EDCTP

Project Information

Grant Code	RIA2020EF-2918
Project Full title	COVID-19 Diagnostics for Africa
Project Acronym	AfriDx
Funding Scheme	EDCTP
Start Date of the Project	1 October 2020
Duration	15 Months
Project Coordinator	(UCAM)
Project Website	https://afridx.ceb.cam.ac.uk/

Deliverable Information

Deliverable Number	3.2
Deliverable Title	Engineered Boon-RT and accompanying expression and purification SOP
Workpackage Number	WP3
WP Leader	UCAM
Authors	Dushanth Seevaratnam (UCAM)
Contributors	UCAM
Reviewers	Lisa Hall (UCAM)
Contractual Deadline	30 April 2021
Actual Delivery Date	31 May 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	24/05/2022	Dushanth Seevaratnam	Final version of document

Total number of pages:	5

*being prepared for open access publication.

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Partner	Contribution to this deliverable
CAM	Engineered and screened Boon-RT. Produced accompanying SOPs.

1 Status of the Deliverable

The deliverable has been completed. The silica-immobilising fluorescent design of the Boon-RT has been synthesised and characterised. This iteration of the enzyme has been delivered to DTU and the plasmid required for local expression of the protein has been delivered to KNUST along with the necessary SOPs.

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

A recombinantly expressed reverse transcriptase has been synthesised with the base silaffin-tag (R5: SSKKSGSYSGSKGSKRRIL) and mCherry template (Figure 2.1). This combination of proteins and tags would allow for low-cost purification (silaffin tag), visual tracking (mCherry), RNA to DNA conversion for efficient nucleic acid amplification (Reverse Transcriptase), and a secondary purification option for additional enzyme characterisation (His-loop).

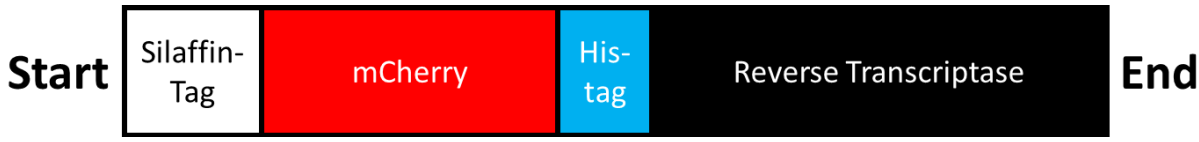

Figure 2.1: Schematic of synthesised Boon-RT.

The Boon-RT was found to be active in solution (shown in Figure 2.2) and while immobilised. It has also been tested with both RT-PCR and RT-LAMP. The plasmid of this synthetic protein has been transferred to KNUST, and purified Boon-RT has been sent to DTU for further characterisation.

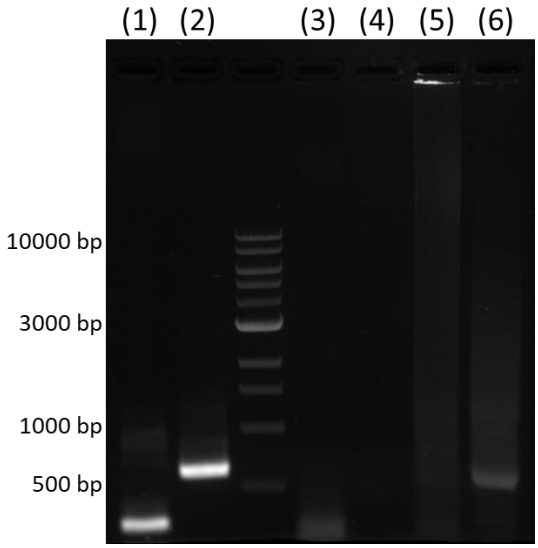

Figure 2.2: PCR activity test of reverse transcriptase (lysed under different conditions) with a desired amplicon size of 240 bp (1,3,5) or 607 bp (2,4,6). Lanes (1,2): Lysozyme with protease inhibitor. Lanes (3,4): Lysis re-suspension buffer followed by a concentration step and a buffer exchange step. Lanes (5,6): Same as Lanes 3 and 4 but with a thermal elution and filtering step to remove aggregated contaminant protein (prior to the concentration step).

3 Description of work performed and obtained results

As of this report we have synthesised and characterised a silaffin-tagged, fluorescently labelled fusion-construct with reverse transcriptase.

3.1 R5-mCh-H10-RTX

Utilising the standard silaffin-tagged mCherry template, a reverse transcriptase was synthesised and expressed in BL21 (DE3) *E. coli*. Unlike the Boon2-BST mentioned in Deliverable D3.1, Boon-RT requires an IPTG expression protocol of 18°C for 18 hours. On the other hand, like Boon2-BST, the recombinant reverse transcriptase expression can be indicated through a change in culture colour and a pink pellet post-induction. In addition, various lysis methods were tested ranging from using only lysozyme to sonication with subsequent thermal elution. It was determined that lysozyme with protease inhibitor was the best approach for enzyme activity, as shown in Figure 2.2.

The Boon-RT was also tested with both RT-PCR and RT-LAMP, shown in Figure 3.1 and 3.2. In either case, the addition of the Boon-RT was significantly more sensitive than the polymerase on its own. Furthermore, Boon-RT and Boon2-BST were observed to have good compatibility with one another.

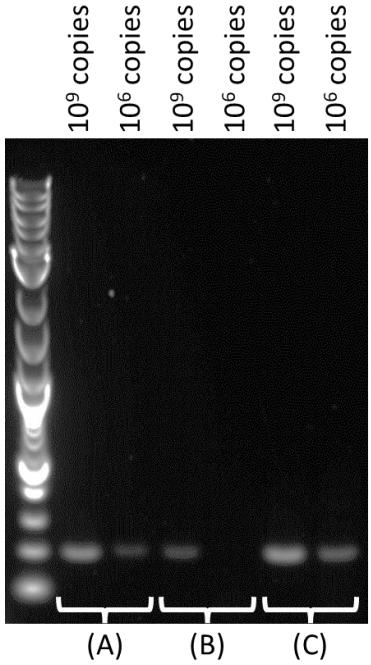

Figure 3.1: Gel electrophoresis of RT-PCR using (A) commercial reverse transcriptase with Q5 DNA polymerase, (B) Only Q5 DNA polymerase, and (C) Boon-RT with Q5 DNA polymerase.

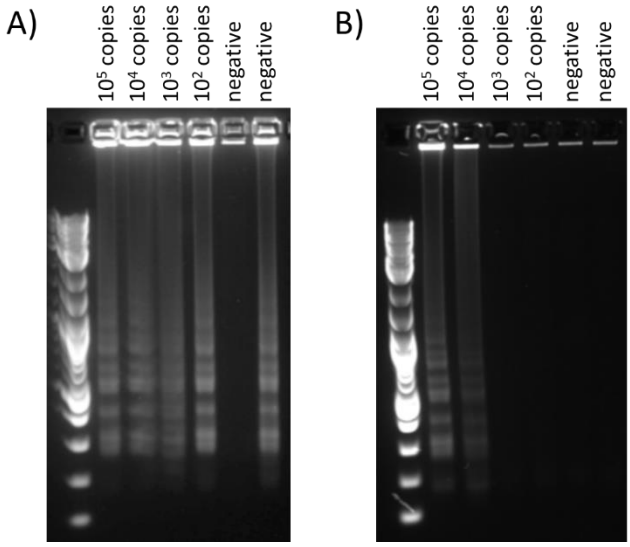

Figure 3.2: Gel electrophoresis of RT-LAMP using (A) Boon-RT and Boon2-BST, and (B) only Boon2-BST.

3.2 Expression and Purification SOP

Both the expression and purification SOP have been completed for the Boon-RT protein. The protocols are available as independent text documentation (WP3) and as part of the manufacturing handbook (WP8).

The titles are the follow:

- WP3.2 - Protein Expression Protocol of Engineered Boon2-BST.pdf
- WP3.2 – Protein Purification Protocol for Engineered Boon2-BST.pdf