Construction of Fusion Proteins by Optimizing Linker Sequences

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A fusion protein contains two or more independent proteins joined together. Fusion proteins have many applications as reporters for simultaneous detection by different methods, such as fluorescence and bioluminescence. In bioscience/biomedical research and diagnostics, fusion proteins are often used to report the expression of a target protein. However, fused proteins may interfere with each other and lead to lower activities than the parent proteins. In this project, I aimed to optimize fusion protein activities by designing different linker sequences between two different luciferases – firefly and Nano luciferase, whose activities can be easily detected and quantitated by an in vitro assay. The linker sequences include both flexible and rigid structures of different sizes. Various DNA constructs that code for the two proteins and different linkers were made by Gibson cloning. The resulting DNA constructs were used as templates for coupled transcription/translation to yield various fusion proteins. Their luciferase activities were then independently measured with a luminator through bioluminescence emission. My data indicate that 1) linkers do in fact affect the activities of two fused proteins, 2) longer linkers reduce the interference, and 3) rigid linkers are better than flexible linkers. These results may be used to construct other fusion proteins with improved activities to achieve sensitive detection of biological targets.

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