Cloning of plasmids harboring anti-PD-L1 scFv and its expresaion in *E. coli*

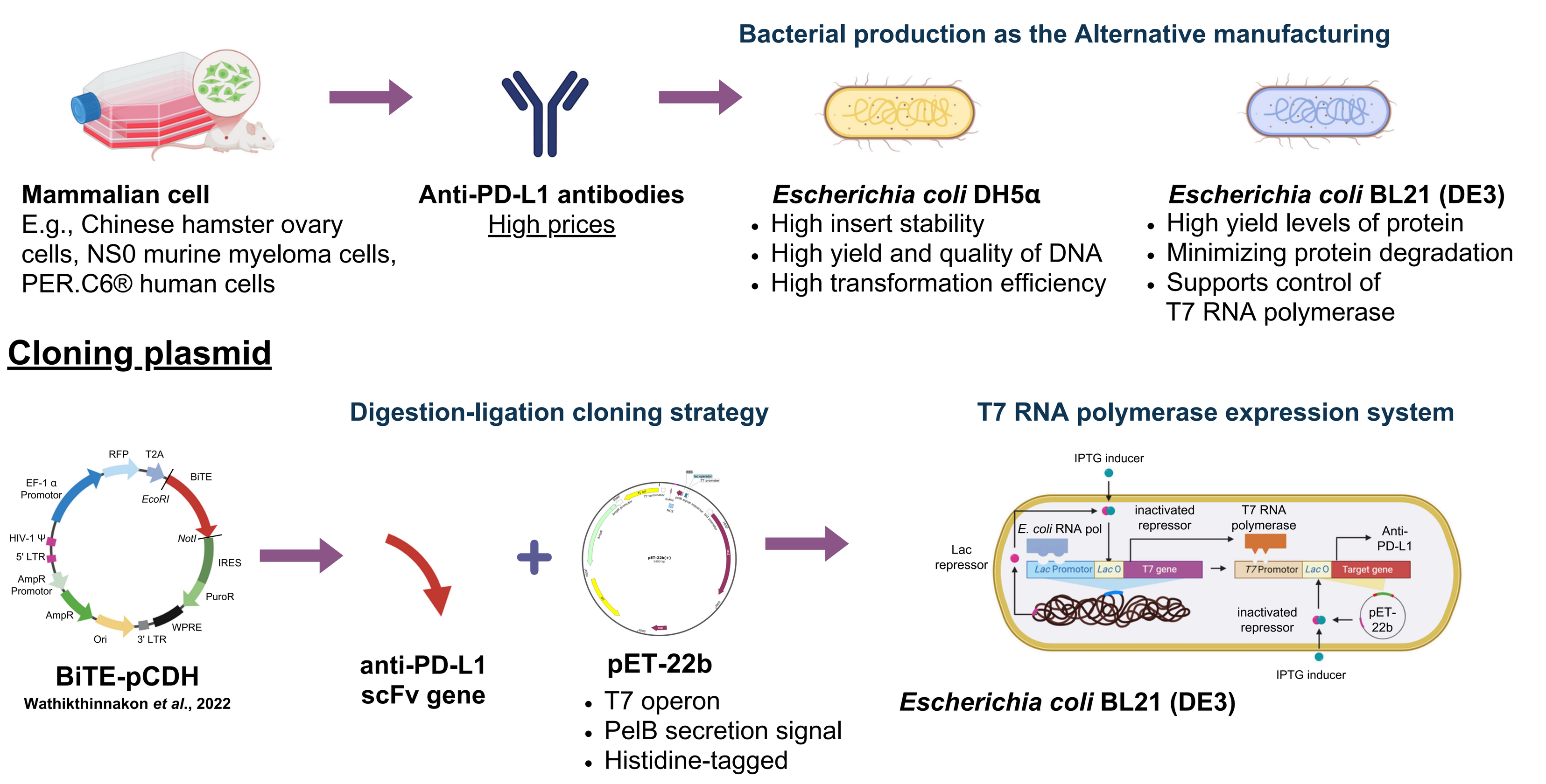
Chonlaphon Chantama[1,*] , Pachara Sattayawat[1,**]

[1] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
e-mail: *chonlaphon_ch@cmu.ac.th, **pachara.sattayawat@cmu.ac.th

Abstract

Immunotherapy is an effective cancer treatment, particularly through the use of checkpoint inhibitors. These inhibitors block protein interactions between cancer cells and T-cell receptors, preventing immune evasion. Currently, various commercial antibodies, such as atezolizumab, have been developed to inhibit PD-L1, an immune checkpoint protein expressed on cancer cells. However, these therapeutic antibodies are expensive due to the high production costs associated with eukaryotic cell cultures. This research aims to clone the anti-PD-L1 scFv gene into the pET-22b plasmid backbone and investigate its expression in *Escherichia coli* as a cost-effective alternative for antibody production. *E. coli* offers advantages such as growth in simple and inexpensive media, ease of genetic modification, and scalability for large-scale production. Following the cloning of anti-PD-L1 scFv into pET-22b and transformation into *E. coli* BL21(DE3), protein expression was confirmed using Coomassie blue staining and western blot analysis with an anti-His-tag antibody. Additionally, the optimal temperature for expression was evaluated, revealing high protein production at relatively low temperatures (16°C and 30°C). These findings indicate that the constructed plasmid successfully expresses anti-PD-L1 scFv, demonstrating its potential for further development in cancer immunotherapy applications.

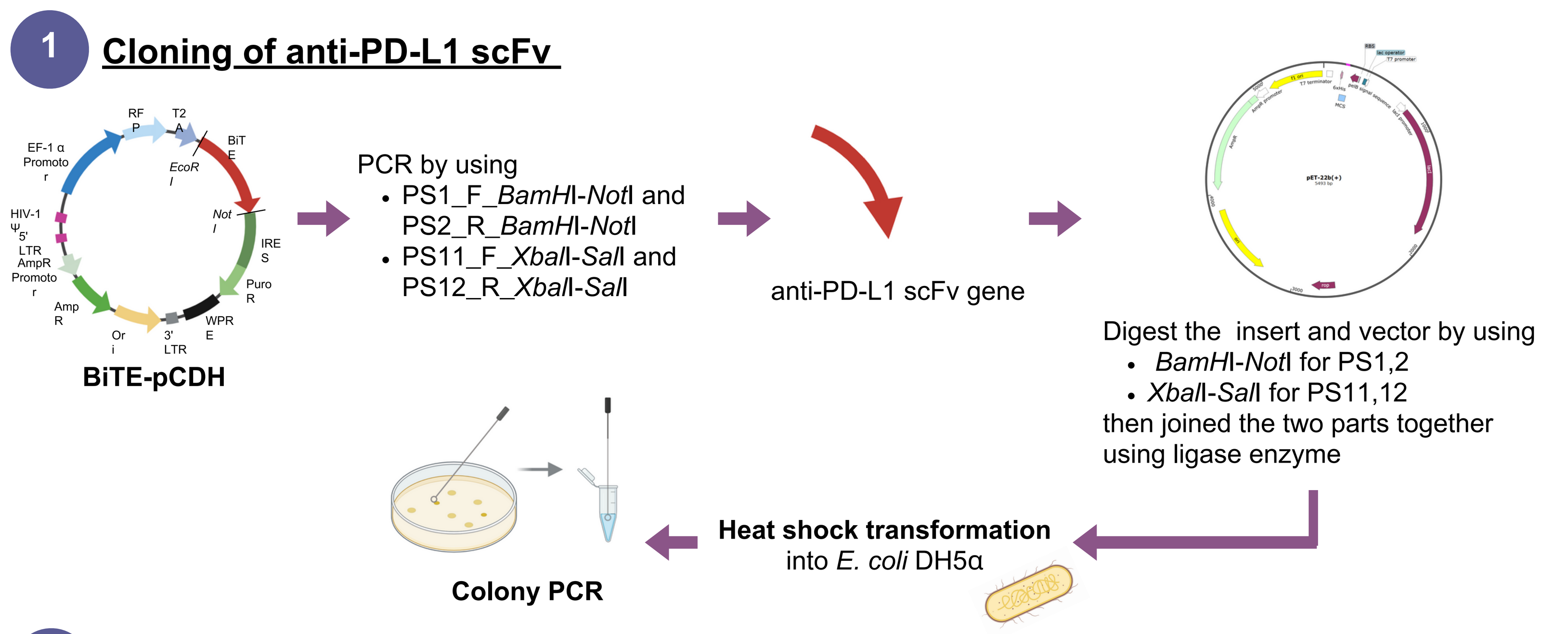
Introduction

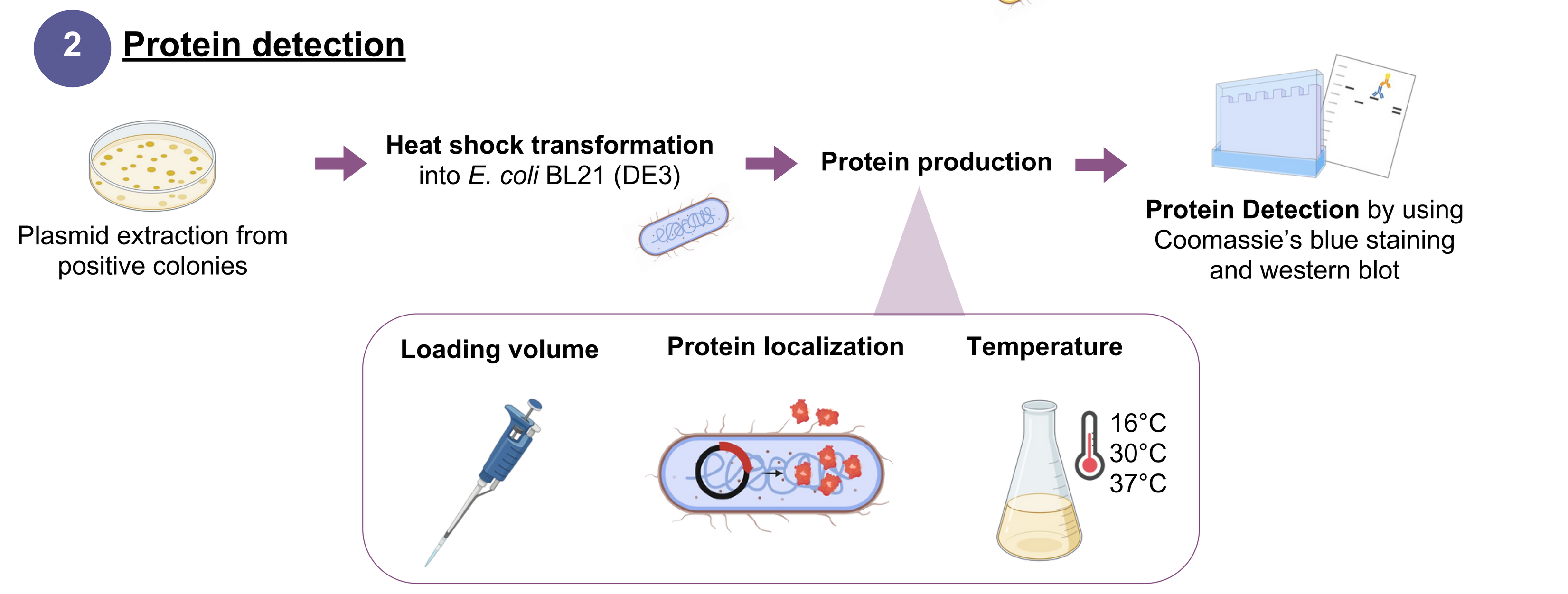


Objectives

- To construct plasmids for anti-PD-L1 scFv in *E. coli* using a digestion-ligation cloning strategy
- To investigate the expression of recombinant anti-PD-L1 scFv in *E. coli*

Materials and method





Discussion and Conclusion

The anti-PD-L1 scFv gene was cloned from the pCDH-BiTE plasmid into the pET-22b expression vector using a digestion-ligation strategy and subsequently transformed into *E. coli* BL21(DE3) via heat shock. This strain of *E. coli* has been shown suitable for large-scale protein protein production. Furthermore, the expression of anti-PD-L1 scFv was confirmed using SDS-PAGE with Coomassie blue staining and western blot analysis with an anti-His-tag antibody, examined using colorimetric and chemiluminescence methods, respectively. The optimal temperature for protein expression was investigated, revealing high production at relatively low temperatures (16°C and 30°C), which improved protein solubility and reduced the formation of insoluble aggregates [1,2] compared to standard cultivation temperatures. Additionally, no translocation of the expressed protein across the membrane to the extracellular environment was observed.

Results

1 Amplification of anti-PD-L1 from pCDH-BiTE

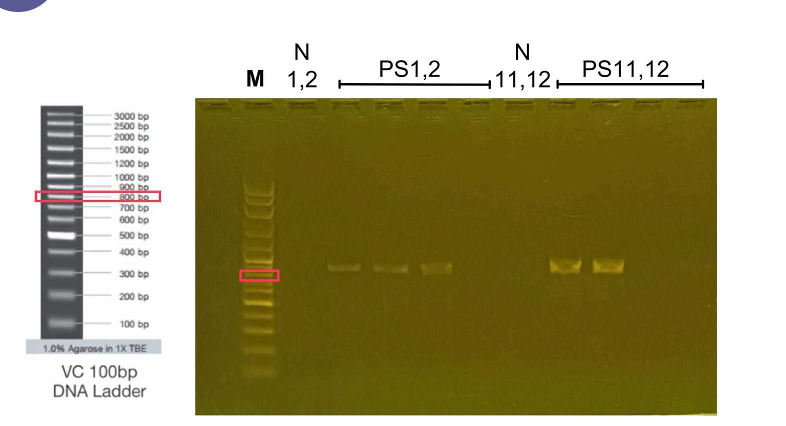


Figure 1: Gel electrophoresis of PCR product from pCDH-BiTE. **M** ; 100 bp DNA ladder, **N1,2** ; Control well for primer PS1,2, **N11,12** ; Control well for primer PS11,12, **PS1,2** ; PCR product well using primer PS1,2, **PS11,12** ; PCR product well using primer PS11,12

2 Digestion and ligation

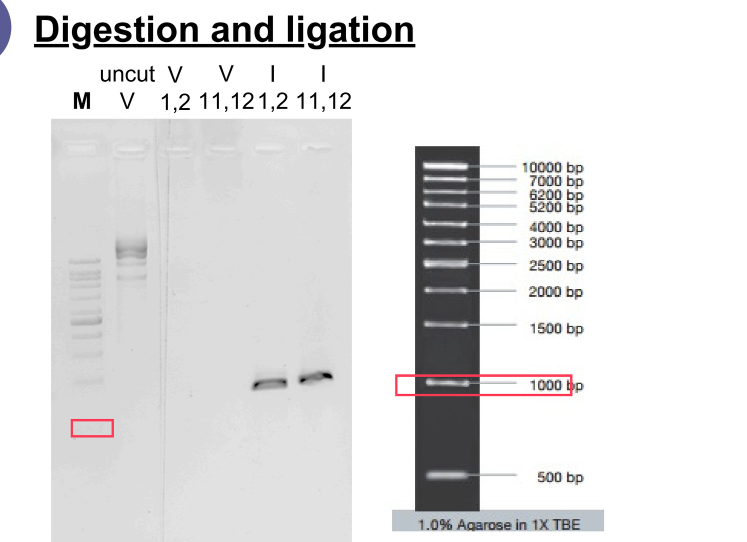


Figure 2: Gel electrophoresis of digestion product. **M** ; 1 kb DNA ladder, **uncut V** ; pET-22b with no restriction enzyme, **V1,2** ; pET-22b with restriction enzyme for primer PS1,2, **V11,12** ; pET-22b with restriction enzyme for primer PS11,12, **I1,2** ; anti-PD-L1 gene with restriction enzyme for primer PS1,2, **I11,12** ; anti-PD-L1 gene with restriction enzyme for primer PS11,12

3 Colony PCR

Figure 3: Colony PCR of the potential transformants. (A) Transformation ligation product in *E. coli* DH5α on LB agar. (B) Gel electrophoresis of colony PCR. **M** ; 100 bp DNA ladder, **N1,2** ; Control well for primer PS1,2, **N11,12** ; Control well for primer PS1,2, **PS1,2** ; PCR product well using primer PS1,2, **PS11,12** ; PCR product well using primer PS11,12

4 Sequencing

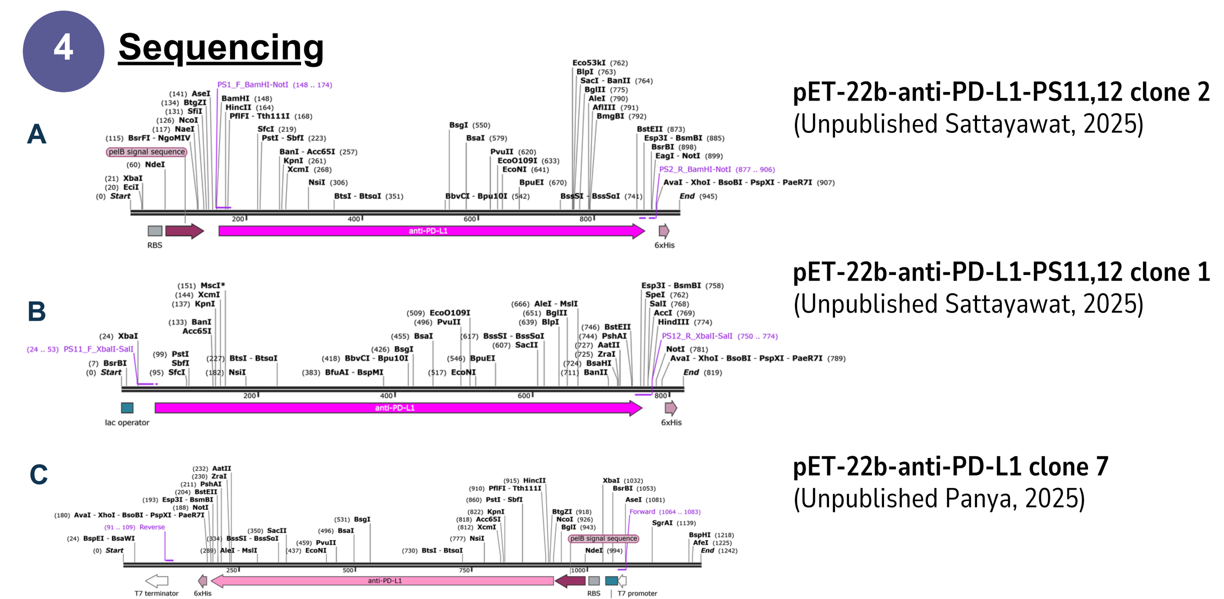


Figure 4: Plasmid sequencing. The PCR products of (A) pET-22b-anti-PD-L1-PS11,12 clone 2 (B) pET-22b-anti-PD-L1-PS11,12 clone 1 (C) pET-22b-anti-PD-L1 clone 7 were sequenced to confirm gene insertion.

5 Protein production and detection

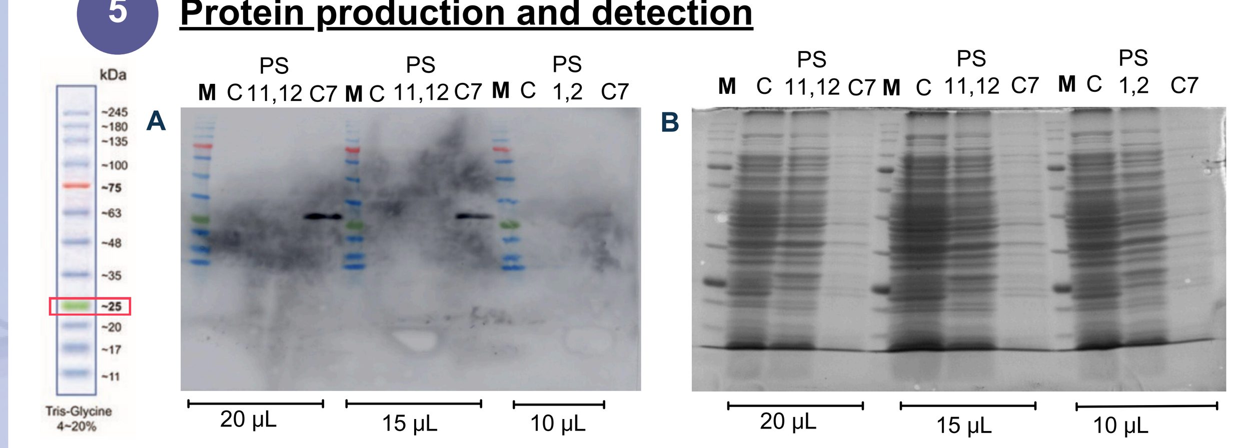


Figure 5: Protein production and detection. Loading volume optimization for SDS-PAGE (A) Western blot (B) Coomassie's blue stain. **M** ; Pre-stained Protein Marker (11~245 kDa), **C** ; Cell lysate of *E. coli* BL21, **PS11,12** ; Cell lysate of *E. coli* BL21 contains pET-22b-anti-PD-L1-PS11,12 clone 1, **clone 7**; Cell lysate of *E. coli* BL21 contains pET-22b-anti-PD-L1 clone 7

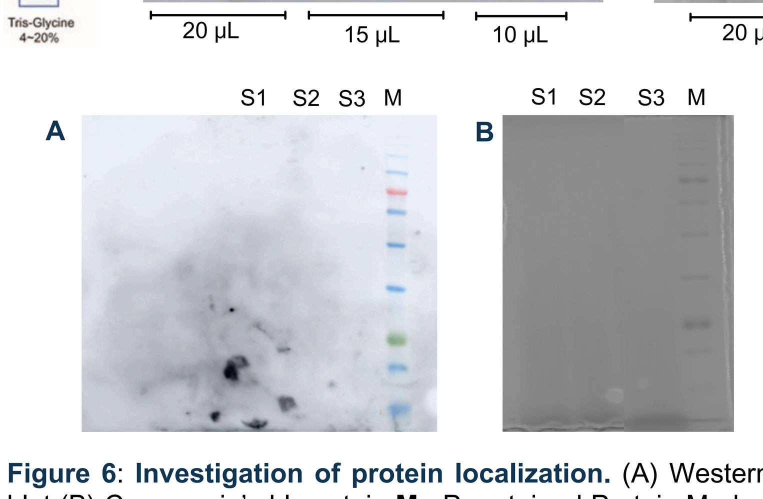


Figure 6: Investigation of protein localization. (A) Western blot (B) Coomassie's blue stain **M** ; Pre-stained Protein Marker (11~245 kDa), **S1** ; Supernatant of *E. coli* BL21, **S2** ; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1 clone 7, **S3**; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1-PS11,12 clone 1

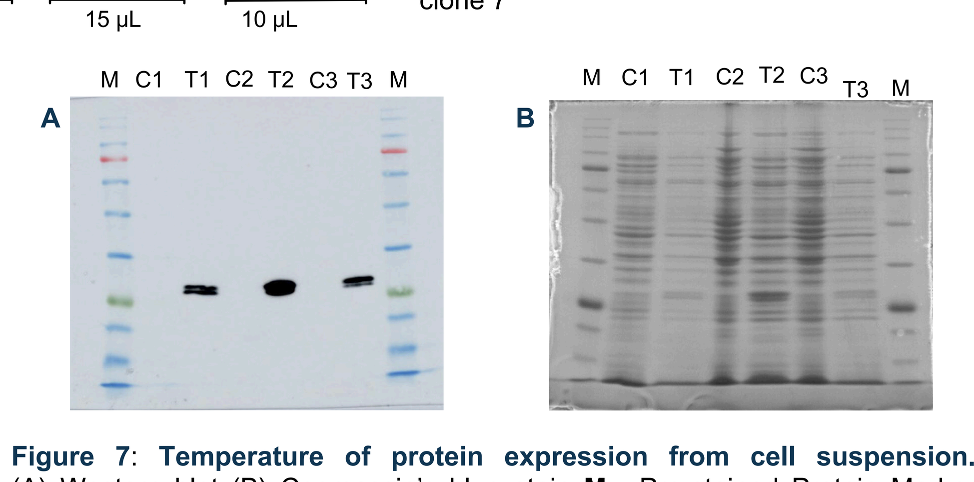


Figure 7: Temperature of protein expression from cell suspension. (A) Western blot (B) Coomassie's blue stain **M** ; Pre-stained Protein Marker (11~245 kDa), **S1** ; Supernatant of *E. coli* BL21, **S2** ; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1 clone 7, **S3**; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1-PS11,12 clone 1

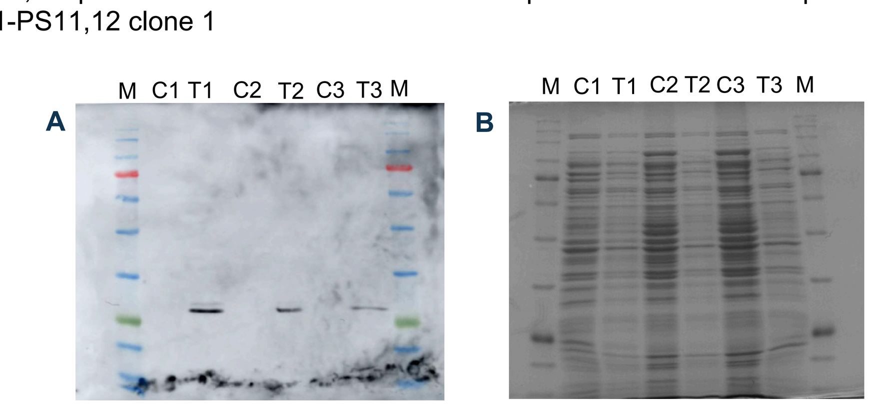


Figure 8: Temperature of protein expression from cell lysate. (A) Western blot (B) Coomassie's blue stain **M** ; Pre-stained Protein Marker (11~245 kDa), **S1** ; Supernatant of *E. coli* BL21, **S2** ; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1 clone 7, **S3**; Supernatant of *E. coli* BL21 contains pET-22b-anti-PD-L1-PS11,12 clone 1

Acknowledgement

- Assistant Professor Pachara Sattayawat, PhD, Project's advisor
- Associate Professor Aussara Punya, PhD and Cell Engineering for Cancer Therapy Research Group, Chiang Mai University
- Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University

Reference

[1] Estabragh, A. M., Sadeghi, H. M. M., & Akbari, V. (2022). Co-Expression of Chaperones for Improvement of Soluble Expression and Purification of An Anti-HER2 scFv in *Escherichia Coli*. *Adv Biomed Res*, 11, 117. https://doi.org/10.4103/abr.abr_351_21

[2] Wang, R., Xiang, S., Feng, Y., Srinivas, S., Zhang, Y., Lin, M., & Wang, S. (2013). Engineering production of functional scFv antibody in *E. coli* by co-expressing the molecule chaperone Skp [Original Research]. *Frontiers in Cellular and Infection Microbiology*, 3. https://doi.org/10.3389/fcimb.2013.00072