Leveraging orthogonal transposase/transposon pairs as an alternative genetic engineering tool in CHO cells

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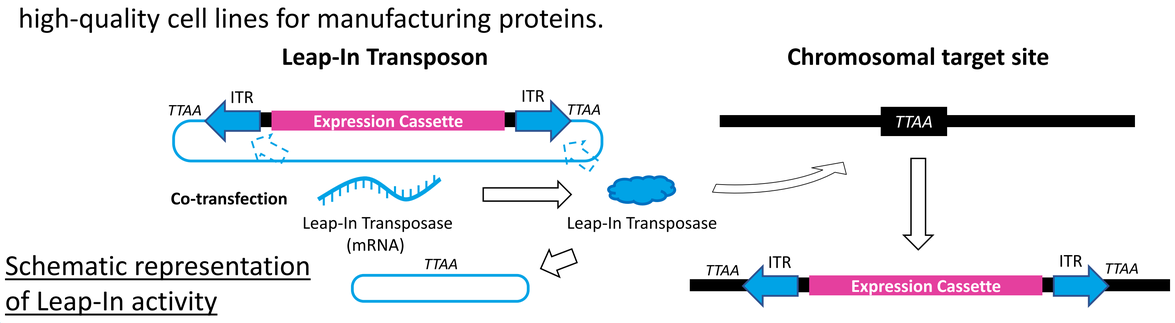
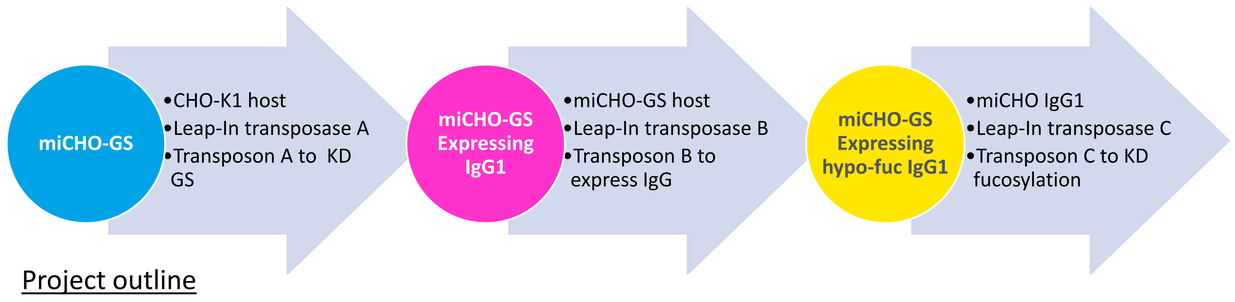
Introduction and Objectives

Transposons ("jumping genes") are genetic elements flanked by inverted terminal repeats (ITRs) that can move from one locus to another via a cut-and-paste mechanism. Discovered by Barbara McClintock (Nobel Prize in 1983), they are among the oldest and most prevalent sequences in biology. Recently, transposon-based platforms have gained prominence, emerging as a new gold standard in cell line development due to their ability to overcome significant challenges associated with traditional random integration systems. ATUM has developed Leap-In Transposase Technology (Leap-In) to efficiently integrate DNA expression cassettes encoding recombinant genes, such as antibodies, Fc-fusions, and bispecifics. Leap-In integrates multiple copies of the expression cassette, typically one copy per locus, distributed across multiple loci within the host genome. This clean, enzymatically driven integration maintains the structural integrity of the expression cassette at each locus, making it an ideal technology for developing high-quality cell lines for manufacturing proteins.



Schematic representation of Leap-In activity

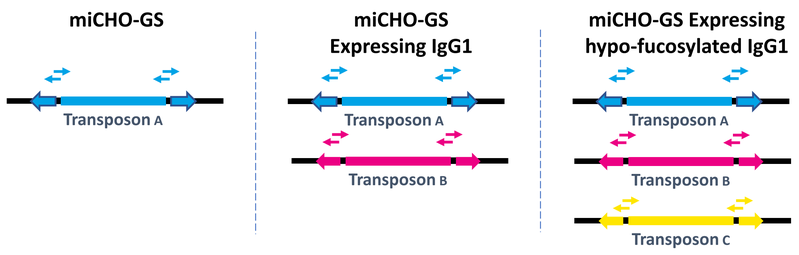


Project outline

In this project, we expand the application of transposon technology beyond conventional cell line development in CHO (Chinese Hamster Ovary) cells and leverage it as a genetic engineering tool to knock-in transgenes for overexpression and to knockdown (KD) endogenous gene expression. We take advantage of the fact that ATUM has developed multiple orthogonal Leap-In Transposases to sequentially deliver three transposons into CHO cells in order to achieve the desired phenotype at each step. We start using Transposon A, which modulates CHO cell metabolism, to generate a new, glutamine synthetase (GS) selectable CHO-K1 host cell line (miCHO-GS) via knockdown of GS gene so the new host does not survive in glutamine-free media. We then integrate Transposon B encoding an IgG biotherapeutic and GS as a selection marker to generate stable and high-expressing clones producing the desired IgG. Finally, using Transposon C, we modulate glycan pathways to develop a clone that produces an IgG biotherapeutic with low overall fucose levels.

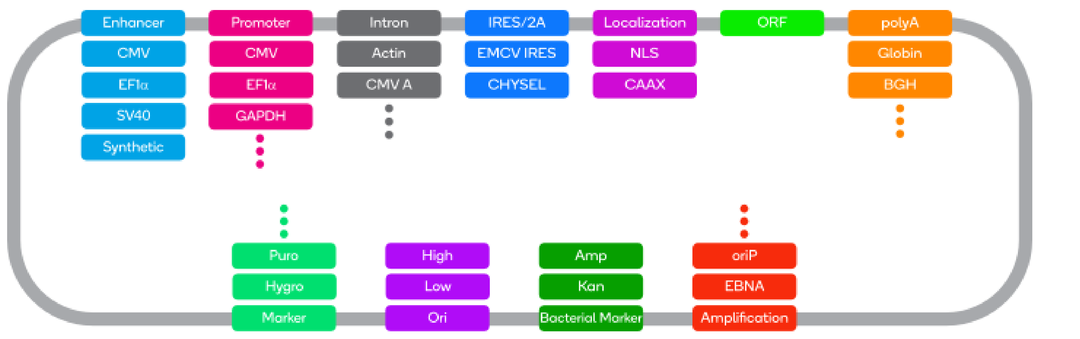
Materials and Methods

The Leap-In Transposases are hyperactive transposases engineered using ATUM's machine learning platform and are regularly used to develop CHO cell lines with predictable expression of different types of biotherapeutic proteins. In this project, we used three orthogonal Leap-In Transposases in combination with ATUM's toolbox for cell line development to knock-in transgenes for overexpression, and ATUM's proprietary miLPN technology to knockdown endogenous gene expression. The genetic characterization of each developed clone was performed by Targeted Locus Amplification (TLA) and Next-Generation Sequencing (NGS) at Cergentis, while fed-batch characterization and product quality analysis were performed at ATUM using ATUM's standard methodologies.



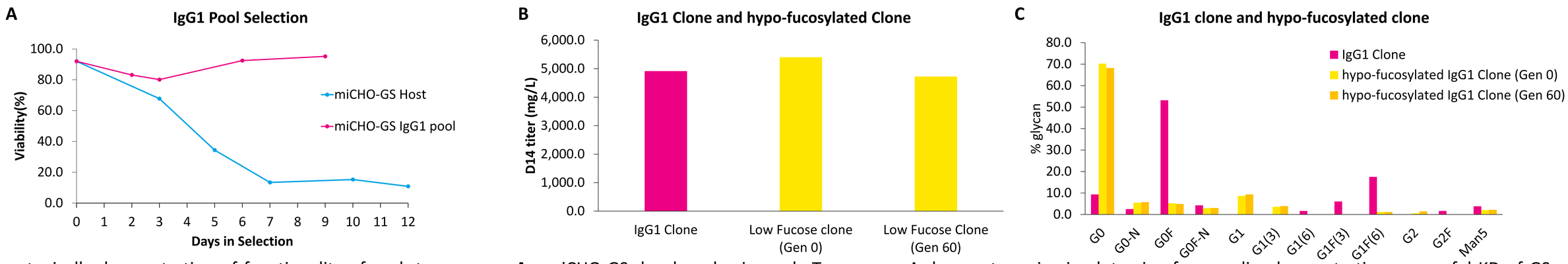
TLA experimental set up.

Two transposon-specific primer sets were designed for each transposon

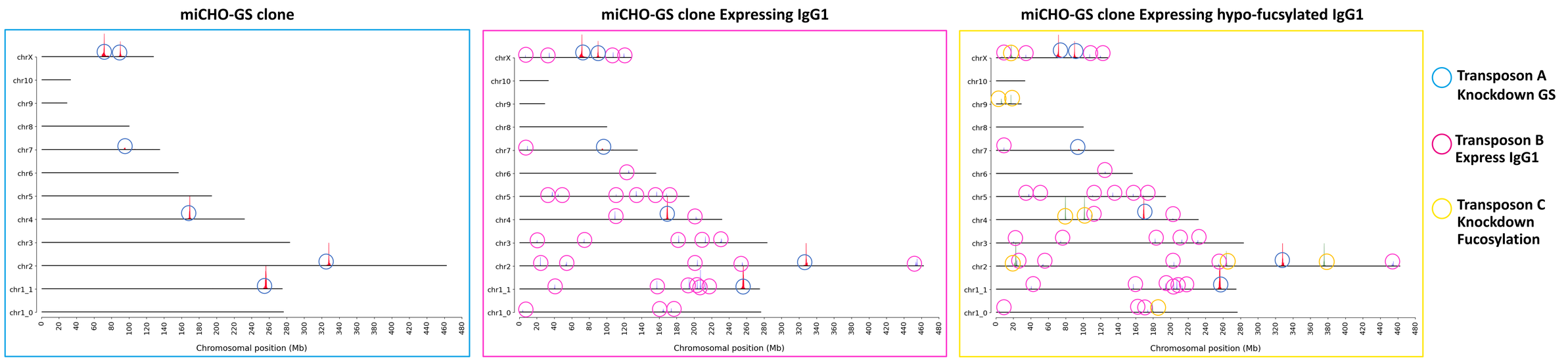


Toolbox for cell line development. ATUM's cell Line development toolbox, combines the Machine Learning (ML)-driven design of genetic circuitry with a comprehensive library of *in vivo* characterized genetic elements. This library includes ATUM's proprietary codon optimization algorithms, selectable markers, promoters, enhancers, insulators, signal peptides, mRNA transport sequences, and other essential components.

Results and Discussion



Phenotypically demonstration of functionality of each transposon. **A** - miCHO-GS developed using only Transposon A does not survive in glutamine-free media, demonstrating successful KD of GS. Survival was re-established when Transposon B was integrated, which included the GS gene as a selection marker**; B** - High-expressing IgG1 with no loss of productivity even upon integration of transposon C to silence of fucosylation, demonstrating successful and stable integration of Transposon B; **C** - Silencing fucosylation with Transposon C leads to a shift into primarily non-fucose glycan species, with overall fucose levels <10%. The stability of the glycan profile even after 60 generations demonstrates the successful and stable integration of Transposon C.



Genome-wide coverage plot showing the stable integration of each orthogonal transposase/transposon system. The TLA analysis in all three clones clearly demonstrated that each transposon was integrated correctly and stably (Transposon A was integrated at 6 genomic loci, Transposon B at 33 loci, and Transposon C at 9 loci). During the sequential rounds of integration, no locus or sequence alterations were observed on existing transposons. The clear demonstration of the stability of the transposons throughout the project, coupled with demonstrations of the functionality/purpose of each – GS knockdown, high IgG1 expression, and the hypo-fucosylation product quality attribute – clearly demonstrates the robustness of the Leap-In platform as a genetic engineering tool.

Conclusions

The potential for Leap-In across various modalities is substantial – in addition to providing robust, high levels of biotherapeutic protein expression, the platform can be leveraged to engineer endogenous metabolic and glycosylation pathways within CHO cells. Moreover, ATUM has multiple orthogonal transposase/transposon systems that can be leveraged to sequentially alter multiple pathways in a robust and stable manner.

ATUM's customers have filed a total of **38** IND's (or equivalent) in **3** jurisdictions (US, EU and China) for molecules produced using Leap-In Technology. ATUM has **45** active Leap-In licensees, including half of top 20 biopharmaceutical companies and has completed over **200** fee-for-service Leap-In projects.

References

Tu *et al*. **Monoclon Antib Immunodiagn Immunother** 2024. Recombinant antibody-producing stable CHOK1 pool stability study. Abbott Laboratories, ATUM

Agostinetto *et al*. **Biotechnol Bioeng** 2022. Rapid cGMP manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools. Merck KGaA, Scripps, IAVI

Schmieder *et al.* **J Biotechnol** 2022. Towards maximum acceleration of monoclonal antibody development: Leveraging transposase-mediated cell line generation to enable GMP manufacturing within 3 months using a stable pool. Boehringer Ingelheim

Wang *et al.* **Biochem Eng J** 2022. An innovative platform to improve asymmetric bispecific antibody assembly, purity, and expression level in stable pool and cell line development Hengenix, ATUM

Rajendran *et al*. **Biotechnol Bioeng** 2022. Accelerating and de-risking CMC development with transposon-derived manufacturing cell lines. ATUM

Lee *et al*. **GEN** 2019. Leap-In transposases and transposons accelerate cell line development ATUM

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