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(54) LOW VOLUME CHEMICAL AND BIOCHEMICAL REACTION SYSTEM

(76) Inventors: **Stevan B. Jovanovich**, Livermore, CA (US); **David J. Roach**, Los Gatos, CA (US)

Correspondence Address:
Amersham Biosciences Corp.
800 Centennial Avenue
Piscataway, NJ 08855 (US)

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(60) Provisional application No. 60/146,732, filed on Aug. 2, 1999.

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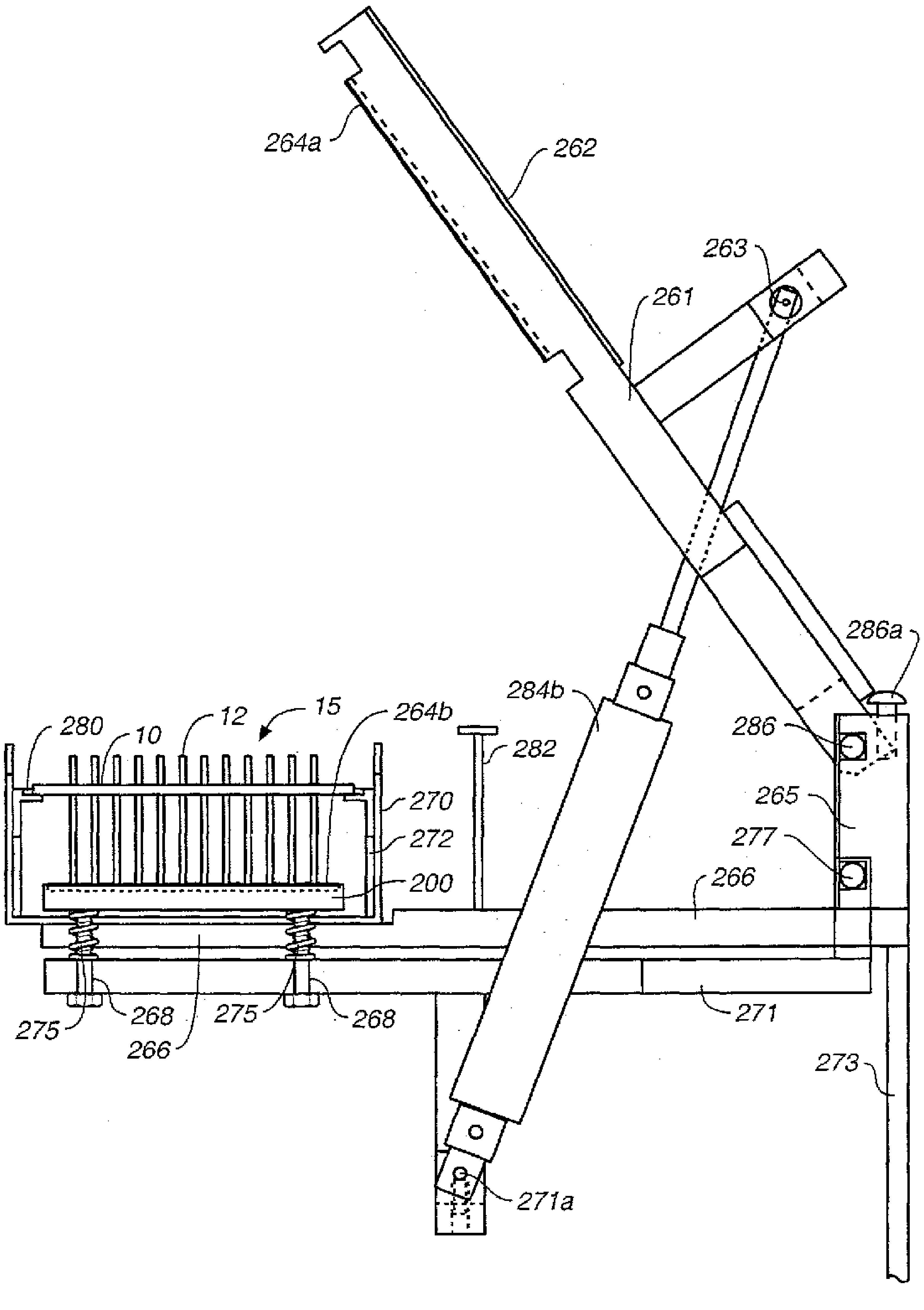
(57) ABSTRACT

A method and device for preparing nanoscale reactions. An automated system utilizes an array of reaction chambers. The ends of the chambers are temporarily sealed with deformable membranes and reactions effected by incubation of temperature cycling. Reaction mixtures may be assembled by using the reaction containers to meter reaction components. After the reaction is finished, the reaction containers may be dispensed onto a substrate and the reaction products analyzed. An automated transfer device may be used for automated transport of the reaction container array or other transportable elements.



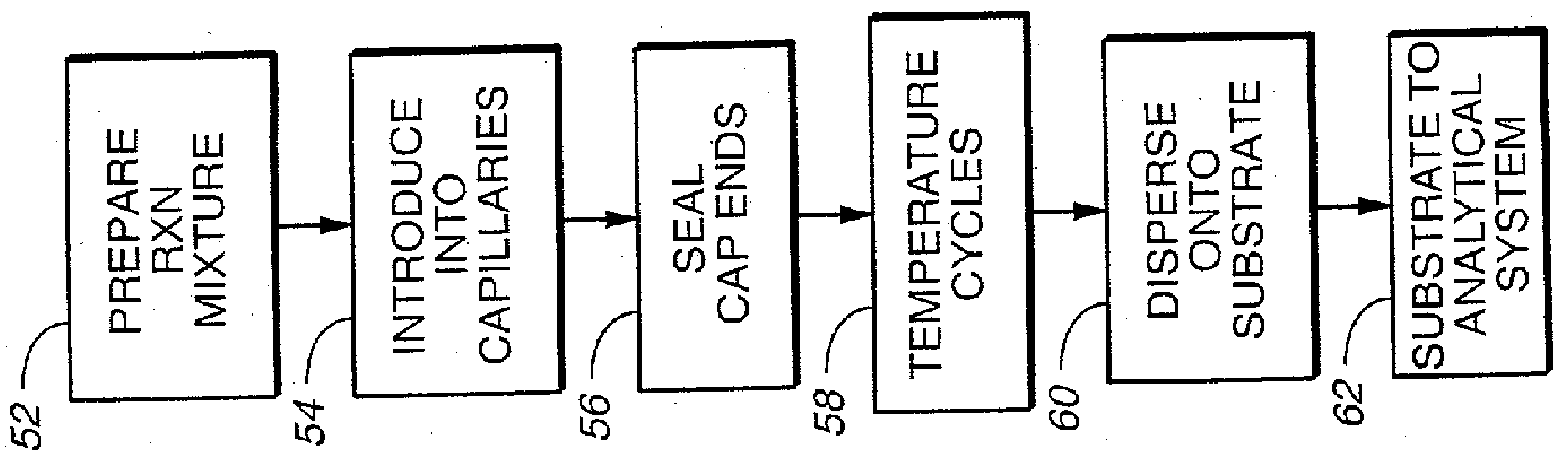
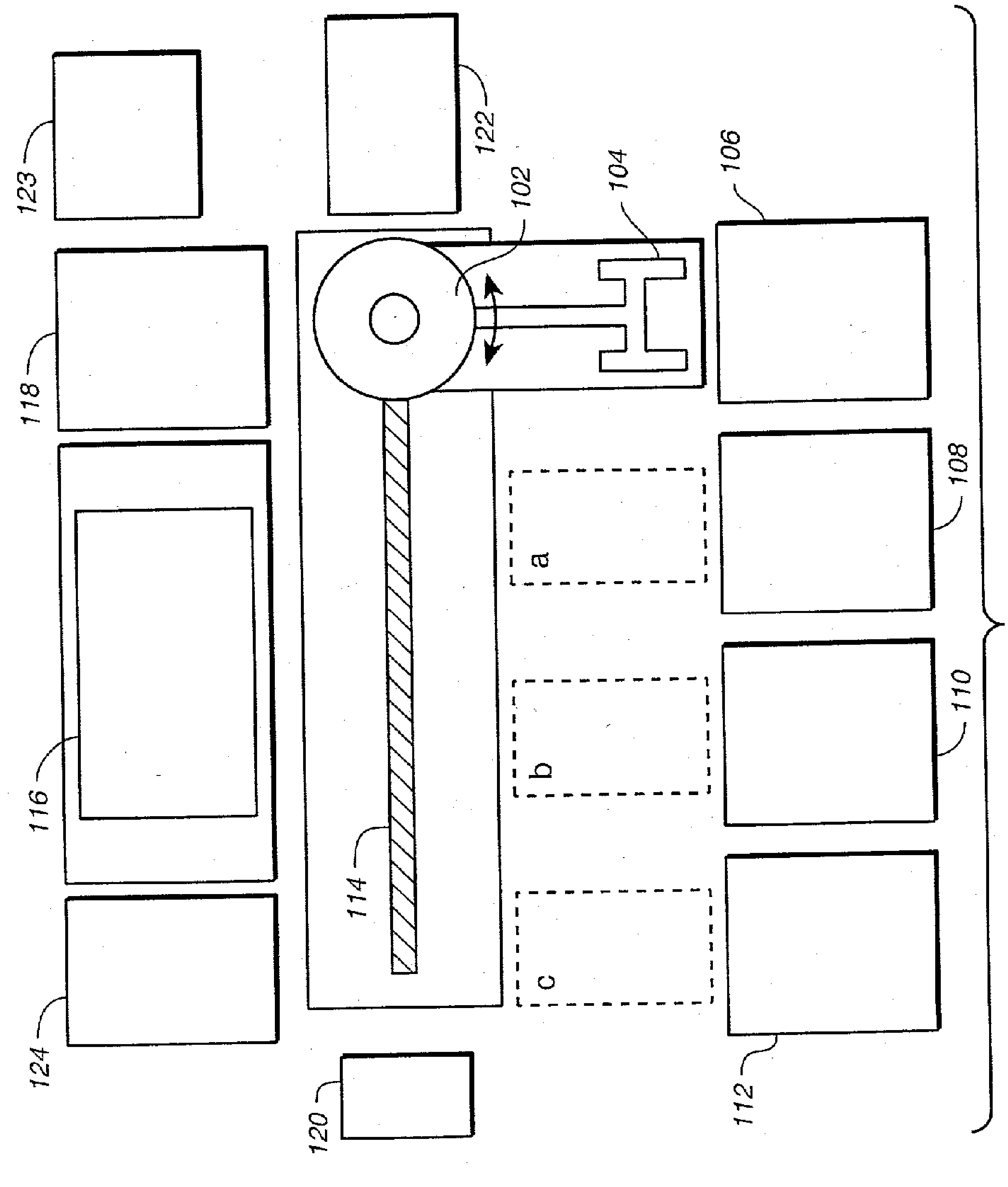

52
PREPARE RXN MIXTURE
54
INTRODUCE INTO CAPILLARIES
56
SEAL CAP ENDS
58
TEMPERATURE CYCLES
60
DISPERSE ONTO SUBSTRATE
62
SUBSTRATE TO ANALYTICAL SYSTEM

FIG._2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122
102
104
106
118
108
a
116
b
110
114
c
124
112
120

FIG._1

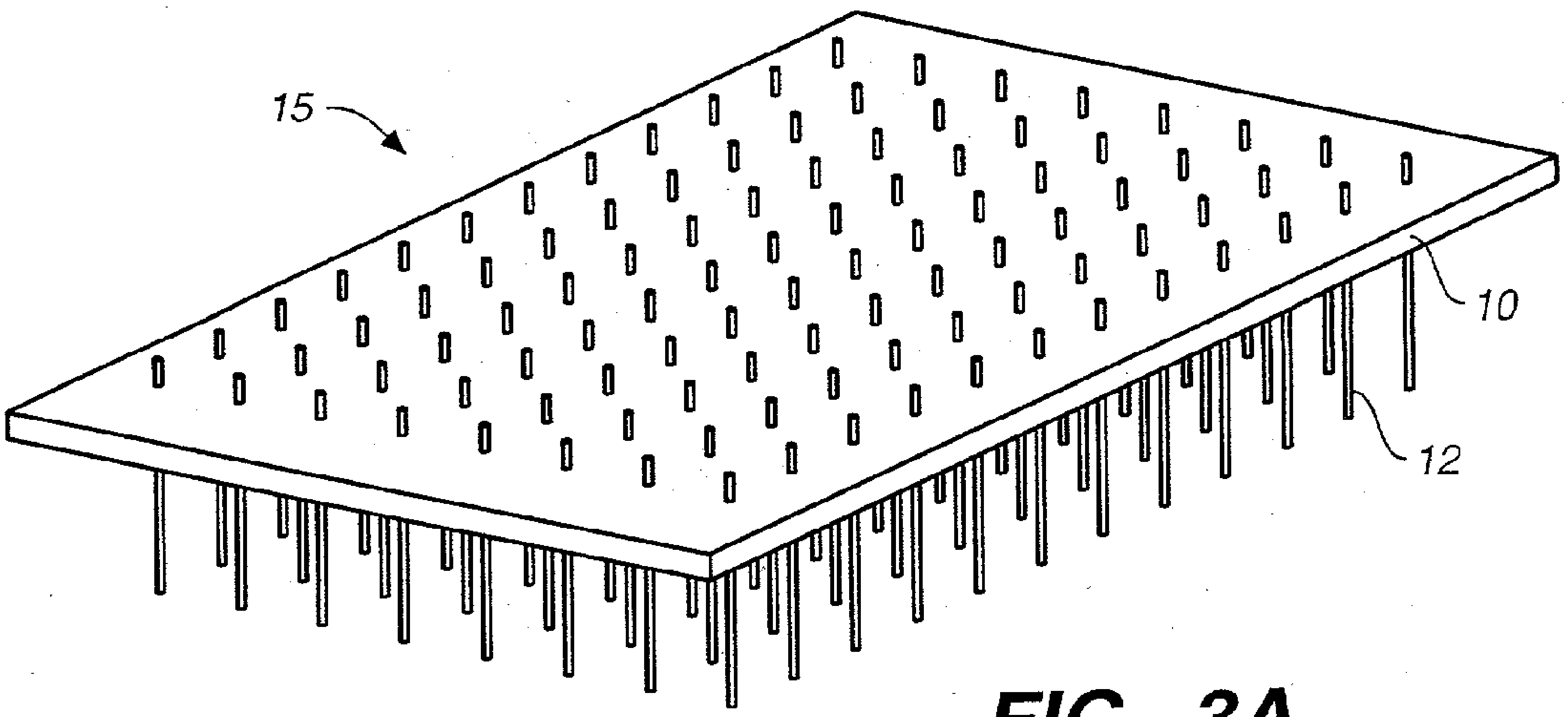


FIG._3A

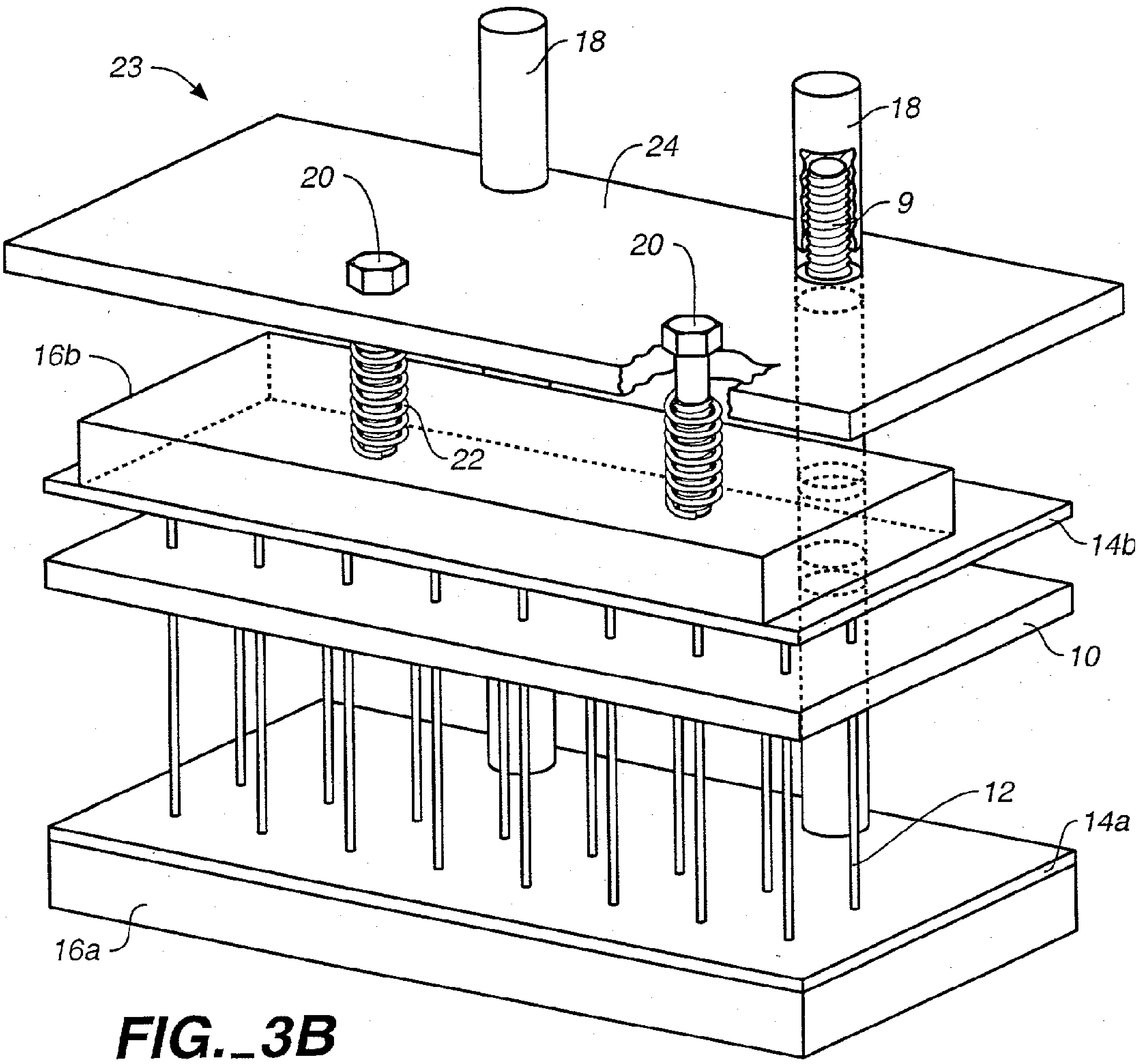


FIG._3B

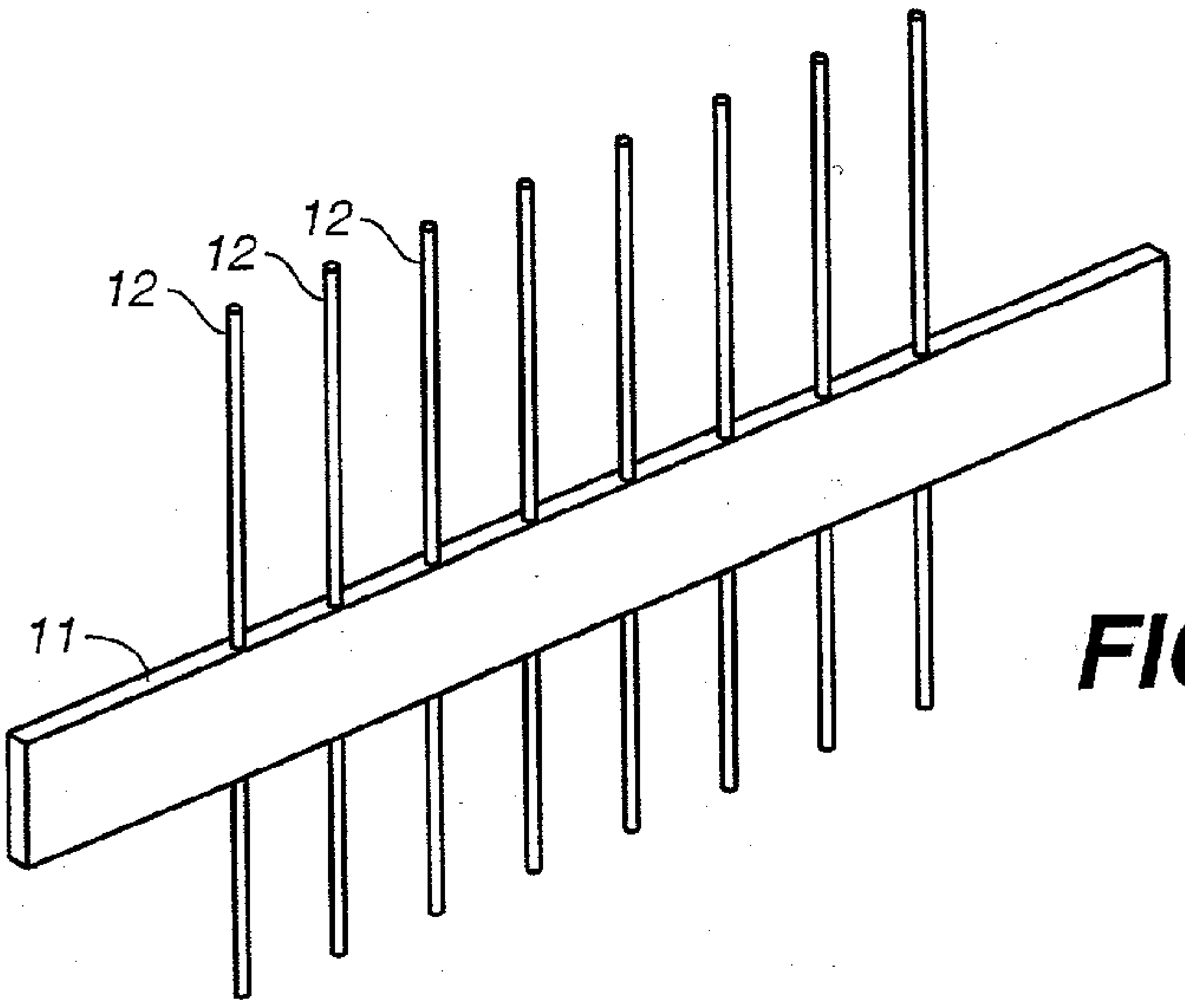


FIG._3C

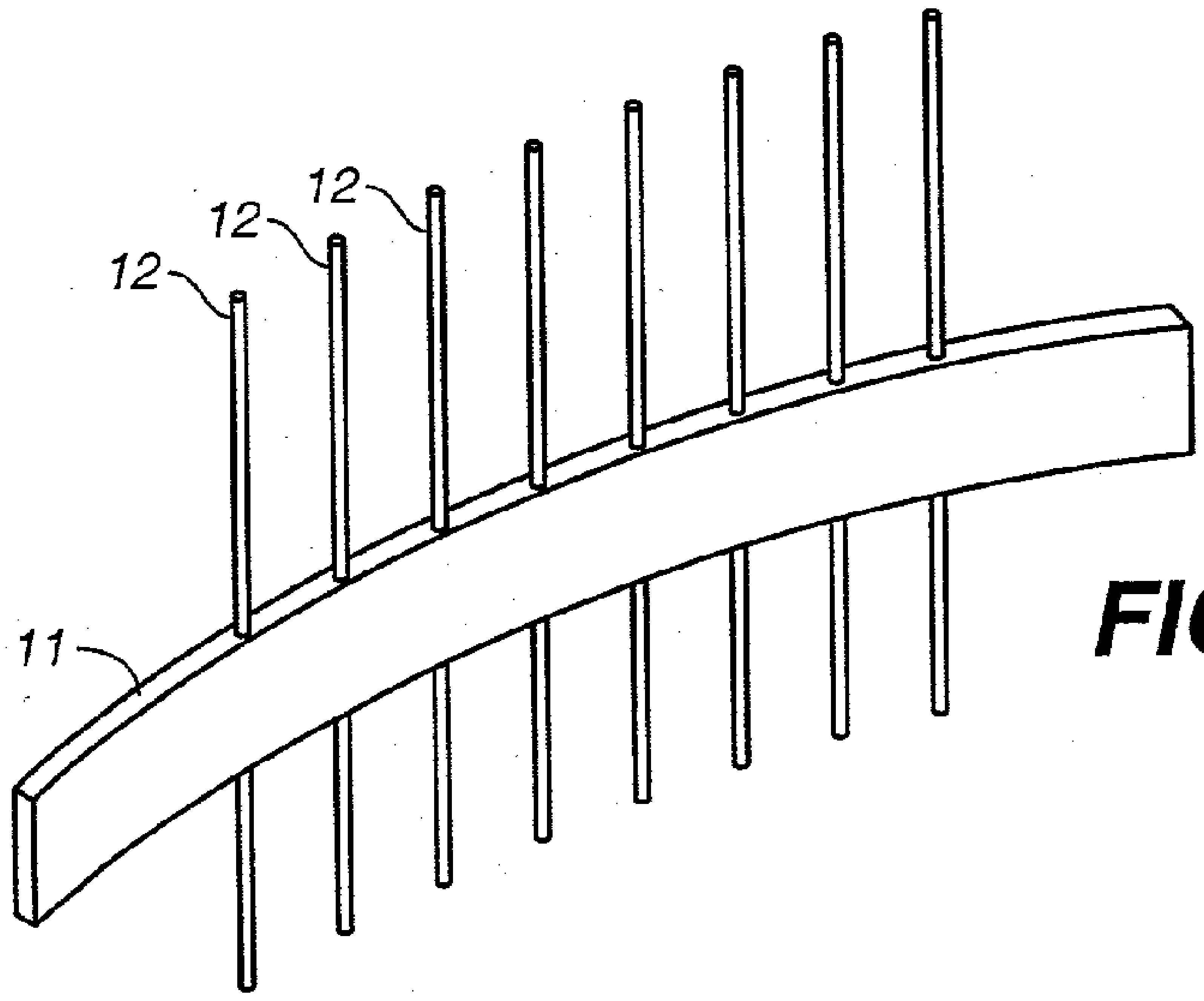


FIG._3D

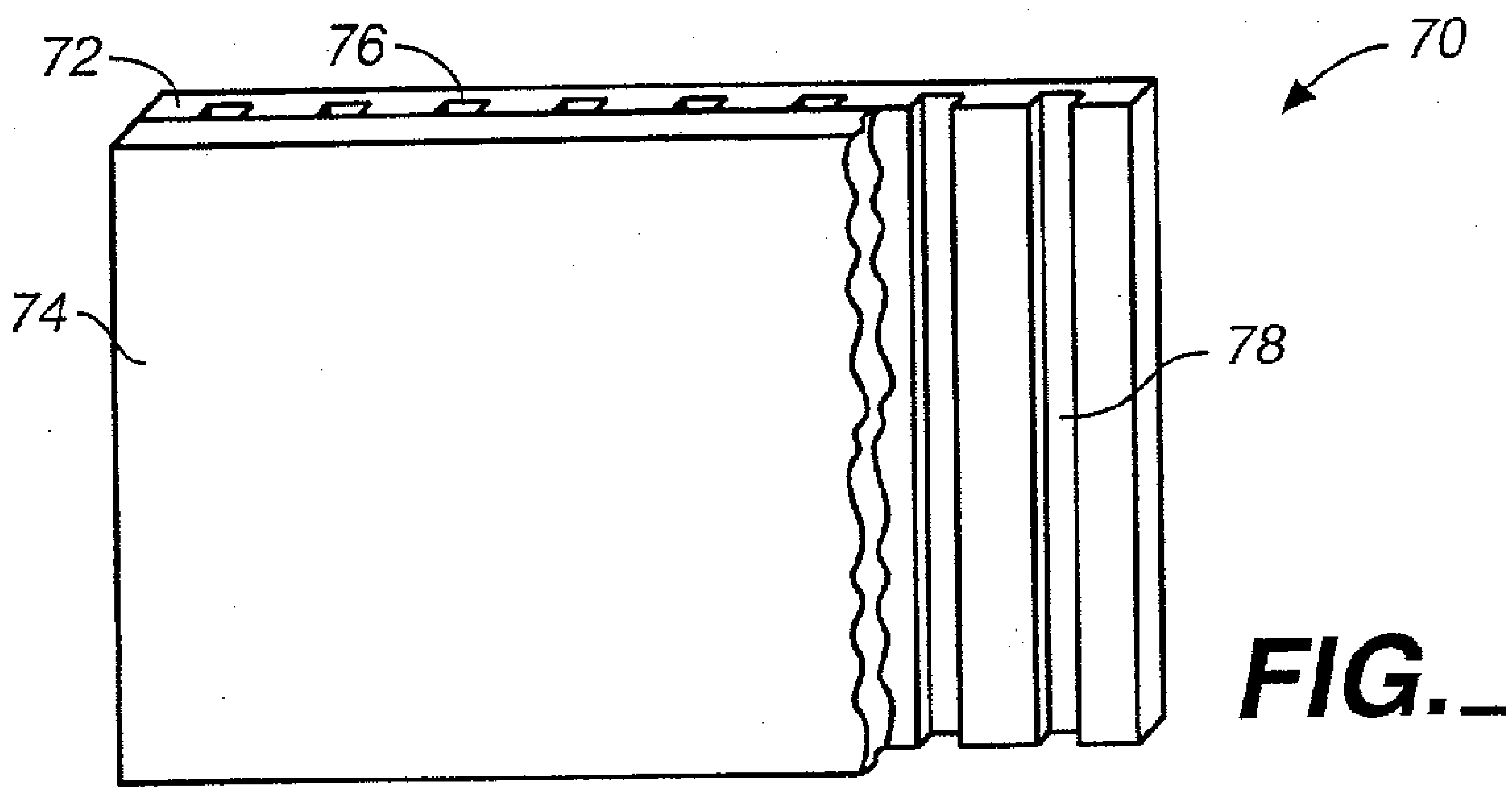


FIG._3E

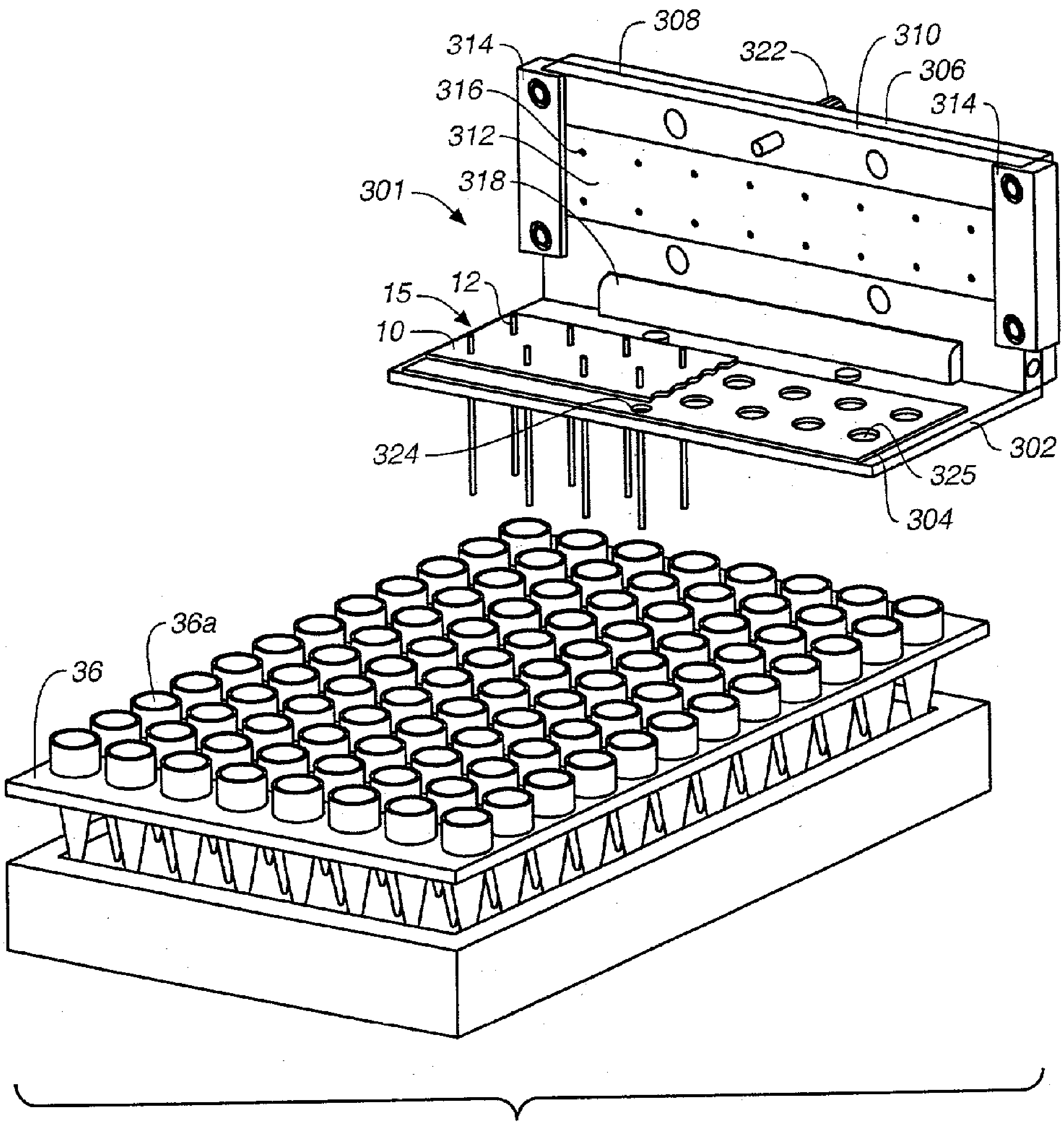
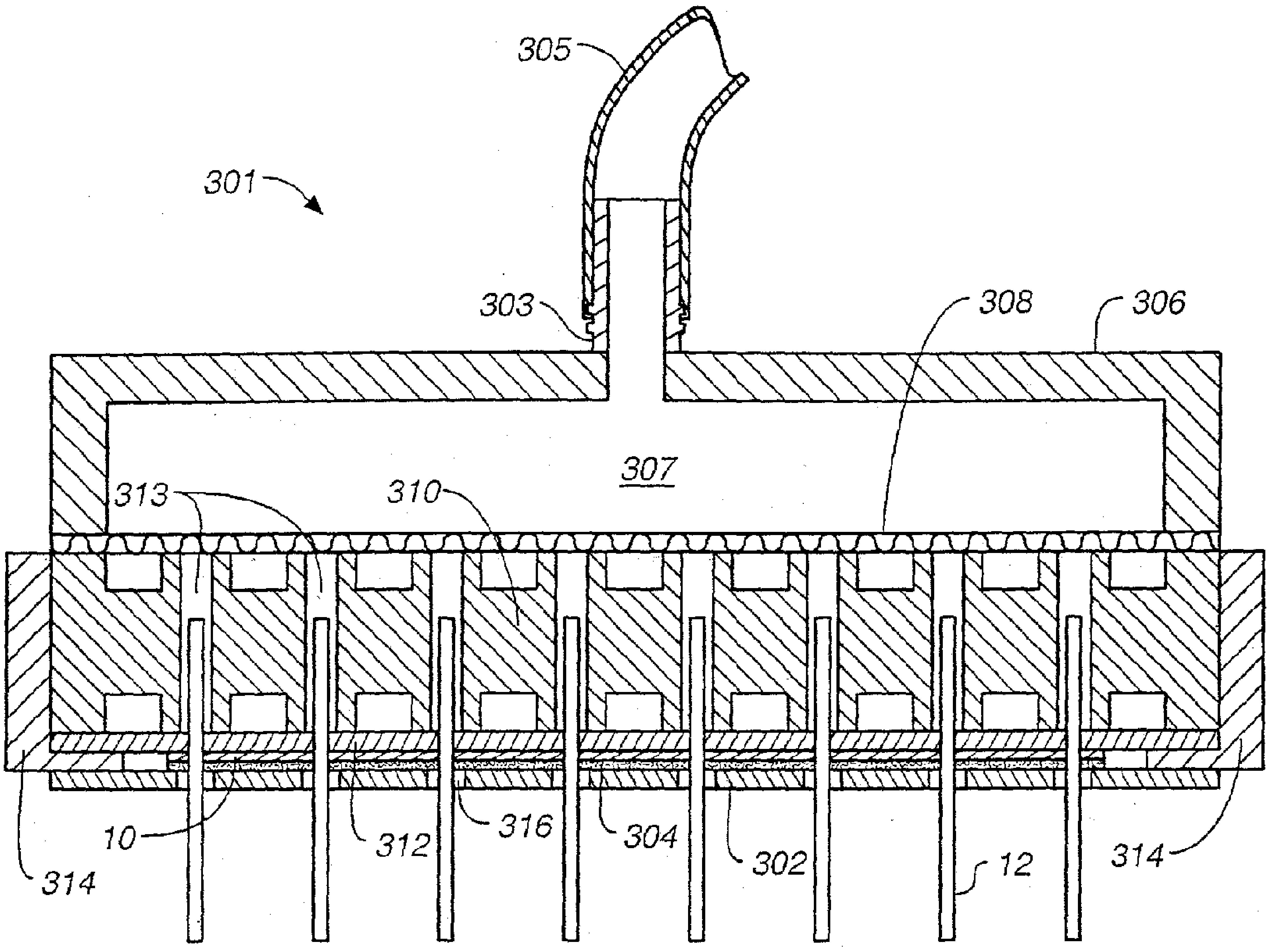

308
314
322
310
306
316
314
312
301
318
15
12
10
324
302
325
304
36a
36

FIG._4A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313
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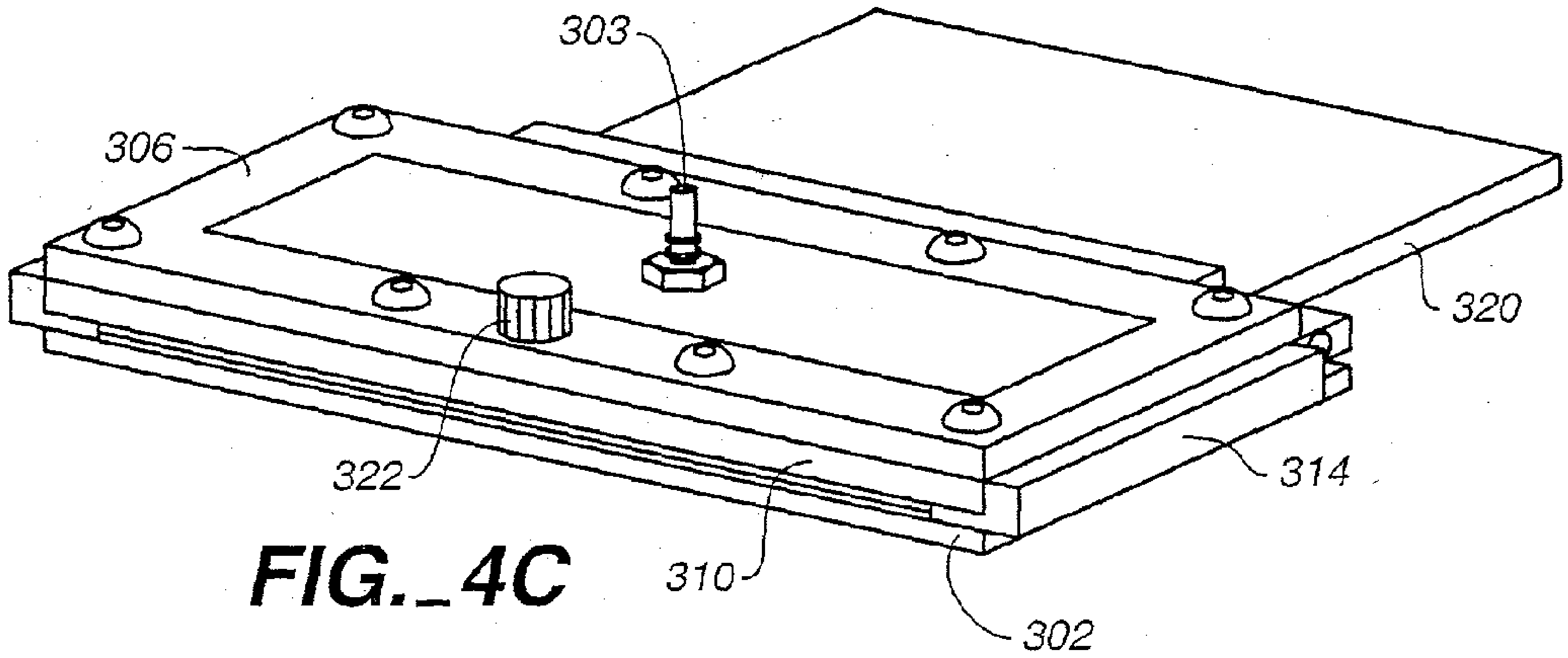
302
12
314
314

FIG._4B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310
302

FIG._4C

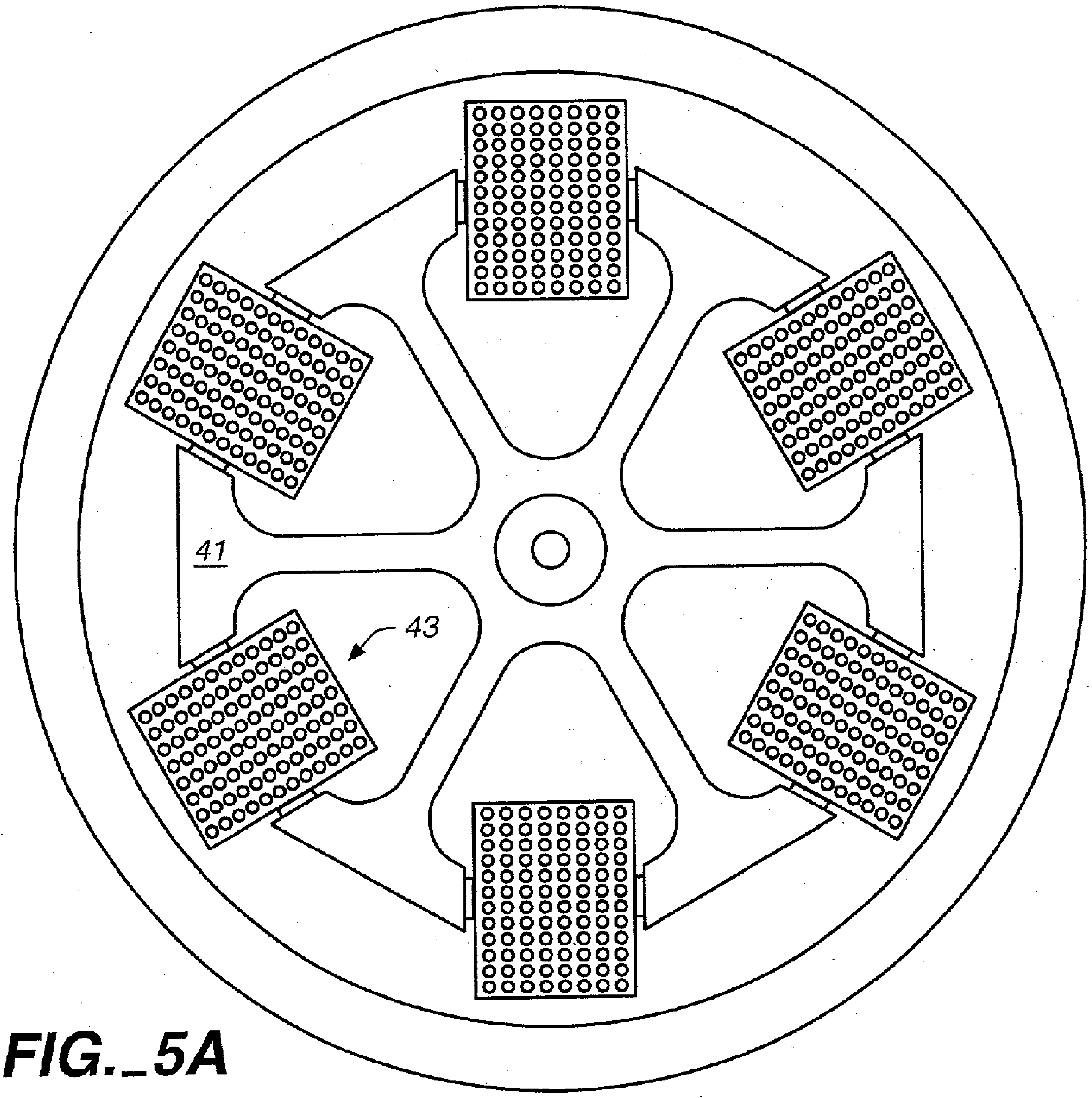


FIG._5A

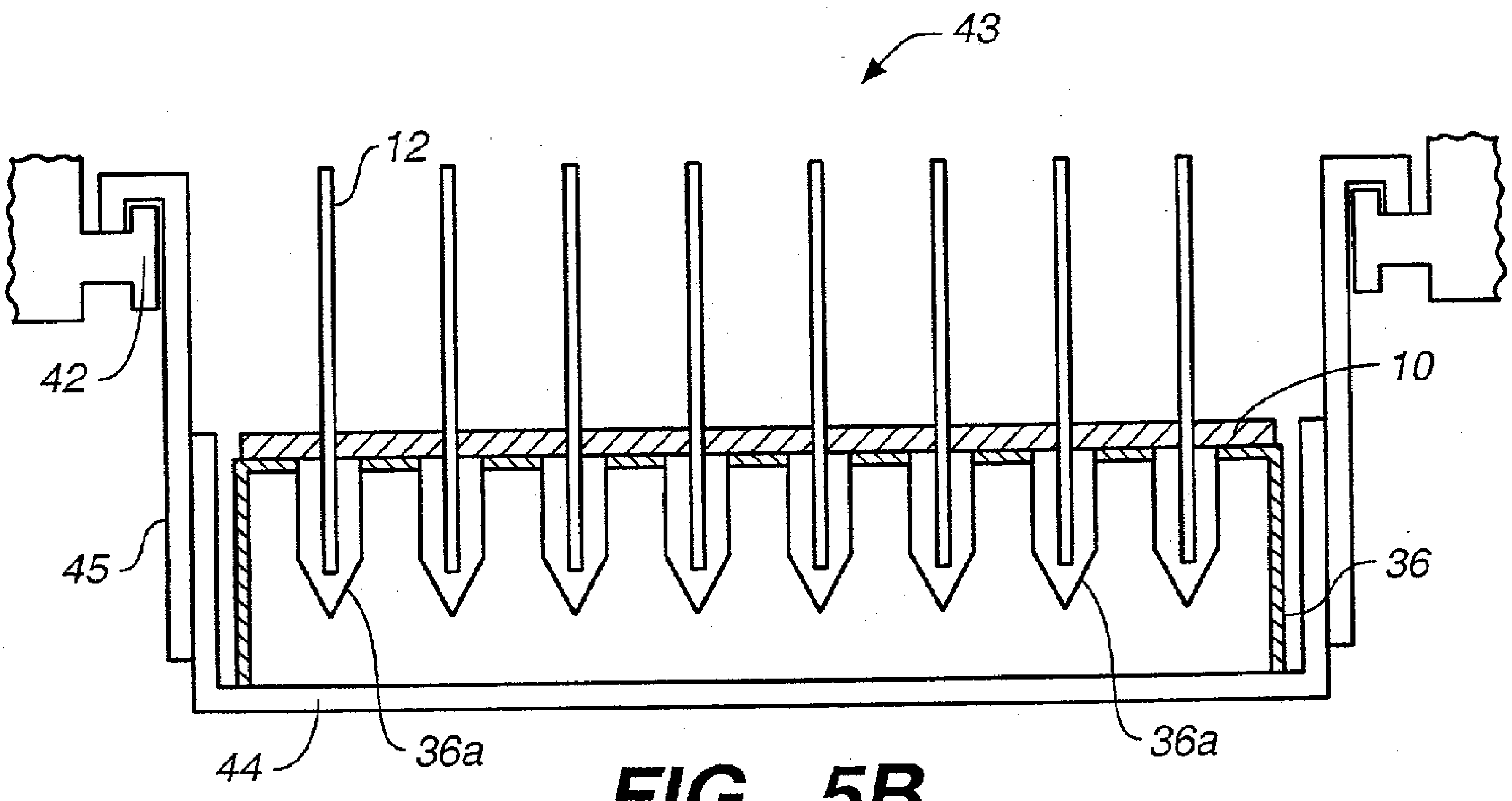
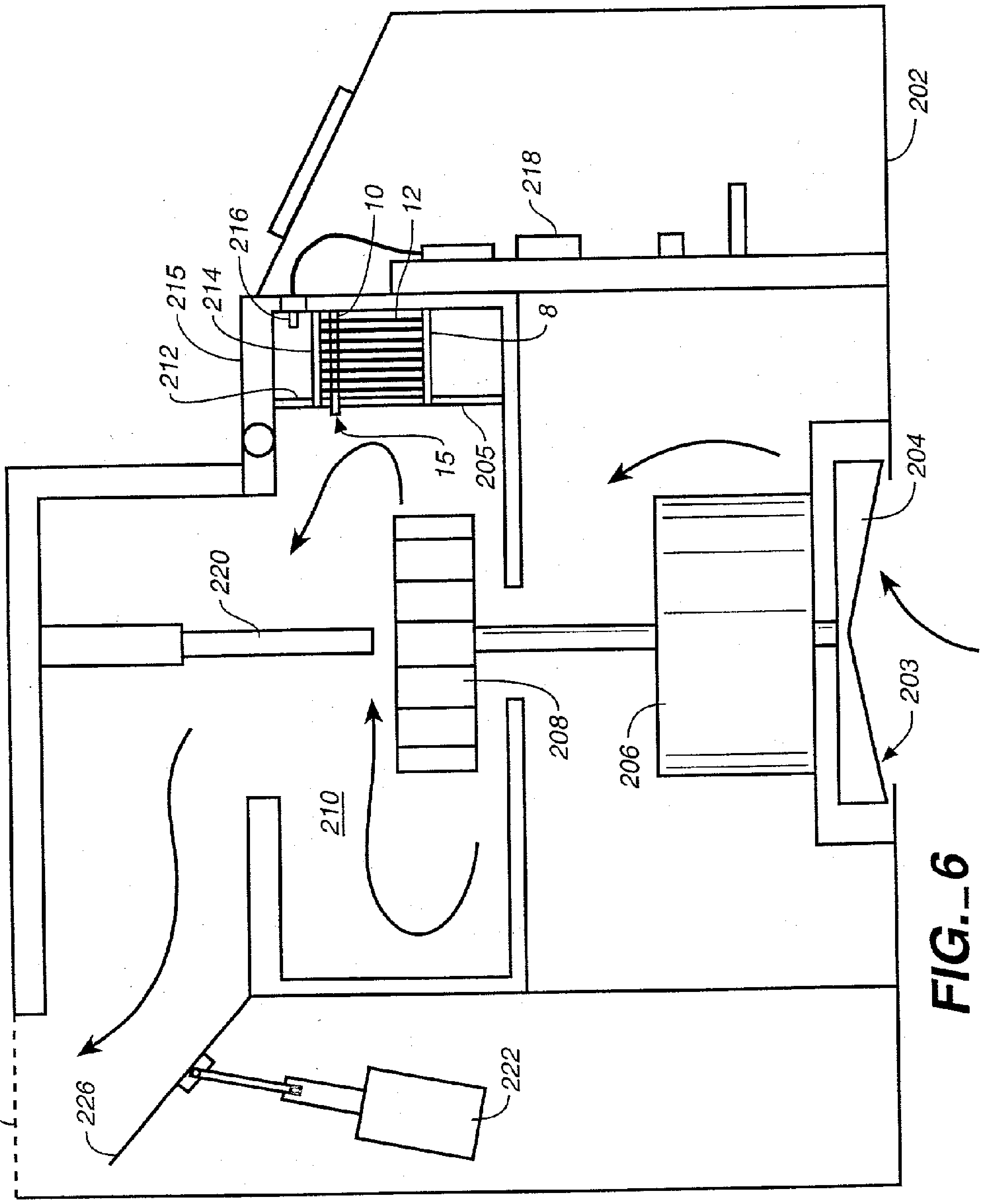


FIG._5B


202
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226
222

FIG._6

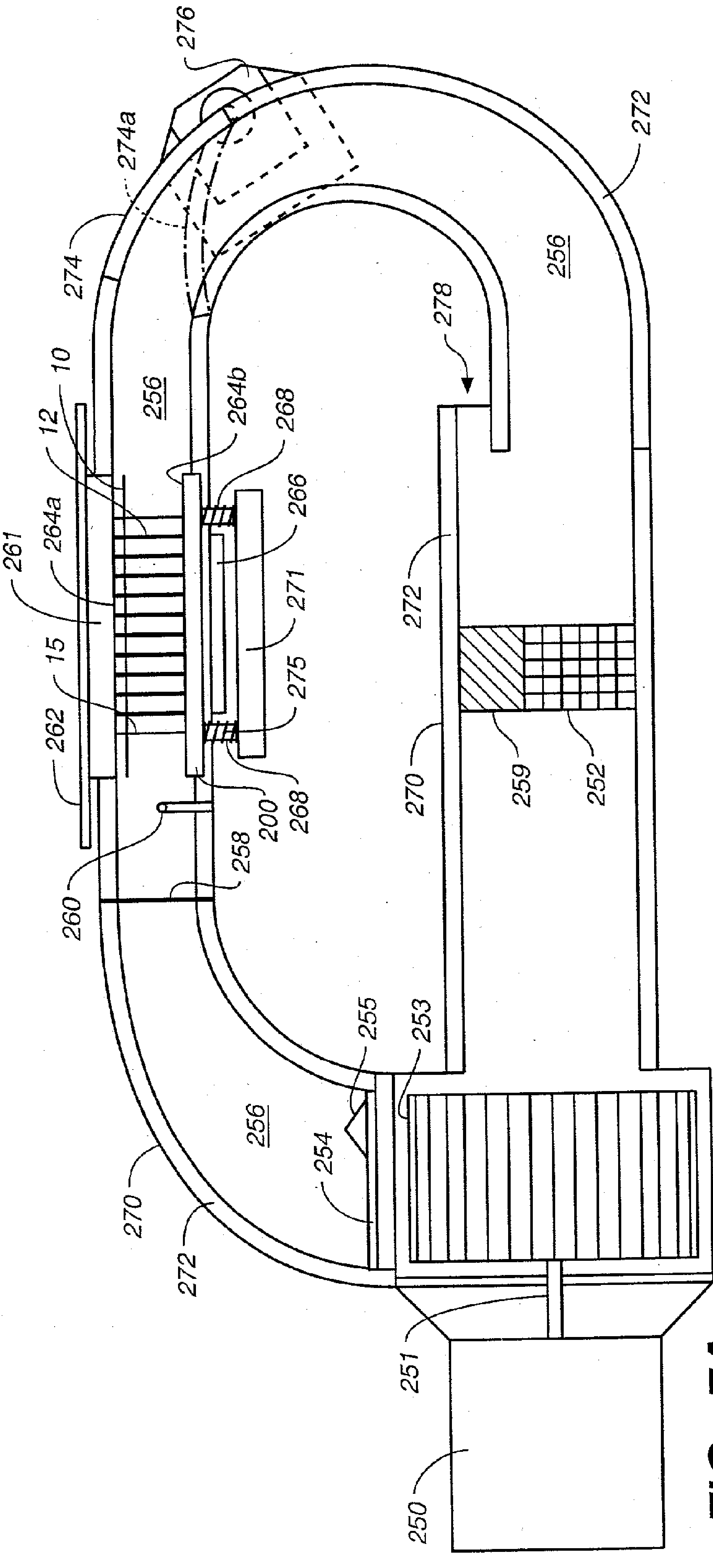
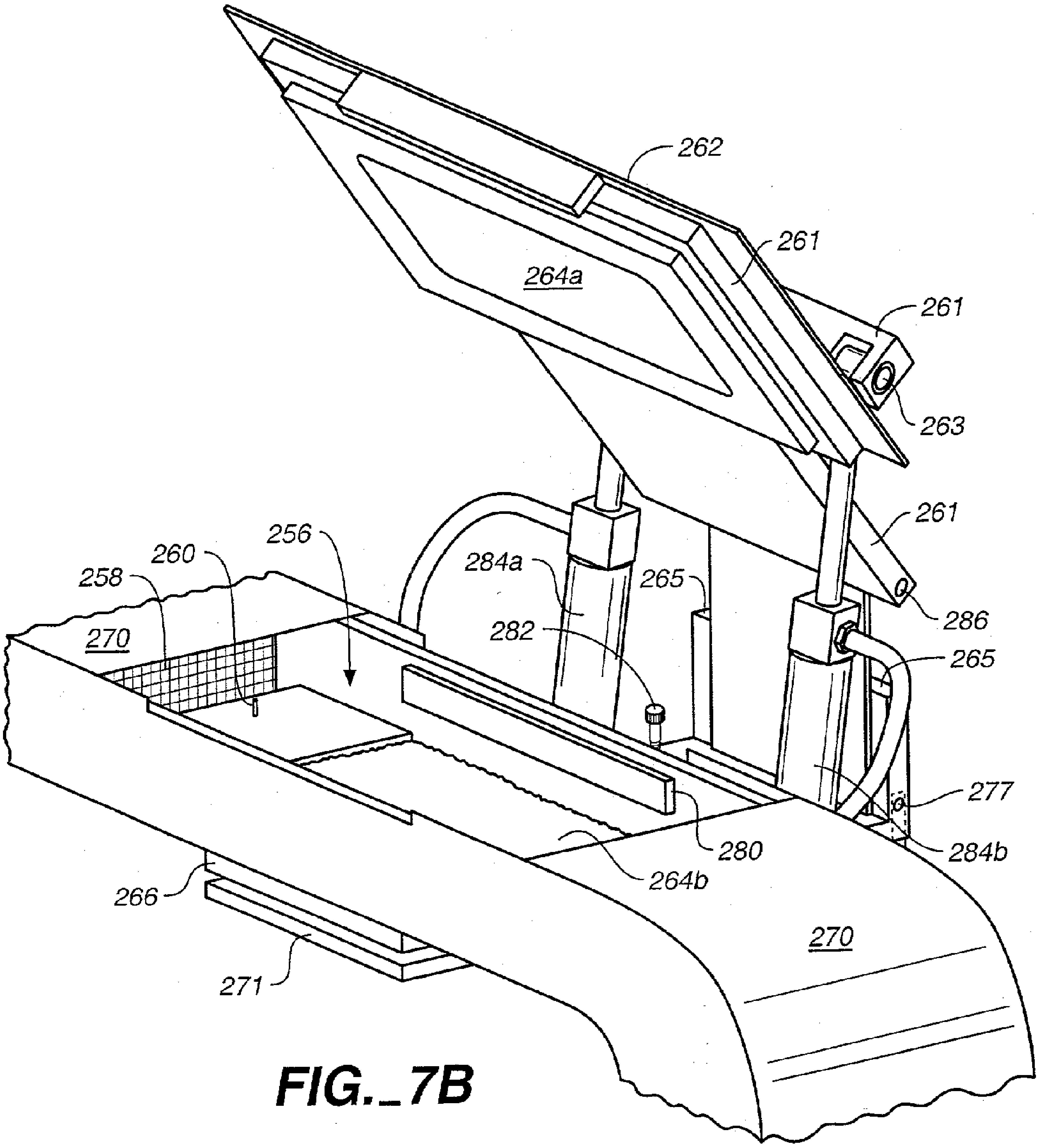

276
274a
274
272
256
278
10
12
256
264b
268
266
264a
261
271
15
275
272
262
268
200
270
259
252
258
260
255
253
256
254
270
272
251
250

FIG._7A


262
261
264a
261
263
261
286
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284a
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282
265
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284b
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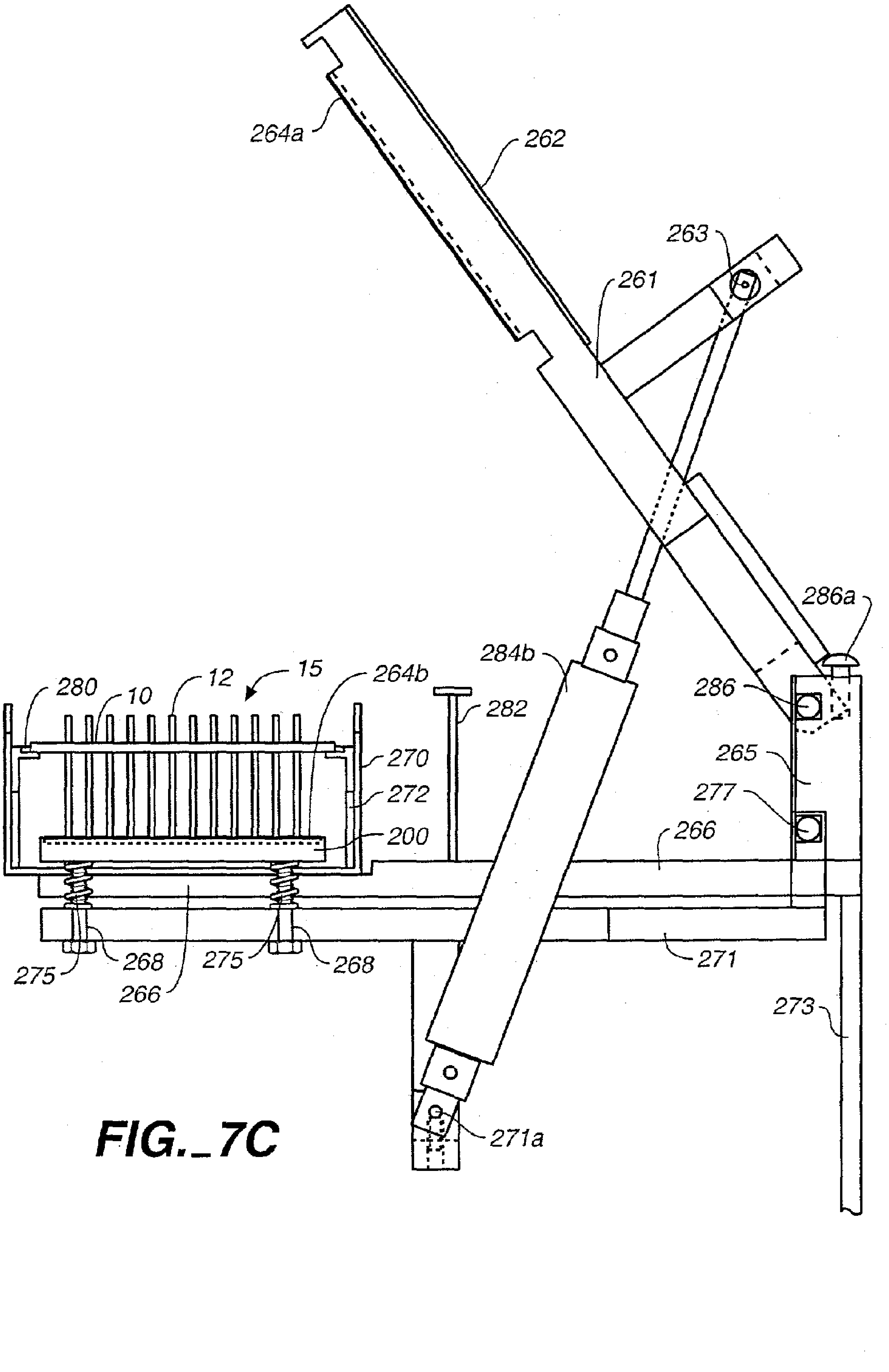
264b
266
270
271

FIG._7B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286a
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264b
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273
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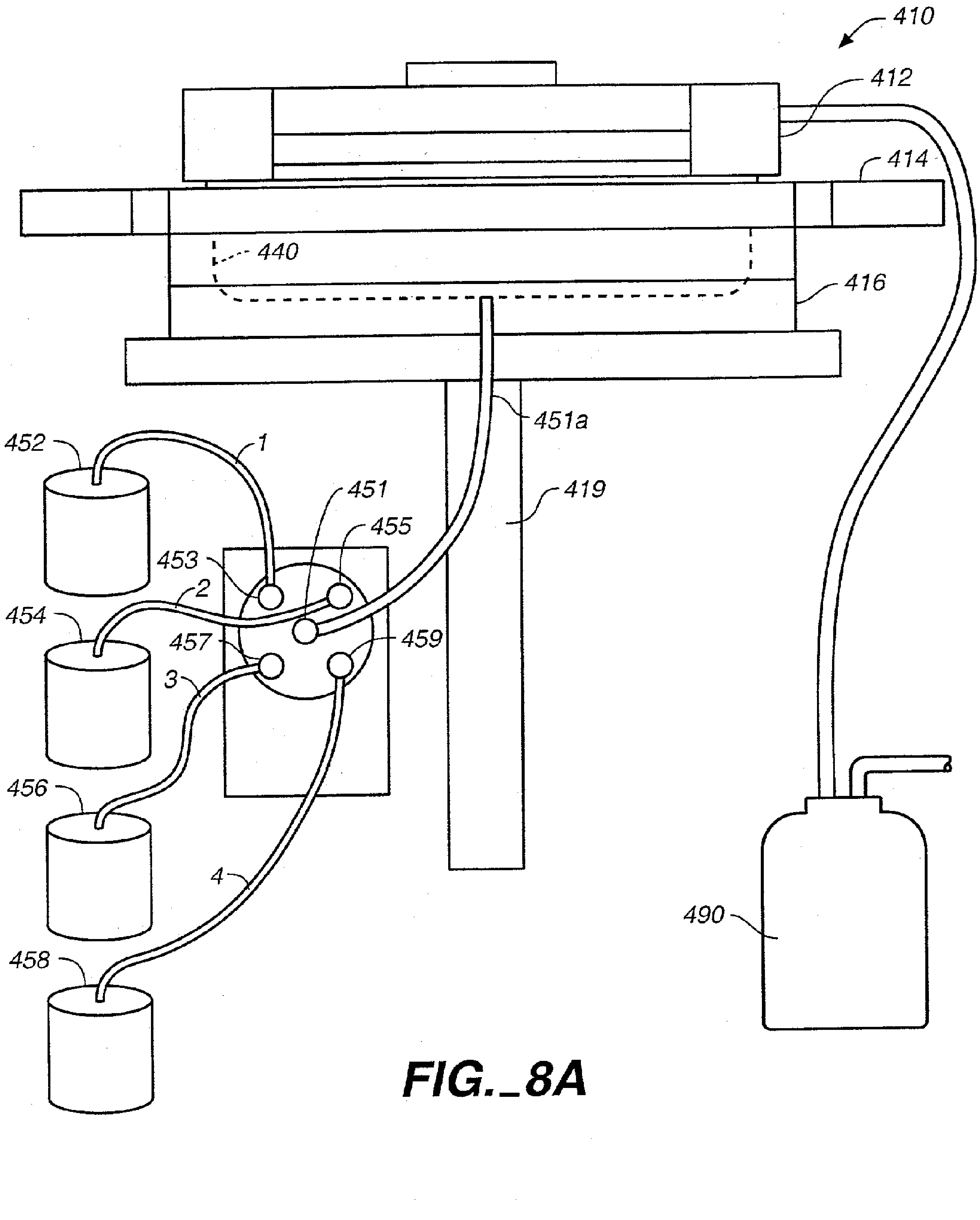
FIG._7C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412
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2
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3
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4
458
490

FIG._8A

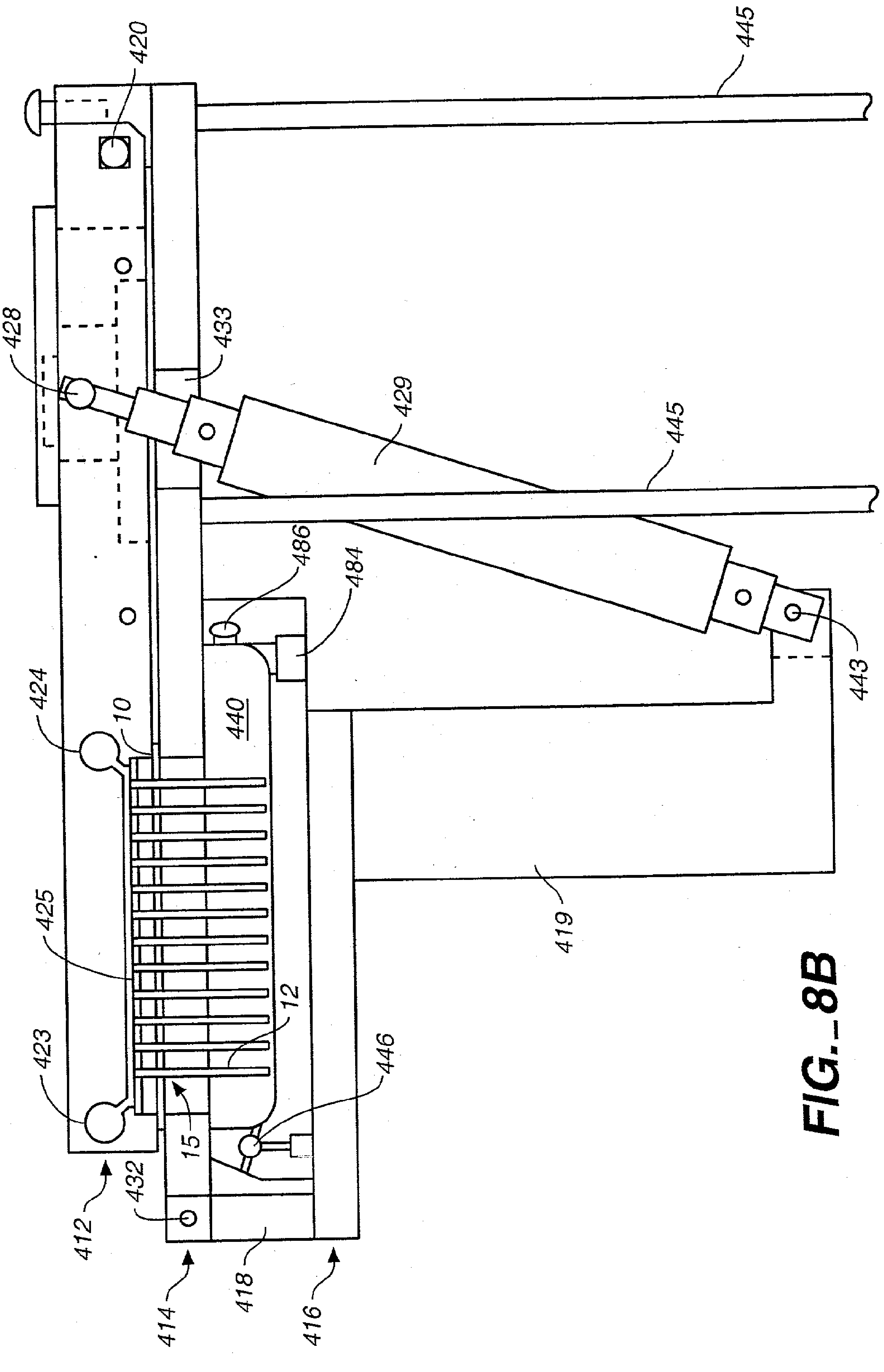
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FIG._8B

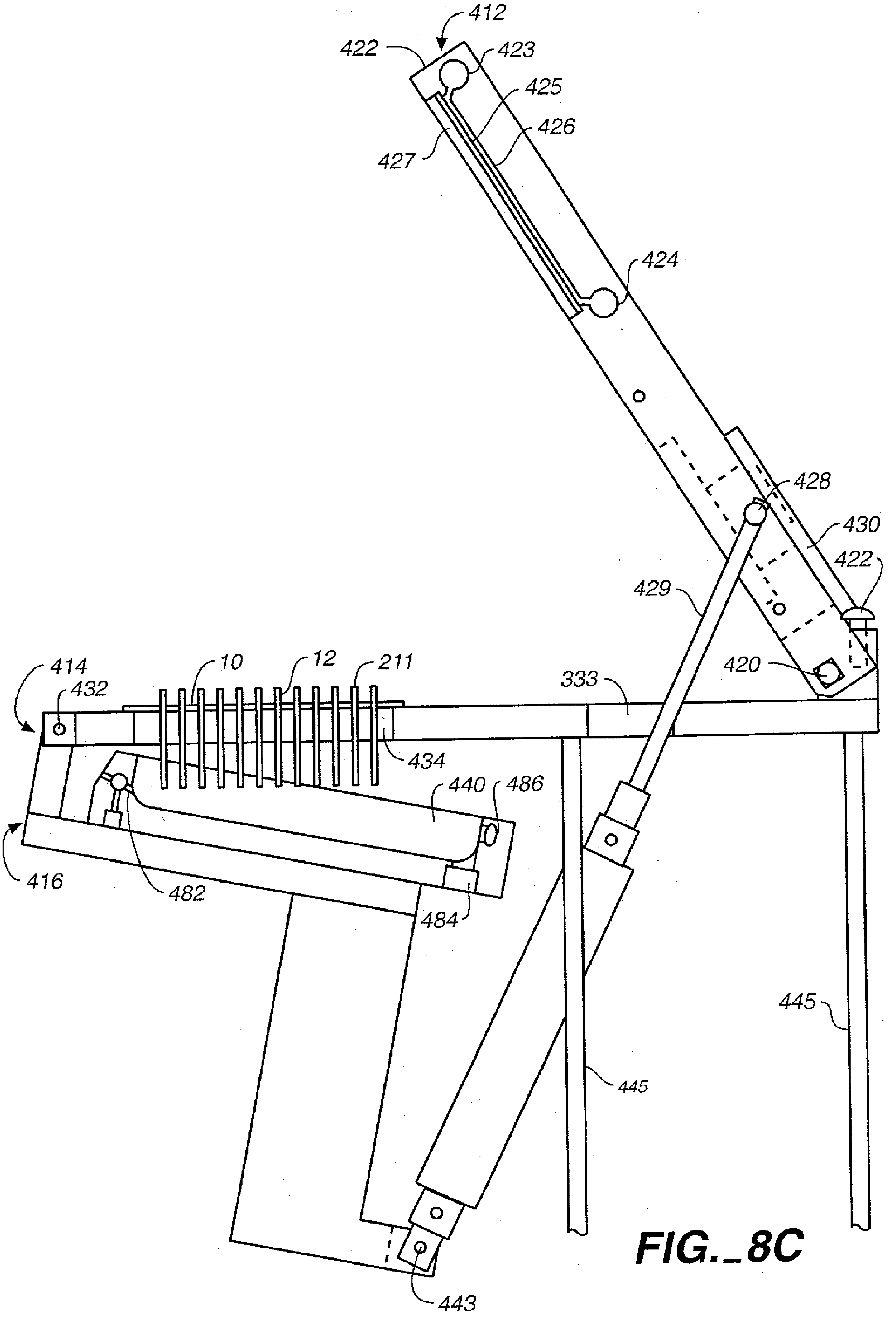
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FIG._8C

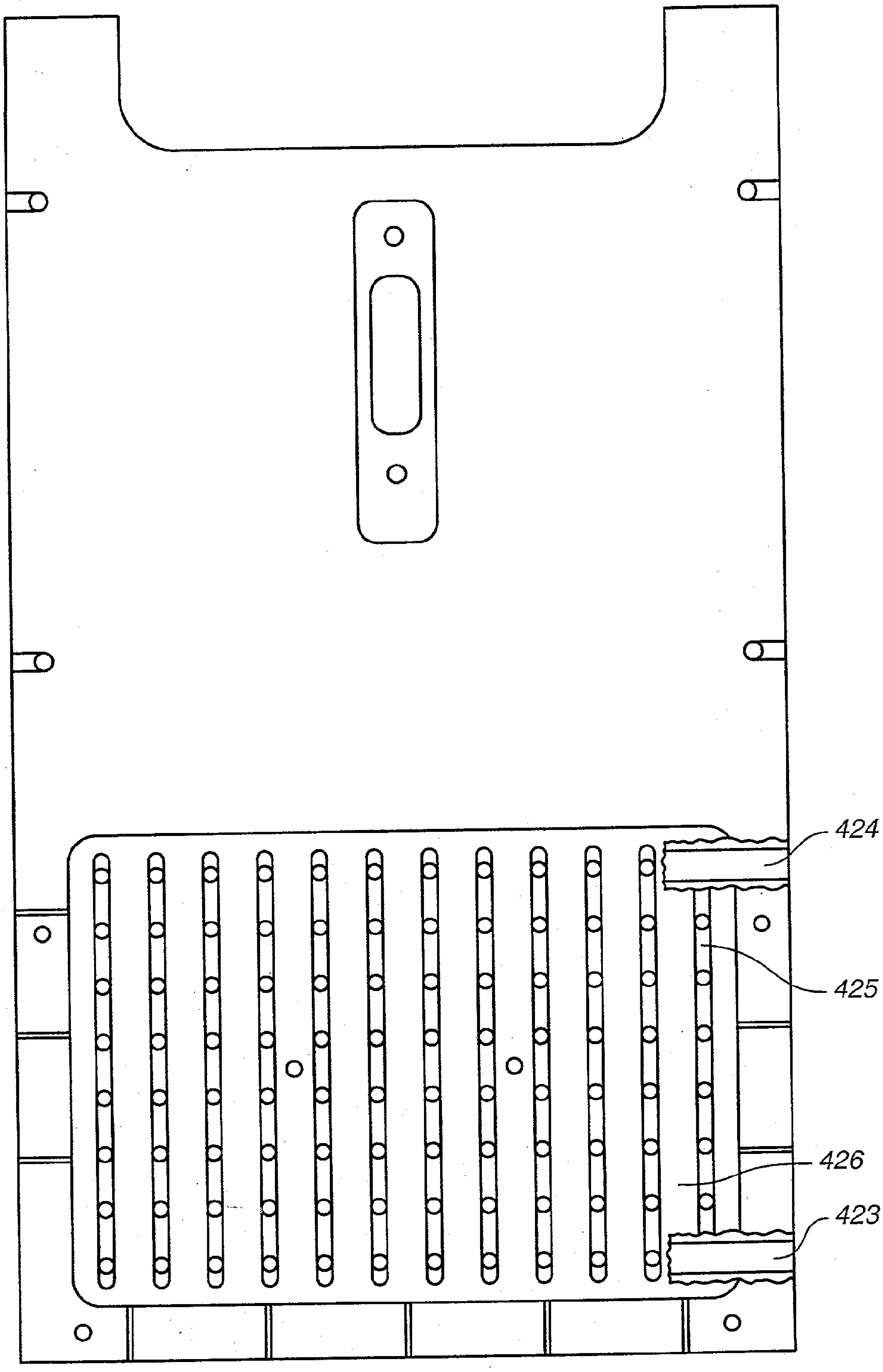
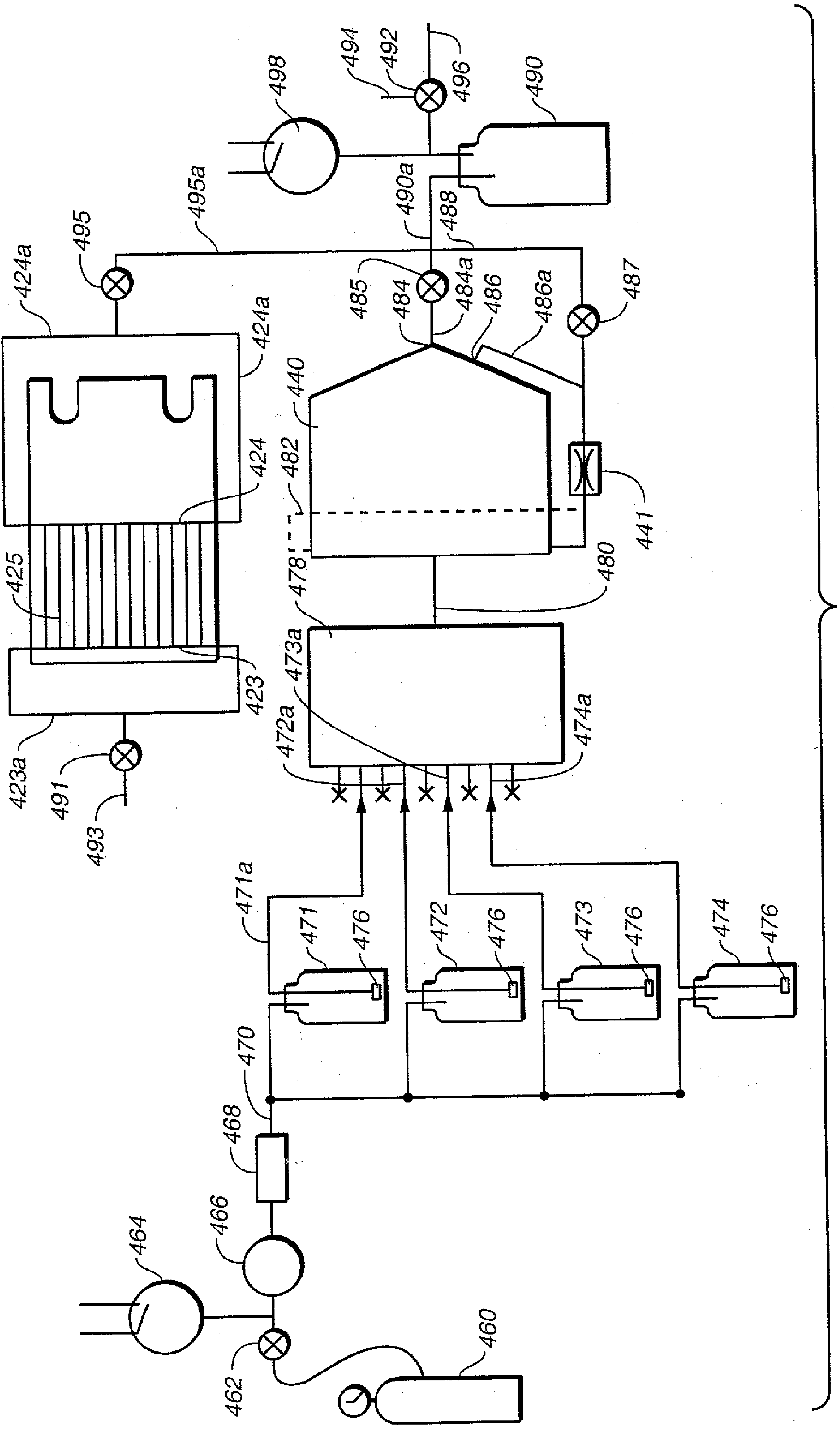


FIG._8D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486a
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482
425
478
441
480
473a
472a
474a
423
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462
460

FIG._8E

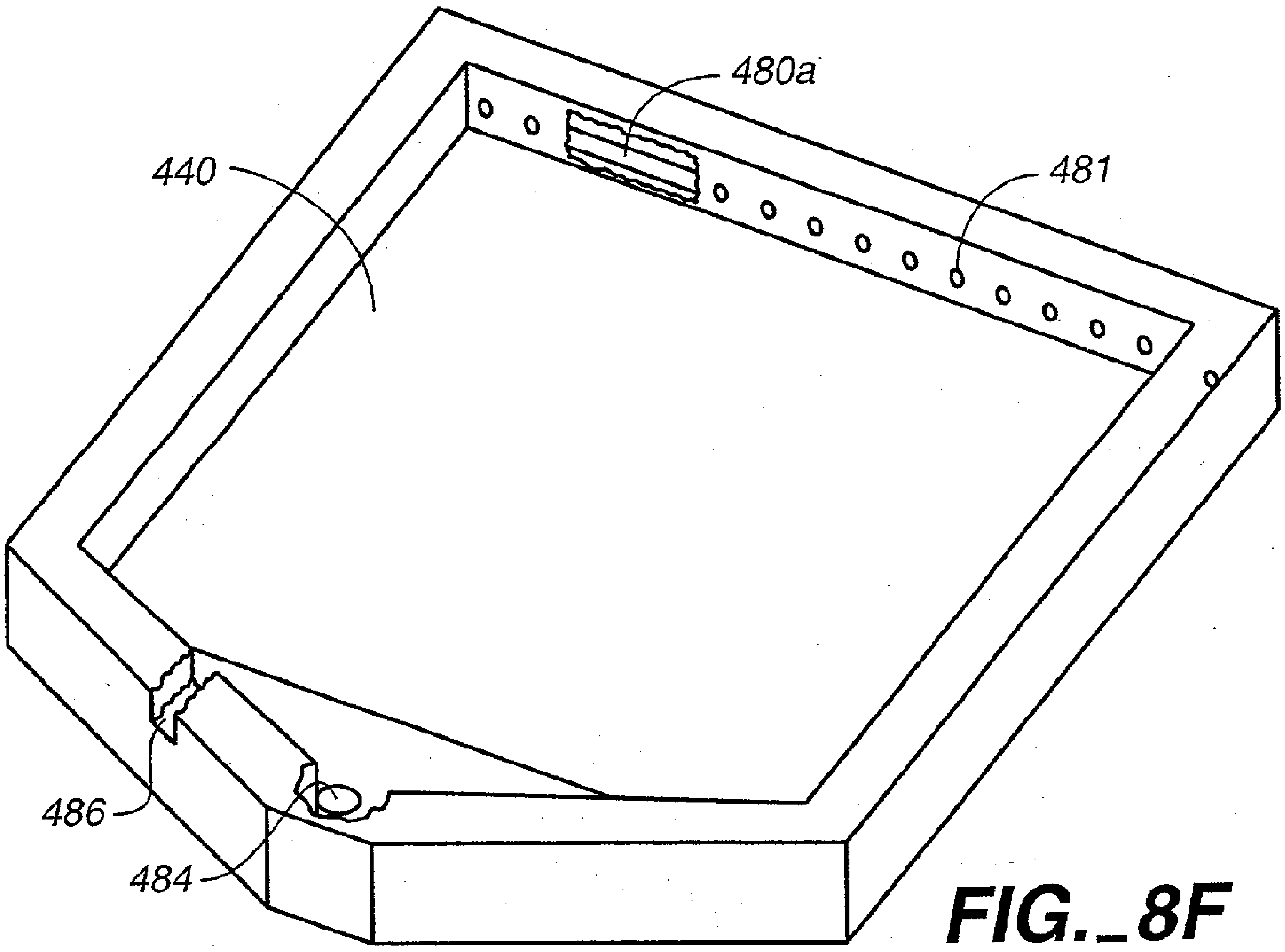
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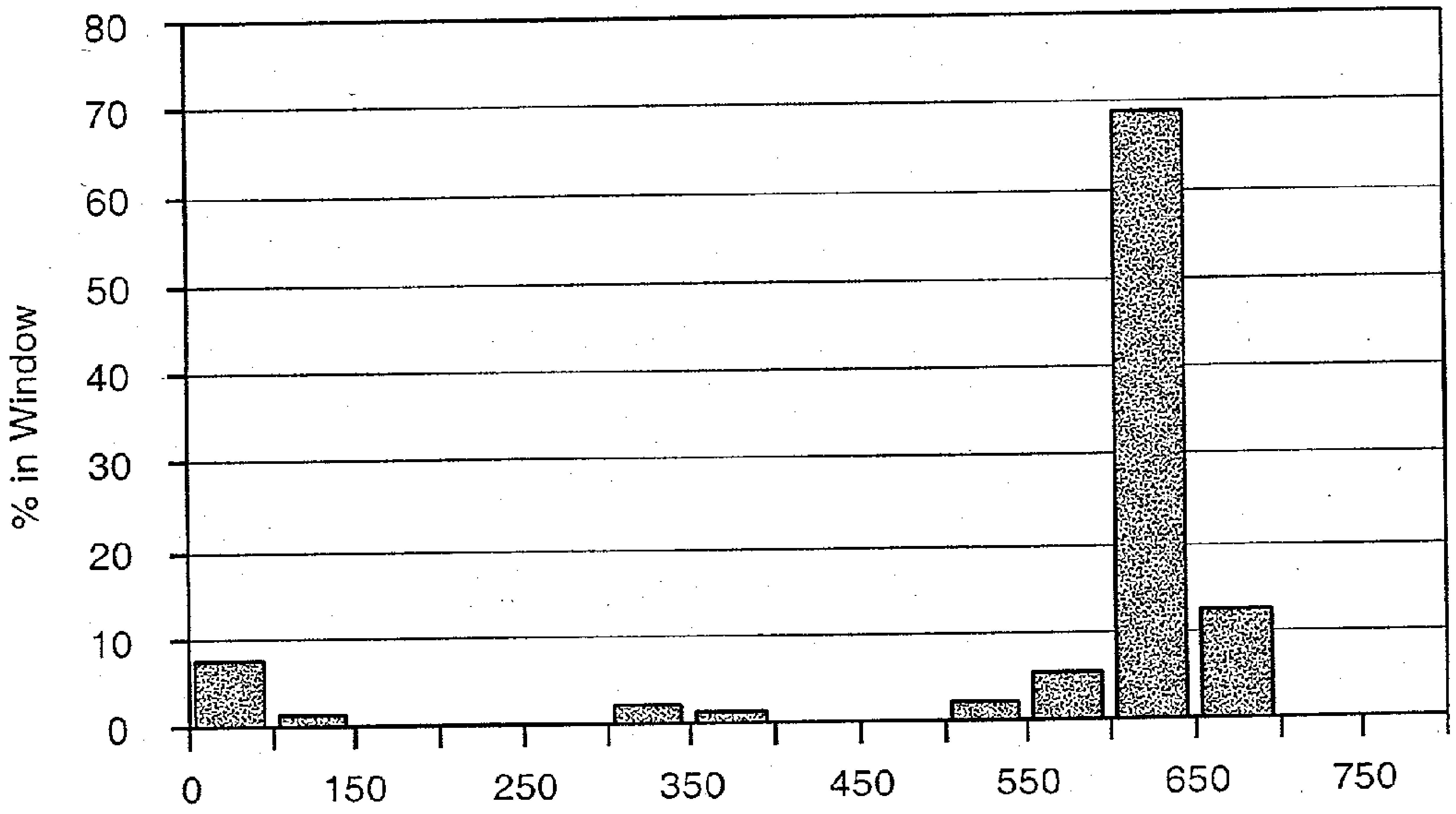
FIG._8F


80
70
60
50
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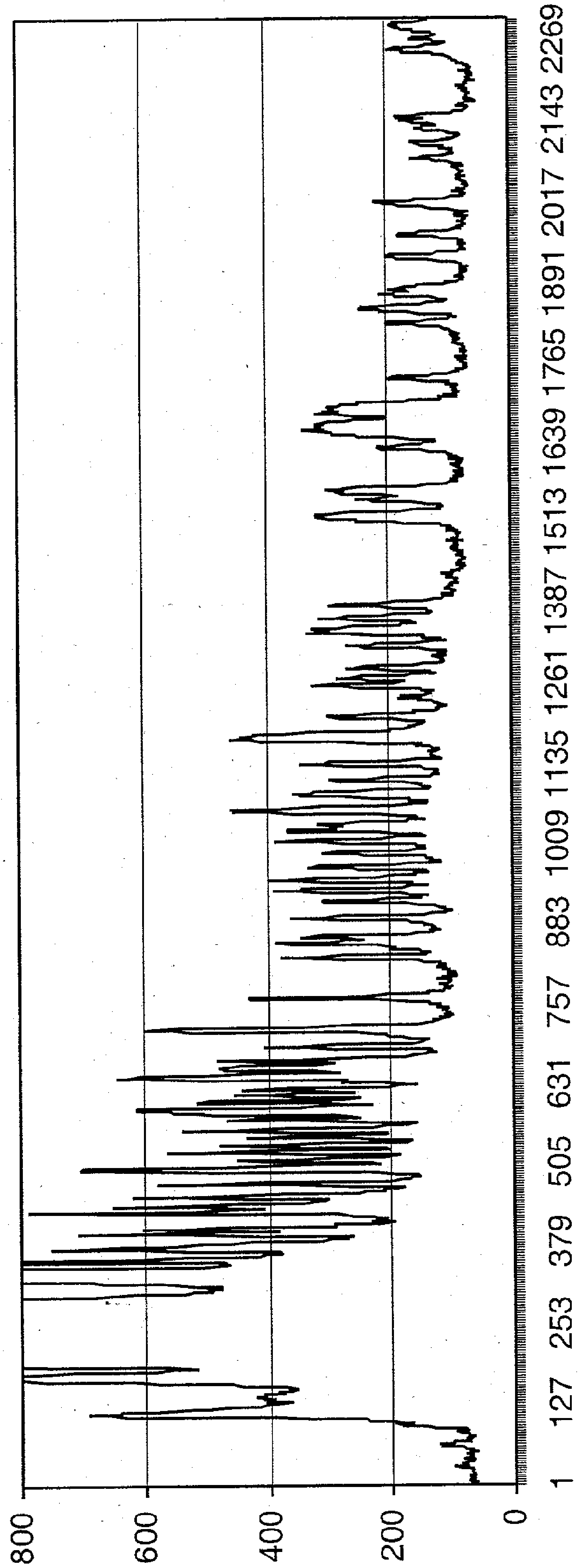
30
20
10
0
% in Window
0
150
250
350
450
550
650
750
Readlength

FIG._9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2143
2269

FIG._10

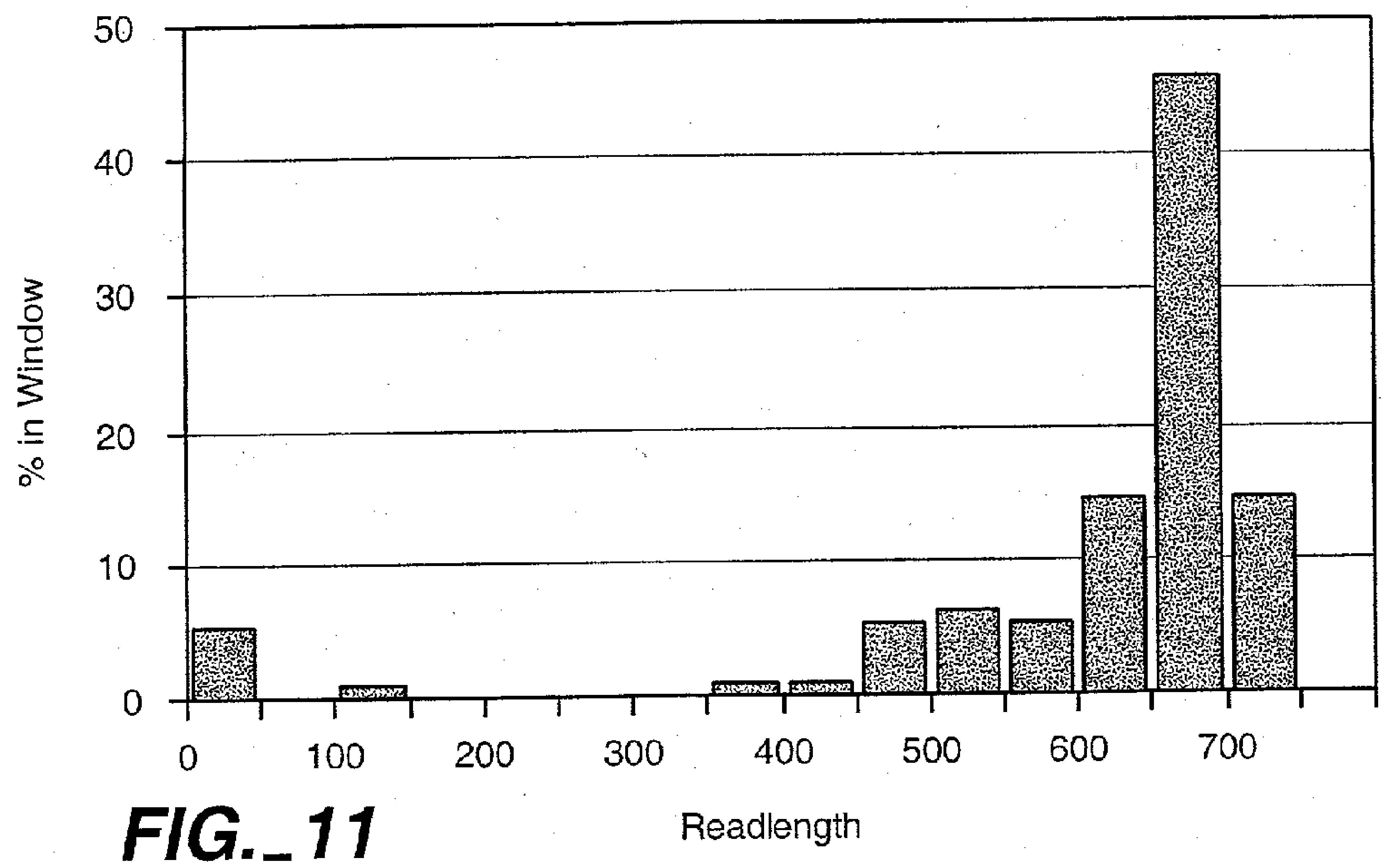

% in Window
50
40
30
20
10
0
0
100
200
300
400
500
600
700
Readlength

FIG._11

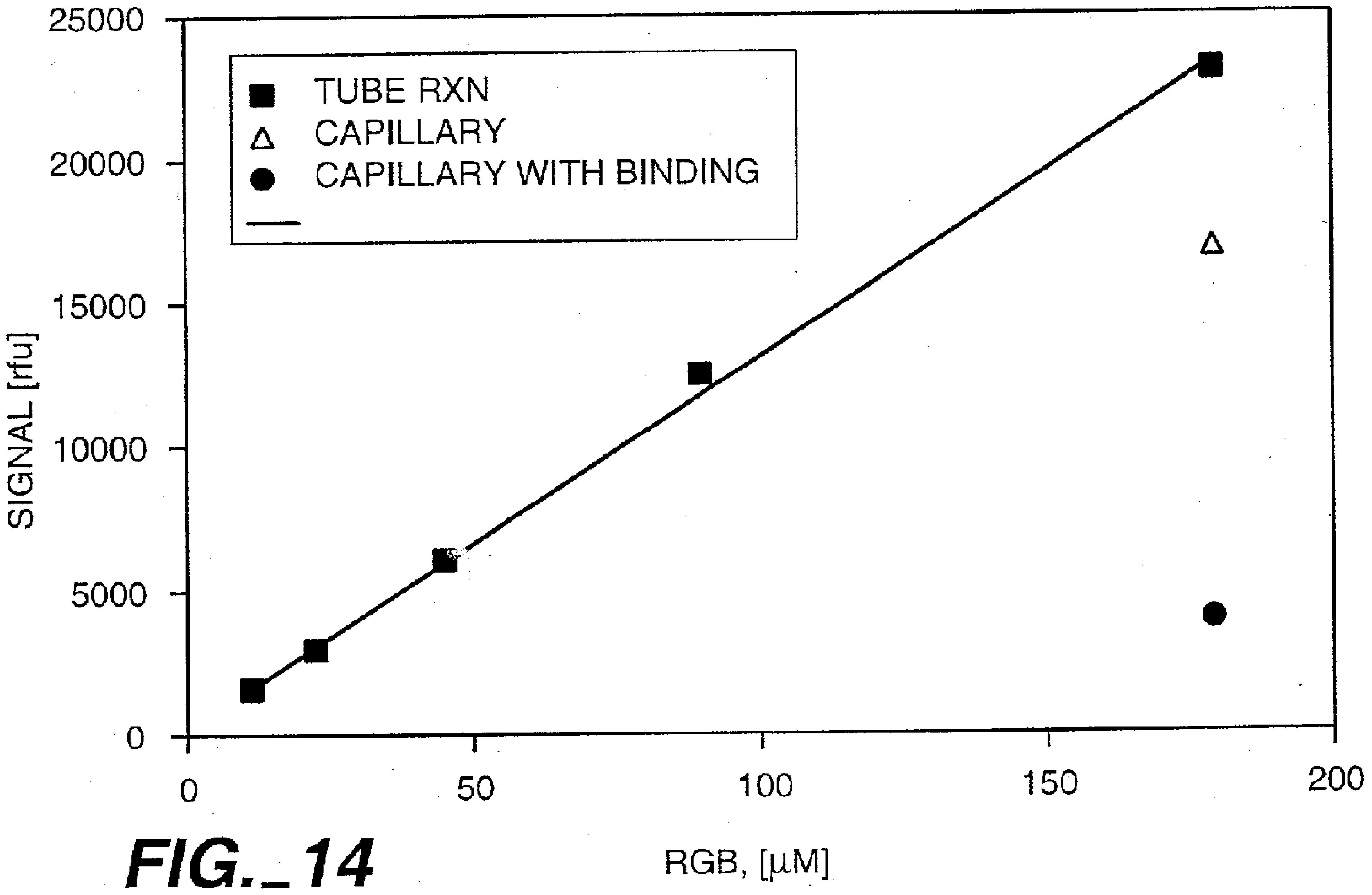

SIGNAL [rfu]
25000
20000
15000
10000
5000
0
■ TUBE RXN
Δ CAPILLARY
● CAPILLARY WITH BINDING
0
50
100
150
200
RGB, [µM]

FIG._14

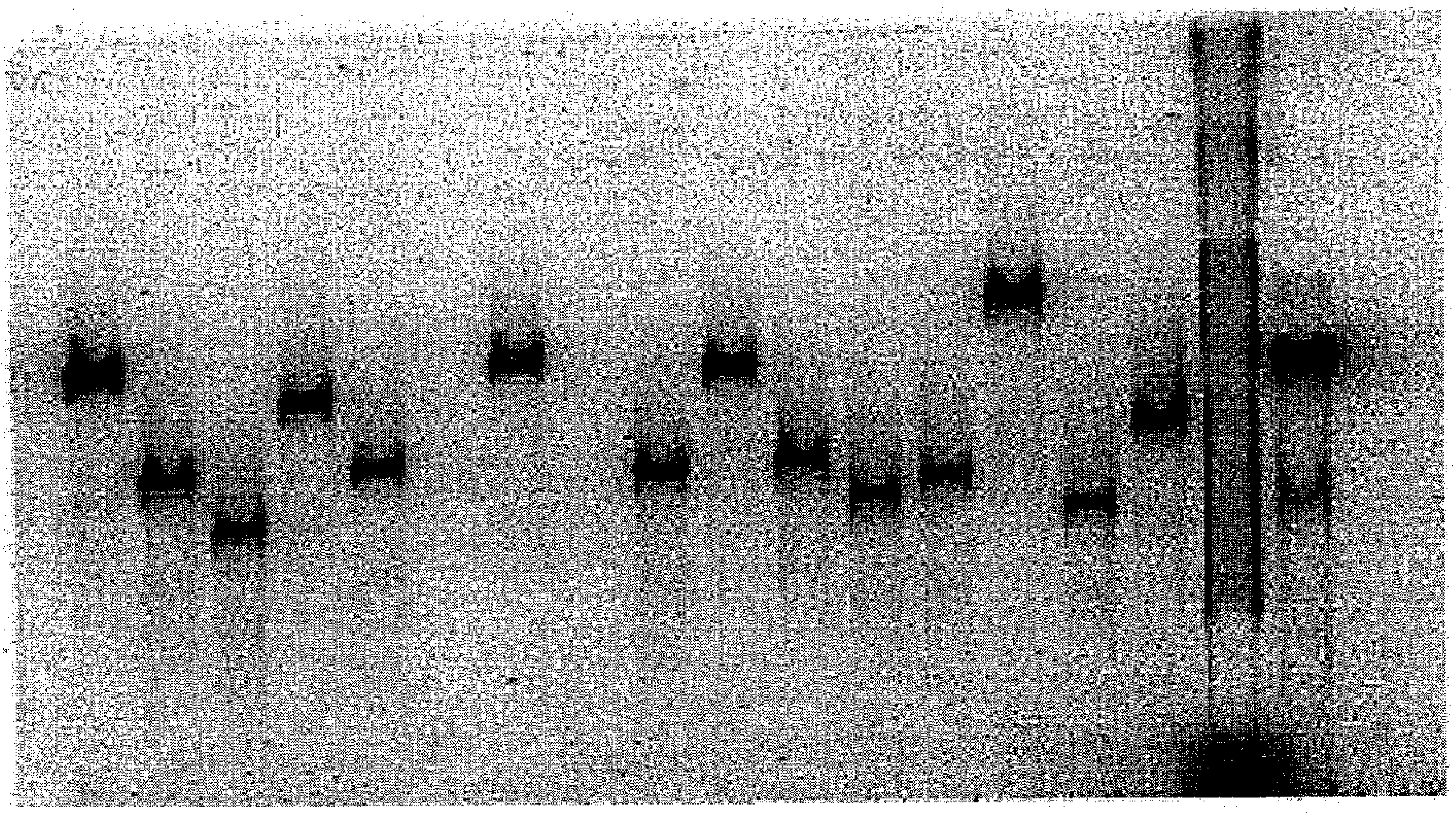


FIG._12A

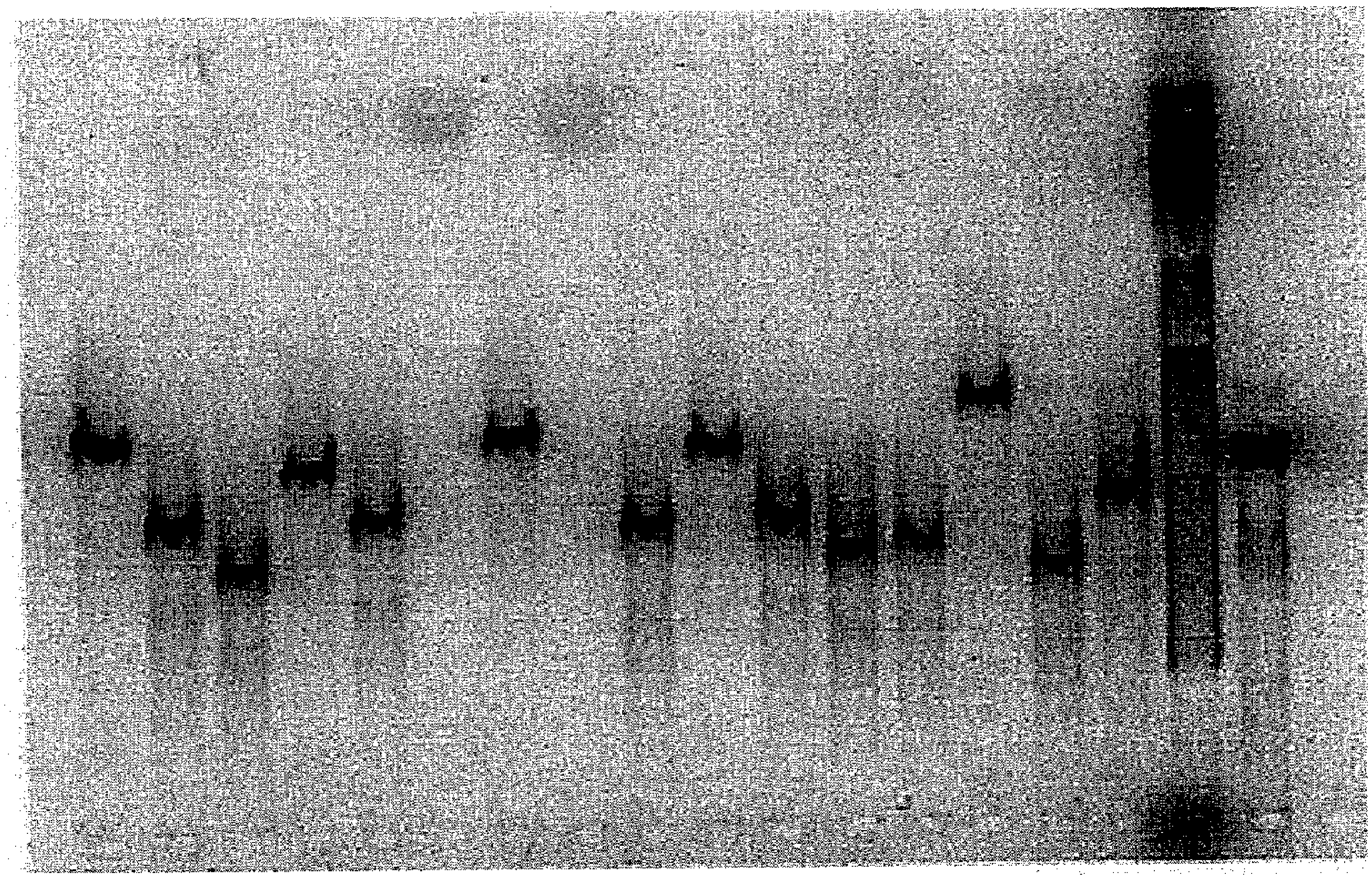


FIG._12B

FIG._13A

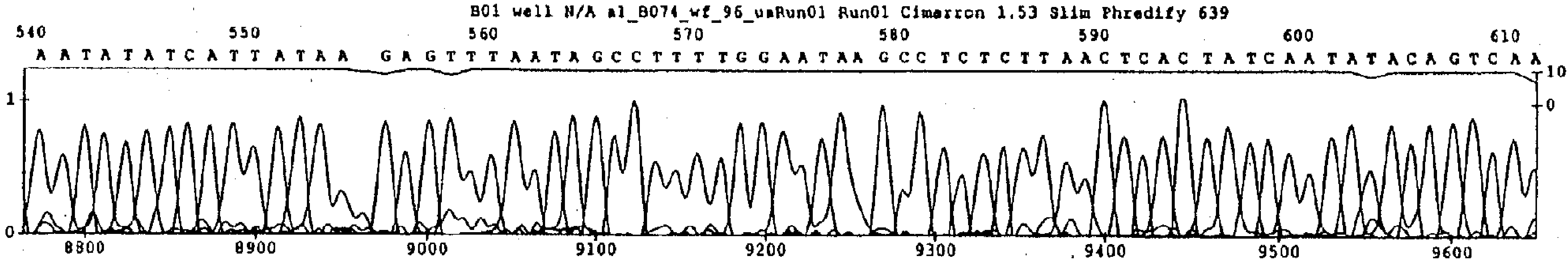


FIG._13B

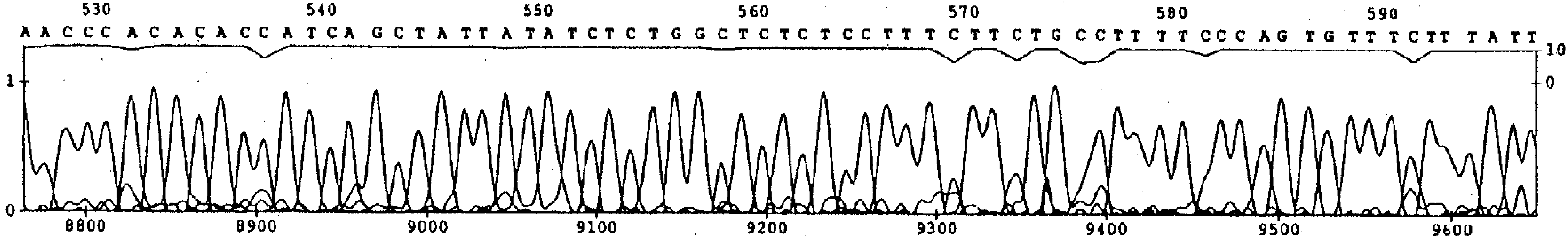


FIG._13C

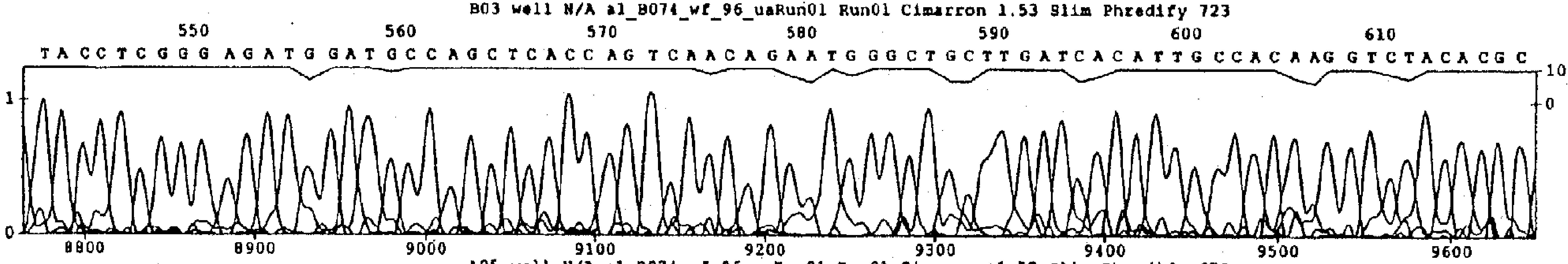


FIG._13D

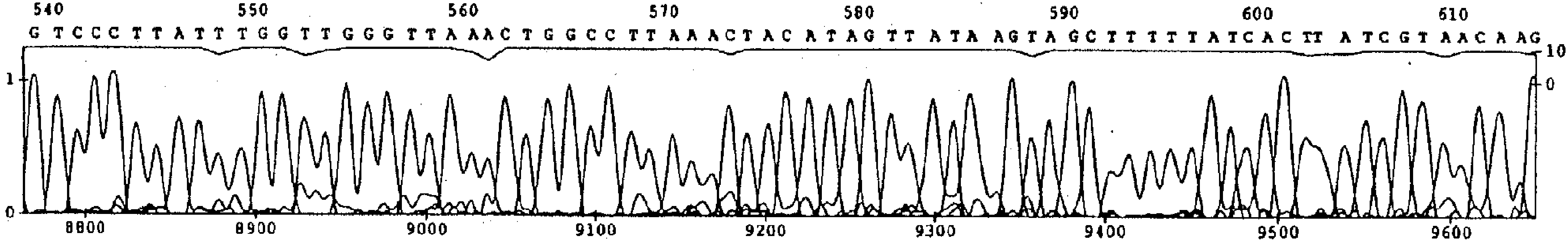
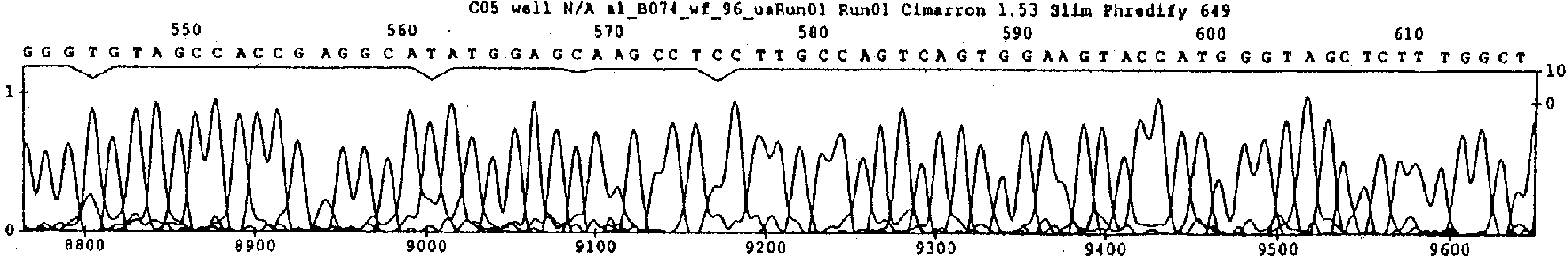


FIG._13E



LOW VOLUME CHEMICAL AND BIOCHEMICAL REACTION SYSTEM

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of U.S. patent application Ser. No. 09/577,199 filed May 23, 2000, and claims priority from U.S. provisional application No. 60/146,732 filed Aug. 2, 1999.

TECHNICAL FIELD OF THE INVENTION

[0002] This invention relates to a method and apparatus for performing small scale reactions. In particular, the instant disclosure pertains to small scale cycling reactions and devices for assembly of sub-microliter reaction mixtures.

BACKGROUND OF THE INVENTION

[0003] The Human Genome Program is a scientific endeavor which is a national priority of the United States. The original goal of the federally funded U.S. effort had been to complete the sequence at ten-fold coverage by the year 2005. A draft, five-fold deep version of the human genome will now be produced by the year 2001. To accomplish this goal, the effort has accelerated to improve sequencing throughput rates and reduce DNA sequencing costs.

[0004] In the late 1970s, Sanger et al. developed an enzymatic chain termination method for DNA sequence analysis that produces a nested set of DNA fragments with a common starting point and random terminations at every nucleotide throughout the sequence. Lloyd Smith, Lee Hood, and others modified the Sanger method to use four fluorescent labels in sequencing reactions enabling single lane separations. This resulted in the creation of the first automated DNA sequencers. More recently, fluorescent energy-transfer dyes have been used to make dye sets that enhance signals by 2- to 10-fold and simplify the optical configuration.

[0005] Automated fluorescent capillary array electrophoresis (CAE) DNA sequencers appear to be the consensus technology to replace slab gels. Capillary gel electrophoresis speeds up the separation of sequencing products and has the potential to dramatically decrease sample volume requirements. The 96-channel CAE instrument, MegaBACE™, which is commercially available from Molecular Dynamics (Sunnyvale, Calif.), uses a laser induced fluorescence (LIF) confocal fluorescence scanner to detect up to an average of about 625 bases per capillary (Phred 20 window) in 90 minute runs with cycle times of two hours. Confocal spatial filtering results in a higher signal-to-noise ratio because superfluous reflections and fluorescence from surrounding materials are eliminated before signal detection at the photomultiplier tube (PMT). Accordingly, sensitivity at the level of subattomoles per sequencing band is attainable. Confocal imaging is also particularly important in capillary electrophoresis in microchip analysis systems where the background fluorescence of a glass or plastic microchip may be much higher than that of fused silica capillaries. Capillary array electrophoresis systems will solve many of the initial throughput needs of the genomic community for DNA analysis. However, low volume sample preparation still presents a significant opportunity to increase throughput and reduce cost.

[0006] While fluorescent DNA sequencers are improving the throughput of DNA sequence acquisition, they have also moved the throughput bottleneck from sequence acquisition back towards sample preparation. In response, rapid methods for preparing sequencing templates and for transposon-facilitated DNA sequencing have been developed as have magnetic bead capture methods that eliminate centrifugation. Thermophilic Archae DNA polymerases have been screened and genetically engineered to improve fidelity, ensure stability at high temperatures, extend lengths, and alter affinities for dideoxynucleotides and fluorescent analogs. These improvements have resulted in lower reagent costs, simpler sample preparation, higher data accuracy, and increased read lengths.

[0007] The sequencing community has also developed higher-throughput methods for preparing DNA templates, PCR reactions, and DNA sequencing reactions. Sample preparation has been increasingly multiplexed and automated using 96- and 384-well microtiter plates, multichannel pipettors, and laboratory robotic workstations. In general, these workstations mimic the manipulations that a technician would perform and have minimum working volumes of about a microliter, although stand-alone multichannel pipettors are being used to manipulate smaller volumes.

[0008] A typical full-scale sample preparation method for DNA shotgun sequencing on capillary systems begins by lysing phage plaques or bacterial colonies to isolate subcloned DNA. Because capillary electrophoresis is more sensitive to impurities in sequencing reactions than slab gels, the subcloned DNA insert is PCR amplified to exponentially increase its concentration in the sample. Next, exonuclease I (ExoI) and arctic shrimp alkaline phosphatase (SAP) are added to perform an enzymatic cleanup reaction to remove primer and excess dNTPs that interfere with cycle sequencing. ExoI is used to degrade the single-stranded primers to dNMPs without digesting double-stranded products. SAP converts dNTPs to dNMPs and reduces the dNTP concentration from 200 :M, as used for the PCR reaction, to less than 0.1 :M for use with fluorescent sequencing. The reaction is performed at 37EC and then heated to 65EC to irreversibly denature the ExoI and SAP.

[0009] Because the PCR amplification produces excess template DNA for cycle sequencing, the ExoI/SAP treated PCR sample can be diluted five-fold before cycle sequencing. This reduces the concentration of contaminants into a range that causes less interference with CAE analysis. Cycle sequencing reagents are added, typically with fluorescently labeled dye primers or terminators and the reaction is thermal cycled to drive linear amplification of labeled fragments. Finally, after cycling, the samples are ethanol precipitated, formamide or another denaturant is added, and the sample is electrokinetically injected into the CAE system.

[0010] This workflow has resulted in a dramatic improvement in the performance of the MegaBACE system and currently appears to be the method of choice for other CAE systems as well. Using actual samples from single plaques and colonies of human genomic random subclones or Expressed Sequence Tags (ESTs), this workflow with linear polyacrylamide as a separation matrix has improved the success rate of samples over 200 base pairs from about 60% to 85-90%, and has improved the average readlength from about 350 to greater than 500 bases. Furthermore, this method has proven to be quite robust.

[0011] While the above sample preparation methods have greatly increased throughput, the cost of reagents remains a major component of the cost of sequencing. CAE requires only subattomoles of sample. Reducing the reaction volume will therefore reduce the cost of DNA sequencing. However, substantial reductions in reaction volume can only be achieved if satisfactory methods can be developed for manipulating and reacting samples and reagents. Ideally, such a method would be automated and configured in order that multiple samples could be produced at one time. Moreover, it would be desirable to integrate such a method as a module capable of interfacing with additional components, such as CAE and a detector for separation and analysis.

[0012] Several devices have been designed to aid in the automation of sample preparation. For example, U.S. Pat. No. 5,720,923 describes a system in which small scale cycling reactions take place in tubes with diameters as small as 1 mm. The tube are subsequently exposed to thermal cycles produced by thermal blocks to effect a desired reaction. Multiple samples may be processed in a single tube by drawing in small amounts of sample, each of which are separated in the tube by a liquid which will not combine with the sample. Fluid moves through the tubes by means of a pump. These features are incorporated into a system which automatically cleans the tubes, moves sample trays having sample containing wells, and brings the tubes into contact with the wells in the sample trays.

[0013] U.S. Pat. No. 5,785,926 discloses a system for transporting small volumes of sample. In this system, at least one capillary tube is used to transport small amounts of sample. A precision linear actuator connected to a computer controlled motor acts as a pneumatic piston to aliquot and dispense liquid using the tube. The sample amount is monitored by an optical sensor that detects the presence of liquid within the capillary segment. The system includes a fluid station containing liquids to be deposited and a positioning device for positioning the transport capillary.

[0014] U.S. Pat. No. 5,897,842 discloses a system for the automated sample preparation using thermal cycling. In this system a reaction mixture is pumped into a capillary tube. One end of the tube is sealed using pressure from an associated pump while the other end is sealed by pressing the tube against a barrier. The pump also serves to move fluid within the tube. Once the ends are sealed, the tube is exposed to thermal cycles. In this system a robotic transfer device moves the tubes between the sample preparation station where the pump loads the components of the reaction mixture into the tubes and the thermal cycling station.

[0015] There is an additional need for an automated system that is able to perform small scale thermal cycling reactions in a highly parallel manner. The system should allow for rapid preparation of cycling reactions with minimal reagents. The combination of reducing the amount of reagents required for a reaction and reducing the time required for a reaction will greatly reduce the overall cost of preparation of cycling reactions.

[0016] Capillary array electrophoresis systems and capillary electrophoresis microchip analytical systems can detect subattomoles of reaction products. It is one object of the invention to disclose a method and system for cycling reactions that operate on a submicroliter scale that takes advantage of the high sensitivity of these analytical systems. This reduction of reaction volume will lower the reagent requirements and cost of each reaction. It is a further object to provide an automated system that is able to reduce the time required for cycling reaction preparation. It is an additional object of the invention to provide a system that may be integrated with current analytical instruments including capillary array electrophoresis systems and electrophoresis chips.

[0017] It is a further object of the invention to provide an automated system for preparing reactions and filling a reaction container using capillary action. This allows metering a quantity of liquid into a capillary tube length of fixed volume without using external force to pump liquids. It is a further object to disclose a reagent metering device which also may act as the reaction container. It is also an object of the invention to provide a system which allow the nanoscale reaction containers to be cleaned and reused, saving material costs.

[0018] It is a further object of the invention to provide a system with highly parallel processing, allowing greater throughput. Preferably the system would match the density of microwell plates. It is also an object of the invention to have an automated system in which a number of different cycling reactions could be performed in parallel using a single temperature regulation source, allowing more efficient use of the thermal cycling apparatus. It is a further object to perform isothermal reactions in a highly parallel manner in submicroliter volumes. It is also an object of the invention to provide an automated reaction preparation system that is able to utilize available automation tools by being compatible with standard plate size formats.

SUMMARY OF THE INVENTION

[0019] The above objects have been achieved through a system and method for preparing cycling reaction mixtures. The system uses a capillary cassette comprised of a number of capillary tube segments arranged in parallel alignment. The tube segments extend through a substrate and are generally positioned with uniform spacing. The capillary cassette may be used both to meter reagents and as a reaction chamber in which the reaction is conducted.

[0020] A reaction mixture containing a nucleic acid sample and reaction reagents for performing a thermal cycling reaction (such as the polymerase chain reaction, ligase chain reaction, or preparing a chain termination sequencing reaction) is introduced into the capillaries of a capillary cassette. In one embodiment each capillary contains a unique nucleic acid sample but the same reaction reagents.

[0021] The reaction mixture may be generated in various manners. In one sample preparation method, sample DNA adheres to the interior of the capillary tubes of the capillary cassette or onto a substrate. The liquid in which the DNA was suspended may be eliminated from the capillary or substrate while the nucleic acid is retained, bound to the capillary or substrate. The reaction reagents may then be introduced into the capillary or substrate, combining the sample and reaction reagents to form an assay mixture. In another sample preparation method, the capillaries in a capillary cassette or the wells in a multiwell plate are coated with dehydrated reaction reagents. The nucleic acid sample is introduced into the capillaries of the capillary cassette or the wells of a multiwell plate and the reaction reagents are dehydrated to form a reaction mixture. If the multiwell plate is used, the reaction mixture is subsequently transferred into the capillaries of a capillary cassette. In another sample preparation method, both the reaction reagents and the nucleic acid sample are metered by the capillaries of a capillary cassette. The capillaries are dipped into the wells of a sample plate and a fixed amount of fluid (defined by the interior volume of the capillary) is drawn into the capillary. The volume of liquid metered by the capillary tubes is dispensed by positive displacement, centrifugal force, or other displacement method into the wells of a microplate. A capillary cassette is used to meter both the reaction reagents in a similar manner and dispense the metered liquids onto a location on a substrate combining the sample and reaction reagents to form a reaction mixture. In any of these reaction mixture preparation methods reaction reagents, nucleic acid sample and assembled reaction mixture are introduced into the capillary tubes of a capillary cassette or drawn into the capillary cassette by capillary action. Liquids may also be introduced into the capillaries by active filling, such as by pressure or vacuum. For example one end of the capillaries may be sealed with a liquid impermeable (hydrophobic), gas permeable membrane. By applying a vacuum force to one side of the membrane, the capillary will fill with liquid to the level of the membrane where hydrophobic forces will prevent further filling of the capillary.

[0022] The capillary cassette filled with the reaction mixture is next sealed by pressing the two ends of the capillary tube segments against deformable membranes. The capillary cassette with ends sealed against deformable membranes is contained within an interior chamber of a temperature cycling device. The temperature cycling device exposes the contents of the capillaries to thermal cycles, causing the thermal cycling reaction to occur. In one embodiment the thermal cycling apparatus is an air thermal cycling device. This device receives the capillary cassette into an interior chamber where the ends of the capillaries are sealed. The temperature changes occur using rapidly flowing air. The temperature of the cycling air may be rapidly lowered by venting air to outside the interior cycling chamber. A thermocouple sensor in the air path of the capillary cassette allows for precise monitoring of the temperature of the reaction mixture. Given the rapid transfer of heat through the capillary and precise temperature sensing allowed by the thermal couple, rapid reaction times are possible. The complete thermal cycling times needed for 30 cycles of denaturing heating followed by a period of lower temperature for extension of a 600-700 base DNA strand are performed in 30 minutes or less and could theoretically be effected in as little as 8 minutes. Following a programmed number of thermal cycles, the capillary cassette is removed from the temperature cycling chamber.

[0023] The reaction mixture is next dispensed from the capillary cassette and transferred onto a substrate. In one embodiment the substrate onto which the completed reaction mixture is dispensed is an analytical chip. Following transfer from the capillary cassette the reaction mixture may be separated and analyzed. Alternatively, the sample may be dispensed into a microplate or other substrate. The substrate may then be placed, manually or by an automated system, in a location where it may be analyzed by capillary array electrophoresis. In addition to electrophoresis, the instant reaction preparation system may also be adapted for use in preparing nucleic acid, protein or other biomolecules for microarray analysis, mass spectrometry analysis or other analysis methods. The capillary cassette may also be used for conducting ELISA or other assays requiring binding to a substrate.

[0024] The use of the present system allows a simplified transition between nanoscale and larger scale preparation steps. For example the PCR step may be performed on a nanoscale in the capillary cassette of the present invention. The resulting products could be dispensed into a microplate well for enzymatic clean-up on a larger scale. Following clean-up, the amplified nucleic acid may be again metered into a nanoscale capillary cassette for subsequent reaction mixture preparation (e.g. cycle sequencing). This achieves a simple transition method from nanoscales to larger scales.

[0025] Depositing the reaction mixtures from the capillary cassette into the wells of a 96 well plate allows subsequent processing by capillary array electrophoresis systems. Post reaction processing is also possible. This could include depositing the reaction mixture into ethanol to precipitate the DNA fragments produced in the reaction or dispensing the reaction mixture into formamide to denature double stranded DNA reaction products.

[0026] Following each use, the capillary cassette may be placed into a capillary cassette washer and washed. Following washing, the capillary cassette may be reused.

[0027] The system can be designed with magazines for holding the sample plates, the multiwell mixing plates, and the plates containing the finished reactions. This would allow the system to continuously operate and prepare reaction mixtures. In addition, an integrated system with a central electronic control would allow for a system which may simultaneously assemble reaction mixtures, perform thermal cycling, and wash capillary cassettes.

[0028] The system is useful in the preparation of sequencing reactions, but may also be used in highly parallel preparation of cell lysing and plasmid extraction, polymerase chain reactions, ligase chain reactions, rolling circle amplification reactions, screening compound libraries for drug discovery or compound activity, protein digestion/sequencing, ELISA, radioimmunoassays and other chemical or biochemical reactions or assays.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** is a schematic of an integrated system for the preparation of cycling reaction products.

[0030] **FIG. 2** is a flow chart illustrating the steps in reaction production using the present system.

[0031] **FIG. 3A** is a perspective view of the capillary cassette of the present invention.

[0032] **FIG. 3B** is a perspective view of the capillary cassette inserted into a capillary cassette holder.

[0033] **FIG. 3C** is a flexible capillary cassette.

[0034] **FIG. 3D** illustrates the capillary cassette of **FIG. 3C** bent to a frame and mating with the wells of an analytical chip.

[0035] **FIG. 3E** shows a two layer substrate with microchannels contained within.

[0036] **FIG. 4A** illustrates the dispense head for dispensing liquid from the capillary cassette.

[0037] **FIG. 4B** shows an internal cross section of an air displacement dispense head of **FIG. 4A**.

[0038] **FIG. 4C** shows the dispense head of **FIG. 4A** with the dispense head closed.

[0039] **FIG. 5A** illustrates a top view of a centrifuge used to move fluid from the capillary cassette of **FIG. 3A**.

[0040] **FIG. 5B** illustrates a cross-section of a rotor arm of **FIG. 5A** holding a swinging microplate bucket.

[0041] **FIG. 6** shows a schematic of an air based thermal cycling device with the capillary cassette and holder shown in **FIG. 3B** inserted into the temperature cycling device.

[0042] **FIG. 7A** shows an internal cross section of an air based thermal cycler with integrated capillary cassette sealing membranes.

[0043] **FIG. 7B** shows a detail of the air based thermocycler of **FIG. 7**A, with the lid raised to illustrate the chamber into which the capillary cassette is inserted.

[0044] **FIG. 7C** shows a cross section of the cassette compartment with the capillary cassette inserted into the internal chamber of the thermal cycler of **FIG. 7A**.

[0045] **FIG. 8A** is a front view of the capillary cassette wash station.

[0046] **FIG. 8B** is a side view of the view of **FIG. 8A** with the wash manifold lowered and the wash rank raised.

[0047] **FIG. 8C** is the view of **FIG. 8B** with the wash manifold raised and the wash tank lowered.

[0048] **FIG. 8D** is an interior cross-section of the wash manifold.

[0049] **FIG. 8E** is a schematic plumbing diagram of the wash station.

[0050] **FIG. 8F** is a top perspective view of the wash tank.

[0051] **FIG. 9** shows a histogram of the percent success versus readlength window for the sequencing analysis of example 2.

[0052] **FIG. 10** is an electropherogram of the reaction products of example 2.

[0053] **FIG. 11** shows a histogram of the percent success versus readlength window for the sequencing analysis of example 3.

[0054] **FIG. 12A** shows a scanned gel image of electrophoretically separated PCR products prepared at full volume.

[0055] **FIG. 12B** show a scanned gel image of electrophoretically separated PCR products prepared at nanoscale (500 nL).

[0056] **FIG. 13** is an electropherogram of analysis of prepared sequencing mixtures.

[0057] **FIG. 14** is a graph comparing signal strength of reaction products prepared in tubes, capillaries, and capillaries using surface binding.

DETAILED DESCRIPTION OF THE INVENTION

[0058] In the present invention, it was realized that a capillary segment could be used both to meter reagents and as a reaction container for preforming temperature cycling reactions. The length of the capillary and the internal diameter (ID) of the bore of the capillary tube define the volume of the interior of the capillary tube segment. Capillaries with a 50-150 um ID are commonly available. The small internal diameter of the capillary tubes allows creation of a reaction container having an interior volume less than one microliter. With the present invention, capillaries with interior volumes from 10-500 nanoliters are adaptable to the preparation of DNA cycle sequencing reactions or any other reaction.

[0059] The process carried out by the present automated system is shown in the flow chart of **FIG. 2**. The process begins by the assembly of the reaction mixture, box **52**, by combination of reagents and a sample nucleic acid. The combined reagents are then introduced into the capillaries of a capillary cassette, box **54**. The ends of the capillaries are next sealed, box **56**. The sealed capillary segments are exposed to thermal cycles, box **58**, which effect the cycling reaction. The capillaries of the capillary cassette are then dispensed onto a substrate, box **60**. The substrate is then transferred to an analytical system for analysis of the reaction mixture, box **62**. Details of this process and the structure of the apparatus for carrying out this process are detailed herein.

[0060] In reference to **FIG. 1**, an automated system is shown for assembly of reaction mixtures, temperature cycling to effect the chemical reaction, and dispensing the volume of the completed reaction mixture onto a substrate for subsequent analysis. In the system an automated robot **102** may move the length of stage **114** and may rotate such that automated robot **102** may be moved in relation to other components of the automated system. The automated robot **102** may be rotated to allow the transfer head **104** on automated robot **102** to access objects on all sides adjacent to stage **114**. The assembly of a reaction mixture would begin by the transfer head **104** picking up a capillary cassette from cassette hotel **106**.

[0061] Capillary cassette **15** is shown in **FIG. 3A**. The capillary cassette is comprised of a number of capillary tubes **12** extending through a substrate **10**. It is preferred that the capillary cassette have at least one row of eight capillary tubes and that the capillary tubes have equal spacing. The capillary cassette shown has substrate **10** with 96 capillary tubes arranged in an 8 by 12 array, with spacing of the tubes matching the spacing of the wells of a 96 well microplate. The length of capillary tubes **12** extending from either side of substrate **10** is unequal. It is preferred that the shorter end of capillary tube segments **12** be shorter than the depth of a microplate well. This allows the short end of capillary tubes **12** to be inserted into the wells of a microplate while substrate **10** rests on the top of the microplate.

[0062] The capillary tubes may be made of any material compatible with the assay and preparation to be performed, but preferred capillary materials include, but are not limited to, glass and silica capillaries, although plastic, metals and other materials may also be used. Capillary tubes of various dimensions may be used, such as 75 um ID capillary tubes or 150 um ID/360 um O.D. capillary tubes.

[0063] The capillary tubes **12** extend through a substrate **10** and preferably are arranged in a uniform pattern. The capillary tubes are of equal length and extend through the substrate in a substantially parallel orientation such that each of the two opposing ends of the capillary tubes **12** are coplanar and the planes defined by the ends of the capillary tubes **12** are substantially parallel to the substrate **10**. The spacing of the capillary tubes may be uniform and selected to match the center to center spacing of wells on a microplate. For example on a standard 96 well microplate the capillary tubes would be arranged with a 9 mm center to center spacing, on a 384 well microplate the capillary tubes **12** would be arranged with a 4.5 mm center to center spacing. Higher density capillary formats, compatible with 1536 well microplates or plates with even higher well density, should also be possible. The capillary tubes **12** are preferably secured within the substrate such that the length of capillary tubes **12** extending from one side of the substrate **10** are shorter than the length of the capillary tube on the opposite side of substrate **10**. The length of the capillary tubes **12** on the shorter side of the substrate may be matched to the depth of wells in a microplate, such that the length of the shorter side is a shorter length than the depth of a well in a microplate. This feature enables the capillary cassette to be inserted into a microplate such that the substrate **10** rests against the top lip of the multiwell plate and the capillaries on one side of the substrate may extend into the multiwell plate without touching the bottom. For example, in a 96 well microplate the capillary tubes may be disposed on a substrate such that the shorter side of the capillary tube extending from the substrate may be inserted into wells in a microplate without the capillary touching the bottom of the well. This ensures that liquid dispensed into a well is clear of the capillary to prevent re-entering the capillary.

[0064] The capillary cassette substrate **10** may be made of a fiberglass board or other rigid or semi-flexible material. The capillary tubes **12** may be inserted through evenly spaced holes in the substrate and secured with adhesive. In one embodiment, the length and width of the substrate are similar to the length and width of a standard 96 well microplate. This simplifies adapting automated systems designed for manipulation of microplates to handle the capillary cassette.

[0065] In some embodiments it may be advantageous to coat the interior of the capillary with various surface coatings such as ionic and non-ionic surfactants. Coatings which may be used include bovine serum albumin (BSA), glycerol, polyvinyl alcohol and Tween 20. The coatings are introduced into the capillary and dried onto the interior surface of the capillary tube. Alternative-ly, covalent modification of the interior surface with silanization or Griganard reaction may be desired. For example, covalent modification of capillary tubes interior surfaces which reduce electroendoosmosis may also be useful in reducing charge surface effects between a capillary interior surface and components of a reaction mixture. U.S. patent application Ser. No. 09/324,892, hereby expressly incorporated by reference for all purposes herein, discloses the use of acryloyldiethanolamine as a covalent capillary coating with advantageous alkaline stability. In addition to this coating, acrylimide or other known coatings may also be used to covalently modify capillary interior surfaces.

[0066] A. Assembly of Reaction Mixture

[0067] Returning to **FIG. 1**, the automated system allows for the combination of reaction reagents and sample DNA using the capillary cassette. The capillary cassette would be taken by transfer head **104** from the cassette hotel **106** and brought into contact with the samples contained in a sample plate at location a. The sample plate is dispensed from sample plate hotel **108**. The sample would be drawn into the capillary tubes of the capillary cassette by capillary action. The internal volume of the capillary tube is defined by the length of the capillary tube and its internal diameter. The capillary cassette of **FIG. 3A** acts as a fixed volume parallel pipettor, allowing a number of capillary tubes to be filled in parallel. Each capillary tube segment will meter a discrete amount of liquid which may be subsequently dispensed.

[0068] Once one end of each capillary is inserted into the sample containing well, a liquid will be drawn into the capillary. This small amount of sample may be combined with other liquids to form a reaction mixture. The sensitivity of analytical instruments such as a capillary array electrophoresis system and the exponential amplification of reaction mixture products enabled by cycling reactions allow for nanoscale reactions and analysis. Very small scale reactions are able to reliably produce reaction mixture products of sufficient quantity for analysis on a capillary array electrophoresis system or a capillary electrophoresis chip. Significantly less reaction reagents are required if a nanoscale reaction mixture is enabled.

[0069] The automated system may be used in various ways to prepare reaction mixtures. A few of the many such methods for use of the system in production of reaction mixtures follow.

[0070] Reaction Mixture Preparation Example 1:

[0071] Metering Reagents with Capillary Cassette and Mixing on a Substrate

[0072] One method to prepare the reaction mixture is to use the pipettor to separately meter the components of a reaction mixture. For example for a PCR mixture, the nucleic acid sample and PCR reagents would be separately metered and dispensed into a single container combining the liquids. In using the automated system of **FIG. 1**, the automated robot **102** moves transfer head **104** containing a capillary cassette to location a where a sample plate is located. The ends of the capillary tubes of the capillary cassette are dipped into the wells. The capillary tubes fill by capillary action, metering a precise amount of sample. The wells of sample plate contain the nucleic acid sample. The DNA sample should be sufficiently dilute such that 5-20 ng of DNA is contained in the 10-10,000 nL volume metered by each capillary tube segment in the capillary cassette.

[0073] **FIG. 4A** shows the capillary cassette transferring fluid samples from a multiwell plate **36** into a capillary cassette **15**. The capillary tube segments **12** on capillary cassette **15** are extended into the wells of multiwell plate **36**. The wells of multiwell plate **36** are conical and liquid in the well will flow to the bottom central area of each well. This allows a small amount of liquid within the well to be positioned such that a capillary inserted into the center of the well and above the bottom of the well will contact the liquid. The capillary tube segments of the capillary cassette then fill by capillary action with the liquid in the wells. It is preferred that the capillary cassette have capillary tube segments which have the same center to center spacing as the wells of the multiwell plate containing the DNA samples. In one embodiment the capillary cassette has the same number of capillary tube segments as the number of wells in a multiwell plate holding samples.

[**0074**] After the capillary cassette is dipped into the nucleic acid sample containing wells, the filled capillary cassette may be moved by transfer head **104** to a dispensing device location **122**. At the dispensing device location **122**, the sample is dispensed onto a substrate. A clean capillary cassette is then retrieved and dipped into a sample plate containing the PCR reagents. As seen earlier, the capillary cassette meters a precise amount of liquid defined by the interior volume of the capillary tubes held in the capillary cassette. The metered amount of reaction reagents may be the same volume as the volume of sample dispensed. The reaction reagents are dispensed from each capillary tube segment onto locations on the mixing substrate containing the nucleic acid sample.

[**0075**] The present reaction mixture assembly may be used for assembly of numerous types of reactions. The same basic method used to assemble the PCR reaction mixture may be adapted to assembly of a cycle sequencing mixture, rolling circle amplification reaction mixture, or other reaction mixtures.

[**0076**] When dispensing the contents into a microplate some care must be taken to mix the sample and reaction reagents in a manner which avoids splattering. A number of different methods have been envisioned to dispense liquid from the capillary cassette.

[**0077**] Capillary Cassette Dispensing Example 1:

[**0078**] Centrifugal force

[**0079**] The first method to dispense the contents of the capillary cassette while avoiding splattering uses a centrifuge to dispense the fluid by centrifugal force. The centrifugal force is applied evenly to all of the capillaries in the capillary cassette such that capillaries independently dispense into microplate wells. The dispensed liquid is drawn by centrifugal force to the bottom of wells in the multiwell plate.

[**0080**] In **FIG.** 5A, the centrifuge **42** is shown having a swinging microplate bucket **43** which may contain a multiwell plate with an inserted capillary cassette. The swinging microplate buckets are held on rotor **41**.

[**0081**] **FIG. 5B** shows a cross-section of swinging microplate bucket **43**. The capillary tubes **12** of the capillary cassette are inserted into wells **36***a* of multiwell plate **36**. The cassette is inserted such that the portion of the capillary tubes **12** extending from the substrate **10** are shorter than the depth of the wells **36***a*. As shown in **FIG. 5**B, the capillary tube **12** extending from substrate **10** do not reach the bottom of the wells **36***a* of multiwell plate **36**. Microplate swinging bucket **43** is comprised of an arm **45** and a platform **44**. An upper end of arm **45** fits onto latch head **42** on rotor **41**. Microplate **36** is positioned on platform **44** of microplate swinging bucket **43**. When the centrifuge is in motion, platform **144** rotates on latch head **42** such that the multiwell plate faces the side wall of the centrifuge and the centrifugal force on the liquid in the capillary tubes dispenses the liquid into the bottom of the wells **36***a* of the multiwell plate **36**. When conical shaped wells are used, the centrifugal force will draw the liquids within the well to the well center, causing the sample to locate at a more precise location The liquid will be displaced from the capillary at fairly low centrifuge speeds.

[**0082**] In **FIG. 1, a** low speed centrifuge may optionally be included in the automated system at the dispensing device location **122**. Automated robot **102** uses transfer head **104** to pick up a nanotiter plate dispensed onto location b by nanotiter plate hotel **110**. The nanotiter plate is transferred by transfer head **104** to the stage of the low speed centrifuge. A capillary cassette is filled with samples or reaction reagents as described and is transferred onto the nanotiter plate on the stage of the low speed centrifuge. The plate and cassette are then spun in the centrifuge, dispensing the liquid from the capillaries into the wells of the nanotiter plate. Once the liquid has been dispensed and the centrifuge has stopped rotating, the capillary cassette may by removed by the transfer head and transferred to the cassette washer **118**. The transfer head **104** can then pick up a clean capillary cassette from the capillary cassette hotel **106**. The clean capillary cassette can be used to meter a second liquid reaction component which is similarly dispensed using the centrifuge. In the automated system the centrifuge includes a sensor associated with the rotor used in conjunction with a rotor braking system to stop the rotor in a position which transfer head **104** can access such a sensor could be magnetic, optical, mechanical, or use other known means of sensing rotor position.

[**0083**] Capillary Cassette Dispensing Example 2:

[**0084**] Air Displacement

[**0085**] A second method of dispensing the liquid contained in the capillary tube segments of a capillary cassette is through the use of an air displacement device. With reference to **FIG. 1, a** nanotiter plate dispensed from nanotiter plate hotel is transferred by transfer head **104** to the dispensing device location **122**. At this location an air dispenser, such as the one pictured in **FIG.** 4A-C is located. Subsequently a capillary cassette is retrieved by transfer head **104**, filled with either sample from a sample multiwell plate or reaction reagents. The capillary cassette is then moved to the dispensing device location **122** and brought into contact with air displacement head. The substrate of the capillary cassette is placed on a receiving platform on the air displacement head. Alternatively, the air displacement head may be joinable to automated transfer robot **102**.

[**0086**] With reference to **FIG.** 4A, the air displacement head **301** is shown with a capillary cassette **15** held on bottom plate **302**. The bottom plate **302** is attached to a manifold assembly by hinge **318**. Capillary cassette substrate **10** is held on foam rubber pad **304** which is secured onto bottom plate **302**. An array of holes **325** extend through foam rubber pad **304** and bottom plate **302** which are spaced to allow the capillary tubes **12** to extend through foam rubber pad **304** and bottom plate **302** when the capillary cassette is positioned on bottom plate **302**. The manifold assembly of the air displacement head is comprised of an upper housing **306**, chamber unit **310** and a set of clamps **314**. Clamps **314** secure membrane **312** to the lower surface of the chamber unit **310**. Membrane **312** forms a seal to the top surface of the capillary cassette **15** when the manifold assembly is closed over the cassette. Membrane **312** has holes **316** corresponding to capillary **12** positions in the cassette when the capillary **12** positions in the cassette when the capillary cassette **15** is placed on bottom plate **302**. When the top manifold of air displacement head **301** is closed against bottom plate **302**, capillary tubes **12** are positioned in capillary tube receiving holes **316** on membrane **312**. When the air displacement head **301** is closed it may be secured by latch **322** which mates with hole **324** to clamp the capillary cassette between the foam rubber pad **304** and membrane **312** resulting in a seal between the top surface of cassette **15** and the membrane **312**.

[**0087**] **FIG. 4B** illustrates a cross sectional view of displacement head **301**. Upper housing **306** is constructed of metal, acrylic or other rigid material. Gas input coupler **303** is disposed on upper housing **301**. When a pressurized gas or vacuum line **305** is attached to gas input coupler **303**, a vacuum or pressure force may be introduced into upper chamber **307**. Held between upper housing **306** and chamber unit **310** is a gas impervious elastic membrane **308**. The area between elastic membrane **308** and upper housing **306** defines upper chamber **307**. Secured onto clamps **314** is membrane **312**. Membrane **312** is pressed against substrate **10** of a capillary cassette inserted into displacement head **301**. Substrate **10** is secured within displacement head **301** by bottom plate **302**. Rubber pad **304** provides a deformable surface which exerts uniform force pressing substrate **10** against membrane **312**. Membrane **312** has an array of holes **316** which allow the capillaries **12** of the capillary cassette to extend through membrane **312**. When a capillary cassette is inserted into air displacement head **301**, the substrate seals holes **316** enclosing lower chamber **313**. When pressurized gas is introduced into chamber **307** by gas line **305**, elastic membrane **308** will be pressed into lower chamber **313**. Membrane **308** is located between upper chamber **307** and lower chambers **313**. Membrane **308** serves both as seal for the upper end of chambers **313** and the chamber displacement actuator when pressure is applied to the upper chamber **307** through coupler **303**. The degree of displacement is dependent on the pressure applied. The resulting air displacement will act to dispense liquid from capillary tubes **12** which extend through the capillary and into the lower chamber **313**. By regulating the amount of pressure applied through line **305**, a consistent displacement force will be applied to each capillary tube. Given the submicroliter volume of the capillary tube segments, fluctuations in the amount of dispensing pressure should not adversely affect displacement from the tubes.

[**0088**] **FIG. 4C** illustrates the closed air displacement head **301**. Upper housing **306** is pulled toward bottom plate **302** by latch **322** in order to compress membrane **312** against the top of the capillary cassette substrate thereby forming a seal. Clamps **314** secure membrane **312** onto chamber unit **310**. Air displacement head **301** is mounted on arm **320**. Arm **320** may extend from automated transfer robot **102** shown in **FIG. 1** or be positioned at dispense location **122**. Pressurized gas may be introduced into upper housing **306** through gas input couple **303**.

[**0089**] This displacement head provides an individual displacement chamber for each of the capillaries dispensed. Although a 16 capillary cassette is depicted, the displacement head may be constructed to dispense capillary cassettes having an array of 96 capillaries or greater capillary densities. The dispensing force applied to each capillary is sufficiently small to allow dispensing directly onto a substrate with the sample dispensed at a discrete location.

[**0090**] Air displacement or centrifugal displacement may be used to dispense liquid from the capillary tube segments in a capillary cassette. It may also be possible to dispense liquid from the capillary tubes using a bank of syringe pumps, applying pressure through a gas permeable/liquid impermeable (hydrophobic) membrane, using electrokinetic dispensing, or other known dispensing means. The air displacement head may also be used to dispense finished reaction mixtures onto a substrate for subsequent analysis.

[**0091**] Reaction Mixture Assembly Example 2:

[**0092**] Dehydrated Reagents

[**0093**] A second method to assemble the reaction mixture is to have the regents required for the reaction stored as a dehydrated coating either on the interior of a capillary or on a substrate, such as within a well of a multiwell plate. If the reaction reagents are dehydrated onto the interior of capillary tube segments in a capillary cassette, introducing a sample into the capillary would cause rehydration, mixing and formation of the reaction mixture. In a similar manner, if the wells of a microplate are coated with the dehydrated reaction reagents, adding a nucleic acid sample into the wells would bring the reaction reagents into solution forming an assay mixture. The sample could be metered with a capillary cassette and dispensed from the capillary cassette by one of the methods set out above. The sample would bring the dehydrated reaction reagents into solution and mix with the sample containing nucleic acid by diffusion. This provides a method to assemble the reaction mixture in a very simple manner, potentially without the need to dispense the capillary tubes with a centrifuge or air displacement device. This could both simplify the reaction processing system and shorten the reaction assembly time.

[**0094**] For PCR, a dehydrated reagent mixture is commercially available, sold as Ready-to-Go® (Amersham Pharmacia Biotechnology, Piscataway, N.J.). The stabilized, dehydrated reagents may be coated onto the interior surface of capillary segments or the interior of the wells of a multiwell plate. The Ready-to-Go® product uses a carbohydrate matrix to stabilize the reaction reagents (DNA polymerase, buffer reagents, dNTPs) in a desiccated state. Bringing the reagents in the Ready-to-Go® mixture into solution with the liquid nucleic acid sample and primers in solution produces the final reaction mixture required for the reaction. The combination of the stabilized Ready-to-Go® compounds, the template DNA, primers, and sufficient water produces a final reaction product. It is contemplated that reagents for chain termination sequencing reactions could also be stored in a desiccated state.

[**0095**] The coating could be applied to surfaces by a number of different methods including vapor phase coating, filling a capillary (by capillary action, pressure filling, etc.) with the Ready-to-Go® mixture and emptying the bulk phase (under vacuum, pressure or other forces), or dipping a substrate (such as a bead) into the reagents and subsequently drying the bead.

[0096] Reaction Mixture Assembly Example 3:

[0097] Nucleic Acid Capture

[0098] A third method of assembly of the reaction mixture is to capture the sample nucleic acid on the surface of a substrate, such as the interior of a capillary tube segment. The sample nucleic acid may be attached onto the surface by a number of methods. These include covalent attachment, DNA hybridization, hydrophobic interactions, electric field, magnetic field, or other chemical or physical forces. Once the sample has been attached, the remaining liquid in which the sample was suspended may evacuated from the capillary or microchip by chemical reaction or physical force. Air displacement or centrifugal dispensing method may be used to empty the capillary, as can a vacuum. The sample nucleic acid would remain on the surface of the substrate. In this single step, the sample nucleic acid may be substantially purified. The reaction reagents may then be combined with the sample nucleic acid, producing the reaction mixture.

[0099] One method to immobilize the nucleic acid sample is to attach the nucleic acid directly to a surface. This may be done by non-covalent modification (such as surface treatment with NaSCN, DMSO, etc.) or covalent linkage. There are a number of different covalent attachment methods for DNA known in the art. For example, an amino group can be attached to the deoxyribose base of DNA and incorporated during a synthetic reaction, such as during PCR amplification of a DNA plasmid insert. The glass or silica of a capillary interior could be silanized and the amino group on the modified DNA would covalently bond to the silanized interior of the capillary. Alternatively, other chemistries are available to covalently immobilize DNA onto a surface. Once the DNA is bound to the surface of a capillary or other substrate, the liquid in which the DNA was suspended may be eliminated from the capillary and the capillary may be filled with reaction reagents.

[0100] An alternative method of attaching a nucleic acid to the interior of the capillaries of a capillary cassette is through affinity chemistry. One common affinity chemistry procedure labels a biomolecule with biotin and then binds the biotinylated biomolecules to avidin or streptavidin. The avidin/streptavidin may be used to link the biotinylated molecules. Nucleic acid labeled with biotin may be subsequently attached to a surface, such as the interior of a capillary tube. This may be accomplished by binding streptavidin to the interior of the capillary.

[0101] One example of the use of affinity chemistry for the binding of DNA to the interior of a capillary is disclosed in U.S. Pat. No. 5,846,727, hereby expressly incorporated herein for all purposes. This reference describes the binding of DNA to the interior surface of the capillary tubes. The technique requires primers labeled with biotin which are combined with dNTPs, a DNA polymerase, and a reaction buffer. This is combined with template DNA, such as plasmids from a DNA library with sub-cloned DNA inserts, to form the reaction mixture. In the present invention a microplate may contain 96 or more reaction mixtures, each with a unique plasmid with a subcloned DNA sequence. This reaction mixture could be assembled by the method stated in reaction mixture assembly example 1: namely the reaction reagents and the plasmid sample could be separately metered and dispensed into a 384 well microtiter plate. In a microplate well the liquids are combined to form a reaction mixture. The reaction mixture is metered into the capillary tube segments of a capillary cassette. The PCR reaction may be effected by temporarily sealing the ends of the capillary tube segments and exposing the capillary cassette to thermal cycles, as described below. The results of the PCR reaction are exponentially amplified copies of the subcloned plasmid DNA insert containing the biotin labeled primer.

[0102] The template DNA containing the biotin labeled primer may then be immobilized on the walls of the capillary tubes of a capillary cassette. The immobilization capillary cassette would have capillary tubes with avidin or streptavidin coated onto the interior surface of each capillary tube. The chemistry for attachment of avidin/streptavidin may be that disclosed in, for example, L. Amankwa et al., "On-Line Peptide Mapping by Capillary Zone Electrophoresis," Anal. Chem., vol. 65, pp. 2693-2697 (1993). The capillary is filled with (3-aniopropyl)trimethoxysilane (3-ATPS), incubated for 30 minutes, and air dried. The dried capillaries in the capillary cassette are next filled with sulfosuccinimidyl-6-(biotinamido)hexonate (NHS-LC biotin) which is again incubated followed by air drying. Avidin or streptavidin in phosphate buffer at 7.4 pH is added to each capillary tube. The avidin binds to the biotin immobilized on the interior of each capillary. The double stranded amplified biotinylated PCR products suspended in a buffer (e.g. Tris-HCl, or EDTA with either NaCl or LiCl at 1-3M added for efficaceous binding) are added to the capillary tube and incubated for 5-10 min. This results in a capillary wall modified as follows: capillary wall-Si—C_3H_6—NH—CO-biotinavidin or streptavidin-amplified oligonucleotide with associated biotin primer.

[0103] Once the DNA is immobilized on the interior surface of the capillary, the contents of the capillary tube may be dispensed in one of the methods described and the DNA would remain bound to the surface of the capillary. This removes debris and other impurities from the DNA presenting a rapid and effective method of DNA purification. The capillary may be rinsed with a buffer for additional purification. The defined area of the interior surface of the capillary provides a known amount of binding sites for the DNA attachment. This provides a simple method for normalization of DNA concentration. The capillary cassette may then be dipped into wells or a reagent reservoir containing the reagents for cycle sequencing. The cycle sequencing reaction can be performed by temporarily sealing the ends of the capillary tubes by pressing each end of the capillary tubes against a deformable membrane. The capillary cassette may then be exposed to thermal cycles which effect the cycle sequencing reaction.

[0104] In this embodiment biotin, rather than avidin or streptavidin, is covalently attached first to the capillary wall. This aids in the regeneration of the capillary cassette for subsequent binding reactions. After completing the cycle sequencing reaction, it would be difficult to remove the amplified biotinylated DNA without also denaturing the avidin protein. By having biotin bound to the interior surface of the capillary the amplified DNA may be easily removed by filling the capillary with phenol or formamide solution at 65-90 degrees C. This denatures the avidin protein without removal of the biotin bound to the interior surface of the capillary. This mixture is then dispensed. The capillary cassette may then again be filled with the avidin containing solution and reused for binding subsequent biotinylated amplified template DNA.

[**0105**] Prior to filling, the capillary tube segments of the capillary cassette may be coated with a variety of compounds. Coating the interior surface of the capillary tube segments with bovine serum albumin (BSA) or polyvinyl alcohol has been shown to improve performance of some reactions, such as preparation of chain termination sequencing reactions.

[**0106**] B. Thermal Cycling

[**0107**] Once the reaction mixture is introduced into the capillary tube segments of the capillary cassette, the ends of the capillaries of the capillary cassette are sealed and the capillary cassette is exposed to temperature cycles. The ends of the capillary cassette capillaries are sealed by pressing each of the ends of the capillary tubes against a deformable membrane. Returning to **FIG. 1**, once the capillary cassette has been filled with the reaction mixture, the ends of the capillaries are sealed and the capillaries are exposed to thermal cycles in thermal cycling device **116**.

[**0108**] In one thermal cycling device, shown in FIGS. 7A-7C, the thermal cycling device has integrated membranes that seal the ends of the capillaries and exposes the capillary cassette to thermal cycles. In this apparatus the means for sealing the ends of the capillaries in the capillary cassette is incorporated into the thermal cycling device.

[**0109**] With reference to **FIG.** 7A, the capillary cassette **15** is held on lip **280** within internal passageway **256** between deformable membranes **264***a* and **264***b*. As seen in **FIG.** 7B, deformable membrane **264***a* is mounted on platform **261**. Lid **262** is secured on platform **261**. Platform **261** is attached by pivot **286** to base **265**. Pneumatics **284***a*, **284***b* are attached at an upper end to platform **261** at pivot **263**. Screw **282** acts as a stop for platform **261** when platform **261** is lowered onto housing **270**, enclosing passageway **256**. Diffuser **258** promotes temperature uniformly of air circulating in internal passageway **256**. Thermocouple **260** measures temperature of the circulating air. The function of pivot **277** and bottom membrane platform **200** is described in conjunction with **FIG. 7C**.

[**0110**] **FIG. 7C** shows a cross section of the capillary cassette holding chamber with capillary cassette **15** inserted into the internal passageway **256**. The capillary cassette could be inserted into this area by automated robot **102** of **FIG. 1** after the capillary tube segments have been filled with the reaction mixture. Capillary cassette **15** is positioned such that substrate **10** rests on ledge **280**. Capillary cassette is positioned such that the ends of capillary tube segments **12** are depressed against top deformable membrane **264***a* and bottom deformable membrane **264***b* when upper platform **261** is lowered over the capillary cassette and lower platform **271** is raised. Notches **262***a*, **262***b* seal along the side lengths of housing **270** when upper platform **261** is lowered to provide a host retaining seal. Screw **282** acts as a stop for upper platform **261** to prevent the platform from lowering so far that capillary tube segments are bowed or damaged. Base platform **266** is secured to post **273** and secured to housing **270**. The lower end of pneumatics, **284***b* is secured at a lower pivot **271***a* to low platform **271**. Extending through lower platform **271** are shoulder screws **268** which extend through housing **270** and stationary platform **266** and are secured to lower platform **200**. When upper platform **261** is lowered by pneumatic **284***b* lower platform **271** is also raised toward housing **270**. When pneumatic cylinders **284***b*, **284***a* are retracted, the pneumatic cylinders move to a vertical orientation. Upper platform **261** is lowered and lower platform **271** is raised slightly in an arc. Lower platform **271** will arc upward on pivot **277** to move to a position substantially parallel to platform **261** when pneumatic cylinder **284***b* is fully retracted. When a capillary cassette **15** is inserted into internal chamber **258** the ability of platform **200** to "float" on springs **275** prevents excess pressure from damaging capillary tubes **12** or membranes **264***a*, **264***b*. Platforms **261** and **200** exert 400 pounds per square inch force on capillary tubes **12** providing sufficient sealing pressure. With upper platform **262** lowered, the capillary tube segments **12** are sealed at each end by deformable membranes **264***a*, **264***b*. Deformable membranes **264***a*, **264***b* may be made of silicon rubber or other deformable material.

[**0111**] Returning to **FIG. 7A, a** motor **250** turns shaft **251** which rotates squirrel cage blower **253**. Blower **253** produces air movement through diffuser **254** to flow into internal passageway **256**. The blower generates sufficient circulation flow that the air flowing through internal passageway **256** circulates at 2000 feet per minute. Diffuser **254** ensures that the heat of the air blown by blower **253** is uniform throughout passageway **256**. Cone **255** on diffuser **254** aids in mixing the flowing air, promotion temperature uniformity through passageway **256**. Diffuser **254** acts to ensure an even flow of air through passageway **256** in the region of the capillary cassette and reduces non-uniformity from wall loss effects in internal passageway **256**.

[**0112**] The internal passageway **256** is defined by outer housing **270**. Outer housing **270** is preferably of rectangular cross section and comprised of sheet metal, plastic or other durable material. The internal surface of outer housing **270** at all locations except for inlet **278** is lined with thermal foam insulation **272**. Insulation **272** prevents excess heating of outer housing **270** and helps retain heat and aids temperature uniformity of the air circulating through internal passageway **256**. After flowing through first diffuser **254** the air flows through second diffuser **258**. Diffusers **254** and **258** promote uniform air flow and temperature uniformity through internal passageway **256**. Past first diffuser **254** internal passageway **256** transitions, to match the dimensions of passageway **256** to accommodate. The heated air flows past thermocouple **260** which is vertically disposed at the center of internal passageway **256** just beyond second diffuser **258**. Thermocouple **260** acts to monitor the temperature within internal passageway **256**. Thermocouple **260** may be a temperature monitoring device inserted into a capillary tube section which extends through outer housing **270** and through foam insulation **272**. Alternatively thermocouple **260** may be selected such that it accurately reflects the internal temperature of a capillary tube.

[**0113**] The air circulating through internal passageway **256** passes thermocouple **260** and flows past the capillary tube segments **12** of capillary cassette **15**. The ends of the capillary tube segments are sealed at their upper end by deformable membrane **264***a* mounted on upper platform **261** which has been lowered to form an air tight seal with housing **270**. The lower end of capillary tube segments **12** are sealed by deformable membrane **264***b*. Deformable membrane **264***b* is mounted on platform **200** which is secured on a bottom surface by shoulder screws **268**. Shoulder screws **268** extend through housing **270** and retained by platform **271**. Springs **275** located between platform **200** and platform **271** provide a biasing force while allowing for platform **200** to float such that deformable membrane is biased against the ends of capillaries **12**. The function of double acting pneumatics act to seal lid **262** and apply force to position platform **271** is described in conjunction with **FIG. 7C**. Lid **262** fits onto housing **270** such that the sheet metal or other material comprising the edge of lid **262** fits on top of housing **270**. Membrane **264***a* is mounted on upper platform **261** such that membrane **264***a* extends into internal passageway **256** at least far enough that membrane **264***a* is even with insulation **272**. As the air travels past capillary tube segments **12**, the length of the capillary tube segments **12** below substrate **10** are rapidly heated and cooled to the temperature of the air rapidly moving through internal passageway **256**.

[**0114**] Door **274** controlled by motor **276** is used in conjunction with thermocouple **260** and heating element **252** to control the temperature within internal passageway **256**. When door **274** is closed, the air circulating within internal passageway will not be exchanged with outside air. As the air continuously passes over heating element **252** the air is rapidly heated until the air comes to the selected temperature. Once thermocouple **260** senses that the temperature is at a selected temperature, heating element **252** may be kept at a lower heat output such that the internal temperature is maintained. If the temperature needs to be rapidly dropped, as in during a thermal cycling reaction, door **274** may be moved to orientation **274***a* by motor **276** with the door **274** moved into internal passageway **256**, allowing all air which has passed capillary cassette **15** to be exhausted to outside internal passageway **256**. It is envisioned that a filter or exhaust duct could be mounted about door **274** to prevent compounds in the circulating air from being exhausted to the environment. The rapidly circulating air will be quickly exhausted to outside of the thermal cycler while ambient air is drawn in through air intake **278**. Air drawn into internal passageway **256** through intake **278** flows through heater **252**. The area through which the air moves is restricted by block **259** positioned above heater **252** within internal chamber **256**. Again the temperature of the air within internal passageway **256** is monitored by thermocouple **260** and when the desired temperature drop has occurred, door **274** will be brought toward housing **270**, reducing air volume drawn through air intake **278**.

[**0115**] By connecting heating element **252**, thermocouple **260** and door motor **276** to an electronic control system, such as a computer controller, this thermal cycler may perform precise air temperature varying sequences. Additional heat is added when needed by heating element **252** and heat is exhausted by opening door **274**, with the temperature result of either action monitored by thermocouple **260**. Exhausting circulating air through door **274** allows air within internal passageway to drop in temperature at a rate greater than 10 degrees per second. The rapid temperature change combined with the rapid transfer of heat to or from the capillaries allows for efficient temperature cycling reactions. For example in reactions using a thermostable polymerase, the denaturing of nucleic acid strands and the annealing of primer to template strands each may take place in one to five seconds. The extension of the primer will require less time to effect since the rapidly circulating air and the thin walls of the capillaries rapidly bring the internal volume of the capillaries to the selected temperature. The thin walls of the capillaries and the small capillary volume enable a rapid temperature change and heat transfer throughout the internal capillary volume. This greatly reduces the preparation time required for each reaction, allowing more efficient use of the thermal cycler and greater throughput in sample preparation. Presently, a 30 cycle PCR amplification may be performed in under 30 minutes. It should be possible to reduce this time to under 8 minutes.

[**0116**] Once the thermal cycling reaction is complete, upper platform **261** may be raised and capillary cassette **15** removed from internal passageway **256**. During the temperature cycling process, the liquid within each capillary tube segment will expand somewhat and some liquid will leak from the capillary and be carried away by the rapidly flowing air. However, such loss is only a few percent of the volume of the capillary tube segment and should not present either a contamination problem or cause enough reaction product loss to materially affect subsequent analysis. In addition, the portion of capillary tube segments **12** located between substrate **10** and deformable membrane **264***a* will receive only poor air flow and will be less likely to rapidly reach the denaturation temperature. However since this length is short, the failure of this area to as rapidly reach the denaturation temperature should not adversely affect the ability of the remaining portion of the capillary from producing sufficient reaction product for subsequent analysis.

[**0117**] An alternative device for sealing the ends of the capillary is a capillary cassette holder which seals the ends of capillary tube segments of a capillary cassette. With reference to **FIG. 3B** the capillary cassette holder is comprised of a pair of parallel deformable membranes **14***a,* **14***b* each secured onto a platform **16***a,* **16***b*. The deformable membranes may be silicon rubber seals, Teflon®, plastics or other resilient, deformable material. A pair of parallel posts **9** extend from bottom platform **16***a* to top support platform **24** where the posts are secured by internally threaded nut **18**. Post **9** passes through platform **24** and nut **18** is retained on an annular lip of platform **24**. Shoulder screws **20** extend through holes in support **24** and are secured to top platform **16***b*. Springs **22** bias the top platform **16***b* against the ends of capillary tube segments **12** while allowing **16***b* to float. The substrate **10** of capillary cassette **15** may be designed to have holes which conform to the spacing and dimension of posts **18** such that capillary cassette **15** may be more easily and securely held within holder **23**.

[**0118**] Once the ends of the capillary cassette are sealed in holder **23**, the combined capillary cassette and holder may be exposed to thermal cycles. The holder shown seals **16** capillaries. However, a holder may be designed to hold capillary cassettes having 96 capillaries or higher densities of capillaries. In addition to capillary cassettes, chips of other substrates may be used as the reaction containers. **FIG. 3E** shows a chip substrate **70** comprised of two bonded substrate layers **72, 74**. One layer **72** has grooves **76** extending the length of the chip. The affixed top substrate **72** encloses a capillary dimension passage **76** with opposite open ends. A liquid reaction mixture may be introduced into the inclosed passage. The ends of these passages may be sealed by pressing the ends against a deformable membrane, as was done with the capillary cassettes. Temperature cycling may require longer times because of greater mass material comprising the chip, but cycling times should still be more rapid than conventional cycling.

[0119] For isothermal reactions, such as rolling cycle amplification, temperature cycling is not required to effect the reaction. Once an isothermal reaction mixture is combined and introduced into a capillary cassette, incubation of the cassette at a reaction temperature will allow the reaction to occur. With reference to **FIG. 1**, the automated transfer device may transfer a capillary cassette into incubator **124** where the capillary cassette is incubated at a selected temperature. A set of deformable membranes may be used to seal the ends of the capillaries during incubation. As was seen in other system components, incubator **124** may be used at the same time as other system components.

[0120] In the case of PCR or chain termination sequencing reactions it is necessary to expose the reaction mixture to temperature cycles. In **FIG. 1** the transfer head **104** moves the capillary cassette into thermocycler **116**. The thermocycling device may be any device which can expose the capillary tube segments of the capillary cassette to temperature cycles. Thermal cycling devices which use water, electric field, heating blocks, or other means may be used. Alternatively, air based thermal cycling devices are rapid and adaptable to the low volume cycling of the present invention.

[0121] A thermal cycling device which uses air as the temperature transfer medium is shown in **FIG. 6**. The reaction mixture is contained in capillary tube segments which have a high surface to volume ratio and small material thickness. This allows very rapid transfer of heat through the walls of the capillary and throughout the liquid reaction mixture. An equilibrium temperature is reached rapidly throughout the liquid in the capillary. The use of air as a heat transfer medium enables the rapid ramping of temperature in the reaction chamber. Rapid circulation of the air ensures rapid and more uniform heating or cooling of the capillary segments and their contents.

[0122] The capillary cassette **15** sealed within holder **8** is inserted through opening **215** in housing **202** of the air based thermal cycler. The holder **8** is supported by housing surface **215** of the thermal cycling chamber **210**. The capillary tubes **12** mounted to substrate **10** are exposed to the air of thermal cycling chamber **210** such that the air may freely flow around capillary tube segments **12**. Thermocouple **216** monitors the temperature of the air moving past capillary tubes **12**.

[0123] In the air based thermal cycling device, paddle **208** driven by motor **206** rapidly circulates air with reaction chamber **210**. The air is rapidly circulated past the capillaries **12** of capillary cassette **15**. Halogen bulb **220** acts as a heat source to heat the air within the thermal cycling chamber **210**. To effect a thermal cycling reaction, the circulating air is held at a selected temperature for a selected period of time. The thermocouple **216** transmits the temperature of the capillary tube segment **12** to microprocessor **218**. To effect the needed temperature changes the microprocessor instructs actuator **222** to open door **226** allowing air to pass through vent **224**. As air passes through vent **224** additional air is drawn into the reaction chamber through air inlet **203** by fan blade **204**. Fan blade **204** is driven by motor **206**. The venting of hot air and replacement with cooler ambient air, combined with the rapid circulation of air by fan **208**, a relatively small thermal cycling chamber **210** and precise measurement of sample temperatures by thermocouple **216** enables rapid temperature ramping. The time required for effecting the thermal cycles is greatly reduced. A typical thermal cycling reaction requires different temperatures for denaturing of nucleic acid strands, annealing of a primer, and extension of a polymerase. The denaturing and annealing steps occur rapidly in a capillary tube where the small internal volume of liquid will rapidly come to equilibrium, while the extension of the DNA molecule takes less than 10 seconds for a 500 base extension. The time required for each thermal cycle of the three temperatures (annealing, extension, denaturing) may be reduced to under 15 seconds by using the rapid heat transfer of the air based thermal cycling apparatus. A program of 30 cycles, each cycle exposing the capillary to three temperatures for varying amounts of time theoretically may be effected in under 8 minutes.

[0124] The use of the capillary cassette in combination with an air based thermal cycler allows additional advantages. The capillary cassette holder temporarily seals the capillary, allowing rapid and simplified sealing of each capillary tube segment. The capillary cassette contains a number of capillary tubes in parallel arrangement, allowing for more efficient use of the thermal cycler and allowing greater sample throughput. Once the thermal cycle is completed the capillary cassette **15** contained with in holder **8** is removed through opening **215**. The capillary cassette **15** is released from the holder and is subsequently dispensed.

[0125] The thermal cyclers of **FIGS. 6 and 7**A-C were illustrated as being used with capillary cassettes. The same devices are adaptable to other containers with opposing ends. For example, a chip-like substrate with a plurality of passageways extending through the chip (as seen in **FIG. 3E**) has, like a capillary cassette, evenly spaced opposed open ends. Several chips could be placed into a thermal cycler with the open ends temporarily sealed and exposed to thermal cycles. The rapid temperature changes may be a bit slower due to increased material thickness. Other containers with opposing open ends may also be used with either temperature cycling device.

[0126] C. Dispensing Completed Reaction Mixture

[0127] Following the completion of the thermal cycling reaction, the prepared reaction mixture is dispensed into a substrate for analysis by an analytical system. As noted above, the capillary cassette may be dispensed by air displacement, centrifugal force, vacuum or any other displacement method. The substrate into which the reaction mixture is displaced may be the wells of a multiwell plate, locations on a planar substrate, or wells which lead into an analytical chip. The reaction mixture, though small, still may produce enough amplified reaction product that dilution is necessary.

[0128] Dispensing Completed Reaction Mixture Example 1:

[0129] Direct Dilution

[0130] In reference to **FIG. 1**, following completion of the temperature cycling process, the capillary cassette may be removed from air thermal cycler **116** by transfer head **104**. The capillary cassette may be moved by transfer head **104** to be placed in a plate dispensed from finished sample hotel **112**. The plate, located at position c, may be a multiwell plate such as a 384 well microplate. The wells of the plate contain a dilution liquid, such as formamide, water, TBE or other selected buffer. The reaction mixture may be dispensed from the capillary tube segments of the capillary cassette by positive displacement, centrifugation, or other dispensing means. The reaction may also be dispensed into a solution for further chemical or biochemical reaction.

[0131] Dispensing Completed Reaction Mixture Example 2:

[0132] Ethanol Precipitation

[0133] Ethanol precipitation may be effected in a dispensing means similar to the means of direct dilution. Transfer head **104** of **FIG. 1** would again take the capillary cassette from air thermal cycler **116** and place the short ends of the capillaries in a multiwell plate located at position c. In this case the wells of the plate would contain an ethanol, such as 90% ethanol chilled to 4EC. The reaction mixture would be dispensed from the capillary cassette into the ethanol by centrifuge, positive displacement, or other dispensing method. The ethanol could then be removed by aspiration or other means. The precipitated DNA could then be resuspended in formamide, water or other suitable diluent. Once the sample plate is prepared, by either direct dilution or ethanol precipitation, the plate may be transferred by transfer head **104** to analytical stage **120**. Analytical stage **120** may feed the sample plate directly into an analytical device, for example a capillary array electrophoresis system, such as MegaBACE™ produced by Molecular Dynamics, Sunnyvale Calif. Alternatively, the analytical stage could direct the product to other systems for further processing. It is also possible to dispense the samples onto a substrate for mass spectrometry analysis, calorimetric analysis, or other analytical methods.

[0134] Dispensing Completed Reaction Mixture Example 3: Dispense Directly into Analytical System

[0135] In the previous two examples the samples were dispensed into multiwell plates. These plates could then be moved manually or robotically onto a stage for analysis by an analytical system. Alternatively the capillary cassette could be dispensed directly into the wells of an analytical device, such as an electrophoresis chip. For example a capillary cassette having 16 capillaries disposed in the substrate in two parallel rows of eight capillaries may dock with 16 wells in an analytical microchip. Such a microchip would have an array of analytical lanes in fluid communication with a sample port.

[0136] The capillary cassette may be designed such that the spacing of the capillaries matches the spacing of the sample reservoir inlets. For example, the capillary cassette illustrated in **FIG. 3C** includes capillaries **12** extending through flexible strip **11**. Flexible strip **11** may be used alone or in combination with other such strips. The orientation of the capillaries in an essentially straight line may be altered by bending strip **11** to form an arc. **FIG. 3D** illustrates strip **11** but allowing capillaries **12** to mate with input ports which is disposed on a substrate in a circular pattern. The liquid in capillaries **12** may then be electrokinetically injected or otherwise dispensed from capillaries **12** into ports of an analytical chip if an appropriate electrode array is used. Strip **13** may be positioned in the curved orientation by pressing strip **13** against a curved form, such as a curved metal block. This may be done by an automated strip mover incorporated into an automated sample preparation system.

[0137] The capillary cassette could be dispensed by air displacement or other dispensing means preferably selected to minimize splattering and bubble formation. Prior to dispensing the prepared reaction mixture into the wells for analysis, a small amount of a dilutant could be added to each analytical microchip well. When the capillary cassette is dispensed, the diluent will dilute the samples in the sample wells. The sub-microliter volume reaction mixtures prepared in the capillary cassette, such as a DNA sequencing reaction product mixture, can readily be integrated with the analytical microchip for sequencing.

[0138] D. Washing Capillary Cassette

[0139] Following each use of a capillary cassette, the capillary cassette may be washed and reused. After the contents of the capillary cassette have been dispensed or a capillary cassette has otherwise been used, the capillary cassette is taken to cassette washer **118** where the cassette is washed. Following washing, the cassette is returned to the cassette hotel **106** where the cassette may be reused.

[0140] With reference to **FIG.** 8A, capillary cassette washer **410** is comprised of wash manifold **412** and wash tank stage **416**. Between wash manifold **412** and wash tank stage **416** is capillary cassette platform **414**. Extending from wash tank stage **416** is leg **419**. In this wash system, a wash liquid is pumped from one or more of containers **452**, **454**, **456**, **458** through respective tubes **1**, **2**, **3**, **4** into respective router inputs **453**, **455**, **457**, **459**. The router directs the selected wash fluid through router outflow **451** through line **451***a* into the wash tank **440**. The fluid is drawn from wash tank **440** through capillary tube segments of a capillary cassette. The capillary cassette substrate is held between wash manifold **412** and wash tank **440** such that if suction is applied to wash manifold **412**, wash fluid will be drawn through capillary tube segments from wash tank **440**. The wash solution is drawn by vacuum through wash manifold **412** and into waste receptacle **490**.

[0141] **FIG. 8E** provides a schematic of the working of the wash station. Nitrogen tank **460** provides a pressure source to direct fluid flow. Opening manual valve **462** allows gas to flow through regulator **466** and through filter **468**. Regulator **466** regulates the pressure from the pressure source. Pressure sensor **464** monitors gas pressure from the nitrogen source, and indicates if gas pressure is below a selected pressure. The pressurized gas flows through filter **468** into line **470**. Pressurized gas line **470** branches into the top of sealed wash bottles **471**, **472**, **473**, and **474**. The pressurized nitrogen pumps the wash liquid within each wash bottle into respective fluid lines **471***a*, **472***a*, **473***a* and **474***a* respectively through an intake filter **476** on each of said respective fluid lines. Each of the sealed wash solution bottles may contain a different wash solution, such as water, alcohol, a buffer or other wash solution. Although four wash bottles are illustrated, the system is adaptable for use with more or fewer wash fluids. In addition, exchange of wash bottles simply requires venting N2 pressure on bottles **471**, **472**, **473**, **474** at value **462**, the removal of the cap from the selected bottle and replacement of the cap with attached pressure and fluid lines into a new or refilled wash fluid bottle. Each of the fluid lines **471***a*, **472***a*, **473***a* and **474***a* terminate in selector valve **478**. According to a preset program, the selector valve routes one of the selected fluids from the input line into valve output line **480**. The valve output line then transports the pressurized liquid into wash tank **440**.

[0142] The capillary tubes in the capillary cassette function as a conduit for transport of fluid from the wash tank **440** into the wash manifold interior **425**. Vacuum source **496** provides a vacuum force once valve **492** is open. When vacuum valve **498** is open, a vacuum force is directed into waste bottle **490** creating negative pressure within line **490***a*. When valve **495** is open, suction will be applied through suction line **490***a*, suction line **495***a* and suction lines **424***a*. As suction is applied through suction ports **424** by suction lines **424***a* the negative pressure through interior wash manifold **425** will draw liquid up through the capillary tube segments extending into wash manifold interior **425**. The liquid will travel through suction passageways **424**, into suction lines **424***a*, past valve **495**, through suction lines **495***a* and **490***a* and into waste bottle **490**.

[0143] **FIG. 8D** illustrates a view of the wash manifold. The bottom of the wash manifold contains holes **426** into which the capillaries are inserted. Wash manifold interior **425** is comprised of lanes joined at a first end to suction passageways **424** and at a second end to purge passageways **423**. When suction is applied through line **424***a* fluid will be drawn through capillaries into the lanes comprising interior **425**, through passageways **424** and into line **424***a*. When the purge valve is opened, air will pass through line **423***a*, through passageway **423**, into interior **425**, and into passageway **424**, clearing interior **425** of any liquid remaining in interior **425**.

[0144] Following a wash procedure, wash tank **440** is lowered relative to the capillary cassette platform such that the ends of the capillary tube segments are not in contact with the liquid in wash tank **440**. The liquid within wash tank **440** is drained through drain **484** which transmits the fluid into drain line **484***a* when value **485** is opened and suction is applied through suction line **490***a*. The fluid within wash tank **440** will then drain into waste bottle **490**.

[0145] Before each wash solution is introduced into wash tank **440**, wash fluid supply line **480** and the wash tank distribution manifold **480***a* are purged to empty the line of any previous liquid. This is effected by opening one of the valves in selector valve **478** and flowing wash fluid through supply line **480** and through bleed lines **482**. Opening valve **487** allows a vacuum force to be transmitted through line **490***a* through line **488** providing suction which in conjunction with fluid pressure is used to purge the distribution manifold through bleed lines **482**. Once wash fluid supply line **480** and distribution manifold are purged, valve **487** is closed and the wash tank is raised and filled. The fill level of wash tank **440** is controlled by a selected wash fluid fill time and wash fluid pressure. Overflow port **486** acts as a safety drain to drain off fluid overfill. If the fluid level within wash tank **440** is too high, liquid will flow from wash tank **440** into overflow port **486** and into line **486***a*. When valve **487** is open, the suction force from line **490***a* and **488** will draw overflow liquid from overflow port **486** into waste bottle **490**. Restriction flow valve **441** limits liquid fluid flow through lines **482**.

[0146] **FIG. 8F** shows the top perspective of wash fluid tank **440**. An input line introduces a wash solution into wash fluid distribution manifold **480***a*. This manifold supplies wash fluid ports **481** which fill tank **440**. The spacing of wash fluid ports **481** aids in uniform filling across the width of tank **440**. The fill time and fluid pressure regulate the amount of fluid filling tank **440**. If excess fluid enters tank **440** it will drain from overflow port **486**.

[0147] To empty the tank, the tank is lowered by the pneumatics as described, and drain **484** is opened. The shape of tank **440** directs fluid to drain **484** when the end of tank **440** containing drain **484** is lowered. This configuration is designed for efficient filling, emptying and purging of tank **440** and associated fill lines.

[0148] Again with reference to **FIG. 8**E, once a wash cycle has been completed, any liquid remaining within wash manifold interior **425** may be eliminated by opening valve **491** while suction is applied through the manifold. Opening valve **491** causes a pulse of air to be drawn in through vent **493**. The air is introduced into wash manifold interior **425** through purge lines **423***a* and is removed by suction lines **424***a*. If the manifold is in contact with a capillaries, the relatively narrow bores of the capillary cassette provide a limited capacity for drawing air through the wash manifold. By opening valve **491**, a much greater amount of air may be drawn through the manifold through purge lines **423***a* which have a much greater capacity for drawing air. This will result in a sudden rush of air drawn through the manifold. This acts to clear the wash manifold of any liquid remaining within the wash manifold interior **425**. Preferably manifold interior **425** is purged before and after raising the wash manifold.

[0149] With reference to **FIG. 8**B, the wash station **410** is shown in side view. The capillary cassette platform **414** is mounted on support legs **445**. The reservoir section, shown in internal cross section has at a back lower end of the reservoir, drain outlet **484**. Upwardly positioned from the drain outlet at the back wall of the tank is overflow outlet **486**. Disposed at the front of the reservoir is reservoir bleed outlet **446**. Each outlet is associated with a respective tube and valve, as described in conjunction with **FIG. 8E**. Each tube carries liquid flowing from an associated outlet when the associated valve is opened and vacuum source applied.

[0150] Capillary cassette platform **414** is held in a fixed position by support legs **445**. Extending downward from the front of capillary cassette platform **414** is hinge **418** with pivot **432**. Attached to a lower end of hinge **418** is wash tank stage **416**. Extending from below wash tank stage **416** is leg **419** which is attached at a lower end by pivot **443** to pneumatic cylinder **429**. At the back end of the stationary capillary cassette platform **414**, the wash manifold is attached at pivot **420**. When pneumatic cylinder **429** is extended from the lower end, wash tank stage **416** will be lowered in an arc away from stationary capillary platform. This occurs when no pressure is applied to **429** and gravity causes the wash tank stage to pivot down. When pneumatic cylinder **429** is extended from the upper end by applied pressure, wash manifold **412** will be raised in an arc away from capillary cassette platform **414**.

[0151] Disposed above capillary cassette platform **414** is wash manifold **412**. The wash manifold has a purge passageway **423** disposed at a front end and a suction passageway **424** disposed toward the back end. The respective lines carrying air to the manifold or removing gas or liquids from the manifold are described in conjunction with **FIG. 8E**.

[0152] With reference to **FIG. 8**C, pneumatic cylinder **429** is shown fully extended from a lower connection pivot **443**

on leg **419**, through hole **333** in capillary cassette platform **414**, to an upper connection at pivot **428** on wash manifold **412**. The extended height of the wash manifold is limited by plate **430** which is secured to the top of manifold **412**. Plate **430** abuts pin **422** on capillary cassette platform **414** when the wash manifold is raised to a selected level and prevents the wash manifold **412** from being raised beyond this level. When suction is applied to wash manifold interior **425** by applying suction through suction passageway **424**, fluid is drawn through capillaries **12** from tank **440**.

[**0153**] The front end of capillary cassette platform **414** is joined at pivot **432** to hinge **418** and wash tank stage **416** and the back end of capillary cassette platform **414** is joined at pivot **420** to wash manifold **412**. Extending through capillary cassette platform **414** is cutout **434**. The dimensions of cutout **434** are such that capillary cassette **15**, when placed on capillary cassette platform **414** has associated capillary tube segments **12** extending through capillary cassette platform **414** while the four edges of capillary cassette substrate **10** are retained on the capillary cassette platform **414** on the edge of cutout **434**. Alignment pins may be added to capillary cassette platform **414** to properly position the capillary cassette.

[**0154**] To effect the cassette wash sequence, an electronic controller implements a sequence of steps. The electronic controller instructs associated controlled devices of the wash station to carry out a programmed wash sequence. The programmed sequence begins with the capillary cassette being placed on the capillary cassette stage by the robotic transfer device. The wash manifold lowers onto the capillary cassette such that the shorter end of capillary tube segments extend into the wash manifold and the opposite end of the capillary tube segments are within the wash liquid in the wash tank once filled. The substrate provides a partial seal between the wash manifold and cassette such that when suction is applied to the capillary tube segments by the wash manifold, fluid will be drawn up into the wash manifold through the capillary tube segments. The wash solution supply line is purged with the first selected solution to clear the previous solution from the line. As noted in relation to **FIG. 8**E, the purge solution is removed through distribution manifold to drain **484** and bleed lines **482** to wash waste line **488** and **490***a* then into waste bottle **490**. The wash tank **440** is then raised and filled with the selected wash solution.

[**0155**] A vacuum is applied to the wash manifold causing the solution in the wash tank to be drawn up through all of the capillary tube segments in the capillary cassette. After the programmed wash duration, the wash tank is drained and lowered. The vacuum force is continued through the wash manifold, drawing air through the capillary tube segments. Once the capillary tube segments are dried, the vacuum line of the wash manifold is turned off. The wash solution supply line is purged with the next wash solution and the steps of raising and filling the wash tank, drawing the wash solution through the capillary tube segments and emptying the wash tank are repeated for each selected solution. The specified sequence may repeat these steps for any number of wash solutions. After the final wash has been completed and the tank emptied, air is drawn through the capillaries by applying a vacuum to the wash manifold, drying the capillary tube segments. Periodically the purge valve **491** is opened and air is drawn through vent **493** into purge lines **423***a* into purge inlets **423**. This draws a blast of air through wash manifold interior **425** and clears the wash manifold interior of any remaining liquid, ensuring that any remaining liquid within the wash manifold will not wick back into the capillaries. The manifold vacuum is then shut off and the manifold is raised, removing the manifold from the capillary cassette. The manifold vacuum is again applied and the purge valve **491** is opened and air is drawn through vent **493** into purge line **423***a* into purge inlet **423**. This ensures that any remaining liquid is removed from the wash manifold interior. The vacuum is then shut off. The washed and dried capillary cassette may then be moved by the transfer robot to a capillary cassette hotel or other location.

[**0156**] System Integration

[**0157**] The components of the system could be integrated in a combined system which allows several elements of the complete system of **FIG. 1** to operate at the same time. For example, electronic control device **123** may be used to send instructions to the components of the integrated system. The electronic control device may be a computer which sends electronic signals to various system components to effect a programmed set of instructions. Elements of the system could operate simultaneously, increasing system efficiency. For example automated robot **102** could retrieve a capillary cassette from cassette hotel **106**, place the capillary cassette in a sample plate at stage a. An amount of sample from the plate is drawn into the capillary tubes by capillary action. The capillary cassette could then be moved to be placed on top of a nanotiter plate such that the short ends of the capillary tube segments are in the wells of the nanotiter plate. The robot **102** could then transfer the combined nanotiter plate/capillary cassette to dispense location **122** for dispensing. The movement of the robot **102**, transfer head **104** and dispensing device located at location **122** are controlled by electronic control device **123**.

[**0158**] At the same time that a reaction mixture is being assembled, the electronic control device could also be sending electronic signals to thermocycler **116**. The vent door, heating element, and thermocouple of thermocycler **116** could be linked to electronic control device **123**, allowing electronic control device **123** to effect a selected temperature cycling procedure by regulating the temperature at which air is cycling within the thermal cycler. This precise monitoring allows the temperature cycling procedure to be effected in a minimum amount of time. Once the thermal cycling procedure is complete, the electronic control device **123** could electronically instruct the thermal cycler to shut off the thermocycler fan and heating element and open the lid pneumatically to allow a capillary cassette to be removed from the interior of the thermal cycler.

[**0159**] While automated robot **102** is moving capillary cassettes to assemble a reaction mixture and the thermocycler is operating, the cassette washer **118** could also be cleaning a capillary cassette. Again the electronic control device **123** could instruct the cassette washer **118** to perform a wash sequence in which a capillary cassette is cleaned with a selected sequence of wash liquids and air dried.

[**0160**] Electronic control device **123** enables each element of the system to be used with maximum efficiency. A single set of instructions to electronic control device **123** could allow assembly of the reaction mixture, thermal cycling of the reaction mixture to effect the desired reaction, dispensing of the completed reaction mixture onto an analytical substrate, movement of the analytical substrate to a stage for processing by an analytical instrument, and cleaning of used capillary cassettes.

[0161] E. Reaction Preparation Examples

[0162] The following examples illustrate the use of the combined reaction preparation systems. The examples are representative of the many different types of reactions that could be effected with the disclosed device or system and are described by 1) dye-primer DNA sequencing, 2) dye-terminator DNA sequencing, 3) PCR amplification, 4) PCR amplification, enzymatic purification, and DNA sequencing, and 5) a general enzymatic reaction.

EXAMPLE 1

Dye-primer DNA Sequencing Analyzed by CAE

[0163] Dye-primer sequencing reactions were performed within a capillary cassette comprised of 96 uncoated 2.8 cm long, 150 μm I.D., 360 O.D. fused-silica capillaries. Dye-primer sequencing reactions were performed by amplifying template DNA with emission-specific primers corresponding to ddT, ddA, ddC, and ddG terminated reactions. The amplification of template was performed as single reactions in each capillary and pooled into a common well for post-reaction processing and analysis. The color-specific primers were based on the M13 -40 FWD primer (5'-FAM-GTTTTCCCAGT*CACGACG-3'), with 5-carboxyfluorescein (FAM) as the donor dye, and a termination-specific fluor attached to the indicated thymine (T*) as the acceptor dye. The thymine was labeled with FAM for ddC-terminated reactions (C-FAM), 6-carboxyrhodamine for ddA reactions (A-REG), N,N,N',N'-tetramethyl-5-carboxyrhodamine for ddG reactions (G-TMR), and 5-carboxy-Xrhodamine for ddT reactions (T-ROX). A master mix for 100 dye-primer sequencing reactions was prepared by combining 65 :L reaction buffer (220 mM Tris-HCl, pH 9.5, 33.2 mM $MgCl_2$), 100 :L dye-primer solution (either 1 μM T-ROX, 1 μM G-TMR, 0.5 μM A-REG, or 0.5 μM C-FAM), 100 ΦL of the corresponding deoxy- and dideoxynucleotide mix (0.94 mM DATP, dCTP, dTTP, 7-deaza-dGTP, with 3.1 uM dideoxynucleotide), 10 ΦL of enzyme (32 U/ΦL ThermoSequenase), and 225 ΦL filtered deionized water. This solution was aliquoted into a 96-well reagent plate prior to mixing with template DNA. The general mixing scheme required the use of two capillary cassettes and a 384-well "mix plate". The first capillary cassette (transfer cassette) was dipped in a solution of template DNA (20 ng/:L M13mp18), and then inverted onto the top of a 384-well "mix plate" with the short ends of the capillaries inserted into the wells. The inverted transfer cassette and mix plate were placed inside a benchtop centrifuge. A balance plate was added to balance the rotor and the centrifuge brought to 3,000×g for 5 seconds. The centrifugation uniformly dispensed the contents of the transfer cassette into individual wells of the 384-well plate. After the centrifuge step, the transfer cassette was transferred to the capillary cassette washer **410** for cleaning, and the mix plate was used for a subsequent centrifuge step for reagent addition.

[0164] To add reagents, a second capillary cassette, (the reaction cassette), was dipped into the wells containing sequencing reagents (prepared as described in the preceding paragraph) and inverted over the wells of the same 384-well plate. The reaction cassette and mix plate were placed in the centrifuge, spun at 3,000×g for 5 seconds, and removed from the centrifuge. At this point each well contained 500 nL of template DNA and 500 nL of sequencing reagents to form the final reaction mixture. The second capillary cassette (used to add reagents) was then dipped into the 1 :L mixture contained in the mix plate, filling the capillaries of the reaction cassette in 500 nL.

[0165] The capillary cassette was inserted into the internal chamber of an air-based thermal cycler, as described herein, where the ends of the capillary segments are sealed by depressing the ends of the capillaries against deformable membrane. After 30 cycles of 95EC for 2 seconds, 55EC for 2 seconds, and 72EC for 60 seconds, the thermal cycler was opened, removing the ends of the capillaries from contact with the deformable membranes. The capillary cassette was removed and placed on top of a 384-well "mix plate" with the short ends of the capillaries inserted into the wells. The capillary cassette and mix plate were placed into a centrifuge, with a balance plate. The reaction products were dispensed by centrifugal force (~2500 g) into a microtiter plate containing 40 μL of 80% isopropyl alcohol. After an initial reaction, the capillaries were washed as described herein. After the four dye-primer reactions had been performed in four individual capillary cassettes and the products pooled into the wells of a microtiter plate, the samples were subsequently centrifuged at 3000×g for 30 minutes. The alcohol was decanted by a gentle inverted spin, and the samples were resuspended in 5 μL of ddH20 for electrokinetic injection and analysis by capillary array electrophoresis.

[0166] Analysis of the DNA sequencing fragments was performed with MegaBACE, a 96-capillary array electrophoresis instrument (Molecular Dynamics, Sunnyvale, Calif.) using scanning confocal laser-induced fluorescence detection. Separations were performed in 62 cm long, 75 μm I.D., 200 μm O.D. fused-silica capillaries with a working separation distance of 40 cm. Electroosmotic flow was reduced by Grignard coupling of a vinyl group to the capillary surface and acrylamide polymerization. The capillaries were filled with a fresh solution of 3% linear polyacrylamide (LPA)(MegaBACE Long Read Matrix, Amersham Life Sciences, Piscataway, N.J.) which was pumped through the capillaries under high-pressure from the anode chamber to individual wells of a 96-well buffer plate contained in the cathode chamber. Each well was filled with 100 :L of Tris-TAPS running buffer (30 mM Tris, 100 mM TAPS, 1 mM EDTA, pH 8.0). The matrix was equilibrated for 20 minutes followed by pre-electrophoresis for 5 minutes at 180 V/cm. Prior to sample injection, the cathode capillary ends and electrodes were rinsed with ddH_2O to remove residual LPA prior to sample injection.

[0167] DNA sequencing samples were electrokinetically injected at constant voltage from a 96-well microtiter plate according to the specified conditions; one preferred injection condition for 500 nL samples is 40 seconds of injection at an applied voltage of 2 kV. After injection, the capillary ends were rinsed with water, the buffer plate was placed in the cathode chamber, and the electrophoresis run was commenced. Separations were typically for 120 minutes at 8 kV. Computer controlled automation of the instrument and data collection was performed using LabBench software

(Molecular Dynamics, Sunnyvale, Calif.). Specific injection and run conditions were tailored to the reaction mixture to be analyzed.

[0168] The reproducibility of the described method for sub-microliter dye-primer cycle sequencing is shown in **FIG. 9**. This histogram shows the percent success versus readlength window and shows that the method is highly reproducible. Over 80 percent of the sequenced DNA inserts had a readlength over 600 bases. Overall, this plate yielded 55,000 high quality bases, with an average readlength of 605 bases.

EXAMPLE 2

Dye-primer DNA Sequencing Analyzed by a CAE Microchip

[0169] In another analysis example, dye-primer reactions performed in the same capillary cassette were analyzed by direct injection into a microfabricated "chip-based" analyzer. In this example, a dye-primer reaction terminated by ddT was performed as described and dispensed into the sample wells of a microchip containing 1.5÷μL of ddH_2O. The electropherogram is featured in **FIG. 10** exemplifying microchip analysis of reactions performed in the described system.

EXAMPLE 3

Dye-terminator Cycle-sequencing with Alcohol Precipitation Purification

[0170] Dye-terminator cycle-sequencing was demonstrated using the capillary cassette system and alcohol precipitation for cleanup prior to capillary array electrophoresis. In this example, the sequencing reaction mix was prepared by mixing 400 μL of sequencing reagents (Dyenamic ET terminator kit, Amersham Pharmacia Biotech, Part 81600) with 100 mL of 5 pmol/μL of M13 -28 FWD primer (5'-TGT AAA ACG ACG GCC AGT-3'). The reaction mix was distributed in 5 μL aliquots to a 96-well "reagent" plate. Mixing of template DNA and sequencing reagents was performed in the same series of steps described in Example 1. A second cassette was used to transfer 500 nL of sequencing reagents from the reagent plate to the wells of the mix plate. This same cassette was then filled by capillary action with the template/reagent mixture.

[0171] The capillary cassette was transferred to the air-based thermal-cycler where the capillaries were sealed between the deformable membranes within the thermal cycler. Thermal cycling was achieved with 30 cycles of 95° C. for 2 s, 55° C. for 2 s, and 60° C. for 60 seconds. After the thermal cycling, the cassette was removed from the cycling chamber and the contents of the capillaries dispensed by centrifugal force (3000×g) into a 96-well plate containing 40 μL of 80% ethanol. The samples were centrifuged at 3000×g for 30 minutes. The alcohol was decanted by a gentle inverted spin, and the samples were resuspended in 5 μL of ddH20 for electrokinetic injection and analysis by capillary array electrophoresis. The cleanup of dye-terminator reactions by alcohol precipitation, the reproducibility of the technique, and the application to "real-world" templates is represented as a histogram of percent success versus readlength in **FIG. 11**. **FIG. 11** demonstrates excellent readlengths and success rates with M13 subclone inserts prepared from the subclone library of a Mouse bacterial artificial chromosome (BAC).

EXAMPLE 4

Dye-terminator Cycle Sequencing with Size-exclusion Purification

[0172] In another example, dye-terminator reactions were performed in 500 nL capillaries as described in Example 3, and the reaction products dispensed into 15 μL of ddH2O by centrifugal force. The 15 mL samples were transferred to a filter plate containing 45 mL of hydrated Sephadex G-50. The samples were centrifuged through the Sephadex matrix at 910×g for 5 minutes and the eluent collected in a clean 96-well injection plate. The samples were electrokinetically injected without further dehydration or processing. For 16 samples, an average readlength of 650 bases was obtained demonstrating the compatibility of sub-microliter dye-terminator sequencing with alcohol and size-exclusion purification.

EXAMPLE 5

PCR Amplification of Plasmid Insert DNA

[0173] The present technology uses the disclosed system for the polymerase chain reaction (PCR) amplification of insert DNA (e.g. subclone inserts from a DNA library). The PCR reaction mixture was prepared by mixing 5 :L of 10 :M of M13 -40 FWD primer (5' GTT TTC CCA GTC ACG AC 3') and 5 :L of 10 NM M13 -40 REV primer (5' GGA TAA CAA TTT CAC ACA GG 3') with 25 :L of 10× GeneAmp buffer, 15 :L of 25 mM $MgCl_2$, 5 :L of AmpliTaq Gold, 2.5 :L of 1 mg/mL bovine serum albumin (BSA), and 67.5 :L of ddH_2O. This mix was aliquoted in equal volumes to sixteen 0.20 mL tubes.

[0174] The reaction was initiated by mixing template DNA with the PCR cocktail using the two-capillary cassette and mix-plate method described. The transfer cassette was dipped into the glycerol stock solutions of a subclone library and dispensed by centrifugal force into the wells of a 384-well plate. A second "reaction" cassette was used to transfer 500 nL of PCR cocktail to the same wells by centrifugal force. The capillaries were subsequently dipped into the combined mixture of template DNA and PCR reagents, filling the capillaries by capillary action. Amplification was effected by placing the capillaries into the cycling chamber and thermally cycling with an activation step of 95° C. for 12 minutes followed by 30 cycles of 64° C. for 4.5 minutes and 95° C. for 5 seconds.

[0175] The PCR products were analyzed by agarose gel electrophoresis and compared with the same subclones amplified by large-volume (25 :L) reactions performed in 0.20 mL tubes. Nanoscale capillary cassette samples were dispensed into 4.5 :L of ddH2O by centrifugal force. Equivalent volume aliquots of full volume reactions were transferred manually using a low volume pipettor. To each 5 :L sample, 1 :L of 6× loading dye was added and the sample quantitatively transferred to the wells of an agarose gel. Agarose gel electrophoresis was performed using a 0.7% agarose gel with 1×Tris-acetate-EDTA buffer, pH 8.0. Samples were separated for **40** minutes at 15 V/cm, stained with Sybr Green II (Molecular Probes, Eugene, Oreg.), and imaged using a two-dimensional fluorescence scanner

(FluorImager, Molecular Dynamics, Sunnyvale, Calif.). The scanned gel image is shown in **FIGS. 12A and 12B**. It can be seen that samples prepared at full-volume **(FIG. 12A)** and 500 nL amplification **(FIG. 12B)** have the same molecular weight distribution. This example demonstrates nanoscale sample preparation can be analyzed by traditional macro-scale analysis such as agarose gel electrophoresis.

EXAMPLE 6

PCR Amplification and Cycle-sequencing

[0176] A preferred mode of preparing cycle sequencing samples using the present invention is to prepare nanoscale PCR samples in the capillary cassette and related instrumentation, perform macroscale ExoI/SAP reactions, and then perform the cycle sequencing in the capillary cassette and related instrumentation. Nanoscale PCR template preparation for DNA sequencing was demonstrated by performing PCR amplification from glycerol stock subclones. Glycerol stock subclones were PCR amplified as described in Example 5. After PCR amplification, the contents of the capillaries were dispensed by centrifugation into the wells of a 96-well plate containing 4.5 :L of 7.5 mU of shrimp alkaline phosphatase (SAP) and 37.5 mU of exonuclease I (ExoI). The PCR products and ExoI/SAP solution were allowed to incubate at 37° C. for 5 minutes to digest the unincorporated primers and to dephosphorylate the unincorporated nucleotides. After an initial incubation, the enzymes were deactivated by heating the solution to 72° C. for 15 minutes.

[0177] The ExoI/SAP treated PCR products were aliquoted to a fresh 384-well mix plate with a transfer capillary cassette and centrifugal dispensing. An equal aliquot of dye-terminator sequencing reagents were added to the 500 nL of purified PCR products using another capillary cassette and centrifugal dispensing. The capillaries were then filled by dipping the capillary cassette into the 1 :L reaction mixture. The template was amplified according to Example 3, dispensed into 40 :L of 80% ethanol and purified as described. Analysis of the sequencing reactions was performed by MegaBACE using electrokinetic injection. Portions of six base-called sequencing electropherograms from subclone templates prepared by nanoscale PCR amplification from glycerol stock solutions and by nanoscale cycle sequencing are shown in **FIG. 13**. By performing PCR in a capillary cassette and subsequently transferring the reaction mixture to a microplate, the present system allows a simplified transition from nanoscale (less than 1 μL volumes) to greater than nanoscale reaction volumes. The present system also allows a simplified transition from macroscale (more than 1 μL volumes) to nanoscale reaction volumes, as shown by utilizing the Exo I/SAP reactions for cycle sequencing in the capillary cassette.

[0178] E. Reaction Preparation Examples

EXAMPLE 7

[0179] Isothermal enzyme assay performed in sub-microliter capillary cassette. The use of the described system for performing general enzyme reactions was demonstrated with a fluoregenic assay of #-galactosidase. The #-galactosidase (#-Gal) catalyzed hydrolysis of resorufin-#-D-#-galactosidase (#-Gal) catalyzed hydrolysis of resorufin-#-D-galactosidase (RBG) was performed within the capillaries of a 96-capillary cassette in which #-Gal hydrolyzes RBG to the fluorophore resorufin.

[0180] A stock solution of 350 micromolar RBG was prepared in 5 mL of 100 mM Tris-HCL, 20 mM KCl, and 2 mM MgCl2 to 5 mg of RBG, vortexing vigorously, and filtering the solution through a 0.40 micron filter. A one-half dilution curve of RBG was prepared from the stock solution. To each 10 microliters of RBG solution prepared in 0.20 mL tubes, 200 ug of #-Gal was added and after briefly mixing, filled into a capillary cassette by capillary action. The cassette was placed in an air-cycler and after 2 minutes at 37 degrees C., the capillary cassette was removed and the contents centrifuged out of the capillaries into a 384-well scan plate containing 5 microliters of 1M sodium bicarbonate. The wells of the scan plate were subsequently filled with 50 microliters of ddH20 and the plate was read by a fluorescent plate reader (Typhoon, Molecular Dynamics). A control aliquot from the enzyme reactions performed in the 0.20 mL tubes was added to the scan plate.

[0181] Solid-phase capture of the #-Gal was also demonstrated with this system by simply filling the cassette with a 20 ug/mL solution of #-Gal, allowing the #-Gal to bind to the capillary surface followed by removing the excess liquid and drying the cassette using the described cassette wash-manifold. After #-Gal binding the capillaries were filled with RBG solution by capillary action. The reaction was performed for 2 minutes at 37° C. and analyzed by dispensing into 1M sodium bicarbonate, diluting the water and scanning using a fluorescent plate reader. The results are summarized in Figure XYZ showing the expected signal versus substrate concentration for the tube reactions, and data points of signal for the pre-mixed enzyme reaction performed in the capillary cassette, and for the capillary-binding #-Gal assay. This example serves to illustrate the compatibility of the described system for performing a range of general enzyme activity and inhibition assays.

What is claimed is:

1. A device for dispensing small quantities of liquid, the device comprising:

a capillary cassette, comprised of a plurality of capillary tube sections disposed in an array through a substrate; and

a capillary cassette dispensing means, said dispensing means receiving said capillary cassette and dispensing an array of capillaries of said capillary cassette to an array of locations.

2. The device of claim 1, wherein said dispensing means is a pressure driven dispenser.

3. The device of claim 2, wherein said pressure driven dispenser includes an enclosed area, wherein one end of said capillary tube sections in said capillary cassette may be sealed in said enclosed area, and wherein the enclosed area enclosing one end of capillaries in said capillary cassette may be pressurized.

4. The device of claim 2, wherein said pressure driven dispenser includes a centrifuge.

5. The device of claim 1, wherein said capillary cassette is an array of 96 capillaries.

6. The device of claim 1, further including a wash station and a means for transferring said capillary cassette between said dispensing means and said wash station.

7. The device of claim 6, wherein said means for transferring said capillary cassette is an automated transfer device.

8. The device of claim 7, wherein said automated transfer device is a robotic arm.

9. The device of claim 6, wherein said wash station includes a housing for receiving a capillary cassette and a liquid introduction and removal system that pumps liquid through said capillary tube sections and evacuates said liquid from said capillary tube sections.

10. The device of claim 1, wherein each capillary tube section of said capillary cassette has an interior volume of less than one microliter.

11. A device for transfer of an amount of liquid from a plurality of locations in a highly parallel manner, the device comprising:

a capillary cassette comprised of a substrate and a two dimensional array of capillary tube sections extending through said substrate, each of said capillary tube sections having a first and a second opposing open ends, said first open ends being coplanar and said second open ends being coplanar;

a capillary cassette dispenser; and

an automated capillary cassette transfer device, wherein said capillary cassette transfer device may move said capillary cassette between said a filling location and said capillary cassette dispenser.

12. The system of claim 11, wherein said substrate is bendable.

13. The system of claim 11, wherein said substrate is curved.

14. The device of claim 11, wherein said capillary cassette dispenser employs a pressure differential to dispense said capillary tube sections in said capillary cassette.

15. The device of claim 12, wherein said dispenser is a centrifuge dispenser.

16. The device of claim 12, wherein said dispenser includes an enclosed area, wherein one end of said capillary tube sections in said capillary cassette may be sealed in said enclosed area, and wherein the enclosed area enclosing one end of capillaries in said capillary cassette may be pressurized.

17. The device of claim 11, wherein said capillary cassette is comprised of a 8 by 12 array of capillary tube sections of equal length extending through a substrate.

18. The device of claim 11, further including a wash station, wherein said automated capillary cassette transfer device is disposed to transfer said capillary cassette between the capillary cassette dispenser and said wash station.

19. The device of claim 16, wherein said wash station has an enclosure that receives said capillary cassette and a fluid distribution manifold for introducing fluid through capillary tube sections of said capillary cassette.

20. The device of claim 11, wherein each capillary tube section in said capillary cassette has an interior volume of less than one microliter.

21. A method to dispense small quantities of fluid in a highly parallel manner, comprising:

filling an array of capillary tube sections in a capillary cassette with fluid at a filling location, moving said capillary cassette to a dispensing location; and simultaneous dispensing said capillary tube sections at said dispensing location.

22. The method of claim 19, further including a step of washing capillary tube sections of said capillary cassette.

23. The method of claim 20, wherein all steps are repeated a plurality of times.

24. The method of claim 19, wherein said filling occurs by capillary action without mechanical force.

25. The method of claim 19, wherein said dispensing is effected by establishing a pressure differential.

26. The method of claim 23, wherein said dispensing is effected by centrifugal force.

27. The method of claim 19, wherein moving said capillary cassette is effected by gripping said capillary cassette by an automated transfer device and moving said capillary cassette to a programmed location.

28. The method of claim 19, where the step of filling said array of capillary tube sections includes filling said capillary tube sections with a volume of fluid less than one microliter.

29. The method of claim 19, wherein all steps are controlled by a central electronic control.

30. A device for transfer of an amount of liquid from a plurality of locations in a highly parallel manner, the device comprising:

a capillary cassette comprised of a substrate and a linear array of capillary tube sections extending through said substrate, each of said capillary tube sections having a first and a second opposing open ends, said first open ends being coplanar and said second open ends being coplanar;

a capillary cassette dispenser; and

an automated capillary cassette transfer device, wherein said capillary cassette transfer device may move said capillary cassette between said a filling location and said capillary cassette dispenser.

31. The system of claim 30, wherein said substrate is bendable.

32. The system of claim 30, wherein said substrate is curved.

33. The device of claim 30, wherein said capillary cassette dispenser employs a pressure differential to dispense said capillary tube sections in said capillary cassette.

34. The device of claim 33, wherein said dispenser is a centrifuge dispenser.

35. The device of claim 33, wherein said dispenser includes an enclosed area, wherein one end of said capillary tube sections in said capillary cassette may be sealed in said enclosed area, and wherein the enclosed area enclosing one end of capillaries in said capillary cassette may be pressurized.

36. The device of claim 30, further including a wash station, wherein said automated capillary cassette transfer device is disposed to transfer said capillary cassette between the capillary cassette dispenser and said wash station.

37. The device of claim 36, wherein said wash station has an enclosure that receives said capillary cassette and a fluid distribution manifold for introducing fluid through capillary tube sections of said capillary cassette.

38. The device of claim 30, wherein each capillary tube section in said capillary cassette has an interior volume of less than one microliter.

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