DEVELOPMENT OF A DUAL LUCIFERASE VECTOR TO STUDY GENE EXPRESSION IN *TRYPANOSOMA CRUZI*

DaRocha W.D.[1,2], Araújo P.R.[2], Mendes-Almeida R.[2], Bartholomeu D.C.[3] and Teixeira S.M.R[2]

[1]Centro Universitário de Belo Horizonte/UniBH and [2]Departamento de Bioquímica e Imunologia and [3]Departamento de Parasitologia, UFMG, Belo Horizonte, Brasil

Similar to other trypanosomatids, *T.cruzi* gene expression is primarily regulated at the post-transcriptional level, usually by mechanisms affecting mRNA stability. To better analyze the contribution of 5' and 3' untranslated sequences (UTR) on gene expression throughout the parasite life cycle, we generated a construct carrying the renilla and firefly luciferase reporter genes as well as the neomycin resistance (Neo^R) gene. Flanking the renilla luciferase gene (RLUC - internal control) and the Neo^R, we placed UTR sequences from constitutively expressed genes (TcP2β or gapdh). Dowstream from the firefly luciferase (FLUC) coding sequence, we inserted 3'UTR+intergenic sequences derived from differentially regulated genes (a-tubulin, amastin and MASP). Transient transfections of epimastigotes with each construct result in FLUC activities that are consistent with previous data on the expression of these genes, with a-tubulin 3'UTR (gene up-regulated in epimastigotes) resulting in the highest levels of luciferase (68% compared to the control plasmid). In contrast, amastin 3'UTR (up-regulated in amastigotes) and MASP 3'UTR (up-regulated in trypomastigotes) resulted in 31% and 7% of FLUC activity, respectively. After drug selection, we were able to generated stable transfectants with all constructs, which are being used to determine the FLUC activity and mRNA levels in all stages of the parasite life cycle. Support: FAPEMIG and HHMI.