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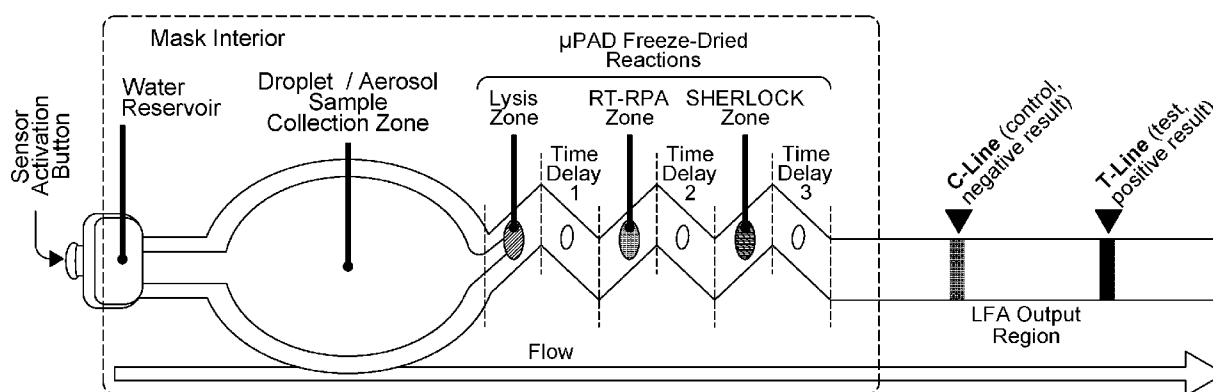


FIG. 28A

(57) Abstract: Embodiments of various aspects described herein are directed to an aqueous solution activated detection device. The device includes a carrier fluid reservoir, a sample collection unit, a sample processing unit including dried synthetic biological components, and a detection unit. The device can be integrated into a face mask for detection of airborne pathogens, viruses and toxins.

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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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AN AIRBORNE PATHOGEN DIAGNOSTIC PLATFORM**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/110,243, filed November 5, 2020, U.S. Provisional Patent Application No. 63/153,232, filed February 24, 2021, and U.S. Provisional Patent Application No. 63/163,357, filed March 19, 2021, each of which is hereby incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under HDTRA1-14-1-0006 awarded by the Department of Defense/Defense Threat Reduction Agency. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present invention relates generally to wearable sensors. More specifically, the present invention relates to a wearable sensing platform based on cell-free synthetic biology reactions added to a face mask.

BACKGROUND

[0004] Synthetic biology has provided control of biological systems and has led to developments in biotechnology and medicine. Modular biosensors, genetic logic gates, and output effectors are a part of customized biological circuits. In parallel, recent developments in wireless technology, wearable electronics, smart materials, and functional fibers including mechanical, electrical and optical properties have included the development of biosensing systems. Even though genetically-encoded sensors have been incorporated into bench-top diagnostics, examples of wearable devices using these tools are limited. Only a few demonstrations of hygroscopically actuated vents and response to induction molecules have been achieved using living engineered bacteria encapsulated in flexible substrates and hydrogels in a wearable format.

[0005] The combination of living engineered bacteria with flexible substrates and hydrogels in a wearable format encounters several limitations, particularly sustaining living organisms within wearable devices for extended periods. In practice, retaining viability and function of wearable sensing systems based on living cells requires nutrient delivery, waste extraction, as well as temperature and gas regulation, all of which involve numerous technological hurdles.

Genetically engineered cells can also pose biocontainment or biohazard concerns, particularly if integrated into consumer-level garments, leading to stringent regulatory pathways in many critical applications. Moreover, continually evolving cell populations suffer mutational pressures over time, resulting in potential loss of the genetic phenotype and function.

[0006] Thus, there is a need for a new approach in synthetic biology to resolve the mismatch between practical requirements of wearable use and operational limitations of available biomolecular circuits for sensing and response. The present disclosure is directed to solving these and other problems.

SUMMARY

[0007] According to one implementation, an aqueous solution activated detection device includes a carrier fluid reservoir, a sample collection unit, a sample processing unit including dried synthetic biological components, and a detection unit.

[0008] According to another implementation, a method of detecting an airborne agent includes; contacting air including the agent to the sample collection unit of the aqueous solution activated detection device, wherein the sample unit collects the agent on a surface of the sample unit; causing a carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the carrier fluid subsequently flowing to the sample processing unit and contacting the dried synthetic biological components; and reading an output from a detection unit, the output indicative of the presence of the airborne agent in the air.

[0009] According to another implementation, a method of making an aqueous solution activated detection device includes: connecting in series a carrier fluid reservoir, a sample collection unit, a sample processing unit, and a detection unit. According to this implementations, an outlet from the carrier fluid reservoir is connected to an inlet of the sample collection unit, an outlet from the sample collection unit is connected to an inlet of the sample processing unit, and an outlet of the sample processing unit is connected to an inlet of the detection unit.

[0010] According to another implementation, a diagnostic system includes a biochemical reaction composed of a combination of reverse transcriptase, nucleic acid-modifying enzymes, amplification enzymes, cell-free lysate, Cas13 or Cas12 enzymes, and other required substrates or reagents for their respective reactions and one or more nucleic acids which may contain complete or partial nucleic acid sequences, including the reverse complement and in DNA or RNA, as disclosed herein.

[0011] According to another implementation, a device for capturing an airborne agent includes a sample collection chamber and a covering. The sample collection chamber captures breath from a subject. The airborne agent is contained in the breath from the subject. The covering is coupled to the sample collection chamber. The covering has a sample collection unit with a porous material.

[0012] According to another implementation, a device for processing a sample includes two or more reaction zones coupled in sequence to each other. The device further includes one or more time delay barriers separating each pair of the two or more reaction zones.

[0013] The above summary is not intended to represent each implementation or every aspect of the present disclosure. Additional features and benefits of the present disclosure are apparent from the detailed description and figures set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The disclosure will be better understood from the following description of exemplary embodiments together with reference to the accompanying drawings.

[0015] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0016] FIGs. 1A-1C illustrate an aqueous solution-activated sensor fabric, according to some implementations of the description. FIG. 1A depicts a top schematic, FIG. 1B depicts an isometric view, and FIG. 1C depicts a detailed view.

[0017] FIG. 2 illustrates an implementation of a fabric based detector, according to some implementations of the description.

[0018] FIGs. 3A-3C depict an implementation of a fabric based detector including a hydrophobic material, according to some implementations of the description. FIG. 3A is a first view and FIG. 3C is a second view, both illustrating the hydrophobic material on a web structure. FIG. 3C depicts a close up schematic view of the hydrophobic material.

[0019] FIGs. 4A-4B depict an implementation of an aqueous solution-activated sensor, according to some implementations of the description.

[0020] FIGs. 5A-5B illustrate another implementation of an aqueous solution-activated sensor, according to some implementations of the description. FIG. 5A is an exploded layer view and FIG. 5B is a front cross-sectional view through one of the chambers.

[0021] FIGs. 6A-6H illustrate wearable cell-free synthetic biology, according to some implementations of the description. FIG. 6A depicts how freeze-dried cell-free reactions can be

embedded in reaction sachets or chambers that are distributed throughout garments for use by soldiers, clinicians, and first responders. FIG. 6B depicts a schematic of the layer-by-layer assembly of the wearable devices. FIG. 6C depicts an array of assembled reaction chambers showing the elasticity (center) and flexibility (right) of the devices. FIG. 6D depicts portals cut into the outermost layer. FIG. 6E-6F, depict various types of synthetic biology circuits can be freeze-dried in these wearable devices, including; constitutively expressed outputs (FIG. 6E), transcription factor-regulated circuits for small molecule detection (FIG. 6F), toehold switches for nucleic acid-sensing (FIG. 6G), and riboswitches to detect various small molecules (FIG. 6H).

[0022] FIGs. 7A-7C depict assembly layers and sample activation of colorimetric wFDCF reactions with constitutive $P_{T7}::LacZ$ module, according to some implementations of the description. FIG. 7B depict the activation of colorimetric wells or reaction chambers with a control rehydration solution. FIG. 7C depict the activation of the reaction chambers with a rehydrating solution including a triggering compound.

[0023] FIGs. 8A-8C depict sample activation of wFDCF colorimetric devices and a bracelet, according to some implementations of the description. FIG. 8A depicts activation of colorimetric Ebola virus DNA toehold wFDCF sensor. FIG. 8B depicts port wicking into reaction chambers containing reaction disks using dd-H₂O fluid splash. FIG. 8C depicts activation of the wearable colorimetric bracelet with four independent Ebola virus DNA toehold sensors.

[0024] FIGs. 9A-9G depict the design and validation of fluorescent and luminescent freeze-dried cell-free synthetic biology wearables, according to some implementations of the description. FIG. 9A details of assembly and activation of fiber-optic based wFDCF module for fluorescence/luminescence output. FIG. 9B top - diagram depicting the layers of the assembled device; bottom - cross-sectional view of the interior of the device. FIG. 9C depicts comparison of fluorescent signal after rehydration of wFDCF constitutive sfGFP template as compared to control. FIG. 9D depicts activation of FDCF riboswitch in a wearable device as compared to a control. FIG. 9E depicts a demonstration of fluorescent aptamer being activated by a substrate as compared to a control. FIG. 9F illustrates luminescence output detected from an HIV toehold sensor with nanoLuciferase operon. FIG. 9G illustrates wearable detection of organophosphate nerve agents.

[0025] FIGs. 10A-10D illustrate that concentrating PURE cell-free reactions increases reaction kinetics, according to some implementations of the description. FIG. 10A is a schematic of reaction concentration through the lyophilization of PUREXPRESS® (New England Biolabs,

Inc., Ipswich, MA) reactions at varying volumes followed by rehydration at a set volume. FIG. 10B depicts representative images of PURE reactions with a LacZ output over one hour, at various concentrations. FIG. 10C depicts quantified PUREXPRESS® reactions with a LacZ output. FIG. 10D depicts the half-maximal values from the curve fitting the data shown in FIG. 10D.

[0026] FIG. 11 depicts Zika DNA Toehold sensor activation in single mercerized cotton thread, according to some implementations of the description.

[0027] FIG. 12 depicts antibiotic resistance sensors for *spa*, *ermA* and *mecA* genes using in-wearable sensor demonstrate specific orthogonality, according to some implementations of the description.

[0028] FIG. 13 depicts POF fabric compatibility with lyophilized transcription-only fluorescent aptamer reactions, according to some implementations of the description.

[0029] FIG. 14 depicts sensor multiplexing using different fluorescent proteins in a single device, according to some implementations of the description.

[0030] FIGs. 15A-15B depict NanoLuciferase (nLuc) luminescence experiments, according to some implementations of the description. FIG. 15A depicts the dynamic response of a wFDCF Lyme disease RNA toehold switch sensor with luminescence output. FIG. 15B depicts the dynamic response of a wFDCF HIV RNA toehold switch sensor with luminescence output in comparison to constitutive P_{T7}::nLuc expression as a positive control.

[0031] FIGs. 16A-16D depict fabrication of polymeric optic fiber (POF) fabric for wFDCF, according to some implementations of the description. FIG. 16A depicts how hydrophilic yarns were weaved along the weft in combination with POFs as warp. FIG. 16B depicts a three-fiber multi-strip design. FIG. 16C depicts a roll of the hydrophilic POF fabric after weaving. FIG. 16D depicts a cut section of the hydrophilic POF fabric with indications in reaction zone and bundle ends.

[0032] FIGs. 17A-17G depict fabrication of textile-based wFDCF sensor patch, according to some implementations of the description. FIG. 17A depicts a cut strip of hydrophilic POF fabric that was laser-etched. FIG. 17B depicts examples of prepared wFDCF fabric-elastomer layers and final assembly into a three-well sensor for garment integration. FIG. 17C depicts a schematic of a POF-fabric-elastomer strip for sensing in a single textile layer including two excitation fibers on the sides of an emission fiber. FIG. 17D depicts a schematic of a double POF-fabric-elastomer strip for sensing with dedicated excitation and emission layers. FIG. 17E depicts a schematic of a single excitation or emission POF-fabric-elastomer layer overlaid on an applied elastomer pattern for creating the impermeable reaction wells or chambers. FIG. 17F depicts a

finalized three-well sensor wFDCF device with heat shrunk POF covers and Luer connectors for interface with a portable spectrometer device. FIG. 17G depicts a top and bottom views of a final three-well sensor wFDCF device.

[0033] FIGs. 18A-18B depict textile substrate compatibility testing using synthetic biology reactions and sample colorimetric reaction, according to some implementations of the description. FIG. 18A depicts samples of eight fabric types selected as part of the textile screening for wFDCF compatibility. FIG. 18B depicts a sample wFDCF colorimetric activation in a cellulose matrix square containing a protein synthesis solution.

[0034] FIGs. 19A-19B depict textile screening using model constitutive $P_{T7}::LacZ$ assay, according to some implementations of the description. FIG. 19A depicts a sample well plate containing BSA blocked and unblocked discs of different textile types after constitutive $P_{T7}::LacZ$ expression following a 12-hour run for reactions containing an protein synthesis solution with plasmid or without plasmid as controls. FIG. 19B depicts examples of qualitative traces of colorimetric signals for these different fabric disks using a plate spectrophotometer.

[0035] FIG. 20 depicts a compilation of normalized functional scoring for colorimetric wFDCF textile screening, according to some implementations of the description.

[0036] FIGs. 21A-21F depict fabrication of wearable microcontroller system with LED illumination and spectrometric capabilities, according to some implementations of the description. FIG. 21A is an exploded isometric view of wearable POF spectrometer components with case and electronics. FIG. 21B is a photograph of an open assembled device. FIG. 21C is a photograph of a fully assembled device ready for imaging. FIG. 21D depict details of a camera used in the device. FIG. 21E is a top view of an assembled device to provide detail of compact electronics arrangement. FIG. 21F depicts the arrangement of a wearable POF spectrometer with wireless connectivity in-garment for wFDCF reaction testing.

[0037] FIGs. 22A-22C depict custom mobile application software, according to some implementations of the description. FIG. 22A depicts a main window of the developed wFDCF sensor mobile application where spectrographic measurements are continuously recorded. FIG. 22B depicts an environmental window of the mobile application displaying geolocation information as well as environmental information. FIG. 22C depicts an excitation window of the application.

[0038] FIGs. 23A-23J depict validation of CRISPR-based FDCF wearable sensors, according to some implementations of the description. FIG. 23A depicts the sensing mechanism of CRISPR-Cas12a system. FIG. 23B depicts a wFDCF *mecA* CRISPR-based sensor exposed to sample containing *mecA* trigger. FIG. 23C depicts wFDCF *spa* CRISPR-based sensor exposed to

spa trigger. FIG. 23D depicts wFDCF *ermA* CRISPR-based sensor exposed to *ermA* trigger. FIG. 23E depicts experimental detection of *mecA* CRISPR-based sensor was statistically distinguishable. FIG. 23F is an orthogonality demonstration of *mecA* / *spa* / *ermA* CRISPR-based multi-sensor wearable. FIG. 23G is a plot depicting the orthogonality. FIG. 23H depicts POF end on light up demonstrating the orthogonality. FIG. 23I depicts garment-level integration of fabric-based wearable synthetic biology sensors. FIG. 23J depicts connection of fabric-based module to wearable POF spectrometer with wireless connectivity capabilities.

[0039] FIG. 24 depicts the limit of detection of wFDCF CRISPR-Cas12a based sensor activated in-fabric, according to some implementations of the description.

[0040] FIG. 25 depicts comparison of Cas13a-based SHERLOCK MRSA RNA-sensing in wFDCF in-fabric prototype against signal in a standardized plate reader, according to some implementations of the description.

[0041] FIGs. 26A-26E depict integrated wFDCF sample Lysis, according to some implementations of the description. FIG. 26A depicts detergent combinations for cellular lysis were tested against CRISPR-Cas12a SHERLOCK reactions. FIG. 26B depicts assembly of the wFDCF with lysis. FIG. 26C depicts in-wearable wFDCF *mecA* sensors containing a lyophilized lysis buffer challenged with intact *E. coli* cells either containing the target *mecA* gene or a negative control plasmid. FIG. 26D depicts some non-ionic surfactants used as freeze-dried lysis reagents: top row left to right Triton X-100, NP-40, and Tween-20; bottom row left to right Brij-58, Brij-C10, and Brij-S20. FIG. 26E depicts some ionic surfactants used as freeze-dried lysis reagents: left to right; sodium dodecyl sulfate, CHAPS hydrate, and sodium deoxycholate.

[0042] FIGs. 27A-27D depict bioinspired sample-wicking for textile-based wFDCF synthetic biology devices, according to some implementations of the description. FIG. 27A is a schematic of the base cover presented for the textile-based wFDCF synthetic biology devices, as well as the underlying biomechanical mechanism of water collection. FIG. 27B depicts a modified cover for the textile-based wFDCF synthetic biology devices with wicking ports. FIG. 27C depicts a five-second time-lapse of the fluid pinning and port wicking exhibited by the device. FIG. 27D is a photograph of an assembled textile-based wFDCF synthetic biology device including the bioinspired port.

[0043] FIG. 28A depicts a sensor, according to some implementations. FIG. 28B depicts a wearable mask, according to some implementations. FIG. 28C depicts freeze-dried lysis and detection components, according to some implementations. FIG. 28D is a plot and FIG. 28E depicts a corresponding Lateral Flow Assay(LFA), according to some implementations. FIG. 28F is a plot and FIG. 28G depicts a corresponding Lateral Flow Assay(LFA), according to some

other implementations. FIG. 28H depicts a breathing simulator, according to some implementations. FIG. 28I is a plot and FIG. 28J depicts a corresponding Lateral Flow Assay(LFA), according to yet another implementation.

[0044] FIGs. 29A and 29B depict implementation of Polyvinyl Alcohol (PVA) time delays for multi-stage wFDCF Reactions, according to some implementations. FIG. 29A depicts testing of the PVA time delays. FIG. 29B shows a representative experiment, from left to right, at 0, 13, 14 and 15 min.

[0045] FIGs. 30A, 30B, 30C1-30C6, 30D-30G show details on the design, performance, and relevant molecular sensor sequences, according to some implementations. FIG. 30A depict sSARS-CoV-2 genomic region targeted by the RT-RPA and SHERLOCK sensor utilized in a face-mask diagnostic of A-version sensors. FIG. 30B depicts a Laser-cut sample collection pad from capillary wicking material. FIG. 30C1-C6 depict steps of the μ PAD construction. FIG. 30D depicts components of a face-mask sensor before assembly. FIG. 30E depicts a fully assembled sensor. FIG. 30F depicts a demonstration of sample flow through face-mask sensor. FIG. 30G depicts the arrangement that allows for the preservation of patient confidentiality.

[0046] FIGs. 31A-31C depict a face-mask diagnostic B-version sensor design and construction, according to some implementations. FIG. 31A depicts a sub-assembly consisting of the sample collection pad, μ PAD (unfolded), and the LFA output strip. FIG. 31B depicts the fully assembled B-version face-mask sensors. FIG. 31C depicts a B-version sensor fully integrated into a face mask.

[0047] FIGs. 32A-32F depicts a breathing simulator, according to some implementations. FIG. 32A depicts a schematic of the modules used in the breathing simulator. FIG. 32B depicts details of a spontaneous breathing generator shown in FIG. 32A. FIG. 32C depicts details of the nebulizer and heating assembly shown in FIG. 32A. FIG. 32D depicts the nebulizer reservoir being filled (left) and the nebulized aerosols exiting the tubing (right). FIG. 32E depicts details of a high-fidelity anatomically precise airway manikin shown in FIG. 32A. FIG. 32F depicts details of the air flow path shown in FIG. 32A.

[0048] FIG. 33A shows a breathalyzer, according to some implementations. FIG. 33B shows a face mask, according to some implementations, for comparison to the breathalyzer shown in FIG. 33A.

[0049] FIG. 34 depicts toehold switches, according to some implementations.

[0050] FIGs. 35A-35U show plots of a LacZ output if screening of a library, according to some implementations (35A control, 35B-35U toehold sensors 1-20 respectively).

[0051] FIGs. 36A-36Q depict a library of gRNAs that were screened to allow Cas13a direct sensing of SARS-CoV-2 vRNA, according to some implementations. Plots of screening data are shown in 36A-36P, a summary plot is shown by 36Q.

[0052] FIG. 37A depicts a reverse transcriptase amplification reaction, according to some implementations. FIG. 37B depicts the experimental setup for screening a library of 11 gRNAs, according to some implementations. FIG. 37C-37M are plots depicting gRNAs activity for the 11 gRNAs screened according to FIG. 37B. FIG. 37N is a summary plot of the data from FIG. 37C-37M.

[0053] FIG. 38A is a diagram depicting an RT-RPA reaction, according to some implementations. FIG. 38B depicts the experimental setup for screening of primer pairs, according to some implementations. FIG. 38C depicts a primer set that allows for rapid detection of viral RNA fragments and full-length genome, according to some implementation. FIG. 38D depicts the optimization of the primer molar ratio for the primer set of FIG. 38C. FIG. 38E depicts the signal over background of the primer set of FIG. 38C.

[0054] FIG. 39A depicts testing of lysis buffer reagents, according to some implementations. FIG. 39B depicts a subset of tested lysis buffer reagents, according to some implementations. FIG. 39C depicts compositions of lysis buffer reagents, according to some implementations.

[0055] While the present disclosure is susceptible to various modifications and alternative forms, specific implementations and embodiments thereof have been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that it is not intended to limit the present disclosure to the particular forms disclosed, but on the contrary, the present disclosure is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure as defined by the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[0056] The present disclosure can be embodied in many different forms. Representative embodiments are shown in the drawings, and will herein be described in detail. The present disclosure is an example or illustration of the principles of the present invention, and is not intended to limit the broad aspects of the disclosure to the embodiments illustrated. To that extent, elements, and limitations that are disclosed, for example, in the Abstract, Summary, and Detailed Description sections, but not explicitly set forth in the claims, should not be incorporated into the claims, singly or collectively, by implication, inference, or otherwise. For purposes of the present detailed description, unless specifically disclaimed, the singular includes the plural and vice versa; and the word “including” means “including without limitation.” Moreover, words of approximation, such as “about,” “almost,” “substantially,” “approximately,”

and the like, can be used herein to mean “at,” “near,” or “nearly at,” or “within 3-5% of,” or “within acceptable manufacturing tolerances,” or any logical combination thereof, for example.

[0057] Embodiments of various aspects described herein are, at least in part, based on the discovery that synthetic biological reactions can be incorporated into wearable devices and fabrics. The synthetic biological reactions can be selected to function as sensors and expand and complement the scope of use available with live biological sensor systems. The various embodiments enable many applications for synthetic biology, allowing utilization in a wide range of wearable substrates (e.g., functional fibers or fabrics) to assess molecular targets difficult to detect through other technologies. The sensors can be used, for example, by first responders, military personnel, and clinicians at risk to exposure to biological pathogens, viruses and chemical toxins.

[0058] Cell-free synthetic biology reactions are self-contained abiotic chemical systems with all the biomolecular components required for efficient transcription and translation. Such systems can be freeze-dried into shelf-stable formats using porous substrates, which allow for robust distribution, storage and use without specialized environmental or biocontainment requirements. Genetically engineered circuits, encoded in DNA or RNA, can be added to freeze-dried, cell-free (FDCF) reactions for activation by simple rehydration. According to some implementations of the disclosure, FDCF genetic circuits are combined with flexible and textile substrates. These can be incorporated and used for the design of practical wearable biosensors. According to some aspects, various wearable freeze-dried, cell-free synthetic biology (wFDCF) sensors for small molecule, nucleic acid, and toxin detection have been made. These sensors can be integrated into flexible multi-material substrates (e.g., silicone elastomers and textiles) using genetically engineered components, including toehold switches, transcriptional factors, riboswitches, fluorescent aptamers, and CRISPR-Cas (e.g., Cas 12a, 13a) complexes.

[0059] FIGs. 1A-1C illustrate an aqueous solution-activated sensor fabric (100), according to some implementations. FIG. 1A depicts a top schematic, FIG. 1B depicts an isometric view, and FIG. 1C depicts a detailed view. The fabric includes an excitation plastic optic fiber (POF) 102, and an emission POF 104 combined with a porous hydrophilic material into a flat web structure 106. The fabric also includes a FDCF synthetic biological component 108 in at least a portion of the web structure. Optionally, the web structure 100 is a woven structure where the excitation POF 102 and emission POF 104 are woven in the warp direction 112, and the hydrophilic material 110 is woven in the weft direction 114.

[0060] The excitation POF 102, and the emission POF 104 include an outer cladding 116. In some implementations, as shown in FIG. 1C, the excitation POF 102 and the emission POF 104

are etched to remove a portion 118 of outer cladding 116. This provides a pathway for light to enter into, or exit out of, the POF along its length.

[0061] FIG. 2 illustrates an implementation of a web structure 200. The web structure 200 is a woven structure including a first layer 202 where the excitation POF 102 is woven in a warp direction 112, and the porous hydrophilic material 110 is woven in the weft direction 114. In some implementations, the excitation POF includes a plurality of substantially parallel POFs. The web structure 200 can optionally include a second layer 204 wherein the emission POF 104 is woven in the warp direction 112, and the porous hydrophilic material is woven in the weft direction 114. In some implementations, the excitation POF 102 includes a plurality of substantially parallel excitation POFs 102. In some implementations, the emission POF 104 includes a plurality of parallel emission POFs 104.

[0062] In some implementations, the FDCF synthetic biological component is spatially contained by being surrounded by patterns of a hydrophobic material. FIG. 3A -3C illustrate one possible configuration. A hydrophobic material 302 is shown in FIG 3A, and shown in outline in FIG. 3B. The synthetic biological component 108 is surrounded by the hydrophobic material 302. The synthetic biological components 108 is absorbed on and in the porous hydrophilic material 110. A port 306 (e.g., a small opening) is included. The port 306 exposes the synthetic biological component 108 to the environment outside of the web structure 106 so that an aqueous solution can enter and make contact with the synthetic biological component 108.

[0063] FIG. 3C depicts a depicts a close up schematic view of the hydrophobic material 302. The hydrophobic material 302 forms a chamber 308, shown as a dashed outline. The port 306 provides a fluid connection to the chamber 308 through a conduit 310. The FDCF synthetic biological components 108 are disposed (e.g., deposited or placed) in the chamber 306 (not shown for clarity). An excitation POF 102 and emission POF 104 are shown passing through the hydrophobic material 302, and through the chamber 308. Additional fibers of POFs can be included. For clarity, the porous hydrophilic material 110 is also not shown. In some implementations, a first end 322 of the excitation POF 102 is treated with a reflective coating. A second end 332 of the excitation POF 102 can be connected to an excitation source, such as an LED light. In some implementations, a first end 324 of the emission POF 104 is treated with a reflective coating. A second end 334 of the emission POF 104 can be connected to a detector.

[0064] In some implementations, the chamber volume is between about 0.1 μL and about 500 μL (e.g., between about 1 and 150 μL). In some implementations, the port 306 is between 0.1 μm^2 and 50 mm^2 (e.g., between 1 μm^2 and 10 mm^2).

[0065] Although illustrated in FIG. 3A-3C as web structure 106, other web structures, such as the web structure 200 (FIG. 2) can also be used. In some implementations, no POFs are used and a top portion 312 of the hydrophobic material 302, all through the hydrophobic material 302 to the chamber 308 (FIG. 3C), is transparent.

[0066] When an aqueous solution contacts the synthetic biological components 108, they are re-hydrated. These can include the various FDCF biological components described herein. If a trigger compound is present in the aqueous solution, a signal output can be observed.

[0067] FIG. 4A and 4B depict an implementation of an aqueous solution-activated sensor 400. FIG. 4A is an exploded perspective layer view and FIG. 4B depicts separated layer of the sensor 400 from a top view. A chamber 402 is formed by a bottom layer 404 of a flexible material, a middle layer 406 of a second flexible material, and a top layer 408 of a third flexible material. The first, second and third flexible materials can have the same or different compositions. The bottom layer 404 defines a bottom wall 414 of the chamber, the area of which is shown in encircled by a dashed line (e.g., the boundary) in FIG. 4B. The boundary defining the bottom wall 414 is provided by a cut out in the middle layer 406. The middle layer 406 defines a side wall 416 of the chamber, by the continuous open space or cut out in the middle layer 406. A top layer 408 defines a top wall of the chamber 418, shown by a dashed outline (opposite an exterior surface 412). Synthetic biological components 108 are disposed in the chamber. A port 410 fluidly connects the exterior surface 412 of the third layer 408 of the flexible material to an interior of the chamber 402. The port is defined by a continuous open space or cut out in the top layer 408. The port 410 allows an aqueous solution in contact with the exterior surface 412 to be wicked to the interior of the chamber 402. The FDCF synthetic biological components are hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components. The rehydrated synthetic biological components are formulated to provide an optical signal transmittable through a light transmitting medium. The optical signal is responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber.

[0068] In some implementations, the top layer 404, or a portion thereof, is a UV-Vis light transmitting medium and provides an optical connection to the interior of the chamber 402. In some implementations, at least of portion of flexible material is opaque to UV-Vis light. In some implementations one or more of the bottom layer 404, the middle layer 406 and top layer 408 include an elastomeric material.

[0069] According to some aspects, a dried lysate is disposed in the chamber. In some implementations, the dried lysate is disposed in the chamber between the port 410 and the FDCF

biological components 108. Optionally, the dried lysate is absorbed on or in a porous hydrophilic material. In some implementations, a dissolvable membrane or dissolvable material is disposed between the dried lysate and the FDCF biological components 108. The dissolvable material can provide a time delay allowing the lysate to act on components, such as cells and viruses, in the aqueous solution. The aqueous solution, and lysates in the aqueous solution, subsequently contact the biological components 108. In some implementations a delay is provided by a tortuous path. For example, a barrier is provided that is made of material that is impermeable to the aqueous solution but has a tortuous channel. The tortuous channel fluidly connects the dried lysate and the FDCF biological compounds. As used herein, tortuous can include a winding path for the channel creating a large distance for the aqueous solution to flow through, and can include constrictions and narrowing restricting. This geometry delays the flow of the aqueous solution through the tortuous channel.

[0070] In some implementations, a porous hydrophilic material is disposed in the chamber 402. In some implementations, the porous hydrophilic material is treated with a blocking agent. The FDCF biological components 108 can be absorbed in or on the hydrophilic material.

[0071] FIG. 5A and 5B illustrate another implementation of an aqueous solution-activated sensor 500. FIG. 5A is an exploded perspective view and FIG. 5B is a front cross-sectional view through one of the chambers. The cross-section is perpendicular to the direction of the parallel POFs 102, 104. Some aspects are similar to the implementation 400 (FIG. 4A, 4B). For example, sensor 500 features a chamber 402 formed in a flexible material by a bottom layer 404 of the flexible material, a middle layer 406a and 406b of the flexible material (a single middle layer 406 is used in the embodiment shown in FIG. 4A), and a top layer 408 of the flexible material. In this implementations, a first UV-Vis light transmitting medium is the emission POF 104, whereas the first UV-Vis light transmitting medium in FIG. 4A is a portion of the top layer 408. In the implementation, a second UV-Vis light transmitting medium is the excitation POF 102. A port 410 fluidly connects the exterior surface of the third layer 408 of the flexible material to an interior of the chamber 402, similar to the implementation shown in FIG. 4A.

[0072] In some implementations, a portion of an outer cladding of the emission POF 104 is removed or etched, as previously described with reference to FIG. 1C. This provides the first optical connection from the interior of the chamber 402. A portion of an outer cladding of the excitation POF 102 can also be removed or etched to provide the second optical connection to the interior of the chamber 402. One end of the emission POF 104 and excitation POF 102 can be connected to a spectrophotometer. For example, in some implementations, the excitation POF 102 is connected to a light source such as an LED light, and in some implementations the

emission POF 104 is connected to a detector, such as a CCD detector. The other end of the emission POF 104 and excitation POF 102 can be treated with a reflective compound to provide a reflective surface.

[0073] In some implementations, light from the reactions enter the POFs through ends that are cut (i.e., transmission through the end of the fiber). Different ways generated light can be absorbed into the POF includes: (a) through the side of the fiber where the cladding has been removed, (b) through the end of the fiber, and/or (c) through some light-focusing material (e.g., some kind of geometric waveguide that can absorb emitted photons and route them to the POF).

[0074] In some implementations, an opaque barrier 504 is inserted between the port 410 and both of the emission POF 104 and excitation POF 104. The opaque barrier is selected to reduce or eliminate light transmission from the port 410 to the emission POF and excitation POF. The optical barrier includes fluid connectivity to the chamber 402, for example shown as a gap 505 in FIG. 5B. Any form of fluid connectivity such as holes and perforations through the optical barrier 504 can be use provided light is eliminated or reduced. For example, in some implementations the light is reduced by at least 80%, at least 90%, at least 95%, or at least 99%, when the optical barrier 504 is used.

[0075] Still referring to the implementation depicted by FIG. 5A-5B, the emission POF 104 and the excitation POF 102 are combined with a porous hydrophilic material. For example, the POFs 102, 104 can be interwoven with the porous hydrophilic material providing a woven fabric 110 as previously described and shown in FIG. 1A-1C and FIG. 2. In some implementations, the emission POF 104 is interwoven with a first portion of porous hydrophilic material providing a first woven fabric (e.g., layer 204 in FIG. 2), and the excitation POF is interwoven with a second portion of the porous hydrophilic material providing a second woven fabric (e.g., layer 202 FIG. 2). FIG. 5B only shows a single hydrophobic material 110 for clarity, but multiple layers of hydrophobic material and POFs is also contemplated as a possible implementation.

[0076] In some implementations, at least a portion of the porous hydrophilic material 110 is embedded in the flexible material. For example, a portion of material 110 indicated as region 506, protrudes out of the layers 406a and 406b. The porous hydrophilic material 110 passes from the chamber 402, through region 405 of layer 406a, 406b, and out of the sensor 500 to region 506.

[0077] In some implementations, the sensor 500 includes a plurality of conical spikes 508 perpendicular to the exterior surface and proximate to the port. The conical spikes aid in collecting and attracting aqueous solutions close to the port 410.

[0078] Some implementations relate to methods for making an aqueous solution-activated sensor. The method includes providing a layer of a first material. For example, the layer of the first material can include the bottom layer 404, as depicted in FIG 4A and 4B, 5A and 5B. A layer of a second material is provided on the top surface of the first material. For example, the layer of the second material can include the middle layer 406, 406a or 406b. A layer of a third material is provided on a top surface of the second material. For example, the layer of the third material can include the top layer 408. The method further includes adding synthetic biological components into the chamber.

[0079] In some implementations, the synthetic biological components are freeze dried after being placed in the chamber 402. In some other implementations, the synthetic biological components are freeze-dried or otherwise dried prior to placement in the chamber 402. In some implementations, the FDCF biological components are absorbed on a porous hydrophilic material. The material can be inserted into the chamber 402 through the port 402, for example, where the top layer 408 is made of an elastomeric material.

[0080] In some implementations, the method includes addition of lysate, optionally absorbed on a porous hydrophilic material. Optionally, a time delay barrier, such as a dissolvable barrier or a barrier having a tortuous channel there through, is placed between the lysate and the biological components.

[0081] In some implementations, the method includes curing any one or more of the first material, the second material, and the third material prior to, during, or after providing the first material, second material, or third material as a layer. For example, any one of the materials can comprise a cross linking polymer that cross-links upon heat curing, exposure to oxygen or after adding an initiator or catalyst.

[0082] In some implementations, the method includes solidifying any one or more of the first material, the second material, and the third material from a molten state prior to, during, or after providing the first material, second material, or third material as a layer. For example, the material can be a thermoplastic which is heated, cast to form one or more layers 404, 405, 406a, 406b, or 408 and then cooled so that it solidifies. In some implementations, the thermoplastic is formed by additive manufacturing such as 3D printed to form the layers. In some implementations the thermoplastic is formed by a subtractive process, such as milling (e.g., CNC machining). In some implementations, one or more of the layers are formed by injection molding.

[0083] In some implementations, the method includes forming, by a polymerization reaction, any one or more of the first material, the second material, and the third material from monomeric

precursors, during, or after providing the first material, second material, or third material as a layer.

[0084] *Hydrophobic materials*

[0085] According to some implementations, any hydrophobic material can be used. For example, a low molecular polymer or oligomer such as a wax. In some implementations, the hydrophobic material is an elastomeric material such as one or more of EPDM, a silicone, a neoprene rubber, a natural rubber, a nitrile rubber, a butyl rubber, a thermoplastic elastomer, or any hydrophobic elastomer. In some implementations, the elastomeric material is a silicone.

[0086] *Porous hydrophilic materials*

[0087] Porous hydrophilic materials can include any material that can be wet by an aqueous solution and adsorbs between 10 wt.% and 1000 wt.% water. For example, materials having hydrophilic or hydrogen bonding groups such as hydroxyls, esters, carboxylates, ketones, amines, amides, sulfates and phosphates. The material can be a fiber that can be formed into a flat shape, including fibers that can be pressed together into a web structure or mesh structure. The material can also be a fiber that is formed into a yarn and then woven into a web structure or pressed together into a mesh structure. Without limitation, the porous hydrophilic material can include one or more of one or more of a cellulose, starch, maltodextrin, glycerin, sugar, sucralose, dextrose, gum arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polyester, polyamide, linen, nylon, or combinations thereof.

[0088] *Chamber for holding FDCF biological components*

[0089] Chambers or reaction chambers, wells or sachets are described herein and refer to a space, for example, where the FDCF biological components are disposed, placed or contained. In some implementations, the chamber volume is between about 0.1 μL and about 500 μL , between about 1 and 150 μL , or between about 1 and 100 μL .

[0090] The chambers include a port or small opening (e.g., FIG. 3C port 306, FIG. 4A-4B, FIG. 5A-5B port 410). In some implementations, the port is between 0.1 μm^2 and 50 mm^2 , such as between 1 μm^2 and 10 mm^2). The port is configured to allow fluid access into the chamber and in some implementations is not self-sealing. The fluid access should be fast, for example within at least five minutes. In some implementations within 1 minute. In some implementations within at least 30 seconds. In some implementations within 10 seconds, within 5 seconds, or within one second. In some implementations, the port has a cover, for example to seal off the chamber from liquids when the sensor device is not in use, is not usable or when the device may be intentionally exposed to a liquid that is not expected to contain a triggering compound. For example, the user may wish to deactivate the sensor by covering the port before the sensor is

immersed in water or when the user is in a wet environment such as in an area with precipitation. The cover can be any form such as a friction fit plug or adhesively attached.

[0091] The sample chambers are impermeable to outside aqueous solutions except through the port opening. The ports are designed for wicking in small volumes, such as from splashes of between with volumes as low as about 1 μL (e.g., between 10 and about 500 μL) at relative humidities between about 20-40%. The chamber and port are also configured to reduce the amount of evaporation once an aqueous solution has entered the chamber. In some implementations the evaporation rate is less than about 1% volume/hr (v/hr). In some implementations, the evaporation rate is less than about 5% v/hr. In some implementations, the evaporation rate is less than about 10% v/hr. In some implementations, the evaporation rate is less than about 15% v/hr. In some implementations, the evaporation rate is less than about 20% v/hr.

[0092] *Biological components for sensors*

[0093] According to the various aspects, FDCF Biological Components are used as circuits that are triggered by a triggering compound to provide a detectable signal. For example, in some implementations, the synthetic biological components provide the optical signal when activated with the triggering compound by synthesizing, activating, or suppressing, a colored, fluorescent or luminescent protein. In some implementations, the synthetic biological components include toehold sensor components, transcription-factor sensor components, aptameric sensor components, enzyme sensor components, antibody sensor components, CRISPR DNA sensor components, CRISPR RNA sensor components, ribonucleoprotein sensor components, and combinations thereof.

[0094] According to some implementations, the biological components can be supplied from a commercial source. For example, cell-free NEB PUREXPRESS® reaction components (New England Biolabs, Inc., Ipswich, MA). The reaction components, such as an A and a B component are combined and diluted with water to a specified concentration according to the manufactures specification for use. It has been found that using a higher concentration than the specified concentration range provides faster kinetics according to some implementations of this disclosure. However, at too high a concentration, the signal kinetics of the reaction are negatively impacted. In some implementations, the rehydrated synthetic biological components have a concentration between 1 and 2.4 times a specified concentration. The reaction kinetics are improved using the higher concentrations, as compared to the specified concentrations, by at least 5%, at least 10%, at least 20%, or at least 50%.

[0095] *CRISPR*

[0096] Microbial Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (CRISPR-Cas) adaptive immune systems contain programmable endonucleases, such as Cas12a Cpf1 (also referred to as Cpf1) and Cas9. Although both Cas12a and Cas9 and target DNA, single effector RNA-guided RNases also have been recently discovered (Shmakov et al., 2015) and characterized (Abudayyeh et al., 2016; Smargon et al., 2017). These programmable endonucleases and RNases provide a platform for specific nucleic acid (DNA or RNA) sensing. DNA-guided endonucleases, such as Cas 12a and Cas9 can be easily and conveniently reprogrammed using CRISPR guide RNA (gRNAs) to cleave target DNAs. RNA-guided RNases, such as C2c2, can be easily and conveniently reprogrammed using CRISPR RNA (crRNAs) to cleave target RNAs.

[0097] Once activated through recognition of the target DNA (e.g., double-stranded DNA) or RNA, many of the CRISPR-Cas endonucleases and RNases exhibit promiscuous non-specific DNase or RNase activity. Thus, after cleavage of the target DNA (e.g., dsDNA) or RNA, the CRISPR-Cas endonucleases and RNases can lead to “collateral” cleavage of any non-targeted DNAs or RNAs present in proximity.

[0098] In general, a CRISPR-Cas or CRISPR system as used in herein refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (“Cas”) genes, including sequences encoding a Cas gene, a *tracr* (trans-activating CRISPR) sequence (e.g. *tracr*RNA or an active partial *tracr*RNA), a *tracr*-mate sequence (encompassing a “direct repeat” and a *tracr*RNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a “spacer” in the context of an endogenous CRISPR system), or “RNA(s)” as that term is herein used (e.g., RNA(s) to guide Cas, such as Cas12a, e.g. CRISPR RNA and transactivating (*tracr*) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system).

[0099] The CRISPR-Cas effector protein can be from an organism from a genus comprising *Streptococcus*, *Campylobacter*, *Nitratifactor*, *Staphylococcus*, *Parvibaculum*, *Roseburia*, *Neisseria*, *Gluconacetobacter*, *Azospirillum*, *Sphaerochaeta*, *Lactobacillus*, *Eubacterium*, *Corynebacter*, *Carnobacterium*, *Rhodobacter*, *Listeria*, *Paludibacter*, *Clostridium*, *Lachnospiraceae*, *Clostridiaridium*, *Leptotrichia*, *Francisella*, *Legionella*, *Alicyclobacillus*, *Methanomethylophilus*, *Porphyromonas*, *Prevotella*, *Bacteroidetes*, *Helcococcus*, *Letospira*,

Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacillus, Methyl obacterium or Acidaminococcus.

[00100] In some implementations, the effector protein can comprise a chimeric effector protein comprising a first fragment from a first effector protein (e.g., a Cpfl) ortholog and a second fragment from a second effector (e.g., a Cpfl) protein ortholog, and wherein the first and second effector protein orthologs are different. At least one of the first and second effector protein (e.g., a Cpfl) orthologs can comprise an effector protein (e.g., a Cpfl) from an organism comprising Streptococcus, Campylobacter, Nitratifactor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacillus, Methyl obacterium or Acidaminococcus; e.g., a chimeric effector protein comprising a first fragment and a second fragment wherein each of the first and second fragments is selected from a Cpfl of an organism comprising Streptococcus, Campylobacter, Nitratifactor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacillus, Methyl obacterium or Acidaminococcus wherein the first and second fragments are not from the same bacteria; for instance a chimeric effector protein comprising a first fragment and a second fragment wherein each of the first and second fragments is selected from a Cpfl of *S. mutans*, *S. agalactiae*, *S. equisimilis*, *S. sanguinis*, *S. pneumoniae*; *C. jejuni*, *C. coli*; *N. salmuginis*, *N. tergaensis*; *S. auricularis*, *S. carnosus*; *N. meningitidis*, *N. gonorrhoeae*; *L. monocytogenes*, *L. ivanovii*; *C. botulinum*, *C. difficile*, *C. tetani*, *C. sordellii*; *Francisella tularensis* 1, *Prevotella albensis*, *Lachnospiraceae* bacterium MC2017 1, *Butyrivibrio proteoclasticus*, *Peregrinibacteria* bacterium GW2011_GWA2_33_10, *Parcubacteria* bacterium GW2011_GWC2_44_17, *Smithella* sp. SCADC, *Acidaminococcus* sp. BV3L6, *Lachnospiraceae* bacterium MA2020, *Candidatus Methanoplasma termitum*, *Eubacterium eligens*, *Moraxella bovoculi* 237, *Leptospira inadai*, *Lachnospiraceae* bacterium ND2006, *Porphyromonas crevioricanis* 3, *Prevotella disiens* and *Porphyromonas macacae*, wherein the first and second fragments are not from the same bacteria.

[00101] In the context of formation of a CRISPR complex, “target sequence” refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence can be DNA or RNA. Generally, the term target nucleic acid refers to a polynucleotide being or comprising the target sequence. In other words, the target nucleic acid can be a polynucleotide or a part of a polynucleotide to which a part of the gRNA, i.e. the guide sequence, is designed to have complementarity and to which the effector function mediated by the complex comprising CRISPR effector protein and a gRNA is to be directed.

[00102] It is noted that the effector protein can be a DNA targeting CRISPR-Cas protein or an RNA targeting CRISPR-Cas protein. Exemplary CRISPR-Cas proteins include, but are not limited to, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Cse1, Cse2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologues thereof, or modified versions thereof.

[00103] The terms “orthologue” (also referred to as “ortholog” herein) and “homologue” (also referred to as “homolog” herein) are well known in the art. By means of further guidance, a “homologue” of a protein as used herein is a protein of the same species which performs the same or a similar function as the protein it is a homologue of. Homologous proteins can but need not be structurally related, or are only partially structurally related. An “orthologue” of a protein as used herein is a protein of a different species which performs the same or a similar function as the protein it is an orthologue of. Orthologous proteins can but need not be structurally related, or are only partially structurally related. Homologs and orthologs can be identified by homology modelling (see, e.g., Greer, *Science* vol. 228 (1985) 1055, and Blundell et al. *Eur J Biochem* vol 172 (1988), 513) or “structural BLAST” (Dey F, Cliff Zhang Q, Petrey D, Honig B. Toward a “structural BLAST”: using structural relationships to infer function. *Protein Sci.* 2013 Apr;22(4):359-66. doi: 10.1002/pro.2225.). See also Shmakov et al. (2015) for application in the field of CRISPR-Cas loci. Homologous proteins can but need not be structurally related, or are only partially structurally related.

[00104] In some embodiments, the effector protein has a sequence homology or sequence identity of at least 60%, more particularly at least 70, such as at least 80%, more preferably at least 85%, even more preferably at least 90%, such as for instance at least 95%, with the wild-type sequence. The skilled person will understand that this includes truncated forms of effector protein whereby the sequence identity is determined over the length of the truncated form.

[00105] The CRISPR-Cas effector protein can be from an organism from a genus comprising Streptococcus, Campylobacter, Nitratifractor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacilus, Methylobacterium or Acidaminococcus.

[00106] In some implementations, the effector protein is a promiscuous non-specific DNase or RNase such as Cas 9, Cas12a, Cas13a, or Cas14. In some implementations, the effector protein is Cas12a, also known as Cpf1 or Cas 13a, also known as C2c2.

[00107] Selection of a promiscuous non-specific DNase or RNase activity Detections is by addition of a short nucleotide sequence that is coupled to a fluorescent reporter and a quencher. Cleavage of the nucleotide allows separation of the quencher from the fluorescent report providing the detectable signal.

[00108] *SHERLOCK*

[00109] SHERLOCK refers to “Specific High-sensitivity Enzymatic Reporter un-LOCKing.” SHERLOCK works by amplifying RNA (or DNA with a reverse transcriptase) using recombinase polymerase amplification (RPA) which is an isothermal nucleic acid amplification. SHERLOCK is useful for biosensors, such as wearable biosensors, because isothermal amplification does not require specialized instrumentation, such as PCR, as it uses a single temperature. The amplified nucleotides are combined with an effector protein (e.g., Cas 13a), a guide RNA that matches the nucleic acid sequence of interest, and a short nucleotide sequence that is coupled to a fluorescent reporter and a quencher. If the target sequence is present in the pool of amplified nucleotides, the non-specific RNase activity of effector protein becomes activated and the RNA reporter will be cleaved resulting in activation of the fluorophore. Therefore, the fluorescent signal is used as an indicator to determine whether the target sequence is present in the original pool of nucleotides. Hence the name “Specific high-sensitivity enzymatic reporter unlocking.”

[00110] *Light-up aptamers*

[00111] Light-up aptamers are RNA aptamers that bind with their cognate fluorogen ligands and activate their fluorescence. A non-hindered fluorogen can be excited and have its energy dissipated by non-radiative pathway such as molecular vibrations (heat). Once tightly bound by an aptamer, the fluorophore is stabilized and radiative fluorescence decay pathways predominate,

leading to a large fluorescence increase. The RNA aptamers can be selected or designed to target specific molecules (trigger compounds) such as small molecules and metabolites. Without the trigger compound, the aptamer region remain unfolded and cannot bind the fluorogen.

[00112] According to some implementations, aptamers MFA, BFR, DIT-Apt1, Spinach, Spinach2, Mango, Broccoli, and dimeric Broccoli can be used. Any cognate fluorogen ligand can be used as the trigger compound. For example, in some implementations, the fluorogen is DFHBI (3,5-difluoro-4-hydroxybenzylidene imidazolinone), Malachite green, Hoechst 1C, DIR, DMHBI, DMABI, 2-HBI, 2-HBI, DFHBI, T01-Biotin, T030Biotin, DFHBI-1T, DFHBI-2T, and PFP-DFHBI.

[00113] *Toehold Switch*

[00114] Toehold switch sensors are synthetic riboregulators that control the translation of a gene via RNA-RNA interactions. They utilize a designed hairpin structure to block gene translation in *cis* by sequestration of the ribosome binding site (RBS) and start codon. Translation is activated upon the binding of a *trans*-acting trigger RNA to the toehold region of the switch, which relieves the sequestration of the RBS and allows translation of the downstream gene. Toehold switch sensors can be designed to bind nearly any RNA sequence. The switch output is described below.

[00115] *Transcription-factor switch*

[00116] Transcriptional factor based biosensors consist of a repressor or activator protein regulating the transcriptional activity of a specific promoter. A *cis*-regulatory DNA sequence (generally called operator or enhancer) adjacent to the promoter is the core DNA element that binds with a TF restricting or enhancing the access of RNA polymerase (RNAP) to the promoter. A repressor binds to the operator and prevents RNAP proceeding forward to decrease transcription an activator binds to the enhancer elements and promotes the formation of more stable RNAP-promoter complex to increase transcription. Apart from the DNA-binding domain, TFs also contain a ligand-binding domain which is the sensor domain that responds to small molecules or environmental stress signal (salt, osmosis, pH, oxygen, redox, light or radiation etc.). Transcriptional activators can be any activators that are coupled to the specific repressor or activator protein used. In some implementations the transcriptional activators are VP 16, VP 64, FapR, FdeR, PcaQ, ArgP, MdcR, Yap1, Gal4, TetR, TrpR, FadR, PhlF, or LexA. The switch output is described below.

[00117] *Riboswitch*

[00118] Riboswitches are RNA-based sensors that utilize chemically induced structural changes in the 5'-untranslated region of mRNA to regulate expression of downstream genes.

Riboswitches are quickly synthesized in vitro, flexible in engineering (both aptamers and expression platforms), and can provide a fast response to recognize elements due to the avoidance of complicated protein–protein interactions, even before considering their high specificity and sensitivity. In some implementations, a typical riboswitch construct includes two domains linked to each other, a sensory domain and the regulatory domain. The aptamer binds to the target ligand and causes sufficient conformational changes or stability changes which then trigger the desired readout in the expression platform (switch output) through different mechanisms depending on the choice of expression control at the translation or transcription level. The switch output is described below.

[00119] *Switch output*

[00120] In some implementations, the switch output can be expression of any protein providing colorimetric, fluorescent or phosphorescent output. In some implementation the protein is selected from one or more of GFP, LacZ, Luciferase, TagBFP, mTagBFP2, Azurite, EBFP2, mKalama1, Sirius, Sapphire, or T-Sapphire; cyan proteins: ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midorishi-cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, far-red proteins; mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, near-IR proteins; TagRFP657, IFP1.4, iRFP, long stokes shift proteins; mKeima Red, LSS-mKate1, LSS-mKate2, and mBeRFP. In some implementations the protein is GFP, LacZ, luciferase or combinations of these.

[00121] Green Fluorescent Protein (GFP) is a single polypeptide gene product of 238 amino acids discovered in the jellyfish *Aequorea victoria*. The protein has a natural green fluorescence. GFP is quite stable and withstands a number of chemical treatments and procedures. GFP requires no biochemical transformation, contrast agent or the use of harmful ionizing radiation in order to be visualized. Enhanced GFP (EGFP) has been engineered to be expressed at higher levels in mammalian cells and to fluoresce more intensely. Cyan Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP) are spectral variants of GFP that allow multiple cell types to be labeled simultaneously.

[00122] The *lacZ* gene encodes beta-galactosidase, which catalyzes the cleavage of lactose to form galactose and glucose. Beta-galactosidase activity can be identified by when incubated with the beta-galactosidase substrate X-gal. Beta-galactosidase cleaves X-gal, a chromogenic substrate, resulting in an insoluble blue dye, thus allowing for the identification of *lacZ* activity.

[00123] Luciferase are a class of oxidative enzymes that produce bioluminescence. Luciferase enzymes isolated from different animal species have inherent variability in light emission. For example, Luciferase enzymes are commercially available from the organisms *Photinus pyralis*, *Luciola cruciate*, *Luciola italic*, *Luciola lateralis*, *Luciola mingrelia*, *Photuris pennsylvanica*, *Pyrophorus plagiophthalmus*, *Phrixothrix hirtus*, *Renilla reniformis*, *Gaussia princeps*, *Cypridina noctiluca*, *Cypridina hilgendorffii*, *Metridia longa*, and *Oplophorus gracilorostris*.

[00124] **Triggering compounds**

[00125] The triggering compounds (e.g., trigger, or trigger compound) can be any compound for which the synthetic biology switch is designed or selected. In some implementations the triggering compound can include natural or synthetic molecules including, but not limited, peptides, oligonucleotides polypeptides, proteins, peptidomimetics, antibodies, antibody fragments (e.g., antigen binding fragments of antibodies), carbohydrate-binding protein, e.g., a lectin, glycoproteins, glycoprotein-binding molecules, amino acids, carbohydrates (including mono-, di-, tri- and poly-saccharides), lipids, steroids, hormones, lipid-binding molecules, cofactors, nucleosides, nucleotides, nucleic acids (e.g., DNA or RNA, analogues and derivatives of nucleic acids, or aptamers), peptidoglycan, lipopolysaccharide, small molecules, and any combinations thereof.

[00126] As used herein, the term “small molecules” refers to natural or synthetic molecules including, but not limited to, amino acids, peptides, peptidomimetics, polynucleotides, aptamers, nucleotide analogs, organic or inorganic compounds (i.e., including heterorganic and organometallic compounds), saccharides (e.g., mono, di, tri and polysaccharides), steroids, hormones, pharmaceutically derived drugs (e.g., synthetic or naturally occurring), lipids, derivatives of these (e.g., esters and salts of these), fragments of these, and conjugates of these. In some implementations the small molecules have a molecular weight less than about 10,000 Da, organic or inorganic compounds having a molecular weight less than about 5,000 Da, organic or inorganic compounds having a molecular weight less than about 1,000 Da, organic or inorganic compounds having a molecular weight less than about 500 Da. In some implementations the small molecule has a molecular weight of less than about 1000 Da.

[00127] In some implementations, the triggering compound can include aptamers. As used herein, the term “aptamer” means a single-stranded, partially single-stranded, partially double-stranded or double-stranded nucleotide sequence capable of specifically recognizing a selected non-oligonucleotide molecule or group of molecules by a mechanism other than Watson-Crick base pairing or triplex formation. Aptamers can include, without limitation, defined sequence segments and sequences comprising nucleotides, ribonucleotides, deoxyribonucleotides,

nucleotide analogs, modified nucleotides and nucleotides comprising backbone modifications, branchpoints and nonnucleotide residues, groups or bridges. The oligonucleotides including aptamers can be of any length, e.g., from about 1 nucleotide to about 100 nucleotides, from about 5 nucleotides to about 50 nucleotides, or from about 10 nucleotides to about 25 nucleotides.

[00128] In some implementation the triggering compound is a component that is extracted or lysed from a microbe. As used interchangeably herein, the terms “microbes” and “pathogens” generally refer to microorganisms, including bacteria, fungi, protozoan, archaea, protists, e.g., algae, and a combination thereof. The term “microbes” also includes pathogenic microbes, e.g., bacteria causing diseases such as plague, tuberculosis and anthrax; protozoa causing diseases such as malaria, sleeping sickness and toxoplasmosis; fungi causing diseases such as ringworm, candidiasis or histoplasmosis; and bacteria causing diseases such as sepsis. The term “microbe” or “microbes” can also encompass non-pathogenic microbes, e.g., some microbes used in industrial applications. In some implementations, the term “microbe” or “microbes” also encompasses fragments of microbes, e.g., cell components of microbes, LPS, and/or endotoxin.

[00129] In some implementations the trigger molecule is a “molecular toxin,” which refers to a compound produced by an organism which causes or initiates the development of a noxious, poisonous or deleterious effect in a host presented with the toxin. Such deleterious conditions may include fever, nausea, diarrhea, weight loss, neurologic disorders, renal disorders, hemorrhage, and the like. Toxins include, but are not limited to, bacterial toxins, such as cholera toxin, heat-labile and heat-stable toxins of *E. coli*, toxins A and B of *Clostridium difficile*, aerolysins, and hemolysins; toxins produced by protozoa, such as *Giardia*; toxins produced by fungi. Molecular toxins can also include exotoxins, i.e., toxins secreted by an organism as an extracellular product, and enterotoxins, i.e., toxins present in the gut of an organism.

[00130] *Lysate*

[00131] According to some implementations a lysate, e.g., a prokaryotic or a eukaryotic cell lysate is used. The lysate can be combined with the FDCF biological components prior to contact with an aqueous solution, or the lysate can be first combined with the aqueous solution. In some implementations, the lysate includes one or more of Triton X-100, NP-40, Tween-20, Brij non-ionic surfactants, CHAPS hydrate, lysozyme, and disaccharides or polysaccharides such as sucrose, mannitolose, or trehalose. In some implementations, the lysate is freeze-dried. In some implemenations the lysate is dried by another method, such as by evaporating the solvent above the freezing temperature (e.g., under vacuum). In some implementations, the lysate does not include a cationic surfactant. In some implementations, the amount of ionic surfactant by weight

of total dry lysate is less than about 20%, less than about 10%, less than about 5%, or less than about 1%.

[00132] *Dissolvable membranes or dissolvable materials*

[00133] In some implementations a dissolvable membrane can be integrated into a sensor, for example, in order to allow control of sample flow. The membrane acts as a time-barrier film, by stopping the sample flow until it is dissolved. The control of sample flow in sensor critical areas, such as cell lysing regions, allows increased exposure time for the lysing reagents to act. This helps to ensure higher sensitivity, reactivity and in some cases reduces false-positive signals.

[00134] Dissolvable membranes contain a water-soluble polymer, sugars such as sucrose, inorganic salts, patterned hydrophobic materials, or other compounds to provide a fluidic delay. In some implementations the water-soluble polymer is a hydroxylpropyl-methylcellulose, polyvinylpyrrolidone, polyvinyl-alcohol (PVA), carboxymethyl-cellulose, polyethylene-oxide, hydroxylpropyl-cellulose, hydroxyethyl-cellulose, methyl-cellulose, pullulan, gelatin, pectin, sodium alginate, maltodextrin, polymerized rosin, and xanthan. In some implementations a plasticizer is added, for example, to improve mechanical properties such as brittleness. In some implementations the plasticizers is glycerol, propylene glycol, poly (ethylene glycol), glycerine, dimethyl phthalate, diacetyl phthalate, dibutyl phthalate, triacetin, castor oil, citrate ether, and triethyl citrate.

[00135] *Blocking agents*

[00136] As used herein a “blocking agent” or “molecular blockers” are compounds used to prevent non-specific interactions. The blocking agent can be a coating on a surface, e.g., of the substrate, that prevents non-specific interactions or fouling of the surface when it is contacted with the test sample. A blocking agent includes a compound that either covalently bonds with the material it is blocking or uses non-covalent interactions to block the material with a desired physiochemical characteristic. Blocking agents can be used to treat any surfaces and materials described herein. In some implementations, the interior or exterior surfaces of sensors are treated with blocking agents. In some implementation, the porous or non-porous hydrophilic materials are treated with blocking agents. In some implementations, hydrophobic materials such as elastomers are treated with blocking agents.

[00137] Non-specific interactions can include any interaction that is not desired between the target molecule (e.g., a triggering compound) and the surface (e.g., a porous hydrophilic material) or between other components in solution. The blocking agent can be a protein, mixture of proteins, fragments of proteins, peptides or other compounds that can passively absorb to the surface in need of blocking. For example, proteins (e.g., BSA and Casein), poloxamers (e.g.,

plurionics), PEG-based polymers and oligomers (e.g., diethylene glycol dimethyl ether), cationic surfactants (e.g., DOTAP, DOPE, DOTMA). Some other examples include commercially available blocking agent or components therein that are available from, for example, Rockland Inc. (Limeric, PA) such as : BBS Fish Gel Concentrate; PBS Fish Gel Concentrate; TBS Fish Gel Concentrate; Blocking Buffer for Fluorescent Western Blotting; BLOTTO; Bovine Serum Albumin (BSA); ELISA Microwell Blocking Buffer; Goat Serum; IPTG (isopropyl beta-D-thiogalactoside) Inducer; Normal Goat Serum (NGS); Normal Rabbit Serum; Normal Rat Serum; Normal Horse Serum; Normal Sheep Serum; Nitrophenyl phosphate buffer (NPP); and Revitablot™ Western Blot Stripping Buffer. In some implementations, the blocking agent is BSA.

[00138] In some embodiments, the blocking agent can be a monomer. In general, a monomer (with a single binding site) has no free binding site after binding to the target-binding agent. For example, saccharide-based monomeric blocking agent. In some embodiments the blocking agent can be a monosaccharide or modification thereof, including, e.g., but not limited to, diose, triose, tetrose, pentose, hexose, heptose, linear chain monosaccharides, open chain monosaccharides, cyclic isomers (e.g., furanose form and pyranose of monosaccharides such as hexose), pyranose, fructose, galactose, xylose, ribose, amino sugars (e.g., but not limited to, galactosamine, glucosamine, sialic acid, N-acetylglucosamine, N-acetyl-muramic acid, sulfosugars (e.g., but not limited to sulfoquinovose).

[00139] *Biological fluids*

[00140] In some implementations, the aqueous solution can include a biological fluid. Exemplary biological fluids can include, but are not limited to, blood (including whole blood, plasma, cord blood and serum), lactation products (e.g., milk), amniotic fluids, sputum, saliva, urine, semen, cerebrospinal fluid, bronchial aspirate, perspiration, mucus, liquefied stool sample, synovial fluid, lymphatic fluid, tears, tracheal aspirate, and any mixtures thereof. In some embodiments, a biological fluid can include a homogenate of a tissue specimen (e.g., biopsy). In one embodiment, an aqueous solution is a suspension obtained from homogenization of a solid sample obtained from a solid organ or a fragment thereof.

[00141] *Optical fibers*

[00142] In some implementations optical fibers are used to transmit excitation or emissions. Optical fibers are waveguide fibers designed for transmission of light. Optical fibers typically include a core surrounded by a transparent cladding material with a lower index of refraction. Light is kept in the core by total internal reflection. Optical fibers can include glass (silica, fluorozirconate, fluoroaluminate, and chalcogenide glasses) or plastic optical fibers

(POF). Glass optical fiber can be made having a high fidelity and low transmission loss, and are often regarded as the fiber of choice for many optical applications, such as communications and long range transmission. For low speed short data links, POFs can often be implemented. POFs also have the advantage of being more flexible than glass optical fibers. POFs are also more economical and the optical fiber of choice for many consumer products, such as digital home appliance networks, home networks and car networks. Being flexible, POFs are rugged and easy to install without fear of damage. POFs generally have a diameter about 8 times that of glass optical fibers.

[00143] In some implementations the optical fiber is a POF. For example, having a poly methyl methacrylate core, or polystyrene core, and having a fluorinated polymer or silicone resin cladding. In some implementations the POFs have a diameter between about 2000 μm and 200 μm , between about 1500 μm and 500 μm , or between about 1200 and about 800 μm .

[00144] In some implementations, one end of the POF is coated with a reflective coating. The coatings ensure light that would escape from the end that is not connected to an emission source or to the detector is not lost. Any reflective coating can be used that reflects at least about 10% of incident light (e.g., at least 20%, at least 50%, at least 80%). For example, reflective coatings can include a metal coating such as gold and silver.

[00145] *Wearable Items*

[00146] The sensors described herein can be configured as, although not limited to, a wearable item. Without limitation these can include a shirt; a jacket; pants; a skirt; a laboratory coat; a full-body garment; an exterior worn armor; a wrist, arm, head or ankle band; a scarf; gloves; socks; shoes or boots; a necklace; a ring; a hat; a helmet; a brooch; a face mask; a patch; or other wearable garments.

[00147] *Devices implementable as, for example, a face covering, mask or air monitor.*

[00148] According to one implementation, an aqueous solution activated detection device includes a carrier fluid reservoir, a sample collection unit, a sample processing unit including dried biological components, and a detection unit. In some implementations, the dried biological components are dried synthetic biological components. In some implementations, the carrier fluid reservoir is connected to the sample collection unit, the sample collection unit is connected to the sample processing unit, and the sample processing unit is connected to the detection unit. In some implementations, the detection device further includes a covering (e.g. a face covering). The carrier fluid reservoir, the sample collection unit, the sample processing unit, and the detection unit are attached to the covering. The sample collection unit is attached to an inner

(e.g., face contacting or facing) surface of the covering for detection of a subject's respiratory infection status. In some implementations, the covering is attached to an outside (e.g., external to the covering) for environmental detection of exposure. In some implementations, the covering is a face mask or a part of a face mask, e.g., as shown in FIG. 33B. In some implementations, the covering is implemented in a breathalyzer. The breathalyzer may have a sample collection chamber (e.g., a tube). For example, a breathalyzer including a tube or mouthpiece as shown as 3314 in FIG. 33A. In operation, the tube is used to engage a subject's mouth and the user exhales into the tube. The covering is positioned in the tube to capture breath from the subject. In some implementations, the tube opens into an expansion chamber, and the covering is located within the expansion chamber. In other implementations, the tube can be adapted for attachment to a nose. In yet other implementations, the detection device can be an air monitor. For example, the breathalyzer can be adapted as an air monitor by attaching a fan to draw ambient air into the tube of the device. In some implementations, the covering is implemented as a nasal cannula. In some implementations, the covering is implemented as part of a helmet.

[00149] According to some implementations, the carrier fluid reservoir is attached to the outer surface of the face mask. In some implementations, the detection unit is hidden during a sample collection when using the device, for example, to protect the subject's privacy. In some implementations, the carrier fluid reservoir includes a valve connecting and controlling the flow of an aqueous solution from the fluid reservoir to the sample collection unit. In some implementations, the valve is a pressure valve configured to open when the pressure difference across the pressure valve exceeds a predefined threshold. For example, the valve opens when the pressure difference is at least 0.5 psi. In some implementations, the valve opens when the pressure difference is at least 1 psi. In some implementations, the valve is configured to open under finger pressure. In some implementations, the valve is configured as a two-way valve. For example, the two-way valve can be a manually or otherwise actuated valve. For example, in some implementations, the two-way valve can be actuated by a solenoid.

[00150] According to some implementations, the fluid reservoir is configured as a blister pack. A blister pack is an impermeable enclosure or sachet containing the fluid. In some implementations, the valve is configured as a puncture zone forming a wall of the blister pack, the puncture zone opening under conditions of an applied pressure to the blister pack (e.g. at least 0.5 psi, or at least 1 psi, finger pressure, or mechanically actuated and optionally electrically controlled such as using a solenoid). In some implementations, the device further comprising a button, the button actuating a spike positioned opposite the puncture zone and puncturing the puncture zone when the button is depressed. (e.g. manually or otherwise actuated as previously

described). In some implementations, the spike is embedded in a compressible material for protecting the puncture zone prior to depressing the button and actuating the spike. In some implementations, a spike is activated by a sliding mechanism. The spike is positioned over the puncture zone and punctures the puncture zone when activated. In some implementations, the carrier fluid reservoir is a pierceable sachet that is configured to be mechanically activated by piercing, cutting, pressure-based rupturing, or a combination thereof.

[00151] According to some implementations, the device further includes a barrier between the fluid reservoir and the control of fluid from the reservoir to the sample collection unit is controlled by opening of the barrier including sliding (e.g. a wall of the barrier), unclamping (e.g., wherein the barrier is a clamp providing a pinch point), and pulling or pushing (e.g., a tube such as a paste tube to direct the fluid).

[00152] In some implementations, the carrier fluid reservoir includes a venting hole configured as a one-way vent. The one-way vent allows ambient air into the carrier fluid reservoir when a pressure differential between an exterior to the carrier fluid reservoir and interior of the pressure reservoir is greater than a threshold value. In some implementations, the interior pressure that allows opening of the one-way vent is at least 0.1 psi, at least 0.5 psi, or at least 1psi. This allows air into reservoir when pressure in the reservoir is at least lower than the atmosphere outside the reservoir. In some implementations, the pressure enters the reservoir due to the elasticity of the reservoir that will tend to restore the reservoir to its original shape if it is compressed. In some implementations, the venting hole material includes a hydrophobic coating that prevents bulk fluid from escaping the reservoir while allowing air to equilibrate between the interior of the reservoir and the external atmosphere.

[00153] According to some implementations, the sample collection zone includes a porous material allowing a carrier fluid to flow (e.g. by capillary action) in a direction from the carrier fluid reservoir to the sample processing unit. In some implementations, the porous material is a hydrophilic material comprises one or more of a cellulose, starch, maltodextrin, glycerine, sugar, sucralose, dextrose, gum Arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polypropylene, polycarbonate, polyester, polyamide, linen, nylon, polyurethane, glass, metal, or a blend thereof. In some implementations, the porous material is a high release media such as a polyurethane high release media (e.g. POREX® porous high release media). Table 1 and FIG. 20 include materials suitable for the porous material. In some implementation, the porous material includes fibers or threads and the majority of the fibers or threads are aligned in a direction pointing from the carrier fluid reservoir to the sample processing unit. For example, in some

implementation, at least 50% (e.g., at 60%, 70%, 80%, 90% by wt.% or alternatively by vol% fibers) of fibers are oriented as in a direction pointing from the carrier fluid reservoir to the sample processing unit, thereby providing a faster aqueous solution flow in this direction. In some implementations, the porous material includes fibers or threads and the majority of the fibers or threads are aligned in a direction orthogonal to the direction pointing from the carrier fluid reservoir to the sample processing unit. For example, in some implementations, at least 50% (e.g., at least 60%, 70%, 80%, 90% by wt.% or alternatively by vol%) of fibers are oriented in a direction orthogonal to the direction pointing from the carrier fluid reservoir to the sample processing unit, thereby providing a slower aqueous solution flow in this direction. The fibers of the porous material can facilitate flow of the carrier fluid in one direction of the porous material compared to orthogonal direction(s). Other implementations are contemplated where the majority of fibers are oriented at any one angle thereby modulating the flow of the solution through the device. In some implementations, the sample collection zone's porous material can be any porous high-release media substrate (e.g. a substrate with adsorption capacity per unit weight <1% of the desired pathogenic target). Porous high-release fiber media can include polyolefin fibers made of a combination 15-85% polypropylene and 15-85% polyethylene as well as fiber additives such as nonionic emulsifiers, antistatic chemicals and mixtures of them.

[00154] In some implementations, the porous material has electrostatic properties for driving separation of molecules in the carrier fluid during flow. In some implementations, the porous material electrostatically binds and sequesters molecules in the carrier fluid during flow. In some implementations, the porous material is covalently modified with biomolecules that alter or bind to molecules in the carrier fluid during flow. In some implementations, the porous material is fully or partially filled with dissolvable material.

[00155] According to some implementations, the sample processing unit of the device includes subunits. In some implementations, the sample processing unit includes a lysis subunit. In some implementations, the sample processing unit includes an amplification subunit. In some implementations, the sample processing unit includes a reporter activation subunit. In some implementations, the lysis subunit includes dry or freeze dried lysis components; the amplification subunit includes dry or freeze-dried nucleic acid, protein or lipid modifying enzyme components such as reverse transcriptase (RT), RNase H, RNA polymerase, oligonucleotide primers, an RT-RPA, RT-LAMP; the reporter activation subunit includes dry or freeze-dried cell-free lysate, cell-free transcription and translation reactions, Cas13 or Cas12 collateral cleavage enzyme, one or more guide RNA, and a corresponding dry or freeze-dried reporter molecule; and the detection unit includes a lateral flow assay (LFA) configured to the

detect the reporter molecule and a cleaved product of the reporter molecule. In some implementations, the lysis subunit includes dry or freeze dried lysis components. In some implementations, the lysis subunit includes freeze dried lysis components. In some implementations, the amplification subunit includes dry or freeze-dried reverse transcriptase (RT), RNase H, RNA polymerase, oligonucleotide primers, an RT-RPA, RT-LAMP. In some implementations, the amplification subunit includes freeze-dried reverse transcriptase (RT), RNase H, RNA polymerase, oligonucleotide primers, an RT-RPA, RT-LAMP. In some implementations, the amplification subunit includes dry or freeze-dried RNase H. In some implementations, the amplification subunit includes dry or freeze-dried RNA. In some implementations, the amplification subunit includes dry or freeze-dried polymerase. In some implementations, the amplification subunit includes dry or freeze-dried oligonucleotide primers. In some implementations, the amplification subunit includes dry or freeze-dried RT-RPA. In some implementations, the amplification subunit includes dry or freeze-dried RT-LAMP. In some implementations, the reporter activation subunit includes dry or freeze-dried cell-free lysate, cell-free transcription and translation reactions, Cas13 or Cas12 collateral cleavage enzyme, one or more guide RNA, and a corresponding dry or freeze-dried reporter molecule. In some implementations, the reporter activation subunit includes freeze-dried cell-free lysate, cell-free transcription and translation reactions, Cas13 or Cas12 collateral cleavage enzyme, one or more guide RNA, and a corresponding dry or freeze-dried reporter molecule. In some implementations, the reporter activation subunit includes dry or freeze-dried cell-free lysate. In some implementations, the reporter activation subunit includes dry or freeze-dried cell-free transcription and translation reactions. In some implementations, the reporter activation subunit includes dry or freeze-dried Cas13 collateral cleavage enzyme, one or more guide RNA, and a corresponding dry or freeze-dried reporter molecule. In some implementations, the reporter activation subunit includes dry or freeze-dried Cas12 collateral cleavage enzyme, one or more guide RNA, and a corresponding dry or freeze-dried reporter molecule. In some implementations the detection unit includes a lateral flow assay (LFA) configured to detect the reporter molecule and a cleaved product of the reporter molecule. In some implementations, the dry or freeze dried lysis components include less than 1% non-ionic surfactants (e.g., less than 0.1%). In some implementations, the LFA includes a sample region in contact with the reporter activation subunit, a control line configured to change color upon contact with the intact reporter molecule, one or more detection lines configured to change color upon contact with the cleaved products of the reporter molecules, and an adsorption zone or well at a position distal from the sample region. In some implementations, the reporter activation subunit includes

Cas12a and FAM-Biotin probe, and the reporter molecule is a FAM-biotin probe. In some implementations, the lysis subunit includes a lysis chamber, the amplification subunit includes an amplification chamber and the reporter activation subunit comprise a reporter activation chamber; first flow channel connecting the lysis chamber and amplification chamber, a second flow channel connecting the amplification chamber to the reporter activation chamber, and a third flow channel connecting the reporter activation chamber to the detection unit. In some implementations, one or more of the first flow channel, the second flow channel and the third flow channel includes a time delay barrier. In some implementations, the first flow channel includes a time delay barrier. In some implementations, the second flow channel includes a time delay barrier. In some implementations, the third flow channel includes a time delay barrier. In some implementations, the time delay barrier is a dissolvable membrane or dissolvable material. In some implementations, the dissolvable membrane includes polyvinyl alcohol (PVA), pullulan, sugars such as sucrose, inorganic salts, patterned hydrophobic solids, or other compounds to provide a fluidic delay based on solubility. In some implementations, the time delay barrier is PVA.

[00156] In some implementations, the dissolvable material is compatible with the biological functioning of reagents found in the lysis chamber, amplification chamber, reporter activation chamber, or detection unit. The dissolvable material can allow spatial segregation of Cas12, Cas13, or Cas14 enzymes from other reactions. In some implementations, the time delay barrier is integrated into the porous material of the device.

[00157] According to some implementations, the lysis subunit, the amplification subunit and the reporter activation subunit is configured as a layered micro-pad (μ PAD) or any other geometrically arranged porous material in which the fluid path is controlled to allow flow between different reaction units. In some implementations, the geometrically arranged porous material or μ PAD includes time delays (e.g., PVA barriers). In some implementations, the μ PAD is in a fold over accordion configuration having the chambers and flow channels defined by a hydrophobic material (e.g. wax). In some implementations, one or more of the chamber subunits and one or more of the flow channels includes a blocking agent disposed on an internal surface of the chambers/flow channels (e.g. BSA/Triton X-100). In some implementations, the one or more chamber and one or more flow channels includes a dissolvable material. In some implementations, the device mass is less than 10g (e.g., less than about 8, 5, or 3 g). For example, in some implementations, the device is light enough to wear as a mask or hold in one hand as a device such as a breathalyzer.

[00158] According to some implementations, the lysis subunit, the amplification subunit, and the reporter activation subunit are geometrically layered porous material in which the fluid path is controlled to allow flow between different reaction units. The layered porous material can be in a stack configuration as opposed to a fold over accordion configuration. Each stack of the lysis subunit

[00159] According to some implementations, the carrier fluid reservoir holds water (e.g. at least 90% water). In some implementations, the carrier fluid reservoir holds nucleases free water.

[00160] According to some implementations, the detection unit provides a visual output, a fluorescent output and/or an electrical output. In some implementations, the detection unit provides a visual output. For example, an LFA showing colored bands. In some implementations, the detection unit provide a fluorescent output. For example, integrated with POFs as described herein (emission and excitation POFs/detectors etc.). In some implementations, the detection unit provides an electrical output. For example, by electrochemical detection using direct electrochemical sensing or through an electrochemical mediator. In some implementations, the unit provides a combination of one or more outputs, such as a visual output an and electrical output; or a visual output and a fluorescent output; or an electrical output and a fluorescent output.

[00161] Although the carrier fluid reservoir, the sample collection unit, the sample processing unit, and the detection unit are individual modules described herein all being attached to or included in the same device, these individual modules do not have to be implemented in the same device. For example, the sample collection unit can be included in a breathalyzer for collecting the airborne sample, and the sample collection unit can be removed from the breathalyzer for further processing to characterize the airborne sample. The sample processing unit can be used for analyzing any sample in a lab and does not need to be incorporated in a same device as the other individual modules.

[00162] *Methods of using the Devices implementable as, for example, a face covering, mask or air monitor.*

[00163] Some implementations include methods of detecting an airborne agent. The methods use the devices implementable as, for example, a face covering, mask or air monitor described herein. The methods include: contacting air including the airborne agent to the sample collection unit of the device, wherein the sample unit collects the airborne agent on a surface of the sample unit; causing a carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the carrier fluid subsequently flowing to the sample processing unit and contacting the dried

synthetic biological components; and reading an output from the detection unit, the output indicative of the presence of the airborne agent in the air.

[00164] According to some implementations, the airborne agent is a pathogen, a virus or a toxin. In some implementations, the airborne agent is a virus. In some implementations, the virus is SARS-CoV-2. In some implementations, the airborne agent is nucleic acid (e.g., environmental DNA or RNA derived from lysed bacteria, viruses, or host cells). In some implementations, the airborne agent is an aerosol (e.g. a colloidal suspensions of the agent suspended in air). In some implementations, the air includes respiratory droplets or other exhalations from a subject. For example, the respiratory droplets or other exhalations comprises the airborne agent.

[00165] In some implementations, contacting the air to the sample collection unit lasts for at least 1 minute prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 5 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 10 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 20 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 30 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 60 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 120 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for less than 120 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for less than 60 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at less than 30 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at less than 20 min prior to causing the carrier fluid to flow to the sample collection unit. In some implementations, contacting the air to the sample collection unit lasts for at least 10 min prior to causing the carrier fluid to flow to the sample collection unit.

[00166] According to some implementations, a positive output for the agent is indicated within 12 hours (e.g. 9, 6, 3 hours) of contacting the air with the sample collection unit. In some implementations, a positive output for the agent is indicated within 9 hours of contacting the air with the sample collection unit. In some implementations, a positive output for the agent is indicated within 6 hours of contacting the air with the sample collection unit. In some implementations, a positive output for the agent is indicated within 3 hours of contacting the air with the sample collection unit. In some implementations, a positive output for the agent is indicated within 1 hour of contacting the air with the sample collection unit. In some implementations, a positive output for the agent is indicated within 30 min of contacting the air with the sample collection unit.

[00167] In some implementations, the carrier fluid includes water or buffer and after causing the carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the fluid flows through the sample collection unit, and the sample processing unit, and the water hydrates at least a portion of dry materials therein when the water reaches the sample collection unit, and the sample processing unit. In some implementations, after causing the carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the fluid flows sequentially through each of the sample collection unit, the sample processing unit, and the detection unit substantially by capillary action. In some implementations, flow through to or through one or more of the units is provided by a pump, such as a micro pump. In some implementations, flow through to or through one or more of the units is at least partially driven by gravity.

[00168] *Methods of making the Devices implementable as, for example, a face covering, mask or air monitor.*

[00169] Some implementations include methods of making an aqueous solution activated detection device. The methods include connecting in series a carrier fluid reservoir, a sample collection unit, a sample processing unit, and a detection unit. In such implementations, an outlet from the carrier fluid reservoir is connected to an inlet of the sample collection unit, an outlet from the sample collection unit is connected to an inlet of the sample processing unit, and an outlet of the sample processing unit is connected to an inlet of the detection unit. The sample processing unit includes dried synthetic biological components. Accordingly, in some implementations, the method includes adding wet biological components to the processing unit and in situ lyophilization (e.g. freeze-drying) of the biological components in the processing unit. In some implementations, the dry biological components are added in a dry state to the processing unit. Some implementations further include positioning a valve at the outlet from the

carrier fluid reservoir. Some implementations further include providing a pressure release vent to the carrier fluid reservoir by punching a hole in a wall of the carrier fluid reservoir and covering the hole with a film of breathable material. For example, the breathable material is a material allowing air to pass therethrough while not allowing larger molecules such as water to pass through. In some implementations, the breathable material is a rayon breathable hydrophobic porous film. Some implementations further include providing a one-way air valve that allows air into the carrier fluid reservoir to offset building up of vacuum. In some implementations, adhesive, gasket, or friction fitting is used to prevent leakage of the carrier fluid.

[00170] According to some implementations, the sample collection unit is made by cutting a porous sheet into a strip, the strip having a central collection region and two opposite distal regions, where a first distal region forms the inlet of the sample collection unit and a second distal region forms an outlet of the sample collection unit. In some implementations, the central collection region is broader (e.g. elliptical in shape) than the first and second distal regions. In some implementations, the porous sheet comprises fibers wherein a majority (e.g. $> 50\%$) of the fibers are aligned in a direction pointing from the first distal region to the second distal region. In some implementations, the porous material includes fibers or threads and the majority (e.g. 50%) of the fibers or threads are aligned in a direction orthogonal to the direction pointing from the first distal region to the second distal region. In some implementations, one side of the sheet includes a hydrophobic barrier. For example, the sheet is covered or coated with a hydrophobic, non-porous or non-absorbent material, or layer of a hydrophobic, non-porous or non-absorbent material.

[00171] According to some implementations, the sample processing unit is configured as a μ PAD and the method further includes: (a) printing a hydrophobic ink or material (e.g. wax) on a hydrophilic sheet (e.g. filter paper), the hydrophobic ink diffusing through the hydrophilic sheet and forming an array of bounding features; (b) defining, in a first column of the array of bounding features (i)-(vii); (i) a first row corresponding to a carrier fluid reservoir to a sample collection unit connection region, (ii) a second row corresponding to a lysis subunit, (iii) a third row corresponding to a fluid outlet from the lysis subunit, (iv) a fourth row corresponding to an amplification subunit, (v) a fifth row corresponding to a fluid outlet from the amplification subunit, (vi) a sixth row corresponding to a reporter activation subunit, (vii) a seventh row corresponding to a fluid outlet from the reporter activation subunit, (viii) an eighth row, and optionally ninth row, corresponding to a detection unit connection region; and (c) folding the first column such that adjoining surfaces of each adjacent row are contacted in an accordion like fashion, thereby providing a rectangular or square μ PAD. Some implementations further include

printing on the hydrophilic sheet with the hydrophobic ink one or more additional columns of the second row, third row, fourth row, fifth row, sixth row, seventh row, eighth row, and optionally ninth row; cutting or otherwise separating each additional column from adjacent columns; and folding each of the additional columns in the accordion like fashion, thereby providing additional μ PAD per additional column.

[00172] In some implementations, one or more of the fluid outlets includes a time delay barrier. For example, a dissolvable polymer can be coated at or on the fluid outlet to form a dissolvable plug. In some implementations, each of the fluid outlets have a volume that is smaller than a volume of each of the subunits.

[00173] In some implementations the method further includes depositing lysate components in the lysis subunit, depositing amplification components in the amplification subunit, and depositing reporter activation components in the reporter activation subunit. In some implementations the method further includes depositing lysate components in the lysis subunit. In some implementations the method further includes depositing amplification components in the amplification subunit. In some implementations the method further includes depositing reporter activation components in the reporter activation subunit. For example, in some implementations, depositing comprises applying the components in an at least partially dissolved state to the units, and removing the water by, for example, drying or freeze drying.

[00174] In some implementations, the method further includes further attaching the carrier fluid reservoir, the sample collection unit, the sample processing unit, and the detection unit to a face mask, wherein the sample collection unit is attached to an inner surface of the face mask. In some implementations, the carrier fluid reservoir is positioned on an outside surface of the face mask, and the outlet from the carrier fluid reservoir, inlet of the sample collection unit, or an area proximate to this inlet and outlet is passed from the outer surface of the face mask, to the inner surface of the face mask through a hole. In some implementations, the carrier fluid reservoir is attached to the inside surface of the face mask. In some implementations, the carrier fluid reservoir is integrated into an interior of the face mask.

[00175] In some implementations of the method, connecting in series includes applying a water proof adhesive to matching surfaces of the outlet of the fluid reservoir and inlet of the collection unit, applying a water proof adhesive to matching surfaces of the outlet from the sample collection unit and inlet of the sample processing unit, and applying a water proof adhesive to matching surfaces of the outlet of the sample processing unit and inlet of the

detection unit, wherein the water proof adhesive bridges any gap between the corresponding surfaces.

[00176] *Diagnostic systems.*

[00177] According to another implementation, a diagnostic system includes a biochemical reaction composed of a combination of reverse transcriptase, nucleic acid-modifying enzymes, amplification enzymes, cell-free lysate, Cas13, Cas12, or Cas14 enzymes, and one or more nucleic acids which may contain complete or partial nucleic acid sequences, including the reverse complement and in DNA or RNA, as disclosed herein (e.g. Table 7). In some implementations, other substrates including ATP, reaction enhancers such as BSA or polyethylene glycol, and freeze drying enhancers such as phosphates and glycine are combined with one or more of the reverse transcriptase, nucleic acid-modifying enzymes, amplification enzymes, cell-free lysate, Cas13, Cas12, or Cas14 enzymes, and one or more nucleic acids. In some implementations, other substrates include ATP. In some implementations, other substrates include reaction enhancers such as BSA. In some implementations, other substrates include freeze drying enhancers such as phosphates, disaccharides such as trehalose and mannose, and glycine.

[00178] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary.

[00179] In one aspect, the present invention relates to the herein described compositions, methods, and respective component(s) thereof, as essential to the invention, yet open to the inclusion of unspecified elements, essential or not ("comprising"). In some embodiments, other elements to be included in the description of the composition, method or respective component thereof are limited to those that do not materially affect the basic and novel characteristic(s) of the invention ("consisting essentially of"). This applies equally to steps within a described method as well as compositions and components therein. In other embodiments, the inventions, compositions, methods, and respective components thereof, described herein are intended to be exclusive of any element not deemed an essential element to the component, composition or method ("consisting of").

[00180] As used herein, the term "small molecules" refers to natural or synthetic molecules including, but not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, aptamers, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds

having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

EXAMPLES

[00181] The following examples illustrate some embodiments and aspects of the invention. It will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be performed without altering the spirit or scope of the invention, and such modifications and variations are encompassed within the scope of the invention as defined in the claims which follow. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The following examples do not in any way limit the invention.

[00182] *Wearable Freeze Dried Cell Free Sensors*

[00183] Colorimetric genetic circuits were embedded into cellulose substrates surrounded by a fluid wicking and containment assembly made of flexible elastomers (FIG. 6A). These prototypes were assembled layer-by-layer to form reaction chambers fluidly connected to top sample portals (FIGs. 6B, 7A). The devices are flexible, elastic, and can rapidly wick in splashed fluids through capillary action (FIGs. 6C, 6D). Pinning geometries throughout the device direct sample fluids towards enclosed hydrophilic paper networks allowing for reaction rehydration (FIGs. 6B and 8B). Using a *lacZ* β -galactosidase operon as the circuit output to hydrolyze chlorophenol red- β -D-galactopyranoside (CPRG), a yellow to purple color change develops upon exposure to a target (FIGs. 7B, 8A).

[00184] Key environmental factors were considered for the design of these prototypes. For instance, sample exposure in the field likely occurs with variable splash volumes (as little as 50-100 μ L), relative humidity (RH, 20-40%), and temperature (20-37°C). Thus, the design was optimized to reduce inhibition of genetic circuit operation due to evaporation or excessive dilution of components. In particular, the devices use impermeable chambers exhibiting low evaporation rates (<20% volume/hour), which also constrains the rehydration volume to ~50 μ L per sensor. In addition, the wFDCF reactions were optimized to generate a higher concentrated reaction upon rehydration. It was found that a 1.5x-concentrated cell-free reaction increased the reaction kinetics to enable signal output at least 10 min faster, ensuring that the desired circuit is

completed before eventual evaporation in the device terminates the reaction (FIG. 10A-10D). The resulting stand-alone colorimetric system is modular and can be used in garments such as bracelets (FIG. 8C).

[00185] Functional testing of this colorimetric wearable platform was performed utilizing four different synthetic biology biosensors with *lacZ* as the output (FIGs. 6E-6H). These various demonstrations include a constitutive *lacZ* expression reaction (FIG. 6E), a transcription factor-regulated circuit using the tetracycline repressor (TetR) (FIG. 6F), a toehold switch for Ebola virus RNA detection (FIG. 6G), and a theophylline riboswitch for small-molecule sensing (FIG. 6H). Genetic circuits using transcriptional regulators are among some of the most common elements used in synthetic biology. The wFDCF TetR sensor demonstrates the capacity of the colorimetric platform for facile integration of well-established genetic modules into a wearable format (FIG. 6F). Similarly, toehold switches have been developed as highly programmable nucleic acid sensors capable of detecting any target RNA. It was shown that a wFDCF Ebola virus RNA toehold sensor in the wearable device is capable of rapid and sensitive detection of biothreats (FIG. 6G). Similar viral or bacterial wearable nucleic acid sensors can be made. Furthermore, a functional theophylline riboswitch wFDCF circuit is functionally validated in these platforms for the environmental detection of small molecules via engineered cis-regulated RNA circuits (FIG. 6H). This specific riboswitch was selected as a model test case, although a plethora of similar riboswitches for various targets have been reported and can be used in a modular fashion. All of the colorimetric wFDCF sensors reported here exhibited visible changes within ~40-60 min after exposure to the respective trigger molecules or inducer, and were performed at ambient conditions of 30-40% RH and 30°C to simulate the average skin surface temperature.

[00186] Expanding on the attractiveness and versatility of textiles as ubiquitous wearable substrates, FDCF synthetic biology systems within wearable woven fabrics and individual threads were made. FIGs. 9A-9G presents various demonstrations of a highly sensitive, textile-based system (FIGs. 9A, 9B) capable of containing and monitoring the activation of wFDCF reactions with fluorescent (FIGs. 9C-9E, 11-14) or luminescent (FIGs. 9F, 15A-15B) outputs. To achieve this, a second wearable platform was made that integrates: (a) hydrophilic threads (85% polyester / 15% polyamide) for cell-free reagent immobilization, (b) patterns of skin-safe hydrophobic silicone elastomers for reaction containment, and (c) inter-weaved polymeric optic fibers (POFs) for signal interrogation (FIGs. 9A, 9B, 16A-16D, 17A-17G). This fabric was chosen as the main immobilization substrate after conducting a compatibility screening of over 100 textiles (e.g., silks, cotton, rayon, linen, hemp bamboo, wool, polyester, polyamide, nylon,

and combination materials) using a lyophilized constitutive *lacZ* cell-free reaction FIGs. 18A, 18B, 19A, 19B, 20). The analysis of sensor outputs was done using a custom-built wearable POF spectrometer (FIGs. 9B, 21A-21F) that could be monitored with a mobile phone application (FIGs. 22A-22C). Using this integrated platform, distributed on-body sensing of various target exposures was performed, as shown in FIGs. 9C-9F. A sample activation through fluid splashing can be seen in FIG. 9A, where the sample wicks through the entry ports with blackout fabrics to rehydrate the freeze-dried, cell-free synthetic biology reactions immobilized within the hydrophilic textile fibers. These fibers are located within the excitation and emission layers of the device as shown in FIG. 9A, 9B. Trigger presence in the splash fluid leads to activation of the sensor circuits, which produce fluorescent or luminescent reporters.

[00187] The versatility of this textile platform in fluorescence mode was first verified using two independent synthetic biology modules upstream of a superfolder green fluorescent protein (sfGFP) operon. These demonstrations included the activation of constitutive sfGFP expression (FIG. 9C) and sensing of theophylline using an inducible riboswitch (FIG. 9D). A third fluorescence demonstration was done via activation of a 49-nucleotide Broccoli aptamer (FIG. 9E) with substrate-specificity to *(Z)*-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1H-imidazol-5(4H)-one (DFHBI-1T), evincing functionality of this emerging class of fluorescent sensors in synthetic biology. Furthermore, demonstrations utilizing luminescence outputs were conducted using a nanoLuciferase operon downstream of an HIV RNA toehold switch (FIG. 9F, 15A), as well as a *B. burgdorferi* RNA toehold switch for the wearable detection of Lyme disease (FIG. 15B).

[00188] Additionally, the operation of this platform was tested for the detection of chemical threats such as organophosphate nerve agents used in chemical warfare and the pesticide industry, both of which constitute prime targets for wearable detection. To achieve this, the POF platform optics for excitation and detection at near-infrared (NIR) fluorescence, generated from a lyophilized acetylcholinesterase (AChE)-choline oxidase (ChOx)-HRP coupled enzyme reaction (FIG. 9G), were modified. In the presence of acetylcholine, this reaction can produce NIR fluorescence that is readily detectable with the wearable prototype. When exposed to an organophosphate AChE inhibitor, the sensor fluorescence is ameliorated as compared to unexposed controls. The wearable nerve agent sensor was validated using paraoxon-ethyl as a nerve agent simulant at levels that are four orders of magnitude lower than the reported lethal dose (LD₅₀) by dermal absorption in mammals (FIG. 9G).

[00189] Despite the single-activation nature of the wFDCF synthetic biology sensors, the presence of fluorescence outputs is continuously monitored to allow for automatic detection of rehydration events containing the desired target. This is achieved by illuminating the wFDCF textile reaction with blue light (447 nm) via etched excitation POFs (FIGs. 9B, 17A). The light emitted from the activated system is then collected by the second set of emission POFs, which exit the fabric weave and bundle into a connection to the optical sensor (FIG. 9B) of the wearable spectrometer (FIGs. 17A-17G, 21A-21F). Signals coming from each of the devices are filtered (FIGs. 17D) and processed to generate temporally and spatially resolved fluorescence images of the POF bundle-ends (510 nm) and averaged pixel intensity traces per channel for quantitative analysis (FIG. 9B). In the case of luminescence demonstrations, all POFs bundles are treated as signal inputs, without the need for sample illumination. All reported wFDCF fluorescence and luminescence sensor replicates ($n \geq 3$) exhibited visible fluorescence or luminescence within 5-20 min after exposure to relevant trigger conditions, at 30-40% RH and 30°C.

[00190] Recent advances in programmable clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) enzymes have enabled the development of new classes of rapid and reliable sensing platforms. The advantages of CRISPR-based systems over existing biosensors include high sensitivity, rapid output, single base-pair resolution, freeze-drying compatibility, and the notable programmability to target any DNA or RNA sequence through interchangeable guide RNAs (gRNAs). Thus, CRISPR-based sensors were integrated into the fluorescence wFDCF platform to demonstrate this detection technique in wearable applications (FIG. 23A). Cas13a and Cas12a were used for the detection of RNA and DNA, respectively. For DNA detection, Cas12a ortholog from *Lachnospiraceae* bacterium (LbaCas12a) was used that displays a non-specific collateral cleavage activity towards single-stranded DNA (ssDNA) after detection of a gRNA-defined double-stranded DNA (dsDNA) target. This Cas12a-based sensor was paired with recombinase polymerase amplification (RPA) and freeze-dried into a one-pot reaction to demonstrate state-of-the-art detection limits for wearable clinical applications. In the presence of a target dsDNA sequence, isothermally generated RPA amplicons activate Cas12a-gRNA complexes. Then, active Cas12a engages in trans-ssDNase activity and cleaves quenched ssDNA fluorophore probes, resulting in a fluorescence output (FIG. 23A). For the wearable CRISPR-based demonstrations, gRNAs were designed against three common resistance markers in *Staphylococcus aureus*: specifically, the *mecA* gene common in methicillin-resistant *S. aureus* (MRSA), the *spa* gene which encodes the protein A virulence factor, and the *ermA* gene conferring macrolide resistance. When tested in

wFDCF format, the RPA-Cas12a sensors displayed detectable signals within 56-78 min ($P < 0.05$) with femtomolar limits of detection (FIGs. 23B-23D). Moreover, using the *mecA* wFDCF sensor (FIG. 23E, 24), it was possible to confirm single-digit femtomolar sensitivity (2.7 fM). Compatibility with RNA inputs and other CRISPR enzymes such as Cas13a, an ortholog from *Leptotrichia wadei* bacterium (LwaCas13a) was also confirmed (FIG. 24), exhibiting similar in-device activation dynamics as that of cell-free reactions conducted in a plate reader. These results suggest that the wearable textile platform could be adapted to achieve sensitivities rivaling that of current laboratory diagnostic tests such as qPCR for monitoring contamination or spread of bacteria and viruses.

[00191] To further demonstrate the modularity of the CRISPR-Cas12a wearable sensors, wFDCF devices containing three orthogonal Cas12a-gRNA complexes in isolated reaction chambers were tested (FIG. 23F). In this experiment, each device was splashed with dd-H₂O containing different targets, each specific to only one Cas12a-gRNA complex. The orthogonal behavior of the CRISPR-based wearable sensors is shown in FIGs. 23G-23H, where higher fluorescence was observed for the cases in which the dsDNA trigger matched the pre-defined Cas12a-gRNA complex at each sensor location. These results suggest the broad applicability of CRISPR-based synthetic biology sensors for multiplexing or logic-gating in wearable synthetic biology applications.

[00192] The wFDCF reactions and networked optical fiber detection system can be integrated into flexible textiles to create an autonomous wearable platform enabling real-time monitoring of environmental exposure and biohazard detection. A jacket was designed that contained a distributed arrangement of wFDCF multi-sensor arrays (FIG. 23I). The various optical fibers carrying the output emission signals can be routed into a single bundle for centralized imaging analysis or interrogated as separate modules, which was demonstrate using a wFDCF CRISPR-Cas12a based MRSA-sensing array containing *spa*, *ermA* and *mecA* sensors that was activated in the wearable prototype with a fluid splash containing 100 fM of *spa* DNA trigger (FIG. 12). Only the well containing the *spa* sensor generated a fluorescent signal upon activation. The platform is also compatible with transcription-only outputs, such as rehydrated fluorescent aptamer reactions (FIG. 13), where the fluorescence signal is monitored by microscopy over time.

[00193] In addition, the optical sensor allows for facile fluorescent output multiplexing simply by using fluorescent proteins with orthogonal emission profiles (FIG. 14). In this example, wFDCF reactions for three constitutively expressed fluorescent output proteins (eForRed,

dTomato, and sfGFP) were used to demonstrate detection of distinguishable output signals in a single bundle. It is possible that additional fluorescent outputs, including orthogonal quenched fluorophore probes for SHERLOCK-based sensors, can be employed to increase the signal multiplexing of this wearable platform. It is also shown that the wFDCF POF system is fully compatible with integrated lyophilized lysis components, allowing for the release and detection of a plasmid-borne *mecA* gene when challenged with intact bacterial cells (FIGs. 26A-26D). Finally, to develop a complete data feedback cycle between the platform and the user, the detector system was integrated with a custom wireless mobile application that enables continuous cloud-based data logging, signal processing, geolocation tracking, and on-the-fly control of various detector components through a smart phone or other networked digital device (FIG. 23J). All images and spectral data presented in FIGs. 9A-9G, 23A-23J were collected and processed using wFDCF devices fully integrated with the wearable spectrometer and mobile phone application. Further details on the hardware (FIGs. 21A-21F) and software design (FIGs. 22A-22C), as well as an implementation of a novel *Opuntia microdasys* bioinspired fluid collection add-on for improved sample harvesting and routing splashes outside of the sensor zones into the wFDCF modules (FIGs. 27A-27D).

[00194] The wearable synthetic biology sensors demonstrated here thus imbue programmable and highly sensitive diagnostic sensing to protective apparel. With the current SARS-CoV-2 pandemic that has led to significant strain on the medical system of all impacted countries and considerable delays in diagnostic testing, the wFDCF system are adapted to key wearable gear, face masks, that have been shown to be critical in reducing the transmission of this highly infectious virus. Although face masks are placed on all incoming patients that are presumptive SARS-CoV-2 carriers, confirmation through burdened laboratory diagnostics may result in delays that could negatively impact rapid triaging or effective contact tracing of patients. Patients suspected of an infectious respiratory disease are fitted with a face mask upon clinical admission as a preventative measure to reduce transmission. Diagnosis is commonly undertaken by nasopharyngeal sampling, which may cause reflexive sneezing and increase exposure risk to clinical workers. Respiratory droplets and aerosols are the transmission routes for respiratory infectious diseases, but their use as a non-invasive diagnostic sample has been underutilized historically. Work on breath-based sensing has focused on the detection of volatile organic compound biomarkers in infected patients using electrochemical sensors or downstream mass spectrometry analysis, which may be challenging to implement on a wide scale. The NIH Rapid Acceleration of Diagnostics (RADx) Initiative has identified SARS-CoV-2 detection from breath sampling technologies as an active area of interest for alleviating testing bottlenecks. Here, is

demonstrated that these freeze-dried synthetic biology sensors can be adapted for a rapid point-of-care SARS-CoV-2 sensor fully integrated into any standard face mask, which takes advantage of the accumulation of virus on the inside of the mask as a result of coughing, talking or normal respiration, as demonstrated in numerous studies. Unlike other current nucleic acid tests (NATs) that require laboratory equipment and trained technicians, the SARS-CoV-2 face-mask NAT sensor describe here requires no power source, operates autonomously without liquid handling, is shelf-stable, functions at near-ambient temperatures, provides a visual output in under 2 hours, and is only ~3 g in weight. All the user has to do is press a button to activate a reservoir containing nuclease-free water.

[00195] The SARS-CoV-2 sensor contains four modular components: a reservoir for hydration, a large surface area collection sample pad, a wax-patterned μ PAD (microfluidic paper-based analytical device), and a lateral flow assay (LFA) strip (FIGs. 28A and 28B). Each module can be oriented on the outside or inside of the face mask, with the exception of the collection pad, which must be positioned on the mask interior facing the mouth and nose of the patient. Capillary action wicks any collected fluid and viral particles from the sample collection pad to the μ PAD, which contains an arrangement of freeze-dried lysis and detection components (FIG. 28C). The use of the μ PAD format allowed us to rapidly prototype and optimize a passively regulated multi-step reaction process. Each reaction zone is separated by polyvinyl alcohol (PVA) time delays that enable tunable incubation times between each reaction, greatly improving the efficiency of the sensor compared to that of a one-pot lyophilized reaction. The first μ PAD reaction zone contains lyophilized lysis reagents including components known to lyse viral membranes. The second μ PAD reaction zone is an RT-RPA reaction zone containing a customized isothermal amplification reaction developed to target a non-overlapping region of the SARS-CoV-2 S gene. The final μ PAD reaction zone contains a Cas12a SHERLOCK sensor with an optimized gRNA for detection of the amplified dsDNA amplicon. In the presence of SARS-CoV-2 derived amplicons, the activated Cas12a enables trans-cleavage of a co-lyophilized 6-FAM-(TTATTATT)-Biotin ssDNA probe. To enable a simple colorimetric visual readout, an integrated LFA strip is used to detect probe cleavage. The output strip orientation is adjustable to preserve patient confidentiality.

[00196] The first, second, and final μ PAD reaction zones are temporally and spatially separated. The μ PAD is merely used as an example but other material can be used. Although three reaction zones are disclosed, any number of reaction zones may be achieved (e.g., two reaction zones, four reaction zones, ten reaction zones, etc.). The spatial separation is achieved with separate reaction zones on separate pieces of material. The temporal separation is achieved

with dissolving membranes. The dissolving membranes allow automated sequence of reactions in order. For example, a first reaction in the first reaction zone ends before a second reaction in the second reaction zone starts. The dissolving membrane separating the first reaction zone and the second reaction zone times the start of the second reaction. This methodology of separating reactions into reaction zones allows attaining higher sensitivity compared to a one-pot reaction where the first, second, and third reactions are mixed together. Ordered reaction as described herein preserves efficiency of each reaction when compared to the one-pot reaction. The spatial separation improves efficiency in labs so that a technician is not required to monitor and move materials between reactions. This methodology is compatible with CRISPR. PVA is used as an example of the dissolving material, but other materials can be used or substituted in cases where PVA may not be inert or may affect the sequenced reactions.

[00197] From activation of the face-mask sensor to a final readout only takes ~1.5 hours. The limit of detection observed for the sensors is 500 copies (17 aM) of SARS-CoV-2 in vitro transcribed (IVT) RNA, which matches that of WHO-endorsed standard laboratory-based RT-PCR assay (FIG. 28D-28E). The sensors also do not cross react to RNA from other commonly circulating human coronavirus strains (HCoVs) (FIG. 28F). Most critically, the hands-off diagnostic reaction proceeds to full completion even at room temperature, which is considered sub-optimal for RT, RPA, and Cas12a activities. The SARS-CoV-2 face-mask sensor was also validated using a precision lung simulator attached to a high-fidelity human airway model (FIGs. 28H, FIG 32E). The target RNA was nebulized to replicate lung emissions with aerosol diameters matching those naturally occurring in breath exhalation plumes. The breath temperature was regulated to 35°C and the relative humidity in the mask microclimate was measured to be 100% RH. Under these realistic simulation conditions, the face-mask sensor was able to detect SARS-CoV-2 vRNA after a breath sample collection period of 30 minutes, with a calculated accumulation of 10⁶-10⁷ vRNA copies on the sample pad, as determined by RT-qPCR (FIG. 28A-28J). Clinical measurements have previously shown that the SARS-CoV-2 breath emission rate of infected patients could reach an output 10³-10⁵ copies/min. This is the first SARS-CoV-2 NAT that is able to achieve high sensitivity and specificity while operating fully at ambient temperature ranges, thus obviating the need for any heating instruments and allowing for integration into a wearable format. The rapid face-mask-integrated SARS-CoV-2 diagnostic presented here can relieve strained medical systems by combining protection and sensing into a simple and easy-to-deploy wearable system, greatly improving patient outcomes. This face-mask system can be adapted to discriminate between SARS-CoV-2 and other

respiratory viruses, as well as different emerging SARS-CoV-2 variants, allowing rapid triaging of patient populations and isolation of specific positive cases to minimize the spread of infection.

[00198] The wFDCF platform are complementary to cell-based synthetic biology sensors. Such living sensors are capable of self-replication, can operate continuously to provide dynamic sensing, and they can actively draw upon environmental resources for energy. However, storage and biocontainment concerns limit their use for wearable technologies. Herein is demonstrated that cell-free synthetic biology systems can be used to build practical wearable biosensors that are shelf-stable, genetically programmable, and highly sensitive.

[00199] The wFDCF sensors are responsive to external rehydration events, such as splashes with contaminated fluids, and withstand inhibitory evaporative and dilutive effects in open-environment conditions (30-40% RH and ~25-30°C). These freeze-dried systems generate measurable colorimetric, fluorescence, or luminescence outputs upon exposure to relevant real-world targets. In the wFDCF POF sensors, continuous monitoring enables rapid alert to an exposure event. The integration of these device designs into garments that are compatible with wireless sensor networks to provide real-time dynamic monitoring of exposure using custom smartphone applications is also demonstrated. Although laboratory testing may be more sensitive, the wFDCF sensors have the distinct advantages of a wearable format, autonomous functioning, and rapid results.

[00200] The presented platform is the first wearable technology demonstrated to detect nucleic acids from potential viral or bacterial pathogens in contaminant fluid samples with sensitivities rivaling those of traditional laboratory tests at ambient temperatures. The wFDCF platform evinces a number of distinct advantages over existing POC diagnostics, which similarly attempt to eliminate the need for time-consuming laboratory tests. Current field-portable POC systems typically use a swabbed or directly applied sample to provide a readout. In contrast to a batch-mode POC sensor, the wFDCF synthetic biology sensors can be networked to provide sensing arrays of lyophilized reactions and lightweight polymer fabrics, thus cloaking the user and continuously generating high-density, real-time outputs without sacrificing comfort or agility in the field. The platform is also designed to operate autonomously, unlike most current POC instruments that require training for use and multiple operations by the user to acquire the final results. This feature removes the need to perform regular exposure checks, freeing those in the field to focus on their core tasks. In comparison to current wearable sensors that primarily employ electronic devices to monitor physiological signals such as heart rate or blood oxygen levels, these modular wearable sensors can detect environmental threats or patient samples

through nucleic acid, protein, or small molecule detection. Although recently electrochemical sensors have been integrated into a wearable format, they only detect chemicals and an easily programmable wearable form for sensitive nucleic acid detection does not exist to date. Finally, the wFDCF components are inexpensive, with cell-free reactions costing only \$0.01-0.03 per μL . Thus, a single 10 mm-diameter sensor would currently only cost $\sim$ \$1 in reagents. The optical fiber textiles are woven from common polymer fibers, and are also inexpensive. At these price points, the wearables could be utilized as disposable protective garments with advanced sensing technology. The sensors are also highly modular and adapted to various form factors, such as clothing.

[00201] Field applications that would greatly benefit from these wFDCF synthetic biology platforms include soldiers and first-responders (e.g., Hazmat personnel, Firemen) operating in environments where a specific chemical or biological threat is suspected. In this situation, the apparel of disposable wFDCF sensors could be used to maintain situational awareness, with continuous spatio-temporal monitoring of exposure and bodily resolution down to centimeters. Another set of potential uses for this platform involves the environmental awareness of clinicians, health workers, and researchers working in high-risk areas. The wearable sensing platforms could enable rapid responses to contagion so that any exposed users could begin decontamination and neutralization procedures immediately. Similarly, wFDCF-enabled coats and gowns in hospitals could provide alerts to prevent the spread of nosocomial infections to vulnerable populations, such as immune-compromised patients or newborns. An additional promising application is patient-worn sensor-enabled wearables such as the face mask presented here that can provide inexpensive, shelf-stable, and labor saving POC diagnostics to rapidly inform clinicians in outbreak events, such as the current COVID-19 pandemic that has rapidly overwhelmed the resources of worldwide medical infrastructures. In another implementation, any animal, such as mammals, can use the wFDCF. For example, a dog associated with a soldiers and first responders can be deployed with or separated with the associated human. In yet other implementations, the wFDCF can be attached to a robot sent in a hazardous environment. In any of the implemenations, the wFDCF can be taken off (e.g., the human, dog, robot) and left to collect and relay or monitor a specific chemical or biological threat.

[00202] *Fabrication of colorimetric synthetic biology wearable modules.*

[00203] Translucent (FIG. 6B top) and opaque (FIG. 6B middle/bottom) layers were made using skin-safe ECOFLEX® silicone elastomer (Smooth-On, Inc, Macungie, PA), precast overnight and laser-cut on a 75W Epilog Legend 36EXT according to the layouts shown in FIG.

6B and 7A. After laser-cutting, the silicone pieces were placed in a warm wash (45°C) with TERGAZYME® detergent (Alconox, Inc., White Plains, NY) for one hour with agitation, followed by three washes in 18-Ω pure water and a final wash in 70% ethanol, before allowing them to air dry. Layers were aligned and bonded together by depositing freshly-made, uncured liquid silicone elastomer and post-curing overnight at 65°C in a well-ventilated oven to obtain the final assembled prototypes. The final assembled elastomer prototypes were thoroughly sprayed with RNase Away Decontaminant (Thermo Fisher Scientific, Waltham, MA) and washed with 70% ethanol twice before being stored in petri dishes.

[00204] For the support matrices housing the cell-free reactions, clean WHATMAN™ No. 4 filter-paper disks (GE Healthcare Lifesciences Inc., Chicago, IL) (FIG. 6B) were punched to obtain cellulose discs with dimensions of 8 mm diameter and 0.5 mm thickness. These disks were incubated overnight in 0.01% DEPC, washed 3x with nuclease-free water, then incubated with 5% bovine serum albumin (BSA; MilliporeSigma, St. Louis, MO) in 50 mM Tris buffer, pH 7.5 for one hour with gentle agitation. The prepared BSA blocked discs were frozen at -80°C and subsequently freeze-dried. These lyophilized BSA-blocked discs were used as a scaffold for the deposition of colorimetric wearable synthetic biology reactions in freeze-dried, cell-free (wFDCF) sensors. The reaction disks saturated with the cell-free reaction components were finally snap-frozen in liquid nitrogen and freeze-dried for 8 - 12 hours in an SP Scientific Freezemobile lyophilizer (SP Industries, Inc., Warminster, PA).

[00205] Freeze-dried reaction disks were then inserted through the wicking ports of the elastomer chambers for assembly. The silicone elastomer chambers in the colorimetric device exhibit three 3 x 5 mm curved wicking ports in each of the four reaction chambers, which allow routes for fluid entry while delaying evaporation of cell-free reaction (FIG. 7A). The device chamber walls were aligned and bonded using uncured elastomer, to prevent flow or lateral diffusion of the reaction after rehydration. The wicking of contaminated fluid through the entry ports is primarily mediated by capillary action. This event then leads to rehydration of the reaction disk containing the chosen FDCF system (FIG. 6A), which marks $t = 0$ in the validation experiments (FIGs. 6H-6K). A magnified photograph of an activated reaction well containing an Ebola virus DNA toehold wFDCF sensor is shown in FIG. 7B, whereas the activation of a fabricated wearable bracelet using the same system is depicted in FIG. 7C. All of the colorimetric wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00206] *Preparation of optimized colorimetric wearable synthetic biology reactions.*

[00207] Each colorimetric wFDCF reaction used for lyophilization, assuming a 50 μL rehydration volume, was a 75 μL cell-free NEB PUREXPRESS® reaction (New England Biolabs, Inc., Ipswich, MA). Thus, each rehydrated reaction is a 1.5x-concentrated cell-free reaction based on the suggested reaction composition indicated by the manufacturer. Each reaction consisted of: 30 μL of PUREXPRESS® Component A, 22.5 μL of PUREXPRESS® Component B, 0.6 mg/mL of chlorophenol red- β -D-galactopyranoside (CPRG; MilliporeSigma, St. Louis, MO), 76 U of RNase Inhibitor (Roche GmbH, Mannheim, Germany), and a DNA template encoding the desired artificial genetic circuit at 5 ng/ μL . For the TetR transcriptional regulation circuit, FPLC- purified recombinant TetR protein was supplemented in the reaction at a concentration of 120 $\mu\text{g/mL}$. During activation of the various wFDCF reactions by rehydration, pure nuclease-free H_2O was used for the constitutive LacZ circuit, 25 $\mu\text{g/mL}$ of anhydrotetracycline (aTc) inducer was used for the TetR-regulated circuit, 300 nM of Ebola viral genome trigger was used for the toehold regulated circuit, and 1 mM of theophylline was used for the riboswitch-regulated circuit. The theophylline riboswitch reactions also included 2-Phenylethyl β -D-thiogalactoside (MilliporeSigma, St. Louis, MO), a β -galactosidase inhibitor, at a final concentration of 250 μM to suppress the background due to leakiness in these genetic circuits. The Ebola RNA genome trigger was acquired by an *in vitro* transcription reaction utilizing the HISCRIBE™ T7 Quick High Yield RNA Synthesis Kit (New England Biolabs, Ipswich, MA), using a DNA template. Each wFDCF reaction was applied to a BSA-blocked cellulose disc inserted into a 2 mL microcentrifuge tube. After the reaction was absorbed into the disc, the tubes were submerged in liquid nitrogen to snap freeze the disc and allowed to lyophilize for 12 hours. All of the colorimetric wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature. The colorimetric wFDCF presented in this work were from distinct sensors, in which each data point is the intensity value of a defined area of the green channel from the color-deconvolution function in ImageJ. The selected area size was kept constant for all sensors.

[00208] *Evaporation and dilution experiments in wearable synthetic biology devices.*

[00209] Evaporation tests were performed by cutting 10 x 10 cm WHATMAN™ No. 4 filter-paper squares and performing the cleaning and BSA blocking as described above for the discs. Each square was freeze-dried with 100 μL of a 1x PUREXPRESS® cell-free reaction with CPRG substrate and a constitutive LacZ plasmid. Various temperature (27-32°C) and fluid exposure conditions were investigated in combination with different coverage ratios of the rehydrated test squares to assess evaporation reduction. Suitable activity of the rehydrated reactions was assessed by visual inspection of the conversion of the colorimetric substrate from

yellow to purple. The port designs shown in FIG. 7A, 8B, 17G were selected empirically due to suitable activation of synthetic biology reactions with reduced evaporation rates (<20% of initial fluid volume in 2 hours) at 30-40% relative humidity.

[00210] *Kinetic enhancement by freeze-dried concentration of cell-free reaction components.*

[00211] Optimization testing of cell-free component concentrations on the kinetics of the reactions was performed by assembling PUREXPRESS® systems, according to the manufacturer's specifications, at various volumes (V_{initial}) and then lyophilizing the reactions in PCR tubes overnight (FIG. 10A). Next, the lyophilized pellets were rehydrated using the same sample volume (V_{final}), so that the tested fold-concentration was ($V_{\text{initial}} / V_{\text{final}}$). PUREXPRESS® concentrations ranging from 1x to 2.5x were tested in replicate by incubation of 10 μL reactions at 30°C for up to 90 minutes, followed by photographic imaging of the colorimetric changes (FIG. 10B) and absorbance measurements at 570 nm (FIG. 10C). The time to half-maximal output signal for each base or concentrated reaction (FIG. 10D) was calculated by a least square fitting of the acquired data.

[00212] *Screening of textiles for freeze-dried cell-free synthetic biology reactions.*

[00213] General compatibility of different textiles to cell-free synthetic biology reactions was tested in 103 different fabrics materials (e.g., silks, cotton, rayon, linen, hemp bamboo, wool, polyester, polyamide, nylon, and combination threads) under activation conditions (FIG. 18A-18B). A detailed list of the textiles used for this substrate screening can be found in Table 1. This compatibility of these textiles to FDCF synthetic biology reactions was compared to samples using WHATMAN™ No. 4 filter paper (GE Healthcare Lifesciences Inc., Chicago, IL) and samples in liquid form without any substrate as seen in FIG. 19A-19B. All tests used a T7RNAP-regulated LacZ circuit for constitutive expression. For this evaluation, fabric samples were identified and cut into 2 x 2 cm squares. Visible particles were removed from the fabrics using an adhesive roller. All fabric squares were cut into 1 x 2 cm pairs and washed thoroughly within 1.5 mL Eppendorf tubes with 1mL dd-H₂O for 30 minutes floating in a sonication bath at 80°C. The washed samples were left to cool to room temperature and then washed with running dd-H₂O for 10 sec. One of each pair of fabric square types was placed in 1.25 mL of a 5% BSA solution for 12 hours. After BSA incubation, the treated fabrics were cleaned with running dd-H₂O for 10 seconds. BSA-blocked and unblocked samples were then placed into fresh Eppendorf tubes with holes in the caps to allow for overnight desiccation of the fabrics at 60°C. Dried BSA blocked and unblocked fabrics were then cut in triplicate with clean 2 mm diameter disk biopsy punchers and placed in their respective slot in flat 384-well black polystyrene plates with a clear

glass bottom (Corning Inc.; Kennebunk ME, Ref#. 3544) for testing. Cell-free PUREXPRESS® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) was combined with a constitutive LacZ template containing 0.6 mg/mL CPRG and spotted (1.8 μ L) on each of the fabric wells. Control wells containing 2 mm disks of Whatman No. 4 filter-paper were also filled with 1.8 μ L constitutive LacZ test reactions, whereas 7 μ L were spotted on empty wells as liquid controls. A transparent adhesive PCR cover compatible with freezing was then placed over the plate and pressed with a roller to seal chambers. A small opening was pierced in each well with a 25-gauge x 5/8 (0.5 mm x 16 mm) BD PrecisionGlide Needle (Becton, Dickinson and Company; Franklin Lakes, NJ, Ref#305122) to allow for sublimation during lyophilization. Prepared plates were wholly immersed into liquid nitrogen for 1 min. A chilled metallic plate (maintained at -80°C with dry ice), was immediately put in contact with the bottom of the scored plates with the sealed frozen samples. A single 15 x 17" Kimwipe (KIMTECH™, Kimberly-Clark Corp., Irving, TX) was placed on top of the plate humidity openings. Then the 384-well test plate with top Kimwipe and the bottom metallic chiller was wrapped with three layers of aluminum foil. The entire wrapped bundle was then placed inside a sealed glass lyophilization chamber and connected to the freeze-drying machine. Lyophilization was performed for two hours. Freeze-dried paper samples were rehydrated with dd-H₂O to the original reaction volume. The colorimetric change was measured after overnight incubation (12 hours) at 37°C using a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in kinetic absorbance readout mode FIG. 19A-19B. Best observed functionality, as measured by the aggregated score shown in FIG. 20, was achieved using a fabric with 85% polyester and 15% polyamide fibers. This substrate was used for all further fluorescence and luminescence experiments, except for the case for a fluorescence Zika DNA Toehold sensing reaction (FIG. 11), which was also tested on a 100% mercerized cotton thread to validate the possibility of running FD-CF reactions at the single fiber level with this natural material commonly used in wound care.

[00214] *Fabrication of fluorescence/luminescence synthetic biology wearable textile module*

[00215] After screening of compatible textiles for freeze-dried, cell-free synthetic biology reactions, the best performing hydrophilic textile substrate (85% polyester / 15% polyamide) was used as weft for a textile inter-woven with a warp made of inert flexible polymeric optic fibers (POF) and polyester support threads. Such POFs were used for distributed optical interrogation of fluorescent or luminescent synthetic biology reactions within this fabric (three fibers per well). Polymeric optic fibers were weaved into this hydrophilic combination fabric using a standard industrial loom (DREAMLUX, Samsara Srl., Milan, IT), according to the design presented in FIGs. 16A-16D. Once fabric samples were manufactured, three-strip arrangements

of this hydrophilic POF fabric were cut to fit the device and laser-etched (5 mm) to disrupt the cladding in the POFs sections within the reaction zones (FIGs. 17A-17G). Black elastomer layers (top and bottom in FIG. 17B) were precast overnight and laser-cut according to the layout shown in FIGs. 17B, 17E. The silicone elastomer chambers in this device exhibit two 3 x 5 mm curved wicking ports that allow for fluid entry while still delaying evaporation within reaction fabric. Uncured black silicone elastomer was stamp-patterned onto the precast layers as well as into the internal POF fabric strips to be aligned and assembled, preventing air bubble formation between device layers and elastomer wicking in reaction zones. Final assembly of the base three-well sensor “patch” can be seen in FIGs. 17B, 17F, 17G. Devices were then placed under vacuum for 15 minutes to remove bubbles and were allowed to cure overnight at 65°C. As with the colorimetric prototypes, the fluorescent POF prototypes were thoroughly sprayed with RNase Away Decontaminant (Thermo Fisher Scientific, Waltham, MA) and washed with 70% ethanol twice before being stored in petri dishes. Once the assembled device was fully cured, POF fibers were separated into excitation and emission bundles and then covered with blackout adhesive fabric as well as black heat shrink tubing (6 mm) to prevent environmental light leakage. Blackout fabric disks (10 mm) made of black polyester knit Item#: 322323 (MoodFabrics Inc. New York, NY) were soaked in RNase Away Decontaminant for 5 minutes, washed thoroughly with 70% ethanol followed by water. The washed blackout fabric was incubated in 0.1% Triton X-100 for 5 minutes (as a wetting agent to enhance the ability of the textile to absorb water) and then excess solution was removed and the fabric pieces allowed to air-dry. The final blackout fabric discs were placed inside the reaction chamber with tweezers to aid in environmental light-blocking over sensing fibers. Finally, quick-turn stainless steel coupling sockets #5194K42 (McMaster-Carr Co., Elmhurst, IL) were added to the ends of the sensor device bundles for connection with the wearable spectrometer. The finalized wFDCF sensor device can be seen in FIGs. 17F, 17G.

[00216] *Hardware / software implementation of wearable POF spectrometer*

[00217] A custom-made wearable spectrometer with internal processing and wireless connectivity modules was fabricated to provide unsupervised sensing of on-body synthetic biology reactions (FIGs. 21A-21F). The device electronics were based on a Raspberry Pi Zero W Version 1.3 architecture (Raspberry Pi Foundation, Cambridge, UK) with connection to a custom shield for battery power, an environmental sensing module, an LED illumination module, and a flexible camera for imaging (FIG. 21A). The Raspberry Pi Zero W was selected as microprocessing for this application, due to its low cost (<\$15.00), small profile/weight (65 x 30 x 5 mm / 12 g), high performance (1 GHz single-core ARM1176JZF-S CPU, 512 MB RAM,

VideoCore IV GPU) and on-board wireless connectivity (802.11 b/g/n LAN, Bluetooth(R) 4.1, Bluetooth Low Energy -BLE). Regulated battery power was achieved using a PiZ-UpTime module, which is an uninterruptible power supply shield for Raspberry Pi Zero (Alchemy Power Inc., Santa Clara, CA), which uses rechargeable a Lithium-Ion 14500 battery (Battery & Power management in FIG. 21A), to reliably provide the charge capacity for 48 hrs of intermittent device operation continuously collecting data at a frequency of one measurement per minute. In-device sensing of temperature, humidity, atmospheric pressure, altitude, total Volatile Organic Compound (TVOC) and eCO₂ was achieved using an I2C environmental CCS811/BME280 Qwiic-Breakout (SPARKFUN ELECTRONICS®, Niwot, CO). The POF illumination module was achieved using a Saber Z4 Luxeon Z 20 mm Square Quad Color Mixing Array LED Module with aluminum base (Quadica Developments Inc. - Luxeon, Alberta, Canada) connected to a 12-Channel 16-bit PWM TLC59711 LED driver with SPI Interface (ADAFRUIT INDUSTRIES®, New York, NY). Four Luxeon Star LEDs were installed in the device with wavelengths 447 nm, 470 nm, 505 nm and 6500 K white (LEDs & Driver in FIG. 21A). An 8.6 mm x 8.6 mm Zero Spy Camera with 2" cable (Raspberry Pi Foundation, Cambridge, UK) was connected to the Raspberry Pi Zero W using a

flat serial interphase connector to provide POF imaging capabilities to the device. A single 5 mm INFINITE© aspherical plastic collimator part#: 191-66041G (Quarton Inc., New Taipei City, Taiwan) with numerical aperture (NA): 0.27 and effective focal length (EFL): 4.96 mm, was placed on top of the camera to allow for magnified POF imaging in proximity to the camera. The wearable spectrometer was covered by a two-part case fabricated using black photoreactive resin and a stereolithography 3D printing method using a Form 2 printer (Formlabs Inc., Summerville, MA) as seen in FIG. 21A. A view of the open device is shown in FIG. 21B, while a closed view is shown in FIG. 21C. This case included geometrical features to fit and align the camera/lens arrangement and the removable 3 mm diameter amber acrylic filter for fluorescence readings (slot arrangement in FIG. 21D). Also, the case features a slot for the 4-LED arrangement, a vent for the environmental sensors (FIG. 21D), as well as female Luer connection (FIG. 21A) to fit quick-turn stainless steel coupling sockets #5194K42 (McMaster-Carr Co., Elmhurst, IL). A top view of the assembled wearable POF spectrometer is shown in FIG. 21E, while the integration of this device within a wearable garment with wFDCF sensors is shown in FIG. 21F. The final volume of the wearable spectrometer device was approximately 235 cm³ with a total weight of around 173.8 grams (6.13 ounces), with a total cost of material and consumable supplies under \$100 USD. Base data-collection software (test version) implemented

in python for control of the Raspberry Pi Zero W within the wearable POF spectrometer was also provided.

[00218] *Preparation of optimized fluorescence wearable synthetic biology reactions.*

[00219] Constitutive sfGFP expression reactions for wFDCF testing (FIG. 9C) were prepared by combining 50 μ L of 1x NEB cell-free PUREXPRESS® in vitro protein synthesis solution with 0.5% Roche Protector RNase Inhibitor and 10 ng/ μ L constitutive PT7-sfGFP plasmid (+) or without as controls (-). Prepared reactions were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O.

[00220] Theophylline riboswitch sensor reactions for wFDCF testing (FIG. 9D) were prepared using 1x NEB cell-free PUREXPRESS® with 10 ng/ μ L Theophylline riboswitch sensor E mRNA in dd-H₂O prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 1 mM theophylline for the positive samples, while 0 mM theophylline was used for controls.

[00221] Dimeric Broccoli fluorescent aptamer sensor reactions for wFDCF testing (FIG. 9E) were prepared using 1.5x NEB cell-free PUREXPRESS® with 25 ng/ μ L of pJL1-F30-2xd-Broccoli aptamer DNA in dd-H₂O. Prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 50 μ M of the substrate (5Z)-5-((3,5-Difluoro-4-hydroxyphenyl)methylene)-3,5-dihydro-2-methyl-3-(2,2,2-trifluoroethyl)-4H-imidazol-4-one (DFHBI-1T; Tocris Bioscience, Minneapolis, MN) substrate for the positive samples, while 0 μ M DFHBI-1T substrate was used for controls.

[00222] Zika RNA Toehold switch sensor reactions for wFDCF testing (FIG. 11) were prepared using 1x NEB cell-free PUREXPRESS® with 33 nM Zika DNA toehold sensor 27B in dd-H₂O. Prepared sensor reactions were quickly deposited in a mercerized cotton thread or paper samples to be snap-frozen and then lyophilized for 4-8 hours within a 384-well plate. Activation of sensors was achieved by rehydration with dd-H₂O spiked with 2 μ M of freshly made Zika trigger RNA for the positive samples, while 0 μ M Zika trigger RNA was used for controls.

[00223] For the wearable nerve agent sensor experiments (FIG. 9G), 50 μ L reactions consisting of 0.5 U/mL acetylcholinesterase (Type V-S from *E. electricus*, MilliporeSigma, St. Louis, MO), 0.1 U/mL of choline oxidase (recombinant *Arthrobacter sp.*, MilliporeSigma, St. Louis, MO), 0.1 mg/mL of freshly prepared horseradish peroxidase (Type VI, MilliporeSigma,

St. Louis, MO), and 125 μM of the fluorescent reporter substrate AMPLITE-IRTM (AAT Bioquest, Sunnyvale, CA) in a final buffer of 10 mM HEPES, pH 8.0 / 1 mg/mL BSA / 1% fish gelatin / 5% trehalose. The reactions were applied to two WHATMANTM No. 4 filter-paper 0.8 cm discs, snap frozen in liquid nitrogen, and lyophilized for at least 12 hours. To test in the fluorescent wearable prototype, the paper discs containing the freeze-dried reactions were inserted into the wearable devices and rehydrated with 75 μL of 50 μM acetylcholine (MilliporeSigma, St. Louis, MO) with or without the nerve agent paraoxon-ethyl (MilliporeSigma, St. Louis, MO). The fluorescent wearable device for the nerve agent was altered for the detection of near-infrared fluorescence by replacing the optical components with excitation using a 627 nm red quad-LED array module (Quadica Developments Inc. - Luxeon, Alberta, Canada). Additionally, the emission camera was substituted with a NoIR Zero Spy Camera without infrared filter, on top of which was positioned three gel transmission filters No. 381, 382 and 383 (Rosco Laboratories Inc., Stamford, CT) to form a dedicated emission filtering stack with <1% cutoff at 660nm and peak transmittance at 740nm. All of the fluorescent wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature. All fluorescent wFDCF presented in this work were from distinct sensors, in which each data point is the integrated value of optical fiber signals from one sensor. Any fiber optic fibers that were 1 SD below the mean of all fibers combined were removed from analysis.

[00224] *Preparation of optimized luminescence wearable synthetic biology reactions.*

[00225] HIV RNA toehold switch sensor reactions for luminescence wFDCF testing (FIG. 9F, 15B) were prepared in 50 μL batches using 20 μL of NEB cell-free PUREXPRESS® Component A, 15 μL NEB Component B, 2.5 μL murine RNase inhibitor (New England Biolabs, Inc., Ipswich, MA), 6 ng/ μL HIV toehold sensor template with a nano luciferase (nLuc) output, 0.5 μL luciferin substrate (Promega Corp., Madison, WI) in dd-H₂O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 10 μM HIV trigger RNA freshly made for the positive samples, while 0 μM HIV trigger RNA was used for controls. The constitutive nLuc control reaction shown as part of FIG. 15B was performed similarly but substituting the toehold switch with a plasmid with a nLuc operon regulated by a T7 promoter.

[00226] *Borrelia burgdorferi* RNA Lyme disease toehold switch sensor reactions for luminescence wFDCF testing (FIG. 15A) were prepared in 50 μL batches using 20 μL of NEB cell-free PUREXPRESS® solA, 15 μL NEB solB, 2.5 μL murine RNase inhibitor, 18 nM *B.*

burgdorferi toehold DNA with luciferase operon, 2.75 μ L luciferin substrate (Promega Corp., Madison, WI) in dd-H₂O. Prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 3 μ M *B. burgdorferi* trigger RNA freshly made for the positive samples, while 0 μ M trigger RNA was used for controls. These wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00227] *Preparation of optimized CRISPR Cas12a-based wearable synthetic biology reactions.*

[00228] CRISPR-based sensor reactions for wFDCF testing in FIG. 23B-23F were prepared using 100 nM Cas12a (New England Biolabs, Ipswich, MA) and 100 nM gRNA, 1x NEB buffer 2.1, 0.45 mM dNTPs, 500 nM of each RPA primer, 1x RPA liquid basic mix (TwistDx Limited, UK), 14 mM MgCl₂, and 5 μ M FAM-IOWA BLACK® FQ quenched ssDNA fluorescent reporter (Integrated DNA Technologies, Coralville, IA) in dd-H₂O. Prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 2.7 fM or 100 fM of *mecA*, *spa* or *ermA* DNA trigger depending on the demonstration. In the sensing performed at 2.7 fM *mecA* trigger, the detection limit is 10,000 copies of DNA per μ L. These wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00229] *Preparation of optimized CRISPR Cas13a-based wearable synthetic biology reactions.*

[00230] Cas13a CRISPR-based sensor reactions for wFDCF testing (FIG. 25) were prepared using 100 nM Cas13a and 100 nM gRNA, 1x NEB buffer 2.1, 0.45 mM dNTP, 14 mM MgCl₂, and 5 μ M FAM-IOWA BLACK® FQ quenched RNA fluorescent reporter (Integrated DNA Technologies, Coralville, IA) in dd-H₂O. Prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 20 nM of MRSA RNA trigger.

[00231] *Preparation of sample lysis-integrated wearable synthetic biology reactions.*

[00232] For wFDCF with integrated lysis reactions, a RNase-free Whatman filter paper disc (8 mm) was filled with concentrated stock solutions that would yield, upon a 50 μ L rehydration volume, 5 mM Tris-HCl (pH 7.5), 1% Triton X-100, 1% NP-40, 0.2% CHAPS, 100 μ g/mL

lysozyme, and 5% sucrose. This was freeze-dried for 4 hours and inserted into the POF wFDCF device below the blackout layer and above a PVA time delay barrier that was sealed around the edges with EcoFlex elastomer to enable an efficient lysis incubation time. All layers containing the lyophilized RPA-Cas12a synthetic biology sensors below the lysis – PVA delay layers were identical to that used in the *mecA* RPA-Cas12a devices shown in FIG. 23B-23E.

[00233] *Garment-level integration of colorimetric synthetic biology sensors.*

[00234] After fabrication of colorimetric synthetic biology wearable module, a bracelet "garment" was achieved simply by gluing the module into an elastic band to be placed in the forearm of a mannequin (FIG. 8C).

[00235] *Garment-level integration of fluorescence/luminescence synthetic biology sensors.*

[00236] After fabrication of at least 12 fluorescence/luminescence synthetic biology wearable modules, a commercially available long-sleeve neoprene wetsuit-type jacket (Eyce Dive & Sail) was modified to integrate an array of wFDCF sensors by sewing these modules in predefined high-splash frequency regions (FIG. 9A, 21F). Reaction modules were covered in the edges with a blackout fabric border with textile adhesive. POF bundles of these modules were sawn internally and directed to a single multi-bundle arrangement for interrogation via the portable spectrometer device (located in a back pocket within the jacket) as seen in FIG. 21F. Base neoprene fabric used for this jacket was of 3mm thickness and treated with a superhydrophobic coating to prevent fluid absorption in places other than the reaction zones. Fabricated wFDCF jacket prototype was specified to fit a medium-sized male torso 36"(chest) by 31"(waist). In-garment sensors were tested on a mannequin at room temperature.

[00237] *Sensor and reporter sequences*

Tables S2 and S3 contain the DNA and RNA sequences of sensors and reporters used in this study. The plasmid construct used for the Zika 27B toehold sensor has been previously described elsewhere. The Lyme disease and HIV toehold sensors with a nanoluciferase output were cloned into the pBW121 plasmid backbone (Addgene plasmid #68779). All other plasmid constructs utilized the pJL1 backbone that has been previously described^{2, 3}. The F30 dimeric Broccoli fluorescent aptamer was subcloned into pJL1 from pET28c-F30-2xdBroccoli which was a gift from Samie Jaffrey (Addgene plasmid #66843; www.n2t.net/addgene:66843; RRID:Addgene_66843). The sequence for the pJL1-sfGFP plasmid can be found on Addgene (Plasmid #69496).

TABLE 1: Fabrics tested – results shown in FIG. 20

ID	Supplier	Supp. ID	Material	Type	Name	Manufacturer Description
0	-	N/A	-	No substrate (control)	No substrate (control)	-
1	Whatman GE	1442-042	Cellulose Paper	42.5mm filter papers	42.5mm filter papers	Ashless filter papers with 0.2mm thickness and 42.5mm diameter.
2	Dharma Trading Co. (DTC)	SBC45	Silk	Spun Silk Broadcloth 45" wide	Spun Silk Broadcloth 45" wide	100% spun silk 23mm, mid-weight flat woven fabric with a crisp hand and slight sheen, drapes nicely.
3	DTC	SD	Silk	Silk Dupion 19mm 45" wide	Silk Dupion 19mm 45" wide	Picture shimmery silk with lots of texture - raised silk slubs - intermittent shiny threads - similar to Shantung. Makes outstanding jackets, suits, coats, etc.
4	DTC	SG36	Silk	Silk Gauze 3mm	Silk Gauze 3mm 36" wide	Wide weave. Very light, very sheer, very beautiful.
		SG45	Silk		Silk Gauze 3mm 45" wide	Wide weave. Very light, very sheer, very beautiful.
		SG55	Silk		Silk Gauze 3mm 55" wide	Wide weave. Very light, very sheer, very beautiful.
5	DTC	SVEL45	Silk	Silk Velvet	Silk Velvet 45" wide	Standard silk, 82% rayon. Dyes and paints super. The ultimate! Use for the Devore technique.
		SVEL55	Silk		Silk Velvet 55" wide	Standard silk, 82% rayon. Dyes and paints super. The ultimate! Use for the Devore technique.
6	DTC	STCHARM	Silk / Spandex	Stretch Charmeuse 12mm 45" wide	Stretch Charmeuse 12mm 45" wide	All die softness and sheen of silk with the comfort of a Stretch fabric. 95% silk & 5% spandex. The low amount of spandex will not the dyeing process.
7	DTC	ST	Silk	Silk Twill 12mm 45" wide	Silk Twill 12mm 45" wide	A very utilitarian durable silk. Great for shirts, handkerchiefs, pillow cases, lamp shades, screens, etc.
8	DTC	SRS45	Silk	Smooth Raw	Smooth Raw Silk 31.5mm	100% Silk 45" width

9	DTC	SG4545	Silk	Silk 31.5mm Silk Gauze 4.5MM 45" wide	Silk Gauze 4.5MM 45" wide	Some like it a bit heavier. Beautiful flowing fabric, great for sewing and gifts.
10	DTC	SK45	Silk	Silk Knit 45" wide	Silk Knit 45" wide	100% Silk Jersey, 3oz/sq.yd approx. 100 g/m2, 22" wide tube (44" wide), soft hand with a nice drape, great for silk underwear garments and scarves.
11	DTC	ORG45 ORG55	Silk	Organza 5.5 mm	Organza 5.5 mm 45" wide	Very sheer, transparent, stiff, gauze-like fabric.
			Silk		Organza 5.5 mm 55" wide	Very sheer, transparent, stiff, gauze-like fabric.
		CBSS36	Silk		Crepe Back Silk Satin (Charmeuse) 19.5mm 36" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
12	DTC	CBSS45	Silk	Crepe Back Silk Satin (Charmeuse)	Crepe Back Silk Satin (Charmeuse) 19.5mm 45" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
					Crepe Back Silk Satin (Charmeuse) 19.5mm 55" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
		CBSS55	Silk			
13	DTC	VRS	Rayon / Silk	Vicose Rayon / Silk Blend	Vicose Rayon / Silk Blend	75% Rayon / 25% Silk blend. Weight equivalent to 18mm. Although fabric is semi-sheer when white. when dyed it is mostly opaque. 58" wide
14	DTC	TAF55	Silk	Silk Taffeta - 14.5mm	Silk Taffeta -14.5mm	100% Silk 55" width
15	DTC	SWSC	Silk	Sand Washed (Sueded) Charmeuse 19.5mm 15" w	Sand Washed (Sueded) Charmeuse 19.5mm 15" wide	Beautiful & desirable! people here are taking up sewing because of it.
16	DTC	SWCDC	Silk	Stone wash Crepe de Chine	Stone wash Crepe de Chine	By stone (sand) washing this heavier weight of Crepe de Chine, one gets a matte silk with a soft, luxurious drape. This is a great weight for any almost article of clothing items like pillows, curtains and such.
17	DTC	SWF	Silk / Wool	Silk / Wool blend 63% silk, 37% wool	Silk / Wool blend 63% silk, 37% wool	Slightly sheer with a diagonal weave almost like a chiffon but sturdier. This material is softer than wool warmer than silk 12.5mm 55" wide.

18	DTC	CHIF16	Silk	Silk Double Chiffon 16mm 45"	Silk Double Chiffon 16mm 45"	With a crepey texture and a low subtle sheen, this is a perfect weight and drape for blouses, dressy slacks, or skirts. Takes dye beautifully
19	DTC	CHIF10	Silk	Silk Chiffon 10 mm, 54"	Silk Chiffon 10 mm, 54"	An elegant, sheer fiber with a soft beautiful drape and a crepe like texture. Heavier and wider than the 8mm.
20	DTC	CHIF44	Silk	Silk Chiffon 8mm	Silk Chiffon 8mm 44"	An elegant, sheer fiber with a soft drape and a crepe like texture.
		CHIF55	Silk		Silk Chiffon 8mm 55"	An elegant, sheer fiber with a soft drape and a crepe like texture.
21	DTC	CDC1236	Silk	Crepe de Chine	Crepe de Chine 12mm 36" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
		CDC1245	Silk		Crepe de Chine 12mm 45" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
		CDC1255	Silk		Crepe de Chine 12mm 55" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
22	DTC	CDC45	Silk	Crepe de Chine	Crepe de Chine 16mm 45"	Slightly crinkled texture with a gentle, graceful drape and very soft hand. Has a smooth surface that lends itself well to all types of painting styles. Ideal for all types of clothing and decorating.
		CDC55	Silk		Crepe de Chine 16mm 55"	Slightly crinkled texture with a gentle, graceful drape and very soft hand. Has a smooth surface that lends itself well to all types of painting styles. Ideal for all types of clothing and decorating.
23	DTC	CCDC45	Silk	Crepe de Chine	Crepe de Chine	100% Silk, Optically Whitened — 16mm, 45" width
24	DTC	CBSSL36	Silk	Crepe Back Silk Satin '	Crepe Back Silk Satin 36"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side, drapes nicely and feels fabulous.
		CBSSL45	Silk		Crepe Back Silk Satin 45"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side, drapes nicely and feels fabulous.
		CBSSL55	Silk		Crepe Back Silk Satin 55"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side,

						drapes nicely and feels fabulous.
25	DTC	HS16	Silk	Silk Habotai	Silk Habotai 16mm 45" wide	The basic "China Silk" but even heavier
26	DTC	HCJ	Hemp / Cotton	Hemp/Cotton Jersey	Hemp/Cotton Jersey	55% Hemp, 45% Cotton — 50z, 61" width
27	DTC	HS1036	Silk	Habotai Silk lomm	Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1045	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1055	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1070	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
28	DTC	HS12	Silk	Silk Habotai	Silk Habotai 12mm 45" wide	The basic China Silk, but heavier
29	DTC	HS836	Silk	Habotai Silk	Habotai Silk 8mm 36" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
		HS845	Silk		Habotai Silk 8mm 45" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
		HS855	Silk		Habotai Silk 8mm 55" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
30	DTC	HS536	Silk	Habotai Silk	Habotai Silk 5mm 36" wide	When they say China Silk this is What they mean. Light Weight and sheer. Everyone likes this stuff.
		HS545	Silk		Habotai Silk 5mm 45" wide	When they say China Silk this is What they mean. Light Weight and sheer. Everyone likes this stuff.
		HS555	Silk		Habotai Silk 5mm 55" wide	When they say China Silk this is What they mean. Light Weight and sheer. Everyone likes this stuff.
31	DTC	FCR4S	Silk	Flat Crepe	Flat Crepe 8mm 45" Wide	A fabric that looks like a combination of the texture

							and drape of crepe and luster of habotai. It is easy to dye and paint and accepts readily all types of painting techniques.
32	DTC	DSAT45	Rayon / Silk	Devore Satin (30% Silk / 70% Rayon)	Devore Satin (30% Silk / 70% Rayon)	Heavy Crepe de Chine 30mm 45"	19 mm 45" wide. Silky, shiny beautiful fabric for "burn-out" (apply "Fiber-Etch" to remove the Rayon threads in a design leaving a lace of silk)
33	DTC	HCDC	Silk	Heavy Crepe de Chine	Heavy Crepe de Chine		Like the 16mm crepe de chine, only heavy enough to take dye with great depth and intensity. With a dull sheen and a soft drape it is perfect for pants, jackets, etc.
34	DTC	JAC9702	Silk	Inkjet Printable Silk fabric	Inkjet Printable Silk fabric		Inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
35	DTC	JAC9703	Silk	Semi-transparent Inkjet Printable Silk fabric	Semi-transparent Inkjet Printable Silk fabric		Thin and semi-transparent inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
36	DTC	PWFC	Wool	Pure Wool Gauze Twill Fabric	Pure Wool Gauze Twill Fabric 45"		100% wool, lightweight with a subtle twill weave, 45" wide, 3.2 oz / linear yard
37	DTC	PWFA	Wool	Pure Wool Gauze Fabric	Pure Wool Gauze Fabric 45"		100% wool, lightweight and semi-sheer, 45" wide, 3.2 oz / linear yard
38	DTC	PHC	Hemp	Hemp Canvas	Hemp Canvas		100% Hemp - 11 oz, 53" width, optically whitened
39	DTC	HWM	Hemp	Hemp Woven Mid-weight	Hemp Woven Mid-weight		100% Hemp - 5.4 oz, 53" width, optically whitened
40	DTC	HSCH	Hemp / Silk	Hemp Silk Charmeuse	Hemp Silk Charmeuse		60% hemp, 40% silk. Silk lends a beautiful shine to one side, while the hemp lends strength to the body. 57" wide
41	DTC	HS	Hemp / Silk	60% Hemp / 40% Silk	60% Hemp / 40% Silk		Natural. 57" wide. A spectacular fabric. Beautiful, soft and interesting. If this fabric was human you would want to marry it. The elegance of silk and the strength of hemp. Vertical textured grain., 2.6 oz/yard
42	DTC	HR	Hemp / Rayon	55% Hemp / 45% Rayon	55% Hemp / 45% Rayon		Natural, 59" wide. Soft and smooth, great for dresses and other clothing items. It washes well. 5.5 oz/yard
43	DTC	HM	Hemp	Hemp Summer	Hemp Summer Cloth		100% Hemp - 70z, 53" width. Optically whitened

44	DTC	HCM	Hemp / Cotton	Cloth Hemp Cotton Muslin	Hemp Cotton Muslin	55% Hemp, 45% Cotton - 4.15z. 53" width. Optically whitened
45	DTC	PTC45/58	Cotton	Pimatex Cotton - PFD	Pimatex Cotton - PFD	High quality, great for sewing fine clothing. For that special occasion blouse, dress shirt or heirloom item. Dyes as well or better than mercerized cotton 133x72 thread count, 45" wide, 3.7 oz/yd.
46	DTC	POP60	Cotton	Poplin	Poplin	100% Cotton tightly woven, medium weight fabric 6 oz per yard Wide. Thread count is 108 threads per inch x 50 threads per inch.
47	DTC	WJ242	Rayon	Rayon Satin, Heavyweight	Rayon Satin, Heavyweight 58"	Satin refers to a particular type of weave which results in a fabric with a very solid, tightly woven look. Optically whitened. 58" wide.
48	DTC	VRL	Rayon	Viscose Rayon Light	Viscose Rayon Light	100% rayon, Optically whitened, 4 oz per sq yd, 45" wide threads per inch x 38 threads per inch.
49	DTC	VCI	Rayon	Viscose (Rayon) Challis (Import)	Viscose (Rayon) Challis (Import)	Often seen in hawaiian shirts and ladies' wrap skirts. Because rayon is pure cellulose, it allows dyes to give very brilliant colors. 58" wide, 4 oz/yd, 90x60 threads.
50	DTC	SCVO	Cotton	Silky Cotton Voile	Silky Cotton Voile	Similar to the Cotton Voile, but with a tighter weave and a nice sheen this fabric has a very smooth silky 1.9 oz/yd sq. 52" wide
51	DTC	SCN	Cotton	Slubby Cotton Knit	Slubby Cotton Knit	100% cotton - 30z. 58" width
52	DTC	BBF	Bamboo Rayon	Bamboo Rayon	Bamboo Rayon	100% bamboo rayon. It's beautiful, soft and luxurious. 3.2 oz /sq yd, 60" wide. Thread count is 76x76 threads per inch.
53	DTC	BAMF	Bamboo Rayon	100% Bamboo Rayon Fleece	100% Bamboo Rayon Fleece	This fabric is really soft. One side is a smooth knit while the flip side is a soft fleece. 14oz per square yd, 60" wide.
54	DTC	SOCJ	Soy Cotton	Soy Organic Cotton Jersey	Soy Organic Cotton Jersey	58% Soy, 37% Organic cotton, 5% Spandex. This is a lovely and extremely soft jersey with a wonderful drape. 6.3oz per square yd, 60" wide.
55	DTC	RSAT45	Rayon	Rayon Satin	Rayon Satin	Satin refers to a particular type of weave which

							results in a fabric with a very solid, tightly woven look. 45" wide. Optically Whitened.
56	DTC	RV045	Rayon	Rayon Voile	Rayon Voile	Rayon Voile	Gorgeous sheen and a soft smooth hand. Weave is loose, somewhat sheer, and drapes well. 45" wide Optically Whitened.
57	DTC	RT55	Rayon	Rayon Twill	Rayon Twill	Rayon Twill	Has perhaps the nicest drape of any of the fabrics, including silks. Strong twill weave that will last. 55" Wide. Optically Whitened.
58	DTC	RL55	Rayon	Rayon Lawn	Rayon Lawn	Rayon Lawn	100% rayon very smooth and has a fine weave. 2.5 oz per square yard, 54/55" wide. Thread count is 90 threads per inch x 88 threads per inch.
59	DTC	RCK	Rayon	Rayon Crinkle	Rayon Crinkle	Rayon Crinkle	beautiful, light crinkled rayon - great fabric for all kinds of clothing. 86 x 44, 4.5 oz. / sq. yd. approx.
60	DTC	QCS	Cotton	Cotton Sateen	Cotton Sateen	Cotton Sateen	Sateen has a smooth finish on one side, kind of a sheen. This softer, finely woven fabric is used for lots more than quilts.
61	DTC	JER40	Cotton	Jersey Cotton	Jersey Cotton	Jersey Cotton	100% Cotton slightly stretchy fabric with a smooth flat face. It's what basic t-shirts are made of. 6.50z per linear yd 58/60" wide.
62	DTC	MUS5B	Cotton	Premier Muslin Bleached	Premier Muslin Bleached	Premier Muslin 60" Bleached	Bleached midweight muslin. 100% Cotton, 3.2 oz per square yard, 60" wide, Thread count is 68 threads per inch x 68 threads per inch
63	DTC	NF	Nylon	Nylon	Nylon	Nylon 58" wide	Untreated dye able nylon. Prepared for printing. Perfect for banners or flags. Stronger outdoors than natural fibers.
64	DTC	NET48	Cotton	Cotton Net Fabric	Cotton Net Fabric	Cotton Net Fabric 48"	100% cotton, slight stiffness disappears when washed, 33yd bolt comes pre-folded in a bag, 48" wide.
65	DTC	NPF	Nylon	Nylon 'Puppet Skin' Fleece	Nylon 'Puppet Skin' Fleece	Nylon 'Puppet Skin' Fleece	100% Nylon - 120z, 54"/56" width.
66	DTC	NVEL	Rayon / Nylon	Rayon/Nylon Velvet	Rayon/Nylon Velvet	Rayon/Nylon Velvet	80% Rayon, 20% Nylon - 4.20z, 45" width.
67	DTC	OCDT	Cotton	Organic Cotton Denim Twill	Organic Cotton Denim Twill	Organic Cotton Denim Twill	This denim is strong and versatile. Anywhere you might use denim or twill. 70z per square yd, 56"

68	DTC	MI60	Rayon	Modal Rayon Jersey	Modal Rayon Jersey	wide. 95% Rayon, Lycra. Rayon jersey is extremely popular in ready to wear at all the best retail stores. 100z / linear yard. 60" wide.
69	DTC	MCCB45	Cotton	Mercerized Combed Cotton Broadcloth	Mercerized Combed Cotton Broadcloth 45" wide	Very finely Woven, 133 x 72 threads per inch. Mercerized to take dyes and paints better. 60" Wide. For batik and fine painting. 3.5 oz/yd sq.
		MCCB60	Cotton		Mercerized Combed Cotton Broadcloth 60" wide	Very finely Woven, 133 x 72 threads per inch. Mercerized to take dyes and paints better. 60" Wide. For batik and fine painting. 3.5 oz/yd sq.
70	DTC	LR	Linen / Rayon	55% Linen / 45% Rayon	55% Linen / 45% Rayon	Want the soft drape of rayon and cool comfort of linen? This is the one fabric for you. The linen look is heavy enough for pant, drapey enough for tops and dresses, 52" wide.
71	DTC	LIN21	Linen	Linen-100% Bleached Linen	Linen-100% Bleached Linen	Beautiful soft bleached fabric. Three different weights available. This one is 3.8 oz per square yard with 52x53 Thread Count, 54" wide.
72	DTC	LIN6	Linen	Linen	Linen	100% Linen, Optically Whitened — 6,80z, 54" width.
73	DTC	LIN	Linen	Linen-100% Bleached Linen	Linen-100% Bleached Linen	Beautiful soft bleached fabric. Three different weights available. This one is 4.70z per square yard with 50x54 Thread Count, 54" wide.
74	DTC	KC118	Cotton	Kona Premium Muslin	Kona Premium Muslin 118"	Midweight cotton sheeting. 100% cotton, 4.2 oz per square yard, 118" wide, Thread count is 130 threads per inch x 70 threads per inch.
75	DTC	mac	Cotton	Kona Cotton - PFD	Kona Cotton - PFD 45" wide	A quilter's dream fabric. also good for soft children's clothing, comfy shirts and dresses. The 60x60 thread count gives this even weave fabric a soft luscious feel and great durability. 45" wide. 44 oz/yd.
			Cotton		Kona Cotton - PFD 60" wide	A quilter's dream fabric. also good for soft children's clothing, comfy shirts and dresses. The 60x60 thread count gives this even weave fabric a soft luscious feel and great durability. 45" wide. 44

						oz/yd.
76	DTC	CVEL	Cotton	Cotton Velveteen - PFD	Cotton Velveteen - PFD	The Velveteen is a woven ICO% cotton with a short close weft pile in imitation Of velvet. Stronger and denser than the rayon Silk velvet and the pile is shorter. 220g/sq meter, 44" wide.
77	DTC	CVO55	Cotton	Cotton Voile	Cotton Voile	Soft, gauzy cotton with a silky wonderful feel. 55" wide 1.9 oz/yd. 80x72 thread count, 30yd bolts.
78	DTC	CST57	Cotton	Cotton Sateen	Cotton Sateen 57"	100% Cotton, mercerized, very soft sheen, 144x66 thread count, 60yd bolts, 57" wide.
79	DTC	CS	Cotton	Cotton Sheeting	Cotton Sheeting	Good quality fabric soft and nice mid-weight. Suitable for all clothing types. 4.20z/sq yd. 55"-58" wide. 60x60 Thread count.
80	DTC	CPOP57	Cotton	Cotton Poplin	Cotton Poplin 57"	100% cotton, mercerized, crisp fabric with a tight weave, 144x68 thread count, 57" wide, bolts are 60 yards.
81	DTC	CPC	Cotton	Cotton Print Cloth Mercerized	Cotton Print Cloth Mercerized	The Best selling cotton fabric. For eyers, dyers, quilters and painters with great quality for finished items (80x80 thread count). Takes dyes and paints particularly well. 45" wide. 3.1 oz/yd
82	DTC	CLF	Cotton	Cotton Lycra PFD	Cotton Lycra PFD	Cotton, Lycra. This is the fabric It's PFD (prepared for dyeing) so it dyes up brilliantly. 60" before shrinkage, 6.3 oz/yd.
83	DTC	CL55	Cotton	Cotton Lawn	Cotton Lawn	100% Cotton, Optically Whitened, 2.0 oz per Square yard. 56/57" wide, thread count 90 threads per inch x 88 threads per inch.
84	DTC	MUS2B	Cotton	Cotton Muslin Bleached	Cotton Muslin 36" - Bleached	Bleached lightweight muslin, with tiny slubs throughout the weave. 100% cotton, oz per square yard, wide. Thread count 68"x68"
85	DTC	7CDB60	Cotton	7 oz'Duck, Bleached	7 oz'Duck, Bleached 60"	Grade "A" Duck. 84 x 29 threads. A little lighter weight. Drapes better than the 10 oz. All dye well. but the natural dyes darker.
			Cotton		7 oz'Duck, Natural 60"	Grade "A" Duck. 84 x 29 threads. A little lighter weight. Drapes better than the 10 oz. All dye well. but the natural dyes darker.

86	DTC	BD-N	Cotton	Bull Denim Natural	Bull Denim Natural	Soft feel and tough as nails. Dyes great. Hats, pants, shorts, bags anywhere you use denim. 60" wide. 10 oz/yd. 50 yard bolts.
		BD-B	Cotton	Bull Denim Natural	Bull Denim bleached	Soft feel and tough as nails. Dyes great. Hats, pants, shorts, bags anywhere you use denim. 60" wide. 10 oz/yd. 50 yard bolts.
87	DTC	CCL	Cotton	Combed Cotton Lawn	Combed Cotton Lawn	This is a soft, high quality semi-sheer lawn suitable for blouses, christening gowns, lingerie, and all your fine sewing needs.
88	DTC	CC110	Cotton	Combed Cotton	Combed Cotton 110"	100% Mercerized Soft Cotton, oz per square yard, 110-112" wide. Thread count is 92 threads per inch x 88 threads per inch.
89	DTC	HCG	Cotton	Heavier Cotton Gauze	Heavier Cotton Gauze	Optically whitened, Soft lightly textured. A heavier weight than the cotton bubble gauze. 3.7 oz per square yd, 50" wide, 48 x48 Thread Count.
90	DTC	FT60	Cotton	French Twill	French Twill 60"	Midweight cotton with a tight diagonal weave, 100% cotton, 4.5 oz per square yard. 60" wide, thread count is 132x61
91	DTC	ESS	Cotton	Essex Linen-PFD	Essex Linen- PFD	Linen 145% Cotton Blend. Slightly textured with a linen look. Optically whitened. 5.5 oz per square yd, 52" wide. 51x49 Thread Count.
92	DTC	CVS55	Cotton	Cotton Voile	Cotton Voile	100% Fully Combed Cotton, Optically whitened, 1.4oz per square yard, 57-58" wide, Thread count is 70 x 70 threads per inch.
93	DTC	CJ60	Cotton	Cotton Jersey PFD	Cotton Jersey 60" PFD	100% cotton jersey. PFD ready to dye. 9.5 oz per linear yd. 6 oz./yd. That is a little heavier than the average T-shirt. Used for skirts, dresses, tops and kids' clothing. 60" wide.
94	DTC	CIL	Cotton	Cotton Interlock	Cotton Interlock	Very warm and soft a little stretchy. Perfect for baby wear. Double knit construction makes it thicker & heavier than single knit. 60z/sq. yd, 62" wide.
95	DTC	CG	Cotton	Cotton Bubble Gauze	Cotton Bubble Gauze	Beautiful white all cotton fabric. like a crinkle gauze. Very neat for skirts, tops, anywhere you

96	DTC	CF60	Cotton	Combed Cotton Fleece	Combed Cotton Fleece	want it light and airy. Heavier gauze is available. 50" wide, 22oz/sq yd.
97	DTC	10CDB60	Cotton	Cotton Duck, Bleached	Cotton Duck, Blch. 10 oz. 45/60"	100% Cotton - 8.70z, 60" width
		10CDB63	Cotton		Cotton Duck, NATURAL. 10 oz. 63"	Grade "A" duck. 76 x 28 threads. Almost has the weight and texture of canvas but the flex and drape of a heavy jacket weight cotton. For strong use. It dyes well, but the natural dyes darker. Grade "A" duck. 76 x 28 threads. Almost has the weight and texture of canvas but the flex and drape of a heavy jacket weight cotton. For strong use. It dyes well, but the natural dyes darker.
98	Jacquard, Rupert, Gibbon & Spider Inc. (JRGSI)	JAC9701	Cotton	Jackard Inkjet Printable Cotton fabric	Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
99	Silhouette of America Inc.	CTNFAB	Cotton	Silhouette Inkjet Printable Cotton fabric	Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
100	LumiGram SARL	LUMW01	Polyester	Lumigram optic fiber fabric (white)	Lumigram optic fiber fabric (white)	Lumigram optic fiber fabric (white). Optic fiber diameter is 0.25mm, with a spacing pitch of 1.2mm, horizontally oriented in a matrix of polyester based synthetic fabric (85% PL, 15% PA6)
101	Wilton Industries Inc.	500523240	Sucralose /Dextrose	Inkjet Printable Sugar sheets	Inkjet Printable Sugar sheets	Dried inkjet sheets made of food starch-modified (corn), maltodextrin, glycerin, sugar, water, food starch-modified (potato), dextrose, cellulose gum, gum arabic, titanium dioxide (color), polysorbate 60, mono and diglycerides of fatty acids, sorbitan monostearate, potassium sorbate (preservative), artificial flavor, citric acid, sucralose.
102	JRGSI	JAC9702	Silk	Dirty Inkjet	Dirty Inkjet Printable Silk	Inkjet silk fabric sheets 1.5"x11" comes with

				Printable Silk fabric	fabric	detachable paper support
103	JRGS	JAC9703	Silk	Dirty Semi-transparent Inkjet Printable Silk fabric	Dirty Semi-transparent Inkjet Printable Silk fabric	Thin and semi-transparent inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
104	JRGS	JAC9701	Cotton	Dirty Inkjet Printable Cotton fabric	Dirty Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
105	Silhouette of America Inc.	CTNFAB	Cotton	Dirty Inkjet Printable Cotton fabric	Dirty Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support

Additional descriptions

[00238] FIGs. 6A-6H depict wearable cell-free synthetic biology. FIG. 6A depicts freeze-dried cell-free reactions can be embedded in reaction sachets or chambers that are distributed throughout garments for use by soldiers, clinicians, and first responders. Upon exposure to an external splash, the reactions are rehydrated, activating dormant synthetic gene circuits that detect pathogens, metabolites, and toxins. FIG. 6B depicts a schematic of the layer-by-layer assembly of the wearable devices. Each layer is fabricated from skin-safe silicone elastomer. The FDCE reactions are embedded in a cellulose matrix placed within each chamber. FIG. 6C depicts an array of assembled reaction chambers showing the elasticity (center) and flexibility (right) of the devices. FIG. 6D depicts portals cut into the outermost layer allow sample access, which is rapidly drawn into the reaction chambers through capillary action. The hydrophobic chamber walls prevent inhibitory dilution through lateral diffusion. FIG. 6E-6F depict various types of synthetic biology circuits can be freeze-dried in these wearable devices, including constitutively expressed outputs (6E), transcription factor-regulated circuits for small molecule detection (6F), toehold switches for nucleic acid-sensing (6G), and riboswitches to detect various small molecules (6H). Each graph depicts color deconvoluted values, $n=3$. Bottom images are representative color images of the wearable device.

[00239] FIGs. 7A-7C depict assembly layers and sample activation of colorimetric wFDCE reactions with constitutive $P_{T7}::LacZ$ module. FIG. 7A depicts the layout of elastomer layers in the colorimetric wFDCE device. FIG. 7B depicts activation of colorimetric prototype reaction chambers using 40 ng/ μ L constitutive LacZ-T7 plasmid in a 50 μ L rehydration splash as compared to FIG. 7C which depicts rehydration with no plasmid. After complete rehydration, PURExpress reactions were conducted at 1.5x concentration. All the reactions were allowed to incubate at 30°C, exposed to the ambient environment, and images were taken every 5 minutes. Color change in one replicate was visible in under 20 min. Each row depicts a representative single-well reaction.

[00240] FIGs. 8A-8C depict sample activation of wFDCE colorimetric devices and bracelet for detection of Ebola virus RNA. FIG. 8A depicts activation of colorimetric Ebola virus DNA toehold wFDCE sensor using a 50 μ L splash of dd-H₂O sample containing 300 nM Ebola RNA trigger as compared to control ($t = 60$ min). FIG. 8B depicts port wicking into reaction chambers containing reaction disks using dd-H₂O fluid splash. Rehydrated paper disks are visibly darker after fluid entry and wicking into the substrate ($t = 1$ sec after splash). FIG. 8C depicts activation of the wearable colorimetric bracelet with four independent Ebola virus DNA toehold sensors (t

= 25 min). Color change in activated sensor disk is distinguishable within 25-60 min after rehydration, as compared to surrounding controls.

[00241] FIGs. 9A-9G depict design and validation of fluorescent and luminescent freeze-dried cell-free synthetic biology wearables. FIG. 9A depicts details of assembly and activation of fiber-optic based wFDCF module for fluorescence/luminescence output, with a schematic of module layers and components of embedded cell-free reactions. Fiber-optic embedded textiles allow excitation of the samples and detection by sensing emission light. A single layer of blackout cover made of polyester fabric is used to prevent the entry of environmental light into the reaction well. Bottom: An example rehydration event over the device shows the aqueous sample being wicked through the portals and blackout fabric and into internal reaction chambers. FIG. 9B top depicts a diagram showing the layers of the assembled device. Contaminated splashes access the interior of the device through portals in the top layer. FIG. 9B bottom depicts a cross-sectional view of the interior of the device, where two layers of hydrophobically patterned fabric inter-woven with polymeric optic fibers are placed in a coplanar arrangement to allow for rehydration of freeze-dried cell-free reaction components as well as to provide light input/output for excitation and emission signals. Excitation POFs are illuminated with a 447-470 nm LED arrangement, and emission fibers are bundled and aligned with an optical sensor containing an amber filter (for fluorescence readings only) and a collimating lens for magnification. The amber filter can be removed from the device in luminescence mode. FIG. 9C depicts a rapid fluorescent signal after rehydration of wFDCF constitutive sfGFP template as compared to control. Fluorescent signal in-device is statistically distinguishable from the control after 11 min ($P < 0.05$). FIG. 9D depicts activation of FDCF riboswitch with 1 mM theophylline in a wearable device as compared to 0 mM theophylline control. Fluorescent signal in-device is statistically distinguishable from the control after 19.5 min ($P < 0.05$). FIG. 9E depicts a wearable demonstration of fluorescent aptamer being activated by the presence of 50 μ M DFHBI-1T substrate as compared to 0 μ M DFHBI-1T control. Fluorescent signal in-device is statistically distinguishable from the control after 24.5 min ($P < 0.05$). FIG. 9F depicts luminescence output detected from an HIV toehold sensor with nanoLuciferase operon. HIV RNA trigger was added at 10 μ M and was statistically distinguishable from the control after 6 min ($P < 0.05$) post-rehydration. FIG. 9G depicts a wearable detection of organophosphate nerve agents using a lyophilized HRP-coupled enzyme sensor rehydrated with 50 mM acetylcholine with and without 3.7 mg/mL paraoxon-ethyl (acetylcholinesterase inhibitor). When the acetylcholinesterase is active, the Amplite-IR substrate is oxidized to generate near-IR fluorescence emission. All images above graphs correspond to time sequences of the recorded POF images in each sensor

demonstration with bundle pictures synchronized with reaction profiles. Each experiment is from three independent reaction chambers each having three fiber optic sensors, for a total of 9 fiber optic outputs. Any fibers that were 1 S.D. below the mean of all nine fiber outputs were excluded from analysis. Scale bars in brightfield images are 250 μm . LED = light-emitting diode, POFs = Polymer Optic Fibers, sfGFP = Superfolder Green Fluorescent Protein, DFHBI-1T = *difluoro-4-hydroxybenzylidene-1,2-dimethyl-1H-imidazol-5(4H)-one*, HIV = Human Immunodeficiency Virus, AChE = Acetylcholinesterase, ChOx = Choline oxidase, HRP = Horseradish peroxidase, NIR = Near Infrared.

[00242] FIGs. 10A-10D depict concentrating PURE cell-free reactions increases reaction kinetics. FIG. 10A depicts a schematic of reaction concentration through the lyophilization of PURExpress reactions at varying volumes followed by rehydration at a set volume. Using this method, synthetic biology reactions can be concentrated to enhance kinetics through molecular crowding effects or greater density of cell-free components per volume. FIG. 10B depicts representative images of PURE reactions with a LacZ output over one hour, at various concentrations. FIG. 10C depicts quantified PURExpress reactions with a LacZ output in triplicate; the error bars denote standard deviation. FIG. 10D depicts the half-maximal values from curve fitting the data shown in FIG. 10D and indicate that the 1.5x concentrated PURE reaction accelerates the signal output by more than 10 minutes. Error bars are smaller than the data points.

[00243] FIG. 11 depicts Zika DNA Toehold sensor activation in single mercerized cotton thread. Sterile mercerized cotton threads ($d = 0.2 \text{ mm}$, $L = 1 \text{ cm}$) taken from a DUKALTM Gauze pad (Dukal Corp., Ronkonkoma, NY) were coiled and deposited into single wells of a flat 384-well black polystyrene plate with a clear glass bottom (Corning Inc., Kennebunk ME). Thread samples were wicked with 2 μL of a solution containing 1x Cell-free PUREXPRESS[®] in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA), adding 33 nM Zika DNA toehold sensor (sfGFP) for sensing. Samples were frozen using liquid nitrogen and lyophilized for 4 hours. For testing, 2 μL of dd-H₂O with 1.2 μM of freshly made Zika RNA trigger was added to each of the freeze-dried samples, and fluorescence was assessed after 60 minutes under a fluorescence digital microscope Dino-Lite Edge AM4115T-GFBW (Dunwell Tech, Inc., Torrance, CA). These reactions were compared to those occurring in 2 mm disks of WHATMANTM No. 4 filter paper (GE Healthcare Lifesciences Inc., Chicago, IL). Zika RNA trigger appears to produce a higher fluorescent signal as compared to PURExpress reactions containing no template and reactions with sensor template but with no trigger.

[00244] FIG. 12 depicts antibiotic resistance sensors for *spa*, *ermA* and *mecA* genes using in-wearable sensor demonstrate specific orthogonality. Only reaction chambers with a Cas12a sensor targeting the *S. aureus* virulence factor-encoding *spa*-gene generates a detectable signal within 30 min.

[00245] FIG. 13 depicts POF fabric compatibility with lyophilized transcription-only fluorescent aptamer reactions. The left panel shows a picture of the fabric; the right panel shows a detail magnified view in. POF fabric treated to eliminate RNases was lyophilized with a fluorescent aptamer reaction containing pJL1-F30-2xd-Broccoli aptamer template and an in vitro transcription reaction (HISCRIBE™ T7 Quick High Yield RNA Synthesis Kit; NEB, Ipswich, MA). The lyophilized in-fabric sensors were activated by rehydration with a fluid splash of dd-H₂O spiked with 50 μM of the substrate (5Z)-5-((3,5-Difluoro-4-hydroxyphenyl)methylene)-3,5-dihydro-2-methyl-3-(2,2,2-trifluoroethyl)-4H-imidazol-4-one (DFHBI-1T; Tocris Bioscience, Minneapolis, MN). Upon rehydration, the in vitro transcription reaction generates an RNA aptamer that binds to the DFHBI-1T substrate, generating fluorescence.

[00246] FIG. 14 depicts sensor multiplexing using different fluorescent proteins can be detected in a single device. The top row depicts cell-free reactions demonstrating different fluorescent protein outputs generated after 30 min at 30°C. All tubes were photographed with illumination using an Invitrogen Safe Imager 2.0 G6600 Blue Light Transilluminator (Carlsbad, CA). The bottom row depicts sensor images of fiber topic bundles in (1) brightfield (intense light is placed over the sensor regions to spatially locate each fiber), (2) image when the sensor is dry, (3) image when wFDCF reaction is hydrated but without plasmid (30 min incubation at 30°C), and (4) image when wFDCF reaction is hydrated but with FP plasmids (30 min incubation at 30°C).

[00247] FIGs. 15A-15B depict additional Nanoluciferase (nLuc) luminescence experiments. FIG. 15A depicts dynamic response of a wFDCF Lyme disease RNA toehold switch sensor with luminescence output. In this experiment, 50 μL reactions consisting of 20 μL of NEB cell-free PUREXPRESS® Component A, 15 μL NEB Component B, 2.5 μL NEB murine RNase inhibitor, 19 μL Lyme disease toehold sensor DNA with nLuc reporter (6 ng/μL), 0.5 μL luciferin substrate (Promega Corp., Madison, WI) and 19 μL dd-H₂O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 3 μM *B. burgdorferi* trigger RNA freshly made for the positive samples, while 0 μM trigger RNA was used for controls. Luminescence signal from toehold sensor in-device is statistically distinguishable from the control after 13 minutes (P<0.05). FIG.15B

depicts dynamic response of a wFDCF HIV RNA toehold switch sensor with luminescence output in comparison to constitutive P_{T7}::nLuc expression as a positive control (+), which was statistically distinguishable from the negative condition after 8 minutes ($P < 0.05$). The HIV toehold reaction was prepared in 50 μ L batches using 20 μ L of NEB cell-free PUREXPRESS® Component A, 15 μ L NEB Component B, 2.5 μ L NEB murine RNase inhibitor, 19 μ L HIV toehold sensor DNA template with a nanoLuciferase reporter (6 ng/ μ L), 0.5 μ L luciferin substrate (Promega Corp., Madison, WI) and 19 μ L dd-H₂O. Prepared sensor reactions (50 μ L per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H₂O spiked with 10 μ M HIV trigger RNA freshly made for the positive samples, while 0 μ M HIV trigger RNA was used for controls. The constitutive P_{T7}::nLuc positive control reaction shown was also prepared similarly, but substituting the toehold switch in the plasmid with a T7 promoter. Results were compared to the same reactions run in a 384-well plate and analyzed using a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in luminescence mode. Activation of constitutive reaction peaked at ~8 minutes, whereas toehold with 10 μ M trigger produced its peak signal at ~15 minutes. Both the wFDCF device tests and the plate reader profiles appeared to be temporally aligned and exhibit analogous signal amplitude differences among reactions.

[00248] FIGs. 16A-16D depict fabrication of polymeric optic fiber (POF) fabric for wFDCF. FIG. 16A depicts clean 0.2 mm hydrophilic yarns made of 85% polyester and 15% polyamide were weaved in VELO style along the weft in combination with 0.25 mm un-etched poly(methyl methacrylate) POFs as warp using a standard industrial loom via Dreamlux's process (Samsara S.R.L., Milan, IT). When etched in specific regions, the cladding of POFs can be disrupted to allow for efficient excitation and emission signal collection from fluorescent or luminescent samples rehydrated within the hydrophilic fibers of the fabric. FIG. 16B depicts a three-fiber multi-strip design was achieved with a POF pitch of ~1 mm and intermediate POFs at 5 mm from the strip center for easy cutting. The reaction zone was cut to be ~30 mm in length. The width of the fabric roll was arbitrary, usually above 1 m depending on the used loom. Free POFs can then be detached from the un-weaved side to be bundled together. 16C, A roll of the hydrophilic POF fabric after weaving. FIG. 16D depicts a cut section of the hydrophilic POF fabric with indications in reaction zone and bundle ends.

[00249] FIGs. 17A-17G depicts a fabrication of textile-based wFDCF sensor patch. FIG. 17A depicts a cut strip of hydrophilic POF fabric was laser-etched (5 mm) to disrupt the POF outer cladding in the POFs sections closest to the reaction zone. FIG. 17B depicts examples of

prepared wFDCF fabric-elastomer layers and final assembly into a three-well sensor for garment integration. POFs in these devices were covered with black heat shrink tubing (6 mm). Top elastomer cover features two 5.19 x 1.85 mm curved sample ports instead of three as in the colorimetric prototypes to reduce direct light leakage on top of the POFs that may cause background light detection. FIG. 17C is a schematic of a POF-fabric-elastomer strip for sensing in a single textile layer including two excitation fibers on the sides of an emission fiber. FIG. 17D is a schematic of a double POF-fabric-elastomer strip for sensing with dedicated excitation and emission layers. This design was the one selected for further experiments due to higher hydrophilic fiber content and capacity to immobilize fluid for lyophilization. FIG. 17E is a schematic of a single excitation or emission POF-fabric-elastomer layer overlaid on an applied elastomer pattern for creating the impermeable reaction chambers. FIG. 17F depicts a finalized three-well sensor wFDCF device with heat shrunk POF covers and Luer connectors for interface with a portable spectrometer device. FIG. 17G depicts top and bottom views of a final three-well sensor wFDCF device. The blackout fabric can be seen through the sample wicking ports and serve to prevent environmental light penetration into reaction chambers.

[00250] FIGs. 18A-18B depict textile substrate compatibility testing using synthetic biology reactions and sample colorimetric reaction. FIG. 18A depicts samples of eight fabric types selected as part of the textile screening for wFDCF compatibility. Bottom icons indicate the environmental and hydration conditions that were monitored over time for analysis. FIG. 18B depicts a sample wFDCF colorimetric activation in a 1 x 1 cm cellulose matrix square containing 75 μ L of NEB cell-free PUREXPRESS® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) with 40 ng/ μ L constitutive pJL1-LacZ plasmid.

[00251] FIGs. 19A-19B depict textile screening using model constitutive P_{T7}::LacZ assay. FIG. 19A depicts a sample 384-well plate containing triplicates of BSA blocked and unblocked 2 mm discs of 30 different textile types after constitutive P_{T7}::LacZ expression following a 12-hour run for reactions containing 1.8 μ L of NEB cell-free PURExpress® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) with 40 ng/ μ L constitutive pJL1-pLacZ plasmid (+) or without plasmid as controls (-). FIG. 19B depicts examples of qualitative traces of colorimetric signals for these different fabric disks using a plate spectrophotometer (420 nm absorbance). While the traces shown here are not normalized across all samples, the increase in signal per cell indicates a color change from yellow to purple. Normalized absorbance values were calculated and used for subsequent analyses.

[00252] FIG. 20 depicts a compilation of normalized functional scoring for colorimetric wFDCF textile screening. A normalized functionality score was calculated for each of the 103

evaluated fabrics tested for compatibility with freeze-dried PURExpress reaction generating a LacZ output. This score was generated by measuring six key parameters: peak absorbance intensity at 420 nm, reaction rate, time to maximum signal, lag-time, fabric fiber density and in-fabric autofluorescence, and then multiplying normalized scores for each of these measurements, penalizing longer times to maximum signal, long lag-times and high autofluorescence. Dotted line indicates aggregated score value for Whatman No. 4 filter paper. The highest average score was observed in fabric ID#:100 containing 85% Polyester / 15% Polyamide fibers.

[00253] FIGs. 21A-21F depict fabrication of wearable microcontroller system with LED illumination and spectrometric capabilities. FIG. 21A depicts an exploded isometric view of wearable POF spectrometer components with case and electronics. The device electronics are based on a Raspberry Pi Zero W Version 1.3 (Raspberry Pi Foundation, Cambridge, UK), assembled with a PiZ-UpTime battery power board (Alchemy Power Inc., Santa Clara, CA), an environmental sensing module, an LED illumination module, and a flexible camera for imaging. FIG. 21B depicts a photograph of an open assembled device. FIG. 21C depicts a photograph of a fully assembled device ready for imaging. FIG. 21D depicts details of camera used in the device as well as the amber fluorescence emission filter and lens for magnification. Slots at the front of the bottom case fit the camera end, the LED arrangement and a vent for the environmental sensors. FIG. 21E depicts a top view of an assembled device to provide detail of compact electronics arrangement. FIG. 21F depicts an arrangement of wearable POF spectrometer with wireless connectivity in-garment for wFDCF reaction testing.

[00254] FIGs. 22A-22C depict custom mobile application software. FIG. 22A depicts a main window of the developed wFDCD sensor mobile application "Biofabrics" where spectrographic measurements are continuously recorded. Display graphs show independent color channels and bottom icons alert features such as Twitter, email, or messaging as a method of alarm in case of sensor activation. FIG. 22B depicts an environmental window of the mobile application depicts geolocation information as well as recorded measurements of temperature (°C), humidity (%) and CO₂ (PPM). FIG. 22C depicts excitation window of the application allows on-the-fly user adjustment of the LED illumination parameters of the four Luxeon Star LEDs installed in the wFDCF device using a Saber Z4 Color Mixing Array (Quadica Developments Inc., Lethbridge, Alberta). LEDs included in the current device were: 447nm, 470nm, 505nm, and 6500K white. This mobile application was developed using blynk.io (Blynk Inc., New York, NY) and the Raspberry Pi communication module. All generated data were recorded in the internal local memory of the wearable device and this application for analysis.

[00255] FIGs. 23A-23J depict validation of CRISPR-based FDCF wearable sensors. FIG. 23A depicts the sensing mechanism of CRISPR-Cas12a system is based on catalytic trans-cleavage of fluorophore-quencher ssDNA probes after activation by an RPA-amplified dsDNA trigger. FIG. 23B depicts wFDCF *mecA* CRISPR-based sensor exposed to sample containing 100 fM *mecA* trigger. FIG. 23C depicts wFDCF *spa* CRISPR-based sensor exposed to 100 fM *spa* trigger. FIG. 23D depicts wFDCF *ermA* CRISPR-based sensor exposed to 100 fM *ermA* trigger. Statistically distinguishable signals ($P < 0.05$) were observed after 72, 56 and 78 min for *mecA*, *spa* and *ermA* sensors respectively. FIG. 23E depicts experimental detection of *mecA* CRISPR-based sensor at 2.7 fM trigger was statistically distinguishable after 75 min ($P < 0.05$), corresponding to 10,000 dsDNA-copies per μL . Each experiment is from three independent wells, each having three fiber optic sensors, for a total of 9 fiber optic outputs. Any fibers that were 1 S.D. below the mean of all nine fiber outputs were excluded from analysis. FIG. 23F depicts an orthogonality demonstration of *mecA* / *spa* / *ermA* CRISPR-based multi-sensor wearable. FIG. 23G-23H depict rehydration only yielded activation of sensors when the Cas12a-gRNA sensor was in the presence of its programmed trigger dsDNA. Scale bars are 250 μm . FIG. 23I depict garment-level integration of fabric-based wearable synthetic biology sensors. Distributed continuous sensing of garment activity can be achieved through multi-bundle imaging. FIG. 23J depict Connection of fabric-based module to wearable POF spectrometer with wireless connectivity capabilities. The spectrometer electronics consist of a Raspberry Pi Zero W with a camera module (Raspberry Pi Foundation, Cambridge, UK), as well as LED illumination, environmental sensing, and custom-fabricated shields for battery power. Smartphone application for visualization and alarm of wFDCF sensor activation was based on the blynk.io platform (Blynk Inc., New York, NY) which provides support for Raspberry Pi communication. This application allows for wireless recording of experiments, control of device parameters, as well as environmental and geolocation information.

[00256] FIG. 24 depict limit of detection of wFDCF CRISPR-Cas12a based sensor activated in-fabric. The wFDCF *mecA* CRISPR-based sensor was exposed to various trigger concentrations containing 100, 27, 10, 2.7 and 1 fM *mecA* trigger, to assess in-fabric reaction fluorescence at $t = 90$ min after fluid entry as compared to controls with a scrambled trigger. Increasing concentrations of trigger lead to an increase in fluorescence signal at the evaluation timepoint as denoted by the recorded mean pixel intensity from POF regions ($n=3$). A statistically significant difference between the negative control and trigger presence was observed at 90 min only for concentrations equal and above that of 2.7 fM of trigger ($P < 0.05$),

which can be considered a limit of detection for this specific trigger, device configuration and evaluation timepoint.

[00257] FIG. 25 depict comparison of Cas13a-based SHERLOCK MRSA RNA-sensing in wFDCF in-fabric prototype against signal in a standardized plate reader. A CRISPR-Cas13a based MRSA SHERLOCK RNA sensor was prepared and freeze-dried over a wearable textile device for testing. This reaction contained Cas13a for ssRNA detection instead of Cas12a for dsDNA detection as reported for the other CRISPR-based sensors. Cell-free reactions were freeze-dried in the wearable devices for 4-8 hours and also freeze-dried in a 384-well plate for comparison in 4 μ L reaction aliquots. All reactions contained RNaseAlert substrate, a quenched fluorophore probe that is cleaved by activated Cas13a (Integrated DNA Technologies, Coralville, IA). The wearable sensor was activated with a fluid splash of dd-H₂O containing 20 nM *mecA* RNA trigger, while the plate samples were rehydrated with the same trigger concentrations to the originally deposited reaction volume (4 μ L). Reactions were monitored at 30°C for 30 minutes using the wearable optical device or and a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in fluorescence mode (Ex. 470 nm / Em. 510 nm). Normalized pixel intensity in the wearable device is comparable in behavior to the results of the kinetic run conducted in the plate reader.

[00258] FIGs. 26A-26D depict integrated wFDCF sample lysis. FIG. 26A depicts detergent combinations for cellular lysis were tested against CRISPR-Cas12a SHERLOCK reactions. Shown are reactions for the SARS-CoV-2 SHERLOCK sensor tested in various detergent dilutions. Based on these results, the 2x dilution was chosen as the optimal lysis buffer. For bacterial samples, the lysis buffer was supplemented with 100 μ g/mL of lysozyme for dissolving peptidoglycan and 5% sucrose to create a hyperosmotic environment. FIG. 26B depicts assembly of the wFDCF with lysis: top to bottom; Blackout fabric layer, Disc containing free-dried lysis reagents and lysozyme, dissolvable PVA time delay bridge (edges sealed with elastomer), freeze-dried RPA/SHERLOCK reactions in layer containing POF emission and POF excitation 26C, In-wearable wFDCF *mecA* sensors containing a lyophilized lysis buffer were challenged with intact *E. coli* cells either containing the target *mecA* gene (+, top images) or a negative control plasmid (-, bottom images). FIG. 26D depicts effectiveness of freeze-dried non-ionic surfactants. The surfactants tested in the top row left to right are Triton X-100, NP-40, and Tween-20. The surfactants tested in the bottom row left to right re Brij-58, Brij-C10, and Brij-S20. All the ionic surfactants show little or no effect on the RFU values. FIG. 26E depicts some ionic surfactants used as freeze-dried lysis reagents. From left to right these are sodium dodecyl sulfate, CHAPS

hydrate, and sodium deoxycholate. Only CHAPs Hydrate shows modest decrease in RFU, Sodium dodecyl sulfate and sodium deoxycholate show immediate impact on RFU.

[00259] FIG. 27A-27D depict bioinspired sample-wicking for textile-based wFDCF synthetic biology devices. FIG. 27A depicts a schematic of the base cover presented for the textile-based wFDCF synthetic biology devices, as well as the underlying biomechanical mechanism of water collection at the areoles of the bunny ears cactus, *Opuntia microdasys*. The high aspect ratio and agglomeration of spikes in these areoles, known as glochids, provide a high wettability gradient, which pins fluid for rapid absorption. FIG. 27B depicts modified cover for the textile-based wFDCF synthetic biology devices with *Opuntia*-inspired wicking ports. The cover features 3D-printed conical spikes (1 mm base diameter) with an aspect ratio of 1:5 arranged concentrically with 1 mm spacing. The cover was fabricated using an elastic photoreactive resin and a stereolithography 3D-printing method using a Form 2 printer (Formlabs Inc., Sommerville, MA), coated with NEVERWET® superhydrophobic coating (NeverWet LLC., Lancaster, PA). Contact angle measurements to confirm hydrophobicity of cover surfaces is also shown. 27C, Five-second time-lapse of the fluid pinning and port wicking exhibited by the device. This demonstration shows that upon fluid splash over the device, fluid rolls through superhydrophobic regions until they encounter the bioinspired ports, which readily pin the fluid, drawing it down into the underlying absorbent fabric layers inside the reaction chamber. FIG. 27D is a photograph of an assembled textile-based wFDCF synthetic biology device including the bioinspired port. Images before and after fluid splash are also shown to evince behavior.

[00260] FIGs. 29A and 29B depict implementation of Polyvinyl Alcohol (PVA) time delays for optimized multi-stage wFDCF Reactions. PVA fluidic time delays allow for wearable multi-stage reactions to occur rather than one-pot lyophilized reactions. As depicted in FIG. 29A, testing of the PVA time delays was performed by applying various PVA mixtures to filter paper, allowing to fully dry overnight, covering with impenetrable PCR tape where a 6 mm hole was punched through and aligned directly on top of the dried PVA region. A test aqueous dyed liquid was applied and PVA dissolution and wicking into the filter paper was monitored over time. FIG. 29B shows a representative experiment using a 50 µL dried time delay consisting of ~67,000 MW PVA (Millipore-Sigma, St. Louis, MO) that allows for a time delay of ~15 minutes. For the A-version sensors, 20% (w/v) PVA was used. For the B-version sensors this was reduced to 18% (w/v) to allow more facile pipetting. No substantial change in delay time was noticed between the 18% and 20% delays.

[00261] FIGs. 30A to 30G show details on the design, performance, and relevant molecular sensor sequences. FIG. 30A depict SARS-CoV-2 genomic region targeted by the RT-RPA and

SHERLOCK sensor utilized in the face-mask diagnostic of the A-version sensors, used for the experiments shown in FIG. 28D-28G. The Cas12a gRNA sensor targets a region (highlighted in green in the multiple sequence alignment) in the Spike protein gene between 22-23k of the SARS-CoV-2 region. An in vitro transcribed RNA portion of the SARS-CoV-2 genome corresponding to 22,772:23,083 was generated from a synthesized DNA fragment and used in testing. The multiple sequence alignment shows the aligned homologous regions from SARS-CoV-2 and the three circulating human coronavirus strains (OC43, HKU1, NL83, and 229E). The sequence alignment was generated using Clustal Omega (EMBL-EBI) and BoxShade (SIB Swiss Institute of Bioinformatics). The shown region corresponds to the amplicon generated from RT-RPA with the F4/R4/R3 primer mix. The sequences for the gRNA, in vitro transcribed RNA targets, and RT-RPA primers are presented in the sequence listings. FIG. 30B depicts a Laser-cut sample collection pad from capillary wicking material. The sample includes a polymeric wicking material was laser-cut with a large sample collection area (55 x 20 mm) that will be positioned inside of the mask to collect respiratory droplets and aerosols for virus detection. FIGs. 30C1-C6 depict images of the μ PAD construction. Steps for construction the μ PAD device portion of the sensor are: 30C1, a solid wax printer is used to print an array of μ PADs on filter paper; 30C2, the printed wax pattern is refluxed by application of a hot press, to fully allow the wax to penetrate the filter paper; 30C3, the individual μ PADs are cut from each sheet; 30C4, polyvinyl alcohol is added to the time delay zones and allowed to dry at room temperature overnight; 30C5, fresh lysis reagents, RT-RPA reactions, and Cas12a SHERLOCK reactions are applied to their respective reaction zones—the μ PAD is lyophilized for a minimum of 4 hours; 30C6, after lyophilization, the μ PAD is folded using RNase-free tweezers to overlap the reaction zones. FIG. 30D depicts components of the face-mask sensor before assembly. From left to right, is depicted a water reservoir with adjustable orientation, a sample collection area with fixed orientation of an absorbent side facing the patient, a μ PAD with adjustable orientation, and a LFA output with adjustable orientation. The water blister (water reservoir) allows for user-activation of the rehydration reaction. The sample collection area absorbs viral particles from the patient. The μ PAD contains the freeze-dried nucleic acid test sensor reactions, separated by PVA time delays. Finally, a lateral flow assay generates a visual output based on Cas12a-based cleavage of a FAM-Biotin probe. The orientation of the water blister reservoir, μ PAD, and LFA components can be adjusted provided the fluidic contacts are properly maintained. These components can also be either placed on the outside or the inside of the face mask. Due to operational requirements, the sample collection pad requires an orientation with the Porex surface facing the patient, and can only be positioned on the inside of the mask. FIG. 30E

depicts a fully assembled sensor. FIG. 30F depicts a demonstration of sample flow through face-mask sensor. Bromophenol blue dye was spotted at random locations throughout the sample collection zone. Upon hydration from the reservoir, a sample front can be clearly seen sweeping across the sample zone and into the μ PAD. FIG. 30G depicts the arrangement that allows for the preservation of patient confidentiality, where the LFA strip for A-version sensors are oriented with the LFA indicator surface facing the mask to hide the output from external view. The clinician must pull the strip back to observe / record the results.

[00262] FIGs. 31A-31C depict SARS-CoV-2 face-mask diagnostic B-version sensor design and construction. The B-version of the SARS-CoV-2 face-mask sensors contain a number of modifications over the A version sensors that optimize robustness and consistency. These sensors were used for the on-simulator mask experiments shown in FIG. 28A-28J FIG. 31A sub-assembly consisting of the sample collection pad, μ PAD (unfolded), and the LFA output strip, highlighting key differences between the B-version and A-version sensor components are: (1) the reservoir connection area is enlarged to ensure adequate flow to collection zones; (2) borders of the sample collection pad is rastered to fuse the porex HRM fiber media with the PET backing material, eliminating delamination; (3) porex HRM fiber media is cut with fibers parallel to the longitudinal axis of the sample collection pad to increase flow speed; (4) lysis buffer composition is altered from the A-version—triton X100 is eliminated and lowering of NP-40 percentage prevents erosion of μ PAD wax layers—CHAPS is increased; (5) PVA time delays were adjusted to 18% (w/v), decreasing viscosity for more consistent application; and (6) border areas not containing wax are blocked using hydrophobic ink to prevent fluidic short-circuits during operation. FIG. 31B depicts the fully assembled B-version face-mask sensors. The indicated changes from A-version sensors are (7) venting holes are punched into the water blister reservoir to eliminate buildup of a vacuum—the holes are overlaid with breathable water-repelling adhesive covers and (8) μ PAD area interfacing with the sample collection pad is tightly sealed to prevent unwanted fluidic short circuits. FIG. 31C depicts a B-version sensor fully integrated into a face mask. The water blister reservoir is positioned as a flap on the outside of the mask, to prevent potential crushing of the blister while the mask is being worn.

[00263] FIGs. 32A-32F depicts a breathing simulator for exhaled emission testing of the SARS-CoV-2 face-mask wearable diagnostic. FIG. 32A depicts a schematic of the key modules used in the breathing simulator. Dotted lines indicate connecting airflow through the different modules via ventilation tubing connectors. Generally, from left to right is depicted: (1) a spontaneous breathing generator; (2) an in-line aerosol producing device (e.g. nebulizer); (3) a heat sleeve, and; (4) an anatomically precise airway manikin. FIG. 32B depicts the spontaneous

breathing generator. To generate spontaneous breathing rhythms, the TESTCHEST® (Organis GmbH, Switzerland) is a full physiologic artificial lung system that can accurately replicate pulmonary mechanics such as lung vital capacity and functional residual capacity. It allows the user to control the respiration rate and tidal volume to simulate complex breathing mechanics. FIG. 32C depicts the nebulizer and heating assembly. To simulate SARS-CoV-2 laden exhalation breath plumes, the AEROGEN® Solo nebulizer system (Aerogen, Inc., Ireland) was used to generate aerosols in the simulated breath stream. The AEROGEN® platform is a medical-grade inhalation medicine device that uses vibrating mesh technology. The AEROGEN®-produced aerosols have a measured size distribution (0.4 - 4.4 microns) that matches the size range of naturally occurring lung aerosols and droplets. A self-regulating thermal pad sleeve was used to heat the simulated breath to maintain a face mask microclimate of 35°C. FIG. 32D depicts photos of the nebulizer reservoir being filled (left) and the nebulized aerosols exiting the tubing (right). FIG. 32E depicts a high-fidelity anatomically precise airway manikin (7-SIGMA Simulation Systems, Minneapolis, MN), which can simulate exhaled breath as it would exit physiologic airway structures and provides realistic fitment of the mask on a patient's face. FIG. 32F depicts a photograph showing the full air flow path from the TESTCHEST® to the AEROGEN® Solo nebulizer, through the heating sleeve, and connecting to the 7-SIGMA manikin.

[00264] Table 2. Comparison to other related technology categories

	Wearable Synthetic Biology (This work)	Current Wearable Devices	Point-of-Care Diagnostics
Wearable Form Factor	YES	YES	NO
Autonomously Functioning	YES	YES	NO
Synthetic Biology Reactions	YES	NO	YES
Molecular Sensing	YES	NO	YES
Pathogen Sensing	YES	NO	YES
Rapid Modular Programmability of Components	YES	NO	YES
Portable Instrumentation	YES	YES	YES

[00265] Table 3. Detailed comparison with other synthetic biology sensor-embedded materials.

	Stretchable Hydrogel-Elastomer Devices with Encapsulated Programmed Cells (Liu, X. et al 2017)	3D Printing of Living Responsive Materials and Devices (Liu, X. et al 2017)	Engineered Bacteria Deposited onto Textiles, Ceramics, and Plastic (Moser, F. et al 2019)	Harnessing the Hygroscopic and Biofluorescent Behaviors of Genetically Tractable Microbial Cells to Design Biohybrid Wearables (Wang, W. et al. 2017)	Programmable CRISPR-Responsive Smart Materials (English, et al. 2020)	wFDCF (This Work)
LOD	100 nM	100 nM	25 mM	n/a	11 aM	17 aM
Sensor Format	Living Cells	Living Cells	Living Cells	Living Cells	Cell-free Freeze-dried Reactions	Cell-free Freeze-dried Reactions
Matrix Material	Hydrogel	Hydrogel	Textiles, Ceramics, Polymer	Polymer	Hydrogel, paper	Textiles, polymer, paper
Shelf-stable	NO	NO	NO	NO	YES	YES
Detection target	Small Molecules	Small Molecules	Small Molecules, Light	Atmospheric Humidity	dsDNA	Hydration, Small Molecules, Proteins, RNA and DNA.
Output	Fluorescent	Fluorescent	Fluorescent	Fluorescent	Fluorescent, Colorimetric, Conductivity	Fluorescent, Colorimetric, Luminescent
Detection time	2 hrs	2-4 hrs	3-18 hrs	minutes	45 min – 24 hrs	6 min – 2 hrs
System Complexity	Simple	Moderate	Moderate	Moderate (Additional Wash Steps)	Moderate	Moderate
Wearable Format	NO	NO	YES	YES	NO	YES

[00266] Table 4. Detailed comparison with point-of-care diagnostic technologies.

Variable	qPCR	Microarrays / Fluorescent barcodes	Next Generation Sequencing (NGS)	Fluoresc e In Situ Hybridizatio n (FISH)	Toehold switches	SHERLOCK / DETECTR	wFDCF (This work)
Cost (USD)	\$0.85	\$250.00	\$23.00	Expensive	Low-Cost	Low-Cost	Low-Cost (~\$100 USD for reusable portable spectrophotometer + ~\$1 USD per sensor)
Ease of Use	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Autonomous Functioning
Readout	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Visual/ Fluorescence	Visual/ Fluorescence	Visual, Fluorescence, Luminescence and Digital
Sensitivity	aM	aM	aM	aM	fM	aM	aM
Time	~3 Hour	1 Day	>1 Day	4-16 Hour	1-4 Hour	~1-3 Hour	6 min - 2 Hour
Multiplex	YES	YES	YES	YES	YES	YES	YES
Wearable Format	NO	NO	NO	NO	NO	NO	YES

Supplemental Descriptions of Breath-Based Diagnostics

[00267] This supplemental description encompasses two main bodies of work: **1)** an engineered platform for breath-based sensing of nucleic acids for POC diagnostics for the detection of pathogens in a patient's respiratory droplets / aerosols. This platform can be integrated with the second component: **2)** freeze-dried compatible programmable nucleic acid test (NAT) reactions that are shelf-stable and can run isothermally at ambient (25 – 35°C) temperatures in an autonomous manner with minimal user intervention. Here, are specifically describe sensors for the detection of the SARS-CoV-2 virus.

[00268] *Shelf-stable and autonomous breath-based sensors*

[00269] Breath-based detection of infection and disease is a convenient, non-invasive, and information rich sample source that is underutilized. Breath samples can be used to detect the infection status of a patient for respiratory infections such as SARS-CoV-2 using a customized inexpensive isothermal nucleic acid test (NAT) based sensor. These sensors can be used in a stand-alone device or integrated into a facemask for a combined sensing and protection device.

[00270] Face masks are effective wearable devices for preventing the spread of infectious respiratory diseases. They work by trapping potentially pathogen-laden droplet and aerosol particles generated by coughing, sneezing, tussis, talking, or breathing. They are often used in tandem with laboratory diagnostics to control disease outbreaks. Patients with infectious respiratory illnesses such as the COVID-19 pandemic present unique challenges to containment. Before hospitals can triage and isolate these patients, diagnostic tests need to be performed to confirm the nature of the infection. Nasal and throat swabbing and laboratory qPCR are the current standard for diagnoses. However, the process from sample acquisition to diagnostic results can take as long as 2-3 days. Lapses in testing may occur as healthcare and laboratory infrastructure is stretched beyond capacity in an acute outbreak, resulting in crippling delays and gaps in monitoring the patient population. These delays result in poor epidemic containment responses and critical delays in executing patient therapies.

[00271] Some embodiments described herein integrate an on-board breath-sensing pathogen diagnostic that can be integrated into a face mask, thus combining both a surveillance as well as a protective function into one system. The inside of the mask collects droplet and aerosol particles as the patient wears the mask. After a certain amount of time, the clinician activates the mask, allowing water from a reservoir to sweep through the sample collection zone and into freeze-dried cell-free reactions embedded in the face mask. The output is visible on the outside of the mask and can be hidden from public view to preserve patient confidentiality. While most of the data presented here are for a face mask integrated system, an alternative embodiment is a

breath-based diagnostic similar to chemical “breathalyzers” that have a mechanism for breath capture.

[00272] FIG. 33A shows a breathalyzer 3302 –based diagnostic. Components such as a carrier fluid reservoir (e.g., a water reservoir or sachet) 3304, a sample collection unit 3306, a sample processing unit including synthetic biological components 3308 (e.g., a μ PAD) and a detection unit 3310 (e.g. an LFA) can be encased in an enclosure 3312. Except for the detection unit 3310 (or at least a visible output of the detection unit), only the general area of the fluid reservoir 3304, sample collection unit 3306, and sample processing unit 3308 is indicated since these are enclosed in enclosure 3312. The breathalyzer includes a mouth piece 3314 which the user blows or breaths into. The sample collection unit 3306 is disposed in case 3302 positioned to direct breath to the sample collection unit 3306, for example at the base of the mouth piece proximate to where the mouth piece 3314 contacts the enclosure 3312. FIG. 33B depicts a face mask 3322 diagnostic for comparison. Similar components are indicated as the fluid reservoir 3324, the sample collection unit 3326, a sample processing unit 3328 and a detection unit 3310. The components of the face mask have been previously described.

[00273] The diagnostic face mask serves to provide a rapid (~1 hour) diagnostic result without any need for hands-on manipulation aside from the activation step. This point-of-care smart personal protective equipment (PPE) ensures that patients can be quickly triaged for proper medical care, while simultaneously protecting healthcare workers and other patients from infectious droplet transmission. This autonomous diagnostic smart-PPE will fill an urgent need for a quick presumptive test (and as a mask preventing viral transmission) to supplement laboratory-based diagnostics without additional burden to hospital staff. Unlike other current SARS-CoV-2 NATs that require cold chain storage, laboratory equipment, and trained technicians, the face mask NAT sensor are freeze-dried, shelf-stable, and fully autonomous point-of-care (POC) devices. A rapid face mask-integrated SARS-CoV-2 diagnostic as presented here can relieve saturated medical systems by combining protection and sensing into a simple and easy-to-deploy wearable system, greatly improving patient outcomes. The face mask system could be further developed to discriminate between SARS-CoV-2 and other respiratory viruses, such as influenza, or for monitoring emerging SARS-CoV-2 mutant strains.

[00274] The results section below detail the design and validation of freeze-dried cell-free SARS-CoV-2 nucleic acid sensors based on various nucleic-acid detection platforms, including transcriptional toeholds, Cas13a, or Cas12a-based switches that work at ambient temperatures. In vitro data is then presented demonstrating the functional performance of the freeze-dried isothermal NAT sensors for detecting SARS-CoV-2 nucleic acids.

[00275] From initial activation of the face mask sensor to a final readout only takes ~40-90 min. The limit of detection observed these sensors is 500 copies (17 aM) of SARS-CoV-2 in vitro transcribed (IVT) RNA, which is comparable to other laboratory-based diagnostics (e.g., RT-PCR) which require trained technicians and a full suite of specialized laboratory equipment. These sensors are specific for SARS-CoV-2 and do not cross react to RNA from other commonly circulating human coronavirus strains (HCoV). Most critically, these mostly hands-off diagnostic reaction proceeds to completion at room temperature, which is the first SARS-CoV-2 NAT that is able to achieve high sensitivity and specificity at ambient temperatures, thus obviating the need for any heating instruments and allowing for integration into a wearable format. Details on the design, performance, and relevant sequences are presented herein.

[00276] All of the sensors presented in this invention disclosure (toeholds, Cas13a, Cas12a) target regions of the SARS-CoV-2 Spike gene (FIG. 30A), which encodes for the primary receptor of the virus that is essential for infectivity. Moreover, this region shows high sequence divergence from other circulating human coronaviruses (HCoVs), allowing for specific discrimination for SARS-CoV-2 and variant strains harboring mutations in the spike gene.

[00277] *Summary - Aspects of a Breath-based NAT Diagnostic Platform*

[00278] The modular sensor inserts can be used in nearly any face mask. These sensor inserts contain the following modules: a source of water, followed by a wicking area that absorbs droplets and aerosols generated by the patient's breath, porous materials containing freeze-dried lysis, isothermal amplification, and toehold or CRISPR-based NAT sensors. An attached lateral flow assay for visual output or optical, electronic, chemical, or biochemical means for assessing the result of the NAT sensor. Each of the four modules are described below. These, and other details, have been previously described, for example with reference to FIG. 30B-30G, 31A-31C, and 33B.

[00279] 1. Water source: This would be an encapsulated sachet containing water or aqueous buffer which can be mechanically or electronically actuated to release the fluid to flow into the remainder of the sensor.

[00280] 2. Breath sample collection area: This area consists of a capillary wicking material which is positioned on the interior of the mask in front of the patient's nose and mouth, for the absorption and collection of viral or bacterial-laden aerosol or droplets generated from breathing, coughing, sneezing, or speaking. The material could be a polymeric, natural fiber, metal, of nonmetal porous material that would support capillary wicking. This sample collection area is positioned and connected downstream of the activatable water source and upstream of the freeze-dried NAT sensors.

[00281] 3. Freeze-dried NAT sensors: The Nucleic Acid Test (NAT) sensors will consist of any combination of sample lysis reactions, isothermal or thermal amplification reactions, and nucleic acid sensing reactions such as toehold switches, Cas13a-SHERLOCK and Cas12a-SHERLOCK reactions. All of these reactions are freeze-dried into a substrate which may or may not be porous. They may also be encapsulated in a dissolvable polymeric matrix. They can be mixed into a single “one-pot” reaction or spatially divided into a series of sequential reactions. As the rehydration front carrying the viral or bacterial samples from the breath sample collection area enter the NAT reaction zones and rehydrate the reactions, fluidic time delays may be positioned between each reaction zone. These time delays consist of dissolvable polymeric, sugar, or inorganic freeze-dried barriers that separate one reaction from another, thus providing a tunable time delay allowing for sufficient completion of one reaction before the next reaction is encountered. The output from the combined NAT may be a visual or fluorescence signal which may require electrochemical or electronic optical detection. In addition, a simple lateral flow assay device connected to the NAT reactions can convert a positive signal to a visual signal detectable by the eye.

[00282] 4. Lateral Flow Assay (LFA) Output: Custom LFAs can be used to generate a colorimetric or fluorescent response by using spatially immobilized affinity molecules, such as antibodies, other proteins, or nucleic acids and a molecular signal generated from the NAT. For example, nucleic acid probes present in Cas12a-SHERLOCK reactions are cleaved when the Cas12a ribonucleoprotein complex attains trans-cleavage DNase activity upon cis-recognition of the target nucleic acid. The cleaved nucleic probes bind to different regions of the LFA, creating a detectable spatial pattern. LFA output is used here as an example, but other output methods can be used as well, whether visual or nonvisual. Furthermore, some LFAs can provide multiple detection bands, where output of the LFA can be multiplexed for detecting different items in one sample.

[00283] *Proof of Concept Experiments*

[00284] Toehold switch sensors were previously described (e.g. FIG. 6A). These are programmable synthetic riboregulators that allow protein expression only when a specific trigger RNA is present. These sensors consist of an mRNA molecule designed to include a hairpin structure that blocks gene translation in cis by sequestration of the ribosome binding site and start codon. Hybridization to a complementary trigger RNA results in secondary structure rearrangement, facilitating ribosomal translation of an output gene (FIG. 6A and FIG. 34A). This technology allows for regulatory control of the various gene outputs (e.g., fluorescent / luminescent proteins, enzymes, or aptamers) to be conditionally dependent on the presence of an

input nucleic acid molecule in the environment. The toehold circuit can be encoded as DNA with a transcriptional promoter with a cell-free system (either a cell lysate or reconstituted system) and the entire reaction can be freeze-dried for shelf-stable storage and transport. A library of toehold sequences was designed and screened to detect regions in the S gene of the SARS-CoV-2 virus (FIG. 35A-35U) to generate a LacZ output, which generates a measurable colorimetric signal in the presence of the substrate chlorophenyl red- β -D-galactopyranoside (CPRG). Several toehold switches (01, 03, and 04—FIG. 35C, 35E and 35F) with significant activation when presented with SARS-CoV-2 viral RNA were identified. All of the sequences for toehold switches in the library are presented in the sequence listings (Table 7).

[00285] Additional description regarding FIG. 34 and 35A-35U is as follows. FIG. 34 depicts toehold switches which are RNA transcripts locked through designed secondary structure. In the presence of a target RNA molecule, strand-invasion driven hybridization unlocks the toehold allowing translational components to express the output gene. FIG. 35A-35U depict plots screening of a library targeting regions in the SARS-CoV-2 S-gene. All output was LacZ (beta-galactosidase enzyme), which generates a colorimetric response using the substrate chlorophenyl red- β -D-galactopyranoside (CPRG). Negative control (EBOV) is an unrelated toehold designed against the Ebola virus. Positive control is LacZ with no toehold. Plots show trigger (blue) and no trigger (orange).

[00286] *Ambient Temperature Cas13a SHERLOCK for SARS-CoV-2.*

[00287] Cas13a-SHERLOCK sensors leverage the ability of Cas13a ribonucleoprotein (RNP) complexes for sensitive detection of RNA nucleic acids. In the presence of a designed crRNA / gRNA, the Cas13a enzyme can be easily programmed to detect different sequences. The key aspect of these enzymes that allow for their use in NAT diagnostics is their unique ability as an activatable nonspecific nuclease. Once the Cas13a-gRNA complex finds its target RNA and cleaves it (known as cis-cleavage), the enzyme undergoes an irreversible conformational change which activates two HEPN domains, allowing it to cleave non-specific RNA molecules in the environment (known as trans-cleavage or collateral cleavage). To achieve a fluorescent sensor, a reporter molecule is designed in which a fluorescent moiety is rendered non-fluorescent through physical coupling to a quenching molecule through an RNA bridge. In the presence of the target RNA and this quenched probe, the cis-activated Cas13a begins to degrade the probe via trans-cleavage and thus generates fluorescence. The probe could be modified to have other chemical moieties at either end to allow for compatibility with various commercially available colorimetric lateral flow assays, obviating the need for a fluorescence detector.

[00288] FIGs. 36A-36Q depict a library of gRNAs that were screened to allow Cas13a direct sensing of SARS-CoV-2 vRNA. The Cas13a sensors with the best switching activity (high signal and low background) for the detection of SARS-CoV-2 were gRNAs 7, 8, 9, and 11 as shown by the summary plot FIG. 36Q. These experiments utilized the Cas13a ortholog from *Leptotrichia wadei*, although other Cas13 enzymes could be used with the same spacer sequences presented here that target the vRNA. All the Cas13a gRNA sequences in the library are presented in the sequence listings (Table 7).

[00289] *Ambient Temperature Cas12a SHERLOCK for SARS-CoV-2*

[00290] For DNA-based detection of SARS-CoV-2, a Cas12a ortholog from *Lachnospiraceae* bacterium (LbaCas12a) was used. The advantages of using this detection method include highly sensitive detection of nucleic acids (activated Cas12a approaches diffusion-limited kinetics), high specificity (capable of single-base pair discrimination), greater stability of the ssDNA output probe, and the ability to operate at room temperature. Upon recognition of the dsDNA amplicon, the cis-activated Cas12a-gRNA complex gains indiscriminate ssDNA nuclease activity, allowing for detection through cleavage of a quenched ssDNA fluorophore probe (FIG. 37A). A library of 11 Cas12a gRNAs for the detection of dsDNA amplicons generated from the SARS-CoV-2 vRNA S-gene was screened. These designed gRNAs contained the requisite 5' TTTN protospacer adjacent motif (PAM) on the DNA strand opposite the target. For this library, each Cas12a-gRNA complex was challenged with water only (negative control) or water spiked with a dsDNA fragment encoding for the entire spike gene region of SARS-CoV-2 and monitored the reactions for fluorescence, indicative of activated Cas12a trans-cleavage of the fluorescent probe (FIG. 37B). The results, presented in FIG. 37C-37N, demonstrate that all of the screened gRNAs designed show switching activity for the detection of SARS-CoV-2 spike gene amplicons. All of the Cas12a gRNA sequences are listed in the sequence listings (Table 7).

[00291] Additional description regarding FIG. 37A-37N is as follows. FIG. 37A depicts that when coupled with a reverse transcriptase amplification reaction, Cas12a SHERLOCK sensors can detect dsDNA amplicons and cleave ssDNA reporters. FIG. 37B depicts the experimental setup for screening a library of 11 gRNAs targeting a dsDNA fragment of the SARS-CoV-2 S-gene for activity. FIG. 37C-37N depict that all gRNAs showed switching activity in the presence of amplicon (green data points), with low signal when no amplicons are present (gray data points). The data is summarized in the plot shown in FIG. 37N.

[00292] *Ambient Temperature Isothermal Amplification of the SARS-CoV-2 S gene fragment using RT-RPA.*

[00293] From the various sensors screened, toeholds, Cas13a-SHERLOCK, and Cas12a-SHERLOCK, the Cas12a sensors were selected as the final sensors for integration into the face mask device due to the following reasons: robust performance, relative stability of the ssDNA probes, and the minimal components needed for operation.

[00294] To use Cas12a sensors for the detection of a virus with an RNA genome such as SARS-CoV-2, the amplicon generation requires a step in which reverse transcriptase (RTase) is paired with an amplification reaction such as PCR, NASBA, LAMP, or RPA. This critical step generates a dsDNA amplicon from the viral RNA that can be detected by the Cas12a RNP (FIG. 38A). The amplification also serves to amplify the nucleic acid target of interest to enhance sensitivity. A key requirement here is the identification of conditions for reverse-transcriptase and isothermal amplification that is capable of sufficiently amplifying the SARS-CoV-2 vRNA at ambient temperatures (25-35°C) that do not require external heating. This is nontrivial, for the following reasons: 1) RNA secondary structure is problematic for efficient probe hybridization for RTase reactions^{11,12}, 2) Viral genomes, especially coronaviruses such as SARS-CoV-2, contain an uncommonly high degree of secondary structure motifs that make probe hybridization very difficult without using a heating step¹³⁻¹⁵, and 3) all commercially available RTases have optimal activities at 42–48°C.

[00295] Several primer combinations were screened under the desired conditions for the detection of SARS-CoV-2 RNA, using an RNA fragment (named F5R11) corresponding to a region of the spike gene for screening. The amplification method chosen was a one-pot RTase (PrimeScript from Takara Bio) and RNase H (from ThermoFisher) reaction paired with RPA (Recombinase polymerase amplification, from TwistDx). The cDNA is generated from the RTase + RNaseH activities and is then amplified by the RPA reaction. RPA was chosen over other isothermal DNA amplification methods due to its capacity for lyophilization, ability for amplification under ambient temperatures, and compatibility with Cas12a-SHERLOCK sensors.

[00296] The majority of the RT-RPA primers screened performed poorly, which is likely due to the aforementioned RNA secondary structure complexity of the SARS-CoV-2 genome. This increased complexity of SARS-CoV-2 RNA genome folding is likely to prevent efficient primer hybridization. However, a set of RT-RPA primers that were able to generate an extremely fast sensor response in a two-pot RT-RPA > Cas12a SHERLOCK reaction (using gRNA-06) were identified. The reaction was able to reach a signal plateau within only 10 minutes, when challenged with the F5R11 RNA fragment. The primer set consists of one forward primer and two reverse primers (F4 / R4 / R3), can successfully amplify full-length SARS-CoV-2 vRNA, and has a determined optimal concentration ratio of (10:10:20 pmols). See FIG. 38B-38E.

[00297] Additional description regarding FIGs. 38A-38E is as follows. FIG. 38A is a diagram of the RT-RPA reaction which isothermally generates DNA amplicons from a viral RNA target. FIG. 38B depicts the experimental setup for screening of primer pairs for successful RT-RPA amplification. FIG. 38C depicts the F4-R4-R3 primer set allows for rapid detection of SARS-CoV-2 RNA fragments and full-length genome. FIG. 38D depicts the optimization of the primer molar ratio for the F4-R4-R3 set. FIG. 38E depicts the signal over background at 30 min.

[00298] The performance of this reaction RT-RPA-Cas12a reaction when containing lysis buffer reagents meant to release nucleic acid targets from viral particles and/or bacterial cells to determine the optimal compatibility with these reagents was also tested, as shown in FIG. 39A. The final determined optimal lysis reagents recipe for compatibility with the freeze-drying process, RT-PCR, and Cas12a SHERLOCK is shown in FIG. 39B and 39C.

[00299] *SARS-CoV-2 Face Mask Sensor Insert Design and Assembly.*

[00300] The design of the sensor to be integrated into any face mask consists of four modular components (e.g. as previously shown by FIG. 30A-30G, 31A-31C): 1) a water sachet or reservoir that can be activated by the clinician to drive the reactions. 2) downstream of the water reservoir is a large sheet of porous material that is the sample collection zone. This module is positioned over the nasal and oral passageways of the patient to collect aerosolized breath samples, such as SARS-CoV-2, that have been shown to be present in exhaled breath. 3) downstream of the sample collection zone is an arrangement of the freeze-dried NAT reaction components -- lysis reagents, RT-RPA for isothermal amplification, and toeholds/Cas12a/Cas13a for nucleic acid detection. These can be arranged as separate reactions or as one-pot reactions. For rapid prototyping, stacked filter paper assemblies that have been patterned with hydrophobic materials to contain the flow were used. These Microfluidic Paper-based Assay Devices (μ PADs) allow us to flexibly reconfigure the freeze-dried reactions and rapidly generate prototype devices for testing. It was found that incorporating time delays in the form of dried polymeric bridges allowed temporal control of each reaction rehydration. The time of the delay could be easily tuned by controlling the amount of dissolvable polymer added. After screening, it was found that time delays made of polyvinyl alcohol (PVA) with an average molecular weight of $\sim 67,000$ kDa efficiently provided delays of 10-15 minutes between each reaction. 4) downstream of the lyophilized reactions / μ PAD a universal commercial lateral flow assay strip was incorporated, which is able to couple cleavage of the Cas12a probe (a positive result) into a visual band pattern for test result output.

[00301] The operation of the fully assembled mask is as so: 1) a presumptive COVID-19 patient is immediately fitted with a mask to prevent infectious spread. 2) the patient's breathing,

coughing or sneezing will allow the sample collection zone to collect viral particles. Previous studies have shown that infected patients can generate up to 10^4 - 10^5 SARS-CoV-2 viral copies per minute during normal breathing. Thus, it can be expected that within 30 minutes, at least 300,000 viral copies would be present on the surface of the sample collection zone. At one hour, 600,000 copies could be expected. 3) at an indicated time after the patient has worn the face mask, the clinician would press a button on the mask that activates the water reservoir by puncturing the sachet. This event would mark the beginning of the sensor activation and is the only hand-on time required from the clinician. 4) the water or buffer would begin to flow by capillary wicking action through the sample collection material, sweeping along any virions along the sample front. 5) the sample would then enter the lysis reaction zone in the μ PAD. The rehydrated detergents and osmotic additives would release the nucleic acid inside the viral particles. 6) after dissolving the first PVA time delay, the sample would then move into and rehydrate the RT-RPA zone, allowing for isothermal amplification of the liberated viral genome, converting the vRNA into a dsDNA signal. 7) after dissolving the second PVA time delay, the sample moves into the final reaction zone containing precomplexed Cas12a-gRNA and a nucleic acid probe. The type of probe may be variable, but here was used a ssDNA probe which has a FAM molecule on one end and a Biotin molecule on the other (FB probe). If the target DNA amplicon from the RT-RPA is present, the Cas12a-gRNA will cis-cleave the target and enter a trans-DNase state allowing it to repeatedly cleave the FB probe. 8) after dissolving the third and last PVA time delay, the sample enters into a LFA in which freely floating gold nanoparticles conjugated with anti-FAM antibodies are freeze-dried. Also on the LFA are areas in which affinity molecules have been immobilized: The “C” band consists of a biotin-binding protein and the “T” band consists of an antibody that binds to the anti-FAM antibody that is conjugated to the gold nanoparticles. In the event of a negative SARS-CoV-2 result, the FB probe is not cleaved, resulting in the gold nanoparticles being tethered to the “C” line due to the FB probe. In the event of a positive result, the FB probe is cleaved and the gold nanoparticles flow past the “C” band and are bound at the “T” line. In the tests of the face mask sensors, it was found that negative results using the FB probe often results in the appearance of “C” and “T” lines, likely due to gold nanoparticles that are able to escape binding at the “C” line. Thus, for the results, the “C/T” ratio was determined based on the relative intensities at these bands to ascertain a negative test result from a positive test result.

[00302] *Fabrication of SARS-CoV-2 diagnostic face mask (A-version).*

[00303] The SARS-CoV-2 in-mask breath diagnostic consists of the sensor assembly containing the lyophilized reactions which was then inserted in an N95-equivalent face mask.

First, capillary wicking material (Porex high release media #36776, thickness = 0.5 mm, density = 0.07 g/cc, porosity = 92%) was laser cut into a shape allowing for an elliptical region approximately 50 x 25 mm that serves as the sample collection area, accumulating viral particles from a patient's respiration, vocalization, and/or reflexive tussis. The laser-cut wicking material is then adhered to a white PET double-adhesive backing material (3M Microfluidic Diagnostic Tape, #9965). One end of the wicking material is adhered to a sterile sealed blister pack containing nuclease-free water. The μ PAD device is created by wax printing hydrophobic patterns onto WHATMAN® Grade 1 chromatographic filter paper (Thermo Fisher Scientific, Waltham, MA) using a Xerox Phaser 8560 solid ink printer. The printed μ PAD sheets were then wax reflowed by hot pressing for 15 sec at 125°C using a Cricut EASYPRESS™ (Cricut Inc., Fork, UT), and then left untouched to cool at room temperature. After wax reflow, the reaction zones have an aperture diameter of 5 mm, while the intervening PVA time delays have an aperture diameter of 3 mm. The PVA time delays were placed onto the time delays zones first, by pipetting 4 μ L of 10% ~67,000 MW PVA (Millipore-Sigma, St. Louis, MO) per each delay layer, and allowing it to dry at room temperature overnight. The lysis buffer, RT-RPA reaction, and the Cas12a SHERLOCK reactions as described below were then added to the respective lysis zones.

[00304] The lysis reaction added to each sensor lysis zone can consist of 0-100 mM Tris-HCl (pH 7.5), 0-5% Triton X-100, 0-5% NP-40, 0-10% CHAPS, 0-500 μ g/mL lysozyme, and/or 0-15% sucrose. The RT-RPA reaction added to the isothermal amplification zone was 15 μ L of a single lyophilized TWISTAMP® lyophilized RPA pellet (TwistDx Limited, UK) that was rehydrated to 50 μ L using a rehydration reaction of 29.6 μ L Twist Rehydration Buffer, 9.6 μ L of a primer mix (see Table S2; RT-RPA-F4, RT-RPA-R4, RT-RPA-R3 primer in the mix are at a ratio of 10 μ M : 10 μ M : 20 μ M). Other primer concentrations and other reaction volumes may be used. Roche Protector RNase Inhibitor, TAKARA PrimeScript Reverse Transcriptase, and Ambion RNase H were all added at 1 μ L each. Nuclease free water was added at 4.4 μ L. Immediately before pipetting onto the reaction zone, 2.5 μ L of 280 mM MgOAc was added to the RT-RPA reaction and thoroughly mixed. For the Cas12a SHERLOCK reaction, 15 μ L of the following reaction was pipetted onto the SHERLOCK reaction zone: 12.3 μ L nuclease-free water, 1.5 μ L of NEB Buffer 2.1, 0.3 μ L 0.5 M DTT, 0.075 μ L of 100 μ M NEB ENGEN® Lba Cas12a, 0.26 μ L of coronavirus S-gene gRNA. Immediately before pipetting onto the reaction zone, 0.6 μ L of the 6-FAM/TTATTATT/Biotin oligo (FB probe, from Integrated DNA Technologies, Inc., Coralville, Iowa) is added and thoroughly mixed. Sequences for all primers, RNA targets, and gRNA are presented in the sequence listing (Table 7).

[00305] All reactions are pipetted onto the reaction zones and the wax-printed sheet was then dipped into liquid nitrogen to freeze all of the embedded reactions and then immediately wrapped in foil and placed on a lyophilizer. After lyophilization for 4 hours, the wax arrays were removed from the lyophilizer and carefully cut into strips. Each strip was folded using sterilized tweezers into an overlapping accordion arrangement, overlapping the reaction zones and time delays to form a μ PAD device. The output end of the laser-cut Porex sample collection section was carefully inserted on top of the lysis zone, while the input end of a Milenia HybriDetect-1 Universal Lateral Flow Assay (TwistDx Limited, UK) was inserted on top of the last PVA time delay. The entire μ PAD section was carefully sandwiched and taped together to compress all of the layers. The entire blister-pack water reservoir – Porex sample collection area – μ PAD – LFA test strip is secured using the double-sided backing to the inside of an N95 equivalent mask, positioning the sample collection area in the region directly in front of the mouth and nose. The LFA test strip is routed to the outside of the mask through a small slit in the mask and the indicator can be oriented to hide the results from external viewing, to ensure patient confidentiality. To access the results in this configuration, the test strip must be bent over to view the results. Lastly, a button was affixed to the outside of the mask directly over the water blister reservoir. The button contains a small spike embedded in a compressible foam double-sided adhesive material. When pressed down, the button pierces the foil on the blister, allowing the nuclease-free water to flow through the same collection zone, the μ PAD reaction zones and time delays, and finally into the LFA indicator strip.

[00306] *Fabrication of SARS-CoV-2 diagnostic face mask (B-version).*

[00307] During development and testing of the face mask sensors, an updated version of the sensors was designed that improved flow throughput and reduced the potential for leakage. The sensors were named “B-version” sensors to distinguish them from the initial prototype sensors, which are designated “A-version” sensors. Wax-printed μ PAD templates were prepared as described above for the A-version sensors. To prevent failure from flow leakage between different layers of the folded μ PAD, unwaxed borders were rendered hydrophobic by drawing over the area with a Super PAP Pen (ThermoFisher, Inc., Waltham MA) and allowed to air dry for at least one hour. The sample collection pads for the B-version sensors were laser-cut from sheets of Porex high release media #36776 with the dominant fiber direction along the long axis of the pad to allow faster flow of the hydration front. The pad geometry was adjusted to enhance water flow by moving the reservoir puncture point to the distal end of the water blister, increasing the pad area in contact with the water reservoir, and reducing the sample collection region. Approximately 2 mm of the outer border of the sample pad was rastered during laser-

cutting to heat seal the Porex material to the PET backing material, preventing delamination. Before assembly, approximately 1 cm of the backing material was peeled away and cut off from the end of the sample pad region that is to be in contact with the reservoir.

[00308] Prior to the addition of the reagents to the μ PAD, each reaction zone area was blocked with 5 mL of 1% BSA + 0.02% Triton X-100 and allowed to air dry for 12 hours to prevent nonspecific adsorption of the biochemical reaction components to the filter paper matrix. PVA at a concentration of 18% (w/v) at a volume of ~ 5 μ L was applied to each time delay zone and allowed to air dry for 24 hours. The lysis buffer for the B-version sensors was reformulated to 10 mM Tris-HCl (pH 7.5), 5% Sucrose, 0.02% NP-40, and 2% CHAPS. The amount of non-ionic surfactants in the lysis buffer were reduced to prevent observed degradation of the wax barrier, an observation made during design and testing of the A-version μ PADs. The CHAPS concentration was increased as it was not found to degrade the wax and this zwitterionic detergent has previously been shown to be effective in lysing coronavirus particles. A volume of 10 μ L of this lysis buffer was added to the μ PAD lysis zone. The RT-RPA and Cas12a SHERLOCK reaction compositions, volumes, and lyophilization parameters were unchanged. During final assembly of the B-version sensor, both the sample pad:: μ PAD and the μ PAD::LFA contact regions were fully sealed using precut sterile aluminum PCR foil seals (#60941-076, VWR Intl., Radnor, PA) to improve contact transfer and prevent any fluidic short-circuiting that may occur from undesired droplet contact to the folded μ PAD edges. To facilitate unimpeded sample flow, venting holes were introduced into the water-containing blister mold to prevent vacuum buildup inside the blister during flow. Three venting holes were punched into the blister surface using an 18-gauge needle and then sealed with a 6-mm adhesive disc of a single-sided rayon breathable hydrophobic porous film (#60941-086 VWR Intl., PA). This allows venting of vacuum while preventing leakage and contamination of the nuclease-free water. For all B-version face-mask-integrated sensors, the water reservoir module was positioned on the exterior of the mask to minimize unwanted contact pressure on the blister pack during wearing of the mask. The sensor activation mechanism is the same as the A-version sensors. To integrate the sensors into the face masks, 1 cm slits were cut into KN-95 masks through which the sensor ends were threaded and subsequently sealed using adhesive.

[00309] *Polymer Time Delays Allow for Efficient Spatial Coordination of Reactions.*

[00310] In between the key reaction zones containing the lyophilized lysis, RT-RPA, and CRISPR sensor reactions, regions of the paper-based μ PAD can be inserted which are designed to act as time delays. Such time delay devices that can be integrated into the paper matrix are useful for functioning of the sensor. Many of the enzymatic reactions of the sensor require a

sequential progression through the different reactions in a multi-step process. Much of this is due to different buffer conditions required for each enzymatic reaction. It was found that the most significant incompatibility occurs when mixing RPA and Cas12a SHERLOCK reactions in a single “one-pot” reaction, which results in poor product yields for both reactions. Alternatively, it was found that the lysis, RTase, RNaseH, and RPA components could be used in a one-pot reaction with minimal impact on the RPA amplicon yield. Hence, a programmable time delay that would regulate the spatial and temporal dynamics of each reaction in the μ PAD was designed using a dissolvable polymeric barrier made of polyvinyl alcohol (PVA) to improve the sensor performance. These PVA time delays are created by applying molten PVA solutions to the paper substrate of the μ PAD and allowing it to completely air dry. The dried PVA barrier acts as a plug to prevent the rehydration front from progressing immediately to the next reaction zone. As the PVA barrier slowly dissolves, this allows the rehydrated reaction preceding the barrier to incubate and execute for a period of time, before the barrier is breached and the flow continues onto the next reaction zone. The timing of the PVA dissolution can be tuned simply by altering the percent solution of the PVA, where there is a positive correlation between the % of PVA and the amount of time delay. For the μ PAD, it was empirically determined that a 15-20% percentage solution of PVA with an average molecular weight of $\sim 67,000$ and 86.7-88.7 mol% hydrolysis (Millipore-Sigma, 81383) was optimal to create the needed time barriers in the SARS-CoV-2 μ PAD modules. When the PVA was dissolved in nuclease-free H₂O at 80°C and ~ 4 μ L of this molten solution was applied to a ~ 12.56 mm² area of the filter pad substrate, a time delay of 15-30 minutes was achieved (Figure 11). This amount of time was determined to be sufficient for each of the reactions to reach an adequate yield.

[00311] *Benchtop Testing of the SARS-CoV-2 Diagnostic Face Mask.*

[00312] Fully assembled A-version sensors against SARS-CoV-2 vRNA were tested on the benchtop to assess the sensitivity and specificity. For FIG. 28F, 28G, 28I, and 28J each data point consisted of a face mask sensor in which a defined amount of synthetic SARS-CoV-2 RNA fragment containing the specific gRNA targeting region of the SARS-CoV-2 spike gene was generated by in vitro transcription using the HISCRIBE™ T7 Quick High Yield RNA Synthesis Kit (NEB, Ipswich, MA) using synthetic DNA templates with a T7 promoter (Integrated DNA Technologies, Inc., Coralville, Iowa, and Twist Bioscience, San Francisco, CA). Corresponding homologous regions to the spike gene for the commonly circulating human coronavirus strains 229E, HKU1, NL63, and OC43 were determined by sequence homology alignment of the respective spike genes and the RNA targets were generated using the same method described above. The indicated amount of target RNA was spotted onto the breath collection zone to

simulate viral deposition. All SARS-CoV-2 face mask sensors were tested at room temperature at ambient humidity. After activation and LFA output formation (~20-30 min), the LFA strips were digitized using the scanner function on a Ricoh MP C3504 on default contrast settings. This ensured equal brightness and contrast across all strips in comparison to photography. Each “T” and “C” output line from each strip was quantified in ImageJ from the raw scanned images without any adjustments to brightness or contrast.

[00313] The results demonstrate that the face mask sensor can reliably detect SARS-CoV-2 nucleic acid signatures with high sensitivity and specificity. The limit of detection observed for these sensors is 500 copies (17 aM) of SARS-CoV-2 in vitro transcribed (IVT) RNA, which matches the sensitivities of current WHO-endorsed standard laboratory-based RT-PCR assays (FIG. 28F and 28G). The limit of detection threshold, +3 S.D. of the no-template control (NTC), is shown as a red dotted line. The sensors also do not cross react to RNA from other commonly circulating human coronavirus strains (HCoV) as shown in FIG. 28I and 28J). Most critically, these hands-off POC diagnostic reaction proceeds to full completion even at room temperature, which is considered sub-optimal for RT, RPA, and Cas12a activities. Current NAT assays for the detection of SARS-CoV-2 require cold-chain storage, a laboratory with specialized equipment, trained personnel, and typical has a 24 to 48 hour turnaround from sample collection to result. In contrast, the sensor is shelf stable, requires no specialized equipment, runs autonomously with minimal user interaction, and returns results in under 90 minutes.

[00314] *Validation of Face Mask Integrated SARS-CoV-2 Sensor on a Patient Simulator.*

[00315] To establish the function of the face mask integrated sensor under clinically relevant parameters, assembled B-version sensors were tested using a custom-assembled breathing simulator (FIG. 28H, 32A-32F). The simulator consisted of four modules that performed the following functions: spontaneous breath generation, aerosol production, heating control, and physiologic airway and head simulation. For the breath generation, the TESTCHEST® lung simulator (Organis GmbH, Switzerland), a highly accurate artificial lung that uses an actuated dual bellows design to replicate lung mechanics such as lung vital capacity and tidal volume, was employed. The TESTCHEST® was connected through ventilator tubing to all other downstream modules for simulated spontaneous breathing. Directly downstream of the TESTCHEST®, was placed an in-line AEROGEN® Solo nebulizer (Aerogen, Inc., Ireland). The AEROGEN® Solo is a medical-grade vibrating-mesh nebulizer for the administration of lung inhalation therapeutics. Previous studies have demonstrated that the nebulizer generates aerosol droplets that are similar in diameter to those that occur naturally from human lung emissions. Furthermore, previous work has used the AEROGEN® system to deliver therapeutic RNA in an

animal model, showing that it can be used to produce transmissible RNA-laden aerosols. The tubing is next wrapped in a temperature-regulated heat pad (Zoo Med Laboratories, Inc., San Luis Obispo, CA) that maintains the output temperature at 35°C. The tubing is connected to a lung input tube in a high-fidelity airway manikin (7-SIGMA Simulation Systems, Minneapolis, MN) that faithfully replicates pulmonary and nasopharyngeal structures as well as head movement ranges. The other simulated lung and the simulated esophagus are clamped shut to direct breath output only through the oral cavity.

[00316] B-version face masks were assembled with the blister pack reservoir and LFAs on the outside of the mask. The face mask tests on the breathing simulator used a spontaneous breathing rate of 12 breaths per minute and a temperature was set to maintain an outflow temperature of 35°C. A 5 mL solution of SARS-CoV-2 F5R11 vRNA IVT target was then nebulized using the AEROGENTM Solo unit. The simulated breath was allowed to collect in the face mask and on the sensor collection pad for a period of 30 minutes, then the sensor was activated on the manikin for processing while maintaining the breathing and heating. The LFA outputs for all sensors were scanned using a Ricoh MP C3504 printer system using default settings. The total amount of aerosolized vRNA collected after 30 minutes on each mask sensor for a given concentration of vRNA IVT target solution was estimated by RT-qPCR analysis of a 6 mm filter paper disc affixed to the sample pad area. Under these realistic simulation conditions, the face-mask sensor was able to detect SARS-CoV-2 vRNA after a breath sample collection period of 30 minutes, with a calculated accumulation of 10^6 - 10^7 vRNA copies on the sample pad, as determined by RT-qPCR (Figure 13C-D). Clinical measurements have previously shown that the SARS-CoV-2 breath emission rate of infected patients could reach an output 10^3 - 10^5 copies/min.

[00317] All patents and other publications identified in the specification and examples are expressly incorporated herein by reference for all purposes. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00318] The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting of the invention. As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including,” “includes,” “having,”

“has,” “with,” or variants thereof, are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[00319] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art. Furthermore, terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art, and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

[00320] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. Numerous changes to the disclosed embodiments can be made in accordance with the disclosure herein, without departing from the spirit or scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described embodiments. Rather, the scope of the invention should be defined in accordance with the following claims and their equivalents.

[00321] Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations, and modifications will occur or be known to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

[00322] Exemplary Alternative embodiments:

[00323] In a first alternative embodiment, a diagnostic system is provided. The diagnostic system, comprising a biochemical reaction including a combination of reverse transcriptase, nucleic acid-modifying enzymes, amplification enzymes, cell-free lysate, Cas13 or Cas12 enzymes, and one or more nucleic acids comprising complete or partial nucleic acid sequences or the reverse complement thereof, wherein the nucleic acids are DNA or RNA corresponding to:

a) Toehold Sequences selected from the group consisting of:

SARS2-TH-01

(CGAUCCCGCGAAAUUAAUACGACUCACUAUAGGGGAUAAUUACCACCAACC
UUAGAAUCAAGAUUGUUAGAGGACUUUAGAACAGAGGAGAUAAAGAUGUCUAAC
AAUCUUACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-02

(CGAUCCCGCGAAAUUAAUACGACUCACUAUAGGGGAUUGUUAGAAUUGCA

AGCUAUAACGCAGCCUGUAAGGACUUUAGAACAGAGGAGAUAAAGAUGUUACAG
GCUGCGACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-03

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGAUAAUACCACCAACC
UUAGAAUCAAGAUUGUUAGAGGACUUUAGAACAGAGGAGAUAAAGAUGUCUAAC
AAUCUUACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-04

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGACCAUUAACAAGGUGUG
CUACCGGCCUGAUAGAUUUCGGACUUUAGAACAGAGGAGAUAAAGAUGGAAAUC
UAUCAGACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-05

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGAACCUUCAACACCAUU
ACAAGGUGUGCUACCGGCCUGGACUUUAGAACAGAGGAGAUAAAGAUGAGGCCG
GUAGCAACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-06

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGCCUUAACACCAUUAC
AAGGUGUGCUACCGGCCUGAGGACUUUAGAACAGAGGAGAUAAAGAUGUCAGGC
CGGUACACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-07

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGAAAACCUUCAACACCA
UUACAAGGUGUGCUACCGGCCGGACUUUAGAACAGAGGAGAUAAAGAUGGCCGGU
AGCACAACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-09

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGCUCUGCCAAAUUGUUG
GAAAGGCAGAAACUUUUUGUGGACUUUAGAACAGAGGAGAUAAAGAUGACAAAA
AGUUUCACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-10

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGCAAGAAUCUCAAGUGU
CUGUGGAUCACGGACAGCAUGGACUUUAGAACAGAGGAGAUAAAGAUGAUGCUG
UCCGUGACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-11

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGCCUGGUUAGAAGUAUU
UGUUCCUGGUGUUAUAACACGGACUUUAGAACAGAGGAGAUAAAGAUGGUGUUA
UAUCACCAGGACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-12

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGUAGAACCUGUAGAAUA
AACACGCCAAGUAGGAGUAAGGACUUUAGAACAGAGGAGAUAAAGAUGUUACUC
CUACUUACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-13

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGCACGUGUUUGAAAAA
CAUUAGAACCUGUAGAAUAAGGACUUUAGAACAGAGGAGAUAAAGAUGUUAUUC
UACAGGACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-14

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGUGCCCGCCGAGGAGA
AUUAGUCUGAGUCUGAUAAACGGACUUUAGAACAGAGGAGAUAAAGAUGGUUAUC
AGACUCACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-15

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGGCCCGCCGAGGAGAAU
UAGUCUGAGUCUGAUAAACUAGGACUUUAGAACAGAGGAGAUAAAGAUGAGUUAU
CAGACUACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-16

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGGCCGAGGAGAAUUAGU
CUGAGUCUGAUAAACUAGCGCGGACUUUAGAACAGAGGAGAUAAAGAUGGCGCUA
GUUAUCACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-17

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGAGGAGAAUUAGUCUG
AGUCUGAUAAACUAGCGCAUAGGACUUUAGAACAGAGGAGAUAAAGAUGUAUGCG
CUAGUUACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-18

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGGACUAGCUACACUACG
UGCCCGCCGAGGAGAAUUAGGGACUUUAGAACAGAGGAGAUAAAGAUGCUAAUU
CUCCUCACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-19

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGAUUGACUAGCUACACU
ACGUGCCCGCCGAGGAGAAUGGACUUUAGAACAGAGGAGAUAAAGAUGAUUCUC
CUCGGCACCAUGAUUACGGAUUCACUGGCCGUC),

SARS2-TH-20

(CGAUCCCGCGAAAUUAUACGACUCACUAUAGGGCAAUGAUGGAUUGACU

AGCUACACUACGUGCCCGCCGGACUUUAGAACAGAGGAGAUAAAGAUGGGCGGG
CACGUAACCAUGAUUACGGAUUCACUGGCCGUC),

b) Cas13 gRNAs selected from the group consisting of:

SARS2-Lwa13-01

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCACCAACCUUAGAA
UCAAGAUUGUUAGA),

SARS2-Lwa13-02

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCAUUAACAAGGUGU
GCUACCGGCCUGAU),

SARS2-Lwa13-03

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACAACCUUCAACACCA
UUACAAGGUGUGCU),

SARS2-Lwa13-04

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACACCAUUAACAAGG
UGUGCUACCGGCCU),

SARS2-Lwa13-05

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACAAAACCUUCAACAC
CAUUACAAGGUGUG),

SARS2-Lwa13-06

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGUAACAAUUA AAAAC
CUUCAACACCAUUA)

SARS2-Lwa13-07

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACAAUUGUUGGAAAG
GCAGAAACUUUUUGU),

SARS2-Lwa13-08

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCAAGAAUCUCAAGU
GUCUGUGGAUCACG),

SARS2-Lwa13-09

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGAAGUAUUUGUUCC
UGGUGUUAUAACAC),

SARS2-Lwa13-10

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGUAGAAUAAACACG
CCAAGUAGGAGUAA),

SARS2-Lwa13-11

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCACGUGUUUGAAA
AACAUUAGAACCUG),

SARS2-Lwa13-12

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGUGCCCGCCGAGGA
GAAUUAGUCUGAGU),

SARS2-Lwa13-13

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCCCGCCGAGGAGA
AUUAGUCUGAGUCU),

SARS2-Lwa13-14

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCCGAGGAGAAUUA
GUCUGAGUCUGAUA),

SARS2-Lwa13-15

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGAGGAGAAUUAGUC
UGAGUCUGAUAACU),

SARS2-Lwa13-16

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCUACACUACGUGC
CCGCCGAGGAGAAU),

SARS2-Lwa13-17

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACACUACGUGCCCG
CCGAGGAGAAUUAG),

SARS2-Lwa13-18

(GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCUACGUGCCCGCCG
AGGAGAAUUAGUCU),

c) Cas12 gRNAs selected from the group consisting of:

SARS2-Lba12a-01

(CGAUCCCGCGAAAUUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUAGUGUUAUGGAGUGUCUCCUACUA),

SARS2-Lba12a-02

(CGAUCCCGCGAAAUUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUACAGGCUGCGUUAUAGCUUGGAAUU),

SARS2-Lba12a-03

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUGCAGAGACAUUGCUGACACUACUG),

SARS2-Lba12a-04

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUUAGGGGCUGAACAUGUCAACAAC),

SARS2-Lba12a-05

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUUAAUAGAGGUGAUGAAGUCAGAC),

SARS2-Lba12a-06

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUCAAGGCUGCGUUAUAGCUUGGAAUU),

SARS2-Lba12a-07

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUAAUCUGAAAUCAUCAGGCCCGUAG)

SARS2-Lba12a-08

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUGCAGAGACAUUGCUGACACUACUG),

SARS2-Lba12a-09

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUGUAGGAGACACUCCAUAACACUUA),

SARS2-Lba12a-10

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUUCUGACUUCAUCACCUCUAAUUAC),

SARS2-Lba12a-11

(CGAUCCCGCGAAAUAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUG
UAGAUAAAAACAUAUAGAACCUGUAGAAUA),

d) Amplification Primers selected from the group consisting of:

RPA-06-F4 (GCAAACCTGGAAAGATTGCTGATTATAATTATAAATTACC)

RPA-06-R4 (CCTGATAGATTTTCAGTTGAAATATCTC)

RPA-06-R3 (CCTTCAACACCATTACAAGGTGTGCTACC).

[00324] In a second alternative embodiment, the diagnostic system according to the first alternative embodiment, wherein the system includes one or more SARS-CoV-2 S-gene amplification primers comprising a fragment of the **Seq1**

(Tcaaacttctaactttagagtcaccaacaagaatctattgttagatttcctaataattacaaactgtgcccttttggtgaagttttaacgccacc agatttgcacatctgtttatgcttgaacaggaagagaatcagcaactgtgttgctgattattctgtcctatataattccgcatcatttccacttttaag tggatggagtgctcctactaaattaaatgatctctgcttactaatgtctatgcagattcatttgaattagaggtgatgaagtcagacaaaatcgcc tccagggcacaactggaaagattgctgattataattataaattaccagatgattttacaggctgcgttatagcttgggaattctaacaatcttgattct aaggttggtggaattataattacctgtatagattgttaggaagtctaattcctaaacctttgagagagatatttcaactgaaatctatcaggccg gtagcacaccttgaatgggtgtgaaggttttaattgttactttcctttacaatcatatggtttcaaccacttatgggtgttggttaccacataca gagtagtagtactttctttgaacttctacatgcaccagcaactgtttgtggacctaaaaagctactaatttggttaaaaacaaatgtgtcaatttc aactcaatgggttaacaggcacaggtgttctactgagtctaacaaaaagttctgcctttccaacaatttggcagagacattgctgacactact gatgctgtccgtgatccacagacacttgagattcttgacattacaccatgttctttggtggtgctcagtggtataacaccaggaacaaatacttca accaggttgctgttctttatcagggtgttaactgcacagaagtccctgttctattcatgcagatcaacttactcctacttggcgtgtttattctaca ggttctaattgttttcaaacacgtgcaggctgttaataggggctgaacatgtcaacaactcatatgagtgtagacataccattgggtgcaggtat atgcgctagtattcaga) nucleic acid sequences or the reverse complement thereof.

[00325] In a third alternative embodiment, the diagnostic system according to the first or second alternative embodiments, wherein the amplification primers are from about 1 nucleotide to about 100 nucleotides, from about 5 nucleotides to about 50 nucleotides, or from about 10 nucleotides to about 25 nucleotides.

[00326] In a fourth alternative embodiment, the diagnostic system according to any one of the first through third alternative embodiments, wherein the biochemical reactions are freeze-dried components.

[00327] SEQUENCE LISTING

Table 5: DNA and RNA sensor sequences used in this study. The sequences presented in this table are SEQ ID NO: 1 to SEQ ID NO: 36 in their order of appearance.

Name	Sequence (5' to 3')	Fig.	Notes
<i>tetO</i>	TCCCTATCAGTGATAGAGATTGACA TCCCTATCAGTGATAGAGATCTAGA AATAATTTTGTTTAACTTTAAGAAG GAGATATACAT	6F	TetR operator region. sfGFP coding region immediately follows.
Zaire Ebola Virus Toehold Switch Sensor	AAGCTGTTGGATATTGTATCAGTCC TTGCTCTGCATGTTATAGTTATGAAC AGAGGAGACATAACATGAACATGC AGAACCATGTAACTTGGCGGCAGC GCAAAGACCATGATTACGGATTCA...	6G	Zaire Strain Ebola Toehold Sensor. DNA template is shown. The start of the LacZ coding sequence is shown in italics.
Zaire Ebola Virus RNA	AUGCAGAGCAAGGACUGAUACAAU AUCCAACAGCUU	6G	RNA gene fragment trigger of the VP30

trigger (ZD)			gene of Zaire ebolavirus
Theophylline Riboswitch Sensor E (Sensor E)	GGTGATACCAGCATCGTCTTGATGC CCTTGGCAGCACCTGCTAAGGAGG TAACAACAAGatgagcaaaggtgaagaa <i>ATG ACCATGATTACGGATTCA...</i>	6G, 9D	Theophylline riboswitch E sequence. DNA template is shown. Coding sequence begins with a 6-aa sfGFP fragment. The start of the LacZ coding sequence is shown in italics.
F30-2xdBroccoli Aptamer	TTGCCATGTGTATGTGGGAGACGGT CGGGTCCATCTGAGACGGTCGGGTC CAGATATTCGTATCTGTCTGAGTAGA GTGTGGGCTCAGATGTCGAGTAGAG TGTGGGCTCCACATACTCTGATGA TCCAGACGGTCGGGTCCATCTGAGA CGGTCGGGTCCAGATATTCGTATCT GTCGAGTAGAGTGTGGGCTCAGATG TCGAGTAGAGTGTGGGCTGGATCAT TCATGGCAAG	9E	F30-dimeric RNA broccoli aptamer sequence. DNA template is shown. Subcloned from Addgene plasmid #66843 (pET28c-F30-2xdBroccoli).
HIV RNA Toehold switch Sensor (#8)	CCCUUUUCUUUUA AAAUUGUGAAU GAUACUGCCAUGGACUUUAGAAC AGAGGAGAUAAAGAUGAUGGCAGU AUUAAACCUUGCGGCAGCGCAAAA G	9F	RNA HIV Toehold Sensor
HIV Virus RNA trigger	AUGGCAGUAUUCAUUCACAAUUUU AAAAGAAAAGGG	9F	RNA gene fragment trigger of the HIV retrovirus
Lyme Disease RNA Toehold switch Sensor	GUUGCAGAUGUAUUCCTCAUCUUUC CCUGAAUUAUUACGGACUUUAGAA CAGAGGAGAUAAAGAUGGUAUAA UUCAGAACCUGGCGGCAGCGCAAA AG	15A	RNA Lyme Toehold Sensor
Lyme Disease (<i>B. burgdorferi</i>) <i>ospC</i> RNA trigger	GGCAAUAUUA AUGACUUUAUUUUU AUUUAUAUCUUGUAUAUUAUUCAGG GAAAGAUGGGAAUACAUCUGCAAA UUCUGCUGAUGAGUCUGUUAAGG GCCUAAUCUACAGAAUAAGUAA AAAAAUUACGGAUUCUAA	15A	RNA gene fragment trigger from <i>B. burgdorferi</i> bacterium (Lyme's Disease)
CRISPR-Cas12a <i>mecA</i> gRNA	GGGUAAUUUCUACUAAGUGUAGAU UUAAGAAGAUGGUAUGUGG	23B- 23E, 24, 12	Cas12a guide RNA targeting single <i>mecA</i> gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a	TTTAATTTTGTAAAGAAGATGGTA TGTGGAAGTTAGATT	23B- 23E,	Double stranded trigger sequence for

<i>mecA</i> dsDNA trigger		24, 12 23B- 23E, 24, 12	Cas12a using <i>mecA</i> gRNA.
<i>mecA</i> RPA primer1 (F)	CATTGATCGCAACGTTCAATTTAATT TTGTTAAAG	23B- 23E, 24, 12	Forward primer for RPA reaction for Cas12a <i>mecA</i> experiments
<i>mecA</i> RPA primer2 (R)	TACGATCAATATGTATGCTTTGGTCT TTCTGCATTCCTG	23B- 23E, 24, 12	Reverse primer for RPA reaction for Cas12a <i>mecA</i> experiments
CRISPR-Cas12a <i>ermA</i> gRNA	GGGUAAUUUCUACUAAGUGUAGAU CUAUUAAUGGUGGAGAUGGA	23B- 23E, 12	Cas12a guide RNA targeting single <i>ermA</i> gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a <i>ermA</i> -dsDNA trigger	GCTTTGGGTTTACTATTAATGGTGG AGATGGATATAAAAA	23B- 23E, 12	Double stranded trigger sequence for Cas12a using <i>ermA</i> gRNA.
<i>ermA</i> RPA primer1 (F)	TCGTTGAGAAGGGATTTGCGAAAAG ATTGC	23B- 23E, 12	Forward primer for RPA reaction for Cas12a <i>ermA</i> experiments
<i>ermA</i> RPA primer2 (R)	GGATGAAAATATAGTGGTGGTACTT TTTTGAGC	23B- 23E, 12	Reverse primer for RPA reaction for Cas12a <i>ermA</i> experiments
CRISPR-Cas12a <i>spa</i> gRNA	GGGUAAUUUCUACUAAGUGUAGAU UGGUAAUGCUUGAGCUUUGU	23B- 23E, 12	Cas12a guide RNA targeting single <i>spa</i> gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a <i>spa</i> dsDNA trigger	TTCACCAGTTTCTGGTAATGCTTGAG CTTTGTTAGCATCT	23B- 23E, 12	Double stranded trigger sequence for Cas12a using <i>spa</i> gRNA.
<i>spa</i> RPA primer1 (F)	AAGAAGCAACCAGCAAACCATGCA GATGC	23B- 23E, 12	Forward primer for RPA reaction for Cas12a <i>spa</i> experiments
<i>spa</i> RPA primer2 (R)	ACCTAACGCTAATGATAATCCACCA AATAC	23B- 23E, 12	Reverse primer for RPA reaction for Cas12a <i>spa</i> experiments
CRISPR-	GAUUUAGACUACCCCAAAAACGAA	25	Cas13a guide RNA

Cas13a MRSA crRNA	GGGGACUAAAACACTCATGCCATAC ATAAATGGATAGACG		(crRNA) targeting single MRSA gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas13a MRSA Trigger	CGUCUAUCCAUUUAUGUAUGGCAU GAGU	25	Single stranded RNA trigger sequence for Cas13a using MRSA crRNA.
Fluorophore-quencher (FQ) reporter	5'-6FAM/TTATT/IowaBlackFQ	23B- 23E, 28C, 24, 12, 26C	Substrate for Cas12a CRISPR-based sensors. When trans-cleaved by cis-activated Cas12a it generates a fluorescent signal.
Zika Toehold switch 27B sensor	TTTCGCTCTATTCTCATCAGTTTCAT GTCCTGTGTCGGACTTTAGAACAGA GGAGATAAAGATGGACACAGGACA CAACCTGGCGGCAGCGCAAAGCTG CGTAAACTG <i>gagc...</i>	11	RNA Zika Toehold Sensor. The start of the sfGFP coding sequence is shown in italics.
Zika Virus RNA trigger	GGGCCAGCACAGUGGGAUGAUCGU UAAUGACACAGGACAUGAAACUGA UGAGAAUAGAGCGAAAGUUGAGAU AACGCCCAAUUCACCAAGAGCCGA AGCCACCCUGGGGGGGUUGGAAG CCUAGGACUUGAUUGUGAACCGAG GACAGG	11	RNA gene fragment trigger of the Zika single-stranded RNA flavivirus
SARS-CoV-2 S-gene Cas12a gRNA Sensor	GGGUAAUUUCUACUAAGUGUAGAU CAGGCUGCGUUAUAGCUUGGAAUU	28C	gRNA targeting region in SARS-CoV-2 Spike gene
RT-RPA-F4	gcaaactggaaagattgctgattataattataaattacc	28C	Primer for SARS-CoV-2 RT-RPA
RT-RPA-R4	cctgatagatttcagttgaaatatctc	28C	Primer for SARS-CoV-2 RT-RPA
RT-RPA-R3	ccttcaacaccattacaaggtgtgtactc	28C	Primer for SARS-CoV-2 RT-RPA
SARS-Cov-2 IVT RNA Target	GGUGAUGAAGUCAGACAAAUCGCU CCAGGGCAAACUGGAAAGAUUGCU GAUUUAUAAUUAUAAAUUACCAGAU GAUUUUACAGGCUGCGUUAUAGCU UGGAAUUCUAACAAUCUUGAUUCU AAGGUUGGUGGUAUUUAUAAUUAC CUGUAUAGAUUGUUUAGGAAGUCU AAUCUCAACCUUUUGAGAGAGAU AUUUCAACUGAAAUCUAUCAGGCC GGUAGCACACCUUGUAAUGGUGUU GAAGGUUUUAUUGUUACUUCCU	28C	S gene RNA fragment trigger for SARS-CoV-2 generated by in vitro transcription from a DNA template

	UUACAAUCAUAUGGUUCCAACCC ACUAAUGGUGUUGGUUACCAACCA		
HCoV-229E IVT RNA Target	GGGGUUA AUGUGUCACAAACCUCU AUUGC UAAUAUAAUUUAUUGCAAC UCUGUUAUUAACAGACUGAGAUGU GACCAGUUGUCCUUUGAUGUACCA GAUGGUUUUUAUUCUACAAGCCCU AUUCAAUCCGUUGAGCUACCUGUG UCUAUUGUGUCGCUACCUGUUUAU CAUAAACAUAACGUUUAUUGUGUUG UACGUUGACUUCAAACCUCAGAGU GGCGGUGGCAAGUGCUUUAACUGU UAUCCUGCUGGUGUAAUAUUAACA CUGGCCAAUUUUA AUGAAACUAAA GGGCCUUUGUGUGUUGACACAUCA CACUUCACUACCAAUAACGUUGCU GUUUAUGCCAAUGUUGGUAGGUGG AGUGCUAGUAUUAACACGGGAAAU UGCCCUUUUUCUUUUGGCAAAGUU AAUAACUUUGUUA AAUUUGGCAGU GUAUGUUUUUCGCUAAAGGAUAUA CCCGGUGGUUGCGCAAUGCCUAUA GUGGCUA AUUGGGCUUAUAGUAAG UACUAUACUAUAGGCUCAUUGUAU GUUUCUUGGAGUGAUGGUGAUGGA AUUACUGGCGUCCCACAA	28C	Homologous S gene RNA fragment trigger from human coronavirus strain 229E, generated by in vitro transcription from a DNA template
HCoV-HKU1 IVT RNA Target	GGGGUUA CUAAUUA AUAAUUAUAAU CCUUCUUCUUGGAAUAGAAGGUAAU GGUUUUA AUAAUUUUA AUUUGAGC UCUCAUAGUGUUGUUUACUCACGU UAUUGUUUUUCUGUUA AUAAUACU UUUUGUCCUUGUGCUAAACCUUCU UUUGCUUCAAGUUGCAAGAGUCAU AAACCACCUUCUGCUUCCUGUCCU AUUGGUACUAAUUAUCGUUCUUGU GAGAGUACUACUGUACUCGACCAC ACUGACUGGUGUAGGUGUUCUUGU UUACCUGAUCCUAUAACUGCUUAU GACCCUAGGUCUUGUUCUAAAAA AAGUCUCUGGUUGGUGUUGGUGAA CAUUGUGCAGGGUUCGGUGUUGAU GAAGAAAAGUGUGGUGUAUUGGAU GGAUCAUAUA AUGUUUCUUGUCUU UGUAGUACUGAUGCCUUUCUAGGU UGGUCUUAUGACACUUGCGUCAGU AACAAACCGUUGUAAUAUUUUUUCU AAUUUUAUUUUA AUGGUAUCAAU AGUGGUACCACUUGUUCUAAUGAU	28C	Homologous S gene RNA fragment trigger from human coronavirus strain HKU1, generated by in vitro transcription from a DNA template

	UUUAUUGCAGCCUAAUACUGAAGUU UUUACUGAUGUUUGUGUUGAUUAC G		
--	--	--	--

HCoV-NL63 IVT RNA Target	GGGGUUA AUGUUAGUGCAACUAAC AUUCAAAACUUACUUUAUUGCGAU UCUCCAUUUGAAAAGUUGCAGUGU GAGCACUUGCAGUUUGGAUUGCAG GAUGGUUUUUUUAUUCUGCAAAUUUU CUUGAUGAUAAUGUUUUUGCCUGAG ACUUAUGUUGCACUCCCCAUUUUAU UAUCAACACACGGACAUAUUUUUU ACUGCAACUGCAUCUUUUUGGUGGU UCUUGUUAUGUUUGUAAACCACAC CAGGUUAAUUAUCUCUUA AUGGU AACACUUCAGUGUGUGUUAGAACA UCUCAUUUUUCAAUUAGGUUAUUU UAUAACCGCGUUAAGAGUGGUUCA CCAGGUGACUCUUC AUGGCACA UAUUUAAAGAGUGGCACUUGUCCA UUUUCUUUUUCUAAGUUAAAUAU UUUCAAAAGUUC AAGACUAUUUGU UUCUCAACCGUCGAAGUGCCUGGU AGUUGUAAUUUCCGCUUGAAGCC ACCUGGCAUUAACAUUCUUAUACU AUUGUUGGUGCUUUGUAUGUUACU UGGUCUGAAGGUAAUUCUAUUACU GGUGUACCUUA	28C	Homologous S gene RNA fragment trigger from human coronavirus strain NL63, generated by in vitro transcription from a DNA template
HCoV-OC43 IVT RNA Target	GGGGCAGGUUUA AUCCUUCUACUU GGAAUAGGAGAUUUGGUUUUACAG AACAAUCUGUUUUUAAGCCUCAAC CUGUAGGUGUUUUUACUCAUCAUG AUGUUGUUUAUGCACAACAUUGUU UUAAAGCUC CCAAAUUCUGUC CGUGUAAAUUGGAUGGGUCUUUGU GUGUAGGUAAUGGUCCUGGUUAUAG AUGCUGGUUAUAAAAAUAGUGGUA UAGGCACUUGUCCUGCAGGUACUA AUUAUUUAACUUGCCAUA AUGCUG CCCAAUGUGAUUGUUUGUGCACUC CCGACCCCAUUA CAUCUAAAUCUA CAGGGCCUUA CAAGUGCCCCCAA CUAAAUACUUAAGUUGGCAUAGGUG AGCACUGUUCGGGUCUUGCUAUUA	28C	Homologous S gene RNA fragment trigger from human coronavirus strain OC43, generated by in vitro transcription from a DNA template

	AAAGUGAUUAUUGUGGAGGUAUC CUUGUACUUGCCAACCACAAGCAU UUUUGGGUUGGUCUGUUGACUCUU GUUUACAAGGGGAUAGGUGUAAUA UUUUUGCUAAUUUUUUUUUGCAUG AUGUAAAUAGUGGUACUACUUGUU CUACUGAUUUACAAAAAUCAAACA CAGACAUAAUUCUUGGUGUUUGUG UUAUUUAU		
Fluorophore- Biotin (FB) reporter	5'-6FAM/TTATTTTATT/Biotin	28C	Reporter molecule used for the SARS- CoV-2 face mask sensor SHERLOCK Reaction. Upon cis- activation, Cas12a collateral activity will cleave the ssDNA linker, separating the 6-carboxyfluorescein from the biotin moiety.

Notes:

1. All toehold sensors and corresponding trigger RNAs for were in vitro transcribed from DNA templates containing the T7 RNA polymerase promoter:
TAATACGACTCACTATA
2. The sequence GGG was added to the 5' end of all toehold sensor RNA and target RNA fragment sequences for efficient expression by T7 RNA polymerase. The Ebola ZD toehold sensor only contains a GG after the T7 promoter. If the RNA sequence began with G or GG, only GG or G, respectively, was added to the 5' end of the sequence.
3. The GGG prefix is not shown in the sensor sequences so that the target RNA binding site can be readily identified, but GGG was always added to the start of each RNA to encourage efficient transcription by the polymerase.
4. The coding sequences of the reporter protein LacZ in the colorimetric sensors were added immediately after the 21-nt linker in the toehold switch RNA sequences starting with the second codon (Threonine) of the wild-type beta-Galactosidase enzyme.
5. Considered Zika virus strains (KU312312, AY632535) have sufficient sequence homology to be detected using the same toehold switch sensors (27B).

Table 6: Reporter sequences used in this study. The sequences presented in this table are SEQ ID NO: 37 to SEQ ID NO: 42 in their order of appearance.

Name (FIG.#)	Sequence (5' to 3')
LacZ Colorimetric Reporter DNA Sequence (6E-6H, 7B,7C, 8A,	ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTG ACTGGGAAAACCCTGGCGTTACCCAACCTTAATCGCCTTGCAGCACA TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGAT CGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTG CCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGT GCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCCTCAAACCTGGCA

10B-10D, 18B,
19A, 19B)

GATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCC
ATTACGGTCAATCCGCCGTTTGTTCACGGAGAATCCGACGGGTT
GTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGG
CCAGACGCGAATTATTTTGTATGGCGTTAACTCGGCGTTTCATCTGT
GGTGAACGGGCGCTGGGTTCGGTTACGGCCAGGACAGTCGTTTGCC
GTCTGAATTTGACCTGAGCGCATTTTACGCGCCGGAGAAAACCGC
CTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAG
ATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTT
GCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACT
CGCTTTAATGATGATTTTACGCCGCGCTGTACTGGAGGCTGAAGTTC
AGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTT
ATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTTCGG
CGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACA
CTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATC
CCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCA
CGCTGATTGAAGCAGAAGCCTGCGATGTTCGGTTTCCGCGAGGTGCG
GATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATT
CGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCA
TGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGA
ACAACCTTTAACGCCGTGCGCTGTTTCGCATTATCCGAACCATCCGCT
GTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAA
GCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCG
ATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAA
TGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCT
GGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCG
CTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGC
GGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACG
CGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTC
CATCAAAAAATGGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATC
CTTTGCGAATACGCCCACGCGATGGGTAAACAGTCTTGGCGGTTTCG
CTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGG
CTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAA
AACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGC
CGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCG
CACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTT
TTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAA
TACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGG
CGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCTCTGGATGT
CGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAG
CCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAA
CCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGG
CAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCG
CGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTG
CATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGC
TTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACCTGCTGACGC
CGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGG
CGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTGGAACGC
TGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAG

	TGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTC ACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAA CCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGT TGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAA CTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATT AGGGCCGCAAGAAAACCTATCCCGACCGCCTTACTGCCGCCTGTTTT GACCGCTGGGATCTGCCATTGTCTAGACATGTATACCCCGTACGTCT TCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATT ATGGCCACACCAAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCG CTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTG CACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATG GGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAAT TCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCA AAAATAA
GFPmut3B Fluorescent Reporter DNA Sequence (11)	ATGCGTAAAGGAGAAGAACTTTTCTACTGGAGTTGTCCCAATTCTTG TTGAATTAGATGGTGTGTTAATGGGCACAAATTTTCTGTCAGTGG AGAGGGTGAAGGTGATGCAACATACGGAAAACCTTACCCTTAAATTT ATTTGCACTACTGGAAAACCTGTTCCGTGGCCAACACTTGTCA CTACTTTCGGTTATGGTGTTCATGCTTTGCGAGATACCCAGATCAC ATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACG TACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACCTACAAGA CACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAAT CGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGA CACAAATTGGAATACAACCTATAACTCACACAATGTATACATCATGG CAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAATTAGAC ACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAAC AAAATACTCCGATTGGCGATGGCCCTGTCCTTTTACCAGACAACCA TTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAG AGAGACCACATGGTCCTTCTTGAGTTTGTAAACCGCTGCTGGGATTA CACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAACGACG AAAACCTACGCTTTAGTAGCTTAA
sfGFP Fluorescent Reporter DNA Sequence (9C, 9D, 23B- 23E)	ATGAGCAAAGGTGAAGAACTGTTTACCGGCGTTGTGCCGATTCTGG TGGAACCTGGATGGCGATGTGAACGGTCACAAATTCAGCGTGCGTG GTGAAGGTGAAGGCGATGCCACGATTGGCAAACCTGACGCTGAAAT TTATCTGCACCACCGGCAAACTGCCGGTGCCGTGGCCGACGCTGGT GACCACCCTGACCTATGGCGTTTCAAGTGTCTTAGTCGCTATCCGGATC ACATGAAACGTCACGATTTCTTTAAATCTGCAATGCCGGAAGGCTA TGTGCAGGAACGTACGATTAGCTTTAAAGATGATGGCAAATATAAA ACGCGCGCCGTTGTGAAATTTGAAGGCGATACCCTGGTGAACCGCA TTGAACTGAAAGGCACGGATTTTAAAGAAGATGGCAATATCCTGG GCCATAAACTGGAATACAACCTTTAATAGCCATAATGTTTATATTAC GGCGGATAAACAGAAAAATGGCATCAAAGCGAATTTTACCGTTTCG CCATAACGTTGAAGATGGCAGTGTGCAGCTGGCAGATCATTATCAG CAGAATACCCCGATTGGTGTGATGGTCCGGTGCTGCTGCCGGATAATC ATTATCTGAGCACGCAGACCGTTCTGTCTAAAGATCCGAACGAAAA AGGCACGCGGGACCACATGGTTCTGCACGAATATGTGAATGCGGC AGGTATTACGTGGAGCCATCCGCAGTTCGAAAAATAA

eforRed Fluorescent Reporter DNA Sequence (14)	ATGAGCGTGATTAAACAGGTGATGAAAACCAAACCTGCATCTGGAA GGCACCGTTAATGGTCATGATTTCCACCATTGAAGGTAAAGGTGAAG GCAAACCGTATGAAGGTCTGCAGCATATGAAAATGACCGTTACCA AAGGTGCACCGCTGCCGTTTAGCGTTCATATTCTGACCCCGAGCCA TATGTATGGTAGCAAACCGTTTAACAAATATCCGGCAGATATCCCG GATTATCACAAACAGAGCTTTCCGGAAGGTATGAGCTGGGAACGT AGCATGATTTTTGAAGATGGTGGTGTGTTGTACCGCAAGCAATCATA GCAGCATTAATCTGCAAGAAAACCTGCTTCATCTACGACGTGAAATT CCATGGTGTTAATCTGCCTCCGGATGGTCCGGTTATGCAGAAAACC ATTGCAGGTTGGGAACCGAGCGTTGAAACCCTGTATGTTTCGTGATG GTATGCTGAAAAGCGATAACCGCCATGGTTTTTAAACTGAAAGGTGG TGGTCATCATCGTGTGGATTTCAAACACCTACAAAGCAAAAAA CCGGTTAAACTGCCGGAATTCCATTTTGTGTAACATCGTCTGGAAC TGACCAAACACGATAAAGATTTTACCACCTGGGATCAGCAAGAAG CAGCAGAAGGTCATTTTAGTCCGCTGCCGAAAGCACTGCCGTAA
dTomato Fluorescent Reporter DNA Sequence (14)	ATGGTGAGCAAGGGCGAGGAGGTCATCAAAGAGTTCATGCGCTTC AAGGTGCGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATC GAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCC AAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGAC ATCCTGTCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGC ACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGG CTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTG ACCGTGACCCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACA AGGTGAAGATGCGCGGCACCAACTTCCCCCCCCGACGGCCCCGTAA GCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTA CCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTGAA GCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGACCATCTAC ATGGCCAAGAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACA CCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGA ACAGTACGAGCGCTCCGAGGGCCGCCACCACCTGTTCTGTACGGC ATGGACGAGCTGTACAAGTAA
Nanoluciferase Luminescent Reporter DNA Sequence (15A, 15B)	ATGGGTCATCACCACCACCATCACGGTGGCGCTAGCGTGTTACAT TGGAGGACTTTGTAGGGGACTGGCGCCAGACAGCGGGCTACAACC TTGATCAGGTTCTGGAGCAGGGAGGTGTAAGTTCACTTTTCCAGAA TTTGGGTGTGAGTGTCACCCCGATCCAACGTATCGTGCTTTCCGGA GAAAATGGGCTGAAGATCGACATCCATGTTATTATTCCTTATGAAG GGCTTAGCGGAGATCAAATGGGCCAAATCGAAAAGATTTTCAAAG TGGTATATCCTGTTGACGACCATCATTTTAAGGTCATTCTGCATTAC GGAACCTTAGTCATCGACGGCGTCACACCTAACATGATTGACTATT TTGGCCGTCCGTATGAGGGCATCGCAGTGTTTGACGGAAAAAAT CACCGTGACAGGGACACTGTGGAACGGCAATAAGATTATCGACGA GCGCCTTATTAACCCAGATGGGTGCGCTTTTATTCCGTGTCATTATA ATGGTGTCACCTGGCTGGCGTTTGTGCGAACGCATCCTGGCATAA

Table 7: Sequences for SARS-CoV-2 Molecular Sensing and Isothermal RT-RPA Amplification. The sequences presented in this table are SEQ ID NO: 43 to SEQ ID NO: 96 in their order of appearance.

Toehold Sensor	Performance	Expressed (RNA) Toehold Sequence
SARS2-TH-01	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG

		GGAUAAUUAACCAACCUUAGAAUCAAGAUU GUUAGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCUAACAAUCUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-02	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGAUUGUUAGAUAUCCAAGCUAAACGCAGC CUGUAAGGACUUUAGAACAGAGGAGAUAAAG AUGUUACAGGCUGCGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-03	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGAUAAUUAACCAACCUUAGAAUCAAGAUU GUUAGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCUAACAAUCUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-04	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGACCAUUAACAAGGUGUGCUACCGGCCUGAU GAUUUCGGACUUUAGAACAGAGGAGAUAAAG AUGGAAAUUCUAUCAGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-05	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGAACCUUCAACACCAUUAACAAGGUGUGCUAC CGGCCUGGACUUUAGAACAGAGGAGAUAAAG AUGAGGCCGGUAGCAACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-06	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGCCUUAACACCAUUAACAAGGUGUGCUACCG GCCUGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCAGGCCGGUACACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-07	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGAAAACCUUCAACACCAUUAACAAGGUGUGCU ACCGGCCGACUUUAGAACAGAGGAGAUAAAG AUGGCCGGUAGCACAACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-09	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGCUCUGCCAAAUUGUUGGAAAGGCAGAAACU UUUUGUGGACUUUAGAACAGAGGAGAUAAAG AUGACAAAAGUUUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-10	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGCAAGAAUCUCAAGUGUCUGUGGAUCACGGA CAGCAUGGACUUUAGAACAGAGGAGAUAAAG AUGAUGCUGUCCGUGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-11	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGCCUGGUUAGAAGUAUUUGUCCUGGUGUU AUAACACGGACUUUAGAACAGAGGAGAUAAA GAUGGUGUUAUAUCACCAGGACCAUGAUUACG GAUUCACUGGCCGUC
SARS2-TH-12	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAGAACCUGUAGAUAUAAACACGCCAAGUAG

		GAGUAAGGACUUUAGAACAGAGGAGAUAAAG AUGUUACUCCUACUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-13	++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGCACGUGUUUGAAAAACAUAAGAACCUGU AGAAUAAGGACUUUAGAACAGAGGAGAUAAA GAUGUUAUUCUACAGGACCAUGAUUACGGAU UCACUGGCCGUC
SARS2-TH-14	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGUGCCCGCCGAGGAGAAUAGUCUGAGUCU GAUAACGGACUUUAGAACAGAGGAGAUAAAG AUGGUUAUCAGACUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-15	+	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGCCCCGCCGAGGAGAAUAGUCUGAGUCUGA UAACUAGGACUUUAGAACAGAGGAGAUAAAG AUGAGUUAUCAGACUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-16	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGCCGAGGAGAAUAGUCUGAGUCUGAUAA CUAGCGCGGACUUUAGAACAGAGGAGAUAAA GAUGGCGCUAGUUAUCACCAUGAUUACGGAU CACUGGCCGUC
SARS2-TH-17	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGAGGAGAAUAGUCUGAGUCUGAUAAUA GCGCAUAGGACUUUAGAACAGAGGAGAUAAA GAUGUAUGCGCUAGUUAUCACCAUGAUUACGGAU UCACUGGCCGUC
SARS2-TH-18	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGGACUAGCUACACUACGUGCCCGCCGAGGAG AAUUAAGGACUUUAGAACAGAGGAGAUAAAG AUGCUAAUUCUCCUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-19	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGAUUGACUAGCUACACUACGUGCCCGCCGAG GAGAAUGGACUUUAGAACAGAGGAGAUAAAG AUGAUUCUCCUCGGCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-20	-	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGCAAUGAUGGAUUGACUAGCUACACUACGUG CCCGCCGGACUUUAGAACAGAGGAGAUAAAGA UGGGCGGGCACGUAACCAUGAUUACGGAUUC CUGGCCGUC
LwaCas13a Sensor		gRNA Sequence
SARS2-Lwa13-01	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCACCAACCUUAGAAUCAAGAUUGUUAGA
SARS2-Lwa13-02	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCAUUAACAAGGUGUGCUACCGGCCUGAU
SARS2-Lwa13-03	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAACCUUACAACCAUUAACAAGGUGUGCU

SARS2-Lwa13-04	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACACACCAUUAACAAGGUGUCUACCGGCCU
SARS2-Lwa13-05	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAAAACCUUCAACACCAUUAACAAGGUGUG
SARS2-Lwa13-06	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUAAACAAUUA AAAACCUUCAACACCAUUA
SARS2-Lwa13-07	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAAUUGUUGGAAAGGCAGAAACUUUUUG U
SARS2-Lwa13-08	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCAAGAAUCUCAAGUGUCUGUGGAUCACG
SARS2-Lwa13-09	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGAAGUAUUUGUUCUGGUGUUUAUAACA C
SARS2-Lwa13-10	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUAGAAUAAACACGCCAAGUAGGAGUA A
SARS2-Lwa13-11	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCACGUGUUUGAAAAACAUAAGAACCUG
SARS2-Lwa13-12	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUGCCCGCCGAGGAGAAUUAAGUCUGAGU
SARS2-Lwa13-13	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCCCGCCGAGGAGAAUUAAGUCUGAGUCU
SARS2-Lwa13-14	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCCGAGGAGAAUUAAGUCUGAGUCUGAU A
SARS2-Lwa13-15	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGAGGAGAAUUAAGUCUGAGUCUGAUAAAC U
SARS2-Lwa13-16	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCUACACUACGUGCCCGCCGAGGAGAAU
SARS2-Lwa13-17	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACACACUACGUGCCCGCCGAGGAGAAUUAAG
SARS2-Lwa13-18	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCUACGUGCCCGCCGAGGAGAAUUAAGUCU
LbaCas12a Sensor		gRNA Sequence
SARS2-Lba12a-01	+++	CGAUCCCGCGAAAUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAGUGUUAU GGAGUGUCUCCUACUA
SARS2-Lba12a-02	+++	CGAUCCCGCGAAAUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAGGCUGC GUUAUAGCUUGGAAUU
SARS2-Lba12a-03	+++	CGAUCCCGCGAAAUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAGCAGAGAC AUUGCUGACACUACUG
SARS2-Lba12a-04	+++	CGAUCCCGCGAAAUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUUAUAGGGGC UGAACAUGUCAACAAC
SARS2-Lba12a-05	+++	CGAUCCCGCGAAAUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUUAUUAAGA

		GGUGAUGAAGUCAGAC
SARS2-Lba12a-06	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUCAGGCUGC GUUAUAGCUUGGAAUU
SARS2-Lba12a-07	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAAACUGAAA UCUAUCAGGCCGGUAG
SARS2-Lba12a-08	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUGCAGAGAC AUUGCUGACACUACUG
SARS2-Lba12a-09	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUGUAGGAGA CACUCCAUAACACUUA
SARS2-Lba12a-10	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUUCUGACUU CAUCACCUCUAAUUAAC
SARS2-Lba12a-11	+++	CGAUCCCGCGAAAUUAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAAAAACAU UAGAACCUGUAGAAUA
RT-RPA Primers		Oligo Sequence
RPA-06-F4	+++	GCAAACCTGGAAAGATTGCTGATTATAATTATAA ATTACC
RPA-06-R4	+++	CCTGATAGATTTTCAGTTGAAATATCTC
RPA-06-R3	+++	CCTTCAACACCATTACAAGGTGTGCTACC
SARS-CoV-2 S-gene amplification primers including any part or fragment of the following nucleic sequences or their respective reverse complementing sequence:		
tcaaacttctaacttttagagtccaaccaacagaatctattgtagatttcctaattatacaaaacttgcccttttggtgaagttttaacgccacca gatttgcattctgtttatgcttggacaggaagagaatcagcaactgtgttgctgattattctgtcctatataattccgcattctttccactttaagt gttatggagtgctcctactaaattaatgatctctgtttactaatgtctatgcagattcatttgtaattagaggtgatgaagtcagacaaatcgct ccaggggcaaactggaaagattgctgattataattataaattaccagatgattttacaggctgcgttatagcttggaaattctaacaatcttgattcta agggttggtggaattataattacctgtatagattgttaggaagtctaattcctcaaaccttttgagagagatatttcaactgaaatctatcaggccgg tagcacacctgtaatggtgtgaagggtttaattgttactttctttacaatcatatggtttccaaccacttatggtgttggttaccacacatacag agtagtagtactttctttgaacttctacatgcaccagcaactgtttgtggacctaataaagtctactaatttggttaaaacaaatgtgtcaattca acttcaatgggttaacaggcacagggtgttcttactgagcttaacaaaagtttctgcctttccaacaatttggcagagacattgctgacactactg atgctgtccgtgatccacagacacttgagattcttgacattacaccatgttcttttggtggtgtcagtggtataacaccaggaacaaataacttctaa ccagggtgctgttctttatcagggtgttaactgcacagaagtcctgttgctattcatgcagatcaacttactcctacttggcgtgtttattctacag gttctaattgttttcaaacacgtgcaggctgttaataaggggtgaacatgtcaacaactcatatgagtggtgacataccattggtgcaggtatat gcgctagttatcaga		
* for the RNA sequences, the respective dsDNA, template ssDNA and reverse complement ssDNA sequences can be used.		

CLAIMS**WHAT IS CLAIMED IS:**

1. An aqueous solution activated detection device, the detection device comprising:
a carrier fluid reservoir;
a sample collection unit;
a sample processing unit including dried synthetic biological components; and
a detection unit.
2. The detection device according to claim 1, wherein the carrier fluid reservoir is connected to the sample collection unit, the sample collection unit is connected to the sample processing unit, and the sample processing unit is connected to the detection unit.
3. The detection device according to claim 1 or 2 further comprising a covering, wherein the carrier fluid reservoir, the sample collection unit, the sample processing unit, and the detection unit are coupled to the covering, and wherein the sample collection unit is attached to an inner surface of the covering for detection of a subject's respiratory infection status or is attached to an outside of the covering for environmental detection of exposure.
4. The detection device according to claim 3, wherein the covering is a face mask.
5. The detection device according to claim 3, wherein the covering is implemented in a breathalyzer.
6. The detection device according to claim 5, wherein the breathalyzer includes a tube for engaging with a mouth of a subject, the covering being positioned in the tube to capture breath from the subject.
7. The detection device according to any one of claims 1-3 and 5, wherein the detection device is an air monitor.
8. The device according to claim 4, wherein the carrier fluid reservoir is attached to the outer surface of the face mask.

9. The device according to any one of claims 1 to 8, wherein the detection unit is hidden during a sample collection when using the device.
10. The device according to any one of claims 1 to 8, wherein the carrier fluid reservoir includes a valve connecting and controlling the flow of an aqueous solution from the fluid reservoir to the sample collection unit.
11. The device according to claim 10, wherein the valve is a pressure valve configured to open when the pressure difference across the pressure valve exceeds a predefined threshold.
12. The device according to claim 11, wherein the predefined threshold is at least 0.5 psi.
13. The device according to any one of claims 10 to 12, wherein the valve is configured as a two-way valve.
14. The device according to any one of claim 10 to 12, wherein the fluid reservoir is configured as a blister pack, and the valve is configured as a puncture zone forming a wall of the blister pack, the puncture zone opening under conditions of an applied pressure to the blister pack.
15. The device according to claim 14, further comprising a button, the button actuating a spike positioned opposite the puncture zone and puncturing the puncture zone when the button is depressed.
16. The device according to claim 15, wherein the spike is embedded in a compressible material for protecting the puncture zone prior to depressing the button and actuating the spike.
17. The device according to any one of claims 1 to 16, where the carrier fluid reservoir is a pierceable sachet configured to be mechanically activated by piercing, cutting, pressure-based rupturing, or a combination thereof.
18. The device according to any one of claims 1 to 17, further comprising a barrier between the fluid reservoir and the control of fluid from the reservoir to the sample collection unit is controlled by opening of the barrier including sliding, unclamping, and pulling or pushing.

19. The device according to any one of claims 1 to 18, wherein the carrier fluid reservoir includes a venting hole configured as a one-way vent allowing ambient air into to carrier fluid reservoir when a pressure differential between an exterior to the carrier fluid reservoir and interior of the pressure reservoir is greater than a threshold value.
20. The device according to claim 19, wherein the venting hole material includes a hydrophobic coating.
21. The device according to any one of claims 1 to 20, wherein the sample collection zone includes a porous material allowing a carrier fluid to flow in a direction from the carrier fluid reservoir to the sample processing unit.
22. The device according to claim 21, wherein the porous material is a hydrophilic material comprising one or more of a cellulose, starch, maltodextrin, glycerine, sugar, sucralose, dextrose, gum Arabic, cotton, wool, silk, rayon, hemp, spandex, lycra, elastane, polypropylene, polycarbonate, polyester, polyamide, linen, nylon, polyurethane, glass, metal, and/or any combination or blends thereof.
23. The device according to claim 22, wherein the porous material is a high release media.
24. The device according to claim 22 or claim 23, wherein the porous material includes at least 50% of fibers and/or threads aligned in a direction pointing from the carrier fluid reservoir to the sample processing unit
25. The device according to claim 22 or claim 23, wherein the porous material includes at least 50% of fibers and/or threads aligned in a direction orthogonal to the direction pointing from the carrier fluid reservoir to the sample processing unit.
26. The device according to any one of claims 1 to 25, wherein the sample processing unit includes:
- a lysis subunit;
 - an amplification subunit, and
 - a reporter activation subunit.

27. The device according to claim 26, wherein the fluid reservoir is connected to the lysis subunit, the lysis subunit is connected to the amplification subunit, the amplification subunit is connected to the reporter activation subunit, and the reporter activation subunit is connected to the detection unit.
28. The device according to claim 26 or claim 27, wherein:
the lysis subunit includes dry or freeze dried lysis components;
the amplification subunit includes dry reverse transcriptase (RT), RNase H, RNA polymerase, oligonucleotide primers, RT-RPA, RT-LAMP;
the reporter activation subunit includes dry cell-free lysate, cell-free transcription and translation reactions, nucleic acid sequences encoding for toehold switch sensors, nucleic acid sequences encoding for riboswitch sensors, Cas14, Cas13 or Cas12 collateral cleavage enzyme, one or more guide RNAs, and a corresponding dry reporter molecule;
and
the detection unit includes a lateral flow assay (LFA) configured to detect the reporter molecule and a cleaved product of the reporter molecule.
29. The device according to claim 28, wherein the lysis subunit includes freeze dried lysis components having less than 1% non-ionic surfactants.
30. The device according to claim 28 or claim 29, wherein the LFA includes a sample region in contact with the reporter activation subunit, a control line configured to change color upon contact with the intact reporter molecule, one or more detection lines configured to change color upon contact with the cleaved products of the reporter molecules, and an adsorption zone at a position distal from the sample region.
31. The device according to any one of claims 26 to 29, wherein the reporter activation subunit includes Cas12a, Cas13a, a toehold switch, a riboswitch, a cell-free biosensor, a FAM-Biotin nucleic acid probe, or any combination thereof, and wherein the reporter molecule is a FAM-biotin probe.
32. The device according to any one of claims 26 to 31, wherein:

the lysis subunit includes a lysis chamber, the amplification subunit includes an amplification chamber and the reporter activation subunit comprise a reporter activation chamber;

a first flow channel connecting the lysis chamber and amplification chamber, a second flow channel connecting the amplification chamber to the reporter activation chamber, and a third flow channel connects the reporter activation chamber to the detection unit; and

one or more of the first flow channel, the second flow channel and the third flow channel including a time delay barrier.

33. The device according to claim 32, wherein the time delay barrier is a dissolvable material.

34. The device according to claim 33, wherein the dissolvable material includes hydrophilic polymers such as polyvinyl alcohol (PVA), pullulan, sugars such as sucrose, inorganic salts, and/or patterned hydrophobic materials.

35. The device according to any one of claims 26 to 34, wherein the lysis subunit, the amplification subunit and the reporter activation subunit are configured as a geometrically arranged porous material in which the fluid path is controlled to allow flow between different reaction units.

36. The device according to claim 35, wherein the activation subunit is configured as a μ PAD.

37. The device according to any one of claims 32 to 36, wherein one or more of the chamber subunits and one or more of the flow channels includes a blocking agent disposed on an internal surface of the chambers/flow channels.

38. The device according to any one of claims 1 to 37, wherein the device mass is less than 10g.

39. The device according to any one of claims 1 to 38, wherein the carrier fluid reservoir contains water free of nucleases.

40. The device according to any one of claims 1 to 39, wherein the detection unit provides a visual output, a fluorescent output and/or an electrical output.
41. The device according to claim 40, wherein the detector is an electrochemical sensor.
42. A method of detecting an airborne agent, the method comprising:
contacting air including the airborne agent to the sample collection unit of the device according to any of claims 1 to 41, wherein the sample unit collects the airborne agent on a surface of the sample unit;
causing a carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the carrier fluid subsequently flowing to the sample processing unit and contacting the dried synthetic biological components; and
reading an output from the detection unit, the output indicative of the presence of the airborne agent in the air.
43. The method of claim 42, wherein the airborne agent is a pathogen, an environmental nucleic acid, a virus, a toxin, a host-derived nucleic acid, a host-derived polypeptide, a host-derived peptide and/or any combination thereof.
44. The method of claim 43, wherein the airborne agent is a virus.
45. The method according to any one of claims 42 to 44, wherein the airborne agent is in an aerosol.
46. The method according to any one of claims 42 to 45, wherein the air includes respiratory droplets and/or other exhalations from a subject.
47. The method according to any one of claims 42 to 46, wherein contacting the air lasts for at least 1 minute prior to causing the carrier fluid to flow to the sample collection unit.
48. The method according to any one of claims 42 to 47, wherein a positive output for the airborne agent is indicated within 12 hours of contacting the air with the sample collection unit.

49. The method according to any one of claims 42 to 48, wherein the carrier fluid includes water and after causing the carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the fluid flows through the sample collection unit and the sample processing unit, and the water hydrates at least a portion of dry materials therein when the water reaches the sample collection unit and the sample processing unit.

50. The method according to any one of claims 42 to 49, wherein after causing the carrier fluid from the carrier fluid reservoir to flow to the sample collection unit, the fluid flows sequentially through each of the sample collection unit, the sample processing unit, and the detection unit substantially by capillary action.

51. A method of making an aqueous solution activated detection device, the method comprising:

connecting in series a carrier fluid reservoir, a sample collection unit, a sample processing unit, and a detection unit;

wherein an outlet from the carrier fluid reservoir is connected to an inlet of the sample collection unit, an outlet from the sample collection unit is connected to an inlet of the sample processing unit, and an outlet of the sample processing unit is connected to an inlet of the detection unit, and

wherein the sample processing unit includes dried synthetic biological components.

52. The method according to claim 51, further comprising positioning a valve at the outlet from the carrier fluid reservoir.

53. The method according to claim 51 or claim 52, further comprising providing a pressure release vent to the carrier fluid reservoir by punching a hole in a wall of the carrier fluid reservoir and covering the hole with a film of breathable material and/or providing a one-way air valve that allows air into the carrier fluid reservoir to offset building up of vacuum.

54. The method according to claim 51 or claim 52, wherein the sample collection unit is made by cutting a porous sheet into a strip, the strip having a central collection region and two opposite distal regions, where a first distal region forms the inlet of the sample collection unit and a second distal region forms an outlet of the sample collection unit.

55. The method according to claim 54, wherein the central collection region is broader than the first and second distal regions.
56. The method according to claim 54 or claim 55, wherein the porous sheet comprises fibers wherein a majority of the fibers are aligned in a direction pointing from the first distal region to the second distal region.
57. The method according to claim 52 or claim 53, wherein the porous sheet includes at least 50% of fibers or threads aligned in a direction orthogonal to the direction pointing from the first distal region to the second distal region.
58. The method according to any one of claims 54 to 57, wherein one side of the porous sheet includes a hydrophobic barrier material.
59. The method according to any one of claims 51 to 58, wherein the sample processing unit is configured as a μ PAD, the method further comprising:
- (a) printing a hydrophobic ink on a hydrophilic sheet, the hydrophobic ink diffusing through the hydrophilic sheet and forming an array of bounding features;
 - (b) defining, in a first column of the array of bounding features;
 - a first row corresponding to a carrier fluid reservoir to a sample collection unit connection region;
 - a second row corresponding to a lysis subunit;
 - a third row corresponding to a fluid outlet from the lysis subunit;
 - a fourth row corresponding to an amplification subunit;
 - a fifth row corresponding to a fluid outlet from the amplification subunit;
 - a sixth row corresponding to a reporter activation subunit;
 - a seventh row corresponding to a fluid outlet from the reporter activation subunit;
 - an eight row, and optionally ninth row, corresponding to a detection unit connection region; and
 - (c) folding the first column such that adjoining surfaces of each adjacent row are in contact in an accordion like fashion, thereby providing a rectangular or square μ PAD.
60. The method according to claim 59, further comprising:

printing on the hydrophilic sheet with the hydrophobic ink one or more additional columns of the second row, third row, fourth row, fifth row, sixth row, seventh row, eighth row, and optionally ninth row;

separating each additional column from adjacent columns; and

folding each of the additional columns in the accordion like fashion, thereby providing additional μ PAD per additional column.

61. The method according to claim 59 or 60, wherein one or more of the fluid outlets includes a time delay barrier.

62. The method according to any of claims 59 to 61, wherein each of the fluid outlets have a volume that is smaller than a volume of each of the subunits.

63. The method according to any of claims 57 to 60 further comprising depositing lysate components in the lysis subunit, depositing amplification components in the amplification subunit, and depositing reporter activation components in the reporter activation subunit.

64. The method according to claim 63, wherein depositing comprises applying the components in an at least partially dissolved state to the units, and removing the water.

65. The method according to any of claims 51 to 64, further comprising attaching the carrier fluid reservoir, the sample collection unit, the sample processing unit, and the detection unit to a face mask, wherein the sample collection unit is attached to an inner surface of the face mask.

66. The method according to claim 65, wherein the carrier fluid reservoir is positioned on an outside surface of the face mask, and the outlet from the carrier fluid reservoir, inlet of the sample collection unit, or an area proximate to the outlet from the carrier fluid reservoir and inlet of the sample collection unit is passed from the outer surface of the face mask, to the inner surface of the face mask through a hole.

67. The method according to any of claims 51 to 65, wherein connecting in series comprise applying a water proof adhesive to matching surfaces of the outlet of the fluid reservoir and inlet of the collection unit, applying a water proof adhesive to matching surfaces of the outlet from the sample collection unit and inlet of the sample processing unit, and applying a water proof

adhesive to matching surfaces of the outlet of the sample processing unit and inlet of the detection unit, wherein the water proof adhesive bridges any gap between the corresponding surfaces.

68. A device for capturing an airborne agent comprising:
a sample collection chamber for capturing breath from a subject, the airborne agent contained in the breath from the subject; and
a covering coupled to the sample collection chamber, the covering having a sample collection unit with a porous material.
69. The device of claim 68, wherein the device is a breathalyzer.
70. The device of claim 69, wherein the breathalyzer includes an expansion chamber and the covering is positioned within the expansion chamber.
71. The device of claim 68 or claim 69, wherein the sample collection chamber is a tube.
72. The device of any one of claims 68 to 71, wherein the sample collection unit is coupled to a carrier fluid reservoir.
73. The device of any one of claims 68 to 72, wherein the sample collection unit is coupled to a sample processing unit including dried biological components and/or dried synthetic biological components.
74. The device of any one of claims 68 to 73, wherein a detection unit is coupled to the sample collection unit.
75. The device of any one of claims 68 to 74, wherein the airborne agent is an aerosol.
76. The device of any one of claims 68 to 75, wherein the porous material is a porous high-release media substrate.

77. The device of claim 76, wherein the porous high-release media substrate is positioned in close proximity to the airborne agent to collect particles containing pathogens or pathogenic material comprised in the airborne agent.

78. A device for processing a sample comprising:
two or more reaction zones coupled in sequence to each other; and
one or more time delay barriers separating each pair of the two or more reaction zones.

79. The device of claim 78, wherein the two or more reaction zones and the one or more time delay barriers are arranged in a stacked configuration, an accordion configuration, a serial configuration, or a parallel configuration.

80. The device of claim 78 or claim 79, wherein the two or more reaction zones include a lysis chamber, an amplification chamber, and a reporter activation chamber.

81. The device of any one of claims 78 or claim 79, wherein the one or more time delay barriers include dissolvable materials.

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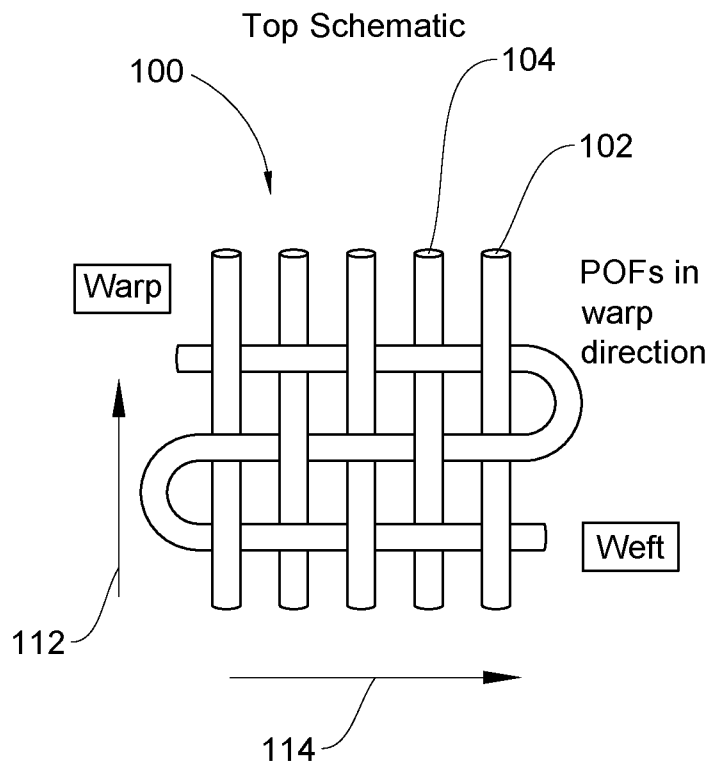


FIG. 1A

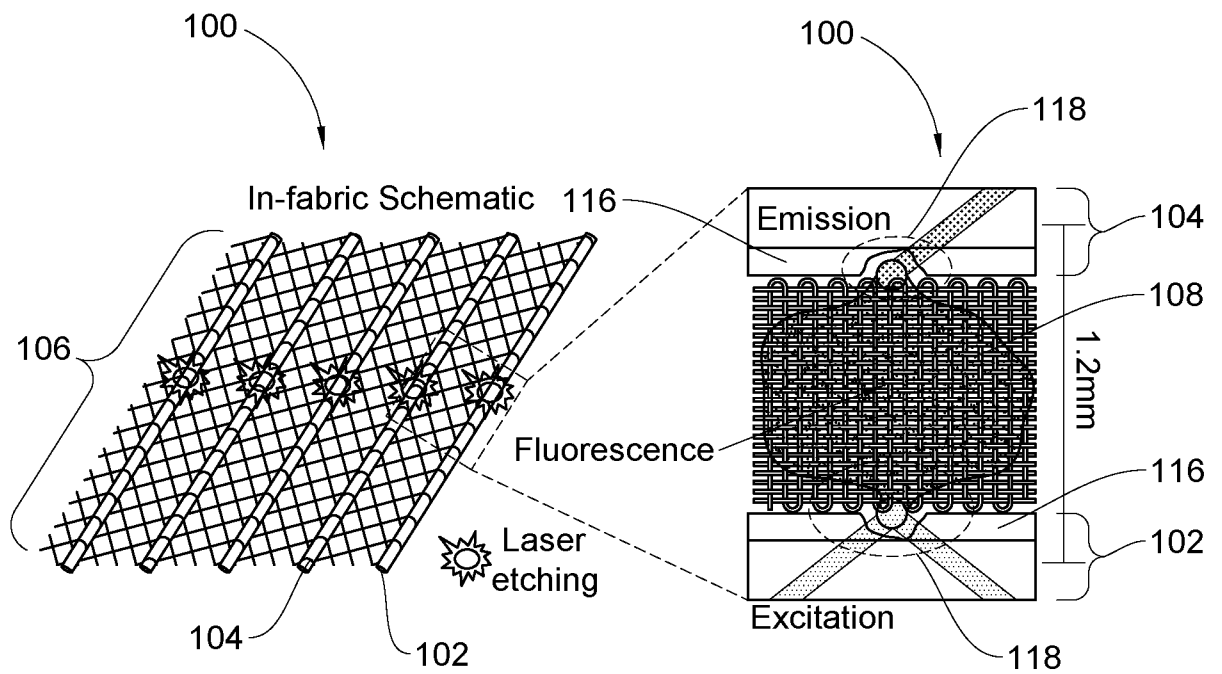


FIG. 1B

FIG. 1C

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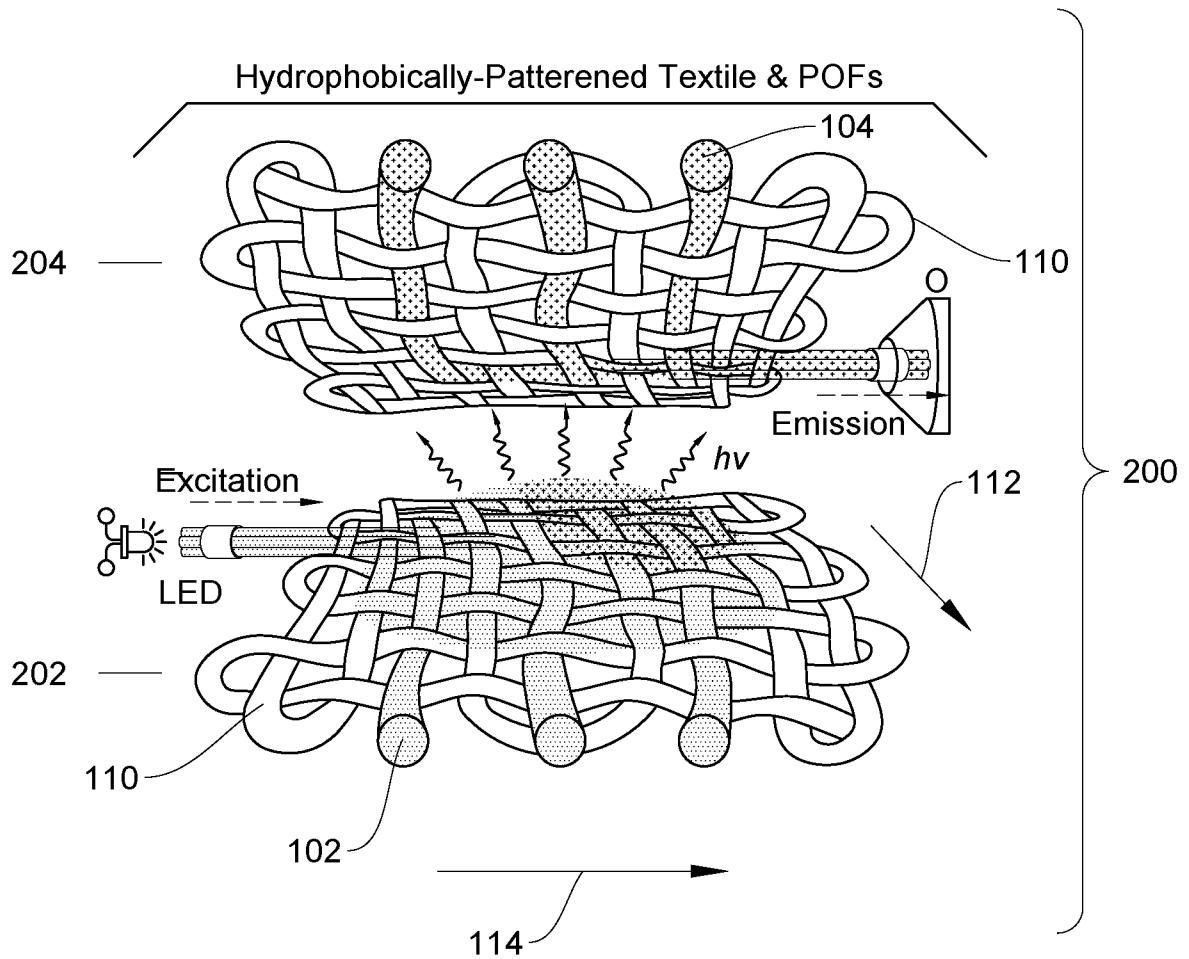


FIG. 2

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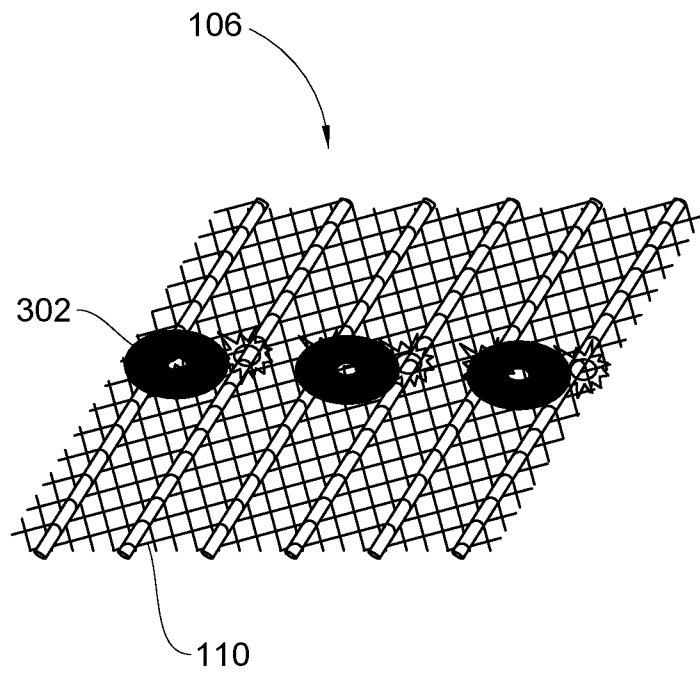


FIG. 3A

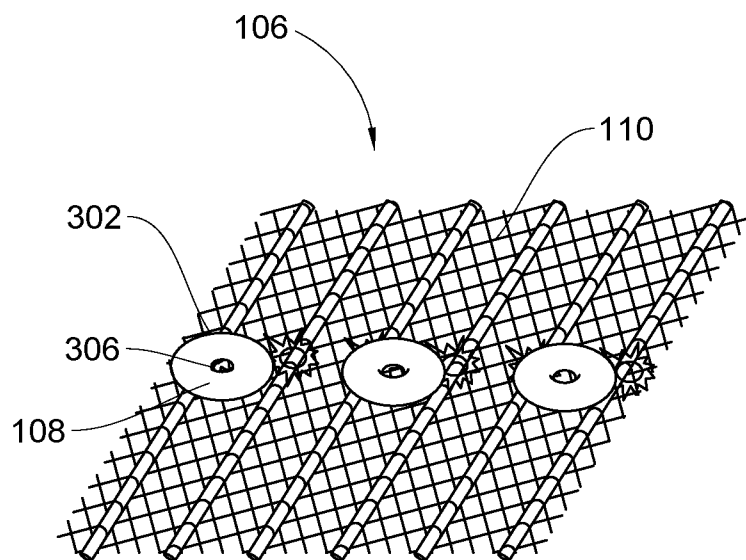


FIG. 3B

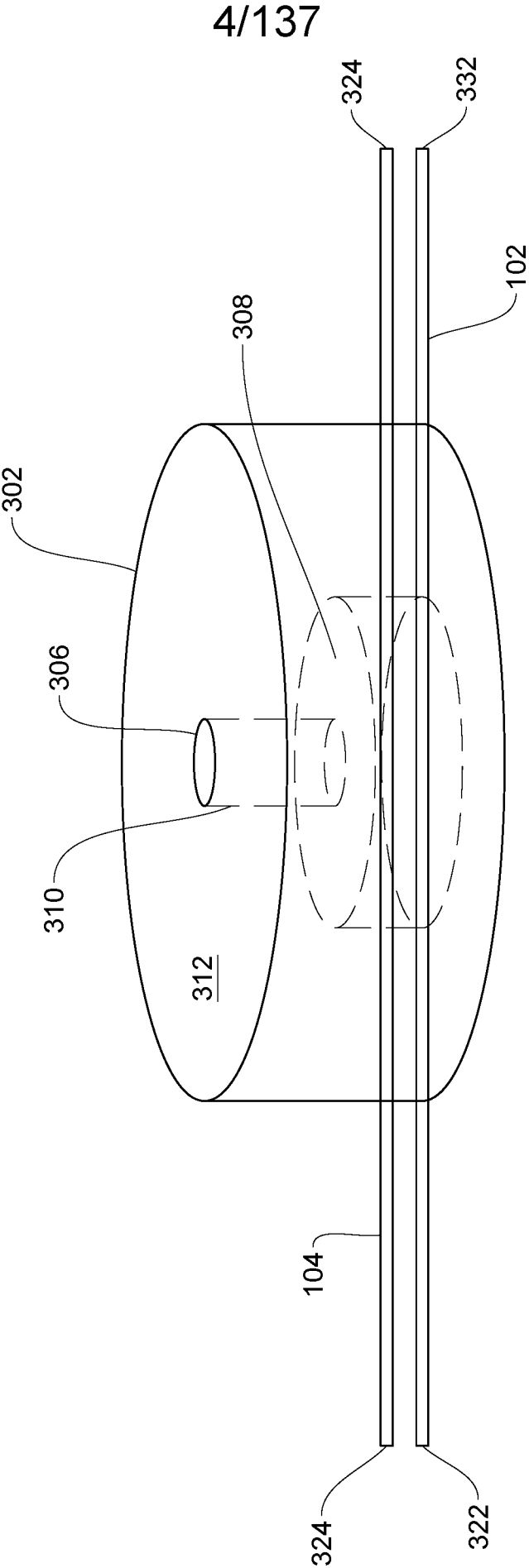


FIG. 3C

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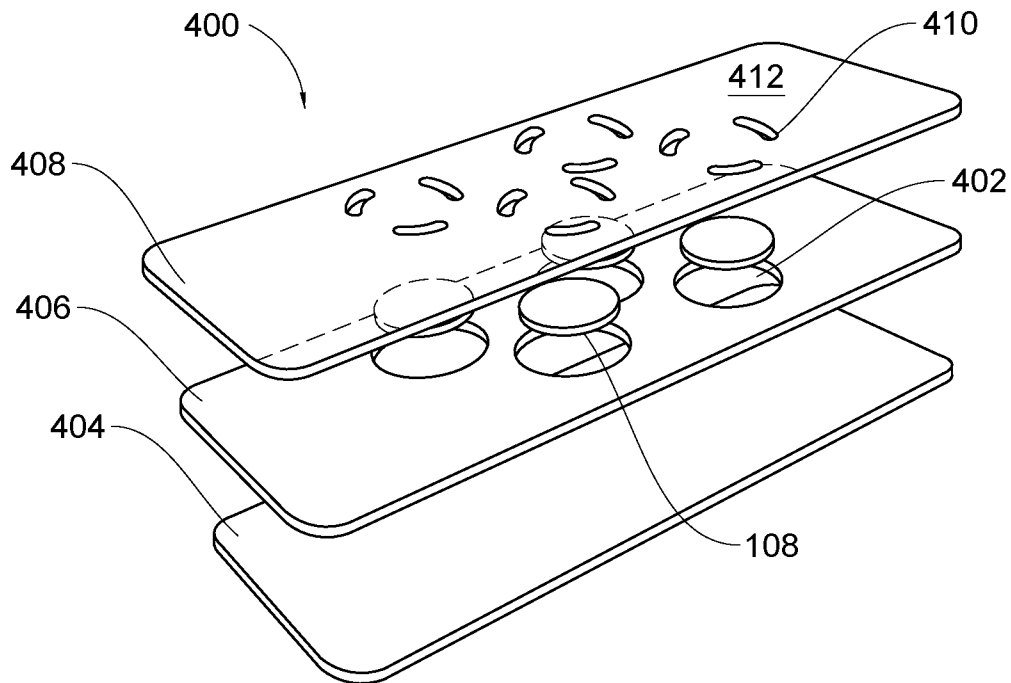


FIG. 4A

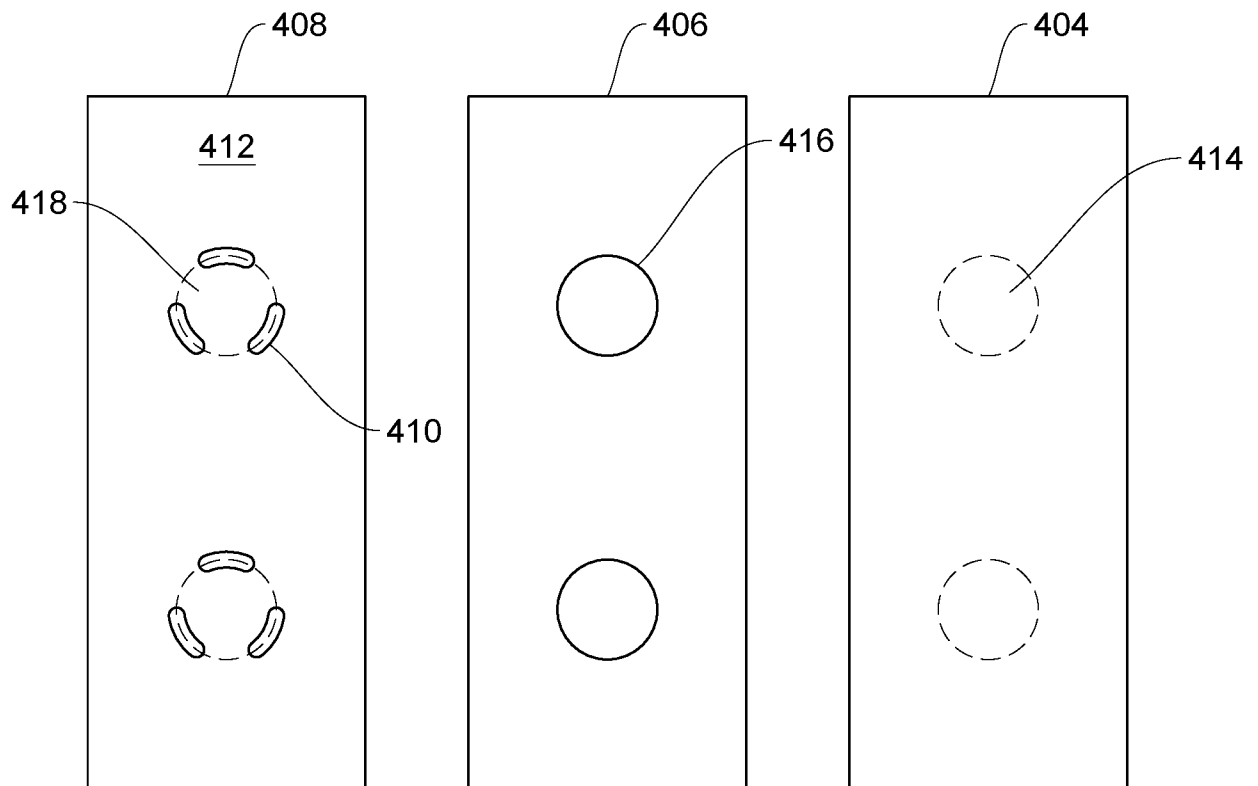


FIG. 4B

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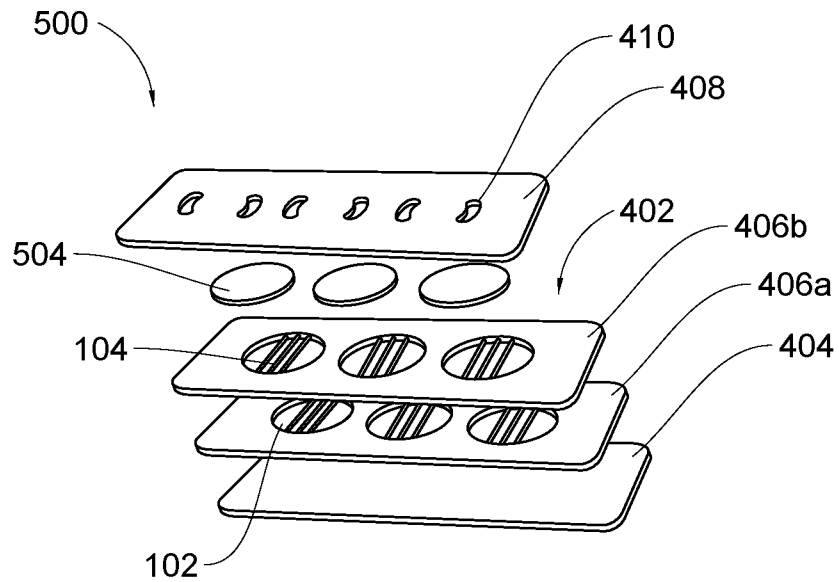


FIG. 5A

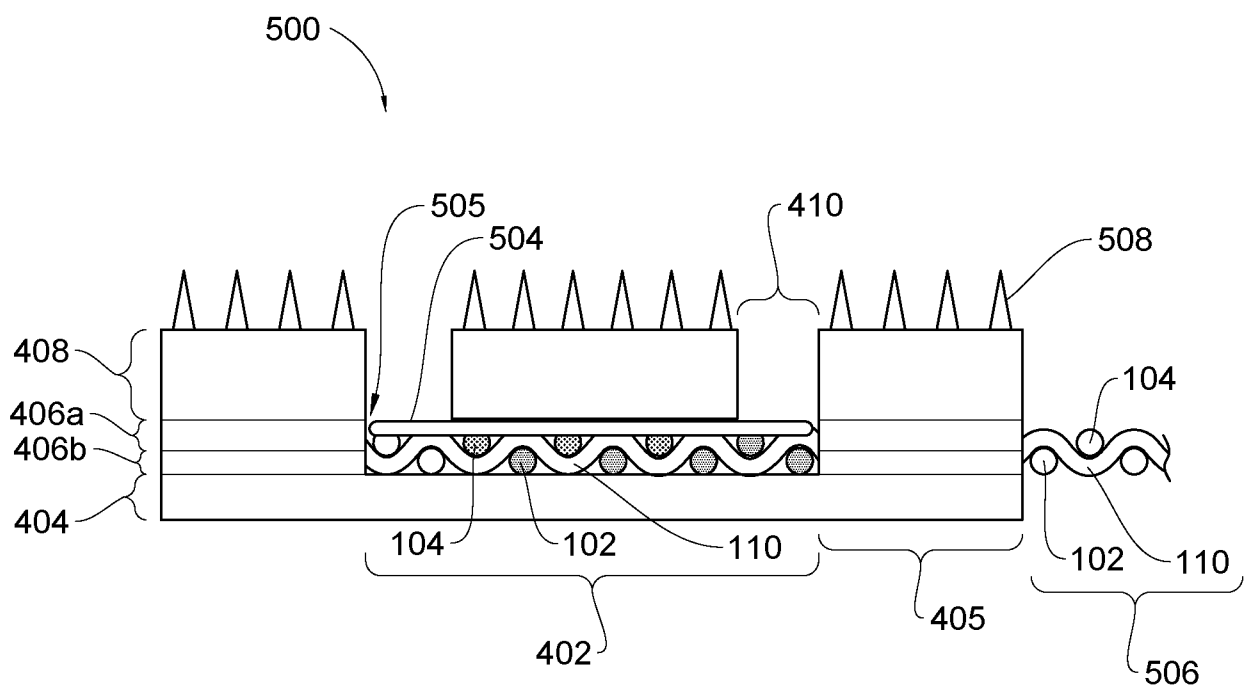


FIG. 5B

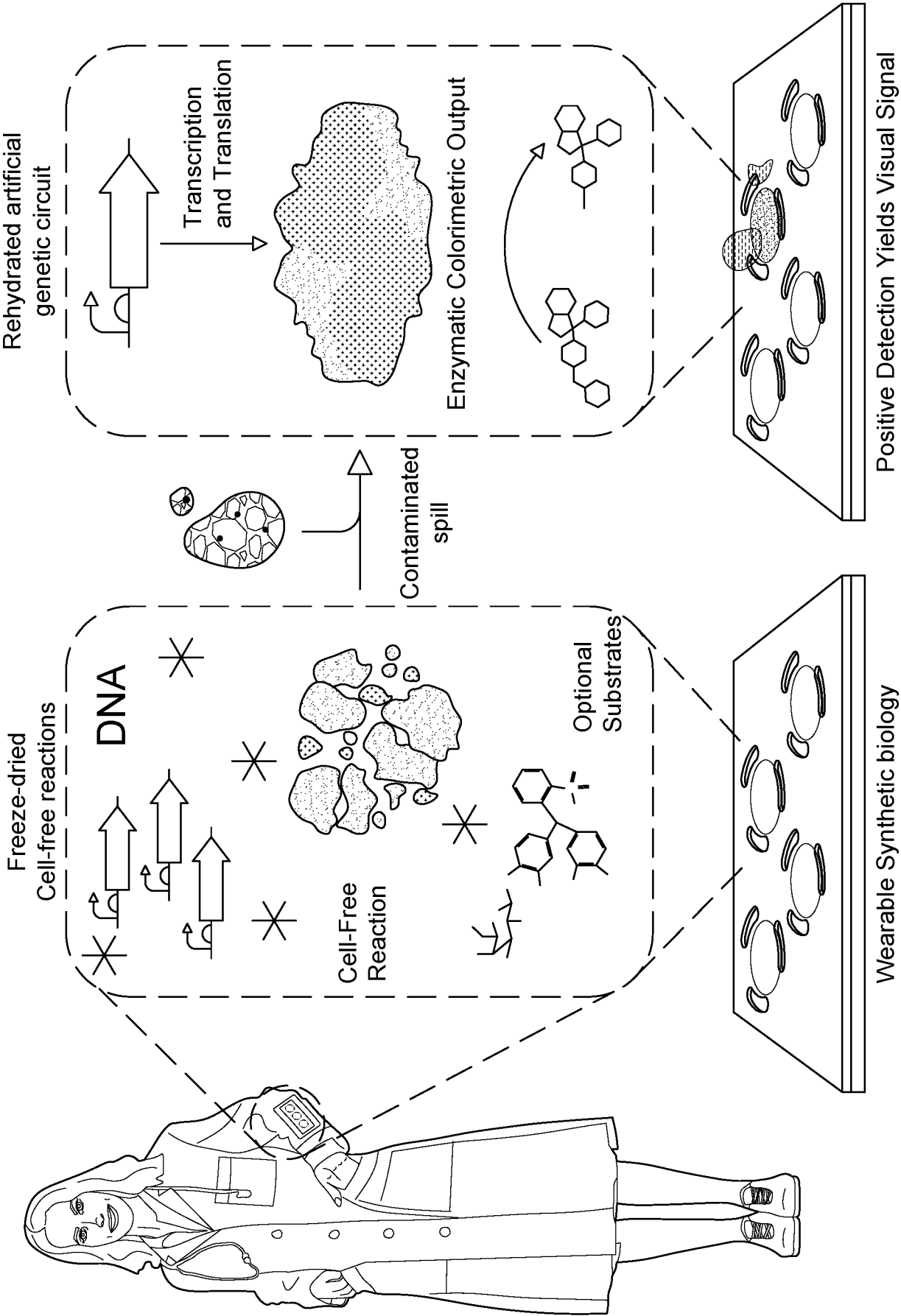


FIG. 6A

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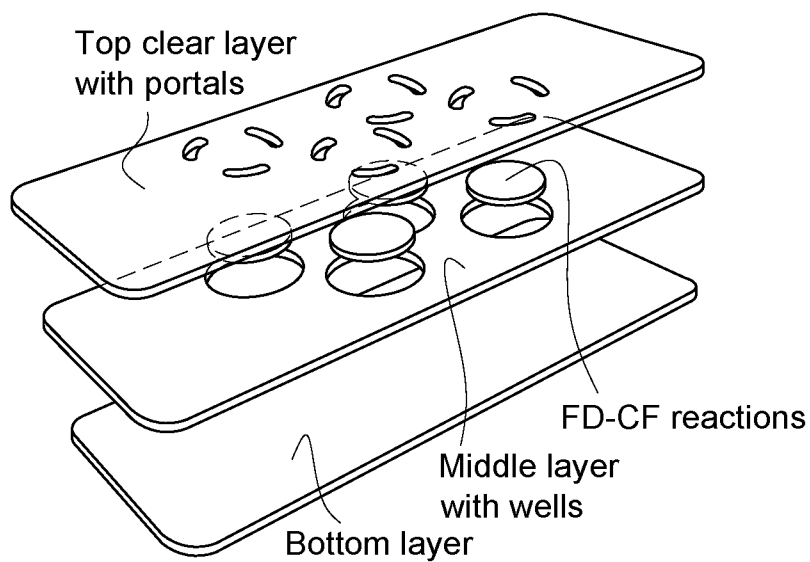


FIG. 6B

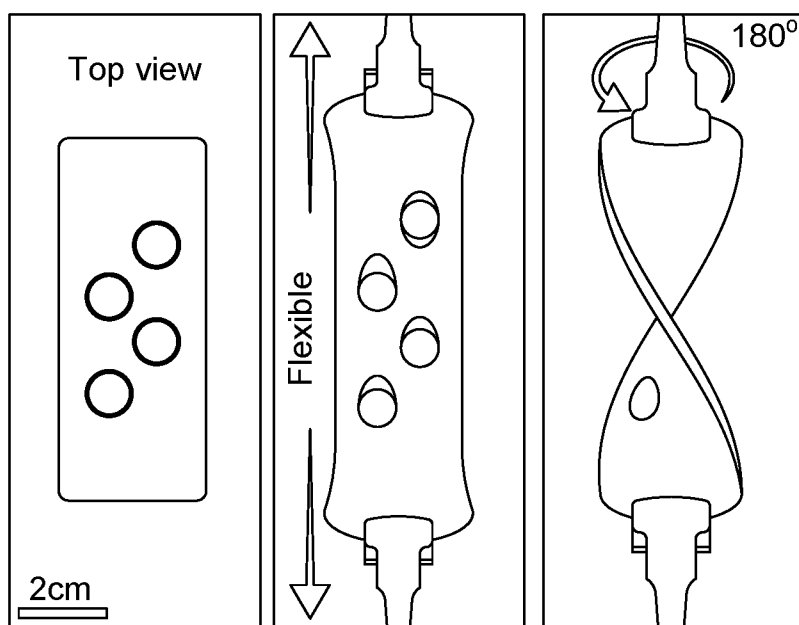


FIG. 6C

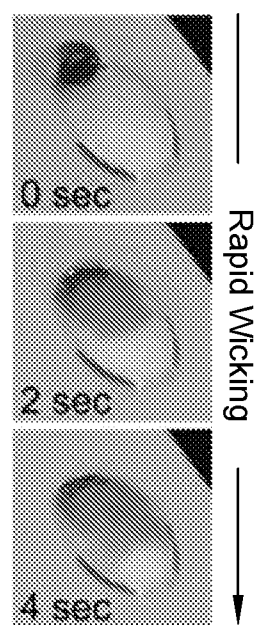


FIG. 6D

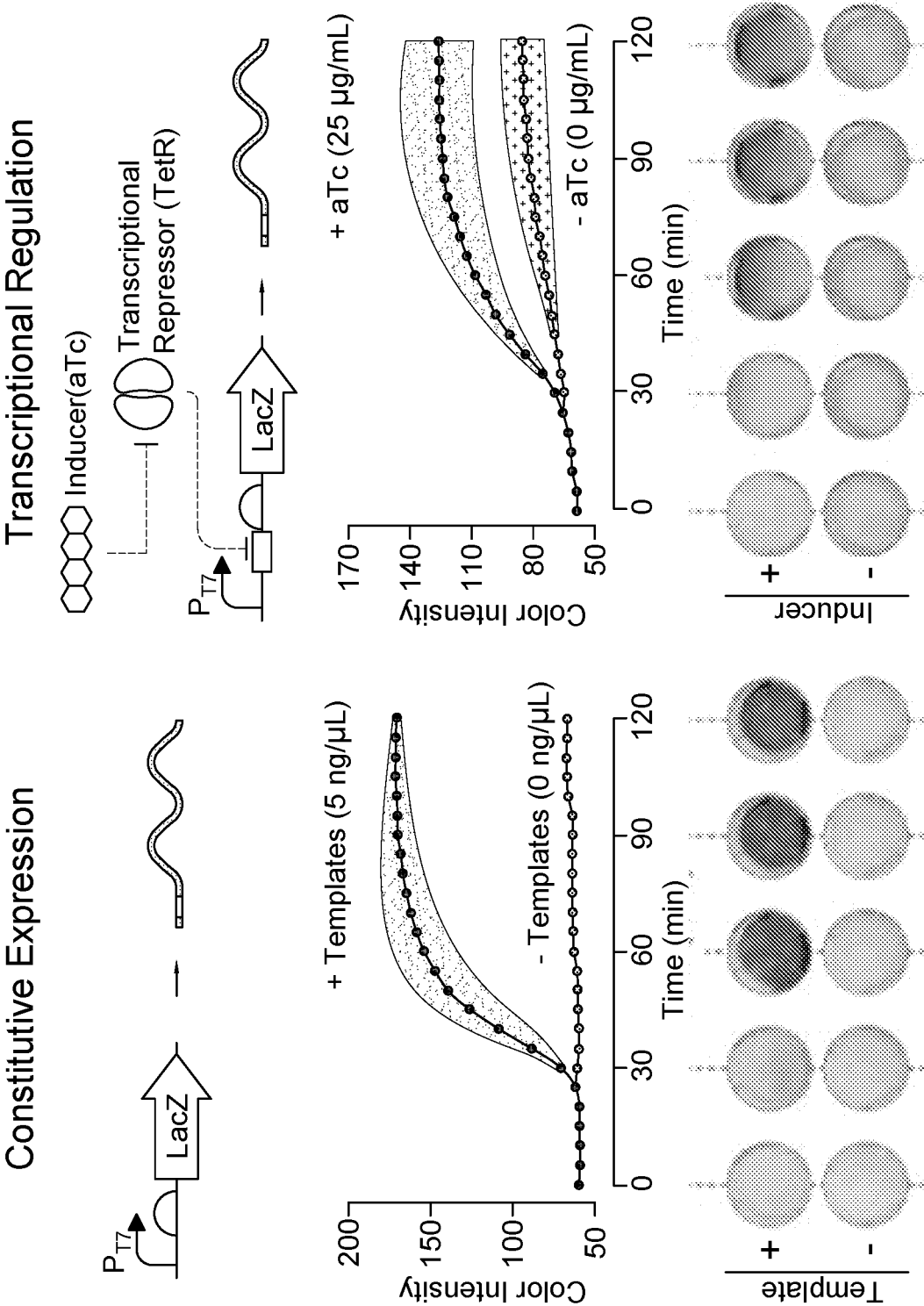
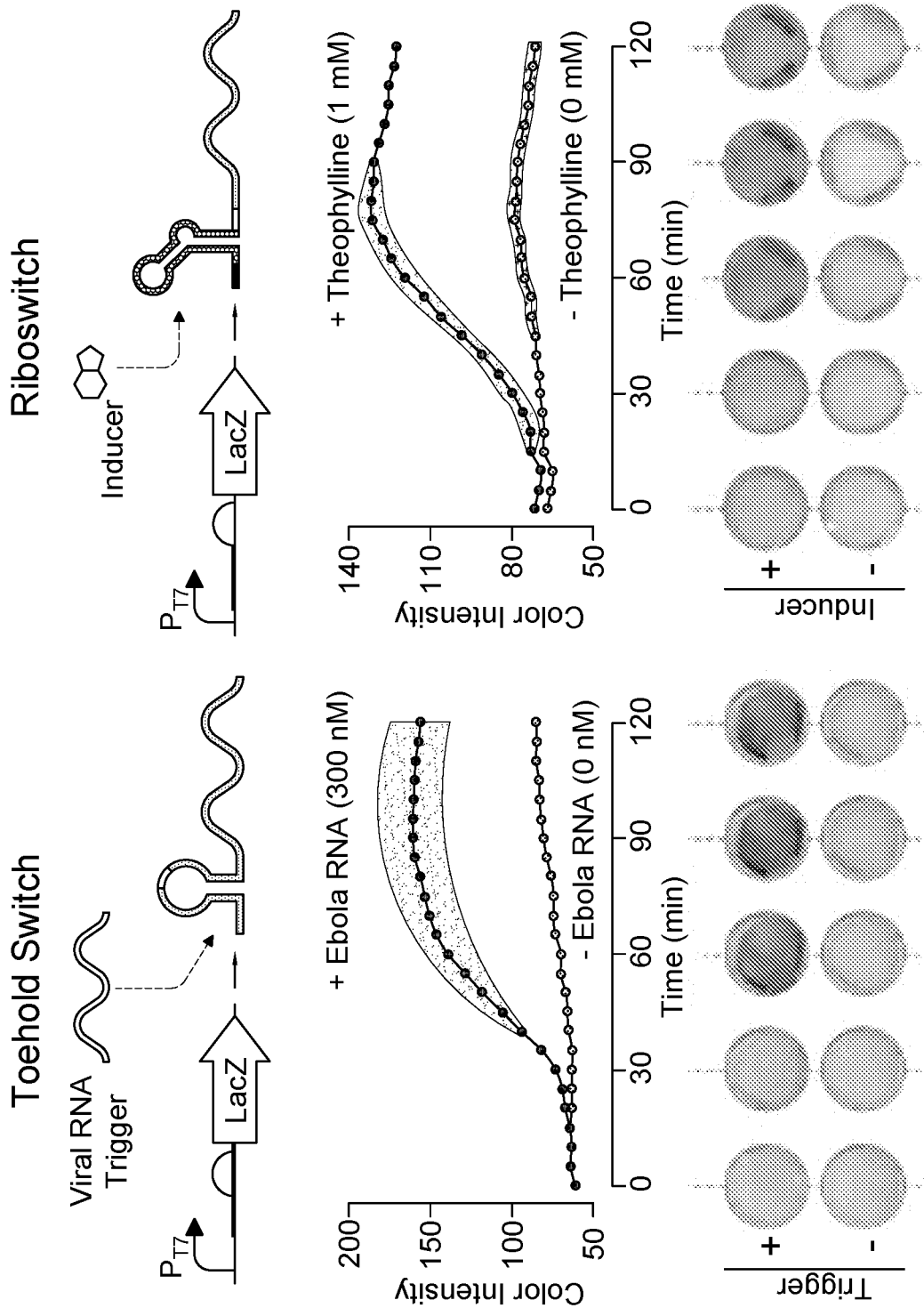


FIG. 6E

FIG. 6F



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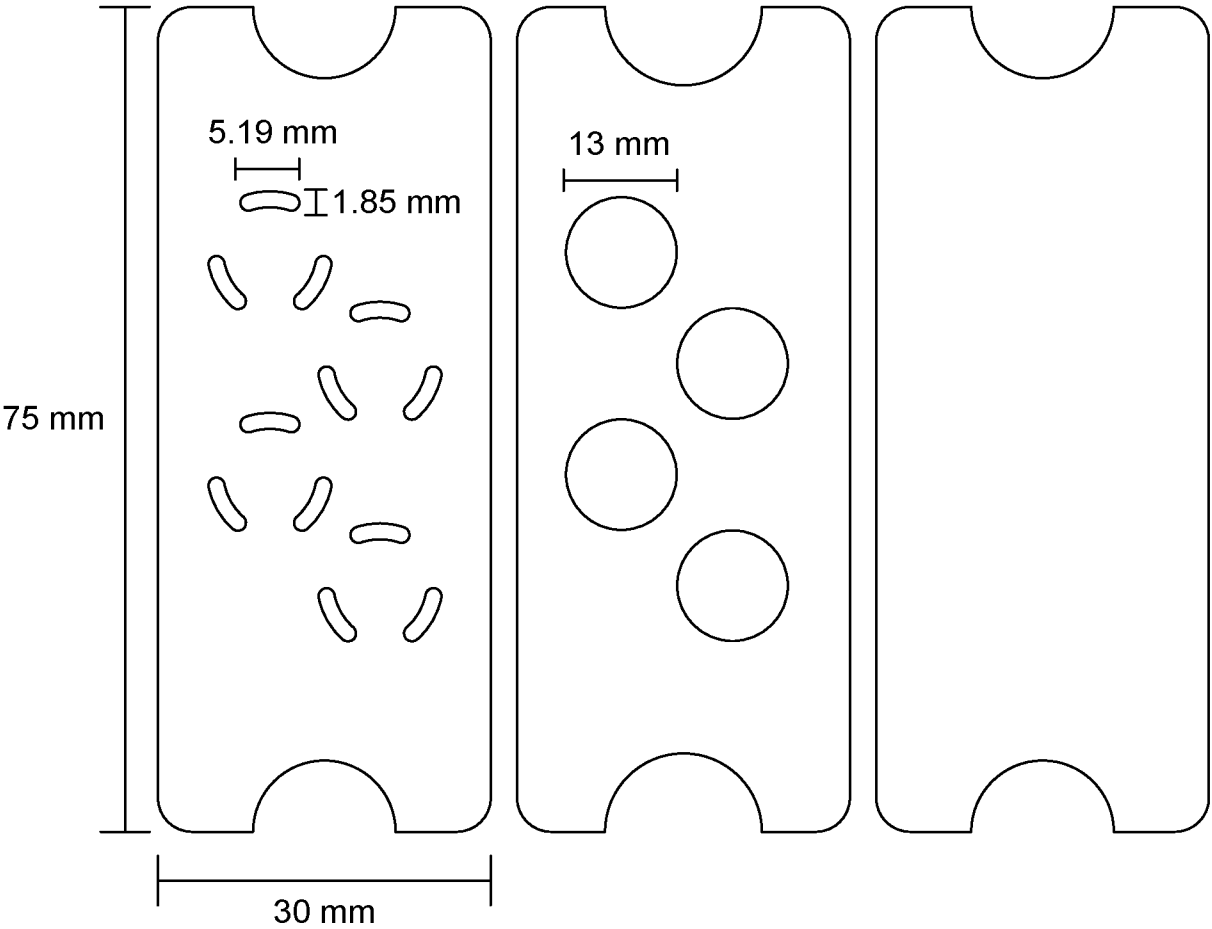


FIG. 7A

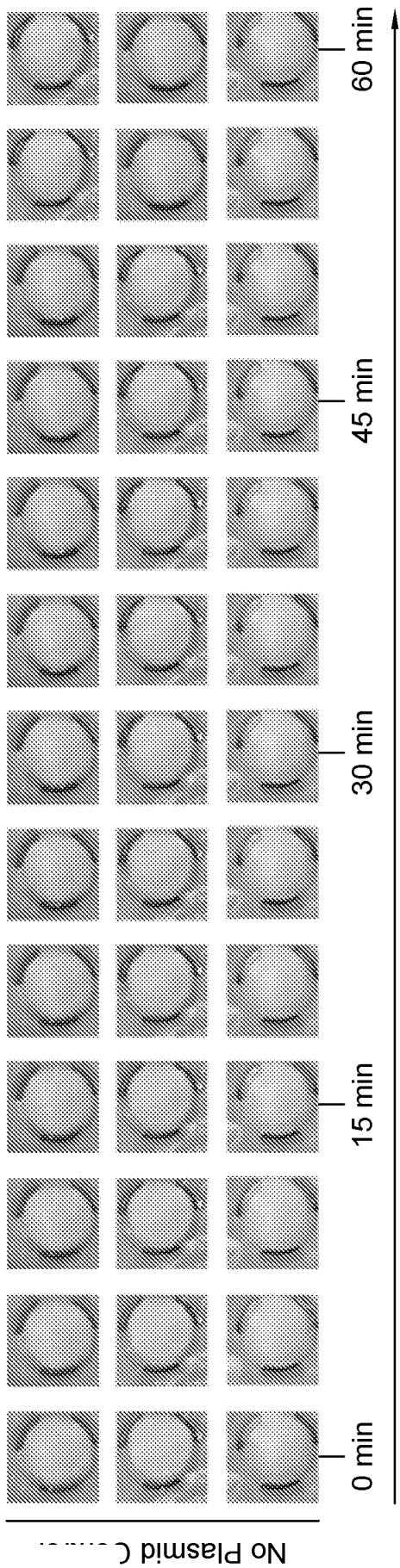


FIG. 7B

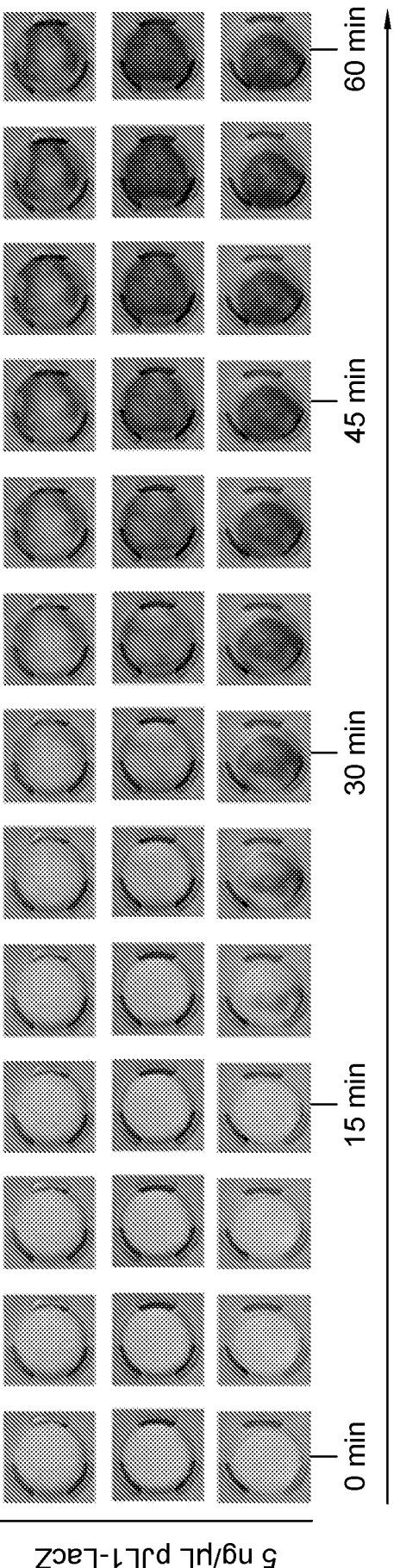


FIG. 7C

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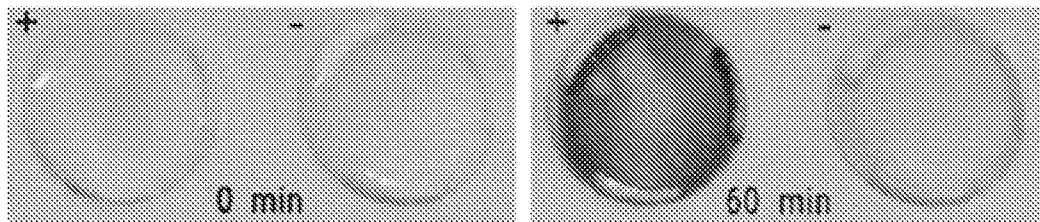


FIG. 8A

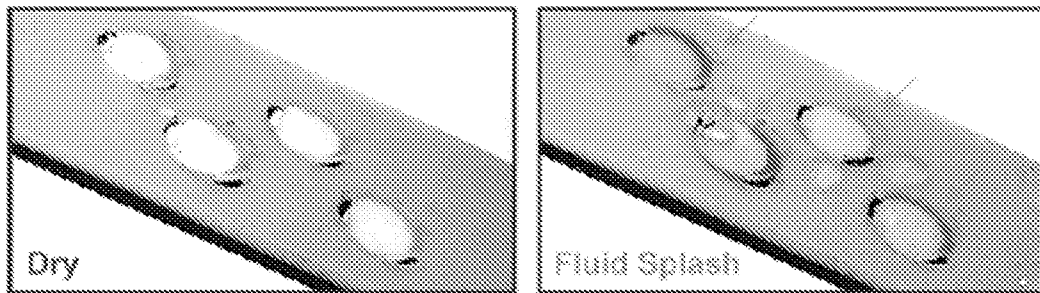


FIG. 8B

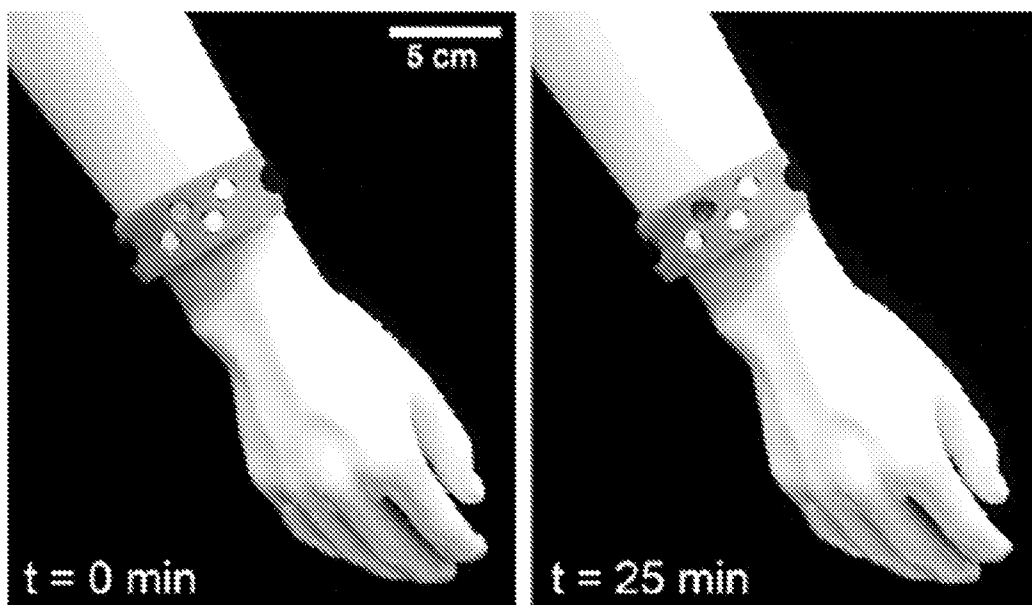


FIG. 8C

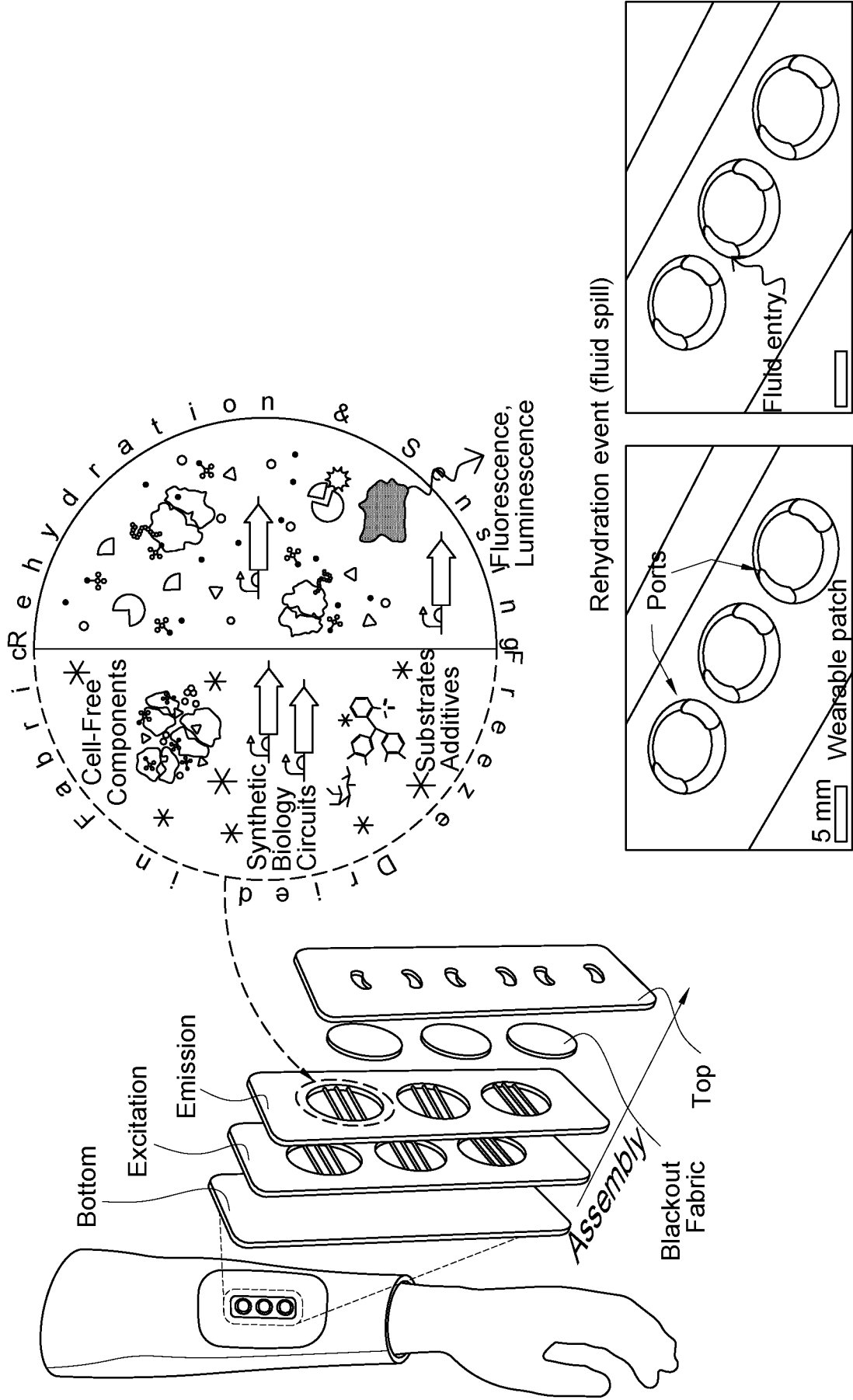


FIG. 9A

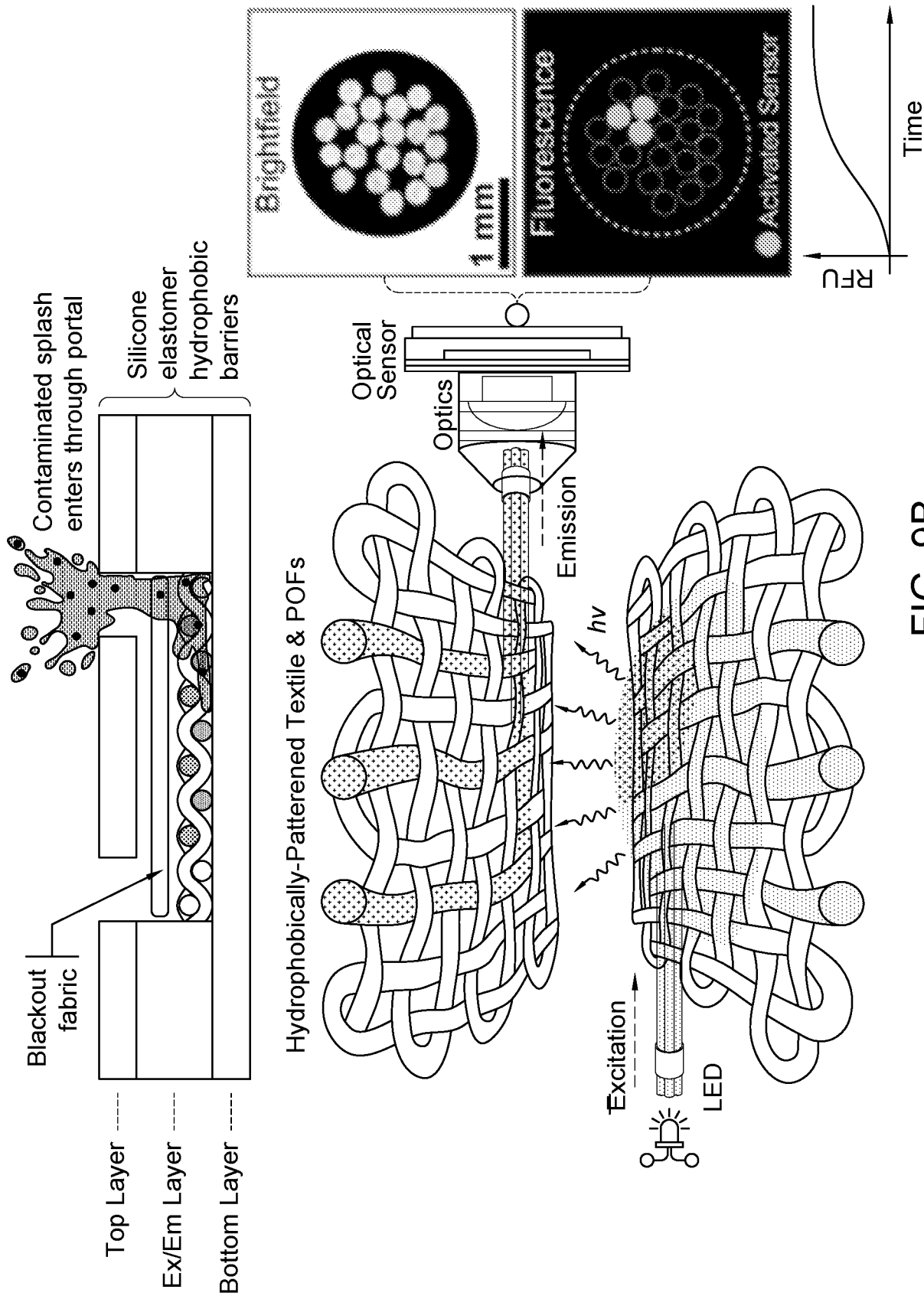


FIG. 9B

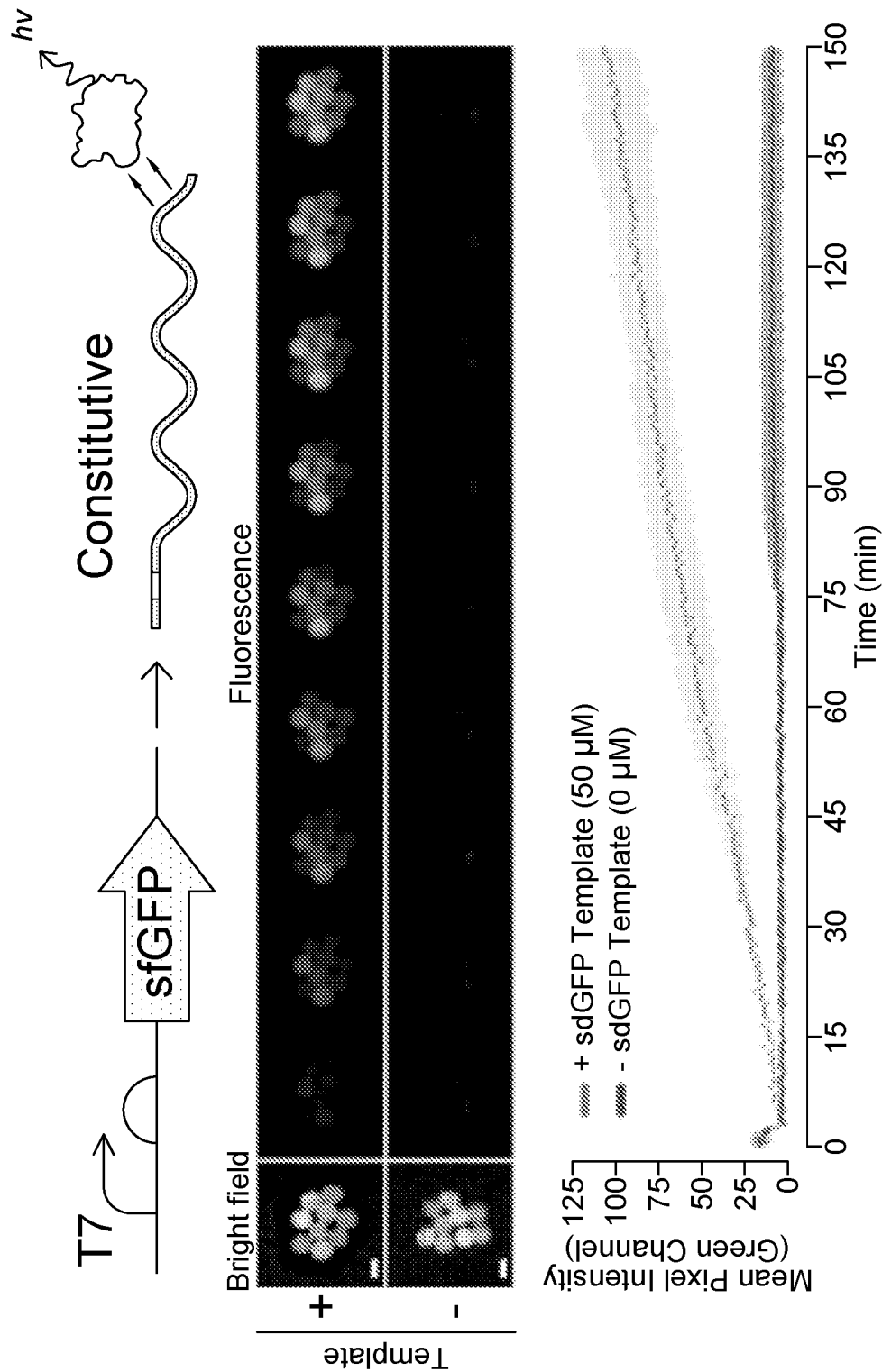


FIG. 9C

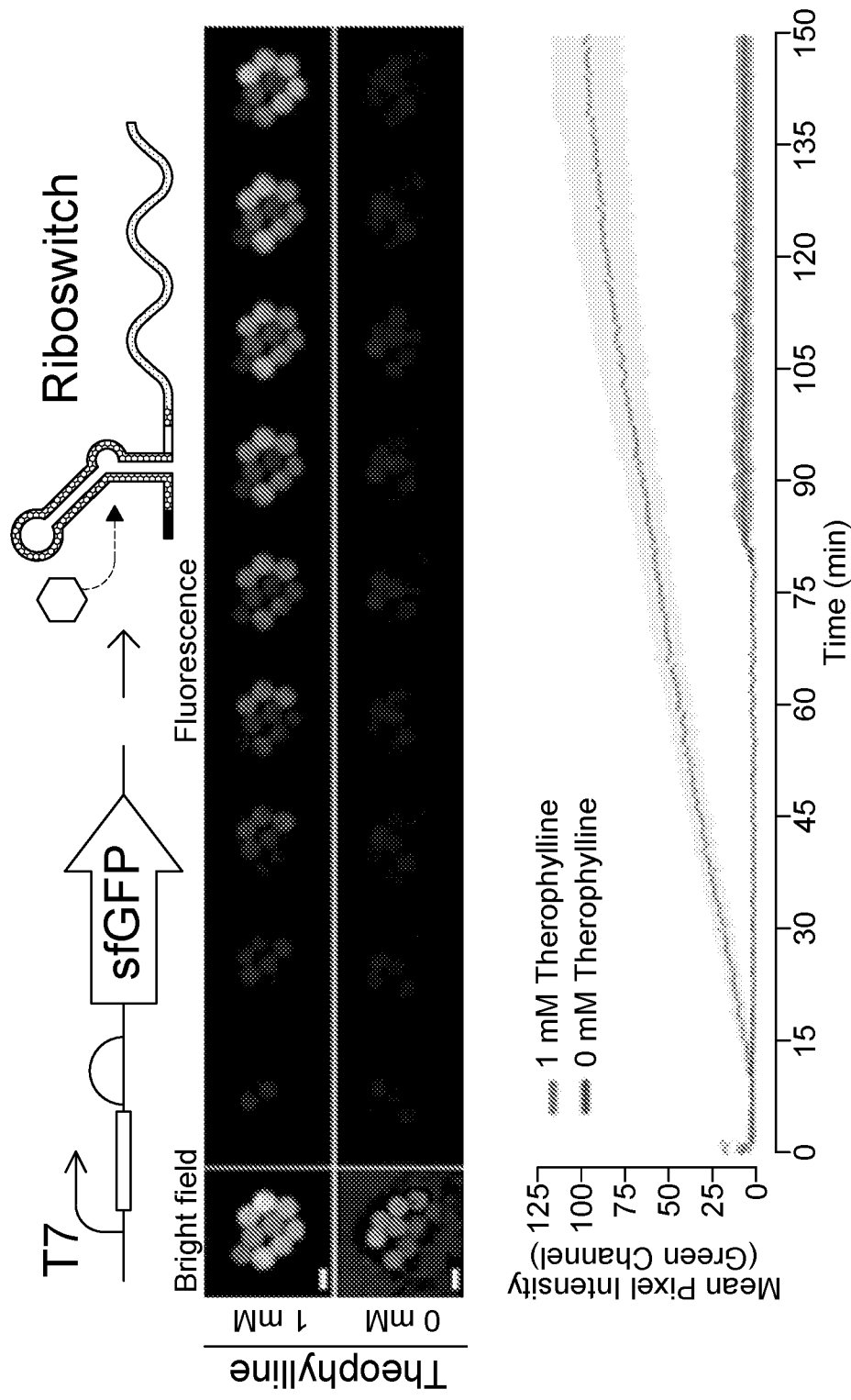


FIG. 9D

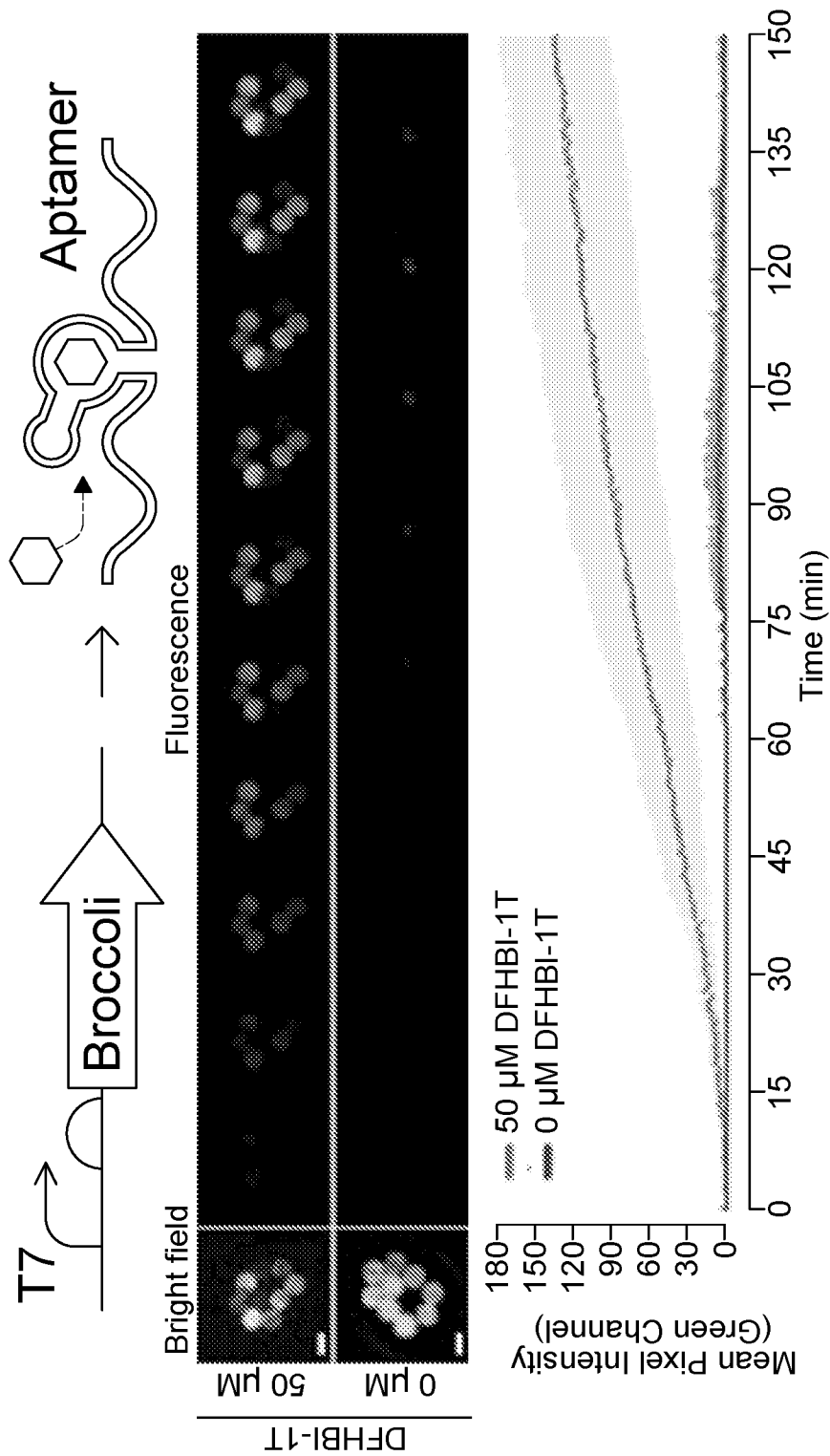


FIG. 9E

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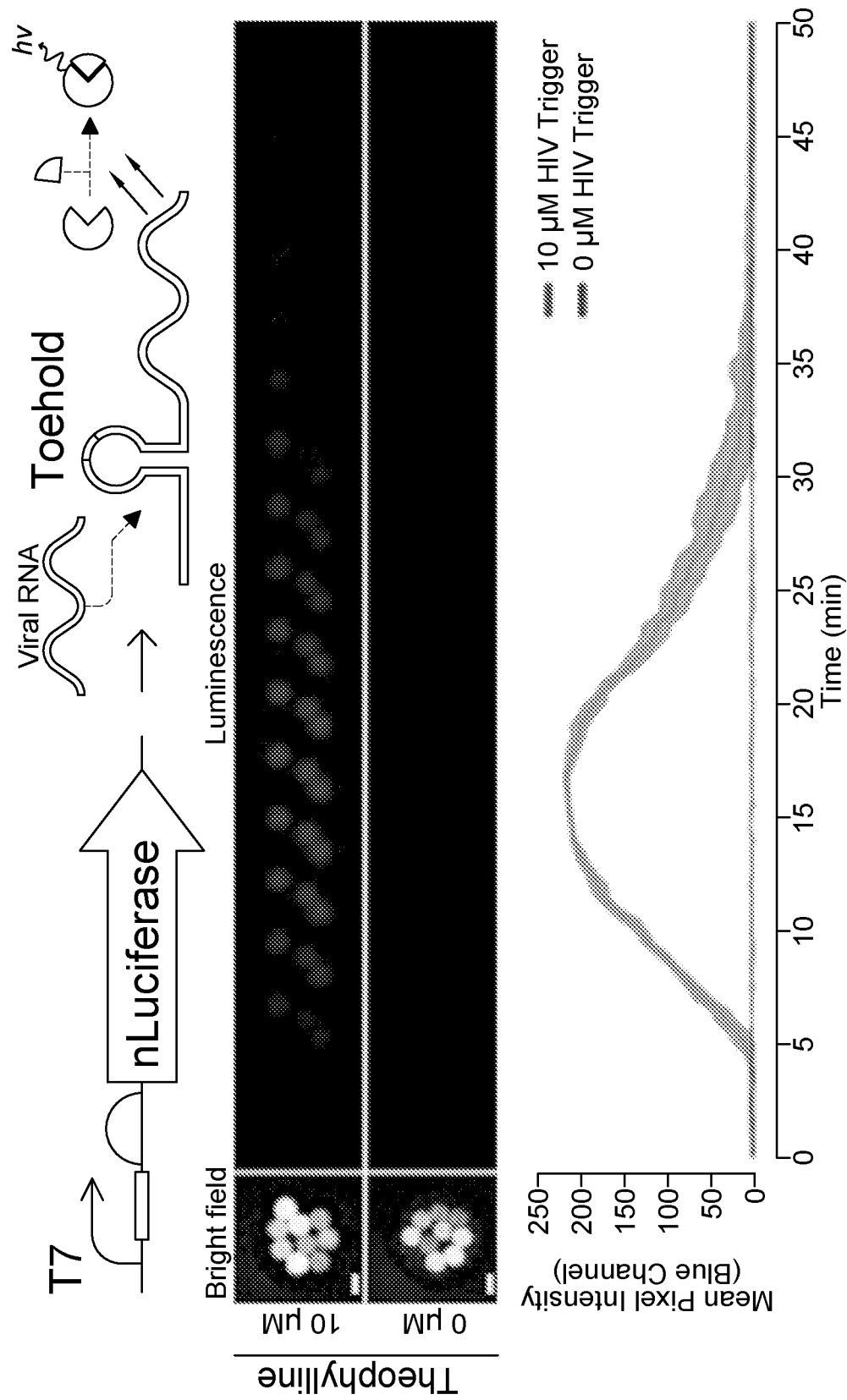


FIG. 9F

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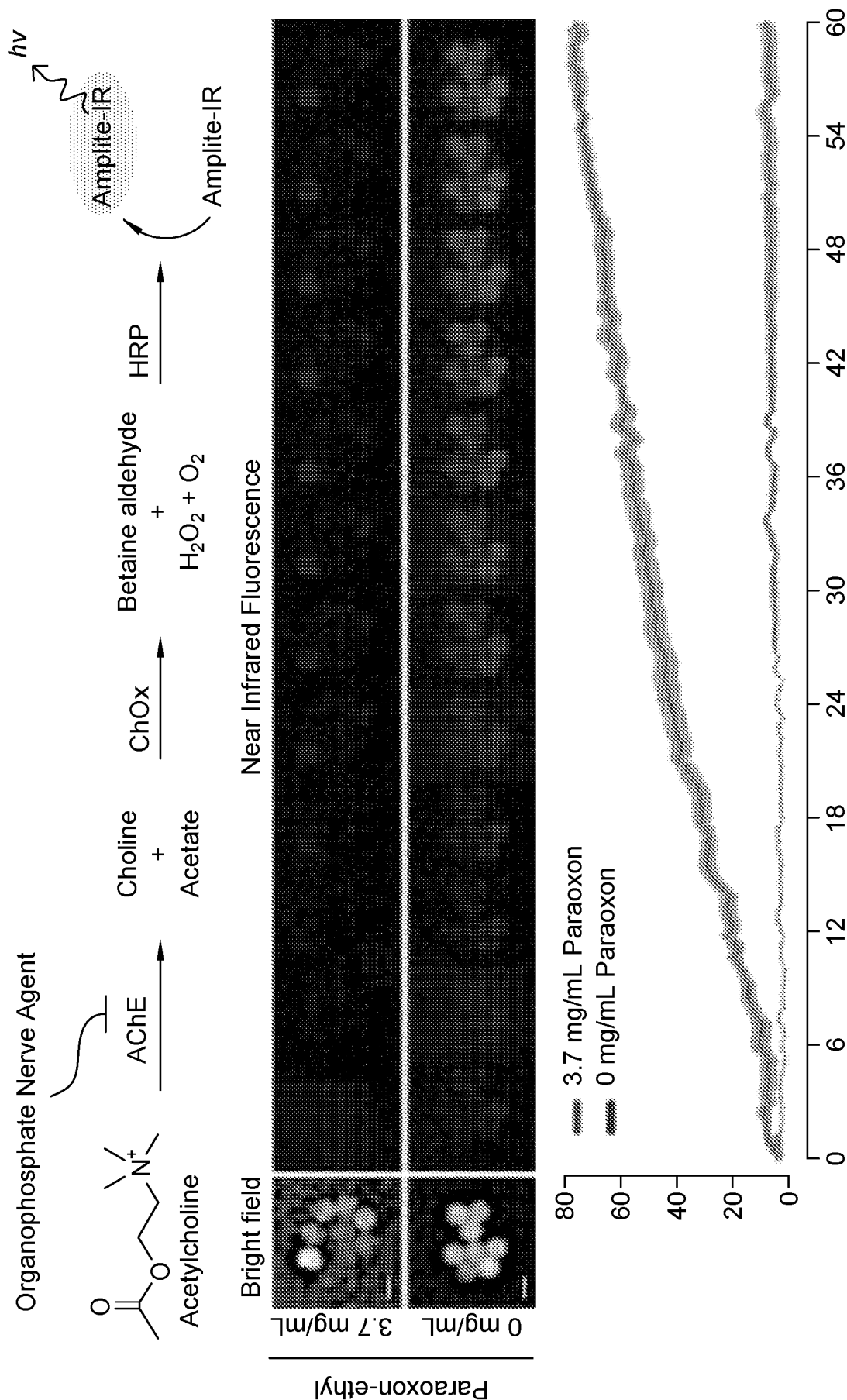


FIG. 9G

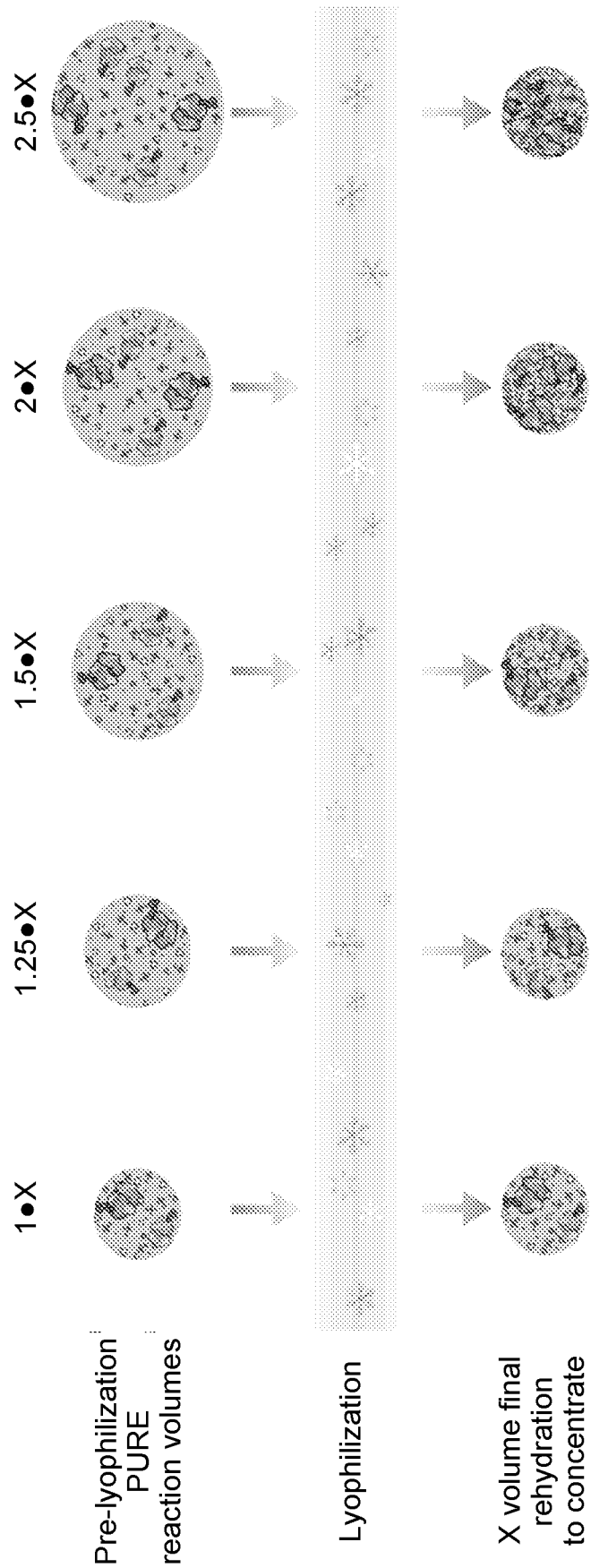


FIG. 10A

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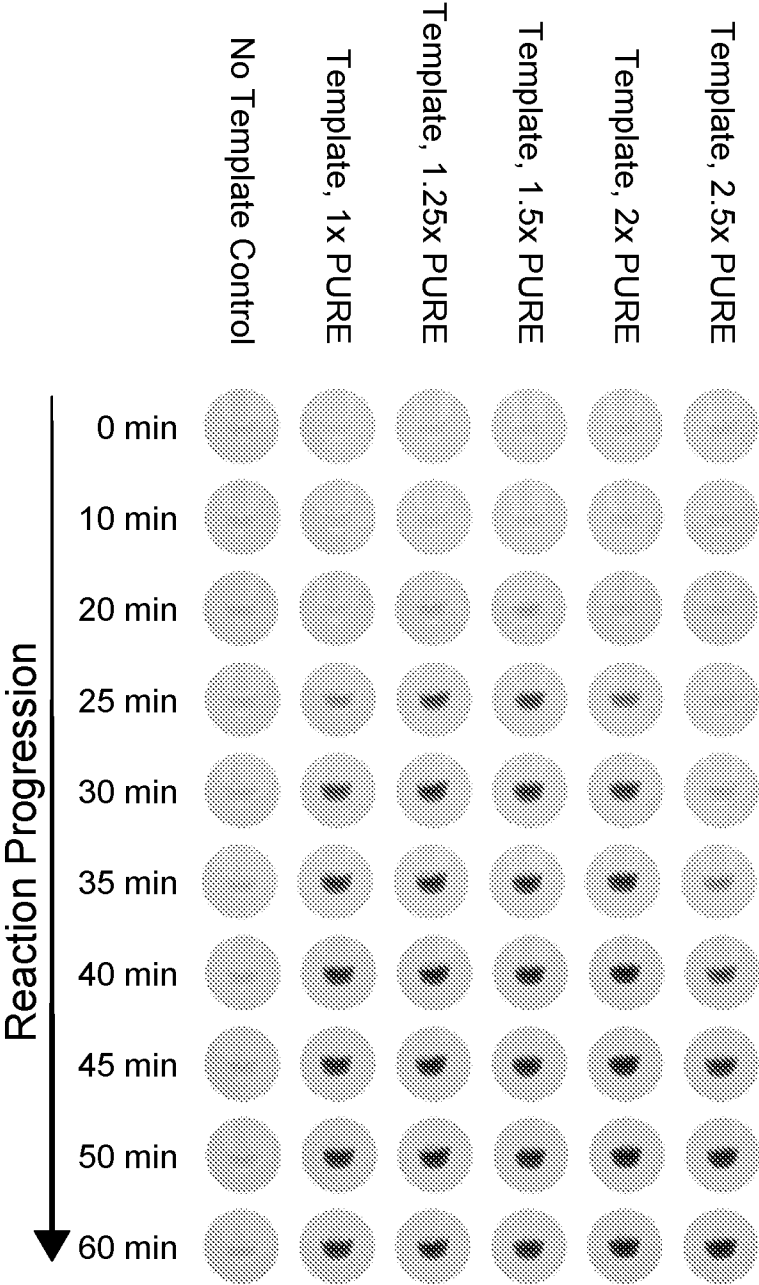


FIG. 10B

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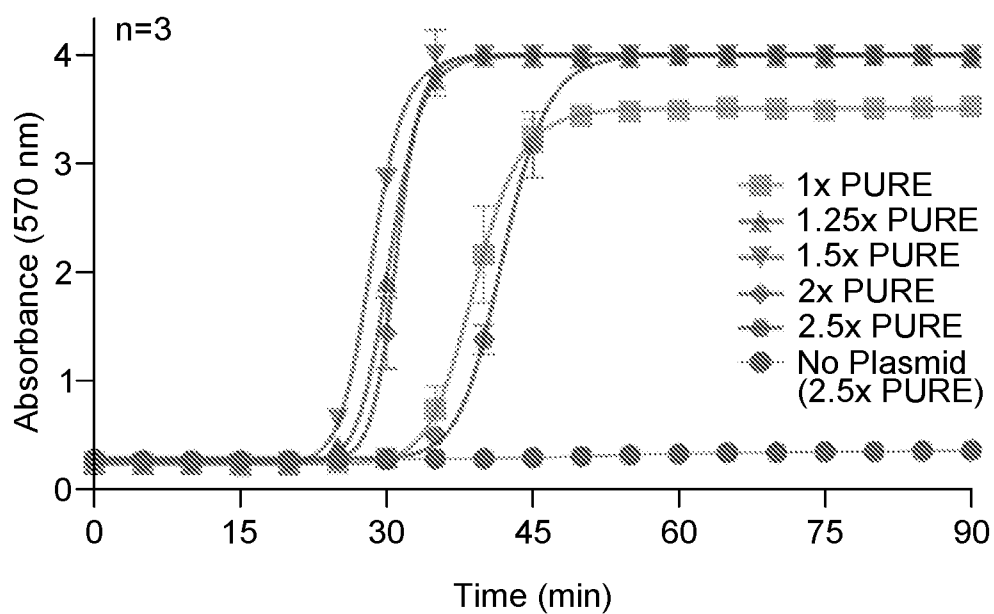


FIG. 10C

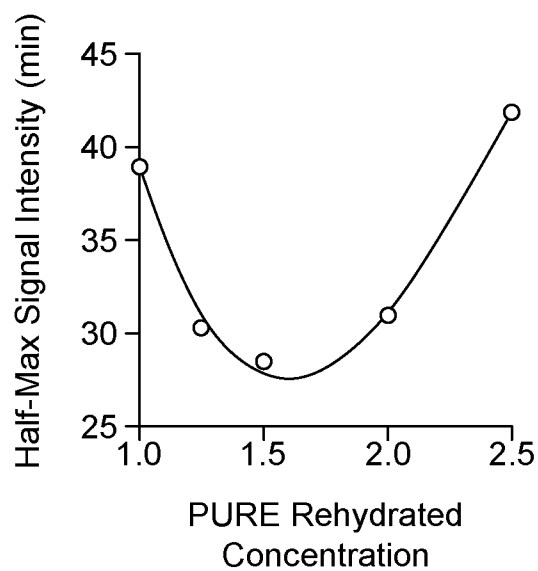


FIG. 10D

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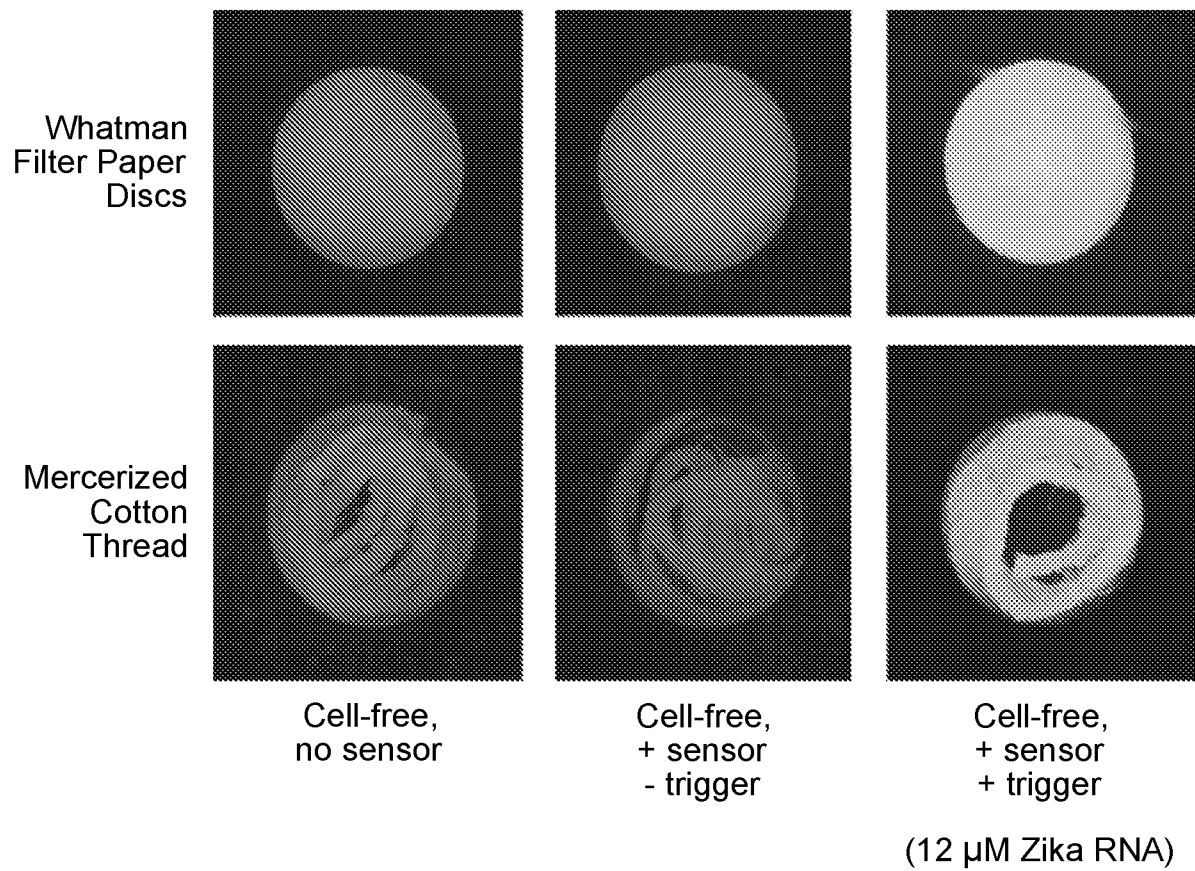


FIG. 11

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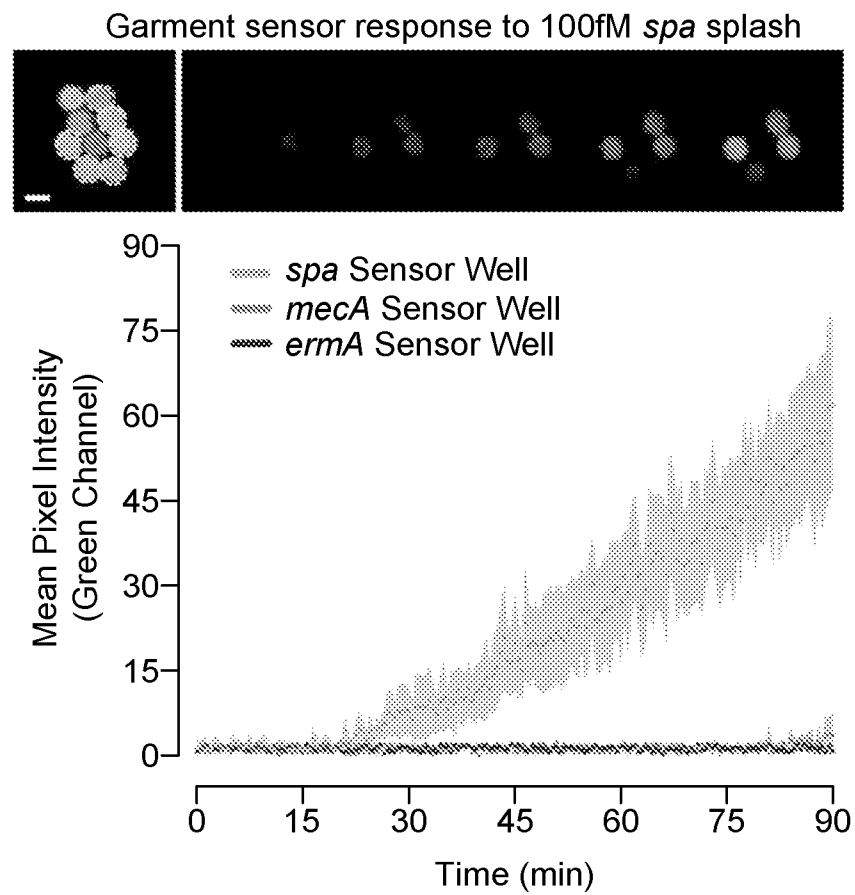


FIG. 12

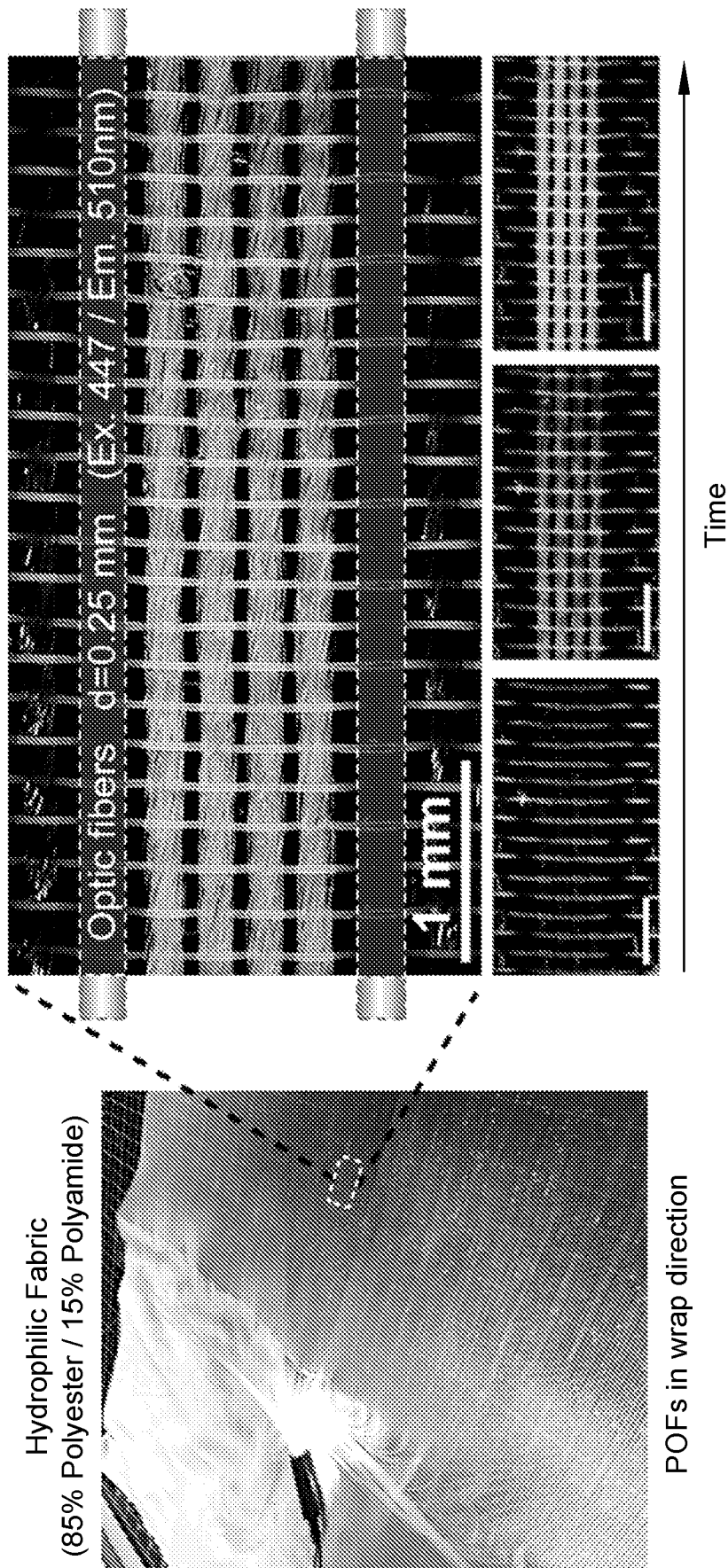


FIG. 13

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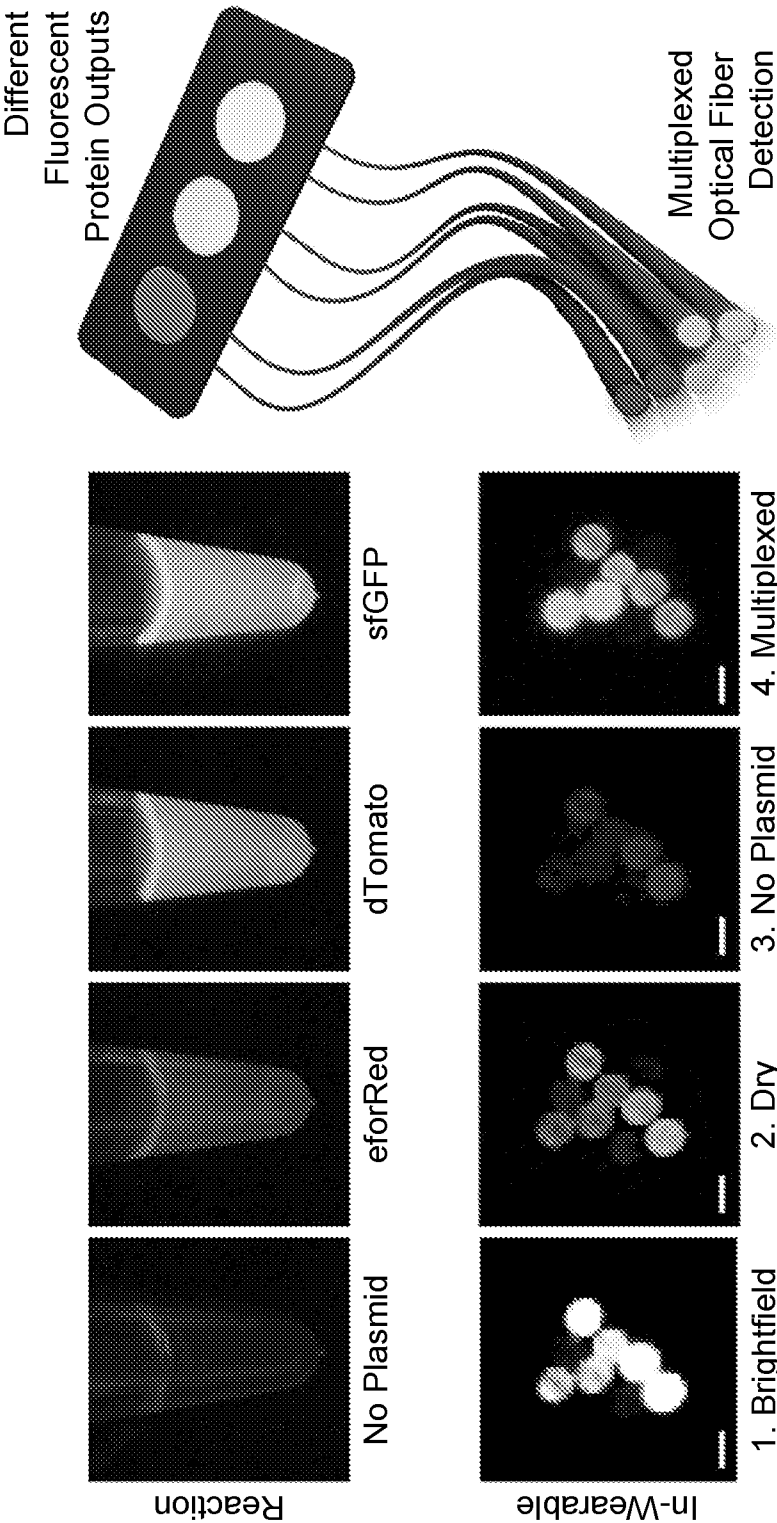


FIG. 14

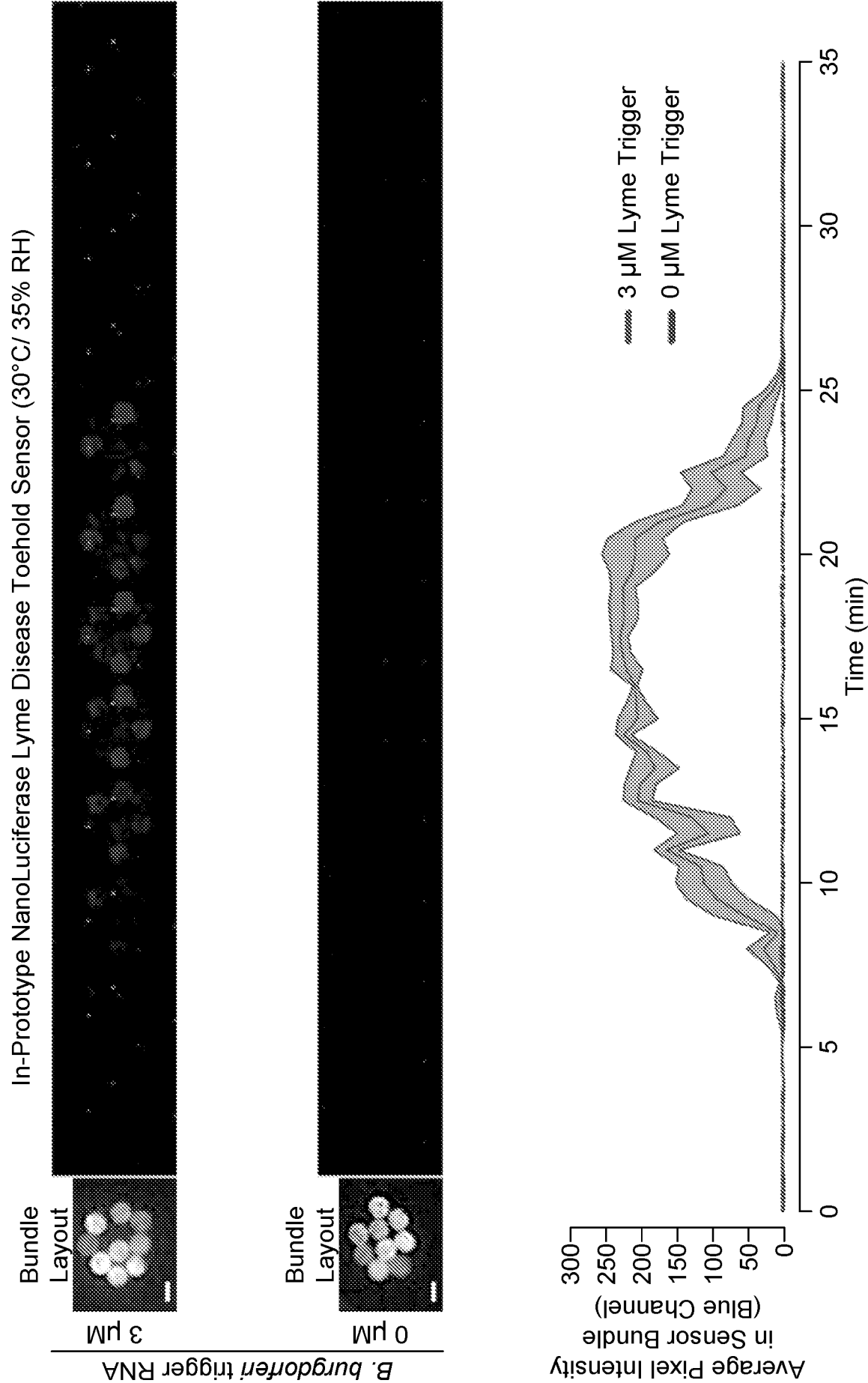


FIG. 15A

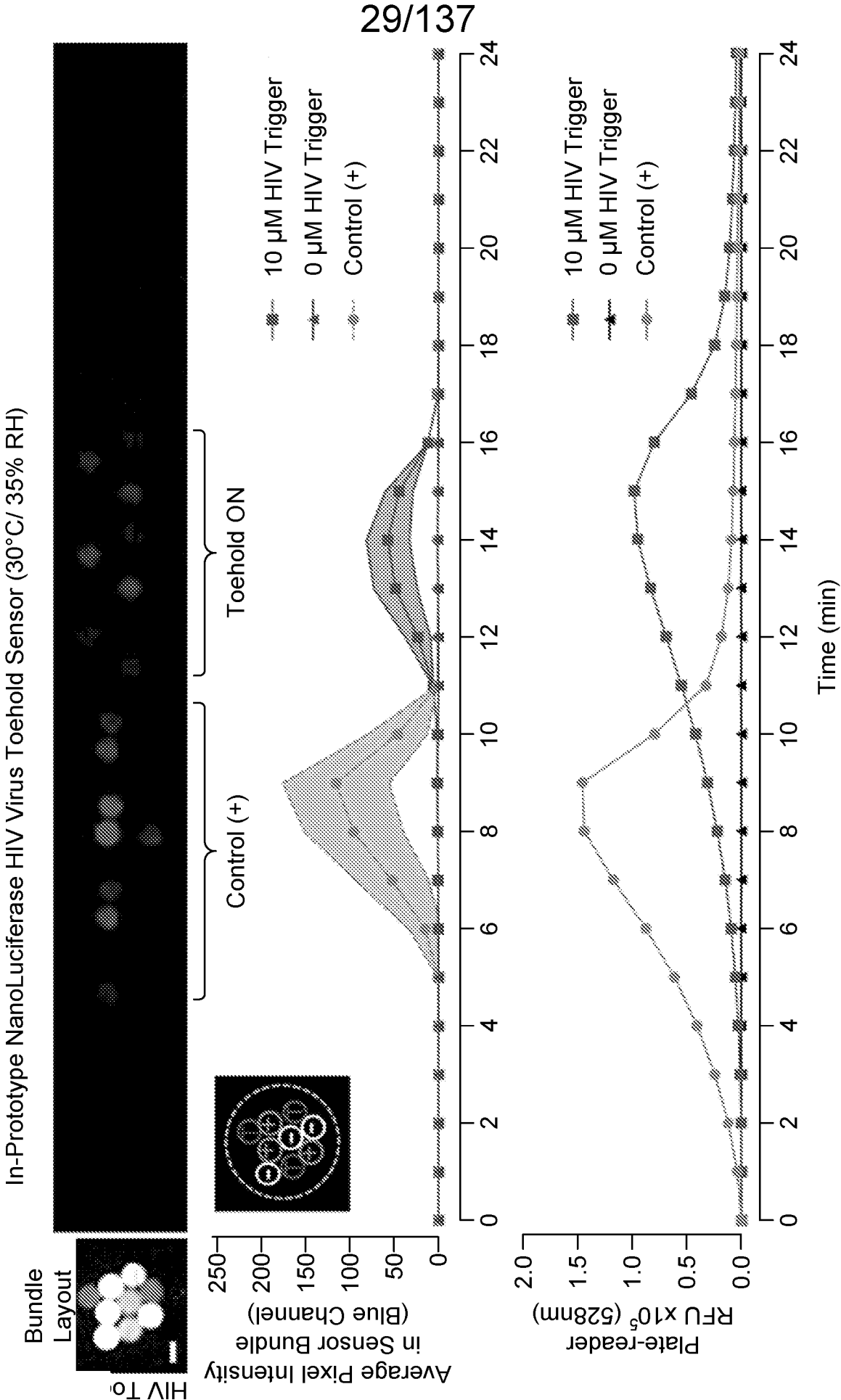


FIG. 15B

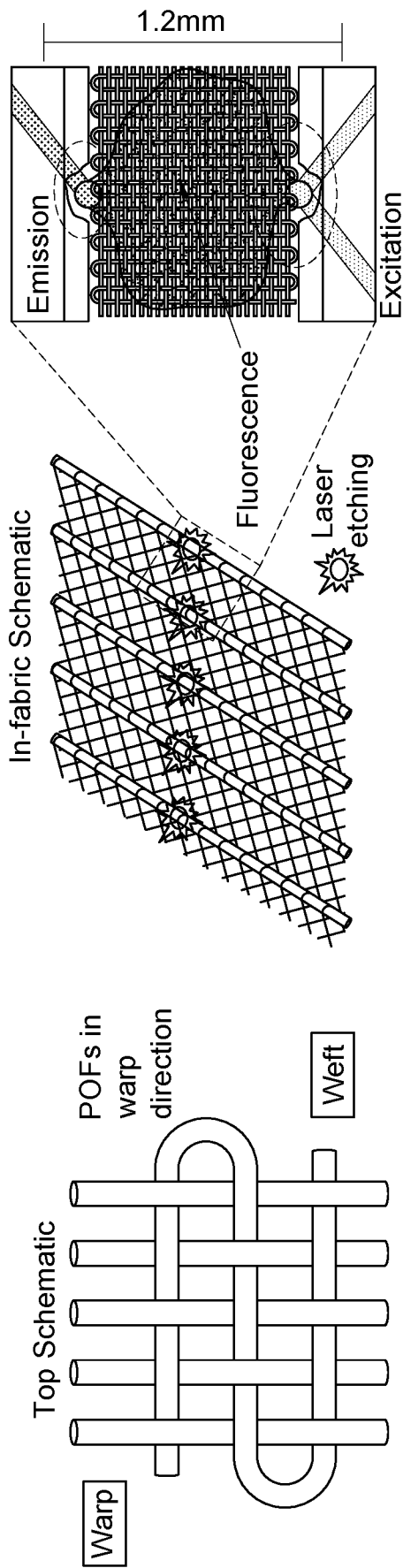


FIG. 16A

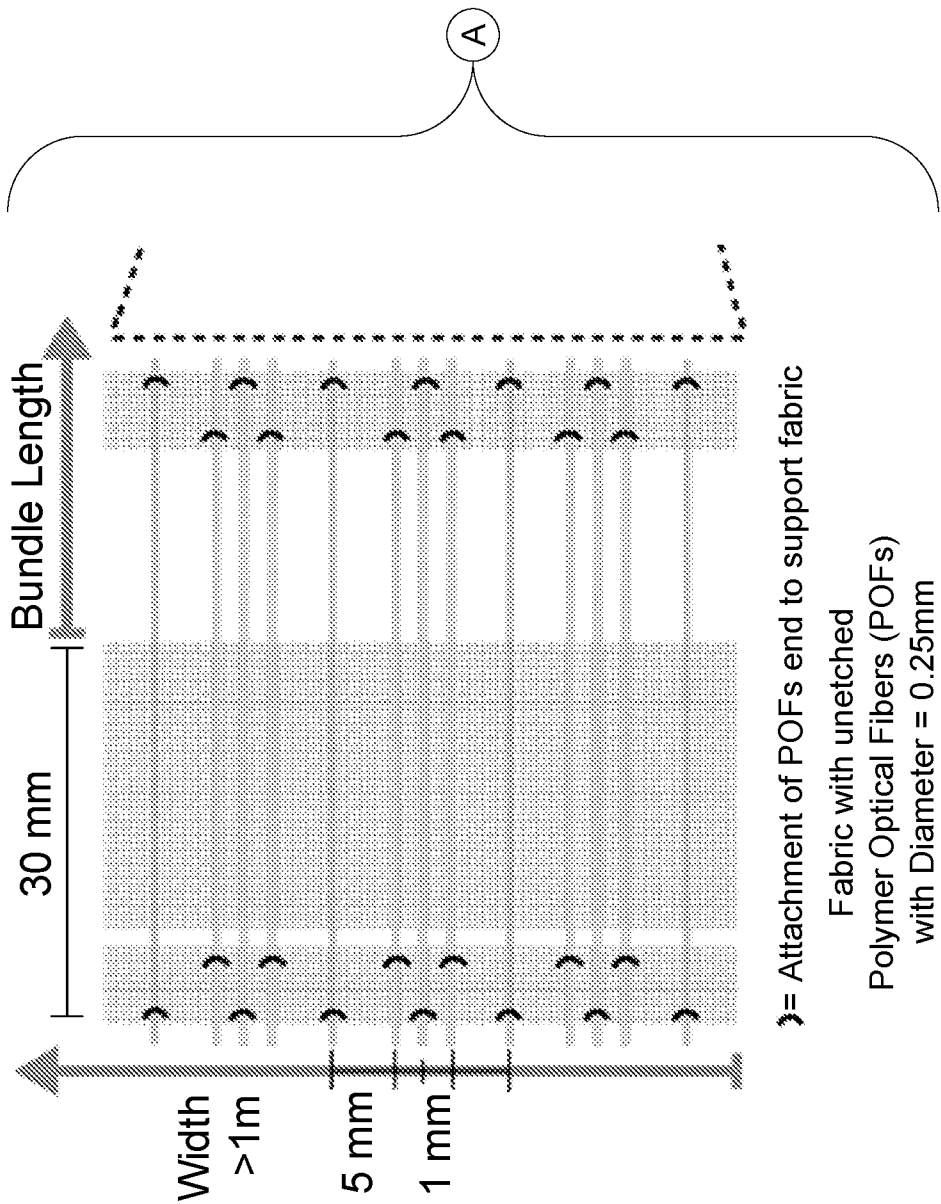


FIG. 16B

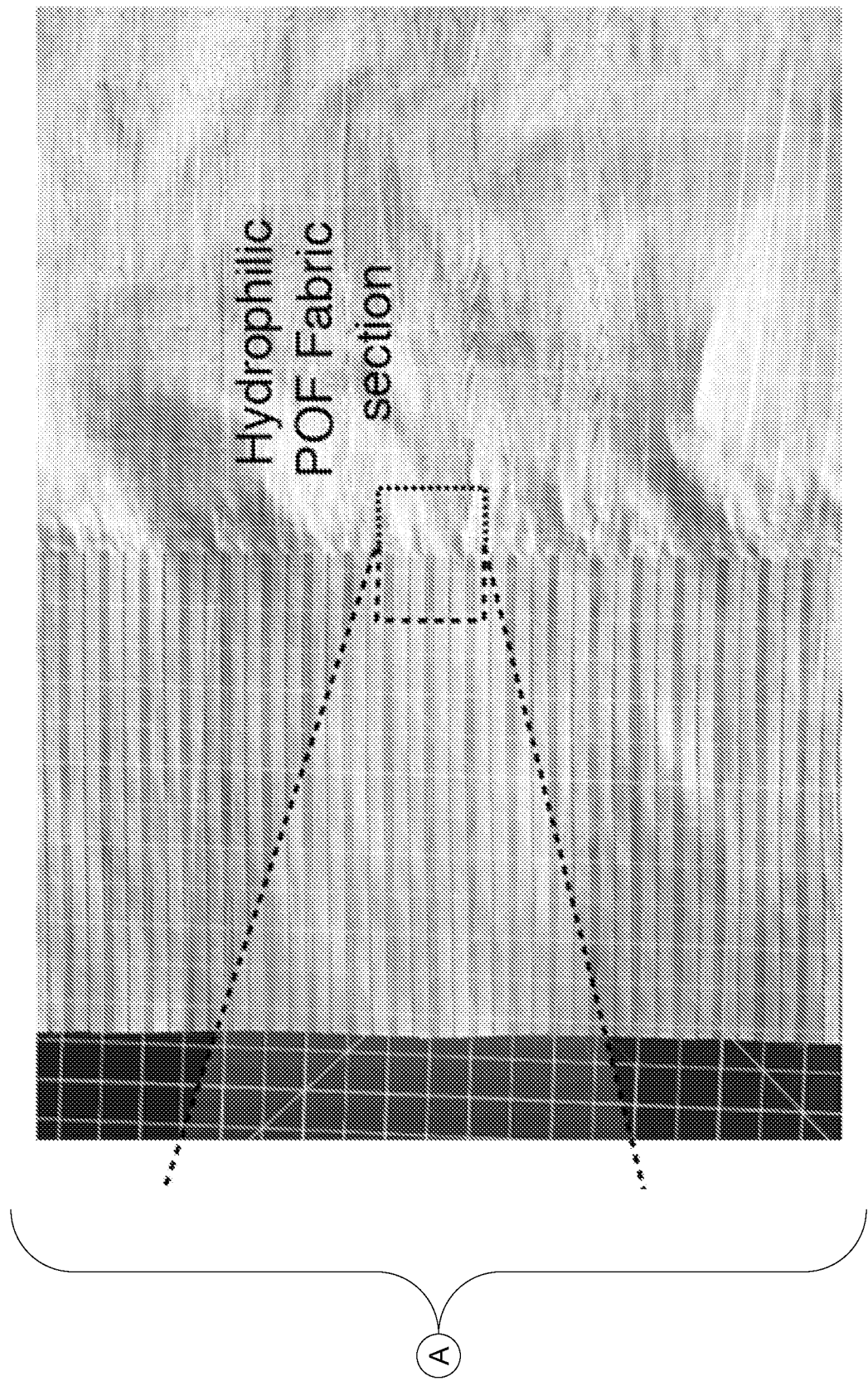


FIG. 16B (CONTINUATION)

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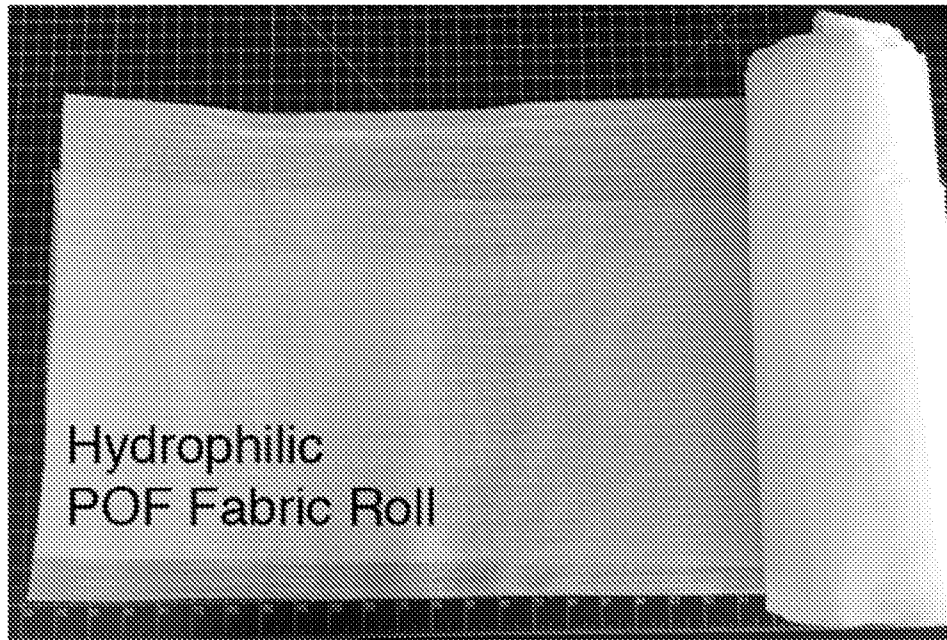


FIG. 16C

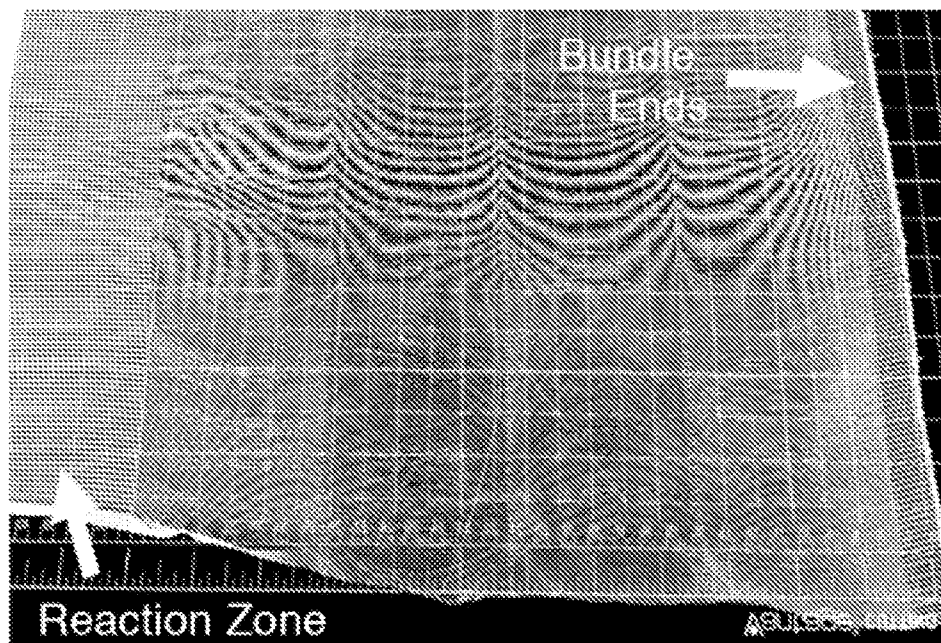


FIG. 16D

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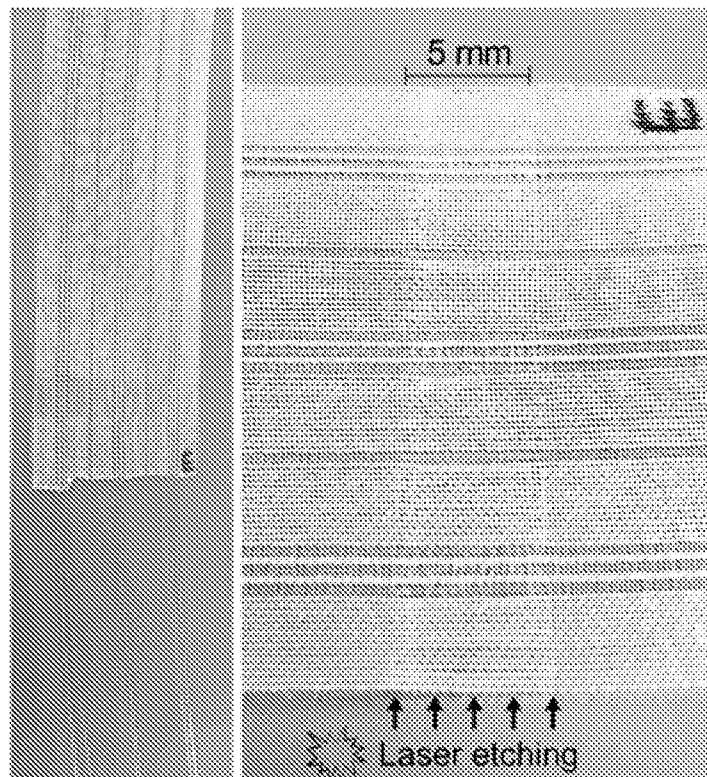
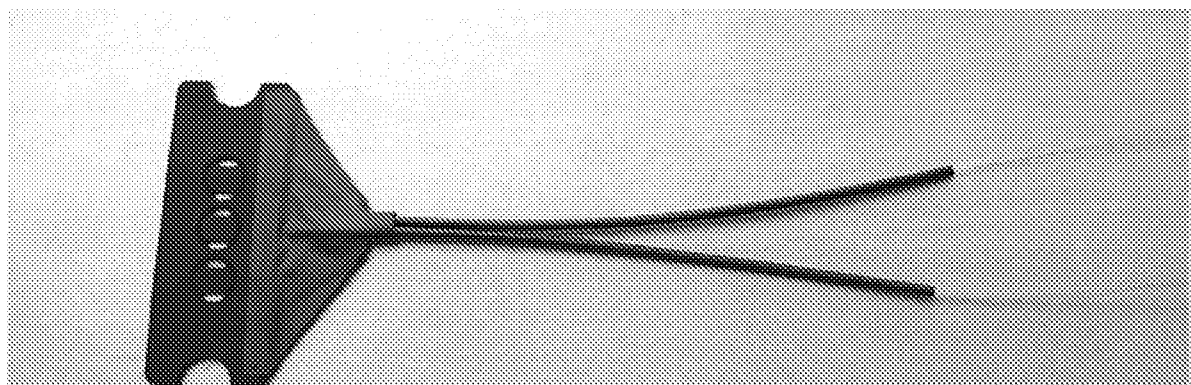
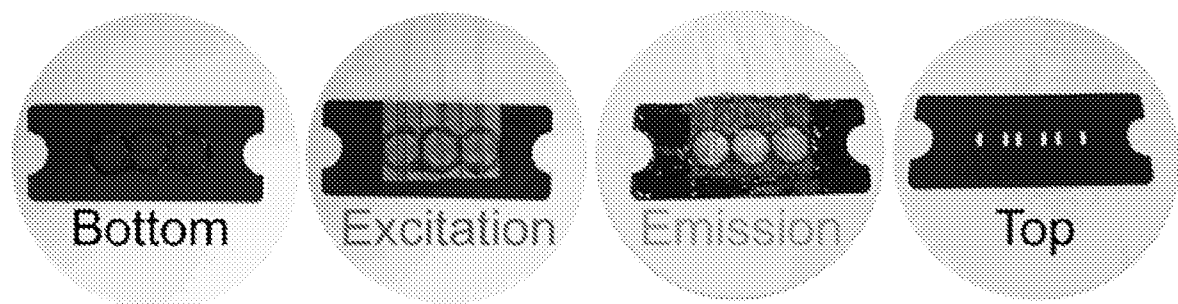


FIG. 17A



Assembled Layers

FIG. 17B

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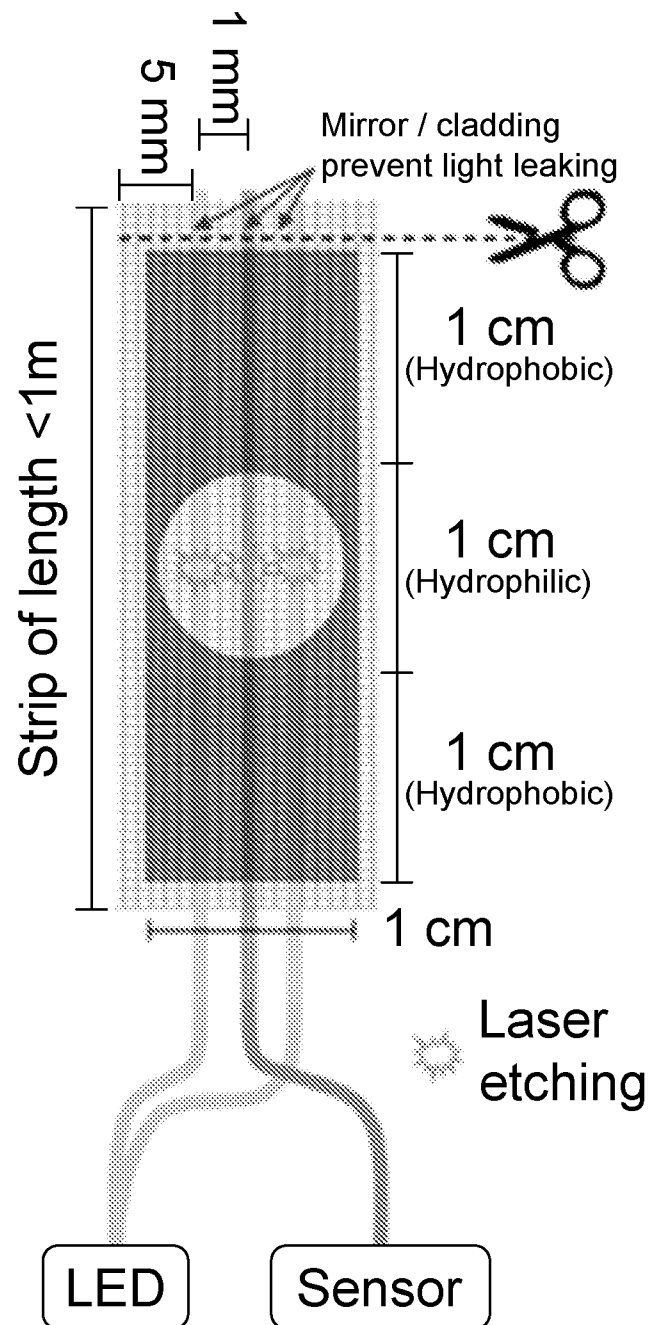


FIG. 17C

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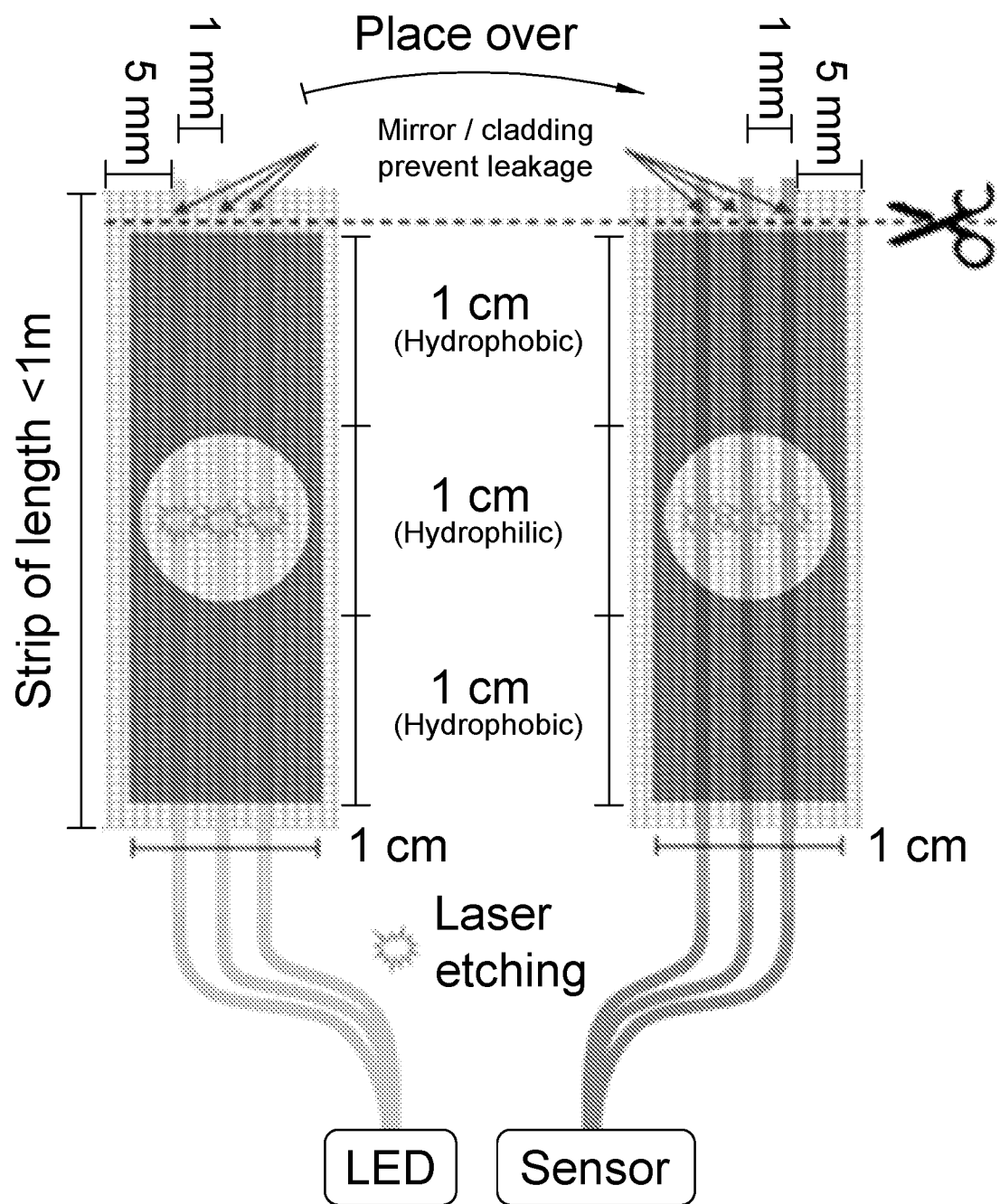


FIG. 17D

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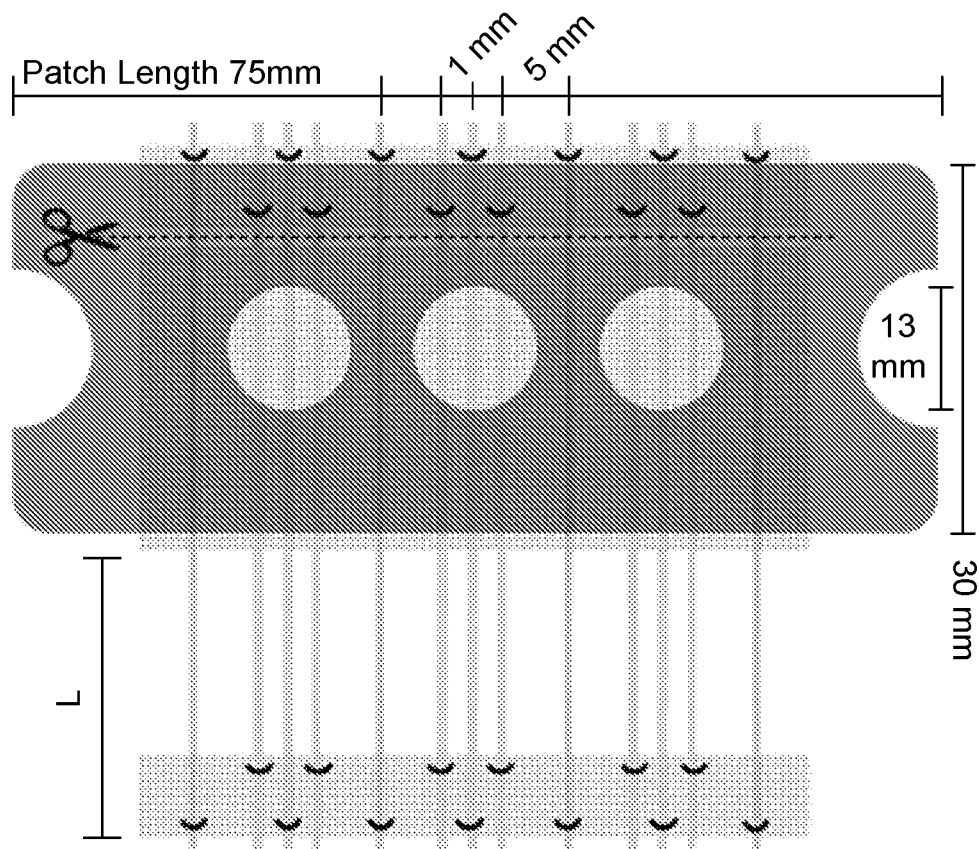


FIG. 17E

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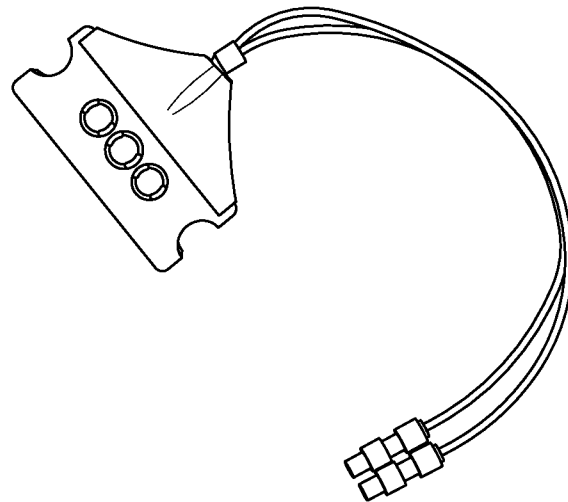


FIG. 17F

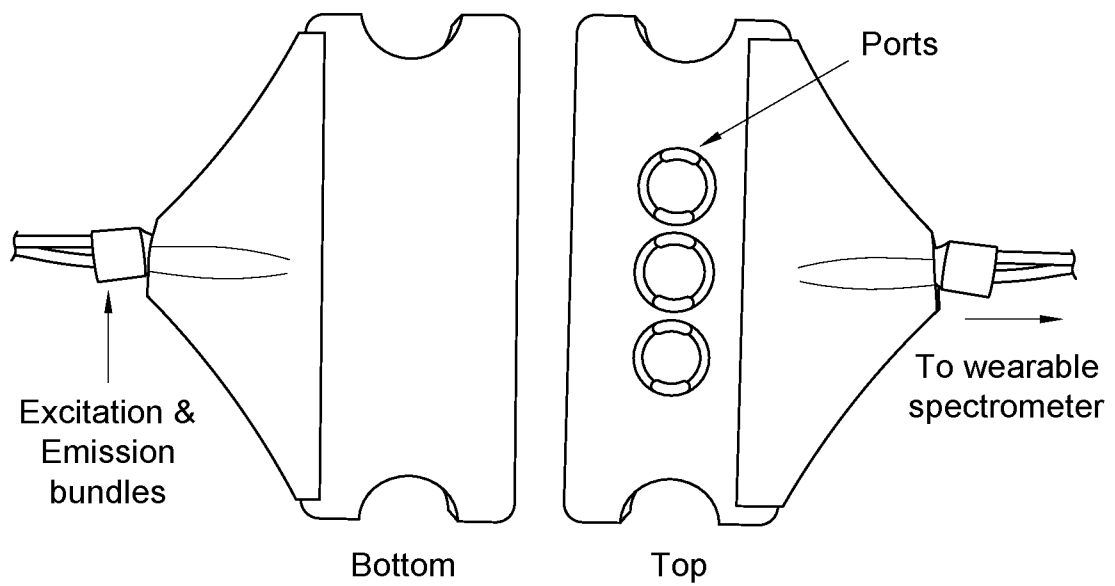


FIG. 17G

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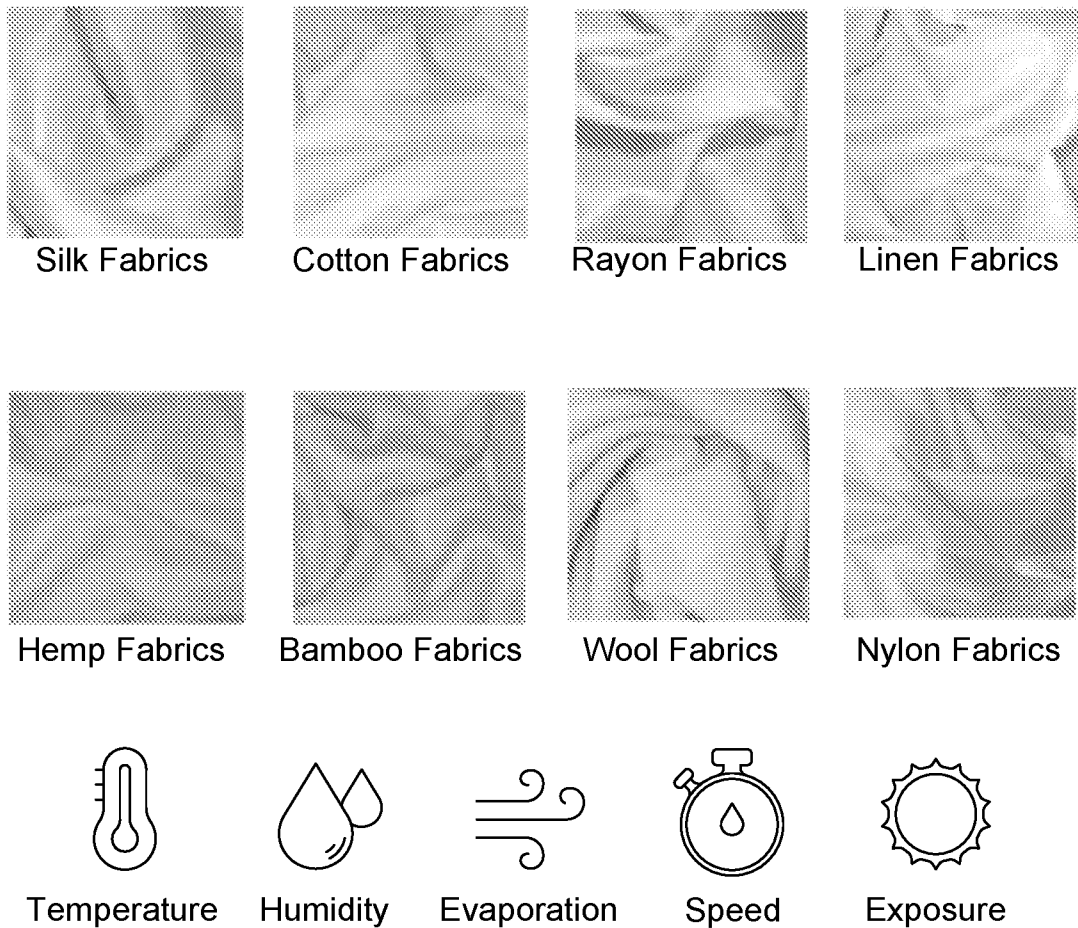


FIG. 18A

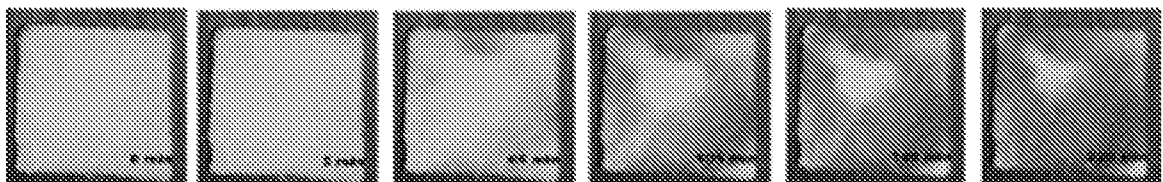


FIG. 18B

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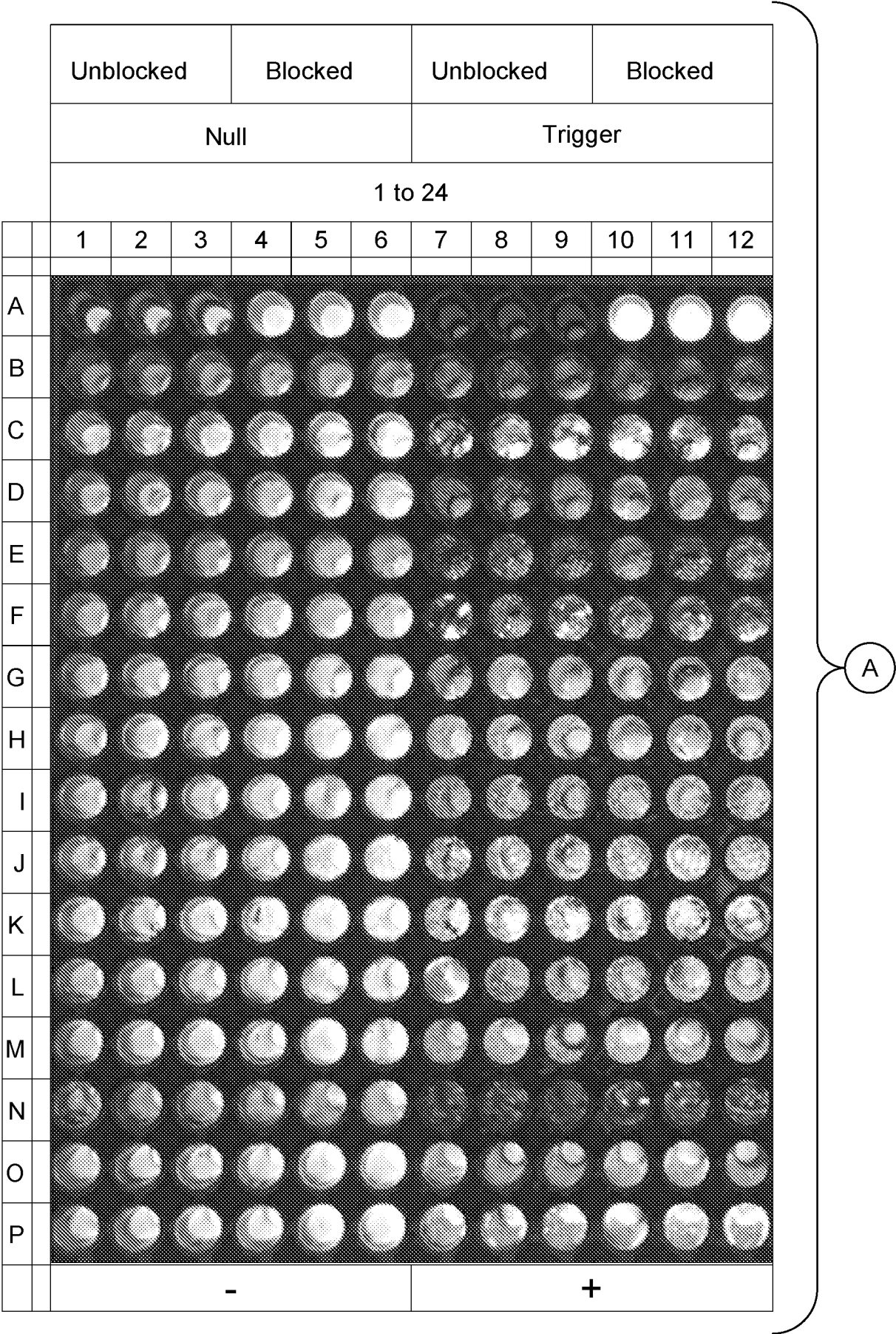


FIG. 19A

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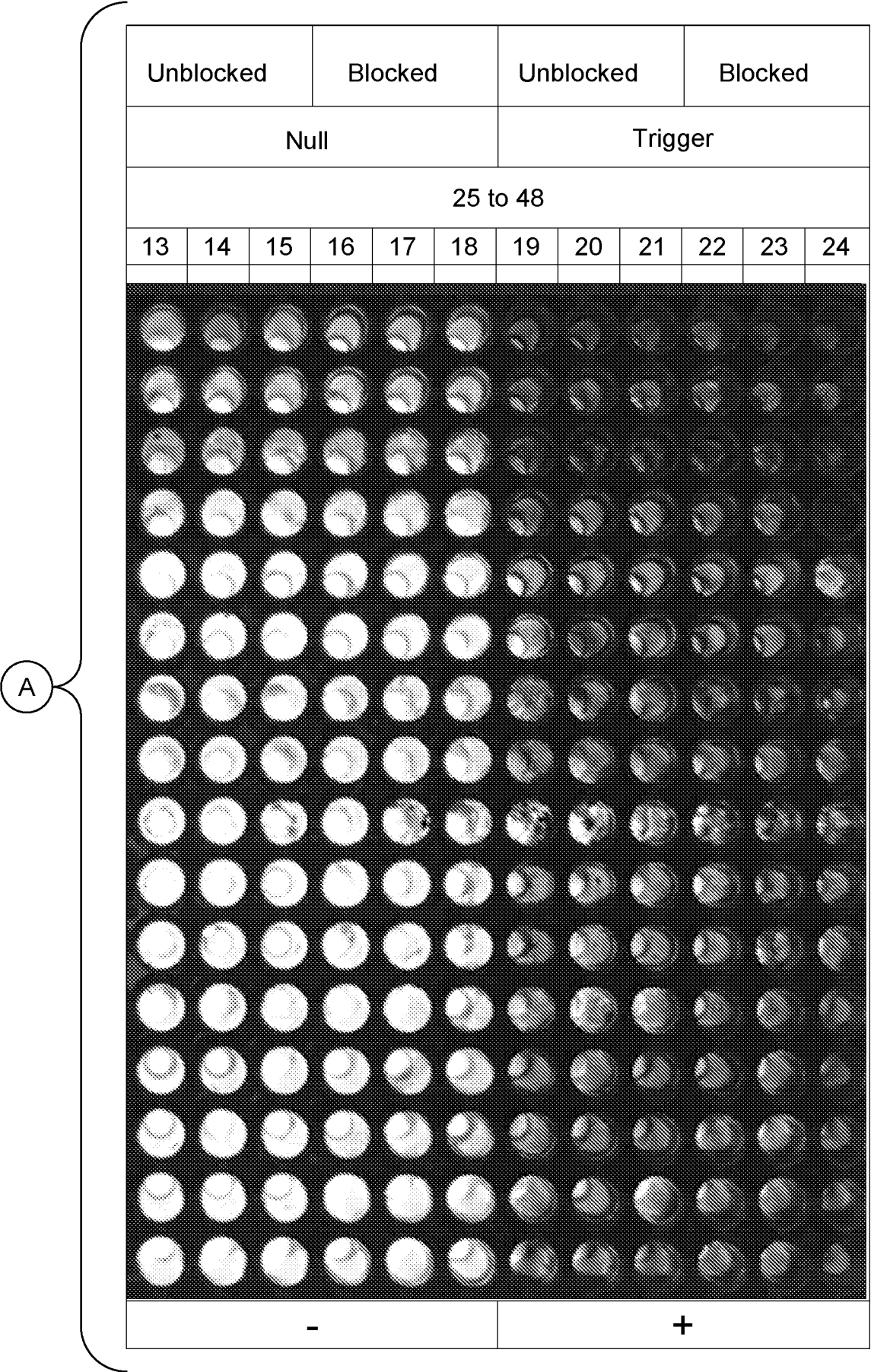


FIG. 19A (CONTINUATION)

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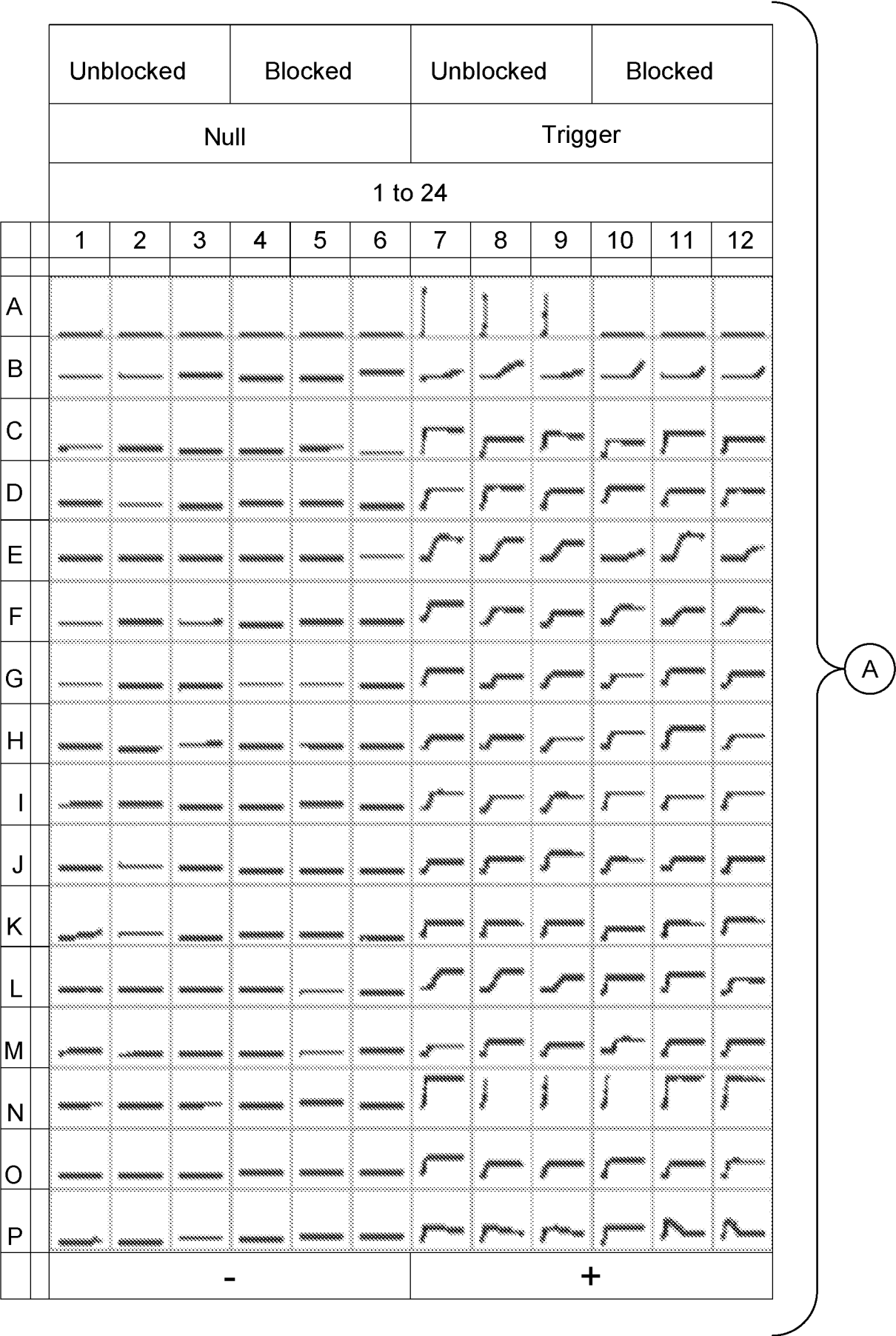


FIG. 19B

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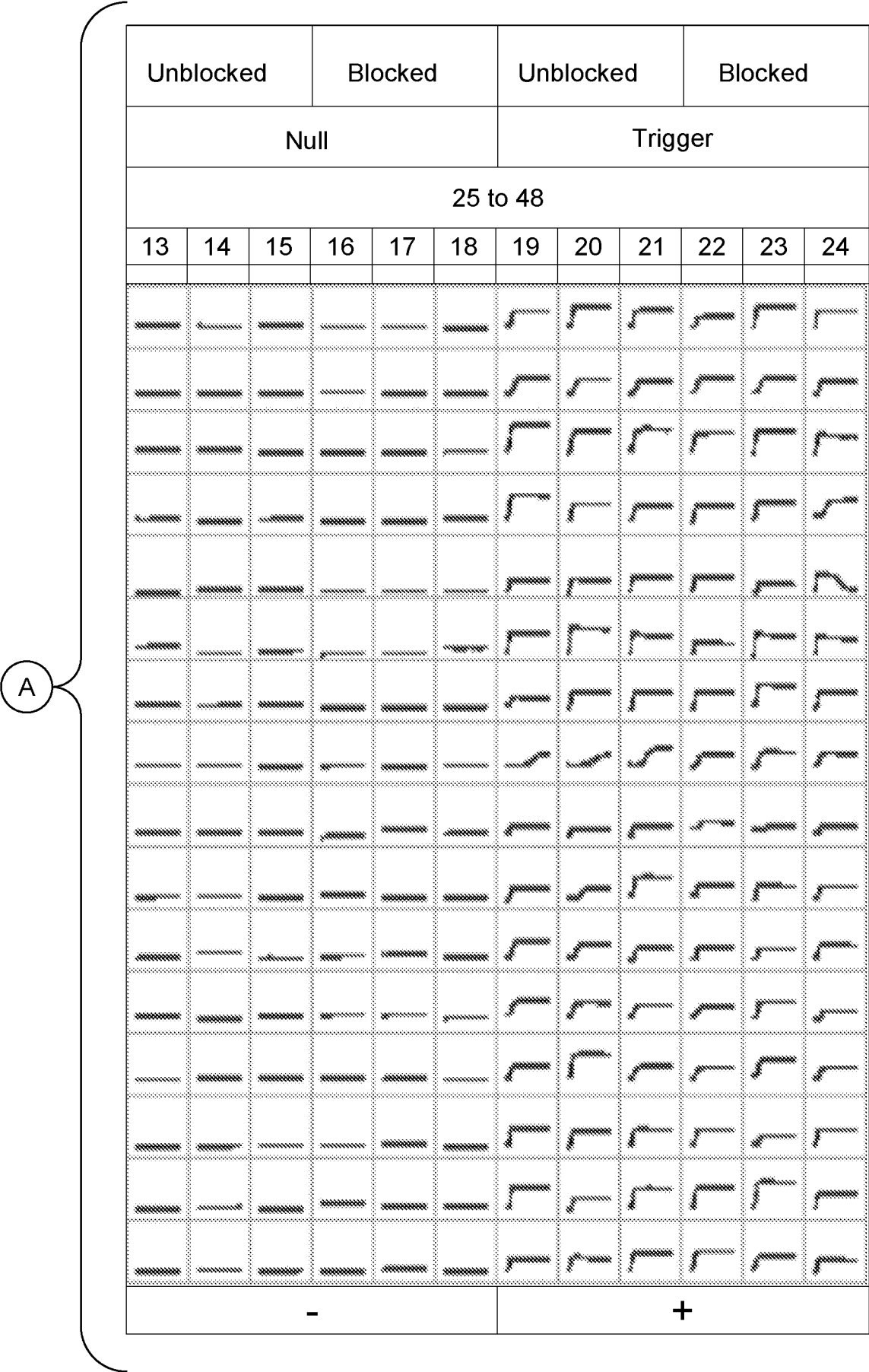


FIG. 19B (CONTINUATION)

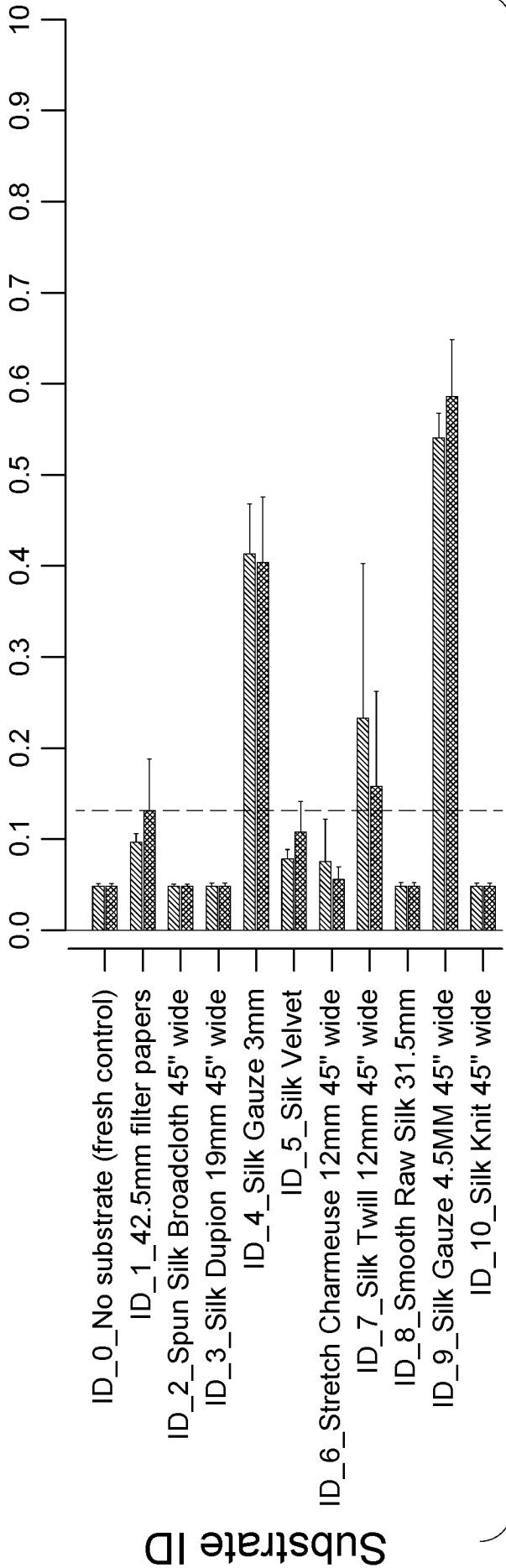
Normalized FDCF Fabric Aggregated Functionality Scoring

Score = Average (A, B, C, D, E, F)
A= Normalized FDCF LacZ Colorimetric Intensity in Fabric
B= Normalized Max FDCF LacZ Reaction Rate in Fabric
C= Normalized Time to Max FDCF LacZ Reaction Rate in Fabric
D= Normalized Lag Time of FDCF LacZ Reaction Rate in Fabric
E= Normalized in-Fabric Fabric Density
F= Normalized in-Fabric Fabric Autofluorescence Intensity
- - - * Grade 4 Whatman Filter Paper Disc Score

Unblocked
5% BSA Blocked

Normalized Score

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A FIG. 20

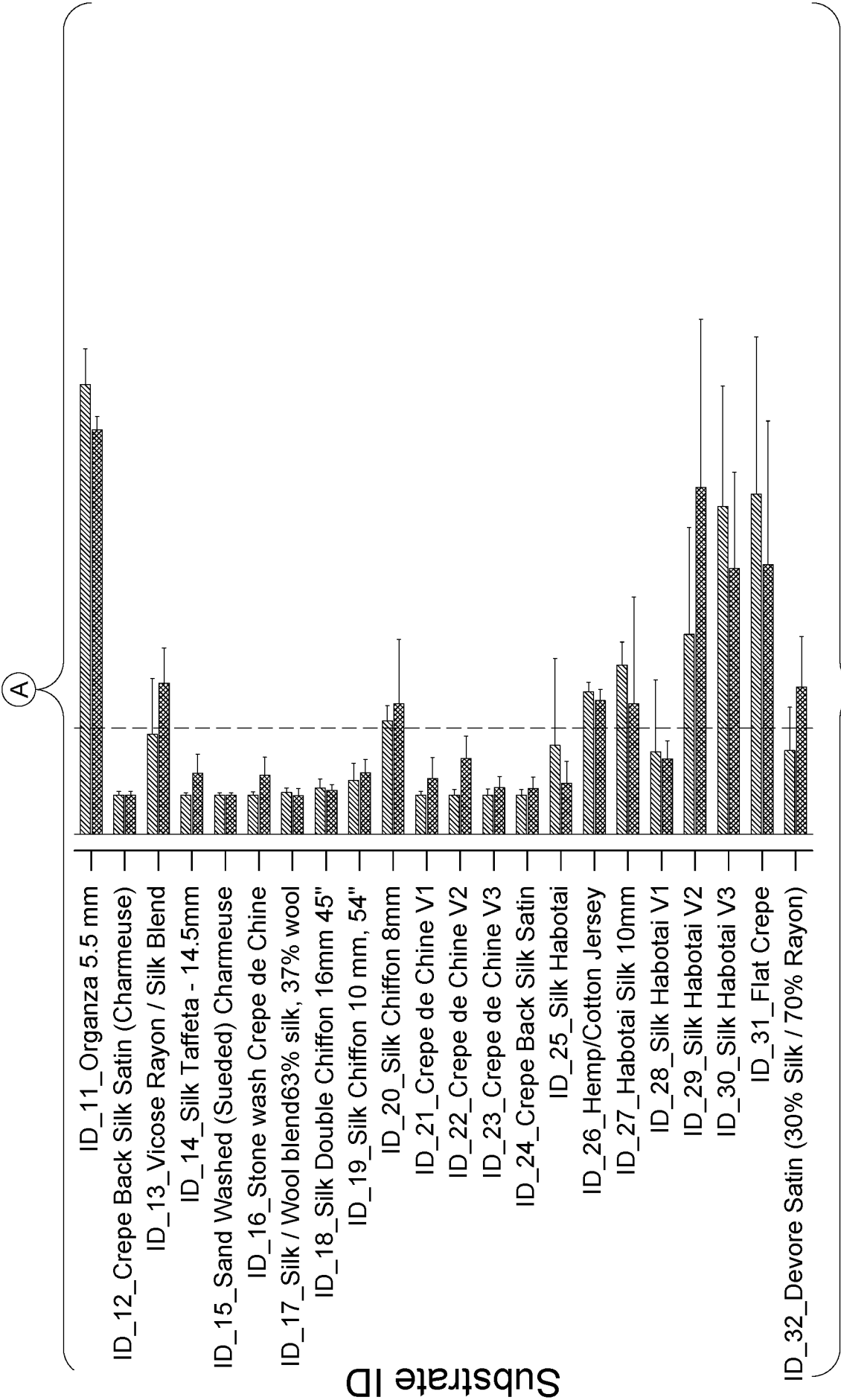


FIG. 20 (CONTINUATION)

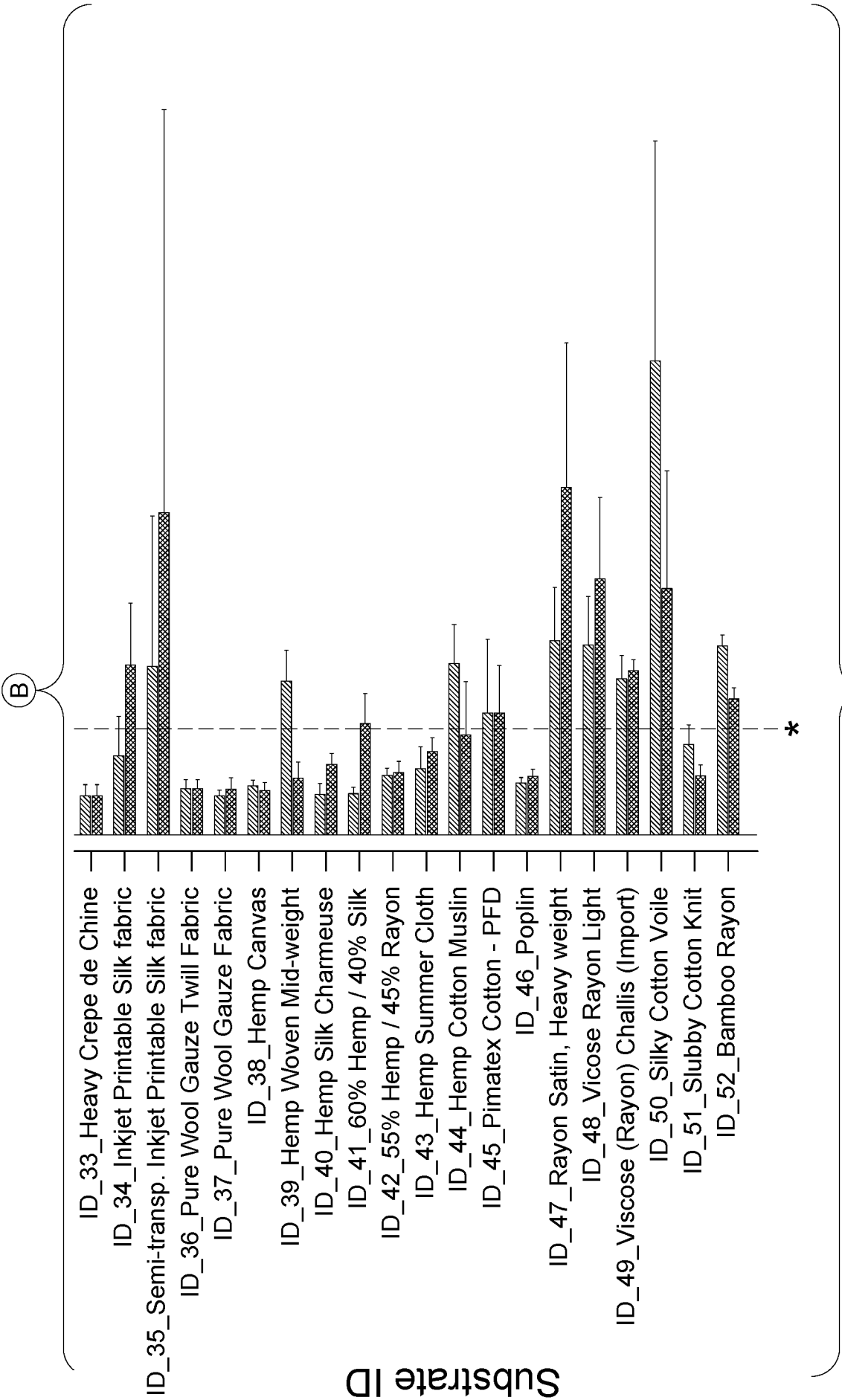
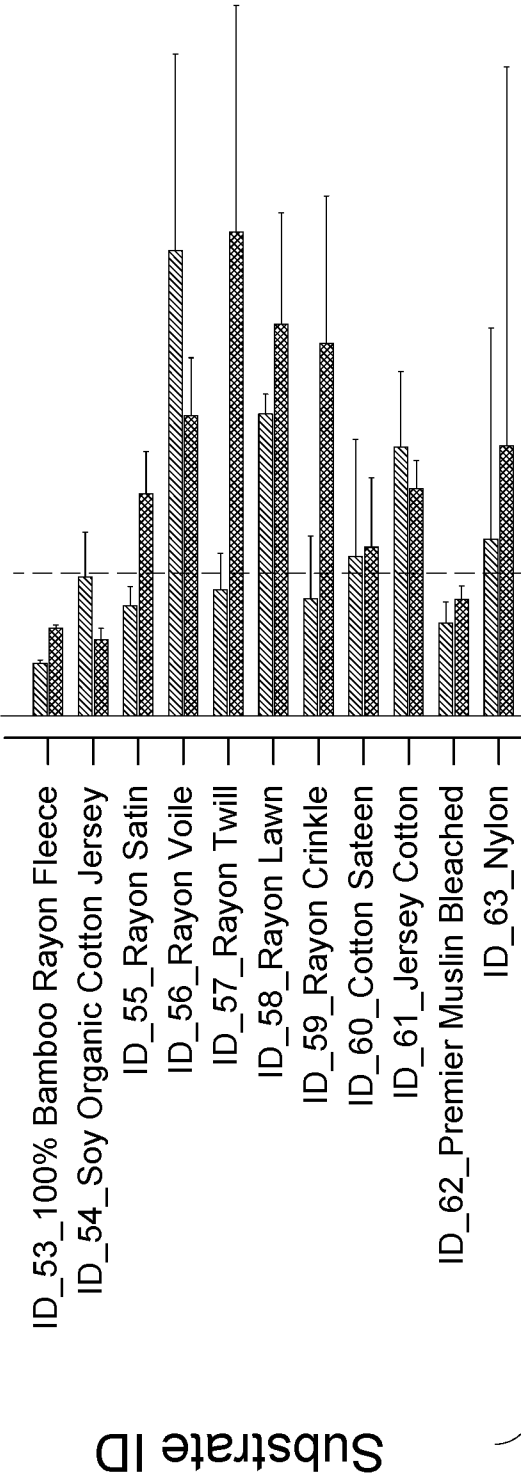


FIG. 20 (CONTINUATION)

C



D

FIG. 20 (CONTINUATION)

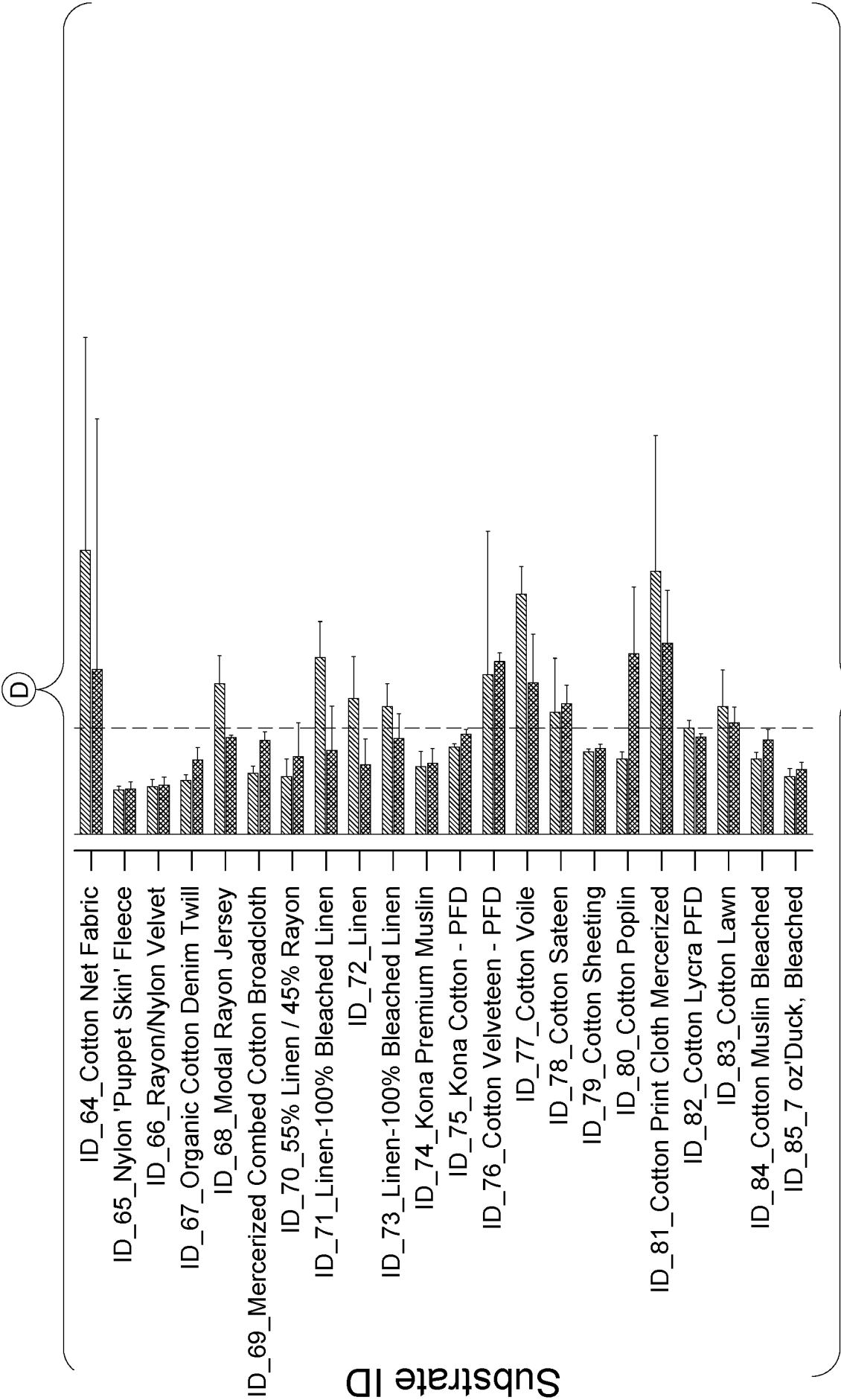


FIG. 20 (CONTINUATION)

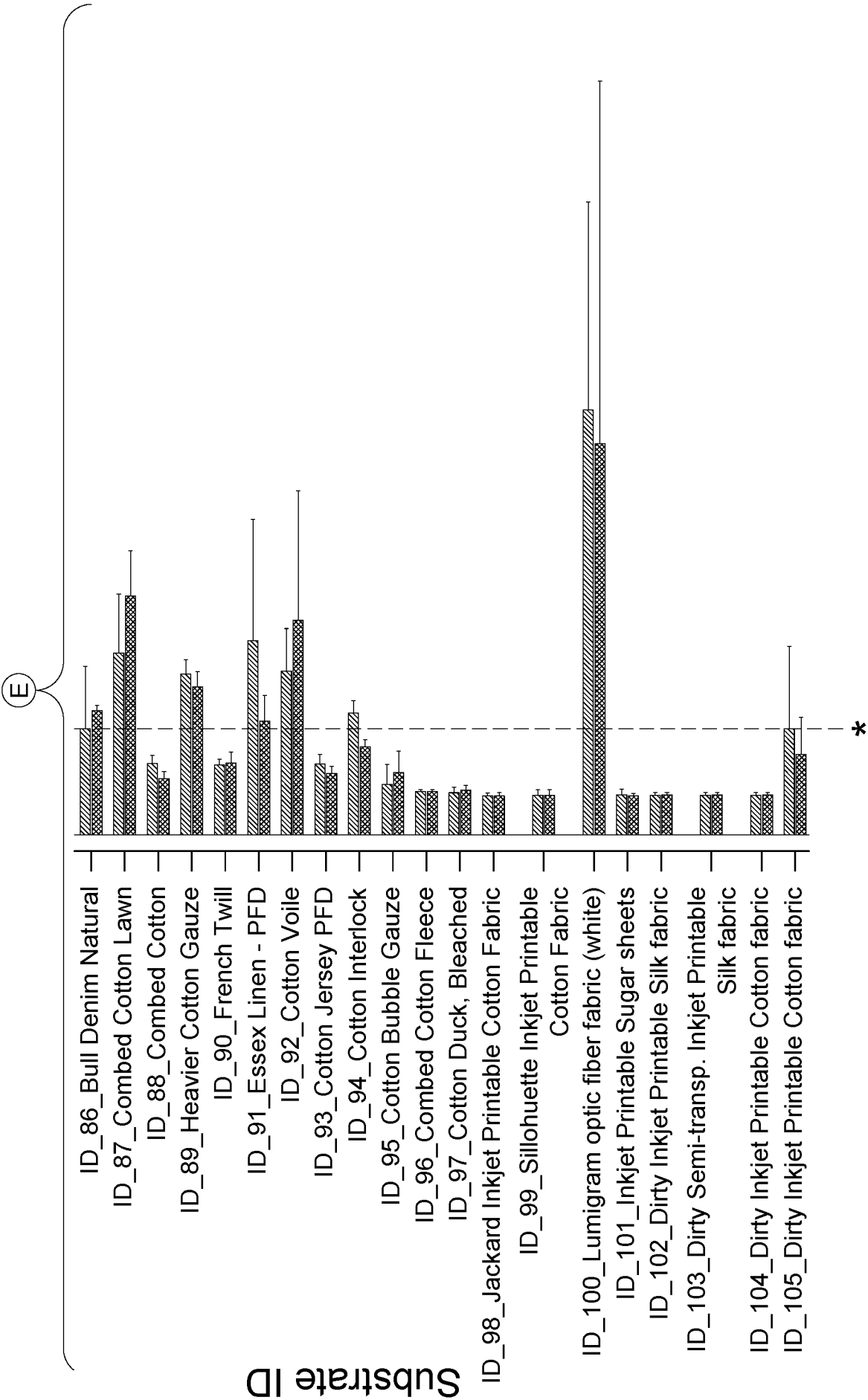


FIG. 20 (CONTINUATION)

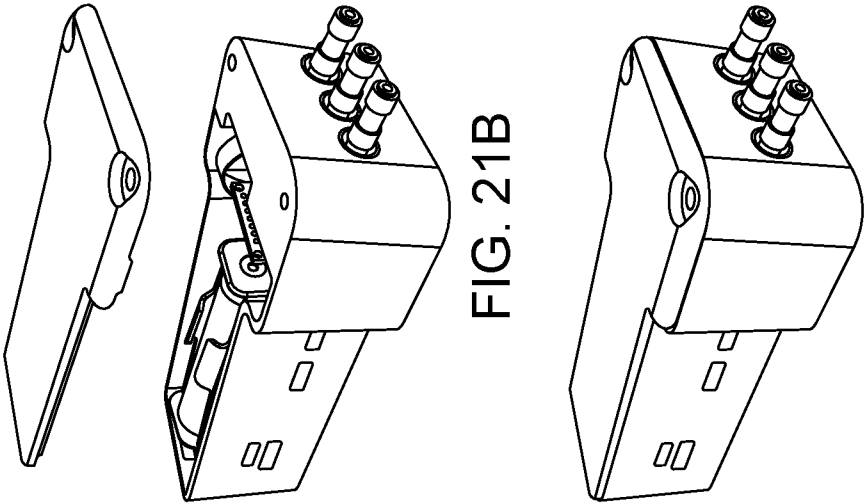


FIG. 21B

FIG. 21C

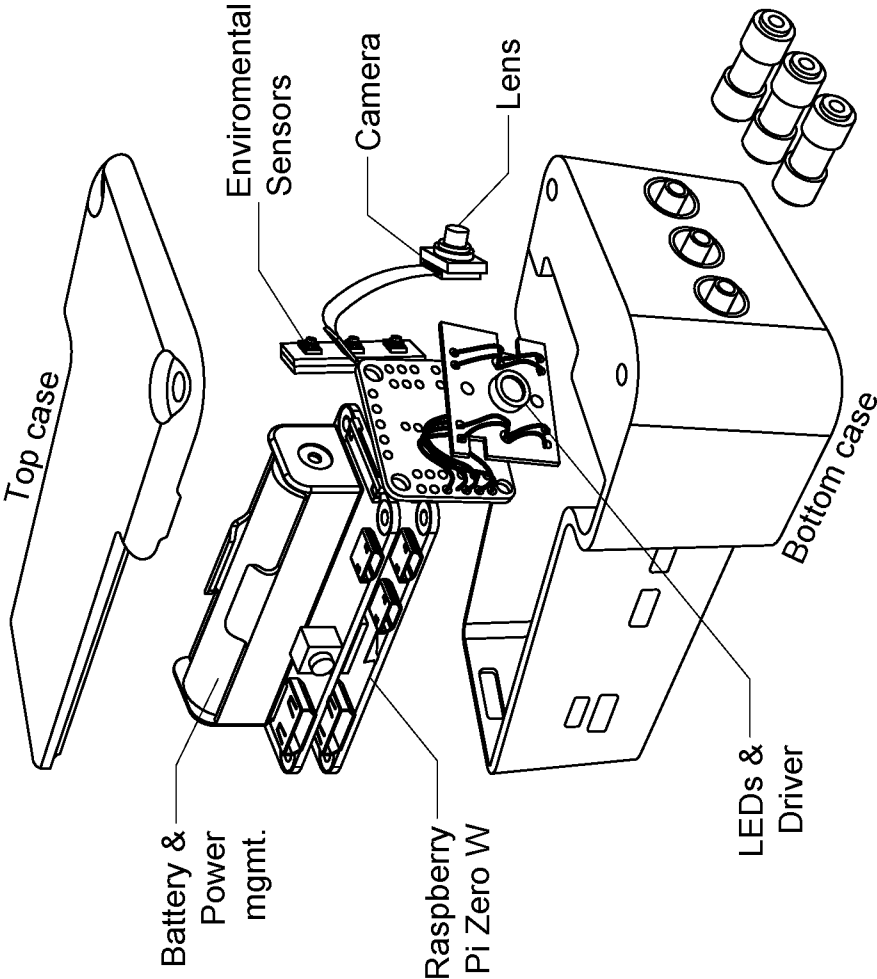


FIG. 21A

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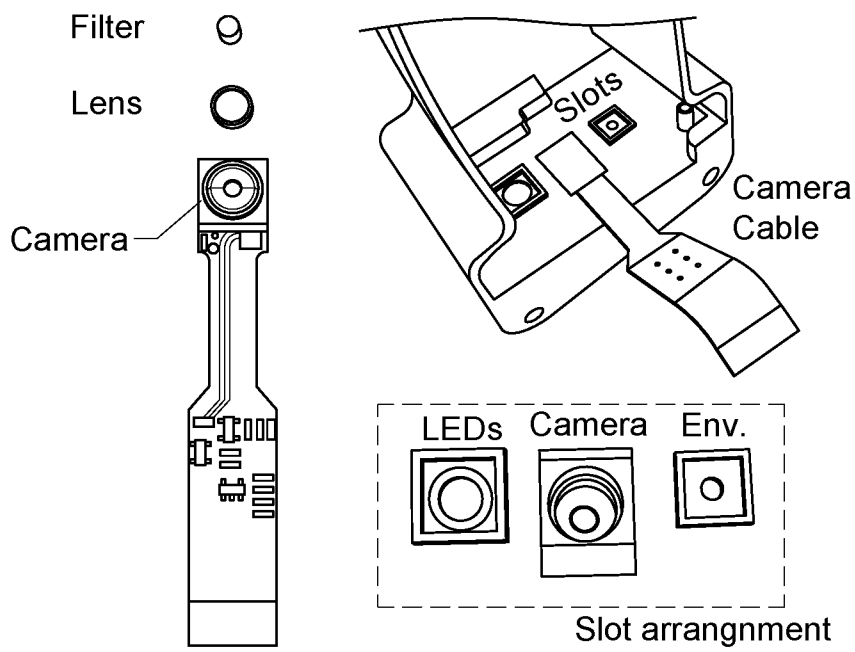


FIG. 21D

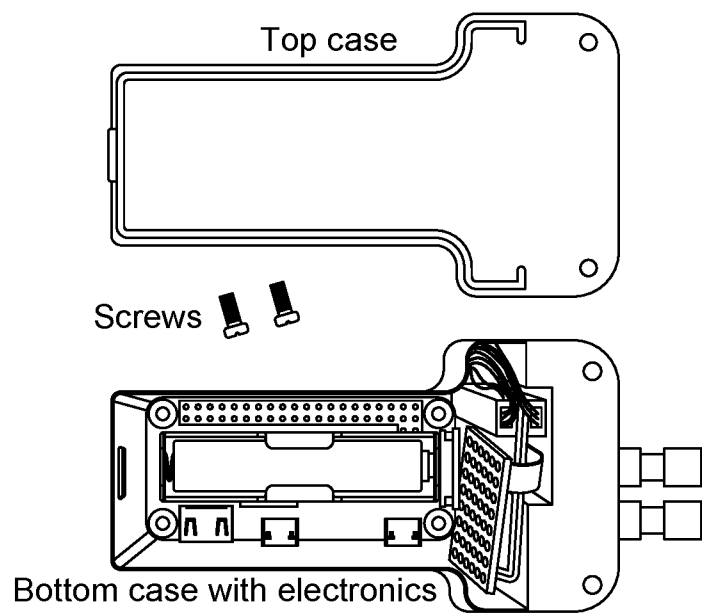


FIG. 21E

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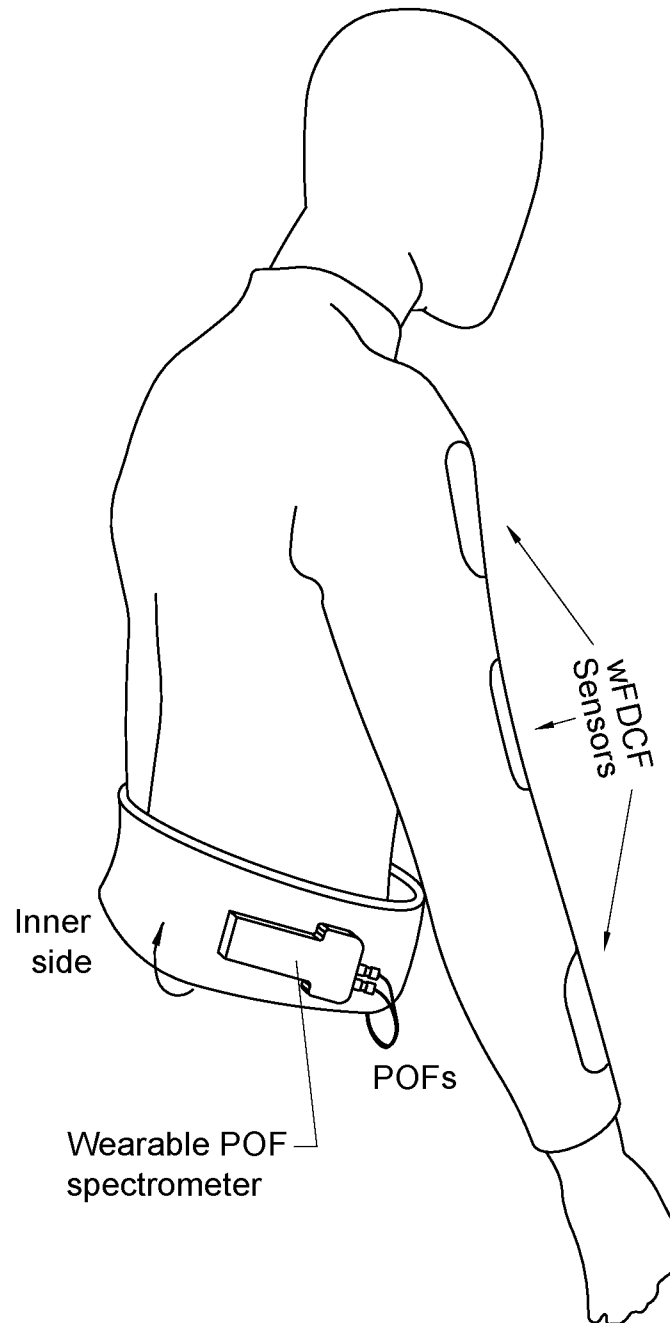


FIG. 21F

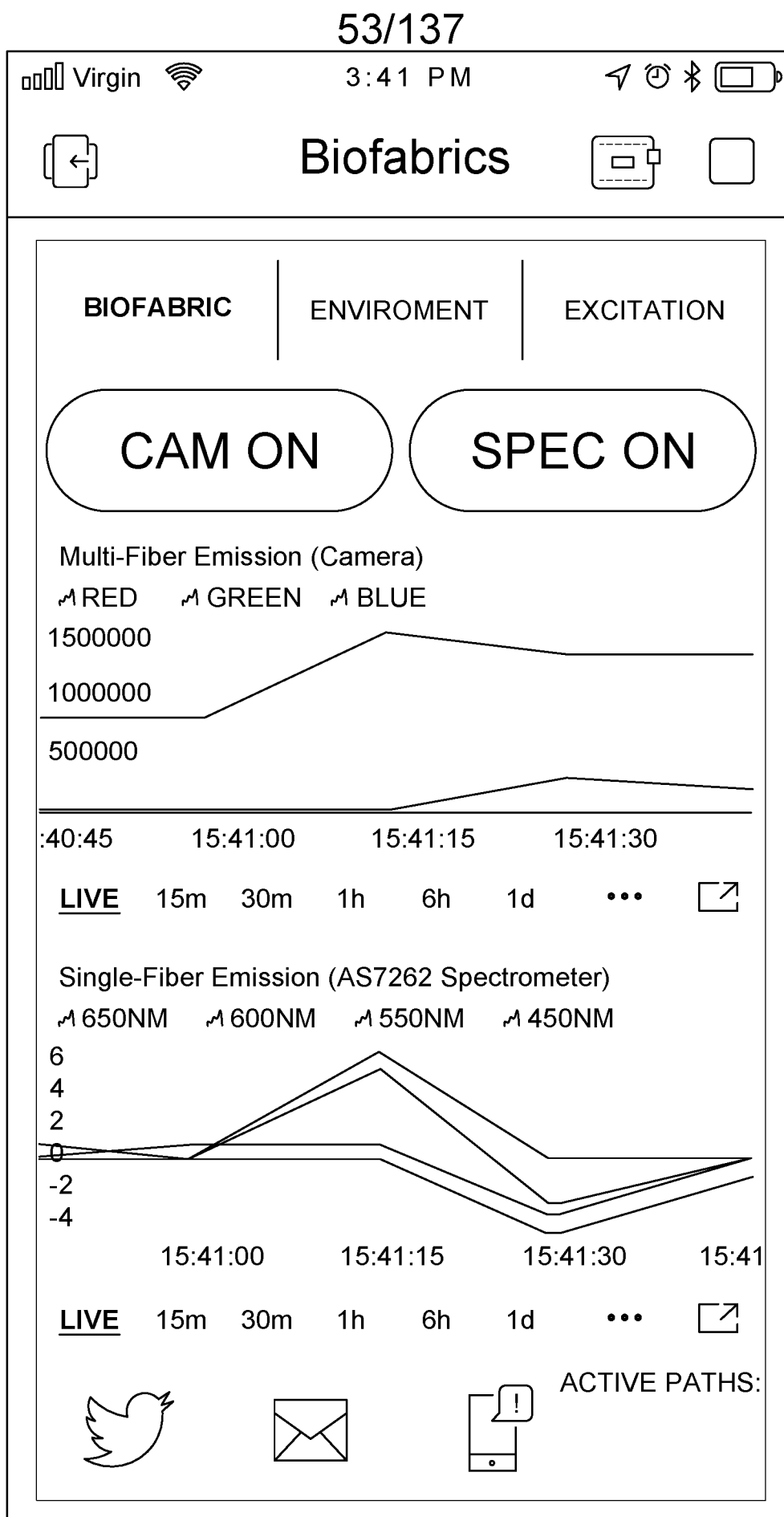


FIG. 22A

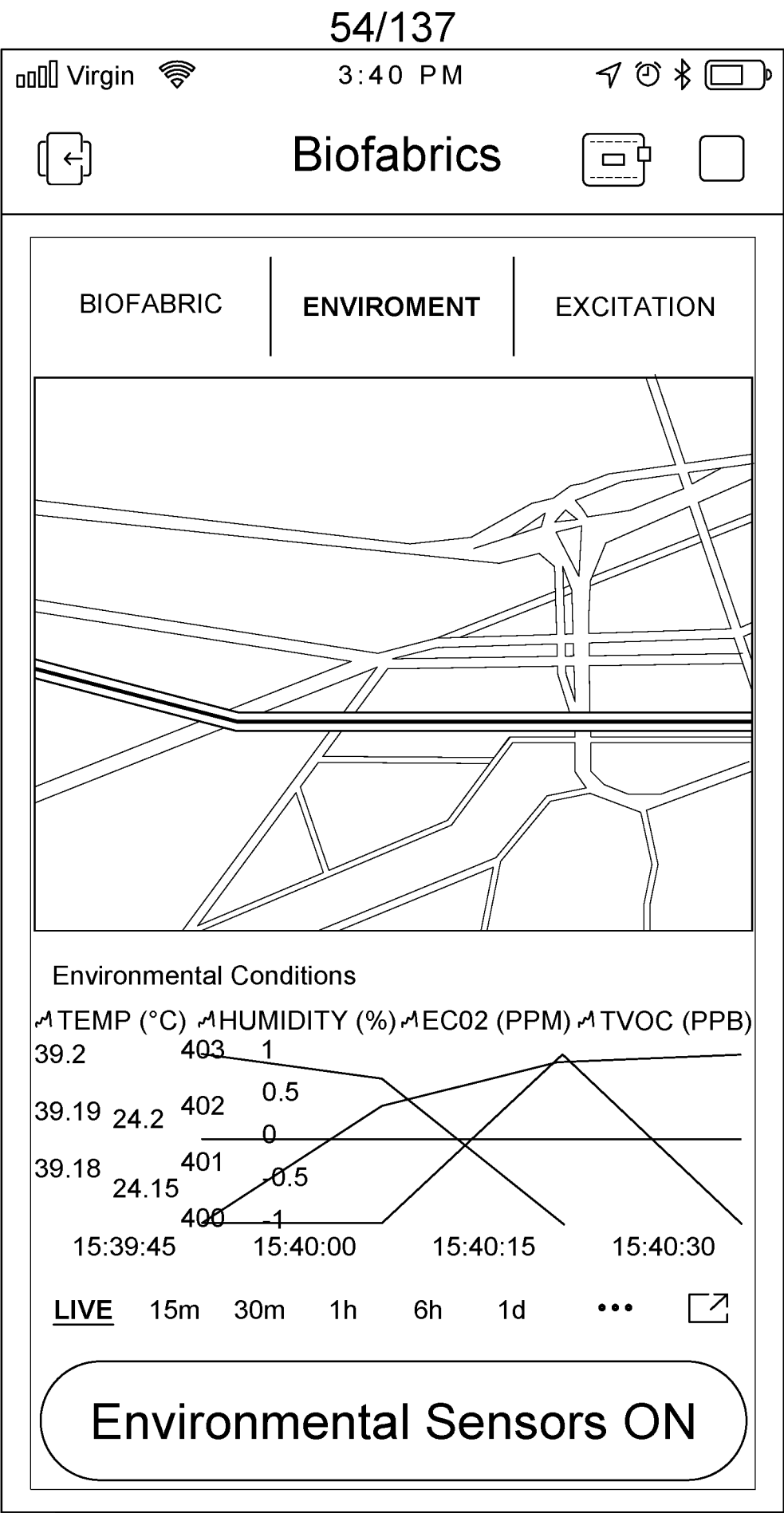


FIG. 22B

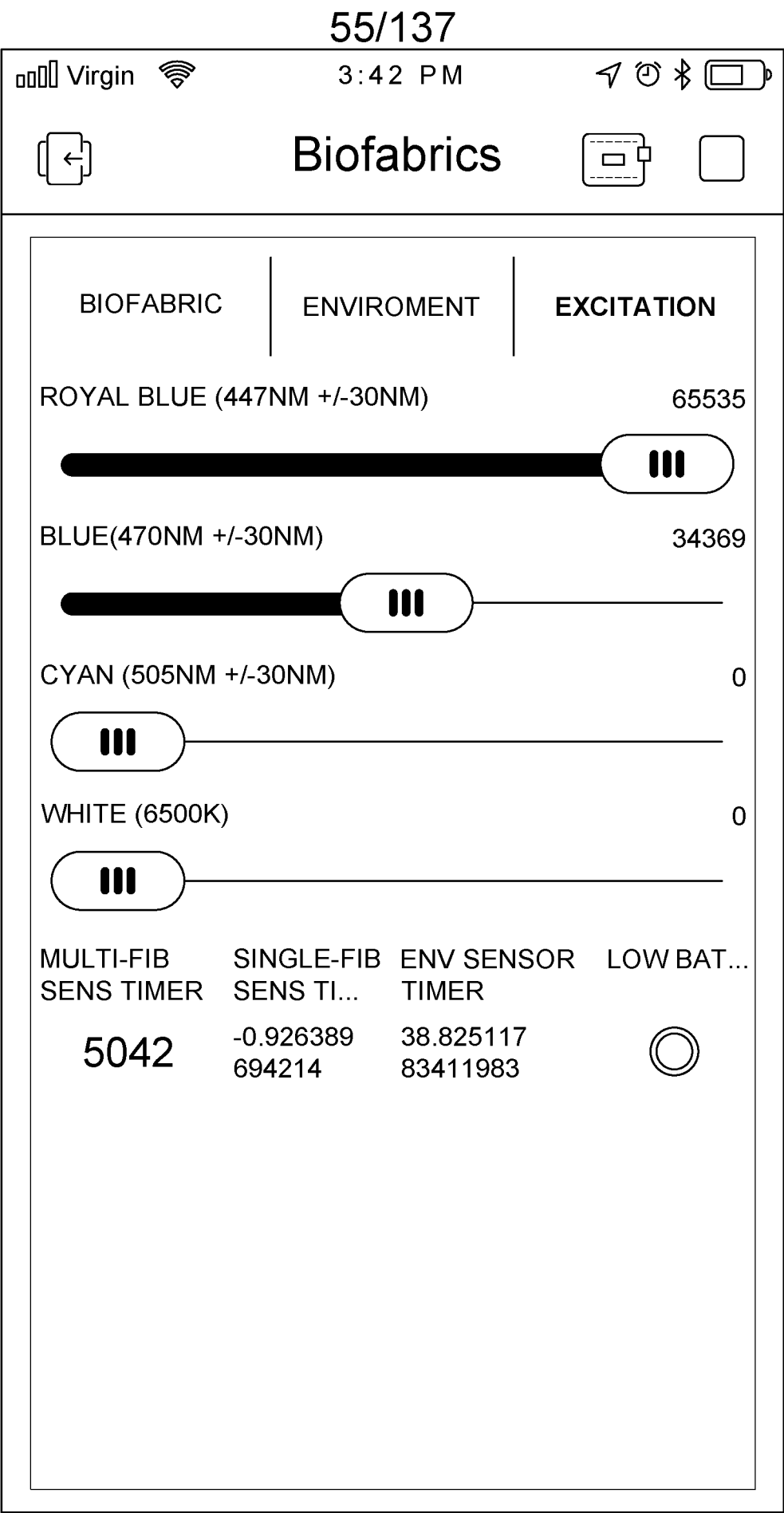


FIG. 22C

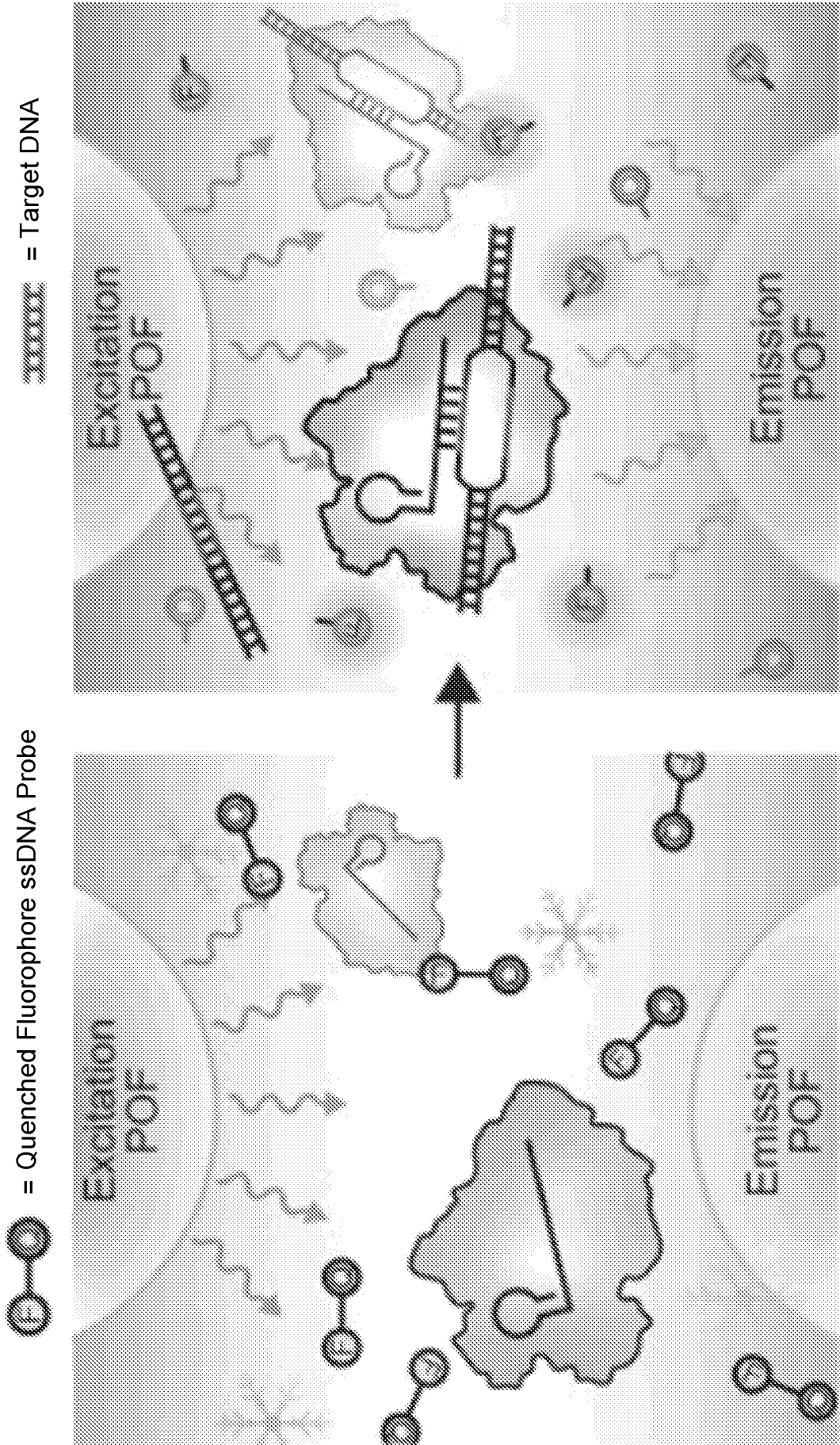


FIG. 23A

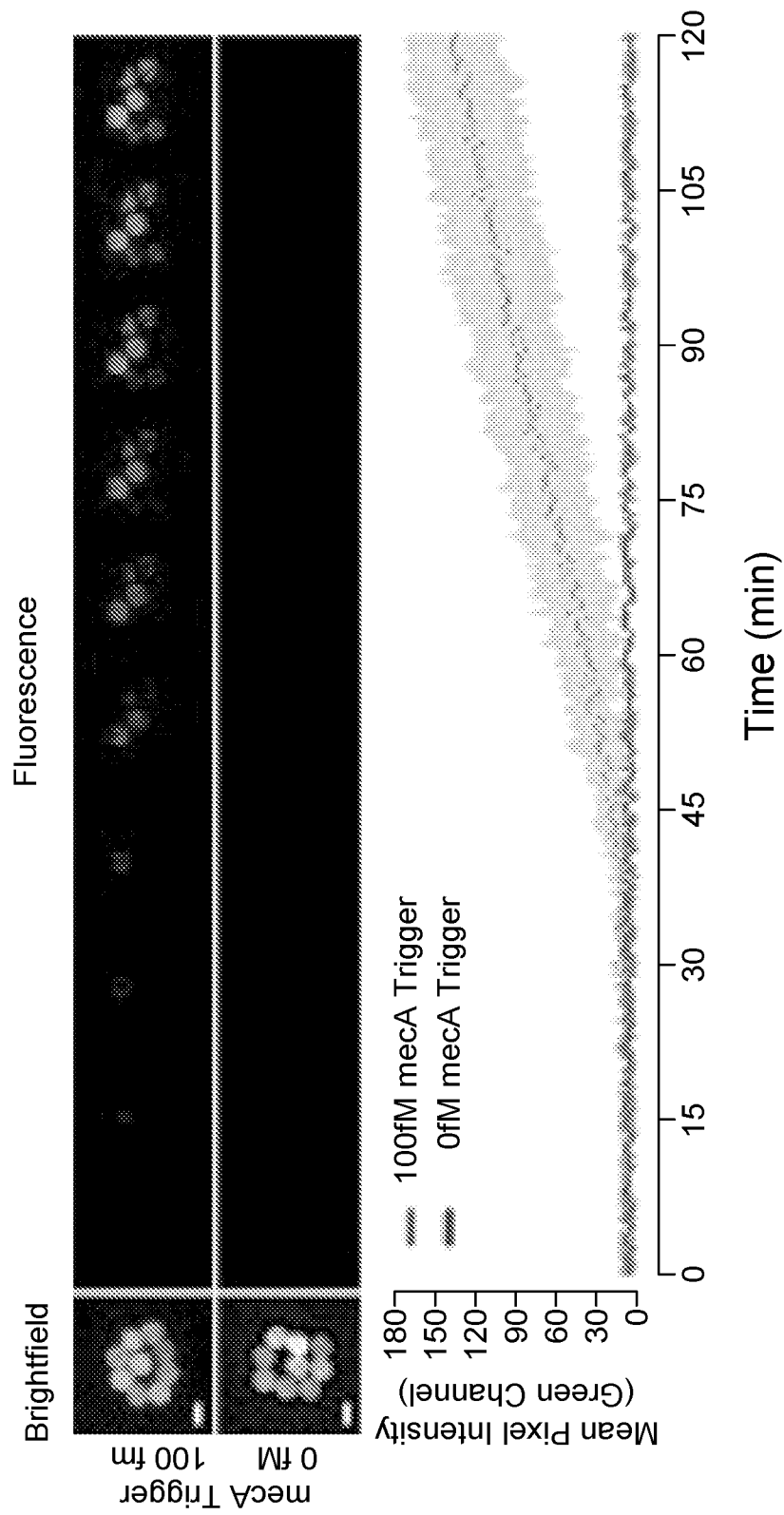


FIG. 23B

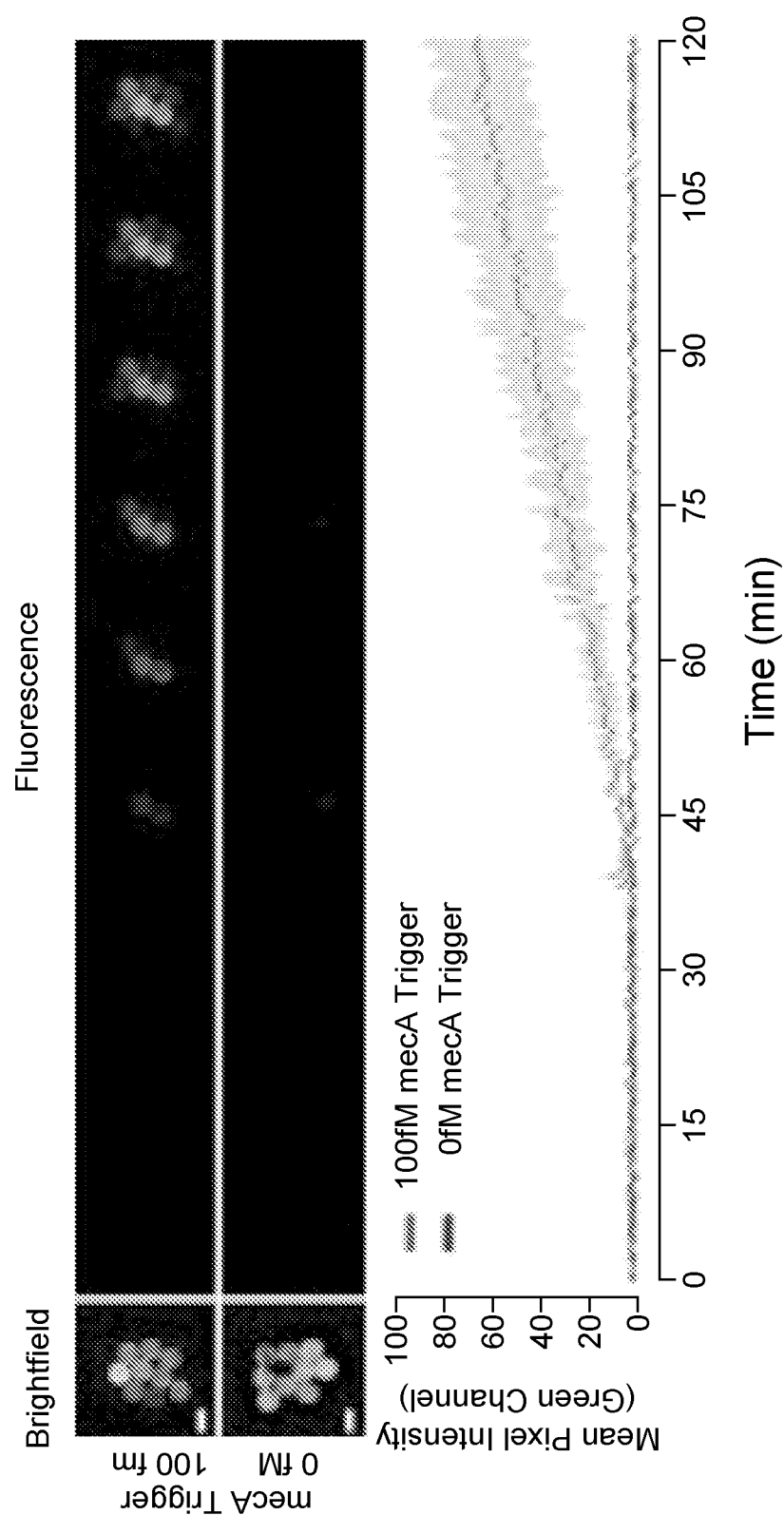


FIG. 23C

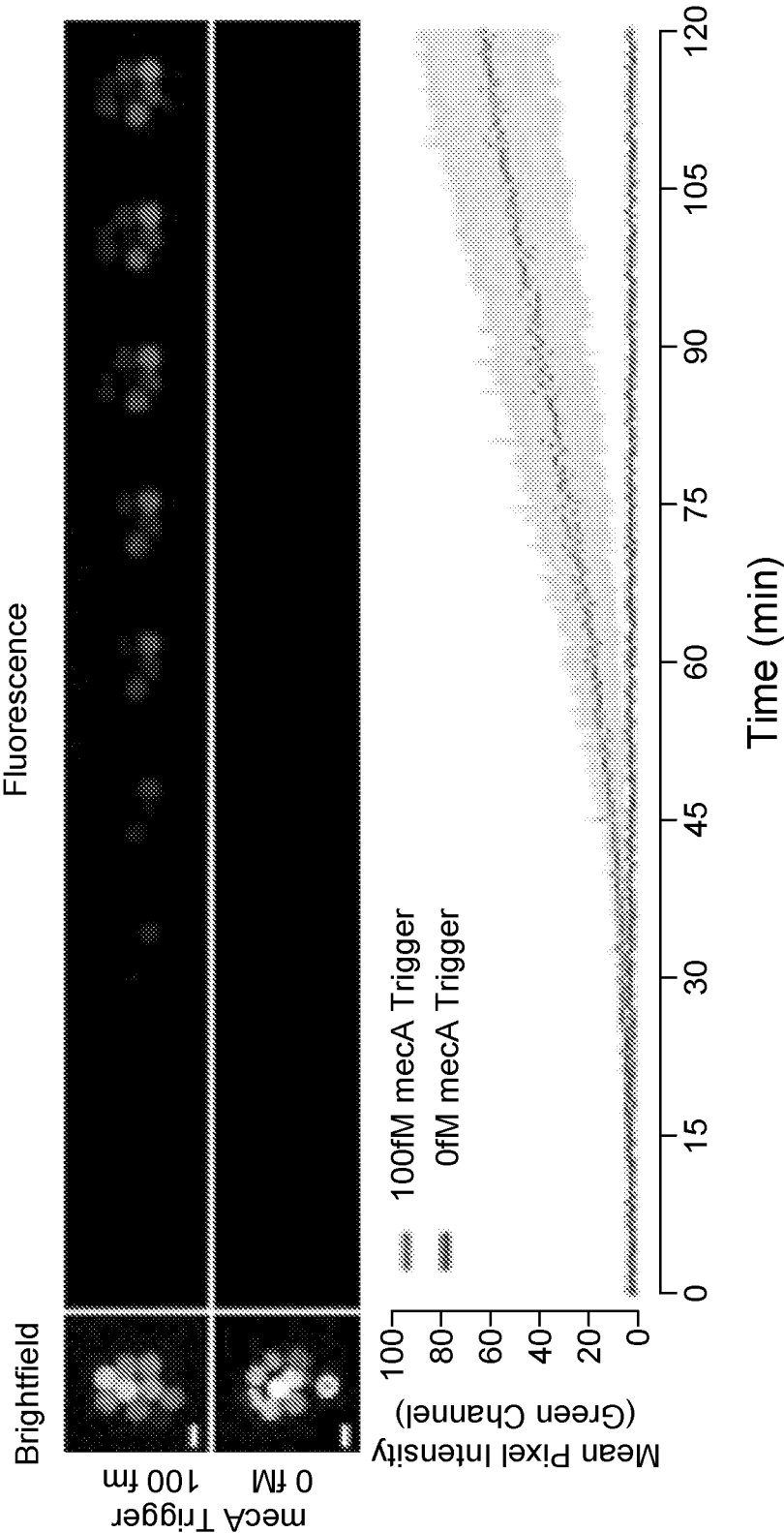


FIG. 23D

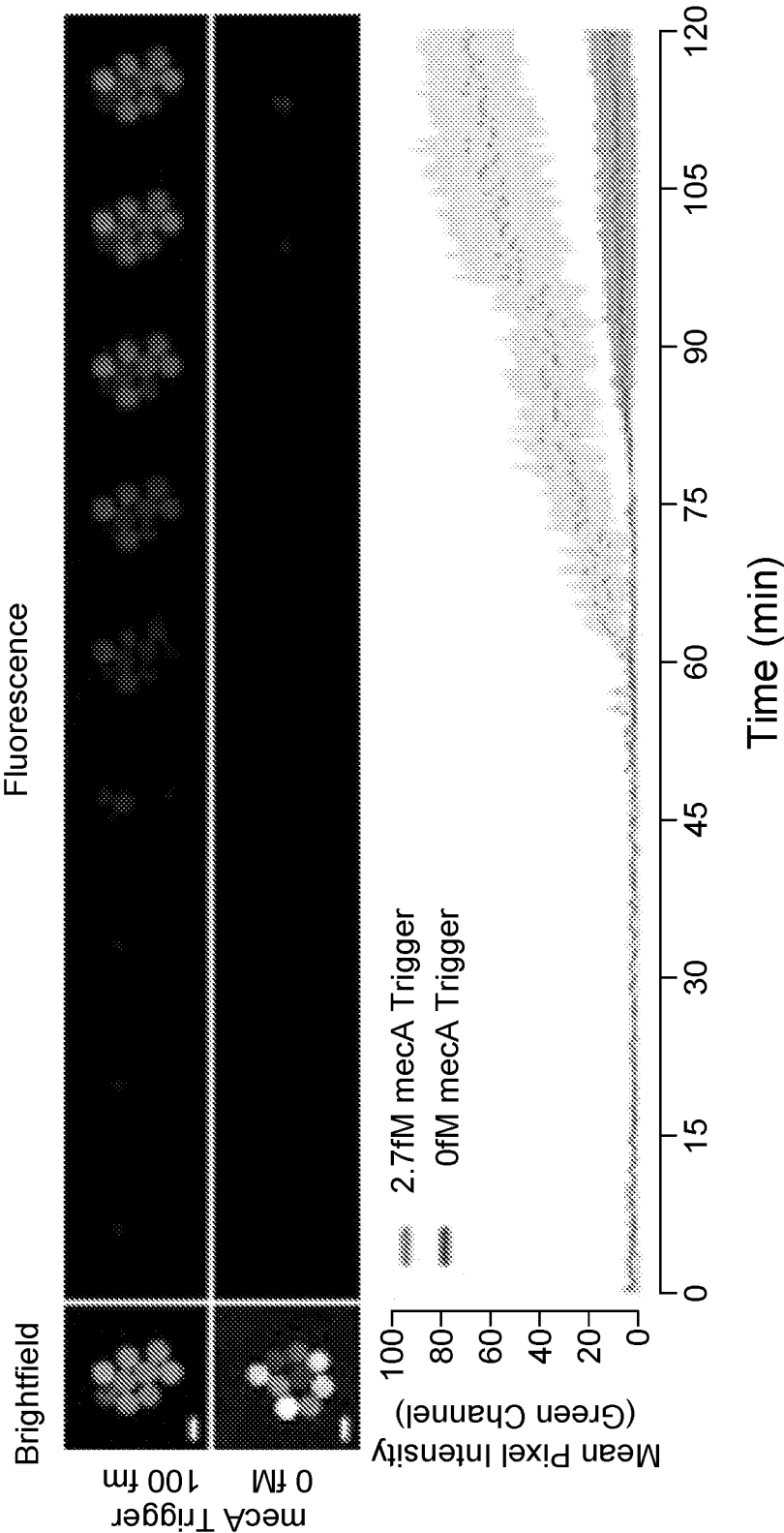


FIG. 23E

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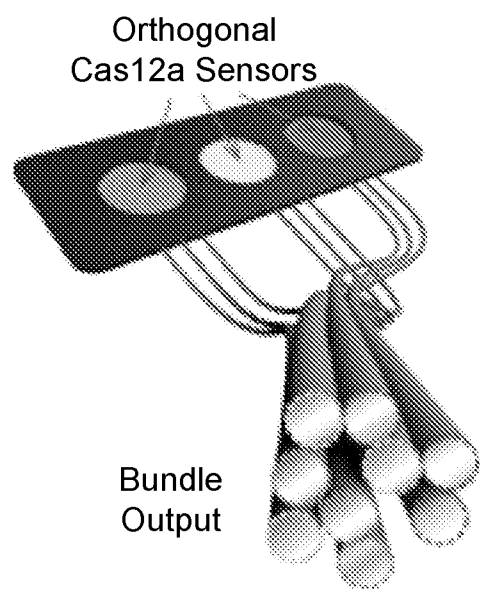


FIG. 23F

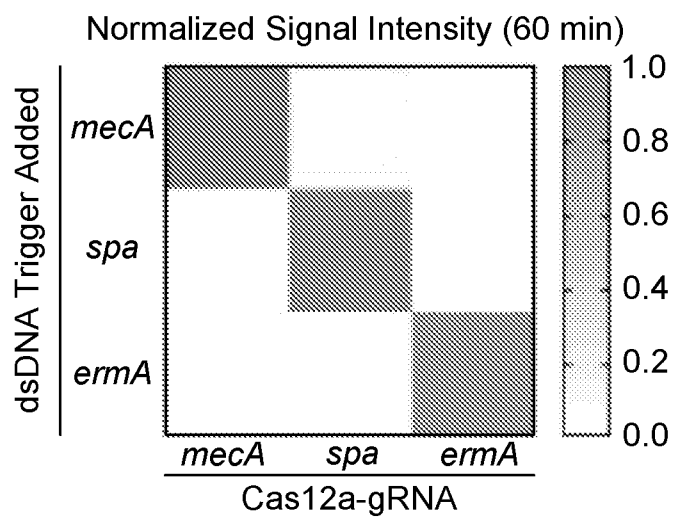


FIG. 23G

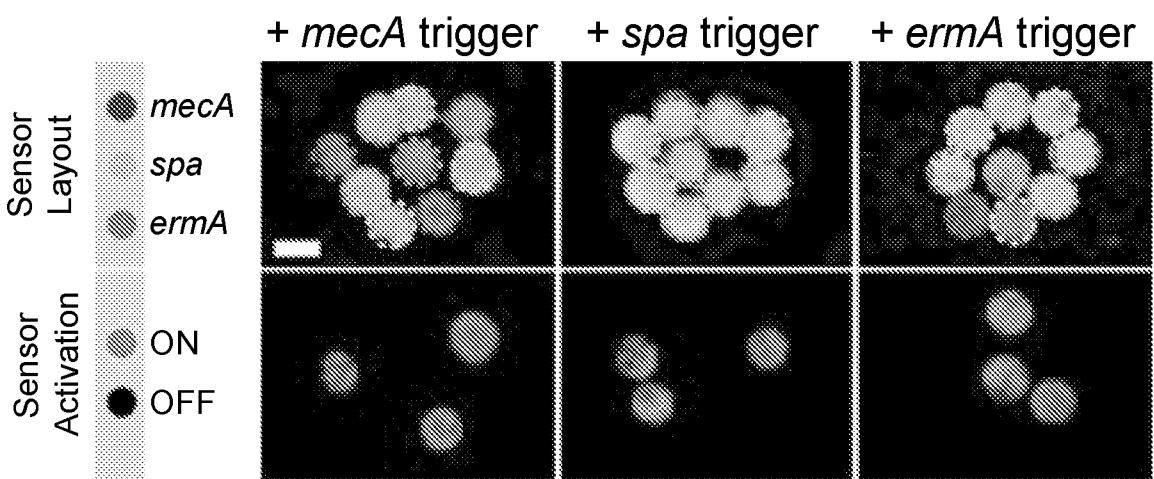


FIG. 23H

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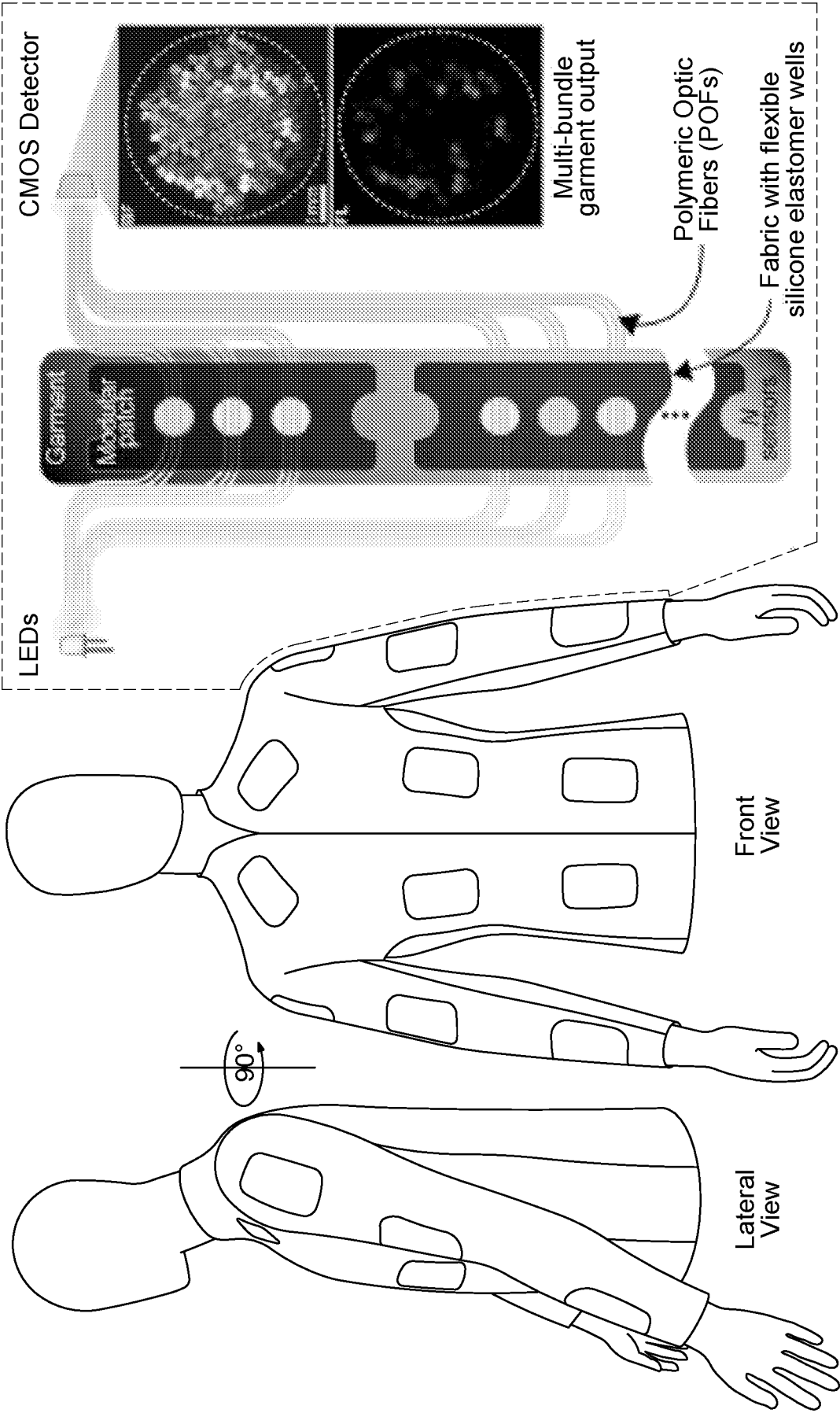


FIG. 23I

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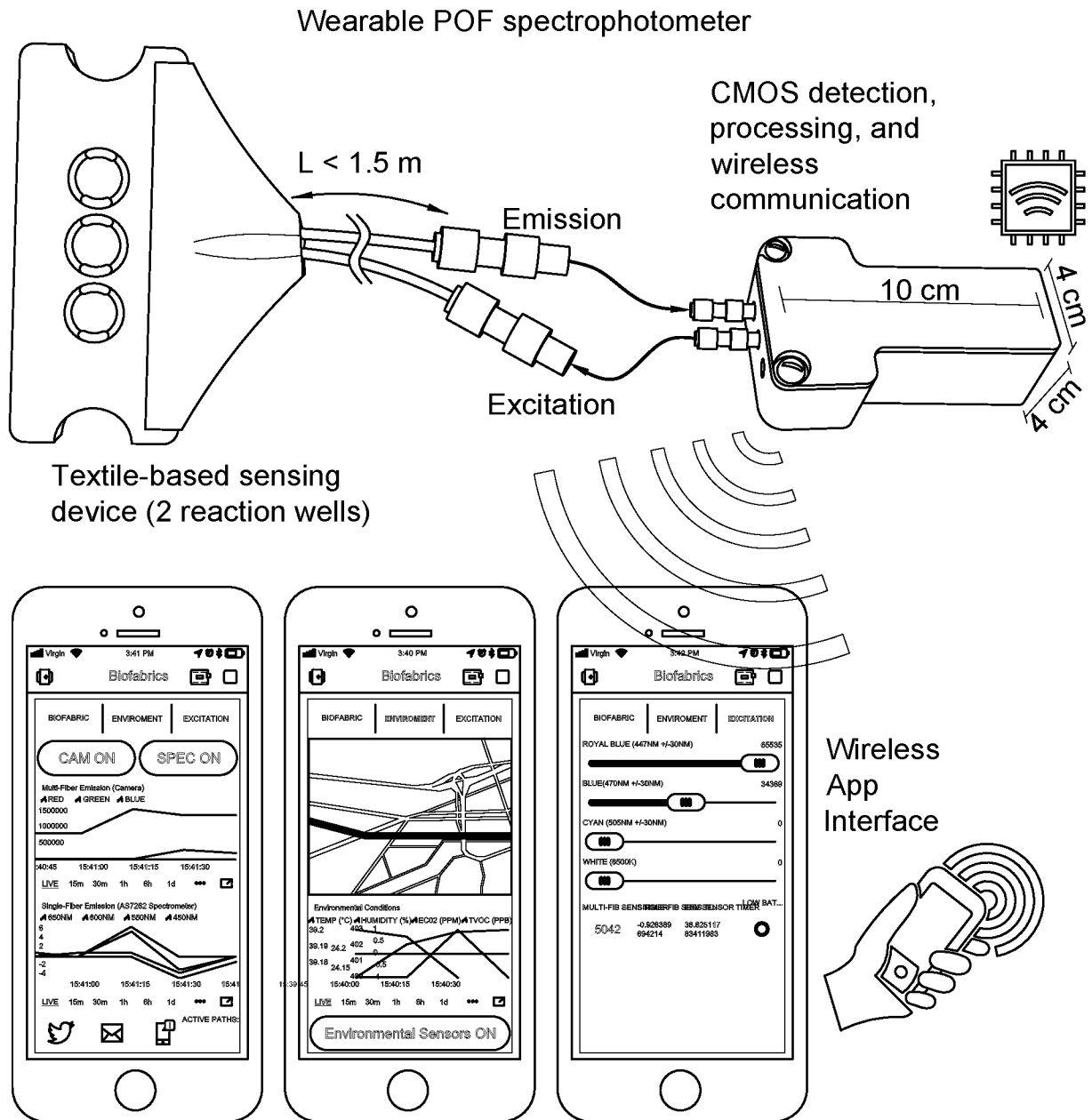


FIG. 23J

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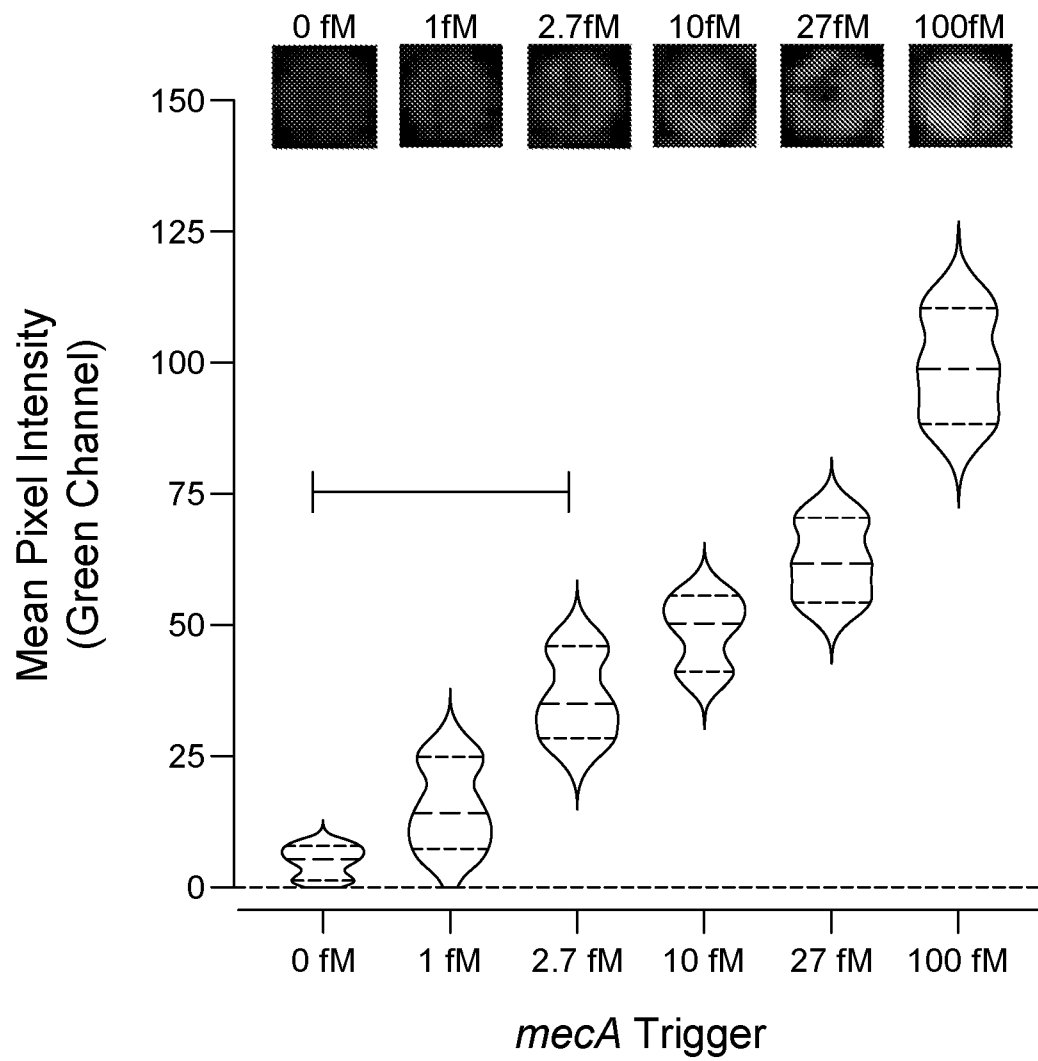


FIG. 24

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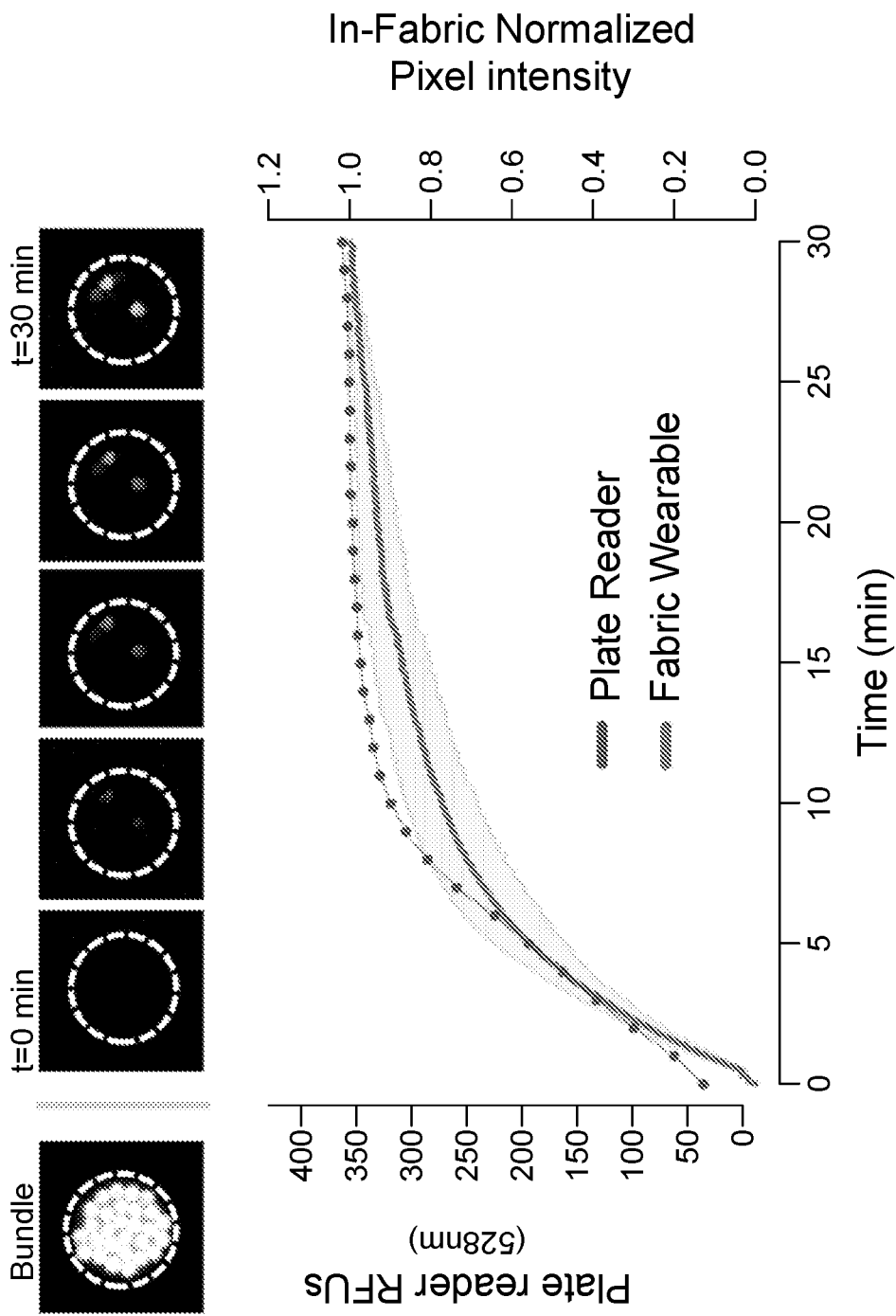


FIG. 25

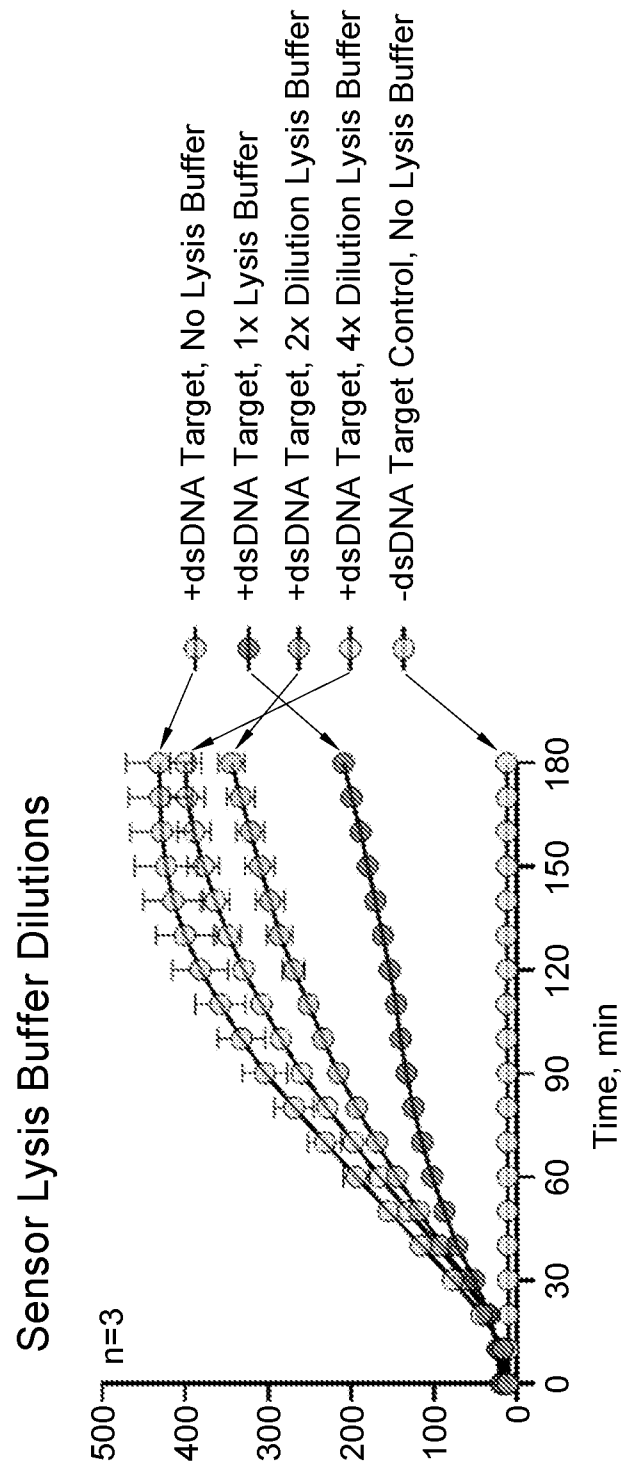


FIG. 26A

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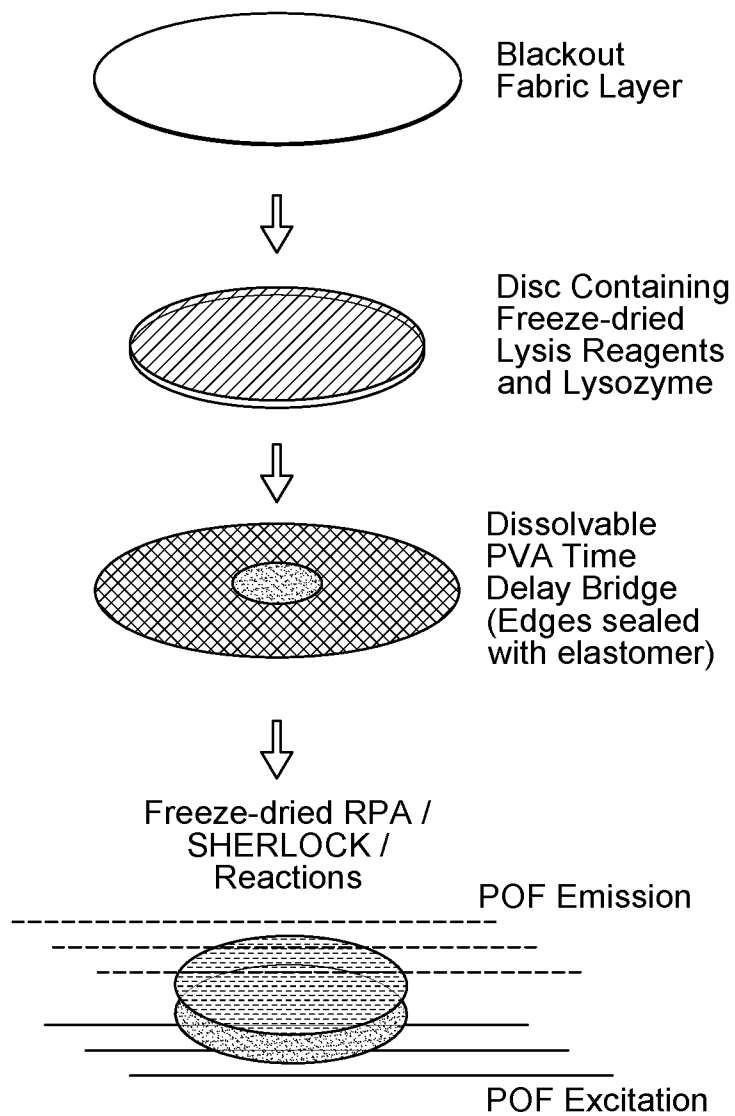


FIG. 26B

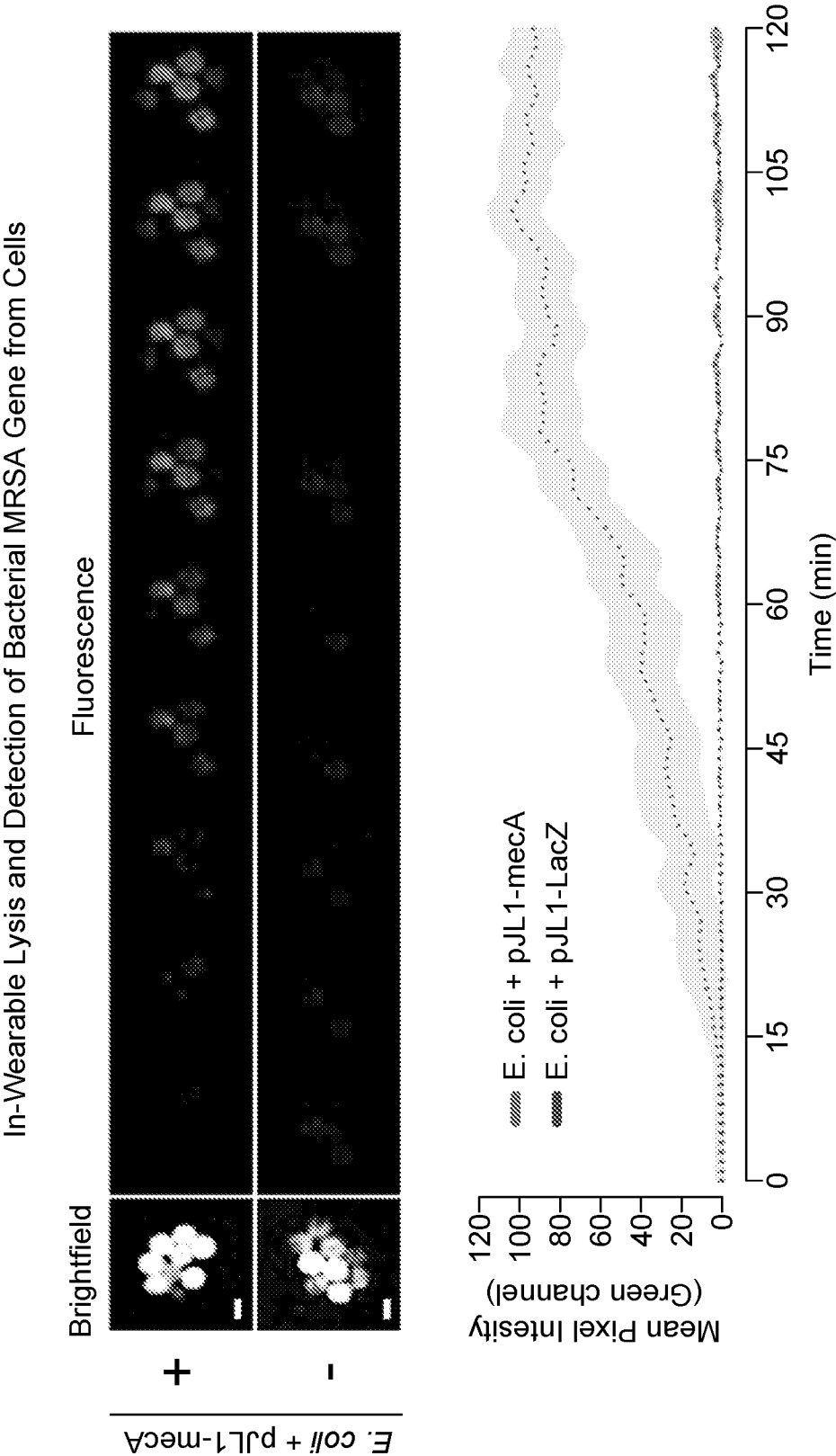


FIG. 26C

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In-wearable freeze-dried lysis reagents for autonomous sample preparation

Cas12a CoV2 Sensor in Non-Ionic Surfactants

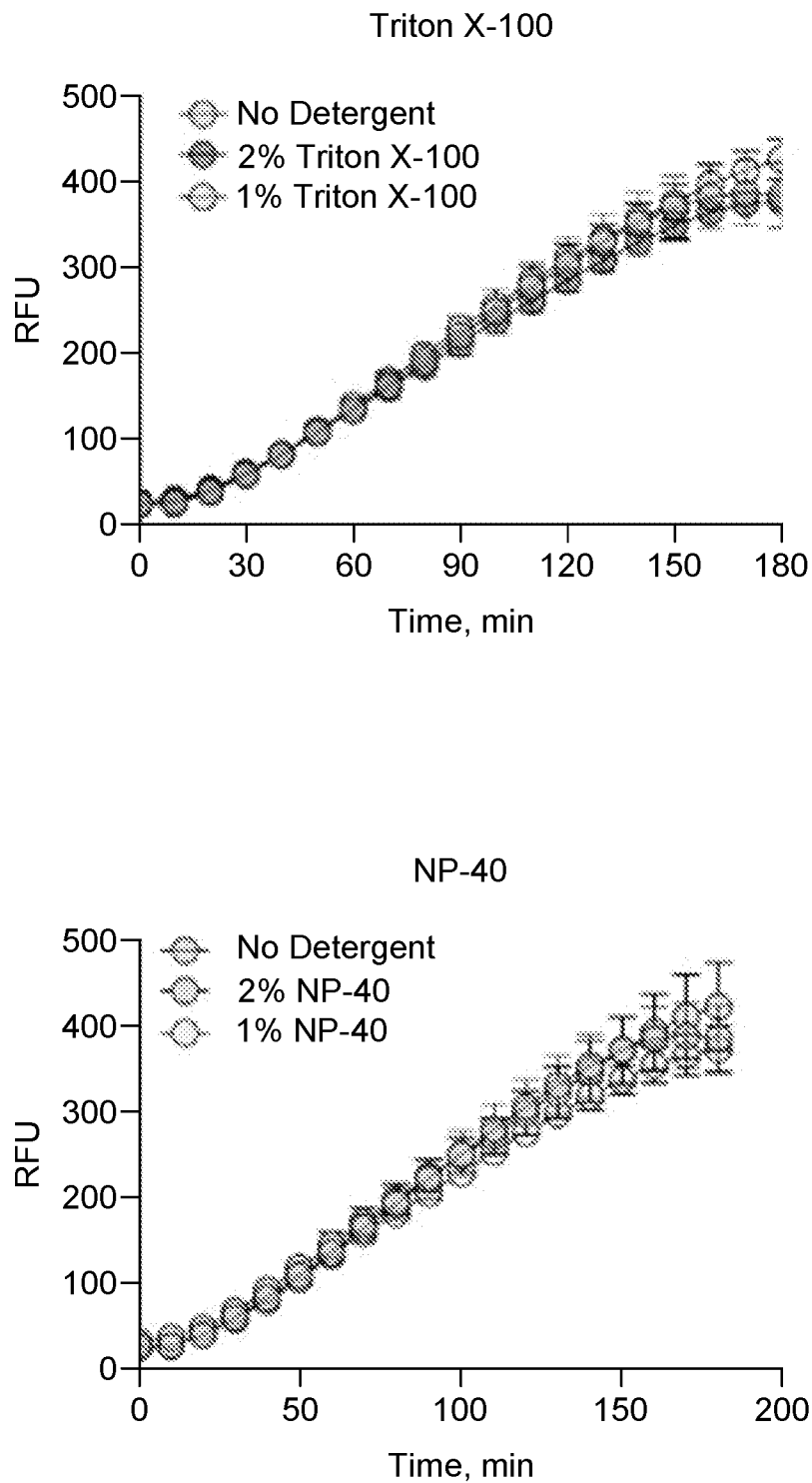


FIG. 26D

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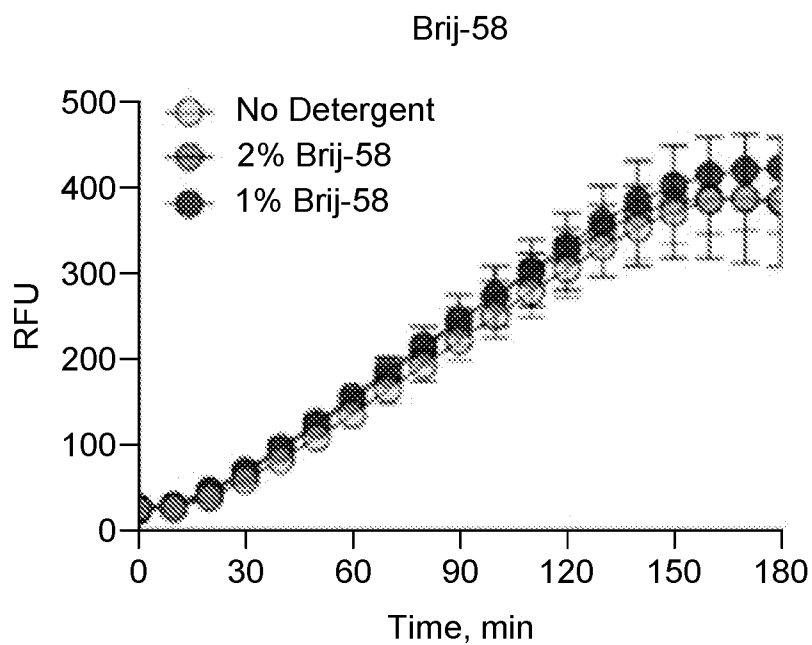
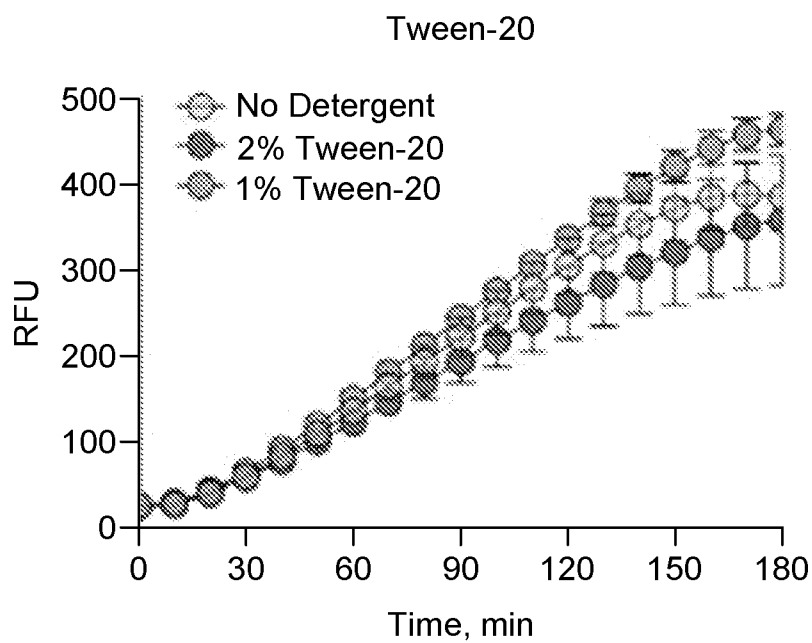


FIG. 26D (CONTINUATION)

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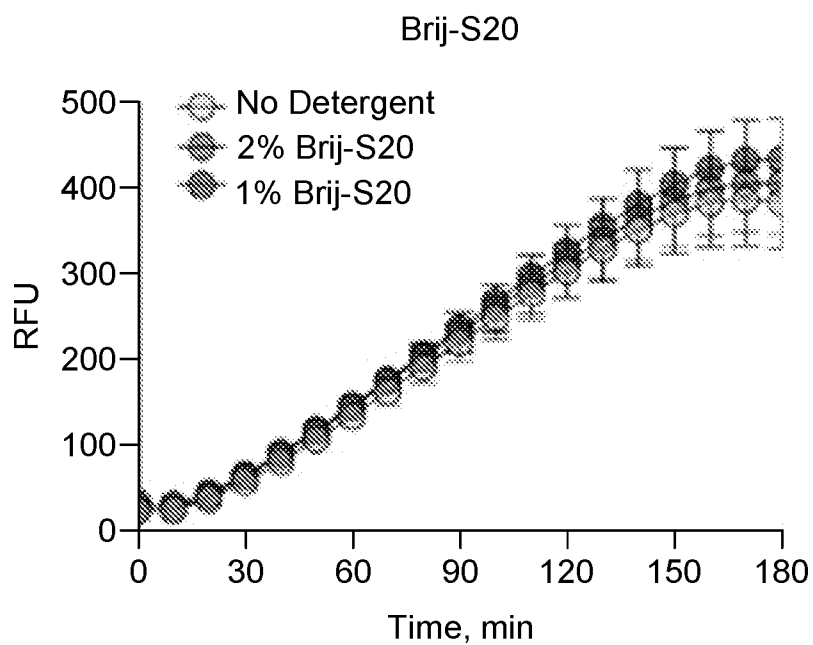
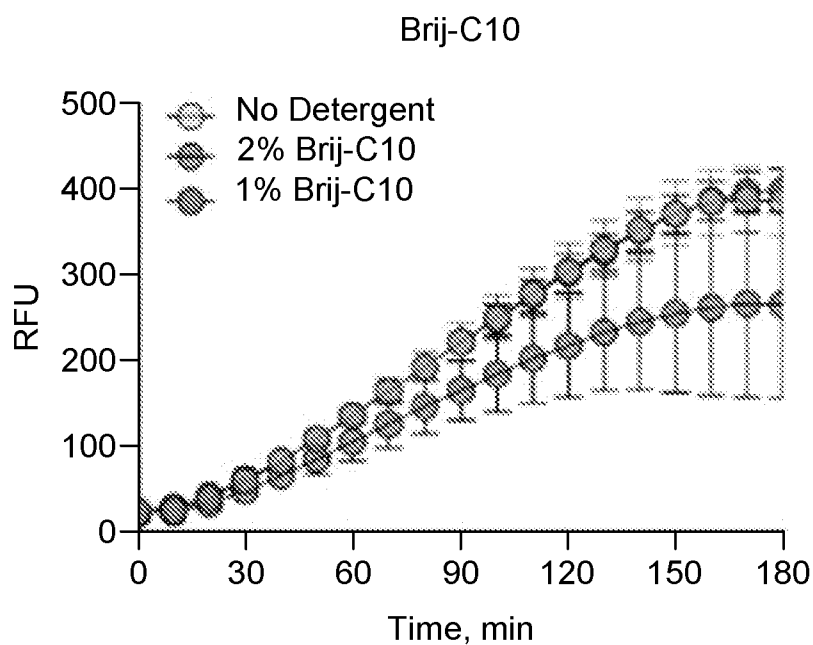


FIG. 26D (CONTINUATION)

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Cas12a CoV2 Sensor in Ionic Surfactants

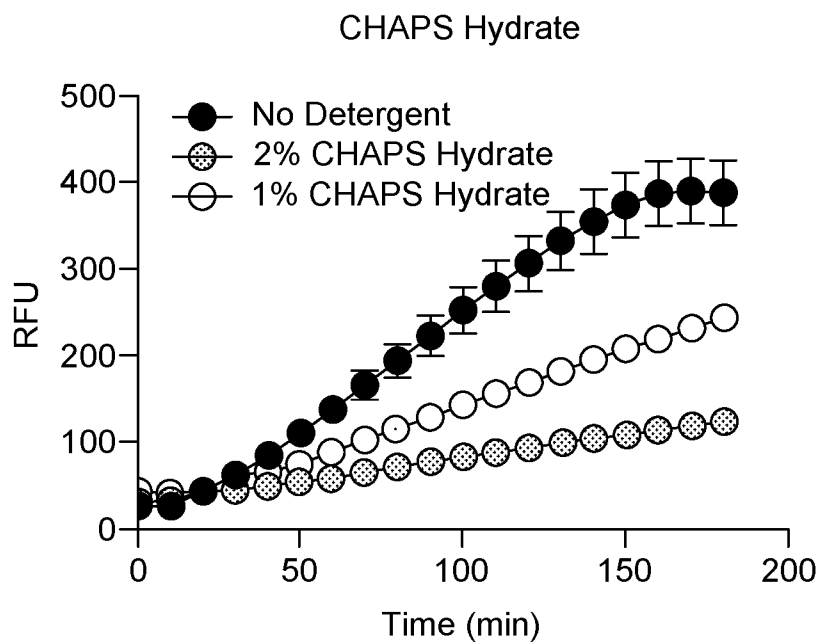
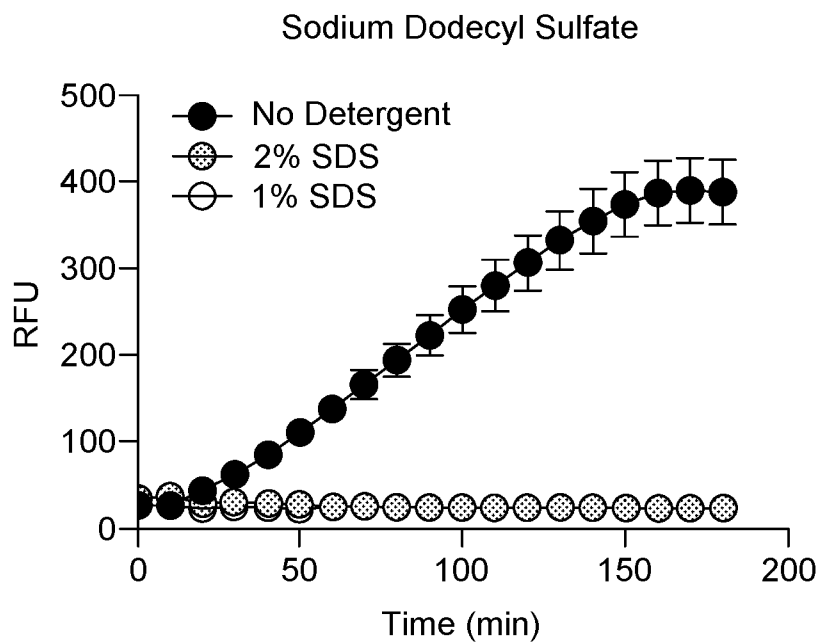


FIG. 26E

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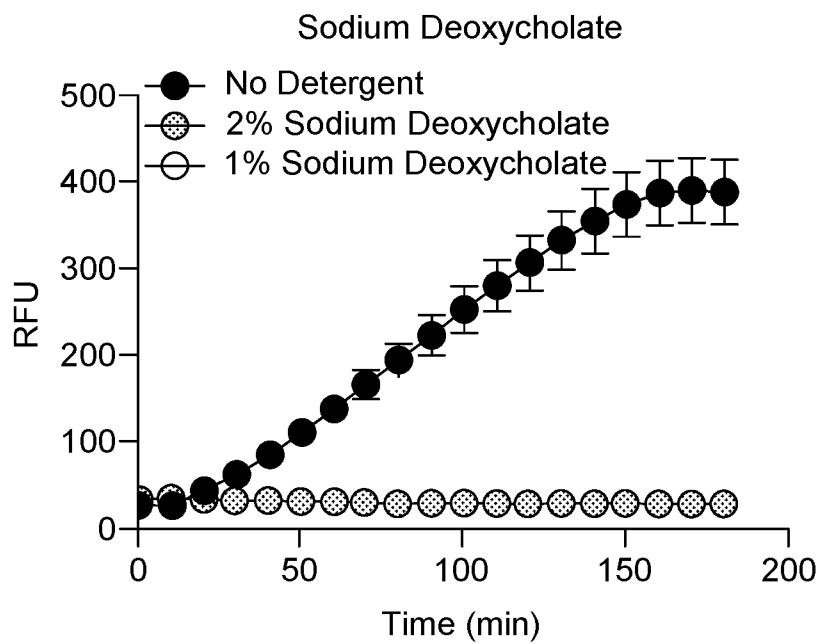


FIG. 26E (CONTINUATION)

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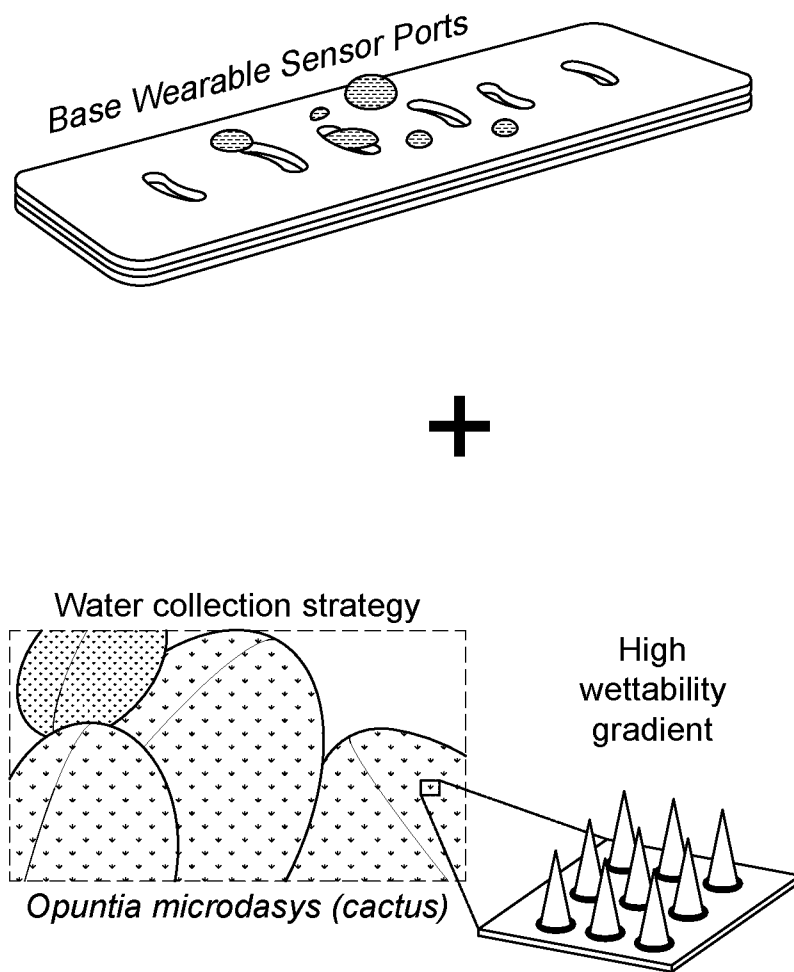


FIG. 27A

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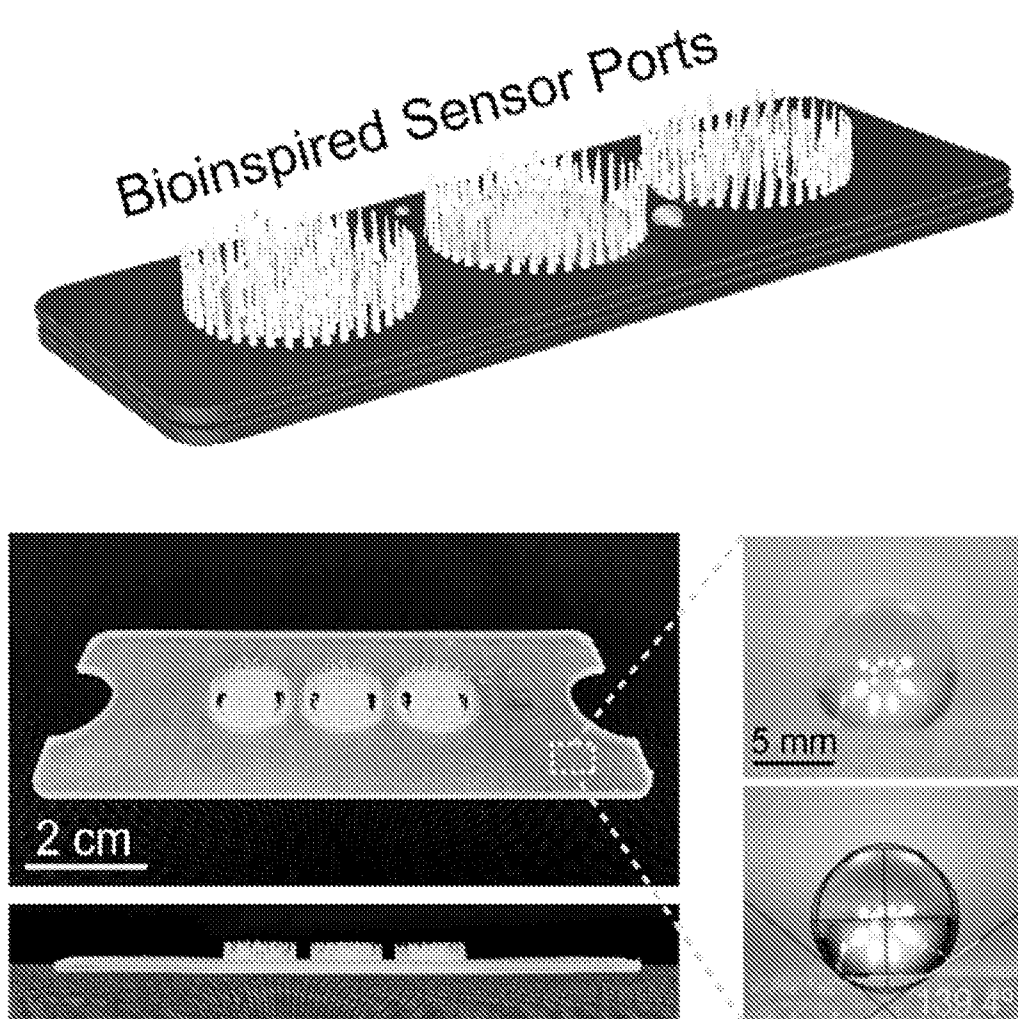
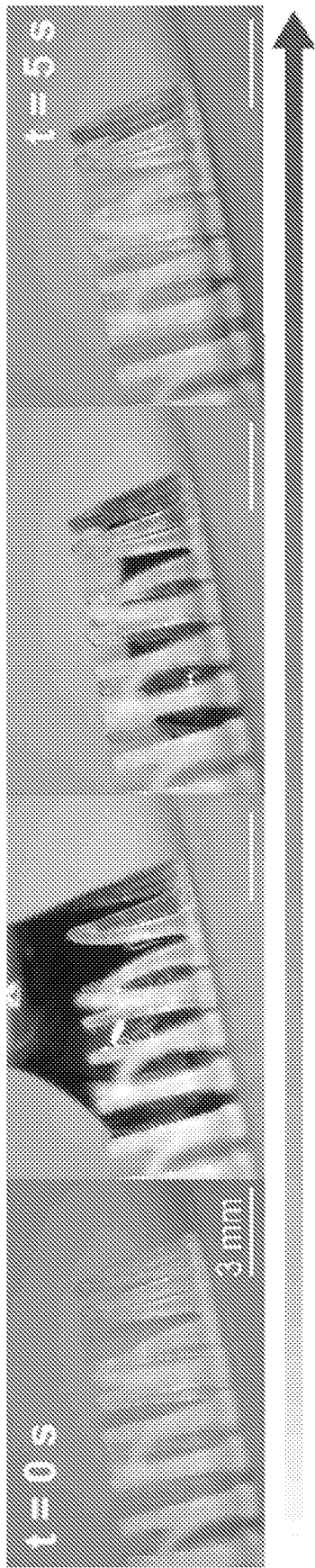


FIG. 27B



Bioinspired rapid sample wicking

FIG. 27C

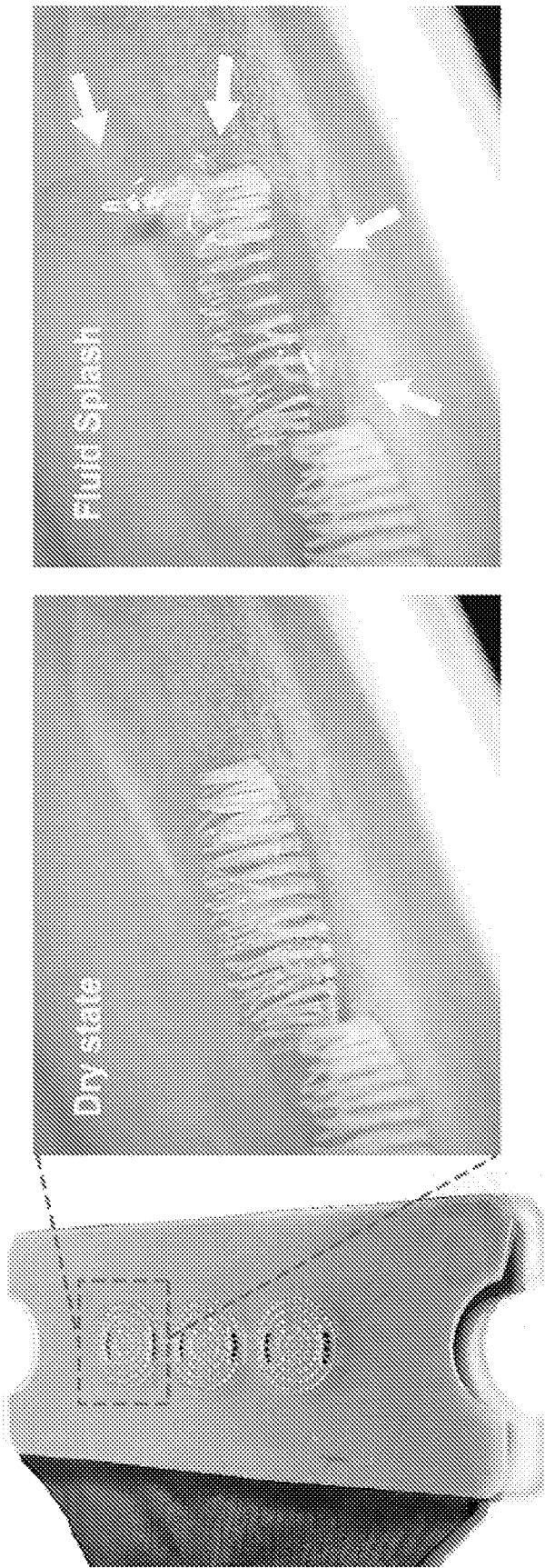


FIG. 27D

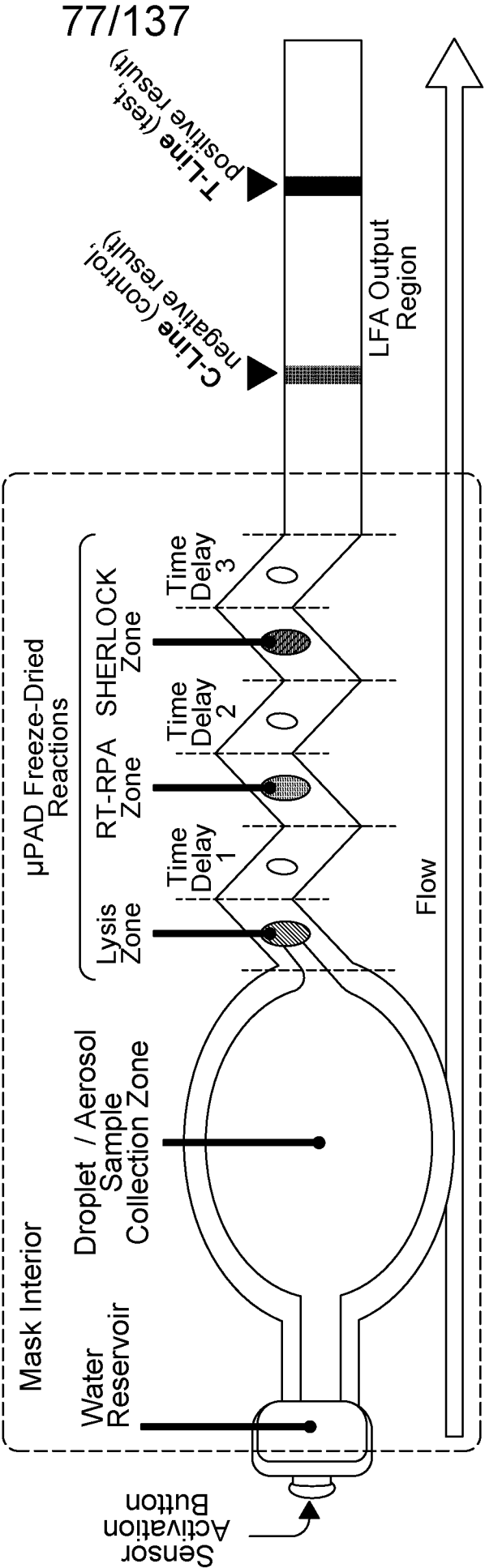


FIG. 28A

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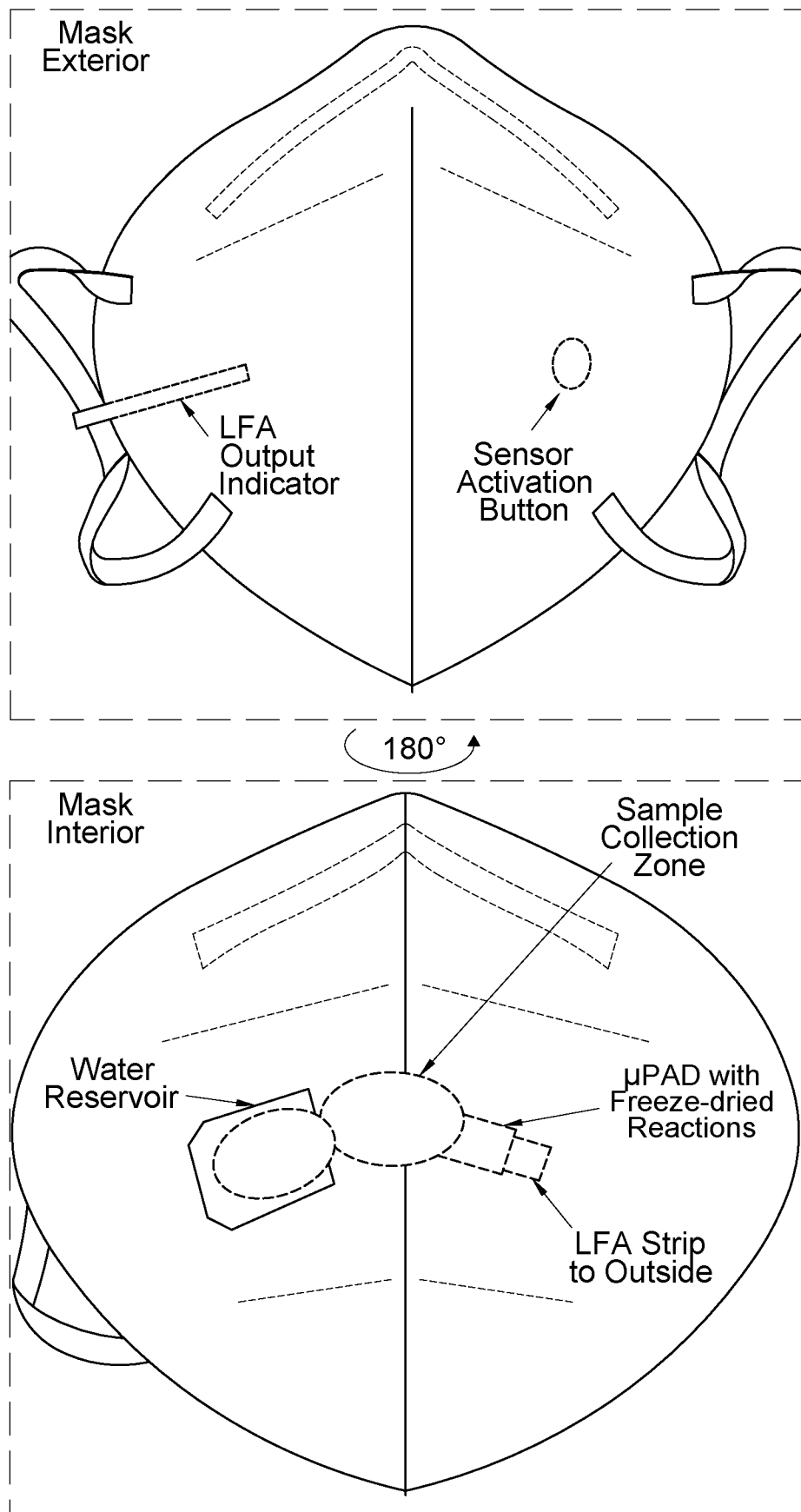


FIG. 28B

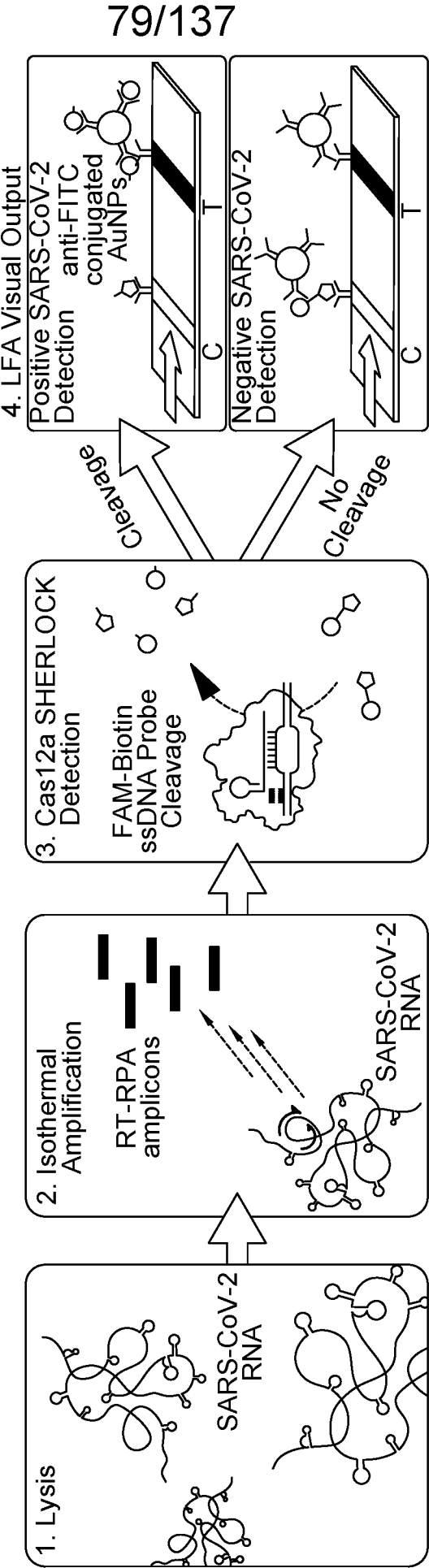


FIG. 28C

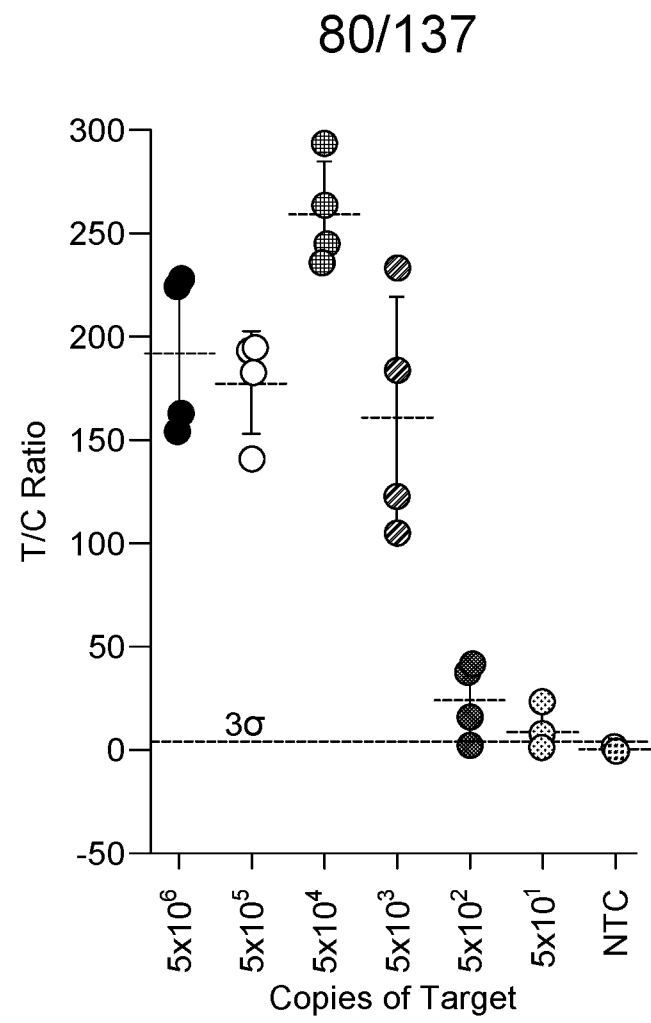


FIG. 28D

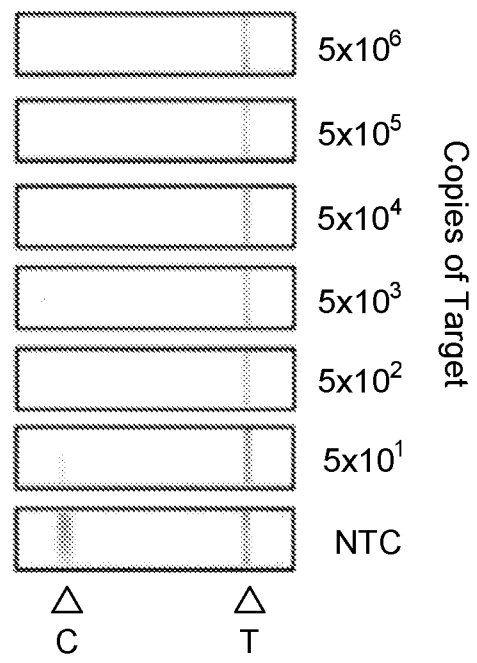


FIG. 28E

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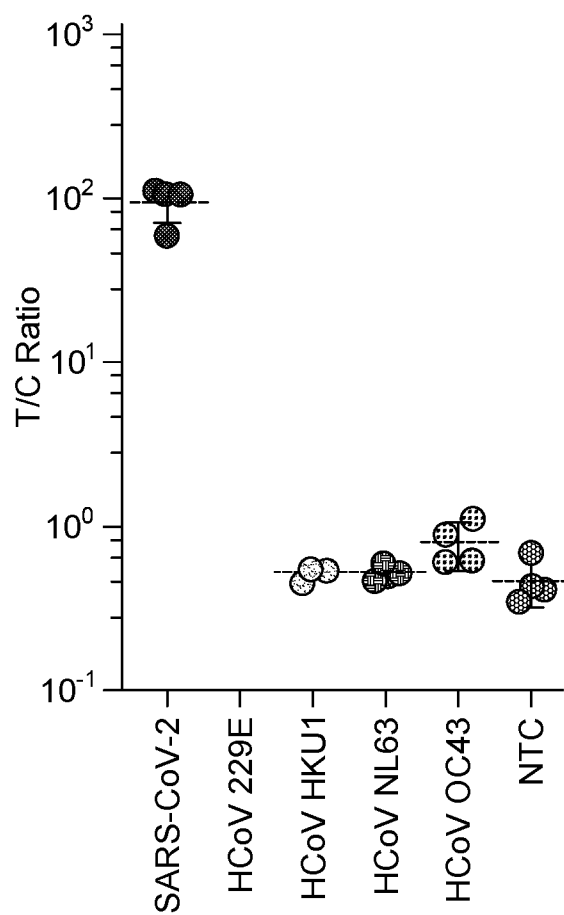


FIG. 28F

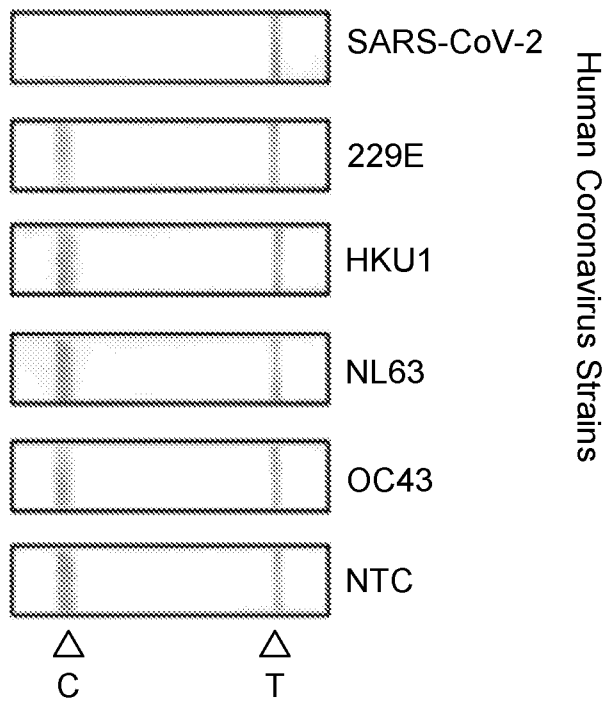


FIG. 28G

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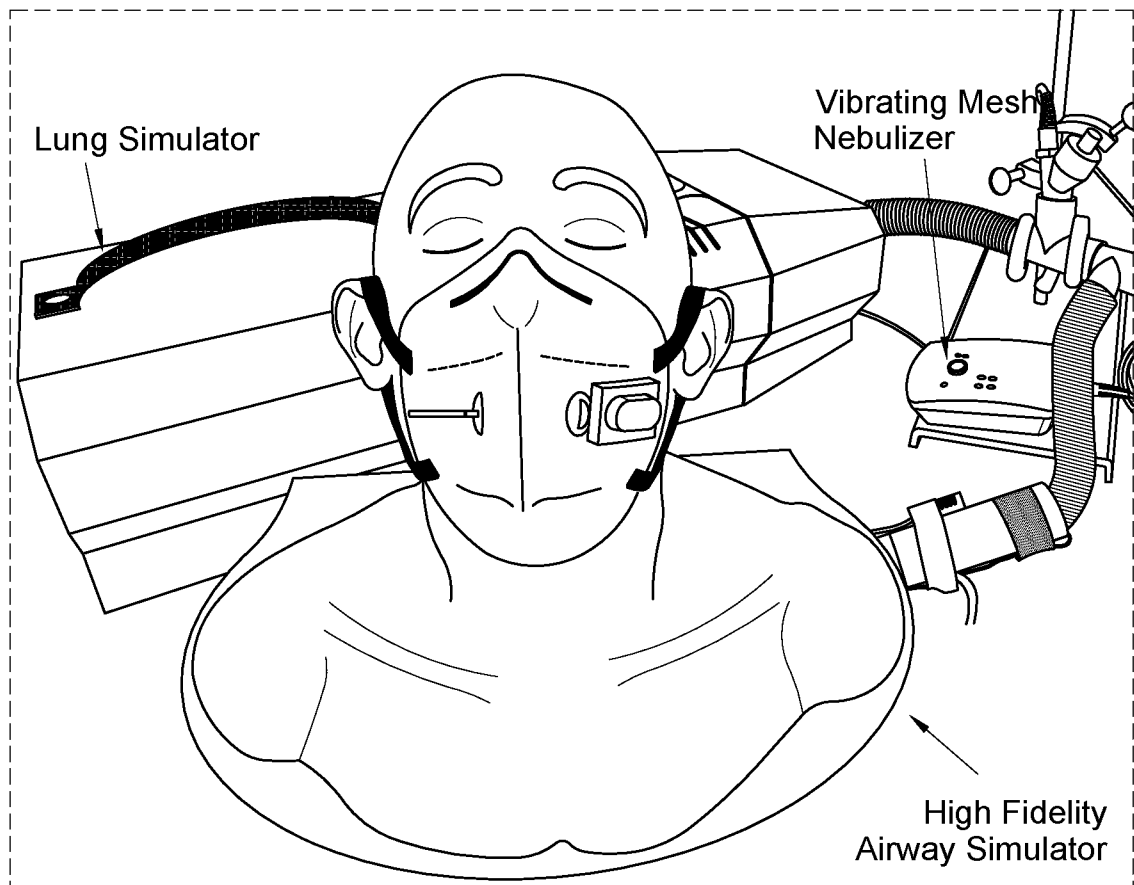


FIG. 28H

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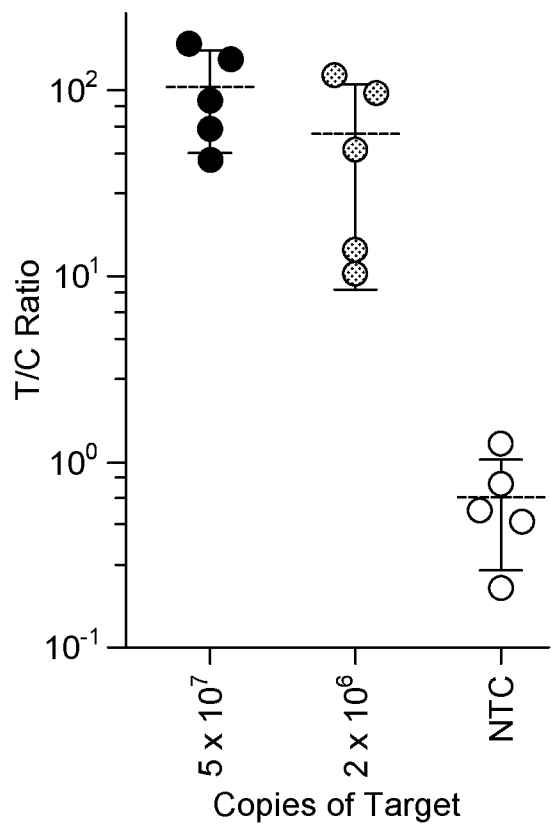


FIG. 28I

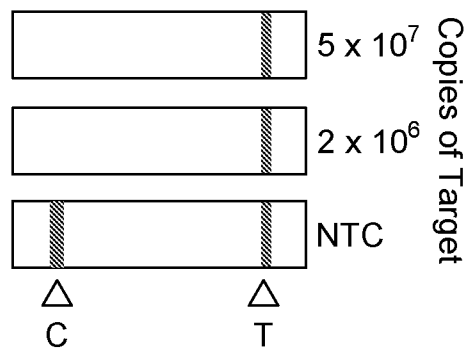


FIG. 28J

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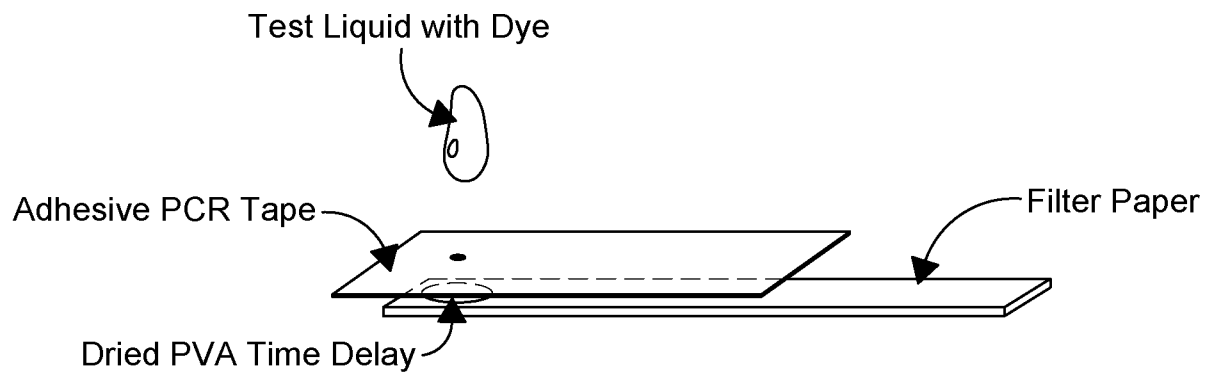


FIG. 29A

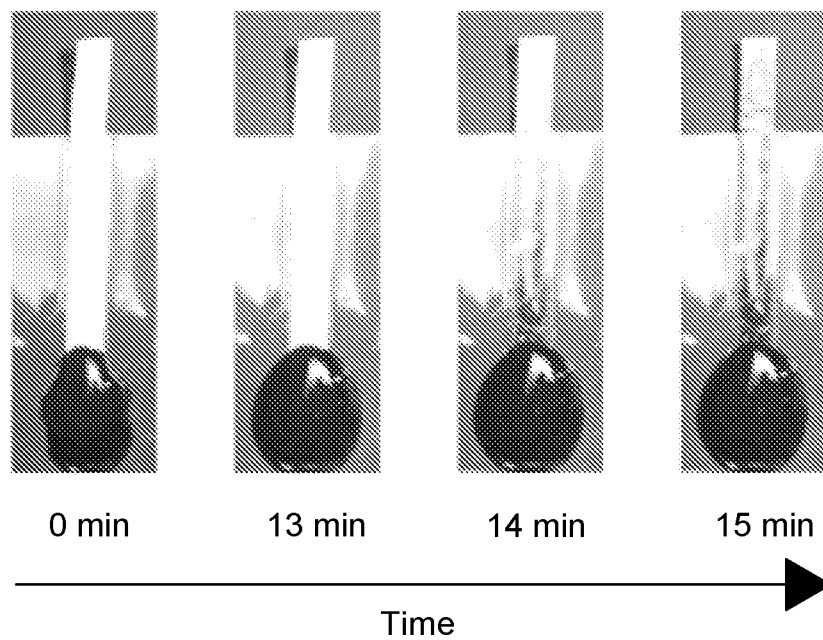


FIG. 29B

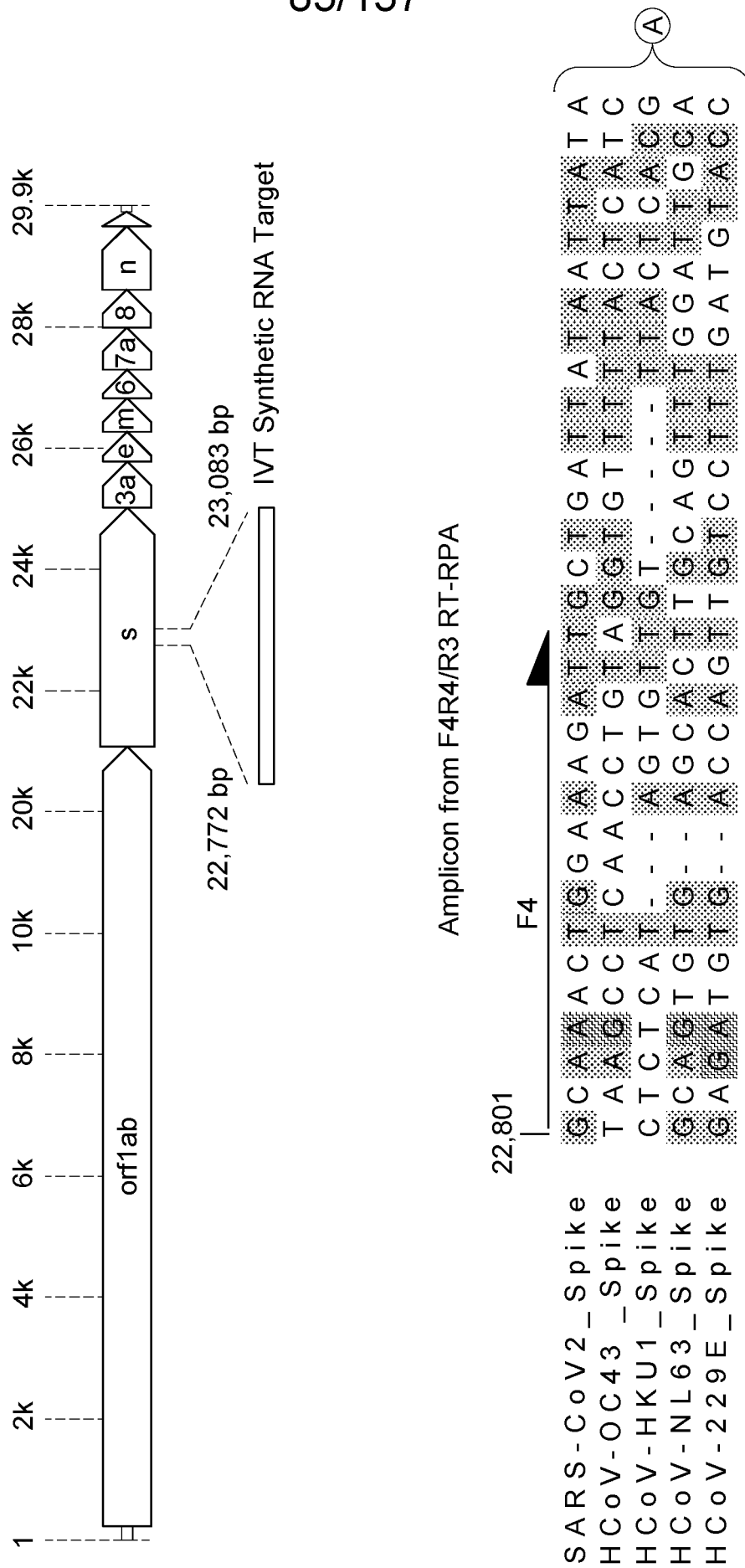


FIG. 30A

[illegible]

FIG. 30A (CONTINUATION)

[illegible]

FIG. 30A (CONTINUATION)

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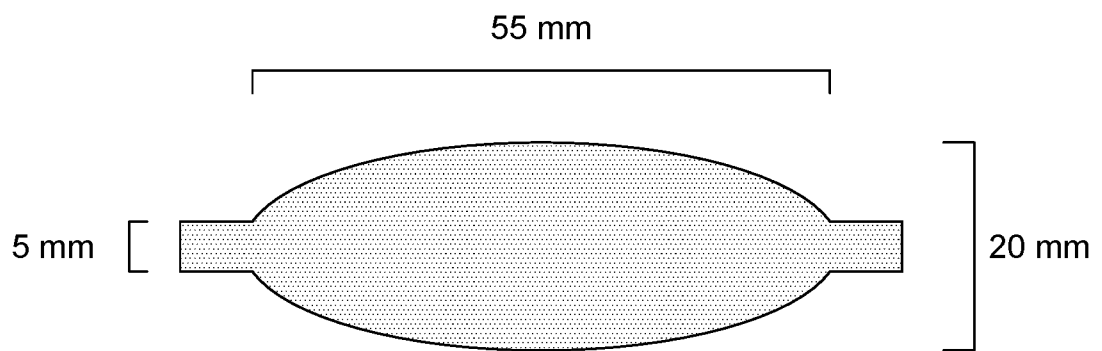
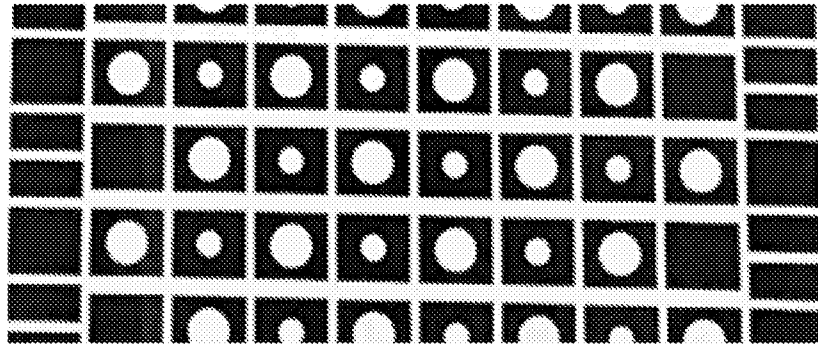


FIG. 30B

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Wax printed patterns for multiple μ PADs.



After wax reflow by hot pressing

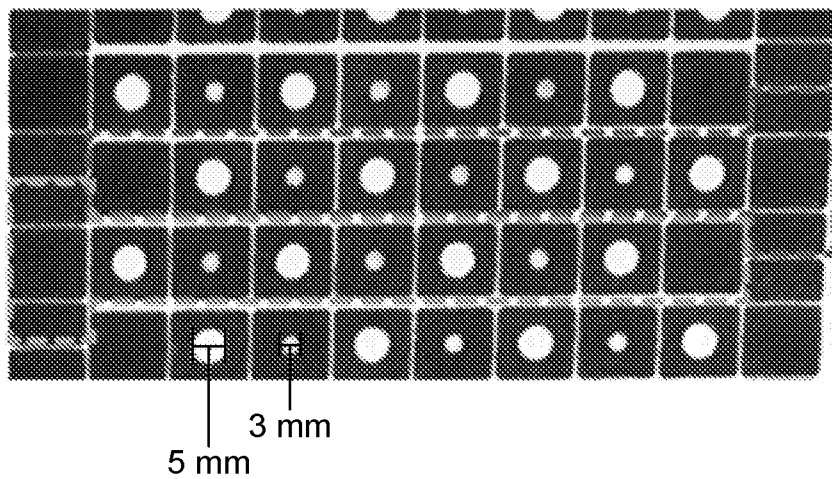


FIG. 30C

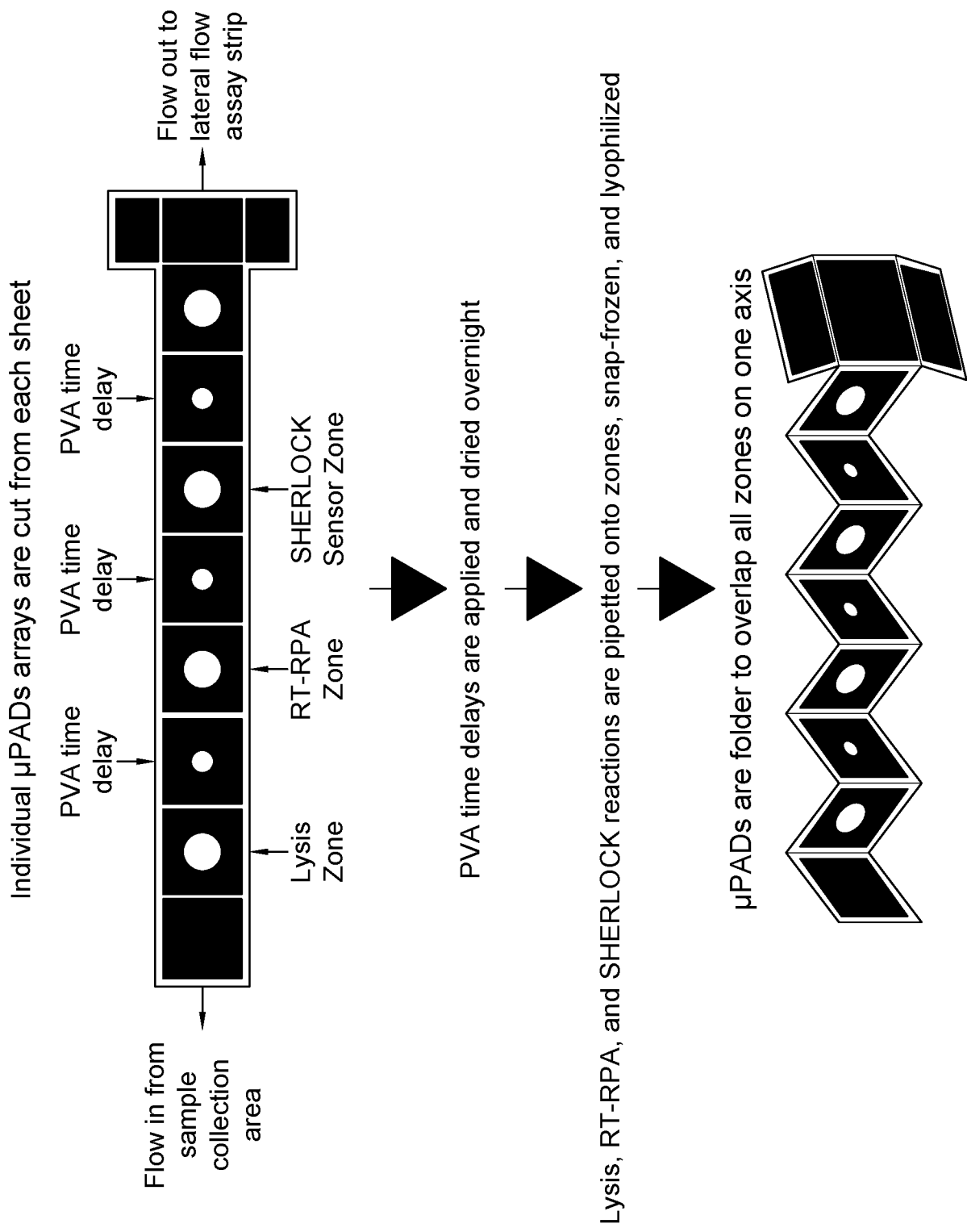


FIG. 30C (CONTINUATION)

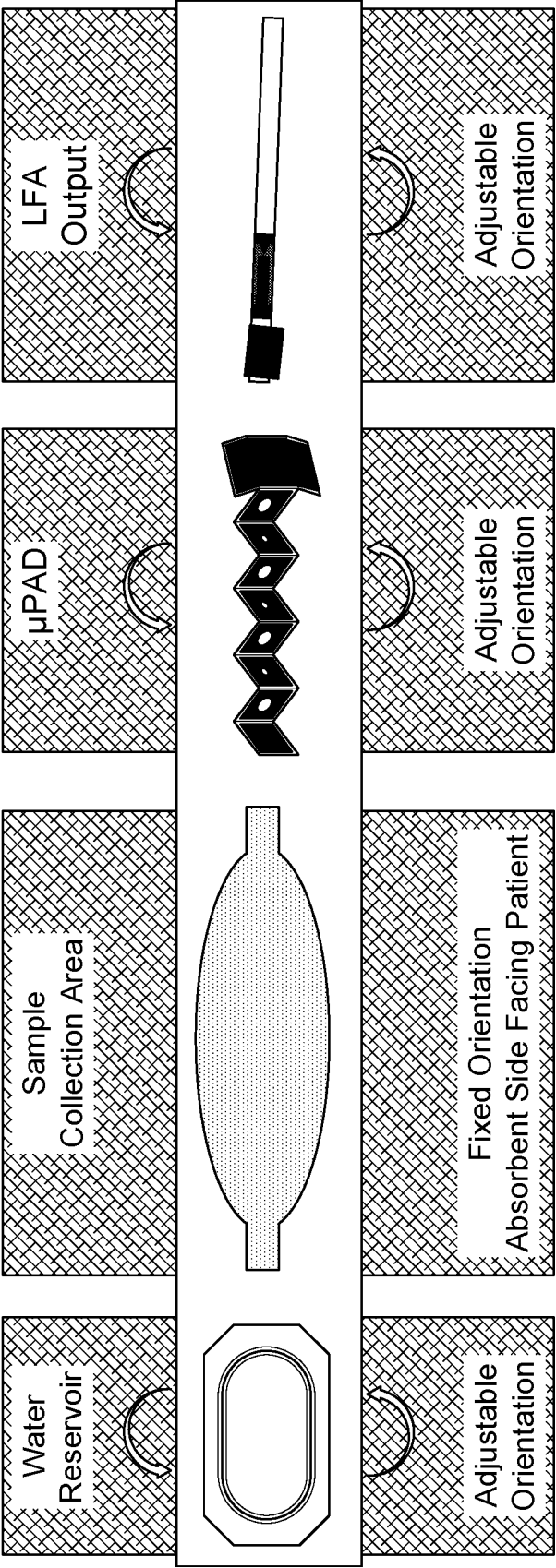


FIG. 30D

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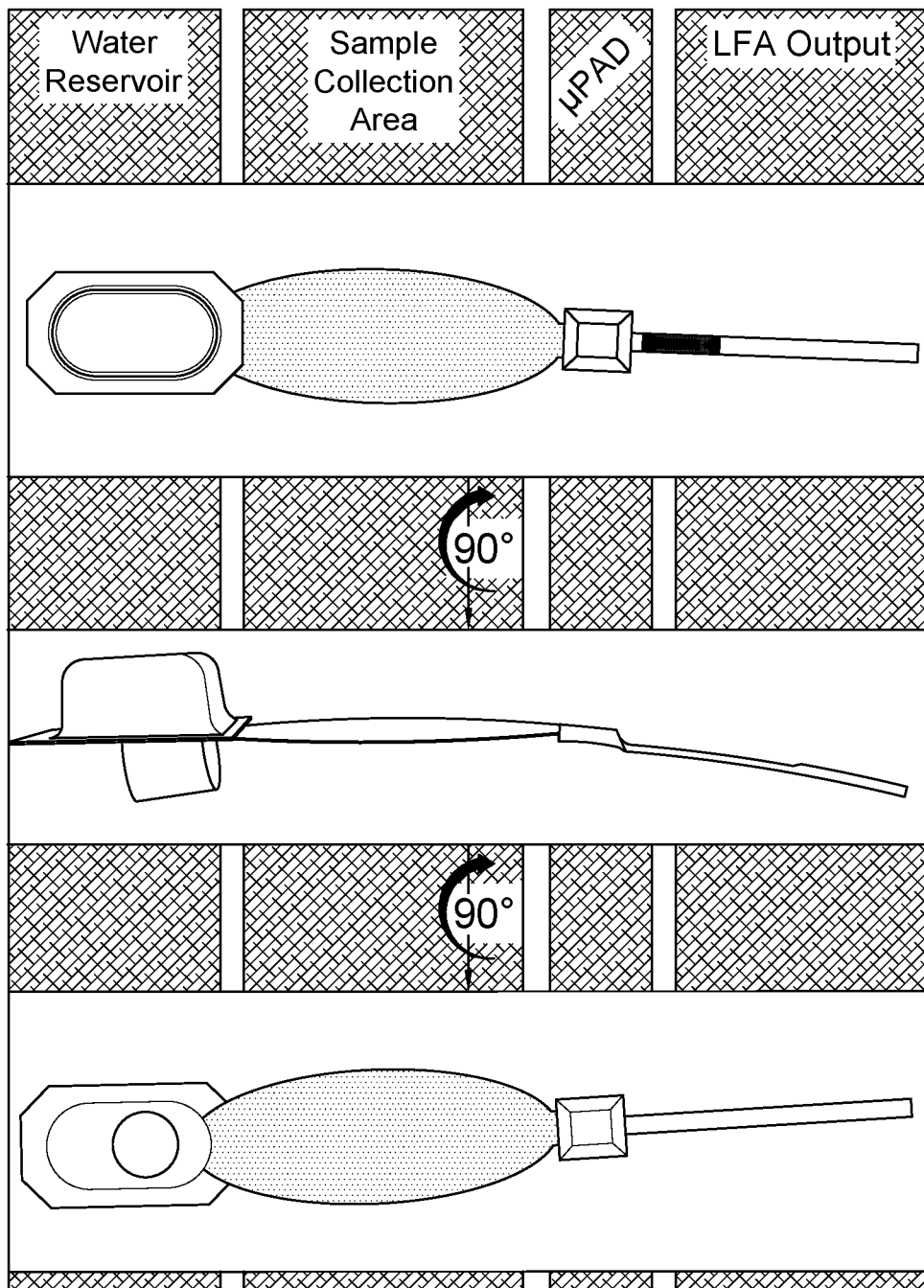


FIG. 30E

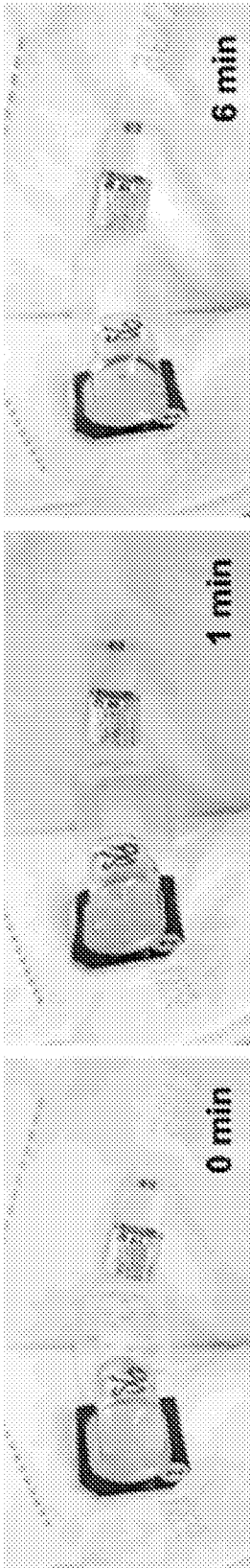


FIG. 30F

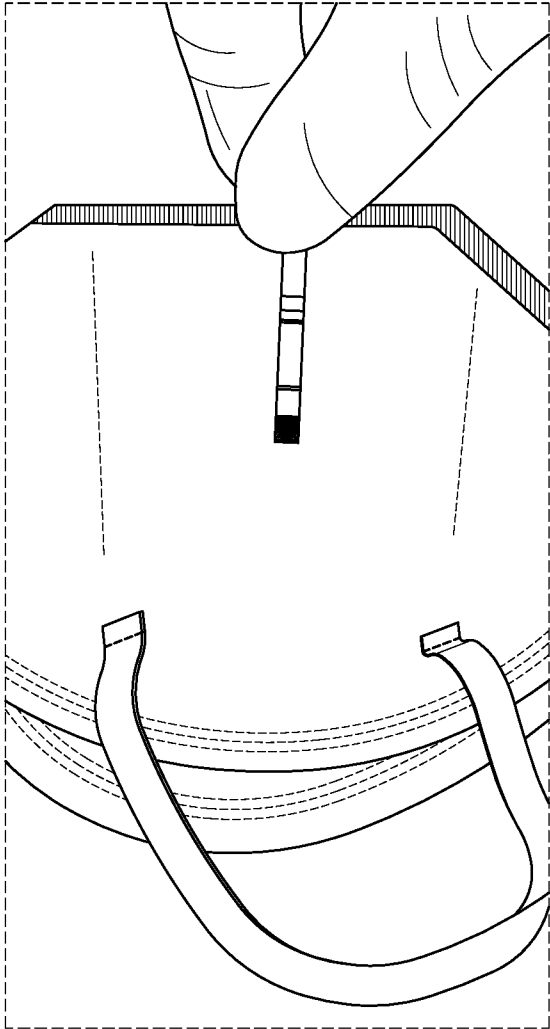


FIG. 30G

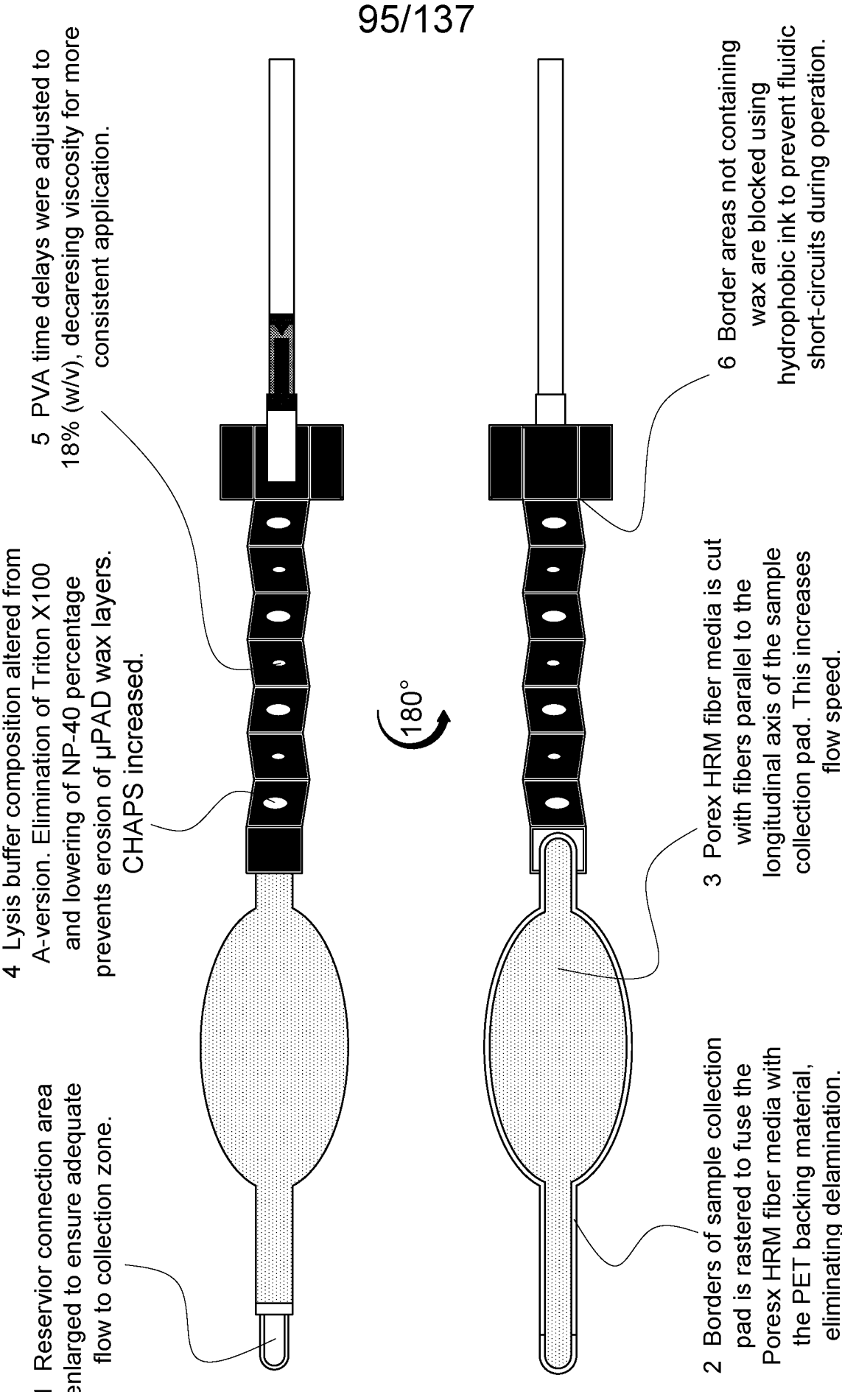


FIG. 31A

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7 Venting holes were punched into the water blister reservoir to eliminate buildup of vacuum.

The holes were overlaid with breathable water-repelling adhesive covers.

8 μ PAD area interfacing with the sample collection pad was tightly sealed to prevent unwanted fluidic short circuits.

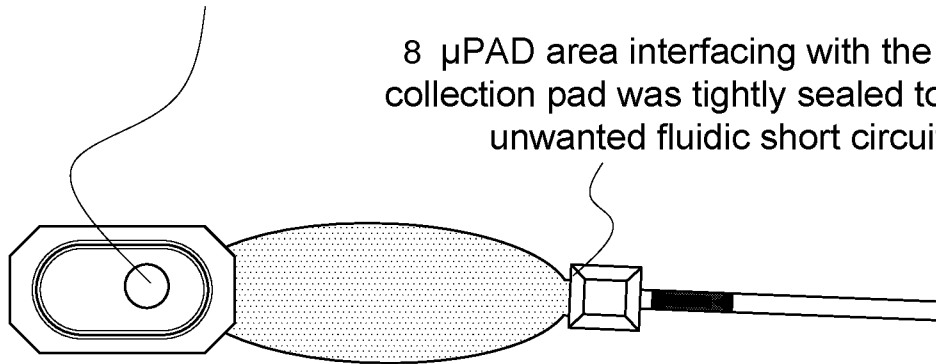


FIG. 31B

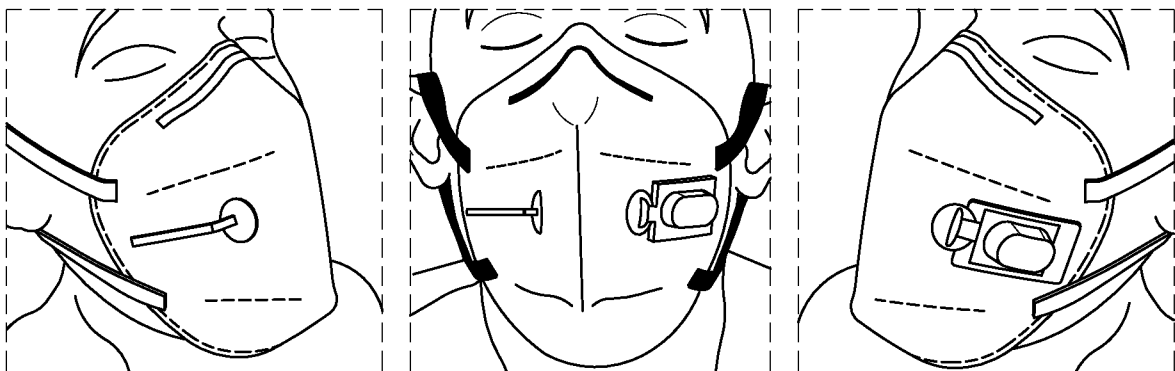


FIG. 31C

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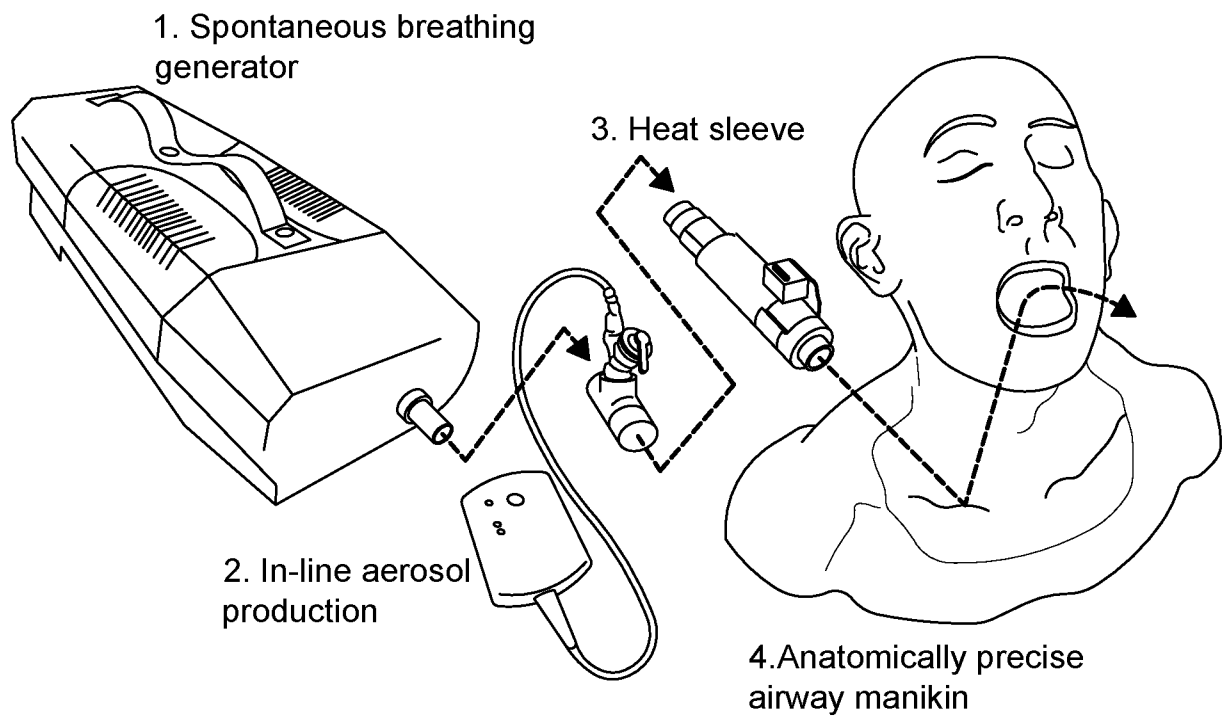
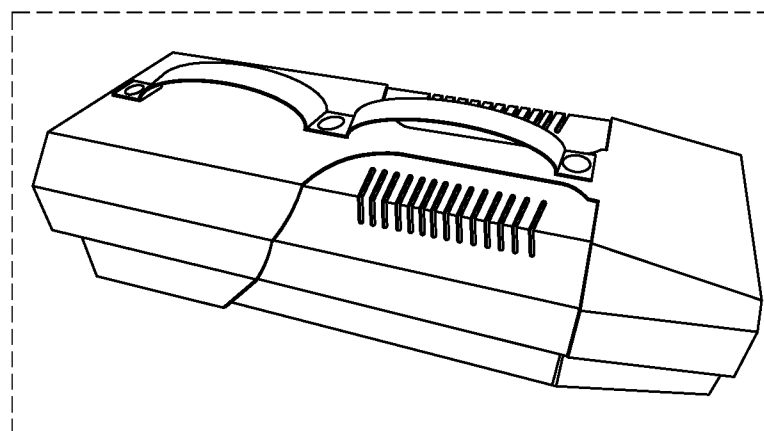
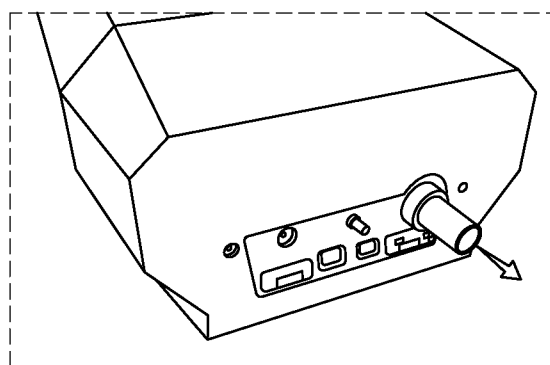


FIG. 32A



TestChest system simulates
spontaneous breathing



TestChest breath output to nebulizer

FIG. 32B

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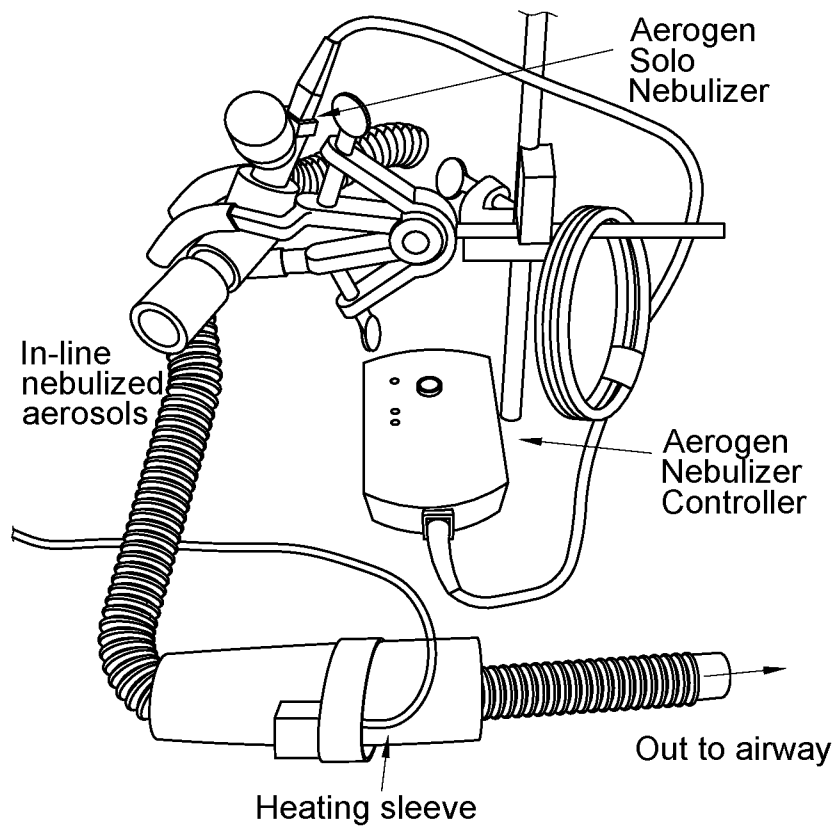


FIG. 32C

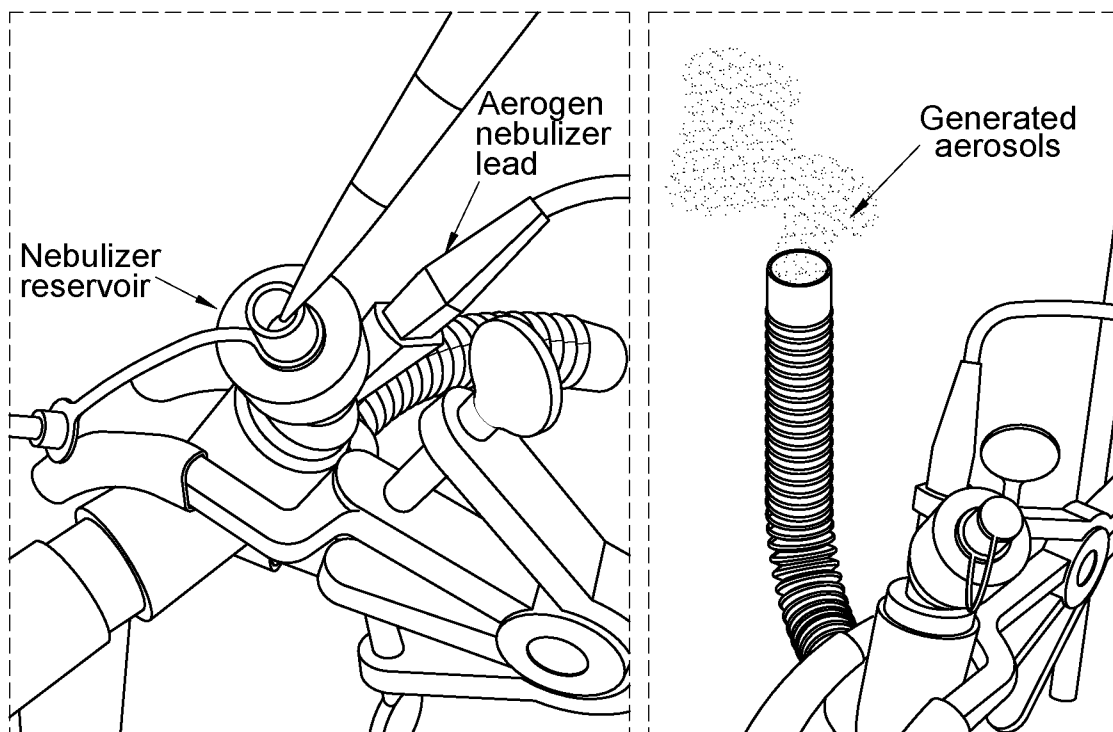


FIG. 32D

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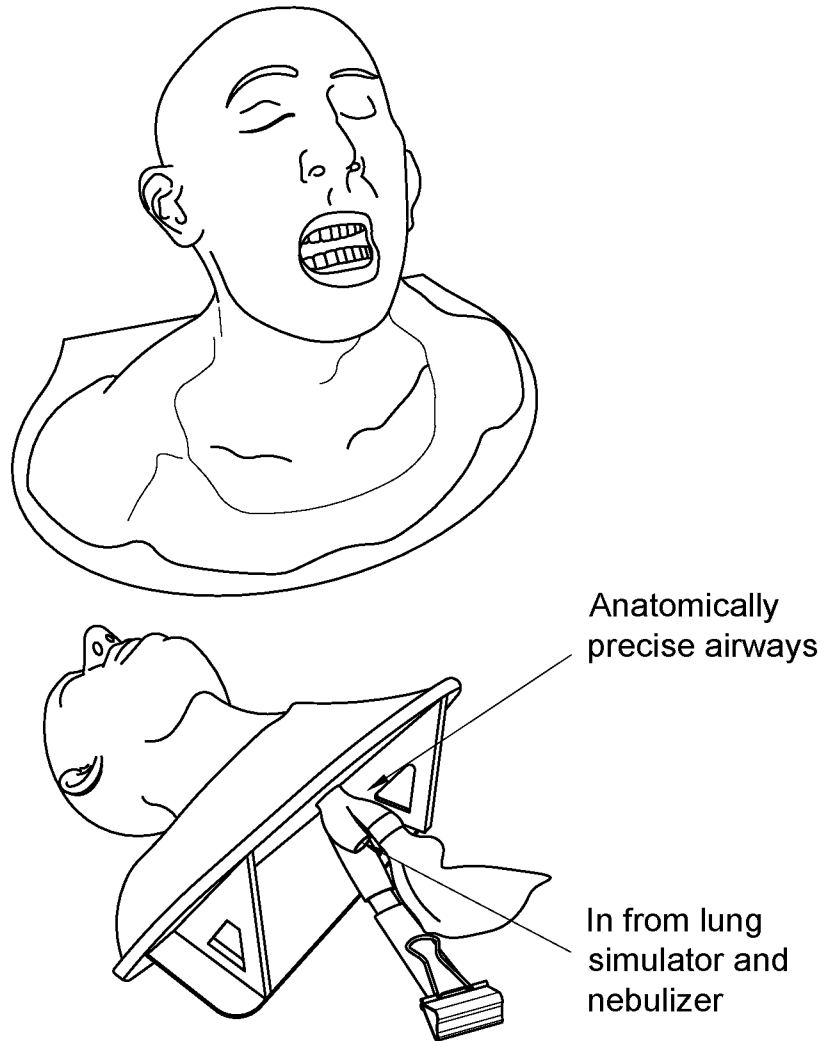


FIG. 32E

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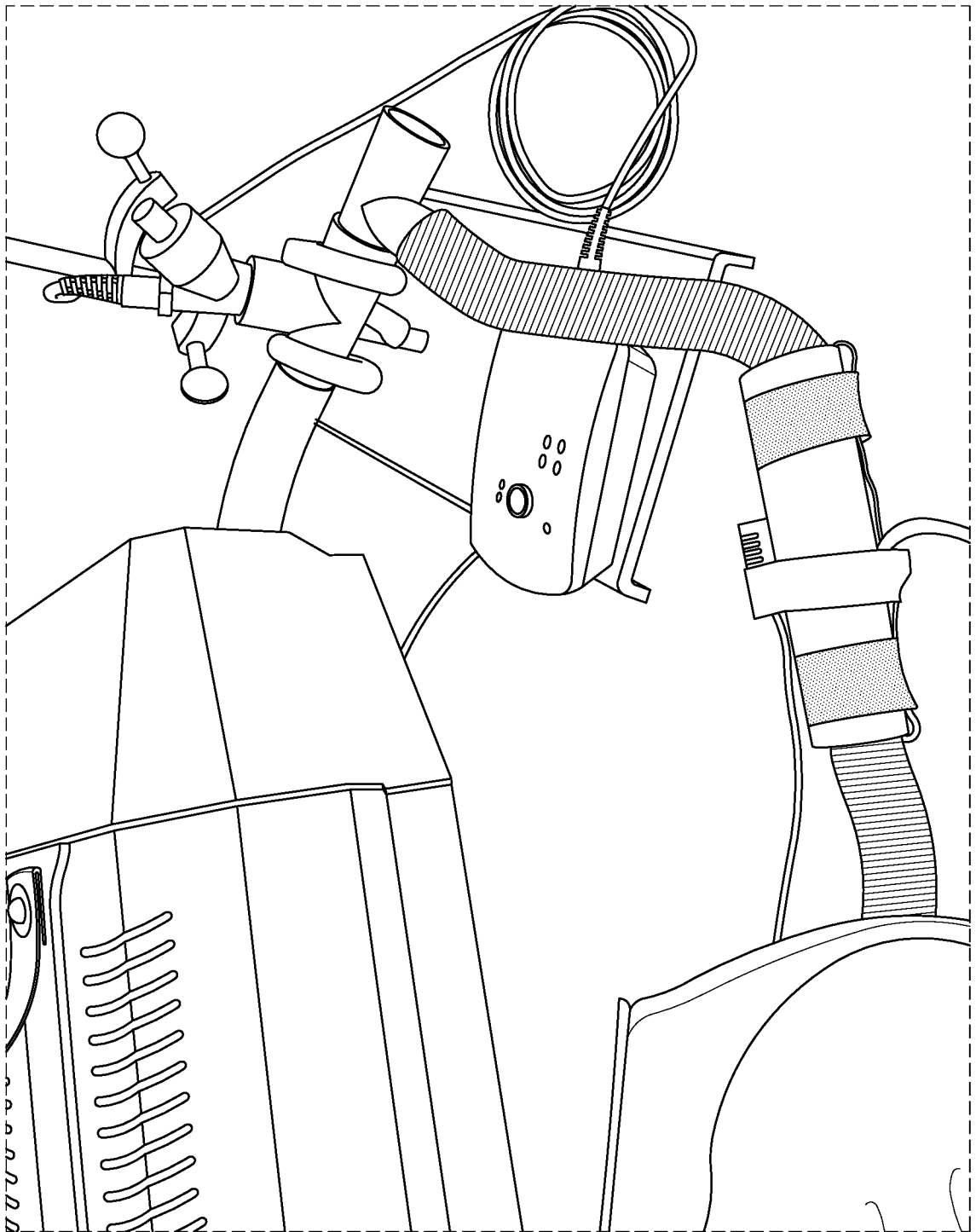


FIG. 32 F

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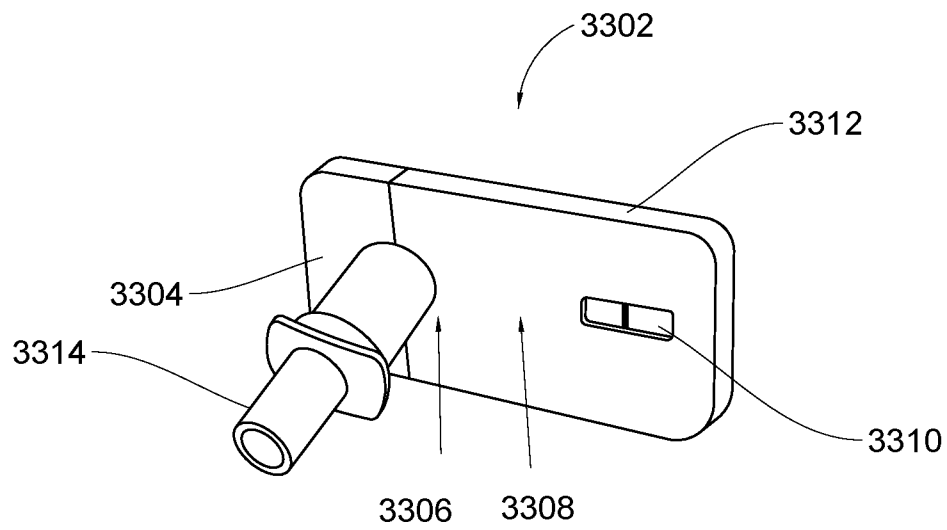


FIG. 33A

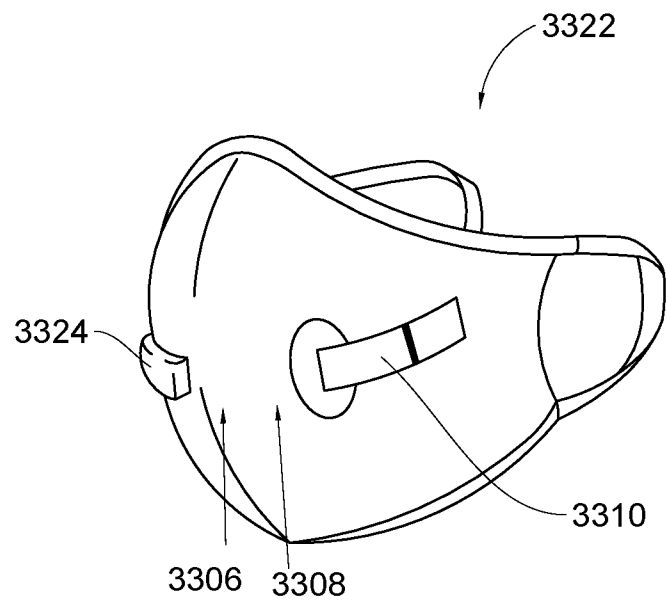


FIG. 33B

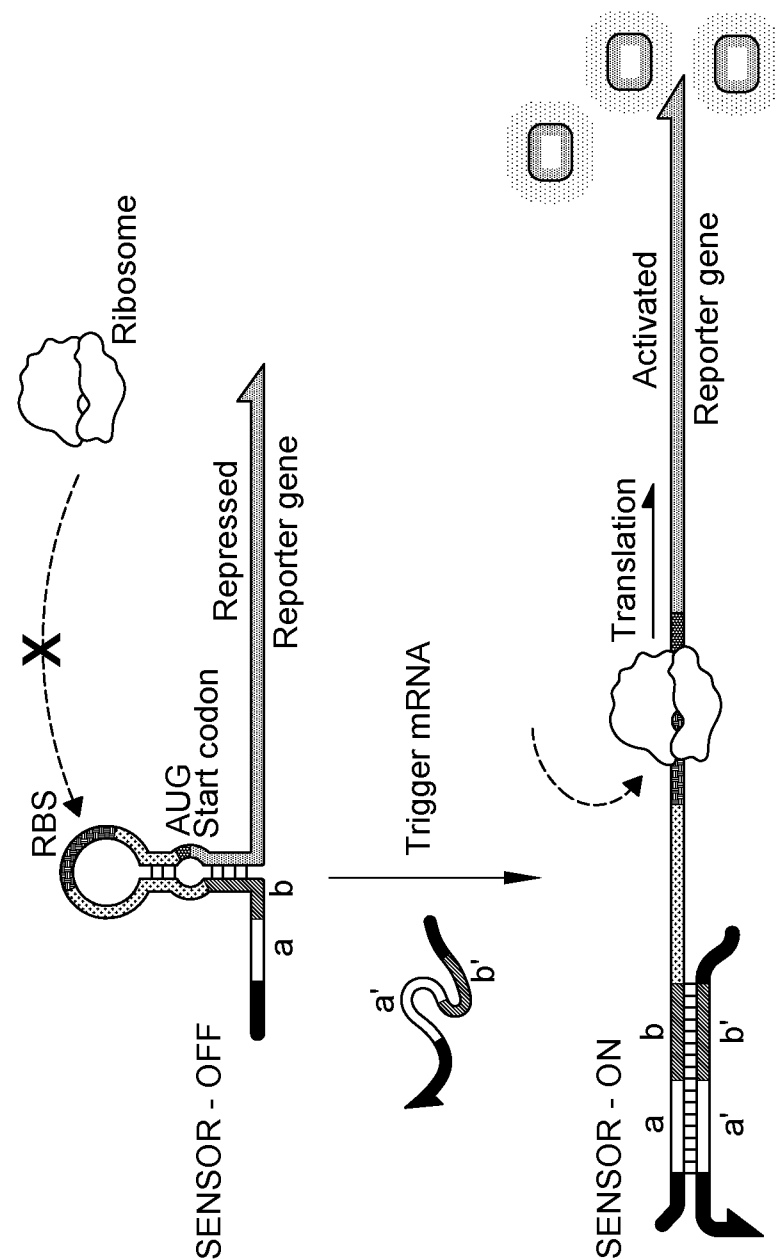


FIG. 34

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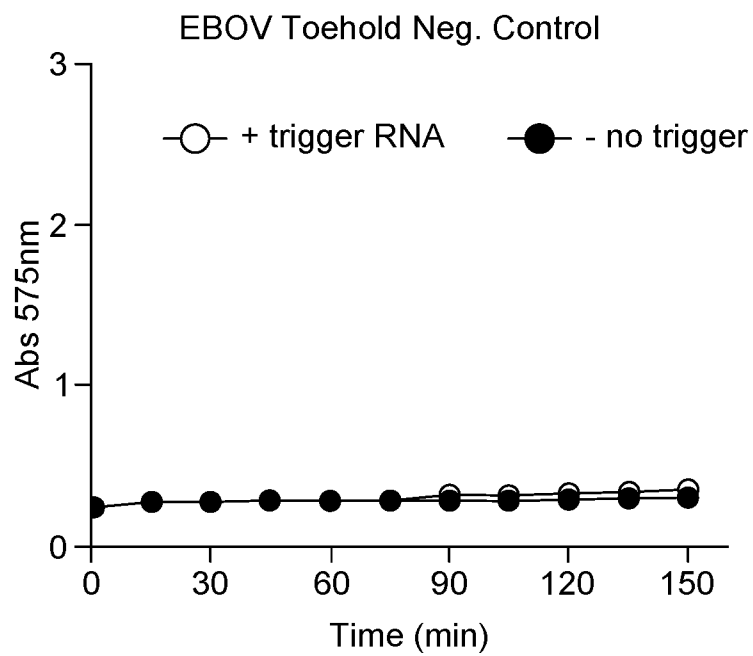


FIG. 35A

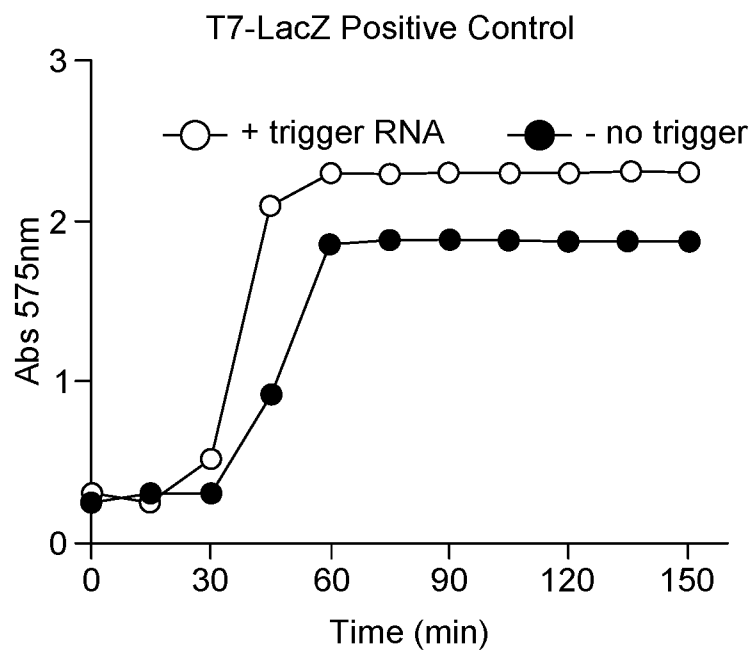


FIG. 35B

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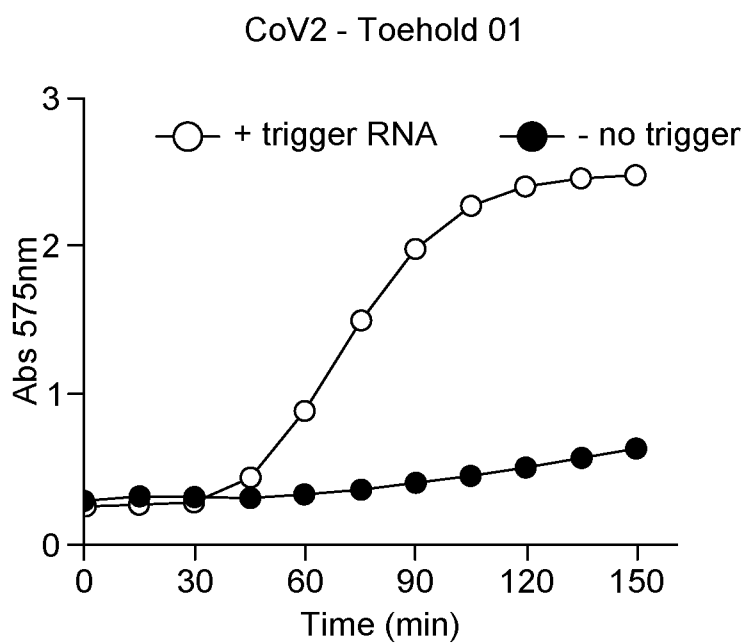


FIG. 35C

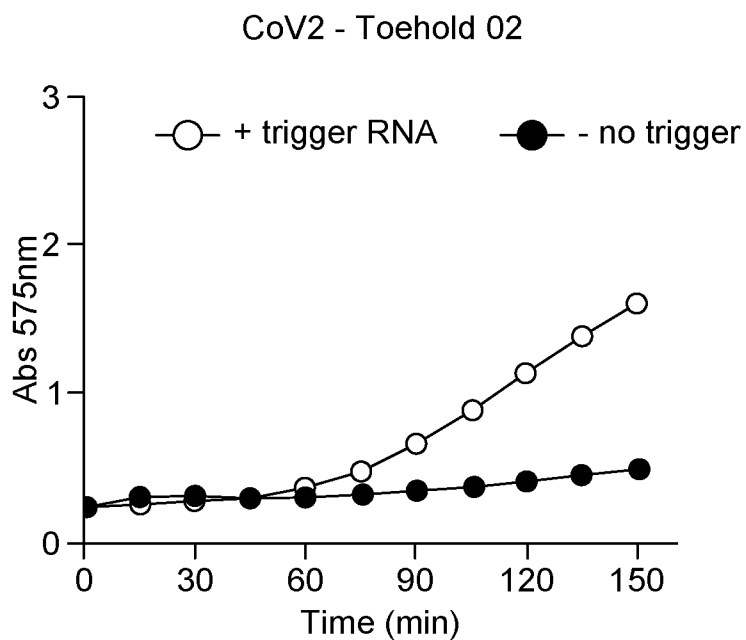


FIG. 35D

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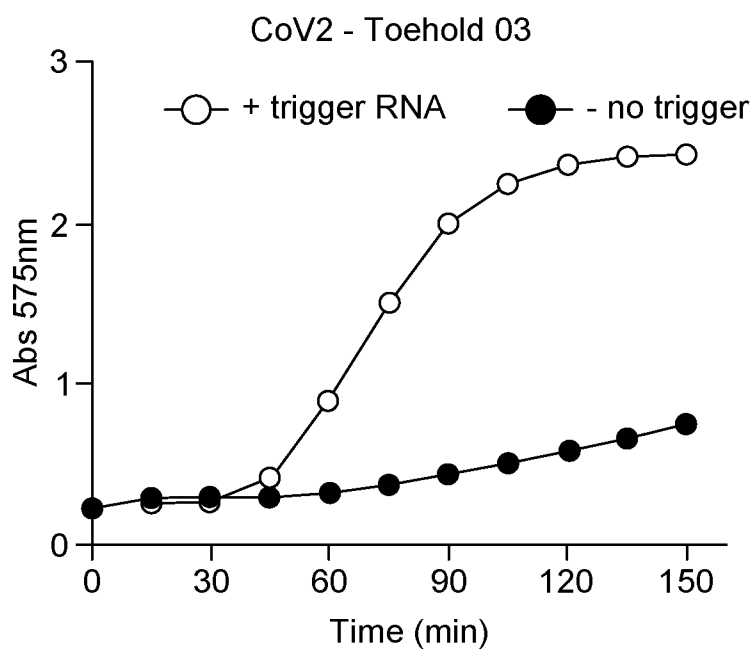


FIG. 35E

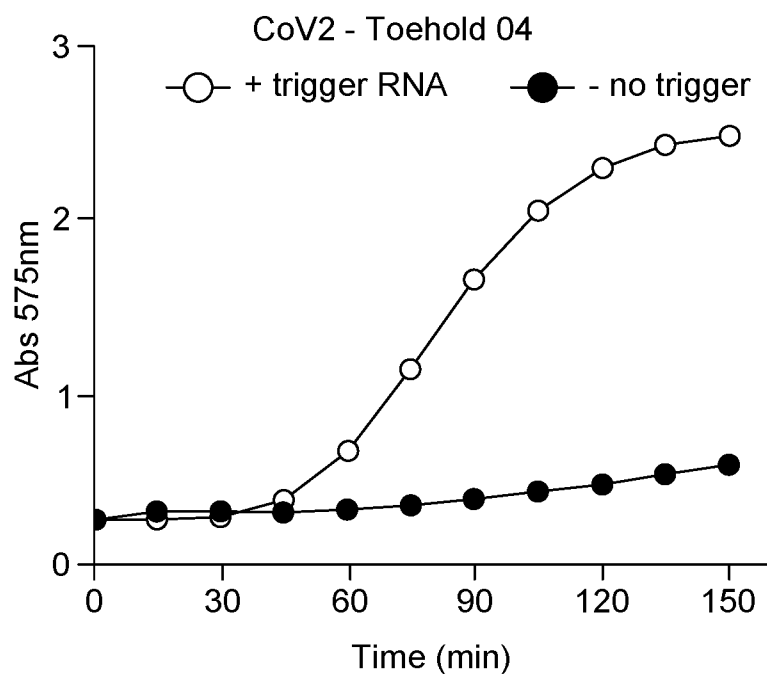


FIG. 35F

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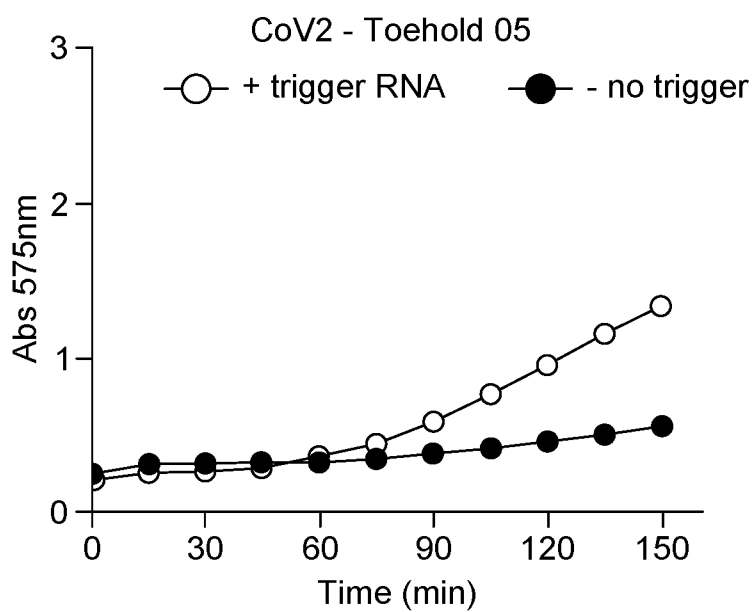


FIG. 35G

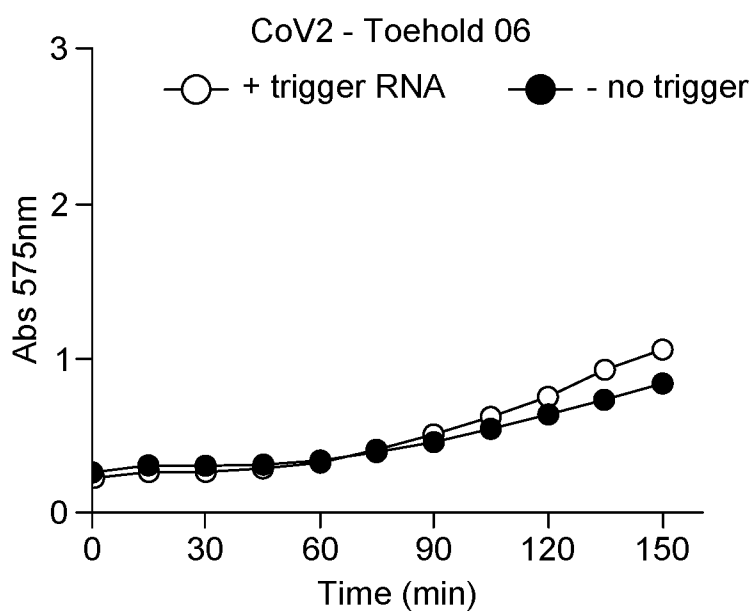


FIG. 35H

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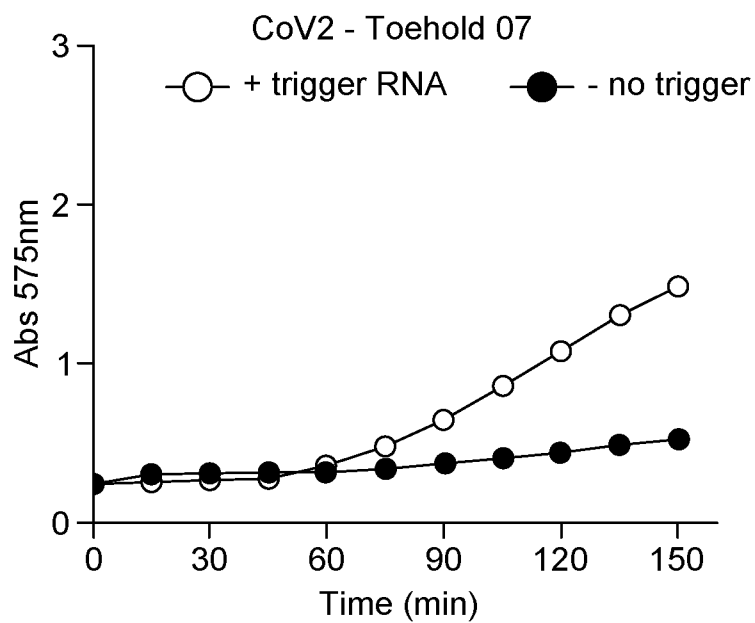


FIG. 35I

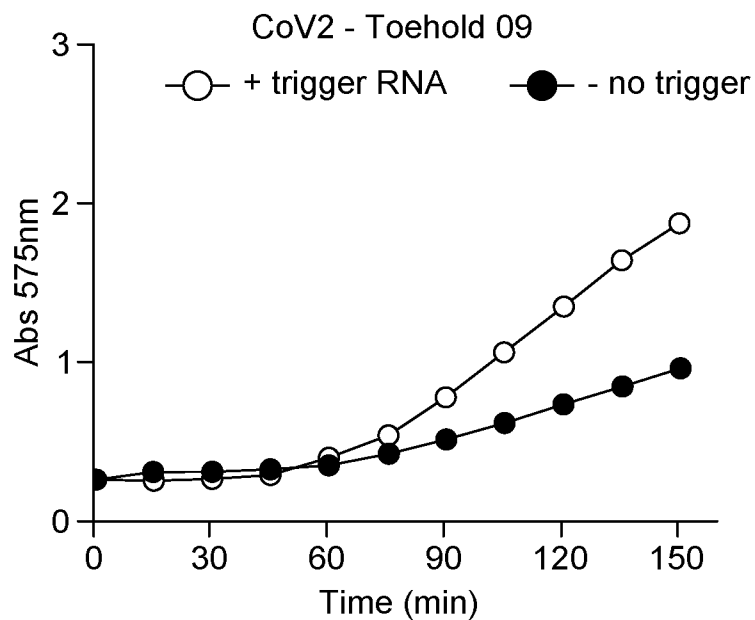


FIG. 35J

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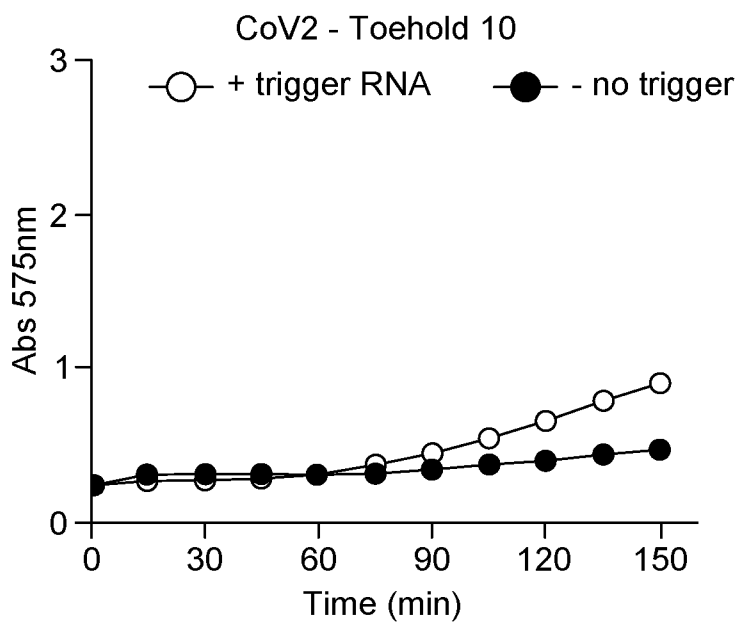


FIG. 35K

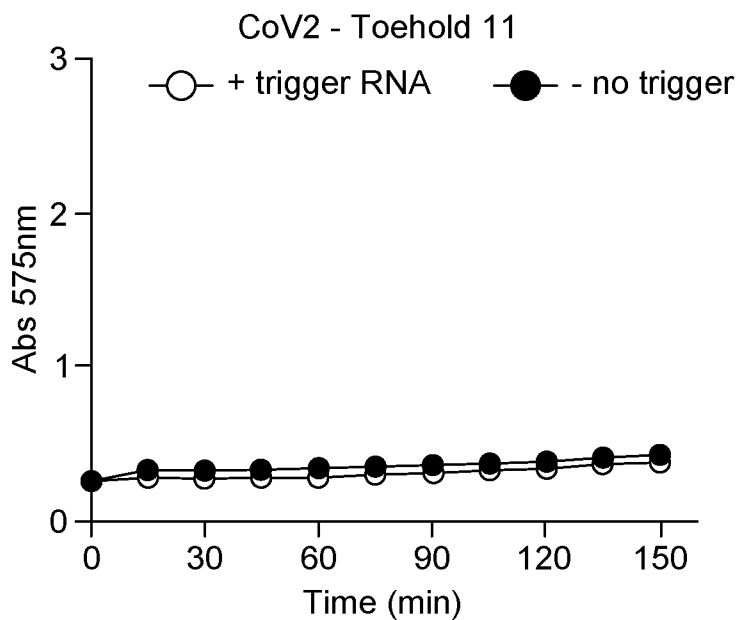


FIG. 35L

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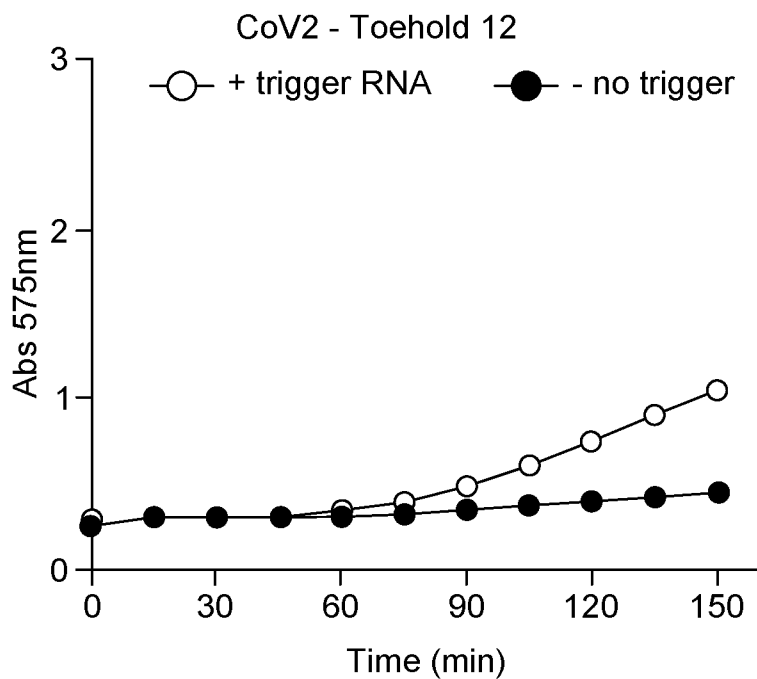


FIG. 35M

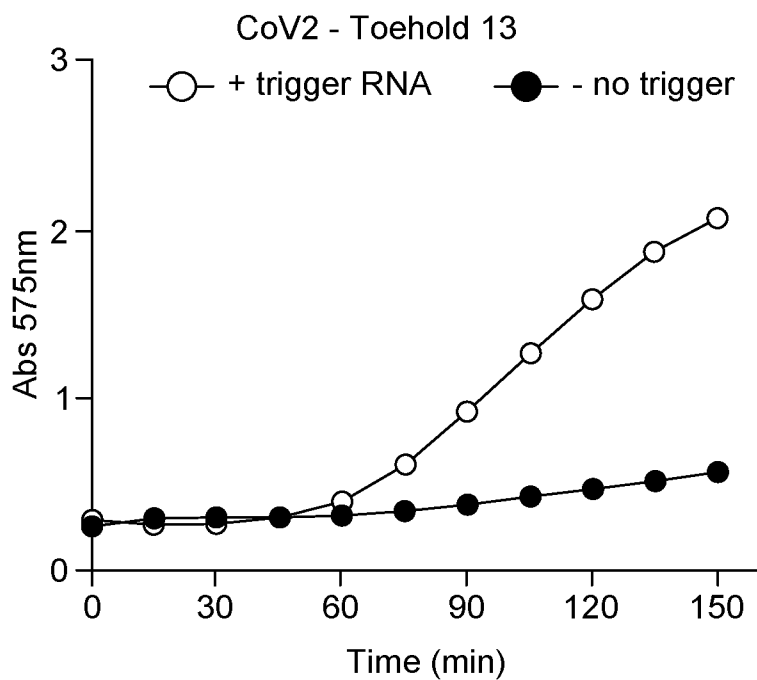


FIG. 35N

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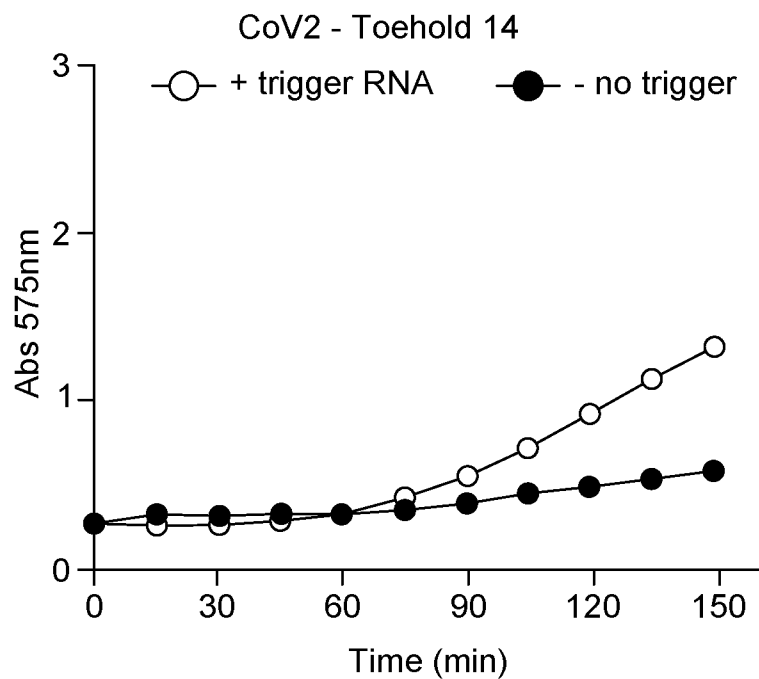


FIG. 35O

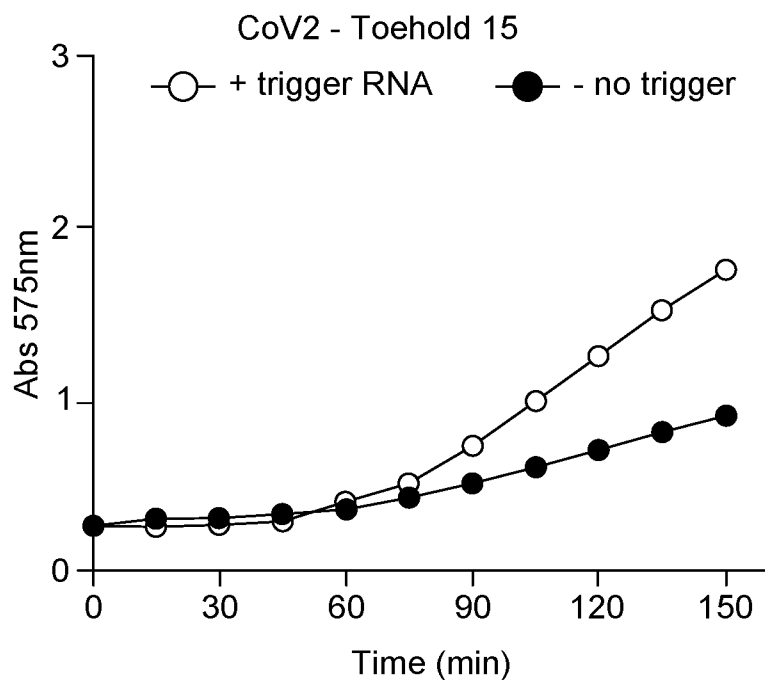


FIG. 35P

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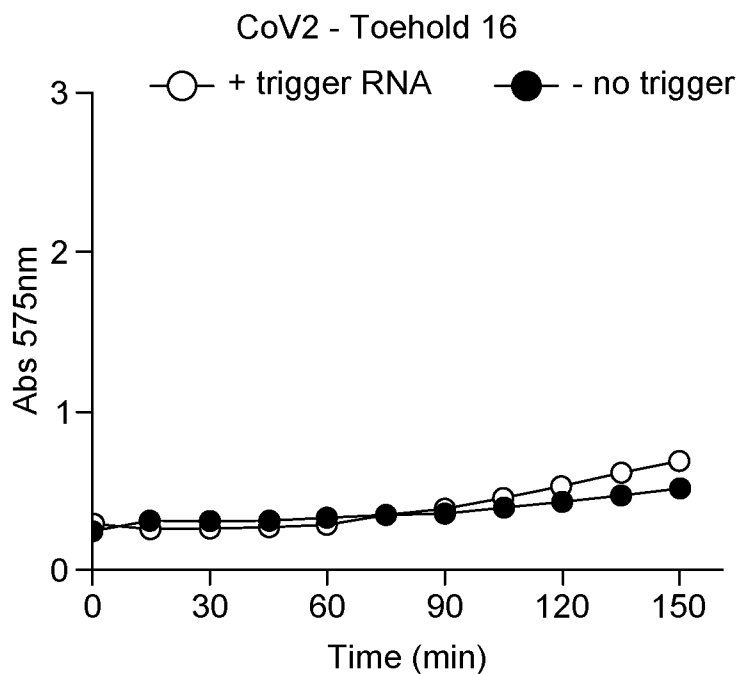


FIG. 35Q

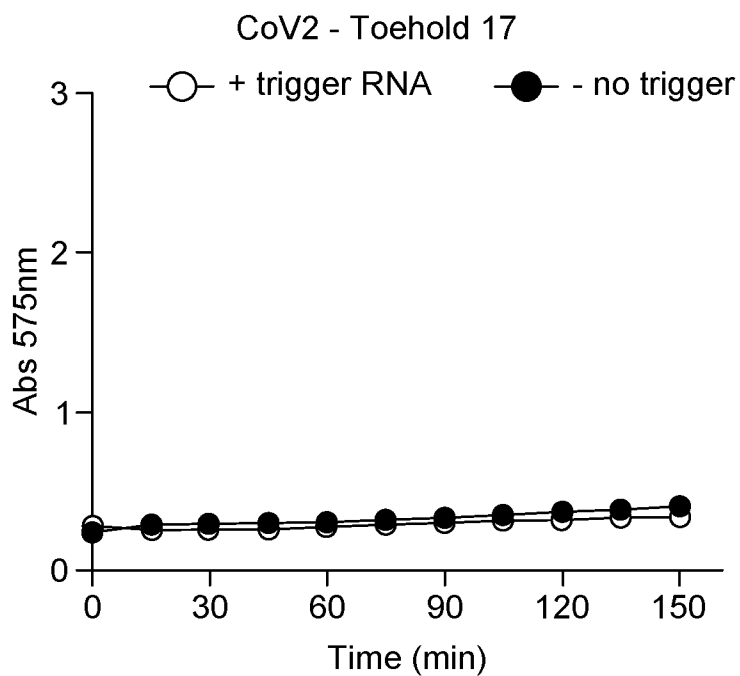


FIG. 35R

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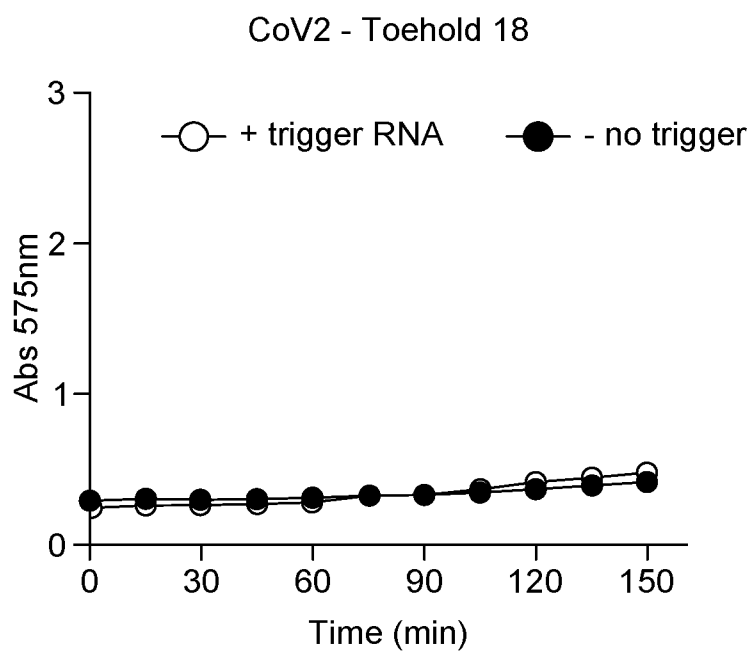


FIG. 35S

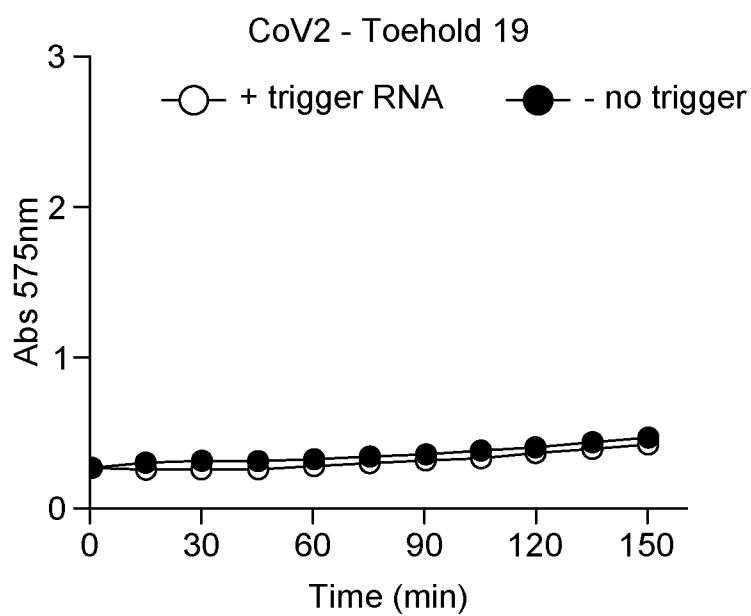


FIG. 35T

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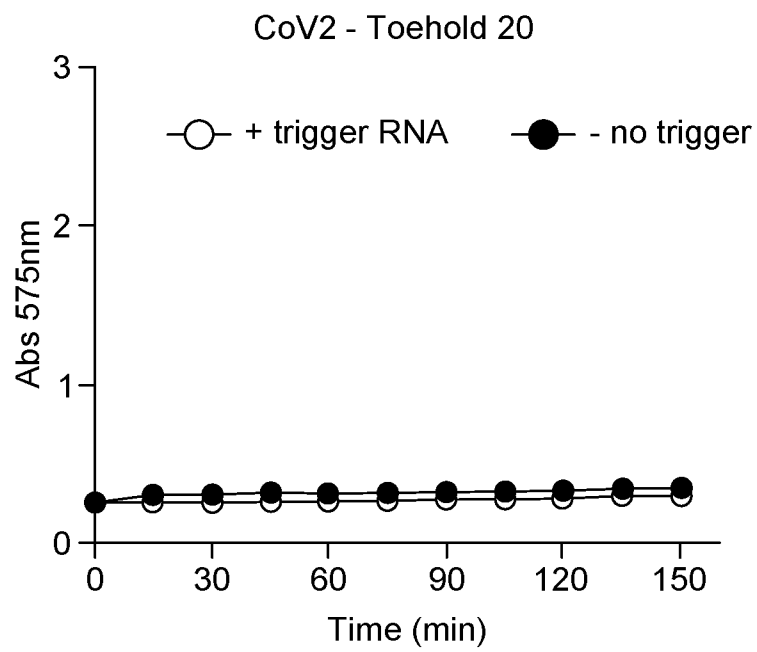


FIG. 35U

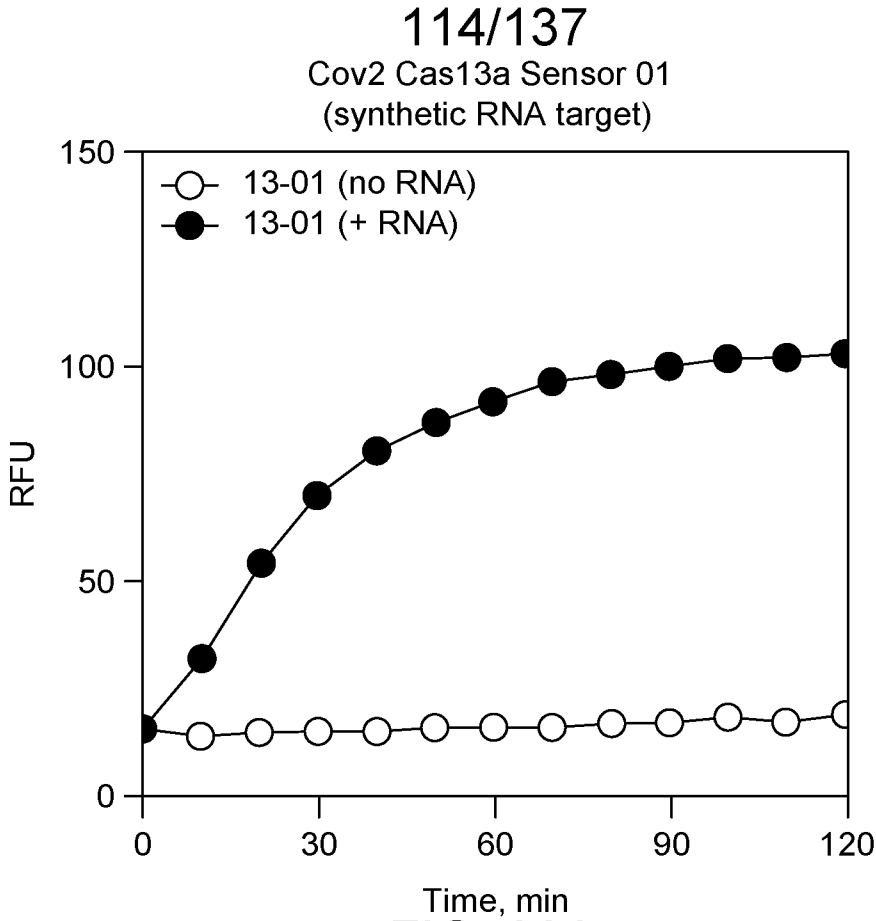


FIG. 36A

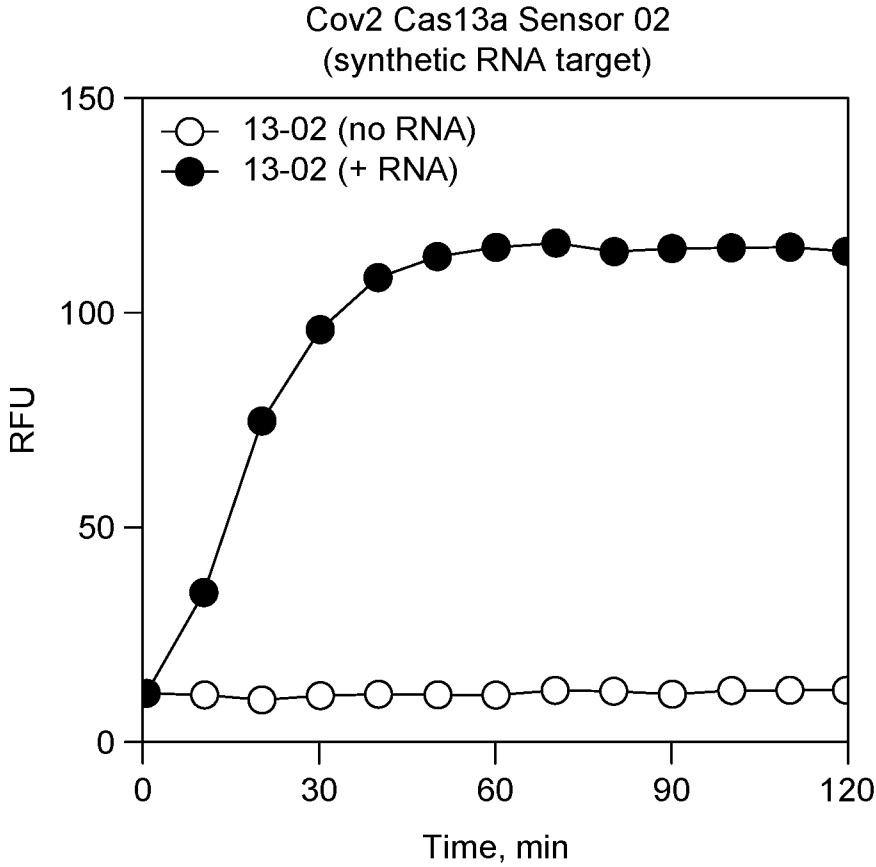


FIG. 36B

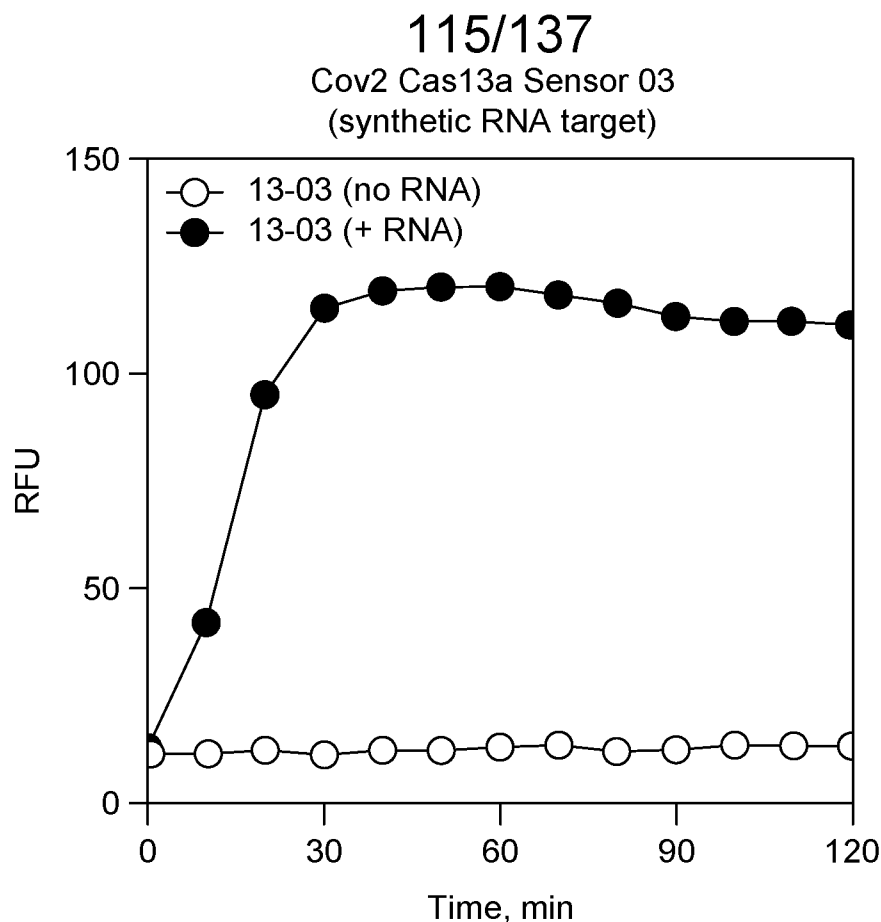


FIG. 36C

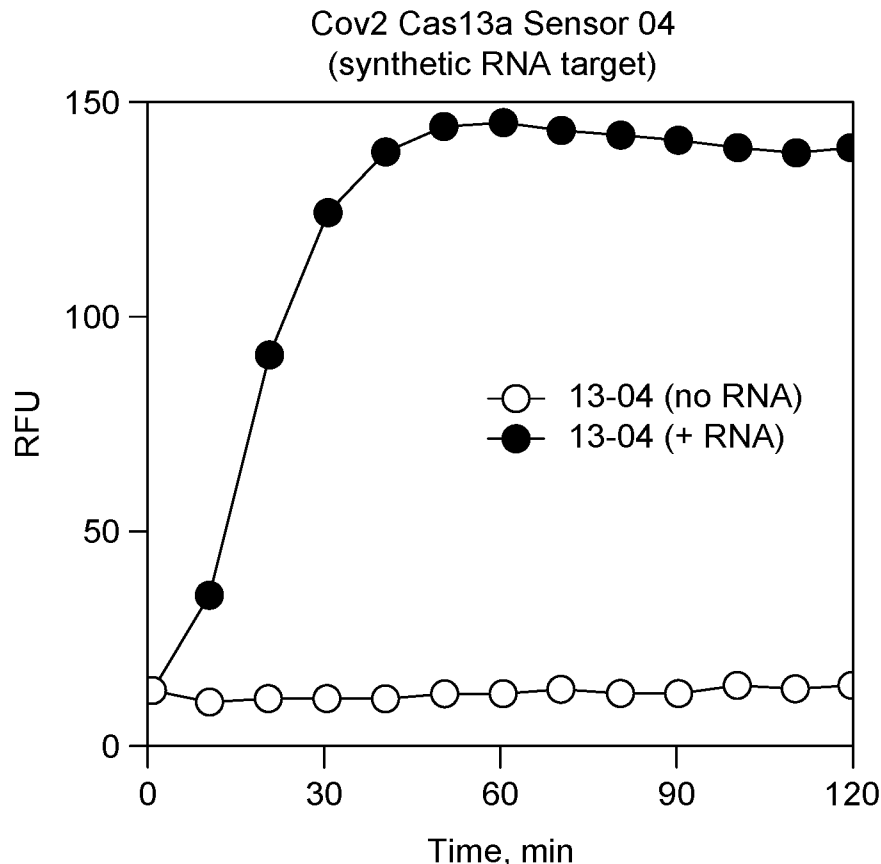


FIG. 36D

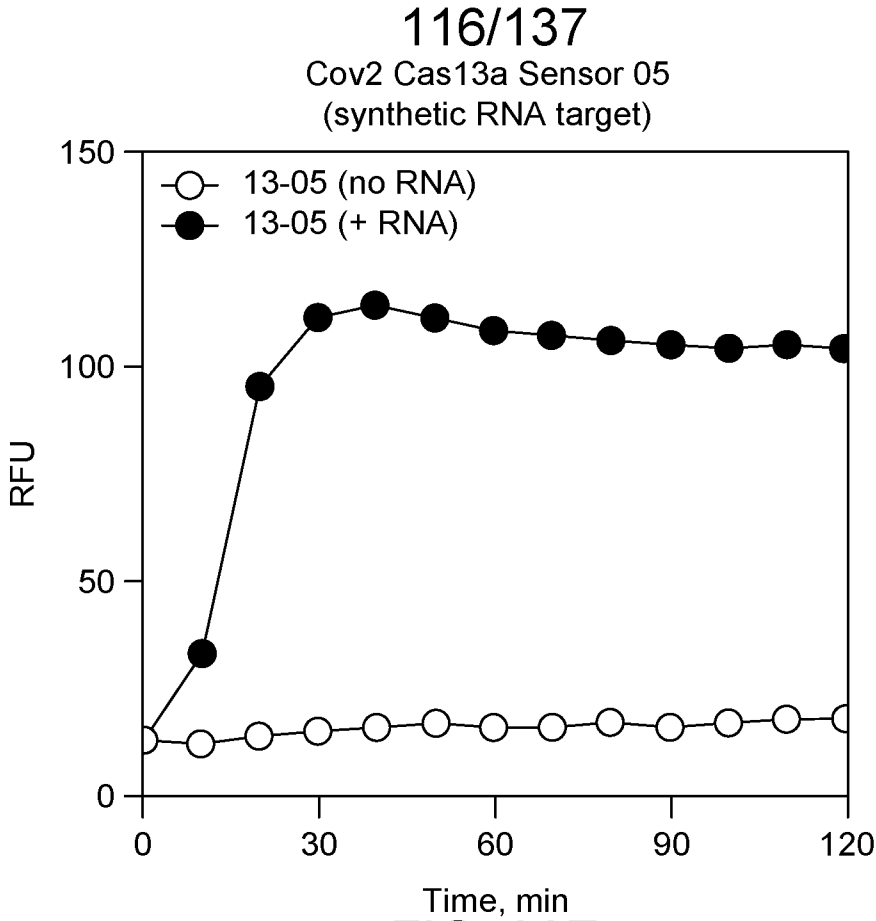


FIG. 36E

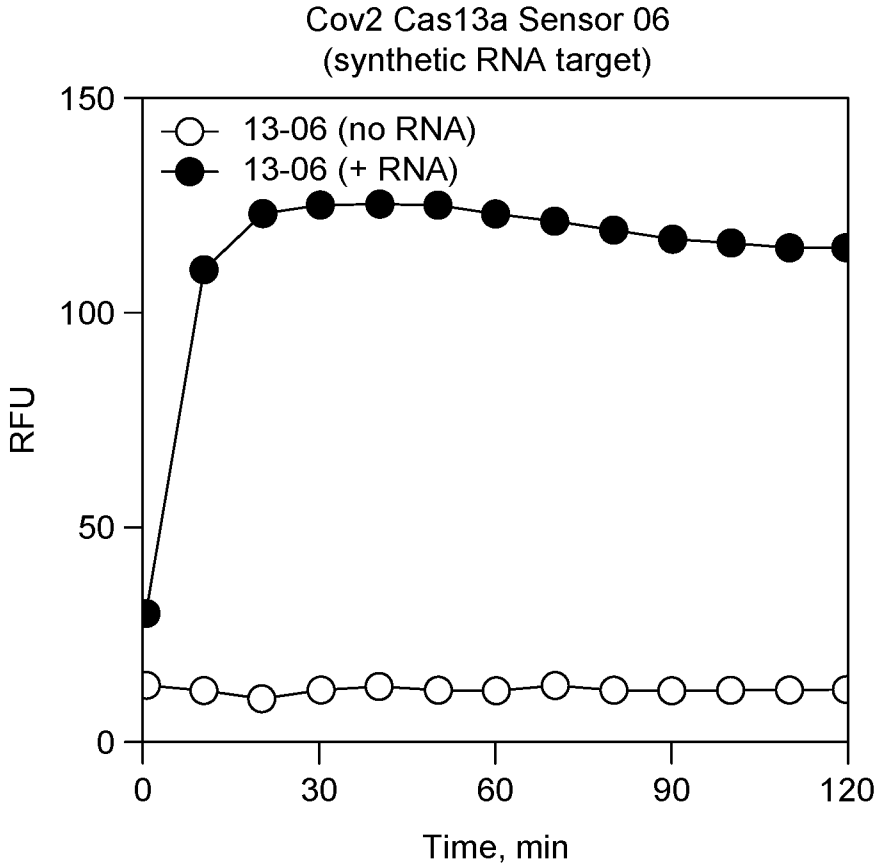


FIG. 36F

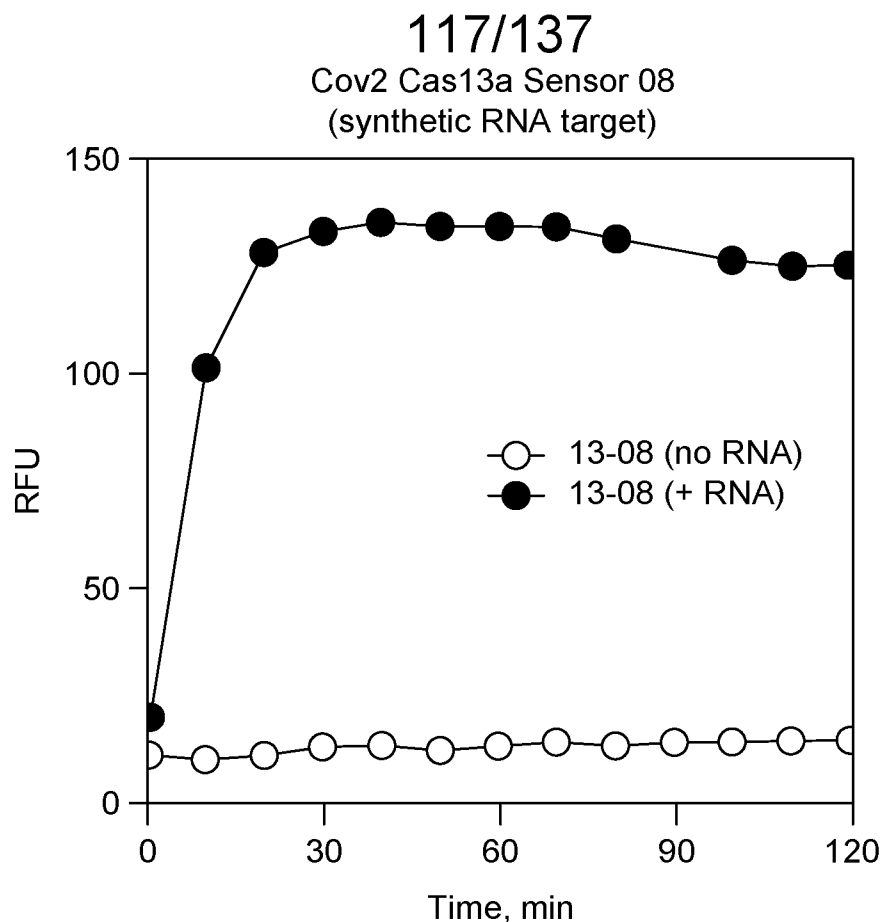


FIG. 36G

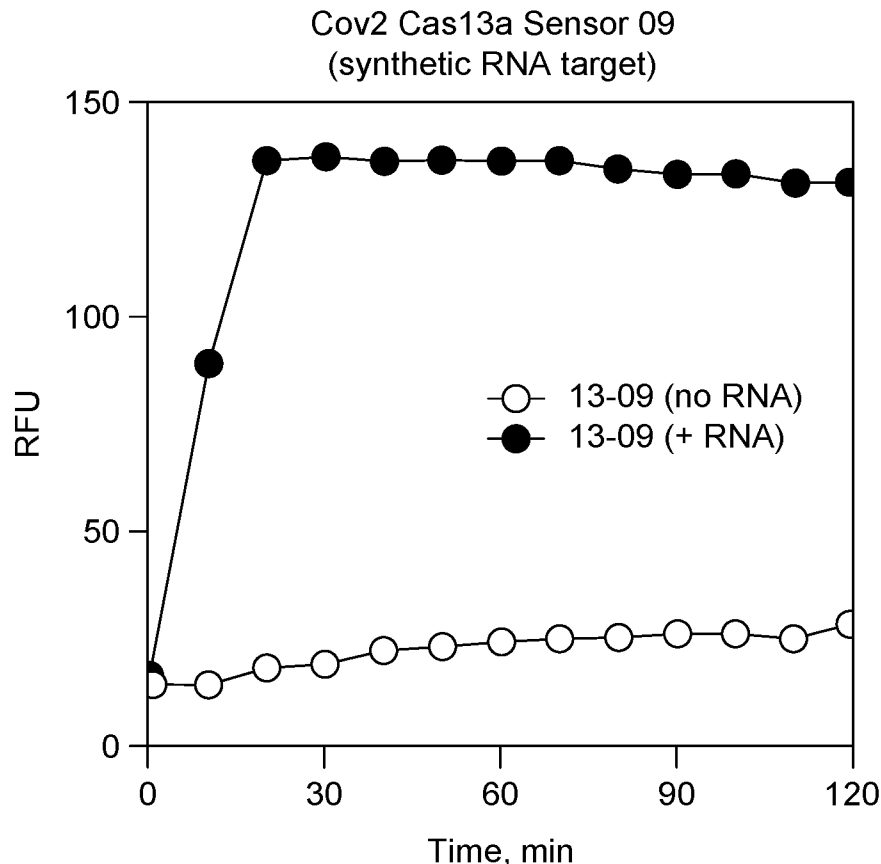


FIG. 36H

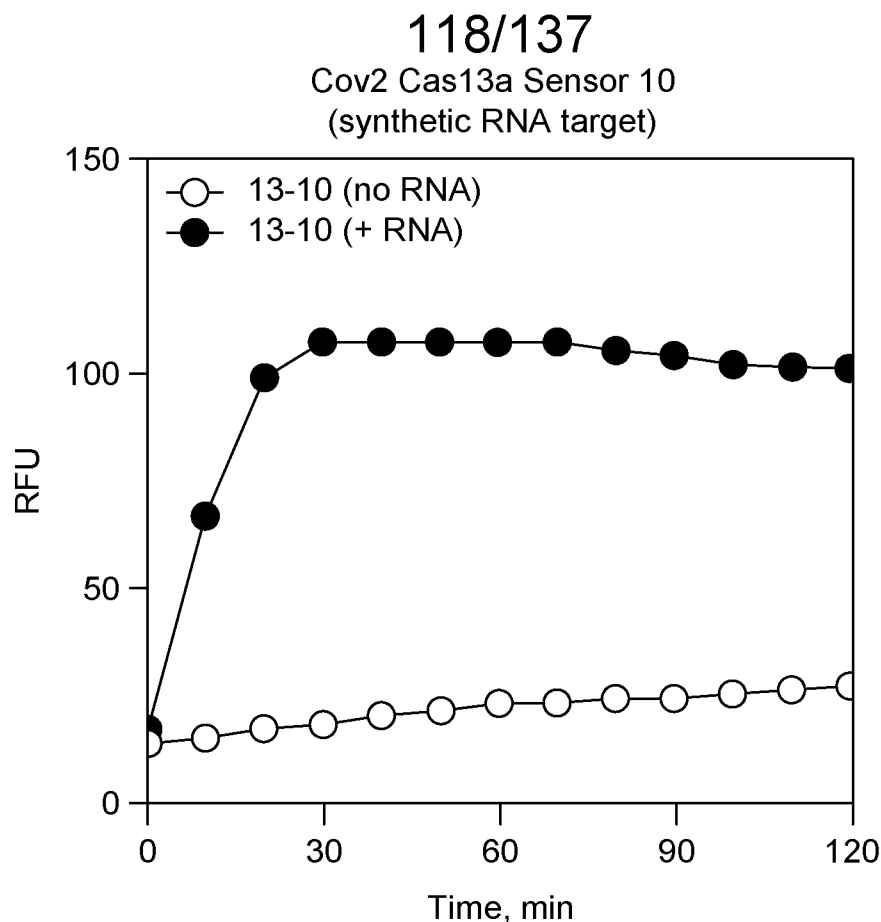


FIG. 36I

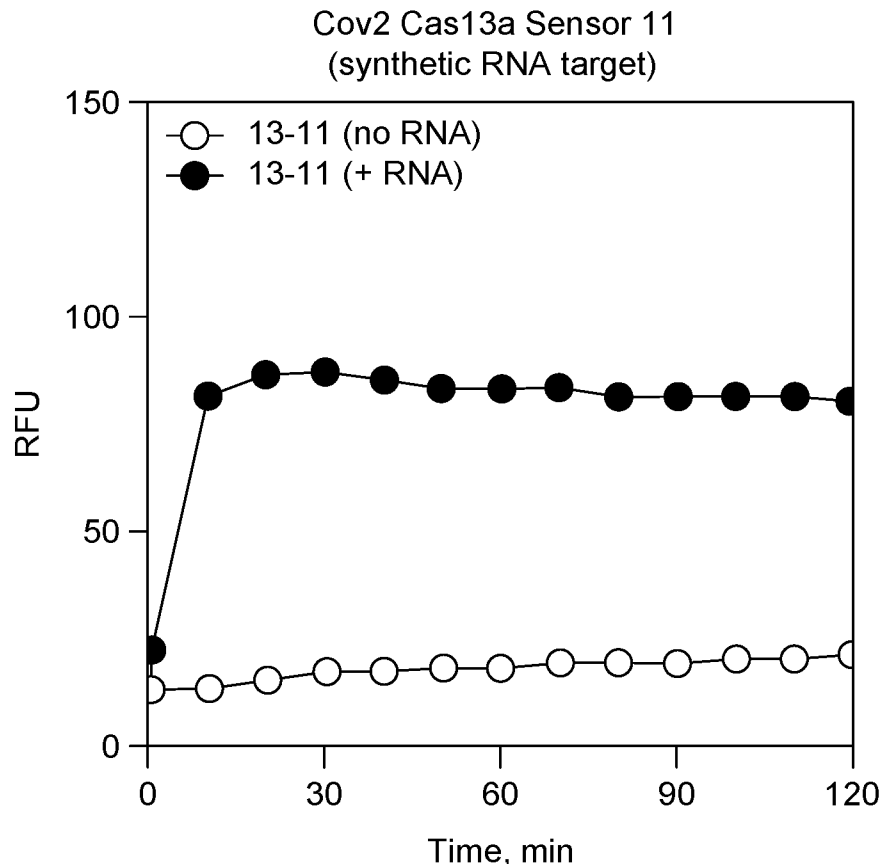


FIG. 36J

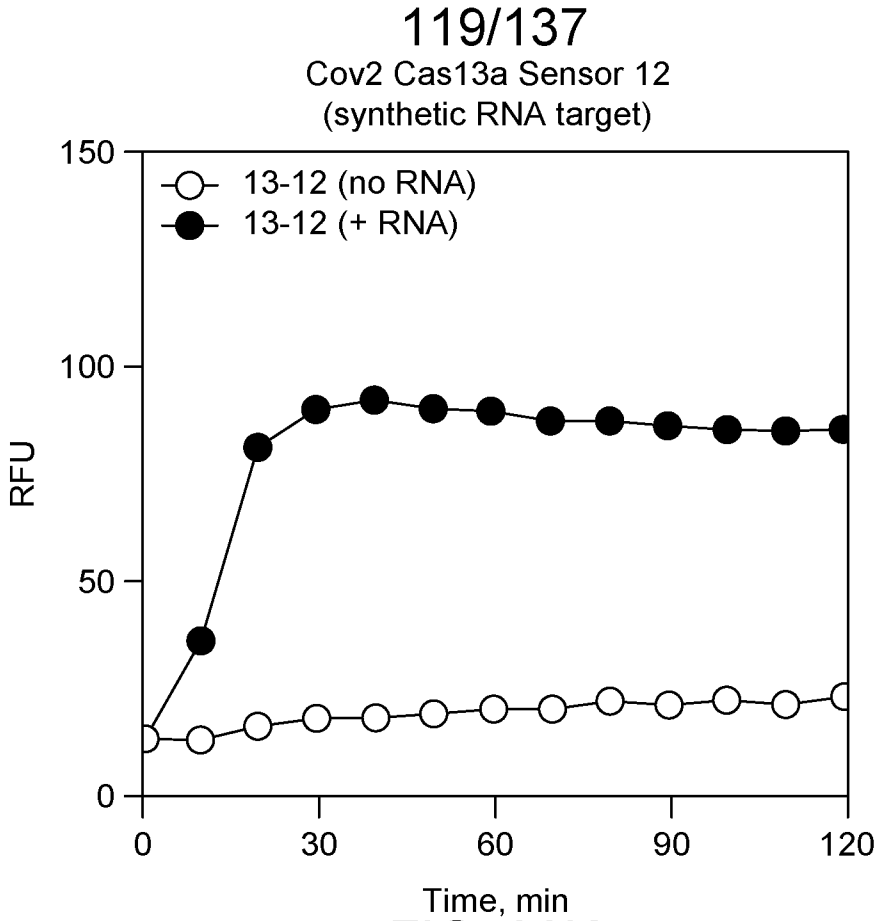


FIG. 36K

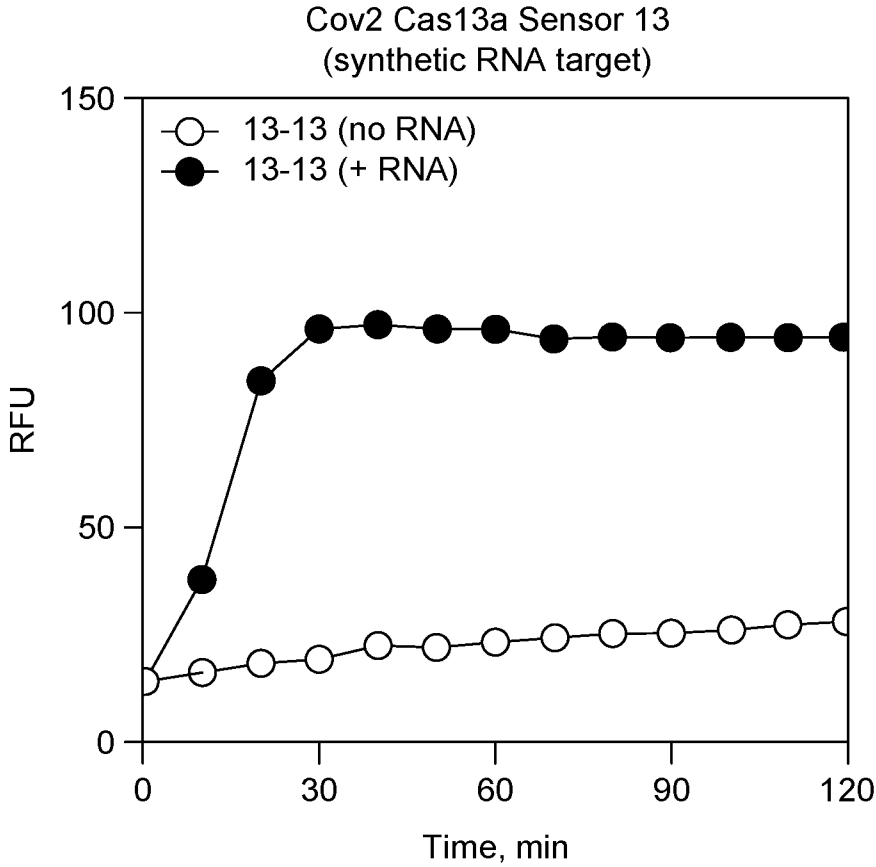


FIG. 36L

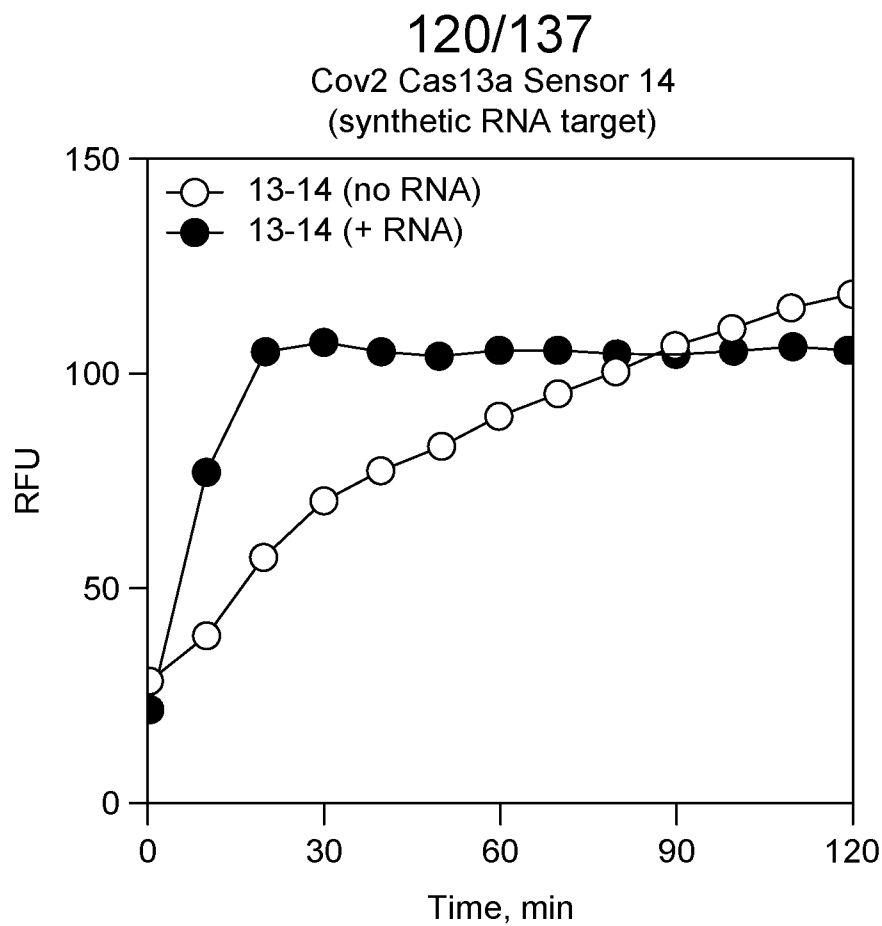


FIG. 36M

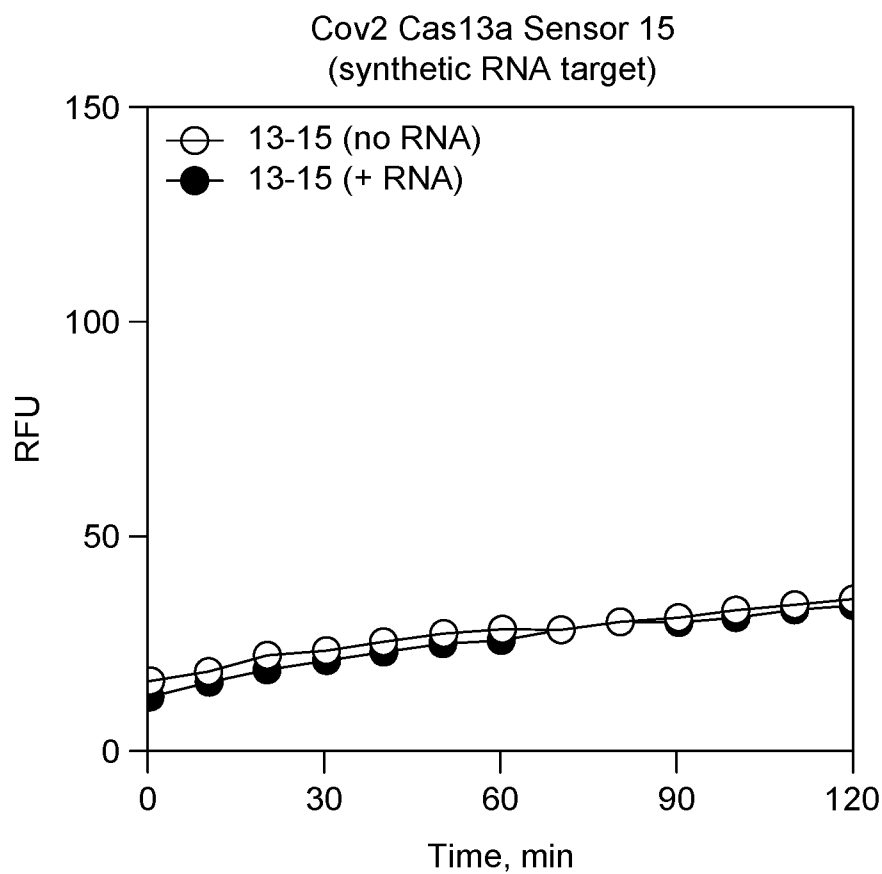


FIG. 36N

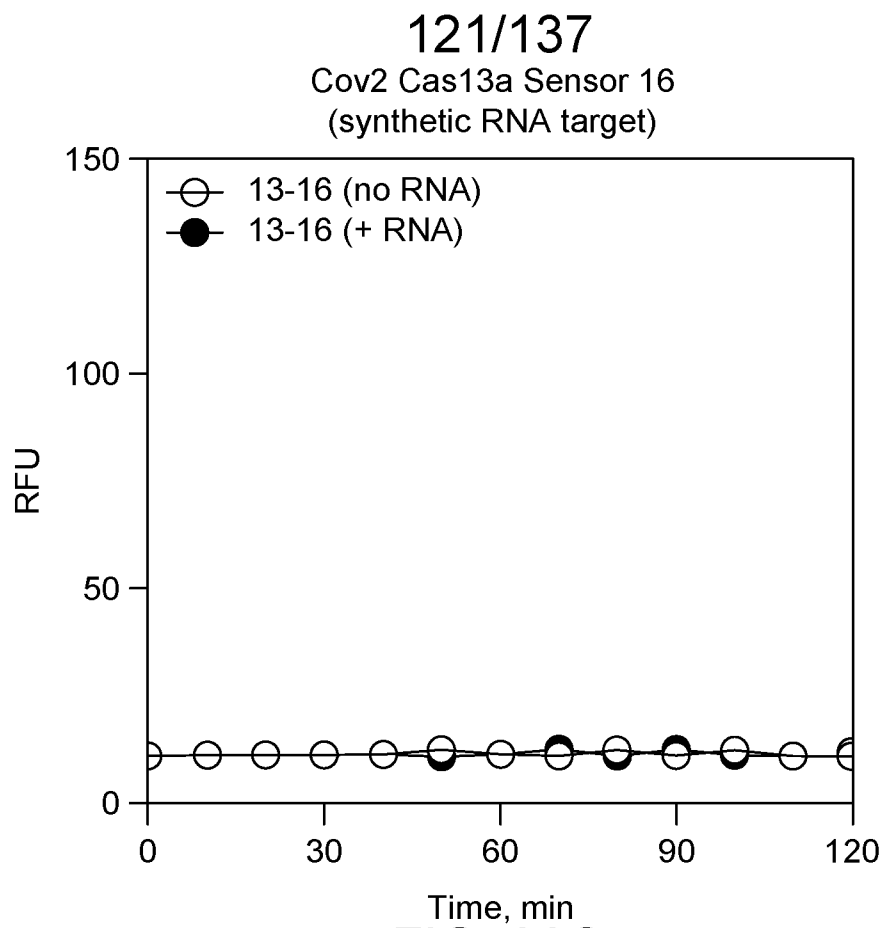


FIG. 36O

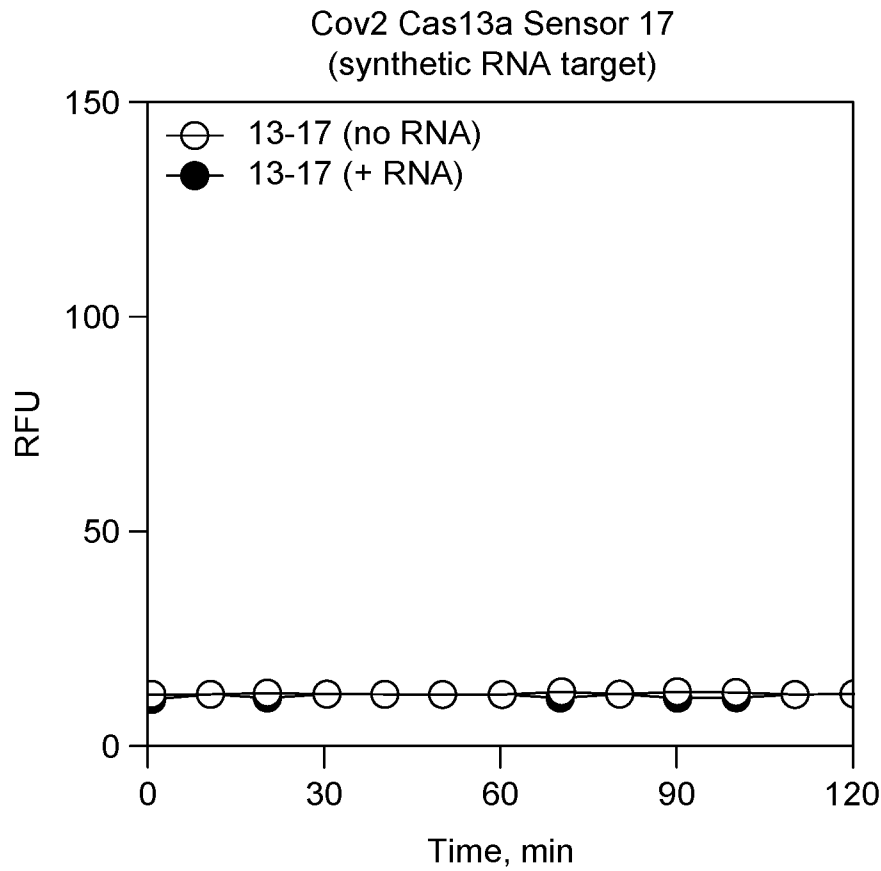


FIG. 36P

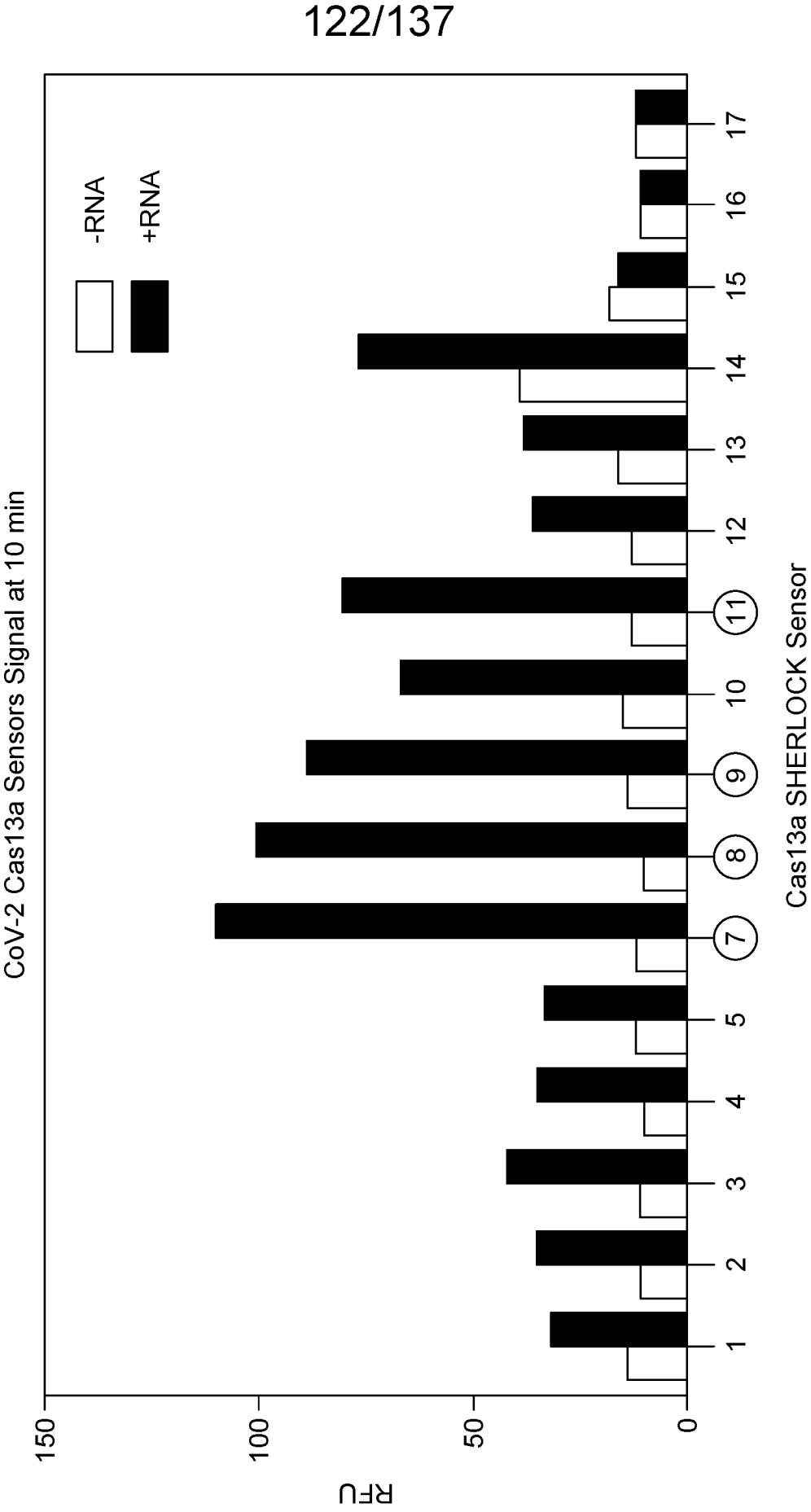


FIG. 36Q

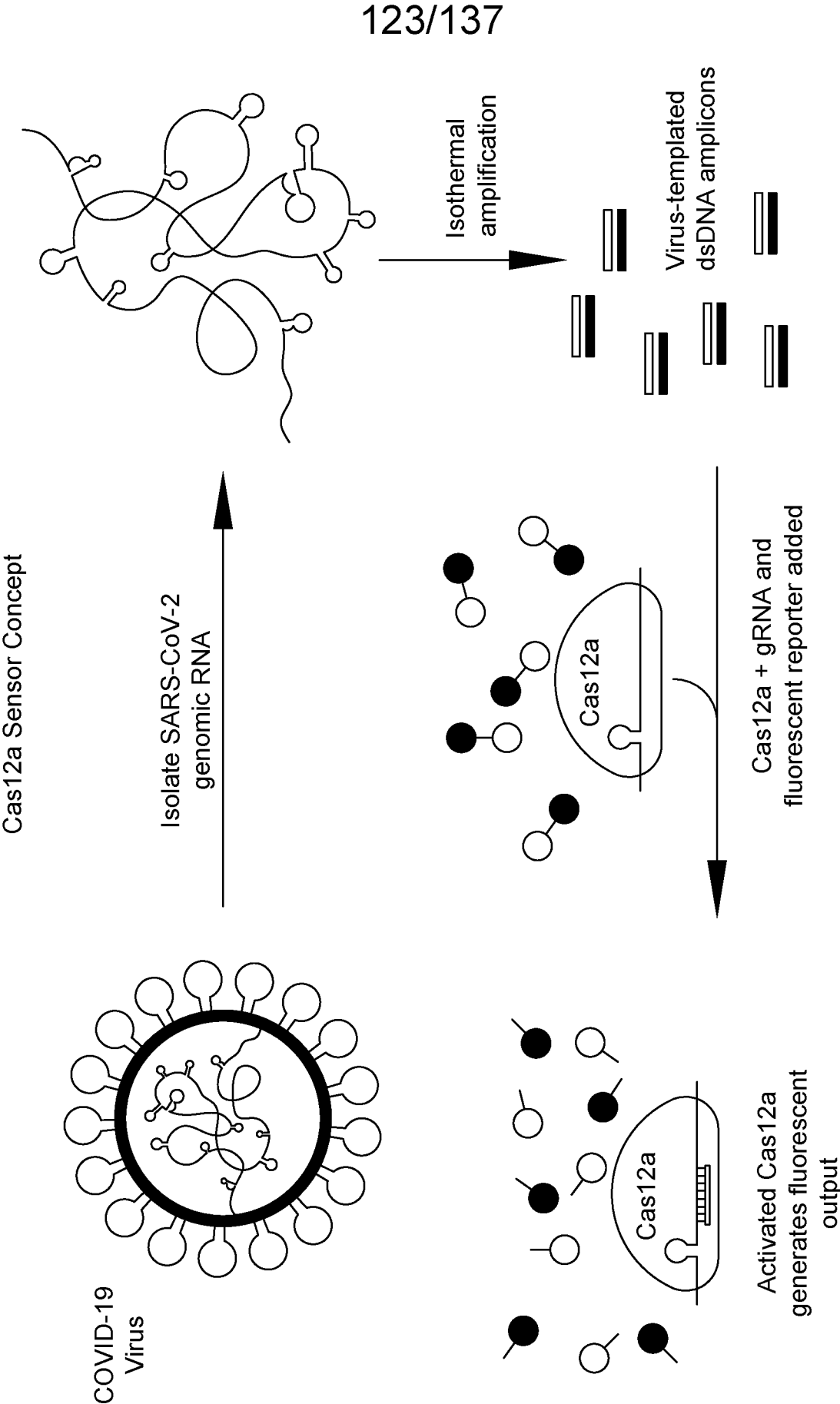


FIG. 37A

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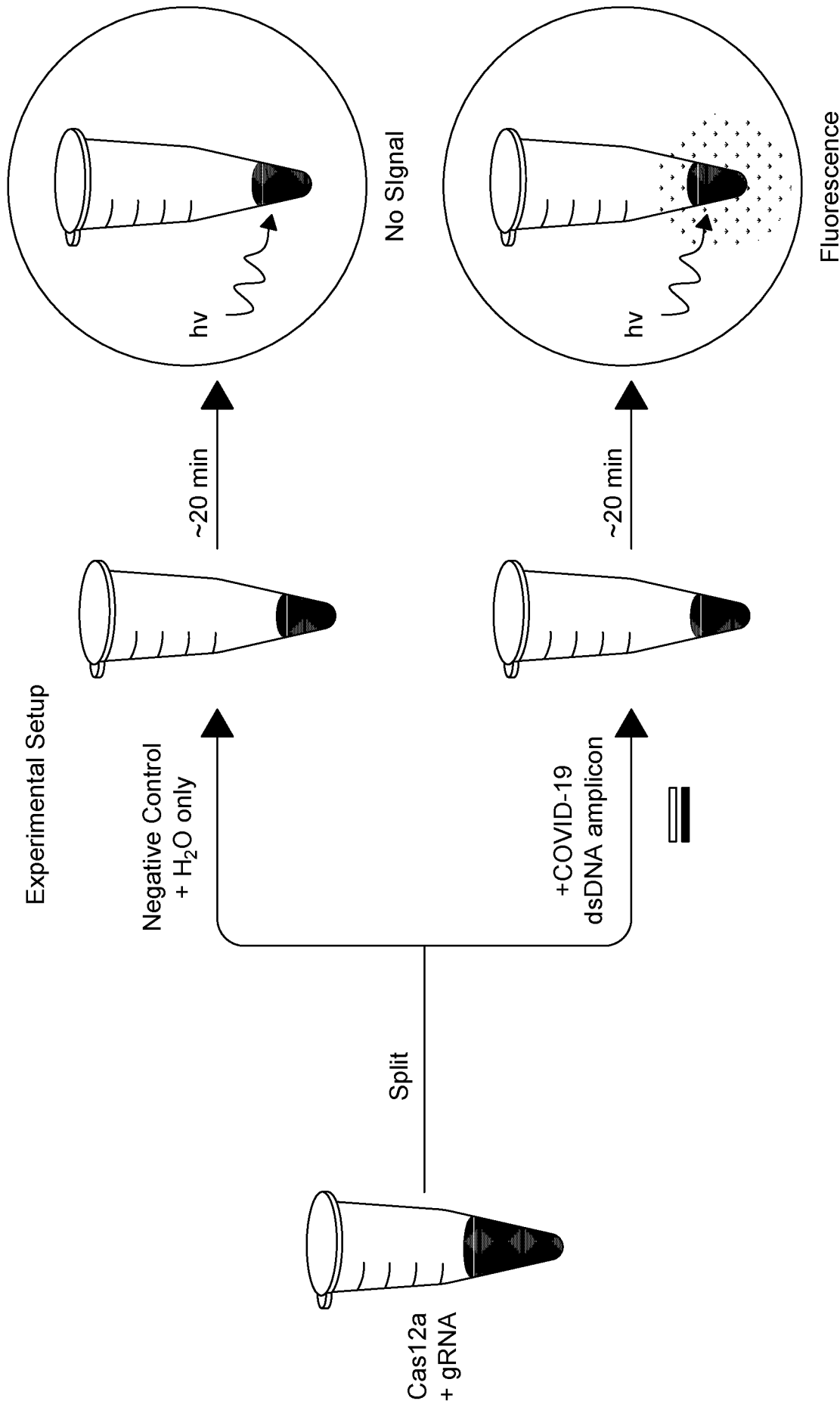


FIG. 37B

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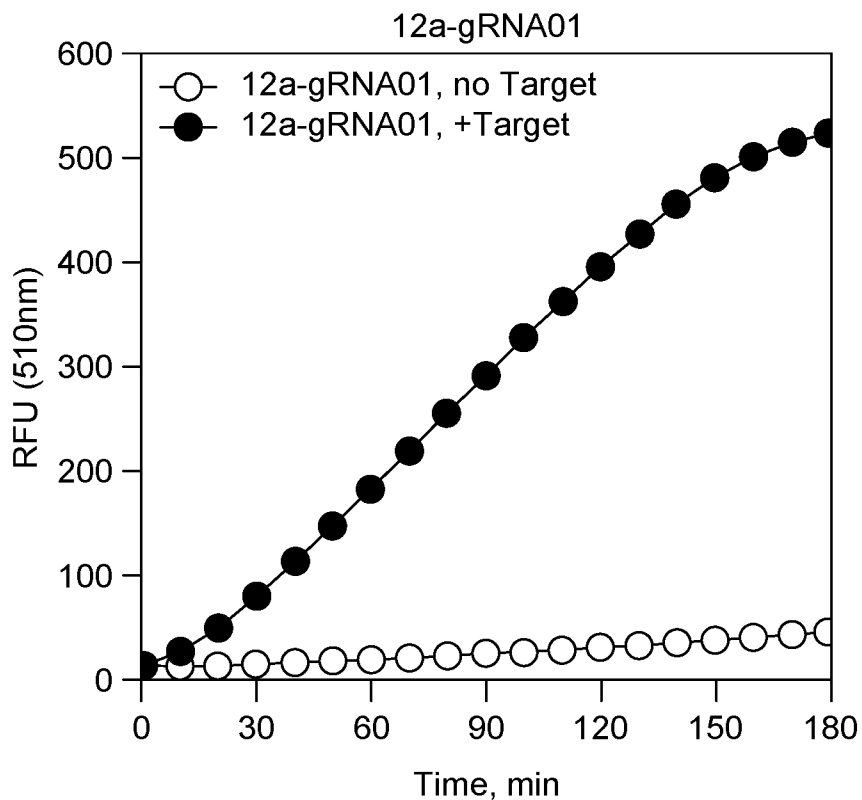


FIG. 37C

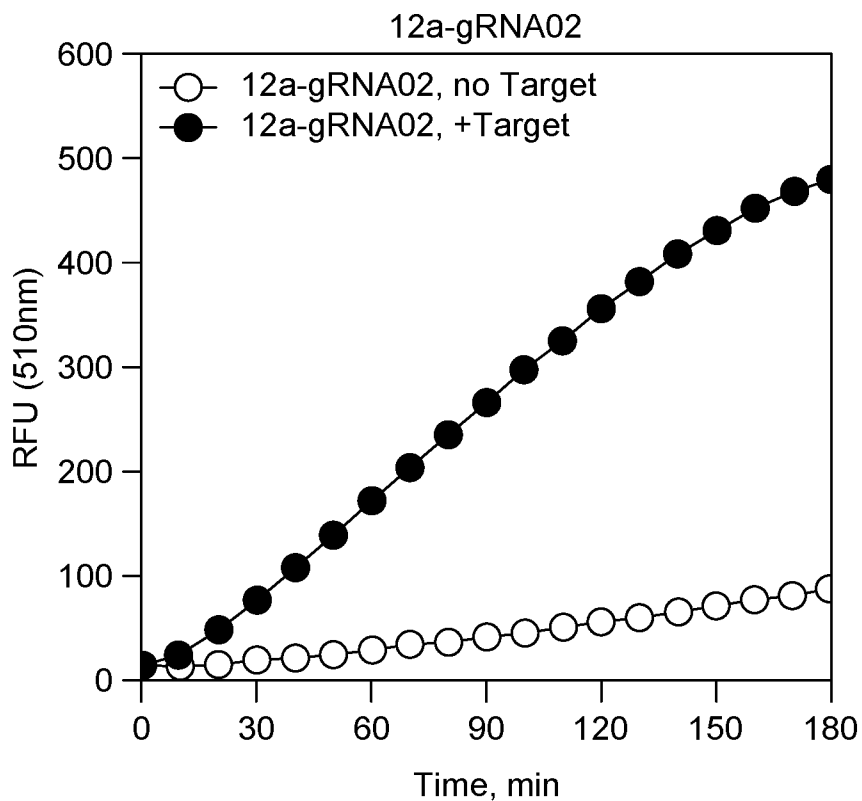


FIG. 37D

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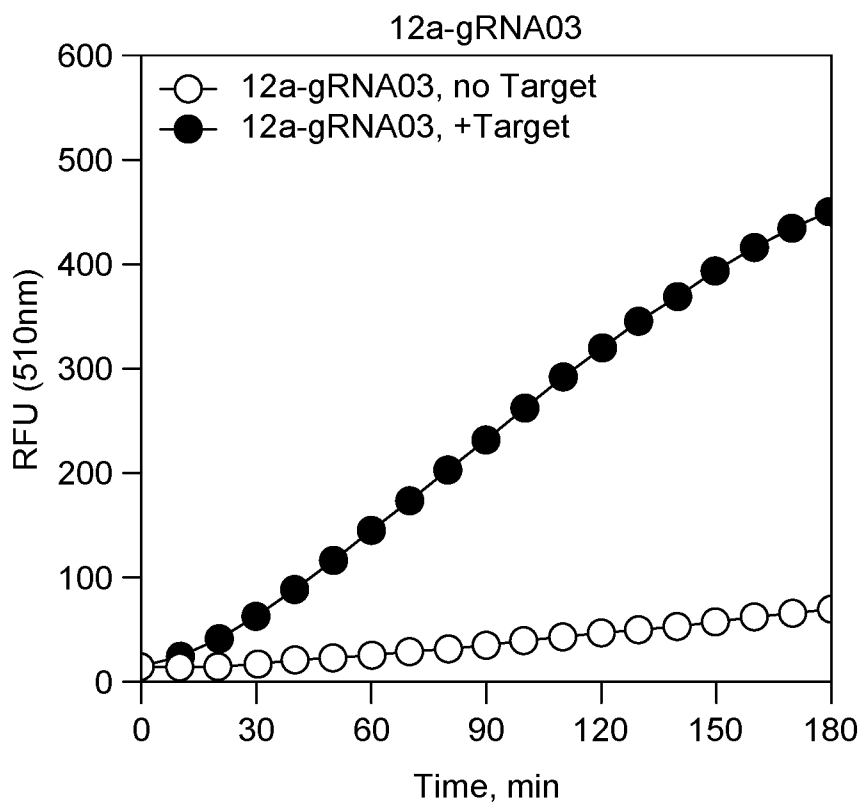


FIG. 37E

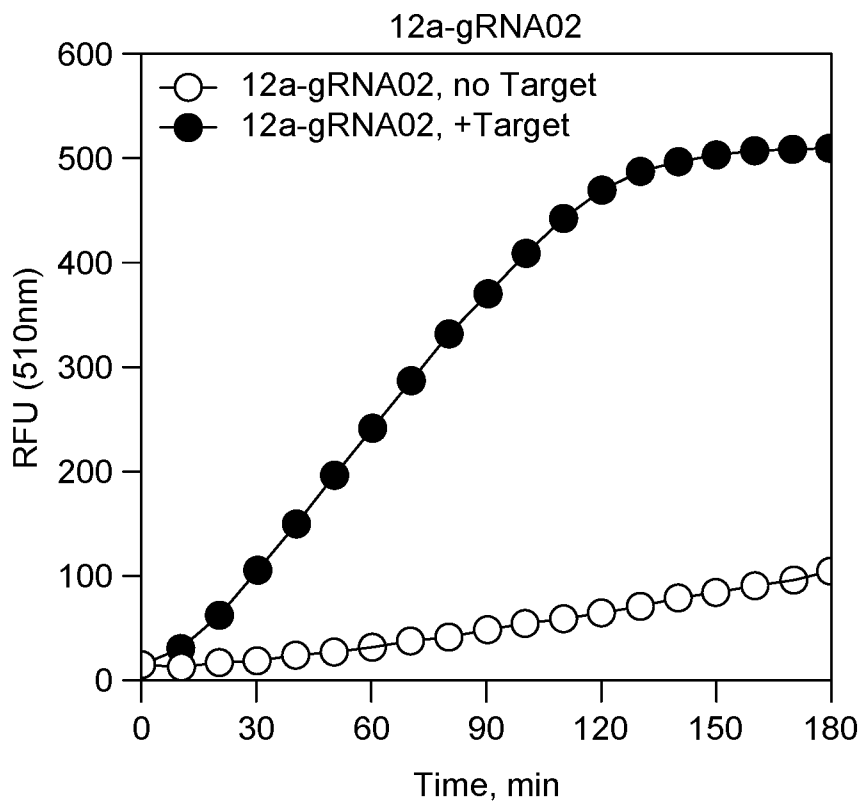


FIG. 37F

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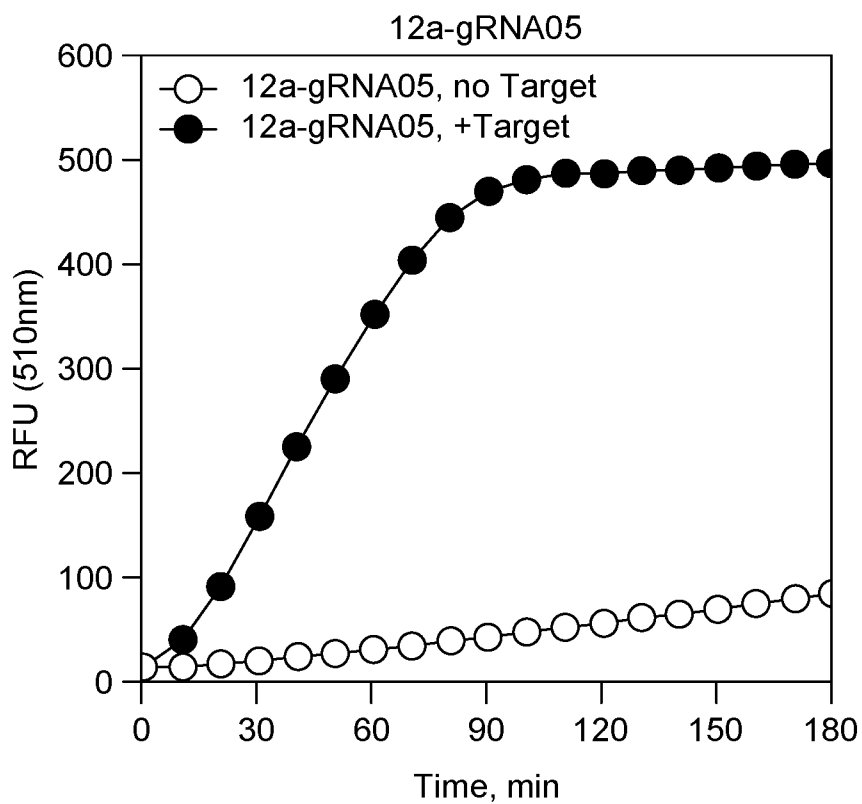


FIG. 37G

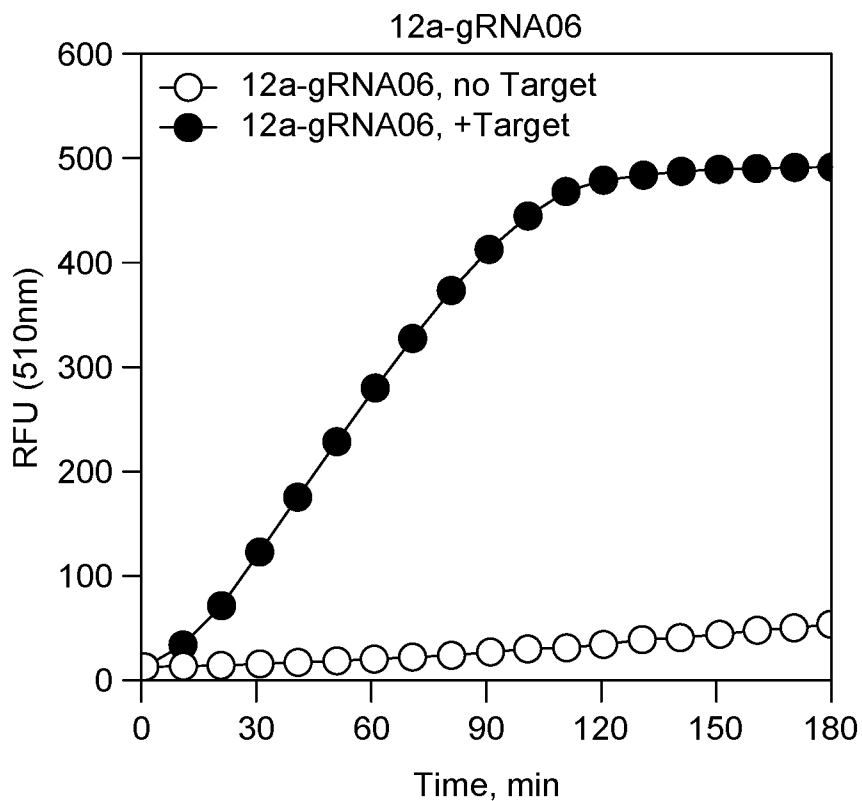


FIG. 37H

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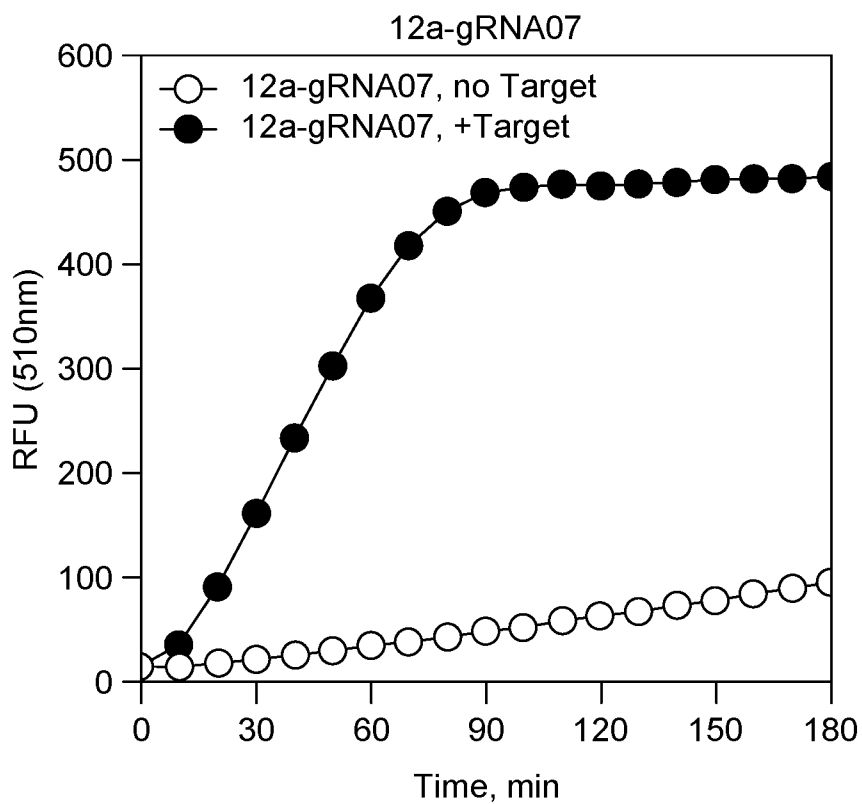


FIG. 37I

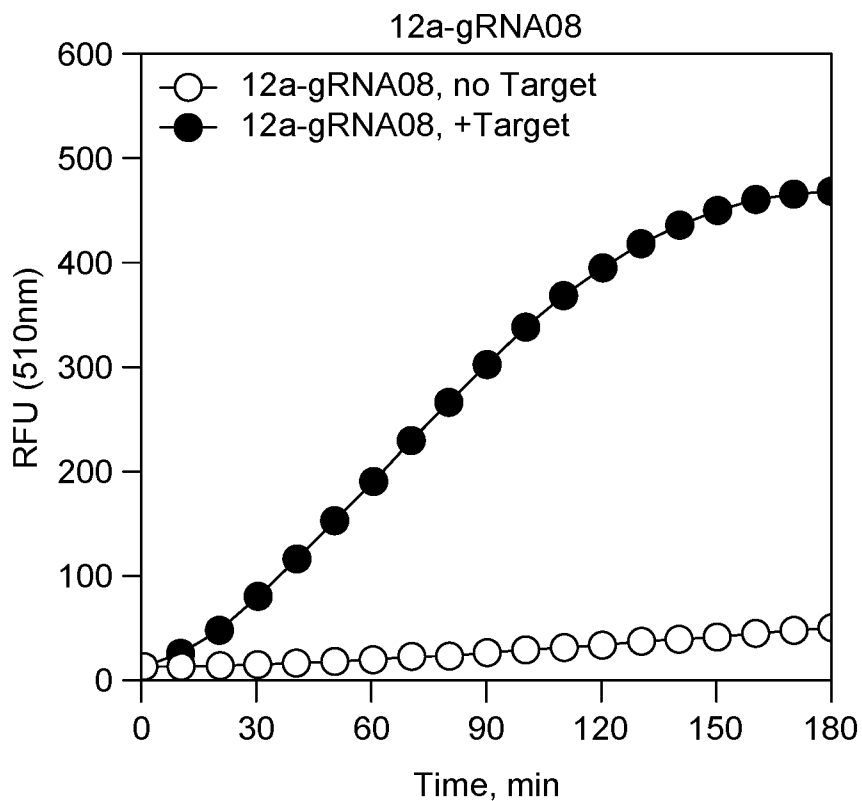


FIG. 37J

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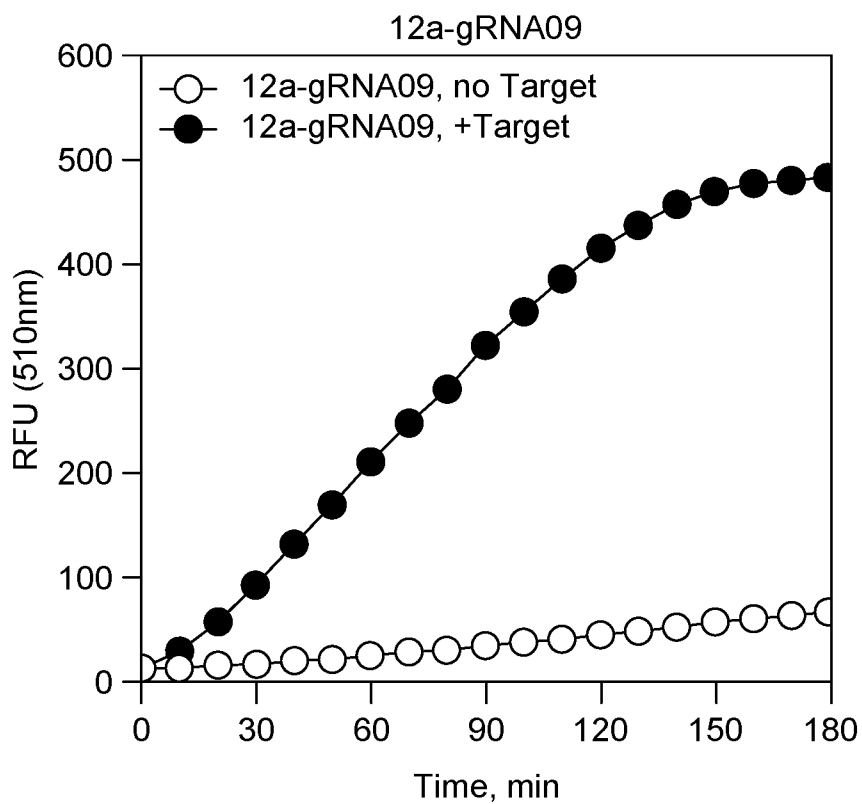


FIG. 37K

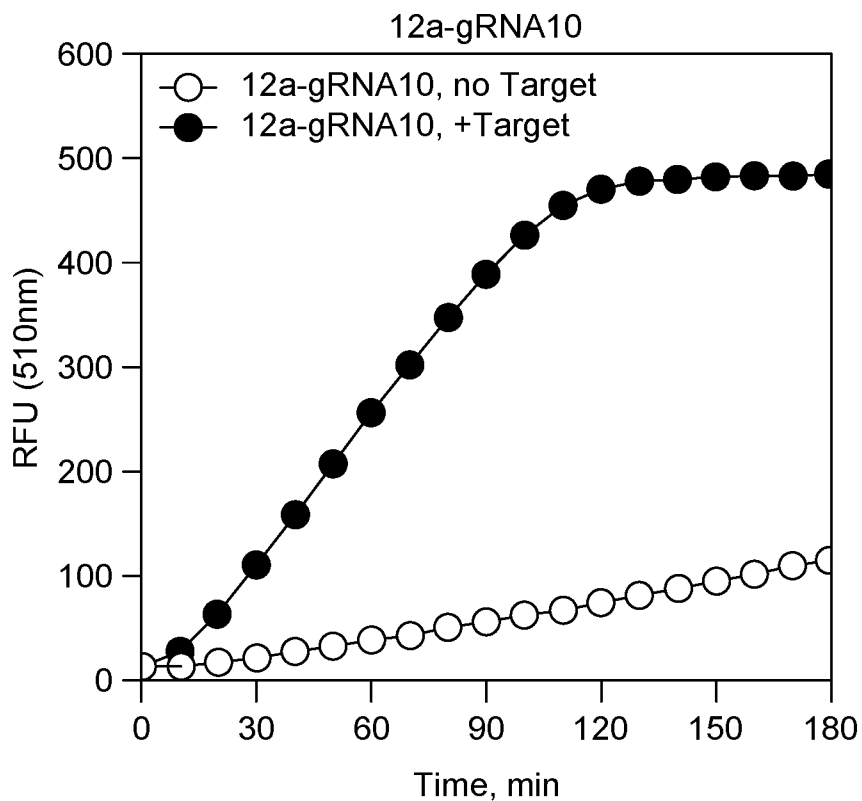
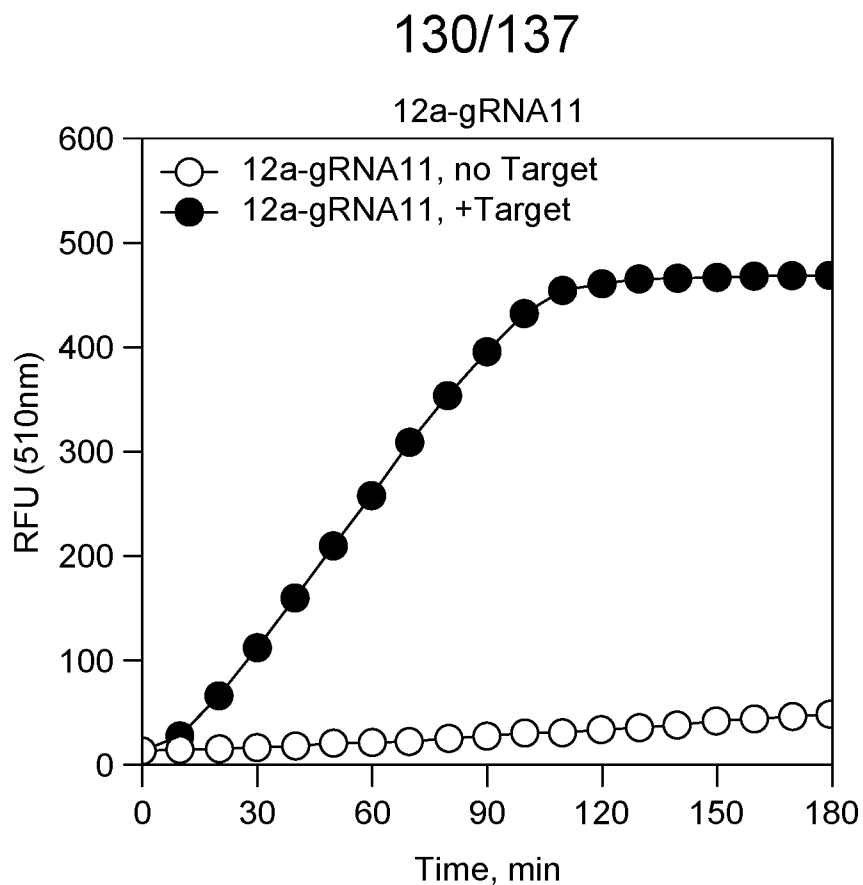
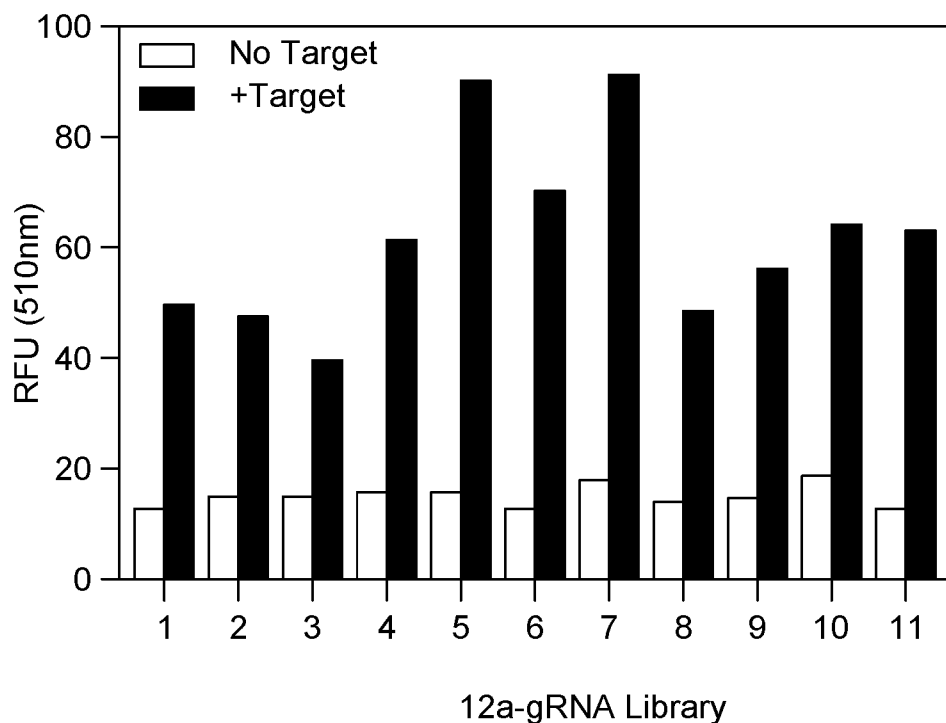


FIG. 37L

**FIG. 37M**

COVID-19 Spike Protein 12a Sensors
Summary - Signal at 20 min

**FIG. 37N**

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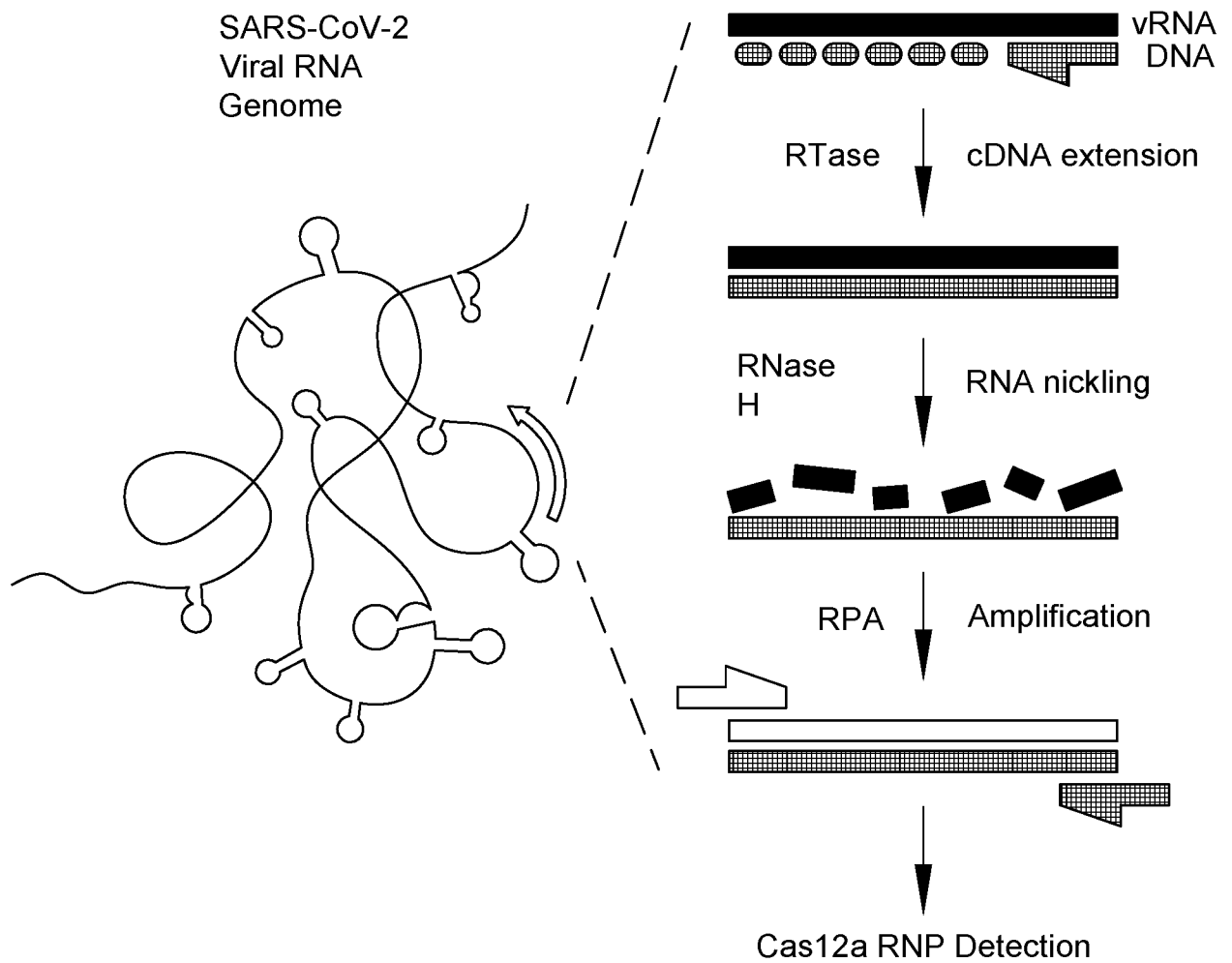
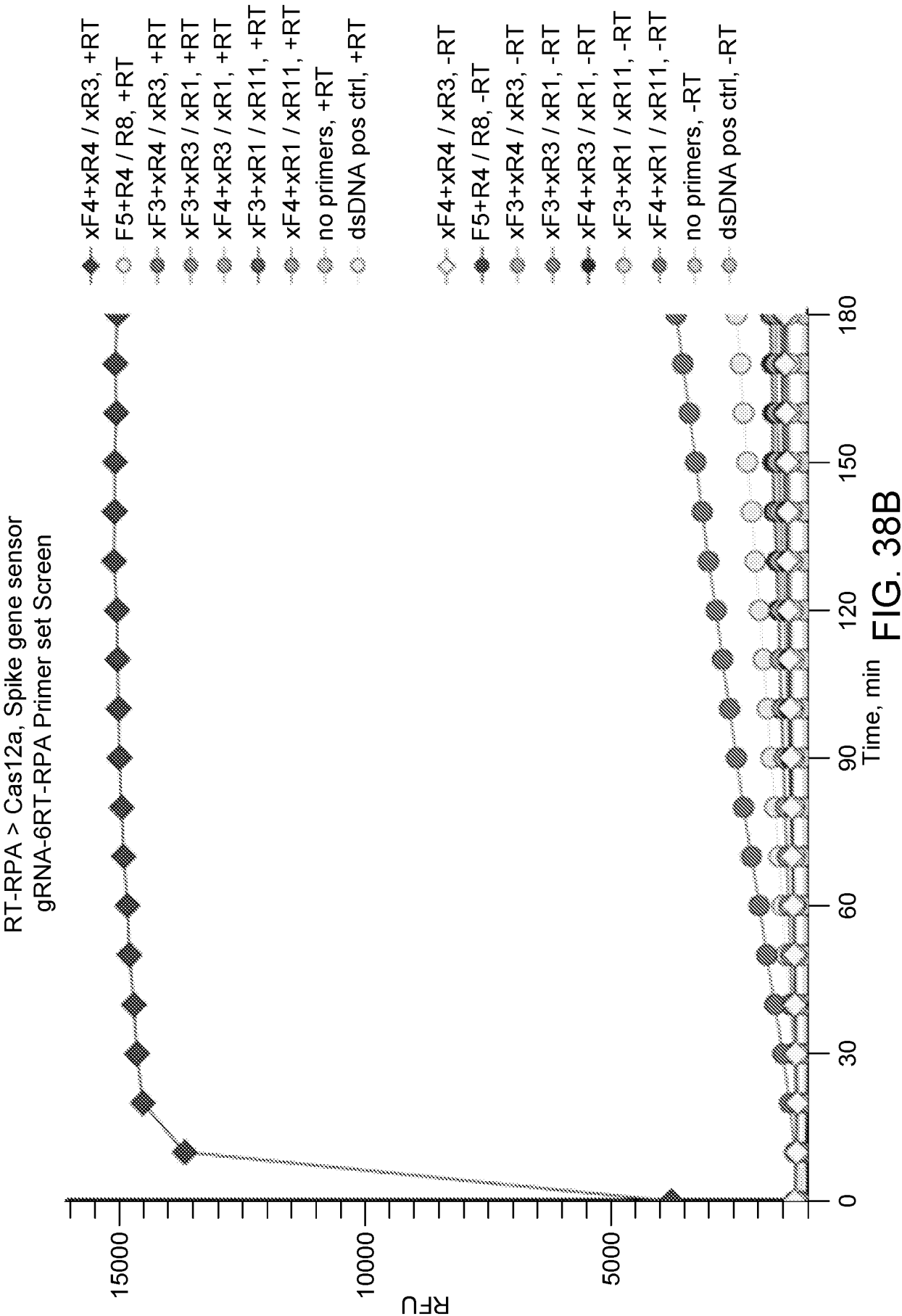


FIG. 38A



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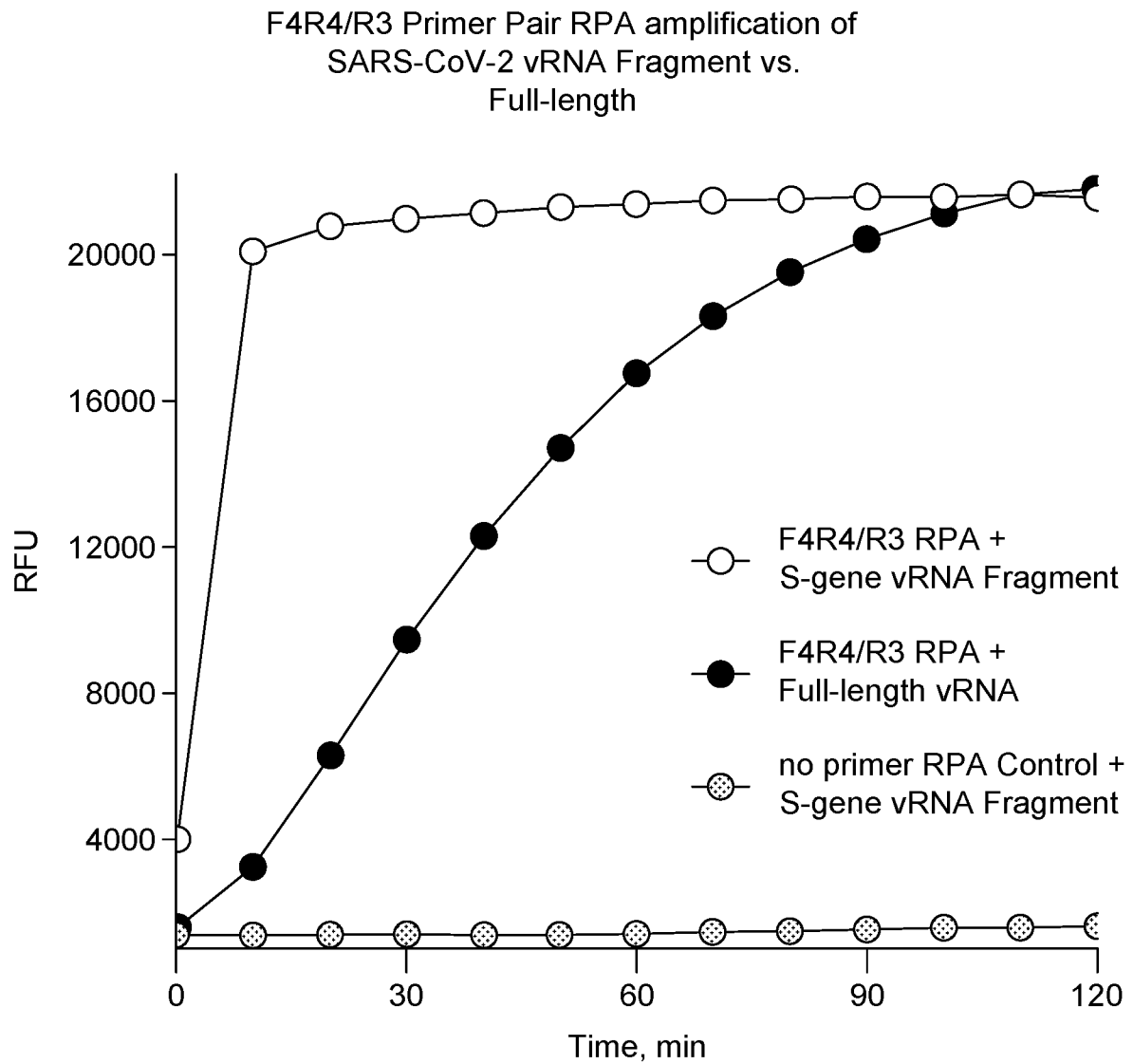


FIG. 38C

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F4R4/R3 Primer RPA Concentration Optimization

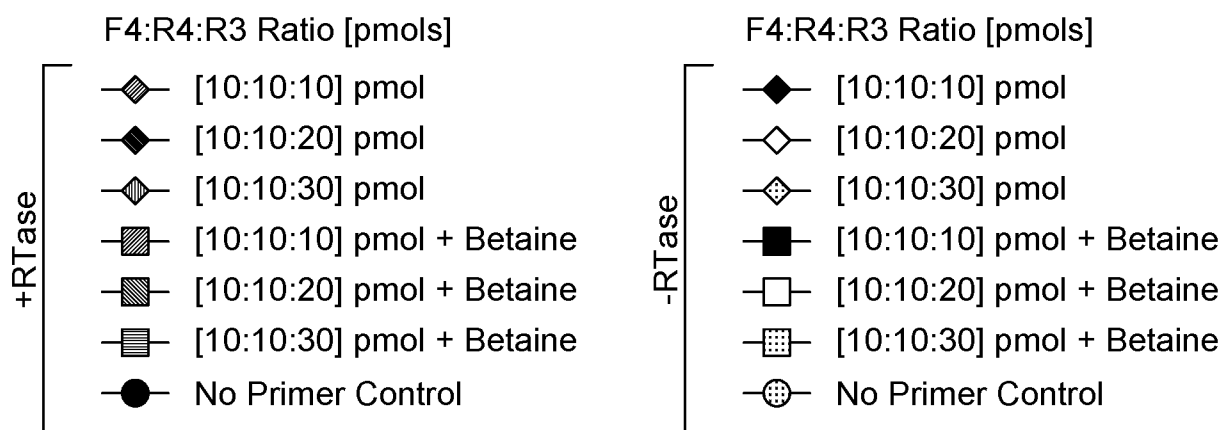
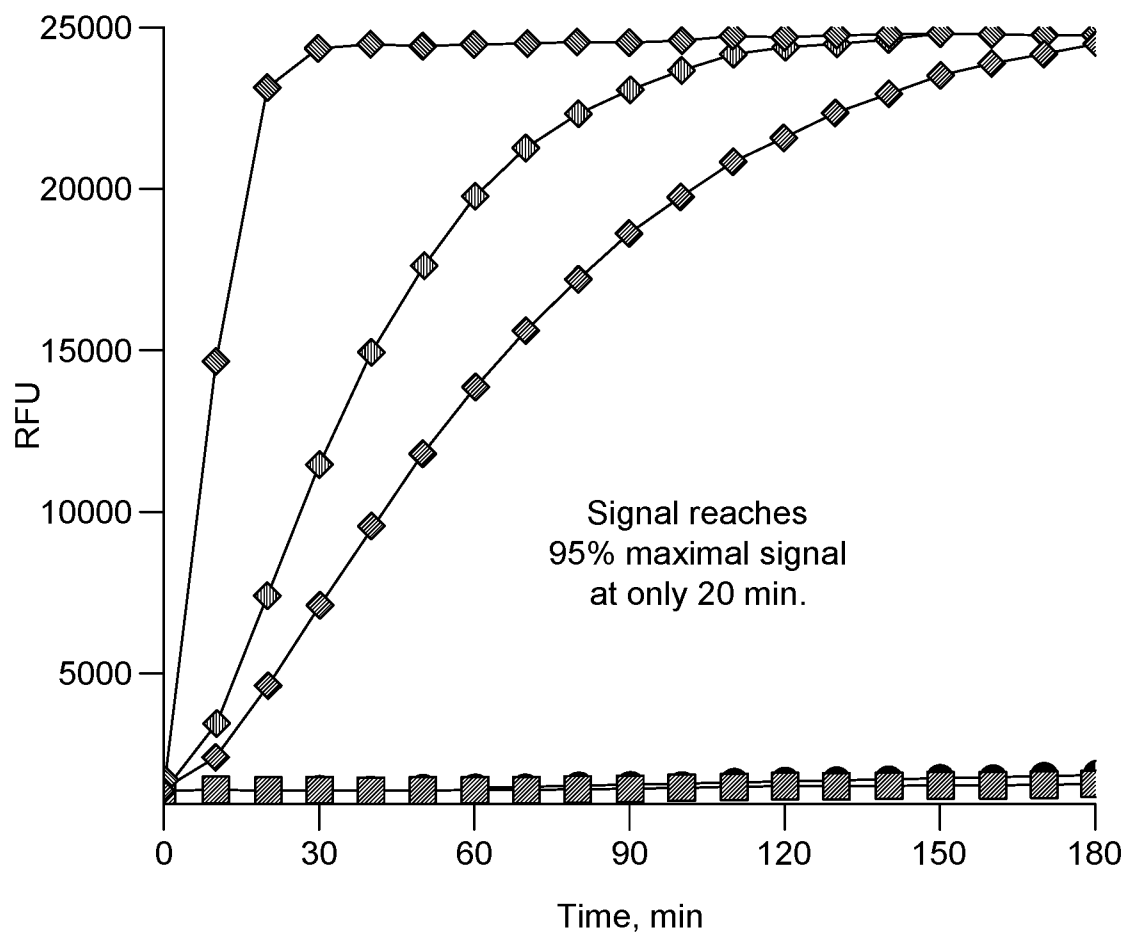


FIG. 38D

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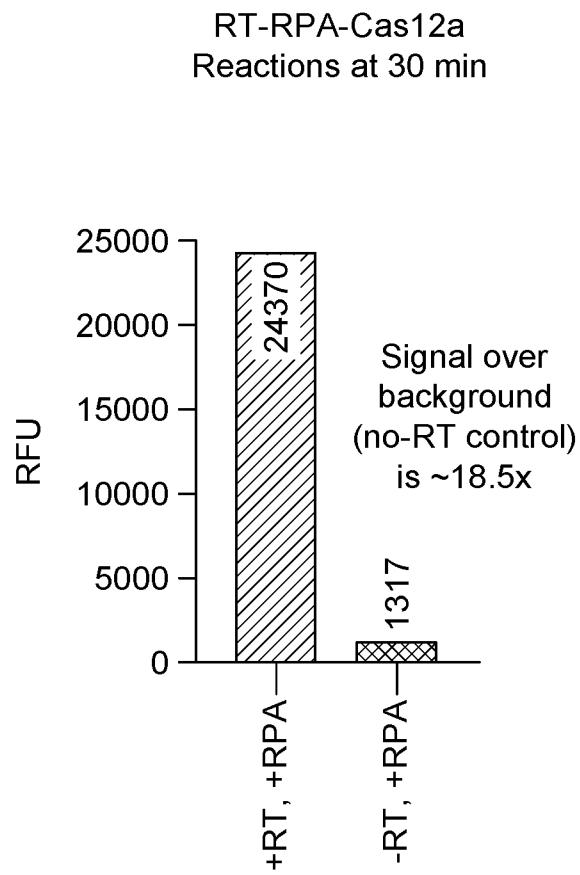


FIG. 38E

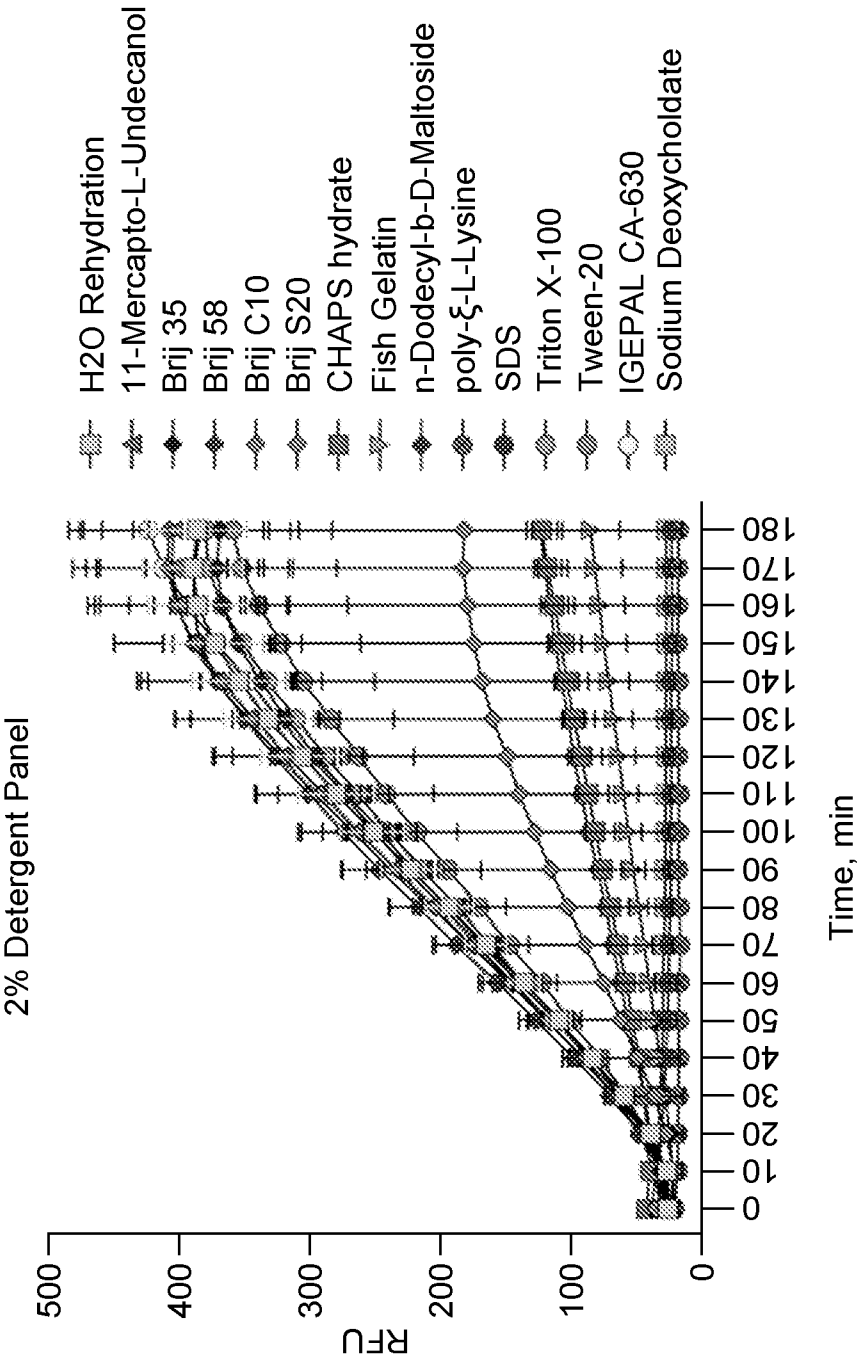


FIG. 39A

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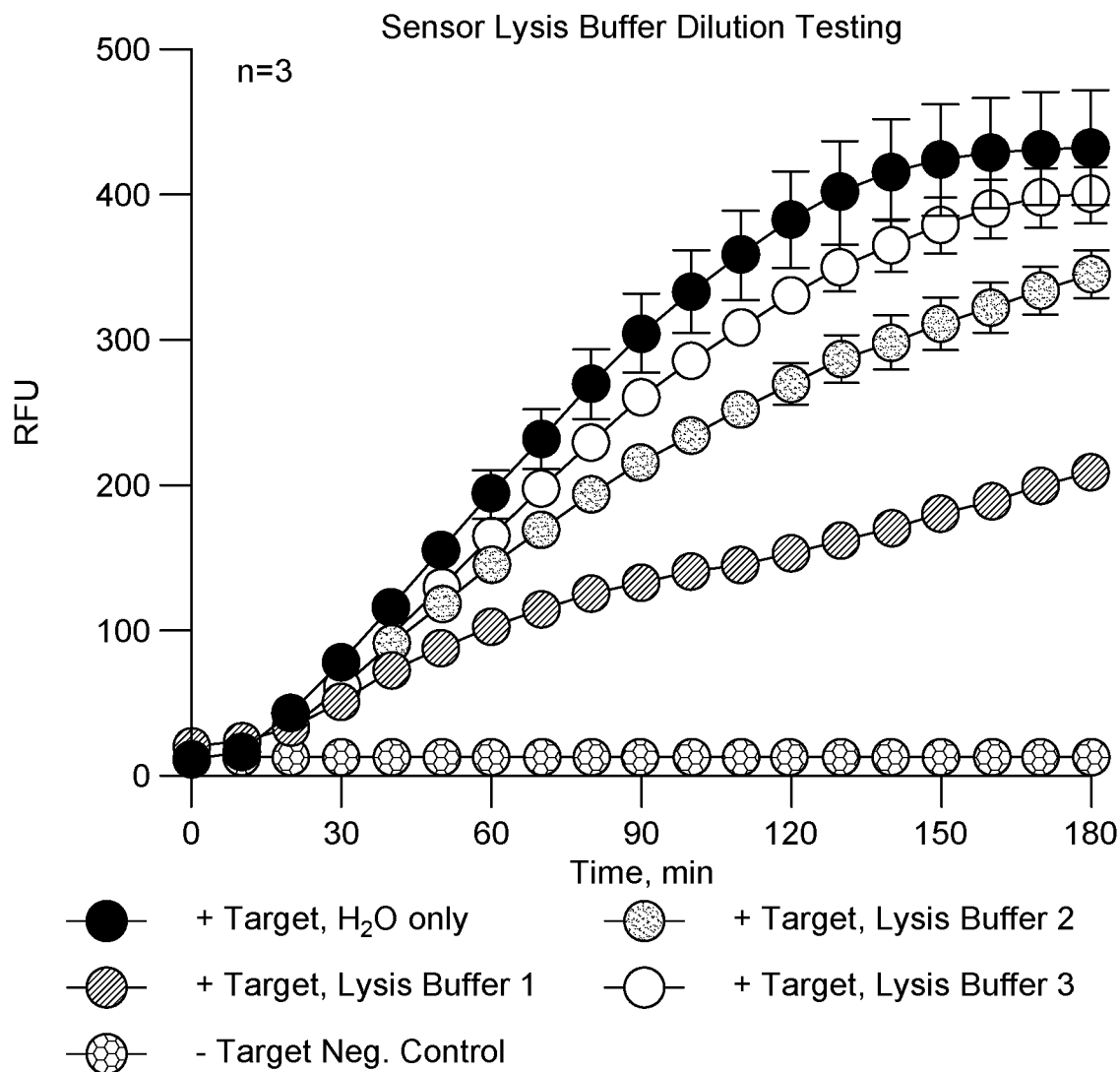


FIG. 39B

Lysis Buffer 2	Lysis Buffer 2B
10 mM Tris, pH 8	10 mM Tris, pH8
1% Triton X-100	0.02% NP-40
1% NP-40	2% CHAPS
0.2% CHAPS	
5% Sucrose	5% Sucrose
0.1% mg/mL Lysozyme*	
* add from fresh stock	

FIG. 39C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/58352

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 5/01; A62B 7/10; G01D 21/00 (2022.01)

CPC - A62B 18/025; A62B 23/025

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 8,240,187 B2 (Colman et al.) 14 August 2012 (14.08.2012) Entire document, especially Abstract, col 5 ln 18-23, 49-56; col 6 ln 3-6, 15-27, 50-55; col 7 ln 61-66	1-4, 8 ----- 5-6
Y --- A	US 2016/0312312 A1 (President and Fellows of Harvard College and Trustees of Boston University) 27 October 2016 (27.10.2016) Entire document, especially Abstract, para [0164]-[0165]; [0243]; [0248]; [0250]; [0324]-[0325]	1-4, 8 ----- 5-6
A	US 2011/0312724 A1 (Facer et al.) 22 December 2011 (22.12.2011) Entire document	1-6, 8
A	US 7,549,349 B2 (Swank et al.) 23 June 2009 (23.06.2009) Entire document	1-6, 8
A	US 2016/0022946 A1 (Deton Corp.) 28 January 2016 (28.01.2016) Entire document	1-6, 8
A	US 6,221,026 B1 (Phillips) 24 April 2001 (24.04.2001) Entire document	1-6, 8

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

05 January 2022

Date of mailing of the international search report

MAR 02 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/58352

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7, 9-50, 56-67, 72-77
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

-- See Extra Sheet --

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 8

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/58352

Continuation of:

Box III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-6 and 8 directed to an aqueous solution activated detection device, the detection device comprising: a carrier fluid reservoir; a sample collection unit; a sample processing unit including dried synthetic biological components; and a detection unit.

Group II: Claims 51-55 directed to a method of making an aqueous solution activated detection device, the method comprising: connecting in series a carrier fluid reservoir, a sample collection unit, a sample processing unit, and a detection unit; wherein an outlet from the carrier fluid reservoir is connected to an inlet of the sample collection unit, an outlet from the sample collection unit is connected to an inlet of the sample processing unit, and an outlet of the sample processing unit is connected to an inlet of the detection unit, and wherein the sample processing unit includes dried synthetic biological components.

Group III: Claims 68-71 directed to a device for capturing an airborne agent comprising: a sample collection chamber for capturing breath from a subject, the airborne agent contained in the breath from the subject; and a covering coupled to the sample collection chamber, the covering having a sample collection unit with a porous material.

Group IV: Claims 78-81 directed to a device for processing a sample comprising: two or more reaction zones coupled in sequence to each other; and one or more time delay barriers separating each pair of the two or more reaction zones.

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group II requires a method of making an aqueous solution activated detection device, the method comprising: connecting in series a carrier fluid reservoir, a sample collection unit, a sample processing unit, and a detection unit, not specifically required by Groups I or III-IV.

Group III requires a device for capturing an airborne agent comprising: a sample collection chamber for capturing breath from a subject, not specifically required by Groups I-II or III.

Group IV requires a device for processing a sample comprising: one or more time delay barriers separating each pair of the two or more reaction zones, not specifically required by Groups I-III.

Common Technical Features:

Groups I and II share the technical feature of an aqueous solution activated detection device, the detection device comprising: a carrier fluid reservoir; a sample collection unit; a sample processing unit including dried synthetic biological components; and a detection unit.

-- See Extra Sheet --

Continuation of:

Box III Observations where unity of invention is lacking

However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is obvious over US 8,240,187 B2 to Colman et al. (hereinafter 'Colman') in view of US 2016/0312312 A1 to President and Fellows of Harvard College and Trustees of Boston University (hereinafter 'President'). Colman discloses a detection device (Abstract - '...There is provided a breath sampling tube including an inner conduit, wherein at least a portion of the inner conduit is non-cylindrical and adapted to store liquids...'; col 6 ln 15-19 - '...The sampling system (100) is adapted for the accurate sampling and analysis of exhaled breath by controlling and reducing the influx of moisture and liquid from exhaled breath and from possible extraneous sources...'), the detection device comprising: a carrier fluid reservoir (col 7 ln 61-66 - '...During normal operation, fluids, which may include the exhaled breath sample and other liquids common to a medical environment, flow into the inlet end (238) of the liquid reservoir (218) through the inlet tube (216), as shown by the direction of fluid flow (250)...'); a sample collection unit (col 6 ln 24-27 - '...The sampling port (110) includes at least one sampling inlet (104) through which the exhaled breath sample is collected and passed into the sampling system (100) through the sampling port (110)...'); and a detection unit (col 5 ln 49-56 - '...In another embodiment, there is provided a breath sampling system including a gas analyzer a self-drying liquid reservoir for a breath sampling tube comprising a housing having a diameter larger than the diameter of the sampling tube; and a wick material essentially within the housing adapted to retain liquid, wherein the reservoir is adapted to permit gas flow through the housing...'), but does not further specifically disclose the device being aqueous solution activated, comprising: a sample processing unit including dried synthetic biological components. President discloses a similar device (para [0164] - '...indicating that the protein is properly folded and active. This surprising discovery offers as yet unavailable methods for sterile and abiotic transportation, storage, and/or safe deployment of novel devices based on synthetic gene networks...'; para [0165] - '...The described devices and methods were developed to exploit this discovery, and can be broadly applicable in areas including, but are not limited to, point-of-care diagnostics, clinical and industrial settings, and consumer goods...'), wherein dried synthetic biological components may be utilized as a means of detection of pathogens via aqueous activation (para [0248] - '...For virus synthesis, a freeze-dried composition comprising a cell-free system and a set of nucleic acids encoding an appropriate set of proteins (e.g., capsids) of the virus of interest can be used. When the freeze-dried composition is activated, e.g., by the addition of water, proteins can be synthesized in vitro and then self-assemble to form the virus. When incorporated into a wound dressing, topical preparation, or implantable device or preparation, the virus would be assembled at the site of application...'; para [0250] - '...The freeze-dried compositions and methods for bacteriophage synthesis can also be used in diagnostic applications, e.g., for the detection of pathogens...'; see instant specification, para [0021] - '...FIG. 6A depicts how freeze-dried cell-free reactions can be embedded in reaction sachets or chambers that are distributed throughout garments for use by soldiers, clinicians, and first responders...'; para [0058] - '...Cell-free synthetic biology reactions are self-contained abiotic chemical systems with all the biomolecular components required for efficient transcription and translation. Such systems can be freeze-dried into shelf-stable formats using porous substrates, which allow for robust distribution, storage and use without specialized environmental or biocontainment requirements...'). It would have therefore been obvious to one of ordinary skill in the art to combine these references, and to create the detection device being aqueous solution activated, comprising: a sample processing unit including dried synthetic biological components, because the use of such dried synthetic biological components can be readily implemented into materials such as clothing, and are are much more cost-efficient than existing means of detection (see President, para [0324] - '...Moreover, rather than competing with technically demanding and expensive lab-based techniques like ELISA, PCR and mass spectrometry, paper-based systems permits the creation of a new, low-cost generation of sensors that could be embedded ubiquitously into daily life (including clothing)...').

As the shared technical features were known in the art at the time of the invention, they cannot be considered common technical features that would otherwise unify the groups. Therefore, Groups I-IV lack unity under PCT Rule 13.