ABSTRACT

The purification of pOTB7 plasmid containing coding sequence of carbonyl reductase1 (CBR1) in cells of *Escherichia coli* (*E. coli*) was done by alkali hydrolysis. The sequence of CBR1 was multiplied by polymerase chain reaction (PCR) and synthesized primers with restriction sites were used. The left primer contained sequence for restrictive endonuclease *Nde*I and the right primer for restrictive endonuclease *Xho*I. Validation of the first step was confirmed by size measurement of the synthesized fragment with following restrictive analysis realized by restrictive endonuclease *Bam*HI.

Prepared sequence CBR1 was cloned into Topo vector, which was transformed into the competent *E. coli* cells. Topo vector was purified by alkali hydrolysis after innidation of *E. coli* cells. The size of cyclic Topo vector without CBR1 and Topo vector with CBR1 coding sequence was verified on agar gel by restrictive endonuclease *Bam*HI. This was the validation of the second step.

Subcloning of coding sequence CBR1 from Topo vector to express vector pET15b was the last step.