

Detecting emerging biological threats with Dial-a-Threat

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Abstract

Many biological detection technologies have limited utility in the field because they require specialized laboratory equipment and training. Thus, the current state of the art for unpowered on-site biological assays remains the lateral flow immunoassay (LFI), despite limited performance, reliance on cold chain storage, and long development times for new threats. Dial-a-Threat aims to maintain the ease-of-use of the LFI while achieving PCR-like performance and the ability to re-target devices in the field within a week.

Detection Technology

This project integrates advancements in cell-free synthetic biology, RNA toehold detection circuits, 3D printing, and 'paperfluidics' to produce a means to perform next-generation biological (and eventually chemical) detection and identification in the field with minimal user burden.

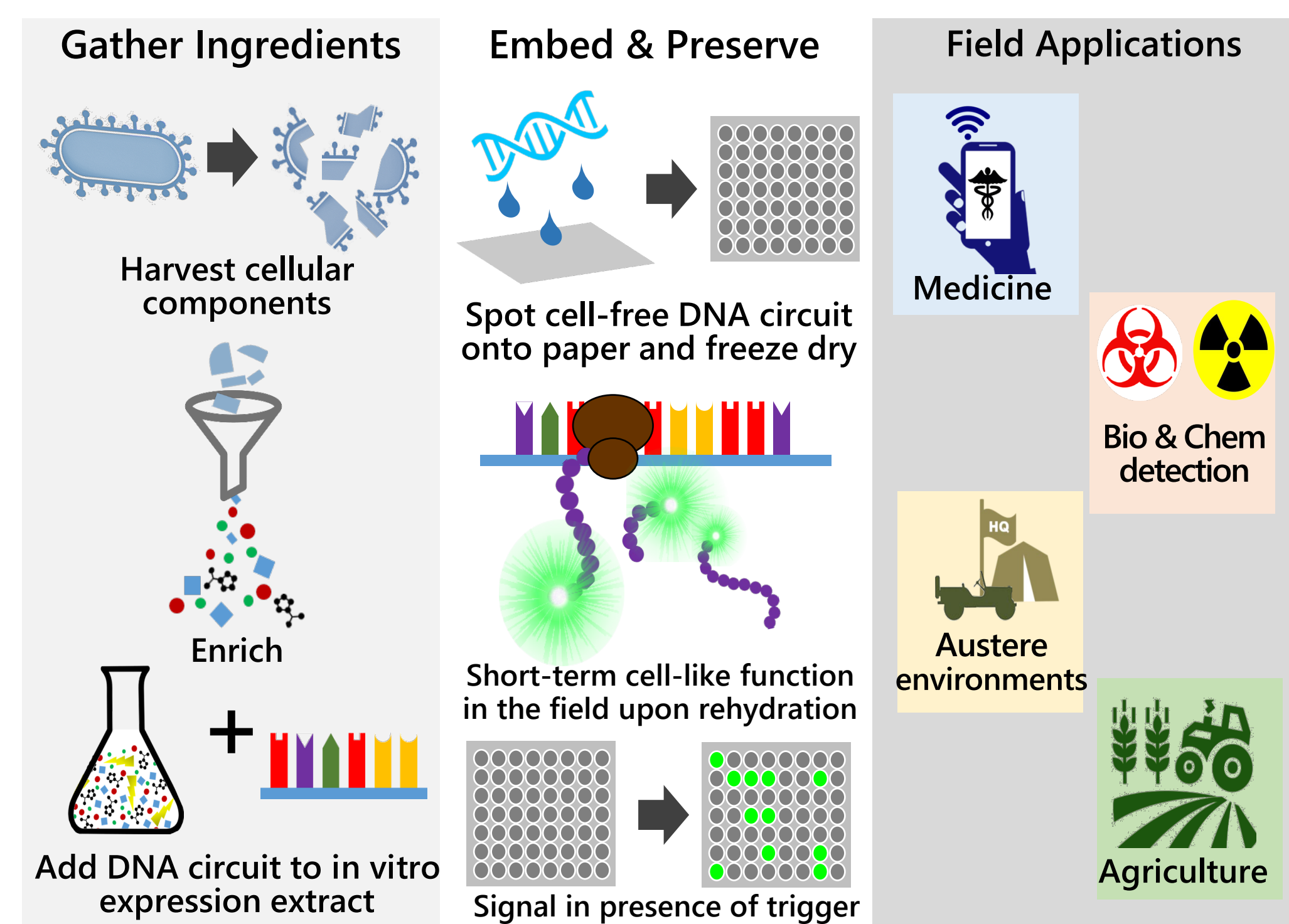


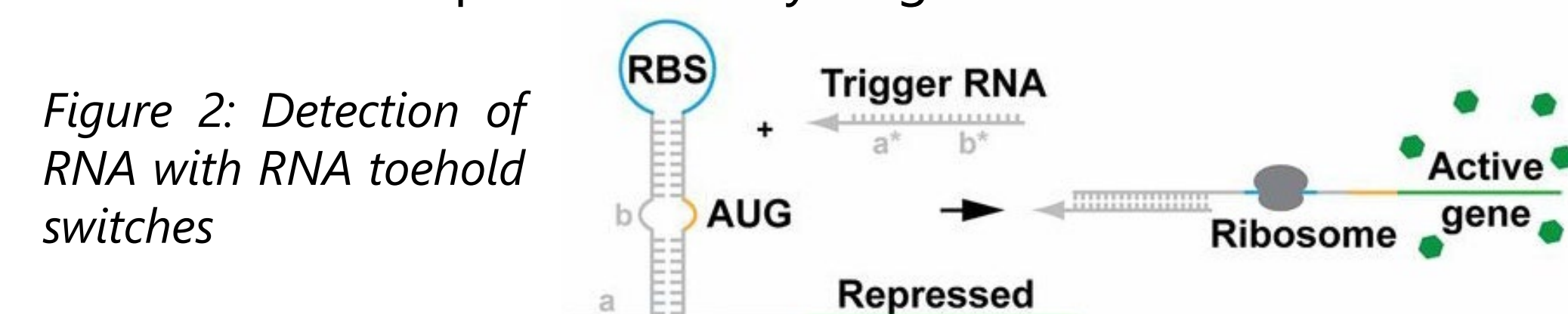
Figure 1: Cell-free expression technology



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Cell-free expression (CFE) technology enables sensors and other genetic functions to be performed outside the confines of the cell (Figure 1). Here we detect nucleic acids using RNA toehold switches of the type first pioneered by Green and Pardee¹. These switches prevent a reporter gene (in our case LacZ) from being expressed until a trigger sequence is present to remove a designed hairpin structure (Figure 2). When paired with a nucleic acid amplification step, these assays can reach sensitivity and specificity performance levels that are similar to PCR but do not require thermocycling and offer colorimetric readouts.



CFE reactions are embedded into wax-printed paper tickets to create individual reaction wells. A major part of the project has been to cut the total reaction time (amplification and CFE reaction) down to under 30 min (Figure 3). Through a series of optimizations, we reduced the CFE reaction time from ~90 min to ~20 min. We also found that using Recombinase Polymerase Amplification (RPA) reactions with a 5 min incubation, we could detect a response in under 25 min, meeting the DTRA-set target of 30 min total time.

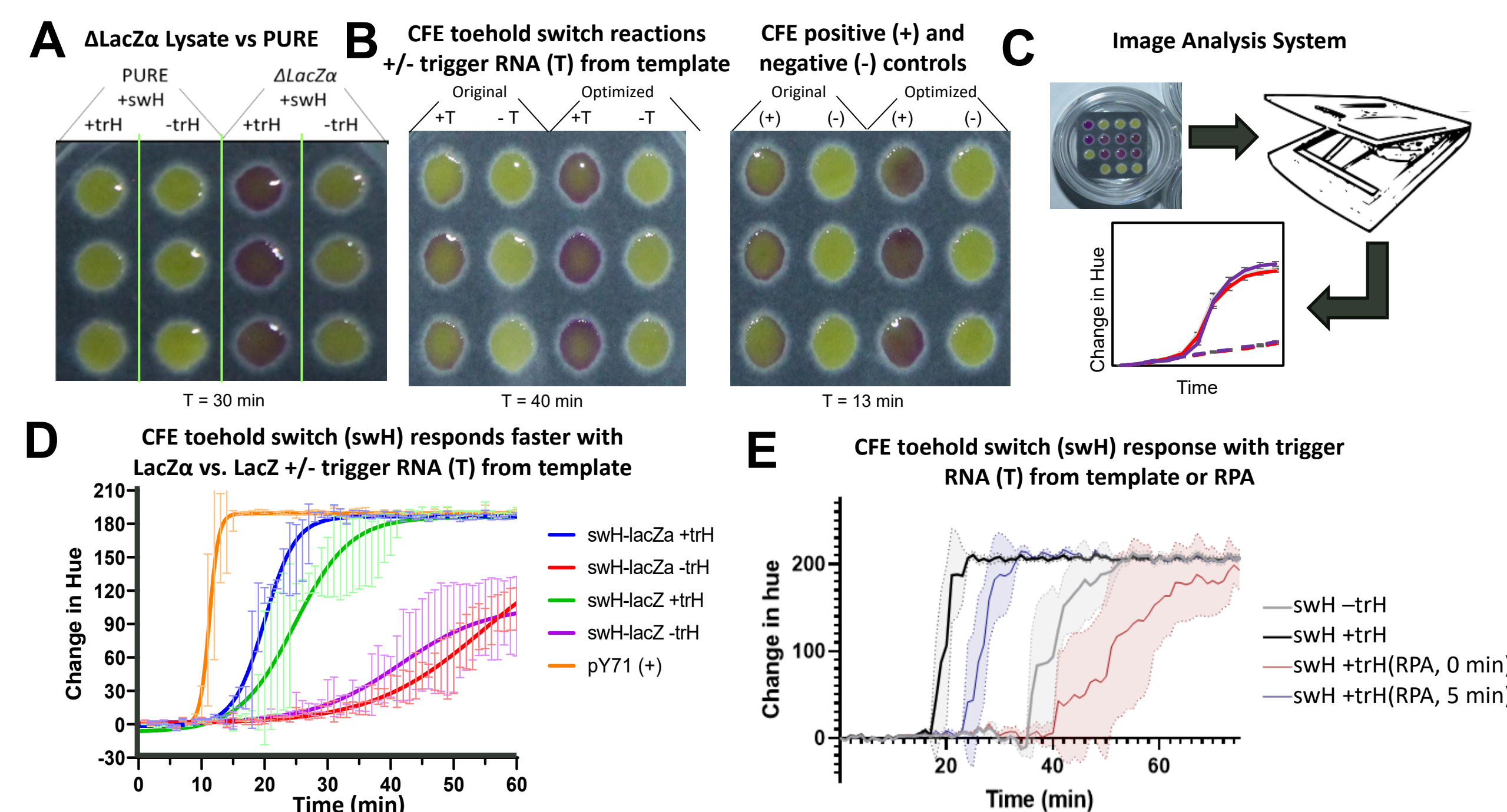


Figure 3: CFE toehold switch reactions detect nucleic acid triggers on paper tickets in under 30 mins. (A) Lysate made from ΔLacZα strain outperforms default PURE system. (B) Optimized reaction compositions improve performance. (C) Image analysis system converts images into hue values. (D) Using LacZα as reporter vs LacZ gets response time to <20 min. (E) Product of RPA reaction with 5 min incubation generates response in <25 min (<30 min total time).

Deployment and Assessment

Reactions are embedded into a user friendly, LFI-like format using paperfluidic devices in 3D-printed housings (Figure 4). The ticket layers are made by wax-printing hydrophobic barriers to create channels, then stacked and compressed together using 3D-printed parts that snap into place. The final layer incorporates a freeze-dried hydrogel that helps to pull fluid through the devices when a sample is added.

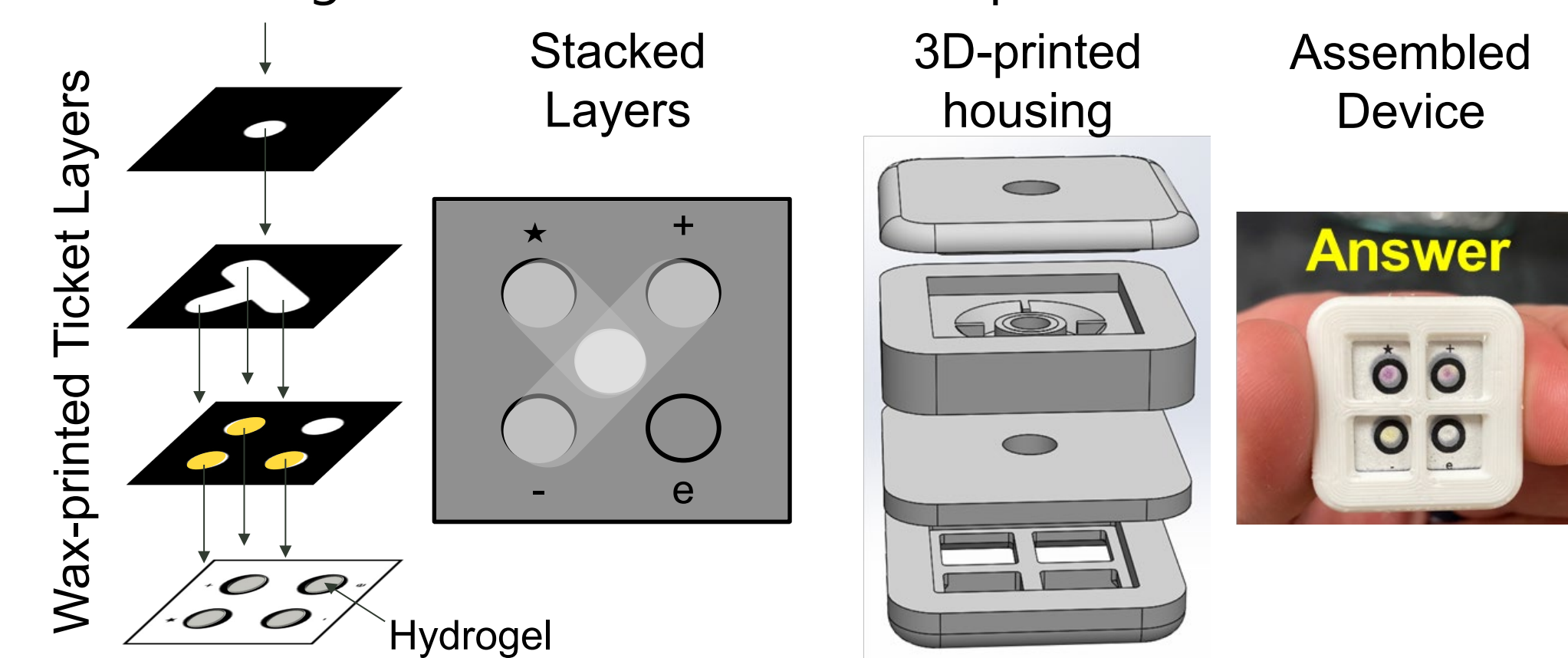


Figure 4: Paperfluidic devices to deploy cell-free sensors

From this first paperfluidic design, we have made continuous improvements based on Warfighter feedback during operational events (CBOA '22, CBOA '23, TD2; Figure 5). The original design was approximately the size of a quarter and found to be too small to be handled by operators wearing protective MOPP gear that includes thick gloves. Using 3D-printing, new housings were printed at the event to provide a handle. Second generation devices included packaging with instruction sets. Current designs further improve handling by using a format similar to a credit card with instructions directly on the test. Additional enhancements include multiplexing up to three threats, a handheld reader to streamline analysis and ATAK integration, and an alternate reporter system visible by night vision.



Figure 5: Field assessment and design progression. (A) Original design. (B) Design with handle to facilitate use with gloves. (C) Warfighter using Dial-a-Threat Device. (D) Pelican case with instruction set. (E) Dial-a-Threat card with 3-threat multiplexing and embedded instructions. (F) Portable reader for automated analysis and ATAK integration. (G) Night vision readout.

Rapid Response to New Threats

As seen during the COVID19 pandemic, traditional LFI assays require months to develop against a new target while PCR assays require only days. Our rapid response pipeline demonstrates that new Dial-a-Threat assays can be designed, screened, and deployed in under a week (Figure 6). Leveraging new benchtop oligo synthesis technologies, it is possible to stockpile blank Dial-a-Threat devices that can be rapidly re-targeted to an emerging threat by in-field functionalization of modules that snap onto the devices.

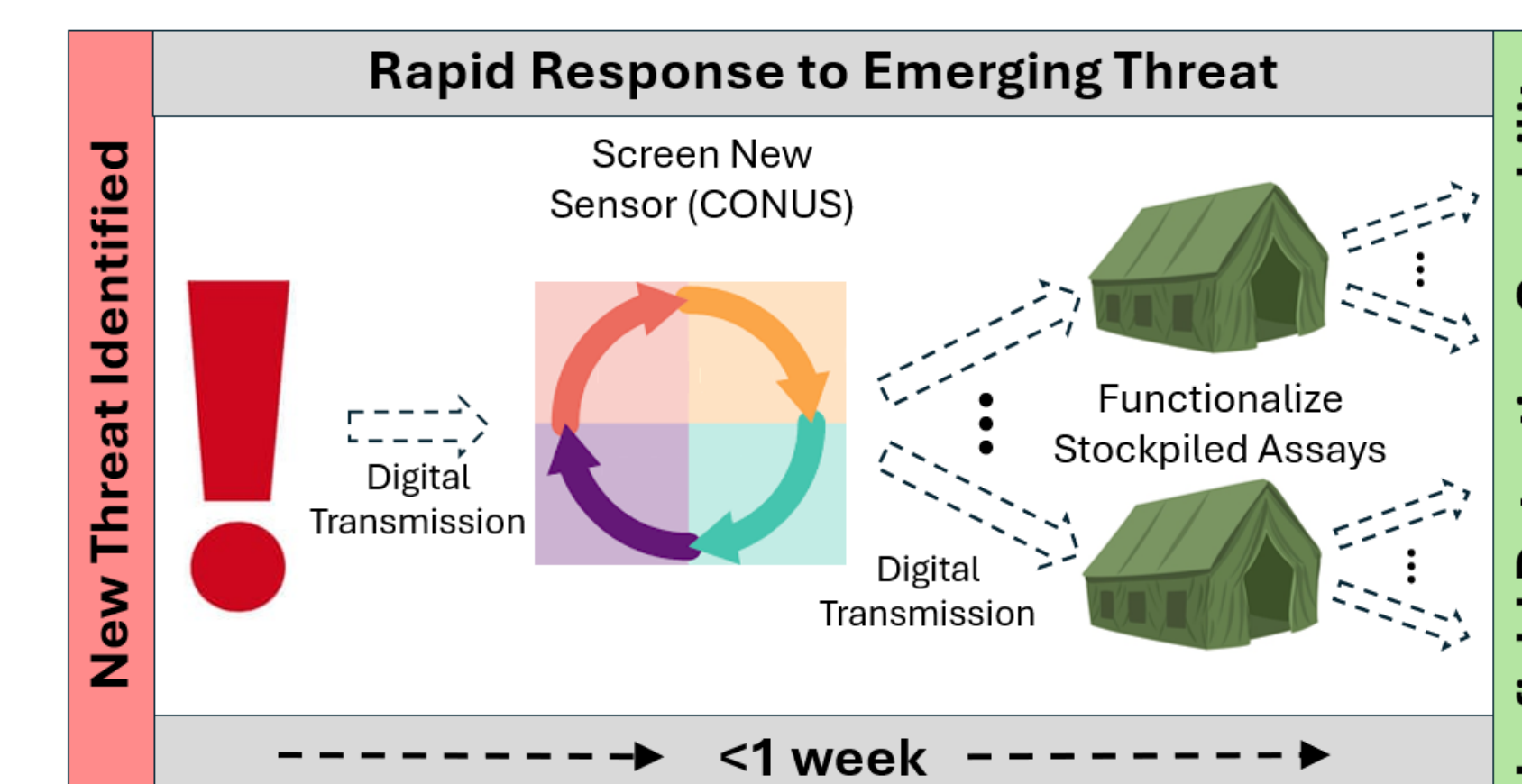


Figure 6: Rapid response to new threats

Beyond Pathogens

The use of CFE allows most natural genetic regulation mechanisms to be exploited as elements in a biological-sensing scheme, meaning that nucleic acids, proteins, and small molecules are all candidates for detection. In addition to the nucleic acid detection work described here, work on detection of other targets in related projects include:

- Heavy metals (current OSD funding)
- Toxins via CFE produced binders (current DTRA funding)
- Toxins via aTF sensors (pending proposals)
- Human performance markers (prior OSD funding)
- Explosives (prior OSD funding)

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