CRISPR-mediated Engineering of CHO Cell Lines: CHO-S and CHO-K1 Case Studies

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Abstract

CHO cells continue to be the gold standard for biotherapeutic development. With the publication of the CHO genome and the advances in CRISPR-mediated gene editing, the engineering of custom CHO cell lines is feasible. Rapid, cost-effective generation of custom CHO lines requires efficient delivery of CRISPR machinery for precise gene editing and high, post engineering cell viability. This poster highlights case studies demonstrating the use of non-viral, CRISPR-mediated genome editing of CHO cells to overcome issues such as protein quality, glycosylation heterogeneity, and product purification including the production of MGAT-deficient CHO cells to produce oligomannose-enriched HIV gp120 for improved vaccine immunogenicity.

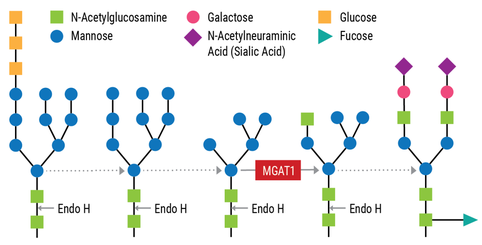
Case Study #1 - Enhancing HIV-1 Vaccine Efficacy & Manufacturing Through CHO Genome Engineering

CRISPR-mediated Inactivation of MGAT1 Enzyme Leads to Oligomannose-enriched gp120 & Improved bN-mAb Binding

RV144 HIV Vaccine Trial
- Demonstrated 31% efficacy
- Used gp120 produced in CHO cells
- gp120 lacked N-linked glycosylation sites key for broadly neutralizing antibodies (bN-mAbs)
- Highly heterogeneous glycosylation interferes with purification

Strategy
- Knockout MGAT1 enzyme in CHO-S genome
- Express gp120 with increased glycan homogeneity enriched for oligomannose residues
- Avenue for improving gp120 manufacturing and vaccine efficacy

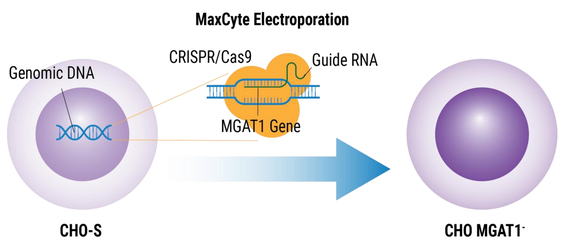
Overview of N-linked Glycosylation Pathway



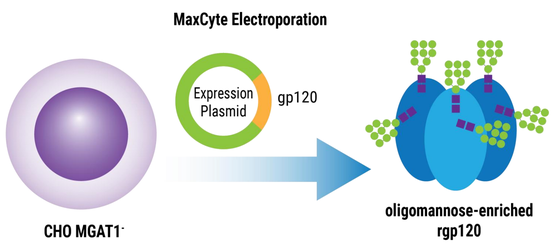
MGAT1 = alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase

CRISPR MGAT1 Editing & Transient gp120 Expression
All experimental details can be found in Materials & Methods of: *(2018) PLoS, 13(8): e0197656& (2018) PLoS Biol, 16(8): e2005817.*

CHO-S Cell Line Engineering



Transient Expression of rgp120



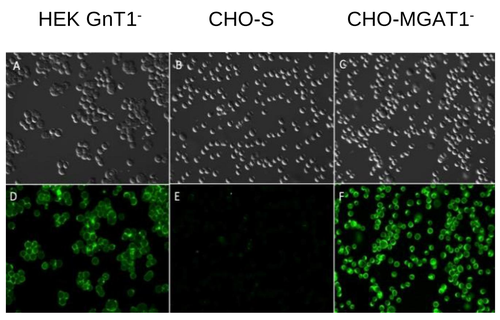


Figure 1: CRISPR-mediated Inactivation of MGAT1 Gene. GNA lectin binds glycan structures with terminal mannose and does not bind complex glycans. CHO-S cells were transfected with a plasmid designed to inactivate the MGAT1 gene by CRISPR/Cas9 gene editing (MGAT CHO). Cells were treated with fluorescein-conjugated GNA to screen for high-mannose glycans in the cell membrane. HEK 293 GnTI− cells that also lack the MGAT1 gene serve as a positive control for MGAT disruption (A & D), while normal CHO-S cells with an intact MGAT1 gene serve as a negative control (B & E). Cells were visualized under 20× magnification using contrast microscopy (A, B, C) or fluorescence (D, E, F). *Figure provided by: Dr. Phil Berman, UCSC.*

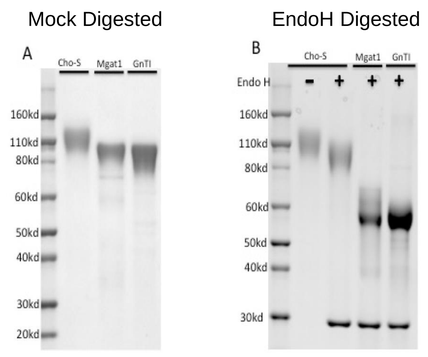


Figure 2: Endoglycosidase Analysis Demonstrates Prevalence of High Mannose Glycans on gp120 Produced in MGAT1⁻ CHO Cells. Immunoaffinity-purified A244-rgp120 recovered from transiently transfected CHO-S, MGAT1⁻ CHO, or HEK GnTI⁻ cells was analyzed by SDS-PAGE following endoglycosidase treatment. Purified gp120s were reduced, denatured, and then treated with Endo H. The digests were then analyzed on 4%±12% tris-glycine SDS-PAGE gels and stained with Coomassie blue dye. A). Mock digested rgp120s; B). rgp120 samples digested with Endo H. Endo H cleaves N-linked high-mannose glycan structures but not complex, sialic acid-containing glycans. The endoglycosidase proteins are visible as bands at 29 kD. *Figure provided by: Dr. Phil Berman, UCSC.*

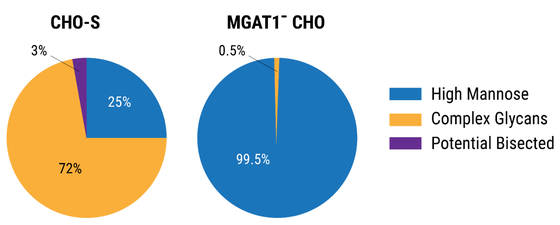


Figure 3: Glycan Profile Analysis Using MALDI-TOF. MALDI-TOF analysis of glycans present on gp120 produced by CHO-S and MGAT1⁻ CHO cell lines. The percentage of high-mannose, complex, and potential bisected N-linked glycans are indicated. Analysis performed by Complex Carbohydrate Research Center at the University of Georgia.

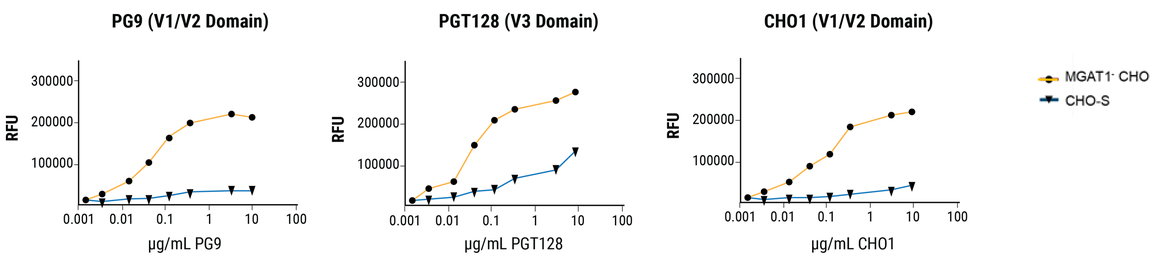
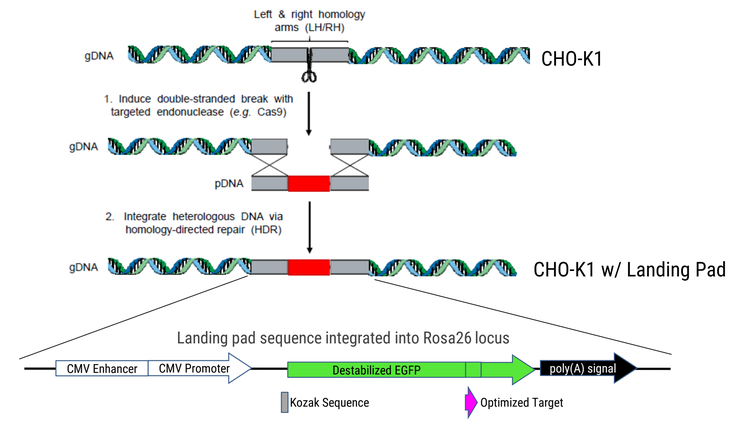


Figure 4: Improved Binding of V1/V2 and V3 Domain bN-mAbs to rgp120 Produced in MGAT1-deficient CHO Cells. A224-rgp120 purified from CHO-S or MGAT1⁻ CHO cells were coated onto microtiter plates and binding of a panel of antibodies with broadly neutralizing capacity examined via fluorescence immunoassays.

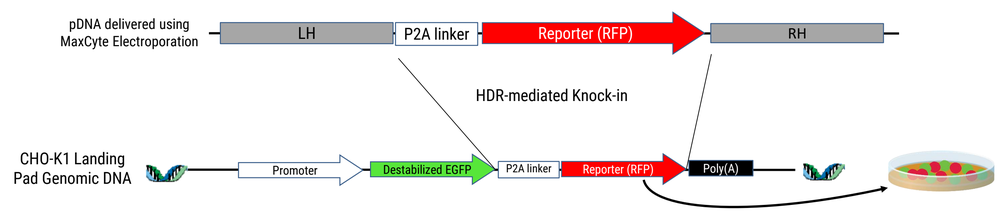
Case Study # 2 – Use of CHO-K1 Cell Line with 'Landing Pad' for Simplified Gene Expression

CRISPR-mediated Knock-in of RFP into CHO-K1 Landing Pad Cell Line

Schematic of CHO-K1 Genome Containing Landing Pad



CRISPR-mediated, Site Specific Integration of RFP Reporter Gene into CHO-K1 Landing Pad



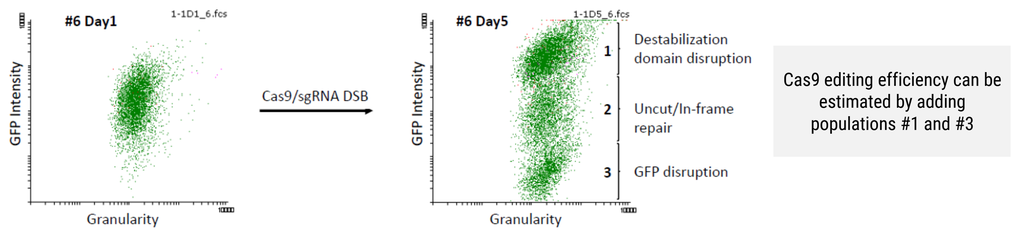


Figure 5: Cas9 Cleavage Efficiency Measured Using Flow Cytometry. 1e7 CHO-K1 cell containing the landing pad were electroporated using MaxCyte electroporation with plasmid encoding Cas9, sgRNA and a circular HDR donor encoding RFP. Cells were examined by flow cytometry on Day 1 and Day 5 post electroporation for GFP expression. *Data from University of Delaware.*

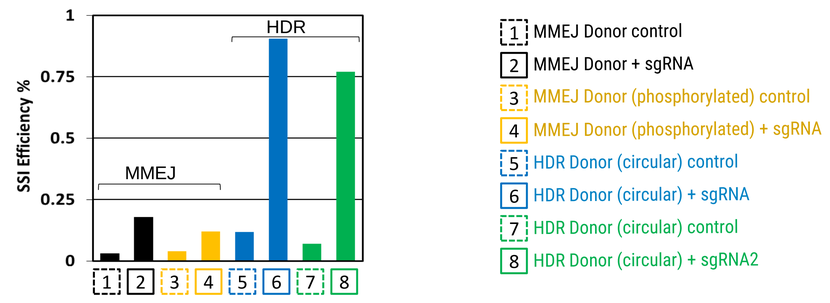


Figure 6: Effect of Donor Type on Site-specific Integration. 1e7 CHO-K1 cell containing the landing pad were electroporated using MaxCyte electroporation with plasmids encoding Cas9 +/- sgRNA and various donors encoding RFP. HDR homology arms were 2 x 850 bp; MMEJ homology arms were 2 x 20 bp. HDR-mediated SSI efficiency was higher than MMEJ-mediated SSI efficiency. *Data from University of Delaware.*

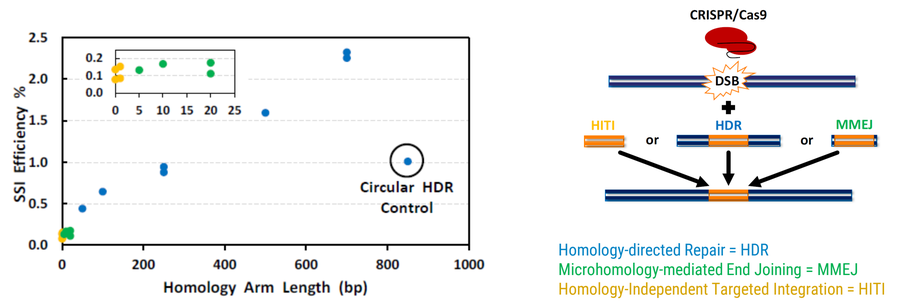


Figure 7: Longer Homology Arm Length Significantly Improves Site-specific Integration Efficiency. 1e7 CHO-K1 cell containing the landing pad were electroporated using MaxCyte electroporation with plasmids encoding Cas9/sgRNA and various donors encoding RFP. Cas9/sgRNA loading fixed at 30µg plus variable donor amount to maintain 1:4:2 Cas9:sgRNA:Donor. HDR homology arms = 50 ≤ x ≤ 850 bp, MMEJ homology arms = 5 ≤ x ≤ 20 bp, NHEJ/HITI homology arms = 0 ± 5' phosphates. *Data from University of Delaware.*

Summary

These case studies demonstrate the broad impacts of MaxCyte's enabling ExPERT™ platform ranging from improving the efficacy of biologics, accelerating protein production and developing innovative new gene editing schemes.

- The MaxCyte platform enables sophisticated, high efficiency, CRISPR-mediated genome editing of CHO cell lines through:
  - High efficiency, high viability transfection
  - High performance transfection of any CHO cell line
  - Efficient delivery of any gene editing machinery (RNPs, mRNA, gRNA, DNA)
- MaxCyte's Flow Electroporation® Technology results in:
  - Precise genome engineering
  - High titer transient protein production
  - Rapid, high titer stable cell line generation

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