aqueous oil matrices through the system show significant problems with the control of the flow rate and depth of the carrier oil. Other methods use miniaturized high-throughput-based PCR chips that incorporate a heater and/or temperature sensor inside the substrate. However, this technique requires complex design and fabrication of the chips.

Thus, there remains a need for systems and methods for PCR amplifying samples that are high-throughput, reduces cost, provides greater analysis flexibility, reduces turnaround time, and improves data quality.

EP 1541237, EP1964610, US7081226 and WO 96/41864 disclose background art.

BRIEF SUMMARY

One aspect of the disclosure features a polymerase chain reaction (PCR) amplification and product detection system, comprising an assembly subsystem that includes a plurality of vessels and one or more liquid dispensing members configured to assemble a collection of aqueous oil matrices, each aqueous oil matrix comprising: 1) an aqueous reaction mix comprising a volume of polynucleotide sample and reagents; and 2) one or two non-miscible oils selected from the group consisting of an encapsulation oil, a carrier oil, and both an encapsulation oil and a carrier oil. As such, components of the aqueous reaction mix do not mix with the one or two non-miscible oils. In this embodiment, each aqueous oil matrix is dispensed by the one or more liquid dispensing members into a vessel for PCR amplification and product detection.

The system also comprises a plurality of heating positions, temperature monitoring positions, and PCR product detection positions. In addition, the system includes a reaction-by-reaction, light-driven photonic heating subsystem comprising a plurality of electromagnetic radiation sources, wherein each vessel is in optical communication with an electromagnetic radiation source when that vessel is in a heating position, and the electromagnetic radiation source emits electromagnetic radiation to that vessel; a reaction-by-reaction temperature monitoring subsystem comprising a plurality of thermal detection devices, wherein each vessel corresponds to a thermal detection device when that vessel is in a temperature monitoring position, and wherein detection device is configured to provide a measuring signal dependent on the temperature of the aqueous oil matrix when the vessel is in the temperature monitoring position; a microcontroller temperature feedback and light source control subsystem communicatively connected to both the photonic heating subsystem and the temperature monitoring subsystem, wherein the microcontroller temperature feedback and light source control subsystem is configured to regulate energy input required for output control and duration of the electromagnetic energy emitted by the electromagnetic radiation

and/or aqueous oil matrices are used to enable the use of smaller volumes of aqueous reaction mix (*e.g.*, less than 5 μL).

Depicted in **Figure 2** is an exemplary configuration of the present system. In this embodiment, the electromagnetic radiation source **201**, *e.g.*, a laser diode emitting infrared radiation, is positioned at the top of vessel **101**. Electromagnetic radiation is carried, or conducted, along an optical path by an optical fiber or light pipe **203** and focused on the aqueous reaction mix volume **104** via a collimator lens **204**.

In an alternative embodiment, an electromagnetic radiation source, *e.g.*, light-emitting diode (LED), is provided for emitting blue light having a spectral wavelength in the range from about 350 nm to about 450 nm (or ultraviolet or violet light having a spectral wavelength from about 100 nm to about 350 nm) to a vessel containing an aqueous oil matrix, wherein the aqueous reaction mixture volume comprises a polynucleotide sample to be analyzed by PCR genotyping. In a preferred embodiment, the aqueous reaction mixture volume is positioned within two non-miscible oils, such as the exemplary aqueous oil matrix depicted in **Figure 1**. In such an embodiment, an LED is positioned within optical communication with vessel and emits electromagnetic radiation having a spectral wavelength of about 450 nm (*i.e.*, blue light). In such an aspects, an LED for emitting the blue light may be positioned above the vessel or at the bottom of the vessel. While water molecules do not readily absorb blue light radiation, the aqueous reaction mix volume can be uniformly heated by the LED light source using plasmon excitable particles in the aqueous reaction mix volume. Plasmon excitable materials that readily absorb blue light can emit heat by a process known in the art as photothermal light-to-heat conversion via photon-electron-phonon coupling. Suitable plasmon excitable particles for absorbing blue light radiation are known in the art and include, but are not limited to particles comprising gold, silver, nickel, platinum, or a combination thereof. Preferably, the plasmon excitable material is gold. The use of gold in photonic PCR is described in detail in Ho Son *et al.* (2015), the content of which is incorporated by reference herein in its entirety. Alternatively, selected walls of the vessels may be coated in plasmon excitable material (*e.g.*, gold particles) by electrolysis or vapor-deposition. In a preferred embodiment, a thin film or layer comprising plasmon excitable material is dispersed in the vessel at or near the bottom of the vessel such that the thin film or layer is within the aqueous oil matrix, but within or underneath the aqueous reaction mix (*see, e.g.*, **Figure 3**). In a more preferred embodiment, a thin film or layer of gold is placed inside the vessel and at the bottom of the vessel such that the thin film or layer is in intimate

fiber optic arrays **715**, **716** and to the aqueous reaction mix of each vessel in the row. Fluorescence emission light from the vessels is conducted back through the fiber optic arrays **715**, **716** to the CCD cameras **710**, **706**, which convert the optical signal intensity to raw fluorescence signals for processing by the control system software. At this vessel position, an optical fiber from the fiber optic array **714** is positioned above each well and conducts black body infrared radiation emitted by each aqueous oil matrix at this vessel station to the fiber optic array plate **713**, which condenses the optical signals for imaging by the thermal imager **704**. The thermal imager **704** converts the signal intensity to temperature information that is used by the control system software to adjust the pulse duration of the electromagnetic radiation sources when the vessels are moved forward to the heating position. This process is repeated up to 40 or more PCR temperature cycles.

In some embodiments, the dichroic filter cubes **702**, **711** and the infrared emission filter **703** enable the temperature measurements and the fluorescence measurements to occur simultaneously by preventing crosstalk by the different spectral wavelengths. In other embodiments, the temperature measurements and the fluorescence measurements are performed sequentially within the available timeframe. Temperature monitoring after the heating step is useful for providing the control system software with temperature information necessary to determine the appropriate energy output required for the next heating step. In some embodiments comprising a temperature monitoring step after the photonic heating step, dynamic temperature feedback during the heating steps is not possible due to rapid heat dissipation. For instance, in some embodiments, the aqueous reaction mix volumes dissipate heat rapidly and exhibit a decrease in temperature by about 20 ºC per second when the heating source is removed. Therefore, in some embodiments, the heating positions comprise insulation to decrease heat loss. In other embodiments, temperature monitoring at the PCR product detection position is used by the control system software to normalize the raw fluorescence data, because it is known in the art that fluorescence light having certain spectral wavelengths can be affected by temperature. However, embodiments comprising a cooling mechanism at the PCR product detection position lower the temperature of the aqueous reaction mix to allow all fluorescence measurements to be taken at approximately the same temperature.

Alternatively, dynamic temperature feedback during the photonic heating steps can be accomplished by placing the laser diodes and the thermal detectors at the same position. However, as discussed above, thermal imaging cannot be performed simultaneously with the heating if the electromagnetic radiation sources emit significant infrared radiation in the same

inexpensive galvo-head mirror scanner can move to over 20 thousand points per second. For fluorescence excitation for both laser line and galvo-head scanning lasers, fluorescence light emitted by the FAM and VIC probes can be sequentially detected by a camera that can integrate over the time required to scan a desired number of wells. A single camera with a suitable 2 wavelength bandpass filter can be employed or two cameras, each with a less expensive single wavelength narrow bandpass filter can be used.

The present disclosure is illustrated by the following examples. The foregoing and following description and the examples are not intended to be limiting but rather are illustrative of the described embodiments. Hence, it will be understood that the present disclosure is not limited to the specific details of the examples.

EXAMPLE 1

Figure 17 illustrates the observed fluorescence from 16 reaction wells after 40 cycles of PCR extracted from a single (200msec exposure) image captured by a ToupTek E3CMOS camera. The fluorescence emission light was filtered through a 565nm +/- 5nm narrow bandpass interference filter before entering the camera lens. The wells were illuminated by an inexpensive 20mW 532nm laser line (Laserlands, Inc.) to provide the VIC excitation light. The collection of 16 samples were a variety of genotypes with homozygous VIC, heterozygous VIC, and VIC negative samples. This variation in genotypes contribute to the variation in height of the peaks. VIC fluorescence is demonstrated here since it is more difficult to cleanly measure VIC fluorescence than FAM fluorescence in typical PCR reactions. Pixel intensity information was extracted from the acquired image using publically available ImageJ image analysis software (imageJ . nih . gov).

When fully automated, using the above camera system, acquiring fluorescence images and extracting fluorescence intensity from the pixels in the images takes only a few seconds. Multiple system embodiments to utilize this approach are feasible. A set of reaction wells could be moved from a location where heating is performed to an adjacent chamber where the cameras can measure fluorescence. Alternatively, the fluorescence detection camera(s) can be positioned next to an IR imaging camera or discrete IR sensors over the same location where wells are heated. The 450nm 1W LEDs used to currently provide heating light energy in prototype systems produce a very bright and wide spectral output that swamps out fluorescence detection even with the best emission blocking filters available. Therefore, in these prototype device embodiments the heating light can't be on while fluorescence measurements are being made. This results in a few seconds of additional cooling of the reaction volumes after each thermo-cycle during fluorescence measurements before the next

heating cycle can begin. While temperatures of the reaction volumes fall initially very fast (generally 6 to 10 degrees per second) from the 95 degree C denaturing temperature when the heating light is extinguished, the rate of temperature drop slows after the first few seconds as the difference between the ambient temperature and the reaction temperature lessens. Even with such a delay in starting the next heating cycle, the additional time required to bring the reaction volume back to the desired annealing temperature to start the next cycle is only 1 or 2 seconds in prototype systems with the reaction volumes specified.

EXAMPLE 2

PCR Product Detection From Light-mediated aqueous oil matrix reactions

Two TAQMAN® probes and custom-designed primer pairs forming a bi-allelic interrogation of a DNA target loci were tested with a light-mediated temperature-cycling in an aqueous oil matrix. The probes and primers were combined with typical TAQMAN® PCR enzymes and reagents in sealed wells and were run through as many as 40 PCR temperature cycles in a water bath thermocycler. After thermocycling, the PCR products were then measured in a commercial fluorescence reader at emission wavelengths for the FAM and VIC dyes that are associated with the two probes for the particular locus alleles. Elevated FAM and/or VIC fluorescence indicates the presence of amplified PCR products for the DNA alleles for which the probes and primers were designed.

For detection of light-mediated PCR products in an aqueous oil matrix, the previously extracted DNA samples were acquired from a routine genotyping production process. These samples constituted known genotypes. Sample 1 was known to produce a PCR product for the selected FAM probe. Sample 2 was known to produce a PCR product for the selected VIC probe. Sample 3 was known to produce a PCR product for both the FAM and VIC probes for the selected specific DNA locus.

A 384-well, clear flat bottom, plate was prepared with the inside bottom of the wells coated with a thin layer of gold emulsion paint. A 0.1mm (0.005 inch) thermocouple was inserted and positioned in the painted well such that the thermocouple bead was located approximately 0.5mm above the gold paint. Aqueous oil matrix PCR reactions were assembled for each of the samples and for a no template control (no DNA added to the reaction) in the prepared wells. Each aqueous oil matrix was composed of 2 μl of an aqueous PCR reaction mix,

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A polymerase chain reaction (PCR) amplification and product detection system, comprising:

a) an assembly subsystem comprising:

i) a plurality of vessels;

ii) one or more liquid dispensing members configured to assemble a collection of aqueous oil matrices, each aqueous oil matrix comprising:

1) an aqueous reaction mix comprising a volume of polynucleotide sample and reagents; and

2) one or two non-miscible oils selected from the group consisting of an encapsulation oil, a carrier oil, and both an encapsulation oil and a carrier oil;

wherein components of the aqueous reaction mix do not mix with the one or two non-miscible oils;

wherein each aqueous oil matrix is dispensed by the one or more liquid dispensing members into a vessel for PCR amplification and product detection;

b) a plurality of heating positions, temperature monitoring positions, and PCR product detection positions;

c) a reaction-by-reaction, light-driven photonic heating subsystem comprising a plurality of electromagnetic radiation sources, wherein each vessel is in optical communication with an electromagnetic radiation source when that vessel is in a heating position, and the electromagnetic radiation source emits electromagnetic radiation to that vessel;

d) a reaction-by-reaction temperature monitoring subsystem comprising a plurality of thermal detection devices, wherein each vessel corresponds to a thermal detection device when that vessel is in a temperature monitoring position, and wherein the detection device is configured to provide a measuring signal dependent on the temperature of the aqueous oil matrix when the vessel is in the temperature monitoring position;

e) a microcontroller temperature feedback and light source control subsystem communicatively connected to both the photonic heating subsystem and the temperature monitoring subsystem, wherein the microcontroller temperature

feedback and light source control subsystem is configured to regulate energy input required for output control and duration of the electromagnetic energy emitted by the electromagnetic radiation source through a cycle of reaction temperatures;

f) a fluorescence detection subsystem comprising:

i) one or more fluorescence excitation light sources;

ii) one or more fluorescence emission light sensing devices;

iii) a plurality of first optical members in optical communication with the one or more fluorescence excitation light sources, wherein each first optical member is configured to provide an optical path for fluorescence excitation light having a first spectral wavelength from the one or more fluorescence excitation light sources to one of said vessels containing the aqueous oil matrix when the vessel is in a PCR product detection position, wherein the volume of the aqueous reaction mix comprises a first reagent capable of excitation by the fluorescence excitation light having the first spectral wavelength when the first reagent hybridizes to the polynucleotide sample, and wherein each first optical member is further configured to provide an optical path for fluorescence emission light from the aqueous reaction mix to the one or more fluorescence emission light sensing devices; and

iv) an active or passive cooling mechanism at the PCR product detection position whereby each of the vessels in the PCR product detection position are cooled; and

g) a mechanical and electronic control system communicatively connected to a positioning device and to the assembly subsystem, wherein mechanical and electronic control system causes the assembly subsystem to assemble the aqueous oil matrices in the plurality of vessels, and wherein the mechanical and electronic control system causes the positioning device to move the plurality of vessels from the assembly subsystem to each of the heating positions, temperature monitoring positions, and PCR product detection positions.

2. The system of Claim 1, further comprising:

a) a plurality of vessel stations, wherein the heating positions, temperature monitoring positions, and PCR product detection positions are at the same vessel stations;

b) a plurality of alternating first vessel stations and second vessel stations, wherein each of the first vessels stations comprise a heating position and a temperature monitoring position, and wherein each of the second vessels stations comprise a PCR product detection position; or

c) a plurality of alternating first vessel stations and second vessel stations, wherein each of the first vessels stations comprise a heating position, and wherein each of the second vessel stations comprise a PCR product detection position and a temperature monitoring position.

3. The system of Claim 1 or Claim 2, wherein:

i) the active or passive cooling mechanism is: (a) a passive heat sink metal comprising aluminum or copper; (b) actively circulating air currents; (c) passively circulating air currents; (d) a Peltier device; (e) a circulating liquid; or (f) any combination of (a), (b), (c), (d) or (e); or

ii) each of the aqueous reaction mix, carrier oil, and encapsulation oil is dispensed into each of the vessels, wherein the carrier oil is non-miscible with both the encapsulation oil and the components of the aqueous reaction mix, optionally
wherein the carrier oil has a density ranging from 1,200 kg/m^3 to 2,000 kg/m^3, the encapsulation oil has a density ranging from 700 kg/m^3 to 990 kg/m^3, and the aqueous reaction mix has a density ranging from 990 kg/m^3 to 1,200 kg/m^3; or

iii) the electromagnetic radiation sources are selected from the group consisting of laser diodes and light emitting diodes, optionally
wherein the electromagnetic radiation sources are laser diodes emitting infrared light having a spectral wavelength in the range from 1,300 nm to 2,200 nm, optionally in the range from 1,300 nm to 1,500 nm.

4. The system of any one of the preceding Claims, wherein each electromagnetic radiation source is configured to uniformly heat the volume of the aqueous reaction mix in

each corresponding vessel when the vessel is in the heating position through the cycle of reaction temperatures comprising: (i) an annealing temperature in the range from 50 ºC to 65 ºC; (ii) an elongation temperature in the range from 65 ºC to 75 ºC; and (iii) a denaturation temperature in the range from 90 ºC to 99 ºC; and wherein the active or passive cooling mechanism is configured to cool each of the vessels to a temperature range from 55 ºC to 65 ºC when the vessel is in the PCR product detection position.

5. The system of Claim 4, wherein the volume of the aqueous reaction mix is less than or equal to 10 μL and wherein the aqueous reaction mix is heated through the cycle of temperatures (i), (ii), and (iii), in less than or equal to 20 seconds, in less than or equal to 15 seconds, or in less than or equal to 10 seconds, optionally wherein the cycle of reaction temperatures in (i), (ii), and (iii) is repeated for an additional 1 to 60 cycles.

6. The system of any one of the preceding Claims, wherein the fluorescence detection subsystem further comprises:

v) a first excitation filter which receives light from the one or more fluorescence excitation light sources and allows passage of the fluorescence excitation light having the first spectral wavelength;

vi) a first emission filter for allowing transmission therethrough of fluorescence emission light to the one or more fluorescence emission light sensing devices from the aqueous reaction mix in response to the fluorescence excitation light having the first spectral wavelength and for substantially blocking transmission of wavelengths other than the wavelengths of the emitted light; or

vii) both v) and vi).

7. The system of any one of the preceding Claims, wherein each first optical member is further configured to provide an optical path for fluorescence excitation light having a second spectral wavelength from the one or more fluorescence excitation light sources to one of said vessels containing the aqueous oil matrix when the vessel is in a PCR product detection position, wherein the volume of the aqueous reaction mix comprises a second reagent capable of excitation by the fluorescence excitation light having the second spectral wavelength when the second reagent hybridizes to the DNA sample, and wherein each first optical member is

further configured to provide an optical path for fluorescence emission light from the aqueous reaction mix to the one or more fluorescence emission light sensing devices.

8. The system of any one of the preceding Claims, wherein the fluorescence detection subsystem further comprises a plurality of second optical members in optical communication with the one or more fluorescence excitation light sources, wherein each second optical member is configured to provide an optical path for fluorescence excitation light having a second spectral wavelength from the one or more fluorescence excitation light sources to one of said vessels containing the aqueous oil matrix when the vessel is in a PCR product detection position, wherein the volume of the aqueous reaction mix comprises a second reagent capable of excitation by the fluorescence excitation light having the second spectral wavelength when the second reagent hybridizes to the DNA sample, and wherein each second optical member is further configured to provide an optical path for fluorescence emission light from the aqueous reaction mix to the one or more fluorescence emission light sensing devices.

9. The system of any one of Claims 1 to 8, wherein the one or more fluorescence emission light sensing devices is a charged-coupled device (CCD) camera, complimentary metal-oxide semiconductor (CMOS) camera, sensor array, or a combination thereof.

10. The system of any one of Claims 1 to 7, wherein each first optical member comprises one or more optical fibers and one or more light emitting diodes.

11. The system of Claim 8, wherein each first optical member comprises one or more optical fibers and one or more light emitting diodes, and wherein each second optical member comprises one or more optical fibers and one or more light emitting diodes.

12. The system of Claim 7 or Claim 8, wherein:

a) the first reagent, the second reagent, or both the first reagent and the second reagent comprises a nucleic acid probe covalently linked to a fluorophore; or

b) the fluorescence detection subsystem further comprises:

v) a first excitation filter which receives light from the one or more fluorescence excitation light sources and allows passage of the fluorescence excitation light having the first spectral wavelength; and

vi) a second excitation filter which receives light from the one or more fluorescence excitation light sources and allows passage of the fluorescence excitation light having the second spectral wavelength, and wherein the second spectral wavelength is different from the first spectral wavelength; and optionally

the fluorescence detection subsystem further comprises:

vii) a first emission filter for allowing transmission therethrough of fluorescence emission light to the one or more fluorescence emission light sensing devices from the aqueous reaction mix in response to the fluorescence excitation light having the first spectral wavelength and for substantially blocking transmission of wavelengths other than the wavelengths of the emitted light; and

viii) a second emission filter for allowing transmission therethrough of fluorescence emission light to the one or more fluorescence emission light sensing devices from the aqueous reaction mix in response to the fluorescence excitation light having the second spectral wavelength and for substantially blocking transmission of wavelengths other than the wavelengths of the emitted light; and optionally wherein:

the one or more fluorescence emission light sensing devices is a CCD camera, CMOS camera, sensor array, or a combination thereof.

13. The system of any one of Claims 1 to 7, wherein:

a) the temperature monitoring subsystem further comprises a thermal infrared sensing device in optical communication with each thermal detection device;

b) each thermal detection device provides a measuring signal dependent on intensity of a received black body infrared radiation; and

c) each thermal detection device is configured to provide a thermal radiation path from the volume of the aqueous reaction mix to the thermal infrared sensing device, optionally wherein:

i) each thermal infrared sensing device is a thermal imager or an infrared sensor, and wherein the plurality of thermal detection devices do not physically contact the aqueous oil matrix;

ii) the positioning device is a belt comprising the plurality of vessels, each vessel comprising a top side and a bottom side, wherein each thermal

detection device corresponds to the bottom side of one of the vessels when the vessel is in the heating position, and wherein each electromagnetic radiation source corresponds to the top side of one of the vessels when the vessel is in the heating position whereby emission of electromagnetic radiation to each vessel and detection of emitted black body infrared radiation from the volume of each aqueous reaction mix occur simultaneously; or

iii) the positioning device is a belt comprising the plurality of vessels, each vessel comprising a top side and a bottom side, wherein each thermal detection device corresponds to the top side of one of the vessels when the vessel is in the heating position, and wherein each electromagnetic radiation source corresponds to the bottom side of one of the vessels when the vessel is in the heating position whereby emission of electromagnetic radiation to each vessel and detection of emitted black body infrared radiation from the volume of each aqueous reaction mix occur simultaneously, optionally

wherein the electromagnetic radiation sources are light emitting diodes having a spectral wavelength in the range from 380 nm to 500 nm and the vessels further comprise a thin layer comprising a transition metal selected from the group consisting of gold, silver, nickel, and platinum optionally wherein the transition metal is gold.

14. The system of any one of Claims 1 to 6, wherein the first reagent comprises a nucleic acid probe covalently linked to a fluorophore.

15. A method for light-mediated PCR amplification and product detection, the method comprising:

a) providing the PCR amplification and product detection system of any one of Claims 1 to 14;

b) assembling a collection of aqueous oil matrices by:

1) aspirating the one or more non-miscible oils, the aspirating step comprising:

(i) aspirating the encapsulation oil from an encapsulation oil input;

(ii) aspirating the carrier oil from a carrier oil input; or

(iii) aspirating the encapsulation oil from an encapsulation oil input and aspirating the carrier oil from a carrier oil input;

2) aspirating a volume of polynucleotide sample from a polynucleotide sample input;

3) aspirating a volume of reagents from a PCR reagent mix input;

4) dispensing the volume of polynucleotide sample, the volume of reagents, and the one or more non-miscible oils into the plurality of vessels, wherein the volume of polynucleotide sample and volume of reagents form an aqueous reaction mix, and wherein components of the aqueous reaction mix do not mix with the one or more non-miscible oils;

wherein steps 1), 2), and 3) are performed in any order or simultaneously, and wherein steps 1), 2), and 3) are performed prior to step 4);

c) uniformly heating each volume of the aqueous oil matrix, wherein the heating comprises:

1) positioning the electromagnetic radiation source in optical communication with each vessel containing the aqueous oil matrix;

2) heating the volume of the aqueous oil matrix until it reaches a temperature in the range of 50 ºC to 65 ºC;

3) further heating the volume of the aqueous oil matrix until it reaches a temperature in the range of 65 ºC to 75 ºC; and

4) further heating the volume of the aqueous oil matrix until it reaches a temperature in the range of 90 ºC to 99 ºC;

d) cooling each aqueous oil matrix by positioning each of the vessels in close proximity to the cooling mechanism until the aqueous oil matrix reaches a temperature in the range of 55 ºC to 65 ºC;

e) measuring the fluorescence emission from each vessel containing the aqueous oil matrix, wherein the measuring comprises:

1) positioning a first optical member in optical communication with each vessel containing the aqueous oil matrix, wherein each first optical member is configured to provide an optical path for fluorescence excitation light having a first spectral wavelength from the one or more fluorescence excitation light sources to one of said vessels containing the aqueous oil matrix when the vessel is in a PCR product detection

position, wherein the volume of the aqueous reaction mix comprises a first reagent capable of excitation by the fluorescence excitation light having the first spectral wavelength when the first reagent hybridizes to the polynucleotide sample, and wherein each first optical member is further configured to provide an optical path for fluorescence emission light from the aqueous reaction mix to the one or more fluorescence emission light sensing devices; and

2) measuring the signal produced by the one or more fluorescence emission light sensing devices from at least one aqueous reaction mix; and

f) repeating c) to e) for a predetermined number of additional cycles.