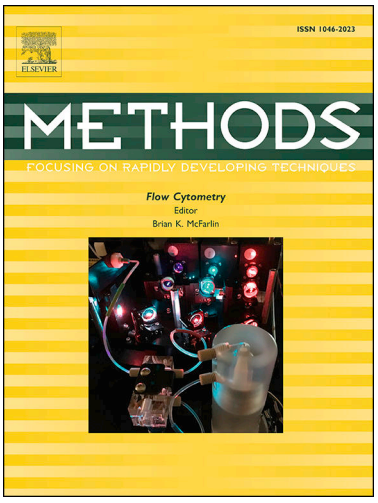
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CRISPR-ENHANCE: An enhanced nucleic acid detection platform using Cas12a

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Abstract

Rapid detection of nucleic acids is essential for clinical diagnosis of a wide range of infectious and non-infectious diseases. CRISPR-based diagnostic platforms are well-established for rapid and specific detection of nucleic acids but suffer from a low detection sensitivity without a target pre-amplification step. Our recently developed detection system, called CRISPR-ENHANCE, employs engineered crRNAs and optimized conditions to achieve a significantly higher sensitivity and enable femtomolar levels of nucleic acid detection even without target pre-amplification. Using the CRISPR-ENHANCE platform and following the methodology detailed in this paper, nucleic acid detection for low copy numbers can be achieved in less than an hour through either a fluorescence-based detection or a lateral flow assay. The step-by-step instructions provided, in addition to describing how to perform both assays, incorporate details on a LAMP/RT-LAMP-based target amplification step to enable detection of RNA, ssDNA and dsDNA. Furthermore, a protocol for in-house expression and purification of LbCas12a using CL7/lm7-based affinity chromatography, which has been used to achieve a high yield and purity of the enzyme in a single-step, is provided.

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1. Introduction

Originally discovered to function as a prokaryotic immune response against viral invasion, CRISPR/Cas (Clustered Regularly Interspaced Palindromic Repeats/ CRISPR-associated) systems have become a critical toolkit in genome editing [1-5]. Prokaryotes incorporate viral DNA into their genome as CRISPR and generate RNA transcripts to bind Cas enzymes, allowing them to specifically target the viral DNA/RNA complementary to the transcript [6]. Such high specificity DNA cleavage offers a multitude of applications in numerous fields of research, industry, and medicine [7-10].

As new CRISPR/Cas systems are being unraveled from all domains of life, a particularly interesting group of Class 2, type V, and VI Cas systems, such as Cas12 and Cas13, show promise for the future of diagnostics in addition to efficient gene editing capabilities. These single effector proteins indiscriminately cleave any single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA), known as trans-cleavage, once bound to the target and having executed target cleavage or cis-cleavage. The trans-cleavage phenomenon has been widely harnessed for the detection of various nucleic acids associated with infectious diseases and cancers (Fig. 1a) [11-18]. DETECTR (DNA endonuclease-targeted CRISPR trans reporter) and SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) are the most prominent CRISPR detection techniques and have been received Emergency Use Authorization (EUA) in the US by the Food and Drug Administration (FDA) [19, 20]. While DETECTR relies on Cas12a to target dsDNA and subsequently activates its trans-cleavage towards ssDNA reporter, SHERLOCK employs Cas13a to achieve the same event but with ssRNA. In general, the detection of nucleic acids within a sample by CRISPR techniques only requires two main components: CRISPR/Cas complex and a reporter. A fluorescence-based or a lateral-based reporter can be used depending on the end-point readout. Often, when low nucleic acid copies are present, an isothermal pre-amplification step such as LAMP/RT-LAMP (Loop-mediated isothermal amplification/ Reserve transcription - Loop-mediated isothermal amplification) or RPA/RT-RPA (Recombinase Polymerase Amplification/ Reserve transcription - Recombinase Polymerase Amplification) is employed to generate detectable amplicons (Fig. 1b).

Here we detail the methods of developing the CRISPR-ENHANCE (**ENH**anced **A**nalysis of **N**ucleic acids with **C**rRNA **E**xtensions) system which utilizes LbCas12a (a Cas12a variant from *Lachnospiraceae bacterium ND2006)* and an engineered guide crRNA (CRISPR RNA) to enhance the trans cleavage capabilities of Cas12a, thus amplifying its ability to detect specific DNA sequences within a sample. The CRISPR-ENHANCE system utilizes a crRNA modified by the addition of 7 DNA nucleotides to the 3' end. This universal 7-mer extension of the crRNA is thought to alter the conformation of LbCas12a after cis cleavage of the target, such that the endonuclease domain remains exposed to the solvent thus enhancing the trans-cleavage [21]. By utilizing LbCas12a target activated trans-cleavage in combination with FRET-based reporter DNA molecules, it is possible to detect trace amounts (pM concentrations) of a target DNA. When incorporated with an isothermal amplification step such as LAMP/RT-LAMP, the ENHANCE is able to detect samples down to low copies comparable to traditional qPCR but in less than an hour of reaction time without requiring the use of specialized equipment (Fig. 1).

ENHANCE provides several advantages over existing methods utilizing wild-type CRISPR/Cas12a. For instance, the 7-mer DNA extension is a simple and universally spacer-independent tag to the 3'-end of crRNA, which works with a wide range of magnesium concentrations, an essential divalent cation for metal-dependent Cas proteins. Here we detail CRISPR detection reaction for ENHANCE working robustly in 3 mM Mg^{2+} condition. More importantly, ENHANCE enhances the sensitivity of detection while slightly increasing the specificity of LbCas12a target binding. As CRISPR-based diagnostics are evolving, ENHANCE can potentially contribute to the rapid development of a fast, sensitive, and low-cost test kit for detecting various diseases.

2. CRISPR-ENHANCE detection workflow

Figure 2 summarizes the CRISPR-ENHANCE workflow. The technique below provides a methodology for the general audience who seeks to produce essential components for the CRISPR-ENHANCE assay.

2.1 Guide design and synthesis

Conserved DNA sequences present in body fluids are prominent biomarkers of various infectious diseases and cancers [22-24]. In order to detect these biomarkers with high specificity and sensitivity, the CRISPR-ENHANCE system relies on the proper design of crRNA. Multiple software platforms are available for generating viable crRNA sequences for particular targets [25]. These generated sequences can then be screened for optimal specificity for the target. In engineering the CRISPR-ENHANCE system, Benchling was utilized to generate all possible crRNAs for Cas12a targeting the various activators tested, such as HIV, HCV, and SAR-CoV-2.

Three protospacer criteria were considered in selecting the optimal crRNA for a given activator: the Protospacer Adjacent Motif (PAM) recognized by LbCas12a, crRNA complimentary protospacer guanine (G)/cytosine (C) content, and length of protospacer. Our prior studies suggest that PAM composed of TTTG is optimally recognized by LbCas12a [21,26]. Improved trans-cleavage was observed when crRNA complimentary protospacer strands contained GC content higher than 50%. Finally, a protospacer length of approximately 20 nucleotides was seen to improve crRNA binding. Since the CRISPR-ENHANCE uses a chimerically DNA-extended crRNA, the sequence can be ordered as oligos through a variety of vendors. Once the optimal crRNA is selected, an extended 7-mer DNA with the sequence 5'-TATTATT-3' is added to the 3'-end of the crRNA to construct the ENHANCE version.

2.2. Recombinant protein expression and purification

Commercial Cas12a is available from New England Biolabs (NEB) for researchers interested in conducting small scale assays. However, for those seeking large scale applications, in-house expression and purification offers a more cost-effective alternative. In synthesizing Cas12a for the CRISPR-ENHANCE system, CL7/IM7 affinity chromatography was used to yield high purity enzymes in a single step (Fig. 3) [27].

2.2.1. LbCas12a-CL7 plasmid construction

Here, we employed a chromatographic technology CL7/Im7 for purification of LbCas12a in a single step. CL7 is a mutated variant of the Colicin E7 DNase (CE7) developed by Trialtus Bioscience. CE7 and its inhibitor Immunity protein (Im7) have a high affinity with Kd of approximately 10^{-4} – 10^{-17} M [25]. To construct a CL7-tagged LbCas12a expression vector, a CL7-tagged plasmid is obtained from TriAltus Bioscience (Plasmid #21) and linearized with HindIII and XhoI restriction enzymes. The resulting linear DNA is purified using a Monarch® DNA Cleanup Kit (New England Biolabs). Next, the LbCas12a gene fragment from the LbCpf1-2NLS plasmid (a gift from Doudna Lab, Addgene # 102566 [28]) is amplified via Polymerase Chain Reaction (PCR) with the following primers:

Forward Primer: CTGAAACTGGTTCTAGCGATGAGCAAACTGGAGAAGTTCACG

Reverse Primer: GTGGTGGTGGTGGTGTTAGTCCACTTTCCGCTTTTTCTTAGGATCT

The PCR product is purified by gel extraction using a Monarch® Gel Extraction Kit from New England Biolabs. The PCR amplified LbCas12a fragment is inserted into the linearized CL7-tagged plasmid and ligated using a NEBHiFi® Builder kit following the manufacturer's procedure. The cloning product is then transformed into DH5α competent cells and inoculated overnight. The final CL7-tagged Lbcas12a plasmids are then extracted from harvested cells using Monarch® Miniprep kit. The presence and orientation of the LbCas12a gene into the CL7-tagged plasmid are confirmed by restriction enzyme assay or Sanger sequencing.

2.2.2. Protein expression

To express LbCas12a, BL21 (DE3) competent cells are transformed with the constructed plasmid via heat shock following the manufacturer's instructions.100 µL of transformed cells are spread on a pre-warmed LB agar plate containing 50 µg/ml Kanamycin and incubated overnight at 37°C. The next day, individual colonies are picked and inoculated in culture tubes containing 15 mL TB broth and Kanamycin. The culture tubes are incubated at 37°C while being shaken at 250 rpm.

In an optional step, small scale test runs with 10 mL of culture are performed to select colonies that express the high amount of LbCas12a. Cells are allowed to grow until OD600 of the culture reaches 0.5-0.6. The 10 mL of the culture is equally divided into 2 x 5 mL aliquots, then 2.5 µL of 1 M IPTG is added to one of the aliquots to induce protein expression. Both the aliquots are incubated at 21°C and 250 rpm for another 3-4 hours. The cells are pelleted by centrifugation, lysed by sonication, and the lysate is used to run a protein gel to determine optimal colonies.

The remaining 5 mL of the original 15 mL culture from an optimal colony, is added to 1.05 L of TB broth. Until an OD600 of 0.5-0.6 is reached, the culture is incubated at 37°C while being shaken at 250 rpm. Approximately 50 mL of culture is stored in 5 mL aliquots before induction with 1 mL of 1 M IPTG. After the induction step, the culture is incubated in a refrigerated shaker at 18°C and 250 rpm for 14-18 hours. Finally, the culture is centrifuged at 10,000 x g for 5 minutes to pellet cells before purification or storage of the cell pellet at -80°C.

2.2.3. LbCas12a purification

All buffers used for protein purification are listed in Appendix B. Purification of LbCas12a from the cell pellet begins with weighing the pellet and resuspending the pelleted cells in lysis buffer (10 mL per 1g cell pellets). The resulting cell suspension is sonicated on ice for 15 minutes (50% power amplitude, 1 second on 1 second off) to disrupt the cells. The lysate is centrifuged at 40,000 x g for 30 minutes at 4°C then filtered through a 0.45 µm filter using a 20 mL syringe.

Chromatography is then used to isolate the desired LbCas12a from other components in the lysate. First, the column is connected to the FPLC and equilibrated using 10 mL of Wash Buffer A at a rate of 0.75 mL/min. The column is then loaded with lysate by direct injection at a rate of 0.3 mL/min. The flow-through lysate can be reloaded for maximum protein binding. The column is then washed three times with 8 mL of Buffer A followed by 8 mL of Buffer B. This cycle is repeated three times. Alternate washing of high-salt and no-salt buffer helps eliminate a majority of contaminants in the column. The column is then eluted using 5 mL of Elution Buffer C containing SUMO protease at 0.2 mL/min for 1.5 hours. Alternatively, the column can be detached from the FPLC and connected to a peristaltic pump. In this case, Elution Buffer C containing SUMO protease is run in a closed-loop cycle.

The eluted protein is then concentrated in a 30 kDa MWCO Sartorius Vivaspin Concentrator. The concentrated eluant is then aliquoted, flash-frozen, and stored at -80°C after the concentration is determined using a nanodrop with the following settings: MW = 151 KDa and extinction coefficient = 181690 M-1cm-1. Optionally, to obtain highly pure LbCas12a an additional size exclusion chromatography can be performed to remove the small amount of SUMO protease in the concentrated eluant. The Im7 column is then denatured by running 10 mL of Denaturing Buffer D at 0.5 mL/min and then regenerated with Refolding Buffer E at 1 mL/min for 1 hour.

2.2.4. LAMP/RT-LAMP reaction

2.2.4.1. LAMP primer design

A set of 6 LAMP primers can be designed using the PrimerExplorer software (available for free at https://primerexplorer.jp/e/) [29]. Often, the software picks LAMP primers, which do not flank the targeting region of the crRNA or overlap with the target region. In this case, it is crucial to manually choose the F1 and B1 primers such that they flank the target region of the crRNA while following parameter constraints such as Tm and GC contents for those primers. To determine optimal primers for the LAMP/RT-LAMP reaction, 4-5 sets of LAMP primers can be screened whenever possible.

2.2.4.2. Sample extraction

For infectious diseases, we recommend using commercially available CDC recommended extraction kits. Use appropriate kits following company's instructions. For cancer detection, optimization of extraction protocol may be required.

2.2.4.3. LAMP/RT-LAMP reaction

Before LAMP/RT-LAMP reaction, we recommend preparing 10X LAMP primers mix by combining 6 primers as shown in Table 1 and aliquot them. The primers can be stored at -20°C for up to 2 years. For the LAMP/RT-LAMP reaction, create the LAMP/RT-LAMP master mix by combining the appropriate amounts of reagents in Table 2. It is recommended to add the LAMP/RT-LAMP master mix in a PCR air-clean workstation. To initiate the reaction, 20 µL of the master mix is transferred to either PCR strips or a 96-well PCR plate. In a clean work area (preferably a biosafety cabinet), add 5 µL of appropriate DNA/RNA templates to each 20 µL reaction. Finally, add 5 µL of the positive template to the positive control well, 5 µL of nuclease-free water to the NTC (non-template control), and incubate the reactions at 65°C for 30 minutes. The first round of detection is the color change of the LAMP reaction (pink if negative, yellow if positive). However, for low copy samples, the color change is not significant.

Table 1

Primer	10x Primer mix concentration
F3	2 µM
B3	2 µM
FIP	16 µM
BIP	16 µM
LF	8 µM
LB	8 µM

Table 2

	1 reaction	1.1N reaction
Warmstart Colorimetric LAMP 2X Master Mix with UDG	12.5 µL	12.5 x 1.1N µL
10x LAMP Primer mix	2.5 µL	2.5 x 1.1N µL
Nuclease-free Water	5 µL	12.5 x 1.1N µL
Total	20 µL	22N µL

2.2.5. CRISPR-ENHANCE reaction for fluorescence reading

The following recipe is used for a 384-well plate format. For a 96-well plate format, the volume of all reagents below should be scaled up 2.5 times. Before performing the CRISPR-ENHANCE reaction, mix LbCas12a, crRNA, and NEBuffer 2.1 to form a LbCas12a:crRNA complex by combining the appropriate volumes of reagents specified in Table 3 for the desired number of reactions. The master mix is incubated at 25°C for 10 minutes and 12 µL

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are transferred to each PCR strip tube. The reporter solution is prepared by adding 0.2 µL of the 100 µM FQ to 25.8 µL of nuclease-free water. For N reactions, a master mix can be made with 10% excess. While the LbCas12:crRNA complex is incubating, 26 µL of the reporter solution is transferred to a 384-well plate. 2 µL of the RT-LAMP/LAMP products are added to each well of the plate by using the adjustable spacing INTEGRA pipette. Immediately, 12 µL of the Cas complex mixture are transferred to each well of the plate again using the adjustable spacing INTEGRA pipette. Finally, the plate is sealed with an optical adhesive film and immediately placed in a microplate reader to obtain a fluorescence reading.

Table 3

	1 reaction	1.1N reaction
3 µM LbCas12a	0.4 µL	0.4 x 1.1N µL
3 µM crRNA	0.8 µL	0.8 x 1.1N µL
10X NEBuffer 2.1	1.2 µL	1.2 x 1.1N µL
Nuclease-free Water	9.6 µL	9.6 x 1.1N µL
Total	12 µL	13.2N µL

2.2.6. CRISPR-ENHANCE reaction for paper strip readout

To obtain paper strip readouts of the CRISPR-ENHANCE reaction, the reagents in Table 4 are combined in the appropriate volumes for the desired number of reactions. 48 µL of the master mix are transferred to each PCR strip tube followed by 2 µL of RT-LAMP/LAMP products. The reaction mixture is incubated for 20 minutes at 37°C. The paper strips are then placed into each tube and a readout is observed after 1-2 minutes.

Table 4

Reagent	1 reaction	1.1N reaction
3 µM LbCas12a	0.5 µL	0.5 x 1.1N µL
3 µM crRNA	1 µL	1 x 1.1N µL
10X NEBuffer 2.1	5 µL	5 x 1.1N µL
5 µM FAM-Biotin reporter	2 µL	2 x 1.1N µL
Nuclease-free Water	39.5 µL	39.5 x 1.1N µL
Total	48 µL	52.8N µL

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2.2.7. Analysis

For the fluorescence-based assay, a sample is considered positive if the fold change of its background-subtracted fluorescence signal in relative fluorescence unit (RFU) to that of the NTC control is greater than or equal to 5 within the first 20 minutes of the reading. The determination of absence or presence of target within the sample can be adjusted based on several factors such as sample type and purity. For lateral flow assay, a sample is positive if there is a clear distinct band appearing on the positive line of the paper strip and/or the control line. The strip can be further analyzed using imageJ to quantify the ratio of positive to the control line. If non-template controls (NTCs) display a positive signal in a fluorescence-based or a lateral flow-based assay, indicating false positives, the most probable cause is the contamination of LAMP/RT-LAMP reaction. In that case, the extraction and LAMP steps should be repeated with a new set of reagents. Also, use a separate workstation or a clean hood for the amplification step that is frequently cleaned with RNase AWAY or 10% bleach. In the protocol above, Warmstart® Colorimetric LAMP 2X Master Mix with UDG (NEB) is recommended for the LAMP reaction as it contains Uracil-DNA Glycosylase (UDG) and UTP, which greatly reduces carry-over contamination.

3. Conclusion

Here we discuss an engineered CRISPR-based detection platform called ENHANCE for improved detection. The CRISPR-based detection system is a robust and specific approach for diagnostics of infectious diseases and cancer. When coupled with an isothermal amplification step, the CRISPR-based detection system provides two separate checkpoints for determination of biomarker presence in the sample. First, the LAMP primer set is designed to specifically recognize the target region and allows for amplification. The CRISPR:LbCas12a complex then binds to amplified targets and executes double-stranded cleavage thus triggering LbCas12a trans-cleavage activity. The ENHANCE approach further intensifies either fluorescence signals or lateral flow readout compared to the wild-type CRISPR/Cas12a system while maintaining its specificity.

As CRISPR-based detection platforms are becoming widely used due to their versatility of adopting different amplification methods and end-point signals, one might wonder how to pick the appropriate techniques suitable for detecting cancer or an infectious disease. LAMP and RPA are powerful isothermal techniques for quickly amplifying nucleic acid targets for CRISPR detection. These two methods are isothermal, and amplification can be achieved using a simple heat block. These are particularly useful for remote areas and home-based settings, where an expensive thermal cycler is not an option. They are also faster than traditional polymerase chain reaction (PCR) with sufficient amplicons being generated for detection in as little as 10 minutes. Therefore, they are often utilized for the development of rapid detection kits. However, these isothermal amplification methods present some limitations. Due to its high sensitivity, LAMP often leads to carry-over contamination when good lab practices cannot be accomplished. For this reason, it is

always recommended that the LAMP is performed in a clean and designated workstation. Also, the LAMP requires 4-6 primers, 2 of which facilitate the formation of stem-loop structures, increases chances for primer-dimer formation. Therefore, an optimal set of LAMP primers must be used to avoid non-template amplification. RPA is an isothermal amplification alternative to LAMP and has been used in SHERLOCK [13-15]. However, RPA is expensive and difficult to scale up for massive testing as it contains multiple enzymes working together to generate amplicons from the nucleic acid template. Additionally, RPA is only offered by a single commercial vendor TwistDx Ltd., which limits the availability of the kit globally.

Fluorescence-based and lateral flow assays provide CRISPR detection platforms an advantage over other traditional diagnostic techniques due to their rapid results and turnaround time. However, with the fluorescence reporter assay, a plate reader or other capable fluorescence reading instruments are needed, which can be quite expensive. This limits the usability of the technique in some remote areas or home-based settings. In these situations, lateral flow assay becomes a powerful alternative to the fluorescence-base reading because it provides visual results on low-cost paper strips in under 2 minutes.

Finally, similar to other Cas12a-based detection methods such as one described by Broughton et al. [12], ENHANCE requires separate steps between isothermal amplification LAMP and CRISPR detection. This leads to a high chance of contamination while the amplified product is transferred from one tube to another. This practice becomes a disadvantage and needs further development. Therefore, besides recommending working in a clean and separate workstation for the LAMP reaction, we have also introduced UDG and UTP in the LAMP master mix to reduce the chances of carry-over contamination. Recent developments also saw a great deal of this problem being fixed such as STOPCovid, which utilizes thermostable AapCas12a to combine with RT-LAMP reagents into a one-pot reaction [16]. The cold chain is a big concern with the deployment and storage of diagnostic kits and samples as it adds significant costs and reliability concerns. To solve these challenges, advanced versions of ENHANCE, including a lyophilized version, are under development. All in all, ENHANCE provides an alternative approach to the development of rapid CRISPR detection towards various diseases.

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The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated, NR-52287.

Appendices

A. Equipment and Reagents

Equipment

1. Water bath (Fisher Scientific, cat no. FSGPD05)
2. Constant temperature incubator (Fisher Brand, cat no. 02-217-753)
3. Culture flask (Nalgene, cat no. 4102-0500)
4. Biological incubator and shaker (Barnstead, cat no. SHKE4000-7)
5. Fisher brand disposable cuvette (Fisher Brand, cat no. 1495-5129)
6. Floor Centrifuge (Beckman Coulter, cat no. BLE1900124)
7. Gel electrophoresis system (Fisher Brand, cat no. S98616)
8. Sonicator (Branson, cat no. ux-04715-04)
9. Shaker/incubator (Thermo Electron Corporation, cat no. 12101)
10. CL7 column (Trialtus Bioscience)
11. Sartorius Vivaspin Concentrator (MWCO=30 KDa) (Sartorius, REF# - VS2021)
12. Microcentrifuge tube (Fisher Brand, cat no. 05408-129)
13. Fast protein liquid chromatography (FPLC) (BioLogic Duoflow, cat no. 7600047)
14. Aluminum foil (Fisher Brand)
15. 0.22 um filter (Restek, cat no. 26144)
16. 20-mL syringe (Fisher Brand, cat no. 1495-5460)
17. Nanodrop (Thermo Scientific, cat no. ND-ONE-W)
18. 96-well plates (Greiner Bioone, cat no. 655-076)
19. 384-well plates (Greiner Bioone, cat no. 5678-1076)
20. Optical adhesive film (USA Scientific, cat no. 2978-2700)
21. Falcon Tubes (Thermo Scientific cat no. 339650)
22. UVP GelStudio Plus (Analytik Jena, cat no. 849-00553-4)

Reagents

1. LbCpf1-2NLS Plasmid (Addgene, 102566)
2. Terrific Broth (Thermo Scientific, 22711022)
3. Plasmid 21 (Trialtus Bioscience)
4. Plasmid Doudna (Trialtus Bioscience)
5. Kanamycin (Fisher, 25389-94-0)
6. DH5α competent E. Coli cells (New England Biolabs, C2987H)
7. BL21 (DE3) competent E. Coli cells (New England Biolabs, C2527H)
8. Lysozyme (Fisher, 12650-88-3)
9. Monarch® PCR&DNA Cleanup Kit (New England Biolabs, T1030L)
10. Monarch® Plasmid Miniprep Kit (New England Biolabs, T1010L)
11. Deoxyribonuclease 1 (bovine pancreas) (Worthington, LS002139)
12. Protease Inhibitor Tablet (Thermo Fisher, A32965)
13. Sodium Chloride (Fisher Chemical, S271-3)
14. Tris-HCl (Fisher Chemical, BP153-500)
15. HEPES (Fisher Bioreagents, BP310-1)
16. TCEP (UBP Bio, P1020-25)

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17. Magnesium Chloride Hexahydrate (Fisher Chemical, BP214-500)
18. Isopropyl ß-D-1-thiogalactopyranoside (Fisher Chemical, BP1620-10)
19. SUMO Protease (Trialtus Bioscience)
20. Glycerol (Fisher Chemical, BP229-1)
21. Antifoam 204 (Sigma Aldrich, 1002923239)
22. Isopropyl β-D-1-thiogalactopyranoside (IPTG, Fisher Chemical, BP1755-10)
23. Ultra-Pure Water (Invitrogen, 10977-615)
24. SurePage Bis-Tris 4-12% (GenScript, M00654)
25. Reducing Agent (Invitrogen, B0009)
26. GenBox Mini Electrophoresis Tank (GenScript, L00780)
27. Bacto-Agar (BD, 214010)
28. Tris-MOPS-SDS Running Buffer (GenScript, M00138)
29. Glacial Acetic Acid (Fisher Chemical, A38-212)
30. Methanol (Fisher Chemical, A454SK-4)
31. Coomassie Blue R-250 (Thermo Scientific, 20278)
32. RNase Away (Molecular Bioproducts, 7002)
33. Sodium Hydroxide (Fisher Chemical, BP359-500)
34. Lysogeny Broth (Fisher Chemical, BP1426-500)
35. Q5-Hot Start High Fidelity Master Mix (M04945)
36. NEBHifi Builder (New England Biolabs, E26215)
37. Forward Primer (Integrated DNA Technologies)
38. Reverse Primer (Integrated DNA Technologies)
39. crRNA (Integrated DNA Technologies)
40. FAM-Quencher Reporter, /56-FAM/TTATT/3IABkFQ/ (Integrated DNA Technologies)
41. FAM-Biotin Reporter, /56-FAM/TTATTATT/3Bio/, (Integrated DNA Technologies)
42. Milenia Genline HybriDetect (Milenia Biotec, 2022-02)

Software

1. Biologic (Biorad)
2. Gen5 (Biotek)
3. ImageJ (National Institutes of Health)
4. VisionWorks (Protein visualization)

B. Buffer Preparation for Protein Purification

Buffer	Reagents	Concentration
Wash Buffer A (pH 7.5)	NaCl	2 M
	Glycerol	5% (v/v)
	Tris-HCl	50 mM
	TCEP	0.5 mM
Wash Buffer B (pH 7.5)	Glycerol	5% (v/v)
	Tris-HCl	50 mM
	TCEP	0.5 mM
Lysis Buffer (pH 7.5)	NaCl	2 M

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	Glycerol	5% (v/v)
	Tris-HCl	50 mM
	TCEP	0.5 mM
	PMSF	1 mM
	Lysozyme	0.25 mg/mL
	Protease inhibitor tablet	1 tablet per 30-40 mL lysate
Elution Buffer C (pH 7.5)	NaCl	0.5 M
	Glycerol	5% (v/v)
	Tris-HCl	50 mM
	TCEP	0.5 mM
Denaturing Buffer D	Guanidine Hydrochloride	6 M
Refolding Buffer E (pH 7.5)	NaCl	0.5 M
	Glycerol	5% (v/v)
	Tris-HCl	50 mM

C. Troubleshooting

Problem	Possible causes	Solutions
Protein expression and purification		
No protein observed on SDS-PAGE gel after elution step	1. Incorrect cloning 2. Protein not bound to column 3. Incorrect colonies	1. Reconstruct the LbCas12a-CL7 fusion plasmid. 2. Perform Sanger sequencing to make sure the CL7 tag and LbCas12a are in frame. 3. Remake Kanamycin solution and pick new colonies.
Low yield of protein	Protein does not bind properly to the column	Decrease the amount of sodium chloride in the bind and wash buffer from 2M to 1M.
Protein with high level of contamination	1. Protein is not well-expressed. 2. Not enough wash of high salt and no salt cycles	1. Optimize IPTG concentration 2. Increase wash step cycles.
LAMP/RT-LAMP reaction		
NTC shows positive	1. Carryover contamination	1. Properly clean workspace with RNAse Away. Perform LAMP/RT-LAMP in a clean designated area such as in a PCR station. Remake all reagents or replace with new reagents. Incorporation of UDG and dUTP in the LAMP master mix reduces contamination.
No amplification product	1. LAMP reagents and primers have degraded. 2. Insufficient time	1. Avoid multiple free-thaw cycles. Remake primer mix and replace LAMP reagents. It is important to include positive control in the assay. 2. Increase reaction time. 3. Optimize reaction temperature

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CRISPR reaction		
Internal Control not detected (QC failure)	1. Sample extraction step failed.	Re-extract samples.
Low Fluorescence signal	1. Low number of copies of the target. 2. CRISPR reagents have degraded.	1. Increase LAMP/RT-LAMP reaction time 2. Increase crRNA:Cas12a annealing time. Replace with new CRISPR reagents.
High background in lateral flow positive line	High concentration of FAM-Biotin reporter	Reduce concentration of the FAM-Biotin reporter.

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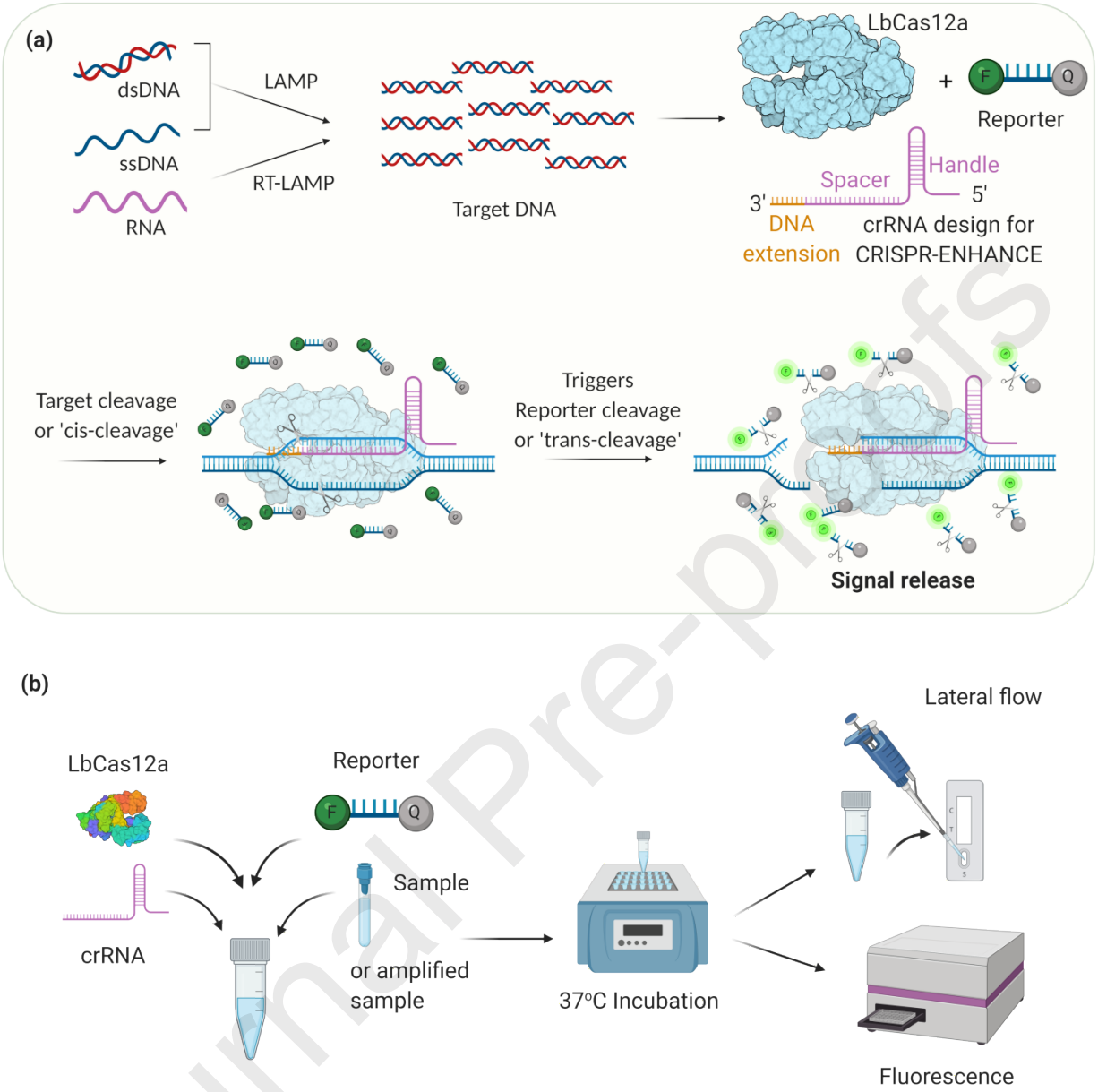


Fig. 1. Schematic diagram for detection using CRISPR-ENHANCE. (**a**) Detection mechanism of CRISPR/Cas12a system. dsDNA or ssDNA sequences can be amplified using LAMP whereas RNA can be amplified using RT-LAMP to make billions of copies of target DNA. The detection assay consists of Cas12a enzyme, a programmable crRNA with a 7-mer DNA extension on the 3' end, and reporter molecules consisting of fluorophore-quencher pairs. Upon PAM recognition and target DNA detection, the CRISPR/Cas system binds to the target DNA to form a R-Loop and performs cis cleavage to cut the target DNA. Cleavage of the target DNA activates collateral cutting of non-specific ssDNA and ssRNA molecules in the vicinity of the Cas enzyme. The reporter molecule shows no signal when intact but emits fluorescence when cleaved by the trans-activated CRISPR-Cas12a complex. (**b**) General CRISPR detection technique. LbCas12a structure is based on PDBID: 5XUS [30]. Created with Biorender.

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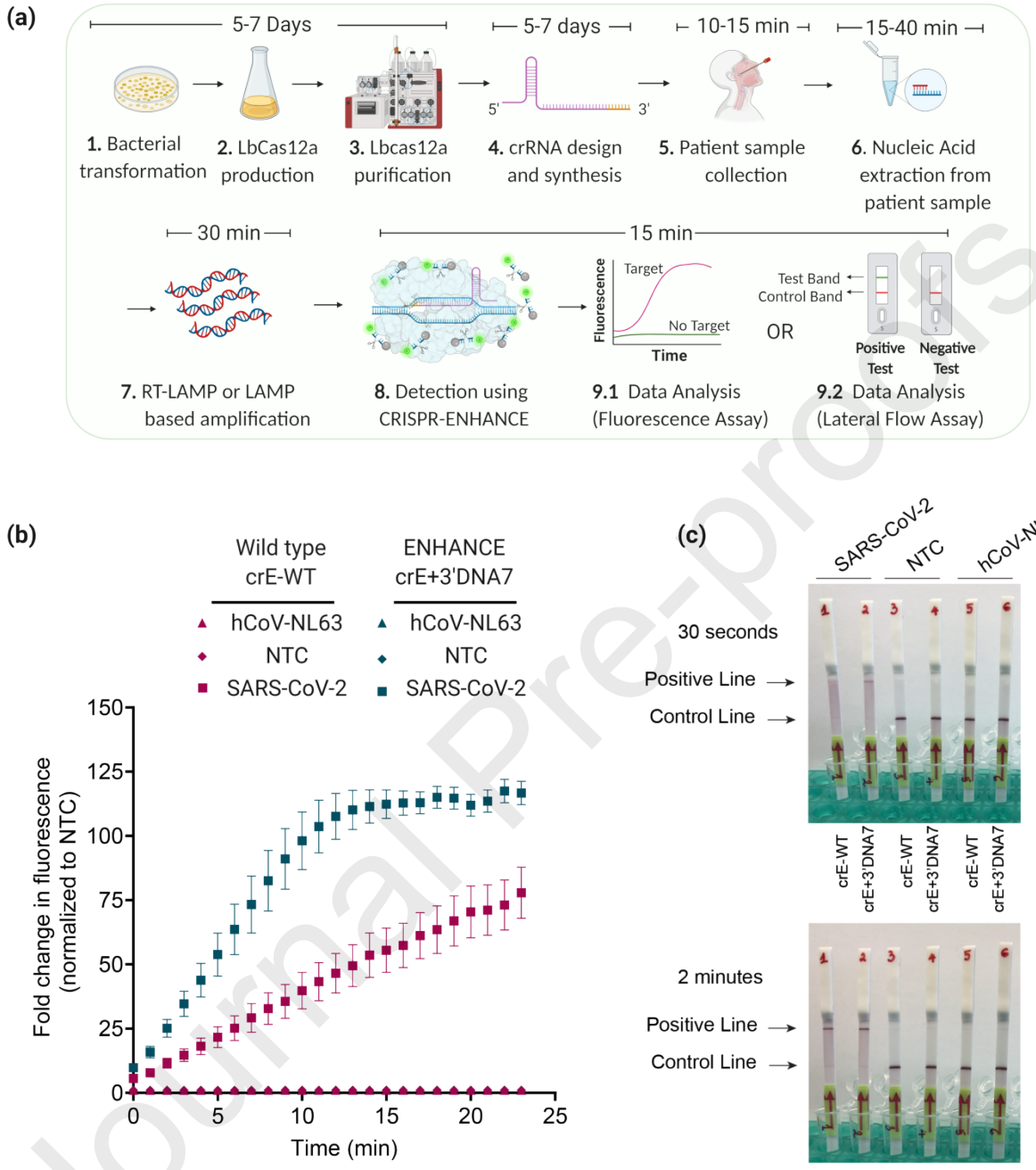


Fig. 2. Workflow for nucleic acid detection using CRISPR-ENHANCE. (**a**) LbCas12a is expressed and purified in house using CL7/Im7 technology followed by sample extraction and CRISPR detection methods. (1) BL21 (DE3) competent E. Coli cells are transformed with CL7-tagged LbCas12a plasmid. (2) Transformed cells are then cultured overnight after induction with IPTG. (3) After 14-18 hours of growth, the cells are then harvested and purified using CL7 affinity FPLC. (4) Guide RNA targeting a particular target DNA sequence is designed and synthesized by Integrated DNA Technologies (IDT). (5) (6). Target nucleic acid is extracted from patient samples

using Center for Disease Control (CDC) recommended commercially available kit (Promega Maxwell RSC Viral Total Nucleic Acid Purification Kit). (7) Extracted nucleic acid are amplified via RT-LAMP. (8) (9) Amplified product is added to CRISPR detection reaction. (**b**) Fold change in fluorescence signal normalized to NTC (non-template control) samples of wild-type and ENHANCE systems towards detecting gamma-irradiated SARS-CoV-2 virus. The gamma-irradiated virus was obtained from BEI Resources, lysed in QuickExtract™ DNA Extraction Solution (Lucigen), and diluted down to 5000 copies/μL. The viral copies were then subjected to RT-LAMP amplification for 30 minutes followed by CRISPR/Cas12a fluorescence reporter detection assay. (**c**) Representation of lateral flow assay on paper strips detecting gamma-irradiated SARS-CoV-2 virus in (b). LbCas12a structure is based on PDBID: 5XUS [30]. Created with Biorender.

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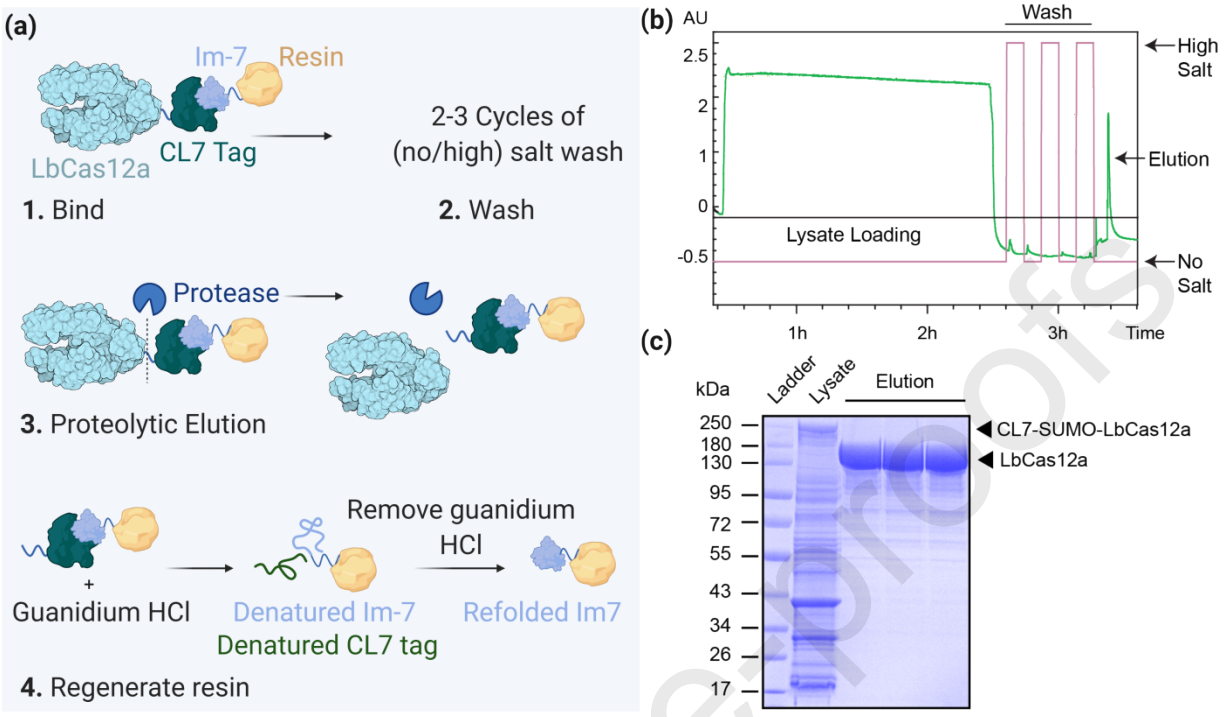


Fig. 3. (**a**) Workflow for CL7/IM7 tag-based protein purification. LbCas12a structure is based on PDBID: 5XUS. (a) The cell lysate is passed through an Im7 column where CL7-tagged LbCas12a binds. The Im7 column is then washed with high salt and no salt buffers for 3-4 cycles. LbCas12a is then eluted by running the elution buffer containing SUMO protease through the column. Finally, the Im7 column is denatured and refolded for next rounds of purification. (**b**) A representation of a chromatogram of LbCas12a purification using the Im7 affinity chromatography. (**c**) SDS-PAGE gel of purified LbCas12a. Created with Biorender.

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