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

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ABSTRACT

Immunotherapy is an effective treatment for cancer, particularly the use of checkpoint inhibitors. These inhibitors block protein binding on the surface of cancer cells with receptors on the surface of T-cells. Currently, various commercial antibodies, such as atezolizumab, have been developed to inhibit PD-L1. However, these therapeutic antibodies are expensive due to the high production costs associated with eukaryotic cell culture. This research aims to clone the anti-PD-L1 scFv gene into the pET-22b plasmid backbone and investigate the protein expression in *Escherichia coli* as an alternative approach for antibody production due to its ability to grow in simple and cost-effective media, ease of genetic modification, and scalability for large-scale production. This research found that 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 investigated, revealing that protein production was high at relatively low temperatures at 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.