SARS-CoV-2 detection with type I-A CRISPR "FALCON" system

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Abstract. CRISPR-Cas systems are currently applied in the detection of infectious diseases as a rapid and accurate alternative for the traditional RT-PCR (reverse-transcription polymerase chain reaction) test. The type I-A CRISPR-Cas nucleic acid detection system has been proven to achieve efficient detection through a 15-minute one-pot reaction at 85°C. For this project, a type I-A system "FALCON" (Fast Agent in Limited COVID Nucleic acid detection) was created to detect SARS-CoV-2 and its variants. The system's guide RNA is designed with the N gene of SARS-CoV-2. When tested with samples containing SARS-CoV-2 pseudovirus, the system's embedded Cas3 enzyme is activated upon guide RNA recognition and collaterally cleaves FQ (fluorophore-quencher) reporters, producing a fluorescent sample where the amount of fluorescence is proportional to the viral load. In addition, FALCON's results are highly consistent with those of RT-PCR but requires much less instrumentation and shorter testing time, and can differentiate among virus variants. FALCON can be developed into commercial SARS-CoV-2 self-testing kits, or used in detection and differentiation of other diseases with a modified guide RNA.

Keywords: infectious diseases, polymerase chain reaction, nucleic acid, detection system

1. Introduction

1.1. CRISPR-Cas mechanism

CRISPR-Cas is a natural defense mechanism found in 50% bacteria and 90% to protect them against phages. CRISPR, short for clustered regular interspaced short palindromic repeats, is a unique portion of the *iap* gene in prokaryotes. Spacers that contain foreign DNA are attached between regular palindromic repeats. The CRISPR section is transcribed and cut into sections containing one repeat and one spacer as the guide RNA. The guide RNA binds to Cas proteins, or CRISPR associate proteins, activating them which enables genome editing. CRISPR systems are categorized into classes 1 and 2 based on the number of effector proteins they contain. Class 1 systems contain multiple effector proteins (cascade), while class 2 systems have a single effector protein (Cas9, Cas12 or Cas13). Once CRISPR systems recognize the PAM sequence in a gene, the "seed" portion of guide RNA unwinds DNA and combines with the targeted strand. This allows the cleaving enzyme (Cas3 in class 1 systems, HNH and RuvC in class 2 systems) to edit the targeted and/or non-targeted strands.

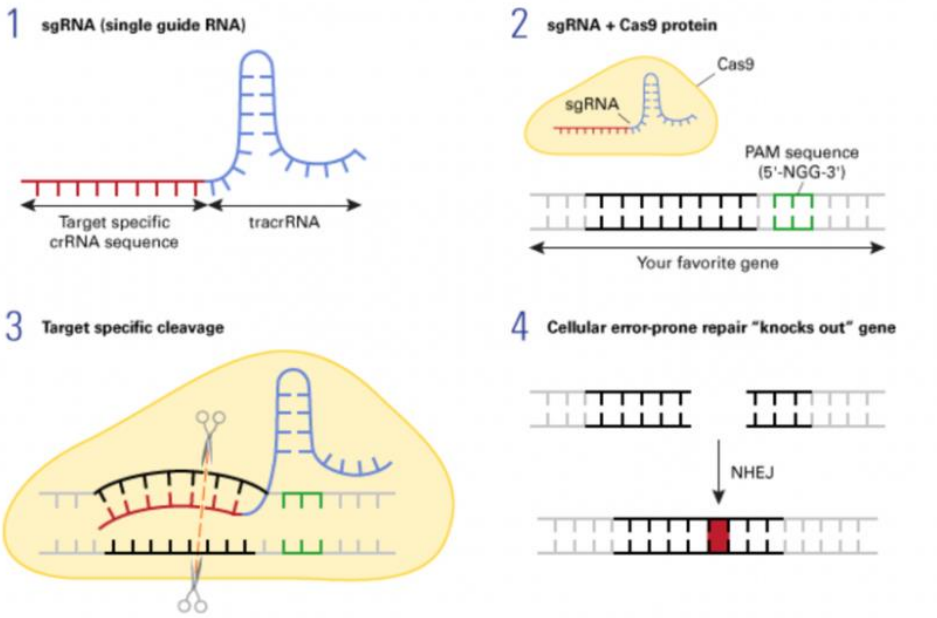

Figure 1. Gene-deleting mechanism of a class 2 CRISPR-Cas9 system. Step 2 displays the system's recognition of a specific PAM sequence. Step 3 displays target-specific cleavage of both the targeted and non-targeted strand (Takara Bio Inc, 2023).

1.2. SARS-CoV-2 pandemic control

The SARS-CoV-2 virus, commonly referred to as COVID-19, has been a major public health concern since its outbreak in 2019. SARS-CoV-2 causes cytokine storms in infected patients, leading to congestion and lung fibrosis, and in severe cases death. Until March 2023, the SARS-CoV-2 pandemic has caused over 6 million deaths worldwide (World Health Organization, 2023). As an RNA virus, SARS-CoV-2 has a high rate of mutation which led to multiple variants including the highly transmissible variants Delta and Omicron.

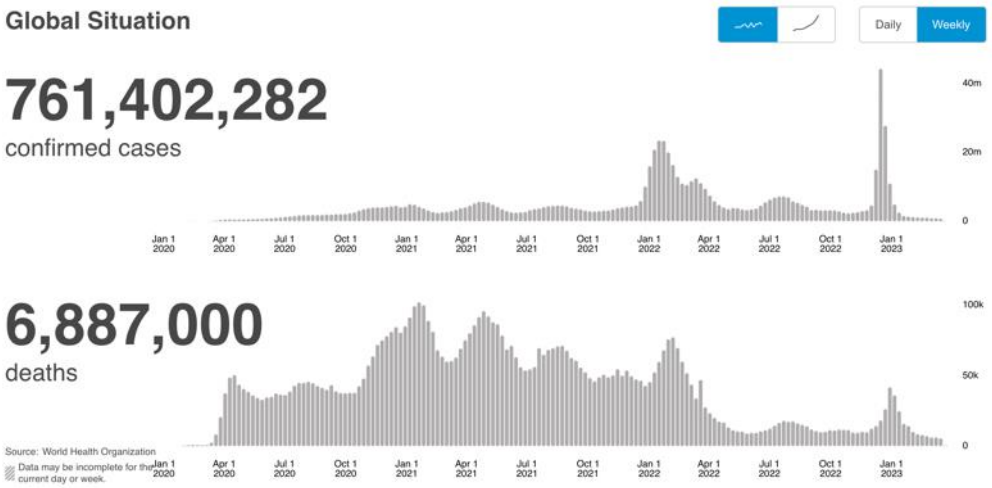

Figure 2. Number of confirmed cases and deaths worldwide from the SARS-CoV-2 pandemic until March 29, 2023 (World Health Organization, 2023).

The control of SARS-CoV-2 outbreak relies on nucleic acid detection: early detection enables subsequent quarantines of positive individuals, which halts the spread of the pandemic if implemented promptly. An SIR model (dividing patients into susceptible, infected, and recovered groups) using neural network modules and available SARS-CoV-2 data has shown a positive correlation between quarantine strength and decreased rate of transmissions (Dandekar, Rackauckas, & Barbastathis, 2020).

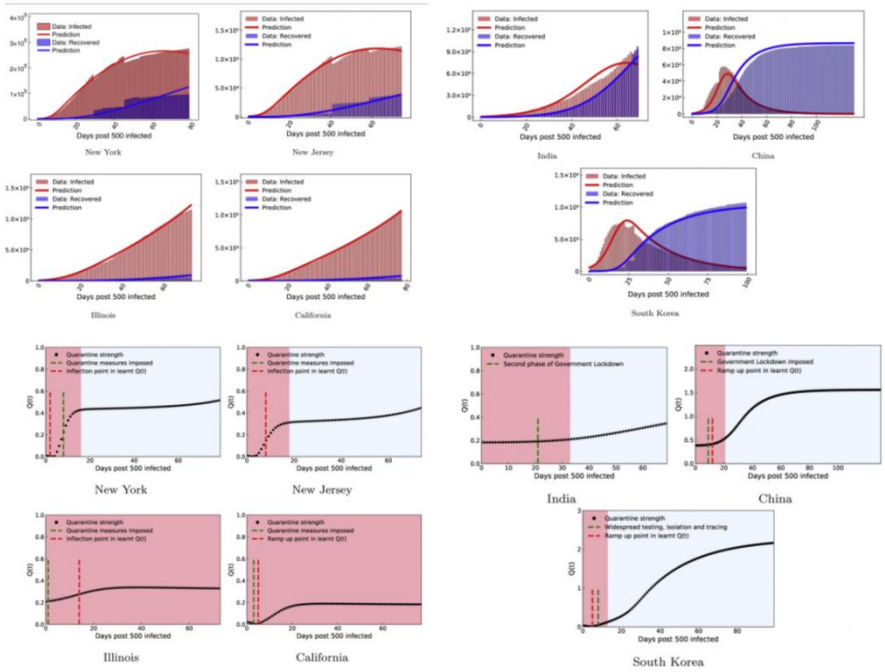

Figure 3. Correlation between quarantine strength and the number of infected and recovered patients in 4 U.S. states and 3 Asian countries with different levels of strictness and times of implementation in quarantine policies. Red regions in the bottom graphs represent inefficient quarantine policies (Dandekar, Rackauckas, & Barbastathis, 2020).

1.2.1. Limitations of current detection methods

The current golden-standard of detection for both RNA and DNA viruses, including SARS-CoV-2, is the RT-PCR (real-time reverse transcriptase polymerase chain reaction) nucleic acid test. The RT-PCR test determines viral presence through the reverse transcription of viral RNA into DNA, an amplification of the recombinant DNA, and a final microarray analysis to determine viral presence by comparing with a template ladder.

Major drawbacks of the RT-PCR method include the long time taken to obtain results (4 to 8 hours), the need for specific equipment, high costs associated with primer design and usage, difficult and indirect result readout, and the inability to determine exact viral load or variant type. The RT-qPCR method which uses fluorescent primers has reduced testing time and improved the result readout by allowing a comparison of fluorescence levels with a baseline. However, both RT-PCR and RT-qPCR still rely heavily on equipment and the design of primers, meaning that they cannot be promptly applied in emergency pandemic outbreaks or in cases of new variants.

Furthermore, the PCR methods have a relatively high rate of false positive and false negative results when conducting detection. An FDA-approved PCR test may still fail to detect 5% of infected patients and produce 2% of false positive results among the uninfected population (Braunstein, Schwartz, Hymel, & Fielding, 2021). Causes of such inaccuracies include sample or equipment contamination, cross-reactions with other viruses, misdetection of vaccinated or recovered populations as positive, and the low sensitivity with samples obtained prior to or several days after symptom onset that have low amounts of viral load. According to a SEIR (Susceptible-Exposed-Infected-Recovered) model, the rate of false negative results is between 40% and 70% among patients that are transmissive but do not display symptoms yet, hence the frequency of testing needed to reduce the rate of transmission increased to once per day if the false negative results are accounted for (Jarvis & Kelley, 2021). This increased frequency of testing combined with the high costs and long testing time for PCR reduces efficiency in disease control.

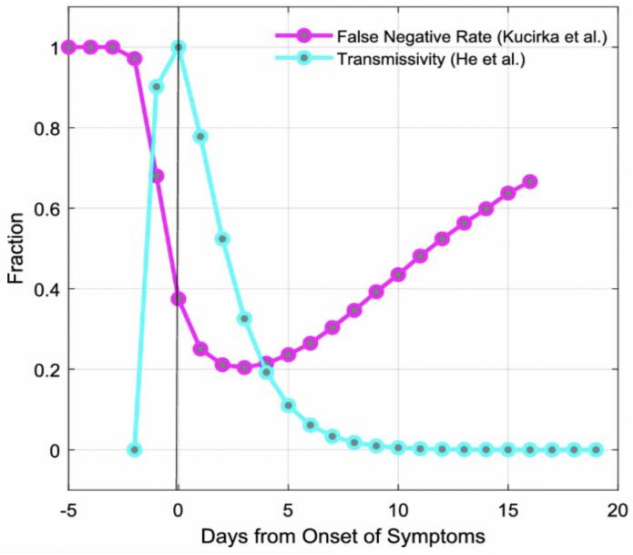

Figure 4: Relationship between SARS-CoV-2 transmissivity and the false negative rate of PCR detection based on the days of symptom onset. At 2 days prior to symptom onset, both transmissivity and false negative rate are above 0.7, meaning that patients at this time are most prone to spreading the virus (Jarvis & Kelley, 2021).

1.3. CRISPR-Cas systems in nucleic acid detection

Most CRISPR systems only cleave a specific strand of DNA that corresponds to the guide RNA, known as the cis-DNA. However, systems such as types V, VI and I-A exhibit collateral activity: with the presence of an activator, the cis-DNA strand, these systems are able to cleave non-specific strands of dsDNA and/or ssDNA known as trans-DNA. CRISPR systems with collateral activity can be designed with guide RNAs containing viral genetic information. Once in contact with a positive sample, the CRISPR system collaterally cleaves FQ reporters (light-emitting fluorophores and light-absorbing quenchers held together by dsDNA) creating a fluorescent sample (Stower, 2018).

1.3.1. Cas12-based DETECTR

CRISPR-Cas12, or type V CRISPR, is a DNA-editing system that exhibits collateral activity with its single effector Cas12 upon target strand recognition. The Cas12a-based DETECTR uses the RT-LAMP assay in sample preamplification, a simultaneously performed reverse transcription of sample RNA and isothermal amplification under a temperature of 62 °C, followed by a one-hour detection of SARS-CoV-2 genome sequence at 37°C. The activated system collaterally cleaves ssDNA FQ reporters, and its results can be interpreted both through sample fluorescence and a colorimetry assay on lateral flow paper (Broughton, Deng, Yu et al.， 2020).

1.3.2. Cas13-based SHERLOCK

CRISPR-Cas13, or type VI CRISPR, has a mechanism similar to that of type V systems but recognizes and edits RNA instead of DNA. The Cas13-based SHERLOCK detection performs sample preamplification through RPA (for DNA samples) or RT-RPA (for RNA samples) at 37°C for 10-30 minutes. A T7 RNA polymerase is attached to the 5’ end of primers during RPA to facilitate the subsequent in vitro transcription into RNA targets. SHERLOCK then performs a separate 30 to 60-minute detection of viral RNA, which results can be interpreted by fluorescence level or lateral flow readout. An improved version SHERLOCKv2 combines RPA, T7 transcription and Cas13 detection into a 60 to 180-minute one-pot reaction, with its detection signal amplified by the addition of the tandem CRISPR typeIII effector nuclease Csm6 to increase efficiency of reporter cleavage (Kellner, Koob, Gootenberg, Abudayyeh, & Zhang, 2020).

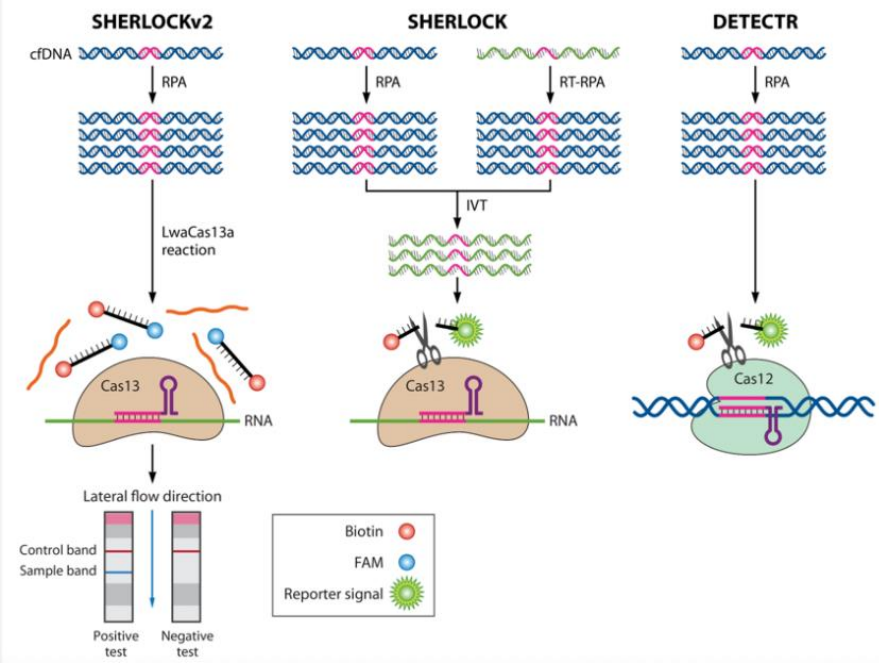

Figure 5. Illustration of DETECTR and SHERLOCK detection mechanisms (Mustafa & Makhawi, 2021)

1.4. Type I-A CRISPR systems

Type I-A CRISPR, a class 1 CRISPR system normally found in thermophilic archaea, contains a cascade formed by Cas11, Cas7, Cas5, and Cas8. Instead of hiring active Cas3 to shred the target strand like other class 1 systems, type I-A contains an embedded Cas 3 divided into the HEL and HD domains whose activity is inhibited by the cascade but can be activated through a conformational change upon target DNA recognition, hence enabling collateral activity and nucleic acid detection

1.4.1. Type I-A "HASTE" system

The "HASTE" (Heat-activated streamlined nucleic acid detection platform) system utilizes the type I-A system of the thermophilic archaea *Pyrococcus furiosus* (Pfu.). Because of its stability under high temperatures, the cell lysis, virus denaturation, and heat activation of HASTE are all performed in a one-pot reaction under 85°C (Hu et al., 2022). HASTE is able to conduct accurate detection without the sample preamplification process that existing type V and VI CRISPR systems require because of the high specificity in target RNA binding due to the interactions between each cascade protein and the crRNA. The lowest detection threshold of HASTE is at the picomolar level for dsDNA and ssDNA targets without amplification, and ssRNA targets are also detectable without reverse transcription but with a 100-fold lower sensitivity. Furthermore, as HASTE is compatible with both DNA and recognizes both DNA and RNA targets, a reverse transcription process is not required for detection of RNA viruses such as SARS-CoV-2 which further increases the efficiency of detection processes.

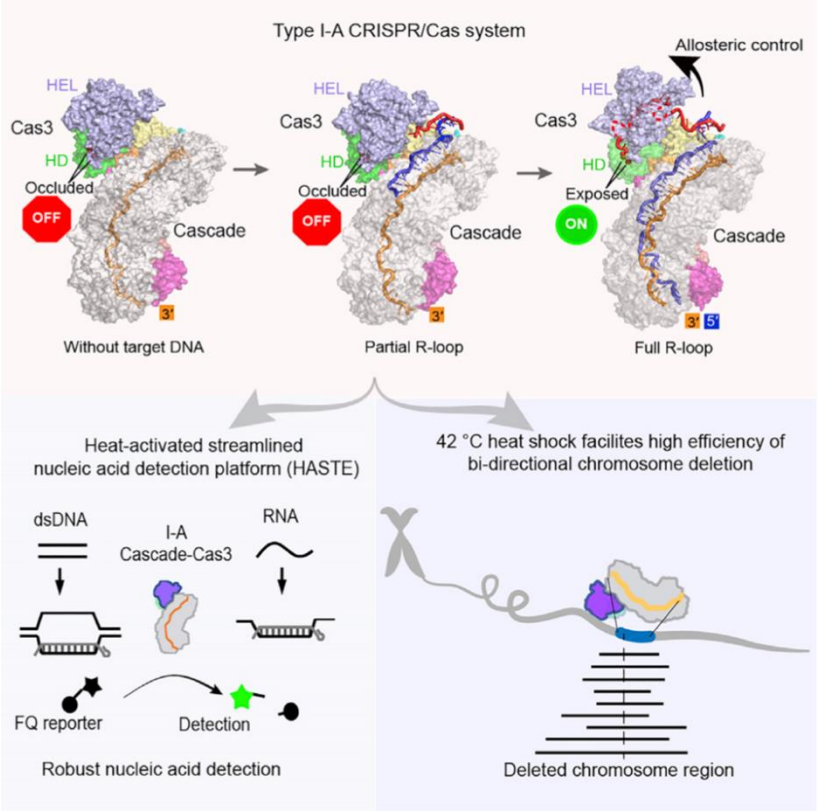

Figure 6. Detection mechanism of type I-A "HASTE" detection platform. As the top portion suggests, the system's collateral activity is enabled through a shape change of the Cas3 HEL and HD domains (Hu et al., 2022).

Materials

Table 1 List of materials used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
E. coli Bl21(DE3) competent cells	NEB	Cat# C2527I
E. coli DH5a competent cells	NEB	Cat# 18265017
SARS-CoV-2 Wuhan strain		
SARS-CoV-2 Delta strain		
SARS-CoV-2 Omicron strain		
Chemicals, peptides, and recombinant proteins		
Modified and unmodified oligos	IDT	N/A
IPTG	GoldBio	Cat # I2481C500
LB broth	Teknova	#L9145
Restriction enzymes	NEB	N/A
iProof high fidelity PCR kit	Biorad	#1725331
Acrylamide	thermal	HC2040
Urea	VWR	57-13-6
Fluorescence stain Cy3 and Cy5	Lumiprobe	11320 and 13320
SYPRO® Orange	Thermo Fisher	S6650
Formamide	VWR	75-12-7
$MnCl_2$	Fisher Scientific	7773-01-5
Strep resin	IBA	2-1201-025

Nickel resin	QIAGEN	Cat. No. / ID: 30250
Copper Grids 1.2/1.3 200 mesh	QUANTIFOIL	Q250-CR1.3
Type I-A Cascade	This study	N/A
Cas3 HEL	This study	N/A
Cas3 HD	This study	N/A
Recombinant DNA		
Complete list of plasmids		
pETDUET-Cas11-Cas7-Cas5-His tag	This study	N/A
pCDFDUET-Twin strep tag-Cas8	This study	N/A
pRSFDUET-crRNA for COVID_Wuhan strain	This study	N/A
pRSFDUET-crRNA for COVID_Delta strain	This study	N/A
pRSFDUET-crRNA for COVID_Omicron strain	This study	N/A
Oligonucleotides		
FQ-ssDNA reporter	This study	
Other		
MonoQ 5/50 GL	Cytiva	Cat# 17516601
HiLoad 16/600 Superdex 200 pg	Cytiva	Cat# 28989335

2. Methods

2.1. Plasmid design

As the functional "HASTE" detection platform was developed with the type I-A CRISPR system of Pfu., a homolog search was conducted with Pfu. Cas8a amino acid sequence as its input. A phylogenetic tree was created, displaying *Thermococcus siculi* (Tsl.) as the closest evolutionary relative of Pfu. The genetic sequences of Tsl. type I-A CRISPR cluster and SARS-CoV-2 Delta and Omicron variants were then obtained. Multiple crRNAs were designed with 5 palindromic repeats and 5 spacers containing SARS-CoV-2 N gene (nucleocapsid gene) sequence instead of the S gene (spike protein gene), since spike proteins are more prone to mutation and are used in vaccines, which may affect the accuracy of detection.

Figure 7. Phylogenetic tree constructed through a 10 homolog search displaying Tsl. (orange box) as the closest evolutionary relative of Pfu. (blue box, original input).

The CRISPR system is synthesized by 5 sets of plasmids: 3 pRSF Duets for Cas3HEL, Cas3HD, and crRNA (the 2 domains of Cas3 are expressed separately to ensure proper protein folding), 1 pET Duet for the Cas11-Cas7-Cas5 cascade, and 1 pCDF Duet for Cas8 which is naturally expressed in lower amounts. Each plasmid contains a unique selection marker providing antibiotic resistance and an affinity tag (Histidine/Strep) which attaches to a specific resin during purification.

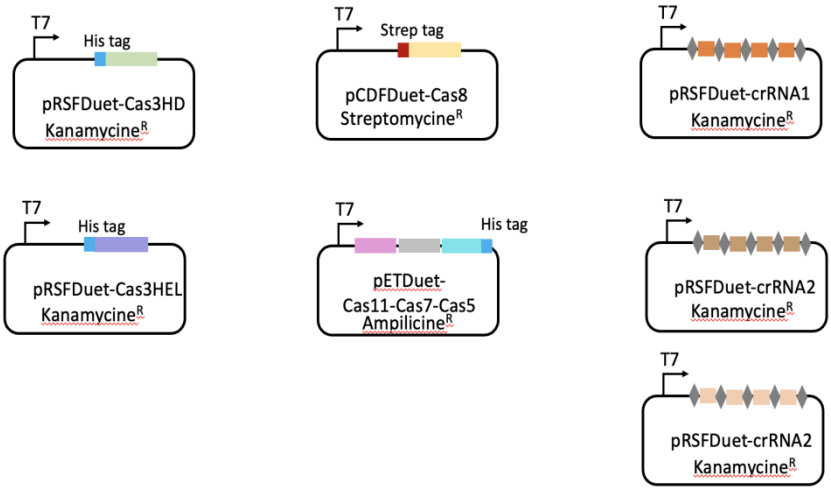

Figure 8. E.Coli plasmids constructed crRNA with chimeric repeat-spacers and Cas proteins with specific affinity tags.

2.1.1. Chimeric Repeat-spacer

The Cas6 enzyme of Tsl. which cleaves the guide RNA after each stem loop was found to be inactive under laboratory conditions due to the large difference in temperature and pressure from its optimum conditions. Hence, the Cas6 of E.Coli was used in this study to ensure proper guide RNA cleavage. A Tsl 5' handle is attached after each repeat-spacer to ensure compatibility with the assembled Tsl. type I-A CRISPR system. This novel technique, known as chimeric repeat-spacer, allows the once-impossible synthesis of functional CRISPR systems from bacteria or archaea living in extreme conditions.

2.2. Bacterial culture & protein overexpression

The E.Coli strand TOP10 was selected for rapid replication of the synthesized plasmids. The cells were induced into competent state through electric shock for plasmid insertion. The cells were then cultured on agarose gel containing antibiotics to filter individuals with successful plasmid insertions. A colony PCR using both the inserted gene and an E.Coli plasmid gene was conducted on randomly selected groups of surviving bacteria to confirm gene insertion and rule out contamination. A plasmid miniprep was conducted to collect replicated plasmids, in which the plasmids containing Cas proteins were then inserted into the DE3 strand cells of E.Coli for protein overexpression. The allolactose IPTG, which removes inhibition of the T7 lactose operator, was induced for an optimal time allow Cas proteins to be expressed in large amounts.

2.3. Protein purification

The collected Cas proteins were purified through tandem purification, in which the protein mixture was first combined with a nickel solution that allows Cas proteins with histidine tags (Cas11-7-5 cascade, Cas3HEL and HD) to attach onto. The attached proteins are separated and washed out by imidazole, and the remaining solution is then added to a resin solution which the strep tag of Cas8 attaches onto with a higher affinity. Cas8 is then washed out by desthiobiotin and combined with the other Cas proteins where the stoichiometric balance (1 Cas3HEL, 1 Cas3HD, 1 Cas8, 7 Cas7, 1 Cas5, 5 Cas11) is maintained because of more efficient purification of Cas8.

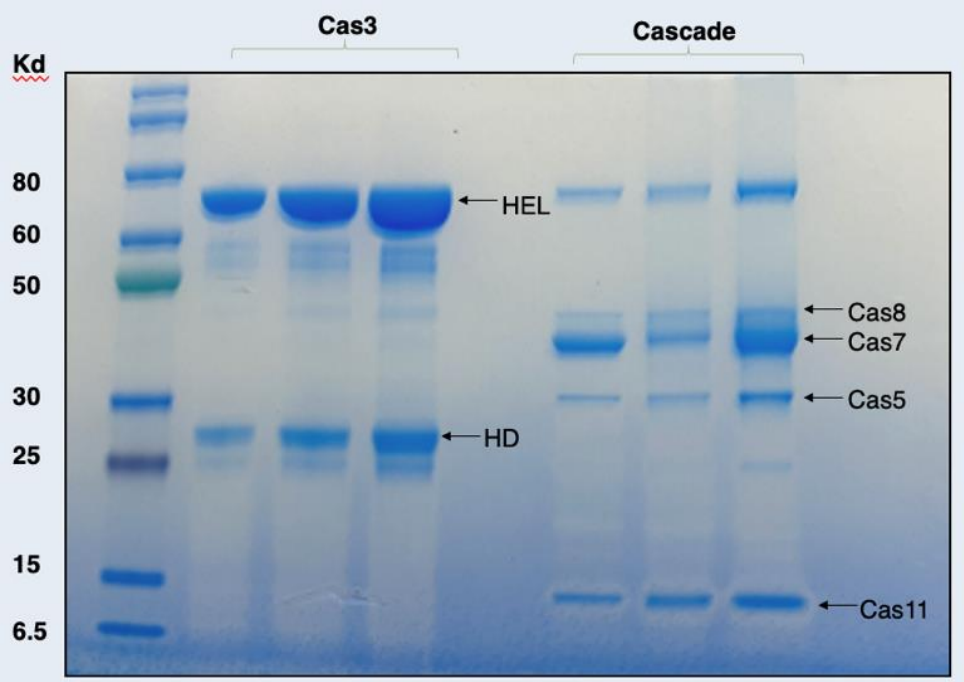

Figure 9. Cas protein purification analysis with Coomassie Blue staining SDS-PAGE. The HEL band is significantly thicker because Coomassie Blue stain has a higher affinity for alkaline proteins (the proportions of Cas3HEL, HD, Cas8, and Cas5 should be equivalent).

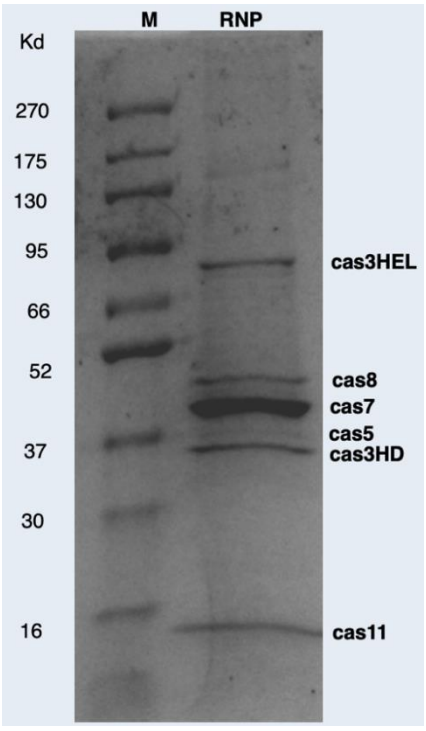

Figure 10. Urea PAGE of assembled CRISPR system where band thickness more accurately reflects Cas protein proportions.

2.4. Downstream analysis

The FALCON system is assembled by combining the Cas proteins with cleaved crRNA. An activity test was first conducted to check for collateral cleavage using 2 experiment groups (target DNA present) and 1 control group (target DNA absent). According to the mechanism of type I-A CRISPR, collateral activity is only enabled via target DNA/RNA recognition, therefore fluorescence should only be seen in positive samples (samples where SARS-CoV-2 genetic information is present).

As figure 12 shows, the activity test proves that downstream cleaved products only appear when a target strand is present.

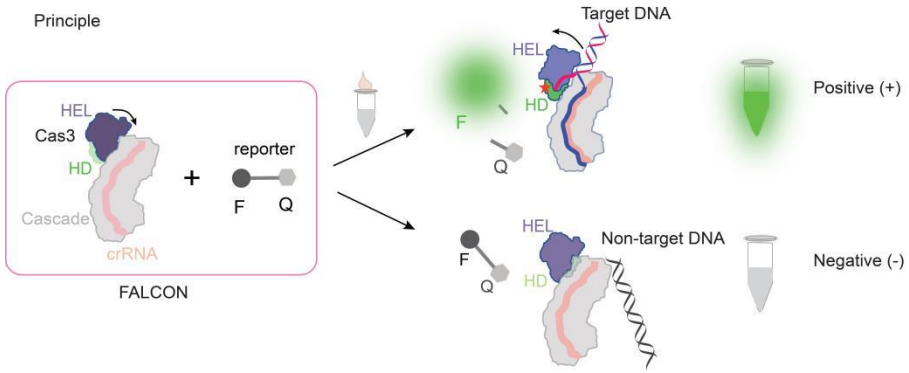

Figure 11. Detection mechanism of "FALCON" system. FQ reporters are only shred in positive samples where a target strand is present.

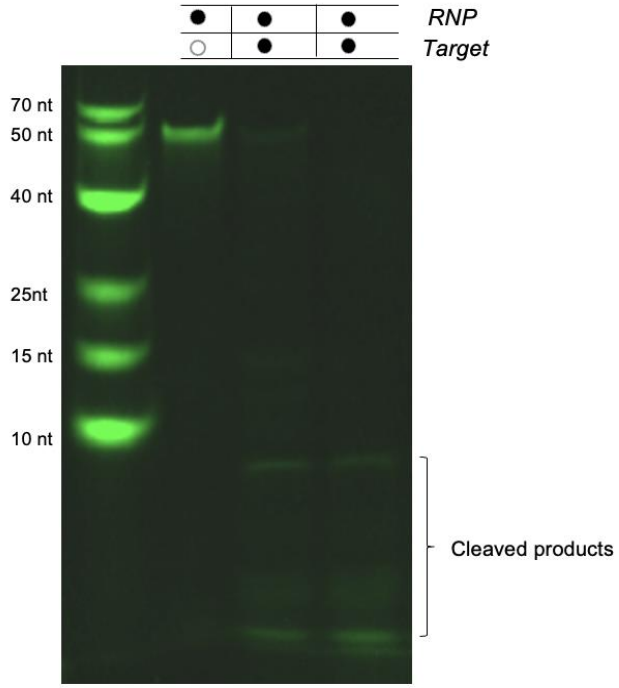

Figure 12. FALCON system activity test with 1 control (no target) and 2 experiment groups (target strand added. FQ reporter cleavage is only seen in experiment groups.

A titration test was then conducted (figure 13) where the pseudovirus concentration was gradually lowered from 20pM to 1pM and the fluorescence level is measured through a 10-minute RT-qPCR reading. At the concentration of 1pM, FALCON still produced detectable fluorescence in the sample (higher than the fluorescence baseline), establishing its detection threshold at the picomolar level whivh eliminates the need of sample preamplification.

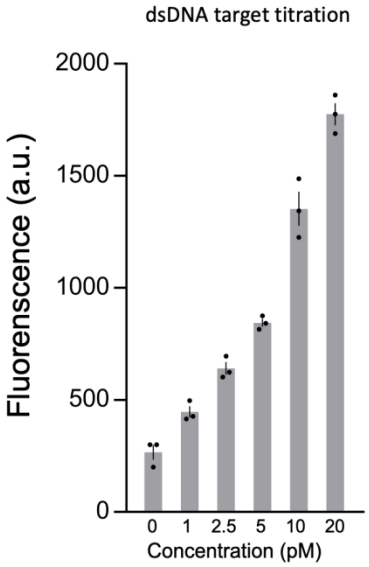

Figure 13. FALCON titration test displaying its lowest detection threshold at 1pM concentration of pseudovirus target

A heat map was also produced through three repeated comparison tests with RT-PCR using the ORF, E and N genes (figure 14). FALCON displayed highly consistent results with those of RT-PCR with no false positivity or false negativity in any of the three tested genes. The heat map also suggests that although FALCON's crRNA is created with SARS-CoV-2 N gene, it remains versatile to detection of multiple genetic sequences that are proof of living virus.

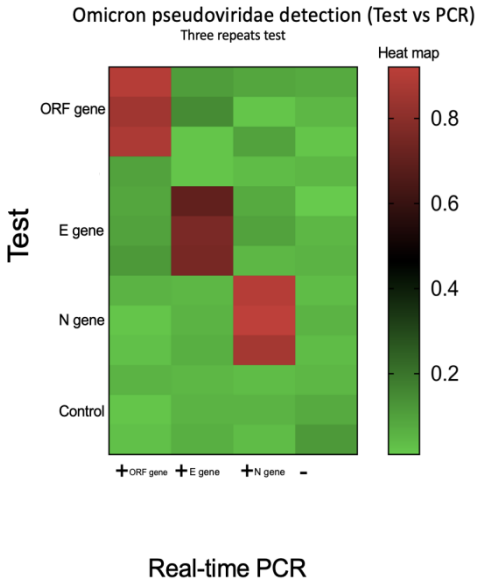

Figure 14. Heat map comparison between FALCON and RT-PCR detection results. Red areas indicate both methods successfully detected the presence of SARS-CoV-2. Green areas indicate both methods did not detect SARS-CoV-2. Black areas indicate inconsistent results, which are not seen in this heat map.

3. Results & Discussions

FALCON displays rapid and accurate detection in a 15-minute one-pot reaction, with results highly consistent with that of RT-PCR but obtained in a significantly shorter testing time (15 minutes vs. above 4 hours) and simpler testing process (one-pot reaction vs. complex reaction with multiple steps in different environments). FALCON has a high sensitivity as shown by its detection threshold

as low as the picomolar level, meaning that it eliminates the need for high quality samples or a separate sample preamplification process. Furthermore, FALCON requires minimal instrumentation as it only needs to be incubated with heat (between 45 to 85 degrees Celsius, 85 as optimal) and its results can be read out by the naked eye or a simple fluorescence detector.

3.1. Advantages over PCR

Two main advantages of FALCON compared to RT-PCR, in addition to the significantly shortened testing time, are FALCON's low cost and high accessibility. The cost to synthesize FALCON is considerably less than that of the primers and instrumentation RT-PCR requires. FALCON also eliminates the need of laboratory conditions for testing, allowing it to be applied in a wider range of conditions, such as during emergency disease outbreaks or in areas without advanced facilities. FALCON further eliminates the need of sample transportation and the associated costs as it can be made into self-detection kits that enable detection at home.

3.2. Advantages over existing CRISPR detection platforms

Compared to type II, type V and type VI CRISPR systems (which include all existing CRISPR-based detection platforms), FALCON's significant advantage is its higher sensitivity due to the interactions between its multiple effector proteins and between the cascade and target DNA. The separate preamplification process (which most current detection systems rely on for accurate detection) is also not required for FALCON, eliminating the need to transfer solutions or alter testing conditions and further lowering testing time. In addition, FALCON's high operating temperature allows for viral DNA/RNA denaturation, preventing cross infections during the testing process.

CRISPR type V				CRISPR type VI				
DETECTR	Cas12a	RPA	10 (RPA) and 60–120 (CRISPR)	–	Cas13	–	NS	NS
Cas14-DETECTR	Cas14 (Cas12f)	PCR	NS (PCR) and 120 (CRISPR)	SHERLOCK	Cas13	NASBA or RPA	132 (NASBA) or 120 (RPA) and 60–180 (CRISPR)	Column-based or crude extraction
HOLMES	Cas12a	PCR	88 (PCR) and 15 (CRISPR)					
CRISPR-materials	Cas12a	RPA	40 (RPA) and 240 (CRISPR)	SHERLOCKv2[b]	Cas13	RPA	60 (RPA) and 60–180 (CRISPR) or 60–180 (one pot)	Column-based or crude extraction
CDetection	Cas12b	RPA	10 (RPA) and 60–180 (CRISPR)					
				SHINE[b]	Cas13	RPA	50 (one pot)	Crude extraction
HOLMESv2	Cas12b	LAMP	40 (LAMP) and 35 (CRISPR) or 120 (one pot)	STOPCovid[b]	Cas12b	LAMP	60 (one pot)	Crude extraction
				CARMEN	Cas13	PCR or RPA	20 (RPA) and 180 (CRISPR)	Column-based
E-CRISPR	Cas12a	–	30–180					

Figure 15. Testing times of existing types V and VI CRISPR detection platforms in minutes (rightmost column), ranging between 1 to 3 hours excluding the time for sample preamplification (Stower, 2018). Hence, FALCON's 15-minute detection significantly outperforms existing systems.

3.3. Limitations

3.3.1. Synthesis and balancing of Cas proteins

Compared to types II, V, and VI CRISPR systems, a limitation of FALCON is that it requires the synthesis of a protein cascade with multiple effector proteins of which stoichiometric balance must be maintained instead of a single effector protein. This means that more plasmids are required to synthesize the Cas proteins for FALCON and hence a more complex plasmid insertion and recollection process and greater chance of error occurrence. Currently, FALCON's stoichiometric balance is well maintained through tandem purification with little to no protein byproduct in the final

assembled system (figure 10). However, the purification step of FALCON still must be conducted as a two-step instead of a one-step process, increasing the time and cost required.

3.3.2. Ability to distinguish variants unconfirmed

In theory, type I-A CRISPR has a high sensitivity and specificity for differences in the target sequence on single nucleotide levels. The current FALCON system is only tested with an Omicron N gene sequence as its crRNA as the Delta strand genetic sequence is currently unavailable in China (where the study was conducted). Since the N gene of the SARS-CoV-2 Delta variant contains a portion directly after the PAM sequence that is not found in the Omicron variants, FALCON's crRNA can be designed using a genetic sequence that contains this portion to distinguish among the 2 variants. This requires synthesis of 2 types of FALCON systems, one containing a crRNA with Delta genetic sequence and one with Omicron genetic sequence. As Cas proteins remain unchanged, only one more set of plasmids with the same design as current crRNA plasmids is required in addition to existing ones. The additional plasmid will not be involved in protein overexpression and therefore does not interfere with current plasmid miniprep and purification processes. To differentiate among variants, 2 samples will be obtained and tested separately with the 2 FALCON systems. The solution that fluoresces (solution with Delta/Omicron crRNA) indicates the type of variant detected in the sample. As the test for variants can be conducted simultaneously, the total testing time is not subject to increase.

3.3.3. Ability to address inaccurate results from RT-PCR unconfirmed

As the heat map (figure 14) suggests, FALCON currently has results consistent with that of RT-PCR and therefore its ability to conduct detections with higher accuracy and flexibility than RT-PCR remains unconfirmed. In subsequent studies, the RT-PCR pseudovirus tests will be run with a larger sample size with false positive and false negative results identified and re-tested by FALCON. In case of little to no inaccurate result occurrence in RT-PCR tests, FALCON will then be tested with samples that mimic situations more prone to resulting in false positives (vaccinated population/recovered patients). Samples that represent these situations can be created with small amounts of viral spike protein resembling vaccine content or virus remains in recovered patients, which FALCON is predicted to identify as negative. As most PCR-tested false negative results are due to the low viral load, FALCON would produce positive results in such situations because of its low detection threshold. Furthermore, FALCON's detection mechanism minimizes the possibility of obtaining false negative results on its own as its target strand recognition is highly specific.

4. Future Implications

4.1. FALCON self-detection kits

FALCON can be made into convenient self-detection kits similar to currently available antigen self-testing kits. The materials required are a preserved FALCON-reporter solution, a heating pack for incubation, materials for sample obtaining (i.e., cotton swabs) and a fluorescence chart for comparison (a higher sample viral load produces a greater fluorescence as shown in figure 13). The minimal instrument requirements of FALCON allow testing to be conducted in any condition where a temperature between 45 to 85 degrees Celsius and light (to excite fluorophores prior to sample addition) are available. The results produced will be either fluorescent (visible to the naked eye) or not. The proportionality between fluorescence level and viral load, however, may not accurately reflect the actual viral load in the patient due to the uncertainty in the quality of self-obtained samples.

4.2. Detection of more pathogens

Existing CRISPR detection platforms (e.g., DETECTR and SHERLOCK) are able to detect for both DNA and RNA viruses in addition to SARS-CoV-2, including HPV (variants 16 and 18), ZIKV (zika virus), PRV (pseudorabies virus), JEV (Japanese encephalitis virus), and DENV (Dengue virus)

(Stower, 2018). FALCON, a prototype developed for specific detection of SARS-CoV-2, can be developed into other models and applied in detections of other pathogens with a modification in its crRNA. Similar to the modifications necessary for differentiation of variants, a new model of FALCON can be synthesized with only a new plasmid for crRNA while all plasmids for Cas proteins remain unchanged. FALCON's high sensitivity would enable detection of a wide range of viruses with a low viral load, meaning that it can be used to detect highly infectious viruses such as HIV and DENV in its early stages and enable proper disease control. FALCON can also be applied as a more efficient alternative to current DNA testing methods for genetic diseases, allowing for accurate diagnosis and early treatment.

4.3. Synthesis of additional archaeal CRISPR systems

The chimeric repeat-spacer technique used in this study can be applied in the study of other archaeal CRISPR systems that are currently unavailable for synthesis due to Cas6 inactivity. Other challenges such as ensuring cascade functionality may still remain, but guide RNA compatibility is guaranteed through the use of chimeric repeat-spacer. Both nucleic acid detection and genome editing functions of archaeal type I-A systems can be further developed with future experiments.

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