

CONSTRUCTION OF SYSTEM ALLOWS TO DISPLAY HETEROLOGOUS PROTEIN ON THE YEAST CELL SURFACE DISPLAY

Fuentes-Rivera, J.N¹, Vicente, E.J¹, Schenberg, A.C.G¹

¹Laboratório de Genética de Microrganismos, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil

Introduction and objective. Display of heterologous proteins on the cell surface of microorganisms have been actively studied and various applications reported, including use as whole-cell biocatalysts, screening of antigens, and development of vaccines. To display proteins on yeast cells, target genes have been fused with C-terminal anchor-domain sequences of native cell-wall of *Saccharomyces cerevisiae*. The aim of this study is to construct recombinant yeast displaying glucoamylase anchored on the yeast cell surface using the C-terminal region of the *S. cerevisiae* Flo1 protein (Flo1p) like domain anchor. To reach this objective, in the first stage there was cloned the C-terminal region of the gene *FLO1* from *S. cerevisiae* genomic DNA. **Results.** Restriction analyses of plasmid isolated from transformant clones allowed us to identify plasmid pGEMT-Flo1. This plasmid contain the fragment encoding C-terminal region of Flo1p from *S. cerevisiae* amplified by PCR. The result of sequence reaction confirmed the correct sequence of fragment Flo1p inserted into pGEMT-Easy vector. Glucoamylase, amplified by PCR, was inserted into pMA91 vector under *PGK* promoter from *S. cerevisiae*. The presence of the insert glucoamilase was revealed by restriction analyses. **Conclusion.** It was possible to cloning of C-terminal region of Flo1p inside *Escherichia coli* DH5a cells, which will be used in the next stage as anchor to fix glucoamylase on the cell wall *S. cerevisiae*.

Key words : Cell surface display, Flo1p, glucoamylase, yeast.

Apoio Financeiro: Companhia Vale do Rio Doce- CVRD