Research Article

Combined Overlap Extension PCR Method for Improved Site Directed Mutagenesis

Hasnain Hussain[1] and Nikson Fatt-Ming Chong[1]

[1]*Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia*

Correspondence should be addressed to Hasnain Hussain; hhasnain@unimas.my

The combined overlap extension PCR (COE-PCR) method developed in this work combines the strengths of the overlap extension PCR (OE-PCR) method with the speed and ease of the asymmetrical overlap extension (AOE-PCR) method. This combined method allows up to 6 base pairs to be mutated at a time and requires a total of 40- 45 PCR cycles. A total of eight mutagenesis experiments were successfully carried out, with each experiment mutating between two to six base pairs. Up to four adjacent codons were changed in a single experiment. This method is especially useful for codon optimization, where doublet or triplet rare codons can be changed using a single mutagenic primer-set, in a single experiment.

1. Introduction

Site Directed Mutagenesis is a technique used for substitution, addition and deletion of specific base sequences in DNA [1]. It is an important tool to generate mutants with altered amino acid sequences for enzyme studies, investigation of the relationship between structure and functions of proteins, and functional analysis of genes or their regulatory sequences [2,3,4]. Altering the amino acid sequence of an enzyme has been used to improve enzyme properties such as catalytic activity, thermostability and chemical tolerance [5,6]. Furthermore, site directed mutagenesis is also used in codon optimization to remedy codon bias during heterologous expression of proteins [7,8].