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강태준 Taejoon Kang

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A CRISPR/Cas12 trans-cleavage reporter enabling label-free colorimetric detection of SARS-CoV-2 and its variants

Authors and Affiliations

Hansol Kim [a,b], Hyowon Jang [b], Jayeon Song [b], Sang Mo Lee [a], Seoyoung Lee [a], Hyung-Jun Kwon [c], Sunjoo Kim [d], Taejoon Kang [b,e], Hyun Gyu Park [a]

[a]Department of Chemical and Biomolecular Engineering (BK 21+ program), Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon, 34141, Republic of Korea
[b]Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 125 Gwahak-ro, Yuseong-gu, Daejeon, 34141, Republic of Korea
[c]Functional Biomaterial Research Center, KRIBB, 181 Ipsin-gil, Jeongeup, Jeollabuk-do, 56212, Republic of Korea
[d]Department of Laboratory Medicine, Gyeongsang National University College of Medicine, 79 Gangnam-ro, Jinju, Gyeongsangnam-do, 52727, Republic of Korea
[e]School of Pharmacy, Sungkyunkwan University (SKKU), 2066 Seobu-ro, Jangan-gu, Suwon, Gyeonggi-do, 16419, Republic of Korea

Corresponding authors : Taejoon Kang, Hyun Gyu Park

Abstract

We present a label-free colorimetric CRISPR/Cas-based method enabling affordable molecular diagnostics for SARS-CoV-2. This technique utilizes 3,3′-diethylthiadicarbocyanine iodide (DISC2(5)) which exhibits a distinct color transition from purple to blue when it forms dimers by inserting into the duplex of the thymidine adenine (TA) repeat sequence. Loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA) was used to amplify target samples, which were subsequently subjected to the CRISPR/Cas12a system. The target amplicons would activate Cas12a to degrade nearby TA repeat sequences, preserving DISC2(5) in its free form to display purple as opposed to blue in the absence of the target. Based on this design approach, SARS-CoV-2 RNA was colorimetrically detected very sensitively down to 2 copies/μL, and delta and omicron variants of SARS-CoV-2 were also successfully identified. The practical diagnostic utility of this method was further validated by reliably identifying 179 clinical samples including 20 variant samples with 100% clinical

sensitivity and specificity. This technique has the potential to become a promising CRISPR-based colorimetric platform for molecular diagnostics of a wide range of target pathogens.

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관련 링크

- 강태준님 전체 논문보기
- 연구자 키워드

연구자 ID

ORCID | 0000-0002-5387-6458

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해당논문 저자보기

김한솔 (KAIST, KRIBB)
박현규 (KAIST)