

EXPRESSION OF LEISHMANIA CHAGASI TOP2 GENE MUTANTS IN E. COLI

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DNA topoisomerase II is a conserved enzyme involved in all metabolic processes related to the DNA and is a target for anti-parasite compounds. Trypanosomatid TOP2 genes have a 3'end terminal nucleotide sequence encoding the amino acids signal sequence for enzyme cellular localization. The goal of this study was to produce deletions on different regions of the *Leishmania chagasi* TOP2 gene (*LchTOP2*) in order to identify this signal sequence on the *LchTOP2* gene. We did deletions on the *EcoRI* restriction site, at the 3592 base pair and on *EcoRV* restriction site at the 1963 base pair. The gene containing these deletions was inserted N-terminally to the EGFP gene and transfected into *E. coli*, B-21 strain and the positive clones were searched after DNA miniprep procedure followed by *Bam*HI, *Bcl*I and *Sma*I enzyme digestions. The restriction products electrophoresis profile were analyzed after Ethidium Bromide staining. The topo II expression was monitored using an Olympus microscope with a 480 nm excitation barrier filter and a 100-W mercury lamp. Two positive clones containing *LchTOP2* were obtained and both were expressing topo II. The next step of this study is to analyze whether these gene deletions affect the enzyme localization in *L. Chagasi*.

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Key words: GFP, DNA topoisomerase II, protein localization, *Leishmania chagasi*.