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Standard method's specificity is improved by nested-splicing by overlap extension PCR

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INTRODUCTION

Splicing by Overlap Extension (SOE) PCR is used to introduce mutations into an enzyme's coding sequence in order to investigate the involvement of particular residues in the structure and function of the protein. The goal of this study was to create a nested-SOE-PCR (N–SOE-PCR) to enhance the specificity of SOE-PCR in producing mutations in a gene. *Bacillus thermocatenulatus* genomic DNA was isolated. Using gene specific and mutagenic specific primers, nested PCR was utilised to amplify *B. thermocatenulatus* lipase gene variants, namely wild type and mutant, followed by cloning in a suitable vector. To summarise, instead of two pairs of primers, three pairs of primers are utilised in the N-SOE-PCR technique to amplify a mutagenic fragment. This might be due to the primers being annealed farther into the amplicon, which enhances efficiency and improves primer attachment. In SOE, placing the primer distant from both ends of an amplicon results in improved binding and affinity in the third round of amplification.

DESCRIPTION

The Overlap Extension Polymerase Chain Reaction (or OE-PCR) is a variant of PCR. It is also referred to as Splicing by overlap extension/splicing by Overhang Extension (SOE) PCR. It is used assemble multiple smaller double stranded DNA fragments into a larger DNA sequence. OE-PCR is widely used to insert mutations at specific points in a sequence or to assemble custom DNA sequence from smaller DNA fragments into a larger polynucleotide. Nested PCR was used to amplify *B. thermocatenulatus* lipase gene variants, namely wild type and mutant, using gene specific and mutagenic specific primers, followed by cloning in a suitable vector. Briefly in N-SOE-PCR method, instead of two pairs of primers, three pairs of primers are used to amplify a mutagenic fragment. Moreover, the first and second PCR products are slightly longer than PCR products in a conventional SOE Site-directed mutagenesis is a technique for altering the DNA of any gene. Many approaches have been developed to enhance mutagenesis efficiency up to this point. The conventional SOE technique was modified in this work to improve the efficiency of mutagenesis of any gene. In the SOE and N-SOE techniques, the anticipated pieces were around 1300 and 1200 bp, respectively. For site-directed mutagenesis, a number of techniques based on Polymerase Chain Reaction (PCR), including overlap extension, have been devised. SOE (splicing by overlap extension) is a potent approach for generating recombinant sequences that does not rely on restriction sites or ligases. The main disadvantage of SOE is its limited selectivity in the third PCR for amplification of the full size mutant fragment. shows a schematic representation of the N-SOE technique. Three pairs of primers are employed in this method. The suggested process is divided into four steps: The target gene is amplified using gene-specific primers (nested primers C and D) and cloned into the vector (pTZ57R/T or pGEM). The specific or nested primers are added to the PCR master mix in the first cycle of the third PCR in the N-SOE technique, which is comparable to conventional PCR, whereas primers are added to the PCR master mix after 10 cycles of the third PCR in the regular SOE method.

CONCLUSION

A fragment is amplified in each of the first and second PCR reactions, respectively. The third PCR amplification used these two PCR results. To summarise, instead of two pairs of primers, three pairs of primers are utilised to amplify a mutagenic fragment in the N-SOE-PCR technique. Furthermore, the first and second PCR products are somewhat longer than those in a standard SOE. To summarise, the N-SOE method increases specificity by using a set of additional primers, adding gene specific primers to the PCR master mix only at the first cycle of the third PCR. The explanation for this was that gene-specific primers were placed in the interior of the fragment, well away from both ends.