Rapid testing and validation of gene-based sensors for the detection of SARS-CoV-2

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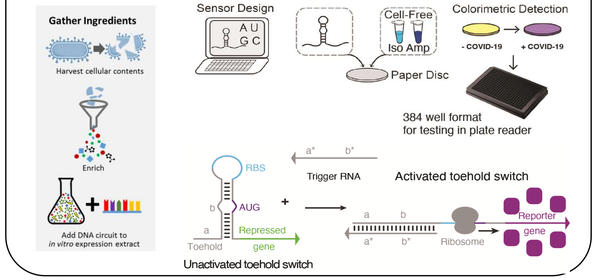
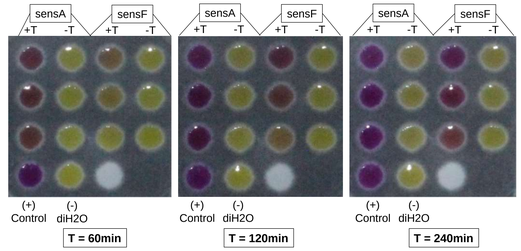
Abstract

The importance of the rapid development and deployment of detection and diagnostic devices in response to novel biological threats has been underscored by the global SARS-CoV-2 pandemic. Gene-based circuit detection of target nucleic acid sequences can be performed using cell-free protein expression systems and has been previously demonstrated for the detection of several biological threats. The use of cell-free systems ultimately enables the exploitation of biological mechanisms without the requirement to keep cells alive and thus enables the creation of a detection platform that is low burden and fieldable. In this work we report the independent validation of gene circuits for the detection of SARS-CoV-2 in a cell-free system. Sensor and primer information was conveyed electronically by our academic collaborators, assembled and amplified in-house and tested on paper-based cell-free tickets for proof of concept validation. We then sought to optimize conditions for the amplification and detection of genomic SARS-CoV-2 RNA. Future work will test this system for the extraction and detection of SARS-CoV-2 RNA in clinical patient samples. The establishment of this capability in our laboratories helps to poise the technology for the rapid response to unknown biological threats that may emerge in the future.

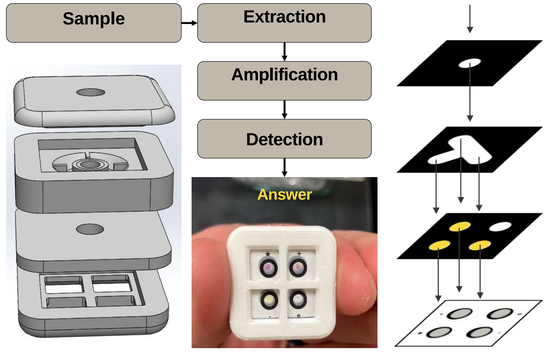
Cell-Free Systems for Biological Detection



Proof-of-Concept Rapid Sensor Test

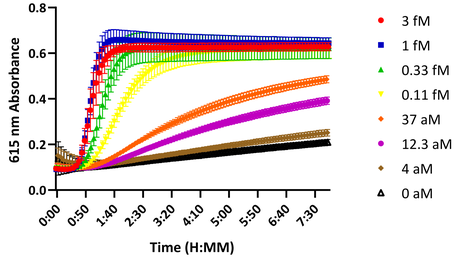


Detector Prototype and Workflow



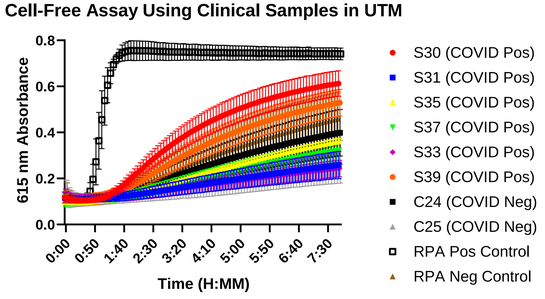
Novel sensors can be easily ported into the Dial-a-Threat device (tested at CBOA 2022), enabling detection of emerging biological agents in an easy to use format.

Amplification and Detection



Amplification of the target SARS-CoV-2 genomic region was conducted using Recombinase Polymerase Amplification (RPA). The target was detected by a toehold switch ("ESensA") in a cell-free reaction.

Detection of SARS-CoV-2 in Patient Samples



Our cell-free assay was able to detect SARS-CoV-2 in UTM-based patient samples. Other matrices produced assay interference that we plan to address in future experiments.

Summary of Patient Sample Results

Sample	COVID Status	Matrix	Ct Values	Ct Mean	Ct Std Dev	Cell-Free Assay Result
B5	Pos	VTM	25.777	25.711	0.094	Neg
			25.644			
B6	Pos	VTM	29.251	29.29	0.055	Neg
			29.329			
B7	Pos	VTM	27.779	27.883	0.146	Pos
			27.986			
B8	Pos	VTM	25.283	25.194	0.126	Pos
			25.105			
F9	Neg	VTM	0	0	0	Neg
			0			
G1	Neg	VTM	0	0	0	Pos
			0			
S30	Pos	UTM	25.541	25.584	0.06	Slight Pos
			25.626			
S31	Pos	UTM	26.255	26.298	0.06	Neg
			26.340			
S33	Pos	UTM	0	0	0	Neg
			0			
S35	Pos	UTM	35.717	35.708	0.014	Neg
			35.698			
S37	Pos	UTM	31.497	31.482	0.022	Neg
			31.466			
S39	Pos	UTM	26.337	26.179	0.224	Slight Pos
			26.021			
C24	Neg	UTM	0	0	0	Neg
			0			
C25	Neg	UTM	0	-	-	Neg
			*37.735			

Summary RT-qPCR (CDC N1 Assay) for each patient sample comparison to results using our cell-free assay. *Possible experimental contamination

Future Directions

Our results show promise that toehold switches can be used in cell-free systems to detect emerging biological threat agents. Computational design of the sensors enables the rapid development of new sensors as they are needed. Future work will aim toward improving the speed and sensitivity of the assay, increasing our repertoire of sensors against a variety of targets, and investigating methods to reduce interference from certain matrix types.

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