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Cloning of ansB gene encoding L-asparginas

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Background and Aims: DNA (rDNA) molecules are DNA sequences that result from the use of laboratory methods (molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. In the present project, cloning of E.coli ansB gene was studied.
Methods: In the present project, cloning oE.coli ansB gene(1047 bps) was studied. Plasmid DNA was extracted from E. coli DH5/pGEM-3Z and DH5/pUC118 by an alkalin lysis method .Chromozomal DNA was extracted fromE.coli PTCC1221 by aphenol-chloroform method .PCR was used to amplify the target gene. The PCR product was purified and precipitated by ethanol.The plasmid and the PCR product were doubly digested with BamHI and EcoRI enzymes .After ligation and transformation, LB agar containing 50 µg/ml and 100µg/ml ampicillin was used to select the transformation. Transformed colonies were picked up and the potential recombinant plasmids were isolated.
Results: Only colonies containing pUC118 drived plasmids grew on LB/ampicillin plates. from 160 potential transformants growing on LB/amp(50µg/ml), coud grow on LB/amp(100µg/ml).Analysis of the plasmids was carried out using restriction mapping by the two enzymes, EcoRI&BamHI.Only a proof for a recombinant plasmid was obtained from the transformant No. 48 which coud produce a double band profile in agarose gel after BamHI and EcoRI double digestion.On band aligned with the plasmids and one band aligned approximately with the 1kb band of the DNA ladder.The latter corresponds to the target gene (ansB) size. Conclutions: Only a proof for a recombinant plasmid was obtained from the transformant No. 48 which coud produce a double band profile in agarose gel after BamHI and EcoRI double digestion.On band aligned with the plasmids and one band aligned approximately with the 1kb band of the DNA ladder.The latter corresponds to the target gene (ansB) size.

Keywords: Gene cloning; ansB; L-Asparginase