Ilia State University

Faculty of Natural Sciences and Medicine

Master's program: Applied Genetics

MASTER'S THESIS

In Silico Construction and Simulation of a Single-Guide GS-Knockout PiggyBac Platform for Anti-PSA Fab Production in CHO Cells

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Abstract

A digital pipeline was developed to engineer Chinese hamster ovary (CHO-K1) cells with a dual-locus glutamine synthetase (GS) knockout combined with PiggyBac-mediated rescue and antibody expression. A single SpCas9 guide RNA was designed using CRISPOR and CHOPCHOP, then filtered for on-target efficiency, specificity, and frameshift probability. BLAST alignment confirmed a perfect match to both *GLUL* loci (Haeussler et al., 2016). Indel outcomes were predicted with inDelphi, showing a 73% likelihood of frameshift disruption (Shen et al., 2018). The selected guide was inserted *in silico* into an all-in-one pX330 vector, and a PiggyBac payload was assembled to carry a codon-optimized GS gene and an anti-PSA Fab (including heavy- and light-chains), each under separate EF-1α promoters. Genome-scale metabolic modelling with the iCHO2291 model predicted complete growth arrest for knockout cells in glutamine-free medium, while full restoration of biomass flux was observed after transgene rescue, confirming the intended selection logic (Schinn et al., 2021). The integrity and orientation of all vectors and cassettes were verified using SnapGene, and no unwanted restriction sites or frameshifts were detected. The full workflow—including guide selection, vector construction, and metabolic validation—was documented in a publicly archived GitHub–Zenodo repository to ensure reproducibility. These results demonstrate that a single-guide, one-vector strategy can be designed *in silico* to shorten cell-line development, lower off-target risk, and align with antibiotic-free regulatory guidelines. The approach should allow rapid laboratory translation and may be adapted for other diagnostic or therapeutic antibody formats.