Research outputs:

**Glyco-engineered CHO cell lines producing alpha-1-antitrypsin and C1 esterase inhibitor with fully humanized N-glycosylation profiles**

Recombinant Chinese hamster ovary (CHO) cells are able to provide biopharmaceuticals that are essentially free of human viruses and have N-glycosylation profiles similar, but not identical, to humans. Due to differences in N-glycan moieties, two members of the serpin superfamily, alpha-1-antitrypsin (A1AT) and plasma protease C1 inhibitor (C1INH), are currently derived from human plasma for treating A1AT and C1INH deficiency. Deriving therapeutic proteins from human plasma is generally a cost-intensive process and also harbors a risk of transmitting infectious particles. Recombinantly produced A1AT and C1INH (rhA1AT, rhC1INH) decorated with humanized N-glycans are therefore of clinical and commercial interest.

Here, we present engineered CHO cell lines producing rhA1AT or rhC1INH with fully humanized N-glycosylation profiles. This was achieved by combining CRISPR/Cas9-mediated disruption of 10 gene targets with overexpression of human ST6GAL1. We were able to show that the N-linked glyco-structures of rhA1AT and rhC1INH are homogeneous and similar to the structures obtained from plasma-derived A1AT and C1INH, marketed as Prolastin®-C and Cinryze®, respectively. rhA1AT and rhC1INH produced in our glyco-engineered cell line showed no detectable differences to their plasma-purified counterparts on SDS-PAGE and had similar enzymatic in vitro activity. The work presented here shows the potential of expanding the glyco-engineering toolbox for CHO cells to produce a wider variety of glycoproteins with fully humanized N-glycan profiles. We envision replacing plasma-derived A1AT and C1INH with recombinant versions and thereby decreasing our dependence on human donor blood, a limited and possibly unsafe protein source for patients.
An Online Compendium of CHO RNA-Seq Data Allows Identification of CHO Cell Line-specific Transcriptomic Signatures

Chinese hamster ovary (CHO) cells can fold, assemble and modify proteins post-translationally to produce human-like proteins; as a consequence, it is the single most common expression systems for industrial production of recombinant therapeutic proteins. A thorough knowledge of cultivation conditions of different CHO cell lines has been developed over the last decade, but comprehending gene or pathway-specific distinctions between CHO cell lines at transcriptome level remains a challenge. To address these challenges, we compiled a compendium of 23 RNA-Seq studies from public and in-house data on CHO cell lines, i.e. CHO-S, CHO-K1 and DG44. Significantly differentially expressed (DE) genes particularly related to subcellular structure and macromolecular categories were used to identify differences between the cell lines. A R-based web application was developed specifically for CHO cell lines to further visualize expression values across different cell lines, and make available the normalized full CHO data set graphically as a CHO research community resource. This study quantitatively categorizes CHO cell lines based on patterns at transcriptomic level and detects gene and pathway specific key distinctions among sibling cell lines. Studies such as this can be used to select desired characteristics across various CHO cell lines. Furthermore, the availability of the data as an internet-based application can be applied to broad range of CHO engineering applications.

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Baicalein reduces oxidative stress in CHO cell cultures and improves recombinant antibody productivity

Oxidative stress that naturally accumulates in the endoplasmic reticulum (ER) as a result of mitochondrial energy metabolism and protein synthesis can disturb the ER function. Because ER has a responsibility on the protein synthesis and quality control of the secreted proteins, ER homeostasis has to be well maintained. When H2O2, an oxidative stress inducer, was added to recombinant Chinese hamster ovary (rCHO) cell cultures, it reduced cell growth, monoclonal antibody (mAb) production, and galactosylated form of mAb in a dose-dependent manner. To find an effective antioxidant for rCHO cell cultures, six antioxidants (hydroxyanisole, N-acetylcysteine, baicalein, berberine chloride, kaempferol, and apigenin) with various concentrations were examined individually as chemical additives to rCHO cell cultures producing mAb. Among these antioxidants, baicalein showed the best mAb production performance. Addition of baicalein significantly reduced the expression level of BiP and CHOP along with reduced reactive oxygen species level, suggesting oxidative stress accumulated in the cells can be relieved using baicalein. As a result, addition of baicalein in batch cultures resulted in 1.7 - 1.8-fold increase in the maximum mAb concentration (MMC), while maintaining the galactosylation of mAb. Likewise, addition of baicalein in fed-batch culture resulted in 1.6-fold increase in the MMC while maintaining the galactosylation of mAb. Taken together, the results obtained here demonstrate that baicalein is an effective antioxidant to increase mAb production in rCHO cells.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, CHO Core, iLoop
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CRISPR/Cas9-multiplexed editing of Chinese hamster ovary B4Gal-T1, 2, 3 and 4 Tailors N-Glycan Profiles of Therapeutics and Secreted Host Cell Proteins

In production of recombinant proteins for biopharmaceuticals, N-glycosylation is often important for protein efficacy and patient safety. IgG with agalactosylated (G0)-N-glycans can improve the activation of the lectin-binding complement system and be advantageous in the therapy of lupus and virus diseases. In this study, the authors aimed to engineer CHO-S cells for the production of proteins with G0-N-glycans by targeting B4Gal-T isoform genes with CRISPR/Cas9. Indel mutations in genes encoding B4Gal-T1, -T2, and -T3 with and without a disrupted B4Gal-T4 sequence resulted in only ≈1% galactosylated N-glycans on total secreted proteins of 3-4 clones per genotype. The authors revealed that B4Gal-T4 is not active in N-glycan galactosylation in CHO-S cells. In the triple-KO clones, transiently expressed erythropoietin (EPO) and rituximab harbored only ≈6% and ≈3% galactosylated N-glycans, respectively. However, simultaneous disruption of B4Gal-T1 and -T3 may decrease cell growth. Altogether, the authors present the advantage of analyzing total secreted protein N-glycans after disrupting galactosyltransferases, followed by expressing recombinant proteins in selected clones with desired N-glycan profiles at a later stage. Furthermore, the authors provide a cell platform that prevalently glycosylates proteins with G0-N-glycans to further study the impact of agalactosylation on different in vitro and in vivo functions of recombinant proteins.

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Enhanced genome editing tools for multi-gene deletion knock-out approaches using paired CRISPR sgRNAs in CHO cells

Since the establishment of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, powerful strategies for engineering of CHO cell lines have emerged. Nevertheless, there is still room to expand the scope of the CRISPR tool box for further applications to improve CHO cell factories. Here, we demonstrate activity of the alternative CRISPR endonuclease Cpf1 in CHO-K1 for the first time and that it can be used in parallel to CRISPR/Cas9 without any interference. Both, Cas9 and Cpf1, can be effectively used for multi-gene engineering with a strategy based on paired single guide RNAs (sgRNAs) for full gene deletions. This strategy also enables the targeting of regulatory regions, which would not respond to the conventional frameshift mutations, as shown by deleting the α-1,6-Fucosyltransferase 8 (FUT8) promoter resulting in a functional knock-out. Fut8 also served as model to verify that deletion efficiency is size-independent (2 - 150 kb). To test the efficiency of multi-gene approaches in combination with gene deletion, clones harboring triple deletions in β-1,4-Galactosyltransferase (B4GALT) isozymes were identified using solely conventional PCR/qPCR. In addition two bicistronic transcription strategies were implemented to enable unequivocal pairing of sgRNAs: a CHO-derived tRNA linker that works for both, Cas9 and Cpf1, as well as paired sgRNAs in an array format, which can be used with Cpf1 due to its RNA processing ability. These strategies broaden the range of application of CRISPR for novel gene editing approaches in CHO cells and also enable the efficient realization of a genome-wide deletion library.

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Impact of CHO Metabolism on Cell Growth and Protein Production: An Overview of Toxic and Inhibiting Metabolites and Nutrients

For over three decades, Chinese hamster ovary (CHO) cells have been the chosen expression platform for the production of therapeutic proteins with complex post-translational modifications. However, the metabolism of these cells is far from...
perfect and optimized, and requires substantial knowhow and process optimization and monitoring to perform efficiently. One of the main reasons for this is the production and accumulation of toxic and growth-inhibiting metabolites during culture. Lactate and ammonium are the most known, but many more have been identified. In this review, we present an overview of metabolites that deplete and accumulate throughout the course of cultivations with toxic and growth inhibitory effects to the cells. We further provide an overview of the CHO metabolism with emphasis to metabolic pathways of amino acids, glutathione (GSH), and related compounds which have growth-inhibiting and/or toxic effect on the cells. Additionally, we survey relevant publications which describe the applications of metabolomics as a powerful tool for revealing which reactions occur in the cell under certain conditions and identify growth-inhibiting and toxic metabolite. We also present a number of resources that describe the cellular mechanisms of CHO and are available on-line. Finally, we discuss the application of this knowledge for bioprocess and medium development and cell line engineering.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Department of Biotechnology and Biomedicine, Network Engineering of Eukaryotic Cell factories
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Method for reducing ammonium and lactate production in cho cells
The present invention relates to modified producer cells for improved production of therapeutic proteins. Specifically, the inventors have found that removing genes involved in amino acid catabolism in Chinese Hamster Ovary (CHO) cells improves the cell growth and viability and likely also the yield of a recombinant therapeutic protein produced by the cells.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Quantitative Modeling of Cell Metabolism, Department of Biotechnology and Biomedicine, Network Engineering of Eukaryotic Cell factories
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Minimizing Clonal Variation during Mammalian Cell Line Engineering for Improved Systems Biology Data Generation

Mammalian cells are widely used to express genes for basic biology studies and biopharmaceuticals. Current methods for generation of engineered cell lines introduce high genomic and phenotypic diversity, which hamper studies of gene functions and discovery of novel cellular mechanisms. Here, we minimized clonal variation by integrating a landing pad for recombinase-mediated cassette exchange site-specifically into the genome of CHO cells using CRISPR and generated subclones expressing four different recombinant proteins. The subclones showed low clonal variation with high consistency in growth, transgene transcript levels and global transcriptional response to recombinant protein expression, enabling improved studies of the impact of transgenes on the host transcriptome. Little variation over time in subclone phenotypes and transcriptomes was observed when controlling environmental culture conditions. The platform enables robust comparative studies of genome engineered CHO cell lines and can be applied to other mammalian cells for diverse biological, biomedical and biotechnological applications.
Modular 5′-UTR hexamers for context-independent tuning of protein expression in eukaryotes

Functional characterization of regulatory DNA elements in broad genetic contexts is a prerequisite for forward engineering of biological systems. Translation initiation site (TIS) sequences are attractive to use for regulating gene activity and metabolic pathway fluxes because the genetic changes are minimal. However, limited knowledge is available on tuning gene outputs by varying TISs in different genetic and environmental contexts. Here, we created TIS hexamer libraries in baker’s yeast *Saccharomyces cerevisiae* directly 5′ end of a reporter gene in various promoter contexts and measured gene activity distributions for each library. Next, selected TIS sequences, resulted in almost 10-fold changes in reporter outputs, were experimentally characterized in various environmental and genetic contexts in both yeast and mammalian cells. From our analyses, we observed strong linear correlations ($R^2 = 0.75–0.98$) between all pairwise combinations of TIS order and gene activity. Finally, our analysis enabled the identification of a TIS with almost 50% stronger output than a commonly used TIS for protein expression in mammalian cells, and selected TISs were also used to tune gene activities in yeast at a metabolic branch point in order to prototype fitness and carotenoid production landscapes. Taken together, the characterized TISs support reliable context-independent forward engineering of translation initiation in eukaryotes.
Revealing key determinants of clonal variation in transgene expression in recombinant CHO cells using targeted genome editing

Generation of recombinant Chinese hamster ovary (rCHO) cell lines is critical for the production of therapeutic proteins. However, the high degree of phenotypic heterogeneity among generated clones, referred to as clonal variation, makes the rCHO cell line development process inefficient and unpredictable. Here, we investigated the major genomic causes of clonal variation. We found: (1) consistent with previous studies, a strong variation in rCHO clones in response to hypothermia (33 vs 37 °C) after random transgene integration; (2) altered DNA sequence of randomly integrated cassettes, which occurred during the integration process, affecting the transgene expression level in response to
hypothermia; (3) contrary to random integration, targeted integration of the same expression cassette, without any DNA alteration, into three identified integration sites showed the similar response of transgene expression in response to hypothermia, irrespective of integration site; (4) switching the promoter from CMV to EF1α eliminated the hypothermia response; and (5) deleting the enhancer part of the CMV promoter altered the hypothermia response. Thus, we have revealed the effects of integration methods and cassette design on transgene expression levels, implying that rCHO cell line generation can be standardized through detailed genomic understanding. Further elucidation of such understanding is likely to have a broad impact on diverse fields that use transgene integration, from gene therapy to generation of production cell lines.

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Using titer and titer normalized to confluence are complementary strategies for obtaining Chinese hamster ovary cell lines with high volumetric productivity of etanercept
The selection of clonally-derived Chinese hamster ovary (CHO) cell lines with the highest production rate of recombinant glycoproteins remains a big challenge during early stages of cell line development. Different strategies using either product titer or product titer normalized to cell number are being used to assess suspension-adapted clones when grown statically in microtiter plates. However, no reported study so far has performed a direct head-to-head comparison of these two early reporters for predicting clone performance. Therefore, we developed a screening platform for high-throughput analysis of titer and confluence of etanercept-producing clones. We then performed an unbiased comparison of clone ranking based on either titer or titer normalized to confluence (TTC). Using two different suspension cultivation vessels, we
demonstrate that titer- or TTC-based ranking gives rise to the selection of clones with similar volumetric productivity in batch cultures. Therefore, a combinatorial titer- and TTC-based ranking is proposed, allowing for selection of distinct clones with both, high integral viable cell density (IVCD) and high specific productivity features, respectively. This contributes to selection of a versatile panel of clones that can be further characterized and from which the final producer clone can be selected that best fits the production requirements.

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Application of CRISPR/Cas9 Genome Editing to Improve Recombinant Protein Production in CHO Cells

Genome editing has become an increasingly important aspect of Chinese Hamster Ovary (CHO) cell line engineering for improving production of recombinant protein therapeutics. Currently, the focus is directed toward expanding the product diversity, controlling and improving product quality and yields. In this chapter, we present our protocol on how to use the genome editing tool Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) to knockout engineering target genes in CHO cells. As an example, we refer to the glutamine synthetase (GS)-encoding gene as the knockout target gene, a knockout that increases the selection efficiency of the GS-mediated gene amplification system.

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Cell Factory Engineering
Rational approaches to modifying cells to make molecules of interest are of substantial economic and scientific interest. Most of these efforts aim at the production of native metabolites, expression of heterologous biosynthetic pathways, or protein expression. Reviews of these topics have largely focused on individual strategies or cell types, but collectively they fall under the broad umbrella of a growing field known as cell factory engineering. Here we condense >130 reviews and key studies in the art into a meta-review of cell factory engineering. We identified 33 generic strategies in the field, all applicable to multiple types of cells and products, and proven successful in multiple major cell types. These apply to three major categories: production of native metabolites and/or bioactives, heterologous expression of biosynthetic pathways, and protein expression. This meta-review provides general strategy guides for the broad range of applications of rational engineering of cell factories.

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CHO glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous N-glycan profiles

Combining the Chinese hamster ovary (CHO) - K1 draft genome1,2, identified CHO glycosyltransferases3 and the power of multiplexing gene knock-outs with CRISPR/Cas94 via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO-S cell lines differing in amount and combination of insertions or deletions (indels) in the targeted genes. Clones harboring 9, 6 and 4 indels were further investigated for growth, Rituximab productivity and secretome N-glycosylation.

This resulted in clones with prolonged viabilities, no changes in N-glycan galactose contents but an increase of matured and sialylated N-glycan structures in the secretome. Additionally we point out, that multiplexing an increasing amount of genes most likely results in clones only revealing a few of all possible combinations of the targets and is highly driven by the sgRNA efficiency which can differ from each other by factor 4, even after FACS sorting.

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Developing a CRISPR/Cas9 screening platform for Chinese hamster ovary cells

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Engineer medium and feed for modulating N-glycosylation of recombinant protein production in CHO cell culture

Chinese hamster ovary (CHO) cells have become the primary expression system for the production of complex recombinant proteins due to their long-term success in industrial scale production and generating appropriate protein N-glycans similar to that of humans. Control and optimization of protein N-glycosylation is crucial, as the structure of N-glycans can largely influence both biological and physicochemical properties of recombinant proteins. Protein N-glycosylation in CHO cell culture can be controlled and tuned by engineering medium, feed, culture process, as well as genetic elements of the cell. In this chapter, we will focus on how to carry out experiments for N-glycosylation modulation through medium and feed optimization. The workflow and typical methods involved in the experiment process will be presented.

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Organisations: Department of Systems Biology, Novo Nordisk Foundation Center for Biosustainability, Network Engineering of Eukaryotic Cell Factories, CHO Cell Line Engineering and Design, CHO Core
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Humanizing recombinant glycoproteins from Chinese hamster ovary cells

With new tools for gene-editing like zinc-fingers, TALENS and CRISPR, it is now feasible totailor-make the N-Glycoforms for therapeutic glycoproteins that have previously been almost impossible. We here demonstrate a case of humanizing a recombinant human glycoprotein that in Wild type (WT) Chinese hamster ovary (CHO) cells are making a very heterogeneous mixture of NGlycans. We speculate that the CHO pattern of N-Glycans would affect half-life and/or efficacy of the glycoprotein in the bloodstream making it unsuitable for human intravenous use, whereas our humanized version would be identical to the native human glycoprotein.

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Improving the secretory capacity of Chinese hamster ovary cells by ectopic expression of effector genes: Lessons learned and future directions

Chinese hamster ovary (CHO) cells are the preferred cell factory for the production of therapeutic glycoproteins. Although efforts primarily within bioprocess optimization have led to increased product titers of recombinant proteins (r-proteins) expressed in CHO cells, post-transcriptional bottlenecks in the biosynthetic pathway of r-proteins remain to be solved. To this end, the ectopic expression of transgenes (effector genes) offers great engineering potential. However, studies on effector genes have in some cases led to inconsistent results. Whereas this can in part be attributed to product specificity, other experimental and cellular factors are likely important contributors to these conflicting results. Here, these factors are reviewed and discussed with the objective of guiding future studies on effector genes.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Network reconstruction of the mouse secretory pathway applied on CHO cell transcriptome data

**Background:** Protein secretion is one of the most important processes in eukaryotes. It is based on a highly complex machinery involving numerous proteins in several cellular compartments. The elucidation of the cell biology of the secretory machinery is of great importance, as it drives protein expression for biopharmaceutical industry, a 140 billion USD global market. However, the complexity of secretory process is difficult to describe using a simple reductionist approach, and therefore a promising avenue is to employ the tools of systems biology.

**Results:** On the basis of manual curation of the literature on the yeast, human, and mouse secretory pathway, we have compiled a comprehensive catalogue of characterized proteins with functional annotation and their interconnectivity. Thus we have established the most elaborate reconstruction (RECON) of the functional secretion pathway network to date, counting 801 different components in mouse. By employing our mouse RECON to the CHO-K1 genome in a comparative genomic approach, we could reconstruct the protein secretory pathway of CHO cells counting 764 CHO components. This RECON furthermore facilitated the development of three alternative methods to study protein secretion through graphical visualizations of omics data. We have demonstrated the use of these methods to identify potential new and known targets for engineering improved growth and IgG production, as well as the general observation that CHO cells seem to have less strict transcriptional regulation of protein secretion than healthy mouse cells.

**Conclusions:** The RECON of the secretory pathway represents a strong tool for interpretation of data related to protein secretion as illustrated with transcriptomic data of Chinese Hamster Ovary (CHO) cells, the main platform for mammalian protein production.
Reprogramming amino acid catabolism in CHO cells with CRISPR-Cas9 genome editing improves cell growth and reduces by-product secretion

CHO cells primarily utilize amino acids for three processes: biomass synthesis, recombinant protein production and catabolism. In this work, we disrupted 9 amino acid catabolic genes participating in 7 different catabolic pathways, to increase synthesis of biomass and recombinant protein, while reducing production of growth-inhibiting metabolic by-products from amino acid catabolism.

Ribosome profiling-guided depletion of an mRNA increases cell growth rate and protein secretion

Recombinant protein production coopts the host cell machinery to provide high protein yields of industrial enzymes or biotherapeutics. However, since protein translation is energetically expensive and tightly controlled, it is unclear if highly expressed recombinant genes are translated as efficiently as host genes. Furthermore, it is unclear how the high expression impacts global translation. Here, we present the first genome-wide view of protein translation in an IgG-producing CHO cell line, measured with ribosome profiling. Through this we found that our recombinant mRNAs were translated as efficiently as the host cell transcriptome, and sequestered up to 15% of the total ribosome occupancy. During cell culture, changes in recombinant mRNA translation were consistent with changes in transcription, demonstrating that transcript levels influence specific productivity. Using this information, we identified the unnecessary resistance marker NeoR to be a highly transcribed and translated gene. Through siRNA knock-down of NeoR, we improved the production- and growth capacity of the host cell. Thus, ribosomal profiling provides valuable insights into translation in CHO cells and can guide efforts to enhance protein production.
Targeted gene integration into site-specific loci can be achieved in Chinese hamster ovary (CHO) cells via CRISPR/Cas9 genome editing technology and the homology-directed repair (HDR) pathway. The low efficiency of HDR often requires antibiotic selection, which limits targeted integration of multiple genes at multiple sites. To improve HDR-mediated targeted integration, while avoiding the use of selection markers, chemical treatment for increased HDR, and fluorescent enrichment of genome-edited cells was assessed in CHO cells. Chemical treatment did not improve HDR-mediated targeted integration. In contrast, fluorescent markers in Cas9 and donor constructs enable FACS enrichment, resulting in a threefold increase in the number of cells with HDR-mediated genome editing. Combined with this enrichment method, large transgenes encoding model proteins (including an antibody) were successfully targeted integrated. This approach provides a simple and fast strategy for targeted generation of stable CHO production cell lines in a rational way.

**General information**

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, iLoop
Contributors: Lee, J. S., Grav, L. M., Pedersen, L. E., Min Lee, G., Kildegaard, H. F.
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A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism

Chinese hamster ovary (CHO) cells dominate biotherapeutic protein production and are widely used in mammalian cell line engineering research. To elucidate metabolic bottlenecks in protein production and to guide cell engineering and bioprocess optimization, we reconstructed the metabolic pathways in CHO and associated them with >1,700 genes in the Cricetulus griseus genome. The genome-scale metabolic model based on this reconstruction, iCHO1766, and cell-line-specific models for CHO-K1, CHO-S, and CHO-DG44 cells provide the biochemical basis of growth and recombinant protein production. The models accurately predict growth phenotypes and known auxotrophies in CHO cells. With the models, we quantify the protein synthesis capacity of CHO cells and demonstrate that common bioprocess treatments, such as histone deacetylase inhibitors, inefficiently increase product yield. However, our simulations show that the metabolic resources in CHO are more than three times more efficiently utilized for growth or recombinant protein synthesis following targeted efforts to engineer the CHO secretory pathway. This model will further accelerate CHO cell engineering and help optimize bioprocesses.
A Markov chain model for N-linked protein glycosylation – towards a low-parameter tool for model-driven glycoengineering

Glycosylation is a critical quality attribute of most recombinant biotherapeutics. Consequently, drug development requires careful control of glycoforms to meet bioactivity and biosafety requirements. However, glycoengineering can be extraordinarily difficult given the complex reaction networks underlying glycosylation and the vast number of different glycans that can be synthesized in a host cell. Computational modeling offers an intriguing option to rationally guide glycoengineering, but the high parametric demands of current modeling approaches pose challenges to their application. Here we present a novel low-parameter approach to describe glycosylation using flux-balance and Markov chain modeling. The model recapitulates the biological complexity of glycosylation, but does not require user-provided kinetic information. We use this method to predict and experimentally validate glycoprofiles on EPO, IgG as well as the endogenous secretome following glycosyltransferase knock-out in different Chinese hamster ovary (CHO) cell lines. Our approach offers a flexible and user-friendly platform that can serve as a basis for powerful computational engineering efforts in mammalian cell factories for biopharmaceutical production.

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Scopus rating (2015): CiteScore 8.2 SJR 3.6 SNIP 1.809
Web of Science (2015): Impact factor 8.201
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BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 7.23 SJR 3.395 SNIP 2.009
Web of Science (2014): Impact factor 6.767
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Case study on human α1-antitrypsin: Recombinant protein titers obtained by commercial ELISA kits are inaccurate

Accurate titer determination of recombinant proteins is crucial for evaluating protein production cell lines and processes. Even though enzyme-linked immunosorbent assay (ELISA) is the most widely used assay for determining protein titer, little is known about the accuracy of commercially available ELISA kits. We observed that estimations of recombinant human α1-antitrypsin (rα1AT) titer by Coomassie-stained SDS-PAGE gels did not correspond to previously obtained titers obtained by a commercially available ELISA kit. This prompted us to develop two independent quantification assays based on biolayer interferometry and reversed-phase high-performance liquid chromatography. We compared the rα1AT titer obtained by these assays with three different off-the-shelf ELISA kits and found that the ELISA kits led to inconsistent results. The data presented here show that recombinant protein titers determined by ELISA kits cannot be trusted per se. Consequently, any ELISA kit to be used for determining recombinant protein titer must be validated by a different, preferably orthogonal method.
CHO On A Detox: Removing By-Product Formation Through Cell Engineering

Chinese Hamster Ovary (CHO) cells are the preferred hosts for the production of therapeutic glycoproteins. However, there is a need for improvement of the bioprocesses towards increased cell growth and higher productivities without compromising the product quality. Efforts to obtain tailor-made products with the desired properties that meet the requirements of regulatory authorities are continuously being made. Of equal relevance is to develop methods to engineer cell lines with improved by-product metabolism.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Department of Biotechnology and Biomedicine, Network Engineering of Eukaryotic Cell factories
Contributors: Pereira, S., Kildegaard, H. F., Andersen, M. R.
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Endoplasmic reticulum-directed recombinant mRNA displays subcellular localization equal to endogenous mRNA during transient expression in CHO cells

When expressing pharmaceutical recombinant proteins in mammalian cells, the protein is commonly directed through the secretory pathway, in a signal peptide-dependent manner, to acquire specific post-translational modifications and to facilitate secretion into the culture medium. One key premise for this is the direction of the mRNA encoding the recombinant protein to the surface of the endoplasmic reticulum (ER) for subsequent protein translocation into the secretory pathway. To evaluate the efficiency of this process in Chinese hamster ovary (CHO) cells, the subcellular localization of recombinant mRNA encoding the therapeutic proteins, erythropoietin (EPO) and Rituximab, was determined. The results show that ER-directed recombinant mRNAs exhibited an efficient recruitment to the ER when compared to an endogenous ER-directed mRNA, with no cytoplasmic translation of ER-directed recombinant proteins observed. These observations indicate that the recombinant mRNA, encoding ER-directed proteins, follows the same distribution pattern as endogenous mRNA directed towards the ER. Furthermore, the previous established fractionation method proves to be an efficient tool to study not only recombinant mRNA localization, but also recombinant protein trafficking between the ER and cytosol in CHO cells.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, CHO Core, Department of Systems Biology, Network Engineering of Eukaryotic Cell factories, Aarhus University
Contributors: Beuchert Kallehauge, T., Kol, S., Andersen, M. R., Kroun Damgaard, C., Min Lee, G., Kildegaard, H. F.
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Glycoprofiling effects of media additives on IgG produced by CHO cells in fed-batch bioreactors

Therapeutic monoclonal antibodies (mAbs) are mainly produced by heterologous expression in Chinese hamster ovary (CHO) cells. The glycosylation profile of the mAbs has major impact on the efficacy and safety of the drug and is therefore an important parameter to control during production. In this study, the effect on IgG N-glycosylation from feeding CHO cells with eight glycosylation precursors during cultivation was investigated. The study was conducted in fed-batch mode in bioreactors with biological replicates to obtain highly controlled and comparable conditions. We assessed charge
heterogeneity and glycosylation patterns of IgG. None of the eight feed additives caused statistically significant changes to cell growth or IgG productivity, compared to controls. However, the addition of 20 mM galactose did result in a reproducible increase of galactosylated IgG from 14% to 25%. On the other hand, addition of 20 mM N-acetyl-D-glucosamine (GlcNAc) reduced relative abundance of galactosylated IgG by 4%. Additionally, supplementation with 10 mM mannose slightly reduced GlcNAc occupancy of IgG. Overall, comparing the effects of IgG glycosylation, by supplementing the cell culture medium with glycosylation precursors during cultivation, revealed an application of these glycosylation precursors for modulating N-glycosylation of IgG.

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- Scopus rating (2015): CiteScore 4.44 SJR 1.632 SNIP 1.355
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- Scopus rating (2014): CiteScore 4.16 SJR 1.612 SNIP 1.395
- Web of Science (2014): Impact factor 4.126
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- Scopus rating (2013): CiteScore 4.44 SJR 1.637 SNIP 1.427
- Web of Science (2013): Impact factor 4.164
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- BFI (2012): BFI-level 2
- Scopus rating (2012): CiteScore 4.04 SJR 1.62 SNIP 1.364
- Web of Science (2012): Impact factor 3.648
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): CiteScore 4.08 SJR 1.668 SNIP 1.481
- Web of Science (2011): Impact factor 3.946
- ISI indexed (2011): ISI indexed yes
Multi-omic profiling of EPO producing Chinese hamster ovary cell panel reveals metabolic adaptation to heterologous protein production

Heterologous protein production in CHO cells imposes a burden on the host cell metabolism and impact cellular physiology on a global scale. In this work, a multi-omics approach was applied to characterize the physiological impact of erythropoietin production, and discover production bottlenecks, in a panel of CHO-K1 cells in batch and chemostat culture.

General information

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Quantitative Modeling of Cell Metabolism, Network Engineering of Eukaryotic Cell factories, Department of Bio and Health Informatics, Genomic Epidemiology, Department of Biotechnology and Biomedicine, Novo Nordisk AS
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Research output: Research - peer-review › Poster – Annual report year: 2017
Multi-omic profiling of EPO-producing CHO cell panel reveals metabolic adaptation to heterologous protein production

The Chinese hamster ovary (CHO) cell line is the predominant mammalian cell factory for production of therapeutic glycoproteins. In this work, we aimed to study bottlenecks in the secretory pathway associated with the production of human erythropoietin (EPO) in CHO cells. In connection to this, we discovered indications of metabolic adaptation of the amino acid catabolism in favor of heterologous protein production. We established a panel of stably EPO expressing CHO-K1 clones spanning a 25-fold productivity range and characterized the clones in batch and chemostat cultures. For this, we employed a multi-omic physiological characterization including metabolic footprinting of amino acids, metabolite fingerprinting of glycolytic intermediates, NAD(P)H-/NAD(P)+ and adenosine nucleotide phosphates. We used qPCR, qRT-PCR, western blots and Affymetrix CHO microarrays to assess EPO gene copy numbers, EPO gene expression, intracellular protein levels and genomewide differential gene expression analysis of genes functionally related to secretory protein processing, respectively. Finally, we generated a network reconstruction of the amino acid catabolism in CHO cells. There construction was utilized as a platform for interpretation of differential gene expression data in a biological meaningful manner. To identify bottlenecks in the protein secretory pathway, we compared EPO gene copy numbers, EPO gene expression levels, intracellular EPO retention and extracellular EPO levels for a high and low producing clone during chemostat culture. The EPO productivity levels were not reflected in EPO gene load, EPO gene expression or intracellular protein retention, indicating that these processes were not limiting EPO productivity. The global gene expression analysis did not identify significant differentially expressed genes related to secretory protein processing. However, when inspecting the gene expression landscape of the aminoacid catabolism, we observed an apparent adaptation in favor of EPO production. That is, we discovered that the gene expression levels of amino acid catabolic genes had adapted to preserve the most abundant aminoacids in EPO in the high producing clone relative to the low producing clone. Based on these data, we speculate that the amino acid metabolism in CHO cells may undergo adaptation in favor of heterologous protein production during long-term cultivation.

Trends and approaches in N-Glycosylation engineering in Chinese hamster ovary cell culture

Chinese hamster ovary (CHO) cells have become the preferred expression system for the production of complex recombinantglycoproteins. It has been historically successful in industrial scale-up application and in generating human-like protein glycosylation. N-glycosylation of recombinant proteins, in particular, of those as drug substances, is extremely concerned in drug development andapproval, as it will largely affect their stability, efficacy, clearance rate and immunogenicity. Therefore to engineering N-glycosylation of CHO cell-derived recombinant proteins are extremely important. Here, we will summarize a group of recent strategies and approaches and come up with case studies for N-glycosylation engineering in CHO cells and show several examples of relevant study cases from our research: 1) media and feed design, 2) culture process optimization, 3) substrate addition, 4) geneticengineering, 5) omics-based characterization, 6) mathematical modelling.

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**Trends and approaches in N-Glycosylation engineering in Chinese hamster ovary cell culture**

Chinese hamster ovary (CHO) cells have become the preferred expression system for the production of complex recombinant glycoproteins. It has been historically successful in industrial scale-up application and in generating human-like protein glycosylation. N-glycosylation of recombinant proteins, in particular, of those as drug substances, is extremely concerned in drug development and approval, as it will largely affect their stability, efficacy, clearance rate and immunogenicity. Therefore to engineering N-glycosylation of CHO cell-derived recombinant proteins are extremely important. Here, we will summarize a group of recent strategies and approaches and come up with case studies for N-glycosylation engineering in CHO cells and show several examples of relevant study cases from our research: 1) media and feed design, 2) culture process optimization, 3) substrate addition, 4) genetic engineering, 5) omics-based characterization, 6) mathematical modelling.

**General information**

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Organisations: Department of Systems Biology, Novo Nordisk Foundation Center for Biosustainability, Network Engineering of Eukaryotic Cell Factories, CHO Cell Line Engineering and Design, CHO Core  
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**Multiplex editing system**

The present invention relates to a multiplex editing system. The system allows multiple editing of nucleic acid sequences such as genomic sequences, such as knockins of genes of interest in a genome, knockouts of genomic sequences and/or allele replacement. Also provided herein are a method for editing nucleic acids and a cell comprising a stably integrated endonuclease.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Core, Glyco-Engineering of CHO, Big Data 2 Knowledge, High Throughput Molecular Bioscience, iLoop, Network Reconstruction in Silico Biology, Department of Systems Biology, CHO Cell Line Engineering and Design, CFB - Core Flow  
Contributors: Pedersen, L. E., Ronda, C., Kildegaard, H. F., Lee, J. S.  
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**A novel low-parameter computational model to aid In-silico glycoengineering**

Glycosylation is a key post-translational modification that can affect critical properties of proteins produced in biopharmaceutical manufacturing, such as stability, therapeutic efficacy or immunogenicity. However, unlike a protein's amino acid sequence, glycosylation is hard to engineer since it does not follow any direct equivalent of a genetic code. Instead, its complex biogenesis in the Golgi apparatus (Figure 1A) integrates a variety of influencing factors most of which are only incompletely understood. Various attempts have been undertaken so far to computationally model the process of glycosylation, but due to the high parametric demand of most of these models, it has been challenging to leverage these
models for glycoengineering purposes. Consequently, industrial glycoengineering is still largely carried out using costly and time-consuming trial-and-error strategies and could greatly benefit from computational models that would better meet the requirements for industrial utilization. Here, we introduce a novel approach combining constraints-based and stochastic techniques to derive a computational model that can predict the effects of gene knockouts on protein glycoprofiles while requiring only minimal a-priori parameter input.

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**CRISPR/Cas9-mediated genome engineering of CHO cell factories: application and perspectives**

Chinese hamster ovary (CHO) cells are the most widely used production host for therapeutic proteins. With the recent emergence of CHO genome sequences, CHO cell line engineering has taken on a new aspect through targeted genome editing. The bacterial clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system enables rapid, easy and efficient engineering of mammalian genomes. It has a wide range of applications from modification of individual genes to genome-wide screening or regulation of genes. Facile genome editing using CRISPR/Cas9 empowers researchers in the CHO community to elucidate the mechanistic basis behind high level production of proteins and product quality attributes of interest. In this review, we describe the basis of CRISPR/Cas9-mediated genome editing and its application for development of next generation CHO cell factories while highlighting both future perspectives and challenges. As one of the main drivers for the CHO systems biology era, genome engineering with CRISPR/Cas9 will pave the way for rational design of CHO cell factories.

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Contributors: Lee, J. S., Grav, L. M., Lewis, N. E., Kildegaard, H. F.
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Glycoengineering of Chinese hamster ovary cells for enhanced erythropoietin N-glycan branching and sialylation

Sialic acid, a terminal residue on complex N-glycans, and branching or antennarity can play key roles in both the biological activity and circulatory lifetime of recombinant glycoproteins of therapeutic interest. In order to examine the impact of glycosyltransferase expression on the N-glycosylation of recombinant erythropoietin (rEPO), a human α2,6-sialyltransferase (ST6Gal1) was expressed in Chinese hamster ovary (CHO-K1) cells. Sialylation increased on both EPO and CHO cellular proteins as observed by SNA lectin analysis, and HPLC profiling revealed that the sialic acid content of total glycans on EPO increased by 26%. The increase in sialic acid content was further verified by detailed profiling of the N-glycan structures using mass spectra (MS) analysis. In order to enhance antennarity/branching, UDP-N-acetylglucosamine: α-1,3-D-mannoside β1,4-N-acetylglucosaminyltransferase (GnTIV/Mgat4) and UDP-N-acetylglucosamine:α-1,6-D-mannoside β1,6-N-acetylglucosaminyltransferase (GnTV/Mgat5), was incorporated into CHO-K1 together with ST6Gal1. Tri- and tetraantennary N-glycans represented approximately 92% of the total N-glycans on the resulting EPO as measured using MS analysis. Furthermore, sialic acid content of rEPO from these engineered cells was increased ~45% higher with tetra-sialylation accounting for ~10% of total sugar chains compared to ~3% for the wild-type parental CHO-K1. In this way, coordinated overexpression of these three glycosyltransferases for the first time in model CHO-K1 cell lines provides a mean for enhancing both N-glycan branching complexity and sialylation with opportunities to generate tailored complex N-glycan structures on therapeutic glycoproteins in the future.

General information

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Web of Science (2014): Impact factor 4.126
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Multi-omic profiling of EPO-producing Chinese hamster ovary cell panel reveals metabolic adaptation to heterologous protein production

Chinese hamster ovary (CHO) cells are the preferred production host for many therapeutic proteins. The production of heterologous proteins in CHO cells imposes a burden on the host cell metabolism and impact cellular physiology on a global scale. In this work, a multi-omics approach was applied to study the production of erythropoietin (EPO) in a panel of CHO-K1 cells under growth-limited and unlimited conditions in batch and chemostat cultures. Physiological characterization of the EPO-producing cells included global transcriptome analysis, targeted metabolome analysis,
including intracellular pools of glycolytic intermediates, NAD(P)H/NAD(P)+, adenine nucleotide phosphates (ANP), and extracellular concentrations of sugars, organic acids, and amino acids. Potential impact of EPO expression on the protein secretory pathway was assessed at multiple stages using quantitative PCR (qPCR), reverse transcription PCR (qRT-PCR), Western blots (WB), and global gene expression analysis to assess EPO gene copy numbers, EPO gene expression, intracellular EPO retention, and differentially expressed genes functionally related to secretory protein processing, respectively. We found no evidence supporting the existence of production bottlenecks in energy metabolism (i.e., glycolytic metabolites, NAD(P)H/NAD(P)+ and ANPs) in batch culture or in the secretory protein production pathway (i.e., gene dosage, transcription and post-translational processing of EPO) in chemostat culture at specific productivities up to 5pg/cell/day. Time-course analysis of high- and low-producing clones in chemostat culture revealed rapid adaptation of transcription levels of amino acid catabolic genes in favor of EPO production within nine generations. Interestingly, the adaptation was followed by an increase in specific EPO productivity.

**General information**

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Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 2  
Scopus rating (2012): CiteScore 4.04 SJR 1.62 SNIP 1.364  
Web of Science (2012): Impact factor 3.648  
ISI indexed (2012): ISI indexed yes
Multi-omic profiling of EPO-producing CHO cell panel reveals metabolic adaptation to heterologous protein production

General information
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Center for Biological Sequence Analysis, Metabolomics Platform, Novo Nordisk AS
Number of pages: 1
Publication date: 2015
Peer-reviewed: Yes
Electronic versions:
One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment.

The CRISPR/Cas9 genome editing technology has previously been shown to be a highly efficient tool for generating gene disruptions in CHO cells. In this study we further demonstrate the applicability and efficiency of CRISPR/Cas9 genome editing by disrupting *FUT8*, *BAK* and *BAX* simultaneously in a multiplexing setup in CHO cells. To isolate Cas9-expressing cells from transfected cell pools, GFP was linked to the Cas9 nuclease via a 2A peptide. With this method, the average indel frequencies generated at the three genomic loci were increased from 11% before enrichment to 68% after enrichment. Despite the high number of genome editing events in the enriched cell pools, no significant off-target effects were observed from off-target prediction followed by deep sequencing. Single cell sorting of enriched multiplexed cells and deep sequencing of 97 clones revealed the presence of four single, 23 double and 34 triple gene-disrupted cell lines. Further characterization of selected potential triple knockout clones confirmed the removal of Bak and Bax protein and disrupted fucosylation activity as expected. The knockout cell lines showed improved resistance to apoptosis compared to wild-type CHO-S cells. Taken together, multiplexing with CRISPR/Cas9 can accelerate genome engineering efforts in CHO cells even further.
Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway

Chinese hamster ovary (CHO) cells are the most widely used mammalian hosts for production of therapeutic proteins. However, development of recombinant CHO cell lines has been hampered by unstable and variable transgene expression caused by random integration. Here we demonstrate efficient targeted gene integration into site-specific loci in CHO cells using CRISPR/Cas9 genome editing system and compatible donor plasmid harboring a gene of interest (GOI) and short homology arms. This strategy has enabled precise insertion of a 3.7-kb gene expression cassette at defined loci in CHO cells following a simple drug-selection, resulting in homogeneous transgene expression. Taken together, the results displayed here can help pave the way for the targeting of GOI to specific loci in CHO cells, increasing the likelihood of generating isogenic cell lines with consistent protein production.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Contributors: Lee, J. S., Beuchert Kallehauge, T., Pedersen, L. E., Kildegaard, H. F.
Number of pages: 11
Publication date: 2015
Peer-reviewed: Yes

Publication information
Journal: Scientific Reports
Volume: 5
Article number: 8572
ISSN (Print): 2045-2322
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.36 SJR 1.533 SNIP 1.245
Versatile microscale screening platform for improving recombinant protein productivity in Chinese hamster ovary cells

Chinese hamster ovary (CHO) cells are widely used as cell factories for the production of biopharmaceuticals. In contrast to the highly optimized production processes for monoclonal antibody (mAb)-based biopharmaceuticals, improving productivity of non-mAb therapeutic glycoproteins is more likely to reduce production costs significantly. The aim of this study was to establish a versatile target gene screening platform for improving productivity for primarily non-mAb glycoproteins with complete interchangeability of model proteins and target genes using transient expression. The platform consists of four techniques compatible with 96-well microplates: lipid-based transient transfection, cell cultivation in microplates, cell counting and antibody-independent product titer determination based on split-GFP complementation. We were able to demonstrate growth profiles and volumetric productivity of CHO cells in 96-half-deepwell microplates comparable with those obtained in shake flasks. In addition, we demonstrate that split-GFP complementation can be used to accurately measure relative titers of therapeutic glycoproteins. Using this platform, we were able to detect target gene-specific increase in titer and specific productivity of two non-mAb glycoproteins. In conclusion, the platform provides a novel miniaturized and parallelisable solution for screening target genes and holds the potential to unravel genes that can enhance the secretory capacity of CHO cells.

General information
Accelerating Genome Editing in CHO Cells Using CRISPR Cas9 and CRISPy, a Web-Based Target Finding Tool

Chinese hamster ovary (CHO) cells are widely used in the biopharmaceutical industry as a host for the production of complex pharmaceutical proteins. Thus, genome engineering of CHO cells for improved product quality and yield is of great interest. Here, we demonstrate for the first time the efficacy of the CRISPR Cas9 technology in CHO cells by generating site-specific gene disruptions in COSMC and FUT8, both of which encode proteins involved in glycosylation. The tested single guide RNAs (sgRNAs) created an indel frequency up to 47.3% in COSMC, while an indel frequency up to 99.7% in FUT8 was achieved by applying lectin selection. All eight sgRNAs examined in this study resulted in relatively high indel frequencies, demonstrating that the Cas9 system is a robust and efficient genome editing methodology in CHO cells. Deep sequencing revealed that 85% of the indels created by Cas9 resulted in frameshift mutations at the target sites, with a strong preference for single base indels. Finally, we have developed a user-friendly bioinformatics tool, named “CRISPy” for rapid identification of sgRNA target sequences in the CHO-K1 genome. The CRISPy tool identified 1,970,449 CRISPR targets divided into 27,553 genes and lists the number of off-target sites in the genome. In conclusion, the proven functionality of Cas9 to edit CHO genomes combined with our CRISPy database have the potential to accelerate genome editing and synthetic biology efforts in CHO cells.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, iLoop, CHO Cell Line Engineering and Design, Research Groups
Pages: 1604-1616
Publication date: 2014
Peer-reviewed: Yes

Publication information
Journal: Biotechnology and Bioengineering (Print)
Volume: 111
Issue number: 8
ISSN (Print): 0006-3592
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.07 SJR 1.372 SNIP 1.186
Web of Science (2017): Impact factor 3.952
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.14 SJR 1.447 SNIP 1.178
Web of Science (2016): Impact factor 4.481
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.44 SJR 1.632 SNIP 1.355
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.16 SJR 1.612 SNIP 1.395
Web of Science (2014): Impact factor 4.126
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.44 SJR 1.637 SNIP 1.427
Web of Science (2013): Impact factor 4.164
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
A Versatile System for USER Cloning-Based Assembly of Expression Vectors for Mammalian Cell Engineering

A new versatile mammalian vector system for protein production, cell biology analyses, and cell factory engineering was developed. The vector system applies the ligation-free uracil-excision based technique – USER cloning – to rapidly construct mammalian expression vectors of multiple DNA fragments and with maximum flexibility, both for choice of vector backbone and cargo. The vector system includes a set of basic vectors and a toolbox containing a multitude of DNA building blocks including promoters, terminators, selectable marker- and reporter genes, and sequences encoding an internal ribosome entry site, cellular localization signals and epitope- and purification tags. USER cloning with FASTs allows rapid swaps of gene, promoter or selection marker in existing plasmids and simple construction of vectors encoding proteins, which are fused to fluorescence-, purification-, localization-, or epitope tags. The mammalian expression vector assembly platform currently allows for the assembly of up to seven fragments in a single cloning step with correct directionality and with a cloning efficiency above 90%. The functionality of basic vectors for FAST assembly was tested and validated by transient expression of fluorescent model proteins in CHO, U-2-OS and HEK293 cell lines. In this test, we
included many of the most common vector elements for heterologous gene expression in mammalian cells, in addition the system is fully extendable by other users. The vector system is designed to facilitate high-throughput genome-scale studies of mammalian cells, such as the newly sequenced CHO cell lines, through the ability to rapidly generate high-fidelity assembly of customizable gene expression vectors.

**General information**
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Center for Biological Sequence Analysis, Systems Biotechnology, Eucaryotic Molecular Cell Biology, Technical University of Denmark
Number of pages: 10
Publication date: 2014
Peer-reviewed: Yes

**Publication information**
Journal: P L o S One
Volume: 9
Issue number: 5
Article number: e96693
ISSN (Print): 1932-6203
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.01 SJR 1.164 SNIP 1.111
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4.15 SJR 1.982 SNIP 1.156
Web of Science (2012): Impact factor 3.73
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
Web of Science (2011): Impact factor 4.092
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.705 SNIP 1.178
Web of Science (2010): Impact factor 4.411
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Engineering amino acid supply pathways in Chinese hamster ovary cells

General information
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Number of pages: 1
Publication date: 2014
Peer-reviewed: No
Event: Poster session presented at 9th Danish Conference on Biotechnology and Molecular Biology, Denmark.
Source: PublicationPreSubmission
Source-ID: 93488748
Research output: Research - peer-review › Journal article – Annual report year: 2014

Protein network reconstruction of CHO cell secretory pathway

General information
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Novo Nordisk AS
Publication date: 2014
Peer-reviewed: Yes
Event: Poster session presented at CHOgenome workshop, Vienna, Austria.
Electronic versions:
Vienna_poster_final.pdf
Source: PublicationPreSubmission
Source-ID: 93296797
Research output: Research - peer-review › Poster – Annual report year: 2014

Protein network reconstruction of CHO cell secretory pathway

General information
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
Contributors: Lund, A. M., Kildegaard, H. F., Andersen, M. R.
Publication date: 2014
Peer-reviewed: Yes
Event: Poster session presented at 9th Danish Conference on Biotechnology and Molecular Biology, Denmark.
Source: PublicationPreSubmission
Source-ID: 93296797
Research output: Research - peer-review › Poster – Annual report year: 2014
Toward genome-scale models of the Chinese hamster ovary cells: incentives, status and perspectives

Bioprocessing of the important Chinese hamster ovary (CHO) cell lines used for the production of biopharmaceuticals stands at the brink of several redefining events. In 2011, the field entered the genomics era, which has accelerated omics-based phenotyping of the cell lines. In this review we describe one possible application of this data: the generation of computational models for predictive and descriptive analysis of CHO cellular metabolism. We describe relevant advances in other organisms and how they can be applied to CHO cells. The immediate implications of the implementation of these methods will be accelerated development of the next generation of CHO cell lines and derived biopharmaceuticals.

General information
State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Symphogen A/S, Novo Nordisk AS
Pages: 437-448
Publication date: 2014
Peer-reviewed: Yes

Publication information
Journal: Pharmaceutical Bioprocessing
Volume: 2
Issue number: 5
ISSN (Print): 2048-9145
Ratings:
Web of Science (2019): Indexed yes
Web of Science (2018): Indexed yes
Web of Science (2017): Indexed yes
Scopus rating (2016): SJR 0.125 SNIP 0
Scopus rating (2015): SJR 0.124 SNIP 0
ISI indexed (2013): ISI indexed no
Original language: English
DOIs: 10.4155/PBP.14.54
Source: PublicationPreSubmission
Source-ID: 102790403
Research output: Research - peer-review > Journal article – Annual report year: 2014

A validated system for ligation-free USER™-based assembly of expression vectors for mammalian cell engineering

The development in the field of mammalian cell factories require fast and high-throughput methods, this means a high need for simpler and more efficient cloning techniques. For optimization of protein expression by genetic engineering and for allowing metabolic engineering in mammalian cells, a new versatile expression vector system was developed. This vector system applies the ligation-free uracilexcision cloning technique to construct mammalian expression vectors of multiple parts and with maximum flexibility.

General information
State: Published
Organisations: Department of Systems Biology, Center for Microbial Biotechnology, Novo Nordisk Foundation Center for Biosustainability, Novozymes AS
Publication date: 2013
Peer-reviewed: Yes
Event: Poster session presented at 23rd ESACT meeting, Lille, France.
Electronic versions:
prod21382433941387.ESACT_poster_final.pdf
URLs:
http://www.lepublicsystemepco.com/events.php?IDManif=640&IDModule=71&IDRub=155
Source: dtu
Source-ID: u::9087
Research output: Research - peer-review > Poster – Annual report year: 2013

RNA-seq based expression analysis of the CHO cell protein secretion pathway

The Chinese hamster ovary (CHO) cell-line is the predominant mammalian industrial cell line being used to produce recombinant therapeutic proteins. Although CHO cells have been used for more than 25 years, the genome sequence was first published in 2011. So far there have been limited studies of the cell biology of the CHO cell and the potential of cell
line engineering. To elucidate the poorly understood cellular processes that control and limit recombinant protein production and secretion, a system-wide study was initiated to identify possible engineering targets relevant for therapeutic protein production.

**General information**

State: Published
Organisations: Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories, Novo Nordisk Foundation Center for Biosustainability, Center for Microbial Biotechnology, Novo Nordisk AS
Publication date: 2013
Peer-reviewed: Yes
Event: Poster session presented at 3rd Annual Next Generation Sequencing Asia Congress, Singapore, Singapore.
Electronic versions:

prod21382433561057.NGS_AsiRNA_seq_based_expression_analysis_of_the_CHO_cell_protein_secretion_pathway.pdf

http://www.ngsasia-congress.com/
Source: dtu
Source-ID: u::9086
Research output: Research - peer-review > Poster – Annual report year: 2013

**The emerging CHO systems biology era: harnessing the ‘omics revolution for biotechnology**

Chinese hamster ovary (CHO) cells are the primary factories for biopharmaceuticals because of their capacity to correctly fold and post-translationally modify recombinant proteins compatible with humans. New opportunities are arising to enhance these cell factories, especially since the CHO-K1 cell line was recently sequenced. Now, the CHO systems biology era is underway. Critical ‘omics data sets, including proteomics, transcriptomics, metabolomics, fluxomics, and glycomics, are emerging, allowing the elucidation of the molecular basis of CHO cell physiology. The incorporation of these data sets into mathematical models that describe CHO phenotypes will provide crucial biotechnology insights. As ‘omics technologies and computational systems biology mature, genome-scale approaches will lead to major innovations in cell line development and metabolic engineering, thereby improving protein production and bioprocessing.

**General information**

State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Johns Hopkins University, Harvard Medical School
Contributors: Kildegaard, H. F., Baycin-Hizal, D., Lewis, N., Betenbaugh, M.
Pages: 1102-1107
Publication date: 2013
Peer-reviewed: Yes

**Publication information**

Journal: Current Opinion in Biotechnology
Volume: 24
Issue number: 6
ISSN (Print): 0958-1669
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 8.45 SJR 3.202 SNIP 2.205
Web of Science (2017): Impact factor 8.38
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.55 SJR 3.367 SNIP 2.115
Web of Science (2016): Impact factor 9.294
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 7.99 SJR 3.113 SNIP 2.137
Web of Science (2015): Indexed yes
A versatile expression vector system for mammalian cell factories

The development of the field of mammalian cell factories requests fast and high-throughput methods which means high need for simpler and more efficient cloning techniques. This project applies the ligation-free USERTM (uracil-specific excision reagent) cloning technique to construct mammalian expression vectors with maximum flexibility.
Exploring the Role of Ubiquitylation in the Mammalian DNA Damage Response

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Kildegaard, H. F.
Number of pages: 105
Publication date: Apr 2009

Publication information
Place of publication: Kgs. Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
ISBN (Print): 87-91-49447-8
Original language: English

USP7 counteracts SCFβTrCP-but not APC/Cdh1-mediated proteolysis of Claspin
Claspin is an adaptor protein that facilitates the ataxia telangiectasia and Rad3-related (ATR)-mediated phosphorylation and activation of Chk1, a key effector kinase in the DNA damage response. Efficient termination of Chk1 signaling in mitosis and during checkpoint recovery requires SCFβTrCP-dependent destruction of Claspin. Here, we identify the deubiquitylating enzyme ubiquitin-specific protease 7(USP7) as a novel regulator of Claspin stability. Claspin and USP7 interact in vivo, and USP7 is required to maintain steady-state levels of Claspin. Furthermore, USP7-mediated deubiquitylation markedly prolongs the half-life of Claspin, which in turn increases the magnitude and duration of Chk1 phosphorylation in response to genotoxic stress. Finally, we find that in addition to the M phase-specific, SCFβTrCP-mediated degradation, Claspin is destabilized by the anaphase-promoting complex (APC) and thus remains unstable in G1. Importantly, we demonstrate that USP7 specifically opposes the SCFβTrCP-but not APC/Cdh1-mediated degradation of Claspin. Thus, Claspin turnover is controlled by multiple ubiquitylation and deubiquitylation activities, which together provide a flexible means to regulate the ATR-Chk1 pathway.

General information
State: Published
Organisations: Danish Cancer Society
Contributors: Kildegaard, H. F., Bekker-Jensen, S., Bartek, J., Lukas, J., Mailand, N.
Number of pages: 7
Pages: 13-19
Publication date: 2009
Peer-reviewed: Yes

Publication information
Journal: The Journal of Cell Biology
Volume: 184
Issue number: 1
ISSN (Print): 0021-9525
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins

Accumulation of repair proteins on damaged chromosomes is required to restore genomic integrity. However, the mechanisms of protein retention at the most destructive chromosomal lesions, the DNA double-strand breaks (DSBs), are poorly understood. We show that RNF8, a RING-finger ubiquitin ligase, rapidly assembles at DSBs via interaction of its FHA domain with the phosphorylated adaptor protein MDC1. This is accompanied by an increase in DSB-associated ubiquitylations and followed by accumulation of 53BP1 and BRCA1 repair proteins. Knockdown of RNF8 or disruption of its FHA or RING domains impaired DSB-associated ubiquitylation and inhibited retention of 53BP1 and BRCA1 at the DSB sites. In addition, we show that RNF8 can ubiquitylate histone H2A and H2AX, and that its depletion sensitizes cells to ionizing radiation. These data suggest that MDC1-mediated and RNF8-executed histone ubiquitylation protects genome integrity by licensing the DSB-flanking chromatin to concentrate repair factors near the DNA lesions.
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
BFI (2009): BFI-level 2
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 25.274 SNIP 5.606
Scopus rating (2007): SJR 25.228 SNIP 5.524
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 23.831 SNIP 5.516
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 25.262 SNIP 5.498
Scopus rating (2004): SJR 25.704 SNIP 5.578
Scopus rating (2003): SJR 28.284 SNIP 5.48
Scopus rating (2002): SJR 25.539 SNIP 5.343
Scopus rating (2001): SJR 29.247 SNIP 5.591
Scopus rating (2000): SJR 47.796 SNIP 6.399
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 43.449 SNIP 6.762
Original language: English
Keywords: DNA , Proteins, Signaling
DOIs:
10.1016/j.cell.2007.09.040
Source: FindIt
Source-ID: 32232052
Research output: Research - peer-review Journal article – Annual report year: 2007

Projects:

**CHO cell line engineering for improved production of challenging therapeutic proteins**

Sergeeva, D., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Nielsen, L. K., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Pedersen, L. E., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Min Lee, G., Supervisor
Grav, L. M., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Fonde
01/09/2017 → 31/08/2020
Award relations: CHO cell line engineering for improved production of challenging therapeutic proteins
Project: PhD

**Regulering af kromatins omstrukturering efter DNA beskadigelse og dennes effekt på DNA reparation og celle cyklus checkpoint**

Kildegaard, H. F., PhD Student
Mortensen, U. H., Main Supervisor
Lukas, J., Supervisor
Workman, C., Examiner
Medema, R., Examiner
Sørensen, C. S., Examiner
Ansat ekstern
01/05/2006 → 01/04/2009
Award relations: Regulering af kromatins omstrukturering efter DNA beskadigelse og dennes effekt på DNA reparation og celle cyklus checkpoint
Project: PhD
Development of novel genome engineering tools to improve CHO cell factories
Julie la Cour Karottki, K., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Lee, J. S., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Nørholm, M., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Lewis, N. E., Supervisor
Samfinansierede - Virksomhed
01/03/2016 → 28/02/2019
Award relations: Development of novel genome engineering tools to improve CHO cell factories
Project: PhD

Development of genome editing tools and identification of targets for CHO cell factory engineering
Pristovsek, N., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Andersen, M. R., Supervisor, Department of Biotechnology and Biomedicine
Hansen, H. G., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Nørholm, M., Examiner, Novo Nordisk Foundation Center for Biosustainability
Andersen, C. R., Examiner
Barron, N., Examiner
Andersen, M. R., Main Supervisor, Department of Biotechnology and Biomedicine
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Andersen, C. R., Examiner
Marie Curie (EU-stipendium)
01/09/2015 → 10/12/2018
Award relations: Development of genome editing tools and identification of targets for CHO cell factory engineering
Project: PhD

Engineering of a by-product-reduced CHO cell line (CleanCHO)
Pereira, S., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Andersen, M. R., Main Supervisor, Department of Biotechnology and Biomedicine
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Jensen, M. K., Examiner, Novo Nordisk Foundation Center for Biosustainability
Åkesson, M. F., Examiner, Department of Systems Biology
Chotteau, V., Examiner
Marie Curie (EU-stipendium)
15/09/2015 → 09/11/2018
Award relations: Engineering of a by-product-reduced CHO cell line (CleanCHO)
Project: PhD

Design of Optimal CHO Protein N-glycosylation Profiles
Amann, T., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Andersen, M. R., Main Supervisor, Department of Biotechnology and Biomedicine
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Mortensen, U. H., Examiner, Department of Biotechnology and Biomedicine
Bolt, G., Examiner
Fischer, S., Examiner
Marie Curie (EU-stipendium)
01/09/2015 → 14/01/2019
Award relations: Design of Optimal CHO Protein N-glycosylation Profiles
Project: PhD

Studies in CHO genomics and transportation networks
Singh, A., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Andersen, M. R., Main Supervisor, Department of Biotechnology and Biomedicine
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Herrgard, M., Examiner, Novo Nordisk Foundation Center for Biosustainability
Kaas, C. S., Examiner, Department of Systems Biology
Borth, N., Examiner
Marie Curie (EU-stipendium)
01/09/2015 → 31/08/2018
Award relations: Studies in CHO genomics and transportation networks
Accelerated and rational design of Improved CHO Cell factories
Grav, L. M., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Andersen, M. R., Supervisor, Department of Biotechnology and Biomedicine
Lee, J. S., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Kaas, C. S., Examiner, Department of Systems Biology
Otte, K., Examiner

01/12/2014 → 18/04/2018
Award relations: Accelerated and rational design of Improved CHO Cell factories

Project: PhD

Developing and implementing high throughput methods for the analysis of glycans
Hansen, A. H., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Madsen, R., Main Supervisor, Department of Chemistry
Harrison, S. J., Supervisor, CFB - Core Flow
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Grundforskningsfonden

01/12/2014 → 17/03/2019
Award relations: Developing and implementing high throughput methods for the analysis of glycans

Project: PhD

Engineering amino acid supply pathways in CHO cells expressing Biopharmaceuticals
Ley, D., PhD Student
Andersen, M. R., Main Supervisor
Kildegaard, H. F., Supervisor
Workman, C., Examiner
Dickson, A. J., Examiner
Müller, C., Examiner, Department of Systems Biology

Institut stipendie (DTU)
15/12/2013 → 25/08/2017
Award relations: Engineering amino acid supply pathways in CHO cells expressing Biopharmaceuticals

Project: PhD

System-wide studies of cell biology of CHO cells
Lund, A. M., PhD Student, Department of Systems Biology
Andersen, M. R., Main Supervisor, Department of Biotechnology and Biomedicine
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Mortensen, U. H., Supervisor, Department of Biotechnology and Biomedicine
Workman, C., Examiner, Department of Biotechnology and Biomedicine

Clarke, C., Examiner
Tolstrup, A. B., Examiner
Clarke, C., Examiner
Tolstrup, A. B., Examiner

Institut stipendie (DTU) Samf.
01/06/2012 → 16/06/2016
Award relations: System-wide studies of cell biology of CHO cells

Project: PhD

Microbial platform for expression of membrane integrated enzymes and sustainable production of high value chemicals
Segaard, K. M., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Nørholm, M., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Nielsen, A. T., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Kildegaard, H. F., Examiner, Novo Nordisk Foundation Center for Biosustainability
Daley, D. O., Examiner
Pedersen, P. A., Examiner
Daley, D. O., Examiner

Institut stipendie (DTU)
01/12/2012 → 27/01/2016
Award relations: Microbial platform for expression of membrane integrated enzymes and sustainable production of high value chemicals
Project: PhD

**microRNA based Chinese hamster ovary cell engineering towards improved recombinant protein N-glycosylation**
Fan, Y., Project Participant, Department of Systems Biology, Novo Nordisk Foundation Center for Biosustainability, Network Engineering of Eukaryotic Cell Factories, CHO Cell Line Engineering and Design
Andersen, M. R., Main Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
01/10/2015 → 31/03/2018
Project: Research

**Accelerated and rational design of improved CHO cell factories for production of biopharmaceuticals**
Grav, L. M., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Lee, J. S., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Andersen, M. R., Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
01/12/2014 → 30/11/2017
Project: Research

**Development of novel genome engineering tools to improve CHO cell factories**
Julie la Cour Karottki, K., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Lee, J. S., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
01/03/2016 → 28/02/2019
Project: Research

**Design of optimal CHO glycosylation profiles**
Amann, T., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Andersen, M. R., Main Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
01/09/2015 → 31/08/2018
Project: Research

**Engineering nutrient and by-product metabolism**
Pereira, S., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Andersen, M. R., Main Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
15/09/2015 → 14/09/2018
Project: Research

**Genomics, transcriptomics and transcription networks**
Singh, A., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Andersen, M. R., Main Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
01/09/2015 → 31/08/2018
Project: Research

**Genome editing tools and target screening**
Pristovsek, N., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Andersen, M. R., Supervisor, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
Hansen, H. G., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
01/09/2015 → 31/08/2018
Project: Research

Enhancement of therapeutic protein production in CHO cells: Coping with the ER stress
FTP post doc stipend
Kwang Ha, T., Project Participant, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Kildegaard, H. F., Supervisor, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
01/04/2016 → 31/03/2019
Project: Research

eCHO Systems: Enhancing CHO by Mammalian Systems Biotechnology
Innovative Training Network (ITN) under Horizon 2020
Andersen, M. R., Project Coordinator, Department of Systems Biology, Network Engineering of Eukaryotic Cell Factories
Kildegaard, H. F., Project Manager, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Pristovsek, N., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Pereira, S., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Amann, T., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Singh, A., PhD Student, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Lohmann, R., Contact Person, Novo Nordisk Foundation Center for Biosustainability
FP7 Contract ID: 642663
Horizon 2020 MSCA ITN
01/01/2015 → 31/12/2018
Keywords: synthetic biotechnology, cell factory optimization, systems biology, industrial biotechnology, biopharmaceuticals
Award relations: Enhancing CHO by Mammalian Systems Biotechnology
Project: Research

Activities:

27th ESACT meeting
Period: 5 May 2019 → 8 May 2019
Helene Faustrup Kildegaard (Organizer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event

27th ESACT meeting
05/05/2019 → 08/05/2019
Activity: Attending an event › Participating in or organising a conference

Period: 18 May 2018
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event

World Advanced Therapies and Regenerative Medicine congress

Period: 24 Apr 2018
Helene Fastrup Kildegaard (Invited speaker)

Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event

Bioprocessing International
23/04/2018 → 25/04/2018
Activity: Talks and presentations › Conference presentations

Novo Nordisk Foundation Cross Cluster Collaboration Event "Talking about CRISPR"

Period: 5 Apr 2018
Helene Fastrup Kildegaard (Organizer)

Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Description
Novo Nordisk Foundation Cross Cluster Collaboration Event "Talking about CRISPR" Copenhagen, Denmark
Degree of recognition: National

Related event

Novo Nordisk Foundation Cross Cluster Collaboration Event "Talking about CRISPR"
05/04/2018 → ...
Activity: Attending an event › Participating in or organising a conference

Towards efficient and controlled genome engineering of CHO Cell Factories. Fujifilm Diosynth Biotechnologies, Billingham, U.K.

Period: 8 Mar 2018
Helene Fastrup Kildegaard (Guest lecturer)

Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation

Fujifilm Diosynth Biotechnologies
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

CRISPR-mediated genome engineering. Symphogen, Copenhagen, Denmark

Period: 2 Mar 2018
Helene Fastrup Kildegaard (Guest lecturer)

Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: Local

Related external organisation

Symphogen A/S
Denmark
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations
What do Chinese hamsters and therapeutics have in common? Wine and Science event, Natural History Museum of Denmark, Copenhagen, Denmark.
Period: 1 Mar 2018
Helene Faustrup Kildegaard (Speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: National

Related event
Wine and Science event
01/03/2018 → …
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

CRISPR-mediated genome engineering. Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, Denmark
Period: 15 Jan 2018
Helene Faustrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related event
NNF event
15/01/2018 → …
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Tools for improved genome engineering of CHO cell factories, KAIST, Korea
Period: 31 Oct 2017
Helene Faustrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation
Korean Advanced Institute of Science and Technology (KAIST)
Daejeon, Korea, Democratic People’s Republic of
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Improving CHO cell factories with CRISPR-mediated genome engineering. 4th Annual BioProNET Science Symposium, University of Warwick, UK
Period: 10 Oct 2017
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related organisation
Improving CHO cell factories with CRISPR-mediated genome engineering. 4th Annual BioProNET Science Symposium, University of Warwick, UK
Kildegaard, H. F. (Invited speaker)
10 Oct 2017
Activity: Talks and presentations › Conference presentations
13th Protein Expression in Animal Cells (PEACe) Conference
Period: 24 Sep 2017 → 28 Sep 2017
Helene Fastrup Kildegaard (Organizer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Description
Organizing Committee Member, 13th PEACe conference, Valencia, Spain
Degree of recognition: International

Related event
13th Protein Expression in Animal Cells (PEACe) Conference
24/09/2017 → 28/09/2017
Valencia, Spain
Activity: Attending an event › Participating in or organising a conference

Girls' Day in Science event. Mærsk Mc-Kinney Møller Science Center, Sørø, Denmark.
Period: 30 Aug 2017
Helene Fastrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: National

Related organisation
Girls' Day in Science event. Mærsk Mc-Kinney Møller Science Center, Sørø, Denmark.
Kildegaard, H. F. (Guest lecturer)
30 Aug 2017
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

CRISPR Tools for CHO Cell Engineering. 9th Bioprocessing Summit, Boston, USA.
Period: 22 Aug 2017
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related organisation
CRISPR Tools for CHO Cell Engineering. 9th Bioprocessing Summit, Boston, USA.
Kildegaard, H. F. (Invited speaker)
22 Aug 2017
Activity: Talks and presentations › Conference presentations

Genome engineering of CHO cell factories. 12th Danish Conference on Biotechnology and Molecular Biology, Vejle, Denmark.
Period: 2 Jun 2017
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event
12th Danish Conference on Biotechnology and Molecular Biology
01/06/2017 → 02/06/2017
Activity: Talks and presentations › Conference presentations
Tools for improved genome engineering of CHO cell factories. 2nd International Advanced Biomanufacturing Conference. Sheffield, UK.
Period: 23 May 2017
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related event
2nd International Advanced Biomanufacturing Conference
22/05/2017 → 23/05/2017
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Towards efficient and controlled genome engineering of CHO Cell Factories. UCB Pharma, Slough, U.K.
Period: 4 May 2017
Helene Fastrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: Local

Related external organisation
UCB Celltech
United Kingdom
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Period: 2 May 2017
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: National

Related event
Ungdommens Naturvidenskabelige Forening (UNF)
02/05/2017 → 02/05/2017
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Period: 25 Apr 2017
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event
Cell Line Development and Engineering
24/04/2017 → 26/04/2017
Activity: Talks and presentations › Conference presentations

Efficient and controlled genome engineering of CHO cell factories. 9th Conference on Recombinant Protein Production (RPP9). Dubrovnik, Croatia.
Period: 24 Apr 2017
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event

7th Recombinant Protein Production (RPP7)
23/04/2017 → 26/04/2017
Activity: Talks and presentations › Conference presentations

CRISPR Technologies, Harvesting the Rare Earth, Overgaden Institute of Contemporary Art.
Period: 24 Feb 2017
Helene Faustrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: National

Related event

Harvesting the Rare Earth by Jacob Remin, Overgaden Institute of Contemporary Art
28/01/2018 → 19/02/2018
Copenhagen, Denmark
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

CHO cell factory engineering, for working towards improved production of therapeutic proteins. 7th Cell Culture World Congress. Munich, Germany.
Period: 22 Feb 2017
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
Degree of recognition: International

Related event

7th Cell Culture World Congress
21/02/2017 → 22/02/2017
Activity: Talks and presentations › Conference presentations

Improving CHO cell factories with CRISPR. European Summit of Industrial Biotechnology. Graz, Austria.
Period: 16 Nov 2016
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
CHO Core

Related external organisation

Unknown external organisation
Activity: Talks and presentations › Conference presentations

Latest advances in applying CRISPR/Cas9 for accelerated and efficient generation of CHO cell factories with improved properties. 2nd Annual Genome Editing Congress. London, UK.
Period: 10 Nov 2016
Helene Faustrup Kildegaard (Invited speaker)
Accelerated genome engineering of CHO cell factories for improved protein production. Bioprocessing Summit. Boston, USA.
Period: 19 Aug 2016
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

CRISPR and CHO cell factories. Johns Hopkins University. Baltimore, USA.
Period: 18 Aug 2016
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Period: 9 Jun 2016
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Generation of desirable CHO cell factories with predictive culture performance using CRISPR/Cas9-mediated genome engineering. Cell Culture Engineering XV. Palm Springs, USA.
Period: 9 May 2016
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design
CRISPR meets CHO cell factories. Club Biotech at University of Natural Resources and Life Sciences (BOKU). Vienna, Austria.
Period: 9 Mar 2016
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations

Application of omics data, screening and genome editing to improve CHO cell factories. 6th annual Cell Culture World Congress. Munich, Germany.
Period: 23 Feb 2016
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations

Period: 3 Feb 2016
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations

Improved CHO cell factories using CRISPR/Cas9 genome editing Technologies. PEGS Europe. Lisbon, Portugal.
Period: 3 Nov 2015
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Description
PEGS Europe

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations

Period: 29 Oct 2015
Helene Faustrup Kildegaard (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
Related external organisation

AstraZeneca Sweden
Montreal, Sweden
Activity: Talks and presentations › Talks and presentations in private or public companies and organisations

Key note PEACe lecture: Application of CRISPR/Cas9-mediated genome engineering for improved protein production in mammalian cells. 12th Protein expression in animal cells (PEACe) conference. San Diego, USA.
Period: 23 Sep 2015
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability

Description
12th Protein expression in animal cells (PEACe) conference. San Diego, USA

Related external organisation

Unknown external organisation
Activity: Talks and presentations › Conference presentations

Period: 31 May 2015
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability

Related event

24th Meeting of the European Society of Animal Cell Technology
31/05/2015 → 03/06/2015
Barcelona, Spain
Activity: Talks and presentations › Conference presentations

Period: 31 May 2015
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability

Related event

24th Meeting of the European Society of Animal Cell Technology
31/05/2015 → 03/06/2015
Barcelona, Spain
Activity: Talks and presentations › Conference presentations

Period: 30 Apr 2015
Helene Fastrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

**Description**
CHo genome tool workshop. University of Sheffield, England

**Related external organisation**

**Unknown external organisation**
Activity: Talks and presentations › Conference presentations

**Optimizing CHO cell line development, design and performance using genome editing Technologies. 5th annual Cell Culture World Congress. Munich, Germany.**
Period: 24 Feb 2015
Helene Fastrup Kildegaard (Invited speaker)

Novo Nordisk Foundation Center for Biosustainability

CHO Cell Line Engineering and Design

**Description**
Cell Culture World Congress. Munich, Germany

**Related external organisation**

**Unknown external organisation**
Activity: Talks and presentations › Conference presentations

**Application of CRISPR Cas9 Technologies to Accelerate Cell Line Development. Informa Cell Line Development and Engineering conference. Dublin, Ireland.**
Period: 11 Feb 2015
Helene Fastrup Kildegaard (Invited speaker)

Novo Nordisk Foundation Center for Biosustainability

CHO Cell Line Engineering and Design

**Description**
Informa Cell Line Development and Engineering conference in Dublin, Ireland

**Related event**

**Informa's Annual Cell Line Development and Engineering Conference**
09/02/2015 → 12/02/2015
Dublin, Ireland
Activity: Talks and presentations › Conference presentations

**Improving CHO Cell Factories Using Systems Biology and Genome Editing Technologies. Cambridge Healthtech Institute’s 14th Annual PepTalk. The protein Science Week. San Diego, USA.**
Period: 22 Jan 2015
Helene Fastrup Kildegaard (Invited speaker)

Novo Nordisk Foundation Center for Biosustainability

CHO Cell Line Engineering and Design

**Description**
Cambridge Healthtech Institute’s 14th Annual PepTalk. The protein Science Week, San Diego, USA

**Related external organisation**

**Unknown external organisation**
Activity: Talks and presentations › Conference presentations
Short course: Genome editing using CRISPR. Cambridge Healthtech Institute's 14th Annual PepTalk. The protein Science Week. San Diego, USA.
Period: 18 Jan 2015
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related external organisation

Unknown external organisation
Activity: Talks and presentations › Conference presentations

Accelerating Genome Editing in CHO Cells using CRISPR Cas9. 9th Danish Conference on Biotechnology and Molecular Biology. Vejle, Denmark.
Period: 22 May 2014
Helene Faustrup Kildegaard (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
CHO Cell Line Engineering and Design

Related event

9th Danish Conference on Biotechnology and Molecular Biology: Symposium on Animal Cell Cultures
22/05/2014 → 23/05/2014
Denmark
Activity: Talks and presentations › Conference presentations

Press clippings:

Interview contribution: Article "CRISPR technologies have changed the ball game" in Biosustain magazine
Helene Faustrup Kildegaard
08/06/2018
Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design

Media contribution (1)

Biosustain magazine
08/06/2018
Denmark
Helene Faustrup Kildegaard
Press/Media: Press / Media

Interview contribution: Article "Tricks of the Trade for Cell Culture optimization" in Genetic Engineering & Biotechnology (GEN)
Helene Faustrup Kildegaard
15/04/2018
Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design

Media contribution (1)

Genetic Engineering & Biotechnology (GEN)
15/04/2018
Denmark
Helene Faustrup Kildegaard
Press/Media: Press / Media

Interview contribution: Article "A new genetic revolution" in Technologist
Helene Faustrup Kildegaard
01/04/2017
Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design
Media contribution (1)
Technologist
01/04/2017
Denmark
Helene Faustrup Kildegaard
Press/Media: Press / Media

Interview contribution: Article "Hamsterceller producerer medicin" in Dynamo
Helene Faustrup Kildegaard
16/09/2016

Subject
Dy
Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design

Media contribution (1)
Dynamo
16/09/2016
Denmark
Helene Faustrup Kildegaard
Press/Media: Press / Media