Project Information

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Project Coordinator	(UCAM)
Project Website	https://afridx.ceb.cam.ac.uk/

Deliverable Information

Deliverable Number	3.3
Deliverable Title	Application of Boon-enzymes in RT-LAMP protocol to detect SARS-Cov-2
Workpackage Number	WP3
WP Leader	KNUST
Authors	Dushanth Seevaratnam (UCAM)
Contributors	UCAM
Reviewers	Lisa Hall (UCAM)
Contractual Deadline	30 April 2021
Actual Delivery Date	31 Dec 2021

Delivery Type

R	Report	
DEM	Demonstrator, pilot, prototype, plan designs, new or revised health policies etc	✓
DEC	Websites, patents filing, press & media actions, etc	
OTHER	Other	

Dissemination Level

PU	Public*	✓
RE	Restricted to a group specified by the consortium.	

Document Log

Version	Date	Author	Description of Change
1.0	31/05/2021	Dushanth Seevaratnam	First Iteration of document
1.1	23/05/2022	Dushanth Seevaratnam	Final version of document

Total number of pages:	4

*being prepared for open access publication

List of content

Partner	Contribution to this deliverable
CAM	Engineered, screened, and delivered Boon-Enzymes. Produced accompanying SOPs.
KNUST	Application of Boon-Enzymes in RT-LAMP

1 Status of the Deliverable

The deliverable has not been completed. The plasmids required for local expression of Boon2-BST and Boon-RT have been delivered to KNUST along with the necessary SOPs and training videos. In addition, local manufacturing of the enzymes has been completed. While preliminary testing with the Boon2-BST has been conducted, experimentation with both Boon-enzymes is still in progress.

2 Summary of the results (max. 1-2 pages)

The recombinant BST2.0 and RT plasmids have been delivered to KNUST for evaluation. The KNUST team were able to transform, express (Figure 2.1) and silica-purify the enzymes using the SOPs provided by Deliverable D3.1 and D3.2. In addition, they were able to successfully detect SARS-Cov-2 using a Boon2-BST LAMP assay.

Figure 2.1: Cell pellets of BL21 (DE3) cells with the Boon2-BST DNA polymerase plasmid after 4 hours of induction using IPTG. The pink colouration indicates the expression of the recombinant protein of interest.

3 Description of work performed and obtained results

As of this report KNUST have successful expressed both Boon2-BST and Boon-RT and have conducted further evaluation on the recombinant BST2.0 DNA polymerase.

3.1 Application of Boon-enzymes in RT-LAMP protocol to detect SARS-Cov-2

The Boon-Enzyme plasmids were transferred to KNUST, where it was transformed into locally available BL21 (DE3) bacteria. The provided SOPs from Deliverable D3.1 and D3.2 were utilised by the KNUST team to express the enzymes, as shown in Figure 2.1 and 3.1.

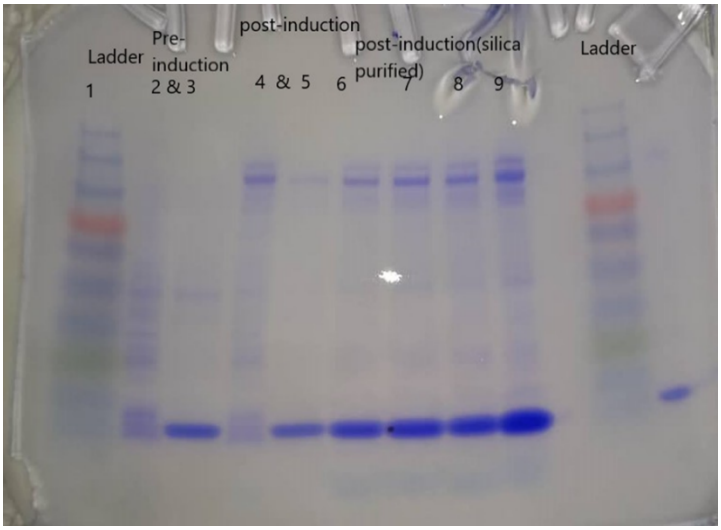
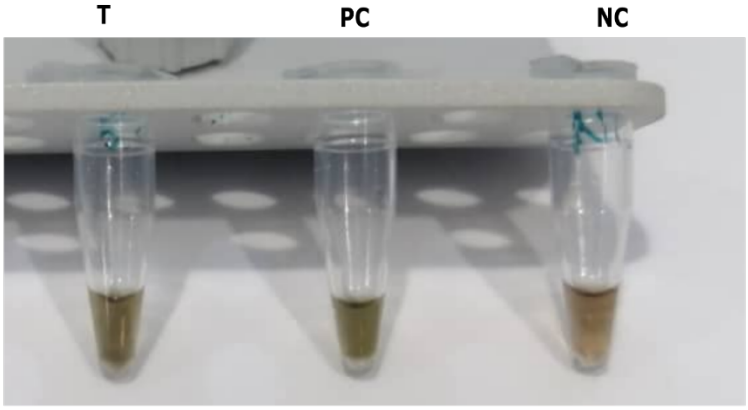


Figure 3.1: SDS-PAGE gel electrophoresis showing the result of silica-purified Boon2-BST DNA polymerase.

SARS-Cov-2 LAMP assays were conducted using silica purified protein, shown in Figure 3.2 and 3.3. Initially, 5 µL of locally produced enzyme was required to match the sensitivity of the commercial assay. However, by iterating with UCAM, the protocol has been updated to improve the overall activity of the enzyme. The current iteration requires only 2 µL of local enzyme per assay.



T:Test = LAMP mastermix + SARS-CoV-2 +ve control + 5ul Bst SiB DNA polymerase
PC: Positive control = LAMP mastermix + SARS-CoV-2 +ve control + commercial Bst DNA pol
NC: Negative control = LAMP mastermix + nuclease free water + commercial Bst DNA pol

Figure 3.2: SARS-CoV-2 LAMP assay using 5 µL of locally produced and purified Boon2-BST DNA polymerase (original protocol).

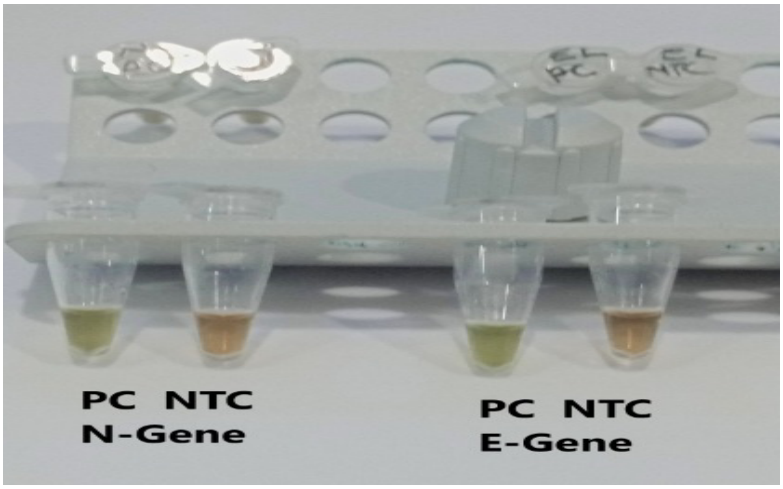


Figure 3.3: SARS-CoV-2 LAMP assay using 2 µL of locally produced and purified Boon2-BST DNA polymerase (modified protocol).

Overall, the evaluation was proven and the initial results promising. Positive and negative samples could be distinguished. The general plasmid to protein path was traversed with little difficulty when set up in KNUST, and the produced enzyme was capable of detecting the virus. Further evaluation will become possible with full sample testing. This was beyond the scope of the workpackage and since new Covid samples are fewer than earlier in the project, it could not be extended to explore the further performance.