(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 July 2011 (21.07.2011)

PCT

(10) International Publication Number
WO 2011/086497 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/IB2011/050103

(22) International Filing Date:
11 January 2011 (11.01.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/294,445 12 January 2010 (12.01.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published**:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2011/086497 A2

(54) **Title**: THREE-STAGE THERMAL CONVECTION APPARATUS AND USES THEREOF

(57) **Abstract**: Disclosed is a multi-stage thermal convection apparatus and uses thereof. In one embodiment, the invention features a three-stage thermal convection apparatus that includes a temperature shaping element for assisting a thermal convection mediated Polymerase Chain Reaction (PCR). The invention has a wide variety of applications including amplifying nucleic acid without cumbersome and expensive hardware associated with many prior devices. In a typical embodiment, the apparatus can fit in the palm of a user's hand for use as a portable, simple to operate, and low cost PCR amplification device.

# THREE-STAGE THERMAL CONVECTION APPARATUS AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATION

The present application claims priority to United States Provisional Application No. 61/294,445 as filed on January 12, 2010, the disclosure of which is incorporated herein by reference.

## FIELD OF THE INVENTION

The present invention features a multi-stage thermal convection apparatus, particularly a three-stage thermal convection apparatus and uses thereof. The apparatus includes at least one temperature shaping element that assists a polymerase chain reaction (PCR). The invention has a wide variety of applications including amplifying a DNA template without the cumbersome and often expensive hardware associated with prior devices. In one embodiment, the apparatus can fit in the palm of a user's hand for use as a portable PCR amplification device.

## BACKGROUND

The polymerase chain reaction (PCR) is a technique that amplifies a polynucleotide sequence each time a temperature changing cycle is completed. See for example, *PCR: A Practical Approach*, by M. J. McPherson, et al., IRL Press (1991), *PCR Protocols: A Guide to Methods and Applications*, by Innis, et al., Academic Press (1990), and *PCR Technology: Principals and Applications for DNA Amplification*, H. A. Erlich, Stockton Press (1989). PCR is also described in many patents, including U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; 4,889,818; 5,075,216; 5,079,352; 5,104,792; 5,023,171; 5,091,310; and 5,066,584.

In many applications, PCR involves denaturing a polynucleotide of interest ("template"), followed by annealing a desired primer oligonucleotide ("primer") to the denatured template. After annealing, a polymerase catalyzes synthesis of a new polynucleotide strand that incorporates and extends the primer. This series of steps: denaturation, primer annealing, and primer extension, constitutes a single PCR cycle. These steps are repeated many times during PCR amplification.

As cycles are repeated, the amount of newly synthesized polynucleotide increases geometrically. In many embodiments, primers are selected in pairs that can anneal to opposite strands of a given double-stranded polynucleotide. In this case, the region between the two annealing sites can be amplified.

There is a need to vary the temperature of the reaction mixture during a multi-cycle PCR experiment. For example, denaturation of DNA typically takes place at about 90°C to about 98°C or a higher temperature, annealing a primer to the denatured DNA is typically performed at about 45°C to about 65°C, and the step of extending the annealed primers with a polymerase is typically performed at about 65°C to about 75°C. These temperature steps must be repeated, sequentially, for PCR to progress optimally.

To satisfy this need, a variety of commercially available devices has been developed for performing PCR. A significant component of many devices is a thermal "cycler" in which one or more temperature controlled elements (sometimes called "heat blocks") hold the PCR sample. The temperature of the heat block is varied over a time period to support the thermal cycling. Unfortunately, these devices suffer from significant shortcomings.

For example, most of the devices are large, cumbersome, and typically expensive. Large amounts of electric power are usually required to heat and cool the heat block to support the thermal cycling. Users often need extensive training. Accordingly, these devices are generally not suitable for field use.

Attempts to overcome these problems have not been entirely successful. For instance, one attempt involved use of multiple temperature controlled heat blocks in which each block is kept at a desired temperature and sample is moved between heat blocks. However, these devices suffer from other drawbacks such as the need for complicated machinery to move the sample between different heat blocks and the need to heat or cool one or a few heat blocks at a time.

There have been some efforts to use thermal convection in some PCR processes. See Krishnan, M. et al. (2002) *Science* 298: 793; Wheeler, E.K. (2004) *Anal. Chem.* 76: 4011-4016; Braun, D. (2004) *Modern Physics Letters* 18: 775-784; and WO02/072267. However, none of these attempts has produced a thermal convection PCR device that is compact, portable, more affordable and with a less significant need for electric power. Moreover, such thermal

convection devices often suffer from low PCR amplification efficiency and limitation in the size of amplicon.

## SUMMARY

The present invention provides a multi-stage thermal convection apparatus, particularly a three-stage thermal convection apparatus and uses thereof. The apparatus generally includes at least one temperature shaping element to assist a polymerase chain reaction (PCR). As described below, a typical temperature-shaping element is a structural and/or positional feature of the apparatus that supports thermal convection PCR. Presence of the temperature shaping element enhances the efficiency and speed of the PCR amplification, supports miniaturization, and reduces need for significant power. In one embodiment, the apparatus readily fits in the palm of a user's hand and has low power requirements sufficient for battery operation. In this embodiment, the apparatus is smaller, less expensive and more portable than many prior PCR devices.

Accordingly, and in one aspect, the present invention features a three-stage thermal convection apparatus adapted to perform thermal convection PCR amplification ("apparatus"). Preferably, the apparatus has at least one of and preferably all of the following elements as operably linked components:

(a) a first heat source for heating or cooling a channel and comprising a top surface and a bottom surface , the channel being adapted to receive a reaction vessel for performing PCR,

(b) a second heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the first heat source,

(c) a third heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the second heat source, wherein the channel is defined by a bottom end contacting the first heat source and a through hole contiguous with the top surface of the third heat source, and further wherein center points between the bottom end and the through hole form a channel axis about which the channel is disposed,

(d) at least one temperature shaping element adapted to assist thermal convection PCR; and

(e) a receptor hole adapted to receive the channel within the first heat source.

Also provided is a method of making the forgoing apparatus which method includes assembling each of (a)-(e) in an operable combination sufficient to perform thermal convection PCR as described herein.

In another aspect of the present invention, there is provided a thermal convection PCR centrifuge ("PCR centrifuge") adapted to perform PCR using at least one of the apparatus as described herein.

Further provided by the present invention is a method for performing a polymerase chain reaction (PCR) by thermal convection. In one embodiment, the method includes at least one of and preferably all of the following steps:

(a) maintaining a first heat source comprising a receptor hole at a temperature range suitable for denaturing a double-stranded nucleic acid molecule and forming a single-stranded template,

(b) maintaining a third heat source at a temperature range suitable for annealing at least one oligonucleotide primer to the single-stranded template,

(c) maintaining a second heat source at a temperature suitable for supporting polymerization of the primer along the single-stranded template; and

(d) producing thermal convection between the receptor hole and third heat source under conditions sufficient to produce the primer extension product.

In another aspect, the invention provides reaction vessels adapted to be received by an apparatus of the invention.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a schematic drawing showing an overhead view of an embodiment of the apparatus. Sectional planes through the apparatus (A-A and B-B) are depicted.

Figures 2A-C are schematic drawings showing sectional views of an apparatus embodiment having a first chamber 100. Figs. 2A-C are cross-sectional views taken along the A-A (Figs. 2A, 2B) and B-B planes (Fig. 2C).

Figures 3A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. Each apparatus has a first 100 and a second 110 chamber of unequal widths with respect to the channel axis 80.

Figures 4A-B are schematic drawings showing a sectional view (A-A) of an embodiment of the apparatus. Fig. 4B shows an expanded view of the region (identified by the dotted circle in Fig. 4A). The apparatus has a first 100, a second 110 and a third 120 chamber. A region between the first and second chambers includes a first thermal brake 130. A region between the second and third chambers includes a second thermal brake 140.

Figures 5A-D are schematic drawings showing channel embodiments of the apparatus (A-A plane).

Figures 6A-J are schematic drawings showing channel embodiments of the apparatus. The plane of section is perpendicular to the channel axis 80.

Figures 7A-I are drawings showing various chamber embodiments of the apparatus. The plane of section is perpendicular to the channel axis 80. Hatched parts represent the second or third heat source.

Figures 8A-P are drawings showing various chamber and channel embodiments of the apparatus. The plane of section is perpendicular to the channel axis 80. Hatched parts represent the second or third heat source.

Figures 9A-B are schematic drawings showing sectional views (A-A plane) of apparatus embodiments. The first chamber 100 is tapered.

Figures 10A-F are schematic drawings showing sectional views (A-A plane) of various apparatus embodiments having a first thermal brake 130. Figures 10B, 10D, and 10F show expanded views of the region identified by the dotted circle shown in Figs. 10A, 10C and 10E, respectively, to illustrate structural details of the first thermal brake 130.

Figures 11A-B are schematic drawings showing a sectional view (A-A) of one embodiment of the apparatus. Fig. 11B illustrates an expanded view of the region identified by

the dotted circle shown in Fig. 11A to highlight locations of the first 130 and second 140 thermal brakes.

Figure 12A is a schematic drawing showing a sectional view (A-A) of one embodiment of the apparatus. The first 20 and second 30 heat sources feature protrusions (23, 24, 33, 34) along the channel axis 80. A first thermal brake 130 is shown below the first chamber 100.

Figure 12B shows a positioning embodiment of the apparatus shown in Figure 12A. The apparatus is tilted (by an angle defined by $\theta_g$) with respect to the direction of gravity.

Figure 13 is a schematic drawing showing a sectional view (A-A) of one embodiment of the apparatus. The receptor hole 73 is asymmetrically disposed around the channel axis 80 and forms a receptor hole gap 74.

Figure 14A is a schematic drawing showing a sectional view (A-A plane) of an embodiment of the apparatus. The first 100 and second 110 chambers are positioned in the second 30 and third 40 heat sources, respectively.

Figure 14B is a schematic drawing showing a sectional view (A-A plane) of an embodiment of the apparatus. The first 100 and second 110 chambers are positioned in the second heat source 30 and a third chamber 120 is positioned in the third heat source 40. The first thermal brake 130 is positioned between the first 100 and second 110 chambers within the second heat source 30.

Figure 14C is a schematic drawing showing a sectional view (A-A) of an embodiment of the apparatus with the first 100 and second 110 chambers positioned in the second 30 and third 40 heat sources, respectively. The first thermal brake 130 is shown below the first chamber 100.

Figures 15A-B are schematic drawings showing sectional views (A-A plane) of apparatus embodiments in which the first chamber 100 is positioned in the third heat source 40. In Figure 15B, the first heat source 20 features protrusions (23, 24) disposed symmetrically about the receptor hole 73.

Figures 16A-C are schematic drawings showing sectional views of an apparatus embodiment. Figs. 16A-C are cross-sectional views taken along the A-A (Figs. 16A-B) and B-B planes (Fig. 16C). The second heat source 30 comprises protrusions (33, 34) disposed symmetrically about the channel axis 80 that extend the length of the first chamber 100.

Figures 17A-C are schematic drawings of an apparatus embodiment taken along the A-A (Figs. 17A-B) and B-B planes (Fig. 17C). The first 20, second 30 and third 40 heat sources include protrusions (23, 24, 33, 34, 43, 44) that are each positioned symmetrically about the channel axis 80.

Figure 18A is a schematic drawings showing a sectional view (A-A) of an embodiment of the apparatus. The apparatus is tilted (by an angle defined by $\theta_g$) with respect to the direction of gravity.

Figure 18B shows an apparatus embodiment in which the channel 70 and the first chamber 100 are tilted with respect to the direction of gravity within the second heat source 30. The direction of gravity remains perpendicular with respect to the heat sources.

Figure 19 is a schematic drawing showing a sectional view (A-A) of one embodiment of the apparatus. In this embodiment, the first heat source 20 features a receptor hole 73 with a receptor hole gap 74.

Figures 20A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. The first heat source 20 includes a receptor hole gap 74. In the embodiment shown by Figure 20B, the receptor hole gap 74 includes a top surface that is inclined with respect to the channel axis 80.

Figures 21A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. The first heat source 20 features a protrusion 23 disposed asymmetrically around the receptor hole 73. In Figure 21A, the protrusion 23 next to the receptor hole 73 has multiple top surfaces one of which has a greater height and is closer to the first chamber 100. In Figure 21B, the protrusion 23 has one top surface that is inclined with respect to the channel axis 80 so that one side has a greater height and is closer to the first chamber 100 than another side opposite to the receptor hole 73.

Figures 22A-D are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. In these embodiments, the first 20 and second 30 heat sources feature protrusions 23 and 33 disposed asymmetrically about the channel axis 80. The protrusions 23 and 33 have a greater height on one side than another side opposite to the channel axis 80. The top end of the protrusion 23 and the bottom end of the protrusion 33 have multiple surfaces (Figs. 22A and 22C) or are inclined with respect to the channel axis 80 (Figs. 22B and 22D). In Figs. 22A and 22B, the first chamber 100 features a bottom end 102 in which a portion is closer to one side of the protrusion 23 than another portion opposite to the channel axis 80. In Figures 22C and 22D, the bottom end 102 of the first chamber 100 is located essentially at a constant distance from the top surface of the protrusion 23.

Figures 23A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. In these embodiments, the first heat source 20 features a protrusion 23 disposed symmetrically around the receptor hole 73 and the second heat source 30 features a protrusion 33 disposed asymmetrically about the channel axis 80. In Figure 23A, the bottom end 102 of the first chamber 100 features multiple surfaces so that a portion of the bottom end 102 that is closer to one side of the protrusion 23 than another portion opposite to the channel axis 80. In Figures 23B, the bottom end 102 of the first chamber 100 is inclined with respect to the channel axis 80 so that a portion of the bottom end 102 is closer to the protrusion 23 than another portion opposite to the channel axis 80.

Figures 24A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. In these embodiments, the second heat source 30 features protrusions 33 and 34 that are disposed asymmetrically about the channel axis 80. The bottom end of the protrusion 33 and the top end of the protrusion 34 are inclined with respect to the channel axis 80 (Fig. 24A) or have multiple surfaces (Fig. 24B). The first chamber 100 features a portion of the bottom end 102 that is closer to the top surface of the first heat source 20 than another portion opposite to the channel axis 80. The top end 101 also features a portion that is closer to the bottom surface of the third heat source 40 than another portion opposite to the channel axis 80.

Figure 25 is a schematic drawing showing a sectional view of an apparatus embodiment taken along the A-A plane showing the first 100 and second 110 chambers disposed asymmetrically about the channel axis 80 within the second heat source 30.

Figure 26 is a schematic drawing showing a sectional view taken along the A-A plane of an apparatus embodiment in which the first chamber 100 includes a wall 103 disposed at an angle with respect to the channel axis 80.

Figures 27A-B are schematic drawings showing sectional views of apparatus embodiments taken along the A-A plane. In these embodiments, the second heat source 30 features protrusions (33, 34) that are disposed asymmetrically about the channel axis 80. The bottom end of the protrusion 33 and the top end of the protrusion 34 are inclined with respect to the channel axis 80 (Fig. 27A) or have multiple surfaces (Fig. 27B). In Figure 27B, the first 20 and third 40 heat sources feature protrusions (23, 24, 43, 44) disposed symmetrically about the channel axis 80. In both Figures 27A and B, a portion of the bottom end 102 of the first chamber 100 is positioned closer to the top surface of the first heat source 20 than another portion opposite to the channel axis 80. Also, the top end 101 has a portion that is positioned closer to the bottom surface of the third heat source 40 than another portion opposite to the channel axis 80.

Figures 28A-B are schematic drawings showing a sectional view of an apparatus embodiment taken alone the A-A plane with the first chamber 100 and the second chamber 110 within the second heat source 30. As shown in Figure 28B, the apparatus features a first thermal brake 130 asymmetrically disposed about the channel 70 and between the first 100 and second 110 chambers with the wall 133 contacting the channel 70 on one side.

Figure 29A is a schematic drawing showing a sectional view of an apparatus embodiment in which the first chamber 100 is within the second heat source 30 and is disposed asymmetrically (off-centered) about the channel 70.

Figures 29B-C are schematic drawings showing sectional views of an apparatus embodiment along the A-A plane. The first chamber 100 is disposed asymmetrically about the channel 70. As shown in Figure 29C, the thermal brake 130 is shown disposed asymmetrically about the channel 70 with the wall 133 contacting the channel 70 on one side.

Figures 30A-B are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30. The first 100 and second 110 chambers are disposed asymmetrically about the channel axis 80. In an expanded view shown in Fig. 30B, the thermal brake 130 is shown disposed symmetrically about the channel 70 between the first 100 and second 110 chambers. The wall 133 of the thermal brake 130 contacts the channel 70.

Figures 30C-D are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30. The first 100 and second 110 chambers are disposed asymmetrically about the channel axis 80. The width of the first chamber 100 perpendicular to the channel axis 80 is smaller than the width of the second chamber 110 along the channel axis 80. In an expanded view shown in Figure 30D, the first thermal brake 130 is shown disposed asymmetrically about the channel 70 with the wall 133 contacting the channel 70 on one side.

Figures 31A-B are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30. The first 100 and second 110 chambers are disposed asymmetrically about the channel axis 80 in opposite directions along the A-A plane. The thermal brake 130 is shown disposed symmetrically about the channel 70 with the wall 133 contacting the channel 70.

Figures 32A-B are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30. The first 100 and second 110 chambers are disposed asymmetrically about the channel axis 80. As shown in Figure 32B, the first thermal brake 130 is also disposed asymmetrically about the channel 70 with the wall 133 contacting the channel 70 on one side.

Figures 32C-D are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80. As shown in Fig. 32D, the first thermal brake 130 is also asymmetrically disposed about the channel 70 with the wall 133 contacting the channel 70 on one side.

Figures 33A-B are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80 in opposite directions along the A-A plane. In an expanded view shown in Figure 33B, the first thermal brake 130 is shown disposed asymmetrically with the wall 133 contacting the channel 70 on one side within the first chamber 100. The second thermal brake 140 is also shown disposed asymmetrically with the wall 143 contacting the channel 70 on one side within the second chamber 110. The top end 131 of the first thermal brake 130 is positioned essentially at the same height as the bottom end 142 of the second thermal brake 140.

Figures 33C-D are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80 in opposite directions along the A-A plane. In an expanded view shown in Figure 33D, the first 130 and second 140 thermal brakes are shown disposed asymmetrically with the walls (133, 143) each contacting the channel 70 on one side. The top end 131 of the first thermal brake 130 is positioned higher than the bottom end 142 of the second thermal brake 140.

Figures 33E-F are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80 in opposite directions along the A-A plane. In an expanded view shown in Figure 33F, the first 130 and second 140 thermal brakes are shown disposed asymmetrically with the walls (133, 143) each contacting the channel 70 on one side. The top end 131 of a first thermal brake 130 is shown positioned lower than the bottom end 142 of the second thermal brake 140.

Figures 34A-B are schematic drawings showing a sectional view of an apparatus embodiment along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80. The top end 101 of the first chamber 100 and the bottom end 112 of the second chamber 110 are inclined (tilted) with respect to the channel axis 80. The wall 103 of the first chamber 100, the wall 113 of the second chamber 110 are each essentially parallel to the channel axis 80. In an expanded view shown in Figure 34B, the first thermal brake 130 is shown inclined (tilted) with respect to the channel axis 80 and the wall 133 contacts the channel 70.

Figures 35A-D are schematic drawings showing sectional views of apparatus embodiments along the A-A plane in which the first 100 and second 110 chambers are within the second heat source 30 and are disposed asymmetrically about the channel axis 80. In Figures 35A-D, the wall 103 of the first chamber 100 and the wall 113 of the second chamber 110 are shown inclined (tilted) with respect to the channel axis 80. In an expanded view shown in Figure 35B, the thermal brake 130 is shown symmetrically disposed about the channel 70 with the wall 133 contacting the channel 70. In an expanded view shown in Figure 35D, the first thermal brake 130 is shown inclined (tilted) with respect to the channel axis 80 with the wall 133 contacting the channel 70.

Figures 36A-C are schematic drawings showing sectional views of various apparatus embodiments taken along the A-A plane in which the first chamber 100 is within the second heat source 30 and the second chamber 110 is within the third heat source 40 (Figs. 36A and C), or the first chamber 100 and the second chamber 110 are within the second heat source 30 and the third chamber 120 is within the third heat source 40 (Fig. 36B). In all figures, the chambers are disposed symmetrically about the channel axis 80. In Figs. 36A-C, the second heat source 30 features a protrusion 33 that defines the first chamber 100 and is disposed symmetrically about the channel axis 80 and the first heat source 20 features protrusions 23 and 24. In Figs. 36A-B, the bottom end 102 of the first chamber 100 contacts the first insulator 50. In Fig. 36C, the bottom end 102 of the first chamber 100 contacts the second heat source 30.

Figures 37A-C are schematic drawings showing sectional views of various apparatus embodiments taken along the A-A plane in which the first chamber 100 is within the second heat source 30 and the second chamber 110 is within the third heat source 40 (Figs. 37A and C) or the first chamber 100 and the second chamber 110 are within the second heat source 30 and the third chamber 120 is within the third heat source 40 (Fig. 37B). In all figures, the chambers are disposed symmetrically about the channel axis 80. Protrusions 23, 24, 33, and 34 are disposed symmetrically about the channel axis 80. In Figures 37A-B, the bottom end 102 of the first chamber 100 contacts the first insulator 50 while in Figure 37C it contacts the second heat source 30.

Figures 38A-C are schematic drawings showing sectional views of various apparatus embodiments taken along the A-A plane. In Figures 38A and C, the first chamber 100 is within

the second heat source 30 and the second chamber 110 is within the third heat source 40, and in Figure 38B the first chamber 100 and the second chamber 110 are within the second heat source 30 and the third chamber 120 is within the third heat source 40. The chambers are disposed symmetrically about the channel axis 80. Protrusions 23, 24, 33, 34, and 43 are disposed symmetrically about the channel axis 80. In Figures 38A-B, the bottom end 102 of the first chamber 100 contacts the first insulator 50 while in Figure 37C it contacts the second heat source 30.

Figure 39 is a schematic drawing showing an overhead view of an embodiment of the apparatus 10 showing first securing element 200, second securing element 210, heating/cooling elements (160a-c), and temperature sensors (170a-c). Various sectional planes are indicated (A-A, B-B, and C-C).

Figures 40A-B are schematic drawings of cross-sectional views of the apparatus embodiment shown in Figure 39 taken along the A-A (Fig. 40A) and B-B (Fig. 40B) planes.

Figure 41 is a schematic drawing of a cross-sectional view of the first securing element 200 taken along the C-C plane.

Figure 42 is a schematic drawing of an overhead view of an apparatus embodiment showing various securing elements, heat source structures, heating/cooling elements, and temperature sensors.

Figures 43A-B are schematic drawings of an overhead view (Fig. 43A) and a cross-sectional view (Fig. 43B) of an apparatus embodiment showing a first housing element 300 defining a third 310 and fourth 320 insulator.

Figures 44A-B are schematic drawings of an overhead view (Fig. 44A) and a cross-sectional view (Fig. 44B) of an apparatus embodiment comprising a second housing element 400 and a fifth 410 and sixth 420 insulator.

Figures 45A-B are schematic drawings of an embodiment of a PCR centrifuge. Fig. 45A shows an overhead view and Fig. 45B shows a cross-sectional view taken along the A-A plane.

Figure 46 is a schematic drawing showing a cross-sectional view of an apparatus embodiment of the PCR centrifuge taken along the A-A plane.

Figures 47A-B are schematic drawings showing an embodiment of a PCR centrifuge comprising a first chamber and a first thermal brake. In Fig. 47A, the plane of section along A-A is through the channel 70. In Fig. 47B, the plane of section along B-B is through the first 200 and second 210 securing means.

Figures 48A-C are schematic drawings showing embodiments of a first (Fig. 48A), second (Fig. 48B) and third (Fig. 48C) heat source for use in the PCR centrifuge shown in Figs. 47A-B. Sectional planes through the apparatus (A-A and B-B) are indicated.

Figures 49A-B are schematic drawings showing an embodiment of a PCR centrifuge comprising no chamber structure. In Fig 49A, the plane of section along A-A is through the channel 70. In Fig. 49B, the plane of section along B-B is through the first 200 and second 210 securing means.

Figures 50A-C are schematic drawings showing embodiments of a first (Fig. 50A), second (Fig. 50B) and third (Fig. 50C) heat source for use in the PCR centrifuge shown in Figs. 49A-B. Sectional planes through the apparatus (A-A and B-B) are indicated.

Figures 51A-D are schematic drawings showing a cross-sectional view of various reaction vessel embodiments.

Figures 52A-J are schematic drawings showing cross-sectional views of various reaction vessel embodiments taken perpendicular to the reaction vessel axis 95.

Figures 53A-C are results of thermal convection PCR using the apparatus of Figure 12A showing amplification of a 373 bp sequence from a 1 ng plasmid sample with three different DNA polymerases from Takara Bio, Finnzymes, and Kapa Biosystems, respectively.

Figures 54A-C are results of thermal convection PCR using the apparatus of Figure 12A showing amplification of three target sequences (with size 177 bp, 960 bp and 1,608 bp, respectively) from 1 ng plasmid samples.

Figure 55 shows results of thermal convection PCR using the apparatus of Figure 12A showing amplification of various target sequences (with size between about 200 bp to about 2 kbp) from 1 ng plasmid samples.

Figures 56A-C are results of thermal convection PCR using the apparatus of Figure 12A showing acceleration of PCR amplification at elevated denaturation temperatures (100°C, 102°C, and 104°C, respectively).

Figures 57A-C are results of thermal convection PCR using the apparatus of Figure 12A showing amplification of three target sequences (with size 363 bp, 475 bp, and 513 bp, respectively) from 10 ng human genome samples.

Figure 58 shows results of thermal convection PCR using the apparatus of Figure 12A showing amplification of various sequences (with size between about 100 bp to about 800 bp) from 10 ng human genome and cDNA samples.

Figure 59 shows results of thermal convection PCR using the apparatus of Figure 12A showing amplification of a 363 bp β-globin sequence from very low copy human genome samples.

Figure 60 shows temperature variations of the first, second and third heat sources of the apparatus of Figure 12A as a function of time when target temperatures were set to 98°C, 70°C, and 54°C, respectively.

Figure 61 shows power consumption of the apparatus of Figure 12A having 12 channels as a function of time.

Figures 62A-E are results of thermal convection PCR using the apparatus of Figure 12B showing acceleration of PCR amplification as a function of the gravity tilting angle. The gravity tilting angle was 0°, 10°, 20°, 30°, and 45° for Figures 62A-E, respectively.

Figures 63A-D are results of thermal convection PCR using the apparatus of Figure 12B showing acceleration of PCR amplification as a function of the gravity tilting angle. The gravity

tilting angle was 0°, 10°, 20°, and 30° for Figures 63A-D, respectively.

Figures 64A-B are results of thermal convection PCR using the apparatus of Figure 12B showing acceleration of PCR amplification as a function of the gravity tilting angle. The gravity tilting angle was 0° for Fig. 64A and 20° for Fig. 64B.

Figure 65 shows results of thermal convection PCR using the apparatus of Figure 12B showing amplification of a 363 bp β-globin sequence from very low copy human genome samples when the gravity tilting angle was introduced.

Figure 66 shows results of thermal convection PCR using the apparatus of Figure 14C showing amplification of a 152 bp sequence from a 1 ng plasmid sample.

Figure 67 shows results of thermal convection PCR using the apparatus of Figure 14C showing amplification of various sequences (with size between about 100 bp to about 800 bp) from 1 ng plasmid samples.

Figures 68A-B are results of thermal convection PCR using the apparatus of Figure 14C showing amplification of 500 bp β-globin (Fig. 68A) and 500 bp β-actin (Fig. 68B) sequences from 10 ng human genome samples.

Figure 69 shows results of thermal convection PCR using the apparatus of Figure 14C showing amplification of a 152 bp sequence from very low copy plasmid samples.

Figures 70A-D are results of thermal convection PCR using the apparatus of Figure 17A showing dependence of PCR amplification as a function of the chamber diameter when the receptor hole depth was about 2 mm. The chamber diameter was about 4 mm for Fig. 70A, about 3.5 mm for Fig. 70B, about 3 mm for Fig. 70C, and about 2.5 mm for Fig. 70D.

Figures 71A-D are results of thermal convection PCR using the apparatus of Figure 17A showing dependence of PCR amplification as a function of the chamber diameter when the receptor hole depth was about 2.5 mm. The chamber diameter was about 4 mm for Fig. 71A, about 3.5 mm for Fig. 71B, about 3 mm for Fig. 71C, and about 2.5 mm for Fig. 71D.

Figures 72A-D are results of thermal convection PCR using the apparatus of Figure 17A showing dependence of PCR amplification as a function of the chamber diameter when the receptor hole depth was about 2 mm and the gravity tilting angle of $10^{o}$ was introduced. The chamber diameter was about 4 mm for Fig. 72A, about 3.5 mm for Fig. 72B, about 3 mm for Fig. 72C, and about 2.5 mm for Fig. 72D.

Figures 73A-D are results of thermal convection PCR using the apparatus of Figure 17A showing dependence of PCR amplification as a function of the chamber diameter when the receptor hole depth was about 2.5 mm and the gravity tilting angle of $10^{o}$ was introduced. The chamber diameter was about 4 mm for Fig. 73A, about 3.5 mm for Fig. 73B, about 3 mm for Fig. 73C, and about 2.5 mm for Fig. 73D.

Figures 74A-F are results of thermal convection PCR using the apparatuses having the first thermal brake, showing dependence of PCR amplification as a function of the position of the first thermal brake along the channel axis. The bottom end of the first thermal brake was positioned at 0 mm (Fig. 74A), about 1 mm (Fig. 74B), about 2.5 mm (Fig. 74C), about 3.5 mm (Fig. 74D), about 4.5 mm (Fig. 74E), and about 5.5 mm (Figure 74F) above the bottom of the second heat source. The thickness of the first thermal brake along the channel axis was about 1 mm.

Figures 75A-E are results of thermal convection PCR using the apparatuses with and without the first thermal brake, showing dependence of PCR amplification as a function of the thickness of the first thermal brake along the channel axis when no gravity tilting angle was used. The thickness of the first thermal brake along the channel axis was 0 mm (Fig. 75A, i.e., without the first thermal brake), about 1 mm (Fig. 75B), about 2 mm (Fig. 75C), about 4 mm (Fig. 75D), and about 5.5 mm (Fig. 75E, i.e., channel only without the chamber structure). The bottom end of the first thermal brake was located on the bottom of the second heat source.

Figures 76A-E are results of thermal convection PCR using the apparatuses with and without the first thermal brake, showing dependence of PCR amplification as a function of the thickness of the first thermal brake along the channel axis when the gravity tilting angle of $10^{o}$ was introduced. The thickness of the first thermal brake along the channel axis was 0 mm (Fig. 76A, i.e., without the first thermal brake), about 1 mm (Fig. 76B), about 2 mm (Fig. 76C), about 4 mm (Fig. 76D), and about 5.5 mm (Fig. 76E, i.e., channel only without the chamber structure).

The bottom end of the first thermal brake was located on the bottom of the second heat source.

Figure 77 shows results of thermal convection PCR using the apparatus of Figure 12A having a symmetric heating structure.

Figures 78A-B show results of thermal convection PCR using the apparatus having an asymmetric receptor hole. The receptor hole was deeper on one side than the opposite side by about 0.2 mm for Fig. 78A and about 0.4 mm for Fig. 78B.

Figure 79 shows results of thermal convection PCR using the apparatus having an asymmetric thermal brake.

Figure 80A-B are schematic drawings showing sectional views of apparatus embodiments having one or more optical detection units 600-603 spaced from the first heat source 20 along the channel axis 80 and sufficient to detect a fluorescence signal from the samples in the reaction vessels 90. The apparatus includes a single optical detection unit 600 to detect the fluorescence signal from multiple reaction vessels (Fig. 80A) or multiple optical detection units 601-603 (Fig. 80B) to detect the fluorescence signal from each reaction vessel. In the embodiments shown in Figs. 80A-B, the optical detection unit detects the fluorescence signal from the bottom end 92 of the reaction vessel 90. The first heat source 20 comprises an optical port 610 positioned about the channel axis 80 between the bottom end 72 of the channel 70 and the first heat source protrusion 24 that provides a path for the excitation and emission of light parallel to the channel axis 80 (shown as upward and downward arrows, respectively).

Figures 81A-B are schematic drawings showing sectional views of apparatus embodiments having one optical detection unit 600 (Fig. 81A) or more than one optical detection units 601-603 (Fig. 81B). Each of optical detection units 600-603 is spaced from the third heat source 40 along the channel axis 80 sufficient to detect a fluorescence signal from the samples located in the reaction vessels 90. In these embodiments, a center part of a reaction vessel cap (not shown) that typically fits to the top opening of the reaction vessel 90 functions as an optical port for the excitation and emission light parallel to the channel axis 80 (shown in Figs. 81A-B as downward and upward arrows, respectively).

Figure 82 is a schematic drawing showing a sectional view of an apparatus embodiment having an optical detection unit 600 spaced from the second heat source 30. In this embodiment, the optical port 610 is positioned in the second heat source 30 along a path perpendicular to the channel axis 80 toward the optical detection unit 600 sufficient to detect a fluorescence signal from the side of the samples in the reaction vessels 90. The optical port 610 provides a path for the excitation and emission light between the reaction vessel 90 and the optical detection unit 600 (shown as left and right pointing arrows or vice versa). A side part of the reaction vessel 90 and a portion of the first chamber 100 along the light path also function as optical port in this embodiment.

Figure 83 is a schematic drawing showing a sectional view of an optical detection unit 600 positioned to detect a fluorescence signal from the bottom end 92 of the reaction vessel 90. In this embodiment, a light source 620, an excitation lens 630, and an excitation filter 640 that are configured to generate an excitation light are located along a direction at a right angle with respect to the channel axis 80, and a detector 650, an aperture or slit 655, an emission lens 660, and an emission filter 670 that are operable to detect an emission light are located along the channel axis 80. A dichrocic beam-splitter 680 that transmits the fluorescence emission and reflects the excitation light is also shown.

Figure 84 is a schematic drawing showing a sectional view of an optical detection unit 600 positioned to detect a fluorescence signal from the bottom end 92 of the reaction vessel 90. In this embodiment, a light source 620, an excitation lens 630, and an excitation filter 640 are positioned to generate an excitation light along the channel axis 80. A detector 650, an aperture or slit 655, an emission lens 660, and an emission filter 670 are positioned to detect an emission light as located along a direction at a right angle with respect to the channel axis 80. A dichrocic beam-splitter 680 that transmits the excitation light and reflects the fluorescence emission is shown.

Figures 85A-B are schematic drawings showing sectional views of an optical detection unit 600 positioned to detect a fluorescence signal from the bottom end 92 of the reaction vessel 90. In these embodiments, a single lens 635 is used to shape the excitation light and also to detect the fluorescence emission. In the embodiment shown in Fig. 85A, the light source 620 and the excitation filter 640 are located along a direction at a right angle to the channel axis 80.

In the embodiment shown in Fig. 85B, the optical elements for detecting the fluorescence emission (650, 655, and 670) are located along a direction at a right angle to the channel axis 80.

Figure 86 is a schematic drawing showing a sectional view of an optical detection unit 600 positioned to detect a fluorescence signal from the top end 91 of the reaction vessel 90. As in Fig. 83, the light source 620, the excitation lens 630, and the excitation filter 640 are located along a direction at a right angle to the channel axis 80, and the detector 650, the aperture or slit 655, the emission lens 660, and the emission filter 670 are located along the channel axis 80. Also shown in this embodiment is a reaction vessel cap 690 sealably attached to the top end 91 of the reaction vessel 90 and including an optical port 695 disposed around a center point of the top end 91 of the reaction vessel 90 and for transmission of the excitation and emission light. The optical port 695 is further defined by the upper part of the reaction vessel cap 690 and the upper part of the reaction vessel 90 in this embodiment.

Figures 87A-B are schematic drawings showing sectional views of reaction vessels 90 with reaction vessel caps 690 and optical ports 695. The reaction vessel cap 690 is sealably attached to the upper part of the reaction vessel 90 and the optical port 695. In these embodiments, the bottom end 696 of the optical port 695 is made to contact the sample when the reaction vessel 90 is sealed with the reaction vessel cap 690. An open space 698 is provided on the side of the bottom end 696 of the optical port 695 and the reaction vessel cap 690 so that the sample can fill up the open space when the reaction vessel 90 is sealed with the reaction vessel cap 690. The sample meniscus is located higher than the bottom end 696 of the optical port 695. In Figs. 87A-B, the optical port 695 is disposed around a center point of the lower part of the reaction vessel cap 690 and is further defined by the lower part of the reaction vessel cap 690 and the upper part of the reaction vessel 90.

Figure 88 is a schematic drawing showing a sectional view of a reaction vessel 90 with an optical detection unit 600 disposed above the reaction vessel 90. The reaction vessel 90 is sealed with the reaction vessel cap 690 having an optical port 695 disposed around a center point of the upper part of the reaction vessel 90 sufficient to make contact with sample. In this embodiment, the excitation light and the fluorescence emission pass through the optical port 695 and reach the sample or vice versa without passing air contained inside the reaction vessel 90.

**DETAILED DESCRIPTION**

The following figure key may help the reader better appreciate the invention including the Drawings and claims:

10: Apparatus embodiment

20: First heat source (bottom stage)

21: Top surface of the first heat source

22: Bottom surface of the first heat source

23: First heat source protrusion (pointing toward the second heat source)

24: First heat source protrusion (pointing toward table)

30: Second heat source (intermediate stage)

31: Top surface of the second heat source

32: Bottom surface of the second heat source

33: Second heat source protrusion (pointing toward the first heat source)

34: Second heat source protrusion (pointing toward the third heat source)

40: Third heat source (top stage)

41: Top surface of the third heat source

42: Bottom surface of the third heat source

43: Third heat source protrusion (pointing toward the second heat source)

44: Third heat source protrusion (pointing away from unit)

50: First insulator (or first insulating gap)

51: First insulator chamber

60: Second insulator (or second insulating gap)

61: Second insulator chamber

70: Channel

71: Top end of the channel/through hole

72: Bottom end of the channel

73: receptor hole

74: receptor hole gap

80: (Center) axis of the channel

90: Reaction vessel

91: Top end of the reaction vessel

92: Bottom end of the reaction vessel

93: Outer wall of the reaction vessel

94: Inner wall of the reaction vessel

95: (Center) axis of the reaction vessel

100: First Chamber

101: Top end of the first chamber, defining an upper limit of the chamber

102: Bottom end of the first chamber, defining a lower limit of the chamber

103: First wall of the first chamber, defining a horizontal limit of the chamber

105: Gap of the first chamber

106: (Center) axis of the first chamber

110: Second Chamber

111: Top end of the second chamber

112: Bottom end of the second chamber

113: First wall of the second chamber

115: Gap of the second chamber

120: Third Chamber

121: Top end of the third chamber

122: Bottom end of the third chamber

123: First wall of the third chamber

125: Gap of the third chamber

130: First thermal brake

131: Top end of the first thermal brake

132: Bottom end of the first thermal brake

133: First wall of the first thermal brake, essentially contacting at least part of the channel

140: Second thermal brake

141: Top end of the second thermal brake

142: Bottom end of the second thermal brake

143: First wall of the second thermal brake, essentially contacting at least part of the channel

160: Heating/cooling elements

160a: Heating (and/or cooling) element of the first heat source

160b: Heating (and/or cooling) element of the second heat source

160c: Heating (and/or cooling) element of the third heat source

170: Temperature Sensors

170a: Temperature sensor of the first heat source

170b: Temperature sensor of the second heat source

170c: Temperature sensor of the third heat source

200: First securing element comprising at least one of following elements

201: Screw or fastener (typically made of a thermal insulator)

202a: Washer or positioning standoff (typically made of a thermal insulator)

202b: Spacer or positioning standoff (typically made of a thermal insulator)

202c: Spacer or positioning standoff (typically made of a thermal insulator)

203a: Securing element of the first heat source

203b: Securing element of the second heat source

203c: Securing element of the third heat source

210: Second securing element (typically made as a wing structure)

- Used to assemble the heat source assembly to the first housing element 300

300: First housing element

310: Third insulator (or third insulating gap)

- Located between the sides of the heat sources and the side walls of the first housing element; and
- Filled with a thermal insulator such as air, a gas, or a solid insulator

320: Fourth insulator (or fourth insulating gap)

- Located between the bottom of the first heat source and the bottom wall of the first housing element; and
- Filled with a thermal insulator such as air, a gas, or a solid insulator

330: Support

400: Second housing element

410: Fifth insulator (or fifth insulating gap)

- Located between the side walls of the first housing element and those of the second housing element; and
- Filled with a thermal insulator such as air, a gas, or a solid insulator

420: Sixth insulator (or sixth insulating gap)

- Located between the bottom wall of the first housing element and that of the second housing element; and
- Filled with a thermal insulator such as air, a gas, or a solid insulator.

500: Centrifuge unit

501: Motor

510: Axis of centrifugal rotation

520: Rotation arm

530: Tilt shaft

600-603: Optical detection units

610: Optical port
620: Light source
630: Excitation lens
635: Lens
640: Excitation filter
650: Detector
655: Aperture or slit
660: Emission lens
670: Emission filter
680: Dichroic beam-splitter
690: Reaction vessel cap
695: Optical port
696: Bottom end of optical port
697: Top end of optical port
698: Open space between inner wall of reaction vessel and side wall of optical port
699: Side wall of optical port

As discussed, and in one embodiment, the present invention features a three-stage thermal convection apparatus adapted to perform thermal convection PCR amplification.

In one embodiment, the apparatus includes as operably linked components the following elements:

(a) a first heat source for heating or cooling a channel and comprising a top surface and a bottom surface , the channel being adapted to receive a reaction vessel for performing PCR,

(b) a second heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the first heat source,

(c) a third heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the second heat source, wherein the channel is defined by a bottom end contacting the first heat source and a through hole contiguous with the top surface of the third heat source, and further wherein center points between the bottom end and

the through hole form a channel axis about which the channel is disposed,
(d) at least one temperature shaping element such as at least one gap or space (e.g., a chamber) disposed around the channel and within at least part of the second or third heat source, the chamber gap being sufficient to reduce heat transfer between the second or third heat source and the channel; and
(e) a receptor hole adapted to receive the channel within the first heat source.

In operation, the apparatus uses multiple heat sources, typically three, four or five heat sources, preferably three heat sources positioned within the apparatus so that each is essentially parallel to the other heat sources in typical embodiments. In this embodiment, the apparatus will generate a temperature distribution suitable for a convection-based PCR process that is fast and efficient. A typical apparatus includes a plurality of channels disposed within the first, second and third heat sources so that a user can perform multiple PCR reactions at the same time. For instance, the apparatus can include at least one or two, three, four, five, six, seven, eight, nine channels up to about ten, eleven or twelve channels, twenty, thirty, forty, fifty or up to several hundred channels extending through the first, second, and third heat sources, with between about eight to about one hundred channels being generally preferred for many invention applications. A preferred channel function is to receive a reaction vessel holding the user's PCR reaction and to provide direct or indirect thermal communication between the reaction vessel and at least one of and preferably all of a) the heat sources, b) the temperature shaping element(s), and c) the receptor hole.

The relative position of each of the three heat sources to the other is an important feature of the invention. The first heat source of the apparatus is typically located on the bottom and maintained at a temperature suitable for nucleic acid denaturation, and the third heat source is typically located on the top and maintained at a temperature suitable for annealing of denatured nucleic acid template with one or more oligonucleotide primers. In some embodiments, the third heat source is maintained at a temperature suitable for both annealing and polymerization. The second heat source is typically located in between the first and third heat sources and maintained at a temperature suitable for polymerization of the primer along the denatured template. Thus in one embodiment, the bottom part of the channel in the first heat source and the top part of the channel in the third heat source are subject to a temperature distribution suitable for the denaturation and annealing steps of the PCR reaction, respectively. In between the top and bottom part of the channel in which the second heat source is located is the transition region in

which most of temperature change from the denaturation temperature of the first heat source (the highest temperature) to the annealing temperature of the third heat source (the lowest temperature) takes place. Thus, in typical embodiments, at least part of the transition region is subject to a temperature distribution suitable for polymerization of the primer along the denaturated template. When the third heat source is maintained at a temperature suitable for both annealing and polymerization, the top part of the channel in the third heat source also provides a temperature distribution suitable for the polymerization step in addition to an upper part of the transition region. Therefore, temperature distribution in the transition region is important for achieving efficient PCR amplification, particularly regarding the primer extension. Thermal convection inside the reaction vessel typically depends on the magnitude and direction of the temperature gradient generated in the transition region, and thus temperature distribution in the transition region is also important for generating suitable thermal convection inside the reaction vessel that is conducive to PCR amplification. Various temperature shaping elements can be used with the apparatus to generate a suitable temperature distribution in the transition region to support fast and efficient PCR amplification.

Typically, each individual heat source is maintained at a temperature suitable for inducing each step of thermal convection PCR. Moreover, and in embodiments in which the apparatus features three heat sources, temperatures of the three heat sources are suitably arranged to induce thermal convection across a sample inside a reaction vessel. One general condition for inducing suitable thermal convection according to the invention is, a heat source maintained at a higher temperature is located at a lower position within the apparatus than a heat source maintained at a lower temperature. Thus in a preferred embodiment, the first heat source is positioned lower in the apparatus than the second or third heat source. In this embodiment, it will be generally preferred to place the second heat source lower in the apparatus than the third heat source. Other configurations are possible provided intended results are achieved.

As discussed, it is an object of the invention to provide an apparatus with at least one temperature shaping element. In most embodiments, each channel of the apparatus will include less than about ten of such elements, for example, one, two, three, four, five, six, seven, eight, nine or ten of the temperature shaping elements for each channel. One function of the temperature shaping element is to provide for efficient thermal convection mediated PCR by providing a structural or positional feature that supports PCR. As will be more apparent from the examples and discussion which follows, such features include, but are not limited to, at least one

gap or space such as a chamber; at least one insulator or insulating gap located between the heat sources; at least one thermal brake; at least one protrusion structure in at least one of the first, second, and third heat sources; at least one asymmetrically disposed structure within the apparatus, particularly in at least one of the channels, first heat source, second heat source, third heat source, gap such as a chamber, thermal brake, protrusion, first and second insulators, or the receptor hole; or at least one structural or positional asymmetry. Structural asymmetry is typically defined in reference to the channel and/or channel axis. An example of positional asymmetry is tilting or otherwise displacing the apparatus with respect to the direction of gravity.

The words "gap" and "space" will often be used herein interchangeably. A gap is a small enclosed or semi-enclosed space within the apparatus that is intended to assist thermal convection PCR. A large gap or large space with a defined structure will be referred to herein as a "chamber". In many embodiments, the chamber will include a gap and be referred to herein as a "chamber gap". A gap may be empty, filled or partially filled with an insulating material as described herein. For many applications, a gap or chamber filled with air will be generally useful.

One or a combination of temperature shaping elements (the same or different) can be used with the invention apparatus. Illustrative temperature shaping elements will now be discussed in more detail.

***Illustrative Temperature Shaping Elements***

*A. Gap or Chamber*

In one embodiment of the present apparatus, each channel will include at least one gap or chamber as the temperature shaping element. In a typical embodiment, the apparatus will include one, two, three, four, five or even six chambers disposed around each channel and within at least one of the second and third heat sources, for example, one, two or three of such chambers for each channel. In this example of the invention, the chamber creates a space between the channel and the second or third heat source that allows the user to precisely control temperature distribution within the apparatus. That is, the chamber assists in shaping the temperature distribution of the channel in the transition region. By "transition region" is meant the region of the channel roughly in between an upper part of the channel that contacts the third heat source and a lower part of the channel that contacts the first heat source. The chamber can be positioned nearly anywhere around the channel provided intended results are achieved. For instance, positioning the chamber (or more than one chamber) within or near the second heat

source, the third heat source or both the second and third heat sources will be useful for many invention applications. In embodiments in which a channel in the apparatus has multiple chambers, each chamber may be separated from the other and may in some instances contact one or more other chambers within the apparatus.

One or a combination of different gap or chamber structures is compatible with the invention. As general requirements, the chamber should generate a temperature distribution in the transition region that fulfills at least one and preferably all of the following conditions: (1) the temperature gradient generated (particularly across the vertical profile of the channel) must be large enough so as to generate a thermal convection across the sample inside the reaction vessel; and (2) the thermal convection thus generated by the temperature gradient must be sufficiently slow (or appropriately fast) so that sufficient time periods can be provided for each step of the PCR process. In particular, it is especially important to make the time period of the polymerization step sufficiently long since the polymerization step typically takes more time than the denaturation and annealing steps. Examples of particular gap or chamber configurations are disclosed below.

If desired, the channel within an invention apparatus may have at least one chamber disposed essentially symmetrically or asymmetrically about the channel axis. In many embodiments, an apparatus with one, two or three chambers will be preferred. The chambers may be disposed in one or a combination of the heat sources, for example, the first heat source, the second heat source, the third heat source, or both the second and third heat sources. For some apparatuses, having one, two, or three chambers within the second heat source or the second and third heat sources will be especially useful. Examples of such chamber embodiments are provided below.

In one embodiment, the chamber will be further defined by what is referred to herein as a "protrusion" from at least one of the first heat source, the second heat source, and the third heat source. In a particular embodiment, the protrusion will extend from the second heat source toward the first heat source in a direction generally parallel to the channel axis. Other embodiments are possible such as including a second protrusion extending from the second heat source to the third heat source generally parallel to the channel axis. Additional embodiments include an apparatus with a protrusion extending from the first heat source toward the second heat source generally parallel to the channel axis. Still further embodiments include an apparatus

with a protrusion extending from the third heat source toward the second heat source also generally parallel to the channel axis. In some embodiments, the apparatus may comprise at least one protrusion that is tilted with respect to the channel axis. In these examples of the invention, it is possible to substantially reduce the volume of the first, second and/or third heat sources as well as the heat transfer between the heat sources while lengthening chamber dimensions along the channel axis. These features have been found to enhance thermal convection PCR efficiency while reducing power consumption.

Figures 2A, 3A, 4A, 9B, 12A, 14A, 15A, and 22A provide a few examples of acceptable chambers for use with the invention. Other suitable chamber structures are disclosed below.

*B. Thermal Brake*

Each channel within an invention apparatus may include one, two, three, four, five, six or more thermal brakes, typically one or two thermal brakes to control the temperature distribution within the apparatus. In many embodiments, the thermal brake will be defined by a top and bottom end and a wall that will be in optional thermal contact with the channel. The thermal brake is typically disposed adjacent or near a wall of the gap or chamber (if present). An undesirable intrusion of a temperature profile from one heat source to another can be controlled and usually reduced by including the thermal brake as a temperature shaping element. As will be described in more detail below, it was found that thermal convection PCR amplification efficiency is sensitive to the position and thickness of the thermal brake. An acceptable thermal brake may be disposed with respect to the channel either symmetrically or asymmetrically.

One or more thermal brakes as described herein may be placed in nearly any position around each channel of the apparatus provided intended results are achieved. Thus in one embodiment, a thermal brake can be positioned adjacent or near a chamber to block or reduce undesired heat flow from an adjacent heat source and achieve suitable PCR amplification.

Figures 10B, 10D, 10F, 11B, 14B, and 14C provide a few examples of suitable thermal brakes for use with the invention. Other suitable thermal brakes are disclosed below.

*C. Positional or Structural Asymmetry*

It was found that thermal convection PCR was faster and more efficient when an invention apparatus included at least one positional or structural asymmetric element, for

example, one, two, three, four, five, six, or seven of such elements for each channel. Such elements can be placed around one or more channels up to the entire apparatus. Without wishing to be bound by theory, it is believed that presence of an asymmetric element within the apparatus increases the buoyancy force in ways that make the amplification process faster and more efficient. It has been found that by introducing at least one positional or structural asymmetry within the apparatus that can cause "horizontally asymmetric heating or cooling" with respect to the channel axis or the direction of gravity, it is possible to assist thermal convection PCR. Without wishing to be bound by theory, it is believed that an apparatus with at least one asymmetric element therein breaks apparatus symmetry with regard to heating or cooling the channel and helps or enhances generation of the buoyancy force so as to make the amplification process faster and more efficient. By a "positional asymmetric element" is meant that a structural element that makes the channel axis or the apparatus tilted with respect to the direction of gravity. By a "structural asymmetric element" is meant that a structural element that is not symmetrically disposed within the apparatus with respect to the channel and/or channel axis.

As discussed, it is necessary to generate a vertical temperature gradient inside a sample fluid in order to generate thermal convection (and also to fulfill the temperature requirements for the PCR process). However, even in the presence of a vertical temperature gradient, the buoyancy force that induces the thermal convection may not be generated if isothermal contours of the temperature distribution are flat (i.e., horizontal) with respect to the direction of gravity (i.e., the vertical direction). Within such a flat temperature distribution, the fluid does not experience any buoyancy force since each part of the fluid has the same temperature (and thus the same density) as other parts of the fluid at the same height. In symmetric embodiments, all the structural elements are symmetric with respect to the channel or channel axis and the direction of gravity is aligned essentially parallel to the channel or channel axis. In such symmetric embodiments, isothermal contours of the temperature distribution inside the channel or the reaction vessel often become nearly or perfectly flat with respect to the gravitational field, and thus it is often difficult to generate the thermal convection that is sufficiently fast. Without wishing to be bound by theory, it is believed that presence of certain perturbations that can induce a fluctuation or instability in the temperature distribution often helps or enhances generation of the buoyancy force and makes the PCR amplification faster and more efficient. For instance, a small vibration that typically exists in usual environment may disturb the near or perfectly flat temperature distribution, or a small structural defect in the apparatus may break the

symmetry of the channel/chamber structure or the reaction vessel structure so as to disturb the near or perfectly flat temperature distribution. In such a perturbed temperature distribution, the fluid can have different temperature for at least part of the fluid as compared to other part of the fluid at the same height, and thus the buoyancy force can be readily generated due to such temperature fluctuation or instability. Such natural or incidental perturbations are usually important in generating the thermal convection in the symmetric embodiments. When a positional or structural asymmetry is present within the apparatus, the temperature distribution within the channel or the reaction vessel can be controllably made uneven at the same height (i.e., horizontally uneven or asymmetric). In the presence of such horizontally asymmetric temperature distribution, the buoyancy force can be readily and usually more strongly generated so as to make the thermal convection PCR faster and more efficient. Useful positional or structural asymmetric elements cause "horizontally asymmetric heating or cooling" of the channel with respect to the channel axis or the direction of gravity.

Asymmetry can be introduced into an invention apparatus by one or a combination of strategies. In one embodiment, it is possible to make an invention apparatus with a positional asymmetry imposed on the apparatus, for example, by tilting the apparatus or the channel with respect to the direction of gravity. Nearly any of the apparatus embodiments disclosed herein can be tilted by incorporating a structure capable of offsetting the channel axis with respect to the direction of gravity. An example of an acceptable structure is a wedge or related inclined shape, or an inclined or tilted channel. See Figures 12B and 18A-B for examples of this invention embodiment.

In other embodiments, at least one of the following elements can be asymmetrically disposed within the apparatus with respect to the channel axis: a) the channel, b) a gap such as a chamber, c) the receptor hole d) the first heat source, e) the second heat source, f) the third heat source; g) the thermal brake; and h) the insulator. Thus in one invention embodiment, the apparatus features a chamber as the structural asymmetric element. In this invention example, the apparatus may include one or more other structural asymmetric elements such as the channel, receptor hole, thermal brake, insulator, or one or more of the heat sources. In another embodiment, the structural asymmetric element is the receptor hole. In yet another embodiment, the structural asymmetric element is the thermal brake or more than one thermal brake. The apparatus may include one or more other asymmetric or symmetric structural elements such as

the first heat source, the second heat source, the third heat source, the chamber, the channel, the insulator etc.

In embodiments in which the first heat source, the second heat source and/or the third heat source features a structural asymmetric element, the asymmetry may reside particularly in a protrusion (or more than one protrusion) that extends generally parallel to the channel axis.

Further examples are provided below. In particular, see Figures 21A-B, 22A-D, 23A-B, 24A-B, 25, 26, and 27A-B.

As discussed, one or both of the channel and chamber can be symmetrically or asymmetrically disposed in the apparatus with respect to the channel axis. See also Figures 6A-J, 7A-I, and 8A-P for examples in which the channel and/or chamber are the symmetric or asymmetric structural element.

It will often be desirable to have an apparatus in which the receptor hole is the structural asymmetric element. Without wishing to be bound to any theory, it is believed that the region between the receptor hole and the bottom end of the chamber or the second heat source is a location in the apparatus where a major driving force for thermal convection flow is generated. As will be readily apparent, this region is where initial heating to the highest temperature (i.e., the denaturation temperature) and transition toward a lower temperature (i.e., the polymerization temperature) take place, and thus the largest driving force should originate from this region.

See, for example, Figures 13 and 21A-B showing asymmetric receptor hole structures.

*D. Insulator and Insulating Gap*

It will often be useful to insulate each of the heat sources from the other to achieve the objects of this invention. As will be apparent from the following discussion, the apparatus can be used with a wide variety of insulators placed in the insulating gaps between each of the heat sources. Thus in one embodiment, a first insulator is placed in the first insulating gap between the first and second heat sources and a second insulator is placed in the second insulating gap between the second and third heat sources. One or a combination of gas or solid insulators having low thermal conductivity can be used. A generally useful insulator for many purposes of the invention is air (having low thermal conductivity of about 0.024 $W \cdot m^{-1} \cdot K^{-1}$ at room

temperature for static air, with a gradual increase with increasing temperature). Although materials that have a thermal conductivity larger than that of static air can be used without significantly reducing the performance of the apparatus other than the power consumption, it is generally preferred to use gas or solid insulators that have a thermal conductivity similar to or smaller than air. Examples of good thermal insulators include, but not limited to, wood, cork, fabrics, plastics, ceramics, rubber, silicon, silica, carbon, etc. Rigid foams made of such materials are particularly useful since they represent very low thermal conductivity. Examples of rigid foams includes, but not limited to, Styrofoam, polyurethane foam, silica aerosol, carbon aerosol, SEAgel, silicone or rubber foam, wood, cork, etc. In addition to air, polyurethane foam, silica aerosol and carbon aerosol are particularly useful thermal insulators to use at elevated temperatures.

In embodiments in which an invention apparatus has the insulating gaps, advantages will be apparent. For instance, a user of the apparatus will have the ability to 1) reduce the power consumption by substantially reducing heat transfer from one heat source to next heat source; 2) control the temperature gradient for generating the driving force (and therefore control the thermal convection) since large temperature change from one heat source to next heat source occurs in the insulating gap regions; and 3) balance heat transfer between the three heat sources so as to simplify the machinery of simultaneously maintaining the temperatures of the three adjacently disposed heat sources and thereby minimize the power consumption. It has been found that larger insulating gaps with low thermal conductivity insulators generally help reducing the power consumption. Use of the protrusion structures is particularly useful for substantially reducing the power consumption since larger average gaps can be provided while independently controlling different regions of each insulating gap (i.e., regions near and distant from the channel, separately). It has been also found that by changing the insulating gaps, particularly in the region near the channel, it is possible to control the speed of the thermal convection and thus the speed of the PCR amplification. Controlling the first insulating gap near the channel region has been found to be particularly useful in modulating the speed of the thermal convection. Moreover, the ratio of the average thickness of the first and second insulating gaps along the channel axis has been found to be very useful in balancing the heat transfer between the three heat sources. Amount of heat transfer between two adjacent heat sources is inversely proportional to the distance between the two heat sources. Therefore, by adjusting the ratio of the average thickness of the first and second insulating gaps, the second heat source located in between the first and third heat sources can be heated near the desired

temperature without power consumption as a result of a balance in the heat transfer between the three heat sources. This makes not only the power consumption of the apparatus substantially reduced but also the temperature control machinery and mechanism required for the apparatus much simple. For many instances, by choosing the average thickness ratio as suitable for the desired temperatures of the three heat sources, the apparatus can be built with using heating elements only without necessity for a cooling element that is typically more power consuming and frequently bulkier. Other advantages of having the insulating gaps will be apparent from the discussion and Examples that follow.

It will be apparent from the following discussion and examples that an invention apparatus may include one or a combination of the foregoing temperature shaping elements. Thus in one embodiment, the apparatus features at least one chamber (e.g., one, two or three chambers) disposed symmetrically about the channel and typically parallel to the channel axis along with the first and second insulators separating the first, second and third heat sources from each other. In this embodiment, the apparatus may further include one or two thermal brakes to further assist thermal convection PCR. In an embodiment in which the apparatus includes two chambers, for instance within the second heat source, each chamber may have the same or different horizontal position with respect to the channel axis. In another embodiment, the second heat source features protrusions extending toward the first and/or third heat sources generally parallel to the channel axis in which the protrusions define the chamber. In this embodiment, the apparatus may further include a protrusion extending from the first heat source to the second heat source; and optionally a protrusion extending from the third heat source toward the second heat source generally parallel to the channel axis. In these embodiments, the second heat source may include no chamber, one chamber, or two chambers disposed symmetrically with respect to the channel axis and the third heat source may include no chamber, one chamber or two chambers disposed symmetrically with respect to the channel axis with the proviso that at least one of the heat sources includes a chamber.

As discussed, it will often be useful to include asymmetric structural element within the apparatus. Thus it is an object of the invention to include within the apparatus a receptor hole that is disposed asymmetrically with respect to the channel axis. In this embodiment, the apparatus may include one or more chambers disposed symmetrically or asymmetrically with respect to the channel axis. Alternatively, or in addition, the apparatus may feature at least one thermal brake that is disposed asymmetrically with respect to the channel axis. In this

embodiment, the apparatus may include one or more chambers disposed symmetrically or asymmetrically with respect to the channel axis. Alternatively, or in addition, the apparatus may feature at least one of the protrusions disposed asymmetrically with respect to the channel axis. In one embodiment, the protrusion extending from the first heat source is disposed asymmetrically about the channel axis while one or both protrusions (and chamber) extending from the second heat source is disposed symmetrically about the channel axis. Alternatively, or in addition, the one or more protrusions (and chamber) of the second heat source can be disposed asymmetrically about the channel axis. In these embodiments, the apparatus may further include a protrusion extending from the third heat source to the second heat source that is disposed symmetrically or asymmetrically with respect to the channel axis.

However, in another embodiment, one or more of the channels up to all of the channels within the apparatus need not include any chamber or gap structure. In this example, the apparatus will preferably include one or more other temperature shaping elements such as tilting the angle of the channel with respect to gravity (an example of positional asymmetry). Alternatively, or in addition, the channel can include a structural asymmetry or be subjected to centrifugal acceleration as provided herein. For instance, see Example 6 and Figure 76E (channel only with the gravity tilting angle of 10°) in comparison with Figure 75E (channel only without the gravity tilting angle).

As will be appreciated, it is possible to have an invention apparatus in which other or further asymmetric elements are present. For example, the apparatus can include two or three chambers in which one or more of the chambers are disposed asymmetrically with respect to the channel axis. In embodiments in which the apparatus includes a single chamber, that chamber may be disposed asymmetrically with respect to the channel axis. Embodiments include an apparatus in which protrusions extending from the second heat source toward each of the first and third heat sources are disposed asymmetrically with respect to the channel axis.

If desired, any of the foregoing invention embodiments can include a positional asymmetry by tilting the device or the channel with respect to the direction of gravity or placing it on a wedge or other inclined shape.

As will be appreciated, nearly any temperature shaping element of an apparatus embodiment (whether symmetrically or asymmetrically disposed within the apparatus with

respect to the channel axis) can be combined with one or more other temperature shaping elements including other structural or positional features of the apparatus so long as intended results are achieved.

As will also be appreciated, the invention is flexible and includes an apparatus in which each channel includes the same or different temperature shaping elements. For example, one channel of the apparatus can have no chamber or gap structures while another channel of the apparatus includes one, two, or three of such chamber or gap structures. The invention is not limited to any channel configuration (or group of channel configurations) so long as intended results are achieved. However, it will often be preferred to have all the channels of an invention apparatus have the same number and type of temperature shaping element to simplify use and manufacturing considerations.

Reference to the following figures and examples is intended to provide greater understanding of the thermal convection PCR apparatus. It is not intended and should not be read as limiting the scope of the present invention.

Turning now to Figures 1 and 2A-C, the apparatus 10 features the following elements as operably linked components:

(a) a first heat source 20 for heating or cooling a channel 70 and comprising a top surface 21 and a bottom surface 22 in which the channel 70 is adapted to receive a reaction vessel 90 for performing PCR;

(b) a second heat source 30 for heating or cooling the channel 70 and comprising a top surface 31 and a bottom surface 32 in which the bottom surface 32 faces the top surface of the first heat source 21,

(c) a third heat source 40 for heating or cooling the channel 70 and comprising a top surface 41 and a bottom surface 42 in which the bottom surface 42 faces the top surface of the second heat source 31, wherein the channel 70 is defined by a bottom end 72 contacting the first heat source 20 and a through hole 71 contiguous with the top surface of the third heat source 41. In this embodiment, center points between the bottom end 72 and the through hole 71 form a channel axis 80 about which the channel 70 is disposed;

(d) at least one chamber disposed around the channel 70 and within at least part of the second 30 or third 40 heat source. In this embodiment, the first chamber

100 includes a chamber gap 105 between the second 30 or third 40 heat source and the channel 70 sufficient to reduce heat transfer between the second 30 or third 40 heat source and the channel 70; and

(e) a receptor hole 73 adapted to receive the channel 70 within the first heat source 20.

By the phrase "operably linked", "operably associated" or like phrase is meant one or more elements of the apparatus that are operationally linked to one or more other elements. More specifically, such an association can be direct or indirect (e.g., thermal), physical and/or functional. An apparatus in which some elements are directly linked and others indirectly (e.g., thermally) linked is within the scope of the present invention.

In the embodiment shown in Figure 2A, the apparatus further includes a first insulator 50 positioned between the top surface 21 of the first heat source 20 and the bottom surface 32 of the second heat source 30. The apparatus further includes a second insulator 60 positioned between the top surface 31 of the second heat source 30 and the bottom surface 42 of the third heat source 40. As will be appreciated, practice of the invention is not limited to having only two insulators present provided the number of insulators is sufficient for intended results to be achieved. That is, the invention may include multiple insulators (e.g. 2, 3 or 4 insulators). In the embodiment shown in Figure 2A, the length of the first insulator 50 along the channel axis 80 is greater than the length of the second insulator 60 along the channel axis 80. In other embodiments, the length of the first insulator 50 may be smaller than or essentially the same as that of the second insulator 60. However, it is generally preferred to have the length of the first insulator 50 greater than that of the second insulator 60. Such embodiment is advantageous in reducing power consumption and facilitating temperature control. In another embodiment, it is preferred to have the length of the second heat source 30 greater than the length of the first heat source 20 or the third heat source 30 along the channel axis 80. Although in other embodiments the length of the second heat source 30 can be smaller or essentially the same as that of the first 20 or third 40 heat source, it is advantageous to have a greater length for the second heat source 30 to achieve a longer path length for the polymerization step.

In one embodiment shown in Figure 2A, the first insulator 50, the second insulator 60 or both insulators 50, 60 are filled with a thermal insulator having a low thermal conductivity. Preferred thermal insulators have a thermal conductivity between about a few tenths of $W \cdot m^{-1} \cdot K^{-}$

$^{1}$ to about 0.01 $W \cdot m^{-1} \cdot K^{-1}$ or smaller. In this embodiment, the length of the first insulator 50 along the channel axis 80 and preferably also that of the second insulator 60 are made to be small, for instance, between about 0.1 mm to about 5 mm, preferably between about 0.2 mm to about 4 mm. In this example of the invention, heat loss from one heat source to an adjacent heat source can be substantially large, resulting in large power consumption in operating the apparatus. For many applications, it will often be preferred to have at least one of the three heat sources (e.g., 20, 30 and 40) isolated from the others, preferably two heat sources thermally isolated from another (e.g., 20 and 30 isolated from each other, 30 and 40 isolated from each other, etc.) with all of the three heat sources (e.g., 20, 30 and 40) thermally isolated from each other being generally preferred for many invention applications. Use of one or more thermal insulators will often be helpful. For instance, use of a thermal insulator in the first 50 and second 60 insulating gaps can often lower power consumption.

Thus in the invention embodiment of the invention shown in Figures 2A-C, the first insulator 50 comprises or consists of a solid or a gas. Alternatively, or in addition, the second insulator 60 includes or consists of a solid or a gas.

Turning again to the apparatus shown in Figures 2A-C, the chamber gap 105 between the chamber wall 103 and the channel 70 inside the second heat source may be partially or totally filled with a thermal insulator such as a gas, solid, or gas-solid combination. Typically useful insulators include air, and gas or solid insulators that have a thermal conductivity similar to or smaller than air. Since one important function of the chamber gap 105 is to control (typically to reduce) heat transfer from the second heat source to the channel inside the second heat source, materials that have a thermal conductivity larger than that of air such as plastics or ceramics can also be used. However, when such higher thermal conductivity materials are used, the chamber gap 105 should be adjusted to be larger compared to the embodiment of using air as an insulator. Similarly, if a material having a lower thermal conductivity than air is used, the chamber gap 105 should be adjusted to be smaller than that of the air insulator embodiment.

In particular, Figures 2A-C show an apparatus embodiment in which air or a gas is used as an insulator in the first insulator 50 and second insulator 60, and the chamber gap 105. The channel structures inside these gaps are depicted with dashed lines to represent invisibility of these structures when air (or a gas) is used as an insulator. If desired to achieve a particular invention objective, the apparatus can be adapted so that a solid insulator is used in the chamber

gap 105. Alternatively, or in addition, the apparatus may include solid insulators in the first insulator 50 and second insulator 60.

Figures 2B and 2C show perspective views of section A-A and B-B of the apparatus as marked in Figure 1. An embodiment in which air or a gas is used as an insulator is shown.

As shown in the embodiment of Figures 1 and 2A-C, the apparatus features twelve channels (sometimes referred herein to as reaction vessel channels). However, more or less channels are possible depending on intended use, for instance, from about one or two to about twelve channels, or between about twelve to several hundred channels, preferably about eight to about one hundred channels. Preferably, each channel is independently adapted to receive a reaction vessel 90 that is typically defined by a bottom end 92 within the first heat source 20 and a top end 91 on the top of the third heat source 41. The channel 70 in the first 20, second 30 and third 40 heat sources typically passes through the first 50 and second 60 insulators. Center points between the top 71 and bottom 72 ends of the channel 70 form an axis of the channel 80 (sometimes referred herein to as channel axis) about which the heat sources and insulators are disposed.

Referring again to the embodiment shown in Figures 1 and 2A-C, the channel 70 is adapted so that the reaction vessel 90 can fit snugly therein i.e., it has a dimensional profile that is essentially the same as that of a lower part of the reaction vessel as depicted in Figure 2A. In the operation, the channel functions as a receptor for receiving a reaction vessel. However as will be explained in more detail below, the structure of the channel 70 can be adjusted and/or moved in relation to the channel axis 80 to provide different thermal contact possibilities between the reaction vessel 90 and one or more of the heat sources 20, 30, and 40.

As an example, the through hole 71 formed in the third heat source can function as a top part of the channel 70. In this embodiment, the channel 70 inside the third heat source 40 is in physical contact with the third heat source 40. That is, a wall of the through hole 71 extending into the third heat source 40 is in physical contact with the reaction vessel 90. In this embodiment, the apparatus can provide efficient heat transfer from the third heat source 40 to the channel 70 and reaction vessel 90.

For many invention applications, it will be generally preferred to have the size of the through hole in the third heat source essentially the same as that of the channel or reaction vessel. However, other through hole embodiments are within the scope of the present invention and are disclosed herein. For example, and referring again to Figures 2A-C, the through hole 71 in the third heat source 40 may be made larger than the size of the reaction vessel 90. However, in such case, heat transfer from the third heat source 40 to the reaction vessel 90 may become less efficient. In this embodiment, it may be useful to lower the temperature of the third heat source 40 for optimal practice of the invention. For most invention applications, it will be generally useful to have the size of the through hole 71 in the third heat source 40 essentially the same size as that of the reaction vessel 90.

In invention embodiments in which the receptor hole 73 has a closed bottom end 72 formed in the first heat source 20, it will often function as a bottom portion of the channel 70. See Figure 2A, for instance. In such an embodiment, the receptor hole 73 of the first heat source 20 has a size essentially the same as that of the bottom part of the reaction vessel 92 which in most embodiments will provide physical contact and efficient heat transfer to the reaction vessel 90. In some invention embodiments, the receptor hole 73 in the first heat source 20 may have a partial chamber structure or a size slightly larger than that of the bottom part of the reaction vessel as will be discussed.

***Chamber Structure and Function***

Turning again to the apparatus shown in Figures 2A-C, the first chamber 100 is symmetrically disposed about the channel 70 and within the second heat source 30. Presence of such a physically non-contacting (but thermally contacting) space within the apparatus 10 provides many benefits and advantages. For example, and without wishing to be bound to any theory, presence of the first chamber 100 provides heat transfer from the second heat source 30 to the channel 70 or the reaction vessel 90 that is desirably less efficient. That is, the chamber 100 reduces heat transfer substantially between the second heat source 30 and the channel 70 or the reaction vessel 90. As will become more apparent from the discussion that follows, this invention feature supports robust and faster thermal convection PCR within the apparatus 10.

While it will often be useful to include a physically non-contacting space within the second heat source 30, it is within the scope of the present invention to include such a space within one or more additional heat sources in the apparatus 10 such as one or both of the first 20

and third 40 heat sources. For example, the first heat source 20 or the third heat source 40 may include one or more chambers intended to reduce heat transfer between one or more of the heat sources and the channel 70 or the reaction vessel 90.

The invention embodiment shown in Figures 2A-C includes a first chamber 100 in the second heat source 20 as a key structural element. In this example of the invention, the first chamber 100 is independently adapted to receive the channel 70 from the top of the second heat source 31 toward the bottom of the second heat source 32 and the top of the first heat source 21. The first chamber 100 is defined by a top end 101 on the top of the second heat source 30, a bottom end 102 on the bottom of the second heat source 30, and the first chamber wall 103 that is disposed around the channel axis 80 and spaced from the channel 70 inside the second heat source 30. The chamber wall 103 surrounds the channel 70 inside the second heat source 20 at a distance, forming a chamber gap 105. The chamber gap 105 between the chamber wall 103 and the channel 70 is preferably in the range between from about 0.1 mm to about 6 mm, more preferably from about 0.2 mm to about 4 mm. The length of the first chamber 100 is between about 1 mm to about 25 mm, preferably between about 2 mm to about 15 mm.

The invention is compatible with a wide variety of heat source and insulator configurations. For instance, the first heat source 20 can have a length larger than about 1 mm along the channel axis 80, preferably from about 2 mm to about 10 mm; the second heat source 30 can have a length between from about 2 mm to about 25 mm along the channel axis 80, preferably from about 3 mm to about 15 mm; the third heat source 40 can have a length larger than about 1 mm along the channel axis 80, preferably from about 2 mm to about 10 mm. As discussed, it will be generally useful to have an apparatus with a first insulator 50 and a second insulator 60. For example, in embodiments without the protrusions, the first insulator 50 can have a length along the channel axis 80 between about 0.2 mm to about 5 mm along the channel axis 80, preferably between about 0.5 mm to 4 mm. The second insulator 60 can have a length along the channel axis 80 between about 0.1 mm to about 3 mm along the channel axis 80, preferably between about 0.2 mm to about 2.5 mm. In other embodiments in which the protrusion structure is present, the first 50 and second 60 insulators can have different lengths along the channel axis 80 depending on the position with respect to the channel 70. For instance, in the region near or around the channel (i.e., within the protrusions), the first insulator 50 can have a length along the channel axis between about 0.2 mm to about 5 mm, preferably between about 0.5 mm to 4 mm, and the second insulator 60 can have a length along the channel axis 80

between about 0.1 mm to about 3 mm, preferably between about 0.2 mm to 2.5 mm. In the region distant from the channel (i.e., outside the protrusion structures), the first insulator 50 can have a length along the channel axis between about 0.5 mm to about 10 mm, preferably between about 1 mm to 8 mm, and the second insulator 60 can have a length along the channel axis 80 between about 0.2 mm to about 5 mm, preferably between about 0.5 mm to 4 mm.

As discussed, an invention apparatus may include multiple chambers (for example, two, three, four, five or more chambers) within at least one of the heat sources such as the second heat source.

In the embodiment shown in Figures 3A-B, the apparatus includes a first chamber 100 positioned entirely within the second heat source 30. In this embodiment, the first chamber 100 includes the chamber top end 101 facing a first chamber bottom end 102 along the channel axis 80. The apparatus further includes a second chamber 110 positioned entirely within the second heat source 30 and in contact with the top end 101 of the first chamber 100. The wall 103 of the first chamber 100 is aligned essentially parallel to the channel axis 80. The second chamber 110 is further defined by the wall 113 positioned essentially parallel to the channel axis 80. The second chamber 110 is further defined by a top end 111 in contact with the top end 31 of the second heat source 30 and a bottom end 112 in contact with the top end 101 of the first chamber 100. As shown, the first chamber 100 and the second chamber 110 include gaps 105 and 115, respectively. In the embodiment shown, each of the top end 111 and bottom end 112 of the second chamber 110 are perpendicular to the channel axis 80. As shown in Fig. 3A, the width or radius of the first chamber 100 from the channel axis 80 is smaller (about 0.9 to 0.3 times smaller) than the width or radius of the second chamber 110 from the channel axis 80. However as shown in the embodiment of Figure 3B, the width or radius of the first chamber 100 from the channel axis 80 is greater (about 1.1 to about 3 times greater) than the width of the second chamber 110 from the channel axis 80.

Turning again to Figures 3A-B, the first chamber 100 and the second chamber 110 provide a highly useful temperature controlling or shaping effect. In these embodiments, the first chamber 100 (Figure 3A) or the second chamber 110 (Figure 3B) has a smaller diameter or width compared to the other chamber. The narrower portion of the second chamber 110 (Figure 3B) or first chamber 100 (Figure 3A) provides more efficient heat transfer from the second heat source 30 compared to the other chamber. In addition, the chamber configuration shown in these

embodiments preferentially blocks heat transfer from a heat source located closer to the narrower portion (e.g., the first heat source 20 in Figure 3A).

Unless otherwise mentioned, embodiments with multiple chambers will be described by numbering the chambers from the first heat source (typically located nearest the bottom of the apparatus). Thus the chamber closest to the first heat source will be designated "first chamber", the next closest chamber to the first heat source will be designated "second chamber", etc.

***Thermal Brake Structure and Function***

Figure 4A shows an invention embodiment with three chambers positioned in one of the heat sources. In particular, the apparatus 10 has the first chamber 100, the second chamber 110 and a third chamber 120 positioned in the second heat source 30. In this embodiment, the third chamber 120 includes a gap 125. The third chamber 120 includes the wall 123 positioned essentially parallel to the channel axis 80. The third chamber 120 is further defined by a top end 121 adjacent to the top 31 of the second heat source. The third chamber 120 is further defined by a bottom end 122 contacting a specific region within the second heat source 30 (see dotted circle in Figure 4A). As shown, the top end 121 and bottom end 122 of the third chamber 120 are perpendicular to the channel axis 80.

Figure 4B is an expanded view of the dotted circle shown in Figure 4A. In particular, the region between the first chamber 100 and the second chamber 110 defines a first thermal brake 130. As mentioned above, the first thermal brake 130 is intended to control the temperature distribution within the apparatus 10. In the embodiment shown, the first thermal brake 130 is defined by a top end 131 and a bottom end 132 and a wall 133 that essentially contacts the channel 70. In this embodiment, a function of the first thermal brake 130 is to reduce or block an undesirable intrusion of a temperature profile from the first heat source 20 to the second heat source 30 and the third heat source 40. Another function of the first thermal brake 130 is to provide an efficient heat transfer between the second heat source 30 and the channel 70 so as to make the channel in that region quickly approach the temperature of the second heat source 30. The first thermal brake 130 is disposed symmetrically about the channel 70.

As shown in Figure 4B, this invention embodiment includes a second thermal brake 140 defined by the region between the second chamber 110 and the third chamber 120. In particular, the second thermal brake 140 is further defined by a top end 141 and a bottom end 142 that

essentially contacts at least part of the channel 70 through a wall 143. An important function of the second thermal brake 140 is further assist in the control of the temperature distribution within the apparatus 10. In this embodiment, the second thermal brake 140 is particularly useful for reducing or blocking an undesirable intrusion of a temperature profile from the third heat source 40 to the second heat source 30 and also for providing an efficient heat transfer between the second heat source 30 and the channel 70 so as to maintain that region at a temperature close to the temperature of the second heat source 30. The second thermal brake 140 is disposed symmetrically about the channel 70.

If desired, at least one of the first chamber 100, the second chamber 110, and the third chamber 120 (or a portion thereof) may include a suitable solid or a gas insulator. Alternatively, or in addition, one or both of the first insulator 50 and/or the second insulator 60 shown may include or consist of a suitable solid or a gas. An example of suitable insulating gas is air.

***Channel Structure***

*A. Vertical Profiles*

The invention is fully compatible with several channel configurations. For example, Figures 5A-D show vertical sections of suitable channel configurations. As shown, the vertical profile of the channel may be shaped as a linear (Figs. 5C-D) or tapered (Fig. 5A-B) channel. In a tapered embodiment, the channel may be tapered either from the top to the bottom or from the bottom to the top. Although various modifications are possible regarding the vertical profile of the channel (e.g., a channel having a side wall that is curved, or tapered with two or more different angles, etc.), it is generally preferred to use a channel that is (linearly) tapered from the top to the bottom because such structure facilitates not only the fabrication process but also introduction of the reaction vessel to the channel. A generally useful taper angle ($\theta$) is in the range between from about $0^o$ to about $15^o$, preferably from about $2^o$ to about $10^o$.

In the embodiments shown in Figures 5A-B, the channel 70 is further defined by an open top 71 and a closed bottom end 72 which ends may be perpendicular to the channel axis 80 (Figure 5A) or curved (Figure 5B). The bottom end 72 may be curved with a convex or concave shape having a radius of curvature equal to or larger than the radius or half width of the horizontal profile of the bottom end. Flat or near flat bottom end with its radius of curvature at least two times larger than the radius or half width of the horizontal profile of the bottom end is more preferred over other shapes since it can provide an enhanced heat transfer for the

denaturation process. The channel 70 is further defined by a height ($h$) along the channel axis 80 and a width ($w1$) perpendicular to the channel axis 80.

For many invention applications, it will be useful to have a channel 70 that is essentially straight (i.e., not bent or tapered). In the embodiments shown in Figures 5C-D, the channel 70 has the open top end 71 and the closed bottom end 72 which may be perpendicular to the channel axis 80 (Figure 5C) or curved (Figure 5D). As in the tapered channel embodiments, the bottom end 72 may be curved with a convex or concave shape and flat or near flat bottom end having a large curvature is typically more preferred. The channel 70 is further defined in these embodiments by a height ($h$) along the channel axis 80 and a width ($w1$) perpendicular to the channel axis 80.

In the channel embodiments shown in Figures 5A-D, the height ($h$) is at least about 5 mm to about 25 mm, preferably 8 mm to about 16 mm for a sample volume of about 20 microliters. Each channel embodiment is further defined by the average of the width ($w1$) along the channel axis 80 which is typically at least about 1 mm to about 5 mm. Each of the channel embodiments shown in Figures 5A-D can be further defined by a vertical aspect ratio which is the ratio of the height ($h$) to the width ($w1$), and a horizontal aspect ratio which is the ratio of the first width ($w1$) to the second width ($w2$) along first and second directions, respectively, that are mutually perpendicular to each other and aligned perpendicular to the channel axis. A generally suitable vertical aspect ratio is between about 4 to about 15, preferably from about 5 to about 10. The horizontal aspect ratio is typically between about 1 to about 4. In embodiments in which the channel 70 is tapered (Figures 5A-B), the width or diameter of the channel changes across the vertical profile of the channel. By way of general guidance, for sample volumes larger or smaller than 20 microliters, the height and width (or diameter) may be scaled by a factor of cubic root or sometimes square root of the volume ratio.

As discussed, the bottom end 72 of the channel may be flat, rounded, or curved as depicted in Figure 5A-D. When the bottom end is rounded or curved, it typically has a convex or concave shape. As discussed, a flat or near flat bottom end is more preferred over other shapes for many invention embodiments. While not wishing to be bound to any theory, it is believed that such a bottom design can enhance heat transfer from the first heat source 20 to the bottom end 71 of the channel 70 so as to facilitate the denaturation process.

None of the foregoing vertical channel profiles are mutually exclusive. That is, a channel that has a first portion that is straight and second portion that is tapered (with respect to the channel axis 80) is within the scope of the present invention.

*B. Horizontal Profiles*

The invention is also compatible with a variety of horizontal channel profiles. An essentially symmetrical channel shape is generally preferred where ease of manufacture is a concern. Figures 6A-J show a few examples of acceptable horizontal channel profiles, each with a designated symmetry. For instance, the channel 70 may have its horizontal shape that is circular (Fig. 6A), square (Fig. 6D), rounded square (Fig. 6G) or hexagonal (Fig. 6J) with respect to the channel axis 80. In other embodiments, the channel 70 may have a horizontal shape that has its width larger than its length (or vice versa). For instance, and as depicted in the middle column of Figure 6B, E and H, the horizontal profile of the channel 70 may be shaped as an ellipsoid (Fig. 6B), rectangular (Fig. 6E), or rounded rectangular (Fig. 6H). This type of horizontal shape is useful when incorporating a convection flow pattern going upward on one side (e.g., on the left hand side) and going downward on the opposite side (e.g., on the right hand side). Due to the relatively larger width profile incorporated compared to the length, interference between the upward and downward convection flows can be reduced, leading to more smooth circulative flow. The channel may have a horizontal shape that has its one side narrower than the opposite side. A few examples are shown on the right column of Figures 6C, F and I. The left side of the channel is depicted to be narrower than the right side for instance. This type of horizontal shape is also useful when incorporating a convection flow pattern going upward on one side (e.g., on the left hand side) and going downward on the opposite side (e.g., on the right hand side). Moreover, when this type of shape is incorporated, speed of the downward flow (e.g., on the right hand side) can be controlled (typically reduced) with respect to the upward flow. Since the convective flow must be continuous within the continuous medium of the sample, the flow speed should be reduced when cross-sectional area becomes larger (or vice versa). This feature is particularly important with regard to enhancing the polymerization efficiency. The polymerization step typically takes place during the downward flow (i.e., after the annealing step), and therefore time period for the polymerization step can be lengthened by making the downward flow slower as compared to that of the upward flow, leading to more efficient PCR amplification.

Thus in one invention embodiment, at least part of the channel 70 (including the entire channel) has a horizontal shape along a plane essentially perpendicular to the channel axis 80. In one invention example, the horizontal shape has at least one reflection ($\sigma$) or rotation symmetry element ($C_x$) in which X is 1, 2, 3, 4, up to $\infty$ (infinity). Nearly any horizontal shape is acceptable provided it satisfies intended invention objectives. Further acceptable horizontal shapes include a circular, rhombus, square, rounded square, ellipsoid, rhomboid, rectangular, rounded rectangular, oval, semi-circular, trapezoid, or rounded trapezoid shape along the plane. If desired, the plane perpendicular to the channel axis 80 can be within the first 20, second 30 or third 40 heat source.

None of the foregoing horizontal channel profiles are mutually exclusive. That is, a channel that has a first portion that is circular, for instance, and a second portion that is semi-circular (with respect to the channel axis 80) is within the scope of the present invention.

***Horizontal Chamber Shape And Position***

As discussed, an apparatus of the invention can include at least one chamber, preferably one, two or three chambers to help control the temperature distribution within the apparatus, for instance, within the transition region of the channel. The channel can have one or a combination of suitable shapes provided intended invention results are achieved.

For instance, Figures 7A-I show suitable horizontal profiles of a chamber (the first chamber 100 is used as an illustration only). In this invention embodiment, the horizontal profile of the chamber 100 may be made into various different shapes although shapes that are essentially symmetric will often be useful to facilitate the fabrication process. For instance, the first chamber 100 may have a horizontal shape that is circular, square, or rounded square as depicted in the left column. See Figures 7A, D, and G. The first chamber 100 may have a horizontal shape that has its width larger than its length (or vice versa), for instance, an ellipsoid, rectangular, or rounded rectangular as depicted in the middle column. The first chamber 100 may have a horizontal shape that has its one side narrower than the opposite side as depicted in the right column. See Figures 7C, F, and I.

As discussed, chamber structure is useful in controlling (typically reducing) the heat transfer from the heat source (typically the second heat source) to the channel or the reaction vessel. Therefore, it is important to change the position of the first chamber 100 relative to that

of the channel 70 depending on the invention embodiment of interest. In one embodiment, the first chamber 100 is disposed symmetrically with respect to the position of the channel 70, i.e., the chamber axis (an axis formed by the center points of the top and bottom end of the chamber, 106) coincides with the channel axis 80. In this embodiment, the heat transfer from the heat source 20, 30 or 40 to the channel is intended to be constant in all directions across the horizontal profile of the channel (at certain vertical location). Therefore, it is preferred to use a horizontal shape of the first chamber 100 that is the same as that of the channel in such embodiments. See Figures 7A-I.

However other embodiments of the chamber structure are within the scope of the present invention. For instance, one or more of the chambers within the apparatus may be disposed asymmetrically with respect to the position of the channel 70. That is the chamber axis 106 formed between the top end and bottom end of a particular chamber may be off-centered, tilted or both off-centered and tilted with respect to the channel axis 80. In this embodiment, one or more of the chamber gaps between the channel 70 and a wall of the chamber will be larger on one side and smaller on the opposite side of that chamber. Heat transfer in such embodiments will be higher in one side of the channel 70 and lower in the opposite side (while it is same or similar in the two opposite sides located along the direction perpendicular to the positions of above two sides). In a particular embodiment, it is preferred to use a horizontal shape of the first chamber 100 that is circular or rounded rectangular. A circular shape is generally more preferred.

Thus in one embodiment of the apparatus, at least part of the first chamber 100 (including the entire chamber) has a horizontal shape along a plane essentially perpendicular to the channel axis 80. See Fig. 7A and Fig. 2A-C, for instance. Typically, the horizontal shape has at least one reflection or rotation symmetry element. Preferred horizontal shapes for use with the invention include those that are circular, rhombus, square, rounded square, ellipsoid, rhomboid, rectangular, rounded rectangular, oval, semi-circular, trapezoid, or rounded trapezoid shape along a plane perpendicular to the channel axis 80. In one embodiment, the plane perpendicular to the channel axis 80 is within the second 30 or third 40 heat source.

It will be appreciated that the foregoing discussion about chamber structure and position will be applicable to more chamber embodiments than the first chamber 100. That is, in an invention embodiment with multiple chambers (e.g., one with the second chamber 110 and/or third chamber 120), these considerations may also apply.

***Asymmetric and Symmetric Channel/Chamber Configurations***

As mentioned, the invention is compatible with a wide variety of channel and chamber configurations. In one embodiment, a suitable channel is disposed asymmetrically with respect to the chamber. Figures 8A-P show some examples of this concept.

In particular, Figures 8A-P show horizontal sections of suitable channel and chamber structures with reference to location of the channel 70 within the chamber 100 (the first chamber 100 is used only for illustrative purposes). Horizontal shapes of the first chamber 100 and channel 70 are shown to be circular or rounded rectangular for instance. The first column (Figs. 8A, E, I and M) shows examples of symmetrically positioned structures. In these embodiments, the chamber axis coincides with the channel axis 70. Therefore, the gap between the first chamber wall (103, solid line) and the channel 70 (dotted line) is the same for the left and right sides, and also for the upper and lower sides, providing a heat transfer from the heat source to the channel that is symmetric in both directions. The second column (Figs. 8B, F, J and N) shows examples of asymmetrically positioned structures. The channel axis 80 is positioned off-centered (to the left hand side) from the chamber axis and the gap between the first chamber wall 103 and the channel 70 is smaller on the left side (while it is the same on the upper and lower sides), providing higher heat transfer from the left side. The third (Figs. 8C, G, K and O) and fourth (Figs. 8D, H, L, and P) columns show other examples of asymmetrically positioned structures that provide more asymmetric heat transfer. The third column (Fig. 8C, G, K and O) shows examples in which the chamber wall is in contact with the channel on one side (the left side). The fourth column (Fig. 8D, H, L, and P) shows examples in which one side (the right side) forms the first chamber 100 while the opposite side (the left side) forms the channel 70. In both examples, heat transfer from the left side is much higher than from the right side. The physically contacting side shown in the third and fourth columns is intended to function as a thermal brake, particularly as an asymmetric thermal brake that provides thermal braking on one side only.

It is thus an object of the invention to provide an apparatus in which at least one of the chambers therein (e.g., one or more of the first chamber 100, second chamber 110, or the third chamber 120) is disposed essentially symmetrically about the channel along a plane that is essentially perpendicular to the channel axis. It is also an object to provide an apparatus in which at least one of the chambers is disposed asymmetrically about the channel and along the plane

that is essentially perpendicular to the channel axis. All or part of a particular chamber(s) can be disposed about the channel axis either symmetrically or asymmetrically as needed. In embodiments in which at least one chamber is disposed asymmetrically about the channel axis, the chamber axis and the channel axis can be off-centered while essentially parallel to each other, tilted or both off-centered and tilted. In a more specific embodiment of the foregoing, at least part of a chamber including the entire chamber is disposed asymmetrically about the channel along a plane perpendicular to the channel axis. In other embodiments, at least part of the channel is located inside the chamber along the plane perpendicular to the channel axis. In one example of this embodiment, at least part of the channel is in contact with the chamber wall along the plane perpendicular to the channel axis. In another embodiment, at least part of the channel is located outside of the chamber along the plane perpendicular to the channel axis and contacting the second or third heat source. For some invention embodiments, the plane perpendicular to the channel axis contacts the second or third heat source.

***Vertical Chamber Shape***

It is also an object of the invention to provide an apparatus in which the second heat source includes at least one chamber, typically one, two or three of same to help control temperature distribution. Preferably, the chamber helps control the temperature gradient of the transition region from one heat source (e.g., the first heat source 20) within the apparatus to another heat source (e.g., the third heat source 40) therein. Various adaptations of the chamber are within the scope of the invention so long as it generates a temperature distribution suitable for the convection-based PCR process of the present invention.

It is an object of the invention to provide an apparatus in which at least part of a chamber (up to and including the entire chamber) is tapered along the channel axis. For instance, and in one embodiment, one or more of the chambers including all of the chambers therein are tapered along the channel axis. In one embodiment, at least part of one or all of the chambers is positioned within the second heat source and has a width ($w$) perpendicular to the channel axis that is greater towards the third heat source than the first heat source. In some embodiments, at least part of the chamber is positioned within the second heat source and has a width ($w$) perpendicular to the channel axis that is greater towards the first heat source than the third heat source. In one embodiment, the apparatus includes the first chamber and the second chamber positioned within the second heat source, the first chamber having a width ($w$) perpendicular to

the channel axis that is larger (or smaller) than the width ($w$) of the second chamber. For some embodiments, the first chamber is facing the first or the third heat source.

***Further Illustrative Apparatus Embodiments***

Suitable heat source, insulator, channel, gap, chamber, receptor hole configurations and PCR conditions are described throughout the present application and may be used as needed with the following invention examples.

*A. Tapered Chamber*

Referring now to Figures 9A-B, the apparatus embodiment features a first chamber 100 that is concentric with the channel. In this example of the invention, the chamber axis (i.e., an axis formed by the centers of the top and bottom end of the chamber) coincides with the channel axis 80. The chamber wall 103 of the first chamber 100 has an angle with respect to the channel axis 80. That is, the chamber wall 103 is tapered from the top end 101 to the bottom end 102 of the first chamber 100 (Fig. 9A). In Figure 9B, the chamber wall 103 is tapered from the bottom end 102 to the top end 101 of the first chamber 100. Such a structure provides a narrow hole on the bottom and a wide hole on the top, or vice versa. For instance, if the bottom part is made narrower, as in Fig. 9A, heat transfer from the bottom part 32 of the second heat source 30 to the channel 70 becomes larger than that from the top part 31 of the second heat source 30. Moreover, the high denaturation temperature typical of the first heat source 20 is more preferentially blocked compared to that of the relatively low annealing temperature of the third heat source 40. If the top part of the second heat source 31 is made narrower, as in Fig. 9B, the effect of the third heat source will be more preferentially blocked.

In the examples shown in Figures 9A-B, the temperature distribution of the channel 70 inside the second heat source 30 can be controlled with the tapered chamber structure. Depending on the temperature property of DNA polymerase used, the temperature conditions inside the second heat source 30 may need to be adjusted using such structure because the polymerization efficiency is sensitive to the temperature conditions inside the second heat source 30. For most widely used *Taq* DNA polymerase or its derivatives, a first chamber wall 103 that is tapered from the top to the bottom is more preferred since optimum temperature of *Taq* DNA polymerase (around 70°C) is closer to the annealing temperature compared to the denaturation temperature in typical operation conditions.

*B. One or Two Chambers, One Thermal Brake*

Referring now to Figure 10A, the apparatus 10 features the first chamber 100 and the second chamber 110 disposed in the second heat source 30 essentially symmetrically about the channel axis 80. In this embodiment, the first chamber 100 is located on the bottom part of the second heat source 30 and the second chamber 110 is located on the top part of the second heat source 30. The apparatus 10 includes the first thermal brake 130 to help provide more active control of the temperature distribution. In this embodiment, the width of the first chamber 100 and the second chamber 110 are about the same. However, the heights of the first chamber 100 and the second chamber 110 can be varied between about 0.2 mm to about 80% or 90% of the length of the second heat source 30 along the channel axis 80, depending on the temperature property of DNA polymerase used as discussed below. Figure 10B provides an expanded view of the first thermal brake 130 defined by the top end 131, bottom end 132, and wall 133 contacting the channel 70. In this embodiment, the location and thickness of the first thermal brake 130 along the channel axis 80 will be defined by the heights of the first 100 and second 110 chambers along the channel axis 80. The thickness of the thermal brake 130 along the channel axis 80 is between about 0.1 mm to about 80% of the height of the second heat source 30 along the channel axis 80, preferably between about 0.5 mm to about 60% of the height of the second heat source 30. The first thermal brake 130 can be located nearly anywhere inside the second heat source in between the first 100 and second 110 chambers, depending on temperature property of DNA polymerase used. It is preferred to locate the first thermal brake 130 closer to the bottom surface 32 of the second heat source 30 if optimum temperature of DNA polymerase used is closer to the annealing temperature of the third heat source 40 than the denaturation temperature of the first heat source 20, or vice versa.

Figure 10C is an example in which the first chamber 100 has a smaller width than the second chamber 110, for instance, about 0.9 to about 0.3 times smaller, preferably about 0.8 to about 0.4 times smaller. An opposite arrangement with the first chamber 100 having a larger width than the second chamber 110 can also be used depending on the temperature property of DNA polymerase used. An expanded view of the first thermal brake 130 is shown in Figure 10D.

In the embodiments shown in Figures 10A-D, the apparatus features the first chamber and the second chamber that are not tapered. In these embodiments, the first chamber is spaced from the second chamber by a length ($l$) along the channel axis 80. In one embodiment, the first chamber, the second chamber, and the second heat source define a first thermal brake contacting

the channel between the first and second chambers with an area and a thickness (or a volume) sufficient to reduce heat transfer from the first heat source or to the third heat source.

Referring to Figures 10E-F, the apparatus features the first chamber 100 disposed symmetrically about the channel axis 80. The first thermal brake 130 is positioned on the bottom of the second heat source 30 between the first chamber 100 and the first insulator 50.

The thickness of the first thermal brake 130 along the channel axis 80 shown in Figures 10E-F is defined by distance from the top end 131 to the bottom end 132 of the first thermal brake 130. Preferably that distance is between from about 0.1 mm to about 80% of the height of the second heat source 30 along the channel axis 80, more preferably about 0.5 mm to about 60% of the height of the second heat source 30.

In this embodiment, the apparatus features the first chamber positioned on the bottom part of the second heat source and the first chamber and the first insulator define the first thermal brake. The first thermal brake contacts the channel between the first chamber and the first insulator with an area and a thickness (or a volume) sufficient to reduce heat transfer from the first heat source. In this embodiment, the first thermal brake comprises a top surface and a bottom surface in which the bottom surface of the first thermal brake is located at about the same height as the bottom surface of the second heat source. This embodiment is particularly useful when using DNA polymerase that has optimum temperature closer to the annealing temperature of the third heat source than the denaturation temperature of the first heat source (e.g., *Taq* DNA polymerase).

*C. One, Two or Three Chambers, Two Thermal Brakes*

As mentioned, it will be useful in some invention embodiments to reduce intrusion of the temperature profile from one or more of the heat sources within the apparatus, for instance from the first and third heat sources. In this embodiment, it will be generally useful to include two thermal brakes.

Referring now to Figure 11A, the apparatus 10 includes the first chamber 100, the first thermal brake 130 and the second thermal brake 140. In this example, the first thermal brake 130 is located on a lower part of the first chamber 100 to block or reduce the heat transfer from the first heat source 20. The second thermal brake 140 is located on an upper part of the first

chamber 100 to further block or reduce heat transfer from the third heat source 40. Figure 11B shows an expanded view of the first thermal brake 130 and the second thermal brake 140 within the apparatus. The thickness of each thermal brake along the channel axis 80 can be varied depending on use. However, each thermal brake 130 and 140 is preferably at least about 0.1 mm, more preferably at least about 0.2 mm. The sum of the thickness of the two thermal brakes 130, 140 is smaller than about 80% of the height of the second heat source along the channel axis, more preferably smaller than about 60% of same. Dimensions of each of thermal brakes 130 and 140 can be the same or different depending on intended use of the apparatus.

Figure 4A shows a related embodiment. In this embodiment, the apparatus 10 includes the first chamber 100, the first thermal brake 130, the second chamber 110, the second thermal brake 140 and the third chamber 120. In this example, the first thermal brake 130 is located on a lower part in between the first chamber 100 and the second chamber 110 to block or reduce the heat transfer from the first heat source 20. The second thermal brake 140 is located on an upper part in between the second chamber 110 and the third chamber 120 to further block or reduce heat transfer from the third heat source 40. Figure 4B shows an expanded view of the first thermal brake 130 and the second thermal brake 140 within the apparatus. The thickness of each thermal brake along the channel axis 80 can be varied depending on use. However, each thermal brake 130 and 140 is preferably at least about 0.1 mm, more preferably at least about 0.2 mm. The sum of the thickness of the two thermal brakes 130, 140 is smaller than about 80% of the height of the second heat source along the channel axis, more preferably smaller than about 60% of same. Dimensions of each of thermal brakes 130 and 140 can be the same or different depending on intended use of the apparatus.

In other embodiments, the apparatus 10 can include two chambers and two thermal brakes in the second heat source. In one embodiment, the first thermal brake is located on the bottom of the second heat source in between the first chamber and the first insulator, and the second thermal brake is located in between the first and second chambers within the second heat source. In another embodiment, the first chamber is located on the bottom of the second heat source and the first thermal brake is located in between the first and second chambers. In this embodiment, the second thermal brake is located on the top of the second heat source in between the second chamber and the second insulator.

*D. One Chamber, First and Second Heat Sources, Protrusion*

In some invention embodiments, it will be useful to manipulate the structure of one or more of the chambers by changing the structure of at least one of the heat sources. For instance, at least one of the first, second and third heat sources can be adapted to include one or more protrusions that defines the gap or chamber and generally extends essentially parallel to the channel or chamber axis. A protrusion may be disposed symmetrically or asymmetrically about the channel or chamber axis. Significant protrusions extend away from one heat source to another heat source within the apparatus. For example, second heat source protrusions extend away from the second heat source in the direction toward the first heat source or the third heat source. In these embodiments, the protrusion contacts the chamber and defines a chamber gap or chamber wall. In a particular embodiment, the width or diameter of the second heat source protrusions along the channel axis is decreased as going away from the second heat source while the width of the first or second insulator adjacent to the protrusion along the channel axis is increased. Each chamber may have the same or different protrusion (including no protrusion). An important advantage of the protrusions is to help reduce the size of the heat sources and lengthen chamber dimensions and insulator or insulating gap dimensions along the channel axis. These and other benefits were found to assist thermal convection PCR in the apparatus while substantially reducing the power consumption of the apparatus.

A particular embodiment of an invention apparatus with protrusions is shown in Figure 12A. The apparatus includes protrusions (33, 34) of the second heat source 30 disposed essentially symmetrically about the channel axis 80. Importantly, there is a gap between the bottom of the second heat source 32 and the top of the first heat source 21. In this embodiment, the first heat source 20 also includes protrusions 23, 24 that are disposed symmetrically about the channel 70 and extending from the first heat source 20 to the second heat source 30 or away from the bottom surface of the first heat source 22. Also in this embodiment, the width or diameter of the first heat source protrusions 23, 24 along the channel axis 80 is reduced as going away from the first heat source 20. The apparatus also includes a thermal brake 130 positioned between the first chamber bottom end 102 and the bottom surface 32 of the second heat source 30. As also shown in Figure 12A, the second heat source 30 includes a protrusion 34 that is disposed symmetrically about the channel 70 and extends from the second heat source 30 to the third heat source 40. Also in this embodiment, there is a gap between the top of the first chamber 101 and the bottom of the third heat source 41.

As is also shown in Figure 12A, the receptor hole 73 is disposed symmetrically about the channel axis 80. In this embodiment, the receptor hole 73 has a width or diameter perpendicular to the channel axis 80 that is about the same as the width or diameter of the channel 70. Alternatively, the receptor hole 73 may have a width or diameter perpendicular to the channel axis 80 that is somewhat larger (for example, about 0.01 mm to about 0.2 mm larger) than the width or diameter of the channel 70.

As discussed, it is an object of the invention to provide an apparatus for performing thermal convection PCR which includes at least one temperature shaping element which in one embodiment can be a positional asymmetry imposed on the apparatus. Figure 12B shows one important example of this embodiment. As shown, the apparatus is tilted at an angle $\theta g$ (tilting angle) with respect to the direction of gravity. This type of embodiments is particularly useful in controlling (typically increasing) speed of the thermal convection PCR. As will be discussed below, increase of the tilting angle typically leads to faster and more robust thermal convection PCR. Other embodiments that include one or more positional asymmetries will be described in more detail below.

The embodiments shown in Figures 12A-B will be particularly suitable for many invention applications including amplification of "difficult" samples such as genomic or chromosomal target sequences or long-sequence target templates (e.g., longer than about 1.5 or 2 kbp). In particular, Figure 12A shows heat sources with a symmetric chamber and channel configuration. The thermal brake 130 effectively blocks protrusion of the high temperature of the first heat source 20 toward inside the first chamber 100 as it is located on the bottom of the second heat source 32. In use, the temperature drops down rapidly in the first insulator region 50 from the high denaturation temperature (about 92°C to about 106°C) of the first heat source 20 to the polymerization temperature (about 75°C to about 65°C) of the second heat source 30. The temperature drop from the second heat source 30 to the third heat source (about 45°C to about 65°C) in the second insulator region 60 is relatively small in typical conditions. Hence, the temperature inside the second heat source 30 becomes more narrowly distributed around the polymerization temperature of the second heat source 30 (due to the early cut off of the high denaturation temperature by the first thermal brake) so that a large volume (and time) inside the second heat source 30 becomes available for the polymerization step.

A major difference between the embodiments shown in Figures 12A and 12B is that the

apparatus of Figure 12B has a tilting angle $\theta g$. The apparatus without the tilting angle (Figure 12A) works well and takes about 15 to 25 min to amplify from a 1 ng plasmid sample and about 25 to 30 min to amplify from a 10 ng human genome sample (3,000 copies) when the structure of the apparatus is optimized. PCR amplification efficiency of the apparatus can be further enhanced if a tilting angle of about $2^{o}$ to about $60^{o}$ (more preferably about $5^{o}$ to about $30^{o}$) is introduced as depicted in Figure 12B. With the gravity tilting angle introduced with this structure (Figure 12B), PCR amplification from a 10 ng human genome sample can be completed in about 20 to 25 min. See Examples 1 and 2 below.

*E. Asymmetric Receptor Hole*

As mentioned, it is an object of the invention to provide an apparatus with at least one temperature shaping element that has horizontal asymmetry. By "horizontal asymmetry" is meant asymmetry along a direction or plane perpendicular to the channel and/or channel axis. It will be apparent that many of the apparatus examples provided herein can be adapted to have a horizontal asymmetry. In one embodiment, the receptor hole is placed asymmetrically in the first heat source with respect to the channel axis sufficient to generate a horizontally asymmetric temperature distribution suitable for inducing a stable, directed convection flow. Without wishing to be bound to theory, it is believed that the region between the receptor hole and the bottom end of the chamber is a location where a major driving force for thermal convection flow can be generated. As will be readily apparent, this region is where initial heating to the highest temperature (i.e., the denaturation temperature) and transition toward a lower temperature (i.e., the polymerization temperature) take place, and thus the largest driving force can originate from this region.

It is thus an object of the invention to provide an apparatus with at least one horizontal asymmetry in which at least one of the receptor holes (for instance, all of them) in the first heat source has a width or diameter larger than the channel in the first heat source. Preferably, the width disparity allows the receptor hole to be off-centered from the channel axis. In this example of the invention, the receptor hole asymmetry produces a gap in which one side of the receptor hole is located closer to the channel compared to the opposite side. It is believed that in this embodiment, the apparatus will exhibit horizontally asymmetric heating from the first heat source to the channel.

An example of such an invention apparatus is shown in Figure 13. As shown, the receptor hole 73 is disposed asymmetrically with respect to the channel axis 80 to form a receptor hole gap 74. That is, the receptor hole 73 is slightly off-centered with respect to the channel axis 80, for instance, by about 0.02 mm to about 0.5 mm. In this example, the receptor hole 73 has a width or diameter perpendicular to the channel axis 80 that is larger than the width or diameter of the channel 70. For example, the width or diameter of the receptor hole 73 can be about 0.04 mm to about 1 mm larger than the width or diameter of the channel 70.

Turning again to the embodiment shown in Figure 13, one side (the left side) of the channel 70 is in contact with the first heat source 20 and the opposite side (the right side) is not in contact with the first heat source 20, to form a receptor hole gap 74. While the invention is compatible with several gap sizes, a typical receptor hole gap can be as small as about 0.04 mm, particularly if the other side is contacted to the channel. In other words, one side is formed as a channel and the opposite side as a small space. In this embodiment, it is believed that one side (the left side) is heated preferentially over the opposite side (the right side), providing a horizontally asymmetric heating directing the upward flow to the preferentially heated side (the left side). A similar effect can be obtained with a receptor hole having a gap from the wall of the receptor hole that is smaller on one side than the opposite side.

As shown in Figure 13, the first protrusion 33 of the second heat source 30 defines a portion 51 of the first insulator 50 (called a first insulator chamber) and the second heat source 30. As shown, the first protrusion 33 also separates the first insulator 50 from the chamber 100 and the channel 70. The second protrusion 34 of the second heat source 30 also defines a portion of the first chamber 100 or the channel 70. In this embodiment, the second protrusion 34 also defines a portion 61 of the second insulator 60 (called a second insulator chamber) and the second heat source 30. In addition, the second protrusion 34 of the second heat source 30 separates the second insulator 60 from the first chamber 100 and the channel 70.

*F. Multiple Chambers, Second And Third Heat Sources*

As discussed, the invention provides an apparatus for performing thermal convection PCR which includes at least one, two or three chambers up to about four or five of such chambers. In one embodiment, one, two or three of such chambers can be symmetrically positioned partially or entirely within the second heat source, the third heat source or both the second and third heat sources. Examples are provided in Figures 14A-C.

In particular, Figure 14A shows an apparatus in which the first chamber 100 is disposed symmetrically within the second heat source 30 and a second chamber 110 is disposed symmetrically within the third heat source 40 (with respect to the channel axis 80). The bottom end 102 of the first chamber 100 contacts the bottom 32 of the second heat source 30. Turning to Figure 14C, the apparatus also shows the first chamber 100 disposed symmetrically within the second heat source 30 and a second chamber 110 is disposed symmetrically within the third heat source 40 (with respect to the channel axis 80). However, the first chamber 100 does not contact the bottom 32 of the second heat source 30. Instead, it has a shorter length with respect to the channel axis 80 i.e., the bottom end 102 of the first heat source 100 contacts the interior of the second heat source 30. In both the embodiments of Figures 14A and 14C, the receptor hole 73 is disposed symmetrically about the channel axis 80. However unlike the embodiment shown in Figure 14A, the apparatus of Figure 14C includes the first thermal brake 130 positioned between the bottom 102 of the first chamber 100 and the bottom 32 of the second heat source. This position of the first thermal brake 130 will be useful for many invention embodiments to reduce or block undesired heat flow from the first heat source 20.

Figure 14B shows an invention embodiment in which the first chamber 100 and the second chamber 110 are disposed symmetrically within the second heat source 30 (with respect to the channel axis 80). This apparatus further includes the third chamber 120 disposed symmetrically within the third heat source 40 (also with respect to the channel axis 80). In this embodiment, the receptor hole 73 is disposed symmetrically about the channel axis 80. In this embodiment, the first thermal brake 130 is positioned between the first chamber 100 and the second chamber 110 to help reduce or block undesired heat flow from the first heat source 20 and/or to the third heat source 40 depending on its thickness and position along the channel axis 80.

*G. One Chamber, Second or Third Heat Source*

Also provided by the invention is an apparatus in which at least one chamber (e.g., one, two or three chambers) is positioned within the third heat source. If desired, the length of at least one of the heat sources along the channel axis can be reduced when compared to the embodiment shown in Figure 2A. Alternatively, and in addition, the length of at least one of the heat sources along the channel axis can be increased.

Turning now to Figure 15A, the first chamber 100 is positioned entirely within the third heat source 40 and it is disposed symmetrically with respect to the channel axis 80. In the embodiment shown in Figure 15B, the first heat source 20 includes a protrusion 23 that is disposed symmetrically about the channel 70, thereby forming a larger insulating gap between the first heat source 20 and the second heat source 30 in the regions between adjacent protrusions 23.

If desired, the third heat source 40 can also include a protrusion 43 that is disposed symmetrically about the channel 70 and extending toward the top 31 of the second heat source 30. In such embodiment, a larger insulating gap can be formed between the second heat source 30 and the third heat source 40 in the regions between adjacent protrusions 43. In these embodiments, the length of the second heat source 30 along the channel axis 80 is larger than about 1 mm, preferably between about 2 mm to about 6 mm, and the length of the third heat source 40 along the channel axis 80 is between about 2 mm to 20 mm, preferably between about 3 mm to about 10 mm. The receptor hole 73 is preferably disposed symmetrically about the channel in Figure 15A. Preferred lengths of the first and second insulators have already been described.

In the embodiment shown in Figures 16A-C, the second heat source 30 includes a protrusion 33 that extends away from the second heat source 20 toward the first heat source 20. The second heat source 20 further includes a protrusion 34 that extends toward the third heat source 40. In this example of the invention, each of the protrusions (33, 34) is disposed symmetrically about the first chamber 100 and channel axis 80. In this embodiment, the protrusion 33 helps define the first chamber 100 or the channel 70, the first insulator 50, and the second heat source 30 , and separate the first insulator 50 from the first chamber 100 or the channel 70. The protrusion 34 helps define the first chamber 100 or the channel 80, the second insulator 60 and the second heat source 30, and separate the second insulator 60 from the first chamber 100 or the channel 70.

In the embodiment shown, the top 101 and bottom 102 ends of the first chamber 100 are essentially perpendicular to the channel axis 80. The length of the first chamber 100 is between about 1 mm to about 25 mm, preferably between about 2 mm to about 15 mm. Additionally, the receptor hole 73 is symmetrically disposed about the channel 70 and channel axis 80.

Referring to the embodiment shown in Figures 17A-C, the first heat source 20 includes a protrusion 23 extending away from the first heat source 20 and toward the second heat source 30. Protrusion 23 and receptor hole 73 are each disposed symmetrically about the channel axis 80. Also in this embodiment, the apparatus 10 features protrusions 33, 34 that extend from the second heat source 30 toward the first heat source 20 or the third heat source 40 and disposed symmetrically about the first chamber 100 and channel axis 80. The apparatus 10 also features a third heat source protrusion 43 that is symmetrically disposed about the first chamber 100 and the channel axis 80. The protrusion 43 extends from the third heat source 40 toward the second heat source 30. In this embodiment, the protrusion 23 helps define the channel 70, the first insulator 50 and the first heat source 20 , and separate the first insulator 50 from the channel 70. The protrusion 43 helps define the channel 80, the second insulator 60 and the third heat source 40, and separate the second insulator 60 from the channel 70. The top end of the first chamber 101 and the bottom end of the first chamber 102 are essentially perpendicular to the channel axis 80. A gap separates the protrusion 23 from the bottom end of the first chamber 102. Another gap separates the top end of the first chamber 101 from the protrusion 43. Additionally, the receptor hole 73 is symmetrically disposed about the channel 70 and channel axis 80.

*H. One Chamber in Second Heat Source, Tilted*

As mentioned, it is an object of the invention to provide an apparatus in which various temperature shaping elements such as one or more of the channel, receptor hole, protrusion (if present), gap such as a chamber, insulators or insulating gaps, and thermal brake are each disposed symmetrically about the channel axis. In use, the apparatus will often be placed on a flat, horizontal surface so that the channel axis will be substantially aligned with the direction of gravity. In such an orientation, it is believed that a buoyancy force is generated by the temperature gradient inside the channel and that the buoyancy force also becomes aligned parallel to the channel axis. It is also believed that the buoyancy force will have its direction opposite to the direction of gravity with a magnitude proportional to the temperature gradient (along the vertical direction). Since the channel and the one or more chambers are symmetrically disposed about the channel axis in this embodiment, it is believed that the temperature distribution (i.e., distribution of the temperature gradient) generated inside the channel should also be symmetric with respect to the channel axis. Therefore, distribution of the buoyancy force should also be symmetric with respect to the channel axis with its direction parallel to the channel axis.

It is possible to introduce a horizontal asymmetry into the apparatus by moving the channel axis away from the direction of gravity. In these embodiments, it is possible to further enhance the efficiency and speed of convection-based PCR within the apparatus. Thus it is an object of the invention to provide an apparatus featuring one or more horizontal asymmetries.

Examples of an invention apparatus with positional horizontal asymmetry are provided by Figures 18A-B.

In Figure 18A, the channel axis 80 is offset with respect to the direction of gravity to give the apparatus a positional horizontal asymmetry. In particular, the channel and chamber are formed symmetrically with respect to the channel axis. However the whole apparatus is rotated (or tilted) by an angle $\theta_g$ with respect to the direction of gravity. In this tilted structure, the channel axis 80 is no longer parallel to the direction of gravity, and thus the buoyancy force generated by the temperature gradient on the bottom of the channel becomes tilted with respect to the channel axis 80 since it is supposed to have a direction opposite to the direction of gravity. Without wishing to be bound to theory, the direction of the buoyancy force makes an angle $\theta_g$ with the channel axis 80 even if the channel/chamber structure is symmetric with respect to the channel axis 80. In this structural arrangement, the upward convection flow will take a route on one side of the channel or the reaction vessel (the left side in the case of Figure 18A) and the downward flow will take a route on the opposite side (i.e., the right side in the case of Figure 18A). Hence, the route or pattern of the convection flow is believed to become substantially locked to one determined by such structural arrangement, therefore the convective flow becomes more stable and not sensitive to small perturbations from environment or small structural defects, leading to more stable convection flow and enhanced PCR amplification. It has been found that introduction of the gravity tilting angle helps enhancing the speed of the thermal convection, thereby supporting faster and more robust convection PCR amplification. The tilt angle $\theta_g$ can be varied between from about $2^o$ to about $60^o$, preferably between about $5^o$ to about $30^o$. This tilted structure can be used in combination with all the symmetric or asymmetric channel/chamber structures provided in the present invention.

The tilt angle $\theta_g$ shown in Figure 18A can be introduced by one or a combination of different element. In one embodiment, the tilt is introduced manually. However it will often be

more convenient to introduce the tilt angle $\theta_g$ by placing the apparatus 10 on an incline, for instance, by placing apparatus 10 on a wedge or similar shaped base.

However for some invention embodiments, it will not be useful to tilt the apparatus 10. Figure 18B shows another approach for introducing the horizontal asymmetry. As shown, one or more of the channel and chambers is tilted with respect to the direction of gravity. That is, the channel axis 80 (and the chamber axis) are offset (by $\theta_g$) with respect to an axis perpendicular to the horizontal surface of the heat sources. In this invention embodiment, the channel axis 80 makes an angle $\theta_g$ with respect to the direction of gravity when the apparatus is placed on a flat, horizontal surface to have its bottom opposite from and parallel to that surface (as would be typical). According to this embodiment, and without wishing to be bound to theory, the buoyancy force generated by the temperature gradient on the bottom of the channel (that is supposed to have a direction opposite to the direction of gravity) will make an angle $\theta_g$ with respect to the channel axis as in the case of the embodiments described above. Such a structural arrangement will make the convection flow going upward on one side (i.e., the left side in the case of Figure 18B) and going downward on the opposite side (i.e., the right side in the case of Figure 18B). The tilt angle $\theta_g$ can be varied preferably between from about $2^o$ to about $60^o$, more preferably between about $5^o$ to about $30^o$. This tilted structure can also be used in combination with all the structural features of the channel and the chamber provided in the present invention.

Nearly any of the apparatus embodiment disclosed herein can be tilted by placing it on a structure capable of offsetting the channel axis 80 between from about 2° to about 60° with respect to the direction of gravity. As mentioned, an example of an acceptable structure is a surface capable of producing an incline such as a wedge or related shape.

*I. One Chamber, Asymmetric Receptor Hole*

As discussed, it is within the scope of the present invention to introduce one or more asymmetries within the first heat source to assist thermal convection PCR. In one embodiment, the receptor hole of the first heat source includes one or more structural asymmetries to achieve this objective.

Referring now to invention apparatus of Figure 19, the receptor hole 73 is disposed asymmetrically about the channel axis 80 to form the receptor hole gap 74. Preferably, the

asymmetry is sufficient to cause uneven heat transfer in a horizontal direction from the first heat source 20 to the channel 70. The receptor hole 73 is thus off-centered with respect to the channel axis 80 (by about 0.02 mm to about 0.5 mm). A further preferred receptor hole 73 has a width or diameter perpendicular to the channel axis 80 that is preferably larger than the width or diameter of the channel 70, for example, about 0.04 mm to about 1 mm larger than the width ($w1$ or $w2$) or diameter of the channel 70. As shown, the second heat source 30 of the apparatus has a constant height along the channel axis 80 in the region around the channel 70.

An even larger asymmetry can be obtained when, as shown in Figure 19, one side of the receptor hole is in contact with the channel. In this embodiment, the asymmetry introduced into the apparatus by the receptor hole 73 helps to drive thermal convection although receptor hole configurations with different gap structures, for instance on two opposing sides of the receptor hole 73 are also within the scope of the present invention. In the particular embodiment shown in Figure 19, one side of the channel 70 (e.g., the left side in the case of Figure 19) is heated preferentially over the opposite side due to a better thermal contact with the first heat source 20, and thus a larger driving force is generated on that side, thereby assisting the upward convection flow to go that route. Width or diameter of the receptor hole 73 in this embodiment may be made at least about 0.04 mm up to about 1 mm larger than the channel 70 and the center of the receptor hole may be positioned off-centered at least about 0.02 mm up to about 0.5 mm.

To enhance asymmetry, it is possible to make one side of the receptor hole deeper than the other with respect to the first heat source (and also closer to the chamber and the second heat source). Referring now to the apparatus shown in Figures 20A-B, the receptor hole 73 has a larger depth on one side of the hole (left side) compared to the side opposite to the channel 70 (right side). In this embodiment, both sides of the receptor hole 73 remain in contact with the channel 70. As shown in Figure 20A, the top portion of the side wall of the receptor hole 73 is removed to form a receptor hole gap 74 defined roughly by the channel 70 and the first heat source 20. The bottom of the receptor hole gap 74 may be perpendicular to the channel axis 80 (Fig. 20A) or it may be disposed at an angle thereto (Fig. 20B). A side wall of the receptor hole gap 74 may be parallel to the channel axis 80 (Fig. 20A) or it may be at an angle thereto (Fig. 20B). In both the embodiments shown in Figs. 20A-B, one side of the channel 70 has a larger depth with respect to the first heat source 20 than the other side with the receptor hole gap 74. Without wishing to be bound to theory, it is believed that the channel side with the larger depth in the embodiments shown in Figs. 20A-B is heated preferentially due to more heat transfer

from the first heat source, generating a larger buoyancy force on that side. It is further believed that by adding such an asymmetric receptor hole 73 and receptor hole gap 74 to the apparatus, there is an increase of the temperature gradient on one side of the channel 70 compared to the opposite side (the temperature gradient is typically inversely proportional to the distance). It is also believed that these features create a larger driving force on one side (e.g., the left side in Figures 20A and B) and support upward thermal convective flow along that side. It will be appreciated that one or a combination of different adaptations of the receptor hole 73 and receptor hole gap 74 are possible to achieve this goal. However, for many invention embodiments, it will be generally useful to make difference in the receptor hole depth on two opposing sides in the range of between from about 0.1 mm up to about 40 to 50% of the receptor hole depth.

*J. One Chamber, Asymmetric or Symmetric Receptor Hole, Protrusions*

Figures 21A-B show further examples of suitable apparatus embodiments in which the receptor hole 73 is disposed about the channel asymmetrically. Portions of the receptor hole are deeper in the first heat source and closer to the chamber or the second heat source than other portions, thereby providing uneven thermal flow toward the second heat source.

In the apparatus shown in Figure 21A, the receptor hole 73 has two surfaces coincident with the top 21 of the first heat source 20. Each surface faces the second heat source 30 and one of the surfaces (the one on the right side in Figure 21A) has a larger gap on one side of the channel 70 compared to the surface opposite the channel 70 (the one on the left side) with respect to the bottom surface 32 of the second heat source 30. That is, one of the surfaces is closer to the bottom 102 of the first chamber 100 or the bottom surface 32 of the second heat source 30 than the other. In this embodiment, both sides of the receptor hole 73 remain in contact with the channel 70. The difference of the receptor hole depth between the two surfaces is preferably in the range of between from about 0.1 mm up to about 40 to 50% of the receptor hole depth. The second heat source 30 features protrusions 33, 34 that are each disposed symmetrically about the channel axis 80. Also in this embodiment, the third heat source 40 includes protrusions 43, 44 disposed symmetrically about the channel axis 80.

Turning to Figure 21B, the receptor hole 73 has a single inclined surface coincident with the top 21 of the first heat source 20. The incline angle is between about $2^{o}$ to about $45^{o}$ with respect to an axis perpendicular to the channel axis 80. In this embodiment, the apex of the

inclined surface is relatively close to the bottom 102 of the first chamber 100. The second heat source 30 features protrusions 33, 34 that are each disposed symmetrically about the channel axis 80. Also in this embodiment, the third heat source includes protrusions 43, 44 that are each disposed symmetrically about the channel axis 80.

In the embodiment shown in Figure 22A-B, the first chamber 100 is disposed asymmetrically about the channel axis 80 sufficient to cause horizontally uneven heat transfer from the second heat source 20 to the channel 70. The receptor hole 73 may also be disposed asymmetrically about the channel 70 as in Figs. 21A-B. In the embodiment shown in Fig. 22A, the first chamber 100 is positioned within the second heat source 30 and has a greater height on one side of the chamber than the other side opposite the channel axis 80. That is, the length between one surface of the top end of the first chamber 101 and one surface of the bottom end of the first chamber 102 is greater (left side of Figure 22A) along the channel axis 80 than the length between another surface of the top end of the first chamber 101 and another surface of the bottom end of the first chamber 102 (right side of Figure 22A). The difference of the chamber height between the two opposing sides is preferably in the range of between from about 0.1 mm up to about 5 mm. There is gap between the bottom 101 of the first chamber 100 (or the bottom surface of the second heat source) and the top end of the receptor hole 73 that is smaller on the left side of the channel 70 than the other side.

Turning to Figure 22B, the bottom end 102 of the first chamber 100 is inclined with respect to an axis perpendicular the channel axis 80 by from about $2^{o}$ to about $45^{o}$. In the example, the apex of the incline is further closer to the receptor hole 73. The top of the receptor hole 73 coincident with the top surface 21 of the first heat source 20 is inclined with respect to the channel axis 80. In this embodiment, the apex of the receptor hole incline is closer to the bottom end of the first chamber 102. That is, there is gap between the bottom of the first chamber 100 (or the bottom surface of the second heat source) and the top end of the receptor hole 73 that is smaller on the left side of the channel 70 than the other side.

The configurations shown in Figures 22A-B provide preferential heating on one side of the channel 70 (i.e., the left side) in the receptor hole 73, and thus initial upward convective flow can start preferentially on that side. However, the second heat source 30 provides preferential cooling on the same side due to the longer chamber length on that side. Therefore, the upward

flow can change its path to the other side depending on the extent of the first chamber asymmetry.

Turning to Figures 22C-D, the length between the top end 101 and the bottom end 102 is greater on one side of the first chamber 100 (the right side) than the other side with respect to the channel axis 80. Here, preferential cooling from the second heat source will be on the right side of the chamber shown in Figures 22C-D. Further asymmetry is provided by the larger depth of the receptor hole 73 on one side of the channel 70 (i.e., the left side of Figs. 22C-D) than the other side. In the receptor hole 73, preferential heating will be on the left side of the channel 70. In this embodiment, a gap between the bottom 102 of the chamber 100 and the top of the receptor hole 73 is essentially constant around the channel 70.

The configurations shown in Figures 22C-D support preferential heating on one side of the channel 70 (i.e., the left side) in the receptor hole 73 and preferential cooling on the opposite side in the first chamber 100, and thus upward convective flow will stay preferentially on the left side.

In the embodiments shown in Figures 22A-D, asymmetry introduced by the chamber configurations is sufficient to cause horizontally uneven heat transfer from the second heat source to the channel. Also in these embodiments, the protrusions 23, 33 are disposed asymmetrically with respect to the channel axis 80 and the protrusion 43 is disposed symmetrically about the channel axis 80. Also in these embodiments, the apparatus includes a first insulator 50 and a second insulator 60 in which the length of the first insulator 50 along the channel axis 80 is greater than the length of the second insulator 60 along the channel axis 80.

Other apparatus embodiments with at least one structural asymmetry are within the scope of the present invention.

For example, and as shown in Figures 23A-B, the bottom end of the first chamber 102, is asymmetrically disposed with respect to the channel axis 80. The length between the top end 101 and the bottom end 102 is greater on one side of the first chamber 100 (the left side of the Figs. 23A-B) than the other side with respect to the channel axis 80. A gap between the bottom of the first chamber 102 and the top of the receptor hole 73 is smaller on one side of the channel 70 (the left side of Figs. 23A-B) than the other side. In these embodiments, the protrusion 23 is

disposed symmetrically about the channel axis 80. Also in these embodiments, there is preferential heating on the right side of the receptor hole 73 (with respect to the channel axis 80) due to the larger gap on that side (since cooling by the second heat source is less significant on that side due to the larger gap) and thus a larger driving force is generated on the right side of the channel 70 and more pronounced upward flow on that side. In addition, the second heat source 30 features a protrusion 33 disposed asymmetrically about the channel axis 80. In this embodiment, the second heat source features a protrusion 34 that is disposed asymmetrically about the channel axis 80. The third heat source includes protrusions 43, 44 that are disposed symmetrically about the channel axis 80. Also in the embodiments shown in Figs. 23A-B, the apparatus includes a first insulator 50 and a second insulator 60 in which the length of the first insulator 50 along the channel axis 80 is greater than the length of the second insulator 60 along the channel axis 80.

Other apparatus embodiments with at least one structural asymmetry are within the scope of the present invention.

In the apparatus embodiments shown in Figure 24A-B, the second heat source 30 features protrusions (33, 34) that are each disposed asymmetrically around the channel axis 80. In the embodiment shown in Figure 24A, the bottom end 102 of the first chamber 100 is inclined by between from about $2^{o}$ to about $45^{o}$ with respect to an axis perpendicular to the channel axis 80 so that a portion of the bottom end 102 is closer to the first heat source 20 than another portion opposite the channel axis 80. In this embodiment, a gap between the bottom end 102 and the first heat source 20 is smaller on one side of the channel axis 80 than the other side. In this embodiment, none of the first 20 and third 40 heat sources includes a protrusion extending toward the second heat source 30. Additionally, the top end of the first chamber 101 is inclined by between about $2^{o}$ to about $30^{o}$ with respect to an axis perpendicular to the channel axis 80.

In Fig. 24B, a surface of the bottom end of the first chamber 102 is positioned closer to the first heat source protrusion 23 than another surface of the bottom end 102. In this embodiment, a gap is smaller between the bottom end 102 of the first chamber 100 and the top of the receptor hole 73 on one side (on left side). Also in Fig. 24B, the third heat source 40 features a protrusion 43 disposed symmetrically about the channel 70. The first chamber 100 features a top end 101 with two surfaces in which one surface is positioned closer to the third heat source protrusion 43 (left side) than the other surface.

In the apparatus embodiments shown in Figures 24A-B, initial upward convective flow is favored along the right side of the channel 70 as a result of preferential heating from the receptor hole 73 on that side (due to less significant cooling by the second heat source as a result of the larger insulating gap on that side). Depending on the extent of the asymmetry on the top part of the first chamber, the upward flow can change its path to the opposite side (i.e., the left side) since preferential cooling from the first heat source 40 takes place on the right side due to the larger second insulating gap on that side. In both embodiments, the length of the first insulator 50 parallel to the channel axis 80 is longer than the length of the second insulator 60 parallel to the channel axis 80.

*K. Asymmetric Chambers*

As discussed, it is an object of the present invention to provide an apparatus within one, two or three chambers in the second heat source, for example. In one embodiment, at least one of the chambers has a horizontal asymmetry. The asymmetry helps create a horizontally asymmetric driving force within the apparatus. For example, and in the embodiment shown in Figure 25, the first chamber 100 and the second chamber 110 are each off-centered from the channel axis 80 along opposite directions. In particular, the top end of the first chamber 101 is positioned at essentially at the same height as the bottom end of the second chamber 112. The first and second chambers may have different width or diameter. Difference of the chamber gap 105, 115 on two opposite sides may be at least about 0.2 mm up to about 4 to 6 mm.

In addition to the off-centered chamber structures exemplified in Figure 25, one or more of the chambers may be made horizontally asymmetric by including structures that are tilted (skewed) with respect to the channel axis 80. For instance, and as shown in Figure 26, the first chamber 100 may be tilted with respect to the channel axis 80. In this embodiment, the first wall of the first chamber 103 is tilted with respect to the channel axis 80 (e.g., at an angle less than about 30° with respect to the channel axis 80). Tilt angle as defined by an angle between the center axis of the chamber (or the chamber wall 103) and the channel axis may be between from about $2^{o}$ to about $30^{o}$, more preferably between from about $5^{o}$ to about $20^{o}$.

In the apparatus embodiments shown in Figures 25 and 26, upward convective flow from the bottom of the channel 70 is favored along the right side of the channel 70 as a result of preferential heating from the receptor hole 73 on that side (due to less significant cooling by the

second heat source as a result of the larger chamber gap on that side). Similarly, downward flow from the top of the channel 70 is favored along the left side of the channel 70 as a result of preferential cooling from the third heat source 40 or the through hole 71 (due to less significant heating by the second heat source 30 as a result of the larger chamber gap on that side).

Referring now to the apparatus embodiments shown in Figure 27A-B, the top end 101 and/or bottom end 102 of the first chamber 100 may be structured to provide different gaps (from the third or first heat source) on two opposite sides of the channel axis 80. For instance, and referring to Figure 27A, the top 101 and/or bottom end 102 of the first chamber 100 may be inclined from about $2^{o}$ to about $30^{o}$ with respect to an axis perpendicular to the chamber axis (or the channel axis 80). Alternatively, the first chamber 100 can have multiple top and bottom end surfaces as shown in Figure 27B.

In the embodiments shown in Figures 27A-B, a gap between the bottom end of the first chamber 102 and the top end of the first heat source 21, and between the top end of the first chamber 101 and the bottom end of the third heat source 42 is different on two opposite sides (i.e., the left and right sides in Figures 27A-B). Hence, similar to the embodiments shown in Figures 25 and 26, upward convective flow from the bottom of the channel 70 is favored along the right side of the channel 70 as a result of preferential heating from the receptor hole 73 on that side (due to less significant cooling by the second heat source as a result of the larger insulating gap on that side). Downward flow from the top of the channel 70 is favored along the left side of the channel 70 as a result of preferential cooling from the third heat source 40 or the through hole 71 (due to less significant heating by the second heat source 30 as a result of the larger insulating gap on that side).

In the embodiments shown in Figures 27A-B, protrusions 33, 34 are disposed asymmetrically about the first chamber 100 with respect to the channel axis 80. Additionally, the receptor hole 73 is disposed symmetrically about the channel axis 80. The embodiment shown in Figure 27B further includes protrusions 23 and 43 disposed symmetrically about the channel axis 80.

*L. Two Chambers, Asymmetric Thermal Brake(s)*

It is an object of the invention to provide an apparatus with one or more thermal brakes, e.g., one, two or three thermal brakes in which one or more of them have horizontal asymmetry.

Referring to the apparatus shown in Figures 28A-B, the first thermal brake 130 has horizontal asymmetry. In this embodiment, the through hole formed in the first thermal brake 130 (that typically is made to fit with the channel) is larger than the channel 70 and off-centered from the channel axis 80 to provide a smaller (or no) gap on one side and a larger gap on the opposite side. Temperature distribution is found to be more sensitive to the asymmetry in the thermal brake compared to the asymmetry in the chamber (i.e., asymmetry in the first chamber wall 103). Preferably, the through hole in the thermal brake may be made at least about 0.1 mm up to about 2 mm larger, and off-centered from the channel axis by at least about 0.05 mm up to about 1 mm.

In embodiments in which the structural asymmetry resides in the first thermal brake 130 or the second thermal brake 140 (or both the first 130 and second 140 thermal brakes), the apparatus can include at least one chamber that is disposed symmetrically or asymmetrically about the channel axis 80. In the embodiment shown in Figure 28A, the first chamber 100 and the second chamber 110 are positioned within the second heat source 30 and disposed symmetrically about the channel axis 80. In this embodiment, the first chamber 100 is spaced from the second chamber 110 by a length *l* along the channel axis 80. A portion of the second heat source 30 contacts the channel 70 to form the first thermal brake 130 sufficient to reduce heat transfer from the first heat source 20 or to the third heat source 40. The first thermal brake 130 is disposed asymmetrically about the channel 70. The first thermal brake 130 contacts one side of the channel 70 between the first 100 and second 110 chambers, the other side of the channel 70 being spaced from the second heat source 30. Figure 28B shows an expanded view of the first thermal brake 130 showing wall 133 contacting the channel 70 on the left side. When the structural asymmetry is associated with one or more of the thermal brakes, the upward and downward convective flow can be favored on one side of the channel or the opposite side with respect to the channel axis depending on the position and thickness of the thermal brakes along the channel axis.

*M. One or Two Asymmetric Chambers with and without Thermal Brake(s)*

Referring to Figure 29A, the first chamber 100 is off-centered with respect to the channel axis 80. In this embodiment, the receptor hole 73 is disposed symmetrically about the channel axis 80 and is of constant depth. The first chamber 100 is off-centered from the channel 70 so that the chamber gap 105 is smaller on one side compared to the opposite side. As shown in Figure 29B, the chamber 100 can be further off-centered from the channel 70 so that one side or wall of the channel 70 makes contact with the chamber wall. In this embodiment, the channel-

forming side (e.g., the left side in Figure 29B) functions as a first thermal brake 130 having its top 131 and bottom 132 ends coincide with the top 101 and bottom 102 end of the first chamber 100. In such an embodiment, heat transfer between the second heat source 30 and the channel 70 is larger on the side where the chamber gap 105 is smaller or does not exist (i.e., the left side in Figures 29A and 29B), thus producing a horizontally asymmetric temperature distribution. Figure 29C provides an expanded view of the first thermal brake 130. An acceptable difference between the chamber gaps on two opposite sides is preferably in the range between from about 0.2 mm to about 4 to 6 mm, and hence the chamber axis is off-centered from the channel axis by at least about 0.1 mm up to about 2 to 3 mm.

It will be appreciated that all or part of a chamber can be made asymmetric with respect to the channel axis 80, for example, all or part of the chamber may be off-centered. For most invention applications, it will be useful to off-center an entire chamber.

It will sometimes be useful to have an invention apparatus with one, two, or three chambers disposed in the second heat source either symmetrically or asymmetrically about the channel axis 80. In one embodiment, the apparatus has a first, second, and third chamber in which one or two of the chambers is disposed asymmetrically about the channel axis 80 and the other chamber is disposed symmetrically about the same axis. In an embodiment in which the apparatus includes a first chamber and second chamber that are each disposed asymmetrically about the channel axis 80, those chambers can reside completely or partially within the second heat source.

Particular examples of this invention embodiment are shown in Figures 30A-D.

In Figure 30A, the first thermal brake 130 contacts part of the height of the channel 70 within the second heat source 30. The first chamber 100 and the second chamber 110 are each positioned in the second heat source 30 and the first chamber 100 is spaced from the second chamber 110 by a length ($l$) along the channel axis 80. In this embodiment, the thermal brake 130 contacts the whole circumference of the channel 70 on the length ($l$) between the first 100 and second 110 chambers. The first chamber 100 and the second chamber 110 are each off-centered from the channel axis 80 in the same horizontal direction. Figure 30B provides an expanded view of the first thermal brake 130 in which wall 133 contacts the channel 70.

Turning to Figure 30C, the first chamber 100 and the second chamber 110 are each off-centered from the channel axis in the same horizontal direction. The first 100 and second 110 chambers can have the same or different width or diameter. In this embodiment, the first thermal brake 130 contacts one side of the channel 70 (i.e., the left side) within the first chamber 100 on a length from the bottom end 132 to the top end 131 of the first thermal brake 130 that is the same as the length of the first chamber 100 along the channel axis 80 in the embodiment shown in Figure 30C. Figure 30D provides an expanded view of the first thermal brake 130 showing wall 133 contacting the channel 70.

In each of the embodiments shown in Figures 30A-D, the receptor hole 73 is disposed symmetrically about the channel 70.

Figure 31A shows an invention embodiment in which the first chamber 100 and the second chamber 110 are each off-centered in opposite directions with respect to the channel axis 80 by about 0.1 mm up to about 2 to 3 mm. The first thermal brake 130 is symmetrically disposed with respect to the channel axis 80. In this embodiment, a portion of the second heat source 30 contacts the channel 70 to form a first thermal brake 130 sufficient to reduce heat transfer from the first heat source 20 or to the third heat source 40. In this example of the invention, the first thermal brake 130 contacts the whole circumference of the channel 70 on a length ($l$) between the first 100 and second 110 chambers. In other embodiments, the first thermal brake 130 can contact the channel 70 on one side, the other side being spaced from the second heat source 30. Figure 31B provides an expanded view of the first thermal brake 130 showing wall 133 contacting the channel 70.

Referring to the embodiment shown in Figures 32A, the first chamber 100 and second chamber 110 are each off-centered with respect to the channel axis 80 in the same horizontal direction (e.g., by about 0.1 mm up to about 2 to 3 mm). In this embodiment, the first thermal brake 130 is asymmetrically disposed with respect to the channel axis 80. The first thermal brake 130 and the chamber wall 103 are off-centered to the same direction. In this embodiment, the first thermal brake 130 contacts the channel 70 on one side (i.e., the left side), the other side being spaced from the second heat source 30. Figure 32B shows an expanded view of the first thermal brake 130.

In Figure 32C, the first chamber 100 and the second chamber 110 are each off-centered with respect to the channel axis 80 in the same horizontal direction and the first thermal brake 130 is off-centered to the opposite direction. In this embodiment, the first thermal brake 130 contacts the channel 70 on one side (i.e., the right side), the other side being spaced from the second heat source 30. Figure 32D shows an expanded view of the first thermal brake 130.

In another invention embodiment, the apparatus has two chambers in the second heat source 30 in which each chamber is off-set from the other in different horizontal directions. Figure 33A shows an example. Here, the first chamber 100 and second chamber 110 within the second heat source 30 are each off-set with respect to the channel axis 80 in opposite horizontal directions (e.g., by about 0.5 mm to about 2 to 2.5 mm). The wall of the first chamber 103 is disposed lower along the channel axis 80 than the wall of the second chamber 113. The wall of the first thermal brake 133 contacts one side of the channel 70 (i.e., the left side) on the lower part of the channel 70 within the first chamber 100, and the wall of the second thermal brake 143 contacts the other side of the channel (i.e., the right side) on the upper part of the channel 70 within the second chamber 110. The top end of the first thermal brake 131 is positioned essentially at the same height as the bottom end of the second thermal brake 142. This arrangement is generally sufficient to cause horizontally uneven heat transfer between the second heat source 30 and the channel 70. Figure 33B shows an expanded view of the first thermal brake 130 and the second thermal brake 140.

Figure 33C shows an invention embodiment in which the top end of the first thermal brake 131 is positioned higher than the bottom end of the second thermal brake 142. The wall of the first thermal brake 133 and the wall of the second thermal brake 143 each contact the channel 70 on one side. Figure 33D shows an expanded view of the first thermal brake 130 and the second thermal brake 140.

Figure 33E shows an embodiment in which the top end of the first thermal brake 131 is positioned lower than the bottom end of the second thermal brake 142. The wall of the first thermal brake 133 and the wall of the second thermal brake 143 each contact the channel 70 on one side. Figure 33F shows an expanded view of the first thermal brake 130 and the second thermal brake 140.

The invention provides other embodiments in which an asymmetry is introduced into

the apparatus by tilting (skewing) one or more of the thermal brakes or the chamber with respect to the channel axis. Referring now to Figure 34A, the top end of the first chamber 101 and the bottom end of the second chamber 112 are each inclined between about 2° to about 45° with respect to an axis perpendicular to the channel axis 80. In this embodiment, the distance between the top end of the first heat source 21 and the bottom end of the first thermal brake 132 is smaller on one side (i.e., the left side) with respect to the channel axis 80, resulting in a temperature gradient that is biased to be larger on that side of the first chamber 100. A similar effect can be expected on the opposite side (i.e., the right side) of the second chamber 110 due to the smaller distance on that side between the bottom end of the third heat source 42 and the top end of the first thermal brake 131. The thermal brake 130 contacts the whole circumference of the channel 70 between the first chamber 100 and the second chamber 110 and at a higher location on one side than the other side. Figure 34B shows an expanded view of the first chamber 100, first thermal brake 130 and the second chamber 110 in which wall 133 contacts the channel 70.

In some invention embodiments, it will be useful to tilt at least one of the chambers with respect to the channel axis (e.g., one, two, or three of the chambers). Indeed, different combinations of the tilted or skewed structures may be adopted to achieve the intended horizontally asymmetric temperature distribution. A few examples are shown in Figures 35A-D.

In particular, Figure 35A shows a case in which the first chamber 100 and the second chamber 110 are each tilted or skewed with respect to the channel axis 80 between about 2° to about 30°. In this embodiment, the first thermal brake 130 is not tilted. Figure 35B shows an expanded view of the first chamber 100, the first thermal brake 130 and the second chamber 110 in which wall 133 contacts the channel 70.

Figure 35C shows an example in which both of the first chamber 100, the second chamber 110, and the first thermal brake 130 are each tilted with respect to the channel axis 80. Each of the first chamber 100 and the second chamber 110 can be tilted or skewed with respect to the channel axis 80 by between about 2° to about 30°. The top end 131 and bottom end 132 of the first thermal brake 130 can be each inclined or tilted by between about 2° to about 45° with respect to an axis perpendicular to the channel axis 80. In this embodiment, the first thermal brake 130 contacts the whole circumference of the channel between the first chamber and the second chamber and at a higher location on one side than the other side.

In the embodiments shown in Figures 31A-B, 32A-D, 33A-F, 34A-B, and 35A-D, the receptor hole 73 is disposed symmetrically about the channel axis 80.

*N. Additional Embodiments*

Additional apparatus embodiments are shown in Figures 36A-C, Figures 37A-C, and Figure 38A-C.

Turning to Figure 36A, the first chamber 100 of the apparatus 10 is within the second heat source 30 and the second chamber 110 is within the third heat source 40. A second heat source protrusion 33 is disposed symmetrically about the channel axis 80. The apparatus 10 further includes a first heat source protrusion 23 disposed symmetrically about the channel axis 80. In this embodiment, the receptor hole 73 is disposed symmetrically about the channel axis 80.

In embodiment shown in Figure 36B, the first chamber 100 of the apparatus 10 and the second chamber 110 are within the second heat source 30. The apparatus further includes a third chamber 120 within the third heat source 40. The apparatus also includes the first thermal brake 130 disposed between the first 100 and second 110 chambers within the second heat source 30. A second heat source protrusion 33 is disposed symmetrically about the channel axis 80. The apparatus further includes a first heat source protrusion 23 disposed symmetrically about the channel axis 80. In this embodiment, the receptor hole 73 is disposed symmetrically about the channel axis 80.

Turning to the embodiment shown in Figure 36C, the bottom of the first chamber 102 is within the second heat source 30. However in the apparatus embodiment shown in Figure 36A, the bottom of the first chamber 102 is coincident with the bottom surface of the second heat source 32. The apparatus shown in Figure 36C includes the first chamber 100 within the second heat source 30 and the second chamber 110 within the third heat source 40. The apparatus further includes the first thermal brake 130 that is disposed on the bottom of the second heat source 30 in between the bottom end of the first chamber 102 and the bottom of the second heat source 32. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

In the embodiments shown in Figures 36A-C, each apparatus further includes a first insulator chamber 51 defined by at least the first heat source 20, the first protrusion of the first heat source 23, the second heat source 30, and the first protrusion of the second heat source 33.

The apparatuses shown in Figures 37A-C further includes a second protrusion of the second heat source 34 disposed symmetrically about the channel axis 80 and a second insulator chamber 61 defined by at least the third heat source 40, the second heat source 30, and the second protrusion of the second heat source 34. In the embodiment shown in Figure 37A, the apparatus includes the first chamber 100 within the second heat source 30 and the second chamber 110 within the third heat source 40. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

Turning to Figure 37B, the apparatus shown features the first chamber 100 and the second chamber 110 positioned within the second heat source 30. The third chamber 120 is within the third heat source 40. The apparatus further includes the first thermal brake 130 located between the first 100 and second 110 chambers within the second heat source 30. In this embodiment, the apparatus 10 includes protrusions (23, 33, 34) that are each disposed symmetrically with respect to the channel axis 80. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

In the embodiments shown in Figures 37A-B, the bottom end of the first chamber 102 contacts the first insulator 50. However in the embodiment shown in Figure 37C, the bottom end of the first chamber 102 is within the second heat source 20 and the first thermal brake 130 is located on the bottom of the second heat source 30 in between the bottom end of the first chamber 102 and the bottom of the second heat source 32. The apparatus shown in Figure 37C also includes protrusions 23, 33, 34 that are each disposed symmetrically about the channel axis 80. Also in the embodiments shown in Figures 37B-C, the first thermal brake 130 is disposed symmetrically with respect to the channel axis 80.

The apparatuses shown in Figures 38A-C further includes a first protrusion of the third heat source 43 disposed symmetrically about the channel axis 80 and a second insulator chamber 61 defined by at least the third heat source 40, the third heat source protrusion 43, the second heat source 30, and the second protrusion of the second heat source 34. In the embodiment shown in Figure 38A, the apparatus includes the first chamber 100 within the second heat source

30 and the second chamber 110 within the third heat source 40. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

In the apparatus embodiment shown in Figure 38B, the first chamber 100 and second chamber 110 are each positioned in the second heat source 30. The third chamber 120 is positioned in the third heat source 40. The apparatus further includes the first thermal brake 130 located between the first 100 and second 110 chambers within the second heat source 30. In this embodiment, the apparatus 10 includes protrusions (23, 33, 34, 43) that are each disposed symmetrically with respect to the channel axis 80. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

In the embodiment shown in Figure 38C, the bottom end of the first chamber 102 is within the second heat source 20 and the first thermal brake 130 is located on the bottom of the second heat source 30 in between the bottom end of the first chamber 102 and the bottom of the second heat source 32. The apparatus shown in Figure 37C also includes protrusions 23, 33, 34, 43 that are each disposed symmetrically about the channel axis 80. The receptor hole 73 is disposed symmetrically with respect to the channel axis 80.

***Manufacture, Use and Temperature Shaping Element Selection***

*A. Heat Sources*

For most invention embodiments, one or more of the heat sources can be made with materials having a relatively low thermal conductivity as compared to materials used for other thermal cycling type apparatuses. Rapid temperature changing process can be usually avoided in the present invention. Therefore, a high temperature uniformity across each of the heat sources (e.g., with a temperature variation smaller than about 0.1°C) can be readily achieved using a material having a relatively low thermal conductivity. The heat sources can be made of any solid material that has a thermal conductivity sufficiently larger than that of the sample or the reaction vessel, for instance, preferably at least about 10 times larger, more preferably at least about 100 times larger. The sample to be heated is mostly water that has a thermal conductivity of 0.58 $W \cdot m^{-1} \cdot K^{-1}$ at room temperature, and the reaction vessel is typically made of a plastic that has a thermal conductivity typically about a few tenths of $W \cdot m^{-1} \cdot K^{-1}$. Therefore, the thermal conductivity of a suitable material is at least about 5 $W \cdot m^{-1} \cdot K^{-1}$ or larger, more preferably at least about 50 $W \cdot m^{-1} \cdot K^{-1}$ or larger. If the reaction vessel is made of a glass or ceramic that has a thermal conductivity larger than that of a plastic, it is preferred to use a material having

somewhat larger thermal conductivity, for instance one having a thermal conductivity larger than about 80 or about 100 $W \cdot m^{-1} \cdot K^{-1}$. Most metals and metal alloys as well as some high thermal conductivity ceramics fulfill such requirement. Although materials having a higher thermal conductivity will generally provide better temperature uniformity across each of the heat sources, aluminum alloys and copper alloys are typically useful materials since they are relatively cheap and easy to fabricate while possessing high thermal conductivity.

The following specifications will be generally useful for making and using apparatus embodiments described herein. The width and length dimensions of the first, second and third heat sources along an axis perpendicular to the channel axis can be selected as any values depending on intended use, for instance, depending on spacing between adjacent channel/chamber structures. The spacing between the adjacent channel/chamber structures can be at least about 2 to 3 mm, preferably between about 4 mm to about 15 mm. It will be generally preferred to use the industry standards, i.e., 4.5 mm or 9 mm spacing. In typical embodiments, the channel/chamber structures are arranged in equally spaced rows and/or columns. In such embodiments, it is preferred to make the width or length (along an axis perpendicular to the channel axis) of each of the heat sources that is at least about the value corresponding to the spacing times the number of rows or columns up to about one to about three spacing larger than this value. In other embodiments, the channel/chamber structures may be arranged in a circular pattern and preferably equally spaced. The spacing in such embodiments is also at least about 2 to 3 mm, preferably about 4 mm to about 15 mm with the industry standards of 4.5 mm or 9 mm spacing more preferred. In these embodiments, it is preferred to have the shape of the heat sources as a donut-like shape typically having a hole in the center. The channel/chamber structures may be positioned on one, two, three, up to about ten concentric circles. Diameter of each concentric circle can be determined by a geometric requirement for intended use, e.g., depending on number of the channel/chamber structures, spacing between adjacent channel/chamber structures in that circle, etc. Outer diameter of the heat sources is preferably at least about one spacing larger than diameter of the largest concentric circle, and inner diameter of the heat sources is preferably at least about one spacing smaller than diameter of the smallest concentric circle.

Length or thickness of the first, second and third heat sources along the channel axis has been already discussed. In the embodiments comprising at least one chamber in the second heat source, the thickness of the first heat source is larger than about 1 mm along the channel axis,

preferably from about 2 mm to about 10 mm. Thickness of the second heat source along the channel axis is between about 2 mm to about 25 mm, preferably between 3 mm to about 15 mm. Thickness of the third heat source along the channel axis is larger than about 1 mm, preferably between about 2 mm to about 10 mm. In other embodiments that include only one chamber that is disposed in the third heat source, the second and third heat sources may have different thickness along the channel axis as compared to the embodiments comprising at least one chamber in the second heat source. For instance, the second heat source has a thickness larger than 1 mm along the channel axis, preferably between about 2 mm to about 6 mm. In these embodiments, thickness of the third heat source along the channel axis is between about 2 to about 20 mm, preferably between about 3 mm to about 10 mm. The first heat source can have a thickness along the channel axis that is within the same range as other embodiments, e.g., larger than about 1 mm, preferably between about 2 mm to about 10 mm.

The channel dimensions can be defined by a few parameters as denoted in Figures 5A-D and 6A-J. The height ($h$) of the channel along the channel axis is at least about 5 mm to about 25 mm, preferably 8 mm to about 16 mm for a sample volume of about 20 microliters. The taper angle ($\theta$) is between from about $0^o$ to about $15^o$, preferably from about $2^o$ to about $10^o$. The width ($w1$) or diameter of the channel (or its average) along an axis perpendicular to the channel axis is at least about 1 mm to about 5 mm. The vertical aspect ratio as defined by the ratio of the height ($h$) to the width ($w1$) is between about 4 to about 15, preferably from about 5 to about 10. The horizontal aspect ratio as defined by the ratio of the first width ($w1$) to the second width ($w2$) along first and second directions, respectively, that are mutually perpendicular to each other and aligned perpendicular to the channel axis, is typically between about 1 to about 4.

The receptor hole has a width or diameter that is in the same range as the channel, i.e., at least about 1 mm to about 5 mm. When the channel is tapered, the width or diameter of the receptor hole is smaller or larger than that of the channel depending on the tapering direction. Depth of the receptor hole is typically at least about 0.5 mm up to about 8 mm, preferably between about 1 mm to about 5 mm.

The chamber typically has a width or diameter along an axis perpendicular to the channel axis that is at least about 1 mm to about 10 or 12 mm, preferably between about 2 mm to about 8 mm. Presence of the chamber structure provide the chamber gap between the channel and the chamber wall that is typically between about 0.1 mm to about 6 mm, more preferably about 0.2

mm to about 4 mm. Length or height of the chamber along the channel axis can vary depending on different embodiments. For instance, if the apparatus comprises one chamber in the second heat source, that chamber can have a height along the channel axis between about 1 mm to about 25 mm, preferably between about 2 mm to about 15 mm. In the embodiments having two or more chambers in the second heat source, the height of each chamber is between about 0.2 mm to about 80% or 90% of the thickness of the second heat source along the channel axis, with the sum of the height of the two or more chambers can be as large as the thickness of the second heat source. In the embodiments having only one chamber that is disposed in the third heat source, the chamber height along the channel axis is in the range between about 0.2 mm up to about 60% or 70% of the thickness of the third heat source along the channel axis.

Dimensions of the thermal brake and the insulators (or insulating gaps) are also very important. Please refer to the general specifications as already provided above.

Although not generally required for optimal use of the invention, it is within the scope of the present invention to provide an apparatus with protrusions 24, 44, or both. See Figure 22C, for example.

It will be appreciated that there usually exists certain tolerance in machining or fabricating mechanical structures. Therefore, in actual practice, the physically contacting holes (e.g., the through hole in the third heat source or the receptor hole in the first heat source in particular embodiments) must be designed to have a positive tolerance with respect to the size of the reaction vessel. Otherwise, the through hole or the channel could be made smaller or equal to the size of the reaction vessel, not allowing proper installation of the reaction vessel to the channel. Practically reliable tolerance for the physically contacting hole is about +0.05 mm in standard fabrication process. Therefore, if two objects are said to be “in physical contact”, it should be interpreted as having a gap between the two contacting objects that is smaller than or equal to about 0.05 mm. If two objects are said to be “not in physical contact”, or “spaced”, it should be interpreted as having a gap between the two objects that is larger than about 0.05 or 0.1 mm.

*B. Use*

Nearly any thermal convection PCR apparatus described herein can be used to perform one or a combination of different PCR amplification techniques. One suitable method includes at least one of and preferably all of the following steps:

(a) maintaining a first heat source comprising a receptor hole at a temperature range suitable for denaturing a double-stranded nucleic acid molecule and forming a single-stranded template,

(b) maintaining a third heat source at a temperature range suitable for annealing at least one oligonucleotide primer to the single-stranded template,

(c) maintaining a second heat source at a temperature suitable for supporting polymerization of the primer along the single-stranded template; and

(d) producing thermal convection between the receptor hole and third heat source under conditions sufficient to produce the primer extension product.

In one embodiment, the method further includes the step of providing a reaction vessel comprising the double-stranded nucleic acid and the oligonucleotide primer(s) in aqueous buffer solution. Typically, the reaction vessel further includes one or more DNA polymerases. If desired, the enzyme may be immobilized. In a more particular embodiment of the reaction method, the method includes a step of contacting (either directly or indirectly) the reaction vessel to the receptor hole, the through hole, and at least one temperature shaping element (typically at least one chamber) disposed within at least one of the second or third heat sources. In this embodiment, the contacting is sufficient to support the thermal convection within the reaction vessel. Preferably, the method further includes a step of contacting the reaction vessel to a first insulator between the first and second heat sources and a second insulator between the second and third heat sources. In one embodiment, the first, second and third heat sources have a thermal conductivity at least about tenfold, preferably about one hundred fold greater than the reaction vessel or aqueous solution therein. The first and second insulators may have a thermal conductivity at least about five fold smaller than the reaction vessel or aqueous solution therein in which the thermal conductivity of the first and second insulators is sufficient to reduce heat transfer between the first, second and third heat sources.

In the step (c) of the foregoing method, the thermal convection fluid flow is produced essentially symmetrically or asymmetrically about the channel axis within the reaction vessel. Preferably, the steps (a)-(d) of the method described above consume less than about 1 W, preferably less than about 0.5 W of power per reaction vessel to produce the primer extension

product. If desired, the power for performing the method is supplied by a battery. In typical embodiments, the PCR extension product is produced in about 15 to about 30 minutes or shorter and the reaction vessel can have a volume of less than about 50 or 100 microliters, for example, less than or equal to about 20 microliters.

In embodiments in which the method is used with a thermal convection PCR centrifuge of the invention, the method further includes the step of applying or impressing a centrifugal force to the reaction vessel conducive to performing the PCR.

In a more specific embodiment of the method for performing PCR by thermal convection, the method includes the steps of adding an oligonucleotide primer, nucleic acid template, and buffer to a reaction vessel received by any of the apparatuses disclosed herein under conditions sufficient to produce a primer extension product. In one embodiment, the method further comprises a step of adding a DNA polymerase to the reaction vessel.

In another embodiment of a method for performing PCR by thermal convection, the method comprising the steps of adding an oligonucleotide primer, nucleic acid template, and buffer to a reaction vessel received by any PCR centrifuge disclosed herein and applying a centrifugal force to the reaction vessel under conditions sufficient to produce a primer extension product. In one embodiment, the method includes the step of adding a DNA polymerase to the reaction vessel.

Practice of the invention is compatible with one or a combination of PCR techniques including quantitative PCR (qPCR), multiplex PCR, ligation-mediated PCR, hot-start PCR, allele-specific PCR among other variations of the amplification technique. The following particular use of the invention is with reference to the embodiment shown in Figures 1 and 2A. As will be appreciated however, the methodology is generally applicable to other embodiments referred to herein.

Referring to Figures 1 and 2A, the first heat source 20 generates a temperature distribution suitable for the denaturation process on the bottom or lower portion of the channel (sometimes referred herein to as a denaturation region). The first heat source 20 is typically maintained at a temperature useful to melt the nucleic acid template of interest (e.g., about 1 fg to about 100 ng of a DNA-based template). In this embodiment, the first heat source 20 should

be maintained at between about 92°C to about 106°C, preferably between about 94°C to about 104°C, and more preferably between about 96°C to about 102°C. As will be appreciated, other temperature profiles may be better suited for optimal practice of the invention depending on recognized parameters such as the nucleic acid of interest, the sensitivity desired, and the speed of which the PCR process should be conducted.

The third heat source 40 generates a temperature distribution suitable for the annealing process on the top or upper portion of the channel (sometimes referred herein to as an annealing region). The third heat source is typically maintained at a temperature between about 45°C to about 65°C, depending, for instance, on the melting temperatures of the oligonucleotide primers used and other parameters known to those with experience in PCR reactions.

The second heat source 30 generates a temperature distribution suitable for the polymerization process in the intermediate region of the channel 70 (sometimes referred herein to as a polymerization region). For many invention applications, the second heat source 30 is typically maintained at a temperature between about 65°C to about 75°C, more preferably between about 68°C to about 72°C, in cases in which *Taq* DNA polymerase or a relatively heat stable derivative thereof is used. If a DNA polymerase that has a different temperature profile of its activity is used, the temperature range of the second heat source can be changed to match with the temperature profile of the polymerase used. See U.S. Pat. No. 7,238,505 and references disclosed therein regarding use of heat sensitive and heat stable polymerases in the PCR process.

See the Examples section for information about use of additional apparatus embodiments.

*C. Selection of Temperature Shaping Elements*

The following section is intended to provide further guidance on the selection and use of temperature shaping elements. It is not intended to limit the invention to a particular apparatus design or use.

Choice of one or a combination of temperature shaping elements for use with an invention apparatus will be guided by the particular PCR application of interest. For instance, properties of the target template are important for selecting temperature shaping element(s) that is/are best suited for a particular PCR application. For instance, the target sequence may be

relatively short or long; and/or the target sequence may have a relatively simple structure (such as in plasmid or bacterial DNA, viral DNA, phage DNA, or cDNA) or a complex structure (such as in genomic or chromosomal DNA). In general, target sequences having longer sequences and/or complex structures are more difficult to amplify and typically require a longer polymerization time. Additionally, longer times for annealing and denaturation are often required. Moreover, the target sequence may be available in a large or small amount. Target sequences in smaller amounts are more difficult to amplify and generally require more PCR reaction time (i.e., more PCR cycles). Other considerations may also be important depending on particular uses. For instance, the PCR apparatus may be used to produce a certain amount of a target sequence for subsequent applications, experiments, or analyses, or else to detect or identify a target sequence from a sample. In further considerations, the PCR apparatus may be used in the laboratory or in the field, or in certain extraordinary environments, for instance, inside a car, a ship, a submarine, or a spaceship; under severe weather conditions, etc.

As discussed, the thermal convection PCR apparatus of the present invention generally provides faster and more efficient PCR amplification than prior PCR apparatuses. Moreover, the invention apparatus has a substantially lower power requirement and a much smaller size than prior PCR apparatuses. For instance, the thermal convection PCR apparatus is typically at least about 1.5 to 2 times faster (preferably about 3 to 4 times faster) and requires at least about 5 times (preferably about ten times to several tens of times) less power for operation with its size or weight at least about 5 to 10 times smaller. Hence, if a suitable design can be selected, users can have an apparatus that can cost much less time, energy, and space.

In order to select a suitable apparatus design, it is important to appreciate the key functions of an intended temperature shaping element. As summarized in Table 1 below, each temperature shaping element has specific functions with regard to the performance of the thermal convection PCR apparatus. For instance, the chamber structure generally increases the speed of the thermal convection within a heat source in which a chamber resides as compared to the structures without the chamber, and the thermal brake generally decreases the speed of the thermal convection as compared to the structures having the chamber structure without the thermal brake. Importantly, however, incorporation of the thermal brake structure in addition to the chamber structure within the second heat source makes the time length or volume of the sample available for the polymerization step larger so that efficiency of the PCR amplification can be increased for target sequences that require a longer polymerization time. Hence, the

chamber structure can be used with or without the thermal brake depending on particular applications as discussed below. As also summarized in Table 1, any one or a combination of the convection accelerating elements (e.g., the positional asymmetry, the structural asymmetry, and the centrifugal acceleration) can be used to increase the speed of the thermal convection regardless of other heat source structures including the channel alone structure (i.e., a structure without the chamber). Hence, at least one or a combination of these convection accelerating elements can be combined with nearly all of the heat source structures in order to enhance the thermal convection speed as needed. As discussed, the invention apparatus requires much less power than prior PCR apparatuses, mainly as a result of eliminating necessity for the thermal cycling process (i.e., the process that changes the temperature of the heat source). As also discussed, a suitable combination of the first and second insulators (i.e., the thickness of the insulating gaps as well as use of a proper thermal insulator) can make the power consumption of the invention apparatus further reduced. Moreover, use of the protrusion structure(s) can still further reduce the power consumption of the invention apparatus substantially (see Examples 1 and 3, for instance) and also to increase the chamber length and thus to increase the polymerization time. Other parameters such as the receptor hole depth and the temperatures of the first, second and third heat sources can also be used to modulate the thermal convection speed and also the time period available for each of the polymerization, annealing and denaturation steps. As discussed below, each of these temperature shaping elements can be used alone or in combination with one or more other elements to construct a particular thermal convection PCR apparatus that is suitable for a particular application.

Table 1. Key Functions of Temperature Shaping Elements

| Temperature Shaping Element | Key Functions |
|---|---|
| Chamber | Increases the thermal convection speed within the heat source in which the chamber resides as compared to the channel alone structure. The smaller the chamber diameter or the chamber gap, the slower is the thermal convection speed. |
| Thermal Brake | Decreases the thermal convection speed when combined with the chamber structure. Typically positioned within the second heat source in combination with at least one chamber and make the time length and volume of the sample available for the polymerization step increase as compared to the chamber only structure. The larger the length of the thermal brake along the channel axis, the slower is the thermal convection speed and the larger time and sample volume becomes available for the polymerization step. |
| Insulator/Insulating gap | Generally required for the multi-stage thermal convection apparatus. Useful to control the thermal convection speed and to |

| | |
|---|---|
| | reduce power consumption. The smaller the length of the insulator along the channel axis, the larger are the power consumption and the driving force for the thermal convection. |
| Protrusion | Useful to reduce power consumption substantially and also to lengthen the chamber length along the channel axis (and thus to increase the time and sample volume available for the polymerization step). |
| Positional Asymmetry | Increases the thermal convection speed and can be incorporated into the invention apparatus as an adjustable structural element so as to provide freedom to control the thermal convection speed within a given design. When used with a structural asymmetry, an adjustable positional asymmetry element can be used as both an accelerating and a decelerating element. |
| Structural Asymmetry | Increases the thermal convection speed. |
| Centrifugal Acceleration | Increases the thermal convection speed while providing freedom to control the thermal convection speed within a given design. Typically used with the positional asymmetry. |

Although many useful apparatus embodiments are provided by the invention, the following combinations are particularly useful and easy to predict the performance of the invention apparatus.

An acceptable thermal convection PCR apparatus for many applications typically includes the channel and the first and second insulators (or the first and second insulating gaps) as basic elements. One or more other temperature shaping elements can be combined to use with these basic elements. An apparatus that uses the channel and the insulators only may not be optimal for some PCR applications. With the channel structure alone, the temperature gradient inside the sample within each heat source may be too small due to efficient heat transfer from the heat sources, and thus thermal convection becomes either too slow or not properly occurring. Use of the chamber structure can remedy this deficiency. As discussed, the speed of the thermal convection within each heat source can be increased by incorporating a chamber structure in that heat source. Thermal convection PCR apparatuses that use the chamber as an additional temperature shaping element are best suited for fast amplification of relatively short target sequences (e.g., shorter than about 1 kbp, preferably shorter than about 500 or 600 bp) having simple structures such as plasmid or bacterial DNA, viral DNA, phage DNA, cDNA, etc. For instance, an apparatus design having a straight chamber in the second heat source with its width or diameter about 3 to 6 mm can deliver PCR amplification of such samples within less than about 25 or 30 min, preferably within less than about 10 to 20 min depending on the amount and size of the target sequence (see Examples 1 and 3, for instance). Further increase of the speed of

the thermal convection PCR could be achieved by incorporating at least one of the convection accelerating elements (e.g., see Examples 2 and 7).

An invention apparatus that includes the chamber (without the thermal brake) is also useful to amplify longer target sequences (e.g., longer than about 1 kbp up to about 2 or 3 kbp) or target sequences having complex structures (e.g., genomic or chromosomal DNAs) as well as the shorter sequences having simple structures. In one type of such embodiments, the chamber(s) resides in the second heat source only or both in the second and third heat sources, and the width or diameter of the chamber located in the second heat source could be reduced (either partially or completely) or an additional chamber having a reduced width or diameter could be incorporated within the second heat source. The reduced chamber width or diameter is typically in the range less than about 3 mm. In such designs, enhanced heat transfer from the second heat source (in the chamber region having the reduced width or diameter) leads to increase of the time length available for the polymerization step, and thus amplification of longer sequences and/or sequences having complex structures becomes efficient. However, use of a reduced chamber width or diameter typically results in decrease of the thermal convection speed. If the convection speed becomes too slow for user's applications, at least one of the convection accelerating elements can be combined to increase the convection speed. In another type of embodiments, the chamber could reside in the third heat source only. In this type of embodiments, use of primers having relatively high melting points (e.g., higher than about 60°C) is typically recommended in order to amplify the different types of the target sequences mentioned above.

As discussed above, the thermal brake is a convection decelerating element and typically makes the polymerization time period longer when combined with the chamber structure typically within the second heat source. Hence, a combination of a thermal brake and the chamber structure within the second heat source is a good design example that can provide a thermal convection speed that is appropriately slow to provide a sufficient polymerization time and also sufficiently fast to deliver fast PCR amplification. As demonstrated in Example 1, a combination of a large width chamber (e.g., the width or diameter of the chamber larger than about 3 mm) and a thin thermal brake (e.g., the length of the thermal brake along the channel axis being less than about 2 mm) is a good example of an apparatus design that can deliver sufficiently fast amplification for both short and long target sequence (e.g., up to about 2 or 3 kbp of plasmid targets) as well as target sequences having complex structures (e.g., up to about 1

kbp or about 800 bp of human genome targets). Importantly, such design provides substantially fast amplification (i.e., within less than about 25 or 30 min, preferably within less than about 10 to 20 min) for the different types of the target sequences without using any of the convection accelerating elements. As also demonstrated, incorporation of a convection accelerating element (e.g., the positional asymmetry in Example 2) can provide further accelerated thermal convection PCR.

Further enhancement of the dynamic range of the thermal convection PCR apparatus can be achieved by incorporating a narrower chamber (e.g., smaller than about 3 mm of the chamber width or diameter) and/or a thermal brake within the second heat source. Use of a chamber having a reduced width or diameter (either partially completely) or a thermal brake within the second heat source leads to enhanced heat transfer from the second heat source to the channel, and hence the thermal convection becomes decelerated. In such decelerated heat source structures, the polymerization time period can be increased so as to amplify longer sequences, for instance, up to about 5 or 6 kbp. However, the total PCR reaction time could be inevitably increased due to a slow thermal convection speed, for instance, about 35 min to up to about 1 hour or longer depending on the size and structure of the target sequence. Any one or more of the convection accelerating elements can also be combined with this type of apparatus designs to increase the speed of the thermal convection PCR as desired.

The convection accelerating elements mentioned above (i.e., the positional asymmetry, the structural asymmetry, and the centrifugal acceleration) can affect the speed of the thermal convection in different degrees. The positional or structural asymmetry can typically enhance the thermal convection speed from about 10% or 20% up to about 3 to 4 times. In the case of the centrifugal acceleration, the enhancement can be made as large as possible, for instance, about 11,200 times at 10,000 rpm when $R$ = 10 cm as discussed. A practically useful range would be up to about 10 to about 20 times enhancement. When any one of these convection accelerating elements is used, the speed of the thermal convection can be increased. Hence, whenever a further increase of the thermal convection speed is needed for the user's applications, such feature can be conveniently incorporated. One particular design that includes at least one of the convection accelerating elements is a heat source structure that does not include the chamber (i.e., the channel only). As demonstrated in Example 6 (see Fig. 76E in comparison with Fig. 75E), use of a convection accelerating element can make the channel alone design operable. Such channel alone design is advantageous since it can provide the time period and volume of

the sample available for the polymerization step that is as largest as possible. However, as discussed, such design delivers a thermal convection speed that is typically too slow. Use of any one or more of the convection accelerating elements can remedy such deficiency by increasing the thermal convection speed as user's demand.

All of the apparatus examples discussed above require much less power than prior PCR apparatuses and can be made as portable devices, i.e., operable with a battery, even without the protrusion structure. As discussed, use of the protrusion structure can reduce the power consumption substantially and thus more recommended if a portable PCR apparatus is essential for the user's applications.

Also, the apparatus designs discussed above can amplify from very low copy number samples (when optimized). For instance, as demonstrated in Examples 1, 2, and 3, target sequences even much less than about 100 copies can be amplified in about 25 min or about 30 min.

Moreover, the apparatus designs discussed above can be used in the laboratory or in the field, or in certain extraordinary conditions, not like many prior PCR apparatuses that can be used only under controlled conditions such as inside a laboratory. For instance, we have tested a few invention apparatuses inside a car while driving and confirmed that fast and efficient PCR amplification can be achieved as inside a laboratory. Furthermore, we also tested a few invention apparatuses under extraordinary temperature conditions (from below about -20°C to above about 40°C) and confirmed fast and efficient PCR amplification regardless of the outside temperatures.

Finally, as exemplified throughout the Examples, the thermal convection PCR apparatuses of the present invention can deliver PCR amplification that is not only fast but also very efficient. Hence, it is demonstrated that the invention apparatuses are generally suitable for nearly all of the diverse different applications of the PCR apparatus while providing enhanced performance with a new feature of a palm-size portable PCR device.

***Apparatus With Housing And Temperature Control Elements***

The invention apparatus referred to above can be used alone or in combination with suitable housing, temperature sensing, and heating and/or cooling elements. In one embodiment shown in Figure 39, the first heat source 20, second heat source 30, and third heat source 40

features at least one first securing element 200 (typically a screw hole) and a second securing element 210 in which each of the elements are adapted to secure the heat sources, the first insulator 50 and second insulator 60 together as a single operable unit. The second securing element 210 is preferably "wing-shaped" to help provide a boundary for additional insulating spaces (see below). Heating and/or cooling elements 160a, 160b, and 160c are each positioned in the first 20, second 30 and third heat sources 40, respectively. Each of the heat sources is typically equipped with at least one heating element. Typically useful heating elements are of resistive heating or inductive heating types. Depending on intended use, one or more of the heat sources can be further equipped with one or more of cooling elements and/or one or more of heating elements. Typically preferred cooling elements are a fan or a Peltier cooler. As well known, the Peltier cooler can function as both a heating and cooling element. It is particularly preferred to use more than one heating elements or both heating and cooling elements in different locations of one or more of the heat sources when a temperature gradient operation is required to provide different temperatures across that heat source. The first 20, second 30 and third heat sources 40 further include temperature sensors 170a, 170b and 170c disposed in each of the heat sources, respectively. For most of the embodiments, each of the heat sources is typically equipped with one temperature sensor. However, in some embodiments such as those with a temperature gradient operation capability in one or more of the heat sources, two or more temperature sensors can be located at different positions of that heat source.

Figures 40A-B provide cross-sectional views of the embodiment shown in Figure 39. In addition to the cross sectional views of the channel and chamber structures, locations of the heating and/or cooling elements are shown as one example. As shown in this example, it is preferred to position the heating and/or cooling elements evenly to each of the heat sources to provide a uniform heating and/or cooling across each of the heat sources. For instance as depicted in Figure 40B, the heating and/or cooling elements are positioned in between each of the channel and chamber structures and equally spaced from each other (see also Figure 42 for instance). The cross-sectional view depicted in Figures 40A, for instance, shows connections (i.e., the circles) between the heating and/or cooling elements from one position in between each of the channel and chamber structures to another. In other types of embodiments such as those with a temperature gradient operation option, two or more of the heating or cooling elements can be used in one or more of the heat sources and positioned to different locations of that heat source to provide a biased heating and/or cooling across that heat source.

In Figure 41, the plane of section is through one of the second securing elements 210 and a first securing element 200. As shown, the first securing element 200 includes a screw 201, washer 202a, securing element of the first heat source 203a, spacer 202b, securing element of the second heat source 203b, spacer 202c, and securing element of the third heat source 203c. Preferably, at least one of and more preferably all of the screw 201, the washer 202a and spacers 202b and 202c are made from a thermal insulator material. Examples include plastics, ceramics, and plastic composites (such as those with carbon or glass fiber). Materials having a high mechanical strength, high melting and/or deflection temperature (e.g., about 100°C or higher, more preferably about 120°C or higher), and low thermal conductivity (e.g., plastics with thermal conductivity smaller than about a few tenths of $W \cdot m^{-1} \cdot 1^{-1}$ or ceramics with thermal conductivity smaller than about a few $W \cdot m^{-1} \cdot K^{-1}$) are more preferred. More specific examples include plastics such as PPS (polyphenylene sulfide), PEEK (polyetherehterketone), Vesper (polyimide), RENY (polyamide), etc. or their carbon or glass composites, and low thermal conductivity ceramics such as Macor, fused silica, zirconium oxide, Mullite, Accuflect, etc.

Figure 42 provides an expanded view of an apparatus embodiment with various securing element and temperature control elements. It will be apparent that in addition to the particular securing structures shown in Figure 42, others are possible. Thus in one embodiment, at least one of the first and/or second securing elements (200, 210) is located in other region(s) of at least one, and preferably all of the first heat source 20, second heat source 30, third heat source 40, first insulator 50, and second insulator 60. That is, although the third heat source 40 is shown to include the second securing element 210, any other or all of the heat sources and/or the insulators could include the second securing element 210. In another embodiment, at least one of the first and/or second securing elements (200, 210) is located in an inner region of at least one, and preferably all of the first heat source 20, second heat source 30, third heat source 40, first insulator 50, and second insulator 60.

Although the forgoing invention embodiments will be generally useful for many PCR applications, it will often be desirable to add protective housing. One embodiment is shown in Figures 43A-B. As shown, the apparatus 10 features a first housing element 300 that surrounds the first heat source 20, the second heat source 30, the third heat source 40, the first insulator 50, and the second insulator 60. In this embodiment, each of the second securing elements 210 has a wing-shaped structure that cooperates with other structural elements of the apparatus 10 to form at least one insulating gap, for example, one, two, three, four, five, six, seven or eight of such

gaps. Each of the gaps can be filled with a suitable insulating material such as those disclosed herein such as a gas or solid insulator. Air will be a preferred insulating material for many applications. Presence of the insulating gap(s) provides advantages such as reducing heat loss from the apparatus 10, thereby lowering power consumption.

Thus in the embodiment shown in Figure 43A-B, the third heat source 40 comprises four second securing elements 210 in which each pair of second securing elements defines a third insulating gap 310. In particular, Figure 43A shows four parts of the third insulating gaps 310 each defined by a first housing element 300 and a pair of the second securing element 210. Figure 43A also shows a fourth insulating gap 320 located between the bottom of the first heat source 20 and the first housing element 300. Also shown is a support 330 for suspending the secured heat sources inside the first housing element 300, thereby helping form the third insulating gap 310 and the fourth insulating gap 320.

It will often be desirable to further house the invention apparatus, for example to provide further protection and insulating gaps. Referring now to Figure 44A-B, the apparatus further includes a second housing element 400 that surrounds the first housing element 300. In this embodiment, the apparatus 10 further includes a fifth insulating gap 410 defined by the first housing element 300 and the second housing element 400. The apparatus 10 can also include a sixth insulating gap 420 located between the bottom of the first housing element 300 and the bottom of the second housing element 400.

If desired, the invention apparatus may further include at least one fan unit to remove heat from the apparatus. In one embodiment, the apparatus comprises a first fan unit positioned above the third heat source 40 to remove heat from the third heat source 40. If desired, the apparatus may further include a second fan unit positioned below the first heat source 20 to remove heat from the first heat source 20.

**Convection PCR Apparatus Incorporating Centrifugal Acceleration**

It is an object of the invention to provide "centrifugal acceleration" as an optional additional feature of the apparatus embodiments described herein. As discussed above, it is believed that thermal convection can be made optimal when a vertical temperature gradient (and optionally or in addition, a horizontally asymmetric temperature distribution when the positional

or structural asymmetry is used) is generated inside a fluid. Proportional to the magnitude of vertical temperature gradient, a buoyancy force is generated that drives a convection flow inside the fluid. Thermal convection generated by an invention apparatus must typically fulfill various conditions for inducing a PCR reaction. For instance, the thermal convection must flow through a plurality of spatial regions sequentially and repeatedly, while maintaining each of the spatial regions at a temperature range suitable for each step of the PCR reaction (i.e., the denaturation, annealing, and polymerization steps). Moreover, the thermal convection must be controlled to have a suitable speed so as to allow suitable time period for each of the three PCR reaction steps.

Without wishing to be bound to any theory, it is believed that thermal convection can be controlled by controlling the temperature gradient, more precisely distribution of the temperature gradient inside the fluid. The temperature gradient ($dT/dS$) depends on temperature difference ($dT$) and distance ($dS$) between two reference positions. Therefore, the temperature difference or distance may be changed to control the temperature gradient. However, in the convection PCR apparatus, neither the temperature (or its difference) nor the distance may be changed easily. The temperature of different spatial regions inside the sample fluid is subject to a specific range as defined by the temperature suitable for each of the three PCR reaction steps. There are not many opportunities to change the temperature of different (typically at least vertically different) spatial regions inside the sample. Furthermore, vertical positions of the different spatial regions (in order to generate a vertical temperature gradient for inducing a buoyant driving force) are usually restricted due to a small volume of the sample fluid. For instance, a typical volume of PCR sample is only about 20 to 50 microliters and sometimes smaller. Such small volumes and space limitations do not allow much freedom to change the vertical positions of the different spatial regions for the PCR reaction.

As discussed, the buoyancy force is proportional to the vertical temperature gradient that in turn depends on temperature difference and distance between two reference points. Further to such dependence, however, the buoyancy force is also proportional to the gravitational acceleration ($g = 9.8$ m/sec$^2$ on Earth). This force field parameter is a constant, a variable that cannot be controlled or changed, but can be only defined by the law of universal gravitation. Therefore, nearly all of the thermal convection based PCR apparatuses rely upon highly restricted special structures, inevitably adapted to gravitational forces.

Use of centrifugal acceleration in accord with the present invention provides a solution for this problem. By making a convection based PCR apparatus subject to a centrifugal acceleration force field, one can control the magnitude of the buoyant driving force regardless of the structure that defines the magnitude of the temperature gradient, thereby controlling the convection speed without much limitation.

Figures 45A-B shows one embodiment of a PCR centrifuge 500 according to the invention. In this example, the apparatus 10 is attached to a rotation arm 520 rotatably attached to motor 501. In this embodiment, the rotation arm 520 includes a tilt shaft 530 for providing freedom of changing the angle between the axis of rotation 510 and the channel axis 80. The PCR centrifuge may include any number of the apparatus 10 provided intended results are achieved, for example, 2, 4, 6, 8, 10 or even 12. The apparatus 10 may or may not include protective housing as discussed above, although having some protective housing will be generally useful.

The tilt shaft 530 is preferably configured to be an angle inducing element capable of tilting the angle of the heat source (more particularly the angle of the channel axis 80) with respect to the rotation axis. Tilt angle can be adjusted depending on the rotation speed (i.e., depending on the magnitude of the centrifugal acceleration) so that the tilt angle between the channel axis 80 and the net acceleration vector depicted in Figure 46 can be adjusted in the range between from about $0^{o}$ to about $60^{o}$. In one embodiment, the angle inducing element in Figure 45A is a rotation shaft (depicted as a circle) in the center of the joint region between the horizontal arm and an arm on which the heat source assembly is located.

In the embodiment shown in Figures 45A-B, the sample fluid inside the reaction vessel placed inside the apparatus 10 is subject to a centrifugal acceleration force in addition to the gravitational acceleration force. See Figure 46. As will be appreciated, the direction of the centrifugal acceleration $g_c$ is perpendicular to (and outward from) the axis of the centrifugal rotation, and its magnitude is given by an equation $g_c = R\,\omega^2$, where $R$ is the distance from the axis of the centrifugal rotation to the sample fluid and $\omega$ is angular velocity in radian/sec. For instance, when $R$ = 10 cm and speed of the centrifugal rotation is 100 rpm (corresponding to $\omega$ = about 10.5 radian/sec), magnitude of the centrifugal acceleration is about 11 $m/sec^2$, similar to the gravitational acceleration on Earth. Since the centrifugal acceleration is proportional to

square of the rotation speed (or square of the angular velocity), the centrifugal acceleration increases quadratically with increase of the rotation speed, for instance, about 4.5 times of the gravitational acceleration at 200 rpm, about 112 times at 1,000 rpm, and about 11,200 times at 10,000 rpm when $R$ = 10 cm. The magnitude of the net force field that acts on the sample fluid can be controlled freely by adopting such centrifugal acceleration. Therefore, the buoyancy force can be controlled (typically increased) as needed so as to make the convection speed as fast as needed. Practically, there are few limitations for inducing the thermal convection to very high flow speed sufficient for very high speed PCR reaction, provided a small vertical temperature gradient can be generated in the sample fluid. Therefore, prior limitations regarding heat source assembly and use can be minimized or avoided when combined with centrifugal acceleration in accord with the invention.

As depicted in Figure 46, the sample fluid is subject to the net force field generated by addition of the centrifugal acceleration and the gravitational acceleration. In a typical embodiment, the channel axis 80 is aligned parallel to the net force field or made to have a tilt angle $\theta_c$ with respect to the net force field. As discussed, presence of the tilt angle is generally preferred in order to make the convection flow stay in a stable route. The tilt angle $\theta_c$ ranges from about $2^o$ to about $60^o$, more preferably about $5^o$ to about $30^o$.

It will be appreciated that the apparatus embodiment used to exemplify the PCR centrifuge 500 is shown in Figures 1 and 2A-C. However, the PCR centrifuge 500 is compatible with use of one or a combination of different invention apparatuses as described herein. In particular, the PCR centrifuge 500 can also be used with nearly any type of heat source structure and reaction vessel described herein provided that a small vertical temperature gradient can be generated inside the sample. For example, nearly any of the heat source structures described above and elsewhere (e.g., WO02/072267 to Benett et al. and U.S Pat. No. 6,783,993 to Malmquist et al.) may be combined with the centrifugal element of the present invention so as to enhance the amplification speed and performance of the apparatus. Moreover, other heat source structures that cannot be made operable (or that cannot be made to provide a high PCR amplification speed) in typical gravitationally driven mode can be made operable when combined with the centrifugal acceleration structure. For instance, a heat source structure that does not include a chamber as described herein but only comprises the channel structure may also be made operable. See PCT/KR02/01900, PCT/KR02/01728 and U.S. Patent No. 7,238,505, for example. In this embodiment, the prior heat source structures without the chamber provides a

temperature distribution inside the second heat source that changes slowly, presumably due to a high heat transfer from the second heat source. A result is a small temperature gradient within the second heat source. With only gravity, thermal convection will be unsatisfactory or too slow for many PCR applications. However, introduction of centrifugal acceleration in accord with the invention can make thermal convection sufficiently fast and stable so as to induce the PCR reaction successfully and efficiently.

In typical operation of the thermal convection PCR centrifuge 500, the axis of rotation 510 is essentially parallel to the direction of gravity. See Figure 46. In this embodiment, the channel axis 80 is essentially parallel to, or tilted with respect to the direction of net force generated by the gravitational force and the centrifugal force. That is, the channel axis 80 can be tilted with respect to the direction of net force generated by the gravitational force and the centrifugal force. For most embodiments, the tilt angle $\theta_c$ between the channel axis 80 and the direction of the net force is between about $2^o$ to about $60^o$. The tilt shaft 530 is adapted to control the angle between the channel axis 80 and the net force. In operation, the axis of rotation 510 is usually located outside of the first 20, second 30, and third 40 heat sources. Alternatively, the axis of rotation 510 is located essentially at or near the center of the first 20, second 30, and third 40 heat sources. In these embodiments, the apparatus 10 includes a plurality of channels 70 that are located concentrically with respect to the axis of rotation 510.

*Circular-shaped Heat Sources*

In another embodiment of the thermal convection PCR centrifuge, one or more of the heat sources has a circular or semi-circular shape. Figures 47A-B, 48A-C, 49A-B, and 50A-C show particular embodiments of such a heat source structure.

Figures 47A-B show vertical sections of a particular embodiment of a centrifugally accelerated convection PCR apparatus. In particular, Figures 47A and 47B show cross-sections along the channel and securing element regions, respectively. The two sections are defined in Figures 48A-C which depict horizontal top view of the first 20, second 30 and third 40 heat sources, respectively. As depicted in Figures 47A-B, the three circular shape heat sources are assembled to form an apparatus embodiment rotatably attached to the rotation axis 510 of a PCR centrifuge 500 through a rotation arm 520. The center of the heat source assembly is positioned concentric with respect to the rotation axis 510 so that the radius of centrifugal rotation is defined by the horizontal length of the rotation arm from the rotation axis to the center of the

channel 70. The three heat sources 20, 30 and 40 are assembled essentially parallel to each other with the top of one heat source facing the bottom of an adjacent heat source. As also depicted, the heat source assembly is oriented with respect to the rotation axis such that the channel axis 80 is aligned either parallel to, or tilted from the net acceleration vector depicted in Figure 46.

The three heat sources depicted in Figures 48A-C are assembled using a set of first securing element comprising a screw 201, spacers or washers 202a-c, and securing apertures 203a-c formed in the heat sources as depicted in Figures 47B. A second securing element 210 formed in the third heat source 40 shown in Figures 47B and 48C is used to install the apparatus within the first housing element 300.

Nearly any of the apparatus embodiments disclosed in the present application (including various channel and chamber structures) can be used with the centrifugally accelerated thermal convection PCR apparatus described herein. However, an apparatus without any chamber structure can also be used. Figures 49A and 50A-C show an example in which each of the heat sources are adapted to provide a channel only, i.e., channel 70 formed as a hole having a closed bottom end in the first heat source 20, and extending through the second heat source 30 to the third heat source 40. As another embodiment, Figure 47A shows a vertical section of an example in which a chamber structure 100 having a first thermal brake 130 on the bottom of the second heat source is used in combination with the channel structure. Figure 48B shows a horizontal top view of the second heat source comprising the chamber 100 and the first thermal brake 130 as used in the example of Figure 47A. The first and third heat sources have the same structures as Figures 50A and 50C, respectively.

In one embodiment of the forgoing thermal convection PCR centrifuge, the device is made portable and preferably operated with a battery. The embodiment shown in Figures 45A-B can be used for high throughput large scale PCR amplification, for example. In this embodiment, the apparatus can be used as a separable module and thus can be easily loaded and unloaded to the centrifuge unit.

## REACTION VESSELS

A suitable channel of the apparatus is adapted to hold a reaction vessel within the apparatus so that intended results can be achieved. In most cases, the channel will have a configuration that

is essentially the same as that of a lower part of the reaction vessel. In this embodiment, the outer profile of the reaction vessel, particularly the lower part, will be essentially identical to the vertical and horizontal profiles of the channel. The upper part of the reaction vessel (i.e., toward the top end) may have nearly any shape depending on intended use. For instance, the reaction vessel may have a larger width or diameter on the upper part to facilitate introduction of a sample and may include a cap to seal the reaction vessel after introduction of a sample to be subjected to thermal convection PCR.

In one embodiment of a suitable reaction vessel, and referring again to Figure 5A-D, the outer profile of the reaction vessel can be identical to the profile of the channel 70 up to the top end 71 of the channel 70. The shape or profile of inside of the reaction vessel may have a shape different from that of outside of the reaction vessel (if wall thickness of the reaction vessel is made to vary). For instance, the outer profile of the horizontal section may be circular while the inner profile is ellipsoidal, or vice versa. Different combinations of outer and inner profiles are possible as far as the outer profile is suitably selected to provide proper thermal contact with the heat sources, and the inner profile is suitably selected for an intended thermal convection pattern. In typical embodiments, however, the reaction vessel has a wall thickness that is about constant or does not vary much, i.e., the inner profile is typically identical or similar to the outer profile of the reaction vessel. Typical wall thickness ranges between from about 0.1 mm to about 0.5 mm, more preferably between from about 0.2 mm to about 0.4 mm, although it can vary depending on the material used.

If desired, the vertical profile of the reaction vessel may also be shaped to form a linear or tapered tube to fit with the channel as shown in Figures 5A-D. When tapered, the reaction vessel may be tapered either from the top to the bottom or from the bottom to the top, although a reaction vessel that is (linearly) tapered from the top to the bottom is generally preferred as in the case of the channel. Typical taper angle $\theta$ of the reaction vessel is in the range between from about $0^{o}$ to about $15^{o}$, more preferably from about $2^{o}$ to about $10^{o}$.

The bottom end of the reaction vessel may also be made flat, rounded, or curved as for the bottom end of the channel depicted in Figures 5A-D. When the bottom end is rounded or curved, it can have a convex or concave shape with its radius of curvature equal to or larger than the radius or half width of the horizontal profile of the bottom end. Flat or near flat bottom end is more preferred over other shapes since it can provide an enhanced heat transfer so as to facilitate

the denaturation process. In such preferred embodiments, the flat or near flat bottom end has a radius of curvature that is at least two times larger than the radius or half width of the horizontal profile of the bottom end.

Also if desired, horizontal profile of the reaction vessel may also be made into various different shapes although a shape having certain symmetry is generally preferred. Figures 6A-J shows a few examples of the horizontal profile of the channel having certain symmetry. An acceptable reaction vessel may be made to fit these shapes. For instance, the reaction vessel may have its horizontal shape that is circular (top, left), square (middle, left), or rounded square (bottom, left) generally the same as that shown for the channel 70 in Figures 6A, D, G, and J. Thus, the reaction vessel may have a horizontal shape that has its width larger than its length (or vice versa), for instance, an ellipsoid (top, middle), rectangular (middle, middle), or rounded rectangular (bottom, middle) that is generally the same as that depicted in the middle column of Figures 6B, E, and H for the channel 70. This type of horizontal shape for the reaction vessel is useful when incorporating a convection flow pattern going upward on one side (e.g., on the left hand side) and going downward on the opposite side (e.g., on the right hand side). Due to the relatively larger width profile incorporated compared to the length, interference between the upward and downward convection flows can be reduced, leading to more smooth circulative flow. The reaction vessel may have a horizontal shape that has its one side narrower than the opposite side. A few examples are shown on the right column of Figures 6A-J for the shape of the channel. In particular, the reaction vessel may be made so that the left side of the reaction vessel is narrower than the right side for instance, as shown in Figures 6C, F and I for the channel 70. This type of horizontal shape is also useful when incorporating a convection flow pattern going upward on one side (e.g., on the left hand side) and going downward on the opposite side (e.g., on the right hand side). Moreover, when this type of shape is incorporated, speed of the downward flow (e.g., on the right hand side) can be controlled (typically reduced) with respect to the upward flow. Since the convective flow must be continuous within the continuous medium of the sample, the flow speed should be reduced when cross-sectional area becomes larger (or vice versa). This feature is particularly important with regard to enhancing the polymerization efficiency. The polymerization step typically takes place during the downward flow (i.e., after the annealing step), and therefore time period for the polymerization step can be lengthened by making the downward flow slower as compared to that of the upward flow, leading to more efficient PCR amplification.

Further examples of suitable reaction vessels are provided in Figures 51A-D. As shown, the reaction vessel 90 includes a top end 91 and a bottom end 92 which ends include center points that define a central reaction vessel axis 95. The reaction vessel 90 is further defined by an outer wall 93 and an inner wall 94 which surround a region for holding a PCR reaction mixture. In Figures 51A-B, the reaction vessel 90 is tapered from the top end 91 to the bottom end 92. A generally useful taper angle ($\theta$) is in the range between from about $0^o$ to about $15^o$, preferably from about $2^o$ to about $10^o$. In the embodiment shown in Figure 51A, the reaction vessel 90 has a flat or near flat bottom end 92 while in the example shown in Figure 52B, the bottom end is curved or rounded. The top 71 and bottom 72 ends of the channel are marked in Figures 51A-D.

Figures 51C-D provide examples of suitable reaction vessels with straight walls from the top end 91 to the bottom end 92. The reaction vessel 90 shown in Figure 51C has a flat or near flat bottom end 92 while in the example shown in Figure 51D, the bottom end is curved or rounded.

Preferably, the vertical aspect ratio of the outer wall 93 of the reaction vessel 90 shown in Figures 51A-D is at least about 4 to about 15, preferably from about 5 to about 10. The horizontal aspect ratio of the reaction vessel is defined by the ratio of the height ($h$) to the width ($w1$) up to the position corresponding to the top end 71 of the channel 70 as in the case of the channel. The horizontal aspect ratio of the outer wall 93 is typically about 1 to about 4. The horizontal aspect ratio is defined by the ratio of the first width ($w1$) to the second width ($w2$) of the reaction vessel along first and second directions, respectively, that are mutually perpendicular to each other and aligned perpendicular to the channel axis. Preferably, the height of the reaction vessel 90 along the reaction vessel axis 95 is at least between about 6 mm to about 35 mm. In this embodiment, the average of the width of the outer wall is between about 1 mm to about 5 mm, and that of the inner wall of the reaction vessel is between about 0.5 mm to about 4.5 mm.

Figures 52A-J show horizontal cross-sectional views of suitable reaction vessels for use with the invention. The invention is compatible with other reaction vessel configurations provided intended results are achieved. Accordingly, the horizontal shape of an acceptable reaction vessel can be one or a combination of circle, semi-circle, rhombus, square, rounded square, ellipsoidal, rhomboid, rectangular, rounded rectangular, oval, triangular, rounded

triangular, trapezoidal, rounded trapezoidal or oblong shape. In many embodiments, the inner wall is disposed essentially symmetrically with respect to the reaction vessel axis. For example, the thickness of the reaction vessel wall can be between about 0.1 mm to about 0.5 mm. Preferably, the thickness of the reaction vessel wall is essentially unchanged along the reaction vessel axis 95.

In one embodiment of the reaction vessel 90, the inner wall 94 is disposed off-centered with respect to the reaction vessel axis 95. For instance, the thickness of the reaction vessel wall is between about 0.1 mm to about 1 mm. Preferably, the thickness of the reaction vessel wall is thinner on one side than the other side by at least about 0.05 or 0.1 mm.

As discussed, bottom end of a suitable reaction vessel can be flat, curved or rounded. In one embodiment, the bottom end is disposed essentially symmetrically with respect to the reaction vessel axis. In another embodiment, the bottom end is disposed asymmetrically with respect to the reaction vessel axis. The bottom end may be closed and can include or consist of a plastic, ceramic or a glass. For some reactions, the reaction vessel may further include an immobilized DNA polymerase. Nearly any reaction vessel described herein can include a cap in sealing contact with the reaction vessel.

In embodiments where a reaction vessel is used with a thermal convection PCR centrifuge of the invention, relatively large forces will be generated by centrifugal rotation. Preferably, the channel and the reaction vessel will have a smaller diameter or width thus having a large vertical profile can be used. The diameter or width of the channel and the outer wall of the reaction vessel is at least about 0.4 mm to up to about 4 to 5 mm, and that of the inner wall of the reaction vessel is at least about 0.1 mm to up to about 3.5 to 4.5 mm.

**Convection PCR Apparatus Comprising An Optical Detection Unit**

It is objective of the invention to provide "optical detection" as an additional feature of the apparatus embodiments described herein. It is important to detect progress or results of the polymerase chain reaction (PCR) during or after the PCR reaction with speed and accuracy. The optical detection feature can be useful for such needs by providing apparatuses and methods for simultaneous amplification and detection of the PCR reaction.

In typical embodiments, a detectable probe that can generates an optical signal as a function of the amount of the amplified PCR product is introduced to the sample, and the optical signal from the detectable probe is monitored or detected during or after the PCR reaction without opening the reaction vessel. The detectable probe is typically a detectable DNA binding agent that changes its optical property depending on its binding or non-binding to DNA molecules or interaction with the PCR reaction and/or the PCR product. Useful examples of the detectable probe include, but not limited to, intercalating dyes having a property of binding to double-stranded DNA and various oligonucleotide probes having detectable label(s).

The detectable probe that can be used with the invention typically changes its fluorescence property such as its fluorescence intensity, wavelength or polarization, depending on the PCR amplification. For instance, intercalating dyes such as SYBR green 1, YO-PRO 1, ethidium bromide, and similar dyes generate fluorescence signal that is enhanced or activated when the dye binds to double-stranded DNA. Hence, fluorescence signal from such intercalating dyes can be detected to monitor the amount of the amplified PCR product. Detection using the intercalating dye is non-specific with regard to the sequence of the double-stranded DNA. Various oligonucleotide probes that can be used with the invention are known in the field. Such oligonucleotide probes typically have at least one detectable label and a nucleic acid sequence that can specifically hybridize to the amplified PCR product or the template. Hence, sequence-specific detection of the amplified PCR product, including allelic discrimination, is possible. The oligonucleotide probes are typically labeled with an interactive label pair such as a pair of two fluorescers or a pair of a fluorescer and a quencher whose interaction (such as "fluorescent resonance energy transfer" or "non-fluorescent energy transfer") is enhanced as the distance between the two labels becomes shorter. Most of the oligonucleotide probes are designed such that the distance between the two interactive labels is modulated depending on its binding (typically a longer distance) or non-binding (typically a shorter distance) to a target DNA sequence. Such hybridization-dependent distance modulation results in change of the fluorescence intensity or change (increase or decrease) of the fluorescence wavelength depending on the amount of the amplified PCR product. In other types of the oligonucleotide probes, the probes are designed to undergo certain chemical reactions during the extension step of the PCR reaction, such as hydrolysis of the fluorescer label due to the 5'-3' nuclease activity of a DNA polymerase or extension of the probe sequence. Such PCR reaction dependent changes of the probes lead to activation or enhancement of a fluorescence signal from the fluorescer so as to signal the change of the amount of the PCR product.

A variety of suitable detectable probes and devices for detecting such probes are described in the following U.S. Pat. Nos. 5,210,015; 5,487,972; 5,538,838; 5,716,784; 5,804,375; 5,925,517; 5,994,056; 5,475,610; 5,602,756; 6,028,190; 6,030,787; 6,103,476; 6,150,097; 6,171,785; 6,174,670; 6,258,569; 6,326,145; 6,365,729; 6,703,236; 6,814,934; 7,238,517, 7,504,241; 7,537,377; as well as non-US counterpart applications and patents.

As used herein, the phrase "optical detection unit" including plural forms means a device(s) for detecting PCR amplification that is compatible with one or more of the PCR thermal convection apparatuses and PCR methods disclosed herein. A preferred optical detection unit is configured to detect a fluorescence optical signal such as when a PCR amplification reaction is in progress. Typically, the device will provide for detection of the signal and preferably quantification thereof without opening at least one reaction vessel of the apparatus to which it is operably attached. If desired, the optical detection unit and one or more of the PCR thermal convection apparatuses of the invention can be configured to relate the amount of amplified nucleic acid in the reaction vessel (i.e., real-time or quantitative PCR amplification). A typical optical detection unit for use with the invention includes one or more of the following components in an operable combination: an appropriate light source(s), lenses, filters, mirrors, and beam-splitter(s) for detecting fluorescence typically in the visible region between from about 400 to about 750 nm. A preferred optical detection unit is positioned below, above and/or to the side of a reaction vessel sufficient to receive and output light for detecting PCR amplification within the reaction vessel.

An optical detection unit is compatible with a thermal convection PCR apparatus of the invention if it supports robust, sensitive and rapid detection of the PCR amplification for which the apparatus is intended. In one embodiment, the thermal convection PCR apparatus includes an optical detection unit that enables detection of an optical property of the sample in the reaction vessel. The optical property to be detected is preferably fluorescence at one or more wavelengths depending on the detectable probe used, although absorbance of the sample is sometimes useful to detect. When fluorescence from the sample is detected, the optical detection unit irradiates the sample (either a portion of, or entire sample) with an excitation light and detects a fluorescence signal from the sample. The wavelength of the excitation light is typically shorter than the fluorescence light. In the case of detecting absorbance, the optical detection unit irradiates the sample with a light (typically at a selected wavelength or with scanning the

wavelength) and the intensity of the light before and after passing through the sample is measured. Fluorescence detection is generally preferred because it is more sensitive and specific to the target molecule to be detected.

Reference to the following figures and descriptions is intended to provide greater understanding of the thermal convection PCR apparatus comprising an optical detection unit for fluorescence detection. It is not intended and should not be read as limiting the scope of the present invention.

Referring to Figures 80A-B, the apparatus embodiments feature one or more optical detection units 600-603 operable to detect a fluorescence signal from the sample in the reaction vessel 90 from the bottom end 92 of the reaction vessel 90 or the bottom end 72 of the channel 70. Shown in Figure 80A is an embodiment in which single optical detection unit 600 is used to detect fluorescence from multiple reaction vessels 90. In this embodiment, a broad excitation beam (shown as upward arrows) is generated to irradiate multiple reaction vessels and a fluorescence signal (shown as downward arrows) from multiple reaction vessels 90 is detected. In this embodiment, a detector 650 (see Fig. 83, for instance) to be used for the fluorescence detection is preferably one that has an imaging capability so that the fluorescence signal from different reaction vessels can be distinguished from the fluorescence image. Alternatively, multiple detectors 650 each of which detects the fluorescence signal from each reaction vessel can be incorporated.

In the embodiment shown in Figure 80B, multiple optical detection units 601-603 are incorporated. In this embodiment, each optical detection unit irradiates the sample in each reaction vessel 90 with an excitation light and detects a fluorescence signal from each sample. This embodiment is advantageous in controlling the profile of the excitation beam for each reaction vessel more precisely and also measuring different fluorescence signal from different reaction vessels independently and simultaneously. This type of embodiment is also advantageous in constructing miniaturized apparatuses since larger optical elements and greater optical paths required for generating a broad excitation beam in the single optical detection unit embodiment can be avoided.

Again referring to Figures 80A-B, when the optical detection unit 600-603 is located on the bottom end 92 of the reaction vessel 90, the first heat source 20 comprises an optical port

610 for each channel 70 to provide a path for the excitation and emission light to the reaction vessel 70. The optical port 610 may be a through hole or an optical element made of (partially or entirely) an optically transparent or semitransparent material such as glass, quartz or polymer materials having such optical property. If the optical port 610 is made as a though hole, the diameter or width of the optical port is typically smaller than that of the bottom end 72 of the channel 70 or the bottom end 92 of the reaction vessel 90. In the embodiments shown in Figures 80A-B, the bottom end 92 of the reaction vessel 90 also works as an optical port. Therefore, it is generally desirable to have all or at least the bottom end 92 of the reaction vessel 90 made of an optically transparent or semitransparent material.

Turning now to Figures 81A-B, the apparatus embodiments feature single optical detection unit 600 (Fig. 81A) or multiple optical detection units 601-603 (Fig. 81B) that are located above the top end 91 of the reaction vessel 90. Again, when a single optical detection unit 600 is incorporated (Fig. 81A), a broad excitation beam (shown as downward arrows) is generated to irradiate the multiple reaction vessels and a fluorescence signal (shown as upward arrows) from the multiple reaction vessels 90 is detected. When multiple optical detection units 601-603 (Fig. 81B) are incorporated, each optical detection unit irradiates the sample in each reaction vessel 90 with an excitation light and detects a fluorescence signal from each sample.

In the embodiments shown in Figures 81A-B, a center part of a reaction vessel cap (not shown) that typically fits to the top end (opening) 91 of the reaction vessel 90 functions as an optical port for the excitation and emission light. Therefore, all or at least the center part of the reaction vessel cap is made of an optically transparent or semitransparent material.

Figure 82 shows an apparatus embodiment that features optical detection units 600 that are located on the side of the reaction vessel 90. In this particular embodiment, the optical port 610 is formed on the side of the second heat source 30. Alternatively, the optical port 610 can be formed any one or more of the first 20, second 30, and third 40 heat sources, and the first 50 and second 60 insulators depending on the position of the fluorescence detection as required by particular application purposes. In this embodiment, a side part of the reaction vessel 90 and a portion of the first chamber 100 along the light path also function as optical port, and thus all or at least the parts of the reaction vessel 90 and the first chamber 100 are made of an optically transparent or semitransparent material. When the optical detection unit 600 is located on the side of the reaction vessel 90, the channels 90 are typically formed in one or two arrays that are

linearly or circularly arranged. Such arrangement of the channels 70 enables to detect a fluorescence signal from every channel 70 or reaction vessel 90 without interference by other channels.

In the embodiments described above, both excitation and fluorescence detection are performed from the same side with respect to the reaction vessel 90, and thus both an excitation part and a fluorescence detection part are located on the same side, typically within a same compartment of an optical detection unit 600-603. For instance, in the embodiments shown in Figs. 80A-B, the optical detection unit 600-603 that contains both parts is located on the bottom end 92 of the reaction vessel 90. Similarly, entire optical detection unit is located above the top end 91 of the reaction vessel 90 in the embodiments shown in Figs. 81A-B, and on the side part of the reaction vessel 90 in the embodiment shown in Fig. 82. Alternatively, the optical detection unit 600-603 may be modified so that the excitation part and the fluorescence detection part are located separately. For instance, the excitation part is located on the bottom (or top) of the reaction vessel 90 and the fluorescence detection part is located on the top (bottom) or side part of the reaction vessel 90. In other embodiments, the excitation part may be located on one side (e.g., left side) of the reaction vessel 90 and the fluorescence detection part may be located another side (e.g., top, bottom, right, front or back side; or a side part other than the excitation side).

The optical detection unit 600-603 typically comprises an excitation part that generates an excitation light with a selected wavelength and a fluorescence detection part that detects a fluorescence signal from the sample in the reaction vessel 90. The excitation part typically comprises a combination of light sources, wavelength selection elements, and/or beam shaping elements. Examples of the light source include, but not limited to, arc lamps such as mercury arc lamps, xenon arc lamps, and metal-halide arc lamps, lasers, and light-emitting diodes (LED). The arc lamps typically generate multiple bands or broad bands of light, and the lasers and LEDs typically generate a monochromatic light or a narrow band light. The wavelength selection element is used to select an excitation wavelength from the light generated by the light source. Examples of the wavelength selection element includes a grating or a prism (for dispersing the light) combined with a slit or an aperture (for selecting a wavelength), and an optical filter (for transmitting a selected wavelength). The optical filter is generally preferred because it can effectively select specific wavelength with compact size and it is relatively cheap. Preferred optical filter is an interference filter having a thin-film coating that can transmit certain band of

light (band-pass filter) or light having wavelength longer (long-pass filter) or shorter (short-pass filter) than certain cut-on value. Acoustic optical filters and liquid crystal tunable filters can be an excellent wavelength selection element since these types of filters can be electronically controlled to change the transmission wavelength with speed and accuracy in a compact size although relatively expensive. A colored filter glass can also be used as a wavelength selection element as a cheap replacement of, or in combination with other types of the wavelength selection element to enhance rejection of undesired light (e.g., IR, UV, or other stray light). Choice of the optical filter depends on the characteristics of the light generated by the light source and the wavelength of the excitation light as well as other geometric requirement of the apparatus such as the size. The beam shaping element is used to shape and guide the excitation beam. The beam shaping element can be any one or combination of lenses (convex or concave), mirrors (convex, concave, or elliptical), and prisms.

The fluorescence detection part typically comprises a combination of detectors, wavelength selection elements, and/or beam shaping elements. Examples of the detector include, but not limited to, photomultiplier tubes (PMT), photodiodes, charge-coupled devices (CCD), and video camera. The photomultiplier tubes are typically most sensitive. Therefore, when the sensitivity is the key issue due to very weak fluorescence signal, the photomultiplier tube can be a suitable choice. However, the photomultiplier tubes are not suitable if a compact size or an imaging capability is required (due to its large size). CCDs, silicon photodiodes, or video cameras intensified with, for example, a microchannel plate can have sensitivity similar to the photomultiplier tubes. If imaging of the fluorescence signal is not required and miniaturization is important as in the embodiments having an optical detection unit for each reaction vessel, photodiodes or CCDs with or without an intensifier can be a good choice since these elements are compact and relatively cheap. If imaging is required as in the embodiments having single optical detection unit for multiple reaction vessels, CCD arrays, photodiode arrays, or video cameras (also with or without an intensifier) can be incorporated. Similar to the excitation part, the wavelength selection element is used to select an emission wavelength from the light collected from the sample and the beam shaping element is used to shape and guide the emission light for efficient detection. Examples of the wavelength selection element and the beam shaping element are the same as those described for the excitation part.

In addition to the optical elements described above, the optical detection unit can comprise a beam-splitter. The beam-splitter is particularly useful if the excitation part and the

fluorescence detection part are located on the same side with respect to the reaction vessel 90. In such embodiments, the paths of the excitation and emission beams (along opposite directions) coincide with each other and thus it becomes necessary to separate the beam paths using a beam-splitter. Typically useful beam-splitters are dichroic beam-splitters or dichroic mirrors that have a thin-film interference coating similar to the thin-film optical filters. Typical beam-splitters reflect the excitation light and transmit the fluorescence light (a long-pass type), or vice versa (a short-pass type).

Referring now to Figs. 83-84, 85A-B, and 86, a few design examples of structure of the optical detection unit 600 are described.

In Figure 83, one embodiment of the optical detection unit 600 is illustrated. In this embodiment, excitation optical elements (620, 630, and 640) are located along a direction at a right angle with respect to the channel axis 80, and fluorescence detection optical elements (650, 655, 660, and 670) are located along the channel axis 80. A dichrocic beam-splitter 680 that transmits the fluorescence emission and reflects the excitation light (i.e., a long-pass type) is located around the middle. As typical, a light generated by the light source 620 is collected by an excitation lens 630 and filtered with an excitation filter 640 to select an excitation light with a desired wavelength. The selected excitation light is then reflected by a dichroic beam-splitter and irradiated to the sample. Fluorescence emission from the sample is collected by an emission lens 660 after passing through the dichroic beam-splitter 680 and an emission filter 670 to select an emission light with a desired wavelength. The fluorescence light thus collected is then focused to an aperture or slit 655 or to a detector 650 to measure the fluorescence signal. The function of the aperture or slit 655 is “spatial filtering” of the emission. Typically, the fluorescence light is focused on or near the aperture or slit 655 and thus a fluorescence image from certain (vertical) location of the sample is formed on the aperture or slit 655. Such optical arrangement enables to collect a fluorescence signal efficiently from a certain limited location inside the sample (e.g., the annealing, extension or denaturation region) while rejecting light from other locations. Use of the aperture or slit 655 is optional depending on the type of the detectable probe used. If the fluorescence signal is subject to be generated from a specific region inside the sample, use of one or more of the aperture or slit 655 is preferred. If the fluorescence signal is subject to be generated regardless of the location inside the sample, use of the aperture or slit 655 may not be necessary or one having a larger opening may be used.

As shown in the embodiment depicted in Figure 84, the optical detection unit 600 may be modified to position the excitation optical elements (620, 630, 640) along the channel axis 80 and the fluorescence detection optical elements (650, 655, 660, and 670) along a direction at a right angle to the channel axis 80. A dichrocic beam-splitter 680 useful for this type of embodiment is a short-pass type that transmits the excitation light and reflects the emission light.

The excitation lens 630 used in the embodiments shown in Figures 83-84 can be replaced with a combination of more than one lenses or a combination of lenses and mirrors. When a combination of such optical elements is used, the first lens (typically a convex lens) is preferably located close to and in front of the light source in order to collect the excitation light efficiently. To further enhance the collection efficiency of the excitation light, a mirror (typically concave or elliptic) may be placed on the back side of the light source. When it is required to make the excitation beam large as in the embodiments having a single optical detection unit 600 for irradiating multiple reaction vessels 90, a concave lens or a convex mirror may be used additionally to expand the excitation beam. In some embodiments, one or more of the optical elements (e.g., one or more of lenses or mirrors) may be placed other locations, e.g., between the reaction vessel 90 and the dichroic beam-splitter 680 or the excitation filter 640. In other aspect, the excitation light is typically shaped to an essentially collinear beam so as to irradiate a larger volume of the sample(s). In some special applications such as when using a multi-photon excitation scheme, the excitation light may be tightly focused to a certain position inside the sample.

The emission lens 660 used in the embodiments shown in Figures 83-84 can also be replaced with a combination of more than one lenses or a combination of lenses and mirrors. When a combination of such optical elements is used, the first lens (typically a convex lens) is preferably located close to the reaction vessel 90 (for instance, between the reaction vessel 90 and the dichroic beam-splitter 680 or the emission filter 670) in order to collect the fluorescence light more efficiently. In some embodiments, one or more of the optical elements (e.g., a lens or a mirror) may be placed other locations, e.g., between the reaction vessel 90 and the dichroic beam-splitter 680 or the emission filter 670.

Figures 85A-B show embodiments in which one lens 635 is used to shape both the excitation beam and the emission beam. Two examples of arranging the excitation optical elements (620 and 640) and the fluorescence detection optical elements (650, 655, and 670) are

shown. The excitation optical elements (620 and 640) are located along a direction at a right angle to the channel axis 80 in Fig. 85A and along the channel axis 80 in Fig. 85B. This type of embodiments using a single lens is useful in miniaturizing the optical detection unit 600 such as in the embodiments of incorporating multiple optical detection units shown in Figures 80B, 81B and 82.

Figure 86 shows one apparatus embodiment in which the optical detection unit 600 is located on the top side of the reaction vessel 90. The arrangement of the optical elements depicted is the same as the embodiment shown in Figure 83. Other types of the optical arrangements (e.g., those shown Figures 84 and 85A-B) can also be incorporated. When the optical detection unit 600 (or the excitation or fluorescence detection part) is located on the top side of the reaction vessel 90, the center part of the reaction vessel cap 690 functions as an optical port 610. Therefore, as discussed, the reaction vessel cap 690 or at least the center part is preferably made of an optically transparent or semitransparent material in this type of embodiments.

Again referring to Figure 86, the reaction vessel 90 and the reaction vessel cap 690 typically has a sealing relationship with each other in order to avoid an evaporative loss of the sample during the PCR reaction. In the reaction vessel embodiment shown in Figure 86, the sealing relationship is made between an inner wall of the reaction vessel 90 and an outer wall of the reaction vessel cap 690. Alternatively, the sealing relationship may be made between an outer wall of the reaction vessel 90 and an inner wall of the reaction vessel cap 690 or between a top surface of the reaction vessel 90 and a bottom surface of the reaction vessel cap 690. In some embodiments, the reaction vessel cap 690 may be a thin-film adhesive tape that is optically transparent or semitransparent. In such embodiments, the sealing relationship is made between a top surface of the reaction vessel 90 and a bottom surface of the reaction vessel cap 690.

The reaction vessel embodiments described above may not be optimal for all uses of the invention. For instance, and as shown in Figure 86, it is typical that the sample meniscus (i.e., a water-air interface) is formed between the sample and the reaction vessel cap 690 (or an optical port part of the reaction vessel cap 690). In operation, water in the sample evaporates and condenses to the inner surface of the reaction vessel cap 690 (or an optical port part of the reaction vessel cap 690) due to the PCR reaction that involves a high temperature process. Such condensed water may, for some applications, interfere somewhat with the excitation beam and

the fluorescence beam, particularly when the optical detection unit is positioned on the top side of the reaction vessel 90.

The reaction vessel embodiments exemplified in Figures 87A-B provide another approach. As shown, a reaction vessel 90 and a reaction vessel cap 690 are designed to have an optical port 695 to contact the sample. A sample meniscus is formed higher than, or about the same height as the bottom surface 696 of the optical port 695. Unlike the typical reaction vessel embodiments described above, the excitation beam and the fluorescence beam are transmitted directly from the optical port 695 to the sample or vice versa without passing through the air or any condensed water inside the reaction vessel 90. Structural requirements for such embodiments are as follows:

Firstly, as shown Figures 87A-B, the reaction vessel cap 690 has a sealing relationship with the upper part of the reaction vessel 90 and also with the optical port 695. As discussed, the sealing between the reaction vessel 90 and the reaction vessel cap 690 can be made at an inner wall of the reaction vessel (as in Figures 87A-B) or at an outer wall or a top end 91 of the reaction vessel 90. The sealing between the reaction vessel cap 690 and the optical port 695 can be made at a top surface 697 (Fig. 87A) or a side wall 699 (Fig. 87B) of the optical port 695. Alternatively the reaction vessel cap 690 and the optical port 695 may be made as one body, preferably using a same or similar optically transparent or semitransparent material.

Additionally, the diameter or width of the optical port 695 (and also that of a wall of the reaction vessel cap 690 if that wall is located near or about the same height as the bottom surface 696 of the optical port 695) is made smaller than the diameter or width of a portion of the inner wall of the reaction vessel 90 that is located near or about the same height as the bottom surface 696 of the optical port 695. Moreover, the bottom surface 696 of the optical port 695 is located lower than, or about the same height as the bottom of the inner part of the reaction vessel cap 690. When these structural requirements are met, an open space 698 is provided between the inner wall of the reaction vessel 90 and the side part of the optical port 695. Therefore, the sample can fill up a portion of the open space to form a sample meniscus above the bottom part 696 of the optical port 695 when the reaction vessel 90 is sealed with the reaction vessel cap 690 to make the bottom of the optical port contact the sample.

In Figure 88, use of the optically non-interfering reaction vessel discussed above is exemplified. As discussed, the bottom 696 of the optical port 695 contacts the sample and the sample meniscus is formed above the bottom 696 of the optical port 695. With an optical detection unit 600 located on the top end 91 of the reaction vessel 90, the excitation beam and the fluorescence beam are transmitted directly from the optical port 695 to the sample or vice versa without passing through the air or any condensed water inside the reaction vessel 90. Such optical structure can greatly facilitate the optical detection feature of the invention.

The following examples are given for purposes of illustration only in order that the present invention may be more fully understood. These examples are not intended to limit in any way the scope of the invention unless otherwise specifically indicated.

**EXAMPLES**

**Materials and Methods**

Three different DNA polymerases purchased from Takara Bio (Japan), Finnzymes (Finland), and Kapa Biosystems (South Africa) were used to test PCR amplification performance of various invention apparatuses. Plasmid DNAs comprising various insert sequences, human genome DNA, and cDNA were used as template DNAs. The plasmid DNAs were prepared by cloning insert sequences with different size into pcDNA3.1 vector. The human genome DNA was prepared from a human embryonic kidney cell (293, ATCC CRL-1573). The cDNA was prepared by reverse transcription of mRNA extracts from HOS or SV-OV-3 cells.

Composition of the PCR mixture was as follows: a template DNA with different amount depending on experiments, about 0.4 μM each of a forward and reverse primer, about 0.2 mM each of dNTPs, about 0.5 to 1 units of DNA polymerase depending on DNA polymerase used, about 1.5 mM to 2 mM of $MgCl_2$ mixed in a total volume of 20 μL using a buffer solution supplied by each manufacturer.

The reaction vessel was made of polypropylene and had structural features as depicted in Figure 51A. The reaction vessel had a tapered cylindrical shape with its bottom end closed and comprised a cap that fits with the inner diameter of the top end of the reaction vessel so as to seal the reaction vessel after introduction of a PCR mixture. The reaction vessel was (linearly) tapered from the top to the bottom end so that the upper part had a larger diameter. The taper

angle as defined in Figure 51A was about 4°. The bottom end of the reaction vessel was made flat in order to facilitate heat transfer from the receptor hole in the first heat source. The reaction vessel had a length from the top end to the bottom end of about 22 mm to about 24 mm, an outer diameter at the bottom end of about 1.5 mm, an inner diameter at the bottom end of about 1 mm, and a wall thickness of about 0.25 mm to about 0.3 mm.

Volume of the PCR mixture used for each reaction was 20 μL. The PCR mixture with 20 μL volume produced a height of about 12 to 13 mm inside the reaction vessel.

All the apparatuses used in the examples below were made operable with a DC power. A rechargeable $Li^+$ polymer battery (12.6 V) or a DC power supply was used to operate the apparatus. The apparatuses used in the examples had 12 (3 x 4), 24 (4 x 6), or 48 (6 x 8) channels that were arranged in an array format with multiple rows and columns as exemplified in Figure 39. The spacing between adjacent channels was made as 9 mm. In the experiments, the reaction vessel(s) containing the PCR mixture sample was introduced into the channel(s) after the three heat sources of the apparatus were heated to desired temperatures. The PCR mixture sample was removed from the apparatus after a desired PCR reaction time and analyzed with agarose gel electrophoresis using ethidium bromide (EtBr) as a fluorescent dye for visualizing amplified DNA bands.

**Example 1. Thermal Convection PCR Using the Apparatus of Figure 12A**

The apparatus used in this example had the structure shown in Figure 12A comprising a channel 70, a first chamber 100, a first thermal brake 130, a receptor hole 73, a through hole 71, protrusions 33, 34 of the second heat source 30, and protrusions 23, 24 of the first heat source 20. The length of the first, second and third heat sources along the channel axis 80 were about 4 mm, about 5.5 mm, and about 4 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 near the channel region (i.e., within the protrusion region) of about 2 mm and about 0.5 mm, respectively. The length of the first and second insulators along the channel axis 80 outside the channel region (i.e., outside the protrusion region) was about 6 mm to about 3 mm (depending on position) and about 1 mm, respectively. The first chamber 100 was located on the upper part of the second heat source 30 and had a cylindrical shape with a length along the channel axis 80 of about 4.5 mm and a diameter of about 4 mm. The first thermal brake 130 was located on the bottom of the second heat source 30 and had a length or thickness along the channel axis 80 of about 1 mm with the wall 133 of the first

thermal brake contacting the whole circumference of the channel 70 or the reaction vessel 90. The depth of the receptor hole 73 along the channel axis 80 was varied between from about 1.5 mm to about 3 mm. In this apparatus, the channel 70 was defined by the through hole 71 in the third heat source 40, the wall 133 of the first thermal brake 130 in the second heat source 30, and the receptor hole 73 in the first heat source 20. The channel 70 had a tapered cylinder shape. Average diameter of the channel was about 2 mm with the diameter at the bottom end (in the receptor hole) being about 1.5 mm. In this apparatus, all the temperature shaping elements including the first chamber, the first thermal brake, the receptor hole, the first and second insulators, and the protrusions were disposed symmetrically with respect to the channel axis.

As presented below, the apparatus used in this example having the structure shown in Figure 12A was found to be efficient enough to amplify from a 10 ng human genome sample (about 3,000 copies) in about 25 to about 30 min without the gravity tilting angle. For a 1 ng plasmid sample, PCR amplification resulted in a detectable amplification in as little as about 6 or 8 min. Hence, this is a good demonstrating example of a symmetric heating structure that can provide an efficient PCR amplification without using the gravity tilting angle. As presented in Example 2, this structure also works better when the gravity tilting angle is introduced. However, a small tilting angle (about $10^{o}$ to $20^{o}$ or smaller) can be sufficient for most applications.

*1.1. PCR Amplification from Plasmid Samples*

Figures 53A-C show PCR amplification results obtained from a 1 ng plasmid DNA template using the three different DNA polymerases (from Takara Bio, Finnzymes, and Kapa Biosystems, respectively) described above. The expected size of the amplicon was 373 bp. The forward and reverse primers used were 5'-TAATACGACTCACTATAGGGAGACC-3' (SEQ ID NO: 1) and 5'-TAGAAGGCACAGTCGAGGCT-3' (SEQ ID NO: 2), respectively. In Figures 53A-C, the left most lane shows DNA size marker (2-Log DNA Ladder (0.1-10.0 kb) from New England BioLabs) and lanes 1 to 5 are results obtained with the thermal convection PCR apparatus at PCR reaction time of 10, 15, 20, 25, and 30 min, respectively, as denoted on the bottom of each Figure. The temperatures of the first, second and third heat sources of the invention apparatus were set to $98^{o}C$, $70^{o}C$, and $54^{o}C$, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. Lane 6 (denoted as C on the bottom) is a result from a control experiment obtained using T1 Thermocycler from Biometra. The same PCR mixture containing the same amount of the plasmid template was used in the control experiment. Total

PCR reaction time of the control experiment including pre-heating (5 min) for hot starting and final extension (10 min) was about 1 hour and 30 min. As shown in Figures 53A-C, the thermal convection apparatus yielded an amplified product at the same size as the control experiment, but in much shorter PCR reaction time (i.e., about 3 to 4 times shorter). PCR amplification reached a detectable level at about 10 to 15 min and became saturated in about 20 or 25 min. As manifested, the three DNA polymerases were found to be nearly equivalent to use with the thermal convection PCR apparatus.

Figures 54A-C show further examples of thermal convection PCR. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. Figures 54A-C are results obtained for amplification from three different plasmid DNA templates with amplicon size of 177 bp, 960 bp, and 1,608 bp, respectively. Amount of the template plasmid used for each reaction was 1 ng. The forward and reverse primers used had the sequences as set forth in SEQ ID NOs: 1 and 2, respectively. As shown, even larger amplicons (about 1 kbp and 1.6 kbp) were amplified in very short reaction time, i.e., to a detectable level in about 20 min and to a saturation level in about 30 min. The short amplicon (177 bp) was amplified in a much shorter reaction time, i.e., to a detectable level in about 10 min and to a saturation level in about 20 min.

Figure 55 shows results of thermal convection PCR amplification obtained from various different plasmid templates with amplicon size between about 200 bp to about 2 kbp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. Amount of the template plasmid used for each reaction was 1 ng. The forward and reverse primers used had the sequences as set forth in SEQ ID NOs: 1 and 2, respectively. The expected size of the amplicon was 177 bp for lane 1; 373 bp for lane 2; 601 bp for lane 3; 733 bp for lane 4; 960 bp for lane 5; 1,608 bp for lane 6; and 1,966 bp for lane 7. PCR reaction time was 25 min for lanes 1-6 and 30 min for lane 7. As shown, nearly saturated product bands were observed for all amplicons in a short reaction time. This result demonstrates that thermal convection PCR is not only fast and efficient, but also has a wide dynamic range.

*1.2. Acceleration of PCR Amplification at Elevated Denaturation Temperature*

The results shown in Figures 56A-C demonstrate acceleration of the thermal convection PCR at elevated denaturation temperatures. The template used was 1 ng plasmid that can yield a

373 bp amplicon. Except for the denaturation temperature, all other experimental conditions including the template and primers used were the same as those used for the experiments presented in Figures 53A-C. While the temperatures of the second and third heat sources were set to 70°C and 54°C, respectively, the temperature of the first heat source was increased to 100°C (Figure 56A), 102°C (Figure 56B), and 104°C (Figure 56C). As shown in Figures 56A-C, increase of the denaturation temperature (i.e., the temperature of the first heat source) resulted in acceleration of PCR amplification. The 373 bp product was barely observable at 8 min reaction time when the denaturation temperature was 100°C (Figure 56A), and it became stronger at the same 8 min reaction time when the denaturation temperature was increased to 102°C (Figure 56B). When the denaturation temperature was further increased to 104°C (Figure 56C), the 373 bp product became observable even at 6 min reaction time.

*1.3. PCR Amplification from Human Genome and cDNA Samples*

Figures 57A-C show three examples of thermal convection PCR for amplification from a human genome sample. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. Amount of the human genome template used for each reaction was 10 ng corresponding to about 3,000 copies only. Figure 57A shows results for amplification of a 363 bp segment of β-globin gene. The forward and reverse primers used for this sequence were 5'-GCATCAGGAGTGGACAGAT-3' (SEQ ID NO: 3) and 5'-AGGGCAGAGCCATCTATTG-3' (SEQ ID NO: 4), respectively. Figure 57B shows results for amplification of a 469 bp segment of GAPDH gene. The forward and reverse primers used in this experiment were 5'-GCTTGCCCTGTCCAGTTAA-3' (SEQ ID NO: 5) and 5'-TGACCAGGCGCCCAATA-3' (SEQ ID NO: 6), respectively. Figure 57C shows results for amplification of a 514 bp segment of β-globin gene. The forward and reverse primers used in this experiment were 5'-TGAAGTCCAACTCCTAAGCCA-3' (SEQ ID NO: 7) and 5'-AGCATCAGGAGTGGACAGATC-3' (SEQ ID NO: 8), respectively.

As shown in Figures 57A-C, the thermal convection PCR from about 3,000 copies of human genome samples yielded amplicons with correct size in very short reaction time. The PCR amplification reached a detectable level in about 20 or 25 min and became saturated in about 25 or 30 min. These results demonstrate that the thermal convection PCR is fast and very efficient for amplifying from low copy number samples.

Figure 58 shows further examples of thermal convection PCR amplification from 10 ng human genome or cDNA samples. PCR reaction time was 30 min. All other experimental conditions were the same as those used for the experiments presented in Figures 57A-C. As shown, all fourteen gene segments with their size ranging from about 100 bp to about 800 bp were successfully amplified in 30 min reaction time. Target genes and corresponding primer sequences are summarized in Table 2 below. Templates used were human genome DNA (10 ng) for lanes 1, 3-5, and 9-14; and cDNA (10 ng) for lanes 2, 6, 7, and 8. The cDNA samples were prepared by reverse transcription of mRNA extracts from HOS (lanes 2 and 7) or SK-OV-3 (lanes 6 and 8) cells.

Table 2. Primer Sequences and Target Genes Used for the Experiments in Figure 58

| Lane No. | Target Gene | Amplicon Size | SEQ ID NO | Primer Sequence |
|---|---|---|---|---|
| 1 | PRPS1 | 99 bp | 9 | 5'-GATCTATTTGGCCTCTCAAA-3' |
| | | | 10 | 5'-CACACAGGTACACACACTTTATT-3' |
| 2 | p53 | 123 bp | 11 | 5'-TGCCCAACAACACCAGC-3' |
| | | | 12 | 5'-CCAAGGCCTCATTCAGCTC-3' |
| 3 | NAIP Exon5 | 132 bp | 13 | 5'-TGCCACTGCCAGGCAATCTAA-3' |
| | | | 14 | 5'-CATTTGGCATGTTCCTTCCAAG-3' |
| 4 | p53 | 152 bp | 15 | 5'-GAAGACCCAGGTCCAGAT-3' |
| | | | 16 | 5'-CTGCCCTGGTAGGTTTTC-3' |
| 5 | CYP27B1 | 168 bp | 17 | 5'-GACAAGGTGAGAGGAGC-3' |
| | | | 18 | 5'-TTAGCTGGACCTCGTCTC-3' |
| 6 | HER2 | 192 bp | 19 | 5'-AGCACTGGGGAGTCTTTGT-3' |
| | | | 20 | 5'-GGGACAGTCTCTGAATGGGT-3' |
| 7 | CDK4 | 284 bp | 21 | 5'-GGTGTTTGAGCATGTAGACCA-3' |
| | | | 22 | 5'-GAACTTCGGGAGCTCGGTA-3 |
| 8 | CD24 | 330 bp | 23 | 5'-TCCAAGCACCCAGCATC-3' |
| | | | 24 | 5'-TGGGGAAATTTAGAAGACGTTTCTTG-3' |
| 9 | β-globin | 363 bp | 3 | 5'-GCATCAGGAGTGGACAGAT-3' |
| | | | 4 | 5'-AGGGCAGAGCCATCTATTG-3' |
| 10 | CR2 | 402 bp | 25 | 5'-AGGTTGGGGTCTTGCCT-3' |
| | | | 26 | 5'-CACCTGTGCTAGACGGTG-3' |
| 11 | PIGR | 433 bp | 27 | 5'-GCCACCTACTACCCAGAGG-3' |
| | | | 28 | 5'-TGATGGTCACCGTTCTGC-3' |
| 12 | GAPDH | 469 bp | 5 | 5'-GCTTGCCCTGTCCAGTTAA-3' |
| | | | 6 | 5'-TGACCAGGCGCCCAATA-3' |
| 13 | β-globin | 514 bp | 7 | 5'-TGAAGTCCAACTCCTAAGCCA-3' |
| | | | 8 | 5'-AGCATCAGGAGTGGACAGATC-3' |
| 14 | β-globin | 830 bp | 3 | 5'-GCATCAGGAGTGGACAGAT-3' |
| | | | 29 | 5'-GGAGAAGATATGCTTAGAACCGA-3' |

Abbreviations used in Table 2 are as follows. PRPS1: phosphoribosyl pyrophosphate synthetase 1; NAIP: NLR family, apoptosis inhibitory protein; CYP27B1: cytochrome P450, family 27, subfamily B, polypeptide 1; HER2: ERBB2, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2; CDK4: cyclin-dependent kinase 4; CR2: complement receptor 2; PIGR: polymeric immunoglobulin receptor; GAPDH: glyceraldehydes 3-phosphate dehydrogenase.

*1.4. PCR Amplification from Very Low Copies of Human Genome Sample*

Figure 59 shows PCR amplification from very low copy number samples using the invention apparatus. Template sample used was human genome DNA extracted from 293 cells. Primers used for this experiment had the sequences as set forth in SEQ ID NOs: 3 and 4. Target sequence was a 363 bp segment of β-globin. PCR reaction time was 30 min. All other experimental conditions including the temperatures of the three heat sources and the depth of the receptor hole were the same as those used for the experiments presented in Figures 57A-C and 58. As denoted on the bottom of Figure 59, amount of the human genome sample used for each reaction was decreased consecutively, starting from 10 ng (about 3,000 copies) to 1 ng (about 300 copies), 0.3 ng (about 100 copies), and 0.1 ng (about 30 copies). As manifested, the thermal convection PCR yielded successful PCR amplification from as little as a 30 copy sample. Single copy samples were also examined for thermal convection PCR amplification. It was found that amplification from a single copy sample was successful with about 30 to 40% probability, likely due to statistical probability associated with chance of sampling a single copy.

*1.5. Temperature Stability and Power Consumption of the Invention Apparatus*

Temperature stability and power consumption of the invention apparatus having the structure shown in Figure 12A were tested. The apparatus used in this experiment had 12 channels (3 x 4) disposed 9 mm apart from each other as shown in Figures 39 and 42. The first, second and third heat sources were each equipped with a NiCr heating wire (160a-c) that was disposed in between the channels as shown in Figure 42. The apparatus also comprised a fan above the third heat source to provide cooling to the third heat source when needed. DC power from a rechargeable $Li^+$ polymer battery (12.6 V) was supplied to each heating wire and controlled by PID (proportional-integral-derivative) control algorism so as to maintain the temperature of each of the three heat sources at a pre-set target value.

Figure 60 shows temperature variations of the first, second and third heat sources when target temperatures were set to 98°C, 70°C, and 54°C, respectively. The ambient temperature

was about 25°C. As shown, the three heat sources reached the target temperatures within less than about 2 min. During about 40 min time span after reaching the target temperatures, the temperatures of the three heat sources were maintained stably and accurately at the target temperatures. Average of the temperature of each heat source during the 40 min time span was within about ±0.05°C with respect to each target temperature. Temperature fluctuations were also very small, i.e., standard deviation of the temperature of each heat source was within about ±0.05°C.

Figure 61 shows power consumption of the invention apparatus having 12 channels. As shown, the power consumption was high in the initial time period (i.e., up to about 2 min) in which rapid heating to the target temperatures took place. After the three heat sources reached the target temperatures (i.e., after about 2 min), the power consumption was reduced to lower values. The large fluctuations observed after about 2 min are result of active control of the power supply to each heat source. Due to such active power control, the temperatures of the three heat sources can be maintained stably and accurately at the target temperatures as shown in Figure 60. Average of the power consumption in the temperature maintaining region (i.e., after about 2 min) was about 4.3 W as denoted in Figure 61. Therefore, power consumption per each channel or each reaction was less than about 0.4 W. Since about 30 min or less time is sufficient for PCR amplification in the invention apparatus, energy cost for completion of one PCR reaction is only about 700 J or less as is equivalent to energy needed to heat up about 2 mL water from room temperature to about 100°C one time.

Invention apparatuses having 24 and 48 channels were also tested. Average power consumption was about 7 to 8 W for the 24 channel apparatus and about 9 to 10 W for the 48 channel apparatus. Hence, power consumption per each PCR reaction was found to be even less for lager apparatuses, i.e., about 0.3 W for the 24 channel apparatus and about 0.2 W for the 48 channel apparatus.

**Example 2. Thermal Convection PCR Using the Apparatus of Figure 12B**

In this example, effect of the gravity tilting angle $\theta_g$ to the thermal convection PCR was examined. The apparatus used in this example had the same structure and dimensions as that used in Example 1 except for incorporation of the gravity tilting angle $\theta_g$ as defined in Figure

12B. The apparatus was equipped with an inclined wedge on the bottom so that the channel axis was tilted by $\theta_g$ with respect to the direction of gravity.

As presented below, introduction of the gravity tilting angle caused the convective flow faster and thus accelerated the thermal convection PCR. It was therefore confirmed that a structural element such as a wedge or leg, or an inclined or tilted channel that can impose a gravity tilting angle to the apparatus or the channel is a useful structural element in constructing an efficient and fast thermal convection PCR apparatus.

*2.1. PCR Amplification from Plasmid Sample*

Figures 62A-E show results of thermal convection PCR as a function of the gravity tilting angle for amplification from a plasmid sample. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. Amount of the template plasmid used for each reaction was 1 ng. The primers used had the sequences as set forth in SEQ ID NOs: 1 and 2. The expected size of the amplicon was 373 bp. Figure 62A shows results obtained at zero gravity tilting angle. Figures 62B-E show results obtained at $\theta_g$ equal to 10°, 20°, 30°, and 45°, respectively. At zero gravity tilting angle (Figure 62A), the amplified product was barely observable at 15 min reaction time and became strong at 20 min. In contrast, the amplified product was observable with a significant intensity at 10 min reaction time when the gravity tilting angle of 10° was introduced (Figure 62B). Further increase of the product band intensity at 10 and/or 15 min reaction time was observed as the gravity tilting angle was increased to 20° (Figure 62C). Above 20° tilting angle (Figures 62D-E), amplification speed was observed to be similar to that observed at 20°.

*2.2. PCR Amplification from Human Genome Sample*

Figures 63A-D show another example that demonstrates the effect of the gravity tilting angle. In this experiment, a 10 ng human genome sample (about 3,000 copies) was used as a template DNA and primers having the sequences as set forth in SEQ ID NOs: 3 and 4 were used. A 363 bp segment of β-globin gene was the target. Other experimental conditions were the same as those used for the experiment presented in Figures 62A-E above. Figures 63A-D show results obtained when $\theta_g$ was set to 0°, 10°, 20°, and 30°, respectively. As shown, the thermal convection PCR was accelerated when the gravity tilting angle was introduced (i.e., Figures

63B-D as compared to Figure 63A). Speed of the PCR amplification was observed to increase as the gravity tilting angle increased. Similar amplification speed was observed at 20° (Figure 63C) and 30° (Figure 63D).

Figures 64A-B show a further example in which primers having high melting temperatures (above 60°C) were used. In this experiment, a 10 ng human genome sample (about 3,000 copies) was used as a template DNA. The forward and reverse primers used had sequences 5'-GCTTCTAGGCGGACTATGACTTAGTTGCG-3' (SEQ ID NO: 30) and 5'-CCAAAAGCCTTCATACATCTCAAGTTGGGGG-3' (SEQ ID NO: 31), respectively. The amplification target was a 521 bp segment of β-actin gene. The temperatures of the first, second and third heat sources were set to 98°C, 74°C, and 64°C, respectively. Depth of the receptor hole along the channel axis was about 2.8 mm. The PCR reaction time was set to 30 min and experiment was performed with duplicate samples (lanes 1 and 2) for each tilting angle. Figures 64A and B show results obtained at $\theta_g = 0°$ and 20°, respectively. As shown, no significant amplification was observed at 0° for both PCR samples (Figure 64A). In contrast, strong product bands were observed when 20° tilting angle was introduced (Figure 64B). Compared to the experiments presented in Figures 63A-D, the temperatures of the third and second heat sources were increased by 10°C and 4°C, respectively, while the temperature of the first heat source was the same. Hence, the thermal convection was slowed down due to the reduced temperature difference between the heat sources. Without using the gravity tilting angle (Figure 64A), the thermal convection PCR became too slow, not enabling fast PCR amplification. However, by introducing the gravity tilting angle (Figure 64B), the thermal convection PCR became sufficiently fast and efficient to yield strong product bands from a low copy human genome sample (about 3,000 copies) in a short reaction time.

*2.3. PCR Amplification from Very Low Copies of Human Genome Sample*

Figure 65 shows results of thermal convection PCR amplification from very low copy human genome samples when the gravity tilting angle was used. The primers used were the same as those used for the experiments presented in Figures 64A-B. Hence, the amplification target was a 521 bp segment of β-actin gene. The temperatures of the first, second and third heat sources were set to 98°C, 74°C, and 60°C, respectively. Depth of the receptor hole along the channel axis was about 2.5 mm. The gravity tilting angle was set to 10° and the PCR reaction

time was set to 30 min. As shown in Figure 65, the thermal convection PCR yielded successful PCR amplification from as little as a 30 copy sample.

**Example 3. Thermal Convection PCR Using the Apparatus of Figure 14C**

The apparatus used in this example had the structure shown in Figure 14C comprising a channel 70, a first chamber 100, a second chamber 110, a first thermal brake 130, a receptor hole 73, and a through hole 71. No protrusion structures were used in this apparatus. The length of the first, second and third heat sources along the channel axis 80 were about 5 mm, about 4 mm, and about 5 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 of about 2 mm and about 1 mm, respectively. The first chamber 100 was located on the upper part of the second heat source 30 and had a cylindrical shape with a length along the channel axis 80 of about 3 mm and a diameter of about 4 mm. The first thermal brake 130 was located on the bottom of the second heat source 30 and had a length or thickness along the channel axis 80 of about 1 mm with the wall 133 of the first thermal brake 130 contacting the whole circumference of the channel 70 or the reaction vessel 90. The second chamber 110 was located on the bottom part of the third heat source 40 and had a cylindrical shape with a diameter of about 4 mm. The length of the second chamber 110 along the channel axis 80 was varied between from about 1.5 mm to about 0.5 mm depending on the depth of the receptor hole 73. The depth of the receptor hole 73 along the channel axis 80 was varied between from about 2 mm to about 3 mm. In this apparatus, the channel was defined by the through hole 71 in the third heat source 40, the wall 133 of the first thermal brake 130 in the second heat source 30, and the receptor hole 73 in the first heat source 20. The channel 70 had a tapered cylinder shape. Average diameter of the channel was about 2 mm with the diameter at the bottom end (in the receptor hole) being about 1.5 mm. In this apparatus, all the temperature shaping elements including the first and second chambers, the first thermal brake, the receptor hole, and the first and second insulators were disposed symmetrically with respect to the channel axis.

*3.1. PCR Amplification from Plasmid Samples*

Figure 66 shows PCR amplification results obtained from a 1 ng plasmid sample using two primers having sequences: 5'-AAGGTGAGATGAAGCTGTAGTCTC-3' (SEQ ID NO: 32) and 5'-CATTCCATTTTCTGGCGTTCT-3' (SEQ ID NO: 33). The expected size of the amplicon was 152 bp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 56°C, respectively. The length of the second chamber along the channel axis

was about 1 mm and the depth of the receptor hole along the channel axis was about 2.5 mm. As shown in Figure 66, the thermal convection PCR yielded successful amplification in as little as 10 min, demonstrating fast and efficient PCR amplification in this type of invention apparatuses.

Figure 67 shows results of thermal convection PCR amplification from various different plasmid templates with amplicon size between about 200 bp to about 2 kbp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 56°C, respectively. The length of the second chamber along the channel axis was about 1.5 mm and depth of the receptor hole along the channel axis was about 2 mm. Amount of the template plasmid used for each reaction was 1 ng. The primers having the sequences as set forth in SEQ ID NOs: 1 and 2 were used. The expected size of the amplicon was 177 bp for lane 1; 373 bp for lane 2; 601 bp for lane 3; 733 bp for lane 4; 960 bp for lane 5; 1,608 bp for lane 6; and 1,966 bp for lane 7. PCR reaction time was 30 min for lanes 1-6 and 35 min for lane 7. As shown, nearly saturated product bands were observed for all amplicons in a short reaction time. These results demonstrate that thermal convection PCR is not only fast and efficient, but also has a wide dynamic range.

*3.2. PCR Amplification from Human Genome Sample*

Figures 68A-B show two examples of thermal convection PCR for amplification from a human genome sample. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 56°C, respectively. The length of the second chamber along the channel axis was about 1 mm and the depth of the receptor hole along the channel axis was about 2.5 mm. Amount of the human genome template used for each reaction was 10 ng corresponding to about 3,000 copies. Figure 68A shows results for amplification of a 500 bp segment of β-globin gene. The forward and reverse primers used for this sequence were 5'-GCATCAGGAGTGGACAGAT-3' (SEQ ID NO: 3) and 5'-CTAAGCCAGTGCCAGAAGA-3' (SEQ ID NO: 34), respectively. Figure 68B shows results for amplification of a 500 bp segment of β-actin gene. The forward and reverse primers used for this sequence had sequences 5'-CGGACTATGACTTAGTTGCG-3' (SEQ ID NO: 35) and 5'-ATACATCTCAAGTTGGGGGA-3' (SEQ ID NO: 36), respectively.

As shown in Figures 68A-B, the thermal convection PCR from about 3,000 copies of human genome samples yielded amplicons with correct size in a short reaction time. Significant amplification was observed in about 20 or 25 min with saturated amplification reached in about

30 min. These results demonstrate high speed and efficiency of the thermal convection PCR for amplification from low copy number samples.

*3.3. PCR Amplification from Very Low Copies of Plasmid Sample*

Figure 69 shows PCR amplification from very low copy number plasmid samples using the invention apparatus. Except for the amount of the plasmid sample, all other experimental conditions including the temperatures of the three heat sources and the depth of the receptor hole were the same as those used for the experiments presented in Figure 66. The template plasmid and the primers used were also the same. The PCR reaction time was 30 min. As denoted on the bottom of Figure 69, amount of the plasmid sample used for each reaction was decreased consecutively, starting from about 10,000 copies (lane 1) to about 1,000 copies (lane 2), 100 copies (lane 3) and 10 copies (lane 4). As manifested, the thermal convection PCR yielded successful PCR amplification from as little as a 10 copy sample. Single copy samples were also examined. It was found that amplification from a single copy sample was successful with about 30 to 40% probability.

*3.4. Temperature Stability and Power Consumption of the Invention Apparatus*

Temperature stability and power consumption of the invention apparatus having the structure shown in Figure 14C were also tested. The apparatus used in this experiment had 48 channels (6 x 8) disposed 9 mm apart from each other. Temperature variations observed for this invention apparatus was slightly larger than the apparatus having the structure shown in Figure 12A that was used for the experiments presented in Example 1 (see Section 1.5 above). Average temperature of each heat source during the temperature maintaining time was within about $\pm 0.1^{\circ}C$ with respect to each of the target temperatures. Temperature fluctuation (i.e., standard deviation) of each heat source was within about $\pm 0.1^{\circ}C$. Average of the power consumption during the temperature maintaining time was between about 15 W to about 20 W depending on the ambient temperature. Compared to the apparatus having the structure shown in Figure 12A, the power consumption was about 1.5 to about 2 times larger as a result of reduced insulating gaps in the absence of the protrusion structures used in the Figure 12A apparatus. These results demonstrate that use of the protrusion structures is efficient in reducing power consumption of the invention apparatus.

**Example 4. Thermal Convection PCR Using the Apparatus of Figure 17A**

The apparatus used in this example had the structure shown in Figure 17A, but without the protrusions 43, 44 of the third heat source 40. The apparatus comprised a channel 70, a first chamber 100, a receptor hole 73, a through hole 71, protrusions 33, 34 of the second heat source 30, and protrusions 23, 24 of the first heat source 20. The first chamber 100 was disposed in the second heat source 30 and no thermal brake structure was used. The length of the first, second and third heat sources along the channel axis 80 were about 4 mm, about 6.5 mm, and about 4 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 near the channel region (i.e., within the protrusion region) of about 1 mm and about 0.5 mm, respectively. The length of the first and second insulators outside the channel region (i.e., outside the protrusion region) was about 6 mm to about 3 mm (depending on position) and about 1 mm, respectively. The first chamber 100 had a cylindrical shape with a length along the channel axis 80 equal to the length of the second heat source along the channel axis 80 (i.e., about 6.5 mm). Diameter of the first chamber 100 was varied from about 4 mm to about 2.5 mm. Depth of the receptor hole 73 along the channel axis was varied between from about 2 mm to about 3 mm. In this apparatus, the channel 70 was defined by the through hole 71 in the third heat source 40 and the receptor hole 73 in the first heat source 20. The channel 70 had a tapered cylinder shape with average diameter of about 2 mm and the diameter at the bottom end (in the receptor hole) of about 1.5 mm. In this apparatus, all the temperature shaping elements including the first chamber, the receptor hole, and the first and second insulators were disposed symmetrically with respect to the channel axis.

In this example, effects of the chamber diameter, the receptor hole depth, and the gravity tilting angle were examined with regard to the speed of the thermal convection PCR.

*4.1. Effects of the Chamber Diameter and the Receptor Hole Depth*

In this example, the thermal convection PCR was examined as a function of the chamber diameter at different receptor hole depths. Template DNA used was a 1 ng plasmid. Two primers having the sequences as set forth in SEQ ID NOs: 1 and 2 were used and the size of the amplicon was 373 bp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively.

Figures 70A-D show results obtained when the diameter of the first chamber was about 4 mm (Figure 70A), about 3.5 mm (Figure 70B), about 3 mm (Figure 70C), and about 2.5 mm (Figure 70D). The depth of the receptor hole along the channel axis was about 2 mm. As shown,

the convection PCR was found to slow down as the diameter of the first chamber was reduced. When the diameter of the first chamber was about 4.0 mm, the PCR product was amplified to a significant level even in 10 min reaction time (Figure 70A). However, more reaction time was needed to reach similar band intensity when the chamber diameter was reduced to about 3.5 mm (Figure 70B) and about 3 mm (Figure 70C). When it was reduced to about 2.5 mm (Figure 70D), no detectable PCR band was observed even after 30 min reaction time. Decrease of the chamber gap between the second heat source and the channel caused more efficient heat transfer between the second heat source and the channel. Thus, temperature gradient inside the channel became smaller at smaller chamber diameter, leading to decrease in the thermal convection speed.

Figure 71A-D show results obtained when the depth of the receptor hole was increased to about 2.5 mm while the diameters of the first chamber remained the same, i.e., about 4 mm (Figure 71A), about 3.5 mm (Figure 71B), about 3 mm (Figure 71C), and about 2.5 mm (Figure 71D). Due to increased heating from the deeper receptor hole, the thermal convection became faster for all different diameters of the first chamber as compared to the results shown in Figures 70A-D. Even when the diameter of the first chamber was the smallest (i.e., about 2.5 mm), the thermal convection PCR became sufficiently fast and efficient to yield a detectable product band in about 15 min reaction time.

The results of this example demonstrate that the chamber diameter or the chamber gap is an important structural element that can be used to control the speed of the thermal convection PCR. It was found that lager chamber diameter leads to faster thermal convection PCR, or vice versa. While it is generally preferred to make the convective flow as fast as possible, it is sometimes preferred to reduce the speed of the convective flow. For instance, some template samples such as templates having long target sequences or certain target genes of genomic DNAs may not be successfully PCR amplified if the convection speed is too fast (due to the large size or certain complex structural limitations). For another instance, DNA polymerase used may have its polymerization speed that is too slow as compared to the speed of the thermal convection PCR. In such cases, use of the chamber structure with different (typically smaller) diameter or chamber gap can be very useful in controlling (typically reducing) the speed of the thermal convection PCR.

*4.2. Effects of the Gravity Tilting Angle*

In this example, the thermal convection PCR of the invention apparatus was further examined by introducing the gravity tilting angle $\theta_g$. Except for the gravity tilting angle, all other experimental conditions including the template DNA and primers used were the same as those used for the example presented in Figures 70A-D and 71A-D.

Figures 72A-D and 73A-D show results obtained when a gravity tilting angle of $10^o$ was introduced. The depth of the receptor hole was about 2.0 mm in Figures 72A-D and about 2.5 mm in Figures 73A-D. As in Figures 70A-D and 71A-D, the diameter of the first chamber was about 4 mm (Figures 72A and 73A), about 3.5 mm (Figures 72B and 73B), about 3 mm (Figures 72C and 73C), and about 2.5 mm (Figures 72D and 73D). As shown, acceleration of the thermal convection PCR was found to be evident when the gravity tilting angle was introduced. However, increase of the thermal convection PCR speed is more pronounced when the depth of the receptor hole was about 2 mm (Figures 72A-D as compared to Figures 70A-D). As compared to the results shown in Figures 70A-D, about 5 min reduction of the PCR reaction time was observed when the chamber diameter was about 4 mm (Figure 72A) and about 3.5 mm (Figure 72B), and about at least 10 to 15 min reduction of the PCR time was observed when the chamber diameter was about 3 mm (Figure 72C) and about 2.5 mm (Figure 72D). When the depth of the receptor hole was about 2.5 mm, only slight increase of the thermal convection PCR speed was observed when the chamber diameter was about 4 mm (Figure 73A as compared to Figure 71A), about 3.5 mm (Figure 73B as compared to Figure 71B), and about 3 mm (Figure 73C as compared to Figure 71C). When the chamber diameter was about 2.5 mm (Figure 73D as compared to Figure 71D), a large reduction (about 10 min reduction) of the PCR reaction time was observed.

The results of this example demonstrate that the gravity tilting angle is an important structural element that can be used to increase the speed of the thermal convection PCR. Moreover, the results suggest that there may be certain limitations (other than the apparatus itself) in speeding up the thermal convection PCR. For instance, the speed of the thermal convection PCR was observed to be about the same in the results shown in Figures 73A-C although the chamber diameter (that was found to affect the convection speed significantly) was changed. Similarly, the results shown in Figures 73A-C were not much different from those shown in Figures 71A-C irrespective of presence or absence of the gravity tilting angle. These results demonstrate that the ultimate speed of the thermal convection PCR can be limited by the

polymerization speed of the DNA polymerase used although the convection speed of the invention apparatus can be increased as fast as desired.

**Example 5. Effects of Position of the First Thermal Brake**

Two types of apparatuses were used in this example. The first apparatus used had the structure shown in Figure 12A comprising a channel 70, a first chamber 100, a first thermal brake 130, a receptor hole 73, a through hole 71, protrusions 33, 34 of the second heat source 30, and protrusions 23, 24 of the first heat source 20. Hence, the first thermal brake 130 was located on the bottom of the second heat source 30 with the first chamber 100 located on the upper part of the second heat source 30 as shown in Figure 12A. The thickness of the first thermal brake 130 along the channel axis 80 was about 1 mm.

The second apparatus used had a structure identical to the structure shown in Figure 12A except for the chamber/thermal brake structure. The second apparatus comprised a first 100 and second 110 chambers located on the bottom and top part of the second heat source 30 and the first thermal brake 130 was located in between the first 100 and second 110 chambers as in the structure shown in Figure 10A. The thickness of the first thermal brake 130 along the channel axis 80 was about 1 mm. The position of the first thermal brake 130 was varied along the channel axis 80.

In both apparatuses, the length of the first, second and third heat sources along the channel axis 80 were about 4 mm, about 6.5 mm, and about 4 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 near the channel region (i.e., within the protrusion region) of about 1 mm and about 0.5 mm, respectively. The length of the first and second insulators outside the channel region (i.e., outside the protrusion region) was about 6 mm to about 3 mm (depending on position) and about 1 mm, respectively. Both the first 100 and second 110 chambers had a cylindrical shape with a diameter of about 4 mm. The first thermal brake 130 had a length or thickness along the channel axis 80 of about 1 mm with the wall 133 of the first thermal brake 130 contacting the whole circumference of the channel 70. Depth of the receptor hole 73 along the channel axis was about 2.8 mm. The channel 70 had a tapered cylinder shape. Average diameter of the channel was about 2 mm with the diameter at the bottom end (in the receptor hole) being about 1.5 mm. In this apparatus, all the temperature shaping elements including the first chamber, the second chamber, the first thermal

brake, the receptor hole, and the first and second insulators were disposed symmetrically with respect to the channel axis.

Template DNA used in this example was a 1 ng plasmid DNA. Two primers having the sequences as set forth in SEQ ID NOs: 1 and 2 were used and the size of the amplicon was 373 bp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively.

Figures 74A-F show results obtained when the position of the first thermal brake was varied along the channel axis. Position of the bottom end 132 of the first thermal brake was varied from the bottom of the second heat source (Figure 74A) to about 1 mm (Figure 74B), about 2.5 mm (Figure 74C), about 3.5 mm (Figure 74D), about 4.5 mm (Figure 74E), or about 5.5 mm (Figure 74F) above the bottom of the second heat source. As shown in Figures 74A-F, the speed of the thermal convection PCR was modulated depending on the position of the first thermal brake along the channel axis. When the first thermal brake was located on the bottom of the second heat source (Figure 74A), the thermal convection PCR yielded relatively slow PCR amplification as compared to other positions. As the first thermal brake was moved up by about up to 3.5 mm (Figures 74B-D), the PCR amplification speed was increased. At the higher positions (Figures 74E-F), a slight decrease of the amplification speed was observed.

The results of this example demonstrate that the position of the thermal brake is a useful structural element that can be used to adjust or control the speed of the thermal convection PCR.

**Example 6. Effects of Thickness of the First Thermal Brake and the Gravity Tilting Angle**

Three types of apparatuses were used in this example. The first apparatus used had the structure shown in Figure 12A comprising a channel 70, a first chamber 100, a first thermal brake 130, a receptor hole 73, a through hole 71, protrusions 33, 34 of the second heat source 30, and protrusions 23, 24 of the first heat source 20. Hence, the first thermal brake 130 was located on the bottom of the second heat source 30 with the first chamber 100 located on the upper part of the second heat source 30 as shown in Figure 12A. The thickness of the first thermal brake along the channel axis was varied.

The second apparatus used had the first chamber only (without the first thermal brake) that is disposed in the second heat source as in the structure shown in Figure 17A. Other structures were identical to those of the first apparatus.

The third apparatus used had no chamber structure with other structures identical to the first apparatus. Hence, the third apparatus had the channel structure only (that works as a thermal brake) without the chamber.

In the three apparatuses, the length of the first, second and third heat sources along the channel axis 80 were about 4 mm, about 5.5 mm, and about 4 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 near the channel region (i.e., within the protrusion region) of about 2 mm and about 0.5 mm, respectively. The length of the first and second insulators outside the channel region (i.e., outside the protrusion region) was about 6 mm to about 3 mm (depending on position) and about 1 mm, respectively. The first chamber 100 had a cylindrical shape with a diameter of about 4 mm. The thermal brake 130 had a length or thickness along the channel axis 80 between about 1 mm to about 5.5 mm (when no chamber was present) with the wall 133 of the first thermal brake 130 contacting the whole circumference of the channel 70. Depth of the receptor hole 73 along the channel axis was about 2.8 mm. The channel 70 had a tapered cylinder shape. Average diameter of the channel was about 2 mm with the diameter at the bottom end (in the receptor hole) being about 1.5 mm. In these apparatuses, all the temperature shaping elements including the first chamber, the first thermal brake, the receptor hole, and the first and second insulators were disposed symmetrically with respect to the channel axis.

Template DNA used in this example was a 1 ng plasmid DNA. Two primers having the sequences as set forth in SEQ ID NOs: 1 and 2 were used and the size of the amplicon was 373 bp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively.

Figures 75A-E show results obtained when the thickness of the first thermal brake along the channel axis was varied. Figure 75A shows the results obtained when no thermal brake was present (i.e., the first chamber only). Figures 75B-E show the results obtained when the thickness of the first thermal brake was about 1 mm (Figure 75B), about 2 mm (Figure 75C), about 4 mm (Figure 75D), and about 5.5 mm (Figure 75E, i.e., channel only without the

chamber structure). As shown, the PCR amplification speed was reduced as the thickness of the first thermal brake was increased. Highest amplification speed was observed when there is no thermal brake (Figure 75A). With the first thermal brake present, the amplification speed was reduced (Figures 75B-E) as compared to the structure without the thermal brake (Figure 75A). As shown, thicker thermal brake imposed "stronger thermal braking", leading to slower PCR amplification. When there was no chamber structure (Figure 75E), no significant PCR amplification was observed as due to the very strong thermal braking by the channel alone structure.

Figures 76A-E show the results obtained when the gravity tilting angle of $10^{o}$ was introduced. Except for the gravity tilting angle, all other experimental conditions were the same as those used for the results presented in Figures 75A-E. Figure 76A shows the results obtained when no thermal brake was present (i.e., the first chamber only). Figures 76B-E show the results obtained when the thickness of the first thermal brake was about 1 mm (Figure 76B), about 2 mm (Figure 76C), about 4 mm (Figure 76D), and about 5.5 mm (Figure 76E, i.e., channel only without the chamber structure). As compared to the results shown in Figures 75A-E in which no gravity tilting angle was introduced, the PCR amplification was accelerated by use of the gravity tilting angle. Even when there is no chamber structure (i.e., the channel structure only, Figure 76E), introduction of the gravity tilting angle enabled successful PCR amplification in about 30 min reaction time. Without the gravity tilting angle, no significant PCR amplification was observed when there is no chamber structure (Figure 75E).

The results of this example demonstrate that the thermal brake, the chamber, and the gravity tilting angle are useful structural elements that can be used to adjust or control the speed of the thermal convection PCR depending on different applications. It was found that the chamber structure and the gravity tilting angle are useful to accelerate the thermal convection PCR while the thermal brake (including its thickness) is useful to decelerate the thermal convection PCR. It was confirmed that the speed of the thermal convection PCR can be modulated as desired by using one or more of such temperature shaping elements.

**Example 7. Thermal Convection PCR Using Apparatuses Having Structural Asymmetry**

Three types of apparatuses were used in this example. The first apparatus used had the structure shown in Figure 12A comprising a channel 70, a first chamber 100, a first thermal brake 130, a receptor hole 73, a through hole 71, protrusions 33, 34 of the second heat source 30,

and protrusions 23, 24 of the first heat source 20. The first thermal brake 130 was located on the bottom of the second heat source 30 with the first chamber 100 located on the upper part of the second heat source 30 as shown in Figure 12A. The thickness of the first thermal brake along the channel axis was about 1 mm. In this apparatus, all the temperature shaping elements including the first chamber, the first thermal brake, the receptor hole, and the first and second insulators were disposed symmetrically with respect to the channel axis.

The second apparatus used had an asymmetric receptor hole having a structure shown in Figure 21A. Half of the receptor hole was made deeper in the first heat source and close to the second heat source compared to the other half opposite to the channel axis. The difference of the receptor hole depth on the two opposite sides was varied to be about 0.2 mm and about 0.4 mm. Other structures of the second apparatus were identical to those of the first apparatus.

The third apparatus used had the first thermal brake that was made asymmetric. The first thermal brake in this apparatus was made to have the structure shown in Figure 28A so that one side of the thermal brake contacted the channel and the opposite side was spaced from the channel. The through hole formed in the first thermal brake was made larger than the diameter of the channel by about 0.4 mm and disposed off-centered with respect to the channel axis by about 0.2 mm. Other structures of the third apparatus including the thickness and position of the first thermal brake along the channel axis were identical to those of the first apparatus.

In the three apparatuses, the length of the first, second and third heat sources along the channel axis 80 were about 4 mm, about 6.5 mm, and about 4 mm, respectively. The first and second insulators (or insulating gaps) had a length along the channel axis 80 near the channel region (i.e., within the protrusion region) of about 1 mm and about 0.5 mm, respectively. The length of the first and second insulators outside the channel region (i.e., outside the protrusion region) was about 6 mm to about 3 mm (depending on position) and about 1 mm, respectively. The first chamber 100 had a cylindrical shape with a diameter of about 4 mm. The thermal brake 130 had a length or thickness along the channel axis 80 of about 1 mm. The depth of the receptor hole 73 along the channel axis was about 2.8 mm. The channel 70 had a tapered cylinder shape. Average diameter of the channel was about 2 mm with the diameter at the bottom end (in the receptor hole) being about 1.5 mm.

Template DNA used in this example was a 1 ng plasmid DNA. Two primers having the sequences as set forth in SEQ ID NOs: 1 and 2 were used and the size of the amplicon was 373 bp. The temperatures of the first, second and third heat sources were set to 98°C, 70°C, and 54°C, respectively.

Figure 77 shows the results obtained with the first apparatus having all the temperature shaping elements that are disposed symmetrically with respect to the channel axis. As shown, a weak product band was observed in 20 min reaction time and nearly saturated strong band was observed after 25 min.

Figures 78A-B show the results obtained with the second apparatus that had the asymmetric receptor hole structure. Difference of the receptor hole depths on the two opposite sides was about 0.2 mm for Figures 78A and about 0.4 mm for Figure 78B. As shown in Figures 78A-B, the PCR amplification became almost two times faster (and efficient) as compared to the results obtained with the symmetric apparatus (Figure 77). As manifested, the small horizontal asymmetry in the receptor hole was sufficient to accelerate the thermal convection PCR dramatically.

Figure 79 shows the results obtained with the third apparatus that had the asymmetric first thermal brake. As shown in Figure 79, the PCR amplification became more than two times faster (and efficient) as compared to the results obtained with the symmetric apparatus (Figure 77). In accord with the results obtained with the second apparatus, the small horizontal asymmetry in the first thermal brake was sufficient to accelerate the thermal convection PCR dramatically.

The results of this example demonstrate that the asymmetric structural elements such as asymmetric receptor hole, asymmetric thermal brake, asymmetric chamber, asymmetric insulators, etc. are useful structural elements. Such asymmetric structural elements can be used alone or in combination with other temperature shaping elements to modulate (typically to increase) the speed of the thermal convection PCR as desired.

The disclosures of all references mentioned herein (including all patent and scientific documents) are incorporated herein by reference. The invention has been described in detail with reference to particular embodiments thereof. However, it will be appreciated that those skilled in

the art, upon consideration of this disclosure, may make modifications and improvements within the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. An apparatus adapted to perform thermal convection PCR comprising:

(a) a first heat source for heating or cooling a channel and comprising a top surface and a bottom surface, the channel being adapted to receive a reaction vessel for performing PCR,

(b) a second heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the first heat source,

(c) a third heat source for heating or cooling the channel and comprising a top surface and a bottom surface, the bottom surface facing the top surface of the second heat source, wherein the channel is defined by a bottom end contacting the first heat source and a through hole contiguous with the top surface of the third heat source, and further wherein center points between the bottom end and the through hole form a channel axis about which the channel is disposed,

(d) at least one temperature shaping element such as a chamber disposed around the channel and within at least part of the second or third heat source, the chamber comprising a chamber gap between the second or third heat source and the channel sufficient to reduce heat transfer between the second or third heat source and the channel; and

(e) a receptor hole adapted to receive the channel within the first heat source.

2. The apparatus of claim 1, wherein the apparatus comprises a first insulator positioned between the top surface of the first heat source and the bottom surface of the second heat source.

3. The apparatus of any of claims 1-2, wherein the apparatus comprises a second insulator positioned between the top surface of the second heat source and the bottom surface of the third heat source.

4. The apparatus of claim 3, wherein the length of the first insulator along the channel axis is greater than the length of the second insulator along the channel axis.

5. The apparatus of any of claims 1-4, wherein the length of the second heat source is greater than the length of the first heat source or the third heat source along the channel axis.

6. The apparatus of any of claims 1-5, wherein the apparatus comprises a first chamber positioned entirely within the second heat source or the third heat source.

7. The apparatus of claim 6, wherein the first chamber is positioned within the second heat source and comprises a first chamber top end facing a first chamber bottom end along the channel axis.

8. The apparatus of claim 7, wherein the apparatus further comprises a second chamber positioned in the second heat source.

9. The apparatus of claim 8, wherein the apparatus further comprises a third chamber positioned in the second heat source.

10. The apparatus of claim 7, wherein the apparatus further comprises a second chamber positioned in the third heat source.

11. The apparatus of claim 8, wherein the apparatus further comprises a third chamber positioned in the third heat source.

12. The apparatus of claim 6, wherein the first chamber is positioned within the third heat source and comprises a first chamber top end facing a first chamber bottom end along the channel axis.

13. The apparatus of any of claims 7-12, wherein the chamber further comprises at least one chamber wall disposed around the channel axis.

14. The apparatus of claim 13, wherein the chamber is further defined by the channel along the channel axis.

15. The apparatus of claim 13, wherein the chamber wall is disposed essentially parallel to the channel axis.

16. The apparatus of any of claims 13-15, wherein the first chamber top end and the first chamber bottom end are each essentially perpendicular to the channel axis.

17. The apparatus of any of claims 2-16, wherein the first insulator comprises a solid or a gas.

18. The apparatus of any of claims 3-17, wherein the second insulator comprises a solid or a gas.

19. The apparatus of any of claims 6-16, wherein at least one chamber comprises a solid or a gas.

20. The apparatus of claim 19, wherein the first insulator and the second insulator comprise a solid or a gas.

21. The apparatus of any of claims 17-20, wherein the gas is air.

22. The apparatus of any of claims 1-21, wherein the channel is further defined by a height ($h$) along the channel axis from the bottom end of the channel to the top end of the through hole.

23. The apparatus of claim 22, wherein the channel is further defined by a first width ($w1$) along a first direction essentially perpendicular to the channel axis.

24. The apparatus of claims 23, wherein the channel is further defined by a second width ($w2$) essentially perpendicular to the first direction and the channel axis.

25. The apparatus of any of claims 23-24, wherein the first and/or second width ($w1$ and/or $w2$) decreases from the top end to the bottom end along the channel axis.

26. The apparatus of claim 25, wherein the first and second width ($w1$ or $w2$) of the channel is defined by a taper angle ($\theta$) of about 0° to about 15°.

27. The apparatus of any of claims 23-24, wherein the first and/or second width ($w1$ and/or $w2$) is essentially unchanged along the channel axis.

28. The apparatus of any of claims 22-27, wherein the bottom end of the channel is rounded, flat or curved.

29. The apparatus of any of claims 22-28, wherein the height ($h$) is at least about 5 mm to about 25 mm.

30. The apparatus of any of claims 22-29, wherein the average of the first or second width ($w1$ or $w2$) along the channel axis is at least about 1 mm to about 5 mm.

31. The apparatus of any of claims 24-30, wherein the vertical aspect ratio of the channel as defined by a ratio of the height ($h$) to the first or second width ($w1$ or $w2$) is about 4 to about 15.

32. The apparatus of any of claims 24-31, wherein the horizontal aspect ratio of the channel as defined by a ratio of the first width ($w1$) to the second width ($w2$) is about 1 to about 4.

33. The apparatus of any of claims 1-32, wherein at least part of the channel has a horizontal shape along a plane essentially perpendicular to the channel axis.

34. The apparatus of claim 33, wherein the horizontal shape has at least one reflection or rotation symmetry element.

35. The apparatus of claim 34, wherein the horizontal shape is a circular, rhombus, square, rounded square, ellipsoid, rhomboid, rectangular, rounded rectangular, oval, semi-circular, trapezoid, or rounded trapezoid shape along the plane.

36. The apparatus of any of claims 33-35, wherein the plane perpendicular to the channel axis is within the first, second or third heat source.

37. The apparatus of any of claims 6-36, wherein at least part of the chamber has a horizontal shape along a plane essentially perpendicular to the channel axis.

38. The apparatus of claim 37, wherein the horizontal shape has at least one reflection or rotation symmetry element.

39. The apparatus of claim 38, wherein the horizontal shape is a circular, rhombus, square, rounded square, ellipsoid, rhomboid, rectangular, rounded rectangular, oval, semi-circular, trapezoid, or rounded trapezoid shape along the plane.

40. The apparatus of any of claims 37-39, wherein the plane perpendicular to the channel axis is within the second or third heat source.

41. The apparatus of any of claims 6-40, wherein the chamber is disposed essentially symmetrically about the channel along a plane perpendicular to the channel axis.

42. The apparatus of any of claims 6-40, wherein at least part of the chamber is disposed asymmetrically about the channel along a plane perpendicular to the channel axis.

43. The apparatus of claim 41 or 42, wherein at least part of the channel is located inside the chamber along the plane perpendicular to the channel axis.

44. The apparatus of claim 42, wherein at least part of the channel is in contact with the chamber wall along the plane perpendicular to the channel axis.

45. The apparatus of claim 42, wherein at least part of the channel is located outside of the chamber along the plane perpendicular to the channel axis and contacting the second or third heat source.

46. The apparatus of any of claims 41-45, wherein the plane perpendicular to the channel axis is within the second or third heat source.

47. The apparatus of any of claims 41-46, wherein at least part of the chamber is tapered along the channel axis.

48. The apparatus of claim 47, wherein at least part of the chamber is positioned within the second heat source and has a width (*w*) perpendicular to the channel axis that is greater towards the third heat source than the first heat source.

49. The apparatus of claim 47, wherein at least part of the chamber is positioned within the second heat source and has a width (*w*) perpendicular to the channel axis that is greater towards the first heat source than the third heat source.

50. The apparatus of any of claims 41-46, wherein the apparatus comprises the first chamber and the second chamber positioned within the second heat source, the first chamber having a width (*w*) perpendicular to the channel axis that is different from the width (*w*) of the second chamber.

51. The apparatus of claim 50, wherein the first chamber is facing the first heat source.

52. The apparatus of any of claims 1-51, wherein the receptor hole is disposed symmetrically about the channel axis.

53. The apparatus of claim 52, wherein the receptor hole has a width perpendicular to the channel axis that is about the same as the width (*w*1 or *w*2) of the channel.

54. The apparatus of claim 52, wherein the receptor hole has a width perpendicular to the channel axis that is about 0.01 mm to about 0.2 mm larger than the width (*w*1 or *w*2) of the channel.

55. The apparatus of any of claims 6-54, wherein the apparatus comprises the first chamber and the second chamber positioned within the second heat source and the first chamber is spaced from the second chamber by a length (*l*) along the channel axis.

56. The apparatus of claim 55, wherein the first chamber, the second chamber, and the second heat source define a first thermal brake contacting the channel between the first and second chambers with an area and a thickness (or a volume) sufficient to reduce heat transfer from the first heat source or to the third heat source.

57. The apparatus of claim 56, wherein the first thermal brake comprises a top surface and a bottom surface.

58. The apparatus of claim 57, wherein the length (*l*) is between from about 0.1 mm to about 80% of the height of the second heat source along the channel axis.

59. The apparatus of any of claims 6-54, wherein the first chamber is positioned in the second heat source and the first chamber and the first insulator define a first thermal brake contacting the channel between the first chamber and the first insulator with an area and a thickness (or a volume) sufficient to reduce heat transfer from the first heat source.

60. The apparatus of claim 59, wherein the first thermal brake comprises a top surface and a bottom surface.

61. The apparatus of claim 60, wherein the bottom surface of the first thermal brake is located at about the same height as the bottom surface of the second heat source.

62. The apparatus of claim 61, wherein the first chamber is spaced from the first insulator by a length (*l*) along the channel axis.

63. The apparatus of claim 62, wherein the length (*l*) is between from about 0.1 mm to about 80% of the height of the second heat source along the channel axis.

64. The apparatus of claim 56, wherein the apparatus further comprises a third chamber positioned inside the second heat source and contacting the top surface of the second heat source.

65. The apparatus of claim 64, wherein the third chamber, the second chamber and the second heat source define a second thermal brake contacting the channel between the second and third chambers with an area and a thickness (or a volume) sufficient to reduce heat transfer to the third heat source.

66. The apparatus of claim 65, wherein the sum of the thickness of the first and second thermal brakes is less than about 80% of the height of the second heat source along the channel axis.

67. The apparatus of any of claims 6-54, wherein the apparatus comprises a first chamber, a first thermal brake positioned between the first chamber and the first insulator, and a second thermal brake positioned between the first chamber and the second insulator in the second heat source, wherein each of the first and second thermal brakes contacts the channel with an area and a thickness (or a volume) sufficient to reduce heat transfer from the first heat source or to the third heat source.

68. The apparatus of any of claims 6-67, wherein the receptor hole is off-centered with respect to the channel axis.

69. The apparatus of claim 68, wherein the receptor hole is off-centered by about 0.02 mm to about 0.5 mm.

70. The apparatus of any of claims 68-69, wherein the receptor hole has a width perpendicular to the channel axis that is larger than the width ($w1$ or $w2$) of the channel.

71. The apparatus of claim 70, wherein the width ($w$) of the receptor hole is about 0.04 mm to about1 mm larger than the width ($w1$ or $w2$) of the channel.

72. The apparatus of any of claims 6-71, wherein the apparatus further comprises a second chamber within the third heat source.

73. The apparatus of claim 72, wherein the chamber walls of the first chamber and the second chamber are positioned on about the same axis.

74. The apparatus of any of claims 72-73, wherein the apparatus further comprises a third chamber within the second heat source.

75. The apparatus of claim 74, wherein the chamber walls of the first, second and third chambers are positioned on about the same axis.

76. The apparatus of claim 75, wherein a thermal brake is positioned between the first and third chambers within the second heat source.

77. The apparatus of any of claims 72-73, wherein the apparatus further comprises a thermal brake positioned between the first chamber and the bottom surface of the second heat source.

78. The apparatus of any of claims 6-67, wherein the first chamber is positioned entirely within the third heat source.

79. The apparatus of any of claims 1-78, wherein the second heat source comprises at least one protrusion extending away from the second heat source.

80. The apparatus of claim 79, wherein the protrusion of the second heat source is essentially parallel to the channel axis and extending toward the first or third heat source.

81. The apparatus of any of claims 79-80, wherein the second heat source comprises a first protrusion extending toward the first heat source and defining a portion of the first chamber or the channel.

82. The apparatus of claim 81, wherein the first protrusion of the second heat source defines a portion of the first insulator and the second heat source.

83. The apparatus of claim 81, wherein the first protrusion of the second heat source separates the first insulator from the chamber or the channel.

84. The apparatus of claim 81, wherein the second heat source further comprises a second protrusion extending toward the third heat source and defining a portion of the chamber or the channel.

85. The apparatus of claim 84, wherein the second protrusion of the second heat source defines a portion of the second insulator and the second heat source.

86. The apparatus of claim 84, wherein the second protrusion of the second heat source separates the second insulator from the chamber or the channel.

87. The apparatus of any of claims 1-86, wherein the first heat source comprises at least one protrusion extending away from the first heat source.

88. The apparatus of claim 87, wherein the protrusion of the first heat source is essentially parallel to the channel axis and extending toward the second heat source or away from the bottom surface of the first heat source.

89. The apparatus of any of claims 87-88, wherein the first heat source comprises a first protrusion extending toward the second heat source and defining a portion of the channel.

90. The apparatus of claim 89, wherein the first protrusion of the first heat source defines a portion of the first insulator and the first heat source.

91. The apparatus of claim 89, wherein the first protrusion of the first heat source separates the first insulator from the channel.

92. The apparatus of claims 89, wherein the first insulator comprises a first insulator chamber defined by at least the first heat source, the first protrusion of the first heat source, the first protrusion of the second heat source and the second heat source.

93. The apparatus of any of claims 1-92, wherein the third heat source comprises at least one protrusion extending away from the third heat source.

94. The apparatus of claim 93, wherein the protrusion of the third heat source is essentially parallel to the channel axis and extending toward the second heat source or away from the top surface of the third heat source.

95. The apparatus of any of claims 93-94, wherein the third heat source comprises a first protrusion extending toward the second heat source and defining a portion of the channel or the chamber.

96. The apparatus of claim 95, wherein the first protrusion of the third heat source defines a portion of the second insulator and the third heat source.

97. The apparatus of claim 95, wherein the first protrusion of the third heat source separates the second insulator from the channel or the chamber.

98. The apparatus of claims 95, wherein the second insulator comprises a second insulator chamber defined by at least the third heat source, the first protrusion of the third heat source, the second protrusion of the second heat source and the second heat source.

99. The apparatus of any of claims 1-98, wherein the apparatus is adapted so that the channel axis is tilted with respect to the direction of gravity.

100. The apparatus of claim 99, wherein the channel axis is perpendicular to the top or bottom surface of any of the first, second, and third heat sources, and the apparatus is tilted.

101. The apparatus of claim 99, wherein the channel axis is tilted from a direction perpendicular to the top or bottom surface of any of the first, second, and third heat sources.

102. The apparatus of claim 99, wherein the tilt is defined by an angle $\theta g$ between the channel axis and the direction of gravity, the tilt angle being between from about 2° to about 60°.

103. The apparatus of any of claims 1-102, wherein the receptor hole is disposed asymmetrically about the channel axis, sufficient to cause horizontally uneven heat transfer from the first heat source to the channel.

104. The apparatus of claim 103, wherein the receptor hole comprises a receptor hole gap that is off-centered with respect to the channel axis (by about 0.02 mm to about 0.5 mm).

105. The apparatus of claim 104, wherein at least part of the receptor hole has a width perpendicular to the channel axis that is larger than the width ($w1$ or $w2$) of the channel.

106. The apparatus of claim 105, wherein the width ($w$) of the receptor hole is about 0.04 mm to about 1 mm larger than the width ($w1$ or $w2$) of the channel.

107. The apparatus of claim 103, wherein the apparatus comprises the receptor hole having a greater depth on one side than the other side along the channel axis.

108. The apparatus of claim 107, wherein the first heat source comprises a first protrusion extending toward the bottom surface of the second heat source and having a greater height on one side than the other side along the channel axis.

109. The apparatus of any of claims 107-108, wherein the second heat source has a constant height along the channel axis in the region around the channel.

110. The apparatus of any of claims 107-108, wherein the second heat source has a greater height along the channel axis on one side than the other side in the region around the channel.

111. The apparatus of any of claims 109-110, wherein the top end of the receptor hole is closer to the bottom surface of the second heat source on one side than the other side along the channel axis.

112. The apparatus of claim 110, wherein the top end of the receptor hole is located at a constant height from the bottom surface of the second heat source along the channel axis.

113. The apparatus of any of claims 6-112, wherein at least part of the chamber is disposed asymmetrically about the channel axis, sufficient to cause horizontally uneven heat transfer from the second or third heat source to the channel.

114. The apparatus of claim 113, wherein the first chamber is positioned within the second heat source and has a great height on one side than the other side along the channel axis, sufficient to cause horizontally uneven heat transfer from the second heat source to the channel.

115. The apparatus of claim 114, wherein the receptor hole has a constant depth around the channel along the channel axis.

116. The apparatus of claim 115, wherein the top end of the receptor hole is closer to the bottom surface of the second heat source on one side than the other side along the channel axis.

117. The apparatus of claim 114, wherein the receptor hole has a greater depth on one side than the other side along the channel axis.

118. The apparatus of claim 117, wherein the top end of the receptor hole is closer to the bottom surface of the second heat source on one side than the other side along the channel axis.

119. The apparatus of claim 117, wherein the top end of the receptor hole is located at a constant height from the bottom surface of the second heat source along the channel axis.

120. The apparatus of any of claims 114-119, wherein the second heat source comprises a first protrusion extending toward the top surface of the first heat source and having a greater height on one side than the other side along the channel axis.

121. The apparatus of any of claims 114-120, wherein the second heat source comprises a second protrusion extending toward the bottom surface of the third heat source and optionally having a greater height on one side than the other side along the channel axis.

122. The apparatus of claim 113, wherein the apparatus comprises the first chamber and the second chambers positioned within the second heat source and each off-centered from the channel axis along opposite directions.

123. The apparatus of claim 122, wherein the top end of the first chamber is positioned at essentially the same height as the bottom end of the second chamber.

124. The apparatus of claim 113, wherein the chamber wall of at least one chamber is tilted with respect to the channel axis.

125. The apparatus of claim 124, wherein the tilt angle is between from about 2° to about 30°.

126. The apparatus of claim 113, wherein at least one of the chambers in the second heat source has a chamber wall that is disposed higher on one side than the other side, sufficient to cause horizontally uneven heat transfer from the second heat source to the channel.

127. The apparatus of any of claims 6-112, wherein the first and second chambers are positioned within the second heat source and disposed symmetrically about the channel axis.

128. The apparatus of claim 127, wherein the first chamber is spaced from the second chamber by a length ($l$) along the channel axis.

129. The apparatus of any of claims 127-128, the apparatus further comprises a portion of the second heat source contacting the channel on the length ($l$) between the first and second chambers, the contact functioning as a thermal brake sufficient to reduce heat transfer from the first heat source or to the third heat source.

130. The apparatus of claim 129, wherein the thermal brake contacts one side of the channel on the length ($l$) between the first and second chambers, the other side of the channel being spaced from the second heat source.

131. The apparatus of claim 113, wherein at least part of the chamber is off-centered with respect to the channel axis by about 0.1 mm to about 3 mm.

132. The apparatus of claim 131, wherein at least part of the chamber has a larger chamber gap on one side than the other side along a direction perpendicular to the channel axis.

133. The apparatus of any of claims 131-132, wherein the apparatus further comprises a portion of the second heat source contacting the channel, the contact functioning as a thermal brake sufficient to reduce heat transfer from the first heat source or to the third heat source.

134. The apparatus of claim 133, wherein the thermal brake contacts the channel on one side, the other side being spaced from the second heat source.

135. The apparatus of claim 134, wherein the thermal brake contacts the entire height of one side of the channel within the second heat source.

136. The apparatus of claim 133, wherein the thermal brake contacts part of the height of the channel within the second heat source.

137. The apparatus of claim 136, wherein the apparatus comprises the first chamber and the second chamber positioned in the second heat source and the first chamber is spaced from the second chamber by a length (*l*) along the channel axis.

138. The apparatus of claim 137, wherein the thermal brake contacts the whole circumference of the channel on the length (*l*) between the first and second chambers.

139. The apparatus of claim 138, wherein the first chamber and the second chamber are off-centered from the channel axis along the same directions.

140. The apparatus of claim 138, wherein the first chamber and the second chamber are off-centered from the channel axis along opposite directions.

141. The apparatus of claim 137, wherein the thermal brake contacts one side of the channel on the length (*l*) between the first and second chambers, the other part of the channel being spaced from the second heat source.

142. The apparatus of claim 136, wherein the top end of the first chamber is positioned at essentially the same height as the bottom end of the second chamber and the thermal brake contacts the channel on one side within the first or second chamber, the other side of the channel being spaced from the second heat source.

143. The apparatus of claim 137, wherein the first chamber and the second chamber are off-centered from the channel axis along the same direction.

144. The apparatus of claim 137, wherein the first chamber and the second chamber are off-centered from the channel axis along opposite directions.

145. The apparatus of any of claims 143-144, wherein the thermal brake contacts one side of the channel on the length (*l*) between the first and second chambers, the other side of the channel being spaced from the second heat source.

146. The apparatus of claim 122, wherein the apparatus comprises a first thermal brake contacting the channel on one side within the first chamber, the other side being spaced from the second heat source.

147. The apparatus of claim 146, wherein the apparatus further comprises a second thermal brake contacting the channel on one side within the second chamber, the other side being spaced from the second heat source.

148. The apparatus of claims 147, wherein the top end of the first thermal brake is positioned at essentially the same height as the bottom end of the second thermal brake.

149. The apparatus of claims 147, wherein the top end of the first thermal brake is positioned higher than the bottom end of the second thermal brake.

150. The apparatus of claims 147, wherein the top end of the first thermal brake is positioned lower than the bottom end of the second thermal brake.

151. The apparatus of claim 137, wherein the top end of the first chamber and the bottom end of the second chamber are each tilted with respect to a direction perpendicular to the channel axis.

152. The apparatus of claim 151, wherein the thermal brake contacts the whole circumference of the channel between the first chamber and the second chamber and at a higher location on one side than the other side.

153. The apparatus of claim 137, wherein the first chamber and the second chamber are each tilted with respect to the channel axis.

154. The apparatus of claim 153, wherein the bottom end of the first chamber and the top end of the second chamber are each essentially perpendicular to the channel axis.

155. The apparatus of claim 154, wherein the thermal brake contacts the whole circumference of the channel between the first chamber and the second chamber.

156. The apparatus of claim 153, wherein the bottom end of the first chamber and the top end of the second chamber are each tilted with respect to a direction perpendicular to the channel axis.

157. The apparatus of claim 156, wherein the thermal brake contacts the whole circumference of the channel between the first chamber and the second chamber and at a higher location on one side than the other side.

158. The apparatus of any of claims 3-157, wherein each of the first heat source, second heat source, and third heat source comprises at least one securing element.

159. The apparatus of claim 158, wherein each of the first insulator and second insulator comprises at least one securing element.

160. The apparatus of any of claims 158-159, wherein the apparatus comprises a first housing element that surrounds the first heat source, second heat source, third heat source, first insulator, and second insulator.

161. The apparatus of claims 160, wherein the apparatus further comprises a second housing element that surrounds the first housing element.

162. The apparatus of any of claims 160-161, wherein the securing elements are adapted to secure the first heat source, second heat source, third heat source, first insulator, and second insulator to each other or to the first housing element.

163. The apparatus of claim 162, wherein at least one of the securing elements is located in an outer region of at least one, preferably all of the first heat source, second heat source, third heat source, first insulator, and second insulator.

164. The apparatus of any of claims 162-163, wherein at least one of the securing elements is located in an inner region of at least one, preferably all of the first heat source, second heat source, third heat source, first insulator, and second insulator.

165. The apparatus of any of claims 158-164, wherein at least one of the first heat source, first insulator, second heat source, second insulator, and third heat source comprises at least one wing structure.

166. The apparatus of claim 165, wherein the wing structure comprises a first, second, third and fourth wing structure.

167. The apparatus of any of claim 165-166, wherein the third heat source comprises the wing structure.

168. The apparatus of any of claims 165-167, wherein the wing structure defines a third insulator between the first, second, and third heat sources and the first housing element.

169. The apparatus of claim 168, wherein the first and the second wing structures define a first part of the third insulator.

170. The apparatus of claim 169, wherein the second and the third wing structures define a second part of the third insulator.

171. The apparatus of claim 170, wherein the third and fourth wing structures define a third part of the third insulator.

172. The apparatus of claim 171, wherein the fourth and the first wing structures define a fourth part of the third insulator.

173. The apparatus of any of claims 169-172, wherein each of the first, second, third and fourth parts of the third insulator are further defined by the first housing element.

174. The apparatus of claim 173, wherein the bottom of the first heat source and the first housing element define a fourth insulator.

175. The apparatus of claim 174, wherein the apparatus further comprises a fifth insulator and/or a sixth insulator that is defined by the first housing element and the second housing element.

176. The apparatus of any of claims 158-175, wherein each of the first, second, and third heat sources comprises at least one heating and/or cooling element.

177. The apparatus of claim 176, wherein each of the first, second, and third heat sources further comprises a temperature sensor.

178. The apparatus of claim 177, wherein the apparatus further comprises at least one fan unit to remove heat from the first, second, and/or third heat sources.

179. The apparatus of claim 178, wherein the apparatus comprises a first fan unit positioned above the third heat source to remove heat from the third heat source.

180. The apparatus of claim 179, wherein the apparatus further comprises a second fan unit positioned below the first heat source to remove heat from the first heat source.

181. The apparatus of any of claims 1-180, wherein the apparatus is adapted to generate a centrifugal force inside the channel so as to modulate the convection PCR.

182. The apparatus of claim 181, wherein the apparatus comprises at least the first, second, and third heat sources rotatably attached to a rotor for rotating the heat sources about an axis of rotation.

183. The apparatus of claim 182, wherein the apparatus comprises a rotation arm attached to the rotor that defines a radius of the centrifugal rotation from the axis of the rotation to the center of the channel.

184. The apparatus of any of claims 182-183, wherein the axis of rotation is essentially parallel to the direction of gravity.

185. The apparatus of any of claims 182-184, wherein the channel axis is essentially parallel to the direction of net force generated by the gravitational force and the centrifugal force.

186. The apparatus of any of claims 182-184, wherein the channel axis is tilted with respect to the direction of net force generated by the gravitational force and the centrifugal force.

187. The apparatus of claim 186, wherein the tilt angle between the channel axis and the direction of the net force is between about $2^{o}$ to about $60^{o}$.

188. The apparatus of any of claims 185-187, wherein the apparatus further comprises a tilt shaft adapted to control the angle between the channel axis and the net force.

189. The apparatus of any of claims 182-188, wherein the axis of rotation is located outside of the first, second, and third heat sources.

190. The apparatus of any of claims 182-188, wherein the axis of rotation is located essentially at the center of the first, second, and third heat sources.

191. The apparatus of claim 190, wherein the apparatus comprises a plurality of channels that are located concentrically with respect to the axis of rotation.

192. The apparatus of claim 191, wherein the first, second, and third heat sources have circular shapes.

193. A PCR centrifuge adapted to perform a polymerase chain reaction (PCR) under centrifugation conditions, the PCR centrifuge comprising the apparatus featured in any one of claims 181-192.

194. A method for performing a polymerase chain reaction (PCR) by thermal convection, the method comprising at least one and preferably all of the following steps:

(a) maintaining a first heat source comprising a receptor hole at a temperature range suitable for denaturing a double-stranded nucleic acid molecule and forming a single-stranded template,

(b) maintaining a third heat source at a temperature range suitable for annealing at least one oligonucleotide primer to the single-stranded template,

(c) maintaining a second heat source at a temperature suitable for supporting polymerization of the primer along the single-stranded template; and

(d) producing thermal convection between the receptor hole and the third heat source under conditions sufficient to produce the primer extension product.

195. The method of claim 194, wherein the method further comprises a step of providing a reaction vessel comprising the double-stranded nucleic acid and the oligonucleotide primer in aqueous solution.

196. The method of claim 195, wherein the reaction vessel further comprises a DNA polymerase.

197. The method of claim 196, wherein the DNA polymerase is an immobilized DNA polymerase.

198. The method of any of claims 195-197, wherein the method further comprises a step of contacting the reaction vessel to the receptor hole and at least one temperature shaping element such as a chamber disposed within at least one of the second or third heat source, the contacting being sufficient to support the thermal convection within the reaction vessel.

199. The method of claim 198, wherein the method further comprises a step of contacting the reaction vessel to a first insulator between the first and second heat sources and a second insulator between the second and third heat sources.

200. The method of claim 199, wherein the first, second and third heat sources have a thermal conductivity at least about tenfold greater than the reaction vessel or aqueous solution therein.

201. The method of claim 200, wherein the first and second insulators have a thermal conductivity at least about five fold smaller than the reaction vessel or aqueous solution therein, wherein the thermal conductivity of the first and second insulators is sufficient to reduce heat transfer between the first, second and third heat sources.

202. The method of any of claims 194-201, wherein the method further comprises a step of producing a fluid flow within the reaction vessel that is essentially symmetric about the channel axis.

203. The method of any of claims 194-201, wherein the method further comprises a step of producing a fluid flow within the reaction vessel that is asymmetric about the channel axis.

204. The method of any of claims 195-203, wherein at least steps (a)-(c) consume less than about 1 W of power per reaction vessel to produce the primer extension product.

205. The method of claim 204, wherein the power for performing the method is supplied by a battery.

206. The method of any of claims 194-205, wherein the PCR extension product is produced in about 15 to about 30 minutes or shorter.

207 The method of any of claims 195-206, wherein the reaction vessel has a volume of less than about 50 microliters.

208 The method of claim 207, wherein the reaction vessel has a volume of less than about 20 microliters.

209. The method of any of claims 194-208, wherein the method further comprises a step of applying a centrifugal force to the reaction vessel conducive to performing the PCR.

210. A method for performing a polymerase chain reaction (PCR) by thermal convection, the method comprising the steps of adding an oligonucleotide primer, nucleic acid template, and buffer to a reaction vessel received by the apparatus of any one of claims 1-192 under conditions sufficient to produce a primer extension product.

211. The method of claim 210, wherein the method further comprises a step of adding a DNA polymerase to the reaction vessel.

212. A method for performing a polymerase chain reaction (PCR) by thermal convection, the method comprising the steps of adding an oligonucleotide primer, nucleic acid template, and buffer to a reaction vessel received by the PCR centrifuge of claim 193 and applying a centrifugal force to the reaction vessel under conditions sufficient to produce a primer extension product.

213. The method of claim 212, wherein the method further comprises a step of adding a DNA polymerase to the reaction vessel.

214. A reaction vessel adapted to be received by the apparatus of claims 1-192 or the PCR centrifuge of claim 193, the reaction vessel comprising a top end, a bottom end, an outer wall and an inner wall, and having a vertical aspect ratio of the outer wall being at least between about 4 to about 15, a horizontal aspect ratio of the outer wall between about 1 to about 4, and a taper angle $\theta$ of the outer wall between about 0° to about 15°.

215. The reaction vessel of claim 214, wherein center points of the top end and the bottom end of the outer wall define a reaction vessel axis.

216. The reaction vessel of claim 215, wherein the height of the reaction vessel along the reaction vessel axis is at least between about 6 mm to about 35 mm.

217. The reaction vessel of claim 216, wherein the average of the width of the outer wall is between about 1 mm to about 5 mm.

218. The reaction vessel of claim 217, wherein the average of the width of the inner wall is about 0.5 mm to about 4.5 mm.

219. The reaction vessel of any of claims 215-218, wherein the outer wall and the inner wall have essentially the same vertical shape along the reaction vessel axis.

220. The reaction vessel of claim 219, wherein the outer wall and the inner wall have essentially the same horizontal shape along a cross-section perpendicular to the reaction vessel axis.

221. The reaction vessel of any of claims 215-218, wherein the outer wall and the inner wall have different vertical shapes along the reaction vessel axis.

222. The reaction vessel of claim 221, wherein the outer wall and the inner wall have different horizontal shapes along a cross-section perpendicular to the reaction vessel axis.

223. The reaction vessel of any of claims 220 and 222, wherein the horizontal shape is one or more of a circular, rhombus, square, rounded square, ellipsoidal, rhomboid, rectangular, rounded rectangular, oval, triangular, rounded triangular, trapezoidal, rounded trapezoidal or oblong shape.

224. The reaction vessel of any of claims 219-223, wherein the inner wall is disposed essentially symmetrically with respect to the reaction vessel axis.

225. The reaction vessel of claim 224, wherein the thickness of the reaction vessel wall is between about 0.1 mm to about 0.5 mm.

226. The reaction vessel of claim 225, wherein the thickness of the reaction vessel wall is essentially unchanged along the reaction vessel axis.

227. The reaction vessel of any of claims 219-223, wherein the inner wall is disposed off-centered with respect to the reaction vessel axis.

228. The reaction vessel of claim 227, wherein the thickness of the reaction vessel wall is between about 0.1 mm to about 1 mm.

229. The reaction vessel of claim 228, wherein the thickness of the reaction vessel wall is thinner on one side than the other side by at least about 0.05 mm.

230. The reaction vessel of any of claims 214-229, wherein the bottom end is flat, curved or rounded.

231. The reaction vessel of claim 230, wherein the bottom end is disposed essentially symmetrically with respect to the reaction vessel axis.

232. The reaction vessel of claim 230, wherein the bottom end is disposed asymmetrically with respect to the reaction vessel axis.

233. The reaction vessel of any of claims 230-232, wherein the bottom end is closed.

234. The reaction vessel of any of claims 214-233, wherein the reaction vessel comprises or consists of a plastic, ceramic or a glass.

235. The reaction vessel of any of claims 214-234 further comprising an immobilized DNA polymerase.

236. The reaction vessel of any of claims 214-235 further comprising a cap in sealing contact with the reaction vessel.

237. The reaction vessel of claim 236, wherein the cap comprises an optical port.

238. The reaction vessel of claim 237 further comprising an open space between the inner wall of the reaction vessel and the side part of the optical port.

239. The apparatus of any of claims 1-192 further comprising at least one optical detection unit.

240. The PCR centrifuge of claim 193, wherein the apparatus in any one of claims 181-192 further comprises at least one optical detection unit.

241. The method of any one of claims 194-209 further comprising the step of detecting the primer extension product in real-time by using at least one optical detection unit.

242. The method of any one of claims 210-213, further comprising the step of detecting the primer extension product in real-time by using at least one optical detection unit.

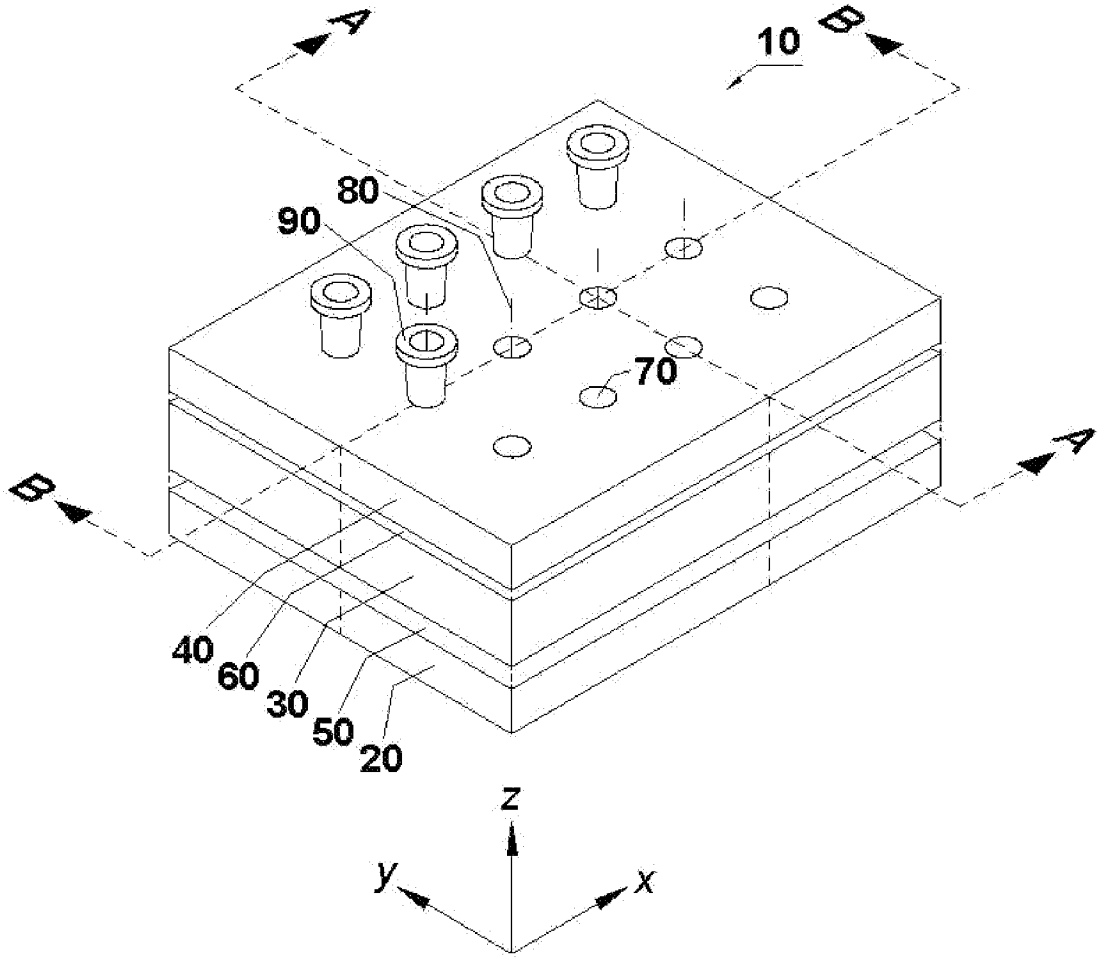


FIG. 1

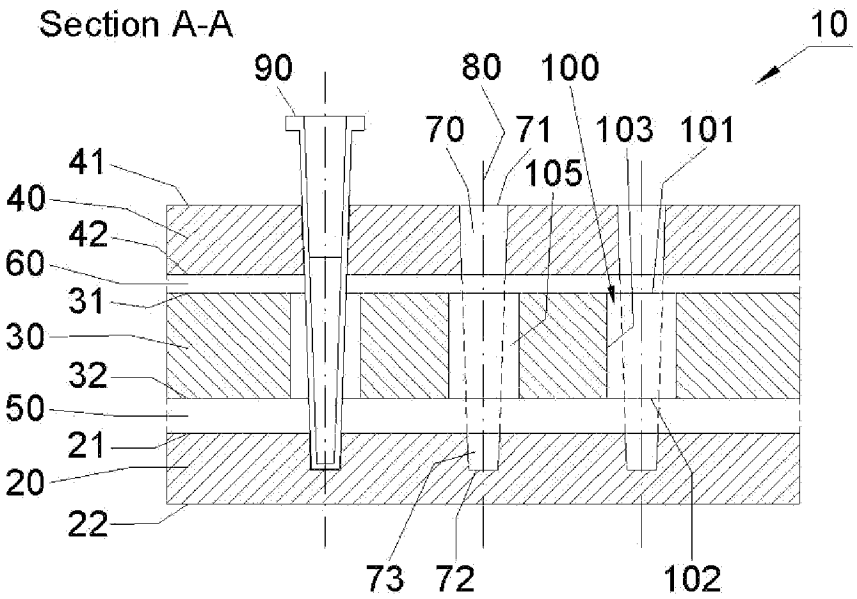
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FIG. 2A

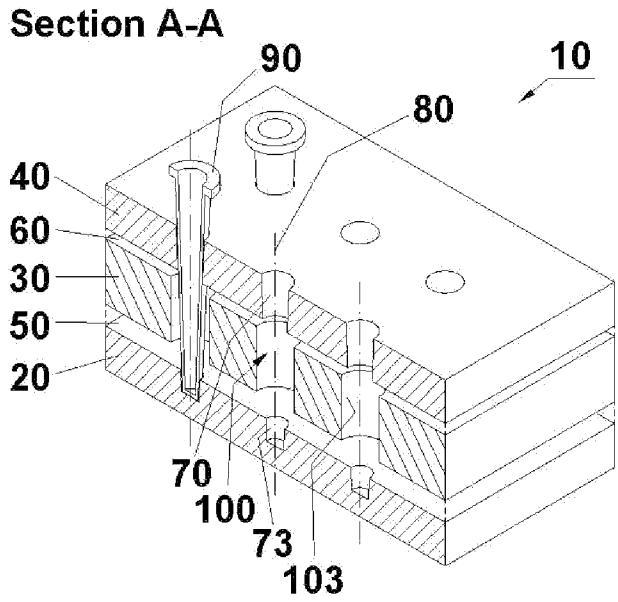
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FIG. 2B

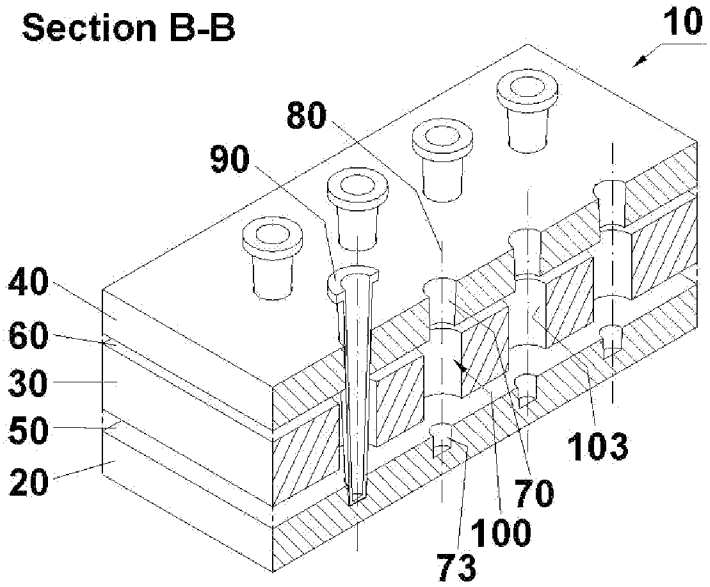
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FIG. 2C

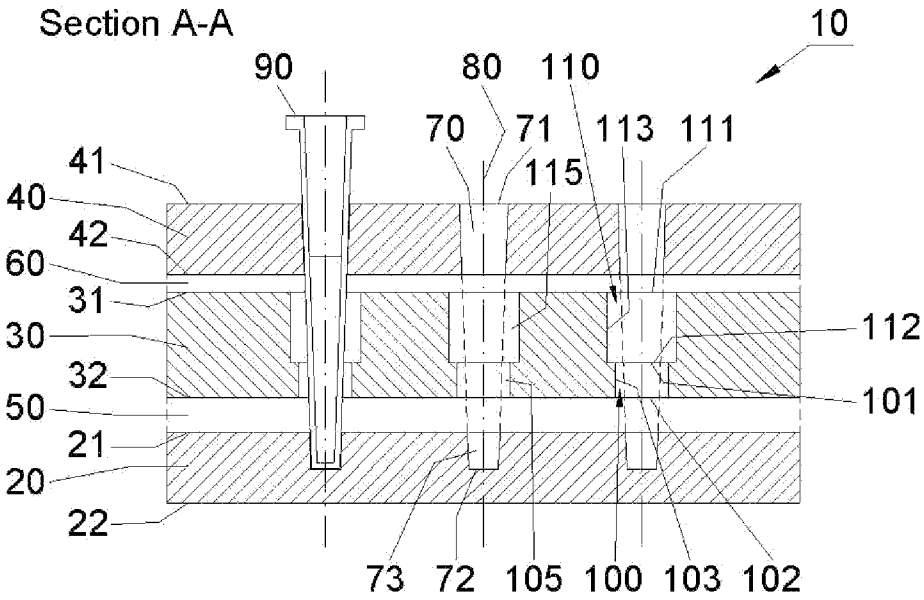
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FIG. 3A

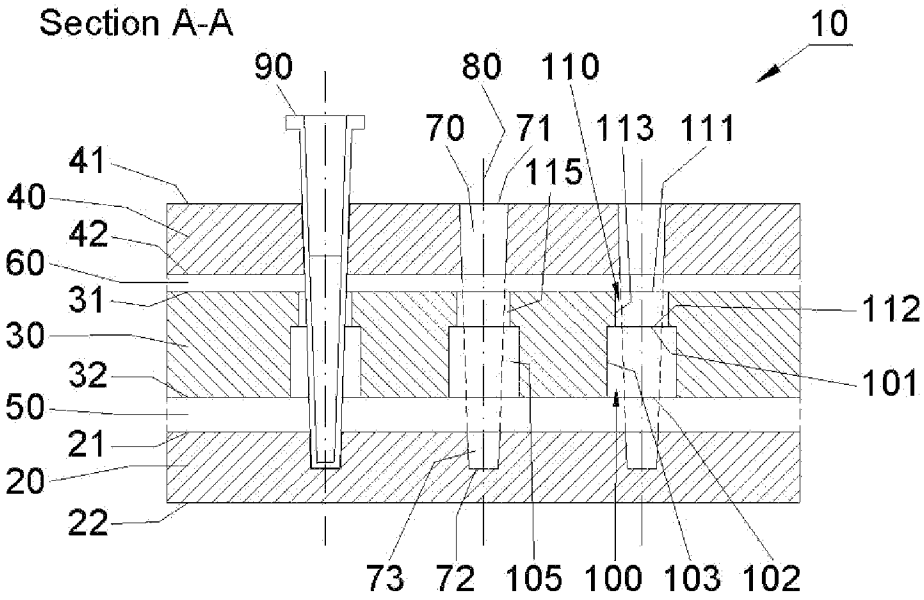
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FIG. 3B

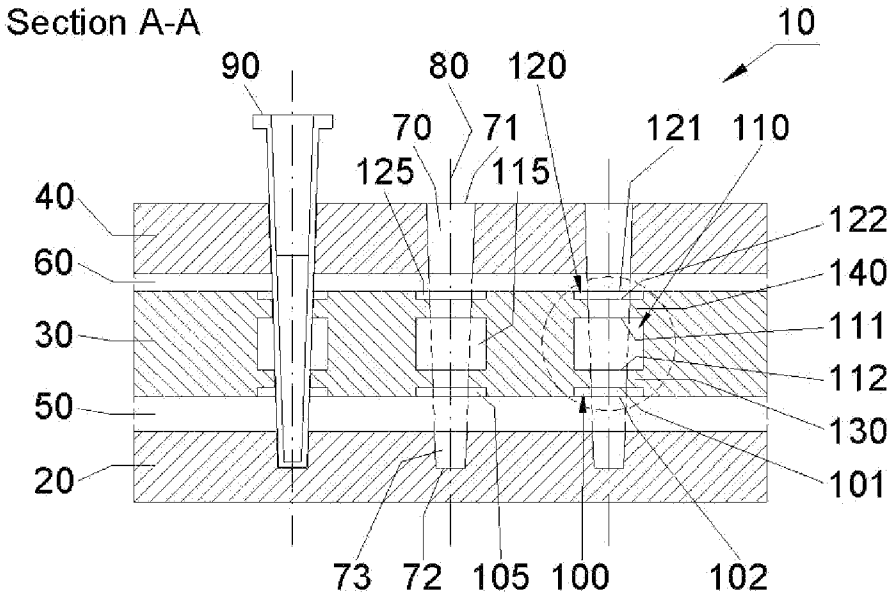
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FIG. 4A

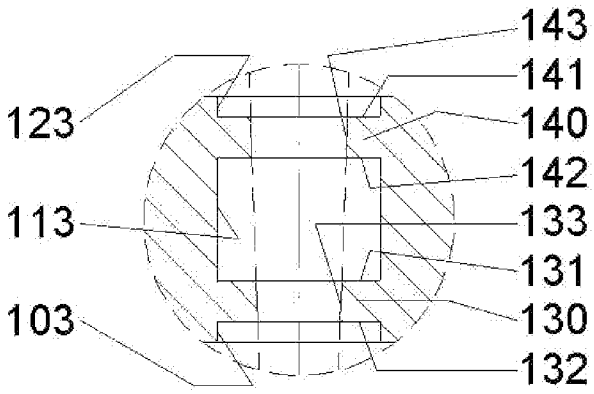
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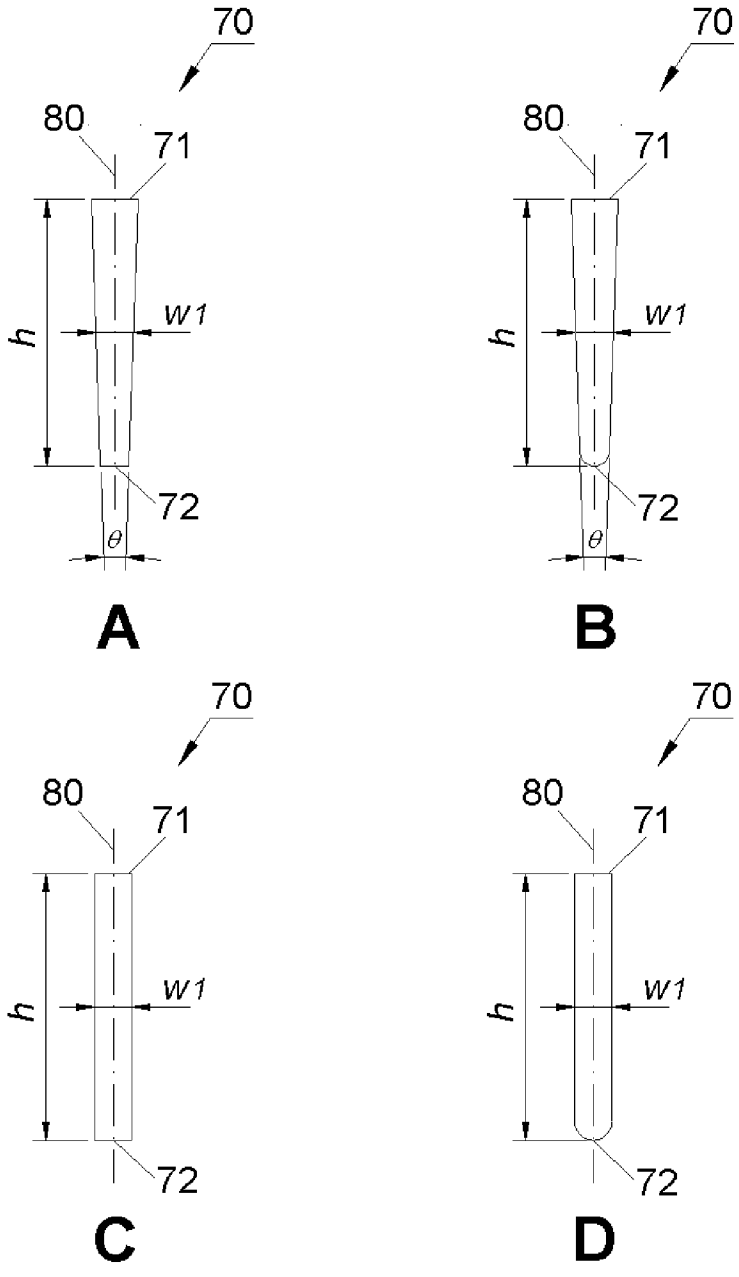
FIG. 4B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W1
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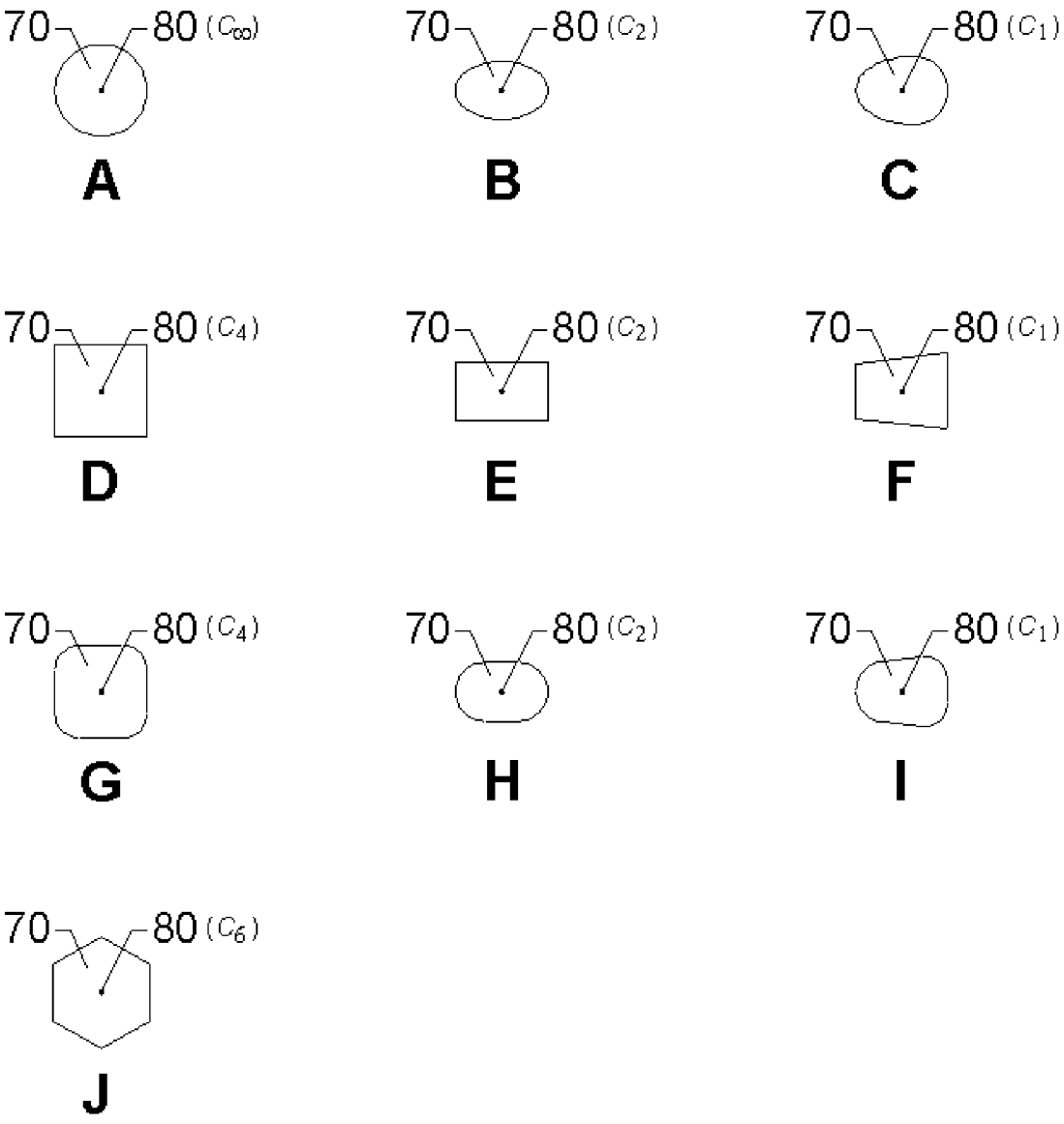
FIG. 5A-D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I
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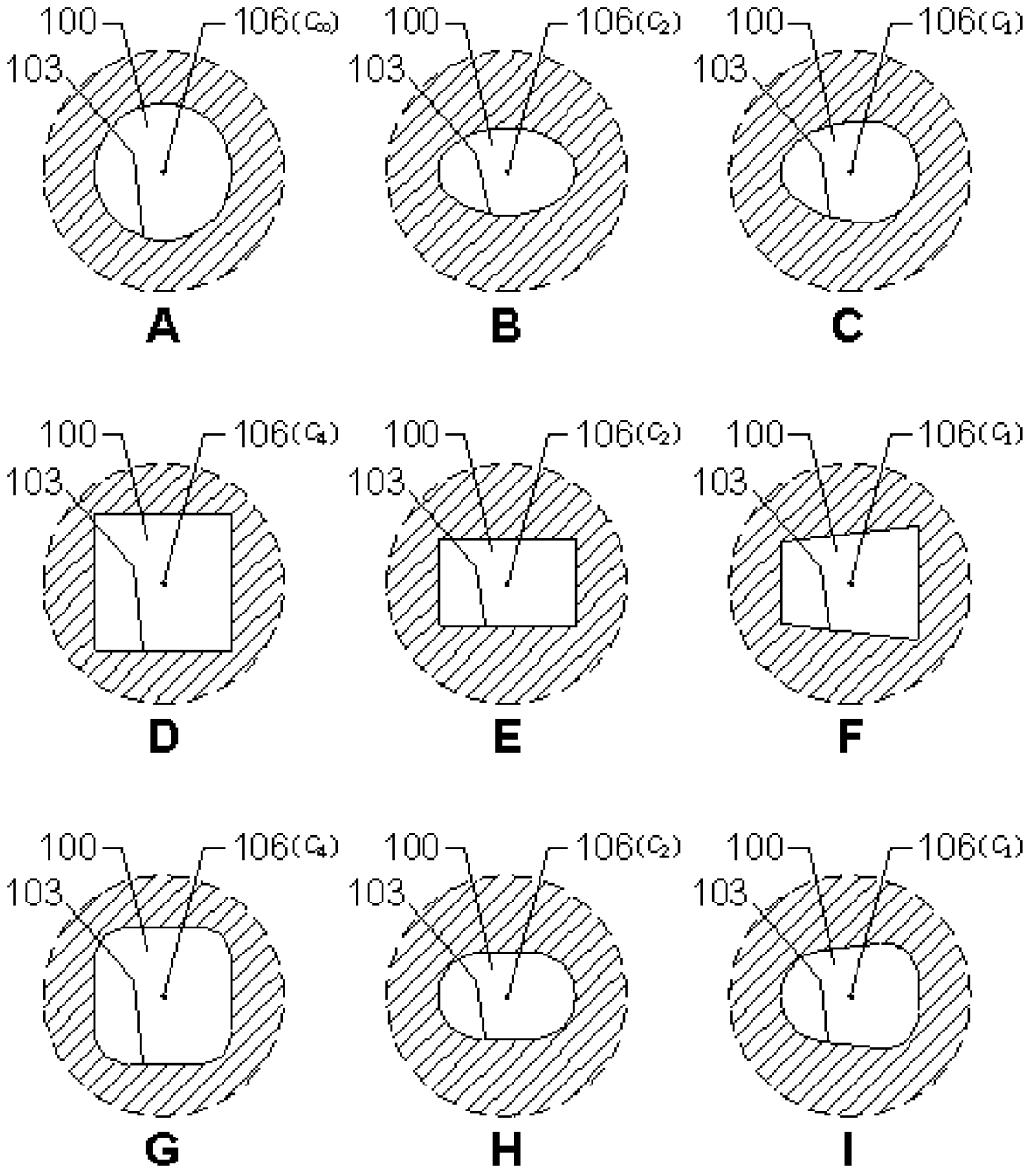
80 ($C_6$)
J

FIG. 6A-J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E
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106(C1)
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106(C2)
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H
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106(C1)
103
I

FIG. 7A-I

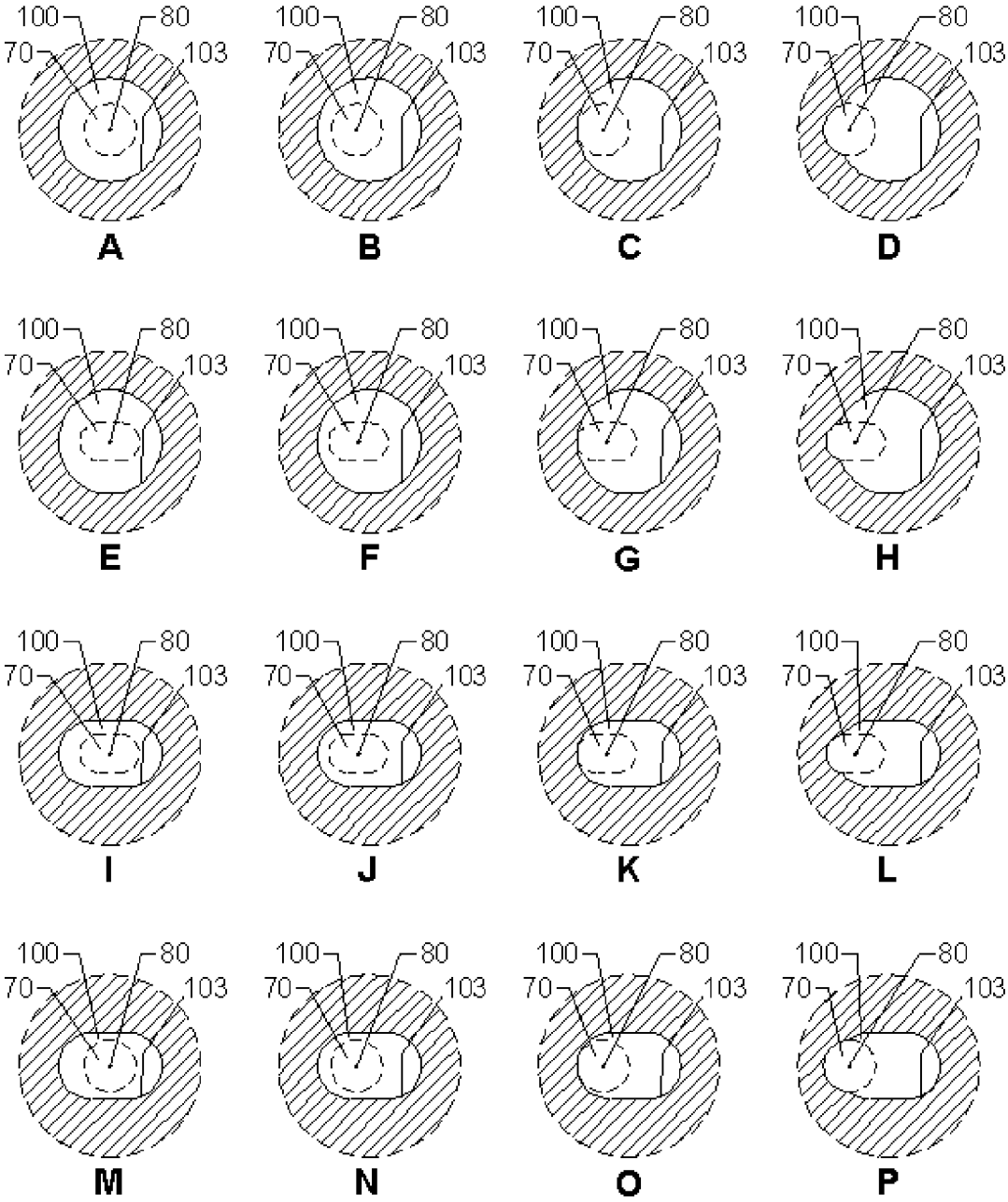


FIG. 8A-P

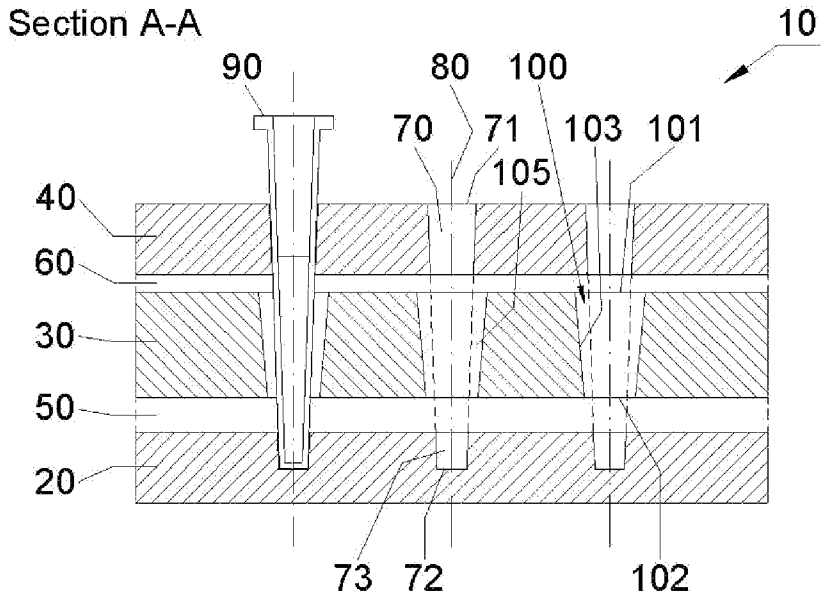
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FIG. 9A

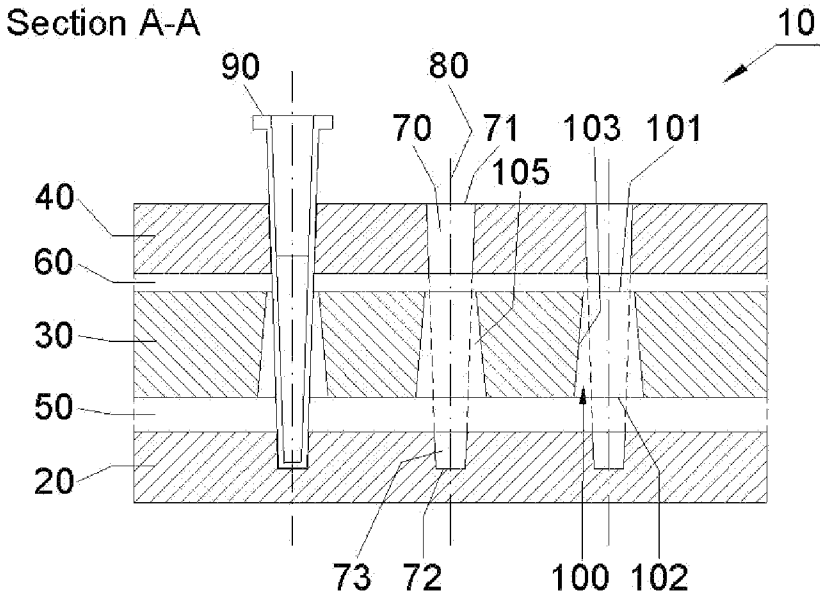
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FIG. 9B

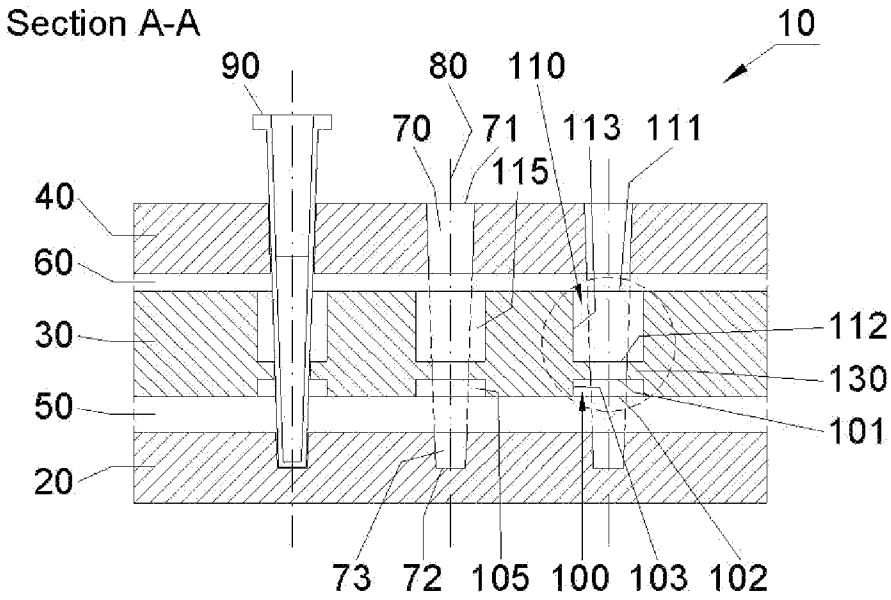


FIG. 10A

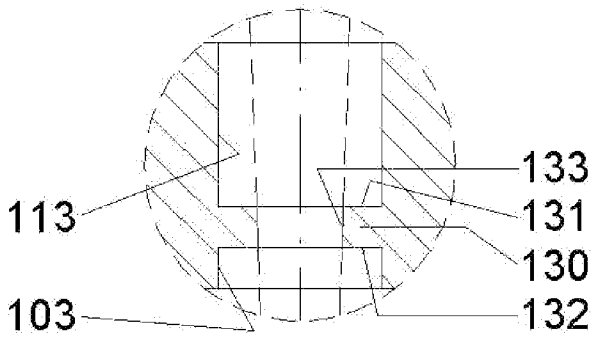


FIG. 10B

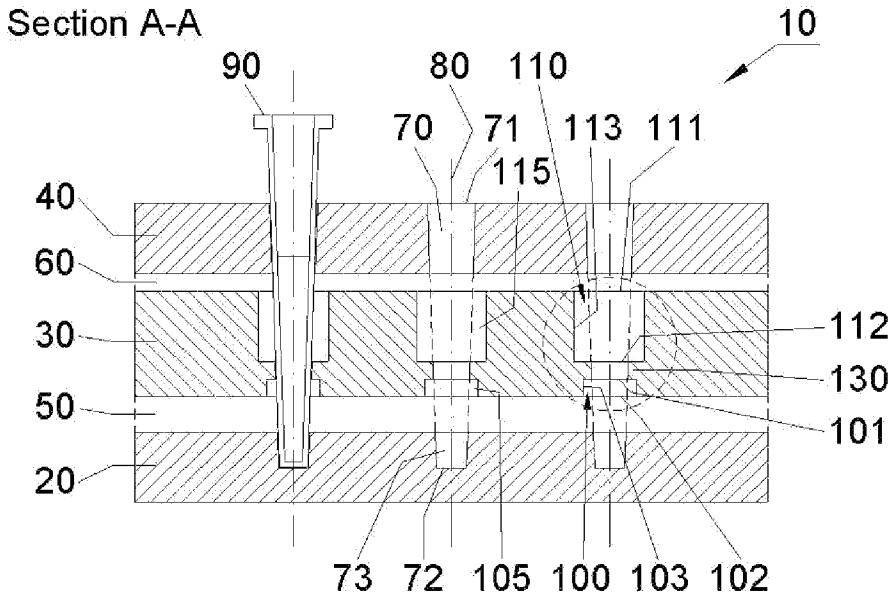


FIG. 10C

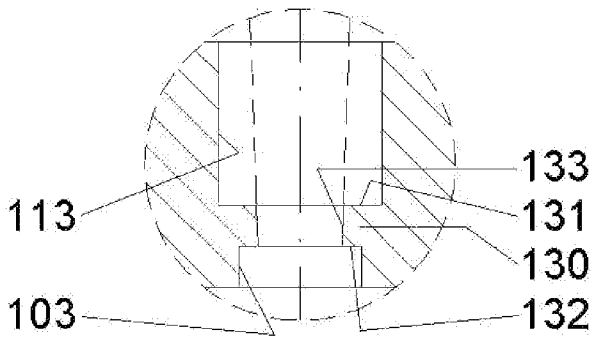


FIG. 10D

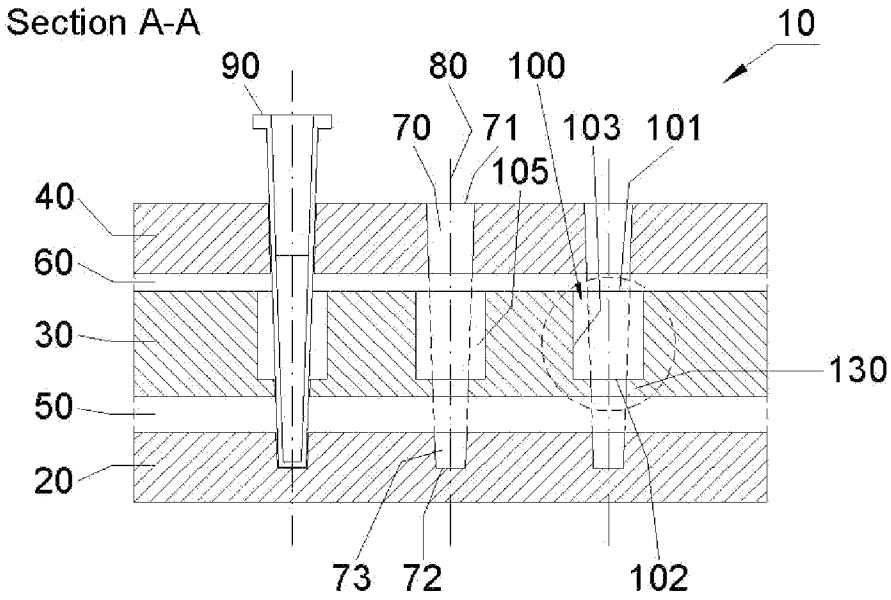


FIG. 10E

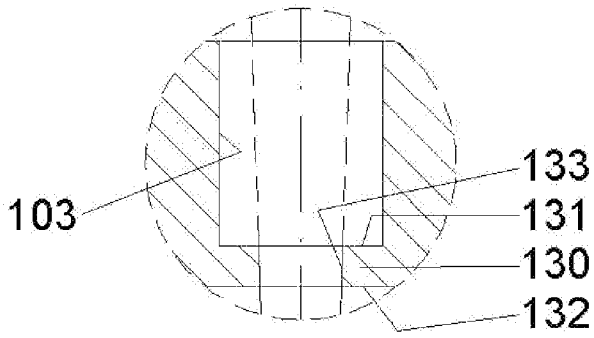


FIG. 10F

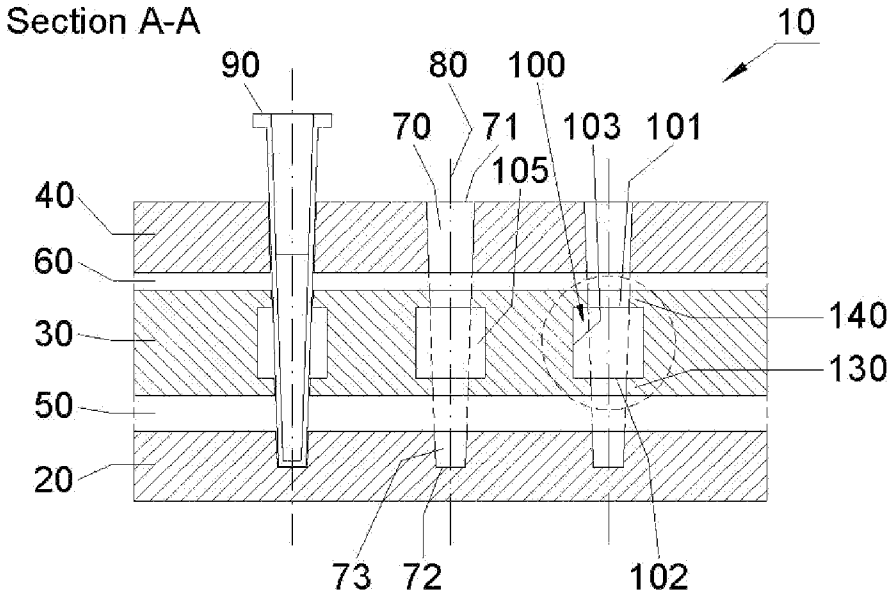


FIG. 11A

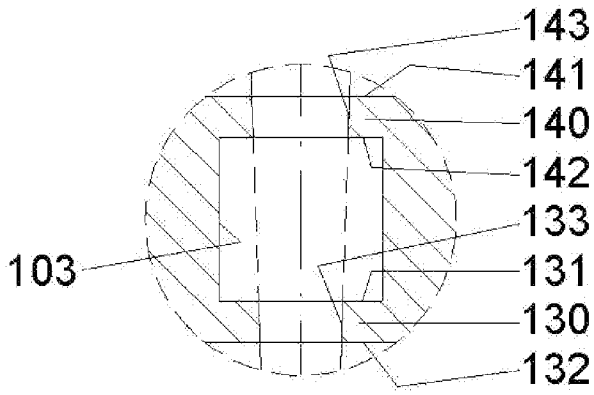


FIG. 11B

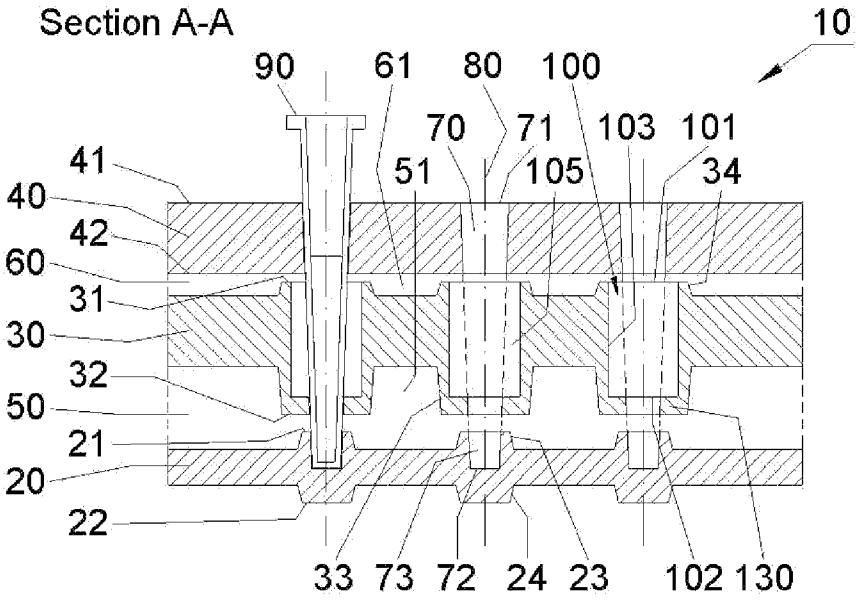


FIG. 12A

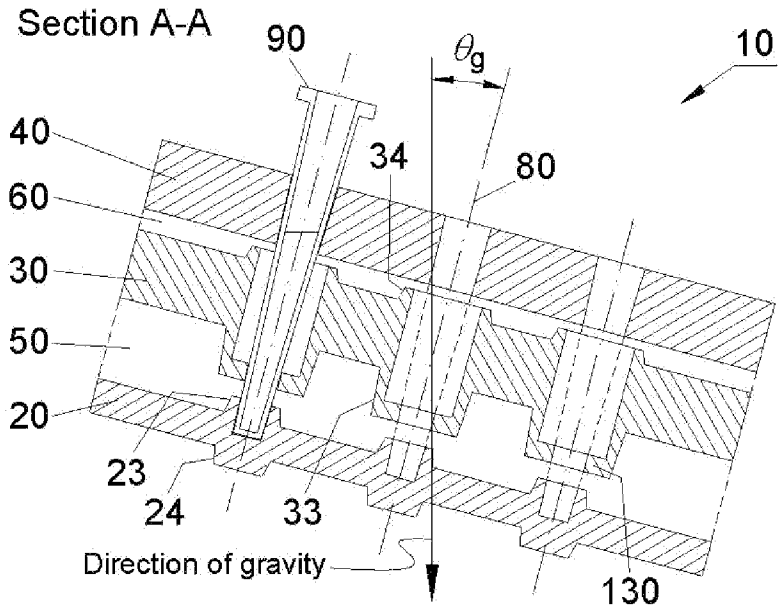


FIG. 12B

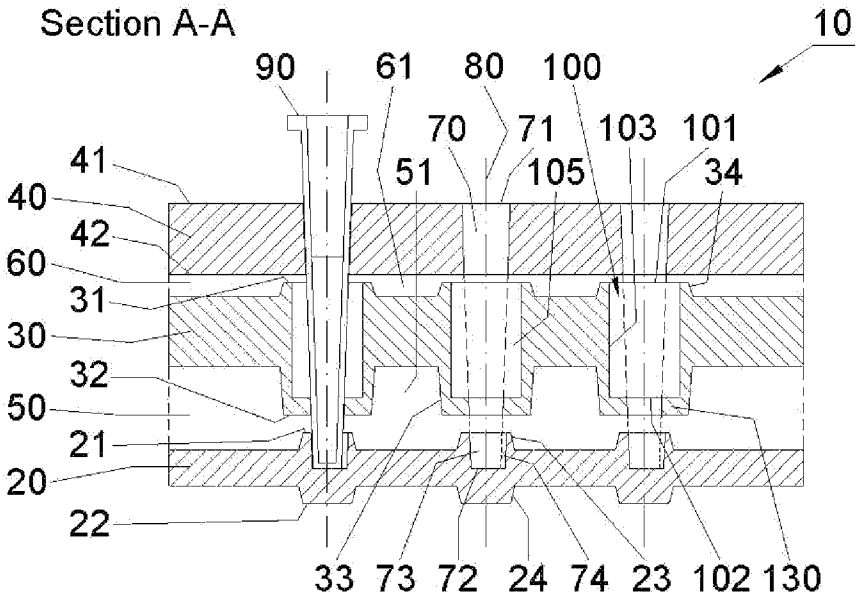
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FIG. 13

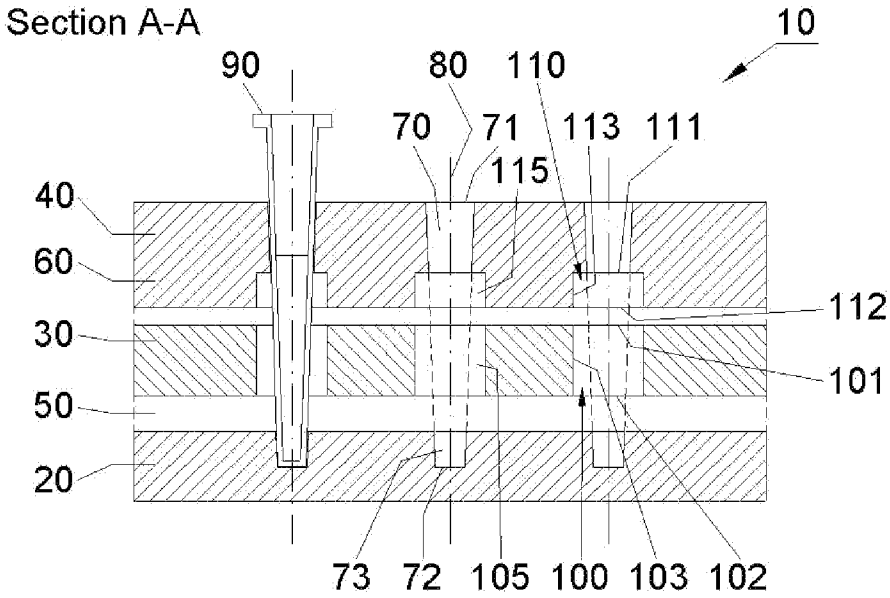


FIG. 14A

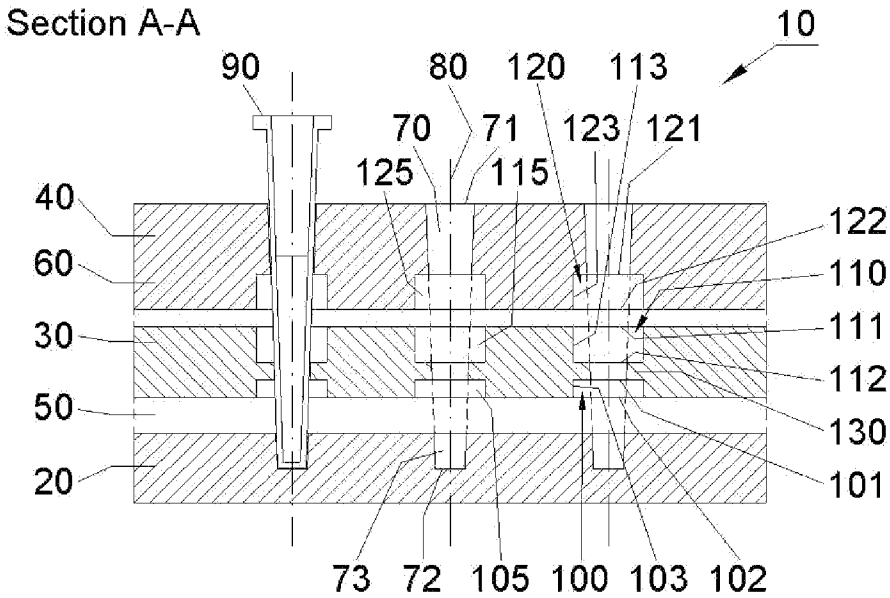


FIG. 14B

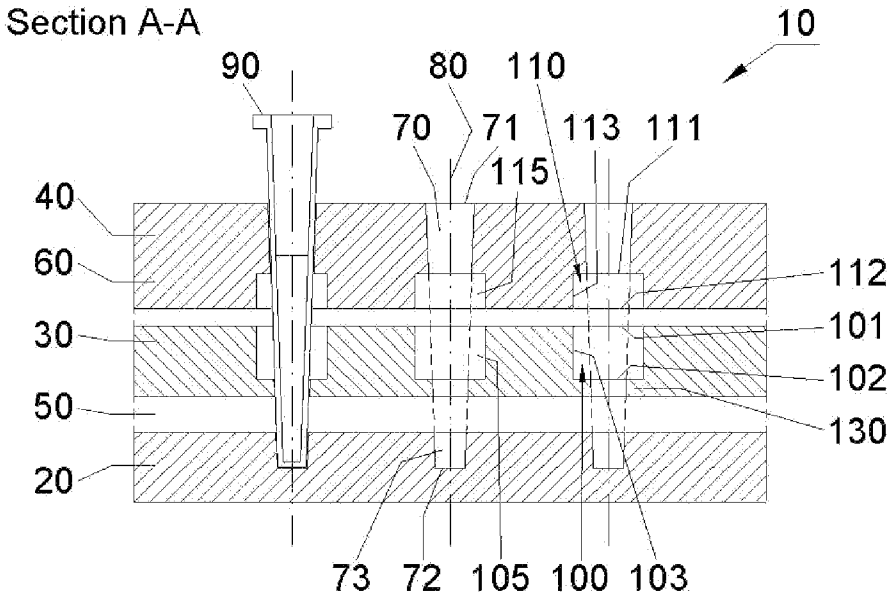
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FIG. 14C

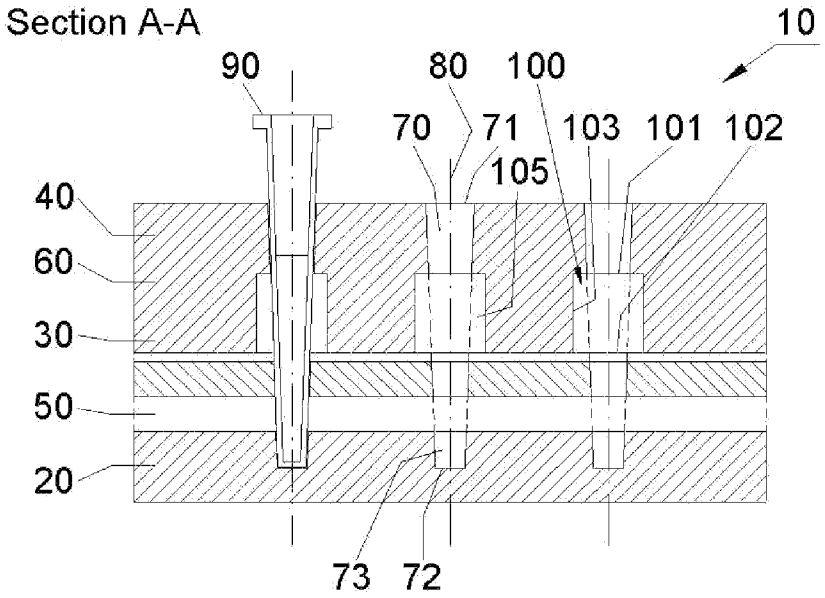


FIG. 15A

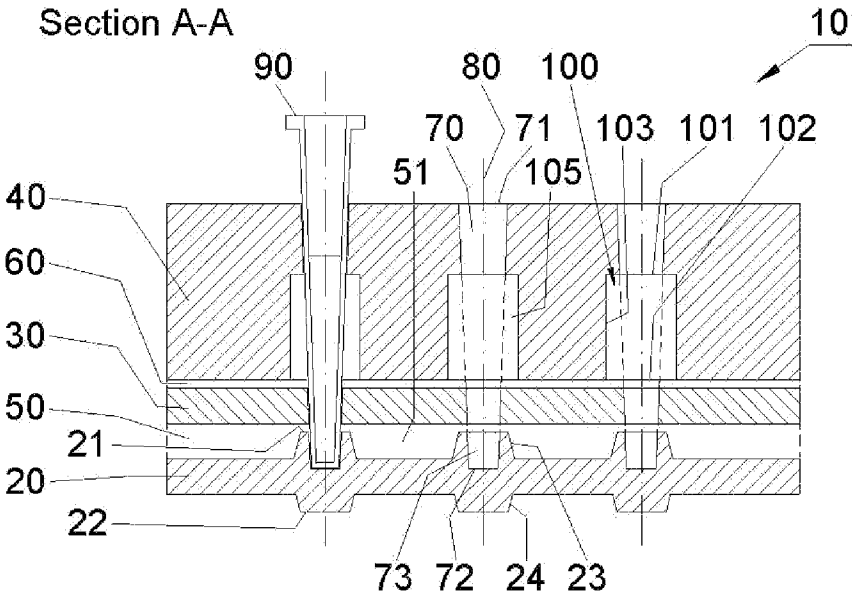


FIG. 15B

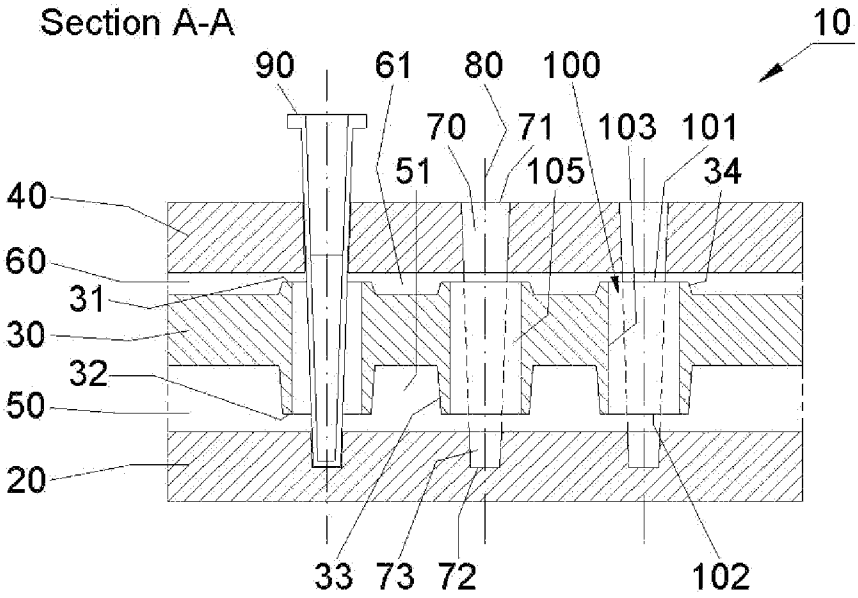
Section A-A
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FIG. 16A

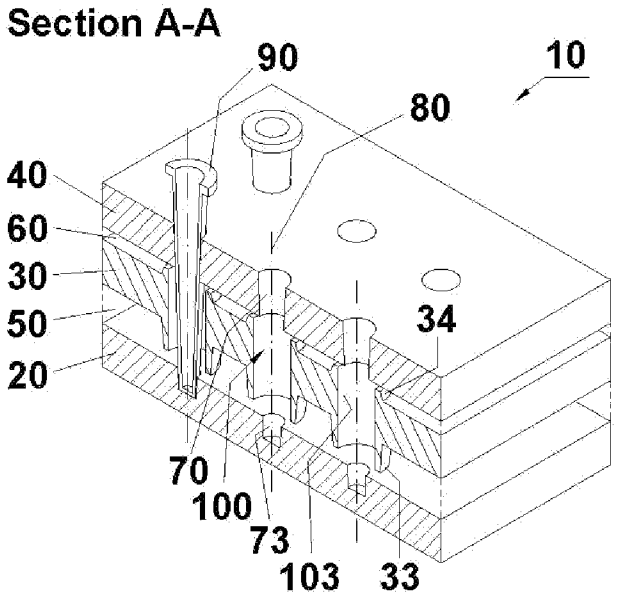


FIG. 16B

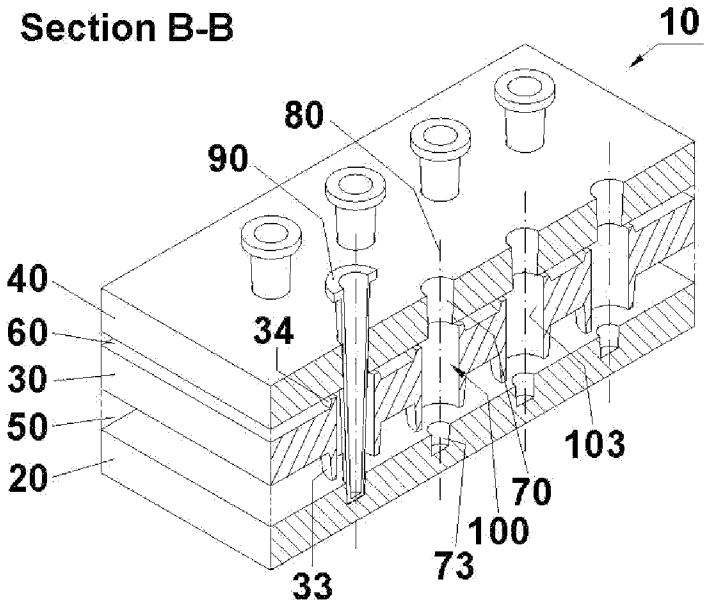


FIG. 16C

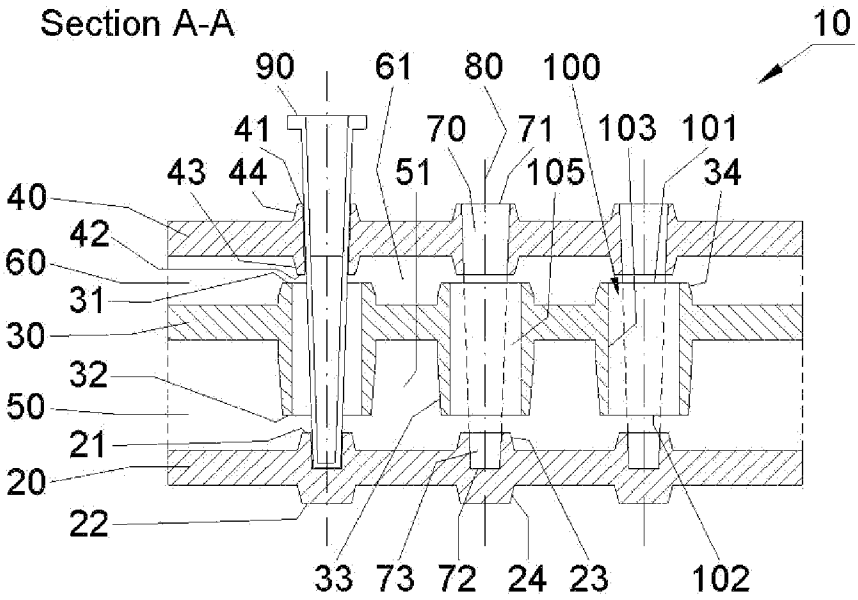
Section A-A
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FIG. 17A

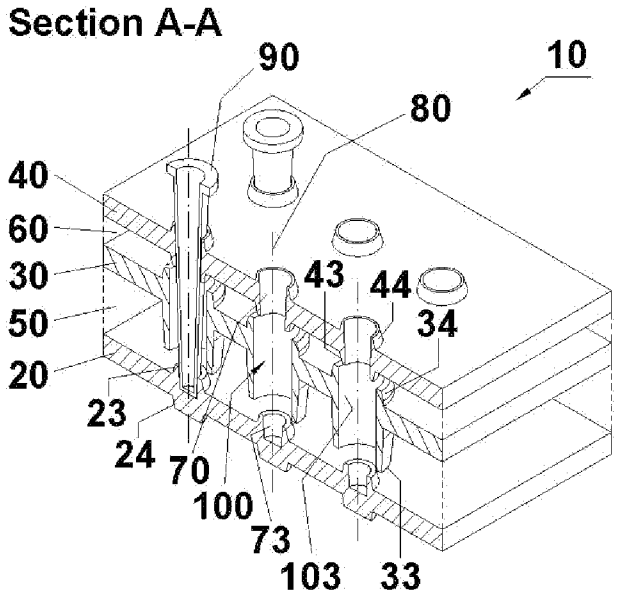


FIG. 17B

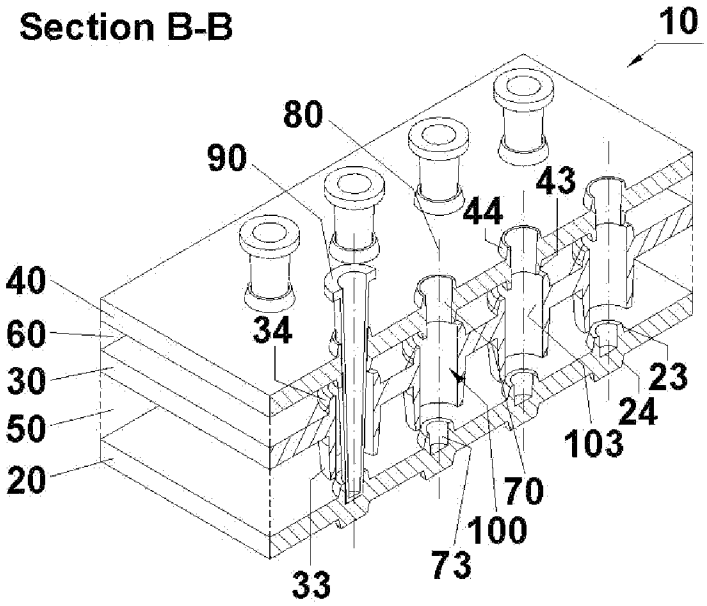


FIG. 17C

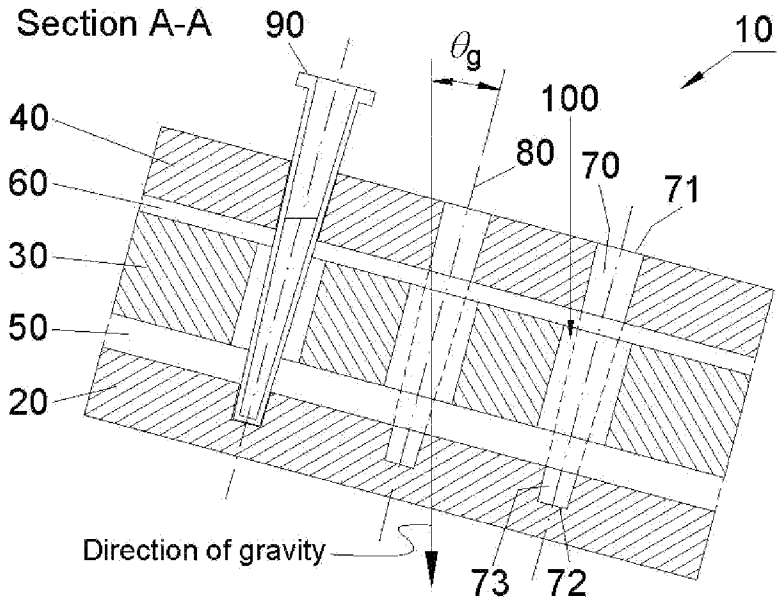


FIG. 18A

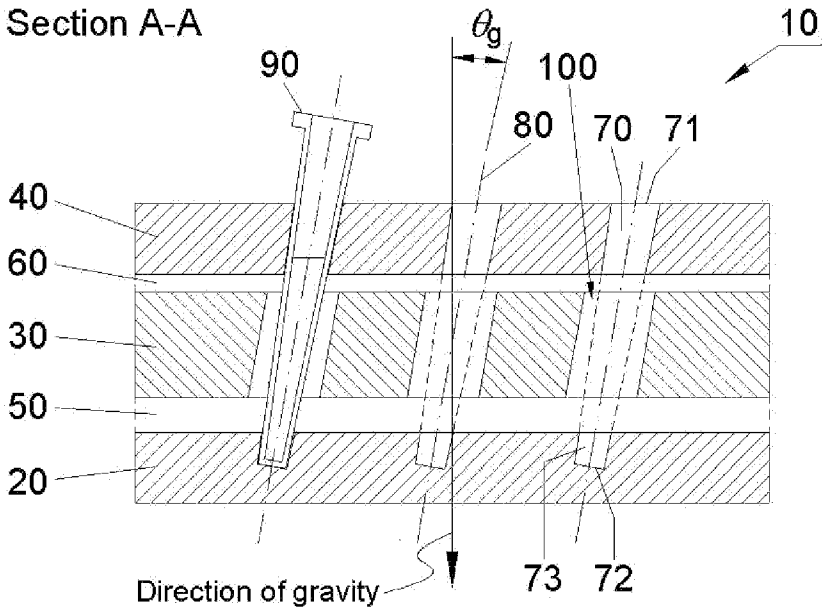


FIG. 18B

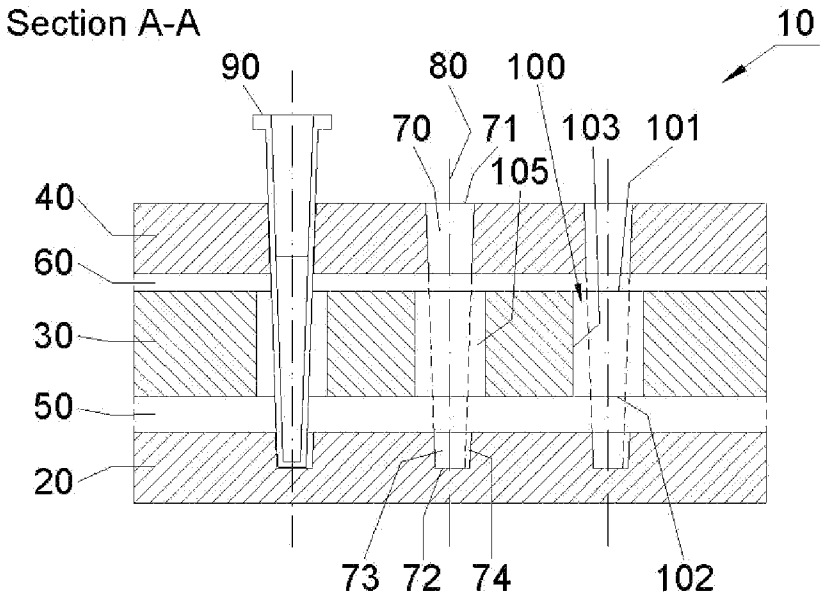
Section A-A
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FIG. 19

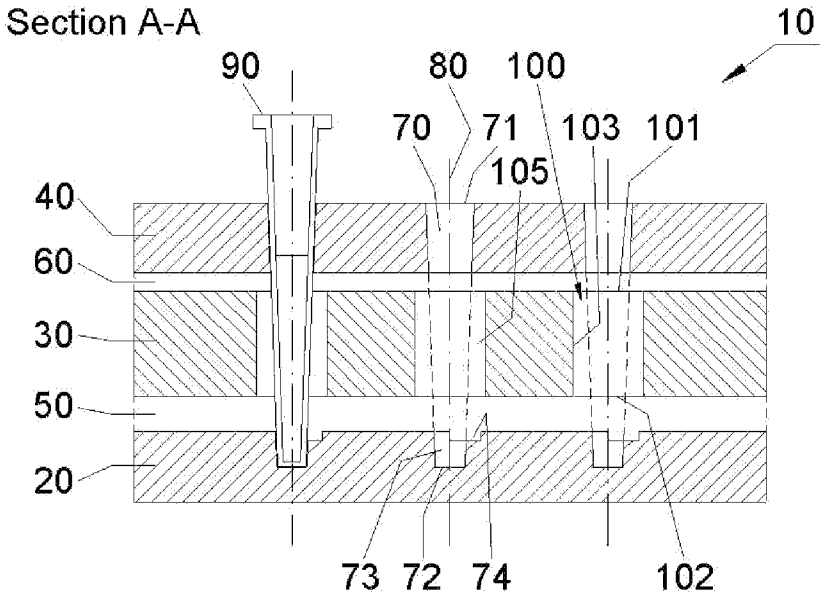


FIG. 20A

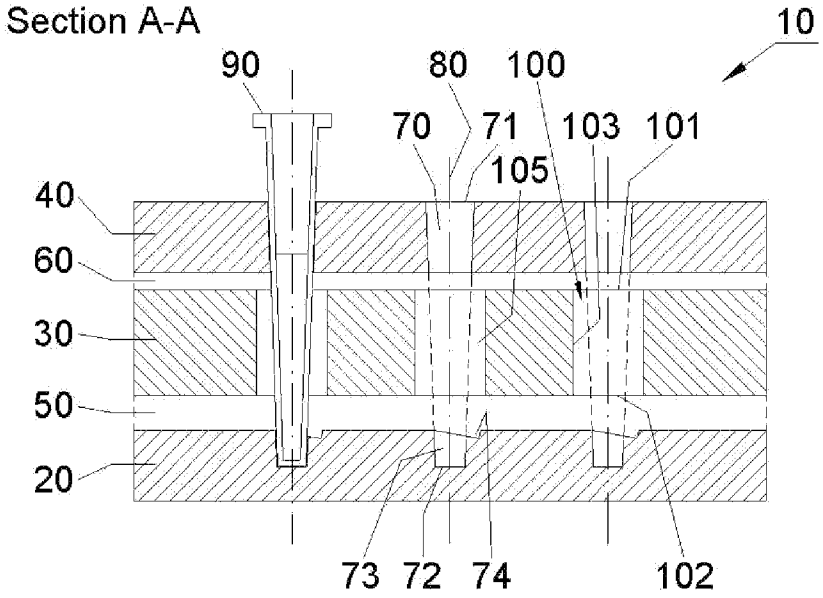


FIG. 20B

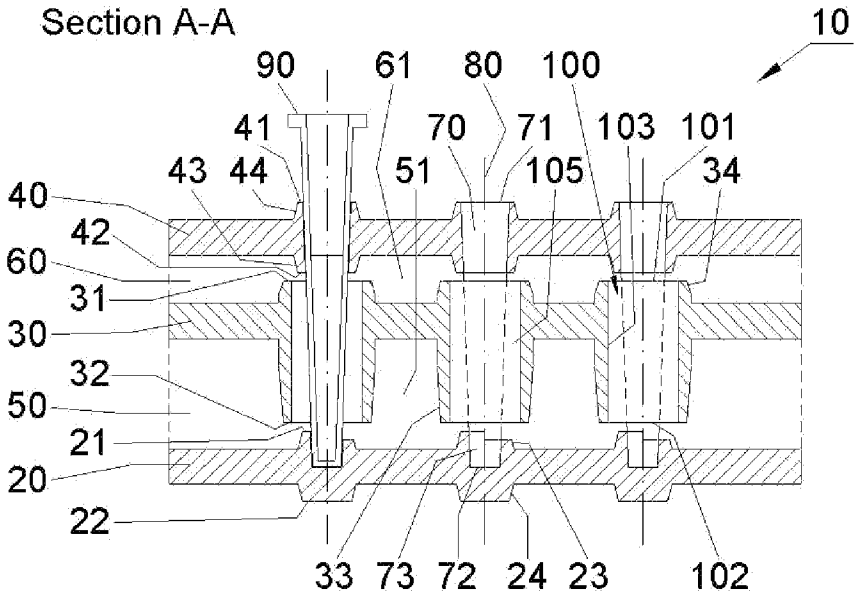


FIG. 21A

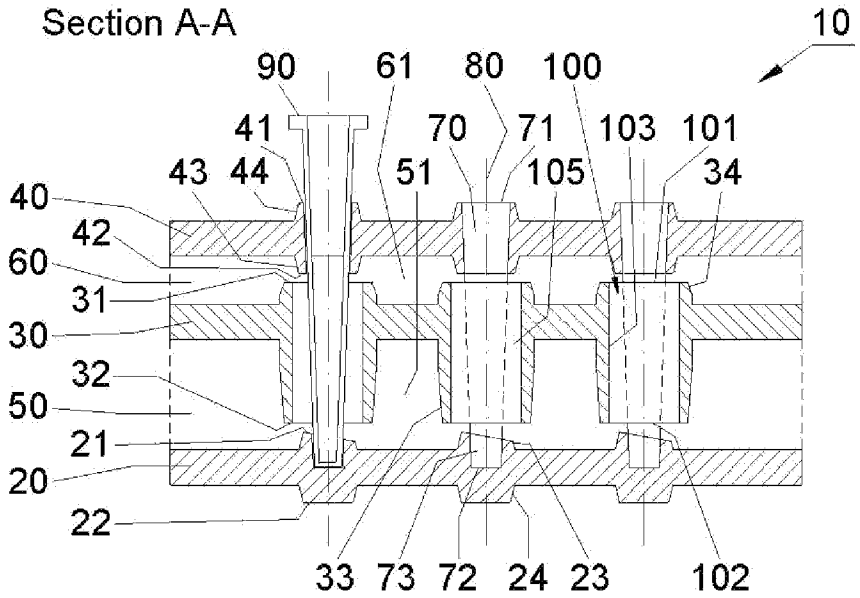


FIG. 21B

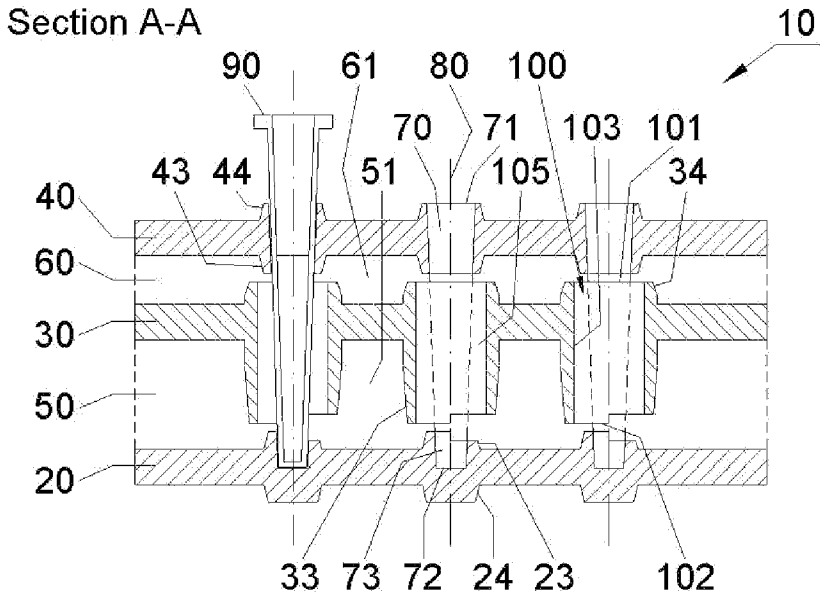


FIG. 22A

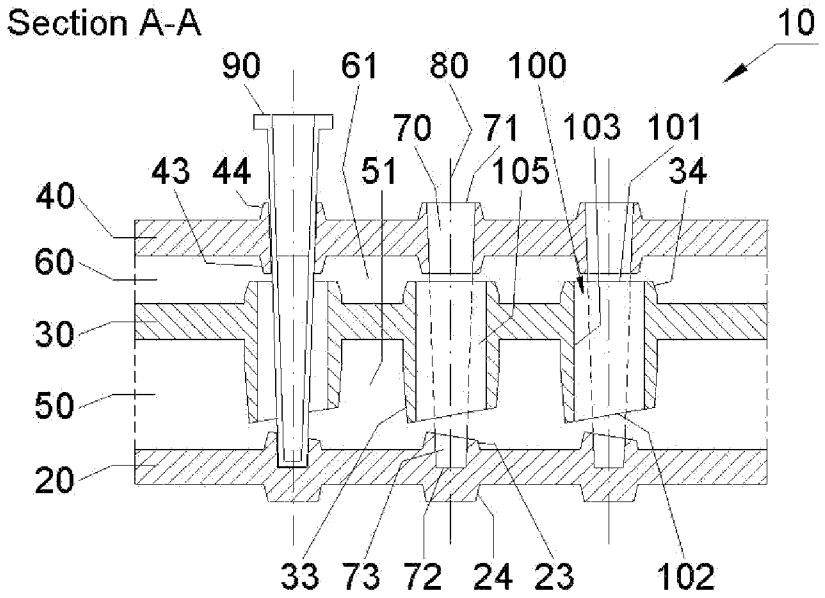


FIG. 22B

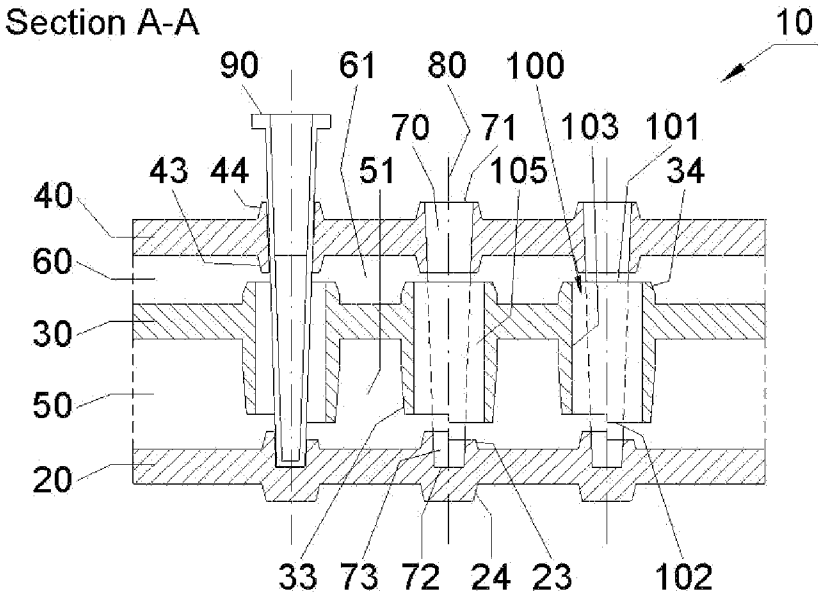


FIG. 22C

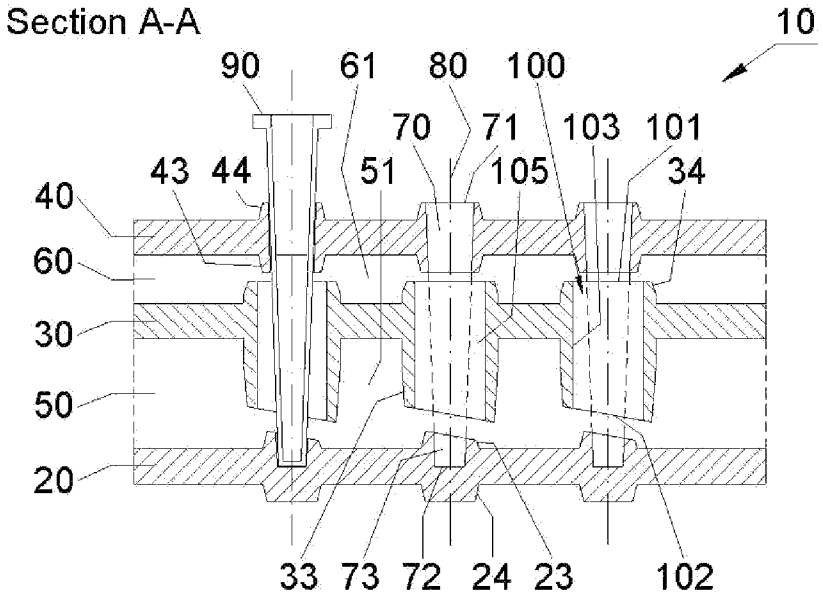


FIG. 22D

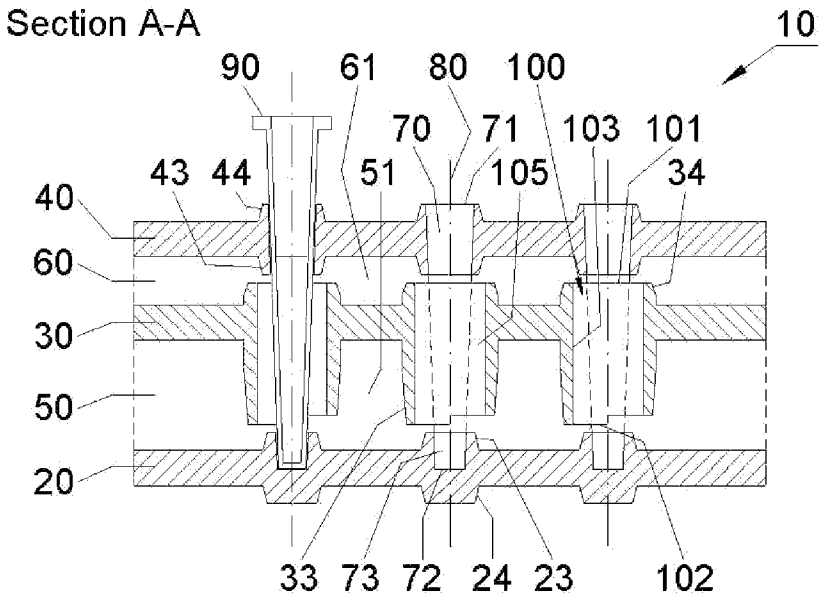


FIG. 23A

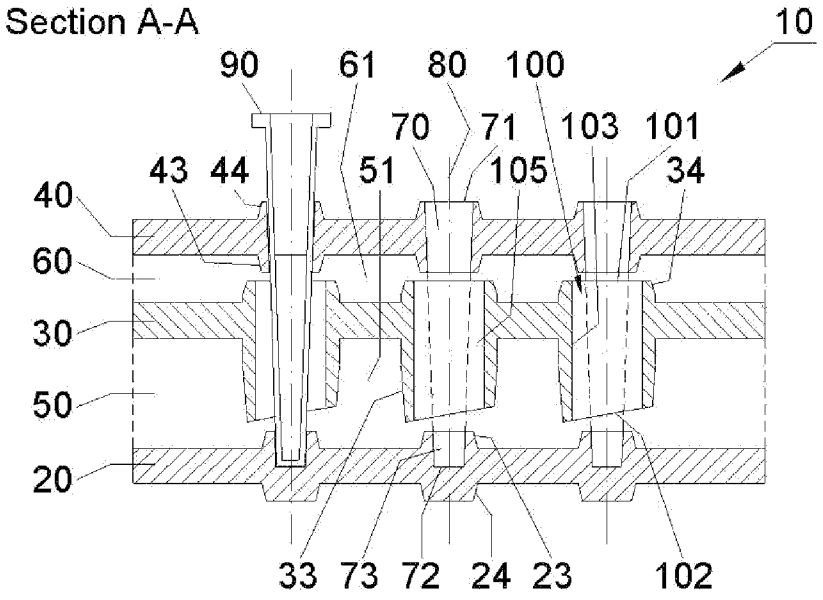


FIG. 23B

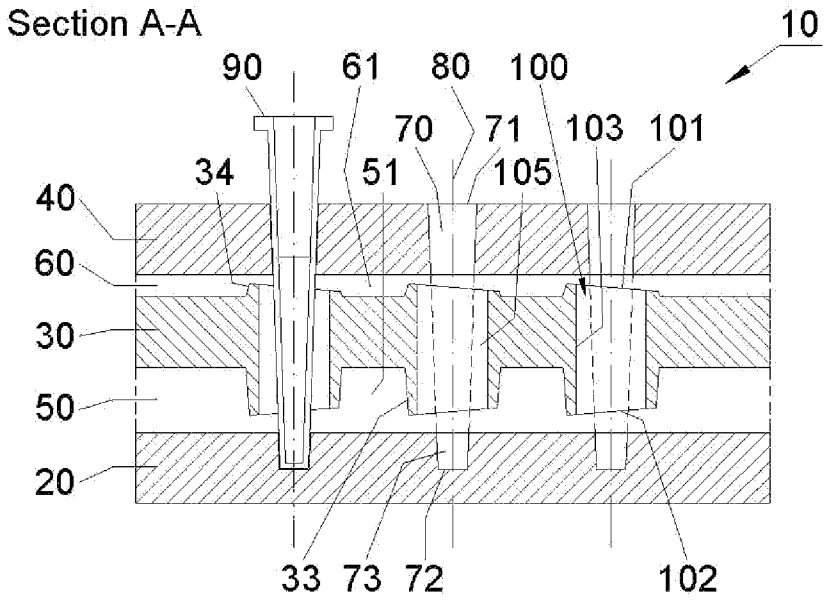


FIG. 24A

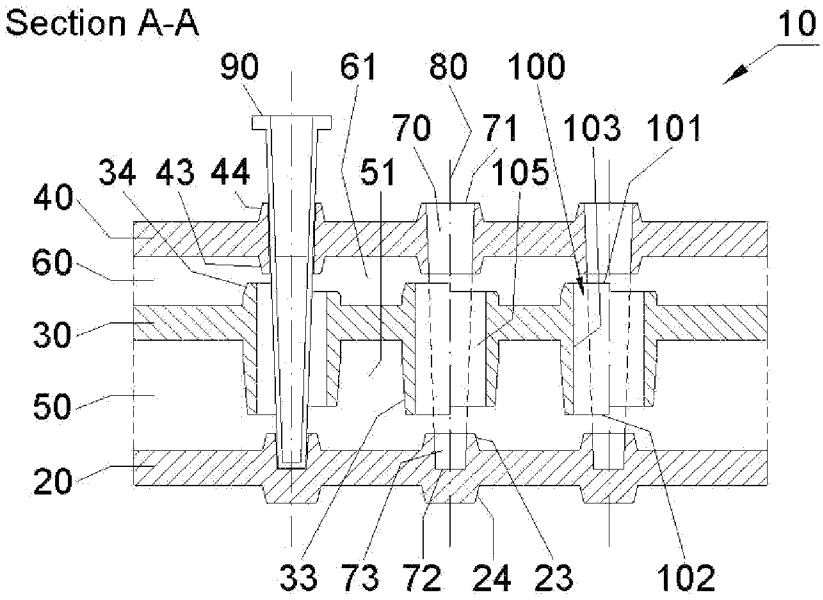


FIG. 24B

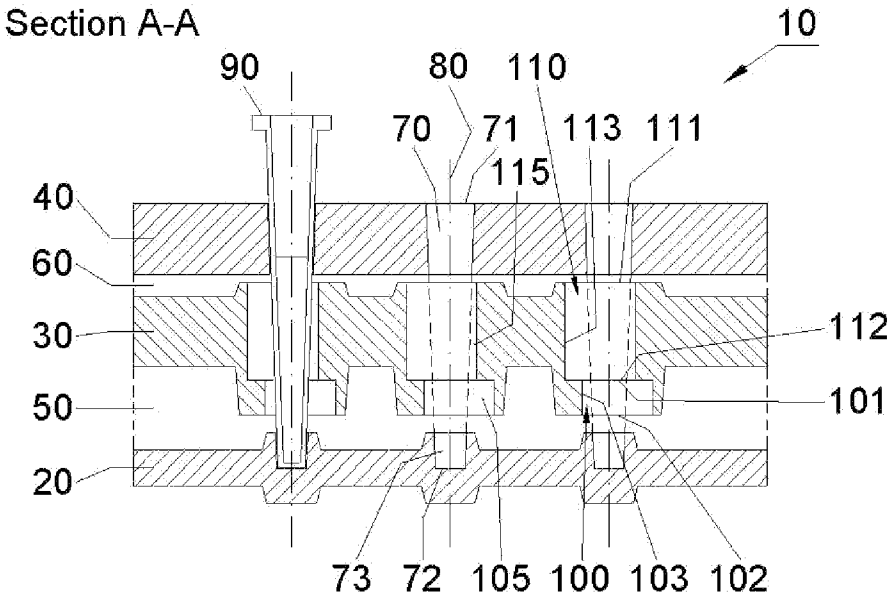
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FIG. 25

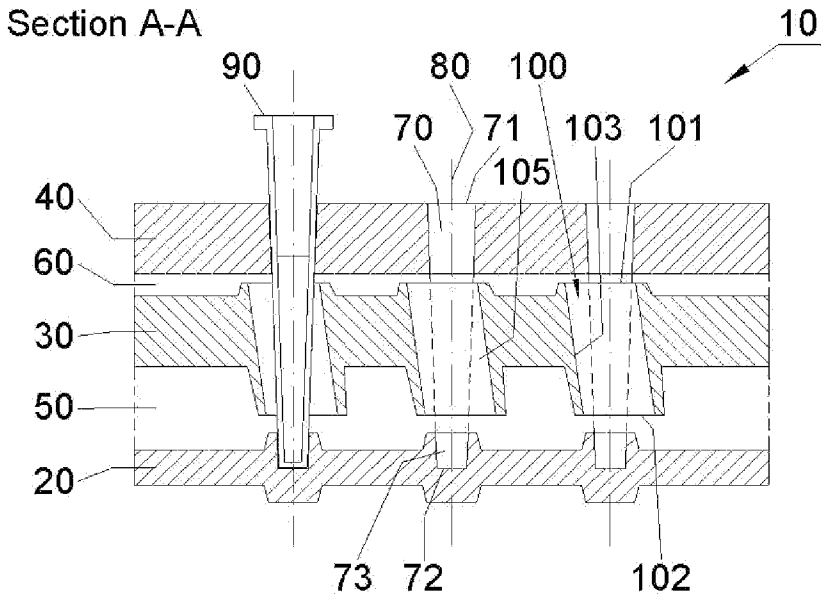
Section A-A
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FIG. 26

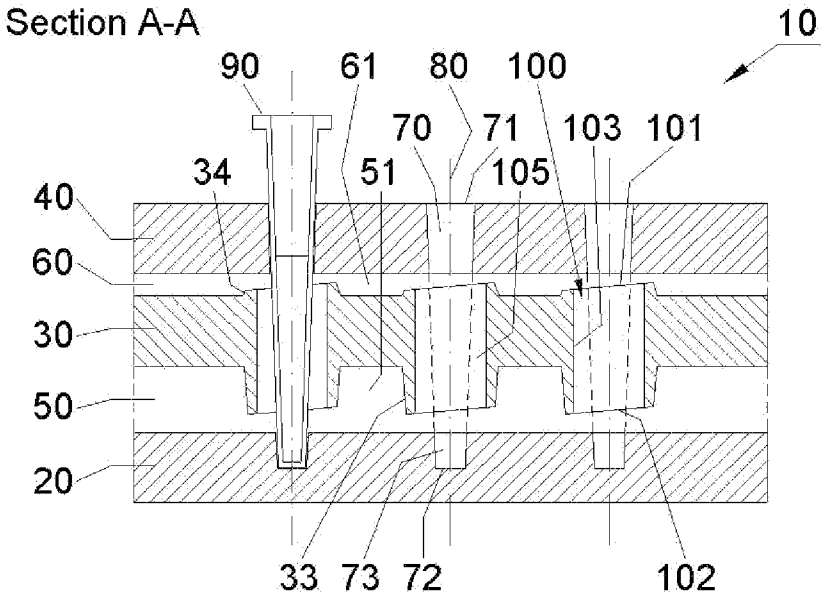



FIG. 27A

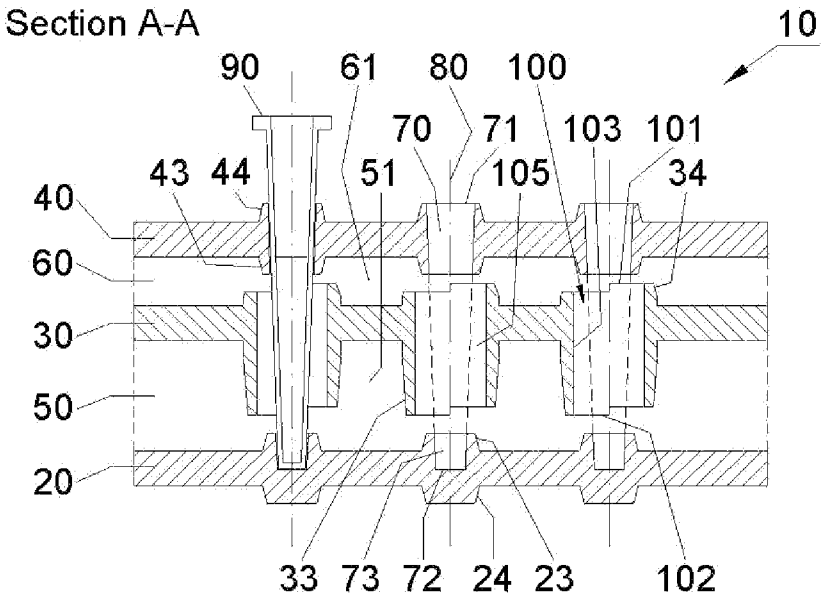



FIG. 27B

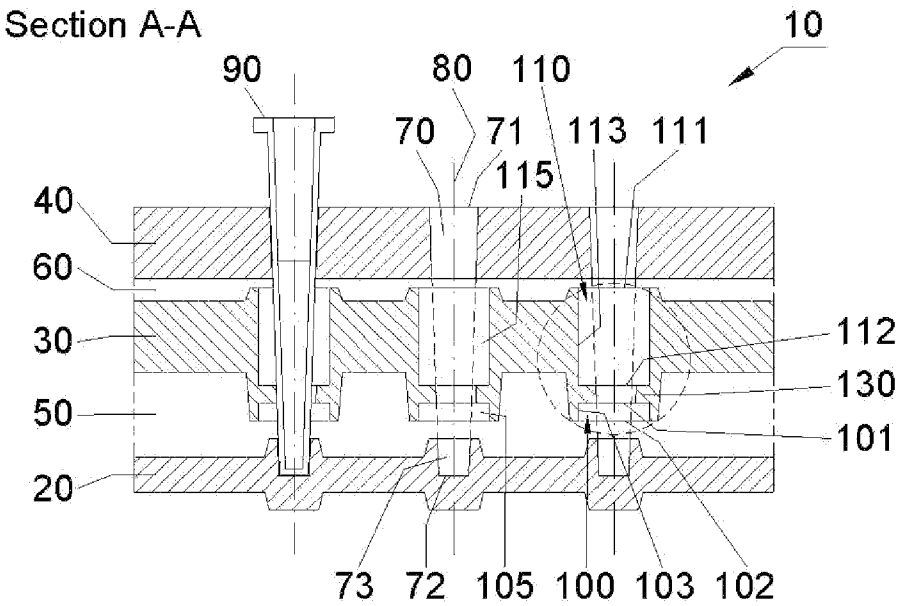



FIG. 28A

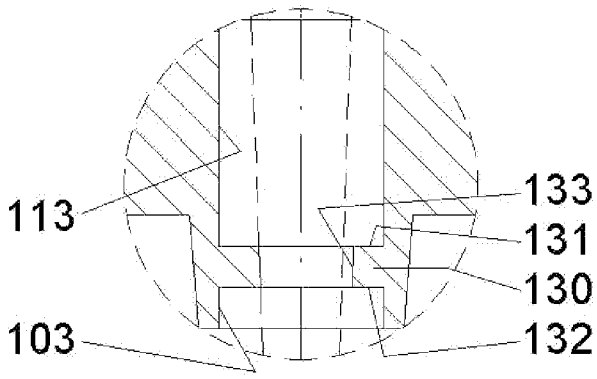



FIG. 28B

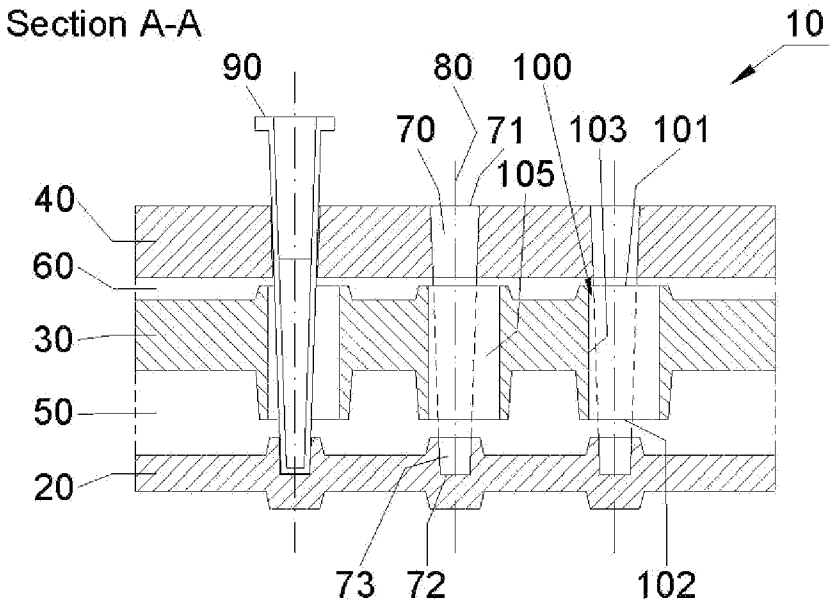



FIG. 29A

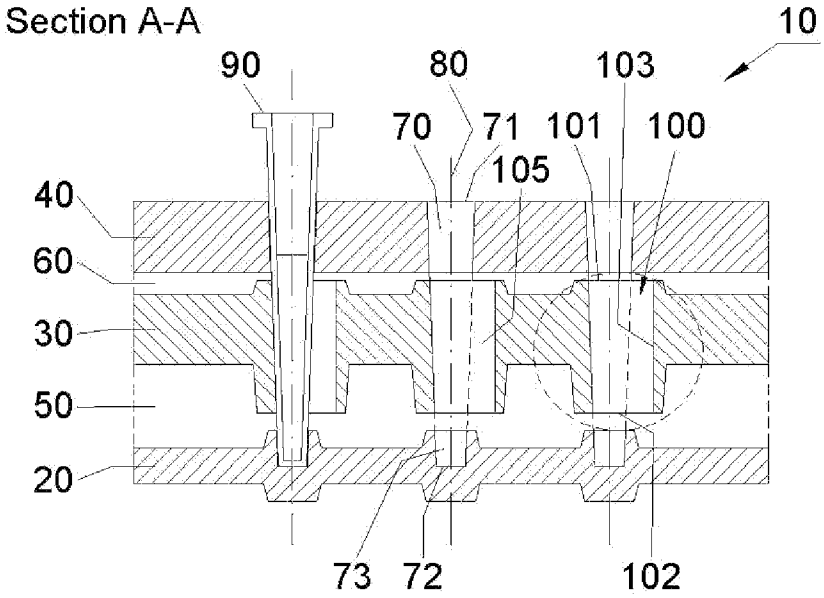



FIG. 29B

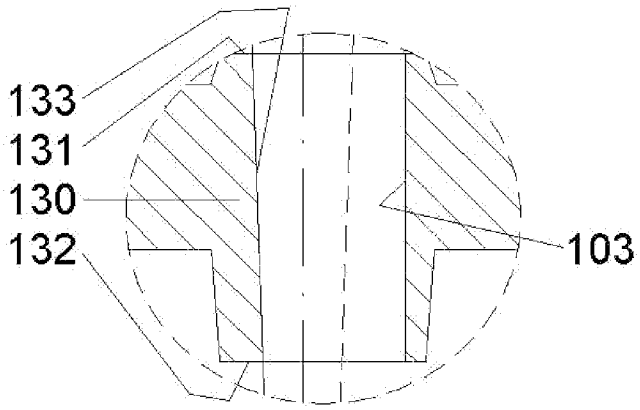



FIG. 29C

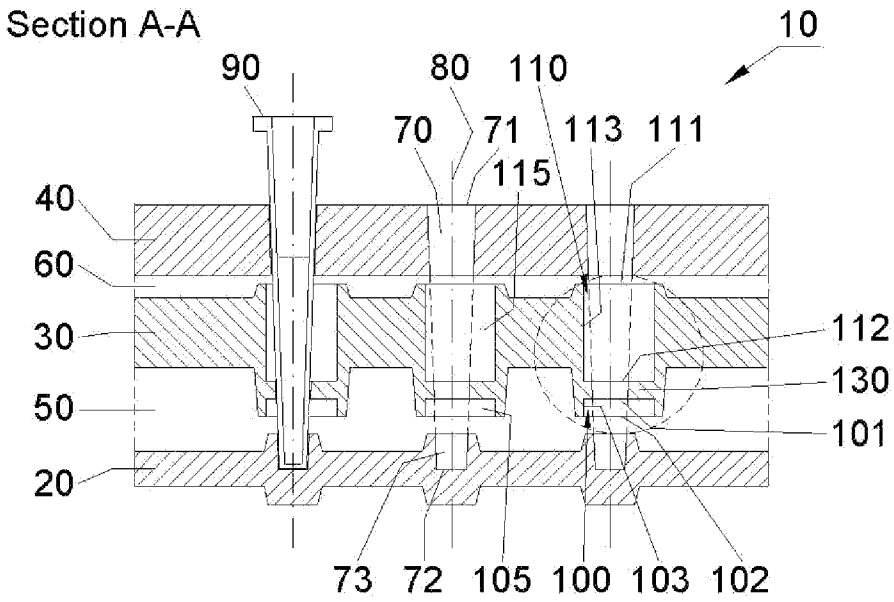



FIG. 30A

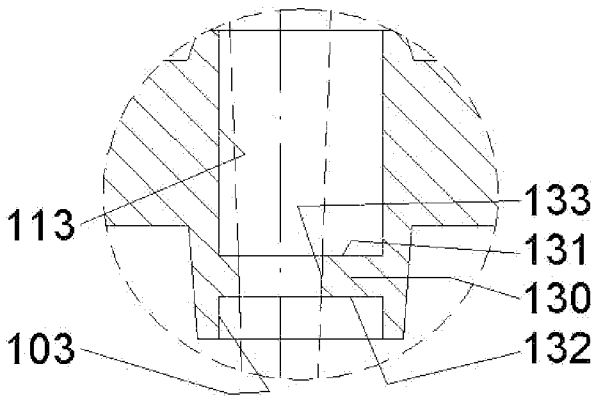



FIG. 30B

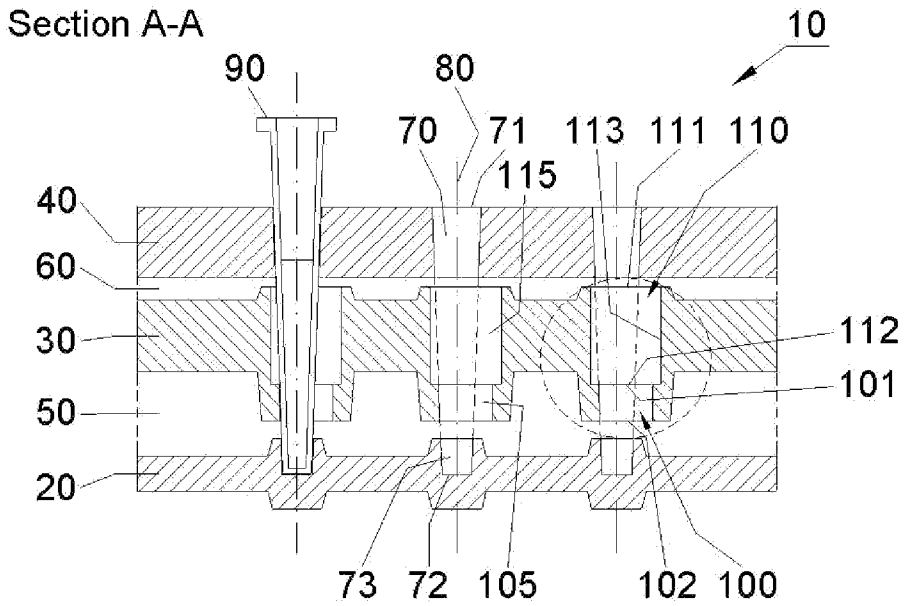



FIG. 30C

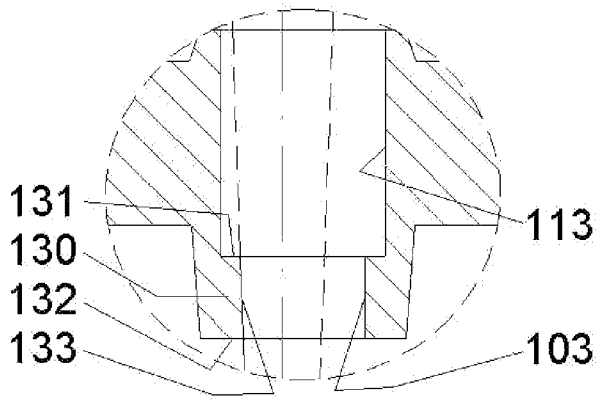



FIG. 30D

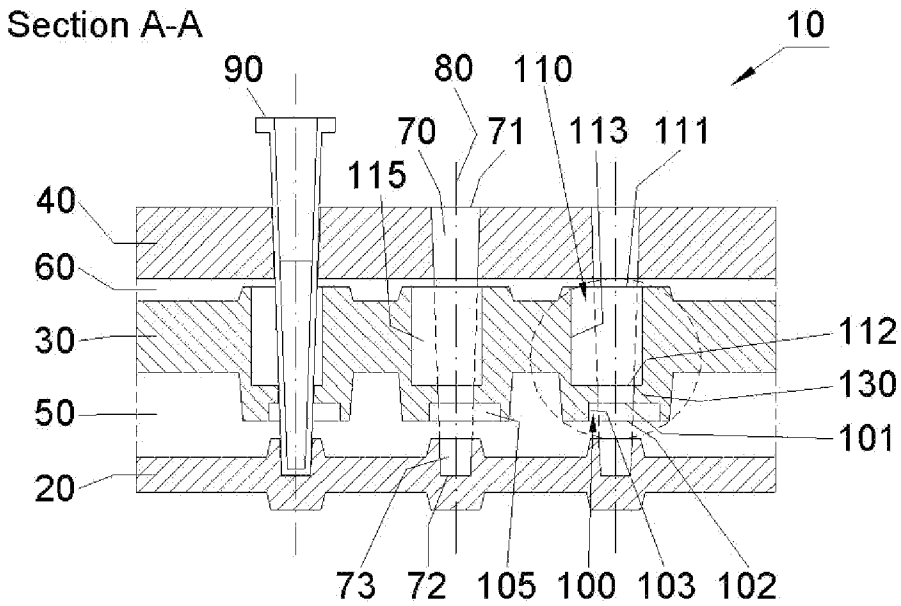



FIG. 31A

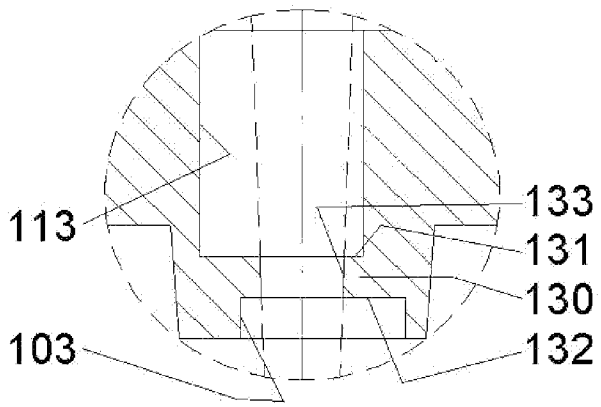



FIG. 31B

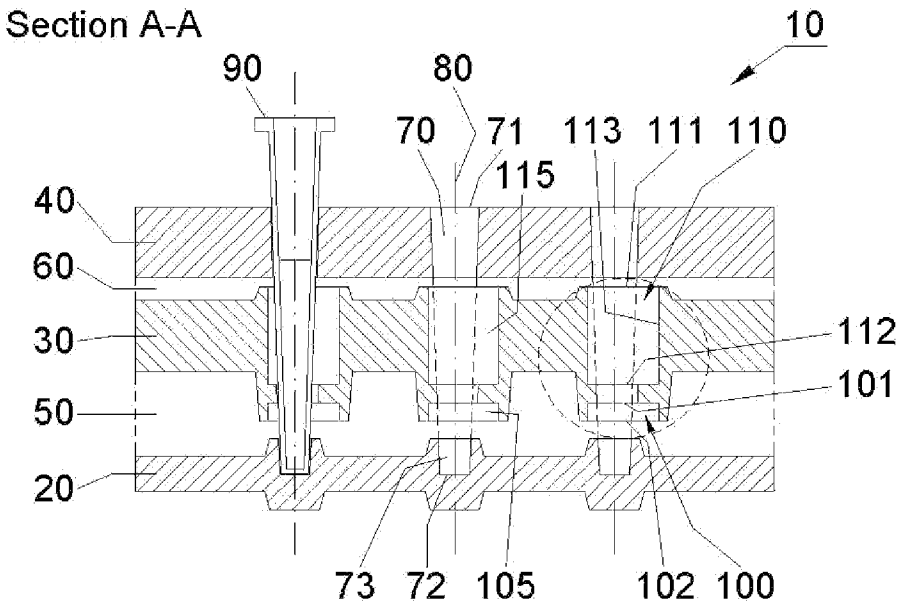



FIG. 32A

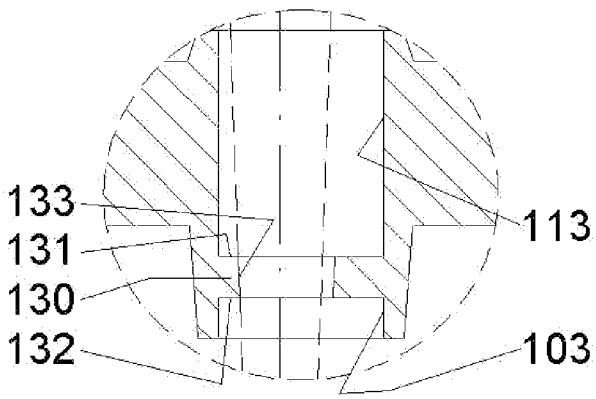



FIG. 32B

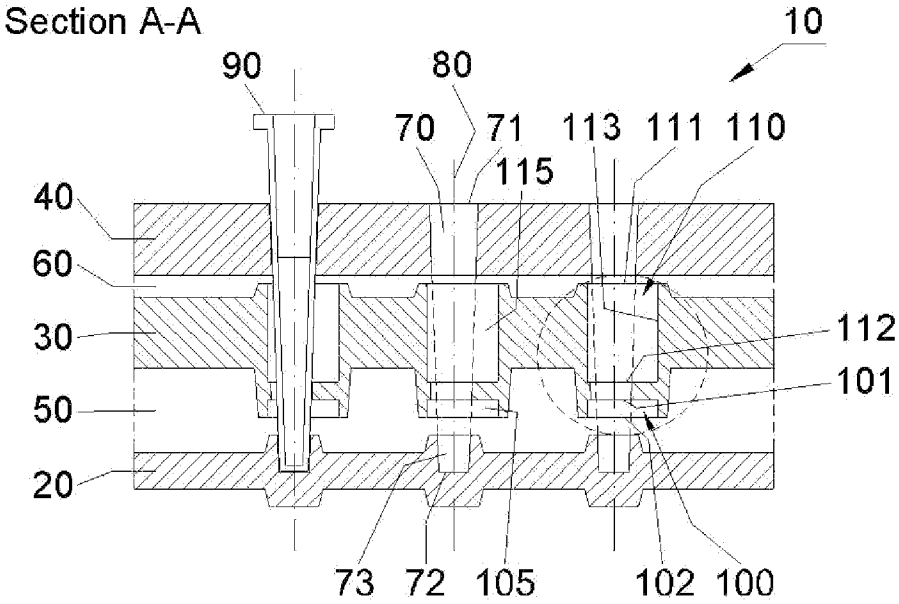



FIG. 32C

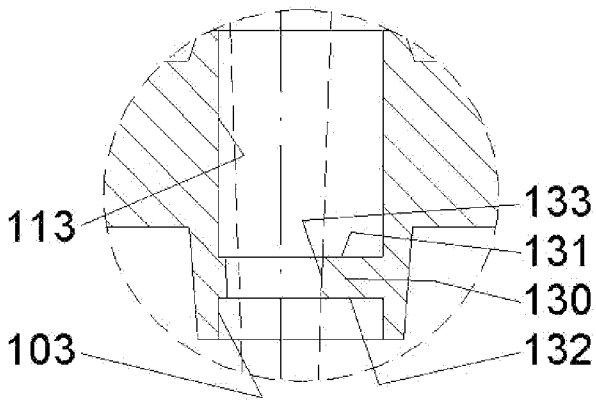



FIG. 32D

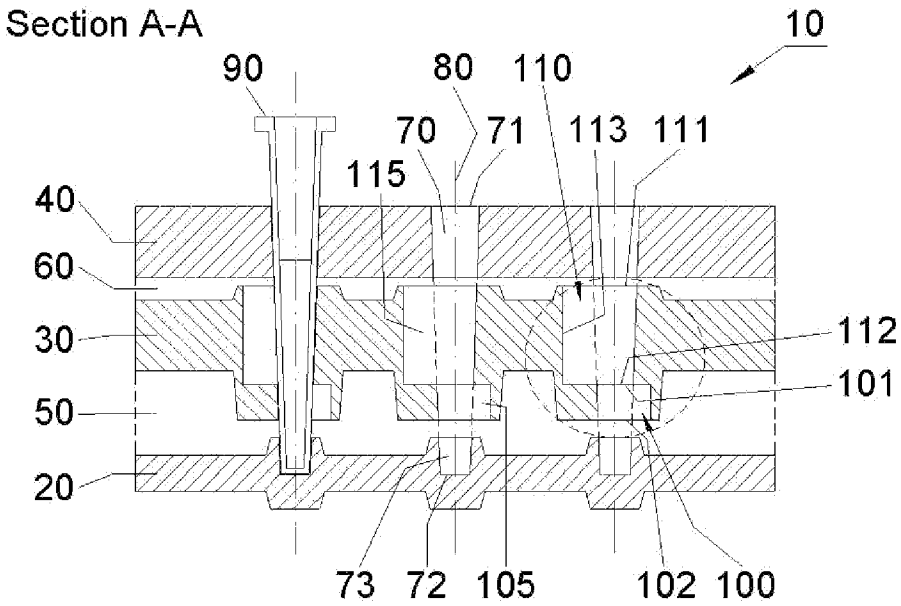



FIG. 33A

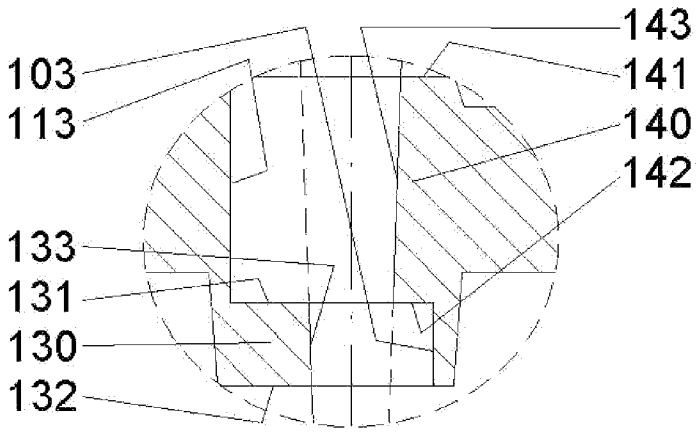



FIG. 33B

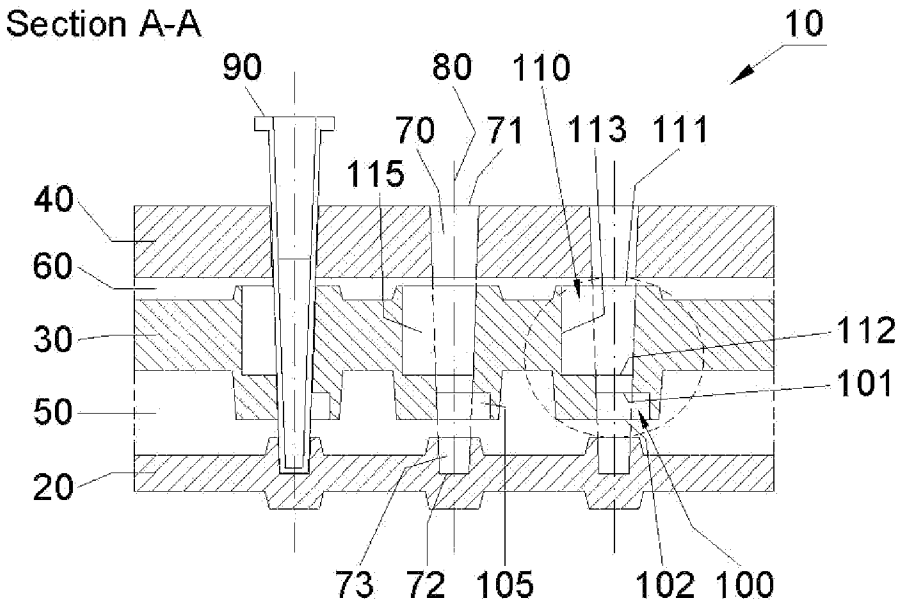



FIG. 33C

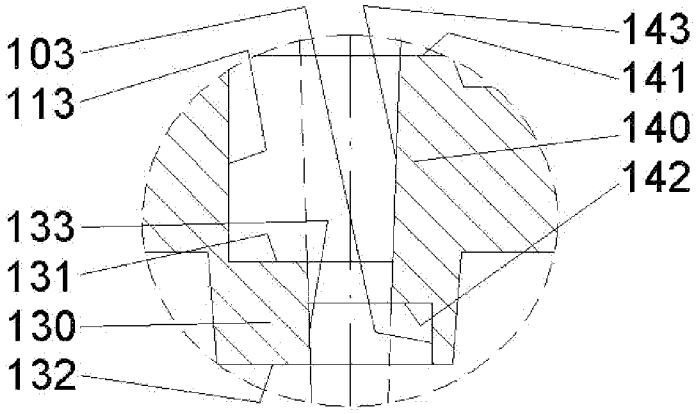



FIG. 33D

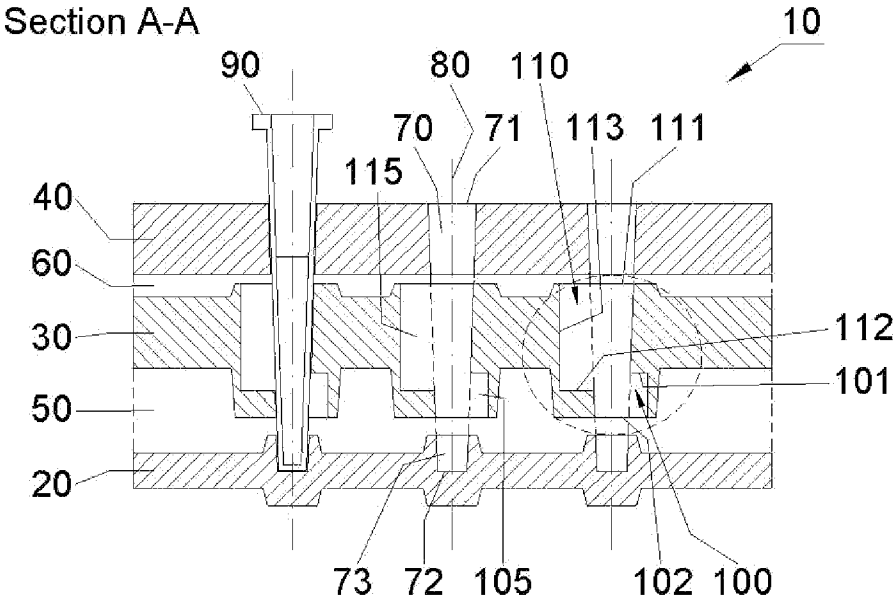



FIG. 33E

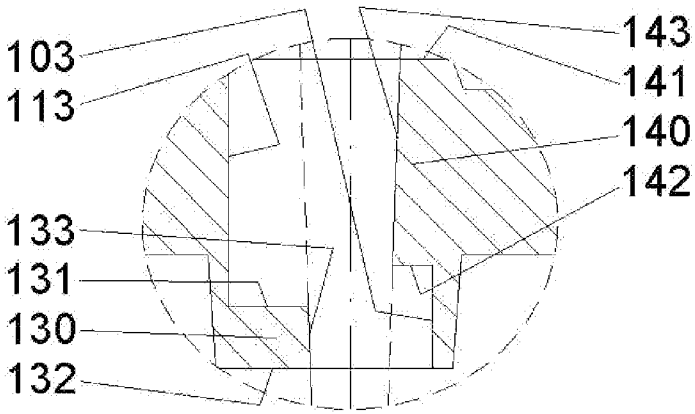



FIG. 33F

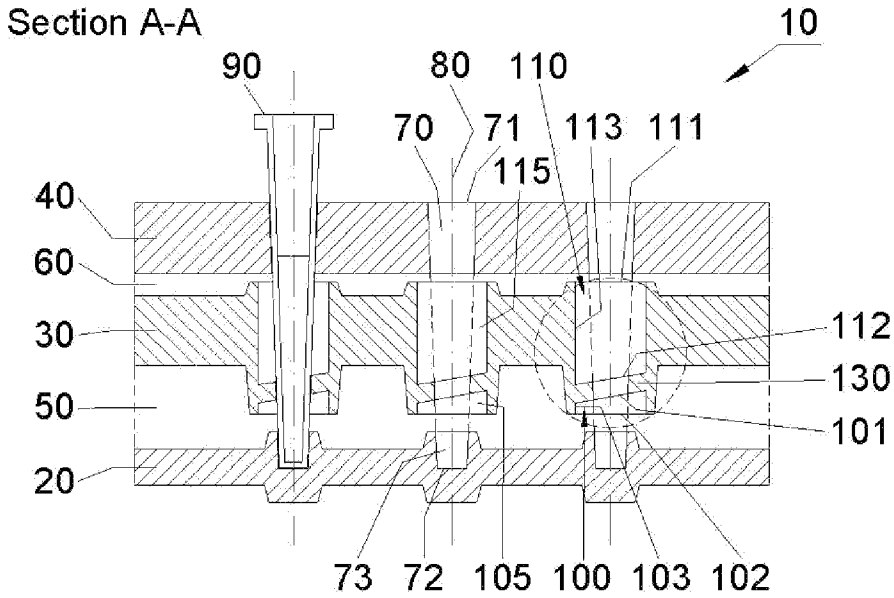



FIG. 34A

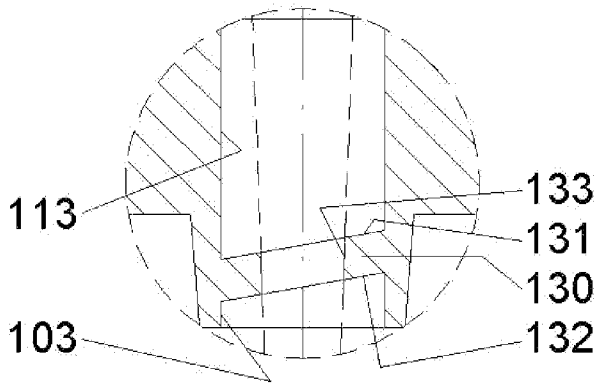



FIG. 34B

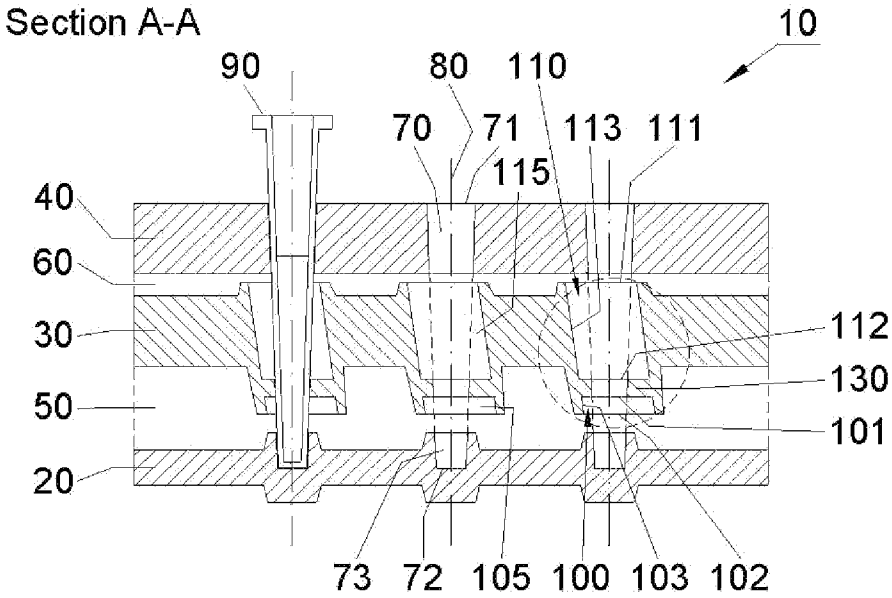



FIG. 35A

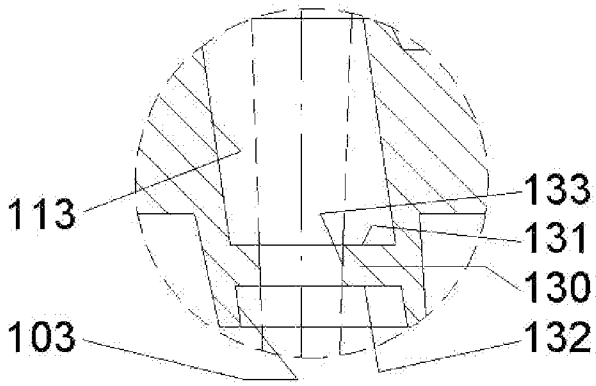



FIG. 35B

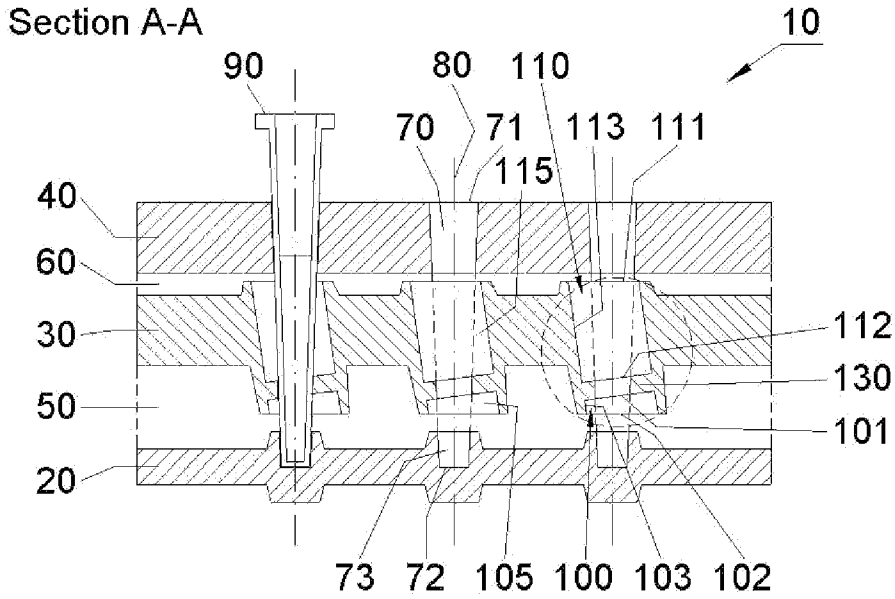



FIG. 35C

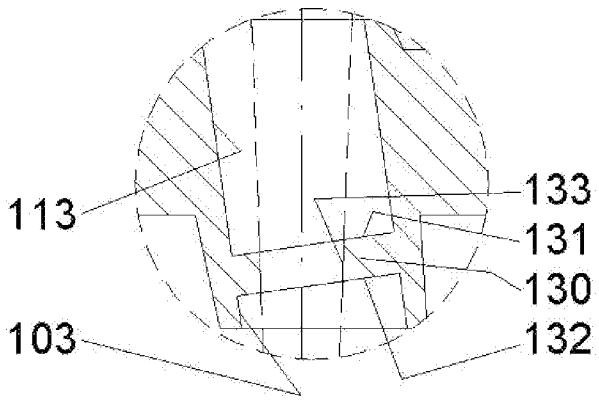



FIG. 35D

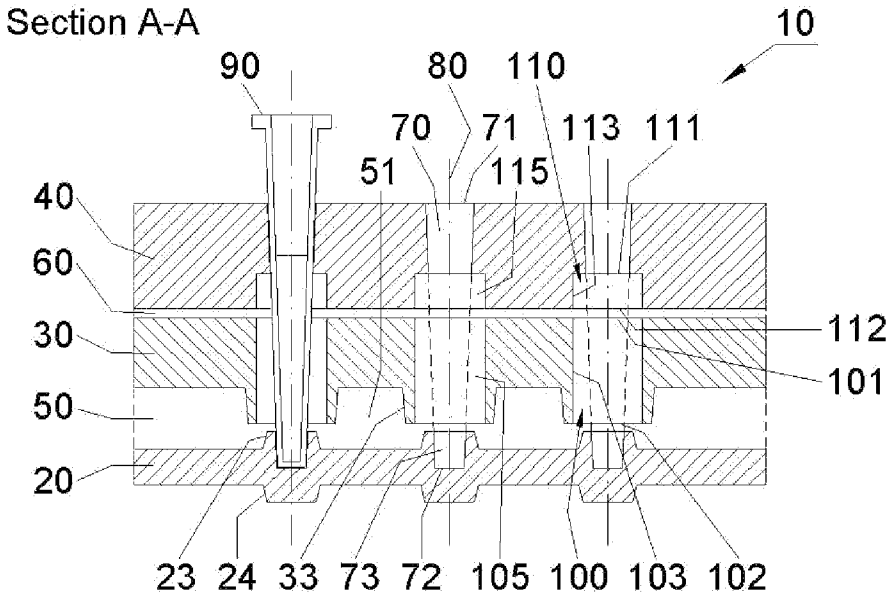



FIG. 36A

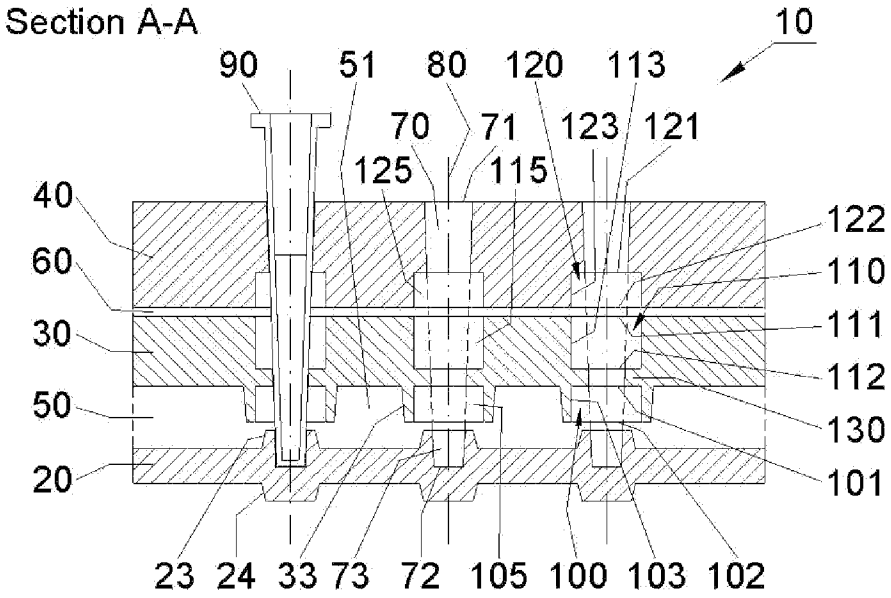
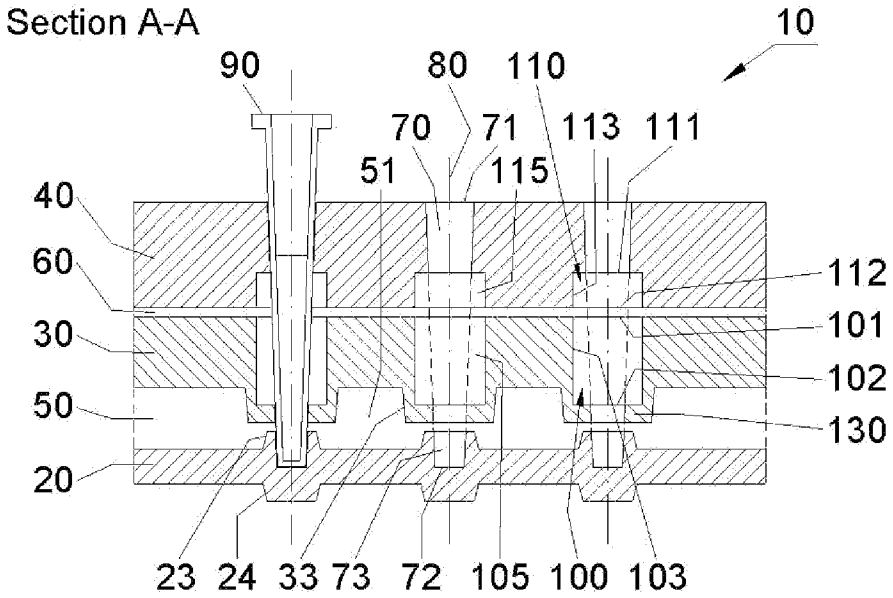



FIG. 36B


Section A-A
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90
80
110
70
71
113
111
51
115
40
60
30
50
20
112
101
102
130
23
24
33
73
72
105
100
103

FIG. 36C

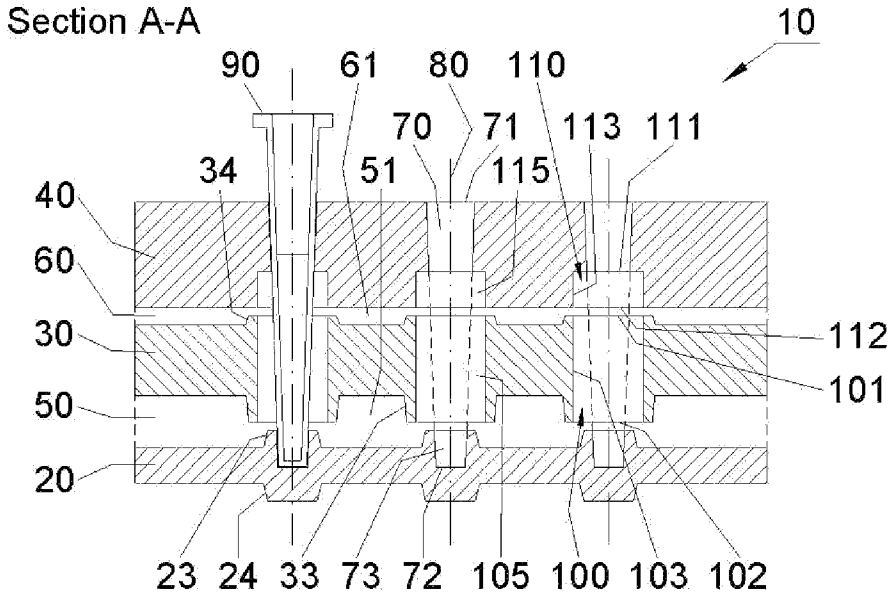


FIG. 37A

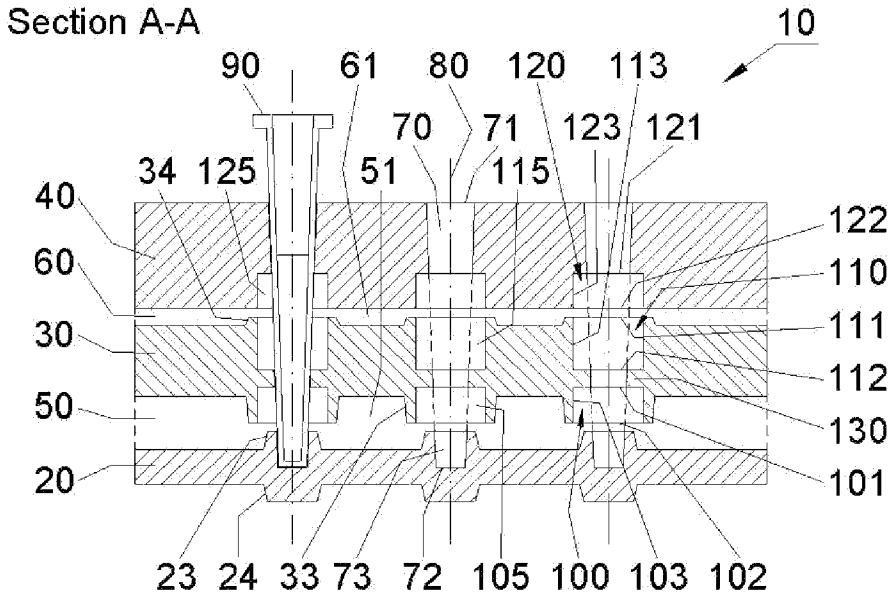


FIG. 37B

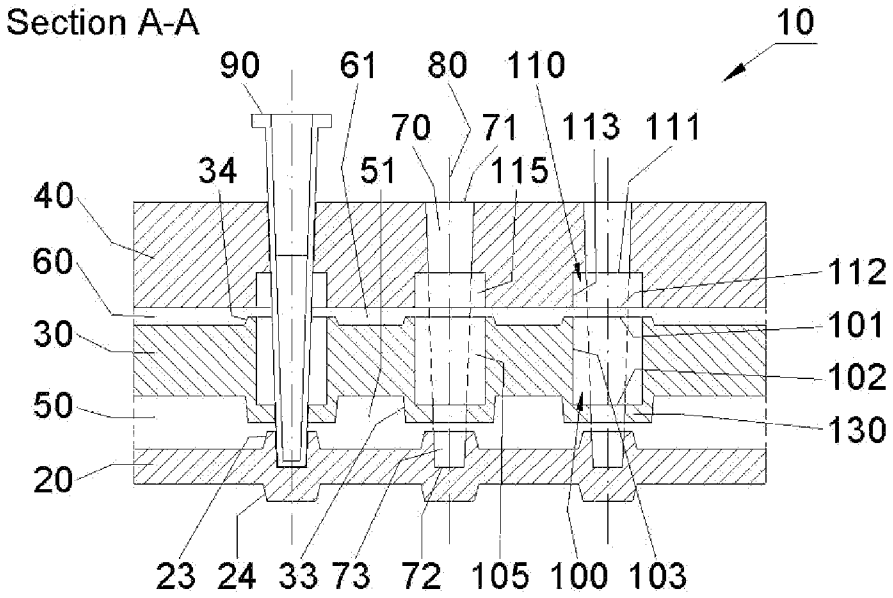
Section A-A
10
90
61
80
110
70
71
113
111
34
51
115
40
60
30
50
20
112
101
102
130
23
24
33
73
72
105
100
103

FIG. 37C

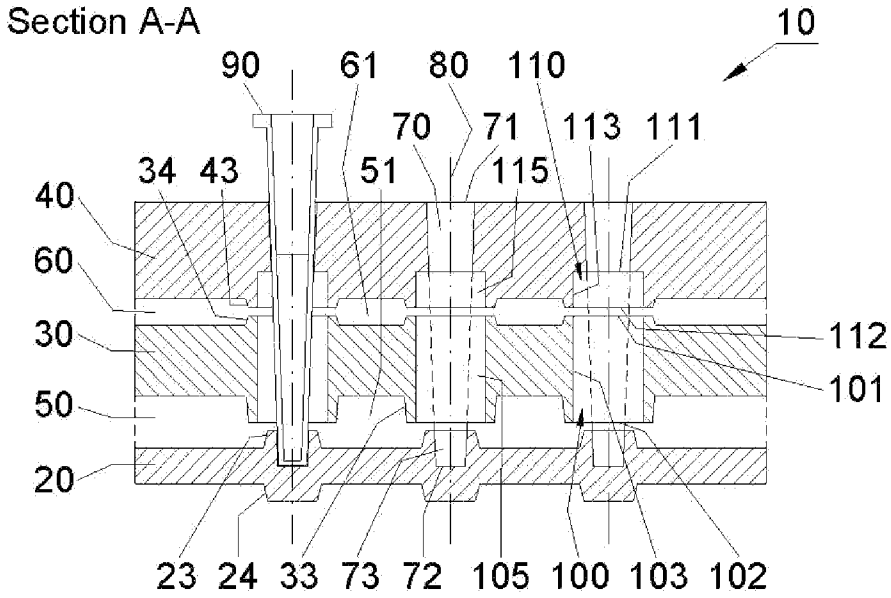


FIG. 38A

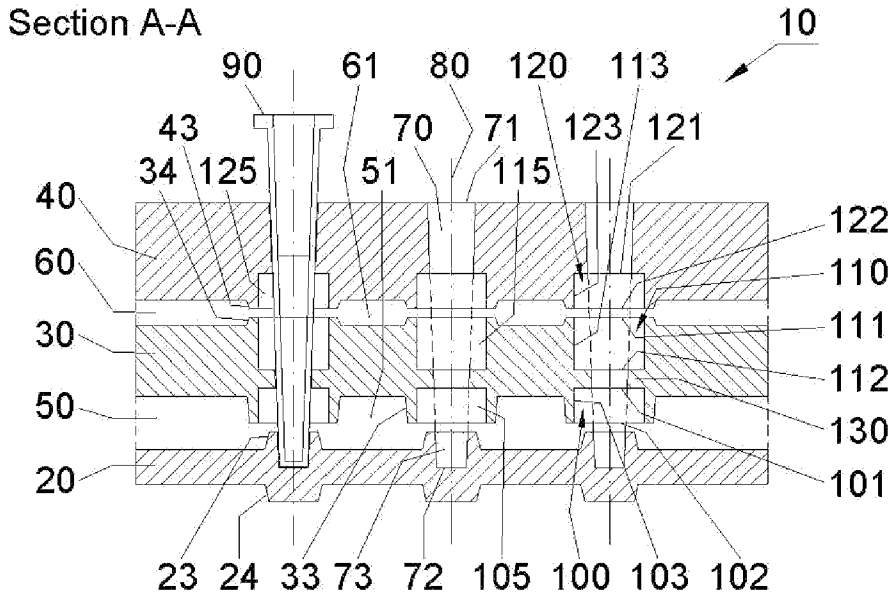


FIG. 38B

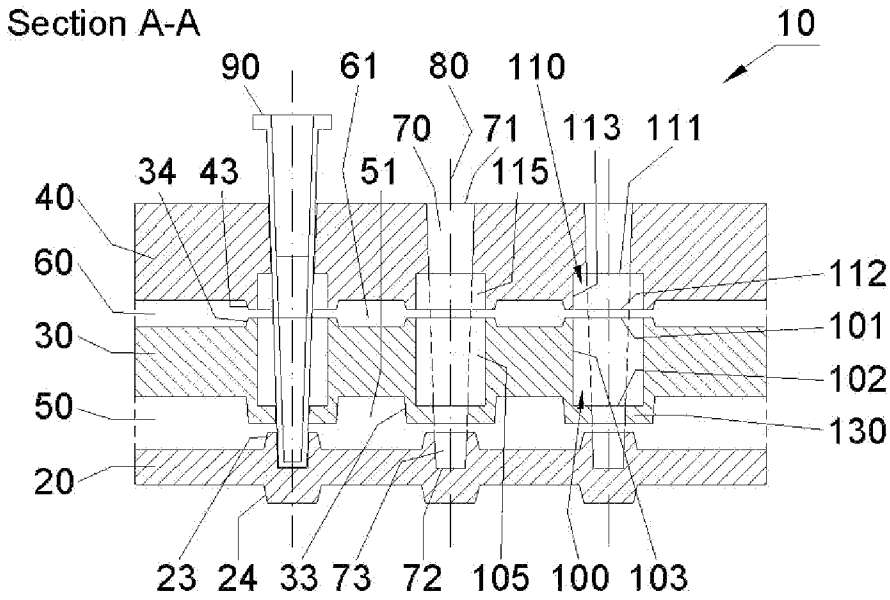
Section A-A
10
90
61
80
110
70
71
113
111
34
43
51
115
40
60
30
50
20
112
101
102
130
23
24
33
73
72
105
100
103

FIG. 38C

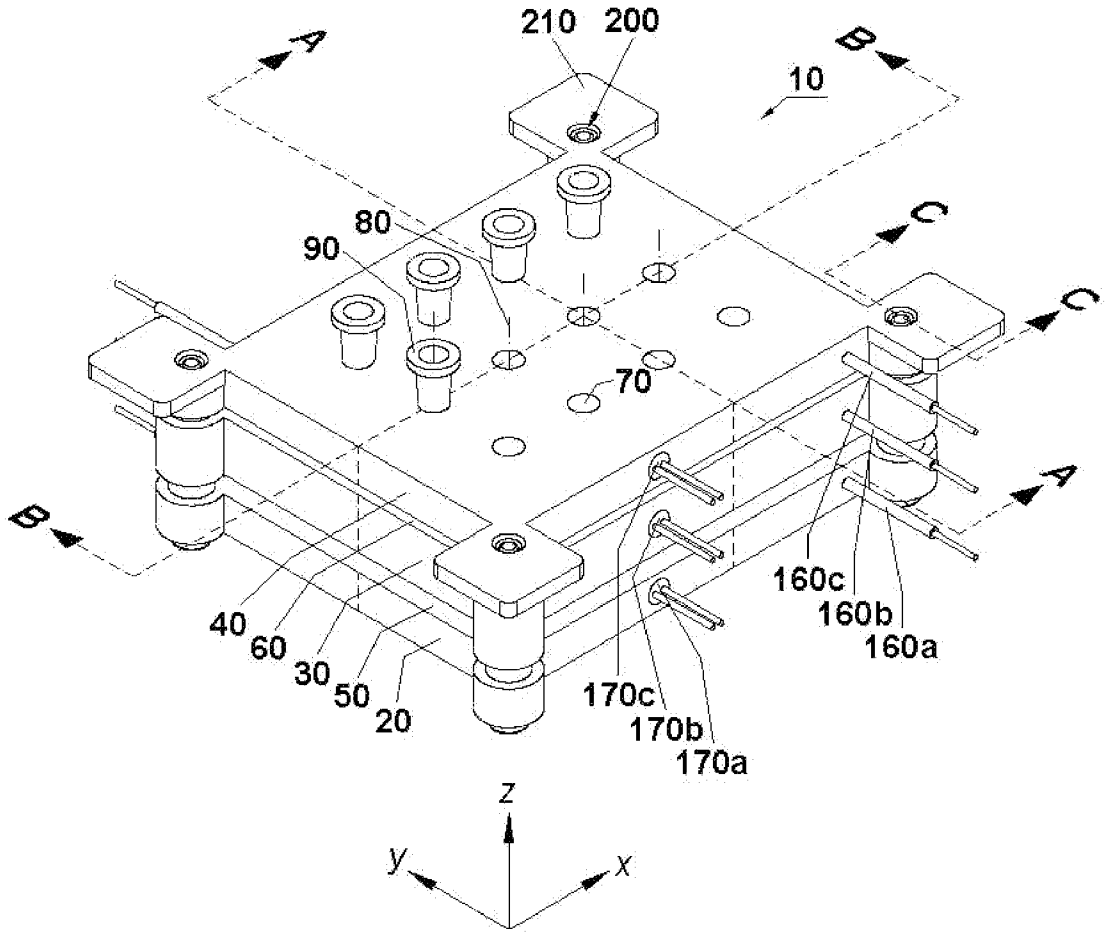
210
200
10
A
B
C
80
90
70
40
60
30
50
20
160c
160b
160a
170c
170b
170a
z
y
x

FIG. 39

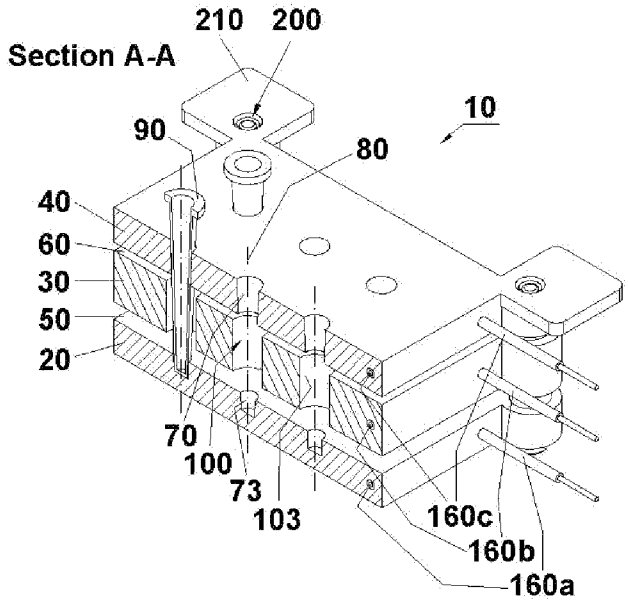


FIG. 40A

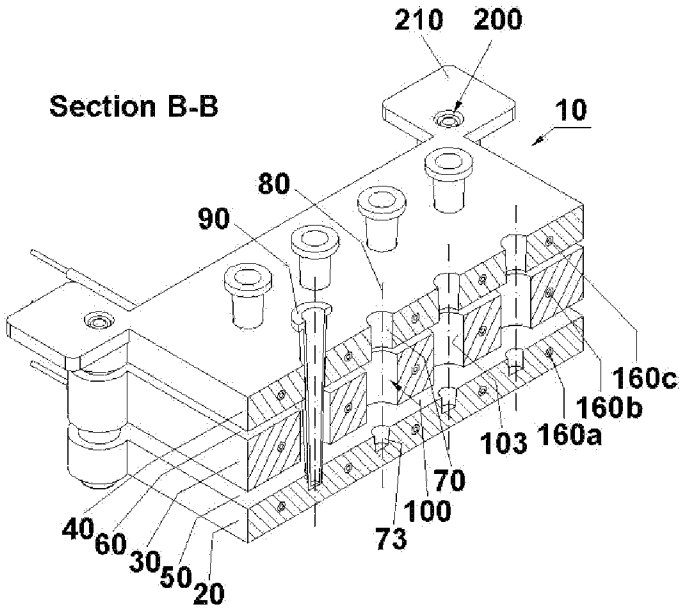


FIG. 40B

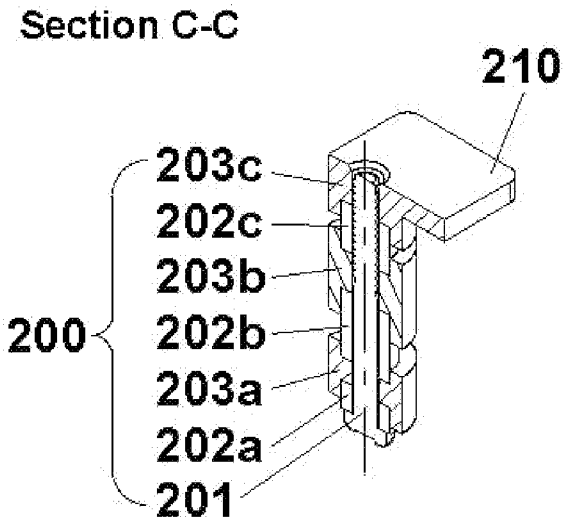


FIG. 41

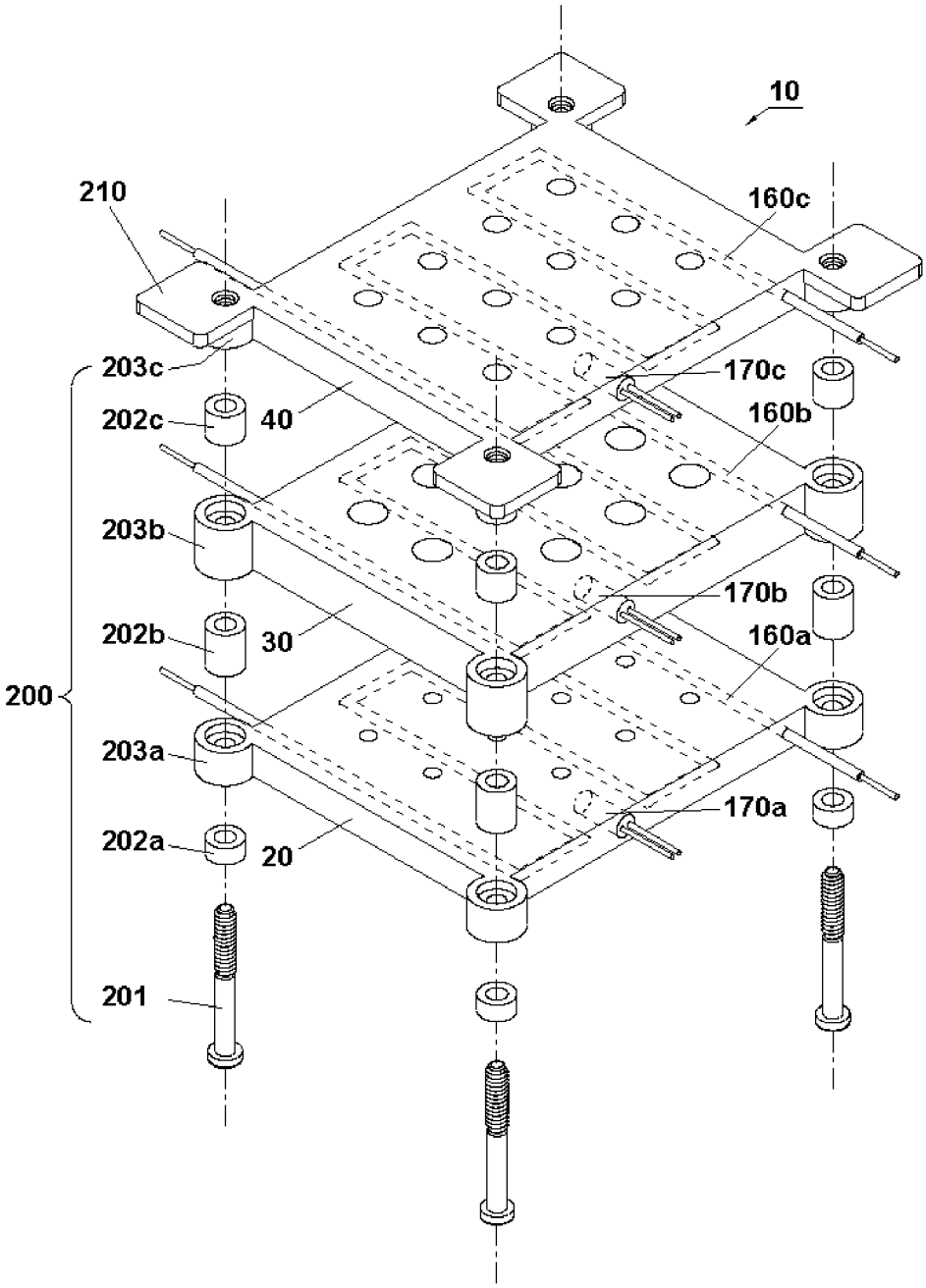
10
210
160c
203c
170c
202c
40
160b
203b
170b
202b
30
160a
200
203a
170a
202a
20
201

FIG. 42

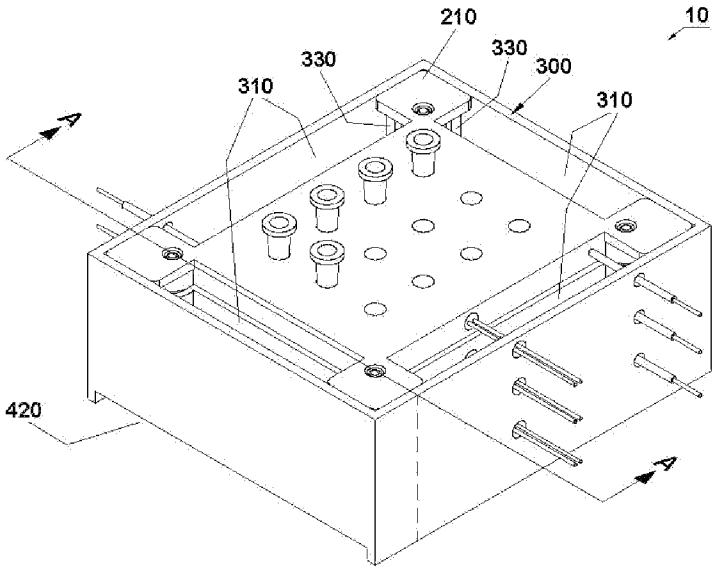


FIG. 43A

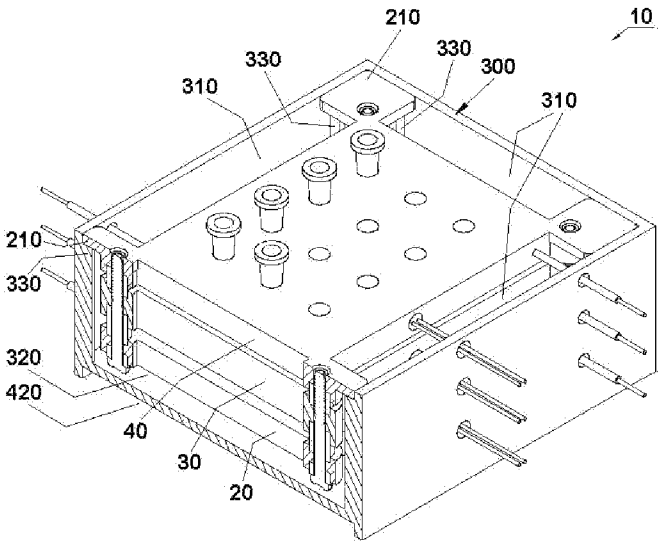


FIG. 43B

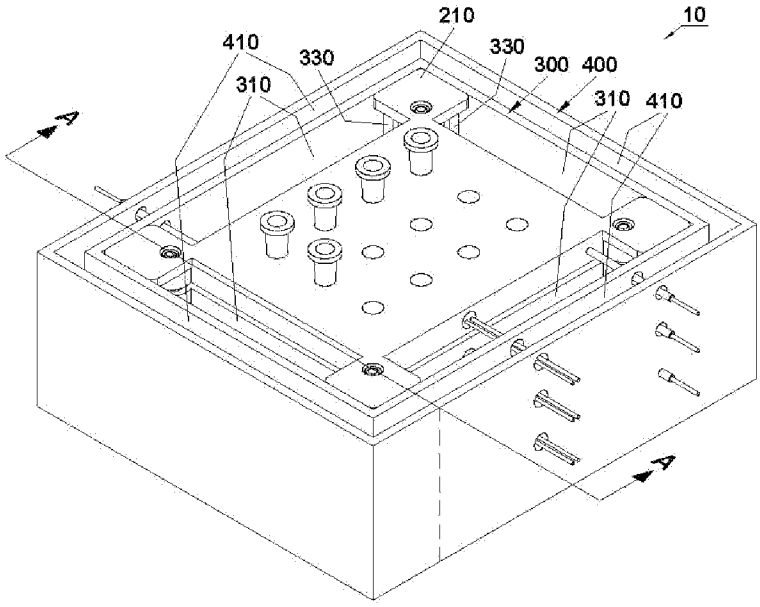


FIG. 44A

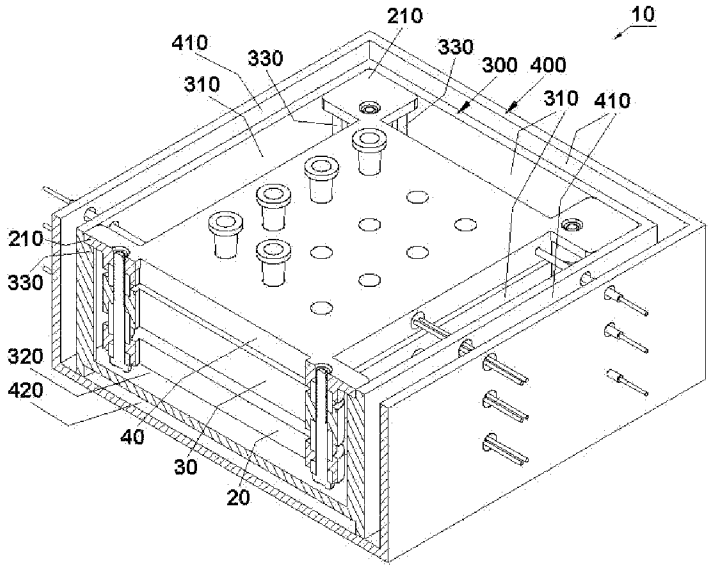


FIG. 44B

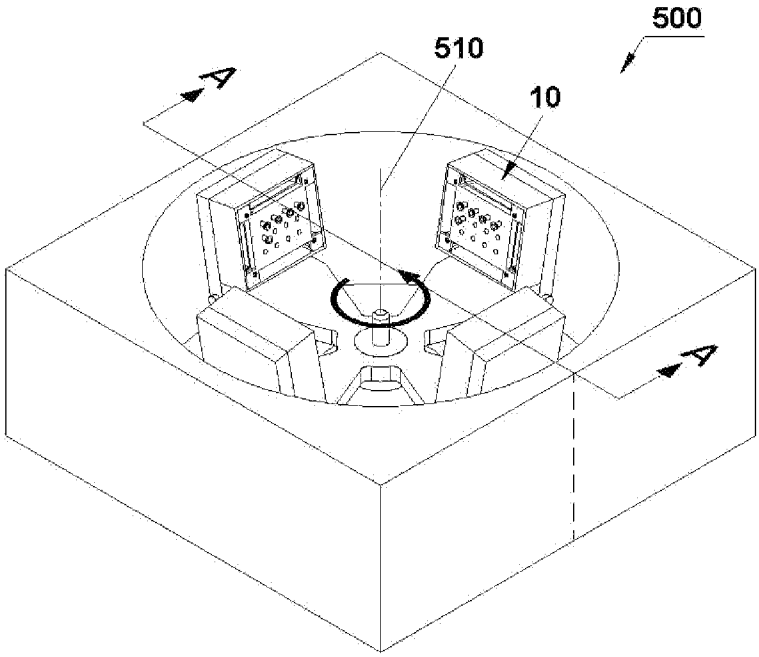


FIG. 45A

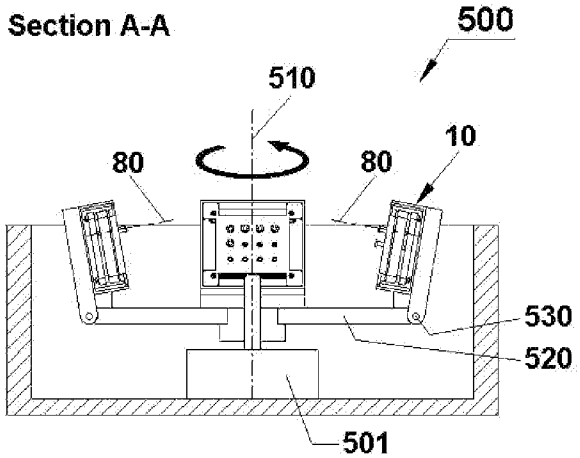


FIG. 45B

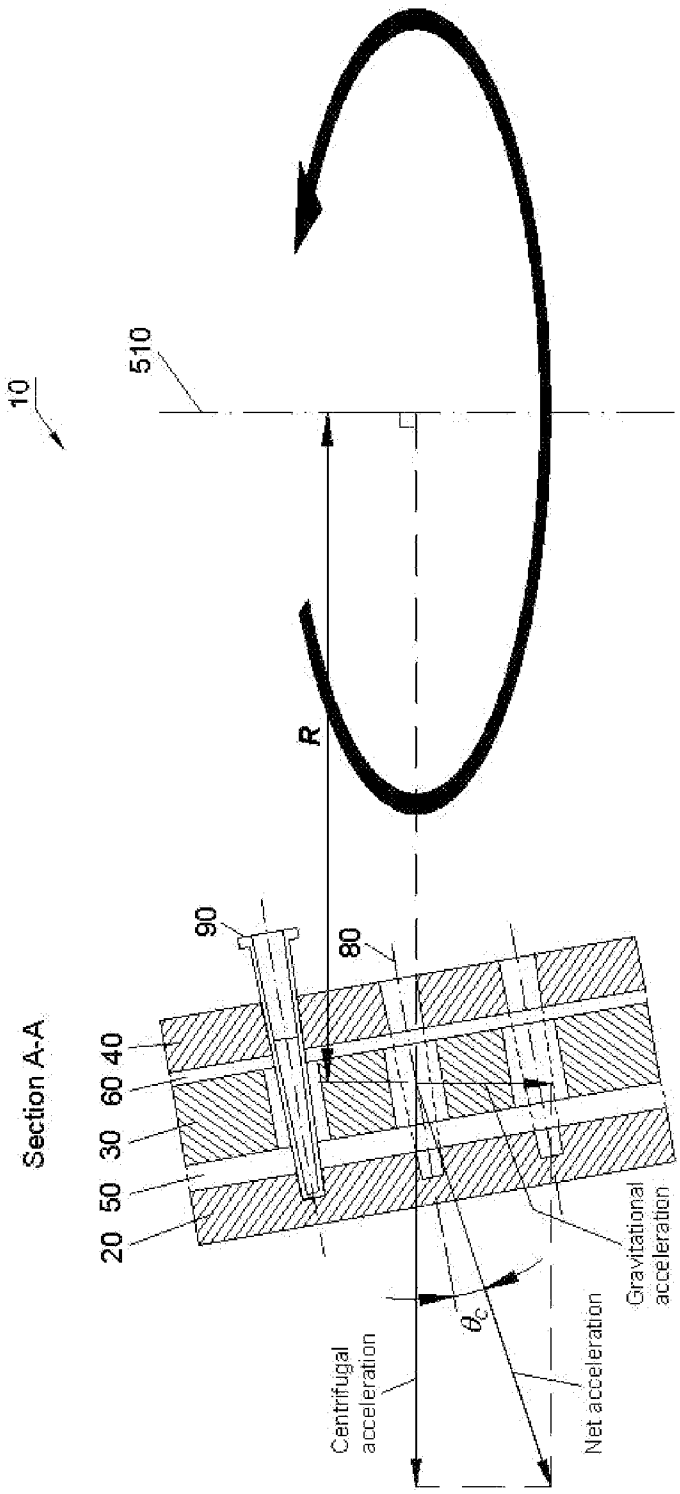
10
510
R
90
80
Section A-A
40
60
30
50
20
Centrifugal acceleration
$\theta_c$
Net acceleration
Gravitational acceleration

FIG. 46

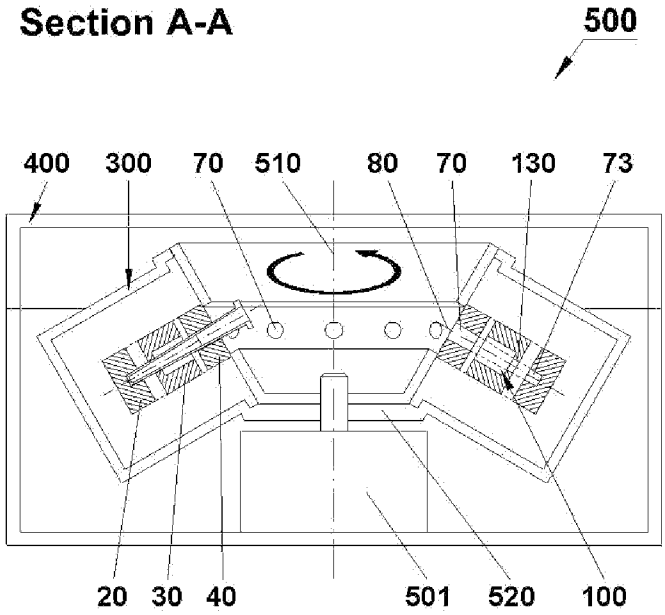


FIG. 47A

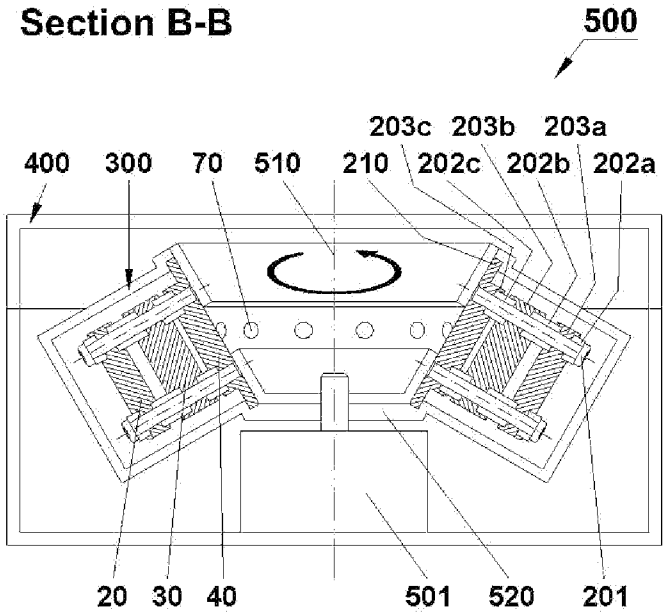


FIG. 47B

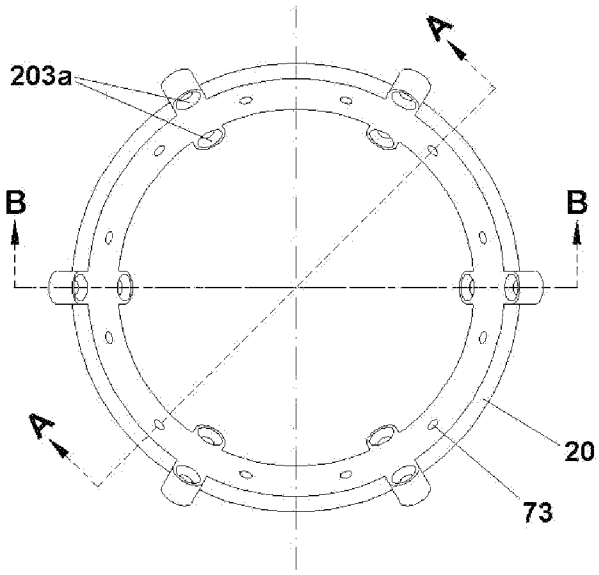


FIG. 48A

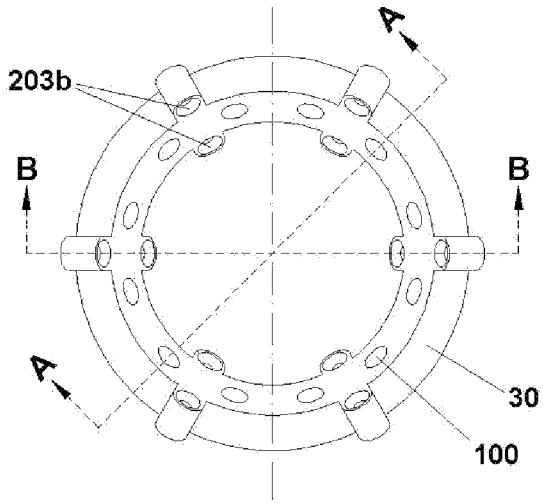


FIG. 48B

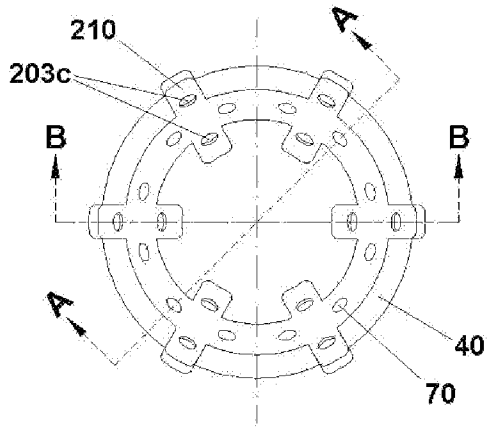


FIG. 48C

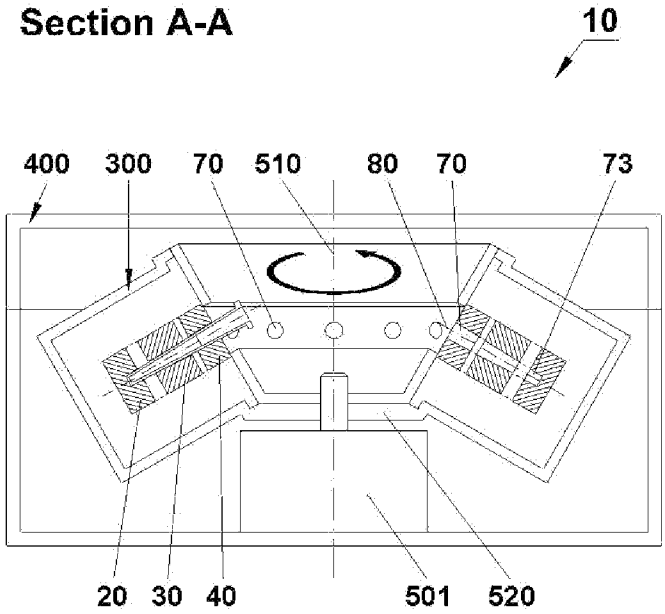


FIG. 49A

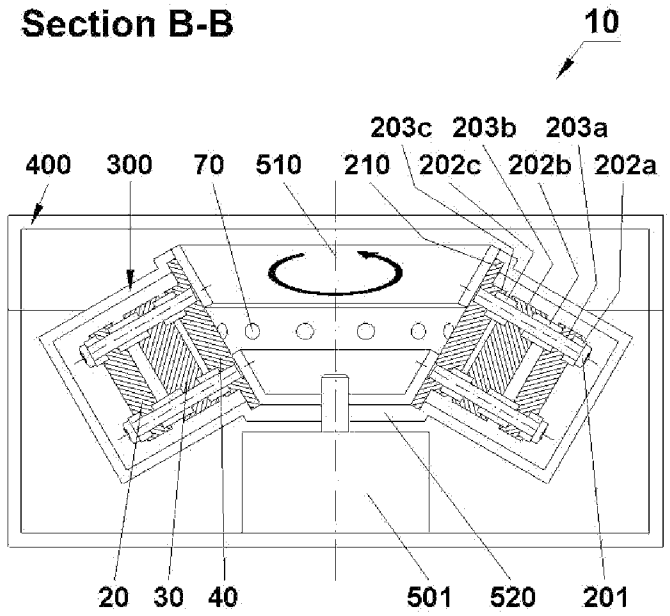


FIG. 49B

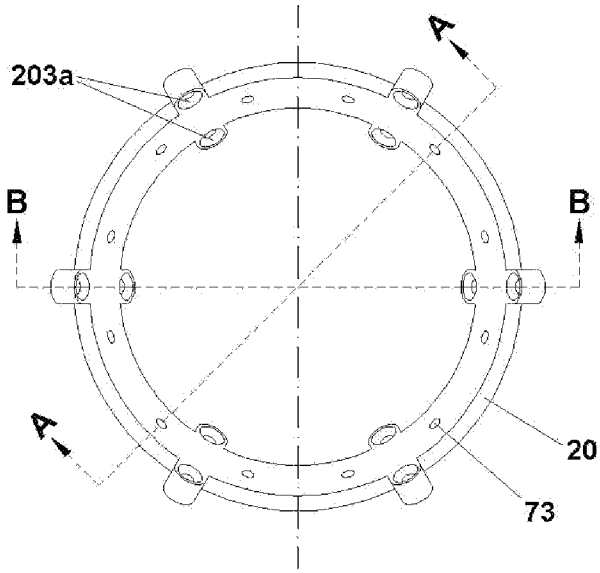


FIG. 50A

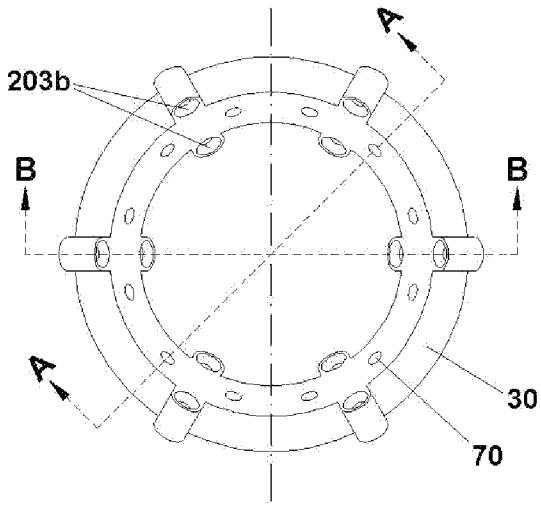


FIG. 50B

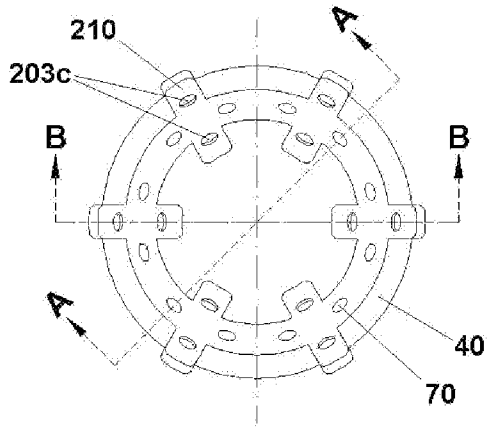


FIG. 50C

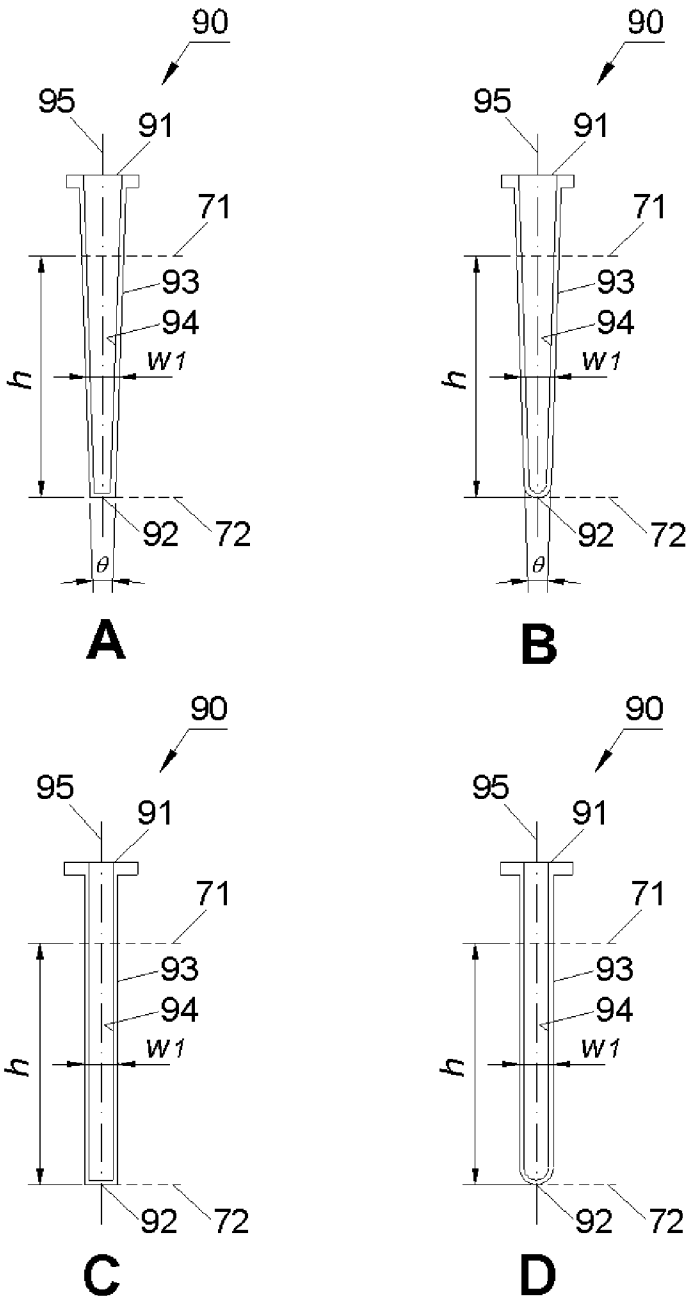


FIG. 51A-D

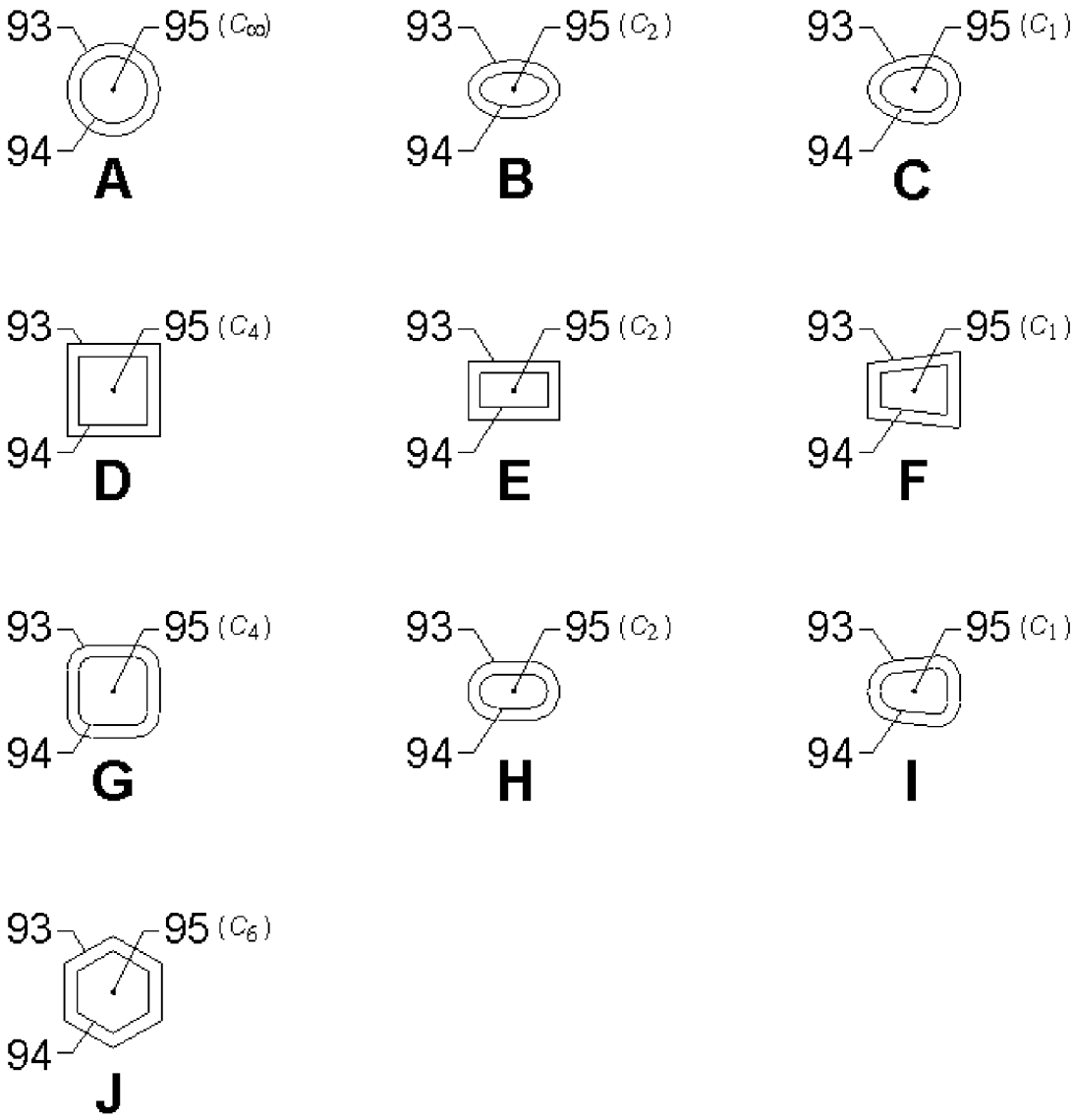
93
95 ($C_\infty$)
94
A
93
95 ($C_2$)
94
B
93
95 ($C_1$)
94
C
93
95 ($C_4$)
94
D
93
95 ($C_2$)
94
E
93
95 ($C_1$)
94
F
93
95 ($C_4$)
94
G
93
95 ($C_2$)
94
H
93
95 ($C_1$)
94
I
93
95 ($C_6$)
94
J

FIG. 52A-J

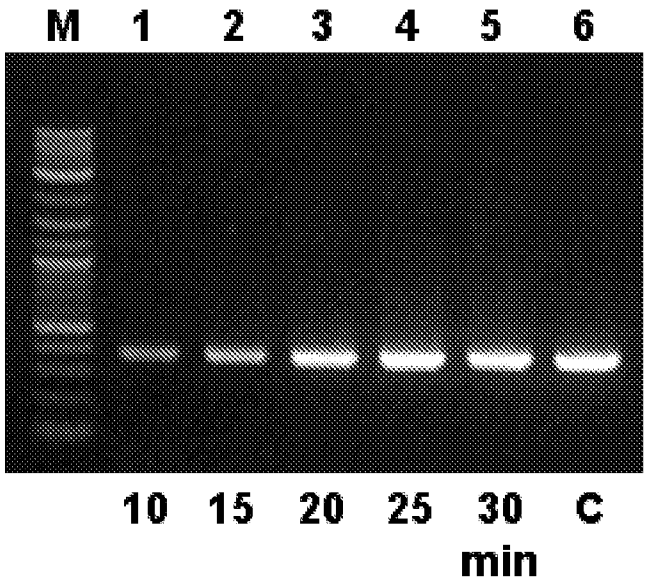


FIG. 53A

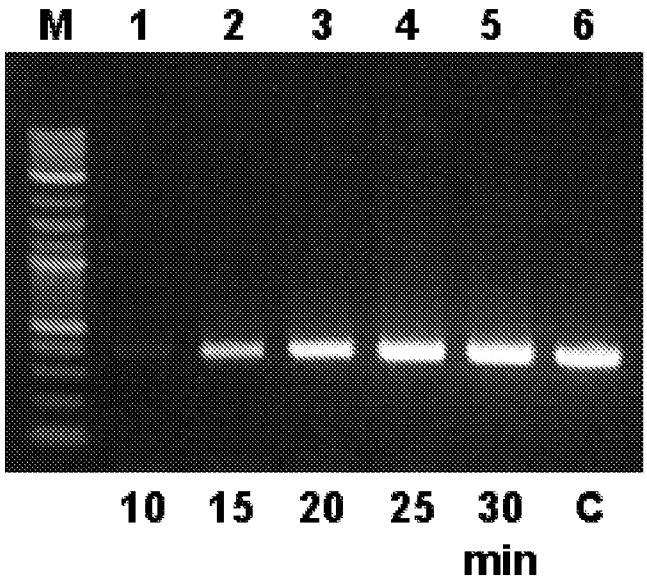


FIG. 53B

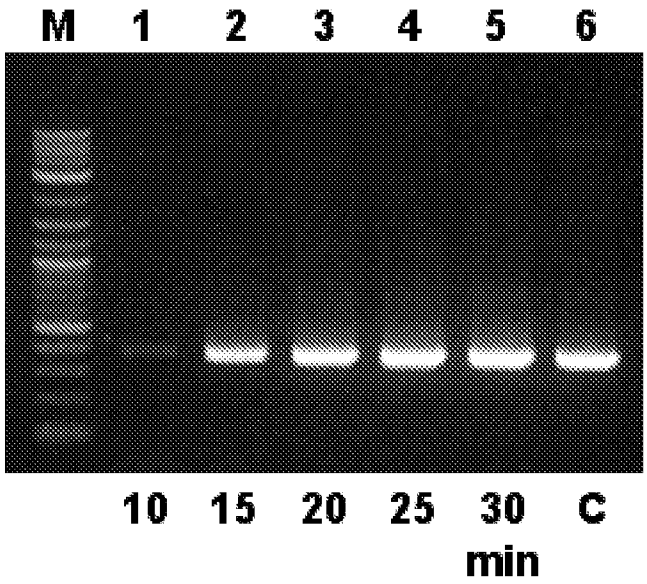


FIG. 53C

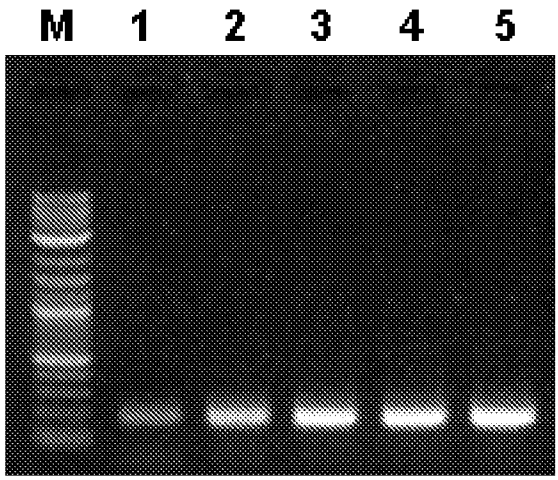


FIG. 54A

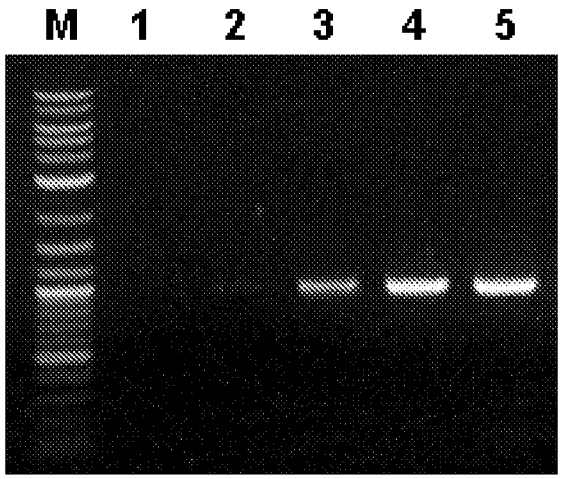


FIG. 54B

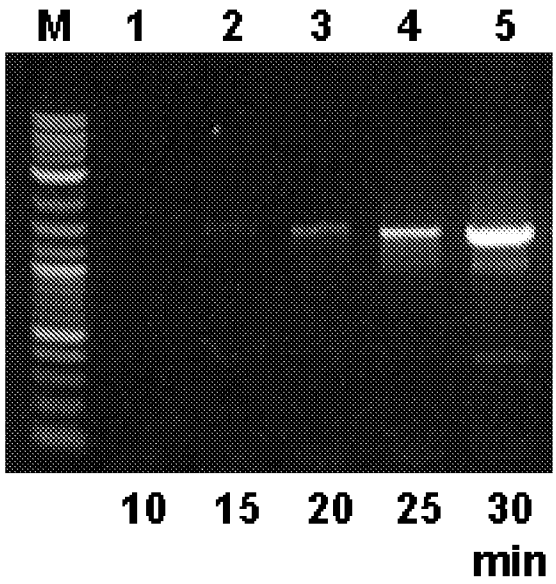


FIG. 54C

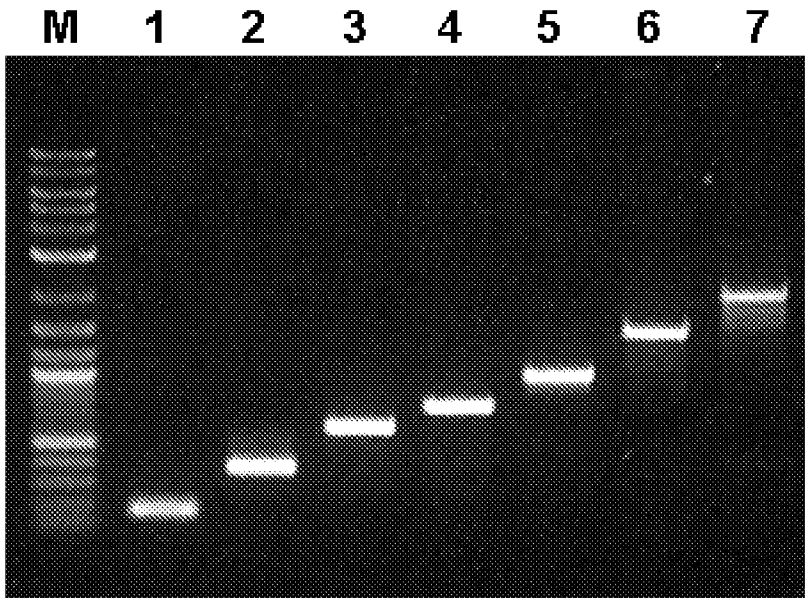


FIG. 55

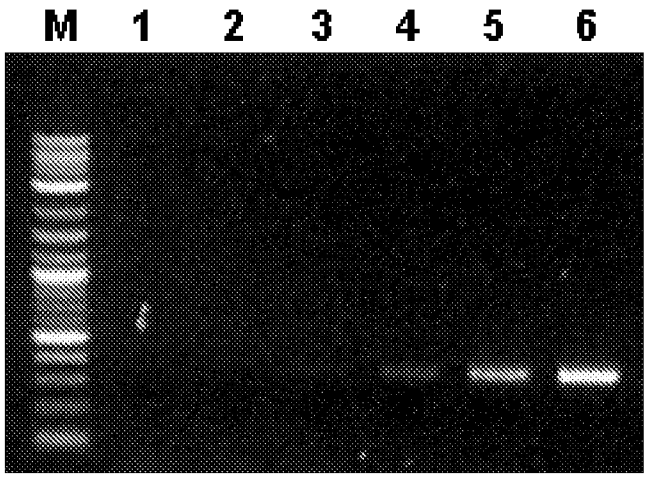


FIG. 56A

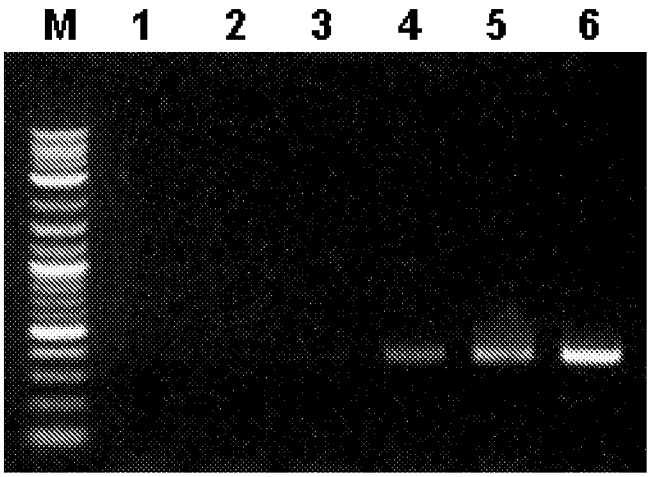


FIG. 56B

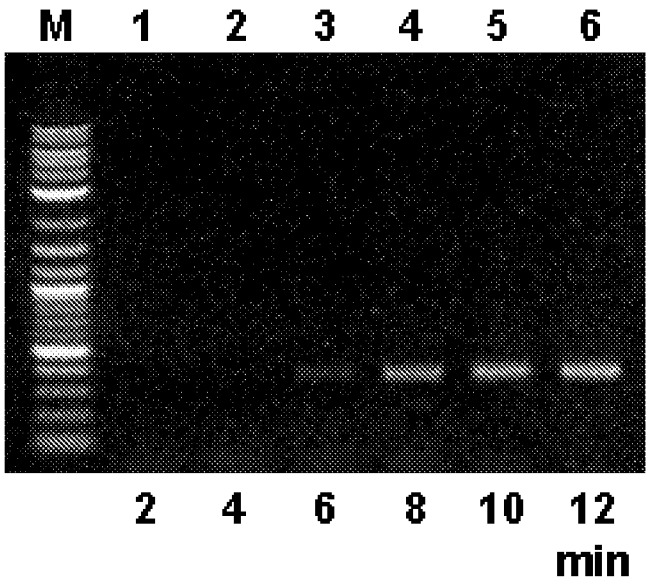


FIG. 56C

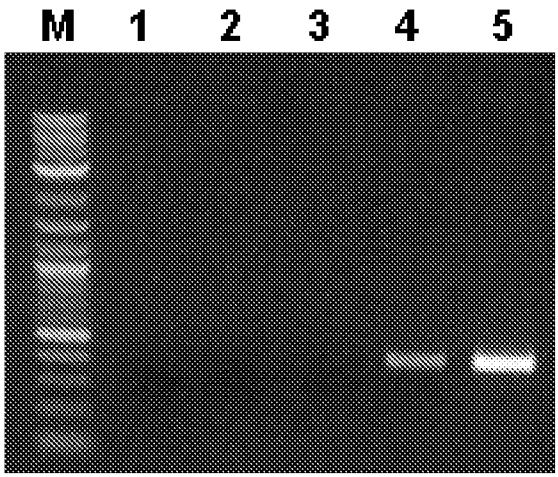


FIG. 57A

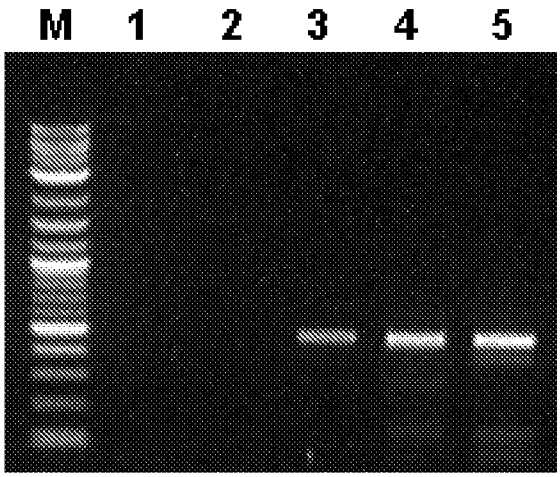


FIG. 57B

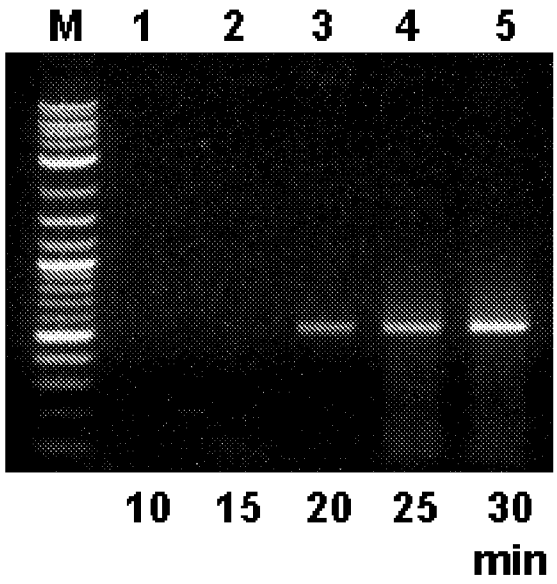


FIG. 57C

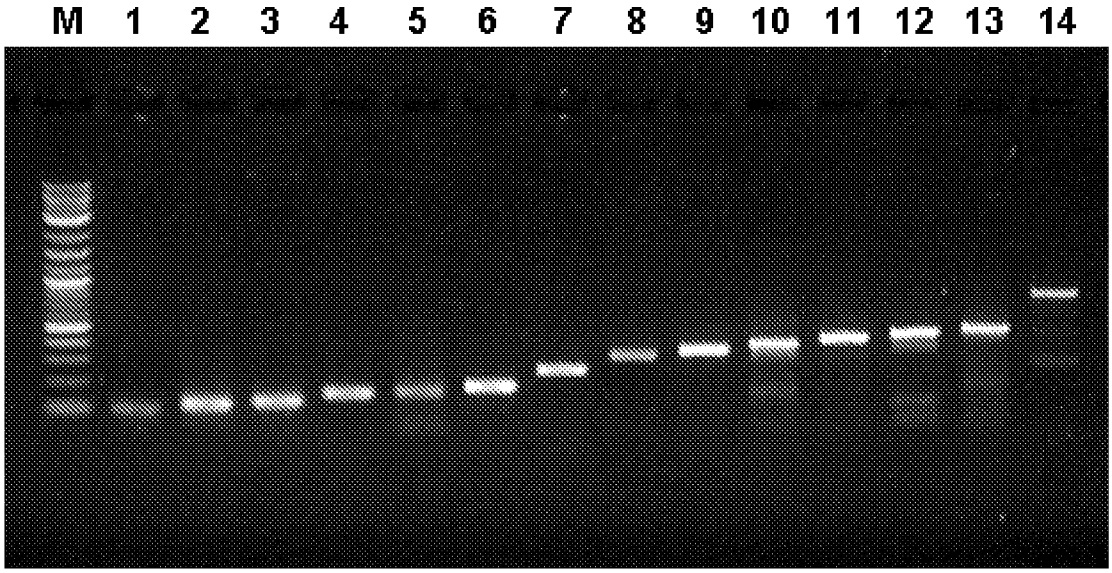
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 58

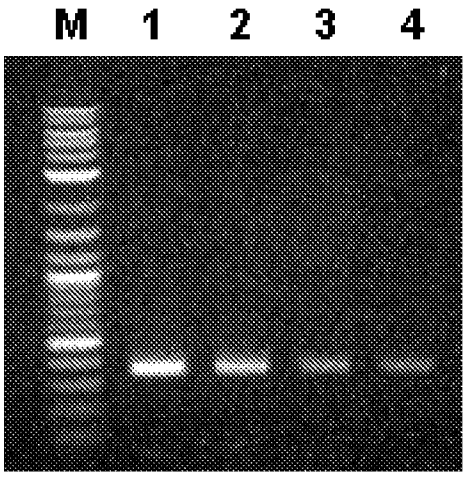
M 1 2 3 4
10 1 0.3 0.1 ng
3,000 300 100 30 copies

FIG. 59

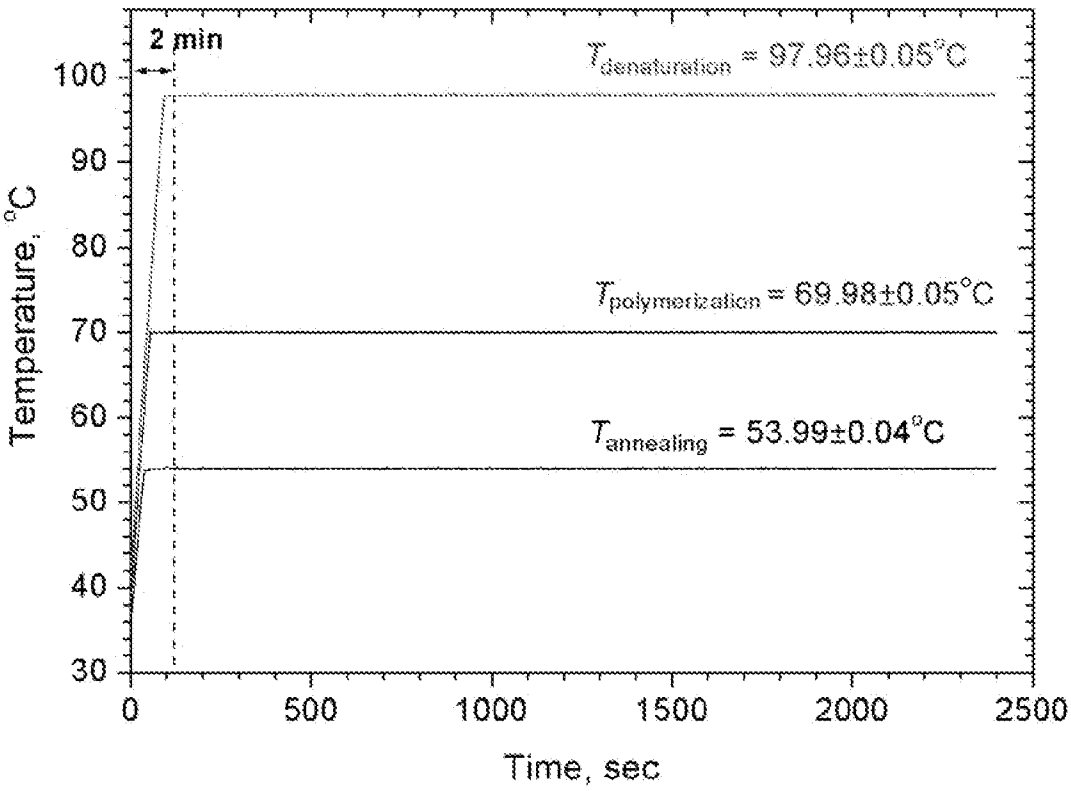
2 min
$T_{denaturation}$ = 97.96±0.05°C
$T_{polymerization}$ = 69.98±0.05°C
$T_{annealing}$ = 53.99±0.04°C
Temperature, °C
100
90
80
70
60
50
40
30
0
500
1000
1500
2000
2500
Time, sec

FIG. 60

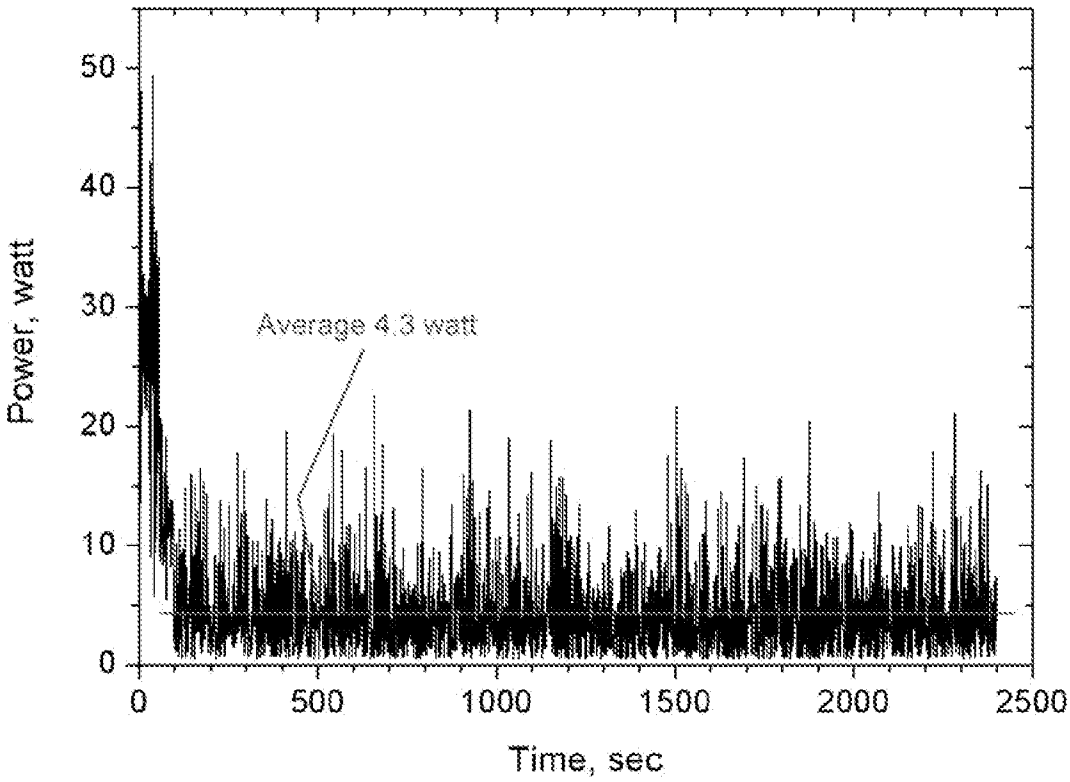
Power, watt
Time, sec
Average 4.3 watt
0
10
20
30
40
50
0
500
1000
1500
2000
2500

FIG. 61

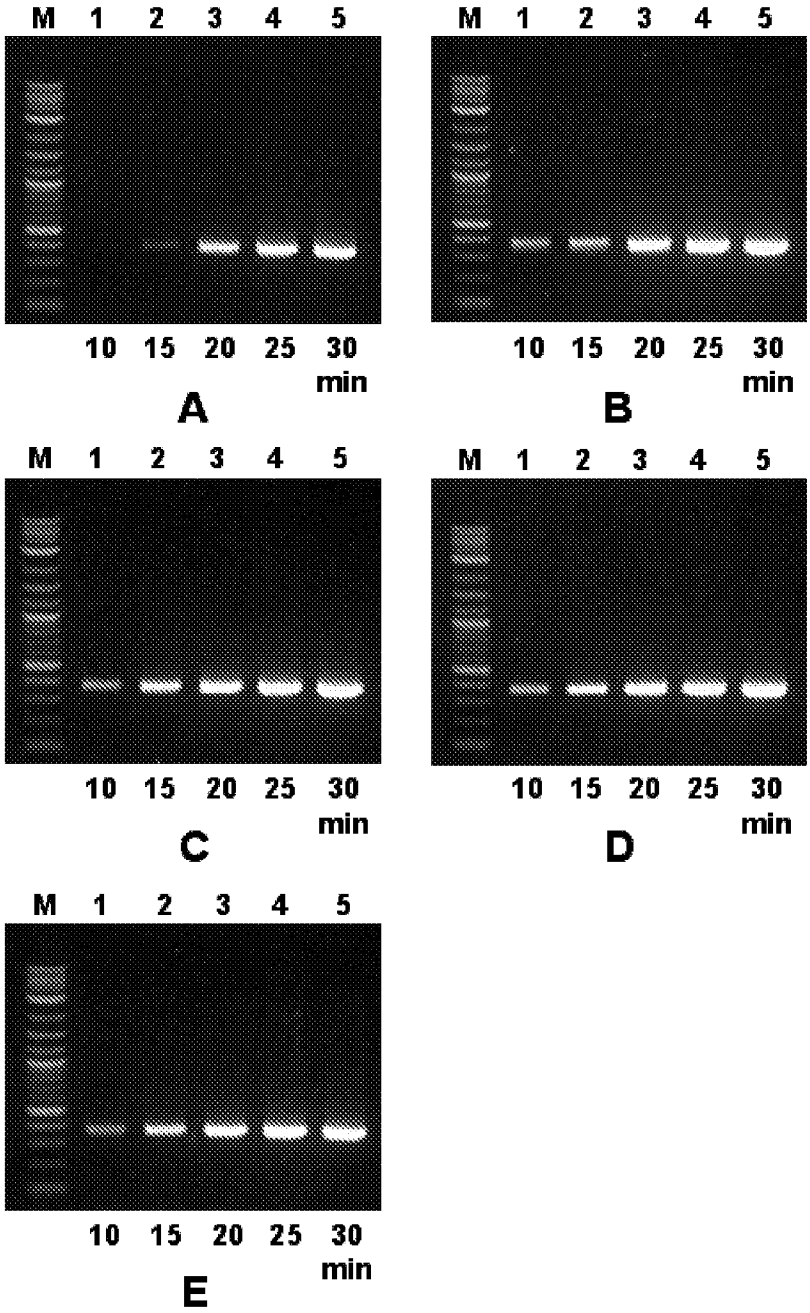
M 1 2 3 4 5
10 15 20 25 30 min
A
M 1 2 3 4 5
10 15 20 25 30 min
B
M 1 2 3 4 5
10 15 20 25 30 min
C
M 1 2 3 4 5
10 15 20 25 30 min
D
M 1 2 3 4 5
10 15 20 25 30 min
E

FIG. 62A-E

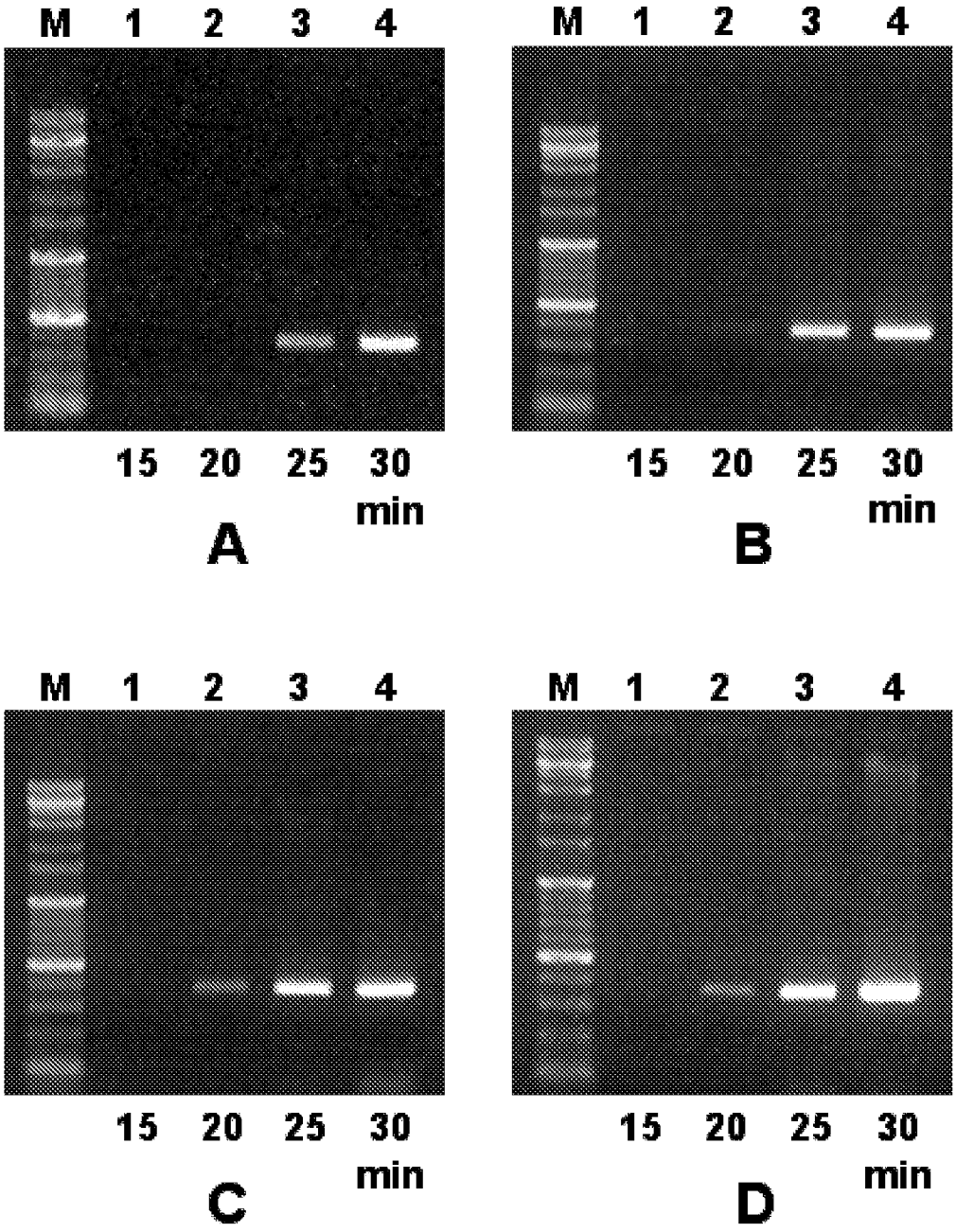
M 1 2 3 4
15 20 25 30
min
A
M 1 2 3 4
15 20 25 30
min
B
M 1 2 3 4
15 20 25 30
min
C
M 1 2 3 4
15 20 25 30
min
D

FIG. 63A-D

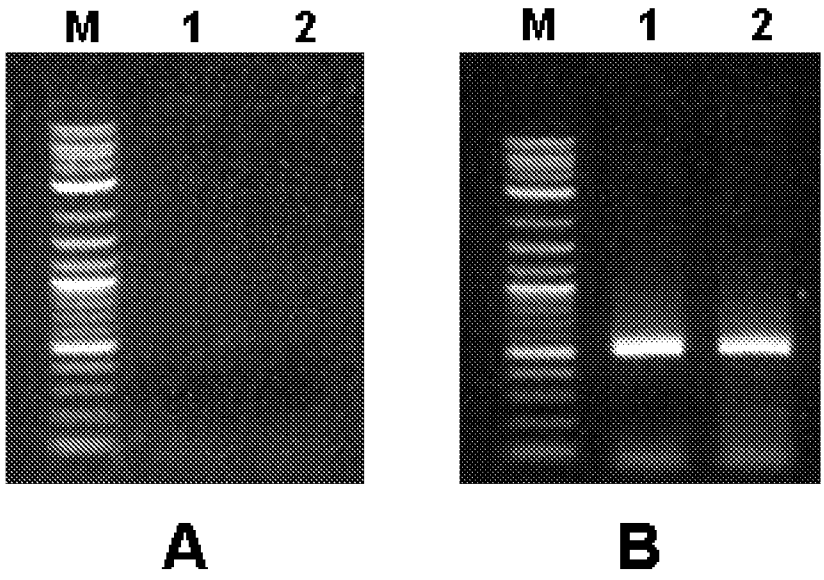


FIG. 64A-B

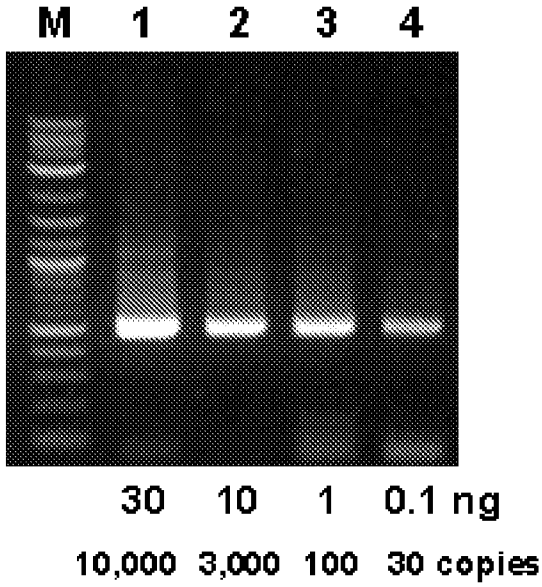


FIG. 65

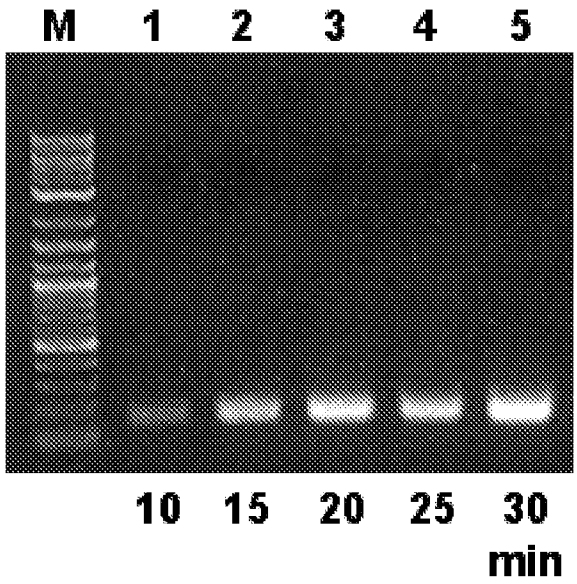


FIG. 66

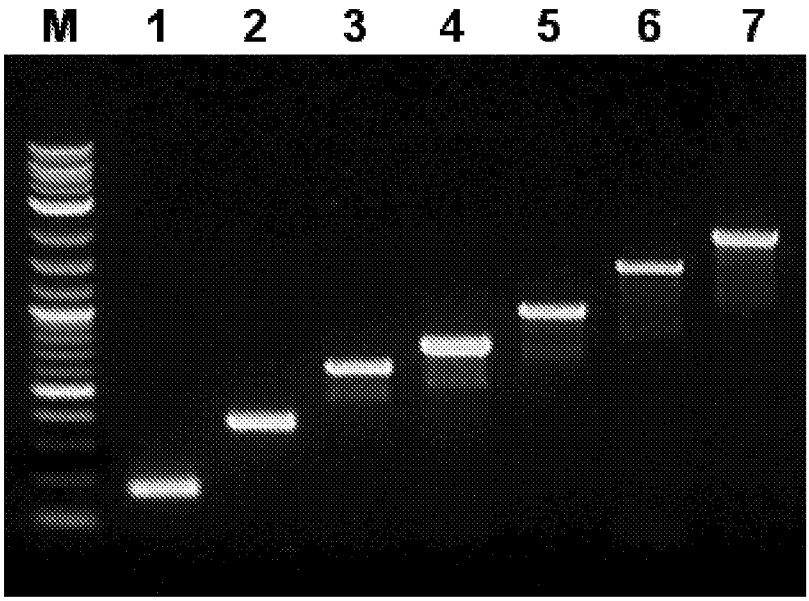


FIG. 67

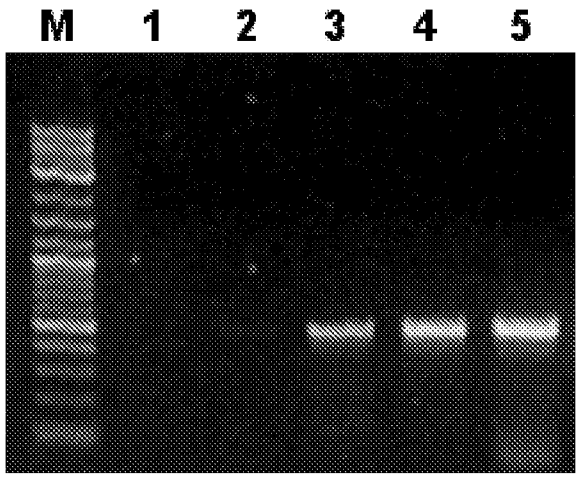


FIG. 68A

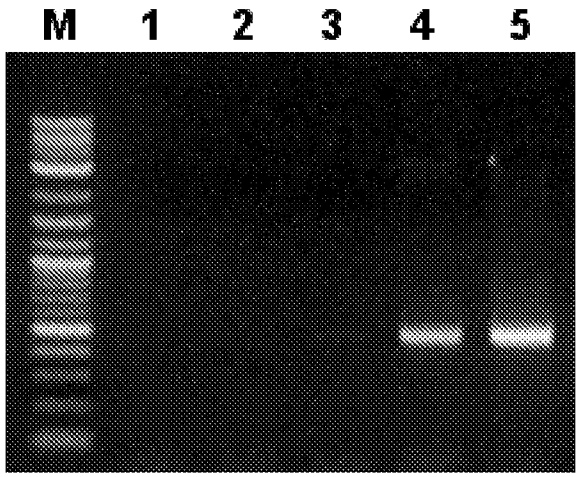


FIG. 68B

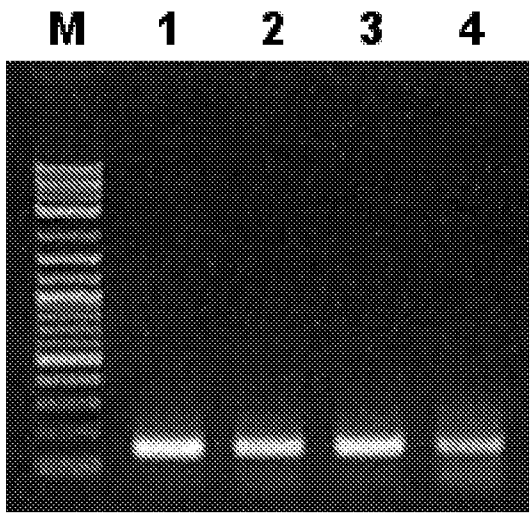


FIG. 69

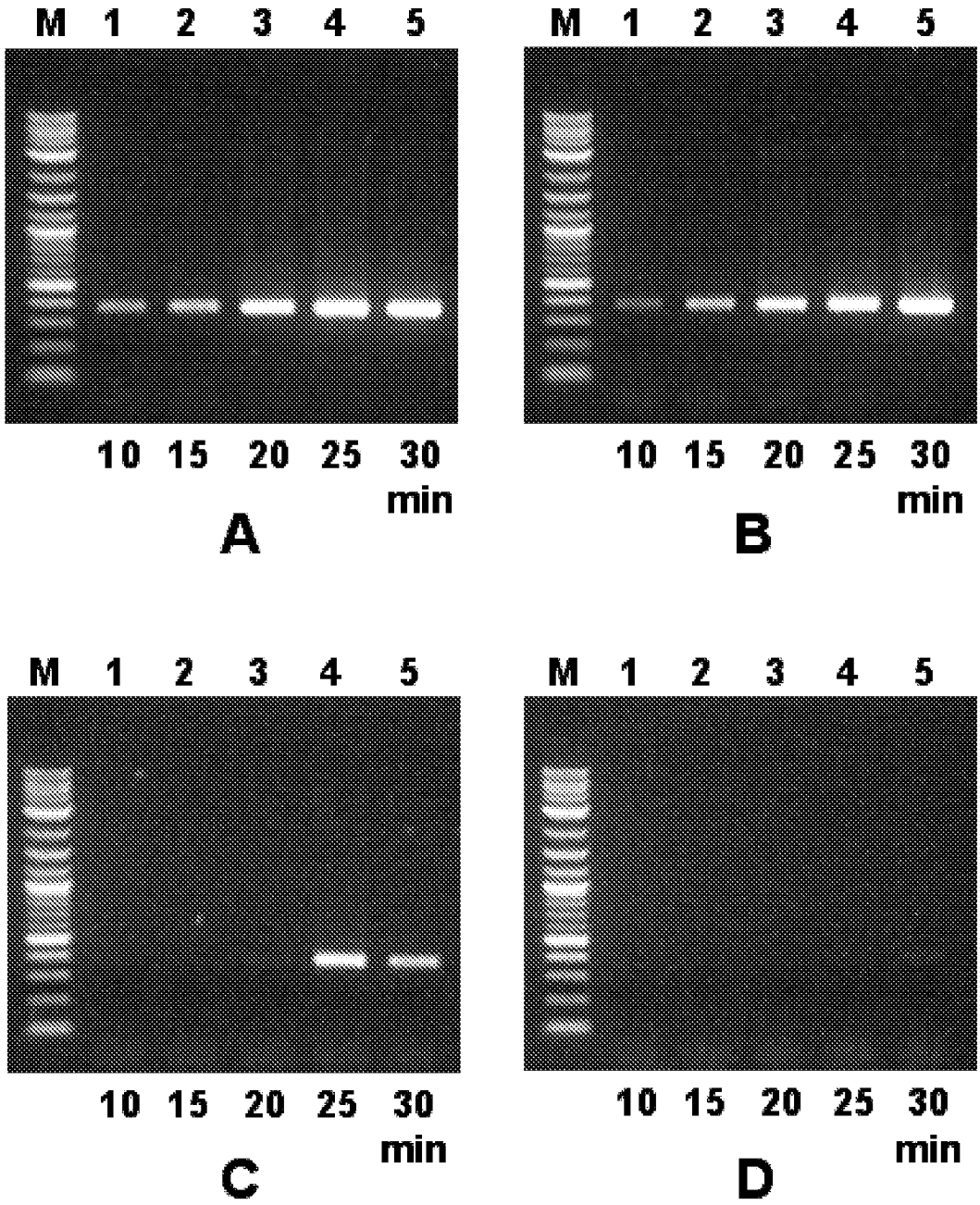
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D

FIG. 70A-D

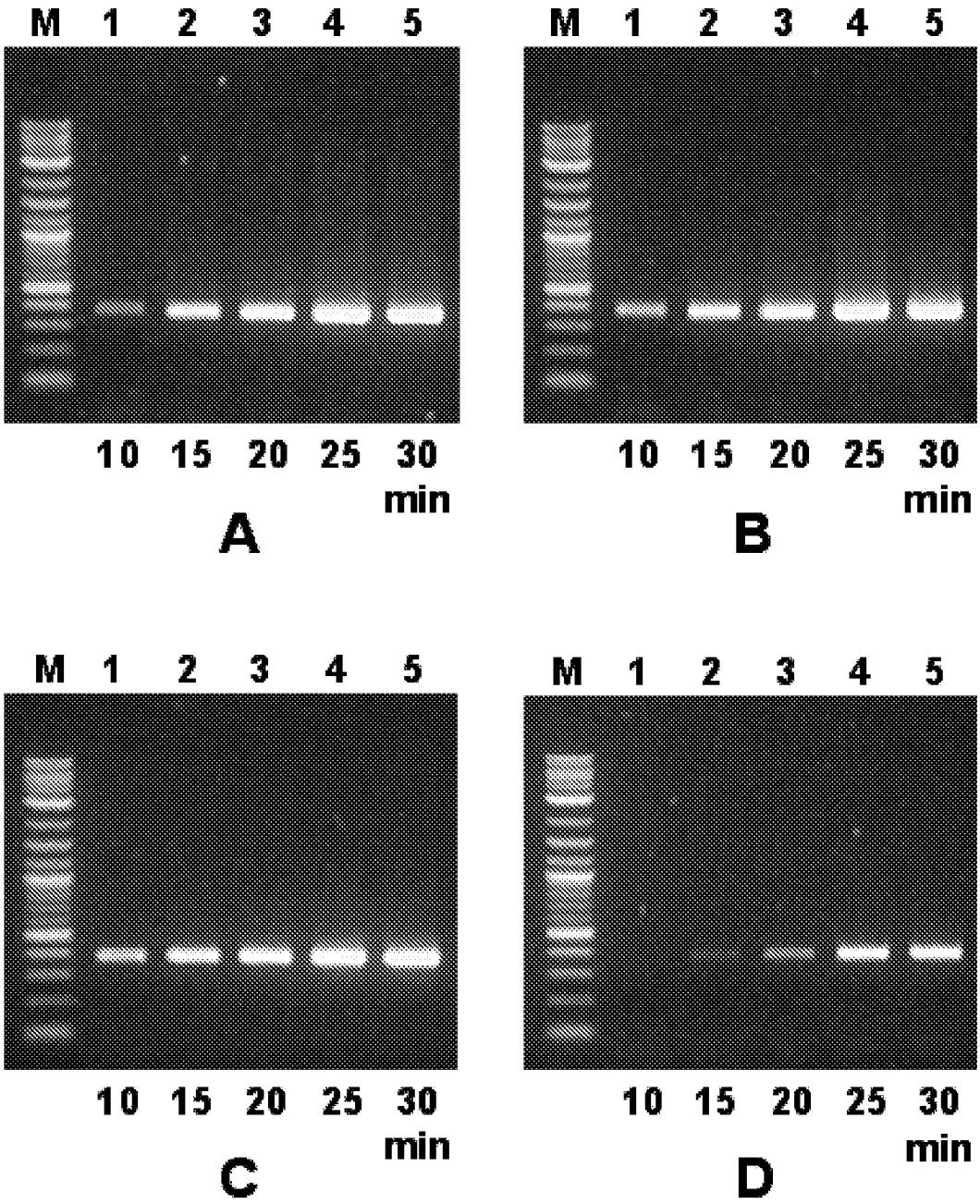
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D

FIG. 71A-D

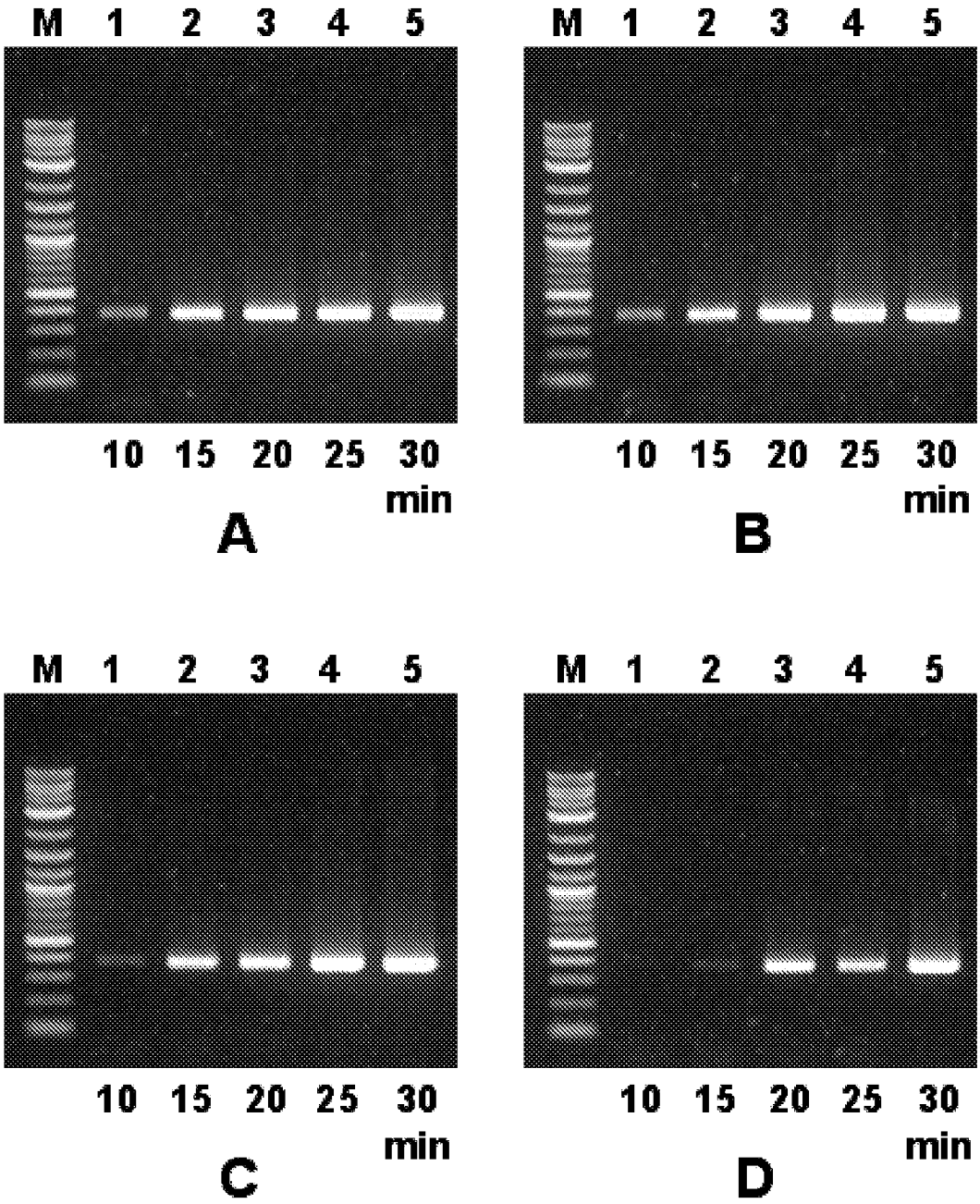
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D

FIG. 72A-D

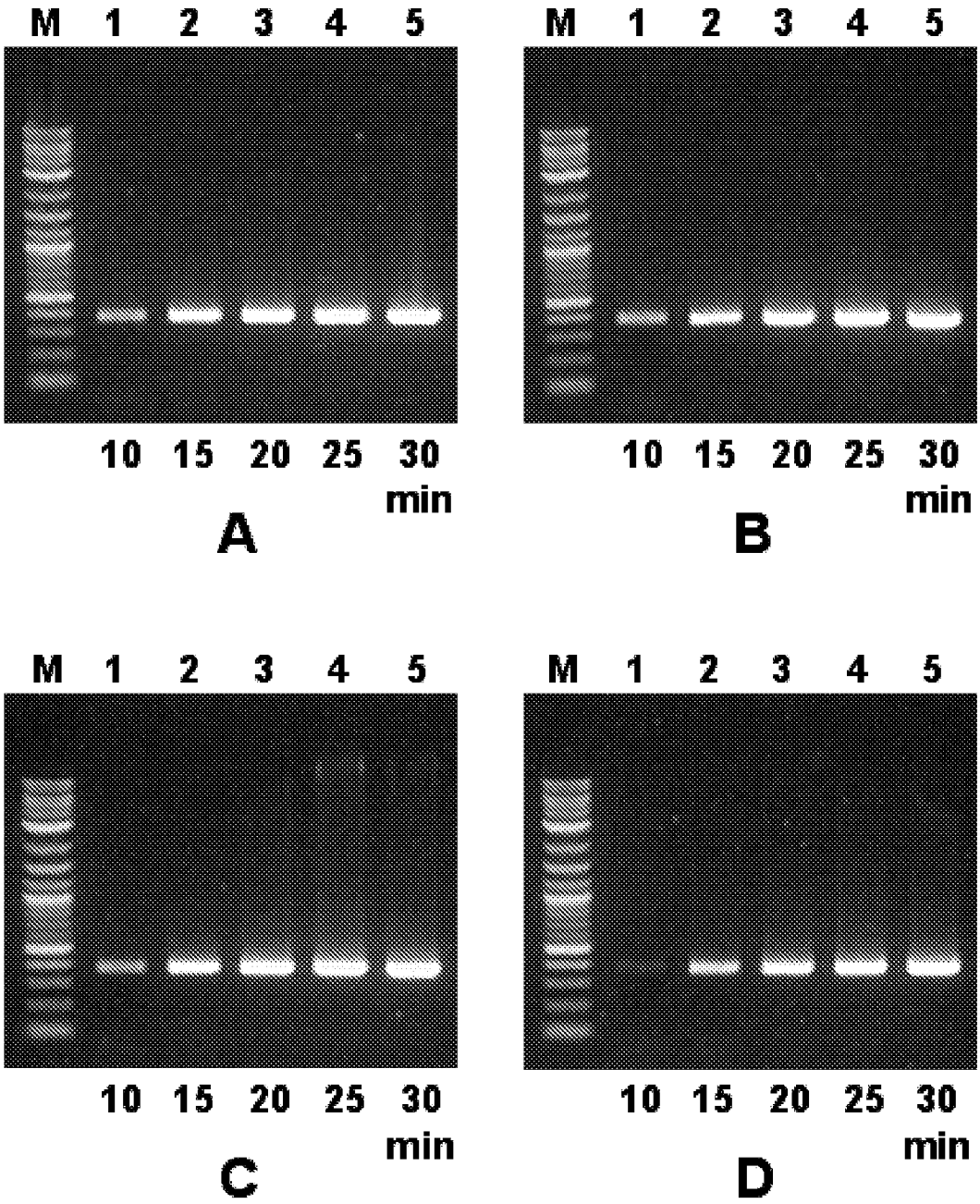
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D

FIG. 73A-D

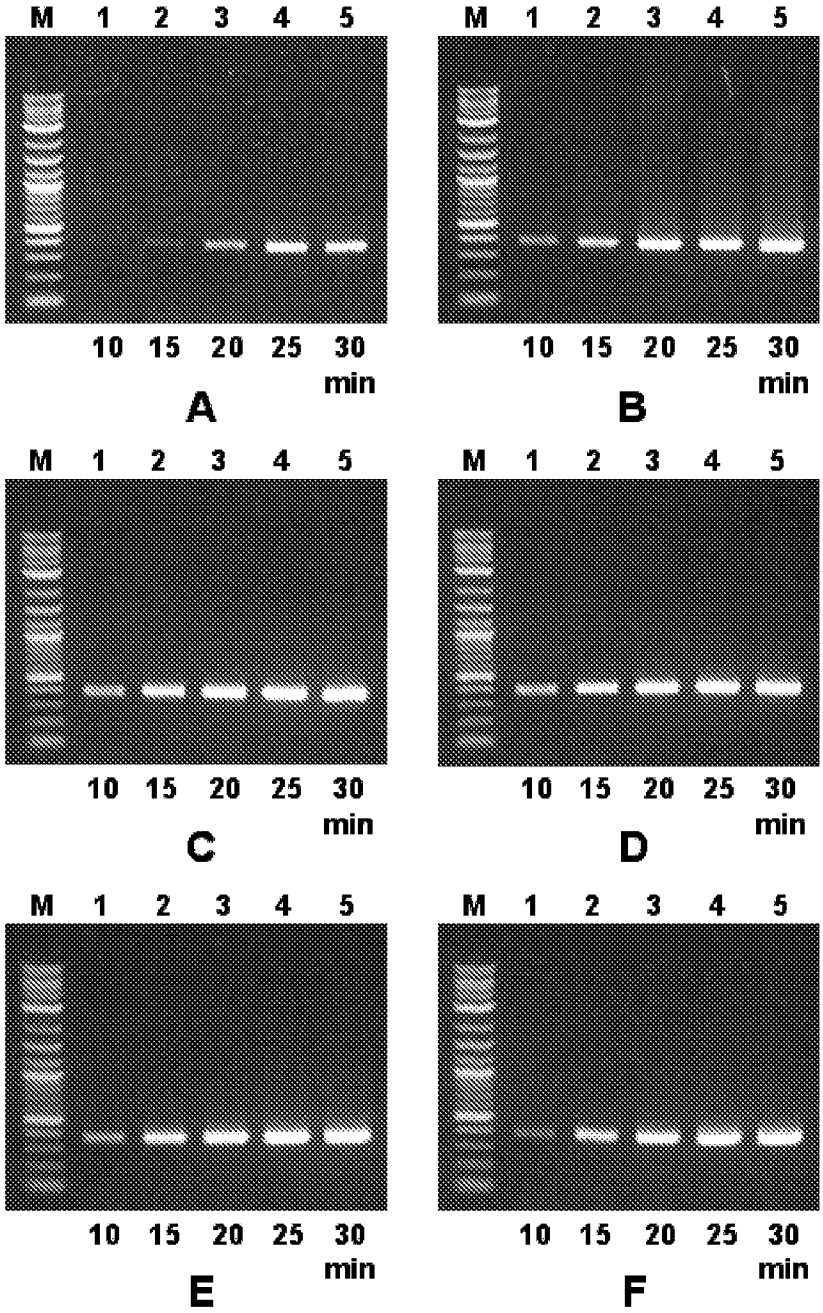
M 1 2 3 4 5
10 15 20 25 30 min
A
M 1 2 3 4 5
10 15 20 25 30 min
B
M 1 2 3 4 5
10 15 20 25 30 min
C
M 1 2 3 4 5
10 15 20 25 30 min
D
M 1 2 3 4 5
10 15 20 25 30 min
E
M 1 2 3 4 5
10 15 20 25 30 min
F

FIG. 74A-F

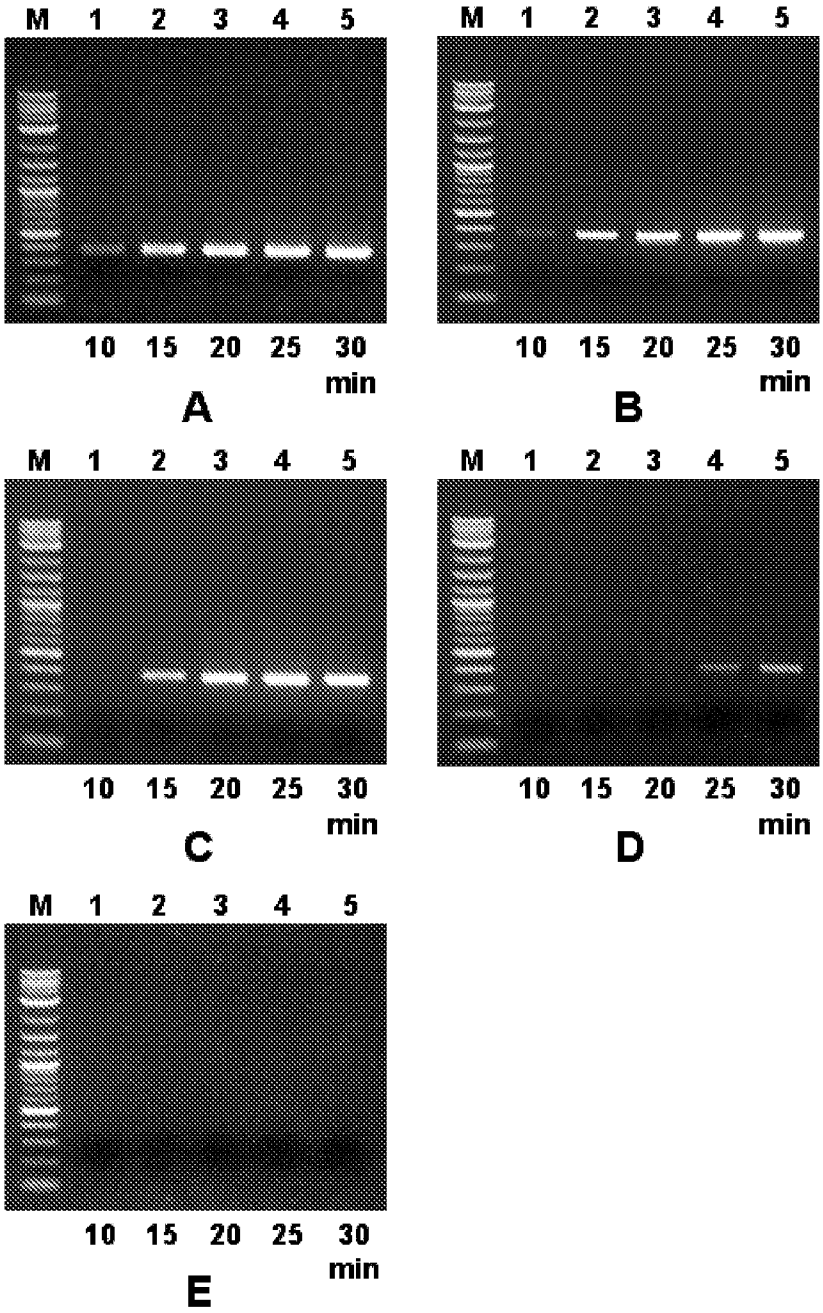
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D
M 1 2 3 4 5
10 15 20 25 30
min
E

FIG. 75A-E

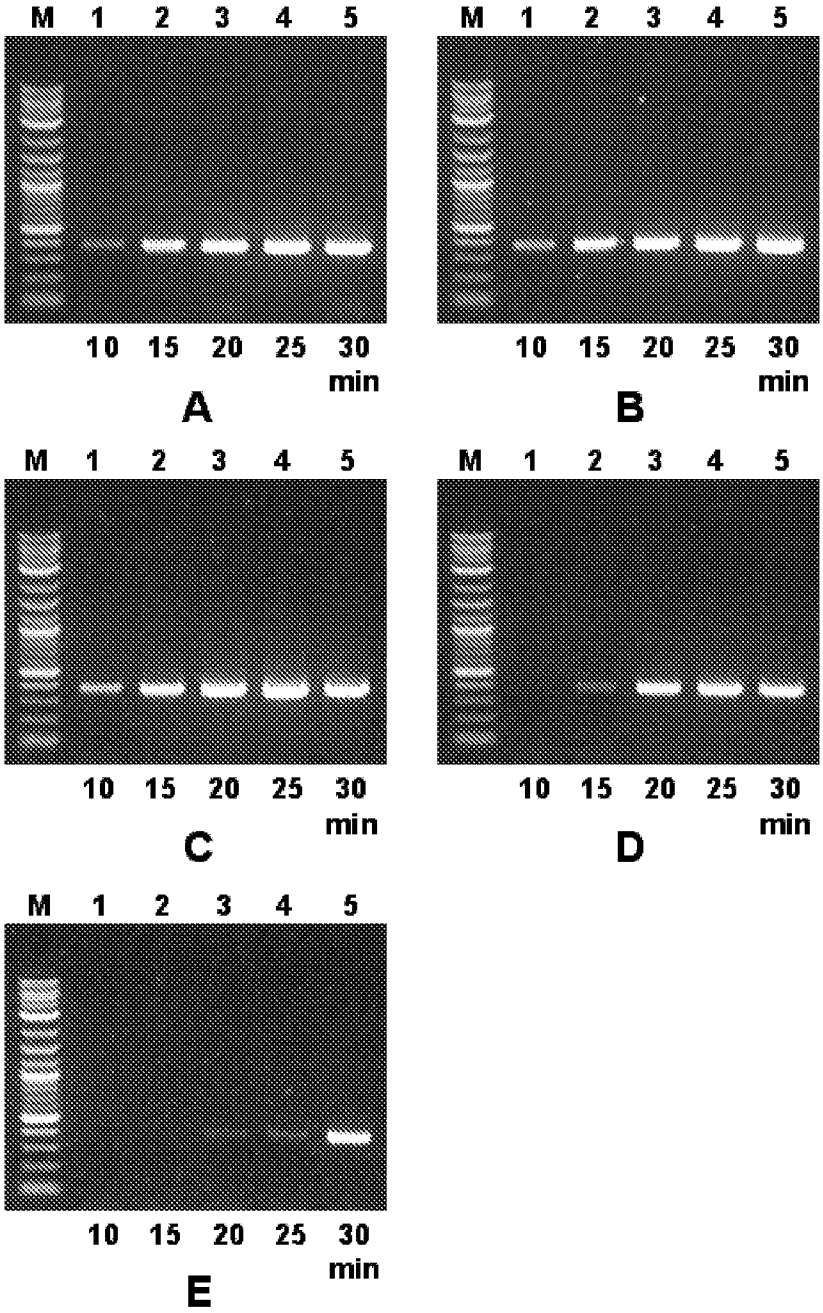
M 1 2 3 4 5
10 15 20 25 30
min
A
M 1 2 3 4 5
10 15 20 25 30
min
B
M 1 2 3 4 5
10 15 20 25 30
min
C
M 1 2 3 4 5
10 15 20 25 30
min
D
M 1 2 3 4 5
10 15 20 25 30
min
E

FIG. 76A-E

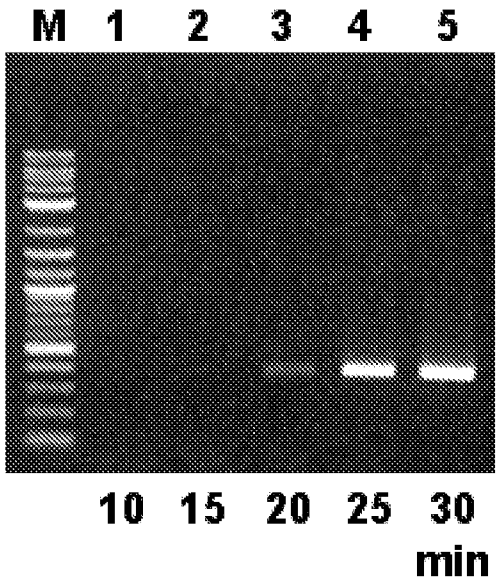


FIG. 77

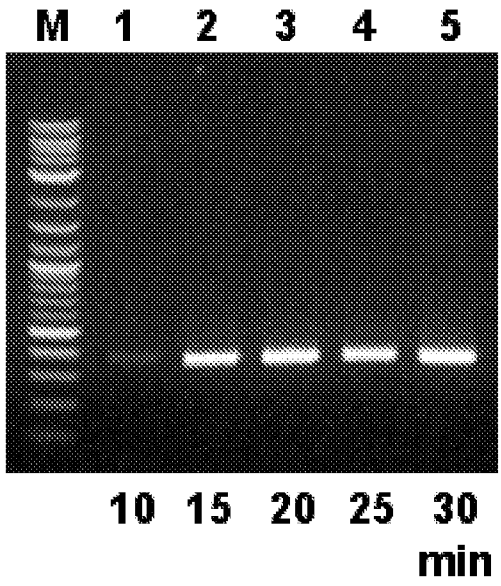


FIG. 78A

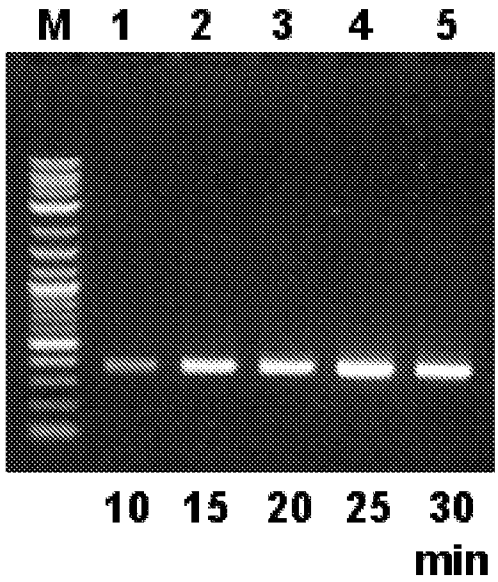


FIG. 78B

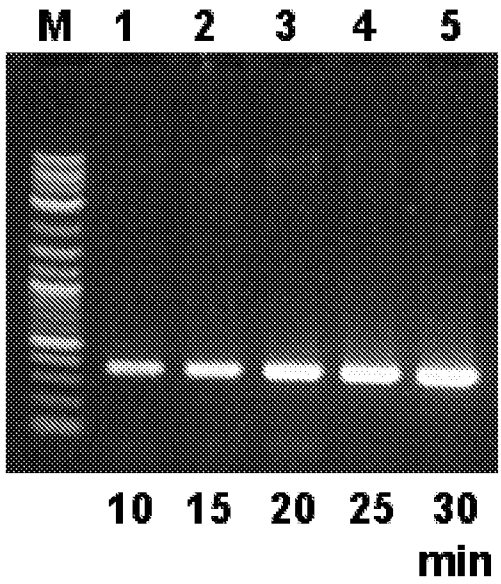


FIG. 79

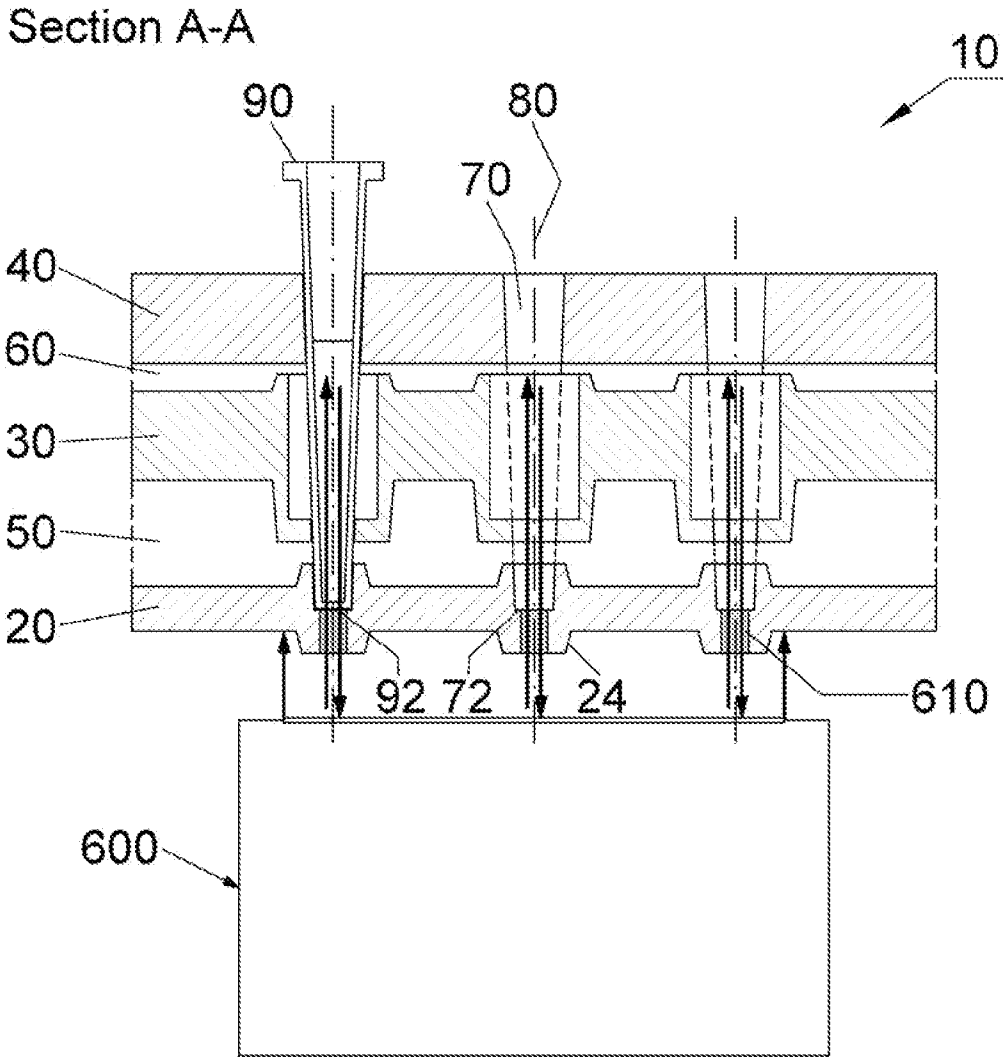


FIG. 80A

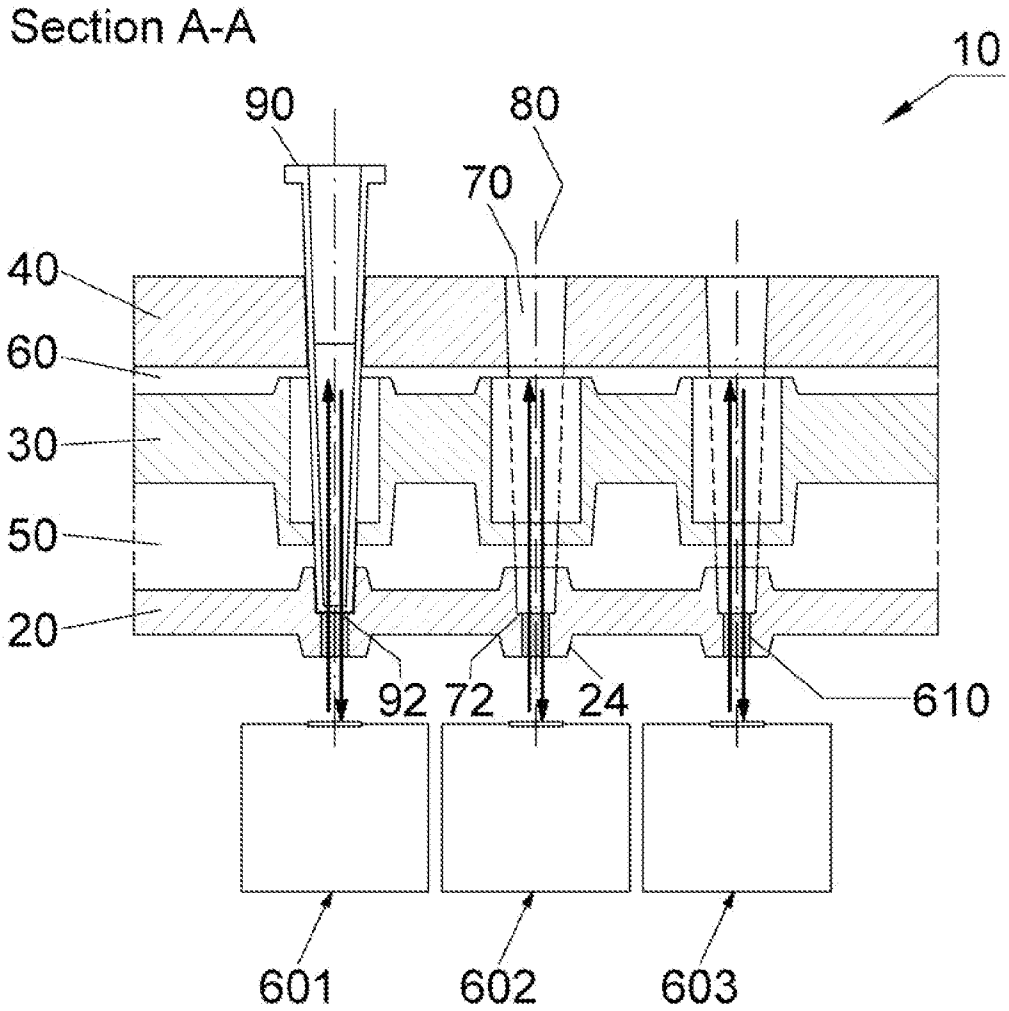


FIG. 80B

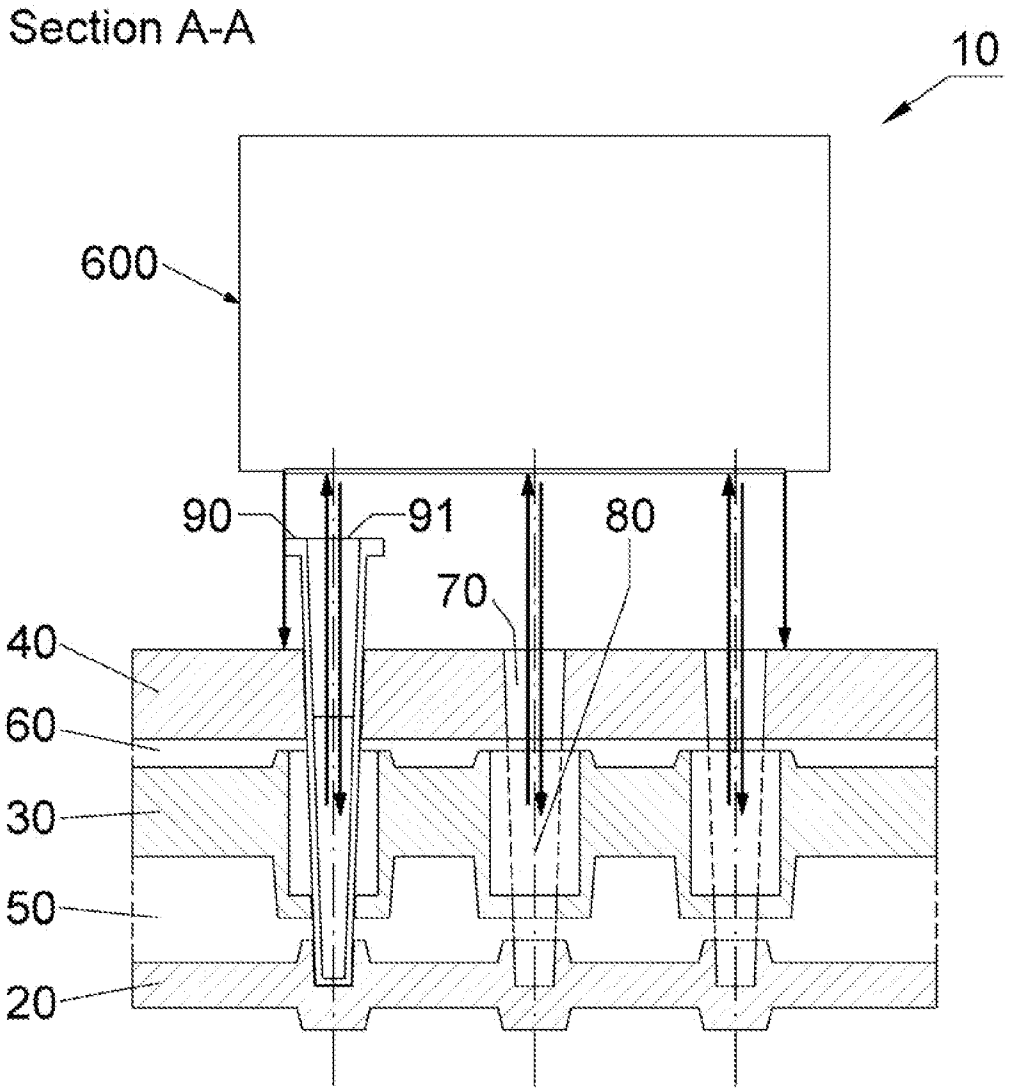


FIG. 81A

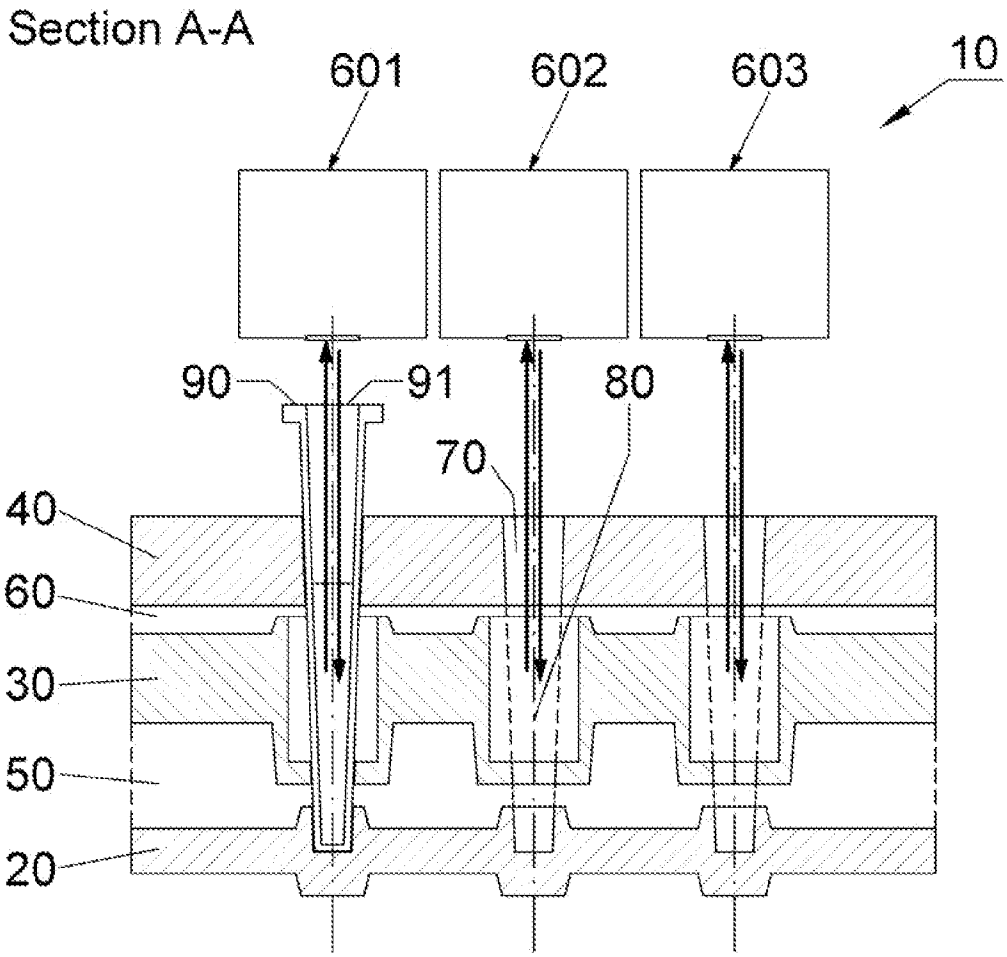


FIG. 81B

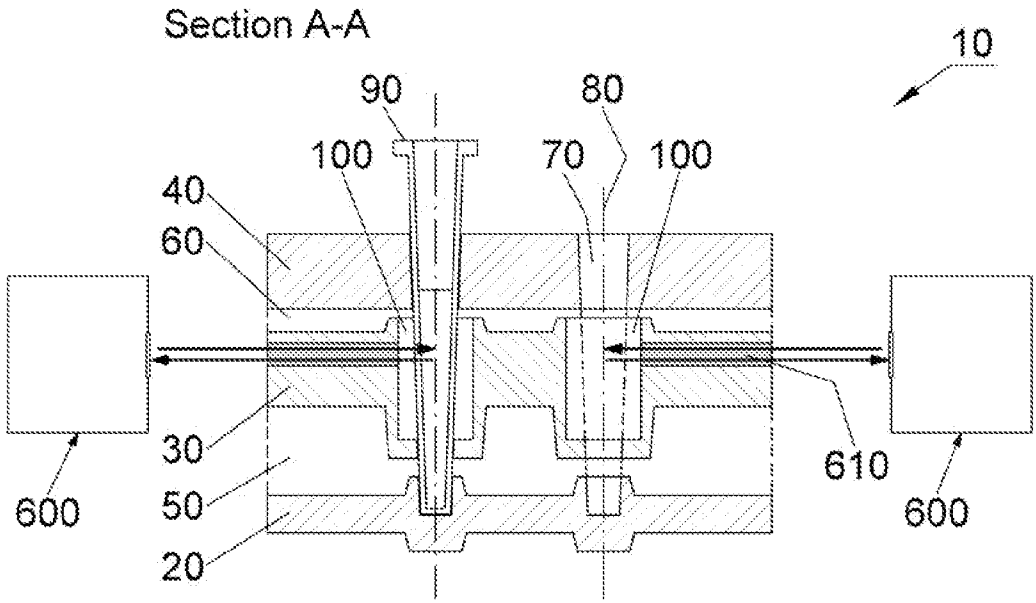


FIG. 82

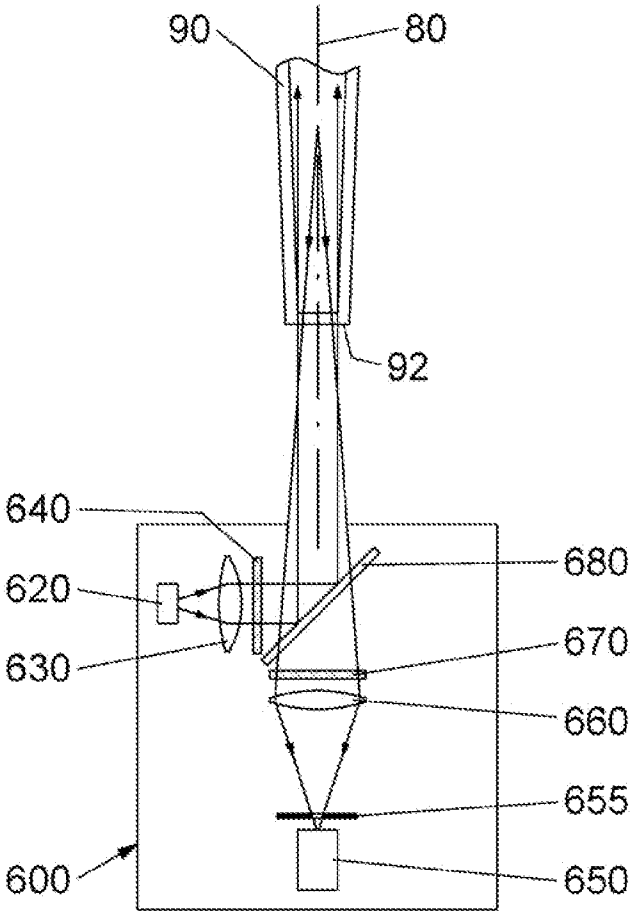


FIG. 83

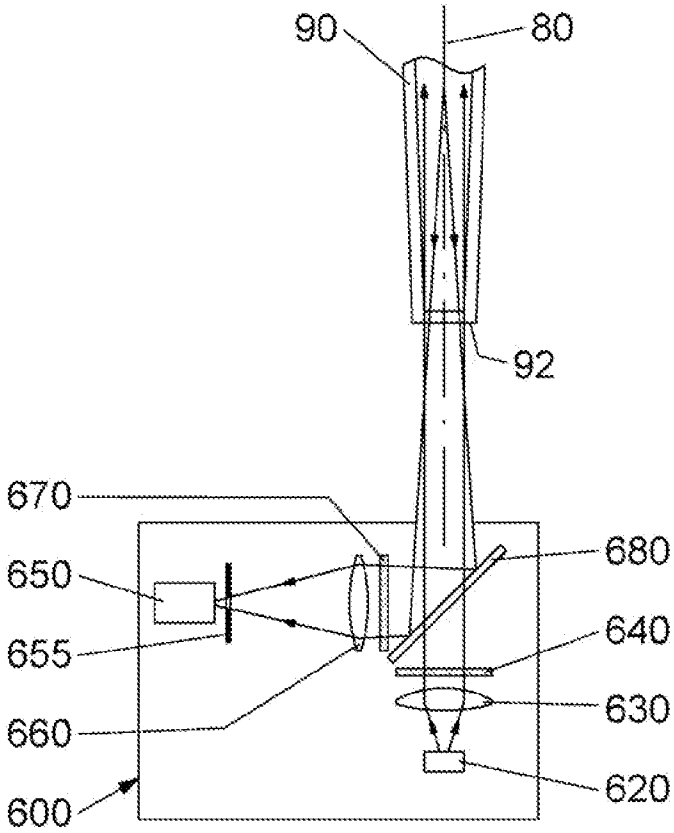


FIG. 84

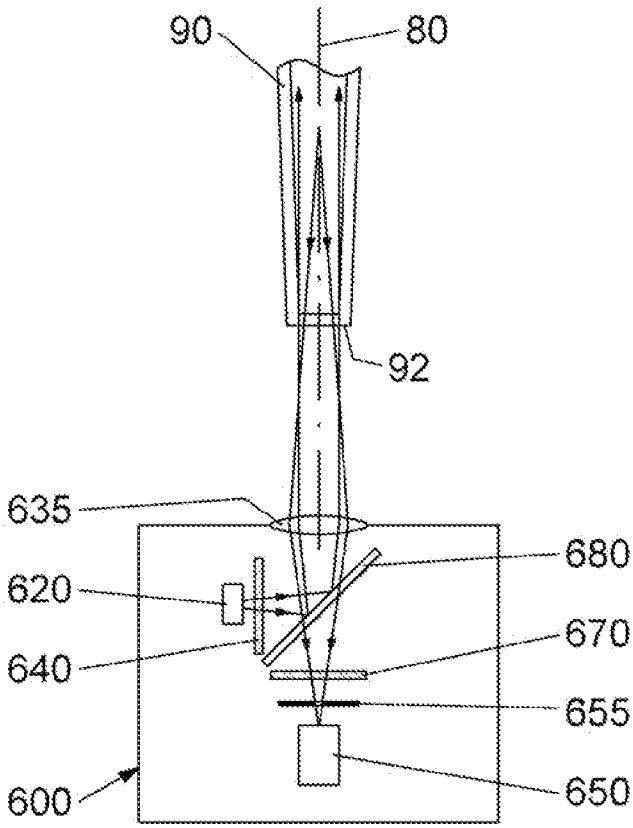


FIG. 85A

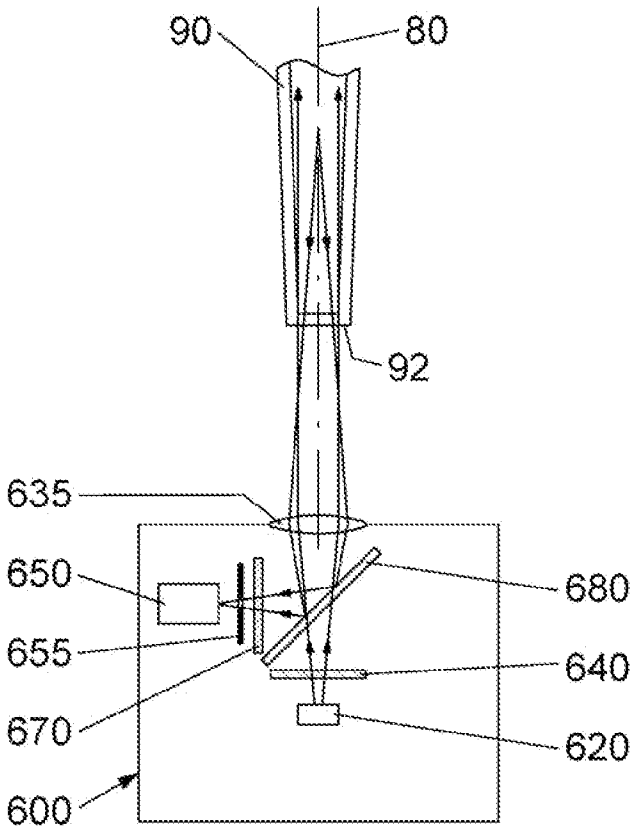


FIG. 85B

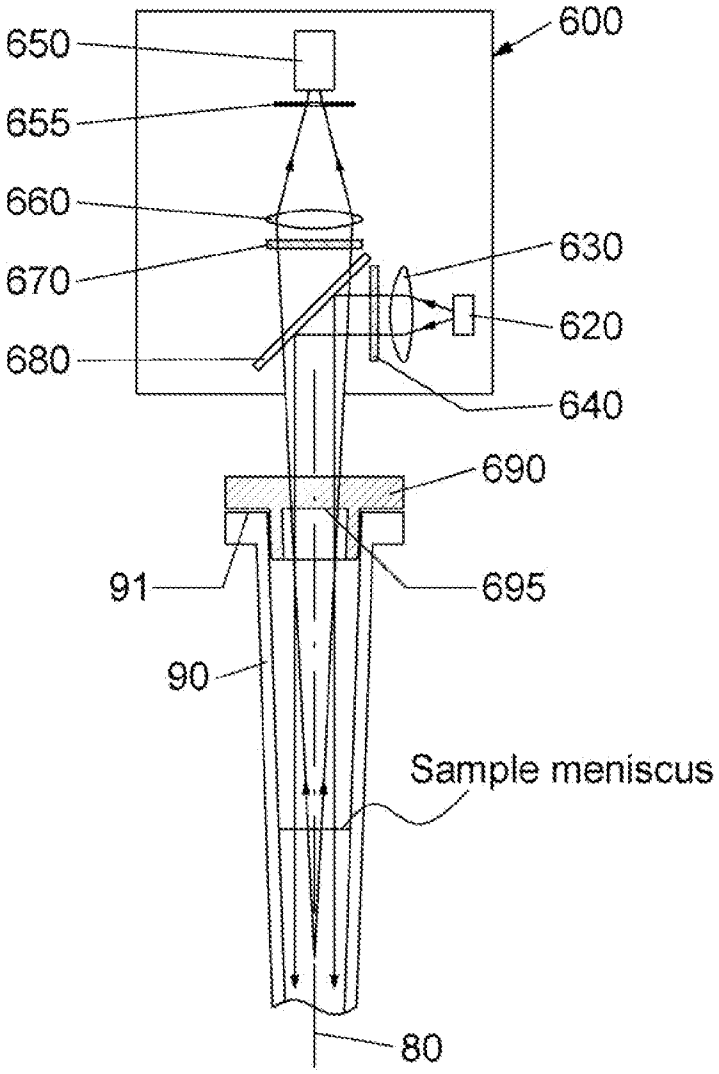
650
600
655
660
630
670
620
680
640
690
91
695
90
Sample meniscus
80

FIG. 86

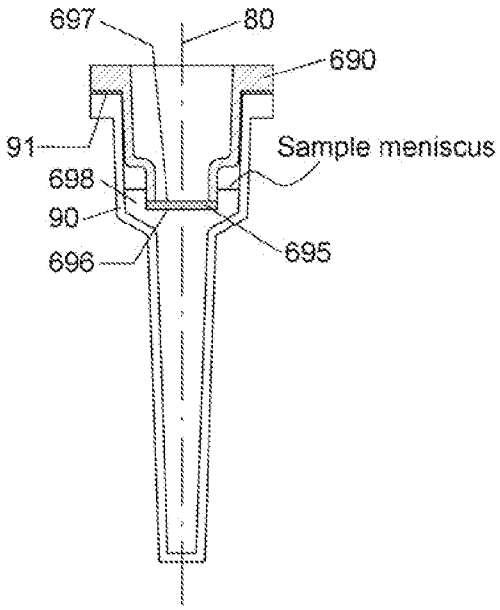


FIG. 87A

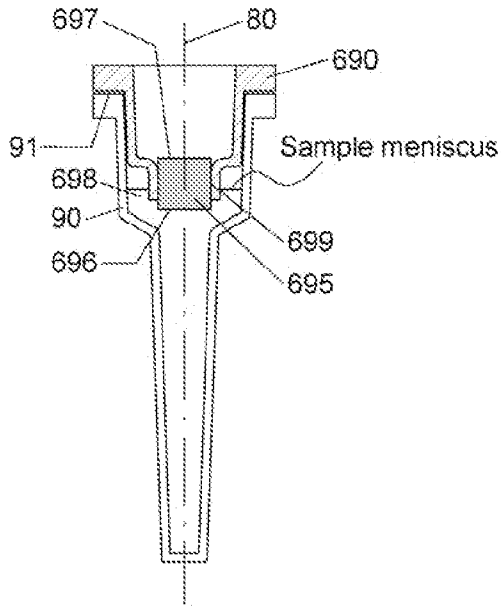


FIG. 87B

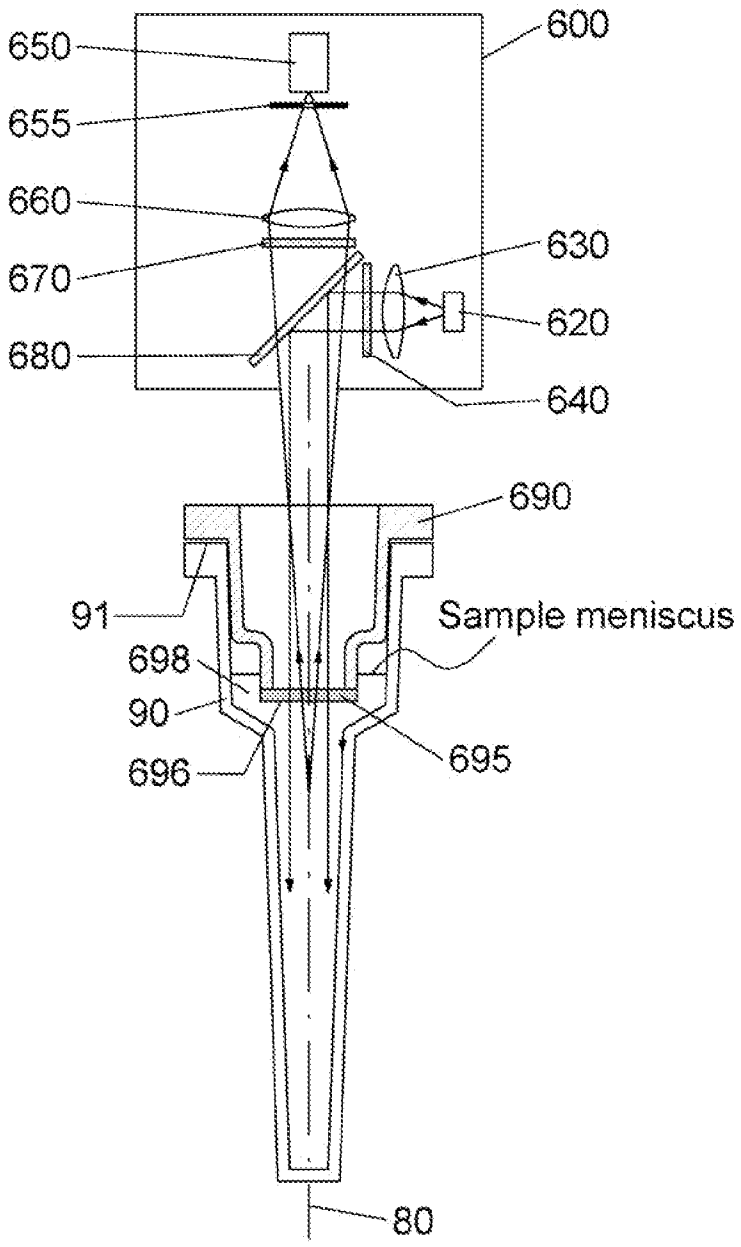
650
600
655
660
630
670
620
680
640
690
91
Sample meniscus
698
90
695
696
80

FIG. 88