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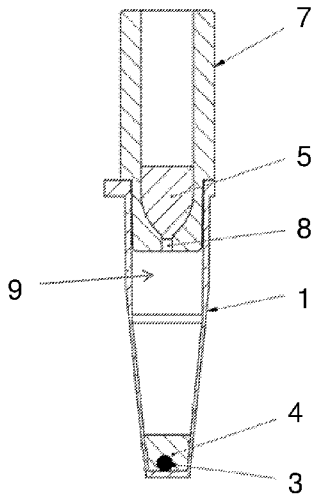

FIG. 3A

(57) Abstract: The present invention relates to a reaction container that is suitable for conducting PCR. In particular, the invention relates to a pre-loaded reaction container comprising a reagent that is overlaid with a viscous barrier fluid, wherein the viscous barrier fluid seals the reagent from the atmosphere. One or more reaction reagents are added to the pre-loaded reaction container and the container is centrifuged to combine the one or more reaction reagents and the pre-loaded reagent. The pre-loaded reagent is stable at temperatures between 10 and 40°C for many months when immersed within the barrier fluid or is sealed from the atmosphere by the barrier fluid. The present invention also relates to a cap for cooperating engagement with a reaction container, the cap comprising an upper portion for receiving a sample and a lower portion adapted to sealingly engage the inlet aperture of the reaction container to thereby define a reaction chamber between the cap and the reaction container. The upper portion and the lower portion of the cap are in fluid communication by a conduit adapted to substantially retain at least a barrier fluid within the reaction chamber.

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REACTION CONTAINER AND CAP ASSEMBLY

[0001] This application claims priority to and the benefit of Australian provisional patent application No. 2015905312 dated 22 December 2015, and Australian provisional patent application No. 2016901589 dated 29 April 2016, both of which are hereby incorporated herein by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to a reaction container and cap assembly suitable for conducting assays. In particular, for conducting nucleic acid amplification reactions, especially PCR, and will be described hereinafter with reference to this application. However, it will be appreciated that the invention is not limited to this particular field of use.

BACKGROUND OF THE INVENTION

[0003] The following discussion of the prior art is provided to place the invention in an appropriate technical context and enable the advantages of it to be more fully understood. It should be appreciated, however, that any discussion of the prior art throughout the specification should not be considered as an express or implied admission that such prior art is widely known or forms part of common general knowledge in the field.

[0004] PCR is a technique involving multiple cycles that results in the geometric amplification of certain polynucleotide sequences each time a cycle is completed. The technique of PCR is well known and is described in many books, including, *PCR: A Practical Approach*, M. J. McPherson, *et al.,* IRL Press (1991), P*CR Protocols: A Guide to Methods and Applications* by Innis, *et al*., Academic Press (1990), and *PCR Technology: Principals and Applications for DNA Amplification* H. A. Erlich, Stockton Press (1989). PCR is also described in many US patents, including US 4,683,195; 4,683,202; 4,800,159; 4,965,188; 4,889,818; 5,075,216; 5,079,352; 5,104,792; 5,023,171; 5,091,310; and 5,066,584.

[0005] The PCR technique typically involves the step of denaturing a polynucleotide, followed by the step of annealing at least a pair of primer oligonucleotides to the denatured polynucleotide*, i.e.,* hybridizing the primer to the denatured polynucleotide template. After the annealing step, an enzyme with polymerase activity (e.g., a DNA polymerase such as Taq polymerase) catalyzes synthesis of a new polynucleotide strand that incorporates the primer oligonucleotide and uses the original denatured polynucleotide as a synthesis template. This series of steps (denaturation, primer annealing, and primer extension) constitutes a PCR cycle.

[0006] As cycles are repeated, the amount of newly synthesized polynucleotide increases geometrically because the newly synthesized polynucleotides from an earlier cycle can serve as templates for synthesis in subsequent cycles. Primer oligonucleotides are typically selected in pairs that can anneal to opposite strands of a given double-stranded polynucleotide sequence so that the region between the two annealing sites is amplified.

[0007] Denaturation of DNA typically takes place at around about 90 ºC to about 95 ºC, annealing a primer to the denatured DNA is typically performed at around about 40 ºC to about 60 ºC, and the step of extending the annealed primers with a polymerase is typically performed at around 70 ºC to about 75 ºC.

[0008] In real-time PCR or quantitative PCR (qPCR), the amount of amplified DNA is monitored at each PCR cycle through the use of a fluorescent agent that binds to DNA (e.g., an intercalating fluorescent dye or a fluorophore-tagged oligonucleotide) and is detectable and quantifiable. By comparing the fluorescence generated during the amplification of a sample with the fluorescence generated during the amplification of multiple dilutions of a known amount of standard DNA, it is possible to calculate the amount of starting DNA in the sample.

[0009] As DNA polymerase is active at room temperature and below, this can lead to nonspecific primer annealing and extension while the PCR reagents (e.g., DNA polymerase, deoxynucleotides, $MgCl_2$, primers, probes, reaction buffers and target DNA) are being mixed. This results in the generation of nonspecific DNA products and lowered product yields. The generation of nonspecific DNA products may be reduced by preventing interaction of the PCR reagents until the first denaturation step is completed. This is commonly referred to as “hot start PCR”.

[0010] There are several known hot start PCR techniques:

- employing DNA polymerase that comprises an inhibitor (either chemical, antibody, aptamer, or otherwise), wherein the inhibitor ensures that the DNA polymerase is not active at ambient temperatures, and wherein the inhibitor is denatured and dissociates from the DNA polymerase during the denaturation step;

- providing a seal (wax, grease or polymer) that is solidified over one of the PCR reagents to exclude it from the other PCR reagents, wherein the seal melts during the denaturation step and allows mixing of the PCR reagents (such a method is described in US5,968,729); or

- preparing the PCR mixture in the absence of magnesium to prevent activation of the DNA polymerase, wherein magnesium is then added to the PCR mixture during the denaturation step by way of a magnesium-containing wax bead that melts when heated.

[0011] These hot start PCR techniques also ensure that the PCR reaction is initiated simultaneously in all reaction tubes, so all reactions are initiated at the same time for gene expression studies.

[0012] There are, however, several disadvantages with the current hot start PCR techniques. In particular, inhibited DNA polymerase, often chemical- or antibody-inhibited, is more expensive than non-inhibited DNA polymerase, and can result in significantly increased costs when large numbers of PCRs are being conducted. Other disadvantages would be known to a person skilled in the art.

[0013] Additionally, condensate may form at the top or side walls of the reaction container during thermal cycling (especially around the edge wells in a 96 well block thermal cyclers, usually between the top edge of the block and the heated plate), resulting in a reduced sample volume and a portion of the sample being no longer being available for fluorescence detection and quantification - which may also compromise the accuracy and reliability of qPCR.

[0014] It is an object of a preferred form of the present invention to provide a reaction container and cap assembly for PCR that prevents the generation of unwanted nonspecific DNA products and enables accurate and reliable qPCR. It is a further object of a preferred form of the present invention to provide an improved reaction container and cap assembly that is particularly suited for automated loading in a thermocycler.

[0015] It is an object of the present invention to overcome or ameliorate one or more of the disadvantages of the prior art, or at least to provide a useful alternative.

SUMMARY OF THE INVENTION

[0016] In one embodiment, the present invention relates to a reaction container that is suitable for hot start PCR and qPCR, wherein the reaction container comprises at least one pre-loaded reagent that is overlaid with, or is embedded within, a viscous barrier fluid, wherein the barrier fluid seals the pre-loaded reagent from contact with the atmosphere. Preferably the pre-loaded reagent is an enzyme with polymerase activity, such as a DNA polymerase or a Taq polymerase or a reverse transcriptase. In some preferred embodiments, the enzyme with polymerase activity does not comprise an inhibitor, and in other embodiments an inhibitor is

also provided with the enzyme. Preferably the enzyme with polymerase activity is Taq polymerase or a Klenow.

[0017] In other embodiments, the pre-loaded reagent comprises nucleic acid amplification reagents, comprising: an enzyme, a nucleic acid, a nucleic acid primer, a buffer, a salt, a deoxyribonucleotide, a biological material, a preservative, an initiator, an acid, a nucleotide, a nucleoside triphosphate, or any combination thereof. In this embodiment, the pre-loaded reagent may optionally comprise an enzyme with polymerase activity, such as a DNA polymerase or a Taq polymerase or a reverse transcriptase.

[0018] Preferably the volume of the reaction container is between 10 to 100µl. Preferably the volume of the reaction container is between 5 to 20 µl, or 20 to 50 µl, or 50 to 100 µl. In other embodiments the reaction container is between 5 to 15 µl, or 15 to 25 µl, or 25 to 35 µl, or 45 to 60 µl, or 60 to 75 µl. In other embodiments the volume of the reaction container is 100µl, 200µl, 300µl, 400µl or 500µl. In other embodiments the volume of the reaction container is up to around 1000µl, or 2000µl, or 3000µl.

[0019] Preferably the volume of the pre-loaded reagent is between 1 and 10 µl. Preferably the volume of the pre-loaded reagent is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 µL. Preferably the volume of the barrier fluid is between 1 to 50 µl. Preferably the volume of the barrier fluid is 1 µl, 2 µl, 3 µl, 4 µl, 5, 10, 15, 20, 25 or 50 µl.

[0020] Preferably the barrier fluid has a viscosity of greater than 500 cSt. In some preferred embodiments the viscosity is between 500 to 1,000 cSt, between 1,000 to 2,000 cSt, between 900 to 1100 cSt, between 2,000 to 3,000 cSt, between 3,000 to 4,000 cSt, between 4,000 to 5,000 cSt, or between 5,000 to 10,000 cSt. In some preferred embodiments the viscosity is between 500 to 600 cSt, or 600 to 700 cSt, or 700 to 800 cSt, or 800 to 900 cSt, or 900 to 1,000 cSt, or 1,000 to 1,200 cSt, or 1,200 to 1,300 cSt, or 1,300 to 1,400 cSt, or 1,400 to 1,500 cSt, or 1,600 to 1,700 cSt, or 1,700 to 1,800 cSt, or 1,800 to 1,900 cSt, or 1,900 to 2,000 cSt. The skilled person will appreciate that “cSt” (centistokes) is a measure of kinematic viscosity (also known as cS) and equals 1 mm^2/sec.

[0021] Preferably the barrier fluid comprises a specific gravity between 0.5 and 0.95 over a temperature range of -20 to 100ºC.

[0022] Preferably the barrier fluid comprises a surface tension between 15 and 50 mN/m. Preferably the barrier fluid surface tension is 20, 25, 30, 35, 40, 45, or 50 mN/m.

[0023] In one embodiment, preferably the barrier fluid is selected such that it remains a fluid over a temperature range of -20 to 100ºC. In other preferred embodiments, the barrier fluid is selected such that it remains a fluid over a temperature range of between room temperature, such as 10 to 20ºC, to 95ºC.

[0024] Preferably the barrier fluid is chosen such that the seal it forms remains substantially coherent over temperatures of -20 to 100 ºC. Preferably the barrier fluid is chosen such that the seal is resistant to mechanical or physical shock. Preferably the barrier fluid is chosen such that it permits transport of a fluid from one side to the other only when a predetermined centrifugal force is applied. For example, in some embodiments a certain thickness of barrier fluid will require a rotation of around 3000rpm to be applied in order to generate sufficient centrifugal force to transport a fluid from one side of the barrier to the other. The centrifugal force required overcomes the resistance of the seal formed from the barrier fluid. The skilled person will appreciate that a relatively thicker volume of barrier fluid will require a relatively higher centrifugal force to be applied in order to permit transport of a fluid from one side of the barrier to the other, and *vice versa*. In other embodiments, rotational speeds of around 1500, 2000, 3000, 4000 or 5000 rpm may be required, depending on the thickness of the barrier fluid and its properties, such as viscosity.

[0025] Preferably the barrier fluid is viscous silicon oil. Silicon oil is preferred because the length of the polymer chain can be controlled to provide a precise predetermined viscosity, such that the viscosity of the silicon oil can be “matched” to the reaction container such that the silicon oil will resist being displaced from the base of the reaction container with a rise in temperature, shaking, vibration, or mechanical shock.

[0026] Alternatively, the barrier fluid could be a paraffin wax. In preferred embodiments, the wax has a melting point in the range of 50 to 60ºC, preferably 53 to 57ºC, preferably 54 to 56ºC, preferably 57 to 60ºC. In further embodiments, the wax has an oil content of below 0.5% and a density of about 0.9 g/cm^3. Suitable waxes can be sourced from Aldrich (CAS: 8002-74-2; Product Code: 327204) or Wexis Parrafin Wax from wexisgp.com (2200045 or 2200046). The preferred waxes of the invention, when in a molten form, have the viscosity, specific gravity, and/or surface tension as described above.

[0027] Alternatively, the barrier fluid may be a silicone wax. In further embodiments, the wax has a molecular weight of about 12,600 amu. In other embodiments, the wax has a melting point in the range of about 40 to 50ºC, preferably 40 to 45ºC, more preferably about 43ºC. In a preferred embodiment, the silicone wax is an active organo silicone wax copolymer. A suitable wax may be sourced from Genesee Polymers Corporation (GP-58). The preferred silicone wax

of the invention, when in a molten form, has the viscosity, specific gravity, and/or surface tension as described above.

[0028] Further alternatively, the barrier fluid may be a grease, petroleum jelly, or other polymer compound. One preferred barrier fluid is petroleum jelly (also known as petrolatum, white petrolatum, and soft paraffin/paraffin wax; CAS number 8009-03-8) which is a semi-solid mixture of hydrocarbons with carbon numbers mainly higher than 25. Petroleum jelly is a mixture of hydrocarbons, having a melting point usually within a few degrees of human body temperature, approximately 37 °C. The preferred greases or petroleum jelly of the invention, when in a molten form, have the viscosity, specific gravity, and/or surface tension as described above.

[0029] In a preferred embodiment, the barrier fluid is transparent or optically clear. In an alternative embodiment, the barrier fluid is translucent. In a further alternative embodiment, the barrier fluid is opaque.

[0030] According to a first aspect the present invention provides a reaction container, comprising:

an upper portion comprising an inlet aperture open to the atmosphere, and

a lower portion for containing at least one pre-loaded reagent,

wherein at least some of the lower portion is configured and/or dimensioned such that when a sufficient volume of a barrier fluid is introduced into the lower potion a seal is formed thereby separating the at least one pre-loaded reagent from contact with the atmosphere.

[0031] In one embodiment of the first aspect, the reaction container includes no pre-loaded reagent, but includes a volume of pre-loaded barrier fluid. Preferably the barrier fluid is chosen such that its predetermined properties co-operate with the configured and/or dimensioned lower portion of said reaction container in order to form said seal, thereby separating the at least one pre-loaded reagent from contact with the atmosphere

[0032] Preferably the lower portion is configured into a waisted section and/or a capillary section and/or a “V”-shaped section and/or a “U”-shaped section and/or a constriction. Preferably the cross-sectional dimension of the configured and/or dimensioned lower portion is between 1mm to 5mm.

[0033] Preferably the barrier fluid is chosen to co-operate with the configuration and/or dimensioning of the lower portion such that at least some of said seal is in the form of an elastic membrane. Preferably the seal is in the form of a resilient barrier plug. Preferably the barrier fluid is selected such that its surface tension substantially maintains the seal when the reaction container is inverted or shaken.

[0034] In one embodiment, a plurality of reaction containers (or pre-loaded reaction containers - see below) are joined together in a circular array. The array can be configured to hold fewer or more than 20 of the reaction containers as required, such as 30, 40, 50, 60, 70, 80, 90, or 100 reaction containers. In one embodiment, the reaction containers are angled at 80 degrees from the horizontal. It will be appreciated that the circular array can be configured with any number of reaction containers as required and to suit the desired application. In one preferred embodiment, the circular array of reaction containers is particularly suited to be loaded into the thermocycler apparatus of PCT Publication WO 2015/054733 (incorporated herein by reference). The reaction container and cap assembly as described herein is particularly suited for automated loading in the thermocycler apparatus of WO 2015/054733, whereby the container and cap assembly can be placed in the thermocycler and loaded by a robot without further manipulation by a user.

[0035] According to a second aspect, the present invention provides a reaction container, comprising:

an upper portion comprising an inlet aperture open to the atmosphere, and

a lower portion containing at least one pre-loaded reagent and a barrier fluid,

wherein at least some of the lower portion is configured and/or dimensioned such that the barrier fluid forms a seal thereby separating the at least one pre-loaded reagent from contact with the atmosphere.

[0036] Preferably the pre-loaded reagent is selected from the group consisting of: an enzyme with polymerase activity, an enzyme, a nucleic acid, a nucleic acid primer, a buffer, a salt, a deoxyribonucleotide, a biological material, a preservative, an initiator, an acid, a nucleotide, a nucleoside triphosphate, or any combination thereof. Preferably, the pre-loaded reagent comprises an enzyme with polymerase activity.

[0037] Preferably the barrier fluid is chosen to co-operate with the configuration and/or dimensioning of the lower portion such that at least some of said seal is in the form of an elastic membrane.

[0038] In some embodiments, a plurality of seals (each comprised of barrier fluid) are configured in the reaction container in separated layers, whereby the pre-loaded reagent is positioned beneath any one of the seals. In one embodiment, the pre-loaded reagent is positioned under one seal and one or more nucleic acid amplification reagents are positioned beneath another seal, thereby separating the pre-loaded reagent from the nucleic acid amplification reagents, and whereby both the pre-loaded reagent and the nucleic acid amplification reagents are separated from contact with the atmosphere. It will be appreciated that more than 2 seals can be provided in order to separate the individual components of the nucleic acid amplification reagents from each other and the pre-loaded reagent. In some embodiments, a target nucleic acid can be provided within the reaction container, the target nucleic acid being separated from the atmosphere, the pre-loaded reagent, and the nucleic acid amplification reagents by use of 3 or more barrier fluid seals. Additionally, the barrier fluid of each individual layer of a plurality of seals can be the same or different, and can be chosen such that the seal permits transport of a fluid from one side to the other only when a predetermined centrifugal force is applied, and/or only when a predetermined temperature is reached during a heating cycle. Combinations of the above will be apparent to the skilled person.

[0039] Preferably the at least one pre-loaded reagent is completely immersed within the seal, or the seal is in overlying relationship with the at least one pre-loaded reagent, thereby separating the at least one pre-loaded reagent from contact with the atmosphere.

[0040] Preferably the barrier fluid is selected such that it is optically clear at wavelengths of light between 400 to 1000 nm over a temperature range of 0 to 100 ºC.

[0041] Preferably the barrier fluid is selected to allow uninterrupted transmission of fluorescent light.

[0042] Preferably the pre-loaded reagent is stable at temperatures between 10 and 40 ºC for up to 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 weeks when immersed within the barrier fluid, or is sealed from the atmosphere by the barrier fluid.

[0043] Preferably the reaction container further comprises a sealingly engageable cap for substantially or completely closing the inlet aperture.

[0044] Preferably the reaction container is formed from a material that is suitable for heating and autoclaving. More preferably the material is a plastics material, such as polycarbonate, polystyrene, high impact polystyrene, polyethylene or polypropylene. Alternatively, the material

may be glass or quartz. Other suitable materials will be known to the persons skilled in the art. Preferably the reaction container is formed from a material that permits uninterrupted transmission of fluorescent light.

[0045] In some embodiments, the reaction container is formed from a material which is adapted to be inductively heated by exposure to electromagnetic energy. Preferably the reaction container is formed from a material comprising ferromagnetic particles such that the reaction container is inductively heatable. Preferably the reaction container is suitable for use in a thermocycler.

[0046] Preferably the reaction container is suitable for use in a thermocycler system and method thereof, for example as described in WO 2015/054733, the contents of which are incorporated herein by reference. Preferably, the thermocycler comprises a rotatable platform formed, at least in part, of a material which is heatable in response to being exposed to electromagnetic energy; and an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform and heat the entire platform substantially simultaneously. Preferably the rotatable platform and/or the reaction wells are formed, at least in part, of a material which is adapted to be inductively heated by exposure to electromagnetic energy. In alternative embodiments, the rotatable platform and/or the reaction wells are in thermal communication with a material which is formed, at least in part, of a material which is adapted to be inductively heated by exposure to electromagnetic energy. The reaction wells are adapted to receive a corresponding plurality of the novel cap and reaction container assemblies of the present invention.

[0047] In use, a target nucleic acid is added to the reaction container along with a mixture of any further required nucleic acid amplification reagents. In this embodiment, the pre-loaded reagent is an enzyme with polymerase activity, such as Taq polymerase. Due to the viscosity of the barrier fluid, which maintains a resilient or elastic seal, the added reagents initially remain above the seal and are physically separated from the pre-loaded reagent. The reaction tube is heated to a temperature that inhibits the formation of nonspecific DNA products and then centrifuged at a speed sufficient to overcome the resistance provided by the seal and to urge the mixture into contact with the reagent. Preferably the temperature that inhibits the formation of nonspecific DNA products is 60 ºC or greater. In some preferred embodiments, the temperature is 65 ºC, 70 ºC, 80 ºC, 85 ºC, 90 ºC, or 95 ºC. Preferably the speed sufficient to bring the mixture into contact with the reagent is greater than 1,000 rpm and up to 5,000 rpm. In some preferred embodiments the speed is 1,000 rpm, 1,500 rpm, 2,000 rpm, 2,500 rpm, 3,000 rpm, 3,500 rpm, 4,000 rpm, 4,500 rpm, or 5,000 rpm. Preferably the reaction

container is sealed or partially sealed during centrifugation with a cap. Preferably the barrier fluid seal remains above the combined mixture and reagent after centrifugation. Preferably the barrier fluid prevents condensate forming at the top or side walls of the reaction container.

[0048] According to a third aspect, the present invention provides a method of amplifying a target nucleic acid, the method comprising:

a) providing a reaction container according to the second aspect;

b) adding a mixture of nucleic acid amplification reagents and the target nucleic acid to the reaction container according to the second aspect, and wherein the mixture of nucleic acid amplification reagents remains above the seal during the adding step and is physically separated from the pre-loaded reagent;

c) heating the reaction container to a temperature that inhibits the formation of nonspecific DNA products;

d) centrifuging the reaction container at a speed sufficient to combine the mixture of nucleic acid amplification reagents with the pre-loaded reagent; and

e) thermally cycling the reaction container to amplify the target nucleic acid.

[0049] In one embodiment, the pre-loaded reagent is an enzyme with polymerase activity, such as Taq polymerase.

[0050] In one embodiment, the pre-loaded reagent is not present in the mixture of nucleic acid amplification reagents. In a further embodiment, the pre-loaded reagent is also present in the mixture of nucleic acid amplification reagents. As an example, the pre-loaded reagent may be a Taq polymerase, which is also present in the mixture of nucleic acid amplification reagents which is added to the reaction container.

[0051] In one embodiment, the temperature that inhibits the formation of nonspecific DNA products is 60 ºC or greater. In some preferred embodiments, the temperature is 65 ºC, 70 ºC, 80 ºC, 85 ºC, 90 ºC, or 95 ºC.

[0052] In one embodiment, the speed sufficient to combine the mixture with the reagent is greater than 1,000 rpm. In some preferred embodiments the speed is 1,000 rpm, 1,500 rpm, 2,000 rpm, 2,500 rpm, 3,000 rpm, 3,500 rpm, 4,000 rpm, 4,500 rpm, or 5,000 rpm.

[0053] In one embodiment, the barrier fluid remains above the combined mixture of nucleic acid amplification reagents and pre-loaded reagent during and/or after step c).

[0054] In one embodiment, the barrier fluid prevents condensate forming at the top or side walls of the reaction container.

[0055] In one embodiment, steps a) to e) are performed in a thermocycler.

[0056] In one embodiment, the thermocycler comprises a rotatable platform formed, at least in part, of a material which is heatable in response to being exposed to electromagnetic energy; and an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform and heat the entire platform substantially simultaneously.

[0057] In one embodiment, amplified target nucleic acid is quantified by inclusion of an agent in the mixture of nucleic acid amplification reagents, wherein the agent fluoresces when bound to or hybridized with the target DNA and wherein the fluorescence is detectable and measurable. Suitable agents can be selected from ethidium bromide or SYBR® Green. Others will be known to the skilled person, such as SYTO 9 as an example of an intercalating dye referred to in the art as saturating dyes.

[0058] In one embodiment, the target nucleic acid is sequenced by inclusion of one or more agents in the mixture of nucleic acid amplification reagents, wherein the one or more agents enable the identification of incorporated nucleotides during amplification of the target nucleic acid.

[0059] According to a fourth aspect, the present invention provides a thermocycler system, the system comprising:

a) a rotatable platform containing at least one reaction container according to the second aspect, wherein the rotatable platform is formed, at least in part, of a material which is heatable in response to being exposed to electromagnetic energy; and

b) an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform, wherein the electromagnetic energy source surrounds a sufficient amount of the rotatable platform in order to heat the entire platform substantially simultaneously.

[0060] According to a fifth aspect, the present invention provides a method of cycling one or more reaction containers according to the second aspect between predetermined temperatures, the method comprising providing the thermocycler system according to the fourth aspect and cyclically:

a) actuating an electromagnetic energy source to heat the entire platform and the reaction container(s) substantially simultaneously,

b) ceasing heating, and

c) contacting the rotatable platform with coolant fluid to cool the platform and the reaction container(s),

thereby thermally cycling the reaction container(s).

[0061] According to a sixth aspect, the present invention provides use of a reaction container according to the first aspect, or a reaction container according to the second aspect in a method of amplifying a target nucleic acid according to the third aspect or a thermocycler system according to the fourth aspect.

[0062] In some preferred embodiments, the enzyme with polymerase activity is Taq polymerase, and the barrier fluid is 1000 cSt silicon oil. The nucleic acid amplification reagents comprise a 'master mix' containing one or more of: reaction buffer, dNTPs (deoxyribonucleotide triphosphate - a generic term referring to the four deoxyribonucleotides: dATP, dCTP, dGTP and dTTP), forward and reverse primer, and hydrolysis probe. The nucleic acid amplification reagents further comprises a nucleic acid target or template. In other embodiments, the barrier fluid is paraffin wax, a grease, petroleum jelly, or other polymer compound. In embodiments where there are a plurality of layers of barrier fluid, the barrier fluid can be chosen from one or more of the above-mentioned types of barrier fluid.

[0063] According to a seventh aspect, the present invention provides a cap for co-operating engagement with the reaction container according to the first aspect, the cap comprising:

an upper portion for receiving a target nucleic acid, and optionally a mixture of nucleic acid amplification reagents; and

a lower portion adapted to sealingly engage said inlet aperture of said reaction container to thereby define a reaction chamber between said cap and said reaction container,

wherein the upper portion and the lower portion of the cap are in fluid communication by a conduit adapted to substantially retain at least a barrier fluid within said reaction chamber.

[0064] In some embodiments the lower portion is adapted to reversibly sealingly engage said inlet aperture of said reaction container, and in other embodiments the lower portion is adapted to irreversibly sealingly engage said inlet aperture of said reaction container. In some embodiments, the cap is integrally formed with the reaction container by a flexible hinge.

[0065] According to an eighth aspect, the present invention provides a cap for co-operating engagement with a reaction container, the reaction container comprising an upper portion comprising an inlet aperture open to the atmosphere and a lower portion for containing at least one pre-loaded reagent, the cap comprising:

an upper portion for receiving a target nucleic acid, and optionally a mixture of nucleic acid amplification reagents; and

a lower portion adapted to sealingly engage said inlet aperture of said reaction container to thereby define a reaction chamber between said cap and said reaction container,

wherein the upper portion and the lower portion of the cap are in fluid communication by a conduit adapted to substantially retain at least a barrier fluid within said reaction chamber.

[0066] Referring to the above aspects, it will be appreciated that, in effect, two mechanisms may be provided for retaining a barrier fluid within the reaction chamber: (a) the reaction container can be configured and/or dimensioned such that when a sufficient volume of the barrier fluid is introduced into the lower portion a seal is formed to separate the at least one pre-loaded reagent from contact with the atmosphere, and (b) the conduit of the cap can be adapted to substantially retain at least the barrier fluid within the reaction chamber. These two mechanisms are advantageous in reducing the possibility of spilling the contents of the reaction chamber. For example, if the reaction chamber, while uncapped, is dropped or otherwise mishandled, the configuration and/or dimensioning of the reaction container may retain the barrier fluid seal and preserve any reagents stored there under (see (a)). Alternatively, if an insufficient volume of barrier fluid is provided in the reaction chamber, for example through user error, the conduit of the cap will provide a secondary barrier to preserve the contents of the reaction chamber (see (b)). This configuration is also advantageous in that

any reagent introduced into the upper portion of the cap will remain there until only until sufficient centrifugal force is applied to draw the reagent(s) through the conduit and into the reaction chamber. The conduit is configured to prevent reagents 'leaking' into the reaction chamber by providing resistance to flow under gravity. Further, the reagents drawn into the reaction chamber will remain above the seal only until sufficient centrifugal force is applied to overcome the resistance provided by the seal and bring the reagent(s) into contact with any reagents underlying the seal (or being held within the volume of the barrier fluid seal).

[0067] Preferably the conduit is configured and/or dimensioned such that a fluid having a predetermined viscosity and/or surface tension remains in the upper portion of the cap until the application of a predetermined centrifugal force is applied which overcomes the resistance to flow presented by the conduit. The fluid is the target nucleic acid and/or the mixture of nucleic amplification reagents and/or a solvent. In one embodiment, the fluid comprises a specific gravity between 0.5 and 0.95 over a temperature range of -20 to 100ºC. In one embodiment the fluid comprises a surface tension between 15 and 50 mN/m. Preferably the barrier fluid surface tension is 20, 25, 30, 35, 40, 45, or 50 mN/m. In one embodiment the fluid has a viscosity of greater than 500 cSt, in particular between 500 to 1,000 cSt, between 1,000 to 2,000 cSt, between 900 to 1100 cSt, between 2,000 to 3,000 cSt, between 3,000 to 4,000 cSt, between 4,000 to 5,000 cSt, or between 5,000 to 10,000 cSt.

[0068] Referring to the seventh or eighth aspects, it will be appreciated that the conduit is a fluid communication conduit adapted to substantially retain at least a barrier fluid within said reaction chamber.

[0069] In one embodiment, the conduit selectively allows the passage of fluids between the upper portion and the lower portion only by the application of sufficient centrifugal force. Preferably the centrifugal force is provided by loading the cap and reaction container in a thermocycler and operating the thermocycler. Preferably the centrifugal force is provided by operating the thermocycler at a speed greater than 1,000 rpm and up to around 5,000 rpm. In some preferred embodiments the speed is about 1,000 rpm, 1,500 rpm, 2,000 rpm, 2,500 rpm, 3,000 rpm, 3,500 rpm, 4,000 rpm, 4,500 rpm, or about 5,000 rpm. Preferably the thermocycler has a rotor diameter of about 7 to 10 cm. In some preferred embodiments, the thermocycler rotor diameter is about 7.0 cm, 7.5 cm, 8.0 cm, 8.5 cm, 9.0 cm, 9.5 cm, or 10.0 cm. The diameter of the thermocycler rotor diameter can be selected along with the rotational speed such as to provide a predetermined G-force which can overcome the resistance to fluid flow provided by the conduit of the cap in order to permit a fluid to be moved from the upper portion of the cap to the reaction container/chamber. By way of example only, at a radius of 7cm G-

forces of between about 80 and 2000G can be generated, and at a radius of 10cm, G-forces of between about 110 and 2800G can be generated. In one embodiment, the configuration of the conduit (size and/or shape) can be selected such that over 80, 100, 150, 200, 250, 300, 350, 400, 500, 750, 1000, 1500, 2000, or 2500G must be applied to move or displace a fluid through the conduit and overcome the resistance to flow that the conduit presents. In a further embodiment, the barrier fluid can be selected such that it is retained within the reaction chamber under normal handling conditions, or in the event of some mechanical shock, e.g. dropping or shaking. It will be appreciated that the conduit can be configured, and the barrier fluid chosen, such that a relatively low centrifugal force will move or migrate a reagent in the upper portion of the cap through the conduit and into the reaction chamber, but will not urge or migrate the reagent through the barrier fluid seal, and that a relatively higher centrifugal force is required to urge the reagent through (or into) the barrier fluid seal. In other embodiments, the conduit can be configured, and the barrier fluid chosen, such that the application of a predetermined centrifugal force will move a reagent in the upper portion of the cap through the conduit and into the reaction chamber, and also urge the reagent through the barrier fluid seal. It will be appreciated that other additives can be incorporated into one or more of the reagents disclosed herein to influence the properties of that reagent, such as a viscosity enhancing agent. The use of a viscosity enhancing agent, such as methyl cellulose, can impart a viscosity similar to the viscosities described above for the barrier fluid, i.e. such as between 500 to 2000 cSt.

[0070] In relation to the seventh and eighth aspects, in one embodiment the pre-loaded reagent is not present in the mixture of nucleic acid amplification reagents but is already present in the reaction chamber/container together with the barrier fluid, as discussed above. It will be appreciated that a target nucleic acid may be added to the cap/reaction container assembly before or after their co-operating engagement for measuring or assaying. The target nucleic acid would not usually be pre-packaged, but may be in certain embodiments.

[0071] In relation to the seventh and eighth aspects, in another embodiment the upper portion of the cap receives a target nucleic acid, whereby the barrier fluid and any other required nucleic acid amplification reagents are pre-loaded in the reaction chamber/container.

[0072] In relation to the seventh and eighth aspects, in another embodiment the upper portion of the cap receives a target nucleic acid, the barrier fluid, and any other required nucleic acid amplification reagents. Only the pre-loaded reagent is pre-loaded in the reaction chamber/container.

[0073] In relation to the seventh and eighth aspects, in another embodiment the upper portion of the cap receives target nucleic acid and any other required nucleic acid amplification reagents. In this embodiment, the pre-loaded reagent and the barrier fluid are pre-loaded in the reaction chamber/container.

[0074] In one embodiment, the reaction container and cap assembly is in the form of a unitary body.

[0075] Other embodiments and combinations of the disclosure above will be apparent to the skilled person. In particular, features of any one of the various described examples, aspects of the invention, or embodiments of the invention may be provided in any combination in any of the other described examples, aspects, or embodiments of the invention.

[0076] In one embodiment, the conduit is an aperture of less than 0.6 mm in diameter. In other embodiments the aperture is less than 0.5, 0.4, 0.3, 0.2, or 0.1 mm. In other embodiments the aperture is between 0.05 to 1 mm, such as 0.05 to 0.1, 0.1 to 0.2, 0.2 to 0.3, 0.3 to 0.4, 0.4 to 0.5, 0.5 to 0.6, 0.6 to 0.75, 0.7 to 0.8, 0.8 to 0.9, 0.9 to 1.0, or 1.0 to 1.2 mm. The aperture can have a cross sectional shape as any one of the following: circular, square, triangular, oval or any other polygon. In other embodiments, the conduit is cylindrical or conical in shape with a height of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, or 5 mm. Preferably the conduit is substantially open to the atmosphere.

[0077] In one embodiment, the conduit is a permeable membrane or a barrier filter. The membrane can be chosen from materials which are hydrophilic or hydrophobic. The material of construction may be polymeric or ceramic. The membrane or barrier filter can be selected to have pore sizes between 100 to 200 micron, 200 to 300 micron, 300 to 400 micron, 400 to 500 micron, 500 to 600 micron, 600 to 700 micron, 700 to 800 micron, 800 to 900 micron, or 900 to 1000 micron. Preferably the pore sizes are below 600 micron.

[0078] The skilled person will appreciate that the conduit is adapted to substantially retain the barrier fluid within the reaction chamber/container even when a sudden force (such as dropping or shaking) is applied to the reaction container, or the reaction container is inverted. The conduit is sized or selected such that the properties of the barrier fluid (e.g. viscosity, specific gravity, surface tension) make it difficult or unlikely that the barrier fluid can pass through the conduit in normal handling. In this way, any viscous barrier fluid (e.g. silicon oil) present in the reaction chamber/container remains therein during normal handling of the reaction container and cap assembly. However, in some embodiments, the barrier fluid may be introduced into the upper portion of the cap and migrated into the reaction chamber/container

by applying a sufficiently high centrifugal force to overcome the resistance to fluid flow provided by the conduit. It will be appreciated that the conduit can be sized and/or selected such that any combination of the pre-loaded reagent, the other nucleic acid amplification reagents (if any), the target nucleic acid and the barrier fluid are all substantially retained within the reaction chamber/container under normal handling.

[0079] Preferably the cap is formed from a material that is suitable for heating and autoclaving. More preferably the material is a plastics material, such as polycarbonate, polystyrene, high impact polystyrene, polyethylene or polypropylene. Alternatively, the material may be glass or quartz. Other suitable materials will be known to the persons skilled in the art. Preferably the cap is formed from a material that permits uninterrupted transmission of fluorescent light. In preferred embodiments, the cap is formed from a material which is the same as the reaction container; however it will be appreciated that the cap and the reaction container may be formed from different materials.

[0080] In one embodiment, at least some of the internal surfaces of the upper portion of the cap have a surface covering of nucleic acid amplification reagents in a 'dehydrated' form, e.g. in a powdered form. It will be appreciated that the addition of a solvent to the upper portion of the cap will solublise the nucleic acid amplification reagents, for example the solvent containing the target nucleic acid. The skilled person will appreciate that the dehydrated/powdered nucleic acid amplification reagents will remain stable over an extended period of time, and therefore the cap can be 'pre-loaded' with nucleic acid amplification reagents for the convenience of a user.

[0081] According to a ninth aspect the present invention provides a cap according to the seventh or eighth aspects, wherein at least some of the internal surfaces of the upper portion of the cap have a surface covering of nucleic acid amplification reagents in a 'dehydrated' or powdered form. In other embodiments, the cap can be 'pre-loaded' with a reagent (e.g. Taq polymerase) or a combination of Taq polymerase and other nucleic acid amplification reagents. In other embodiments the cap can be pre-loaded with reaction reagents that are lyophilised.

[0082] In some embodiments, at least some of the internal surfaces of the upper portion of the cap also have a surface covering of the pre-loaded reagent in a 'dehydrated' or powdered form.

[0083] In some embodiments, the 'dehydrated' or powdered reagents are adhered to the internal surface of the upper portion of the cap by electrostatic forces. In other embodiments,

the 'dehydrated' or powdered reagents are adhered to the internal surface of the upper portion of the cap by van der Waals forces. In some embodiments, the upper portion of the cap has a disposable removable foil seal, disposable lid, or plastic film as an additional means to preserve any dehydrated reagents during handling and transport, whereby the user removes the seal, lid, or film prior to use.

[0084] According to a tenth aspect, the present invention provides a reaction container and cap assembly, comprising:

a cap according to the seventh aspect sealingly engaged to a reaction container according to the first aspect.

[0085] According to an eleventh aspect, the present invention provides a reaction container and cap assembly, comprising:

a cap according to the eighth aspect sealingly engaged to a reaction container comprising an upper portion comprising an inlet aperture open to the atmosphere and a lower portion for containing at least one reagent.

[0086] It will be appreciated that the cap and reaction container assemblies of the tenth or eleventh aspects may be reversibly sealingly engaged. Alternatively, they may be bonded together to form a single assembly. Alternatively, they may be formed as a unitary body.

[0087] In one embodiment, the cap comprises a lip to selectively reversibly sealingly engage the reaction container. In one embodiment, the reaction container comprises a lip to selectively reversibly sealingly engage the cap. In one embodiment, the cap and the reaction container both comprise a lip to selectively reversibly sealingly engage one another.

[0088] In one embodiment, the cap and the reaction container comprise screw threads to selectively reversibly sealingly engage one another. For example, the cap can comprise an external screw thread and the reaction container can comprise an internal screw thread. Alternatively, the cap comprises the internal screw thread and the reaction container comprises the external screw thread.

[0089] According to a twelfth aspect, the present invention provides a reaction container and cap assembly according to the tenth aspect, wherein the reaction container contains a barrier fluid.

[0090] According to a thirteenth aspect, the present invention provides a reaction container and cap assembly according to the eleventh aspect, wherein the reaction container contains a barrier fluid.

[0091] In relation to the twelfth and thirteenth aspects, in some preferred embodiments a pre-loaded reagent is also present in the reaction chamber/container together with the barrier fluid.

[0092] In relation to the twelfth and thirteenth aspects, in some further preferred embodiments at least one nucleic acid amplification reagent is also present in the reaction chamber/container together with the barrier fluid and the pre-loaded reagent. The pre-loaded reagent and at least one nucleic acid amplification reagent may be in distinct and separate layers, which are separated by intervening layers of barrier fluid.

[0093] In relation to the twelfth and thirteenth aspects, in some further preferred embodiments at least some of the internal surfaces of the upper portion of the cap (and/or the upper portion of the reaction container) have a surface covering of nucleic acid amplification reagents in a 'dehydrated' or powdered form. In some embodiments, at least some of the internal surfaces of the upper portion of the cap (and/or the upper portion of the reaction container) also have a surface covering of the pre-loaded reagent in a 'dehydrated' or powdered form. In some embodiments, the 'dehydrated' or powdered particles are adhered to the internal surface of the upper portion of the cap (and/or the upper portion of the reaction container) by electrostatic forces. In other embodiments, the 'dehydrated' or powdered particles are adhered to the internal surface of the upper portion of the cap (and/or the upper portion of the reaction container) by van der Waals forces.

[0094] In relation to the twelfth and thirteenth aspects, in some further preferred embodiments at least some of the internal surfaces of the upper portion of the cap (and/or the upper portion of the reaction container) have a surface covering of at least one nucleic acid amplification reagent in a 'dehydrated' or powdered form, and the pre-loaded reagent is present in the reaction chamber/container together with the barrier fluid. Other embodiments and combinations will be apparent to the skilled person.

[0095] According to a fourteenth aspect, the present invention provides a method of amplifying a target nucleic acid comprising:

a) providing the reaction container and cap assembly according to the twelfth or thirteenth aspects, and

b) adding a mixture comprising at least a target nucleic acid to the reaction container and cap assembly, wherein a pre-loaded reagent is pre-loaded in the reaction chamber/container together with a barrier fluid and wherein the mixture remains above the barrier fluid, which physically separates the pre-loaded reagent and the mixture;

c) heating the reaction container to a temperature that inhibits the formation of nonspecific DNA products;

d) centrifuging the reaction container and cap assembly at a speed sufficient to combine the mixture with the pre-loaded reagent; and

e) thermally cycling the reaction container and cap assembly to amplify the target nucleic acid.

[0096] In one embodiment, the mixture further comprises at least one nucleic acid amplification reagent.

[0097] In one embodiment, the addition of the mixture in step (b) solublises at least one nucleic acid amplification reagent provided on the internal surfaces of the upper portion of the cap (and/or the upper portion of the reaction container) in a 'dehydrated' or powdered form.

[0098] In one embodiment, the temperature that inhibits the formation of nonspecific DNA products is 60 ºC or greater. In some preferred embodiments, the temperature is 65 ºC, 70 ºC, 80 ºC, 85 ºC, 90 ºC, or 95 ºC.

[0099] In one embodiment, the speed sufficient to combine the mixture with the reagent is greater than 1,000 rpm. In some preferred embodiments the speed is 1,000 rpm, 1,500 rpm, 2,000 rpm, 2,500 rpm, 3,000 rpm, 3,500 rpm, 4,000 rpm, 4,500 rpm, or 5,000 rpm.

[00100] In one embodiment, the barrier fluid remains above the combined mixture and the pre-loaded reagent during and/or after step d).

[00101] In one embodiment, the barrier fluid prevents condensate forming at the top or side walls of the reaction container.

[00102] In one embodiment, steps a) to e) are performed in a thermocycler.

[00103] In one embodiment, the thermocycler comprises a rotatable platform formed, at least in part, of a material which is heatable in response to being exposed to electromagnetic

energy; and an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform and heat the entire platform substantially simultaneously.

[00104] In one embodiment, amplified target nucleic acid is quantified by inclusion of an agent in the mixture, wherein the agent fluoresces when bound to or hybridized with the target nucleic acid and wherein the fluorescence is detectable and measurable.

[00105] In one embodiment, the target nucleic acid is sequenced by inclusion of one or more agents in the mixture, wherein the one or more agents enable the identification of incorporated nucleotides during amplification of the target nucleic acid.

[00106] It will be appreciated that, in one embodiment, the mixture undergoes an incubation reaction above the barrier fluid before being combined with the mixture with the pre-loaded reagent in step d).

[00107] Alternatively, the mixture must be combined with the pre-loaded reagent to cause an incubation reaction during or after step d).

[00108] In a preferred embodiment, the method provides an Uracil N-glycosylase (UNG) activation to remove potential PCR amplification contamination.

[00109] In another preferred embodiment, the method provides a Reverse Transcription (RT) for the conversion of RNA to cDNA.

[00110] According to a fifteenth aspect, the present invention provides a thermocycler system, the system comprising:

a) a rotatable platform containing at least one reaction container and cap assembly according to the twelfth or thirteenth aspects, wherein the rotatable platform is formed, at least in part, of a material which heatable in response to being exposed to electromagnetic energy; and

b) an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform, wherein the electromagnetic energy source surrounds a sufficient amount of the rotatable platform in order to heat the entire platform substantially simultaneously.

[00111] According to a sixteenth aspect, the present invention provides a method of cycling a reaction container and cap assembly according to the twelfth or thirteenth aspects between

predetermined temperatures, the method comprising providing the thermocycler system according to the fifteenth aspect and cyclically:

a) actuating an electromagnetic energy source to heat the entire platform and one or more reaction container and cap assemblies substantially simultaneously,

b) ceasing heating, and

c) contacting the rotatable platform with coolant fluid to cool the platform and the reaction container and cap assemblies,

thereby thermally cycling the reaction container and cap assemblies.

[00112] According to a seventeenth aspect, the present invention provides use of a reaction container and cap assembly according to the twelfth or thirteenth aspects in a method of amplifying a target nucleic acid according to the fourteenth aspect, in a thermocycler system according to the fifteenth aspect, or in a method of cycling a reaction container and cap assembly according to the sixteenth aspect.

[00113] According to an eighteenth aspect, the present invention provides a reaction container, comprising:

an upper portion comprising an inlet aperture open to the atmosphere, and

a lower portion containing:

a first pre-loaded reagent,

a first barrier fluid positioned over said first pre-loaded reagent, thereby substantially sealing said first pre-loaded reagent in the lower portion,

a second pre-loaded reagent positioned above said first barrier fluid, and

a second barrier fluid positioned over said second pre-loaded reagent,

wherein at least some of the lower portion is configured and/or dimensioned such that the first and/or second barrier fluid forms a seal thereby separating any underlying pre-loaded reagent from contact with the atmosphere.

[00114] In one embodiment, the first pre-loaded reagent and/or second pre-loaded reagent are selected independently from the group consisting of: an enzyme with polymerase activity

(such as a DNA polymerase, Taq polymerase or reverse transcriptase), an enzyme, a nucleic acid, a nucleic acid primer, a buffer, a salt, a deoxyribonucleotide, a biological material, a preservative, an initiator, an acid, a nucleotide, a nucleoside triphosphate, or combinations thereof.

[00115] In one embodiment, the first barrier fluid and/or second barrier fluid may be selected independently from the group consisting of: a silicon oil, a paraffin wax, grease, petroleum jelly, other polymer compounds, or combinations thereof.

[00116] In one embodiment, the first pre-loaded reagent is a Taq DNA polymerase. Alternatively, in another embodiment, the first pre-loaded reagent is a PCR reagent.

[00117] In one embodiment, the first barrier fluid is a wax.

[00118] In one embodiment, the second pre-loaded reagent is a PCR reagent. Alternatively, in another embodiment, the second pre-loaded reagent is a reverse transcriptase (RT). The present invention is suitable for reverse transcription, and all RNA based qPCR.

[00119] In one embodiment, the second barrier fluid is a silicon oil.

[00120] In one embodiment, the lower portion contains a third pre-loaded reagent and/or a third barrier fluid positioned above said second barrier fluid.

[00121] According to a nineteenth aspect, the present invention provides a reaction container and cap assembly, comprising:

a cap according to the seventh, eighth or ninth aspect sealingly engaged to a reaction container according to the eighteenth aspect.

[00122] It will be appreciated that the reaction container according to the eighteenth aspect is a pre-loaded reaction container.

[00123] There are many advantages which the present invention provides. For example, the reaction container can be supplied to users pre-capped, with nucleic acid amplification reagents provided in the cap (without the polymerase reagent or Taq provided in the cap), and the polymerase reagent or Taq is provided under the barrier fluid layer in the container. This configuration is particularly useful in robotics systems, as the sample can be extracted and loaded by the robot into the cap. The reaction sequences can then be commenced automatically. All other prior art systems that require some form of capping which in a robotics setting is not easy or straightforward.

[00124] In relation to the cap and reaction container assembly referred to above, an alternative to using a silicon oil as the barrier fluid is to utilise a wax in the lower portion of the reaction container to achieve the same result as a silicon oil.

[00125] The cap of the invention, the reaction container of the invention, or the assembly of the container and cap, is particularly suited for use in conducting nucleic acid amplification reactions such as the polymerase chain reaction (PCR) and the ligase chain reaction (LCR). However, it will be appreciated that the cap/container/assembly of the invention can be used in a wide variety of systems which require multiple or cyclic chemical reactions to produce a desired product. In relation to PCR, preferably the sample to be analysed or reacted is a nucleic acid such as DNA or RNA containing sample. Other components of the sample will typically include oligonucleotide primers, deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), and at least one of a thermostable DNA polymerase, enzymatically active fragments thereof, an enzymatically active derivative thereof and a reverse transcriptase.

[00126] The preferred thermocycler also includes monitoring means to assess the progress of the reaction occurring in the reaction container. Typically, this monitoring means will be a fluorescence detector, spectrophotometer, or photometer. This is particularly useful in monitoring the progress of a number of enzymatic reactions where a change in optical density or fluorescence of the product is observed. Such monitoring means is very useful in monitoring PCR reactions. In this case an intercalating dye, such as ethidium bromide or SYBR® Green, would be added to the reaction mix. When the dye binds to double stranded DNA there is fluorescence. Accordingly, by monitoring the degree of fluorescence in the reaction mixture an assessment as to the number of doublings which have occurred can be made. Alternatively, fluorescently labelled probes that hybridize to the DNA could be used. Other methods would be known to the skilled person.

[00127] Cycling between various predetermined temperatures can be automated. This would involve one or more of: actuation of the inductor, turning off or pulsing the inductor, increasing the speed of the rotatable platform, decreasing the speed of the rotatable platform, holding the rotational speed of the platform constant, reducing the rotational speed of the platform to zero or near zero, admitting coolant gas to cool the platform, reducing the flow of coolant gas to zero, controlling the temperature of the platform to a predetermined temperature, changing the platform temperature from one predetermined temperature to another (either colder to hotter, or vice versa), etc. Further, in the situation where monitoring means are provided and the

reaction has reached a suitable point, refrigerated gas may be pumped into the chamber thereby cooling the reaction mixture to a sub-ambient temperature.

[00128] The skilled addressee will understand that the invention comprises the embodiments and features disclosed herein as well as all combinations and/or permeations of the disclosed embodiments and features.

BRIEF DESCRIPTION OF THE DRAWINGS

[00129] Preferred embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings.

[00130] Figure 1 is a side section view showing:

A. a reaction container according to the invention having a substantially V-shaped lower portion;

B. a reaction container according to the invention comprising DNA polymerase overlaid with silicon oil of about 1000 cSt, wherein the silicon oil seals the Taq DNA polymerase from the atmosphere;

C. a reaction container according to the invention comprising DNA polymerase overlaid with silicon oil of about 1000 cSt and further comprising a PCR mixture comprising deoxynucleotides, $MgCl_2$, primers, probes, reaction buffers and target DNA, wherein the silicon oil physically separates the DNA polymerase from the PCR mixture; and

D. a reaction container according to the invention wherein, following heating to 60 ºC and centrifugation at greater than about 1,000 rpm, the PCR mixture is combined with the DNA polymerase and the silicon oil overlays the resultant mixture, reducing the formation of condensate.

[00131] Figure 2 is a perspective view showing reaction containers according to the invention with sealingly engagable closures fitted thereto.

[00132] Figure 3 is a side section view showing an embodiment of the reaction container and cap assembly according to the invention, wherein the cap conduit is an aperture, wherein:

A. the assembly is shown prior to assaying, the upper portion of the cap containing nucleic acid amplification reagents (PCR reagents) and the lower portion of the container containing a silicon oil and a DNA polymerase mixture; and

B. the assembly shown after assaying, the lower portion of the container containing a silicon oil overlaying nucleic acid amplification reagents (PCR reagents) and a DNA polymerase mixture.

[00133] Figure 4 is a side section view showing an embodiment of the reaction container and cap assembly according to the invention, wherein the cap conduit is an barrier filter, wherein:

A. the assembly is shown prior to assaying, the upper portion of the cap containing nucleic acid amplification reagents (PCR reagents) and the lower portion of the container containing a silicon oil and a DNA polymerase mixture; and

B. the assembly shown after assaying, the lower portion of the container containing a silicon oil overlaying nucleic acid amplification reagents (PCR reagents) and a DNA polymerase mixture.

[00134] Figure 5 is a graph showing the results of the real-time amplification curves for the Taq DNA Polymerase under silicon oil barrier fluid test, as discussed below.

[00135] Figure 6 is a graph showing the results of the normalised real-time amplification curves for high and low concentration templates under the cap test, as discussed below.

[00136] Figure 7 is a graph showing the results of the normalised amplification curves for the dehydrated reagent in cap test, as discussed below.

[00137] Figure 8 is a graph showing positive amplification of GAPDH mRNA (#1) that was reverse transcribed into cDNA using an RT enzyme under oil and then amplified using uninhibited Taq polymerase that was under a wax layer. No amplification of the GAPDH product was observed for the -RT (#2) and NTC (#3) controls

DEFINITIONS

[00138] In describing and claiming the present invention, the following terminology has been used in accordance with the definitions set out below. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one having ordinary skill in the art to which the invention pertains.

[00139] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive

sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of 'including, but not limited to'.

[00140] The terms 'preferred' and 'preferably' refer to embodiments of the invention that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the invention.

[00141] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein are to be understood as modified in all instances by the term 'about'.

[00142] The recitation of a numerical range using endpoints includes all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc.).

[00143] The prior art referred to in this specification is incorporated herein by reference.

PREFERRED EMBODIMENT OF THE INVENTION

[00144] The present invention will now be described with reference to the following examples which should be considered in all respects as illustrative and non-restrictive.

[00145] A reaction container 1 having a "V" shaped lower portion 2 (Figure 1A) is loaded with 1 µl or 1 Unit of Taq DNA polymerase 3 that does not comprise an inhibitor. The 1 µl liquid volume is then overlaid with 2-3 µL of high viscosity silicon oil 4 having viscosity of about 1000 cSt) (Figure 1B). The surface tension of the silicon oil 4 ensures the layered liquids cannot escape from the reaction container and maintains the integrity of the layered liquids even when a sudden force (such as dropping or shaking) is applied to the reaction container 1 or the reaction container 1 is inverted. This enables reaction containers to be shipped loose in bags. Silicon oil 4 has no optical transition and does not affect the DNA melt curve data, thereby making it suitable for qPCR.

[00146] The remaining PCR reagents 5 (e.g., dNTPs, $MgCl_2$, primers, probes, reaction buffers and sample) are added to the reaction container 1. These reagents 5 sit on top of the silicon oil layer 4 and are physically separated from the Taq DNA polymerase 3 that sits at the bottom of the container 1 (Figure 1C), which in the case of Figures 1B and 1C is shown as a droplet, but could instead be a layer at the base of the reaction container 1.

[00147] The reaction container 1 is heated to 60 ºC and centrifuged at a speed of greater than about 1,000 rpm. This has the effect of mixing all the aqueous volumes together at 60 ºC, which is equivalent to a "Hot start" qPCR reaction (Figure 1D).

[00148] The silicon oil 4 overlays the PCR reagents 5 (Figure 1D) and ensures that all of the PCR reagents 5 remains at the bottom of the container 1 during thermal cycling, preventing the formation of condensate at the top or side walls of the container 1. This keeps all the reaction mixture in the optical detection path and in the thermal cycling volume, and prevents:

- spread in the high resolution melts of identical samples; and/or
- spread in the Cq values of identical samples when measuring gene expression.

[00149] This prevents the introduction of errors in melt temperatures or Cq amplification points, which would affect the validity of the Gene Expression or DNA melt data.

[00150] The reaction container 1 enables the use of an inexpensive non-"hot-start" Taq DNA polymerase 3 in conjunction with silicon oil 4 to provide a physical barrier "hot-start". The silicon oil 4 ensures the Taq DNA polymerase 3 stays at the lower portion of the container 1 and prevents it from drying out.

[00151] The Taq DNA polymerase 3 can be supplied in liquid form (1-2 µl) so there is no need for lyophilisation. Lyophilised reagents typically do not have the same performance (usually sub-optimal) as wet reagents. Certain qPCR enhancers are burnt off during the drying process, and the lyophilised form is susceptible to humidity and must be packed in Mylar pouches. Additionally, it can be difficult to re-suspend the lyophilised pellet and "glassing" components must be added to the mix to ensure the pellet remains stuck to the bottom of a qPCR reaction tube. Lyophilising reagents is a process that requires substantial in-house know-how and significantly adds to the cost of a pre-packaged, room temperature stable reagent solution.

[00152] Liquid Taq DNA polymerase is stable at 37 ºC for in excess of 8 weeks, so it can be shipped in a standard post pack and no ice or cooling to 4 ºC is required.

[00153] Figure 2 shows the reaction container 1 sealed with a closure 6.

[00154] Figures 3 and 4 show embodiments of the reaction container 1 and cap 7 assembly according to the invention.

[00155] As shown in Figures 3A and 4A, the upper portion of the cap 7 is provided with one or more PCR reagents 5 which can be dispensed, via a conduit 8, into the reaction container 1. The conduit 8 selectively allows the passage of fluids between the upper portion of the cap 7 and the lower portion of the cap 7 only by the application of sufficient centrifugal force. The reaction container 1 is pre-loaded with silicon oil 4 and a DNA polymerase 3. In other embodiments, the container 1 may also be pre-loaded with further PCR reagents and/or a target nucleic acid.

[00156] Figures 3B and 4B illustrate the invention of Figures 3A and 4A, respectively, once the reaction container 1 and cap 7 assembly is subject to centrifugation. When a sufficiently high centrifugal force is applied to overcome the resistance provided by the conduit 8, for example through use with a thermocycler, the PCR reagents 5 will be drawn through the conduit 8 into the reaction chamber 9 for assaying by the thermocycler.

[00157] The conduit 8 is adapted to retain at least the silicon oil 4 within the reaction chamber 9. In Figure 3, the conduit 8 is an aperture of less than 0.6 mm in diameter. In Figure 4, the conduit 8 is a barrier filter with a pore size below 600 micron. In other embodiments, it will be appreciated that the barrier filter may be a permeable membrane.

[00158] In other embodiments, the reaction chamber 9 may either be pre-loaded with any combination of the silicon oil 4, the PCR reagents 5 and the DNA polymerase 3. In other embodiments, any combination of silicon oil 4, the PCR reagents 5 and the DNA polymerase 3 are provided to the chamber 9 through the conduit 8 of the cap.

[00159] The cap 7 and reaction container 1 are reversibly sealingly engagable, such that a user may unseal the cap 7 from the reaction container 1 to access the contents of the reaction container 1.

[00160] This reaction container 1 and cap 7 assembly provides manufacturers the ability to produce transportable reaction tubes for assays which are pre-loaded with appropriate compounds, and/or caps which are pre-loaded with appropriate compounds, wherein the apparatus is ready-to-use immediately after transport and with minimal risk of spilling the compounds.

[00161] A skilled person would appreciate that this assembly may simplify the assaying process, as less set-up time is required for loading the tubes with the appropriate compounds.

EXAMPLES

Physical test

[00162] Samples of 1 µl of an analogue to a typical DNA polymerase storage buffer reagent (100 mM KCl, 10 mM Tris-HCl, 0.5% Tween 20 and 50% glycerol), were loaded into empty reaction containers 1 according to the present invention. A volume of 5 µL of silicone oil barrier fluid 4 having a viscosity of 1000 cSt was then loaded on top of the buffer reagent, and the reaction containers 1 were subjected to the following tests discussed below. Control samples of reaction containers 1 with the same buffer reagent, but without a barrier fluid 4, were also tested.

[00163] A group of sample reaction containers 1 ($n = 20$) were held inverted for a period of 48 hours. A second group of sample reaction containers 1 were dropped from a height of 1.5 m to a solid concrete floor. In both the inversion and drop tests there was no observed movement in the storage buffer reagent from its initial position at the bottom of the reaction containers 1 under the silicone oil barrier fluid 4. Conversely, the storage buffer reagent was found to be displaced in the control samples. This demonstrates that the high viscosity of the silicone oil barrier fluid 4 provides a sufficient surface tension which co-operates with the dimensions of the reaction container 1 such that the storage buffer reagent sample is retained during periods of force capable of displacing liquid from reaction containers 1 that did not contain the silicone oil barrier fluid 4.

Taq under silicone oil barrier fluid test

[00164] An amount of 1 µL of 1 U Taq DNA Polymerase 3 (New England Biosciences, USA) under 5 µL of 1000 cSt silicone oil barrier fluid 4 were prepared into empty reaction containers 1 according to the present invention. A master mix containing 1X ThermoPol Reaction buffer, 0.2 mM dNTPs, 0.3 µM forward and reverse primer each, and 0.1 µM FAM-labelled hydrolysis probe was also prepared and set aside. The target was a synthetic gene referred to as Shh created as a gBlock template (IDT, USA). One micro-litre of template was loaded at a high ($1x10^6$ copies/µL) or low (1000 copies/µL) concentration into sample of the master mix and loaded on top of the silicone oil barrier fluid 4. A 'no template control' (NTC) was used as negative control; and reactions containing the Taq polymerase 3 within the master mix were used as positive controls for both high and low concentrations of template. The reaction containers were spun at 1000 rpm to combine the Taq polymerase 3 and the master mix. Reactions were run on a commercial thermocycler (the 'Mic cycler' from BMS; http://mic-qpcr.com/, as described in PCT Publication WO 2015/054733) using the following conditions:

initial hold at 95°C for 5 min, followed by 40 cycles at 95°C for 10 seconds and 60° for 30 seconds, acquiring at the end of anneal on the 'green channel'.

[00165] Figure 5 shows the results of the real-time amplification curves for the Taq DNA Polymerase 3 under silicone oil barrier fluid 4 samples (#1 and #2) using either a high (1 x 10^6 copies/µL; #1) or low (1000 copies/µL; #2) concentration of template. There was a 3-cycle delay in take-off compared with the positive control samples (#3 and #4). None of the NTCs (#5) amplified.

[00166] The results demonstrated the feasibility of running Taq DNA Polymerase 3 under silicone oil barrier fluid 4 in the reaction container 1. The delay in cycle take-off was attributed to a lack of mixing at the start.

Cap test

[00167] A cap 7 and a reaction container 1 assembly according to the present invention was tested using the same assay described above for the Taq DNA Polymerase 3 under silicone oil barrier fluid 4 tests. The caps 7 were pre-assembled to reaction containers 1 prior to loading a qPCR master mix. The cap 7 was designed to be in fluid communication with the reaction container 1 by a conduit 8.

[00168] The master mix, including the Taq DNA Polymerase 3, was loaded into the cap 7. Either a high or low concentration of template was loaded into a cap 7 containing master mix. NTCs were used as negative controls and were run alternating with samples containing template. Reaction containers 1 with master mix loaded conventionally and enclosed using conventional caps, were used as positive controls. All experimental reaction containers 1 contained 1000 cSt silicone oil barrier fluid 4 pre-loaded into the reaction container 1. The cap 7 a reaction container 1 assemblies according to the present invention were spun to 3000 rpm for 60 seconds at room temperature within the Mic cycler, prior to the initial denaturation of the template. Observations were made post run to ensure that the reaction contents had entered the reaction container 1 from the caps 7, and were under the silicone oil barrier fluid 4.

[00169] Ten successive runs, on the same thermocycler instrument, were performed to evaluate for any contamination during the run by looking for any signs of amplification in the NTCs. Swabs of the thermocycler rotor and surrounding surfaces were taken to check for any further signs of contamination. A quarantined thermocycler instrument (never used to run the Shh assay) was used as a control for the swab tests. Positive controls were run in another thermocycler instrument as a control to rule out potential contamination due to conditions independent of the cap 7 assemblies.

[00170] Figure 6 shows the normalised real-time amplification curves for high (1×10^6 copies/µL; #1) and low (10 copies/µL; #2) concentration templates using the cap assembly 7 to load solutions into the reaction container 1. None of the NTCs (#3) amplified, thereby demonstrating no contamination issues.

[00171] It was observed that a positive amplification signal occurred for both the high and low concentration test cases. There was no amplification observed in any of the NTCs for any of the 10 runs undertaken. All positive control samples amplified with similar results to the test cases. None of the swab samples of the thermocycler rotor or surrounding surfaces for the test case, control, or quarantined thermocycler instruments produced amplification curves. The results demonstrated the feasibility of using the cap assembly 7 to load samples effectively into a reaction container 1 with no observable issues associated with contamination.

Dehydrated reagent in cap test

[00172] Six micro-litres of a primer and probe mix (5 µM primer and 1 µM probe) were loaded into a number of caps 7 according to the present invention. The caps 7 were then placed into a hermetically sealed chamber with desiccant to dehydrate the mixture. Once dehydrated, the caps 7 were assembled onto reaction containers 1 containing 1 µL of 1 U Taq DNA polymerase 3 under 5 µL of 1000 cSt silicone oil barrier fluid 4. Twenty-three micro-litres of master mix described for the Shh assay (without primers and probe), was added to caps 7 containing the dehydrated oligonucleotides. Either a high or low concentration of template was added, or water as a negative control, to a cap 7 (1 µL). Standard reaction containers containing all the reaction components were used as positive controls. The samples were spun at 3000 rpm for 60 seconds prior to the initial denaturation of the template. The reactions were carried out using the conditions described for Shh above.

[00173] Figure 7 shows the normalised amplification curves for both cap 7 and reaction container 1 assembly samples containing dehydrated primers and probe (#1 and #2) and positive controls (#3 and #4) for both high and low concentrations of template. There was no amplification observed in any of the NTCs (#5).

[00174] Positive amplification of the dehydrated test samples was observed for both high and low concentration templates. No amplification was observed for NTCs. There was a two-cycle delay in Cq values for the test samples compared with the positive control samples. The results demonstrate feasibility in dehydrating oligonucleotides into the cap 7 and using the cap 7 with a reaction container 1 having Taq DNA Polymerase 3under a silicone oil barrier fluid 4.

The small difference in cycle number was put down to a minor lack of mixing during reconstitution of the oligonucleotide mix at the start.

One step reverse transcription (RT-qPCR) test

[00175] The feasibility of running a two-stage process of reverse transcription (RT)-qPCR within a cap 7 and reaction container 1 assembly according to the present invention was also tested. A plurality of reaction containers 1 were loaded with the following: 1 U of Taq DNA polymerase 3 loaded underneath 5 µL of a solid wax barrier (stage 2); 50 U of AMV reverse transcriptase (RT-enzyme) loaded above the wax barrier and covered with 5 µL of 1000 cSt silicone oil barrier fluid 4 (stage 1). Caps 7, containing dehydrated primers, were assembled on top of the reaction containers 1.

[00176] Master mix containing buffer, dNTP, SYBR Green and water, to run the RT-qPCR, was loaded into the caps 7. Ten nanograms per micro-litre of human total RNA was added to the caps 7. Water was used as a negative control. Primers were designed to target the human glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Samples were spun down in the Mic thermocycler at 3000 rpm for 60 seconds at room temperature to allow the master mix-template-primer solution to combine with the RT-enzyme under the silicone oil barrier fluid 4. Samples were heated to 40°C to start the reverse transcription reaction. After 10 min, the samples were heated to 95°C, deactivating the RT-enzyme and melting the wax, and then spun at 3000 rpm to allow the RT reaction to combine in with the Taq DNA polymerase under the wax-oil barrier. Samples were cycled 40 times at 95°C for 10 seconds and 60°C for 20 seconds. Fluorescence was acquired at anneal on the 'green channel'.

[00177] Figure 8 shows the positive amplification of GAPDH mRNA (#1) that was reverse transcribed into cDNA using the RT enzyme under oil and then amplified using uninhibited Taq polymerase that was under a wax layer. No amplification of the GAPDH product was observed for the -RT (#2) and NTC (#3) controls. These results demonstrated the ability to use a two-stage barrier protocol to achieve one step RT-qPCR without the need to use an inhibited Taq polymerase.

[00178] The skilled person will appreciated that an alternative comprises a 'one step' RT-qPCR using both the Taq (antibody inhibited) and reverse transcriptase under barrier fluid (oil).

[00179] Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms. In particular features of any one of the various described examples may be provided in any combination in any of the other described examples.

CLAIMS

1. A cap for co-operating engagement with a reaction container, the reaction container comprising an upper portion comprising an inlet aperture open to the atmosphere and a lower portion for containing at least one pre-loaded reagent, the cap comprising:

an upper portion for receiving a target nucleic acid, and optionally a mixture of nucleic acid amplification reagents; and

a lower portion adapted to sealingly engage said inlet aperture of said reaction container to thereby define a reaction chamber between said cap and said reaction container,

wherein the upper portion and the lower portion of the cap are in fluid communication by a conduit adapted to substantially retain at least a barrier fluid within said reaction chamber.

2. The cap according to claim 1 wherein the lower portion of said cap is adapted to be reversibly sealingly engagable with the upper portion of said reaction container.

3. The cap according to claim 1 or claim 2 wherein said conduit is adapted to selectively allow the passage of fluids between the upper portion and the lower portion of the cap by the application of sufficient centrifugal force.

4. The cap according to claim 3 wherein said centrifugal force is provided by rotating said cap and said reaction container at a speed greater than about 1,000 rpm and up to around 5,000 rpm.

5. The cap according to claim 4 wherein the rotation is provided by a thermocycler having a rotor diameter of about 7 to 10 cm

6. The cap according to any one of claims 3-5 wherein the conduit is adapted to selectively allow the passage of fluids between the upper portion and the lower portion of the cap by the application of sufficient G-forces.

7. The cap according to claim 6 wherein said G-forces are selected from about 80, 100, 150, 200, 250, 300, 350, 400, 500, 750, 1000, 1500, 2000, or 2500G.

8. The cap according to any one of claims 1-7 wherein the reaction container is pre-loaded with at least one barrier fluid.

9. The cap according to any one of claims 1-8 wherein the barrier fluid is selected from viscous silicon oil, paraffin wax, silicone wax, grease, or petroleum jelly.

10. The cap according to any one of claims 1-9 wherein the barrier fluid has a viscosity of greater than 500 cSt and up to around 2000 cSt.

11. The cap according to any one of claims 1-10 wherein the barrier fluid has a specific gravity between 0.5 and 0.95 over a temperature range of -20 to 100ºC.

12. The cap according to any one of claims 1-11 wherein the barrier fluid has a surface tension between 15 and 50 mN/m.

13. The cap according to any one of claims 1-12 wherein the barrier fluid is selected such that it remains a fluid over a temperature range of 20 to 95ºC.

14. The cap according to any one of claims 1-13 wherein the barrier fluid is selected such that has a melting point in the range of 50 to 60ºC.

15. The cap according to any one of claims 1-14 wherein the barrier fluid is transparent.

16. The cap according to any one of claims 1-14 wherein the barrier fluid is translucent.

17. The cap according to any one of claims 1-14 wherein the barrier fluid is opaque.

18. The cap according to any one of claims 1-17 wherein the reaction container is pre-loaded with at least one pre-loaded reagent.

19. The cap according to claim 18 wherein the pre-loaded reagent is not present in the mixture of nucleic acid amplification reagents received by the upper portion of the cap.

20. The cap according to any one of claims 1-19 wherein the pre-loaded reagent is an enzyme with polymerase activity.

21. The cap according to claim 20 wherein the enzyme with polymerase activity is selected from: a DNA polymerase, a Taq polymerase, a reverse transcriptase, or a Klenow.

22. The cap according to any one of claims 1-21 wherein the nucleic acid amplification reagents are selected from: an enzyme, a nucleic acid, a nucleic acid primer, a buffer, a salt, a deoxyribonucleotide, a biological material, a preservative, an initiator, an acid, a nucleotide, a nucleoside triphosphate, or any combination thereof.

23. The cap according to any one of claims 1-22 wherein the conduit is an aperture.

24. The cap according to claim 23 wherein the aperture is of less than 0.6 mm in diameter, or between 0.05 to 1 mm in diameter.

25. The cap according to any one of claims 1-22 wherein the conduit is a permeable membrane or a barrier filter.

26. The cap according to claim 25 wherein the permeable membrane or a barrier filter is hydrophobic.

27. The cap according to claims 25 or claim 26 wherein the permeable membrane or a barrier filter is polymeric or ceramic.

28. The cap according to any one of claims 25-27 wherein the permeable membrane or a barrier filter has a pore size between 600 to 1000 micron.

29. The cap according to any one of claims 1-28 wherein the cap is formed from a material that is suitable for heating and autoclaving.

30. The cap according to any one of claims 1-29 wherein the cap is formed from a material that permits uninterrupted transmission of fluorescent light.

31. The cap according to claim 29 or 30 wherein the material is a plastics material selected from polycarbonate, polystyrene, high impact polystyrene, polyethylene or polypropylene, or is glass or quartz.

32. The cap according to any one of claims 1-31 wherein the cap is formed from a material which is the same as the reaction container.

33. The cap according to any one of claims 1-32 wherein at least some of the internal surfaces of the upper portion of the cap have a surface covering of nucleic acid amplification reagents in a dehydrated or powdered form.

34. The cap according to claim 33 wherein the surface covering of nucleic acid amplification reagents comprises Taq polymerase.

35. The cap according to claim 33 wherein the surface covering of nucleic acid amplification reagents comprises a combination of Taq polymerase and other nucleic acid amplification reagents.

36. The cap according to any one of claims 1-32 wherein the at least some of the internal surfaces of the upper portion of the cap have a surface covering of the pre-loaded reagent in a dehydrated or powdered form.

37. The cap according to any one of claims 33-36 wherein the surface covering of nucleic acid amplification reagents or pre-loaded reagent is adhered to the internal surface of the upper portion of the cap by electrostatic forces or van der Waals forces.

38. The cap according to any one of claims 1-37 wherein the upper portion of the cap has a disposable removable foil seal, disposable lid, or plastic film.

39. The cap according to any one of claims 1-38 wherein at least some of the lower portion of the reaction container is configured and/or dimensioned such that when a sufficient volume of a barrier fluid is introduced into the lower portion of the reaction container a seal is formed thereby separating any pre-loaded reagent from contact with the atmosphere.

40. The cap according to claim 39 wherein the barrier fluid is chosen to co-operate with the configuration and/or dimensioning of the lower portion of the reaction container such that at least some of said seal is in the form of an elastic membrane.

41. A reaction container and cap assembly, comprising:

a cap according to any one of the preceding claims sealingly engaged to a reaction container comprising an upper portion comprising an inlet aperture open to the atmosphere and a lower portion for containing at least one reagent.

42. The assembly according to claim 41 wherein the cap and reaction container assembly is adapted to be reversibly sealingly engaged.

43. The assembly according to claim 41 or 42 wherein the reaction container contains a barrier fluid.

44. The assembly according to claim 43 wherein a pre-loaded reagent is also present in the reaction container together with the barrier fluid.

45. The assembly according to claim 44 wherein the reaction container contains a barrier fluid, at least one nucleic acid amplification reagent, and the pre-loaded reagent.

46. A method of amplifying a target nucleic acid comprising:

a) providing the reaction container and cap assembly according to any one of claims 41 to 45, and

b) adding a mixture comprising at least a target nucleic acid to the reaction container and cap assembly, wherein a pre-loaded reagent is pre-loaded in the reaction chamber/container together with a barrier fluid and wherein the mixture remains above the barrier fluid, which physically separates the pre-loaded reagent and the mixture;

c) heating the reaction container to a temperature that inhibits the formation of nonspecific DNA products;

d) centrifuging the reaction container and cap assembly at a speed sufficient to combine the mixture with the pre-loaded reagent; and

e) thermally cycling the reaction container and cap assembly to amplify the target nucleic acid.

47. The method according to claim 46 wherein the mixture further comprises at least one nucleic acid amplification reagent.

48. The method according to claim 47 wherein the addition of the mixture in step (a) solubilises at least one nucleic acid amplification reagent provided on the internal surfaces of the upper portion of the cap in a dehydrated or powdered form.

49. The method according to any one of claims 46-48 wherein temperature that inhibits the formation of nonspecific DNA products is 60 ºC or greater.

50. The method according to any one of claims 46-49 wherein the speed sufficient to combine the mixture with the reagent is greater than 1,000 rpm.

51. The method according to any one of claims 46-50 wherein the barrier fluid remains above the combined mixture and the pre-loaded reagent during and/or after step c).

52. The method according to any one of claims 46-51 wherein the barrier fluid prevents condensate forming at the top or side walls of the reaction container.

53. The method according to any one of claims 46-52 wherein steps a) to d) are performed in a thermocycler.

54. The method according to claim 53 wherein the thermocycler comprises a rotatable platform formed, at least in part, of a material which is heatable in response to being exposed to electromagnetic energy; and an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform and heat the entire platform substantially simultaneously.

55. The method according to any one of claims 46-54 wherein amplified target nucleic acid is quantified by inclusion of an agent in the mixture, wherein the agent fluoresces when bound to or hybridized with the target nucleic acid and wherein the fluorescence is detectable and measurable.

56. The method according to any one of claims 46-55 wherein the target nucleic acid is sequenced by inclusion of one or more agents in the mixture, wherein the one or more agents enable the identification of incorporated nucleotides during amplification of the target nucleic acid.

57. The method according to any one of claims 46-56 wherein the mixture undergoes an incubation reaction above the barrier fluid before being combined with the mixture with the pre-loaded reagent in step d).

58. The method according to any one of claims 46-56 wherein the mixture is combined with the pre-loaded reagent to cause an incubation reaction during or after step d).

59. The method according to any one of claims 46-58 including the step of uracil n-glycosylase activation to remove potential PCR amplification contamination.

60. The method according to any one of claims 46-58 including reverse transcription (RT) for the conversion of RNA to cDNA.

61. A thermocycler system, the system comprising:

a) a rotatable platform containing at least one reaction container and cap assembly according to the any one of claims 41 to 45, wherein the rotatable platform is formed, at least in part, of a material which heatable in response to being exposed to electromagnetic energy; and

b) an electromagnetic energy source adapted to direct electromagnetic energy at the rotatable platform, wherein the electromagnetic energy source surrounds a

sufficient amount of the rotatable platform in order to heat the entire platform substantially simultaneously.

62. A method of cycling a reaction container and cap assembly according to any one of claims 41 to 45 between predetermined temperatures, the method comprising providing the thermocycler system according to claim 61 and cyclically:

a) actuating an electromagnetic energy source to heat the entire platform and the reaction container and cap assemblies substantially simultaneously,

b) ceasing heating, and

c) contacting the rotatable platform with coolant fluid to cool the platform and the reaction container and cap assemblies,

thereby thermally cycling the reaction container and cap assemblies.

63. Use of a reaction container and cap assembly according to any one of claims 41 to 45 in a method of amplifying a target nucleic acid according to any one of claims 46 to 60, in a thermocycler system according to claim 61, or in a method of cycling a reaction container and cap assembly according to claim 62.

64. A reaction container, comprising:

an upper portion comprising an inlet aperture open to the atmosphere, and

a lower portion containing:

a first pre-loaded reagent,

a first barrier fluid positioned over said first pre-loaded reagent, thereby sealing said first pre-loaded reagent in the lower portion,

a second pre-loaded reagent positioned above said first barrier fluid, and

a second barrier fluid positioned over said second pre-loaded reagent,

wherein at least some of the lower portion is configured and/or dimensioned such that the second barrier fluid forms a seal thereby separating the at least one pre-loaded reagent from contact with the atmosphere.

65. The container according claim 64 wherein the first pre-loaded reagent and/or second pre-loaded reagent may be selected independently from the group consisting of: an enzyme with polymerase activity, optionally a DNA polymerase, Taq polymerase or reverse transcriptase, an enzyme, a nucleic acid, a nucleic acid primer, a buffer, a salt, a deoxyribonucleotide, a biological material, a preservative, an initiator, an acid, a nucleotide, a nucleoside triphosphate, or combinations thereof.

66. The container according claim 64 or claim 65 wherein the first barrier fluid and/or second barrier fluid may be selected independently from the group consist of; a silicon oil, a paraffin wax, a silicone wax, grease, petroleum jelly, other polymer compounds, or combinations thereof.

67. The container according any one of claims 64-66 wherein the first pre-loaded reagent is a Taq DNA polymerase.

68. The container according any one of claims 64-66 wherein the first pre-loaded reagent is a PCR reagent.

69. The container according any one of claims 64-68 wherein the first barrier fluid is a wax.

70. The container according any one of claims 64-69 wherein the second pre-loaded reagent is a PCR reagent.

71. The container according any one of claims 64-69 wherein the second pre-loaded reagent is a reverse transcriptase.

72. The container according any one of claims 64-71 wherein the second barrier fluid is a silicon oil.

73. The container according claim 64 wherein the first pre-loaded reagent is a Taq DNA polymerase, the first barrier fluid is a wax, the second pre-loaded reagent is a PCR reagent, and the second barrier fluid is a silicon oil.

74. The container according claim 64 wherein the first pre-loaded reagent is a Taq DNA polymerase, the first barrier fluid is a wax, the second pre-loaded reagent is a reverse transcriptase, and the second barrier fluid is a silicon oil.

75. The container according any one of claims 64-74 wherein the lower portion contains a third pre-loaded reagent and/or a third barrier fluid positioned above said second barrier fluid.

76. A reaction container and cap assembly, comprising:

a cap according to any one of claims 1-40 sealingly engaged to a reaction container according to any one of claims 64-75.

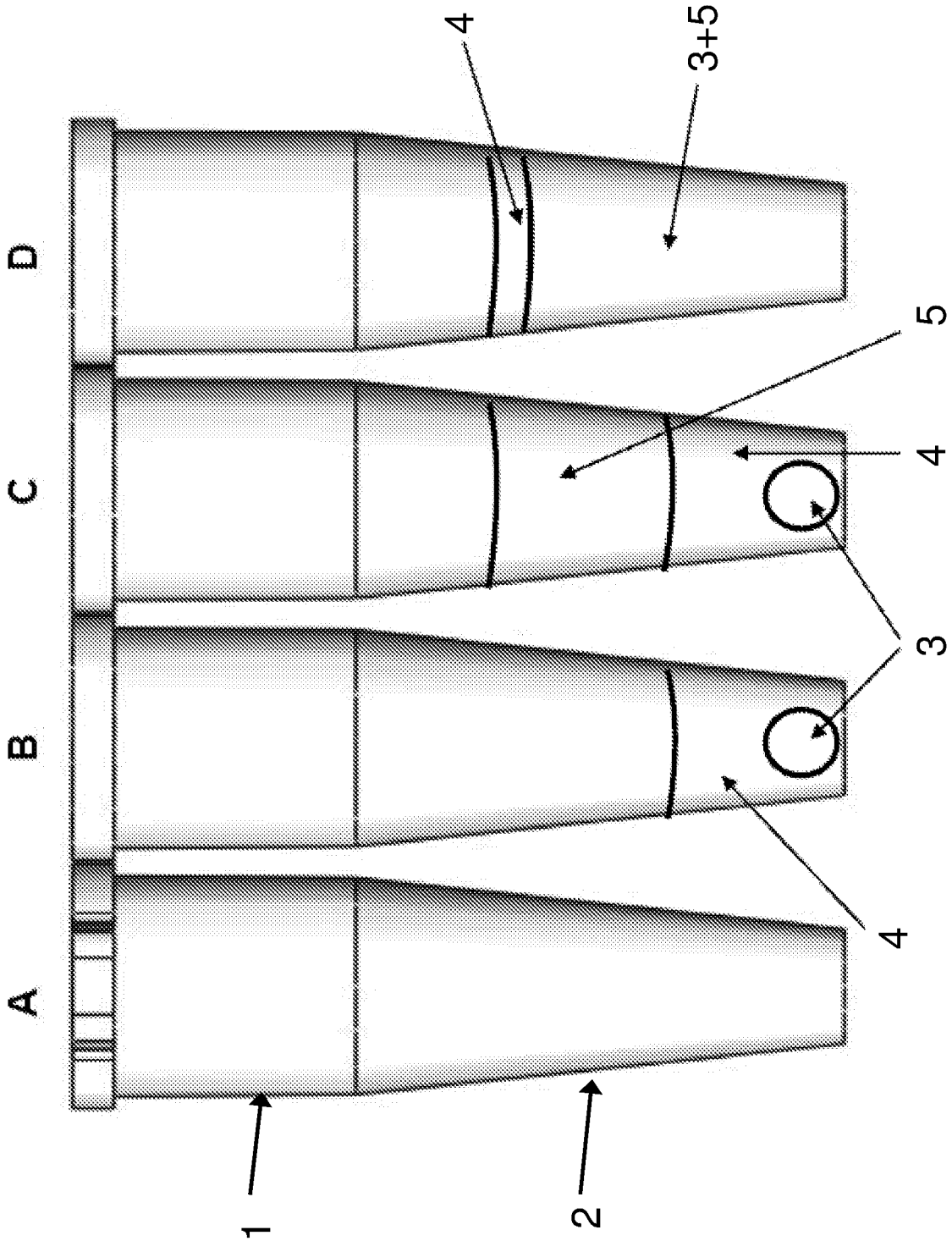


FIG. 1

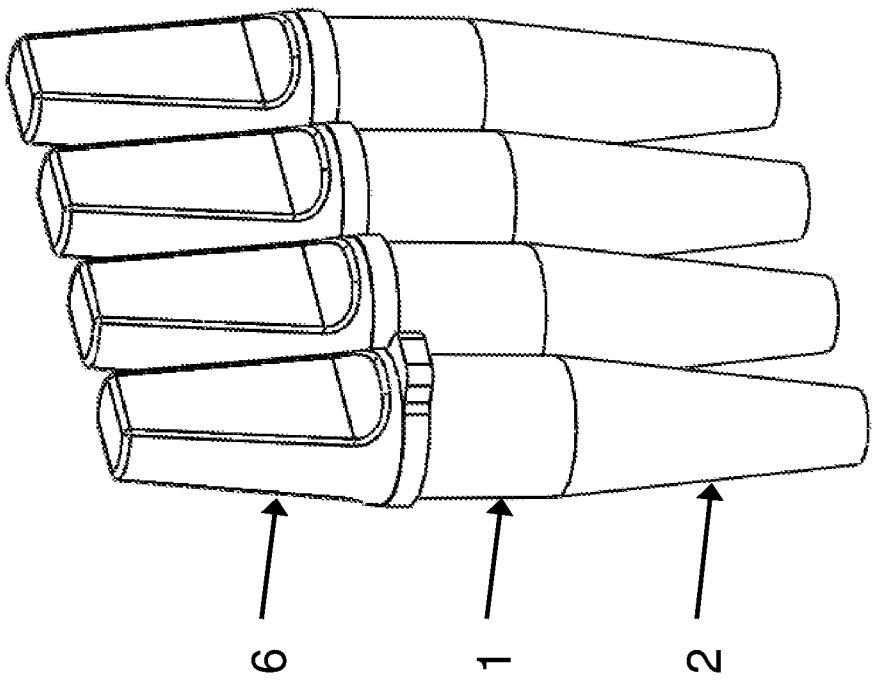


FIG. 2

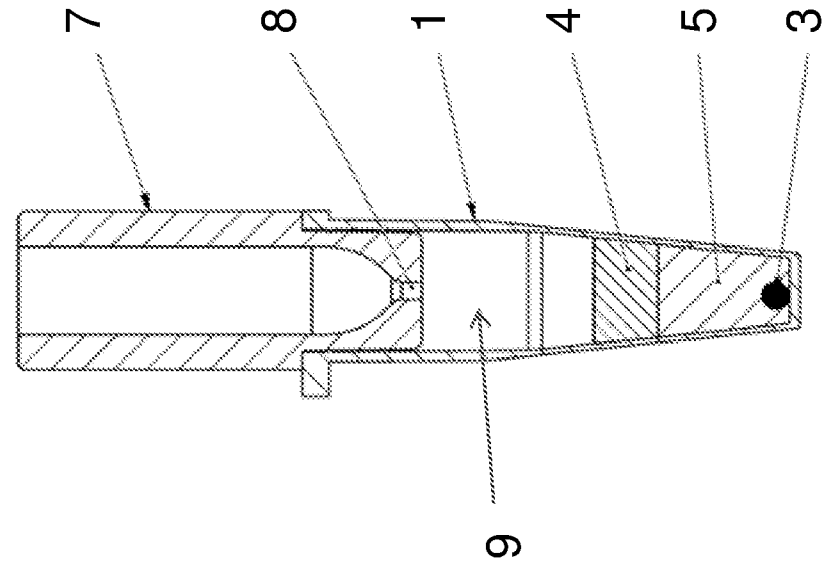


FIG. 3B

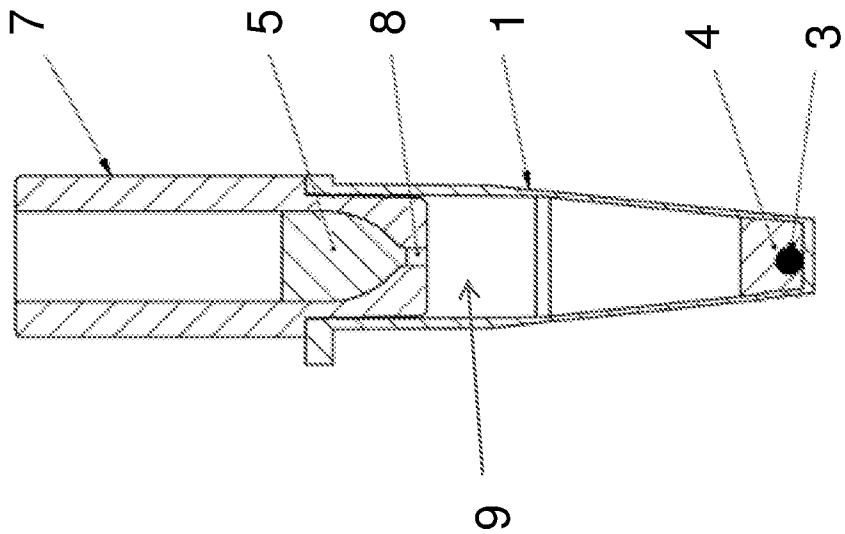


FIG. 3A

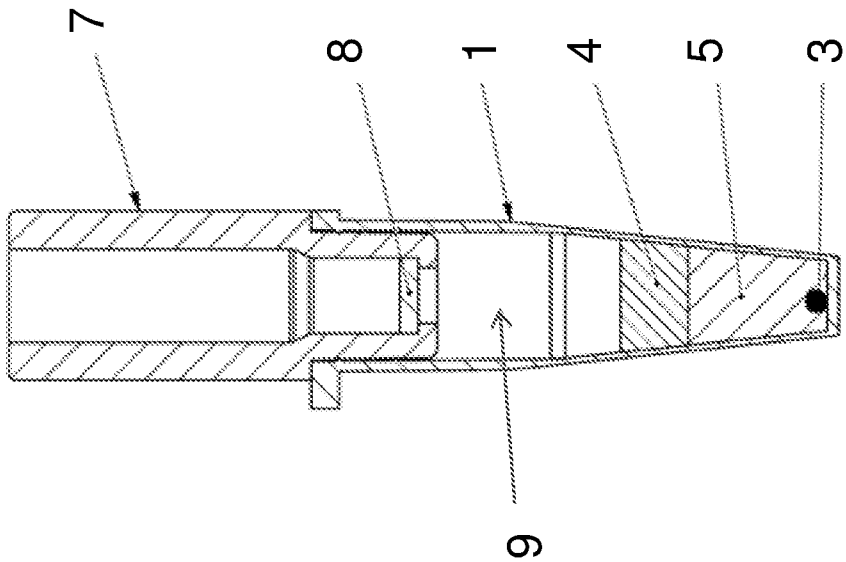


FIG. 4B

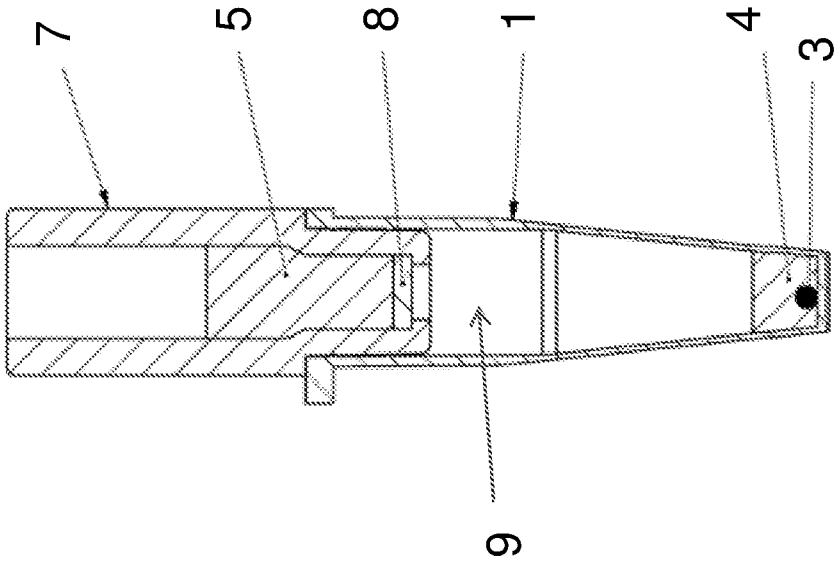
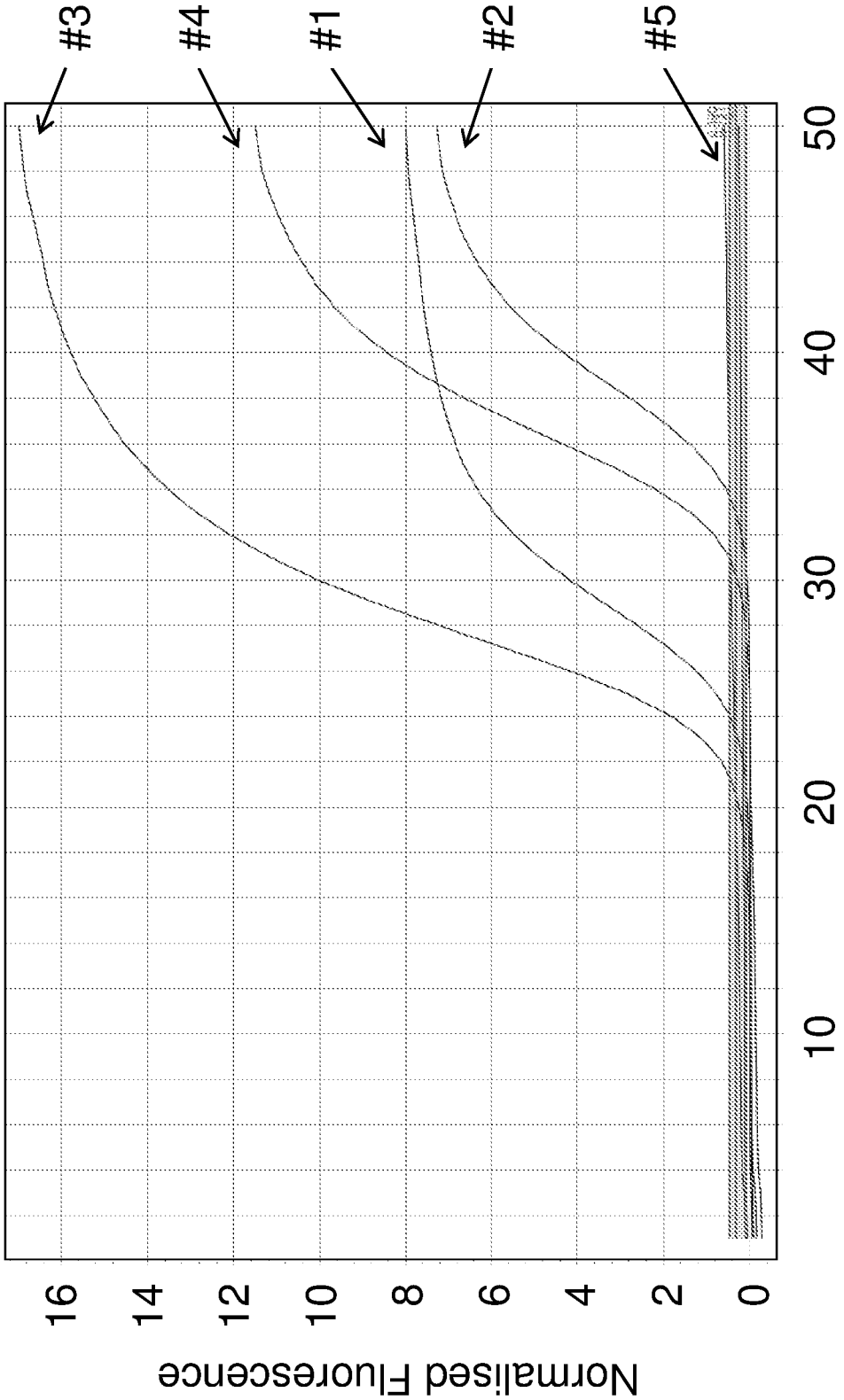


FIG. 4A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#4
#1
#2
#5
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2
4
6
8
10
12
14
16
10
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30
40
50
Cycles

FIG. 5

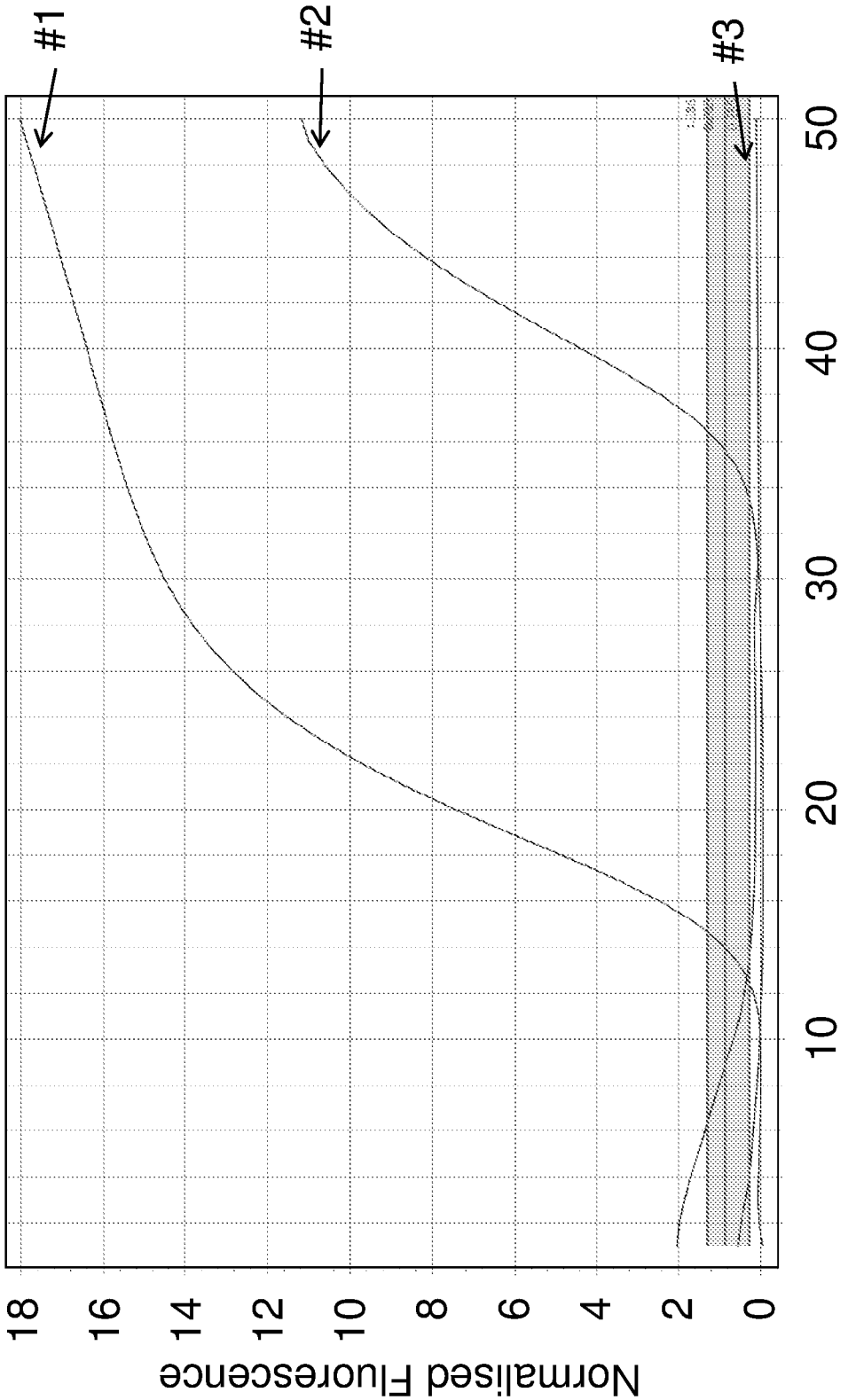
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FIG. 6

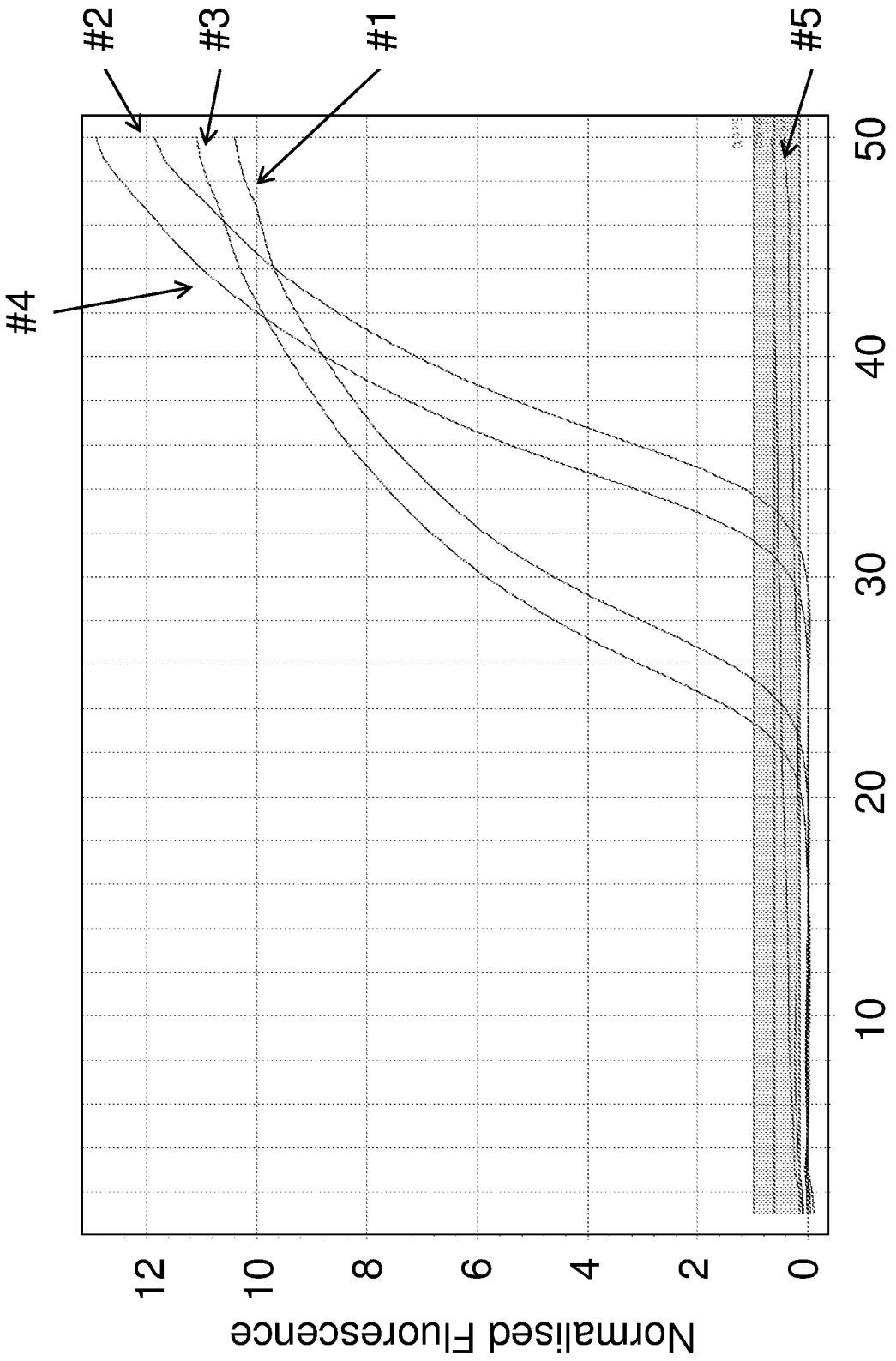
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FIG. 7

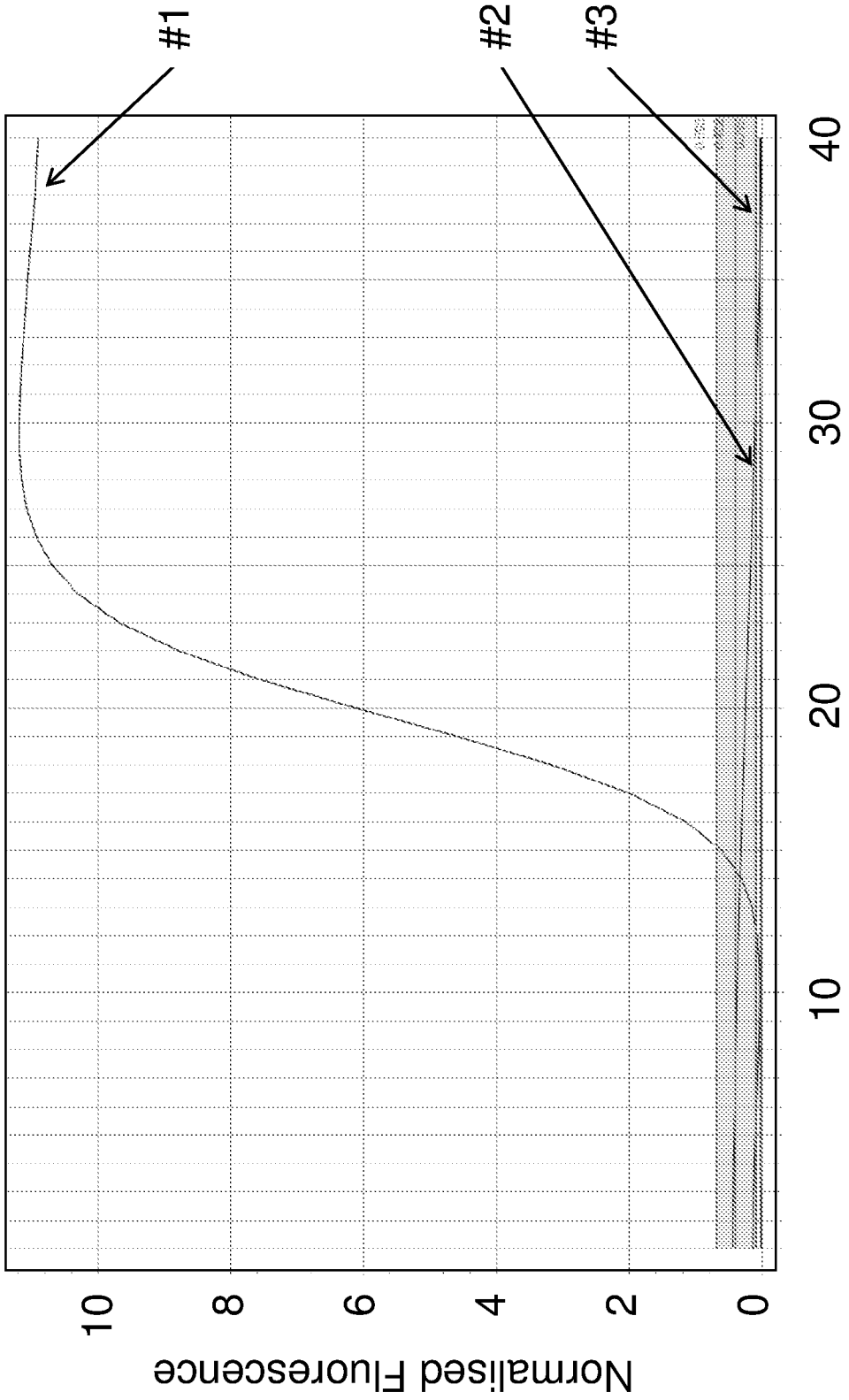

FIG. 8