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(54) **Strip for multiparametrics assays**

Streifen für multiparametrische Assays

Bande pour analyses multiparamétriques

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Description

Field of the invention

[0001] The present invention is related to an assay strip having a 8-well format for performing biological reactions between target molecules and capture molecules, wherein these capture molecules present are preferably fixed upon the inner surface of a well of the strip in the form of a micro-array.

[0002] The present invention is also related to multiple strips for performing multiple assays in a 96-well format frame.

Background of the invention

[0003] Biological samples are difficult to analyze, because of the complexity of the molecules being present in biological organisms and the large number of molecules having similar chemical or physical characteristics. On the other side, there is a need to perform analyses on materials as small as possible given the fact that, in many analyses, the amount of material available is very limited. The miniaturized assays for multiple components are now performed on a miniaturization scale using micro-arrays.

[0004] Currently, micro-array assays are performed on an individual basis along with the different steps of the method from the incubation with the sample to the scanning of the array and the data analysis. These methods are not adapted for handling multiple samples simultaneously. The ability to do so would be advantageous for routine assays requiring automated procedure, (preferably upon multi-wells plate) such as in clinical diagnostic laboratories or in high throughput screening.

[0005] Assays in molecular biology require reaction temperature being high for controlling the specificity when working with polynucleotides reaction. At these high temperatures, evaporation will occur, if devices used for performing these reactions are not perfectly sealed. Furthermore, a possible deformation of substrates bearing the reactive molecules at high temperatures will also hamper the specific detection of multiple targets at the end of the assay.

State of the art

[0006] US patent 7,390,463 describes a fluidic device compatible with multi-well plate format, having capture molecule immobilized at the bottom of the well. Fluidic modules comprising channels can be inserted into individual wells of the plate in order to seal the wells. US patent 5,874,219 provides methods for processing multiple biological chip assays by providing a biological chip plate comprising a contiguous substrate with a plurality of probe arrays. Surrounding the probe arrays, the support comprises a material that is resistant to the flow of liquid thereby forming a plurality of test wells, each test

well defining a space for an introduction of a sample. These probe arrays are made of small oligonucleotides which are hybridized at low temperature usually comprised between about 25°C and about 30°C.

[0007] The US patent 5,545,531 provides methods for making a device for processing multiple biological chip assays comprising the steps of providing a wafer comprising on its surface a plurality of probe arrays and applying a material resistant to a flow of a liquid sample so as to surround the probe arrays, thereby creating test wells.

[0008] The US patent application 20040191810 proposes a plate having conical wells for holding immersed micro-arrays. The plate comprises a body with a plurality of conical wells having a frame for the attachment of the arrays. The conical wells also contain inlets and outlets. This frame has perforations along the sides of the micro-arrays that interact with the inlets and outlets to facilitate liquid pipetting and control bubbles. An optically clear window is attached to the body for possible detection. These micro-arrays are first produced and then have to be inserted inside the well. The wells are completely open.

[0009] The US patent application 20040171167 proposes alternative methods for analysis of micro-arrays by packaging micro-array suspended in a well of a well plate. This configuration was proposed for easier handling as there is no micro-array at the bottom of the wells. Here also the array is first manufactured and then inserted inside the wells.

[0010] The US patent 7,220,573 describes an array assay device comprising a base dimensioned to hold a substrate having at least one array, a cover dimensioned to cover said substrate and a snap-fit for holding said cover and said base together and to enclose a space between said cover and said base. Preferably, this array assay device is substantially vapor and fluid tight, when the snap-fit hold said base and said cover together.

[0011] However, such device is not compatible with multi-well format for performing biological reaction between target molecule and capture molecule.

[0012] Mendoza et al. (1999, Biotechniques, 27, 778-80 782-6, 788) provides biochips in multi-well format for the detection of protein wherein the solid support is made of an optically flat, glass plate containing 96 wells formed by an enclosing hydrophobic Teflon mask. Each of these wells contains at its bottom four identical 36-element arrays (144 elements per well) comprising 8 different antigens and a marker protein.

Aims of the invention

[0013] A first aim of the present invention is to provide an assay strip, a plate comprising multiple strips or an apparatus comprising strip(s), for performing multiple assays in parallel and possibly on multiple target molecules, possibly present (simultaneously) in a (biological) sample, in a miniaturized format that does not present the

drawbacks of the state of the art.

[0014] A preferred aim of the present invention is to propose such assay including a sample injection, an incubation even at high temperature and a washing in a optimized device which avoids cross contamination between wells, avoids a bubble formation inside the wells and allows an easy, efficient and reproductive detection of target molecules bound on corresponding capture molecules thereafter.

Summary of the invention

[0015] The present invention is related to an assay strip C compatible with multi-well plate format and adaptable upon a frame D of a multi-well plate for performing a series of biological reaction between capture and target molecules, comprising:

- a strip A consisting of at least two wells 2, preferably three, four, five, six, seven or eight (adjacent) wells 2, each well 2 having an external bottom surface, preferably surrounded by a ring of at least 1 mm high, a flat inner bottom surface 5 (the surface 5 which is present inside the well 2 at the bottom of the well 2) upon which capture molecules are immobilized, and an open top surface 3, adapted to receive a cap 6, having preferably a rough surface, wherein these wells 2 are preferably linked to each other by attachments 4,
- a cap strip B comprising at least two (adjacent) and preferably, three, four, five, six, seven or eight (adjacent) caps 6, wherein these caps are preferably linked to each other by attachments 9 located at the top part 7 of these caps 6, wherein each cap 6 comprises at least one inlet channel 10 and at least one outlet channel 11, wherein there is a trapping well (15) around these inlet and outlet channels 10, 11

wherein this said cap strip B seals the strip A and wherein side walls of the cap 6 comprise a pad 14 to seal the cap 6 inside the well 2, to form a chamber having a height of less than five mm, preferably less than two mm and even less than one mm and

wherein this pad 14 has a thermal expansion coefficient which is higher than the thermal expansion coefficient of the well 2.

[0016] Preferably, in the assay strip according to the invention, the inlet of the cap strip B comprises a conical part and the inlet channel 10 of the cap strip B forms an angle lower than 85° and more preferably lower than 75° with the flat inner bottom surface 5 of the well 2 upon which capture molecules are immobilized.

[0017] Advantageously, the inlet 10 and outlet 11 channels are linked by a ditch 13 at the bottom part 8 of the cap 6 and the adjacent wells are linked to each other by attachments located at or close to the top surface 3 of the wells 2. In the assay strip according to the invention, the flat inner bottom surface 5 of all wells upon which

capture probes are immobilized is preferably in the same focal plan. Preferably, the flat inner bottom surface 5 of each well is being separated from the external observation surface by an optically transparent solid support for detection of target molecules along an observation angle (θ_{ob}) being a forbidden angle, and wherein said solid support has a refractive index higher than 1.33. More preferably, in the assay strip, each well 3 has an external flat bottom surface which is not parallel to the flat inner bottom surface 5 and forms an angle between about 90° and about 62° with the normal to said flat inner bottom surface 5 preferably an angle between 85° and 70° with the normal to said flat inner bottom surface 5.

[0018] According to a preferred embodiment, a side face of the solid support used for the detection of target molecules along the forbidden angle is between 0° and 36° preferably between 5° and 30° more preferably between 10° and 25° with the normal to the flat inner bottom surface 5.

[0019] According to another embodiment, the well 2 walls of the assay strip according to the invention have an opening angle of about 1.5° with the normal to the flat inner bottom surface 5.

[0020] Another aspect of the present invention, is related to an assay strip which further comprises an adapter F comprising at least two supercaps 23, preferably eight supercaps 23, each supercap having at least an inlet 24 fitting inside the cap strip inlet channel 10 or outlet channel 11 and having a conical part and possibly wherein the inlet 24 has preferably a length which is equal to the depth of the well 2 minus 0.5 and preferably minus 0.2 mm.

[0021] Another aspect of the invention is related to a multi-well plate (having frames preferably with squared shape E complementary to the well 2 with a multi-well chassis D, preferably having 96 frames (twelve x eight) for inserting, preferably at least one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve assay strip (s) C, (preferably composed of eight wells 2 sealed by eight caps 6).

[0022] The multi-well chassis D further comprises attachment feature(s) 17 complementary to attachment feature(s) 16 of the strip C and/or attachment feature(s) 19 complementary to mounting foot(s) 18 of the strip C.

[0023] The invention also relates to a multi-well plate for performing a series of biological reactions between capture molecules and (corresponding) target molecules, comprising:

- 96 wells 2, each well 2 comprising a (flat) inner bottom surface 5 upon which capture molecules are immobilized and an open top surface 3 adapted to receive a cap 6, wherein adjacent wells are preferably linked to each other by attachments 4,
- a well cap comprising 96 caps 6, each cap 6 comprising at least one inlet channel 10 and at least one outlet channel 11, wherein there is a trapping well (15) around these inlet and outlet channels 10, 11

and wherein adjacent caps are preferably linked to each other by attachments 9 located at the top part 7 of the caps,

- wherein side walls of the cap 6 comprise a pad 14 to seal these cap 6 inside these well 2, to form a chamber having a height of less than five mm, preferably less than two mm and even less than one mm,
- and wherein this pad 14 has a thermal expansion coefficient which is higher than the thermal expansion coefficient of the well 2.

[0024] Still another aspect of the invention is related to a method for a detection and/or quantification of a target molecule performed by the following steps:

- inserting a (biological) sample (solution) containing a target (biological) molecule into the strip or the multi-wall plate according to the invention and containing immobilized (corresponding) capture (biological) molecules at flat inner bottom surface 5 of wells 2;
- sealing the strip A with the cap strip B to form an assay strip C;
- incubating the assay strip C in reaction condition for a binding (reaction) between the said target molecule and its (corresponding) capture molecule;
- optionally removing the sample solution through the cap strip B and inserting washing solution in these wells 2;
- optionally removing the cap strip B from the strip A;
- optionally incorporating reagents generating a signal resulting from the binding between the target molecule and its corresponding capture molecule in the wells 2 to provide a detectable signal;
- measuring this signal (at the target molecule location i.e. fixed upon its corresponding capture molecule) to detect and/or quantify the said target molecule in the said sample (solution).

[0025] In the method according to the invention, the capture molecule binding the target molecule is present on an optically transparent solid support surface having a refractive index n_1 , said solid support being in contact with a medium having refractive index n_2 , whereby $n_1 > n_2$, said method comprising the steps of:

- a. illuminating the target molecule, thereby causing the target molecule to emit light;
- b. detecting light emitted from the target molecule through said support at an observation angle θ_{ob} relative to the normal to the solid support surface, such that $90^\circ > \theta_{ob} > \sin^{-1}(n_2/n_1)$.

[0026] According to another preferred embodiment, the target molecule is a polynucleotide sequence synthesized in the wells by copying and/or amplifying a polynucleotide sequence present in the sample solution inserted into the wells.

[0027] Another aspect of the invention is related to an apparatus, (preferably a high throughput screening apparatus of one or more target molecules) which comprise the assay strip (C) or the multi-well plate according to the invention.

[0028] A last aspect of the invention is a kit of part comprising the strip A comprising capture molecules (probes) fixed (immobilized) upon the strip A (onto the inner bottom surface of the well) and cap strip B of the assay strip C or the multi well plate according to the invention and possibly sealing means for the cap strip, one or more washing solution(s) and one or more detecting solution(s).

[0029] The present invention will be described in more details in following detailed description in reference to the enclosed figures presented as non-limiting illustrations of the various embodiments of the present invention.

Short description of the figures and tables

[0030]

Figure 1 represents a 3D view of a preferred assay strip C of the invention comprising a strip (A) and a cap strip B. The assay strip C is preferably inserted into frames E of a multi-well chassis D.

Figure 2 represents different views (top, bottom, side) of the strip A of figure 1 as well as attachment features 16, 18 which are complementary to attachment features 17, 19 of the multi-well chassis D and allow its insertion into the multi-well plate. The strip A has a discontinuous bottom surface 1 and comprises 8 wells 2, each well 2 comprising a (flat) bottom surface 5 upon which capture molecules are immobilized and an open top surface 3 adapted to receive a cap. The strip also preferably comprises asymmetric extremities or holding pads 22, 22' in order to allow only one orientation into the multi-well plate. Adjacent wells are linked by attachments 4 located at or close to the top surface 3 of the wells. The bottom surface of the wells 2 also comprises preferably an o-ring 20 of polymer of at least 1 mm to avoid scratches of such surface due to contact with various tables surfaces.

Figure 3 represents different views (top, bottom, side) of the cap strip B of figure 1 comprising 8 caps 6, each cap 6 comprising at least one inlet channel 10 and at least one outlet channel 11 which are linked by an open channel 13 at the bottom surface 8 of the cap. Adjacent caps are linked by attachments 9

located at the top surface 7 of the caps. The cap strip also preferably comprises asymmetric extremities or holding pads 12, 12' in order to fit with the strip A allow only one orientation into the multi-well plate. Advantageously, to avoid solution to go out of the wells, the top surface 7 of the cap strip B comprises a depression 15 around the inlet and outlet channels 10, 11 where the exceeding solution is trapped. The side walls of the cap 6 comprise a pad 14 to seal the cap 6 inside the well 2, to form a closed and tight chamber.

Figure 4 represents different views (top, bottom, side) of the multi-well plate of figure 1 comprising a multi-well chassis D and frames E. The chassis comprises asymmetric attachment features 17, 19 which are complementary to the attachment features 16, 18 of the strip A.

Figure 5 represents different views (top, side) of the cap strip B of figure 1 comprising eight caps 6, which is superimposed with an adapter. F allowing automatic pipetting of solution into the assay strip. The adapter F comprises eight supercaps 23, each supercap 23 comprising at least one conical part 24 (inlet and/or outlet) which corresponds to the position of inlet channel 10 and outlet channel 11 of the cap 6. Adjacent subcaps are linked by attachments 9 located at the top surface 7 of the adapter F. The adapter F also preferably comprises asymmetric extremities in order to fit with the cap strip B and allow only one orientation into the multi-well plate. Once the adapter F is fixed on the cap strip B, they form a adapted cap B' which allows automatic pipetting. Alternatively, the cap strip B and the adapter F may be fused into a single part.

Figure 6 represents the results of the post-PCR analysis of GMO RRS 0.1% by hybridization on a microarray present at the bottom surface of wells being part of the strip of the invention. The experiment was performed in 6 wells in parallel. The genetic elements detected in the 6 wells were P35S, Tnos, EPSPS, Lectin and Rbcl which is in complete agreement with the element present in the GMO RRS.

Detailed description

[0031] The strip assay according to the invention is configured in order to obtain a chamber that is vapor and fluid tight. Therefore, the side wall(s) of the cap 6 comprise a pad (or feature 14) to seal the cap 6 inside the well 2, so that the (flat) bottom surface 5 of the well 2 and the bottom surface 8 of the cap 6 are essentially parallel to each other.

In a preferred embodiment, the cap strip is sealable and removable from the strip.

[0032] Advantageously, the cap strip is made of a flex-

ible material such as thermoplastic elastomer (TPE) to better seal the wells. Preferably the (linear) thermal expansion coefficient of the cap strip material is higher by at least 5 and better 10 and even better more than 20 $10^{-6}/K$ at $20^{\circ}C$ in $m/m.K \times 10^{-6}$ than the thermal expansion coefficient of the well material. The thermal expansion coefficient is a fractional change in length per degree of temperature change. It is usually defined as followed:

$$\alpha = \frac{1}{L_0} \frac{\partial L}{\partial T}$$

where L_0 is the original length, L the new length, and T the temperature.

The linear thermal expansion is the one-dimensional length change with temperature.

$$\frac{\Delta L}{L_0} = \alpha_L \Delta T$$

The linear thermal expansion coefficient is related to the volumetric thermal expansion.

The change in volume with temperature can be written:

$$\frac{\Delta V}{V_0} = \alpha_V \Delta T$$

For exactly isotropic materials, the volumetric thermal expansion coefficient is very closely approximated as three times the linear coefficient.

$$\frac{\Delta V}{V_0} = 3\alpha \Delta T$$

[0033] Preferably, the top surface 3 of the wells 2 has a rough surface to better seal the caps 6. The presently used surface topography specification is based on comparisons of e. g. polymer masters which should meet the quantitative requirements of the German VDI scale of surface textures (VDI 3400: June 1975). This scale is a logarithmic scaling of peak-to-valley and average-height amplitudes of the surface texture as measured on tools of typically VDI 12 to 45, which indicate the average roughness. Roughness is preferably VDI 33.

[0034] Advantageously, the cap strip B has been designed to avoid bubble formation either during the intro-

duction of the sample and/or during the incubation. The diameter of the outlet channel 11 is at least 1 mm preferably about 1.2 mm to allow bubble to get out of the chamber. Furthermore, the inlet channel 10 and outlet channel 11 are preferably linked by a ditch 13 or guiding area located on the bottom part 8 of the cap 6 in order to guide bubble to the exit (outlet channel 11). This ditch is preferably not flat with the upper part being oriented to the outlet and the lower part to the inlet channel, so as to guide the air outside the chamber when injecting solution (through the inlet channel).

[0035] One of the problem solved by the present invention is the possibility to use an automate for injecting the solution in the wells. The strip is best designed in order to fit the pipette in the inlet and inject liquids without touching the array, preferably the inlet of the cap strip B comprises a conical (injection) part so that it can accommodate a pipette being inserted into such conical part. Preferably the tip of the conical part is sticking out in the well chamber and more preferably is close to, but not touching the surface. This particular feature is well adapted for high or middle throughput use of such a strip which indeed allows several assays to be performed in parallel.

[0036] Washing is also best obtained in an automatic process by having a pipette in the inlet and/or in the outlet opening comprising preferentially a conical part so that it can aspirate the liquid of the wells. Preferably the inlet channel 10 of the cap strip B forms an angle lower than 85° and preferably lower than 75° with the flat inner bottom surface 5 of the well 2 upon which capture molecules are immobilized. In this way injection flow will be directed on a side of the surface, where the capture molecules and the targets are not fixed, so as to avoid any possible detachment by the injecting flow.

[0037] The invention also includes the possibility for having a cover or adapter F having an appropriate injection part which fit into the cap inlet and being placed on the cap strip during the injection and/or the aspiration of the liquids from the wells. The cover is designed as such as an injection part does not touch the surface of the wells containing the capture probes, but is made such as to be very close to this surface. The liquid influx is best dispersed by solid part(s) being present in the injection section of the inlet channel and which splits the liquid flux. Also preferred is the orientation of the liquid influx on the side of the well in order to avoid direct flux of injection liquid on immobilized target molecules. Preferably, the outflow of the liquid from the injection inlet is oriented with angle lower than 85° and better lower than 75° compared to the inner flat surface of the well comprising the immobilized probes.

[0038] In a preferred embodiment, the assay strip C comprises an adapter F comprising at least two supercaps 23, preferably eight supercaps 23, each supercap having at least an inlet 24 fitting inside the cap strip inlet channel 10 or outlet channel 11 and having a conical shape (figure 5). Preferably the inlet 24 has a length which is equal to the depth of the well 2 minus 0.5 and

preferably minus 0.2 mm.

[0039] According to a preferred embodiment, the capture molecules (probes) are immobilized (fixed) on the (flat) inner bottom surface 5 of the well 2 in discrete regions in the form of a micro-array having a density of at least 4, preferably 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 400, 600, 1000 or more discrete region per cm^2 . Advantageously, the sealing of the cap strip B into the strip A prevent or avoid drying out of this array.

[0040] Preferably, this (flat) inner bottom surface of all wells upon which capture molecules are immobilized are in the same focal plan.

Also preferably, each well has an external (flat) surface which is parallel to the (flat) inner surface and all surfaces are in the same focal plan.

[0041] Advantageously, the distance (tolerance) between this micro-array comprising capture molecules and the well 2 side walls is higher than 1 mm, for allowing an efficient delivery of capture molecules upon this (flat) bottom surface 5 of the well 2 by a spotting arrayer. The distance of at least 1 mm also avoids border effects during incubation with the sample (capillary movements along the walls. Preferably, the pitch between adjacent wells 2 is about 9 mm.

[0042] The assay strip C of the invention allows many tests upon many arrays to be set up and processed together; because they allow much higher throughput of test samples and greatly improves the efficiency for performing assays on biological micro-arrays.

[0043] The assay strip C allows proper formation of a tight chamber resulting from the sealing of two complementary parts strip A, cap strip B by avoiding the use of glue that can interfere with biological reaction which is difficult to remove from the support and which can induce a fluorescent background.

[0044] Advantageously, The volume of liquid above capture molecules present on a (flat)bottom surface 5 of the well 2 is small and remains homogeneous on the overall bottom surface 5 comprising these immobilized capture molecules during the different processing steps, this requirement improves the reproducibility of the assay, particularly when these capture molecules are present in a micro-array format.

[0045] A sealing of a cap strip B on a strip A, to form the assay strip C of the invention is also configured to avoid contamination of a sample solution contained in a first well by another sample solution, possibly contained in an adjacent well. The bottom surface 5 of the wells 2 has preferably a square shape with round shape corner to avoid capillary effects bringing solution out of the well. The corner radius is preferably of about 1.5 mm. Advantageously, to avoid solution to go out of the wells, the top surface 7 of the cap strip B comprises a trapping well 15 around the inlet and outlet channels 10, 11 where the exceeding solution is trapped.

[0046] The assay strip C according to the invention allows easy access to the bottom surface 5 of fixed by capture molecules as required for the scanning of the

array. Also the chamber can be dissociated and re-associated according to the constraints of the fabrication or of the multiple steps of a detection method. These sealing and opening step have to be easily performed either manually or by using a robotic automate that can be also used for the formation of the array on this bottom surface 5 and for a scanning of this bottom flat surface 5 comprising the capture molecules.

[0047] The assay strip C can be subjected to a plurality of processing steps, including genetic amplification such as PCR, hybridization, washing, staining and scanning steps. The cap strip B can be removed after a sample incubation, thus making the strip A easily accessible for successive working and labelling either manually or by using an automatic device.

[0048] Preferably, adjacent wells 2 of the strip A are preferably linked to each other by attachments located at or close to the top surface 3 of the wells 2 in order to allow a tight insertion into a multi-well plate.

[0049] The assay strip C according to the invention is compatible with a multi-well format. Therefore, another aspect of the present invention is related to a multi-well plate comprising a multi-well chassis D and at least one, preferably twelve assay strip(s) C according to the invention.

[0050] Preferably, this chassis D comprises 96 square frames E (12 x 8) for inserting up to 12 assay strip(s) C made of square shape wells 2 according to the invention. Dimension of the chassis D are preferably about 85.5 mm (W) x 127.8 mm (L) x 14.8 mm (H).

The chassis D is made of a polymer being preferably resistant at 100°C. The polymer is preferably transparent polycarbonate.

[0051] Advantageously, this frame E has squared shape (format) which is obtained at low cost, but allow an easy insertion of the assay strip(s) C, especially the wells 2 of strip(s) A into the frame E of the plate. Preferably, the square is about 8 x 8 mm. The chassis D has preferably an asymmetric shape for receiving the assay strip C in a single orientation. For example it may present insertion features 17 and 19 fitting with complementary features 16 and 18 of the strip A in a predetermined position into the chassis D as provided in figures 2 and 4. Preferably, the extremities or holding pads 22, 22' of the strip A and/or the extremities or holding pads 12, 12' of the cap strip B have different shapes (i.e. one pad is square and the other one is circle) to fit with this requirement. Advantageously, the square pad may be used to apply a code bar. This insertion system provides an adequate alignment of the top surfaces of different assay strips C positioned in a same plane above the frames E.

[0052] The extremities of the strip A may comprise a mounting foot 18 to maintain strip A in the multi-well chassis D. Advantageously, the mounting foot 18 is inserted in the attachment feature 19 of the multi-well chassis D. Furthermore, the extremities of the strip A are asymmetric and one of them comprises the attachment feature 16 that is complementary to the attachment feature 17 of

the multi-well chassis D, as provided in figure 2.

[0053] The assay strip according to the invention is also adapted for easy automation of reaction inside these strips. The assay strips C are aligned in the same orientation in the multi-well chassis D by means of the mounting foot 18 inserted in the attachment feature 19 of the multi-well chassis D and also by means of complementary attachment features 16 and 17. Advantageously, as the bottom surface of the strip A is discontinuous, each well 2 is easily inserted and maintained in the frames E of the multi-well chassis D. These particularities allow an alignment of the assay strips C in the multi-well chassis D. A correct alignment of the assay strips C is important for an efficient positioning of automatic handling elements into the assay devices C and also for obtaining an efficient detection of target molecules fixed in their capture molecules. Examples of automatic handling elements are: pipette tips which are inserted into the inlet 10 and outlet 11 channels of the strip caps B. Automation of the different steps of the process makes the assays much more reproducible and thus leads to a better quantification of the tests which is a requirement of assays on micro-arrays where the quantification of the results are obtained by the detection of signals on very small surfaces.

[0054] Advantageously, the multi-well chassis contains 12 inserted assay strips, each one being inserted into the chassis by means of asymmetrical extremity. This feature was found useful for stable and unambiguous insertion of the assay strip and to have all flat surfaces aligned on the same height so that they are in the same focus for the detection step.

[0055] Advantageously, the number of assay strips C inserted in the multi-well chassis D is adapted according to the customer need and the number of samples to be processed in parallel.

[0056] Preferably, when inserted into the frames E of the multi-well chassis D, the distance between the external bottom surface of the assay strip C or the wells 2 of the strip A and a support surface (table surface) is of at least 1 mm. When the assay strip C according to the invention is incorporated in the chassis D, the surface of the chassis D in contact with a support (table surface) is preferably aligned with the external bottom surface 1 of the strip A or of the well 2. This surface 1 is opposite to the inner bottom surface 5 comprising the immobilized capture molecules.

[0057] To avoid any contact between the external bottom surface 1 of the strip A or of the well 2 and a solid support, the strip A is inserted in the multi-well plate at a position which avoid any contact between this external bottom surface 1 and the solid support preferably, in this position the external bottom surface is located at the distance of at least 1 mm from this solid support surface. Preferably, the contact is made only between the solid support surface and the chassis D of the multi-well plate.

[0058] The bottom surface 1 of strip A or of the well 2 may present also an ring format 20 of polymer in a height

of at least 1 mm. This feature avoids scratches of the external bottom 1 of the well 2 due to contact with various tables surfaces. An ring 20 of polymer of at least 1 mm high surrounding the external bottom surface 1 of the well 2 is depicted in figure 2.

[0059] For an easy production, the wells 2 of the strip A and the caps 6 of the cap strip B have preferably a square shape for an easy introduction into the frames E of the multi-well chassis D. However, other shapes or others sections of the wells could be selected by the person skilled in the art.

[0060] The assay strip C according to the invention may further comprises means for sealing inlet and outlet channels 10, 11 at the top surface 7 of the cap strip B so as to obtain a sealing of inlet port and an outlet port of this inlet channel and outlet channel. Sealing typically comprise the aluminium sealing foil, plastic foils or plugs.

[0061] In the assay strip according to the invention, the capture molecules are selected from the group consisting of nucleotide sequences (polynucleotide sequences or oligonucleotide sequences), proteins, peptides (preferably antibodies or hypervariable portions thereof) polysaccharides or a mixture thereof. These capture molecules are capable of binding complementary target molecules which are preferably selected from the group consisting of nucleotide sequences, proteins, peptides, haptens or a mixture thereof.

[0062] Advantageously, the capacity volume of the chamber formed in the assay strip C according to the invention is comprised between about 10 μ l and about 200 μ l, preferably about 10.0 μ l.

[0063] In a preferred embodiment, the capacity volume of the well 2, when not sealed by the cap is comprised between about 100 μ l and about 1000 μ l.

[0064] In a preferred embodiment, the wells 2 of the strip A have a square shape to maximize the spotting area of the capture molecules, particularly when they are spotted in the form of a micro-array. Preferably, the well walls have an opening angle of about 1.5° with the normal to the (flat) inner bottom surface 5.

[0065] Round corners of the walls are preferred to avoid capillary effects both in open well 2 or closed well 2 with cap strip B. Preferably, the (flat) bottom surface 5 of the well 2 has a square shape with the corners being curved and having a curve shape corresponding to a circle of 1.5 mm of radius.

[0066] The assay strip C according to the invention is made of two parts: the strip A and the cap strip B which are advantageously made of different polymeric materials. Preferably, the strip A is made of a rigid polymer while the cap strip B is made of flexible polymer.

[0067] The strip A is preferably made of a rigid low fluorescence material, preferably selected from the list consisting of cycloolefin, polycarbonate, polyacrylate and polyethylene, more preferably a cycloolefin polymer.

[0068] The cap strip B is preferably made of a flexible polymer, preferably thermoplastic elastomer (TPE).

[0069] The bottom surface 5 onto which the capture

molecules are immobilized is preferably transparent to light and distortion free for purposes of imaging the surface. In the preferred embodiment, the transmission of the light of wavelength between 400 and 600 nm is higher than 50% and even higher than 90% and even higher than 99%. This material should preferably also be non-fluorescent in order to minimize the background signal level and allow detection of low level signals from low intensity features on the surface. Typically the intrinsic fluorescence of the material should be lower than 10 % and even lower than 1% of the fluorescent emitted by the location where the target is bound.

[0070] The capture molecules are preferably covalently immobilized on Zeonex™ (330R) using the chemistry described in the EP Patent application 06112775.9. incorporated herein by reference. The well B bottom thickness is preferably of less than 1 mm with 50 μ m tolerance between the first well and the last well of one strip.

[0071] Advantageously, in the strip A according to the invention the bottom surface 5 of the well 2 is about 40 mm².

[0072] Preferably, the bottom surface 5 of the well 2 has a square shape with a length of about 6 mm and height of about 4 mm with a tolerance of 50 μ m. Preferably, such surface comprises round shape corners (having preferably a radius of about 1.5 mm) to avoid capillary effect.

[0073] Preferably, the strip A has width of about 8 mm, a length of about 82 mm and height of about 5 mm (without the o-ring 20) or 6 mm (with the o-ring 20) with a 100 μ m tolerance.

[0074] Preferably, the cap strip B has width of about 8 mm, a length of about 82 mm and height of about 1 mm. Preferably, the top surface 7 of the cap strip comprises at least one inlet channel 10 and at least one outlet channel 11 which are separated from each other by a pitch of about 4.5 mm. The diameter of inlet channel 10 or outlet channel 11 is about 1.2 mm.

[0075] When the strip A is sealed with the cap strip B (assay strip C) it prevents less than 10% evaporation of liquid present in the chamber after 24 hours incubation at 65°C, preferably less than 5% evaporation of liquid volume. Furthermore, the sealed assay strip C resists to agitation and may be incubated in adequate temperature in a mixing apparatus like the thermomixer from Eppendorf AG (Hamburg, Germany).

[0076] The target molecules may be labeled to allow their detection. The labelled associated detections are numerous. A review of the different labelling molecules is given in W0 97/27317. The most frequently used and preferred labels are fluorochromes like Cy3, Cy5 and Cy7.

[0077] Radioactive labelling, cold labelling or indirect labelling with small molecules recognised thereafter by specific ligands (streptavidin or antibodies) are common methods. The resulting signal of target fixation on the array is either fluorescent, colorimetric, diffusion, electroluminescent, bio- or chemiluminescent, magnetic,

electric like impedometric or voltametric (US-A-5,312,527).

[0078] A preferred label is the use of the gold labelling of the bound target in order to obtain resonance light scattering (RLS) detection or silver staining which is then easily detected and quantified by a scanner. Gold particles of 10-30 nm are required for silver amplification while particles of 40-80 nm are required for direct detection of gold particles by RLS or by Photothermal Heterodyne Imaging.

[0079] In a preferred method, gold particles of 10-30 nm are amplified by silver enhancement preferably using the silverquant analysis platform including the Silverquant kit for detection, the Silverquant Scanner for slide scanning and Silverquant Analysis software for image quantification and data analysis (Eppendorf, Germany). Due to the non linear detection of the presence of silver, the data analysis requires a linearization of data before data processing. The data are then processed according to the invention. An algorithm of curve fitting is applied to a positive detection curve spotted on the array. Then each spot signal is linearized in 'concentration units' using the fitting curve.

[0080] Assays on biological reactions are performed according by the use of the strip and cap.strip according to the present invention and having the feature as provided here above in the following way. The solution containing the target to be detected and/or quantified are inserted into the strip containing immobilized capture molecules and comprising a cap strip (B) of the assay strip (C) according to any of the preceding embodiments. The cap strips are then sealed and the strip incubated under the selected reaction conditions for the binding reaction between the target and its capture molecule to take place. The sample solution is removed preferably through the cap strip and washing solution inserted in the well. Optionally reagents are incorporated into the wells to provide a detecting signal and the signal at the target location is detected in order to detect and/or quantify the presence of the target in the sample solution.

[0081] The detection of the array based signal can be performed by direct illumination of the bound target and detection of an emitted or transmitted or refracted or absorbed light.

[0082] The light emitted from the bound target molecule can be also detected at a forbidden angle for the light emitted from the soluble target molecule.

[0083] This method allows detecting and/or quantifying a biological target molecule present on an (optically transparent) solid support surface having a refractive index n_1 , this solid support being in contact with a medium having refractive index n_2 , whereby $n_1 > n_2$, this method comprising the steps of a) illuminating the target molecule, thereby causing the target molecule to emit light and b) detecting light emitted from the target molecule through said support at an observation angle θ_{obin} relative to the normal to the said solid support surface in the support, such that $90^\circ > \theta_{\text{obin}} > \sin^{-1}(n_2/n_1)$.

[0084] Preferably the observation angle (θ_{ob}) is within the forbidden angle range, but close to the critical angle (θ_c) to be able to reconstitute the image of the support. This is especially useful when different capture molecules are present on the surface of the support and have to be differentiated from each other as in the case of microarray. Preferably the observation angle is lower than 85° , more preferably lower than 80° and still more preferably lower than 70° . In a preferred embodiment, the observation angle is within the forbidden angle and in the range of the critical angle plus 10° , preferably plus 5° and more preferably plus 3° . In a particular application, the observation angle is between about 62.4° and about 65° when the solid support has a refractive index of around 1.5 as for glass. For glass or material having similar refractive index, the critical angle θ_c is $\sin^{-1}(1.33/1.5) = \sin^{-1}(0.887) = 62.4^\circ$. The required observation angle θ_{obin} is given by the formula $90^\circ > \theta_{\text{obin}} > \theta_c$. In other words, the observation angle should be at least 62.4° .

[0085] Preferably, the (flat) inner bottom surface 5 of each well is being separated from an external observation surface by an optically transparent solid support for detection of target molecules along an observation angle (θ_{ob}) being a forbidden angle, and wherein this solid support has a refractive index higher than 1.33.

[0086] Preferably each well 2 of a strip A has an external flat bottom surface which is not parallel to the (flat) inner bottom surface 5 and forms an angle of between 90° and 62° with the normal to this (flat) inner bottom surface 5.

[0087] In another embodiment, the observation angle is within the forbidden angle and is such that the signal coming from a solution is not significantly detected compared to an emitted light of the bound target molecules.

[0088] Also preferably, a side face of the solid support used for the detection of target molecules along the forbidden angle is between about 0 and about 36° with the normal to the (flat) inner bottom surface 5.

[0089] In another embodiment, the well presents a ridge channel on the opposite side of the support compared to the observation area having immobilized capture molecules.

[0090] In still another embodiment, the illuminating and/or the detecting light are in the Total Internal Reflection (TIR) method using the support as the wave guide.

[0091] In another embodiment the invention provides an apparatus for carrying out the process of the invention requiring: an optically transparent solid support having refractive index n_1 designed to comprise, when in use, at least one target molecule bound on capture molecules present on said solid support surface (bound target molecule) and wherein the refractive index of the solid support is higher than the refractive index n_2 of the medium, where the binding of the target molecule on the capture molecule occurs; a light source to produce a light beam directed on the target molecule; a detector for measuring light emitted from the target molecule, said emitted light being detected through said optically transparent solid

support at an observation angle θ_{obin} relative to the normal to the said solid support surface in the support, such that $90^\circ > \theta_{\text{obin}} > \sin^{-1}(n_2/n_1)$.

[0092] In a preferred embodiment, in the method of the invention, the detection of the signals at the target molecule location is performed online during the target molecule binding to its capture molecule.

[0093] In another embodiment, the target molecule is a polynucleotide sequence synthesized in the wells by copying and/or amplifying a polynucleotide sequence present in the sample solution inserted into the wells.

[0094] In a preferred embodiment, the first and preferably the second removals of the solutions from the well after the incubation are performed with the cap strip still present on the strip and the following liquid handlings are then performed without the cap strips being present on the strips.

[0095] Preferably, the assay method of this invention automates the handling steps so as to allow multiple assays to be performed concurrently. Accordingly, this invention employs automated fluid handling systems for concurrently performing the assay steps in each device. Fluid handling allows uniform treatment of samples in the devices. Microtiter robotic and fluid-handling devices are available commercially, for example, from Tecan AG, Biotek or Eppendorf (Eppendorf AG).

[0096] In a preferred embodiment, handling of the assay strip C is performed by using a (multi)pipette or syringe and/or 96-wells plate format automate. Samples solutions are preferably introduced into the assay strip C through inlet port of an inlet channel 10. The air present in the chamber 1 of the device C is moved out of the chamber by the outlet channel 11. Preferably the inlet port of the inlet channel 10 and the air duct port of the outlet channel 11 are located in a depression on the cap strip where the exceeding solution is trapped if needed when injecting a too high volume of solution thus avoiding contamination from one strip to the other. The steps after the incubation step with the sample are preferably performed without removing the cap strip B by just removing sealing means applied on the inlet elements of the cap strip top surface 7.

[0097] Drying of the surface area comprising the capture molecules is also possible using the assay strip of the invention. Air aspiration is performed by one of the port.

[0098] Just before analyzing the surface comprising the capture molecules for evidence of reaction between target and capture molecules, the cap strip B is preferably removed to allow a scanning of the well 2 bottom surface 5 from the top.

[0099] Scanning of multiple assay strips C inserted into the multi-well chassis D may be performed by several means.

[0100] Scanners adapted for multi-well plate reading may be used. These scanners have usually a limited focusing tolerance. Alternatively, the scanning may be performed by using a scanner having independent focusing

system for each assay strip.

[0101] Asymmetric holding pads 22, 22' of the strip A as depicted in figure 1 are advantageously used to insert the strip into the multi-well plate chassis D having complementary structures. These support structures may be used to mount or position the strip assays C in the same orientation into the multi-well chassis D. Each frame E of the multi-well chassis D matches the shape of a well of the strip A that has preferably a square shape to avoid rotation of the assay strip C once positioned into the frame E. Asymmetric and unidirectional positioning of the assay strips C into multi-well chassis allows unique horizontal orientation. The assay strips C are also aligned vertically into the frames E due to the presence of discontinuous bottom surface 1 of the strip A allowing each well to be inserted into an individual frame and making the overall structure rigid and stable. Thus, the assay strips which are inserted in the multi-well chassis D may be easily aligned by an automatic handling device or on detection or imaging system. This feature ensures proper orientation and alignment for liquid handling and scanning of the target molecules bound to complementary capture molecules.

[0102] The plate of the invention can be introduced into a holder in the fluid-handling device. This robotic device is programmed to set appropriate reaction conditions, such as temperature, add samples to the well 2 of assay strip C through inlet channel 10, incubate the test samples for an appropriate time, remove un-reacted samples, wash the wells 2, add substrates as appropriate and perform detection assays. The particularity of the reaction conditions depends upon the purpose of the assay. For example, the assay may involve testing whether a sample contains target molecules that react to a probe under a specified set of reaction conditions. In this case, the reaction conditions are chosen accordingly.

[0103] Preferably, removals of the solutions from the wells 2 after the incubation step is performed with the cap strip (B) present on the strip (A) and the next liquid handlings are then performed in the strips (A) in absence of the cap strips (B).

[0104] In still another aspect, the present invention is related to an apparatus (high throughput screening apparatus) which comprises the assay strip C or the multi-well plate comprising the assay strip C or the well(s) 2 of the assay strip C according to the invention, and possibly multi pipettes and other element of a 96 well plate format or an automate.

[0105] Another aspect of the present invention is related to an apparatus (high throughput screening apparatus) which comprise the assay strip C and the multi-well plate according to the invention, and a 96-well format PCR thermocycler. An example of compatible PCR cycler is the Mastercycler® (Eppendorf AG). Such apparatus allow automated PCR and/or hybridization on capture molecules into the assay strip C of the invention. Additionally, a multi-well plate cover (preferably in plastic) may be used between the assay strip C and the heating

cover of the thermocycler to exert a homogeneous pressure over all assay strips and maintain a tight sealing during the PCR.

Example 1: micro-array spotting at the bottom (flat) surface of the strip A of the assay strip C

1. Preparation of cycloolefin polyaldehyde strip A

[0106] Aldehyde functions were introduced into a cycloolefin (Zeonex™ 330R) strip A using the chemistry described in the EP Patent application 07106545.2 incorporated herein by reference using 0.1% of dextran polyaldehyde.

[0107] The dimensions of the strip A were the following: 8.07 mm (W) x 82 mm (L) x 4.9 mm (H) without bottom o-ring 20 (5.9 mm with o-ring). The strip A comprises 8 wells 2.

2. Capture molecule immobilization

[0108] The capture probes of the array were those of the DualChip GMO kit (Eppendorf AG, Hamburg, Germany) and were described in the EP Patent application 05447115.6 incorporated herein by reference. The capture molecules (probes) were single stranded polynucleotide which were aminated on the 5' end. These probes were able to identify genetically modified events by screening simultaneously multiple genetic elements:

P35S, T-nos, Pnos-nptII, Pat, CryIAb, EPSPS, Invertase (Maize), Lectin (Soybean), Cruciferin (Rapeseed) and Rbcl (Plant universal). The array also includes a control element CaMV.

The spotting protocol described by Schena et al. (1996 PNAS. USA 93:10614) was followed for the grafting of aminated DNA to the aldehyde derivatized glass. The aminated capture nucleotide sequences were spotted from solutions at concentrations of 3 µM. Capture nucleotide sequences were printed with a home made robotic device using 250 µm diameter plain pins. The spots have around 400 µm in diameter and the volume dispensed is about 0.5 nl. After washing, the wells were dried at room temperature and stored at 4 °C until used.

3. PCR

[0109] In order to amplify the sequences of the genetic elements that could be detected on the DualChip GMO, 4 PCR reactions were processed according to the kit manual (Eppendorf AG, Hamburg, Germany). The primers used and PCR conditions were also described in EP Patent application 05447115.6.

PCR A: Tnos, P35S

PCR B: Pnos-nptII, CaMV, PCR control

PCR C: Pat, CryIAb, EPSPS

PCR D: Maize, Soybean, Rapeseed and plant

[0110] The PCR were performed in a final volume of 25 µl containing: 1X Buffer Biotools including 2 mM MgCl₂, 0.2 µM of each primer with one of the primer being biotinylated, 200 µM of each dATP, dCP, dGTP and 400 µM of dUTP, 1.25 U of Taq DNA polymerase Biotools, 0.5 U of UNG (ref 71960, USB, Cleveland, Ohio, USA) and containing 100 ng of Genomic DNA that was extracted from RRS 0.1% sample (ERM-BF910f, IRMM, Geel, Belgium) using a CTAB-based method (Rogers and Bendich, 1985) and quantified using the "Quant-it™ PicoGreen dsDNA assay kit" (Invitrogen, USA) as described in the manual.

[0111] DNA sample were from RRS GMO which contains the following 5 genetic elements: P35S, T-nos, EPSPS, Lectin (Soybean), and Rbcl (Plant universal).

[0112] Samples were first incubated at 22°C for 10 min and then denatured at 94 °C for 5 min. Then 35 cycles of amplification were performed consisting of 30 sec at 94 °C, 40 sec at 56 °C and 1 min at 72 °C and a final extension step of 10 min at 72 °C.

4. Hybridization and Colorimetric detection

[0113] Six wells 2 of a strip A having fixed at its bottom surface a GMO array were hybridized with the 4 PCR products according to the to the kit manual (Eppendorf AG, Hamburg, Germany).

[0114] For each well, 9 µl of each PCR product was mixed in a 1.5 ml microtube with 5 µl of SensiHyb solution and 4 µl of hybridization control. 5 µl of 0.5 N NaOH was added and incubated for 5 min at room temperature. Then 50 µl of Genomic HybriBuffer was added and 100 µl of the hybridization mix solution was injected inside a well 2 of a strip A of the invention. The strip A was sealed with the cap strip B and hybridization was performed for 1h at 60°C. The hybridization was immediately followed by the detection step. The colorimetric detection was performed using the Silverquant detection kit (Eppendorf, Hamburg, Germany).

[0115] The cap strip B was removed from the strip A and wells were washed 2 times with Post Hybridization Buffer for 1 min and 3 times with Washing Buffer for 1 min. The wells were incubated for 10 min at temperature in Pre-Blocking Buffer and for 45 min at room temperature in the Diluted Gold-Conjugate (100 µl/well). After incubation, the wells were washed 4 times with Washing Buffer for 1 min, then once with Rinsing Buffer for 1 min. Then, an equal volume of Silverquant A and B solutions were incubated in the wells for 5 min at room temperature. After incubation, the wells were washed 2 times with distilled water for 30 sec at room temperature and air dried. The strip A was then inserted into the holder adapter of the Silverquant scanner (Eppendorf, Hamburg, Germany) and scanning of the array was performed according

to the instruction manual. The Silverquant analysis software (Eppendorf, Hamburg, Germany) was used for processing the data of the array. The result is presented in figure 6. The five genetic elements (P35S, T-nos, EP-SPS, Lectin, and Rbcl) present in RRS were effectively detected on the array present in the 6 wells of the assay strip of the invention.

Claims

1. An assay strip (C) compatible with multi-well format, comprising:

- a strip (A) consisting of at least two wells (2), each well (2) having an external bottom surface, a flat inner bottom surface (5) upon which capture molecules are immobilized, and an open top surface (3), adapted to receive a cap (6), wherein the said wells (2) are linked to each other by attachments (4)
- a cap strip (B) comprising at least two caps (6), wherein the said caps are linked to each other by attachments (9) located at the top part (7) of the said caps (6), wherein each cap (6) comprises at least one inlet channel (10) and at least one outlet channel (11), wherein there is a trapping well (15) around said inlet and outlet channels (10, 11) on the top part (7) of the cap strip (B), wherein the said cap strip (B) seals the strip (A) and wherein said side walls of the cap (6) comprise a pad (14) to seal the cap (6) inside the well (2), to form a chamber having a height of less than five mm and, wherein the said pad (14) has a thermal expansion coefficient which is higher than the thermal expansion coefficient of the well (2).

2. The assay strip of claim 1, wherein the capture molecules are immobilized onto the inner bottom surface (5) of the well (2) in discrete regions in the form of a micro-array having a density of at least 4 discrete regions per cm².

3. The assay strip of claims 1 or 2, wherein the inlet (10) and the outlet (11) channels are linked by a ditch (13) at the bottom part (8) of the cap (6).

4. The assay strip of claims 1 to 3, wherein the inner bottom surface (5) of all wells upon which capture molecules are immobilized is in the same focal plane.

5. The assay strip of claim 4, wherein the well (2) walls have an opening angle of 1.5° with the normal to the inner bottom surface (5).

6. The assay strip of claims 1 to 5, further comprising an adapter (F) comprising at least two supercaps (23), each supercap having at least an inlet (24) fitting inside the cap strip inlet channel (10) or outlet channel (11) and having a conical part.

7. The assay strip of claim 6, wherein the inlet (24) has a length which is equal to the depth of the well (2) minus 0.5 mm.

8. A multi-well plate having frames, (E) and comprising :

- a multi-well chassis (D), attachment feature(s) (17) complementary to attachment feature(s) (16) of the assay strip (C) and/or attachment feature(s) (19) complementary to mounting foot(s) (18) of the assay strip (C) and,
- at least one and up to twelve assay strips (C), according to the claims 1 to 7.

9. A multi-well plate for performing a series of biological reactions between capture and target molecules, comprising assay strips (C) of claims 1 to 7 comprising:

- 96 wells (2), each well (2) comprising a flat inner bottom surface (5) upon which capture molecules are immobilized and an open top surface (3) adapted to receive a cap (6), wherein adjacent wells are linked to each other by attachments (4)
- a well cap comprising 96 caps (6), each cap (6) comprising at least one inlet channel (10) and at least one outlet channel (11), wherein there is a trapping well (15) around said inlet and outlet channels (10, 11) on the top part of the cap strip and wherein adjacent caps are linked to each other by junctions (9) located at the top part (7) of the caps,
- wherein side walls of the cap (6) comprise a pad (14) to seal the cap (6) inside the well (2), to form a chamber having a height of less than five mm,
- and wherein the said pad (14) has a coefficient of thermal expansion which is higher than the thermal expansion coefficient of the well (2).

10. A method for a detection and/or quantification of a target molecule performed by the following steps:

- inserting a sample solution containing the said target molecule into the strip (A) according to any of the preceding claims 1 to 7 or the multi-well plate according to the claims 8 or 9 and containing immobilized capture molecules at inner bottom surface (5) of wells (2);
- sealing the strip (A) with the cap strip (B) to

- form an assay strip (C);
 - incubating the assay strip (C) in reaction condition for a binding between the target molecule and its capture molecule;
 - measuring a signal to detect and/or quantify the target molecule in the sample solution. 5
11. The method of Claim 10, which further comprises after the incubating step the following steps of: 10
- removing the sample solution through the cap strip (B) and inserting a washing solution in the wells (2),
 - removing the cap strip (B) from the strip (A) and
 - incorporating reagents generating a signal resulting from the binding between the target molecule and its corresponding capture molecule in the wells (2) to provide a detectable signal. 15
12. The method of claim 10 or 11, wherein the target molecule is bound to its capture molecule present on an optically transparent solid support surface having a refractive index n_1 , said solid support being in contact with a medium having refractive index n_2 , whereby $n_1 > n_2$, said method comprising the steps of: 20
- a) illuminating the target molecule, thereby causing the target molecule to emit light;
 b) detecting light emitted from the target molecule through said support at an observation angle θ_{ob} relative to the normal to the solid support surface, such that $90^\circ > \theta_{ob} > \sin^{-1}(n_2/n_1)$. 25 30
13. The method of claims 10 to 12, wherein the target molecule is a polynucleotide sequence synthesized in the wells by copying and/or amplifying a polynucleotide sequence present in the sample solution inserted into the wells. 35
14. A high throughput screening apparatus of target molecule(s), which comprises the assay strip (C) according to any of the preceding claims 1 to 7 or the multiwell plate according to the claims 8 or 9. 40
15. A kit of part comprising: 45
- the strip (A) and cap strip (B) of the assay strip (C) according to any of the preceding claims 1 to 7 or the multi well plate according to the claims 8 or 9, sealing means for the cap strip, washing solution(s) and/or detecting solution(s). 50
- Patentansprüche** 55
1. Mehrfachmuldenformat-kompatibler Probenstreifen (C), umfassend:
- einen Streifen (A) bestehend aus mindestens zwei Mulden (2), wobei jede Mulde (2) aufweist:
 eine äußere Bodenfläche,
 eine flache innere Bodenfläche (5), auf welcher Fängermoleküle immobilisiert sind,
 eine obere Freifläche (3), die zur Aufnahme einer Kappe (6) ausgelegt ist,
 wobei die Mulden (2) durch Befestigungen (4) miteinander verbunden sind,
 - einen Kappenstreifen (B), der mindestens zwei Kappen (6) umfasst, wobei die Kappen durch Befestigungen, die sich am oberen Teil (7) der Kappen (6) befinden, miteinander verbunden sind, wobei jede Kappe (6) mindestens einen Einlasskanal (10) und mindestens einen Auslasskanal (11) umfasst, wobei eine Auffangmulde (15) um die Ein- und Auslasskanäle (10, 11) auf dem oberen Teil (7) des Kappenstreifens (B) vorhanden ist,
 wobei der Kappenstreifen (B) den Streifen (A) verschließt, und wobei die Seitenwände der Kappe (6) einen Unterbau (14) umfassen, um die Kappen (6) innerhalb der Mulde (2) abzuschließen, um eine Kammer mit einer Höhe von weniger als fünf mm zu bilden, und
 wobei der Unterbau (14) einen Wärmeausdehnungskoeffizienten aufweist, der höher als der Wärmeausdehnungskoeffizient der Mulde (2) ist.
2. Probenstreifen nach Anspruch 1, wobei die Fängermoleküle auf der inneren Bodenfläche (5) der Mulde (2) in getrennten Regionen in der Form eines Mikroarrays mit einer Dichte von mindestens 4 getrennten Regionen pro cm^2 immobilisiert sind.
3. Probenstreifen nach Anspruch 1 oder 2, wobei der Einlass (10)- und die Auslass (11)-Kanäle durch einen Graben (13) am unteren Teil (8) der Kappe (6) verbunden sind.
4. Probenstreifen nach Anspruch 1 bis 3, wobei die innere Bodenfläche (5) aller Mulden, auf welcher Fängermoleküle immobilisiert sind, in derselben Fokalebene liegt.
5. Probenstreifen nach Anspruch 4, wobei die Mulden (2)-Wände einen Öffnungswinkel von $1,5^\circ$ mit der Normalen auf die innere Bodenfläche (5) aufweisen.
6. Probenstreifen nach Anspruch 1 bis 5, ferner umfassend einen Adapter (F), der mindestens zwei Oberkappen (23) umfasst, wobei jede Oberkappe mindestens einen Einlass (24) aufweist, der in den Kappenstreifeneinlasskanal (10) oder den Kappenstreifenauslasskanal (11) passt und einen konischen Teil

- aufweist.
7. Probenstreifen nach Anspruch 6, wobei der Einlass (24) eine Länge aufweist, die gleich der Tiefe der Mulde (2) minus 0,5 mm ist. 5
 8. Mehrfachmuldenplatte mit Rahmen (E) und umfassend:
 - ein Mehrfachmulden-Chassis (D), Befestigungsmerkmal(e) (17) komplementär zu Befestigungsmerkmal(en) (16) des Probenstreifens (C) und/oder Befestigungsmerkmal(e) (19) komplementär zu Montagebein(en) (18) des Probenstreifens (C), und 10
 - mindestens einen und bis zu zwölf Probenstreifen (C) nach Anspruch 1 bis 7. 15
 9. Mehrfachmuldenplatte zum Durchführen einer Reihe von biologischen Reaktionen zwischen Fänger- und Zielmolekülen, umfassend Probenstreifen (C) nach Anspruch 1 bis 7, umfassend: 20
 - 96 Mulden (2), wobei jede Mulde (2) eine flache innere Bodenfläche (5), auf welcher Fängermoleküle immobilisiert sind, und eine obere Freifläche (3) umfasst, die zur Aufnahme einer Kappe (6) ausgelegt ist, wobei benachbarte Mulden durch Befestigungen (4) miteinander verbunden sind, 25
 - eine Muldenkappe, die 96 Kappen (6) umfasst, wobei jede Kappe (6) mindestens einen Einlasskanal (10) und mindestens einen Auslasskanal (11) umfasst, wobei eine Auffangmulde (15) um die Ein- und Auslasskanäle (10, 11) auf dem oberen Teil des Kappenstreifens vorhanden ist, und wobei benachbarte Kappen durch Verbindungen (9), die sich am oberen Teil (7) der Kappen befinden, miteinander verbunden sind, 30
 - wobei Seitenwände der Kappe (6) einen Unterbau (14) umfassen, um die Kappe (6) innerhalb der Mulde (2) abzuschließen, um eine Kammer mit einer Höhe von weniger als fünf mm zu bilden, 35
 - und wobei der Unterbau (14) einen Wärmeausdehnungskoeffizienten aufweist, der höher als der Wärmeausdehnungskoeffizient der Mulde (2) ist. 40
 10. Verfahren für einen Nachweis und/oder eine Quantifizierung eines Zielmoleküls, das durch die folgenden Schritte durchgeführt wird: 50
 - Einführen einer Probenlösung, die das Zielmolekül enthält, in den Streifen (A) nach einem der vorhergehenden Ansprüche 1 bis 7 oder die Mehrfachmuldenplatte nach Anspruch 8 oder 9 und Enthalten immobilisierter Fängermoleküle 55
- an einer inneren Bodenfläche (5) von Mulden (2);
- Verschließen des Streifens (A) mit dem Kappenstreifen (B), um einen Probenstreifen (C) zu bilden;
 - Inkubieren des Probenstreifens (C) unter Reaktionsbedingungen für eine Bindung zwischen dem Zielmolekül und seinem Fängermolekül;
 - Messen eines Signals, um das Zielmolekül in der Probenlösung nachzuweisen und/oder zu quantifizieren.
11. Verfahren nach Anspruch 10, ferner umfassend nach dem Inkubationsschritt die folgenden Schritte:
 - Entnehmen der Probenlösung durch den Kappenstreifen (B) und Einführen einer Waschlösung in die Mulden (2),
 - Entnehmen des Kappenstreifens (B) aus dem Streifen (A) und
 - Zugeben von Reagenzien, die ein Signal erzeugen, das aus der Bindung zwischen dem Zielmolekül und seinem entsprechenden Fängermolekül in den Mulden (2) resultiert, um ein nachweisbares Signal bereitzustellen.
 12. Verfahren nach Anspruch 10 oder 11, wobei das Zielmolekül an sein Fängermolekül gebunden wird, das auf einer optisch transparenten, festen Trägerfläche mit einem Brechungsindex n_1 vorhanden ist, wobei der feste Träger mit einem Medium mit einem Brechungsindex n_2 in Kontakt ist, wobei $n_1 > n_2$, und das Verfahren die folgenden Schritte umfasst:
 - a) Beleuchten des Zielmoleküls, um **dadurch** zu bewirken, dass das Zielmolekül Licht ausstrahlt;
 - b) Erfassen von Licht, das vom Zielmolekül durch den Träger in einem Beobachtungswinkel θ_{ob} in Bezug auf die Normale auf die feste Trägerfläche ausgestrahlt wird, derart dass $90^\circ > \theta_{ob} > \sin^{-1}(n_2/n_1)$.
 13. Verfahren nach Anspruch 10 bis 12, wobei das Zielmolekül eine Polynucleotidsequenz ist, die in den Mulden durch Kopieren und/oder Amplifizieren einer Polynucleotidsequenz synthetisiert wird, die in der Probenlösung vorhanden ist, die in die Mulden eingeführt wird.
 14. Vorrichtung zum Hochdurchsatz-Screening von Zielmolekül(en), die den Probenstreifen (C) nach einem der vorhergehenden Ansprüche 1 bis 7 oder die Mehrfachmuldenplatte nach Anspruch 8 oder 9 umfasst.
 15. Teilekit, umfassend:

- den Streifen (A) und den Kappenstreifen (B) des Probenstreifens (C) nach einem der vorhergehenden Ansprüche 1 bis 7 oder die Mehrfachmuldenplatte nach Anspruch 8 oder 9, Verschlussmittel für den Kappenstreifen, Waschlösung(en) und/oder Nachweislösung (en).

Revendications

1. Bandelette de dosage (C) compatible avec un format multipuits, comprenant :

- une bande (A) constituée d'au moins deux puits (2), chaque puits (2) ayant une surface inférieure externe, une surface inférieure interne plane (5) sur laquelle des molécules de capture sont immobilisées, et une surface supérieure ouverte (3), adaptée pour recevoir une coiffe (6), dans laquelle lesdits puits (2) sont liés les uns aux autres par des attaches (4) ;
- une bande-coiffe (B) comprenant au moins deux coiffes (6), où lesdites coiffes sont liées les unes aux autres par des attaches (9) situées au niveau de la partie supérieure (7) desdites coiffes (6), où chaque coiffe (6) comprend au moins un canal d'entrée (10) et au moins un canal de sortie (11), où un puits de piégeage (15) se trouve autour desdits canaux d'entrée et de sortie (10, 11) sur la partie supérieure (7) de la bande-coiffe (B), dans laquelle ladite bande-coiffe (B) scelle la bandelette (A) et dans laquelle lesdites parois latérales de la coiffe (6) comprennent une pastille (14) pour sceller la coiffe (6) à l'intérieur du puits (2), afin de former une chambre ayant une hauteur inférieure à cinq mm et dans laquelle ladite pastille (14) présente un coefficient de dilatation thermique qui est supérieur au coefficient de dilatation thermique du puits (2).

2. Bandelette de dosage selon la revendication 1, dans laquelle les molécules de capture sont immobilisées sur la surface inférieure interne (5) du puits (2) dans des régions discrètes sous la forme d'un microréseau ayant une densité d'au moins quatre régions discrètes par cm².
3. Bandelette de dosage selon la revendication 1 ou 2, dans laquelle les canaux d'entrée (10) et de sortie (11) sont liés par une rainure (13) au niveau de la partie inférieure (8) de la coiffe (6).
4. Bandelette de dosage selon les revendications 1 à 3, dans laquelle la surface inférieure interne (5) de

tous les puits sur lesquels des molécules de capture sont immobilisées se trouve dans le même plan focal.

5. Bandelette de dosage selon la revendication 4, dans laquelle les parois de puits (2) présentent un angle d'ouverture de 1,5° par rapport à la normale à la surface inférieure interne (5).
6. Bandelette de dosage selon les revendications 1 à 5, comprenant en outre un adaptateur (F) comprenant au moins deux supercoiffes (23), chaque supercoiffe ayant au moins une entrée (24) s'adaptant à l'intérieur du canal d'entrée (10) ou du canal de sortie (11) de bande-coiffe et ayant une partie conique.
7. Bandelette de dosage selon la revendication 6, dans laquelle l'entrée (24) a une longueur qui est égale à la profondeur du puits (2) moins 0,5 mm.
8. Plaque multipuits comportant des cadres (E) et comprenant :
- un châssis multipuits (D), un ou plusieurs éléments d'attache (17) complémentaires à un ou plusieurs éléments d'attache (16) de la bandelette de dosage (C) et/ou un ou plusieurs éléments d'attache (19) complémentaires d'un ou plusieurs pieds de montage (18) de la bandelette de dosage (C), et
- au moins une et jusqu'à douze bandelettes de dosage (C), selon les revendications 1 à 7.
9. Plaque multipuits pour réaliser une série de réactions biologiques entre des molécules de capture et des molécules cibles, comprenant des bandelettes de dosage (C), selon les revendications 1 à 7, comprenant :
- 96 puits (2), chaque puits (2) comprenant une surface inférieure interne plane (5) sur laquelle des molécules de capture sont immobilisées et une surface supérieure ouverte (3) adaptée pour recevoir une coiffe (6), où les puits adjacents sont liés les uns aux autres par des attaches (4),
- une coiffe de puits comprenant 96 coiffes (6), chaque coiffe (6) comprenant au moins un canal d'entrée (10) et au moins un canal de sortie (11), où un puits de piégeage (15) se trouve autour desdits canaux d'entrée et de sortie (10, 11) sur la partie supérieure de la bande-coiffe et où des coiffes adjacentes sont liées les unes aux autres par des jonctions (9) situées au niveau de la partie supérieure (7) des coiffes,
- dans laquelle des parois latérales de la coiffe (6) comprennent une pastille (14) pour sceller

- la coiffe (6) à l'intérieur du puits (2), afin de former une chambre ayant une hauteur inférieure à cinq mm,
 - et dans laquelle ladite pastille (14) présente un coefficient de dilatation thermique qui est supérieur au coefficient de dilatation thermique du puits (2). 5
- 10.** Procédé de détection et/ou de quantification d'une molécule cible réalisé par les étapes suivantes consistant à : 10
- insérer une solution d'échantillon contenant ladite molécule cible dans la bandelette (A) selon l'une quelconque des revendications 1 à 7 précédentes ou la plaque multipuits selon la revendication 8 ou 9 et contenant des molécules de capture immobilisées au niveau d'une surface inférieure interne (5) de puits (2) ; 15
 - sceller la bandelette (A) avec la bande-coiffe (B) pour former une bandelette de dosage (C) ; 20
 - incubé la bandelette de dosage (C) dans des conditions de réaction pour une liaison entre la molécule cible et sa molécule de capture ; 25
 - mesurer un signal pour détecter et/ou quantifier la molécule cible dans la solution d'échantillon.
- 11.** Procédé selon la revendication 10, qui comprend en outre après l'étape d'incubation les étapes suivantes consistant à : 30
- retirer la solution d'échantillon à travers la bande-coiffe (B) et insérer une solution de lavage dans les puits (2),
 - retirer la bande-coiffe (B) de la bandelette (A) et 35
 - incorporer les réactifs générant un signal résultant de la liaison entre la molécule cible et sa molécule de capture correspondante dans les puits (2) pour fournir un signal détectable. 40
- 12.** Procédé selon la revendication 10 ou 11, dans lequel la molécule cible est liée à sa molécule de capture présente sur une surface de support solide optiquement transparente ayant un indice de réfraction n_1 , ledit support solide étant en contact avec un milieu 45 ayant un indice de réfraction n_2 , où $n_1 > n_2$, ledit procédé comprenant les étapes consistant à :
- a) illuminer la molécule cible, amenant ainsi la molécule cible à émettre de la lumière ; 50
 - b) détecter la lumière émise par la molécule cible à travers ledit support selon un angle d'observation θ_{ob} par rapport à la normale à la surface de support solide, de sorte que $90^\circ > \theta_{ob} > \sin^{-1}(n_2/n_1)$. 55
- 13.** Procédé selon les revendications 10 à 12, dans lequel la molécule cible est une séquence de polynu-
- cléotides synthétisée dans les puits par copie et/ou amplification d'une séquence de polynucléotides présente dans la solution d'échantillon insérée dans les puits.
- 14.** Appareil de criblage à haut rendement en molécule (s) cible(s), qui comprend la bandelette de dosage (C) selon l'une quelconque des revendications 1 à 7 précédentes ou la plaque multipuits selon les revendications 8 ou 9.
- 15.** Nécessaire de pièce comprenant :
- la bandelette (A) et la bande-coiffe (B) de la bandelette de dosage (C) selon l'une quelconque des revendications 1 à 7 précédentes ou la plaque multipuits selon les revendications 8 ou 9, des moyens d'obturation pour la bande-coiffe, une(des) solution(s) de lavage et/ou une(des) solution(s) de détection.

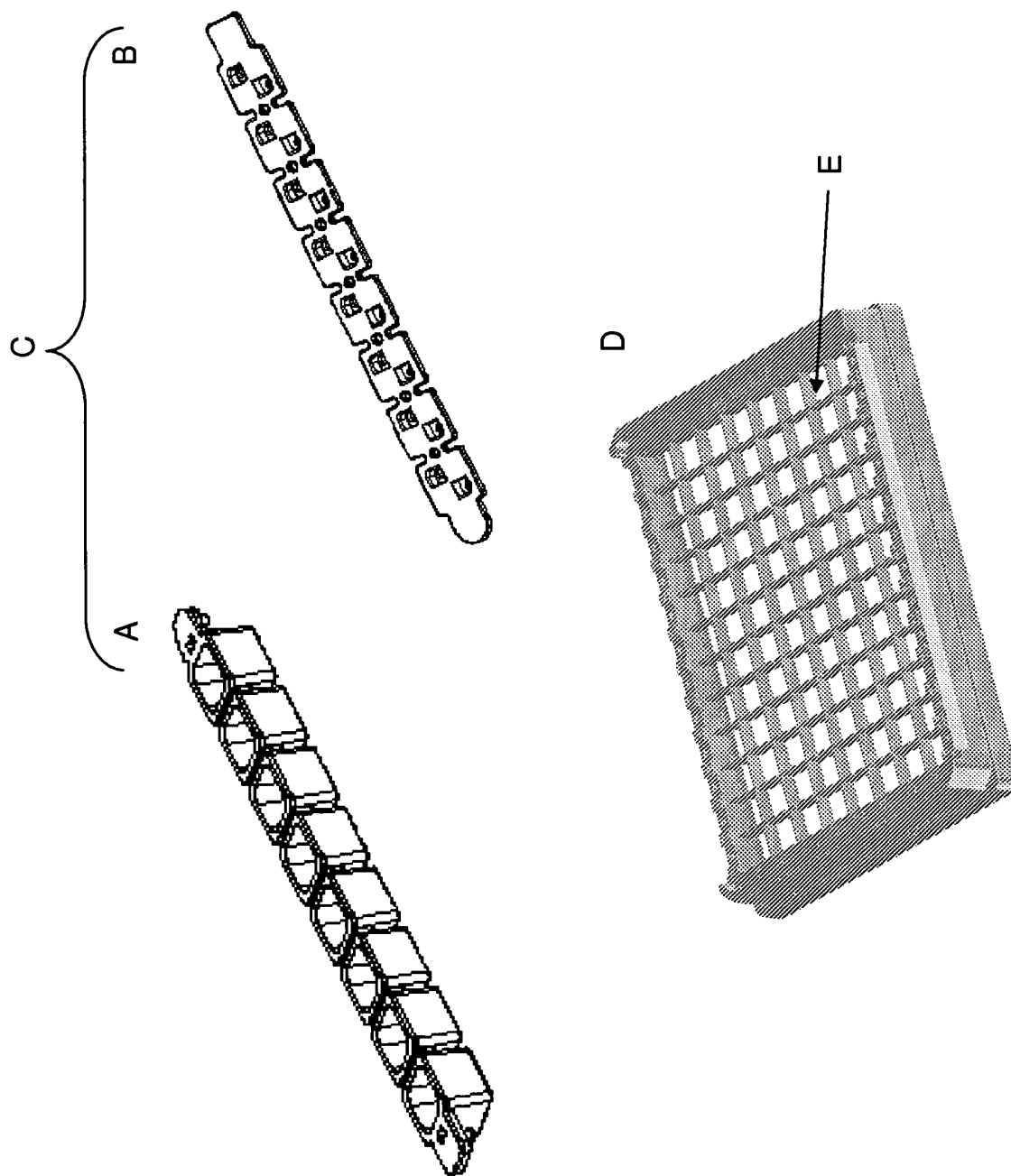


Fig. 1

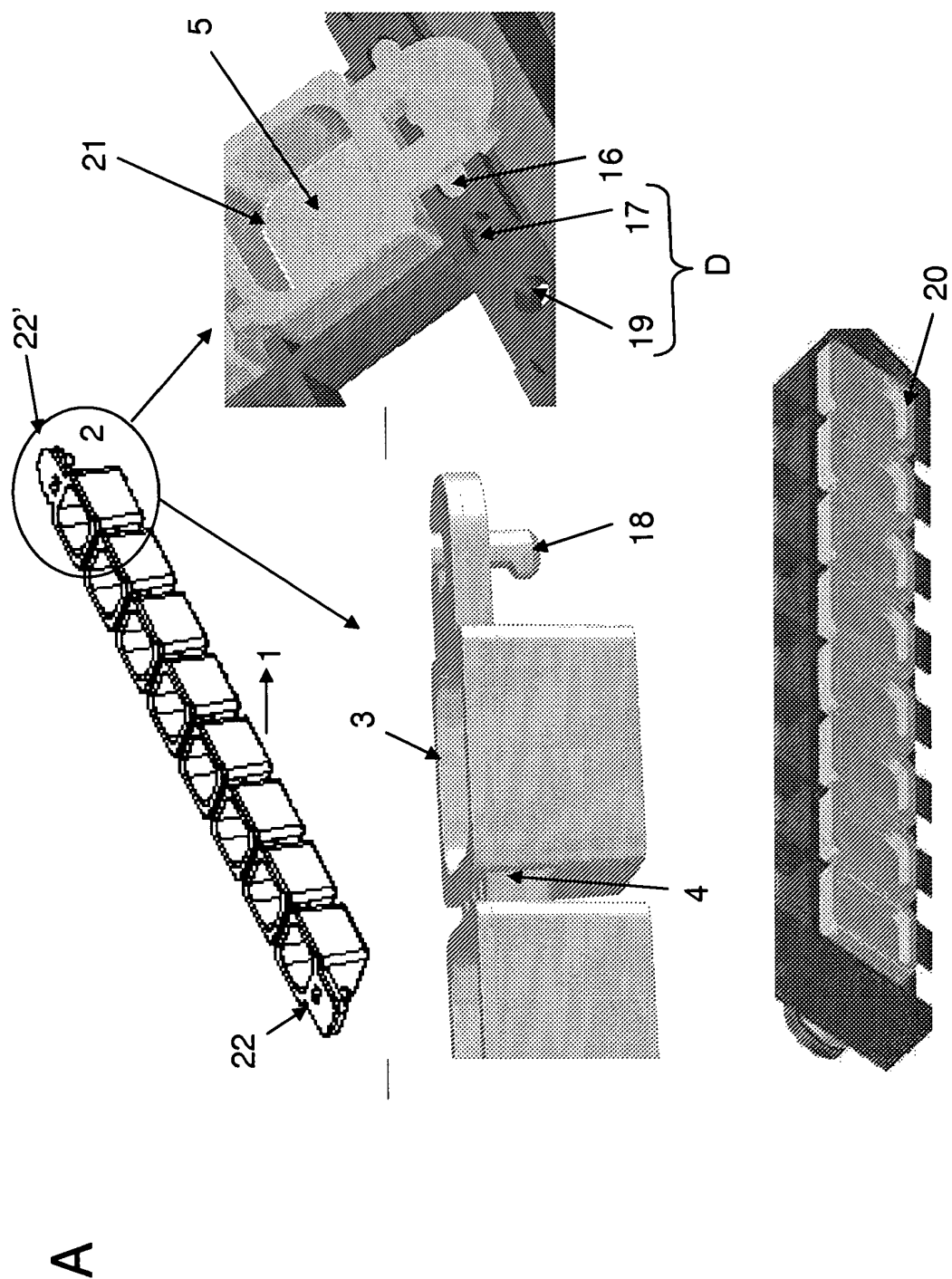


Fig. 2

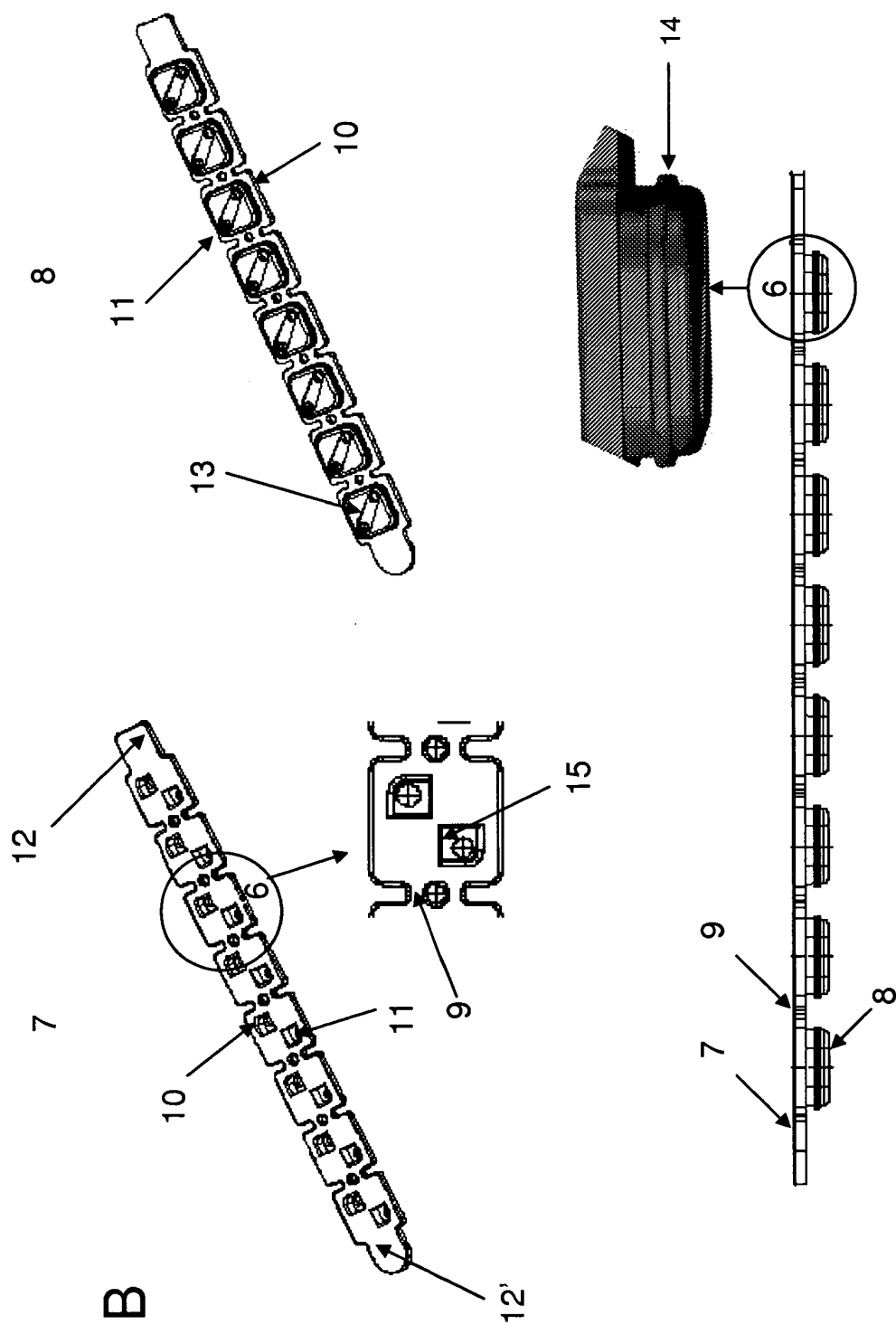


Fig. 3

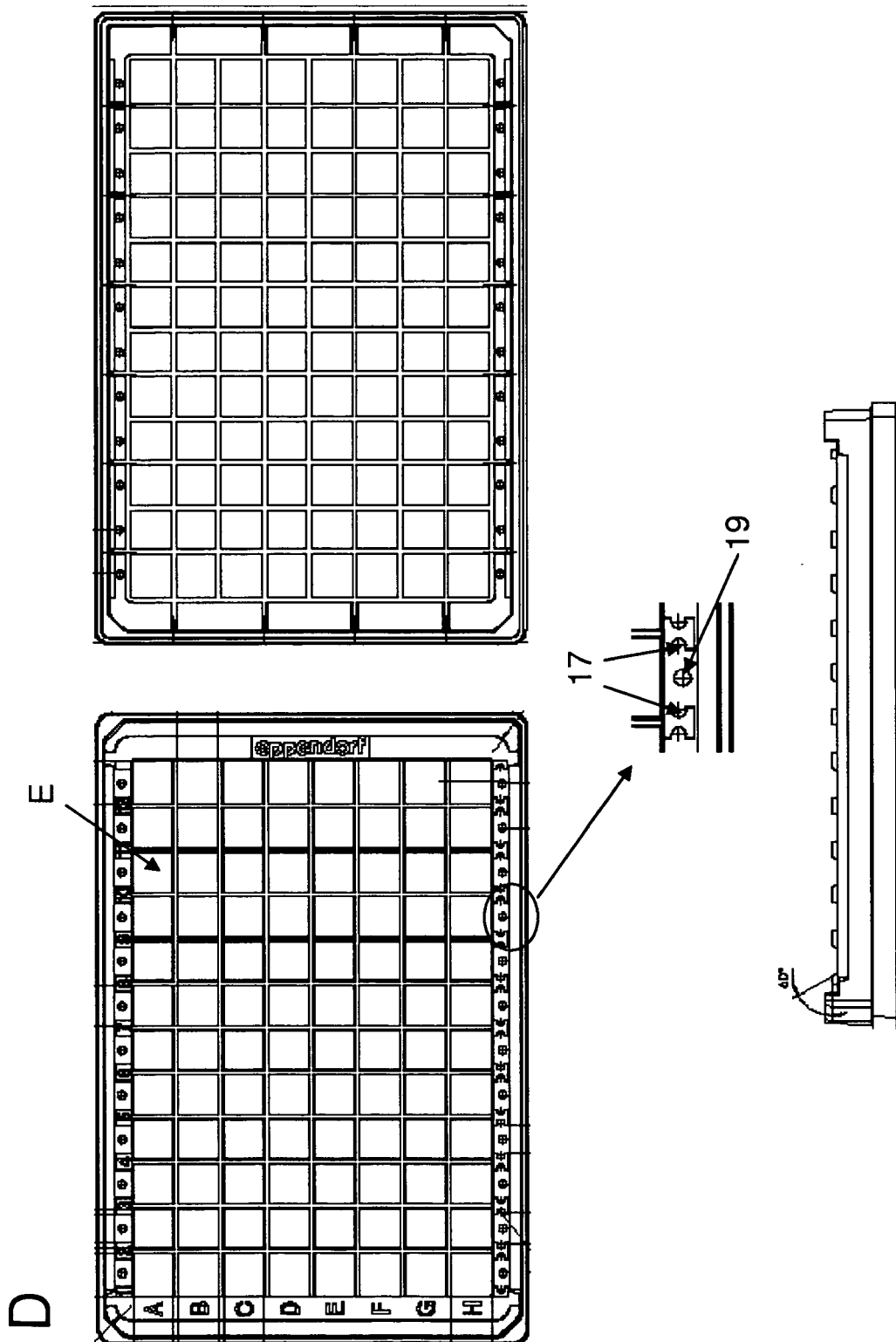


Fig. 4

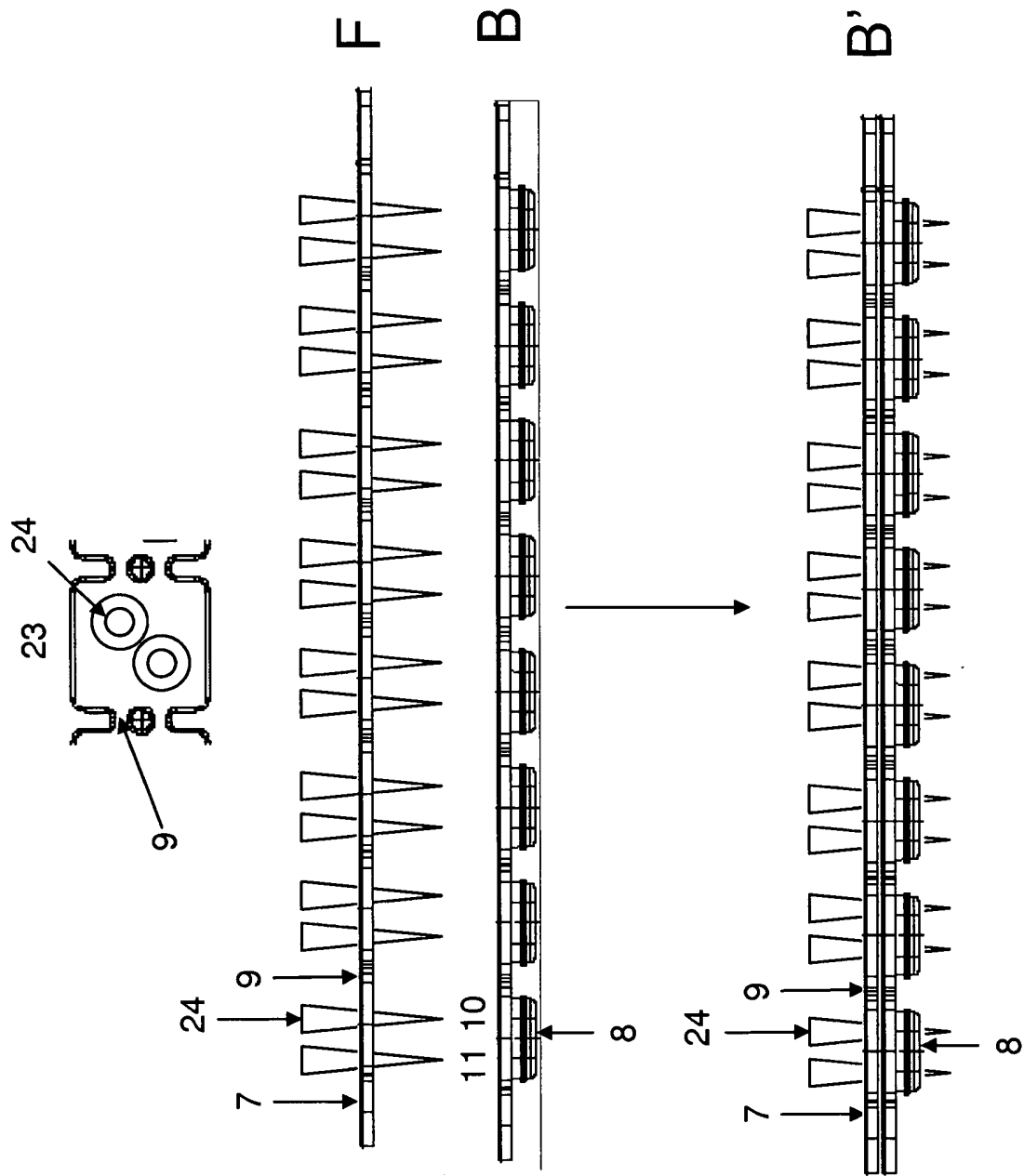


Fig. 5

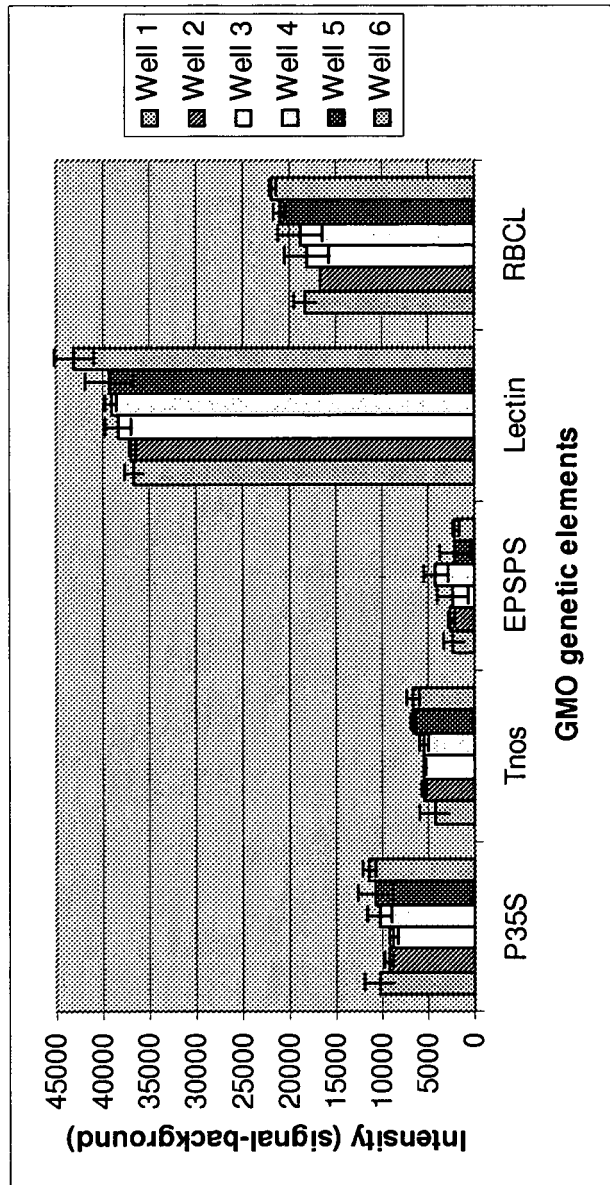


Fig. 6

REFERENCES CITED IN THE DESCRIPTION

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