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G2J J35X1

(56) Documents Cited
EP 0902271 A EP 0848814 A
EP 0353592 A US 5473437 A

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(54) Abstract Title
Assay Apparatus

(57) Assay apparatus 1 comprises a reading sensor 6 and lamp 7. Directly beneath the sensor 6 and lamp 7 is a circular filter wheel 8 mounted for rotation on spindle 9 and including filter apertures 10 to restrict light transmission to preselected wavelengths. The filter wheel 8 can be rotated in a stepwise fashion to bring diametrically opposed filter apertures 10 into register with the reading sensor 6 and lamp 7. Beneath the filter wheel 8, two upright tubes 12, 13 are linked by diagonally extending tubes 14, 15. Extending from the upright tubes 12,13 are two rotatable optical fibre devices 16. The series of tubes contain optical fibres and combined with optical fibre devices 16 provide two light paths carrying light from the lamp 7 to a plurality of reaction vessels 3 and two light paths carrying light from said vessels to the sensor 6. In use, the lamp 7 is turned on and one of the optical fibre devices 16 is turned to a blank position so it is not aligned with an aperture 20, while the other optical fibre device is positioned over one of said apertures 20, which are arranged radially with the path of the devices. Light from the lamp passes through the optical fibre device 16 into an optical fibre and is then absorbed by a sample in one of the vessels 3, while light emitted from the sample returns to the reading sensor 6. The device is rotated through a complete cycle and then set to a blank position while the process is repeated with the other device 16.

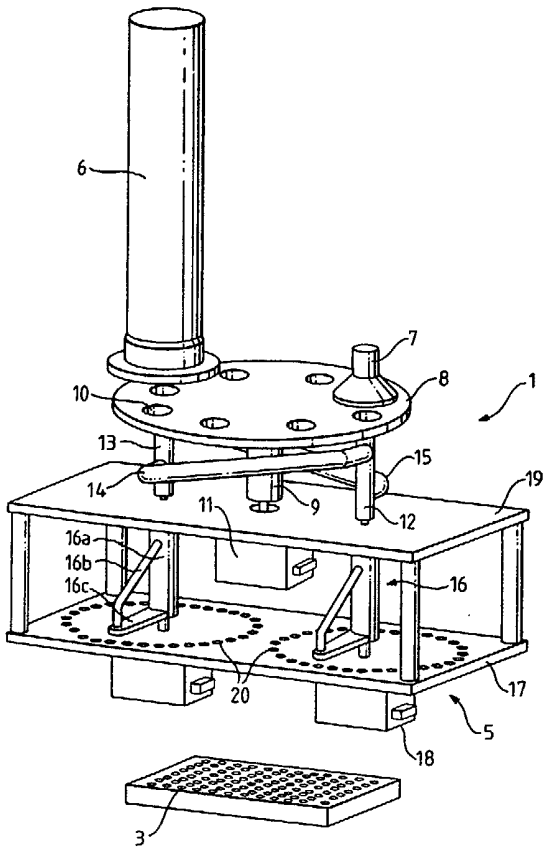

FIG. 1

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

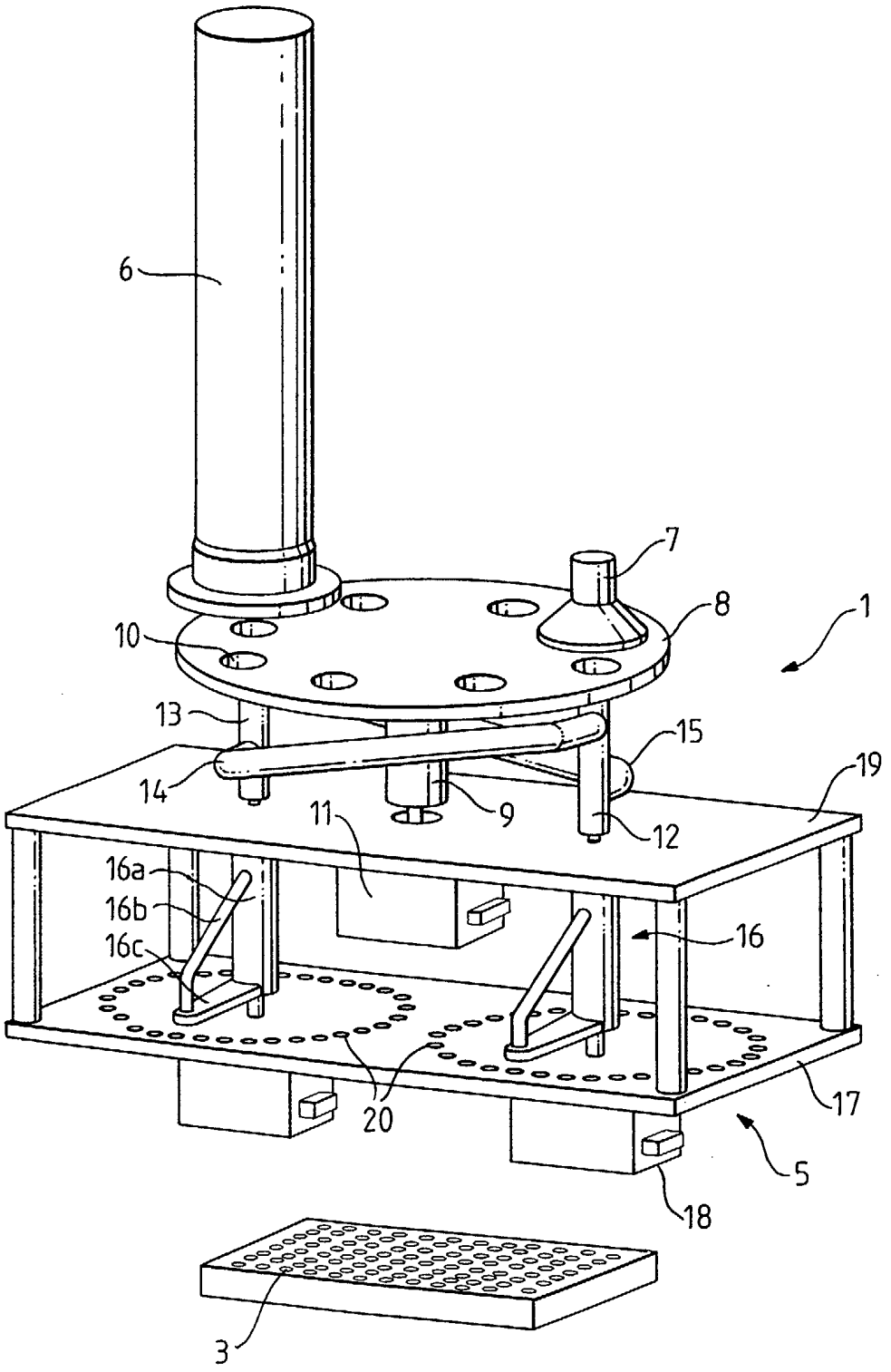

FIG. 1

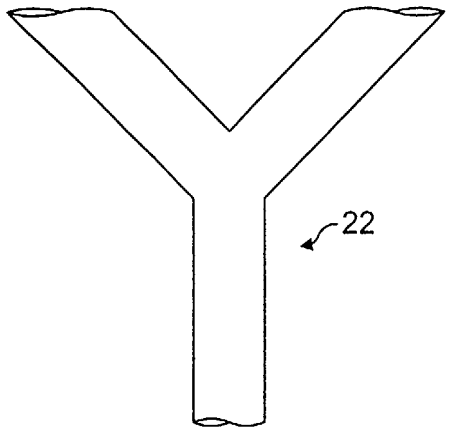

FIG. 2

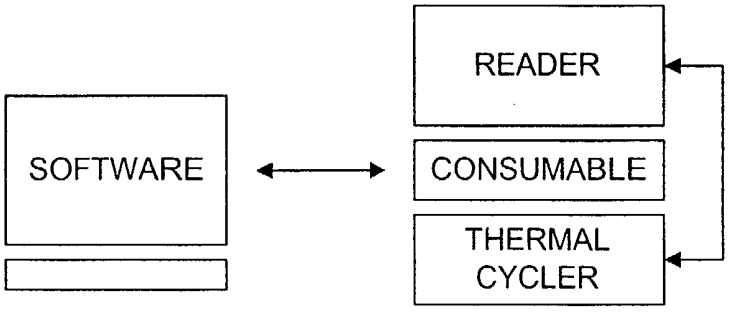

FIG. 3

ASSAY APPARATUS

This application relates to assay apparatus and a method of performing an assay, and in particular relates to assay apparatus suitable for the performance of quantitative PCR and a method of performing quantitative PCR.

Many assay techniques involve the measurement of light for the detection and/or quantification of products of reactions. Such assay procedures can be time consuming and labour intensive to perform normally and automation of such techniques is therefore highly desirable. For example, due to its particularly labour intensive nature, the provision of automated systems for the performance of the polymerase chain reaction was an entirely obvious step and a number of devices exist, some of which are capable of providing information on the quantity of target DNA being produced through detection of light. Such techniques are well known and include the FRET process in which a flourescent label is a added to the primers and/or probes whose emission changes when it is bound/integrated into the double stranded DNA amplified product. By measuring the amount of light emitted at a specific wavelength a relative measure is given of how much amplified target DNA is in the reaction vessel. In another technique, by adding SYBR green (a dye which fluoresces when bound to double-stranded DNA) the total amount of DNA present can be directly measured.

In the prior art a number of automated systems from which light producing assay techniques can be run are in existence. However, all existing devices are complicated and expensive. It is an object of the present invention to seek to mitigate problems such as this.

According to the invention there is provided assay apparatus, including a first light path for applying light to a reaction vessel, and a second light path for receiving light from the reaction vessel, and means for operatively connecting the paths to each of a

plurality of reaction vessels for transmission of light thereto and therefrom. This arrangement offers a considerable simplification in engineering terms over existing systems leading to reduced cost and increased reliability in operation.

It is preferred that the first light path and the second light path comprise optical fibres. The optical fibres may be disposed within a tube, and the tube may include a bifurcated section, the optical fibres of the first light path and the second light path passing separately through the respective bifurcations.

In a preferred embodiment, there may be two light paths for applying light to two reaction vessels simultaneously, and two light paths for receiving light from the said reaction vessels.

It is preferred that the means for connecting the paths to the reaction vessels comprises an optical fibre operatively connected to the light paths, and means to move the optical fibre for transmission of light to and from each vessel.

It is preferred that the optical fibre is mounted for rotation and the apparatus may be provided with a stepper motor for the said rotation.

The apparatus may include plate means comprising a plurality of apertures corresponding to the number of reaction vessels, the apertures being arranged in a circle to register with the optical fibre for light transmission therewith.

The apparatus may include a light source and a reading sensor and may further include optical filter means for disposal between the light source and the first light path and the second light path and reading sensor for variation of the wavelength of transmitted light.

Each aperture in the plate means may include an optical fibre for connection to a reaction vessel or lid therefor.

The apparatus may include a microtitre plate and lid therefor.

According to a second aspect of the invention there is provided a quantitative polymerase chain reaction device, comprising apparatus as set out hereinabove, a thermal cycler and a computer device.

According to a third aspect of the invention there is provided a method of performing an assay, including the use of apparatus as hereinabove defined.

According to a fourth aspect of the invention there is provided a method of performing PCR, including the use of a device as defined hereinabove. The method may be a quantitative method.

The invention will further be described by way of example only and with reference to the following figures, in which:

Figure 1 is a perspective view of apparatus according to the invention;

Figure 2 is a side view of an alternative part of apparatus according to the invention; and

Figure 3 is a block diagram illustrating a quantitative PCR device according to the invention.

Referring to the Figures assay apparatus 1 comprises a first light path 2 for applying light to a reaction vessel 3, and a second light path 4 for receiving light from the

reaction vessel, and means 5 for operatively connecting the paths to each of a plurality of reaction vessels 3 for transmission of light therefrom.

In Figure 1 the assay apparatus 1 is shown provided with a reading sensor 6 and lamp 7 which are fixed in place by any suitable means. Directly beneath the sensor 6 and lamp 7 is a circular filter wheel 8 mounted for rotation on spindle 9 and including filter apertures 10. Spindle 9 is attached to a stepper motor 11 which is controllable to rotate the filter wheel 8 in stepwise fashion to bring diametrically opposed filter apertures 10 into register with the reading sensor 6 and lamp 7. The filter apertures 10 are provided with filters to allow transmission of light at preselected wavelengths.

Beneath the filter wheel 8 there is provided an arrangement of tubes which house the first and second light paths 2,4. In this embodiment, two substantially parallel, upright (in use) tubes 12,13 are linked by diagonally extending tubes 14,15. In this embodiment of the assay apparatus 1, two first light paths and two second light paths are provided. Each light path comprises a plurality of optical fibres; thus, taking for example upright tube 12, one set of first light path fibres pass straight down from the top to the bottom of the tube (as viewed) and a second group of first light path tubes pass along diagonally extending tube 14 and down to the bottom (as viewed) of upright tube 13. Similarly, a first set of second light path fibres pass directly up upright tube 13 and a second set of second light path fibres pass from the bottom of upright tube 12 into diagonally extending tube 15 and up to the top of upright tube 12.

Means 5 for connecting the paths 2,4 to each of a plurality of reaction vessels is provided in the form of rotatable optical fibre devices 16, perforated plates 17 and stepper motors 18. Each rotatable optical fibre device 16 comprises a spindle 16a mounted for rotation between perforated plate 17 and top plate 19 by a stepper motor 18. Spindle 16a includes a conduit 16b extending to the side and downwardly from near its top and attached at its base by bracket 16c. An optical fibre is situated inside conduit 16b which is in operative connection for transmission of light with an upright

tube 12,13. Perforated plate 17 is provided with two sets of apertures 20, each set arranged in a circle corresponding in radius to the distance from spindle 16a to the lower end of conduit 16b, each set having one blank. Each aperture 20 can be linked to a reaction well in a microtitre plate or the corresponding aperture in a heated lid for use on a microtitre plate by for example optical fibres, one per aperture 20.

Figure 2 is an illustration of an alternative form of tube to accommodate the first and second light paths 2,4. Tube 22 consists of a "Y" shape wherein optical fibres of the first light path 2 pass from the stem of the "Y" into one of its bifurcations, and optical fibres of the second light path 4 pass from the stem into the other.

Figure 3 is a block diagram representing a configuration for a quantitative PCR machine which includes assay apparatus 1 according to the invention, a thermal cycler with consumable, and a computer running appropriate software. The thermal cycler can consist of a full size 96 x 0.2ml block and a suitable unit would have the following functional specifications:

1. The thermal cycler will be programmable by the application program and be able to go to any temperature between 4^0C and 99°C with the set point resolution of 0.1°C.

2. The heating rate measured between 60°C and 80°C on a 50°C-90°C second will be greater than 2.0°C per second.

3. The cooling rate measured between 80°C and 60°C on a 90°C-50°C will be greater than 1.0°C per second.

4. The temperature uniformity, defined as the temperature measured between the hottest and coldest wear on the plate will be less than 1.0°C.

5. The temperature accuracy, defined as the average temperature of all of the wells will be less than ± 2.5°C of the true temperature.

In use, the apparatus 1 is used to determine the quantity of target DNA being made in real time during PCR. The desired reaction regimen is programmed into a suitable thermal cycler control device such as a P.C. which controls the thermal cycler device to heat and cool the reaction vessel. In the vessels, as the reaction progresses, fluorescence is produced. The amount of fluorescence, and thus the amount of target DNA being produced in a vessel is read by positioning one of the optical fibre devices 16a over the blank in its set of apertures 20. The lamp 7 is turned on, and the other optical fibre device 16a is positioned over one aperture 20 in its respective set. The light from the lamp 7 passes through the first light path 2 in tube 12, into the optical fibre devices 16a. For the optical fibre device 16a positioned over the blank the light stops there. For the other, the light passes through the aperture 20 in the plate 17 and down a fibre (not shown) to a well in the microtitre plate. Emitted light from the well travels back up the fibre, through the optical fibre device 16a and then through the second light path in upright tube 12 to tube 13 and thence to the reader 6, where it is measured. This procedure is repeated until the optical fibre device 16a has performed a full rotation at which point it is positioned over its blank and the process is repeated for the other optical fibre device 16a.

CLAIMS:

1. Assay apparatus, comprising a first light path for applying light to a reaction vessel, and a second light path for receiving light from the reaction vessel, and means for operatively connecting the paths to each of a plurality of reaction vessels for transmission of light thereto and therefrom.

2. Apparatus according to claim 1, the first light path and the second light path comprising optical fibres.

3. Apparatus according to claim 2, the optical fibres being disposed within a tube.

4. Apparatus according to claim 3, the tube including a bifurcated section, the optical fibres of the first light path and the second light path passing through the respective bifurcations.

5. Apparatus according to any preceding claim, there being two light paths for applying light to two reaction vessels simultaneously, and two light paths for receiving light from the said reaction vessels.

6. Apparatus according to any preceding claim, the means for connecting the paths to the said reaction vessels comprising a optical fibre operatively connected to the light paths, and means to move the optical fibre for transmission of light to and from each vessel.

7. Apparatus according to claim 6, the optical fibre being mounted for rotation.

8. Apparatus according to claim 7, including a stepper motor for rotation of the optical fibre.

9. Apparatus according to claim 7 or claim 8, including plate means comprising a plurality of apertures corresponding to the number of reaction vessels, the apertures being arranged in a circle to register with the optical fibre for light transmission therewith.

10. Apparatus according to any preceding claim, including a light source and a reading sensor.

11. Apparatus according to any preceding claim, including optical filter means for disposal between the light source and the first light path, and the second light path and reading sensor, for control of the wavelength of transmitted light.

12. Apparatus according to claim 11, each aperture including an optical fibre for connection to a reaction vessel or lid therefor.

13. Apparatus according to any preceding claim, including a microtitre plate and lid therefor.

14. Assay apparatus, substantially as hereinbefore described with reference to the accompanying drawings.

15. A quantitative polymerase chain reaction device, comprising apparatus according to any one of claims 1 to 14, a thermal cycler and a computer device.

16. A method of performing an assay, including the use of apparatus as claimed in any one of claims 1 to 14.

17. A method of performing PCR, including the use of apparatus as claimed in any one of claims 1 to 14, or the device of claim 15.

18. A method according to claim 17, wherein the method is a quantitative method.

Application No: GB 0213400.5
Claims searched: 1 to 18
Examiner: Matthew Perkins
Date of search: 21 February 2003

Patents Act 1977 : Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance	
X	1, 2, 5, 6, 10, 11, 16, 17 & 18	EP 0902271	(BECTON DICKINSON) See abstract, figures and paragraphs 4, 15 to 20 and 58 to 59
X	1, 2, 5, 6, 10 & 16	US 5473437	(BECTON DICKINSON) See column 3 lines 32 to 46 and column 4 lines 24 to 53
A	-	EP 0353592	(ABBOTT LAB) Abstract
A	-	EP 0848814	(SFRI) Abstract

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[V]:

G1A, G2J

Worldwide search of patent documents classified in the following areas of the IPC[7]:

G01N

The following online and other databases have been used in the preparation of this search report:

Online: WPI, EPODOC, PAJ