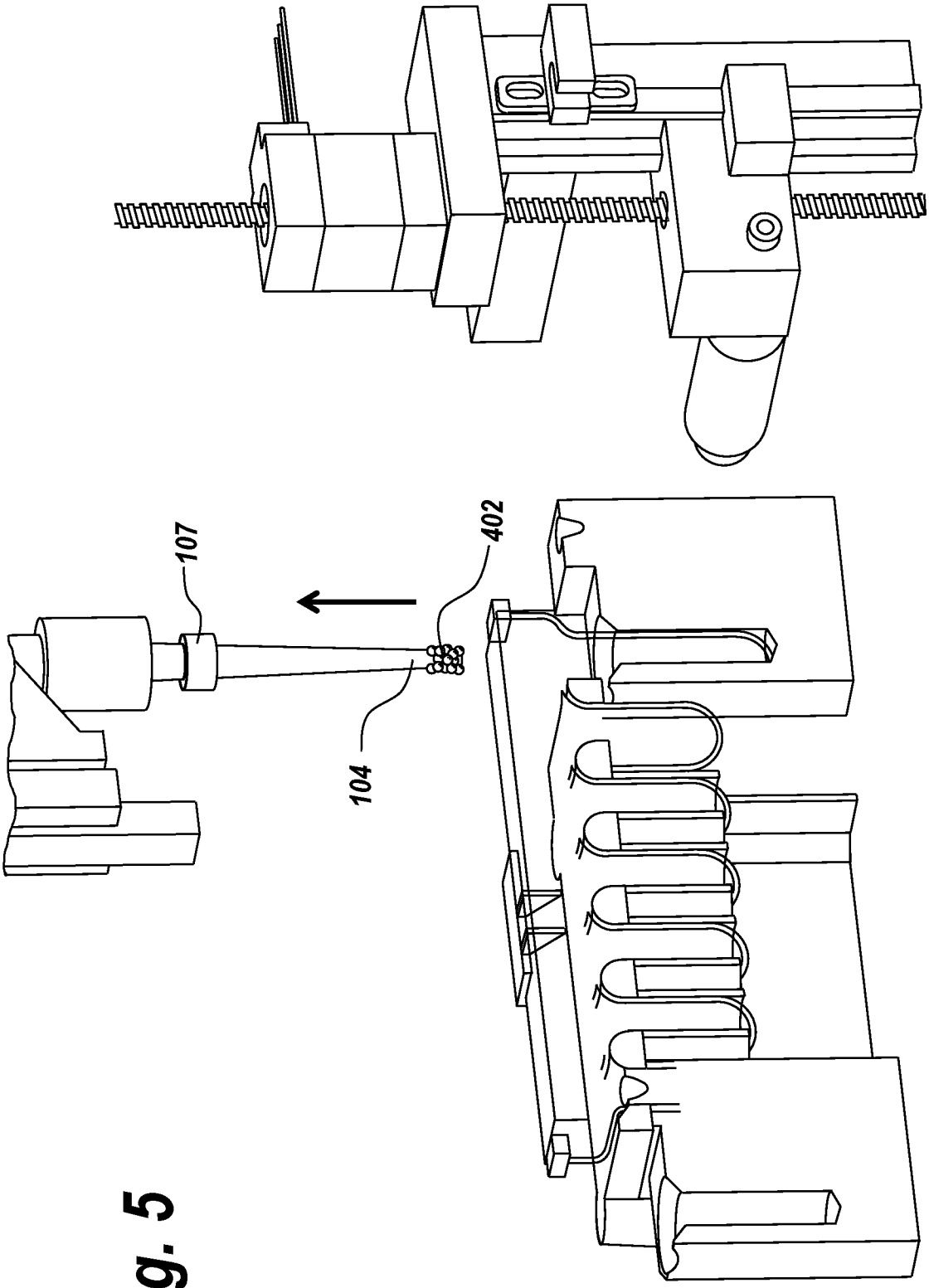

Fig. 5

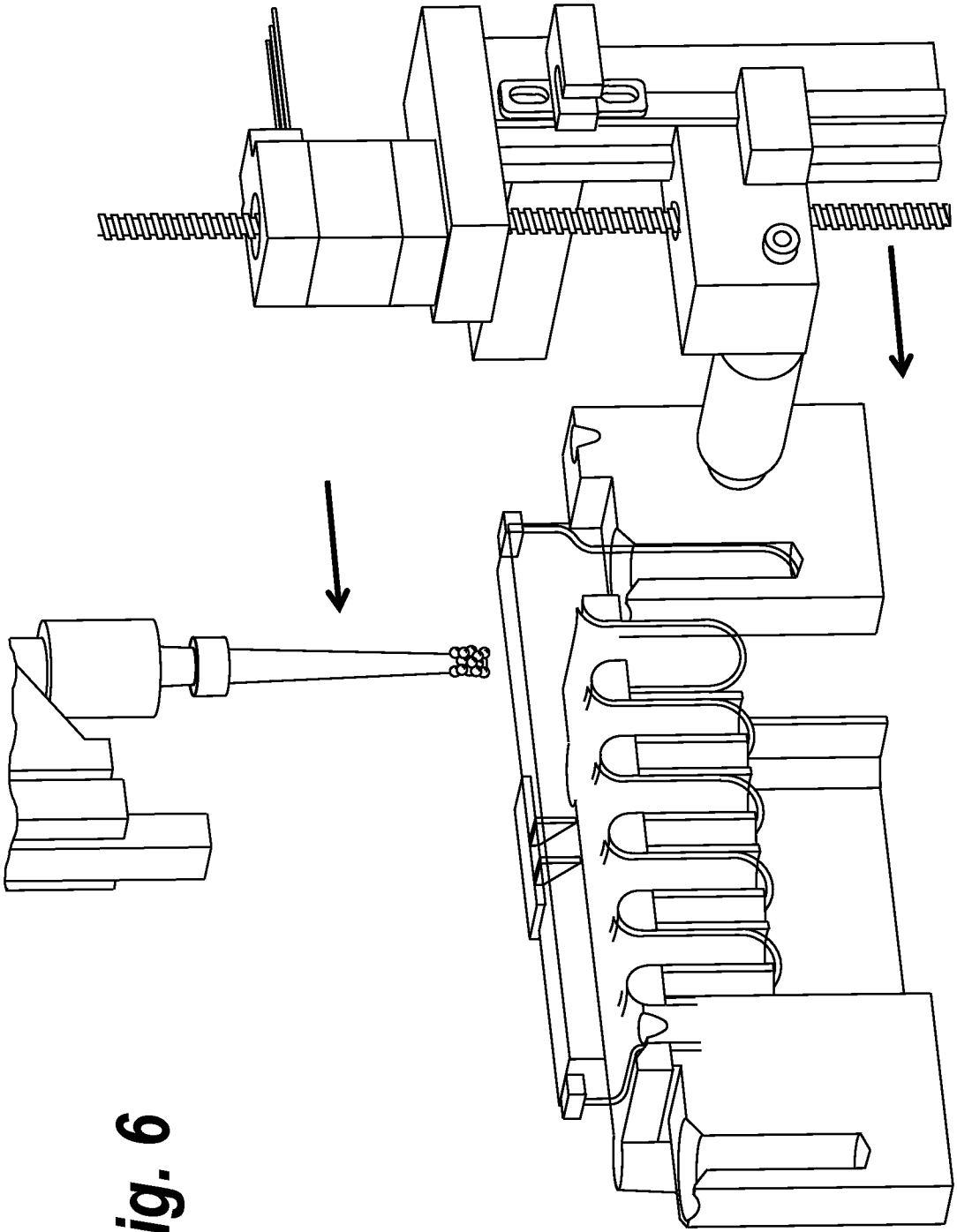

Fig. 6

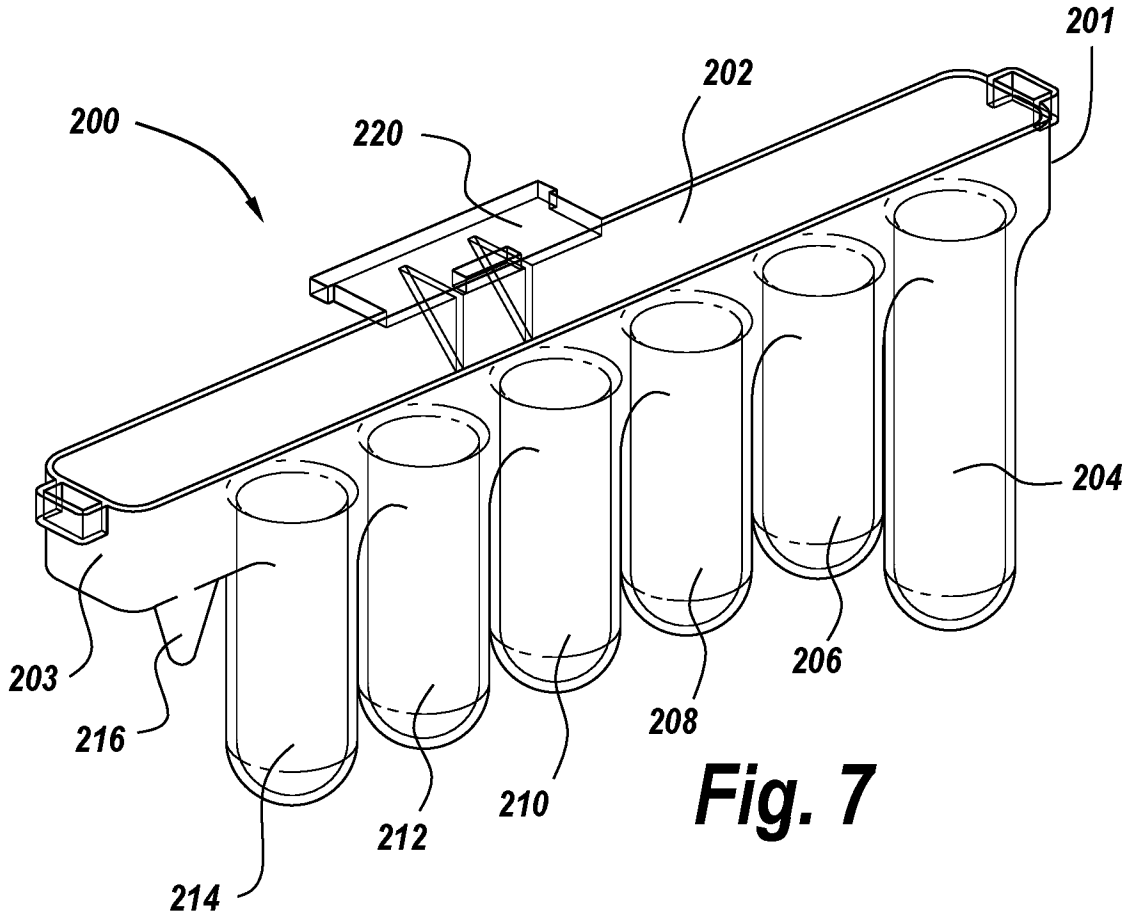

Fig. 7

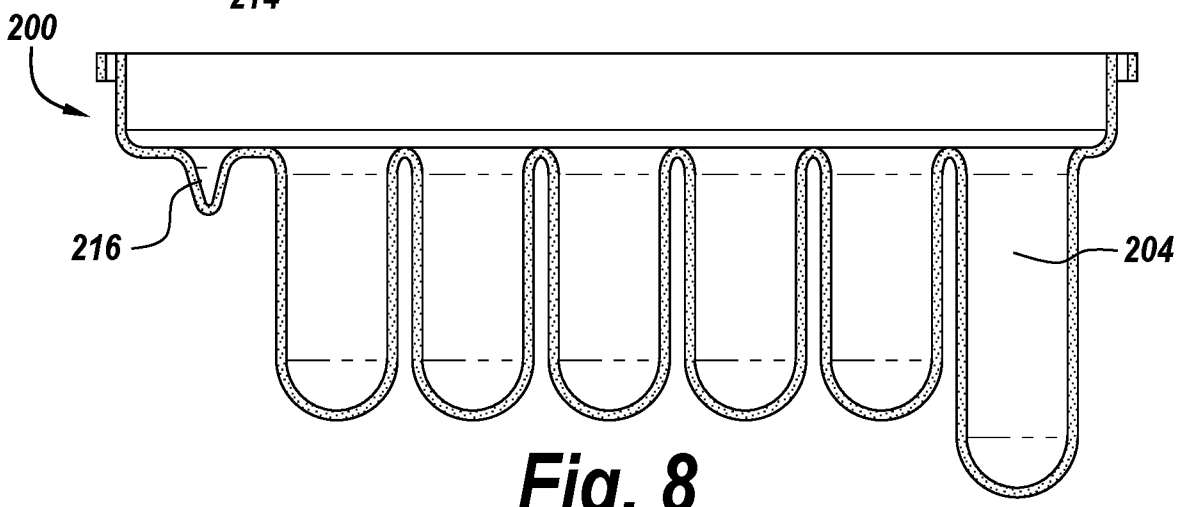

Fig. 8

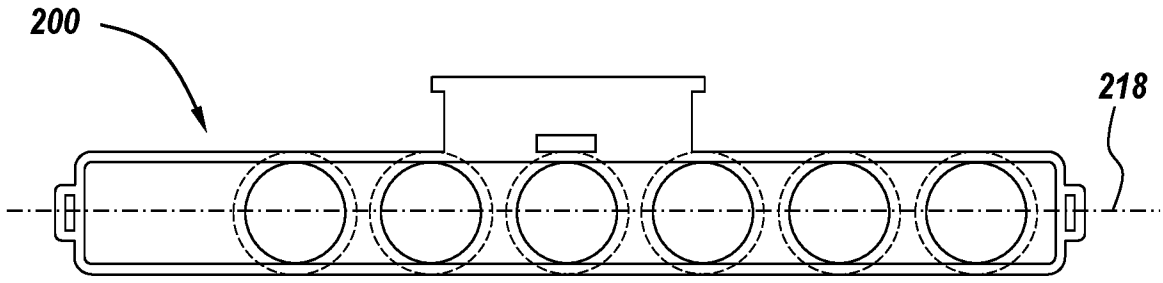

Fig. 9

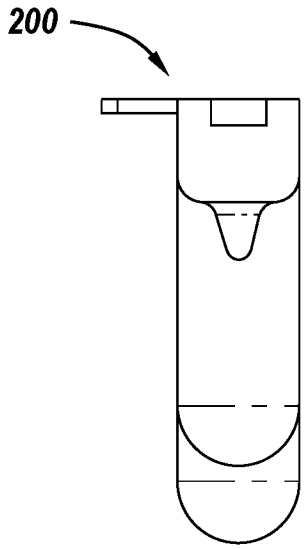

Fig. 10

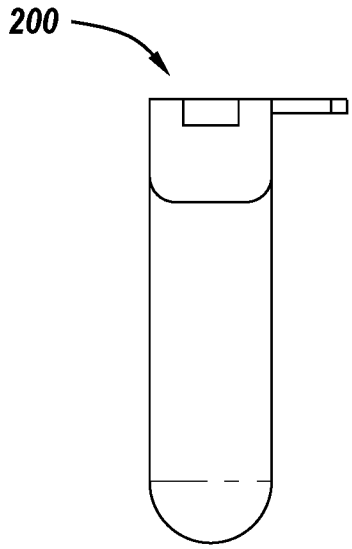

Fig. 11

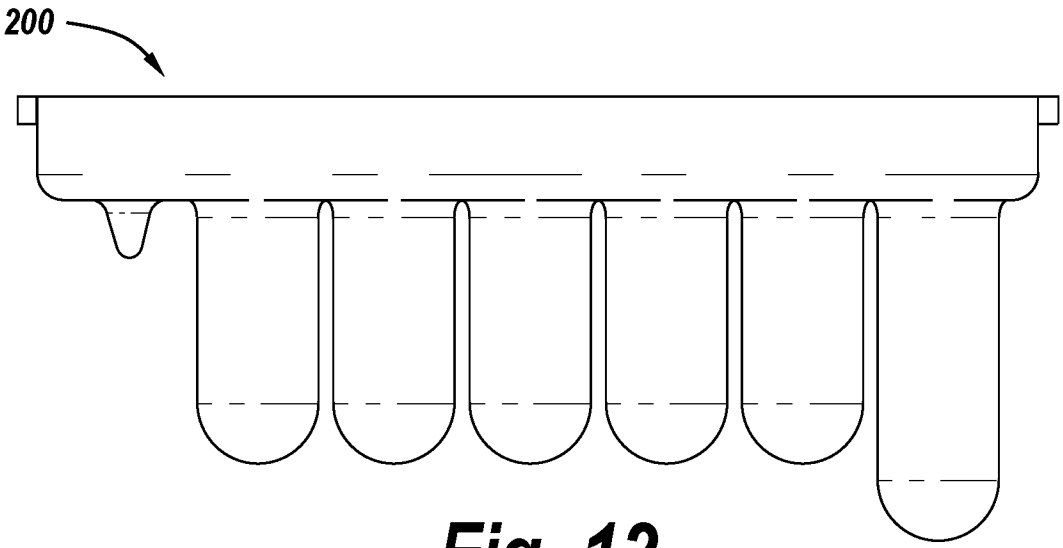

Fig. 12

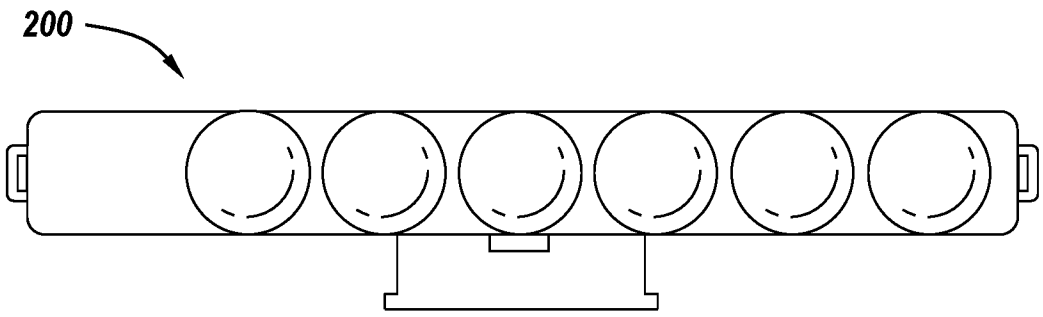

Fig. 13

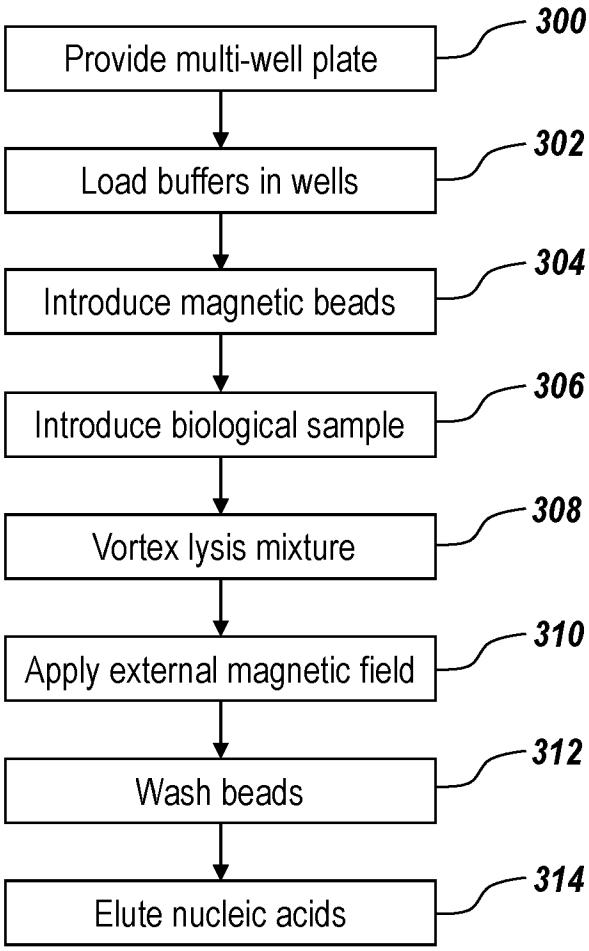
Provide multi-well plate
300
Load buffers in wells
302
Introduce magnetic beads
304
Introduce biological sample
306
Vortex lysis mixture
308
Apply external magnetic field
310
Wash beads
312
Elute nucleic acids
314

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/46743

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-14 directed to a multi-well plate.

Group II: Claims 15-32 directed to a sample lysis and nucleic acid extraction apparatus.
*Note: see Note below.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

--- Continued in Supplemental Box ---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-14

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/46743

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12M 1/00, C12M 1/34, C12Q 1/68, G01N 35/00 (2020.01)

CPC - G01N 35/00, G01N 35/028, G01N 35/1002, G01N 35/1065

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0034845 A1 (NORTHWESTERN UNIVERSITY) 07 February 2013 (07.02.2013), entire document, especially Fig. 1, 2, 3, 4; para[0153]; para[0170]; para[0125]; para[0194];	1-4, 6-10, 13-14
X	US 2013/0196422 A1 (Beckman Coulter, Inc.) 01 August 2013 (01.08.2013), entire document, especially Fig. 4(a)-1; para[0187]; para[0181]; para[0275]; para[0748]; para[0320]; para[0287]; para[0288]; para[0807]; para[0286]; para[0279]; para[0309]; para[0289]; para[0290]; para[0291];	1-2, 4-14
A	US 2018/0169658 A1 (QuanDx Inc.) 21 June 2018 (21.06.2018), entire document	1-14
A	US 8,691,149 B2 (Fritchie et al.) 08 April 2014 (08.04.2014), entire document	1-14
A	US 2012/0135394 A1 (Kim et al.) 31 May 2012 (31.05.2012), entire document	1-14
A	US 9,428,746 B2 (AKONNI BIOSYSTEMS, INC.) 30 August 2016 (30.08.2016), entire document	1-14
A	US 2011/0092691 A1 (Euting et al.) 21 April 2011 (21.04.2011), entire document	1-14

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2020 (23.10.2020)	07 JAN 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 20/46743

--- Continuation of Box No. III Observations where unity of invention is lacking ---

SPECIAL TECHNICAL FEATURES

The invention of Group I includes the special technical feature of a multi-well plate including a series of plural wells extending from a floor of a body member and having an interior volume and an aperture in the body member, the aperture extending into the respective well interior volume not required by the claims of Group II.

The invention of Group II includes the special technical feature of a sample lysis and nucleic acid extraction including a base, a vertically aligned rotor mixer comprising a magnetic tip, a rotor mixer vertical actuator, and a rotor mixer horizontal actuator, not required by the claims of Group I.

COMMON TECHNICAL FEATURES

Groups I and II share the common technical features of a multi-well plate for performing nucleic acid isolation, comprising: a first end and an opposite second end of the multi-well plate, a series of plural process wells, comprising: a lysis well proximate the first end, at least one wash well intermediate the first and second end, and an elution well proximate the second end.

However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2013/0034845 A1 to NORTHWESTERN UNIVERSITY (hereinafter 'NORTHWESTERN'), which NORTHWESTERN discloses a multi-well plate for performing nucleic acid isolation (Fig. 1, 2 - see multi-well plate for performing nucleic acid isolation; para[0153]; para[0170]; para[0125]), comprising: a first end and an opposite second end of the multi-well plate (Fig. 1, 2 - see first end of plate adjacent lysis buffer well, and see second end of plate adjacent elution buffer well), a series of plural process wells (Fig. 1, 2 - see series of plural process wells), comprising: a lysis well proximate the first end (Fig. 1, 2 - see lysis well comprising chamber 1 proximate the first end of the body; para[0170], 'lysis buffer in chamber 1'), at least one wash well intermediate the first and second end (Fig. 1, 2 - see wash well comprising chamber 2 intermediate the first and second ends; para[0170], 'various wash buffers in chambers 2-5'), and an elution well proximate the second end (Fig. 1, 2 - see elution well comprising chamber 6 proximate the second end of the plate; para[0170], 'elution buffer in chamber 6').

As the common technical features were known in the art at the time of the invention, these cannot be considered special technical feature that would otherwise unify the groups.

Therefore, Groups I-II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

*Note:
Claim 1: Regarding claim 1, the term "the floor" is confusing and lacks proper antecedent basis, and accordingly, for purposes of this opinion, has been interpreted to be the term "a floor."