Development of a Simple and Efficient Method for Site-Specific Mutagenesis

Huang, Allen

Site-specific mutagenesis, also known as site-directed mutagenesis, is the specific and intentional introduction of mutations into a DNA sequence. This technique has many applications in research, including producing a rationally designed protein and investigating the activity and function of a DNA sequence or protein. In recent years, cloning technology has rapidly advanced mutagenesis techniques, but many of the modern methods still involve steps that are time-consuming or require expensive reagents. In this project, I designed a novel site-specific mutagenesis system with mutagenic primers that is fast and efficient. It does not require template DNA digestion before transformation, instead diluting the template to low enough levels such that it will become insignificant during transformation. Because the parent was green fluorescent protein (GFP), measurement of successful mutation did not require sequencing but rather visual analysis of the different types of fluorescent proteins synthesized. Starting from GFP, I was able to create non-fluorescent protein from 1 point mutation, blue fluorescent protein from 2 mutations, and yellow fluorescent protein from 3 mutations. The efficiency of mutation was measured against the length of the primer overlap. It was determined that longer overlap regions promote more efficient transformation. This simple and efficient method for inducing mutations can be broadly applied to fields including protein engineering and rational protein design.

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