CONSTRUTION, CLONING AND EXPRESSION OF THE ETS DOMAIN OF HUMAN *FEV* GENE

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The Ets family of proteins is composed of transcription factors that are involved in a variety of developmental processes and cellular responses to extra cellular signals. These transcription factors have a conserved sequence, the Ets domain, which mediates specific binding to DNA. The Fifth Ewing Variant (FEV) is an Ets factor whose expression was only detected in prostate and small intestine of adults. It binds DNA regulatory regions and acts as transcriptional repressor, thus affecting expression of target genes. Two domains were identified as responsible for FEV-mediated repression: the Ets domain and the carboxy-terminal alanine-rich domain. The objectives of this work are to perform sequence-specific DNA binding kinetic studies and protein crystallization for structural analysis of the FEV transcription factor. To that end, the region of the gene that encodes the Ets domain of FEV was synthetically constructed by extension of overlapping oligonucleotides by a DNA polymerase in PCRs. This fragment was cloned into the pCR-Blunt vector and subcloned into the pET23a(+) expression vector. Different *Escherichia coli* strains are being used in expression assays with the aim of overexpressing the protein. This work represents an important step for the three-dimensional structure determination and studies of FEV bound to DNA.