Project Information

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Deliverable Information

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Deliverable Title	Engineered Boon-scFV and accompanying expression and purification SOP
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WP Leader	UCAM
Authors	Dushanth Seevaratnam (UCAM)
Contributors	UCAM
Reviewers	Lisa Hall (UCAM)
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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
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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-scFv. Produced accompanying SOPs.

1 Status of the Deliverable

The deliverable has been completed. A fluorescently tagged design of the Boon-scFv has been synthesised and characterised. In addition, the associated expression and purification SOPs have been produced and distributed.

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

A recombinantly expressed single-chain variable fragment (scFv) (targeting human Immunoglobulin A/G/M) has been synthesised with an internal mCherry (for protein tracking), a pectate lyase B (pelB) leader sequence (for improved productivity), a His-tag (for purification), and a FLAG-tag (for specific identification), as shown in Figure 2.1.

Figure 2.1: Schematic of synthesised Boon-scFV.

Roughly 2.5 – 4 mg of Boon-scFv could be harvested from 50 mL of *E. coli* culture and were observed to be stable even at high concentrations (up to 8 mg/mL), as shown in Figure 2.2. In addition, the Boon-scFv was found to be highly selective, binding only to human IgG when screened against various targets (Figure 2.3).

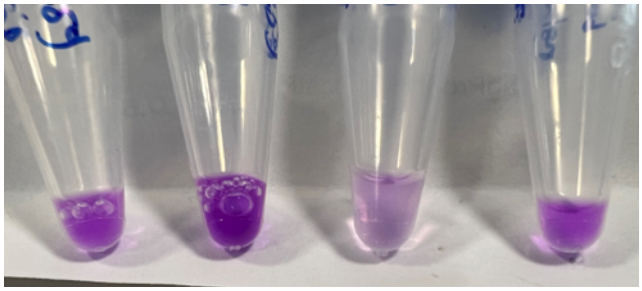

Figure 2.2: Purified and concentrated Boon-scFv.

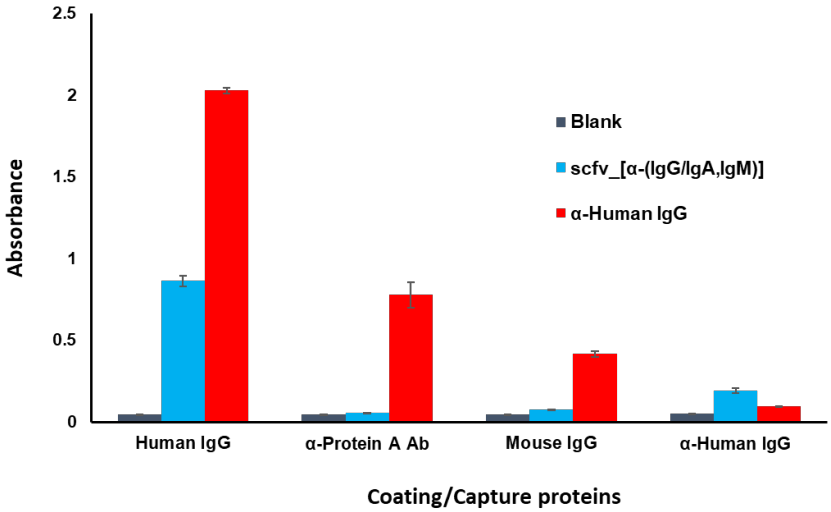

Figure 2.3: Immunoassay comparing selectivity of the Boon-scFv (blue) with commercial anti-human IgG (red)

3 Description of work performed and obtained results

As of this report we have synthesised and characterised a fluorescently labelled scFv with good solubility and selectivity.

3.1 Recombinant Expression of Boon-scFv

Tracking mCherry fluorescence, Boon-scFv expression was found to be highly temperature dependent and required lower temperature (18 - 25 °C) for overexpression, as shown in Figure 3.1. However, unlike the proteins described in WP3.1 and WP3.2, Boon-scFv was hindered by its solubility. Switching *E. coli* strains from BL21 (DE3) to C43(DE3) (a cell line for effective expression of toxic and/or membrane proteins) greatly increased solubility with a slight cost in expression.

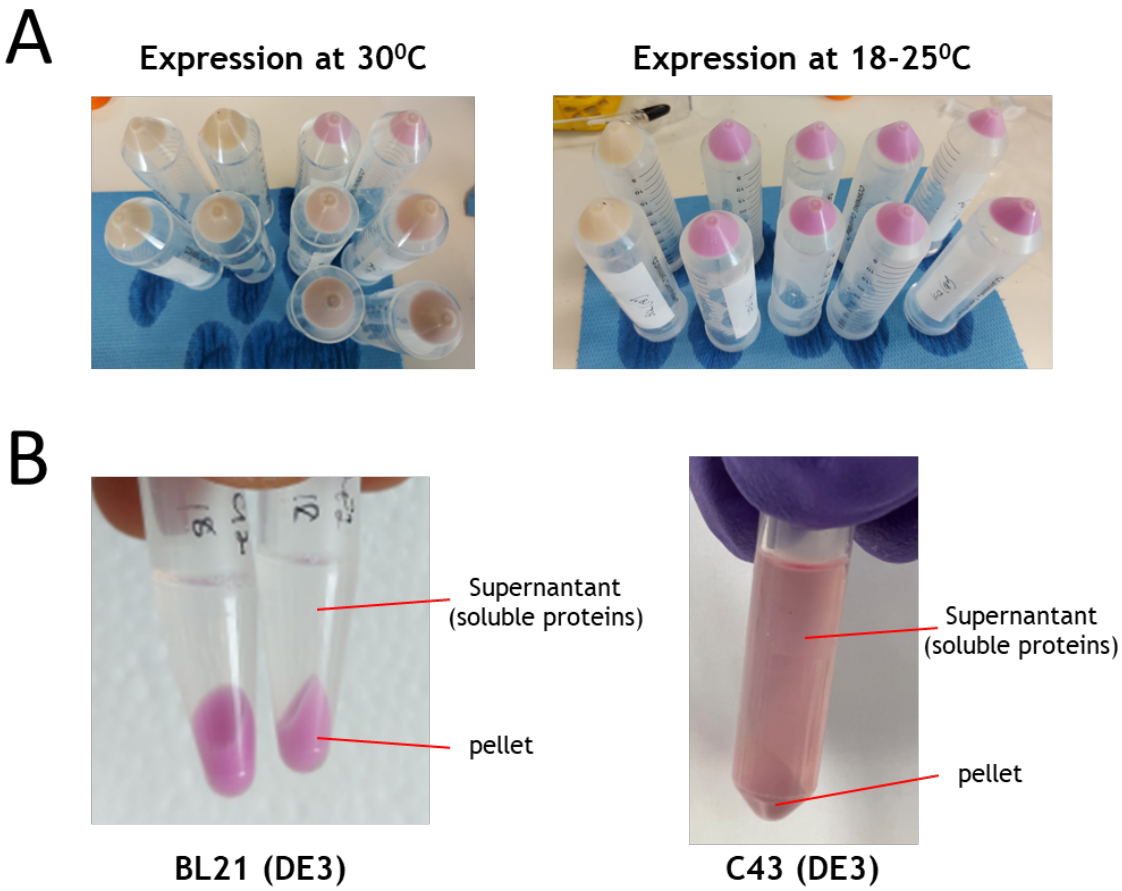

*Figure 3.1: **A** Cell pellet comparison between Boon-scFv expressed at 30 °C and 18 - 25 °C in BL21 (DE3) cells. **B** Lysis comparison between Boon-scFv expressed in BL21 (DE3) and C43 (DE3).*

In addition, Boon-scFv expressed better in autoinduction media rather than in LB broth with ITPG (Figure 3.2). Lastly, all 3 components of the Boon-scFv were observed to be functional. Despite being located between the heavy and light chains, the mCherry correctly folded leading to the pink colour of the protein without inhibiting the selectivity of the scFv (Figure 2.3) and the His-tag was still accessible for Nickel-resin purification.

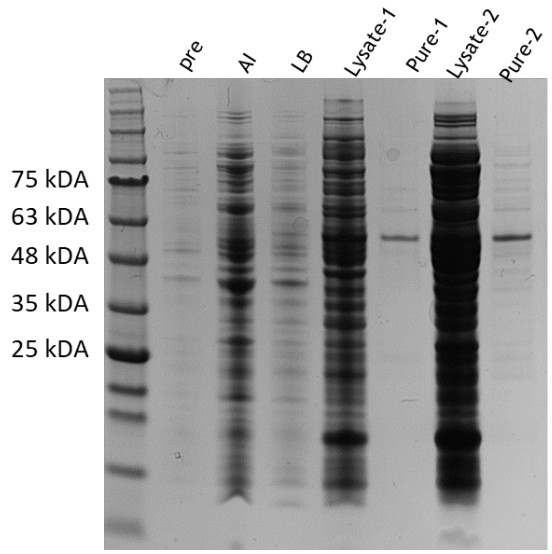

Figure 3.2: SDS-PAGE for Boon-scFv (57 kDA). The lanes display proteins collected from the pre-induced cell pellet (pre), autoinduction cell pellet (AI), IPTG induced LB broth cell pellet (LB), cell lysates from autoinduction cultures and the Nickel-resin purified Boon-scFv.

3.2 Expression and Purification SOP

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

The titles are the follow:

- WP3.5 - Protein Expression Protocol of Engineered Boon-scFv.pdf
- WP3.5 – Protein Purification Protocol for Engineered Boon-scFv.pdf