Development of a versatile cassette for directional genome walking using cassette ligation-mediated PCR and its utility in the cloning of complete genes from environmental genetic material: a case study of Family VII bacterial lipolytic genes.

Nthangeni M.B.* Tlou M.G, Ramagoma F and Litthauer D

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, P. O. BOX 339, BLOEMFONTEIN, 9300, SOUTH AFRICA

Since the invention of the PCR technology, adaptation techniques to clone DNA fragments flanking the known sequence continue to be developed. We describe a perfectly annealed cassette available in almost unlimited quantities with variable sticky- and blunt-end restriction enzyme recognition sites for efficient restriction and ligation with the restricted target genomic DNA. The cassette provides a 200 bp sequence, which is used to design a variety of cassette-specific primers. The dephosphorylation prevents cassette self-ligation and creates a nick at the cassette: target genome DNA ligation site suppressing unspecific PCR amplifications. We introduce the single-strand amplification PCR (SSA-PCR) technique where a lone known locus-specific primer is firstly used to enrich the targeted template DNA strand resulting in significant PCR product specificity during the second round conventional nested PCR. The distance between the known locus-specific primer and the nearest location of the restriction enzyme used determined the length of the obtained PCR product. We demonstrated the utility of the technique as a cost-effective method during PCR-based prospecting for novel genes by using Family VII carboxylesterase genes as a case study. We used the technique to clone novel carboxylesterase genes from environmental genetic materials using partly known gene fragments as known loci.