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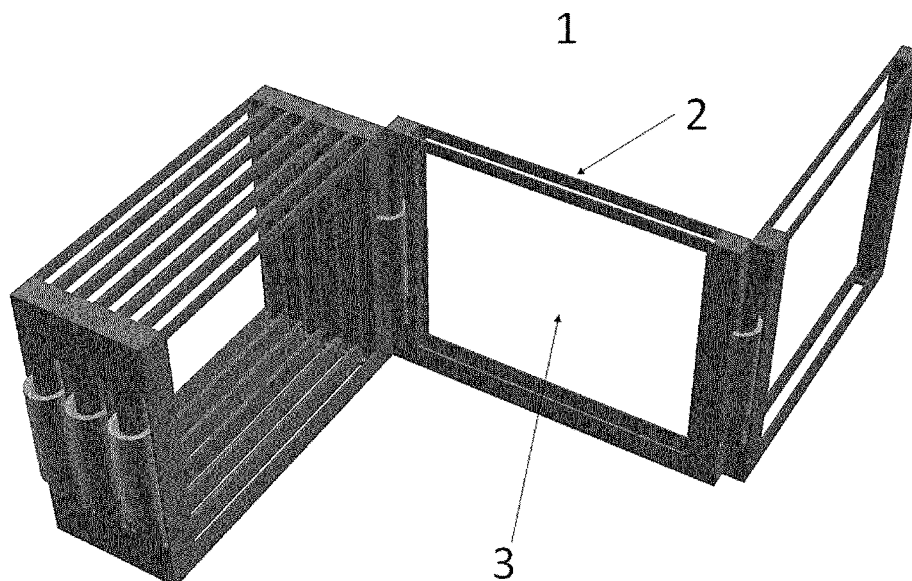
FIG. 2



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(57) Abstract: The present invention relates to an electrophoresis assembly, particularly useful for performing high-throughput elec¬trophoresis, as well as to methods for performing electrophoresis. Also disclosed is a frame for holding a separation matrix, and a system comprising an electrophoresis assembly, as well as uses thereof in electrophoretic methods. The methods allow for real-time imaging and simultaneous imaging and analysis.

[Continued on next page]

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Electrophoresis assembly

Technical field

The present invention relates to an electrophoresis assembly, particularly useful for performing high-throughput electrophoresis, as well as to methods for performing electrophoresis. Also disclosed is a frame for holding a separation matrix, and a system comprising an electrophoresis assembly, and uses thereof for performing electrophoresis. The methods allow for real-time imaging and simultaneous imaging and analysis.

Background

Biological experimentation, in particular in the field of molecular biology, often requires separation of molecules, in particular macromolecules such as nucleic acid molecules, proteins, amino acids or carbohydrates, for analytical purposes. A common separation procedure involves electrophoresis, where molecules are separated by charge and/or size via mobility through a separating matrix, for example a gel, in the presence of an electrical field.

Gel separating matrices are typically made by pouring a liquid phase into a mold and letting the matrices solidify. In slab gel electrophoresis, outcroppings in plastic material form combs embedded in the top of the separating matrix. These allow the formation of sample loading wells when the combs are removed after solidification of the matrix. In order to separate the molecules, the gel matrix is placed in appropriate electrophoresis buffers, and an electrical field is generated.

The loading of samples suspected of containing molecules which it is desirable to separate and extract into the combs is a time consuming task, which it is difficult to perform in a high throughput manner. To prevent samples from mixing with the buffers used for electrophoresis, dense solutions are often added to the samples prior to loading into the wells.

After loading of the samples, an electrical field is generated by electrodes in electrical contact with the separation matrix. The field allows charged molecules to migrate towards the electrode of opposite charge.

The migration distances for the separated molecular species depend on their relative mobility through the separation matrix. Mobility of each species depends on hydrodynamic size and molecular charge. Proteins are often electrophoresed under conditions where each protein is complexed with a detergent or other material that imparts a negative charge to proteins in the sample. The detergent causes most or all of the proteins to migrate in the same direction (towards the electrophoresis anode). Samples may be stained prior to, during, or after a separation run to visualize the nucleic acids or proteins within the gel. The location of the various components in the gel is determined using ultraviolet light absorbance, autoradiography, fluorescence, chemiluminescence, or any other well-known means of detection. To determine the molecular weight and relative concentration of unknown nucleic acids or proteins, the band positions and intensities are typically compared to known molecular standards.

Blotting is a process used to transfer molecules from an electrophoresis matrix to a membrane for further analysis, such as Southern, Northern, Western or Eastern blotting. Traditionally the separation matrix containing the electrophoresed biological material is removed from the electrophoresis apparatus and placed in a blotting sandwich. The blotting sandwich generally consists of buffer saturated sponges and paper pads; a gel containing the separated biologicals; a suitable transfer membrane that is in intimate contact with the separating matrix; and another layer of buffer saturated paper pads and sponges. In electroblotting, electrotransfer electrodes and buffer may provide an electrical field to move the molecules out of the separating matrix and into the membrane.

It is difficult to perform electrophoresis in a high-throughput manner. Methods and devices are needed to perform electrophoresis in a high-throughput manner.

Summary

The invention is as defined in the claims.

Provided herein is an electrophoresis assembly (1), comprising two or more frames (2) for holding a separation matrix such as a gel cassette (4), wherein each frame comprises at least one inner frame (3), and wherein the two or more frames are attached together, such that in a first configuration the two or more frames abut each other in a longitudinal direction, and in a second configuration the two or more frames are in extension of each other.

Also provided is a method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;
ii) Providing at least one separation matrix (4);
iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;
v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;
vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;
vii) Placing the electrophoresis assembly in a chamber (5) comprising at least a first pair of electrodes (6) at opposite ends of the chamber, wherein the electrophoresis assembly is contacted with said electrodes, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);
viii) applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

Also provided is a method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;
ii) Providing at least one separation matrix (4);
iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;
v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

vii) Placing the electrophoresis assembly in a chamber (5) comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);

viii) Applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

Also provided herein is a frame (2) for holding a separation matrix (4), wherein the frame comprises at least one inner frame (3) on a lateral side, and wherein the frame is configured to be attached to another identical frame (2), such that in a first configuration the two frames abut each other in a longitudinal direction, and in a second configuration the two frames are in extension of each other.

Also provided herein is a system comprising:

i) an electrophoresis assembly (1) as described herein;

ii) a liquid-tight chamber (5) configured to hold a fluid and the electrophoresis assembly, said chamber comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction.

Description of the drawings

Figure 1: An embodiment of an electrophoresis assembly (1) comprising 8 frames (2), wherein the frames (2) each comprise one inner frame (3) and an opening in an upper side (10). The assembly is shown in the first configuration, where the frames abut each other in a longitudinal direction. In this embodiment, each frame comprises a hinge (11) on the right or left side.

Figure 2: The electrophoresis assembly **(1)** of figure **1** is shown in a configuration where two frames are in extension of each other, and the remaining 6 frames are still in a configuration where they abut each other in a longitudinal direction.

5 **Figure 3:** The electrophoresis assembly **(1)** of figure **1** is shown in the first configuration. Separation matrices (4) (here gel cassettes) are loaded into the frames (2), so that the wells for sample loading are located in the upper end of the assembly.

Figure 4: The electrophoresis assembly (1) of figure 3, fully loaded with separation
10 matrices (4).

Figure 5: The electrophoresis assembly (1) of figure 4, shown in a second configuration, where the frames (2) are in extension of each other.

15 **Figure 6:** The electrophoresis assembly (1) of figure 5, wherein the two frames located at the extremities are connected to form a closed perimeter. In this particular embodiment, the closed perimeter has a square shape.

Figure 7: The electrophoresis assembly (1) of the previous figures is placed in a
20 chamber (5) comprising a pair of electrodes (6). The chamber in this embodiment is equipped with detection means (7) (here a camera) and an additional compartment (8) configured to hold a light source (9).

Detailed description

25 *Definitions*

Charge The term as used herein refers to an electrical charge, in particular of a molecule or of an electrode. An electrical charge has an absolute value, which is the numerical value of the electrical charge, and a sign - i.e. the charge is either positive or negative.

30

Electrophoresis assembly

The present disclosure relates to an electrophoresis assembly comprising two or more frames for holding a separation matrix such as a gel cassette, wherein each frame

comprises at least one inner frame, and wherein the two or more frames are attached together, such that in a first configuration the two or more frames abut each other in a longitudinal direction, and in a second configuration the two or more frames are in extension of each other. Preferably, each frame is configured to hold a separation matrix, i.e. a frame alone can hold a separation matrix. Preferably, each frame comprises, in addition to the at least one inner frame, an opening in an upper end, in which a separation matrix can be inserted to be held by the frame, and further comprises a hinge or a slidable connection, which allows two or more frames to be attached together in such a way that in a first configuration the two or more frames abut each other in a longitudinal direction, and in a second configuration the two or more frames are in extension of each other. The electrophoresis assembly disclosed herein allows for easy sample loading, and can be used in high-throughput methods. In particular, the electrophoresis assembly allows for high-throughput analysis and recovery of the parts of the matrix suspected of containing the molecules which it is desirable to analyse or use further.

The term “abutting each other in a longitudinal direction” in relation to two frames refers herein to the fact that two frames abut each other on their biggest lateral side, i.e. the side that defines the biggest plane of the frame.

The electrophoresis assembly comprises two or more frames. The frames are suitable for holding a separation matrix such as a gel cassette. In other words, the frames when holding the separation matrix surround the matrix. Preferably, one frame holds one separation matrix. The frame may however also be configured to hold more than one separation matrix.

Preferably, the frame comprises an opening in an upper end allowing the insertion of a separation matrix therein. In other words, the frame is hollow and can contain a separation matrix. The frame may be designed as a box or a cassette, i.e. in some embodiments the frame can be opened to insert the separation matrix therein, and may be equipped with locking means allowing the frame to be in tight contact, e.g. fluid-tight contact, around the separation matrix.

The frames may be designed in such a way that they may provide structural support to the separation matrix, which can be useful when the separation matrix or gel is fragile. The frames need not be transparent; the at least one inner frame they comprise should

however preferably be transparent or allow light transmission for proper monitoring of sample migration. Preferably, the frames are made of a material which is structurally stable under the conditions commonly used for electrophoresis. Preferably, the material from which the frame is manufactured is inert, and/or does not oxidise when contacted with fluids such as electrophoresis buffers, and/or does not rust easily. Materials suitable for manufacturing electrophoresis assemblies are known to the skilled person.

The electrophoresis assembly of the present disclosure is preferably reusable.

The frames are preferably of a rectangular or square shape. The dimensions of the frame are preferably such that a separation matrix such as a gel cassette can be introduced in the frame, and maintained therein in such a manner that the separation matrix does not move once inserted. Accordingly, the frame preferably has at least one opening for inserting the separation matrix therein, e.g. on the upper side. Preferably, the lower side of the frame is at least partially closed so that the separation matrix does not simply slide through the frame. In some embodiments, the lower side of the frame comprises an opening, which allows for better contact between the gel and the liquids such as electrophoresis buffers used for sample migration. Preferably, the dimensions of the opening in the lower side are smaller than the corresponding dimensions of the separation matrix, so that the matrix is held in place.

Each frame comprises at least one inner frame on at least one side of the frame, preferably on a lateral side of the frame. The inner frame allows visualisation and optionally access to the separation matrix through the lateral side of the frame when it is placed inside the frame, for example for cutting out a piece of the separation matrix. Accordingly, the inner frame should preferably be transparent. In some embodiments, the inner frame is a cavity. In other embodiments, the inner frame is made of a transparent material, preferably an inert material, which allows for visualisation of sample migration. In some embodiments, each frame comprises at least one inner frame on only one side. In other embodiments, each frame comprises at least one inner frame on each side. The at least one inner frame may be two inner frames, such as three inner frames, for example four inner frames, such as five inner frames, for example six inner frames, such as seven inner frames, for example eight inner frames, such as nine inner frames, for example ten inner frames, such as eleven inner frames, for examples twelve inner frames, or more.

The dimensions of the inner frame should be such that they allow visualisation and optionally access to the lateral side of the separation matrices held in the electrophoresis assembly. Preferably, the inner frame has dimensions such that the separation matrix introduced in the frame is held in place and does not slide out of the frame. The dimensions of the inner frame are thus preferably smaller than the dimensions of the lateral side of the frame in which it is located.

The frames of the electrophoresis assembly are attached together in such a way that the electrophoresis assembly can be arranged in different configurations. In a first configuration, the two or more frames abut each other in a longitudinal direction, i.e. the biggest lateral side of two adjacent frames are adjacent and parallel to one another along the entire surface of their biggest lateral side. This first configuration is represented in figure 1. In a second configuration, the two or more frames are in extension of each other. This is represented in figures 5 and 6. The frames thus preferably comprise a hinge or a slidable connection, and the two or more frames are pivotably hinged to one another via said hinge or are slidably connected to one another via said slidable connection.

In other words, in a first configuration, when each frame of the electrophoresis assembly holds a separation matrix, at least one lateral side of the separation matrix is not directly accessible, as it is covered by the separation matrix held in the adjacent frame. In a second configuration, when each frame of the electrophoresis assembly comprises a separation matrix, both lateral sides of the separation matrix are directly accessible. The term “lateral side” herein refers to a side of the separation matrix on which migration of samples throughout or after electrophoresis can be monitored, and/or to a side of the separation matrix from which a piece of the separation matrix suspected of containing molecules of interest can be isolated. The lateral side is usually the biggest lateral side.

The electrophoresis assembly can be arranged in any configuration going from the first configuration to the second configuration. In order to allow the electrophoresis assembly to move from the first configuration to the second configuration or vice versa, the two or more frames are mobile in respect to one another. For example, two adjacent frames can be pivotably hinged to one another, e.g. via the hinge. Alternatively, two adjacent frames can be slidably connected to one another, e.g. via the slidable connection. Preferably, two adjacent frames are directly connected to one

another, i.e. they are in direct contact to one another and the attachment does not require the presence of an additional element beside the hinge or the slidable connection.

5 Thus in some embodiments, the frame comprises a hinge. The frame defines a rectangle comprising four sides: an upper side (where the separation matrix is introduced), a lower side (holding the separation matrix), a right side and a left side. The hinge should be located on the right side and/or on the left side of the frame. The hinge should preferably not be located on the upper side or on the lower side. The 10 hinge may be one hinge, two hinges, three hinges or more.

Where the frame(s) comprise(s) a hinge on one side, it may be advantageous for the frame to also include additional connection means on another side, for example magnets. The frame may thus comprise a hinge on at least one side, through which it 15 can be connected to another identical frame, and may further comprise additional connection means such as magnets on another side. The additional connection means may be located on the side of the frame opposite to the hinge, and/or they may be located on the upper and/or the lower end of the frame. Such additional connection means may help maintain the several frames comprised in the electrophoresis 20 assembly in place to facilitate loading of the separation matrices and/or samples, as they may prevent the creation of gaps between the frames.

Preferably, the additional connection means are magnets. The magnets are preferably coated with isolating material as is known in the art to prevent them from interfering 25 with or getting damaged by the electrical fields applied to the frame when in use.

In other embodiments, the frame comprises a slidable connection, for example a ridge, a groove or a slit, which allows the frame to be slidably connected to another frame. Preferably, the slidable connection is located on the upper and/or on the lower side of 30 the frame. This allows two frames connected via a slidable connection to slide in relation to one another.

The first configuration corresponds to a closed configuration. This configuration is particularly advantageous for loading samples onto the separation matrices held within 35 the assembly, as will be detailed herein below.

The second configuration corresponds to an open configuration. This configuration is particularly advantageous for monitoring migration of the samples through the separation matrix, and for extracting parts of the samples, as will be detailed herein below, for example by extracting parts of the separation matrix suspected of containing
5 the molecules of interest.

It is preferred that the two or more frames of the electrophoresis assembly all have the same dimensions. At any rate, the two or more frames preferably have at least the same height. Optionally, the two or more frames preferably also have the same
10 thickness and/or the same width.

Separation matrices, such as gel cassettes, can for example have the following dimensions: 12 cm in width; 0.6 cm in thickness; 11 cm in height. Electrophoresis assemblies suitable for holding such separation matrices can aptly have the following
15 dimensions: 13 to 14 cm in width or more; 0.85 cm in thickness or more; 11.25 cm in height or more.

In some embodiments, the electrophoresis assembly described herein comprises frames configured to match a separation matrix, e.g. a gel cassette, such as a standard
20 separation matrix. Standard separation matrices are well-known in the art. For example, a standard separation matrix has 96 wells, 192 wells, 384 wells, 768 wells or 1536 wells. Thus, in some embodiments, the electrophoresis assembly in the first configuration has such dimensions that the upper part of the assembly, to which samples are loaded when the electrophoresis assembly holds one or more separation
25 matrices, matches the dimensions of a 96 well plate, a 192 well plate, a 384 well plate, a 768 well plate or a 1536 well plate. In other words, when separation matrices are inserted in the electrophoresis assembly, the spacing between the wells of the separation matrices when the electrophoresis assembly is in the first configuration match the spacings between the dispenser tips of standard manual or automatic liquid
30 handlers as are known in the art. In this manner, high-throughput loading of the samples to the separation matrices may be achieved.

In some embodiments, the electrophoresis assembly may further comprise a removable cap. The cap is preferably located on the upper side of the frame. It can be
35 opened when samples are to be loaded in the separation matrix held within the frame, and closed once sample loading is finished, thus preventing the samples from flowing

out of the wells when handling the electrophoresis assembly. Suitable caps may be for example silicone strips. The cap may also or alternatively be used together with loading of the separation matrix in the electrophoresis assembly as detailed below.

5 In some embodiments, the electrophoresis assembly comprising at least two frames, such as at least three frames, such as at least four frames, such as at least five frames, such as at least six frames, such as at least seven frames, such as at least eight frames, such as at least nine frames, such as at least ten frames, such as at least eleven frames, such as at least twelve frames, or more.

10

The electrophoresis assembly when in the second configuration can form a closed perimeter. In such embodiments, the electrophoresis assembly comprises at least three frames, and the two frames located in the extremities of the electrophoresis assembly can be connected to one another; the assembly can thus form a closed
15 perimeter. Depending on the number of frames within the electrophoresis assembly, the closed perimeter thus formed can adopt various shapes. An electrophoresis assembly comprising three frames will have an essentially triangular shape. An electrophoresis assembly comprising four frames, or a number of frames which is a multiple of four, can have an essentially square shape. An electrophoresis assembly
20 comprising an even number of frames greater than four, in particular an even number which is not a multiple of four, can have an essentially regular shape. Uneven numbers of frames will result in other shapes.

The electrophoresis assembly can however also be used in configurations where the
25 frames when in extension of each other, i.e. in the second configuration, do not form a closed perimeter.

The skilled person will realise that the frames can each hold two separation matrices, provided that the frames then comprise at least one inner frame on each side, such
30 that each separation matrix in one frame is accessible for visualisation when the electrophoresis assembly is in the second configuration.

Method for performing electrophoresis

The electrophoresis assembly described herein is particularly useful for performing
35 electrophoresis and optionally blotting. The electrophoresis assembly can

advantageously be used in high-throughput methods, resulting in shorter handling times, as well as facilitated handling and analysis. Accordingly, all the methods described herein are suitable for high-throughput and automated handling, e.g. automated sample loading.

Thus is also provided herein a method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;
ii) Providing at least one separation matrix (4);
iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;
v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;
vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;
vii) Placing the electrophoresis assembly in a chamber (5) comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);
viii) Applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

Also disclosed herein is a method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;

ii) Providing at least one separation matrix (4);

iii) Loading the at least one separation matrix in the electrophoresis assembly;

iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5 v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

vii) Placing the electrophoresis assembly in a chamber (5) comprising at least a
10 first pair of electrodes (6) at opposite ends of the chamber, wherein the electrophoresis assembly is contacted with said electrodes, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber
15 optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);

viii) applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

20

The electrophoresis assembly is preferably as described herein. In some embodiments, each frame comprises an opening in an upper end and further comprises a hinge or a slidable connection, as described herein above.

25 Molecules of interest

The present methods can be used to perform electrophoretic separation of molecules of interest.

Molecules of interest can be any molecule of interest comprised or suspected of being
30 comprised in a sample of interest. The molecules of interest can thus be biomolecules, for example macromolecules such as nucleic acid molecules, for example DNA or RNA, proteins or carbohydrates. The molecules of interest can also be smaller molecules, such as amino acids, small peptides and monosaccharides or polysaccharides.

35

The molecules of interest can be comprised within a sample, and it may be desirable to perform electrophoresis in order to separate the molecules of interest from the remaining molecules comprised within the sample. The sample may be a biological sample or a synthetic sample, for example the product of a polymerase chain reaction (PCR); in this case the desired product of the PCR reaction can be separated from the other compounds comprised within the reaction such as primers, enzymes, and template nucleic acid.

Separation matrix

In step ii) of the methods disclosed herein, a separation matrix is provided. The choice of separation matrix will typically be dictated by the nature of the molecules to be separated in the matrix. The skilled person knows how to choose a separation matrix suitable for given molecules of interest. Separation matrices comprise gel cassettes suitable for electrophoresis as is known in the art.

In embodiments where the molecules of interest are nucleic acids, such as DNA or RNA, the separation matrix may be a gel cassette. Suitable gel cassettes for performing electrophoretic separation of nucleic acid molecules are known in the art, and include e.g. agarose gels. The concentration of agarose in the gel is typically determined by the expected size of the molecules of interest. For example, smaller molecules of interest can advantageously be separated in agarose gels of lower concentration than bigger molecules of interest.

For protein separation, the separation matrix may also be a gel cassette, typically a polyacrylamide gel cassette. Protein separation may involve denaturing methods and native methods. SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) is a denaturing method, where proteins are separated according to their electrophoretic mobility in the denatured state, as a function of the length of the polypeptide chain and its charge. SDS binding to the polypeptide confers an even distribution of charge per unit mass and thus results in separation by approximate size during electrophoresis.

Native or non-denaturing gels allows for separation of proteins in their folded state according to their electrophoretic mobility, which in this case is a function not only of the charge-to-mass ratio but also on the physical size and shape of the protein. Several

types of non-denaturing gels are commonly used for protein separation: blue native PAGE (BN-PAGE), clear native PAGE or native PAGE (CN-PAGE) and quantitative native PAGE (QPNC-PAGE).

In BN-PAGE, the Coomassie blue dye provides the necessary charges to the proteins for electrophoretic separation. CN-PAGE separates acidic water-soluble and membrane proteins in polyacrylamide gradient gels. No charged dye is used, so the electrophoretic mobility depends on the intrinsic charge of the proteins. Migration distance is a function of protein charge, size and gel pore size. QPNC-PAGE is used to separate folded protein complexes.

For separation of carbohydrates, several types of separation matrices exist. Silica matrices can e.g. be used. Alternatively, alkaline polyacrylamide gels or gradient polyacrylamide gels can be employed.

For separation of amino acids, capillary gel electrophoresis is commonly used.

Separation matrices often comprise wells in one end, typically the upper end. Such wells are configured to allow loading of the samples, as is known in the art, in the upper end of the separation matrix. When the electrical field promoting migration of the samples through the separation matrix is generated in a direction parallel to the general orientation of the gel, samples are forced from the well into the separation matrix, where the molecules they contain are separated as described above and as otherwise known in the art.

In step iii) of the present methods, the at least one separation matrix is placed or loaded in the electrophoresis assembly. In practice, the separation matrix is inserted in one frame - in some embodiments however it may be of interest to load two separation matrices in the same frame. As described herein above, the frames of the electrophoresis assembly are preferably of such dimensions that they allow a separation matrix to be inserted therein, while at the same time if needed providing structural support for the separation matrix. Loading the separation matrices in the electrophoresis assembly may be performed while the electrophoresis assembly is in

the first configuration, in the second configuration, or in any configuration between the first and the second configurations.

It will typically be practical to insert the separation matrix in the appropriate frame in a liquid form, as is known in the art. The electrophoresis assembly may in such case also comprise a cap, for example a silicon cap, which will allow the formation of wells in the upper end of the separation matrix. As is known in the art, the separation matrix should solidify before samples are loaded thereto and before performing electrophoresis.

In some embodiments, the separation matrix already has incorporated therein a dye or a marker allowing visualisation of the samples as migration progresses.

Loading of samples

The samples comprising or suspected of comprising the molecules of interest must then be loaded into the separation matrices, preferably in wells as described above. In order to facilitate sample loading, the samples may be mixed with high density solutions, as is known in the art, to prevent the samples from diffusing out of the wells.

The electrophoresis assembly disclosed herein allows for easy sample loading, optionally in a high-throughput manner. In step iv) of the method, the electrophoresis assembly, in which separation matrices have been inserted, is arranged in a first configuration wherein the two or more frames it comprises abut each other in a longitudinal direction. When in this configuration, the sample loading pockets or wells present in the upper end of the separation matrices are in close vicinity. This allows for easy pipetting of the sample, and may in some embodiments allow use of a manual or automatic liquid handler.

For example, in a specific embodiment, the electrophoresis assembly comprises eight frames for holding eight separation matrices with 12 wells each. When in the first configuration, such an electrophoresis assembly may advantageously match the dimensions of a 96-well plate. In other words, the wells of the eight separation matrices are placed in such a manner that the spacing therebetween corresponds to the spacing between the wells of a 96-well plate. Liquid handlers having corresponding sizes thus enable easy and rapid sample loading; for example, the samples to be loaded on the gel may be provided in a 96-well plate, and easy transfer of the samples from the plate

to the electrophoresis assembly can thus be achieved with a liquid handler. It will be obvious to the skilled person that less than eight frames (and less than eight separation matrices) may also be used, but the spacing may still aptly match that of the dispensing ends of a liquid handler. Conversely, the electrophoresis assembly may comprise more than eight frames and still be easily handled if it matches a liquid handler, since individual loading of the samples is thus avoided.

Similarly, in a specific embodiment, the electrophoresis assembly comprises 12 frames for holding 12 separation matrices with 16 wells each. The electrophoresis assembly thus matches the layout of a 192-well plate.

In another specific embodiment, the electrophoresis assembly comprises 16 frames for holding 16 separation matrices with 24 wells each. The electrophoresis assembly thus matches the layout of a 384-well plate.

In another specific embodiment, the electrophoresis assembly comprises 16 frames for holding 16 separation matrices with 48 wells each. The electrophoresis assembly thus matches the layout of a 768-well plate.

In another specific embodiment, the electrophoresis assembly comprises 32 frames for holding 32 separation matrices with 48 wells each. The electrophoresis assembly thus matches the layout of a 1536-well plate.

The present electrophoresis assembly can be scaled up as follows. It may be impractical or difficult to load separation matrices with many wells. In order to reduce the volume of the electrophoresis assembly, the spacing between the wells may be as on a 96 well plate or a 192 well plate, and the frames may be scaled up in one or both directions. It may also be advantageous to have two separation matrices within one frame, as described herein. In such embodiments, where a frame comprises two separation matrices, it may be advantageous to also include an intercalator between the two frames. The term intercalator refers here to a thin sheet or plate of a material which preferably does not allow transmission of light, in order to facilitate imaging as it will block the signal from one of the separation matrices and thus allow proper imaging of the other separation matrices. The material of which the intercalator is made can be for example black plastic, and the intercalator has a thickness such that it effectively blocks light transmission.

The skilled person will be able to easily scale up the number and/or dimensions of frames and/or wells to meet his/her needs.

5 Dyes/stains

The samples may be mixed with a dye or a marker prior to loading, in order to facilitate monitoring of sample migration through the separation matrix in a later step. In some embodiments, several dyes or markers are used.

10 Preferably, the sample, or at least the molecules of interest contained in the sample, is stained or dyed using common stains or dyes known in the art. As noted above, it is also possible to include the dye in the conductive fluid, if it is undesirable or impractical to add it to the sample directly.

15 For staining of nucleic acid molecules such as DNA or RNA, common dyes include: SYBR® Green, e.g. SYBR® Green I or II, RedSafe™ stain, SYBR® Gold, oxazole yellow, thiazole orange, PicoGreen®, Safe-Green™, ethidium bromide, and others. For staining of proteins, stains and dyes include: Instant-Bands treatment buffer, Coomassie-based stains, silver stains, negative staining with insoluble metal salts such 20 as copper or zinc salts, fluorescent stains, tetramethylrhodamine (TRITC), and others. For staining of amino acids, ninhydrin (2,2-dihydroxyindane-1 ,3-dione) is a suitable stain. For staining of carbohydrates, 2-aminoacridone (AMAC), 2-aminobenzoic acid (2-AA), 7-amino-1 ,3-naphthalene disulfonic acid (ANDS), 8-aminonaphthalene-1 ,3,6-trisulfonic acid (ANTS), and 9-aminopyrene-1 ,4,6-trisulfonic acid (APTS), among 25 others, are suitable.

Alternatively, in some embodiments, the samples can be marked during electrophoresis, as will be described in more details below.

30 Loading of the electrophoresis assembly in an electrophoresis chamber

Once the samples are loaded, the configuration of the electrophoresis assembly is modified, so that the electrophoresis is arranged in the second configuration. This is to allow easy placing of the electrophoresis assembly in a chamber, where electrophoresis is to be performed. The electrophoresis chamber is suitable for

performing electrophoresis. In other words, the electrophoresis chamber is preferably fluid-tight, i.e. it can hold a fluid such as the conductive fluid without leakage.

Conductive fluid

The electrophoresis chamber preferably comprises a conductive fluid suitable for performing sample migration, e.g. by electrophoresis. The present methods may however also be performed in an electrophoresis chamber devoid of conductive fluid, e.g. in a process of dry or semi-dry electrophoresis. Separation matrices typically contain a high percentage of conductive fluid, which may be sufficient for separating the molecules in the samples without actually submerging the separation matrix in conductive fluid. In such embodiments, the electrodes used for separating the samples are brought into contact with the upper and the lower part of the separation matrix.

In embodiments where the chamber comprises a conductive fluid, contact may be established between the electrodes via the conductive fluid, by placing the electrodes in the conductive fluid in which the electrophoresis assembly is at least partly immersed. The chamber may comprise the conductive fluid prior to placing the electrophoresis assembly in the chamber, or the electrophoresis assembly may be placed in the chamber before the conductive fluid is added thereto. It is to be understood that the chamber does not have to be completely filled with the conductive fluid. The amount of conductive fluid is sufficient if the conductive fluid is in contact at least with the bottom of the sample loading pockets or wells of the separation matrices comprised in the electrophoresis assembly once the assembly has been placed in the chamber, so that there is electrical contact between the samples and the conductive fluid. In other words, once the electrophoresis assembly has been placed in the chamber and conductive fluid has been added thereto, the electrophoresis is at least partially immersed in the conductive fluid in such a way that there is electrical contact between the samples loaded in the separation matrices contained in the electrophoresis assembly and the conductive fluid contained in the chamber.

Conductive fluids suitable for performing electrophoresis are known to the skilled person. In general, a buffer should be chosen with a pKa close to the desired pH; most often, buffers with a pKa in the range of 7-9 are suited for most electrophoretic purposes. Suitable electrophoresis buffers may be one of the following: TAE buffer, TBE buffer, Tris-Glycine SDS PAGE buffer, or Tris-Tricine SDS PAGE buffer.

In some embodiments, the conductive fluid may comprise a dye or a marker allowing for staining or visualisation of the samples. For example, RedSafe or ethidium bromide may be added to buffers used in electrophoresis migration of nucleic acids, as is known in the art. Adding a dye or a stain to the conductive fluid may be desirable in some embodiments, and renders the addition of a dye or a stain to the sample unnecessary.

Preferably, in order to facilitate monitoring of sample migration in a later step, the chamber is made of a transparent material, which allows visualisation of sample migration, in particular when the separation matrix is illuminated by a light source.

Electrodes

The electrophoresis chamber is further equipped with at least a first pair of electrodes. These electrodes are located at opposite ends of the chamber, and are capable of generating a first electrical field in a first direction corresponding to the direction of migration of the samples throughout electrophoresis. In other words, the electrical field and the separation matrices when the electrophoresis has been placed in the chamber are parallel to one another. The electrical field preferably allows migration of the samples from the top of the separation matrix to the bottom of the separation matrix.

As described above, in embodiments where the chamber comprises a conductive fluid, the first pair of electrodes is contacted with the conductive fluid, e.g. by immersion as is known in the art. In such embodiments, the separation matrix is contacted with the electrodes of the first pair indirectly, i.e. via the conductive fluid. In embodiments where the chamber does not comprise a conductive fluid, e.g. for dry or semi-dry electrophoresis, the separation matrix is contacted with the electrodes of the first pair directly, e.g. one electrode is placed at the upper end of the separation matrix and the other electrode is placed at the lower end of the separation matrix.

Light source

The chamber may also be equipped with a light source, which may be placed in another additional compartment, or in the same additional compartment as the detection means. Preferably, the walls of the additional compartment allow passage of the light emitted by the light source. The light source should be placed in such a way that the light it emits falls on the side of the separation matrix, through the inner frame, where sample migration can be visualised. For example, the direction of the light may

be perpendicular to the plane of the separation matrix corresponding to sample migration.

The light source preferably emits light which is suitable for monitoring sample migration. The skilled person knows which type of light is useful depending on which kind of separation is run, and/or depending on the type of dye/stain or marker used for visualisation.

Examples of suitable lights include UV light, blue light and laser.

Detection means

Additional elements may be comprised in the chamber. In some embodiments, the chamber is equipped with detection means, e.g. imaging equipment such as a camera. In order to extend the life-time of the detection means, these may be contained in an additional compartment, which may be fluid tight and preferably devoid of fluid. The additional compartment preferably has walls which allow the detection means to visualise and detect sample migration in the adjacent electrophoresis compartment. The detection means should preferably be placed with an orientation such that they can monitor sample migration. The detection means may be capable of doing so in real time.

Analysis means

The chamber may also comprise analysis means, which can be connected to the detection means. Such analysis means may be a computer or another automated system, for example a smartphone or a tablet, which can collect data from the detection means and provide an output to the user - such output may give valuable information as to the progress of migration.

Additional elements

The chamber may be further equipped with one or more input valves and one or more output valves. These may be useful to facilitate the introduction and/or removal of various fluids to and from the chamber, for example the conductive fluid and/or rinsing fluid and/or transfer fluid. The valves may be automatic or manual valves.

Migration of samples

In a final step, a current is applied between the at least two electrodes, thereby allowing migration of the samples through the separation matrix.

5 In some embodiments, the method further comprises a step of monitoring migration of the samples through the matrix. This can be achieved for example with the naked eye, or using suitable detection means as described above.

In some embodiments, monitoring is performed in real-time, i.e. in a continuous manner. In other embodiments, monitoring is performed at punctual times, for example every second, every 10 seconds, every 30 seconds, every minute, every second minute, every 5 minutes. A decision may be made by the user (or by appropriate automated means) to interrupt the electrical field by interrupting the current between the at least two electrodes if the samples have migrated sufficiently. Preferably, the current is interrupted before the molecules of interest have migrated so far through the separation matrix that there is a risk that they might exit the matrix at the lower end. Migration should proceed for a period of time sufficient to allow proper separation of the molecules of interest from the other molecules contained in the sample, as is known in the field.

Monitoring of migration may require the presence of a dye or a stain either in the sample or in the conductive fluid, as explained herein above.

Electroblotting

In some embodiments, the method, once the molecules of interest have been separated from the remaining molecules in the samples, may comprise a step of performing electroblotting. In practice, the conductive fluid is removed from the chamber. The chamber may be washed by passing a rinsing fluid through it, once or several times, before a transfer fluid is introduced in the chamber, in such a manner that the electrophoresis assembly - and hence the separation matrices comprised therein - is at least partially immersed in said transfer fluid. Electroblotting is performed as is known in the art, by transferring molecules from a gel cassette suspected of containing the molecules of interest to a membrane such as an electroblotting membrane. In embodiments where electroblotting is to be performed in the chamber, the chamber is equipped with another pair of electrodes (herein also termed

electroblotting pair of electrodes) located at opposite ends of the chamber or on each side of the separation matrix, where said electroblotting pair of electrodes can generate an electrical field (herein termed electroblotting field) in a direction perpendicular to the first electrical field generated by the first pair of electrodes when a current is applied thereto. This allows transfer of the molecules, including the molecules of interest, from the separation matrix to the membrane.

Alternatively, the separation matrix or matrices comprising or suspected of comprising molecules of interest can be removed from the electrophoresis assembly, and transferred to an electroblotting apparatus as is known in the art. In this case, electroblotting is performed outside the chamber.

Electroblotting methods are known in the art and involve various types of membranes, which are generally determined by the type of molecules to be transferred thereto.

In embodiments where the molecules are proteins, the electroblotting is a Western blot, and the membrane is preferably a nitrocellulose membrane, a nylon membrane or a polyvinylidene difluoride membrane. The electroblotting may also be a Far-Western blot, typically to study protein-protein interactions, or an Eastern blotting, typically to study post-translational modifications.

In embodiments where the molecules are nucleic acid molecules such as DNA molecules or RNA molecules, the membrane is aptly selected from a nitrocellulose membrane, a polyvinylidene difluoride membrane, a filter paper membrane or a nylon membrane. DNA molecules can be transferred by Southern blot, and RNA molecules by Northern blot or reverse Northern blot, as is known in the art.

In embodiments where the molecules are e.g. glycoplipids, Far-Eastern blots are typically used.

During or after transfer, the molecules may be marked as is known in the art, in order to enable proper analysis of the membrane. Accordingly, in some embodiments, the method further comprises a step of analysing the membrane after transfer. Analysis of the membrane may comprise one or more of: detection of the presence of the molecules of interest, lack of detection of the presence of the molecules of interest,

quantification of the amount of the molecules of interest, for example by Western blot, Southern blot or Northern blot.

Extraction of the molecules of interest from the separation matrix

5 It may be desirable to extract the molecules of interest from the separation matrix for further analysis. Accordingly, in some embodiments, the method may further comprise a step of extracting the molecules of interest from the separation matrix, for example for analytical purposes, or because the molecules are to be used for experimental purposes. For example, if the molecules of interest are the product of a polymerase 10 chain reaction or a transcript from a given gene, the molecules after extraction and optionally purification may be sequenced to determine their abundance, to verify the absence of unwanted mutations, or to verify the presence of desired gene editing events such as the introduction of a mutation, of a marker or of a tag. If the molecules of interest are proteins, it may be desirable to further analyse the proteins of interest, 15 for example to determine their abundance, or their crystal structure.

Extraction of the molecules of interest from the separation matrix will often require that a piece of the separation matrix, to which the molecules of interest are expected to have migrated, be separated from the matrix, for example by cutting. Thus in some 20 embodiments the method further comprises the step of cutting one or more pieces of the separation matrix suspected of containing the molecules of interest with cutting means. The cutting can be done manually, in which case the cutting means may be a scalpel, a knife, a tip such as a pipette tip used on the broad end or a tip having an end which has a square shape or a rectangular shape. The cutting may also be done in an 25 automated manner, for example using a robot equipped with appropriate cutting means, such as the means described above.

In some embodiments, extraction of the molecules of interest can advantageously be performed as described in application “Methods for extraction of molecules” assigned 30 to same applicant and having the same filing date as the present application. The methods described therein are also suitable for high-throughput. They require that the molecules of interest have a positive or a negative charge, which will most often be the case for molecules of interest which can be electrophoretically separated in a separation matrix as described herein.

35

First, the sample is placed in a first compartment comprising a conductive fluid and a second electrode pair with a first electrode and a second electrode.

The first compartment is a fluid-tight container, such as a standard microcentrifuge tube, for example an Eppendorf tube, as is known in the art to be suitable for sample handling.

The first compartment comprises a second electrode pair comprising a first electrode and a second electrode. These electrodes do not need to be attached to the first compartment, but may be placed therein in such a way that the lower ends of both electrodes are below the upper end of the first compartment. In this way, both electrodes can be contacted with a conductive fluid comprised within the first compartment, in such a manner that an electrical field can be generated in the conductive fluid when an electrical current is applied to the electrodes.

Each electrode of the second pair may be divided in two parts: an upper part (or insulated part), which is insulated as is known in the art, for example by a plastic coating, and a lower part (or conductive part) which is the electrode as such and which may generate a field.

In preferred embodiments, the first electrode of the second pair is longer than the second electrode of the second pair. When placed in the first compartment, the first electrode will thus be closest to the bottom of the first compartment, while the second electrode will be farthest from the bottom of the first compartment.

The conductive fluid is as known in the art, and may for example be a buffer such as an electrophoresis buffer, for example TAE buffer, TBE buffer, Tris-Glycine SDS PAGE buffer, or Tris-Tricine SDS PAGE buffer.

Once the sample and the electrodes of the second pair have been placed in the first compartment comprising an appropriate volume of conductive fluid as described above, a current (termed the second current) is applied to the first and second electrodes of the second pair to generate an electrical field (termed the second electrical field) in the conductive fluid. The second electrical field is generated in such a way that the first electrode of the second pair becomes charged with a charge of the same sign as the molecules to be extracted, and the second electrode of the second

pair becomes charged with a charge of the opposite sign. For example, if the molecules have a positive charge, the first electrode of the second pair becomes positively charged and the second electrode of the second pair becomes negatively charged. If the molecules have a negative charge, the first electrode of the second pair becomes negatively charged, and the second electrode of the second pair becomes positively charged.

Once the electrodes of the second pair are charged, the charged molecules will thus migrate to the second electrode, which has a charge opposite to the charge of the molecules.

The second electrode pair (i.e. the first and the second electrodes) is then removed from the first compartment and placed in a second compartment; the second electrode has attached thereto at least part of the charged molecules.

The second compartment comprises a constricted volume around at least the first electrode when the third electrode pair is placed therein. The term “constricted volume” refers to the lower part of the second compartment, which has a smaller diameter than the upper part of the first compartment. This will allow concentration of the molecules in a smaller volume than using a “normal” compartment, such as a standard microcentrifuge tube. Preferably, the constricted volume is not around the second electrode when the second electrode pair is placed in the second compartment.

In some embodiments the second compartment may be the same as the first compartment.

The second compartment comprises a suitable elution buffer. A third electrical field is generated in the elution buffer by applying a third current to the second electrode pair. The third current may have a direction opposite to that of the second current, so that the third electrical field has a direction opposite to that of the second electrical field. In this way, the charges of the electrodes of the second pair are reversed: the first electrode of the second pair now has a charge opposite to the charge it had under the second electrical field, and the second electrode of the second pair now has a charge opposite to the charge it had under the second electrical field. As a consequence, the charged molecules are released from the second electrode of the second pair and the

matrix if present, and migrate electrophoretically to the first electrode of the second pair, which now has a charge of the opposite sign as the charged molecules.

Alternatively, the charges of the molecules of interest may be inverted, for example by the use of suitable buffers. In this case, the charges of the electrodes of the second pair should not be reversed. Migration of the molecules from the second electrode of the second pair to the first electrode of the second pair will be enabled by the reversal of charges of the molecules to be extracted.

Once the electrodes of the second pair are charged, the charged molecules will thus migrate to the first electrode of the second pair, which has a charge opposite to the charge of the molecules.

It is to be noted that applying the third electrical current is not an absolute requirement for recovering the charged molecules of interest. In some embodiments, it may be sufficient to interrupt the second electrical field, whereby the charged molecules will be released from the second electrode of the second pair. The use of a third electrical current may however result in faster extraction.

Finally, the charged molecules are collected. This is done by interrupting the third electrical field and collecting the elution buffer from the second compartment. Because the electrodes of the second pair are no longer charged when the third current is interrupted, the charged molecules are released from the first electrode and are now present in the elution buffer. The elution buffer comprising the charged molecules can then be collected. The charged molecules have thus been extracted.

In one specific embodiment, is provided herein a provided herein a method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;
ii) Providing at least one separation matrix (4);
iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

5 vii) Placing the electrophoresis assembly in a chamber (5) comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction, wherein 10 the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);

15 viii) Applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled;

ix) Cutting one or more pieces of the separation matrix suspected of containing the molecules of interest with cutting means, wherein the molecules of interest are charged;

20 x) Recovering the molecules of interest from the one or more pieces of the separation matrix, wherein this step comprises the steps of:

1) placing the one or more pieces of the separation matrix in a first compartment, wherein the first compartment comprises a conductive fluid such as an electrophoresis buffer, and a second electrode pair 25 comprising a first electrode and a second electrode,

2) generating a second electrical field in the conductive fluid by applying a second current to the first and second electrodes of the second pair, such that the first electrode becomes of the second pair charged with the same charge as the molecules and the second electrode of the 30 second pair becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode of the second pair, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix or a polyvinylidene difluoride 35 matrix,

3) removing the electrodes of the second pair from the first compartment, and placing the electrodes of the second pair in a second compartment, wherein the second compartment comprises a constricted volume around at least the first electrode of the second pair, and the second compartment comprises an elution buffer in contact with the first and the second electrodes of the second pair,
4) optionally generating a third electrical field in the elution buffer by applying a fourth current between the two electrodes of the second pair, such that the charges of the electrodes of the second pair are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode of the second pair,
5) optionally interrupting the third electrical field; and
6) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules,

whereby the molecules are extracted.

The electrophoresis assembly and the frames may be as described herein.

Frame for holding a gel cassette

Also provided herein is a frame for holding a separation matrix, wherein the frame comprises at least one inner frame on a lateral side, and wherein the frame is configured to be attached to another identical frame, such that in a first configuration the two frames abut each other in a longitudinal direction, and in a second configuration the two frames are in extension of each other. Preferably, the frame comprises also an opening on an upper side, in which a separation matrix can be inserted, as described herein above. Preferably, the frame comprises a hinge or a slidable connection, whereby the frame is configured to be pivotably hinged to another identical frame via said hinge, or slidably connected to another identical frame via said slidable connection, such that in a first configuration the two frames abut each other in a longitudinal direction, and in a second configuration the two frames are in extension of each other.

The frame may be as described herein above.

System comprising an electrophoresis assembly

Also provided herein is a system comprising:

i) an electrophoresis assembly (1) as described herein;

ii) a liquid-tight chamber (5) configured to hold a fluid and the electrophoresis assembly, said chamber comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction.

The system is suitable for performing electrophoresis.

In some embodiments, the liquid-tight chamber further comprises at least one compartment configured to hold one or more detection means and/or at least one compartment configured to hold a light source. The detection means and the light source may be as described herein above. The compartments configured for holding the detection means or the light source may be as described herein above.

The system may further comprise one or more containers configured to hold a fluid such as a conductive fluid, a transfer fluid and/or a rinsing fluid, where the containers are connected to the chamber via one or more input and/or output valves. The valves may be manual or automatic, as described herein above.

Items

1. An electrophoresis assembly (1), comprising two or more frames (2) for holding a separation matrix such as a gel cassette (4), wherein each frame comprises at least one inner frame (3), and wherein the two or more frames are attached together, such that in a first configuration the two or more frames abut each other in a longitudinal direction, and in a second configuration the two or more frames are in extension of each other.

2. The electrophoresis assembly according to item 1, wherein the two or more frames are pivotably hinged to one another.

3. The electrophoresis assembly according to any one of the preceding items, wherein the two or more frames are slidably connected to one another.

4. The electrophoresis assembly according to any one of the preceding items, wherein each frame comprises at least one inner frame (3) and an opening in an upper end (10) and further comprises a hinge (11) or a slidable connection, and wherein the two or more frames are pivotably hinged to one another via said hinge or slidably connected to one another via said slidable connection.

5. The electrophoresis assembly according to any one of the preceding items, wherein the two or more frames all have the same dimensions.

6. The electrophoresis assembly according to any one of the preceding items, configured to match a standard separation matrix when in the first configuration, optionally wherein the standard separation matrix is a 96-well, a 192-well, a 384-well, a 768-well or a 1536-well separation matrix.

7. The electrophoresis assembly according to any one of the preceding items, wherein the electrophoresis assembly is reusable.

8. The electrophoresis assembly according to any one of the preceding items, further comprising a removable cap such as a silicone strip.

9. The electrophoresis assembly according to any one of the preceding items, wherein the frame has two or more inner frames.

10. The electrophoresis assembly according to any one of the preceding items, wherein the at least one inner frame is on one or both sides of the frame.

11. The electrophoresis assembly according to any one of the preceding items, wherein the inner frame is a cavity in the frame.

12. The electrophoresis assembly according to any one of the preceding items, wherein the inner frame consists of a material which allows transmission of light, such as UV light, laser light or blue light.

13. The electrophoresis assembly according to any one of the preceding items, wherein the two frames located in the extreme positions of the electrophoresis

assembly when in the first configuration can be connected to one another such that the electrophoresis assembly forms a closed perimeter.

14. The electrophoresis assembly according to any one of the preceding items, wherein the frame is manufactured of an inert, UV-resistant material such as plastic.

15. A method for performing electrophoresis, comprising the steps of:
i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;
ii) Providing at least one separation matrix (4);
iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;
v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;
vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;
vii) Placing the electrophoresis assembly in a chamber (5) comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);
viii) Applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

16. The method according to item 15, further comprising a step ix) of monitoring the migration of the samples via the detection means.

17. The method according to any one of items 15 to 16, wherein monitoring is performed in real-time.

18. The method according to any one of items 15 to 17, wherein the samples and/or the separation matrices comprise one or more dyes allowing monitoring the migration of the molecules.

19. The method according to item 18, wherein the light source (9) is UV light, blue light or laser light.

20. The method according to any one of items 15 to 19, wherein the electrophoresis assembly (1) is placed in the chamber (5) such that the light source (9) can illuminate the separation matrix (4) through the inner frame (3).

21. The method according to any one of items 15 to 20, wherein the electrophoresis assembly (1) is placed in the chamber (5) such that the detection means (7) can detect a signal from the separation matrix (4).

22. The method according to any one of items 15 to 21, wherein the chamber contains an input valve and/or an output valve allowing for the conductive fluid and/or rinsing fluid to flow in and/or out of the chamber, respectively.

23. The method according to any one of items 15 to 22, wherein the input valve and/or the output valve are independently selected from a manual valve and an automatic valve.

24. The method according to any one of items 15 to 23, wherein the detection means (7) are connected to analysis means.

25. The method according to any one of items 15 to 24, wherein the chamber (5) is made of a transparent material to allow visualisation of the migration of the samples when the separation matrix (4) is illuminated.

26. The method according to any one of items 15 to 25, wherein the molecules are nucleic acid molecules, such as DNA or RNA molecules, proteins, amino acids, carbohydrates such as sugar molecules.

27. The method according to any one of items 15 to 26, wherein the method after migration through the separation matrix (4) further comprises the steps of:
    a) removing the conductive fluid from the chamber (5);
    b) introducing a transfer fluid into the chamber (5), such that the electrophoresis assembly (1) is at least partially immersed in said transfer fluid; and
    c) transferring molecules from a separation matrix suspected of containing the molecules of interest to a membrane.

28. The method according to item 27, wherein the step of transferring the molecules from the separation matrix (4) to the membrane comprises performing capillary transfer and/or applying an electroblotting field in a second direction, wherein the second direction is perpendicular to the first direction.

29. The method according to item 28, wherein the electroblotting field is generated by at least another pair of electrodes located at opposite ends of the chamber (5), wherein the other pair of electrodes is an electroblotting pair of electrodes.

30. The method according to any one of items 27 to 29, wherein the molecules are proteins and the membrane is suitable for performing a Western blot, such as a nitrocellulose membrane, a nylon membrane or a polyvinylidene difluoride membrane.

31. The method according to any one of items 27 to 29, wherein the molecules are nucleic acid molecules such as DNA molecules or RNA molecules and the membrane is suitable for performing a Northern blot or a Southern blot, such as a nitrocellulose membrane, a polyvinylidene difluoride membrane, a filter paper membrane or a nylon membrane.

32. The method according to any one of items 27 to 31, wherein the membrane is provided in the electrophoresis assembly (1) and is in contact with at least part of the surface of the separation matrix (4) throughout electrophoresis.

33. The method according to any one of items 27 to 32, wherein the membrane is contacted with the separation matrix (4) suspected of containing the molecules of interest after electrophoresis.

34. The method according to any one of items 27 to 33, further comprising a step of analysing the membrane.

35. The method according to item 34, wherein the step of analysing the membrane comprises one or more of: detection of the presence of the molecules of interest, lack of detection of the presence of the molecules of interest, quantification of the amount of the molecules of interest, for example by Western blot, Southern blot or Northern blot.

36. The method according to any one of items 15 to 35, further comprising a step of extracting the molecules of interest from the separation matrix (4).

37. The method according to any one of items 15 to 36, wherein the method further comprises the step of cutting one or more pieces of the separation matrix (4) suspected of containing the molecules of interest with cutting means.

38. The method according to item 37, wherein the cutting means is a squared-end tip, a rectangular-end tip or a circular-end tip such as a pipette tip.

39. The method according to any one of items 37 to 38, wherein the method further comprises the step of recovering the molecules of interest from the one or more pieces of the separation matrix (4).

40. The method according to any one of items 36 to 39, wherein the molecules of interest have a positive or a negative charge and the step of extracting the molecules of interest from the separation matrix (4) comprises:

1) placing the one or more pieces of the separation matrix in a first compartment, wherein the first compartment comprises a conductive fluid such as an electrophoresis buffer and a second electrode pair comprising a first electrode and a second electrode,
2) generating a second electrical field in the conductive fluid by applying a second current to the first and second electrodes of the second pair, such that the first

electrode of the second pair becomes charged with the same charge as the molecules and the second electrode of the second pair becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode of the second pair, and optionally attached to said second electrode via a silica matrix,

3) removing the electrodes of the second pair from the first compartment, and placing the electrodes of the second pair in a second compartment, wherein the second compartment comprises a constricted volume around at least the first electrode of the second pair, and the second compartment comprises an elution buffer in contact with the first and the second electrodes of the second pair,
4) optionally generating a third electrical field in the elution buffer by applying a third current between the two electrodes, such that the charges of the electrodes of the second pair are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode of the second pair,
5) optionally interrupting the third electrical field; and
6) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules,

whereby the molecules are extracted.

41. The method according to item 40, wherein the first electrode of the second pair is longer than the second electrode of the second pair.

42. The method according to any one of items 40 to 41, wherein the first and second electrodes of the second pair are attached in an upper end to a frame, optionally wherein the frame comprises at least one hole (10) located next to the upper end of the first electrode and/or the upper end of the second electrode of the second pair, said hole being configured to allow a solution to flow through.

43. The method according to item 42, wherein the hole is a valve of which the opening and closing can be controlled, such as a solenoid valve.

44. The method according to any one of the items 36 to 43, wherein the method is a high-throughput method.

45. A frame (2) for holding a separation matrix (4), wherein the frame comprises at least one inner frame (3) on a lateral side, and wherein the frame is configured to be attached to another identical frame (2), such that in a first configuration the two frames abut each other in a longitudinal direction, and in a second configuration the two frames are in extension of each other.

46. The frame according to item 45, wherein the frame further comprises an opening on an upper side, said opening being configured for inserting the separation matrix therein, and wherein the frame further comprises a hinge or a slidable connection, whereby the frame is configured to be pivotably hinged to another identical frame (2) via said hinge, or slidably connected to another identical frame (2) via said slidable connection.

47. The frame according to any one of items 45 to 46, wherein the frame (2) is as defined in any one of items 1 to 44.

48. A system comprising:

i) an electrophoresis assembly (1) according to any one of items 1 to 14;

ii) a liquid-tight chamber (5) configured to hold a fluid and the electrophoresis assembly, said chamber comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction.

49. The system according to item 48, wherein the frame further comprises an opening on an upper side, said opening being configured for inserting the separation matrix therein, and wherein the frame further comprises a hinge or a slidable connection, whereby the frame is configured to be pivotably hinged to another identical frame (2) via said hinge, or slidably connected to another identical frame (2) via said slidable connection.

50. The system according to any one of items 48 to 49, wherein the liquid-tight chamber further comprises at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9).

51. The system according to any one of items 48 to 49, further comprising one or more containers configured to hold a fluid such as a conductive fluid or a transfer fluid, said one or more containers being connected to the liquid-tight chamber (5) via one or more input and/or output valves.

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52. The electrophoresis assembly according to any one of items 1 to 14, the frame of any one of items 45 to 46 or the system of any one of items 48 to 51, for use in a method for performing electrophoresis.

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Claims

1. An electrophoresis assembly (1), comprising two or more frames (2), each frame being configured for holding a separation matrix such as a gel cassette (4), wherein each frame comprises at least one inner frame (3) and an opening in an upper end (10) and further comprises a hinge (11) or a slidable connection, and wherein the two or more frames are pivotably hinged to one another via said hinge or slidably connected to one another via said slidable connection, such that in a first configuration the two or more frames abut each other in a longitudinal direction, and in a second configuration the two or more frames are in extension of each other.

2. The electrophoresis assembly according to claim 1, wherein the two or more frames all have the same dimensions.

3. The electrophoresis assembly according to any one of the preceding claims, configured to hold a standard separation matrix when in the first configuration, optionally wherein the standard separation matrix is a 96-well, a 192-well, a 384-well, a 768-well or a 1536-well separation matrix.

4. The electrophoresis assembly according to any one of the preceding claims, wherein the electrophoresis assembly is reusable.

5. The electrophoresis assembly according to any one of the preceding claims, further comprising a removable cap such as a silicone strip.

6. The electrophoresis assembly according to any one of the preceding claims, wherein the frame has two or more inner frames.

7. The electrophoresis assembly according to any one of the preceding claims, wherein the at least one inner frame is on one or both sides of the frame.

8. The electrophoresis assembly according to any one of the preceding claims, wherein the inner frame consists of a material which allows transmission of light, such as UV light, laser light or blue light.

9. The electrophoresis assembly according to any one of the preceding claims, wherein the two frames located in the extreme positions of the electrophoresis assembly when in the first configuration can be connected to one another such that the electrophoresis assembly forms a closed perimeter.

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10. The electrophoresis assembly according to any one of the preceding claims, wherein the inner frame is a cavity in the frame.

11. The electrophoresis assembly according to any one of the preceding claims,
10 wherein the frame is manufactured of an inert, UV-resistant material such as plastic.

12. A method for performing electrophoresis, comprising the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames
15 (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;

ii) Providing at least one separation matrix (4);

iii) Loading the at least one separation matrix in the electrophoresis assembly;

iv) Arranging the electrophoresis assembly in a first configuration wherein the
20 two or more frames abut each other in a longitudinal direction;

v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

25 vii) Placing the electrophoresis assembly in a chamber (5) comprising at least a first pair of electrodes (6) at opposite ends of the chamber, wherein the electrophoresis assembly is contacted with said electrodes, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each
30 separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);

viii) Applying a current between the at least two electrodes,
35 whereby migration of the samples through the separation matrix is enabled.

13. The method according to claim 12, wherein the chamber (5) comprises a conductive fluid and the electrophoresis assembly is placed in the chamber such that it is at least partly immersed in said conductive fluid, and wherein the first pair of electrodes is contacted with the conductive fluid.

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14. The method according to any one of claims 12 to 13, further comprising a step ix) of monitoring the migration of the samples via the detection means, optionally wherein monitoring is performed in real-time.

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15. The method according to any one of claims 12 to 14, wherein the samples and/or the separation matrices comprise one or more dyes allowing monitoring the migration of the molecules.

16. The method according to any one of claims 12 to 15, wherein the light source (9) is UV light, blue light or laser light.

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17. The method according to any one of claims 12 to 16, wherein the electrophoresis assembly (1) is placed in the chamber (5) such that the light source (9) can illuminate the separation matrix (4) through the inner frame (3).

20

18. The method according to any one of claims 12 to 17, wherein the electrophoresis assembly (1) is placed in the chamber (5) such that the detection means (7) can detect a signal from the separation matrix (4).

25

19. The method according to any one of claims 12 to 18, wherein the chamber contains an input valve and/or an output valve allowing for the conductive fluid and/or rinsing fluid to flow in and/or out of the chamber, respectively.

20. The method according to any one of claims 12 to 19, wherein the input valve and/or the output valve are independently selected from a manual valve and an automatic valve.

30

21. The method according to any one of claims 12 to20, wherein the detection means (7) are connected to analysis means.

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22. The method according to any one of claims 12 to 21, wherein the chamber (5) is made of a transparent material to allow visualisation of the migration of the samples when the separation matrix (4) is illuminated.

5 23. The method according to any one of claims 12 to 22, wherein the step of transferring the molecules from the separation matrix (4) to the membrane comprises performing capillary transfer and/or applying an electroblotting field in a second direction, wherein the second direction is perpendicular to the first direction.

10

24. The method according to any one of claims 12 to 23, wherein the electroblotting field is generated by at least another pair of electrodes located at opposite ends of the chamber (5), wherein the other pair of electrodes is an electroblotting pair of electrodes.

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25. The method according to any one of claims 12 to 24, wherein the molecules are proteins and the membrane is suitable for performing a Western blot, such as a nitrocellulose membrane, a nylon membrane or a polyvinylidene difluoride membrane.

20

26. The method according to any one of claims 12 to 25, wherein the molecules of interest are nucleic acid molecules such as DNA molecules or RNA molecules and the membrane is suitable for performing a Northern blot or a Southern blot, such as a nitrocellulose membrane, a polyvinylidene difluoride membrane, a
25 filter paper membrane or a nylon membrane.

27. The method according to any one of claims 12 to 26, wherein the membrane is provided in the electrophoresis assembly (1) and is in contact with at least part of the surface of the separation matrix (4) throughout electrophoresis.

30

28. The method according to any one of claims 12 to 27, wherein the membrane is contacted with the separation matrix (4) suspected of containing the molecules of interest after electrophoresis.

35 29. The method according to any one of claims 12 to 28, further comprising a step of analysing the membrane.

30. The method according to any one of claims 12 to 29, wherein the step of analysing the membrane comprises one or more of: detection of the presence of the molecules of interest, lack of detection of the presence of the molecules of interest, quantification of the amount of the molecules of interest, for example by Western blot, Southern blot or Northern blot.

31. The method according to any one of claims 12 to 30, further comprising a step of extracting the molecules of interest from the separation matrix (4).

32. The method according to any one of claims 12 to 31, wherein the method further comprises the step of cutting one or more pieces of the separation matrix (4) suspected of containing the molecules of interest with cutting means, preferably wherein the cutting means is a squared-end tip, a rectangular-end tip or a circular-end tip such as a pipette tip.

33. The method according to claim 32, wherein the method further comprises the step of recovering the molecules of interest from the one or more pieces of the separation matrix (4).

34. The method according to any one of claims 12 to 33, wherein the molecules of interest have a positive or a negative charge and the step of extracting the molecules of interest from the separation matrix (4) comprises:
1) placing the one or more pieces of the separation matrix in a first compartment, wherein the first compartment comprises a conductive fluid such as an electrophoresis buffer and a second electrode pair comprising a first electrode and a second electrode,
2) generating a second electrical field in the conductive fluid by applying a second current to the first and second electrodes of the second pair, such that the first electrode of the second pair becomes charged with the same charge as the molecules and the second electrode of the second pair becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode of the second pair, and optionally attached to the second electrode via matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix or a polyvinylidene difluoride matrix,

3) removing the electrodes of the second pair from the first compartment, and placing the electrodes of the second pair in a second compartment, wherein the second compartment comprises a constricted volume around at least the first electrode of the second pair, and the second compartment comprises an elution buffer in contact with the first and the second electrodes of the second pair,
4) optionally generating a third electrical field in the elution buffer by applying a third current between the two electrodes of the second pair, such that the charges of said electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode of the second pair,
5) interrupting the third electrical field; and
6) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules,

whereby the molecules are extracted.

35. The method according to any one of claims 12 to 23, wherein the molecules are nucleic acid molecules, such as DNA or RNA molecules, proteins, amino acids, carbohydrates such as sugar molecules.

36. The method according to any one of claims 12 to 35, wherein the method after migration through the separation matrix (4) further comprises the steps of:
   a) removing the conductive fluid from the chamber (5);
   b) introducing a transfer fluid into the chamber (5), such that the electrophoresis assembly (1) is at least partially immersed in said transfer fluid; and
   c) transferring molecules from a separation matrix suspected of containing the molecules of interest to a membrane.

37. The method according to any one of claims 23 to 36, wherein the first electrode of the second pair is longer than the second electrode of the second pair.

38. The method according to any one of claims 23 to 37, wherein the first and second electrodes of the second pair are attached in an upper end to a frame, optionally wherein the frame comprises at least one hole (10) located next to the upper end of the first electrode and/or the upper end of the second electrode of the second pair, said hole being configured to allow a solution to

flow through, preferably wherein the hole is a valve of which the opening and closing can be controlled, such as a solenoid valve.

39. The method according to any one of claims 12 to 38, wherein each frame comprises an opening in an upper end (10) and further comprises a hinge (11) or a slidable connection, and wherein the two or more frames are pivotably hinged to one another via said hinge or slidably connected to one another via said slidable connection.

40. The method according to any one of claims 12 to 39, wherein the electrophoresis assembly is the electrophoresis assembly according to any one of claims 1 to 10.

41. A frame (2) for holding a separation matrix (4), wherein the frame comprises at least one inner frame (3) on a lateral side and an opening on an upper side, said opening being configured for inserting the separation matrix therein, and wherein the frame further comprises a hinge or a slidable connection, whereby the frame is configured to be pivotably hinged to another identical frame (2) via said hinge, or slidably connected to another identical frame (2) via said slidable connection, such that in a first configuration the two frames abut each other in a longitudinal direction, and in a second configuration the two frames are in extension of each other.

42. The frame according to claim 41, wherein the frame (2) is as defined in any one of claims 1 to 40.

43. A system comprising:

i) an electrophoresis assembly (1) according to any one of claims 1 to 11;

ii) a liquid-tight chamber (5) configured to hold a fluid and the electrophoresis assembly, said chamber comprising at least a first pair of electrodes (6) at opposite ends of the chamber, said electrodes being capable of generating a first electrical field in a first direction.

44. The system according to claim 43, wherein the liquid-tight chamber further comprises at least one compartment configured to hold one or more detection

means (7) and/or at least one compartment (8) configured to hold a light source (9).

45. The system according to any one of claims 43 to 44, further comprising one or more containers configured to hold a fluid such as a conductive fluid or a transfer fluid, said one or more containers being connected to the liquid-tight chamber (5) via one or more input and/or output valves.

46. The electrophoresis assembly according to any one of claims 1 to 11, the frame of any one of claims 41 to 42 or the system of any one of claims 43 to 45, for use in a method for performing electrophoresis.

FIG. 1

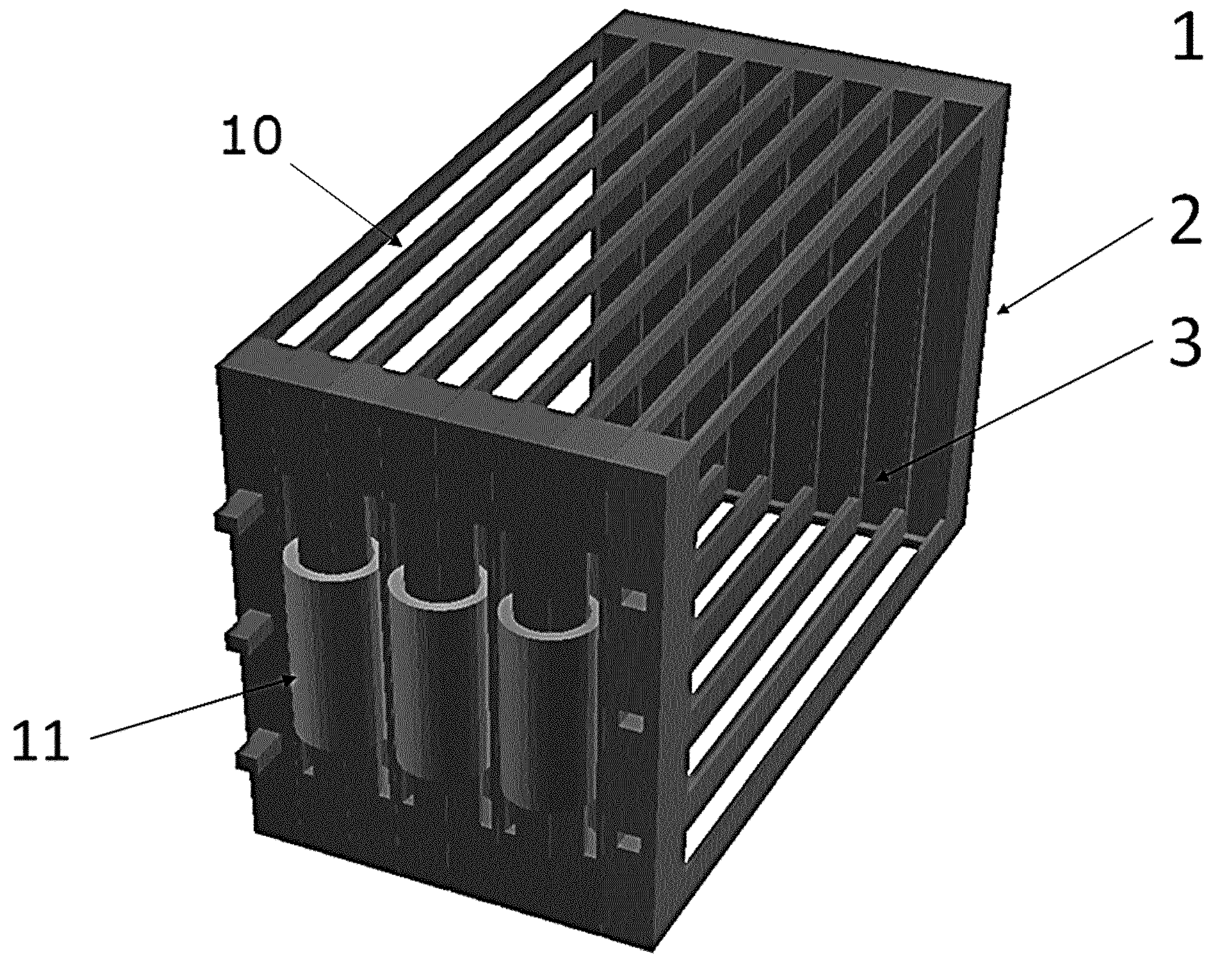


FIG. 2

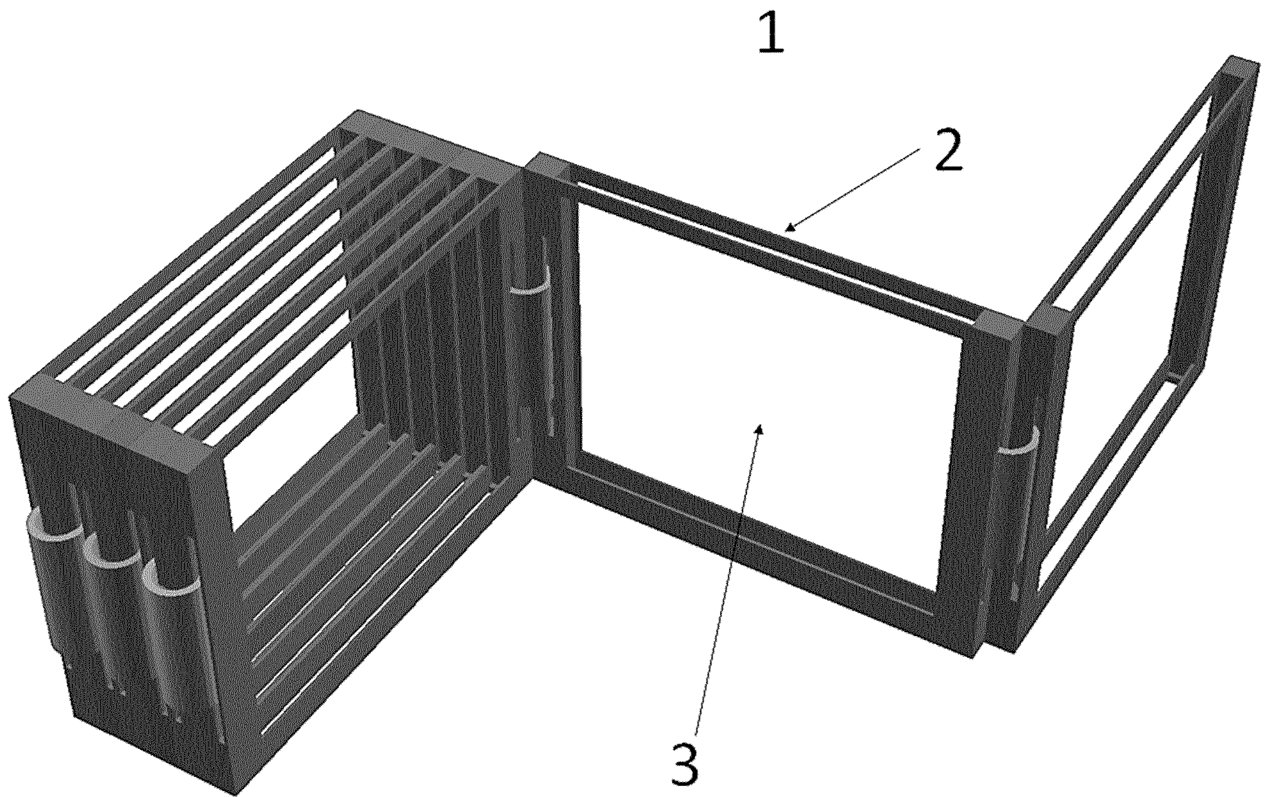


FIG. 3

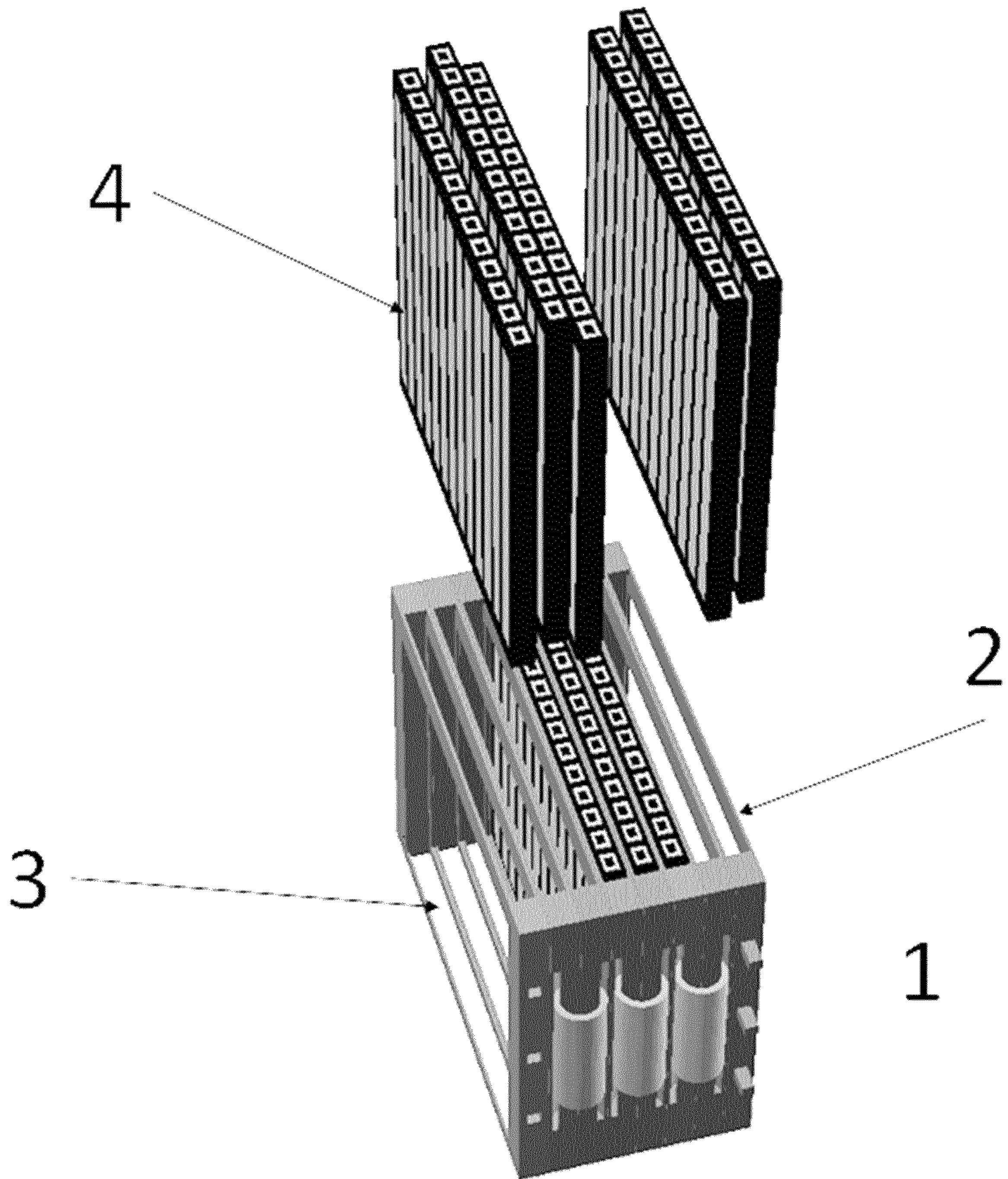


FIG. 4

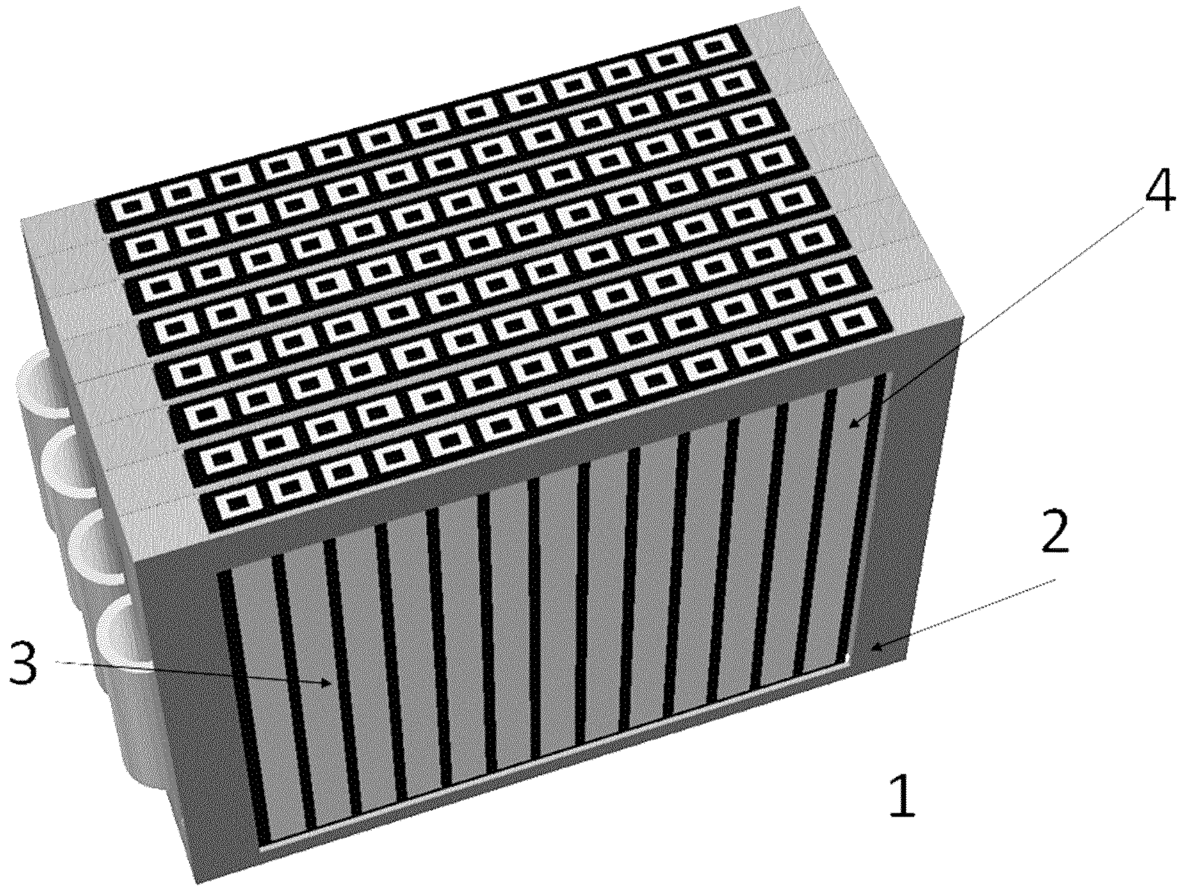


FIG. 5

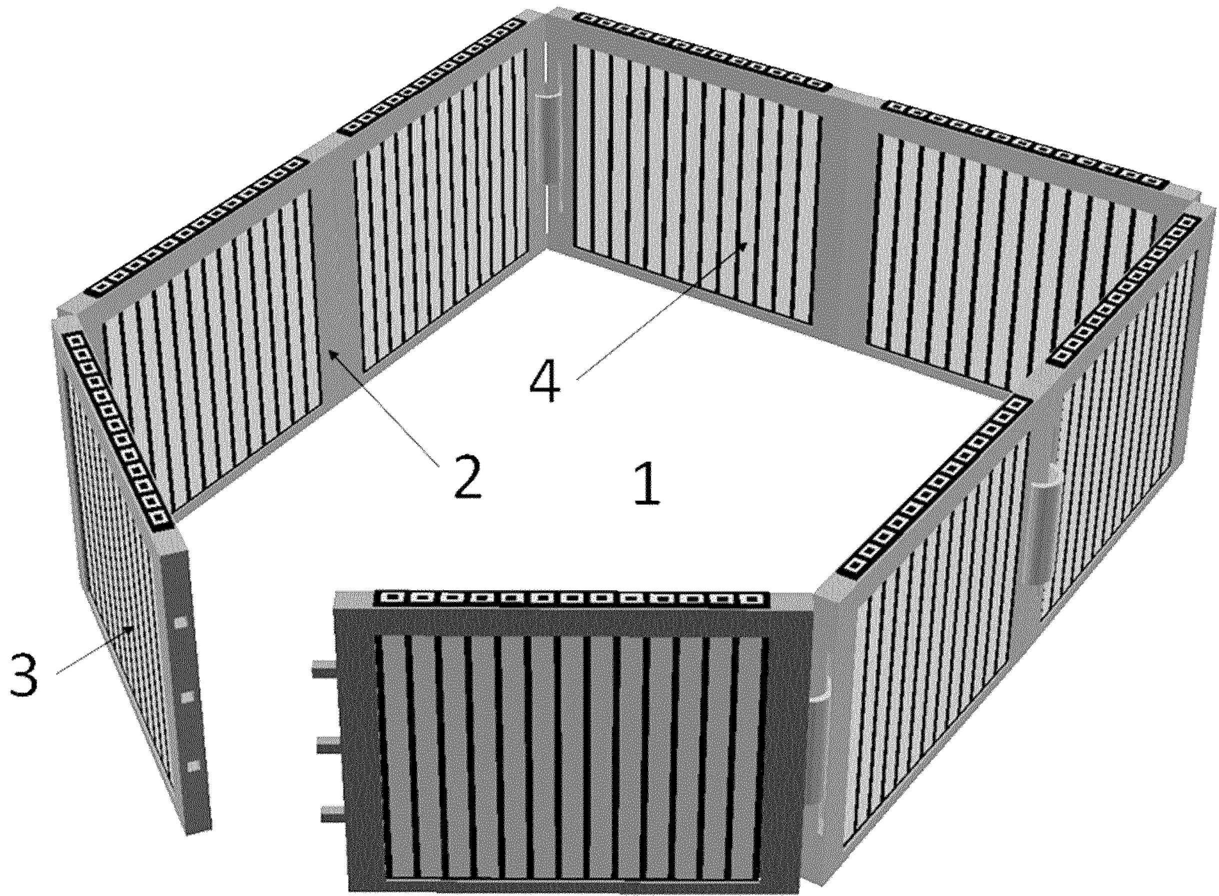


FIG. 6

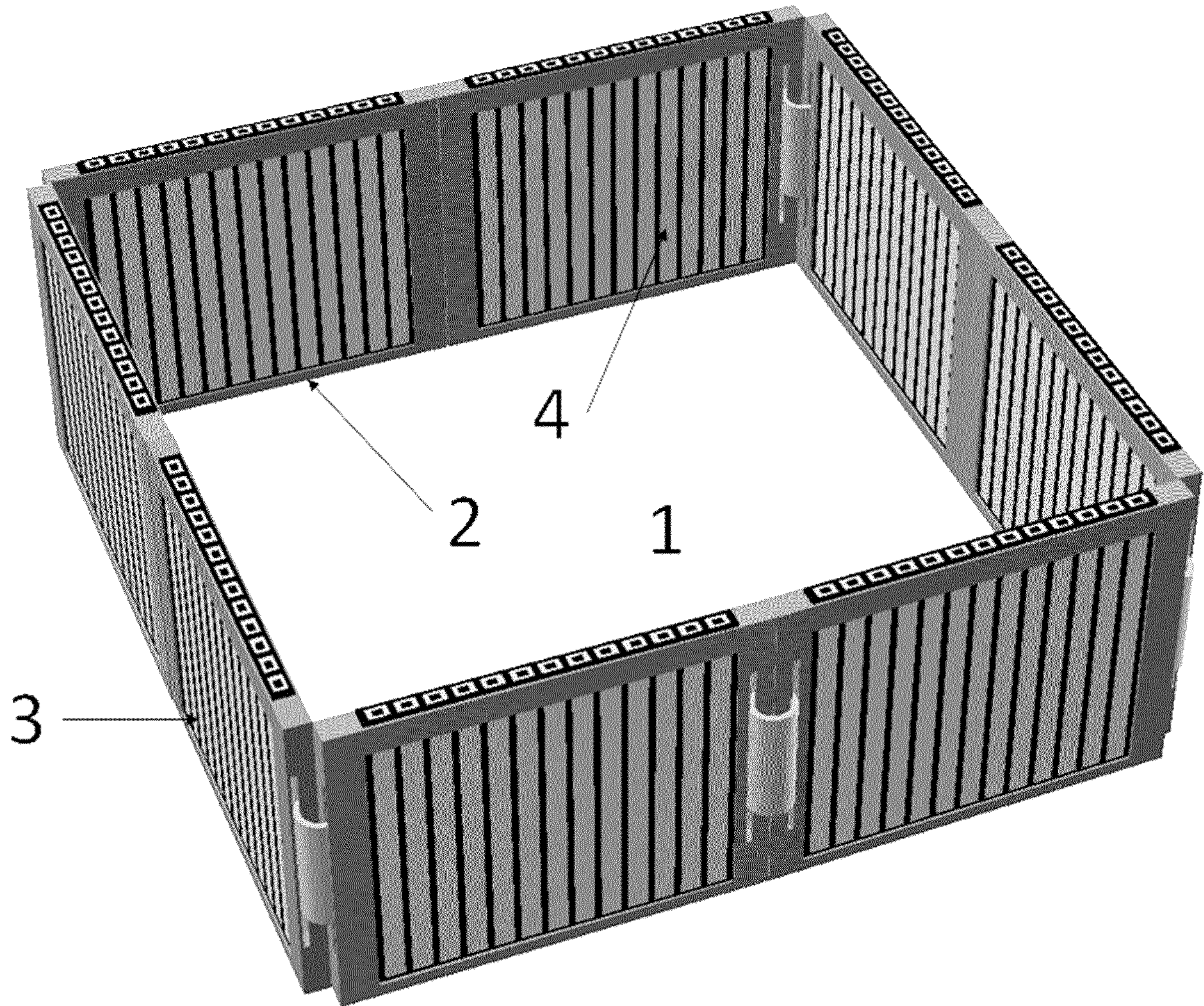
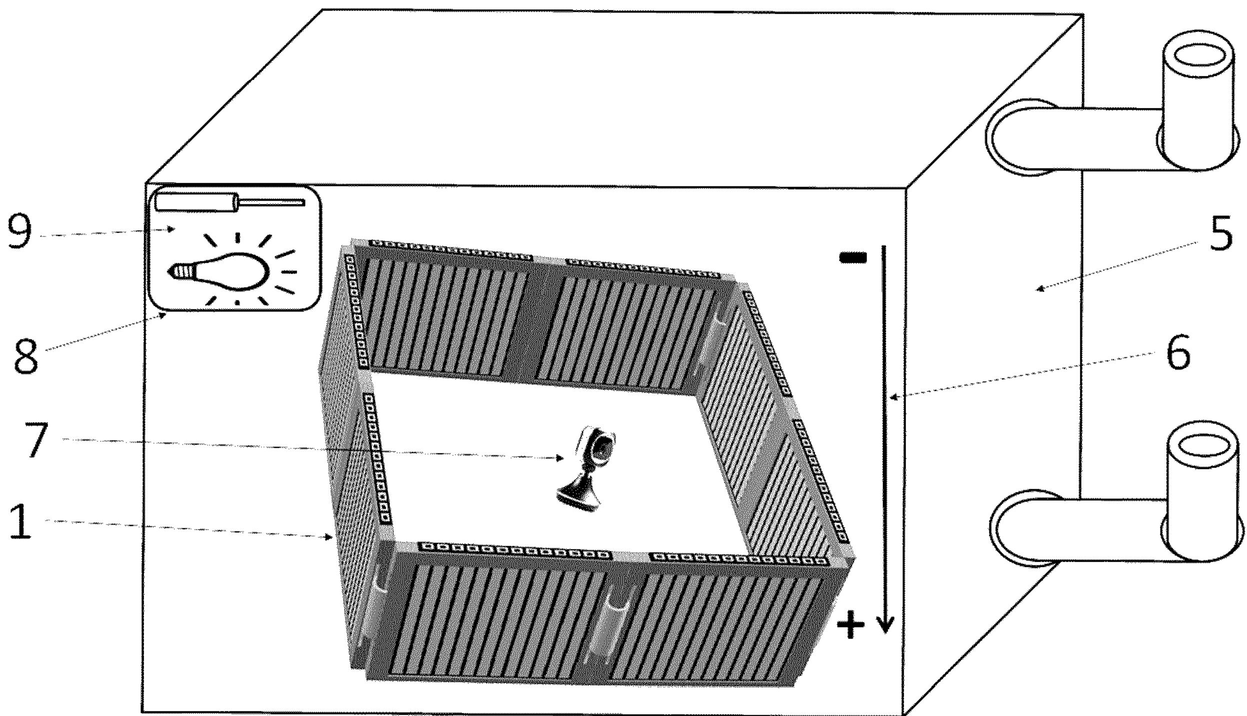


FIG. 7



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/078335

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/447
ADD. C07K1/26

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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2004/195103 A1 (ZHOU DEMING [US]) 7 October 2004 (2004-10-07) paragraph [0047]; claim 1; figure 2 figure 20 paragraph [0047]	1-46
X	WO 2005/098408 A1 (C B S SCIENT CO INC [US]; SCOTT CHARLES B [US] ET AL.) 20 October 2005 (2005-10-20)	41,42
A	page 8, lines 10-14; figures 8B, 8C, 9 page 12, lines 1-9	1-40, 43-46
A	CN 203 540 581 U (CHINA NAT RICE RES INST) 16 April 2014 (2014-04-16) claims 1,2; figure 2	1-46

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[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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"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

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"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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Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2020	21/01/2020

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1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/106832 A2 (SAGE SCIENCE INC [US]; MAGNANT GARY [US]; FINNEY MICHAEL [US]; BARBERA) 20 September 2007 (2007-09-20) claims 1,11; figure 12 -----	1-46
A	WO 2015/079048 A1 (GE HEALTHCARE BIO SCIENCES AB [SE]) 4 June 2015 (2015-06-04) figure 6a -----	1-46

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/078335

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		WO 2007106832 A2	20-09-2007
WO 2015079048 A1	04-06-2015	NONE	