Plating cells and corresponding picking colonies

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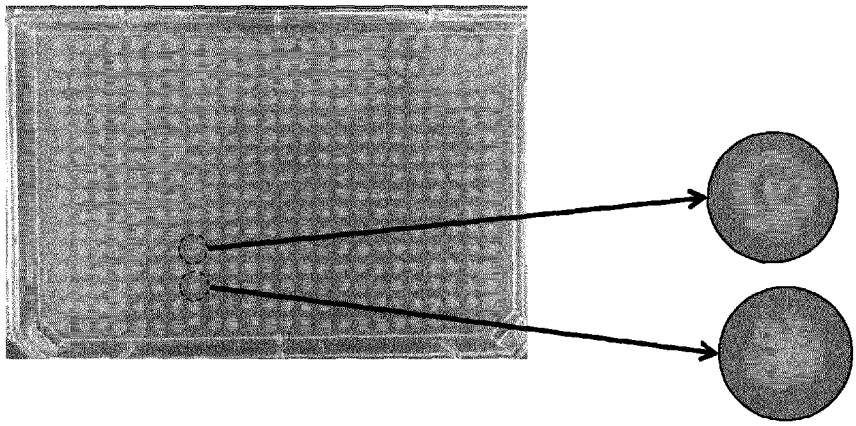

FIG. 6B

(57) Abstract: There is presented a method for plating cells and for picking corresponding colonies, said method comprising for each predetermined position (x_i, y_i) in a plurality of spatially distributed predetermined positions (X, Y) on a surface (344) of a solid element where each predetermined position is predetermined with respect to the solid element, plating (110) one or more cells (342), growing (120) a colony (446) from the one or more cells, picking (130) the colony (446), wherein said picking is based on the predetermined spatially distributed position (x_i, y_i).

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PLATING CELLS AND CORRESPONDING PICKING COLONIES

FIELD OF THE INVENTION

The present invention relates to a method for plating cells and picking corresponding colonies and in particular relates to a method for plating cells and picking corresponding colonies based on spatially predetermined positions and a corresponding device and computer program product.

BACKGROUND OF THE INVENTION

Colony picking is a technique which may be used to collect microbial colonies (starting each from 1 single clone), and re-suspend these colonies in liquid media, in order to perform further studies on these individual clones (e.g. High Throughput screening assays to evaluate the clones). The colony picking can be done by hand, or automatically, e.g. by using an instrument dedicated to colony picking, such as an instrument comprising a camera, a light table and image processing software. The main concept of automated colony picking may typically be seen as taking an image of, e.g., an agar plate with colonies, analysing the image using dedicated software that will detect the colonies to be picked, and then picking the colonies at the positions detected in the image.

An improved method would be advantageous, and in particular a more efficient method and/or a method which would enable simplifying necessary equipment would be advantageous.

SUMMARY OF THE INVENTION

It may be seen as an object of the present invention to provide a method for plating cells and picking corresponding colonies that solves the above mentioned problems of the prior art with efficiency and/or enabling implementation with relatively simple devices.

It is a further object of the present invention to provide an alternative to the prior art.

Thus, the above described object and several other objects are intended to be obtained in a first aspect of the invention by providing a method for plating cells, such as in order to enable growing colonies originating from a single cell, such as plating single cells, and for picking corresponding colonies, said method comprising:

- for each predetermined position (which may be understood to be each predetermined spatially distributed position) in a plurality of spatially distributed predetermined positions on a surface of a solid element, such as an Omnitray™, where each predetermined position is predetermined with respect to the solid element,
    i. plating one or more cells,
    ii. growing, such as proliferating, a colony, such as a microbial colony, from the one or more cells, such as the one or more cells subject to plating,
    iii. picking the colony, wherein said picking is based, such as exclusively based, on the predetermined spatially distributed position (which may be understood to correspond to the predetermined position).

The invention is particularly, but not exclusively, advantageous for obtaining a method for plating and picking, which enables dispensing with imaging and image analysis, since both plating and picking is carried out based on the same set of predetermined spatially distributed positions. Another potential advantage may be that picking may be carried out very fast (such as in a time-efficient manner). Another potential advantage may be that it enables dispensing with the need for imaging equipment and image analysis software. Another possible advantage may be that it enables plating and picking with relatively simple equipment. Another possible advantage may be that it enables plating and picking with relatively simple equipment, such as enables plating with a cell sorter and picking with a liquid handler. In an embodiment plating is carried out with a cell sorter and/or picking is carried out with a liquid handler.

'Plating' is understood as is common in the art and may in particular be understood to comprise placing the one or more cells on the predetermined position on the surface of the solid element.

In an embodiment there is presented a method wherein the plating comprises placing one or more containers at positions corresponding to the plurality of spatially distributed predetermined positions, and dispensing the one or more cells from said one or more containers. An advantage of this may be that it dispenses with the need for electrostatic stream translation, since the container, such a dispensing nozzle, is placed at each spatially distributed position before dispensing.

'Growing colonies originating from a single cell' is understood as is common in the art, where growing may alternatively be referred to as proliferating. For example a single cell may be plated on an agar surface and incubated so as to allow growth and the formation of a colony.

'Picking' is understood as is common in the art and may in particular be understood to comprise obtaining one or more cells from the colony by removing the one or more cells from the colony.

By 'corresponding colonies' may be understood that each colony (at each predetermined position) corresponds to the one or more cells plated at that predetermined position. Thus, each colony corresponds to a defined set of one or more cells.

By 'for each predetermined position in a plurality of spatially distributed predetermined positions' may be understood that some action (e.g., plating or picking) is carried out for each predetermined position. Each action, which corresponds to a plurality of actions (i.e., at least one action for each predetermined position in a plurality of spatially distributed predetermined positions), may be carried out sequentially (one after the other), in parallel (all at once) or a combination of these two options (such as carrying out half of the

plurality of actions in parallel in a first step and then afterwards carrying out the other half in parallel in a second step).

It may generally be understood that a "predetermined position" is a "predetermined spatially distributed position" and that "predetermined position" and "predetermined spatially distributed position" may be used interchangeably.

By 'a solid element' may be understood any solid element, such as an OmniTray™, a planar surface (such as a planar surface with dimensions 128 mm x 86 mm), a surface with topographical features (such as a multi-well plate, such as a 96 well plate, such as 384 well plate, such as 1596 well plate). The solid element may in particular be understood to be a solid medium.

By 'where each predetermined position is predetermined with respect to the solid element' may be understood that the coordinates of the predetermined positions may be determined once the coordinates (such as position and orientation) of the solid element is determined. This may be advantageous with respect to a situation where predetermined positions are predetermined merely with respect to each other, since then identification of the predetermined positions requires determination of at least one predetermined position.

'Colony' is understood as is common in the art and may in particular be understood to comprise two or more conspecific cells living in close association with, or connected to, one another. In the context of the present application, 'colony' may in particular be understood to be a 'microbial colony' defined as a cluster of cells growing on the surface of a solid medium cultured from a single cell. Because the colony is clonal, with all organisms in it descending from a single ancestor (assuming no contamination), they are genetically identical, except for any mutations.

By 'based on the predetermined spatially distributed position' may be understood that the picking relies on the information relating to the predetermined spatially distributed position. By 'exclusively based on' may be understood that no further

information is relied on, such as no information obtained from optical methods, such as imaging and image analysis.

In an embodiment there is presented a method wherein said picking is based exclusively on the predetermined spatially distributed position. An advantage of this may be that it facilitates expedient processing since no further information needs to be obtained. Another advantage of this may be that it facilitates simplification of the equipment employed, because it dispenses with the need for additional equipment, e.g., imaging means and image processing means. Since said picking in based exclusively on the predetermined spatially distributed position, such as without imaging, picking may be described as being carried out blindly (such as relying exclusively on the information in the predetermined spatially distributed position).

In an embodiment there is presented a method wherein said picking is based on the predetermined spatially distributed position as obtained from a database. An advantage of this may be that it facilitates efficient storage and handling of the relevant information (relating to the predetermined spatially distributed position), which can for example be stored in a database and provided (such as sent) to means for plating and provided (such as sent) to means for picking.

In a further embodiment there is presented a method wherein the method comprises (such as before plating, growing and picking):

- Sorting cells by
  - providing a first group of cells,
  - providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,

and wherein the one or more cells, such as the one or more cells which are plated, belong to the second group of cells. An advantage of this may be that all plated cells have been sorted and belong to the second group of cells. Thus, if colonies based on cells from the second group of cells are of interest, then all colonies can be picked. Since all colonies originate from cells from the second group of cells, a step of checking which colonies originate from the second group of cells is rendered superfluous.

In a further embodiment there is presented a method wherein the sorting is fluorescence-activated cell sorting (FACS). An advantage of this may be that FACS is efficient. Another advantage may be that it enables using an apparatus (FACS machine), which is readily available in many laboratories and industrial facilities.

In an embodiment there is presented a method wherein plating is carried out with a single cell dispenser. An advantage of this may be that plating may be carried out in an automated manner. Another advantage may be that it enables using a single cell dispenser, which may be readily available in many laboratories and research facilities. By 'single cell dispenser' is understood an instrument capable of dispensing a single cell (such as a volume of liquid with a single cell).

It is understood that both and each of a FACS machine and a single cell dispenser may be equipped with x-y translators, such as an automated x-y table for controlling relative x-y position between the solid element and the dispensing unit (such as nozzle). This may enable automated, spatially resolved plating of single cells.

In a further embodiment there is presented a method wherein the single cell dispenser obtains the plurality of spatially distributed predetermined positions from a database. An advantage of this may be that it facilitates an efficient way of storing and handling the information relating to the plurality of spatially distributed predetermined positions.

In an embodiment there is presented a method wherein picking is carried out with a liquid handler. An advantage of this may be that it enables using a liquid handler, which may be readily available in many laboratories and research facilities. 'Liquid handler' is understood as is common in the art, and more particularly as automated liquid handling robots being a class of devices that include automated pipetting systems for dispensing liquid from a pipette tip and aspirating liquids from tubes or wells into a pipette tip.

In a further embodiment there is presented a method wherein the liquid handler obtains the plurality of spatially distributed predetermined positions from a database. An advantage of this may be that it facilitates an efficient way of storing and handling the information relating to the plurality of spatially distributed predetermined positions.

In an embodiment there is presented a method wherein plating is carried out with a cell sorter and picking is carried out with a liquid handler. An advantage of this may be that a method for (optionally automated) plating and picking may be realized with equipment, which is readily available in many laboratories and research facilities.

In a further embodiment there is presented a method wherein the single cell dispenser and the liquid handler obtains the plurality of spatially distributed predetermined positions from a database, such as from the same database. An advantage of this may be that it facilitates an efficient way of storing and handling the information relating to the plurality of spatially distributed predetermined positions.

In an embodiment there is presented a method wherein optical data, such as fluorescence data or colorimetric data or absorption data, is obtained optically, such as obtained subsequent to growing, based on said spatially distributed predetermined positions and optionally sequentially from one or more of the spatially distributed predetermined positions. An advantage of this may be that the optical data may yield information relevant for selecting which colonies to pick. In a further embodiment there is presented a method wherein said picking is furthermore based on the optical data. An advantage of this may be that it may enable dispensing with sorting before plating.

In an embodiment there is presented a method wherein said picking is not based on optical data, such as not based on images. An advantage of this may be that it facilitates expedient processing since no optical data needs to be obtained. Another advantage of this may be that it facilitates simplification of the equipment employed, because it dispenses with the need for additional equipment, e.g.,

imaging means and image processing means. Since said picking is not based on optical data, such as not based on images, picking may be described as being carried out blindly (such as relying exclusively on the information in the predetermined spatially distributed position).

In an embodiment there is presented a method wherein the method does not comprise

- providing an image at an image sensor, such as a two-dimensional image sensor, and analysing a corresponding image obtained at the image sensor, such as does not comprise providing an image at an image sensor and/or does not comprise analysing a corresponding image obtained at the image sensor, such as consists of non-optical methods.

A possible advantage of this may be that the method may be more efficient (since there will be no time and resources spent on imaging and image analysis).

In an embodiment there is presented a method wherein the plurality of spatially distributed predetermined positions corresponds to a regular grid, such as a Cartesian grid or a hexagonal grid or a rectilinear grid or a curvilinear grid. In a particular embodiment, the Cartesian grid is corresponding to well positions (for any one of a 96 well plate, a 384 well plate, a 1536 well plate, a 3456 well plate) as defined by ANSI (the American National Standards Institute) and Society for Laboratory Automation and Screening (SLAS) in ANSI SLAS 4-2004 (R2012) (formerly recognized as ANSI/SBS 4-2004) which is hereby incorporated by reference in entirety.

In an embodiment there is presented a method wherein the surface of the solid element is topographically modified according to a pattern corresponding to the plurality of spatially distributed predetermined positions, such as the surface of the solid element having corrugations (e.g., corrugations impressed in a planar surface of the solid element, such as impressed in a planar agar surface of the solid element), such as the surface of the solid element comprising wells (such as microwells), such as the surface of the solid element comprising wells with growth medium, such as agar with nutrients. An advantage of topographical modifications

may be better safety since colonies will be less likely to grow into each other, such as end up overlapping.

In an embodiment there is presented a method wherein the solid element comprises:

- A partitioning element, such as a room divider, and
- A solid medium, such as a solid growth medium, such as agar, such as agar with nutrients,

wherein the partitioning element is arranged at least partially within the solid medium and wherein partitions of partitioning element is arranged according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y). An advantage of this may be that it facilitates partitioning the solid medium, so that a colony on one side of the partitioning element or a part of the partitioning element cannot overlap with a colony on another side of the partitioning element or a part of the partitioning element. By having the partitions of partitioning element arranged according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y), it may be facilitated that plating of one or more cells and growing and picking of a colony may take place on different sides of the partitioning element, so that the colonies are kept separate from each other by the partitioning element.

The medium may be agar. The medium may be a growth medium, such as agar with nutrients. The volume of the solid medium may be within 10-100 mL, such as within 20-80 mL such as within 25-75 mL, such as 40 mL.

In general, advantages may include the flexibility (as a liquid culture), the reduced or eliminated influence of evaporation (as a solid culture), enabling high throughput (as a solid culture), reducing or eliminating risk of cross-contamination, enabling automated (such as fully automated) workflows, enabling low volumes (relevant in case of expensive compounds) and easy monitoring (as a liquid culture).

In a further embodiment there is presented a method wherein the partitioning element is arranged at least partially within the solid growth medium so as to

provide a plurality of exposed areas of the surface of the solid growth medium where each exposed area within the plurality of exposed areas of the surface of the growth medium are separated from one or more other exposed areas by the partitioning element. The number of exposed areas must be at least 10, such as at least 25, such as at least 75 (for example 96), such as at least 100, such as at least 250, such as 384.

In a further embodiment there is presented a method wherein the method comprises providing the solid element by:

- Placing a liquid phase medium in a container,
- Placing the partitioning element in the container, and
- Solidifying the medium.

Placing the partitioning element in the container may take place before or after placing the liquid phase medium in a container. Placing the partitioning element in the container may take place before or after solidifying the liquid medium.

In a further embodiment there is presented a method wherein:

- Placing a liquid phase medium in a container, and
- Placing the partitioning element in the container (optionally with locking pins for fixating the partitioning element to the container),

is prior to:

- Solidifying the medium.

An advantage of this may be that when placing the partitioning element in the container when the medium is in a liquid phase before solidifying the medium, there will be little or no stress on the medium and/or the partitioning element. Another possible advantage may be that it requires less force on the partitioning element.

In a further embodiment there is presented a method wherein the liquid phase medium is a liquid phase growth medium, such as agar with nutrients.

In a further embodiment there is presented a method wherein subsequent to:

- Placing a liquid phase medium in a container, such as wherein the liquid phase medium is neutral liquid agar,

- Placing the partitioning element in the container, and
- Solidifying the medium,

the method comprises for one or more exposed areas:

- Adding a substance or composition, such as a nutrient and/or an antibiotic, specifically to the one or more exposed areas, such as wherein the substance or composition is then absorbed into the medium.

An advantage of this may be that different substances or compositions may be added to different exposed areas. This may be advantageous for providing different conditions for different exposed areas.

In an embodiment there is presented a method wherein the time for picking each colony is 1 second or less. By 'the time for picking each colony' may be understood the total time for picking divided by the number of picked colonies, where the total time is given by the time it takes to fill picking tips with medium, pick the colonies, dispense colonies, discard tips.

According to a second aspect of the invention, there is presented an integrated device for sorting and plating cells and for picking corresponding colonies, said device comprising:

- A sorter, such as a FACS, for sorting cells by
  i. providing a first group of cells,
  ii. providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,
- a plating unit, such as a single cell dispenser with an X-Y table, arranged for
  i. for each predetermined position in a plurality of spatially distributed predetermined positions on a surface of a solid element, where each predetermined position is predetermined with respect to the solid element, plating one or more cells from the second group of cells,
- a picking unit, such as a liquid handler, arranged for
  i. for each predetermined position in the plurality of spatially distributed predetermined positions on a surface of a solid

element, picking each colony, wherein said picking is based on the predetermined spatially distributed position.

In an embodiment there is presented a device wherein the device does not comprise image forming optics and an image sensor, such as does not comprise image forming optics, such as consists of non-optical elements. An advantage of this may be that the device is kept relatively simple.

In a third aspect, the invention relates to a computer program product having instructions which, when executed cause a device, such as a device according to the second aspect to perform a method according to the first aspect.

In a third aspect, the invention relates to a system comprising

- a device according to the second aspect, and
- a solid element comprising:
    i. A partitioning element, such as a room divider, and
    ii. A solid medium, such as a solid growth medium,

  wherein the partitioning element is arranged at least partially within the solid medium and wherein partitions of partitioning element is arranged according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y).

The system may be advantageous for providing a device for plating and picking according to a pattern corresponding to a plurality of spatially distributed predetermined positions (X, Y), where the picking and plating may be carried out on the solid element, which may be provided in a simple manner and/or which inhibits cross-contamination.

The first, second and third aspect of the present invention may each be combined with any of the other aspects. These and other aspects of the invention will be apparent from and elucidated with reference to the embodiments described hereinafter.

BRIEF DESCRIPTION OF THE FIGURES

The method for plating, growing and picking according to the invention will now be described in more detail with regard to the accompanying figures. The figures show one way of implementing the present invention and is not to be construed as being limiting to other possible embodiments falling within the scope of the attached claim set.

FIGS. 1-2 shows flow-charts of embodiments of the invention,

FIGS. 3-4 are schematics showing a method for plating cells and for picking corresponding colonies,

FIG. 5 demonstrates plating of TOP10 E.coli strain cells with a cell sorter with a single cell dispenser,

FIG. 6A shows colonies grown in a second test using a different type of E.coli strain,

FIG. 6B shows the colonies from FIG. 6A after picking,

FIG. 7 shows deep well plates,

FIG. 8 shows schematics demonstrating colony overlap and topographical modification.

FIG. 9 shows a method wherein optical data, being absorption data, is obtained optically,

FIG. 10 shows a method wherein optical data, being fluorescence data, is obtained optically,

FIG. 11 shows a layout of plating on a surface of a solid element where only a few colonies are considered as positive,

FIG. 12 is a schematic illustration of a picking embodiment,

FIG. 13 illustrates quadrants of, e.g., a solid element in the form of an agar plate.

FIG. 14 shows a figure which demonstrates plating of yeast cells.

FIG. 15 is a schematic with a partitioning element.

FIGs. 16-19 shows photographs from an example.

FIG. 20 shows a graph relating to the Example referred to in FIGs .16-19.

FIG. 21 shows photographs taken after the experiment of Figs. 16-20.

FIG. 22 shows Yeast cells plated directly on agar plates (OmniTray™).

FIG. 23 shows analysis with a flow cytometer after picking cells of FIG. 22.

DETAILED DESCRIPTION OF AN EMBODIMENT

FIG. 1A shows a flow-chart of an embodiment of the invention, and more particularly a method 100a for plating cells and for picking corresponding colonies, said method comprising:

- for each predetermined position in a plurality of spatially distributed predetermined positions (X, Y) on a surface of a solid element where each predetermined position is predetermined with respect to the solid element, where the plurality of spatially distributed predetermined positions (X, Y) may be obtained from a database 107,
    i. plating 110 one or more cells,
    ii. growing 120 a colony from the one or more cells,
    iii. picking 130 the colony, wherein said picking is based on the predetermined spatially distributed position.

FIG. 1B shows a method 100b similar to the method 100a of FIG. 1A, except that before plating 110, growing 120 and picking 130, a step of sorting 104 has been added, more particularly sorting 104 cells by

- providing a first group of cells,
- providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,

and wherein the one or more cell belong to the second group of cells.

FIG. 1C shows a method 100c similar to the method 100a of FIG. 1A, except that a step of selecting 129, such as selecting based on optical data, has been added after plating 110 and growing 120, but before picking 130. The step of selecting 129 is also based on the plurality of spatially distributed predetermined positions (X, Y), such as optical data obtained by optical probing specifically and exclusively at the plurality of spatially distributed predetermined positions (X, Y).

FIG. 2 shows in more detail a method 200 according to an embodiment of the invention in a flow-chart with start 200, sorting 204, obtaining 206 from a database 207 a plurality of spatially distributed predetermined positions (X, Y)

and the number N of spatially distributed predetermined positions, setting 208 an index *i* to unity, plating 210 by – for index *i* – plating 212 at predetermined position (x_i, y_i), increment *i* with unity, repeat if $i < N$, if $i = N$ then growing 220 colonies from the plated cells, setting 228 index *i* to unity, picking 230 by – for index *i* – picking 232 at predetermined position (x_i, y_i), increment *i* with unity, repeat if $i < N$, if $i = N$ then end 238.

FIGS. 3-4 are schematics showing a method for plating cells and for picking corresponding colonies, said method comprising plating (110) one or more cells (342) on a surface (344) of a solid element from a container being a pipette tip of a single cell dispenser 340, growing (120) a colony (446) from the one or more cells on the surface (344) of a solid element, picking (130) the colony (446) from the a surface (344) of a solid element with a pipette tip of a liquid handler 448. The 'x' and 'y' denote the x- and y-axes respectively.

FIG. 5 demonstrates plating of TOP10 E.coli strain cells with a cell sorter (where cell sorting was carried out previously using mCherry and GFP) with a single cell dispenser (BD FACSAria™ cell sorter, BD Biosciences, San Jose, CA), and more particularly plating a various number of cells on an on an OmniTray™ agar plate (corresponding to the solid element) at a plurality of spatially distributed predetermined positions, which corresponds to a 384 well plate format. The figures shows plating 1 (columns 1-4, 13-16), 2 (columns 5-8, 17-20) or 10 (columns 9-12, 21-24) cells per "virtual well" (with a virtual well corresponding to one spatially distributed predetermined position in the 384 well plate format pattern on the OmniTray™ agar plate). The cells have been FACS sorted prior to plating. All cells grew, demonstrating that plating a single cell on an agar plate works. The figure also demonstrates that the single cells (in columns 1-4 and 13-16) are well aligned.

Plating may be carried out according to an example embodiment as given below:

i. Cells are inoculated in a tube for overnight culture in an incubator (culture volume depends on the number of plates to be generated)
ii. OD is measured using a standard cuvette spectrometer

iii. Sample is diluted (so as to ensure enough cells are present in the starting FACS tube) in a FACS tube with FACS Flow buffer, or sterile PBS (optionally add HEPES to get a better survival rate) as follows:
- If measured OD is 4, 400 microliter of Sample in culture media + 1000 microliter FACS Flow/PBS
- If measured OD is 8, 200 microliter of Sample in culture media + 1000 microliter FACS Flow/PBS
- If measured OD is 12, 100 microliter of Sample in culture media + 1000 microliter FACS Flow/PBS

iv. FACS tube is loaded on the FACS and the acquisition can start

v. Population to be plated is identified using the FACS Diva Software

vi. OmniTray™ with agar is loaded on the FACS

vii. The selected population is then sorted by the FACS according to the desired plate layout (e.g., according to Fig. 5 a standard SBS 384 plate).

For plating using FACS and a single cell dispenser, plating is handled by the FACS software. The user may select a type of plate (6, 12, 24, 48, 96 or 384) which may in effect yield the plurality of spatially distributed predetermined positions (corresponding to the layout of the wells of the selected type of well plate), and for each well the amount of cells to be plated (for example 1).

FIG. 6A shows colonies grown in a second test using a different type of E.coli strain (W). As the first test showed 100 % viability on the plated single cells, this time only one single cell was plated in each of the 384 virtual wells, still using the cell sorter with single cell dispenser. Cells were incubated 26 hours. The figure shows that the colonies are distributed regularly wherein the plurality of spatially distributed predetermined positions corresponds to a regular Cartesian grid.

FIG. 6B shows the colonies from FIG. 6A after picking 96 colonies at a time with a 96 pipettes pipetting head (VANTAGE Liquid Handling System™ with a 96 Multi-Probe Head, Hamilton) without relying on any imaging or image processing). For most of the colonies the pick was perfect (see, e.g., the upper magnification) or nearly perfect (see, e.g., the lower magnification).

Picking may be carried out by a liquid handler according to an example embodiment as given below:

i. 1 source OmniTray™ with agar and 384 colonies + 4 empty destination DeepWell plates + one trough containing culture media are loaded on the robot. For pipetting, 4 tip boxes used for picking (10 microliter tips Hamilton) + 1 tip box used to pipette media (1000 microliter tips Hamilton) from the trough to the deepwell plates are also needed

ii. The pipettor loads the tips on the 96 head to pipette media, aspirates and dispense from media trough to each deepwell plates a cultivation volume defined by the user (500 microliter in our case)

iii. Tips are discarded

iv. The pipettor loads the 1st set of 96 tips on the 96 head to pick the colonies

iv. The pipetting head goes over the media trough to aspirate a small pre-volume of media (8 microliter in our case), used to ensure the "agar stopper" is pushed in the liquid (see FIG. 12)

v. Optionally, an airgap can be aspirated (a few microliter) to ensure no liquid leaks from the tip on the agar plate.

vi. The pipettor head goes over to a virtual quadrant (see FIG. 13) 1 of the agar plate, and pick the 96 corresponding colonies. A trick to ensure robot picks the colony is to define a fix pipetting height so the tips go into the agar 1-2 mm deep (e.g. if the agar layer height is 6 mm, pipetting 0 microliter at 4-5 mm height will work). Some robots may not like a "Pipette 0 microliter" command, so in this case the user should select the lowest volume accepted by the robot

vii. The pipettor head goes over destination deepwell plate 1 and empty the tips (pre-volume + optional airgap + "agar volume" if different than 0 microliter.

viii. Tips are discarded

ix. Repeated from step iv. to process the other 3 quadrants as follow:
-Colonies picked from quadrant 1 go in Deepwell plate 1 (A1 in A1, A3 in A2, A5 in A3, etc.)
-Colonies picked from quadrant 2 go in Deepwell plate 2 (A2 in A1, A4 in A2, A6 in A3, etc.)

-Colonies picked from quadrant 3 go in Deepwell plate 3 (B1 in A1, B3 in A2, B5 in A3, etc.)
-Colonies picked from quadrant 4 go in Deepwell plate 4 (B2 in A1, B4 in A2, B6 in A3, etc.)

FIG. 12 is a schematic illustration of a picking embodiment.

FIG. 13 illustrates quadrants of, e.g., a solid element in the form of an agar plate.

According to another embodiment a method for picking may be given as:

i. aspirating a volume (for example 10 microliter) of liquid (such as medium) into each of one or more liquid handler pipettes,
ii. move the one or more liquid handler pipettes into physical contact with the colony (and optionally request liquid handler software to aspirate 0 microliter into each of one or more liquid handler pipettes), so that one or more cells from each colony gets stuck on or within a corresponding pipette,
iii. remove the one or more liquid handler pipettes from physical contact with the colony,
iv. move the one or more liquid handler pipettes into a position above a surface or one or more containers (such as one or more wells) for receiving picked colony cells,
v. dispense from each of the one or more liquid handler pipettes a volume (for example 10 microliter) of liquid, so as to dispense with the volume one or more of the cells which got stuck on or within the pipette tip.

FIG. 7 shows four 96 deep well plates, which were incubated with the colonies from FIGS. 6A-B after picking all the colonies. All wells showed growth demonstrating the viability of the cells after sorting with FACS, plating with the single cell dispenser of the FACS, picking with the 96 pipettes pipetting head, dispensing into deep wells and incubating.

The time for picking is calculated to be slightly less than 1 second per colony, more particularly 0.98 seconds per colony.

FIG. 8 shows in the left side a schematic demonstrating that a first colony 846A may grow into and overlap with a second colony 846B. The figure shows in the right side another schematic wherein the surface of the solid element is topographically modified according to a pattern corresponding to the plurality of spatially distributed predetermined positions, more particularly wherein the surface of the solid element comprises wells with walls 848 and growth medium in the wells whereupon colonies 846C and 846D can grow safely shielded from each other by the wall 848.

In general, in embodiments of the invention the surface of the solid element may be planar, such as an OmniTray™, or be topographically modified, such as according to any other type of SBS plate (e.g. 96 well plate). Wells may be pre-filled with agar, then single cells are seeded, colonies are grown then picked. Wells actually adds extra safety because they may help avoid overlapping of the colonies (thus ensuring picking the right colony and not the neighbour colony or a mixture of both). In case some colonies grow faster than others it can also be useful with wells as the physical walls between each well will prevent the colonies to overgrow/overlap.

FIG. 9 shows a method wherein optical data, being absorption data, is obtained optically, subsequent to growing, based on said spatially distributed predetermined positions (X, Y). More particularly, the figure shows incident light 950 into a plurality of wells, and photometers 954 at the spatially distributed predetermined positions (X, Y), which measure ca. 0 % of incident light 952A due to the presence of a huge colony in the first well from the left side, ca. 50 % of incident light 952B due to the presence of a huge colony in the second well from the left side, ca. 80 % of incident light 952C due to the presence of a small colony in the third well from the left side, ca. 100 % of incident light 952D due to the presence of a no colony (negative control) in the fourth well from the left side,

In case a laboratory is not equipped with a cell sorter, such as FACS, a standard single cell dispenser can also do the job of plating. Then, cells are not sorted prior

to plating, but then an evaluation of the colonies can be done using, e.g., a standard plate reader after the colonies have grown, e.g., as follows:

1. Single cells are plated on a normal deep well plate using any single cell dispenser. One single cell is plated per well, prefilled with agar.

2. Colonies are growing.

3. When colonies are grown, the plate is read using a standard micro plate reader.

4. Positive colonies are then picked, e.g., using channels instead of pipetting head.

In general, if measurement method is optical density (OD) or colorimetry then the plate needs to have a transparent bottom. If the readout is fluorescence, then the walls have to be non-transparent (to avoid well to well "false positives", e.g. if well A1 is not fluorescent, if A2 is florescent and if the walls are transparent, A1 may emit a signal because of A2 proximity). If a combination of OD and fluorescent signal is measured, then plate should have transparent bottom (to allow OD) and non-transparent walls (to avoid neighbor wells to generate false positives).

OD measurement of such transparent plates prefilled with agar and with single cells seeded in each well could allow estimating the size of a colony, such as could also be used to monitor growth rate on agar.

It is noted that on pipetting robots (liquid handlers), 2 types of pipetting tools are used: Pipetting head is a head with 96, 384 or even 1536 channels, all pipetting the same volumes at the same time, and each channel always goes to the same well, e.g., using a 96 pipetting head, channel A1 will always and only pipette from/to A1 in a 96 well plate, A1, A2, B1 and B4 from/to a 384 well plate, or A1->A4, B1->B4, C1->C4, and D1->D4 from/to a 1536 well plate, etc. Then the other tool used to pipette is called channel. Most of the cases there are 8

channels, but some instruments can have less, sometimes only 1, or up to 16. An advantage of the channels is that they can pipette different volumes simultaneously, and they are not restricted to a few wells in the plates. Any of the channels can pipette from/to any well of a plate.

As for a pick using a head, the channels will pick the colonies going to a defined position of the plates (The center of the well) but this time according to a worklist created after analysis of the reader output file. Only the fluorescent / colored / positive colonies will be picked. This is slower than picking all of them as we would do with the head, but this will ensure this technique can be used without a prior cell sorting step, e.g., in a laboratory not equipped with a FACS. But it can still be much faster than a single picking tool, as the channels can also work simultaneously and pick from 1 to 8 positive colonies per plate column (in case of a 96 well plate) or 1-16 (in case of a 384 well plate). Still there is no need to have image analysis. It may even give precious information regarding the size of the colonies and may allow to determine growth rates by doing many measurements with a time interval.

FIG. 10 shows a method wherein optical data, being fluorescence data, is obtained optically, subsequent to growing, based on said spatially distributed predetermined positions (X, Y). More particularly, the figure shows incident light into a plurality of wells, and emittted fluorescent light 956 due to the presence of a huge colony in the third well from the left side, and no fluorescent light from the negative control in the fourth well from the left side.

FIG. 11 shows a layout of plating on a surface of a solid element where only a few colonies are considered as positive (grey), such as the positive colonies determined using optical data as obtained in FIG. 9 or FIG. 10. The channels can still pick colonies simultaneously (3 in column 1; 5 in column 3; 8 in column 7; 1 in column 10; 1 in column. 11). After the plate has been read, the worklist can be generated so the robot knows where to pick. For this pattern, 18 colonies will be picked in 3 steps as follows (in case of a standard pipetting robot incl. 8 channels):

Step 1:

1. Channel 1 to 8 load tips
2. Channel 1 picks B1, Channel 2 picks D1, channel 3 picks E1, all 3 at the same time, then the robot arm holding the channels moves 2 x 9 mm to the right to be above column 3, then
3. Channel 4 picks A3, channels 5 to 8 pick C3 to F3
4. Channels 1 to 8 dispense picked colonies in destination plate (all at the same time)
5. Tips are discarded

Step 2:

1. Channel 1 to 8 load tips
2. Channels 1 to 8 pick column 7 all wells at the same time (channel 1 -> A7, channel 2 -> B7, …, channel 8 ->H7)
3. Channels 1 to 8 dispense picked colonies in destination plate (all at the same time)
4. Tips are discarded

Step 3:

1. Channels 1 & 2 load tips
2. Channel 1 picks D10
3. Channel 2 picks D11
4. Channels 1 & 2 dispense picked colonies in destination plate (all at the same time)
5. Tips are discarded

Fig. 1 illustrates an example use of an embodiment of the present invention, more particularly colony picking coupled to clone identification, separation and analysis at single cell levels.

More particularly, the figure demonstrates plating of yeast cells, and more still particularly shows colonies of yeast cells on an OmniTray™. Yeast cells were plated on an OmniTray™ using a BD FACSAria™ cells sorter and the Omnitray™ was placed in an incubator for 3 days, whereafter the image (photograph inset in the lower left corner) was obtained (it is noted that the photograph is not necessary for the process, and is obtained purely for demonstration purposes).

The process illustrated in figure 14 relates to:

- Isolating 91 single clones (out of 96 plated cells, 91 gave colonies),
- pre-culturing the picked colonies and
- splitting the pre-culture plate in 2 sub culture plates (one + ligand, and one – ligand)
- treating.

Then an analysis was performed, here on 2 control conditions showing that the picked colonies were responding to both conditions (with or without ligand), and this demonstrates that the present embodiment is suitable for such workflows.

This experiment (figure 14) show that an embodiment of the invention also works with Yeast (note that another example in the present application was done with E.coli strains). It also shows that this way of doing colony picking can be integrated in a workflow and can be used on a daily base with good hit rate (such as demonstrated 100 % in the E.coli example and 95 % in the example using Yeast).

The histograms on the right hand side show GFP fluorescence, where the vertical axis shows 'counts' and the horizontal axis is a logarithmic scale of wavelength in arbitrary units.

FIG. 15 is a schematic with a partitioning element, which in the figure is referred to as an AgarGrid. Subfigure 1 shows an empty container, which in the figure is an OmniTray™. The portioning may correspond to the plurality of spatially distributed predetermined positions (X, Y). In subfigure 2, there is placed liquid medium (which in the figure is liquid agar) in the container. In subfigure 3 the partitioning element is placed in the liquid medium so that upon solidifying the medium, there is provided a plurality of exposed areas of the surface of the solid

growth medium where each exposed area within the plurality of exposed areas of the surface of the growth medium are separated from one or more other exposed areas by the partitioning element. In subfigure 4 there is for specifically for each of the exposed areas added a substance or composition (which in the figure is referred to as "media"), such as a nutrient and/or an antibiotic. In subfigure 5 the plate (container, partitioning element and solid medium) is shown ready for inoculation or plating. In subfigure 6 the exposed areas are inoculated. In subfigure 7 growth is monitored via optical density (OD) or fluorescence. In subfigure 8 liquid is added to compensate feed and/or compensate evaporation. In subfigure 9 colonies are picked, such as picked based on the plurality of spatially distributed predetermined positions (X, Y). In the present example with FIGs. 14-21, picking was carried out with a mechanical hand pipette.

FIGs. 16-19 shows photographs from an example.

FIG. 16 shows a container, being an OmniTray™, with liquid medium, being liquid neutral agar.

FIG. 17 shows a partitioning element being inserted into the liquid medium. Afterwards, the liquid medium is allowed to cool down and solidify.

FIG. 18 shows that resulting wells (corresponding to a plurality of exposed areas) have even levels, see for example the three indicated wells.

FIG. 19 shows that 32 wells (2 columns) were used for this example. Wells A1 to N2 have been inoculated manually with cells, wells 01 to P2 are used as blank (only treated with water). The assembly was placed on a screening robot for 32 hours, incubated at 37°C, and a robot was pipetting 1 microliter of media (water for the 4 blank wells) to feed the cells every 20 minutes. This liquid addition was also helping to keep the agar layer as regular as possible by reducing evaporation effect. Every hour the assembly was moved by the robot in a microtiter plate reader and absorbance was measured. It is noted that if the partitioning element is in a standard format, e.g., compatible with SBS format OmniTrays™, then standard equipment, such as microtiter plate readers may be applicable.

FIG. 20 shows a graph corresponding to an experiment, which with respect to the experiment described in connection with FIGs. 16-19 is different but similar. More particularly, the difference is that wells A1 to N2 have been inoculated manually with cells, wells O1 to O2 are used as negative controls (only treated with media without inoculation) and P1 to P2 are used as blank (only treated with water). The graph was made by deducting T_0 reading (being a reading at time t = 0 hours) to every measurement to avoid any influence of different level of agar (even if all the wells have the same agar height, the reader is sensitive enough to measure differences), and then also deducting the negative controls (no inoculation but media addition), to remove the absorbance of the media (yellow colour) measured by the reader. Each curve thus corresponds to one of the 28 inoculated wells. Another important parameter is that the blanks stayed at the same level during the entire experiment, meaning the height of agar was kept by adding the same amount of water that was evaporated over time. By using this calculation (Value-T_0-negative) it is possible to monitor the amount of cells present on the surface of the agar. This graph shows the 28 samples are following the same trend. It is noted that to avoid confusion, data between 5 hours and 22 hours (x-axis shows time in units of hours) have been covered, because it would appear misleading, because another piece of labware was tested on the same robot, using a shaker and this shaker created vibrations and these vibrations made the data inconsistent between 5 hours and 22 hours after the experiment was started, because during that time span the shaker was on.

FIG. 21 shows photographs taken after the experiment of Figs. 16-19 was finished and the partitioning element was removed from the container (the OmniTray™) for a visual control.

FIG. 22 shows Yeast cells plated directly on agar plates (OmniTray™) using the BD FACSAria™ cell sorter in a 96-position format, and after the trays were placed in an incubator for 3 days. The cells were FACS sorted prior to plating.

FIG. 23 shows analysis with a flow cytometer after picking cells of FIG. 22 with a pin inoculator and pre-culturing. Cells were grown in liquid medium on 96-well microtiter plates (transfer with 96-pin inoculator) and analysed on flow cytometer.

FIGs. 22-23 demonstrates that embodiments also works with Yeast.

In embodiments E1-E15 of the invention, there is presented:

E1. A method for plating cells and for picking corresponding colonies, said method comprising:

- for each predetermined position (x_i, y_i) in a plurality of spatially distributed predetermined positions (X, Y) on a surface (344) of a solid element where each predetermined position is predetermined with respect to the solid element,
    i. plating (110) one or more cells (342),
    ii. growing (120) a colony (446) from the one or more cells,
    iii. picking (130) the colony (446), wherein said picking is based on the predetermined spatially distributed position (x_i, y_i).

E2. A method according to any one of the preceding embodiments, wherein the method comprises:

- Sorting (104) cells by
    i. providing a first group of cells,
    ii. providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,

and wherein the one or more cell belong to the second group of cells.

E3. A method according to any one of the preceding sorting embodiments, wherein the sorting (104) is fluorescence-activated cell sorting.

E4. A method according to any one of the preceding embodiments, wherein plating (110) is carried out with a single cell dispenser (340).

E5. A method according to any one of the preceding embodiments, wherein picking (130) is carried out with a liquid handler (448).

E6. A method according to any one of the preceding embodiments, wherein plating (110) is carried out with a cell sorter (340) and picking is carried out with a liquid handler (448).

E7. A method according to any one of the preceding embodiments, wherein optical data is obtained optically based on said spatially distributed predetermined positions (X, Y).

E8. A method according to embodiment E7, wherein said picking (130) is furthermore based on the optical data.

E9. A method according to any one of the preceding embodiments, wherein the method does not comprise

- providing an image at an image sensor and analysing a corresponding image obtained at the image sensor.

E10. A method according to any one of the preceding embodiments, wherein the plurality of spatially distributed predetermined positions (X, Y) corresponds to a regular grid.

E11. A method according to any one of the preceding embodiments, wherein the surface (344) of the solid element is topographically modified according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y)

E12. A method according to any one of the preceding embodiments, wherein the time for picking (130) each colony (446) is 1 second or less.

E13. An integrated device for sorting (104) and plating (110) cells and for picking (130) corresponding colonies, said device comprising:

- A sorter for sorting cells by

i. providing a first group of cells,

ii. providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,

- a plating unit arranged for
  i. for each predetermined position (x_i, y_i) in a plurality of spatially distributed predetermined positions (X, Y) on a surface of a solid element, where each predetermined position is predetermined with respect to the solid element, plating (110) one or more cells from the second group of cells,
- a picking unit arranged for
  i. for each predetermined position (x_i, y_i) in the plurality of spatially distributed predetermined positions (X, Y) on a surface of a solid element, picking (130) each colony, wherein said picking is based on the predetermined spatially distributed position (x_i, y_i).

E14. A device according to any one of the preceding embodiment E13, wherein the device does not comprise image forming optics and an image sensor.

E15. Computer program product having instructions which, when executed cause a device to perform a method according to any one of embodiments E1-E12.

For the above embodiments E1-E15, it may be understood that reference to preceding 'embodiments' may refer to preceding embodiments within embodiments E1-E15. It may furthermore be understood that any of the embodiments E1-E15 may be combined with any other embodiment disclosed in this application.

Although the present invention has been described in connection with the specified embodiments, it should not be construed as being in any way limited to the presented examples. The scope of the present invention is set out by the accompanying claim set. In the context of the claims, the terms "comprising" or

"comprises" do not exclude other possible elements or steps. Also, the mentioning of references such as "a" or "an" etc. should not be construed as excluding a plurality. The use of reference signs in the claims with respect to elements indicated in the figures shall also not be construed as limiting the scope of the invention. Furthermore, individual features mentioned in different claims, may possibly be advantageously combined, and the mentioning of these features in different claims does not exclude that a combination of features is not possible and advantageous.

CLAIMS

1. A method for plating cells and for picking corresponding colonies, said method comprising:
   - for each predetermined position (x_i, y_i) in a plurality of spatially distributed predetermined positions (X, Y) on a surface (344) of a solid element where each predetermined position is predetermined with respect to the solid element,
     i. plating (110) one or more cells (342),
     ii. growing (120) a colony (446) from the one or more cells,
     iii. picking (130) the colony (446), wherein said picking is based on the predetermined spatially distributed position (x_i, y_i).

2. A method according to any one of the preceding claims, wherein said picking is based exclusively on the predetermined spatially distributed position (x_i, y_i).

3. A method according to any one of the preceding claims, wherein said picking is based on the predetermined spatially distributed position (x_i, y_i) as obtained from a database (107).

4. A method according to any one of the preceding claims, wherein the method comprises:
   - Sorting (104) cells by
     iii. providing a first group of cells,
     iv. providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,

   and wherein the one or more cell belong to the second group of cells.

5. A method according to claim 4, wherein the sorting (104) is fluorescence-activated cell sorting.

6. A method according to any one of the preceding claims, wherein plating (110) is carried out with a single cell dispenser (340).

7. A method according to claim 6, wherein the single cell dispenser (340) obtains the plurality of spatially distributed predetermined positions (X, Y) from a database (107).

8. A method according to any one of the preceding claims, wherein picking (130) is carried out with a liquid handler (448).

9. A method according to claim 8, wherein the liquid handler (448) obtains the plurality of spatially distributed predetermined positions (X, Y) from a database (107).

10. A method according to any one of the preceding claims, wherein plating (110) is carried out with a cell sorter (340) and picking (130) is carried out with a liquid handler (448).

11. A method according to claim 10, wherein the single cell dispenser (340) and the liquid handler (448) each obtains the plurality of spatially distributed predetermined positions (X, Y) from a database (107).

12. A method according to any one of the preceding claims, wherein plating (110) is carried out with a plating unit and picking (130) is carried out with a picking unit, wherein the picking unit and the plating unit each obtains the plurality of spatially distributed predetermined positions (X, Y) from a database (107).

13. A method according to any one of the preceding claims, wherein optical data is obtained optically based on said spatially distributed predetermined positions (X, Y).

14. A method according to claim 13, wherein said picking (130) is furthermore based on the optical data.

15. A method according to any one of the preceding claims, wherein said picking is not based on optical data, such as imaging.

16. A method according to any one of the preceding claims, wherein the method does not comprise
    - providing an image at an image sensor and analysing a corresponding image obtained at the image sensor.

17. A method according to any one of the preceding claims, wherein the plurality of spatially distributed predetermined positions (X, Y) corresponds to a regular grid.

18. A method according to any one of the preceding claims, wherein the surface (344) of the solid element is topographically modified according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y).

19. A method according to any one of the preceding claims, wherein the solid element comprises:
    - A partitioning element, such as a room divider, and
    - A solid medium, such as a solid growth medium,

    wherein the partitioning element is arranged at least partially within the solid medium and wherein partitions of partitioning element is arranged according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y).

20. A method according to claim 19, wherein the partitioning element is arranged at least partially within the solid growth medium so as to provide a plurality of exposed areas of the surface of the solid growth medium where each exposed area within the plurality of exposed areas of the surface of the growth medium are separated from one or more other exposed areas by the partitioning element.

21. A method according to any one of claims 19-20, wherein the method comprises providing the solid element by:
    - Placing a liquid phase medium in a container,
    - Placing the partitioning element in the container, and
    - Solidifying the medium.

22. A method according to claim 21, wherein:
    - Placing a liquid phase medium in a container, and
    - Placing the partitioning element in the container,

    is prior to:

    Solidifying the medium.

23. A method according to any one of claims 21-22, wherein the liquid phase medium is a liquid phase growth medium.

24. A method according to any one of claims 21-23, wherein subsequent to:
    - Placing a liquid phase medium in a container,
    - Placing the partitioning element in the container, and
    - Solidifying the medium,

    the method comprises for one or more exposed areas:
    - Adding a substance or composition, such as a nutrient and/or an antibiotic, specifically to the one or more exposed areas.

25. A method according to any one of the preceding claims, wherein the time for picking (130) each colony (446) is 1 second or less.

26. An integrated device for sorting (104) and plating (110) cells and for picking (130) corresponding colonies, said device comprising:
    - A sorter for sorting cells by
        i. providing a first group of cells,
        ii. providing a second group of cells by selecting one or more cells in the first group of cells, which fulfil a sorting criteria,
    - a plating unit arranged for

i. for each predetermined position (x_i, y_i) in a plurality of spatially distributed predetermined positions (X, Y) on a surface of a solid element, where each predetermined position is predetermined with respect to the solid element, plating (110) one or more cells from the second group of cells,

- a picking unit arranged for
  i. for each predetermined position (x_i, y_i) in the plurality of spatially distributed predetermined positions (X, Y) on a surface of a solid element, picking (130) each colony, wherein said picking is based on the predetermined spatially distributed position (x_i, y_i).

27. A device according to claim 26, wherein the device does not comprise image forming optics and an image sensor

28. A system comprising
    - a device according to any one of claims 26-27, and
    - a solid element comprising:
      i. A partitioning element, such as a room divider, and
      ii. A solid medium, such as a solid growth medium,

    wherein the partitioning element is arranged at least partially within the solid medium and wherein partitions of partitioning element is arranged according to a pattern corresponding to the plurality of spatially distributed predetermined positions (X, Y).

29. Computer program product having instructions which, when executed cause a device to perform a method according to any one of claims 1-25.

FIG. 1A

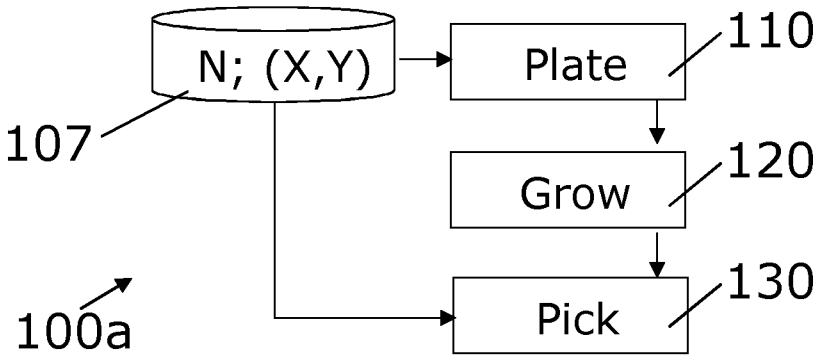


FIG. 1B

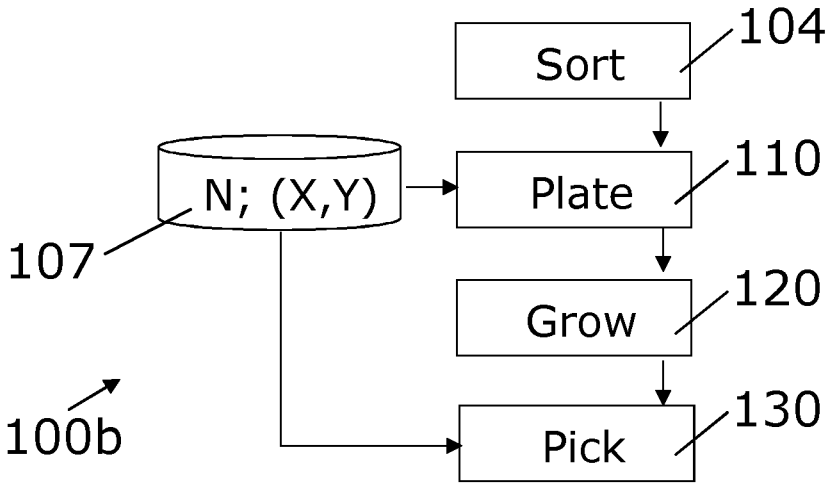
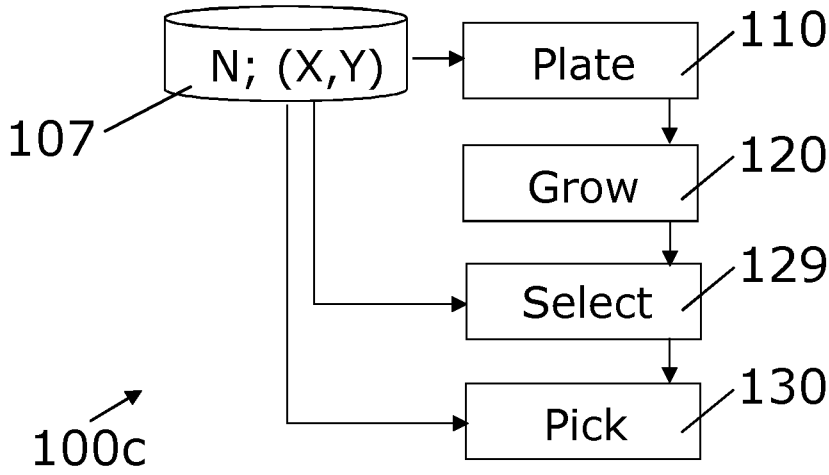


FIG. 1C



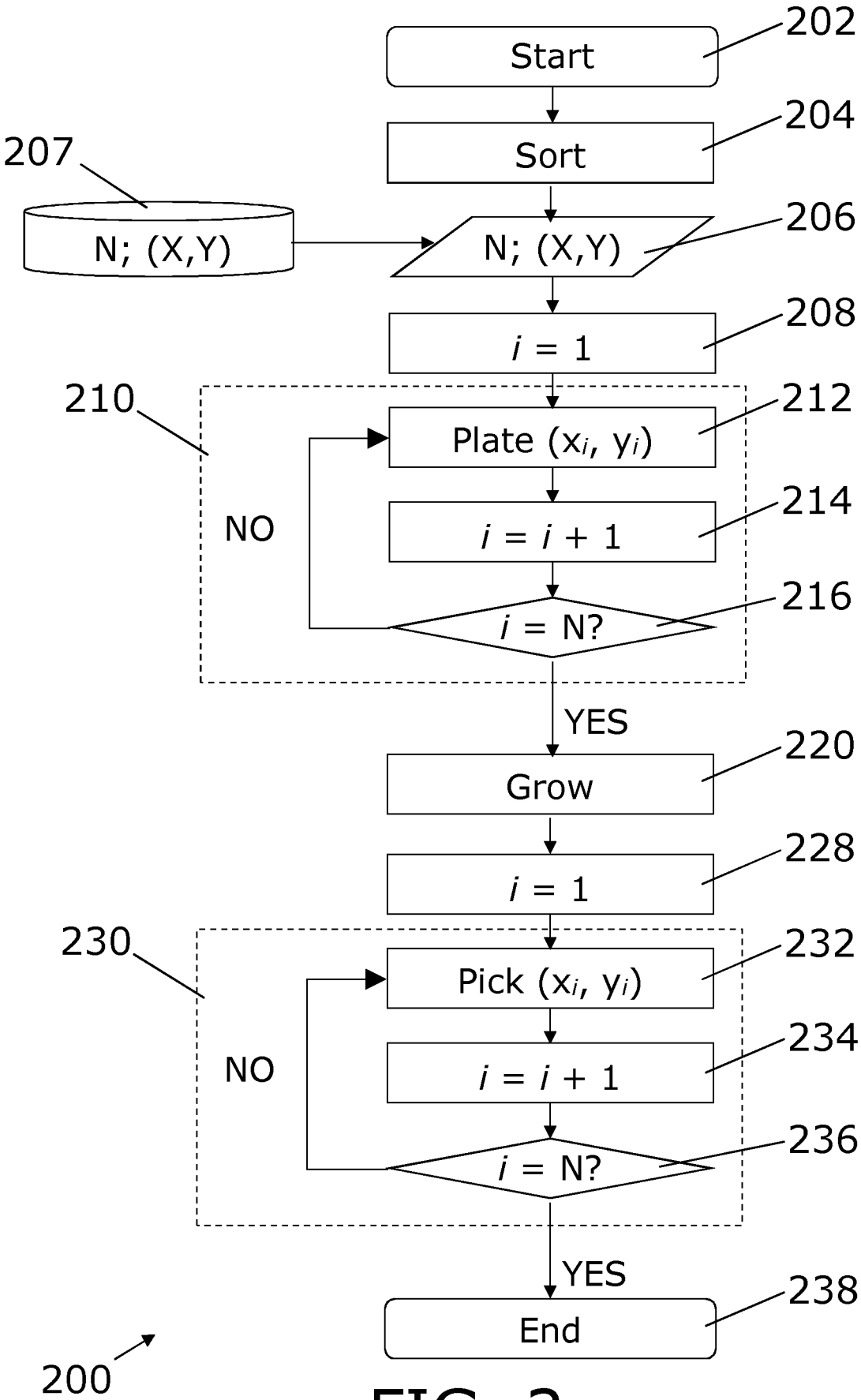

Start
202
Sort
204
207
N; (X,Y)
N; (X,Y)
206
i = 1
208
210
Plate (xi, yi)
212
NO
i = i + 1
214
i = N?
216
YES
Grow
220
i = 1
228
230
Pick (xi, yi)
232
NO
i = i + 1
234
i = N?
236
YES
End
238
200

FIG. 2

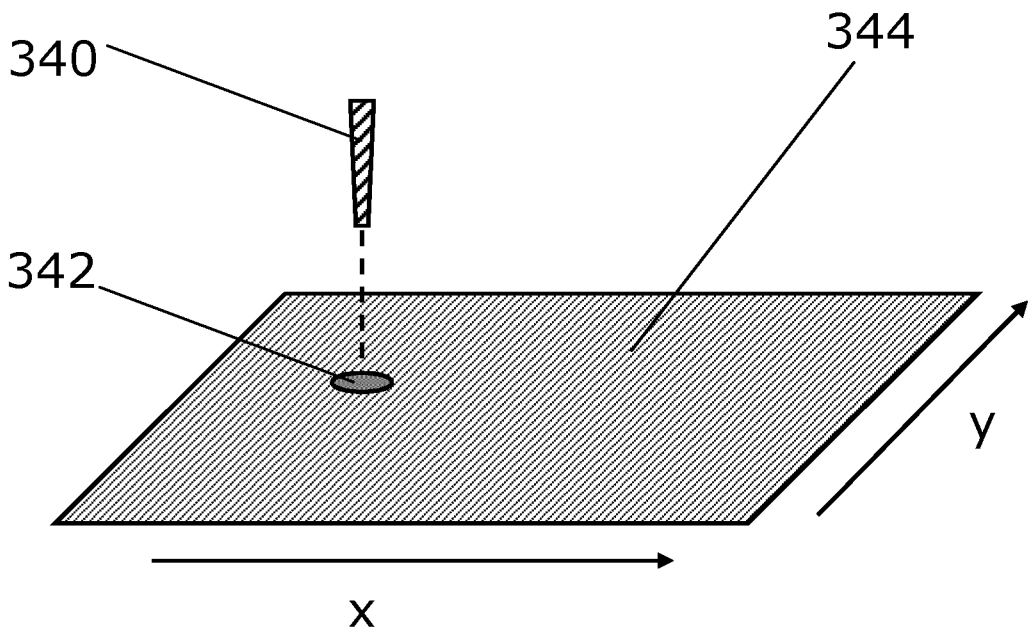


FIG. 3

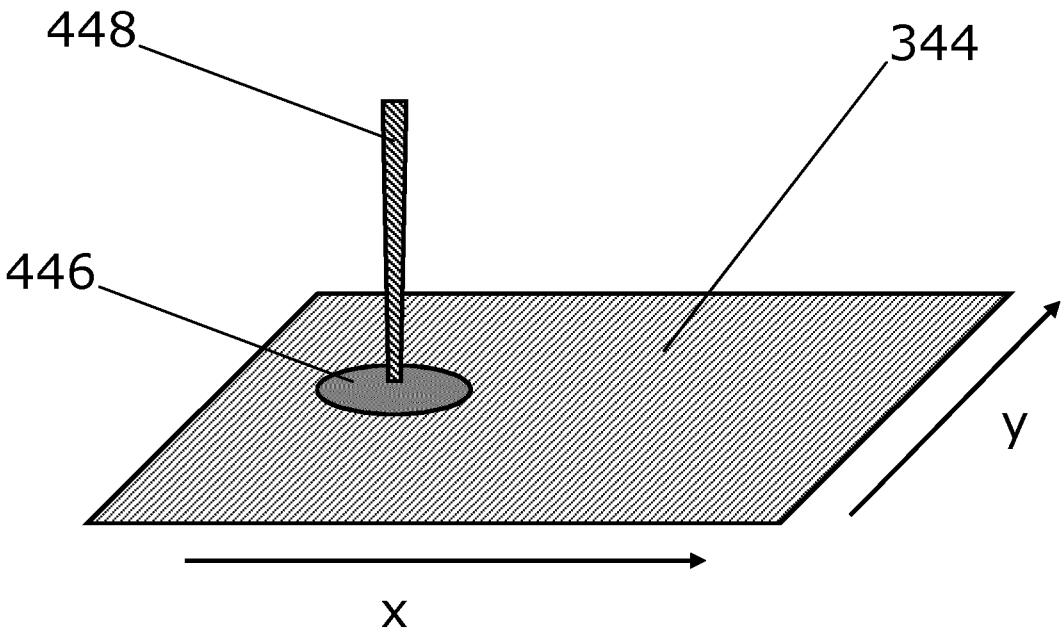


FIG. 4

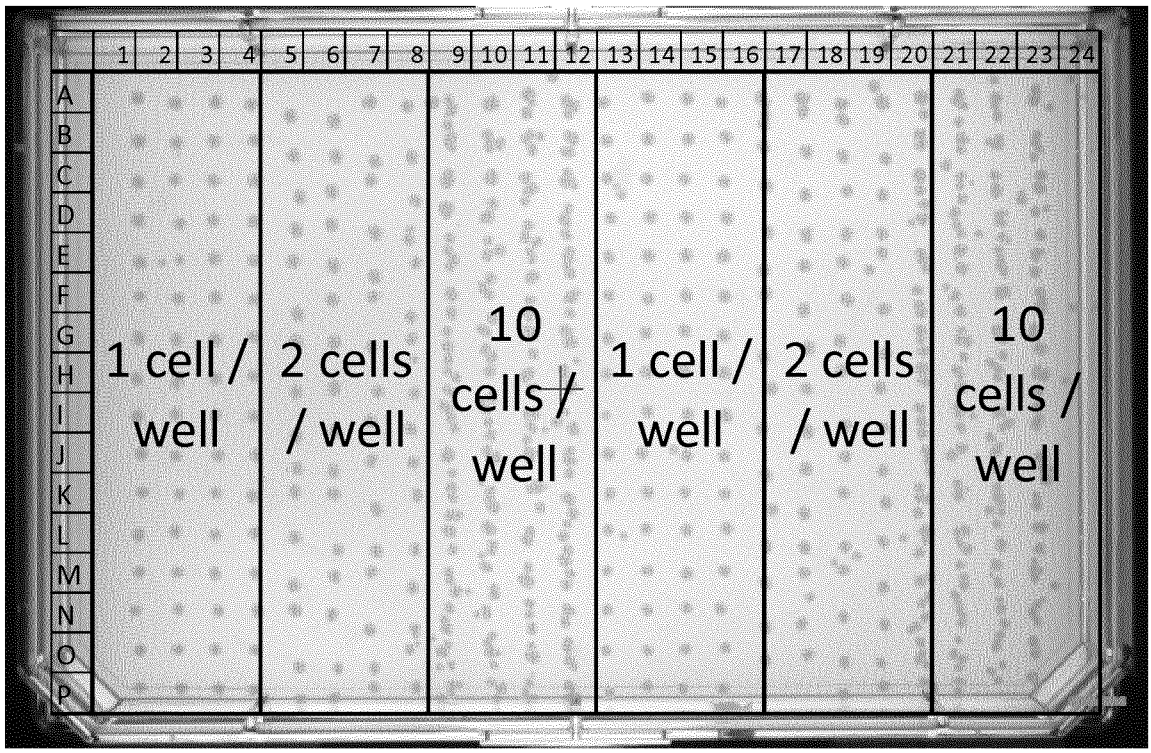

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A B C D E F G H I J K L M N O P
1 cell / well
2 cells / well
10 cells / well
1 cell / well
2 cells / well
10 cells / well

FIG. 5

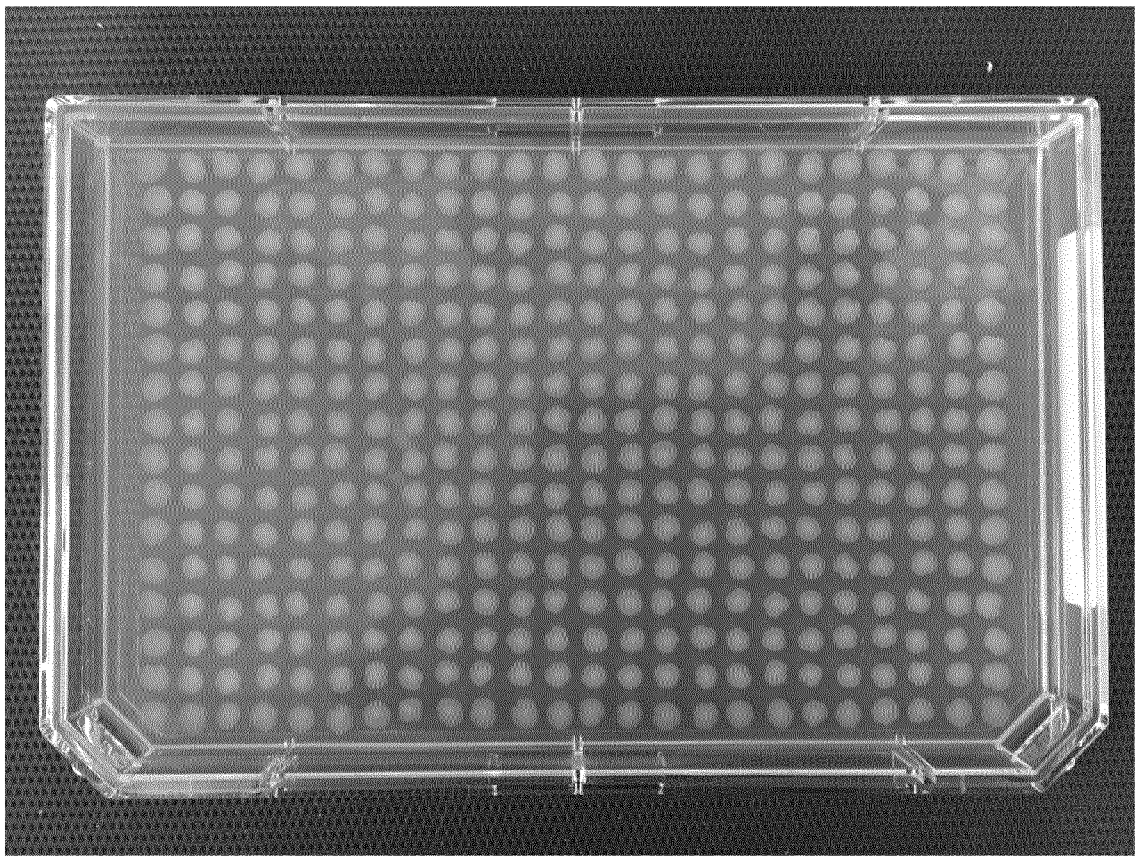


FIG. 6A

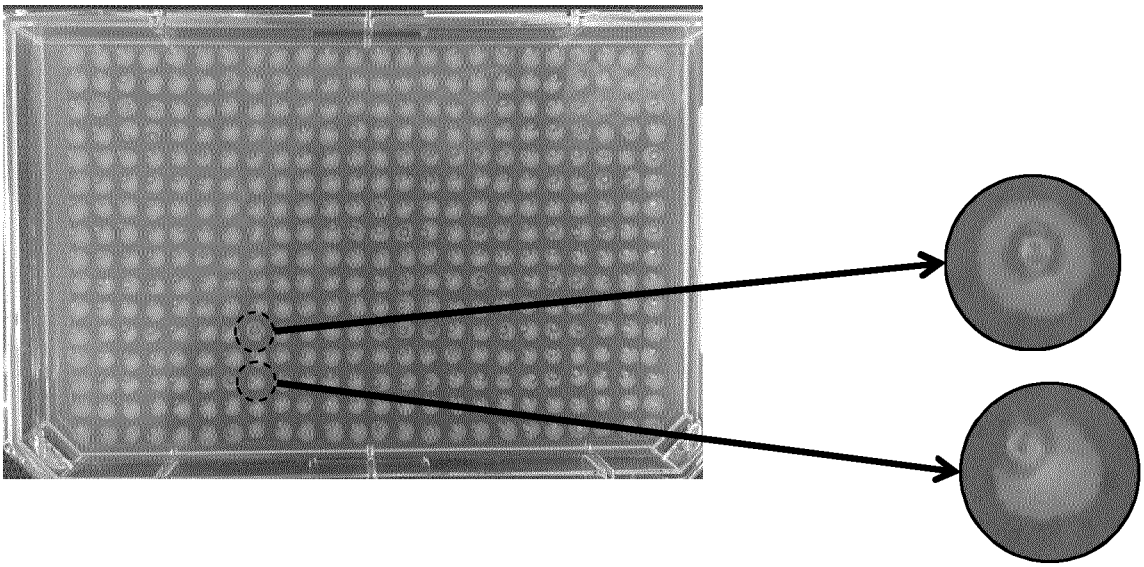


FIG. 6B

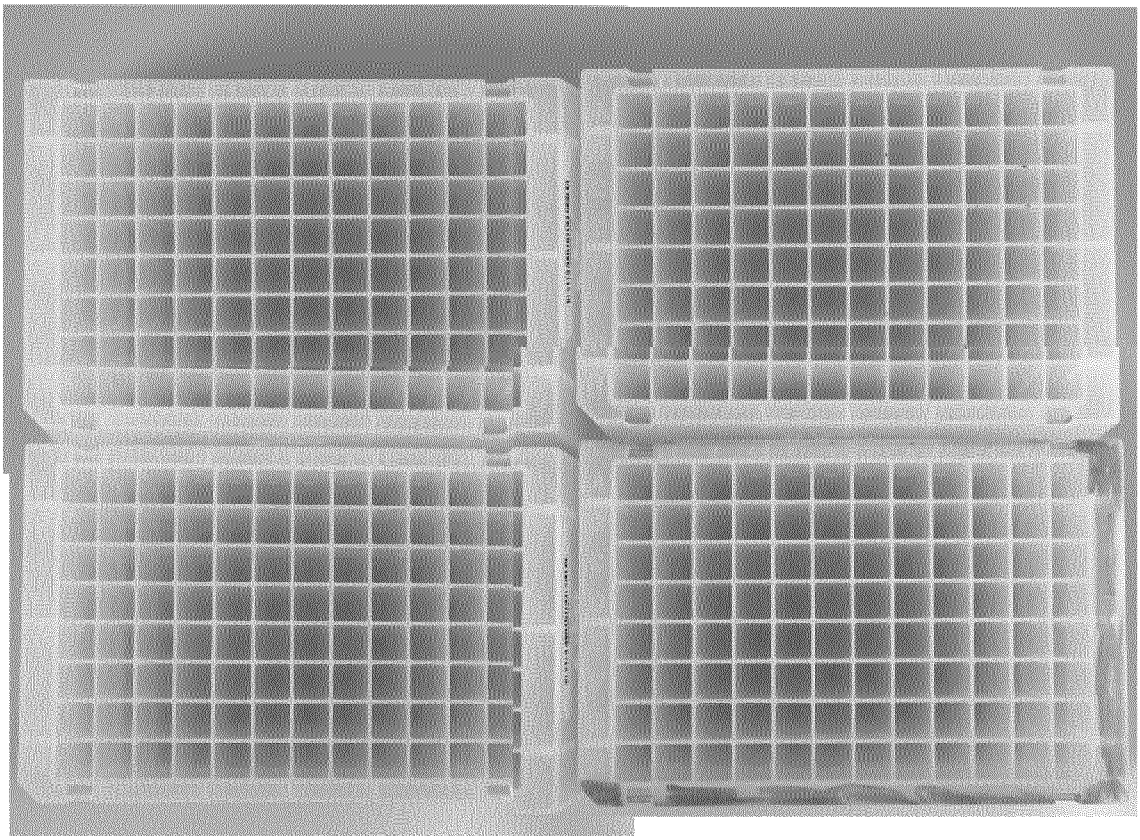


FIG. 7

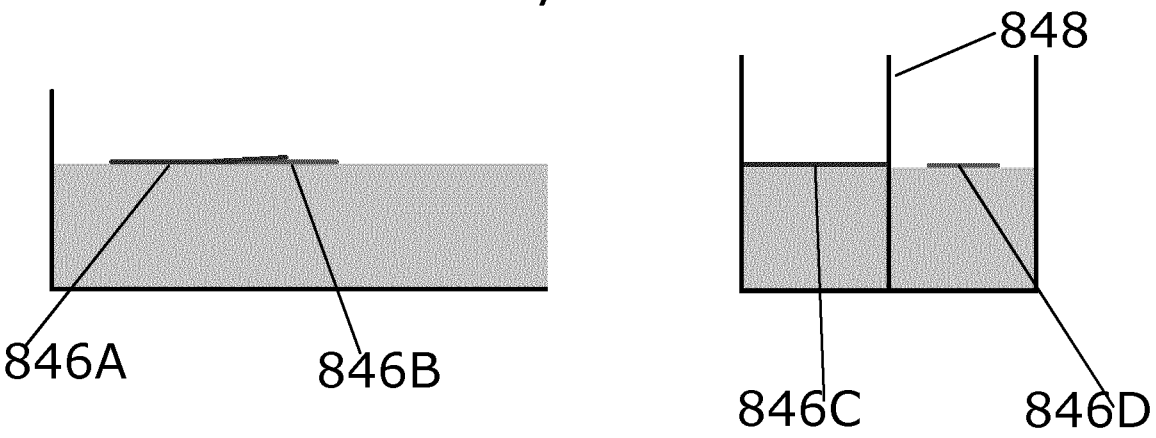


FIG. 8

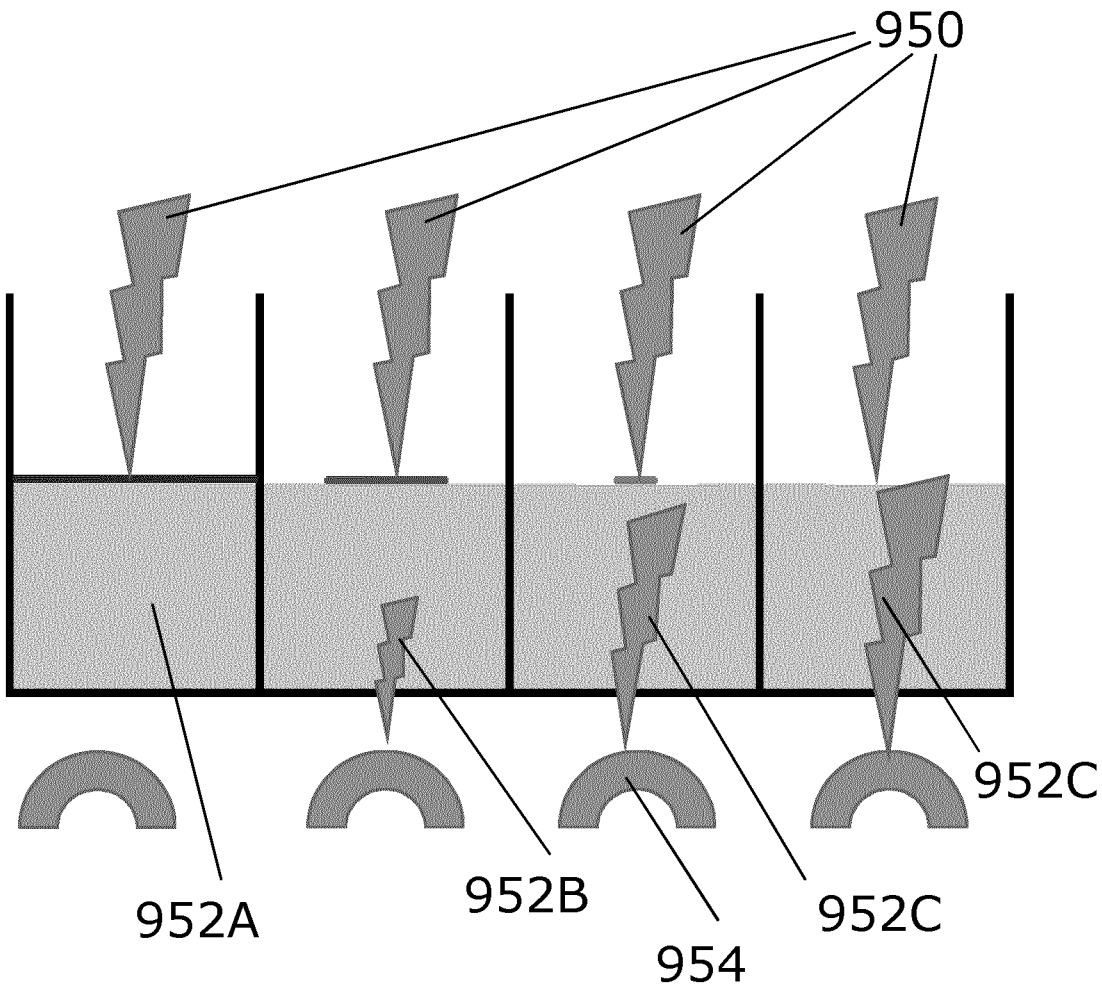


FIG. 9

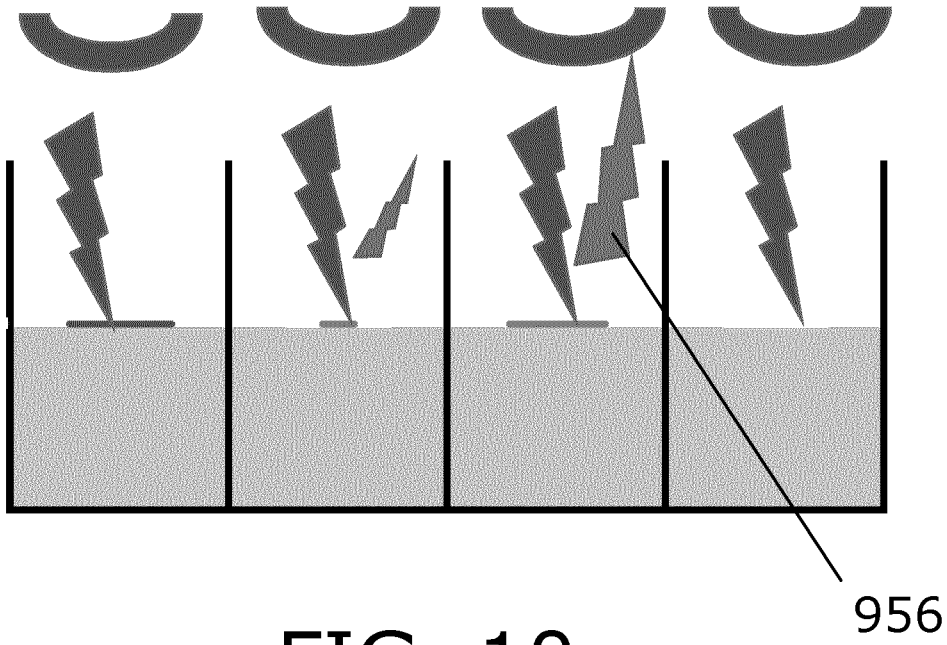


FIG. 10

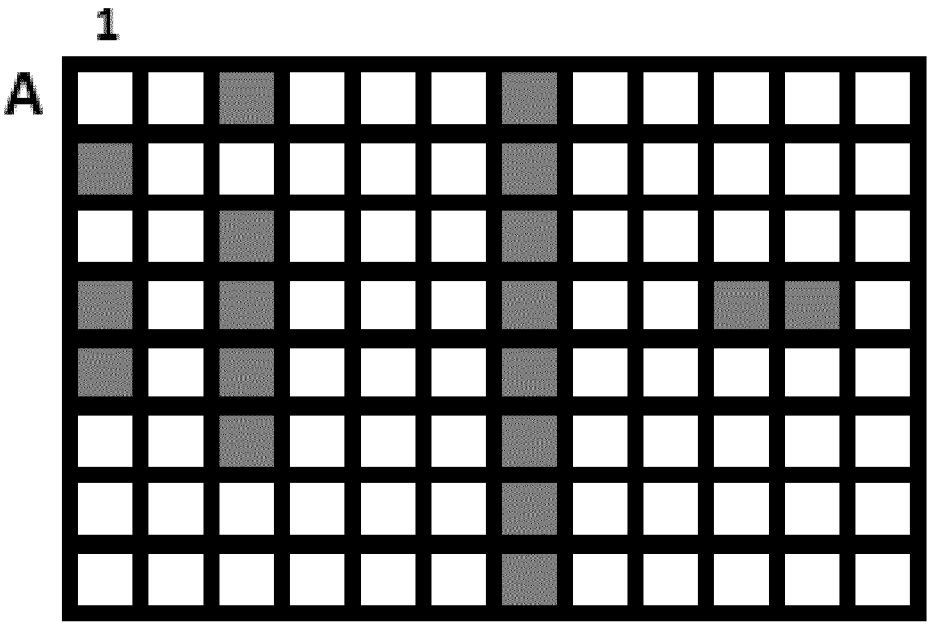


FIG. 11

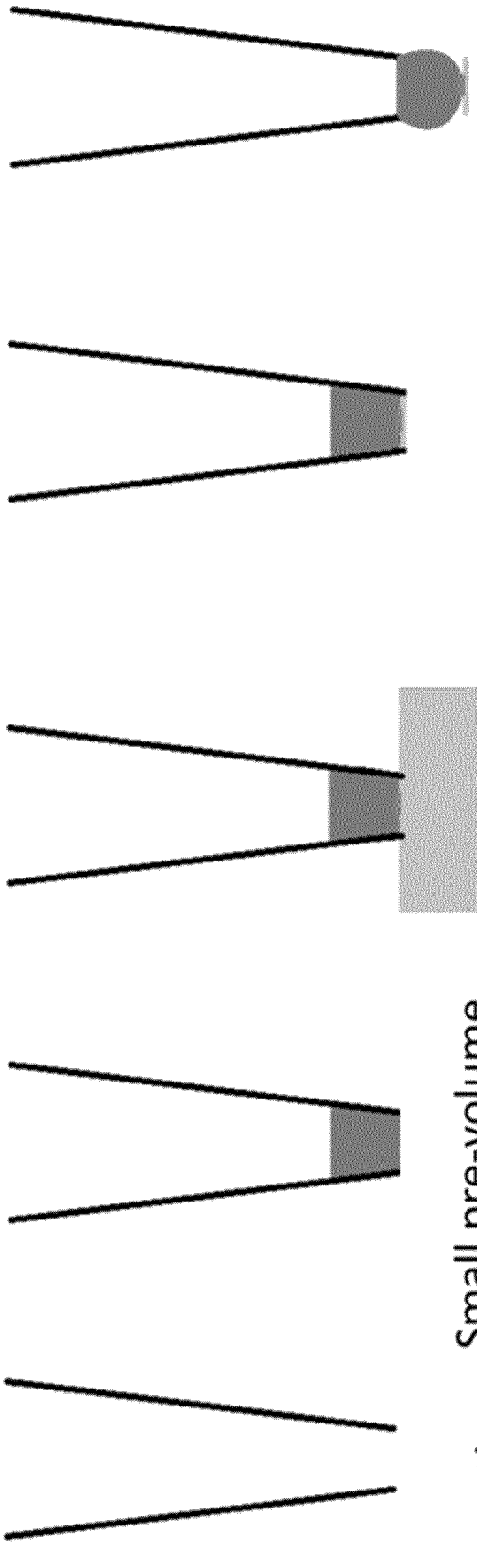

Empty tip loaded
Small pre-volume pipetted before picking in the agar
Colony pick
"Agar stopper" is pushed in the destination well when the pre-volume is dispensed

FIG. 12

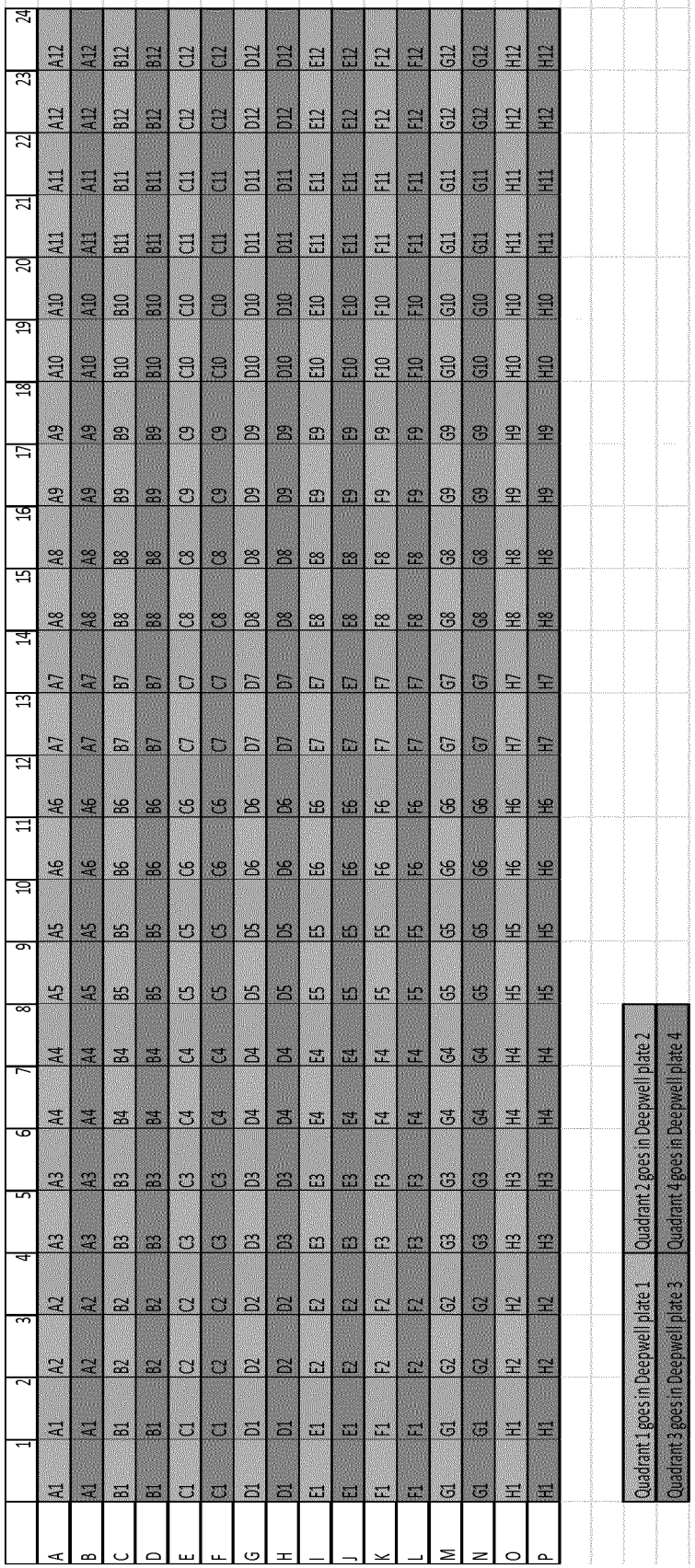

Quadrant 1 goes in Deepwell plate 1
Quadrant 2 goes in Deepwell plate 2
Quadrant 3 goes in Deepwell plate 3
Quadrant 4 goes in Deepwell plate 4

FIG. 13

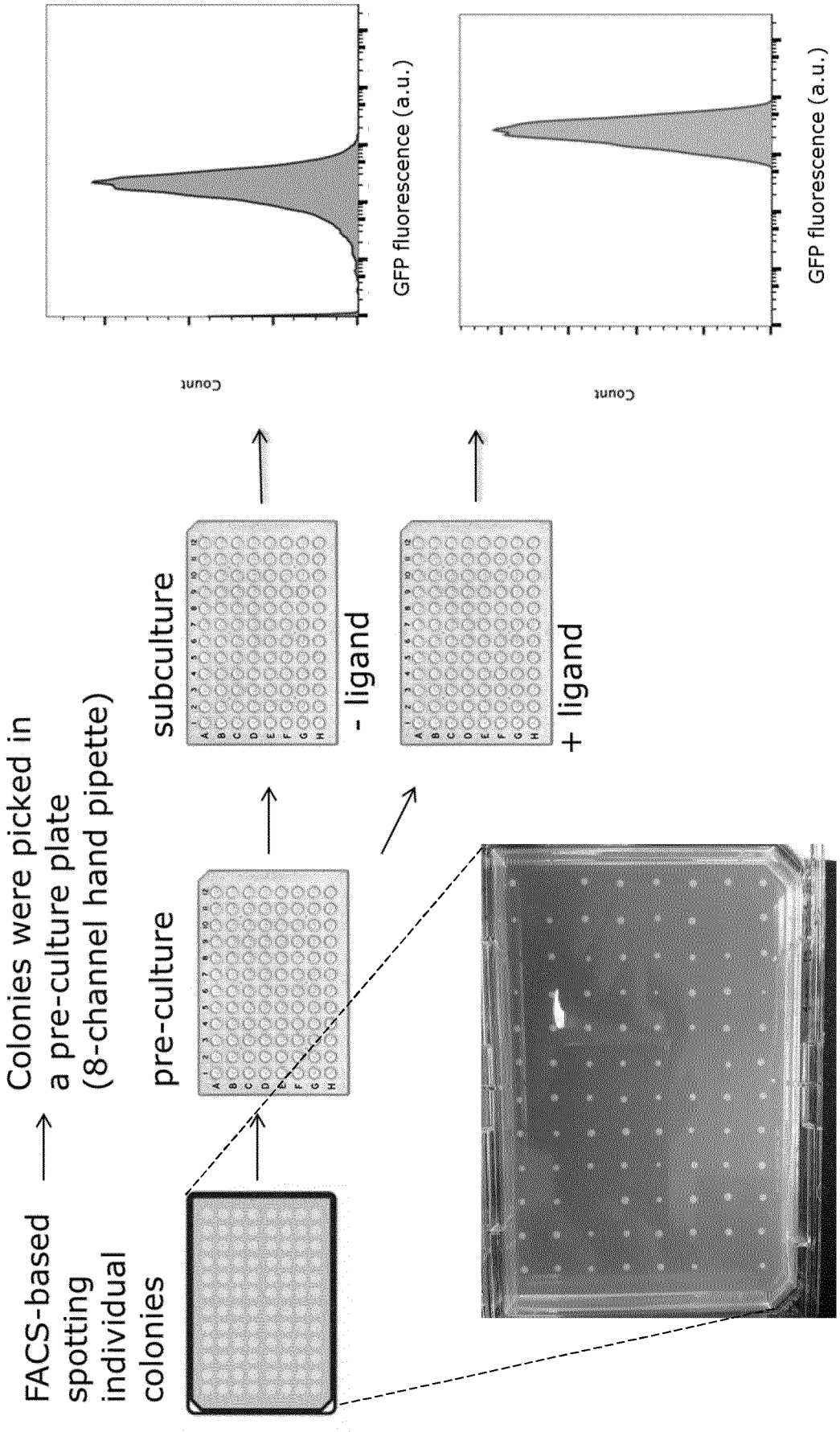

FACS-based spotting individual colonies
Colonies were picked in a pre-culture plate (8-channel hand pipette)
pre-culture
subculture
- ligand
+ ligand
Count
GFP fluorescence (a.u.)
Count
GFP fluorescence (a.u.)

FIG. 14

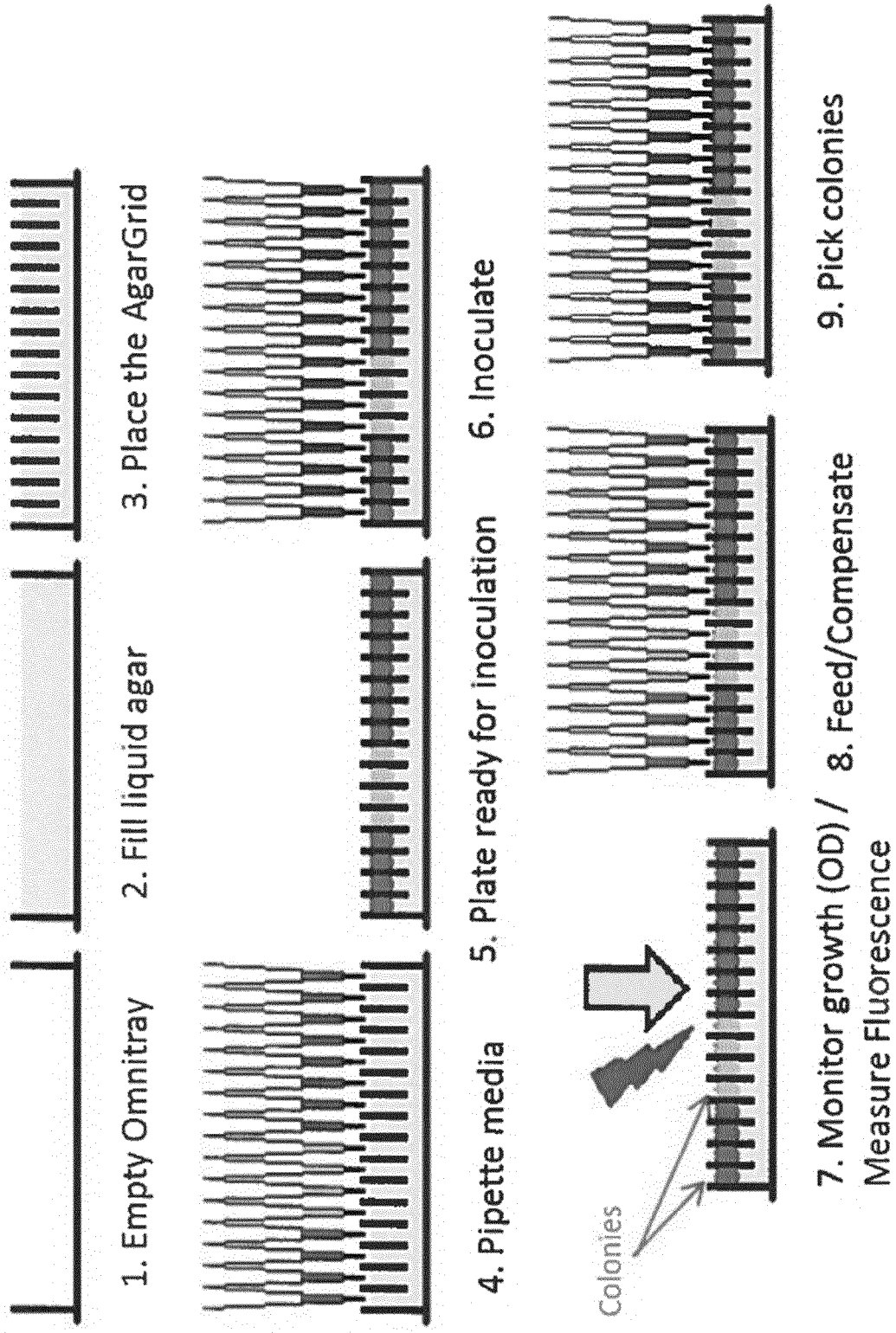

1. Empty Omnitray
2. Fill liquid agar
3. Place the AgarGrid
4. Pipette media
5. Plate ready for inoculation
6. Inoculate
7. Monitor growth (OD) / Measure Fluorescence
8. Feed/Compensate
9. Pick colonies
Colonies

FIG. 15

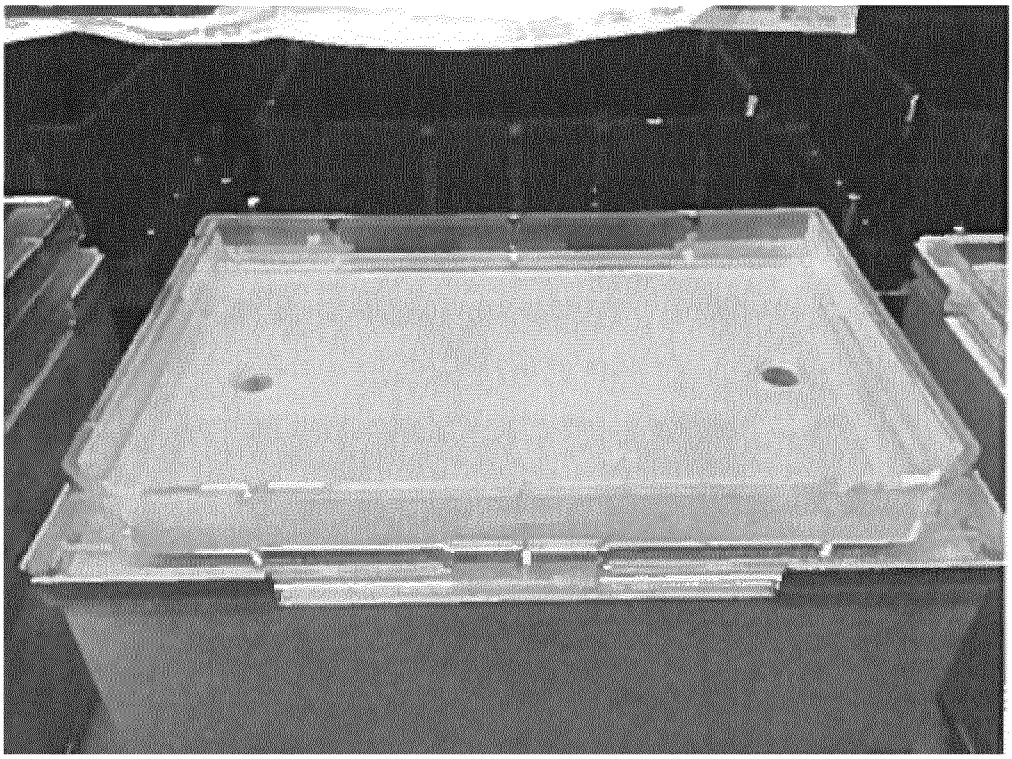


FIG. 16

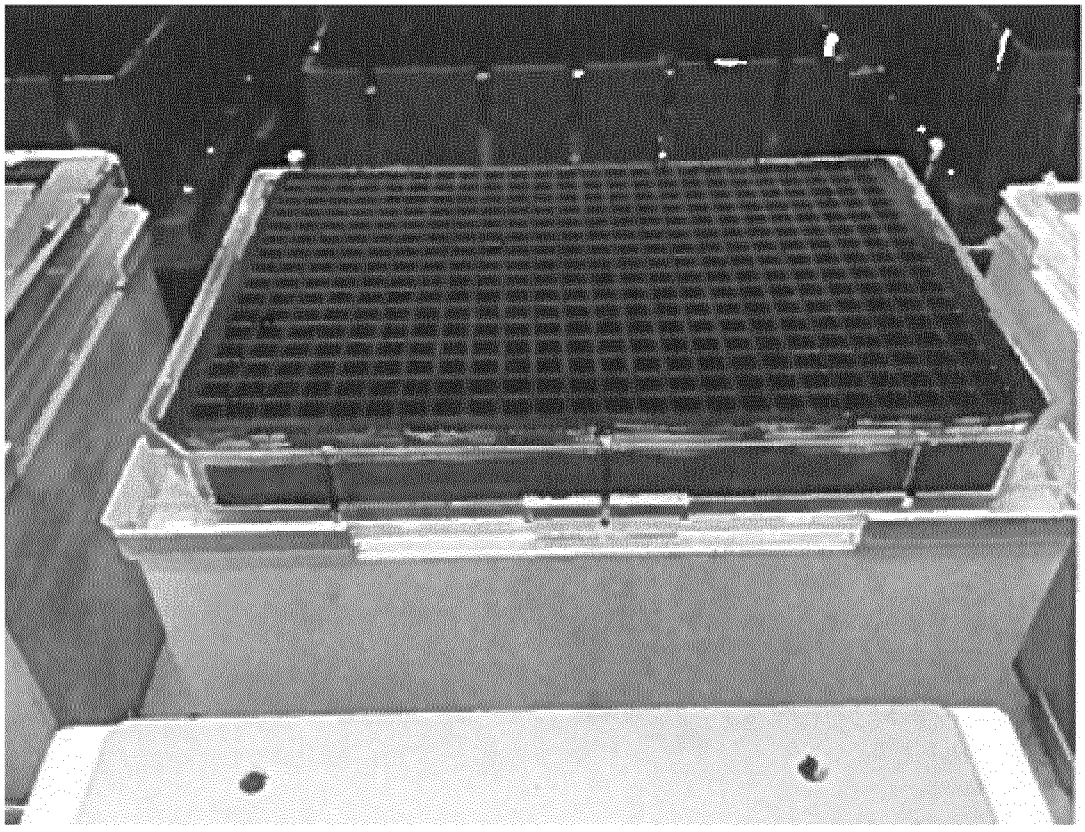


FIG. 17

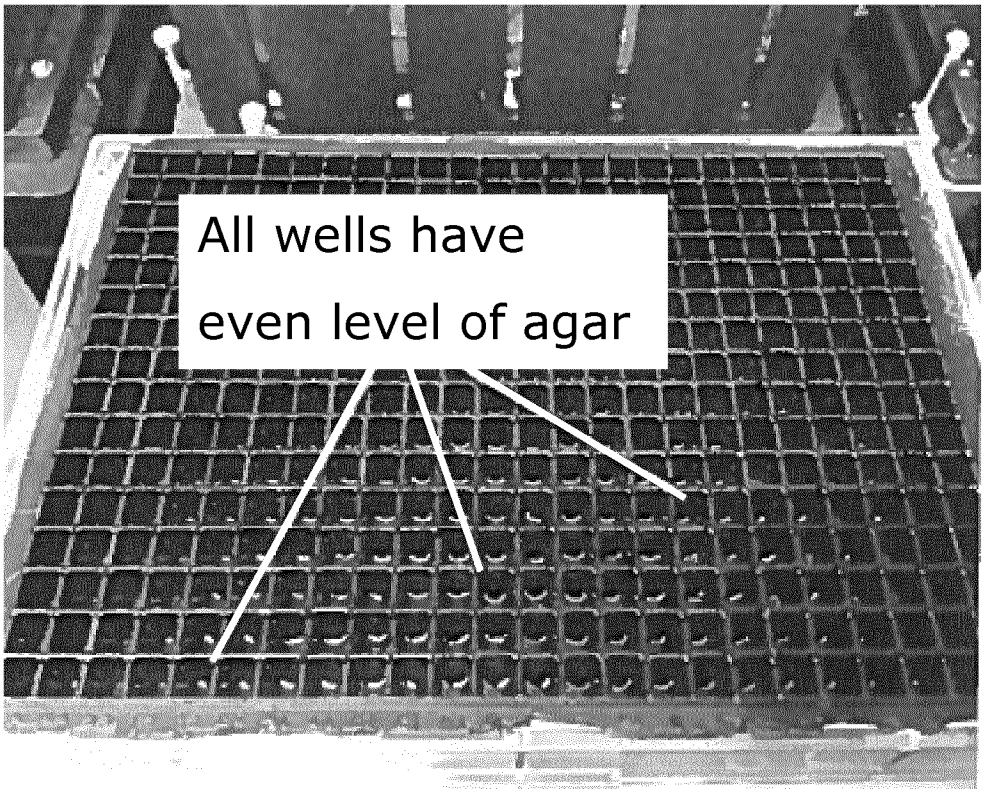

All wells have
even level of agar

FIG. 18

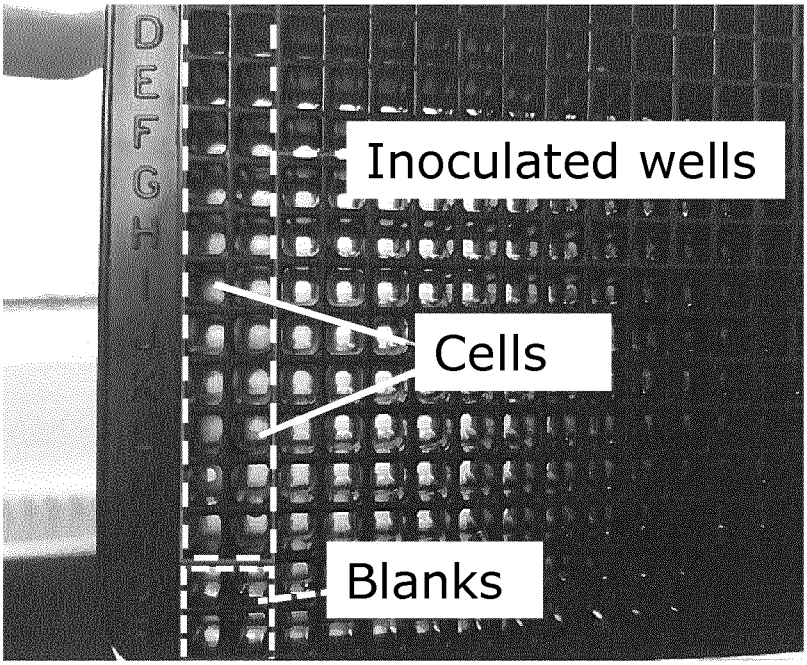

D E F G H
Inoculated wells
Cells
Blanks

FIG. 19

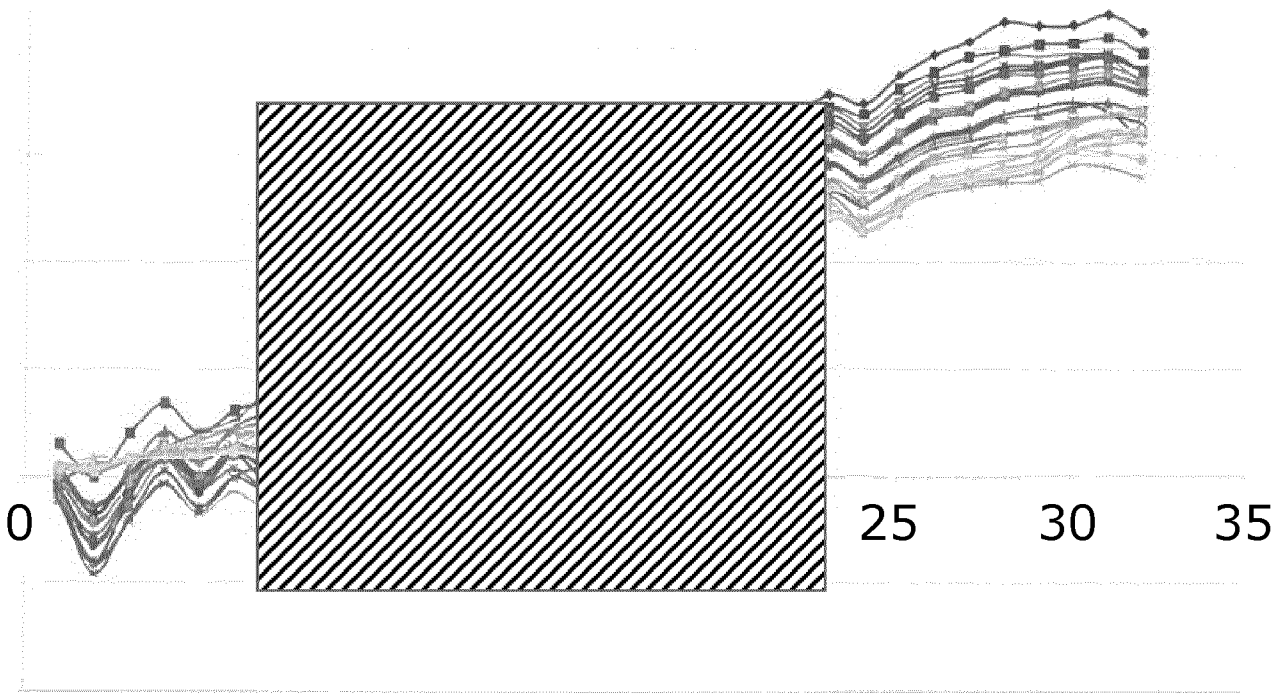


FIG. 20

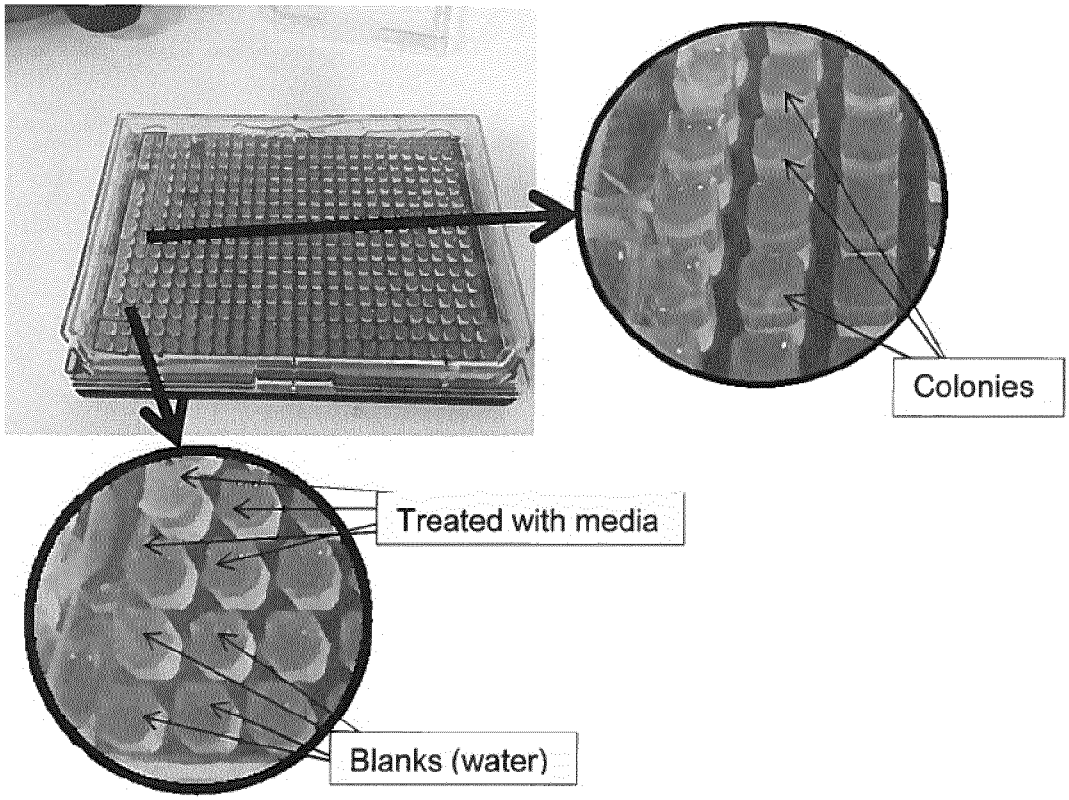


FIG. 21

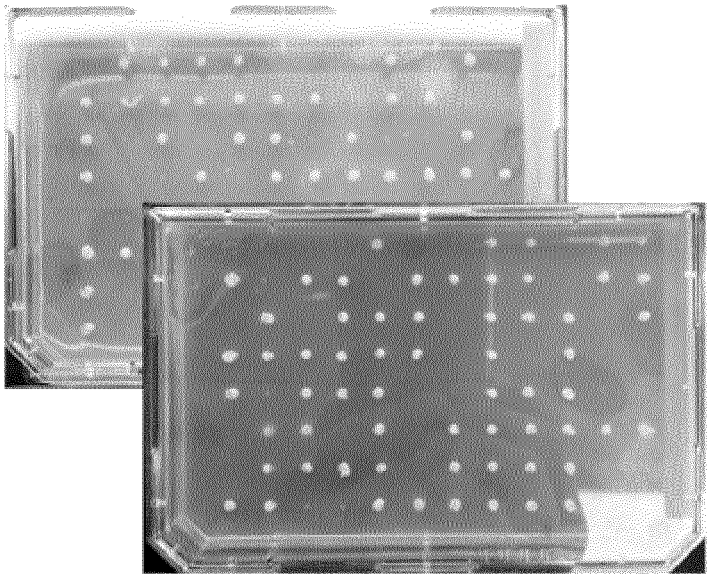


FIG. 22

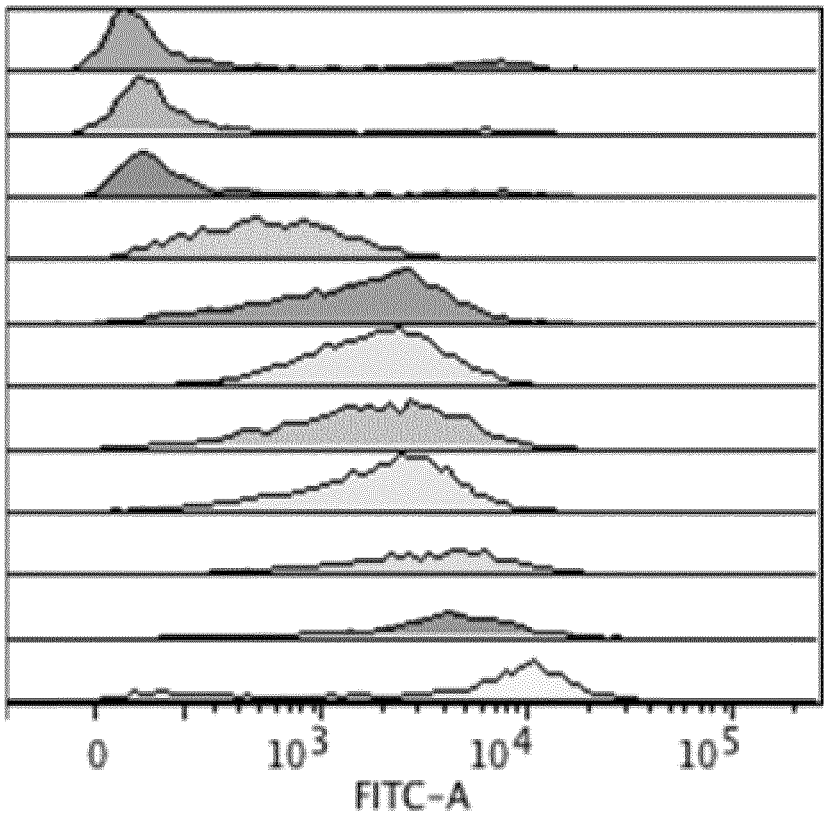


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/067925

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/24 G06T7/00 G06K9/00 G01N1/28
ADD.

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)
C12Q G06T G06K 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.
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X	US 2004/092001 A1 (BEDINGHAM WILLIAM [US] ET AL) 13 May 2004 (2004-05-13) the whole document paragraphs [0005] - [0012], [0019], [0020], [0025], [0050] - [0057]; claims 13-31; figures 1-6 ----- -/--	1-29

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2017	13/09/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Boiangiu, Clara

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Y	WO 03/067904 A2 (UNIV NORTH CAROLINA [US]; MAGNUSON TERRY R [US]; CHEN YIJING [US]; YEE) 14 August 2003 (2003-08-14) the whole document	1-29
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/067925

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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International application No
PCT/EP2017/067925

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International application No
PCT/EP2017/067925

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