

Replacement Sheet

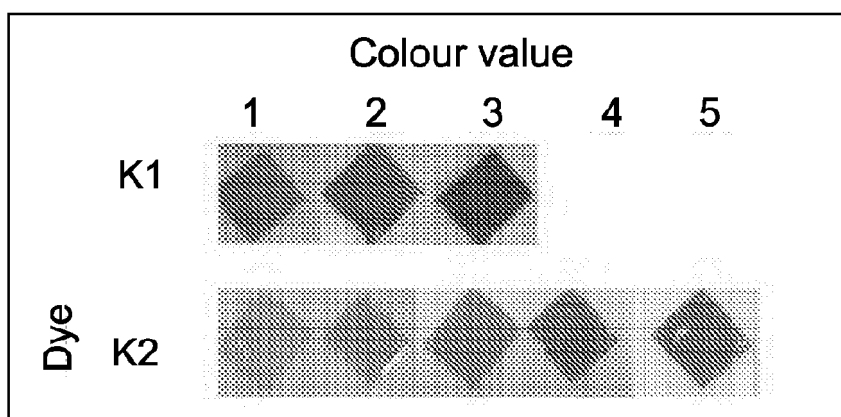


FIG. 1

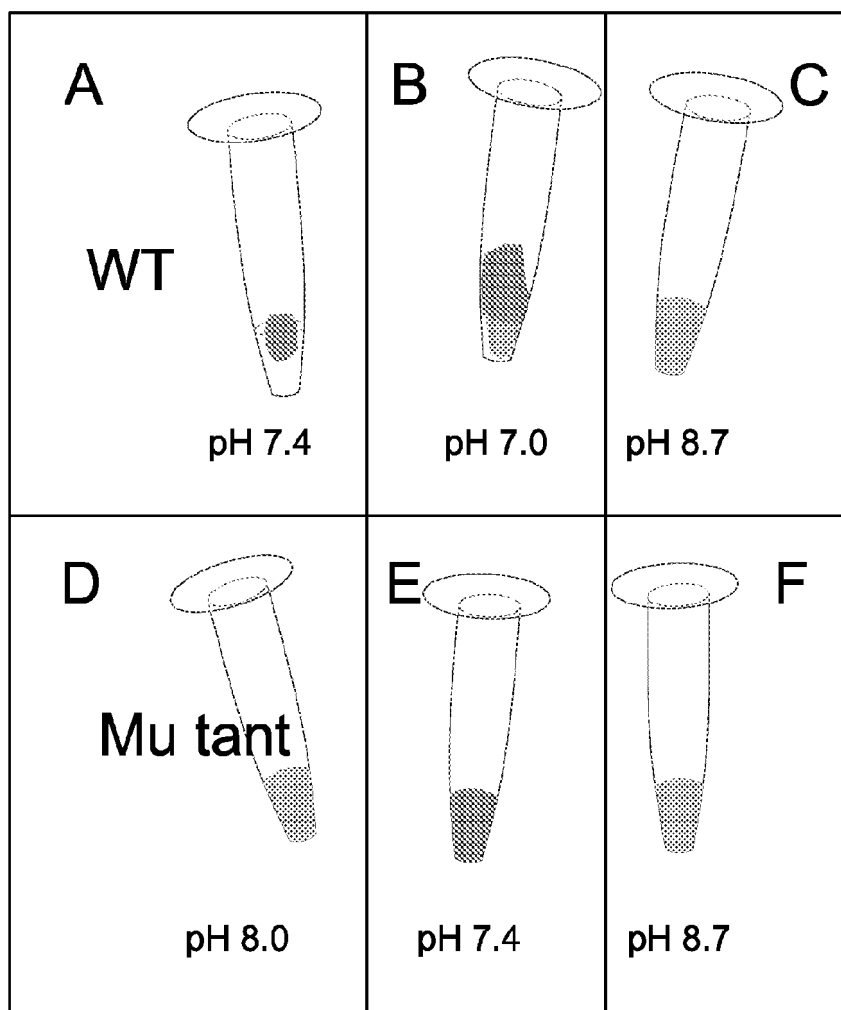


FIG. 2

Replacement Sheet

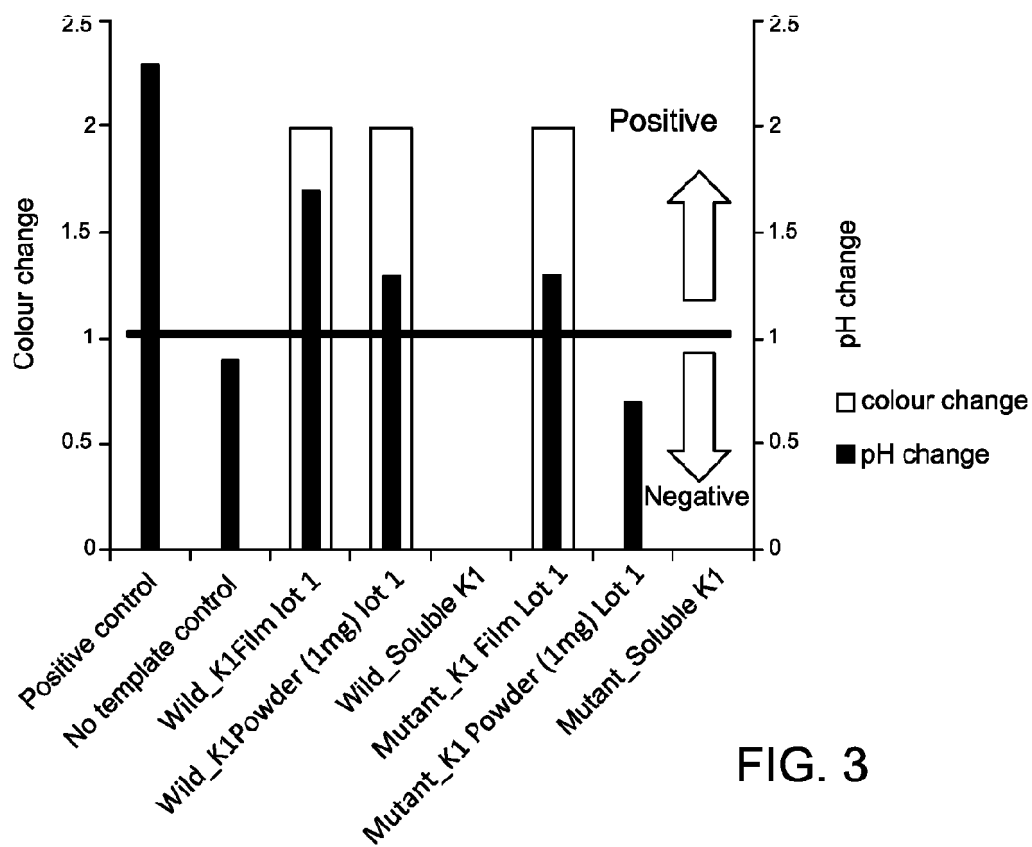


FIG. 3

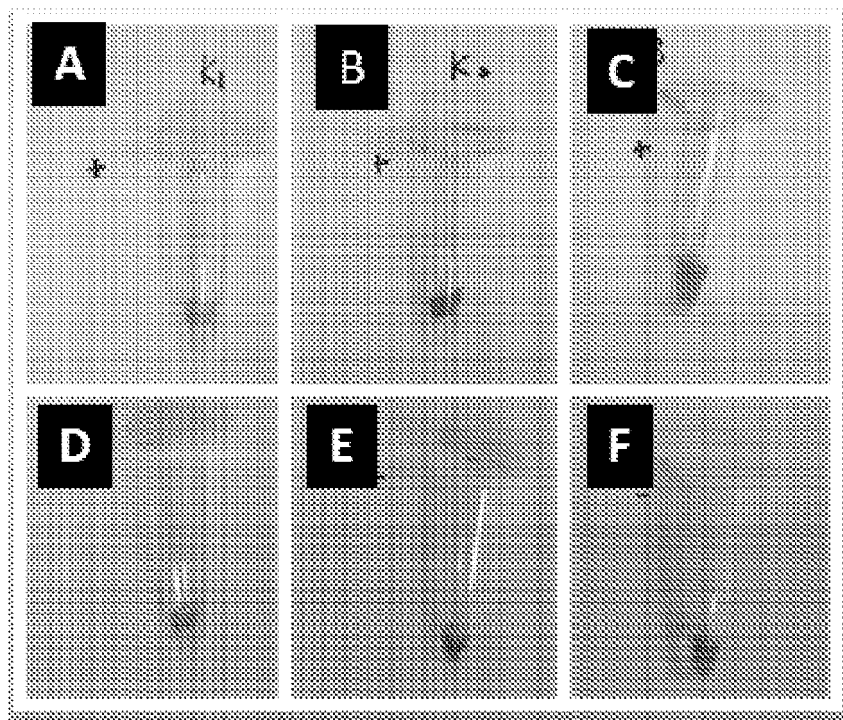


FIG. 4

Replacement Sheet

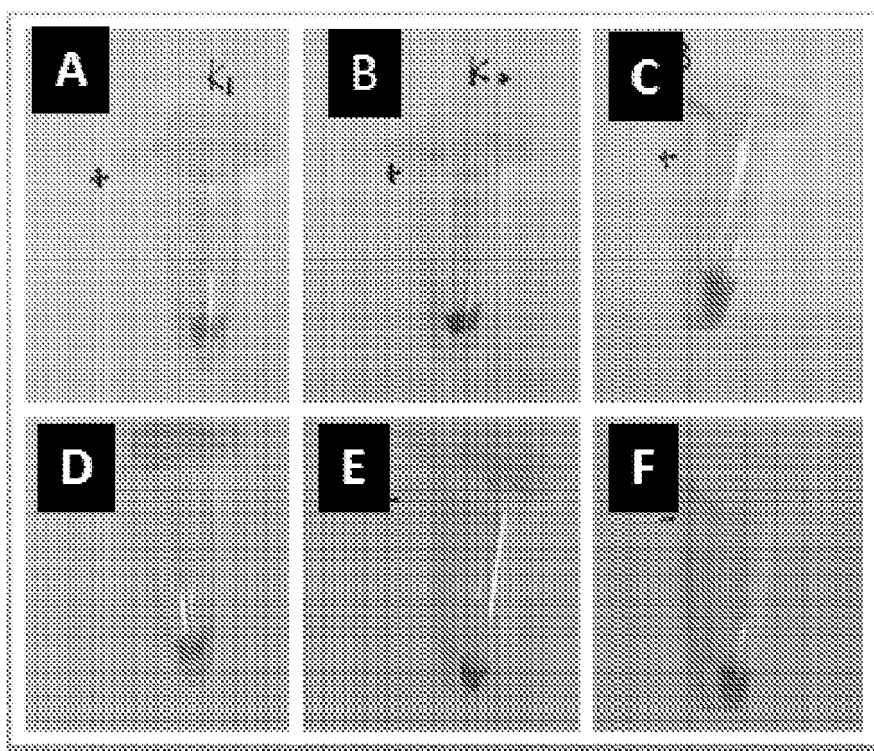


FIG. 5

Replacement Sheet

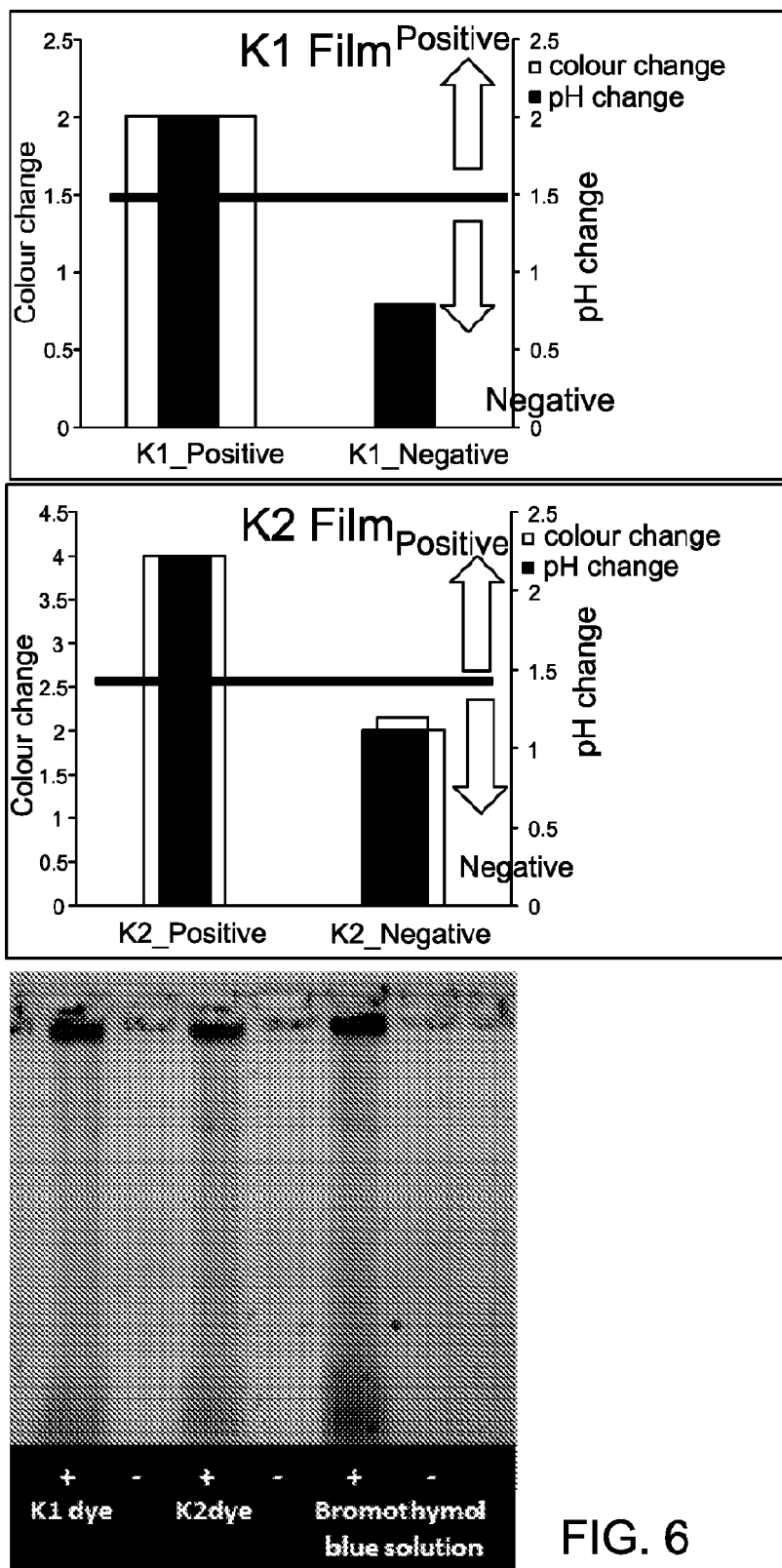


FIG. 6

Replacement Sheet

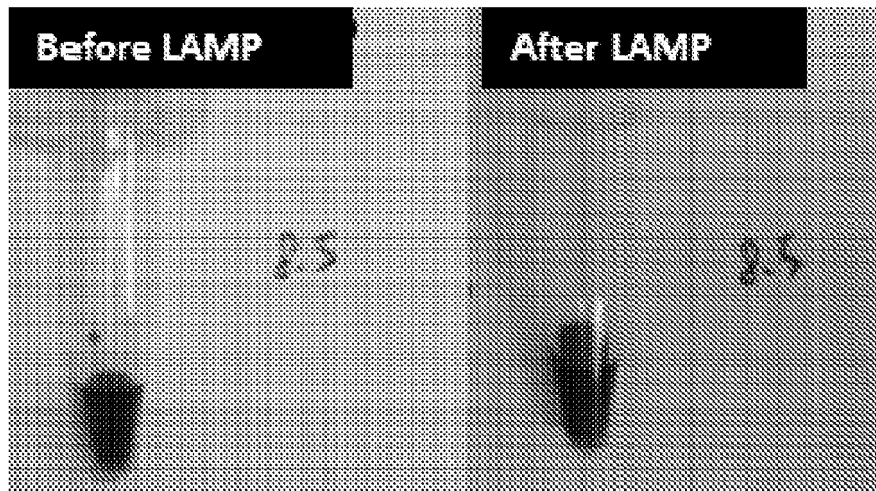


FIG. 7

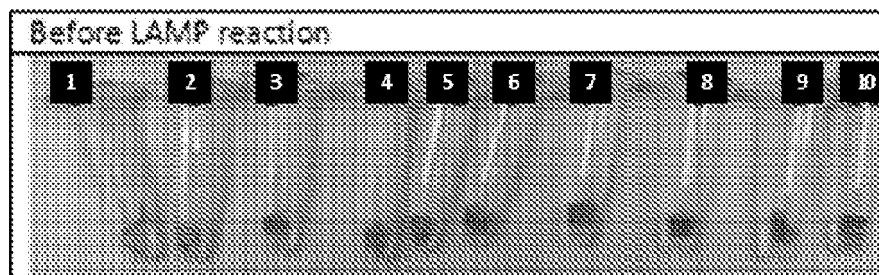


FIG. 8

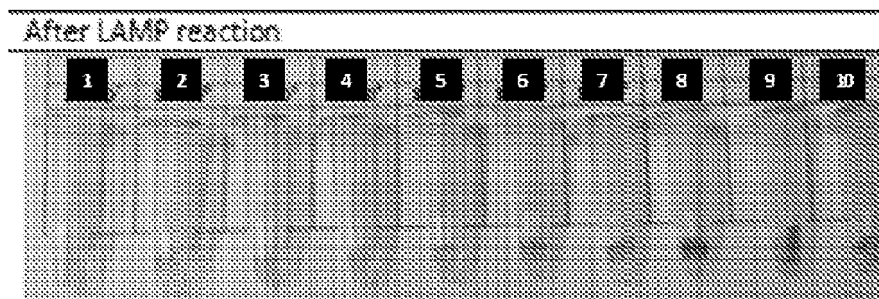


FIG. 9

Replacement Sheet

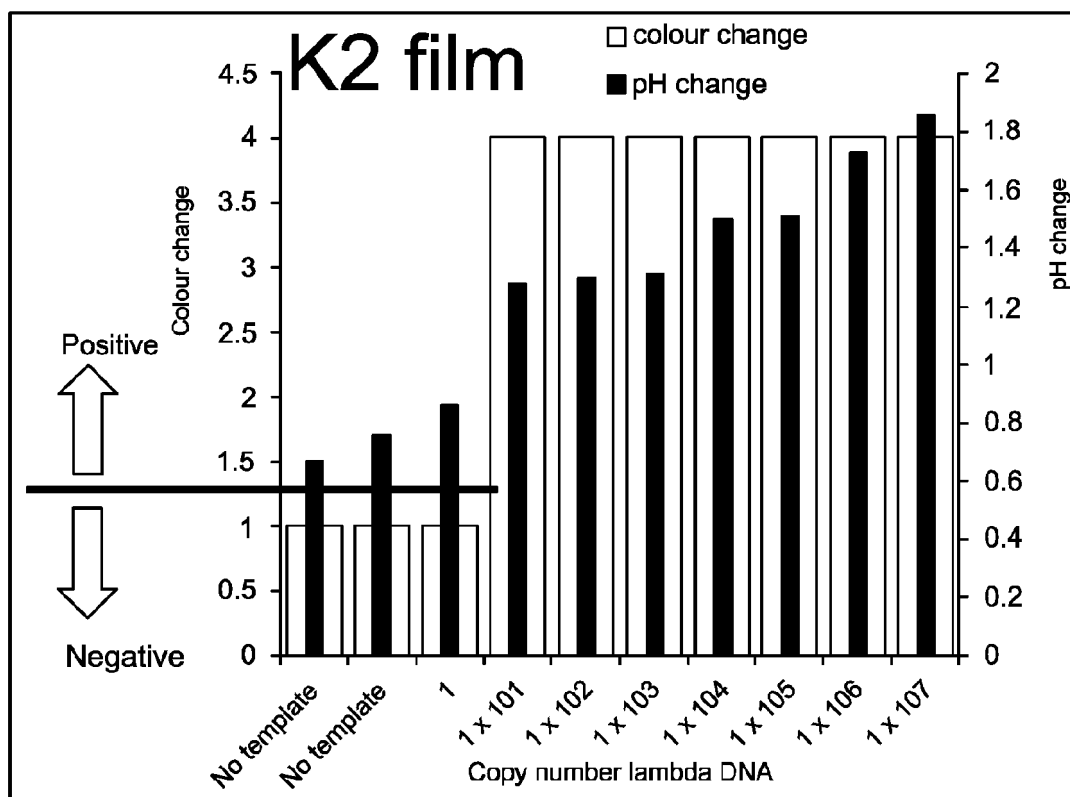


FIG. 10

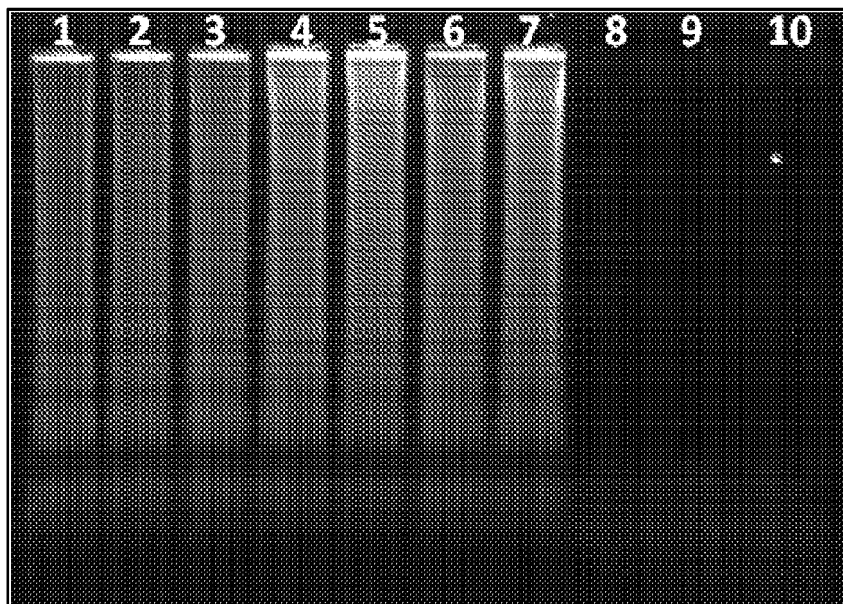


FIG. 11

Replacement Sheet

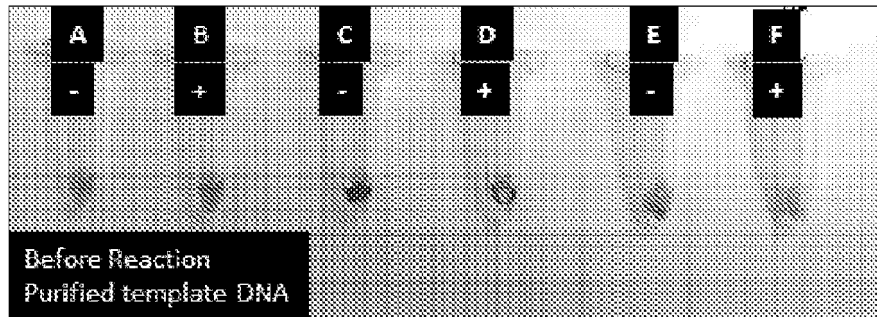


FIG. 12

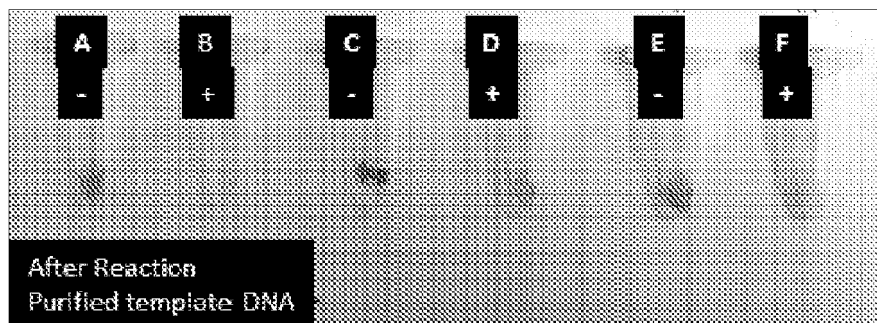


FIG. 13

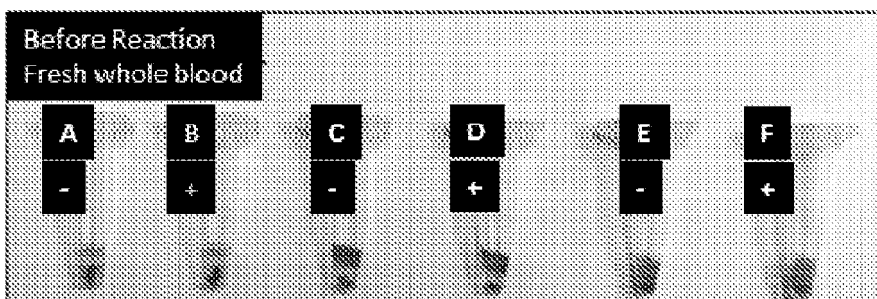


FIG. 14

Replacement Sheet

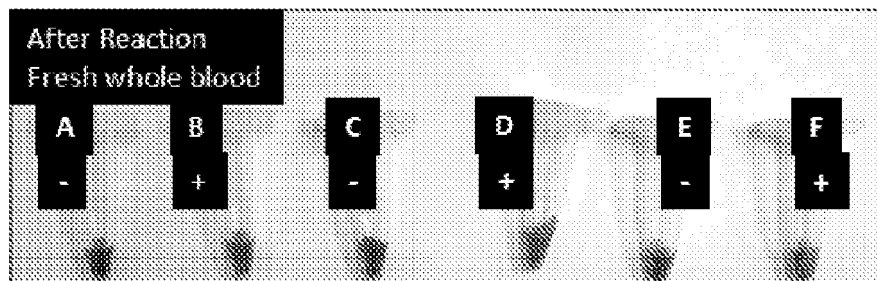


FIG. 15

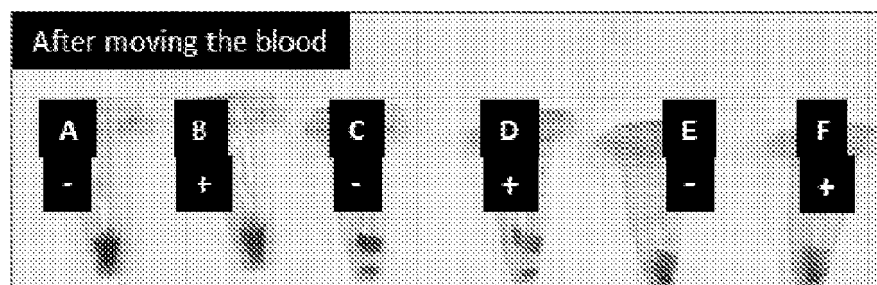


FIG. 16

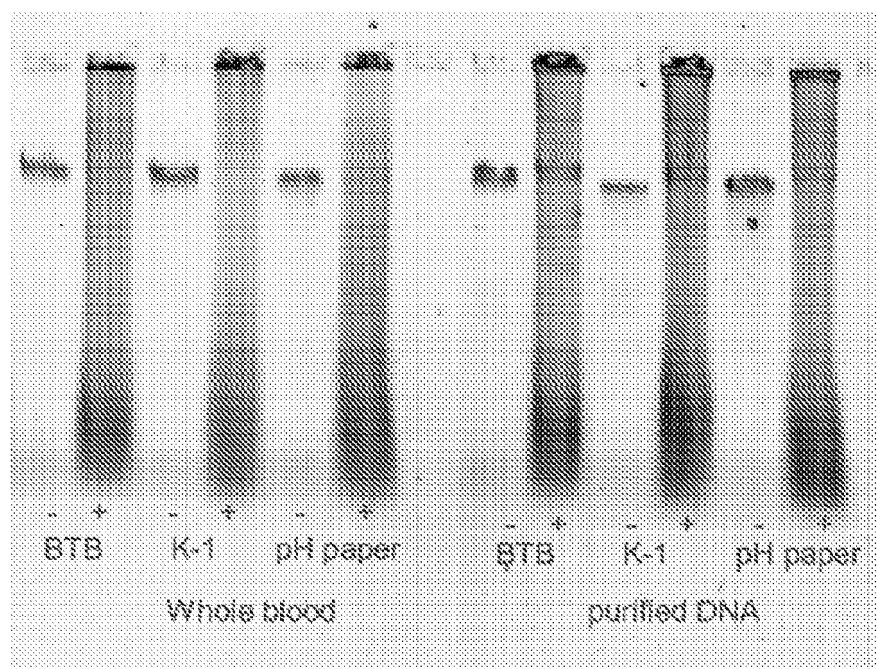


FIG. 17

Replacement Sheet

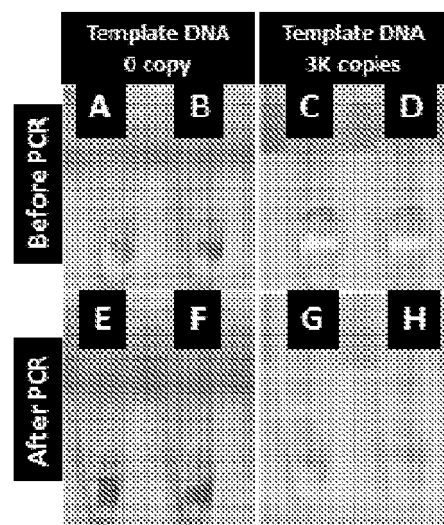


FIG. 18

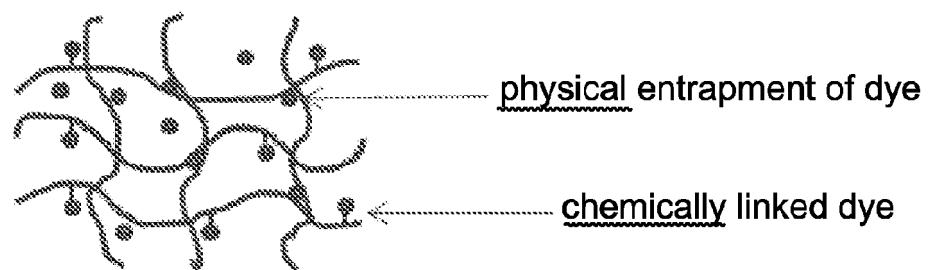


FIG. 19

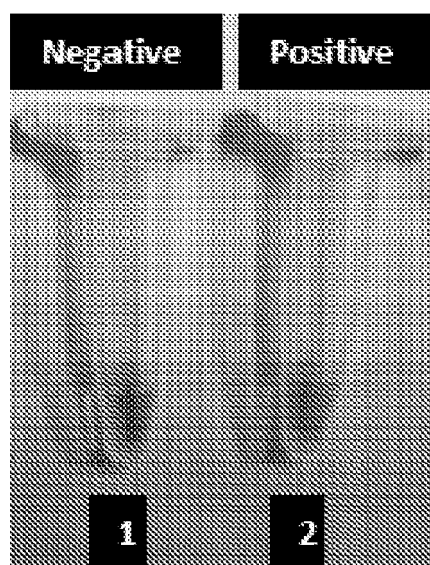


FIG. 20

Replacement Sheet

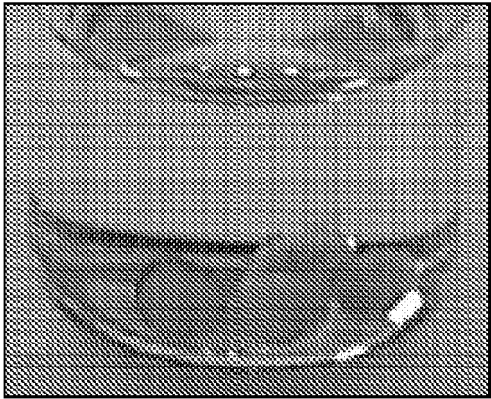
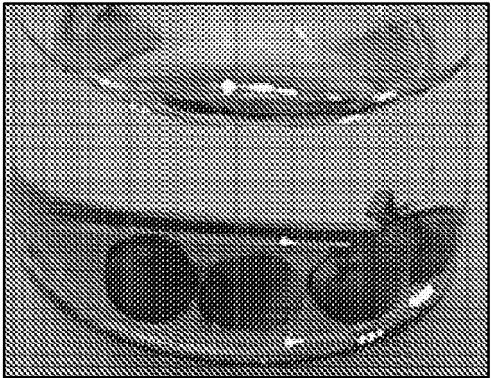
pH responsive dye on polymer ball	
pH 7	pH 8.5
	

FIG. 21

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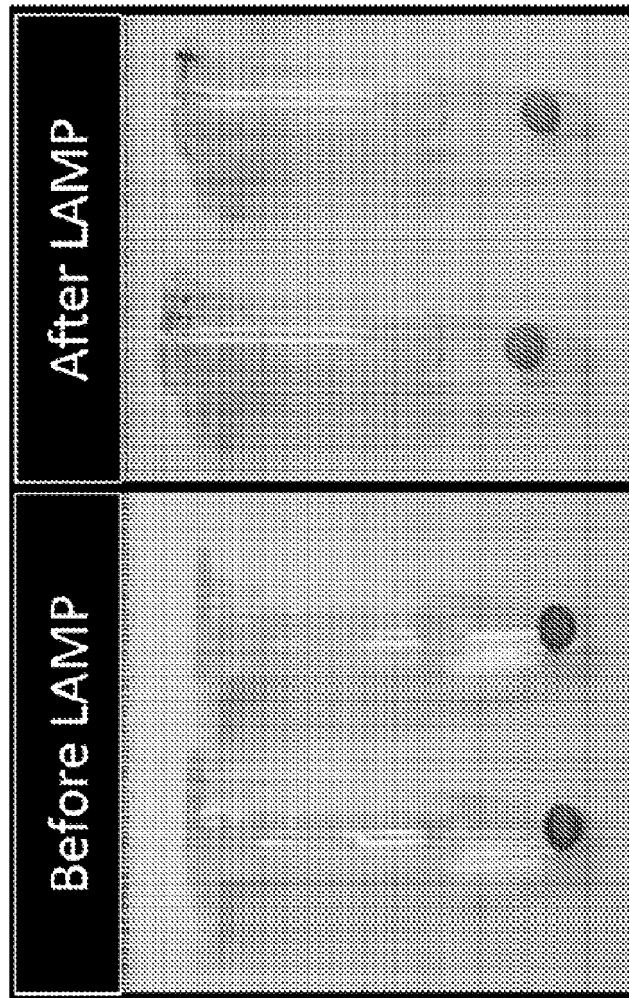
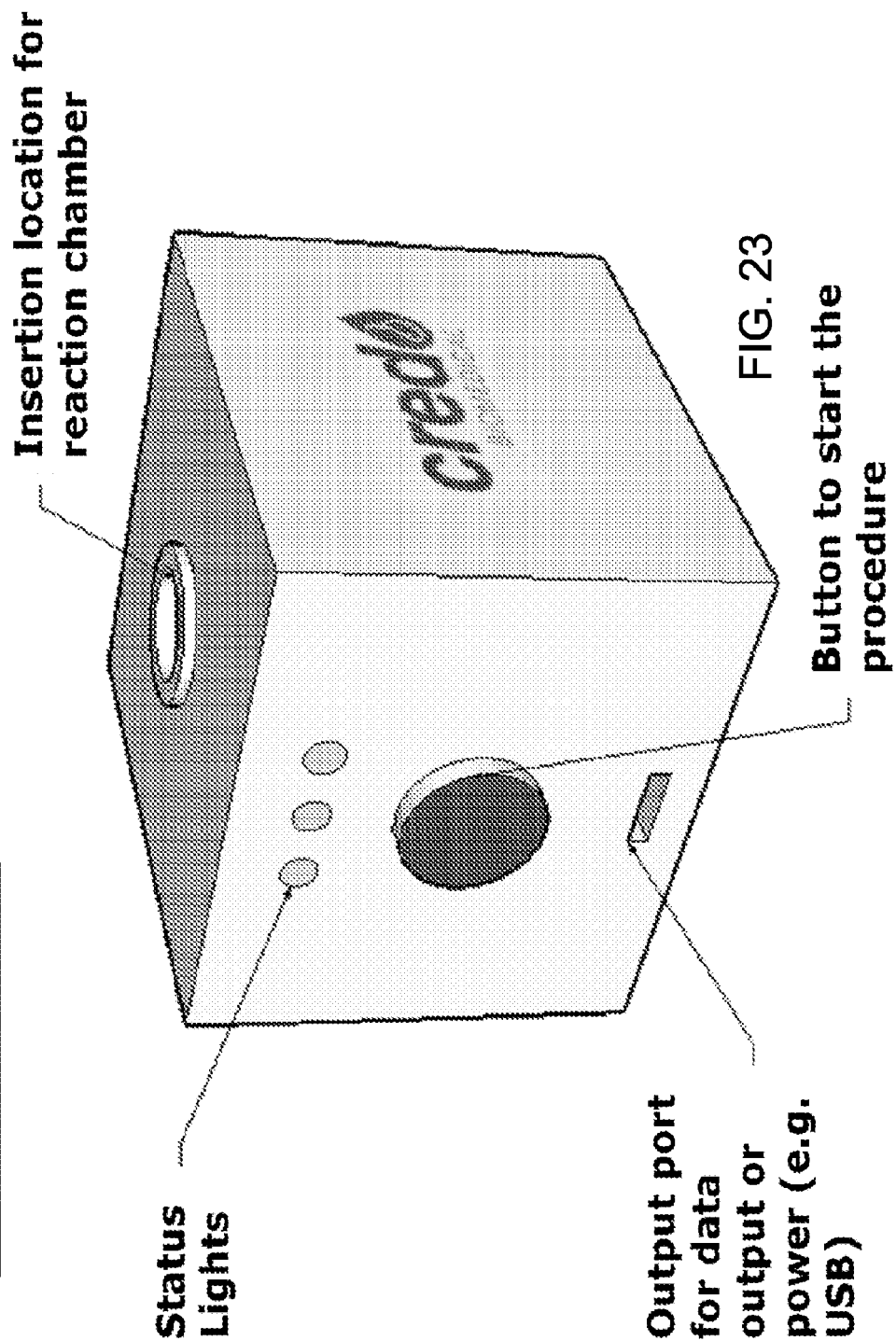


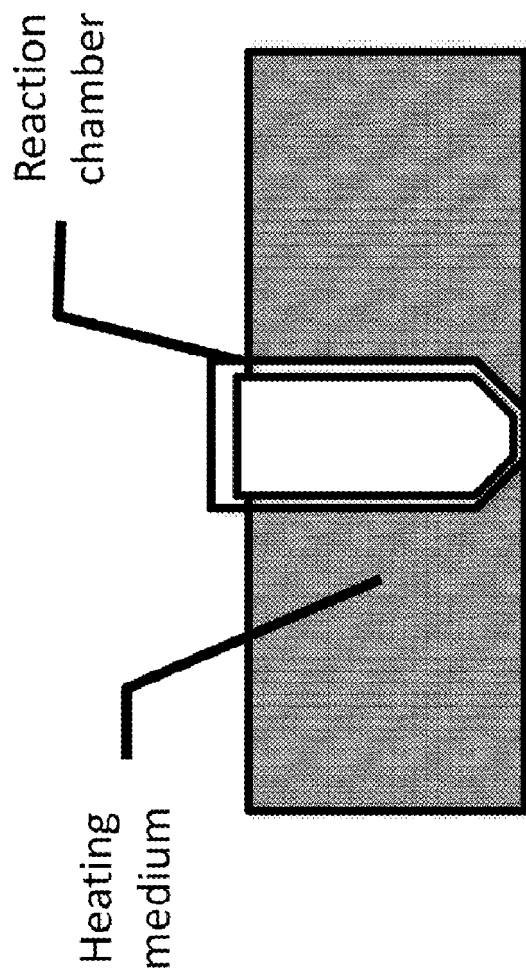
FIG. 22

Replacement Sheet

SAMPLE 3D SKETCH



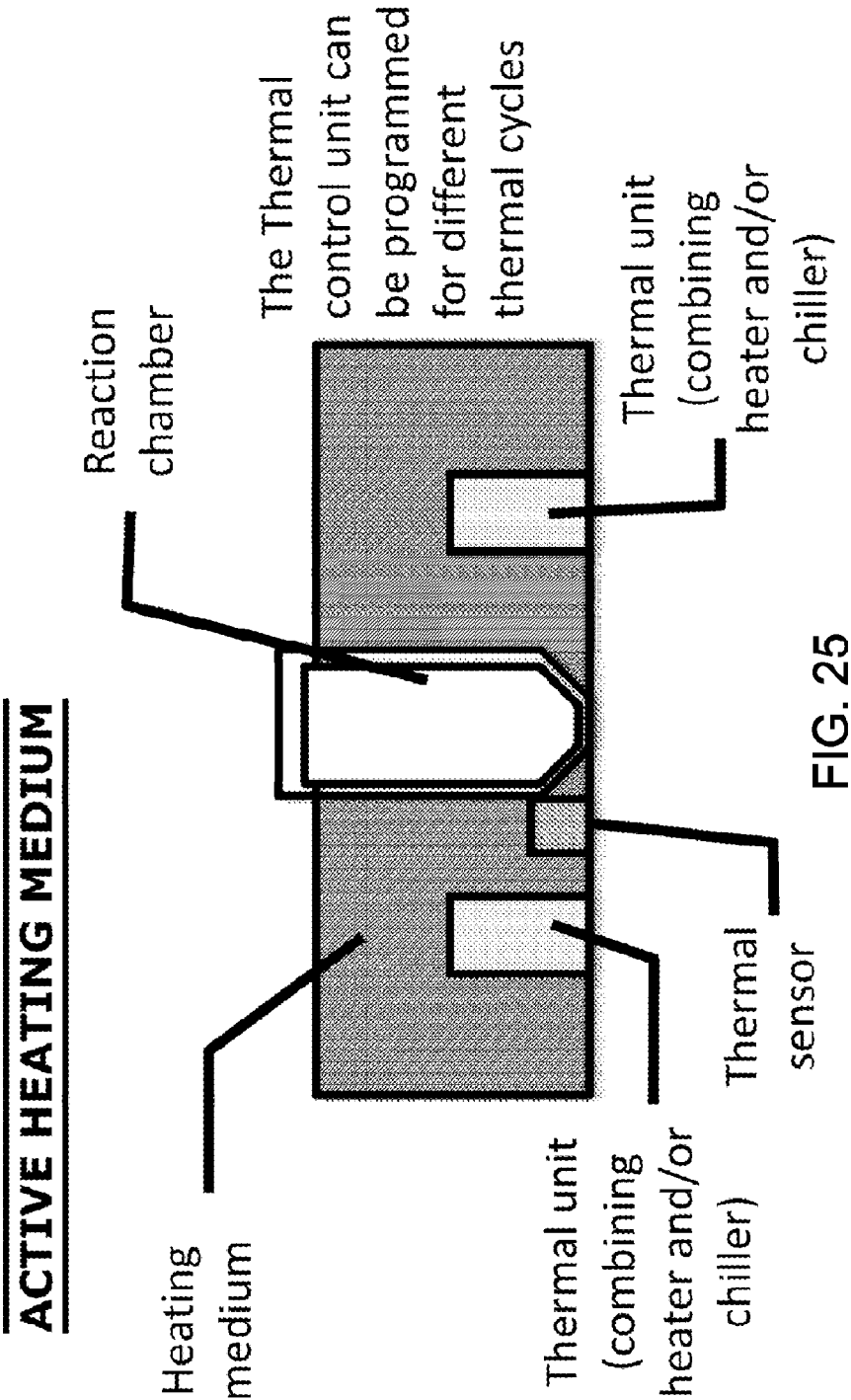
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INTERCHANGEABLE HEATING MEDIUM

The medium can be heated, then applied to the reaction chamber (example: heating medium can be wax)

FIG. 24

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Result is read
visually through a
opening or by
taking the
reaction chamber
out of the heating
medium

Note: This result
readout
embodiment can
be combined with
different
embodiment of
heating medium

RESULT READOUT1

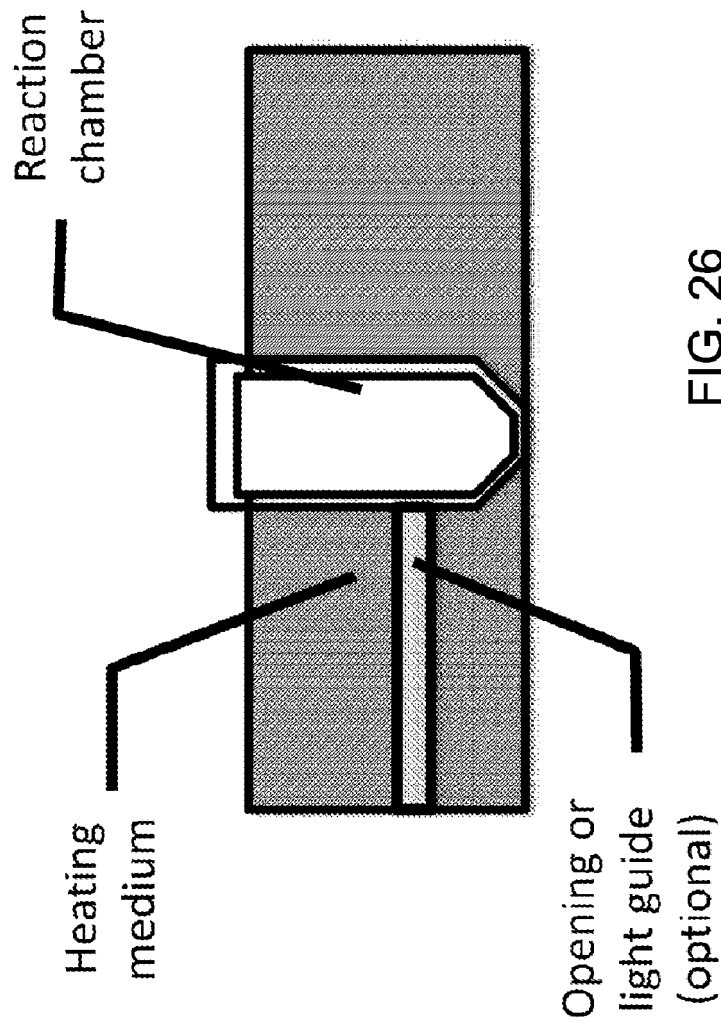


FIG. 26

Replacement Sheet

- Result is read visually through a opening or by taking the reaction chamber out of the heating medium
- In this embodiment, a lens is added for easier reading
- Note: This result readout embodiment can be combined with different embodiment of heating medium

RESULT READOUT2

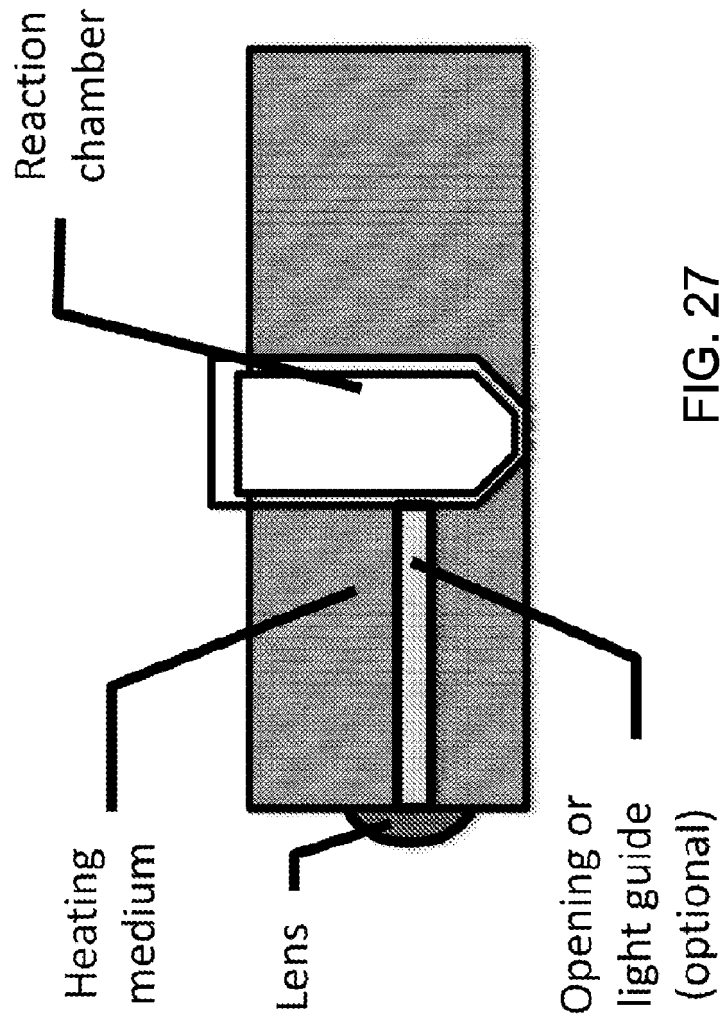


FIG. 27

RESULT READOUT3

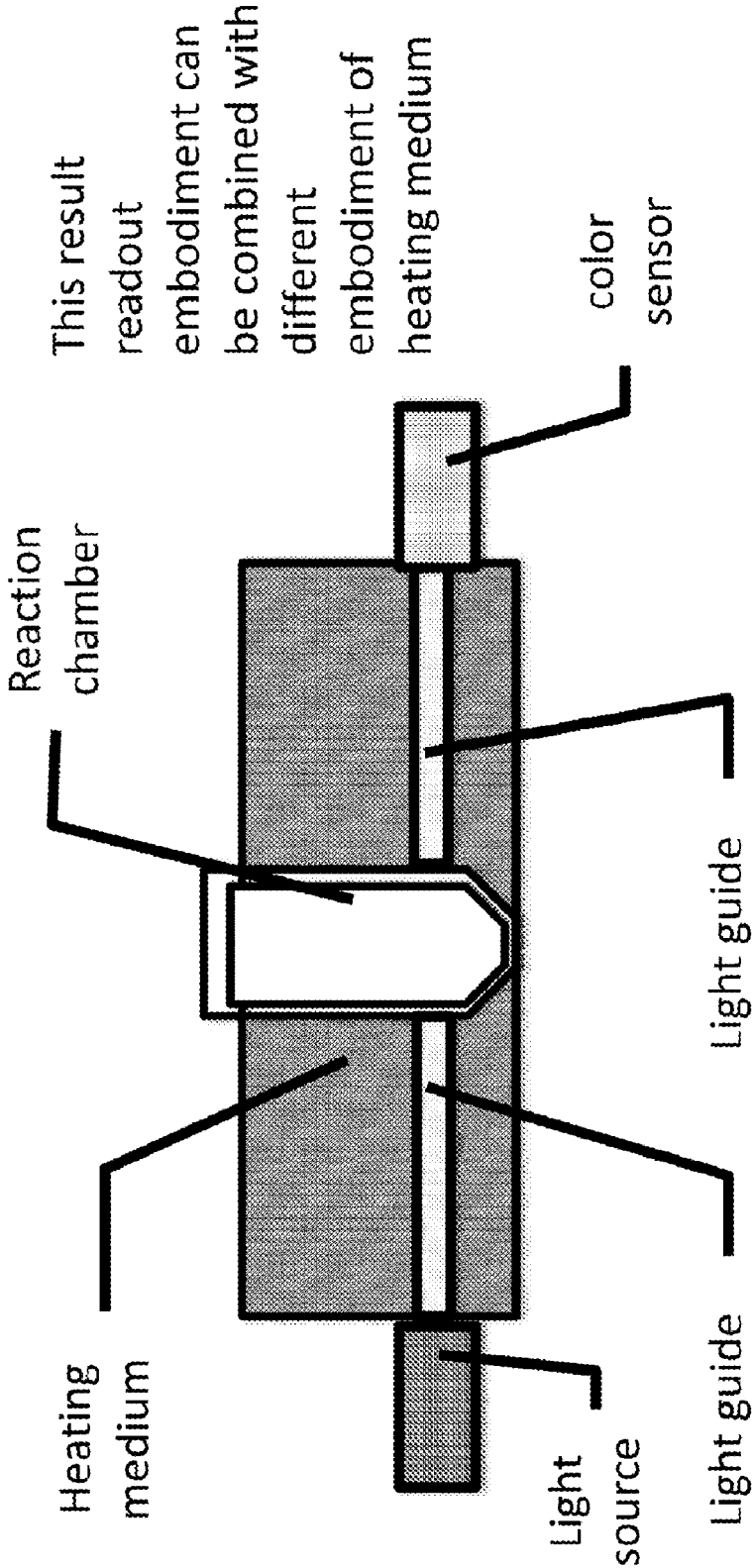
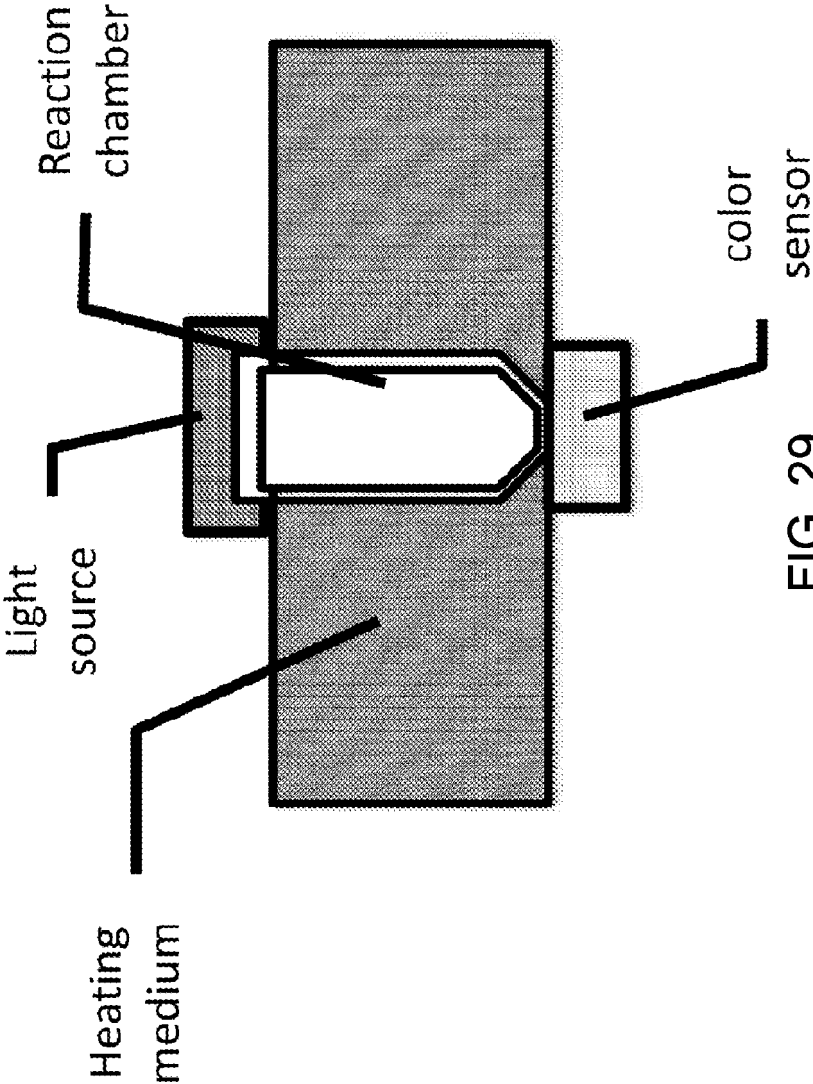


FIG. 28

Replacement Sheet

RESULT READOUT4



This result readout embodiment can be combined with different embodiments of heating medium

FIG. 29

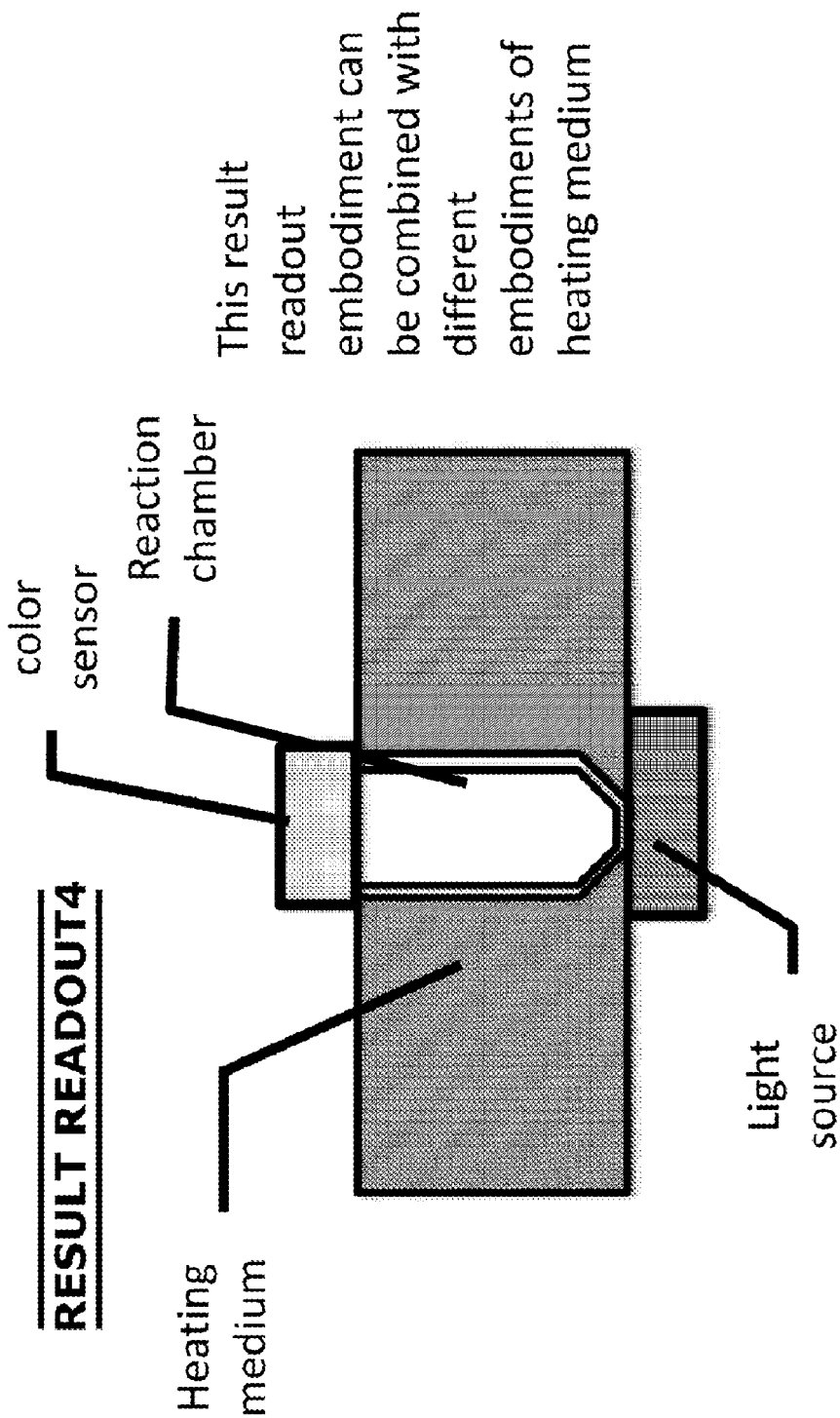


FIG. 30

Replacement Sheet

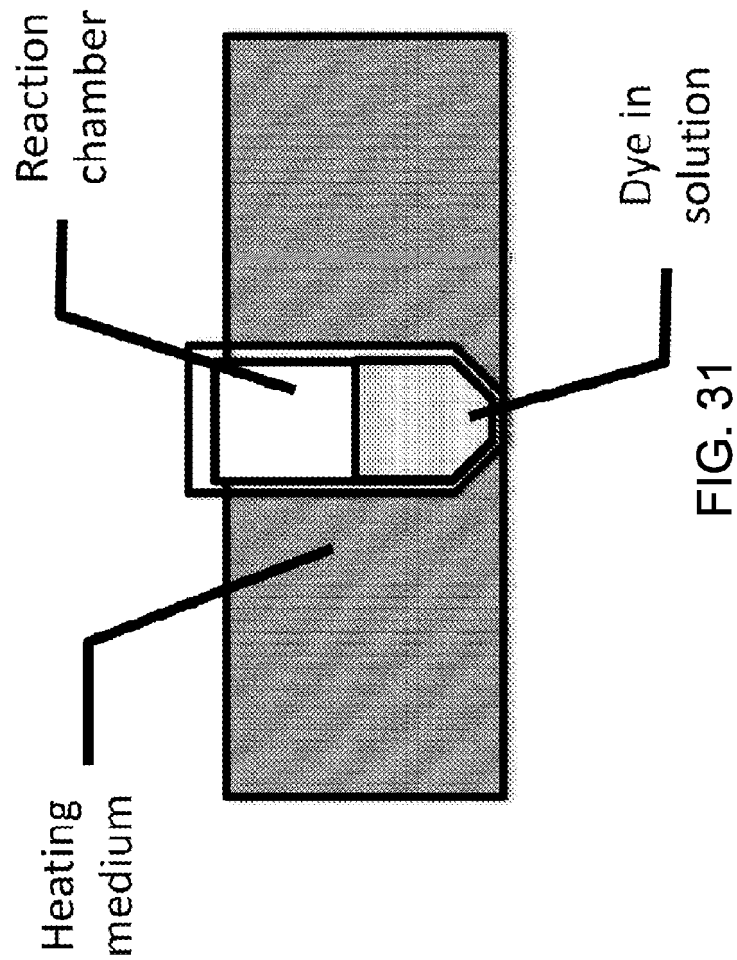
DYE IMMOBILIZING SUBSTRATE 1

FIG. 31

This dye immobilizing substrate embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrate

Replacement Sheet

DYE IMMOBILIZING SUBSTRATE2

- The entire hydrogel bead will change color
- This dye immobilizing substrate embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrate

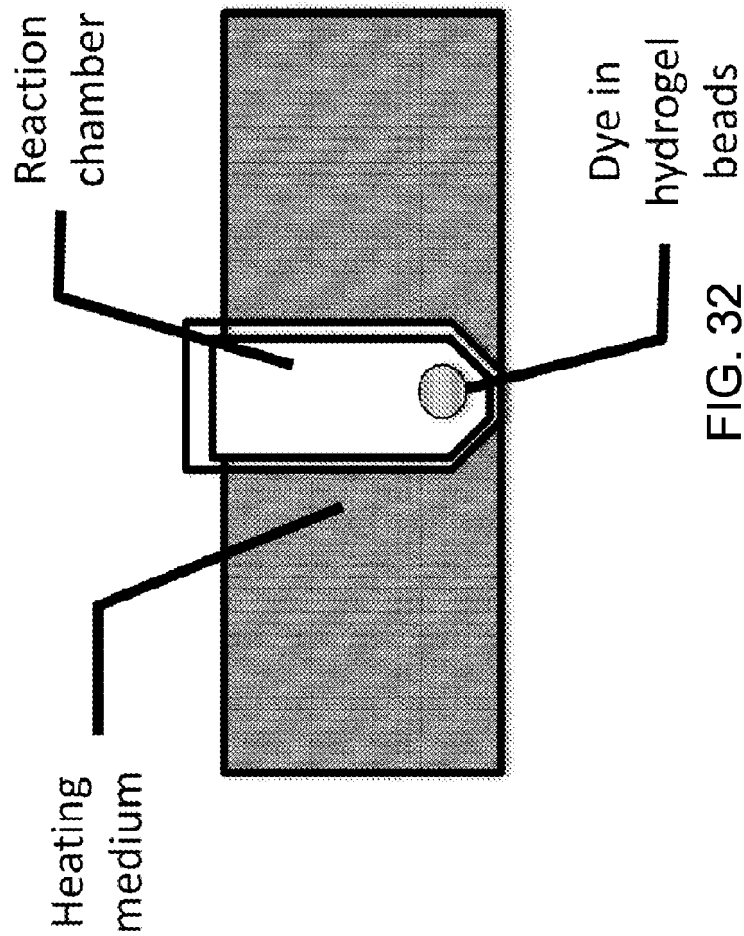


FIG. 32

Replacement Sheet

DYE IMMOBILIZING SUBSTRATE3

- The dye on the bead's surface will change color
- This dye immobilizing substrate embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrate

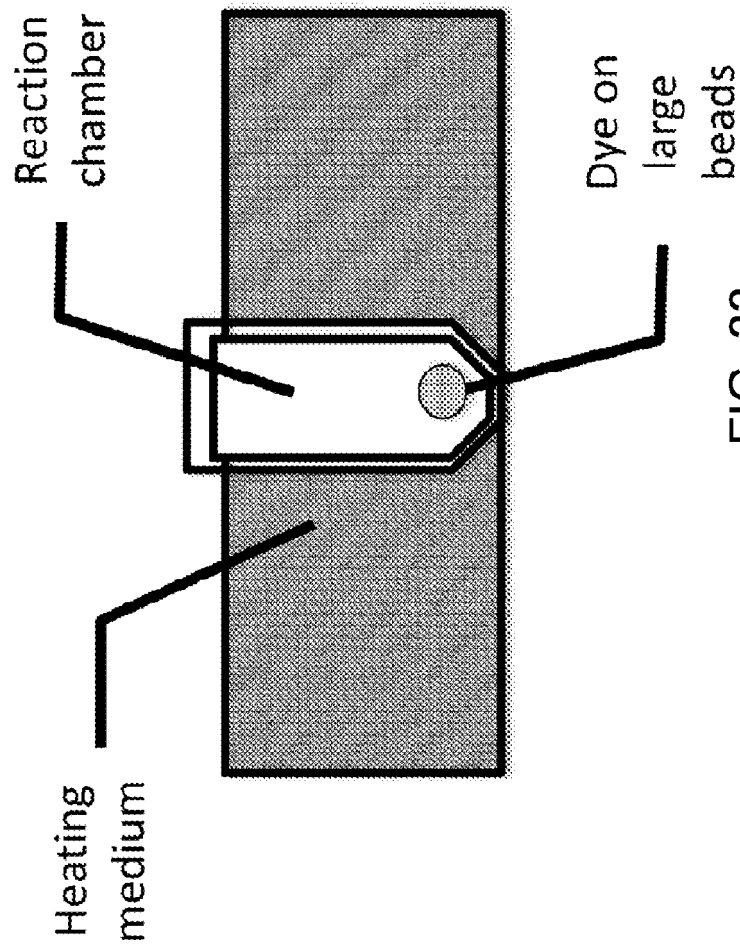


FIG. 33

Replacement Sheet

DYE IMMOBILIZING SUBSTRATE

- The dye on the bead's surface will change color
- This dye immobilizing embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrate

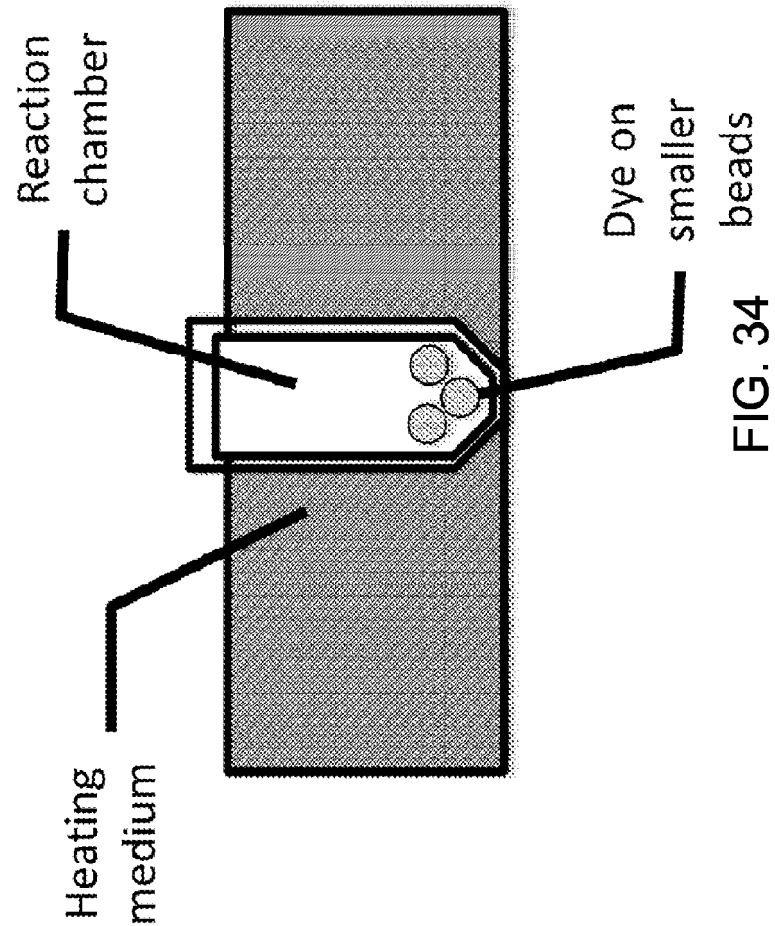


FIG. 34

Replacement Sheet

DYE IMMOBILIZING SUBSTRATES

- The dye on the bead's surface will change color
- This dye immobilizing substrate embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrate

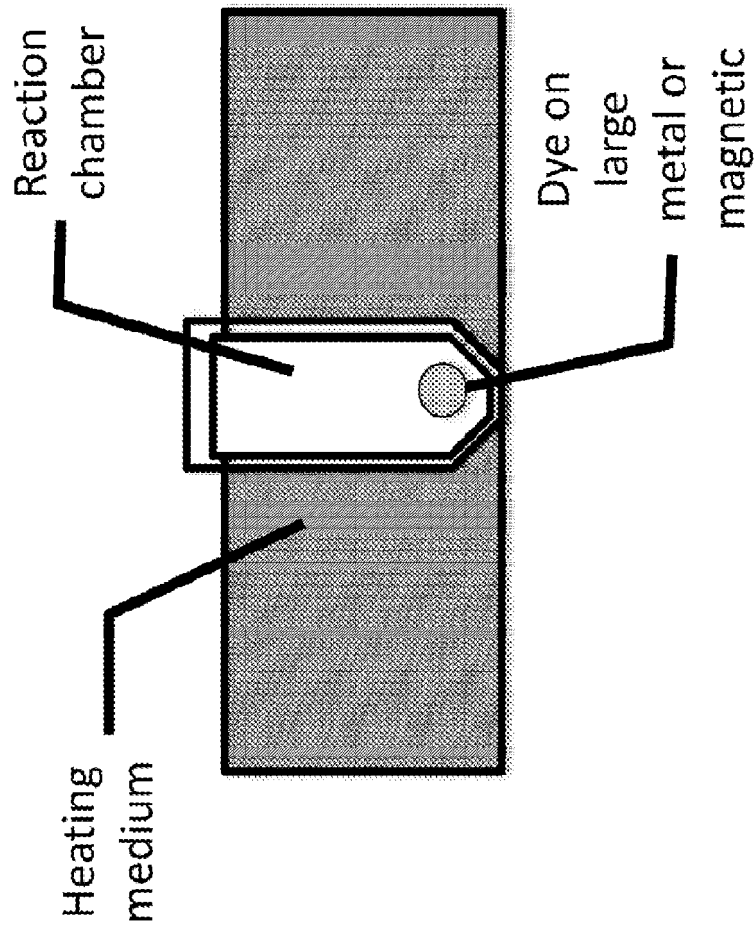
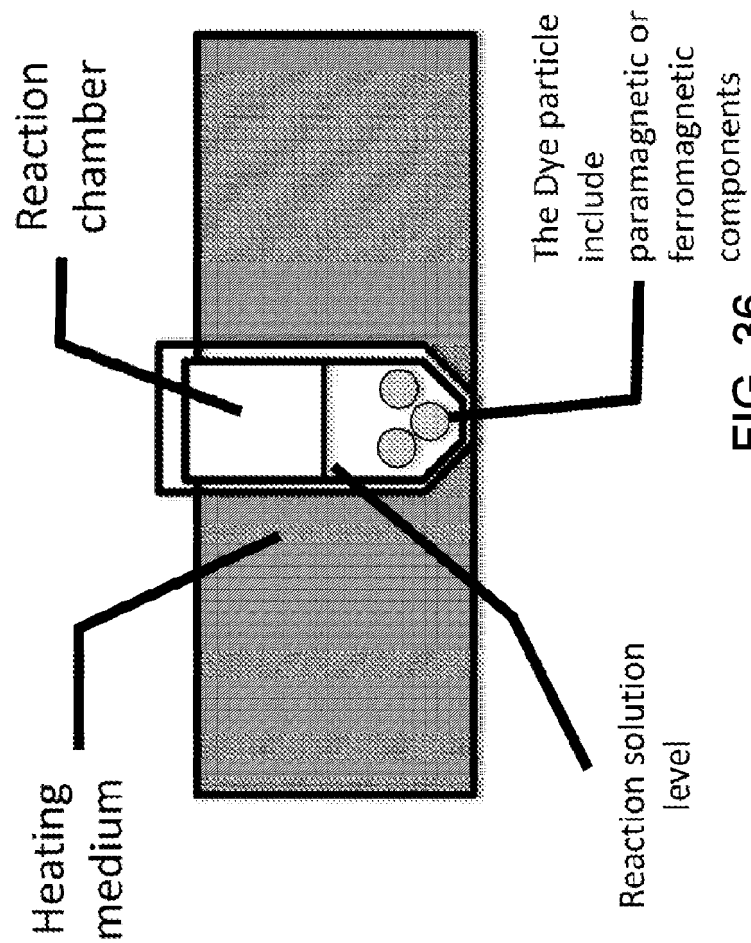


FIG. 35 beads

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DYE IMMOBILIZING SUBSTRATE6

- The dye on the bead's surface will change color
- This dye immobilizing substrate embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates

**FIG. 36**

Replacement Sheet

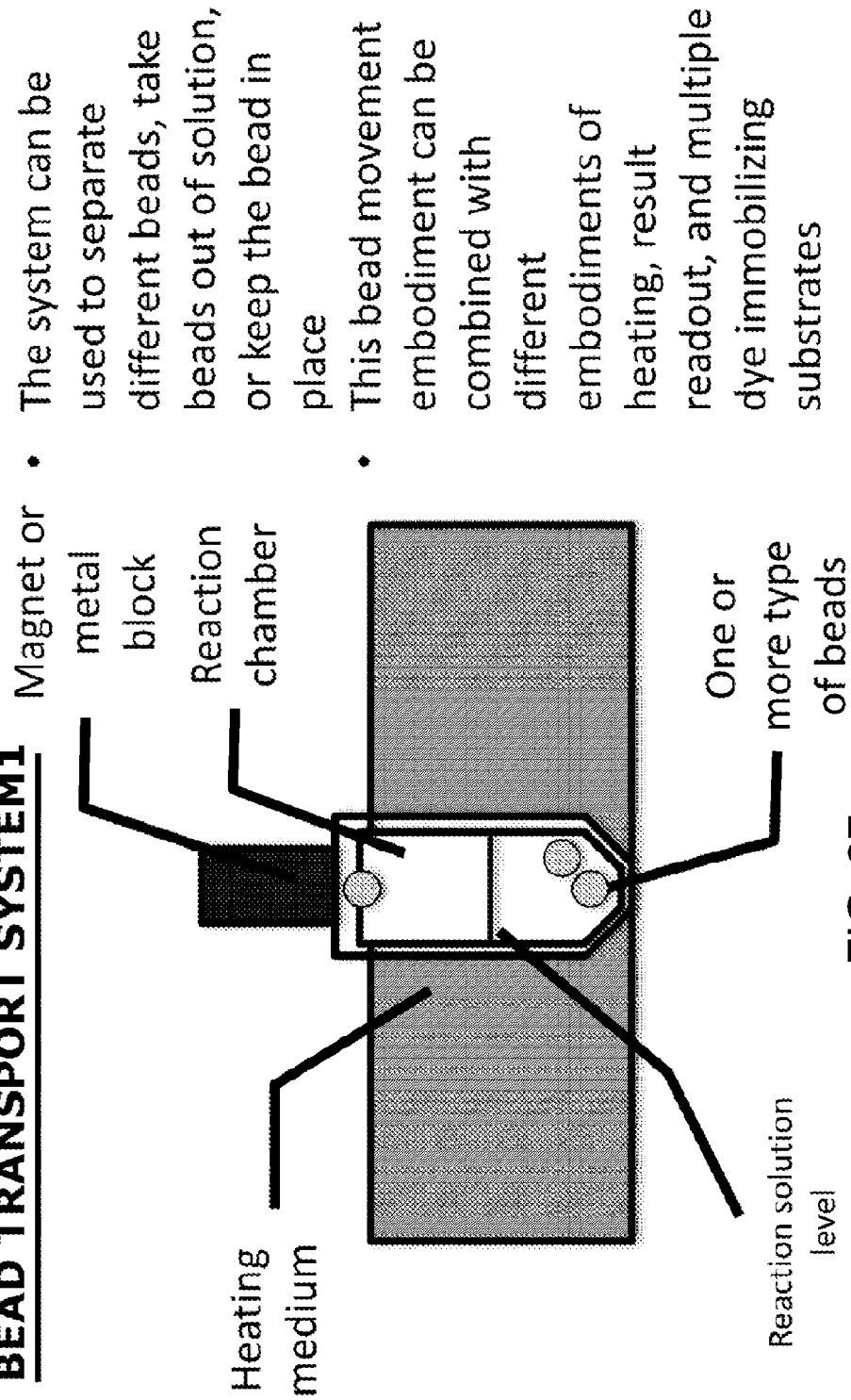
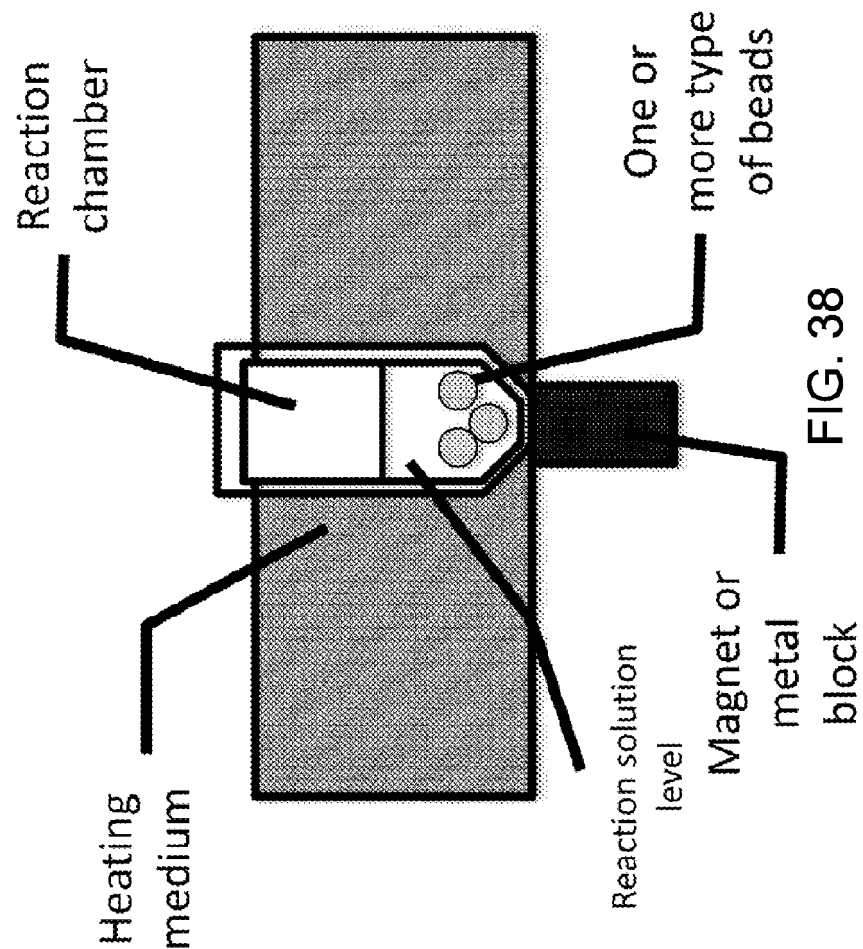
BEAD TRANSPORT SYSTEM1

FIG. 37

Replacement Sheet

- The system can be used to separate different beads, take beads out of solution, or keep the bead in place
- This bead movement embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates

BEAD TRANSPORT SYSTEM2



Replacement Sheet

- The system can be used to separate different beads, take beads out of solution, or keep the bead in place
- This bead movement embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates

BEAD TRANSPORT SYSTEM3

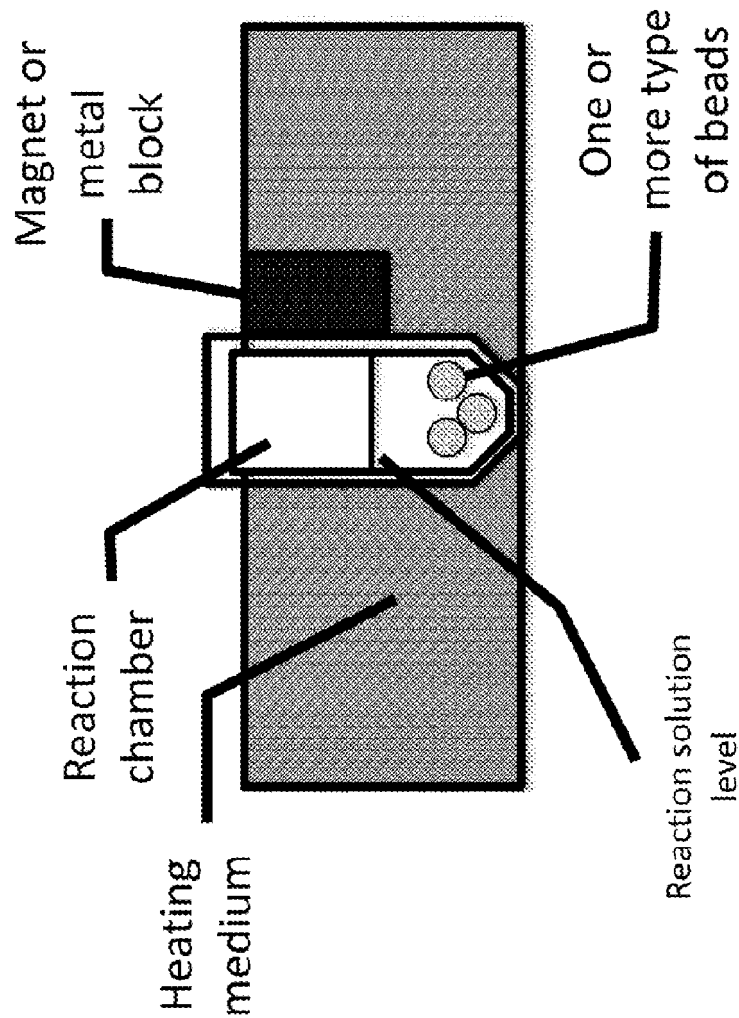
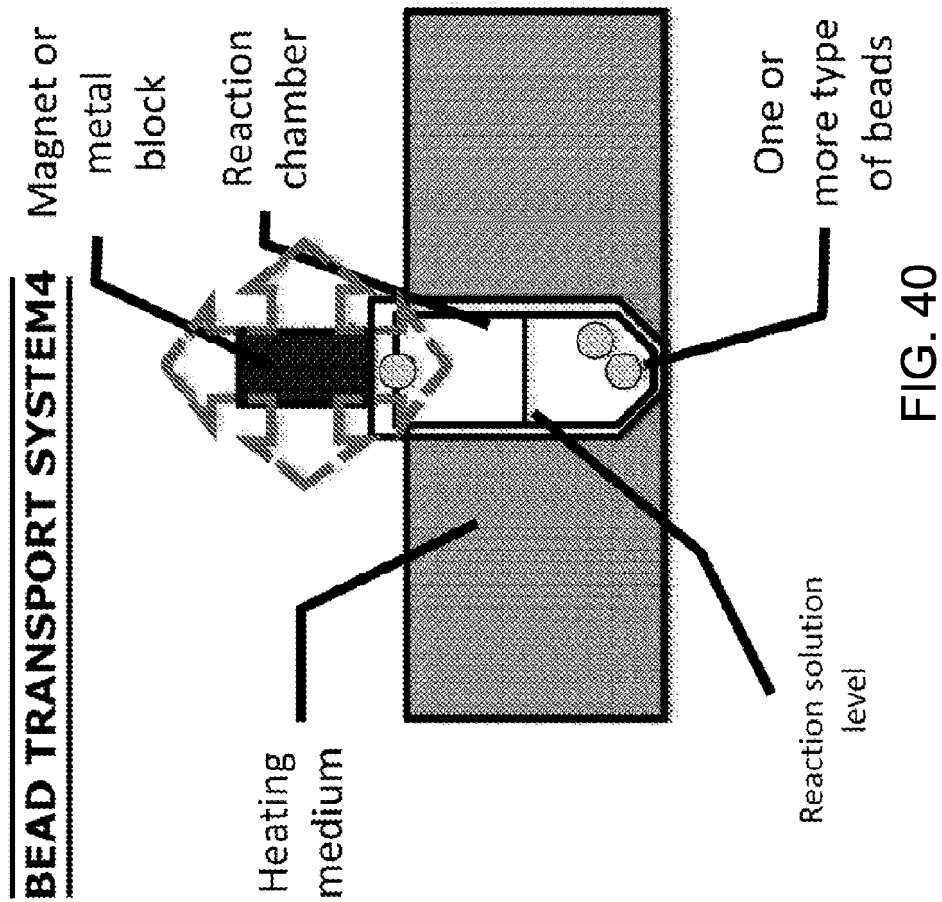


FIG. 39

Replacement Sheet

- In this embodiment, the magnet or metal block can move to place after the reaction is completed
- The system can be used to separate different beads, take beads out of solution, or keep the bead in place
- This bead movement embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates



Replacement Sheet

- In this embodiment, the magnet or metal block can move to place after the reaction is completed
- The system can be used to separate different beads, take beads out of solution, or keep the bead in place
- This bead movement embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates

BEAD TRANSPORT SYSTEM5

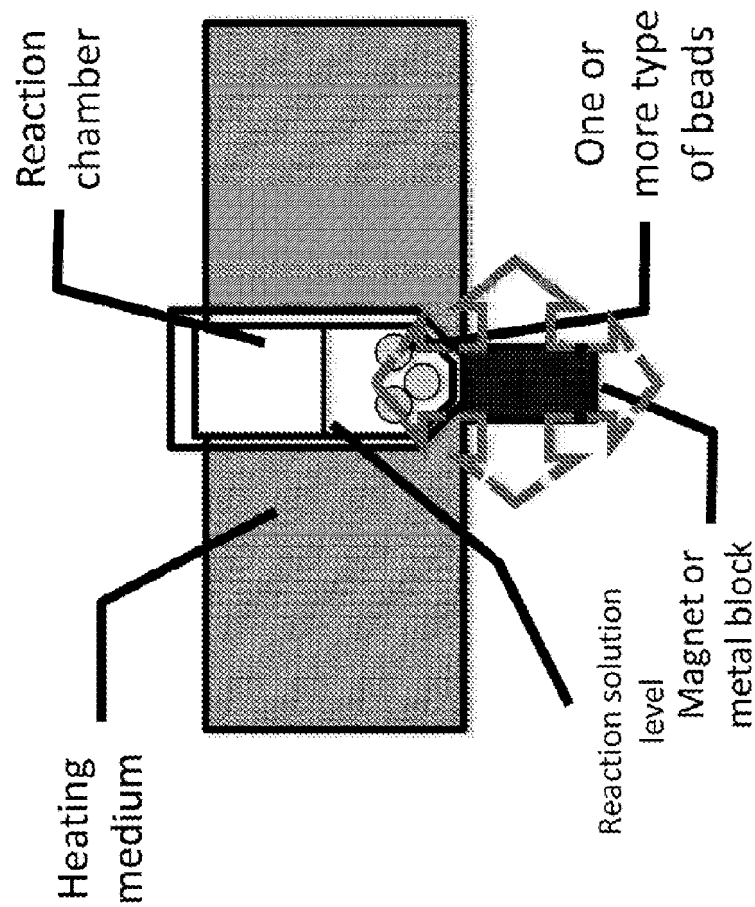


FIG. 41

Replacement Sheet

- In this embodiment, the magnet or metal block can move to place after the reaction is completed
- The system can be used to separate different beads, take beads out of solution, or keep the bead in place
- This bead movement embodiment can be combined with different embodiments of heating, result readout, and multiple dye immobilizing substrates

BEAD TRANSPORT SYSTEM6

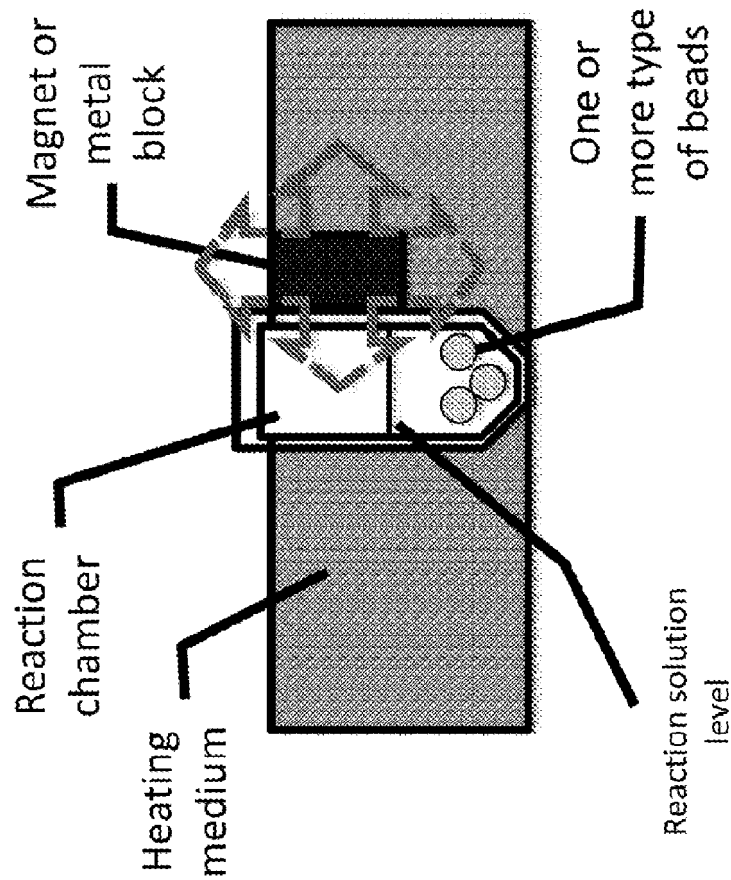


FIG. 42

Replacement Sheet

- in this embodiment, the heating medium is heated by an active thermal unit
- Inside the reaction chamber, there is a dye in solution which indicate if the starting pH is within range
- After the reaction, a magnetic block is move to the top of the cap to attract the metal beads, leaving behind the non-magnetic beads and solution
- The light source is then turned on, and the color of the bead is read by the sensor

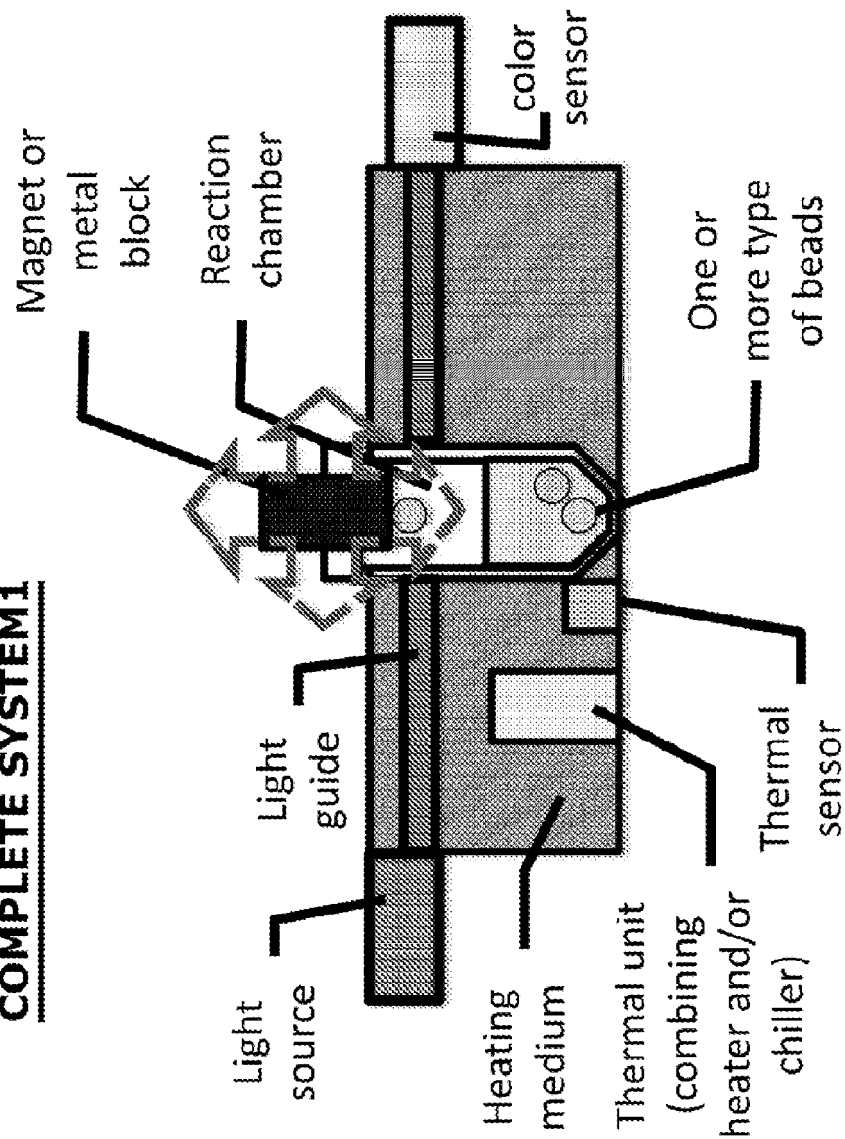
COMPLETE SYSTEM1

FIG. 43

Replacement Sheet

Embodiment:MMP1 SNP detection with saliva

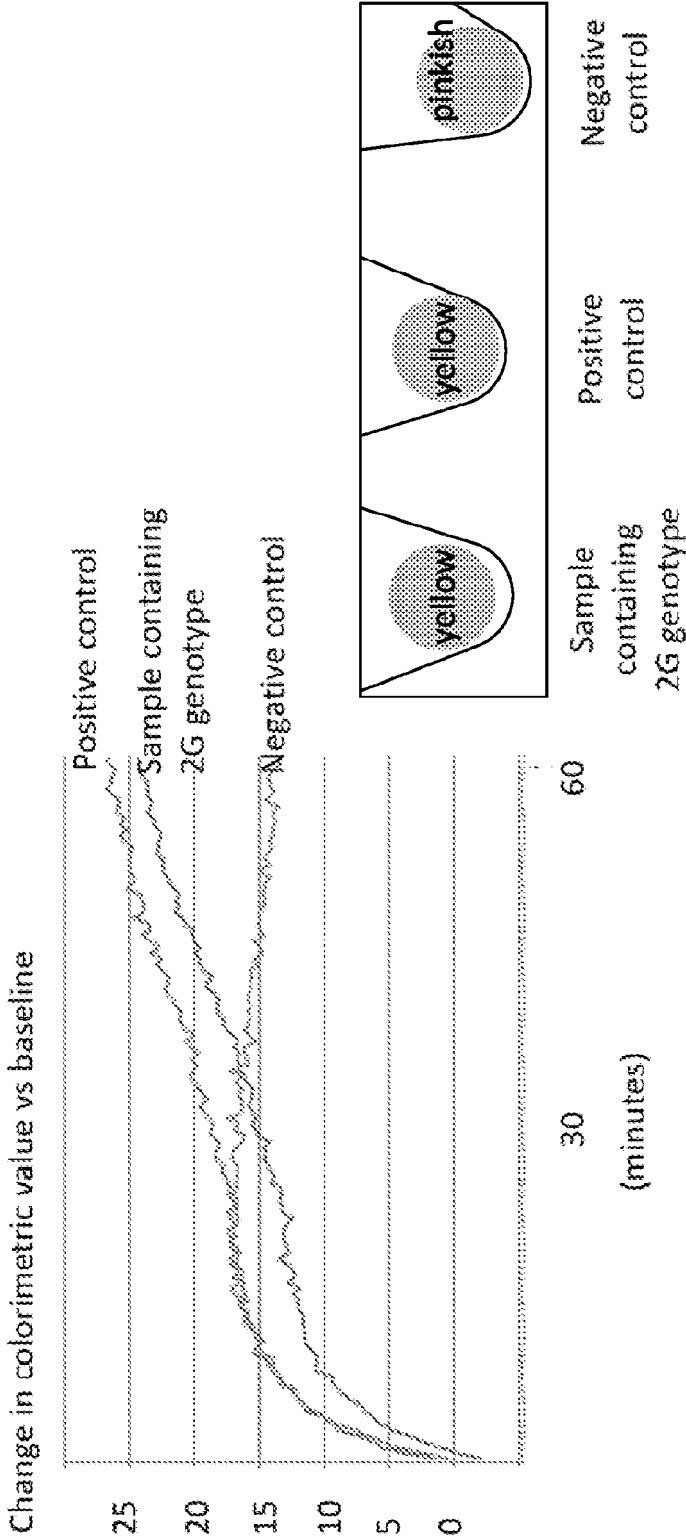


FIG. 44

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MMP1 SNP result using real-time LAMP with florescence
(SYBER GREEN)

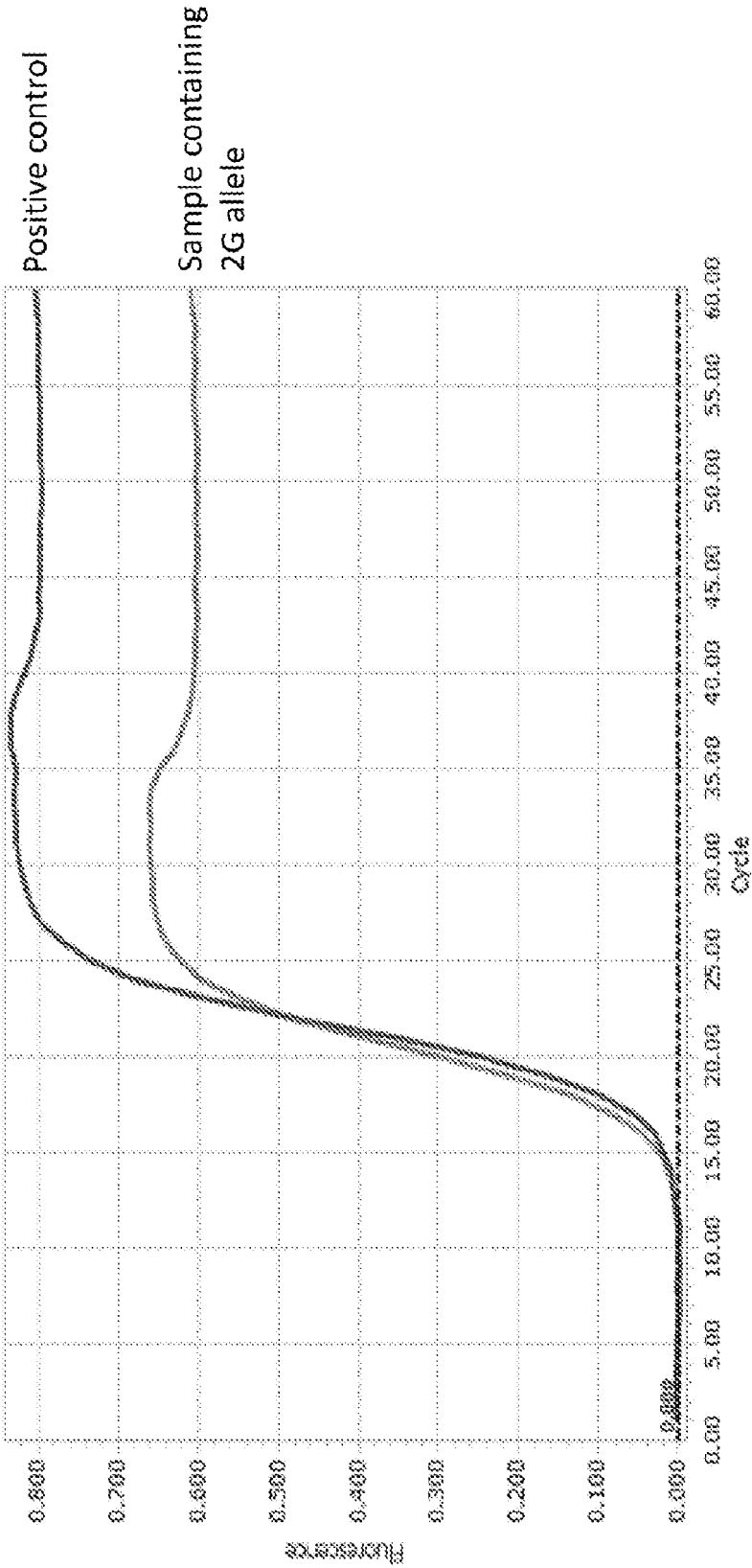


FIG. 45

MMP1 primer sequences used in reaction

MMP1: rs1799750

MMP1-2G	LF	CTCAAATCAATCCAAGA
	LB	CTACAAATTCCTCATCTAAGTG
	F3	CCCTTATGGATTCCCTGTTTC
	B3	TGAGAATGCTCTCCCATCT
	F	GGATATGACTTATCATATCTTACTCATAAACAAATAC
	B	TCCTTTCTAATTATTAACTACCCCTCTTGAACTCACATG
MMP1-Positive Control	LF	CTCAAATCAATCCAAGA
	LB	TTTCCTCATCTAAGTGGC
	F3	CCCTTATGGATTCCCTGTTTC
	B3	TGAGAATGCTCTCCCATCT
	F	GATATGACTTATCTCAAAATATCTTACTCATAAACAAATAC
	B	CTTTCTAATTATTAACTACAATACCCCTCTTGAACTCACATG
MMP1-Negative control	LF	CTCAAATCAATCCAAGA
	LB	CTACAAATTCCTCATCTAAGTG
	F3	CCCTTATGGATTCCCTGTTTC
	B3	TGAGAATGCTCTCCCATCT
	F	GGATATGACTTATCATATCTTACTCATAAACAAATAC
	B	TCCTTTCTAATTATTAACTACCCCTCTTGAACTCACATG

FIG. 46

Replacement Sheet

MMP1 reaction reagent: 25 ul volume
(colorimetric display)

	Final
Bst DNA polymerase large fragment	8U
DNA	1ul of Lyse
Primers FIP & BIP	1.6 uM
Primers F3 & B3	0.2 uM
Primers LF & LB	0.8 uM
dNTP	1.4mM each
MgSO ₄	10mM
(NH ₄) ₂ SO ₄	10mM
KCl	10mM
Trizma	1.5mM
Triton X-100	0.1%
Betaine	1 M

Lysis buffer: 400 ul

component	final
NaOH	25 mM
EDTA	0.2 mM

Negative control's DNA is 1ul of Lysis buffer without any sample

FIG. 47

MMP1 reaction reagent: 25 ul volume
(florescence)

	Final
Bst DNA polymerase large fragment	8U
DNA	1ul of Lyse
Primers FIP & BIP	1.6 uM
Primers F3 & B3	0.2 uM
Primers IF & IB	0.8 uM
dNTP	1.4mM each
MgSO ₄	10mM
(NH ₄) ₂ SO ₄	10mM
KCl	10mM
Trizma	1.5mM
Triton X-100	0.1%
Betaine	1 M
SYBR Green	0.1X (1/200,000 dilution)

Lysis buffer: 400 ul

component	final
NaOH	25 mM
EDTA	0.2 mM

FIG. 48

Replacement Sheet

Sample preparation with Blood

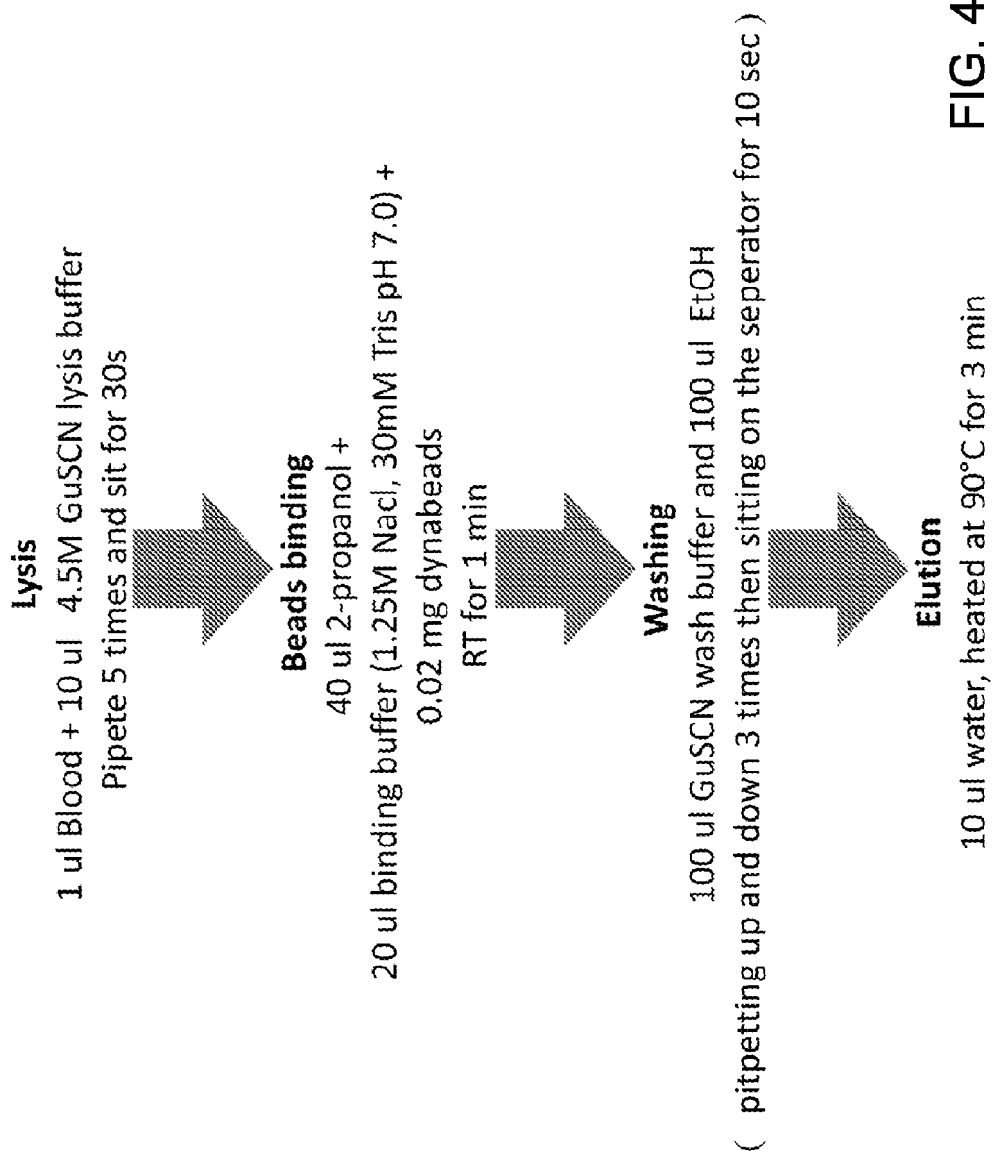


FIG. 49

Replacement Sheet

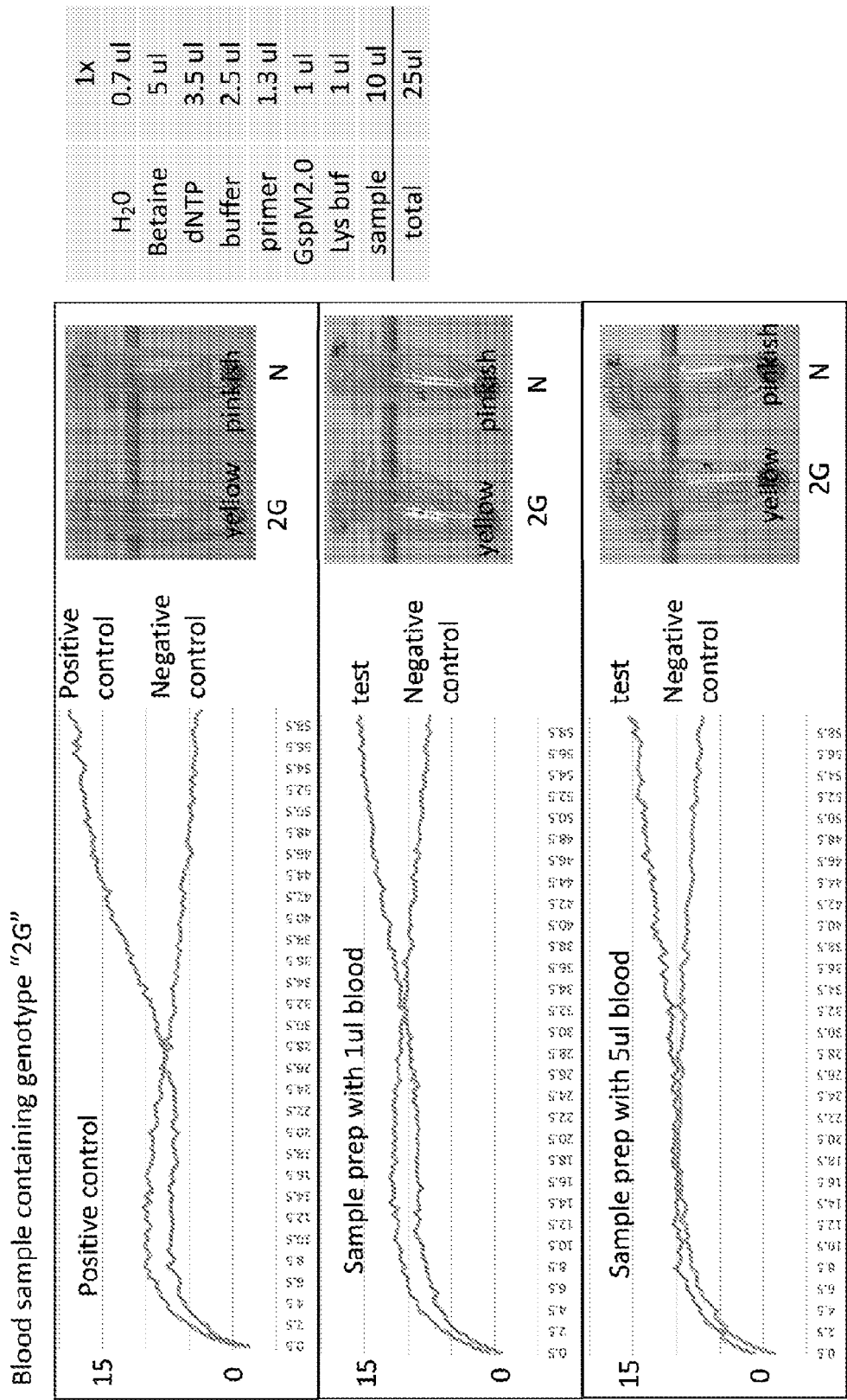


FIG. 50

- In this example, the heating medium is heated by an active thermal unit
- Inside the reaction chamber, there can be a dye in solution which indicate if the starting pH is within range
- Magnetic block can be used in the sample prep step
- The light source is turned on, and the color of the bead is read by the color sensor

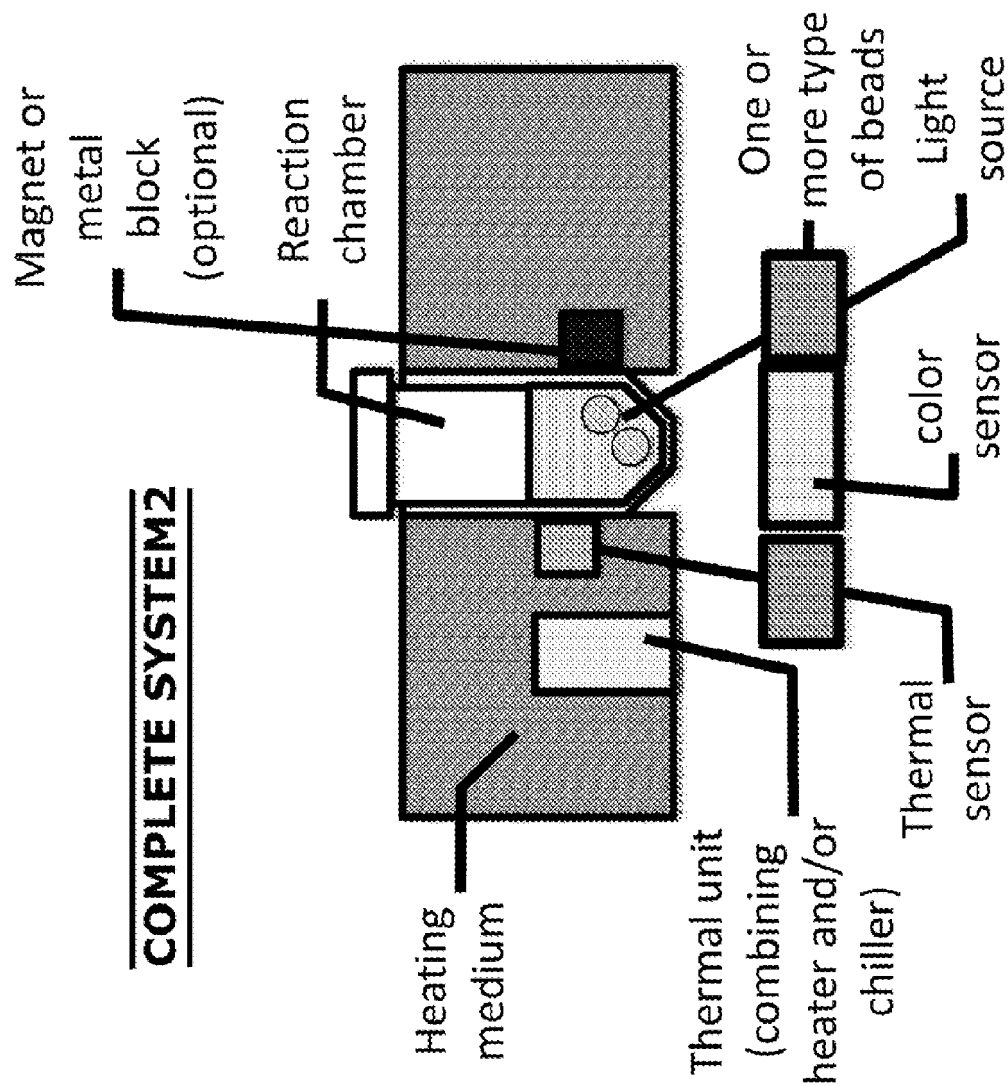


FIG. 51