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Nanopore device and a method for nucleic acid analysis

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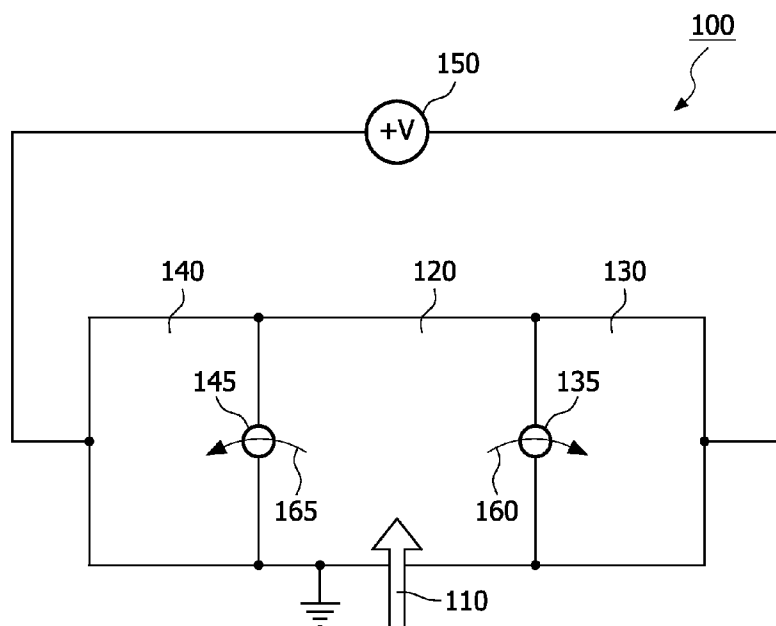


FIG. 1

(57) Abstract: A nanopore device is described wherein is provided a sample input (110), an input chamber (120), and first and second sample chambers (130, 140) connected to the input chambers (120) via first and second nanopores (135, 145).

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Nanopore device and a method for nucleic acid analysis

FIELD OF THE INVENTION

The invention relates to the field of determining information from nucleic acid material, for example genetic material such as DNA. In particular, the invention relates to the field of rapid nucleic acid analysis by means of a nanopore device.

- 5 Specifically the invention relates to a nanopore device, arranged to cooperate with a sample of genetic material, the nanopore device comprising
- an input chamber, for containment of the sample of nucleic acid
 - a first chamber connected to the input chamber by a first nanopore
 - means to apply an electrical potential difference across the first nanopore, the
- 10 electrical potential being arranged to draw a first fragment of the sample of genetic material through the first nanopore
- an electrolytic solution which is capable of flow through the first nanopore under the influence of the electrical potential difference, the flow detectable as a first ionic current, changes in the first ionic current being indicative of detection of a first target
- 15 molecule by the first nanopore

BACKGROUND OF THE INVENTION

The structure and composition of nucleic acid material, including genetic material, especially gene sequencing and the identification of genetic markers associated with

20 specific disease, is becoming more important. Within biomedical and pharmaceutical industries and associated research, more emphasis is being placed on accurate and speedy gathering of information. In particular the genetic patterns connected with disease or reactions to medications should be accessed quickly with an aim to provide genetic information for point-of-care applications. Methods and devices are being developed to

25 facilitate this.

It has been discovered that biosensor elements can be capable of identifying individual DNA strands with single base resolution, a base being one of four possible types of molecules (A, C, G or T) used to encode information in the DNA. In particular, so-called nanopores are utilized. These are apertures in a thin layer, typically 100nm or smaller,

formed in many possible geometrical shapes. Segments of the genetic material to be sequenced are passed through or forced through the aperture. The shape of the samples or the temporary binding of the molecules of the samples with the molecules of the nanopore cause registration in the detection device characteristic of the various bases, thereby
5 facilitating sequencing. A nanopore may be biological or synthetic in origin. Each nanopore is specific for a desired detection and can therefore be made into a detector for a specific gene sequence, such as that associated with a particular cancer mutation, or for an SNP (single nucleotide polymorphism), for example associated with a drug response in anaesthesia or chemotherapy. The use of nanopores allows rapid detection compared with
10 current sequencing devices.

US patent application 2007/0054276 discusses polynucleotide analysis systems and methods of nanopore analysis, and how to rapidly determine the sequence of a nucleic acid molecule for identifying genetic mutations and polymorphisms. It discloses the concept of nanopore sequencing as based in the property of physically sensing the individual
15 nucleotides or physical changes in the environment of the nucleotides (e.g. electric current) within an individual polynucleotide as it traverses through a nanopore aperture. The SNP can be identified using the nanopore analysis system to measure an electronic signature (e.g. ion current or tunneling current) of the modified target polynucleotides, the electronic signatures of modified and non-modified molecules being distinguishable. The nanopore detection
20 system used comprises electronic equipment capable of measuring the electronic characteristics of the interaction between nanopore aperture in a structure of the nanopore detection system and the polynucleotide. A computer system controls the electronic measurement and handles the data produced. Volume, shape or charges on each monomer can affect conductance in a characteristic way. A voltage gradient is applied to the nanopore
25 device to draw the target polynucleotide from one side of the aperture to the other.

US patent application 2007/0048745, from the same applicant, further discloses devices, systems and methods for nanopore analysis of polymers.

Papers in J. Amer. Chem. Soc. 128 (2006) 1705 and Nature Biotech 19 (2001) 636, disclose the use of biological nanopores for sequence specific detection of individual
30 DNA strands using engineered nanopores. Binding of the DNA strand molecules to molecules in the nanopore causes changes in an ionic current flowing through the nanopore.

Present work focuses on a nanopore detection and readout for a specific gene sequence. The devices make only one specific measurement on a sample.

A problem with these current devices and methods is that acquiring information for more than one target sequence is slow, requiring repetitive measurements.

It is thus an object of the invention to provide an improved device which can provide more sequencing information, faster.

5

SUMMARY OF THE INVENTION

According to the invention this object is achieved by provision of

- a second chamber connected to the input chamber by a second nanopore

- the electrical potential difference being applied across the second nanopore

10 equal to, and common with, the electrical potential difference across the first nanopore,

- the electrical potential across the second nanopore being arranged to draw a

second fragment of the sample of nucleic acid through the second nanopore and to influence the electrolytic solution to flow through the second nanopore,

- the flow detectable as a second ionic current, changes in the second ionic

15 current being indicative of detection of a second target molecule by the second nanopore.

The invention allows for incorporation of a second chamber into the nanopore device. This allows two measurements to be performed simultaneously thereby obtaining sequencing information faster.

An additional advantage of this invention is that the second nanopore receives
20 input fragments from the same input chamber as the first nanopore, therefore the samples being processed by the first and second nanopores are from the same source and have no aging effects or other processing differences which may influence the sequencing results.

Examples of possible samples input to the device are polynucleotides such as DNA, RNA or uRNA. In certain instances, such as with biopsy samples, it is very disadvantageous if the

25 sample must be split in two halves in order to run two different sequencing detections. There is the possibility that the different components of the sample may not be properly distributed between the two halves. A single sample input for both measurements removes such inaccuracies. The nucleic acid sample is not, however, restricted to natural nucleic acids. Synthetic or artificial nucleic acids may also be used as samples.

30

Yet another advantage of this invention is that the electrical potential difference being applied across the first and second nanopores is the same. This consistency improves the functioning of the device and the reliability of the measurements obtained. The fragments passing through each nanopore are subject to the same forces and the electrolytic

solution is drawn through the nanopores at the same rate under electrical field with similar properties.

The electrical potential difference may be applied from the same voltage supply or different voltage supplies. The voltage supply may be internal or external to the device.

The invention is described in terms of a first and a second chamber, but is not limited to two chambers as there could be a third, fourth, fifth or nth number of chambers connected to the input chamber via a third, fourth, fifth or nth number of nanopores, all made subject to the same electrical potential.

The nanopores may be biological or synthetic. Each nanopore is by definition designed to detect specific target molecules. (For example the paper by Howorka, Cheley and Bayley in Nature Biotech 19 (2001) 636 discusses how binding of single stranded DNA (ssDNA) molecules to tethered DNA stand is achieved by covalent attachment within the lumen of an alpha-hemolysin pore of an individual DNA oligonucleotide). The active detection component of the nanopore may, for example, be a natural or an artificial nucleic acid. In the invention, however, the nanopores used in the various chambers may be the same as each other, completely different from each other or a weighted distribution of specific nanopores for measurements dependent on the application for which the nanopore device is used.

Biological nanopores and synthetic nanopores operate in different ways to alter the flow of an ionic current, changes in which register the detection of the presence of target molecules in sample fragments. Biological nanopores temporarily close on detection of a target molecule due to bonding between the sample fragment and the nanopore detection molecules. The bonding stops the flow of electrolytic solution through the nanopore thereby halting the flow of charge and therefore the ionic current. Synthetic nanopores are typically formed by an etched hole in a substrate, such as silicon, which becomes blocked due to the physical presence of an attached molecule only present when a target molecule is detected. Biological pores have proved useful for a range of experiments but exhibit some disadvantages such as fixed size and limited stability. External factors, such as temperature and stress can trigger this instability. Fabrication of nanopores from solid state materials allows greater control over diameter and channel length of the nanopore, and surface properties.

The connection between the input chamber and a first chamber is described above as a single nanopore but it should be understood that a connection made by a plurality

of nanopores in parallel, all with equal access to the input chamber sample fragments, should not be excluded.

The nanopore device according to the invention is arranged to cooperate with a sample of nucleic acid. However, the nanopore device is also capable of cooperating with samples of other materials including, but not limited to, proteins, peptides, sugars, glycolipids, lipids and synthetic polymers or molecules.

A "sample" in the context of the present invention may originate from a biological source. Encompassed are biological fluids such as lymph, urine, cerebral fluid, bronchoalveolar lavage fluid (BAL), blood, saliva, serum, faeces or semen. Also encompassed are tissues, such as epithelium tissue, connective tissue, bones, muscle tissue such as visceral or smooth muscle and skeletal muscle, nervous tissue, bone marrow, cartilage, skin, mucosa or hair.

A "sample" in the context of the present invention may also be a sample originating from an environmental source, such as a plant sample, a water sample, a soil sample, or may be originating from a household or industrial source or may also be a food or beverage sample.

A "sample" in the context of the present invention may also be a sample originating from a biochemical or chemical reaction or a sample originating from a pharmaceutical, chemical, or biochemical composition.

The amount of sample is preferably 1000 μ l or less, more preferably 500 μ l or less, even more preferably 100 μ l or less, most preferably 50 μ l or less.

Where appropriate, as for instance in the case of solid samples or viscous suspensions, the sample may need to be solubilized, homogenized, or extracted with a solvent prior to use in the present invention in order to obtain a liquid sample. A liquid sample hereby may be a solution or suspension.

Liquid samples may be subjected to one or more pre-treatments prior to use in the present invention. Such pre-treatments include, but are not limited to dilution, filtration, centrifugation, pre-concentration, sedimentation, dialysis, lysis, elution, extraction and precipitation.

Pre-treatments may also include the addition of chemical or biochemical substances to the solution, such as acids, bases, buffers, salts, solvents, reactive dyes, detergents, emulsifiers, chelators, enzymes, chaotropic agents.

The nanopore detection device may be applied in many circumstances but in particular is useful for:

- quantitative detection of a known sequence, so-called “PCR-less” detection (PCR being a polymerase chain reaction), where the data of interest is the amount of a certain nucleotide sequence or gene present and the nanopore is setup to detect this specific sequence.

5 - SNP (single nucleotide polymorphism) detection, where the nanopores are arranged to detect mutations in a sequence

- sequencing where the end goal is to determine the base pair sequence of a nucleic acid.

In a further embodiment of the invention, the nanopore device further
10 comprises a third chamber connected to the input chamber by a third nanopore, an electrical potential difference being applied across the third nanopore equal to, and common with, the electrical potential difference across the first nanopore, the electrical potential across the third nanopore being arranged to draw a third fragment of the sample of nucleic acid through the
15 the flow detectable as a third ionic current by the current detector, changes in the third ionic current being indicative of detection of a control target molecule for validating device measurement.

For some types of sample fragments, certain sequences are either always present or never present. To measure such sequences, or confirm their absence, gives a
20 positive or negative indicator to support the other measurements taking place on the sample fragments. This improves the quality of the overall measurement result.

In a further embodiment of the invention, a correlating means is arranged to correlate detections indicated by changes in at least two of the ionic currents.

Each ionic current is representative of detection of a specific target molecule.
25 The nanopore device may be arranged to detect several different target molecules using different nanopores. However, the detection of individual molecules may be more relevant or interesting if these detections occur in relation to detections of another molecule. Thus by correlating the changes in the ionic currents, extra information may be extracted.

An example of this would be the correlation over time between changes in a
30 first and second ionic current to determine the frequency of occurrence of mutations as compared to normal genetic sequence. This would be relevant in the field of oncology, for example.

The correlating means could also be used to compare detection of positive or negative indicators (for example the third ionic current) with sample detections (for example the first and/or second ionic currents) to confirm valid device functioning.

In a further embodiment of the invention, the first target molecule is a selected wild type gene sequence and the second target molecule is a specific mutation in the same gene sequence, for relative detection of the statistical occurrence of the mutation.

This is a particular embodiment of the device directed to the study of two types of DNA which is important for the development of new strategies in cancer therapies. The basic sample to be analysed is comprised of at least two types of DNA, wild and mutated. The mutated sample may comprise mutations such as SNP (single nucleotide polymorphisms), deletion, or insertion of nucleotides. The device of the invention can be used to perform a simple quantitative detection of the balance at which these species occur in the sample. The extent to which a specific mutated gene (e.g. a tumour suppressor gene or oncogene) occurs in the population of cancer cells can be analysed. This could be a very important diagnostic tool in determining therapeutic decisions and allow personalised treatment – for example the mutated gene could be a proven drug target, or resection margins in surgery free from cancerous cells would be determined.

Specifically, when combined with correlation of detections of first and second target molecules, wild and mutated DNA respectively, 10 binding events for wild type sample fragment could correspond to only 3 binding events for mutated sample fragment. Investigation of this information by correlating detections over a time period of for example 1 hour, would then prompt the conclusion that 3/13 or approximately 23% of the sample studied contains defective mutated material. This figure would be relevant for a clinical decision.

In a further embodiment of the invention, at least one of the nanopores is a biological nanopore.

In a further embodiment of the invention, the biological nanopore is arranged in cooperation with a lipid bilayer.

A method based on biological nanopores according to the invention, would be applicable to a small sequencing device for simple, rapid and inexpensive mutation detection, particularly at point of care.

In another aspect of the invention, there is provided a method of manufacturing a nanopore device comprising the steps of :

- providing an input chamber for containing a sample of nucleic acid

- providing a first chamber connected to the input chamber by a first nanopore
- providing an electrolytic solution capable of flow through the first nanopore under the influence of an electric potential difference

5 nanopore

- providing a second chamber connected to the input chamber by means of a second nanopore and in that

- arranging the means to apply the electrical potential difference across the second nanopore equal to and common with the electrical potential across the first nanopore.

10 The invention allows for incorporation of a second chamber into the nanopore device. This allows two measurements to be performed simultaneously thereby obtaining sequencing information faster.

An advantage of this invention is that the electrical potential difference being applied across the first and second nanopores is the same. This consistency improves the functioning of the device and the reliability of the measurements obtained. The fragments passing through each nanopore are subject to the same forces and the electrolytic solution is drawn through the nanopores at the same rate under electrical field with similar properties.

15 The electrical potential difference may be applied from the same voltage supply or different voltage supplies. The voltage supply may be internal or external to the device.

The invention is described in terms of a first and a second chamber, but is not limited to two chambers as there could be a third, fourth, fifth or nth number of chambers connected to the input chamber via a third, fourth, fifth or nth number of nanopores, all made subject to the same electrical potential.

25 The nanopores may be biological or synthetic. Each nanopore is by definition designed to detect specific target molecules. The active detection component of the nanopore may, for example, be a natural or an artificial nucleic acid. In the invention, however, the nanopores used in the various chambers may be the same as each other, completely different from each other or a weighted distribution of specific nanopores for measurements dependent on the application for which the nanopore device is used.

30 Biological pores have proved useful for a range of experiments but exhibit some disadvantages such as fixed size and limited stability. External factors, such as temperature and stress can trigger this instability. Fabrication of nanopores from solid state

materials allows greater control over diameter and channel length of the nanopore, and surface properties.

The connection between the input chamber and a first chamber is described above as a single nanopore but it should be understood that a connection made by a plurality of nanopores in parallel, all with equal access to the input chamber sample fragments, should not be excluded.

In another aspect of the invention, there is provided a method of using a nanopore device wherein a wild gene sequence is detected by means of the first nanopore and a specific mutation of said gene sequence is detected by means of the second nanopore

This is a particular embodiment of the device directed to the study of two types of DNA which is important for the development of new strategies in cancer therapies. The basic sample to be analysed is comprised of two types of DNA, wild and mutated, and the device of the invention can be used to perform a simple quantitative detection of the balance at which these species occur in the sample. The extent to which a specific mutated gene (e.g. a tumour suppressor gene or oncogene) occurs in the population of cancer cells can be analysed. This could be a very important diagnostic tool in determining therapeutic decisions and allow personalised treatment – for example the mutated gene could be a proven drug target, or resection margins in surgery free from cancerous cells would be determined.

In another aspect of the invention there is provided a method of correlating detections indicated by changes in at least one of the first or second ionic currents and the third ionic current to generate a signal indicative of a valid device measurement.

The correlating means could also be used to compare detection of positive or negative indicators (for example the third ionic current) with sample detections (for example the first and/or second ionic currents) to confirm valid device functioning.

For some types of sample fragments, certain sequences are either always present or never present. To measure such sequences, or confirm their absence, gives a positive or negative indicator to support the other measurements taking place on the sample fragments. This improves the quality of the overall measurement result.

In another aspect of the invention, there is provided a method of correlating detection indicated by changes in at least two of the ionic currents to generate a comparative relation between ionic currents.

An example of this would be the correlation over time between changes in a first and second ionic current to determine the frequency of occurrence of mutations as

compared to normal genetic sequence. This would be relevant in the field of oncology, for example.

As an example, when combined with correlation of detections of first and second target molecules, wild and mutated DNA respectively, 10 binding events for wild type sample fragment could correspond to only 3 binding events for mutated sample fragment. Investigation of this information by correlating detections over a time period of for example 1 hour, would then prompt the conclusion that 3/13 or approximately 23% of the sample studied contains defective mutated material. This figure would be relevant for a clinical decision.

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BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be further elucidated with reference to the drawings.

Figure 1 illustrates a nanopore device according to the invention.

Figure 2 illustrates a further embodiment of the invention comprising means to perform a control on the validity of device measurement

15

Figure 3 illustrates a further embodiment of the invention comprising a plurality of chambers

Figure 4 illustrates a further embodiment of the invention where the chambers are arranged in the form of an array

20

Figure 5 illustrates a method of manufacturing a nanopore device according to the invention

Figure 6 illustrates a method of using a nanopore device according to the invention.

Where features are consistent between drawings the numbering of reference signs has been kept consistent.

25

DETAILED DESCRIPTION OF EMBODIMENTS

Figure 1 depicts a nanopore device 100 according to the invention. The device comprises a sample input 110 for inputting a sample of nucleic acid material (not shown), such as DNA, RNA or uRNA, which is prepared into small fragments (not shown), into an input chamber 120. For DNA these small fragments have a negative electric charge. The input chamber 120 is arranged to be connected to a first sample chamber 130 via a first nanopore 135 and to a second sample chamber 140 via a second nanopore 145. The nanopores 135, 145 further comprise specific detection molecules (not shown) which are

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tailored to the required detection function of the nanopore. An electrolytic solution (not shown) is present in the chambers 120, 130, 140 and comprises charged particles. Means to apply an electrical potential difference 150 are arranged so that an electrical potential difference is present across both nanopores 135, 145. (This means to apply an electrical potential difference is shown here as internal to the nanopore device 100, but this should not be construed as limiting as an external means may also be used if desired). The electrical potential difference across the second nanopore 145 is equal to and common with the electrical potential difference across the first nanopore 135. The effect of the electrical potential difference is to draw ions of the electrolytic solution through the nanopores 135, 145 and to also draw small fragments of the sample individually through the nanopores 135, 145. Usually the sample fragments are negatively charged, as for DNA, which results in the means to apply an electrical potential being arranged so that the input chamber 120 side of the nanopores 135, 145 is at an earth potential and the first 130 and second 140 chamber sides of the nanopores are at a positive potential in order to draw the charged sample through the nanopores 135, 145 in the correct direction, here indicated by arrows 160 and 165. (However, this is only one example of the possible arrangements of the means to apply electrical potential. It will be understood that a negative to positive voltage drop may be achieved in other ways, and that depending on the sample to be analysed, a reverse electrical potential may be suitable). As the electrolytic solution is drawn through each of the nanopores 135 and 145, an ionic current is generated. Thus flow through the first nanopore 135 gives rise to a first ionic current and flow through the second nanopore 145 gives rise to a second ionic current. Detection of target molecules by the nanopores 135, 145 results in a change in this ionic current. For the present example of DNA sequencing, the biological nanopore will temporarily close on detection thereby blocking the flow of the electrolytic solution and temporarily stopping the ionic current. This ionic current and flow stoppage is detected by a means for current detection (not shown).

The commonality of the sample input chamber 120 allows different measurements and detections to be performed on the same sample in the same time period. This is beneficial for consistency of measurements results and avoids issues of splitting samples which can affect the statistical distributions of target molecules within the sample. Further the application of a common electrical potential difference ensures different measurements performed by the different nanopores are performed under the same external conditions. This aids reliability and accuracy of the data determined by the nanopore device.

Note that the input chamber 120 and first 130 and second 140 chambers are shown in figure 1 as being linear and two dimensional in layout and square in shape. This should not be construed as limiting as the invention allows for any shape of chambers 120 130 140 and for the chambers to be arranged also in three dimensions, depending on the requirements of the nanopore device 100.

Figure 2 shows a further embodiment of the invention comprising a nanopore device 200. This device is an extension of nanopore device 100 and comprises the same features of this device. Nanopore device 200 is, however, augmented by the addition of a third chamber 210 which is connected to the input chamber 120 by a third nanopore 220.

This nanopore allows flow of the electrolytic solution (not shown) and passage of sample fragments (not shown) in the direction of the arrow 230. (Again, this is the direction for the specific example as described in figure 1 and should not be construed as limiting for the nanopore device 200). The nanopore 220 operates in the same manner as nanopores 135, 145, as previously described, and gives rise to a third ionic current for detection.

In this embodiment the third nanopore 220 comprises a detection molecule or mechanism to detect a specific sequence which is always present in a sample fragment regardless of genetic mutation. Thus this nanopore registers a detection for the passage of each sample fragment passing through it. This gives a positive result which can be used as a validity check for functioning of the nanopore device 200. The result can be input to a correlating means (not shown) for computed comparison with the detections from the other nanopores 135 145.

Such a validity check using the third chamber setup is not restricted to a positive measurement result. The third nanopore 220 may also be engineered to detect a sequence where a negative detection result is expected, depending on the application of the nanopore device 200.

Again, the shape of the chambers 130, 140, 210 are not limited to the shape and design as illustrated, and the layout may be arranged in two or three dimensions.

Figure 3 illustrates another possible embodiment of the invention wherein the nanopore device 300 comprises multiple chambers 311, 312, 313, 314, 315, 316, 317, 318 around a central input chamber 320. Each of the chambers 311, 312, 313, 314, 315, 316, 317, 318 is independent of the other chambers but each chamber is connected to the input chamber 320 by a nanopore, there being provided for each chamber a single nanopore 341, 342, 343, 344, 345, 346, 347, 348, respectively. (The invention should not be construed as being limited to a single nanopore as shown here, or in any other embodiment, but may be

arranged to have multiple nanopore openings in parallel to connect each chamber to the input chamber). The nanopores 341, 342, 343, 344, 345, 346, 347, 348 are subject to the same electrical potential difference and are arranged to operate in the same manner as explained previously. (Means to apply an electrical potential difference are not shown). Ionic currents are generated for each nanopore, via electrolytic solution flow through the nanopores (not shown), which are detected and changes in these currents indicate detection of target molecules in the sample (not shown).

Figure 4 depicts a further embodiment of the invention where the nanopore device 400 comprises an array 405 comprising individual chambers 410, 411, 412, 413, 414, 415, 416, 417, 418 each remotely connected to an input chamber 430 with sample input 440 via nanopores 420, 421, 422, 423, 424, 425, 426, 427, 428, respectively. The same electrical potential difference is arranged to be applied across the nanopores 420, 421, 422, 423, 424, 425, 426, 427, 428 from a common means to apply an electrical potential (not shown).

Nanopore device 400 further comprises connection means, here shown as individual conduits 450, 451, 452, 453, 454, 455, 456, 457, 458, 459 but not limited to this specific embodiment, to guide samples from the input chamber to the individual chambers 410, 411, 412, 413, 414, 415, 416, 417, 418, respectively. The operation of the nanopores and means associated with this operation (e.g. ionic current detection) are as described previously. The array 405 may be supported on a substrate (not shown) which may also comprises means (not shown) to direct the flow of sample between input 430 and chambers 410, 411, 412, 413, 414, 415, 416, 417, 418.

Figure 5 illustrates a method of manufacturing a nanopore device according to the invention. The method involves providing an input chamber for containing a sample of nucleic acid 510, providing a first chamber connected to the input chamber by a first nanopore 520, providing an electrolytic solution capable of flow through the first nanopore under the influence of an electric potential difference 530, providing a means to apply an electrical potential difference across the first nanopore 540, providing a second chamber connected to the input chamber by means of a second nanopore 550 and arranging the means to apply the electrical potential difference across the second nanopore equal to and common with the electrical potential across the first nanopore 560.

Figure 6 illustrates a method of using a nanopore device according to the invention. This method involves detecting a wild gene sequence by means of the first nanopore 610 and detecting a specific mutation of said gene sequence by means of the second nanopore 620.

This method is especially important for determining the ration of mutated genes in a sample which can be indicative for progress of a particular cancer disease or indicative of a best treatment for personalized care.

List of reference numerals :

- 100 nanopore device
- 110 sample input
- 5 120 input chamber
- 130 first chamber
- 135 first nanopore
- 140 second chamber
- 145 second nanopore
- 10 150 means to apply an electrical potential difference
- 160 arrow indicating direction of movement of a first sample fragment through the first nanopore under the influence of the electrical potential difference
- 165 arrow indicating direction of movement of a second sample fragment through the second nanopore under the influence of the electrical potential difference
- 15 200 nanopore device
- 210 third chamber
- 220 third nanopore
- 230 arrow indicating direction of movement of a third sample fragment through the third nanopore under the influence of the electrical potential difference
- 20 300 nanopore device
- 311...318 first to eighth chambers
- 320 input chamber
- 330 sample input
- 341...348 first to eighth nanopores
- 25 400 nanopore device
- 405 array
- 410...418 first to ninth chambers
- 420 to 428 first to ninth nanopores
- 430 input chamber
- 30 440 sample input
- 450 first to ninth conduits
- 510 method step
- 520 method step
- 530 method step

540 method step

550 method step

560 method step

610 method step

5 620 method step

CLAIMS:

1. A nanopore device (100), arranged to cooperate with a sample of nucleic acid, the nanopore device comprising

- an input chamber (120), for containment of the sample of nucleic acid
- a first chamber (130) connected to the input chamber by a first nanopore

5 (135)

- means to apply an electrical potential difference (150) across the first nanopore (135), the electrical potential being arranged to draw (160) a first fragment of the sample of nucleic acid through the first nanopore (135)

10 (135) under the influence of the electrical potential difference, the flow detectable as a first ionic current, changes in the first ionic current being indicative of detection of a first target molecule by the first nanopore (135)

characterized in that, the nanopore device further comprises :

15 nanopore (145)

- a second chamber (140) connected to the input chamber by a second

(145) equal to, and common with, the electrical potential difference across the first nanopore (135),

20 draw (165) a second fragment of the sample of nucleic acid through the second nanopore (145) and to influence the electrolytic solution to flow through the second nanopore (145),

- the flow detectable as a second ionic current, changes in the second ionic current being indicative of detection of a second target molecule by the second nanopore (145).

25

2. A nanopore device (200) as claimed in claim 1 further comprising a third chamber (210) connected to the input chamber (120) by a third nanopore (220), an electrical potential difference being applied across the third nanopore (220) equal to, and common with, the electrical potential difference across the first nanopore (135), the electrical potential

across the third nanopore (220) being arranged to draw (230) a third fragment of the sample of nucleic acid through the third nanopore (220) and to influence the electrolytic solution to flow through the third nanopore (220), the flow detectable as a third ionic current by the current detector, changes in the third ionic current being indicative of detection of a control target molecule for validating device measurement.

3. A nanopore device (100) as claimed in claims 1 or 2 further comprising a correlating means arranged to correlate detections indicated by changes in at least two of the ionic currents.

4. A nanopore device (100) as claimed in any of the above claims wherein the first target molecule is a selected wild gene sequence and the second target molecule is a specific mutation in the same gene sequence, for relative detection of the statistical occurrence of the mutation.

5. A nanopore device (100) as claimed in any of the above claims wherein at least one of the nanopores is a biological nanopore.

6. A nanopore device as claimed in claim 5 wherein the biological nanopore is arranged in cooperation with a lipid bilayer.

7. A method of manufacturing a nanopore device comprising the steps of :
providing an input chamber for containing a sample of nucleic acid (510)
providing a first chamber connected to the input chamber by a first nanopore

(520)
providing an electrolytic solution capable of flow through the first nanopore under the influence of an electric potential difference (530)

providing a means to apply an electrical potential difference across the first nanopore (540)

characterized in that the method further comprises the steps of

providing a second chamber connected to the input chamber by means of a second nanopore (550) and in that

arranging the means to apply the electrical potential difference across the

second nanopore equal to and common with the electrical potential across the first nanopore (560).

8. A method of using a nanopore device as claimed in claim 1, comprising the
5 steps of:
detecting a wild gene sequence by means of the first nanopore (610)
detecting a specific mutation of said gene sequence by means of the second
nanopore (620).
- 10 9. A method of using a nanopore device as claimed in claim 2 comprising the
steps of :
correlating detections indicated by changes in at least one of the first or second
ionic currents and the third ionic current to generate a signal indicative of a valid device
measurement.
- 15 10. A method of using a nanopore device as claimed in claim 1 or 2 comprising
the steps of :
correlating detection indicated by changes in at least two of the ionic currents
to generate a comparative relation between ionic currents.

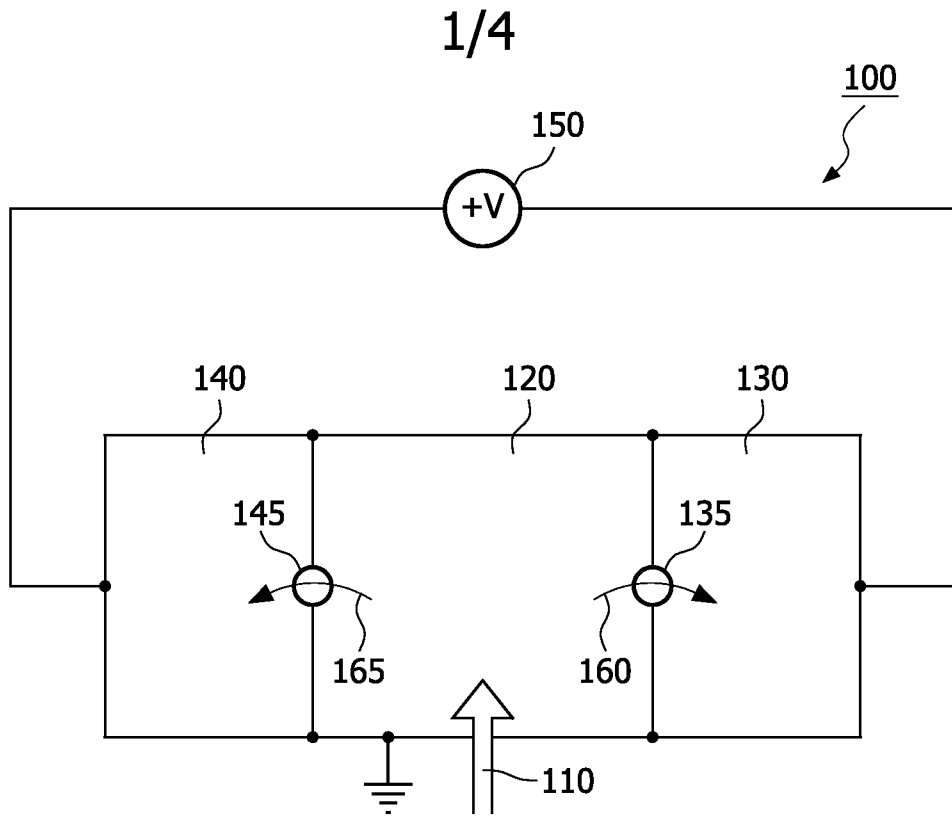


FIG. 1

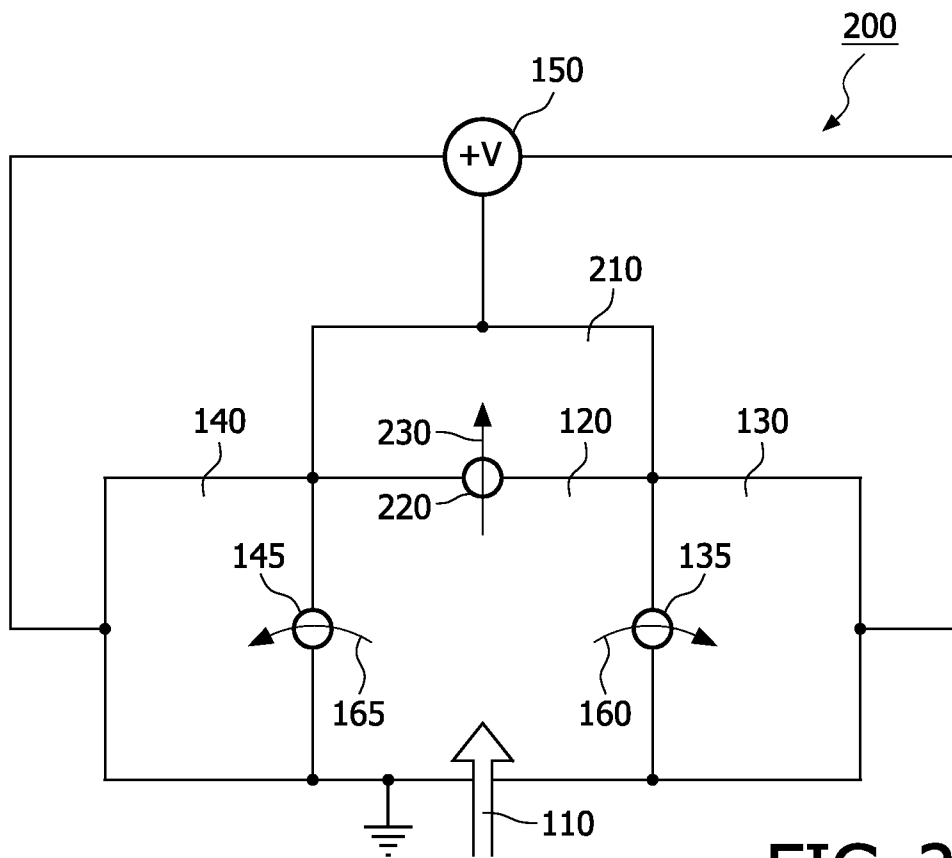


FIG. 2

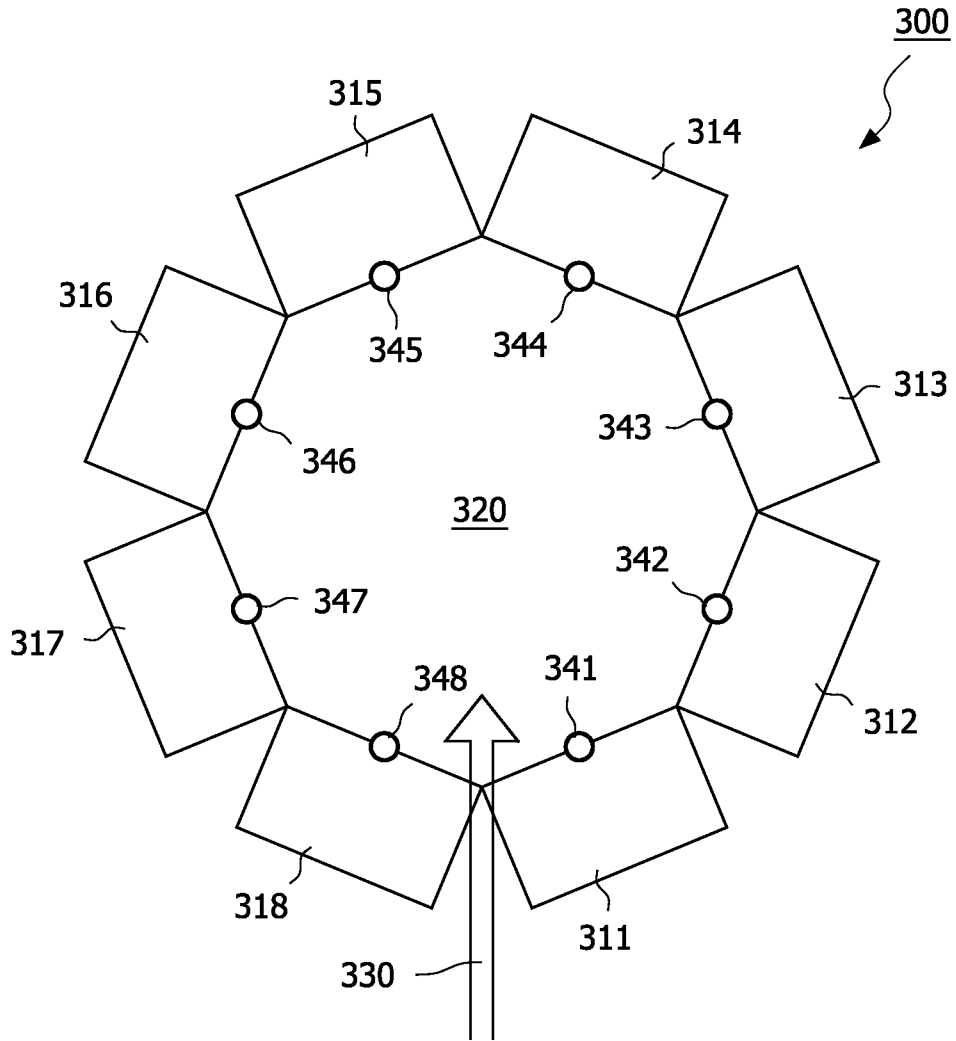


FIG. 3

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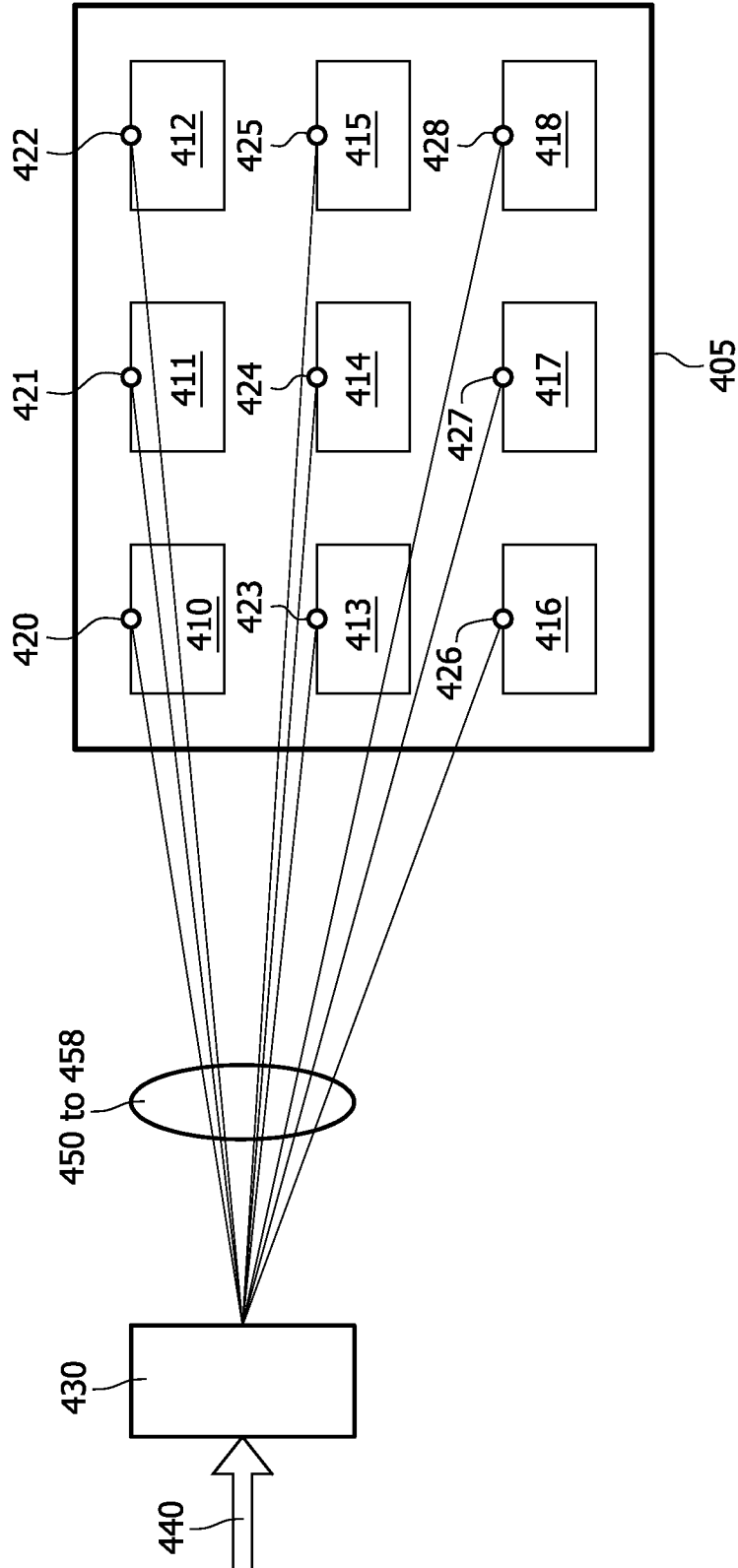


FIG. 4

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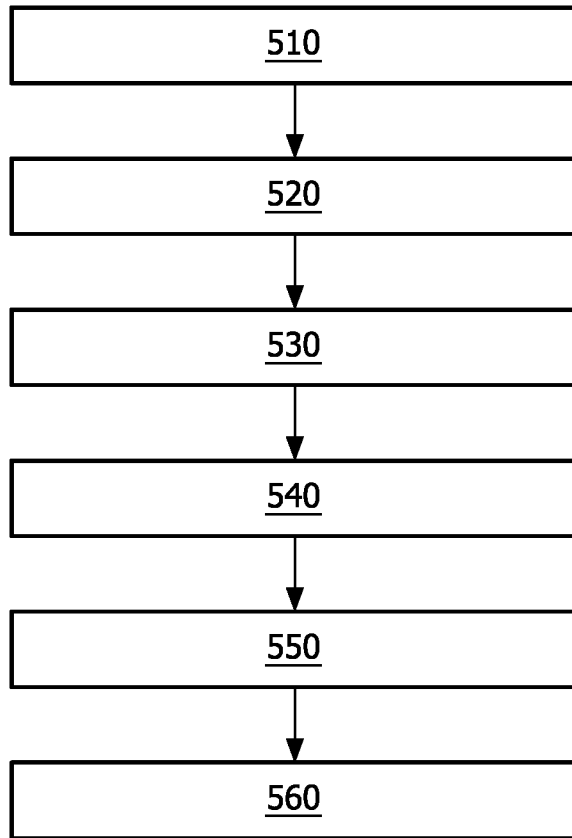


FIG. 5

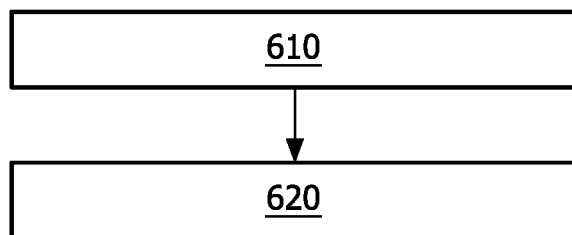


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2009/050635

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N15/12

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)
G01N

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 practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 645 628 A (AGILENT TECHNOLOGIES INC [US]) 12 April 2006 (2006-04-12)	1-7, 9, 10
Y	paragraphs [0096] - [0099]; figures 4, 5	8
Y	US 2008/119366 A1 (SAUER JON R [US] ET AL) 22 May 2008 (2008-05-22)	8
	paragraphs [0094] - [0096]; figure 26	
A	DE 10 2006 035072 A1 (WESTFAELISCHE WILHELMS UNI MUE [DE]) 31 January 2008 (2008-01-31)	1-10
	figures 8, 9	
	----- -/--	

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

* Special categories of cited documents :

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Date of the actual completion of the international search

6 April 2009

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/050635

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DANIEL BRANTON ET AL: "The potential and challenges of nanopore sequencing" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 26, no. 10, 1 January 2008 (2008-01-01), pages 1146-1153, XP002507807 ISSN: 1087-0156 the whole document</p>	1-10
A	<p>HENRIQUEZ R R ET AL: "The resurgence of Coulter counting for analyzing nanoscale objects" ANALYST, ROYAL SOCIETY OF CHEMISTRY, vol. 129, 1 January 2004 (2004-01-01), pages 478-482, XP002488755 ISSN: 0003-2654 the whole document</p>	1-10
A	<p>ASTIER YANN ET AL: "Toward single molecule DNA sequencing: direct identification of ribonucleoside and deoxyribonucleoside 5'-monophosphates by using an engineered protein nanopore equipped with a molecular adapter" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US, vol. 128, no. 5, 8 February 2006 (2006-02-08), pages 1705-1710, XP002503447 ISSN: 0002-7863 cited in the application the whole document</p>	1-10
A	<p>HOWORKA S ET AL: "Sequence-specific detection of individual DNA strands using engineered nanopores" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 19, no. 7, 1 July 2001 (2001-07-01), pages 636-639, XP002510816 ISSN: 1087-0156 cited in the application the whole document</p>	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2009/050635

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