the supporting member 838. A channel 831 may be formed in the supporting member 838 coupling the pump 836 to the cavity 832. The channel 831 may be configured to allow the fluid to pass from the pump 836 to the cavity 832. The pump 836 may be positioned away from the cavity 832 to allow light to be directed through the cavity during use. The supporting member 838 and the pump 836 may be formed from a silicon substrate, a plastic material, or photoresist material. The pump 836 may be configured to pump fluid to the cavity via the channel, as depicted by the arrows in FIG. 32. When the fluid reaches the cavity 832, the fluid may flow past the particle 834 and out through the bottom of the cavity. An advantage of using pumps is that better flow through the channels may be achieved. Typically, the channels and cavities may have a small volume. The small volume of the cavity and channel tends to inhibit flow of the fluid through the cavity. By incorporating a pump, the flow of fluid to the cavity and through the cavity may be increased, allowing more rapid testing of the fluid sample. While a diaphragm based pump system is depicted in FIG. 33, it should be understood that electrode based pumping systems may also be incorporated into the sensor array to produce fluid flows.

In another embodiment, a pump may be coupled to a supporting member for analyzing analytes in a fluid stream, as depicted in FIG. 34. A channel 842 may couple a pump 846 to multiple cavities 844 formed in a supporting member 840. The cavities 842 may include sensing particles 848. The pump may be configured to create a flow of the fluid through the channel 842 to the cavities 848. In one embodiment, the cavities may inhibit the flow of the fluid through the cavities 844. The fluid may flow into the cavities 844 and past the particle 848 to create a flow of fluid through the sensor array system. In this manner a single pump may be used to pass the fluid to multiple cavities. While a diaphragm pump system is depicted in FIG. 33, it should be understood that electrode pumping systems may also be incorporated into the supporting member to create similar fluid flows..

In another embodiment, multiple pumps may be coupled to a supporting member of a sensor array system. In one embodiment, the pumps may be coupled in series with each other to pump fluid to each of the cavities. As depicted in FIG. 35, a first pump 852 and a second pump 854 may be coupled to a supporting member 850. The first pump 852 may be coupled to a first cavity 856. The first pump may be configured to transfer fluid to the first cavity 856 during use. The cavity 856 may be configured to allow the fluid to pass through the cavity to a first cavity outlet channel 858. A second pump 854 may also be coupled to the supporting member 850. The second pump 854 may be coupled to a second cavity 860 and the first cavity outlet channel 858. The second pump 854 may be configured to transfer fluid from the first cavity outlet channel 858 to the second cavity 860. The pumps may be synchronized such that a steady flow of fluid through the cavities is obtained. Additional pumps may be coupled to the second cavity outlet channel 862 such that the fluid may be pumped to additional cavities. In one embodiment, each of the cavities in the supporting member is coupled to a pump configured to pump the fluid stream to the cavity.

In another embodiment, multiple electrode based pumps may be incorporated into the sensor array system. The pumps may be formed along the channels which couple the cavities. . As depicted in FIG. 36, a plurality of cavities 870 may be formed in a supporting member 872 of a sensor array. Channels 874 may also be formed in the supporting member 872 interconnecting the cavities 870 with each other. An inlet channel 876 and an outlet channel 877, which allow the fluid to pass into and out of the sensor array, respectively, may also be formed. A series of electrodes 878 may be positioned over the channels 874, 876, and 877. The electrodes may be used to form an electroosmosis pumping system or an electrohydrodynamic pumping system. The electrodes may

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2801

be coupled to a controller 880 which may apply the appropriate voltage to the appropriate electrodes to produce a flow of the fluid through the channels. The pumps may be synchronized such that a steady flow of fluid through the cavities is obtained. The electrodes may be positioned between the cavities such that the electrodes do not significantly interfere with the application of light to the cavities.

In some instances it may be necessary to add a reagent to a particle before, during or after an analysis process. Reagents may include receptor molecules or indicator molecules. Typically, such reagents may be added by passing a fluid stream which includes the reagent over the sensor array. In an embodiment, the reagent may be incorporated into the sensor array system which includes two particles. In this embodiment, a sensor array system 900 may include two particles 910 and 920 for each sensing position of the sensor array, as depicted in FIG. 37. The first particle 910 may be positioned in a first cavity 912. The second particle 920 may be positioned in a second cavity 922. In one embodiment, the second cavity is coupled to the first cavity via a channel 930. The second particle includes a reagent which is at least partially removable from the second particle 920. The reagent may also be configured to modify the first particle 910, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with an analyte during use. The reagent may be added to the first cavity before, during or after a fluid analysis. The reagent is preferably coupled to the second particle 920. The a portion of the reagent coupled to the second particle may be decoupled from the particle by passing a decoupling solution past the second particle. The decoupling solution may include a decoupling agent which will cause at least a portion of the reagent to be at released by the particle. A reservoir 940 may be formed on the sensor array to hold the decoupling solution.

A first pump 950 and a second pump 960 may also be coupled to the supporting member 915. The first pump 950 may be configured to pump fluid from a fluid inlet 952 to the first cavity 912 via channel 930. The fluid inlet 952 is the location where the fluid, which includes the analyte, is introduced into the sensor array system. A second pump 950 may be coupled to the reservoir 940 and the second cavity 922. The second pump 960 may be used to transfer the decoupling solution from the reservoir to the second cavity 922. The decoupling solution may pass through the second cavity 922 and into first cavity 912. Thus, as the reagent is removed the second particle it may be transferred to the first cavity912, where the reagent may interact with the first particle 910. The reservoir may be refilled by removing the reservoir outlet 942, and adding additional fluid to the reservoir 940. While diaphragm based pump systems are depicted in FIG. 37, it should be understood that electrode based pumping systems may also be incorporated into the sensor array to produce fluid flows.

The use of such a system is described by way of example. In some instances it may be desirable to add a reagent to the first particle prior to passing the fluid which includes the analyte to the first particle. The reagent may be coupled to the second particle and placed in the sensor array prior to use, typically during construction of the array. A decoupling solution may be added to the reservoir before use. A controller 970 may also be coupled to the system to allow automatic operation of the pumps. The controller 970 may be configured to initiate the analysis sequence by activating the second pump 960, causing the decoupling solution to flow from the reservoir 940 to the second cavity 922. As the fluid passes through the second cavity 922, the decoupling solution may cause at least some of the reagent molecules to be released from the second particle 920. The decoupling solution may be passed out of the second cavity 922 and into the first cavity 912. As the solution passes through the first cavity, some of the reagent molecules may be captured by the first particle 910. After a sufficient number of molecules

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2802

have been captured by the first particle 910, flow of fluid thorough the second cavity 922 may be stopped. During this initialization of the system, the flow of fluid through the first pump may be inhibited.

After the system is initialized, the second pump may be stopped and the fluid may be introduced to the first cavity. The first pump may be used to transfer the fluid to the first cavity. The second pump may remain off, thus inhibiting flow of fluid from the reservoir to the first cavity. It should be understood that the reagent solution may be added to the first cavity while the fluid is added to the first cavity. In this embodiment, both the first and second pumps may be operated substantially simultaneously.

Alternatively, the reagent may be added after an analysis. In some instances, a particle may interact with an analyte such that a change in the receptors attached to the first particle occurs. This change may not, however produce a detectable signal. The reagent attached to the second bead may be used to produce a detectable signal when it interacts with the first particle, if a specific analyte is present. In this embodiment, the fluid is introduced into the cavity first. After the analyte has been given time to react with the particle, the reagent may be added to the first cavity. The interaction of the reagent with the particle may produce a detectable signal. For example, an indicator reagent may react with a particle which has been exposed to an analyte to produce a color change on the particle. Particle which have not been exposed to the analyte may remain unchanged or show a different color change.

As shown in FIG. 1, a system for detecting analytes in a fluid may include a light source 110, a sensor array 120 and a detector 130. The sensor array 120 is preferably formed of a supporting member which is configured to hold a variety of particles 124 in an ordered array. A high sensitivity CCD array may be used to measure changes in optical characteristics which occur upon binding of the biological/chemical agents. Data acquisition and handling is preferably performed with existing CCD technology. As described above, colorimetric analysis may be performed using a white light source and a color CCD detector. However, color CCD detectors are typically more expensive than gray scale CCD detectors.

In one embodiment, a gray scale CCD detector may be used to detect colorimetric changes. In one embodiment, a gray scale detector may be disposed below a sensor array to measure the intensity of light being transmitted through the sensor array. A series of lights (e.g., light emitting diodes) may be arranged above the sensor array. In one embodiment, groups of three LED lights may be arranged above each of the cavities of the array. Each of these groups of LED lights may include a red, blue and a green light. Each of the lights may be operated individually such that one of the lights may be on while the other two lights are off. In order to provide color information while using a gray scale detector, each of the lights is sequentially turned on and the gray scale detector is used to measure the intensity of the light passing through the sensor array. After information from each of the lights is collected, the information may be processed to derive the absorption changes of the particle.

In one embodiment, the data collected by the gray scale detector may be recorded using 8 bits of data. Thus, the data will appear as a value between 0 and 255. The color of each chemical sensitive element may be represented as a red, blue and green value. For example, a blank particle (i.e., a particle which does not include a receptor) will typically appear white. When each of the LED lights (red, blue and green) are operated the CCD detector will record a value corresponding to the amount of light transmitted through the cavity. The intensity of the light may be compared to a blank particle, to determine the absorbance of a particle with respect to the LED light which is used. Thus, the red, green and blue components may be recorded individually without the use of a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2803

color CCD detector. In one embodiment, it is found that a blank particle exhibits an absorbance of about 253 when illuminated with a red LED, a value of about 250 when illuminated by a green LED, and a value of about 222 when illuminated with a blue LED. This signifies that a blank particle does not significantly absorb red, green or blue light. When a particle with a receptor is scanned, the particle may exhibit a color change, due to absorbance by the receptor. For example, it was found that when a particle which includes a 5-carboxyfluorescein receptor is subjected to white light, the particle shows a strong absorbance of blue light. When a red LED is used to illuminate the particle, the gray scale CCD detector may detect a value of about 254. When the green LED is used, the gray scale detector may detect a value of about 218. When a blue LED light is used, a gray scale detector may detect a value of about 57. The decrease in transmittance of blue light is believed to be due to the absorbance of blue light by the 5-carboxyfluorescein. In this manner the color changes of a particle may be quantitatively characterized using a gray scale detector.

As described above, after the cavities are formed in the supporting member, a particle may be positioned at the bottom of a cavity using a micromanipulator. This allows the location of a particular particle to be precisely controlled during the production of the array. The use of a micromanipulator may, however, be impractical for production of sensor array systems. An alternate method of placing the particles into the cavities may involve the use of a silk screen like process. A series of masking materials may be placed on the upper surface of the sensor array prior to filling the cavities. The masking materials may be composed of glass, metal or plastic materials. A collection of particles may be placed upon the upper surface of the masking materials and the particles may be moved across the surface. When a cavity is encountered, a particle may drop into the cavity if the cavity is unmasked. Thus particles of known composition are placed in only the unmasked regions. After the unmasked cavities are filled, the masking pattern may be altered and a second type of particles may be spread across the surface. Preferably, the masking material will mask the cavities that have already been filled with particle. The masking material may also mask other non-filled cavities. This technique may be repeated until all of the cavities are filled. After filling the cavities, a cover may be placed on the support member, as described above, to inhibit the displacement and mixing of the particles. An advantage of such a process is that it may be more amenable to industrial production of supporting members.

Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as the presently preferred embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2804

What is claimed is:

1. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

2. The system of claim 1, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

3. The system of claim 1, wherein the system comprises a plurality of particles positioned within the cavity.

4. The system of claim 1, wherein the light source comprises a light emitting diode.

5. The system of claim 1, wherein the light source comprises a white light source.

6. The system of claim 1, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is positioned below a bottom surface of the supporting member, and wherein the top cover layer is positioned above the upper surface of the supporting member, and wherein the bottom layer and the top cover layer are positioned such that the particle is substantially contained within the cavity by the bottom layer and the top cover layer.

7. The system of claim 6, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

8. The system of claim 1, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is coupled to a bottom surface of the supporting member, and wherein the top cover layer is coupled to a top surface of the supporting member; and wherein both the bottom layer and the top cover layer are coupled to the supporting member such that the particle is substantially contained within the cavity by bottom layer and the top cover layer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2805

9. The system of claim 8, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

10. The system of claim 1, wherein the sensor array further comprises a bottom layer coupled to the supporting member, and wherein the supporting member comprises silicon, and wherein the bottom layer comprises silicon nitride.

11. The system of claim 1, wherein the sensor array further comprises a sensing cavity formed on a bottom surface of the sensor array.

12. The system of claim 1, wherein the supporting member is formed from a plastic material, and wherein the sensor array further comprises a top cover layer, the top cover layer being coupled to the supporting member such that the particle is substantially contained within the cavity, and wherein the top cover layer is configured to allow the fluid to pass through the top cover layer to the particle, and wherein both the supporting member and the top cover layer are substantially transparent to light produced by the light source.

13. The system of claim 1, further comprising a fluid delivery system coupled to the supporting member.

14. The system of claim 1, wherein the detector comprises a charge-coupled device.

15. The system of claim 1, wherein the detector comprises an ultraviolet detector.

16. The system of claim 1, wherein the detector comprises a fluorescence detector.

17. The system of claim 1, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

18. The system of claim 1, wherein the particle ranges from about 0.05 micron to about 500 microns.

19. The system of claim 1, wherein a volume of the particle changes when contacted with the fluid.

20. The system of claim 1, wherein the particle comprises a metal oxide particle.

21. The system of claim 1, wherein the particle comprises a metal quantum particle.

22. The system of claim 1, wherein the particle comprises a semiconductor quantum particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2806

23. The system of claim 1, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

24. The system of claim 23, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

25. The system of claim 23, wherein the receptor molecule produces the signal in response to the pH of the fluid.

26. The system of claim 23, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

27. The system of claim 23, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of a carbohydrate.

28. The system of claim 23, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

29. The system of claim 23, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

30. The system of claim 23, wherein the receptor comprises a polynucleotide.

31. The system of claim 23, wherein the receptor comprises a peptide.

32. The system of claim 23, wherein the receptor comprises an enzyme.

33. The system o f claim 23, wherein the receptor comprises a synthetic receptor.

34. The system of claim 23, wherein the receptor comprises an unnatural biopolymer.

35. The system of claim 23, wherein the receptor comprises an antibody.

36. The system of claim 23, wherein the receptor comprises an antigen.

37. The system of claim 1, wherein the analyte comprises phosphate functional groups, and wherein the particle is configured to produce the signal in the presence of the phosphate functional groups.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2807

38. The system of claim 1, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

39. The system of claim 1, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

40. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising a plurality of cavities formed within the supporting member, wherein the supporting member comprises silicon;
a plurality of particles, the particles comprising a receptor molecule covalently linked to a polymeric resin, wherein the particles are positioned within the cavities, and wherein each of the particles is configured to produce a signal when the particle interacts with the analyte during use; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

41. The system of claim 40, wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

42. The system of claim 40, wherein each cavity is configured to hold a single particle.

43. The system of claim 40, wherein each cavity is configured to hold a plurality of particles.

44. The system of claim 40, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is positioned below a bottom surface of the supporting member, and wherein the top cover layer is positioned above the upper surface of the supporting member, and wherein the bottom layer and the top cover layer are positioned such that the particle is substantially contained within the cavity by the bottom layer and the top cover layer.

45. The system of claim 44, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

46. The system of claim 40, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is coupled to a bottom surface of the supporting member, and wherein the top cover layer is coupled to a top surface of the supporting member; and wherein both the bottom layer and the top cover layer are coupled to the supporting member such that the particle is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2808

substantially contained within the cavity by bottom layer and the top cover layer.

47. The system of claim 46, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

48. The system of claim 40, wherein the sensor array further comprises a bottom layer coupled to the supporting member, and wherein the bottom layer comprises silicon nitride.

49. The system of claim 40, wherein the sensor array further comprises a sensing cavity formed on a bottom surface of the sensor array.

50. The system of claim 40, further comprising a fluid delivery system coupled to the supporting member.

51. The system of claim 40, wherein the detector comprises a charge-coupled device.

52. The system of claim 40, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

53. The system of claim 40, wherein the particle ranges from about 0.05 micron to about 500 microns.

54. The system of claim 40, wherein a volume of the particle changes when contacted with the fluid.

55. The system of claim 40, wherein the polymeric bead comprises a polystyrene-polyethylene glycol-divinyl benzene resin.

56. The system of claim 40, wherein the receptor molecule produces the signal in response to the pH of the fluid.

57. The system of claim 40, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

58. The system of claim 40, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of the carbohydrate.

59. The system of claim 40, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

60. The system of claim 40, wherein the particles further comprises an indicator, wherein the indicator is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2809

associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

61. The system of claim 40, wherein the receptor comprises a polynucleotide.

62. The system of claim 40, wherein the receptor comprises a peptide.

63. The system of claim 40, wherein the receptor comprises an enzyme.

64. The system o f claim 40, wherein the receptor comprises a synthetic receptor.

65. The system of claim 40, wherein the receptor comprises an unnatural biopolymer.

66. The system of claim 40, wherein the receptor comprises an antibody.

67. The system of claim 40, wherein the receptor comprises an antigen.

68. The system of claim 40, wherein the particles produce a detectable pattern in the presence of the analyte.

69. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

70. The sensor array of claim 69, further comprising a plurality of particles positioned within the cavity.

71. The sensor array of claim 69, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

72. The sensor array of claim 69, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter.

73. The sensor array of claim 69, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter, and wherein the cavity is configured to substantially contain the particle.

74. The sensor array of claim 69, wherein the supporting member comprises a plastic material.

75. The sensor array of claim 69, wherein the supporting member comprises a silicon wafer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2810

76. The sensor array of claim 75, wherein the cavity extends through the silicon wafer.

77. The sensor array of claim 75,wherein the cavity is substantially pyramidal in shape and wherein the sidewalls of the cavity are substantially tapered at an angle of between about 50 to about 60 degrees.

78. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer.

79. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon dioxide, silicon nitride, or silicon oxide/silicon nitride multilayer stacks.

80. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon nitride.

81. The sensor array of claim 75, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

82. The sensor array of claim 75, further comprising a plurality of cavities formed in the silicon wafer, and wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

83. The sensor array of claim 69, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavity.

84. The sensor array of claim 69, further comprising an inner surface coating, wherein the inner surface coating is configured to inhibit dislodgment of the particle.

85. The sensor array of claim 69, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

86. The sensor array of claim 85, wherein the detector is a semiconductor based photodetector.

87. The sensor array of claim 85, wherein the detector is a Fabry-Perot type detector.

88. The sensor array of claim 85, further comprising an optical fiber coupled to the detector, wherein the optical fiber is configured to transmit optical data to a microprocessor.

89. The sensor array of claim 69, further comprising an optical filters coupled to a bottom surface of the sensor array.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2811

90. The sensor array of claim 69, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

91. The sensor array of claim 90, wherein the barrier layer comprises a substantially transparent cover plate positioned over the cavity, and wherein the cover plate is positioned a fixed distance over the cavity such that the fluid can enter the cavity.

92. The sensor array of claim 91, wherein the barrier layer comprises plastic, glass, quartz, silicon oxide, or silicon nitride.

93. The sensor array of claim 69, further comprising a plurality of particles positioned within a plurality of cavities formed in the supporting member.

94. The sensor array of claim 69, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

95. A sensor array for detecting an analyte in a fluid comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member;
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte.

96. The sensor array of claim 95, wherein a plurality of particles is positioned within each of the cavities.

97. The sensor array of claim 95, wherein the particles comprise a receptor molecule coupled to a polymeric bead.

98. The sensor array of claim 95, wherein the cavity extends through the supporting member.

99. The sensor array of claim 95,wherein the cavity is substantially pyramidal in shape and wherein the sidewalls of the cavity are substantially tapered at an angle of between about 50 to about 60 degrees.

100. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the supporting member.

101. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon dioxide, silicon nitride, or silicon oxide/silicon nitride multilayer stacks.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2812

102. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon nitride.

103. The sensor array of claim 95, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

104. The sensor array of claim 95, further comprising a plurality of cavities formed in the silicon wafer, and wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

105. The sensor array of claim 95, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavities.

106. The sensor array of claim 95, further comprising an inner surface coating, wherein the inner surface coating is configured to inhibit dislodgment of the particle.

107. The sensor array of claim 95, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

108. The sensor array of claim 107, wherein the detector is a semiconductor based photodetector.

109. The sensor array of claim 107, wherein the detector is a Fabry-Perot type detector.

110. The sensor array of claim 107, further comprising an optical fiber coupled to the detector, wherein the optical fiber is configured to transmit optical data to a microprocessor.

111. The sensor array of claim 95, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

112. The sensor array of claim 111, wherein the barrier layer comprises a cover plate positioned over the cavity, and wherein the cover plate is positioned a fixed distance over the cavity such that the fluid can enter the cavity.

113. The sensor array of claim 111, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavities, and wherein the barrier layer comprises a cover plate positioned upon an upper surface of the supporting member, and wherein the cover plate inhibits passage of the fluid into the cavities such that the fluid enters the cavities via the channels.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2813

114. The sensor array of claim 111, wherein the barrier layer comprises plastic, glass, quartz, silicon oxide, or silicon nitride.

115. A method for forming a sensor array configured to detect an analyte in a fluid, comprising:
forming a cavity in a supporting member, wherein the supporting member comprises a silicon wafer;
placing a particle in the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
forming a cover upon a portion of the supporting member, wherein the cover is configured to inhibit dislodgment of the particle from the cavity.

116. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer.

117. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer with a wet hydroxide etch.

118. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer such that sidewalls of the cavity are tapered at an angle from about 50 degrees to about 60 degrees.

119. The method of claim 115, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

120. The method of claim 115, further comprising forming a substantially transparent layer upon a bottom surface of the silicon wafer below the cavity.

121. The method of claim 115, further comprising forming a substantially transparent layer upon a bottom surface of the silicon wafer, wherein the cavity extends through the silicon wafer and wherein the substantially transparent layer is positioned to support the particle.

122. The method of claim 115, wherein the substantially transparent layer comprises silicon nitride.

123. The method of claim 115, wherein the cover comprises plastic, glass, quartz, silicon nitride, or silicon oxide.

124. The method of claim 115, wherein forming the cover comprises coupling the cover to the silicon wafer at a distance above the silicon wafer substantially less than a width of the particle.

125. The method of claim 115, further comprising etching channels in the silicon wafer prior to forming the cover on the silicon wafer, wherein forming the cover comprises placing the cover against the upper

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2814

surface of the silicon wafer, and wherein the channels are configured to allow the fluid to pass through the silicon wafer to and from the cavities.

126. The method of claim 115, further comprising coating an inner surface of the cavity with a material to increase adhesion of the particle to the inner surface of the cavity.

127. The method of claim 115, further comprising coating an inner surface of the cavity with a material to increase reflectivity of the inner surface of the cavity.

128. The method of claim 115, further comprising forming an optical detector upon a bottom surface of the supporting member below the cavity.

129. The method of claim 115, further comprising forming a sensing cavity upon a bottom surface of the supporting member below the cavity.

130. The method of claim 129, wherein forming the sensing cavity comprises:
forming a barrier layer upon a bottom surface of the silicon wafer;
forming a bottom diaphragm layer upon the barrier layer;
forming etch windows extending through the bottom diaphragm layer;
forming a sacrificial spacer layer upon the bottom diaphragm layer;
removing a portion of the spacer layer;
forming a top diaphragm layer; and
removing a remaining portion of the spacer layer.

131. The method of claim 130, further comprising filling a portion of the sensing cavity with a sensing substrate.

132. The method of claim 115, further comprising forming an optical filter upon the bottom surface of the supporting member.

133. The method of claim 115, further comprising forming a plurality of cavities in the silicon wafer.

134. The method of claim 115, wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

135. A sensor array produced by the method of claim 115.

136. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2815

monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

137. The method of claim 136, wherein the spectroscopic change comprises a change in absorbance of the particle.

138. The method of claim 136, wherein the spectroscopic change comprises a change in fluorescence of the particle.

139. The method of claim 136, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

140. The method of claim 136, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

141. The method of claim 136, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

142. The method of claim 136, wherein the analyte is an anion, and wherein the spectroscopic change is produced in response to the presence of the anion in the fluid.

143. The method of claim 136, wherein the analyte is a DNA molecule, and wherein the spectroscopic change is produced in response to the presence of the DNA molecule in the fluid.

144. The method of claim 136, wherein the analyte is a protein, and wherein the spectroscopic change is produced in response to the presence of the protein in the fluid.

145. The method of claim 136, wherein the analyte is a metabolite, and wherein the spectroscopic change is produced in response to the presence of the metabolite in the fluid.

146. The method of claim 136, wherein the analyte is a sugar, and wherein the spectroscopic change is produced in response to the presence of the sugar in the fluid.

147. The method of claim 136, wherein the analyte is a bacteria, and wherein the spectroscopic change is produced in response to the presence of the bacteria in the fluid.

148. The method of claim 136, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2816

149. The method of claim 148, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

150. The method of claim 148, wherein the indicator is a fluorescent indicator.

151. The method of claim 136, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

152. The method of claim 136, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

153. The method of claim 136, wherein monitoring the spectroscopic change is performed with a CCD device.

154. The method of claim 136, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

155. A sensor array for detecting an analyte in a fluid comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member;
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte.

156. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member; and
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte at least one particle positioned within a cavity of a supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

157. The method of claim 156, wherein the spectroscopic change comprises a change in absorbance of the particle.

158. The method of claim 156, wherein the spectroscopic change comprises a change in reflectance of the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2817

159. The method of claim 156, wherein the spectroscopic change comprises a change in fluorescence of the particle.

160. The method of claim 156, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

161. The method of claim 156, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

162. The method of claim 156, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

163. The method of claim 156, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

164. The method of claim 156, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

165. The method of claim 156, wherein the indicator is a fluorescent indicator.

166. The method of claim 156, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

167. The method of claim 156, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

168. The method of claim 156, wherein monitoring the spectroscopic change is performed with a CCD device.

169. The method of claim 156, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

170. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising at least one particle coupled to the sensor array, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2818

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

171. A sensor array for detecting an analyte in a fluid comprising:
at least one particle coupled to the sensor array, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

172. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle coupled to a supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

173. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte;
wherein the cavities are configured to allow the fluid to pass through the supporting member during use.

174. The sensor array of claim 173, further comprising a plurality of particles positioned within the cavity.

175. The sensor array of claim 173, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

176. The sensor array of claim 173, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter.

177. The sensor array of claim 173, wherein the cavity is configured to substantially contain the particle.

178. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable.

179. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

180. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2819

a bottom layer coupled to the supporting member, wherein the bottom layer is coupled to a bottom surface of the supporting member and wherein the cover layer is removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

181. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein an opening is formed in the cover layer substantially aligned with the cavity, and wherein an opening is formed in the bottom layer substantially aligned with the cavity.

182. The sensor array of claim 173, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

183. The sensor array of claim 173, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

184. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is configured to support the particle, and wherein an opening is formed in the cover layer substantially aligned with the cavity.

185. The sensor array of claim 173, further comprising a removable cover layer coupled to the supporting member.

186. The sensor array of claim 173, wherein the supporting member comprises a plastic material.

187. The sensor array of claim 173, wherein the supporting member comprises a silicon wafer.

188. The sensor array of claim 173, wherein the supporting member comprises a dry film photoresist material.

189. The sensor array of claim 173, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

190. The sensor array of claim 173, wherein an inner surface of the cavity is coated with a reflective material.

191. The sensor array of claim 173, further comprising channels in the supporting member, wherein the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2820

channels are configured to allow the fluid to flow through the channels into and away from the cavity.

192. The sensor array of claim 173, further comprising a plurality of additional particles positioned within a plurality of additional cavities formed in the supporting member.

193. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member, wherein the cavity is configured such that the fluid entering the cavity passes through the supporting member during use;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

194. The system of claim 193, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

195. The system of claim 193, wherein the system comprises a plurality of particles positioned within the cavity.

196. The system of claim 193, wherein the light source comprises a light emitting diode.

197. The system of claim 193, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

198. The system of claim 193, wherein the light source comprises a white light source.

199. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable.

200. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting ,member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2821

201. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is coupled to a bottom surface of the supporting member and wherein the cover layer is removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

202. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein an opening is formed in the cover layer substantially aligned with the cavity, and wherein an opening is formed in the bottom layer substantially aligned with the cavity.

203. The system of claim 193, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

204. The system of claim 193, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

205. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is configured to support the particle, and wherein an opening is formed in the cover layer substantially aligned with the cavity.

206. The system of claim 193, further comprising a removable cover layer.

207. The system of claim 193, wherein the supporting member comprises a plastic material.

208. The system of claim 193, wherein the supporting member comprises a silicon wafer.

209. The system of claim 193, wherein the supporting member comprises a dry film photoresist material.

210. The system of claim 193, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

211. The system of claim 193, wherein an inner surface of the cavity is coated with a reflective material.

212. The system of claim 193, further comprising channels in the supporting member, wherein the channels

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2822

are configured to allow the fluid to flow through the channels into and away from the cavity.

213. The system of claim 193, wherein the detector comprises a charge-coupled device.

214. The system of claim 193, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

215. The system of claim 193, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

216. The system of claim 215, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

217. The system of claim 215, wherein the receptor molecule produces the signal in response to the pH of the fluid.

218. The system of claim 215, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

219. The system of claim 215, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of a carbohydrate.

220. The system of claim 215, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

221. The system of claim 215, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

222. The system of claim 215, wherein the receptor comprises a polynucleotide.

223. The system of claim 215, wherein the receptor comprises a peptide.

224. The system of claim 215, wherein the receptor comprises an enzyme.

225. The system o f claim 215, wherein the receptor comprises a synthetic receptor.

226. The system of claim 215, wherein the receptor comprises an unnatural biopolymer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2823

227. The system of claim 215, wherein the receptor comprises an antibody.

228. The system of claim 215, wherein the receptor comprises an antigen.

229. The system of claim 193, wherein the analyte comprises phosphate functional groups, and wherein the particle is configured to produce the signal in the presence of the phosphate functional groups.

230. The system of claim 193, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

231. The system of claim 193, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

232. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
a pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the cavity;
wherein a channel is formed in the supporting member, the channel coupling the pump to the cavity such that the fluid flows through the channel to the cavity during use.

233. The sensor array of claim 232, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

234. The sensor array of claim 232, wherein the supporting member comprises a plastic material.

235. The sensor array of claim 232, wherein the supporting member comprises a silicon wafer.

236. The sensor array of claim 232, wherein the supporting member comprises a dry film photoresist material.

237. The sensor array of claim 232, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2824

238. The sensor array of claim 232, wherein an inner surface of the cavity is coated with a reflective material.

239. The sensor array of claim 232, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

240. The sensor array of claim 232, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

241. The sensor array of claim 232, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use, wherein the barrier layer comprises a transmission electron microscope grid.

242. The sensor array of claim 232, further comprising a plurality of particles positioned within a plurality of cavities formed in the supporting member.

243. The sensor array of claim 232, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

244. The sensor array of claim 232, wherein the pump comprises a diaphragm pump.

245. The sensor array of claim 232, wherein the pump comprises an electrode pump.

246. The sensor array of claim 232 wherein the pump comprises a piezoelectric pump.

247. The sensor array of claim 232, wherein the pump comprises a pneumatic activated pump.

248. The sensor array of claim 232, wherein the pump comprises a heat activated pump.

249. The sensor array of claim 232, wherein the pump comprises a peristaltic pump.

250. The sensor array of claim 232, wherein the pump comprises an electroosmosis pump.

251. The sensor array of claim 232, wherein the pump comprises an electrohydrodynamic pump.

252. The sensor array of claim 232, wherein the pump comprises an electroosmosis pump and an electrohydrodynamic pump.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2825

253. The sensor array of claim 232, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

254. The sensor array of claim 232, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

255. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member, a pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the cavity, and wherein a channel is formed in the supporting member, the channel coupling the pump to the cavity such that the fluid flows through the channel to the cavity during use;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

256. The system of claim 255, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

257. The system of claim 255, wherein the light source comprises a light emitting diode.

258. The system of claim 255, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

259. The system of claim 255, wherein the light source comprises a white light source.

260. The system of claim 255, wherein the supporting member comprises a plastic material.

261. The system of claim 255, wherein the supporting member comprises a silicon wafer.

262. The system of claim 255, wherein the supporting member comprises a dry film photoresist material.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2826

263. The system of claim 255, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

264. The system of claim 255, wherein an inner surface of the cavity is coated with a reflective material.

265. The system of claim 255, further comprising a barrier layer coupled to the supporting member, wherein the barrier layer is positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

266. The system of claim 255, wherein the pump comprises a diaphragm pump.

267. The system of claim 255, wherein the pump comprises an electrode pump.

268. The system of claim 255 wherein the pump comprises a piezoelectric pump.

269. The system of claim 255, wherein the pump comprises a pneumatic activated pump.

270. The system of claim 255, wherein the pump comprises a heat activated pump.

271. The system of claim 255, wherein the pump comprises a peristaltic pump.

272. The system of claim 255, wherein the pump comprises an electroosmosis pump.

273. The system of claim 255, wherein the pump comprises an electrohydrodynamic pump.

274. The system of claim 255, wherein the pump comprises an electroosmosis pump and an electrohydrodynamic pump.

275. The system of claim 255, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

276. The system of claim 255, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

277. The system of claim 255, wherein the detector comprises a charge-coupled device.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2827

278. The system of claim 255, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

279. The system of claim 278, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

280. The system of claim 278, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

281. The system of claim 278, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

282. The system of claim 278, wherein the receptor comprises a polynucleotide.

283. The system of claim 278, wherein the receptor comprises a peptide.

284. The system of claim 278, wherein the receptor comprises an enzyme.

285. The system o f claim 278, wherein the receptor comprises a synthetic receptor.

286. The system of claim 278, wherein the receptor comprises an unnatural biopolymer.

287. The system of claim 278, wherein the receptor comprises an antibody.

288. The system of claim 278, wherein the receptor comprises an antigen.

289. The system of claim 255, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

290. The system of claim 255, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2828

291. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein a first cavity and a second cavity are formed within the supporting member;
a first particle positioned within the first cavity;
a second particle positioned within the second cavity, wherein the second particle comprises a reagent, wherein a portion of the reagent is removable from the second particle when contacted with a decoupling solution, and wherein the reagent is configured to modify the first particle, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with the analyte during use;
a first pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the first cavity;
a second pump coupled to the supporting member, wherein the second pump is configured to direct the decoupling solution towards the second cavity;
wherein a first channel is formed in the supporting member, the first channel coupling the first pump to the first cavity such that the fluid flows through the first channel to the first cavity during use, and
wherein a second channel is formed in the supporting member, the second channel coupling the second cavity to the first cavity such that the decoupling solution flows from the second cavity through the second channel to the first cavity during use.

292. The sensor array of claim 291, wherein the first particle comprises a receptor molecule coupled to a first polymeric resin, and wherein the second particle comprises an indicator molecule coupled to a second polymeric resin.

293. The sensor array of claim 291, wherein the first particle comprises an indicator molecule coupled to a first polymeric resin, and the second particle comprises a receptor molecule coupled to a second polymeric resin.

294. The sensor array of claim 291, wherein the first particle comprises a first polymeric resin configured to bind to the receptor molecule, and wherein the second particle comprises the receptor molecule coupled to a second polymeric resin.

295. The sensor array of claim 291, wherein the supporting member comprises a plastic material.

296. The sensor array of claim 291, wherein the supporting member comprises a silicon wafer.

297. The sensor array of claim 291, wherein the supporting member comprises a dry film photoresist material.

298. The sensor array of claim 291, wherein the supporting member comprises a plurality of layers of a dry

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2829

film photoresist material.

299. The sensor array of claim 291, wherein an inner surface of the first cavity is coated with a reflective material.

300. The sensor array of claim 291, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the first cavity.

301. The sensor array of claim 291, further comprising a plurality of additional particles positioned within a plurality of additional cavities formed in the supporting member, and wherein the second cavity is coupled to the additional cavities such that the reagent may be transferred from the second particle to the additional cavities during use.

302. The sensor array of claim 291, wherein the first and second pumps comprise a diaphragm pump.

303. The sensor array of claim 291, wherein the first and second pumps comprise an electrode pump.

304. The sensor array of claim 291, wherein the first pump comprises a diaphragm pump or an electrode pump and wherein the second pump comprises a diaphragm pump or an electrode pump.

305. The sensor array of claim 291, wherein the first cavity is substantially tapered such that the width of the first cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the first cavity is substantially less than a width of the first particle, and wherein the second cavity is substantially tapered such that the width of the second cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the second cavity is substantially less than a width of the second particle.

306. The sensor array of claim 291, wherein a width of a bottom portion of the first cavity is substantially less than a width of a top portion of the first cavity, and wherein the width of the bottom portion of the first cavity is substantially less than a width of the first particle, and wherein a width of a bottom portion of the second cavity is substantially less than a width of a top portion of the second cavity, and wherein the width of the bottom portion of the second cavity is substantially less than a width of the second particle.

307. The sensor array of claim 291, further comprising a reservoir coupled to the second pump, the reservoir configured to hold the decoupling solution.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2830

308. A system for detecting an analyte in a fluid comprising:

a light source;

a sensor array, the sensor array comprising:

a supporting member; wherein a first cavity and a second cavity are formed within the supporting member;

a first particle positioned within the first cavity;

a second particle positioned within the second cavity, wherein the second particle comprises a reagent, wherein a portion of the reagent is removable from the second particle when contacted with a decoupling solution, and wherein the reagent is configured to modify the first particle, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with the analyte during use;

a first pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the first cavity;

a second pump coupled to the supporting member, wherein the second pump is configured to direct the decoupling solution towards the second cavity;

wherein a first channel is formed in the supporting member, the first channel coupling the first pump to the first cavity such that the fluid flows through the first channel to the first cavity during use, and wherein a second channel is formed in the supporting member, the second channel coupling the second cavity to the first cavity such that the decoupling solution flows from the second cavity through the second channel to the first cavity during use; and

a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

309. The system of claim 308, wherein the sensor array further comprises a plurality of additional particles positioned within a plurality of additional cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid, and wherein the second cavity is coupled to the additional cavities such that the reagent may be transferred from the second particle to the additional cavities during use.

310. The system of claim 308, wherein the light source comprises a light emitting diode.

311. The system of claim 308, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

312. The system of claim 308, wherein the light source comprises a white light source.

313. The system of claim 308, wherein the first particle comprises a receptor molecule coupled to a first

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2831

polymeric resin, and wherein the second particle comprises an indicator molecule coupled to a second polymeric resin.

314. The system of claim 308, wherein the first particle comprises an indicator molecule coupled to a first polymeric resin, and the second particle comprises a receptor molecule coupled to a second polymeric resin.

315. The system of claim 308, wherein the first particle comprises a first polymeric resin configured to bind to the receptor molecule, and wherein the second particle comprises the receptor molecule coupled to a second polymeric resin.

316. The system of claim 308, wherein the supporting member comprises a plastic material.

317. The system of claim 308, wherein the supporting member comprises a silicon wafer.

318. The system of claim 308, wherein the supporting member comprises a dry film photoresist material.

319. The system of claim 308, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

320. The system of claim 308, wherein an inner surface of the first cavity is coated with a reflective material.

321. The system of claim 308, wherein the first and second pumps comprise a diaphragm pump.

322. The system of claim 308, wherein the first and second pumps comprise an electrode pump.

323. The system of claim 308, wherein the first pump comprises a diaphragm pump or an electrode pump and wherein the second pump comprises a diaphragm pump or an electrode pump.

324. The system of claim 308, wherein the first cavity is substantially tapered such that the width of the first cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the first cavity is substantially less than a width of the first particle, and wherein the second cavity is substantially tapered such that the width of the second cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the second cavity is substantially less than a width of the second particle.

325. The system of claim 308, wherein a width of a bottom portion of the first cavity is substantially less

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2832

than a width of a top portion of the first cavity, and wherein the width of the bottom portion of the first cavity is substantially less than a width of the first particle, and wherein a width of a bottom portion of the second cavity is substantially less than a width of a top portion of the second cavity, and wherein the width of the bottom portion of the second cavity is substantially less than a width of the second particle.

326. The system of claim 308, wherein the sensor array further comprises a reservoir coupled to the second pump, the reservoir configured to hold the decoupling solution.

327. The system of claim 308, wherein the analyte comprises bacteria, and wherein the first particle is configured to produce the signal in the presence of the bacteria.

328. A method for forming a sensor array configured to detect an analyte in a fluid, comprising:
forming a cavity in a supporting member, wherein the cavity is configured to allow the fluid to pass through the supporting member;
placing a particle in the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
placing a cover upon a portion of the supporting member, wherein the cover is configured to inhibit dislodgment of the particle from the cavity.

329. The method of claim 328, further comprising forming a substantially transparent layer upon a bottom surface of the supporting member below the cavity, wherein the bottom layer is configured to inhibit the displacement of the particle from the cavity while allowing the fluid to pass through the supporting member.

330. The method of claim 328, further comprising forming an optical detector upon a bottom surface of the supporting member below the cavity.

331. The system of claim 328, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

332. The method of claim 328, further comprising forming channels in the supporting member wherein the channels are configured to allow the fluid to pass through the supporting member to and from the cavity.

333. The method of claim 328, further comprising forming a pump on the supporting member, the pump being configured to pump the fluid to the cavity.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2833

334. The method of claim 328, further comprising forming additional cavities in the supporting member and further comprising placing additional particles in the additional cavities.

335. The method of claim 328, further comprising forming a cover, wherein forming the cover comprises:
forming a removable layer upon the upper surface of the supporting member;
forming a cover upon the removable layer;
forming support structures upon the supporting member, the support structures covering a portion of the cover; and
dissolving the removable layer.

336. The method of claim 335, wherein the cover layer is formed prior to forming the cavity.

337. The method of claim 335, wherein forming the cover further comprises forming openings in the cover, wherein the openings are substantially aligned with the cavity.

338. The method of claim 328, wherein the particles are placed in the cavities using a micromanipulator.

339. The method of claim 328, further comprising forming additional cavities within the supporting member, and further comprising placing additional particles in the additional cavities, wherein placing the additional particles in the additional cavities comprises:
placing a first masking layer on the supporting member, wherein the first masking layer covers a first portion of the additional cavities such that passage of a particle into the first portion of the additional cavities is inhibited, and wherein the first masking layer a second portion of the cavities substantially unmasked,;
placing the additional particles on the supporting member; and
moving the additional particles across the supporting member such that the particles fall into the second portion of the cavities.

340. The method of claim 339, further comprising:
removing the first masking layer;
placing a second masking layer upon the supporting member, wherein the second masking layer covers the second portion of the cavities and a portion of the first portion of the cavities while leaving a third portion of the cavities unmasked;
placing additional particles on the supporting member; and
moving the additional particles across the supporting member such that the particle fall into the third portion of the cavities.

341. The method of claim 328, wherein forming the cover comprises coupling the cover to the supporting member at a distance above the supporting member substantially less than a width of the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2834

342. The method of claim 328, wherein the supporting member comprises a silicon wafer.

343. The method of claim 342, wherein forming the cavity comprises anisotropically etching the silicon wafer.

344. The method of claim 342, wherein forming the cavity comprises anisotropically etching the silicon wafer such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

345. The method of claim 328, wherein the supporting member comprises a dry film photoresist material.

346. The method of claim 328, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

347. The method of claim 346, wherein forming the cavity comprises:
etching a first opening through a first dry film photoresist layer, the first opening having a width substantially less than a width of the particle;
placing a second dry film photoresist layer upon the first dry film photoresist layer;
etching a second opening through the second dry film photoresist layer, the second opening being substantially aligned with the first opening, wherein a width of the second opening is substantially greater than the width of the first opening.

348. The method of claim 347, wherein the second dry film photoresist layer comprises a thickness substantially greater than a width of the particle.

349. The method of claim 345, further comprising forming a reflective layer upon the inner surface of the cavity.

350. The method of claim 328, wherein the supporting material comprises a plastic material.

351. The method of claim 350, wherein the cavity is formed by drilling the supporting material.

352. The method of claim 350, wherein the cavity is formed by transfer molding the supporting member.

353. The method of claim 350, wherein the cavity is formed by a punching device.

354. A sensor array produced by the method of claim 328.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2835

355. A sensor array produced by the method of claim 342.

356. A sensor array produced by the method of claim 345.

357. A sensor array produced by the method of claim 346.

358. A sensor array produce by the method of claim 350.

359. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member, wherein the cavity is configured such that the fluid entering the cavity passes through the supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

360. The method of claim 359, wherein the spectroscopic change comprises a change in absorbance of the particle.

361. The method of claim 359, wherein the spectroscopic change comprises a change in fluorescence of the particle.

362. The method of claim 359, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

363. The method of claim 359, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

364. The method of claim 359, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

365. The method of claim 359, wherein the analyte is an anion, and wherein the spectroscopic change is produced in response to the presence of the anion in the fluid.

366. The method of claim 359, wherein the analyte is a DNA molecule, and wherein the spectroscopic change is produced in response to the presence of the DNA molecule in the fluid.

367. The method of claim 359, wherein the analyte is a protein, and wherein the spectroscopic change is produced in response to the presence of the protein in the fluid.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2836

368. The method of claim 359, wherein the analyte is a metabolite, and wherein the spectroscopic change is produced in response to the presence of the metabolite in the fluid.

369. The method of claim 359, wherein the analyte is a sugar, and wherein the spectroscopic change is produced in response to the presence of the sugar in the fluid.

370. The method of claim 359, wherein the analyte is a bacteria, and wherein the spectroscopic change is produced in response to the presence of the bacteria in the fluid.

371. The method of claim 359, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

372. The method of claim 371, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

373. The method of claim 371, wherein the indicator is a fluorescent indicator.

374. The method of claim 359, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

375. The method of claim 359, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

376. The method of claim 359, wherein monitoring the spectroscopic change is performed with a CCD device.

377. The method of claim 359, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

378. The method of claim 359, wherein monitoring the spectroscopic change comprises:
directing a red light source at the particle;
detecting the absorbance of red light by the particle;
directing a green light source at the particle;
detecting the absorbance of green light by the particle;
directing a blue light source at the particle; and
detecting the absorbance of blue light by the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2837

379. A sensor array for detecting an analyte in a fluid comprising:
at least one particle coupled to a supporting member, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

380. The sensor array of claim 379, wherein the particle is coupled to the supporting member with via an adhesive material.

381. The sensor array of claim 379, wherein the particle are coupled to the supporting member via a gel material.

382. The sensor array of claim 379, wherein the particle is suspended in a gel material, the gel material covering a portion of the supporting member, and wherein a portion of the particle extends from the upper surface of the gel.

383. The sensor array of claim 379, further comprising a cover positioned above the particle.

384. The sensor array of claim 379, further comprising a cover coupled to the supporting member, positioned above the particle, wherein a force exerted by the cover on the particle inhibits the displacement of the particle from the supporting member.

385. The sensor array of claim 379, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

386. The sensor array of claim 379, wherein the supporting member comprises a plastic material.

387. The sensor array of claim 379, wherein the supporting member comprises a dry film photoresist material.

388. The sensor array of claim 379, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

389. The sensor array of claim 379, wherein the supporting member comprises glass.

390. The sensor array of claim 379, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the particle.

391. The sensor array of claim 379, further comprising a plurality of particles coupled to the supporting member.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2838

392. The sensor array of claim 379, wherein the supporting member is composed of a material substantially transparent to visible light.

393. The sensor array of claim 379, wherein the supporting member is composed of a material substantially transparent to ultraviolet light.

394. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising at least one particle coupled to a supporting member, wherein the particle is configured to produce a signal when the particle interacts with the analyte, and wherein the supporting member is substantially transparent to a portion of light produced by the light source; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

395. The system of claim 394, wherein the system comprises a plurality of additional particles coupled to the supporting member, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

396. The system of claim 394, wherein the light source comprises a light emitting diode.

397. The system of claim 394, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

398. The system of claim 394, wherein the light source comprises a white light source.

399. The system of claim 394, wherein the particle is coupled to the supporting member with via an adhesive material.

400. The system of claim 394, wherein the particle are coupled to the supporting member via a gel material.

401. The system of claim 394, wherein the particle is suspended in a gel material, the gel material covering a portion of the supporting member, and wherein a portion of the particle extends from the upper surface of the gel.

402. The system of claim 394, wherein the sensor array further comprises a cover positioned above the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2839

403. The system of claim 394, wherein the sensor array further comprises a cover coupled to the supporting member, positioned above the particle, wherein a force exerted by the cover on the particle inhibits the displacement of the particle from the supporting member.

404. The system of claim 394, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

405. The system of claim 394, wherein the supporting member comprises a plastic material.

406. The system of claim 394, wherein the supporting member comprises a dry film photoresist material.

407. The system of claim 394, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

408. The system of claim 394, wherein the supporting member comprises glass.

409. The system of claim 394, wherein the supporting member is composed of a material substantially transparent to ultraviolet light.

410. The system of claim 394, wherein the detector comprises a charge-coupled device.

411. The system of claim 394, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

412. The system of claim 394, wherein the system comprises a plurality of particles coupled to the supporting member, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2840

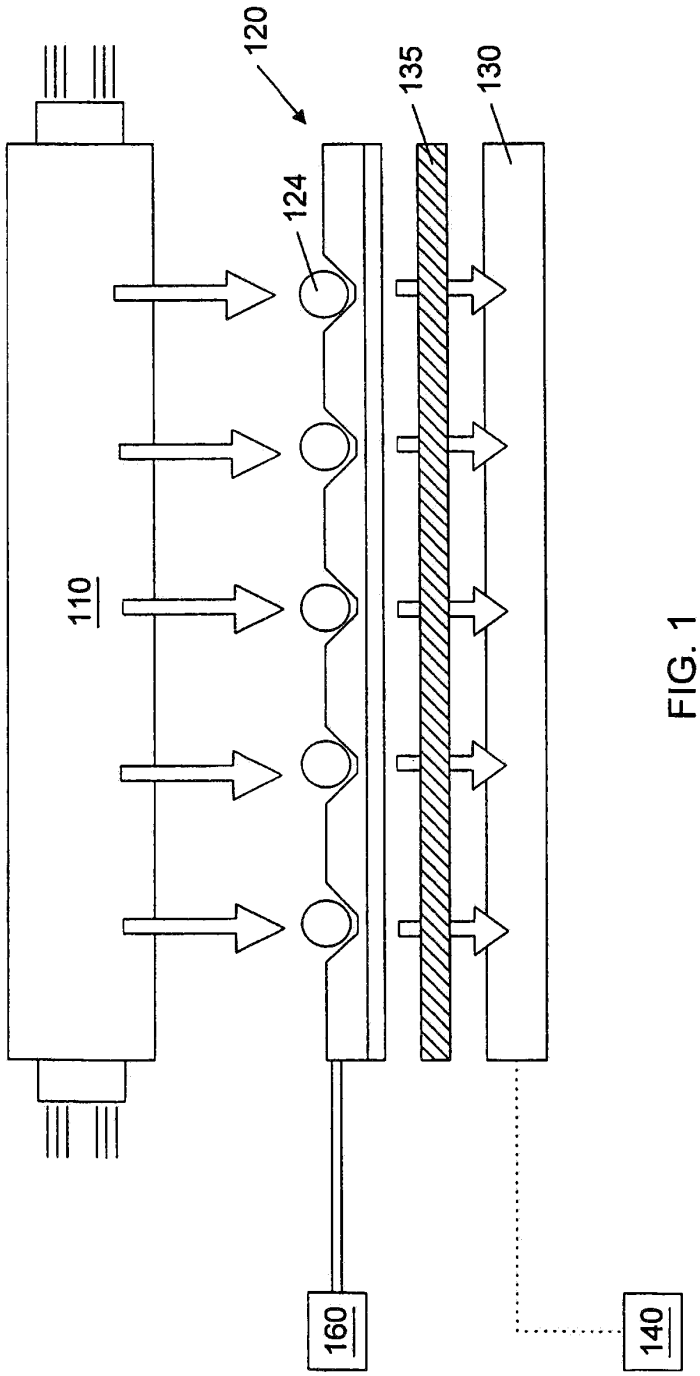


FIG. 1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2841

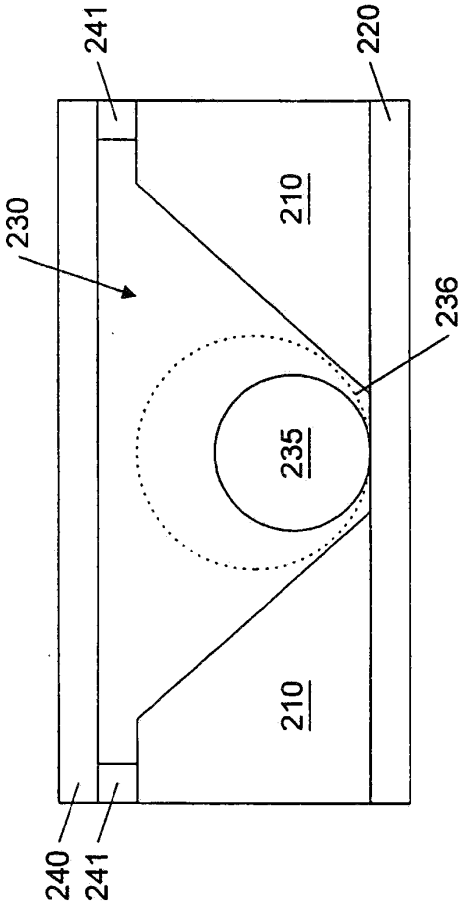


FIG. 2

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2842

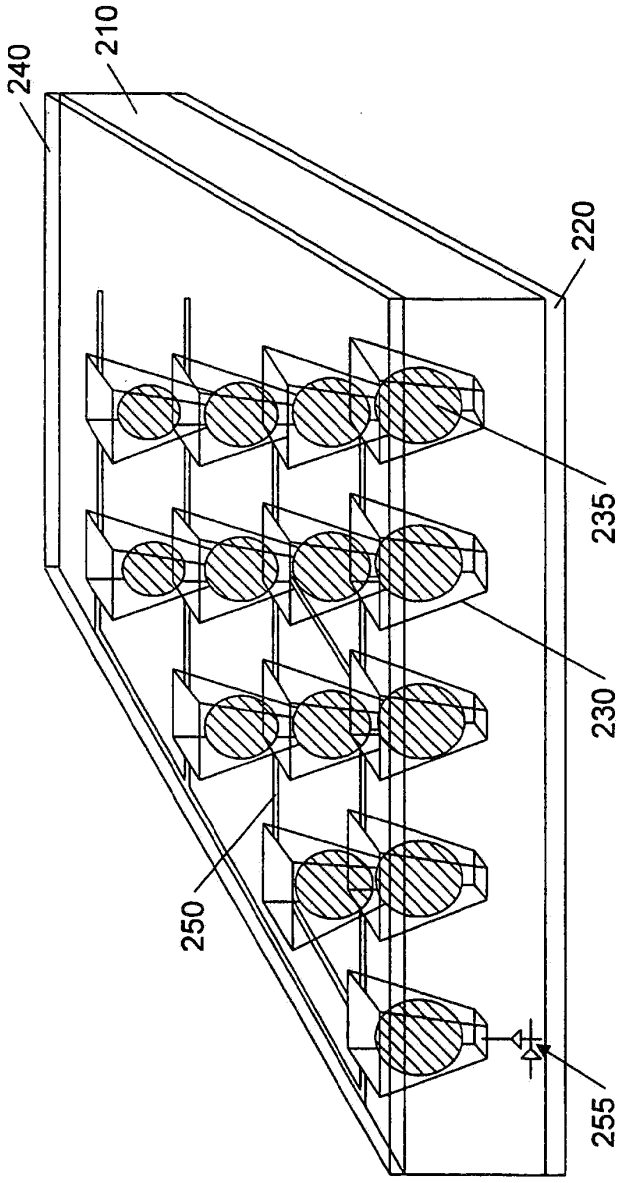


FIG. 3

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2843

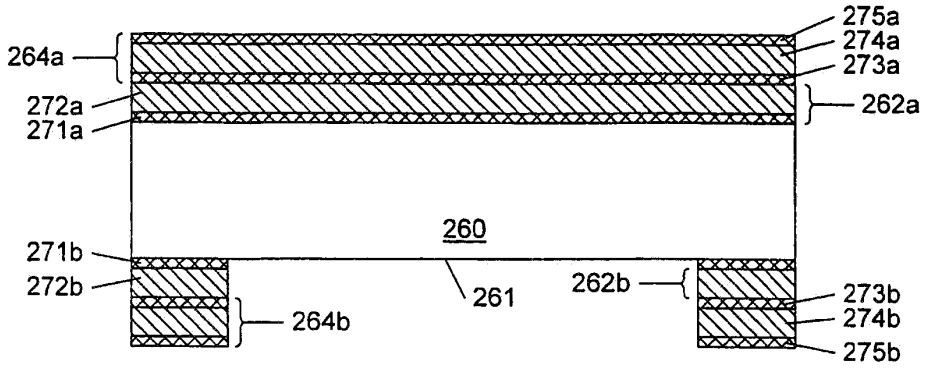


FIG. 4A

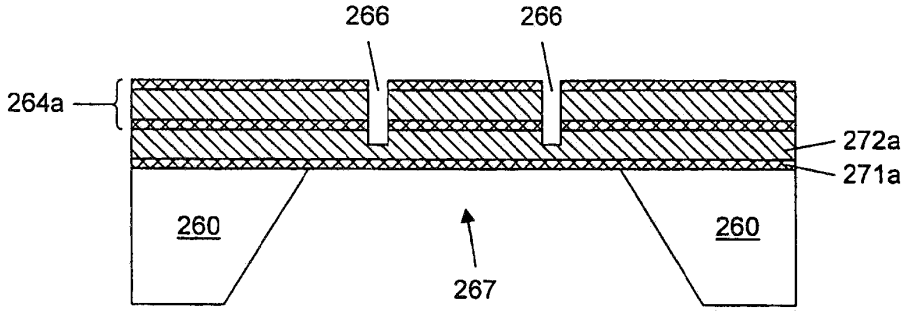


FIG. 4B

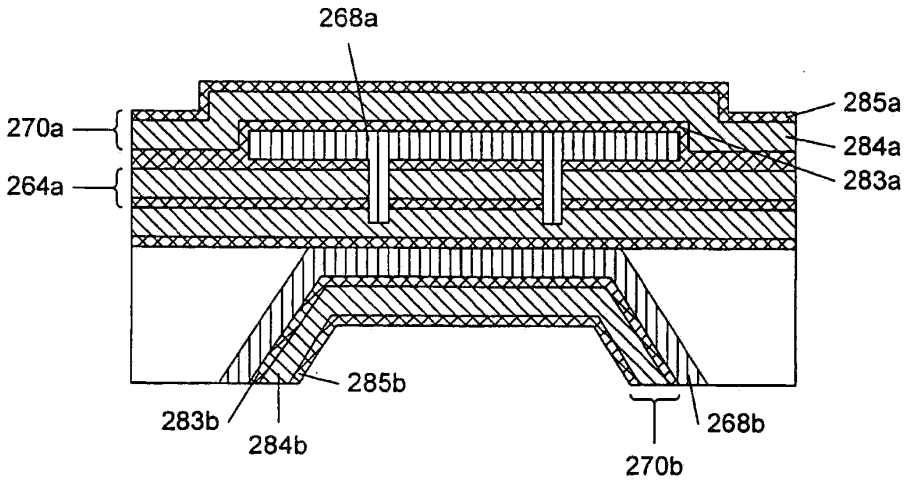


FIG. 4C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2844

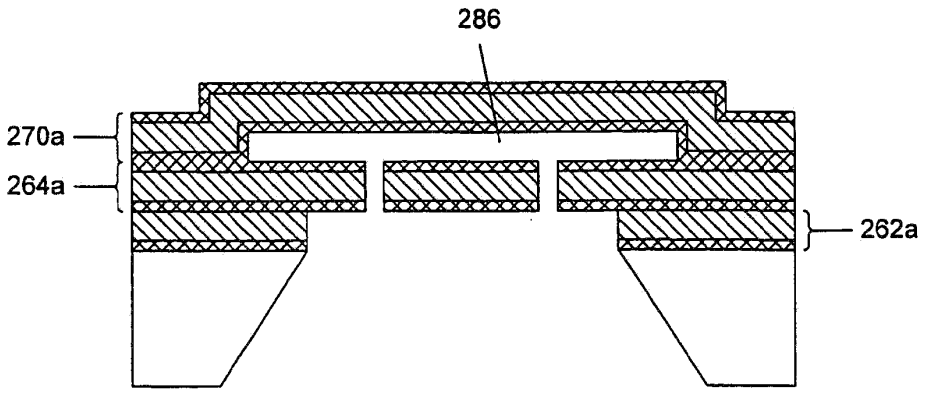


FIG. 4D

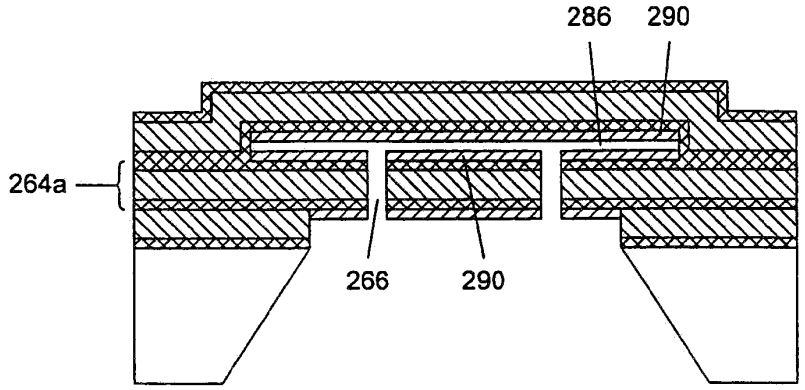


FIG. 4E

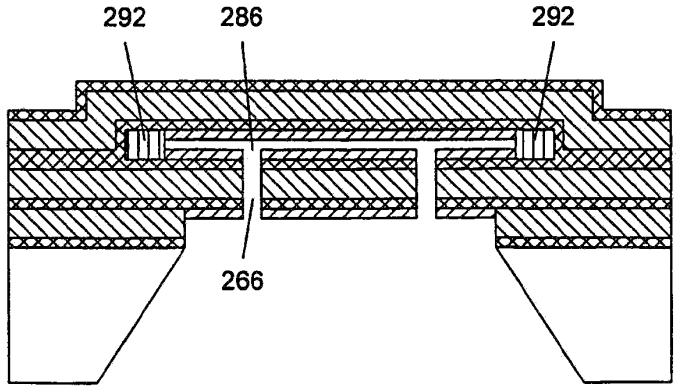


FIG. 4F

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2845

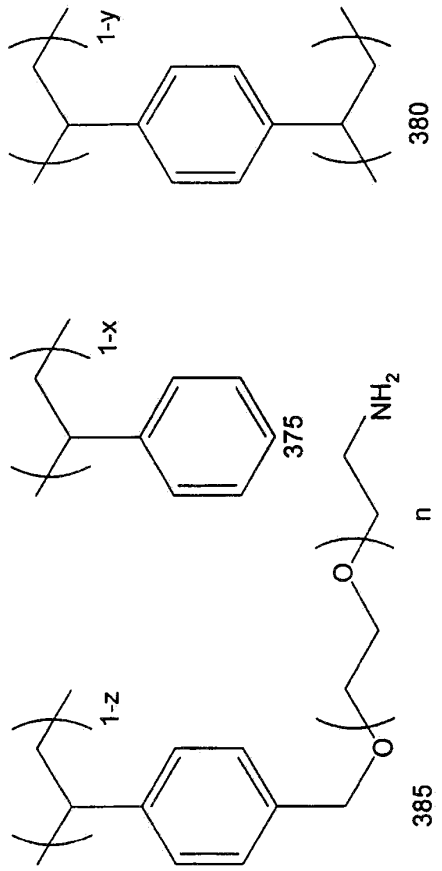


FIG. 5

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2846

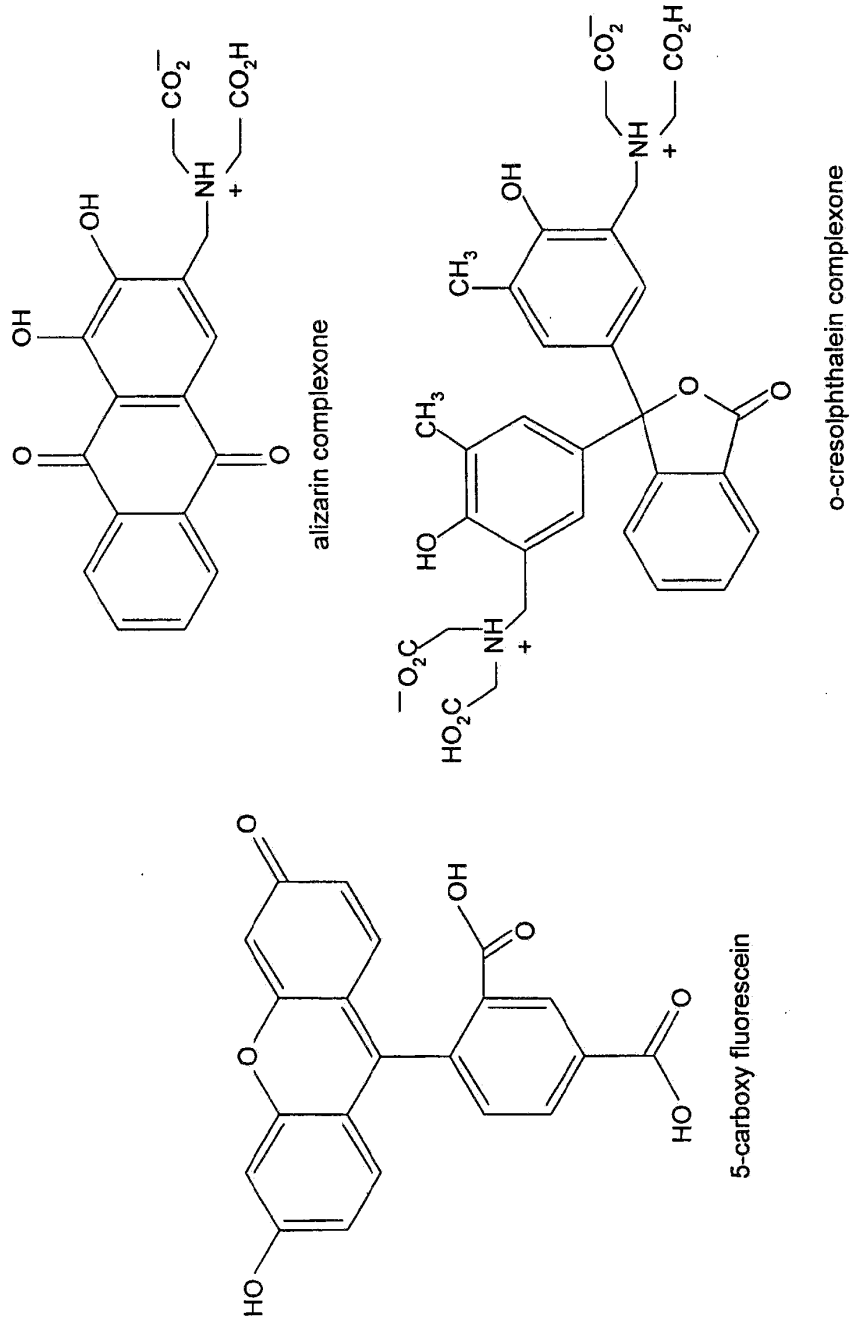


FIG. 6

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2847

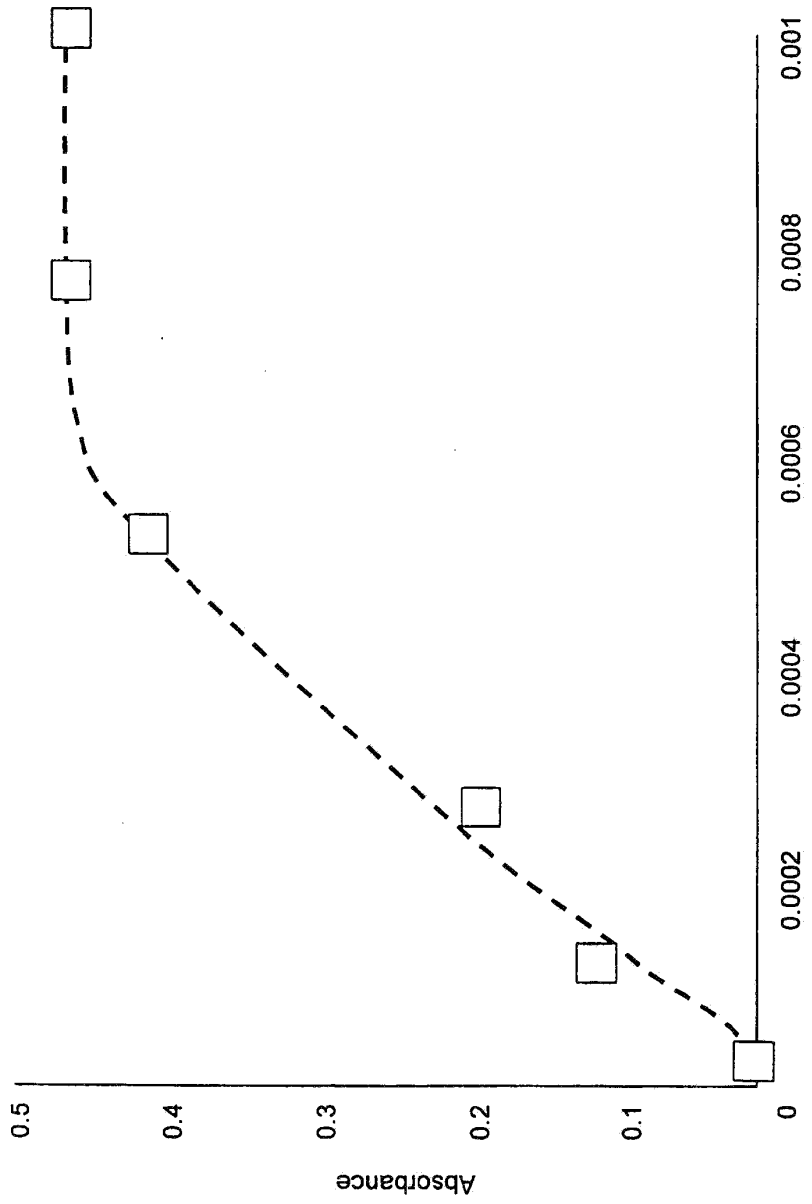


FIG. 7

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2848

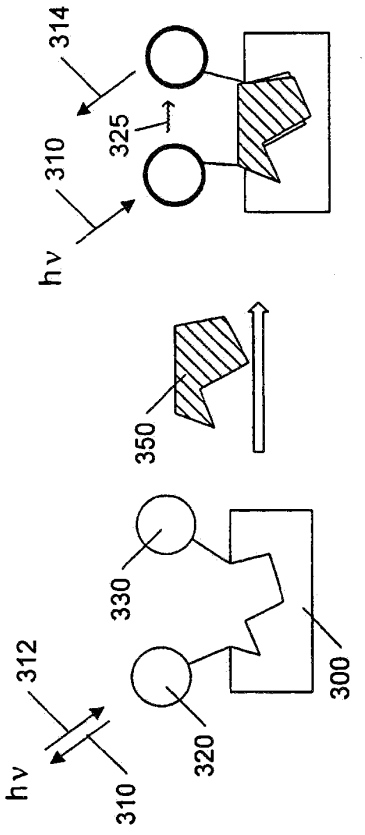


FIG. 8

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2849

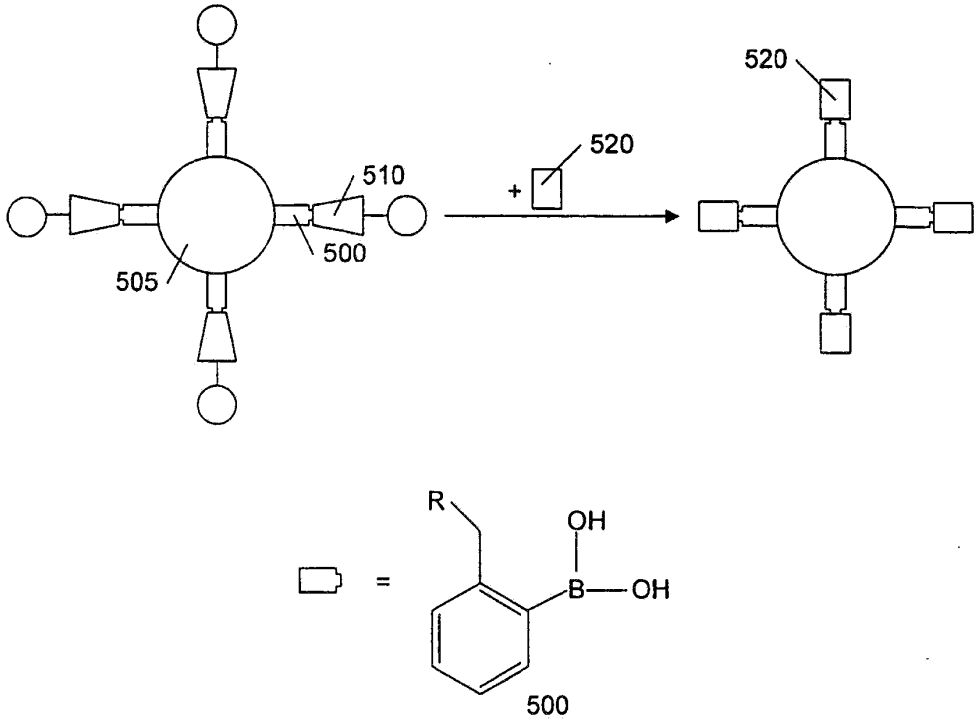
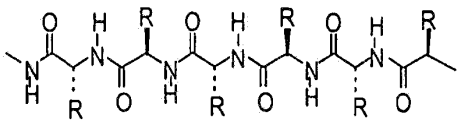
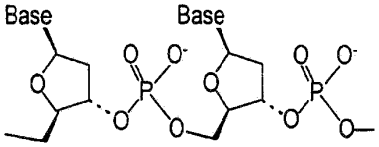


FIG. 9

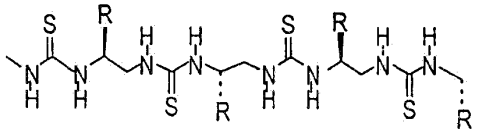
Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2850



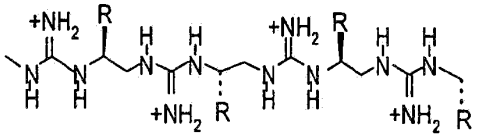
Peptides



Nucleotides



Polythioureas



Polyguanidiniums

FIG. 10

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2851

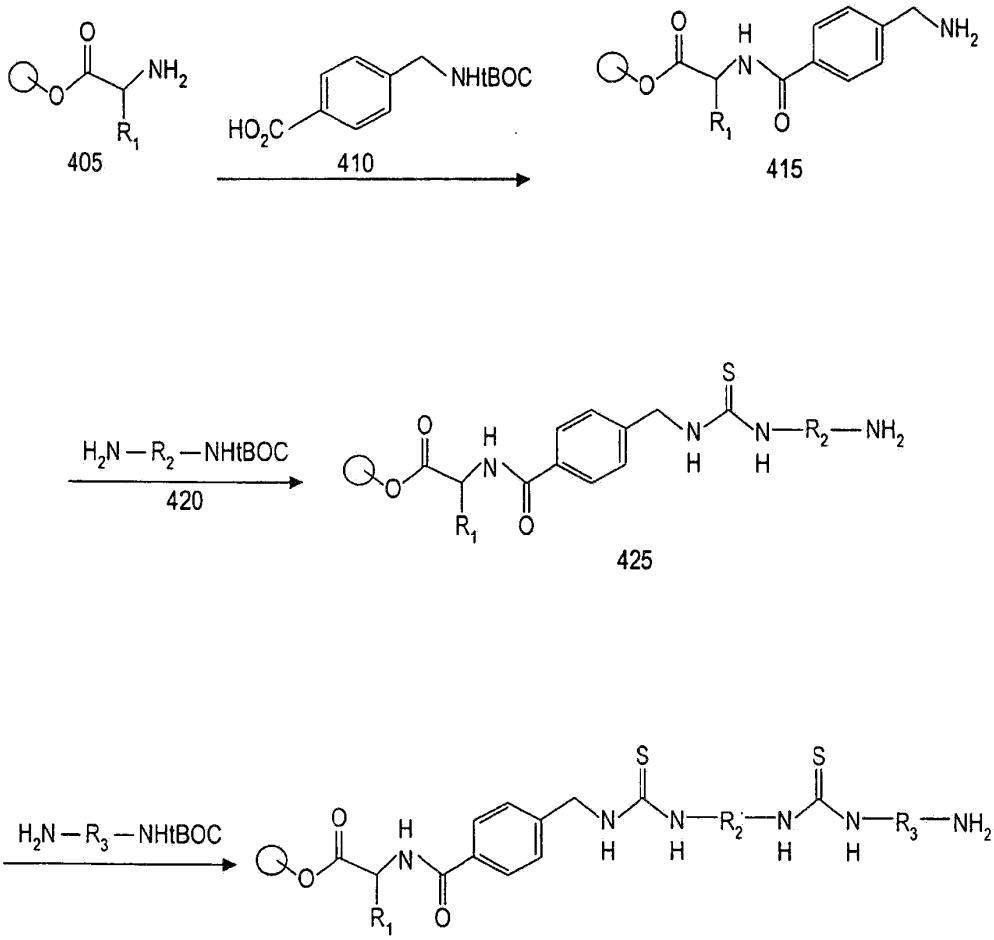


FIG. 11

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2852

13/44

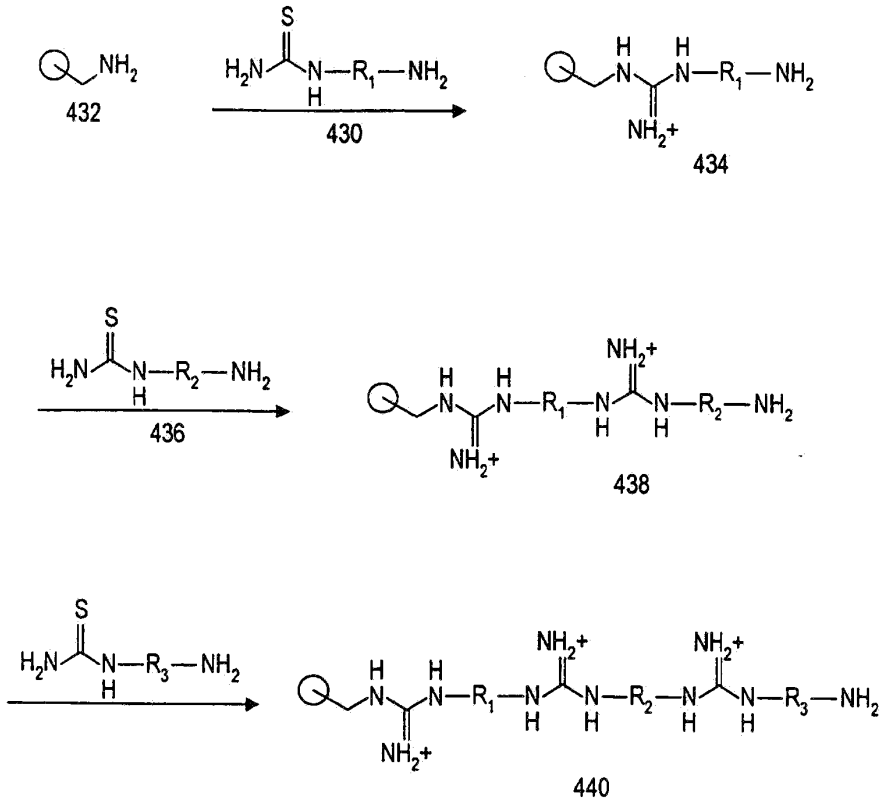


FIG. 12

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2853

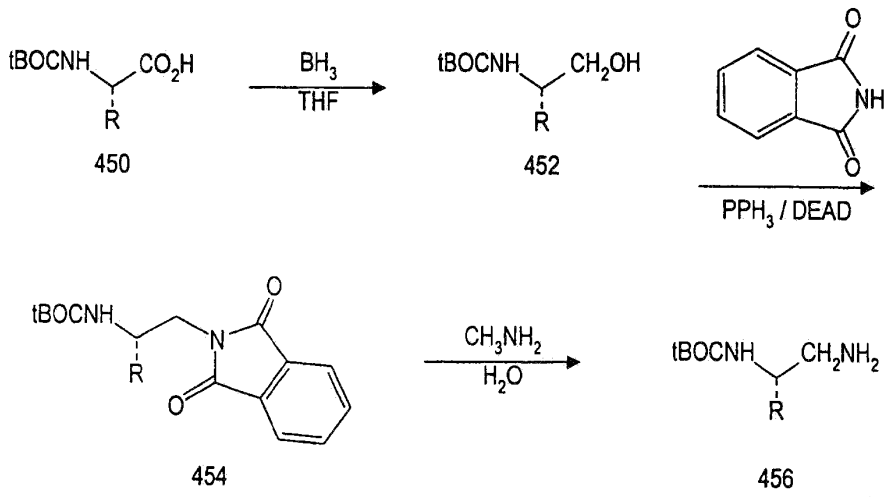
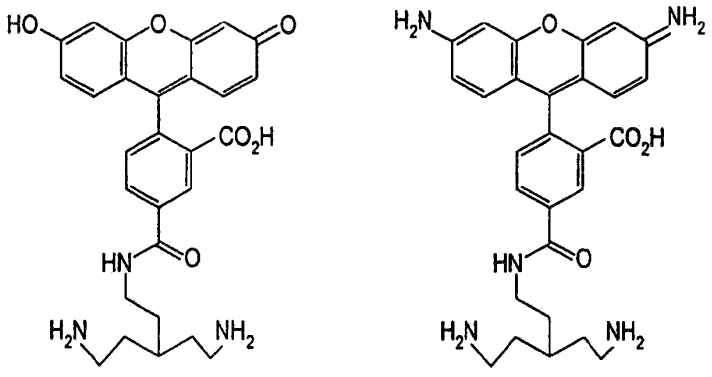


FIG. 13

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2854



470 475

FIG. 14

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2855

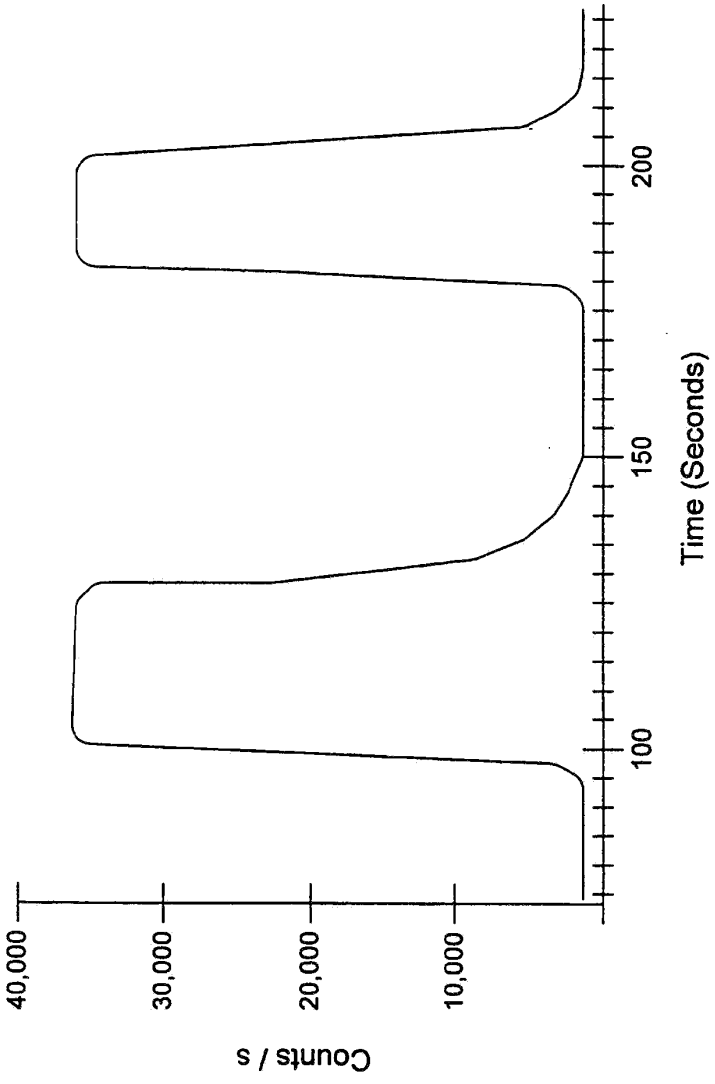


FIG. 15

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2856

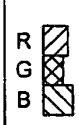
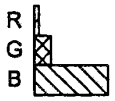
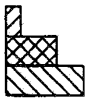
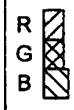
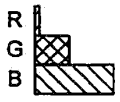
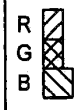
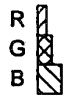
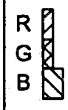
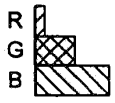
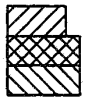
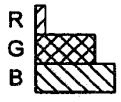
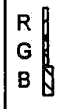
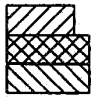
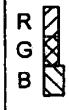
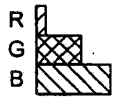
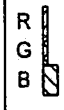
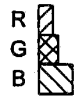
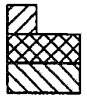
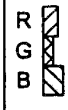
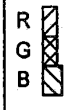
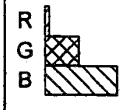
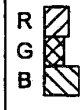
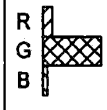
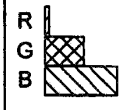
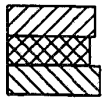
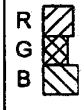
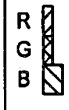
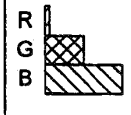
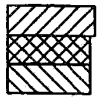
RESIN: pH	Ion	Blank	Alizarin	o-Cresol-phthalein	Fluorescein	Alizarin-Ce^{3+} complex
2	none					
2	Ca^{2+}					
7	none					
7	Ca^{2+}					
7	F^-					
12	none					
12	Ca^{2+}					
12	F^-					

FIG. 16

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2857

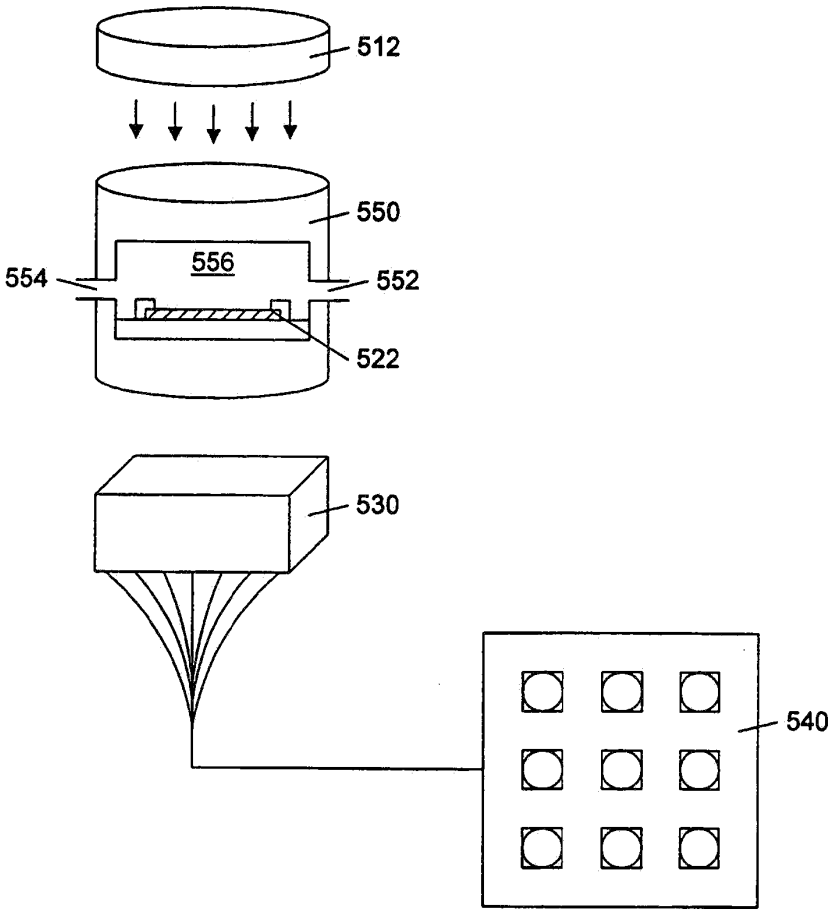


FIG. 17

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2858

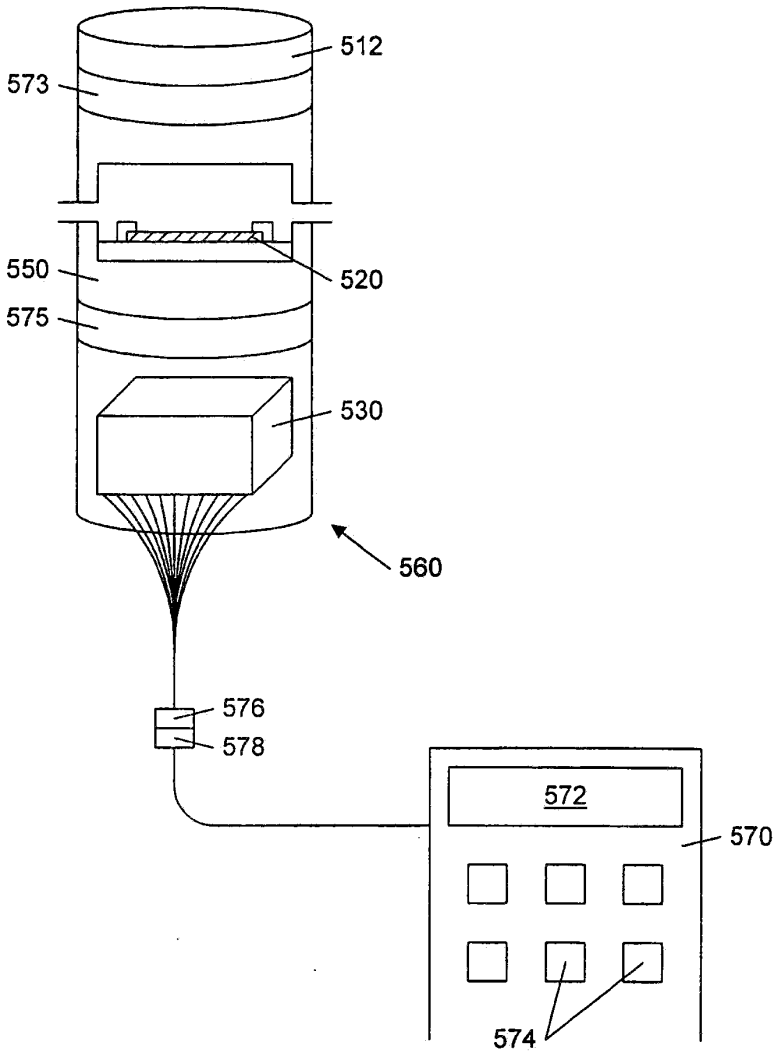


FIG. 18

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2859

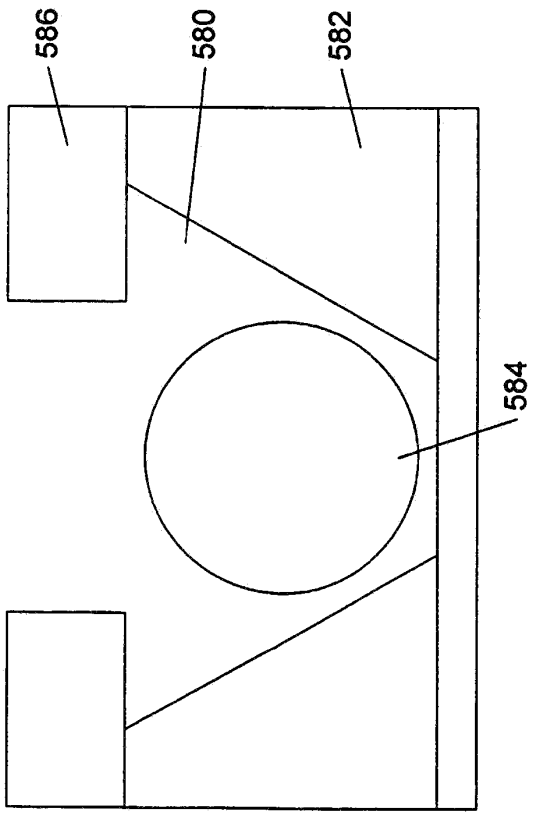


FIG. 19

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2860

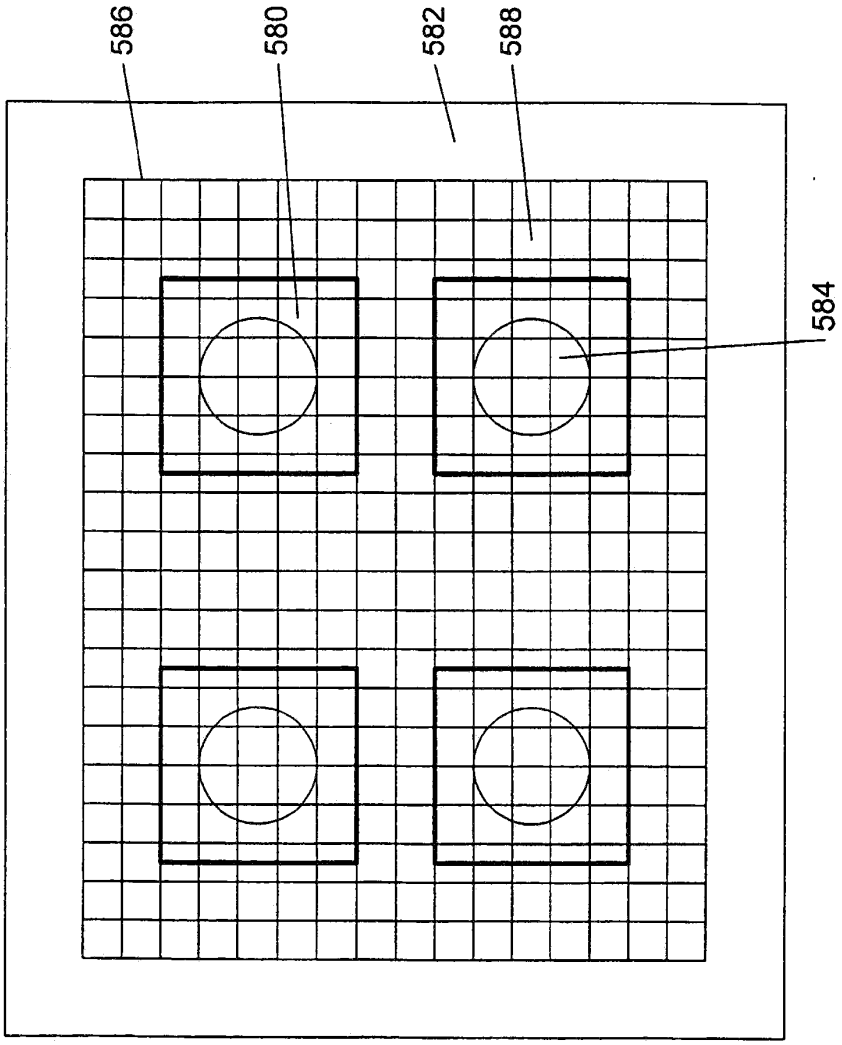


FIG. 20

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2861

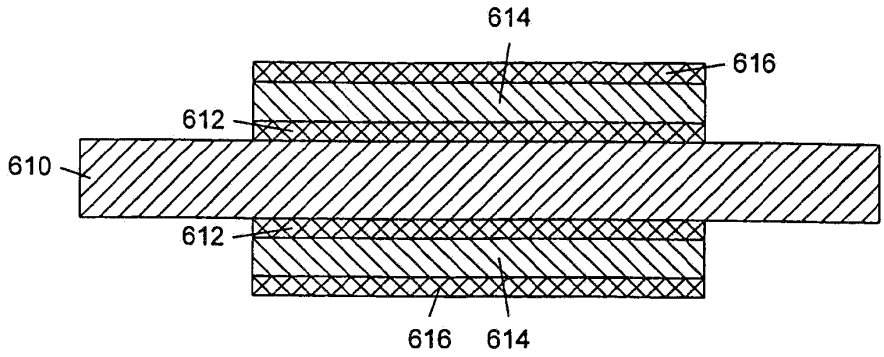


FIG. 21A

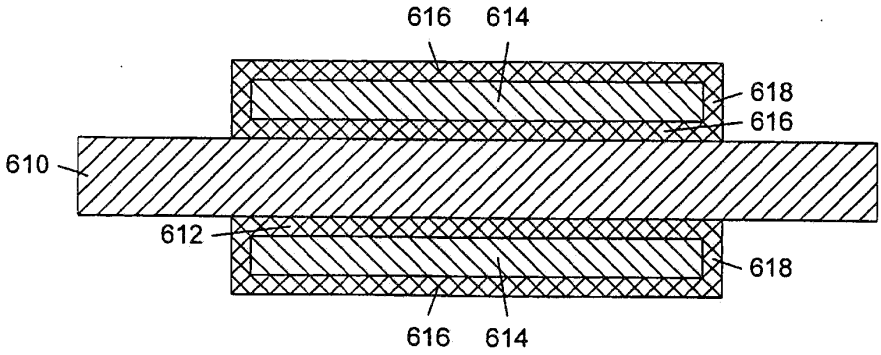


FIG. 21B

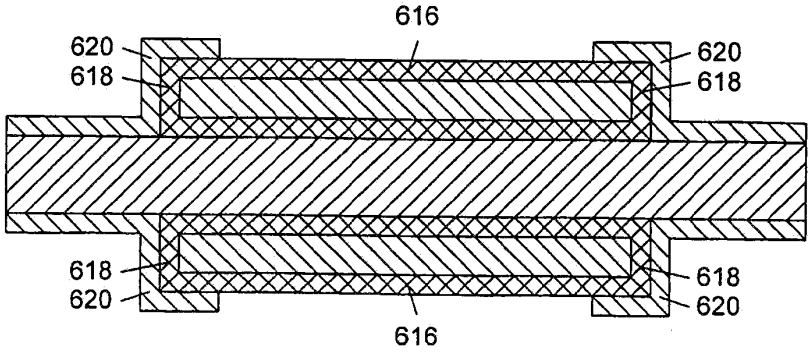


FIG. 21C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2862

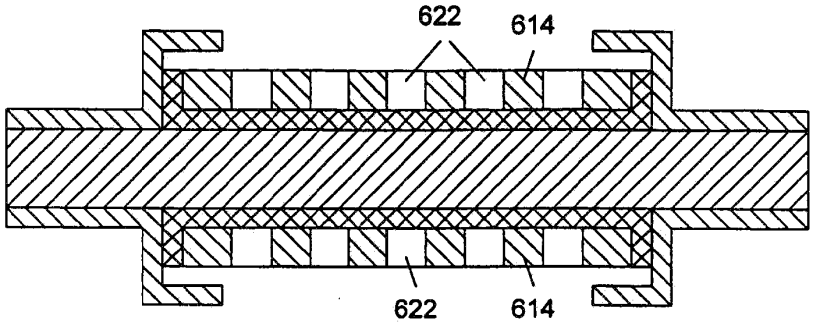


FIG. 21D

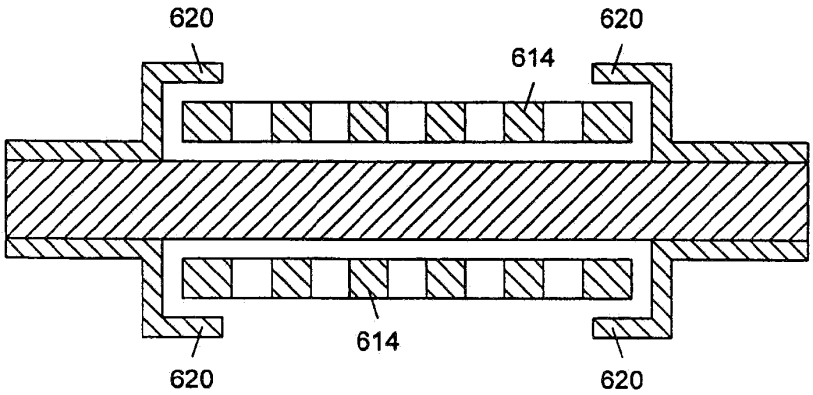


FIG. 21E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2863

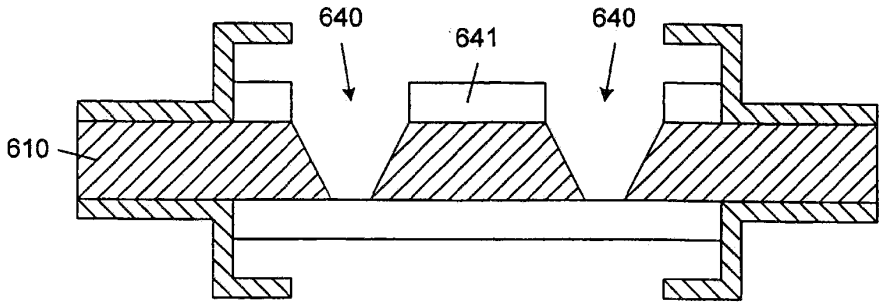


FIG. 21F

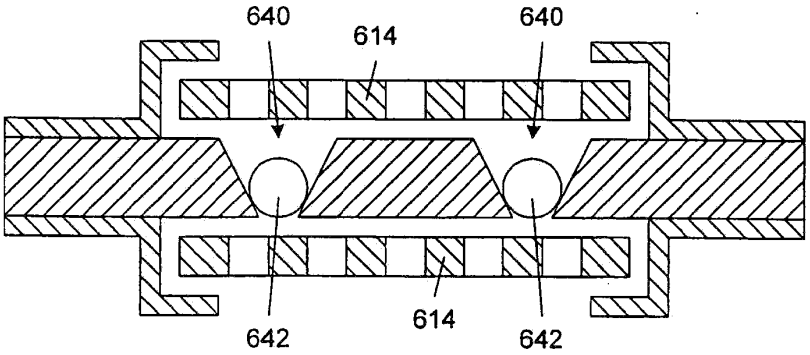


FIG. 21G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2864

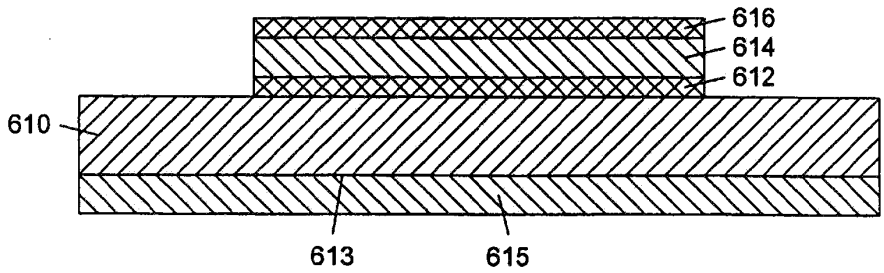


FIG. 22A

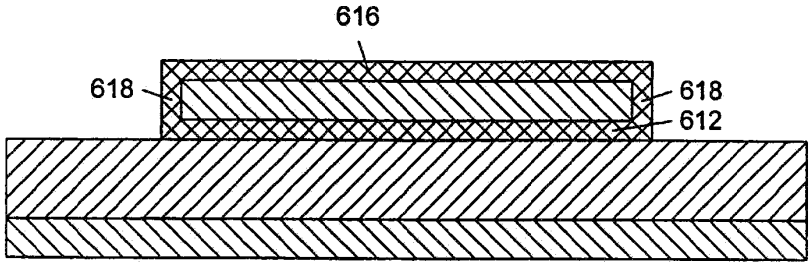


FIG. 22B

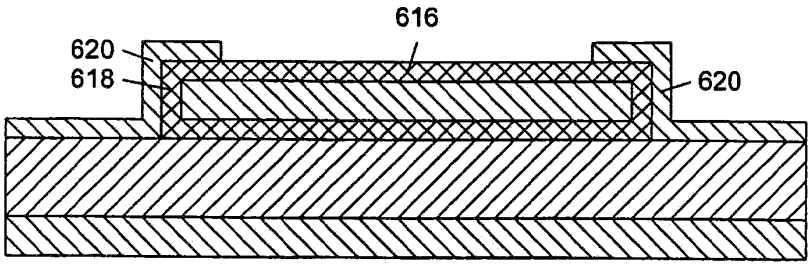


FIG. 22C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2865

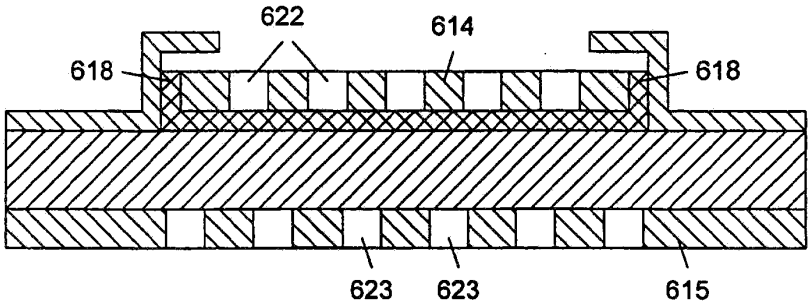


FIG. 22D

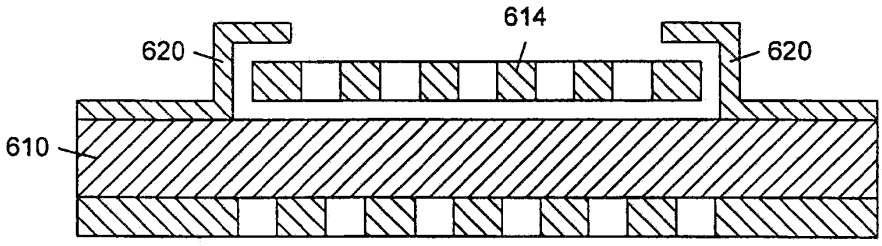


FIG. 22E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2866

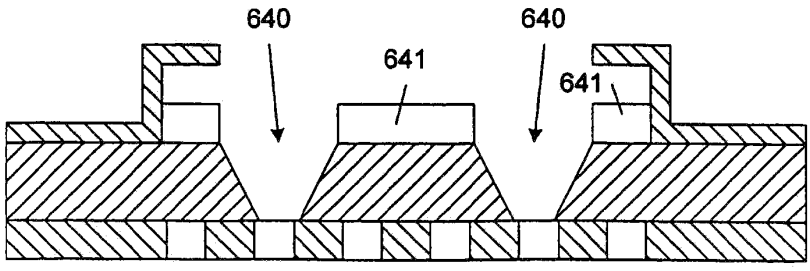


FIG. 22F

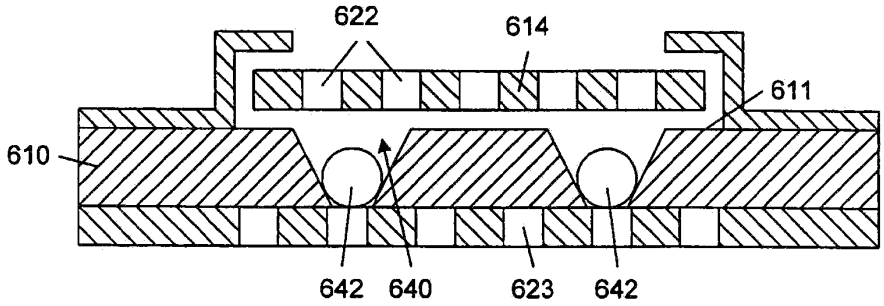


FIG. 22G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2867

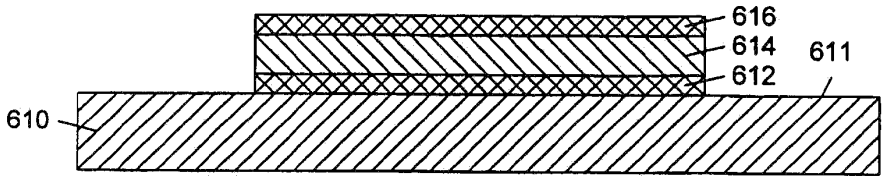


FIG. 23A

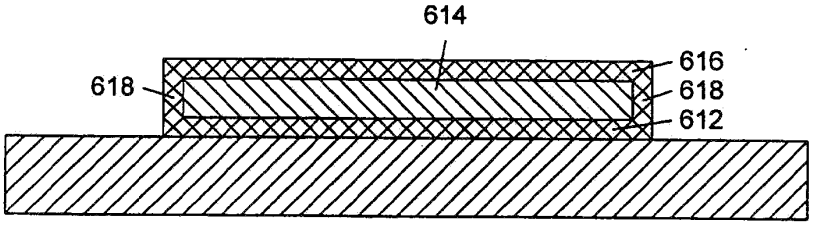


FIG. 23B

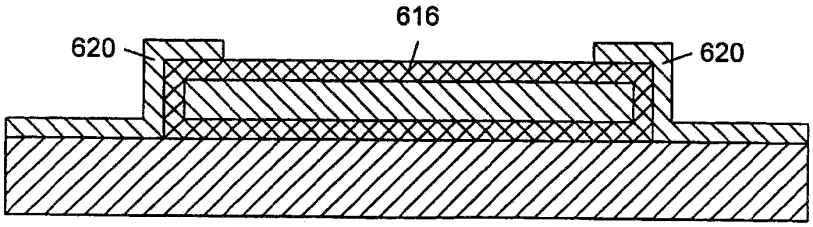


FIG. 23C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2868

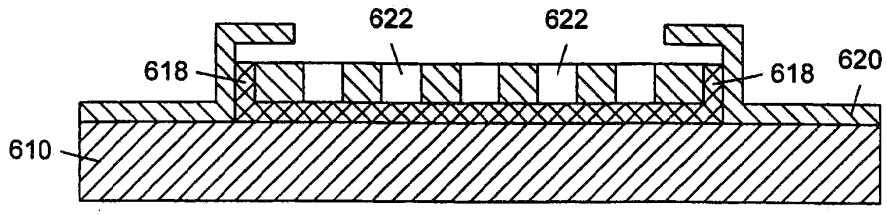


FIG. 23D

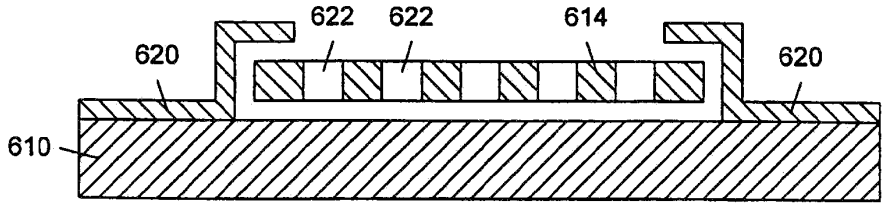


FIG. 23E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2869

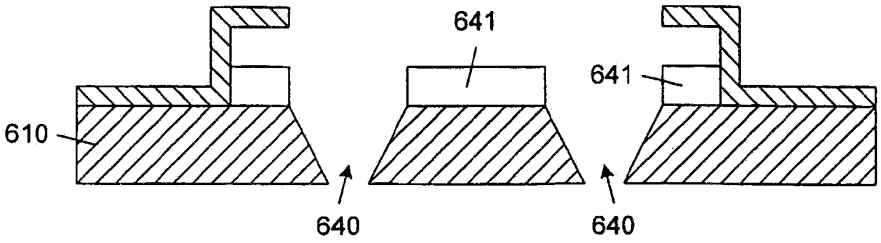


FIG. 23F

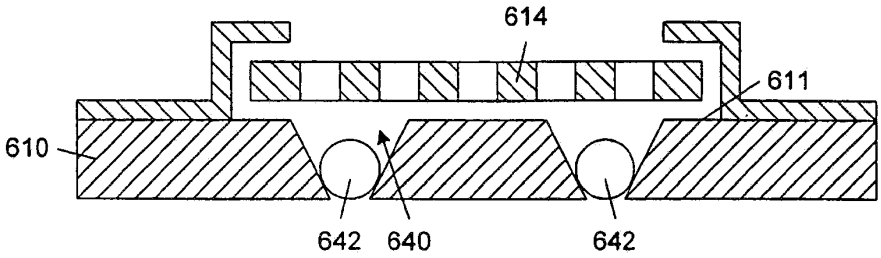


FIG. 23G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2870

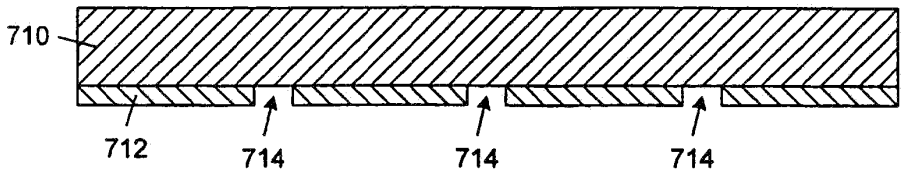


FIG. 24A

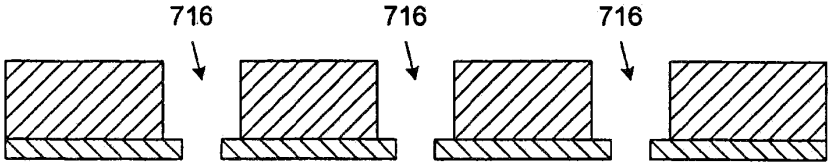


FIG. 24B

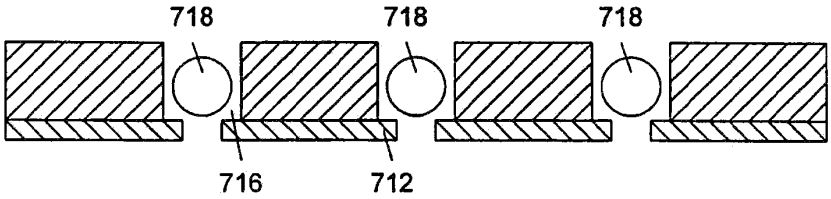


FIG. 24C

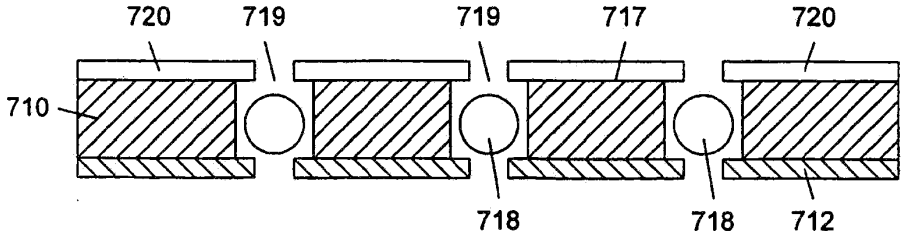


FIG. 24D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2871

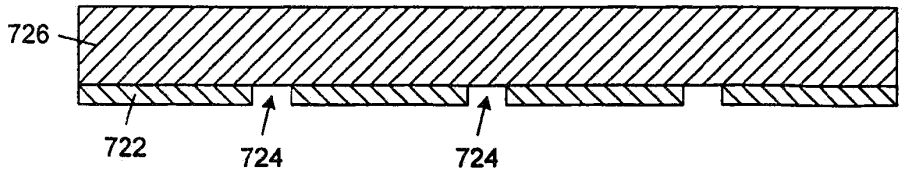


FIG. 25A

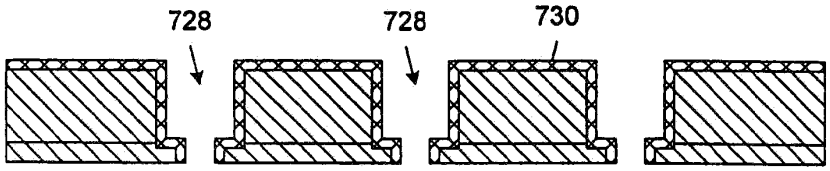


FIG. 25B

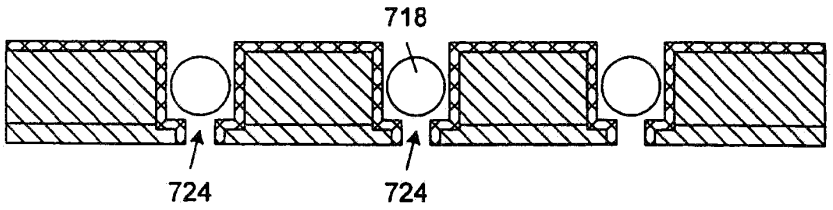


FIG. 25C

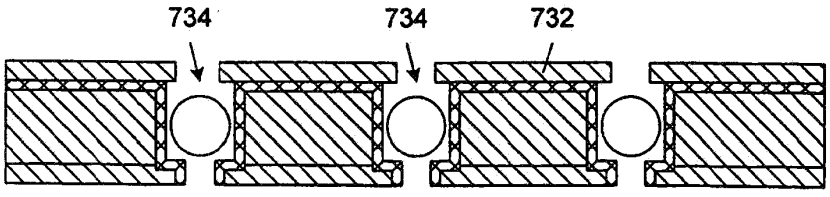


FIG. 25D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2872

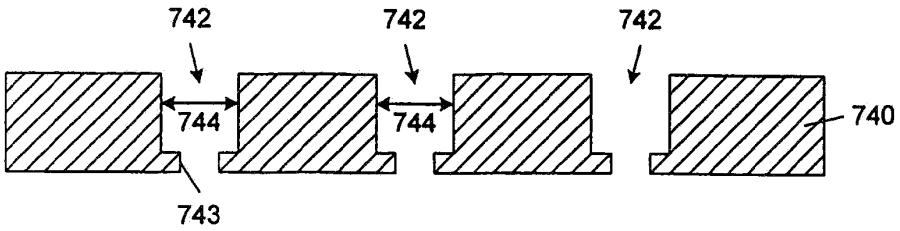


FIG. 26A

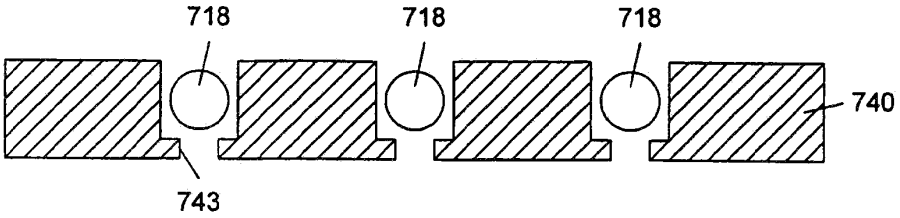


FIG. 26B

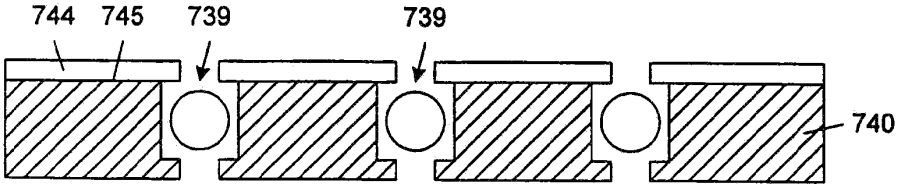


FIG. 26C

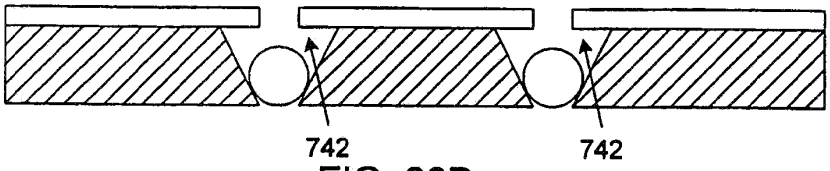


FIG. 26D

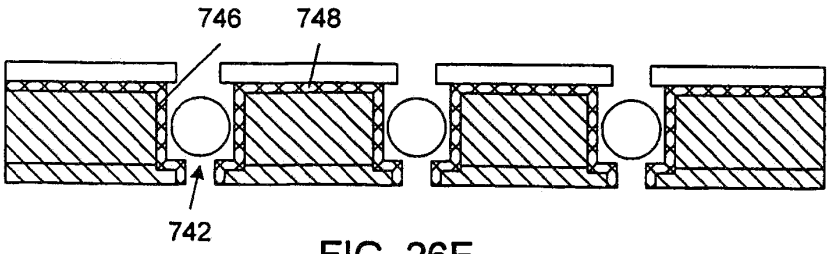


FIG. 26E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2873

34/44

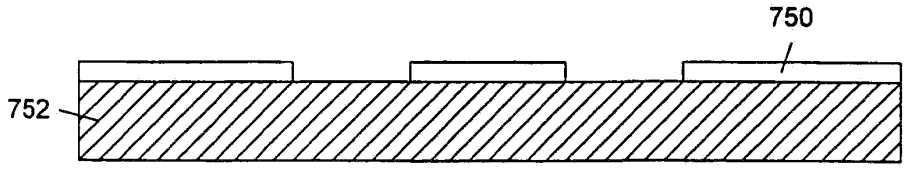


FIG. 27A

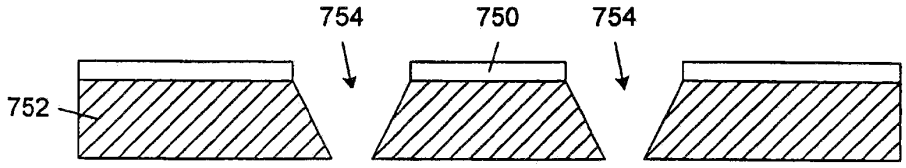


FIG. 27B

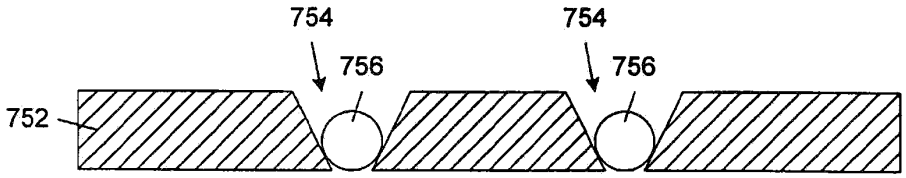


FIG. 27C

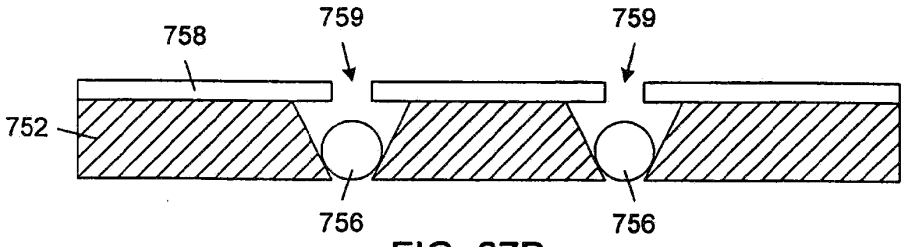


FIG. 27D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2874

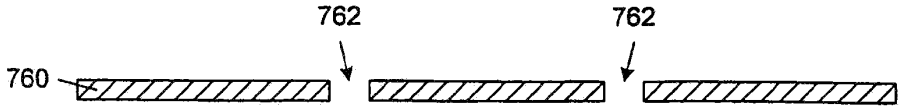


FIG. 28A

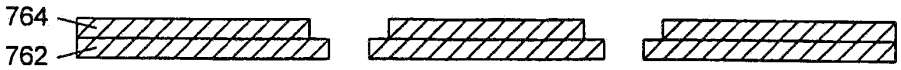


FIG. 28B

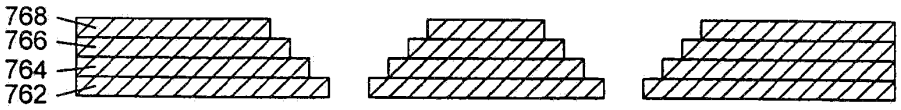


FIG. 28C

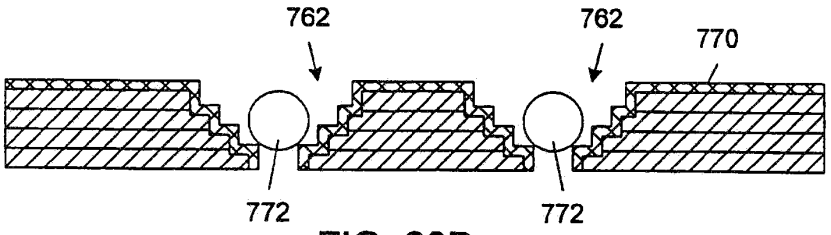


FIG. 28D

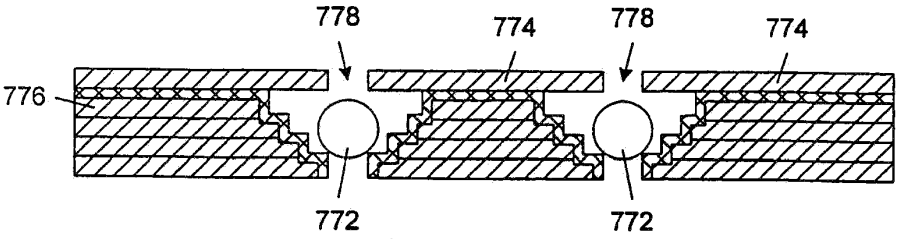


FIG. 28E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2875

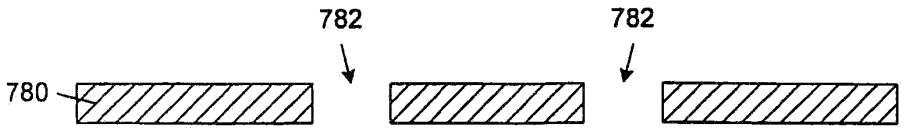


FIG. 29A

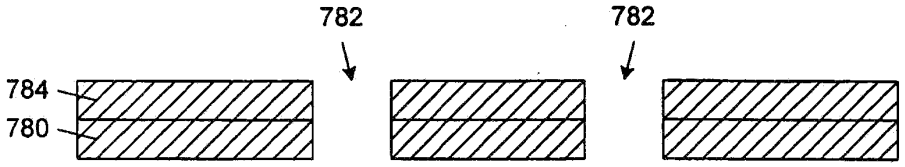


FIG. 29B

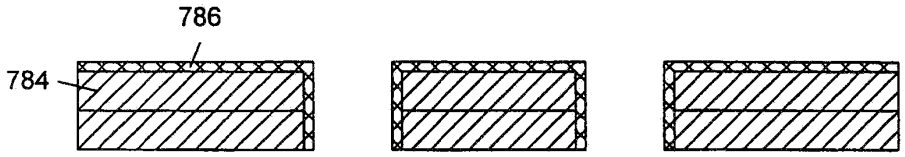


FIG. 29C

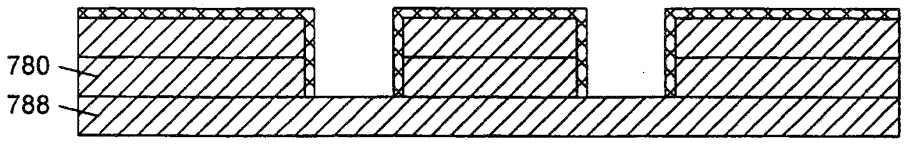


FIG. 29D

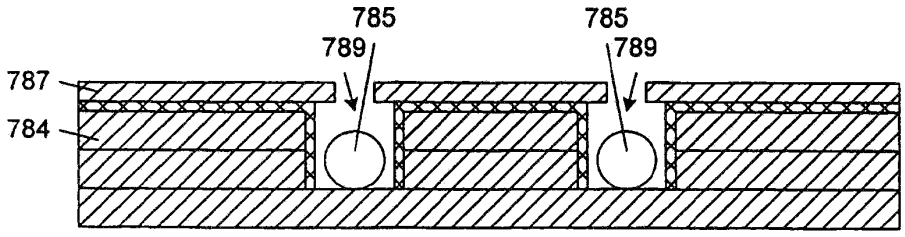


FIG. 29E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2876

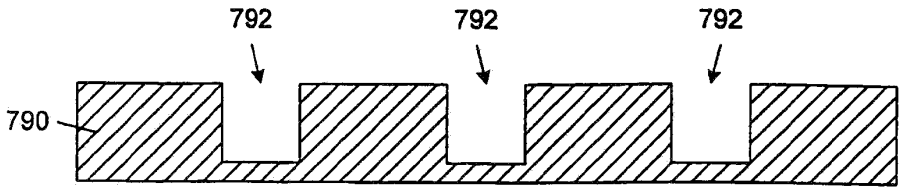


FIG. 30A

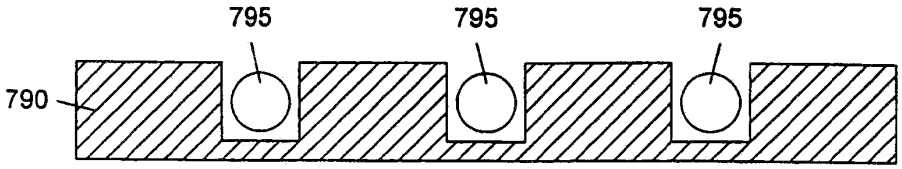


FIG. 30B

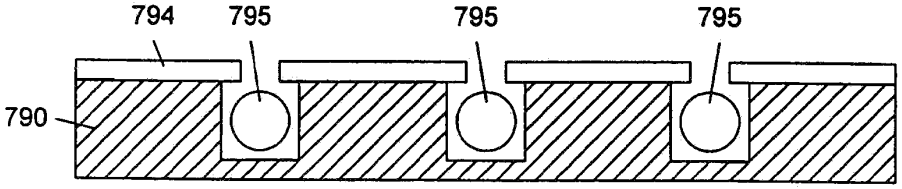


FIG. 30C

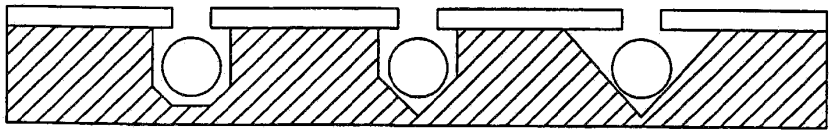


FIG. 30D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2877

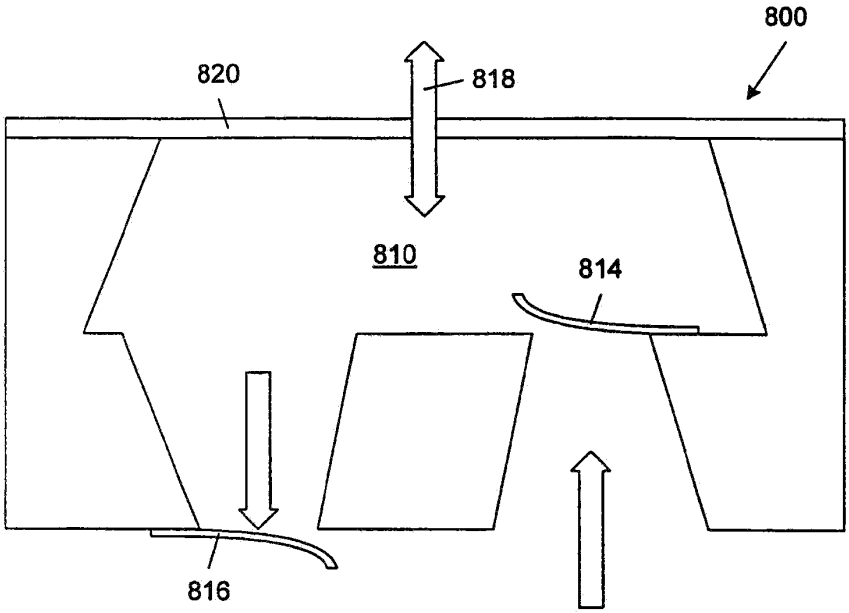


FIG. 31

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2878

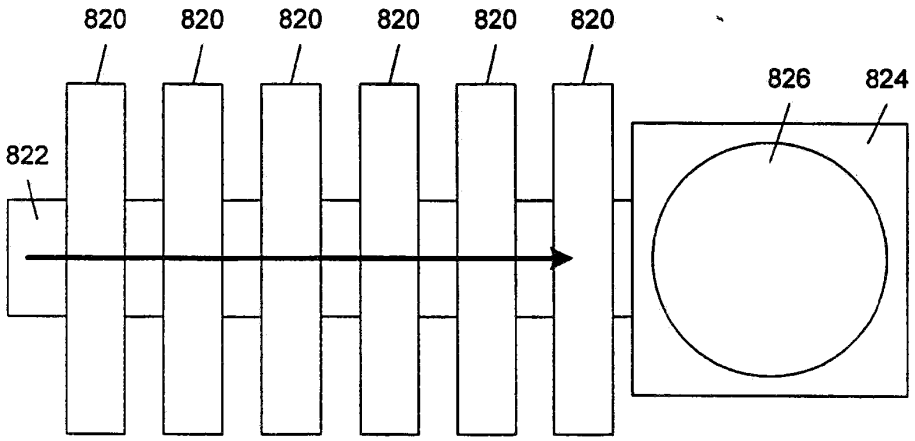


FIG. 32

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2879

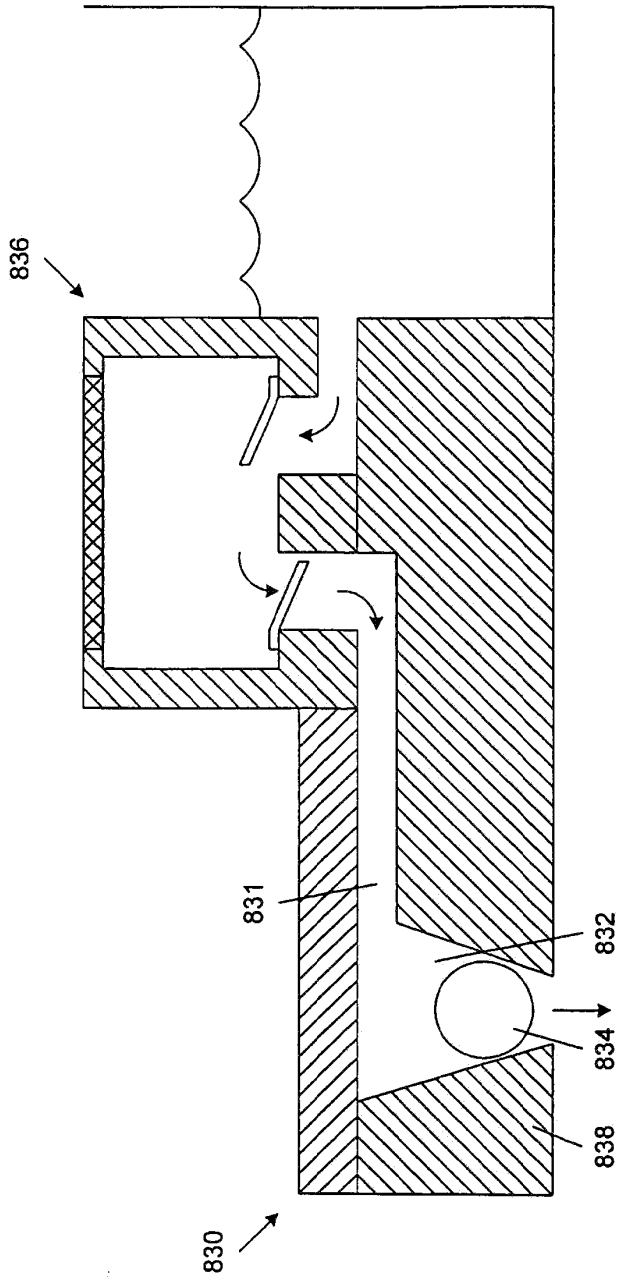


FIG. 33

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2880

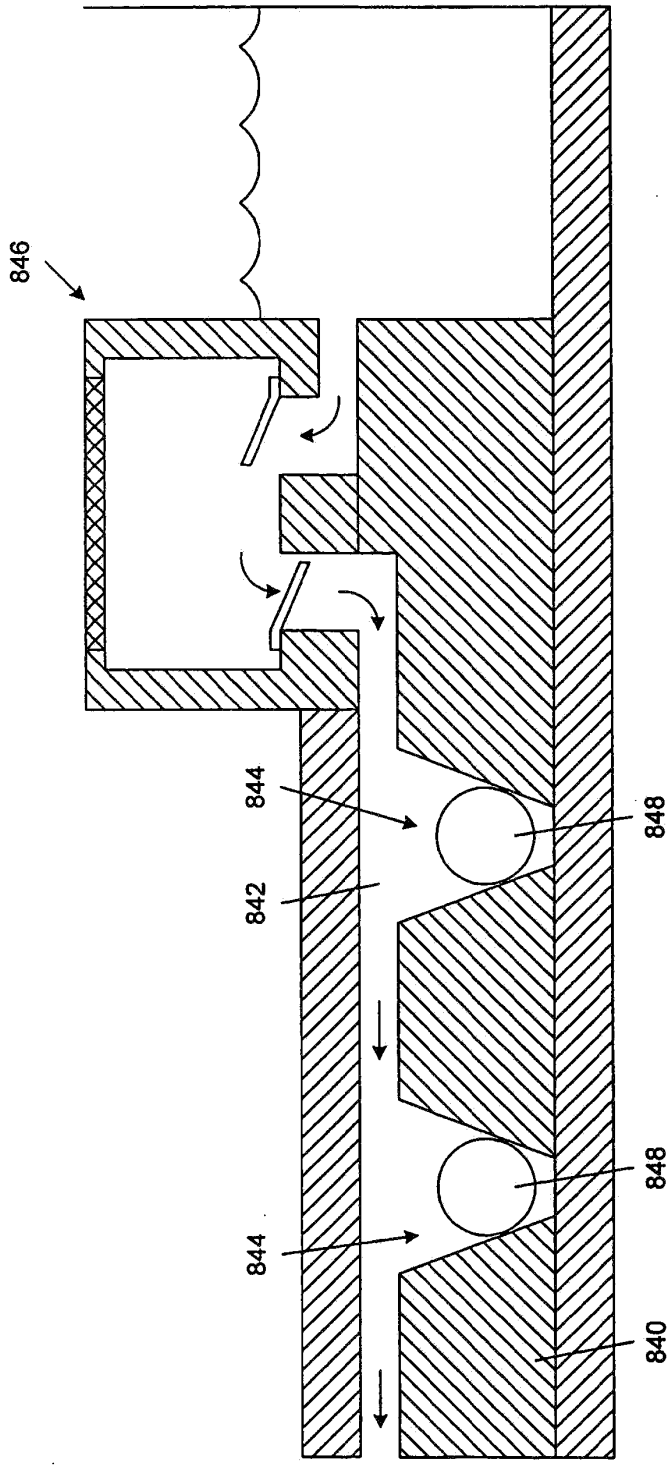


FIG. 34

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2881

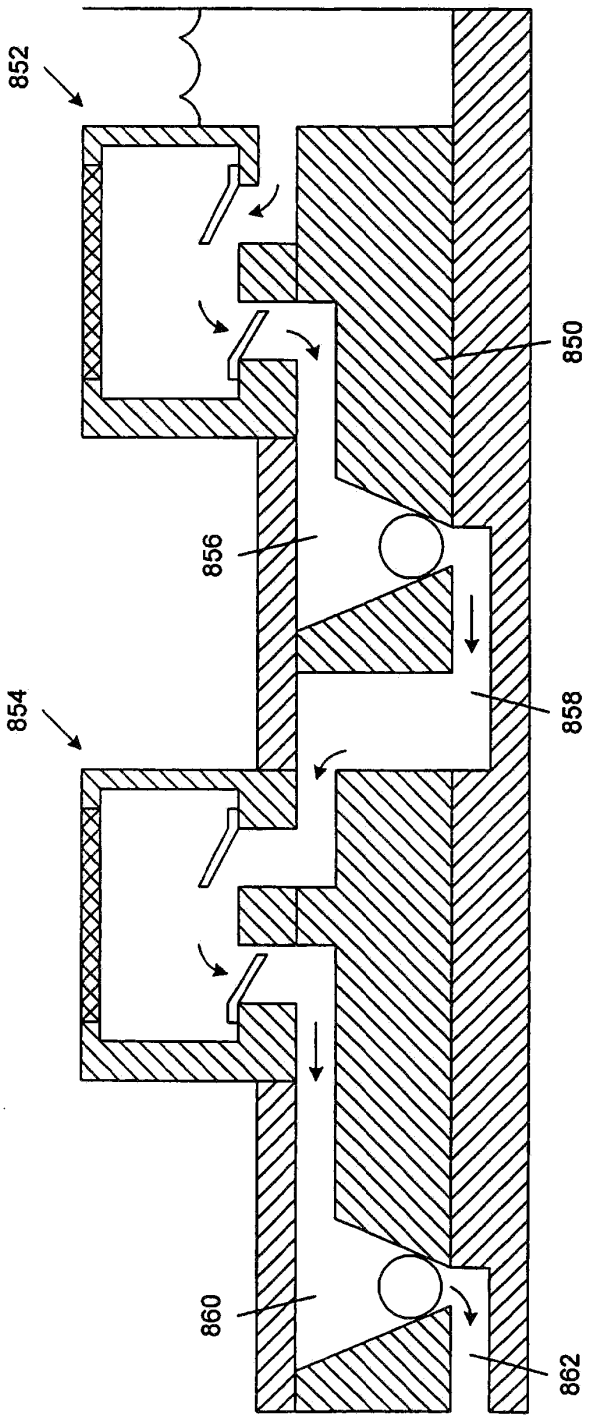


FIG. 35

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2882

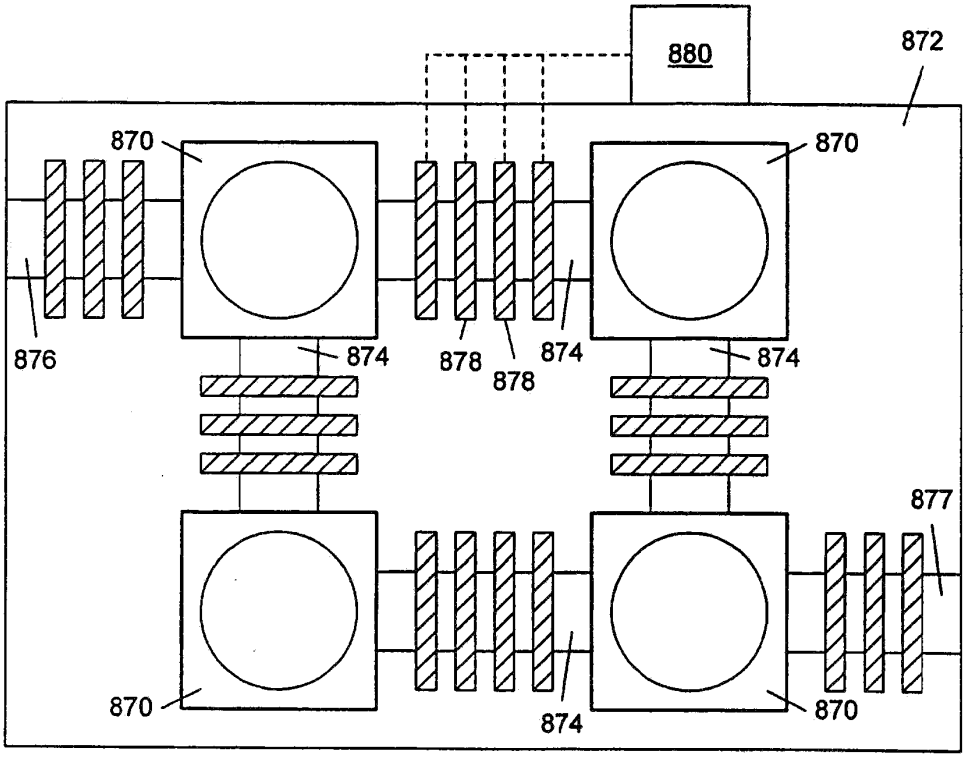


FIG. 36

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2883

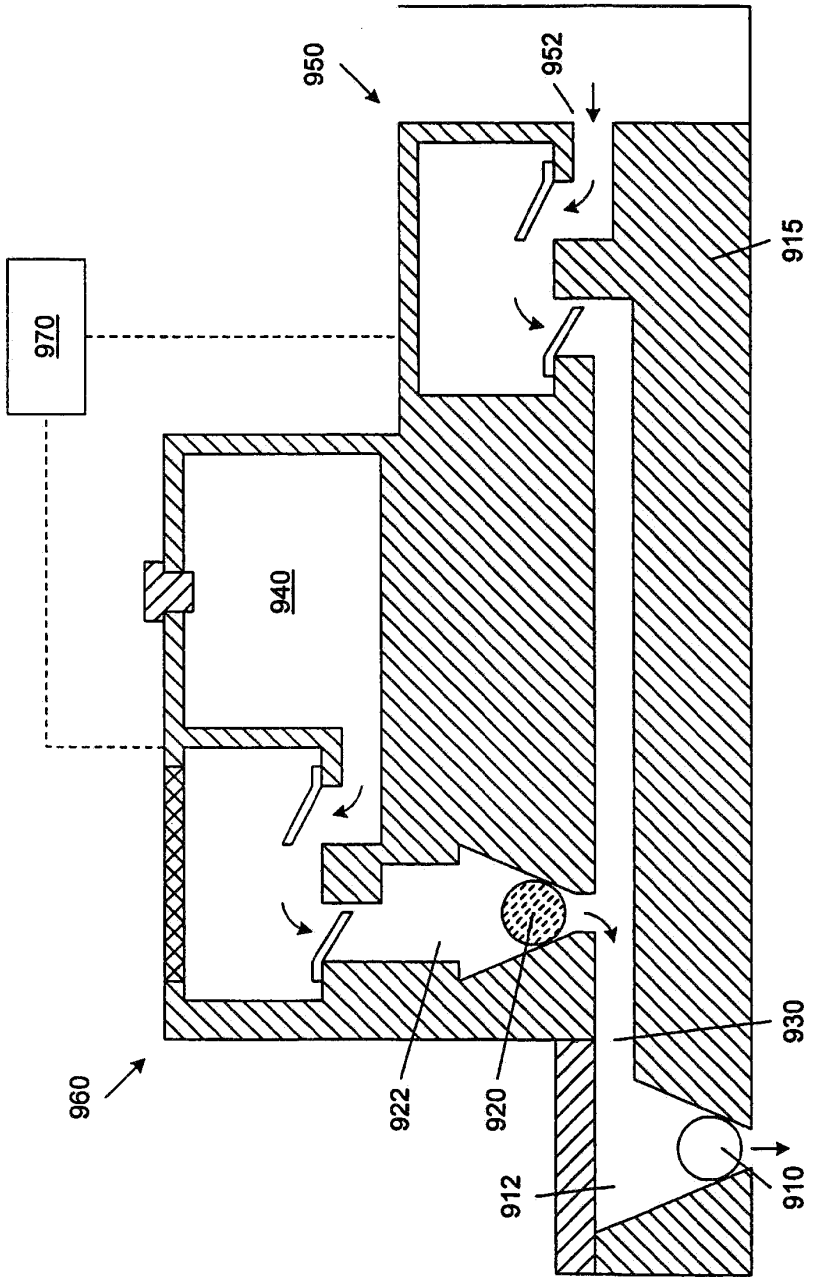


FIG. 37

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2884

INTERNATIONAL SEARCH REPORT

International Application No
F /US 99/16162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64 G01N21/77

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)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 99 18434 A (TUFTS COLLEGE ;WALT DAVID R (US); DICKINSON TODD A (US)) 15 April 1999 (1999-04-15) page 8, line 28 -page 9, line 7 page 9, line 13 - line 21 page 10, line 23 - line 35 page 11, line 2 - line 4 page 12, line 5 - line 9 page 13, line 8 - line 17 -/--	1-5,10, 11,14, 16-20, 23-28, 30-33, 35,36, 38-42, 48,49, 51-59, 61-64, 66-74, 84,95, 106-108

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 1999	26/11/1999

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Navas Montero, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2885

INTERNATIONAL SEARCH REPORT

International Application No
P../US 99/16162

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page 15, line 11 - line 17 page 16, line 31 - line 38 page 17, line 16 - line 19 page 17, line 23 - line 37 page 19, line 9 - line 16 page 19, line 24 - line 27 page 20, line 22 - line 30 page 21, line 3 - line 12 page 24, line 6 - line 20 page 25, line 24 - line 30 page 26, line 12 - line 18 page 27, line 2 - line 5 page 27, line 6 - line 14 page 29, line 16 - line 18 page 30, line 31 - line 36 table 3 page 33, line 10 -page 34, line 2 page 36, line 28 - line 34 figures 5-8 ---	
X,P	WO 98 53300 A (BRENNER SYDNEY ;LYNX THERAPEUTICS INC (US); BRIDGHAM JOHN (US); CO) 26 November 1998 (1998-11-26) page 2, line 6 - line 29 page 3, line 31 - line 36 page 5, line 18 - line 33 page 6, line 1 - line 5 page 6, line 13 - line 17 page 7, line 20 - line 23 page 7, line 27 - line 31 page 10, line 10 - line 14 page 11, line 36 -page 12, line 9 page 22, line 22 - line 28 figures 1-4 ---	1,6-9, 12,13, 40, 43-47, 50,69, 70,83, 95,96, 105
X,P	WO 98 40726 A (TUFTS COLLEGE ;MICHAEL KARRI C (US); WALT DAVID R (US)) 17 September 1998 (1998-09-17) page 5, line 18 - line 31 page 6, line 24 - line 27 page 7, line 5 - line 15 page 19, line 15 -page 20, line 26 figures 4-7 --- -/--	1,69, 84-86, 89, 93-95, 97,100, 101, 106-108

1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2886

INTERNATIONAL SEARCH REPORT

International Application No
P../US 99/16162

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 35189 A (UNIV WASHINGTON) 25 September 1997 (1997-09-25) page 2, line 19 - line 22 page 9, line 15 - line 18 page 8, line 26 - line 29 page 10, line 19 - line 27 figures 1,4,5 ---	69,75, 76,81, 103
X,P	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 03, 31 March 1999 (1999-03-31) & JP 10 332593 A (HITACHI LTD), 18 December 1998 (1998-12-18) abstract ---	95,99, 104
X,P	WO 99 17139 A (CLARK HEATHER ;UNIV MICHIGAN (US); BARKER SUSAN (US); KOPELMAN ' RAO) 8 April 1999 (1999-04-08) page 3, line 23 -page 4, line 2 page 9, line 20 - line 26 figures 1,3 ---	1,40,69
X,P	US 5 866 430 A (GROW ANN E) 2 February 1999 (1999-02-02) column 1, line 11 - line 19 column 1, line 36 - line 46 column 18, line 27 - line 50 column 19, line 54 -column 20, line 11 column 20, line 51 - line 57 column 21, line 17 - line 25 column 21, line 52 - line 60 column 43, line 28 - line 56 column 51, line 25 - line 41 figures 1,5,6 -----	1,40,95

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2887

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/16162

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 115-412
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2888

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99/16162

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 115-412

In view of the large number of claims presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely 1-114.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P../US 99/16162

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9918434 A	15-04-1999	AU 1269599 A	27-04-1999
WO 9853300 A	26-11-1998	AU 7715598 A	11-12-1998
WO 9840726 A	17-09-1998	AU 6464898 A	29-09-1998
WO 9735189 A	25-09-1997	US 5747349 A AU 4000197 A	05-05-1998 10-10-1997
JP 10332593 A	18-12-1998	NONE	
WO 9917139 A	08-04-1999	AU 9580798 A	23-04-1999
US 5866430 A	02-02-1999	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :		(11) International Publication Number:
G01N 33/50	A2	WO 00/47996
		(43) International Publication Date: 17 August 2000 (17.08.00)

(21) International Application Number: PCT/US00/03375

(22) International Filing Date: 9 February 2000 (09.02.00)

(30) Priority Data:
60/119,323 9 February 1999 (09.02.99) US

(71) Applicant: ILLUMINA, INC. [US/US]; Suite 200, 9390 Towne Centre Drive, San Diego, CA 92121 (US).

(72) Inventors: STUELPNAGEL, John, R.; 38 Briggs Avenue, Encinitas, CA 92024 (US). CHEE, Mark, S.; 155 15th Street #24, Del Mar, CA 92014 (US). DICKINSON, Todd, A.; Apartment 1133, 3435 Lebon Drive, San Diego, CA 92122 (US). GUNDERSON, Kevin; 1543 Juniper Hill Drive, Encinitas, CA 92024 (US).

(74) Agents: BREZNER, David, J. et al.; Flehr Hohbach Test Albritton & Herbert LLP, Suite 3400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: AUTOMATED INFORMATION PROCESSING IN RANDOMLY ORDERED ARRAYS

(57) Abstract

The invention relates to the use of a computer system to compare images generated from a randomly ordered array. This system preserves the relative position of each site within the array so that the same site can be compared in different images.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

AUTOMATED INFORMATION PROCESSING IN RANDOMLY ORDERED ARRAYS

FIELD OF THE INVENTION

The invention relates to the use of a computer system to compare images generated from a randomly ordered array. This system preserves the relative position of each site within the array so that the same site can be compared in different images.

BACKGROUND OF THE INVENTION

There are a number of assays and sensors for the detection of the presence and/or concentration of specific substances in fluids and gases. Many of these rely on specific ligand/antiligand reactions as the mechanism of detection. That is, pairs of substances (i.e. the binding pairs or ligand/antiligands) are known to bind to each other, while binding little or not at all to other substances. This has been the focus of a number of techniques that utilize these binding pairs for the detection of the complexes. These generally are done by labeling one component of the complex in some way, so as to make the entire complex detectable, using, for example, radioisotopes, fluorescent and other optically active molecules, enzymes, etc.

Of particular use in these sensors are detection mechanisms utilizing luminescence or fluorescence. Recently, the use of optical fibers and optical fiber strands in combination with light absorbing dyes for chemical analytical determinations has undergone rapid development, particularly within the last decade. The use of optical fibers for such purposes and techniques is described by Milanovich et al., "Novel Optical Fiber Techniques For Medical Application", Proceedings of the SPIE 28th Annual International Technical Symposium On Optics and Electro-Optics, Volume 494, 1980; Seitz, W.R., "Chemical Sensors Based On Immobilized Indicators and Fiber Optics" in *C.R.C. Critical Reviews In Analytical Chemistry*, Vol. 19, 1988, pp. 135-173; Wolfbeis, O.S., "Fiber Optical Fluorosensors In Analytical Chemistry" in *Molecular Luminescence Spectroscopy, Methods and Applications* (S. G.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2893

Schulman, editor), Wiley & Sons, New York (1988); Angel, S.M., *Spectroscopy 2* (4):38 (1987); Walt, et al., "Chemical Sensors and Microinstrumentation", *ACS Symposium Series*, Vol. 403, 1989, p. 252, and Wolfbeis, O.S., *Fiber Optic Chemical Sensors*, Ed. CRC Press, Boca Raton, FL, 1991, 2nd Volume.

When using an optical fiber in an *in vitro/in vivo* sensor, one or more light absorbing dyes are located near its distal end. Typically, light from an appropriate source is used to illuminate the dyes through the fiber's proximal end. The light propagates along the length of the optical fiber; and a portion of this propagated light exits the distal end and is absorbed by the dyes. The light absorbing dye may or may not be immobilized; may or may not be directly attached to the optical fiber itself; may or may not be suspended in a fluid sample containing one or more analytes of interest; and may or may not be retainable for subsequent use in a second optical determination.

Once the light has been absorbed by the dye, some light of varying wavelength and intensity returns, conveyed through either the same fiber or collection fiber(s) to a detection system where it is observed and measured. The interactions between the light conveyed by the optical fiber and the properties of the light absorbing dye provide an optical basis for both qualitative and quantitative determinations.

Of the many different classes of light absorbing dyes which conventionally are employed with bundles of fiber strands and optical fibers for different analytical purposes are those more common compositions that emit light after absorption termed "fluorophores" and those which absorb light and internally convert the absorbed light to heat, rather than emit it as light, termed "chromophores."

Fluorescence is a physical phenomenon based upon the ability of some molecules to absorb light (photons) at specified wavelengths and then emit light of a longer wavelength and at a lower energy. Substances able to fluoresce share a number of common characteristics: the ability to absorb light energy at one wavelength λ_{ab}; reach an excited energy state; and subsequently emit light at another light wavelength, λ_{em}. The absorption and fluorescence emission spectra are individual for each fluorophore and are often graphically represented as two separate curves that are slightly overlapping. The same fluorescence emission spectrum is generally observed irrespective of the wavelength of the exciting light and, accordingly, the wavelength and energy of the exciting light may be varied within limits; but the light emitted by the fluorophore will always provide the same emission spectrum. Finally, the strength of the fluorescence signal may be measured as the quantum yield of light emitted. The fluorescence quantum yield is the ratio of the number of photons emitted in comparison to the number of photons initially absorbed by the fluorophore. For more detailed information regarding each of these characteristics, the following references are recommended: Lakowicz, J. R., *Principles of Fluorescence Spectroscopy*, Plenum Press, New York, 1983; Freifelder, D., Physical Biochemistry,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2894

second edition, W. H. Freeman and Company, New York, 1982; "Molecular Luminescence Spectroscopy Methods and Applications: Part I" (S.G. Schulman, editor) in *Chemical Analysis*, vol. 77, Wiley & Sons, Inc., 1985; *The Theory of Luminescence*, Stepanov and Gribkovskii, Iliffe Books, Ltd., London, 1968.

In comparison, substances which absorb light and do not fluoresce usually convert the light into heat or kinetic energy. The ability to internally convert the absorbed light identifies the dye as a "chromophore." Dyes which absorb light energy as chromophores do so at individual wavelengths of energy and are characterized by a distinctive molar absorption coefficient at that wavelength. Chemical analysis employing fiber optic strands and absorption spectroscopy using visible and ultraviolet light wavelengths in combination with the absorption coefficient allow for the determination of concentration for specific analyses of interest by spectral measurement. The most common use of absorbance measurement via optical fibers is to determine concentration which is calculated in accordance with Beers' law; accordingly, at a single absorbance wavelength, the greater the quantity of the composition which absorbs light energy at a given wavelength, the greater the optical density for the sample. In this way, the total quantity of light absorbed directly correlates with the quantity of the composition in the sample.

Many of the recent improvements employing optical fiber sensors in both qualitative and quantitative analytical determinations concern the desirability of depositing and/or immobilizing various light absorbing dyes at the distal end of the optical fiber. In this manner, a variety of different optical fiber chemical sensors and methods have been reported for specific analytical determinations and applications such as pH measurement, oxygen detection, and carbon dioxide analyses. These developments are exemplified by the following publications: Freeman, et al., *Anal Chem.* 53:98 (1983); Lippitsch et al., *Anal. Chem. Acta.* 205:1, (1988); Wolfbeis et al., *Anal. Chem.* 60:2028 (1988); Jordan, et al., *Anal. Chem.* 59:437 (1987); Lubbers et al., *Sens. Actuators* 1983; Munkholm et al., *Talanta* 35:109 (1988); Munkholm et al., *Anal. Chem.* 58:1427 (1986); Seitz, W. R., *Anal. Chem.* 56:16A-34A (1984); Peterson, et al., *Anal. Chem.* 52:864 (1980): Saari, et al., *Anal. Chem.* 54:821 (1982); Saari, et al., *Anal. Chem.* 55:667 (1983); Zhujun et al., *Anal. Chem. Acta.* 160:47 (1984); Schwab, et al., *Anal. Chem.* 56:2199 (1984); Wolfbeis, O.S., "Fiber Optic Chemical Sensors", *Ed. CRC Press, Boca Raton, FL*, 1991, 2nd Volume; and Pantano, P., Walt, D.R., *Anal. Chem.*, 481A-487A, Vol. 67, (1995).

More recently, fiber optic sensors have been constructed that permit the use of multiple dyes with a single, discrete fiber optic bundle. U.S. Pat. Nos. 5,244,636 and 5,250,264 to Walt, *et al.* disclose systems for affixing multiple, different dyes on the distal end of the bundle, the teachings of each of these patents being incorporated herein by this reference. The disclosed configurations enable separate optical fibers of the bundle to optically access individual dyes. This avoids the problem of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2895

deconvolving the separate signals in the returning light from each dye, which arises when the signals from two or more dyes are combined, each dye being sensitive to a different analyte, and there is significant overlap in the dyes' emission spectra.

U.S.S.N.s 08/818,199 and 09/151,877 describe array compositions that utilize microspheres or beads on a surface of a substrate, for example on a terminal end of a fiber optic bundle, with each individual fiber comprising a bead containing an optical signature. Since the beads go down randomly, a unique optical signature is needed to "decode" the array; i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art. Once the array is loaded with the beads, the array can be decoded, or can be used, with full or partial decoding occuring after testing, as is more fully outlined below.

The use of fiducials for the registration of sequential images has been used in screen printing (U.S. Patent No. 5,129,155) and in implants in the human body (U.S. Patent No. 4,991,579) and in various image processing (see U.S. Patent Nos. 5,245,676 and 5,129,014).

Accordingly, it is an object of the present invention to provide biosensors comprising random arrays, generally comprising beads distributed at discrete sites on the surface of a substrate, that utilize computer systems and fiducials to allow comparison of sequential data images of the arrays.

SUMMARY OF THE INVENTION

In accordance with the objects outlined above, the present invention provides array compositions comprising a substrate with a surface comprising discrete sites, at least one fiducial, and a population of microspheres comprising at least a first and a second subpopulation. Each subpopulation comprises a bioactive agent, and the microspheres are distributed on said surface. Each subpopulation may optionally comprise a unique optical signature, an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated, or both.

In an additional aspect, the invention provides compositions comprising a computer readable memory to direct a computer to function in a specified manner. The computer readable memory comprises an acquisition module for receiving a data image of a random array comprising a plurality of discrete sites, a registration module for registering a data image, and a comparison module for comparing registered data images. Each module comprises computer code for carrying out its function. The

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2896

registration module may utilize any number of fiducials, including a fiducial fiber when the substrate comprises a fiber optic bundle, a fiducial microsphere, or a fiducial template generated from the random array.

In a further aspect, the invention provides methods of making the array compositions of the invention comprising forming a surface comprising individual sites on a substrate, distributing microspheres on the surface such that the individual sites contain microspheres, and incorporating at least one fiducial onto the surface. When the array has complete rotational freedom, at least two fiducials are preferred in the array to allow for correction of rotation.

In an additional aspect, the invention provides methods for comparing separate data images of a random array. The methods comprise using a computer system to register a first data image of the random array to produce a registered first data image, using the computer system to register a second data image of the random array to produce a registered second data image, and comparing the first and the second registered data images to determine any differences between them.

In a further aspect, the invention provides methods of decoding a random array composition comprising providing a random array composition as outlined herein. A first plurality of decoding binding ligands is added to the array composition and a first data image is created. A fiducial is used to generate a first registered data image. A second plurality of decoding binding ligands is added to the array composition and a second data image is created. The fiducial is used to generate a second registered data image. A computer system is used to compare the first and the second registered data image to identify the location of at least two bioactive agents.

In an additional aspect, the invention provides methods of determining the presence of a target analyte in a sample. The methods comprise acquiring a first data image of a random array composition, and registering the first data image to create a registered first data image. The sample is then added to the random array and a second data image is acquired from the array. The second data image is registered to create a registered second data image. Then the first and the second registered data images are compared to determine the presence or absence of the target analyte. Optionally, the data acquisition may be at different wavelengths.

FIGURES

Figure 1 illustrates a fiber optic bundle with fiducial fibers.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2897

Figure 2 illustrates the components of a multi-multi fiber including fiducial markers, optical fiber bundles (multi fiber) and the components of a single optical fiber (mono fiber).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the use of randomly ordered arrays comprising a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities are distributed on a substrate comprising a patterned surface of discrete sites that can bind the individual microspheres. The beads are generally put onto the substrate randomly, i.e. each bead goes down arbitrarily or indescriminately on to a site. This allows the synthesis of the candidate agents (i.e. compounds such as nucleic acids and antibodies) to be divorced from their placement on an array, i.e. the candidate agents may be synthesized on the beads, or on a different substrate and then put onto the beads, and then the beads are randomly distributed on a patterned surface.

However, the random placement of the beads means that all or part of the array must be "decoded" after synthesis; that is, after the array is made, a correlation of the location of an individual site on the array with the bead or candidate agent at that particular site can be made. This encoding/decoding can be done in a number of ways, as is generally described in 60/090,473; 09/189,543; 08/944,850; 08/818,199; 09/151,877; and 08/851,203, all of which are hereby expressly incorporated by reference in their entirety. These methods include: (1) "encoding" the beads with unique optical signatures, generally fluorescent dyes, that can be used to identify the chemical functionality on any particular bead; (2) using a decoding binding ligand (DBL), generally directly labeled, that binds to either the bioactive agent or to identifier binding ligands (IBLs) attached to the beads; (3) the use of positional decoding, for example by either targeting the placement of beads (for example by using photoactivatible or photocleavable moieties to allow the selective addition of beads to particular locations), or by using either sub-bundles or selective loading of the sites, as are more fully outlined below; (4) the use of selective decoding, wherein only those beads that bind to a target are decoded; or (5) combinations of any of these. In some cases, as is more fully outlined below, this decoding may occur for all the beads, or only for those that bind a particular target analyte. Similarly, this may occur either prior to or after addition of a target analyte.

This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art.

Once the identity (i.e. the actual agent) and location of each microsphere in the array has been fixed, the array is exposed to samples containing the target analytes, although as outlined below, this can be

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2898

done prior to, during or after the assay as well. The target analytes will bind to the bioactive agents as is more fully outlined below, and results in a change in an optical signal of a particular bead.

The present invention is directed to compositions and methods that allow comparisons of sequential data images taken during decoding and assay analysis. That is, in the broadest sense, the invention provides computer systems comprising processors and computer readable memory that allow the storage and analysis of multiple captured images of the same array, whether to compare a decoding image and an experimental image, several experimental images or several decoding images. That is, a first data image is taken of a random array, and using either a fiducial template or an external fiducial, the data image is registered. A second data image is then taken and registered, and the two registered data images can now be compared, as is more fully outlined below.

In a preferred embodiment, the present invention provides a variety of "registration" techniques that allow the comparison of a variety of these images in a uniform and reliable way. That is, in order to compare multiple data images from an array comprising a plurality of unique sites, it is important that the correct individual sites be compared during analysis. In a highly complex and small system, methods are needed to ensure that a first site in a first data image is correctly matched to the first site in a second data image. Accordingly, the present invention provides the incorporation of one or more reference features, also referred to herein as "markers" or "fiducials" or "registration points", that allow this registration from image to image. It is generally preferred to have a number of spatially separated fiducials so that small amounts of skew and reduction/enlargement can be determined and taken into account.

As is further described below, these fiducials can take a number of forms. For example, when the random array comprises beads, the fiducial may be a bead with a unique optical signature or other characteristic (Figure 1). When the random array comprises a fiber optic bundle, the fiducial may be a fiber element with a unique shape or optical properties. Alternatively, the substrate may have other types of physical fiducials, such one or more defined edges that have characteristic optical properties that can be either spaced along the edge(s) or comprise the entire edge (Figure 2). Alternatively, the fiducials may be an inherent characteristic of the array; for example, small irregularities in the sites (features) of the array can be exploited to serve as fiducials, generating a "fiducial template".

Accordingly, the present invention provides random array compositions comprising at least a first substrate with a surface comprising individual sites. By "random" array herein is meant an array that is manufactured under conditions that results in the identification of the agent in at least some, if not all, of the sites of the array being initially unknown; that is, each agent is put down arbitrarily on a site of the array in a generally non-reproducible manner. What is important in random arrays, and what

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2899

makes the present invention so useful, is that random arrays generally require at least one, and generally several "decoding" steps that produce data images that must be compared. In addition, while the techniques of the invention can be used on a variety of random arrays, the discussion below is directed to the use of arrays comprising microspheres that are laid down randomly on a surface comprising discrete sites. However, as will be appreciated by those in the art, other types of random arrays, i.e. those not containing beads, may also utilize the methods of the invention.

By "array" herein is meant a plurality of candidate agents in an array format; the size of the array will depend on the composition and end use of the array. Arrays containing from about 2 different bioactive agents (i.e. different beads) to many millions can be made, with very large fiber optic arrays being possible. Generally, the array will comprise from two to as many as a billion or more, depending on the size of the beads and the substrate, as well as the end use of the array, thus very high density, high density, moderate density, low density and very low density arrays may be made. Preferred ranges for very high density arrays (all numbers are per cm^2) are from about 10,000,000 to about 2,000,000,000, with from about 100,000,000 to about 1,000,000,000 being preferred. High density arrays range about 100,000 to about 10,000,000, with from about 1,000,000 to about 5,000,000 being particularly preferred. Moderate density arrays range from about 10,000 to about 100,000 being particularly preferred, and from about 20,000 to about 50,000 being especially preferred. Low density arrays are generally less than 10,000, with from about 1,000 to about 5,000 being preferred. Very low density arrays are less than 1,000, with from about 10 to about 1000 being preferred, and from about 100 to about 500 being particularly preferred. In some embodiments, the compositions of the invention may not be in array format; that is, for some embodiments, compositions comprising a single bioactive agent may be made as well. In addition, in some arrays, multiple substrates may be used, either of different or identical compositions. Thus for example, large arrays may comprise a plurality of smaller substrates.

In addition, one advantage of the present compositions is that particularly through the use of fiber optic technology, extremely high density arrays can be made. Thus for example, because beads of 200 µm or less (with beads of 200 nm possible) can be used, and very small fibers are known, it is possible to have as many as 40,000 or more (in some instances, 1 million) different fibers and beads in a 1 mm^2 fiber optic bundle, with densities of greater than 25,000,000 individual beads and fibers (again, in some instances as many as 100 million) per 0.5 cm^2 obtainable.

By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of beads and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates is very large. Possible substrates include, but are not limited to,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2900

glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, Teflon ®, etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. In general, the substrates allow optical detection and do not themselves appreciably fluoresce.

Generally the substrate is flat (planar), although as will be appreciated by those in the art, other configurations of substrates may be used as well; for example, three dimensional configurations can be used, for example by embedding the beads in a porous block of plastic that allows sample access to the beads and using a confocal microscope for detection. Similarly, the beads may be placed on the inside surface of a tube, for flow-through sample analysis to minimize sample volume. Preferred substrates include optical fiber bundles as discussed below, and flat planar substrates such as glass, polystyrene and other plastics and acrylics.

In addition, as is more fully outlined below, the substrate may include a coating, edging or sheath of material, generally detectable, that defines a substrate edge that may serve as one or more fiducials.

In a preferred embodiment, the substrate is an optical fiber bundle or array, as is generally described in U.S.S.N.s 08/944,850 and 08/519,062, PCT US98/05025, and PCT US98/09163, all of which are expressly incorporated herein by reference. Preferred embodiments utilize preformed unitary fiber optic arrays. By "preformed unitary fiber optic array" herein is meant an array of discrete individual fiber optic strands that are co-axially disposed and joined along their lengths. The fiber strands are generally individually clad. However, one thing that distinguished a preformed unitary array from other fiber optic formats is that the fibers are not individually physically manipulatable without intentionally treating the preformed unitary array with agents that separate them, for example treating a preformed array susceptible to acid with an acid such that the interstitial material is etched and thus the individual cores can be separated. However, absent these intentional treatments, one strand generally cannot be physically separated at any point along its length from another fiber strand.

At least one surface of the substrate is modified to contain discrete, individual sites for later association of microspheres. These sites may also be referred to in some embodiments as "features". These sites may comprise physically altered sites, i.e. physical configurations such as wells or small depressions in the substrate that can retain the beads, such that a microsphere can rest in the well, or the use of other forces (magnetic or compressive), or chemically altered or active sites, such as chemically functionalized sites, electrostatically altered sites, hydrophobically/ hydrophilically functionalized sites, spots of adhesive, etc.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2901

The sites may be a pattern, i.e. a regular design or configuration, or randomly distributed. A preferred embodiment utilizes a regular pattern of sites such that the sites may be addressed in the X-Y coordinate plane. "Pattern" in this sense includes a repeating unit cell, preferably one that allows a high density of beads on the substrate. However, it should be noted that these sites may not be discrete sites. That is, it is possible to use a uniform surface of adhesive or chemical functionalities, for example, that allows the attachment of beads at any position. That is, the surface of the substrate is modified to allow attachment of the microspheres at individual sites, whether or not those sites are contiguous or non-contiguous with other sites. Thus, the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead, or alternatively, the surface of the substrate is modified and beads may go down anywhere, but they end up at discrete sites.

In a preferred embodiment, the surface of the substrate is modified to contain wells, i.e. depressions in the surface of the substrate. This may be done as is generally known in the art using a variety of techniques, including, but not limited to, photolithography, stamping techniques, molding techniques and microetching techniques. As will be appreciated by those in the art, the technique used will depend on the composition and shape of the substrate.

In a preferred embodiment, physical alterations are made in a surface of the substrate to produce the sites. In a preferred embodiment, the substrate is a fiber optic bundle and the surface of the substrate is a terminal end of the fiber bundle, as is generally described in 08/818,199 and 09/151,877, both of which are hereby expressly incorporated by reference. In this embodiment, wells are made in a terminal or distal end of a fiber optic bundle comprising individual fibers. In this embodiment, the cores of the individual fibers are etched, with respect to the cladding, such that small wells or depressions are formed at one end of the fibers. The required depth of the wells will depend on the size of the beads to be added to the wells.

Generally in this embodiment, the microspheres are non-covalently associated in the wells, although the wells may additionally be chemically functionalized as is generally described below, cross-linking agents may be used, or a physical barrier may be used, i.e. a film or membrane over the beads.

In a preferred embodiment, the surface of the substrate is modified to contain chemically modified sites, that can be used to attach, either covalently or non-covalently, the microspheres of the invention to the discrete sites or locations on the substrate. "Chemically modified sites" in this context includes, but is not limited to, the addition of a pattern of chemical functional groups including amino groups, carboxy groups, oxo groups and thiol groups, that can be used to covalently attach microspheres, which generally also contain corresponding reactive functional groups; the addition of a pattern of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2902

adhesive that can be used to bind the microspheres (either by prior chemical functionalization for the addition of the adhesive or direct addition of the adhesive); the addition of a pattern of charged groups (similar to the chemical functionalities) for the electrostatic attachment of the microspheres, i.e. when the microspheres comprise charged groups opposite to the sites; the addition of a pattern of chemical functional groups that renders the sites differentially hydrophobic or hydrophilic, such that the addition of similarly hydrophobic or hydrophilic microspheres under suitable experimental conditions will result in association of the microspheres to the sites on the basis of hydroaffinity. For example, the use of hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, "pattern" in this sense includes the use of a uniform treatment of the surface to allow attachment of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be appreciated by those in the art, this may be accomplished in a variety of ways.

In a preferred embodiment, the compositions of the invention further comprise a population of microspheres. By "population" herein is meant a plurality of beads as outlined above for arrays. Within the population are separate subpopulations, which can be a single microsphere or multiple identical microspheres. That is, in some embodiments, as is more fully outlined below, the array may contain only a single bead for each bioactive agent; preferred embodiments utilize a plurality of beads of each type.

By "microspheres" or "beads" or "particles" or grammatical equivalents herein is meant small discrete particles. The composition of the beads will vary, depending on the class of bioactive agent and the method of synthesis. Suitable bead compositions include those used in peptide, nucleic acid and organic moiety synthesis, including, but not limited to, plastics, ceramics, glass, polystyrene, methylstyrene, acrylic polymers, paramagnetic materials, thoria sol, carbon graphited, titanium dioxide, latex or cross-linked dextrans such as Sepharose, cellulose, nylon, cross-linked micelles and teflon may all be used. *"Microsphere Detection Guide"* from Bangs Laboratories, Fishers IN is a helpful guide.

The beads need not be spherical; irregular particles may be used. In addition, the beads may be porous, thus increasing the surface area of the bead available for either bioactive agent attachment or tag attachment. The bead sizes range from nanometers, i.e. 100 nm, to millimeters, i.e. 1 mm, with beads from about 0.2 micron to about 200 microns being preferred, and from about 0.5 to about 5 micron being particularly preferred, although in some embodiments larger or smaller beads may be used.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2903

It should be noted that a key component of the invention is the use of a substrate/bead pairing that allows the association or attachment of the beads at discrete sites on the surface of the substrate, such that the beads do not move during the course of the assay.

Each microsphere comprises a bioactive agent, although as will be appreciated by those in the art, there may be some microspheres which do not contain a bioactive agent, depending the on the synthetic methods. By "candidate bioactive agent" or "bioactive agent" or "chemical functionality" or "binding ligand" herein is meant as used herein describes any molecule, *e.g.*, protein, oligopeptide, small organic molecule, coordination complex, polysaccharide, polynucleotide, etc. which can be attached to the microspheres of the invention. It should be understood that the compositions of the invention have two primary uses. In a preferred embodiment, as is more fully outlined below, the compositions are used to detect the presence of a particular target analyte; for example, the presence or absence of a particular nucleotide sequence or a particular protein, such as an enzyme, an antibody or an antigen. In an alternate preferred embodiment, the compositions are used to screen bioactive agents, i.e. drug candidates, for binding to a particular target analyte.

Bioactive agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Bioactive agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The bioactive agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Bioactive agents are also found among biomolecules including peptides, nucleic acids, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Particularly preferred are nucleic acids and proteins.

Bioactive agents can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification and/or amidification to produce structural analogs.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2904

In a preferred embodiment, the bioactive agents are proteins. By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and norleucine are considered amino acids for the purposes of the invention. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradations.

In one preferred embodiment, the bioactive agents are naturally occurring proteins or fragments of naturally occuring proteins. Thus, for example, cellular extracts containing proteins, or random or directed digests of proteinaceous cellular extracts, may be used. In this way libraries of procaryotic and eukaryotic proteins may be made for screening in the systems described herein. Particularly preferred in this embodiment are libraries of bacterial, fungal, viral, and mammalian proteins, with the latter being preferred, and human proteins being especially preferred.

In a preferred embodiment, the bioactive agents are peptides of from about 5 to about 30 amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to about 15 being particularly preferred. The peptides may be digests of naturally occurring proteins as is outlined above, random peptides, or "biased" random peptides. By "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus forming a library of randomized bioactive proteinaceous agents.

In a preferred embodiment, a library of bioactive agents are used. The library should provide a sufficiently structurally diverse population of bioactive agents to effect a probabilistically sufficient range of binding to target analytes. Accordingly, an interaction library must be large enough so that at least one of its members will have a structure that gives it affinity for the target analyte. Although it is difficult to gauge the required absolute size of an interaction library, nature provides a hint with the immune response: a diversity of 10^7-10^8 different antibodies provides at least one combination with sufficient affinity to interact with most potential antigens faced by an organism. Published in vitro selection techniques have also shown that a library size of 10^7 to 10^8 is sufficient to find structures with affinity for the target. Thus, in a preferred embodiment, at least 10^6, preferably at least 10^7, more

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2905

preferably at least 10^8 and most preferably at least 10^9 different bioactive agents are simultaneously analyzed in the subject methods. Preferred methods maximize library size and diversity.

In a preferred embodiment, the library is fully randomized, with no sequence preferences or constants at any position. In a preferred embodiment, the library is biased. That is, some positions within the sequence are either held constant, or are selected from a limited number of possibilities. For example, in a preferred embodiment, the nucleotides or amino acid residues are randomized within a defined class, for example, of hydrophobic amino acids, hydrophilic residues, sterically biased (either small or large) residues, towards the creation of cysteines, for cross-linking, prolines for SH-3 domains, serines, threonines, tyrosines or histidines for phosphorylation sites, etc., or to purines, etc.

In a preferred embodiment, the bioactive agents are nucleic acids (generally called "probe nucleic acids" or "candidate probes" herein). By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage, *et al.*, Tetrahedron, **49**(10):1925 (1993) and references therein; Letsinger, J. Org. Chem., **35**:3800 (1970); Sprinzl, *et al.*, Eur. J. Biochem., **81**:579 (1977); Letsinger, *et al.*, Nucl. Acids Res., **14**:3487 (1986); Sawai, *et al.*, Chem. Lett., 805 (1984), Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); and Pauwels, *et al.*, Chemica Scripta, **26**:141 (1986)), phosphorothioate (Mag, *et al.*, Nucleic Acids Res., **19**:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu, *et al.*, J. Am. Chem. Soc., **111**:2321 (1989)), O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem. Soc., **114**:1895 (1992); Meier, *et al.*, Chem. Int. Ed. Engl., **31**:1008 (1992); Nielsen, Nature, **365**:566 (1993); Carlsson, *et al.*, Nature, **380**:207 (1996), all of which are incorporated by reference)). Other analog nucleic acids include those with positive backbones (Denpcy, *et al.*, Proc. Natl. Acad. Sci. USA, **92**:6097 (1995)); non-ionic backbones (U.S. Patent Nos. 5,386,023; 5,637,684; 5,602,240; 5,216,141; and 4,469,863; Kiedrowshi, *et al.*, Angew. Chem. Intl. Ed. English, **30**:423 (1991); Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); Letsinger, *et al.*, Nucleosides & Nucleotides, **13**:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker, *et al.*, Bioorganic & Medicinal Chem. Lett., **4**:395 (1994); Jeffs, *et al.*, J. Biomolecular NMR, **34**:17 (1994); Tetrahedron Lett., **37**:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins, *et al.*, Chem. Soc. Rev., (1995) pp. 169-176). Several nucleic acid

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2906

analogs are described in Rawls, C & E News, June 2, 1997, page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments; for example, PNA is particularly preferred. In addition, mixtures of naturally occurring nucleic acids and analogs can be made. Alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made. The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthanine, hypoxanthanine, isocytosine, isoguanine, and base analogs such as nitropyrrole and nitroindole, etc.

As described above generally for proteins, nucleic acid bioactive agents may be naturally occuring nucleic acids, random nucleic acids, or "biased" random nucleic acids. For example, digests of procaryotic or eukaryotic genomes may be used as is outlined above for proteins.

In general, probes of the present invention are designed to be complementary to a target sequence (either the target analyte sequence of the sample or to other probe sequences, as is described herein), such that hybridization of the target and the probes of the present invention occurs. This complementarity need not be perfect; there may be any number of base pair mismatches that will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under the selected reaction conditions. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2907

occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, supra, and Tijssen, supra.

The term 'target sequence" or grammatical equivalents herein means a nucleic acid sequence on a single strand of nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA and rRNA, or others. It may be any length, with the understanding that longer sequences are more specific. As will be appreciated by those in the art, the complementary target sequence may take many forms. For example, it may be contained within a larger nucleic acid sequence, i.e. all or part of a gene or mRNA, a restriction fragment of a plasmid or genomic DNA, among others. As is outlined more fully below, probes are made to hybridize to target sequences to determine the presence or absence of the target sequence in a sample. Generally speaking, this term will be understood by those skilled in the art.

In a preferred embodiment, the bioactive agents are organic chemical moieties, a wide variety of which are available in the literature.

In a preferred embodiment, each bead comprises a single type of bioactive agent, although a plurality of individual bioactive agents are preferably attached to each bead. Similarly, preferred embodiments utilize more than one microsphere containing a unique bioactive agent; that is, there is redundancy built into the system by the use of subpopulations of microspheres, each microsphere in the subpopulation containing the same bioactive agent.

As will be appreciated by those in the art, the bioactive agents may either be synthesized directly on the beads, or they may be made and then attached after synthesis. In a preferred embodiment, linkers are used to attach the bioactive agents to the beads, to allow both good attachment, sufficient flexibility to allow good interaction with the target molecule, and to avoid undesirable binding reactions.

In a preferred embodiment, the bioactive agents are synthesized directly on the beads. As is known in the art, many classes of chemical compounds are currently synthesized on solid supports, such as peptides, organic moieties, and nucleic acids. It is a relatively straightforward matter to adjust the current synthetic techniques to use beads.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2908

In a preferred embodiment, the bioactive agents are synthesized first, and then covalently attached to the beads. As will be appreciated by those in the art, this will be done depending on the composition of the bioactive agents and the beads. The functionalization of solid support surfaces such as certain polymers with chemically reactive groups such as thiols, amines, carboxyls, etc. is generally known in the art. Accordingly, "blank" microspheres may be used that have surface chemistries that facilitate the attachment of the desired functionality by the user. Some examples of these surface chemistries for blank microspheres include, but are not limited to, amino groups including aliphatic and aromatic amines, carboxylic acids, aldehydes, amides, chloromethyl groups, hydrazide, hydroxyl groups, sulfonates and sulfates.

These functional groups can be used to add any number of different candidate agents to the beads, generally using known chemistries. For example, candidate agents containing carbohydrates may be attached to an amino-functionalized support; the aldehyde of the carbohydrate is made using standard techniques, and then the aldehyde is reacted with an amino group on the surface. In an alternative embodiment, a sulfhydryl linker may be used. There are a number of sulfhydryl reactive linkers known in the art such as SPDP, maleimides, α-haloacetyls, and pyridyl disulfides (see for example the 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference) which can be used to attach cysteine containing proteinaceous agents to the support. Alternatively, an amino group on the candidate agent may be used for attachment to an amino group on the surface. For example, a large number of stable bifunctional groups are well known in the art, including homobifunctional and heterobifunctional linkers (see Pierce Catalog and Handbook, pages 155-200). In an additional embodiment, carboxyl groups (either from the surface or from the candidate agent) may be derivatized using well known linkers (see the Pierce catalog). For example, carbodiimides activate carboxyl groups for attack by good nucleophiles such as amines (see Torchilin et al., Critical Rev. Therapeutic Drug Carrier Systems, 7(4):275-308 (1991), expressly incorporated herein). Proteinaceous candidate agents may also be attached using other techniques known in the art, for example for the attachment of antibodies to polymers; see Slinkin et al., Bioconj. Chem. 2:342-348 (1991); Torchilin et al., *supra*; Trubetskoy et al., Bioconj. Chem. 3:323-327 (1992); King et al., Cancer Res. 54:6176-6185 (1994); and Wilbur et al., Bioconjugate Chem. 5:220-235 (1994), all of which are hereby expressly incorporated by reference). It should be understood that the candidate agents may be attached in a variety of ways, including those listed above. What is important is that manner of attachment does not significantly alter the functionality of the candidate agent; that is, the candidate agent should be attached in such a flexible manner as to allow its interaction with a target.

Specific techniques for immobilizing enzymes on microspheres are known in the prior art. In one case, NH_2 surface chemistry microspheres are used. Surface activation is achieved with a 2.5%

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2909

glutaraldehyde in phosphate buffered saline (10 mM) providing a pH of 6.9. (138 mM NaCl, 2.7 mM, KCl). This is stirred on a stir bed for approximately 2 hours at room temperature. The microspheres are then rinsed with ultrapure water plus 0.01% tween 20 (surfactant) -0.02%, and rinsed again with a pH 7.7 PBS plus 0.01% tween 20. Finally, the enzyme is added to the solution, preferably after being prefiltered using a 0.45µm amicon micropure filter.

In some embodiments, the microspheres may additionally comprise identifier binding ligands for use in certain decoding systems. By "identifier binding ligands" or "IBLs" herein is meant a compound that will specifically bind a corresponding decoder binding ligand (DBL) to facilitate the elucidation of the identity of the bioactive agent attached to the bead. That is, the IBL and the corresponding DBL form a binding partner pair. By "specifically bind" herein is meant that the IBL binds its DBL with specificity sufficient to differentiate between the corresponding DBL and other DBLs (that is, DBLs for other IBLs), or other components or contaminants of the system. The binding should be sufficient to remain bound under the conditions of the decoding step, including wash steps to remove non-specific binding. In some embodiments, for example when the IBLs and corresponding DBLs are proteins or nucleic acids, the dissociation constants of the IBL to its DBL will be less than about 10^{-4}-10^{-6} M^{-1}, with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} -10^{-9} M^{-1} being particularly preferred.

IBL-DBL binding pairs are known or can be readily found using known techniques. For example, when the IBL is a protein, the DBLs include proteins (particularly including antibodies or fragments thereof (FAbs, etc.)) or small molecules, or vice versa (the IBL is an antibody and the DBL is a protein). Metal ion- metal ion ligands or chelators pairs are also useful. Antigen-antibody pairs, enzymes and substrates or inhibitors, other protein-protein interacting pairs, receptor-ligands, complementary nucleic acids, and carbohydrates and their binding partners are also suitable binding pairs. Nucleic acid - nucleic acid binding proteins pairs are also useful. Similarly, as is generally described in U.S. Patents 5,270,163, 5,475,096, 5,567,588, 5,595,877, 5,637,459, 5,683,867,5,705,337, and related patents, hereby incorporated by reference, nucleic acid "aptomers" can be developed for binding to virtually any target; such a aptomer-target pair can be used as the IBL-DBL pair. Similarly, there is a wide body of literature relating to the development of binding pairs based on combinatorial chemistry methods.

In a preferred embodiment, the IBL is a molecule whose color or luminescence properties change in the presence of a selectively-binding DBL. For example, the IBL may be a fluorescent pH indicator whose emission intensity changes with pH. Similarly, the IBL may be a fluorescent ion indicator, whose emission properties change with ion concentration.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2910

Alternatively, the IBL is a molecule whose color or luminescence properties change in the presence of various solvents. For example, the IBL may be a fluorescent molecule such as an ethidium salt whose fluorescence intensity increases in hydrophobic environments. Similarly, the IBL may be a derivative of fluorescein whose color changes between aqueous and nonpolar solvents.

In one embodiment, the DBL may be attached to a bead, i.e. a "decoder bead", that may carry a label such as a fluorophore.

In a preferred embodiment, the IBL-DBL pair comprise substantially complementary single-stranded nucleic acids. In this embodiment, the binding ligands can be referred to as "identifier probes" and "decoder probes". Generally, the identifier and decoder probes range from about 4 basepairs in length to about 1000, with from about 6 to about 100 being preferred, and from about 8 to about 40 being particularly preferred. What is important is that the probes are long enough to be specific, i.e. to distinguish between different IBL-DBL pairs, yet short enough to allow both a) dissociation, if necessary, under suitable experimental conditions, and b) efficient hybridization.

In a preferred embodiment, as is more fully outlined below, the IBLs do not bind to DBLs. Rather, the IBLs are used as identifier moieties ("IMs") that are identified directly, for example through the use of mass spectroscopy.

In a preferred embodiment, the microspheres comprise an optical signature that can be used to identify the attached bioactive agent, as is generally outlined in U.S.S.N.s 08/818,199 and 09/151,877, both of which are hereby incorporated by reference. That is, each subpopulation of microspheres comprise a unique optical signature or optical tag that can be used to identify the unique bioactive agent of that subpopulation of microspheres; a bead comprising the unique optical signature may be distinguished from beads at other locations with different optical signatures. As is outlined herein, each bioactive agent will have an associated unique optical signature such that any microspheres comprising that bioactive agent will be identifiable on the basis of the signature. As is more fully outlined below, it is possible to reuse or duplicate optical signatures within an array, for example, when another level of identification is used, for example when beads of different sizes are used, or when the array is loaded sequentially with different batches of beads.

In a preferred embodiment, the optical signature is generally a mixture of reporter dyes, preferably fluorescent. By varying both the composition of the mixture (i.e. the ratio of one dye to another) and the concentration of the dye (leading to differences in signal intensity), matrices of unique tags may be generated. This may be done by covalently attaching the dyes to the surface of the beads, or alternatively, by entrapping the dye within the bead. The dyes may be chromophores or phosphors

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2911

but are preferably fluorescent dyes, which due to their strong signals provide a good signal-to-noise ratio for decoding. Suitable dyes for use in the invention include, but are not limited to, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, and others described in the 1989-1991 Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

In a preferred embodiment, the encoding can be accomplished in a ratio of at least two dyes, although more encoding dimensions may be added in the size of the beads, for example. In addition, the labels are distinguishable from one another; thus two different labels may comprise different molecules (i.e. two different fluors) or, alternatively, one label at two different concentrations or intensity.

In a preferred embodiment, the dyes are covalently attached to the surface of the beads. This may be done as is generally outlined for the attachment of the bioactive agents, using functional groups on the surface of the beads. As will be appreciated by those in the art, these attachments are done to minimize the effect on the dye.

In a preferred embodiment, the dyes are non-covalently associated with the beads, generally by entrapping the dyes in the bead matrix or pores of the beads. Fluorescent dyes are generally preferred because the strength of the fluorescent signal provides a better signal-to-noise ratio when decoding. Additionally, encoding in the ratios of the two or more dyes, rather than single dye concentrations, is preferred since it provides insensitivity to the intensity of light used to interrogate the reporter dye's signature and detector sensitivity.

In one embodiment, the dyes are added to the bioactive agent, rather than the beads, although this is generally not preferred.

In one embodiment, the microspheres do not contain an optical signature.

In a preferred embodiment, the present invention does not rely solely on the use of optical properties to decode the arrays. However, as will be appreciated by those in the art, it is possible in some embodiments to utilize optical signatures as an additional coding method, in conjunction with the present system. Thus, for example, as is more fully outlined below, the size of the array may be effectively increased while using a single set of decoding moieties in several ways, one of which is the use of optical signatures one some beads. Thus, for example, using one "set" of decoding molecules, the use of two populations of beads, one with an optical signature and one without, allows the effective

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2912

doubling of the array size. The use of multiple optical signatures similarly increases the possible size of the array.

In a preferred embodiment, each subpopulation of beads comprises a plurality of different identifier binding ligands ("IBLs"). By using a plurality of different IBLs to encode each bioactive agent, the number of possible unique codes is substantially increased. That is, by using one unique IBL per bioactive agent, the size of the array will be the number of unique IBLs (assuming no "reuse" occurs, as outlined below). However, by using a plurality of different IBLs per bead, n, the size of the array can be increased to 2^n, when the presence or absence of each IBL is used as the indicator. For example, the assignment of 10 IBLs per bead generates a 10 bit binary code, where each bit can be designated as "1" (IBL is present) or "0" (IBL is absent). A 10 bit binary code has 2^{10} possible variants However, as is more fully discussed below, the size of the array may be further increased if another parameter is included such as concentration or intensity; thus for example, if two different concentrations of the IBL are used, then the array size increases as 3^n. Thus, in this embodiment, each individual bioactive agent in the array is assigned a combination of IBLs, which can be added to the beads prior to the addition of the bioactive agent, after, or during the synthesis of the bioactive agent, i.e. simultaneous addition of IBLs and bioactive agent components.

Alternatively, when the bioactive agent is a polymer of different residues, i.e. when the bioactive agent is a protein or nucleic acid, the combination of different IBLs can be used to elucidate the sequence of the protein or nucleic acid.

Thus, for example, using two different IBLs (IBL1 and IBL2), the first position of a nucleic acid can be elucidated: for example, adenosine can be represented by the presence of both IBL1 and IBL2; thymidine can be represented by the presence of IBL1 but not IBL2, cytosine can be represented by the presence of IBL2 but not IBL1, and guanosine can be represented by the absence of both. The second position of the nucleic acid can be done in a similar manner using IBL3 and IBL4; thus, the presence of IBL1, IBL2, IBL3 and IBL4 gives a sequence of AA; IBL1, IBL2, and IBL3 shows the sequence AT; IBL1, IBL3 and IBL4 gives the sequence TA, etc. The third position utilizes IBL5 and IBL6, etc. In this way, the use of 20 different identifiers can yield a unique code for every possible 10-mer.

The system is similar for proteins but requires a larger number of different IBLs to identify each position, depending on the allowed diversity at each position. Thus for example, if every amino acid is allowed at every position, five different IBLs are required for each position. However, as outlined above, for example when using random peptides as the bioactive agents, there may be bias built into

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2913

the system; not all amino acids may be present at all positions, and some positions may be preset; accordingly, it may be possible to utilize four different IBLs for each amino acid.

In this way, a sort of "bar code" for each sequence can be constructed; the presence or absence of each distinct IBL will allow the identification of each bioactive agent.

In addition, the use of different concentrations or densities of IBLs allows a "reuse" of sorts. If, for example, the bead comprising a first agent has a 1X concentration of IBL, and a second bead comprising a second agent has a 10X concentration of IBL, using saturating concentrations of the corresponding labelled DBL allows the user to distinguish between the two beads.

In a preferred embodiment, the compositions of the invention further comprise at least one fiducial. By "fiducial" or "marker" or "registration point" herein is meant a physical reference feature or characteristic that allows precise comparisons of sequential data images of an array. The use of fiducials is useful for a variety of reasons. In general, the assays involve monitoring of objects, i.e. bioactive agents, located at spatially distinct locations (features) over the course of several data image frames taken over time. Any shifting that occurs from frame to frame complicates the analysis of the agents. By incorporating permanent fiducials into the assay structure, each data image can be aligned, either manually or automatically, to allow accurate comparison of the images, and control for translation (i.e. a shift in an X-Y direction) and/or rotation as well as reduction or enlargement of the image. In addition, when fluorescence based assays are used (either for decoding or analyte assaying or both), in any given image, a particular region or feature may or may not emit fluorescence, depending on the label characteristics and the wavelength being interrogated, or the presence or absence of an analyte or DBL, etc. The presence of fluorescence is detected as a positive change in feature intensity with respect to the background intensity, which is then used to draw a software "segment" over the core. In situations where the core is dark, i.e. no fluorescence is detected at that particular feature, it is difficult to accurately draw the segment over the core.

Accordingly, in a preferred embodiment, at least one fiducial is incorporated into the array. In a preferred embodiment, a plurality of fiducials are used, with the ideal number depending on the size of the array (i.e. features per fiducial), the density of the array, the shape of the array, the irregularity of the array, etc. In general, at least three non-linear fiducials are used; that is, three fiducials that define a plane (i.e. are not in a line) are used. In addition, it is preferred to have at least one of the fiducials be either on or close to the periphery of the array.

In a preferred embodiment, the substrate is a fiber optic bundle and the fiducial is a fiducial fiber. As will be appreciated by those in the art, the characteristics of a fiducial fiber may vary widely. For

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2914

example, in a preferred embodiment, the fiducial fibers may have unique or special optical properties; for example, fiducial fibers made of stock glass that exhibits broad fluorescence across the visible range of the spectrum; glasses are available in a wide range of compositions, and often possess intrinsic fluorescence due to the presence of impurities or dopants in the glass material. In a preferred embodiment, the fiducial fibers may have a different shape or size, or both, from the other fibers of the array. In addition, as is true for all the fiducial techniques herein, it is often preferred to have different fiducials have different characteristics, i.e. asymmetry among the fiducials, to allow for an extra level of registration. For example, in an square array format with fiducials at the corners, one of the four fiducials could be of a different shape or size than the other three or positionally offset.

The fiducial fibers may each be labeled with the same or with different labels. In a preferred embodiment a fiber is coated with a single label. In addition, multiple fibers to be incorporated into an array are labeled with the same label. In an alternative embodiment, each of a plurality of fiducial fibers is labeled with a different or discrete label. In a preferred embodiment a fiducial fiber comprises a detectable label, such as a dye, fluorescent organic dye or fluorescent inorganic particles such as quantum dots.

Arrays comprising fiducial fibers are generally made in a variety of ways.

In one embodiment, the fiber is doped with fluorescent organic dyes or fluorescent inorganic particles such as quantum dots at the melting stage, prior to the machining of the glass into rods for drawing.

In a preferred embodiment, the glass rod material is dipped into or covered with a solution of a label such as a fluorescent dopant material. Preferred embodiments utilize inorganic nanoparticles (quantum dots) as they are small in size, exhibit high fluoresence quantum efficiencies, and are extremely photostable over long period of time (i.e. resistant to photobleaching). The fluorescent characteristics of quantum dots are known to be directly related to the size of the particles. In a perferred embodiment, a polydisperse collection of particles is employed to give rise to broad absorbtive and emissive properties.

In another embodiment predoped fibers such as terbium-doped fibers are used as a foundation for the fiducial, and a label such as a fluorescent particle is added to the exterior of these core glasses to give rise to a fiber that exhibits fluorescent properties of both the internal and external dopants.

Following the coating of the outside of either of the above-described core bars with a label, the bar is then inserted into a cladding tube of lower refractive index and drawn. By cladding the coated fiber, excitation light can be made to propagate down the core, exciting any fluorescent material present

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2915

either in the core itself or, in this case, at the interface between the core and the clad. The fluorescence is then coupled into the core and light-guided back up the core to the proximal face of the fiber where it is detected, for example by a CCD camera.

In an alternative embodiment each of a plurality of fibers are incorporated into an array and each is coated with a different label. By increasing the number of labels incorporated into fiducial fibers, the number and complexity of labels and/or registers increases.

In a preferred embodiment, the fiducial is a fiducial bead of the random array. Similar to the fiducial fibers, fiducial beads may be added to the random array in any position. Thus, for example, a few fiducial beads may be added to the array prior to or simultaneously with the addition of the beads comprising bioactive agents. In this case the fiducial beads may go down randomly on the array. Alternatively, when wells are used, targeted addition of fiducial beads may be done; for example, by creating larger wells in defined locations (for example by using a few larger fibers and etching techniques), large fiducial beads may be laid down in certain sites. As above for fiducial fibers, the detectable properties of the fiducial beads may be different than the properties of the beads comprising agents. In addition, when randomly laid down fiducial beads are used, it should be noted that an advantage of the resulting array is that the fiducial pattern is essentially a "signature" of the individual array. That is, since the likelihood of two arrays containing the same spatial arrangement of fiducial beads is very low, individual arrays can be visually distinguished, serving as a sort of internal "label" for the array.

In another embodiment, the marker bead comprises no label, while the remaining beads or microspheres are labeled. Thus, the absence of a label serves to identify the marker bead on the array.

In a preferred embodiment an array comprises at least one marker bead, although more than one marker bead is particularly preferred.

The marker bead(s) can be added to the substrate or array at any time prior to, simultaneously with or following the addition of other microspheres.

In a preferred embodiment, the fiducial is a defined edge or edges of the substrate. As will be appreciated by those in the art, this may be done in a variety of ways. In one embodiment, a coating or sheath of fiducial material, such as highly fluorescent glass, is incorporated into the array composition. The fiducial material can have any number of physical characteristics to allow registration; for example, a "stripe" of fiducial material may have notches, dark impurities, or other

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2916

identifying features along its length. Alternatively, the fiducial material is placed in discrete spots or discrete shapes; basically, any orientation that allows translation, rotation, enlargement or reduction of images to be detected can be used.

In a preferred embodiment, an exogeneous fiducial is not used; rather, inherent characteristics of the array are used. That is, rather than incorporate a special feature into the array to serve as a fiducial, the inherent variability of the features of an array is used to create a sort of "fiducial template". In this embodiment, an image of the array is taken under conditions in which all the features are illuminated evenly and can be differentiated from one another. For example, the surface of the substrate can be illuminated with white light in such a way that all the features are illuminated evenly. This finds particular use when the substrate is a fiber optic bundle with etched wells, in that the illumination angle and intensity is chosen such that the light reflecting off the beads differs in intensity from the light reflecting off the cladding and spacer material. Preferred embodiments utilize polarized light or light impinging at various angles. Alternatively, the surface of the array may be contacted with a fluorescent solution, allowing fluorescence to be collected equally by all the features.

In a preferred embodiment, the invention provides the use of an image produced by a randomly ordered array to identify and/or label the array. When forming a random array, many, but not all, of the microwells on an array are filled with microspheres. The filled versus unfilled sites on the array are randomized; thus, an image or a composite of images of an array that details the filled from unfilled locations on the array serves as a unique identifier of the array. Thus, the image of a particular array is statistically different and distinct from an image or a composite of images of another array even though the different arrays have functional equivalence. By "statistically different" is meant that although there is a theoretical probability that two arrays may be similar, the probability is so small as to be insignificant or unimportant.

In one embodiment, the arrays have at least one subpopulation of microspheres. The pattern on the array created by the random assembly of microspheres on the array serves to identify the particular array. The image of the array registers the location of each bead such that composite images taken from the array can be compared directly. For example, an image produced by an array after exposure to a first substance can be directly compared with the same array exposed to a second substance. Alternatively, a single population of microspheres can be analyzed by multiple wavelengths and directly compared.

In another embodiment, these arrays have two or more subpopulations represented in each array. Because the arrays are assembled randomly, the individual locations of beads representing each subpopulation are randomized. Thus, an image or a composite of images that registers the location of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2917

each bead in a particular subpopulation will be statistically different from an image or composite of images of another array even though the different arrays have functional equivalence. Likewise, the image or composite of images for another subpopulation within the same array will be statistically different from an image or composite of images of another array.

In addition, the number of beads of each subpopulation that actually populate a given array can vary. The specific number of beads within a subpopulation on an array is defined by a Poisson distribution. The variation in number of beads representing a subpopulation adds another dimension to identifying individual arrays.

The recognition that functionally equivalent arrays result in different images affords one the possibility of using that difference to "fingerprint" each array. Essentially, each random array has a built in method for identifying and tracking that array.

Thus, the invention facilitates the use of innate features within a random array to identify and track a specific array. The ability to identify and track a specific array has important functionality ain quality control monitoring, inventory monitoring, performance monitoring and use monitoring. For example, the ability to identify an array will allow one to determine when it is used and whether it is reused.

In a preferred embodiment, the template image is used to define a "grid" which is placed upon the data images. The use of a template image to define the location of features is optional, although currently preferred. Using standard image processing software such as Image Pro (Media Cybernetics) a template is built based on this grid. This type of software allows the user to create simultaneous software segments to calculate the mean feature intensity over a region of interest using a simple, one step segmentation function. This software-based fiducial template can then be mapped onto each data image in the assay protocol to allow data collection for each region for each data image. See for example U.S. Patent No. 5,768,412. This allows the location of each array feature to be defined.

Once the microspheres comprising the candidate agents and the unique tags are generated, they are added to the substrate to form an array. In general, the methods of making the arrays and of decoding the arrays is done to maximize the number of different candidate agents that can be uniquely encoded. The compositions of the invention may be made in a variety of ways. In general, the arrays are made by adding a solution or slurry comprising the beads to a surface containing the sites for attachment of the beads. This may be done in a variety of buffers, including aqueous and organic solvents, and mixtures. The solvent can evaporate, and excess beads removed.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2918

It should be noted that not all sites of an array may comprise a bead; that is, there may be some sites on the substrate surface which are empty. In addition, there may be some sites that contain more than one bead, although this is not preferred.

It should additionally be noted that in some cases, empty sites can serve as fiducials. That is, consistently "dark" sites can also be used as fiducials. This finds particular use when the filling efficiencies of the array are high; that is, when most sites contain a bead. In addition, the dark sites also can be used to "fingerprint" the array as described above. That is, the image of light and dark sites serves to define or identify a particular array. This image also serves to register the array for comparison purposes.

In some embodiments, for example when chemical attachment is done, it is possible to attach the beads in a non-random or ordered way. For example, using photoactivatible attachment linkers or photoactivatible adhesives or masks, selected sites on the array may be sequentially rendered suitable for attachment, such that defined populations of beads are laid down.

The arrays of the present invention are constructed such that information about the identity of the candidate agent is built into the array, such that the random deposition of the beads in the fiber wells can be "decoded" to allow identification of the candidate agent at all positions. This may be done in a variety of ways, and either before, during or after the use of the array to detect target molecules.

Thus, after the array is made, it is "decoded" in order to identify the location of one or more of the bioactive agents, i.e. each subpopulation of beads, on the substrate surface. In general, both decoding and the experimental assay to determine the presence or absence of a target analyte, both of which are described below, requires the comparison of sequential data images to determine the differences between two data images. In general, this is done by taking a first or initial data image, using the fiducial to create a registered first data image, subjecting the array to decoding conditions and taking a second data image. The same fiducial is used to create a registered second data image, and then the two registered images can be compared. In this context, a "data image" includes a primary data image or a reduction of the image; for example, the image may be reduced to a set of X-Y coordinates with corresponding intensity values.

In a preferred embodiment, this is done using a computer system comprising a processor and a computer readable memory. The computer readable memory comprises an acquisition module that comprises computer code that can receive a data image from a random array and a registration module comprising computer code that can register the data image using at least one fiducial, including a fiducial template, to generate a registered data image. This registered data image can

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2919

then be stored in a storage module as needed. This same computer code, or different code, if required, can be used to receive additional data images and generate additional registered data images, which also can be stored. The computer readable memory further comprises a comparison module comprising computer code that can compare the registered data images to determine the differences between them, to allow both decoding of the array and target analyte detection. That is, when decoding is done, the comparison of at least two registered data images allows the identification of the location of at least two unique bioactive agents on the array.

Thus, using the systems described herein, a random array is decoded. In a preferred embodiment, a selective decoding system is used. In this case, only those microspheres exhibiting a change in the optical signal as a result of the binding of a target analyte are decoded. This is commonly done when the number of "hits", i.e. the number of sites to decode, is generally low. That is, the array is first scanned under experimental conditions in the absence of the target analytes. The sample containing the target analytes is added, and only those locations exhibiting a change in the optical signal are decoded. For example, the beads at either the positive or negative signal locations may be either selectively tagged or released from the array (for example through the use of photocleavable linkers), and subsequently sorted or enriched in a fluorescence-activated cell sorter (FACS). That is, either all the negative beads are released, and then the positive beads are either released or analyzed in situ, or alternatively all the positives are released and analyzed. Alternatively, the labels may comprise halogenated aromatic compounds, and detection of the label is done using for example gas chromatography, chemical tags, isotopic tags mass spectral tags.

In a preferred embodiment, atomic force microscopy (AFM) is used to decode the array. In this embodiment, an AFM tip, comprising a DBL, is positioned at the site to be decoded, that comprises an IBL. The force of interaction between the IBL/DBL is measured using AFM. IN addition, since AFM has atomic resolution, a variety of other physical characteristics, including physical size and shape can be used for decoding. For example, different "shaped" molecules could be used as IBLs; in this embodiment, the AFM tip can comprise a DBL or just a moiety that can detect different surfaces. In addition, AFM could be used as "nanotweezers" to deliver or recover beads to and from specific locations on the array.

As will be appreciated by those in the art, this may also be done in systems where the array is not decoded; i.e. there need not ever be a correlation of bead composition with location. In this embodiment, the beads are loaded on the array, and the assay is run. The "positives", i.e. those beads displaying a change in the optical signal as is more fully outlined below, are then "marked" to distinguish or separate them from the "negative" beads. This can be done in several ways, preferably using fiber optic arrays. In a preferred embodiment, each bead contains a fluorescent dye. After the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2920

assay and the identification of the "positives" or "active beads", light is shown down either only the positive fibers or only the negative fibers, generally in the presence of a light-activated reagent (typically dissolved oxygen). In the former case, all the active beads are photobleached. Thus, upon non-selective release of all the beads with subsequent sorting, for example using a fluorescence activated cell sorter (FACS) machine, the non-fluorescent active beads can be sorted from the fluorescent negative beads. Alternatively, when light is shown down the negative fibers, all the negatives are non-fluorescent and the the postives are fluorescent, and sorting can proceed. The characterization of the attached bioactive agent may be done directly, for example using mass spectroscopy.

Alternatively, the identification may occur through the use of identifier moieties ("IMs"), which are similar to IBLs but need not necessarily bind to DBLs. That is, rather than elucidate the structure of the bioactive agent directly, the composition of the IMs may serve as the identifier. Thus, for example, a specific combination of IMs can serve to code the bead, and be used to identify the agent on the bead upon release from the bead followed by subsequent analysis, for example using a gas chromatograph or mass spectroscope.

Alternatively, rather than having each bead contain a fluorescent dye, each bead comprises a non-fluorescent precursor to a fluorescent dye. For example, using photocleavable protecting groups, such as certain ortho-nitrobenzyl groups, on a fluorescent molecule, photoactivation of the fluorochrome can be done. After the assay, light is shown down again either the "positive" or the "negative" fibers, to distinquish these populations. The illuminated precursors are then chemically converted to a fluorescent dye. All the beads are then released from the array, with sorting, to form populations of fluorescent and non-fluorescent beads (either the positives and the negatives or vice versa).

In an alternate preferred embodiment, the sites of attachment of the beads (for example the wells) include a photopolymerizable reagent, or the photopolymerizable agent is added to the assembled array. After the test assay is run, light is shown down again either the "positive" or the "negative" fibers, to distinquish these populations. As a result of the irradiation, either all the positives or all the negatives are polymerized and trapped or bound to the sites, while the other population of beads can be released from the array.

In a preferred embodiment, the location of every bioactive agent is determined using decoder binding ligands (DBLs). As outlined above, DBLs are binding ligands that will either bind to identifier binding ligands, if present, or to the bioactive agents themselves, preferably when the bioactive agent is a nucleic acid or protein.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2921

In a preferred embodiment, as outlined above, the DBL binds to the IBL.

In a preferred embodiment, the bioactive agents are single-stranded nucleic acids and the DBL is a substantially complementary single-stranded nucleic acid that binds (hybridizes) to the bioactive agent, termed a decoder probe herein. A decoder probe that is substantially complementary to each candidate probe is made and used to decode the array. In this embodiment, the candidate probes and the decoder probes should be of sufficient length (and the decoding step run under suitable conditions) to allow specificity; i.e. each candidate probe binds to its corresponding decoder probe with sufficient specificity to allow the distinction of each candidate probe.

In a preferred embodiment, the DBLs are either directly or indirectly labeled. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic, electrical, thermal; and c) colored or luminescent dyes; although labels include enzymes and particles such as magnetic particles as well. Preferred labels include luminescent labels. In a preferred embodiment, the DBL is directly labeled, that is, the DBL comprises a label. In an alternate embodiment, the DBL is indirectly labeled; that is, a labeling binding ligand (LBL) that will bind to the DBL is used. In this embodiment, the labeling binding ligand-DBL pair can be as described above for IBL-DBL pairs.

Accordingly, the identification of the location of the individual beads (or subpopulations of beads) is done using one or more decoding steps comprising a binding between the labeled DBL and either the IBL or the bioactive agent (i.e. a hybridization between the candidate probe and the decoder probe when the bioactive agent is a nucleic acid). After decoding, the DBLs can be removed and the array can be used; however, in some circumstances, for example when the DBL binds to an IBL and not to the bioactive agent, the removal of the DBL is not required (although it may be desirable in some circumstances). In addition, as outlined herein, decoding may be done either before the array is used to in an assay, during the assay, or after the assay.

In one embodiment, a single decoding step is done. In this embodiment, each DBL is labeled with a unique label, such that the the number of unique tags is equal to or greater than the number of bioactive agents (although in some cases, "reuse" of the unique labels can be done, as described herein; similarly, minor variants of candidate probes can share the same decoder, if the variants are encoded in another dimension, i.e. in the bead size or label). For each bioactive agent or IBL, a DBL is made that will specifically bind to it and contains a unique tag, for example one or more fluorochromes. Thus, the identity of each DBL, both its composition (i.e. its sequence when it is a nucleic acid) and its label, is known. Then, by adding the DBLs to the array containing the bioactive

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2922

agents under conditions which allow the formation of complexes (termed hybridization complexes when the components are nucleic acids) between the DBLs and either the bioactive agents or the IBLs, the location of each DBL can be elucidated. This allows the identification of the location of each bioactive agent; the random array has been decoded. The DBLs can then be removed, if necessary, and the target sample applied.

In a preferred embodiment, the number of unique labels is less than the number of unique bioactive agents, and thus a sequential series of decoding steps are used. To facilitate the discussion, this embodiment is explained for nucleic acids, although other types of bioactive agents and DBLs are useful as well. In this embodiment, decoder probes are divided into **n** sets for decoding. The number of sets corresponds to the number of unique tags. Each decoder probe is labeled in **n** separate reactions with **n** distinct tags. All the decoder probes share the same **n** tags. The decoder probes are pooled so that each pool contains only one of the **n** tag versions of each decoder, and no two decoder probes have the same sequence of tags across all the pools. The number of pools required for this to be true is determined by the number of decoder probes and the **n**. Hybridization of each pool to the array generates a signal at every address. The sequential hybridization of each pool in turn will generate a unique, sequence-specific code for each candidate probe. This identifies the candidate probe at each address in the array. For example, if four tags are used, then 4 X **n** sequential hybridizations can ideally distinguish 4^n sequences, although in some cases more steps may be required. After the hybridization of each pool, the hybrids are denatured and the decoder probes removed, so that the probes are rendered single-stranded for the next hybridization (although it is also possible to hybridize limiting amounts of target so that the available probe is not saturated. Sequential hybridizations can be carried out and analyzed by subtracting pre-existing signal from the previous hybridization).

An example is illustrative. Assuming an array of 16 probe nucleic acids (numbers 1-16), and four unique tags (four different fluors, for example; labels A-D). Decoder probes 1-16 are made that correspond to the probes on the beads. The first step is to label decoder probes 1-4 with tag A, decoder probes 5-8 with tag B, decoder probes 9-12 with tag C, and decoder probes 13-16 with tag D. The probes are mixed and the pool is contacted with the array containing the beads with the attached candidate probes. The location of each tag (and thus each decoder and candidate probe pair) is then determined. The first set of decoder probes are then removed. A second set is added, but this time, decoder probes 1, 5, 9 and 13 are labeled with tag A, decoder probes 2, 6, 10 and 14 are labeled with tag B, decoder probes 3, 7, 11 and 15 are labeled with tag C, and decoder probes 4, 8, 12 and 16 are labeled with tag D. Thus, those beads that contained tag A in both decoding steps contain candidate probe 1; tag A in the first decoding step and tag B in the second decoding step contain candidate probe 2; tag A in the first decoding step and tag C in the second step contain candidate probe 3; etc.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2923

In one embodiment, the decoder probes are labeled in situ; that is, they need not be labeled prior to the decoding reaction. In this embodiment, the incoming decoder probe is shorter than the candidate probe, creating a 5' "overhang" on the decoding probe. The addition of labeled ddNTPs (each labeled with a unique tag) and a polymerase will allow the addition of the tags in a sequence specific manner, thus creating a sequence-specific pattern of signals. Similarly, other modifications can be done, including ligation, etc.

In addition, since the size of the array will be set by the number of unique decoding binding ligands, it is possible to "reuse" a set of unique DBLs to allow for a greater number of test sites. This may be done in several ways; for example, by using some subpopulations that comprise optical signatures. Similarly, the use of a positional coding scheme within an array; different sub-bundles may reuse the set of DBLs. Similarly, one embodiment utilizes bead size as a coding modality, thus allowing the reuse of the set of unique DBLs for each bead size. Alternatively, sequential partial loading of arrays with beads can also allow the reuse of DBLs. Furthermore, "code sharing" can occur as well.

In a preferred embodiment, the DBLs may be reused by having some subpopulations of beads comprise optical signatures. In a preferred embodiment, the optical signature is generally a mixture of reporter dyes, preferably fluoroscent. By varying both the composition of the mixture (i.e. the ratio of one dye to another) and the concentration of the dye (leading to differences in signal intensity), matrices of unique optical signatures may be generated. This may be done by covalently attaching the dyes to the surface of the beads, or alternatively, by entrapping the dye within the bead. The dyes may be chromophores or phosphors but are preferably fluorescent dyes, which due to their strong signals provide a good signal-to-noise ratio for decoding. Suitable dyes for use in the invention include, but are not limited to, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, and others described in the 6th Edition of the Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

In a preferred embodiment, the encoding can be accomplished in a ratio of at least two dyes, although more encoding dimensions may be added in the size of the beads, for example. In addition, the labels are distinguishable from one another; thus two different labels may comprise different molecules (i.e. two different fluors) or, alternatively, one label at two different concentrations or intensity.

In a preferred embodiment, the dyes are covalently attached to the surface of the beads. This may be done as is generally outlined for the attachment of the bioactive agents, using functional groups on the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2924

surface of the beads. As will be appreciated by those in the art, these attachments are done to minimize the effect on the dye.

In a preferred embodiment, the dyes are non-covalently associated with the beads, generally by entrapping the dyes in the pores of the beads.

Additionally, encoding in the ratios of the two or more dyes, rather than single dye concentrations, is preferred since it provides insensitivity to the intensity of light used to interrogate the reporter dye's signature and detector sensitivity.

In a preferred embodiment, a spatial or positional coding system is done. In this embodiment, there are sub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an "area code", that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique tags can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.

In alternative embodiments, additional encoding parameters can be added, such as microsphere size. For example, the use of different size beads may also allow the reuse of sets of DBLs; that is, it is possible to use microspheres of different sizes to expand the encoding dimensions of the microspheres. Optical fiber arrays can be fabricated containing features with different fiber diameters or cross-sections; alternatively, two or more fiber optic bundles, each with different cross-sections of the individual fibers, can be added together to form a larger bundle; or, fiber optic bundles with fiber of the same size cross-sections can be used, but just with different sized beads. With different diameters, the largest wells can be filled with the largest microspheres and then moving onto progressively smaller microspheres in the smaller wells until all size wells are then filled. In this manner, the same dye ratio could be used to encode microspheres of different sizes thereby expanding the number of different oligonucleotide sequences or chemical functionalities present in the array. Although outlined for fiber optic substrates, this as well as the other methods outlined herein can be used with other substrates and with other attachment modalities as well.

In a preferred embodiment, the coding and decoding is accomplished by sequential loading of the microspheres into the array. As outlined above for spatial coding, in this embodiment, the optical signatures can be "reused". In this embodiment, the library of microspheres each comprising a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2925

different bioactive agent (or the subpopulations each comprise a different bioactive agent), is divided into a plurality of sublibraries; for example, depending on the size of the desired array and the number of unique tags, 10 sublibraries each comprising roughly 10% of the total library may be made, with each sublibrary comprising roughly the same unique tags. Then, the first sublibrary is added to the fiber optic bundle comprising the wells, and the location of each bioactive agent is determined, generally through the use of DBLs. The second sublibrary is then added, and the location of each bioactive agent is again determined. The signal in this case will comprise the signal from the "first" DBL and the "second" DBL; by comparing the two matrices the location of each bead in each sublibrary can be determined. Similarly, adding the third, fourth, etc. sublibraries sequentially will allow the array to be filled.

In a preferred embodiment, codes can be "shared" in several ways. In a first embodiment, a single code (i.e. IBL/DBL pair) can be assigned to two or more agents if the target analytes different sufficiently in their binding strengths. For example, two nucleic acid probes used in an mRNA quantitation assay can share the same code if the ranges of their hybridization signal intensities do not overlap. This can occur, for example, when one of the target sequences is always present at a much higher concentration than the other. Alternatively, the two target sequences might always be present at a similar concentration, but differ in hybridization efficiency.

Alternatively, a single code can be assigned to multiple agents if the agents are functionally equivalent. For example, if a set of oligonucleotide probes are designed with the common purpose of detecting the presence of a particular gene, then the probes are functionally equivalent, even though they may differ in sequence. Similarly, if classes of analytes are desired, all probes for different members of a class such as kinases or G-protein coupled receptors could share a code. Similarly, an array of this type could be used to detect homologs of known genes. In this embodiment, each gene is represented by a heterologous set of probes, hybridizing to different regions of the gene (and therefore differing in sequence). The set of probes share a common code. If a homolog is present, it might hybridize to some but not all of the probes. The level of homology might be indicated by the fraction of probes hybridizing, as well as the average hybridization intensity. Similarly, multiple antibodies to the same protein could all share the same code.

Once made, the compositions of the invention find use in a number of applications. In a preferred embodiment, the compositions are used to probe a sample solution for the presence or absence of a target analyte, including the quantification of the amount of target analyte present. By "target analyte" or "analyte" or grammatical equivalents herein is meant any atom, molecule, ion, molecular ion, compound or particle to be either detected or evaluated for binding partners. As will be appreciated by those in the art, a large number of analytes may be used in the present invention; basically, any target

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2926

analyte can be used which binds a bioactive agent or for which a binding partner (i.e. drug candidate) is sought.

Suitable analytes include organic and inorganic molecules, including biomolecules. When detection of a target analyte is done, suitable target analytes include, but are not limited to, an environmental pollutant (including pesticides, insecticides, toxins, etc.); a chemical (including solvents, polymers, organic materials, etc.); therapeutic molecules (including therapeutic and abused drugs, antibiotics, etc.); biomolecules (including hormones, cytokines, proteins, nucleic acids, lipids, carbohydrates, cellular membrane antigens and receptors (neural, hormonal, nutrient, and cell surface receptors) or their ligands, etc); whole cells (including procaryotic (such as pathogenic bacteria) and eukaryotic cells, including mammalian tumor cells); viruses (including retroviruses, herpesviruses, adenoviruses, lentiviruses, etc.); and spores; etc. Particularly preferred analytes are nucleic acids and proteins.

In a preferred embodiment, the target analyte is a protein. As will be appreciated by those in the art, there are a large number of possible proteinaceous target analytes that may be detected or evaluated for binding partners using the present invention. Suitable protein target analytes include, but are not limited to, (1) immunoglobulins; (2) enzymes (and other proteins); (3) hormones and cytokines (many of which serve as ligands for cellular receptors); and (4) other proteins.

In a preferred embodiment, the target analyte is a nucleic acid. These assays find use in a wide variety of applications.

In a preferred embodiment, the probes are used in genetic diagnosis. For example, probes can be made using the techniques disclosed herein to detect target sequences such as the gene for nonpolyposis colon cancer, the BRCA1 breast cancer gene, P53, which is a gene associated with a variety of cancers, the Apo E4 gene that indicates a greater risk of Alzheimer's disease, allowing for easy presymptomatic screening of patients, mutations in the cystic fibrosis gene, cytochrome p450s or any of the others well known in the art.

In an additional embodiment, viral and bacterial detection is done using the complexes of the invention. In this embodiment, probes are designed to detect target sequences from a variety of bacteria and viruses. For example, current blood-screening techniques rely on the detection of anti-HIV antibodies. The methods disclosed herein allow for direct screening of clinical samples to detect HIV nucleic acid sequences, particularly highly conserved HIV sequences. In addition, this allows direct monitoring of circulating virus within a patient as an improved method of assessing the efficacy of anti-viral therapies. Similarly, viruses associated with leukemia, HTLV-I and HTLV-II, may be

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2927

detected in this way. Bacterial infections such as tuberculosis, chlamydia and other sexually transmitted diseases, may also be detected.

In a preferred embodiment, the nucleic acids of the invention find use as probes for toxic bacteria in the screening of water and food samples. For example, samples may be treated to lyse the bacteria to release its nucleic acid, and then probes designed to recognize bacterial strains, including, but not limited to, such pathogenic strains as, *Salmonella*, *Campylobacter*, *Vibrio cholerae*, *Leishmania*, enterotoxic strains of *E. coli*, and Legionnaire's disease bacteria. Similarly, bioremediation strategies may be evaluated using the compositions of the invention.

In a further embodiment, the probes are used for forensic "DNA fingerprinting" to match crime-scene DNA against samples taken from victims and suspects.

In an additional embodiment, the probes in an array are used for sequencing by hybridization.

The present invention also finds use as a methodology for the detection of mutations or mismatches in target nucleic acid sequences. For example, recent focus has been on the analysis of the relationship between genetic variation and phenotype by making use of polymorphic DNA markers. Previous work utilized short tandem repeats (STRs) as polymorphic positional markers; however, recent focus is on the use of single nucleotide polymorphisms (SNPs), which occur at an average frequency of more than 1 per kilobase in human genomic DNA. Some SNPs, particularly those in and around coding sequences, are likely to be the direct cause of therapeutically relevant phenotypic variants. There are a number of well known polymorphisms that cause clinically important phenotypes; for example, the apoE2/3/4 variants are associated with different relative risk of Alzheimer's and other diseases (see Cordor et al., Science 261(1993). Multiplex PCR amplification of SNP loci with subsequent hybridization to oligonucleotide arrays has been shown to be an accurate and reliable method of simultaneously genotyping at least hundreds of SNPs; see Wang et al., Science, 280:1077 (1998); see also Schafer et al., Nature Biotechnology 16:33-39 (1998). The compositions of the present invention may easily be substituted for the arrays of the prior art.

In a preferred embodiment, the compositions of the invention are used to screen bioactive agents to find an agent that will bind, and preferably modify the function of, a target molecule. As above, a wide variety of different assay formats may be run, as will be appreciated by those in the art. Generally, the target analyte for which a binding partner is desired is labeled; binding of the target analyte by the bioactive agent results in the recruitment of the label to the bead, with subsequent detection.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2928

In a preferred embodiment, the binding of the bioactive agent and the target analyte is specific; that is, the bioactive agent specifically binds to the target analyte. By "specifically bind" herein is meant that the agent binds the analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. However, as will be appreciated by those in the art, it will be possible to detect analytes using binding which is not highly specific; for example, the systems may use different binding ligands, for example an array of different ligands, and detection of any particular analyte is via its "signature" of binding to a panel of binding ligands, similar to the manner in which "electronic noses" work. This finds particular utility in the detection of chemical analytes. The binding should be sufficient to remain bound under the conditions of the assay, including wash steps to remove non-specific binding, although in some embodiments, wash steps are not desired; i.e. for detecting low affinity binding partners. In some embodiments, for example in the detection of certain biomolecules, the dissociation constants of the analyte to the binding ligand will be less than about 10^{-4}-10^{-6} M^{-1}, with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} -10^{-9} M^{-1} being particularly preferred.

Generally, a sample containing a target analyte (whether for detection of the target analyte or screening for binding partners of the target analyte) is added to the array, under conditions suitable for binding of the target analyte to at least one of the bioactive agents, i.e. generally physiological conditions. The presence or absence of the target analyte is then detected. As will be appreciated by those in the art, this may be done in a variety of ways, generally through the use of a change in an optical signal. This change can occur via many different mechanisms. A few examples include the binding of a dye-tagged analyte to the bead, the production of a dye species on or near the beads, the destruction of an existing dye species, a change in the optical signature upon analyte interaction with dye on bead, or any other optical interrogatable event.

In a preferred embodiment, the change in optical signal occurs as a result of the binding of a target analyte that is labeled, either directly or indirectly, with a detectable label, preferably an optical label such as a fluorochrome. Thus, for example, when a proteinaceous target analyte is used, it may be either directly labeled with a fluor, or indirectly, for example through the use of a labeled antibody. Similarly, nucleic acids are easily labeled with fluorophor, for example during PCR amplification as is known in the art. Alternatively, upon binding of the target sequences, a hybridization indicator may be used as the label. Hybridization indicators preferentially associate with double stranded nucleic acid, usually reversibly. Hybridization indicators include intercalators and minor and/or major groove binding moieties. In a preferred embodiment, intercalators may be used; since intercalation generally only occurs in the presence of double stranded nucleic acid, only in the presence of target hybridization will the label light up. Thus, upon binding of the target analyte to a bioactive agent, there is a new optical signal generated at that site, which then may be detected.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2929

Alternatively, in some cases, as discussed above, the target analyte such as an enzyme generates a species that is either directly or indirectly optical detectable.

Furthermore, in some embodiments, a change in the optical signature may be the basis of the optical signal. For example, the interaction of some chemical target analytes with some fluorescent dyes on the beads may alter the optical signature, thus generating a different optical signal.

As will be appreciated by those in the art, in some embodiments, the presence or absence of the target analyte may be done using changes in other optical or non-optical signals, including, but not limited to, surface enhanced Raman spectroscopy, surface plasmon resonance, radioactivity, etc.

Again, as outlined above for decoding, the assay for the presence or absence of a target analyte utilizes sequential processing of data images using a computer system. Thus, in a preferred embodiment, a first data image of a random array is acquired using an acquisition module of the computer system. This initial data image may be decoded, i.e. the location of some or all of the bioactive agents may be known, or decoding may occur either during or after the assay. A registration module of the computer system is used to create a registered first data image, using either an exogeneous fiducial or a fiducial template generated by acquiring a template data image as outlined herein, for example by evening illuminating the array. The sample is then added to the array, and a second data image is acquired using the acquisition module. The fiducial and registration module are then used to create a registered second data image. A comparision module of the computer system is then used to compare the registered data images to determine the presence or absence of said target analyte.

The assays may be run under a variety of experimental conditions, as will be appreciated by those in the art. A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding. Various blocking and washing steps may be utilized as is known in the art.

In a preferred embodiment, two-color competitive hybridization assays are run. These assays can be based on traditional sandwich assays. The beads contain a capture sequence located on one side (upstream or downstream) of the SNP, to capture the target sequence. Two SNP allele-specific probes, each labeled with a different fluorophor, are hybridized to the target sequence. The genotype

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2930

can be obtained from a ratio of the two signals, with the correct sequence generally exhibiting better binding. This has an advantage in that the target sequence itself need not be labeled. In addition, since the probes are competing, this means that the conditions for binding need not be optimized. Under conditions where a mismatched probe would be stably bound, a matched probe can still displace it. Therefore the competitive assay can provide better discrimination under those conditions. Because many assays are carried out in parallel, conditions cannot be optimzed for every probe simultaneously. Therefore, a competitive assay system can be used to help compensate for non-optimal conditions for mismatch discrimination.

In a preferred embodiment, dideoxynucleotide chain-termination sequencing is done using the compositions of the invention. In this embodiment, a DNA polymerase is used to extend a primer using fluorescently labeled ddNTPs or other chain terminating nucleotides. The 3' end of the primer is located adjacent to the SNP site. In this way, the single base extension is complementary to the sequence at the SNP site. By using four different fluorophors, one for each base, the sequence of the SNP can be deduced by comparing the four base-specific signals. This may be done in several ways. In a first embodiment, the capture probe can be extended; in this approach, the probe must either be synthesized 5'-3' on the bead, or attached at the 5' end, to provide a free 3' end for polymerase extension. Alternatively, a sandwich type assay can be used; in this embodiment, the target is captured on the bead by a probe, then a primer is annealed and extended. Again, in the latter case, the target sequence need not be labeled. In addition, since sandwich assays require two specific interactions, this provides increased stringency which is particularly helpful for the analysis of complex samples.

In addition, primer extension is possible; extension of a primer bound to template in liquid phase is followed by capture of the extended primer on the array.

In addition, when the target analyte and the DBL both bind to the agent, it is also possible to do detection of non-labelled target analytes via competition of decoding.

In a preferred embodiment, the methods of the invention are useful in array quality control. Prior to this invention, no methods have been described that provide a positive test of the performance of every probe on every array. Decoding of the array not only provides this test, it also does so by making use of the data generated during the decoding process itself. Therefore, no additional experimental work is required. The invention requires only a set of data analysis algorithms that can be encoded in software.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2931

The quality control procedure can identify a wide variety of systematic and random problems in an array. For example, random specks of dust or other contaminants might cause some sensors to give an incorrect signal-this can be detected during decoding. The omission of one or more agents from multiple arrays can also be detected. An advantage of this quality control procedure is that it can be implemented immediated prior to the assay itself, and is a true functional test of each individual sensor. Therefore any problems that might occur between array assembly and actual use can be detected. In applications where a very high level of confidence is required, and/or there is a significant chance of sensor failure during the experimental procedure, decoding and quality control can be conducted both before and after the actual sample analysis.

In a preferred embodiment, the arrays can be used to do reagent quality control. In many instances, biological macromolecules are used as reagents and must be quality controlled. For example, large sets of oligonucleotide probes may be provided as reagents. It is typically difficult to perform quality control on large numbers of different biological macromolecules. The approach described here can be used to do this by treating the reagents (formulated as the DBLs) as variable instead of the arrays.

In a preferred embodiment, the methods outlined herein are used in array calibration. For many applications, such as mRNA quantitation, it is desirable to have a signal that is a linear response to the concentration of the target analyte, or, alternatively, if non-linear, to determine a relationship between concentration and signal, so that the concentration of the target analyte can be estimated. Accordingly, the present invention provides methods of creating calibration curves in parallel for multiple beads in an array. The calibration curves can be created under conditions that simulate the complexity of the sample to be analyzed. Each curve can be constructed independently of the others (e.g. for a different range of concentrations), but at the same time as all the other curves for the array. Thus, in this embodiment, the sequential decoding scheme is implemented with different concentrations being used as the code "labels", rather than different fluorophores. In this way, signal as a response to concentration can be measured for each bead. This calibration can be carried out just prior to array use, so that every probe on every array is individually calibrated as needed.

In a preferred embodiment, the methods of the invention can be used in assay development as well. Thus, for example, the methods allow the identification of good and bad probes; as is understood by those in the art, some probes do not function well because they do not hybridize well, or because they cross-hybridize with more than one sequence. These problems are easily detected during decoding. The ability to rapidly assess probe performance has the potential to greatly reduce the time and expense of assay development.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2932

Similarly, in a preferred embodiment, the methods of the invention are useful in quantitation in assay development. Major challenges of many assays is the ability to detect differences in analyte concentrations between samples, the ability to quantitate these differences, and to measure absolute concentrations of analytes, all in the presence of a complex mixture of related analytes. An example of this problem is the quantitation of a specific mRNA in the presence of total cellular mRNA. One approach that has been developed as a basis of mRNA quantitation makes use of a multiple match and mismatch probe pairs (Lockhart et al., 1996), hereby incorporated by reference in its entirety. While this approach is simple, it requires relatively large numbers of probes. In this approach, a quantitative response to concentration is obtained by averaging the signals from a set of different probes to the gene or sequence of interest. This is necessary because only some porbes respond quantitatively, and it is not possible to predict these probes with certainty. In the absence of prior knowledge, only the average response of an appropriately chosen collection of probes is quantitative. However, in the present invention, that can be applied generally to nucleic acid based assays as well as other assays. In essence, the approach is to identify the probes that respond quantitatively in a particular assay, rather than average them with other probes. This is done using the array calibration scheme outlined above, in which concentration-based codes are used. Advantages of this approach include: fewer probes are needed; the accuracy of the measurement is less dependent on the number of probes used; and that the response of the sensors is known with a high level of certainty, since each and every sequence can be tested in an efficient manner. It is important to note that probes that perfomr well are selected empiriically, which avoids the difficulties and uncertainties of predicting probe performance, particularly in complex sequence mixtures. In contrast, in experiments described to date with ordered arrays, relatively small numbers of sequences are checked by perfomring quantitative spiking experiments, in which a known mRNA is added to a mixture.

All references cited herein are incorporated by reference in their entirety.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2933

CLAIMS

We claim:

1. An array composition comprising:

a) a substrate with a surface comprising discrete sites; and

b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; and

c) at least one fiducial;

wherein said microspheres are distributed on said surface.

2. An array composition according to claim 1 wherein each subpopulation comprises a unique optical signature.

3. An array composition according to claim 1 wherein each subpopulation comprises an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

4. An array composition according to claim 1 wherein said substrate is a fiber optic bundle and said fiducial is a fiducial fiber.

5. An array composition according to claim 1 wherein said substrate is a fiber optic bundle, said array comprises at least three non-linear fiducials, and each of said fiducials is a fiducial fiber.

6. An array composition according to claim 5 wherein at least one of said fiducial fibers has a different shape from the others.

7. An array composition according to claim 1 wherein said fiducial is a defined edge of said substrate.

8. An array composition according to claim 1 wherein said fiducial is a fiducial bead.

9. An array composition according to claim 1 wherein said bioactive agents are nucleic acids.

10. An array composition according to claim 1 wherein said bioactive agents are proteins.

11. An array composition according to claim 1 further comprising a computer readable memory comprising:

a) computer code that receives a first data image; and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2934

b) computer code that registers said first data image using said fiducial to generate a first registered data image.

12. An array composition according to claim 11 wherein said computer readable memory further comprises:

a) computer code that receives a second data image;

b) computer code that registers said second data image using said fiducial to generate a second registered data image; and

c) computer code that compares said first and said second data image.

13. A composition comprising a computer readable memory to direct a computer to function in a specified manner, said computer readable memory comprising:

a) an acquisition module for receiving a data image of a random array comprising a plurality of discrete sites;

b) a registration module for registering a data image; and

c) a comparison module for comparing registered data images.

14. A composition according to claim 13 wherein said random array comprises a fiber optic bundle and said registration module utilizes a fiducial fiber for registration.

15. A composition according to claim 13 wherein said random array comprises microspheres and said registration module utilizes a fiducial microsphere for registration.

16. A composition according to claim 13 wherein said registration module utilizes a fiducial template for registration.

17. A composition according to claim 13 further comprising a random array comprising:

a) a substrate with a surface comprising discrete sites; and

b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface.

18. A method of making an array composition comprising:

a) forming a surface comprising individual sites on a substrate;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2935

b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising a bioactive agent; and

c) incorporating at least one fiducial onto said surface.

19. A method according to claim 18 wherein said subpopulations further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

20. A method according to claim 18 wherein said subpopulations further comprise an optical signature such that the identification of the bioactive agent can be elucidated.

21. A method according to claim 18 wherein said substrate is a fiber optic bundle and said fiducial is a fiducial fiber.

22. A method according to claim 18 wherein said substrate is a fiber optic bundle, said array comprises at least three non-linear fiducials, and each of said fiducials is a fiducial fiber.

23. A method according to claim 122 wherein at least one of said fiducial fibers has a different shape from the others.

24. A method according to claim 18 wherein said fiducial is a defined edge of said substrate.

25. A method according to claim 18 wherein said fiducial is a fiducial bead.

26. A method according to claim 18 wherein said bioactive agents are nucleic acids.

27. A method according to claim 18 wherein said bioactive agents are proteins.

28. A method for comparing separate data images of a random array comprising:

a) using a computer system to register a first data image of said random array to produce a registered first data image;

b) using said computer system to register a second data image of said random array to produce a registered second data image; and

c) comparing said first and said second registered data image to determine any differences between them.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2936

29. A method according to claim 28 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

30. A method according to claim 28 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

31. A method according to claim 28 wherein the the registration of said first data image utilizes a fiducial template.

32. A method of decoding a random array composition comprising

a) providing a random array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface;

b) adding a first plurality of decoding binding ligands to said array composition and creating a first data image;

c) using a fiducial to generate a first registered data image;

d) adding a second plurality of decoding binding ligands to said array composition and creating a second data image;

e) using said fiducial to generate a second registered data image; and

f) using a computer system to compare said first and said second registered data image to identify the location of at least two bioactive agents.

33. A method according to claim 32 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

34. A method according to claim 32 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

35. A method according to claim 32 wherein the the registration of said first data image utilizes a fiducial template.

36. A method according to claim 32 wherein said bioactive agents are proteins.

37. A method according to claim 32 wherein said bioactive agents are nucleic acids.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2937

38. A method of determining the presence of a target analyte in a sample comprising:

a) acquiring a first data image of a random array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent;

wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres;

b) registering said first data image to create a registered first data image;

c) contacting said random array composition with said sample;

d) acquiring a second data image from said array with said sample;

e) registering said second data image to create a registered second data image; and

f) comparing said first and said second registered data images to determine the presence or absence of said target analyte.

39. A method according to claim 38 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

40. A method according to claim 38 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

41. A method according to claim 38 wherein the the registration of said first data image utilizes a fiducial template.

42. A method according to claim 38 wherein said bioactive agents are proteins.

43. A method according to claim 38 wherein said bioactive agents are nucleic acids.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2938

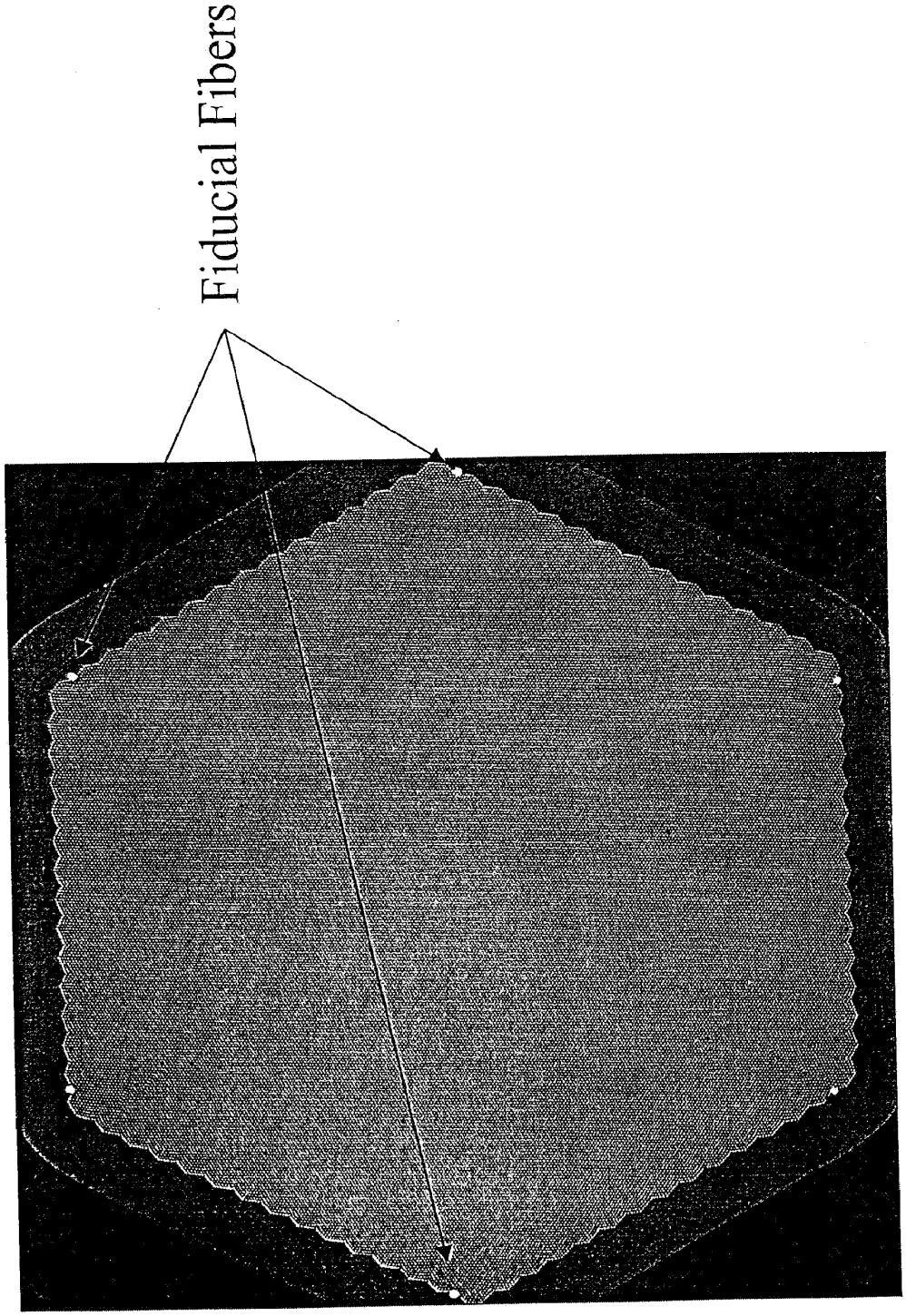


Figure 1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2939

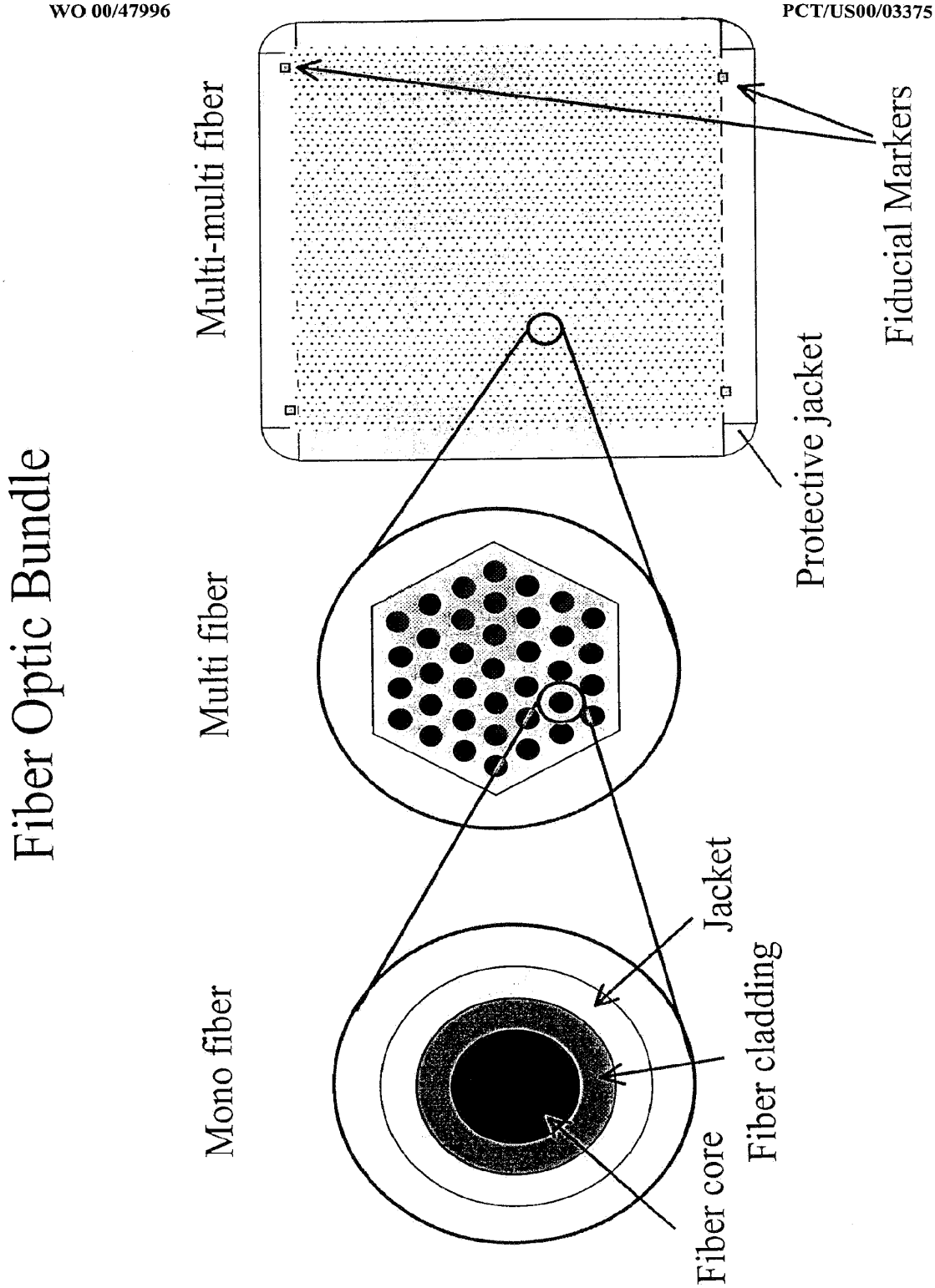


Figure 2

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2940

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 21/64, 21/77 | A1

(11) International Publication Number: **WO 00/04372**

(43) International Publication Date: 27 January 2000 (27.01.00)

(21) International Application Number: PCT/US99/16162

(22) International Filing Date: 16 July 1999 (16.07.99)

(30) Priority Data:

60/093,111	16 July 1998 (16.07.98)	US
09/287,248	7 April 1999 (07.04.99)	US

(71) Applicant: THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors: MCDEVITT, John, T.; 4812 Eagle Feather Drive, Austin, TX 78735 (US). ANSLYN, Eric, V.; 8323 Young Lane, Austin, TX 78737 (US). SHEAR, Jason, B.; 4433 Sacred Arrow Drive, Austin, TX 78735 (US). NEIKIRK, Dean, P.; 6604 Auburnhill, Austin, TX 78723 (US).

(74) Agent: CONLEY, ROSE & TAYON, P.C.; Suite 320, 816 Congress Avenue, Austin, TX 78701-2443 (US).

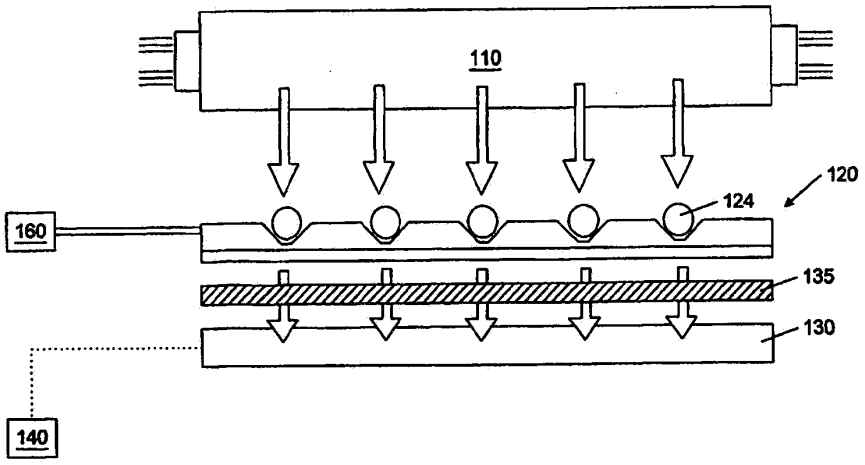
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SENSOR ARRAYS FOR THE MEASUREMENT AND IDENTIFICATION OF MULTIPLE ANALYTES IN SOLUTIONS



(57) Abstract

A system for the rapid characterization of multi-analyte fluids, in one embodiment, includes a light source, a sensor array, and a detector. The sensor array is formed from a supporting member into which a plurality of cavitites may be formed. A series of chemically sensitive particles microspheres are, in one embodiment positioned within the cavities. The particles may be configured to produce a signal when a receptor coupled to the particle interacts with the analyte. Using pattern recognition techniques, the analytes within a multi-analyte fluid may be characterized.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2942

TITLE: SENSOR ARRAYS FOR THE MEASUREMENT AND IDENTIFICATION OF MULTIPLE ANALYTES IN SOLUTIONS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Research leading to this invention was federally supported, in part, by grant No. 1R01GM57306-01 entitled "The Development of an Electronic Tongue" from the National Institute of Health and the U.S. Government has certain rights to this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method and device for the detection of analytes in a fluid. More particularly, the invention relates to the development of a sensor array system capable of discriminating mixtures of analytes, toxins, and/or bacteria in medical, food/beverage, and environmental solutions.

2. Brief Description of the Related Art

The development of smart sensors capable of discriminating different analytes, toxins, and bacteria has become increasingly important for clinical, environmental, health and safety, remote sensing, military, food/beverage and chemical processing applications. Although many sensors capable of high sensitivity and high selectivity detection have been fashioned for single analyte detection, only in a few selected cases have array sensors been prepared which display solution phase multi-analyte detection capabilities. The advantages of such array systems are their utility for the analysis of multiple analytes and their ability to be "trained" to respond to new stimuli. Such on site adaptive analysis capabilities afforded by the array structures make their utilization promising for a variety of future applications. Array based sensors displaying the capacity to sense and identify complex vapors have been demonstrated recently using a number of distinct transduction schemes. For example, functional sensors based on Surface Acoustic Wave (SAW), tin oxide (SnO_2) sensors, conductive organic polymers, and carbon black/polymer composites have been fashioned. The use of tin oxide sensors, for example, is described in U.S. Patent No. 5,654,497 to Hoffheins et al. These sensors display the capacity to identify and discriminate between a variety of organic vapors by virtue of small site-to-site differences in response characteristics. Pattern recognition of the overall fingerprint response for the array serves as the basis for an olfaction-like detection of the vapor phase analyte species. Indeed, several commercial "electronic noses" have been developed recently. Most of the well established sensing elements are based on SnO_2 arrays which have been derivatized so as to yield chemically distinct response properties. Arrays based on SAW crystals yield extremely sensitive responses to vapor, however, engineering challenges have prevented the creation of large SAW arrays having multiple sensor sites. To our knowledge, the largest SAW device reported to date possesses only 12 sensor elements. Additionally, limited chemical diversity and the lack of understanding of the molecular features of such systems makes their expansion into more complex analysis difficult.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2943

Other structures have been developed that are capable of identifying and discriminating volatile organic molecules. One structure involves a series of conductive polymer layers deposited onto metal contacting layers. When these sensors are exposed to volatile reagents, some of the volatile reagents adsorb into the polymer layers, leading to small changes in the electrical resistance of these layers. It is the small differences in the behavior of the various sites that allows for a discrimination, identification, and quantification of the vapors. The detection process takes only a few seconds, and sensitivities of part-per-billion can be achieved with this relatively simple approach. This "electronic nose" system is described in U.S. Patent No. 5,698,089 to Lewis et al. which is incorporated by reference as if set forth herein.

Although the above described electronic nose provides an impressive capability for monitoring volatile reagents, the system possesses a number of undesirable characteristics that warrant the development of alternative sensor array systems. For example, the electronic nose can be used only for the identification of volatile reagents. For many environmental, military, medical, and commercial applications, the identification and quantification of analytes present in liquid or solid-phase samples is necessary. Moreover, the electronic nose systems are expensive (e.g., the Aromascan system costs about $50,000/unit) and bulky ($\geq 1 ft^3$). Furthermore, the functional elements for the currently available electronic nose are composed of conductive polymer systems which possess little chemical selectivity for many of the analytes which are of interest to the military and civilian communities.

One of the most commonly employed sensing techniques has exploited colloidal polymer microspheres for latex agglutination tests (LATs) in clinical analysis. Commercially available LATs for more than 60 analytes are used routinely for the detection of infectious diseases, illegal drugs, and early pregnancy tests. The vast majority of these types of sensors operate on the principle of agglutination of latex particles (polymer microspheres) which occurs when the antibody-derivatized microspheres become effectively "cross-linked" by a foreign antigen resulting in the attachment to, or the inability to pass through a filter. The dye-doped microspheres are then detected colorimetrically upon removal of the antigen carrying solution. However, the LATs lack the ability to be utilized for multiple, real time analyte detection schemes as the nature of the response intrinsically depends on a cooperative effect of the entire collection of microspheres.

Similar to the electronic nose, array sensors that have shown great analytical promise are those based on the "DNA on a chip" technology. These devices possess a high density of DNA hybridization sites that are affixed in a two-dimensional pattern on a planar substrate. To generate nucleotide sequence information, a pattern is created from unknown DNA fragments binding to various hybridization sites. Both radiochemical and optical methods have provided excellent detection limits for analysis of limited quantities of DNA. (Stimpson, D. I.; Hoijer, J. V.; Hsieh, W.; Jou, C.; Gardon, J.; Theriault, T.; Gamble, R.; Baldeschwieler, J.D. Proc. Natl. Acad. Sci. USA 1995, 92, 6379). Although quite promising for the detection of DNA fragments, these arrays are generally not designed for non-DNA molecules, and accordingly show very little sensitivity to smaller organic molecules. Many of the target molecules of interest to civilian and military communities, however, do not possess DNA components. Thus, the need for a flexible, non-DNA based sensor is still desired. Moreover, while a number of prototype DNA chips containing up to a few thousand different nucleic acid probes have been described, the existing technologies tend to be difficult to expand to a practical size. As a result, DNA chips may be prohibitively expensive for practical uses.

A system of analyzing fluid samples using an array formed of heterogeneous, semi-selective thin films

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2944

which function as sensing receptor units is described in U.S. Patent No. 5,512,490 to Walt et al., which is incorporated by reference as if set forth herein. Walt appears to describe the use of covalently attached polymeric "cones" which are grown via photopolymerization onto the distal face of fiber optic bundles. These sensor probes appear to be designed with the goal of obtaining unique, continuous, and reproducible responses from small localized regions of dye-doped polymer. The polymer appears to serve as a solid support for indicator molecules that provide information about test solutions through changes in optical properties. These polymer supported sensors have been used for the detection of analytes such as pH, metals, and specific biological entities. Methods for manufacturing large numbers of reproducible sensors, however, has yet to be developed. Moreover, no methods for acquisitions of data streams in a simultaneous manner are commercially available with this system. Optical alignment issues may also be problematic for these systems.

A method of rapid sample analysis for use in the diagnostic microbiology field is also desirable. The techniques now used for rapid microbiology diagnostics detect either antigens or nucleic acids. Rapid antigen testing is based on the use of antibodies to recognize either the single cell organism or the presence of infected cell material. Inherent to this approach is the need to obtain and characterize the binding of the antibody to unique structures on the organism being tested. Since the identification and isolation of the appropriate antibodies is time consuming, these techniques are limited to a single agent per testing module and there is no opportunity to evaluate the amount of agent present.

Most antibody methods are relatively insensitive and require the presence of 10^5 to 10^7 organisms. The response time of antibody-antigen reactions in diagnostic tests of this type ranges from 10 to 120 minutes, depending on the method of detection. The fastest methods are generally agglutination reactions, but these methods are less sensitive due to difficulties in visual interpretation of the reactions. Approaches with slower reaction times include antigen recognition by antibody conjugated to either an enzyme or chromophore. These test types tend to be more sensitive, especially when spectrophotometric methods are used to determine if an antigen-antibody reaction has occurred. These detection schemes do not, however, appear to allow the simultaneous detection of multiple analytes on a single detector platform.

The alternative to antigen detection is the detection of nucleic acids. An approach for diagnostic testing with nucleic acids uses hybridization to target unique regions of the target organism. These techniques require fewer organisms (10^3 to 10^5), but require about five hours to complete. As with antibody-antigen reactions this approach has not been developed for the simultaneous detection of multiple analytes.

The most recent improvement in the detection of microorganisms has been the use of nucleic acid amplification. Nucleic acid amplification tests have been developed that generate both qualitative and quantitative data. However, the current limitations of these testing methods are related to delays caused by specimen preparation, amplification, and detection. Currently, the standard assays require about five hours to complete. The ability to complete much faster detection for a variety of microorganisms would be of tremendous importance to military intelligence, national safety, medical, environmental, and food areas.

It is therefore desirable that new sensors capable of discriminating different analytes, toxins, and bacteria be developed for medical/clinical diagnostic, environmental, health and safety, remote sensing, military, food/beverage, and chemical processing applications. It is further desired that the sensing system be adaptable to

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2945

the simultaneous detection of a variety of analytes to improve throughput during various chemical and biological analytical procedures.

SUMMARY OF THE INVENTION

Herein we describe a system and method for the analysis of a fluid containing one or more analytes. The system may be used for either liquid or gaseous fluids. The system, in some embodiments, may generate patterns that are diagnostic for both the individual analytes and mixtures of the analytes. The system in some embodiments, is made of a plurality of chemically sensitive particles, formed in an ordered array, capable of simultaneously detecting many different kinds of analytes rapidly. An aspect of the system is that the array may be formed using a microfabrication process, thus allowing the system to be manufactured in an inexpensive manner.

In an embodiment of a system for detecting analytes, the system, in some embodiments, includes a light source, a sensor array, and a detector. The sensor array, in some embodiments, is formed of a supporting member which is configured to hold a variety of chemically sensitive particles (herein referred to as "particles") in an ordered array. The particles are, in some embodiments, elements which will create a detectable signal in the presence of an analyte. The particles may produce optical (e.g., absorbance or reflectance) or fluorescence/phosphorescent signals upon exposure to an analyte. Examples of particles include, but are not limited to functionalized polymeric beads, agarous beads, dextrose beads, polyacrylamide beads, control pore glass beads, metal oxides particles (e.g., silicon dioxide (SiO_2) or aluminum oxides (Al_2O_3)), polymer thin films, metal quantum particles (e.g., silver, gold, platinum, etc.), and semiconductor quantum particles (e.g., Si, Ge, GaAs, etc.). A detector (e.g., a charge-coupled device "CCD") in one embodiment is positioned below the sensor array to allow for the data acquisition. In another embodiment, the detector may be positioned above the sensor array to allow for data acquisition from reflectance of the light off of the particles.

Light originating from the light source may pass through the sensor array and out through the bottom side of the sensor array. Light modulated by the particles may pass through the sensor array and onto the proximally spaced detector. Evaluation of the optical changes may be completed by visual inspection or by use of a CCD detector by itself or in combination with an optical microscope. A microprocessor may be coupled to the CCD detector or the microscope. A fluid delivery system may be coupled to the supporting member of the sensor array. The fluid delivery system, in some embodiments, is configured to introduce samples into and out of the sensor array.

In an embodiment, the sensor array system includes an array of particles. The particles may include a receptor molecule coupled to a polymeric bead. The receptors, in some embodiments, are chosen for interacting with analytes. This interaction may take the form of a binding/association of the receptors with the analytes. The supporting member may be made of any material capable of supporting the particles, while allowing the passage of the appropriate wavelengths of light. The supporting member may include a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity.

In an embodiment, the optical detector may be integrated within the bottom of the supporting member, rather than using a separate detecting device. The optical detectors may be coupled to a microprocessor to allow evaluation of fluids without the use of separate detecting components. Additionally, a fluid delivery system may

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2946

also be incorporated into the supporting member. Integration of detectors and a fluid delivery system into the supporting member may allow the formation of a compact and portable analyte sensing system.

A high sensitivity CCD array may be used to measure changes in optical characteristics which occur upon binding of the biological/chemical agents. The CCD arrays may be interfaced with filters, light sources, fluid delivery and micromachined particle receptacles, so as to create a functional sensor array. Data acquisition and handling may be performed with existing CCD technology. CCD detectors may be configured to measure white light, ultraviolet light or fluorescence. Other detectors such as photomultiplier tubes, charge induction devices, photo diodes, photodiode arrays, and microchannel plates may also be used.

A particle, in some embodiments, possess both the ability to bind the analyte of interest and to create a modulated signal. The particle may include receptor molecules which posses the ability to bind the analyte of interest and to create a modulated signal. Alternatively, the particle may include receptor molecules and indicators. The receptor molecule may posses the ability to bind to an analyte o f interest. Upon binding the analyte of interest, the receptor molecule may cause the indicator molecule to produce the modulated signal. The receptor molecules may be naturally occurring or synthetic receptors formed by rational design or combinatorial methods. Some examples of natural receptors include, but are not limited to, DNA, RNA, proteins, enzymes, oligopeptides, antigens, and antibodies. Either natural or synthetic receptors may be chosen for their ability to bind to the analyte molecules in a specific manner.

In one embodiment, a naturally occurring or synthetic receptor is bound to a polymeric bead in order to create the particle. The particle, in some embodiments, is capable of both binding the analyte(s) of interest and creating a detectable signal. In some embodiments, the particle will create an optical signal when bound to an analyte of interest.

A variety of natural and synthetic receptors may be used. The synthetic receptors may come from a variety of classes including, but not limited to, polynucleotides (e.g., aptamers), peptides (e.g., enzymes and antibodies), synthetic receptors, polymeric unnatural biopolymers (e.g., polythioureas, polyguanidiniums), and imprinted polymers. Polynucleotides are relatively small fragments of DNA which may be derived by sequentially building the DNA sequence. Peptides include natural peptides such as antibodies or enzymes or may be synthesized from amino acids. Unnatural biopolymers are chemical structure which are based on natural biopolymers, but which are built from unnatural linking units. For example, polythioureas and polyguanidiniums have a structure similar to peptides, but may be synthesized from diamines (i.e., compounds which include at least two amine functional groups) rather than amino acids. Synthetic receptors are designed organic or inorganic structures capable of binding various analytes.

In an embodiment, a large number of chemical/biological agents of interest to the military and civilian communities may be sensed readily by the described array sensors. Bacteria may also be detected using a similar system. To detect, sense, and identify intact bacteria, the cell surface of one bacteria may be differentiated from other bacteria, or genomic material may be detected using oligonucleic receptors. One method of accomplishing this differentiation is to target cell surface oligosaccharides (i.e., sugar residues). The use of synthetic receptors which are specific for oligosaccharides may be used to determine the presence of specific bacteria by analyzing for cell surface oligosaccharides.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2947

BRIEF DESCRIPTION OF THE DRAWINGS

The above brief description as well as further objects, features and advantages of the methods and apparatus of the present invention will be more fully appreciated by reference to the following detailed description of presently preferred but nonetheless illustrative embodiments in accordance with the present invention when taken in conjunction with the accompanying drawings in which:

FIG. 1 depicts a schematic of an analyte detection system;

FIG. 2 depicts a particle disposed in a cavity;

FIG. 3 depicts a sensor array;

FIG. 4A-F depicts the formation of a Fabry-Perot cavity on the back of a sensor array;

FIG. 5 depicts the chemical constituents of a particle;

FIG. 6 depicts the chemical formulas of some receptor compounds;

FIG. 7 depicts a plot of the absorbance of green light vs. concentration of calcium (Ca^{+2}) for a particle which includes an o-cresolphthalein complexone receptor;

FIG. 8 depicts a schematic view of the transfer of energy from a first indicator to a second indicator in the presence of an analyte;

FIG. 9 depicts a schematic of the interaction of a sugar molecule with a boronic acid based receptor.

FIG. 10 depicts various synthetic receptors;

FIG. 11 depicts a synthetic pathway for the synthesis of polythioureas;

FIG. 12 depicts a synthetic pathway for the synthesis of polyguanidiniums;

FIG. 13 depicts a synthetic pathway for the synthesis of diamines from amino acids;

FIG. 14 depicts fluorescent diamino monomers;

FIG. 15 depicts a plot of counts/sec. (i.e., intensity) vs. time as the pH of a solution surrounding a particle coupled to *o*-cresolphthalein is cycled from acidic to basic conditions;

FIG. 16 depicts the color responses of a variety of sensing particles to solutions of Ca^{+2} and various pH levels;

FIG. 17 depicts an analyte detection system which includes a sensor array disposed within a chamber;

FIG. 18 depicts an integrated analyte detection system;

FIG. 19 depicts a cross-sectional view of a cavity covered by a mesh cover;

FIG. 20 depicts a top view of a cavity covered by a mesh cover;

FIG. 21A-G depicts a cross-sectional view of a series of processing steps for the formation of a sensor array which includes a removable top and bottom cover;

FIG. 22A-G depicts a cross-sectional view of a series of processing steps for the formation of a sensor array which includes a removable top and a stationary bottom cover;

FIG. 23A-G depicts a cross-sectional view of a series of processing steps for the formation of a sensor array which includes a removable top;

FIG. 24A-D depicts a cross-sectional view of a series of processing steps for the formation of a silicon based sensor array which includes a top and bottom cover with openings aligned with the cavity;

FIG. 25A-D depicts a cross-sectional view of a series of processing steps for the formation of a photoresist based sensor array which includes a top and bottom cover with openings aligned with the cavity;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2948

FIG. 26A-E depicts a cross-sectional view of a series of processing steps for the formation of a plastic based sensor array which includes a top and bottom cover with openings aligned with the cavity;

FIG. 27A-D depicts a cross-sectional view of a series of processing steps for the formation of a silicon based sensor array which includes a top cover with openings aligned with the cavity and a tapered cavity;

FIG. 28A-E depicts a cross-sectional view of a series of processing steps for the formation of a photoresist based sensor array which includes a top cover with openings aligned with the cavity and a tapered cavity;

FIG. 29A-E depicts a cross-sectional view of a series of processing steps for the formation of a photoresist based sensor array which includes a top cover with openings aligned with the cavity and a bottom cover;

FIG. 30A-D depicts a cross-sectional view of a series of processing steps for the formation of a plastic based sensor array which includes a top cover with openings aligned with the cavity and a bottom cover;

FIG. 31 depicts a cross-sectional view of a schematic of a micropump;

FIG. 32 depicts a top view of an electrohydrodynamic pump;

FIG. 33 depicts a cross-sectional view of a sensor array which includes a micropump;

FIG. 34 depicts a cross-sectional view of a sensor array which includes a micropump and channels which are coupled to the cavities;

FIG. 35 depicts a cross-sectional view of a sensor array which includes multiple micropumps each micropump being coupled to a cavity;

FIG. 36 depicts a top view of a sensor array which includes multiple electrohydrodynamic pumps;

FIG. 37 depicts a cross-sectional view of a sensor array which includes a system for delivering a reagent from a reagent particle to a sensing cavity.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Herein we describe a system and method for the simultaneous analysis of a fluid containing multiple analytes. The system may be used for either liquid or gaseous fluids. The system may generate patterns that are diagnostic for both individual analytes and mixtures of the analytes. The system, in some embodiments, is made of a combination of chemically sensitive particles, formed in an ordered array, capable of simultaneously detecting many different kinds of analytes rapidly. An aspect of the system is that the array may be formed using a microfabrication process, thus allowing the system to be manufactured in an inexpensive manner.

SYSTEM FOR ANALYSIS OF ANALYTES

Shown in FIG. 1 is an embodiment of a system for detecting analytes in a fluid. The system, in some embodiments, includes a light source 110, a sensor array 120 and a detector 130. The light source 110 may be a white light source or light emitting diodes (LED). In one embodiment, light source 110 may be a blue light emitting diode (LED) for use in systems relying on changes in fluorescence signals. For colorimetric (e.g., absorbance) based systems, a white light source may be used. The sensor array 120, in some embodiments, is formed of a supporting member which is configured to hold a variety of particles 124. A detecting device 130 (e.g., a charge-coupled device "CCD") may be positioned below the sensor array to allow for data acquisition. In another embodiment, the detecting device 130 may be positioned above the sensor array.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2949

Light originating from the light source 110, in some embodiments, passes through the sensor array 120 and out through the bottom side of the sensor array. The supporting member and the particles together, in some embodiments, provide an assembly whose optical properties are well matched for spectral analyses. Thus, light modulated by the particles may pass through the sensor array and onto the proximally spaced detector 130. Evaluation of the optical changes may be completed by visual inspection (e.g., with a microscope) or by use of a microprocessor 140 coupled to the detector. For fluorescence measurements, a filter 135 may be placed between supporting member 120 and detector 130 to remove the excitation wavelength. A fluid delivery system 160 may be coupled to the supporting member. The fluid delivery system 160 may be configured to introduce samples into and out of the sensor array.

In an embodiment, the sensor array system includes an array of particles. Upon the surface and within the interior region of the particles are, in some embodiments, located a variety of receptors for interacting with analytes. The supporting member, in some embodiments, is used to localize these particles as well as to serve as a microenvironment in which the chemical assays can be performed. For the chemical/biological agent sensor arrays, the particles used for analysis are about 0.05 - 500 microns in diameter, and may actually change size (e.g., swell or shrink) when the chemical environment changes. Typically, these changes occur when the array system is exposed to the fluid stream which includes the analytes. For example, a fluid stream which comprises a non-polar solvent, may cause non-polar particles to change in volume when the particles are exposed to the solvent. To accommodate these changes, it is preferred that the supporting member consist of an array of cavities which serve as micro test-tubes.

The supporting member may be made of any material capable of supporting the particles, while allowing the passage of the appropriate wavelength of light. The supporting member is also made of a material substantially impervious to the fluid in which the analyte is present. A variety of materials may be used including plastics, glass, silicon based materials (e.g., silicon, silicon dioxide, silicon nitride, etc.) and metals. In one embodiment, the supporting member includes a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity. Alternatively, a plurality of particles may be contained within a single cavity.

In an embodiment, the supporting member may consist of a strip of plastic which is substantially transparent to the wavelength of light necessary for detection. A series of cavities may be formed within the strip. The cavities may be configured to hold at least one particle. The particles may be contained within the strip by a transparent cover which is configured to allow passage of the analyte containing fluid into the cavities.

In another embodiment, the supporting member may be formed using a silicon wafer as depicted in FIG. 2. The silicon wafer 210 may include a substantially transparent layer 220 formed on the bottom surface of the wafer. The cavities 230, in one embodiment, are formed by an anisotropic etch process of the silicon wafer. In one embodiment, anisotropic etching of the silicon wafer is accomplished using a wet hydroxide etch. Photolithographic techniques may be used to define the locations of the cavities. The cavities may be formed such that the sidewalls of the cavities are substantially tapered at an angle of between about 50 to 60 degrees. Formation of such angled cavities may be accomplished by wet anisotropic etching of <100> silicon. The term "<100> silicon" refers to the crystal orientation of the silicon wafer. Other types of silicon, (e.g., <110> and <111> silicon) may lead to steeper angled sidewalls. For example, <111> silicon may lead to sidewalls formed at about 90

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2950

degrees. The angled sides of the cavities in some embodiments, serve as "mirror layers" which may improve the light collection efficiency of the cavities. The etch process may be controlled so that the formed cavities extend through the silicon wafer to the upper surface of transparent layer 220. While depicted as pyramidal, the cavities may be formed in a number of shapes including but not limited to, spherical, oval, cubic, or rectangular. An advantage to using a silicon wafer for the support member, is that the silicon material is substantially opaque to the light produced from the light source. Thus, the light may be inhibited from passing from one cavity to adjacent cavities. In this manner, light from one cavity may be inhibited from influencing the spectroscopic changes produced in an adjacent cavity.

The silicon wafer, in some embodiments, has an area of approximately 1 cm^2 to about 100 cm^2 and includes about 10^1 to about 10^6 cavities. In an embodiment, about 100 cavities are formed in a ten by ten matrix. The center to center distance between the cavities, in some embodiments, is about 500 microns. Each of the cavities may include at least one particle.

The transparent layer 220 may serve as a window, allowing light of a variety of wavelengths to pass through the cavities 230 and to the detector. Additionally, the transparent layer may serve as a platform onto which the individual particles 235 may be positioned. The transparent layer may be formed of silicon dioxide (SiO_2), silicon nitride (Si_3N_4) or silicon dioxide/silicon nitride multi-layer stacks. The transparent layer, in some embodiments, is deposited onto the silicon wafer prior to the formation of the cavities.

The cavities 230 may be sized to substantially contain a particle 235. The cavities are, in some embodiments, larger than a particle. The cavities are, in some embodiments, sized to allow facile placement and removal of the particle within the cavities. The cavity may be substantially larger than the particle, thus allowing the particle to swell during use. For example, a particle may have a size as depicted in FIG. 2 by particle 235. During use, contact with a fluid (e.g., a solvent) may cause the particle to swell, for example, to a size depicted as circle 236. In some embodiments, the cavity is sized to allow such swelling of the particle during use. A particle may be positioned at the bottom of a cavity using, e.g., a micromanipulator. After a particle has been placed within the cavity, a transparent cover plate 240 may be placed on top of the supporting member to keep the particle in place.

When forming an array which includes a plurality of particles, the particles may be placed in the array in an ordered fashion using the micromanipulator. In this manner, a ordered array having a predefined configuration of particles may be formed. Alternatively, the particles may be randomly placed within the cavities. The array may subsequently undergo a calibration test to determine the identity of the particle at any specified location in the supporting member.

The transparent cover plate 240, in some embodiments, is coupled to the upper surface of the silicon wafer 220 such that the particles are inhibited from becoming dislodged from the cavity. The transparent cover plate, in some embodiments, is positioned a fixed distance above the silicon wafer, as depicted in FIG. 2, to keep the particle in place, while allowing the entrance of fluids into the cavities. The transparent cover plate, in some embodiments, is positioned at a distance above the substrate which is substantially less than a width of the particle. The transparent cover plate may be made of any material which is substantially transparent to the wavelength of light being utilized by the detector. The transparent cover plate may be made of plastic, glass, quartz, or silicon dioxide/silicon nitride.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2951

In one embodiment, the transparent cover plate 240, is a thin sheet of glass (e.g., a microscope slide cover slip). The slide may be positioned a fixed distance above the silicon wafer. Support structures 241 (See FIG. 2) may be placed upon the silicon wafer 210 to position the transparent cover plate 240. The support structures may be formed from a polymer or a silicon based material. In another embodiment, a polymeric substrate is coupled to the silicon wafer to form the support structures 241 for the transparent cover plate 240. In an embodiment, a plastic material with an adhesive backing (e.g., cellophane tape) is positioned on the silicon wafer 210. After the support structures 241 are placed on the wafer the transparent cover plate 240 is placed upon the support structures. The support structures inhibit the transparent cover sheet from contacting the silicon wafer 200. In this manner, a channel is formed between the silicon wafer and the transparent cover plate which allow the fluid to pass into the cavity, while inhibiting displacement of the particle by the fluid.

In another embodiment, the transparent cover plate 240 may be fastened to the upper surface of the silicon wafer, as depicted in FIG. 3. In this embodiment, the fluid may be inhibited from entering the cavities 230 by the transparent cover plate 240. To allow passage of the fluid into the cavities, a number of channels 250 may be formed in the silicon wafer. The channels, in one embodiment, are oriented to allow passage of the fluid into substantially all of the cavities. When contacted with the fluid, the particles may swell to a size which may plug the channels. To prevent this plugging, the channels may be formed near the upper portion of the cavities, as depicted in FIG 3. The channels, in one embodiment, are formed using standard photolithographic masking to define the regions where the trenches are to be formed, followed by the use of standard etching techniques. A depth of the cavity may be such that the particle resides substantially below the position of the channel. In this way, the plugging of the channels due to swelling of the particle may be prevented.

The inner surfaces of the cavities may be coated with a material to aid the positioning of the particles within the cavities. In one embodiment, a thin layer of gold or silver may be used to line the inner surface of the cavities. The gold or silver layer may act as an anchoring surface to anchor particles (e.g., via alkylthiol bonding). In addition, the gold or silver layer may also increase the reflectivity of the inner surface of the cavities. The increased reflectance of the surface may enhance the analyte detection sensitivity of the system. Alternatively, polymer layers and self-assembled monolayers formed upon the inner surface of the cavities may be used to control the particle adhesion interactions. Additional chemical anchoring methods may be used for silicon surfaces such as those based on siloxane type reagents, which may be attached to Si-OH functionalities. Similarly, monomeric and polymeric reagents attached to an interior region of the cavities can be used to alter the local wetting characteristics of the cavities. This type of methodology can be used to anchor the particles as well as to alter the fluid delivery characteristics of the cavity. Furthermore, amplification of the signals for the analytes may be accomplished with this type of strategy by causing preconcentration of appropriate analytes in the appropriate type of chemical environment.

In another embodiment, the optical detector may be integrated within the bottom transparent layer 220 of the supporting member, rather than using a separate detecting device. The optical detectors may be formed using a semiconductor-based photodetector 255. The optical detectors may be coupled to a microprocessor to allow evaluation of fluids without the use of separate detecting components. Additionally, the fluid delivery system may also be incorporated into the supporting member. Micro-pumps and micro-valves may also be incorporated into the silicon wafer to aid passage of the fluid through the cavities. Integration of detectors and a fluid delivery system

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2952

into the supporting member may allow the formation of a compact and portable analyte sensing system. Optical filters may also be integrated into the bottom membrane of the cavities. These filters may prevent short wavelength excitation from producing "false" signals in the optical detection system (e.g., a CCD detector array) during fluorescence measurements.

A sensing cavity may be formed on the bottom surface of the support substrate. An example of a sensing cavity that may be used is a Fabry-Perot type cavity. Fabry-Perot cavity-based sensors may be used to detect changes in optical path length induced by either a change in the refractive index or a change in physical length of the cavity. Using micromachining techniques, Fabry-Perot sensors may be formed on the bottom surface of the cavity.

Figures 4A-F depict a sequence of processing steps for the formation of a cavity and a planar top diaphragm Fabry-Perot sensor on the bottom surface of a silicon based supporting member. A sacrificial barrier layer 262a/b is deposited upon both sides of a silicon supporting member 260. The silicon supporting member 260 may be a double-side polished silicon wafer having a thickness ranging from about 100 μm to about 500 μm, preferably from about 200 μm to about 400 μm, and more preferably of about 300 μm. The barrier layer 262a/b may be composed of silicon dioxide, silicon nitride, or silicon oxynitride. In one embodiment, the barrier layer 262a/b is composed of a stack of dielectric materials. As depicted in FIG 4A, the barrier layer 262 a/b is composed of a stack of dielectric materials which includes a silicon nitride layer 271a/b and a silicon dioxide layer 272a/b. Both layers may be deposited using a low pressure chemical vapor deposition ("LPCVD") process. Silicon nitride may be deposited using an LPCVD reactor by reaction of ammonia (NH_3) and dichlorosilane ($SiCl_2H_2$) at a gas flow rate of about 3.5:1, a temperature of about 800 □C, and a pressure of about 220 mTorr. The silicon nitride layer 271a/b is deposited to a thickness in the range from about 100 Å to about 500 Å, preferably from 200 Å to about 400 Å, and more preferably of about 300 Å. Silicon dioxide is may be deposited using an LPCVD reactor by reaction of silane (SiH_4) and oxygen (O_2) at a gas flow rate of about 3:4, a temperature of about 450 □C, and a pressure of about 110 mTorr. The silicon dioxide layer 272a/b is deposited to a thickness in the range from about 3000 Å to about 7000 Å, preferably from 4000 Å to about 6000 Å, and more preferably of about 5000 Å. The front face silicon dioxide layer 272a, in one embodiment, acts as the main barrier layer. The underlying silicon nitride layer 271a acts as an intermediate barrier layer to inhibit overetching of the main barrier layer during subsequent KOH wet anisotropic etching steps.

A bottom diaphragm layer 264a/b is deposited upon the barrier layer 262a/b on both sides of the supporting member 260. The bottom diaphragm layer 264a/b may be composed of silicon nitride, silicon dioxide, or silicon oxynitride. In one embodiment, the bottom diaphragm layer 264 a/b is composed of a stack of dielectric materials. As depicted in FIG 4A, the bottom diaphragm layer 264a/b is composed of a stack of dielectric materials which includes a pair of silicon nitride layers 273a/b and 275a/b surrounding a silicon dioxide layer 274a/b. All of the layers may be deposited using an LPCVD process. The silicon nitride layers 273a/b and 275a/b have a thickness in the range from about 500 Å to about 1000 Å, preferably from 700 Å to about 800 Å, and more preferably of about 750 Å. The silicon dioxide layer 274a/b has a thickness in the range from about 3000 Å to about 7000 Å, preferably from 4000 Å to about 6000 Å, and more preferably of about 4500 Å.

A cavity which will hold the particle may now be formed in the supporting member 260. The bottom diaphragm layer 264b and the barrier layer 262b formed on the back side 261 of the silicon supporting member 260

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2953

are patterned and etched using standard photolithographic techniques. In one embodiment, the layers are subjected to a plasma etch process. The plasma etching of silicon dioxide and silicon nitride may be performed using a mixture of carbontetrafluoride (CF_4) and oxygen (O_2). The patterned back side layers 262b and 264b may be used as a mask for anisotropic etching of the silicon supporting member 260. The silicon supporting member 260, in one embodiment, is anisotropically etched with a 40% potassium hydroxide ("KOH") solution at 80 □C to form the cavity. The etch is stopped when the front side silicon nitride layer 271a is reached, as depicted in FIG 4B. The silicon nitride layer 271a inhibits etching of the main barrier layer 272a during this etch process. The cavity 267 may be formed extending through the supporting member 260. After formation of the cavity, the remaining portions of the back side barrier layer 262b and the diaphragm layer 264b may be removed.

Etch windows 266 are formed through the bottom diaphragm layer 264a on the front side of the wafer. A masking layer (not shown) is formed over the bottom diaphragm layer 264a and patterned using standard photolithographic techniques. Using the masking layer, etch windows 266 may be formed using a plasma etch. The plasma etching of silicon dioxide and silicon nitride may be performed using a mixture of carbontetrafluoride (CF_4) and oxygen (O_2). The etching is continued through the bottom diaphragm layer 264a and partially into the barrier layer 262a. In one embodiment, the etching is stopped at approximately half the thickness of the barrier layer 262a. Thus, when the barrier layer 262a is subsequently removed the etch windows 266 will extend through the bottom diaphragm layer 264a, communicating with the cavity 267. By stopping the etching at a midpoint of the barrier layer, voids or discontinuities may be reduced since the bottom diaphragm is still continuous due to the remaining barrier layer.

After the etch windows 266 are formed, a sacrificial spacer layer 268a/b is deposited upon the bottom diaphragm layer 264a and within cavity 267, as depicted in FIG. 4C. The spacer layer may be formed from LPCVD polysilicon. In one embodiment, the front side deposited spacer layer 268a will also at least partially fill the etch windows 266. Polysilicon may be deposited using an LPCVD reactor using silane (SiH_4) at a temperature of about 650 □C. The spacer layer 268a/b is deposited to a thickness in the range from about 4000 Å to about 10,000 Å, preferably from 6000 Å to about 8000 Å, and more preferably of about 7000 Å. The preferred thickness of the spacer layer 268a is dependent on the desired thickness of the internal air cavity of the Fabry-Perot detector. For example, if a Fabry-Perot detector which is to include a 7000 Å air cavity between the top and bottom diaphragm layer is desired, a spacer layer having a thickness of about 7000 Å would be formed. After the spacer layer has been deposited, a masking layer for etching the spacer layer 268a (not shown) is used to define the etch regions of the spacer layer 268a. The etching may be performed using a composition of nitric acid (HNO_3), water, and hydrogen fluoride (HF) in a ratio of 25:13:1, respectively, by volume. The lateral size of the subsequently formed cavity is determined by the masking pattern used to define the etch regions of the spacer layer 268a.

After the spacer layer 268a has been etched, the top diaphragm layer 270a/b is formed. The top diaphragm 270a/b, in one embodiment, is deposited upon the spacer layer 268a/b on both sides of the supporting member. The top diaphragm 270a/b may be composed of silicon nitride, silicon dioxide, or silicon oxynitride. In one embodiment, the top diaphragm 270a/b is composed of a stack of dielectric materials. As depicted in FIG. 4C, the top diaphragm 270a/b is composed of a stack of dielectric materials which includes a pair of silicon nitride layers 283a/b and 285a/b surrounding a silicon dioxide layer 284a/b. All of the layers may be deposited using an LPCVD process. The silicon nitride layers 283a/b and 285a/b have a thickness in the range from about 1000 Å to about

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2954

2000 Å, preferably from 1200 Å to about 1700 Å, and more preferably of about 1500 Å. The silicon dioxide layer 284a/b has a thickness in the range from about 5000 Å to about 15,500 Å, preferably from 7500 Å to about 12,000 Å, and more preferably of about 10,500 Å.

After depositing the top diaphragm 270a/b, all of the layers stacked on the bottom face of the supporting member (e.g., layers 268b, 283b, 284b, and 285b) are removed by multiple wet and plasma etching steps, as depicted in FIG. 4D. After these layers are removed, the now exposed portions of the barrier layer 262a are also removed. This exposes the spacer layer 268a which is present in the etch windows 266. The spacer layer 268 may be removed from between the top diaphragm 270a and the bottom diaphragm 264a by a wet etch using a KOH solution, as depicted in FIG. 4D. Removal of the spacer material 268a, forms a cavity 286 between the top diaphragm layer 270a and the bottom diaphragm layer 264a. After removal of the spacer material, the cavity 286 may be washed using deionized water, followed by isopropyl alcohol to clean out any remaining etching solution.

The cavity 286 of the Fabry-Perot sensor may be filled with a sensing substrate 290, as depicted in FIG. 4E. To coat the cavity 286 with a sensing substrate 290, the sensing substrate may be dissolved in a solvent. A solution of the sensing substrate is applied to the supporting member 260. The solution is believed to rapidly enter the cavity 286 through the etched windows 266 in the bottom diaphragm 264a, aided in part by capillary action. As the solvent evaporates, a thin film of the sensing substrate 290 coats the inner walls of the cavity 286, as well as the outer surface of the bottom diaphragm 264a. By repeated treatment of the supporting member with the solution of the sensing substrate, the thickness of the sensing substrate may be varied.

In one embodiment, the sensing substrate 290 is poly(3-dodecylthiophene) whose optical properties change in response to changes in oxidation states. The sensing substrate poly(3-dodecylthiophene) may be dissolved in a solvent such as chloroform or xylene. In one embodiment, a concentration of about 0.1 g of poly(3-dodecylthiophene)/mL is used. Application of the solution of poly(3-dodecylthiophene) to the supporting member causes a thin film of poly(3-dodecylthiophene) to be formed on the inner surface of the cavity.

In some instances, the sensing substrate, when deposited within a cavity of a Fabry-Perot type detector, may cause stress in the top diaphragm of the detector. It is believed that when a sensing polymer coats a planar top diaphragm, extra residual stress on the top diaphragm causes the diaphragm to become deflected toward the bottom diaphragm. If the deflection becomes to severe, sticking between the top and bottom diaphragms may occur. In one embodiment, this stress may be relieved by the use of supporting members 292 formed within the cavity 286, as depicted in FIG. 4F. The supporting members 292 may be formed without any extra processing steps to the above described process flow. The formation of supporting members may be accomplished by deliberately leaving a portion of the spacer layer within the cavity. This may be accomplished by underetching the spacer layer (e.g., terminating the etch process before the entire etch process is finished). The remaining spacer will behave as a support member to reduce the deflection of the top diaphragm member. The size and shape of the support members may be adjusted by altering the etch time of the spacer layer, or adjusting the shape of the etch windows 266.

In another embodiment, a high sensitivity CCD array may be used to measure changes in optical characteristics which occur upon binding of the biological/chemical agents. The CCD arrays may be interfaced with filters, light sources, fluid delivery and micromachined particle receptacles, so as to create a functional sensor array. Data acquisition and handling may be performed with existing CCD technology. Data streams (e.g., red, green, blue for colorimetric assays; gray intensity for fluorescence assays) may be transferred from the CCD to a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2955

computer via a data acquisition board. Current CCDs may allow for read-out rates of 10^5 pixels per second. Thus, the entire array of particles may be evaluated hundreds of times per second allowing for studies of the dynamics of the various host-guest interaction rates as well as the analyte/polymer diffusional characteristics. Evaluation of this data may offer a method of identifying and quantifying the chemical/biological composition of the test samples. CCD detectors may be configured to measure white light, ultraviolet light or fluorescence. Other detectors such as photomultiplier tubes, charge induction devices, photodiode, photodiode arrays, and microchannel plates may also be used. It should be understood that while the detector is depicted as being positioned under the supporting member, the detector may also be positioned above the supporting member. It should also be understood that the detector typically includes a sensing element for detecting the spectroscopic events and a component for displaying the detected events. The display component may be physically separated from the sensing element. The sensing element may be positioned above or below the sensor array while the display component is positioned close to a user.

In one embodiment, a CCD detector may be used to record color changes of the chemical sensitive particles during analysis. As depicted in FIG. 1, a CCD detector 130 may be placed beneath the supporting member 120. The light transmitted through the cavities is captured and analyzed by the CCD detector. In one embodiment, the light is broken down into three color components, red, green and blue. To simplify the data, each color is recorded using 8 bits of data. Thus, the data for each of the colors will appear as a value between 0 and 255. The color of each chemical sensitive element may be represented as a red, blue and green value. For example, a blank particle (i.e., a particle which does not include a receptor) will typically appear white. For example, when broken down into the red, green and blue components, it is found that a typical blank particle exhibits a red value of about 253, a green value of about 250, and a blue value of about 222. This signifies that a blank particle does not significantly absorb red, green or blue light. When a particle with a receptor is scanned, the particle may exhibit a color change, due to absorbance by the receptor. For example, it was found that when a particle which includes a 5-carboxyfluorescein receptor is subjected to white light, the particle shows a strong absorbance of blue light. The CCD detector values for the 5-carboxyfluorescein particle exhibits a red value of about 254, a green value of about 218, and a blue value of about 57. The decrease in transmittance of blue light is believed to be due to the absorbance of blue light by the 5-carboxyfluorescein. In this manner, the color changes of a particle may be quantitatively characterized. An advantage of using a CCD detector to monitor the color changes is that color changes which may not be noticeable to the human eye may now be detected.

The support array may be configured to allow a variety of detection modes to be practiced. In one embodiment, a light source is used to generate light which is directed toward the particles. The particles may absorb a portion of the light as the light illuminates the particles. The light then reaches the detector, reduced in intensity by the absorbance of the particles. The detector may be configure to measure the reduction in light intensity (i.e., the absorbance) due to the particles. In another embodiment, the detector may be placed above the supporting member. The detector may be configured to measure the amount of light reflected off of the particles. The absorbance of light by the particles is manifested by a reduction in the amount of light being reflected from the cavity. The light source in either embodiment may be a white light source or a fluorescent light source.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2956

CHEMICALLY SENSITIVE PARTICLES

A particle, in some embodiments, possess both the ability to bind the analyte of interest and to create a modulated signal. The particle may include receptor molecules which posses the ability to bind the analyte of interest and to create a modulated signal. Alternatively, the particle may include receptor molecules and indicators. The receptor molecule may posses the ability to bind to an analyte o f interest. Upon binding the analyte of interest, the receptor molecule may cause the indicator molecule to produce the modulated signal. The receptor molecules may be naturally occurring or synthetic receptors formed by rational design or combinatorial methods. Some examples of natural receptors include, but are not limited to, DNA, RNA, proteins, enzymes, oligopeptides, antigens, and antibodies. Either natural or synthetic receptors may be chosen for their ability to bind to the analyte molecules in a specific manner. The forces which drive association/recognition between molecules include the hydrophobic effect, anion-cation attraction, and hydrogen bonding. The relative strengths of these forces depend upon factors such as the solvent dielectric properties, the shape of the host molecule, and how it complements the guest. Upon host-guest association, attractive interactions occur and the molecules stick together. The most widely used analogy for this chemical interaction is that of a "lock and key". The fit of the key molecule (the guest) into the lock (the host) is a molecular recognition event.

A naturally occurring or synthetic receptor may be bound to a polymeric resin in order to create the particle. The polymeric resin may be made from a variety of polymers including, but not limited to, agarous, dextrose, acrylamide, control pore glass beads, polystyrene-polyethylene glycol resin, polystyrene-divinyl benzene resin, formylpolystyrene resin, trityl-polystyrene resin, acetyl polystyrene resin, chloroacetyl polystyrene resin, aminomethyl polystyrene-divinylbenzene resin, carboxypolystyrene resin, chloromethylated polystyrene-divinylbenzene resin, hydroxymethyl polystyrene-divinylbenzene resin, 2-chlorotrityl chloride polystyrene resin, 4-benzyloxy-2'4'- dimethoxybenzhydrol resin (Rink Acid resin), triphenyl methanol polystyrene resin, diphenylmethanol resin, benzhydrol resin, succinimidyl carbonate resin, p-nitrophenyl carbonate resin, imidazole carbonate resin, polyacrylamide resin, 4-sulfamylbenzoyl-4'- methylbenzhydrylamine-resin (Safety-catch resin), 2-amino-2-(2'-nitrophenyl) propionic acid-aminomethyl resin (ANP Resin), p-benzyloxybenzyl alcohol-divinylbenzene resin (Wang resin), p-methylbenzhydrylamine-divinylbenzene resin (MBHA resin), Fmoc-2,4-dimethoxy-4'-(carboxymethyloxy)-benzhydrylamine linked to resin (Knorr resin), 4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin (Rink resin), 4-hydroxymethyl-benzoyl-4'- methylbenzhydrylamine resin (HMBA-MBHA Resin), p-nitrobenzophenone oxime resin (Kaiser oxime resin), and amino-2,4-dimethoxy-4'-(carboxymethyloxy)-benzhydrylamine handle linked to 2-chlorotrityl resin (Knorr-2-chlorotrityl resin). In one embodiment, the material used to form the polymeric resin is compatible with the solvent in which the analyte is dissolved. For example, polystyrene-divinyl benzene resin will swell within non-polar solvents, but does not significantly swell within polar solvents. Thus, polystyrene-divinyl benzene resin may be used for the analysis of analytes within non-polar solvents. Alternatively, polystyrene-polyethylene glycol resin will swell with polar solvents such as water. Polystyrene-polyethylene glycol resin may be useful for the analysis of aqueous fluids.

In one embodiment, a polystyrene-polyethylene glycol-divinyl benzene material is used to form the polymeric resin. The polystyrene-polyethylene glycol-divinyl benzene resin is formed from a mixture of polystyrene 375, divinyl benzene 380 and polystyrene-polyethylene glycol 385, see FIG. 5. The polyethylene glycol portion of the polystyrene-polyethylene glycol 385, in one embodiment, may be terminated with an amine.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2957

The amine serves as a chemical handle to anchor both receptors and indicator dyes. Other chemical functional groups may be positioned at the terminal end of the polyethylene glycol to allow appropriate coupling of the polymeric resin to the receptor molecules or indicators.

The chemically sensitive particle, in one embodiment, is capable of both binding the analyte(s) of interest and creating a detectable signal. In one embodiment, the particle will create an optical signal when bound to an analyte of interest. The use of such a polymeric bound receptors offers advantages both in terms of cost and configurability. Instead of having to synthesize or attach a receptor directly to a supporting member, the polymeric bound receptors may be synthesized *en masse* and distributed to multiple different supporting members. This allows the cost of the sensor array, a major hurdle to the development of mass-produced environmental probes and medical diagnostics, to be reduced. Additionally, sensor arrays which incorporate polymeric bound receptors may be reconfigured much more quickly than array systems in which the receptor is attached directly tot he supporting member. For example, if a new variant of a pathogen or a pathogen that contains a genetically engineered protein is a threat, then a new sensor array system may be readily created to detect these modified analytes by simply adding new sensor elements (e.g., polymeric bound receptors) to a previously formed supporting member.

In one embodiment, a receptor, which is sensitive to changes in the pH of a fluid sample is bound to a polymeric resin to create a particle. That is, the receptor is sensitive to the concentration of hydrogen cations (H^+). The receptor in this case is typically sensitive to the concentration of H^+ in a fluid solution. The analyte of interest may therefore be H^+. There are many types of molecules which undergo a color change when the pH of the fluid is changed. For example, many types of dyes undergo significant color changes as the pH of the fluid medium is altered. Examples of receptors which may be used to monitor the pH of a fluid sample include 5-carboxyfluorescein and alizarin complexone, depicted in FIG. 6. Each of these receptors undergoes significant color changes as the pH of the fluid is altered. 5-carboxyfluorescein undergoes a change from yellow to orange as the pH of the fluid is increased. Alizarin complexone undergoes two color changes, first from yellow to red, then from red to blue as the pH of the fluid increases. By monitoring the change in color caused by dyes attached to a polymeric particle, the pH of a solution may be qualitatively and, with the use of a detector (e.g., a CCD detector), quantitatively monitored.

In another embodiment, a receptor which is sensitive to presence of metal cations is bound to a polymeric particle to create a particle. The receptor in this case is typically sensitive to the concentration of one or more metal cations present in a fluid solution. In general, colored molecules which will bind cations may be used to determine the presence of a metal cation in a fluid solution. Examples of receptors which may be used to monitor the presence of cations in a fluid sample include alizarin complexone and *o*-cresolphthalein complexone, see FIG. 6. Each of these receptors undergoes significant color changes as the concentration of a specific metal ion in the fluid is altered. Alizarin complexone is particularly sensitive to lanthanum ions. In the absence of lanthanum, alizarin complexone will exhibit a yellow color. As the concentration of lanthanum is increased, alizarin complexone will change to a red color. *o*-Cresolphthalein complexone is particularly sensitive to calcium ions. In the absence of calcium, *o*-cresolphthalein complexone is colorless. As the concentration of calcium is increased, *o*-cresolphthalein complexone will change to a blue color. By monitoring the change in color of metal cation sensitive receptors attached to a polymeric particle, the presence of a specific metal ion may be qualitatively and, with the use of a detector (e.g., a CCD detector), quantitatively monitored.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2958

Referring to FIG. 7, a graph of the absorbance of green light vs. concentration of calcium (Ca^{+2}) is depicted for a particle which includes an *o*-cresolphthalein complexone receptor. As the concentration of calcium is increased, the absorbance of green light increases in a linear manner up to a concentration of about 0.0006 M. A concentration of 0.0006 M is the solubility limit of calcium in the fluid, thus no significant change in absorbance is noted after this point. The linear relationship between concentration and absorbance allows the concentration of calcium to be determined by measuring the absorbance of the fluid sample.

In one embodiment, a detectable signal may be caused by the altering of the physical properties of an indicator ligand bound to the receptor or the polymeric resin. In one embodiment, two different indicators are attached to a receptor or the polymeric resin. When an analyte is captured by the receptor, the physical distance between the two indicators may be altered such that a change in the spectroscopic properties of the indicators is produced. A variety of fluorescent and phosphorescent indicators may be used for this sensing scheme. This process, known as Forster energy transfer, is extremely sensitive to small changes in the distance between the indicator molecules.

For example, a first fluorescent indicator 320 (e.g., a fluorescein derivative) and a second fluorescent indictor 330 (e.g., a rhodamine derivative) may be attached to a receptor 300, as depicted in FIG. 8. When no analyte is present short wavelength excitation 310 may excite the first fluorescent indicator 320, which fluoresces as indicated by 312. The short wavelength excitation, however, may cause little or no fluorescence of the second fluorescent indicator 330. After binding of analyte 350 to the receptor, a structural change in the receptor molecule may bring the first and second fluorescent indicators closer to each other. This change in intermolecular distance may allow the excited first indicator 320 to transfer a portion of its fluorescent energy 325 to the second fluorescent indicator 330. This transfer in energy may be measured by either a drop in energy of the fluorescence of the first indicator molecule 320, or the detection of increased fluorescence 314 by the second indicator molecule 330.

Alternatively, the first and second fluorescent indicators may initially be positioned such that short wavelength excitation, may cause fluorescence of both the first and second fluorescent indicators, as described above. After binding of analyte 350 to the receptor, a structural change in the receptor molecule may cause the first and second fluorescent indicators to move further apart. This change in intermolecular distance may inhibit the transfer of fluorescent energy from the first indicator 320 to the second fluorescent indicator 330. This change in the transfer of energy may be measured by either a drop in energy of the fluorescence of the second indicator molecule 330, or the detection of increased fluorescence by the first indicator molecule 320.

In another embodiment, an indicator ligand may be preloaded onto the receptor. An analyte may then displace the indicator ligand to produce a change in the spectroscopic properties of the particles. In this case, the initial background absorbance is relatively large and decreases when the analyte is present. The indicator ligand, in one embodiment, has a variety of spectroscopic properties which may be measured. These spectroscopic properties include, but are not limited to, ultraviolet absorption, visible absorption, infrared absorption, fluorescence, and magnetic resonance. In one embodiment, the indicator is a dye having either a strong fluorescence, a strong ultraviolet absorption, a strong visible absorption, or a combination of these physical properties. Examples of indicators include, but are not limited to, carboxyfluorescein, ethidium bromide, 7-dimethylamino-4-methylcoumarin, 7-diethylamino-4-methylcoumarin, eosin, erythrosin, fluorescein, Oregon Green 488, pyrene, Rhodamine Red, tetramethylrhodamine, Texas Red, Methyl Violet, Crystal Violet, Ethyl Violet, Malachite green,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2959

Methyl Green, Alizarin Red S, Methyl Red, Neutral Red, *o*-cresolsulfonephthalein, *o*-cresolphthalein, phenolphthalein, Acridine Orange, *B*-naphthol, coumarin, and *a*-naphthionic acid. When the indicator is mixed with the receptor, the receptor and indicator interact with each other such that the above mentioned spectroscopic properties of the indicator, as well as other spectroscopic properties may be altered. The nature of this interaction may be a binding interaction, wherein the indicator and receptor are attracted to each other with a sufficient force to allow the newly formed receptor-indicator complex to function as a single unit. The binding of the indicator and receptor to each other may take the form of a covalent bond, an ionic bond, a hydrogen bond, a van der Waals interaction, or a combination of these bonds.

The indicator may be chosen such that the binding strength of the indicator to the receptor is less than the binding strength of the analyte to the receptor. Thus, in the presence of an analyte, the binding of the indicator with the receptor may be disrupted, releasing the indicator from the receptor. When released, the physical properties of the indicator may be altered from those it exhibited when bound to the receptor. The indicator may revert back to its original structure, thus regaining its original physical properties. For example, if a fluorescent indicator is attached to a particle that includes a receptor, the fluorescence of the particle may be strong before treatment with an analyte containing fluid. When the analyte interacts with the particle, the fluorescent indicator may be released. Release of the indicator may cause a decrease in the fluorescence of the particle, since the particle now has less indicator molecules associated with it.

An example of this type of system is illustrated by the use of a boronic acid substituted resin 505 as a particle. Prior to testing, the boronic acid substituted resin 505 is treated with a sugar 510 which is tagged with an indicator (e.g., resorufin) as depicted in FIG. 9. The sugar 510 binds to the boronic acid receptor 500 imparting a color change to the boronic substituted resin 505 (yellow for the resorufin tagged sugar). When the boronic acid resin 505 is treated with a fluid sample which includes a sugar 520, the tagged sugar 510 may be displaced, causing a decrease in the amount of color produced by the boronic acid substituted resin 505. This decrease may be qualitatively or, with the use of a detector (e.g., a CCD detector), quantitatively monitored.

In another embodiment, a designed synthetic receptor may be used. In one embodiment, a polycarboxylic acid receptor may be attached to a polymeric resin. The polycarboxylic receptors are discussed in U.S. patent application serial no. 08/950,712 which is incorporated herein by reference.

In an embodiment, the analyte molecules in the fluid may be pretreated with an indicator ligand. Pretreatment may involve covalent attachment of an indicator ligand to the analyte molecule. After the indicator has been attached to the analyte, the fluid may be passed over the sensing particles. Interaction of the receptors on the sensing particles with the analytes may remove the analytes from the solution. Since the analytes include an indicator, the spectroscopic properties of the indicator may be passed onto the particle. By analyzing the physical properties of the sensing particles after passage of an analyte stream, the presence and concentration of an analyte may be determined.

For example, the analytes within a fluid may be derivatized with a fluorescent tag before introducing the stream to the particles. As analyte molecules are adsorbed by the particles, the fluorescence of the particles may increase. The presence of a fluorescent signal may be used to determine the presence of a specific analyte. Additionally, the strength of the fluorescence may be used to determine the amount of analyte within the stream.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2960

RECEPTORS

A variety of natural and synthetic receptors may be used. The synthetic receptors may come from a variety of classes including, but not limited to, polynucleotides (e.g., aptamers), peptides (e.g., enzymes and antibodies), synthetic receptors, polymeric unnatural biopolymers (e.g., polythioureas, polyguanidiniums), and imprinted polymers., some of which are generally depicted in FIG. 10. Natural based synthetic receptors include receptors which are structurally similar to naturally occurring molecules. Polynucleotides are relatively small fragments of DNA which may be derived by sequentially building the DNA sequence. Peptides may be synthesized from amino acids. Unnatural biopolymers are chemical structure which are based on natural biopolymers, but which are built from unnatural linking units. Unnatural biopolymers such as polythioureas and polyguanidiniums may be synthesized from diamines (i.e., compounds which include at least two amine functional groups). These molecules are structurally similar to naturally occurring receptors, (e.g., peptides). Some diamines may, in turn, be synthesized from amino acids. The use of amino acids as the building blocks for these compounds allow a wide variety of molecular recognition units to be devised. For example, the twenty natural amino acids have side chains that possess hydrophobic residues, cationic and anionic residues, as well as hydrogen bonding groups. These side chains may provide a good chemical match to bind a large number of targets, from small molecules to large oligosaccharides. Amino acid based peptides, polythioureas, and polyguanidiniums are depicted in FIG. 10.

Techniques for the building of DNA fragments and polypeptide fragments on a polymer particle are well known. Techniques for the immobilization of naturally occurring antibodies and enzymes on a polymeric resin are also well known. The synthesis of polythioureas upon a resin particle may be accomplished by the synthetic pathway depicted in FIG. 11. The procedure may begin by deprotection of the terminal tBoc protecting group on an amino acid coupled to a polymeric particle. Removal of the protecting group is followed by coupling of the rigid spacer 410 to the resulting amine 405 using diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole hydrate (HOBT). The spacer group may inhibit formation of a thiazolone by reaction of the first amino acids with subsequently formed thioureas. After the spacer group is coupled to the amino acid, another tBoc deprotection is performed to remove the spacer protecting group, giving the amine 415. At this point, monomer may be added incrementally to the growing chain, each time followed by a tBoc deprotection. The addition of a derivative of the diamine 420 (e.g., an isothiocyanate) to amine 415 gives the mono-thiourea 425. The addition of a second thiourea substituent is also depicted. After the addition of the desired number of monomers, a solution of benzylisothiocyanate or acetic anhydride may be added to cap any remaining amines on the growing oligomers. Between 1 to 20 thioureas groups may be formed to produce a synthetic polythiourea receptor.

The synthesis of polyguanidiniums may be accomplished as depicted in FIG. 12. In order to incorporate these guanidinium groups into the receptor, the coupling of a thiourea with a terminal amine in the presence of Mukaiyama's reagent may be utilized. The coupling of the first thiourea diamine 430 with an amino group of a polymeric particle gives the mono-guanidinium 434. Coupling of the resulting mono-guanidinium with a second thiourea diamine 436 gives a di-guanidinium 438. Further coupling may create a tri-guanidinium 440. Between 1 to 20 guanidinium groups may be formed to produce a synthetic polyguanidinium receptor.

The above described methods for making polythioureas and polyguanidiniums are based on the incorporation of diamines (i.e., molecules which include at least two amine functional groups) into the oligomeric

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2961

receptor. The method may be general for any compound having at least two amino groups. In one embodiment, the diamine may be derived from amino acids. A method for forming diamines from amino acids is shown in FIG. 13. Treatment of a protected amino acid 450 with borane-THF reduces the carboxylic acid portion of the amino acid to the primary alcohol 452. The primary alcohol is treated with phthalimide under Mitsunobu conditions (PPh$_3$/DEAD). The resulting compound 454 is treated with aqueous methylamine to form the desired monoprotected diamine 456. The process may be accomplished such that the enantiomeric purity of the starting amino acid is maintained. Any natural or synthetic amino acid may be used in the above described method.

The three coupling strategies used to form the respective functional groups may be completely compatible with each other. The capability to mix linking groups (amides, thioureas, and guanidiniums) as well as the side chains (hydrophobic, cationic, anionic, and hydrogen bonding) may allow the creation of a diversity in the oligomers that is beyond the diversity of receptors typically found with natural biological receptors. Thus, we may produce ultra-sensitive and ultra-selective receptors which exhibit interactions for specific toxins, bacteria, and environmental chemicals. Additionally, these synthetic schemes may be used to build combinatorial libraries of particles for use in the sensor array.

In an embodiment, the indicator ligand may be incorporated into synthetic receptors during the synthesis of the receptors. The ligand may be incorporated into a monomeric unit, such as a diamine, that is used during the synthesis of the receptor. In this manner, the indicator may be covalently attached to the receptor in a controlled position. By placing the indicator within the receptor during the synthesis of the receptor, the positioning of the indicator ligand within the receptor may be controlled. This control may be difficult to achieve after synthesis of the receptor is completed.

In one embodiment, a fluorescent group may be incorporated into a diamine monomer for use in the synthetic sequences. Examples of monomeric units which may be used for the synthesis of a receptor are depicted in FIG. 14. The depicted monomers include fluorescent indicator groups. After synthesis, the interaction of the receptor with the analyte may induce changes in the spectroscopic properties of the molecule. Typically, hydrogen bonding or ionic substituents on the fluorescent monomer involved in analyte binding have the capacity to change the electron density and/or rigidity of the fluorescent ring system, thereby causing observable changes in the spectroscopic properties of the indicator. For fluorescent indicators such changes may be exhibited as changes in the fluorescence quantum yield, maximum excitation wavelength, and/or maximum emission wavelength. This approach does not require the dissociation of a preloaded fluorescent ligand, which may be limited in response time by $k_{(off)}$). While fluorescent ligands are shown here, it is to be understood that a variety of other ligand may be used including colorimetric ligands.

In another embodiment, two fluorescent monomers for signaling may be used for the synthesis of the receptor. For example, compound 470 (a derivative of fluorescein) and compound 475 (a derivative of rhodamine), depicted in FIG. 14, may both be incorporated into a synthetic receptor. Compound 470 contains a common colorimetric/fluorescent probe that will, in some embodiments, send out a modulated signal upon analyte binding. The modulation may be due to resonance energy transfer to compound 475. When an analyte binds to the receptor, structural changes in the receptor may alter the distance between monomeric units 470 and 475. It is well known that excitation of fluorescein can result in emission from rhodamine when these molecules are oriented correctly. The efficiency of resonance energy transfer from monomers 470 to 475 will depend strongly upon the presence of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2962

analyte binding; thus, measurement of rhodamine fluorescence intensity (at a substantially longer wavelength than fluorescein fluorescence) may serve as an indicator of analyte binding. To greatly improve the likelihood of a modulatory fluorescein-rhodamine interaction, multiple rhodamine tags may be attached at different sites along a receptor molecule without substantially increasing background rhodamine fluorescence (only rhodamine very close to fluorescein will yield appreciable signal). This methodology may be applied to a number of alternate fluorescent pairs.

In an embodiment, a large number of chemical/biological agents of interest to the military and civilian communities may be sensed readily by the described array sensors including both small and medium size molecules. For example, it is known that nerve gases typically produce phosphate structures upon hydrolysis in water. The presence of molecules which contain phosphate functional groups may be detected using polyguanidiniums. Nerve gases which have contaminated water sources may be detected by the use of the polyguanidinium receptors described above.

In order to identify, sense, and quantitate the presence of various bacteria using the proposed micro-machined sensor, two strategies may be used. First, small molecule recognition and detection may be exploited. Since each bacteria possesses a unique and distinctive concentration of the various cellular molecules, such as DNA, proteins, metabolites, and sugars, the fingerprint (i.e., the concentration and types of DNA, proteins, metabolites, and sugars) of each organism is expected to be unique. Hence, the analytes obtained from whole bacteria or broken down bacteria may be used to determine the presence of specific bacteria. A series of receptors specific for DNA molecules, proteins, metabolites, and sugars may be incorporated into an array. A solution containing bacteria, or more preferably broken down bacteria, may be passed over the array of particles. The individual cellular components of the bacteria may interact in a different manner with each of the particles. This interaction will provide a pattern within the array which may be unique for the individual bacteria. In this manner, the presence of bacteria within a fluid may be determined.

In another embodiment, bacteria may be detected as whole entities, as found in ground water, aerosols, or blood. To detect, sense, and identify intact bacteria, the cell surface of one bacteria may be differentiated from other bacteria. One method of accomplishing this differentiation is to target cell surface oligosaccharides (i.e. sugar residues). Each bacterial class (gram negative, gram positive, etc.) displays a different oligosaccharide on their cell surfaces. The oligosaccharide, which is the code that is read by other cells giving an identification of the cell, is part of the cell-cell recognition and communication process. The use of synthetic receptors which are specific for oligosaccharides may be used to determine the presence of specific bacteria by analyzing for the cell surface oligosaccharides.

In another embodiment, the sensor array may be used to optimize which receptor molecules should be used for a specific analyte. An array of receptors may be placed within the cavities of the supporting member and a stream containing an analyte may be passed over the array. The reaction of each portion of the sensing array to the known analyte may be analyzed and the optimal receptor determined by determining which particle, and therefore which receptor, exhibits the strongest reaction toward the analyte. In this manner, a large number of potential receptors may be rapidly scanned. The optimal receptor may then be incorporated into a system used for the detection of the specific analyte in a mixture of analytes.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2963

It should be emphasized that although some particles may be purposefully designed to bind to important species (biological agents, toxins, nerve gasses, etc.), most structures will possess nonspecific receptor groups. One of the advantages associated with the proposed sensor array is the capacity to standardize each array of particles via exposure to various analytes, followed by storage of the patterns which arise from interaction of the analytes with the particles. Therefore, there may not be a need to know the identity of the actual receptor on each particle. Only the characteristic pattern for each array of particles is important. In fact, for many applications it may be less time consuming to place the various particles into their respective holders without taking precautions to characterize the location associated with the specific particles. When used in this manner, each individual sensor array may require standardization for the type of analyte to be studied.

On-site calibration for new or unknown toxins may also be possible with this type of array. Upon complexation of an analyte, the local microenvironment of each indicator may change, resulting in a modulation of the light absorption and/or emission properties. The use of standard pattern recognition algorithms completed on a computer platform may serves as the intelligence factor for the analysis. The "fingerprint" like response evoked from the simultaneous interactions occurring at multiple sites within the substrate may be used to identify the species present in unknown samples.

The above described sensor array system offers a number of distinct advantages over exiting technologies. One advantage is that "real time" detection of analytes may be performed. Another advantage is that the simultaneous detection of multiple analytes may be realized. Yet another advantage is that the sensor array system allows the use of synthetic reagents as well as biologically produced reagents. Synthetic reagents typically have superior sensitivity and specificity toward analytes when compared to the biological reagents. Yet another advantage is that the sensor array system may be readily modified by simply changing the particles which are placed within the sensor array. This interchangability may also reduce production costs.

EXAMPLES

1. The determination of pH using a chemically sensitive particle.

Shown in FIG. 15 is the magnitude of the optical signal transmitted through a single polymer particle derivatized with o-cresolphthalein. Here, a filter is used to focus the analysis on those wavelengths which the dye absorbs most strongly (i.e., about 550 nm). Data is provided for the particle as the pH is cycled between acid and basic environments. In acidic media (i.e., at times of 100-150 seconds and 180-210 seconds), the particle is clear and the system yields large signals (up to greater than 300,000 counts) at the optical detector. Between times of 0-100 and 150-180 seconds, the solution was made basic. Upon raising the pH (i.e., making the solution more basic), the particle turns purple in color and the transmitted green light is greatly diminished. Large signal reductions are recorded under such circumstances. The evolution of the signal changes show that the response time is quite rapid, on the order of 10 seconds. Furthermore, the behavior is highly reproducible.

2. The simultaneous detection of Ca^{+2}, Ce^{+3}, and pH by a sensor array system.

The synthesis of four different particles was accomplished by coupling a variety of indictor ligands to a polyethylene glycol-polystyrene ("PEG-PS") resin particle. The PEG-PS resin particles were obtained from Novabiochem Corp., La Jolla, Ca. The particles have an average diameter of about 130 μm when dry and about

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2964

250 μm when wet. The indicator ligands of fluorescein, *o*-cresolphthalein complexone, and alizarin complexone were each attached to PEG-PS resin particles using a dicyclohexylcarbodiimide (DCC) coupling between a terminal resin bound amine and a carboxylic acid on the indicator ligand.

These synthetic receptors, localized on the PEG-PS resin to create sensing particles, were positioned within micromachined wells formed in silicon/silicon nitride wafers, thus confining the particles to individually addressable positions on a multicomponent chip. These wells were sized to hold the particles in both swollen and unswollen states. Rapid introduction of the test fluids can be accomplished using these structures while allowing spectrophotometric assays to probe for the presence of analytes. For the identification and quantification of analyte species, changes in the light absorption and light emission properties of the immobilized resin particles can be exploited, although only identification based upon absorption properties are discussed here. Upon exposure to analytes, color changes for the particles were found to be 90% complete within one minute of exposure, although typically only seconds were required. To make the analysis of the colorimetric changes efficient, rapid, and sensitive, a charge-coupled-device (CCD) was directly interfaced with the sensor array. Thus, data streams composed of red, green, and blue (RGB) light intensities were acquired and processed for each of the individual particle elements. The red, blue, and green responses of the particles to various solutions are graphically depicted in FIG. 16.

The true power of the described bead sensor array occurs when simultaneous evaluation of multiple chemically distinct bead structures is completed. A demonstration of the capacity of five different beads is provided in FIG. 16. In this case, blank, alizarin, o-cresol phthalein, fluorescein, and alizarin-Ce3+ complex derivatized beads serve as a matrix for subtle differentiation of chemical environments. The blank bead is simply a polystyrene sphere with no chemical derivatization. The bead derivatized with o-cresolphthalein responds to Ca+2 at pHs values around 10.0. The binding of calcium is noted from the large green color attenuation noted for this dye while exposed to the cation. Similarly, the fluorescein derivatized bead acts as a pH sensor. At pHs below 7.4 it is light yellow, but at higher pHs it turns dark orange. Interesting, the alizarin complexone plays three distinct roles. First, it acts as a proton sensor yielding a yellow color at pHs below 4.5, orange is noted at pHs between 4.5 and 11.5, and at pHs above 11.5 a blue hue is observed. Second, it functions as a sensor for lanthanum ions at lower pHs by turning yellow to orange. Third, the combination of both fluoride and lanthanum ions results in yellow/orange coloration.

The analysis of solutions containing various amount of Ca^{+2} or F^- at various pH levels was performed using alizarin complexone, *o*-cresolphthalein complexone, 5-carboxy fluorescein, and alizarin-Ce^{3+} complex. A blank particle in which the terminal amines of a PEG-PS resin particle have been acylated was also used. In this example, the presence of Ca^{+2} (0.1 M $Ca(NO_3)_2$) was analyzed under conditions of varying pH. The pH was varied to values of 2, 7, and 12, all buffered by a mixture of 0.04 M phosphate, 0.04 M acetate, and 0.04 M borate. The RGB patterns for each sensor element in all environments were measured. The bead derivatized with o-cresolphthalein responds to Ca^{+2} at pH values around 12. Similarly, the 5-carboxy fluorescein derivatized bead acts as a pH sensor. At pHs below 7.4 it is light yellow, but at higher pHs it turns dark orange. Interesting, the alizarin complexone plays three distinct roles. First, it acts as a proton sensor yielding a yellow color at pHs below 4.5, orange is noted at pHs between 4.5 and 11.5, and at pHs above 11.5 a blue hue is observed. Second, it functions as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2965

a sensor for lanthanum ions at lower pHs by turning yellow to orange. Third, the combination of both fluoride and lanthanum ions results in yellow/orange coloration.

This example demonstrates a number of important factors related to the design, testing, and functionality of micromachined array sensors for solution analyses. First, derivatization of polymer particles with both colorimetric and fluorescent dyes was completed. These structures were shown to respond to pH and Ca^{2+}. Second, response times well under 1 minute were found. Third, micromachined arrays suitable both for confinement of particles, as well as optical characterization of the particles, have been prepared. Fourth, integration of the test bed arrays with commercially available CCD detectors has been accomplished. Finally, simultaneous detection of several analytes in a mixture was made possible by analysis of the RGB color patterns created by the sensor array.

3. The detection of sugar molecules using a boronic acid based receptor.

A series of receptors were prepared with functionalities that associate strongly with sugar molecules, as depicted in FIG. 9. In this case, a boronic acid sugar receptor 500 was utilized to demonstrate the functionality of a new type of sensing scheme in which competitive displacement of a resorufin derivatized galactose sugar molecule was used to assess the presence (or lack thereof) of other sugar molecules. The boronic acid receptor 500 was formed via a substitution reaction of a benzylic bromide. The boronic acid receptor was attached to a polyethylene glycol-polystyrene ("PEG-PS") resin particle at the "R" position. Initially, the boronic acid derivatized particle was loaded with resorufin derivatized galactose 510. Upon exposure of the particle to a solution containing glucose 520, the resorufin derivatized galactose molecules 510 are displaced from the particle receptor sites. Visual inspection of the optical photographs taken before and after exposure to the sugar solution show that the boron substituted resin is capable of sequestering sugar molecules from an aqueous solution. Moreover, the subsequent exposure of the colored particles to a solution of a non-tagged sugar (e.g., glucose) leads to a displacement of the bound colored sugar reporter molecule. Displacement of this molecule leads to a change in the color of the particle. The sugar sensor turns from dark orange to yellow in solutions containing glucose. The particles were also tested in conditions of varying pH. It was noted that the color of the particles changes from dark orange to yellow as the pH is varied from low pH to high pH.

FURTHER IMPROVEMENTS

Shown in FIG. 17 is an embodiment of a system for detecting analytes in a fluid. In one embodiment, the system includes a light source 512, a sensor array 522, a chamber 550 for supporting the sensor array and a detector 530. The sensor array 522 may include a supporting member which is configured to hold a variety of particles. In one embodiment, light originating from the light source 512 passes through the sensor array 522 and out through the bottom side of the sensor array. Light modulated by the particles may be detected by a proximally spaced detector 530. While depicted as being positioned below the sensor array, it should be understood that the detector may be positioned above the sensor array for reflectance measurements. Evaluation of the optical changes may be completed by visual inspection (e.g., by eye, or with the aid of a microscope) or by use of a microprocessor 540 coupled to the detector.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2966

In this embodiment, the sensor array 522 is positioned within a chamber 550. The chamber 550, may be configured to allow a fluid stream to pass through the chamber such that the fluid stream interacts with the sensor array 522. The chamber may be constructed of glass (e.g, borosilicate glass or quartz) or a plastic material which is transparent to a portion of the light from the light source. If a plastic material is used, the plastic material should also be substantially unreactive toward the fluid. Examples of plastic materials which may be used to form the chamber include, but are not limited to, acrylic resins, polycarbonates, polyester resins, polyethylenes, polyimides, polyvinyl polymers (e.g., polyvinyl chloride, polyvinyl acetate, polyvinyl dichloride, polyvinyl fluoride, etc.), polystyrenes, polypropylenes, polytetrafluoroethylenes, and polyurethanes. An example of such a chamber is a Sykes-Moore chamber, which is commercially available from Bellco Glass, Inc., in New Jersey. Chamber 550, in one embodiment, includes a fluid inlet port 552 and a fluid outlet port 554. The fluid inlet 552 and outlet 554 ports are configured to allow a fluid stream to pass into the interior 556 of the chamber during use. The inlet and outlet ports may be configured to allow facile placement of a conduit for transferring the fluid to the chamber. In one embodiment, the ports may be hollow conduits. The hollow conduits may be configured to have an outer diameter which is substantially equal to the inner diameter of a tube for transferring the fluid to or away from the chamber. For example, if a plastic or rubber tube is used for the transfer of the fluid, the internal diameter of the plastic tube is substantially equal to the outer diameter of the inlet and outlet ports.

In another embodiment, the inlet and outlet ports may be Luer lock style connectors. Preferably, the inlet and outlet ports are female Luer lock connectors. The use of female Luer lock connectors will allow the fluid to be introduced via a syringe. Typically, syringes include a male Luer lock connector at the dispensing end of the syringe. For the introduction of liquid samples, the use of Luer lock connectors may allow samples to be transferred directly from a syringe to the chamber 550. Luer lock connectors may also allow plastic or rubber tubing to be connected to the chamber using Luer lock tubing connectors.

The chamber may be configured to allow the passage of a fluid sample to be substantially confined to the interior 556 of the chamber. By confining the fluid to a small interior volume, the amount of fluid required for an analysis may be minimized. The interior volume may be specifically modified for the desired application. For example, for the analysis of small volumes of fluid samples, the chamber may be designed to have a small interior chamber, thus reducing the amount of fluid needed to fill the chamber. For larger samples, a larger interior chamber may be used. Larger chambers may allow a faster throughput of the fluid during use.

In another embodiment, depicted in FIG. 18, a system for detecting analytes in a fluid includes a light source 512, a sensor array 522, a chamber 550 for supporting the sensor array and a detector 530, all enclosed within a detection system enclosure 560. As described above, the sensor array 522 is preferably formed of a supporting member which is configured to hold a variety of particles. Thus, in a single enclosure, all of the components of an analyte detection system are included.

The formation of an analyte detection system in a single enclosure may allow the formation of a portable detection system. For example, a small controller 570 may be coupled to the analyte detection system. The controller 570 may be configured to interact with the detector and display the results from the analysis. In one embodiment, the controller includes a display device 572 for displaying information to a user. The controller may also include input devices 574 (e.g., buttons) to allow the user to control the operation of the analyte detection

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2967

system. For example, the controller may control the operation of the light source 512 and the operation of the detector 530.

The detection system enclosure 560, may be interchangeable with the controller. Coupling members 576 and 578 may be used to remove the detection system enclosure 560 from the controller 570. A second detection system enclosure may be readily coupled to the controller using coupling members 576 and 578. In this manner, a variety of different types of analytes may be detecting using a variety of different detection system enclosures. Each of the detection system enclosures may include different sensor arrays mounted within their chambers. Instead of having to exchange the sensor array for different types of analysis, the entire detection system enclosure may be exchanged. This may prove advantageous, when a variety of detection schemes are used. For example a first detection system enclosure may be configured for white light applications. The first detection system enclosure may include a white light source, a sensor that includes particles that produce a visible light response in the presence of an analyte, and a detector sensitive to white light. A second detection system enclosure may be configured for fluorescent applications, including a fluorescent light source, a sensor array which includes particles which produce a fluorescent response on the presence of an analyte, and a fluorescent detector. The second detection system enclosure may also include other components necessary for producing a proper detection system. For example, the second detection system may also include a filter for preventing short wavelength excitation from producing "false" signals in the optical detection system during fluorescence measurements. A user need only select the proper detection system enclosure for the detection of the desired analyte. Since each detection system enclosure includes many of the required components, a user does not have to make light source selections, sensor array selections or detector arrangement selections to produce a viable detection system.

In another embodiment, the individual components of the system may be interchangeable. The system may include coupling members 573 and 575 that allow the light source and the detector, respectively, to be removed from the chamber 550. This may allow a more modular design of the system. For example, an analysis may be first performed with a white light source to give data corresponding to an absorbance/reflectance analysis. After this analysis is performed the light source may be changed to a ultraviolet light source to allow ultraviolet analysis of the particles. Since the particles have already been treated with the fluid, the analysis may be preformed without further treatment of the particles with a fluid. In this manner a variety of tests may be performed using a single sensor array.

In one embodiment, the supporting member is made of any material capable of supporting the particles, while allowing the passage of the appropriate wavelength of light. The supporting member may also be made of a material substantially impervious to the fluid in which the analyte is present. A variety of materials may be used including plastics (e.g., photoresist materials, acrylic polymers, carbonate polymers, etc.), glass, silicon based materials (e.g., silicon, silicon dioxide, silicon nitride, etc.) and metals. In one embodiment, the supporting member includes a plurality of cavities. The cavities are preferably formed such that at least one particle is substantially contained within the cavity. Alternatively, a plurality of particles may be contained within a single cavity.

In some embodiments, it will be necessary to pass liquids over the sensor array. The dynamic motion of liquids across the sensor array may lead to displacement of the particles from the cavities. In another embodiment, the particles are preferably held within cavities formed in a supporting member by the use of a transmission electron microscope ("TEM") grid. As depicted in FIG. 19, a cavity 580 is formed in a supporting member 582.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2968

After placement of a particle 584 within the cavity, a TEM grid 586 may be placed atop the supporting member 582 and secured into position. TEM grids and adhesives for securing TEM grids to a support are commercially available from Ted Pella, Inc., Redding, CA. The TEM grid 586 may be made from a number of materials including, but not limited to, copper, nickel, gold, silver, aluminum, molybdenum, titanium, nylon, beryllium, carbon, and beryllium-copper. The mesh structure of the TEM grid may allow solution access as well as optical access to the particles that are placed in the cavities. FIG. 20 further depicts a top view of a sensor array with a TEM grid 586 formed upon the upper surface of the supporting member 582. The TEM grid 586 may be placed on the upper surface of the supporting member, trapping particles 584 within the cavities 580. As depicted, the openings 588 in the TEM grid 586 may be sized to hold the particles 584 within the cavities 580, while allowing fluid and optical access to cavities 580.

In another embodiment, a sensor array includes a supporting member configured to support the particles, while allowing the passage of the appropriate wavelength of light to the particle. The supporting member, in one embodiment, includes a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity. The supporting member may be configured to substantially inhibit the displacement of the particles from the cavities during use. The supporting member may also be configured to allow the passage of the fluid through cavities, e.g., the fluid may flow from the top surface of the supporting member, past the particle, and out the bottom surface of the supporting member. This may increase the contact time between the particle and the fluid.

Figures 21A-G depict a sequence of processing steps for the formation of a silicon based supporting member which includes a removable top cover and bottom cover. The removable top cover may be configured to allow fluids to pass through the top cover and into the cavity. The removable bottom cover may also be configured to allow the fluid to pass through the bottom cover and out of the cavity. As depicted in FIG. 21A, a series of layers may be deposited upon both sides of a silicon substrate 610. First removable layers 612 may be deposited upon the silicon substrate. The removable layers 612 may be silicon dioxide, silicon nitride, or photoresist material. In one embodiment, a layer of silicon dioxide 612 is deposited upon both surfaces of the silicon substrate 610. Upon these removable layers, covers 614 may be formed. In one embodiment, covers 614 are formed from a material that differs from the material used to form the removable layers 612 and which is substantially transparent to the light source of a detection system. For example, if the removable layers 612 are formed from silicon dioxide, the cover may be formed from silicon nitride. Second removable layers 616 may be formed upon the covers 614. Second removable layers 616 may be formed from a material that differs from the material used to form the covers 614. Second removable layers 616 may be formed from a material similar to the material used to form the first removable layers 612. In one embodiment, first and second removable layers 612 and 616 are formed from silicon dioxide and covers 614 are formed from silicon nitride. The layers are patterned and etched using standard photolithographic techniques. In one embodiment, the remaining portions of the layers are substantially aligned in the position where the cavities are to be formed in the silicon substrate 610.

After the layers have been etched, spacer structures may be formed on the sidewalls of the first removable layers 612, the covers 614, and the second removable layers 616, as depicted in FIG. 21B. The spacer structures may be formed from the same material used to form the second removable layers 616. In one embodiment, depositing a spacer layer of the appropriate material and subjecting the material to an anisotropic etch may form the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2969

spacer structures. An anisotropic etch, such as a plasma etch, employs both physical and chemical removal mechanisms. Ions are typically bombarded at an angle substantially perpendicular to the semiconductor substrate upper surface. This causes substantially horizontal surfaces to be removed faster than substantially vertical surfaces. During this etching procedure the spacer layers are preferably removed such that the only regions of the spacer layers that remain may be those regions near substantially vertical surfaces, e.g., spacer structures 618.

After formation of the spacer structures 618, cover support structures 620, depicted in FIG. 21C, may be formed. The cover support structures may be initially formed by depositing a support structure layer upon the second removable layer 616 and spacer structures 618. The support structure layer is then patterned and etched, using standard photolithography, to form the support structures 620. In one embodiment, the support structures are formed from a material that differs from the removable layers material. In one embodiment, the removable layers may be formed from silicon dioxide while the support structures and covers may be formed from silicon nitride.

Turning to FIG. 21 D, the second removable layers 616 and an upper portion of the spacer structures 618 are preferably removed using a wet etch process. Removal of the second removable layers leaves the top surface of the covers 614 exposed. This allows the covers to be patterned and etched such that openings 622 are formed extending through the covers. These openings 622 may be formed in the covers 614 to allow the passage of fluid through the cover layers. In one embodiment, the openings 622 are formed to allow fluid to pass through, while inhibiting displacement of the particles from the subsequently formed cavities.

After the openings 622 have been formed, the remainder of the first removable layers 612 and the remainder of the spacer structures 618 may be removed using a wet etch. The removal of the removable layers and the spacer structures creates "floating" covers 614, as depicted in FIG. 21E. The covers 614 may be held in proximity to the silicon substrate 610 by the support structures 620. The covers 614 may now be removed by sliding the covers away from the support structures 620. In this manner removable covers 614 may be formed.

After the covers 614 are removed, cavities 640 may be formed in the silicon substrate 610, as depicted in FIG. 21F. The cavities 640 may be formed by, initially patterning and etching a photoresist material 641 to form a masking layer. After the photoresist material 641 is patterned, the cavities 640 may be etched into the silicon substrate 610 using a hydroxide etch, as described previously.

After the cavities 640 are formed, the photoresist material may be removed and particles 642 may be placed within the cavities, as depicted in FIG. 21G. The particles 642, may be inhibited from being displaced from the cavity 640 by placing covers 614 back onto the upper and lower faces of the silicon substrate 610.

In another embodiment, a sensor array may be formed using a supporting member, a removable cover, and a secured bottom layer. FIGS. 22 A-G depict a series of processing steps for the formation of a silicon based supporting member which includes a removable top cover and a secured bottom layer. The removable top cover is preferably configured to allow fluids to pass through the top cover and into the cavity. As depicted in FIG. 22A, a series of layers may be deposited upon both sides of a silicon substrate 610. A first removable layer 612 may be deposited upon the upper face 611 of the silicon substrate 610. The removable layer 612 may be silicon dioxide, silicon nitride, or photoresist material. In one embodiment, a layer of silicon dioxide 612 is deposited upon the silicon substrate 610. A cover 614 may be formed upon the removable layer 612 of the silicon substrate 610. In one embodiment, the cover 614 is formed from a material that differs from the material used to form the removable layer 612 and is substantially transparent to the light source of a detection system. For example, if the removable

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2970

layer 612 is formed from silicon dioxide, the cover layer 614 may be formed from silicon nitride. In one embodiment, a bottom layer 615 is formed on the bottom surface 613 of the silicon substrate 610. In one embodiment, the bottom layer 615 is formed from a material that is substantially transparent to the light source of a detection system. A second removable layer 616 may be formed upon the cover 614. Second removable layer 616 may be formed from a material that differs from the material used to form the cover layer 614. Second removable layer 616 may be formed from a material similar to the material used to form the first removable layer 612. In one embodiment, first and second removable layers 612 and 616 are formed from silicon dioxide and cover 614 is formed from silicon nitride. The layers formed on the upper surface 611 of the silicon substrate may be patterned and etched using standard photolithographic techniques. In one embodiment, the remaining portions of the layers formed on the upper surface are substantially aligned in the position where the cavities are to be formed in the silicon substrate 610.

After the layers have been etched, spacer structures may be formed on the side walls of the first removable layer 612, the cover 614, and the second removable layer 616, as depicted in FIG. 22B. The spacer structures may be formed from the same material used to form the second removable layer 616. In one embodiment, the spacer structures may be formed by depositing a spacer layer of the appropriate material and subjecting the spacer layer to an anisotropic etch. During this etching procedure the spacer layer is preferably removed such that the only regions of the spacer layer which remain may be those regions near substantially vertical surfaces, e.g., spacer structures 618.

After formation of the spacer structures 618, cover support structures 620, depicted in FIG. 22C, may be formed upon the removable layer 616 and the spacer structures 618. The cover support structures 620 may be formed by depositing a support structure layer upon the second removable layer 616 and spacer structures 618. The support structure layer is then patterned and etched, using standard photolithography, to form the support structures 620. In one embodiment, the support structures are formed from a material that differs from the removable layer materials. In one embodiment, the removable layers may be formed from silicon dioxide while the support structures and cover may be formed from silicon nitride.

Turning to FIG. 22 D, the second removable layer 616 and an upper portion of the spacer structures 618 may be removed using a wet etch process. Removal of the second removable layer leaves the top surface of the cover 614 exposed. This allows the cover 614 to be patterned and etched such that openings 622 are formed extending through the cover 614. These openings 622 may be formed in the cover 614 to allow the passage of fluid through the cover. In one embodiment, the openings 622 are formed to allow fluid to pass through, while inhibiting displacement of the particle from a cavity. The bottom layer 615 may also be similarly patterned and etched such that openings 623 may be formed extending thorough the bottom layer 615.

After the openings 622 and 623 are formed, the first removable layer 612 and the remainder of the spacer structures 618 may be removed using a wet etch. The removal of the removable layers and the spacer structures creates a "floating" cover 614, as depicted in FIG. 22E. The cover 614 may be held in proximity to the silicon substrate 610 by the support structures 620. The cover 614 may now be removed by sliding the cover 614 away from the support structures 620. In this manner a removable cover 614 may be formed.

After the cover 614 is removed, cavities 640 may be formed in the silicon substrate 610, as depicted in FIG. 22F. The cavities 640 may be formed by, initially patterning and etching a photoresist material 641 to form a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2971

masking layer. After the photoresist material 614 is patterned, the cavities 640 may be etched into the silicon substrate 610 using a hydroxide etch, as described previously.

After the cavities 640 are formed, the photoresist material may be removed and particles 642 may be placed within the cavities, as depicted in FIG. 22G. The particles 642, may be inhibited from being displaced from the cavity 640 by placing cover 614 back onto the upper face 611 of the silicon substrate 610. The bottom layer 615 may also aid in inhibiting the particle 642 from being displaced from the cavity 640. Openings 622 in cover 614 and openings 623 in bottom layer 615 may allow fluid to pass through the cavity during use.

In another embodiment, a sensor array may be formed using a supporting member and a removable cover. FIGS. 23A-G depict a series of processing steps for the formation of a silicon based supporting member which includes a removable cover. The removable cover is preferably configured to allow fluids to pass through the cover and into the cavity. As depicted in FIG. 23A, a series of layers may be deposited upon the upper surface 611 of a silicon substrate 610. A first removable layer 612 may be deposited upon the upper face 611 of the silicon substrate 610. The removable layer 612 may be silicon dioxide, silicon nitride, or photoresist material. In one embodiment, a layer of silicon dioxide 612 is deposited upon the silicon substrate 610. A cover 614 may be formed upon the removable layer 612. In one embodiment, the cover is formed from a material which differs from the material used to form the removable layer 612 and which is substantially transparent to the light source of a detection system. For example, if the removable layer 612 is formed from silicon dioxide, the cover 614 may be formed from silicon nitride. A second removable layer 616 may be formed upon the cover 614. Second removable layer 616 may be formed from a material that differs from the material used to form the cover 614. Second removable layer 616 may be formed from a material similar to the material used to form the first removable layer 612. In one embodiment, first and second removable layers 612 and 616 are formed from silicon dioxide and cover 614 is formed from silicon nitride. The layers formed on the upper surface 611 of the silicon substrate may be patterned and etched using standard photolithographic techniques. In one embodiment, the remaining portions of the layers formed on the upper surface are substantially aligned in the position where the cavities are to be formed in the silicon substrate 610.

After the layers have been etched, spacer structures 618 may be formed on the side walls of the first removable layer 612, the cover layer 614, and the second removable layer 616, as depicted in FIG. 23B. The spacer structures 618 may be formed from the same material used to form the second removable layer 616. In one embodiment, the spacers may be formed by depositing a spacer layer of the appropriate material upon the second removable layer and subjecting the material to an anisotropic etch. During this etching procedure the spacer layer is preferably removed such that the only regions of the spacer layer which remain may be those regions near substantially vertical surfaces, e.g., spacer structures 618.

After formation of the spacer structures 618, cover support structures 620, depicted in FIG. 23C, may be formed upon the removable layer 616 and the spacer structures 618. The cover support structure may be formed by initially depositing a support structure layer upon the second removable layer 616 and spacer structures 618. The support structure layer is then patterned and etched, using standard photolithography, to form the support structures 620. In one embodiment, the support structures 620 are formed from a material that differs from the removable layer materials. In one embodiment, the removable layers may be formed from silicon dioxide while the support structure and cover layer may be formed from silicon nitride.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2972

Turning to FIG. 23D, the second removable layer 616 and an upper portion of the spacer structures 618 may be removed using a wet etch process. Removal of the second removable layer leaves the top surface of the cover 614 exposed. This allows the cover 614 to be patterned and etched such that openings 622 are formed extending through the cover 614. These openings 622 may be formed in the cover 614 to allow the passage of fluid through the cover 614.

After the openings 622 are formed, the remainder of the first removable layer 612 and the remainder of the spacer structures 618 may be removed using a wet etch. The removal of the removable layers and the spacer structures creates a "floating" cover 614, as depicted in FIG. 23E. The cover 614 is preferably held in proximity to the silicon substrate 610 by the support structures 620. The cover 614 may now be removed by sliding the cover 614 away from the support structures 620. In this manner a removable cover 614 may be formed.

After the cover 614 is removed, cavities 640 may be formed in the silicon substrate 610, as depicted in FIG. 23F. The cavities 640 may be formed by initially depositing and patterning a photoresist material 641 upon the silicon support 610. After the photoresist material 614 is patterned, the cavities 640 may be etched into the silicon substrate 610 using a hydroxide etch, as described previously. The etching of the cavities may be accomplished such that a bottom width of the cavity 643 is less than a width of a particle 642. In one embodiment, the width of the bottom of the cavity may be controlled by varying the etch time. Typically, longer etching times result in a larger opening at the bottom of the cavity. By forming a cavity in this manner, a particle placed in the cavity may be too large to pass through the bottom of the cavity. Thus, a supporting member that does not include a bottom layer may be formed. An advantage of this process is that the processing steps may be reduced making production simpler.

After the cavities 640 are formed, the photoresist material may be removed and particles 642 may be placed within the cavities, as depicted in FIG. 23G. The particles 642, may be inhibited from being displaced from the cavity 640 by placing cover 614 back onto the upper face 611 of the silicon substrate 610. The narrow bottom portion of the cavity may also aid in inhibiting the particle 642 from being displaced from the cavity 640.

Figures 24A-d depict a sequence of processing steps for the formation of a silicon based supporting member which includes a top partial cover and a bottom partial cover. The top partial cover and bottom partial covers are, in one embodiment, configured to allow fluids to pass into the cavity and out through the bottom of the cavity. As depicted in FIG. 24A, a bottom layer 712 may be deposited onto the bottom surface of a silicon substrate 710. The bottom layer 712 may be silicon dioxide, silicon nitride, or photoresist material. In one embodiment, a layer of silicon nitride 712 is deposited upon the silicon substrate 710. In one embodiment, openings 714 are formed through the bottom layer as depicted in FIG. 24A. Openings 714, in one embodiment, are substantially aligned with the position of the cavities to be subsequently formed. The openings 714 may have a width that is substantially less than a width of a particle. Thus a particle will be inhibited from passing through the openings 714.

Cavities 716 may be formed in the silicon substrate 710, as depicted in FIG. 24B. The cavities 716 may be formed by initially depositing and patterning a photoresist layer upon the silicon substrate 710. After the photoresist material is patterned, cavities 716 may be etched into the silicon substrate 710 using a number of etching techniques, including wet and plasma etches. The width of the cavities 716 is preferably greater than the width of a particle, thus allowing a particle to be placed within each of the cavities. The cavities 716, in one

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2973

embodiment, are preferably formed such that the cavities are substantially aligned over the openings 714 formed in the bottom layer.

After the cavities have been formed, particles 718 may be inserted into the cavities 716, as depicted in FIG. 24C. The etched bottom layer 712 may serve as a support for the particles 718. Thus the particles 718 may be inhibited from being displaced from the cavities by the bottom layer 712. The openings 714 in the bottom layer 712 may allow fluid to pass through the bottom layer during use.

After the particles are placed in the cavities, a top layer 720 may be placed upon the upper surface 717 of the silicon substrate. In one embodiment, the top layer 720 is formed from a material is substantially transparent to the light source of a detection system. The top layer may be formed from silicon nitride, silicon dioxide or photoresist material. In one embodiment, a sheet of photoresist material is used. After the top layer 620 is formed, openings 719 may be formed in the top layer to allow the passage of the fluid into the cavities. If the top layer 720 is composed of photoresist material, after depositing the photoresist material across the upper surface of the silicon substrate, the openings may be initially formed by exposing the photoresist material to the appropriate wavelength and pattern of light. If the top layer is compose of silicon dioxide or silicon nitride the top layer 720 may be developed by forming a photoresist layer upon the top layer, developing the photoresist, and using the photoresist to etch the underlying top layer.

Similar sensor arrays may be produced using materials other than silicon for the supporting member. For example, as depicted in FIG 25 A-D, the supporting member may be composed of photoresist material. In one embodiment, sheets of photoresist film may be used to form the supporting member. Photoresist film sheets are commercially available from E. I. du Pont de Nemours and Company, Wilmington, DE under the commercial name RISTON. The sheets come in a variety of sizes, the most common having a thickness ranging from about 1 mil. (25 μm) to about 2 mil. (50 μm).

In an embodiment, a first photoresist layer 722 is developed and etched such that openings 724 are formed. The openings may be formed proximate the location of the subsequently formed cavities. Preferably, the openings have a width that is substantially smaller than a width of the particle. The openings may inhibit displacement of the particle from a cavity. After the first photoresist layer 720 is patterned and etched, a main layer 726 is formed upon the bottom layer. The main layer 720 is preferably formed from a photoresist film that has a thickness substantially greater than a typical width of a particle. Thus, if the particles have a width of about 30 μm, a main layer may be composed of a 50 μm photoresist material. Alternatively, the photoresist layer may be composed of a multitude of photoresist layers placed upon each other until the desired thickness is achieved, as will be depicted in later embodiments.

The main photoresist layer may be patterned and etched to form the cavities 728, as depicted in FIG. 25B. The cavities, in one embodiment, are substantially aligned above the previously formed openings 724. Cavities 728, in one embodiment, have a width which is greater than a width of a particle.

For many types of analysis, the photoresist material is substantially transparent to the light source used. Thus, as opposed to a silicon supporting member, the photoresist material used for the main supporting layer may be substantially transparent to the light used by the light source. In some circumstances, the transparent nature of the supporting member may allow light from the cavity to migrate, through the supporting member, into a second cavity. This leakage of light from one cavity to the next may lead to detection problems. For example, if a first

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2974

particle in a first cavity produces a fluorescent signal in response to an analyte, this signal may be transmitted through the supporting member and detected in a proximate cavity. This may lead to inaccurate readings for the proximately spaced cavities, especially if a particularly strong signal is produced by the interaction of the particle with an analyte.

To reduce the occurrence of this "cross-talk", a substantially reflective layer 730 may be formed along the inner surface of the cavity. In one embodiment, the reflective layer 730 is composed of a metal layer which is formed on the upper surface of the main layer and the inner surface of the cavity. The metal layer may be deposited using chemical vapor deposition or other known techniques for depositing thin metal layers. The presence of a reflective layer may inhibit "cross-talk" between the cavities.

After the cavities 728 have been formed, particles 718 may be inserted into the cavities 728, as depicted in FIG. 25C. The first photoresist layer 722 may serve as a support for the particles 718. The particles may be inhibited from being displaced from the cavities by the first photoresist layer 722. The openings 724 in the first photoresist layer 722 may allow fluid to pass through the bottom layer during use.

After the particles 728 are placed in the cavities 728, a top photoresist layer 732 may be placed upon the upper surface of the silicon substrate. After the cover layer is formed, openings 734 may be formed in the cover layer to allow the passage of the fluid into the cavities.

In another embodiment, the supporting member may be formed from a plastic substrate, as depicted in FIG. 26A-D. In one embodiment, the plastic substrate is composed of a material which is substantially resistant to the fluid which includes the analyte. Examples of plastic materials which may be used to form the plastic substrate include, but are not limited to, acrylic resins, polycarbonates, polyester resins, polyethylenes, polyimides, polyvinyl polymers (e.g., polyvinyl chloride, polyvinyl acetate, polyvinyl dichloride, polyvinyl fluoride, etc.), polystyrenes, polypropylenes, polytetrafluoroethylenes, and polyurethanes. The plastic substrate may be substantially transparent or substantially opaque to the light produced by the light source. After obtaining a suitable plastic material 740, a series of cavities 742 may be formed in the plastic material. The cavities 740 may be formed by drilling (either mechanically or with a laser), transfer molding (e.g., forming the cavities when the plastic material is formed using appropriately shaped molds), or using a punching apparatus to punch cavities into the plastic material. In one embodiment, the cavities 740 are formed such that a lower portion 743 of the cavities is substantially narrower than an upper portion 744 of the cavities. The lower portion 743 of the cavities may have a width substantially less than a width of a particle. The lower portion 743 of the cavities 740 may inhibit the displacement of a particle from the cavity 740. While depicted as rectangular, with a narrower rectangular opening at the bottom, it should be understood that the cavity may be formed in a number of shapes including but not limited to pyramidal, triangular, trapezoidal, and oval shapes. An example of a pyramidal cavity which is tapered such that the particle is inhibited from being displaced from the cavity is depicted in FIG. 25D.

After the cavities 742 are formed, particles 718 may be inserted into the cavities 742, as depicted in FIG. 26B. The lower portion 743 of the cavities may serve as a support for the particles 718. The particles 718 may be inhibited from being displaced from the cavities 742 by the lower portion 743 of the cavity. After the particles are placed in the cavities 740, a cover 744 may be placed upon the upper surface 745 of the plastic substrate 740, as depicted in FIG. 26C. In one embodiment, the cover is formed from a film of photoresist material. After the cover

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2975

744 is placed on the plastic substrate 740, openings 739 may be formed in the cover layer to allow the passage of the fluid into the cavities.

In some circumstances a substantially transparent plastic material may be used. As described above, the use of a transparent supporting member may lead to "cross-talk" between the cavities. To reduce the occurrence of this "cross-talk", a substantially reflective layer 748 may be formed on the inner surface 746 of the cavity, as depicted in FIG. 26E. In one embodiment, the reflective layer 748 is composed of a metal layer which is formed on the inner surface of the cavities 742. The metal layer may be deposited using chemical vapor deposition or other techniques for depositing thin metal layers. The presence of a reflective layer may inhibit cross-talk between the cavities.

In another embodiment, a silicon based supporting member for a sensing particle may be formed without a bottom layer. In this embodiment, the cavity may be tapered to inhibit the passage of the particle from the cavity, through the bottom of the supporting member. FIG. 27A-D, depicts the formation of a supporting member from a silicon substrate. In this embodiment, a photoresist layer 750 is formed upon an upper surface of a silicon substrate 752, as depicted in FIG. 27A. The photoresist layer 750 may be patterned and developed such that the regions of the silicon substrate in which the cavities will be formed are exposed.

Cavities 754 may now be formed, as depicted in FIG. 27B, by subjecting the silicon substrate to an anisotropic etch. In one embodiment, a potassium hydroxide etch is used to produced tapered cavities. The etching may be controlled such that the width of the bottom of the cavities 750 is less than a width of the particle. After the cavities have been etched, a particle 756 may be inserted into the cavities 754 as depicted in FIG. 27C. The particle 756 may be inhibited from passing out of the cavities 754 by the narrower bottom portion of the cavities. After the particle is positioned within the cavities 754, a cover 758 may be formed upon the silicon substrate 752, as depicted in FIG. 27D. The cover may be formed of any material substantially transparent to the light produced by the light source used for analysis. Openings 759 may be formed in the cover 758 to allow the fluid to pass into the cavity from the top face of the supporting member 752. The openings 759 in the cover and the opening at the bottom of the cavities 754 together may allow fluid to pass through the cavity during use.

In another embodiment, a supporting member for a sensing particle may be formed from a plurality of layers of a photoresist material. In this embodiment, the cavity may be tapered to inhibit the passage of the particle from the cavity, through the bottom of the supporting member. FIGS. 28A-E depict the formation of a supporting member from a plurality of photoresist layers. In an embodiment, a first photoresist layer 760 is developed and etched to form a series of openings 762 which are positioned at the bottom of subsequently formed cavities, as depicted in FIG. 28A. As depicted in FIG. 28B, a second layer of photoresist material 764 may be formed upon the first photoresist layer 760. The second photoresist layer may be developed and etched to form openings substantially aligned with the openings of the first photoresist layer 760. The openings formed in the second photoresist layer 764, in one embodiment, are substantially larger than the layers formed in the first photoresist layer 760. In this manner, a tapered cavity may be formed while using multiple photoresist layers.

As depicted in FIG. 28C, additional layers of photoresist material 766 and 768 may be formed upon the second photoresist layer 764. The openings of the additional photoresist layers 766 and 768 may be progressively larger as each layer is added to the stack. In this manner, a tapered cavity may be formed. Additional layers of photoresist material may be added until the desired thickness of the supporting member is obtained. The thickness

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2976

of the supporting member, in one embodiment, is greater than a width of a particle. For example, if a layer of photoresist material has a thickness of about 25 μm and a particle has a width of about 100 μm, a supporting member may be formed from four or more layers of photoresist material. While depicted as pyramidal, the cavity may be formed in a number of different shapes, including but not limited to, rectangular, circular, oval, triangular, and trapezoidal. Any of these shapes may be obtained by appropriate patterning and etching of the photoresist layers as they are formed.

In some instances, the photoresist material may be substantially transparent to the light produced by the light source. As described above, the use of a transparent supporting member may lead to "cross-talk" between the cavities. To reduce the occurrence of this "cross-talk", a substantially reflective layer 770 may be formed along the inner surface of the cavities 762, as depicted in FIG. 28D. In one embodiment, the reflective layer is composed of a metal layer which is formed on the inner surface of the cavities 762. The metal layer may be deposited using chemical vapor deposition or other techniques for depositing thin metal layers. The presence of a reflective layer may inhibit "cross-talk" between the cavities.

After the cavities 762 are formed, particles 772 may be inserted into the cavities 762, as depicted in FIG. 28D. The narrow portions of the cavities 762 may serve as a support for the particles 772. The particles 772 may be inhibited from being displaced from the cavities 762 by the lower portion of the cavities. After the particles 772 are placed in the cavities 762, a cover 774 may be placed upon the upper surface of the top layer 776 of the supporting member, as depicted in FIG. 28E. In one embodiment, the cover 774 is also formed from a film of photoresist material. After the cover layer is formed, openings 778 may be formed in the cover 774 to allow the passage of the fluid into the cavities.

In another embodiment, a supporting member for a sensing particle may be formed from photoresist material which includes a particle support layer. FIGS. 29A-E depict the formation of a supporting member from a series of photoresist layers. In an embodiment, a first photoresist layer 780 is developed and etched to form a series of openings 782 which may become part of subsequently formed cavities. In another embodiment, a cavity having the appropriate depth may be formed by forming multiple layers of a photoresist material, as described previously. As depicted in FIG. 29B, a second photoresist layer 784 may be formed upon the first photoresist layer 780. The second photoresist layer 784 may be patterned to form openings substantially aligned with the openings of the first photoresist layer 782. The openings formed in the second photoresist layer 784 may be substantially equal in size to the previously formed openings. Alternatively, the openings may be variable in size to form different shaped cavities.

For reasons described above, a substantially reflective layer 786 may be formed along the inner surface of the cavities 782 and the upper surface of the second photoresist layer 784, as depicted in FIG. 29C. In one embodiment, the reflective layer is composed of a metal layer. The metal layer may be deposited using chemical vapor deposition or other techniques for depositing thin metal layers. The presence of a reflective layer may inhibit "cross-talk" between the cavities.

After the metal layer is deposited, a particle support layer 788 may be formed on the bottom surface of the first photoresist layer 780, as depicted in FIG. 29D. The particle support layer 788 may be formed from photoresist material, silicon dioxide, silicon nitride, glass or a substantially transparent plastic material. The particle support

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2977

layer 788 may serve as a support for the particles placed in the cavities 782. The particle support layer, in one embodiment, is formed from a material that is substantially transparent to the light produced by the light source.

After the particle supporting layer 788 is formed, particles 785 may be inserted into the cavities 782, as depicted in FIG. 29E. The particle support layer 788 may serve as a support for the particles. Thus the particles 785 may be inhibited from being displaced from the cavities by the particle support layer 788. After the particles 785 are placed in the cavities 782, a cover 787 may be placed upon the upper surface of the second photoresist layer 784, as depicted in FIG. 29E. In one embodiment, the cover is also formed from a film of photoresist material. After the cover is formed, openings 789 may be formed in the cover 787 to allow the passage of the fluid into the cavities. In this embodiment, the fluid is inhibited from flowing through the supporting member. Instead, the fluid may flow into and out of the cavities via the openings 789 formed in the cover 787.

A similar supporting member may be formed from a plastic material, as depicted in FIGS. 30A-D. The plastic material may be substantially resistant to the fluid which includes the analyte. The plastic material may be substantially transparent or substantially opaque to the light produced by the light source. After obtaining a suitable plastic substrate 790, a series of cavities 792 may be formed in the plastic substrate 790. The cavities may be formed by drilling (either mechanically or with a laser), transfer molding (e.g., forming the cavities when the plastic substrate is formed using appropriately shaped molds), or using a punching machine to form the cavities. In one embodiment, the cavities extend through a portion of the plastic substrate, terminating proximate the bottom of the plastic substrate, without passing through the plastic substrate. After the cavities 792 are formed, particles 795 may be inserted into the cavities 792, as depicted in FIG. 30B. The bottom of the cavity may serve as a support for the particles 795. After the particles are placed in the cavities, a cover 794 may be placed upon the upper surface of the plastic substrate 790, as depicted in FIG. 30C. In one embodiment, the cover may be formed from a film of photoresist material. After the cover 794 is formed, openings 796 may be formed in the cover to allow the passage of the fluid into the cavities. While depicted as rectangular, is should be understood that the cavities may be formed in a variety of different shapes, including triangular, pyramidal, pentagonal, polygonal, oval, or circular. It should also be understood that cavities having a variety of different shapes may be formed into the same plastic substrate, as depicted in FIG. 30D.

In one embodiment, a series of channels may be formed in the supporting member interconnecting some of the cavities, as depicted in FIG. 3. Pumps and valves may also be incorporated into the supporting member to aid passage of the fluid through the cavities. A schematic figure of a diaphragm pump 800 is depicted in FIG. 31. Diaphragm pumps, in general, include a cavity 810, a flexible diaphragm 812, an inlet valve 814, and an outlet valve 816. The flexible diaphragm 812, during use, is deflected as shown by arrows 818 to create a pumping force. As the diaphragm is deflected toward the cavity 810 it may cause the inlet valve 814 to close, the outlet valve 816 to open and any liquid which is in the cavity 810 will be forced toward the outlet 816. As the diaphragm moves away from the cavity 810, the outlet valve 816 may be pulled to a closed position, and the inlet valve 814 may be opened, allowing additional fluid to enter the cavity 810. In this manner a pump may be used to pump fluid through the cavities. It should be understood that the pump depicted in FIG. 31 is a generalized version of a diaphragm based pump. Actual diaphragm pumps may have different shapes or may have inlet and outlet valves which are separate from the pumping device.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2978

In one embodiment, the diaphragm 810 may be made from a piezoelectric material. This material will contract or expand when an appropriate voltage is applied to the diaphragm. Pumps using a piezoelectric diaphragms are described in U.S. Patent Nos. 4,344,743, 4,938,742, 5,611,676, 5,705,018, and 5,759,015, all of which are incorporated by reference. In other embodiments, the diaphragm may be activated using a pneumatic system. In these systems, an air system may be coupled to the diaphragm such that changes in air density about the diaphragm, induced by the pneumatic system, may cause the diaphragm to move toward and away from the cavity. A pneumatically controlled pump is described in United States Patent No. 5,499,909 which is incorporated by reference. The diaphragm may also be controlled using a heat activated material. The diaphragm may be formed from a temperature sensitive material. In one embodiment, the diaphragm may be formed from a material which is configured to expand and contract in response to temperature changes. A pump system which relies on temperature activated diaphragm is described in United States Patent No. 5,288,214 which is incorporated by reference.

In another embodiment, an electrode pump system may be used. FIG. 32 depicts a typical electrode based system. A series of electrodes 820 may be arranged along a channel 822 which may lead to a cavity 824 which includes a particle 826. By varying the voltage in the electrodes 820 a current flow may be induced in the fluid within the channel 822. Examples of electrode based systems include, but are not limited to, electroosmosis systems, electrohydrodynamic systems, and combinations of electroosmosis and electrohydrodynamic systems.

Electrohydrodynamic pumping of fluids is known and may be applied to small capillary channels. In an electrohydrodynamic system electrodes are typically placed in contact with the fluid when a voltage is applied. The applied voltage may cause a transfer in charge either by transfer or removal of an electron to or from the fluid. This electron transfer typically induces liquid flow in the direction from the charging electrode to the oppositely charged electrode. Electrohydrodynamic pumps may be used for pumping fluids such as organic solvents.

Electroosmosis, is a process which involves applying a voltage to a fluid in a small space, such as a capillary channel, to cause the fluid to flow. The surfaces of many solids, including quartz, glass and the like, become variously charged, negatively or positively, in the presence of ionic materials, such as for example salts, acids or bases. The charged surfaces will attract oppositely charged (positive or negative) counterions in aqueous solutions. The application of a voltage to such a solution results in a migration of the counterions to the oppositely charged electrode, and moves the bulk of the fluid as well. The volume flow rate is proportional to the current, and the volume flow generated in the fluid is also proportional to the applied voltage. An electroosmosis pump system is described in United States Patent No. 4,908,112 which is incorporated by reference.

In another embodiment, a combination of electroosmosis pumps and electrohydrodynamic pumps may be used. Wire electrodes may be inserted into the walls of a channel at preselected intervals to form alternating electroosmosis and electrohydrodynamic devices. Because electroosmosis and electrohydrodynamic pumps are both present, a plurality of different solutions, both polar and non-polar, may be pump along a single channel. Alternatively, a plurality of different solutions may be passed along a plurality of different channels connected to a cavity. A system which includes a combination of electroosmosis pumps and electrohydrodynamic pumps is described in United States Patent No. 5,632,876 which is incorporated by reference.

In an embodiment, a pump may be incorporated into a sensor array system, as depicted in FIG. 32. A sensor array 830 includes at least one cavity 832 in which a particle 834 may be placed. The cavity 832 may be configured to allow fluid to pass through the cavity during use. A pump 836 may be incorporated onto a portion of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2979

the supporting member 838. A channel 831 may be formed in the supporting member 838 coupling the pump 836 to the cavity 832. The channel 831 may be configured to allow the fluid to pass from the pump 836 to the cavity 832. The pump 836 may be positioned away from the cavity 832 to allow light to be directed through the cavity during use. The supporting member 838 and the pump 836 may be formed from a silicon substrate, a plastic material, or photoresist material. The pump 836 may be configured to pump fluid to the cavity via the channel, as depicted by the arrows in FIG. 32. When the fluid reaches the cavity 832, the fluid may flow past the particle 834 and out through the bottom of the cavity. An advantage of using pumps is that better flow through the channels may be achieved. Typically, the channels and cavities may have a small volume. The small volume of the cavity and channel tends to inhibit flow of the fluid through the cavity. By incorporating a pump, the flow of fluid to the cavity and through the cavity may be increased, allowing more rapid testing of the fluid sample. While a diaphragm based pump system is depicted in FIG. 33, it should be understood that electrode based pumping systems may also be incorporated into the sensor array to produce fluid flows.

In another embodiment, a pump may be coupled to a supporting member for analyzing analytes in a fluid stream, as depicted in FIG. 34. A channel 842 may couple a pump 846 to multiple cavities 844 formed in a supporting member 840. The cavities 842 may include sensing particles 848. The pump may be configured to create a flow of the fluid through the channel 842 to the cavities 848. In one embodiment, the cavities may inhibit the flow of the fluid through the cavities 844. The fluid may flow into the cavities 844 and past the particle 848 to create a flow of fluid through the sensor array system. In this manner a single pump may be used to pass the fluid to multiple cavities. While a diaphragm pump system is depicted in FIG. 33, it should be understood that electrode pumping systems may also be incorporated into the supporting member to create similar fluid flows..

In another embodiment, multiple pumps may be coupled to a supporting member of a sensor array system. In one embodiment, the pumps may be coupled in series with each other to pump fluid to each of the cavities. As depicted in FIG. 35, a first pump 852 and a second pump 854 may be coupled to a supporting member 850. The first pump 852 may be coupled to a first cavity 856. The first pump may be configured to transfer fluid to the first cavity 856 during use. The cavity 856 may be configured to allow the fluid to pass through the cavity to a first cavity outlet channel 858. A second pump 854 may also be coupled to the supporting member 850. The second pump 854 may be coupled to a second cavity 860 and the first cavity outlet channel 858. The second pump 854 may be configured to transfer fluid from the first cavity outlet channel 858 to the second cavity 860. The pumps may be synchronized such that a steady flow of fluid through the cavities is obtained. Additional pumps may be coupled to the second cavity outlet channel 862 such that the fluid may be pumped to additional cavities. In one embodiment, each of the cavities in the supporting member is coupled to a pump configured to pump the fluid stream to the cavity.

In another embodiment, multiple electrode based pumps may be incorporated into the sensor array system. The pumps may be formed along the channels which couple the cavities. . As depicted in FIG. 36, a plurality of cavities 870 may be formed in a supporting member 872 of a sensor array. Channels 874 may also be formed in the supporting member 872 interconnecting the cavities 870 with each other. An inlet channel 876 and an outlet channel 877, which allow the fluid to pass into and out of the sensor array, respectively, may also be formed. A series of electrodes 878 may be positioned over the channels 874, 876, and 877. The electrodes may be used to form an electroosmosis pumping system or an electrohydrodynamic pumping system. The electrodes may

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2980

be coupled to a controller 880 which may apply the appropriate voltage to the appropriate electrodes to produce a flow of the fluid through the channels. The pumps may be synchronized such that a steady flow of fluid through the cavities is obtained. The electrodes may be positioned between the cavities such that the electrodes do not significantly interfere with the application of light to the cavities.

In some instances it may be necessary to add a reagent to a particle before, during or after an analysis process. Reagents may include receptor molecules or indicator molecules. Typically, such reagents may be added by passing a fluid stream which includes the reagent over the sensor array. In an embodiment, the reagent may be incorporated into the sensor array system which includes two particles. In this embodiment, a sensor array system 900 may include two particles 910 and 920 for each sensing position of the sensor array, as depicted in FIG. 37. The first particle 910 may be positioned in a first cavity 912. The second particle 920 may be positioned in a second cavity 922. In one embodiment, the second cavity is coupled to the first cavity via a channel 930. The second particle includes a reagent which is at least partially removable from the second particle 920. The reagent may also be configured to modify the first particle 910, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with an analyte during use. The reagent may be added to the first cavity before, during or after a fluid analysis. The reagent is preferably coupled to the second particle 920. The a portion of the reagent coupled to the second particle may be decoupled from the particle by passing a decoupling solution past the second particle. The decoupling solution may include a decoupling agent which will cause at least a portion of the reagent to be at released by the particle. A reservoir 940 may be formed on the sensor array to hold the decoupling solution.

A first pump 950 and a second pump 960 may also be coupled to the supporting member 915. The first pump 950 may be configured to pump fluid from a fluid inlet 952 to the first cavity 912 via channel 930. The fluid inlet 952 is the location where the fluid, which includes the analyte, is introduced into the sensor array system. A second pump 950 may be coupled to the reservoir 940 and the second cavity 922. The second pump 960 may be used to transfer the decoupling solution from the reservoir to the second cavity 922. The decoupling solution may pass through the second cavity 922 and into first cavity 912. Thus, as the reagent is removed the second particle it may be transferred to the first cavity912, where the reagent may interact with the first particle 910. The reservoir may be refilled by removing the reservoir outlet 942, and adding additional fluid to the reservoir 940. While diaphragm based pump systems are depicted in FIG. 37, it should be understood that electrode based pumping systems may also be incorporated into the sensor array to produce fluid flows.

The use of such a system is described by way of example. In some instances it may be desirable to add a reagent to the first particle prior to passing the fluid which includes the analyte to the first particle. The reagent may be coupled to the second particle and placed in the sensor array prior to use, typically during construction of the array. A decoupling solution may be added to the reservoir before use. A controller 970 may also be coupled to the system to allow automatic operation of the pumps. The controller 970 may be configured to initiate the analysis sequence by activating the second pump 960, causing the decoupling solution to flow from the reservoir 940 to the second cavity 922. As the fluid passes through the second cavity 922, the decoupling solution may cause at least some of the reagent molecules to be released from the second particle 920. The decoupling solution may be passed out of the second cavity 922 and into the first cavity 912. As the solution passes through the first cavity, some of the reagent molecules may be captured by the first particle 910. After a sufficient number of molecules

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2981

have been captured by the first particle 910, flow of fluid thorough the second cavity 922 may be stopped. During this initialization of the system, the flow of fluid through the first pump may be inhibited.

After the system is initialized, the second pump may be stopped and the fluid may be introduced to the first cavity. The first pump may be used to transfer the fluid to the first cavity. The second pump may remain off, thus inhibiting flow of fluid from the reservoir to the first cavity. It should be understood that the reagent solution may be added to the first cavity while the fluid is added to the first cavity. In this embodiment, both the first and second pumps may be operated substantially simultaneously.

Alternatively, the reagent may be added after an analysis. In some instances, a particle may interact with an analyte such that a change in the receptors attached to the first particle occurs. This change may not, however produce a detectable signal. The reagent attached to the second bead may be used to produce a detectable signal when it interacts with the first particle, if a specific analyte is present. In this embodiment, the fluid is introduced into the cavity first. After the analyte has been given time to react with the particle, the reagent may be added to the first cavity. The interaction of the reagent with the particle may produce a detectable signal. For example, an indicator reagent may react with a particle which has been exposed to an analyte to produce a color change on the particle. Particle which have not been exposed to the analyte may remain unchanged or show a different color change.

As shown in FIG. 1, a system for detecting analytes in a fluid may include a light source 110, a sensor array 120 and a detector 130. The sensor array 120 is preferably formed of a supporting member which is configured to hold a variety of particles 124 in an ordered array. A high sensitivity CCD array may be used to measure changes in optical characteristics which occur upon binding of the biological/chemical agents. Data acquisition and handling is preferably performed with existing CCD technology. As described above, colorimetric analysis may be performed using a white light source and a color CCD detector. However, color CCD detectors are typically more expensive than gray scale CCD detectors.

In one embodiment, a gray scale CCD detector may be used to detect colorimetric changes. In one embodiment, a gray scale detector may be disposed below a sensor array to measure the intensity of light being transmitted through the sensor array. A series of lights (e.g., light emitting diodes) may be arranged above the sensor array. In one embodiment, groups of three LED lights may be arranged above each of the cavities of the array. Each of these groups of LED lights may include a red, blue and a green light. Each of the lights may be operated individually such that one of the lights may be on while the other two lights are off. In order to provide color information while using a gray scale detector, each of the lights is sequentially turned on and the gray scale detector is used to measure the intensity of the light passing through the sensor array. After information from each of the lights is collected, the information may be processed to derive the absorption changes of the particle.

In one embodiment, the data collected by the gray scale detector may be recorded using 8 bits of data. Thus, the data will appear as a value between 0 and 255. The color of each chemical sensitive element may be represented as a red, blue and green value. For example, a blank particle (i.e., a particle which does not include a receptor) will typically appear white. When each of the LED lights (red, blue and green) are operated the CCD detector will record a value corresponding to the amount of light transmitted through the cavity. The intensity of the light may be compared to a blank particle, to determine the absorbance of a particle with respect to the LED light which is used. Thus, the red, green and blue components may be recorded individually without the use of a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2982

color CCD detector. In one embodiment, it is found that a blank particle exhibits an absorbance of about 253 when illuminated with a red LED, a value of about 250 when illuminated by a green LED, and a value of about 222 when illuminated with a blue LED. This signifies that a blank particle does not significantly absorb red, green or blue light. When a particle with a receptor is scanned, the particle may exhibit a color change, due to absorbance by the receptor. For example, it was found that when a particle which includes a 5-carboxyfluorescein receptor is subjected to white light, the particle shows a strong absorbance of blue light. When a red LED is used to illuminate the particle, the gray scale CCD detector may detect a value of about 254. When the green LED is used, the gray scale detector may detect a value of about 218. When a blue LED light is used, a gray scale detector may detect a value of about 57. The decrease in transmittance of blue light is believed to be due to the absorbance of blue light by the 5-carboxyfluorescein. In this manner the color changes of a particle may be quantitatively characterized using a gray scale detector.

As described above, after the cavities are formed in the supporting member, a particle may be positioned at the bottom of a cavity using a micromanipulator. This allows the location of a particular particle to be precisely controlled during the production of the array. The use of a micromanipulator may, however, be impractical for production of sensor array systems. An alternate method of placing the particles into the cavities may involve the use of a silk screen like process. A series of masking materials may be placed on the upper surface of the sensor array prior to filling the cavities. The masking materials may be composed of glass, metal or plastic materials. A collection of particles may be placed upon the upper surface of the masking materials and the particles may be moved across the surface. When a cavity is encountered, a particle may drop into the cavity if the cavity is unmasked. Thus particles of known composition are placed in only the unmasked regions. After the unmasked cavities are filled, the masking pattern may be altered and a second type of particles may be spread across the surface. Preferably, the masking material will mask the cavities that have already been filled with particle. The masking material may also mask other non-filled cavities. This technique may be repeated until all of the cavities are filled. After filling the cavities, a cover may be placed on the support member, as described above, to inhibit the displacement and mixing of the particles. An advantage of such a process is that it may be more amenable to industrial production of supporting members.

Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as the presently preferred embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2983

What is claimed is:

1. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

2. The system of claim 1, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

3. The system of claim 1, wherein the system comprises a plurality of particles positioned within the cavity.

4. The system of claim 1, wherein the light source comprises a light emitting diode.

5. The system of claim 1, wherein the light source comprises a white light source.

6. The system of claim 1, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is positioned below a bottom surface of the supporting member, and wherein the top cover layer is positioned above the upper surface of the supporting member, and wherein the bottom layer and the top cover layer are positioned such that the particle is substantially contained within the cavity by the bottom layer and the top cover layer.

7. The system of claim 6, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

8. The system of claim 1, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is coupled to a bottom surface of the supporting member, and wherein the top cover layer is coupled to a top surface of the supporting member; and wherein both the bottom layer and the top cover layer are coupled to the supporting member such that the particle is substantially contained within the cavity by bottom layer and the top cover layer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2984

9. The system of claim 8, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

10. The system of claim 1, wherein the sensor array further comprises a bottom layer coupled to the supporting member, and wherein the supporting member comprises silicon, and wherein the bottom layer comprises silicon nitride.

11. The system of claim 1, wherein the sensor array further comprises a sensing cavity formed on a bottom surface of the sensor array.

12. The system of claim 1, wherein the supporting member is formed from a plastic material, and wherein the sensor array further comprises a top cover layer, the top cover layer being coupled to the supporting member such that the particle is substantially contained within the cavity, and wherein the top cover layer is configured to allow the fluid to pass through the top cover layer to the particle, and wherein both the supporting member and the top cover layer are substantially transparent to light produced by the light source.

13. The system of claim 1, further comprising a fluid delivery system coupled to the supporting member.

14. The system of claim 1, wherein the detector comprises a charge-coupled device.

15. The system of claim 1, wherein the detector comprises an ultraviolet detector.

16. The system of claim 1, wherein the detector comprises a fluorescence detector.

17. The system of claim 1, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

18. The system of claim 1, wherein the particle ranges from about 0.05 micron to about 500 microns.

19. The system of claim 1, wherein a volume of the particle changes when contacted with the fluid.

20. The system of claim 1, wherein the particle comprises a metal oxide particle.

21. The system of claim 1, wherein the particle comprises a metal quantum particle.

22. The system of claim 1, wherein the particle comprises a semiconductor quantum particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2985

23. The system of claim 1, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

24. The system of claim 23, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

25. The system of claim 23, wherein the receptor molecule produces the signal in response to the pH of the fluid.

26. The system of claim 23, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

27. The system of claim 23, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of a carbohydrate.

28. The system of claim 23, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

29. The system of claim 23, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

30. The system of claim 23, wherein the receptor comprises a polynucleotide.

31. The system of claim 23, wherein the receptor comprises a peptide.

32. The system of claim 23, wherein the receptor comprises an enzyme.

33. The system o f claim 23, wherein the receptor comprises a synthetic receptor.

34. The system of claim 23, wherein the receptor comprises an unnatural biopolymer.

35. The system of claim 23, wherein the receptor comprises an antibody.

36. The system of claim 23, wherein the receptor comprises an antigen.

37. The system of claim 1, wherein the analyte comprises phosphate functional groups, and wherein the particle is configured to produce the signal in the presence of the phosphate functional groups.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2986

38. The system of claim 1, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

39. The system of claim 1, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

40. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising a plurality of cavities formed within the supporting member, wherein the supporting member comprises silicon;
a plurality of particles, the particles comprising a receptor molecule covalently linked to a polymeric resin, wherein the particles are positioned within the cavities, and wherein each of the particles is configured to produce a signal when the particle interacts with the analyte during use; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

41. The system of claim 40, wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

42. The system of claim 40, wherein each cavity is configured to hold a single particle.

43. The system of claim 40, wherein each cavity is configured to hold a plurality of particles.

44. The system of claim 40, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is positioned below a bottom surface of the supporting member, and wherein the top cover layer is positioned above the upper surface of the supporting member, and wherein the bottom layer and the top cover layer are positioned such that the particle is substantially contained within the cavity by the bottom layer and the top cover layer.

45. The system of claim 44, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

46. The system of claim 40, wherein the sensor array further comprises a bottom layer and a top cover layer, wherein the bottom layer is coupled to a bottom surface of the supporting member, and wherein the top cover layer is coupled to a top surface of the supporting member; and wherein both the bottom layer and the top cover layer are coupled to the supporting member such that the particle is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2987

substantially contained within the cavity by bottom layer and the top cover layer.

47. The system of claim 46, wherein the bottom layer and the top cover layer are substantially transparent to light produced by the light source.

48. The system of claim 40, wherein the sensor array further comprises a bottom layer coupled to the supporting member, and wherein the bottom layer comprises silicon nitride.

49. The system of claim 40, wherein the sensor array further comprises a sensing cavity formed on a bottom surface of the sensor array.

50. The system of claim 40, further comprising a fluid delivery system coupled to the supporting member.

51. The system of claim 40, wherein the detector comprises a charge-coupled device.

52. The system of claim 40, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

53. The system of claim 40, wherein the particle ranges from about 0.05 micron to about 500 microns.

54. The system of claim 40, wherein a volume of the particle changes when contacted with the fluid.

55. The system of claim 40, wherein the polymeric bead comprises a polystyrene-polyethylene glycol-divinyl benzene resin.

56. The system of claim 40, wherein the receptor molecule produces the signal in response to the pH of the fluid.

57. The system of claim 40, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

58. The system of claim 40, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of the carbohydrate.

59. The system of claim 40, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

60. The system of claim 40, wherein the particles further comprises an indicator, wherein the indicator is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2988

associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

61. The system of claim 40, wherein the receptor comprises a polynucleotide.

62. The system of claim 40, wherein the receptor comprises a peptide.

63. The system of claim 40, wherein the receptor comprises an enzyme.

64. The system o f claim 40, wherein the receptor comprises a synthetic receptor.

65. The system of claim 40, wherein the receptor comprises an unnatural biopolymer.

66. The system of claim 40, wherein the receptor comprises an antibody.

67. The system of claim 40, wherein the receptor comprises an antigen.

68. The system of claim 40, wherein the particles produce a detectable pattern in the presence of the analyte.

69. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

70. The sensor array of claim 69, further comprising a plurality of particles positioned within the cavity.

71. The sensor array of claim 69, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

72. The sensor array of claim 69, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter.

73. The sensor array of claim 69, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter, and wherein the cavity is configured to substantially contain the particle.

74. The sensor array of claim 69, wherein the supporting member comprises a plastic material.

75. The sensor array of claim 69, wherein the supporting member comprises a silicon wafer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2989

76. The sensor array of claim 75, wherein the cavity extends through the silicon wafer.

77. The sensor array of claim 75,wherein the cavity is substantially pyramidal in shape and wherein the sidewalls of the cavity are substantially tapered at an angle of between about 50 to about 60 degrees.

78. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer.

79. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon dioxide, silicon nitride, or silicon oxide/silicon nitride multilayer stacks.

80. The sensor array of claim 75, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon nitride.

81. The sensor array of claim 75, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

82. The sensor array of claim 75, further comprising a plurality of cavities formed in the silicon wafer, and wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

83. The sensor array of claim 69, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavity.

84. The sensor array of claim 69, further comprising an inner surface coating, wherein the inner surface coating is configured to inhibit dislodgment of the particle.

85. The sensor array of claim 69, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

86. The sensor array of claim 85, wherein the detector is a semiconductor based photodetector.

87. The sensor array of claim 85, wherein the detector is a Fabry-Perot type detector.

88. The sensor array of claim 85, further comprising an optical fiber coupled to the detector, wherein the optical fiber is configured to transmit optical data to a microprocessor.

89. The sensor array of claim 69, further comprising an optical filters coupled to a bottom surface of the sensor array.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2990

90. The sensor array of claim 69, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

91. The sensor array of claim 90, wherein the barrier layer comprises a substantially transparent cover plate positioned over the cavity, and wherein the cover plate is positioned a fixed distance over the cavity such that the fluid can enter the cavity.

92. The sensor array of claim 91, wherein the barrier layer comprises plastic, glass, quartz, silicon oxide, or silicon nitride.

93. The sensor array of claim 69, further comprising a plurality of particles positioned within a plurality of cavities formed in the supporting member.

94. The sensor array of claim 69, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

95. A sensor array for detecting an analyte in a fluid comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member;
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte.

96. The sensor array of claim 95, wherein a plurality of particles is positioned within each of the cavities.

97. The sensor array of claim 95, wherein the particles comprise a receptor molecule coupled to a polymeric bead.

98. The sensor array of claim 95, wherein the cavity extends through the supporting member.

99. The sensor array of claim 95,wherein the cavity is substantially pyramidal in shape and wherein the sidewalls of the cavity are substantially tapered at an angle of between about 50 to about 60 degrees.

100. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the supporting member.

101. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon dioxide, silicon nitride, or silicon oxide/silicon nitride multilayer stacks.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2991

102. The sensor array of claim 95, further comprising a substantially transparent layer positioned on a bottom surface of the silicon wafer, wherein the substantially transparent layer comprises silicon nitride.

103. The sensor array of claim 95, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

104. The sensor array of claim 95, further comprising a plurality of cavities formed in the silicon wafer, and wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

105. The sensor array of claim 95, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavities.

106. The sensor array of claim 95, further comprising an inner surface coating, wherein the inner surface coating is configured to inhibit dislodgment of the particle.

107. The sensor array of claim 95, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

108. The sensor array of claim 107, wherein the detector is a semiconductor based photodetector.

109. The sensor array of claim 107, wherein the detector is a Fabry-Perot type detector.

110. The sensor array of claim 107, further comprising an optical fiber coupled to the detector, wherein the optical fiber is configured to transmit optical data to a microprocessor.

111. The sensor array of claim 95, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

112. The sensor array of claim 111, wherein the barrier layer comprises a cover plate positioned over the cavity, and wherein the cover plate is positioned a fixed distance over the cavity such that the fluid can enter the cavity.

113. The sensor array of claim 111, further comprising channels in the supporting member, wherein the channels are configured to allow the fluid to flow through the channels into and away from the cavities, and wherein the barrier layer comprises a cover plate positioned upon an upper surface of the supporting member, and wherein the cover plate inhibits passage of the fluid into the cavities such that the fluid enters the cavities via the channels.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2992

114. The sensor array of claim 111, wherein the barrier layer comprises plastic, glass, quartz, silicon oxide, or silicon nitride.

115. A method for forming a sensor array configured to detect an analyte in a fluid, comprising:
forming a cavity in a supporting member, wherein the supporting member comprises a silicon wafer;
placing a particle in the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
forming a cover upon a portion of the supporting member, wherein the cover is configured to inhibit dislodgment of the particle from the cavity.

116. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer.

117. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer with a wet hydroxide etch.

118. The method of claim 115, wherein forming the cavity comprises anisotropically etching the silicon wafer such that sidewalls of the cavity are tapered at an angle from about 50 degrees to about 60 degrees.

119. The method of claim 115, wherein the silicon wafer has an area of about 1 cm^2 to about 100 cm^2.

120. The method of claim 115, further comprising forming a substantially transparent layer upon a bottom surface of the silicon wafer below the cavity.

121. The method of claim 115, further comprising forming a substantially transparent layer upon a bottom surface of the silicon wafer, wherein the cavity extends through the silicon wafer and wherein the substantially transparent layer is positioned to support the particle.

122. The method of claim 115, wherein the substantially transparent layer comprises silicon nitride.

123. The method of claim 115, wherein the cover comprises plastic, glass, quartz, silicon nitride, or silicon oxide.

124. The method of claim 115, wherein forming the cover comprises coupling the cover to the silicon wafer at a distance above the silicon wafer substantially less than a width of the particle.

125. The method of claim 115, further comprising etching channels in the silicon wafer prior to forming the cover on the silicon wafer, wherein forming the cover comprises placing the cover against the upper

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2993

surface of the silicon wafer, and wherein the channels are configured to allow the fluid to pass through the silicon wafer to and from the cavities.

126. The method of claim 115, further comprising coating an inner surface of the cavity with a material to increase adhesion of the particle to the inner surface of the cavity.

127. The method of claim 115, further comprising coating an inner surface of the cavity with a material to increase reflectivity of the inner surface of the cavity.

128. The method of claim 115, further comprising forming an optical detector upon a bottom surface of the supporting member below the cavity.

129. The method of claim 115, further comprising forming a sensing cavity upon a bottom surface of the supporting member below the cavity.

130. The method of claim 129, wherein forming the sensing cavity comprises:
forming a barrier layer upon a bottom surface of the silicon wafer;
forming a bottom diaphragm layer upon the barrier layer;
forming etch windows extending through the bottom diaphragm layer;
forming a sacrificial spacer layer upon the bottom diaphragm layer;
removing a portion of the spacer layer;
forming a top diaphragm layer; and
removing a remaining portion of the spacer layer.

131. The method of claim 130, further comprising filling a portion of the sensing cavity with a sensing substrate.

132. The method of claim 115, further comprising forming an optical filter upon the bottom surface of the supporting member.

133. The method of claim 115, further comprising forming a plurality of cavities in the silicon wafer.

134. The method of claim 115, wherein from about 10 to about 10^6 cavities are formed in the silicon wafer.

135. A sensor array produced by the method of claim 115.

136. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2994

monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

137. The method of claim 136, wherein the spectroscopic change comprises a change in absorbance of the particle.

138. The method of claim 136, wherein the spectroscopic change comprises a change in fluorescence of the particle.

139. The method of claim 136, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

140. The method of claim 136, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

141. The method of claim 136, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

142. The method of claim 136, wherein the analyte is an anion, and wherein the spectroscopic change is produced in response to the presence of the anion in the fluid.

143. The method of claim 136, wherein the analyte is a DNA molecule, and wherein the spectroscopic change is produced in response to the presence of the DNA molecule in the fluid.

144. The method of claim 136, wherein the analyte is a protein, and wherein the spectroscopic change is produced in response to the presence of the protein in the fluid.

145. The method of claim 136, wherein the analyte is a metabolite, and wherein the spectroscopic change is produced in response to the presence of the metabolite in the fluid.

146. The method of claim 136, wherein the analyte is a sugar, and wherein the spectroscopic change is produced in response to the presence of the sugar in the fluid.

147. The method of claim 136, wherein the analyte is a bacteria, and wherein the spectroscopic change is produced in response to the presence of the bacteria in the fluid.

148. The method of claim 136, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2995

149. The method of claim 148, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

150. The method of claim 148, wherein the indicator is a fluorescent indicator.

151. The method of claim 136, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

152. The method of claim 136, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

153. The method of claim 136, wherein monitoring the spectroscopic change is performed with a CCD device.

154. The method of claim 136, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

155. A sensor array for detecting an analyte in a fluid comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member;
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte.

156. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising:
a supporting member, wherein the supporting member comprises a silicon wafer, and wherein a plurality of cavities are formed within the supporting member; and
a plurality of particles, at least one particle being positioned in each of the cavities, wherein the particles are configured to produce a signal when the particles interact with the analyte at least one particle positioned within a cavity of a supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

157. The method of claim 156, wherein the spectroscopic change comprises a change in absorbance of the particle.

158. The method of claim 156, wherein the spectroscopic change comprises a change in reflectance of the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2996

159. The method of claim 156, wherein the spectroscopic change comprises a change in fluorescence of the particle.

160. The method of claim 156, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

161. The method of claim 156, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

162. The method of claim 156, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

163. The method of claim 156, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

164. The method of claim 156, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

165. The method of claim 156, wherein the indicator is a fluorescent indicator.

166. The method of claim 156, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

167. The method of claim 156, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

168. The method of claim 156, wherein monitoring the spectroscopic change is performed with a CCD device.

169. The method of claim 156, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

170. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising at least one particle coupled to the sensor array, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2997

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

171. A sensor array for detecting an analyte in a fluid comprising:
at least one particle coupled to the sensor array, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

172. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle coupled to a supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

173. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte;
wherein the cavities are configured to allow the fluid to pass through the supporting member during use.

174. The sensor array of claim 173, further comprising a plurality of particles positioned within the cavity.

175. The sensor array of claim 173, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

176. The sensor array of claim 173, wherein the particle has a size ranging from about 0.05 micron to about 500 microns in diameter.

177. The sensor array of claim 173, wherein the cavity is configured to substantially contain the particle.

178. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable.

179. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

180. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2998

a bottom layer coupled to the supporting member, wherein the bottom layer is coupled to a bottom surface of the supporting member and wherein the cover layer is removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

181. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein an opening is formed in the cover layer substantially aligned with the cavity, and wherein an opening is formed in the bottom layer substantially aligned with the cavity.

182. The sensor array of claim 173, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

183. The sensor array of claim 173, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

184. The sensor array of claim 173, further comprising a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is configured to support the particle, and wherein an opening is formed in the cover layer substantially aligned with the cavity.

185. The sensor array of claim 173, further comprising a removable cover layer coupled to the supporting member.

186. The sensor array of claim 173, wherein the supporting member comprises a plastic material.

187. The sensor array of claim 173, wherein the supporting member comprises a silicon wafer.

188. The sensor array of claim 173, wherein the supporting member comprises a dry film photoresist material.

189. The sensor array of claim 173, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

190. The sensor array of claim 173, wherein an inner surface of the cavity is coated with a reflective material.

191. The sensor array of claim 173, further comprising channels in the supporting member, wherein the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
2999

channels are configured to allow the fluid to flow through the channels into and away from the cavity.

192. The sensor array of claim 173, further comprising a plurality of additional particles positioned within a plurality of additional cavities formed in the supporting member.

193. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member, wherein the cavity is configured such that the fluid entering the cavity passes through the supporting member during use;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

194. The system of claim 193, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

195. The system of claim 193, wherein the system comprises a plurality of particles positioned within the cavity.

196. The system of claim 193, wherein the light source comprises a light emitting diode.

197. The system of claim 193, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

198. The system of claim 193, wherein the light source comprises a white light source.

199. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable.

200. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting ,member and a bottom layer coupled to the supporting member, wherein the cover layer and the bottom layer are removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3000

201. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is coupled to a bottom surface of the supporting member and wherein the cover layer is removable, and wherein the cover layer and the bottom layer include openings that are substantially aligned with the cavities during use.

202. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein an opening is formed in the cover layer substantially aligned with the cavity, and wherein an opening is formed in the bottom layer substantially aligned with the cavity.

203. The system of claim 193, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

204. The system of claim 193, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

205. The system of claim 193, wherein the sensor array further comprises a cover layer coupled to the supporting member and a bottom layer coupled to the supporting member, wherein the bottom layer is configured to support the particle, and wherein an opening is formed in the cover layer substantially aligned with the cavity.

206. The system of claim 193, further comprising a removable cover layer.

207. The system of claim 193, wherein the supporting member comprises a plastic material.

208. The system of claim 193, wherein the supporting member comprises a silicon wafer.

209. The system of claim 193, wherein the supporting member comprises a dry film photoresist material.

210. The system of claim 193, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

211. The system of claim 193, wherein an inner surface of the cavity is coated with a reflective material.

212. The system of claim 193, further comprising channels in the supporting member, wherein the channels

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3001

are configured to allow the fluid to flow through the channels into and away from the cavity.

213. The system of claim 193, wherein the detector comprises a charge-coupled device.

214. The system of claim 193, wherein the detector comprises a semiconductor based photodetector, and wherein the detector is coupled to the sensor array.

215. The system of claim 193, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

216. The system of claim 215, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

217. The system of claim 215, wherein the receptor molecule produces the signal in response to the pH of the fluid.

218. The system of claim 215, wherein the analyte comprises a metal ion, and wherein the receptor produces the signal in response to the presence of the metal ion.

219. The system of claim 215, wherein the analyte comprises a carbohydrate, and wherein the receptor produces a signal in response to the presence of a carbohydrate.

220. The system of claim 215, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

221. The system of claim 215, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

222. The system of claim 215, wherein the receptor comprises a polynucleotide.

223. The system of claim 215, wherein the receptor comprises a peptide.

224. The system of claim 215, wherein the receptor comprises an enzyme.

225. The system o f claim 215, wherein the receptor comprises a synthetic receptor.

226. The system of claim 215, wherein the receptor comprises an unnatural biopolymer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3002

227. The system of claim 215, wherein the receptor comprises an antibody.

228. The system of claim 215, wherein the receptor comprises an antigen.

229. The system of claim 193, wherein the analyte comprises phosphate functional groups, and wherein the particle is configured to produce the signal in the presence of the phosphate functional groups.

230. The system of claim 193, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

231. The system of claim 193, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

232. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein at least one cavity is formed within the supporting member;
a particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
a pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the cavity;
wherein a channel is formed in the supporting member, the channel coupling the pump to the cavity such that the fluid flows through the channel to the cavity during use.

233. The sensor array of claim 232, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

234. The sensor array of claim 232, wherein the supporting member comprises a plastic material.

235. The sensor array of claim 232, wherein the supporting member comprises a silicon wafer.

236. The sensor array of claim 232, wherein the supporting member comprises a dry film photoresist material.

237. The sensor array of claim 232, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3003

238. The sensor array of claim 232, wherein an inner surface of the cavity is coated with a reflective material.

239. The sensor array of claim 232, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the cavity.

240. The sensor array of claim 232, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

241. The sensor array of claim 232, further comprising a barrier layer positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use, wherein the barrier layer comprises a transmission electron microscope grid.

242. The sensor array of claim 232, further comprising a plurality of particles positioned within a plurality of cavities formed in the supporting member.

243. The sensor array of claim 232, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

244. The sensor array of claim 232, wherein the pump comprises a diaphragm pump.

245. The sensor array of claim 232, wherein the pump comprises an electrode pump.

246. The sensor array of claim 232 wherein the pump comprises a piezoelectric pump.

247. The sensor array of claim 232, wherein the pump comprises a pneumatic activated pump.

248. The sensor array of claim 232, wherein the pump comprises a heat activated pump.

249. The sensor array of claim 232, wherein the pump comprises a peristaltic pump.

250. The sensor array of claim 232, wherein the pump comprises an electroosmosis pump.

251. The sensor array of claim 232, wherein the pump comprises an electrohydrodynamic pump.

252. The sensor array of claim 232, wherein the pump comprises an electroosmosis pump and an electrohydrodynamic pump.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3004

253. The sensor array of claim 232, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

254. The sensor array of claim 232, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

255. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member, a pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the cavity, and wherein a channel is formed in the supporting member, the channel coupling the pump to the cavity such that the fluid flows through the channel to the cavity during use;
a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use; and
a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

256. The system of claim 255, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

257. The system of claim 255, wherein the light source comprises a light emitting diode.

258. The system of claim 255, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

259. The system of claim 255, wherein the light source comprises a white light source.

260. The system of claim 255, wherein the supporting member comprises a plastic material.

261. The system of claim 255, wherein the supporting member comprises a silicon wafer.

262. The system of claim 255, wherein the supporting member comprises a dry film photoresist material.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3005

263. The system of claim 255, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

264. The system of claim 255, wherein an inner surface of the cavity is coated with a reflective material.

265. The system of claim 255, further comprising a barrier layer coupled to the supporting member, wherein the barrier layer is positioned over the cavity, the barrier layer being configured to inhibit dislodgment of the particle during use.

266. The system of claim 255, wherein the pump comprises a diaphragm pump.

267. The system of claim 255, wherein the pump comprises an electrode pump.

268. The system of claim 255 wherein the pump comprises a piezoelectric pump.

269. The system of claim 255, wherein the pump comprises a pneumatic activated pump.

270. The system of claim 255, wherein the pump comprises a heat activated pump.

271. The system of claim 255, wherein the pump comprises a peristaltic pump.

272. The system of claim 255, wherein the pump comprises an electroosmosis pump.

273. The system of claim 255, wherein the pump comprises an electrohydrodynamic pump.

274. The system of claim 255, wherein the pump comprises an electroosmosis pump and an electrohydrodynamic pump.

275. The system of claim 255, wherein the cavity is substantially tapered such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

276. The system of claim 255, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

277. The system of claim 255, wherein the detector comprises a charge-coupled device.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3006

278. The system of claim 255, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

279. The system of claim 278, wherein the polymeric resin comprises polystyrene-polyethylene glycol-divinyl benzene.

280. The system of claim 278, wherein the particles further comprises a first indicator and a second indicator, the first and second indicators being coupled to the receptor, wherein the interaction of the receptor with the analyte causes the first and second indicators to interact such that the signal is produced.

281. The system of claim 278, wherein the particles further comprises an indicator, wherein the indicator is associated with the receptor such that in the presence of the analyte the indicator is displaced from the receptor to produce the signal.

282. The system of claim 278, wherein the receptor comprises a polynucleotide.

283. The system of claim 278, wherein the receptor comprises a peptide.

284. The system of claim 278, wherein the receptor comprises an enzyme.

285. The system o f claim 278, wherein the receptor comprises a synthetic receptor.

286. The system of claim 278, wherein the receptor comprises an unnatural biopolymer.

287. The system of claim 278, wherein the receptor comprises an antibody.

288. The system of claim 278, wherein the receptor comprises an antigen.

289. The system of claim 255, wherein the analyte comprises bacteria, and wherein the particle is configured to produce the signal in the presence of the bacteria.

290. The system of claim 255, wherein the system comprises a plurality of particles positioned within a plurality of cavities, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3007

291. A sensor array for detecting an analyte in a fluid comprising:
a supporting member; wherein a first cavity and a second cavity are formed within the supporting member;
a first particle positioned within the first cavity;
a second particle positioned within the second cavity, wherein the second particle comprises a reagent, wherein a portion of the reagent is removable from the second particle when contacted with a decoupling solution, and wherein the reagent is configured to modify the first particle, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with the analyte during use;
a first pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the first cavity;
a second pump coupled to the supporting member, wherein the second pump is configured to direct the decoupling solution towards the second cavity;
wherein a first channel is formed in the supporting member, the first channel coupling the first pump to the first cavity such that the fluid flows through the first channel to the first cavity during use, and
wherein a second channel is formed in the supporting member, the second channel coupling the second cavity to the first cavity such that the decoupling solution flows from the second cavity through the second channel to the first cavity during use.

292. The sensor array of claim 291, wherein the first particle comprises a receptor molecule coupled to a first polymeric resin, and wherein the second particle comprises an indicator molecule coupled to a second polymeric resin.

293. The sensor array of claim 291, wherein the first particle comprises an indicator molecule coupled to a first polymeric resin, and the second particle comprises a receptor molecule coupled to a second polymeric resin.

294. The sensor array of claim 291, wherein the first particle comprises a first polymeric resin configured to bind to the receptor molecule, and wherein the second particle comprises the receptor molecule coupled to a second polymeric resin.

295. The sensor array of claim 291, wherein the supporting member comprises a plastic material.

296. The sensor array of claim 291, wherein the supporting member comprises a silicon wafer.

297. The sensor array of claim 291, wherein the supporting member comprises a dry film photoresist material.

298. The sensor array of claim 291, wherein the supporting member comprises a plurality of layers of a dry

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3008

film photoresist material.

299. The sensor array of claim 291, wherein an inner surface of the first cavity is coated with a reflective material.

300. The sensor array of claim 291, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the first cavity.

301. The sensor array of claim 291, further comprising a plurality of additional particles positioned within a plurality of additional cavities formed in the supporting member, and wherein the second cavity is coupled to the additional cavities such that the reagent may be transferred from the second particle to the additional cavities during use.

302. The sensor array of claim 291, wherein the first and second pumps comprise a diaphragm pump.

303. The sensor array of claim 291, wherein the first and second pumps comprise an electrode pump.

304. The sensor array of claim 291, wherein the first pump comprises a diaphragm pump or an electrode pump and wherein the second pump comprises a diaphragm pump or an electrode pump.

305. The sensor array of claim 291, wherein the first cavity is substantially tapered such that the width of the first cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the first cavity is substantially less than a width of the first particle, and wherein the second cavity is substantially tapered such that the width of the second cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the second cavity is substantially less than a width of the second particle.

306. The sensor array of claim 291, wherein a width of a bottom portion of the first cavity is substantially less than a width of a top portion of the first cavity, and wherein the width of the bottom portion of the first cavity is substantially less than a width of the first particle, and wherein a width of a bottom portion of the second cavity is substantially less than a width of a top portion of the second cavity, and wherein the width of the bottom portion of the second cavity is substantially less than a width of the second particle.

307. The sensor array of claim 291, further comprising a reservoir coupled to the second pump, the reservoir configured to hold the decoupling solution.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3009

308. A system for detecting an analyte in a fluid comprising:

a light source;

a sensor array, the sensor array comprising:

a supporting member; wherein a first cavity and a second cavity are formed within the supporting member;

a first particle positioned within the first cavity;

a second particle positioned within the second cavity, wherein the second particle comprises a reagent, wherein a portion of the reagent is removable from the second particle when contacted with a decoupling solution, and wherein the reagent is configured to modify the first particle, when the reagent is contacted with the first particle, such that the first particle will produce a signal when the first particle interacts with the analyte during use;

a first pump coupled to the supporting member, wherein the pump is configured to direct the fluid towards the first cavity;

a second pump coupled to the supporting member, wherein the second pump is configured to direct the decoupling solution towards the second cavity;

wherein a first channel is formed in the supporting member, the first channel coupling the first pump to the first cavity such that the fluid flows through the first channel to the first cavity during use, and wherein a second channel is formed in the supporting member, the second channel coupling the second cavity to the first cavity such that the decoupling solution flows from the second cavity through the second channel to the first cavity during use; and

a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

309. The system of claim 308, wherein the sensor array further comprises a plurality of additional particles positioned within a plurality of additional cavities, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid, and wherein the second cavity is coupled to the additional cavities such that the reagent may be transferred from the second particle to the additional cavities during use.

310. The system of claim 308, wherein the light source comprises a light emitting diode.

311. The system of claim 308, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

312. The system of claim 308, wherein the light source comprises a white light source.

313. The system of claim 308, wherein the first particle comprises a receptor molecule coupled to a first

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3010

polymeric resin, and wherein the second particle comprises an indicator molecule coupled to a second polymeric resin.

314. The system of claim 308, wherein the first particle comprises an indicator molecule coupled to a first polymeric resin, and the second particle comprises a receptor molecule coupled to a second polymeric resin.

315. The system of claim 308, wherein the first particle comprises a first polymeric resin configured to bind to the receptor molecule, and wherein the second particle comprises the receptor molecule coupled to a second polymeric resin.

316. The system of claim 308, wherein the supporting member comprises a plastic material.

317. The system of claim 308, wherein the supporting member comprises a silicon wafer.

318. The system of claim 308, wherein the supporting member comprises a dry film photoresist material.

319. The system of claim 308, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

320. The system of claim 308, wherein an inner surface of the first cavity is coated with a reflective material.

321. The system of claim 308, wherein the first and second pumps comprise a diaphragm pump.

322. The system of claim 308, wherein the first and second pumps comprise an electrode pump.

323. The system of claim 308, wherein the first pump comprises a diaphragm pump or an electrode pump and wherein the second pump comprises a diaphragm pump or an electrode pump.

324. The system of claim 308, wherein the first cavity is substantially tapered such that the width of the first cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the first cavity is substantially less than a width of the first particle, and wherein the second cavity is substantially tapered such that the width of the second cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the second cavity is substantially less than a width of the second particle.

325. The system of claim 308, wherein a width of a bottom portion of the first cavity is substantially less

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3011

than a width of a top portion of the first cavity, and wherein the width of the bottom portion of the first cavity is substantially less than a width of the first particle, and wherein a width of a bottom portion of the second cavity is substantially less than a width of a top portion of the second cavity, and wherein the width of the bottom portion of the second cavity is substantially less than a width of the second particle.

326. The system of claim 308, wherein the sensor array further comprises a reservoir coupled to the second pump, the reservoir configured to hold the decoupling solution.

327. The system of claim 308, wherein the analyte comprises bacteria, and wherein the first particle is configured to produce the signal in the presence of the bacteria.

328. A method for forming a sensor array configured to detect an analyte in a fluid, comprising:
forming a cavity in a supporting member, wherein the cavity is configured to allow the fluid to pass through the supporting member;
placing a particle in the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte; and
placing a cover upon a portion of the supporting member, wherein the cover is configured to inhibit dislodgment of the particle from the cavity.

329. The method of claim 328, further comprising forming a substantially transparent layer upon a bottom surface of the supporting member below the cavity, wherein the bottom layer is configured to inhibit the displacement of the particle from the cavity while allowing the fluid to pass through the supporting member.

330. The method of claim 328, further comprising forming an optical detector upon a bottom surface of the supporting member below the cavity.

331. The system of claim 328, wherein a width of a bottom portion of the cavity is substantially less than a width of a top portion of the cavity, and wherein the width of the bottom portion of the cavity is substantially less than a width of the particle.

332. The method of claim 328, further comprising forming channels in the supporting member wherein the channels are configured to allow the fluid to pass through the supporting member to and from the cavity.

333. The method of claim 328, further comprising forming a pump on the supporting member, the pump being configured to pump the fluid to the cavity.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3012

334. The method of claim 328, further comprising forming additional cavities in the supporting member and further comprising placing additional particles in the additional cavities.

335. The method of claim 328, further comprising forming a cover, wherein forming the cover comprises:
forming a removable layer upon the upper surface of the supporting member;
forming a cover upon the removable layer;
forming support structures upon the supporting member, the support structures covering a portion of the cover; and
dissolving the removable layer.

336. The method of claim 335, wherein the cover layer is formed prior to forming the cavity.

337. The method of claim 335, wherein forming the cover further comprises forming openings in the cover, wherein the openings are substantially aligned with the cavity.

338. The method of claim 328, wherein the particles are placed in the cavities using a micromanipulator.

339. The method of claim 328, further comprising forming additional cavities within the supporting member, and further comprising placing additional particles in the additional cavities, wherein placing the additional particles in the additional cavities comprises:
placing a first masking layer on the supporting member, wherein the first masking layer covers a first portion of the additional cavities such that passage of a particle into the first portion of the additional cavities is inhibited, and wherein the first masking layer a second portion of the cavities substantially unmasked,;
placing the additional particles on the supporting member; and
moving the additional particles across the supporting member such that the particles fall into the second portion of the cavities.

340. The method of claim 339, further comprising:
removing the first masking layer;
placing a second masking layer upon the supporting member, wherein the second masking layer covers the second portion of the cavities and a portion of the first portion of the cavities while leaving a third portion of the cavities unmasked;
placing additional particles on the supporting member; and
moving the additional particles across the supporting member such that the particle fall into the third portion of the cavities.

341. The method of claim 328, wherein forming the cover comprises coupling the cover to the supporting member at a distance above the supporting member substantially less than a width of the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3013

342. The method of claim 328, wherein the supporting member comprises a silicon wafer.

343. The method of claim 342, wherein forming the cavity comprises anisotropically etching the silicon wafer.

344. The method of claim 342, wherein forming the cavity comprises anisotropically etching the silicon wafer such that the width of the cavity narrows in a direction from a top surface of the supporting member toward a bottom surface of the supporting member, and wherein a minimum width of the cavity is substantially less than a width of the particle.

345. The method of claim 328, wherein the supporting member comprises a dry film photoresist material.

346. The method of claim 328, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

347. The method of claim 346, wherein forming the cavity comprises:
etching a first opening through a first dry film photoresist layer, the first opening having a width substantially less than a width of the particle;
placing a second dry film photoresist layer upon the first dry film photoresist layer;
etching a second opening through the second dry film photoresist layer, the second opening being substantially aligned with the first opening, wherein a width of the second opening is substantially greater than the width of the first opening.

348. The method of claim 347, wherein the second dry film photoresist layer comprises a thickness substantially greater than a width of the particle.

349. The method of claim 345, further comprising forming a reflective layer upon the inner surface of the cavity.

350. The method of claim 328, wherein the supporting material comprises a plastic material.

351. The method of claim 350, wherein the cavity is formed by drilling the supporting material.

352. The method of claim 350, wherein the cavity is formed by transfer molding the supporting member.

353. The method of claim 350, wherein the cavity is formed by a punching device.

354. A sensor array produced by the method of claim 328.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3014

355. A sensor array produced by the method of claim 342.

356. A sensor array produced by the method of claim 345.

357. A sensor array produced by the method of claim 346.

358. A sensor array produce by the method of claim 350.

359. A method of sensing an analyte in a fluid comprising:
passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member, wherein the cavity is configured such that the fluid entering the cavity passes through the supporting member;
monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

360. The method of claim 359, wherein the spectroscopic change comprises a change in absorbance of the particle.

361. The method of claim 359, wherein the spectroscopic change comprises a change in fluorescence of the particle.

362. The method of claim 359, wherein the spectroscopic change comprises a change in phosphorescence of the particle.

363. The method of claim 359, wherein the analyte is a proton atom, and wherein the spectroscopic change is produced when the pH of the fluid is varied, and wherein monitoring the spectroscopic change of the particle allows the pH of the fluid to be determined.

364. The method of claim 359, wherein the analyte is a metal cation, and wherein the spectroscopic change is produced in response to the presence of the metal cation in the fluid.

365. The method of claim 359, wherein the analyte is an anion, and wherein the spectroscopic change is produced in response to the presence of the anion in the fluid.

366. The method of claim 359, wherein the analyte is a DNA molecule, and wherein the spectroscopic change is produced in response to the presence of the DNA molecule in the fluid.

367. The method of claim 359, wherein the analyte is a protein, and wherein the spectroscopic change is produced in response to the presence of the protein in the fluid.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3015

368. The method of claim 359, wherein the analyte is a metabolite, and wherein the spectroscopic change is produced in response to the presence of the metabolite in the fluid.

369. The method of claim 359, wherein the analyte is a sugar, and wherein the spectroscopic change is produced in response to the presence of the sugar in the fluid.

370. The method of claim 359, wherein the analyte is a bacteria, and wherein the spectroscopic change is produced in response to the presence of the bacteria in the fluid.

371. The method of claim 359, wherein the particle comprises a receptor coupled to a polymeric resin, and further comprising exposing the particle to an indicator prior to passing the fluid over the sensor array.

372. The method of claim 371, wherein a binding strength of the indicator to the receptor is less than a binding strength of the analyte to the receptor.

373. The method of claim 371, wherein the indicator is a fluorescent indicator.

374. The method of claim 359, further comprising treating the fluid with an indicator prior to passing the fluid over the sensor array, wherein the indicator is configured to couple with the analyte.

375. The method of claim 359, wherein the analyte is bacteria and further comprising breaking down the bacteria prior to passing the fluid over the sensor array.

376. The method of claim 359, wherein monitoring the spectroscopic change is performed with a CCD device.

377. The method of claim 359, further comprising measuring the intensity of the spectroscopic change, and further comprising calculating the concentration of the analyte based on the intensity of the spectroscopic change.

378. The method of claim 359, wherein monitoring the spectroscopic change comprises:
directing a red light source at the particle;
detecting the absorbance of red light by the particle;
directing a green light source at the particle;
detecting the absorbance of green light by the particle;
directing a blue light source at the particle; and
detecting the absorbance of blue light by the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3016

379. A sensor array for detecting an analyte in a fluid comprising:
at least one particle coupled to a supporting member, wherein the particle is configured to produce a signal when the particle interacts with the analyte.

380. The sensor array of claim 379, wherein the particle is coupled to the supporting member with via an adhesive material.

381. The sensor array of claim 379, wherein the particle are coupled to the supporting member via a gel material.

382. The sensor array of claim 379, wherein the particle is suspended in a gel material, the gel material covering a portion of the supporting member, and wherein a portion of the particle extends from the upper surface of the gel.

383. The sensor array of claim 379, further comprising a cover positioned above the particle.

384. The sensor array of claim 379, further comprising a cover coupled to the supporting member, positioned above the particle, wherein a force exerted by the cover on the particle inhibits the displacement of the particle from the supporting member.

385. The sensor array of claim 379, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

386. The sensor array of claim 379, wherein the supporting member comprises a plastic material.

387. The sensor array of claim 379, wherein the supporting member comprises a dry film photoresist material.

388. The sensor array of claim 379, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

389. The sensor array of claim 379, wherein the supporting member comprises glass.

390. The sensor array of claim 379, further comprising a detector coupled to the bottom surface of the supporting member, wherein the detector is positioned below the particle.

391. The sensor array of claim 379, further comprising a plurality of particles coupled to the supporting member.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3017

392. The sensor array of claim 379, wherein the supporting member is composed of a material substantially transparent to visible light.

393. The sensor array of claim 379, wherein the supporting member is composed of a material substantially transparent to ultraviolet light.

394. A system for detecting an analyte in a fluid comprising:
a light source;
a sensor array, the sensor array comprising at least one particle coupled to a supporting member, wherein the particle is configured to produce a signal when the particle interacts with the analyte, and wherein the supporting member is substantially transparent to a portion of light produced by the light source; and
a detector configured to detect the signal produced by the interaction of the analyte with the particle;
wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

395. The system of claim 394, wherein the system comprises a plurality of additional particles coupled to the supporting member, and wherein the system is configured to substantially simultaneously detect a plurality of analytes in the fluid.

396. The system of claim 394, wherein the light source comprises a light emitting diode.

397. The system of claim 394, wherein the light source comprises a red light emitting diode, a blue light emitting diode, and a green light emitting diode.

398. The system of claim 394, wherein the light source comprises a white light source.

399. The system of claim 394, wherein the particle is coupled to the supporting member with via an adhesive material.

400. The system of claim 394, wherein the particle are coupled to the supporting member via a gel material.

401. The system of claim 394, wherein the particle is suspended in a gel material, the gel material covering a portion of the supporting member, and wherein a portion of the particle extends from the upper surface of the gel.

402. The system of claim 394, wherein the sensor array further comprises a cover positioned above the particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3018

403. The system of claim 394, wherein the sensor array further comprises a cover coupled to the supporting member, positioned above the particle, wherein a force exerted by the cover on the particle inhibits the displacement of the particle from the supporting member.

404. The system of claim 394, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

405. The system of claim 394, wherein the supporting member comprises a plastic material.

406. The system of claim 394, wherein the supporting member comprises a dry film photoresist material.

407. The system of claim 394, wherein the supporting member comprises a plurality of layers of a dry film photoresist material.

408. The system of claim 394, wherein the supporting member comprises glass.

409. The system of claim 394, wherein the supporting member is composed of a material substantially transparent to ultraviolet light.

410. The system of claim 394, wherein the detector comprises a charge-coupled device.

411. The system of claim 394, wherein the particle comprises a receptor molecule coupled to a polymeric resin.

412. The system of claim 394, wherein the system comprises a plurality of particles coupled to the supporting member, and wherein the plurality of particles produce a detectable pattern in the presence of the analyte.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3019

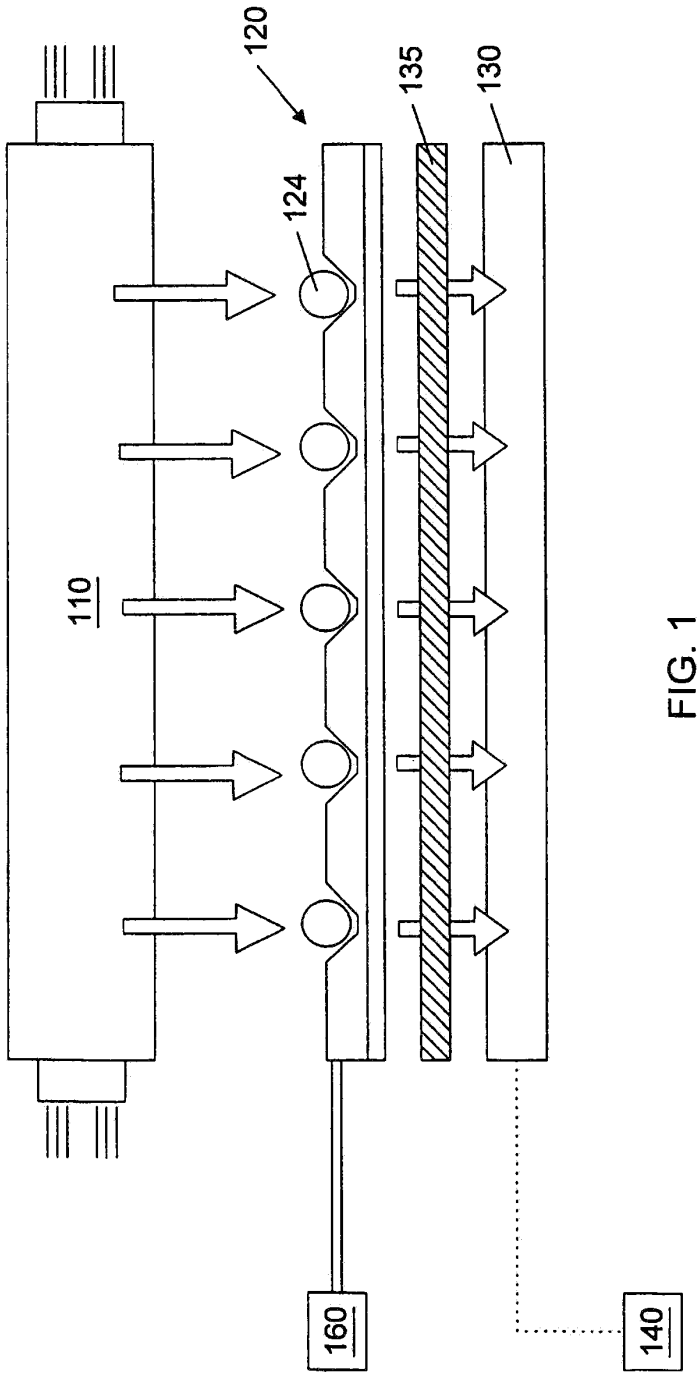


FIG. 1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3020

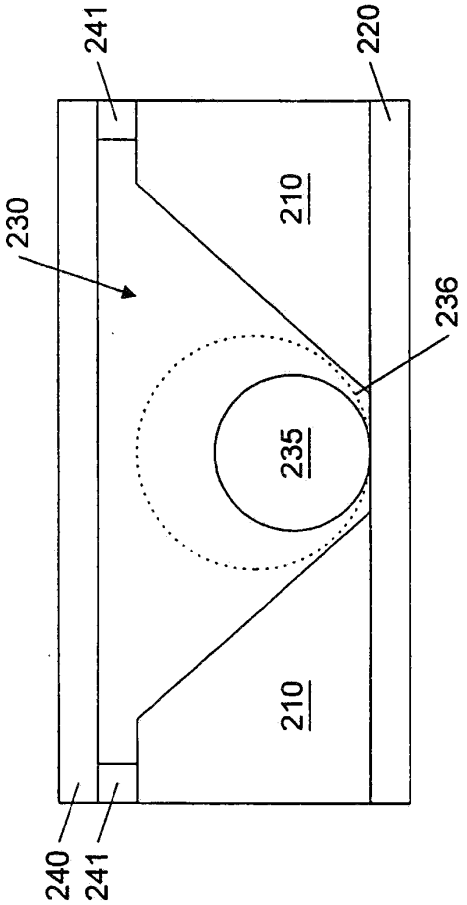


FIG. 2

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3021

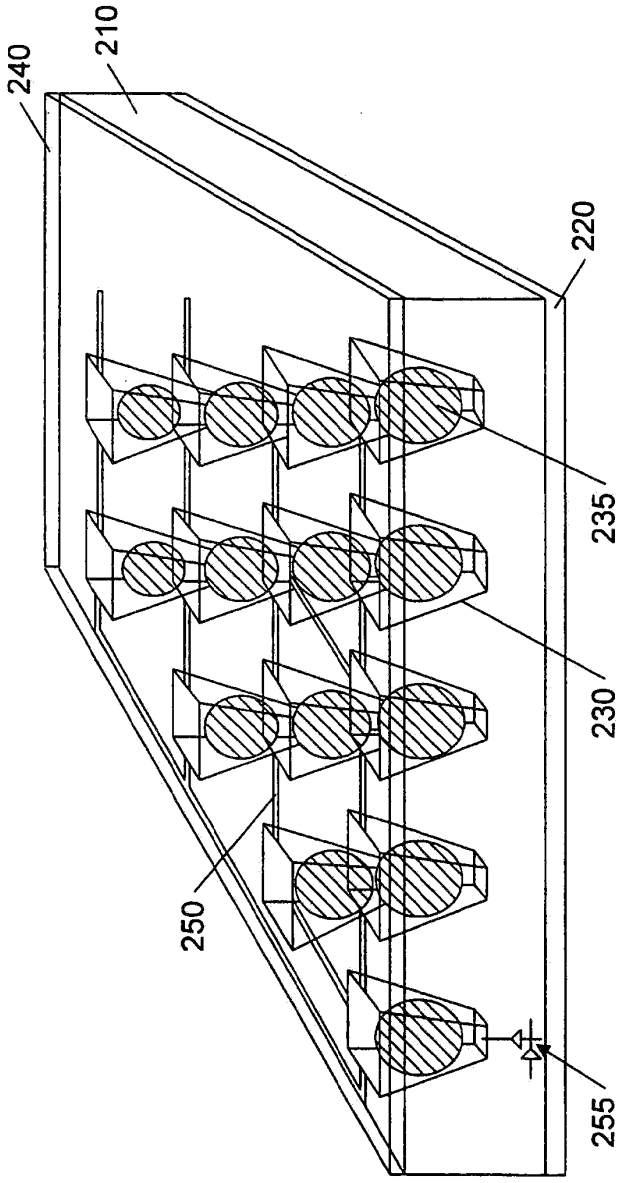


FIG. 3

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3022

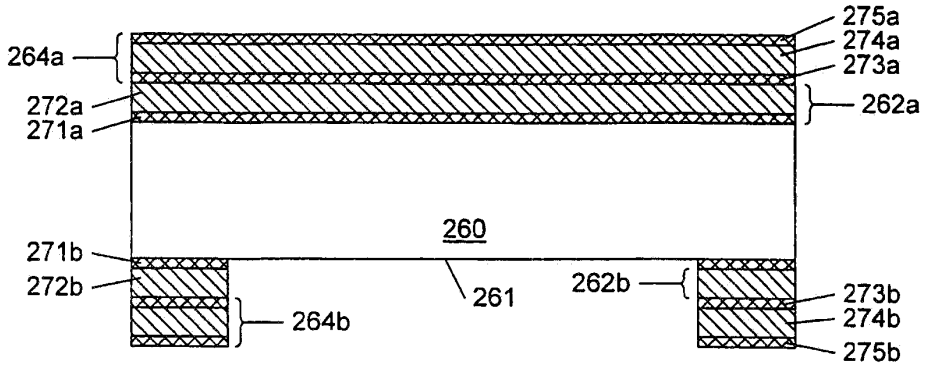


FIG. 4A

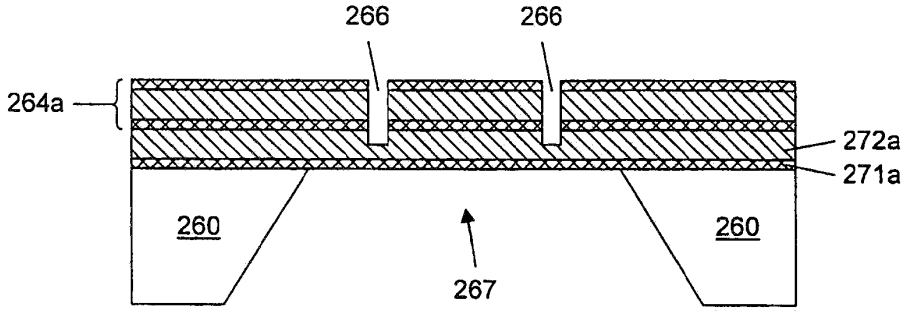


FIG. 4B

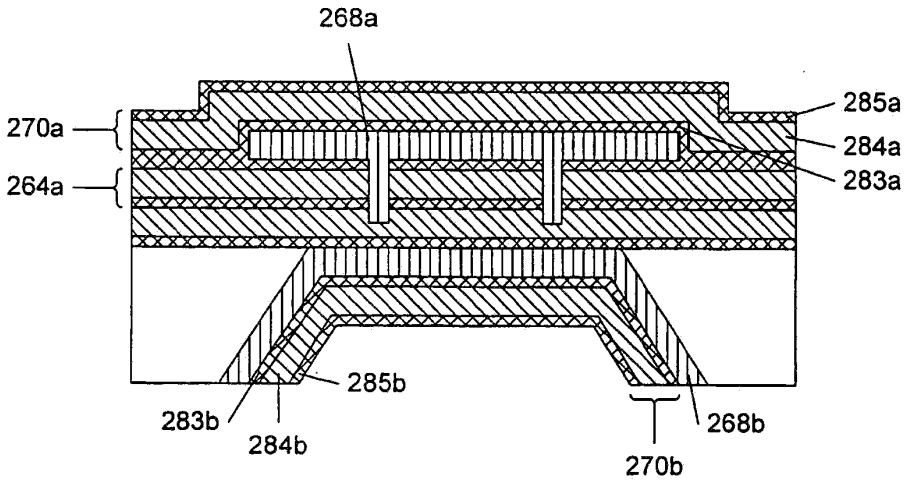


FIG. 4C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3023

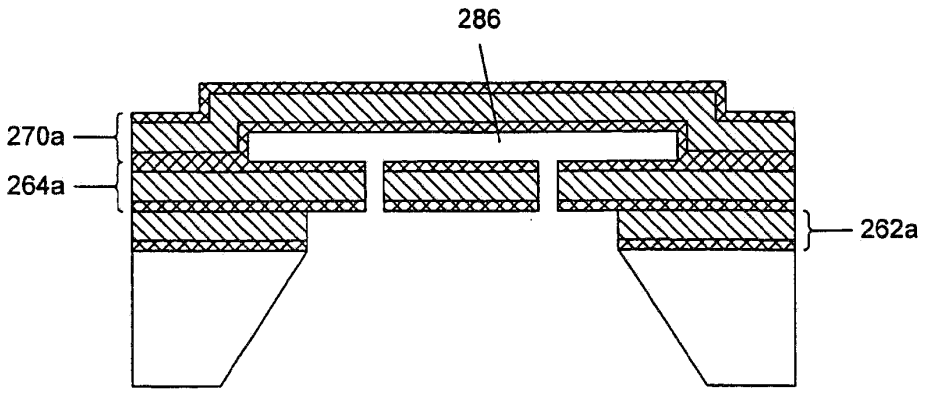


FIG. 4D

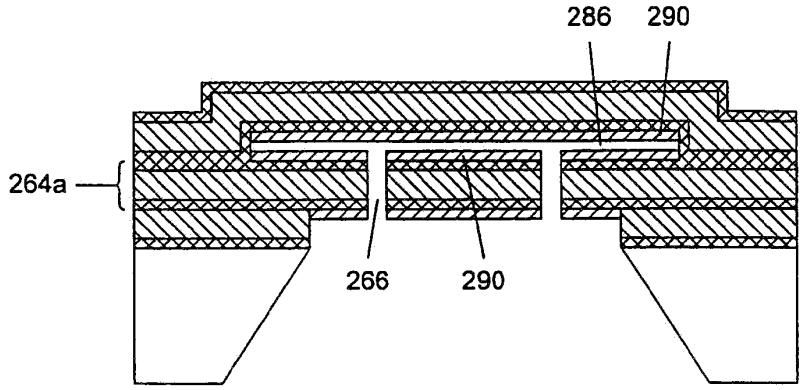


FIG. 4E

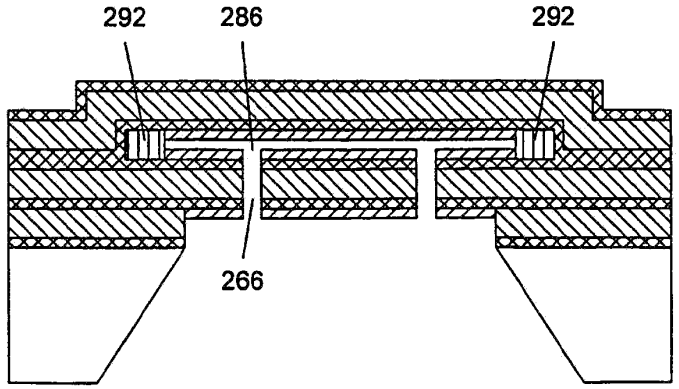


FIG. 4F

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3024

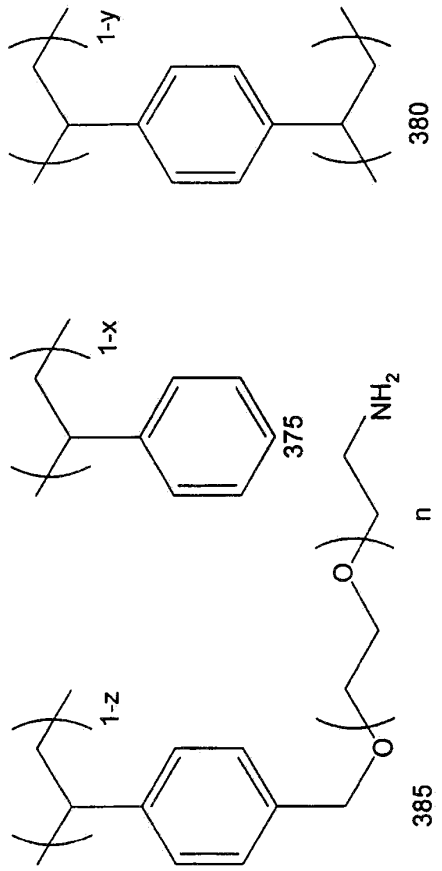


FIG. 5

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3025

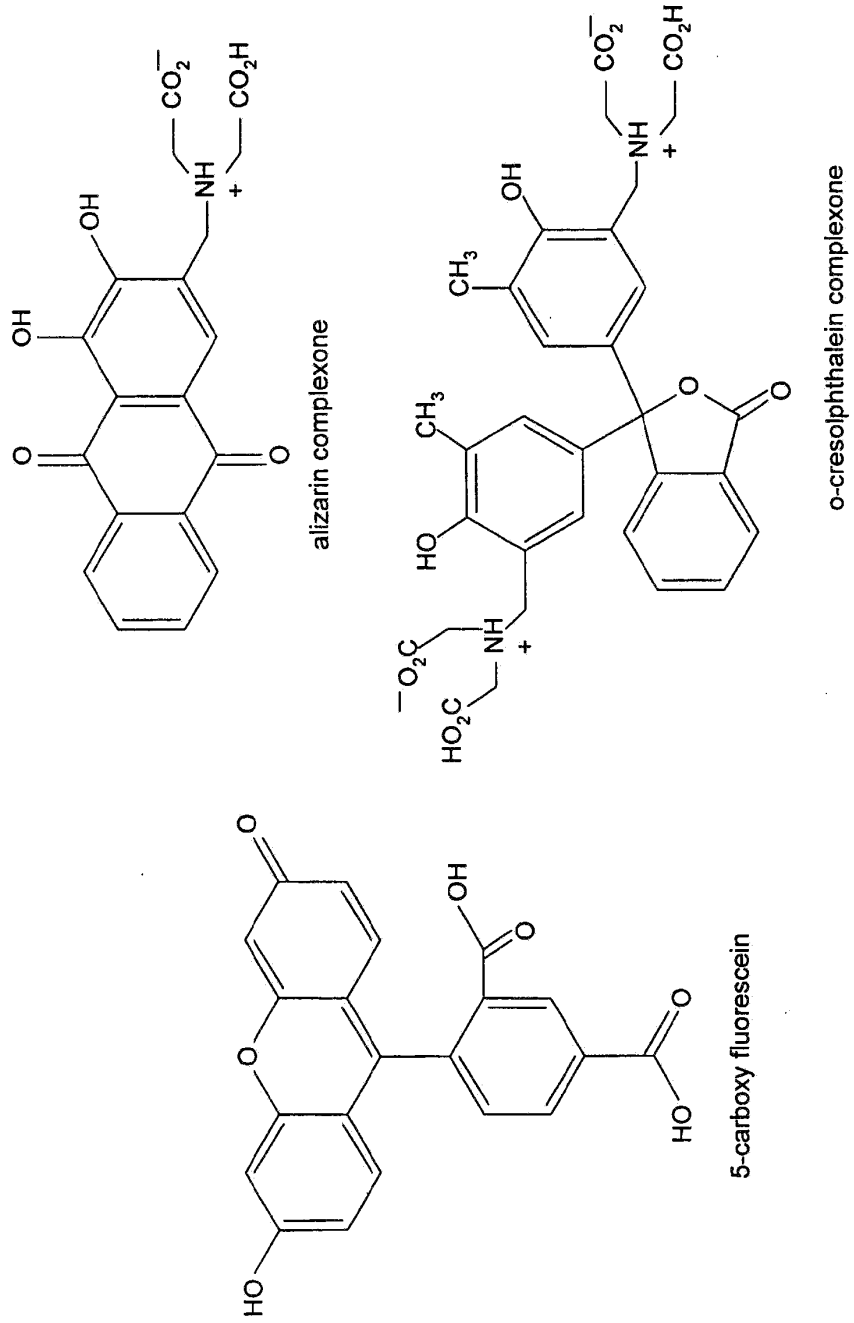


FIG. 6

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3026

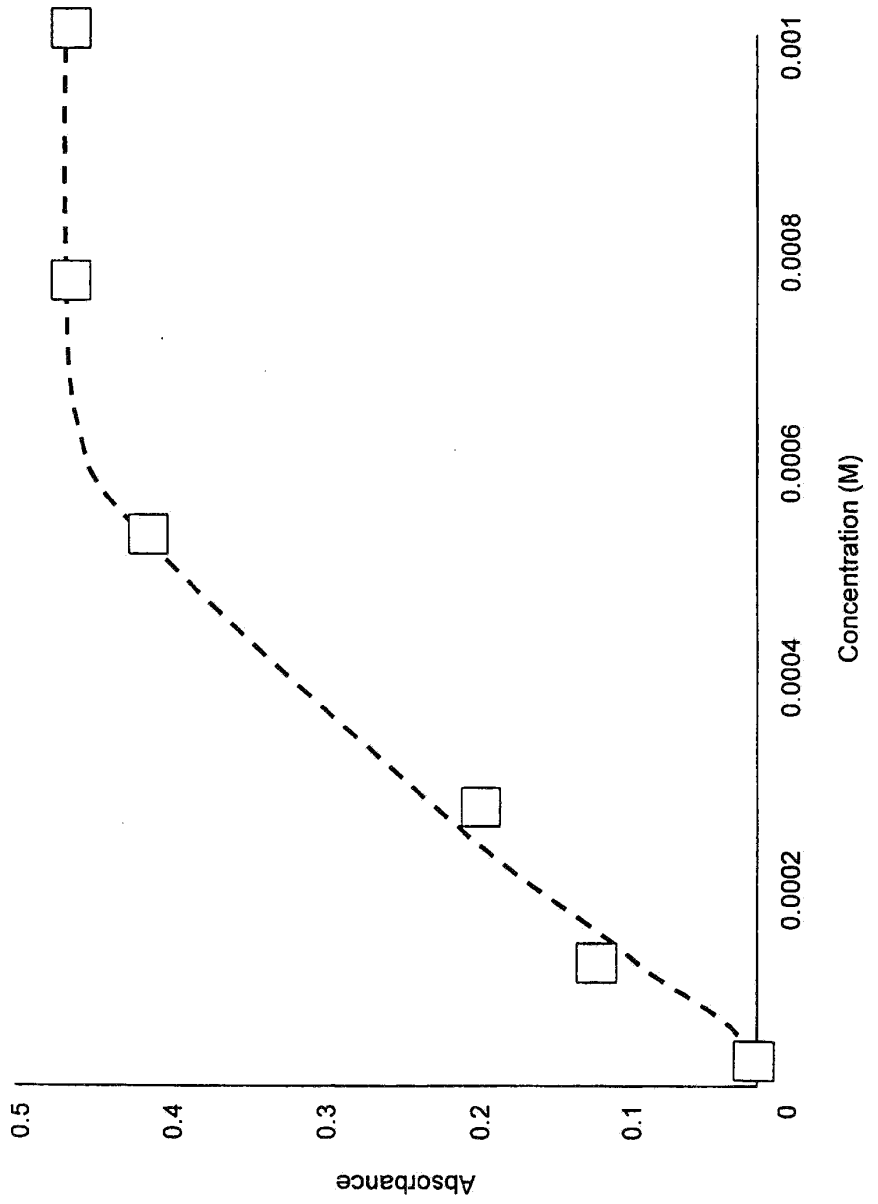


FIG. 7

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3027

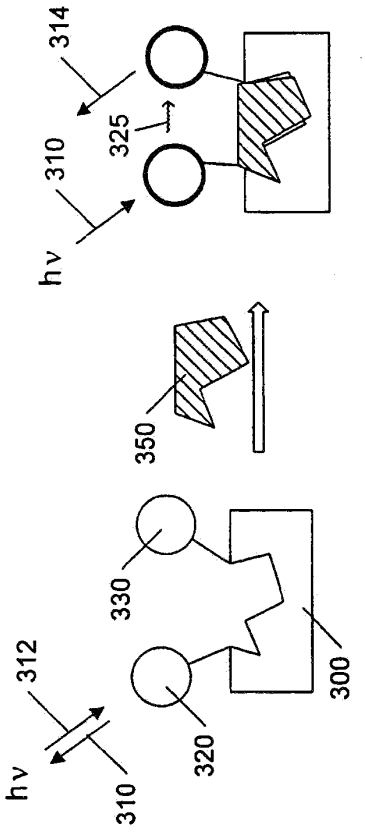


FIG. 8

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3028

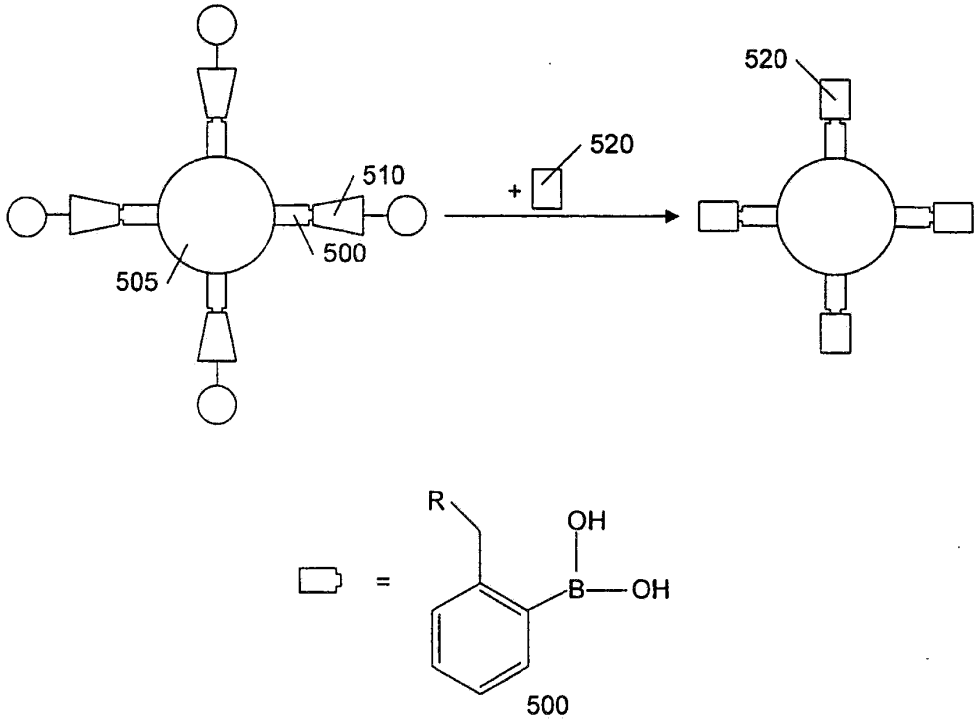
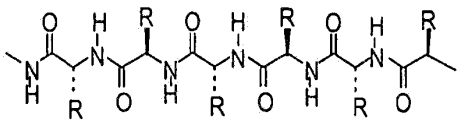
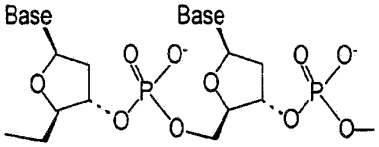


FIG. 9

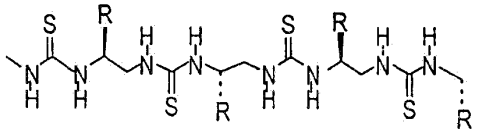
Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3029



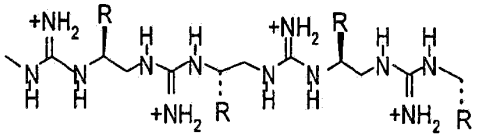
Peptides



Nucleotides



Polythioureas



Polyguanidiniums

FIG. 10

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3030

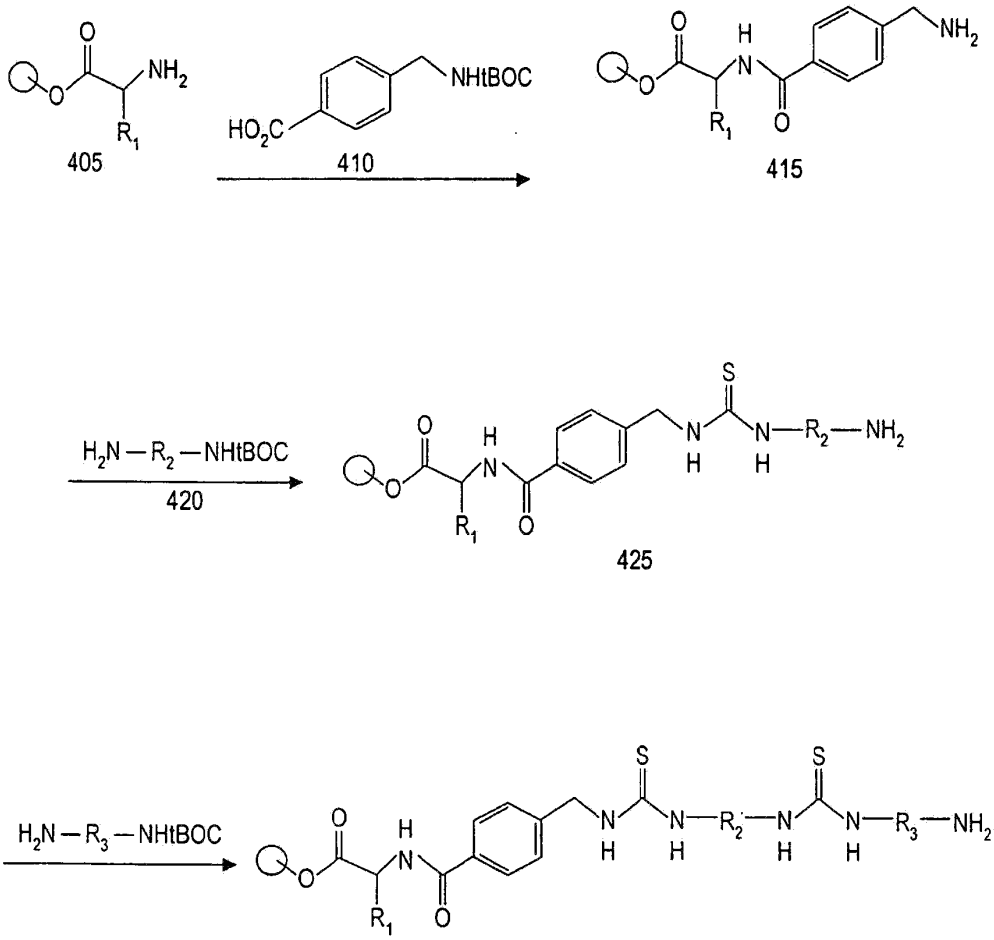


FIG. 11

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3031

13/44

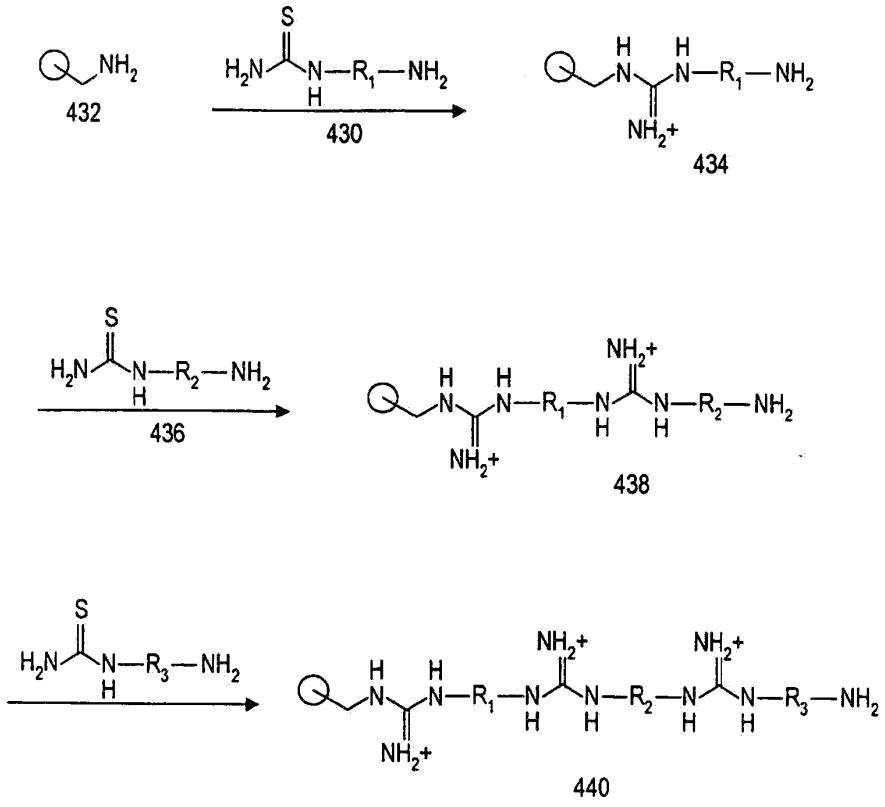


FIG. 12

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3032

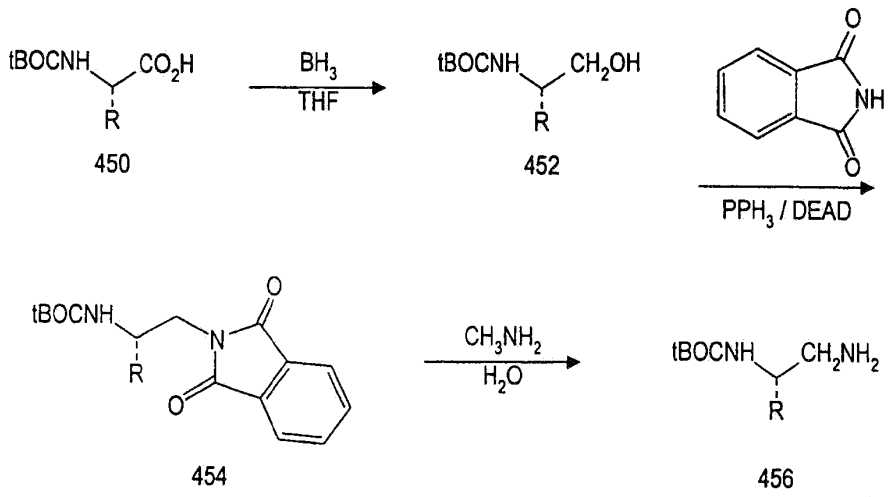
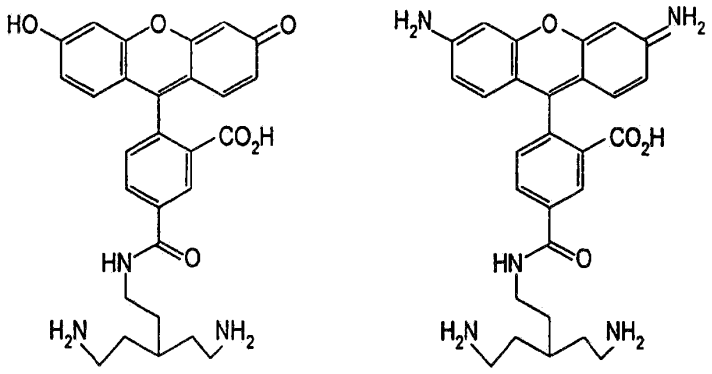


FIG. 13

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3033



470 475

FIG. 14

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3034

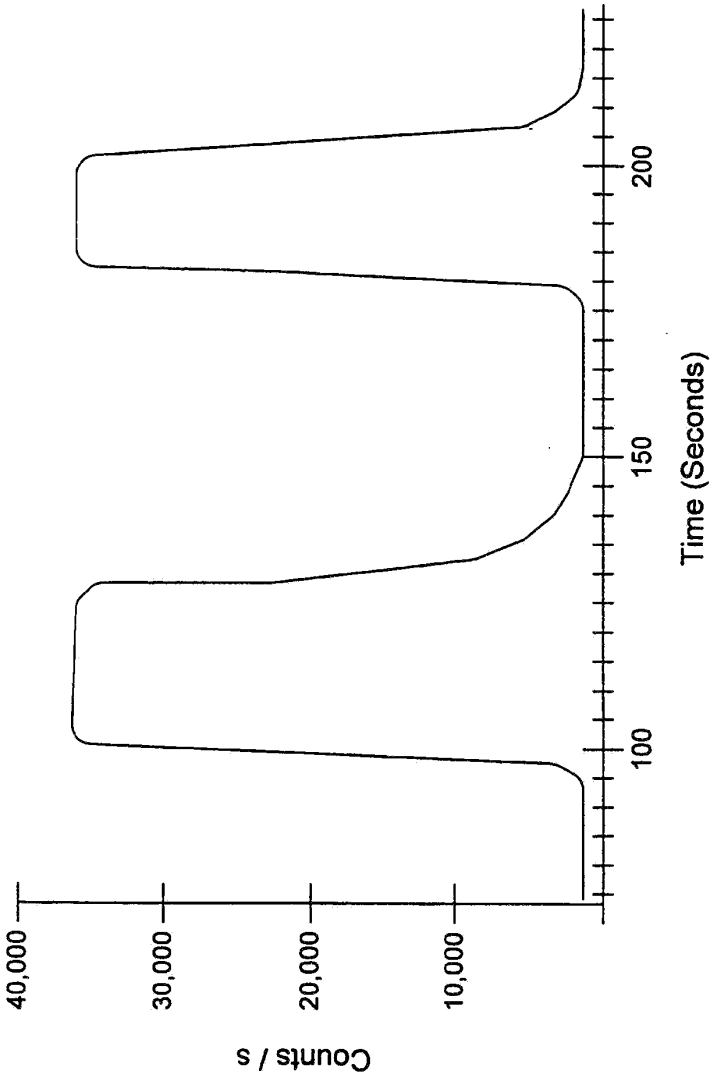


FIG. 15

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3035

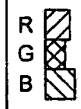
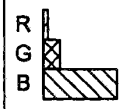
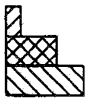
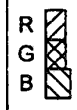
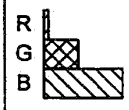
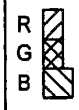
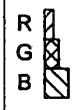
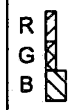
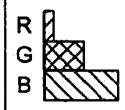
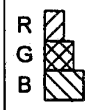
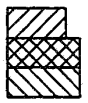
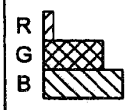
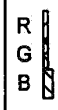
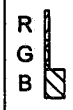
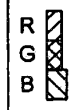
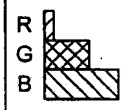
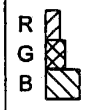
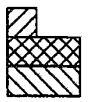
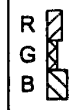
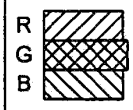
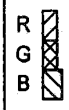
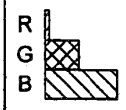
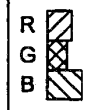
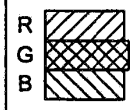
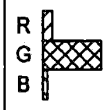
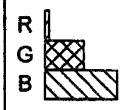
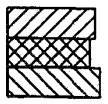
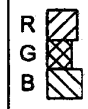
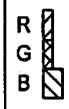
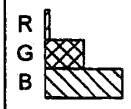
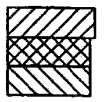
RESIN: pH	Ion	Blank	Alizarin	o-Cresol-phthalein	Fluorescein	Alizarin-Ce^{3+} complex
2	none					
2	Ca^{2+}					
7	none					
7	Ca^{2+}					
7	F^-					
12	none					
12	Ca^{2+}					
12	F^-					

FIG. 16

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3036

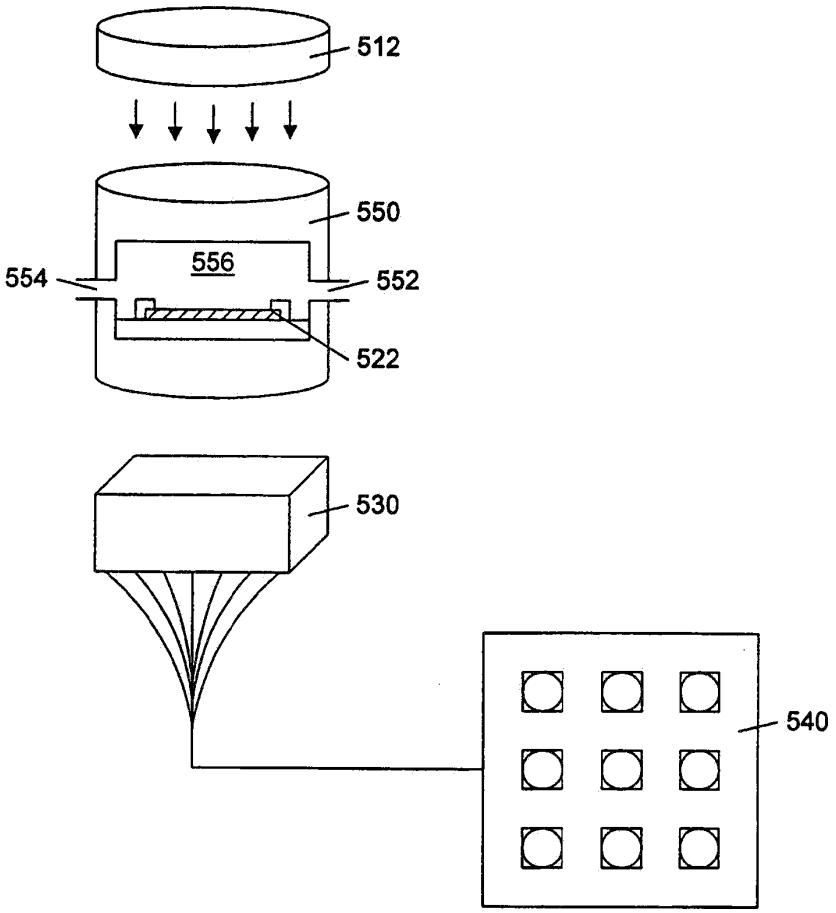


FIG. 17

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3037

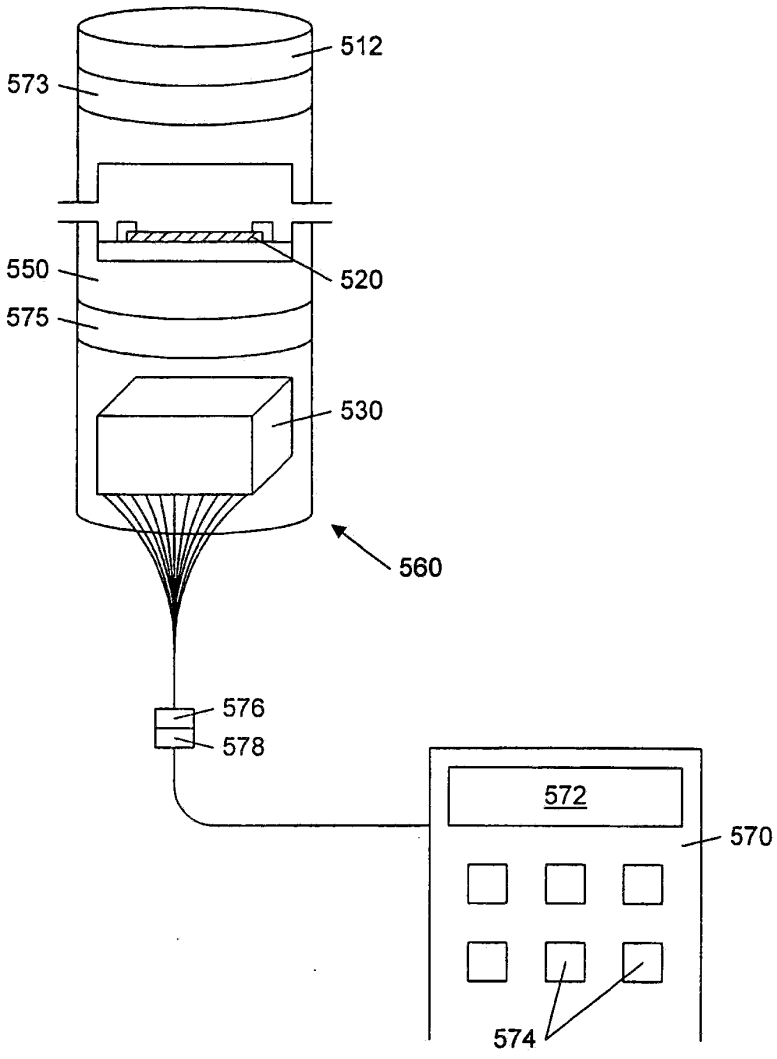


FIG. 18

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3038

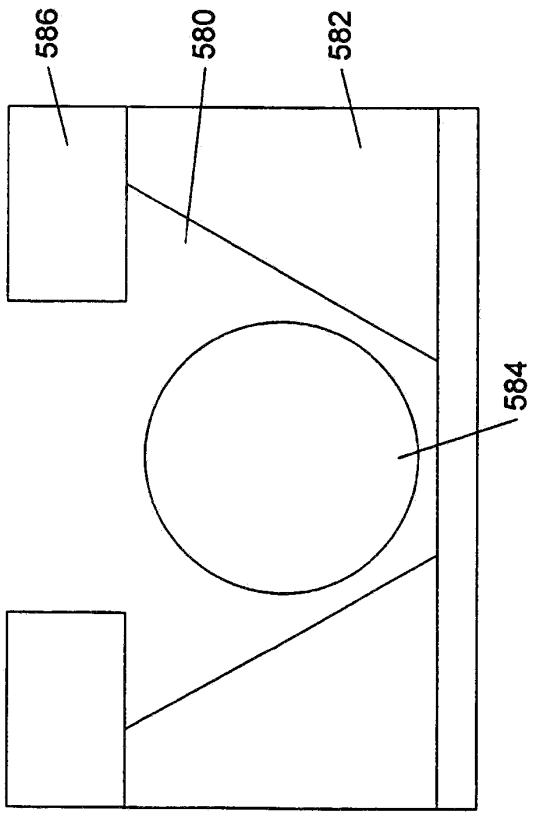


FIG. 19

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3039

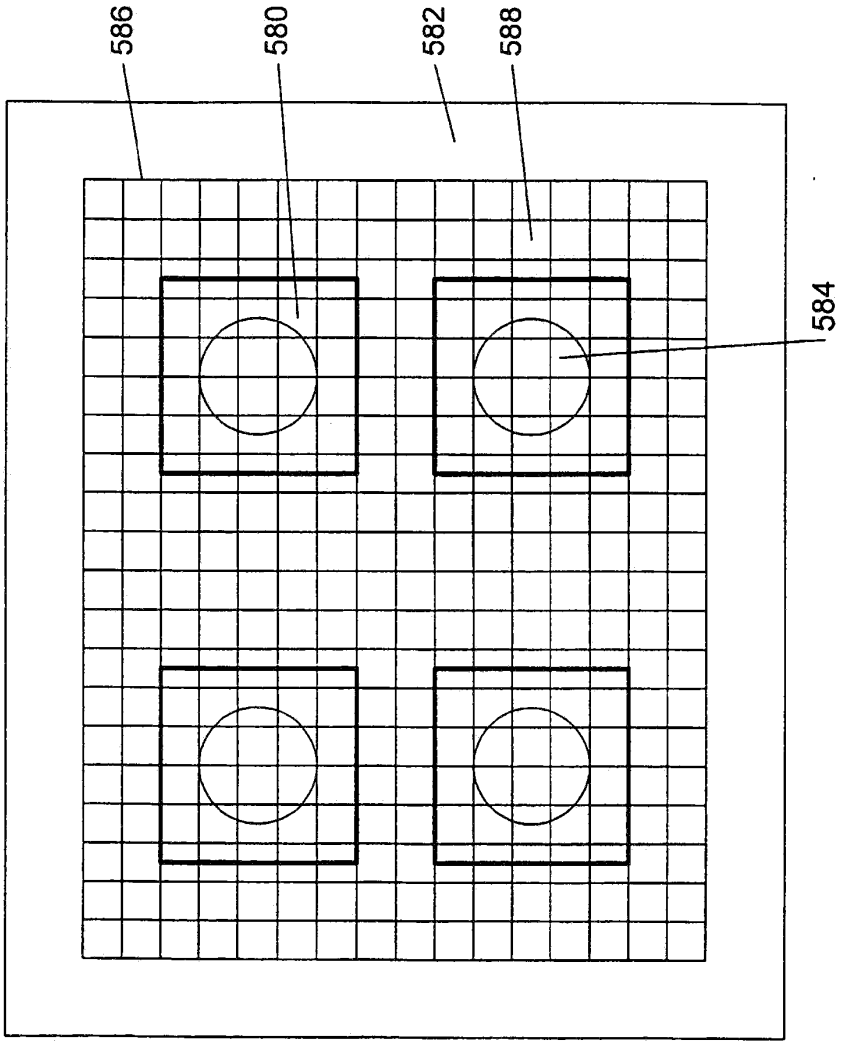


FIG. 20

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3040

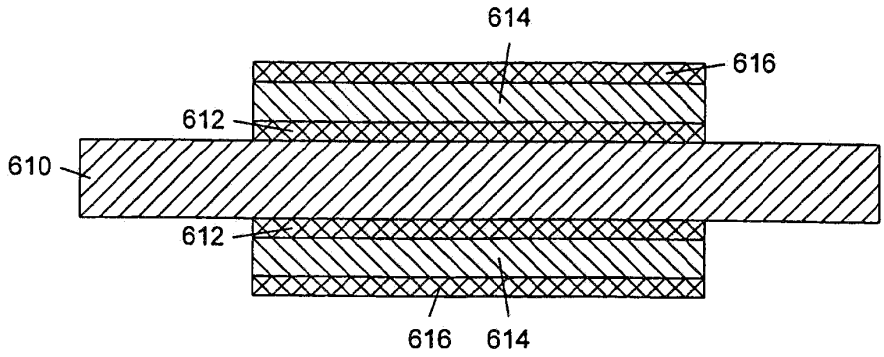


FIG. 21A

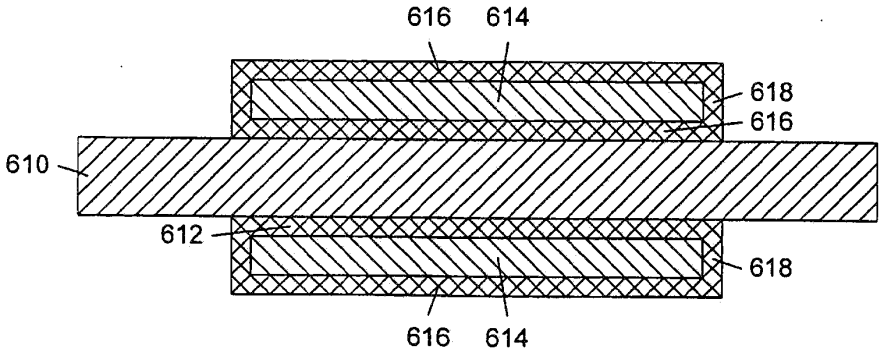


FIG. 21B

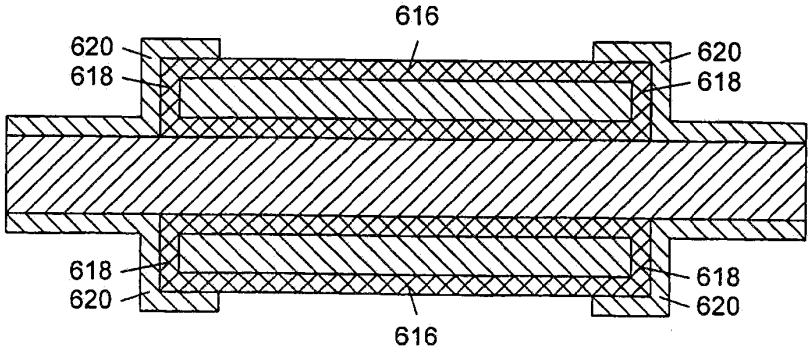


FIG. 21C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3041

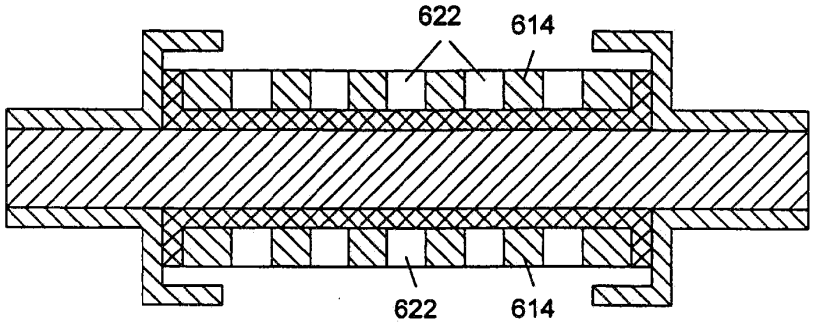


FIG. 21D

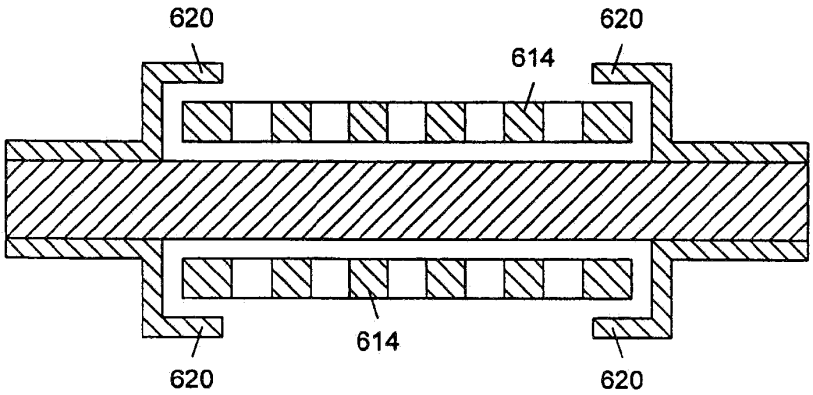


FIG. 21E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3042

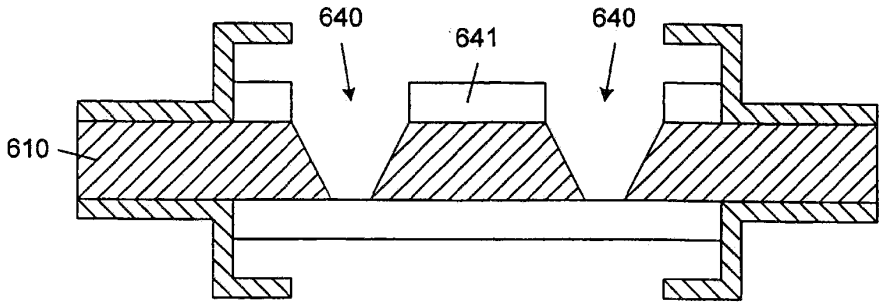


FIG. 21F

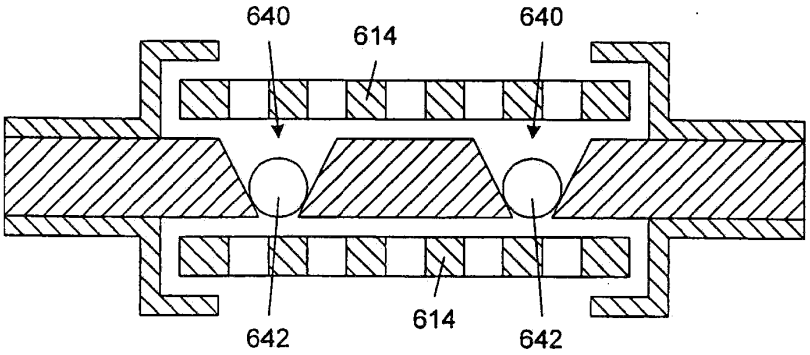


FIG. 21G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3043

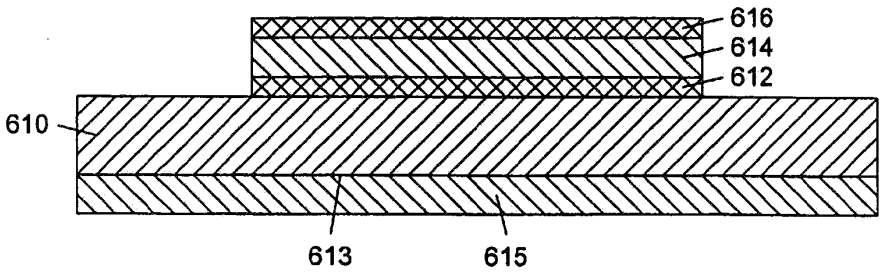


FIG. 22A

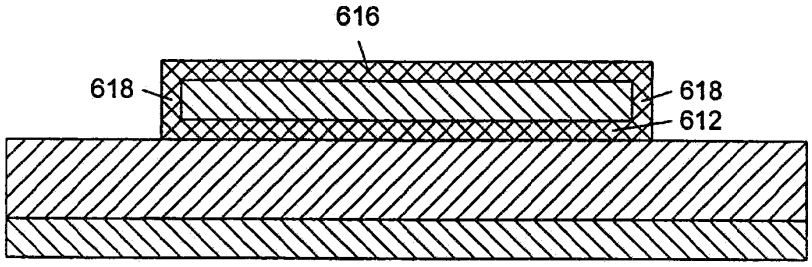


FIG. 22B

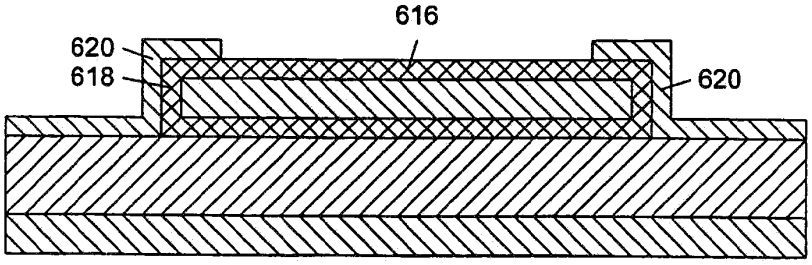


FIG. 22C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3044

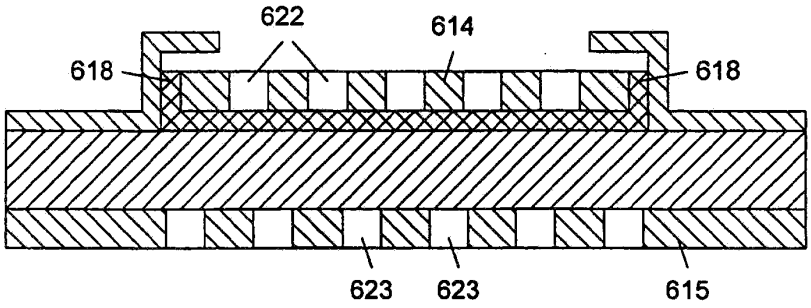


FIG. 22D

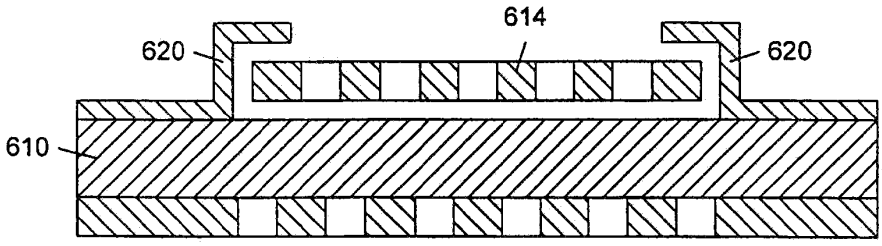


FIG. 22E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3045

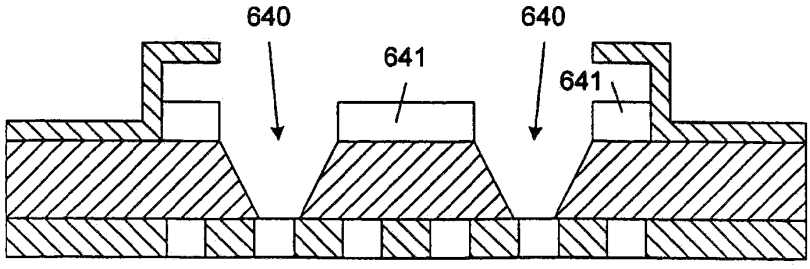


FIG. 22F

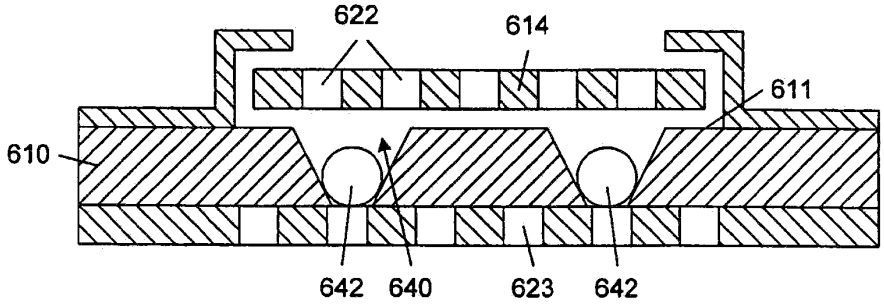


FIG. 22G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3046

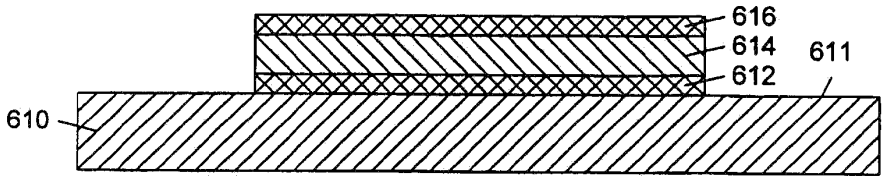


FIG. 23A

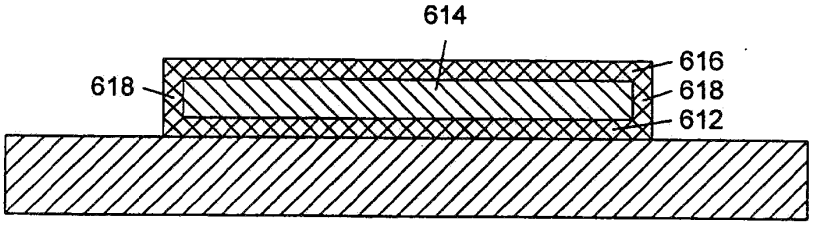


FIG. 23B

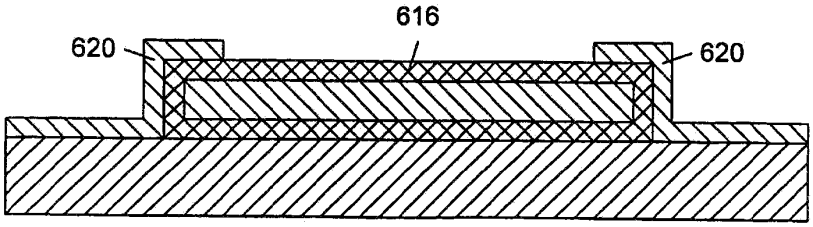


FIG. 23C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3047

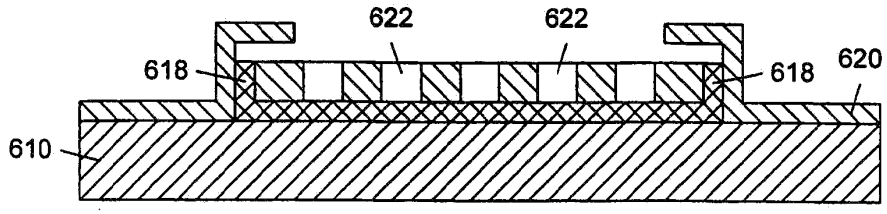


FIG. 23D

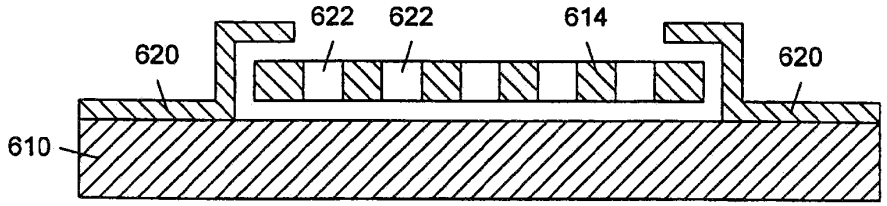


FIG. 23E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3048

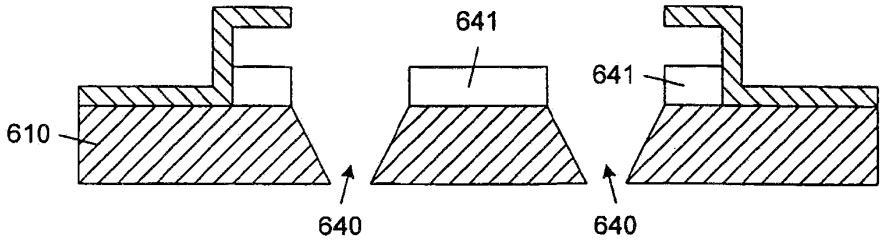


FIG. 23F

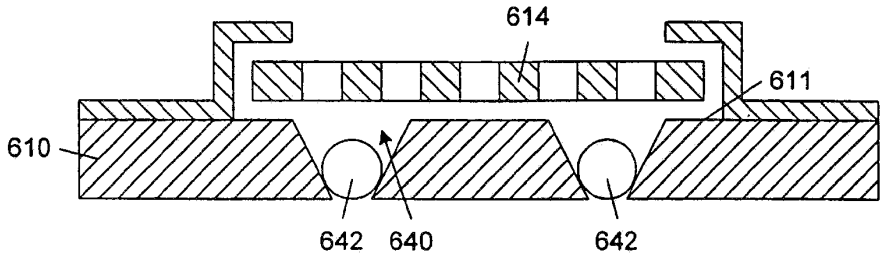


FIG. 23G

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3049

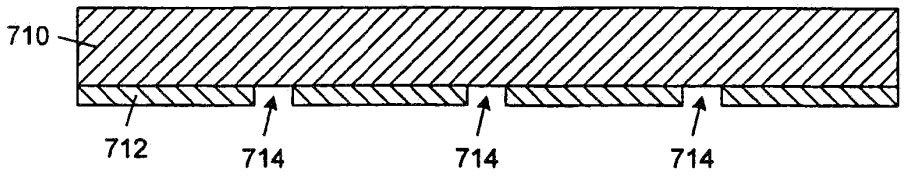


FIG. 24A

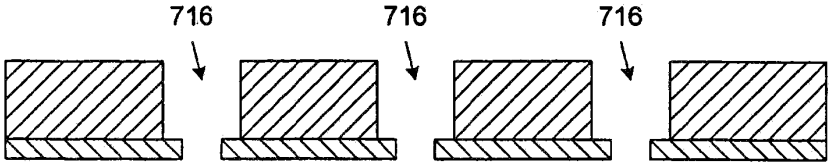


FIG. 24B

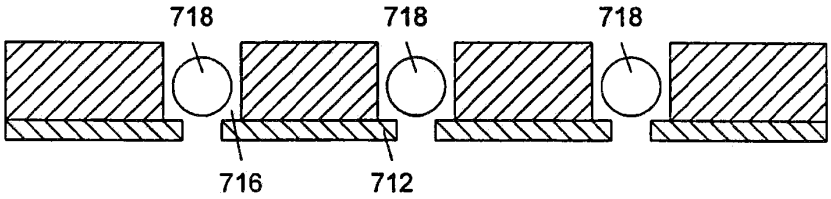


FIG. 24C

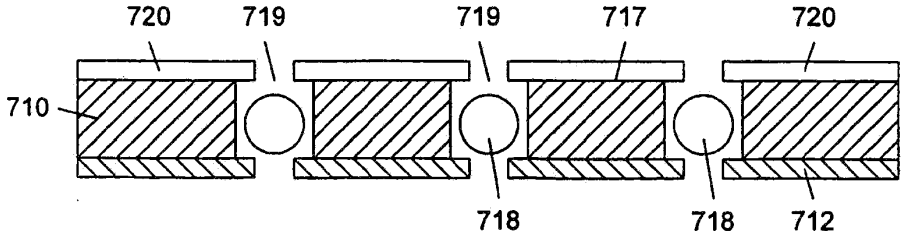


FIG. 24D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3050

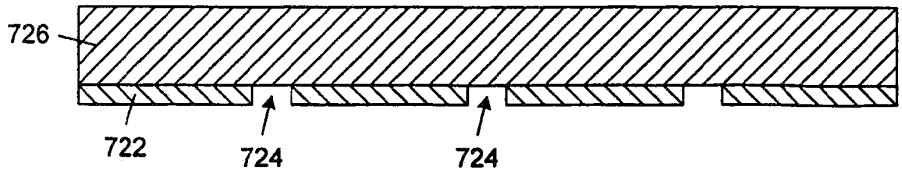


FIG. 25A

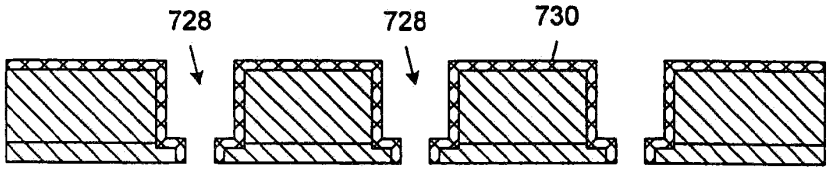


FIG. 25B

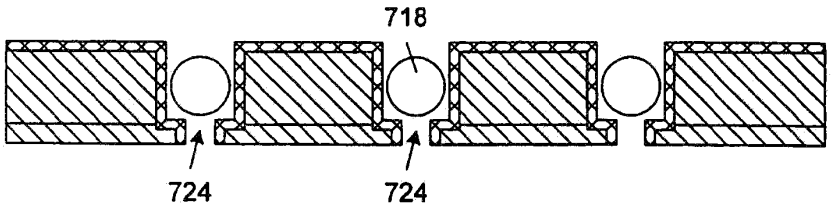


FIG. 25C

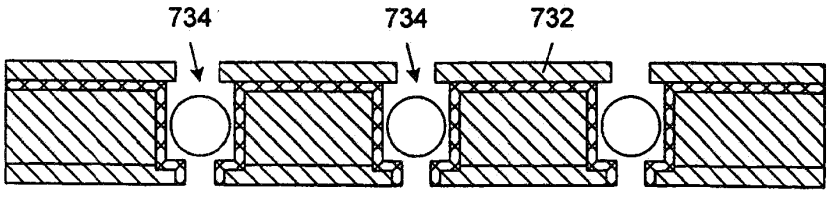


FIG. 25D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3051

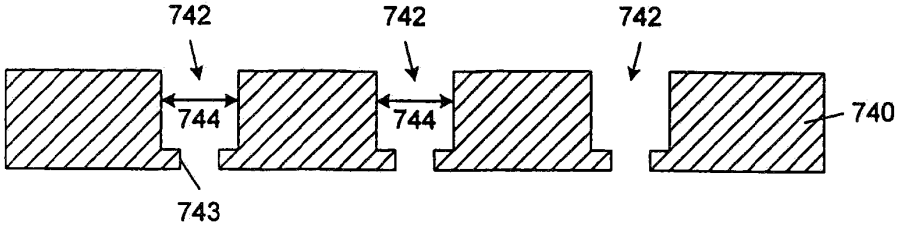


FIG. 26A

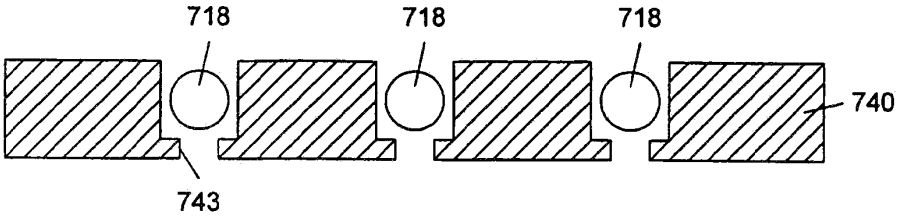


FIG. 26B

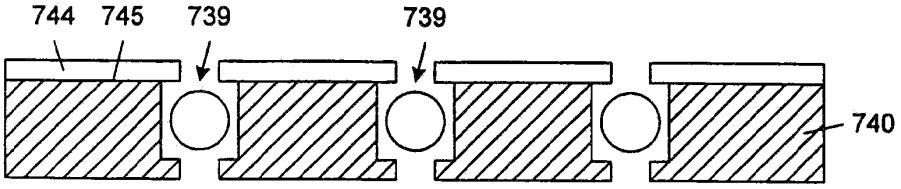


FIG. 26C

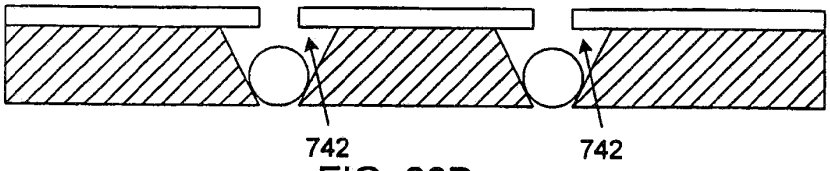


FIG. 26D

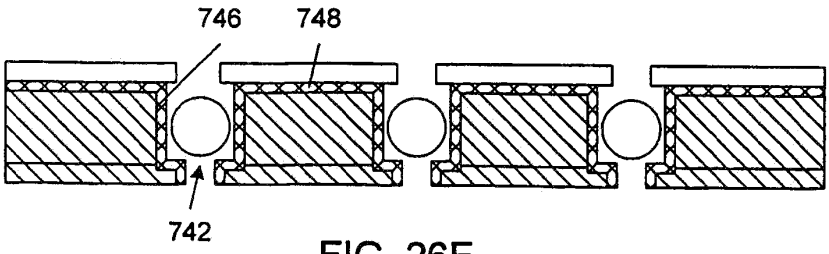


FIG. 26E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3052

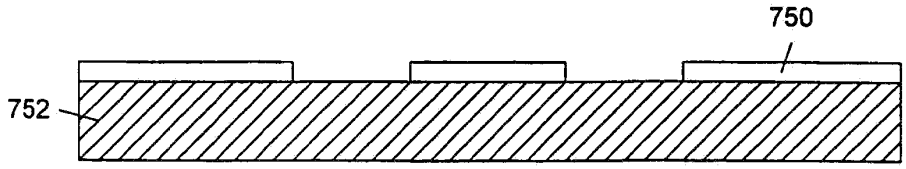


FIG. 27A

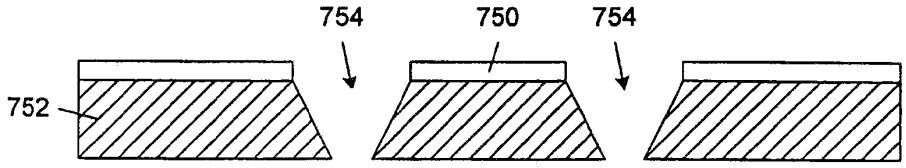


FIG. 27B

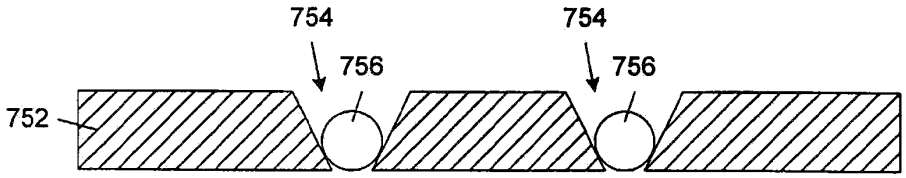


FIG. 27C

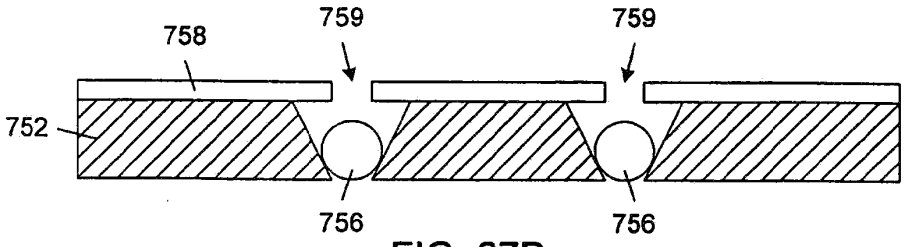


FIG. 27D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3053

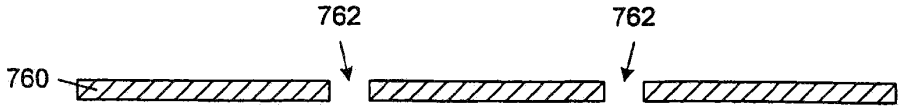


FIG. 28A

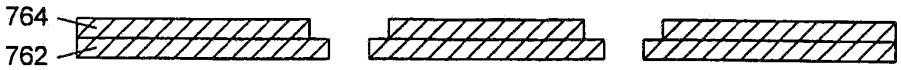


FIG. 28B

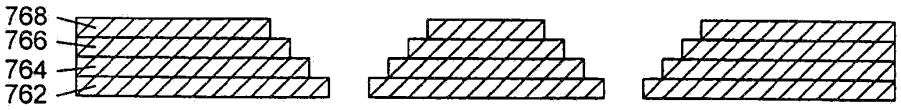


FIG. 28C

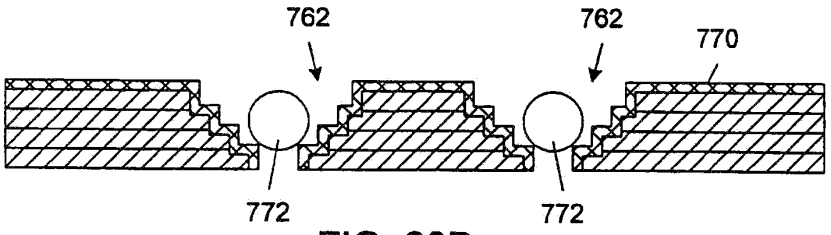


FIG. 28D

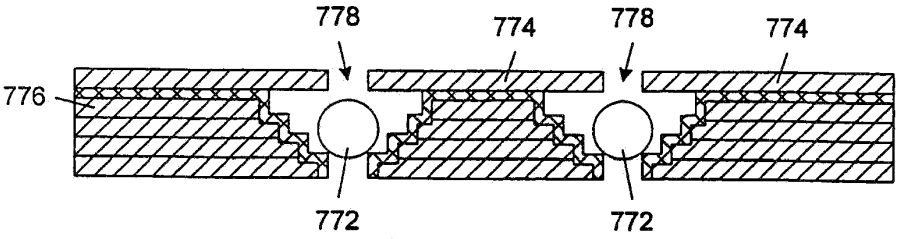


FIG. 28E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3054

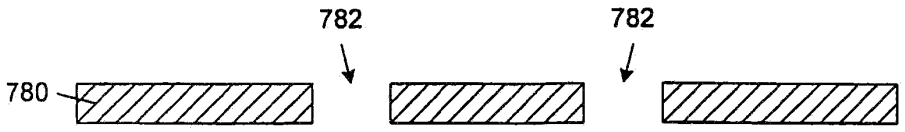


FIG. 29A

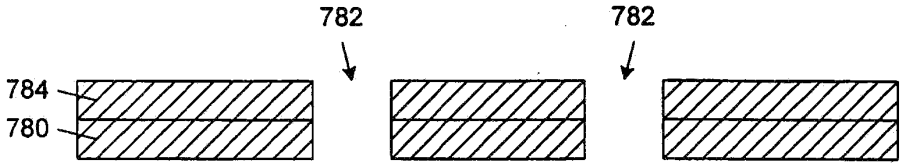


FIG. 29B

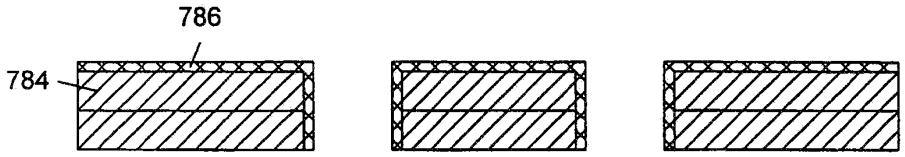


FIG. 29C

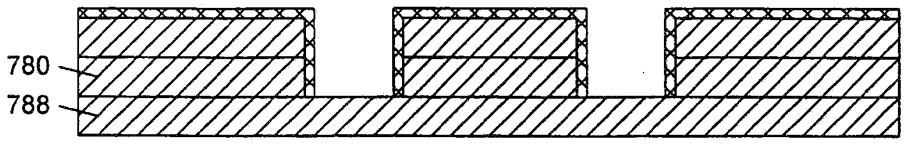


FIG. 29D

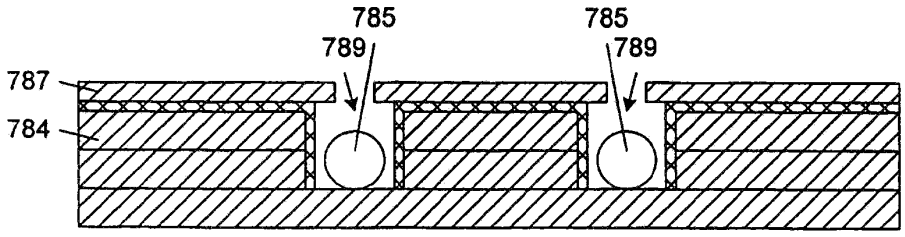


FIG. 29E

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3055

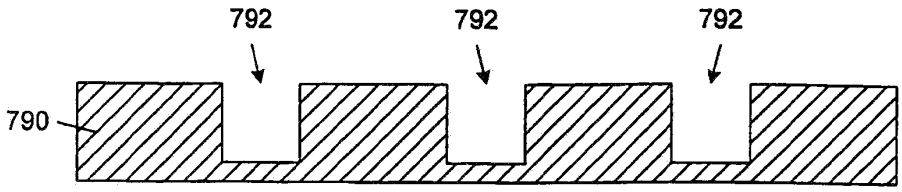


FIG. 30A

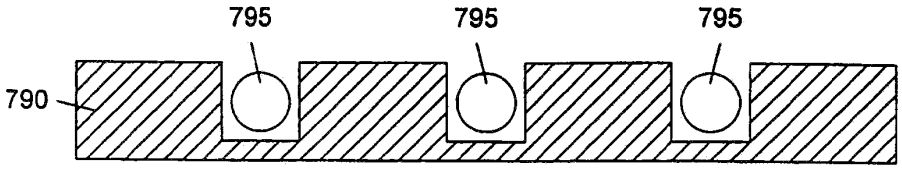


FIG. 30B

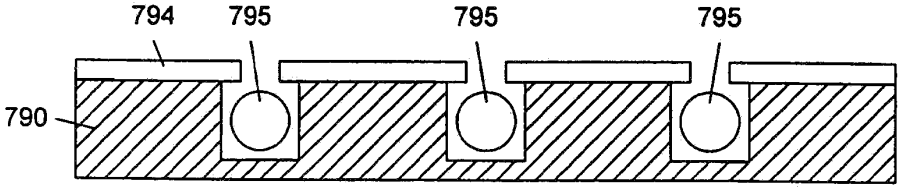


FIG. 30C

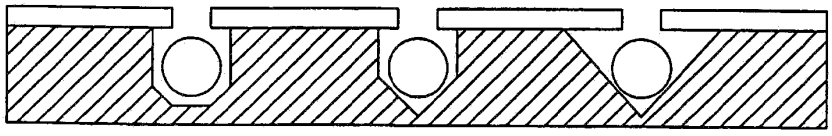


FIG. 30D

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3056

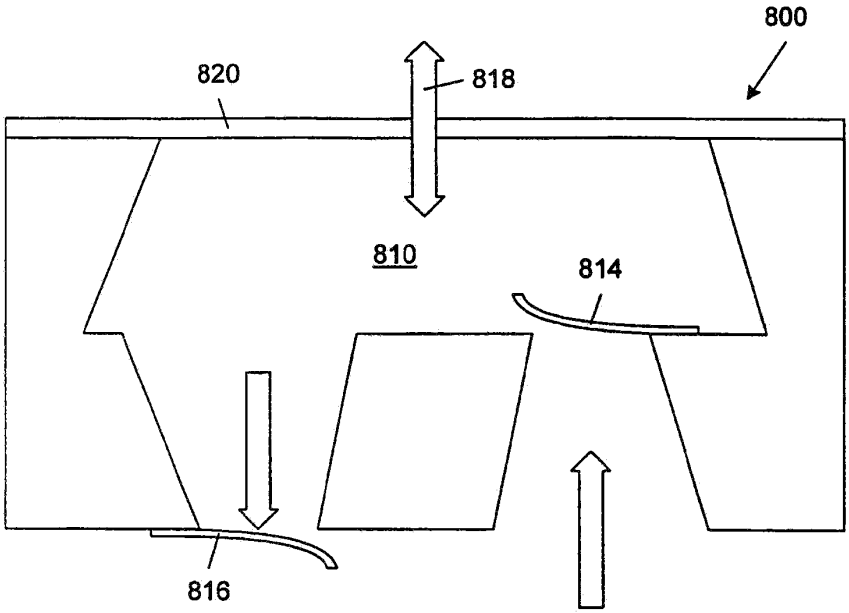


FIG. 31

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3057

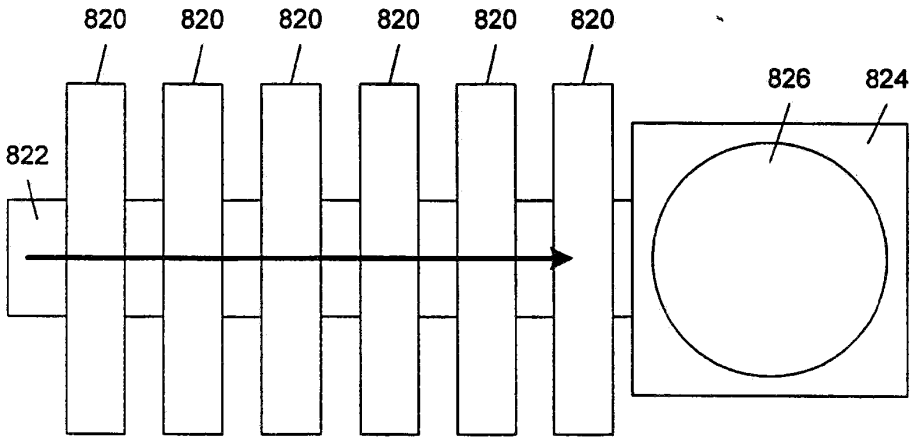


FIG. 32

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3058

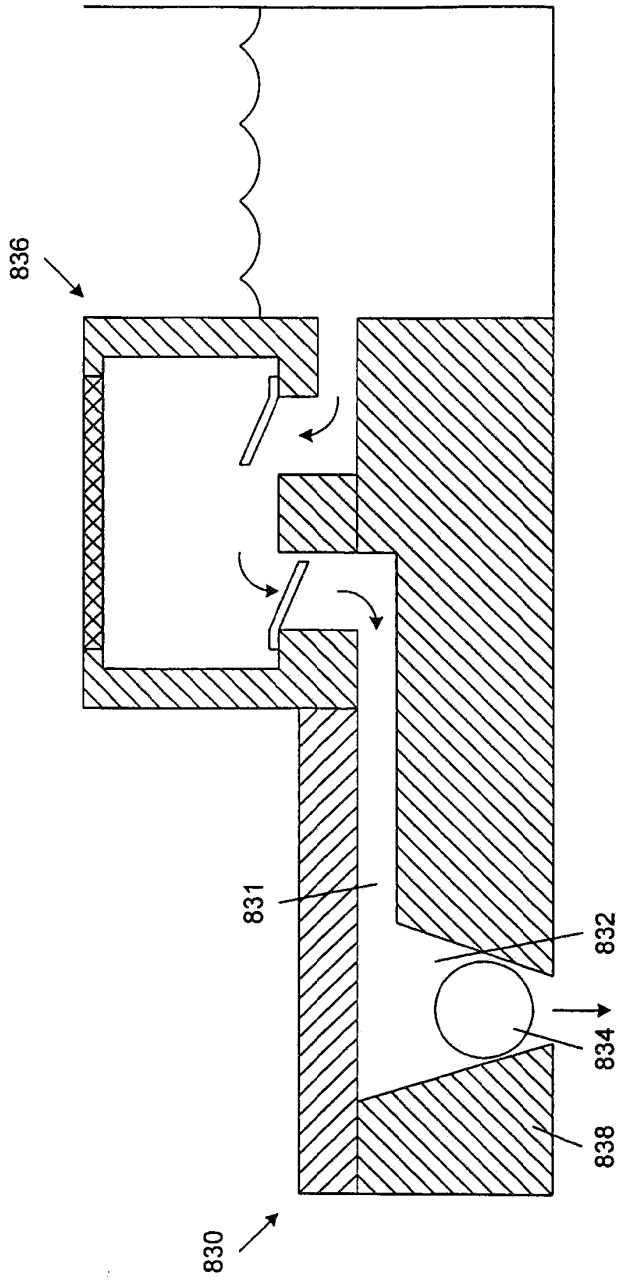


FIG. 33

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3059

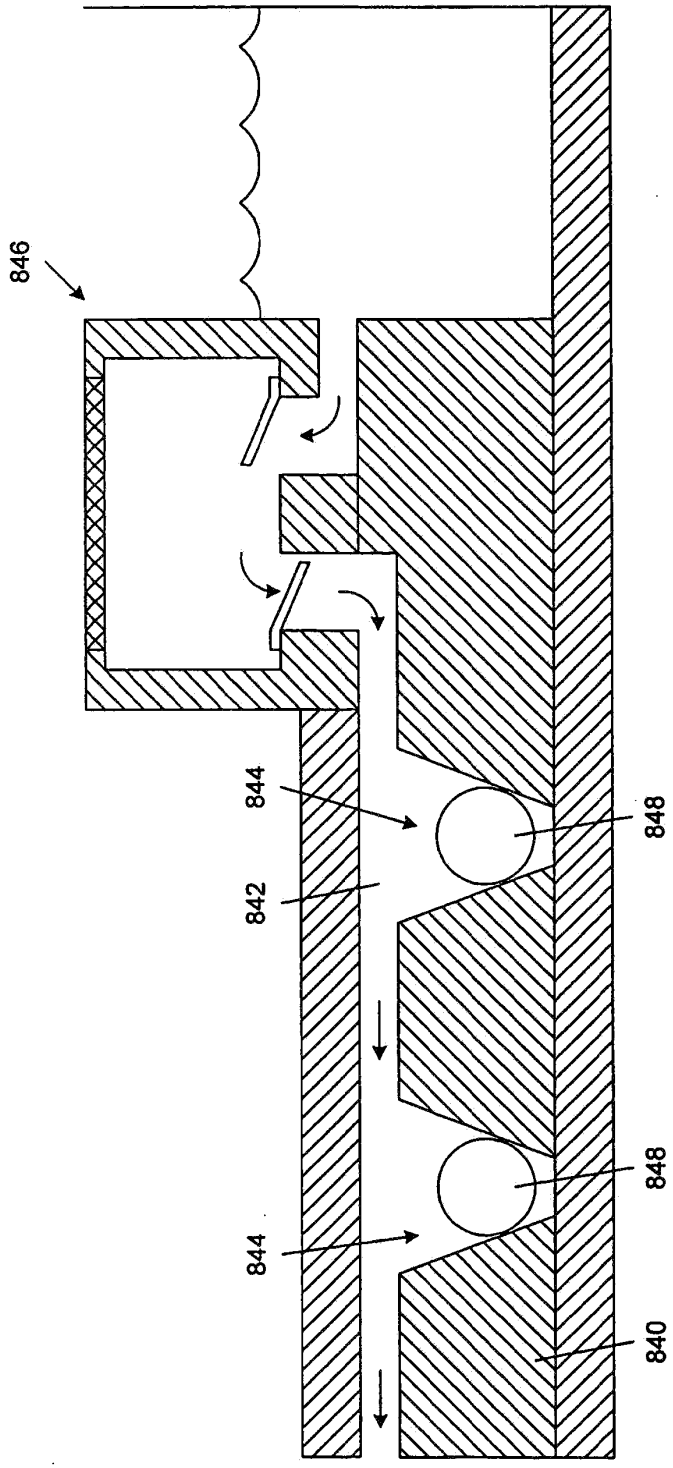


FIG. 34

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3060

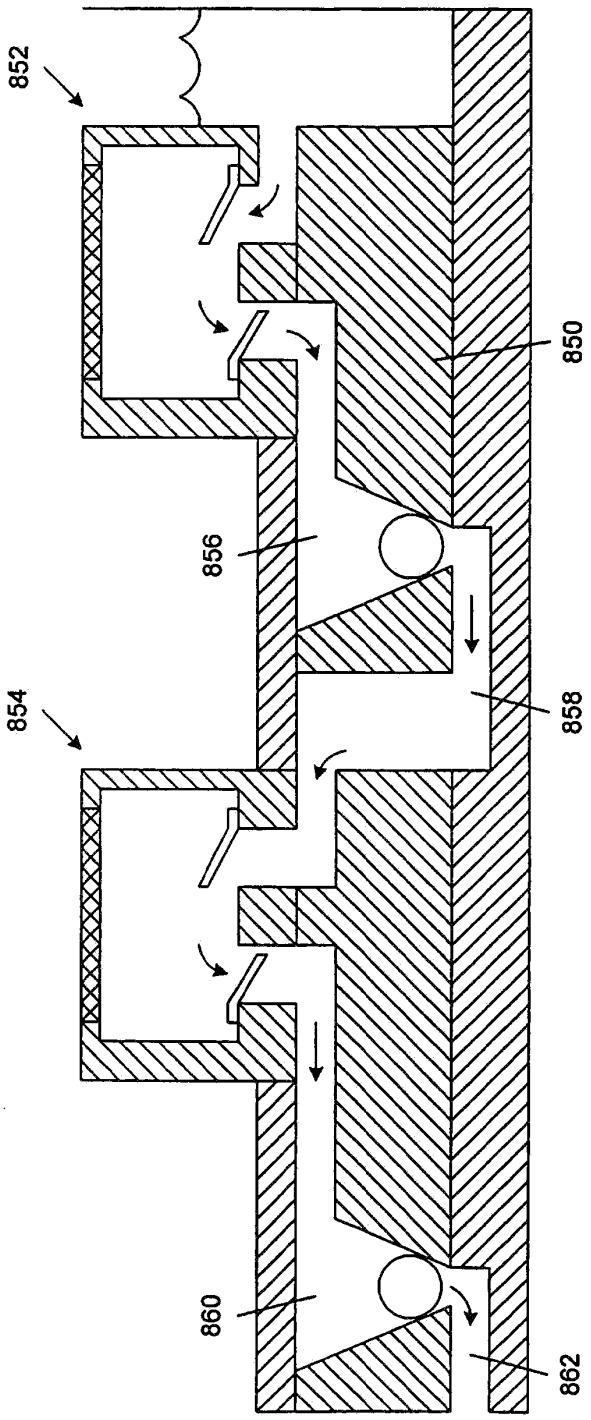


FIG. 35

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3061

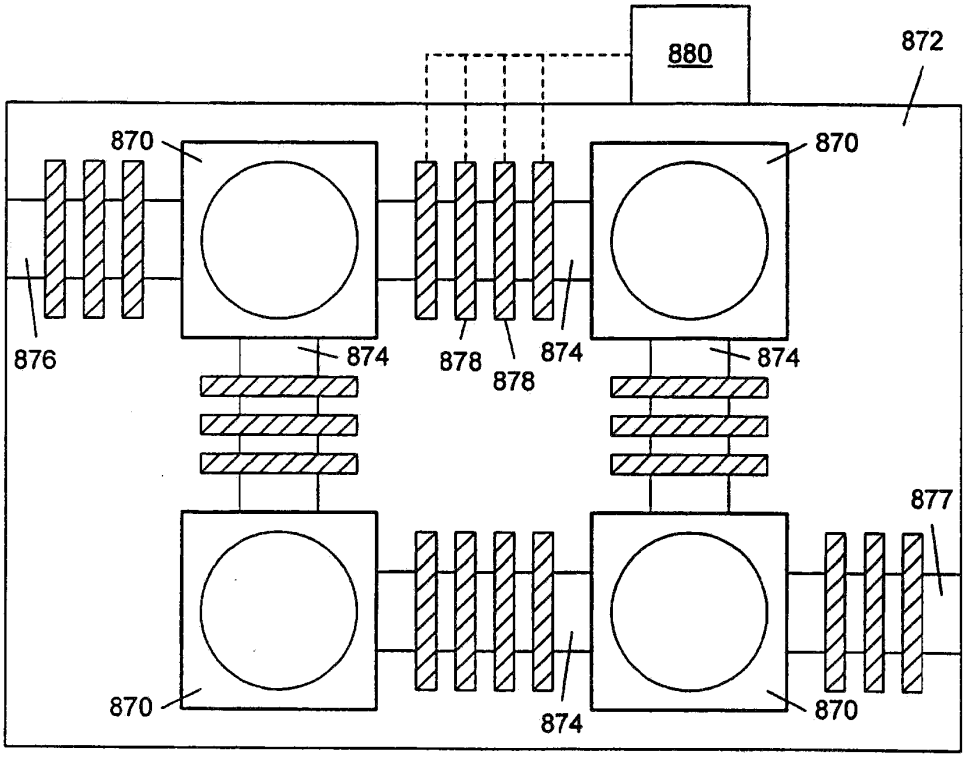


FIG. 36

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3062

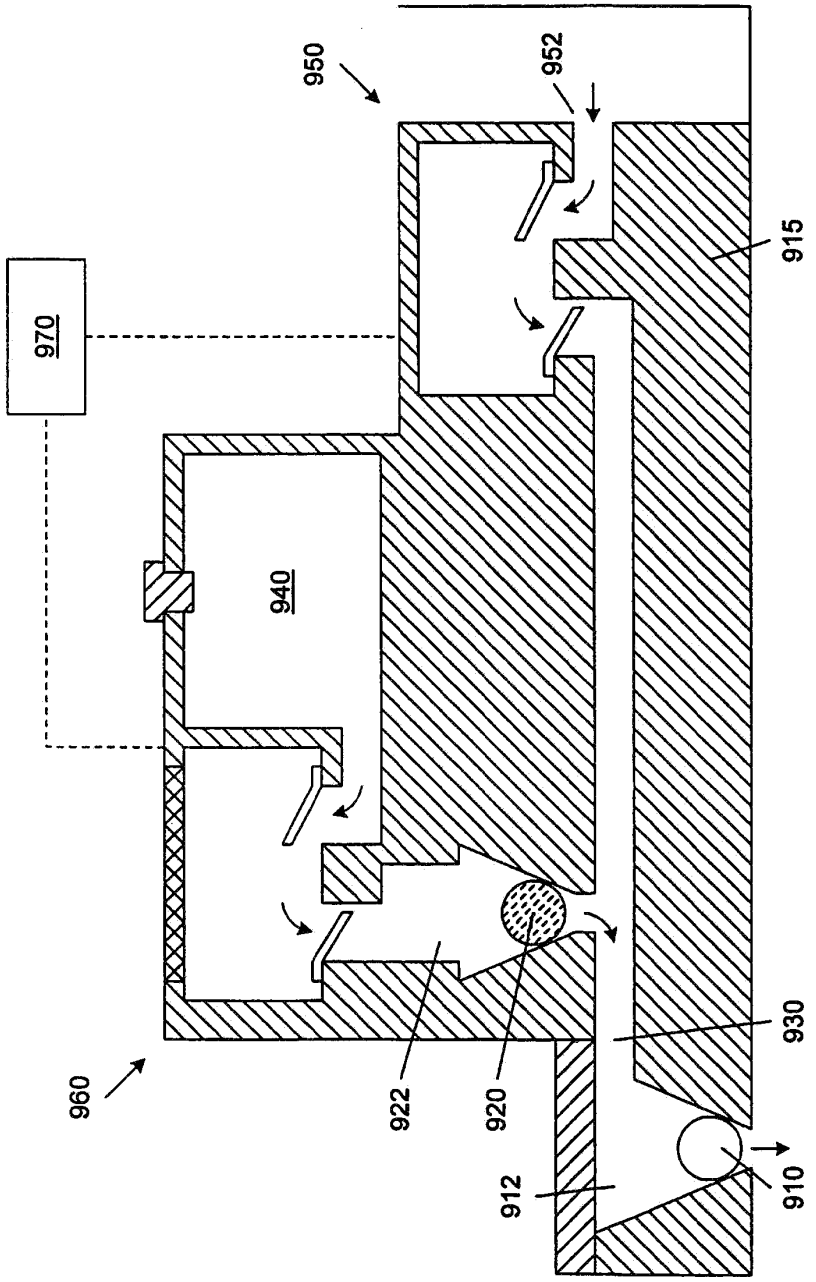


FIG. 37

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3063

INTERNATIONAL SEARCH REPORT

International Application No
F /US 99/16162

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64 G01N21/77

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)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 99 18434 A (TUFTS COLLEGE ;WALT DAVID R (US); DICKINSON TODD A (US)) 15 April 1999 (1999-04-15) page 8, line 28 -page 9, line 7 page 9, line 13 - line 21 page 10, line 23 - line 35 page 11, line 2 - line 4 page 12, line 5 - line 9 page 13, line 8 - line 17 -/--	1-5,10, 11,14, 16-20, 23-28, 30-33, 35,36, 38-42, 48,49, 51-59, 61-64, 66-74, 84,95, 106-108

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 1999	26/11/1999

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, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Navas Montero, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3064

INTERNATIONAL SEARCH REPORT

International Application No
P../US 99/16162

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page 15, line 11 - line 17 page 16, line 31 - line 38 page 17, line 16 - line 19 page 17, line 23 - line 37 page 19, line 9 - line 16 page 19, line 24 - line 27 page 20, line 22 - line 30 page 21, line 3 - line 12 page 24, line 6 - line 20 page 25, line 24 - line 30 page 26, line 12 - line 18 page 27, line 2 - line 5 page 27, line 6 - line 14 page 29, line 16 - line 18 page 30, line 31 - line 36 table 3 page 33, line 10 -page 34, line 2 page 36, line 28 - line 34 figures 5-8 ---	
X,P	WO 98 53300 A (BRENNER SYDNEY ;LYNX THERAPEUTICS INC (US); BRIDGHAM JOHN (US); CO) 26 November 1998 (1998-11-26) page 2, line 6 - line 29 page 3, line 31 - line 36 page 5, line 18 - line 33 page 6, line 1 - line 5 page 6, line 13 - line 17 page 7, line 20 - line 23 page 7, line 27 - line 31 page 10, line 10 - line 14 page 11, line 36 -page 12, line 9 page 22, line 22 - line 28 figures 1-4 ---	1,6-9, 12,13, 40, 43-47, 50,69, 70,83, 95,96, 105
X,P	WO 98 40726 A (TUFTS COLLEGE ;MICHAEL KARRI C (US); WALT DAVID R (US)) 17 September 1998 (1998-09-17) page 5, line 18 - line 31 page 6, line 24 - line 27 page 7, line 5 - line 15 page 19, line 15 -page 20, line 26 figures 4-7 --- -/--	1,69, 84-86, 89, 93-95, 97,100, 101, 106-108

1

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3065

INTERNATIONAL SEARCH REPORT

International Application No
P../US 99/16162

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 35189 A (UNIV WASHINGTON) 25 September 1997 (1997-09-25) page 2, line 19 - line 22 page 9, line 15 - line 18 page 8, line 26 - line 29 page 10, line 19 - line 27 figures 1,4,5 ---	69,75, 76,81, 103
X,P	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 03, 31 March 1999 (1999-03-31) & JP 10 332593 A (HITACHI LTD), 18 December 1998 (1998-12-18) abstract ---	95,99, 104
X,P	WO 99 17139 A (CLARK HEATHER ;UNIV MICHIGAN (US); BARKER SUSAN (US); KOPELMAN ' RAO) 8 April 1999 (1999-04-08) page 3, line 23 -page 4, line 2 page 9, line 20 - line 26 figures 1,3 ---	1,40,69
X,P	US 5 866 430 A (GROW ANN E) 2 February 1999 (1999-02-02) column 1, line 11 - line 19 column 1, line 36 - line 46 column 18, line 27 - line 50 column 19, line 54 -column 20, line 11 column 20, line 51 - line 57 column 21, line 17 - line 25 column 21, line 52 - line 60 column 43, line 28 - line 56 column 51, line 25 - line 41 figures 1,5,6 -----	1,40,95

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3066

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/16162

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 115-412
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3067

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /16162

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 115-412

In view of the large number of claims presently on file, which render it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely 1-114.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P../US 99/16162

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9918434 A	15-04-1999	AU 1269599 A	27-04-1999
WO 9853300 A	26-11-1998	AU 7715598 A	11-12-1998
WO 9840726 A	17-09-1998	AU 6464898 A	29-09-1998
WO 9735189 A	25-09-1997	US 5747349 A AU 4000197 A	05-05-1998 10-10-1997
JP 10332593 A	18-12-1998	NONE	
WO 9917139 A	08-04-1999	AU 9580798 A	23-04-1999
US 5866430 A	02-02-1999	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number
WO 03/054142 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/34612

(22) International Filing Date: 30 October 2002 (30.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

69/335,949	30 October 2001 (30.10.2001)	US
10/122,706	11 April 2002 (11.04.2002)	US
10/154,515	23 May 2002 (23.05.2002)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	US 60/335,949 (CIP)
Filed on	30 October 2001 (30.10.2001)
US	US 10/122,706 (CIP)
Filed on	11 April 2002 (11.04.2002)
US	US 10/154,515 (CIP)
Filed on	23 May 2002 (23.05.2002)

(71) Applicant *(for all designated States except US)*: **454 CORPORATION** [US/US]; 20 Commercial Street, Branford, CT 06405 (US).

(72) Inventors; and

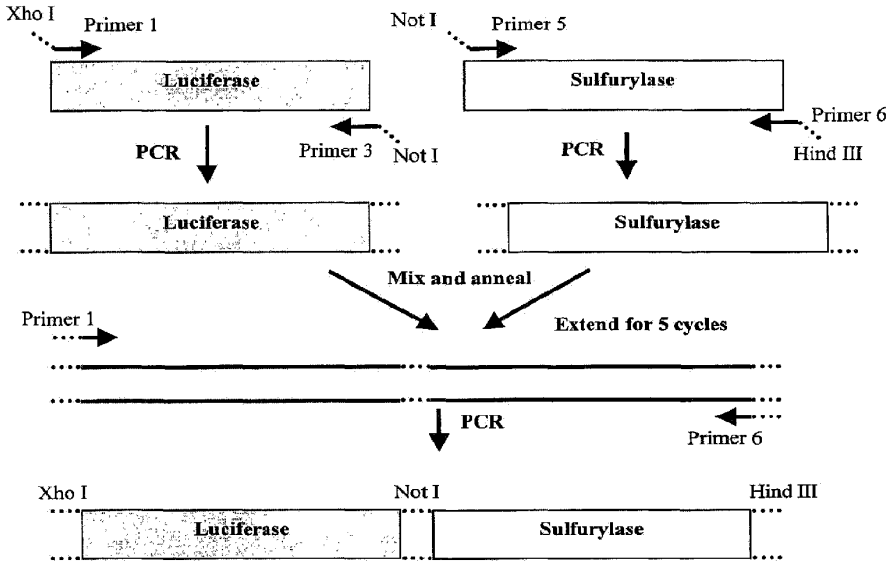
(75) Inventors/Applicants *(for US only)*: **SRINIVASAN, Maithreyan** [IN/US]; 275 Circular Avenue, Unit 2C, Hamden, CT 06516 (US). **REIFLER, Michael** [US/US]; 905 Mix Avenue, Apartment T3, Hamden, CT 06514 (US).

(74) Agent: **ELRIFI, Ivor, R.**; Mintz, Levin Cohn, Ferris, Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: NOVEL SULFURYLASE-LUCIFERASE FUSION PROTEINS AND THERMOSTABLE SULFURYLASE



(57) Abstract: The present invention relates to the field of DNA recombinant technology. More specifically, this invention relates to fusion proteins comprising an ATP generating polypeptide joined to a polypeptide that converts ATP into a detectable entity. Accordingly, this invention focuses on sulfurylase-luciferase fusion proteins. This invention also relates to pharmaceutical compositions containing the fusion proteins and methods for using them.

WO 03/054142 A2

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

NOVEL SULFURYLASE-LUCIFERASE FUSION PROTEINS AND THERMOSTABLE SULFURYLASE

FIELD OF THE INVENTION

The invention relates generally to fusion proteins that are useful as reporter proteins, in particular to fusion proteins of ATP sulfurylase and luciferase which are utilized to achieve an efficient conversion of pyrophosphate (PPi) to light. This invention also relates to a novel thermostable sulfurylase which can be used in the detection of inorganic pyrophosphate, particularly in the sequencing of nucleic acid.

BACKGROUND OF THE INVENTION

ATP sulfurylase has been identified as being involved in sulfur metabolism. It catalyzes the initial reaction in the metabolism of inorganic sulfate (SO_4^{-2}); see *e.g.*, Robbins and Lipmann, 1958. *J. Biol. Chem.* **233**: 686-690; Hawes and Nicholas, 1973. *Biochem. J.* **133**: 541-550). In this reaction SO_4^{-2} is activated to adenosine 5'-phosphosulfate (APS). ATP sulfurylase is also commonly used in pyrophosphate sequencing methods. In order to convert pyrophosphate (PPi) generated from the addition of dNMP to a growing DNA chain to light, PPi must first be converted to ATP by ATP sulfurylase.

ATP produced by an ATP sulfurylase can also be hydrolyzed using enzymatic reactions to generate light. Light-emitting chemical reactions (*i.e.*, chemiluminescence) and biological reactions (*i.e.*, bioluminescence) are widely used in analytical biochemistry for sensitive measurements of various metabolites. In bioluminescent reactions, the chemical reaction that leads to the emission of light is enzyme-catalyzed. For example, the luciferin-luciferase system allows for specific assay of ATP. Thus, both ATP generating enzymes, such as ATP sulfurylase, and light emitting enzymes, such as luciferase, could be useful in a number of different assays for the detection and/or concentration of specific substances in fluids and gases. Since high physical and chemical stability is sometimes required for enzymes involved in sequencing reactions, a thermostable enzyme is desirable.

Because the product of the sulfurylase reaction is consumed by luciferase, proximity between these two enzymes by covalently linking the two enzymes in the form of a fusion protein would provide for a more efficient use of the substrate. Substrate channeling is a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3072

phenomenon in which substrates are efficiently delivered from enzyme to enzyme without equilibration with other pools of the same substrates. In effect, this creates local pools of metabolites at high concentrations relative to those found in other areas of the cell. Therefore, a fusion of an ATP generating polypeptide and an ATP converting peptide could benefit from the phenomenon of substrate channeling and would reduce production costs and increase the number of enzymatic reactions that occur during a given time period.

All patents and publications cited throughout the specification are hereby incorporated by reference into this specification in their entirety in order to more fully describe the state of the art to which this invention pertains.

SUMMARY OF THE INVENTION

The invention provides a fusion protein comprising an ATP generating polypeptide bound to a polypeptide which converts ATP into an entity which is detectable. In one aspect, the invention provides a fusion protein comprising a sulfurylase polypeptide bound to a luciferase polypeptide. This invention provides a nucleic acid that comprises an open reading frame that encodes a novel thermostable sulfurylase polypeptide. In a further aspect, the invention provides for a fusion protein comprising a thermostable sulfurylase joined to at least one affinity tag.

In another aspect, the invention provides a recombinant polynucleotide that comprises a coding sequence for a fusion protein having a sulfurylase poylpeptide sequence joined to a luciferase polypeptide sequence. In a further aspect, the invention provides an expression vector for expressing a fusion protein. The expression vector comprises a coding sequence for a fusion protein having: (i) a regulatory sequence, (ii) a first polypeptide sequence of an ATP generating polypeptide and (iii) a second polypeptide sequence that converts ATP to an entity which is detectable. In an additional embodiment, the fusion protein comprises a sulfurylase polypeptide and a luciferase polypeptide. In another aspect, the invention provides a transformed host cell which comprises the expression vector. In an additional aspect, the invention provides a fusion protein bound to a mobile support. The invention also includes a kit comprising a sulfurylase-luciferase fusion protein expression vector.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3073

The invention also includes a method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising: (a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added; (b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released; (c) separately recovering each of the feedstocks from the polymerization environment; and (d) measuring the amount of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer. In one embodiment, the amount of inorganic pyrophosphate is measured by the steps of: (a) adding adenosine-5'-phosphosulfate to the feedstock; (b) combining the recovered feedstock containing adenosine-5'-phosphosulfate with an ATP generating polypeptide-ATP converting polypeptide fusion protein such that any inorganic pyrophosphate in the recovered feedstock and the adenosine-5'-phosphosulfate will react to the form ATP and sulfate; (c) combining the ATP and sulfate-containing feedstock with luciferin in the presence of oxygen such that the ATP is consumed to produced AMP, inorganic pyrophosphate, carbon dioxide and light; and (d) measuring the amount of light produced.

In another aspect, the invention includes a method wherein each feedstock comprises adenosine-5'-phosphosulfate and luciferin in addition to the selected nucleotide base, and the amount of inorganic pyrophosphate is determined by reacting the inorganic pyrophosphate feedstock with an ATP generating polypeptide-ATP converting polypeptide fusion protein thereby producing light in an amount proportional to the amount of inorganic pyrophosphate, and measuring the amount of light produced.

In another aspect, the invention provides a method for sequencing a nucleic acid, the method comprising: (a) providing one or more nucleic acid anchor primers; (b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3074

reaction chambers have a center to center spacing of between 5 to 200 μm; (c) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (e) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of a ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the sequence of the nucleic acid.

In one aspect, the invention provides a method for sequencing a nucleic acid, the method comprising: (a) providing at least one nucleic acid anchor primer; (b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites; (c) annealing a first amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (e) annealing a second amount of a sequencing primer to one or more copies of the covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, when the predetermined nucleotide triphosphate is incorporated onto the 3' end of the sequencing primer, to yield a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of a ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the sequence of the nucleic acid at each reaction site that contains a nucleic acid template.

In another aspect, the invention includes a method of determining the base sequence of a plurality of nucleotides on an array, the method comprising the steps of: (a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, (b) adding an activated nucleotide 5'-triphosphate

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3075

precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates; (c) determining whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands through detection of a sequencing byproduct with a ATP generating polypeptide-ATP converting polypeptide fusion protein, thus indicating that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and

(e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

In one aspect, the invention includes a method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising: (a) introducing a plurality of template nucleic acid polymers into a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, each reaction chamber having a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added; (b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released; (c) detecting the formation of inorganic pyrophosphate with an ATP generating polypeptide-ATP

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3076

converting polypeptide fusion protein to determine the identify of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

In one aspect, the invention provides a method of identifying the base in a target position in a DNA sequence of sample DNA including the steps comprising: (a) disposing sample DNA within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, said DNA being rendered single stranded either before or after being disposed in the reaction chambers, (b) providing an extension primer which hybridizes to said immobilized single-stranded DNA at a position immediately adjacent to said target position; (c) subjecting said immobilized single-stranded DNA to a polymerase reaction in the presence of a predetermined nucleotide triphosphate, wherein if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer then a sequencing reaction byproduct is formed; and (d) identifying the sequencing reaction byproduct with a ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the nucleotide complementary to the base at said target position.

The invention also includes a method of identifying a base at a target position in a sample DNA sequence comprising: (a) providing sample DNA disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, said DNA being rendered single stranded either before or after being disposed in the reaction chambers; (b) providing an extension primer which hybridizes to the sample DNA immediately adjacent to the target position; (c) subjecting the sample DNA sequence and the extension primer to a polymerase reaction in the presence of a nucleotide triphosphate whereby the nucleotide triphosphate will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position, said nucleotide triphosphate being added either to separate aliquots of sample-primer mixture or successively to the same sample-primer mixture; (d) detecting the release of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein to indicate which nucleotide is incorporated.

In one aspect, the invention provides a method of identifying a base at a target position in a single-stranded sample DNA sequence, the method comprising: (a) providing an extension primer which hybridizes to sample DNA immediately adjacent to the target position, said sample DNA disposed within a plurality of cavities on a planar surface, each

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3077

cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 um, said DNA being rendered single stranded either before or after being disposed in the reaction chambers; (b) subjecting the sample DNA and extension primer to a polymerase reaction in the presence of a predetermined deoxynucleotide or dideoxynucleotide whereby the deoxynucleotide or dideoxynucleotide will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position, said predetermined deoxynucleotides or dideoxynucleotides being added either to separate aliquots of sample-primer mixture or successively to the same sample-primer mixture, (c) detecting any release of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein to indicate which deoxynucleotide or dideoxynucleotide is incorporated;characterized in that, the PPi-detection enzyme(s) are included in the polymerase reaction step and in that in place of deoxy- or dideoxy adenosine triphosphate (ATP) a dATP or ddATP analogue is used which is capable of acting as a substrate for a polymerase but incapable of acting as a substrate for a said PPi—detection enzyme.

In another aspect, the invention includes a method of determining the base sequence of a plurality of nucleotides on an array, the method comprising: (a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, (b) converting PPi into light with an ATP generating polypeptide-ATP converting polypeptide fusion protein; (c) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device; (d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions; (e) determining a light intensity for each of said discrete regions from the corresponding electrical signal; (f) recording the variations of said electrical signals with time.

In one aspect, the invention provides a method for sequencing a nucleic acid, the method comprising:(a) providing one or more nucleic acid anchor primers; (b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm;(c) converting PPi into a detectable entity with the use of an ATP generating polypeptide-ATP converting

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3078

polypeptide fusion protein; (d) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device; (e) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions; (f) determining a light intensity for each of said discrete regions from the corresponding electrical signal; (g) recording the variations of said electrical signals with time.

In another aspect, the invention includes a method for sequencing a nucleic acid, the method comprising: (a) providing at least one nucleic acid anchor primer; (b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites; (c) converting PPi into a detectable entity with an ATP generating polypeptide-ATP converting polypeptide fusion protein; (d) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device; (e) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions; (f) determining a light intensity for each of said discrete regions from the corresponding electrical signal; (g) recording the variations of said electrical signals with time.

In another aspect, the invention includes an isolated polypeptide comprising an amino acid sequence selected from the group consisting of: (a) a mature form of an amino acid sequence of SEQ ID NO: 2; (b) a variant of a mature form of an amino acid sequence of SEQ ID NO: 2; an amino acid sequence of SEQ ID NO: 2; (c) a variant of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 5% of amino acid residues from said amino acid sequence; (d) and at least one conservative amino acid substitution to the amino acid sequences in (a), (b), (c) or (d). The invention also includes an antibody that binds immunospecifically to the polypeptide of (a), (b), (c) or (d).

In another aspect, the invention includes an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: (a) a mature form of an amino acid sequence of SEQ ID NO: 2; (b) a variant of a mature form of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 5% of the amino acid residues from

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3079

the amino acid sequence of said mature form; (c) an amino acid sequence of SEQ ID NO: 2; (d) a variant of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 5% of amino acid residues from said amino acid sequence; (e) and a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e). In a further aspect, the invention provides a nucleic acid molecule wherein the nucleic acid molecule comprises nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than **20%** of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence; an isolated second polynucleotide that is a complement of the first polynucleotide; (b) and a nucleic acid fragment of (a) or (b). The invention also includes a vector comprising the nucleic acid molecule of (a) or (b). In another aspect, the invention includes a cell comprising the vector.

In a further aspect, the invention includes a method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising: (a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added; (b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released; (c) separately recovering each of the feedstocks from the polymerization environment; and (d) measuring the amount of PPi with an ATP sulfurylase and a luciferase in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3080

In another aspect, the invention provides a method for sequencing a nucleic acid, the method comprising: (a) providing one or more nucleic acid anchor primers; (b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities in an array on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm and at least 400,000 discrete sites; (c) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (e) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of an ATP sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid.

In another aspect, the invention provides a method for sequencing a nucleic acid, the method comprising: (a) providing at least one nucleic acid anchor primer; (b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites; (c) annealing a first amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (e) annealing a second amount of a sequencing primer to one or more copies of the covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, when the predetermined nucleotide triphosphate is incorporated onto the 3' end of the sequencing primer, to yield a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of a thermostable sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid at each reaction site that contains a nucleic acid template.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3081

In a further aspect, the invention includes a method of determining the base sequence of a plurality of nucleotides on an array, the method comprising: (a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, (b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates; (c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands through detection of a sequencing byproduct with a thermostable sulfurylase and luciferase, thus indicating that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and (e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is one embodiment for a cloning strategy for obtaining the luciferase-sulfurylase sequence.

FIG. 2A and 2B show the preparative agarose gel of luciferase and sulfurylase as well as sulfurylase-luciferase fusion genes.

FIG. 3 shows the results of experiments to determine the activity of the luciferase-sulfurylase fusion protein on NTA-agarose and MPG-SA solid supports.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3082

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a fusion protein containing an ATP generating polypeptide bound to a polypeptide which converts ATP into an entity which is detectable. As used herein, the term "fusion protein" refers to a chimeric protein containing an exogenous protein fragment joined to another exogenous protein fragment. The fusion protein could include an affinity tag to allow attachment of the protein to a solid support or to allow for purification of the recombinant fusion protein from the host cell or culture supernatant, or both.

In a preferred embodiment, the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote. The eukaryote could be an animal, plant, fungus or yeast. In some embodiments, the animal is a mammal, rodent, insect, worm, mollusk, reptile, bird and amphibian. Plant sources of the polypeptides include but are not limited to *Arabidopsis thaliana*, *Brassica napus*, *Allium sativum*, *Amaranthus caudatus*, *Hevea brasiliensis*, *Hordeum vulgare*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *Oryza sativum*, *Pisum sativum*, *Populus trichocarpa*, *Solanum tuberosum*, *Secale cereale*, *Sambucus nigra*, *Ulmus americana* or *Triticum aestivum*. Examples of fungi include but are not limited to *Penicillum chrysogenum*, *Stachybotrys chartarum*, *Aspergillus fumigatus*, *Podospora anserina* and *Trichoderma reesei*. Examples of sources of yeast include but are not limited to *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida lypolitica*, *Candida utilis*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Candida spp.*, *Pichia spp.* and *Hansenula spp.*.

The prokaryote source could be bacteria or archaea. In some embodiments, the bacteria is *E. coli*, *B. subtilis*, *Streptococcus gordonii*, flavobacteria or green sulfur bacteria. In other embodiments, the archaea is *Sulfolobus*, *Thermococcus*, *Methanobacterium*, *Halococcus*, *Halobacterium* or *Methanococcus jannaschii*.

The ATP generating polypeptide can be a ATP sulfurylase, hydrolase or an ATP synthase. In a preferred embodiment, the ATP generating polypeptide is ATP sulfurylase. In one embodiment, the ATP sulfurylase is a thermostable sulfurylase cloned from *Bacillus stearothermophilus* (Bst) and comprising the nucleotide sequence of SEQ ID NO:1. This putative gene was cloned using genomic DNA acquired from ATCC (Cat. No. 12980D). The gene is shown to code for a functional ATP sulfurylase that can be expressed as a fusion

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3083

protein with an affinity tag. The disclosed Bst sulfurylase nucleic acid (SEQ ID NO:1) includes the 1247 nucleotide sequence. An open reading frame (ORF) for the mature protein was identified beginning with an ATG codon at nucleotides 1-3 and ending with a TAA codon at nucleotides 1159-1161. The start and stop codons of the open reading frame are highlighted in bold type. The putative untranslated regions are underlined and found upstream of the initiation codon and downstream from the termination codon.

Bst Thermostable Sulfurylase Nucleotide Sequence (SEQ ID NO:1)

GTTATGAAC**ATG**AGTTTGAGCATTCCGCATGGCGGCACATTGATCAACCGTTGGAATCCG 60
GATTACCCAATCGATGAAGCAACGAAAACGATCGAGCTGTCCAAAGCCGAACTAAGCGAC 120
CTTGAGCTGATCGGCACAGGCGCCTACAGCCCGCTCACCGGGTTTTTAACGAAAGCCGAT 180
TACGATGCGGTCGTAGAAACGATGCGCCTCGCTGATGGCACTGTCTGGAGCATTCCGATC 240
ACGCTGGCGGTGACGGAAGAAAAAGCGAGTGAACTCACTGTCGGCGACAAAGCGAAACTC 300
GTTTATGGCGGCGACGTCTACGGCGTCATTGAAATCGCCGATATTTACCGCCCGGATAAA 360
ACGAAAGAAGCCAAGCTCGTCTATAAAACCGATGAACTCGCTCACCCGGGCGTGCGCAAG 420
CTGTTTGAAAAACCAGATGTGTACGTCGGCGGAGCGGTTACGCTCGTCAAACGGACCGAC 480
AAAGGCCAGTTTGCTCCGTTTTATTTCGATCCGGCCGAAACGCGGAAACGATTTGCCGAA 540
CTCGGCTGGAATACCGTCGTCGGCTTCCAAACACGCAACCCGGTTCACCGCGCCCATGAA 600
TACATTCAAAAATGCGCGCTTGAAATCGTGGACGGCTTGTTTTTAAACCCGCTCGTCGGC 660
GAAACGAAAGCGGACGATATTCCGGCCGACATCCGGATGGAAAGCTATCAAGTGCTGCTG 720
GAAAACTATTATCCGAAAGACCGCGTTTTCTTGGGCGTCTTCCAAGCTGCGATGCGCTAT 780
GCCGGTCCGCGCGAAGCGATTTTCCATGCCATGGTGCGGAAAAACTTCGGCTGCACGCAC 840
TTCATCGTCGGCCGCGACCATGCGGGCGTCGGCAACTATTACGGCACGTATGATGCGCAA 900
AAAATCTTCTCGAACTTTACAGCCGAAGAGCTTGGCATTACACCGCTCTTTTTCGAACAC 960
AGCTTTTATTGCACGAAATGCGAAGGCATGGCATCGACGAAAACATGCCCGCACGACGCA 1020
CAATATCACGTTGTCCTTTCTGGCACGAAAGTCCGTGAAATGTTGCGTAACGGCCAAGTG 1080
CCGCCGAGCACATTCAGCCGTCCGGAAGTGGCCGCCGTTTTGATCAAAGGGCTGCAAGAA 1140
CGCGAAACGGTCACCCCGTCGACACGC**TAA**AGGAGGAGCGAGATGAGCACGAATATCGTT 1200
TGGCATCATACATCGGTGACAAAAGAAGATCGCCGCCAACGCAACGG 1247

The Bst sulfurylase polypeptide (SEQ ID NO:2) is 386 amino acid residues in length and is presented using the three letter amino acid code.

Bst Sulfurylase Amino Acid Sequence (SEQ ID NO:2)

Met Ser Leu Ser Ile Pro His Gly Gly Thr Leu Ile Asn Arg Trp Asn
1 5 10 15

Pro Asp Tyr Pro Ile Asp Glu Ala Thr Lys Thr Ile Glu Leu Ser Lys
20 25 30

Ala Glu Leu Ser Asp Leu Glu Leu Ile Gly Thr Gly Ala Tyr Ser Pro
35 40 45

Leu Thr Gly Phe Leu Thr Lys Ala Asp Tyr Asp Ala Val Val Glu Thr

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3084

50 55 60

Met Arg Leu Ala Asp Gly Thr Val Trp Ser Ile Pro Ile Thr Leu Ala
65 70 75

Val Thr Glu Glu Lys Ala Ser Glu Leu Thr Val Gly Asp Lys Ala Lys
80 85 90 95

Leu Val Tyr Gly Gly Asp Val Tyr Gly Val Ile Glu Ile Ala Asp Ile
100 105 110

Tyr Arg Pro Asp Lys Thr Lys Glu Ala Lys Leu Val Tyr Lys Thr Asp
115 120 125

Glu Leu Ala His Pro Gly Val Arg Lys Leu Phe Glu Lys Pro Asp Val
130 135 140

Tyr Val Gly Gly Ala Val Thr Leu Val Lys Arg Thr Asp Lys Gly Gln
145 150 155

Phe Ala Pro Phe Tyr Phe Asp Pro Ala Glu Thr Arg Lys Arg Phe Ala
160 165 170 175

Glu Leu Gly Trp Asn Thr Val Val Gly Phe Gln Thr Arg Asn Pro Val
180 185 190

His Arg Ala His Glu Tyr Ile Gln Lys Cys Ala Leu Glu Ile Val Asp
195 200 205

Gly Leu Phe Leu Asn Pro Leu Val Gly Glu Thr Lys Ala Asp Asp Ile
210 215 220

Pro Ala Asp Ile Arg Met Glu Ser Tyr Gln Val Leu Leu Glu Asn Tyr
225 230 235

Tyr Pro Lys Asp Arg Val Phe Leu Gly Val Phe Gln Ala Ala Met Arg
240 245 250 255

Tyr Ala Gly Pro Arg Glu Ala Ile Phe His Ala Met Val Arg Lys Asn
260 265 270

Phe Gly Cys Thr His Phe Ile Val Gly Arg Asp His Ala Gly Val Gly
275 280 285

Asn Tyr Tyr Gly Thr Tyr Asp Ala Gln Lys Ile Phe Ser Asn Phe Thr
290 295 300

Ala Glu Glu Leu Gly Ile Thr Pro Leu Phe Phe Glu His Ser Phe Tyr
305 310 315

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3085

Cys Thr Lys Cys Glu Gly Met Ala Ser Thr Lys Thr Cys Pro His Asp
320 325 330 335

Ala Gln Tyr His Val Val Leu Ser Gly Thr Lys Val Arg Glu Met Leu
340 345 350

Arg Asn Gly Gln Val Pro Pro Ser Thr Phe Ser Arg Pro Glu Val Ala
355 360 365

Ala Val Leu Ile Lys Gly Leu Gln Glu Arg Glu Thr Val Thr Pro Ser
370 375 380

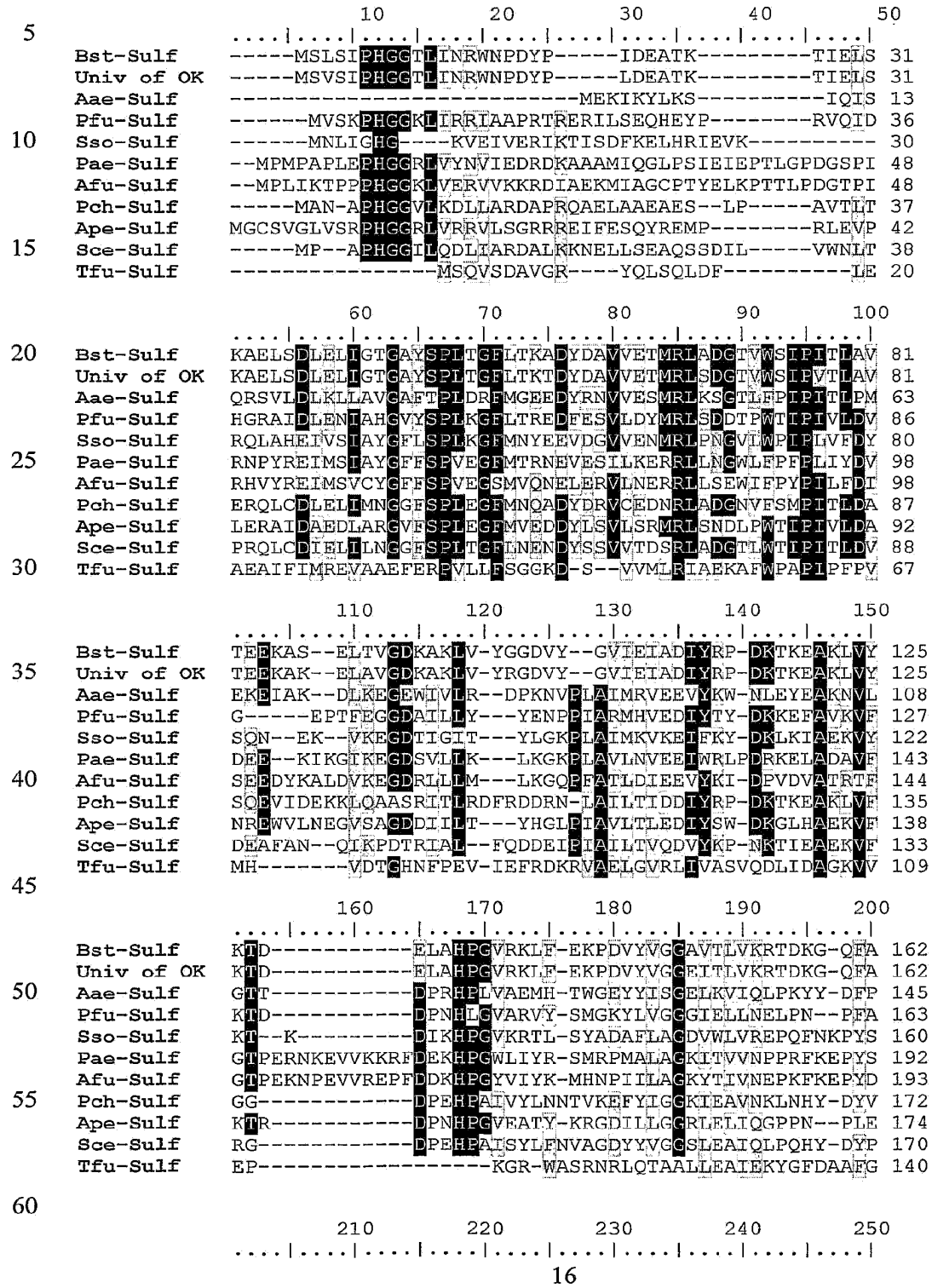
Thr Arg
385

In one embodiment, the thermostable sulfurylase is active at temperatures above ambient to at least 50°C. This property is beneficial so that the sulfurylase will not be denatured at higher temperatures commonly utilized in polymerase chain reaction (PCR) reactions or sequencing reactions. In one embodiment, the ATP sulfurylase is from a thermophile. The thermostable sulfurylase can come from thermophilic bacteria, including but not limited to, *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

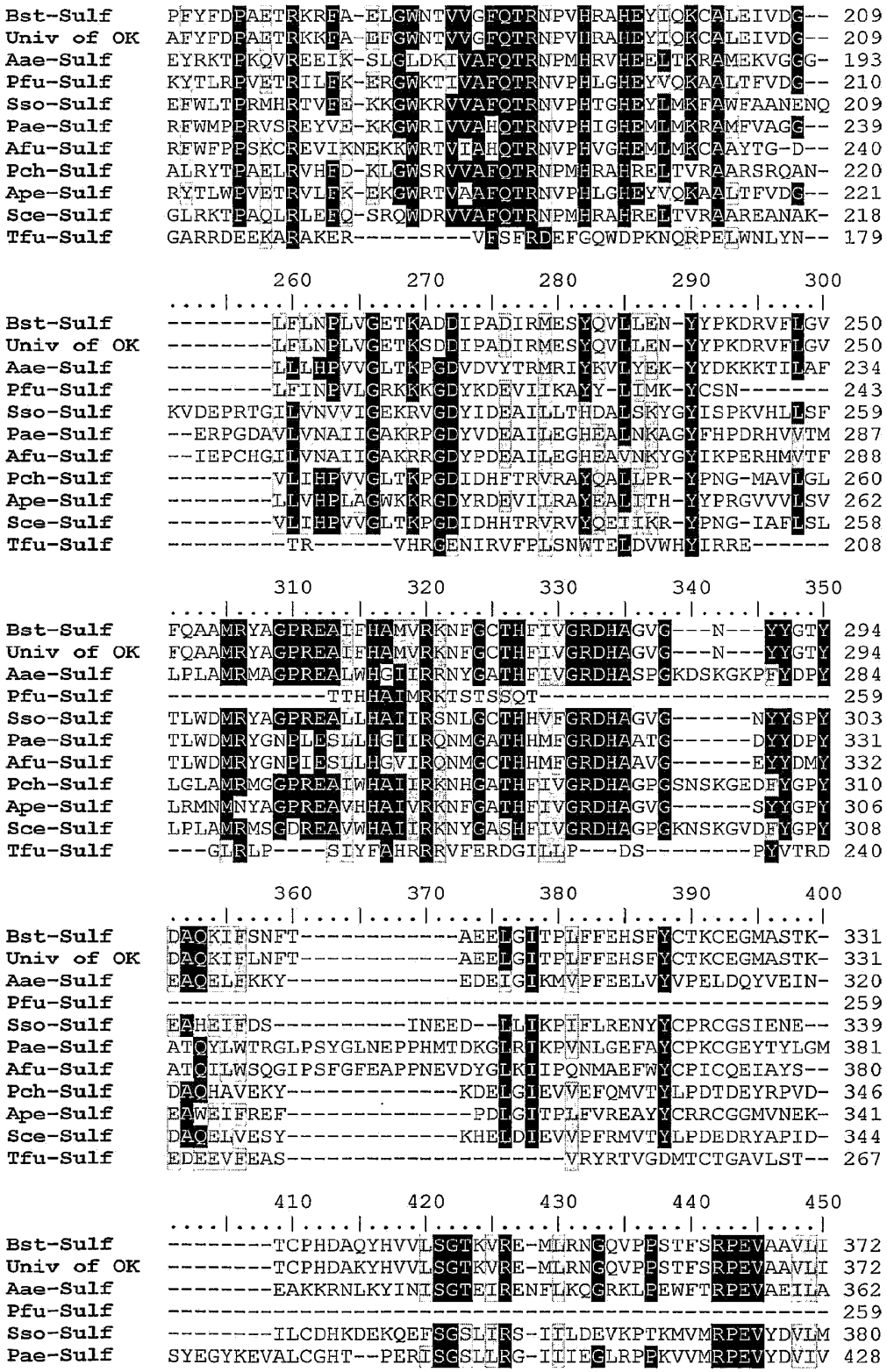
The homology of twelve ATP sulfurylases can be shown graphically in the ClustalW analysis in Table1. The alignment is of ATP sulfurylases from the following species: *Bacillus stearothermophilus* (Bst), University of Oklahoma – Strain 10 (Univ of OK), *Aquifex aeolicus* (Aae), *Pyrococcus furiosus* (Pfu), *Sulfolobus solfataricus* (Sso), *Pyrobaculum aerophilum* (Pae), *Archaeoglobus fulgidus* (Afu), *Penicillium chrysogenum* (Pch), *Aeropyrum pernix* (Ape), *Saccharomyces cerevisiae* (Sce), and *Thermomonospora fusca* (Tfu).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3086

Table 1: ClustalW Analysis of ATP Sulfurylase Amino Acid Sequence



Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3087



Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3088

```
Afu-Sulf    --------ENCGHTDAKQKFSGSFLRG-MVAEGVFPPRVVMRPEVYKQIV 421
Pch-Sulf    --------QVPAG-VKTLNISGTELRR-RLRSGAHIPEWFSYPEVVKILR 386
Ape-Sulf    --------VCPHGDEYRVRISGTRLRE-MLGRGERPPEYMMRPEVADAII 382
Sce-Sulf    --------QIDTTKTRTLNISGTELRR-RLRVGGEIPEWFSYPEVVKILR 385
Tfu-Sulf    --------AT-TLDEVIAEIAATRITE---RGQTRADDRGSEAAMEERKR 305

                      460       470       480       490       500
                    ....|....|....|....|....|....|....|....|....|
Bst-Sulf    KGLQER-----------------ETVT----------------PSTR--- 386
Univ of OK  KGLQER-----------------ETVA----------------PSAR--- 386
Aae-Sulf    ETYVPKHKQGFCVWLTGLPCAGKSTIA-EILATMLQARGRKVTLLDGDVV 411
Pfu-Sulf    -------------------------------------------------- 259
Sso-Sulf    KAAEQYGFGS--------PFVTEEYLE--------------KRQSILG-- 406
Pae-Sulf    KWWRVYGY----------PYVTDKYLR--------------IKEQELEVE 454
Afu-Sulf    KWWKVYNY----------PFVNRKYLE--------------LKNKELEID 447
Pch-Sulf    ESNPPRATQGFTIFLTGYMNSGKDAIARALQVTLNQQGGRSVSLLLGDTV 436
Ape-Sulf    SHPDPFI----------------------------------------- 389
Sce-Sulf    ESNPPRPKQGFSIVLGNSLTVSREQLSIALLSTFLQFGGGRYYKIFEHNN 435
Tfu-Sulf    EGYF-------------------------------------------- 309

                      510       520       530       540       550
                    ....|....|....|....|....|....|....|....|....|
Bst-Sulf    -------------------------------------------------- 386
Univ of OK  -------------------------------------------------- 386
Aae-Sulf    RTHLSRGLGFSKEDRITNILRVGFVASEIVKHNGVVICALVSPYRSARN- 460
Pfu-Sulf    -------------------------------------------------- 259
Sso-Sulf    -------------------------------------------------- 406
Pae-Sulf    L------------------------------------------------- 455
Afu-Sulf    LPAMEVPKA--------------------------------------- 456
Pch-Sulf    RHELSSELGFTREDRHTNIQRIAFVATELTRAGAAVIAAPIAPYEESRKF 486
Ape-Sulf    -------------------------------------------------- 389
Sce-Sulf    KTELLSLI----------------QDFIGSGSGLIIP--NQWEDD--- 462
Tfu-Sulf    -------------------------------------------------- 309

                      560       570       580       590       600
                    ....|....|....|....|....|....|....|....|....|
Bst-Sulf    -------------------------------------------------- 386
Univ of OK  -------------------------------------------------- 386
Aae-Sulf    QVRNMMEEGKFIEVFVDAPVEVCEERDVKGLYKKAKEGLIKGFTGVDDPY 510
Pfu-Sulf    -------------------------------------------------- 259
Sso-Sulf    -------------------------------------------------- 406
Pae-Sulf    -------------------------------------------------- 455
Afu-Sulf    -------------------------------------------------- 456
Pch-Sulf    ARDAVSQAGSFFLVHVATPLEHCEQSDKRGIYAAARRGEIKGFTGVDDPY 536
Ape-Sulf    -------------------------------------------------- 389
Sce-Sulf    -KDSVVGKQNVYLLDTSS------------------------------- 479
Tfu-Sulf    -------------------------------------------------- 309

                      610       620       630
                    ....|....|....|....|....|....|..
Bst-Sulf    ------------------------------------ 386 (SEQ ID NO:2)
Univ of OK  ------------------------------------ 386 (SEQ ID NO:22)
Aae-Sulf    EPPVAPEVRVDTTKLTPEESALKILEFLKKEGFIKD- 546 (SEQ ID NO:23)
Pfu-Sulf    ------------------------------------ 259 (SEQ ID NO:24)
Sso-Sulf    ------------------------------------ 406 (SEQ ID NO:25)
Pae-Sulf    ------------------------------------ 455 (SEQ ID NO:26)
Afu-Sulf    ------------------------------------ 456 (SEQ ID NO:27)
Pch-Sulf    ETPEKADLVVDFSKQSVRSIVHEIILVLESQGFLERQ 573 (SEQ ID NO:28)
Ape-Sulf    ------------------------------------ 389 (SEQ ID NO:29)
Sce-Sulf    ----SADIQLESADEPISHIVQKVVLFLEDNGFFVF- 511 (SEQ ID NO:30)
Tfu-Sulf    ------------------------------------ 309 (SEQ ID NO:31)
```


Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3089

A thermostable sulfurylase polypeptide is encoded by the open reading frame ("ORF") of a thermostable sulfurylase nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO:1 due to degeneracy of the genetic code and thus encode the same thermostable sulfurylase proteins as that encoded by the nucleotide sequences shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2. In addition to the thermostable sulfurylase nucleotide sequence shown in SEQ ID NO:1 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the thermostable sulfurylase polypeptides may exist within a population (*e.g.*, the bacterial population). Such genetic polymorphism in the thermostable sulfurylase genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a thermostable sulfurylase protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the thermostable sulfurylase genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the thermostable sulfurylase polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the thermostable sulfurylase polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding thermostable sulfurylase proteins from other species, and thus that have a nucleotide sequence that differs from the sequence SEQ ID

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3090

NO:1 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the thermostable sulfurylase cDNAs of the invention can be isolated based on their homology to the thermostable sulfurylase nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. The invention further includes the nucleic acid sequence of SEQ ID NO:1 and mature and variant forms thereof, wherein a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 11% of the nucleotides in the coding sequence differ from the coding sequence.

Another aspect of the invention pertains to nucleic acid molecules encoding a thermostable sulfurylase protein that contains changes in amino acid residues that are not essential for activity. Such thermostable sulfurylase proteins differ in amino acid sequence from SEQ ID NO:2 yet retain biological activity. In separate embodiments, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 96%, 97%, 98% or 99% homologous to the amino acid sequence of SEQ ID NO:2. An isolated nucleic acid molecule encoding a thermostable sulfurylase protein homologous to the protein of SEQ ID NO: 2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NO:2 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3091

histidine). Thus, a predicted non-essential amino acid residue in the thermostable sulfurylase protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a thermostable sulfurylase coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for thermostable sulfurylase biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

The thermostable sulfurylase nucleic acid of the invention includes the nucleic acid whose sequence is provided herein, or fragments thereof. The invention also includes mutant or variant nucleic acids any of whose bases may be changed from the corresponding base shown herein while still encoding a protein that maintains its sulfurylase-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

A thermostable sulfurylase nucleic acid can encode a mature thermostable sulfurylase polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3092

present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "isolated" nucleic acid molecule, as utilized herein, is one, which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated thermostable sulfurylase nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3093

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 as a hybridization probe, thermostable sulfurylase molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to thermostable sulfurylase nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3094

identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 89% identity over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of thermostable sulfurylase polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for a thermostable sulfurylase polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions in SEQ ID NO:1, as well as a polypeptide possessing thermostable sulfurylase biological activity. Various biological activities of the thermostable sulfurylase proteins are described below.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3095

The thermostable sulfurylase proteins of the invention include the sulfurylase protein whose sequence is provided herein. The invention also includes mutant or variant proteins any of whose residues may be changed from the corresponding residue shown herein while still encoding a protein that maintains its sulfurylase-like activities and physiological functions, or a functional fragment thereof. The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention. This invention also includes a variant or a mature form of the amino acid sequence of SEQ ID NO:2, wherein one or more amino acid residues in the variant differs in no more than 4% of the amino acic residues from the amino acid sequence of the mature form.

Several assays have been developed for detection of the forward ATP sulfurylase reaction. The colorimetric molybdolysis assay is based on phosphate detection (see *e.g.*, Wilson and Bandurski, 1958. *J. Biol. Chem.* **233**: 975-981), whereas the continuous spectrophotometric molybdolysis assay is based upon the detection of NADH oxidation (see *e.g.*, Seubert, *et al.*, 1983. *Arch. Biochem. Biophys.* **225**: 679-691; Seubert, *et al.*, 1985. *Arch. Biochem. Biophys.* **240**: 509-523). The later assay requires the presence of several detection enzymes.

Suitable enzymes for converting ATP into light include luciferases, *e.g.*, insect luciferases. Luciferases produce light as an end-product of catalysis. The best known light-emitting enzyme is that of the firefly, *Photinus pyralis* (*Coleoptera*). The corresponding gene has been cloned and expressed in bacteria (see *e.g.*, de Wet, *et al.*, 1985. *Proc. Natl. Acad. Sci. USA* **80**: 7870-7873) and plants (see *e.g.*, Ow, *et al.*, 1986. *Science* **234**: 856-859), as well as in insect (see *e.g.*, Jha, *et al.*, 1990. *FEBS Lett.* **274**: 24-26) and mammalian cells (see *e.g.*, de Wet, *et al.*, 1987. *Mol. Cell. Biol.* 7: 725-7373; Keller, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* **82**: 3264-3268). In addition, a number of luciferase genes from the Jamaican click beetle, *Pyroplorus plagiophihalamus* (*Coleoptera*), have recently been cloned and partially characterized (see *e.g.*, Wood, *et al.*, 1989. *J. Biolumin. Chemilumin.* **4**: 289-301; Wood, *et al.*, 1989. *Science* **244**: 700-702). Distinct luciferases can sometimes produce light of different wavelengths, which may enable simultaneous monitoring of light emissions at different wavelengths. Accordingly, these aforementioned characteristics are unique, and add new dimensions with respect to the utilization of current reporter systems.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3096

Firefly luciferase catalyzes bioluminescence in the presence of luciferin, adenosine 5'-triphosphate (ATP), magnesium ions, and oxygen, resulting in a quantum yield of 0.88 (see *e.g.,* McElroy and Selinger, 1960. *Arch. Biochem. Biophys.* **88**: 136-145). The firefly luciferase bioluminescent reaction can be utilized as an assay for the detection of ATP with a detection limit of approximately $1x10^{-13}$ M (see *e.g.,* Leach, 1981. *J. Appl. Biochem.* **3**: 473-517). In addition, the overall degree of sensitivity and convenience of the luciferase-mediated detection systems have created considerable interest in the development of firefly luciferase-based biosensors (see *e.g.,* Green and Kricka, 1984. *Talanta* **31**: 173-176; Blum, *et al.,* 1989. *J. Biolumin. Chemilumin.* **4**: 543-550).

The development of new reagents have made it possible to obtain stable light emission proportional to the concentrations of ATP (see *e.g.,* Lundin, 1982. Applications of firefly luciferase In; *Luminescent Assays* (Raven Press, New York). With such stable light emission reagents, it is possible to make endpoint assays and to calibrate each individual assay by addition of a known amount of ATP. In addition, a stable light-emitting system also allows continuous monitoring of ATP-converting systems.

In a preferred embodiment, the ATP generating-ATP converting fusion protein is attached to an affinity tag. The term "affinity tag" is used herein to denote a peptide segment that can be attached to a polypeptide to provide for purification or detection of the polypeptide or provide sites for attachment of the polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract or a biotin carboxyl carrier protein (BCCP) domain, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), substance P, Flag.™. peptide (Hopp et al., Biotechnology 6:1204-1210, 1988; available from Eastman Kodak Co., New Haven, Conn.), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, N.J.).

As used herein, the term "poly-histidine tag," when used in reference to a fusion protein refers to the presence of two to ten histidine residues at either the amino- or carboxy-terminus of a protein of interest. A poly-histidine tract of six to ten residues is preferred. The poly-histidine tract is also defined functionally as being a number of consecutive histidine

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3097

residues added to the protein of interest which allows the affinity purification of the resulting fusion protein on a nickel-chelate or IDA column.

In some embodiments, the fusion protein has an orientation such that the sulfurylase polypeptide is N-terminal to the luciferase polypeptide. In other embodiments, the luciferase polypeptide is N-terminal to the sulfurylase polypeptide. As used herein, the term sulfurylase-luciferase fusion protein refers to either of these orientations. The terms "amino-terminal" (N-terminal) and "carboxyl-terminal" (C-terminal) are used herein to denote positions within polypeptides and proteins. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide or protein to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a protein is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete protein.

The fusion protein of this invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or "sticky"-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). The two polypeptides of the fusion protein can also be joined by a linker, such as a unique restriction site, which is engineered with specific primers during the cloning procedure. In one embodiment, the sulfurylase and luciferase polypeptides are joined by a linker, for example an ala-ala-ala linker which is encoded by a Not1 restriction site.

In one embodiment, the invention includes a recombinant polynucleotide that comprises a coding sequence for a fusion protein having an ATP generating polypeptide sequence and an ATP converting polypeptide sequence. In a preferred embodiment, the recombinant polynucleotide encodes a sulfurylase-luciferase fusion protein. The term "recombinant DNA molecule" or "recombinant polynucleotide" as used herein refers to a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3098

DNA molecule which is comprised of segments of DNA joined together by means of molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed from a recombinant DNA molecule.

In one aspect, this invention discloses a sulfurylase-luciferase fusion protein with an N-terminal hexahistidine tag and a BCCP tag. The nucleic acid sequence of the disclosed N-terminal hexahistidine-BCCP luciferase-sulfurylase gene (His6-BCCP L-S) gene is shown below:

His6-BCCP L-S Nucleotide Sequence (SEQ ID NO:3):

```
ATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGGAAGCGCCAGCAGCA   60
GCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGTTGGTACTTTCTACCGCACCCCA  120
AGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACCCTG  180
TGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTACCGTG  240
AAAGCAATTCTGGTCGAAAGTGGACAACCGGTAGAATTTGACGAGCCGCTGGTCGTCATC  300
GAGGGATCCGAGCTCGAGATCCAAATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCG  360
CCATTCTATCCTCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGA  420
TACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACG  480
TACGCGGAATACTTCGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTG  540
AATACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCAATTCTTTATGCCGGTG  600
TTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATGAACGT  660
GAATTGCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTGTTTCCAAAAAGGGG  720
TTGCAAAAAATTTTGAACGTGCAAAAAAAATTACCAATAATCCAGAAAATTATTATCATG  780
GATTCTAAAACGGATTACCAGGGATTTCAGTCGATGTACACGTTCGTCACATCTCATCTA  840
CCTCCCGGTTTTAATGAATACGATTTTGTACCAGAGTCCTTTGATCGTGACAAAACAATT  900
GCACTGATAATGAATTCCTCTGGATCTACTGGGTTACCTAAGGGTGTGGCCCTTCCGCAT  960
AGAACTGCCTGCGTCAGATTCTCGCATGCCAGAGATCCTATTTTTGGCAATCAAATCATT 1020
CCGGATACTGCGATTTTAAGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACA 1080
CTCGGATATTTGATATGTGGATTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTG 1140
TTTTTACGATCCCTTCAGGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCTATTT 1200
TCATTCTTCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAAATT 1260
GCTTCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGGTTGCAAAACGCTTCCAT 1320
CTTCCAGGGATACGACAAGGATATGGGCTCACTGAGACTACATCAGCTATTCTGATTACA 1380
CCCGAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTCCATTTTTTGAAGCGAAG 1440
GTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAATCAGAGAGGCGAATTATGTGTC 1500
AGAGGACCTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATT 1560
GACAAGGATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACACTTC 1620
TTCATAGTTGACCGCTTGAAGTCTTTAATTAAATACAAAGGATATCAGGTGGCCCCCGCT 1680
GAATTGGAATCGATATTGTTACAACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTT 1740
CCCGACGATGACGCCGGTGAACTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACG 1800
ATGACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGAAAAAGTTG 1860
CGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAACTCGACGCA 1920
AGAAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAAAGTCCAAATTGGCGGCC 1980
GCTATGCCTGCTCCTCACGGTGGTATTCTACAAGACTTGATTGCTAGAGATGCGTTAAAG 2040
AAGAATGAATTGTTATCTGAAGCGCAATCTTCGGACATTTTAGTATGGAACTTGACTCCT 2100
AGACAACTATGTGATATTGAATTGATTCTAAATGGTGGGTTTTCTCCTCTGACTGGGTTT 2160
TTGAACGAAAACGATTACTCCTCTGTTGTTACAGATTCGAGATTAGCAGACGGCACATTG 2220
TGGACCATCCCTATTACATTAGATGTTGATGAAGCATTTGCTAACCAAATTAAACCAGAC 2280
ACAAGAATTGCCCTTTTCCAAGATGATGAAATTCCTATTGCTATACTTACTGTCCAGGAT 2340
```


Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3099

GTTTACAAGCCAAACAAAACTATCGAAGCCGAAAAAGTCTTCAGAGGTGACCCAGAACAT 2400
CCAGCCATTAGCTATTTATTTAACGTTGCCGGTGATTATTACGTCGGCGGTTCTTTAGAA 2460
GCGATTCAATTACCTCAACATTATGACTATCCAGGTTTGCGTAAGACACCTGCCCAACTA 2520
AGACTTGAATTCCAATCAAGACAATGGGACCGTGTCGTAGCTTTCCAAACTCGTAATCCA 2580
ATGCATAGAGCCCACAGGGAGTTGACTGTGAGAGCCGCCAGAGAAGCTAATGCTAAGGTG 2640
CTGATCCATCCAGTTGTTGGACTAACCAAACCAGGTGATATAGACCATCACACTCGTGTT 2700
CGTGTCTACCAGGAAATTATTAAGCGTTATCCTAATGGTATTGCTTTCTTATCCCTGTTG 2760
CCATTAGCAATGAGAATGAGTGGTGATAGAGAAGCCGTATGGCATGCTATTATTAGAAAG 2820
AATTATGGTGCCTCCCACTTCATTGTTGGTAGAGACCATGCGGGCCCAGGTAAGAACTCC 2880
AAGGGTGTTGATTTCTACGGTCCATACGATGCTCAAGAATTGGTCGAATCCTACAAGCAT 2940
GAACTGGACATTGAAGTTGTTCCATTCAGAATGGTCACTTATTTGCCAGACGAAGACCGT 3000
TATGCTCCAATTGATCAAATTGACACCACAAAGACGAGAACCTTGAACATTTCAGGTACA 3060
GAGTTGAGACGCCGTTTAAGAGTTGGTGGTGAGATTCCTGAATGGTTCTCATATCCTGAA 3120
GTGGTTAAAATCCTAAGAGAATCCAACCCACCAAGACCAAAACAAGGTTTTTCAATTGTT 3180
TTAGGTAATTCATTAACCGTTTCTCGTGAGCAATTATCCATTGCTTTGTTGTCAACATTC 3240
TTGCAATTCGGTGGTGGCAGGTATTACAAGATCTTTGAACACAATAATAAGACAGAGTTA 3300
CTATCTTTGATTCAAGATTTCATTGGTTCTGGTAGTGGACTAATTATTCCAAATCAATGG 3360
GAAGATGACAAGGACTCTGTTGTTGGCAAGCAAAACGTTTACTTATTAGATACCTCAAGC 3420
TCAGCCGATATTCAGCTAGAGTCAGCGGATGAACCTATTTCACATATTGTACAAAAAGTT 3480
GTCCTATTCTTGGAAGACAATGGCTTTTTTGTATTTTAA 3519

The amino acid sequence of the disclosed His6-BCCP L-S polypeptide is presented using the three letter amino acid code (SEQ ID NO:4).

His6-BCCP L-S Amino Acid Sequence (SEQ ID NO:4)

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Glu
1 5 10 15

Ala Pro Ala Ala Ala Glu Ile Ser Gly His Ile Val Arg Ser Pro Met
20 25 30

Val Gly Thr Phe Tyr Arg Thr Pro Ser Pro Asp Ala Lys Ala Phe Ile
35 40 45

Glu Val Gly Gln Lys Val Asn Val Gly Asp Thr Leu Cys Ile Val Glu
50 55 60

Ala Met Lys Met Met Asn Gln Ile Glu Ala Asp Lys Ser Gly Thr Val
65 70 75 80

Lys Ala Ile Leu Val Glu Ser Gly Gln Pro Val Glu Phe Asp Glu Pro
85 90 95

Leu Val Val Ile Glu Gly Ser Glu Leu Glu Ile Gln Met Glu Asp Ala
100 105 110

Lys Asn Ile Lys Lys Gly Pro Ala Pro Phe Tyr Pro Leu Glu Asp Gly

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3100

115 120 125

Thr Ala Gly Glu Gln Leu His Lys Ala Met Lys Arg Tyr Ala Leu Val
130 135 140

Pro Gly Thr Ile Ala Phe Thr Asp Ala His Ile Glu Val Asn Ile Thr
145 150 155 160

Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala Glu Ala Met Lys
165 170 175

Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val Cys Ser Glu Asn
180 185 190

Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu Phe Ile Gly Val
195 200 205

Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg Glu Leu Leu Asn
210 215 220

Ser Met Asn Ile Ser Gln Pro Thr Val Val Phe Val Ser Lys Lys Gly
225 230 235 240

Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro Ile Ile Gln Lys
245 250 255

Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly Phe Gln Ser Met
260 265 270

Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe Asn Glu Tyr Asp
275 280 285

Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile Ala Leu Ile Met
290 295 300

Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val Ala Leu Pro His
305 310 315 320

Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp Pro Ile Phe Gly
325 330 335

Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val Val Pro Phe His
340 345 350

His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu Ile Cys Gly Phe
355 360 365

Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu Phe Leu Arg Ser

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3101

370 375 380

Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val Pro Thr Leu Phe
385 390 395 400

Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr Asp Leu Ser Asn
405 410 415

Leu His Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser Lys Glu Val Gly
420 425 430

Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile Arg Gln Gly Tyr
435 440 445

Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr Pro Glu Gly Asp
450 455 460

Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe Phe Glu Ala Lys
465 470 475 480

Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val Asn Gln Arg Gly
485 490 495

Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly Tyr Val Asn Asn
500 505 510

Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly Trp Leu His Ser
515 520 525

Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe Phe Ile Val Asp
530 535 540

Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln Val Ala Pro Ala
545 550 555 560

Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile Phe Asp Ala Gly
565 570 575

Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu Pro Ala Ala Val
580 585 590

Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys Glu Ile Val Asp
595 600 605

Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu Arg Gly Gly Val
610 615 620

Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly Lys Leu Asp Ala

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3102

625 630 635 640

Arg Lys Ile Arg Glu Ile Leu Ile Lys Ala Lys Lys Gly Gly Lys Ser
645 650 655

Lys Leu Ala Ala Ala Met Pro Ala Pro His Gly Gly Ile Leu Gln Asp
660 665 670

Leu Ile Ala Arg Asp Ala Leu Lys Lys Asn Glu Leu Leu Ser Glu Ala
675 680 685

Gln Ser Ser Asp Ile Leu Val Trp Asn Leu Thr Pro Arg Gln Leu Cys
690 695 700

Asp Ile Glu Leu Ile Leu Asn Gly Gly Phe Ser Pro Leu Thr Gly Phe
705 710 715

Leu Asn Glu Asn Asp Tyr Ser Ser Val Val Thr Asp Ser Arg Leu Ala
720 725 730 735

Asp Gly Thr Leu Trp Thr Ile Pro Ile Thr Leu Asp Val Asp Glu Ala
740 745 750

Phe Ala Asn Gln Ile Lys Pro Asp Thr Arg Ile Ala Leu Phe Gln Asp
755 760 765

Asp Glu Ile Pro Ile Ala Ile Leu Thr Val Gln Asp Val Tyr Lys Pro
770 775 780

Asn Lys Thr Ile Glu Ala Glu Lys Val Phe Arg Gly Asp Pro Glu His
785 790 795

Pro Ala Ile Ser Tyr Leu Phe Asn Val Ala Gly Asp Tyr Tyr Val Gly
800 805 810 815

Gly Ser Leu Glu Ala Ile Gln Leu Pro Gln His Tyr Asp Tyr Pro Gly
820 825 830

Leu Arg Lys Thr Pro Ala Gln Leu Arg Leu Glu Phe Gln Ser Arg Gln
835 840 845

Trp Asp Arg Val Val Ala Phe Gln Thr Arg Asn Pro Met His Arg Ala
850 855 860

His Arg Glu Leu Thr Val Arg Ala Ala Arg Glu Ala Asn Ala Lys Val
865 870 875

Leu Ile His Pro Val Val Gly Leu Thr Lys Pro Gly Asp Ile Asp His

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3103

880 885 890 895

His Thr Arg Val Arg Val Tyr Gln Glu Ile Ile Lys Arg Tyr Pro Asn
900 905 910

Gly Ile Ala Phe Leu Ser Leu Leu Pro Leu Ala Met Arg Met Ser Gly
915 920 925

Asp Arg Glu Ala Val Trp His Ala Ile Ile Arg Lys Asn Tyr Gly Ala
930 935 940

Ser His Phe Ile Val Gly Arg Asp His Ala Gly Pro Gly Lys Asn Ser
945 950 955

Lys Gly Val Asp Phe Tyr Gly Pro Tyr Asp Ala Gln Glu Leu Val Glu
960 965 970 975

Ser Tyr Lys His Glu Leu Asp Ile Glu Val Val Pro Phe Arg Met Val
980 985 990

Thr Tyr Leu Pro Asp Glu Asp Arg Tyr Ala Pro Ile Asp Gln Ile Asp
995 1000 1005

Thr Thr Lys Thr Arg Thr Leu Asn Ile Ser Gly Thr Glu Leu Arg Arg
1010 1015 1020

Arg Leu Arg Val Gly Gly Glu Ile Pro Glu Trp Phe Ser Tyr Pro Glu
1025 1030 1035

Val Val Lys Ile Leu Arg Glu Ser Asn Pro Pro Arg Pro Lys Gln Gly
1040 1045 1050 1055

Phe Ser Ile Val Leu Gly Asn Ser Leu Thr Val Ser Arg Glu Gln Leu
1060 1065 1070

Ser Ile Ala Leu Leu Ser Thr Phe Leu Gln Phe Gly Gly Gly Arg Tyr
1075 1080 1085

Tyr Lys Ile Phe Glu His Asn Asn Lys Thr Glu Leu Leu Ser Leu Ile
1090 1095 1100

Gln Asp Phe Ile Gly Ser Gly Ser Gly Leu Ile Ile Pro Asn Gln Trp
1105 1110 1115

Glu Asp Asp Lys Asp Ser Val Val Gly Lys Gln Asn Val Tyr Leu Leu
1120 1125 1130 1135

Asp Thr Ser Ser Ser Ala Asp Ile Gln Leu Glu Ser Ala Asp Glu Pro

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3104

1140 1145 1150

Ile Ser His Ile Val Gln Lys Val Val Leu Phe Leu Glu Asp Asn Gly
1155 1160 1165

Phe Phe Val Phe
1170

Accordingly, in one aspect, the invention provides for a fusion protein comprising a thermostable sulfurylase joined to at least one affinity tag. The nucleic acid sequence of the disclosed N-terminal hexahistidine-BCCP Bst ATP Sulfurylase (His6-BCCP Bst Sulfurylase) gene is shown below:

His6-BCCP Bst Sulfurylase Nucleotide Sequence (SEQ ID NO:5)

```
ATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGGAAGCGCCAGCAGCA    60
GCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGTTGGTACTTTCTACCGCACCCCA   120
AGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACCCTG   180
TGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTACCGTG   240
AAAGCAATTCTGGTCGAAAGTGGACAACCGGTAGAATTTGACGAGCCGCTGGTCGTCATC   300
GAGGGATCCGAGCTCGAGATCTGCAGCATGAGCGTAAGCATCCCGCATGGCGGCACATTG   360
ATCAACCGTTGGAATCCGGATTACCCAATCGATGAAGCAACGAAAACGATCGAGCTGTCC   420
AAAGCCGAACTAAGCGACCTTGAGCTGATCGGCACAGGCGCCTACAGCCCGCTCACCGGG   480
TTTTTAACGAAAGCCGATTACGATGCGGTCGTAGAAACGATGCGCCTCGCTGATGGCACT   540
GTCTGGAGCATTCCGATCACGCTGGCGGTGACGGAAGAAAAAGCGAGTGAACTCACTGTC   600
GGCGACAAAGCGAAACTCGTTTATGGCGGCGACGTCTACGGCGTCATTGAAATCGCCGAT   660
ATTTACCGCCCGGATAAAACGAAAGAAGCCAAGCTCGTCTATAAAACCGATGAACTCGCT   720
CACCCGGGCGTGCGCAAGCTGTTTGAAAAACCAGATGTGTACGTCGGCGGAGCGGTTACG   780
CTCGTCAAACGGACCGACAAAGGCCAGTTTGCTCCGTTTTATTTCGATCCGGCCGAAACG   840
CGGAAACGATTTGCCGAACTCGGCTGGAATACCGTCGTCGGCTTCCAAACACGCAACCCG   900
GTTCACCGCGCCCATGAATACATTCAAAAATGCGCGCTTGAAATCGTGGACGGCTTGTTT   960
TTAAACCCGCTCGTCGGCGAAACGAAAGCGGACGATATTCCGGCCGACATCCGGATGGAA  1020
AGCTATCAAGTGCTGCTGGAAAACTATTATCCGAAAGACCGCGTTTTCTTGGGCGTCTTC  1080
CAAGCTGCGATGCGCTATGCCGGTCCGCGCGAAGCGATTTTCCATGCCATGGTGCGGAAA  1140
AACTTCGGCTGCACGCACTTCATCGTCGGCCGCGACCATGCGGGCGTCGGCAACTATTAC  1200
GGCACGTATGATGCGCAAAAAATCTTCTCGAACTTTACAGCCGAAGAGCTTGGCATTACA  1260
CCGCTCTTTTTCGAACACAGCTTTTATTGCACGAAATGCGAAGGCATGGCATCGACGAAA  1320
ACATGCCCGCACGACGCACAATATCACGTTGTCCTTTCTGGCACGAAAGTCCGTGAAATG  1380
TTGCGTAACGGCCAAGTGCCGCCGAGCACATTCAGCCGTCCGGAAGTGGCCGCCGTTTTG  1440
ATCAAAGGGCTGCAAGAACGCGAAACGGTCGCCCCGTCAGCGCGCTAA              1488
```

The amino acid sequence of the His6-BCCP Bst Sulfurylase polypeptide is presented using the three letter amino acid code in Table 6 (SEQ ID NO:6).

His6-BCCP Bst Sulfurylase Amino Acid Sequence (SEQ ID NO:6)

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Glu
1 5 10 15

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3105

Ala Pro Ala Ala Ala Glu Ile Ser Gly His Ile Val Arg Ser Pro Met
20 25 30

Val Gly Thr Phe Tyr Arg Thr Pro Ser Pro Asp Ala Lys Ala Phe Ile
35 40 45

Glu Val Gly Gln Lys Val Asn Val Gly Asp Thr Leu Cys Ile Val Glu
50 55 60

Ala Met Lys Met Met Asn Gln Ile Glu Ala Asp Lys Ser Gly Thr Val
65 70 75 80

Lys Ala Ile Leu Val Glu Ser Gly Gln Pro Val Glu Phe Asp Glu Pro
85 90 95

Leu Val Val Ile Glu Gly Ser Glu Leu Glu Ile Cys Ser Met Ser Val
100 105 110

Ser Ile Pro His Gly Gly Thr Leu Ile Asn Arg Trp Asn Pro Asp Tyr
115 120 125

Pro Ile Asp Glu Ala Thr Lys Thr Ile Glu Leu Ser Lys Ala Glu Leu
130 135 140

Ser Asp Leu Glu Leu Ile Gly Thr Gly Ala Tyr Ser Pro Leu Thr Gly
145 150 155 160

Phe Leu Thr Lys Ala Asp Tyr Asp Ala Val Val Glu Thr Met Arg Leu
165 170 175

Ala Asp Gly Thr Val Trp Ser Ile Pro Ile Thr Leu Ala Val Thr Glu
180 185 190

Glu Lys Ala Ser Glu Leu Thr Val Gly Asp Lys Ala Lys Leu Val Tyr
195 200 205

Gly Gly Asp Val Tyr Gly Val Ile Glu Ile Ala Asp Ile Tyr Arg Pro
210 215 220

Asp Lys Thr Lys Glu Ala Lys Leu Val Tyr Lys Thr Asp Glu Leu Ala
225 230 235 240

His Pro Gly Val Arg Lys Leu Phe Glu Lys Pro Asp Val Tyr Val Gly
245 250 255

Gly Ala Val Thr Leu Val Lys Arg Thr Asp Lys Gly Gln Phe Ala Pro
260 265 270

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3106

Phe Tyr Phe Asp Pro Ala Glu Thr Arg Lys Arg Phe Ala Glu Leu Gly
275 280 285

Trp Asn Thr Val Val Gly Phe Gln Thr Arg Asn Pro Val His Arg Ala
290 295 300

His Glu Tyr Ile Gln Lys Cys Ala Leu Glu Ile Val Asp Gly Leu Phe
305 310 315 320

Leu Asn Pro Leu Val Gly Glu Thr Lys Ala Asp Asp Ile Pro Ala Asp
325 330 335

Ile Arg Met Glu Ser Tyr Gln Val Leu Leu Glu Asn Tyr Tyr Pro Lys
340 345 350

Asp Arg Val Phe Leu Gly Val Phe Gln Ala Ala Met Arg Tyr Ala Gly
355 360 365

Pro Arg Glu Ala Ile Phe His Ala Met Val Arg Lys Asn Phe Gly Cys
370 375 380

Thr His Phe Ile Val Gly Arg Asp His Ala Gly Val Gly Asn Tyr Tyr
385 390 395 400

Gly Thr Tyr Asp Ala Gln Lys Ile Phe Ser Asn Phe Thr Ala Glu Glu
405 410 415

Leu Gly Ile Thr Pro Leu Phe Phe Glu His Ser Phe Tyr Cys Thr Lys
420 425 430

Cys Glu Gly Met Ala Ser Thr Lys Thr Cys Pro His Asp Ala Gln Tyr
435 440 445

His Val Val Leu Ser Gly Thr Lys Val Arg Glu Met Leu Arg Asn Gly
450 455 460

Gln Val Pro Pro Ser Thr Phe Ser Arg Pro Glu Val Ala Ala Val Leu
465 470 475 480

Ile Lys Gly Leu Gln Glu Arg Glu Thr Val Ala Pro Ser Ala Arg
485 490 495

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an ATP generating polypeptide and an ATP converting

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3107

polypeptide, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3108

design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce a fusion protein.

The recombinant expression vectors of the invention can be designed for expression of the fusion protein in prokaryotic or eukaryotic cells. For example, a sulfurylase-luciferase fusion protein can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein.

In another embodiment, the ATP generating-ATP converting fusion protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, the fusion protein can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3109

include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. The invention also includes a kit comprising a sulfurylase-luciferase fusion protein expression vector.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3110

A host cell can be any prokaryotic or eukaryotic cell. For example, the sulfurylase-luciferase fusion protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding ORFX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) the fusion protein. Accordingly, the invention further provides methods for producing the fusion protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding the fusion protein has been introduced) in a suitable medium such that the fusion protein is produced. In another embodiment, the method further comprises isolating the fusion protein from the medium or the host cell.

The invention also includes a fusion protein bound to a mobile support. In a preferred embodiment, the fusion gene is a sulfurylase-luciferase fusion gene. In another embodiment,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3111

the mobile support is bound to strepavidin. The mobile support could be a bead or optical fiber. In a preferred embodiment, the bead is a nickel-agarose bead or a MPG-Streptavidin bead. In one embodiment, the sulfurylase-luciferase fusion protein is bound to the beads in a 1:3 ratio of protein to bead. It can be attached to the solid support via a covalent or non-covalent interaction. In general, any linkage recognized in the art can be used. Examples of such linkages common in the art include any suitable metal (*e.g.*, Co^{2+}, Ni^{2+})-hexahistidine complex, a biotin binding protein, *e.g.*, NEUTRAVIDIN™ modified avidin (Pierce Chemicals, Rockford, IL), streptavidin/biotin, avidin/biotin, glutathione S-transferase (GST)/glutathione, monoclonal antibody/antigen, and maltose binding protein/maltose, and pluronic coupling technologies. Samples containing the appropriate tag are incubated with the sensitized substrate so that zero, one, or multiple molecules attach at each sensitized site.

Acetyl-CoA carboxylase (ACCase) catalyzes the first committed step in de novo fatty acid biosynthesis. It belongs to a group of carboxylases that use biotin as cofactor and bicarbonate as a source of the carboxyl group. There are two types of ACCase: prokaryotic ACCase (e.g., *E. coli*, *P. aeruginosa*, *Anabaena, Synechococcus* and probably pea chloroplast) in which the three functional domains: biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP) and carboxyltransferase (CT) are located on separable subunits and eukaryotic ACCase (e.g., rat, chicken, yeast, diatom and wheat) in which all the domains are located on one large polypeptide. It is known that a BCCP as a subunit of acetyl CoA carboxylase from *E. coli* is biotinated at the Lys residue at the 122-position by the action of biotin holoenzyme synthetase in *E. coli* (Journal of Biological Chemistry, 263, 6461 (1988)). In a preferred embodiment of this invention, the fusion protein is bound to a BCCP domain which is then utilized for binding avidins; therefore, it can bind to a streptavidin mobile support. One biotin-(strept-)avidin-based anchoring method uses a thin layer of a photoactivatable biotin analog dried onto a solid surface. (Hengsakul and Cass, 1996. *Bioconjugate Chem.* 7: 249-254). The biotin analog is then exposed to white light through a mask, so as to create defined areas of activated biotin. Avidin (or streptavidin) is then added and allowed to bind to the activated biotin. The avidin possesses free biotin binding sites which can be utilized to "anchor" the biotinylated proteins through a biotin-(strept-)avidin linkage.

Alternatively, the fusion protein can be attached to the solid support with a biotin derivative possessing a photo-removable protecting group. This moiety is covalently bound

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3112

to bovine serum albumin (BSA), which is attached to the solid support, *e.g.*, a glass surface. See Pirrung and Huang, 1996. *Bioconjugate Chem.* 7: 317-321. A mask is then used to create activated biotin within the defined irradiated areas. Avidin may then be localized to the irradiated area, with a biotinylated sulfurylase-luciferase fusion protein subsequently attached through a BSA-biotin-avidin-biotin link.

Another method of attachment is with the use of a pluronics based attachment. Pluronics attach to hydrophobic surfaces by virtue of the reaction between the hydrophobic surface and the polypropylene oxide. The remaining polyethylene oxide groups extend off the surface, thereby creating a hydrophilic environment. Nitrilotriacetic acid (NTA) can be conjugated to the terminal ends of the polyethylene oxide chains to allow for hexahistidine tagged proteins to be attached.

This invention provides methods of sequencing which utilize and ATP generating polypeptide-ATP converting polypeptide fusion protein for detection. In a preferred embodiment, the nucleotide sequence of the sequencing product is determined by measuring inorganic pyrophosphate (PPi) liberated from a nucleotide triphosphate (dNTP) as the dNMP is incorporated into an extended sequence primer. This method of sequencing is termed Pyrosequencing™ technology (PyroSequencing AB, Stockholm, Sweden). It can be performed in solution (liquid phase) or as a solid phase technique. Various sequencing methods, including PPi sequencing methods, are described in, *e.g.,* WO9813523A1, Ronaghi, *et al.,* 1996. *Anal. Biochem.* 242: 84-89, and Ronaghi, *et al.*, 1998. *Science* 281: 363-365 (1998), US patent 6,274,320 and the patent application USSN 10/104,280 which was filed on March 21, 2001 (21465-501CIP3). These disclosures of sequencing are incorporated herein in their entirety, by reference.

Pyrophosphate released under these conditions can be detected enzymatically (*e.g.,* by the generation of light in the luciferase-luciferin reaction). Such methods enable a nucleotide to be identified in a given target position, and the DNA to be sequenced simply and rapidly while avoiding the need for electrophoresis and the use of potentially dangerous radiolabels.

The invention also provides a method for sequencing nucleic acids which generally comprises (a) providing one or more nucleic acid anchor primers and a plurality of single-stranded circular nucleic acid templates disposed within a plurality of reaction chambers or cavities; (b) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3113

complex; (c) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (d) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid; (e) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and (f) identifying the PPi sequencing reaction byproduct with the use of an ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the sequence of the nucleic acid. In one embodiment, a dATP or ddATP analogue is used in place of deoxy- or dideoxy adenosine triphosphate. This analogue is capable of acting as a substrate for a polymerase but incapable of acting as a substrate for a PPi-detection enzyme. This method can be carried out in separate parallel common reactions in an aqueous environment.

In another aspect, the invention includes a method of determining the base sequence of a plurality of nucleotides on an array, which generally comprises (a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface; (b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates; (c) utilizing an ATP generating polypeptide-ATP converting polypeptide fusion protein to detect whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands in which incorporation of the nucleoside 5'-triphosphate precursor indicates that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3114

incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and (e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

The anchor primers of the invention generally comprise a stalk region and at least one adaptor region. In a preferred embodiment the anchor primer contains at least two contiguous adapter regions. The stalk region is present at the 5' end of the anchor primer and includes a region of nucleotides for attaching the anchor primer to the solid substrate.

The adaptor region(s) comprise nucleotide sequences that hybridize to a complementary sequence present in one or more members of a population of nucleic acid sequences. In some embodiments, the anchor primer includes two adjoining adaptor regions, which hybridize to complementary regions ligated to separate ends of a target nucleic acid sequence. In additional embodiments, the adapter regions in the anchor primers are complementary to non-contiguous regions of sequence present in a second nucleic acid sequence. Each adapter region, for example, can be homologous to each terminus of a fragment produced by digestion with one or more restriction endonucleases. The fragment can include, *e.g.*, a sequence known or suspected to contain a sequence polymorphism. Additionally, the anchor primer may contain two adapter regions that are homologous to a gapped region of a target nucleic acid sequence, *i.e.*, one that is non-contiguous because of a deletion of one or more nucleotides. When adapter regions having these sequences are used, an aligning oligonucleotide corresponding to the gapped sequence may be annealed to the anchor primer along with a population of template nucleic acid molecules.

The anchor primer may optionally contain additional elements such as one or more restriction enzyme recognition sites, RNA polymerase binding sites, *e.g.*, a T7 promoter site, or sequences present in identified DNA sequences, *e.g.*, sequences present in known genes. The adapter region(s) may also include sequences known to flank sequence polymorphisms. Sequence polymorphisms include nucleotide substitutions, insertions, deletions, or other rearrangements which result in a sequence difference between two otherwise identical nucleic acid sequences. An example of a sequence polymorphism is a single nucleotide polymorphism (SNP).

In general, any nucleic acid capable of base-pairing can be used as an anchor primer. In some embodiments, the anchor primer is an oligonucleotide. As utilized herein the term

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3115

oligonucleotide includes linear oligomers of natural or modified monomers or linkages, *e.g.*, deoxyribonucleosides, ribonucleosides, anomeric forms thereof, peptide nucleic acids (PNAs), and the like, that are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions. These types of interactions can include, *e.g.*, Watson-Crick type of base-pairing, base stacking, Hoogsteen or reverse-Hoogsteen types of base-pairing, or the like. Generally, the monomers are linked by phosphodiester bonds, or analogs thereof, to form oligonucleotides ranging in size from, *e.g.*, 3-200, 8-150, 10-100, 20-80, or 25-50 monomeric units. Whenever an oligonucleotide is represented by a sequence of letters, it is understood that the nucleotides are oriented in the 5' $\longrightarrow$ 3' direction, from left-to-right, and that the letter "A" donates deoxyadenosine, the letter "T" denotes thymidine, the letter "C" denotes deoxycytosine, and the letter "G" denotes deoxyguanosine, unless otherwise noted herein. The oligonucleotides of the present invention can include non-natural nucleotide analogs. However, where, for example, processing by enzymes is required, or the like, oligonucleotides comprising naturally occurring nucleotides are generally required for maintenance of biological function.

Anchor primers are linked to the solid substrate at the sensitized sites. They can be linked by the same method of linkage as described for the fusion protein to the solid support. A region of a solid substrate containing a linked primer is referred to herein as an anchor pad. Thus, by specifying the sensitized states on the solid support, it is possible to form an array or matrix of anchor pads. The anchor pads can be, *e.g.*, small diameter spots etched at evenly spaced intervals on the solid support. The anchor pads can be located at the bottoms of the cavitations or wells if the substrate has been cavitated, etched, or otherwise micromachined as discussed above.

In one embodiment, the anchor primer is linked to a particle. The anchor primer can be linked to the particle prior to formation of the extended anchor primer or after formation of the extended anchor primer.

Each sensitized site on a solid support is potentially capable of attaching multiple anchor primers. Thus, each anchor pad may include one or more anchor primers. It is preferable to maximize the number of pads that have only a single productive reaction center (*e.g.*, the number of pads that, after the extension reaction, have only a single sequence extended from the anchor primer). This can be accomplished by techniques which include, but are not limited to: *(i)* varying the dilution of biotinylated anchor primers that are washed

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3116

over the surface; (*ii*) varying the incubation time that the biotinylated primers are in contact with the avidin surface; (*iii*) varying the concentration of open- or closed-circular template so that, on average, only one primer on each pad is extended to generate the sequencing template; or (*iv*) reducing the size of the anchor pad to approach single-molecule dimensions (< 1 μm) such that binding of one anchor inhibits or blocks the binding of another anchor (*e.g.* by photoactivation of a small spot); or (*v*) reducing the size of the anchor pad such that binding of one circular template inhibits or blocks the binding of a second circular template.

In some embodiments, each individual pad contains just one linked anchor primer. Pads having only one anchor primer can be made by performing limiting dilutions of a selected anchor primer on to the solid support such that, on average, only one anchor primer is deposited on each pad. The concentration of anchor primer to be applied to a pad can be calculated utilizing, for example, a Poisson distribution model.

In order to maximize the number of reaction pads that contain a single anchor primer, a series of dilution experiments are performed in which a range of anchor primer concentrations or circular template concentrations are varied. For highly dilute concentrations of primers, primers and circular templates binding to the same pad will be independent of each other, and a Poisson distribution will characterize the number of anchor primers extended on any one pad. Although there will be variability in the number of primers that are actually extended, a maximum of 37% of the pads will have a single extended anchor primer (the number of pads with a single anchor oligonucleotide).

In other embodiments multiple anchor primers are attached to any one individual pad in an array. Limiting dilutions of a plurality of circular nucleic acid templates (described in more detail below) may be hybridized to the anchor primers so immobilized such that, on average, only one primer on each pad is hybridized to a nucleic acid template. Library concentrations to be used may be calculated utilizing, for example, limiting dilutions and a Poisson distribution model.

The nucleic acid templates that can be sequenced according to the invention, *e.g.*, a nucleic acid library, in general can include open circular or closed circular nucleic acid molecules. A "closed circle" is a covalently closed circular nucleic acid molecule, *e.g.*, a circular DNA or RNA molecule. An "open circle" is a linear single-stranded nucleic acid molecule having a 5' phosphate group and a 3' hydroxyl group. In one embodiment, the single stranded nucleic acid contains at least 100 copies of nucleic acid sequence, each copy

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3117

covalently linked end to end. In some embodiments, the open circle is formed *in situ* from a linear double-stranded nucleic acid molecule. The ends of a given open circle nucleic acid molecule can be ligated by DNA ligase. Sequences at the 5' and 3' ends of the open circle molecule are complementary to two regions of adjacent nucleotides in a second nucleic acid molecule, *e.g.*, an adapter region of an anchor primer, or to two regions that are nearly adjoining in a second DNA molecule. Thus, the ends of the open-circle molecule can be ligated using DNA ligase, or extended by DNA polymerase in a gap-filling reaction. Open circles are described in detail in Lizardi, U.S. Pat. No. 5,854,033. An open circle can be converted to a closed circle in the presence of a DNA ligase (for DNA) or RNA ligase following, *e.g.*, annealing of the open circle to an anchor primer.

If desired, nucleic acid templates can be provided as padlock probes. Padlock probes are linear oligonucleotides that include target-complementary sequences located at each end, and which are separated by a linker sequence. The linkers can be ligated to ends of members of a library of nucleic acid sequences that have been, *e.g.*, physically sheared or digested with restriction endonucleases. Upon hybridization to a target-sequence, the 5'- and 3'-terminal regions of these linear oligonucleotides are brought in juxtaposition. This juxtaposition allows the two probe segments (if properly hybridized) to be covalently-bound by enzymatic ligation (*e.g.*, with T4 DNA ligase), thus converting the probes to circularly-closed molecules which are catenated to the specific target sequences (see *e.g.*, Nilsson, *et al.*, 1994. *Science* 265: 2085-2088). The resulting probes are suitable for the simultaneous analysis of many gene sequences both due to their specificity and selectivity for gene sequence variants (see *e.g.*, Lizardi, *et al.*, 1998. *Nat. Genet.* 19: 225-232; Nilsson, *et al.*, 1997. *Nat. Genet.* 16: 252-255) and due to the fact that the resulting reaction products remain localized to the specific target sequences. Moreover, intramolecular ligation of many different probes is expected to be less susceptible to non-specific cross-reactivity than multiplex PCR-based methodologies where non-cognate pairs of primers can give rise to irrelevant amplification products (see *e.g.*, Landegren and Nilsson, 1997. *Ann. Med.* 29: 585-590).

A starting library can be constructed comprising either single-stranded or double-stranded nucleic acid molecules, provided that the nucleic acid sequence includes a region that, if present in the library, is available for annealing, or can be made available for annealing, to an anchor primer sequence. For example, when used as a template for rolling

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3118

circle amplification, a region of a double-stranded template needs to be at least transiently single-stranded in order to act as a template for extension of the anchor primer.

Library templates can include multiple elements, including, but not limited to, one or more regions that are complementary to the anchor primer. For example, the template libraries may include a region complementary to a sequencing primer, a control nucleotide region, and an insert sequence comprised of the sequencing template to be subsequently characterized. As is explained in more detail below, the control nucleotide region is used to calibrate the relationship between the amount of byproduct and the number of nucleotides incorporated. As utilized herein the term "complement" refers to nucleotide sequences that are able to hybridize to a specific nucleotide sequence to form a matched duplex.

In one embodiment, a library template includes: (*i*) two distinct regions that are complementary to the anchor primer, (*ii*) one region homologous to the sequencing primer, (*iii*) one optional control nucleotide region, (*iv*) an insert sequence of, *e.g.*, 30-500, 50-200, or 60-100 nucleotides, that is to be sequenced. The template can, of course, include two, three, or all four of these features.

The template nucleic acid can be constructed from any source of nucleic acid, *e.g.*, any cell, tissue, or organism, and can be generated by any art-recognized method. Suitable methods include, *e.g.*, sonication of genomic DNA and digestion with one or more restriction endonucleases (RE) to generate fragments of a desired range of lengths from an initial population of nucleic acid molecules. Preferably, one or more of the restriction enzymes have distinct four-base recognition sequences. Examples of such enzymes include, *e.g.*, Sau3A1, MspI, and TaqI. Preferably, the enzymes are used in conjunction with anchor primers having regions containing recognition sequences for the corresponding restriction enzymes. In some embodiments, one or both of the adapter regions of the anchor primers contain additional sequences adjoining known restriction enzyme recognition sequences, thereby allowing for capture or annealing to the anchor primer of specific restriction fragments of interest to the anchor primer. In other embodiments, the restriction enzyme is used with a type IIS restriction enzyme.

Alternatively, template libraries can be made by generating a complementary DNA (cDNA) library from RNA, *e.g.*, messenger RNA (mRNA). The cDNA library can, if desired, be further processed with restriction endonucleases to obtain a 3' end characteristic of a specific RNA, internal fragments, or fragments including the 3' end of the isolated RNA.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3119

Adapter regions in the anchor primer may be complementary to a sequence of interest that is thought to occur in the template library, *e.g.*, a known or suspected sequence polymorphism within a fragment generated by endonuclease digestion.

In one embodiment, an indexing oligonucleotide can be attached to members of a template library to allow for subsequent correlation of a template nucleic acid with a population of nucleic acids from which the template nucleic acid is derived. For example, one or more samples of a starting DNA population can be fragmented separately using any of the previously disclosed methods (*e.g.*, restriction digestion, sonication). An indexing oligonucleotide sequence specific for each sample is attached to, *e.g.*, ligated to, the termini of members of the fragmented population. The indexing oligonucleotide can act as a region for circularization, amplification and, optionally, sequencing, which permits it to be used to index, or code, a nucleic acid so as to identify the starting sample from which it is derived.

Distinct template libraries made with a plurality of distinguishable indexing primers can be mixed together for subsequent reactions. Determining the sequence of the member of the library allows for the identification of a sequence corresponding to the indexing oligonucleotide. Based on this information, the origin of any given fragment can be inferred.

Libraries of nucleic acids are annealed to anchor primer sequences using recognized techniques (see, *e.g.*, Hatch, *et al.*, 1999. *Genet. Anal. Biomol. Engineer.* 15: 35-40; Kool, U.S. Patent No. 5,714, 320 and Lizardi, U.S. Patent No. 5,854,033). In general, any procedure for annealing the anchor primers to the template nucleic acid sequences is suitable as long as it results in formation of specific, *i.e.*, perfect or nearly perfect, complementarity between the adapter region or regions in the anchor primer sequence and a sequence present in the template library.

A number of *in vitro* nucleic acid amplification techniques may be utilized to extend the anchor primer sequence. The size of the amplified DNA preferably is smaller than the size of the anchor pad and also smaller than the distance between anchor pads.

The amplification is typically performed in the presence of a polymerase, *e.g.*, a DNA or RNA-directed DNA polymerase, and one, two, three, or four types of nucleotide triphosphates, and, optionally, auxiliary binding proteins. In general, any polymerase capable of extending a primed 3'-OH group can be used a long as it lacks a 3' to 5' exonuclease activity. Suitable polymerases include, *e.g.*, the DNA polymerases from *Bacillus stearothermophilus*, *Thermus acquaticus*, *Pyrococcus furiosis*, *Thermococcus litoralis*, and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3120

Thermus thermophilus, bacteriophage T4 and T7, and the *E. coli* DNA polymerase I Klenow fragment. Suitable RNA-directed DNA polymerases include, *e.g.*, the reverse transcriptase from the Avian Myeloblastosis Virus, the reverse transcriptase from the Moloney Murine Leukemia Virus, and the reverse transcriptase from the Human Immunodeficiency Virus-I.

A number of *in vitro* nucleic acid amplification techniques have been described. These amplification methodologies may be differentiated into those methods: (*i*) which require temperature cycling - polymerase chain reaction (PCR) (see *e.g.*, Saiki, *et al.*, 1995. *Science* 230: 1350-1354), ligase chain reaction (see *e.g.*, Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189-193; Barringer, *et al.*, 1990. *Gene* 89: 117-122) and transcription-based amplification (see *e.g.*, Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177) and (*ii*) isothermal amplification systems - self-sustaining, sequence replication (see *e.g.*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878); the Qβ replicase system (see *e.g.*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197-1202); strand displacement amplification Nucleic Acids Res. 1992 Apr 11;20(7):1691-6.; and the methods described in PNAS 1992 Jan 1;89(1):392-6; and NASBA J Virol Methods. 1991 Dec;35(3):273-86.

Isothermal amplification also includes rolling circle-based amplification (RCA). RCA is discussed in, *e.g.*, Kool, U.S. Patent No. 5,714,320 and Lizardi, U.S. Patent No. 5,854,033; Hatch, *et al.*, 1999. *Genet. Anal. Biomol. Engineer.* 15: 35-40. The result of the RCA is a single DNA strand extended from the 3' terminus of the anchor primer (and thus is linked to the solid support matrix) and including a concatamer containing multiple copies of the circular template annealed to a primer sequence. Typically, 1,000 to 10,000 or more copies of circular templates, each having a size of, *e.g.*, approximately 30-500, 50-200, or 60-100 nucleotides size range, can be obtained with RCA.

In vivo, RCR is utilized in several biological systems. For example, the genome of several bacteriophage are single-stranded, circular DNA. During replication, the circular DNA is initially converted to a duplex form, which is then replicated by the aforementioned rolling-circle replication mechanism. The displaced terminus generates a series of genomic units that can be cleaved and inserted into the phage particles. Additionally, the displaced single-strand of a rolling-circle can be converted to duplex DNA by synthesis of a complementary DNA strand. This synthesis can be used to generate the concatemeric duplex molecules required for the maturation of certain phage DNAs. For example, this provides the principle pathway by which λ bacteriophage matures. RCR is also used *in vivo* to generate

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3121

amplified rDNA in *Xenopus* oocytes, and this fact may help explain why the amplified rDNA is comprised of a large number of identical repeating units. In this case, a single genomic repeating unit is converted into a rolling-circle. The displaced terminus is then converted into duplex DNA which is subsequently cleaved from the circle so that the two termini can be ligated together so as to generate the amplified circle of rDNA.

Through the use of the RCA reaction, a strand may be generated which represents many tandem copies of the complement to the circularized molecule. For example, RCA has recently been utilized to obtain an isothermal cascade amplification reaction of circularized padlock probes *in vitro* in order to detect single-copy genes in human genomic DNA samples (see Lizardi, *et al.,* 1998. *Nat. Genet.* 19: 225-232). In addition, RCA has also been utilized to detect single DNA molecules in a solid phase-based assay, although difficulties arose when this technique was applied to *in situ* hybridization (see Lizardi, *et al.,* 1998. *Nat. Genet.* 19: 225-232).

If desired, RCA can be performed at elevated temperatures, *e.g.*, at temperatures greater than 37° C, 42° C, 45° C, 50° C, 60° C, or 70° C. In addition, RCA can be performed initially at a lower temperature, *e.g.*, room temperature, and then shifted to an elevated temperature. Elevated temperature RCA is preferably performed with thermostable nucleic acid polymerases and with primers that can anneal stably and with specificity at elevated temperatures.

RCA can also be performed with non-naturally occurring oligonucleotides, *e.g.*, peptide nucleic acids. Further, RCA can be performed in the presence of auxiliary proteins such as single-stranded binding proteins.

The development of a method of amplifying short DNA molecules which have been immobilized to a solid support, termed RCA has been recently described in the literature (see *e.g.,* Hatch, *et al.,* 1999. *Genet. Anal. Biomol. Engineer.* 15: 35-40; Zhang, *et al.,* 1998. *Gene* 211: 277-85; Baner, *et al.,* 1998. *Nucl. Acids Res.* 26: 5073-5078; Liu, *et al.,* 1995. *J. Am. Chem. Soc.* 118: 1587-1594; Fire and Xu, 1995. *Proc. Natl. Acad. Sci. USA* 92: 4641-4645; Nilsson, *et al.,* 1994. *Science* 265: 2085-2088). RCA targets specific DNA sequences through hybridization and a DNA ligase reaction. The circular product is then subsequently used as a template in a rolling circle replication reaction.

RCA driven by DNA polymerase can replicate circularized oligonucleotide probes with either linear or geometric kinetics under isothermal conditions. In the presence of two

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3122

primers (one hybridizing to the + strand, and the other, to the - strand of DNA), a complex pattern of DNA strand displacement ensues which possesses the ability to generate $1x10^9$ or more copies of each circle in a short period of time (*i.e.,* less-than 90 minutes), enabling the detection of single-point mutations within the human genome. Using a single primer, RCA generates hundreds of randomly-linked copies of a covalently closed circle in several minutes. If solid support matrix-associated, the DNA product remains bound at the site of synthesis, where it may be labeled, condensed, and imaged as a point light source. For example, linear oligonucleotide probes, which can generate RCA signals, have been bound covalently onto a glass surface. The color of the signal generated by these probes indicates the allele status of the target, depending upon the outcome of specific, target-directed ligation events. As RCA permits millions of individual probe molecules to be counted and sorted, it is particularly amenable for the analysis of rare somatic mutations. RCA also shows promise for the detection of padlock probes bound to single-copy genes in cytological preparations.

In addition, a solid-phase RCA methodology has also been developed to provide an effective method of detecting constituents within a solution. Initially, a recognition step is used to generate a complex h a circular template is bound to a surface. A polymerase enzyme is then used to amplify the bound complex. RCA uses small DNA probes that are amplified to provide an intense signal using detection methods, including the methods described in more detail below.

Other examples of isothermal amplification systems include, *e.g.*, (*i*) self-sustaining, sequence replication (see *e.g.,* Guatelli, *et al.,* 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), (*ii*) the Qβ replicase system (see *e.g.,* Lizardi, *et al.,* 1988. *BioTechnology* 6: 1197-1202), and (*iii*) nucleic acid sequence-based amplification (NASBA™; see Kievits, *et al.,* 1991. *J. Virol. Methods* 35: 273-286).

Amplification of a nucleic acid template as described above results in multiple copies of a template nucleic acid sequence covalently linked to an anchor primer. In one embodiment, a region of the sequence product is determined by annealing a sequencing primer to a region of the template nucleic acid, and then contacting the sequencing primer with a DNA polymerase and a known nucleotide triphosphate, *i.e.*, dATP, dCTP, dGTP, dTTP, or an analog of one of these nucleotides. The sequence can be determined by detecting a sequence reaction byproduct, as is described below.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3123

The sequence primer can be any length or base composition, as long as it is capable of specifically annealing to a region of the amplified nucleic acid template. No particular structure for the sequencing primer is required so long as it is able to specifically prime a region on the amplified template nucleic acid. Preferably, the sequencing primer is complementary to a region of the template that is between the sequence to be characterized and the sequence hybridizable to the anchor primer. The sequencing primer is extended with the DNA polymerase to form a sequence product. The extension is performed in the presence of one or more types of nucleotide triphosphates, and if desired, auxiliary binding proteins.

The method comprises the steps of: (a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added; (b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released; (c) separately recovering each of the feedstocks from the polymerization environment; and (d) measuring the amount of inorganic pyrophosphate by utilizing an ATP generating polypeptide-ATP converting polypeptide fusion protein in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

The sequence primer can be any length or base composition, as long as it is capable of specifically annealing to a region of the amplified nucleic acid template. No particular structure is required for the sequencing primer so long as it is able to specifically prime a region on the amplified template nucleic acid. Preferably, the sequencing primer is complementary to a region of the template that is between the sequence to be characterized and the sequence hybridizable to the anchor primer. The sequencing primer is extended with the DNA polymerase to form a sequence product. The extension is performed in the presence of one or more types of nucleotide triphosphates, and if desired, auxiliary binding proteins.

This invention also includes a method wherein the amount of inorganic pyrophosphate is measured by (a) adding adenosine-5'-phosphosulfate to the feedstock; combining the recovered feedstock containing adenosine-5'-phosphosulfate with an ATP generating

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3124

polypeptide-ATP converting polypeptide fusion protein such that any inorganic pyrophosphate in the recovered feedstock and the adenosine-5'-phosphosulfate will first react to the form ATP and sulfate and then react with luciferin in the presence of oxygen such that the ATP is consumed to produced AMP, inorganic pyrophosphate, carbon dioxide and light; and (b) measuring the amount of light produced. In a preferred embodiment, the template polymer and ATP generating polypeptide-ATP converting polypeptide fusion protein are immobilized on a solid support.

The invention also includes a method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising: (a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added; (b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released; (c) separately recovering each of the feedstocks from the polymerization environment; and (d) measuring the amount of PPi with a thermostable sulfurylase and a luciferase in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer. In one embodiment, the thermostable sulfurylase and the luciferase are joined in a fusion protein. In another embodiment, the thermostable sulfurylase is joined to an affinity tag.

The invention further provides a method for sequencing a nucleic acid, the method comprising: (a) providing one or more nucleic acid anchor primers; (b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200; (c) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3125

circular nucleic acid template; (e) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of a thermostable sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid. In one embodiment, the thermostable sulfurylase and the luciferase are joined in a fusion protein. In another embodiment, the thermostable sulfurylase is joined to an affinity tag.

Also included in the invention is a method for sequencing a nucleic acid, the method comprising: (a) providing at least one nucleic acid anchor primer; (b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites; (c) annealing a first amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex; (d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template; (e) annealing a second amount of a sequencing primer to one or more copies of the covalently linked complementary nucleic acid; (f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, when the predetermined nucleotide triphosphate is incorporated onto the 3' end of the sequencing primer, to yield a sequencing reaction byproduct; and (g) identifying the sequencing reaction byproduct with the use of a thermostable sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid at each reaction site that contains a nucleic acid template. In one embodiment, the thermostable sulfurylase and the luciferase are joined in a fusion protein. In another embodiment, the thermostable sulfurylase is joined to an affinity tag.

The invention also includes a method of determining the base sequence of a plurality of nucleotides on an array, the method comprising: (a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, (b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3126

mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates; (c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands through detection of a sequencing byproduct with a thermostable sulfurylase and luciferase, thus indicating that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and (e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors. In one embodiment, the thermostable sulfurylase and the luciferase are joined in a fusion protein. In another embodiment, the thermostable sulfurylase is joined to an affinity tag.

The invention will be further illustrated in the following non-limiting examples. There are several abbreviations which will be used in the following examples: FUS stands for fusion gene, S stands for sulfurylase, L stands for luciferase, TL stands for thermostable luciferase, X stands for XhoI, H stands for HindIII, N stands for NotI and B stands for BamHI. For example, FUS-L/S X F means a primer for the fusion gene, luciferase-sulfurylase Xho Forward and so forth. Primers 1 through 6 are for the L or TL to S fusions and primers 7 through 13 are for the S to L or TL fusions.

EXAMPLES

Example 1: Cloning Strategy for Obtaining the Bst Sulfurylase Gene

Gene specific primers, which incorporated restriction site linkers, were designed based on the sequence for a putative ATP sulfurylase from *Bacillus stearothermophilus* in

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3127

ERGO, a curated database of genomic DNA made available on the World Wide Web by Integrated Genomics which included the *Bacillus stearothermophilus* Genome Sequencing Project at the University of Oklahoma (NSF Grant #EPS-9550478). The forward primer utilized was 5'-CCC TTC TGC AGC ATG AGC GTA AGC ATC CCG CAT GGC GGC ACA TTG-3' (SEQ ID NO: 7) and the reverse primer used was 5'-CCC GTA AGC TTT TAG CGC GCT GAC GGG GCG ACC GTT TCG CGT TCT TG-3' (SEQ ID NO:8). The reaction mix for PCR amplification contained 5.0 uL 10X polymerase buffer (Clontech, Cat. #8714), 2.0 uL 5 M betaine (Sigma, Cat. #B0300), 1.0 uL dNTP mix (10 mM each dATP, dCTP, dGTP, dTTP), 0.8 uL Advantage 2 polymerase (Clontech, Cat. #8714), 0.2 uL Advantage-HF 2 polymerase (Clontech, Cat. #K1914), 10 pmol forward primer, 10 pmol reverse primer, 100 ng (or less) *Bst* genomic DNA (ATCC, Cat. #12980D), and enough distilled water to make total volume of 50 uL. As little as 1 ng *Bst* genomic DNA was sufficient to yield PCR product. The PCR amplification of Bst ATP sulfurylase gene from genomic DNA consisted of an initial step at 96°C for 3 min, then 35 cycles of 96°C for 15 sec, 60°C for 30 sec, 72°C for 6 min, a finishing step at 72°C for 10 min and finally 14°C until removal. The PCR product was cleaned using QIAquick PCR Purification Kit (QIAGEN).

Example 2: Cloning Strategy for Obtaining the Sulfurylase-Luciferase Fusion Protein

All chemicals were purchased from Sigma unless noted otherwise. Racemically pure D-luciferin was ordered from Pierce. The assay buffer for measuring ATP sulfurylase and luciferase activities contained *Taq* polymerase. A polymerase chain reaction (PCR)-mediated approach was utilized to link the open reading frames (ORFs) of luciferase and sulfurylase. The cloning strategy is outlined in Fig. 1. Briefly, it involved the amplification of luciferase and sulfurylase ORFs by PCR, using primers that contain convenient restriction sites (XhoI and HindIII) to clone the fusion gene into an expression vector, in-frame and, the design of a rare restriction site (Not I) at the junction of the two polypeptides so that other versions of luciferase, such as thermostable luciferase (TL), and sulfurylase can be conveniently swapped to obtain either sulfurlyase-luciferase (S-L) or luciferase-sulfurlyase (L-S) fusion proteins. A Not I site was used to fuse the variable heavy chain of antibodies to luciferase to generate a viable fusion protein. These primers were also designed in such a way that the primers that form part of the junction of the two ORFs contain sufficient overlapping regions of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3128

nucleotides. For example, the 5' end of FUS-L/S Not R contains deoxynucleotides in an anti-parallel orientation that encode the N-terminal 10 amino acids of yeast sulfurylase. Thus, a PCR product generated using this primer would anneal to the 5' end of yeast sulfurylase ORF and would generate the fusion protein, L-S.

The products in boxes were obtained by PCR as elaborated in Fig.2. As shown in Fig. 3, the PCR products were subjected to electrophoresis. The PCR products were then purified, digested with Xho I and Hind III and subcloned into Xho I/Hind III digested pRSETA-BCCP. pRSETA-BCCP is a derivative of pRSET A (Invitrogen) in which the sequence between *Nhe*I and *Bam*HI restriction sites has been replaced by the portion of the biotin carboxyl carrier protein (BCCP) gene from *E. coli* (GenBank accession #M80458) that codes for residues 87-165. The 87- amino acid BCCP domain was obtained by PCR and cloned into the NheI and Bam HI sites of pRSETA to obtain pRSETA-BCCP. The ligated fusion protein and pRSETA-BCCP were transformed into BL21DE3 and TOP10 cells. BL21DE3 cells yielded colonies for L-S and TOP10 cells yielded colonies for TL-S.

The following list of primers was used to construct the fusion proteins:

PRIMER NO	TITLE	NUCLEIC ACID SEQUENCE	SEQ ID NO
1	FUS-L/S X F	CCCC CTC GAG ATC CAA ATG GAA GAC GCC AAA AAC ATA AAG AAA GGC CC	9
2	FUS-TL/S X F	CCCC CTC GAG ATC CAA ATG GCT GAC AAA AAC ATC CTG TAT GGC CC	10
3	FUS-L/S Not R	TTG TAG AAT ACC ACC GTG AGG AGC AGG CAT AGC GGC CGC CAA TTT GGA CTT TCC GCC CTT CTT GGC C	11
4	FUS-TL/S Not R	TTG TAG AAT ACC ACC GTG AGG AGC AGG CAT AGC GGC CGC ACC GTT GGT GTG TTT CTC GAA CAT C	12
5	FUS-S-Not F	GCG GCC GCT ATG CCT GCT CCT CAC GGT GGT ATT CTA C	13
6	FUS-S-Hind III R	CCCC AAG CTT TTA AAA TAC AAA AAA GCC ATT GTC TTC CAA GAA TAG GAC	14
7	FUS-S/L B F	CCCC GGA TCC ATC CAA ATG CCT GCT CCT CAC GGT GGT ATT CTA CAA GAC	15
8	FUS-S/L R	GGGCCTTTCTTTATGTTTTTGGCGTCTTCCAT AGC GGC CGC AAA TAC AAA AAA GCC ATT GTC	16
9	FUS-L- F	GCG GCC GCT ATG GAA GAC GCC AAA AAC ATA AAG AAA GGC CC	17
10	FUS-L-N-R	CCCC CCA TGG TTA CAA TTT GGA CTT TCC GCC CTT CTT GGC C	18
11	FUS-S/TL R	GG GCC ATA CAG GAT GTT TTT GTC AGC CAT AGC GGC CGC AAA TAC AAA AAA GCC ATT GTC	19

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3129

12	FUS-TL-F	GCG GCC GCT ATG GCT GAC AAA AAC ATC CTG TAT GGC CC	20
13	FUS-TL-H-R	CCCC AAG CTT CTA ACC GTT GGT GTG TTT CTC GAA CAT CTG ACG C	21

These primers were utilized to perform PCR. The following PCR condition was used.

PCR condition

96°C for 3:00; 96°C for 0:15; 76°C for 0:30; -1°C per cycle; 72°C for 6:00;
For 15 cycles; 96°C for 0:15; 60°C for 0:30; 72°C for 6:00;
For 29 cycles; 72°C for 10:00;
14°C forever

Example 3: Cloning of the His6-BCCP Bst ATP Sulfurylase Fusion Protein

The Bst-affinity tagged fusion construct is a derivative of pRSETA in which the NheI-XhoI fragment has been replaced by the BCCP domain and the ATP sulfurylase is inserted after the BCCP domain.

Briefly, the BstSulf PCR product, as described in Example 1, was double-digested with *Pst*I and *Hind*III, isolated on a 1% agarose/TAE gel, purified using QIAEXII (QIAGEN) and ligated into the large *Pst*I/*Hind*III fragment of pRSETA-BCCP using the Quick Ligation Kit from NEB according to manufacturer's instructions. As mentioned in Example 2, pRSETA-BCCP is a derivative of pRSET A (Invitrogen) in which the sequence between *Nhe*I and *Bam*HI restriction sites has been replaced by the portion of the biotin carboxyl carrier protein (BCCP) gene from *E. coli* (GenBank accession #M80458) that codes for residues 87-165. 2 uL ligation reaction was used to transform 50 uL TOP10 competent cells (Invitrogen) and plated on LB-Ap plates. Sequencing of plasmid insert from ten clones was used to determine the consensus sequence for the ATP sulfurylase gene from ATCC 12980.

The plasmid pRSETA-BCCP-BstSulf was transformed into the *E. coli* expression host BL21(DE3)pLysS (Novagen) and the induction expression of BstHBSulf was carried out according to the maufacturer's instructions. The cells were harvested and stored as frozen pellets. The pellets were lysed using BugBuster plus Benzonase according to manufacturer's instructions and protein was purified on a 20 mL column packed with Chelating Sepharose Fast Flow (Amersham, Cat. #17-0575-02) and charged with nickel (II). Protein was eluted

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3130

using a 0-500 mM imidazole gradient. Analysis by SDS-PAGE showed a single band of the correct size.

Example 4: Binding enzymes to beads

The BCCP domain enables the *E. coli* to add a single biotin molecule onto a specific lysine residue. Hence these fusion proteins can be bound to solid supports that contain streptavidin. TL-S was successfully cloned into a TA vector. 25 µl of MPG-Streptavidin (CPG, Inc.) or Nickel-agarose (Qiagen) were taken in a 1.5 ml tube and placed on a magnet. The supernatant was removed and the beads were resuspended in 25 µg of His6-BCCP-sulfurylase and 75 µg of His6-BCCP-luciferase. To test the fusion protein, 100 µl of dialyzed fusion protein was bound to the 25 µl of beads. The beads were allowed to mix at room temperature for 1hr, washed with assay buffer (25mM Tricine (pH 7.8), 5mM MgAcetate, 1mM DTT, 1mM EDTA, and 1 mg/ml BSA) and assayed for enzyme activities with 1mM PPi, 4mM APS and 300 mM D-luciferin. With the nickel-agarose beads, the EDTA was omitted from the assay buffer.

As shown in Fig. 4, these fusion proteins displayed activity on both the NTA- Agarose and MPG-SA beads. S:L 1:3 represents sulfurylase and luciferase bound individually to beads in a 1:3 ratio. Ni-Ag and MPG-SA are nickel-agarose and MPG-Streptavidin beads, respectively. PL is Promega luciferase, which does not have a polyhistidine or a biotin tag on it and hence serves as a negative control. Fraction 19 contains the fusion protein and is active on both kinds of beads. This suggests that the fusion protein was synthesized with a poly-histidine tag and a biotin molecule on the BCCP domain of the fusion protein.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3131

What is claimed is:

1. A fusion protein comprising an ATP generating polypeptide bound to a polypeptide which converts ATP to an entity that is detectable.

2. The fusion protein of claim 1 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

3. The fusion protein of claim 2 wherein the ATP sulfurylase is a thermostable sulfurylase comprising the nucleotide sequence of SEQ ID NO:1.

4. The fusion protein of claim 3 wherein the nucleotide sequence encodes the polypeptide sequence of SEQ ID NO:2.

5. The fusion protein of claim 3 wherein the thermostable sulfurylase is active at room temperature.

6. The fusion protein of claim 2 wherein the ATP sulfurylase is from a thermophile.

7. The fusion protein of claim 6 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

8. The fusion protein of claim 1 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3132

9. The fusion protein of claim 8 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

10. The fusion protein of claim 9 wherein the animal is selected from the group consisting of mammal, rodent, insect, worm, mollusk, reptile, bird and amphibian.

11. The fusion protein of claim 9 wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Brassica napus*, *Allium sativum*, *Amaranthus caudatus*, *Hevea brasiliensis*, *Hordeum vulgare*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *Oryza sativum*, *Pisum sativum*, *Populus trichocarpa*, *Solanum tuberosum*, *Secale cereale*, *Sambucus nigra*, *Ulmus americana* or *Triticum aestivum*.

12. The fusion protein of claim 9 wherein the fungus is *Penicillum chrysogenum*, *Stachybotrys chartarum*, *Aspergillus fumigatus*, *Podospora anserina*, *Trichoderma reesei* and *Riftia pachyptila*.

13. The fusion protein of claim 9 wherein the yeast is *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida lypolitica*, *Candida utilis*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Candida spp.*, *Pichia spp.* and *Hansenula spp.*.

14. The fusion protein of claim 8 wherein the prokaryote is bacteria or archaea.

15. The fusion protein of claim 14 wherein the bacteria selected from the group consisting of *E. coli*, *B. subtilis*, *Streptococcus gordonii*, flavobacteria and green sulfur bacteria.

16. The fusion protein of claim 14 wherein the archaea is selected from the group consisting of *Sulfolobus*, *Thermococcus*, *Methanobacterium*, *Halococcus*, *Halobacterium* and *Methanococcus jannaschii*.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3133

17. The fusion protein of claim 1 wherein the detectable entity is selected from the group consisting of chemiluminescence, bioluminescence and fluorescence.

18. The fusion protein of claim 1 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

19. The fusion protein of claim 18 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

20. The fusion protein of claim 1 which further comprises an affinity tag.

21. The fusion protein of claim 20 wherein the affinity tag is selected from the group consisting of N-terminal poly-histidine, BCCP, protein A, glutathione S transferase, substance P and streptavidin binding peptide.

22. The fusion protein of claim 1 wherein the polypeptides are joined by a linker.

23. The fusion protein of claim 22 wherein the linker is an ala-ala-ala linker.

24. The fusion protein of claim 1 wherein the ATP generating polypeptide is N-terminal to the ATP converting polypeptide.

25. The fusion protein of claim 1 wherein the ATP converting polypeptide is N-terminal to the ATP generating polypeptide.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3134

26. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3 and 5.

27. An isolated polypeptide comprising an amino acid sequence selected from the group consising of SEQ ID NOs:2, 4 and 6.

28. A fusion protein comprising a sulfurylase polypeptide bound to a luciferase polypeptide and at least one affinity tag.

29. The fusion protein of claim 28 wherein the fusion protein comprises the sequence of SEQ ID NO:4.

30. The fusion protein of claim 28 wherein the fusion protein is encoded by a nucleic acid comprising the sequence of SEQ ID NO:3.

31. A fusion protein comprising a thermostable sulfurylase bound to at least one affinity tag.

32. The fusion protein of claim 31 wherein the fusion protein comprises the sequence of SEQ ID NO: 6.

33. The fusion protein of claim 31 wherein the fusion protein is encoded by a nucleic acid comprising the sequence of SEQ ID NO:5.

34. A recombinant polynucleotide that comprises a coding sequence for a fusion protein having an ATP generating polypeptide sequence and an ATP converting polypeptide sequence.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3135

35. The recombinant polynucleotide sequence of claim 34 wherein the ATP generating polypeptide is ATP sulfurylase.

36. The recombinant polynucleotide sequence of claim 34 wherein the ATP converting polypeptide is luciferase.

37. The recombinant polynucleotide of claim 34 wherein the ATP generating polypeptide is N-terminal to the ATP converting polypeptide.

38. The recombinant polynucleotide of claim 34 wherein the ATP converting polypeptide is N-terminal to the ATP generating polypeptide.

39. An expression vector for expressing a fusion protein, said vector comprising a coding sequence for a fusion protein having: (i) a regulatory sequence, (ii) a first polypeptide sequence of an ATP generating polypeptide and (iii) a second polypeptide sequence that converts ATP to an entity which is detectable.

40. The expression vector of claim 39 wherein the vector further comprises an affinity tag.

41. The expression vector of claim 39 wherein the ATP generating polypeptide is ATP sulfurylase.

42. The expression vector of claim 39 wherein the ATP converting polypeptide is luciferase.

43. The expression vector of claim 39 wherein the regulatory element is an enhancer or a promoter.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3136

44. The expression vector of claim 43 wherein the promoter is a constitutive promoter or an inducible promoter.

45. A transformed host cell which contains the expression vector of claim 39.

46. The transformed host cell of claim 45 wherein the host cell is a eukaryotic cell.

47. The transformed host cell of claim 46 wherein the eukaryotic cell is human, rat or mouse.

48. The transformed host cell of claim 45 wherein the host cell is a prokaryotic cell.

49. The transformed host cell of claim 48 wherein the prokaryotic cell is bacteria.

50. A purified fusion protein expressed by cells transformed with an expression vector of claim 39.

51. The fusion protein of claim1 bound to a mobile support.

52. The fusion protein of claim 51 wherein the fusion protein is attached by a covalent or non-covalent interaction.

53. The fusion protein of claim 52 wherein the fusion protein is attached by a linkage selected from the group consisting of a metal, a CO^{2+}-hexahistidine complex, a Ni^{2+}-hexahistidine complex, a biotin binding protein, a glutathione S-transferase/glutathione

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3137

complex, a monoclonal antibody/antigen complex, a maltose binding protein/maltose complex and pluronic coupling.

54. The fusion protein of claim 53 wherein the biotin binding protein is selected from the group consisting of NEUTRAVIDIN™ modified avidin, streptavidin and avidin.

55. The fusion protein of claim 51 wherein the mobile support is selected from the group consisting of a bead, optical fiber and glass surface.

56. The fusion protein of claim 55 wherein the bead is a nickel-agarose bead or a MPG-Streptavidin bead.

57. The fusion protein of claim 51 wherein the fusion protein is bound to the mobile support in a 1:3 ratio of protein to mobile support.

58. The fusion protein of claim 51 wherein the fusion protein is a sulfurylase-luciferase fusion protein.

59. A method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising:

(a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added;

(b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3138

(c) separately recovering each of the feedstocks from the polymerization environment; and

(d) measuring the amount of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

60. The method of claim 59 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

61. The method of claim 60 wherein the ATP sulfurylase is a thermostable sulfurylase.

62. The method of claim 60 wherein the ATP sulfurylase is from a thermophile.

63. The method of claim 62 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

64. The method of claim 59 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

65. The method of claim 64 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

66. The method of claim 59 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3139

67. The method of claim 66 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

68. The method of claim 59 which further comprises an affinity tag.

69. A method according to claim 59, wherein the amount of inorganic pyrophosphate is measured by

(a) adding adenosine-5'-phosphosulfate to the feedstock;

(b) combining the recovered feedstock containing adenosine-5'-phosphosulfate with an ATP generating polypeptide-ATP converting polypeptide fusion protein such that any inorganic pyrophosphate in the recovered feedstock and the adenosine-5'-phosphosulfate will react to the form ATP and sulfate;

(c) combining the ATP, sulfate, and said fusion protein-containing feedstock with luciferin in the presence of oxygen such that the ATP is consumed to produced AMP, inorganic pyrophosphate, carbon dioxide and light; and

(d) measuring the amount of light produced.

70. The method of claim 69 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

71. The method of claim 70 wherein the ATP sulfurylase is a thermostable sulfurylase.

72. The method of claim 70 wherein the ATP sulfurylase is from a thermophile.

73. The method of claim 72 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3140

74. The method of claim 69 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

75. The method of claim 74 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

76. The method of claim 69 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

77. The method of claim 76 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

78. The method of claim 69 which further comprises an affinity tag.

79. The method according to claim 59 wherein each feedstock comprises adenosine-5'-phosphosulfate and luciferin in addition to the selected nucleotide base, and the amount of inorganic pyrophosphate is determincd by reacting the inorganic pyrophosphate is determined by reacting the inorganic pyrophosphate-containing feedstock with an ATP generating polypeptide-ATP converting polypeptide fusion protein thereby producing light in an amount proportional to the amount of inorganic pyrophosphate, and measuring the amount of light produced.

80. The method of claim 79 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

81. The method of claim 80 wherein the ATP sulfurylase is a thermostable sulfurylase.

82. The method of claim 80 wherein the ATP sulfurylase is from a thermophile.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3141

83. The method of claim 82 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

84. The method of claim 79 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

85. The method of claim 84 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

86. The method of claim 79 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

87. The method of claim 86 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

88. The method of claim 79 which further comprises an affinity tag.

89. A method for sequencing a nucleic acid, the method comprising:

(a) providing one or more nucleic acid anchor primers;

(b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm;

(c) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3142

(d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;

(e) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid;

(f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and

(g) identifying the sequencing reaction byproduct with the use of an ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the sequence of the nucleic acid.

90. The method of claim 89 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

91. The method of claim 90 wherein the ATP sulfurylase is a thermostable sulfurylase.

92. The method of claim 90 wherein the ATP sulfurylase is from a thermophile.

93. The method of claim 92 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

94. The method of claim 89 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

95. The method of claim 94 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3143

96. The method of claim 89 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

97. The method of claim 96 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

98. The method of claim 89 which further comprises an affinity tag.

99. A method for sequencing a nucleic acid, the method comprising:

(a) providing at least one nucleic acid anchor primer;

(b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites;

(c) annealing a first amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex;

(d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;

(e) annealing a second amount of a sequencing primer to one or more copies of the covalently linked complementary nucleic acid;

(f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, when the predetermined nucleotide triphosphate is incorporated onto the 3' end of the sequencing primer, to yield a sequencing reaction byproduct; and

(g) identifying the sequencing reaction byproduct with the use of an ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the sequence of the nucleic acid at each reaction site that contains a nucleic acid template.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3144

100. The method of claim 99 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

101. The method of claim 100 wherein the ATP sulfurylase is a thermostable sulfurylase.

102. The method of claim 100 wherein the ATP sulfurylase is from a thermophile.

103. The method of claim 102 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

104. The method of claim 99 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

105. The method of claim 104 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

106. The method of claim 99 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

107. The method of claim 106 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

108. The method of claim 99 which further comprises an affinity tag.

109. A method of determining the base sequence of a plurality of nucleotides on an array, the method comprising:

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3145

(a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm,

(b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates;

(c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands through detection of a sequencing byproduct with an ATP generating polypeptide-ATP converting polypeptide fusion protein, thus indicating that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and

(d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and

(e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

110. The method of claim 109 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

111. The method of claim 110 wherein the ATP sulfurylase is a thermostable sulfurylase.

112. The method of claim 110 wherein the ATP sulfurylase is from a thermophile.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3146

113. The method of claim 112 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

114. The method of claim 109 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

115. The method of claim 114 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

116. The method of claim 109 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

117. The method of claim 116 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

118. The method of claim 109 which further comprises an affinity tag.

119. A method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising:

(a) introducing a plurality of template nucleic acid polymers into a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, each reaction chamber having a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3147

(b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released;

(c) detecting the formation of inorganic pyrophosphate with an ATP generating polypeptide-ATP converting polypeptide fusion protein to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

120. The method of claim 119 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

121. The method of claim 120 wherein the ATP sulfurylase is a thermostable sulfurylase.

122. The method of claim 120 wherein the ATP sulfurylase is from a thermophile.

123. The method of claim 122 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

124. The method of claim 119 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

125. The method of claim 124 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

126. The method of claim 119 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3148

127. The method of claim 126 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

128. The method of claim 119 which further comprises an affinity tag.

129. A method of identifying the base in a target position in a DNA sequence of sample DNA, wherein:

(a) sample DNA is disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, said DNA being rendered single stranded either before or after being disposed in the reaction chambers,

(b) an extension primer is provided which hybridizes to said immobilized single-stranded DNA at a position immediately adjacent to said target position;

(c) said immobilized single-stranded DNA is subjected to a polymerase reaction in the presence of a predetermined nucleotide triphosphate, wherein if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer then a sequencing reaction byproduct is formed; and

(d) identifying the sequencing reaction byproduct with an ATP generating polypeptide-ATP converting polypeptide fusion protein, thereby determining the nucleotide complementary to the base at said target position.

130. The method of claim 129 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

131. The method of claim 130 wherein the ATP sulfurylase is a thermostable sulfurylase.

132. The method of claim 130 wherein the ATP sulfurylase is from a thermophile.

133. The method of claim 132 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus*

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3149

caldolyticus, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

134. The method of claim 129 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

135. The method of claim 134 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

136. The method of claim 129 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

137. The method of claim 136 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

138. The method of claim 129 which further comprises an affinity tag.

139. A method of identifying a base at a target position in a sample DNA sequence comprising:

(a) providing sample DNA disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm, said DNA being rendered single stranded either before or after being disposed in the reaction chambers;

(b) providing an extension primer which hybridizes to the sample DNA immediately adjacent to the target position;

(c) subjecting the sample DNA sequence and the extension primer to a polymerase reaction in the presence of a nucleotide triphosphate whereby the nucleotide triphosphate will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3150

in the target position, said nucleotide triphosphate being added either to separate aliquots of sample-primer mixture or successively to the same sample-primer mixture; and

(d) detecting the release of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein to indicate which nucleotide is incorporated.

140. The method of claim 139 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

141. The method of claim 140 wherein the ATP sulfurylase is a thermostable sulfurylase.

142. The method of claim 140 wherein the ATP sulfurylase is from a thermophile.

143. The method of claim 142 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

144. The method of claim 139 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

145. The method of claim 144 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

146. The method of claim 139 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

147. The method of claim 146 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3151

148. The method of claim 139 which further comprises an affinity tag.

149. A method of identifying a base at a target position in a single-stranded sample DNA sequence, the method comprising:
(a) providing an extension primer which hybridizes to sample DNA immediately adjacent to the target position, said sample DNA disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 um, said DNA being rendered single stranded either before or after being disposed in the reaction chambers;
(b) subjecting the sample DNA and extension primer to a polymerase reaction in the presence of a predetermined deoxynucleotide or dideoxynucleotide whereby the deoxynucleotide or dideoxynucleotide will only become incorporated and release pyrophosphate (PPi) if it is complementary to the base in the target position, said predetermined deoxynucleotides or dideoxynucleotides being added either to separate aliquots of sample-primer mixture or successively to the same sample-primer mixture,
(c) detecting any release of PPi with an ATP generating polypeptide-ATP converting polypeptide fusion protein to indicate which deoxynucleotide or dideoxynucleotide is incorporated;
characterized in that, the PPi-detection enzyme(s) are included in the polymerase reaction step and in that in place of deoxy- or dideoxy adenosine triphosphate (ATP) a dATP or ddATP analogue is used which is capable of acting as a substrate for a polymerase but incapable of acting as a substrate for a said PPi—detection enzyme.

150. The method of claim 149 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

151. The method of claim 150 wherein the ATP sulfurylase is a thermostable sulfurylase.

152. The method of claim 150 wherein the ATP sulfurylase is from a thermophile.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3152

153. The method of claim 152 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

154. The fusion protein of claim 149 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

155. The method of claim 154 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

156. The method of claim 149 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

157. The method of claim 156 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

158. The method of claim 149 which further comprises an affinity tag.

159. A method of determining the base sequence of a plurality of nucleotides on an array, the method comprising:

(a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm,

(b) converting PPi into light with a an ATP generating polypeptide-ATP converting polypeptide fusion protein;

(c) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3153

(d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions;

(e) determining a light intensity for each of said discrete regions from the corresponding electrical signal;

(f) recording the variations of said electrical signals with time.

160. The method of claim 159 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

161. The method of claim 160 wherein the ATP sulfurylase is a thermostable sulfurylase.

162. The method of claim 160 wherein the ATP sulfurylase is from a thermophile.

163. The method of claim 162 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

164. The method of claim 159 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

165. The method of claim 164 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

166. The method of claim 159 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3154

167. The method of claim 166 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

168. The method of claim 159 which further comprises an affinity tag.

169. Method for sequencing a nucleic acid, the method comprising:

(a) providing one or more nucleic acid anchor primers;

(b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm;

(c) converting PPi into a detectable entity with the use of an ATP generating polypeptide-ATP converting polypeptide fusion protein;

(d) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device;

(e) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions;

(f) determining a light intensity for each of said discrete regions from the corresponding electrical signal;

(g) recording the variations of said electrical signals with time.

170. The method of claim 169 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

171. The method of claim 170 wherein the ATP sulfurylase is a thermostable sulfurylase.

172. The method of claim 170 wherein the ATP sulfurylase is from a thermophile.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3155

173. The method of claim 172 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

174. The method of claim 169 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

175. The method of claim 174 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

176. The method of claim 169 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

177. The method of claim 176 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

178. The method of claim 169 which further comprises an affinity tag.

179. A method for sequencing a nucleic acid, the method comprising:

(a) providing at least one nucleic acid anchor primer;

(b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites;

(c) converting PPi into a detectable entity with an ATP generating polypeptide-ATP converting polypeptide fusion protein;

(d) detecting the light level emitted from a plurality of reaction sites on respective portions of an optically sensitive device;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3156

(e) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other regions;

(f) determining a light intensity for each of said discrete regions from the corresponding electrical signal;

(g) recording the variations of said electrical signals with time.

180. The method of claim 179 wherein the ATP generating polypeptide is selected from the group consisting of ATP sulfurylase, hydrolase and ATP synthase.

181. The method of claim 180 wherein the ATP sulfurylase is a thermostable sulfurylase.

182. The method of claim 180 wherein the ATP sulfurylase is from a thermophile.

183. The method of claim 182 wherein the thermophile is a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

184. The method of claim 179 wherein the ATP generating polypeptide and ATP converting polypeptide are from a eukaryote or a prokaryote.

185. The method of claim 184 wherein the eukaryote is selected from the group consisting of animal, plant, fungus and yeast.

186. The method of claim 179 wherein the ATP converting polypeptide is selected from the group consisting of luciferase, ecto-nucleoside diphosphate kinase and ATPase.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3157

187. The method of claim 186 wherein the luciferase is selected from the group consisting of *Photinus pyralis*, *Pyroplorus plagiophihalamus* (*Coleoptera*), *Luciola cruciata* and *Luciola lateralis*.

188. The method of claim 179 which further comprises an affinity tag.

189. A kit comprising a sulfurylase-luciferase fusion protein expression vector as claimed in claim 39.

190. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence of SEQ ID NO: 2;

(b) a variant of a mature form of an amino acid sequence of SEQ ID NO: 2;

(c) an amino acid sequence of SEQ ID NO: 2;

(d) a variant of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 4% of amino acid residues from said amino acid sequence; and

(e) an amino acid sequence of (a), (b), (c) or (d) further containing one or more conservative amino acid substitutions.

191. The polypeptide of claim 190 wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence of SEQ ID NO: 2.

192. The polypeptide of claim 190 wherein the amino acid sequence of said variant comprises one or more conservative amino acid substitution.

193. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence of SEQ ID NO: 2;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3158

(b) a variant of a mature form of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 4% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence of SEQ ID NO: 2;

(d) a variant of an amino acid sequence of SEQ ID NO: 2, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 4% of amino acid residues from said amino acid sequence;

(e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 4% of amino acid residues from said amino acid sequence; and

(f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).

194. The nucleic acid molecule of claim 193 wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

195. The nucleic acid molecule of claim 193 wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

196. The nucleic acid molecule of claim 193 wherein the nucleic acid molecule comprises nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 11% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

(b) an isolated second polynucleotide that is a complement of the first polynucleotide; and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3159

(c) a nucleic acid fragment of (a) or (b).

197. A vector comprising the nucleic acid molecule of claim 196.

198. The vector of claim 197, further comprising a promoter operably-linked to said nucleic acid molecule.

199. A cell comprising the vector of claim 197.

200. An antibody that binds immunospecifically to the polypeptide of claim 190.

201. A method for determining the nucleic acid sequence in a template nucleic acid polymer, comprising:

(a) introducing the template nucleic acid polymer into a polymerization environment in which the nucleic acid polymer will act as a template polymer for the synthesis of a complementary nucleic acid polymer when nucleotides are added;

(b) successively providing to the polymerization environment a series of feedstocks, each feedstock comprising a nucleotide selected from among the nucleotides from which the complementary nucleic acid polymer will be formed, such that if the nucleotide in the feedstock is complementary to the next nucleotide in the template polymer to be sequenced said nucleotide will be incorporated into the complementary polymer and inorganic pyrophosphate will be released;

(c) separately recovering each of the feedstocks from the polymerization environment; and

(d) measuring the amount of PPi with a thermostable sulfurylase and a luciferase in each of the recovered feedstocks to determine the identity of each nucleotide in the complementary polymer and thus the sequence of the template polymer.

202. The method of claim 201 wherein said thermostable sulfurylase comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence of SEQ ID NO: 2.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3160

203. The method of claim 201 wherein the thermostable sulfurylase is derived from a thermophilic bacteria selected from the group consisting of *Bacillus stearothermophilus*, *Thermus thermophilus*, *Bacillus caldolyticus*, *Bacillus subtilis*, *Bacillus thermoleovorans*, *Pyrococcus furiosus*, *Sulfolobus acidocaldarius*, *Rhodothermus obamensis*, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

204. The method of claim 201 wherein the thermostable sulfurylase and the luciferase are joined in a fusion protein.

205. The method of claim 201 wherein the thermostable sulfurylase is joined to an affinity tag.

206. A method for sequencing a nucleic acid, the method comprising:

(a) providing one or more nucleic acid anchor primers;

(b) providing a plurality of single-stranded circular nucleic acid templates disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200;

(c) annealing an effective amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex;

(d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;

(e) annealing an effective amount of a sequencing primer to one or more copies of said covalently linked complementary nucleic acid;

(f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct; and

(g) identifying the sequencing reaction byproduct with the use of a thermostable sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3161

207. The method of claim 206 wherein said thermostable sulfurylase comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence of SEQ ID NO: 2.

208. The method of claim 206 wherein the thermostable sulfurylase is derived from a thermophilic bacteria selected from the group consisting of Bacillus stearothermophilus, Thermus thermophilus, Bacillus caldolyticus, Bacillus subtilis, Bacillus thermoleovorans, Pyrococcus furiosus, Sulfolobus acidocaldarius, Rhodothermus obamensis, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

209. The method of claim 206 wherein the thermostable sulfurylase and the luciferase are joined in a fusion protein.

210. The method of claim 206 wherein the thermostable sulfurylase is joined to an affinity tag.

211. A method for sequencing a nucleic acid, the method comprising:

(a) providing at least one nucleic acid anchor primer;

(b) providing a plurality of single-stranded circular nucleic acid templates in an array having at least 400,000 discrete reaction sites;

(c) annealing a first amount of the nucleic acid anchor primer to at least one of the single-stranded circular templates to yield a primed anchor primer-circular template complex;

(d) combining the primed anchor primer-circular template complex with a polymerase to form an extended anchor primer covalently linked to multiple copies of a nucleic acid complementary to the circular nucleic acid template;

(e) annealing a second amount of a sequencing primer to one or more copies of the covalently linked complementary nucleic acid;

(f) extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, when the predetermined nucleotide triphosphate is incorporated onto the 3' end of the sequencing primer, to yield a sequencing

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3162

reaction byproduct; and

(g) identifying the sequencing reaction byproduct with the use of a thermostable sulfurylase and a luciferase, thereby determining the sequence of the nucleic acid at each reaction site that contains a nucleic acid template.

212. The method of claim 211 wherein said thermostable sulfurylase comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence of SEQ ID NO: 2.

213. The method of claim 211 wherein the thermostable sulfurylase is derived from a thermophilic bacteria selected from the group consisting of Bacillus stearothermophilus, Thermus thermophilus, Bacillus caldolyticus, Bacillus subtilis, Bacillus thermoleovorans, Pyrococcus furiosus, Sulfolobus acidocaldarius, Rhodothermus obamensis, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

214. The method of claim 211 wherein the thermostable sulfurylase and the luciferase are joined in a fusion protein.

215. The method of claim 211 wherein the thermostable sulfurylase is joined to an affinity tag.

216. A method of determining the base sequence of a plurality of nucleotides on an array, the method comprising:

(a) providing a plurality of sample DNAs, each disposed within a plurality of cavities on a planar surface, each cavity forming an analyte reaction chamber, wherein the reaction chambers have a center to center spacing of between 5 to 200 μm,

(b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber, each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3163

template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates;

(c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands through detection of a sequencing byproduct with a thermostable sulfurylase and luciferase, thus indicating that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor; and

(d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition; and

(e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors.

217. The method of claim 216 wherein said thermostable sulfurylase comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence of SEQ ID NO: 2.

218. The method of claim 216 wherein the thermostable sulfurylase is derived from a thermophilic bacteria selected from the group consisting of Bacillus stearothermophilus, Thermus thermophilus, Bacillus caldolyticus, Bacillus subtilis, Bacillus thermoleovorans, Pyrococcus furiosus, Sulfolobus acidocaldarius, Rhodothermus obamensis, *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Aeropyrum pernix*, *Pyrobaculum aerophilum*, *Pyrococcus abyssi*, *Penicillium chrysogenum*, *Sulfolobus solfataricus* and *Thermomonospora fusca*.

219. The method of claim 216 wherein the thermostable sulfurylase and the luciferase are joined in a fusion protein.

220. The method of claim 216 wherein the thermostable sulfurylase is joined to an affinity tag.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3164

FIGURE 1

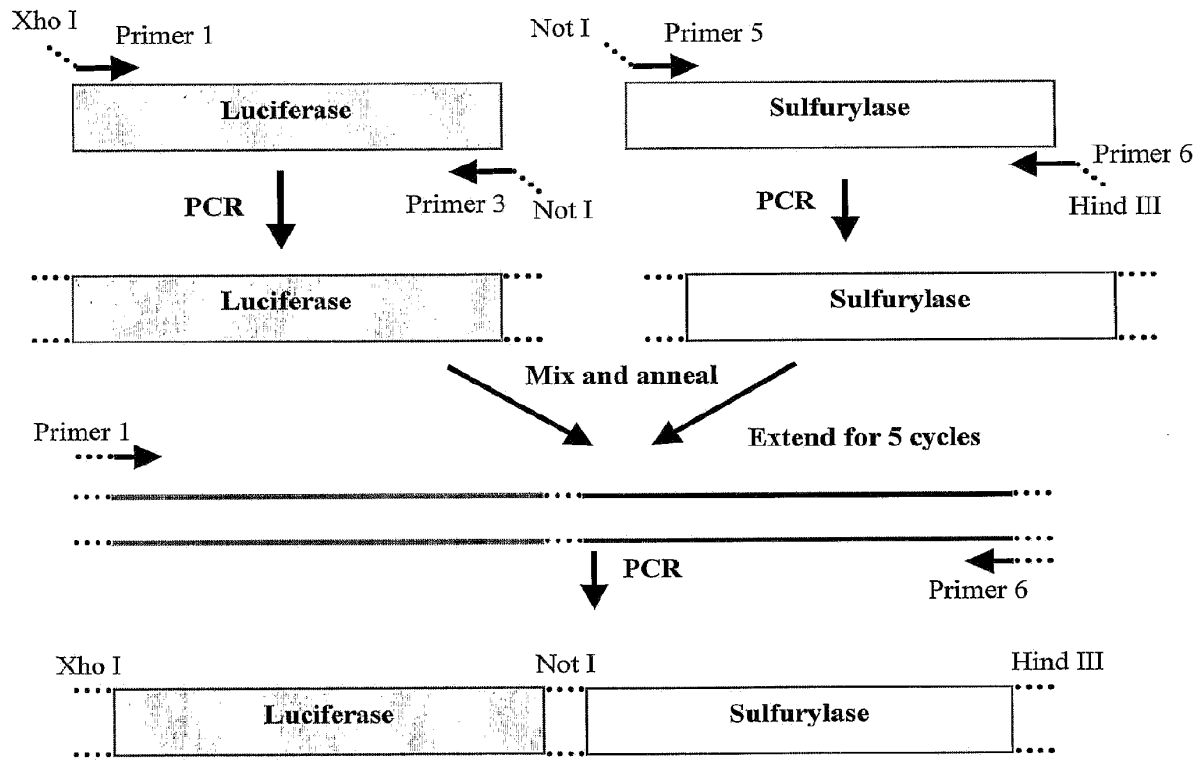
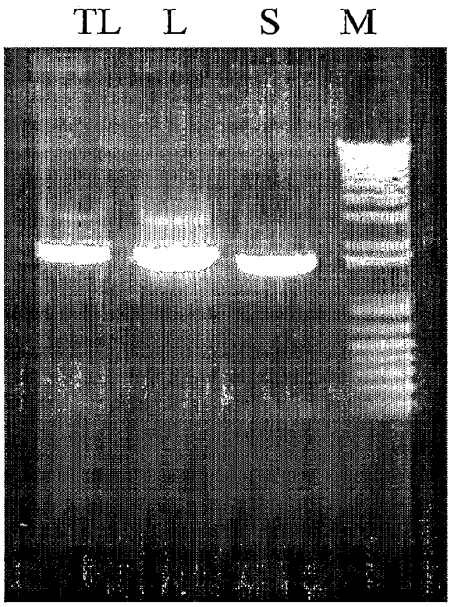
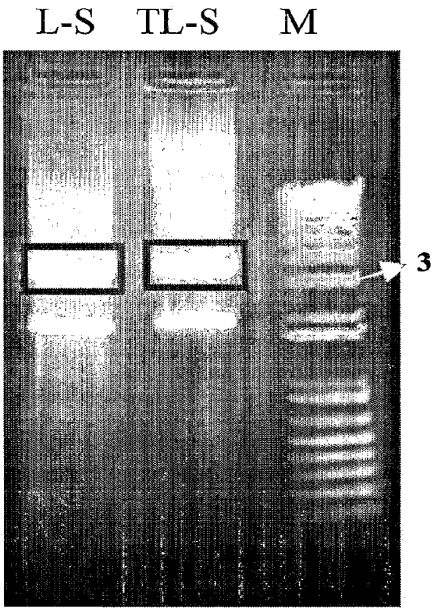

Xho I
Primer 1
Not I
Primer 5
Luciferase
Sulfurylase
Primer 6
PCR
Primer 3
Not I
PCR
Hind III
Luciferase
Sulfurylase
Mix and anneal
Primer 1
Extend for 5 cycles
PCR
Primer 6
Xho I
Not I
Hind III
Luciferase
Sulfurylase

Figure 2

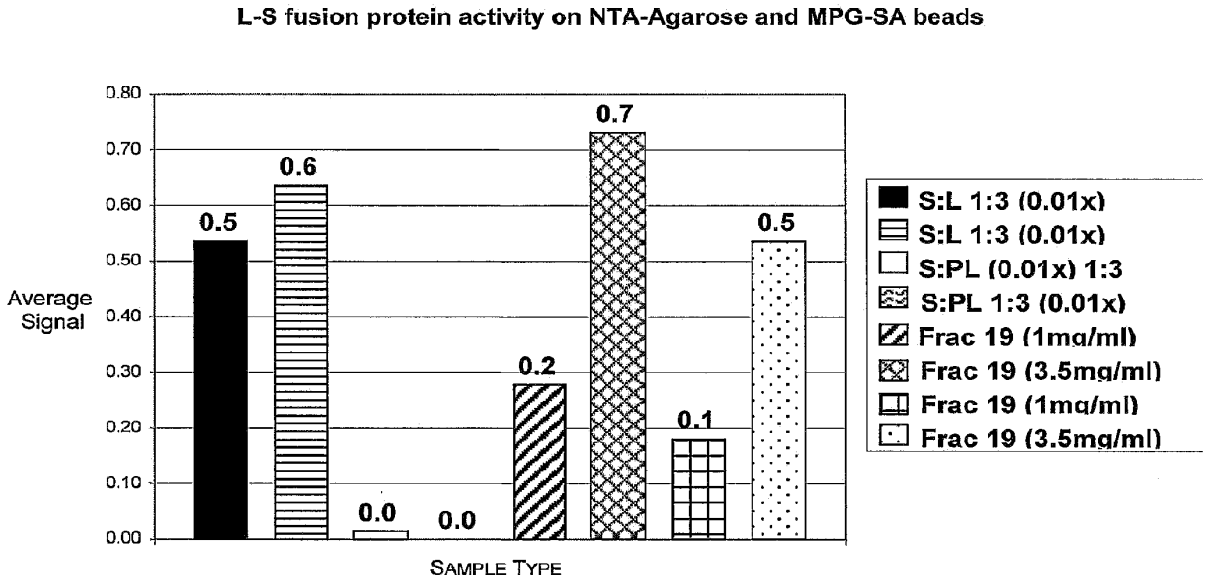


2A



2B

FIGURE 3


L-S fusion protein activity on NTA-Agarose and MPG-SA beads
Average Signal
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.5
0.6
0.0
0.0
0.2
0.7
0.1
0.5
SAMPLE TYPE
S:L 1:3 (0.01x)
S:L 1:3 (0.01x)
S:PL (0.01x) 1:3
S:PL 1:3 (0.01x)
Frac 19 (1mg/ml)
Frac 19 (3.5mg/ml)
Frac 19 (1mg/ml)
Frac 19 (3.5mg/ml)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2005 (17.03.2005)

PCT

(10) International Publication Number
WO 2005/023414 A1

(51) International Patent Classification[7]: B01J 19/00

(21) International Application Number:
PCT/US2004/027599

(22) International Filing Date: 25 August 2004 (25.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/651,568 29 August 2003 (29.08.2003) US

(71) Applicant *(for all designated States except US)*: **ILLUMINA, INC.** [US/US]; 9885 Towne Centre Drive, San Diego, California 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ELLMAN, Brett** [US/US]; 1736 Westminster Drive, Cardiff, California 92007 (US). **LEBL, Michal** [CZ/US]; 4532 Mercurio Street, San Diego, California 92130 (US). **JONES, Aaron** [US/US]; 11140 Caminito Vista Pacific, San Diego, California 92131 (US). **FAMBRO, Steve** [US/US]; 2012 Ladera Court, Carlsbad, California 92009 (US). **HEINER, David** [US/US]; 8967 January Place, San Diego, California 92122 (US).

(74) Agents: **SILVA, Robin, M.** et al.; Dorsey & Whitney LLP, 4 Embarcadero Center, Suite 3400, San Francisco, CA 94111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

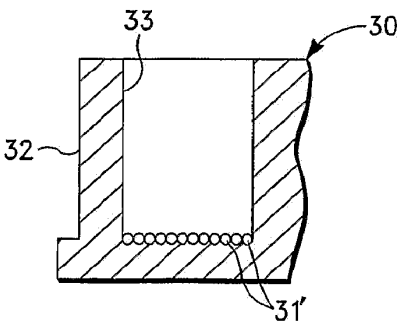
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for all designations*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF FORMING AND USING SOLID-PHASE SUPPORT



(57) Abstract: A method of forming a solid-phase support (30), the method including the steps of providing a substrate (32) having a reaction vessel (33), dispensing a particle (31) in the reaction vessel (33), and permanently bonding the particle in the substrate (32) within the reaction vessel (33). The particle (31) may include a microbead. The particle (31) may include controlled pore glass. A method of synthesis is also disclosed that includes including the steps of providing a solid-phase support (30) including a particle (31) embedded to the substrate (32) adjacent a surface of substrate, the particle (31) being functionalized to covalently attach an intermediate compound of a synthetic reaction, dispensing a liquid including a reagent to the solid-phase support (31) to effect the synthetic reaction, and removing the liquid from the solid-phase support (31) by centrifugation, whereby the intermediate compound remains attached to the substrate (32) by the particle (31).

WO 2005/023414 A1

Attorney Docket No. 33493/RMS/VEJ

METHOD OF FORMING AND USING SOLID-PHASE SUPPORT

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under Grant No. 9 R44 HG02805-02 awarded by National Human Genome Research Institute. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] This invention relates, in general, to a solid-phase support for high-throughput synthesis, and more particularly to substrates having particles attached thereto, the solid-phase support being capable of withstanding centrifugal forces generated during high-throughput synthesis.

Description of Related Art

[0003] Solid-phase synthesis of organic molecules is the method of choice for preparation of libraries and compound megaarrays, which are currently being applied for screening in the quest to find new drugs or pharmaceutical lead compounds, i.e., compounds which exhibit a particular biological activity of pharmaceutical interest. These leads can serve as a starting point for the selection and synthesis of a drug compound, which in addition to the particular biological activity of interest have pharmacologic and toxicologic properties suitable for administration to animals, including humans.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3169

[0004] Solid-phase synthesis requires repetitions of several relatively simple operations of addition of reagents, incubation and separation of liquid-phases. In solid-phase synthesis, final compounds are synthesized attached to solid-phase supports that permit the use of mechanical means to separate intermediate, partially-synthesized compounds between synthesis steps. Various designs of manual and automated instruments for combinatorial synthesis utilizing solid-phase synthesis have appeared in patent and non-patent literature. For example, U.S. Patent Nos. 5,202,418 and 5,338,831, both to Lebl et al., each describe a method of performing multiple synthesis of peptides on a solid carrier. U.S. Patent No. 5,342,585, also to Lebl et al., describes an apparatus for multiple syntheses of peptides on solid support. U.S. Patent No. 6,045,755, also to Lebl, et al., describes an apparatus and a method for combinatorial chemistry synthesis. The entire contents of the above patents are incorporated herein by this reference.

[0005] Some known instruments have employed centrifugation for the separation of solid and liquid-phases in order to provide devices that are capable of high-throughput synthesis. Such devices can utilize solid-phase particles, for example, macrobeads or microbeads, as a solid-phase support to which intermediate and final compounds are attached. Such solid-phase particles are generally suspended within a reaction vessel, which reaction vessel is subjected to centrifugal forces to separate the liquid and solid-phases. For example, U.S. Patent No. 6,121,054 to Lebl, describes a method for separation of liquid and solid-phases for solid-phase organic synthesis in which a slurry of beads are suspended in a solvent. Similarly, U.S. Patent Application Publication No. US 2002/0044894 A1 to Lebl et al. describes an oligonucleotide synthesizer that utilizes centrifugation to separate the liquid-phase from beads. Generally, such instruments employ mechanical means to retain the solid-phase beads within a reaction vessel, for example, a well of a microtiter plate, during centrifugation. The entire contents of the above patent and publication are incorporated herein by this reference.

[0006] One means to retain the solid-phase beads is to tilt the reaction vessel to form pockets from which the solid-phase cannot be removed by centrifugal force. See, e.g.,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3170

U.S. Patent No. 6,121,054. Alternatively, mesh or other frit materials can be employed to enclose and restrain the beads within the reaction vessel. See, e.g., U.S. Patent Application Publication No. US 2002/0044894 A1. Disadvantageously, such retention means increase the mechanical complexity of the instruments and/or the reaction vessels.

[0007] What is needed is a solid-phase support for high-throughput synthesis that overcomes the above and other disadvantages of known instruments employing solid-phase particles suspended within reaction vessels.

BRIEF SUMMARY OF THE INVENTION

[0008] In summary, one aspect of the present invention is directed to a method of forming a solid-phase support, the method including the steps of providing a substrate having a reaction vessel, dispensing a particle in the reaction vessel, and permanently bonding the particle in the substrate within the reaction vessel. The particle may include a microbead. The particle may include controlled pore glass. The dispensing step may include brushing the particle into a recess of a dispenser plate. The substrate may include a microtiter plate including a plurality of microtiter wells. The dispensing plate may include a plurality of the recesses and each of the microtiter the wells may define a respective reaction vessel that is aligned with a respective the recess. The dispensing step may further include contacting the dispensing plate and the microtiter plate such that the recess is aligned with the reaction vessel. The dispensing step may further include pivoting the dispensing plate into engagement with the microtiter plate. The dispensing step may further include providing a first dispensing pressure to the recess facilitating transfer of the particle from the recess to the reaction vessel.

[0009] In one embodiment, bonding can include embedding a particle in a substrate. Bonding can also include heating the substrate. The embedding step may further include heating the substrate, thereby increasing the temperature of a portion of the substrate, and in the case of multi-well plate at least a portion of each the reaction

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3171

vessels, to the melting point of the substrate. The bonding step may further include providing a second bonding pressure to the reaction vessel biasing the particle against the reaction vessel. The bonding step may further include heating the substrate, thereby increasing the temperature of a portion of the substrate adjacent a bottom surface of each the reaction vessel to the melting point of the substrate. The bonding step may further include providing a second bonding pressure to the reaction vessel biasing the particle against the bottom of the reaction vessel. The method may include dispensing a plurality of the particles to the reaction vessel, thereby bonding one or more of the particles into the substrate within the reaction vessel. The one or more of the particles may remain nonbonded to the substrate. The method may further include the step of removing the one or more nonbonded particles from the reaction vessel. The providing step may include providing a bottomless-well plate and a rigid sheet, the method further including providing a layer of hot-melt adhesive to the rigid sheet to form a laminate sheet, applying the laminate sheet to a bottom surface of the bottomless-well plate thereby forming the substrate having a reaction vessel. The bonding step may include embedding the particle in the layer of hot-melt adhesive.

[0010] Another aspect of the present invention is directed to a method of synthesis including the steps of providing a solid-phase support including a particle bonded to the substrate adjacent a surface of substrate, the particle being functionalized to covalently attach an intermediate compound of a synthetic reaction, dispensing a liquid including a reagent to the solid-phase support to effect the synthetic reaction, and removing the liquid from the solid-phase support by centrifugation, whereby the intermediate compound remains attached to the substrate by the particle. The solid-phase support may include a reaction vessel and wherein the particle may be bonded the substrate and in the reaction vessel. The solid-phase support may include a microtiter plate. The particle may include controlled pore glass. The providing step may include heating the substrate and bonding the particle in the substrate, thereby making the solid-phase support. The removing step may include centrifugation around an axis of rotation, wherein the surface of the substrate may be oriented away

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3172

from the axis of rotation and wherein the particles may remain bonded in the surface. The particle may include 1400 Angstrom controlled pore glass.

[0011] A further aspect of the present invention is directed to a method of making a solid-phase support, including heating a substrate, thereby forming a molten substrate, contacting the molten substrate with a particle, and allowing the molten substrate to cool in the presence of the particle, thereby making a solid-phase support including the particle bonded in the substrate.

[0012] Yet another aspect of the present invention is directed to a method of separating a liquid and solid-phase, including providing a solid-phase support, the solid phase support including a particle embedded in a surface of a substrate, and removing the liquid from the solid-phase support by centrifugation around an axis of rotation, wherein the surface of the substrate may be oriented away from the axis of rotation and wherein the particles remain embedded in the surface.

[0013] An object of the present invention is to provide a method of forming and using a solid-phase support.

[0014] Another object of the present invention is to provide a substrate having a solid-phase support attached thereto, in which the substrate and support are capable of withstanding centrifugal forces generated during high-throughput synthesis.

[0015] Yet another object of the present invention is to provide a method of forming a substrate of the above character.

[0016] The solid-phase support of the present invention has other features and advantages which will be apparent from or are set forth in more detail in the accompanying drawings, which are incorporated in and form a part of this specification, and the following Detailed Description of the Invention, which together serve to explain the principles of the present invention.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3173

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 is a perspective view of a solid-phase support for high-throughput synthesis in accordance with the present invention.

[0018] FIG. 2 is an enlarged, fragmented, cross-sectional view of the solid-phase support of FIG. 1 schematically illustrating particles disposed in the well of the substrate prior to bonding of the particles to the substrate.

[0019] FIG. 3 is an enlarged, fragmented, cross-sectional view of the solid-phase support of FIG. 1 schematically illustrating particles disposed in the well of the substrate following bonding but prior to removal of excess particles from the well.

[0020] FIG. 4 is an enlarged, fragmented, cross-sectional view of the solid-phase support of FIG. 1 schematically illustrating particles bonded to the well of the substrate.

[0021] FIG. 5 is a schematic view of a workstation for applying particles to a substrate, which figure shows particles which have been loaded on an applicator.

[0022] FIG. 6 is a schematic view of the workstation of FIG. 5 in which a reclaim tray has been disengaged from the applicator.

[0023] FIG. 7 is a schematic view of the workstation of FIG. 5 in which a substrate has been mounted on a heating element.

[0024] FIG. 8 is a schematic view of the workstation of FIG. 5 in which the applicator has been pivoted from a loading position to a bonding position resting against the substrate.

[0025] FIG. 9 is a schematic view of the workstation of FIG. 5 in which the applicator has been returned to its loading position and the substrate has been removed from the heating element.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3174

[0026] FIG. 10 is a cross-sectional view of another substrate for high-throughput synthesis in accordance with the present invention.

[0027] FIG. 11 is an enlarged perspective view of particles embedded in a substrate in accordance with the present invention.

[0028] FIG. 12 is a perspective view of the solid-phase support of FIG. 1 mounted on rotor assembly of a high-throughput synthesis apparatus.

DETAILED DESCRIPTION OF THE INVENTION

[0029] Reference will now be made in detail to particular embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention will be described in conjunction with particular embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover alternatives, modifications and equivalents, which can be included within the spirit and scope of the invention as defined by the appended claims.

[0030] The present invention is directed to a solid-phase support for separation of liquid and solid phase components of a two-phase system such as a synthetic or analytical system. A solid-phase support of the invention can be used for high-throughput synthesis including, but not limited to, solid-phase combinatorial chemistry synthesis of organic molecules. In particular, the present invention is directed to substrates particularly suited for separation of components using a centrifuge. For example, the substrates are particularly suited for use with high-throughput synthesis ("HTS") apparatus which utilize a centrifugal rotor assembly for the step-wise addition and removal of solid-phase and liquid-phase solutions, and the separation and removal of the solid-phase synthetic reactions, as is described in U.S. Patent No. 6,121,054 to Lebl ("the Lebl '054 patent"), and in U.S. Patent Application Publication No. US 2002/0044894 A1 to Lebl et al. ("the Lebl '894 publication"), the entire contents of which patent and publication are incorporated by this reference.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3175

The present invention is also useful for other phase separation systems such as those based on removal of a liquid phase from a solid phase by pipetting or aspiration.

[0031] Accordingly, the invention provides a method of separating a liquid and solid-phase. The method includes the steps of (a) providing a solid-phase support having a particle embedded in a surface of a substrate; and (b) removing the liquid from the solid-phase support by centrifugation around an axis of rotation, wherein the surface of the substrate is oriented away from the axis of rotation and wherein the particles remain embedded in the surface.

[0032] By way of introduction, combinatorial chemistry synthesis protocols prescribe the stepwise, sequential addition of building blocks to intermediate and/or partially synthesized intermediate compounds in order to synthesize a final compound. In solid-phase synthesis, final compounds are synthesized attached to solid-phase supports that permit the use of simple mechanical means to separate intermediate, partially-synthesized intermediate compounds between synthetic steps.

[0033] In a particular embodiment, a solid-phase support of the present invention is used to synthesize nucleic acids. By "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that can have alternate backbones, comprising, for example, phosphoramide (Beaucage et al., Tetrahedron 49(10):1925 (1993) and references therein; Letsinger, J. Org. Chem. 35:3800 (1970); Sprinzl et al., Eur. J. Biochem. 81:579 (1977); Letsinger et al., Nucl. Acids Res. 14:3487 (1986); Sawai et al, Chem. Lett. 805 (1984), Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); and Pauwels et al., Chemica Scripta 26:141 91986)), phosphorothioate (Mag et al., Nucleic Acids Res. 19:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu et al., J. Am. Chem. Soc. 111:2321 (1989), O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3176

Soc. 114:1895 (1992); Meier et al., Chem. Int. Ed. Engl. 31:1008 (1992); Nielsen, Nature, 365:566 (1993); Carlsson et al., Nature 380:207 (1996), all of which are incorporated by reference). Other analog nucleic acids include those with positive backbones (Denpcy et al., Proc. Natl. Acad. Sci. USA 92:6097 (1995); non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi et al., Angew. Chem. Intl. Ed. English 30:423 (1991); Letsinger et al., J. Am. Chem. Soc. 110:4470 (1988); Letsinger et al., Nucleoside & Nucleotide 13:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker et al., Bioorganic & Medicinal Chem. Lett. 4:395 (1994); Jeffs et al., J. Biomolecular NMR 34:17 (1994); Tetrahedron Lett. 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins et al., Chem. Soc. Rev. (1995) pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone can be done to increase the stability and half-life of such molecules in physiological environments.

[0034] The nucleic acids (sometimes referred to herein as oligonucleotides) can be synthesized using a variety of possible synthetic reactions. In a particular embodiment, phosphoramidite chemistry is used, with enzymatic techniques and techniques based on photodeprotection useful as well. In addition, any number of nucleic acid analogs and labeled nucleic acids can be made and used. See for example Oligonucleotides and Analogs: A Practical Approach, Ed. F. Eckstein, IRL Press, 1991, hereby incorporated by reference in its entirety.

[0035] An exemplary solid-phase combinatorial protocol is that for the synthesis of peptides attached to polymer resin, which proceeds according to Lam et al., 1991, A new type of synthetic peptide library for identifying ligand-binding activity, Nature

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3177

354:82-84. U.S. Pat. No. 5,510,240 to Lam et al. for Method of screening a peptide library; Lam et al., 1994, Selectide technology: Bead-binding screening. Methods: Companion to Methods in Enzymology 6:372-380. Another exemplary protocol is that for the synthesis of benzodiazepine moieties, which proceeds according to Bunin et al., 1992, A general and expedient method for the solid phase synthesis of 1,4-benzodiazepine derivatives, J. Amer. Chem. Soc., 114:10997-10998. U.S. Pat. No. 5,288,514 to Ellman for Solid phase and combinatorial synthesis of benzodiazepine compounds on a solid support. Also, for protocols for the addition of N-substituted glycines to form peptoids, see, e.g., Simon, et al., 1992, Peptoids: A modular approach to drug discovery. Proc. Natl. Acad. Sci. USA, 89:9367-9371; Zuckermann et al., 1992, Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. J. Amer. Chem. Soc., 114:10646-10647; WO PCT94/06,451 to Moos et al. for Synthesis of N-substituted polyamide monomers, useful as solvents, additives for food, enzyme inhibitors etc. Approaches for synthesis of small molecular libraries were recently reviewed by, e.g., Krchnak and Lebl, 1996, Synthetic library techniques: Subjective (biased and generic) thoughts and views, Molecular Diversity, 1:193-216; Ellman, 1996, Design, synthesis, and evaluation of small-molecule libraries, Account. Chem. Res., 29:132-143; Armstrong et al., 1996, Multiple-component condensation strategies for combinatorial library synthesis, Account. Chem. Res., 29:123-131.; Fruchtel et al., 1996, Organic chemistry on solid supports, Angew. Chem. Int. Ed., 35:17-42; Thompson et al., 1996, Synthesis and application of small molecule libraries, Chem. Rev., 96:555-600; Rinnova et al., 1996, Molecular diversity and libraries of structures: Synthesis and screening, Collect. Czech. Chem. Commun., 61: 171-231; Hermkens et al., 1996, Solid-phase organic reactions: A review of the recent literature, Tetrahedron, 52:4527-4554. Exemplary building blocks and reagents are amino acids, other organic acids, aldehydes, alcohols, and so forth, as well as bifunctional compounds, such as those given in Krchnak and Lebl, 1996, Synthetic library techniques: Subjective (biased and generic) thoughts and views, Molecular Diversity, 1:193-216. All of these references are hereby expressly incorporated by reference.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3178

[0036] In one embodiment, peptides are synthesized. By "peptide" herein is meant at least two amino acids joined via a peptide bond. The peptide may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradations. The stepwise solid phase synthesis of peptides is well known. An exemplary solid-phase combinatorial protocol is that for the synthesis of peptides attached to polymer resin, which proceeds according to Lam et al., 1991, Nature 354:82-84; U.S. Patent 5,510,240; Lam et al., 1994, Selectide technology: Bead-binding screening. Methods: A Companion to Methods in Enzymoloqy 6:372-380. Another exemplary protocol is that for the synthesis of benzodiazepine moieties, which proceeds according to Bunin et al., 1992, J. Amer. Chem. Soc., 114:10997-10998 and U.S. Patent 5,288,514. Also, for protocols for the addition of N-substituted glycines to form peptoids, see, e.g., Simon, et al., 1992, Proc. Natl. Acad. Sci. USA, 89:9367-9371; Zuckermann et al., 1992, J. Amer. Chem. Soc., 114:10646-10647; WO PCT94/06,451 to Moos et al.; Approaches for synthesis of small molecular libraries were recently reviewed by, e.g., Krchnak and Lebl, 1996, Molecular Diversity, 1:193-216; Ellman, 1996, Account. Chem. Res., 29:132-143; Armstrong et al., 1996, Account. Chem. Res., 29:123-131.; Fruchtel et al., 1996, Anaew. Chem. Int. Ed., 35:17-42; Thompson et al., 1996, Chem. Rev., 96:555-600; Rinnova et al., 1996, Collect. Czech. Chem. Commun., 61: 171-231; Hermkens et al., 1996, Tetrahedron, 52:4527-4554. All of these references are hereby expressly incorporated by reference.

[0037] One should appreciate however that a solid-phase support of the present invention is similarly applicable to other chemical protocols having similar functional steps. For example, components of the present invention can be applied to appropriate liquid-phase, combinatorial chemistry synthesis protocols, to other solid- or liquid-phase chemical protocols, or to any combination thereof. Such synthesis

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3179

protocols include, but are not limited to organic syntheses such as peptide synthesis, polymer synthesis, and small molecule synthesis.

[0038] A solid-phase support of the invention can also be used for an analytical system in which it is desired to separate a liquid-phase from a solid-phase. Exemplary analytical systems include, without limitation, a binding assay such as an assay for detecting nucleic acid hybridization, binding of a protein to a ligand, binding of an antibody to an epitope, binding of a cell to another cell, binding of a molecule to a cell or subcellular component or binding between cells or subcellular components; an enzyme activity assay such as a kinetic assay or inhibition assay; or a cellular activity assay such as detection of response of a cell to a particular molecule.

[0039] The invention provides a method of making a solid-phase support. The method includes the steps of (a) heating a substrate, thereby forming a molten substrate; (b) contacting the molten substrate with a particle; and (c) allowing the molten substrate to cool in the presence of the particle, thereby making a solid-phase support having the particle embedded in the substrate.

[0040] As used herein the term "molten" is intended to mean transformed from a solid state to a state that is at least partially liquid. A molten substrate can be one that is converted from a solid state to a fully liquid state or to a state that is partially liquid. A molten substrate in a partially liquid state includes, for example, a substrate that has a liquid surface and solid core. A substrate can be transformed from a solid state to a liquid state, for example, by application of heat, pressure, chemical agents or a combination thereof.

[0041] As used herein, the term "embedded" is intended to mean placed at least partially within a substrate, thereby interrupting the surface of the substrate. In accordance with the invention, a particle embedded in a substrate can be fully or partially surrounded by the substrate, so long as the particle interrupts the surface of the substrate and can be accessed by a liquid applied to the surface of the substrate.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3180

[0042] Turning now to the drawings, wherein like components are designated by like reference numerals throughout the various figures, attention is directed to FIG. 1, which illustrates a solid-phase support for high-throughput synthesis, generally designated by the numeral 30. In accordance with the present invention, one or more particles 31 are securely attached to a substrate 32 thus enabling the use of the particles in apparatus which utilize centrifuge technology to separate the liquid-phase from the solid-phase such as those used for high-throughput synthesis ("HTS").

[0043] As used herein, the term "substrate" is intended to mean a material capable of forming a solid base. A material included in the term can have any of a variety of properties depending upon the desired application of the invention including for example, porous, nonporous, rigid, elastic, pliable, malleable, low temperature melting, high temperature melting, and/or chemically resistant to one or more solvents commonly used in the reactions set forth herein. The substrate can be formed of a variety of materials including, but not limited to, plastic; polypropylene, polyethylene, polybutylene, polyurethane, nylon, polymer such as acrylic, acrylonitrile butadiene styrene (ABS), ULTEM (Polyetherimide), acetal copolymer, PROPYLUX HS (heat stabilized polypropylene), RADEL A (polyethersulfone), RADEL R (polyarylethersulfone), UDEL (polysulfone), NORYL PPO (polyphenylene oxide & styrene), Polycarbonate, UHMW-PE (ultra high molecular weight polyethylene), Polyetheretherketone (PEEK), polyphenylene sulfide (PPS, TECHTRON or RYTON), polyolefin or polystyrene; metal such as aluminum, iron, steel or an alloy; other materials such as glass or silicon, or derivatives or combinations of these or other suitable materials. The material of the substrate is typically formed of a material that is capable of achieving a state wherein it is malleable or pliable such that particles can be bonded to it, as discussed below. The material of the substrate can also be in a state where it is rigid such that it can withstand forces applied during synthetic or analytical processes. As set forth below, substrates useful in the invention include, for example, those that can be heated to a molten state that is malleable or pliable such that when loaded with particles and cooled to a rigid state a solid-phase substrate is formed.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3181

[0044] Substrate 32 can include one or more reaction vessels or wells 33. One or more particles 31 can be disposed within each well 33 and affixed to substrate 32. Such configuration allows synthetic reactions to be performed within one or more wells of the substrate. In particular, liquid reagents can be added to each well, reacted within each well, and then the residual liquid can be removed from each well, for example, via centrifugation, inversion of the wells, and/or aspiration. In one embodiment, the substrate is a multi-well plate that includes a plurality of wells disposed in arrays. Exemplary multi-well plates include, for example, microplates, microtiter plates or n-well plates where "n" is the number of wells including, for example, 8, 16, 96, 384 or 1536-wells. As used herein, the term "microplate" is intended to mean a multi-well plate that has dimensions and properties consistent with the definition provided by the Society for Biomolecular Screening (Danbury, CT, USA).

[0045] One will appreciate that the substrate and the wells can be configured in a variety of ways. The shape of the wells can vary depending on the particular application and/or desired fluid dynamics. For example, the depth and diameter of the wells can be U-shaped, V-shaped, or flat bottomed. The wells can be substantially vertical, inclined inwardly (e.g., toward a centrifugal axis) and/or inclined outwardly (e.g., away from a centrifugal axis). Furthermore, the sidewall of the well can be cylindrical, conical, flat, tapered inwardly or outwardly, or have any other desired geometry. One should also appreciate that the shape of the substrate itself can also vary. For example, the shape of the substrate can be polygonal (e.g., rectangular), regular, irregular, arcuate or round.

[0046] As noted above, one or more particles can be affixed to the substrate within each well. The particles can be embedded into the substrate while the substrate is heated to an elevated temperature approximating the melting point of the substrate. In particular embodiments, external mechanical pressure can be applied to the particles while the substrate is heated. Alternatively, embedding can be allowed to occur due to gravity in the absence of externally applied mechanical pressure. As the substrate cools, the particles will typically be permanently bonded to the substrate, which bond

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3182

is capable of withstanding forces typically used to remove liquids such as centrifugal force, aspiration, vacuum, or gravity. Alternatively, a particle can be adhesively bonded to the internal surface of the well utilizing a thermoplastic material having a relatively low melting point (i.e., lower than that of the substrate), a two-part epoxy, and/or other suitable means. One or more particles can be bonded to a well bottom as exemplified in FIG. 2 through FIG. 4. Those skilled in the art will recognize that particles can be bonded to all or part of a well surface including, without limitation, the bottom, sides, subsections thereof or combinations thereof.

[0047] Accordingly, the invention provides a solid-phase support having a particle bonded to a substrate capable of withstanding centrifugal forces directed opposite the surface of the substrate to which the particle is bonded including, for example, centrifugal forces of at least about 10 x *g*, 100 x *g*, 500 x *g*, 1,000 x *g*, 5,000 x *g*, 10,000 x *g*, or 100,000 x *g*.

[0048] Although the invention has been exemplified above with particles bonded to wells, those skilled in the art will recognize that a solid-phase support useful in the invention need not include wells. For example, a solid-phase substrate of the invention can include a flat or planar substrate to which particles are bonded. Alternatively, particles can be bonded to a raised area of a substrate or protrusion. For example, particles can be bonded to protruding "pins" that are configured in an array having dimensions similar to a microtiter plate such that the pins can be dipped into a microtiter plate for delivery of synthetic reactants or analytical reagents. An array of pins can be centrifuged or aspirated to remove liquid from the bonded beads using methods similar to those exemplified herein with respect to microtiter plate-based solid-phase supports.

[0049] As used herein, the term "particle" is also intended to mean a substance having a size or diameter small enough to fit within the area of a substrate to which it is bonded. Exemplary particles include, without limitation, a "microparticle" or "nanoparticle" or "bead" or "microbead" or "microsphere." Particles useful in the invention include, for example, beads such as microbeads measuring 30 microns to

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3183

300 microns in diameter and/or macrobeads measuring larger than 300 microns in diameter. A particle can be made of glass, plastic, polystyrene, resin, gel, agarose, sepharose, and/or other suitable materials.

[0050] Typically, particles will have a melting temperature that is higher than that of the substrate to which they are being bonded when the bonding method includes melting of the substrate. In particular embodiments, a low-melting-point polymer can be placed in contact with particles and a substrate to which they are to be bonded, thus providing an adhesive. There are other types of beads on which chemical synthesis can be performed, which can also be used in the invention.

[0051] In particular embodiments, a particle can be controlled pore glass ("CPG"). CPG having any of a variety of particle sizes and pore sizes can be used in the invention including, for example, CPG with a median particle size falling within about 37 to 177 microns and a median pore size falling within about 65 to 3300 Angstroms. A particularly useful CPG has a median particle size and pore size of about 100 microns and about 1400 Angstroms, respectively. Those skilled in the art will recognize that CPG is typically obtained with a median particle size specification and there can be variation within a lot. For example, a lot of 100-micron CPG can include members be as small as about 75 or as large as about 125 microns. It will be understood that the above described ranges, as with all ranges described herein, are intended to include individual integer and non integer values therein.

[0052] CPG can have a surface modification or it can be unmodified. Unmodified CPG is typically used as a porous bed material in chromatography columns. Its surface can be derivatized to provide reactive functional groups for synthesis using surface modifications methods known in the art.

[0053] A particle can be functionalized in order to covalently attach molecules, cells, other particles or other materials. Exemplary molecules that can be attached include intermediate compounds (or final compounds) of a synthetic reaction. As will be appreciated by those in the art, functionalization of a particle will depend on the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3184

synthetic reactions to be performed. The functionalization of solid support surfaces such as certain polymers with chemically reactive groups such as thiols, amines, carboxyls, etc. is generally known in the art. Some examples of these surface chemistries for subsequent addition of building blocks during synthetic reactions include, but are not limited to, amino groups including aliphatic and aromatic amines, carboxylic acids, aldehydes, amides, chloromethyl groups, hydrazide, hydroxyl groups, sulfonates and phosphates.

[0054] These functional groups can be used to add any number of different building block moieties to the particles, generally using known chemistries, including, but not limited to the use of amino-functionalized supports, sulfhydryl linkers, etc. There are a number of sulfhydryl reactive linkers known in the art such as SPDP, maleimides, α-haloacetyls, and pyridyl disulfides (see for example the 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference). Similarly, amino groups on the building blocks and on the surface can be attached using linkers; for example, a large number of stable bifunctional groups are well known in the art, including homobifunctional and heterobifunctional linkers (see Pierce Catalog and Handbook, pages 155-200). In an additional embodiment, carboxyl groups (either from the surface or from the building block) can be derivatized using well-known linkers (see the Pierce catalog). For example, carbodiimides activate carboxyl groups for attack by good nucleophiles such as amines (see Torchilin et al., Critical Rev. Therapeutic Drug Carrier Systems, 7(4):275-308 (1991), expressly incorporated herein). In addition, useful methods include systems that allow post-synthesis cleavage from the reaction vessels.

[0055] The configuration of the particles affixed to the substrate in accordance with the present invention allows the use of solid-phase support 32 with methods that utilize centrifuges for liquid-phase and solid-phase separation, such as the ones described by the Lebl '054 patent, and with apparatus that utilize centrifugal rotors for liquid-phase and solid-phase separation, such as the ones described by the Lebl '894 publication. For example, FIG. 12 illustrates a solid-phase support mounted on an apparatus 34 similar to that described in the Lebl '894 publication, which apparatus is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3185

configured for high-throughput, multi-protocol combinatorial syntheses. The apparatus includes a rotor assembly 35 for rotatably supporting one or more solid-phase supports. Preferably, the rotor assembly includes a plate holder 36 that releasably secures the solid-phase support to the rotor assembly.

[0056] Rotor assembly 35 is supported for rotation about a centrifugal axis 37 which extends substantially vertically. Apparatus 34 also includes a motor drivingly connected to rotor assembly 35 in order to spin the rotor assembly for centrifugation in a manner similar to that described in the Lebl '894 publication. Apparatus 34 is adapted for synthesizing oligomers in each of a plurality of the reaction vessels disposed in the substrate. Thusly, the apparatus can also be equipped with a liquid delivery system (not shown) that is capable of delivering one or more liquids to one or more wells of the microtiter plate, in a manner similar to that also described in the Lebl '894 publication. In addition to the components mentioned above, the apparatus can also comprise additional components. For example, the centrifuge can comprise a sensor to signal the computer and liquid distribution system when a set of reaction vessels is in a particular orientation, and a motor to rotate the rotor into the correct orientation for liquid delivery, also in control of the computer. Furthermore, in the case of adjustable tilt rotors, the centrifuge can utilize a control and a sensor to control the degree of tilt.

[0057] Turning now to centrifugation and liquid-phase removal, rotor assembly 35 typically is activated to centrifuge the solid-phase support, exemplified in the illustrated embodiment, microtiter plates 32. The microtiter plates can be held in a fixed angle with respect to the rotor and with respect to vertical, as described in U.S. Patent No. 6,045,755 to Lebl et al. ("the Lebl '755 patent"), the entire contents of which patent is incorporated herein by this reference, as well as the Lebl '054 patent. Alternatively, the microtiter plates can be dynamically adjusted such that the angle of the microtiter plates varies during centrifugation, as described in the Lebl '894 publication.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3186

[0058] As particles are permanently affixed to the substrate in accordance with the present invention, the solid-phase support can be centrifuged in any of a variety of orientations. For example, solid-phase support 32 allows a "zero-tilt" centrifugation, that is, centrifugal removal of the liquid-phase from microtiter plates that are horizontally oriented during centrifugation. Accordingly, the solid-phase support of the present invention need not be tilted in such a manner to create "pockets" within the wells to retain the particles, namely because particles are securely attached to the substrate. Since particles are bonded to the substrate, a solid-phase substrate of the invention can be centrifuged in a reverse-tilt orientation in which the opening of a reaction vessel is oriented away from the axis of rotation. Thus, substantially all of the liquid in the vessel can be expelled due to centrifugation, while the bonded particles are maintained in the vessel. Furthermore, a solid-phase support of the invention need not have vessels with walls for containing particles or liquid-phase during centrifugation. Thus, particles can be attached to a portion of a substrate that is not in a vessel such as on a flat surface or on a protruding feature. The solid-phase supports of the present invention beneficially allow for the use of less complex high-throughput synthesizers, for example, by reducing the number of components of the rotor assembly, and simplifying the maintenance of the rotor assembly.

[0059] A method of forming solid-phase support 32, in accordance with the present invention, will now be described. In one embodiment, solid-phase particles in the form of controlled pore glass ("CPG") are bonded to the well bottoms of the substrate. For example, the method of the present invention can be utilized to bond a relatively equal amount of 1000 or 1400 Angstrom CPG to the bottom well surfaces of a 384-well polypropylene microtiter plate, for example, 384-well plates of the type sold by Robbins Scientific of Sunnyvale, California.

[0060] In a particular embodiment, approximately one to three grams of CPG can be initially applied to each microtiter plate and approximately 0.2 mg per well is bonded to the substrate. Preferably, the well is overloaded with CPG, that is, more CPG is provided in each well than is necessary to cover the bottom surface of the well. Not all of the CPG will be bonded to the plate, however, most of the excess non-bonded

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3187

CPG is recoverable, as will become apparent below. One will appreciate that the actual amount of CPG, or other particle used will vary depending upon various factors including, but not limited to, the geometry, size and number of the wells present in the substrate. Accordingly, an amount of particles that overload a well or other area of a substrate or, alternatively, an amount of particles that sparsely load or load only a portion of a well or other area of a substrate can be applied.

[0061] Turning now to FIG. 5, the method of the present invention can be performed at a workstation 38 which includes a heating assembly 39 for warming the substrate and a solid-phase particle applicator 40 for dispensing and applying the solid-phase particles to the substrate. In a particular embodiment, the applicator is complementary in shape to substrate 32, and in the embodiment illustrated in FIG. 5, has an array of recesses 41 that correspond to the arrangement of wells 33 of a 384-well microtiter plate.

[0062] Recesses 41 are fluidly connected to a vacuum source 42 for generating a negative pressure within the recesses, which negative pressure facilitates loading CPG into the recesses and temporarily restraining the CPG within the recesses, as will become apparent below. Preferably, the bottoms of the recesses, which are fluidly connected to the vacuum source, are lined with a filter mesh or frit material to retain the CPG within the recesses. Recesses 41 of the applicator are also fluidly connected to a pressurized fluid source 43 that supplies fluid into the recesses and generates a fluid pressure for dispensing the CPG from recesses 41 into wells 33 when applicator 40 is positioned over substrate 32, as well become apparent below. Applicator 40 further includes a pressure plate assembly 44 that is also fluidly connected to pressurized fluid source 43 and is configured to bias substrate 32 against heating assembly 39 the applicator is positioned over the substrate. In one embodiment, pressure plate assembly 44 is a single-acting cylinder assembly that is configured to seat against and bias substrate 32 when a bonding pressure is supplied to the pressure plate assembly. A control unit 45 is provided which is used to control the temperature of heating assembly 39, activation of vacuum source 42, as well as the dispensing and bonding pressures of applicator 40. Preferably, the pressurized fluid source is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3188

compressed air, however, one will appreciate that other pressurized fluids can be used.

[0063] In one embodiment, the control unit is adjusted to set the heating assembly at a desired temperature, that is, the approximate melting point of the substrate material. In the illustrated embodiment, the heating assembly is set to approximately 235°C to 240°C, however, one will appreciate that the desired temperature will vary depending upon the melting point of the particular substrate and particles being used. An infrared thermometer or other suitable means can be utilized to measure the true temperature of the heating assembly immediately adjacent the substrate such as the microtiter plate exemplified in FIG. 5. In the illustrated embodiment, the true temperature is approximately 270°C (+/-5°C). Again, one will appreciate that the actual temperature can vary depending upon various factors, including the material of the microtiter plate and/or the configuration of the heating assembly.

[0064] Next, control unit can 45 be adjusted to set the desired vacuum as well as the desired "Dispense" and "Bond" pressures of the applicator. In one embodiment, the vacuum is set to approximately 20 SCFH, the dispense pressure is set to approximately 30 psi and the bond pressure to approximately 60 psi, however, one will appreciate that the actual vacuum and pressures necessary will vary depending upon various factors including, but not limited to, the configuration and materials of the substrate.

[0065] In the illustrated embodiment, applicator 40 is pivotally mounted on the workstation and located immediately adjacent heating assembly 39. Applicator 40 pivots about a pivot axis 46 between a first loading position (FIG. 5) and a second bonding position (FIG. 8). Such a pivotal configuration readily allows CPG to be loaded on a dispenser plate 47 of the applicator in the loading position. By way of example, approximately one to three grams of CPG can be poured onto the dispenser plate and thoroughly spread over the recesses of the dispenser plate with a foam brush 48 or other suitable means. Excess CPG can be brushed from the dispenser plate into a CPG reclaim tray 49 with the foam brush (FIG. 5), thus minimizing waste of CPG.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3189

The reclaim tray can then be removed (FIG. 6) and the excess CPG can be placed in a reclaim container 50 for later use.

[0066] The amount of particles adhered to a substrate can be influenced by altering any of a variety of conditions used for bonding. Exemplary conditions that can be varied to alter particle bonding include, without limitation, the amount of time the substrate is heated, the amount of pressure applied to the particles and substrate or a combination thereof. In a particular embodiment, a polypropylene microtiter plate can be heated to its melting point for about 20 to 40 seconds and CPG pressed with sufficient force to deform well depth by about 0.02 to 0.125 inches.

[0067] As shown in FIG. 7, a release sheet 51 can be placed on the heating assembly in order to facilitate removal of the microtiter plate from the heating assembly after bonding has taken place. In one embodiment, a sheet of aluminum foil is placed on the heating element, however, one will appreciate that other suitable means can be used. Next, a microtiter plate can be placed on the heating element, as well as the release sheet in the event that one is used.

[0068] With microtiter plate 32 in place on heating assembly 39, applicator 40 can be pivoted from its loading position to the bonding position and latched in place, as shown in FIG. 8. The vacuum within recesses 41 applied by vacuum source 42 facilitate restraint of the CPG within the recesses when the applicator is pivoted and latched in place. In one embodiment, the control unit is activated causing the dispensing and bonding cycle to commence. First, control unit 45 will terminate the vacuum allowing the CPG to fall under the force of gravity from recesses 41 into wells 33. The control unit may further activate the dispensing pressure to further facilitate travel of the CPG from the recesses of the dispensing plate into the wells of the microtiter plate. Meanwhile, a hot plate 52 can move upwardly and immediately adjacent microtiter plate 32 in order to heat the bottom of the microtiter plate, and particularly, the bottom surfaces of wells 33 to a temperature approximating the melting point of the material of the microtiter plate. At this time, the control unit can be used to increase the fluid pressure within the pressure plate assembly 44 thus

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3190

firmly seating the pressure plate assembly on the microtiter plate 32 and biasing the microtiter plate downwardly against the heating assembly to facilitate heating of the microtiter plate.

[0069] When the temperature of the substrate material adjacent the well bottoms increases, the well bottoms become malleable and pliable. Once the well bottoms are warm and pliable, CPG 31 is embedded into the bottoms of wells 33 under the force of gravity and bonded with microtiter plate 32, thus permanently bonding the CPG to the microtiter plate.

[0070] Typically, for a solid-phase support of the invention at least a portion of a particle is embedded in a substrate. The embedded portion can be less than one-half of the particle embedded within the substrate, and more preferably, less than 25%. The embedded portion can be at least 1%, 5%, 10%, 25% or 50% or more. In one embodiment, only a nominal portion of the CPG is embedded within the substrate, as shown in FIG. 11. In this embodiment, CPG 31 is embedded in the bottom of well 33 at a depth of less than approximately 10 microns but, nonetheless, provides a bond that is sufficient to withstand the forces that the particle is subjected to during centrifugation. Such configuration advantageously leaves the majority of the CPG surface exposed, thus maximizing the surface area upon which synthetic reactions may take place.

[0071] Once the bonding cycle is complete, the applicator can be unlatched and returned to the loading position. The microtiter plate, along with the release sheet, can then be removed from the heating assembly and allowed to cool on a work surface of the workstation (FIG. 9). Once the microtiter plate is cool, the release sheet can be readily removed, typically falling away from the microtiter plate with gravity alone.

[0072] Any excess CPG, that is, any CPG that has not been bonded to the microtiter plate can be removed from the microtiter plate and stored in a CPG reclaim container for later use. In this regard, the microtiter plate can be tapped against the reclaim

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3191

container to dislodge excess CPG. If desired, the entire microtiter plate can also be vacuumed to further remove any remaining excess CPG from the microtiter plate. The microtiter plate can also be visually inspected to evaluate CPG coverage.

[0073] In operation and use, the CPG bonded microtiter plate 32 can be used upon various HTS apparatuses including, but not limited to, apparatus 34 for solid-phase combinatorial synthesis which typically proceeds according to the following steps. In a first step, the substrate can be mounted on the rotor assembly. As one or more solid-phase supports are already affixed to the substrate within each well, there is no need to charge the wells with a solid-phase support as is typically done with other microtiter plate-based methods (e.g., charging each well with a slurry of microbeads suspended in a solvent). The solid-phase can be preconditioned with an appropriate solvent, if desired, and the first of the plurality of building blocks or a linker moiety can be covalently linked to the solid-phase supports. Subsequently, a plurality of building block addition steps can be performed, all of which involve repetitive execution of the following or similar sub-steps, and in a sequence chosen to synthesize a desired compound. First, a sufficient quantity of a solution, which contains the building block moiety selected for addition, can be dispensed into the well so that the building block moiety is present in a molar excess to the intermediate compound present in the well. A sub-step reaction can be triggered and promoted by activating reagents and other reagents and solvents, which are also added to the well. The well can then be incubated at a controlled temperature for a time, typically between 5 second and 24 hours, sufficient for the building block addition reaction to go to substantial completion. Optionally, during this incubation, the well can be intermittently agitated, stirred, or the liquid contents otherwise moved to increase the volume of liquid contacting the surface area of the bonded particles. Finally, in a last sub-step of building block addition, the well containing the solid-phase support with attached intermediate compound can be prepared for addition of the next building block by removing the spent reaction fluid and thoroughly washing and reconditioning the solid-phase support.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3192

[0074] Washing typically involves one to seven cycles of adding and removing a wash solvent. Optionally, during the addition steps, multiple building blocks can be added to one well in order to synthesize multiple compounds attached to one solid-phase support. After the desired number of building block addition steps, the final compound is present in the well and attached to the solid-phase support. The final compounds can be utilized either directly attached to their synthetic solid-phase support, or alternatively, can be cleaved from their support. In the latter case, the linker moiety attaching the compound to the solid-phase support can be cleaved in a variety of ways, and the final compound, or library of compounds can be extracted from the reaction vessel into a liquid-phase.

[0075] The substrate and methods of the present invention are advantageously useful for the manual or automated preparation of combinatorial libraries or megaarrays of compounds by fluorous phase organic synthesis. As is well known to those skilled in the art, such combinatorial libraries or megaarrays have numerous uses, in particular, for the selection of pharmaceutical lead compounds, for the optimization of pharmaceutical lead compounds and for the identification and/or isolation of pharmaceutical drugs. The methods and apparatus of the invention for liquid/solid-phase separation can also advantageously be used for parallel extraction and purification of compound arrays synthesized or obtained by other methods. Other applications in analytical chemistry (extraction, desalting or other means of parallel preparations of samples), biochemistry (parallel processing of samples) are envisioned.

[0076] The affixed-support configuration of the substrate also enables centrifuge-HTS apparatus to run with higher accelerations and velocities compared to systems in which particles are free-floating in a reaction vessel, thus providing quicker cycle times. In particular, the solid-phase support of the present invention is capable of withstanding significant acceleration because any likelihood of particle loss is significantly reduced and/or prevented with the particles securely bonded to the substrate.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3193

[0077] The solid-phase support and method of the present invention also obviates the need to design a specific 384 microtiter plate having specific well geometries intended to contain loose liquid-phase particles, as is the case with other centrifugal HTS systems. Bonding the solid-phase supports directly to the substrate of the present invention has resulted in the increased and consistent production of high-quality oligonucleotides even when used in combination with existing centrifuge synthesis technology.

[0078] In one embodiment of the present invention, solid-phase support 32a is similar to solid-phase support 32 described above but includes a laminate sheet 53 bonded to a bottomless-well plate 54 as shown in FIG. 10. Like reference numerals have been used to describe like components of solid-phase support 32 and solid-phase support 32a. In this embodiment, the laminate sheet includes a rigid sheet 55 having a layer of hot melt adhesive 56 in which particles 31a are embedded. For example, the laminate sheet can be in the form of a "heat-sealing sheet" that is commonly used to close the contents of a microtiter plate. In operation and use, solid-phase support 32a can be used in substantially the same manner as solid-phase support 32 discussed above.

[0079] In another embodiment, the substrate can be formed of a porous polymeric material. Preferably, the porosity of the material allows the substrate to support the liquid-phase within the wells under the normal force of gravity but does not support the liquid-phase under the higher forces of centrifugation. For example, the substrate can be formed of a porous polymeric material including, but not limited to, polyethylene, polypropylene, and other suitable materials. Such porous polymeric materials are typically available in sheets, rods, tubes, and molded shapes and can be machined to form the wells. Porosity of the material typically depends on the specific material and can be as low as 7 μm. As the solid-phase supports are affixed to the substrate, the porosity of the substrate need not be less than the maximum physical dimension of a solid-phase support, as is the case with prior substrates in which the solid-phase supports are merely disposed within the wells. One will appreciate that, in the event the substrate bottom is heated to a temperature approximating the melting

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3194

point of the substrate material in order to affix the solid-phase supports, the porosity of the well bottom can be substantially impaired or lost. Provided that the sides of the well do not reach the melting point, the porosity of the sides of the well remain porous in such a manner that would facilitate the removal of the liquid-phase.

[0080] Alternatively, a solid-phase support can be provided with one or more openings adjacent to the bottom of, and in fluid communication with, the wells to facilitate expulsion of the liquid-phase from the wells. For example, the opening can be a round aperture or one or more slits that would allow expulsion of the liquid-phase during centrifugation or other separation procedure including, but not limited to, vacuum, pressure, venturi vacuum, blotting, and other suitable means. Preferably, the opening or openings are dimensioned such that the bulk of the liquid-phase within the wells remains within the wells until a pressure differential is applied to the liquid-phase within the wells. Alternatively, larger openings can be utilized with a discrete filter mesh or frit extending across the opening, which mesh or frit is provided to retain the liquid-phase The filter mesh or frit is sufficiently fine to retain the liquid-phase within the well under the normal force of gravity but sufficiently course to allow the passage of the liquid-phase through the opening upon the application of a pressure differential.

[0081] For convenience in explanation and accurate definition in the appended claims, the terms "upwardly", "bottom", and "side" are used to describe features of the present invention with reference to the positions of such features as displayed in the figures.

[0082] In many respects the modifications of the various figures resemble those of preceding modifications and the same reference numerals followed by subscript "a" designate corresponding parts.

[0083] The foregoing descriptions of specific embodiments of the present invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3195

many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application, to thereby enable others skilled in the art to best utilize the invention and various embodiments with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the Claims appended hereto and their equivalents.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3196

WHAT IS CLAIMED IS:

1. A method of forming a solid-phase support, said method comprising the steps:

providing a substrate having a reaction vessel;

dispensing a particle in said reaction vessel; and

permanently bonding said particle to said substrate within said reaction vessel.

2. The method of claim 1, wherein said permanently bonding step includes embedding said particle in said substrate.

3. The method of claim 2, wherein less than one-half of said particle is embedded into said substrate.

4. The method of claim 1, wherein said particle comprises a microbead.

5. The method of claim 1, wherein said particle comprises controlled pore glass.

6. The method of claim 1, wherein said dispensing step includes brushing said particle into a recess of a dispenser plate.

7. The method of claim 6, wherein said substrate comprises a microtiter plate including a plurality of microtiter wells.

8. The method of claim 7, wherein said dispensing plate comprises a plurality of said recesses and each said microtiter well defining a respective reaction vessel that is aligned with a respective said recess.

9. The method of claim 7, wherein said dispensing step further comprises contacting said dispensing plate and said microtiter plate such that said recess is aligned with said reaction vessel.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3197

10. The method of claim 9, wherein said dispensing step further includes pivoting said dispensing plate into engagement with said microtiter plate.

11. The method of claim 10, wherein said dispensing step further includes providing a first dispensing pressure to said recess facilitating transfer of said particle from said recess to said reaction vessel.

12. The method of claim 11, wherein said permanently bonding step includes heating said substrate.

13. The method of claim 12, wherein said permanently bonding step further includes heating said substrate, thereby increasing the temperature of a portion of said substrate of each said reaction vessel to the melting point of said substrate.

14. The method of claim 13, wherein said permanently bonding step further includes providing a second bonding pressure to bias said substrate against a heating element..

15. The method of claim 12, wherein said permanently bonding step further includes heating said substrate, thereby increasing the temperature of a portion of said substrate adjacent a bottom surface of each said reaction vessel to the melting point of said substrate.

16. The method of claim 15, wherein said permanently bonding step further includes providing a second bonding pressure to said reaction vessel biasing said particle against the bottom of said reaction vessel.

17. The method of claim 1, comprising dispensing a plurality of said particles to said reaction vessel, whereby said permanently bonding further includes

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3198

embedding one or more of said particles into said substrate within said reaction vessel.

18. The method of claim 17, wherein one or more of said particles remain nonbonded to said substrate.

19. The method of claim 18, wherein said method further comprises the step of removing said one or more nonbonded particles from said reaction vessel.

20. The method of claim 1, wherein said providing step comprises providing a bottomless-well plate and a rigid sheet, said method further including providing a layer of hot-melt adhesive to said rigid sheet to form a laminate sheet, applying said laminate sheet to a bottom surface of said bottomless-well plate thereby forming said substrate having a reaction vessel.

21. The method of claim 20, wherein said permanently bonding step comprises embedding.

22. The method of claim 20, wherein said permanently bonding step comprises embedding said particle in said layer of hot-melt adhesive.

23. A method of synthesis comprising:

providing a solid-phase support comprising a particle bonded to said substrate adjacent a surface of substrate, said particle being functionalized to covalently attach an intermediate compound of a synthetic reaction;

dispensing a liquid comprising a reagent to said solid-phase support to effect said synthetic reaction; and

removing said liquid from said solid-phase support by centrifugation, whereby said intermediate compound remains attached to said substrate by said particle.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3199

24. The method of claim 23, wherein said particle is embedded in said substrate.

25. The method of claim 23, wherein said solid-phase support comprises a reaction vessel and wherein said particle is embedded in said substrate and in said reaction vessel.

26. The method of claim 24, wherein less than one-half of said particle is embedded into said substrate.

27. The method of claim 24, wherein said solid-phase support comprises a microtiter plate.

28. The method of claims 27, wherein said particle comprises controlled pore glass.

29. The method of claim 23, wherein said providing step comprises heating said substrate and embedding said particle in said substrate, thereby making said solid-phase support.

30. The method of claim 29, wherein said removing step comprises centrifugation around an axis of rotation, wherein said surface of said substrate is oriented away from said axis of rotation and wherein said particles remain bonded in said surface.

31. The method of claim 30, wherein said particle comprises 1400 Angstrom controlled pore glass.

32. A method of making a solid-phase support, comprising:
heating a substrate, thereby forming a molten substrate;
contacting said molten substrate with a particle; and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
3200