



## Robust SARS-CoV-2 detection for COVID-19 diagnosis: enhancing LAMP assays with single-strand DNA binding proteins

*“The expertise of the Durham University team in protein production and characterisation enabled us to explore a wide range of auxiliary proteins for biotechnology” ArcticZymes*

**PROJECT AIMS:** The speed, accuracy and accessibility of viral testing — including SARS-CoV-2 testing — needs to be improved. This project aimed to use a technology called loop-mediated isothermal amplification (LAMP) in single-step viral testing. Specifically, the study sought to optimize the production of single-strand DNA binding (SSB) proteins, which stabilise single strand nucleic acids, and test them in LAMP assays to detect genetic material from viruses, including SARS-CoV-2.

**OUTCOMES:** First, the recombinant expression of the 12 target SSB proteins was optimized to enable large-scale purification. Then the stability of the 12 SSB proteins in the presence of several metals was assessed using thermal shift assays. The thermal stability of the SSB target proteins was increased by MgSO<sub>4</sub> and a number of lanthanides. These experiments established the optimal conditions for producing and storing the target SSB proteins. Then the stabilising effect of the metal-optimised SSBs on nucleic acids in reverse transcriptase-LAMP assays was evaluated using commercially available RNA from M21 virus and SARS-CoV-2 samples. This experiment showed that SSBs significantly improved the separation of virus-positive and virus-negative samples.

**KEY MESSAGE:** Using metal-optimised SSBs in reverse transcriptase-LAMP assays improves the detection of viral RNA

- Change in technology readiness level: 3 to 3/4

	1	2	3	4	5	6	7	8	9	10	11	12
A	62.8 75.8	62.6 75.2	51.4	N/S	54.9	57.6	58.7	63.4	67.6	62.8 75.6	72.5	65.7 71.2
B	74.5	74.5	74.1	73.0	73.9	71.2	87.9	85.1	84.0	82.2	80.8	63.8 78.8
C	78.3	76.1	75.8	75.3	75.0	63.1 74.7	81.8	61.7 80.7	61.8 80.1	62.1 79.4	62.6 78.8	63.4 78.4
D	78.3	77.4	76.6	75.5	74.4	76.5	79.9	76.4	73.5	72.0	74.9	63.5 74.3
E	73.3	73.4	70.0	63.0 73.4	74.4	62.9 73.8	67.3 74.6	64.7	73.5	74.3	62.4 74.5	58.6 71.7
F	60.3 74.2	74.0	62.7 76.3	82.5	80.2	80.8	78.0	73.1	72.0	81.5	63.1 77.2	74.7
G	71.0	63.2	58.4	72.1	63.1 76.5	63.1 76.5	62.4 74.4	85.9	84.0	86.8	86.9	84.2
H	87.4	88.0	88.5	88.5	88.5	62.1 73.4	67.0	64.3 75.7	62.6 76.0	38.6 86.5	63.2 76.6	60.8

An example of a thermal shift analysis of the stabilizing effects of anions, cations and metals on one of the SSB target proteins. doi: 10.3791/58666.

### OUTCOMES & NEXT STEPS

- Further funding secured from the MRC Confidence in Concept program to move the project towards community testing
- Additional funding obtained from Durham University Covid-19 research fund
- Application submitted to the 2020 TDRF call