Abstract

PCR method has recently become a key element for the synthesis of genes. In this work two-step DNA synthesis based on PCR was used in order to obtain 5′-UTR of the PSM-E splicing variant of glutamate carboxypeptidase II (GCPII) which should serve as a standard for qPCR in real time. Another aim of this work was to clone this sequence into the plasmid PCRII-TOPO PSM-E for the purpose of protein expression in insect cells in the baculovirus expression system. In the first PCR the middle section of the 5′-UTR sequence was synthesized. This product was subsequently amplified in the second PCR using the first and last oligonucleotide. The product of the second PCR was ligated into the plasmid pUC19 and E. coli was transformed by this construct. Sequence correctness was confirmed by sequencing analysis. Subcloning of the insert from pUC19 to PCRII-TOPO PSM-E was carried out and the sequence was checked by sequencing analysis again. The obtained construct was used as a standard in qPCR in real time in order to determine the concentration of the samples of mRNA from the prostate tissues of oncologic patients.