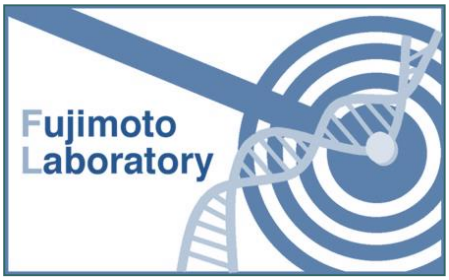
STUDIES ON SITE-DIRECTED MUTAGENESIS USING PHOTO CHEMICAL DNA EDITING



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Abstract

Site directed mutagenesis is a method for inducing specific and intentional changes to DNA sequence with the help of enzymes or other means. One of the most common methods for achieving site directed mutagenesis is use of a template and a primer with mismatch of one or some nucleotide in the sequence and thus amplifying it using Polymerase chain reaction.

Besides chemical and enzymatic methods for inducing site-directed mutation in the DNA, there are various photochemical methods available. One of the major methods require the use of a photo reactive molecule, cyanovinyl carbazole (^{CNV}K) which when incorporated in the DNA strand can selectively crosslink with the cytosine moiety of the complimentary strand and lead to base pair mutation by causing deamination of the cytosine moiety to uracil. DNA photo-cross-linking has proved to be a vital tool for detection, regulation and manipulation of DNA and RNA. The photo-cross-linking, which is due to the presence of ^{CNV}K, is highly sequence specific and can be simply achieved by photo irradiation and heating.

The jellyfish based green fluorescent protein was extensively engineered and manipulated to produce a vast palette of coloured fluorescent proteins. This led to introduction of mutated GFP to various shades of cyan, orange, red, yellow and blue fluorescent proteins. Despite the origin and manipulations, the average size of fluorescence proteins is ~25kD. The fluorescence of the protein majorly depends on the structure of the protein moiety and the incorporated chromophore. In most of the fluorescent proteins, the chromophore consists of only a few amino acids, which are located near the center of the β-barrel. The amino acids present as the chromophore vary at position 65, and majorly, Tyr66 and Gly67 are highly conserved among the GFP-like proteins. The mutation at these sites and the sites adjacent to them could lead to change in the fluorescent properties and the spectral properties of the fluorescent proteins.

From three different fluorescent protein genomes, EGFP, EYFP, and DsRed, 11 mutations in all were identified and carried out by enzymatic methods. The proteins from these mutations when synthesized and analyzed for their fluorescent properties revealed that most of the mutations in EGFP and EYFP had no effect on the fluorescent properties of the protein. Whereas, in the DsRed, proteins, the mutations had prominent effect on the fluorescent properties. Wherein, one of the mutants showed fluorescent in the yellow region of the UV-Vis spectra, the other two lost their fluorescent properties completely. DsRed Q66R and DsRed Y67C, having the most prominent change in fluorescent properties were used for the photo-induced site directed mutagenesis, in which they were mutated back to wild type.

The genomic DNA and ODN were mixed in the molar ratio of 1:25 in buffer solution. The mixture was first denatured at 98ºC and then irradiated by 366nm UV radiation at 56ºC for one hour. After which, the sample was irradiated by 312 nm UV radiation at 80ºC. The restriction enzyme analysis of the final products, using BsaWI for DsRed Q66R and TspRI for DsRed Y67C, was carried out. The mutant genome types were converted back to the wild type after the photoreaction and confirmed by sequencing of the products obtained after the photoreaction and comparing them with the sequence of wild type genome.

These experiment concluded that the site-directed mutagenesis using photo-responsive ODN containing ^{CNV}K is a viable process for the purpose. The reaction takes place in a single vial and in fewer steps as compared to conventional enzymatic method. It also does not require use of bacteria for plasmid replication.