**Introduction**

The demand for efficient, scalable production of therapeutic and diagnostic monoclonal antibodies (mAbs) has intensified the need for high-performing mammalian expression systems, particularly Chinese hamster ovary (CHO) cells. CHO cells remain the most widely used platform in biopharmaceutical manufacturing due to their capability to perform complex post-translational modifications, their robust performance in large-scale bioreactors, and their established regulatory acceptance.

Among genetic enhancements aimed at improving CHO cell lines, knockout of the glutamine synthetase (GS/GLUL) gene has emerged as a valuable strategy. GS-knockout (GS-KO) enables metabolic selection under glutamine-deprived conditions, enriching cells that have stably integrated expression vectors containing a functional GS gene. This method significantly reduces dependency on antibiotic-based selection and enhances clone stability. When combined with a transposon-based integration system, such as PiggyBac—which provides stable, efficient, and predictable genomic insertion—GS-KO represents a powerful genetic-engineering approach for next-generation antibody expression systems.

This thesis proposes a fully *in silico* pipeline designed to facilitate the creation and evaluation of a GS-knockout CHO cell line integrated with a PiggyBac vector system for the expression of a diagnostic anti-PSA monoclonal antibody. The antibody, derived from clone 5D5A5, specifically targets prostate-specific antigen (PSA), an established biomarker used extensively in prostate cancer diagnostics. By performing comprehensive computational analyses and optimization, this project aims to rigorously assess each genetic design aspect before any costly laboratory implementation occurs.

Adopting a design-build-test-learn (DBTL) framework, the thesis initially focuses on identifying high-efficiency and high-specificity CRISPR guides for the GS knockout using CHO genomic databases and CRISPOR-based scoring algorithms (Haeussler et al., 2016). Subsequently, the project explores guide safety profiling, indel spectrum predictions, vector architecture design, antibody sequence codon optimization, and an *in silico* scoring method for evaluating expression cassette performance. The pipeline then incorporates genome-scale metabolic modeling using the iCHO1766 metabolic network, providing predictive insights into the effects of GS knockout on CHO cell metabolism, growth rates, and monoclonal antibody production. Finally, the project includes a techno-economic assessment to quantify the expected improvements in productivity and reductions in manufacturing costs resulting from the engineered cell line.

This research addresses a practical biotechnology challenge: engineering a more productive, economically viable CHO platform through computational predictions and optimizations before substantial investment in laboratory validation. Furthermore, the resulting computational workflow offers a modular, reproducible pipeline adaptable for engineering other therapeutic proteins and antibodies.