CLONING PROPOSALS FOR LARGE DNA FRAGMENTS: AN EXPERIENCE WITH UROPATHOGENIC *ESCHERICHIA COLI* ALPHA-HEMOLYSIN AND CYTOTOXIC NECROTIZING FACTOR 1 TOXINS

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Every year nearly 150 million people are affected by urinary tract infection (UTI). Notoriously, about 60 to 80% of all cases of UTI are caused by uropathogenic *Escherichia coli* (UPEC). UPEC presents two toxins with specific and significant roles in the pathogenesis: alpha-hemolysin (HlyA) and cytotoxic necrotizing factor type 1 (CNF1). HlyA is responsible for cellular lyse by host membrane pore formation. CNF1 activates the Rho GTPases, leading to cytoskeleton disruption. Thus, this work aims to obtain these toxins through cloning techniques, as a first step to improve the diagnosis and treatment of UTI by antibody development. The genes of those toxins are similar in size, both are around 3 kb, and presented a challenge from our past cloning experience. Thus, different approaches were proposed for enhancing the cloning efficiency. First, the pET20b vector was selected, for its peptide signal sequence, to be inserted into *E. coli* DH5a and BL21(DE3) pLysE host, directing the toxin to periplasmatic region with a controlled level of expression for a soluble arrangement. Also, the pQE30 plasmid was chosen, for its T5 promoter, in the *E. coli* XL1-Blue host, allowing proteins to be produced in a lower rate for high-efficiency protein expression. The *hlyA* was obtained with gene amplification of the prototype strain UPEC J96 using two set of primers, containing the restriction enzymes BamHI and NotI or KnpI for pET20b and pQE30 cloning, respectively. For the polymerase chain reaction different conditions were tested, the Platinum High-Fidelity Taq (Thermo Scientific) amplifying genomic DNA at a 60 ºC extension temperature showed to be the most efficient condition. Afterwards, *hlyA* was cloned into a linearized pET20b using 1:3 vector:insert ratio, and chemocompetent *E. coli* DH5a strain was successfully transformed. The plasmids were extracted with the QIAprep Spin Miniprep kit (Qiagen) for restriction analysis and sequencing, however in both assay the presence of *hlyA* was not detected. For the CNF1 toxin, a synthetic gene was manufactured by Genescript in pUC57. The *cnf1* was also cloned in pET20b and inserted into DH5a strains as described for *hlyA*. Also, both toxins genes will be inserted in another vector, the pQE30, and *E. coli* XL1-Blue will be chemotransformed for protein expression.

Keywords: Uropathogenic *Escherichia coli*, alpha-hemolysin, cytotoxic necrotizing factor 1, gene cloning, large DNA.

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