Optimized Expression Condition of SARS-CoV-2 Polymerase Nsp12

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SARS-CoV-2 non-structural protein 12 (Nsp12) is a viral polymerase and a key drug target. The in vitro production of pure Nsp12 protein is crucial for advancing drug and treatment strategies. Currently, Nsp12 is produced using an E. coli expression system and purified by chromatography; however, a contaminating protein is difficult to separate from Nsp12 using this purification process. In this study, we aimed to identify the non-target protein and explore and optimize the expression conditions of SARS-CoV-2 Nsp12 to obtain high-purity Nsp12. Mass spectrometry was used to identify the non-target protein. Based on our previous study that tested various growth parameters according to existing methods, we optimized Nsp12 expression in E. coli by varying parameters such as the rotational speed and cooling rate. Two different cooling rates under the control of two different cooling methods were also examined, and differences in protein expression and proportion resulting from these two cooling processes were evaluated. Data were analyzed by using the t-test and GraphPad Prism software. The results showed that the non-target protein is ArnA,and the cooling rate is the main factor that affects the expression and purity of Nsp12. Under a mild cooling rate, the generation and the proportion of ArnA decreased, and ArnA was easily eluted by the washing buffer. This study presented an optimized expression and purification protocol for producing high-quality, pure Nsp12 protein. The research provides a foundation for drug selection against Nsp12 and enzyme activity determination and offers a new approach to optimize protein expression.

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