

# **Study and Evolution Cloning of the Gene Encoding the L-AsparaginaseII from E. coli in B. subtilis**

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## **Abstract**

**Background and purpose:** *L-asparaginaseII* (E.C. 3.5.1.1) has effective usage for treatment of acute lymphoblastic leukemia. This enzyme is isolated from bacterial sources and it is commercially available as an anti-cancer drug. This enzyme catalyzes the hydrolysis of L-asparagine to ammonium and aspartate. Unlike Normal cells, the cancer cells strongly require L-Asparagine leading to destruction of these cells. The propose of this project is cloning of *L-asparaginaseII* gene from *E. coli* in *B. subtilis* to produce the mass of this enzyme.

**Materials and methods:** *L-asparaginaseII* gene is isolated from *E. coli* by PCR approach. The amplified fragment and expression shuttle vector pMR12 are digested by *HindIII* and *BamHI* enzymes. The ligation between DNA fragment and the cloning shuttle vector is done by standard method. In the next step recombinant vector is transferred to *E. coli* JM101 by cold calcium chloride treatment and finally introduced into *B. subtilis* by a chemical method.

**Results:** in this experiment, *ansB* gene is isolated from *E. coli* by PCR and cloned into the expression shuttle vector pMR12. Existence of gene with 1047 bp length is confirmed by enzymatic analysis and PCR reaction. Then recombinant vector cloned in *E. coli* at first and then in *B. subtilis*. Finally the plasmid extracted and compared with the band of expression shuttle vector containing *ansB* gene to confirm.

**Conclusions:** In this study, we tried to clone the *ansB* gene in *B. subtilis* using expression shuttle vector pMR12. This is the first report of *ansB* gene cloning in *B. subtilis*.

**Key words:** *L-Asparaginase*, *E. coli*, *B. subtilis*, Cloning, pMR12 .

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