BIO.18 - Genomic integration as a way to stable high producers CHO cells

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Introduction: Therapeutic protein produced in mammal cells is a promissing market estimated worldwide in US$ 500 by until 2020.

Objective: Our main goal was to develop a proprietary system to increase expression levels of the gene of interest. This system was based in ExpiCHO-S cells genetic manipulation based on Transposon integration, epigenetic expression regulation by insulators and fluorescent gene reporter co-expression controlled by IRES (Internal Ribosome Enter Site), resulting in bicystronic mRNA.

Methodology: This methodology is based on DNA genomic integration by sleeping beauty transposase. Transfections were made with 25 µg of PEI (polyethylenimine) and 12,5 µg of total DNA (75% of DNA of interest and 25% of transposase DNA) for a total of 10^7 cells. Two days after, transfectants were selected by FACS, an antibiotic independent process, using GFP as the reporter gene (controled by IRES). Two more sortings rounds were necessary to stablish a 100% transfectant stable population. Cell culture supernatant was collected, and protein of interest was analysed by Western blot and ELISA.

Results: After genomic integration by transposase, transgene expression was stable for more than 20 cell passages and also after three consecutive freeze and thaw cicles. There was a clear relationship between protein of interest expression level and GFP fluorescence level. Working directly with FACS selected populations (without cell cloning) the range of protein expression was about 0,5g/L in fed-batch bioreactors.

Conclusion: Since (i) transposase mediated genomic integration showed as a reliable method to produce stable recombinant cells (without any selective pressure) and (ii) that the most fluorescent cells are the higher producers, we are now optimizing a cloning protocol in order to find the best balance between cell growth and protein expression and reach protein titers higher than 1g/L in fed-batch bioreactors. We are currently improving these recombinant gene expression system to produce any complex protein of interest in a CHO cell model and place IBMP as a Biotechnology Reference Center.

Keywords: Therapeutic protein; CHO cell; Expression system