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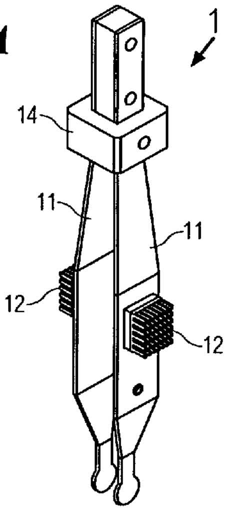
FIG. 1A



(57) **Abstract**: The present invention provides an apparatus for conducting electroporation with minimal thermal effect on a treated sample. The apparatus has an electroporation module for receiving the sample and executing electroporation thereon; and a cooling system for enhancing dissipation of the heat generated by the electroporation module. In one embodiment, the electroporation module comprises a pair of electrodes, and the cooling system has two cooling units, each of which is directly attached to one of the electrode. In another embodiment, the electroporation module comprises an array of electroporation wells accommodated in an enclosure, and the cooling system is designed to thermally control the ambient temperature in the enclosure.

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APPARATUS FOR ELECTROPORATION WITH COOLING SYSTEM

Field of the Invention

[0001] The present invention relates to electroporation technology, in particular to electroporation apparatuses with a cooling system.

Background of the Invention

[0002] Electroporation is a well-established technique for moving exogenous molecules, such as nucleic acids, drugs, and other compounds, across membranes including cell membranes and the membranes that form liposomes and other lipid-encapsulated vesicles. It involves the external application of electric field of suitable strength across a biological sample, for instance, a suspension of cells. While the mechanism by which electroporation functions is not fully understood, it is generally agreed that in context of living cells, the electric field induces reversible/irreversible structural changes in the cell membrane's lipid bilayer, leading to the formation of transient/permanent pores in the membrane that permit exogenous molecules to enter the cell by diffusion. Under optimal electroporation conditions, these pores reseal after a short period of time and cells will recover and continue to grow.

[0003] Electroporation is executed with an electroporator, an apparatus that applies pulses of a high-voltage electric field to the biological sample via a pair of electrodes, namely a cathode and an anode. It is believed that pore formation in the membrane occurs at the cell poles, the sites on the cell membranes that directly face the electrodes and thus experience the highest transmembrane electric field potential. The outcome of pore formation is dependent on energizing parameters such as pulse amplitude, duration, wave form shape, and repetition rate, in addition to the type and inherent nature of the biological sample.

[0004] Although electroporation is able to increase membrane permeability to exogenous molecules, it may also cause detrimental side effects on the cells, which can be extensive and irreversible. Permanent permeabilization of cell membrane due to excessive exposure to the electric field results in changes in cell homeostasis and cell death. In the context of therapeutic gene transfer, such deleterious impact seriously hampers the clinical efficacy, because of the resulting low cell viability.

[0005] One of the contributing factors to the electroporation-induced cell damage is the thermal effect due to Joule heating. Cells are sensitive to the change of temperature. The effects of elevated temperatures on biological materials are reviewed in the reference titled 'Bioengineering Heat Transfer, Advances in Heat Transfer' volume 32, published by Academic Press in 1992. The amount of heat produced during an electroporation procedure is not negligible. Intensive research has been performed to examine the thermal effects and to investigate the relevant parameters during electroporation including electrode geometry, pulse frequency and heat dissipation through the electrodes. See 'Thermal effects during electroporation: theoretical and experimental considerations', Roumanian Biotechnological Letters Vol. 9, No. 2, 2004, pp1587-1590; and 'Theoretical analysis of the thermal effects during in vivo tissue electroporation', Bioelectrochemistry Vol. 61, 2003, pp99-107.

[0006] Aware of the thermal effects associated with electroporation, scientists have made great efforts to optimize the experimental electric pulse parameters in order to maximize the amount of molecules transferred across the cell membrane while minimizing the damage to the cells or tissues. However, little has been done to address the problem by improving the functionality of the conventional electroporator, in particular by speeding up the dissipation of the generated heat.

Summary of the Invention

[0007] The present invention provides an apparatus for electroporation with a thermal cooling system. The apparatus comprises an electroporation module for receiving a sample and executing electroporation thereon; and a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation.

[0008] According to one aspect of the present invention, the electroporation module comprises a pair of spaced electrodes operable to be connected to an energy source, for directing an electric current through the sample. The cooling system comprises a first and second cooling units, each attached to one of the electrodes for conducting heat therefrom and dissipating heat into ambient air.

[0009] In one embodiment, each of the cooling units comprises a solid-state cooling element.

[0010] In another embodiment, the solid-state cooling element is a Peltier element.

[0011] In yet another embodiment, each of the cooling units further comprises a heat sink thermally coupled to the solid-state cooling element for transferring heat away therefrom.

[0012] According to another aspect of the present invention, the apparatus further comprises a base for detachably supporting a chambered slide and the pair of electrodes, whereby the pair of electrodes is operable to execute electroporation on the sample loaded in the chambered slide.

[0013] In one embodiment, the cooling system further comprises a third cooling unit disposed on the base for conducting heat from the supported chambered slide.

[0014] In other embodiment, the apparatus further comprises an adjusting means for controlling the space between the electrodes.

[0015] According to yet another aspect of the present invention, there is provided an apparatus for large-scale electroporation. The apparatus further comprises a housing that defines an enclosure for accommodating the electroporation module. The electroporation module comprises a plurality of wells for receiving and energizing multiple electroporation cuvettes so as to execute electroporation on the sample loaded therein; and an circuit for electronically connecting each well to an external energy source. The cooling system is operable to control the ambient temperature within the enclosure.

[0016] In one embodiment, the housing comprises a first and second side ends in communication with the external air, and a fist and second vertical structures disposed in the housing for creating the enclosure between the first and second side ends. The cooling system comprises a first and second cooling units embedded in the first and second vertical structures respectively.

[0017] In another embodiment, each of the first and second cooling units comprises a solid-state cooling element that has a first surface facing the enclosure for transferring heat away therefrom, and a second surface facing one of the side ends of the housing for dissipating heat to the external air.

[0018] In yet another embodiment, each of the first and second cooling units further comprises a first heat sink having a first end in contact with ambient air in the enclosure and a second end thermally coupled to the first surface of the solid-state cooling element, thereby being operable to transfer heat from the ambient air within the enclosure to the solid-state cooling element; and a second heat sink having a first end in contact with the external air and a second end thermally coupled to the second surface of the solid-state cooling element, thereby being operable to transfer the heat from the solid-state cooling element to the external air.

[0019] In a further embodiment, each of the first and second cooling units further comprises a first radial fan attached to the first end of the first heat sink; and a second radial fan attached to the first end of the second heat sink; both of which are operable to create air convection to facilitate heat exchange.

[0020] In yet another embodiment, the cooling system further comprises a third cooling unit located within the housing and below the electroporation module. In one embodiment, the third cooling unit is a radial fan for creating air convection below the electroporation module.

[0021] The objectives and advantages of the present invention will become apparent from the following detailed description of embodiments thereof in connection with the accompanying drawings.

Brief Description of the Drawings

[0022] Preferred embodiments according to the present invention will now be described with reference to the Figures accompanied herein, in which like reference numerals denote like elements.

[0023] FIG. 1A shows an isometric perspective view of an apparatus for electroporation in accordance with one embodiment of the present invention.

[0024] FIG. 1B shows a cross sectional perspective view of the apparatus in FIG. 1A.

[0025] FIG. 2A shows an assembled view of an apparatus for electroporation in accordance with another embodiment of the present invention.

[0026] FIG. 2B shows an exploded perspective view of the apparatus in FIG. 2A.

[0027] FIG. 2C shows a top plane view of the apparatus in FIG. 2A.

[0028] FIG. 2D shows a cross sectional view of the apparatus in FIG. 2C.

[0029] FIG. 3A shows an assembled perspective view of an apparatus for electroporation in accordance with yet another embodiment of the present invention.

[0030] FIG. 3B shows a partially exploded perspective view of the apparatus in FIG. 3A.

[0031] FIG. 3C shows a top plane view of the apparatus in FIG. 3A.

[0032] FIG. 3D shows a cross section view of the apparatus in FIG. 3C along axis J-J.

[0033] FIG. 4A shows an exploded perspective view of the electroporation array assembly and the first cooling units of the apparatus in FIG. 3A.

[0034] FIG. 4B shows a partial enlarged perspective view of FIG. 4A.

[0035] FIG. 5A shows an enlarged isometric perspective view of one of the cooling units in FIG. 3A.

[0036] FIG. 5B shows an exploded perspective view of the cooling unit in FIG. 5A.

Detailed Description of the Invention

[0037] The present invention may be understood more readily by reference to the following detailed description of certain embodiments of the invention.

[0038] Now referring to FIG. 1, there is provided an apparatus **1** for electroporation, particularly for *in vivo* application, in accordance with one embodiment of the present invention. FIG. 1A shows an isometric perspective view of the apparatus **1**; FIG. 1B a cross sectional perspective view. The apparatus **1** comprises a pair of spaced electrodes **11** for applying electric pulses to a sample positioned therebetween, and a first and second cooling units **12** attached to one of the electrodes **11** respectively for dissipating heat therefrom into ambient environment.

[0039] Each electrode **11** has a stretching body **112**, a first end **114** and a second end **116**. The first ends **114** of electrodes are housed in an electronically insulated holder **14**, and are kept apart by an electrically insulated spacer **15**. Three electronically insulated pins **142** are lodged to fix the electrodes **11**, spacer **15** and holder **14** together for preventing displacement thereof. A corresponding female socket (not shown in FIG. 1A and 1B) with electrical connection outlets is attached onto the distal end of the holder **14** for detachably connecting the electrodes **11** to an external power source via electrical wiring of appropriate length. The second ends **116** of electrodes are operable to be in direct contact with the biological sample, e.g. animal tissues, and are configured to be substantially parallel to one another, so as to create a uniform electric field in between when energized. The bodies **112** of electrodes are coated with an electrical insulating material.

[0040] In one embodiment as shown in FIG. 1A, the second end **116** of each electrode **11** is of a disk shape. In another embodiment, the second end **116** of each electrode **11** is of a needle shape. In yet another embodiment, the second end **116** of each electrode **11** is oval shaped.

[0041] In one embodiment, the distance between the second ends **116** of the electrodes is adjustable.

[0042] As shown in FIG. 1B, the cooling units **12** are attached to the outer surfaces of the electrode bodies **112**, being away from the second ends **116** for a pre-determined distance so as to avoid any contact with the biological sample. Each cooling unit **12** includes a solid-state cooling element **122** and a heat sink **124**. The solid-state cooling element **122** has a first surface in direct contact with the electrode body **112**, and a second surface with the base **124a** of the heat sink **124.** A plurality of fins **124b** extends from the heat sink base **124a** for increasing the heat exchange surface with the ambient environment.

[0043] In one embodiment, the solid-state cooling **122** is a Peltier element, which includes two lead wires (not shown in FIG. 1A and 1B) for electronically connecting to an external power source. In operation, when a DC voltage is applied to the Peltier element **122**, the first surface of the Peltier element will cool to a pre-set temperature, e.g. room temperature, and the second surface will heat. Heat generated in the biological sample during the electroporation process will be transferred to the second end **116** of each electrode **11**, conducted along the electrode body **112** to the coupled cooling unit **12**, where the heat is transformed from the first surface of the Peltier element **122** to the second surface against the temperature gradient, and eventually dissipated to the ambient air via the heat sink **124**. Through instant removal of the generated heat, the detrimental thermal effect of the electroporation on the treated biological sample is minimized.

[0044] It is to be understood that the solid sate cooling element **122** is not limited to Peltier element. Other cooling devices, such as thermotunneling heat pump, may be applicable as well.

[0045] In one embodiment, the cooling units **12** are coupled to the electrodes **11** via heat conducting adhesive means. In another embodiment, the cooling units **12** are fixed to the electrodes **11** via mechanical means such as screws and nuts.

[0046] In yet another embodiment, the cooling units **12** do not have the heat sinks **124** attached to the solid-state cooling elements **122**. Instead, the second surface of each solid-state cooling element **122** is directly exposed to the ambient environment.

[0047] Now referring to FIG. 2, there is provided an apparatus **2** for electroporation, particular for applying electroporation to cells grown in a culture chamber, in accordance with another embodiment of the present invention. FIG. 2A shows an assembled perspective view of the apparatus **2**; FIG. 2B an exploded perspective view; FIG. 2C a top plane view; FIG. 2D a cross sectional view. The apparatus **2** comprises a planar base **24**, a pair of electrodes **22** detachably disposed thereon, adjusting means **25** for controlling the inter-electrode distance, a first and second cooling units **22** attached to one of the electrodes respectively, and a third cooling unit **23** (not shown in FIG. 2A) installed on the base **24**.

[0048] The base **24** is rectangular and dimensioned to house the electrodes **22** and at least one chambered slide **20** simultaneously. A typical chambered slide **20**, as shown in FIG. 2A and 2B, comprises a rectangular glass slide **202**, and two leak-proof cell culture chambers **204** formed thereon. The upper surface of the glass slide **202** within the chamber **204** may be chemically pre-treated to facilitate cell adhesion.

[0049] The base **24** further has a mounting plate **242** fixed thereon for providing an elevated stage, and two vertical supporting structures **244** disposed at two ends of the mounting plate **242** for supporting the adjusting means **25**. The adjusting means **25** comprises a guide rod **252** and a threaded shaft **254**, which are parallel to each other in the horizontal direction. The thread shaft **254** is rotably connected to the vertical supporting structures **244** via bearings **256**. A driving knob **258** is coupled to one end of the shaft **254** for actuating the rotation thereof. The outer thread along the longitudinal axis of the shaft **254** is so configured that a first half of which is in a first direction and a second half of which is in the opposite direction.

[0050] Two electronically insulated holders **246** are arranged to engage the electrodes **21** with the adjusting means **25**. Each holder **246** has a first through hole **246a** for engaging with the guide rod **25**, a second through hole **246b** with inner thread matching with the outer thread on one half of the thread shaft **254**, and a planar top surface **246c** for connecting to and supporting the first end **214** of electrode **21**. The inter-electrode distance is adjustable through controlling linear displacement of the two holders **246** along the longitudinal axes of the threaded shaft **254** and guide rod **252** by rotating the thread shaft **254**.

[0051] The configuration of each electrode **21** is similar to the earlier embodiment shown in FIG. 1, except that the first end **214** is so configured to be detachably mounted onto the top surface **246c** of the holders, and that the second end **216** is so dimensioned as to apply an electric field cross the width and depth of the chambers **204**. A user is able to choose electrodes **21** of optimal configuration, e.g. geometry, size, material, tip surface size, to match with chambers **204** of distinct size and shape.

[0052] Still referring to FIG. 2A and 2B, the first and second cooling units **22** are attached to the outer surfaces of the electrodes **21** respectively, and have identical configurations to the ones **11** in the earlier embodiment shown in FIG. 1. The position of each cooling unit **22** relative to the second end **216** of electrode is so configured that the cooling unit **22** is able to instantly and optimally absorb electroporation-generated heat from the second end **216** of electrode without contaminating the biological sample in the chamber **204**.

[0053] Now referring to FIG. 2B and 2D, a recess **246** is carved in the base **24** at the position for mounting the chambered slide **20**. The third cooling unit **23** is disposed therein, which forms a planar surface with the base **24** to support the chambered slide, and functions to absorb and dissipate heat from the chambered slide **20** during electroporation.

[0054] In one embodiment, the third cooling unit **23** is a Peltier element. In another embodiment, the size of the third cooling unit **23** is the same as the chambered slide **20**.

[0055] Now referring to FIG. 3, there is provided an apparatus **3** for high through put *ex vivo* electroporation, in accordance with another embodiment of the present invention. FIG. 3A shows an assembled view of the apparatus **3**; FIG. 3B a partially exploded perspective view; FIG. 3C a top plane view; FIG. 3D a cross sectional view. The apparatus **3** comprises a housing **36** defining an internal enclosure **362**, an electroporation array assembly **37** disposed in the enclosure **362** for receiving multiple electroporation cuvettes **30** and carrying out electroporation therein; and a plurality of cooling units **32, 33** for controlling ambient temperature in the enclosure **362**.

[0056] The housing **36** is of a rectangular configuration, having two side openings **364** in communication with the surrounding air and a pivotal lid **366** installed in the top surface. Two vertical structures **368** are disposed in the housing **36** for creating the internal enclosure **362** between the two side openings **364** and beneath the lid **366**, where the electroporation array assembly **37** is accommodated. The array assembly **37** has twenty-five sample wells **373** arranged in 5 by 5 grid for holding multiple electroporation cuvettes **30**. One cooling unit **33** is installed within the enclosure **362** and beneath the array assembly **37**, whilst two cooling units **32** are mounted on each vertical structure **368**.

[0057] Now referring to FIG. 4A, there are provided an exploded perspective view of the array assembly **37** and the cooling units **33**. The array assembly **37** comprises five pairs of side blocks **371** and one bottom block **372** to form the sample wells **373** (not shown in FIG. 4A), and an operational circuit **374** (now shown in FIG. 4A) for electronically connecting each sample well **373** to an external power source (not shown in FIG. 4A).

[0058] Each side block **371** has five recesses **375** carved in a row. FIG. 4B provides an enlarged view of one of such recesses **375**. The recess **375** has a leaf spring **376** disposed therein. The leaf spring **376** has a first end **376a** electronically connected to the operational circuit **374** (not shown in FIG. 4B), and a second end **376b** for biasing against the electrode **31** (not shown in FIG. 4B) embedded in the sidewall of the loaded conventional eletroporation cuvette **30** (not shown in FIG. 4B) in order to supply the electric power thereto.

[0059] It is to be understood by a person skilled in the art that the number of sample wells **373** can be varied and scalable depending on the specific application requirements.

[0060] Referring back to FIG. 4A, the operational circuit **374** (not shown in FIG. 4A) is electronically connected to the sample wells **373** by a matrix of link bars **377a** and link rods **377b** that are integrated with the side blocks **371** and bottom blocks **372** via a plurality of tie bars **378a** and lock plates **378b**.

[0061] In accordance with one embodiment, a controller is provided to regulate the operational circuit **374** so that the sample wells can be energized independently from one other. In other embodiment, the controller is operable to direct a series of pulse trains generated by the external energy source to pre-selected sample wells **313** in a pre-determined sequence.

[0062] Now referring back to FIGs. 3B and 4A, the array assembly **37** is supported and elevated by a pair of brackets **365** disposed in the housing **36**, thereby creating a space beneath the bottom block **372**. The cooling unit **33** is installed therein. In one embodiment, the cooling unit **33** is a radial fan, the rotation of which creates an artificially induced air convection within the space to enhance heat dissipation.

[0063] The four cooling units **32** are identical. FIG. 5A and 5B provide an enlarged isometric view and an exploded view of one of the cooling unit **32**. The cooling unit **32** comprises a solid-state cooling element **322**, two heat sinks **324** sandwiching the solid-state cooling element **322**; and two radial fans **326** connected to the heat sinks **324** at distal ends. The configuration of the heat sinks **324** is identical to the one in the earlier embodiments shown in FIG. 1 and FIG. 2.

[0064] Now referring back to FIG. 3D, each solid-state cooling element **322** is embedded in one of the vertical structures **368**, having a first surface **322a** facing towards the enclosure **362** and a second surface **322b** opposite thereto. In one embodiment, the solid-state cooling element **322** is a Peltier element. When electrical current is supplied thereto via two lead wires, heat will be transferred from the first **322a** to the second **322b** surface. Radial fans propel ambient air towards the heat sinks **324** that are in turn thermally coupled to the Peltier elements **322**. In this way, heat generated within the enclosure **362** during electroporation process will be instantly dissipated to the surrounding air outside the housing **36**.

[0065] It is to be understood by a person skilled in the art that the number of cooling units **32** can be varied depending on the specific application requirements.

[0066] While the present invention has been described with reference to particular embodiments, it will be understood that the embodiments are illustrative and that the invention scope is not so limited. Alternative embodiments of the present invention will become apparent to those having ordinary skill in the art to which the present invention pertains. Such alternate embodiments are considered to be encompassed within the spirit and scope of the present invention. Accordingly, the scope of the present invention is described by the appended claims and is supported by the foregoing description.

Claims

1. An apparatus comprising:

an electroporation module for receiving a sample and executing electroporation thereon; and

a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation.

2. The apparatus in accordance with claim 1, wherein the electroporation module comprises a pair of spaced electrodes operable to be connected to an energy source, for directing an electric current through the sample.

3. The apparatus in accordance with claim 2, wherein the cooling system comprises a first and second cooling units, each attached to one of the electrodes for conducting heat therefrom and dissipating heat into ambient air.

4. The apparatus in accordance with claim 3, wherein each of the cooling units comprises a solid-state cooling element.

5. The apparatus in accordance with claim 4, wherein the solid-state cooling element is a Peltier element.

6. The apparatus in accordance with claim 4, wherein each of the cooling units further comprises a heat sink thermally coupled to the solid-state cooling element for transferring heat away therefrom.

7. The apparatus in accordance with claim 2, wherein each of the electrodes comprises:

a first end electronically connected to the energy source;

a body covered with electrically insulated coating; and a second end in contact with the sample for directing the electric current therethrough,

wherein each of the cooling units is attached to the body at a pre-determined distance from the second end, so as to optimally conduct heat without contaminating the sample.

8. The apparatus in accordance with claim 2, wherein the space between the electrodes is adjustable.

9. The apparatus in accordance with claim 3, which further comprises:

a base for detachably supporting a chambered slide and the pair of electrodes, whereby the pair of electrodes are operable to execute electroporation on the sample loaded in the chambered slide.

10. The apparatus in accordance with claim 9, wherein the cooling system further comprises a third cooling unit disposed on the base for conducting heat from the supported chambered slide.

11. The apparatus in accordance with claim 10, wherein the base has a recess for housing the third cooling unit; and

wherein the third cooling unit is so dimensioned to form a planar supporting surface with the base for supporting the chambered slide, and so positioned to be beneath the chambered slide.

12. The apparatus in accordance with claim 10, wherein the third cooling unit comprises a solid-state cooling element.

13. The apparatus in accordance with claim 12, wherein the solid-state cooling element is a Peltier element.

14. The apparatus in accordance with claim 9, which further comprises an adjusting means for controlling the space between the electrodes, the adjusting means having:

a first and second support structures mounted on the base,

a threaded shaft and a guide rod, both being supported by the first and second support structures, and aligned with each other in the horizontal direction; wherein the threaded shaft has a first half that is threaded in a first direction and a second half that is threaded in a second direction; and

a first and second holders for releasably holding the pair of electrodes in parallel, wherein each holder has a first bore that is internally threaded so as to engage with and maneuver on one half of the threaded shaft, and a second bore for engaging with the guide rod.

15. The apparatus in accordance with claim 1, which further has a housing that defines an enclosure for accommodating the electroporation module; and

wherein the cooling system is operable to control the ambient temperature within the enclosure.

16. The apparatus in accordance with claim 15,

wherein the housing comprises

a first and second side ends in communication with the external air; and

a fist and second vertical structures disposed in the housing for creating the enclosure between the first and second side ends; and

wherein the cooling system comprises:

a first and second cooling units embedded in the first and second vertical structures respectively.

17. The apparatus in accordance with claim 16, wherein each of the first and second cooling units comprises a solid-state cooling element that has a first surface facing the enclosure for transferring heat away therefrom, and a second surface facing one of the side ends of the housing for dissipating heat to the external air.

18. The apparatus in accordance with claim 17, wherein each of the solid-state cooling elements is a Peltier element.

19. The apparatus in accordance with claim 17, wherein each of the first and second cooling units further comprises:

a first heat sink having a first end in contact with ambient air in the enclosure and a second end thermally coupled to the first surface of the solid-state cooling element, thereby being operable to transfer heat from the ambient air within the enclosure to the solid-state cooling element; and

a second heat sink having a first end in contact with the external air and a second end thermally coupled to the second surface of the solid-state cooling element, thereby being operable to transfer the heat from the solid-state cooling element to the external air.

20. The apparatus in accordance with claim 19, wherein each of the first and second cooling units further comprises:

a first radial fan attached to the first end of the first heat sink; and

a second radial fan attached to the first end of the second heat sink;

both of which are operable to create air convection to facilitate heat exchange.

21. The apparatus in accordance with claim 13, wherein the cooling system comprises a third cooling unit located within the housing and below the electroporation module.

22. The apparatus in accordance with claim 21, wherein the third cooling unit is a radial fan for creating air convection below the electroporation module.

23. The apparatus in accordance with claim 13, wherein the electroporation module comprises:

a plurality of wells for receiving and energizing multiple electroporation cuvettes so as to execute electroporation on the sample loaded therein; and

an circuit for electronically connecting each well to an external energy source.

24. The apparatus in accordance with claim 23, which further comprises a controller for regulating the circuit so that each well is operable to be independently energized with pre-set electric parameters and in a pre-determined order.

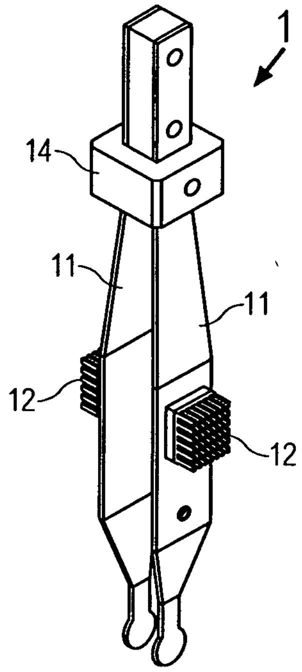
1
14
11
11
12
12

FIG. 1A

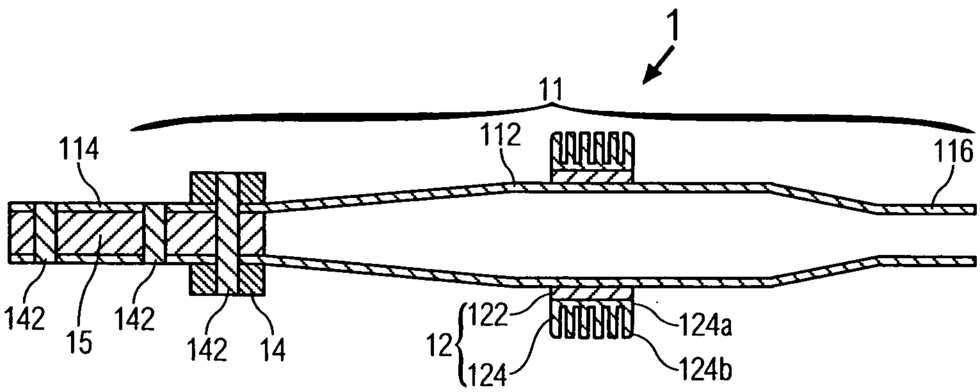
1
11
114
112
116
142
15
142
142
14
12
122
124
124a
124b

FIG. 1B

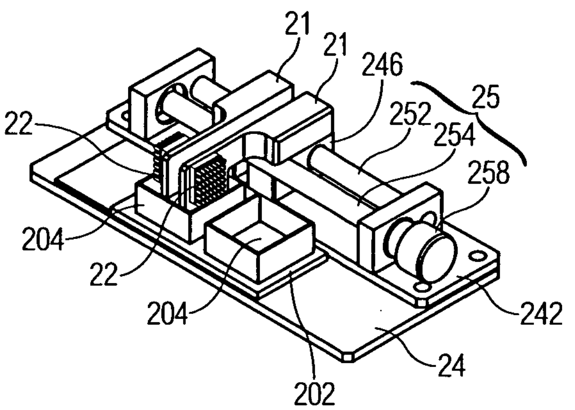
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FIG. 2A

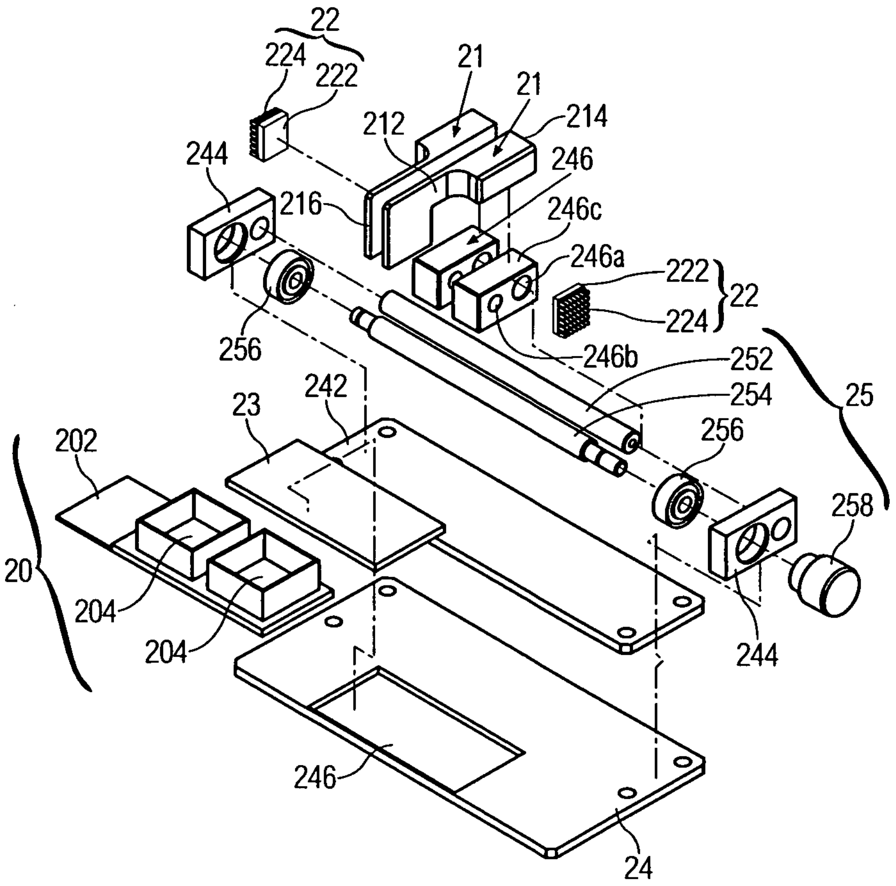
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FIG. 2B

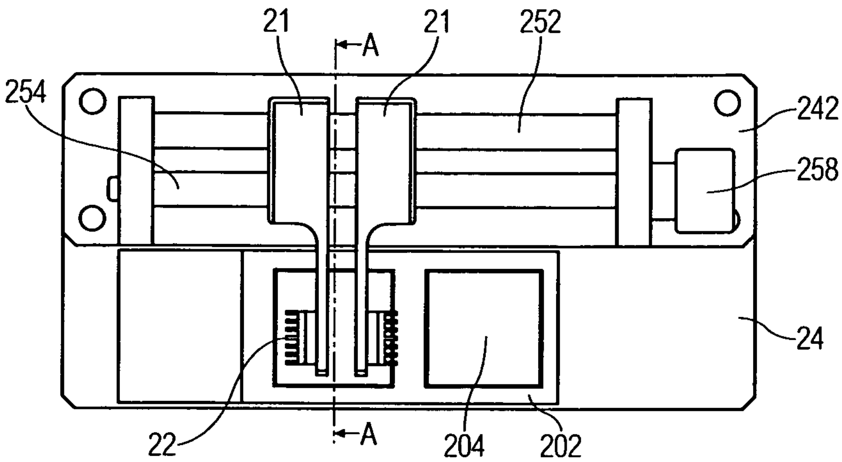

FIG. 2C

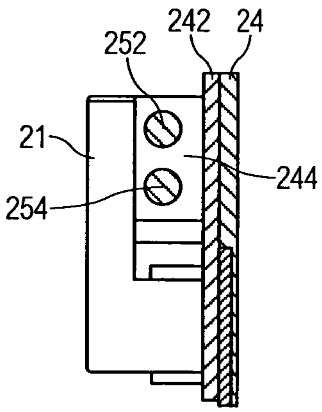

FIG. 2D

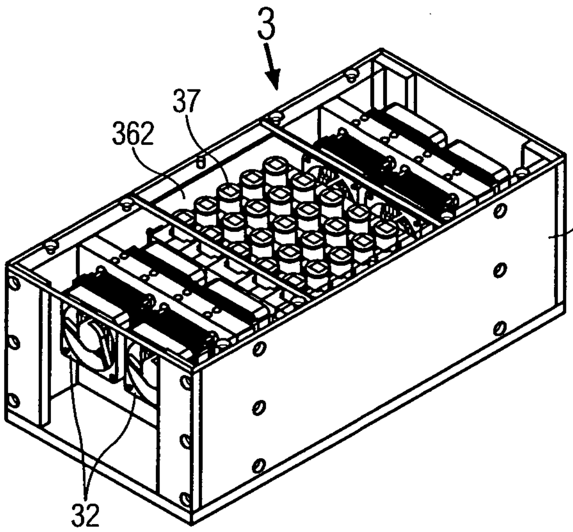
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FIG. 3A

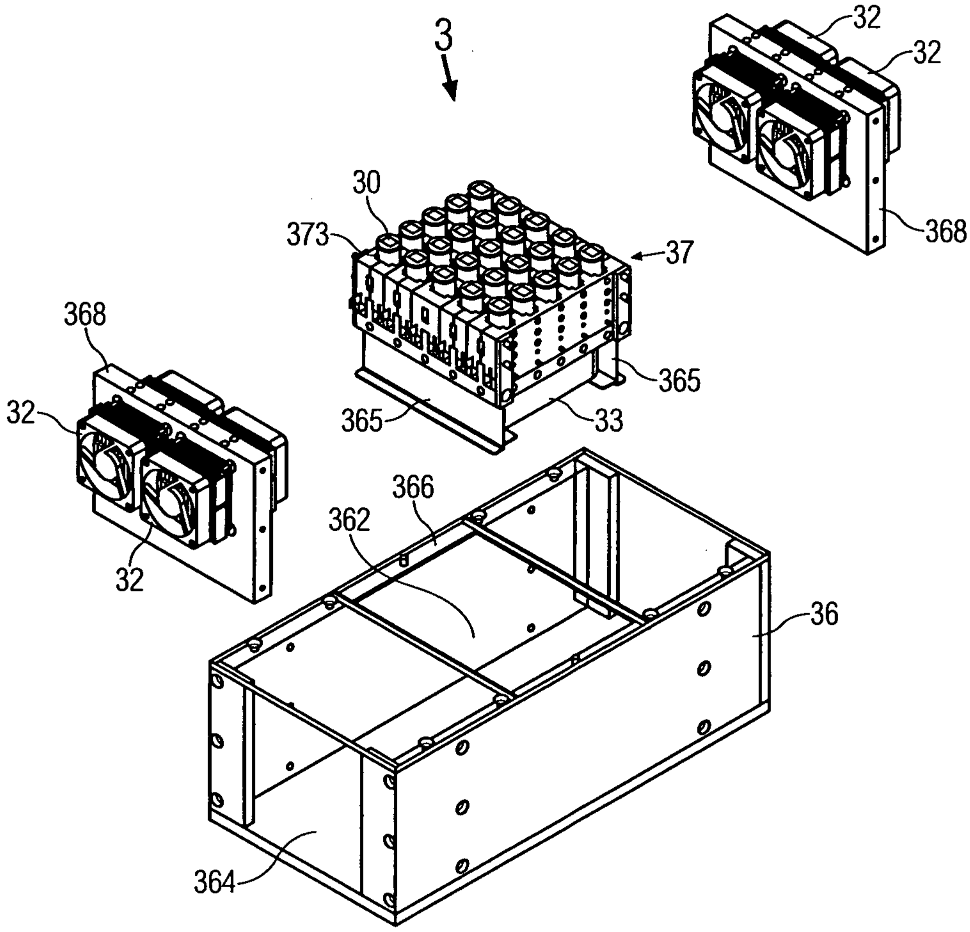
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FIG. 3B

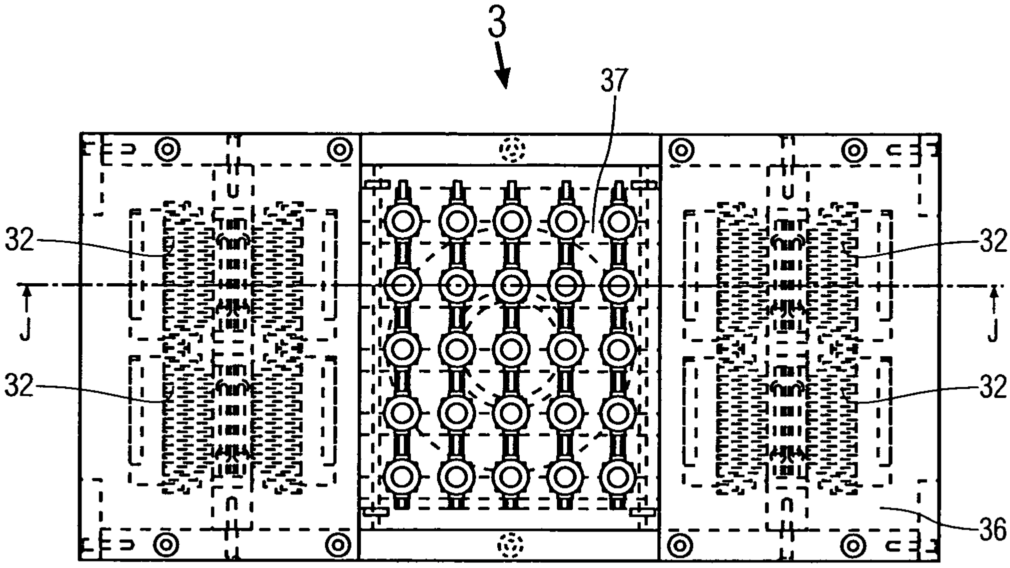
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FIG. 3C

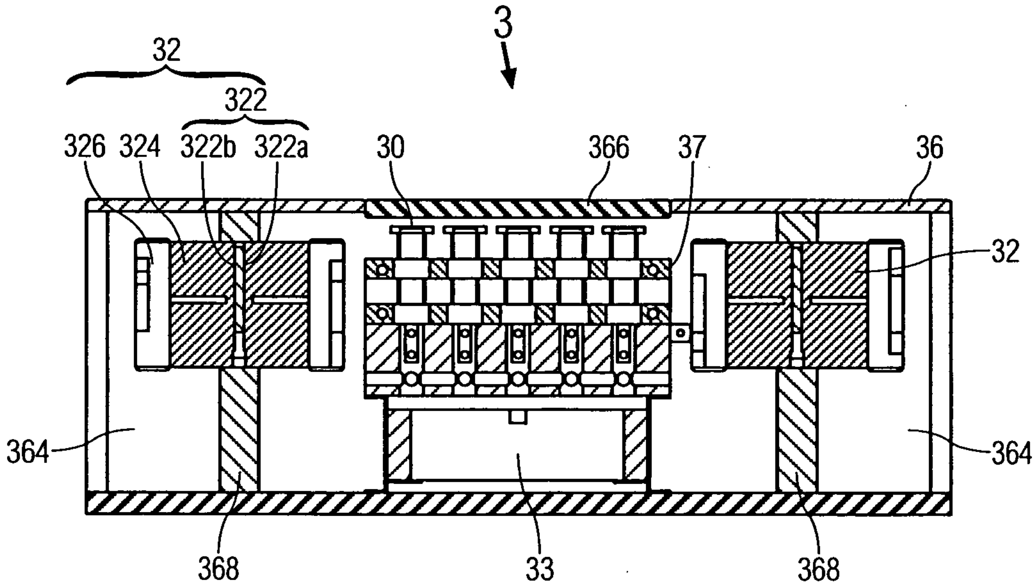
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FIG. 3D

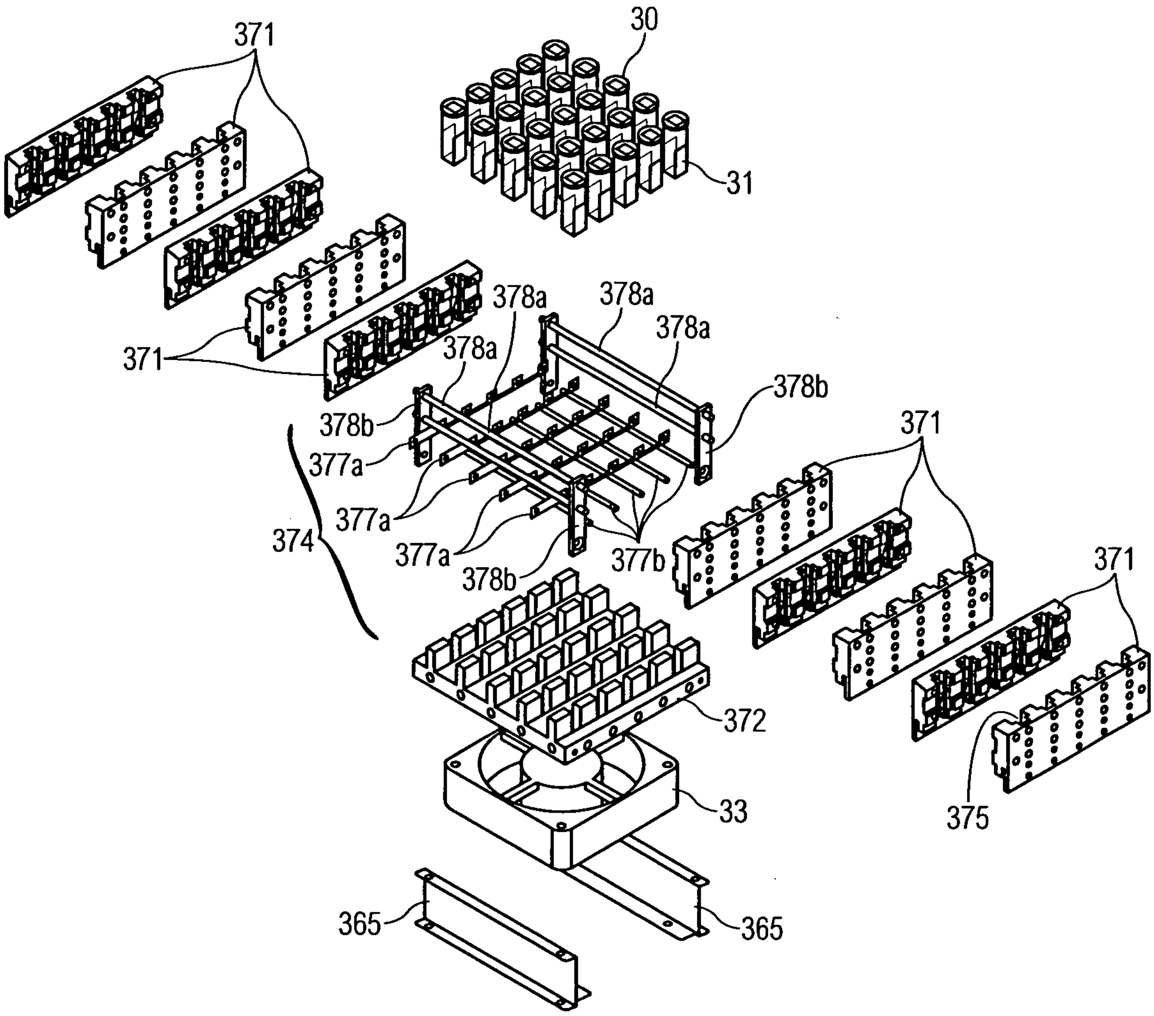
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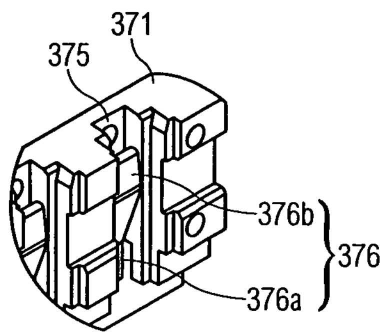
FIG. 4A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376a

FIG. 4B

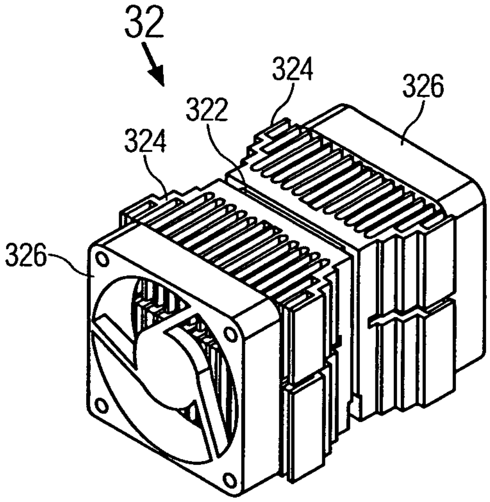

FIG. 5A

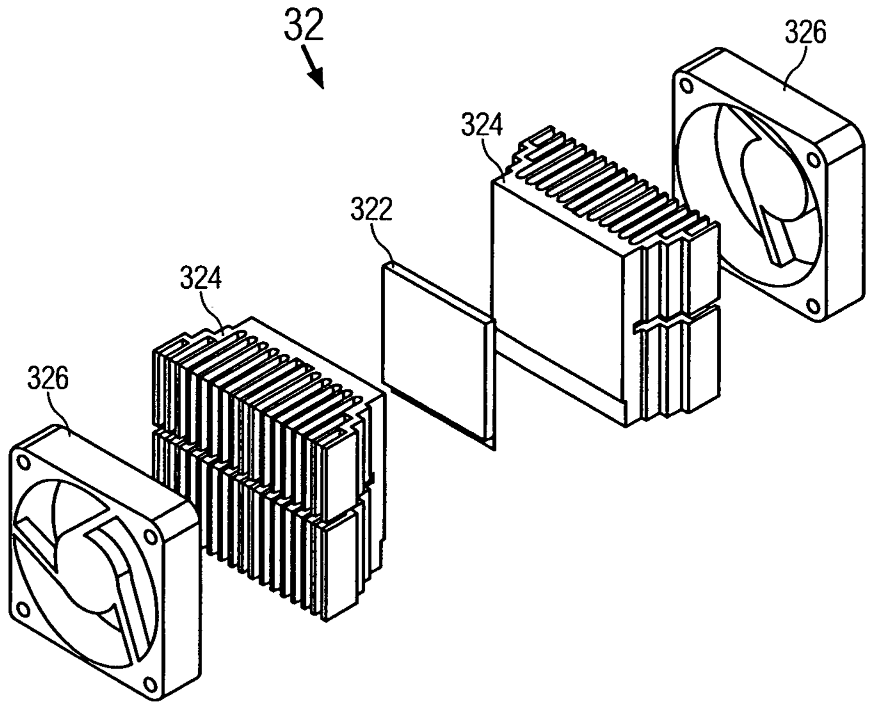

FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2008/000108

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61N 1/30 (2006.01)	*C12N 15/87* (2006.01)	*C12M 1/42* (2006.01)	*C12N 13/00* (2006.01)
A61N 1/18 (2006.01)	*C12N 1/12* (2006.01)	*A61M 5/00* (2006.01)	*C12N 1/21* (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

DWPI and IPC A 61N, C12N 13-, C12M, A61M 5- and keywords and phrases (electroporation, electroporesis, electroinjection, electro transfection, electrotransfection, electropermeabilization, temperature, heat, thermal, decrease, low, diminish, dissipate, away, manage, cool, cooling, cold, frozen, ice, Peltier, solid state, sink, heat sink, fan) and the like.

ESP@cenet and similar keywords and phrases. 'PubMed' and 'Google' search engine with keywords electroporation and cooling. USPTO via 'Google Patents' search engine with similar keywords.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2000/023563 A1 (GENETRONICS, INC.) 27 April 2000 Abstract; Figures 1A – 1F and Figures 2A-4; elements (40), (42), (44), (56), (58), (120), (122), pages 8 to 17; Figures 8-11 and related text on page 25.	1-9, 15, 16-19

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 May 2008	12 MAY 2008

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2003/018751 A2 (MAXCYTE, INC.) 06 March 2003 Abstract; Figures 1-4 and related text; Figure 13 –and related text page 52, line 13-page 53, line 15; Figure 14; see also page 57; see page 62, lines 30-page 66, line 25; Table 2 'ELECTRICAL COMPONENTS' –'PELTIER COOLER WITH FAN,' Figure 7;– see pages 34-67.	1-4, 8-9, 15
X	WO 2003/095019 A2 (PRODUCTION TECHNOLOGY CORPORATION) 20 November 2003 Para [0005]-[0029], esp. para[0015]-[0016]; Figures 1-5; Figure 10 and related text; para [0096]-[0099]; a cooler (92); a temperature sensor (88); a temperature controller (90).	1-4, 8-9, 15
X	WO 2004/031353 A2 (MAXCYTE INC.) 15 April 2004 Figures 1- 4, 4A-B, especially Figure 3, electrodes (12), (14); a cooling element (17); gap spacers (18), (20); walls (22), (24), insulation walls (28), (30), Pages 16, line 24-page 18, line 25.	1-3, 8, 15
X	US 7029916 B2 (DZEKUNOV ET AL.) 18 April 2006 Abstract, Table 2 and related cooling methods and elements, esp. part named 'PELTIER COOLER WITH FAN,' Figures 6-7, columns 25-28; column 33, line 54-column 40, line 52.	1-4, 8-9, 15
X	US 5720921 A (MESEROL) 24 February 1998 Abstract, Figures 1-2, Figures 4-5, Figures 9-10, Figures 14-16 and related text, especially column 16, lines 41- column 18, line 32, esp. column 18, lines 4-12; column 21, line 63-66; column 22, line 24-47; column 23, line 35-52.	1-2, 15
X	Becker S.M. and Kuznetsov A.V.'Thermal damage reduction associated with *in vivo* skin electroporation: A numerical investigation justifying aggressive pre-cooling,' International Journal of Heat and Mass Transfer, 2007, Vol. (50), Issues 1-2, pp. 105-116. Abstract; Figure 1 and parts 'Model Geometry,' 'Mathematical model,' 'Results and Discussion,' esp. 'Thermal solution' esp. Figure 10 and related text; Figures 3-9, 11-12, part 'Conclusions. '	1-2, 15
X	WO 2005/0100540 A1 (MOLECULAR TRANSFER, INC.) 27 October 2005 Abstract, Figures 1, 2A, 2B, 3-6; para[0008]-para[0010]; para[0019]-para[0030].	1-2

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	0023563	AU	62666/99	CA	2344325	CN	1324398
		EP	1121416	US	6150148	US	2002068338
WO	03018751	CA	2459697	EP	1456345	US	7141425
		US	7186559	US	2003073238	US	2004197883
WO	03095019	AU	2003239443	US	2004029240		
WO	2004031353	AU	2003277109	CA	2497649	EP	1565555
		US	2004115784				
US	7029916	US	2003059945				
US	5720921	AU	53050/96	CA	2214800	CA	2363548
		CN	1195997	EP	0814855	JP	2007007430
		US	6074605	US	6773669	US	2005019311
		WO	9628199				
WO	20050100540	NONE					

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX